FFA4 inverse agonism and Piezo1 agonism regulate cellular mechanics of 3T3-L1 adipocytes

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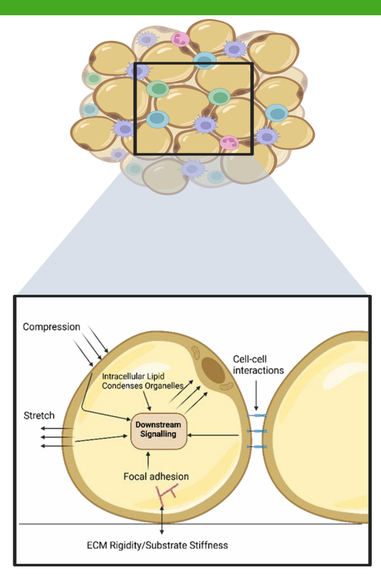
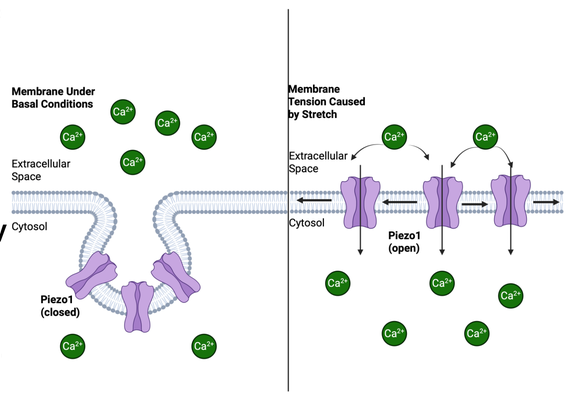
Adipocyte Mechanotransduction

- Adipocytes (fat cells) play critical role in energy metabolism in the body
- These cells store energy in the form of glycerol and fatty acids which are released and used as energy in a fasted state
- Dysregulation of adipocytes leads to imbalances in energy metabolism as commonly seen in metabolic diseases such as obesity and diabetes.
- However, one essential pathway that is understudied is mechanotransduction, a signalling pathway that is activated by mechanical stimulation



Piezo1: a mechanosensitive ion channel

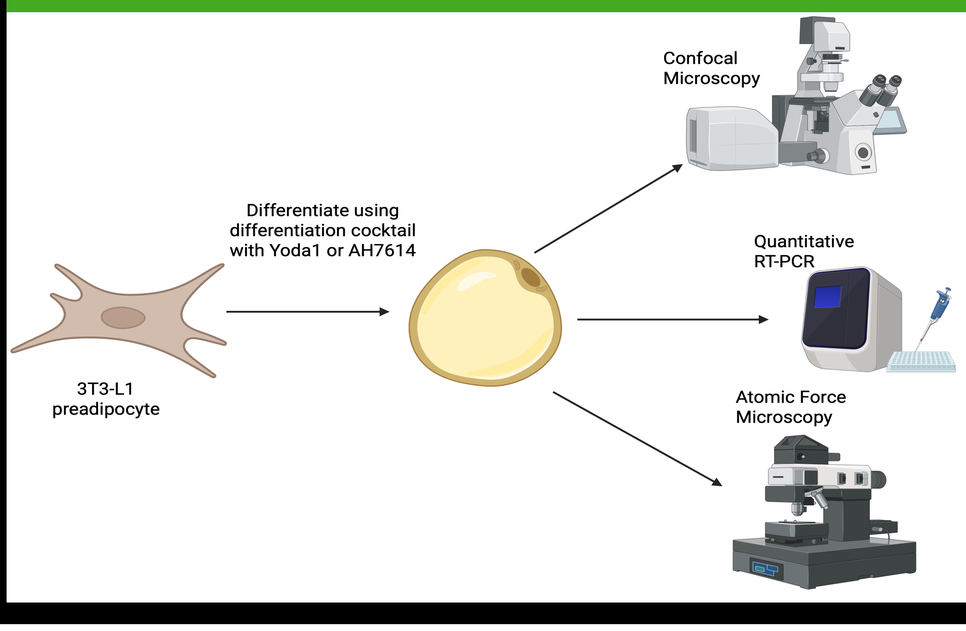
- Activated by straightening of membrane curvature/membrane tension in response to mechanical stimuli
- Trimeric protein with propeller-like structure
- Non-selective but predominantly calcium ion channel
- Expressed universally but predominately in mesenchymal-derived cells
- Can be activated by agonist, Yoda1



Aims

- Evaluate the effect of FFA4 inverse agonism by AH7614 on adipogenesis, total lipid accumulation and cellular mechanics during adipocyte differentiation in 3T3-L1 adipocytes
- Investigate Piezo1 agonism by Yoda1 on its role on adipogenesis and metabolite-sensing GPCR expression in 3T3-L1 adipocytes

Methods



Results – FFA4 inverse agonism reduces 3T3-L1 differentiation, increases adipocyte stiffness and inhibits F-actin remodelling

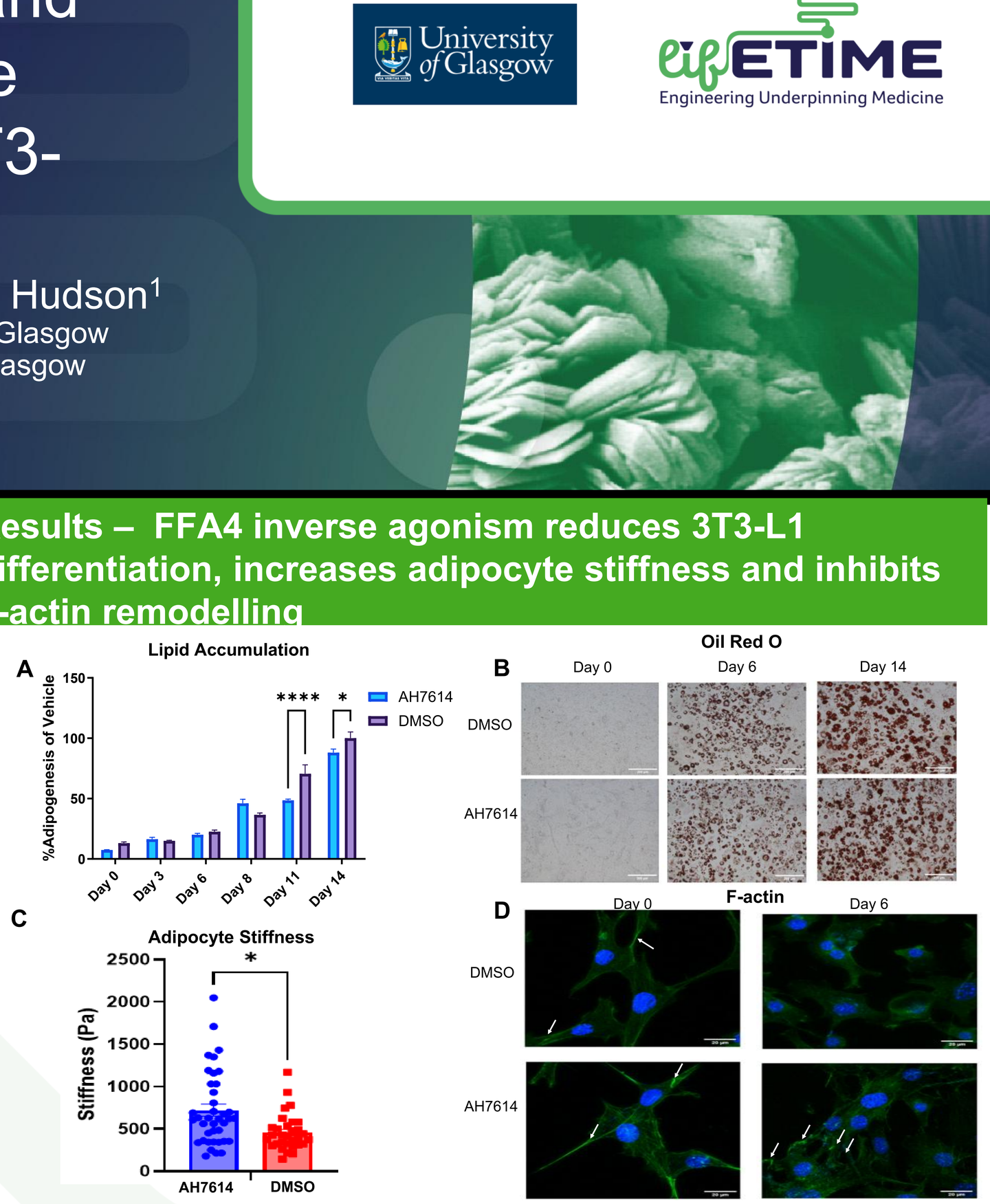


Figure 1: FFA4 inverse agonism reduces 3T3-L1 differentiation, increases adipocyte stiffness and inhibits F-actin remodelling. (A) Quantification of lipid accumulation using Oil Red O lipid stain during differentiaton with/without 10μM AH7614 . (B) Images at x20 magnification of 3T3-L1 cells during differentiation with/without 10 μM AH7614 (C) Young's Modulus of Day 14 3T3-L1 adipocytes following differentiation with/without 10μM AH7614 (D) F-actin staining of 3T3-L1 adipocytes at different time points treated with 10μM AH7614 or untreated. Green – Phalloidin, Blue – DAPI

Results – Piezo1 agonism modulates 3T3-L1 adipogenesis and modulates metabolite-sensing GPCR expression

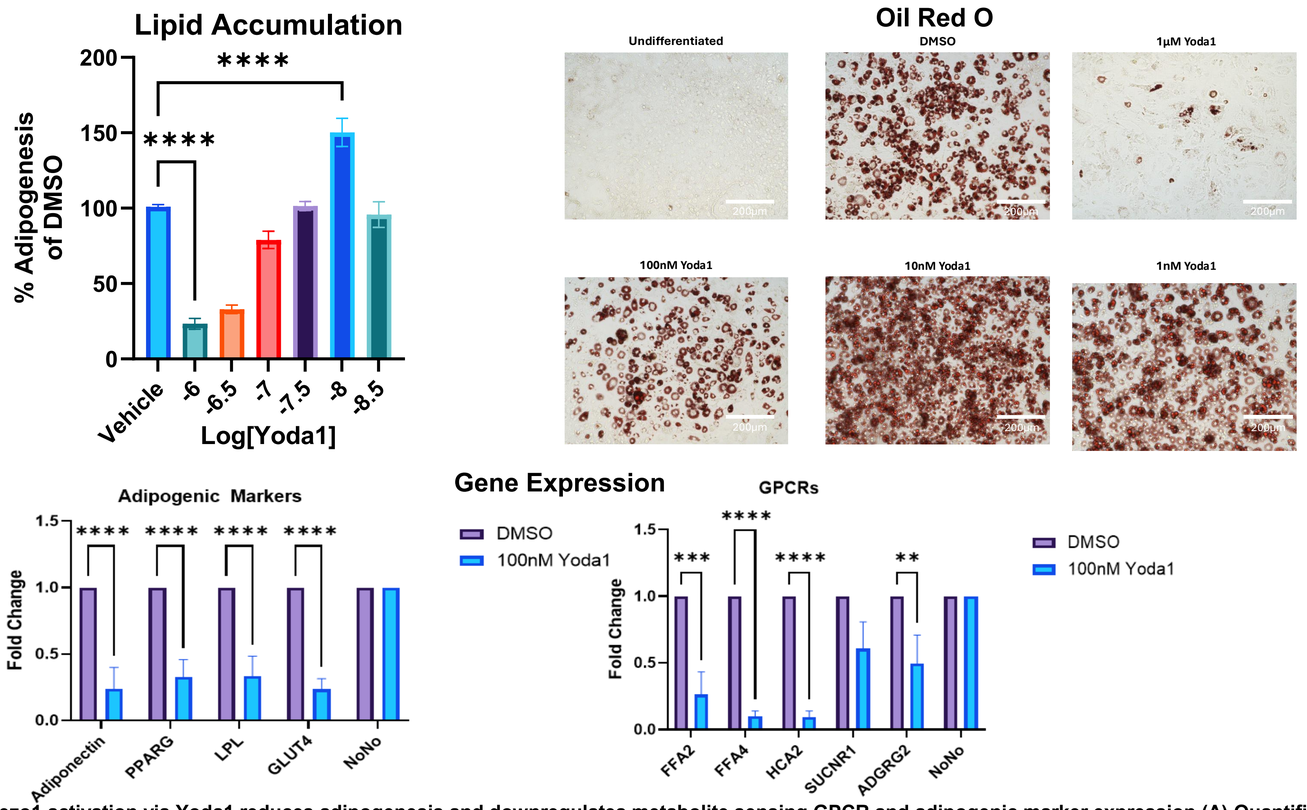


Figure 2: Piezo1 activation via Yoda1 reduces adipogenesis and downregulates metabolite sensing GPCR and adipogenic marker expression (A) Quantification of Oil-Red O stained 3T3-L1 adipocytes at day 14 treated with 100nM Yoda1compared with vehicle treated, normalised to vehicle-treated 3T3-L1 adipocytes, ****p<0.001(n=3) (B) Brightfield images of Oil Red O stained 3T3-L1 preadipocytes, vehicle treated 3T3-L1 adipocytes at day 14, and 3T3-L1 adipocytes at day 14 treated with varying concentrations of Yoda1 at x20 magnification. Scale bar = 200μm (C) Fold change of gene expression of adipogenic markers and metabolite-sensing GPCRs in 100nM Yoda1-treated and untreated day 14 3T3-L1 adipocytes. relative to untreated adipocytes

Conclusion and Future Work

- FFA4 inverse agonism inhibits 3T3-L1 adipogenesis throughout differentiation and inhibits F-actin remodelling
- Piezo1 agonism modulates adipogenesis with high concentrations of Yoda1 inhibits adipogenesis, with lower concentrations of Yoda1 increases adipogenesis
- Future work will focus on dual treatment of Yoda1 and AH7614 to investigate potential link between Piezo1 activation and FFA4 basal activity

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