S01 - Regulation of Contraction by Thick and Thin Filament			
Invited speaker	Luca Fusi King's College London	*Visualising thin- and thick-filament activation across regulatory zones of the sarcomere*	Myosin-dependent regulation of sarcomere contractility is a major determinant of force production and contraction kinetics in striated muscle and represents a key therapeutic target in muscle disease. Myosin-containing thick filaments are organised into P-, C-, and D-zones defined by Myosin-Binding Protein-C (MyBP-C) and titin super-repeats, yet how myosin activity is regulated across these domains remains unclear. Proposed interactions between MyBP-C and the actin-containing thin filament in the C-zone may also influence local thin-filament activation, but their functional significance remains debated. We recently developed a fluorescence polarisation microscopy (FPM) approach to directly visualise structural changes in the myofilaments across these regulatory zones in isolated myofibrils. Rabbit psoas myofibrils were exchanged with bifunctional rhodamine probes attached to either troponin-C (TnC) or the myosin regulatory light chain (RLC), allowing spatially resolved measurements of troponin and myosin conformation with ~400 nm resolution. We found that myosin motors in relaxed myofibrils are preferentially folded within the C-zone, providing direct in situ evidence that MyBP-C stabilises the myosin OFF state. Titin-based passive tension and calcium activation induced zone-specific OFF-to-ON transitions, with motors in the P- and C-zones displaying greater OFF-state stability than those in the D-zone. Using TnC probes, we further show that myosin-dependent structural changes in the thin filament propagate from the A-band to the I-band during contraction, consistent with a force-dependent activation mechanism. Together, these findings reveal zone-specific regulation of thin and thick filaments in skeletal muscle and establish FPM as a powerful tool for investigating disease-associated alterations in myofilament regulation.
Invited speaker	Neil Kad University of Kent, Canterbury	*Imaging the molecular origins of the cardiac reserve and its implications for cardiac disease*	Contraction of striated cardiac muscle is regulated through dual filament control mechanisms. In β-cardiac myosin, motor heads exist in either a biochemically ON or disordered relaxed (DRX) state, which is available for actin interaction, or an OFF super-relaxed (SRX) state, which is functionally inactive. While the SRX state is increasingly recognised as a critical regulator of contractility and energetic efficiency, its spatial distribution along the thick filament has remained largely unresolved. To address this question, we spatially mapped myosin activity within cardiac left ventricular sarcomeres using single-molecule imaging of fluorescently labelled ATP. By localising individual nucleotide turnover events to defined regions of the thick filament, we directly quantified the distribution of DRX and SRX myosin populations in situ. We found that SRX occupancy was highest in the C-zone, where 51.5% of myosin heads resided in the SRX state, compared with 46.1% in the D-zone and an overall filament average of 44.3%. These findings reveal pronounced regional heterogeneity in thick filament regulation, consistent with structurally mediated control of myosin availability. Building on this work, we are developing a high-resolution, high-throughput mapping platform capable of assigning activity with crown-level (~15 nm) spatial precision. This approach will enable near-molecular-scale localisation of myosin regulatory states along the filament backbone. Together, these studies establish quantitative spatial mapping of β-cardiac myosin regulatory states in healthy and diseased conditions, providing a powerful framework for understanding dual filament regulation with unprecedented spatial resolution.
Contributed talk	Weikang M Illinois Institute of Technology	*Porcine Left Atrial and Ventricular Thick Filaments Exhibit Distinct Resting Structures and Calcium-dependent Responses*	The heart maintains systemic perfusion through the coordinated function of its four chambers. Each chamber has distinct structural, functional, and molecular properties tailored to its role in circulation, which may result in chamber-specific differences in myofilament structure and regulation between atria and ventricles. To test this hypothesis, we employed muscle mechanics and X-ray diffraction to investigate functional and structural differences in porcine left atrial (LA) and left ventricular (LV) tissue. Here, we report the first X-ray diffraction study of atrial tissue, demonstrating that under resting conditions, myosin filaments in LA adopted a more ON-like, structurally distinct configuration compared with those in LV. Under contracting conditions, LV generated greater force and exhibited higher sinusoidal stiffness than LA across multiple calcium concentrations. LA showed faster kTR than in LV, with no calcium-dependence, in contrast to the calcium-dependence of kTR seen in LV. Structurally, the distinct myosin head configuration seen in the relaxed LA persisted during contraction. Furthermore, using the troponin inhibitor MYK-7660 to inhibit active contraction, we showed that, unlike LV, LA showed no direct calcium-dependent thick filament activation, reconciling discrepancies between fast rat and slow porcine ventricular myocardium regarding calcium's role in thick filament regulation. Altogether, our study reveals that LA myosin filaments adopt a molecular architecture and regulatory mechanism distinct from their LV counterparts, suggesting that myosin filament structure and regulation have evolved differently to meet the unique functional demands of each cardiac chamber. Moreover, understanding atrial-specific regulatory mechanisms provides new insights into therapeutic strategies for atrial diseases.

Contributed talk	Belinda Bullard Department of Biology, University of York	*Tropomyosin as a stretch sensor in the activation of insect flight muscle*	Insect flight muscle (IFM) is activated by rapid stretches produced by alternating contractions of opposing muscles in the thorax. High frequency contractions occur at constant priming levels of calcium and the stiffness of flight muscle ensures that small length changes are rapidly transmitted to thick and thin filaments. Troponin bridges, observed between thick and thin filaments by EM and X-ray diffraction in relaxed Lethocerus muscle, are possible stretch-sensors. The bridges contact troponin and are midway between target areas for force-producing crossbridges on actin. The tropomyosin-troponin complex (Tm-Tn) is likely to bind to bridging myosin-S1. However, it is Tm in the complex that binds S1, not Tn. There are two isoforms of Tm in IFM: Tm1 binds to S1 with nanomolar affinity and Tm2 does not bind to S1. Because they form homodimers in the IFM, the two Tm isoforms could act independently. EM images show that, when added to isolated thick filaments, the complex binds to the filaments with a periodicity of ~40 nm. Tm-Tn or Tm1 alone often cross link thick filaments, which is observed in both EM and AFM (tapping mode) images. Tm1 and Tm2 have residues that occur as mutations causing cardiomyopathy. The mutation D230N in Tm1 would stabilise and E62Q in Tm2 would destabilise the blocked state on actin. In our model for activation of IFM, Tn-bridges move Tm1 to an activating position in response to stretch, followed by rapid movement of Tm2 to an activating position. The associated Tn would prevent activation of relaxed fibres.
P01.01	Belinda Bullard Department of Biology, University of York	*Tropomyosin as a stretch sensor in the activation of insect flight muscle*	Insect flight muscle (IFM) is activated by rapid stretches produced by alternating contractions of opposing muscles in the thorax. High frequency contractions occur at constant priming levels of calcium and the stiffness of flight muscle ensures that small length changes are rapidly transmitted to thick and thin filaments. Troponin bridges, observed between thick and thin filaments by EM and X-ray diffraction in relaxed Lethocerus muscle, are possible stretch-sensors. The bridges contact troponin and are midway between target areas for force-producing crossbridges on actin. The tropomyosin-troponin complex (Tm-Tn) is likely to bind to bridging myosin-S1. However, it is Tm in the complex that binds S1, not Tn. There are two isoforms of Tm in IFM: Tm1 binds to S1 with nanomolar affinity and Tm2 does not bind to S1. Because they form homodimers in the IFM, the two Tm isoforms could act independently. EM images show that, when added to isolated thick filaments, the complex binds to the filaments with a periodicity of ~40 nm. Tm-Tn or Tm1 alone often cross link thick filaments, which is observed in both EM and AFM (tapping mode) images. Tm1 and Tm2 have residues that occur as mutations causing cardiomyopathy. The mutation D230N in Tm1 would stabilise and E62Q in Tm2 would destabilise the blocked state on actin. In our model for activation of IFM, Tn-bridges move Tm1 to an activating position in response to stretch, followed by rapid movement of Tm2 to an activating position. The associated Tn would prevent activation of relaxed fibres.
P01.02	Laurin Hanft University of Missouri	*Afterload Dependence of Cardiac Function Is Regulated by Cardiac Myosin Binding Protein C (cMyBP C)*	The sarcomeric protein, cardiac myosin binding protein-C (cMyBP-C), binds to myosin in the thick filament and plays a key role in regulating cardiac myocyte contraction. Our laboratory has shown that permeabilized cardiac myocytes lacking cMyBP-C exhibit disproportionately faster shortening velocities and greater power at high loads. Also, high-resolution X-ray diffraction studies of cardiac trabeculae demonstrate that myosin cross-bridges within the cMyBP-C zone are the most active during loaded contractions. Together, these findings implicate cMyBP-C as a molecular load sensor. We tested the hypothesis that cMyBP-C is a tunable load sensor that matches cardiac afterload with left ventricular (LV) performance. We compared the afterload dependence of LV power in isolated hearts from mice lacking cMyBP-C (KO) and from transgenic mice expressing wild type cMyBP-C (WT), pseudo-phosphorylated cMyBP-C (t3SD), or non-phosphorylatable cMyBP-C (t3SA). cMyBP-C KO hearts exhibited minimal changes in LV power across afterloads ranging from 40 to 60 mmHg. In contrast, WT hearts showed a markedly steeper afterload dependence of LV power. We next examined whether cMyBP-C mediated load sensing is modulated by phosphorylation. Hearts expressing pseudo-phosphorylated cMyBP-C (t3SD) displayed a steep afterload dependence of LV power, whereas hearts expressing non-phosphorylatable cMyBP-C (t3SA) exhibited a blunted afterload response. These findings indicate that cMyBP-C acts as a tunable molecular load sensor that couples myofilament power generation to cardiac hemodynamics. Ongoing studies are testing whether load sensing can be restored in isolated cMyBP-C KO hearts using myosin inhibitors that mimic cMyBP-C function, and whether these mechanisms extend to in vivo hearts.
P01.03	Anthony Hessel University of Muenster	*Increasing I-Band Titin Compliance Shifts the Structure and Function of Intact Rat EDL Muscle During Peak Twitch and Tetanic Contraction Towards a Less Active State*	Skeletal muscle contraction is not merely an all-or-nothing response but is instead regulated by various sarcomeric proteins. The titin protein is a long filament that extends across the half-sarcomere as a freely-extensible viscoelastic element between the thin and thick filaments in the I-band, and runs along the thick filament backbone in the A-band. Current evidence points towards a role for I-band titin as a regulator of contractile performance, partially determined by the viscoelastic properties. To explore this feature, we studied intact rat extensor digitorum longus (EDL) muscle with a spontaneous global knockout of RBM20, leading to a longer and more compliant I-band titin. Twitch and maximal tetanus contractions were produced in parallel with time-resolved (5 ms frames) small-angle X-ray diffraction to track thick filament structures. Results indicate that homozygous muscle presents mechanical and structural signatures of impaired thick filament activation, and this is more pronounced in twitch than in tetanus. We further report on the activation-dependence of the A-band titin-based ~3.9 nm reflection (e.g., M11) that arises in the C-zone from periodic titin kinks, though to stabilization of some myosin heads in the docked / OFF configuration. We report that the titin M11 reflections undergo a noticeable change with activation, where one periodicity disappears (~3.90 nm) while another emerges (~3.97 nm), in relation to the relative activation state, suggesting an activation-dependent change in the A-band titin configuration. Taken together, our results demonstrate that modifying I-band viscoelastic properties can alter contraction performance by regulating, but not impairing, thick filament contraction machinery.

P01.04	Venus Joumaa University of Calgary	*Preservation of force following active stretch in damaged skinned muscle fibres: a perplexing observation*	Introduction: The deficit in isometric force after repetitive eccentric muscle contractions has been extensively studied. However, whether this force loss affects force after active stretch remains unknown. Isometric contractions are primarily associated with actin-myosin cross-bridges, while titin is thought to contribute to the peak and steady-state forces during and following active stretching. Therefore, damage of the contractile system may affect isometric and force after active stretching differentially. Purpose: Investigate the loss in the peak and steady-state forces following active stretch in damaged skinned muscle fibres. Methods: Rabbit psoas skinned muscle fibres (n=11) were activated at an average sarcomere length (SL) of 2.4µm, exposed to a damaging eccentric stretch that consisted of an active stretch to an average SL of 4.0µm, held until steady-state force was reached, and then deactivated (ActiveStretch 1). This protocol was repeated three additional times (ActiveStretch 2, 3 and 4). Peak and steady-state forces following active stretch to a SL of 4.0µm were compared between ActiveStretch 1, 2, 3 and 4. Results and Discussion: Despite a substantial deficit in isometric force at a SL of 2.4µm (160±11 vs. 50±4, 41±3, and 35±3kPa in ActiveStretch 1, 2, 3, and 4, respectively), peak and steady-state forces following active stretch to a SL of 4.0µm were mostly preserved (peak: 268±19 vs. 269±21, 258±20 and 247±19kPa; steady-state: 156±12 vs. 166±13, 164±13 and 161±12kPa). This suggests that the structures responsible for force production are different between isometric contractions and following active stretch, and that these structures are affected differentially by damage.
P01.05	Vincenzo Lombardi University of Florence	*Dual Filament Regulation of the afterload contraction of the mammalian heart*	The cardiac output is regulated by mechanisms in both thin, actin-containing filaments and thick, myosin-containing filaments. In the thin filament a Ca2+-dependent structural change in regulatory proteins troponin and tropomyosin makes actin available for myosin motor attachment; in the thick filament myosin motors must be released from their folded (OFF) state on the filament surface, to attach to actin and hydrolyse ATP. Using X-ray diffraction from electrically paced intact trabeculae and papillary muscles from rat ventricle (temperature 27°C) it has been shown that switching ON of motors by thick filament mechano-sensing (Linari et al. 2015, Nature 528:276-279) occurs starting from the periphery of the thick filament and saturates well in advance with respect to the measured maximum isometric twitch force Tp (90 kPa, Morotti et al. 2024, PNAS 121:e2410893121). Meanwhile, first attachments occur from the central 1/3 of the half-thick filament (containing the myosin binding protein C), notwithstanding the filament periphery is already switched ON. Higher forces are associated to further motor attachments from progressively more peripheral thick filament regions, in agreement with near-neighbour cooperative thin filament activation. Here this hierarchical regionalization of cardiac performance regulation is tested during shortening under different afterloads (20-60 kPa), a proxy of the task of the ventricle pumping blood against the aortic pressure. We find, as distinctive features, that (i) the force-dependent progression of motor attachments towards thick filament periphery leads that measured in isometric contraction and (ii) the conformational dispersion of attached motors increases as expected from working motors. Supported by MUR (Italy).
P01.06	Weikang Ma Illinois Institute of Technology	*Porcine Left Atrial and Ventricular Thick Filaments Exhibit Distinct Resting Structures and Calcium-dependent Responses*	The heart maintains systemic perfusion through the coordinated function of its four chambers. Each chamber has distinct structural, functional, and molecular properties tailored to its role in circulation, which may result in chamber-specific differences in myofilament structure and regulation between atria and ventricles. To test this hypothesis, we employed muscle mechanics and X-ray diffraction to investigate functional and structural differences in porcine left atrial (LA) and left ventricular (LV) tissue. Here, we report the first X-ray diffraction study of atrial tissue, demonstrating that under resting conditions, myosin filaments in LA adopted a more ON-like, structurally distinct configuration compared with those in LV. Under contracting conditions, LV generated greater force and exhibited higher sinusoidal stiffness than LA across multiple calcium concentrations. LA showed faster kTR than in LV, with no calcium-dependence, in contrast to the calcium-dependence of kTR seen in LV. Structurally, the distinct myosin head configuration seen in the relaxed LA persisted during contraction. Furthermore, using the troponin inhibitor MYK-7660 to inhibit active contraction, we showed that, unlike LV, LA showed no direct calcium-dependent thick filament activation, reconciling discrepancies between fast rat and slow porcine ventricular myocardium regarding calcium's role in thick filament regulation. Altogether, our study reveals that LA myosin filaments adopt a molecular architecture and regulatory mechanism distinct from their LV counterparts, suggesting that myosin filament structure and regulation have evolved differently to meet the unique functional demands of each cardiac chamber. Moreover, understanding atrial-specific regulatory mechanisms provides new insights into therapeutic strategies for atrial diseases.
P01.07	Lorenzo Marcucci Padova University	*Beyond diffusion: modeling dextran effects on SRX estimation in skinned muscle fiber mantATP chasing*	The Super Relaxed State (SRX) of myosin, with a low ATP-turnover conformation, is a key determinant of muscle energetics and contractility. The mantATP chasing technique has become a widely adopted tool to quantify SRX and Disordered Relaxed State (DRX) myosin populations in skeletal muscle fibers, which preserve native thick filament architecture and cooperative interactions. In our previous work, we demonstrated that nonspecific mantATP binding and nucleotide diffusion properties significantly affect the reliability of SRX/DRX population estimates in skinned fiber preparations, and we developed a reaction-diffusion model to account for these confounding factors. Here, we extend this framework to investigate the role of dextran, used in skinned fiber studies to restore physiological lattice spacing and filament geometry. Dextran increases the viscosity of the surrounding medium, which is expected to alter the kinetics of mantATP diffusion and thus the fluorescence decay; however, dextran may also independently affect SRX stability, an effect that would otherwise be confounded by the diffusion component. By combining experimental mantATP chasing traces collected from four fiber conditions — control and myosin-depleted fibers, each with and without dextran — with our reaction-diffusion model, we aim to disentangle these two contributions. Our results suggest that accounting for this additional physical component may further improve the accuracy of SRX/DRX population estimates, with implications for both basic research and clinical interpretation of myosin resting states.

P01.08	Joy Pajarla Yale University	*A model that includes cardiac thick filament cooperativity produces realistic Ca2+-dependent myofilament dynamics*	Structural and biochemical studies have characterized an additional regulatory state of myosin in striated muscle in which the heads fold back into a “super-relaxed” (SRX), or “off,” state. Myosin must emerge from SRX before binding to actin, creating a regulatory step. Recent cryo-EM-derived thick filament structures suggest that SRX in a given myosin head could be disrupted by forces transmitted from neighboring myosins as they bind actin and undergo powerstroke. We used a computational model of myofilament activation to explore the consequences of thick filament cooperativity and whether its inclusion improves model behavior. We represented thick filament cooperativity by assuming that the energy required to exit SRX is lowered when either neighboring head is bound to actin. This coupling was implemented in a Markov model of myofilament activation that includes cooperative thin filament regulation by Ca2+. The model simulated Ca2+-dependent steady-state force and force redevelopment after slack/restretch (ktr). Realistic steady-state force-pCa curves were obtained under three cooperative regimes: thin-only, thick-only, and combined thick/thin cooperativity. However, only the combined thick/thin parameter set predicted a realistic ktr-pCa curve. A systematic evaluation of Hill coefficient as a function of thick and thin filament cooperative strength revealed synergy between the two forms of cooperativity. Running the combined model with a Ca2+ transient as input yielded realistic twitch contractions.The inability of simpler models with only one form of cooperativity to simultaneously explain force development and ktr dynamics strongly supports the physiological relevance and existence of the synergy between thin and thick filament cooperativity.
P01.09	Daniel Zornow Kruse Aarhus University	*Effects of thick-filament modulation by piperine and mavacamten on work capacity and glycogen depletion in response to work-matched stretch-shortening contractions in isolated slow rat skeletal muscle*	Background: Changes in the proportions of sequestered OFF and disordered OFF myosin heads may be induced pharmacologically. However, the contractile and metabolic effects of pharmacological modulation of skeletal-muscle myosin are relatively unexplored. Thus, this study aimed to determine the effects of myosin activation/inhibition on dynamic contractility and associated glycogen depletion in slow skeletal muscle. Methods: Contralateral pairs of soleus muscles from 4-week-old Wistar rats were isolated and exposed to piperine (activation) or mavacamten (inhibition), compared to vehicle treatment (DMSO). Isometric and dynamic contractility were assessed at baseline as well as after incubation and fatigue. After incubation, muscles were fatigued by a varying number of stretch-shortening contractions (SSC) at 30 Hz stimulation (number of contractions: piperine, 375; vehicle, 450; mavacamten, 525), matching the accumulated force AUC between groups. Immediately after the final assessment of contractility muscles were snap-frozen in liquid nitrogen for analysis of whole-muscle glycogen. Preliminary results: Overall, the accumulated force AUC from the fatigue protocol was successfully matched between all groups. Accordingly, piperine increased the force AUC per SSC, while mavacamten decreased it, compared to vehicle. The fatigue-induced reductions in isometric fused tetanic force were significantly larger (~18%-point) in the piperine group, while mavacamten did not change, compared to vehicle. Interestingly, this large functional decline in the piperine group did not coincide with changes in whole-muscle glycogen depletion levels compared to vehicle treatment. Preliminary conclusion: Piperine-induced myosin activation improved contractile work, but increased fatigue rate without significantly altering whole muscle glycogen usage in slow rat muscles.
P01.10	Nicoletta Piroddi University of Florence	*Comparison of the Inhibition Mechanisms of Fast Skeletal and Slow Cardiac Contractile Properties by Aficamten and Mavacamten in the 15–25°C Temperature Range*	Mavacamten and Aficamten reduce myosin ATPase activity and contractility by binding to distinct sites on the myosin motor, suggesting different mechanisms that converge on reducing the number of functionally available cross-bridge motors. We characterized the effects of Aficamten and Mavacamten on maximal isometric force development in myofibrils from fast skeletal rabbit psoas (MyHC-1) and slow cardiac minipig ventricle (MyHC-7), calcium-activated and relaxed by rapid solution switching at 15 or 25°C and exposed to selected micromolar concentrations of the inhibitors. Relaxed MyHC-1 and MyHC-7 myofibrillar and skinned-fiber ATPase activity was spectroscopically assayed at 25°C by malachite green phosphate or NADH-coupled assay with both drugs producing similar inhibitory effects. Both drugs reversibly inhibited force generation with markedly different kinetics. Mavacamten induced rapid force decline, whereas Aficamten caused slow, temperature-dependent decrease correlating with the resting ATPase of the myosin isoform present. Notably, the rate of force development was reduced by Aficamten to a lesser extent than by Mavacamten, particularly in slow ventricular myofibrils. These findings indicate that the two drugs inhibit contractility through distinct mechanisms. Aficamten primarily acts by preventing the formation of strongly bound actomyosin states, preferentially affecting slowly cycling myosin heads. In contrast, Mavacamten acts on post-powerstroke states, inducing rapid cross-bridge detachment. Under the hypothesis that Mavacamten also promotes sequestration of myosin heads into OFF states, the rapid kinetics of both inhibition and recovery observed in jump experiments suggest that the ON/OFF transition is fast and does not represent the rate-limiting step for re-entry into force-generating states within the cross-bridge cycle.

S02 - Excitation-Contraction Coupling

Invited speaker	Elena Lilliu Medical University of Vienna, AT	*Phasic store-operated calcium entry in skeletal muscle*	Store-operated Ca^{2+} entry (SOCE) in skeletal muscle fibres occurs across the t-tubular membrane when sarcoplasmic reticulum (SR) Ca^{2+} depletion activates the Ca^{2+} sensor Stim1, leading to opening of Orai1 channels and influx of extracellular Ca^{2+}. Impaired SOCE is associated with increased muscle fatigue and the development of various myopathies. A phasic form of SOCE (pSOCE) has been demonstrated during individual action potentials; however, its molecular basis and physiological role are poorly understood. We measured pSOCE in mechanically skinned fast- and slow-twitch rodent muscle fibres by simultaneously recording Ca^{2+}-dependent fluorescence from the t-tubular system (rhod-5N) and the cytosol (fluo-4) using high-speed confocal microscopy, while activating excitation–contraction (EC) coupling via electrical field stimulation. In EDL muscle fibres expressing a non-Ca^{2+}-conducting Cav1.1 channel mutant (N617D), pSOCE was indistinguishable from wild type. Muscle-specific deletion of Stim1 significantly reduced pSOCE, whereas selective knockout of Stim1L—a Stim1 splice variant proposed to mediate preformed Stim1–Orai1 clusters—had no effect. We identified fibre type–specific differences in pSOCE regulation: compared with fast-twitch EDL fibres, slow-twitch soleus fibres exhibited greater sensitivity to pSOCE activation despite releasing less Ca^{2+} from the SR upon action potentials. Together, our data support a molecular model in which pSOCE is activated by Stim1 but not STIM1L, and is independent on Cav1.1. The consistent presence of pSOCE across muscle types and species highlights its potential importance in skeletal muscle Ca^{2+} homeostasis. Enhanced pSOCE sensitivity in slow-twitch fibres suggests its potential role in improving maintenance of Ca^{2+} balance and protection against disease states.

Invited speaker	Marta Campiglio Innsbruck, AT	*The Mystery of Skeletal Muscle EC Coupling: The Missing Link Between Voltage Sensing and RyR1 Activation*	Excitation–contraction (EC) coupling in skeletal muscle depends on the coordinated action of two calcium channels: the voltage-gated calcium channel CaV1.1 in the transverse (T)-tubules and the ryanodine receptor RyR1 in the sarcoplasmic reticulum (SR). Membrane depolarization induces a conformational change in CaV1.1 that is mechanically transmitted to RyR1, triggering calcium release from the SR and initiating muscle contraction. Unlike cardiac muscle, where RyR2 activation depends on calcium influx through CaV1.2, skeletal muscle EC coupling is independent of calcium entry and instead relies on direct communication between CaV1.1 and RyR1. Although skeletal and cardiac muscles employ homologous sets of EC coupling proteins, they differ in their isoforms and regulatory mechanisms. A key distinction is the presence of STAC3, a skeletal muscle-specific adaptor protein that is essential for EC coupling and has no cardiac paralog, prompting the hypothesis that STAC3 might serve as a molecular linker between the two channels. e will discuss findings from our laboratory that challenge this model and instead highlight recent data identifying the domains and interactions that are essential for communication between CaV1.1 and RyR1 during skeletal muscle EC coupling.
Contributed talk	Theresa Hofmann Universitätskliniku m Münster, DE	*Trdn-as directs m6A-dependent transcriptional termination for accurate triadin isoform switching, preventing aberrant dyads and cardiomyopathy*	Heart failure is a leading cause of mortality and impaired cardiac excitation-contraction coupling represents a major driver of myocardial dysfunction. Long non-coding RNAs (lncRNAs) have emerged as important regulators of cardiac gene expression, but their contribution to calcium handling remains poorly understood. Here, we identify the lncRNA TRDN-AS as a critical regulator of cardiac triadin isoform specification. Reduced TRDN-AS expression in human cardiomyopathy, as well as genetic abrogation of Trdn-as in human iPSC-derived cardiomyocytes and mice, induces a switch from the cardiac TRDN/TRISK32 isoform to the skeletal muscle-associated TRDN/TRISK95 isoform. Mechanistically, transcription of Trdn-as in cis promotes RNA polymerase II stalling at the 3′ end of the cardiac Trdn locus, thereby facilitating proximal polyadenylation and formation of the cardiac TRDN/TRISK32 transcript. This process depends on the m6A methyltransferase METTL3, which enforces transcriptional termination and isoform selection. We further demonstrate that altered TRDN isoform composition remodels the cardiac calcium release complex, impairs calcium handling, disrupts dyad ultrastructure, prolongs the QT interval and promotes dilated cardiomyopathy in mice and humans.
Contributed talk	Vincent Jacquemond CNRS-Univ Lyon UMR5261 - Inserm U1315, FR	*Excitation-contraction coupling dysfunction in muscle fibers from a mouse model of spinal muscular atrophy*	Spinal muscular atrophy (SMA) is a genetic disorder due to mutations in the SMN1 gene, leading to loss of motor neurons and consequent muscle weakness and atrophy. We questioned whether the SMA muscle phenotype is associated with defective Ca2+ signaling and EC coupling. For this, we used the mouse model combining homozygous deletion of exon-7 in the mouse Smn gene and 2 human SMN2 transgenes inserted in tandem. This model presents early motor weakness and a reduced survival rate that can be increased up to ~30 days by a single nusinersen-like ASO ICV injection. Single muscle fibers were isolated from the FDB and interosseus muscles of 23-28 days-old mice. SMA muscle fibers presented with reduced diameter and length. There was no associated alteration of the t-tubule network but the spatial organization of the mitochondria network exhibited an increased density of longitudinally-oriented elements. No reproducible change in the occurrence of spontaneous Ca2+ release events was identified in SMA fibers. Conversely, detection of voltage-clamp-activated Ca2+ transients revealed that the maximum sarcoplasmic reticulum (SR) Ca2+ release flux through ryanodine receptors was depressed by about 50% with no change in voltage-dependency. The peak value of Ca2+ current through t-tubule CaV1.1 channels was also depressed by a similar extent. STORM-3D-super-resolution imaging of CaV1.1 immuno-labeling showed that CaV1.1 clusters were larger in the SMA fibers (by 38 %) but contained a reduced density (by 22 %) of CaV1.1 locations in each cluster. Results establish that EC coupling dysfunction is contributing to muscle weakness in SMA. Camille Dauvois1, Céline Malleval1, Ludivine Rotard1, Jonathan Schreiber1, Rasha Slika2, K. Monier1, Bruno Allard1, Frédéric Charbonnier2, Vincent Jacquemond1
P02.01	Theresa Hofmann Universitätskliniku m Münster	*Trdn-as directs m6A-dependent transcriptional termination for accurate triadin isoform switching, preventing aberrant dyads and cardiomyopathy*	Heart failure is a leading cause of mortality and impaired cardiac excitation-contraction coupling represents a major driver of myocardial dysfunction. Long non-coding RNAs (lncRNAs) have emerged as important regulators of cardiac gene expression, but their contribution to calcium handling remains poorly understood. Here, we identify the lncRNA TRDN-AS as a critical regulator of cardiac triadin isoform specification. Reduced TRDN-AS expression in human cardiomyopathy, as well as genetic abrogation of Trdn-as in human iPSC-derived cardiomyocytes and mice, induces a switch from the cardiac TRDN/TRISK32 isoform to the skeletal muscle-associated TRDN/TRISK95 isoform. Mechanistically, transcription of Trdn-as in cis promotes RNA polymerase II stalling at the 3′ end of the cardiac Trdn locus, thereby facilitating proximal polyadenylation and formation of the cardiac TRDN/TRISK32 transcript. This process depends on the m6A methyltransferase METTL3, which enforces transcriptional termination and isoform selection. We further demonstrate that altered TRDN isoform composition remodels the cardiac calcium release complex, impairs calcium handling, disrupts dyad ultrastructure, prolongs the QT interval and promotes dilated cardiomyopathy in mice and humans.
P02.02	Vincent Jacquemond CNRS-Univ Lyon 1 UMR5261 - Inserm U1315	*Excitation-contraction coupling dysfunction in muscle fibers from a mouse model of spinal muscular atrophy*	Spinal muscular atrophy (SMA) is a genetic disorder due to mutations in the SMN1 gene, leading to loss of motor neurons and consequent muscle weakness and atrophy. We questioned whether the SMA muscle phenotype is associated with defective Ca2+ signaling and EC coupling. For this, we used the mouse model combining homozygous deletion of exon-7 in the mouse Smn gene and 2 human SMN2 transgenes inserted in tandem. This model presents early motor weakness and a reduced survival rate that can be increased up to ~30 days by a single nusinersen-like ASO ICV injection. Single muscle fibers were isolated from the FDB and interosseus muscles of 23-28 days-old mice. SMA muscle fibers presented with reduced diameter and length. There was no associated alteration of the t-tubule network but the spatial organization of the mitochondria network exhibited an increased density of longitudinally-oriented elements. No reproducible change in the occurrence of spontaneous Ca2+ release events was identified in SMA fibers. Conversely, detection of voltage-clamp-activated Ca2+ transients revealed that the maximum sarcoplasmic reticulum (SR) Ca2+ release flux through ryanodine receptors was depressed by about 50% with no change in voltage-dependency. The peak value of Ca2+ current through t-tubule CaV1.1 channels was also depressed by a similar extent. STORM-3D-super-resolution imaging of CaV1.1 immuno-labeling showed that CaV1.1 clusters were larger in the SMA fibers (by 38 %) but contained a reduced density (by 22 %) of CaV1.1 locations in each cluster. Results establish that EC coupling dysfunction is contributing to muscle weakness in SMA.

P02.03	Alain Lacampagne Inserm / Cnrs / Montpellier University	*Acute ciguatoxin exposure disrupts cardiac RyR2 function, inducing arrhythmias and acute heart failure phenotype: implications for an emerging climate-sensitive cardiotoxic disease*	Ciguatera is a foodborne illness caused by ciguatoxins, marine phycotoxins produced by benthic dinoflagellates. It represents the most prevalent non-bacterial seafood poisoning worldwide, with an estimated 50,000 to 100,000 new cases annually, although it remains largely underreported. The syndrome is highly polymorphic, combining gastrointestinal, neurological, cutaneous, and inflammatory manifestations. Among its clinical features, cardiovascular complications such as bradycardia, hypotension, and arrhythmias suggest that ciguatoxins directly affect cardiac ion handling and excitation-contraction coupling. While sodium channel dysfunction is frequently proposed as a primary mechanism, the type 2 ryanodine receptor (RyR2), the principal sarcoplasmic reticulum calcium release channel central to cardiac contraction and also expressed in the brain, emerges as a potential critical target. In this study, we examined the cardiotoxic effects of purified ciguatoxin-1B and ciguatoxin-3C using human induced pluripotent stem cell-derived ventricular cardiomyocytes and in vivo Wistar rat models. We evaluated intracellular calcium homeostasis, excitation-contraction coupling, and RyR2 function under toxin exposure. Ciguatoxins induced a marked intracellular calcium leak, increasing basal calcium levels and promoting a gain-of-function of RyR2, with higher opening probability and reduced closing time. These alterations impaired contractility in vitro. In vivo, ciguatoxin-1B rapidly triggered arrhythmias and after 24 hours of administration, echocardiography revealed reduced contractile performance and altered relaxation, indicative of acute heart failure both consistent with cellular observations. These findings identify RyR2 dysfunction as a central mechanism of ciguatoxin cardiotoxicity, highlighting ciguatera as a growing, climate-sensitive cardiovascular health concern.
P02.04	Elena Lilliu Medical University of Vienna	*Phasic Store-operated Calcium Entry in skeletal muscle*	Store-operated Ca^{2+} entry (SOCE) in skeletal muscle fibres occurs across the t-tubular membrane when sarcoplasmic reticulum (SR) Ca^{2+} depletion activates the Ca^{2+} sensor Stim1, leading to opening of Orai1 channels and influx of extracellular Ca^{2+}. Impaired SOCE is associated with increased muscle fatigue and the development of various myopathies. A phasic form of SOCE (pSOCE) has been demonstrated during individual action potentials; however, its molecular basis and physiological role are poorly understood. We measured pSOCE in mechanically skinned fast- and slow-twitch rodent muscle fibres by simultaneously recording Ca^{2+}-dependent fluorescence from the t-tubular system (rhod-5N) and the cytosol (fluo-4) using high-speed confocal microscopy, while activating excitation–contraction (EC) coupling via electrical field stimulation. In EDL muscle fibres expressing a non-Ca^{2+}-conducting Cav1.1 channel mutant (N617D), pSOCE was indistinguishable from wild type. Muscle-specific deletion of Stim1 significantly reduced pSOCE, whereas selective knockout of Stim1L—a Stim1 splice variant proposed to mediate preformed Stim1–Orai1 clusters—had no effect. We identified fibre type–specific differences in pSOCE regulation: compared with fast-twitch EDL fibres, slow-twitch soleus fibres exhibited greater sensitivity to pSOCE activation despite releasing less Ca^{2+} from the SR upon action potentials. Together, our data support a molecular model in which pSOCE is activated by Stim1 but not STIM1L, and is independent on Cav1.1. The consistent presence of pSOCE across muscle types and species highlights its potential importance in skeletal muscle Ca^{2+} homeostasis. Enhanced pSOCE sensitivity in slow-twitch fibres suggests its potential role in improving maintenance of Ca^{2+} balance and protection against disease states.
P02.05	Zoltán Singlár University Of Debrecen	*CB1R blockade improves force generation and motor coordination without interference with calcium signaling in mdx mice*	Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by dystrophin deficiency, leading to sarcolemmal instability, elevated intracellular Ca^{2+} concentrations, and progressive muscle degeneration. Increased Ca^{2+} drives mitochondrial dysfunction and oxidative stress, key contributors to disease pathology. The ECS comprises cannabinoid receptors (CB1R, CB2R), endogenous ligands, and associated metabolic enzymes. CB1R is expressed in skeletal muscle and modulates mitochondrial metabolism and myogenic processes. Recent evidence suggests that CB1R signaling (over activity) may worsen dystrophic phenotype. Pharmacological inhibition of CB1Rs, with the inverse agonist rimonabant has been shown to delay the disease onset, to improve regeneration and reduce inflammation. Here, 12-weeks-old control and mdx mice were administered rimonabant for two weeks. Following this chronic treatment functional assessment revealed a mild rescue in grip strength, and a significant improvement in rotarod performance in mdx mice compared to control, indicating improved motor performance and coordination. In isolated flexor digitorum brevis (FDB) fibers, excitation-contraction coupling was mainly preserved, with no change in voltage-dependent Ca^{2+} release. We also evaluated the effect of acute rimonabant treatment on the Ca^{2+} homeostasis of FDB fibers and the results align with the findings of the chronic application. These results suggest that CB1R signaling modulate muscle force development independently of excitation-contraction coupling, contributing to functional muscle improvement. Targeting ECS signaling may therefore represent promising modifier pathway, but not a root cause cure for DMD. Keywords: skeletal muscle, endocannabinoid system, CB1 receptor, Duchenne muscular dystrophy, calcium signaling

P02.06	Mónika Sztretye University of Debrecen	*Evaluation of the Interplay Between Statins and the Endocannabinoid System in Skeletal Muscle*	Statins are widely prescribed pharmacological agents with proven cardiovascular and metabolic benefits. Despite their efficacy, statin therapy is increasingly associated with skeletal muscle adverse effects ranging from mild myalgia to severe myopathy and muscle weakness, which lead in some cases to treatment discontinuation. The endocannabinoid system (ECS) plays an important role in regulating energy metabolism, mitochondrial function, calcium homeostasis, and inflammatory processes in skeletal muscle. Statin-induced muscle toxicity has been linked to mitochondrial dysfunction, disturbances in calcium handling, and impaired excitation–contraction coupling; however, the mechanisms underlying individual susceptibility remain poorly understood. Notably, several of these pathways overlap with molecular mechanisms implicated in muscle atrophy and sarcopenia. This overlap suggests that interactions between statins and ECS signaling may represent a previously unrecognized vulnerability pathway influencing muscle health. In the present study, we investigated in three different genetically modified CB1R murine models (Tamoxifen inducible muscle-specific CB1R knockdown, global CB1R knockout, and mitochondrial CB1R knockout) the potential interaction between statin treatment and ECS signaling as a mechanistic contributor to statin-associated muscle impairment. Statin therapy was applied via oral gavage at a weight-adjusted dosage of 20 mg/kg over four weeks. To assess functional and physiological changes, we employed in vivo behavioral and performance assays, including grip strength, hang test, and Rota-Rod performance, as well as in vitro skeletal muscle force measurements. We have also studied calcium transients using whole-cell patch clamp combined with confocal microscopy. Elucidating the interplay between statins and ECS may identify potential therapeutic targets to mitigate muscle dysfunction.
S03 - Cytoskeleton			
Invited speaker	Henk Granzier University of Arizona, Tucson, US	*Z-disk Thickness Regulation is Independent of Titin and Nebulin Z-repeats*	The Z-disk plays a crucial role in transmitting forces between sarcomeres. Its thickness varies from ~ 50 nm in fast skeletal muscles to ~100 nm in cardiac and slow skeletal muscles. It has been hypothesized that titin and nebulin, which span the Z-disk, may act as regulators of Z-disk thickness. This is based on the presence of variable numbers of repeat motifs: titin contains up to seven Z-repeats (Zr), with Zr1-3 and Zr7 found in all striated muscles, and Zr4-6 expressed only in cardiac and slow skeletal muscles—where Z-disks are thickest. Similarly, nebulin includes 18 Zr, with more expressed in muscles that have thicker Z-disks. To test whether titin and nebulin determine Z-disk thickness, we generated mouse models lacking all titin Zr (TtnΔ8-14), another lacking nebulin's differentially expressed Zr (NebΔ152-160), as well as a double mutant to explore co-regulation. Unexpectedly, electron microscopy revealed that Z-disk thickness in the EDL (fast), soleus (slow), and cardiac muscle of TtnΔ8-14 mice was unchanged compared to wild-type. In the NebΔ152-160 model, the number of nebulin Zr in the soleus matched that of the EDL, yet Z-disk thickness remained soleus-like—again contradicting expectations. The double mutant similarly retained normal soleus Z-disk structure. Collectively, these findings refute the hypothesis that titin and nebulin determine Z-disk thickness. Although overall sarcomere structure was maintained, TtnΔ8-14 skeletal muscle had increased susceptibility to eccentric contraction-induced force loss, and subsequent sarcolemmal damage as evidenced by Evans blue dye uptake. The implications of our findings will be discussed.
Invited speaker	Nicole Dubois Icahn School of Medicine at Mount Sinai, NY, US	*Mechanisms of local translation at the sarcomere and the role of LARP1 in cardiac growth*	Sarcomeres are architecturally complex structures that require highly orchestrated assembly, maintenance, and growth during cardiac development. Once studied primarily as contractile structures, sarcomeres are increasingly recognized to have important signaling and regulatory roles in cardiomyocytes. Recent evidence highlights high rates of protein turnover and enrichment of mRNA and translational machinery at the Z-disc, suggestive of local translation. Our lab recently profiled the local sarcomeric Z-disc protein interactome and found enrichment of several RNA-binding proteins (RBPs). From this screen we identified the family of La-related proteins (LARPs). We find LARPs to exhibit distinct spatial expression patterns in mouse and human cardiomyocytes, with enrichment at key structures such as the sarcomere, mitochondria and the intercalated disc. eCLIP analysis for LARP1 and LARP4B identified shared and unique targets and shows binding of LARP1 to TOP mRNAs. HCR analysis for select LARP1 TOP mRNA targets shows that they too are localized at the sarcomere, spatially overlapping with LARP1. Loss of LARP1 in hPSCs does not affect their differentiation to cardiomyocytes, but results in smaller cardiomyocytes and reduced contractile force in engineered heart tissues. Whole body KO of LARP1 in mice results in lethality shortly before birth with reduced overall embryo size, further supporting a critical role for LARP1 in cell and organ growth. We propose that local translation at key structures such as sarcomeres, mitochondria and the intercalated disc is regulated via localized RBPs, and that LARP1 plays a critical role through its regulation of TOP mRNAs in response to different growth signals.
Contributed talk	Matthew Stroud King's College London, UK	*Harnessing nucleoskeleton remodelling to mitigate muscle decline*	Age-related decline in skeletal muscle structure and function can be mitigated by regular exercise. However, the precise mechanisms that govern this are not fully understood. The nucleus plays an active role in translating forces into biochemical signals (mechanotransduction), with nuclear lamina protein Lamin A regulating nuclear shape, nuclear mechanics, and ultimately gene expression. Defective Lamin A expression causes muscle pathologies and premature ageing syndromes, but the roles of nuclear structure and function in physiological ageing and in exercise adaptations remain obscure. Here, we isolated single muscle fibres and carried out detailed morphological and functional analyses on myonuclei from young and older exercise-trained individuals. Strikingly, myonuclei from trained individuals were more spherical, less deformable, and contained a thicker nuclear lamina than untrained individuals. Complementary to this, exercise resulted in increased levels of Lamin A and increased myonuclear stiffness in mice. We conclude that exercise is associated with myonuclear remodelling, independently of age, which may contribute to the preservative effects of exercise on muscle function throughout the lifespan.

Contributed talk	Mónika Gönczi University of Debrecen, HU	*Alteration of septin expression, co-localization of cytoskeletal proteins, cell cycle progression, and calcium handling in Septin7 knockdown myogenic cell line*	Myogenesis and skeletal muscle regeneration are strictly regulated processes, where the division of myoblasts, their migration to the appropriate location, and their fusion into myotubes are key points. Previously, we have demonstrated that cytoskeletal Septin7 plays a crucial role in controlling myoblast proliferation and their differentiation into multinucleated myotubes. This study aimed to describe the changes in the expression of other septins, co-localization of septins with other cytoskeletal proteins, and cell cycle progression in Septin7-knockdown (S7-KD) myogenic cell cultures. Calcium homeostasis of proliferating and differentiated control cultures was also compared to S7-KD C2C12 cells. RNA sequence analysis of the samples displayed extensive molecular and cellular alterations, including disrupted expression of structural and myogenic regulatory proteins, confirming previously observed reduced capacity for proliferation and differentiation. Septin7-knockdown also impaired cell cycle progression with increased G2/M phase arrest. Moreover, the decreased co-localization of Septin7 with Septin5, Septin9, actin, and tubulin highlights the importance of Septin7 in maintaining cytoskeletal organization and protein interactions essential for myogenesis. Functional analyses further revealed significantly impaired calcium signaling in both proliferating myoblasts and differentiated myotubes, likely resulting from altered expression of P2X7 receptors and voltage-gated calcium channels. Altogether, these results emphasize the pivotal role of Septin7 in coordinating cytoskeletal integrity, cell cycle regulation, and calcium homeostasis during myogenesis and skeletal muscle regeneration.
P03.01	Mónika Gönczi University Of Debrecen	*Alteration of septin expression, co-localization of cytoskeletal proteins, cell cycle progression, and calcium handling in Septin7 knockdown myogenic cell line*	Myogenesis and skeletal muscle regeneration are strictly regulated processes, where the division of myoblasts, their migration to the appropriate location, and their fusion into myotubes are key points. Previously, we have demonstrated that cytoskeletal Septin7 plays a crucial role in controlling myoblast proliferation and their differentiation into multinucleated myotubes. This study aimed to describe the changes in the expression of other septins, co-localization of septins with other cytoskeletal proteins, and cell cycle progression in Septin7-knockdown (S7-KD) myogenic cell cultures. Calcium homeostasis of proliferating and differentiated control cultures was also compared to S7-KD C2C12 cells. RNA sequence analysis of the samples displayed extensive molecular and cellular alterations, including disrupted expression of structural and myogenic regulatory proteins, confirming previously observed reduced capacity for proliferation and differentiation. Septin7-knockdown also impaired cell cycle progression with increased G2/M phase arrest. Moreover, the decreased co-localization of Septin7 with Septin5, Septin9, actin, and tubulin highlights the importance of Septin7 in maintaining cytoskeletal organization and protein interactions essential for myogenesis. Functional analyses further revealed significantly impaired calcium signaling in both proliferating myoblasts and differentiated myotubes, likely resulting from altered expression of P2X7 receptors and voltage-gated calcium channels. Altogether, these results emphasize the pivotal role of Septin7 in coordinating cytoskeletal integrity, cell cycle regulation, and calcium homeostasis during myogenesis and skeletal muscle regeneration.
P03.02	Carol Gregorio Icahn School of Medicine at Mount Sinai	*The N-terminal actin-binding site of Lmod2 promotes controlled pointed end elongation*	Muscle contraction is a regulated process driven by the sliding of actin-thin filaments over myosin-thick filaments. Leiomodin 2 (Lmod2) is an actin filament length regulator and essential for life since human mutations and complete loss of Lmod2 in mice lead to dilated cardiomyopathy and death. Lmods are critical for the assembly and maintenance of thin filaments in striated muscles by allowing thin filament elongation at their pointed ends. Lmod2's elongation function has been linked to both actin binding sites 2 and 3 (ABS2 and 3) while the existence and function of an N-terminal actin-binding site (ABS1) has been debated. To elucidate the little-known role of this site in Lmod2, we created a unique mutant (F64D/L69D/W72D/W73D: Lmod2-QM) predicted to decrease binding of ABS1 to actin. We analyzed the effect of the mutations using several in vitro, cellular and in vivo assays. By disrupting the interaction of Lmod2 ABS1 with actin in isolated cardiomyocytes and in mice, we engineered a "super Lmod2" that results in remarkably longer thin filaments. Structural analysis determined that ABS1 of Lmod2 binds to actin through a disordered region and an amphipathic α-helix. Analysis of the mutated ABS1 revealed that the helix is destroyed and binding to actin is maintained only in the N-terminal disordered region of Lmod2 ABS1. These discoveries support a model of controlled thin filament pointed end elongation by Lmod2 and provide direct evidence of, as well as the structural and functional mechanistic basis for, Lmod2's physiological leaky cap activity.
P03.03	Miklós Kellermayer Semmelweis University	*Titin's PEVK domain is a Liquid-Liquid Phase Separating Protein*	Titin is a giant filamentous protein that spans the half muscle sarcomere between the Z- and M-lines and provides elasticity, structural template and mechanosensor function. Titin's I-band section contains a heavily spliced domain called PEVK, named so due to its propensity of prolines, glutamates, valines and lysines. Even though PEVK has been shown to contain structural motifs such as poly-proline helices, by large it is an intrinsically disordered protein (IDP). IDPs display a tendency for liquid-liquid phase separation (LLPS), a reversible demixing process characterized by the formation of liquid droplets. Here we hypothesized that PEVK might also display LLPS. To test our hypothesis, we carried out in vivo experiments and molecular dynamics (MD) simulations. The 733-residue-long middle part of the full-length PEVK was inserted into a construct containing, C-terminally from the PEVK, the genes encoding mCherry and the photodimerizing protein Cry2. Cultured Drosophila S2R+ cells were transfected with the construct, and protein expression was induced by the addition of 1 mM copper-sulfate. Photoactivation and fluorescence imaging were carried out by using confocal and TIRF microscopy. We found that, upon a few-second illumination with 488-nm laser light, mCherry positive droplets formed rapidly in the cytoplasm. The droplets often fused together and evaded the mechanical probing by an AFM cantilever tip, thereby revealing their liquid nature. MD simulations suggested that PEVK indeed displays self-interaction, and that calcium enhances the effect. Altogether, our results demonstrate that titin's PEVK domain is a protein capable of LLPS, which may have yet-to-be-revealed implications for muscle function.

P03.04	Wolfgang Linke University of Münster	*Novex-3 titin defines a structural and signaling hub at the cardiac Z-disk and is dysregulated in heart failure*	Novex-3 (Nx3) is an exceptionally small titin isoform whose sarcomeric roles remain poorly understood. We employed a multimodal approach, including microscopy, immunoblotting, quantitative PCR, total-RNA sequencing, and ribosome profiling of normal and diseased mouse and human cardiomyocytes, to define key properties of Nx3, such as its abundance relative to full-length titin, sarcomeric localization, protein interactions, and functional relevance. We find that Nx3 is constitutively expressed from fetal development through adulthood. In adult heart, Nx3 makes up ~20-25% of total titin protein versus ~8-14% at the transcript level. We propose that Nx3 adopts a non-linear configuration within the sarcomere: its N-terminus is anchored at the Z-disk, while the proximal portion of its unique (Ttn-exon-48) region, enriched in coiled-coil motifs, engages laterally with adjacent titin/Nx3 molecules at the Z-I-band interface. Its monomeric C-terminus extends toward the A-band but remains confined to the Z-I-band region. This architecture confers enhanced stability and flexibility to the Z-disk. Protein-interaction studies, including yeast two-hybrid screening and co-immunoprecipitation, identified Pin1 and TBC1D4 as binding partners of the Nx3 C-terminus, suggesting participation in signaling networks regulating cardiomyocyte metabolism. Genetic ablation of Nx3 in human induced pluripotent stem cell-derived cardiomyocytes indicates that, although dispensable for sarcomere assembly, Nx3 is required for optimal Z-disk organization and mechanical performance. In end-stage human dilated cardiomyopathy, hearts exhibit dysregulated Nx3 expression, with reduced protein abundance relative to non-failing controls and focal Z-disk disruption, likely contributing to impaired contractile function. We conclude that Nx3 regulates Z-disk stability and modulates signaling pathways that optimize cardiac performance.
P03.05	Andrea Balogh-Molnar Semmelweis University	*Ion-Dependent Modulation of Titin Proteolysis Reveals the Physiological Relevance of Short Titin forms*	Titin is the largest known human protein and a key structural component of the sarcomere, contributing to passive elasticity, structural integrity, and force transmission in striated muscle. Besides the full-length T1 isoform, a shorter T2 form is consistently observed in a muscle-specific ratio, though it is traditionally considered a proteolytic degradation product of T1 with unclear physiological relevance. This study investigated post-mortem titin proteolysis and its potential regulation to clarify the origin and significance of T2 titin. Whole muscle extracts from striated muscles were incubated under varying conditions, and proteolysis was modulated using protease inhibitors, chelating agents, and different concentrations of calcium ions. Samples were analyzed using vertical agarose gel electrophoresis optimized for high-molecular-weight proteins. Native muscle samples showed stable, muscle-specific T1:T2 ratios consistent with previous reports, and neither tissue excision nor homogenization altered this distribution. Proteolytic degradation was strongly temperature-dependent, increasing at 37°C compared to 4°C. Protease inhibitors and chelators partially reduced titin degradation, while near-complete inhibition was achieved in chemically skinned samples under specific conditions. Conversely, elevated calcium concentrations enhanced proteolysis. These findings suggest that T2 titin is not solely an artifact of sample handling but likely reflects a regulated, naturally occurring form of titin shaped by ion-dependent proteolytic mechanisms, potentially indicating a functional role in muscle physiology.
P03.06	Nóra Beatrix Máthé Faculty of Medicine, University of Debrecen	*The role of Septin expression and function in human skeletal muscle cell cultures*	Septins are cytoskeletal GTP-binding proteins involved in cell division, membrane organization, cytoskeletal remodeling, and regulating differentiation. It has been previously shown that Septin7 is one of the key proteins in regulating cytoskeletal septin assembly in mouse-originated skeletal muscle cell line, and has a significant impact on physiological organization, force generation, and also on the regeneration program of skeletal muscle in mice. However, their role in human skeletal muscle cells remains insufficiently characterized. In the present study, we investigated septin expression in human skeletal muscle cell cultures (HSkMc). Samples from cell cultures were collected at the proliferative blast stage and at specific phases of differentiation. The gene expression was analyzed by qPCR, while protein expression was examined by Western blot. Quantitative PCR analysis demonstrated the expression of several septin family members in human skeletal muscle cells. Their expression patterns changed between proliferative and differentiated states. In parallel, the expression of key myogenic markers varied across differentiation stages, supporting the progression of myogenic differentiation in the examined cultures. Western blot analysis confirmed the presence of septins in HSkMc samples. Confocal microscopic analysis of immunostained samples confirmed progressive differentiation in both fetal and adult HSkMc cultures, with clear morphological changes from blast cells to elongated, aligned myotubes during later differentiation stages. Overall, our results show that specific septins are expressed in HSkMc cultures and show stage- and cell type-dependent expression during myogenic differentiation. These findings suggest that septins may contribute to the regulation of human skeletal muscle cell structure and differentiation.
P03.07	Jolanta Nowak Nencki Institute Of Experimental Biology PAS	*INVOLVEMENT OF UNCONVENTIONAL MYOSIN VI IN AORTIC MORPHOLOGY AND FUNCTION*	Unconventional myosin VI (MVI), a unique actin-based motor protein, plays an important role in both skeletal and cardiac muscle functions. In earlier studies, we demonstrated that its loss leads to the age-progressing cardiomyopathy together with disturbances in hindlimb muscle function and metabolism. We also found that loss of MVI correlates with reduced levels of vascular endothelial growth factor A (VEGFA) in the heart, suggesting a possible role of MVI in vascularization and/or vessel function. Based on these findings, we investigated the role of MVI in aorta morphology and organization. We performed studies on thoracic aortas isolated from Snell's waltzer mice (MVI knockouts) and heterozygous (WT) littermates at 3-months and 12-months of age. Morphometric analyses revealed age-related alteration in the aortic wall, ranging from stenosis to dilation. MVI is present both in the endothelium and smooth muscle compartments of the WT aortas and its level decreases with age, similarly to the cardiac and skeletal muscle. Using TEM we observed several changes in aorta structural compartments. Immunofluorescent staining of the aorta revealed age-related accumulation of PECAM1 (platelet/endothelial cell adhesion protein 1) in the tunica intima, indicating that MVI may contribute to the production and/or secretion of inflammatory mediators, hormones and factors (including NO), involved in regulation of vascular contraction and relaxation. Furthermore, we identified endothelial nitric oxide synthase (eNOS) as a potential MVI partner in the heart. Together, these data indicate important role(s) of MVI in vascularization and blood vessel function, and further research is needed to elucidate mechanisms behind these observations.

P03.08	Katarzyna Robaszkiewicz Kazimierz Wielki University	*Regulation of gelsolin-dependent actin filament dynamics by tropomyosin isoforms Tpm2*	Actin filament dynamics is regulated by tropomyosin (Tpm) which controls actin interactions and activity of other proteins, including actin-severing protein gelsolin (GSN). Mammalian cells express ~40 Tpm isoforms and three GSN isoforms: GSN-a (plasma), GSN-b (cytoplasmic), and GSN-c (cytoplasmic and nuclear). To date, Tpm isoform-dependent regulation of GSN isoforms has not been investigated. This study aimed to: (1) characterize the Ca^{2+} sensitivity of the GSN isoforms; and (2) determine the regulatory effects of cytoplasmic Tpm2 isoforms on GSN-b function. To address these aims, human recombinant GSN and Tpm2 (Tpm2.1, Tpm2.3, Tpm2.4) isoforms were analyzed using biochemical and fluorescence microscopy assays. The study showed that among the GSN isoforms, GSN-b has the highest affinity for actin, and its actin-binding and depolymerizing activities are the most sensitive to Ca^{2+}. The presence of Tpm2 isoforms decreased the Ca^{2+} sensitivity of GSN-b binding to actin, but strongly increased its binding cooperativity. Tpms differentially regulated GSN-b activity, with Tpm2.1 and Tpm2.4 enhancing GSN-b binding to actin. In contrast, Tpm2.3 inhibited actin binding but facilitated actin depolymerization. Isothermal titration calorimetry revealed that GSN-b binds Tpms with different affinities, with Tpm2.3 showing the highest and Tpm2.1 the lowest K_a. In summary, the biochemical properties of GSN isoforms differ significantly. In addition, the activity of GSN-b is differentially modulated by Tpm2 isoforms, underscoring their importance in regulating actin cytoskeleton dynamics. Funded by the National Science Centre, Poland (WEAVE-UNISONO project no. 2022/04/Y/NZ5/00064) and was co-financed by the Polish Minister of Science under the program “Regional Initiative of Excellence”.
P03.09	Małgorzata Siatkowska Kazimierz Wielki University	*Modeling Osteosarcoma Actin Dynamics In Vitro: TPM2 Downregulation Facilitates Cofilin-Mediated Dissociation of Fascin Bundles*	Osteosarcoma progression is characterized by radical actin remodeling driven by the upregulation of cofilin-1 and fascin-1 alongside the downregulation of TPM2-encoded tropomyosins (Tpms) [Arlt MJ et al., 2019; Li ZF et al., 2020; Meng Y et al., 2023]. This study provides a mechanistic basis for this process, analyzing how the loss of Tpm-mediated control enables an aggressive, hyper-dynamic cytoskeleton. High-speed co-sedimentation showed that by binding to actin cofilin-1 displaces Tpm isoforms in a concentration-dependent manner. While Tpms slightly reduced cofilin's affinity for actin, they paradoxically facilitated cofilin recruitment into fascin-actin complex. In low-speed co-sedimentation assays involving fascin-1 titration, cofilin-1 reduced bundling efficiency to 50%. At lower fascin-1 concentrations, Tpms synergistically enhanced this inhibition, acting as co-facilitators of bundle destabilization. At higher fascin levels, bundling stabilized at 40-50%, with fascin-1 remaining in the pellets, indicating that cofilin-1 impairs bundle integrity rather than preventing initial association. Using isothermal titration calorimetry, direct interaction between cofilin-1 and fascin-1 was identified, establishing a novel regulatory layer to their functional antagonism. These findings suggest that in healthy cells, Tpm2 acts as a "gatekeeper," maintaining ordered structures and preventing excessive fascin-mediated protrusions. In osteosarcoma, Tpm2 downregulation removes this barrier, allowing the direct antagonism between upregulated cofilin-1 and fascin-1 to drive rapid actin turnover. This dynamic cycle of bundle formation and severing provides the mechanical plasticity required for the formation of invasive filopodia and invadopodia, ultimately fueling the metastatic potential of tumor cells. This work was funded by the National Science Centre, Poland (WEAVE-UNISONO project no. 2022/04/Y/NZ5/00064).
P03.10	Matt Stroud King's College London	*Harnessing nucleoskeleton remodelling to mitigate muscle decline*	Age-related decline in skeletal muscle structure and function can be mitigated by regular exercise. However, the precise mechanisms that govern this are not fully understood. The nucleus plays an active role in translating forces into biochemical signals (mechanotransduction), with nuclear lamina protein Lamin A regulating nuclear shape, nuclear mechanics, and ultimately gene expression. Defective Lamin A expression causes muscle pathologies and premature ageing syndromes, but the roles of nuclear structure and function in physiological ageing and in exercise adaptations remain obscure. Here, we isolated single muscle fibres and carried out detailed morphological and functional analyses on myonuclei from young and older exercise-trained individuals. Strikingly, myonuclei from trained individuals were more spherical, less deformable, and contained a thicker nuclear lamina than untrained individuals. Complementary to this, exercise resulted in increased levels of Lamin A and increased myonuclear stiffness in mice. We conclude that exercise is associated with myonuclear remodelling, independently of age, which may contribute to the preservative effects of exercise on muscle function throughout the lifespan.
P03.11	Laszlo Szabo University of Debrecen	*The effect of simulated space conditions on the cytoskeletal septin system and mechanotransduction via Piezo1*	An intact cytoskeletal network and preserved plasma membrane integrity are essential for normal skeletal muscle function. Septins, particularly Septin7, are key cytoskeletal components involved in intracellular organization, membrane stability, and regulation of interactions between the plasma membrane, cytoskeletal elements, and mechanosensitive receptors such as the Piezo1 ion channel. These structural and functional interactions are critical for maintaining cellular rigidity and mechanotransduction. However, environmental stressors associated with spaceflight, including microgravity and ionizing radiation, may disrupt these processes. This study aimed to investigate how simulated space conditions influence the interaction between septin7 and the Piezo1 mechanosensitive ion channel. C2C12 mouse myoblasts were exposed separately to simulated microgravity and proton irradiation. Western blot analysis revealed no significant changes in total protein expression levels under either condition. However, confocal immunofluorescence microscopy demonstrated marked alterations in the intracellular organization of both proteins. Under simulated microgravity, Septin7 formed thinner filamentous structures, while the actin cytoskeleton remained largely unaffected. In addition, treated cells exhibited increased cellular circularity, indicating altered cell morphology. Following proton irradiation, the septin scaffold appeared fragmentated. Both simulated microgravity and irradiation also modified the intracellular localization of Piezo1. Overall, simulated space conditions induced structural changes in the septin cytoskeletal network and modified Piezo1 localization without affecting total protein expression levels. These findings suggest a dynamic relationship between the septin system and mechanotransduction pathways, which may contribute to cellular adaptation under space-related environmental stress.

S04 - Muscle Structure			
Invited speaker	Charlie Scarff University of Leeds, UK	*Myosin in Motion: Mechanisms of Molecular Modulation*	Small-molecule modulators of myosin are emerging as promising therapies for muscle disease, yet their mechanisms of action remain incompletely understood. Using cryo-electron microscopy, we capture myosin in motion at different stages of its chemomechanical cycle, revealing how small molecules reshape the structural landscape of the motor. These studies provide new insights into the molecular basis of myosin regulation, therapeutic modulation, and the structural transitions that underpin force generation. Together, our findings improve our understanding of how myosin functions in muscle and provide a structural framework for interpreting how small molecules influence contractility.
Invited speaker	Roberto Dominguez University of Pennsylvania, US	*A Structural–Functional Perspective on Thin Filament Length Regulation*	In non-muscle cells, actin filaments display variable lengths and rapid turnover, with subunit addition occurring predominantly at the barbed end and dissociation at the pointed end. The situation is strikingly different in striated muscle sarcomeres, where the actin thin filaments are highly uniform in length, despite substantial subunit turnover occurring at both ends and, more prominently, at the pointed end. Filament length in sarcomeres is tightly regulated by multiple proteins, including the molecular ruler nebulin, the barbed-end capper CapZ, and the pointed-end capper tropomodulin (Tmod), which functions synergistically with tropomyosin (Tpm). Together, CapZ and Tmod–Tpm restrict subunit exchange at both filament ends. Recent studies in cells and animal models have identified yet another player, leiomodin-2 (Lmod2), as an additional regulator proposed to promote pointed-end elongation and thereby contribute to thin filament length maintenance. This activity makes leiomodin the only known eukaryotic factor capable of driving pointed-end elongation, although its molecular mechanism remains unknown. I will describe a series of cryo-electron microscopy structures that address the capping mechanisms of CapZ and Tmod–Tpm, and support a stepwise elongation mechanism for Lmod2, in which two Lmod2 molecules alternate at the pointed end while mediating actin subunit recruitment. These findings establish a molecular framework for pointed-end elongation in muscle sarcomeres and provide insight into dilated cardiomyopathy–causing mutations in Lmod2. I will also discuss ongoing work on additional factors that regulate filament length in skeletal muscle sarcomeres.
Contributed talk	Alicia Peschel Max Planck Institute of Molecular Physiology, Dortmund, DE	*Structural investigation of the jellyfish sarcomere by cryo-electron tomography*	The sarcomere is the basic contractile unit of skeletal muscle, built from highly organized assemblies of thick and thin filaments. While sarcomere organization has been well characterized in insects and vertebrates (bilaterian animals), jellyfish (cnidarians) represent one of the oldest muscle systems in the animal kingdom, with transcriptomic evidence suggesting their striated muscle evolved independently. To investigate cnidarian sarcomere architecture, we prepared myofibrils from Pelagia noctiluca striated muscle and applied cryo-electron tomography (cryo-ET) coupled with focused ion beam (FIB)-milling. Our results reveal both unexpected organizational features and striking evolutionary conservations, offering new insights into the deep origins of striated muscle and the fundamental principles governing sarcomere architecture.
Contributed talk	Aditya Aniruddha Sane IBDM Marseille, FR	*DNA-PAINT imaging reveals a thick filament subdivision in Drosophila sarcomeres required to power flight*	Sarcomeres, the contractile units of muscles, are super-assemblies of actin and myosin filaments linked by titin and ordered by crosslinking proteins. To date, the native ultrastructure of sarcomeres, particularly at the Z-disc and M-band, remains unresolved. Here, we combined DNA-PAINT super-resolution with nanobodies recognizing specific protein domains to uncover the ultrastructure of Drosophila flight muscle sarcomeres. Imaging at 10 nm resolution, we confirmed that the two titin homologs Sallimus and Projectin are linearly arranged in a staggered organization: Sallimus spans from the Z-disc to the distal end of the A-band, where it overlaps with Projectin. To reveal details of the Z-disc, we localized α-Actinin, Zasp52, Zasp66, F-actin, and Sallimus N-terminus and found that all Z-disc components, including the F-actin overlap, form an 80 nm thick block. Surprisingly, Sallimus N-termini anchor near this block center, but Sallimus proteins from neighboring sarcomeres do not overlap. Imaging the M-band cross-linker Obscurin revealed that its C-terminus locates at the M-band center, while its N-terminus extends toward myosin motor domains. Finally, we discovered, Projectin instructs an unexpected subdivision of the A-band: the myosin-binding protein Stretchin decorates only its central region, excluding the distal region where Projectin is located. Importantly, removing Projectin selectively from flight muscles affects A-band architecture but does not prevent the subdivision, and causes flightlessness. A similar thick-filament subdivision in C- and D-zones is present in mammals, suggesting additional commonalities between insect and mammalian sarcomere architectures. Together, our data provide detailed insight into the insect flight muscle sarcomere architecture required to power flight.

P04.01	Sonia Calabrò University of Padova	*Neuromuscular development is aberrant in germ-free mice, and post-weaning colonization selectively rescues skeletal muscle but not peripheral nerve defects*	The gut microbiota (GM) has recently emerged as a positive modulator of skeletal muscle homeostasis, but its interaction with the other components of the neuromuscular systems remains underinvestigated. To expand our understanding of the gut-muscle axis in a more system view, we analyzed skeletal muscles, neuromuscular junctions (NMJs) and peripheral nerves in mouse models with distinct GM profiles: germ-free (GF), gnotobiotic (colonized with 12 known bacterial strains, Oligo-Mouse-Microbiota 12, or OMM12), conventional (with a complex GM, CGM) and EX-GF mice (recolonized, after weaning, with a complex GM). Compared to CGM mice, GF mice displayed atrophic skeletal muscles, increased basal autophagy, imbalanced protein turnover, and an altered proteomic profile, with deregulation of proteins involved in oxidative metabolism and sarcomeric organization. Moreover, they exhibited fragmented NMJs endplates with enlarged presynaptic areas as well as smaller hypermyelinated peripheral nerves with elongated nodes of Ranvier and an altered gene expression profile. While recolonization of GF mice with the simplified OMM12 bacterial community did not correct most neuromuscular abnormalities, EX-GF mice showed complete recovery of muscle and NMJ postsynaptic alterations. However, they failed to rescue molecular and structural aberrancies of the NMJ presynaptic compartment and somatic nerves. Overall, our data demonstrate that neuromuscular development is perturbed under GM depletion and suggest that both microbial richness and diversity as well as the timing of colonization are critical determinants for proper neuromuscular maturation, with early-life GM exposure being likely necessary for eliciting dynamic responses also of the peripheral nervous system.
P04.02	Dana Cizkova Charles University, Faculty of Medicine in Hradec Kralove	*Nestin re-expression in the heart of adult and aged spontaneously hypertensive rats: Comparison to conplastic SHR-mtBN strain and co-localization with connexin 43*	Nestin, a remarkable intermediate filament protein, is detected in several stem, progenitor and developing cells and re-expressed in the adult tissues in processes recapitulating the development such as skeletal muscle regeneration or angiogenesis. In the heart nestin is identified early prenatally in rodents and unexplainedly in the adult diseased human and rodent myocardium, e.g. in the hypertrophic heart of the spontaneously hypertensive rats (SHR), as reported in our previous study. In this work nestin was immunohistochemically detected in the hearts of the SHR-mtBN conplastic rat strain to uncover influence of the normotensive Brown Norway rat mitochondrial genome on its re-expression. Next, in the SHR myocardium nestin was co-localized with connexin 43 to reveal any differences in the intercalated discs gap junction pattern between nestin+ and nestin- cardiomyocytes. Nestin+ cardiac muscle cells were slightly more abundant in the SHR than in the SHR-mtBN rats. In the aged SHR, nestin was detected in cardiomyocytes evenly located in left ventricle and interventricular septum, whereas in the SHR-mtBN strain it was expressed more in groups of cells in certain regions. Thus, less frequent nestin re-expression and different distribution reflect lower intensity of myocardial cytoskeletal remodelling in the SHR-mtBN rats that are more resistant to hypoxia. Connexin 43 pattern did not differ substantially between nestin+ and nestin- cardiomyocytes. Only in the aged SHR, connexin 43 expression was more irregular and situated at the lateral cell membranes, regardless of nestin appearance. So, nestin re-expression is likely unrelated to the regulation of myocardial impulse conduction.
P04.03	Michael Cowley Stellenbosch University	*From Quiescence to Activation: CLEM-based evidence of Three-Dimensional Remodeling of Human Satellite Cells Following Exercise*	Acute eccentric exercise induces activation of satellite cells (SCs) within skeletal muscle. Current understanding of SC morphology is largely derived from two-dimensional electron microscopy and immunofluorescence studies. We aimed to investigate the three-dimensional SC morphology in human skeletal muscle during quiescence to activation transition following acute eccentric exercise. Muscle biopsies were obtained from the vastus lateralis muscle at baseline and 24h post-exercise. SC activation state was determined using frozen sections and immunohistochemistry staining for Pax7 and DLK1. After immunolabelled cells were localized, the sections were processed and embedded in Epon resin for subsequent volume electron microscopy imaging. Then, correlative light and electron microscopy (CLEM) was employed to superimpose labelling and ultrastructure of the cells. As proof of concept to characterize activated stem cell morphology, Three-dimensional reconstructions were generated to quantify elongation index, flattening ratio, and sphericity index as measures of cellular morphology. Preliminary observations demonstrate that quiescent Pax7+ SC exhibit a highly elongated and flattened morphology within their niche between the basal lamina and sarcolemma. In contrast, early activated DLK1+ SCs appear morphologically condensed, seen with chromatin condensation, with reduced elongation and increased cellular thickness, presenting with a rounded shape. Ongoing analysis may illustrate whether comparable morphological adaptations occur in SCs from contralateral unexercised muscle. These findings aim to establish a structural framework for defining SC activation state and provide novel insight into the ultrastructural adaptations associated with human skeletal muscle remodeling following exercise. This work also aims to provide a reproducible workflow for further CLEM analysis with muscle tissue.
P04.04	Alicia Cuber Caballero King's College London	*Super-resolution fluorescence microscopy to obtain a unified M-band model of titin*	Cryo-electron tomography of cardiac myofibrils has shown the structure of titin in unprecedented detail, revealing for the first time its differential alpha/beta conformations and placing the kinase domains 43nm apart at the first and fourth levels of myosin motor domains (Tamborrini et al, 2023). The latest model of M-band titin produced from this data, however, disagrees with published immuno-electron microscopy data used in previous models (Obermann et al, 1996; Fukuzawa et al, 2008). All three models suggest that titin crosses the centre of the M-band (albeit a non-crossing U-turn was considered by Obermann), resulting in a region of anti-parallel overlap, but could not pinpoint which regions cross the M1 line. While the interaction of titin with some M-band proteins has been established, the structural arrangement of these complexes is still unknown. We have used a wide range of antibodies targeting the C-terminal domain of titin and other M-band proteins to obtain nanometre-scale resolution images using DNA-PAINT. We have resolved the ~43nm separation between the alpha and beta m2 domains, establishing that titin is not cleaved by calpain-3 at the m1 domain, as recently suggested. Additionally, we have identified the first conformation-specific antibody to titin, binding only to the A170 domain of alpha-titin. We have also resolved and found the position of other M-Band titin domains, for which only a single band could be found, possibly corresponding to alpha-titin. Here, we put these findings in the context of previously published data and propose an updated model for M-band titin.

P04.05	Philipp Galuska, Universität Wien	*From Dynamic Z-Bodies to Ordered Z-Discs: Molecular Mechanisms of Early Myofibrillogenesis*	Z-discs define the boundaries of sarcomeres, the contractile subunits of striated muscle. Their highly ordered structure arises from numerous interacting proteins, yet molecular mechanisms governing early Z-disc assembly remain poorly understood. According to the premyofibril model, Z-discs originate from precursor structures termed Z-bodies. Using a combination of in vitro reconstitution, quantitative biophysics, and imaging of hiPSC-derived cardiomyocytes (hiPSC-CMs), we investigated how Z-body formation is initiated and transitions to mature Z-disc organisation. Filamin C bound and crosslinked F-actin into dense planar networks in vitro and localized to early actin-based structures in vivo, supporting a role as early cytoskeletal scaffold during myofibrillogenesis. Thin filament proteins, including tropomyosin and troponin components, were incorporated into early actin structures, while TNNT2 perturbation impaired Z-disc formation. Using purified proteins, we further reconstituted Z-body-like assemblies in vitro through FATZ-1 liquid–liquid phase separation (LLPS) along actin filaments. ACTN2 promoted FATZ-1 condensate growth, while ZASP and Filamin C acted as opposing modulators of condensate size and dynamics. However, in hiPSC-derived cardiomyocytes FATZ-2 was only weakly enriched in early Z-bodies, whereas ZASP and ACTN2 strongly localized to nascent assembly sites during early myofibrillogenesis. Interestingly, ZASP and ACTN2 formed droplet-like assemblies in vitro, identifying ZASP-ACTN2 complexes as possible central organising nodes for early Z-body formation, while FATZ contributes later to maturation. Together, these data support a modular model in which Filamin C establishes an actin scaffold, ZASP-ACTN2 condensates nucleate Z-bodies, and FATZ-driven LLPS contributes to subsequent Z-disc maturation — assigning distinct physical and temporal roles to each component in sarcomere biogenesis.
P04.06	Mathias Gautel King's College London	*Titin kinase: dead or alive?*	The giant muscle proteins titin and obscurin contain kinase domains whose physiological roles remain unclear. Titin kinase (TK), located at the innermost layers of myosin crossbridges and there associated with the myosin S2 segment and regulatory light chains, has been proposed to be either an unusual active kinase or an inactive pseudokinase. To assess these possibilities, we combined evolutionary, structural and functional analyses. Protein kinase activity depends on conserved side chains that coordinate protein substrate, ATP and co-factor binding and catalysis. These functional groups are homologous or conserved between TK and active kinases such as twitchin kinase (TwK) and canonical myosin-light-chain kinase. In TK, they are strongly conserved across chordates, from agnatha, cartilaginous and bony fishes to amphibians, reptiles, birds and mammals. This level of conservation is uncommon across pseudokinases, which typically lack residues essential for catalysis. In experimental structures and computational models, homologous and conserved residues in TK and related kinases align within 1–2.3 Å, consistent with structural compensation. To test the effect of sequence differences near the ATP-binding pocket, we introduced a titin-like hydrophobic motif (YMAK) into TwK's FAAK. TwK carrying the mutant FMAK motif remained catalytically active, showing that this substitution is compatible with kinase activity. Modelling further suggested that some other TK-like substitutions introduced into TwK create steric clashes, highlighting the importance of local structural context. Overall, our findings suggest that titin kinase, although unusual, retains key features required for catalysis across evolution; news of its death might be premature.
P04.07	Utku Gulbulak University of Kentucky	*MyoProfiler: Open-source software to analyze patterns of fluorescently labeled sarcomeric proteins*	MyoProfiler is open-source software that was developed to extract and analyze striated patterns of fluorescently labeled contractile proteins. It combines image analysis and segmentation to quantify sarcomere length, localization, and structural metrics within the user selected region of interest. The software calculates metrics automatically and accelerated the team's previous workflow by a factor of 10. The investigators have used the software to analyze fluorescence patterns associated with myosin, regulatory light chain, myosin binding proteins, myomesin, and desmin. MyoProfiler extracts intensity profiles from experimental images. Sarcomere length is calculated from the distance between adjacent Z lines. The full-width of A-band patterns can also be measured. The straightness of Z lines can be quantified via the tortuosity metric which is defined as the ratio of a curve's length to the straight-line distance between the curve's endpoints. In this case, the curves are smoothing splines fitted to each Z-line. The Z-lines are identified by segmenting the raw image to obtain binary objects and then linking adjacent objects appropriately through an iterative process. MyoProfiler analyzes each channel in multi-layered images to better support colocalization studies. Raw data, metadata, and analysis workflows are combined into a single open-format file to improve reproducibility. The software has a user-friendly interface and works with proprietary microscope image formats, including ND2 files, as well as open-formats, such as PNG and TIF.
P04.08	Jagannath Maharana Max-Planck Institute for Molecular Physiology	*Molecular architecture of the native muscle M-band*	Every heartbeat depends on the precise mechanical organization of the sarcomere, the fundamental contractile unit of striated muscle. At its centre, the M-band crosslinks antiparallel myosin filaments into a cohesive thick filament lattice. Mutations in M-band-associated proteins cause a broad spectrum of cardiomyopathies and muscular dystrophies, yet how its components are arranged within native muscle has remained unresolved. Using cryo-electron tomography, we determine the in situ molecular architecture of the M-band, uncovering unexpected organizational principles governing its constituent components and establishing a structural framework for understanding M-band-related muscle disease.

P04.09	Francis Pacagnelli University of Western São Paulo	*Muscle-Specific Inhibition of MicroRNA Function Alters Extracellular Matrix and Inflammatory Pathways During Disuse-Induced Skeletal Muscle Atrophy*	Disuse-induced skeletal muscle atrophy markedly increases the risk of frailty, falls, and mortality. Although microRNAs(miRNAs) are recognized as critical post-transcriptional regulators of muscle mass and remodeling, their global contribution to muscle responses during disuse and subsequent recovery remains incompletely understood due to limitations of existing animal models that fail to fully inhibit miRNA function.Purpose:We evaluated the role of miRNAs in regulating molecular pathways underlying skeletal muscle atrophy following disuse, using a reversible and muscle-specific miRNA inhibition model. Methods: We generated an inducible, skeletal muscle–specific mouse model (HSA-T6B) that inhibits miRNA function under doxycycline induction. Adult HSA-T6B mice were subjected to 14 days of disuse-induced atrophy. T6B mice, which lacks the reverse tetracycline-controlled transactivator (rtTA), were used as controls.Gastrocnemius muscles were collected and analyzed by bulk RNA sequencing. Differential gene expressions were assessed using DESeq2, followed by gene ontology enrichment.Results: Atrophied muscles with impaired miRNA activity showed upregulation of extracellular matrix (ECM) including genes associated with matrix organization (Fn1: 2.3-fold, adj.p=0.001; Adamts19: 3.25-fold, adj.p=0.04), collagen deposition (Col2a1:1.8-fold, adj.p=0.02; Col1a2: 2.22-fold, adj.p=0.001) and tissue remodeling (Mmp2: 1.60-fold, adj.p=0.01; Mmp9: 2.48-fold, adj.p<0.001). Inflammatory and immune-related pathways were markedly enhanced, with enrichment of cytokine–chemokine signaling (Il1b: 5.30-fold, adj.p=0.003; Ccl22: 4.43-fold, adj.p<0.001), immune cell recruitment (Sele:3.05-fold, adj.p=0.01; S100a8: 2.77-fold, adj.p=0.03), and chemotaxis-associated processes (Chga: 4.03-fold, adj.p=0.01).Conclusions: Muscle-specific inhibition of miRNA function significantly reshapes the molecular landscape of disuse-induced skeletal muscle atrophy, promoting exaggerated extracellular matrix remodeling and heightened inflammatory signaling.These findings demonstrate that miRNAs are key regulators of structural and immune responses during muscle disuse.
P04.10	Alicia Peschel Max Planck Institute of Molecular Physiology Dortmund	*Structural investigation of the jellyfish sarcomere by cryo-electron tomography*	The sarcomere is the basic contractile unit of skeletal muscle, built from highly organized assemblies of thick and thin filaments. While sarcomere organization has been well characterized in insects and vertebrates (bilaterian animals), jellyfish (cnidarians) represent one of the oldest muscle systems in the animal kingdom, with transcriptomic evidence suggesting their striated muscle evolved independently. To investigate cnidarian sarcomere architecture, we prepared myofibrils from Pelagia noctiluca striated muscle and applied cryo-electron tomography (cryo-ET) coupled with focused ion beam (FIB)-milling. Our results reveal both unexpected organizational features and striking evolutionary conservations, offering new insights into the deep origins of striated muscle and the fundamental principles governing sarcomere architecture.
P04.11	Momcilo Prodanovic Filamentech D.O.O. Beograd	*PREDICTING EQUATORIAL X-RAY FIBER DIFFRACTION PATTERNS DURING CARDIAC TWITCH*	Small-angle X-ray fiber diffraction provides structural information from intact striated muscle. Equatorial reflections up to the 4,0 order incorporate information about thin and thick filament structure and crossbridge distributions across the sarcomere lattice. We extend MUSICO-X simulations to predict a complete set of equatorial intensities from pig cardiac trabeculae throughout a twitch contraction. Thick filament electron density distributions are built from the cryo-EM structure of the human cardiac thick filament (PDB: 8G4L), covering the C-zone with myosin heavy and light chains, cMyBP-C, and titin, and extended geometrically to include the P-zone, D-zone, and central bare zone. We calculate thin filament electron density distributions from PDB structures of actin, tropomyosin, and the troponin complex. At rest, most myosin heads occupy a quasi-ordered parked state (PS) held close to the thick filament backbone, with a fraction of heads transitioning to actin-bound, force-generating configurations as calcium activation progresses during the twitch. We calibrate MUSICO-Fiber simulations against experimental twitch force recordings from pig cardiac trabeculae, obtaining time-resolved distributions of myosin heads across the parked, unbound, and actin-bound states. MUSICO-X uses these distributions to simulate time-resolved 2D projected electron density distributions and predict equatorial reflection intensities up to the 4,0 order, enabling direct comparison with experimental patterns recorded synchronously with force. Fitting the full reflection series during the twitch lets us simultaneously track thick filament backbone geometry, myosin head redistribution, and thin filament lattice spacing. This approach enables more detailed characterization of sarcomeric structural dynamics during a cardiac twitch that mechanical data alone cannot provide.
P04.12	Michael Radke MDC-Berlin	*The M-Band of the Sarcomere- more than an anchor / Exploring the sarcoremic M-Band with Titin-BioID knock-in mice*	To decipher the interaction network of titin at the sarcomeric M-band, we generated a titin-bioID M-band knock-in mouse model. For this purpose, a biotin ligase was fused to the C-terminal region of titin. Following successful ES cell targeting, the titin M-band knock-in model was validated by PCR, Western blotting, and immunofluorescence analyses. Phenotypic characterization revealed no apparent abnormalities. Mass spectrometric analyses enabled the identification of biotinylated proteins as interaction partners of titin at the M-band. In addition to well-established structural interaction partners of titin, including obscurin, myomesin, and myosin, we identified further proteins involved in energy metabolism and signal transduction pathways. Moreover, Streptavidine-based pulldown experiments allowed the enrichment of additional candidate proteins that may constitute part of the interaction network associated with titins M-band. The localization of biotinylation sites on titin and its interacting proteins provides new opportunities to further resolve the structural organization of the M-band. Our findings confirm that the M-band region of titin plays a critical role in sarcomeric structure, metabolism, and signaling.

P04.13	Martin Rees King's College London	*The generation and validation of a de novo-designed titin binder for use in fluorescence microscopy*	Skeletal muscle development and regeneration require coordinated myoblast differentiation and fusion into multinucleated myotubes. Although myogenesis has been extensively studied, many underlying molecular mechanisms remain unclear. Our study shows that loss of unconventional myosin VI (MVI) markedly impairs myotube formation, resulting in abnormal, spindle-shaped myotubes with centrally located nuclei, indicating defective differentiation and fusion. MVI-knockout (MVI-KO) differentiating myoblasts exhibit significantly increased expression of the fusogenic proteins Myomaker and Myomerger. These changes are accompanied by elevated reactive oxygen species (ROS) levels and a decreased GSH/GSSG ratio, indicating disrupted redox balance. Although the link between ROS and fusion is not fully defined, increased oxidative stress in MVI-KO cells may contribute to altered Myomaker expression. We also observed increased levels of nuclear factor erythroid 2-related factor 2 (NRF2), a key regulator of redox homeostasis. However, fractionation analysis revealed abnormal intracellular distribution: despite higher total NRF2 levels, the protein was mainly retained in the cytoplasm, with reduced nuclear accumulation compared to controls. This suggests impaired nuclear transport of NRF2 in the absence of MVI, potentially compromising activation of the antioxidant response. Overall, our findings indicate that MVI is an important regulator of redox homeostasis during myogenic differentiation and may influence myoblast fusion through ROS-dependent regulation of fusion machinery and NRF2 signaling. These effects are probably associated with mitochondrial dysfunction in MVI-KO myogenic cells. This work was supported by National Science Center, Poland (Grant number: 2022/47/D/NZ3/02737).
P04.14	Aditya Aniruddha Sane Ibdm (Marseille)	*DNA-PAINT imaging reveals a thick filament subdivision in Drosophila sarcomeres required to power flight*	Sarcomeres, the contractile units of muscles, are super-assemblies of actin and myosin filaments linked by titin and ordered by crosslinking proteins. To date, the native ultrastructure of sarcomeres, particularly at the Z-disc and M-band, remains unresolved. Here, we combined DNA-PAINT super-resolution with nanobodies recognizing specific protein domains to uncover the ultrastructure of Drosophila flight muscle sarcomeres. Imaging at 10 nm resolution, we confirmed that the two titin homologs Sallimus and Projectin are linearly arranged in a staggered organization: Sallimus spans from the Z-disc to the distal end of the A-band, where it overlaps with Projectin. To reveal details of the Z-disc, we localized α-Actinin, Zasp52, Zasp66, F-actin, and Sallimus N-terminus and found that all Z-disc components, including the F-actin overlap, form an 80 nm thick block. Surprisingly, Sallimus N-termini anchor near this block center, but Sallimus proteins from neighboring sarcomeres do not overlap. Imaging the M-band cross-linker Obscurin revealed that its C-terminus locates at the M-band center, while its N-terminus extends toward myosin motor domains. Finally, we discovered, Projectin instructs an unexpected subdivision of the A-band: the myosin-binding protein Stretchin decorates only its central region, excluding the distal region where Projectin is located. Importantly, removing Projectin selectively from flight muscles affects A-band architecture but does not prevent the subdivision, and causes flightlessness. A similar thick-filament subdivision in C- and D-zones is present in mammals, suggesting additional commonalities between insect and mammalian sarcomere architectures. Together, our data provide detailed insight into the insect flight muscle sarcomere architecture required to power flight.
P04.15	Morgan Seffrood University of Arizona	*Structural Dynamics of Slow Skeletal MyBP-C N-terminal Domains: Effects of Phosphorylation and Splicing in the N-terminal Linker*	Slow skeletal myosin binding protein-C (sMyBP-C) is a key sarcomeric regulator of muscle contractility through interactions between its N-terminal domains and actin and myosin. sMyBP-C is phosphorylated at the Pro/Ala-rich linker (PAL) in the N-terminus in response to β-adrenergic stimulation, enhancing muscle function; however, the structural and mechanistic effects of phosphorylation remain poorly understood. To address this, we examined phosphorylation-dependent regulation of the sMyBP-C N-terminal fragment (sC1-C2). Cosedimentation and time-resolved fluorescence resonance energy transfer (TR-FRET)-based binding assays demonstrate that phosphorylation reduces sC1-C2 binding to actin. To define the underlying structural changes, we used TR-FRET to measure distance distributions within the N-terminus. In the unphosphorylated state, the inherently flexible PAL is, unexpectedly, closely anchored to the C1 domain, and structured C1 and C2 domains, though separated by the M-domain, are also in close proximity. Phosphorylation increases the distance between sites within the PAL, the PAL and C1, and surprisingly, the C1 and C2 domains, indicating phosphorylation induces conformational rearrangement. We also examined a splice variant lacking 25 amino acids from the PAL, including the phosphorylation site, that differentially modulates thin-filament motility. FRET revealed a modest decrease in the PAL interprobe distance in this short isoform, suggesting the 25 amino acids are not extended in the long isoform. Overall, these findings show that phosphorylation and alternative splicing alter the N-terminal conformation and dynamics of sMyBP-C. This work provides a structural basis for how sMyBP-C phosphorylation and isoform diversity might tune muscle contractility, with broad implications for sarcomeric regulation in health and disease.
P04.16	Victoria Trinkaus Max-Planck-Institut für Molekulare Physiologie	*Structural Basis of Cardiac Thick Filament Activation*	The systolic output of the heart is modulated by its diastolic filling volume, which is described by the Frank-Starling law of the heart. The cellular basis of this law is length-dependent activation of the sarcomere. Despite the central importance of thick filament activation in this process and its association with hypertrophic and titin-associated dilated cardiomyopathies, the underlying molecular mechanism remains unknown. Here, we combine cryo-electron tomography with molecular simulations to demonstrate how stretching of titin results in the activation of the thick filament. We determine an in situ structure of the cardiac thick filament in its activated rigor state and find that, compared to the inactivated state, the myosin coiled coils and titin chains elongate and rotate along the filament axis. This leads to a compaction of the filament backbone. Physiologically relevant forces acting on titin are sufficient to trigger this process. The conformational changes result in altered interfaces and steric clashes between titin, myosin-binding protein C (MyBP-C) and the myosin heads. Together with the direct transmission of stress from titin to the S2 domain of myosin, this displaces the heads from their resting state in an unforeseen manner, activating the thick filament. Due to the individual structural arrangement of the three myosin crowns, displacement is non-uniform, adding an additional layer to the hierarchy of activation. Our results provide important molecular details underlying the Frank-Starling law and provide a foundation for future research on heart diseases.

P04.17	Andreas Unger University Muenster	*Microscopical characterization of Novex-3 titin to assess its functional role in cardiomyocytes under physiological and disease states.*	Titin, the largest human protein, is essential for the structure and function of sarcomeres in striated muscle, including the myocardium. Spanning from the Z-disk to the M-band, titin maintains sarcomere integrity and regulates cardiomyocyte (CM) elasticity, contractility, and mechanosignaling. Novex-3 (Nx3) is a uniquely truncated titin isoform expressed in all striated muscles. It shares its N-terminus with full-length titin isoforms but contains exon 48 (ex48), which introduces a premature stop codon and generates the smallest titin isoform (~650 kDa). Despite its likely importance, Nx3 remains poorly characterized. Although its C-terminus may interact with cytoskeletal proteins such as obscurin, its precise sarcomeric localization and functional role are unclear. Here, we comprehensively investigate Nx3 in the heart, focusing on its sarcomeric role. Using custom anti-Nx3 antibodies, confocal and electron microscopy, interaction assays, a murine Nx3-deletion model, and CRISPR/Cas9-mediated knockout in hiPSC-CMs, we assess its localization, binding partners, and effects on sarcomere organization. We also analyzed Nx3 expression in failing human hearts compared with non-failing donor hearts. Together, our findings establish the structural and functional significance of Nx3 in cardiac biology and support its involvement in heart disease.
P04.18	Dorjana Zerbo Šporin University of Primorska, Faculty of Health Science	*Slower Gait Speed as a Predictor of Increased Frailty Risk in Nursing Home Residents*	Skeletal muscle aging contributes to age-related frailty and sarcopenia, increasing the risk of adverse outcomes, including falls, disability, hospitalization, and mortality. This cross-sectional study examined whether gait speed can identify the frail phenotype among older adults living in nursing homes. The study included 94 residents from Slovenian nursing homes, with a mean age of 83.6 ± 7.9 years; 68% were women. Frailty status was evaluated using Fried's Frailty Phenotype criteria, while gait speed was assessed over a 4.5 m walking distance. Relative risk analysis determined whether reduced gait speed was associated with an increased likelihood of frailty. Most participants were classified as pre-frail (63%) or frail (16%), while 21% were non-frail. Compared with non-frail residents, individuals in the frail group had significantly slower gait speed, with a mean difference of 0.21 m/s (U = 331.5; $p < 0.001$). A gait speed of ≤ 0.457 m/s was associated with a 40% higher risk of frailty (RR = 1.40; 95% CI: 1.15–1.70; $p = 0.0008$). These findings indicate that gait speed may serve as a simple, easily applicable screening tool for identifying nursing home residents at increased risk of frailty. Individuals identified as at risk should be referred to appropriate preventive or therapeutic interventions. Šporin, M., & Zerbo Šporin, D. (2025). Slow gait speed is associated with frailty in older adults from nursing homes. Kinesiologia Slovenica: Scientific Journal on Sport, 31(2), 167–176.

S05 - Data Science in Muscle: from -omics to Physiology			
Invited speaker	Manuel Mayr Imperial College London, UK	The proteomics landscape of dilated cardiomyopathy: assessing the interplay of comorbidities and pathogenic variants	In the largest cardiac proteomics analysis to date (n=198), left ventricular samples from patients with ischemic heart disease (IHD, n=65) and dilated cardiomyopathy (DCM, n=114) were analyzed by proteomics and compared to non-failing controls (n=19). In both IHD and DCM, we observed a marked rise in ventricular levels of atrial natriuretic factor (ANF). Four clusters of DCM patients could be identified with comorbidities (hypertension, atrial fibrillation), clinical parameters (sex, body mass index, hematocrit, left ventricular ejection fraction), titin truncating variants and circulating ANF levels as defining clinical characteristics. Among all proteins identified, ventricular ANF levels exhibited the highest inter-patient variability in DCM patients and correlated most strongly with the accumulation of versican. This correlation was confirmed using spatial transcriptomics with versican being expressed by fibroblasts in close proximity to strained cardiomyocytes (ANF+) in both DCM and IHD. Ventricular ANF expression increased >2-fold in mice with a point mutation preventing versican degradation (VcanE441A). This is consistent with a versican-rich matrix inducing re-expression of fetal gene programmes. Unlike normal adult hearts, left ventricles in human fetal hearts displayed high expression of ANF and versican. Thus, our results unveil a novel link between versican and ANF induction, implicating the extracellular matrix in a coordinated cardiomyocyte strain response, leading to re-expression of fetal genes in failing hearts.
Invited speaker	Marcus Krueger University of Cologne, DE	*Spatial proteomics identifies FXYD6 as a dual-site protein of neuromuscular junction in the diaphragm*	Morphological studies of the diaphragm have provided a detailed view of its architecture, which consists of parallel skeletal muscle fibers and a central ring of neuronal innervation that includes neuromuscular junction (NMJ) units. NMJs are disease-vulnerable synapses; thus, analysis of the NMJ is essential to understand its function in both healthy and disease-related conditions. The diaphragm was analyzed by spatial proteomics and 115 proteins were enriched at the NMJ. Comparison of the protein signatures of the NMJ and myotendinous junction (MTJ) revealed 31 shared proteins, suggesting partially conserved structures between these junctions. Key mediators of synaptic transmission and extracellular matrix organization were observed among the NMJ-enriched components, which indicates the molecular complexity and regulatory potential of the NMJ. A focused study of the uncharacterized NMJ protein FXYD6 demonstrate enhanced FXYD6 expression in type IIa fibers of the diaphragm, which exhibit a unique balance of oxidative and glycolytic capacity. FXYD6 interacts with Na^+/K^+-ATPase subunits in the diaphragm, which supports the function of FXYD6 in the ionic homeostasis required for continuous, fatigue-resistant contraction. Overall, the dataset provides a comprehensive molecular atlas of the NMJ in the diaphragm and opens new opportunities to dissect the synaptic mechanisms underlying respiratory function and neuromuscular diseases.

Contributed talk	Alexandra Winant University of Copenhagen, DK	*Integrated single-myofiber multi-omics links inflammatory state to myosin energetics in ICU-acquired weakness*	Skeletal muscle dysfunction is a pervasive complication of critical illness that worsens survival and recovery. This rapid ICU-acquired weakness (ICU-AW) is incompletely explained by admission diagnosis, treatment protocols, or measures of muscle atrophy. ICU-AW has a predominantly myogenic origin involving targeted sarcomeric protein degradation, yet the underlying pathophysiological mechanisms remain poorly understood. We developed an integrative single-fiber approach combining myosin dynamics measurements with next-generation sequencing to generate high-dimensional multi-omic and functional data from individual muscle fibers. ATP-chase experiments assess myosin dynamics and ATP turnover, followed by sequential isolation of transcriptomic, epigenomic, and proteomic data from the same myofiber. This strategy links sarcomeric functional states with their underlying molecular regulation. Despite marked donor-level heterogeneity, integrated analysis revealed a conserved myofiber state enriched in ICU-AW, characterized by inflammatory and chemotactic gene programs, intracellular structural remodeling, and bioenergetic adaptation. Nineteen features were significantly altered at both RNA and protein levels, linking an inflammatory transcriptional landscape to a proteomic shift toward mitochondrial and translational machinery and away from membrane-associated signaling. Functionally, these fibers displayed selectively disrupted myosin dynamics, implicating altered myosin energetics in muscle dysfunction. These findings define a discrete, disease-associated myofiber state and establish an integrative single-fiber framework for connecting multi-omic heterogeneity to molecular motor function in complex human disease.
Contributed talk	Cinzia Barresi Medical University of Vienna, AT	*Analysis of the MuSK interactome identifies a novel regulator of neuromuscular junction development*	The neuromuscular junction (NMJ) is the synapse between motor neurons and skeletal muscle fibers that coordinates muscle contraction and movement. At the mature NMJ, acetylcholine receptors (AChRs) are clustered in the postsynaptic membrane in opposition to the presynaptic motor terminal ensuring efficient neurotransmission. The key signaling molecule during NMJ formation and maintenance is the muscle-specific receptor tyrosine kinase MuSK. Loss of MuSK is lethal and impairment of MuSK signaling causes neuromuscular disorders. MuSK activation is triggered by the nerve-derived signal Agrin and its co-receptor Lrp4, which promote MuSK dimerization and activation. This initiates a signaling cascade involving the docking protein Dok-7, which recruits the adaptor proteins Crk and Crk-L, ultimately driving the clustering of acetylcholine receptors (AChRs) at the postsynaptic membrane. Although MuSK activation by Agrin, Lrp4, and Dok-7 is well characterized, the temporal organization of downstream signaling remains unclear. Here, we define the MuSK proximity proteome using TurboID-based proximity labeling combined with mass spectrometry. We identified 74 MuSK-associated proteins, including known substrates and novel interactors. Among early signaling components, the adaptor Crk-L emerged as a central hub linking MuSK to small GTPase pathways. We further identified Rapgef1 as a Crk-L-associated effector required for Agrin-dependent AChR phosphorylation and clustering. Rapgef1 loss in adult muscle led to NMJ fragmentation. We propose that these defects result from reduced cytoskeletal anchorage of AChRs due to compromised Rapsyn/AChR interaction. These findings uncover Rapgef1 as a key regulator of MuSK-mediated signaling and highlight dynamic mechanisms governing NMJ formation and maintenance.
P05.01	Maral Azodi Deutsches Herzzentrum der Charité (DHZC)	*Altered FHL2 signaling following titin N2B deletion in human iPSC-derived cardiomyocytes*	Titin's cardiac-specific N2B domain functions as an important signaling hub through its interaction with FHL2 (Four-and-a-Half LIM Domain Protein 2). Interestingly, N2BKO mouse cardiomyocytes exhibit reduced FHL2 expression, cardiac atrophy and fail to develop hypertrophy in response to β-adrenergic stimulation. In contrast, FHL2 knockout mice show exaggerated cardiac hypertrophy following β-adrenergic activation. Together, these contrasting phenotypes highlight an unresolved role for the N2B–FHL2 signaling axis in cardiac stress adaptation. To address this, we generated N2B-deficient human induced pluripotent stem cell-derived cardiomyocytes (N2BKO hiPSC-CMs) using CRISPR/Cas9 genome editing. Similarly to mice, FHL2 protein expression was significantly downregulated in N2BKO hiPSC-CMs as compared to WT. By immunofluorescence imaging, FHL2 protein was predominantly localized in the sarcomere of WT hiPSC-CMs but in the nucleus of N2BKO hiPSC-CMs. Consistently, N2BKO hiPSC-CMs showed increased expression of p21 mRNA, a reported downstream target of FHL2. Following 72 hours of isoproterenol treatment, N2BKO hiPSC-CMs exhibited reduced cell size, increased nuclear accumulation of FHL2, and elevated p21 expression relative to untreated WT, untreated N2BKO, and isoproterenol-treated WT hiPSC-CMs. In addition, siRNA-induced knockdown of FHL2 in WT hiPSC-CMs resulted in an exaggerated hypertrophic growth in response to isoproterenol, supporting the observation that the balance between sarcomere and nuclear localization is crucial for the regulatory role of FHL2 in β-adrenergic hypertrophic signaling. These results show that hiPSC-CMs can recapitulate key features of the N2B–FHL2 signaling axis and can help disentangle the contrasting results on FHL2-dependent hypertrophic signaling and clarify the role of titin N2B domain.

P05.02	Kristina Bardova Institute Of Physiology, Czech Academy of Sciences	*Physiological Consequences of Muscle Non-Shivering Thermogenesis: Delayed Regulation and Contractile Dysfunction*	Thermogenesis significantly contributes to energy expenditure and may affect propensity to obesity. In cold environments, multiple thermogenic mechanisms maintain stable body temperature, including uncoupled respiration in brown adipose tissue. We previously showed that obesity-resistant A/J mice rely more on skeletal muscle-based thermogenesis than C57BL/6 (B6) mice, potentially involving altered efficiency of muscle calcium pumping via sarcolipin. We proposed that the slower induction and inhibition of this muscle non-shivering thermogenesis may create a negative energy balance, potentially preventing obesity. Here, we validate this concept by comparing A/J and B6 mice during 2 weeks of cold exposure, with and without a high-fat diet. Cold exposure causes an immediate rise in energy expenditure and a transient drop in body temperature. Normalization is slower in A/J mice, reflecting gradual buildup of thermogenic capacity, and circadian rhythmicity of body temperature is disrupted for longer. Upon return to thermoneutrality, mice tend to overheat, suggesting an inability to rapidly suppress thermogenesis. Muscle transcriptomic and lipido-metabolomic analyses reveal robust adaptations in cold-exposed A/J mice, including upregulation of the pentose phosphate pathway, de novo lipogenesis, and fatty acid content — changes less pronounced in B6 mice. Sarcolipin and related genes are more highly expressed in A/J mice after cold adaptation. Consistent with sarcolipin reducing muscle contraction efficiency, cold-adapted A/J mice show higher energy expenditure during treadmill running, unlike B6 mice. These findings confirm a prominent role of skeletal muscle in A/J thermogenesis, characterized by slower activation and interference with the energetic cost of physical activity. Supported by CSF (24-10994S)
P05.03	Cinzia Barresi Medical University of Vienna	*Analysis of the MuSK interactome identifies a novel regulator of neuromuscular junction development*	The neuromuscular junction (NMJ) is the synapse between motor neurons and skeletal muscle fibers that coordinates muscle contraction and movement. At the mature NMJ, acetylcholine receptors (AChRs) are clustered in the postsynaptic membrane in opposition to the presynaptic motor terminal ensuring efficient neurotransmission. The key signaling molecule during NMJ formation and maintenance is the muscle-specific receptor tyrosine kinase MuSK. Loss of MuSK is lethal and impairment of MuSK signaling causes neuromuscular disorders. MuSK activation is triggered by the nerve-derived signal Agrin and its co-receptor Lrp4, which promote MuSK dimerization and activation. This initiates a signaling cascade involving the docking protein Dok-7, which recruits the adaptor proteins Crk and Crk-L, ultimately driving the clustering of acetylcholine receptors (AChRs) at the postsynaptic membrane. Although MuSK activation by Agrin, Lrp4, and Dok-7 is well characterized, the temporal organization of downstream signaling remains unclear. Here, we define the MuSK proximity proteome using TurboID-based proximity labeling combined with mass spectrometry. We identified 74 MuSK-associated proteins, including known substrates and novel interactors. Among early signaling components, the adaptor Crk-L emerged as a central hub linking MuSK to small GTPase pathways. We further identified Rapgef1 as a Crk-L-associated effector required for Agrin-dependent AChR phosphorylation and clustering. Rapgef1 loss in adult muscle led to NMJ fragmentation. We propose that these defects result from reduced cytoskeletal anchorage of AChRs due to compromised Rapsyn/AChR interaction. These findings uncover Rapgef1 as a key regulator of MuSK-mediated signaling and highlight dynamic mechanisms governing NMJ formation and maintenance.
P05.04	André Mourão Batista, IPEN São Paulo	*Raman spectroscopy and machine learning in the classification of muscle fiber types*	The classification of skeletal muscle fibers (Types I, IIa, and IIx) is essential for understanding muscle physiology and pathologies. Traditionally, this classification relies on laborious immunofluorescence assays targeting myosin heavy chain isoforms, which fail to capture the full structural and metabolic complexity of the cells. To overcome these limitations, this study investigates the use of Raman spectroscopy combined with Machine Learning (ML) as an innovative, label-free approach to differentiate fiber types based on intrinsic biochemical signatures. We analyzed 250 mapped fibers from the soleus muscle of five mice. After applying spectral interpolation and evaluating seven predictive models, a nonlinear SVM-RBF model demonstrated the best predictive performance. By implementing a majority voting strategy and selecting specific spectral regions corresponding to key vibrational bands of proteins and lipids, the model achieved an impressive balanced accuracy of 94.4%. The results showed that slow-twitch (Type I) fibers possess highly distinct spectral signatures. However, significant chemical overlap was observed among fast-twitch fibers, with misclassifications occurring primarily between types IIa and IIx. This difficulty in discrimination reflects the biological reality of muscle tissue, in which fast fibers operate within a continuous dynamic spectrum, frequently exhibiting hybrid profiles. Ultimately, Raman spectroscopy combined with machine learning proves to be a highly effective and label-free analytical tool for muscle fiber typing. This consolidation paves the way for advanced in situ diagnostics, enabling monitoring of conditions such as atrophy, hypertrophy, and sarcopenia at the cellular level.
P05.05	Anderson Z. Freitas Nuclear and Energy Research Institute	*Phenotypic Characterization of Skeletal Muscle Fibers by Raman Spectroscopy: A Molecular Signature-Based Approach*	The identification of skeletal muscle fiber types using specific antibodies remains a classical and widely adopted approach in histological analyses. However, alternative methods requiring reduced sample preparation, shorter processing times, and lower experimental costs are still highly desirable. This study investigated the potential of Raman spectroscopy as a high-precision, low-cost alternative tool for fiber phenotyping in mice soleus muscle. By using a 473 nm laser and Principal Component Analysis (PCA), it was possible to clearly differentiate oxidative (Type I) from glycolytic (Type II) fibers, focusing on the spectral region of 1500 to 1800 cm^{-1}. Detailed analysis of bandwidths and Gaussian fits revealed significant molecular differences among fiber types. A progressive increase in the phenylalanine/tyrosine band area was observed following the order Type I < Type IIa < Type IIx fibers. In the Amide I region, a conformational transition was identified in the proteins, characterized by an increase in α-helix structures (1657 cm^{-1}) and a reduction in β-sheet structures (1685 cm^{-1}) during the transition from Type I to Type IIx fibers. In addition, the Width at Half Height (HWHM) of the band at 1604 cm^{-1} allowed a clear separation between the subtypes, highlighting unique chemical signatures related to free amino acids and protein synthesis. Although there are inherent diagnostic limitations to band broadening, the results demonstrate that Raman spectroscopy can provide information into intrafiber protein conformational changes, supporting its application as a suitable technique for the biochemical and pathophysiological characterization of muscle tissue.

P05.06	Isabel Martin Universität Konstanz	*Proximity-dependent labelling to identify mechanosensory signalling cascades of titin-like kinases*	Sensing and responding to mechanical signals is fundamentally important for healthy muscle tissue. One of the key structures in muscle sarcomeres required for mechanical responses are filaments of the titin-like family, namely titin and obscurin in humans. Dysregulation of mechanosensing on these filaments can lead to muscle disease such as dilated cardiomyopathy, muscular dystrophy, or exercise intolerance. Although the importance of these filaments for mechanical sensing has been known for a long time, the exact molecular mechanisms underlying this process remain understudied. Kinase domains located on these filaments have been the focus of many recent studies, and their mechanism of mechanosensing involves stretch-induced conformational changes. However, how this signal is subsequently transmitted from the sarcomere to other cellular compartments, such as the mitochondria to regulate energy requirements or the nucleus to induce expression of protein isoforms geared to withstand mechanical stress, is still unknown. To date, only a few interaction partners of mechanosensory muscle kinases have been identified. This suggests that many additional interaction partners remain undiscovered, especially since these kinases are proposed to act as important signalling nodes for mechanical signals. Using the model organism C.elegans, which contains homologues of titin and obscurin –namely, Twitchin, TTN-1, and UNC-89– whose kinase domains share high similarity with the human proteins, we aim to identify signalling cascades mediated by mechanoreceptory kinases. For this, we apply an innovative in vivo proximity-based labelling approach using a Nanobody-BioID-fusion enzyme and genome edited C.elegans to reveal kinase interaction partners that may uncover potential drug targets.
P05.07	Glen Pyle Dalhousie Medicine	*Sex differences in the cardiac effects of chronic pain*	Chronic pain impacts over 2 billion people globally. Despite higher rates and severity, females experience delays in diagnosis and receive inadequate pain management. Chronic pain is a cardiovascular disease risk, with a greater impact on females. The underlying mechanisms connecting pain and cardiovascular disease are unknown, as is the impact of sex. We used the spared nerve injury model of neuropathic pain in mice to induce chronic pain and investigated the cardiac effects in both sexes. Mice recovered for 5 or 30d after surgery and were subjected to echocardiography. Hearts were extracted for proteomic analysis using LC-MS/MS. Molecular function was assessed with an actomyosin ATPase assay. Echocardiography identified sex differences by 5d post-surgery, with males showing a decrease in left ventricular systolic diameter, and females a reduction in left ventricular volume. Cardiac myofilament activation was increased in females at 5d and remained elevated at 30d, whereas males showed no changes. Proteomic analysis revealed greater changes in male samples, with 291 proteins changing expression by 50% or more at 5d and 296 at 30d. By contrast, 199 and 183 proteins were altered in female samples at the same timepoints. Proteins related to energy production and molecular function of small molecule and nucleotide binding exhibited the greatest sex differences. This is the first investigation of cardiac changes associated with chronic pain, identifying sex-dependent differences in cardiac function and protein expression to provide mechanistic insight into the unique cardiovascular risk profiles of females and males with chronic pain.
P05.08	Glen Pyle Dalhousie Medicine	*Laying the Foundation for Cardiovascular Disease: Sex-Dependent Effects of Early Life and Environmental Stress on Oxidative Stress in the Heart*	Early life stressors (ELS) like neglect are associated with higher risks for cardiovascular diseases (CVD) later in life. ELS including poverty expose children and adolescents to higher levels of environmental CVD risk factors like bisphenol S (BPS). The mechanisms by which ELS and environmental stress drive CVD separately and in combination are unknown. We used maternal separation with early weaning (MSEW) alone and in combination with BPS (250 nM) in the drinking water to represent biological and environmental ELS. BPS depressed maximum cardiac myofilament ATPase activity by 12% in female mice ($p=0.02$ vs control), and MSEW reduced activity by a further 10% ($p<0.01$ vs control). The combination of MSEW + BPS yielded effects similar to MSEW alone ($p<0.01$ vs control). Cardiac myofilament function in males was not significantly affected in any group. Immunoblotting (N=3 per group) for the oxidative stress marker nitrotyrosine found reduced levels in male and female samples, except for MSEW + BPS males where levels increased. Antioxidant enzymes including catalase, glutathione peroxidase, and xanthine oxidase were reduced or unchanged in male samples, while females showed higher levels in conjunction with increased Nrf2 expression. p62 expression was higher in female samples across most groups, as was the autophagy marker beclin, while opposite trends were observed in males. Bax and Bcl-2 levels remained unchanged or modestly reduced, indicating no significant apoptosis in any group. These results demonstrate sex-dependent changes in oxidative stress and antioxidant defence mechanisms with ELS and BPS that impact cardiac molecular function.
P05.09	Fernando Ribeiro Uppsala University	*Protecting the Diaphragm from Acute Disuse: MuRF1 as a Promising Therapeutic Target*	Background: Mechanical inactivity rapidly drives diaphragm contractile dysfunction and atrophy, affecting respiratory function and increasing risks of morbidity and mortality in clinical conditions such as phrenic nerve injury or mechanical ventilation. Muscle RING-finger protein-1 (MuRF1) consists of an E3-ubiquitin ligase targeting key myofibrillar proteins for degradation. MuRF1 is found rapidly upregulated during diaphragm stress induced by denervation and disuse. We investigated the effects of a small-molecule targeting MuRF1 (MyoMed-205) against denervation-induced diaphragm dysfunction and atrophy, and explored the underlying mechanisms. Methods: Wistar rats underwent 12h of unilateral diaphragm denervation (UDD) and received either placebo (vehicle) or MyoMed-205 (50 mg/kg bw, i.v.), compared with Sham controls. Diaphragm muscle functional and structural properties, as well as global gene expression (bulk RNA-seq) and protein expression (Western blotting) analyses, were performed. Results: UDD induced diaphragm contractile dysfunction and fiber atrophy (I, IIa and IIb/x). Importantly, MyoMed-205 protected against both UDD-induced fiber contractile dysfunction and atrophy. Transcriptomic profiling revealed that UDD perturbed pathways controlling myofiber ultrastructure, calcium handling, mitochondrial function, proteolysis, and tissue remodeling. Conversely, MyoMed-205 enhanced activation of pathways controlling sarcomere integrity, antioxidant defense, chaperone-mediated unfolded protein response, and muscle growth via PI3K-Akt-mTOR signaling. MyoMed-205 also mitigated FoxO1 and HDAC4 activation, suppressed MuRF2 upregulation, and attenuated intramuscular fat deposition and pro-fibrotic gene expression triggered by UDD. Conclusions: Small-molecule targeting of MuRF1 (MyoMed-205) protects diaphragm structure and function from acute denervation stress through a coordinated transcriptional and protein-level molecular response. These findings establish MuRF1 as a promising therapeutic target against disuse-associated diaphragm wasting and weakness.

P05.10	Maira Rossi University of Pavia	*Single-nucleus transcriptomics reveals myonuclear state remodeling and altered cell-cell communication during human disuse muscle atrophy.*	Five healthy young men (18–29 years) underwent 21 days of bed rest (BR), a well-established model of whole-body deconditioning, followed by 21 days of active recovery (AR). Quadriceps maximal voluntary contraction, muscle cross-sectional area, and voluntary activation were reduced after BR and recovered following AR. We performed single-nucleus RNA sequencing on vastus lateralis biopsies collected at baseline (BR0), after 10 (BR10) and 21 days (BR21) of BR, and after recovery (AR). Unsupervised clustering identified 19 major cell populations consistent with known skeletal muscle cell types. Analysis of myonuclei revealed two principal transcriptional clusters corresponding to slow (MYH7+) and fast (MYH2+/MYH1+) myonuclei. Within fast myonuclei, MYH2+, MYH1+, and hybrid MYH2+/MYH1+ nuclei occupied distinct transcriptional regions associated with different gene ontology signatures. Bed rest induced marked remodeling of myonuclear composition and transcriptional states. In particular, MYH2+ nuclei decreased substantially, whereas MYH1+ and hybrid MYH2+/MYH1+ nuclei increased. Notably, nuclei expressing the same MYH isoform displayed distinct transcriptional profiles across time points, indicating that disuse modifies myonuclear functional states beyond canonical fiber-type identity. RNA velocity analysis identified dynamic transcriptional trajectories associated with disuse and recovery, enabling identification of genes most responsive to unloading and reloading in each myonuclear population. To investigate intercellular communication, we applied CellChat analysis across all nuclear populations, revealing an overall reduction in both the number and strength of cell-cell interactions during BR, particularly involving fibro-adipogenic progenitor (FAP) cells. These findings demonstrate that human disuse atrophy involves coordinated remodeling of myonuclear transcriptional programs and intercellular signaling networks.
P05.11	Nejc Umek Faculty of Medicine, University of Ljubljana	*A Skeletal Muscle-Adapted Workflow for Xenium Spatial Transcriptomics Analysis*	Spatial transcriptomics enables gene expression analysis while preserving tissue spatial context. Imaging-based platforms such as Xenium (10x Genomics) provide subcellular resolution, making them suitable for highly structured tissues such as skeletal muscle. However, skeletal muscle presents major computational challenges due to its large multinucleated fibers and complex spatial organization. Standard cell-centric analysis workflows are not well suited to this tissue, particularly for accurate muscle fiber segmentation and reliable transcript-to-fiber assignment. Existing computational tools are often not optimized for these tissue-specific characteristics or for Xenium datasets. We therefore developed a computational pipeline specifically adapted for skeletal muscle, incorporating muscle fiber segmentation, fiber-level quality control, and spatially informed clustering. For workflow development and evaluation, we used Xenium spatial transcriptomic data generated from tibialis anterior muscle samples of a well-characterized polygenic mouse model of obesity and leanness comprising 8 lean and 8 obese male mice. To account for the structural complexity of skeletal muscle, the workflow distinguishes between fibre-level and nucleus-level analysis, combining muscle fibre segmentation and transcript-to-fibre assignment with nucleus-centered analysis of non-muscle cells. Benchmarking was performed using synthetic and experimental datasets to evaluate segmentation accuracy, transcript assignment performance, and reproducibility across technical replicates. Preliminary downstream analyses, including differential gene expression and spatial pattern analysis, are currently underway. The proposed pipeline addresses an important methodological gap by enabling spatial transcriptomics analysis optimized for the unique structural organization of skeletal muscle, which is not adequately captured by standard cell-centric workflows.
P05.12	Alexandra Winant University of Copenhagen	*Integrated single-myofiber multi-omics links inflammatory state to myosin energetics in ICU-acquired weakness*	Skeletal muscle dysfunction is a pervasive complication of critical illness that worsens survival and recovery. This rapid ICU-acquired weakness (ICU-AW) is incompletely explained by admission diagnosis, treatment protocols, or measures of muscle atrophy. ICU-AW has a predominantly myogenic origin involving targeted sarcomeric protein degradation, yet the underlying pathophysiological mechanisms remain poorly understood. We developed an integrative single-fiber approach combining myosin dynamics measurements with next-generation sequencing to generate high-dimensional multi-omic and functional data from individual muscle fibers. ATP-chase experiments assess myosin dynamics and ATP turnover, followed by sequential isolation of transcriptomic, epigenomic, and proteomic data from the same myofiber. This strategy links sarcomeric functional states with their underlying molecular regulation. Despite marked donor-level heterogeneity, integrated analysis revealed a conserved myofiber state enriched in ICU-AW, characterized by inflammatory and chemotactic gene programs, intracellular structural remodeling, and bioenergetic adaptation. Nineteen features were significantly altered at both RNA and protein levels, linking an inflammatory transcriptional landscape to a proteomic shift toward mitochondrial and translational machinery and away from membrane-associated signaling. Functionally, these fibers displayed selectively disrupted myosin dynamics, implicating altered myosin energetics in muscle dysfunction. These findings define a discrete, disease-associated myofiber state and establish an integrative single-fiber framework for connecting multi-omic heterogeneity to molecular motor function in complex human disease.

S06 - Muscle Energetics

Invited speaker	Eili Tranheim Kase University of Oslo, NO	*The SGLT2 inhibitor empagliflozin promotes increased fatty acid oxidation in skeletal muscle cells*	In this study, we investigated the potential of the sodium-glucose cotransporter 2 (SGLT2) inhibitor empagliflozin (EMPA) to modify energy metabolism in differentiated human primary skeletal muscle cells and mouse C2C12 skeletal muscle cells. Treatment of human myotubes with EMPA for 96 h decreased glucose oxidation as well as oxidation and uptake of acetoacetate, while complete oxidation of fatty acids and leucine was increased. There were no EMPA-induced changes in glucose, fatty acid, or leucine uptake, nor was lactate concentration in the culture medium affected. EMPA treatment increased phosphorylation of AMP-activated protein kinase (AMPK) and its downstream target acetyl-CoA carboxylase (ACC), without altering the expression of selected metabolic genes. Mitochondrial respiration and glycolytic function in mouse myotubes were assessed using a Seahorse XF assay. EMPA reduced basal respiration and glycolysis under standard conditions, however under conditions promoting fatty acid utilization, maximal respiration and spare respiratory capacity was enhanced. Insulin-induced phosphorylation of Akt was unaffected by EMPA treatment in human myotubes. In summary, EMPA treatment for 96 h of skeletal muscle cells in vitro induced metabolic shifts characterized by enhanced fatty acid and leucine catabolism, reduced glucose and acetoacetate metabolism, and suppressed glycolysis, potentially via AMPK activation.
Invited speaker	Nejc Umek University of Ljubljana, SLO	*A muscle-enriched intracellular lipid-remodelling pathway regulates skeletal muscle energetics and whole-body energy expenditure*	Skeletal muscle is a major contributor to resting energy expenditure, yet the intracellular lipid pathways that tune this function remain incompletely understood. We investigated a muscle-enriched intracellular lipid-remodelling pathway associated with oxidative muscle characteristics using genetically divergent mouse models, in vivo loss- and gain-of-function experiments, myocellular bioenergetic assays, muscle transcriptomics, and complementary human skeletal muscle datasets. Across independent models, lowering pathway activity increased whole-body energy expenditure, preserved lean tissue relative to fat during nutritional challenge, and shifted muscle gene expression toward oxidative metabolism. Conversely, increasing pathway activity in skeletal muscle cells and adult skeletal muscle reduced mitochondrial respiratory capacity and favoured an energy-conserving phenotype. By integrating whole-animal physiology with muscle-specific models, the work distinguished changes in energy expenditure from differences in food intake and from secondary consequences of altered adiposity. These reciprocal effects indicate that the pathway is not simply a marker of metabolic state, but a regulator of mitochondrial performance within muscle. Human datasets supported relevance beyond the mouse models. Pathway expression differed across skeletal muscles and was dynamically regulated in states of altered energetic demand, including acute exercise. Together, the findings identify a muscle-linked lipid-remodelling mechanism that couples intracellular membrane-lipid biology to mitochondrial respiration, whole-body energy expenditure, and body composition, highlighting skeletal muscle lipid remodelling as one of the determinants of energy-dissipating versus energy-conserving muscle states.
Contributed talk	Jana Disch IMSB Universität Stuttgart, DE	*A computer model of integrated glycolytic and mitochondrial ATP synthesis to uncover proton dynamics in active skeletal muscle fibers*	The foundation for the broad functional range of skeletal muscle performance is a highly flexible metabolic network that can handle 100-fold changes in ATP demand. To obtain causal insight into the complex role of myoplasmic pH dynamics and the mitochondrial pH gradient in the regulation of enzyme kinetics associated with energy supply in skeletal muscle during exercise, a detailed model of mitochondrial metabolism and trans-membrane transport was integrated into our existing computational framework of glycolytic ATP production. The proposed model consists of a set of differential-algebraic equations describing the thermodynamically constrained kinetics of a biochemical reaction network that is strictly feedback regulated. Simulations replicated experimentally observed in vivo Pi, PCr, pH and lactate dynamics, supporting the reported 1:1 ratio of lactate production and glycolytic proton generation in (resting) muscle under ischaemia. Notably, the framework resolves the contribution of individual enzyme reactions to the overall pH dynamics, demonstrating that lactate production catalysed by lactate dehydrogenase (LDH) is not the cause of muscle acidification during exercise. Conversely, LDH counteracts free proton production by ATPase and glycolytic reactions (e.g., glyceraldehyde 3-phosphate dehydrogenase) showcasing that common notions, such as “lactate production is responsible for myoplasmic acidification” can be misleading without additional context. The proposed model affords asking mechanistic questions about muscle function in complex settings with accumulation of glycolytic intermediates in the presence of oxidative ATP synthesis from pyruvate that have been difficult, if not impossible to address experimentally. As such, it provides a simulation platform to capture and test hypotheses in muscle physiology.

Contributed talk	Maria Jolanta Rędowicz Nencki Institute of Experimental Biology, Warszawa, PL	*Myosin VI Regulates Myoblast Fusion Through Redox-Dependent Mechanisms*	Skeletal muscle development and regeneration require coordinated myoblast differentiation and fusion into multinucleated myotubes. Although myogenesis has been extensively studied, many underlying molecular mechanisms remain unclear. Our study shows that loss of unconventional myosin VI (MVI) markedly impairs myotube formation, resulting in abnormal, spindle-shaped myotubes with centrally located nuclei, indicating defective differentiation and fusion. MVI-knockout (MVI-KO) differentiating myoblasts exhibit significantly increased expression of the fusogenic proteins Myomaker and Myomerger. These changes are accompanied by elevated reactive oxygen species (ROS) levels and a decreased GSH/GSSG ratio, indicating disrupted redox balance. Although the link between ROS and fusion is not fully defined, increased oxidative stress in MVI-KO cells may contribute to altered Myomaker expression. We also observed increased levels of nuclear factor erythroid 2-related factor 2 (NRF2), a key regulator of redox homeostasis. However, fractionation analysis revealed abnormal intracellular distribution: despite higher total NRF2 levels, the protein was mainly retained in the cytoplasm, with reduced nuclear accumulation compared to controls. This suggests impaired nuclear transport of NRF2 in the absence of MVI, potentially compromising activation of the antioxidant response. Overall, our findings indicate that MVI is an important regulator of redox homeostasis during myogenic differentiation and may influence myoblast fusion through ROS-dependent regulation of fusion machinery and NRF2 signaling. These effects are probably associated with mitochondrial dysfunction in MVI-KO myogenic cells. This work was supported by National Science Center, Poland (Grant number: 2022/47/D/NZ3/02737).
P06.01	Takayuki Akimoto Waseda University	*The miR-23 cluster microRNAs control muscle mass and slow fiber formation*	MicroRNAs (miRNAs) are short non-coding RNAs that suppress gene expression at the post-transcriptional level. We previously reported that the miR-23~27~24 cluster miRNAs are abundant in skeletal muscle (Wada et al., J Biol Chem, 2011), although they are not classified as muscle-specific miRNAs (myomiRs). We also reported that some of the miRNAs in the miR-23 cluster are responsive to exercise (Russell et al., 2013). In this study, we generated muscle-specific miR-23a/b cluster knockout (MyoD-miR-23KO) mice to determine the role of these miRNAs in skeletal muscle. MyoD-miR-23KO mice exhibited phenotypic changes characterized by reduced muscle mass, which resulted in decreased body weight, along with increased myosin heavy chain type I expression in fast muscle. The proliferative capacity of myoblasts isolated from MyoD-miR-23KO mice was comparable to that of control mice; however, their differentiation capacity was impaired. In addition, MEF2 transactivation activity was higher in MyoD-miR-23KO mice than in control mice. Spatial transcriptomic analysis of KO embryos identified several genes potentially responsible for these phenotypes. Together, these findings suggest that the miR-23~27~24 cluster plays an important role in regulating muscle mass and slow-fiber formation.
P06.02	Jana Disch Institut für Modellierung und Simulation Biomechanischer Systeme (IMSB), Universität Stuttgart	*A computer model of integrated glycolytic and mitochondrial ATP synthesis to uncover proton dynamics in active skeletal muscle fibers*	The foundation for the broad functional range of skeletal muscle performance is a highly flexible metabolic network that can handle 100-fold changes in ATP demand. To obtain causal insight into the complex role of myoplasmic pH dynamics and the mitochondrial pH gradient in the regulation of enzyme kinetics associated with energy supply in skeletal muscle during exercise, a detailed model of mitochondrial metabolism and trans-membrane transport was integrated into our existing computational framework of glycolytic ATP production. The proposed model consists of a set of differential-algebraic equations describing the thermodynamically constrained kinetics of a biochemical reaction network that is strictly feedback regulated. Simulations replicated experimentally observed in vivo Pi, PCr, pH and lactate dynamics, supporting the reported 1:1 ratio of lactate production and glycolytic proton generation in (resting) muscle under ischaemia. Notably, the framework resolves the contribution of individual enzyme reactions to the overall pH dynamics, demonstrating that lactate production catalysed by lactate dehydrogenase (LDH) is not the cause of muscle acidification during exercise. Conversely, LDH counteracts free proton production by ATPase and glycolytic reactions (e.g., glyceraldehyde 3-phosphate dehydrogenase) showcasing that common notions, such as “lactate production is responsible for myoplasmic acidification” can be misleading without additional context. The proposed model affords asking mechanistic questions about muscle function in complex settings with accumulation of glycolytic intermediates in the presence of oxidative ATP synthesis from pyruvate that have been difficult, if not impossible to address experimentally. As such, it provides a simulation platform to capture and test hypotheses in muscle physiology.
P06.03	Alexandra Dorn Amsterdam UMC	*Fast skeletal myosin inhibition improves muscle energetics in a mouse model for hypercontractile Nemaline myopathy type 6*	Nemaline myopathy type 6 (NEM6) is a congenital myopathy caused by variants in Kelch-repeat-and-BTB-(POZ)-Domain-Containing-13 (KBTBD13). NEM6 patients experience muscle weakness, stiffness and slowness associated with sarcomere-based hypercontractility. Additionally, NEM6 is associated with mitochondrial dysfunction characterized by cores, which are devoid of NADH activity. In this study, we aimed to target sarcomere-based hypercontractility in NEM6, using the myosin II inhibitor EDG-4131. We first determined the acute effect of EDG-4131 on contractility in isolated intact muscle fibers of NEM6 mice. We found a dose-dependent reduction in percentage of shortening, concluding that EDG-4131 can effectively inhibit contraction in NEM6 fibers. Additionally, EDG-4131 was administered chronically as preventive treatment in mice, starting from 1 months of age, when mice do not yet show the NEM6 phenotype, up to 3 months of age, aiming particularly at enhancing muscle energetics. Chronic EDG-4131 treatment in NEM6 mice resulted in a significant reduction in the prevalence of cores and improved mitochondrial respiration. This study highlights the potential of myosin inhibition in NEM6 and other skeletal muscle myopathies with a hypercontractile phenotype

P06.04	Matthias Ernst Medical University of Vienna	*Empagliflozin Improves Mitochondrial Structure and Oxidative Metabolism in Pressure Overload-Induced Cardiac Remodeling*	Background: Pressure overload-induced left ventricular hypertrophy (LVH) is characterized by adverse cardiac remodeling and mitochondrial dysfunction. Sodium–glucose cotransporter-2 inhibitors (SGLT2i) improve heart failure outcomes, but their effects on cardiac muscle ultrastructure and mitochondrial function remain incompletely understood. Purpose: To determine whether empagliflozin (EMPA) reverses established LVH and improves mitochondrial structure and function in pressure-overloaded hearts. Methods: Male (n=59) and female (n=53) A/J mice underwent transverse aortic constriction (TAC). Four weeks later, mice received EMPA (30 mg/kg/day) or vehicle for an additional four weeks. Cardiac structure and function were assessed by serial echocardiography. LV tissue underwent transmission electron microscopy, high-resolution mitochondrial respirometry, bulk RNA sequencing, and untargeted metabolomics with integrated pathway analysis. Results: TAC induced marked hypertrophy and systolic dysfunction in both sexes (both $p<0.001$), which were significantly attenuated by EMPA treatment ($p<0.001$). Ultrastructural analysis demonstrated mild mitochondrial impairment and increased ER vesicles in TAC hearts, whereas EMPA-treated hearts showed preserved mitochondrial integrity and sex-specific lipid vesicle remodeling, particularly in males. High-resolution respirometry revealed increased complex I-, complex II-, and fatty acid oxidation-supported respiration, alongside reduced non-mitochondrial respiration in EMPA-treated hearts (all $p<0.05$). Integrated transcriptomic and metabolomic analyses identified mitochondrial fatty acid β-oxidation and oxidative phosphorylation as the dominant regulated pathways following EMPA treatment (FDR<0.05). Conclusion: EMPA promotes reverse remodeling in established pressure overload-induced LVH through coordinated improvement of cardiac muscle ultrastructure and mitochondrial oxidative metabolism. These findings support restoration of mitochondrial function as a central mechanism underlying the cardioprotective effects of SGLT2 inhibition.
P06.05	Aneta Fenclová Charles University	*Myokine levels during early stages of mild cold acclimation in rat skeletal muscle*	INTRODUCTION: Our recent studies showed that mild cold acclimation (9 °C) reduces myocardial infarction size after only 3 days, however the effect of mild cold on myokines production by skeletal muscle is not known under these conditions. Release of myokines, by skeletal muscle during muscle contraction and sever cold exposure has been described. Myokines regulate energy metabolism by mobilizing energy stores and mediating communication between skeletal muscle and other organs via the bloodstream. They also exert additional beneficial effects, including autocrine support of muscle regeneration. The aim of this study was to determine whether mild cold acclimation affects the expression of the interleukin-6 (IL-6) and fibronectin type III domain-containing protein 5 (FNDC5), and proteins involved in glucose metabolism in skeletal muscle. METHODS: Adult male Wistar rats (350 g) were used. Controls were housed at 23± 1°C, while cold-exposed groups were at 9±1 °C for 4-12-24 hours, 3 and 10 days. After acclimation, m. soleus was isolated from deeply anesthetized animals. Total and spatial protein expression was assessed by western blotting and quantitative immunofluorescence microscopy. RESULTS: Acute cold exposure caused oscillations in IL-6 protein levels during 10 days of cold acclimation in homogenates, while the FNDC5 decreased after 10 days in cold group. Expression of GLUT4 and AMPK, which may be influenced by IL-6, was also evaluated. Although elevated circulating irisin levels are reported under cold conditions, muscle contraction (i.e. shivering), absent in our experimental conditions, is required for its expression.
P06.06	Torri Heiser University of Calgary	*Energy Consumption After Active Stretch in Single Skinned Muscle Fibres*	Residual force enhancement (rFE) describes an increased steady-state force following active stretch compared to a purely isometric reference contraction (Abbott & Aubert, 1952). Evidence suggests that titin contributes to rFE. If so, this additional force would require no additional energy (ATP) consumption. However, few studies have systematically investigated muscle force and energetics simultaneously in the rFE state (Curtain & Woledge, 1979; Joumaa & Herzog, 2013). The study objective was to determine the ATP cost in the rFE state compared to a purely isometric reference contraction for two stretch magnitudes. We hypothesized that ATP cost per unit of force is decreased in rFE compared to the corresponding reference state and that the reduction in ATP cost increases with greater stretch magnitude. Skinned rabbit psoas fibres were used to measure the ATP consumption during steady-state isometric contractions at 3.5µm (n=12) and 3.7µm (n=12), and at steady-state following active stretch from 2.6µm to 3.5µm and 2.6µm to 3.7µm. Force and ATP cost were measured synchronously (804A, Aurora Scientific) (de Tombe & Stienen,1995). rFE at 3.5µm was 7.0±3.0% and the reduction in ATP cost per unit of force was 4.0±4.4% compared to the isometric reference contraction. rFE at 3.7µm was 21.0±8.3% and the reduction in ATP cost per unit of force was 18.0±7.2% compared to the isometric reference contraction. These results support a passive element, not additional attached cross-bridges as the mechanism for rFE. Titin-actin interactions have been suggested to occur upon muscle activation and stretch, resulting in rFE without increased in ATP consumption.

P06.07	Filip Jezek University of Michigan	*A Mechanistic, ATP-Dependent Cross-Bridge Model of Cardiac Muscle Mechanics Under Energetic Stress*	Heart failure is accompanied by an energetic deficit — reduced ATP, elevated ADP, lowered PCr/ATP — that compromises cross-bridge cycling. Linking sarcomere mechanics to mitochondrial ATP supply [1] requires an accurate sarcomere model. The model of Beard et al. [2] reproduces force-velocity data at saturating ATP but encodes nucleotide dependence through a single step — ATP-dependent detachment — which cannot capture differences across ATP concentrations. Using multiple datasets from mouse right-ventricular trabeculae (Baker laboratory) — force-velocity and slack-restretch protocols at 8 mM (saturating) and 2 mM (compromised) ATP — we observe a dissociation: isometric force rises modestly (~20%) while force redevelopment (ktr) after slack slows substantially (~40–50%). Additional features (Vmax, force-velocity profile, peak restretch force...) further constrain the model. Where ATP modulates only detachment, force and ktr share one rate constant and cannot dissociate this far. We present a six-state strain-discretized model with explicit nucleotide pools: two disordered-relaxed states by nucleotide, two strain-sensitive attached states (pre- and post-power-stroke), and two super-relaxed states. A variant where ATP acts only on detachment reproduces the force increase but underpredicts the ktr reduction; reproducing both requires ATP (and ADP) to act on more than one step. With these mechanisms, the model qualitatively reproduces the high- versus low-ATP differences. These results expose a gap in current cross-bridge models and constrain which ATP-dependent transitions fit the data, providing a foundation for coupling to mitochondrial ATP-production models [1] and evaluating interventions in heart failure. [1] Collins et al., bioRxiv 2026. [2] Beard et al., Biophys. J. 2022.
P06.08	Ulrik Mjaaseth University of Oslo	*All-trans retinoic acid alters lipid metabolism in human myotubes in part through modulation of mitochondrial function*	Objectives: Retinoid signaling in human skeletal muscle remains incompletely understood. We investigated how all-trans retinoic acid (ATRA) alters lipid metabolism, gene expression, and mitochondrial function in human myotubes. Methods: Primary human myoblasts from multiple donors were differentiated into myotubes and treated with ATRA or vehicle. Fatty acid uptake and oxidation were assessed using radiolabeled oleic acid. Incorporation into intracellular lipid classes was measured after incubation with radiolabeled oleic acid or acetoacetate, followed by filtration of lysates through hydrophobic MultiScreen HTS plates and scintillation counting. Mitochondrial function was evaluated by measuring oxidative and reserve capacity following FCCP uncoupling. RNA was isolated for RT-qPCR analysis, alongside transcriptomic and proteomic profiling using RNA sequencing and untargeted proteomics followed by enrichment analyses. Statistical analyses were performed in R using models accounting for donor variability and multiple testing; significance was defined as $P < 0.05$. Results: ATRA increased fatty acid uptake, oxidation, and incorporation into intracellular lipid classes. FCCP uncoupling revealed increased maximum oxidative and reserve capacity. RT-qPCR showed increased PDK4 and PLIN2 expression. Transcriptomics revealed upregulation of lipid metabolism and mitochondrial remodeling genes, including FASN, ACSL5, ACACA, SREBF1, and MTFR2. Proteomics showed 78 upregulated mitochondrial proteins involved in oxidation, respiratory chain assembly, and nucleotide synthesis. Integrated analyses identified canonical retinoid-responsive genes, including CRABP2 and CYP26B1, alongside lipid metabolism regulators. Conclusions: ATRA enhances fatty acid uptake, oxidation, and intracellular lipid incorporation in human myotubes, partly through mitochondrial and metabolic gene network modulation. These findings support retinoid signaling as a regulator of skeletal muscle lipid metabolism.
P06.09	Kathy Myburgh Stellenbosch University	*Maturation-Dependent Characterisation of C2C12 Myotube Structure and its Contractile Responsiveness to Electrical Pulse Stimulation*	Responsiveness of C2C12 myotubes to electrical stimulation (EPS) may be influenced by myotube maturation. This study characterised the structural maturation of C2C12 myotubes over 15 days of differentiation, and to assess how maturation influences metabolic and structural responsiveness to EPS. Myotubes were cultured in 2% horse serum on matrix coated plates and analysed at 5, 10 and 15 days for morphological and structural changes. Myosin heavy chain and desmin were assessed using western blotting and immunofluorescence. Myotube ultrastructure was examined by transmission electron microscopy. Twitch (12 V, 1 Hz with 2 ms pulses) and tetanic pulses (12 V, 30 Hz with 5 s pulses every 5 s) were administered for 24 hours. Physiological responses were evaluated by measuring lactate and lactate dehydrogenase release over these 24 hours. Myotube length increased progressively (days 10 and 15 differed from day 5 by $p=0.03$ and $p=0.002$ respectively). Desmin increased significantly by day 6 of differentiation and MHC by day 11. Improved filament organisation was observed in the ultrastructure over time. Lactate release in response to stimulation was consistent with twitch contraction regardless of maturation stage ($p<0.05$). The magnitude was greater with tetanic contractions determined at each differentiation stage (between $p<0.01$ and $p<0.0001$). There was little evidence of membrane damage assessed by LDH release (only on Day 5 with twitch contraction $p=0.04$). These findings established the relationship between myotube maturation and lactate release in response to electrical stimulation. The two contraction modes exhibited different responses which has implications for modelling exercise in vitro.

P06.10	Maria Jolanta Rędowicz Nencki Institute of Experimental Biology, Polish Academy Of Sciences	*Myosin VI Regulates Myoblast Fusion Through Redox-Dependent Mechanisms*	Skeletal muscle development and regeneration require coordinated myoblast differentiation and fusion into multinucleated myotubes. Although myogenesis has been extensively studied, many underlying molecular mechanisms remain unclear. Our study shows that loss of unconventional myosin VI (MVI) markedly impairs myotube formation, resulting in abnormal, spindle-shaped myotubes with centrally located nuclei, indicating defective differentiation and fusion. MVI-knockout (MVI-KO) differentiating myoblasts exhibit significantly increased expression of the fusogenic proteins Myomaker and Myomerger. These changes are accompanied by elevated reactive oxygen species (ROS) levels and a decreased GSH/GSSG ratio, indicating disrupted redox balance. Although the link between ROS and fusion is not fully defined, increased oxidative stress in MVI-KO cells may contribute to altered Myomaker expression. We also observed increased levels of nuclear factor erythroid 2-related factor 2 (NRF2), a key regulator of redox homeostasis. However, fractionation analysis revealed abnormal intracellular distribution: despite higher total NRF2 levels, the protein was mainly retained in the cytoplasm, with reduced nuclear accumulation compared to controls. This suggests impaired nuclear transport of NRF2 in the absence of MVI, potentially compromising activation of the antioxidant response. Overall, our findings indicate that MVI is an important regulator of redox homeostasis during myogenic differentiation and may influence myoblast fusion through ROS-dependent regulation of fusion machinery and NRF2 signaling. These effects are probably associated with mitochondrial dysfunction in MVI-KO myogenic cells. This work was supported by National Science Center, Poland (Grant number: 2022/47/D/NZ3/02737).
P06.11	Anja Srpčič University Medical Centre Ljubljana	*IL-6 expression modulates the signalling and metabolic response to recombinant IL-6 in primary human myoblasts*	Myoblasts, the precursors of skeletal muscle, secrete interleukin-6 (IL-6) and respond to both intrinsic IL-6 and extrinsic IL-6 secreted by pro-inflammatory immune cells and extramuscular tissues. IL-6 activates the JAK/STAT signalling pathway and regulates proliferation, differentiation, and metabolism, resulting in physiological effects such as skeletal muscle hypertrophy, and pathological effects such as atrophy associated with chronic inflammation. We studied whether interactions between intrinsic and extrinsic IL-6 affect JAK/STAT signalling and metabolism in myoblasts. Gene silencing was used to reduce IL-6, STAT1, and STAT3 expression in primary human myoblasts, and intrinsic IL-6 was blocked in the cell culture medium with neutralising anti-IL-6 antibodies (NAbIL6). The response to extrinsic (recombinant human) IL-6 (rhIL-6) was assessed by measuring levels of phosphorylated STAT1 (pSTAT1 (Tyr701)) and STAT3 (pSTAT3 (Tyr705)) using immunoblotting, and by measuring glucose and oleic acid metabolism with radio-labelled substrates. Reduction of IL-6 secretion by gene silencing increased rhIL-6-induced pSTAT1 and pSTAT3 levels, which was further enhanced by the addition of NAbIL6. STAT3 gene silencing increased both total and rhIL-6-induced pSTAT1 levels, while STAT1 gene silencing did not affect total or pSTAT3 levels. RhIL-6 stimulation increased glucose and oleic acid uptake and complete oxidation, while IL-6 gene silencing decreased these in basal conditions and limited the metabolic response observed in rhIL-6-stimulated myoblasts. Taken together, basal secretion of intrinsic IL-6 by primary myoblasts decreases activation of the JAK/STAT signalling pathway by rhIL-6, while promoting both basal and rhIL-6-induced glucose and oleic acid metabolic activity.
P06.12	Katarina Miš University Of Ljubljana, Faculty Of Medicine	*Department of Metabolism and Endocrinology of Skeletal Muscle at the Alliance4Life Virtual Research Center*	The Department of Metabolism and Endocrinology of Skeletal Muscle at the Alliance4Life Virtual Research Center (VRC) is dedicated to research on skeletal muscle, particularly its metabolic and endocrine functions. The Alliance4Life VRC, as part of the A4L_BRIDGE project (funded by the European Union under Grant Agreement No. 101136453), was established in 2025 to strengthen scientific collaboration and knowledge exchange among leading health research institutions across Central and Eastern Europe and beyond. The VRC's mission is to create a thriving international scientific network that empowers researchers, accelerates innovation, facilitates societal outreach, and drives societal impact through collaborative biomedical research, education, training, and communication initiatives. The virtual Department of Metabolism and Endocrinology of Skeletal Muscle, one of the three founding VRC departments, provides a platform for networking among researchers focused on skeletal muscle as a metabolic tissue, a target for metabolic hormones, and a source of signalling molecules such as myokines, particularly in the context of physical inactivity, ageing, and metabolic and other non-communicable chronic disorders. By promoting collaborative research, the Department of Metabolism and Endocrinology of Skeletal Muscle aims to facilitate new discoveries in skeletal muscle physiology, pathophysiology, and pharmacology, ultimately leading to the translation of fundamental scientific findings into therapeutic strategies for conditions such as diabetes, obesity, metabolic syndrome, and sarcopenia. Collaboration with other VRC departments, professional organisations, and patient and citizens' associations ensures a multidisciplinary and holistic approach to addressing these complex health challenges.

S07 - Muscle Mechanobiology

Invited speaker	Dominic del Re Rutgers New Jersey Medical School, US	*Novel insights into the regulation of metabolic adaptation and dysfunction in the heart*	Mechanisms of metabolic regulation are central to cardiac homeostasis and responses to stress that drive heart failure. Neurofibromin 2 (NF2) is a tumor suppressor that influences a wide range of cellular functions yet remains understudied in the heart. We employed conditional NF2 cKO mice to investigate the role of cardiomyocyte NF2 in the development of heart failure in response to chronic stress. We unexpectedly identified an adaptive function of NF2 during pressure overload as NF2 cKO mice had exacerbated cardiac pathology. RNAseq revealed the downregulation of genes associated with fatty acid metabolism in NF2 cKO hearts. Cardiac fatty acid oxidation was impaired, and lipid accumulation was enhanced, in NF2 cKO mice. Complementary approaches, including unbiased proteomics and AAV-mediated gene expression, were used to elucidate downstream mechanistic insights. Additionally, NF2 and fatty acid oxidation genes were downregulated in human heart failure biopsies, providing clinical relevance to our findings. Our study provides evidence that NF2 signaling drives a novel mechanism that facilitates metabolic gene expression and promotes energetic resilience during chronic stress.
Invited speaker	Giancarlo Forte King's College London, UK	*Force-regulated alternative splicing in the failing human heart*	How cells convert physical forces into biological responses is a fundamental question in both development and disease. Mechanical cues shape tissue architecture during embryogenesis and are equally important in cardiovascular disease, where pathological extracellular matrix (ECM) remodelling alters the mechanical environment of cardiac cells and drives organ failure. While mechanotransduction has traditionally been viewed through the lens of transcriptional regulation, emerging evidence suggests that post-transcriptional mechanisms may represent a critical endpoint of force sensing. Our work has identified the RNA-binding protein heterogeneous nuclear ribonucleoprotein C (hnRNPC), a spliceosome-associated factor, as a key mediator linking mechanical stress to RNA metabolism in the human heart. We found that ischemic and chronic cardiac pathologies are associated with altered hnRNPC expression. Importantly, pathological ECM remodelling induces the relocalisation of hnRNPC from its nuclear compartment to the sarcomeric z-disc. At this site, hnRNPC interacts with sites of active local translation, where it might participate further in the processing of mRNA. Furthermore, the absence of hnRNPC from the nucleus affects the splicing of numerous transcripts involved in mechanotransduction pathways and cardiovascular disease. Altogether, these findings demonstrate that mechanical cues regulate RNA metabolism through hnRNPC relocalisation and identify force-regulated alternative splicing as a previously unrecognised mechanism by which the failing heart senses and adapts to changes in its mechanical environment.
Contributed talk	Annika J. Klotz University of Muenster, DE	*Loss of titin-based elasticity elevates end-diastolic pressure through geometric remodelling*	Introduction: Increased titin-based myocardial stiffness is a recognised driver of diastolic dysfunction in HFpEF, and titin softening is being explored therapeutically. However, the consequences of titin softening on ventricular pressure remain unclear. We therefore investigated the haemodynamic effects of in vivo titin cleavage in titin-cleavage (TC) mice using a Laplace-based framework to distinguish myocardial stiffness from geometric determinants of left ventricular (LV) pressure. Methods: Titin cleavage was induced in homozygous TC mice carrying a TEV-site within I-band titin via AAV9-mediated TEV protease expression; AAV9-GFP served as control. LV pressure-volume analyses were performed 6 days post-injection. Titin cleavage was quantified by SDS-PAGE, passive mechanics assessed in permeabilized trabeculae, and MRI data analysed for LV geometry. Results: TEV-treated hearts exhibited robust titin cleavage (38.5±4.2%) and markedly reduced elastic stress ($p=0.0009$) without changes in viscous stress ($p=0.9746$). In vivo analyses revealed diastolic dysfunction with a small-volume, high-pressure phenotype, including reduced end-diastolic ($p=0.0020$) and stroke ($p=0.0008$) volumes, ~150% elevated end-diastolic pressure ($p=0.0003$), prolonged tau ($p=0.0039$), and increased EDPVR ($p=0.0006$) and ESPVR ($p=0.0110$). Preload responsiveness was impaired, with a reduced Frank-Starling slope ($p=0.0222$) and delayed preload adaptation ($p=0.0003$). MRI showed reduced LV radius ($p=2.1\times10\text{-}5$) and increased wall thickness ($p=4.6\times10\text{-}6$) without changes in LV mass ($p=0.9927$). Laplace decomposition revealed that geometric remodelling increased end-diastolic and end-systolic pressure by 89% ($p=2.3\times10\text{-}5$) and 181% ($p=1.5\times10\text{-}5$), respectively. Conclusion: Loss of titin-based elasticity induces diastolic dysfunction with elevated end-diastolic pressure predominantly through geometric remodelling, challenging the stiffness-centric paradigm of diastolic dysfunction and raising safety considerations for titin-targeting therapies in HFpEF.
Contributed talk	Luuk Kemna Amsterdam University Medical Centre, NL	*Dynamic loading of Engineered Heart Tissue: measuring workloads*	Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) and Engineered Heart Tissues (EHTs) have emerged as valuable in vitro models to study cardiac physiology and disease. Current EHT platforms typically consist of hiPSC-CMs embedded in a hydrogel matrix, casted between deformable pillars with fixed positions. Spontaneous tissue contraction creates pillar deflection used to measure contractile force and basic contraction/relaxation kinetics. Despite advances in platform designs that add the ability to continuously measure and integrate electrical stimulation, current EHT platforms are limited in their ability to replicate dynamic loading conditions of the heart in vivo. We present functional data obtained from the implementation of EHTs in the IonOptix MyoClamp. hiPSC-CMs (day 30) were used to cast EHTs in a fibrin/fibrinogen matrix. At EHT day 20-45, EHTs were taken off their pillars and mounted in the Myoclamp system. In this system, simultaneous control of preload and afterload while measuring force and tissue length results in the generation of real-time force-length workloops, analogous to pressure-volume (PV) loops of the heart. We showcase the ability to obtain, with high reproducibility, force-strain relationships from which length dependent activation and passive stiffness are derived. Additionally, by gradually increasing preload and afterload while measuring force-length work loops, EDPVR and ESPVR relations, stroke length, performed work, and detailed, phase-specific, contraction/relaxation kinetics are evaluated. EHT measurements in the Myoclamp platform allow us to reproduce key aspects of cardiac ventricular loading. This next generation model bridges the gap between traditional in vitro cardiac models and in vivo cardiac physiology.

P07.01	Vigdis Aas Oslo Metropolitan University	*Extracellular vesicles from electrical pulse-stimulated human skeletal muscle cells can be taken up by hepatoma HepG2 cells and improve fatty acid oxidation*	Regular exercise is first-line therapy for preventing and treating lifestyle diseases, such as obesity, type 2 diabetes and metabolic dysfunction-associated steatotic liver disease. Contracting skeletal muscle releases signaling factors, including extracellular vesicles (EVs) which may mediate intercellular communication. This study explored whether EVs derived from electrical pulse-stimulated (EPS) human skeletal muscle cells can be taken up by recipient liver cells and convey exercise-like effects. Satellite cells isolated from muscle biopsy samples (m. vastus lateralis) obtained from healthy donors were differentiated into myotubes. Myotubes were exposed to chronic low-frequency EPS for 24 hours, after which EVs were isolated from cell culture media. Size and concentration of small EVs (sEVs) were characterized by nanoparticle tracking analysis. Uptake of fluorescent-labelled EVs in target cells was examined using laser scanning confocal microscopy. The effect of EVs on hepatocytes was assessed by energy substrate oxidation, insulin-stimulated phosphorylation of Akt and gene expression. Myotube-derived EVs were internalized by HepG2 hepatoma cells. EV size and concentration were unaffected by EPS. EVs from EPS-treated myotubes increased oleic acid oxidation, both in control and insulin resistant HepG2 cells (exposed to palmitic acid). However, insulin-stimulated phosphorylation of Akt was not affected by EPS-EVs. Effects of EPS-EVs on recipient cell glucose metabolism and gene expression are being studied. This exploratory study demonstrated that EVs derived from EPS-treated myotubes were taken up by recipient cells, and the effects observed on HepG2 cells indicate a role of EVs in transmitting an exercise effect to liver cells.
P07.02	Elisabeth Barton University of Florida	*Fibro-adipogenic precursors are required for load-induced muscle hypertrophy*	Maintenance and adaptation of skeletal muscle mass relies on the balance of anabolic and catabolic pathways in muscle fibers, but mononuclear cells surrounding the fibers may modulate the changes in fiber properties. The fibro-adopogenic precursors (FAPs), which are mesenchymal stromal cells in muscle, have been shown to be important for muscle maintenance and regenerative capacity, yet whether they are required for muscle hypertrophy has not been examined thoroughly. We utilized mice in which FAPs were genetically deleted, as well as mice in which FAPs could not produce insulin-like growth factor I (IGF-I), and compared their response to increased mechanical loading to control mice. Using synergist ablation to drive hypertrophy in the soleus muscle, we observed a robust positive response in both male and female control mice. However, mice lacking FAPs or FAP-IGF-i production had significantly blunted responses, indicated by lower muscle mass, smaller muscle fibers, and decreased protein synthesis, with no change in muscle satellite cell content. Further, soleus specific forces were lower in mice lacking FAPs after synergist ablation. These data support that FAPs are required for functional muscle hypertrophy caused by changed in mechanical load, and that IGF-I from FAPs is a significant contributor to the hypertrophic response.
P07.03	Beatrix Dienes University of Debrecen	*Piezo1 Regulates the Skeletal Muscle Length–Tension Relationship Through Channel-Independent Mechanotransduction*	Piezo1 is widely recognized as a mechanosensitive Ca^{2+}-permeable ion channel, yet its contribution to adult skeletal muscle function remains poorly understood. Here, we identify Piezo1 as a previously unrecognized regulator of skeletal muscle mechanical performance, linking mechanosensing to length-dependent force generation. To investigate the functional role of Piezo1 in adult skeletal muscle, we combined pharmacological modulation with electrophysiological, calcium influx, and ex vivo force measurements in isolated muscle fibers and intact skeletal muscles. Piezo1 activity was manipulated using the agonist Yoda1 and the antagonist Dooku1 in wild-type muscle, and results were validated in muscles with reduced Piezo1 expression following anti-Piezo1 shRNA treatment. Functional responses were further compared with dystrophin-deficient mdx muscle to determine the contribution of cytoskeletal integrity. Piezo1 activation had no detectable effect on force generation. In contrast, its inhibition significantly reduced contractile force at stretched lengths, indicating a selective role in length-dependent force regulation. This effect was independent of extracellular Ca^{2+}, suggesting that Piezo1 contributes to muscle mechanics beyond its canonical ion-conducting activity. Importantly, the effect was diminished following Piezo1 downregulation and was absent in mdx muscle, demonstrating dependence on intact dystrophin-associated cytoskeletal organization. Together, these findings identify Piezo1 as a novel mechanobiological regulator of skeletal muscle force generation. Our results support a model in which Piezo1 contributes to muscle mechanical adaptation through cytoskeleton-dependent mechanisms, revealing a previously unrecognized layer of force regulation and expanding current understanding of skeletal muscle mechanobiology. (University of Debrecen Scientific Research Bridging Fund (DETKA) (B.D.))
P07.04	Marta Gawor Nencki Institute of Experimental Biology PAS, Warsaw, Poland	*Myosin VI regulates chromatin organisation and transcription in myoblasts*	Skeletal muscle cells experience substantial mechanical load, requiring coordinated regulation of nuclear architecture, chromatin organisation and gene expression. Cell nucleus serves as a mechanosensing platform, allowing for swift adaptation to mechanical stimuli. Impaired mechanical resilience of the nucleus may have severe repercussions for genome integrity and, consequently, for cell survival. While nuclear actin and myosins are emerging as important regulators of chromatin structure and transcription, their roles in muscle cell nuclei remain poorly understood. Myosin VI is an unconventional actin-based motor previously implicated in chromatin organisation and transcriptional regulation in cancer cells. Here, we identify a new nuclear role for myosin VI in myoblasts. Using myosin VI depletion, we show that loss of myosin VI disrupts myonuclear morphology and alters chromatin organisation. These changes are associated with altered epigenetic chromatin marks and impaired transcriptional progression, indicating that myosin VI contributes to the maintenance of a transcriptionally competent nuclear environment. We further assess how myosin VI downregulation affects nuclear stiffness and cellular mechanics, linking chromatin-level changes to mechanoadaptation in myoblasts. Previous studies from our laboratory have shown that loss of myosin VI disrupts myogenic growth, differentiation and muscle metabolism. Current findings extend this role by placing myosin VI within the regulatory network that coordinates chromatin remodelling, gene expression and nuclear organisation. We propose that myosin VI acts as a nuclear organiser in myoblasts, coupling transcriptional regulation with the mechanical demands of muscle cell function.

P07.05	Áron Gere University of Debrecen	*The effects of microgravity and proton irradiation on the biophysical properties, Piezo1-induced mechanotransduction and myogenic differentiation of C2C12 cells*	Skeletal muscle function depends not only on biochemical cues but also on the continuous integration of mechanical signals required to maintain contractile performance, structural integrity, and regenerative capacity. Muscle atrophy and weakness during spaceflight have traditionally been attributed to microgravity-induced unloading. However, increasing evidence suggests that impaired muscle regeneration and altered mechanotransduction may also be directly influenced by additional space-related stressors. In addition to microgravity, ionizing radiation constitutes a major nongravitational stressor during spaceflight. In this study, we evaluated the effects of simulated space environmental conditions on C2C12 skeletal muscle cells. Proton irradiation was carried out using an MCG-20 cyclotron at HUN-REN ATOMKI. Microgravity environment was simulated by an AirBus 2.0 3D clinostat. Western blot analysis was performed to evaluate target protein expression. Fluorescence immunocytochemistry and microscopy were used to determine fusion indices and membrane fluidity. Calcium transients were recorded using the Fura-2-AM fluorescent ratiometric calcium-sensitive dye. Proton irradiation decreased cell membrane fluidity in a dose-dependent manner and significantly hindered the pharmacological responsiveness of Piezo1 channels, resulting in decreased calcium transients. It also impeded the proliferation and differentiation capacity of skeletal muscle cells, which could not be salvaged by activating the Piezo1 channel. Simulated microgravity diminished the formation of myotubes and altered the expression of myogenic regulatory factors, implicating modified differentiation dynamics. Our findings highlight the mission-critical environmental factors of space travel and may support the development of future protective strategies. Reduced mechanical loading is also relevant in bedridden and elderly patients, further supporting the translational value of this research.
P07.06	Luuk Kemna Amsterdam University Medical Centre	*Dynamic loading of Engineered Heart Tissue: measuring workloads*	Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) and Engineered Heart Tissues (EHTs) have emerged as valuable in vitro models to study cardiac physiology and disease. Current EHT platforms typically consist of hiPSC-CMs embedded in a hydrogel matrix, casted between deformable pillars with fixed positions. Spontaneous tissue contraction creates pillar deflection used to measure contractile force and basic contraction/relaxation kinetics. Despite advances in platform designs that add the ability to continuously measure and integrate electrical stimulation, current EHT platforms are limited in their ability to replicate dynamic loading conditions of the heart in vivo. We present functional data obtained from the implementation of EHTs in the IonOptix MyoClamp. hiPSC-CMs (day 30) were used to cast EHTs in a fibrin/fibrinogen matrix. At EHT day 20-45, EHTs were taken off their pillars and mounted in the Myoclamp system. In this system, simultaneous control of preload and afterload while measuring force and tissue length results in the generation of real-time force-length workloops, analogous to pressure-volume (PV) loops of the heart. We showcase the ability to obtain, with high reproducibility, force-strain relationships from which length dependent activation and passive stiffness are derived. Additionally, by gradually increasing preload and afterload while measuring force-length work loops, EDPVR and ESPVR relations, stroke length, performed work, and detailed, phase-specific, contraction/relaxation kinetics are evaluated. EHT measurements in the Myoclamp platform allow us to reproduce key aspects of cardiac ventricular loading. This next generation model bridges the gap between traditional in vitro cardiac models and in vivo cardiac physiology.
P07.07	Taiki Ino University of Calgary	*SARCOMERE LENGTH AND FORCE BEHAVIOR WITH LOCAL DEACTIVATION*	Muscles produce active and passive force. Active force is generated through cross-bridge cycling, while passive force in single myofibrils depends virtually exclusively on titin. Within a myofibril, sarcomeres are arranged in-series transmitting identical forces. When the active force between sarcomeres in a myofibril differs, passive forces need to compensate for this difference in which can be achieved by length changes of sarcomeres. This adjustment of sarcomere length can lead to so-called sarcomere “popping”, resulting in muscle injury. We deactivated single sarcomeres in an otherwise fully activated myofibril to test if deactivated sarcomeres “pop” when active force is eliminated. However, deactivated sarcomeres elongated by only 0.7μm from an average length of 2.5μm to 3.2μm. At 3.2 μm, rabbit psoas sarcomeres generate a small passive force, insufficient to explain the remaining myofibrillar force. In a reverse experiment, we examined length changes in single sarcomeres that were never activated, while the remaining sarcomeres were activated maximally. Interestingly, in these experiments, sarcomeres that were never activated consistently “popped”. We propose that upon activation, sarcomeres form a linked actin-titin framework that provides increased passive force, thus preventing overstretching of sarcomeres when sarcomeres are deactivated. In contrast, when a sarcomere was not activated, and all remaining sarcomeres in a myofibril were activated, popping occurs, presumably to allow for increased passive force to match the force of the active sarcomere. In conclusion, we provide evidence that there is an in-built safety mechanism in active sarcomeres that provides stability in sarcomere length and prevents popping/injury in active muscle.

P07.08	Masataka Kawai University of Iowa	*The Effect of Temperature on Force Generation Step – Overview*	It has been known that an increase in temperature increases active force in vertebrate striated muscles. The rate constant of the force-generation step r4 was measured to increase with temperature to result in the increased number of force-generating cross-bridges (CB) by sinusoidal analysis, caged phosphate (Pi) experiments, and temperature jump studies. The active force vs. temperature plot can be explained by this mechanism, assuming that CB force does not change with temperature; this result does not depend on the stiffness measurement. In vitro motility assay also demonstrated that force/CB does not change with temperature (Kawai et al 2006). From the slope of the Arrhenius plot (Log K4 vs 1/T), the enthalpy change $\Delta H°=124\pm9$kJ·mol-1 was observed. This plot is somewhat curved, from which the heat capacity change $\Delta Cp=-6.4\pm1.8$kJ·K-1mol-1 was observed. $\Delta H°$ and ΔCp are linearly related to the accessible surface area changes of the hydrophobic area ΔAHP and ionic area ΔAI in the following equations: $\Delta AHP=\zeta\ \Delta H°+\gamma\Delta Cp$ $\Delta AI=\xi\Delta H°+\varphi\Delta Cp$ where $\alpha=0.188\pm0.008$kJ· K-1mol-1nm-2, $\beta=0.110\pm0.010$kJ· K-1mol-1nm-2, and $\psi=14.6\pm1.2$kJ·mol-1nm-2 were experimentally determined (Murphy & Gill 1991). Consequently, $\xi=1/\psi=0.0685\pm0.0056$kJ-1mol·nm2, $\varphi=85\xi=5.82\pm0.48$kJ-1Kmol·nm2, and $\zeta\equiv\beta/\alpha\psi=0.0401\pm0.0052$kJ-1mol·nm2. From these ΔAHP and ΔAI are calculated: $\Delta AHP=-51\pm12$nm2 and $\Delta AI=-29\pm11$nm2, totaling -80 ± 6nm2. The hydrophobic interaction area of actin and myosin based on structural studies is ~15 nm2 (30% of observation). The remaining 70% must come from the closing of the large myosin cleft as proposed (Murphy et al 1996). The cleft closure on force generation was later observed (Holmes et al, 2004; Klebl et al 2025).
P07.09	Annika J. Klotz University of Muenster	*Loss of titin-based elasticity elevates end-diastolic pressure through geometric remodelling*	Introduction: Increased titin-based myocardial stiffness is a recognised driver of diastolic dysfunction in HFpEF, and titin softening is being explored therapeutically. However, the consequences of titin softening on ventricular pressure remain unclear. We therefore investigated the haemodynamic effects of in vivo titin cleavage in titin-cleavage (TC) mice using a Laplace-based framework to distinguish myocardial stiffness from geometric determinants of left ventricular (LV) pressure. Methods: Titin cleavage was induced in homozygous TC mice carrying a TEV-site within I-band titin via AAV9-mediated TEV protease expression; AAV9-GFP served as control. LV pressure-volume analyses were performed 6 days post-injection. Titin cleavage was quantified by SDS-PAGE, passive mechanics assessed in permeabilized trabeculae, and MRI data analysed for LV geometry. Results: TEV-treated hearts exhibited robust titin cleavage (38.5±4.2%) and markedly reduced elastic stress (p=0.0009) without changes in viscous stress (p=0.9746). In vivo analyses revealed diastolic dysfunction with a small-volume, high-pressure phenotype, including reduced end-diastolic (p=0.0020) and stroke (p=0.0008) volumes, ~150% elevated end-diastolic pressure (p=0.0003), prolonged tau (p=0.0039), and increased EDPVR (p=0.0006) and ESPVR (p=0.0110). Preload responsiveness was impaired, with a reduced Frank-Starling slope (p=0.0222) and delayed preload adaptation (p=0.0003). MRI showed reduced LV radius (p=2.1×10-5) and increased wall thickness (p=4.6×10-6) without changes in LV mass (p=0.9927). Laplace decomposition revealed that geometric remodelling increased end-diastolic and end-systolic pressure by 89% (p=2.3×10-5) and 181% (p=1.5×10-5), respectively. Conclusion: Loss of titin-based elasticity induces diastolic dysfunction with elevated end-diastolic pressure predominantly through geometric remodelling, challenging the stiffness-centric paradigm of diastolic dysfunction and raising safety considerations for titin-targeting therapies in HFpEF.
P07.10	Meriem Matouk University of Versailles Saint Quentin En Yvelines	*No evidence of the presence of dystrophin protein in rodent muscle stem cells*	Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by the loss of full-length dystrophin at the sarcolemma, resulting in myofiber fragility, degeneration, and impaired regeneration. In addition to myofiber-intrinsic pathology, muscle stem cell (MuSC) dysfunction has been proposed to contribute to disease progression. In particular, previous studies have suggested that dystrophin is asymmetrically localized within activated MuSCs, where it may regulate stem cell polarity, asymmetric division, and self-renewal. However, the dynamics and origin of dystrophin localization in MuSCs remain poorly defined. Here, we revisited dystrophin expression and subcellular distribution in MuSCs using a dystrophin-EGFP reporter mouse combined with tdTomato-based lineage tracing of satellite cells. Across quiescent and activated states, dystrophin protein was undetectable in MuSCs despite robust Dmd transcript expression. This absence was consistently confirmed by in vivo imaging, histological analyses, fluorescence-activated cell sorting, and capillary electrophoresis. Dystrophin signal observed adjacent to MuSCs originated from the neighboring sarcolemma and did not display reproducible polarized enrichment relative to satellite cell position. These findings challenge the current model in which dystrophin acts as a cell-intrinsic determinant of MuSC polarity and asymmetric division. While our data do not question the importance of stem cell polarity in muscle regeneration nor the existence of MuSC dysfunction in DMD, they strongly argue against dystrophin as a direct regulator of these processes within satellite cells. Together, our study refines the current understanding of DMD pathophysiology and suggests that restoration of dystrophin expression in muscle stem cells may not be required for effective regenerative therapies.

P07.11	Beatrice Pistolato, Italian Institute of Technology	*Proprioception: the 'sixth sense' that guides octopus during motion*	Proprioception, the 'sixth sense', provides information about body position and movement. Octopus arms have virtually infinite degrees of freedom, and these animals developed unique control strategies to enable fast stereotypical and reflex motions that do not require a brain-centred feedback control. In this study, we investigated with a bottom-up approach, the involvement and role of proprioception in arm stereotypical motions. Through proteomic and bioinformatic analysis we identified the presence of two Piezo-like mechanosensitive ion channel isoforms, X1 (A0A7E6FJ03) and X2 (A0A6P7TA58) in the arm musculature. Immunofluorescence analysis revealed a regularly spaced distribution of Piezo-channels on the muscles, possibly localized in correspondence with calcium stores. Biomechanical experiments on isolated arm muscle preparations, showed the presence of stretch activation, a myogenic activity typical of proprioception-based responses. We show that this phenomenon is calcium-dependent and is reversibly blocked in the presence of GsMTx4, a known Piezo-channel blocker. We then performed biomechanical and electrophysiological recordings in whole-arm and show that stretch responses are transmitted from the muscle to the arm nervous system via a dense neurite network. A second mechanism is mediated by a complex circuit network at the arm level. In conclusion, we demonstrated the presence of at least two mechanisms of proprioceptive response: one embedded in the arm musculature and a second one engaging the arm nervous system and possibly reaching higher brain central level. Our study thus represents the first physiological assessment of octopus proprioception and opens the way to further investigations into its role brain-centred complex motion.
P07.12	Roberto Silva-Rojas Centro Nacional de Investigaciones Cardiovasculares (CNIC)	*Myofiber reprogramming and muscle rejuvenation by titin cleavage*	The physiological role of biological systems is defined by its function. In skeletal muscle, this is governed by the contractile capacity of sarcomeres, its functional identity element. Here, we propose sarcomeres as a plastic unit tuneable with a protease system directed to titin. We show that inducing a proteolytic cleavage in titin interferes directly in the functional identity of skeletal muscle fibers, destabilizing them into a dedifferentiated state. This state is characterized by the downregulation of terminally differentiated gene programs and the reactivation of core developmental and myogenic pathways, at the epigenetic, transcriptomic and proteomic levels, resembling a precursor-like identity. To determine the potential reversibility of this scenario, we further engineered a doxycycline-controlled system to cleave titin. This tool allowed us to prove the reversibility of both the titin cleavage and the myofiber dedifferentiation. Notably, this partial and reversible reprogramming yielded a rejuvenation of the DNA methylome when applied in ageing skeletal muscles.
P07.13	Emrulla Spahiu Hannover Medical School, Institute of Molecular And Cell Physiology	*Calmodulin-mediated modulation of cardiac myosin function*	Calmodulin (CaM) is the main calcium sensor found in all eukaryotic cells, orchestrating numerous vital cellular functions. In mammalian hearts, CaM plays a critical role in cardiac contraction cycle. With a large set of target proteins in cardiomyocytes, we hypothesized that CaM may have direct impact on cardiac myosin function, the major motor protein responsible for the cardiac contraction. Here, we investigated effects of CaM on atrial and ventricular myosin functions using in vitro motility assay. We further examined Ca2+ dependent regulation of CaM activity. CaM significantly enhanced actin gliding speed of ventricular myosins in a dose-dependent manner (~17% at 2 µM CaM, and ~46% at 10 µM CaM). Atrial myosin exhibited a calcium-dependent modulation of function, where 2 µM CaM increased gliding speed by ~16% consistently at high [Ca2+]. To elucidate the mechanism of CaM's modulation of myosin function, we performed myosin co-sedimentation assay. We incubated myosin with CaM under the same buffer and temperature conditions as in the motility assay and collected myosin pellet to test for co-precipitation. SDS-PAGE analysis indicates that a portion of CaM co-precipitated with both atrial and ventricular myosins. Notably, the supernatant fraction consisting of the unbound protein components contained free MLC1 proteins, suggesting a possible replacement of MLC1 from MyHCs by CaM, offering a possible explanation of the augmented myosin function observed as increased actin filament speed. These data support a model in which CaM modulates cardiac myosin function by association with myosin, fine-tuning myosin activity in a chamber specific manner.
P07.14	Chris Tiessen University Of Calgary	*Isometric Contraction Induces Passive Force Enhancement in Skeletal Muscle*	Passive force enhancement (PFE) is the increase in steady-state passive force following eccentric stretch and subsequent deactivation of a muscle. This intrinsic property of skeletal muscle has been shown at all levels of hierarchical organization, from single sarcomeres to intact in vivo muscles. PFE is related to residual force enhancement (RFE), the steady-state increase in active isometric force production following eccentric stretch and is thus only known to occur following eccentric contraction and subsequent deactivation. We hypothesized PFE may also occur following isometric contraction. We tested rabbit psoas myofibrils and skinned fibres. We found passive force production increased significantly following isometric activation and subsequent deactivation in myofibrils (50.6% PFE at steady-state) and skinned fibres (22.9% PFE at steady-state). We noted that PFE following isometric activation increased as a function of stretch length, suggesting the origin of PFE is a spring-element (titin?). We used a unique immunolabelling model to perform real-time tracking of titin's PEVK in myofibrils to confirm if titin may indeed be responsible for PFE following isometric contraction. Labelled myofibrils and unlabelled myofibrils showed identical mechanical properties. We found titin's PEVK was significantly longer during PFE. We approximated the mechanical effect of the longer PEVK region during PFE using a modified worm-like chain model and found the elongated PEVK accounts for ~68.9% of PFE. We conclude that isometric contractions induce PFE, implicating PFE as more relevant to movement than previously understood. Second, we suggest titin's PEVK is longer during PFE, supporting titin-actin binding as a major contributor to PFE.

P07.15	Tim J. van der Zee KU Leuven	*Computational cross-bridge model explains why muscle stiffness is reduced after shortening*	For reasons that have remained unclear, skeletal muscle's high initial resistance to stretch is temporarily reduced after shortening. This history-dependent short-range stiffness modulation may arise from shortening-induced detachment of actomyosin cross-bridges that act as in-parallel springs when attached. In addition to cross-bridge detachment, shortening may also cause myosin heads to transition to a super-relaxed state from which actomyosin binding is no longer possible. After shortening, myosin heads may be slow to transition back to a state in which actomyosin binding is possible, explaining why short-range stiffness reductions persist for up to 0.1 s after shortening. As it remains challenging to assess myosin's state during contraction, we used computational models to probe the mechanisms underlying muscle's mechanical response to stretch. Specifically, we tested whether the addition of myosin's super-relaxed state to a cross-bridge model better explained the temporal recovery of muscle's short-range stiffness following shortening, at a range of activation levels previously assessed in skinned rat soleus muscle fibers (n = 11). We quantified model accuracy using the root-mean-square deviation between the modelled and the measured ratio between the short-range stiffness before and after shortening. Short-range stiffness ratios predicted by a cross-bridge model incorporating myosin's super-relaxed state better matched empirical values than those predicted by a model lacking this state (root-mean-square deviation: 0.11 versus 0.14). The model with super-relaxed state also had a lower AIC score (ΔAIC = -42), indicating an improved model complexity-accuracy trade-off. These computational results suggest that myosin's super-relaxed state contributes to muscle's history-dependent mechanical response to stretch.
P07.16	Apostolos Papandreou National and Kapodistrian University of Athens	*Systemic Adaptations to Different High-Intensity Interval Training Modalities in National-Level Flatwater Kayak Athletes*	Background: High-intensity interval training (HIIT) and sprint interval training (SIT) are powerful stimuli for exercise-induced adaptations. Various aspects of the adaptational processes may be associated with or influenced by inflammation, hypoxia, angiogenesis, or oxidative balance mechanisms and be differentially triggered following different high-intensity interval training modalities. Purpose: This study examined and compared important systemic factors associated with these mechanisms following different high-intensity training modalities in competitive kayak athletes. Methods: Thirty-two national-level kayak athletes were randomly assigned to four different conditions and completed a six-week intervention period consisting of either HIIT (110% VO_2peak), SIT (140% VO_2peak), combined training (COMB: HIIT + SIT + continuous training), or a control condition. Circulating interleukin-6 (IL-6), vascular endothelial growth factor-A (VEGF-A), hypoxia-inducible factor-1α (HIF-1α), and total antioxidant capacity (TAC) were measured before and after the intervention period, and potential post-training changes were analysed using two-way repeated measures ANOVA. Results: Significant reductions in IL-6 were observed following HIIT ($p<0.01$) and SIT ($p<0.05$), while TAC increased after both HIIT ($p<0.05$) and SIT ($p<0.01$), with the largest increase being observed in the SIT group. Differential responses were revealed following the training modalities regarding the angiogenesis-related factors; VEGF-A increased significantly only in the HIIT group ($p<0.01$), while interestingly, HIF-1α exhibited a significant increase following SIT ($p<0.01$) and a significant decrease after the combined training ($p<0.05$). Conclusion: These findings suggest that different high-intensity kayak training modalities might differentially trigger systemic chronic adaptations that involve inflammation-, oxidative stress- and angiogenesis-related mechanisms.

S08 - Cardiomyopathies			
Invited speaker	Gavin Oudit University of Alberta, Edmonton, CA	*Prognostic Utility of Cardiovascular Magnetic Resonance-Based Phenotyping in Patients With Muscular Dystrophy*	Background: The presence of cardiomyopathy in patients with muscular dystrophy is common and linked to adverse outcomes. We evaluated the prevalence and burden of cardiomyopathy in adult patients with muscular dystrophy using cardiovascular magnetic resonance (CMR). Methods and Results: We prospectively enrolled 148 patients with dystrophinopathies (including heterozygotes), LGMD, and type 1 myotonic dystrophy (median age, 36.0 years; 51 [34.5%] women) over 7.7 years in addition to an age- and sex–matched healthy control cohort (n=50). CMR markers, including 3-dimensional strain and fibrosis, were assessed for their respective association with major adverse cardiac events. Markers of contractile performance were reduced across all muscular dystrophy groups. In particular, the dystrophinopathies cohort experienced reduced left ventricular ejection fraction (LVEF) and high burden of replacement fibrosis. Patients with type 1 MD showed a 26.8% relative reduction in LV mass with corresponding reduction in chamber volumes. Eighty-two major adverse cardiac events occurred over a median follow-up of 5.2 years. Although LVEF was associated with major adverse cardiac events (adjusted hazard ratio [aHR], 3.0) after adjusting for covariates, peak 3-dimensional strain amplitude demonstrated greater predictive value (minimum principal amplitude: aHR, 5.5; maximum principal amplitude: aHR, 3.3; circumferential amplitude: aHR, 3.4]). Minimum principal strain yielded incremental prognostic value beyond LVEF for association with major adverse cardiac events (change in $\chi 2=13.8$). Conclusions: Cardiac dysfunction is across all muscular dystrophy subtypes; however, subtypes demonstrate distinct phenotypic profiles. Myocardial deformation analysis highlights unique markers of principal strain that improve risk assessment over other strain markers, LVEF, and late gadolinium enhancement.

Invited speaker	Attila Kiss Medical University Vienna, AT	*Tenascin-C Drives Cardiomyopathy and Fibrotic Remodeling in Duchenne Muscular Dystrophy*	Background: Duchenne Muscular Dystrophy (DMD) is an X-linked genetic disorder characterized by progressive muscle weakness and cardiomyopathy. This study investigates the causative pathophysiological role of Tenascin-C (TN-C) upregulation in DMD-associated cardiovascular complications. Methods: To address this, mdx TN-C knockout (mdx TN-C KO) mice were generated and cardiac and vascular function were evaluated. Quantitative proteomics was performed to define molecular alterations in dystrophic mouse hearts. Circulating TN-C levels were quantified in DMD patients and healthy controls and correlated with cardiac fibrosis and cardiomyopathy assessed by cardiac MRI. Complementarily, DMD hiPSC-derived cardiomyocytes were used to further evaluate the translational impact of TN-C. The direct effects of TN-C were further examined in human cardiac fibroblasts, and HUVECs. Results: TN-C levels are elevated in DMD patient plasma, negatively correlating with left ventricular function and fibrosis. Mdx TN-C KO mice show reduced fibrosis, preserved cardiac function, and partial restoration of mitochondrial metabolism, with improved fatty acid oxidation and reduced glycolysis. In vitro, TN-C activated p38 MAPK and Hsp47 dependent epigenetic changes in cardiac fibroblasts. Consistently, patient-derived DMD cardiac sheets exposed to TN-C exhibit faster beat rates, electrical and mechanical instability, and increased collagen deposition in vitro. Conclusions: TN-C is one of a key driver of cardiomyopathy in DMD, linking fibrosis and mechanical instability, inflammation, and metabolic dysfunction. Elevated plasma TN-C levels in patients correlate with ventricular dysfunction and fibrosis. These findings establish TN-C as a potential biomarker and therapeutic target for DMD-cardiomyopathy.
Contributed talk	Callum Dark Murdoch Children's Research Institute, Melbourne, AU	*Making mature muscle: Investigating metabolic and developmental pathways for improving the maturation of bioengineered muscle*	Recent advances in the development of 3D bioengineered human muscle tissues have allowed high-throughput investigation of functional outputs of human skeletal muscle. Despite this, 3D skeletal muscle tissues generated from induced pluripotent stem cells (iPSCs) are still considered immature, showing underdeveloped contractile mechanisms, neonatal transcriptomic profiles, and less active metabolic states. Little is known about what specific factors drive skeletal muscle maturation, let alone how they can be used to mature bioengineered tissues. This project aims to identify novel factors involved in the maturation of skeletal muscle for the purpose of producing more physiologically relevant iPSC-derived 3D bioengineered muscle tissues. To generate these tissues, we use an iPSC line containing an mScarlet tagged reporter of alpha-actinin-2 (ACTN2), a sarcomeric Z-Disc protein shown to increase in expression with muscle age. Functional analysis of 3D skeletal muscle tissues allows us to measure contractile force, including twitch and tetanus strength, twitch/tetanus ratios, and endurance. To investigate novel factors that may improve muscle tissue maturation, we have performed an initial compound screen in 2D myotubes using a library of ~1800 compounds targeting diverse molecular pathways and metabolic processes. In this preliminary screen we identified 20 compounds that increase either ACTN2 expression (Z-score>2) or myotube area (Z-score>2) and decrease embryonic myosin, MYH3. These compounds will undergo functional follow up in 3D skeletal muscle tissues. From this study, we aim to identify culture conditions that can produce functionally mature 3D skeletal muscle tissues and thus create a more representative platform for investigating muscle disease.
Contributed talk	Tania Incitti AstraZeneca AB, SE	*Advanced in vitro modelling of human skeletal muscle enables physiologically relevant target validation*	Obesity and type 2 diabetes are linked to impaired skeletal muscle insulin sensitivity, metabolic inflexibility, and altered substrate use. As anti-obesity therapies become widely used, it is important to understand potential implications for muscle quality and function, ensuring weight loss does not compromise muscle integrity. However, major translational challenges remain in preclinical research. For instance, existing human skeletal muscle 2D in vitro models differ widely in their ability to reproduce mature myofibre architecture and insulin-responsive metabolism, limiting their value for mechanistic studies and target validation. To address this gap, we have identified advanced human 3D skeletal muscle platforms that support the generation of more physiologically relevant muscle tissue, and engineered 3D muscle tissues from both primary human myoblasts and iPSC-derived myogenic cells. We benchmarked platform performance with reference compounds known to affect muscle biology, enabling direct comparison of responsiveness, maturity, and reproducibility across cell sources and 3D systems. We are now expanding this framework by supporting the functional data on force generation and insulin-stimulated glucose uptake with molecular characterization and high-content imaging. To assess disease relevance, we will compare tissues derived from diseased donors or healthy donors under defined stimuli and pharmacological treatments, and we will use the platforms to evaluate compounds in our development pipeline. By linking compound responses to functional outcomes across platforms and cell sources, this work aims to establish a robust benchmarking framework and improve the translational utility of human skeletal muscle models in metabolic indications.

P08.01	Rostyslav Bubnov Clinical hospital Pheophania, Kyiv	*Multimodal Ultrasound-Guided Dry Needling in Chemotherapy-Associated Neuropathic–Myofascial Pain: Clinical and Functional Neuromuscular Outcomes in Opioid-Refractory Oncology Patients*	Background: Chemotherapy-associated pain syndromes result from overlapping mechanisms including chemotherapy-induced peripheral neuropathy (CIPN), autonomic dysregulation, and secondary myofascial dysfunction, leading to complex neuropathic–myofascial pain often resistant to pharmacological treatment. Objective: To evaluate clinical and neuromuscular effects of ultrasound-guided dry needling (US-DN) in chemotherapy-associated neuropathic–myofascial pain using a multimodal ultrasound assessment approach. Methods: A prospective observational case series included 10 oncology patients (mean age 62 years) with opioid-refractory neuropathic–myofascial pain following chemotherapy. All patients demonstrated clinical features of CIPN combined with musculoskeletal dysfunction, including impaired mobility, postural imbalance, and focal myofascial hyperalgesia. A multimodal ultrasound protocol was applied, including peripheral nerve ultrasound for structural neuropathic changes, musculoskeletal ultrasound for trigger points and fascial alterations, and dynamic cervical–suboccipital imaging assessing neuromuscular motion patterns and myodural interface behavior. Patients with structural metastatic compression were excluded from intervention targeting. All patients underwent US-guided dry needling of identified myofascial trigger points combined with individualized rehabilitation. Results: US-DN produced rapid and clinically significant pain reduction in most patients, with several achieving near-complete pain relief after 1–2 sessions and reduced opioid requirements. Ultrasound assessment demonstrated peripheral nerve echotextural abnormalities consistent with CIPN, focal trigger-point activity, and fascial stiffness. Dynamic imaging revealed altered cervical–suboccipital neuromuscular patterns suggestive of dysregulated muscle tone and central sensitization features. Post-intervention imaging showed partial normalization of muscle–fascial mobility and improved dynamic symmetry in responders. Conclusion: Multimodal ultrasound enables identification of combined neuropathic and myofascial contributors to chemotherapy-related pain. Ultrasound-guided dry needling may modulate both clinical symptoms and neuromuscular function.
P08.02	Callum Dark Murdoch Children's Research Institute	*Making mature muscle: Investigating metabolic and developmental pathways for improving the maturation of bioengineered muscle*	Recent advances in the development of 3D bioengineered human muscle tissues have allowed high-throughput investigation of functional outputs of human skeletal muscle. Despite this, 3D skeletal muscle tissues generated from induced pluripotent stem cells (iPSCs) are still considered immature, showing underdeveloped contractile mechanisms, neonatal transcriptomic profiles, and less active metabolic states. Little is known about what specific factors drive skeletal muscle maturation, let alone how they can be used to mature bioengineered tissues. This project aims to identify novel factors involved in the maturation of skeletal muscle for the purpose of producing more physiologically relevant iPSC-derived 3D bioengineered muscle tissues. To generate these tissues, we use an iPSC line containing an mScarlet tagged reporter of alpha-actinin-2 (ACTN2), a sarcomeric Z-Disc protein shown to increase in expression with muscle age. Functional analysis of 3D skeletal muscle tissues allows us to measure contractile force, including twitch and tetanus strength, twitch/tetanus ratios, and endurance. To investigate novel factors that may improve muscle tissue maturation, we have performed an initial compound screen in 2D myotubes using a library of ~1800 compounds targeting diverse molecular pathways and metabolic processes. In this preliminary screen we identified 20 compounds that increase either ACTN2 expression (Z-score>2) or myotube area (Z-score>2) and decrease embryonic myosin, MYH3. These compounds will undergo functional follow up in 3D skeletal muscle tissues. From this study, we aim to identify culture conditions that can produce functionally mature 3D skeletal muscle tissues and thus create a more representative platform for investigating muscle disease.
P08.03	Marta Hanczar Amsterdam UMC	*Muscle-microenvironment-in-a-dish: a novel model reveals that endothelial cells modulate muscle contractility and gene expression*	Diabetic myopathy and microvascular dysfunction are important complications for patients with type 2 diabetes. Recent research suggests that muscle microvasculature and surrounding adipose tissue can impact muscle function, potentially contributing to muscle dysfunction. Here we developed a model to test whether endothelial cells and diabetic adipokines in the muscle microenvironment can impact muscle function. We created a muscle-microenvironment-in-a-dish model by casting mature mouse flexor digitorum brevis (FDB) fibers in a gel and using the transwell system for endothelial cell co-culture. After the co-culture period, contractility was measured in the Multicell Ionoptix System. Our results show that endothelial cells shorten contraction and relaxation time after 2 hours of co-culture. These effects are even stronger after 48h of co-culture, with the addition of a significant increase in muscle fiber percentage shortening . Bulk RNA-seq showed that upon co-culture muscle fibers increase the expression of genes related to muscle metabolism and calcium handling, such as secretin. Incubation of endothelial cells with palmitic acid and TNFα reversed the co-culture effect in FDB's showing a significantly lower percentage of shortening, and longer contraction and relaxation times compared to both the co-culture and baseline conditions. In conclusion, this study shows that endothelial cells in muscle microenvironment increase muscle contractility and change gene transcription. After exposure to known diabetic adipokines the endothelial cells worsen muscle contractility, showing that impacted microvascular function can contribute to diabetic myopathy. This offers potential treatment strategies for this complication by targeting endothelial function.

P08.04	Tania Incitti AstraZeneca AB	*Advanced in vitro modelling of human skeletal muscle enables physiologically relevant target validation.*	Obesity and type 2 diabetes are linked to impaired skeletal muscle insulin sensitivity, metabolic inflexibility, and altered substrate use. As anti-obesity therapies become widely used, it is important to understand potential implications for muscle quality and function, ensuring weight loss does not compromise muscle integrity. However, major translational challenges remain in preclinical research. For instance, existing human skeletal muscle 2D in vitro models differ widely in their ability to reproduce mature myofibre architecture and insulin-responsive metabolism, limiting their value for mechanistic studies and target validation. To address this gap, we have identified advanced human 3D skeletal muscle platforms that support the generation of more physiologically relevant muscle tissue, and engineered 3D muscle tissues from both primary human myoblasts and iPSC-derived myogenic cells. We benchmarked platform performance with reference compounds known to affect muscle biology, enabling direct comparison of responsiveness, maturity, and reproducibility across cell sources and 3D systems. We are now expanding this framework by supporting the functional data on force generation and insulin-stimulated glucose uptake with molecular characterization and high-content imaging. To assess disease relevance, we will compare tissues derived from diseased donors or healthy donors under defined stimuli and pharmacological treatments, and we will use the platforms to evaluate compounds in our development pipeline. By linking compound responses to functional outcomes across platforms and cell sources, this work aims to establish a robust benchmarking framework and improve the translational utility of human skeletal muscle models in metabolic indications.
P08.05	Thorsten Jonas Optics11 Life	*A contractility platform for 3D engineered muscle tissues*	Models and assays used in preclinical drug development largely determine the success of clinical trials. Findings in 2D cell cultures or lab animals often fail to translate to humans due to differences in genetics, physiology, and the lack of a realistic 3D tissue environment. To enhance translatability, in vitro models that accurately replicate human tissue physiology and function are needed. In muscle biology and disease, contractility serves as a key functional readout that cannot be fully assessed using biochemical or structural methods. This necessitates not only a physiologically relevant 3D muscle model but also an accurate method for measuring its functional performance. The Cuore system addresses this need. It is a versatile multi-well platform designed for culturing and electrically stimulating 3D engineered muscle tissues, while precisely quantifying contraction forces and kinetics. Compatible with primary, immortalized, and iPSC-derived cell lines, Cuore enables the development of patient-specific in vitro models. Its customizable stimulation protocols allow researchers to explore muscle maturation, exercise, fatigue, and other physiologically relevant conditions. Cuore's optical interferometry-based readout provides absolute and precise force measurements with single-micronewton and millisecond resolution, and key contractility parameters are automatically extracted, using the proprietary analysis software. Additionally, the system can be combined with imaging readouts and biochemical tissue post-processing. We demonstrate the broad application of the Cuore platform across skeletal, cardiac, and smooth muscle fields. Cuore a powerful platform for modelling muscle pathophysiology and evaluating therapeutic efficacy in vitro, leading to enhanced translatability of preclinical studies and more informed, data-driven decisions in drug development.
P08.06	Kathy Myburgh Stellenbosch University	*Myoblast Derived Small EVs Loaded with fluorescent miR-26: A Therapeutic Model for Small EV Uptake by Skeletal Muscle Myoblasts In Vitro and Subsequent Myogenic Response to Cargo*	Small extracellular vesicles (EVs) released by skeletal muscle influence myogenesis in a cargo-dependent manner. Among the muscle-specific microRNAs (myomiRs), miR-206 is a key regulator. This study hypothesised that miR-206 loaded into small EVs would be protected from degradation and effectively delivered to recipient cells. Small EVs were generated from proliferating C2C12 myoblasts. Nanoparticle Tracking Analysis confirmed the majority of isolated particles were within the expected size range (mean diameters below 200 nm). Small EVs were further characterised using a lipid bilayer membrane stain (Cell Mask Orange), transmission electron microscopy and western blotting to detect TSG101, Alix, and CD81. Small RNA fractions were isolated from both C2C12 cells and small EVs to compare endogenous C2C12 miR-206 expression and EV miR-206 cargo before and after loading. Loading with a fluorescent miR-206 mimic (50 pmol) using Exo-fect resulted in 358-fold ($p<0.0001$) higher miR-206 EV cargo. Loading efficiency was 98.5%. Confocal microscopy confirmed uptake of miR-206-loaded small EVs by recipient C2C12 cells over a 16 h period. Intracellular miR-206 levels increased 177-fold compared to untreated cells. Treatment with miR-206 inhibitor-loaded small EVs reduced cellular miR-206 levels (0.25-fold). miR-206-loaded small EVs reduced myoblast proliferation and levels of Pax7 expression. During differentiation, miR-206 delivery promoted myogenic fusion, with treated cells displaying the highest number of fused nuclei per field of view (35, n=3). This study demonstrated that C2C12 myoblast-derived small EVs can be efficiently loaded with miR-206 for delivery to recipient cells without degradation. This supports their potential as a delivery platform for therapeutic miRNA cargo.

P08.07	Malte Rinn University Medical Center Göttingen	*Human Myomatrix Muscle Organoids Capture Collagen VI-Related Dystrophy Phenotypes*	Background: Collagen VI-related dystrophy (COLVI-RD) comprises a spectrum of congenital muscular disorders caused by pathogenic variants in COL6A1, COL6A2, or COL6A3, ranging from mild Bethlem myopathy to severe Ullrich congenital muscular dystrophy (UCMD). Hallmarks include progressive muscle weakness, contractures, and respiratory impairment. Collagen VI is a key component of the skeletal muscle extracellular matrix (myomatrix), where it supports tissue integrity and cell-matrix interactions. Its deficiency disrupts extracellular matrix organization and signaling and is associated with impaired mechanotransduction, mitochondrial dysfunction, and defective autophagy. However, the mechanisms by which defects in collagen VI-producing interstitial fibroblasts contribute to myofiber dysfunction remain incompletely understood. Methods and Results: To address this, we developed myomatrix muscle organoids (MMOs), a human skeletal muscle model based on cell-autonomous extracellular matrix production. Induced pluripotent stem cells (iPSCs) were gene-edited to carry a UCMD-associated COL6A1 mutation (c.930+189C>T) or a COL6A1 knockout and differentiated into MMOs. Single-nucleus RNA sequencing revealed an organotypic cellular composition, including NEB+ myofibers, PAX7+ satellite-like cells, PRRX1+/FAP+/PDGFRA+ fibroblasts, vascular cells, and a small MAPT+ neuronal population. COL6A1/2 expression was enriched in fibroblast and satellite-like cells and reduced in knockout MMOs. Functionally, collagen VI-deficient organoids showed significantly decreased contractile force compared to wild-type controls. Conclusion: Together, these findings establish MMOs as a relevant human platform to study COLVI-RD and fibroblast-driven extracellular matrix contributions to muscle dysfunction.
P08.08	Jiahao Zhao Medizinische Hochschule Hannover (MHH)	*Antifibrotic Effects of LNA-21, MEG3 on Human Lung Tissue Using a Living Pulmonary Punch Preparation Model*	Background: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by excessive extracellular matrix deposition and irreversible loss of lung function. Current therapies slow progression but cannot reverse fibrosis, underscoring the need for novel treatments. Aims: To evaluate non-coding RNA therapies targeting microRNA-21 (LNA-21) and MEG3 as antifibrotic strategies in human ex vivo lung tissue. Methods: Human lung tissue was sectioned into 300-µm slices and punched into 8 mm biopsies, cultured at 37°C, 5% CO_2 for 48–96 hours. Treatments included LNA-21 (100 nM) and MEG3 (10 µM). Cell viability was assessed by LDH assay, fibrotic gene expression (ACTA2, VIM, POSTN) by qPCR, and collagen deposition by Picrosirius Red staining. Results: LDH levels were slightly reduced across treatment groups, confirming retained tissue viability. LNA-21 significantly downregulated miR-21 and fibrotic marker genes ACTA2, VIM, and POSTN at 48 hours. MEG3 treatment markedly suppressed fibrotic gene expression, demonstrating potent antifibrotic activity. Picrosirius Red staining showed a non-significant trend in collagen changes, likely reflecting ECM remodeling time-lag. Conclusions: Both LNA-21 and MEG3 effectively suppress fibrotic markers at the transcriptional level in a human ex vivo lung punch model. Future work will optimize the model and further explore MEG3–miR-21 signaling interactions.

S09 - Skeletal Myopathies

Invited speaker	Andreas Roos University Duisburg-Essen, DE	*The clinical, genetic and pathophysiological landscape of protein aggregattion myopathies*	Protein aggregation myopathies comprise a rapidly expanding group of inherited and acquired neuromuscular disorders characterized by the accumulation of misfolded proteins within skeletal muscle fibers. Despite this common pathological hallmark, they display remarkable clinical, genetic, and molecular heterogeneity, with disease onset ranging from infancy to late adulthood and considerable phenotypic variability even within individual entities. Advances in genomic medicine have identified pathogenic variants in genes encoding proteins with diverse cellular functions that ultimately converge on impaired proteostasis. These disorders can be broadly classified into defects affecting post-translational protein modification (e.g., GNE), molecular chaperones and protein folding (e.g., SIL1, CRYAB), structural and cytoskeletal proteins (e.g., MYH7, FLNC), nuclear matrix proteins (e.g., MATR3), and the ubiquitin-proteasome and autophagy-lysosomal pathways (e.g., VCP, VMA21, LAMP2). Although functionally distinct, these proteins disrupt common pathways regulating protein quality control, aggregate formation, and degradation. Proteostasis failure is also central to acquired toxic myopathies. Drugs such as chloroquine and clozapine impair ER-Golgi trafficking and autophagy-lysosomal function, recapitulating mechanisms that lead to vacuolar and protein aggregate myopathies and providing valuable insights into disease pathogenesis. This lecture will provide a comprehensive overview of the clinical, genetic, and molecular landscape of protein aggregation myopathies. By comparing phenotypes ranging from isolated distal myopathies to multisystem proteinopathies, it will highlight shared mechanisms of proteostasis failure and discuss the dynamic evolution of protein aggregates, from early misfolded species to mature inclusions, and their contribution to muscle fiber degeneration and emerging therapeutic strategies.

Invited speaker	Alessandra Ruggieri Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, IT	*From PLIN4 Aggregates to Aggrephagy: Lessons for Muscle Disorders*	PLIN4-related myopathy is a rare autosomal dominant disorder first described in 2020 in a large Italian family, characterized by distal lower limb weakness with frequent involvement of the upper limbs and scapular and pelvic girdle muscles, in the absence of significant cardiac or respiratory manifestations. Since then, five additional families and one sporadic case have been reported in China and Spain, all carrying expansions of the same 33-amino acid repeat, albeit of variable lengths. More recently, a second pathogenic expansion, a 14-repeat duplication, was identified in an Italian patient. Clinical manifestations are heterogeneous, ranging from predominant distal to more proximal and severe phenotypes. The broad clinical and genetic spectrum of PLIN4-related myopathy, coupled with the technical challenges of detecting repeat expansions, likely contributes to underdiagnosis and suggests that additional affected individuals remain to be identified. Despite this clinical and genetic variability, PLIN4-related myopathy is consistently characterized by perilipin 4 aggregation within muscle fibers. These aggregates trigger the activation of key aggrephagy mediators, progressively disrupting myofiber architecture and contractile function, thereby highlighting impaired protein quality control as a central pathogenic mechanism. We extended these findings to myofibrillar myopathies, another group of protein aggregate disorders, demonstrating that aggrephagy activation correlates with disease severity. Aggrephagy dysregulation has also been reported in FHL1-related rigid spine syndrome, sporadic inclusion body myositis (sIBM), and oculopharyngeal muscular dystrophy. Current evidence implicates aggrephagy dysfunction as a central driver of PLIN4-related myopathy and potentially other protein aggregate myopathies, highlighting new opportunities for biomarker discovery and therapeutic intervention.
Contributed talk	Fernando Ribeiro Uppsala University, SE	*Myosin Post-Translational Modifications Associated With Critical Illness Myopathy*	Background: Critical illness myopathy (CIM) is a common and devastating consequence of critical care, causing dramatic loss of skeletal muscle mass and function in intensive care unit (ICU) patients. Functional deficits often exceed the loss in muscle mass and myosin content. However, the mechanisms underlying the loss of force remain elusive. Methods: Myosin dysfunction was investigated in six neuro-ICU patients exposed to a 12-day mechanical ventilation and immobilization period using mass spectrometry-based proteomics and molecular dynamics simulations. Results: Previous single muscle fiber analyses revealed decreased fiber size and specific force from the 1st to the 12th days in all ICU patients. A subset of myosin-expressing fibers exhibiting a complete loss of contractile function was identified in three of the six ICU patients despite similar atrophy levels (~30%, $p<0.05$) after 12 days. All fibers had decreased specific force after 12 days of mechanical ventilation, but 9% to 21% of the fibers were non-force-generating. The decline in muscle specific force was linked to 27 post-translational myosin modifications, including oxidation, ubiquitination, acetylation, and methylation. Molecular dynamics simulations suggested oxidation-induced rigidity of the myosin head, which was predicted to compromise the flexibility of key functional (i.e. actin-binding and converter) domains. Non-force-generating fibers exhibited a unique proteomic signature predicted to further enhance myosin head exposure and rigidity. Conclusion: In addition to muscle wasting and myosin loss, abnormal myosin post-translational modifications contribute to ICU-acquired muscle weakness in critically ill patients, including the development of muscle fibers incapable of generating contractile force.
Contributed talk	Patryk Konieczny Adam Mickiewicz University, PL	*DP71 uncouples early survival from functional differentiation in dystrophin-deficient muscle*	Duchenne muscular dystrophy is caused by mutations in the DMD gene, which encodes multiple dystrophin isoforms. Although all patients lack full-length dystrophin DP427, many retain the shorter isoform DP71, whose role in skeletal muscle remains unclear. We investigated whether DP71 modulates the disease phenotype in the absence of DP427 and its paralog utrophin. DP71 expression, splicing and promoter activity were analysed in human and mouse models. Functional effects of selective DP427 loss or combined DP427/DP71 depletion were assessed in differentiating human myotubes using functional assays and transcriptomic profiling, together with rescue experiments using DP71 splice variants. DP71 was enriched in myoblasts and showed greater splice diversity in human than in murine models. In DP427-deficient myotubes, DP71 was transcriptionally upregulated, and this response was further enhanced by utrophin depletion. Increased DP71 expression was associated with larger myotube areas, improved viability, reduced ROS and lower intracellular calcium during early differentiation. Transcriptomic analyses showed that, compared with complete dystrophin deficiency, DP427-deficient cells retaining DP71 displayed weaker induction of structural and stress-responsive genes, but preserved metabolic, ribosomal and proteostasis-related programmes. Utrophin depletion selectively impaired structural gene expression without reproducing the full transcriptional profile of complete dystrophin loss. These findings identify DP71 as an inducible modulator of early adaptation in dystrophin-deficient muscle. DP71 supports cellular homeostasis and buffers stress-associated phenotypes but does not restore full structural or functional differentiation. Our data suggest that early survival and functional maturation can be uncoupled in dystrophin-deficient muscle, with DP71 and utrophin making distinct contributions to this process.

P09.01	Wilson Agyapong Hannover Medical School	*Mavacamten elicits conserved and model-specific responses across cardiac models of hypertrophic cardiomyopathy*	Background: Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease and is characterized by myocardial hypertrophy, fibrosis, myocyte disarray, and impaired cardiac function. Mavacamten (MAVA), a selective cardiac myosin inhibitor, improves outcomes in patients with hypertrophic obstructive cardiomyopathy (HOCM) by reducing hypercontractility. However, its functional and molecular effects across different cardiac model systems remain unclear. Methods: We investigated MAVA responses in healthy porcine cardiomyocytes and living myocardial slices (LMS), HOCM patient-derived LMS, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), and cardiac organoids. Long-term cultured LMS enabled continuous force measurements, while SarcTrack and MUSCLEMOTION were used to assess contractile dynamics. Results: MAVA reduced contractile force across all investigated models, confirming its negative inotropic effect. In primary cardiac tissues, force reduction was accompanied by minor changes in time-to-peak contraction and relaxation time, whereas contraction and relaxation velocities were decreased. In contrast, engineered human HOCM models showed accelerated contraction and relaxation kinetics following treatment, indicating model-dependent responses to myosin inhibition. Transcriptomic analyses revealed alterations in pathways related to sarcomere organization, cytoskeletal remodeling, mechanosensing, and cellular metabolism, suggesting that the effects of MAVA extend beyond direct modulation of contractile proteins. Among the differentially expressed genes, MYBPH was consistently upregulated in living myocardial slices following MAVA treatment. Conclusions: Mavacamten exerts conserved force-reducing effects across diverse cardiac models while eliciting distinct kinetic and transcriptional responses depending on the experimental system. These findings highlight both shared and model-specific adaptations to cardiac myosin inhibition and provide new insight into the molecular effects of MAVA in HCM.
P09.02	Melissa Amerudin King's College London	*Truncated titin is expressed in a cell-specific and muscle-specific manner in vivo*	Titinopathies represent a diverse collection of cardiac and musculoskeletal disorders that arise from mutations in TTN, the gene encoding the giant sarcomeric protein titin. Amongst these, truncating variants (TTNtvs) in the A-band are most commonly associated with dilated cardiomyopathy, but their underlying mechanisms of disease remain controversial. Is it the deficit of full-length titin ('haploinsufficiency'), or the direct, negative influence of the TTNtv ('poison-peptide'), or both, that impairs sarcomeric stability, protein handling and thus the contractile capacity of the myocyte? To investigate the functional consequences of TTN variants, several CRISPR/Cas9-engineered mouse lines carrying patient-derived mutations in TTN were generated. Homozygous inheritance of truncated TTN alleles is embryonic lethal, yet heterozygotes mature to adulthood and exhibit overtly normal behaviour at baseline. However, specialised gel electrophoresis and RT-qPCR revealed a trend towards reduced expression of full-length and total titin mRNA in adult TTNtv-bearing mice. Direct assessment of TTNtv protein expression was performed with confocal microscopy using a custom TTNtv-specific antibody. Strikingly, not only was truncated titin observed in a regional fashion, but its pattern of expression varied between different striated muscles – with its integration into some cardiomyocytes, but its uncoupling from the skeletal muscle sarcomere. Thus far, these results support a dual TTNtv-mediated pathomechanism, whereby loss of wild-type titin and persistence of mutant titin may disrupt local sarcomeric integrity and provoke muscle-specific dysfunction.
P09.03	Mamta Amrute Hannover Medical School	*Insights into Mavacamten's Effect on the Actomyosin Crossbridge Cycle at the Single-Molecule Level*	Mavacamten (Mava), a cardiac myosin inhibitor, is approved for symptomatic obstructive hypertrophic cardiomyopathy (oHCM) in adult patients. Previous studies have shown that Mava promotes the super-relaxed state of myosin, characterized by slow ATP turnover, and stabilizes the folded-back conformation of myosin heads, highlighting its primary action on 'OFF-states' of myosin. However, its effects on actively cycling actomyosin cross-bridges ('ON-states') remained poorly understood. Here, using human beta-cardiac myosins in optical trapping-based single-molecule measurements, we identified two distinct populations of actomyosin binding events in the presence of Mava: (1) single-step interactions (P1), typical of conventional myosin II, and (2) two-substep events (P2). P1 events revealed a significantly reduced stroke size, while the actomyosin detachment rate remained largely unchanged compared to control. P2 events exhibited a faster transition rate during the initial binding phase, whereas the second substep- corresponding to a distinct AM state – displayed a detachment rate identical to that of P1 events. Notably, increasing ATP concentrations selectively shortened the lifetime of the second substep, but had no effect on the first, implying that the initial actomyosin-bound state is ATP-insensitive. Collectively, these findings reveal that Mava delays the powerstroke by stabilizing the pre-powerstroke state for ß-cardiac myosin. Myosin adopts different conformations in the Mava-bound state, eliciting variable effects on kinetic and mechanical outcomes. Understanding how Mava fine-tunes myosin's chemomechanical cycle is critical for its use in treating HCM patients. Myosin function modulators are crucial for identifying distinct states of the cross-bridge cycle that underlie the basic mechanisms of cardiac function.

P09.04	Ana Isabel Antunes Gonçalves Medical University of Vienna	*Blocking IL-6 Reverses Multi-Organ Dysfunction in Cancer Cachexia*	Introduction/Background: Cachexia is an inflammatory catabolic syndrome causing loss of skeletal muscle and fat. It is common in end-stage heart failure but its effects on cardiac and vascular function in cancer associated cachexia still remain unclear. Both conditions are linked through elevated IL6 levels that are strongly associated poorer clinical outcomes, highlighting IL-6 as a potential shared therapeutic target. Methods: Male BALB/c mice (10–12 weeks old) were injected subcutaneously in the right flank with 1×103 cells/µL mouse colon-26 carcinoma (C26) or PBS as control (n=8, all groups). On day 3, C26 mice were randomized to receive IL-6 antibody (AB; Bioxcell) or PBS every second day. Cardiac function, grip strength, and body composition were assessed. Multi-organ molecular fingerprints were assessed by different approaches. Results: C26 tumour-bearing mice show body weight loss >5% (22.18 vs 26.45 g), decreased grip strength (73.66 vs 99.96 g), systemic inflammation, and anaemia. C26 mice exhibited cardiac edema, cardiomyocyte atrophy, endothelial dysfunction, and renal metabolic impairment, including reduced mitochondrial OXPHOS capacity (22.46 vs 49.22 pmol/s·mL). Endothelial dysfunction was reflected by impaired NO-mediated relaxation (ACh EC50: −7.33 vs −6.91, $p < 0.001$). These alterations were partially reversed by anti-IL-6 treatment. Conclusion: These findings indicate that targeting IL-6 markedly improves the cachectic phenotype and enhances cardiac and renal function, metabolism, and endothelial function, supporting IL-6 inhibition as a therapeutic strategy for cancer-associated cachexia and cardiometabolic dysfunction. Funding : This study was supported by FWF Austria (PAT 1863824)
P09.05	Annie Brong University of Maryland, Baltimore	*Deciphering the source of sex differences in the Obscn-ΔIg58/59 mouse model exhibiting cardiac remodeling and arrhythmia*	Immunoglobulin domains 58 and 59 (Ig58/59) of the multitasking muscle giant obscurin interact with several essential mediators of sarcomere structure and function; consequently, mutations in the Ig58/59 module have been linked to human cardiomyopathy. To explore the pathophysiological significance of this region, we excised these two domains from the obscurin gene in our ObscnΔIg58/59 mouse model. Prominent arrhythmias appear in ObscnΔIg58/59 males by 6-months and augment with aging, accompanied by maladaptive remodeling of both upper and lower heart chambers. However, the hearts of female ObscnΔIg58/59 mice exhibit minimal electrical and functional alterations. We hypothesized that estrogen signaling insulates female ObscnΔIg58/59 hearts from disease and that deprivation of both endogenous estrogens and dietary phytoestrogens could incite dormant pathologies. Indeed, ovariectomy (OVX) and soy-free diet prompt age-specific cardiac remodeling in ObscnΔIg58/59 females, with adaptive hypertrophy apparent at 3-months and maladaptive dilation evident at 15-months. Soy-free, OVX ObscnΔIg58/59 females also exhibit pronounced arrhythmias by 15-months of age or under acute β-adrenergic stress at 3-months of age. In stark contrast, male ObscnΔIg58/59 mice fed a soy-free diet show diminished arrhythmic burden and cardiac remodeling, demonstrating that soy feeding elicits diametric outcomes in our model. We are currently investigating whether infusion of the nonaromatizable androgen, dihydrotesterone, can accelerate remodeling and arrhythmogenesis in soy-free, OVX ObscnΔIg58/59 females, as we posit that the relative absence of androgens provides residual protection to these females. Collectively, our work in both sexes emphasizes the profound capacity of phytoestrogens to alter cardiomyopathic phenotypes and challenges the hegemony of estrogenic cardioprotection.
P09.06	Kenneth Campbell University of Kentucky	*Extracellular fibrosis and low phosphorylation of myofilament proteins predict diastolic deceleration time in a cohort of 90 patients*	Diastolic dysfunction is an important clinical problem that remains poorly understood. It is categorized in clinical settings through detailed analysis of echocardiograms. Relatively few studies have investigated potential relationships between patients' diastolic function and the biochemical status of their myocardium. Myocardial samples were procured from the middle transmural region of the left ventricular free wall from a cohort of 90 patients who had advanced heart failure or were organ donors. 80% of the patients had an ejection faction below 40%. 67% of the cohort had an elevated left atrial pressure consistent with diastolic dysfunction. Experiments performed with the samples included histological staining for collagen, gel electrophoresis to quantify titin isoforms and the relative phosphorylations of troponin and regulatory light chain, and western blotting to determine the relative phosphorylation of myosin binding protein-C. In myocardium from patients with heart failure, fibrosis was elevated and regulatory proteins were less phosphorylated than in samples from organ donor. No strong relationships were observed between any biochemical measure and the E to A, E to e', or isovolumic relaxation time indices of diastolic function. The deceleration time of mitral flow valve quantifies how long pressure takes to equilibrate across the valve during diastole. This clinical parameter was negatively correlated with myocardial collagen content and positively correlated with the relative phosphorylation of troponin I and myosin binding protein-C. Ongoing studies are using multiscaled computer modeling to investigate the physiological and pathophysiological significance of these new findings.
P09.07	Stuart Campbell Yale University	*Effects of cMyBP-C Loss on the Contractile Behavior of Isometrically Loaded Human Engineered Heart Tissue*	cMyBP-C is a structural and regulatory protein located in the cardiac sarcomere. Its known binding interactions with both actin and myosin give it the potential to modulate muscle contraction in complex ways, with implications for both normal and diseased states. To better understand the fundamental roles of cMyBP-C in human cardiac function, we studied isometric twitch behavior of human engineered heart tissues (EHTs) under conditions of normal and haploinsufficient cMyBP-C expression. An iPSC line was generated from an HCM patient homozygous for an MYBPC3 truncating mutation (MYBPC3+/-). CRISPR/Cas9 was used to create an isogenic mutation-corrected line that restored the wildtype MYBPC3 sequence (WT). Cardiomyocytes were differentiated from the respective iPSC lines and seeded into decellularized porcine myocardial slices to form EHTs. EHTs were mounted to a physiological testing apparatus and paced at 1-1.5 Hz while measuring force and intracellular Ca2+ transients. EHTs were also stretched to lengths spanning -5 to +10% of the baseline (culture) length to obtain the isometric force-length relationship. MYBPC3+/- EHTs (~50% loss of cMyBP-C) showed isometric twitch force values that were significantly higher than WT, while simultaneously having faster relaxation time and an overall shorter twitch duration. Length-dependent activation of MYBPC3+/- EHTs was blunted when compared with WT, while exhibiting a steeper diastolic force-length relationship. Ca2+ transients in the mutant EHTs showed only mild differences relative to WT. Collectively, these results support simultaneous roles of cMyBP-C in both sequestering myosin heads during diastole and at shorter muscle lengths while also binding to actin to prolong systole.

P09.08	Olivier Cazorla Phymedexp Inserm - Cnrs – Univ Montpellier	*Right Heart dysfunctions of experimental-induced exacerbated emphysema: bisoprolol misses the target*	The mechanisms linking Chronic Obstructive Pulmonary Disease (COPD) progression to right heart failure (RHF) are unclear, and beta-blocker use in COPD is debated. Our prior work in a rat elastase-LPS (ELA-LPS) emphysema model showed left ventricular diastolic dysfunction (HFpEF), partially improved by the beta1-blocker bisoprolol. Preliminary right heart dysfunction signs prompted this study investigating how lung inflammation and emphysema affects pulmonary vasculature, right heart function, and whether bisoprolol's benefit on the left heart extends to the right heart and pulmonary circulation. Male Wistar rats received four weekly intratracheal 4U-elastase doses followed by LPS to induce exacerbation (ELA-LPS), with littermate controls. Five weeks post-exacerbation, ELA-LPS caused severe emphysema, pulmonary vascular remodeling, and hyper-contractile pulmonary arteries. The right ventricle (RV) developed diastolic dilation, hypertrophy, and elevated systolic pressure despite preserved contractility. RV cardiomyocytes showed increased stiffness, heightened myofilament Ca^{2+} sensitivity, and impaired Ca^{2+} reuptake, leading to poor relaxation. Right atrial hypertrophy, dilatation, reduced ejection fraction, arrhythmias, and low heart rate variability indicated RHF. RV transcriptomics revealed hypertrophy, dysregulation in metabolism and circadian pathways. Bisoprolol, started one day post-LPS, prevented autonomic dysfunction but failed to mitigate RV structural, functional, or transcriptomic remodeling. The ELA-LPS model demonstrates significant autonomic and structural RHF, highlighting the complex link between emphysema exacerbation and HFpEF. Bisoprolol's inability to improve RV dysfunction suggests pulmonary hypertension plays a key role in this mixed heart failure model, mirroring human clinical observations. This underscores the intricate pathophysiology of COPD-HF comorbidity, necessitating multidisciplinary therapies targeting pulmonary vascular, metabolic, and cardiac functions.
P09.09	Chahida Chaami University of Copenhagen	*Exploring the biochemical potency for diabetic failing hearts (HFpEF)*	Heart failure with preserved ejection fraction (HFpEF) accounts for more than half of all cases and is frequently associated with cardiometabolic conditions such as type 2 diabetes mellitus (T2DM). Although the underlying molecular mechanisms remain poorly defined, emerging evidence points to a role for cardiac myosin, the most abundant protein in the heart. In this study, we investigated whether T2DM alters myosin super-relaxed (SRX) state regulation in HFpEF. Left ventricular (LV) tissue was obtained from donor hearts (n=5), HFpEF patients without T2DM (HFpEF_DM-, n=5), and HFpEF patients with T2DM (HFpEF-DM+, n=5). In parallel, LV samples from obese (n=8) and lean (n=8) ZSF1 rats were analyzed. Myosin biochemical SRX was assessed in permeabilized cardiac muscle strips using Mant-ATP chase experiments. HFpEF-DM+ samples exhibited a greater proportion of myosin in the SRX state and significantly prolonged ATP turnover time compared to donor controls. Consistent with these findings, obese ZSF1 rats showed an increased SRX fraction and slower ATP turnover relative to lean controls. These results indicate that HFpEF is associated with altered myosin energetic states, with further shifts toward SRX in the presence of T2DM. Ongoing studies are aimed at identifying the underlying mechanisms, with a particular focus on myosin post-translational modifications. The ZSF1 rat model appears well suited for mechanistic investigation of diabetic HFpEF.
P09.10	Weronika Ficerman-Kuta Nencki Institute of Experimental Biology, Polish Academy of Sciences	*The role of unconventional myosin VI in the differentiation and energy metabolism of cardiac mesenchymal stem/stromal cells*	Unconventional myosin VI (MVI) plays a recognized role in cardiomyopathy (CM) pathogenesis, as evidenced by the development of CM in Snell's waltzer mice (MVI-KO), natural MVI knockouts. Our group previously demonstrated that the observed phenotype is associated with increased cell proliferation in newborn (P0) hearts. Within the heart, cardiac mesenchymal stem/stromal cells (cMSCs) display proliferative capacity and the c-Kit+ population is capable of differentiation into cardiomyocytes, endothelial cells, and vascular smooth muscle cells. Since cardiomyopathy progression is associated with impaired cardiac regeneration and metabolic disturbances, we investigated the role of MVI in cardiac differentiation and energy metabolism of cMSCs isolated from P4 heterozygous (WT) and MVI-KO hearts. Single-cell RNA sequencing of cMSCs identified changes in molecular pathways associated with cell differentiation and cardiac development in MVI-KO cMSCs. In line with these findings, our preliminary data indicated altered cardiomyogenic differentiation potential of MVI-KO cMSCs. Furthermore, functional assessment with the use of Seahorse Mito Stress Test suggested altered mitochondrial respiration in MVI-KO cells. Western Blot analysis of oxidative phosphorylation (OXPHOS) complexes demonstrated a tendency toward elevated levels of complexes II and III in MVI-KO cMSCs. In addition, alterations in the mitochondrial network structure were observed in cardiomyocytes isolated from P6 MVI-KO mice. Taken together, our results indicate that lack of MVI could affect both differentiation-related processes and energy balance, highlighting a multifaceted role of MVI in cardiac physiology. Research was supported by Preludium Bis 3 grant (UMO-2021/43/O/NZ5/02028) and by Miniatura-7 grant (2023/07/X/NZ3/00597) from the National Science Centre, Poland.
P09.11	Natalia Georgiadou University of Leeds	*Understanding heart failure phenotypes: effects on cardiomyocyte organisation*	Skeletal muscle cells experience substantial mechanical load, requiring coordinated regulation of nuclear architecture, chromatin organisation and gene expression. Cell nucleus serves as a mechanosensing platform, allowing for swift adaptation to mechanical stimuli. Impaired mechanical resilience of the nucleus may have severe repercussions for genome integrity and, consequently, for cell survival. While nuclear actin and myosins are emerging as important regulators of chromatin structure and transcription, their roles in muscle cell nuclei remain poorly understood. Myosin VI is an unconventional actin-based motor previously implicated in chromatin organisation and transcriptional regulation in cancer cells. Here, we identify a new nuclear role for myosin VI in myoblasts. Using myosin VI depletion, we show that loss of myosin VI disrupts myonuclear morphology and alters chromatin organisation. These changes are associated with altered epigenetic chromatin marks and impaired transcriptional progression, indicating that myosin VI contributes to the maintenance of a transcriptionally competent nuclear environment. We further assess how myosin VI downregulation affects nuclear stiffness and cellular mechanics, linking chromatin-level changes to mechanoadaptation in myoblasts. Previous studies from our laboratory have shown that loss of myosin VI disrupts myogenic growth, differentiation and muscle metabolism. Current findings extend this role by placing myosin VI within the regulatory network that coordinates chromatin remodelling, gene expression and nuclear organisation. We propose that myosin VI acts as a nuclear organiser in myoblasts, coupling transcriptional regulation with the mechanical demands of muscle cell function.

P09.12	Larissa Hartmann University Ulm /Uniklinik Ulm (Innere Medizin II)	*Pharmacological Inhibition of Valosin-Containing Protein (VCP) Triggers Cardiac Hypertrophy and Heart Failure in Vivo*	Valosin-containing protein (VCP) is an ATPase playing a critical role in various cellular processes, including protein degradation, organelle function, and maintenance of cellular homeostasis. In humans, mutations in the VCP gene are linked to multisystem proteinopathies, neurodegenerative diseases, cancer, and have been associated with cardiac dysfunction and hypertrophic remodeling. However, the specific role of VCP in cardiomyocyte function and cardiac proteostasis remain poorly studied. In this study, we investigated the effects of pharmacological Vcp inhibition in zebrafish embryos using CB-5083, a selectice Vcp ATPase inhibitor. CB-5083-treated embryos developed cardiac and skeletal muscle abnormalities, including myofibrillar disorganization, impaired cardiac function, and locomotor defects, closely resembling CRISPR/Cas9-mediated vcp knockout phenotypes. Early developmental inhibition (24–72 hpf) resulted in severe cardiac dysfunction and increased apoptosis, whereas late developmental inhibition (72–120 hpf) induced ventricular hypertrophy while preserving contractile function, suggesting a developmental stage-dependent effect of Vcp inhibition. Furthermore, Vcp inhibition was associated with markers of disrupted proteostasis, suggesting UPS dysfunction and ER-stress activation. Together, these findings establish a zebrafish model of VCP-related cardiomyopathy, underscore the importance of VCP in cardiac proteostasis and hypertrophic remodeling, and provide novel insights into disease mechanisms and potential therapeutic targets.
P09.13	Bogdan Iorga Hannover Medical School (MHH)	*Primary effects of the HCM-causing myosin mutation G716R on human ventricular myofibril function and modulation by mavacamten*	The primary effects of the hypertrophic cardiomyopathy-causing myosin mutation G716R on sarcomere function are not yet defined. Often HCM is associated with a hypercontractile ventricular phenotype. Therefore, a myosin ATPase inhibitor called mavacamten was developed to attenuate sarcomere-driven contractility. We investigated the effect of this mutation and tested how mavacamten influence force-related parameters in patient and donor (as control) human ventricular myofibrils (hvMFs). Patient-hvMFs and donor-hvMFs developed similar isometric forces at maximal and intermediate calcium activation levels. Kinetics of force re-development and of biphasic relaxation were slower (smaller rate constant ktr, larger half-time of relaxation t1/2rel) in patient-hvMFs compared to donor-hvMFs. Mavacamten (0.6 μM) inhibited force less at saturating than at intermediate [Ca2+] in donor-hvMFs and it was opposite in patient-hvMFs. The drug reduced ktr and t1/2rel in donor-hvMFs, while its effect in patient-hvMFs was smaller for these two parameters. Compared to donor-hvMFs investigated in absence of mavacamten, impairments caused by the mutation on ktr and on t1/2rel persisted in patient-hvMFs also in the presence of the drug. Our results suggest that HCM-causing myosin mutation G716R does not evoke gain-of-function in human ventricular myofibrils, and mavacamten may interfere in a different manner with the mutant myosin than it does with the wild-type myosin isoform.
P09.14	Jan Karchňák, Charles University	*MIRO1 SHAPES HEART'S RESPONSE TO I/R INJURY AND FIBROSIS*	Background & Aims: Miro1 is a GTPase located at the outer mitochondrial membrane. It is responsible for binding mitochondria to cell motors and thus functions in mitochondrial dynamics. Miro1 also plays a role in energy metabolism by facilitating the formation of functional cristae, which is necessary for efficient oxidative phosphorylation. The present study aims to analyse Miro1 role in cardiac ischemic tolerance and fibrosis. Methodology: C57BL/6Gt mice with Miro-1 knockout (KO) and their wild-type (WT) controls (4–5 months old; 12-15 weeks after tamoxifen induction) were used. The sensitivity of male hearts to global ischemia was assessed ex vivo using Langendorff apparatus, the extent of fibrosis was quantified based on histological Sirius Red staining, mitochondrial respiration was measured via oxygraphy (Oroboros O2K), and protein analyses was done using western blot. Results: The analyses showed significantly increased extent of myocardial infarction in Miro-1 KO hearts after ischemia/reperfusion injury ex vivo and exhibited a significantly higher degree of fibrosis. Mitochondrial respiration analyses revealed significant increase in complex I respiration in the male KO group. Surprisingly, there was no effect of Miro-1 deletion on HK1 and GLUT4 expression, but female exhibited a significantly higher expression of GLUT4 transporter than males. Conclusion: Our data suggest that Miro-1 deletion reduces the heart's resistance to ischemia and increasing cardiac fibrosis. There are also expressed differences in oxidative and glycolytic metabolism between male and female hearts. Further studies are needed. This work is funded via AZV project number NW25-02-00459.

P09.15	Xaver Koenig Medical University of Vienna	*IL-6-Dependent Remodelling of Cardiac Ca^{2+} Handling in the C26 Colon Carcinoma Model of Cancer Cachexia*	Cancer cachexia is a multifactorial syndrome characterized by progressive loss of skeletal and cardiac muscle mass, yet the mechanisms underlying cachexia-induced cardiac dysfunction remain poorly understood. We investigated the contribution of tumour-derived IL-6 to cardiac remodelling using the C26 colon carcinoma mouse model and an IL-6 knockdown variant C26shIL6. While tumour growth was comparable between groups, only C26 mice developed cachexia, exhibiting marked body, skeletal muscle, adipose tissue, and heart weight loss. These changes were absent in C26shIL6 mice, confirming a central role for tumour-derived IL-6. Despite preserved systolic function, echocardiography revealed mild diastolic dysfunction in cachectic hearts without evidence of fibrosis, inflammation, or capillary density, suggesting a cardiomyocyte-intrinsic mechanism. Cardiomyocytes from C26 mice displayed cellular atrophy, reduced L-type Ca^{2+} current density, impaired plasma membrane Ca^{2+} extrusion, and enhanced electrically evoked Ca^{2+} transients with accelerated decay, consistent with increased SERCA activity. These alterations were absent in C26shIL6 mice, indicating IL-6 dependence. Proteomic and RNAseq analyses identified changes in cardiac Ca^{2+}-handling at the level of the plasma membrane and sarcoplasmic reticulum. These molecular changes coincided with a metabolic shift from fatty acid oxidation toward glycolysis, suggesting reduced energetic efficiency in cachectic hearts. Elevated circulating IL-6 and soluble IL-6 receptor levels were associated with increased cardiac STAT3 phosphorylation, supporting activation of IL-6 trans-signalling. Together, these findings identify tumour-derived IL-6 as a key driver of cardiomyocyte Ca^{2+} handling defects and metabolic remodelling during cancer cachexia, providing mechanistic insight into cachexia-associated cardiac dysfunction and highlighting IL-6 signalling as a potential therapeutic target.
P09.16	Christopher Lewis University of Copenhagen	*Leveraging AI for the Design of Novel Therapies Targeting Myosin Heavy Chain for the Treatment of Inherited Cardiomyopathies*	The inherited cardiomyopathies hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) both have an incidence rate of 1 in 250 people, making them the most common genetic cardiac conditions in the world. Despite this, medical therapies for their treatment have been elusive, with only one approved drug for HCM and zero for DCM. Recent advances in our understanding of the molecular mechanisms of these conditions have demonstrated that the dysregulation of myosin heavy chain (MYH7) resting conformational state is highly prevalent. By utilizing an AI drug discovery platform, we have been able to synthesize novel compounds which are in silico predicted to bind to both MYH7 and sarcomeric accessory proteins in areas of the protein which control its resting conformation. We further test these compounds using high-throughput screening methods to identify hits and further optimize these toward lead molecules. By leveraging AI tools to conduct in silico screening, we have been able to conduct a drug discovery project on a scale previously inaccessible to academic field and advancing our progress towards critical novel treatments for inherited cardiomyopathies.
P09.17	Wolfgang Linke University of Münster	*Selective titin cleavage primarily inhibits cardiac elastic recoil to induce diastolic dysfunction and proliferation of fibroblasts*	Titin, the largest human protein, forms the elastic sarcomeric backbone, providing passive stiffness and length-dependent activation in cardiomyocytes. Whereas titin mutations cause inherited cardiomyopathies, ischemic and chemotherapy-induced injury also provoke proteolytic cleavage of titin's elastic segment ("T2" titin). To test the effects of titin stiffness loss, we selectively cleaved cardiac titin springs in the titin-cleavage mouse model through cardiac-specific expression of tobacco etch virus protease (TEV) via tail-vein injection of AAV9-TEV plasmid, with AAV9-GFP as control. We found that titin cleavage began at day (D)3-4 post-AAV9-TEV injection, reaching ~40% and 55-60% at D6 and D10-13, respectively. On tissue sections, anti-cTEV antibody revealed a mosaic pattern of titin-cleaved and uncleaved cardiomyocytes at a proportion consistent with biochemical results. Multimodal analysis, including cMRI, echocardiography, and microscopy, demonstrated that titin cleavage reduces chamber size and impairs ventricular filling, resulting in a restrictive phenotype and diastolic dysfunction but largely preserved systolic dysfunction already at D6. Mechanical assays of isolated cardiomyocytes revealed diminished restoring forces causing loss of elastic recoil. In-vivo cleavage disrupted junctions, including integrin linkages and connexin-43 gap junctions, widened inter-myocyte space without hypertrophy or hyperplasia, and drove fibroblast activation, followed by fibrosis. Transcriptomic and proteomic analyses confirmed the activation of cell-matrix crosstalk and ECM remodeling, besides cytoskeletal reorganization and enhanced protein quality control. However, compensatory mechanisms failed, causing decompensated heart failure by D13. Thus, proteolytic cleavage of elastic titin perturbs cardiac mechanical homeostasis mainly by impairing elastic recoil and disrupting intra- and intercellular crosslinks, driving diastolic dysfunction, matrix remodeling, and heart disease.
P09.18	Elise Melhedegaard University of Copenhagen	*Altered sarcomeric structure and function in canine myxomatous mitral valve disease*	Myxomatous mitral valve disease (MMVD) is a degenerative condition characterized by mitral valve thickening and loss of its structural integrity. The underlying molecular mechanisms remain unexplored. Hence, in this study, we investigated whether the papillary muscles, related to mitral valves (and their sarcomeres), play a role in the molecular and cellular disease pathogenesis. For that, we isolated thin papillary strips from 26 dogs (16 MMVD, 10 control donors) and ran a combination of targeted proteomics, immunolabeling, X-ray diffraction, and mechanics experiments. We observed a unique and unusual ubiquitination site on actin monomers from the MMVD animals, which, to our knowledge, has not been found in any other studies before. This PTM alteration was accompanied by reduced actin to myosin ratio, shorter thin filament lengths, and decreased interfilament lattice spacing. In parallel, we observed preserved permeabilized maximal force, but increased calcium sensitivity in MMVD-dogs compared to control donors. Overall, these results indicate clear and strong papillary muscle remodeling in the context of MMVD, which is likely to impair valve function over time. These findings warrant the design of a sarcomeric-centered therapy for MMVD.

P09.19	Maya Noureddine University of Birmingham	*Integrated Functional and Structural Analysis of Cardiomyopathy Mechanisms Reveals Protein Destabilisation in an Alpha-actinin-2 Variant*	Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder characterised by diastolic dysfunction, arrhythmias, and sudden cardiac death. HCM is associated with variants in Z-disk proteins, including alpha-actinin-2 (ACTN2), a key protein that cross-links actin filaments. Although previous studies have identified functional consequences of HCM-linked ACTN2 variants, underlying disease mechanisms remain poorly understood. Here, we combined functional and structural approaches to investigate cardiomyopathy mechanisms by studying the previously identified ACTN2 variant, M228T, using induced-pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) and recombinant protein biophysical analyses. Mutant iPSC-CMs showed fetal-gene programme upregulation, with imaging revealing aggregation of ACTN2 and sarcomeric markers. Cells exhibited developmental delays, reduced contractility and lower bioenergetic demand. Optical mapping demonstrated delayed calcium transients and shortened action potentials, consistent with pro-arrhythmic phenotypes. To further investigate these functional changes, biophysical analyses were performed. Actin-binding assays revealed increased binding affinity, while thermolysin proteolytic digestion showed accelerated degradation of M228T. Mutant protein also displayed reduced solubility and thermal stability, consistent with protein destabilisation. Techniques assessing oligomeric state indicated increased aggregation propensity in M228T. Protein destabilisation was further examined in iPSC-CMs, where biochemical fractionation highlighted reduced protein levels. Pharmacological inhibition of the protein degradation pathways, ubiquitin-proteosome system (UPS) and autophagy-lysosomal pathway (ALP), revealed altered protein degradation dynamics following ALP inhibition in M228T cells. Mutant cells also revealed upregulation of autophagy markers, suggesting dysregulation. Overall, our multipronged analyses identified protein destabilisation as a central mechanism by which M228T impairs protein structure and function. Finally, autophagy dysregulation emerged as a potential target for future investigations in HCM.
P09.20	Marta Rocha Medical University of Vienna	*Impaired cardiac function, stress-intolerance, protein aggregation, and intercalated disc derangement: hallmarks of plectin-related cardiomyopathy*	Plectin, a versatile cytolinker protein, is essential for cellular integrity and function in various tissues, including the heart. Mutations in the human plectin gene (PLEC) cause epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) and are associated with several cardiac disease manifestations. However, plectin's role in normal and diseased hearts remains poorly understood. To understand the consequences of plectin deficiency in the heart, cardiac tissue and/or isolated cardiomyocytes from conditional, striated muscle-specific plectin knockout (MCK-Cre/cKO) mice and wild-type controls was analysed using morphological, ultrastructural and biochemical approaches. Cardiac function was evaluated by echocardiography and impact of mechanical stress was accessed through an isolated working heart approach with a pump function protocol. Plectin ablation led to marked alterations of the desmin intermediate filament (IF) network, including loss of its cross-striated pattern at Z-discs and accumulation of desmin-positive protein aggregates. Plectin-deficient hearts further displayed increased fibrosis as well as aberrant and fragmented intercalated discs (IDs). Functionally, MCK-Cre/cKO mice exhibited impaired left ventricular function, with significantly reduced ejection fraction, fractional shortening, and cardiac output compared to wild-type controls. Under mechanical stress, isolated plectin-deficient hearts exhibited a significantly faster decay in dp/dtmax and dp/dtmin, and reached null aortic flow at a significantly lower afterload, indicating reduced cardiac stress tolerance. Together, our findings demonstrate that plectin is critical for maintaining cardiac cytoarchitecture and mechanical function, and elucidate the molecular aspects underlying the observed pathologies in plectin-related diseases.
P09.21	Tim Scholz Hannover Medical School	*The Hypertrophic Cardiomyopathy associated myosin mutation R723G increases myosin motor domain stiffness*	With a prevalence of 1:500 to 1:200 in the human population hypertrophic cardiomyopathy (HCM) is the most frequent hereditary cardiac disease. Mutations in several sarcomeric proteins such as the frequently affected cardiac myosin binding protein-C and β-myosin heavy chain have been shown to be associated with HCM. Reports of an increased rigor stiffness of slow-twitch muscle fibers of HCM patients with the mutation R723G located in the converter of the β-myosin motor domain suggested an increased myosin molecule stiffness. However, in mainly heterozygous patients, wild type and mutated myosin coexist, thus, complicating the functional characterization of mutation effects. This raised the question whether we could test for possible mutation-associated changes in β-myosin molecular stiffness directly, i.e., at the level of individual, patient-derived myosin molecules. We investigated the interaction of individual β-myosin molecules with actin filaments in vitro in a three-bead optical trap approach. In these experiments, individual actomyosin interactions can be recorded and the stiffness of the connecting myosin molecule can be determined. We found that the HCM-associated mutation R723G enhanced the molecular stiffness of both, patient-derived slow skeletal myosin motor domains and full-length β-cardiac myosin molecules. Both rigor stiffness as well as the stiffness of the ADP state of R723G β-cardiac myosin molecules were increased compared to donor myosin. This confirms and extends previous studies on slow-twitch muscle fibers from R723G HCM patients, on expressed artificial truncated myosin constructs and structural simulations. We conclude that increased stiffness of β-myosin molecules may contribute to the development of R723G-associated HCM symptoms.

P09.22	Yimin Shen James Black Centre 125 Coldharbour Lane	*PSAT1 mediates cardiac fibroblast phenotypic switching via metabolic remodeling in pressure overload induced myocardial fibrosis*	Cardiovascular disease is the leading cause of death worldwide, with ventricular remodeling representing a key pathological process in heart failure, particularly characterized by myocardial fibrosis. Abnormal activation of cardiac fibroblasts (CFs) and metabolic remodeling are critical contributors to the progression of myocardial fibrosis. We previously established a pressure overload-induced myocardial fibrosis mouse model using transverse aortic constriction (TAC). Metabolomic analysis at 7 days post-TAC revealed decreased levels of glucose, α-ketoglutarate (α-KG) and pyruvate, whereas serine biosynthesis was significantly upregulated compared to the Sham group. RNA-seq further demonstrated significant upregulation of phosphoserine aminotransferase 1 (Psat1) expression after TAC. PSAT1 is a key enzyme in the serine biosynthesis pathway and also catalyzes the conversion of glutamate to α-KG. We hypothesize that PSAT1 promotes cardiac fibroblast activation through metabolic reprogramming. This study aims to investigate the underlying mechanism of PSAT1 in myocardial fibrosis. Mechanical stretch significantly increased PSAT1 and cardiac fibrosis markers expression in primary neonatal mouse cardiac fibroblasts. Knockdown of Psat1 altered the expression of genes enriched in pathways associated with cell adhesion, cytoskeletal organization and extracellular matrix formation, suggesting that PSAT1 mediates cardiac fibroblast differentiation under mechanical stress. Furthermore, metabolomic analysis indicated that PSAT1 regulated fibroblast function through modulation of oxidative phosphorylation. In summary, PSAT1 expression is significantly upregulated in response to mechanical stress. Activated PSAT1 promotes oxidative phosphorylation in cardiac fibroblasts, thereby facilitating the phenotypic transition of fibroblasts into myofibroblasts. These findings identify PSAT1 as a metabolic regulator of cardiac fibroblast activation and a potential therapeutic target for myocardial fibrosis.
P09.23	Egor Shepelev Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences	*Cardiomyopathy-associated variants R105H and D254N affect Tpm1.1 structure and function*	The Tpm1.1 isoform is the predominant tropomyosin isoform expressed in the heart, and mutations in the gene encoding this protein are often associated with inherited cardiomyopathies. We examined the effects of the disease-associated R105H and D254N mutations on the properties of Tpm1.1 molecule. To investigate protein stability, we used differential scanning calorimetry. The mutations had opposing effects: R105H destabilized the N-terminal region of the Tpm molecule, whereas D254N enhanced the stability of the C-terminal region. According to viscosity measurements, R105H promoted, while D254N impaired, continuous strand formation by Tpm1.1 (0.357 ± 0.003 and 0.2703 ± 0.002 vs. 0.318 ± 0.003 mPa·s for WT, respectively). In addition, D254N markedly reduced the affinity of Tpm1.1 for F-actin in cosedimentation experiments (K50% = 4.37 ± 0.11 μM vs. 2.71 ± 0.08 μM for WT). Changes in the regulatory properties of Tpm were assessed using an in vitro motility assay. The D254N variant increased the maximum sliding velocity of thin filaments by 30% and enhanced the Ca^{2+} sensitivity of actomyosin interaction by 0.61 pCa units, whereas R105H only decreased pCa50 by 0.28 units. We also found that R105H, and to a lesser extent D254N, promoted Tpm aggregation. These results demonstrate that distinct alterations in Tpm properties can lead to the same pathological outcome—hypertrophic cardiomyopathy. Further studies are required to elucidate the relationship between altered actin affinity and the regulatory properties of the D254N variant of Tpm1.1, and to determine whether protein aggregation is the sole pathogenic mechanism underlying the effects of R105H.
P09.24	Andreu Slushchev Russia, Moscow, Research Centre of Biotechnology, Russian Academy of Sciences	*Pseudophosphorylation modulates the functional properties of cardiomyopathy-associated tropomyosin mutants.*	LVNC (left ventricular noncompaction) is a severe cardiac disorder associated with mutations in genes encoding sarcomeric proteins. One such gene is TPM1, which encodes Tpm1.1, the predominant tropomyosin isoform in heart. One potential strategy for counteracting the effects of these mutations involves Tpm phosphorylation. We generated recombinant Tpm variants carrying the S283D substitution and investigated the effects of pseudophosphorylation on the properties of LVNC-associated mutant Tpms. Differential scanning calorimetry demonstrated that the introduction of the S283D substitution did not affect Tpm stability. At the same time, viscometry data showed that pseudophosphorylation increased the viscosity of mutant Tpm solutions. The most pronounced effect of the S283D substitution was observed for the I130V and D219V mutants, for which viscosity increased approximately 2.5–4-fold. According to cosedimentation assays, the S283D substitution did not alter the actin-binding properties of WT Tpm; however, it significantly affected the affinity of LVNC-associated mutants for actin. Data obtained using the in vitro motility assay demonstrated that pseudophosphorylation induced by the S283D substitution does not exert a universal effect on Tpm function. The most pronounced effects were observed for the D159N variant. This mutation reduced the maximal sliding velocity by nearly twofold and simultaneously decreased calcium sensitivity. Introduction of the S283D substitution into this variant resulted in an almost complete restoration of both parameters. The S283D substitution acts as a factor capable of selectively modulating the structural and functional properties of cardiomyopathy-associated Tpm variants. This study was supported by the Russian Science Foundation (Grant No. 22-14-00059-P).

P09.25	Gemma Toerien-Howie Cardiff University	*Investigating anti-arrhythmic properties of the myofilament desensitiser mavacamten in a model of hypertrophic cardiomyopathy*	Hypertrophic cardiomyopathy (HCM) is a leading cause of sudden cardiac death in young adults, affecting 1 in 500 people in the UK. Sudden death typically results from fatal arrhythmias arising from structural and cellular alterations, caused by mutations in sarcomeric proteins. These mutations increase myofilament Ca2+ sensitivity, strengthening contractions but disrupting Ca2+ handling. In a HCM mouse model, pretreatment with myofilament inhibitor Blebbistatin reduced myofilament Ca2+ sensitivity and provided anti-arrhythmic effects in HCM hearts(Doi:10.1172/JCI36642). Mavacamten, a recently approved therapy for HCM, directly targets cardiac sarcomere proteins by reducing actin-myosin cross-bridge formation. This reduces the contractile strength and myofilament Ca2+ sensitivity; providing relief from heart failure-like symptoms. We hypothesised that Mavacamten could decrease the risk of arrhythmic events in HCM, similar to Blebbistatin. To study this, we used a TPM1-mutant HCM mouse model which expresses a point mutation in α-tropomyosin. Monophasic action potentials (AP) recorded at the apex of whole hearts ex vivo, showed a reduced repolarisation gradient (70-90%) in sinus rhythm compared to wild-type (WT) mice. This decrease in repolarisation gradient occasionally allowed for ventricular ectopic beats (VEBs) to propagate. These extra beats, when severe, led to sustained arrhythmias. However, when treated with Mavacamten the decreased repolarisation gradient and presence of VEBs was reversed. In untreated HCM cells loaded with Fluo-4AM, duration of the Ca2+ transient increased compared to WT, whereas Mavacamten reversed the effects of HCM. Resulting changes in both Ca2+ transient and AP morphology suggests in addition to current applications of Mavacamten, it could provide further anti-arrhythmic effects.
P09.26	Tianbang Wang Hannover Medical School	*Probing the Functional Effect of cardiac modulators on the Actomyosin Crossbridge Cycle using Optical Tweezer-based Measurements*	Omecamtiv mecarbil (OM), a myotrope, improves systolic function in patients with heart failure exhibiting a reduced ejection fraction (HFrEF). While OM has been shown to improve the contractile performance by destabilizing the auto-inhibited conformation, the mechanistic details of how OM modulates individual steps of the actomyosin cross-bridge cycle are not fully understood. We employed high-resolution optical-trap based single-molecule assays to assess OM's effects on human ß-cardiac myosin isolated from human donor tissue samples - ensuring direct physiological relevance. Investigation of the mechanical force generation, using histogram-shift method revealed that the power stroke was almost absent in the presence of OM. Ensemble-average analysis confirmed that both first powerstroke associated to Pi release and second power stroke associated with ADP release was near-zero, indicating blocked force generation. Notably, increasing [ATP] did not decrease the actomyosin interaction lifetime, indicating impaired ATP-induced AM dissociation. A lower IC50 estimated using actin filament gliding assay suggested high affinity of the drug towards human cardiac myosin. We further compared the effects of OM with Mavacamten (Mava), a cardiac myosin inhibitor that is used to treat symptomatic obstructive hypertrophic cardiomyopathy (oHCM). The two drugs - OM and Mava - binds to the same binding pocket between the converter region and N-terminus of myosin but exhibit diverse effects on the contractile force and the diastolic function. I will present the mechanistic effects that explain how OM and Mava differentially regulate distinct conformational states of myosin, leading to their distinct impact on the overall cardiac performance.

S10 - Innovations in Human Disease Models

Invited speaker	Francesco Saverio Tedesco UCL/Francis Crick Institute, London	*Engineering human skeletal myogenesis foradvanced disease and therapy modelling*	Muscular dystrophies are severe inherited neuromuscular disorders characterised by progressive muscle wasting, loss of mobility, and premature death, for which effective treatments remain unavailable. A major barrier to therapeutic development is the lack of robust human-relevant models that faithfully recapitulate disease pathology. To address this challenge, we engineered three-dimensional (3D) human skeletal muscle models capable of reproducing structural and functional defects affecting key tissue compartments implicated in muscular dystrophies, including the sarcolemma, nuclear envelope, and extracellular matrix (ECM). These tissues were generated from primary human myoblasts and fibroblasts, as well as induced pluripotent stem cells (iPSCs) differentiated into myogenic, neural, and vascular progenitors. Combined with biocompatible biomaterials, these cells formed aligned, contractile muscle constructs incorporating ECM, vascular networks, and motor neurons. The resulting engineered muscles recapitulated key morphological and functional features of native human skeletal muscle, providing a high-fidelity platform for modelling severe congenital muscular dystrophies. Using this approach, we reproduced disease-associated phenotypes such as nuclear envelope abnormalities in laminopathies. To further demonstrate the versatility of the platform, we developed additional 3D models of severe congenital muscular dystrophies and myopathies, identifying robust morphological and functional readouts with translational potential for therapeutic development. Finally, we extended this technology to evaluate clinically relevant genetic therapies. Together, these studies establish a versatile human platform for disease modelling, therapeutic testing, and precision medicine in neuromuscular disorders.

Invited speaker	Lavinia Ginistrelli IMBA, Vienna	*Dissecting the human fetal cardiac injury response using cardioids*	Human cardiac injury responses are governed by dynamic interacting processes that are difficult to resolve. While regeneration in the adult heart is limited, fetal mammalian hearts regenerate through coordinated remodeling and proliferation supported by a pro-regenerative immune environment, hyaluronan-rich extracellular matrix (ECM), and immature cardiomyocytes, including trabecular subtypes. Here, we establish a modular human cardioid injury platform to dissect these interactions. By developing trabecular cardioids and comparing them to left ventricular cardioids, we show that macrophage infiltration is determined not solely by their subtype but also by cardiomyocytes identity. Upon injury, anti-inflammatory macrophages specifically migrate to the lesion, clear debris and promote ECM deposition. We further demonstrate that trabecular cardioids display distinct injury response compared to left ventricular cardioids, showing reduced injury size. This injury response is ECM-dependent and characterized by cytoskeletal remodeling and injury-induced cardiomyocyte proliferation, involving YAP and WNT signaling. Finally, we tested whether we could promote injury repair also in left ventricular cardioids using the signaling involved in trabecular injury response. While exogenous WNT induces only cardiomyocyte proliferation, YAP activation is sufficient to promote proliferation and repair. These findings define coordinated immune-ECM-cardiomyocyte interactions governing human regenerative competence and establish cardioids as a platform to mechanistically resolve remodeling and proliferative components of cardiac repair.
Contributed talk	Ana Isabel Antunes Gonçalves Medical University of Vienna	*Blocking IL-6 Reverses Multi-Organ Dysfunction in Cancer Cachexia*	Introduction/Background: Cachexia is an inflammatory catabolic syndrome causing loss of skeletal muscle and fat. It is common in end-stage heart failure but its effects on cardiac and vascular function in cancer associated cachexia still remain unclear. Both conditions are linked through elevated IL6 levels that are strongly associated poorer clinical outcomes, highlighting IL-6 as a potential shared therapeutic target. Methods: Male BALB/c mice (10–12 weeks old) were injected subcutaneously in the right flank with 1×103 cells/µL mouse colon-26 carcinoma (C26) or PBS as control (n=8, all groups). On day 3, C26 mice were randomized to receive IL-6 antibody (AB; Bioxcell) or PBS every second day. Cardiac function, grip strength, and body composition were assessed. Multi-organ molecular fingerprints were assessed by different approaches. Results: C26 tumour-bearing mice show body weight loss >5% (22.18 vs 26.45 g), decreased grip strength (73.66 vs 99.96 g), systemic inflammation, and anaemia. C26 mice exhibited cardiac edema, cardiomyocyte atrophy, endothelial dysfunction, and renal metabolic impairment, including reduced mitochondrial OXPHOS capacity (22.46 vs 49.22 pmol/s·mL). Endothelial dysfunction was reflected by impaired NO-mediated relaxation (ACh EC50: −7.33 vs −6.91, $p < 0.001$). These alterations were partially reversed by anti-IL-6 treatment. Conclusion: These findings indicate that targeting IL-6 markedly improves the cachectic phenotype and enhances cardiac and renal function, metabolism, and endothelial function, supporting IL-6 inhibition as a therapeutic strategy for cancer-associated cachexia and cardiometabolic dysfunction. Funding : This study was supported by FWF Austria (PAT 1863824)
Contributed talk	Wolfgang Linke University of Münster	*Selective titin cleavage primarily inhibits cardiac elastic recoil to induce diastolic dysfunction and proliferation of fibroblasts*	Titin, the largest human protein, forms the elastic sarcomeric backbone, providing passive stiffness and length-dependent activation in cardiomyocytes. Whereas titin mutations cause inherited cardiomyopathies, ischemic and chemotherapy-induced injury also provoke proteolytic cleavage of titin's elastic segment ("T2" titin). To test the effects of titin stiffness loss, we selectively cleaved cardiac titin springs in the titin-cleavage mouse model through cardiac-specific expression of tobacco etch virus protease (TEV) via tail-vein injection of AAV9-TEV plasmid, with AAV9-GFP as control. We found that titin cleavage began at day (D)3-4 post-AAV9-TEV injection, reaching ~40% and 55-60% at D6 and D10-13, respectively. On tissue sections, anti-cTEV antibody revealed a mosaic pattern of titin-cleaved and uncleaved cardiomyocytes at a proportion consistent with biochemical results. Multimodal analysis, including cMRI, echocardiography, and microscopy, demonstrated that titin cleavage reduces chamber size and impairs ventricular filling, resulting in a restrictive phenotype and diastolic dysfunction but largely preserved systolic dysfunction already at D6. Mechanical assays of isolated cardiomyocytes revealed diminished restoring forces causing loss of elastic recoil. In-vivo cleavage disrupted junctions, including integrin linkages and connexin-43 gap junctions, widened inter-myocyte space without hypertrophy or hyperplasia, and drove fibroblast activation, followed by fibrosis. Transcriptomic and proteomic analyses confirmed the activation of cell-matrix crosstalk and ECM remodeling, besides cytoskeletal reorganization and enhanced protein quality control. However, compensatory mechanisms failed, causing decompensated heart failure by D13. Thus, proteolytic cleavage of elastic titin perturbs cardiac mechanical homeostasis mainly by impairing elastic recoil and disrupting intra- and intercellular crosslinks, driving diastolic dysfunction, matrix remodeling, and heart disease.

P10.01	Cristian Romeo Revnic Umf Carol Davila	*THE IMPACT OF ISCHEMIA REPERFUSSION UPON YOUNG AND OLD RAT HEART PHYSIOLOGY AND ULTRASTRUCTURE*	To evaluate the effect of stress conditions imposed by45minutes ischemia followed by 60 minutes reperfusion on isolated rat heart of different ages ,upon physiological parameters: heart rate(HR),coronary flow (CF)and left ventricle developed pressure (LVDP) ,ultrastructure and to assay ventricular cells for apoptosis .12 female Wistar rats aged 6 and respectively 32 month old were anesthetized i.p. with Na Phenobarbital 60 mg/ kg body weight and Heparin 300U i.p. After abolishment of reflexes the hearts were excised and placed in ice cold perfusion medium and quikly mounted at a constant pressure (75 mmHg)in Langendorff perfusion system .Physiological parameters: , (H.R),(C F.) and LVPD .were evaluated following ischemia reperfusion and TACS Apoptotic DNA Laddering kit was used to assay heart cells for apoptosis after 60 minutes reperfusion. Electron microscopy (EM) examinations of rat heart ultrastructure were performed following ischemia-reperfusion. There is a constant decrease in HR during reperfusion in both groups, at the end higher values were recorded than in young.(CF) is variable in time in aging rats in comparison with young ones where is a slow decrease during experiment. LVPD is net elevated in aging rats but, with fluctuations in time .Internucleosomal fragmentation and DNA laddering displaying has been observed (EM) examinations pointed out greater damage in old hearts in terms of myofiber tears, mitochondrial and sarcoplasmic reticulum disruption. It is possible that due to aging process the rat heart is trying to cope with new condition imposed by experiment by increasing the heart rate in order to pump the fluid.
P10.02	Fleur Van Brakel Amsterdam UMC	*The effect of non-invasive ventilation on the respiratory muscle pump in end-stage COPD*	To breathe effectively, one requires not only intact lungs but also strong respiratory muscles, known as "the respiratory muscle pump", to move air in and out. In 25% of patients with chronic obstructive pulmonary disease (COPD), the respiratory muscle pump fails, resulting in chronic hypercapnic respiratory failure (CHRF). A substantial and growing proportion of COPD patients with CHRF now receive chronic nocturnal non-invasive ventilation (NIV) at home, which may increases health-related quality of life. However, not all patients experience benefits. In fact, over time patients can become progressively more reliant on NIV, which can rapidly deteriorate the physical health. In a very selected group of COPD patients, lung transplantation (LTx) is offered. In cases where NIV is used as a "bridge to lung transplant," the decline in physical health may result in removal from the transplant waiting list, or, if transplantation occurs, a challenging and prolonged postoperative recovery period, and prolonged hospital admittance. We propose a novel concept in which these clinical observations are caused by chronic NIV-induced injury of the respiratory muscle pump. We have previously shown that invasive mechanical ventilation rapidly (within 2-3 days) causes respiratory muscle pump dysfunction, contributing to weaning failure (PMID: 39083588). Moreover NIV limits patient triggering of a breath, rendering the respiratory muscles inactive and thus highly susceptible to atrophy and dysfunction (PMID: 39137747). Pilot data indicates that diaphragm myofibers isolated from biopsies obtained during LTx have a severely impaired force response to Ca2+ in COPD patients compared to control patients, supporting our hypothesis.
P10.03	Carlos Acosta Nencki Institute of Experimental Biology	*MK5 is required Skeletal Muscle adaptations to Exercise: a special focus on Sarcopenia*	Skeletal muscle (SKM) is a major site of nutrient storage, energy expenditure, and locomotion, making it essential for human health. Obesity, particularly in older adults, predisposes individuals to and worsens several diseases, including diabetes, cancer, and musculoskeletal disorders such as sarcopenia and dynapenia. These conditions increase the risk of falls and fractures and contribute to reduced quality of life, loss of independence, and mortality. Sarcopenia is characterized by reduced muscle quantity and quality. The complex mitochondrial network of SKM is markedly altered in both obesity and sarcopenia; therefore, therapies aimed at restoring mitochondrial function may represent an important strategy to prevent or ameliorate sarco-obesity. We previously demonstrated that the complex formed by Extracellular Signal-Regulated Kinase 3 (ERK3) and MAP Kinase-Activated Protein Kinase 5 (MK5) regulates energy expenditure and mitochondrial function in adipose and skeletal muscle tissues. To further investigate its role in aging and muscle physiology, we developed gain- and loss-of-function mouse models with MK5 overexpression or SKM-specific MK5 deletion, together with primary cells for in vitro studies. Our results show that young MK5-deficient mice exhibit reduced body weight gain and weaker grip strength. With aging, this phenotype becomes more pronounced, as MK5-deficient mice display impaired oxidative and endurance adaptations to chronic endurance exercise (CEE), muscle atrophy, and reduced metabolic flexibility. Conversely, MK5-overexpressing mice show greater body weight gain, stronger grip strength, and increased skeletal muscle mass after CEE. Fatigue resistance also improved significantly after training, with greater reliance on oxidative metabolism during activity.

P10.04	Lorenza Brocca University of Pavia	*Oxidative stress and proteostatic impairment characterize COPD skeletal muscle without a detectable inflammatory cytokine response*	Chronic obstructive pulmonary disease (COPD) is associated with exercise intolerance resulting not only from pulmonary limitations, but also from peripheral skeletal muscle dysfunction. Among peripheral factors, mitochondrial and metabolic impairments are considered major contributors and may be promoted by chronic inflammation. Although systemic inflammation is a common feature of COPD, evidence for local skeletal muscle inflammation remains inconsistent. Therefore, this study aimed to investigate skeletal muscle inflammation and its potential contribution to mitochondrial dysfunction and muscle atrophy in COPD. Seven COPD patients (70±4 years; FEV1= 50.4±7.9% predicted, severe-to-very severe airflow obstruction) and 5 healthy controls underwent vastus lateralis muscle biopsy. COPD patients showed preserved single muscle fiber Cross-Sectional-Area and reduced capillary density. Elevated pIKKαβ and pNF-κBp65 levels indicated NF-κB pathway activation, although no increases in NF-kB-transcribed cytokines were detected. Significant increases in NRF2 and carbonylated protein accumulation were observed alongside upregulation of MuRF1 and Atrogin-1 and impaired autophagic flux, indicated by elevated Beclin-1, LC3BII/LC3BI ratio, and p62 protein without changes in p62 mRNA. Increased pAMPK and decreased PGC-1α levels suggested energetic stress and impaired mitochondrial biogenesis, despite unchanged mitochondrial content, dynamics, mitochondrial respiratory chain complexes and ex-vivo mitochondrial respiration. These findings suggest that NF-κB activation in COPD skeletal muscle is not associated with a detectable local inflammatory cytokine response and that mitochondrial function and muscle fiber size appear relatively preserved despite evidence of energetic and redox stress. The accumulation of oxidized proteins, alongside impaired autophagic flux, suggests compromised muscle proteostasis, with potential consequences for muscle function in COPD patients.
P10.05	Claude Collet INRAE	*DIAMIDE INSECTICIDES INTERFERE WITH CALCIUM HOMEOSTASIS IN MOUSE AND HONEY BEE MUSCLE CELLS*	Synthetic insecticides have been clearly identified as a major driver of a worrying worldwide decline in insect diversity. The global market sales and field usage of diamides, a recent neurotoxic insecticide family increased substantially in Europe. In vitro, anthranilic and phthalic diamides disrupt intracellular calcium homeostasis in peripheral neurons, skeletal muscle cells and cardiomyocytes of the honey bee Apis mellifera. Calcium conductances are also decreased at the plasma membrane of central neurons and muscle cells exposed to diamides. At the tissular level, these insecticides compromise cardiac rhythmicity and contraction kinetics as well as force production from leg muscles of bees. At high doses, compatible with field recommendations, they are lethal to bees and different toxicity levels were demonstrated, depending on exposed body parts. These results call into question the relevance and accuracy of mandatory toxicological methods in their approval process. At low doses, a single contact exposure modifies bees behaviour in a persistent manner, which may create a durable vulnerability in the field. These long-lasting behavioural deficits may be ascribed partially to the multiple toxic effects observed in the heart, muscles and peripheral and central nervous systems, as well as other tissues where calcium is involved in the modulation of vital functions. Diamides also alter calcium homeostasis in skeletal muscle fibers isolated from wild-type mice and mice carrying a mutation in the ryanodine receptor. This may necessitate a reassessment of their toxicity in a wide range of pathological contexts.
P10.06	Alexandra Dorn Amsterdam UMC	*Hypercontractility at the sarcomere level contributes to muscle stiffness in patients harboring a novel variant in TPM3*	Variants in the TPM3 gene encoding α-tropomyosin-3 have been linked to congenital myopathies characterized by muscle weakness. Here, we report two related patients, a father (P1) and daughter (P2), harboring a novel missense mutation c.289G>A (p.Glu97Lys) in TPM3, resulting in muscle stiffness. Histological characteristics of P1 revealed rods and cores consistent with core-rod myopathies. Electron microscopy images revealed heavy deposits of z-line material and hypertrophic fibers. To test whether this novel TPM3 variant affects sarcomere function, we studied the contractile performance of permeabilized myofibers isolated from both patients biopsies. The results obtained from the permeabilized myofibers isolated from P1 showed that the force normalized to cross-sectional area was decreased in both slow- and fast-twitch fibers compared to controls. In both fiber types, the calcium sensitivity of force generation was increased, and the relaxation kinetics were slower compared to controls. Results obtained from the biopsy of P2 showed no differences in normalized force compared to control myofibers. The calcium sensitivity of force and the relaxation kinetics were affected only in the fast-twitch fibers. These findings indicate that the changes at the sarcomere level (e.g. increased calcium-sensitivity of force generation and impaired relaxation kinetics), could contribute to the hypercontractile phenotype experienced by patients with the novel TPM3 c.289G>A variant. The heterogeneity of the results obtained from both patients could be caused by the age difference between the patients and the disease progression. This study provides evidence for the pathogenicity of the TPM3 c.289G>A variant and broadens the knowledge on TPM3-related myopathies.

P10.07	Karla P. Garcia-Pelagio Universidad Nacional Autonoma de Mexico	*Pelvic radiotherapy induces musculoskeletal dysfunction in geriatric rats*	Radiotherapy (RT) is an effective treatment for cancer; however, the musculoskeletal system is inevitably exposed to radiation during treatment leading to adverse structural and functional alterations. There are currently no established preventive or therapeutic strategies to mitigate these effects. Therefore, preclinical models are essential for investigating musculoskeletal injury. Geriatric ovariectomized (OVX) Wistar rats were irradiated in the pelvic region following a fractionation scheme of 4 Gy (2 Gy × 2 fractions). Muscle-specific force was evaluated in situ in the vastus lateralis (VL) and tibialis anterior (TA) muscles. Bone microarchitecture of the hip and femoral head was analyzed using micro-computed tomography. To correlate muscle structure and function, histological and ultrastructural analyses were performed. Biomechanical properties of the sarcolemma were assessed with elastimetry. Analyses were done in control, OVX, and OVX plus irradiated (OVX+irr) groups (n=7 per/group) one day after irradiation. A significant reduction in muscle strength was observed in the VL muscle of OVX+irr rats, with force decreasing up to 3-fold compared with control and OVX groups. Bone microarchitecture was also markedly deteriorated in OVX+irr animals, including reductions in volumetric bone density, trabecular thickness, and cortical density. Histological and ultrastructural analyses revealed alterations in the contractile apparatus, sarcolemma, and extracellular matrix in both OVX and OVX+irr muscles. Overall, irradiation negatively affected muscle function, bone quality, and tissue structural integrity. RT remains an indispensable cancer treatment modality but these findings highlight the importance of minimizing collateral damage to the musculoskeletal system to preserve functionality and promote recovery following treatment.
P10.08	Romane Idoux PGNM-INMG	*A Bethlem myopathy zebrafish model reveals CaV1.1 as the missing link between collagen VI deficiency and muscle dysfunction*	Bethlem myopathy (BM) is an incurable muscle disease characterized by progressive muscle weakness and joint contractures. BM is caused by mutations in genes encoding collagen VI (ColVI), an extracellular matrix protein produced by muscle interstitial fibroblasts. A major unresolved question is how defects in ColVI present outside muscle fibers lead to dysfunction within the fibers. In this study, we investigated excitation–contraction coupling in isolated fast skeletal muscle fibers from one-year-old zebrafish carrying an exon-skipping mutation (col6a1Δex14) that is the most frequently found in BM patients. Mutant fish showed an age-dependent loss of ColVI deposition, associated with basement membrane disorganization and intracellular accumulation of ColVI. Muscle action potentials were unchanged compared with wild-type fish. However, the function of dihydropyridine receptors (DHPRs), which control calcium release from the sarcoplasmic reticulum (SR), was altered in mutant fibers. DHPR charge movements were reduced, and their voltage dependence was shifted toward more negative membrane potentials. As a result, calcium release was also shifted toward more negative membrane potentials, promoting an elevated SR calcium leak at rest. This was evidenced by higher calcium spark activity and enhanced calcium release during prolonged low-level depolarizations. In addition, DHPR α1S subunits were abnormally distributed within the T-tubule network of mutant fibers. Altogether, these alterations result in reduced muscle force and impaired swimming performance. These findings suggest that ColVI deficiency alters DHPR function, promoting a pathogenic calcium leak from the SR. DHPRs may therefore represent the missing link through which extracellular matrix defects trigger intracellular muscle dysfunction in BM.
P10.09	Per Harald Jonson Folkhälsan	*SUMOylation studies on SMPX*	Small Muscle Protein X-linked (SMPX) is a small (9 kDa) proline-rich protein predominantly expressed in skeletal muscle, particularly in slow fibres, and in the heart. Missense variants in SMPX cause distal myopathy, whereas loss-of-function cause hearing loss. While SMPX has been linked to actin cytoskeletal dynamics, the molecular functions of SMPX in muscle is not fully characterized. To understand the functions of SMPX, a yeast two-hybrid interaction screen was performed against a skeletal muscle cDNA library. Putative interactors included components of the SUMO (Small Ubiquitin-like Modifier) conjugation machinery, namely the SUMO E2 ligase UBE2I (UBC9) and the E3 ligases PIAS1, PIAS2, and PIAS3, suggesting that SMPX is linked to SUMOylation or SUMOylated itself. Coexpression experiments of SMPX, SUMO ligases and wild-type or deconjugation-deficient SUMO constructs confirmed that SMPX can be modified with both SUMO1 and SUMO2. While SUMO1 conjugation can be mediated by PIAS2, SUMO2 conjugation appears to depend on other E3 ligase(s). Interestingly, SUMO coexpression dramatically increased the expression level of SMPX, suggesting that SUMOylation modifies SMPX turnover. Using site-directed mutagenesis, we mapped the main SUMO1 conjugation site to residue K77 of SMPX, situated within a consensus SUMOylation motif, and identified additional residues (K81 and K85) that can be SUMOylated at least in an overexpression setup. Further studies are required to determine the importance of SUMOylation for SMPX function and possible implications on the disease mechanisms of SMPX myopathy. We are characterizing the impact of myopathy-causing variants on SMPX SUMOylation, and how SUMOylation affects SMPX turnover, solubility and localization.

P10.10	Abraha Kahsay Umea University	*Role of Fhl5 in Extraocular muscle resistance to muscular dystrophies*	Muscular dystrophies are a heterogeneous group of diseases leading to muscle atrophy and premature death. Remarkably, the extraocular muscles (EOMs) are spared across muscular dystrophies despite sharing the same gene defect that is causing dystrophy in the other muscles. This resistance suggests that EOMs withhold therapeutic potential; however, the basis behind their resistance remains unknown. RNA sequencing analysis comparing zebrafish EOMs and trunk muscle identified a set of genes highly enriched in EOMs, among which Four and a half LIM domain 5 (FHL5) was one of the most significantly upregulated candidates. To investigate the potential role in EOM resistance, we characterized the spatial and temporal expression of fhl5 in zebrafish and examined its function using both knockout and overexpression approaches in a dystrophic zebrafish model. Expression analysis revealed that fhl5 is specifically expressed in the EOMs and pharyngal muscles. However, RT-PCR detected fhl5 transcripts as early as 24 hpf, prior to EOM development, suggesting a role in early muscle development. Furthermore, fhl5 expression persisted into adulthood, indicating sustained activity throughout development. Preliminary characterization of fhl5 knockout zebrafish revealed no effect on survival of the fish. Stable knockout lines are currently being established to enable detailed investigation of potential phenotypes related to EOM structure and function. In parallel, fhl5 is being overexpressed under a muscle specific promoter in a dystrophic zebrafish model, and its effect on disease pathology is currently under investigation. This study will clarify whether Fhl5 contributes to the intrinsic resistance of EOMs to muscular dystrophy.
P10.11	Tom Kerkhoff Amsterdam UMC	*The effects of serum, containing myositis-specific autoantibodies, on muscle fiber contractility in Immune-Mediated Necrotizing Myopathy*	Background – Immune-mediated necrotizing myopathy (IMNM) is the most severe myositis subtype in terms of muscle weakness. Immunosuppressive therapies are often insufficient. IMNM is associated with myositis-specific autoantibodies (MSAs); anti-SRP and anti-HMGCR, which have been suggested to play a pathogenic role. We have previously shown that the force generating capacity of non-necrotic muscle fibers from untreated IMNM patients is impaired. This may be caused by serological constituents; more specifically IgGs and MSAs, this is however unknown. Methods – Serum was collected from treatment naive IMNM patients and healthy controls. Subsequently, IgG was isolated from the serum to obtain total IgG and IgG-depleted serum. Healthy intact murine muscle fibers from the FDB muscle were isolated. Fibers were exposed to (IgG-depleted) serum for 2 hours, permeabilized and single fiber measurements were performed. Results – Maximum normalized force of healthy muscle fibers is lower after a 2-hour serum exposure from IMNM patients compared to healthy controls. Fibers exposed to IgG-depleted patient serum exposure did not replicate the differences as seen after serum exposure, suggesting that IgGs contribute to the sarcomere dysfunction. Conclusion – Serum of IMNM patients has a direct impact on muscle fiber contractility. Since the preliminary data suggest that IgG depleted serum does not affect muscle contractility, it is likely that the IgG of these patients cause the observed effect. To further examine that MSAs play a role in impaired muscle fiber contractility, experiments are ongoing with the IgG and IgG-depleted serum of these patients followed up with purified MSA exposure.
P10.12	Patryk Konieczny Adam Mickiewicz University	*DP71 uncouples early survival from functional differentiation in dystrophin-deficient muscle*	Duchenne muscular dystrophy is caused by mutations in the DMD gene, which encodes multiple dystrophin isoforms. Although all patients lack full-length dystrophin DP427, many retain the shorter isoform DP71, whose role in skeletal muscle remains unclear. We investigated whether DP71 modulates the disease phenotype in the absence of DP427 and its paralog utrophin. DP71 expression, splicing and promoter activity were analysed in human and mouse models. Functional effects of selective DP427 loss or combined DP427/DP71 depletion were assessed in differentiating human myotubes using functional assays and transcriptomic profiling, together with rescue experiments using DP71 splice variants. DP71 was enriched in myoblasts and showed greater splice diversity in human than in murine models. In DP427-deficient myotubes, DP71 was transcriptionally upregulated, and this response was further enhanced by utrophin depletion. Increased DP71 expression was associated with larger myotube areas, improved viability, reduced ROS and lower intracellular calcium during early differentiation. Transcriptomic analyses showed that, compared with complete dystrophin deficiency, DP427-deficient cells retaining DP71 displayed weaker induction of structural and stress-responsive genes, but preserved metabolic, ribosomal and proteostasis-related programmes. Utrophin depletion selectively impaired structural gene expression without reproducing the full transcriptional profile of complete dystrophin loss. These findings identify DP71 as an inducible modulator of early adaptation in dystrophin-deficient muscle. DP71 supports cellular homeostasis and buffers stress-associated phenotypes but does not restore full structural or functional differentiation. Our data suggest that early survival and functional maturation can be uncoupled in dystrophin-deficient muscle, with DP71 and utrophin making distinct contributions to this process.

P10.13	Sarah Konze Hannover Medical School	*hiPSC-derived skeletal muscle cell model of N-acetylneuraminate pyruvate lyase (NPL) - linked myopathy*	The enzyme NPL (N-acetylneuraminate pyruvate lyase) is involved in the breakdown of sialic acids, which are important components of cell surfaces and glycoproteins. It catalyzes the conversion of N-acetylneuraminic acid into pyruvate and N-acetylmannosamine, thereby contributing to cellular metabolism. A defect in the NPL enzyme can impair sialic acid degradation, leading to an accumulation of metabolic intermediates and disturbances in cellular function. Importantly, a patient with NPL deficiency has been reported to present with muscle weakness, and both NPL-deficient zebrafish and mouse models exhibit muscle defects, suggesting an important role of NPL in muscle function and maintenance. To study the effects of NPL deficiency in a human model, we have analysed human induced pluripotent stem cell-derived skeletal muscle cells (hiPSC-SkMs) with a full knockout of NPL and the corresponding isogenic control. We observed impaired myofibrillar alignment and altered contraction parameters in myotubes at day 7 of differentiation, whereas only minor changes in sugar metabolites were detected. Taken together, our data suggest that NPL deficiency also affects muscle function in our hiPSC model. However, due to the immature state of the derived hiPSC-SkMs, these effects remain subtle, indicating that further maturation is required to more accurately recapitulate the pathomechanisms observed in the animal models.
P10.14	Paulina Kościelniak-Wawro Adam Mickiewicz University	*Small activating RNAs targeting the UTRN promoter upregulate utrophin in DMD-deficient human cell lines*	Duchenne muscular dystrophy (DMD) lacks a broadly effective therapy, despite substantial progress in dystrophin directed and gene replacement approaches, including AAV delivered microdystrophin. Utrophin, an autosomal dystrophin paralogue encoded by UTRN, can functionally compensate for dystrophin deficiency, but pharmacological utrophin upregulation has so far not achieved successful clinical translation, following the failure of small molecule utrophin modulators in clinical trials. RNA activation (RNAa) using small activating RNAs (saRNAs) represents a clinically investigated modality for endogenous gene induction, with emerging evidence in early phase human studies. Here, we explored whether saRNAs targeting the UTRN locus can upregulate utrophin in human cellular models of DMD. We designed a panel of saRNAs complementary to regulatory sequences within the UTRN promoter region and performed preliminary screening in human cell lines, including DMD knockout models. UTRN transcript levels were quantified by RT qPCR, and the most active saRNA candidates were selected based on reproducible increases in UTRN mRNA compared with non targeting controls. Across multiple cell models, saRNAs targeting distinct promoter regions induced a consistent elevation of UTRN transcript abundance, demonstrating that utrophin expression can be upregulated in DMD deficient backgrounds through saRNA mediated transcriptional activation. These preliminary data support saRNA mediated activation of UTRN as a rational, redosable and clinically tractable RNA based strategy to enhance utrophin expression in DMD. Ongoing work focuses on expanding the panel of UTRN targeting saRNAs and characterising the magnitude, durability and functional consequences of utrophin upregulation at the protein and cellular level in disease relevant muscle cell models.
P10.15	Joanna Moraczewska, Kazimierz Wielki University in Bydgoszcz	*Dysregulation of Thin Filament Stability and Length Maintenance by Myopathy-Linked Mutations in TPM2*	Tropomyosin (Tpm) is a regulatory protein that polymerizes along both sides of the actin filament. In striated muscle, together with the troponin complex (Tn), Tpm regulates actomyosin interactions, stabilizes the filament, and controls cofilin-2 (Cof2)- and tropomodulin (Tmod)-dependent filament length maintenance. We hypothesized that myopathy-causing mutations in Tpm-encoding genes contribute to disease development by disrupting filament stability and length. To address this possibility, we used fluorescence microscopy and biochemical assays to compare the effects of four disease-associated substitutions in Tpm2.2 – hypercontractile D20H and E181K, and hypocontractile E41K and N202K, on thin filament stabilization against myosin-generated force and Cof2-dependent depolymerization in the absence and presence of Tn ($+Ca^{2+}$) and Tmod1. Filaments bound in rigor (no ATP) to HMM-coated coverslips had similar lengths in the absence and presence of Tpm2.2, Tn, and Tmod1. Under ATP-driven actomyosin interactions, the filaments became shorter. Acceleration of ATPase activity by Tn ($+Ca^{2+}$) caused further shortening of filaments containing the hypercontractile Tpm2.2 variants, both in the absence and presence of Tmod1. For analysis of Cof2-dependent depolymerization kinetics, coverslips were coated with lipids to eliminate stress imposed on actin by myosin. Both Tpm2.2–Tn ($+Ca^{2+}$) and Tmod1 strongly protected filaments from Cof2-induced depolymerization, most likely by stabilizing Tpm2.2 on the filament. In the absence of Tmod1, hypocontractile variants further increased filament resistance to depolymerization. However, when Tmod1 was bound, most mutations accelerated Cof2-induced depolymerization. In conclusion, disease-causing Tpm2.2 variants differentially alter thin filament stability and length maintenance depending on the regulatory proteins associated with the filament.

P10.16	Albert Bálint Papp University of Debrecen	*Impaired skeletal muscle regeneration in the absence of adenosine A2A receptor*	Skeletal muscle regeneration is a complex biological process that requires coordinated interactions between muscle stem cells (satellite cells) and immune cells, particularly macrophages. Following muscle injury, large amounts of adenosine are released as danger-associated molecules, influencing both immune and satellite cell functions through adenosine receptors. In this study, we investigated skeletal muscle regeneration in adenosine A2A receptor (A2AR) wild-type (WT) and knockout (KO) male mice. Injury to the tibialis anterior muscle was induced by cardiotoxin injection, and regeneration was monitored for three weeks. We assessed overall muscle morphology, fusion index, and cross-sectional area (CSA) of regenerating muscle fibers, and analyzed the expression of myogenic differentiation markers and growth factors by qPCR. In addition, infiltrating macrophage subpopulations and their cytokine profiles were characterized by flow cytometry and qPCR. In the absence of A2AR, regenerating muscle fibers exhibited reduced fusion and smaller CSA. A2AR deficiency was associated with higher ratio of infiltrating inflammatory macrophages during regeneration. Furthermore, regenerating muscle tissue and infiltrating leukocytes showed decreased expression of the growth factors Igf1 and Gdf3, alongside elevated levels of Il1β and Il6. Although the enhanced inflammatory environment may accelerate certain regenerative processes, the reduced production of growth factors impairs myoblast fusion and muscle fiber growth, ultimately resulting in defective muscle regeneration. These findings demonstrate that A2AR signaling is essential for proper skeletal muscle regeneration and may provide a basis for the future development of locally administered A2AR agonist therapies to promote muscle healing.
P10.17	Fernando Ribeiro Uppsala University	*Myosin Post-Translational Modifications Associated With Critical Illness Myopathy*	Background: Critical illness myopathy (CIM) is a common and devastating consequence of critical care, causing dramatic loss of skeletal muscle mass and function in intensive care unit (ICU) patients. Functional deficits often exceed the loss in muscle mass and myosin content. However, the mechanisms underlying the loss of force remain elusive. Methods: Myosin dysfunction was investigated in six neuro-ICU patients exposed to a 12-day mechanical ventilation and immobilization period using mass spectrometry-based proteomics and molecular dynamics simulations. Results: Previous single muscle fiber analyses revealed decreased fiber size and specific force from the 1st to the 12th days in all ICU patients. A subset of myosin-expressing fibers exhibiting a complete loss of contractile function was identified in three of the six ICU patients despite similar atrophy levels (~30%, $p<0.05$) after 12 days. All fibers had decreased specific force after 12 days of mechanical ventilation, but 9% to 21% of the fibers were non-force-generating. The decline in muscle specific force was linked to 27 post-translational myosin modifications, including oxidation, ubiquitination, acetylation, and methylation. Molecular dynamics simulations suggested oxidation-induced rigidity of the myosin head, which was predicted to compromise the flexibility of key functional (i.e. actin-binding and converter) domains. Non-force-generating fibers exhibited a unique proteomic signature predicted to further enhance myosin head exposure and rigidity. Conclusion: In addition to muscle wasting and myosin loss, abnormal myosin post-translational modifications contribute to ICU-acquired muscle weakness in critically ill patients, including the development of muscle fibers incapable of generating contractile force.
P10.18	Cristiana Sazzi, Università Di Pavia	*Fibro-adipogenic progenitors from hindlimb-suspended mice induce myotube atrophy in vitro*	Maintenance of skeletal muscle mass is essential for health. Increasing evidence suggests that mechanisms regulating muscle homeostasis extend beyond myofibres and involve resident stromal cells, including fibro-adipogenic progenitors (FAPs). In addition to their established role in supporting muscle regeneration, FAPs depletion has been associated with skeletal muscle atrophy, suggesting a potential contribution to muscle mass maintenance. This study aimed to investigate whether disuse induces FAPs to acquire a pro-atrophic secretory phenotype capable of promoting myotube atrophy in vitro. Hindlimb muscles were collected from adult male mice subjected to 14 days of hindlimb suspension (HLS) and from ground control mice. Following enzymatic digestion, FAPs were isolated by flow cytometry, cultured, and their conditioned media (CM) collected. C2C12 myotubes were exposed to FAP-derived CM, and morphological, transcriptomic, and proteomic analyses were performed. Portions of the same muscle samples were processed for immunohistochemical analyses. HLS muscles displayed a significant reduction in fibre cross-sectional area together with an increased proportion of PDGFRα$^+$ cells ($p<0.001$), a canonical marker of FAPs, compared with controls. Myotubes exposed to CM derived from HLS-FAPs exhibited a significantly reduced diameter compared with those treated with control-FAP CM ($p<0.05$). Preliminary protein expression analysis revealed reduced phosphorylation levels of AKT and p70S6K, suggesting impairment of the Akt/mTOR signalling pathway. Ongoing analyses will further characterize disuse-induced FAP remodelling and identify the factors underlying their pro-atrophic effects. These preliminary findings suggest that FAPs contribute to the skeletal muscle response to disuse and may promote muscle mass loss through paracrine mechanisms.

P10.19	Vincenzo Sorrentino University of Siena	*miR-486 exerts protective effects in Ryr1-IT mice, a mild model of RYR1-related myopathies (RYR1-RM)*	Mutations in RYR1, which encodes the skeletal muscle ryanodine receptor, are the leading cause of malignant hyperthermia and the most common cause of RYR1-related myopathies (RYR1-RM), a heterogeneous group of congenital muscle disorders characterized by muscle weakness ranging from mild to severe forms. Although impaired Ca^{2+} signaling is a hallmark of RYR1-RM, the mechanisms linking altered calcium homeostasis to muscle hypotrophy and wasting remain incompletely understood. Given the role of muscle-specific microRNAs (myomiRs) in regulating muscle growth, regeneration, and homeostasis, we investigated the therapeutic potential of miR-486, which has previously been shown to improve muscle function in mdx mice and in a skeletal muscle cachexia model. . To determine whether similar benefits could be achieved in RYR1-RM, we crossed transgenic mice overexpressing miR-486 with Ryr1-IT mice, a well-established mild model of RYR1-RM harboring the I4895T mutation in Ryr1. In vivo analyses showed that miR-486 overexpression improved muscle function, as assessed by hanging, grip strength, and treadmill tests, while ex vivo nerve-induced contraction studies of the gastrocnemius revealed improved contractile kinetics, indicating enhanced muscle fiber performance and excitation–contraction coupling. RNA-sequencing analysis revealed that miR-486 most strongly modulated genes involved in mitochondrial oxidative phosphorylation and ATP production, followed by immediate early genes and protein homeostasis and ubiquitin–proteasome system genes. Further studies are required to assess whether these transcriptional changes translate into altered mitochondrial bioenergetics and contribute to the functional benefits observed in Ryr1I4895T mice, further supporting the therapeutic potential of miR-486 in RYR1-related myopathies.
P10.20	Ewa Stępniak-Konieczna Poznan University of Life Sciences	*Targeted RNA activation of MBNL1 corrects spliceopathy in myotonic dystrophy and provides a framework for dual-paralog therapeutic enhancement.*	Functional depletion of Muscleblind-like (MBNL) proteins by sequestration on CUG-expanded RNA is a central driver of myotonic dystrophy type 1 (DM1), causing widespread spliceopathy. We developed a targeted RNA activation (RNAa) strategy to restore endogenous MBNL1 expression using promoter-specific small activating RNAs (saRNAs). Two lead duplexes targeting the predominant MBNL1 promoter (P2) activated transcription through an AGO2-dependent, DNA-targeted mechanism that promoted RNA polymerase II recruitment and productive elongation. This increased MBNL1 protein 1.3–3-fold in DM1 fibroblasts and myoblasts, sufficient to correct multiple disease-relevant splicing biomarkers without increasing nuclear RNA foci, apoptosis, or overt cytotoxicity. Mechanistically, activation was driven by the antisense saRNA strand, while sense-strand off-target activity could be minimized by rational chemical modification; promoter-associated transcripts, including lncRNA MBNL1-AS1, were dispensable, supporting a direct promoter-targeting mode of action. These findings establish endogenous MBNL1 activation as a precise, reversible, and potentially safer alternative to exogenous overexpression, with advantages for tunable dosing and chemical optimization. Given the tissue-specific expression of MBNL paralogs and the disease-modifying contribution of MBNL2, this platform provides a strong rationale for future combinatorial strategies aimed at coordinated enhancement of MBNL1 and MBNL2 to broaden tissue coverage and strengthen multisystem therapeutic benefit in DM1. This study was supported by funding from the National Science Centre, Poland, under grants no. 2020/37/B/NZ5/01263 (to E.S.-K.) and 2022/46/E/NZ5/00088 (to E.S.-K.).
P10.21	Brigitta Tillmann University Of Debrecen	*The expression of cytoskeletal septins and SOCE complex proteins during spontaneous muscle regeneration in mdx mice*	Duchenne muscular dystrophy is an X chromosome-linked recessive skeletal muscle disorder caused by the mutation of the DMD gene, which encodes the protein dystrophin. In the absence of functional dystrophin, the sarcolemma becomes highly susceptible to mechanical stress, resulting in micro-injuries upon contraction. However, mdx mice, the animal models of DMD have milder phenotype caused by a spontaneous regeneration phase between 8-12 weeks of age. During this period, satellite cells are activated, undergoing extensive proliferation and differentiation, muscle mass is largely restored, and functional capacity is substantially preserved. Septins are known as the fourth component of the cytoskeleton. Previous research suggests that septins, especially Septin7, are involved in skeletal muscle regeneration and development. We want to examine whether cytoskeletal septins are involved in the spontaneous regeneration process in young mdx mice. Our experimental results show that the expression of several septin proteins, as well as the components of store-operated calcium entry, STIM1 and Orai1, are significantly modified during the regeneration phase. These findings indicate that cytoskeletal protein dynamical assembly and changes of SOCE are essential mediators of the spontaneous regeneration of skeletal muscle in the dystrophic animal model. Examining the localization of these proteins in flexor digitorum brevis single muscle fibers using fluorescent immunostaining, we detected an altered myofibrillar pattern of Septin7. These results suggest a complex functional role of septins in the spontaneous regeneration process of dystrophic skeletal muscle, presumably through the activation of satellite cells, proliferation of committed muscle progenitor cells, and differentiation of myoblasts into multinucleated myotubes.

P10.22	Ya Wen Jiaxing Hospital of Jiaxing University	*Rescuing the Diaphragm: Human Bone Marrow Mesenchymal Stromal Cells (BM-MSCs)-Derived Extracellular Vesicles Reverse Ventilator-Induced Diaphragm Dysfunction and Reactivate Satellite Cells*	Background: Mechanical ventilation (MV) sustains life in critically ill patients—yet progressively destroys the very muscle required to breathe independently. Ventilator-induced diaphragm dysunction (VIDD) is the principal barrier to weaning, yet no approved treatment exists. We hypothesized that ventilator-induced lung injury (VILI) drives systemic inflammation that directly impairs diaphragm muscle, and tested whether extracellular vesicles (EVs) from human bone marrow mesenchymal stromal cells (BM-MSCs) could arrest this process and restore respiratory muscle function. Methods: Sprague-Dawley rats underwent five days of controlled MV with neuromuscular blockade and deep sedation in a unique experimental ICU (ExICU) model. A single intavenous EV dose was administered at ventilation onset. Diaphragm single-fiber CSA and specific force, lung histopathology, and integrated multiomics (transcriptomics, proteomics, metabolomics) of lung, BAL fluid, and diaphragm were assessed alongside cellular deconcolution of bulk RNA-seq data. Results: Controlled MV devastated the diaphragm: fiber CSA and specific force each collapsed by 50%, accompanied by alveolar injury and systemic inflammatory activation. A single EV dose reversed diaphragm atrophy and force loss, curtailed alveolar ectasia, and normalised immune, proteomic, and metabolic dysregulation across lung and respiratory muscle. Cellular deconvolution revealed recovery of both slow- and fast-twitch myofiber populations alongside reactivation of muscle satellite cells—evidence of genuine regeneration, not merely blunted damage. Conclusion: To our knowledge, this is the first intervention to simultaneously mitigate VILI and VIDD. BM-MSC-derived EV therapy offers a compelling, clinically translatable strategy to restore respiratory muscle function and fibre-type composition, accelerate ventilator weaning, and reduce the burden of prolonged critical care.
P10.23	Xiang Zhang Jiaxing Hospital of Jiaxing University	*Longitudinal transcriptomics reveals the role of ferroptosis in the progression of critical illness myopathy*	Background: Critical illness myopathy (CIM) causes profound skeletal muscle atrophy and prolonged ventilator dependence in critically ill patients. Systemic inflammation and mechanical silencing are established drivers, but core molecular mechanisms remain unclear. Ferroptosis, an iron-dependent, lipid peroxidation-driven regulated cell death, mediates multiple muscle-wasting disorders, but its role in CIM remains uncharacterized. Methods: Ten neuro-ICU patients on long-term controlled mechanical ventilation were prospectively followed for 12 days. Serial tibialis anterior biopsies collected at six time points were subjected to bulk RNA-seq. Subsequently, single-cell deconvolution was performed to predict cell-type composition and alterations along with the progression of CIM. Phenotype-associated gene modules were screened via weighted gene co-expression network analysis (WGCNA). Known ferroptosis driver genes were retrieved from the FerrDb V3 database and intersected with network-derived hub genes, and candidate axes were verified in pathologically stressed myotubes differentiated from human skeletal muscle satellite cells. Results: All patients developed progressive CIM with preferential myosin loss and >65% contractile force loss. Longitudinal transcriptomics revealed progressive loss of type II myofibers and increase of inflammation, accompanied by enhanced expression of ferroptosis drivers and markers like hub genes HSPA5 and HMOX1. Ultimately, two core hub gene axes from network analysis, XBP1–HSPA5 and FOSL1–HMOX1, were overexpressed in ferroptosis-induced myotubes, while gene knockdown reverse such expression changes. Conclusion: This study establishes progressive ferroptosis as a critical molecular driver of CIM via XBP1–HSPA5 and FOSL1–HMOX1 axes. Our findings provide a novel mechanistic framework for ICU-acquired myopathy, with these two axes as promising actionable therapeutic targets.
P10.24	Ietizia Zullo Irccs Ospedale Policlinico San Martino	*Effects of Physical Exercise in a Zebrafish Model of Neuroinflammation*	Inflammatory processes are involved in a range of neuromuscular dysfunctions leading to progressive motor impairment. A significant body of literature currently highlights the role of physical exercise as a non-pharmacological intervention in neuromuscular diseases. However, the mechanisms through which exercise preserves motor performance remain poorly understood. Our study aims to clarify how prolonged exercise training influences disease onset, severity, and motor decline in a zebrafish model of neuroinflammation. To induce neuroinflammation, adult zebrafish (Tubingen) aged 5–6 months were injected subcutaneously with 1 mg/ml MOG in Complete Freund's Adjuvant (CFA), or alternatively with CFA alone. Animals were scored daily for up to 15 days to assess clinical symptoms, swimming abilities, and mortality. MOG-injected animals showed an early onset of symptoms, including increased immobility, reduced swimming activity, and a slanted body posture rather than a horizontal one. Additionally, biomechanical characterization of the muscles after MOG injection revealed an increased twitch-to-tetanus ratio, indicative of impaired muscle force summation. CFA-injected animals showed no clinical alterations. Two additional groups underwent a 15-day Resistance Training protocol prior to injection. MOG-injected animals altered their swimming kinematics by adopting a strategy that optimized resistance to water flow, possibly as a compensatory response to the ongoing neuroinflammatory process. This investigation lays the foundation for further studies examining the combined effects of pharmacological treatment and physical exercise on neuromuscular disease progression. Taken together, our findings provide important insights into how treatment effectiveness and patient quality of life may be improved through a simple and cost-effective intervention.