High-Throughput Automated Patch Clamp Method to Enable Drug Discovery on the Force-Sensing Ion Channel Piezo1

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1 Introduction

Piezo1 is a multimodal mechanosensitive ion channel involved in vascular development, erythrocyte function, and cancer progression, making it a key pharmacological target. However, mechanically activating Piezo1 at scale is challenging, as conventional patch clamp electrophysiology is low-throughput and difficult to standardize.

M-Stim is an automated patch clamp approach that uses rapid, high-rate flow in a 384-well format to deliver controlled mechanical stimuli, reproducibly evoking Piezo1 currents. We used M-Stim to profile Piezo1 inhibitors in overexpressing and endogenously expressing cell lines, enabling faster, standardized compound screening in biologically relevant models.

2 M-Stim approach

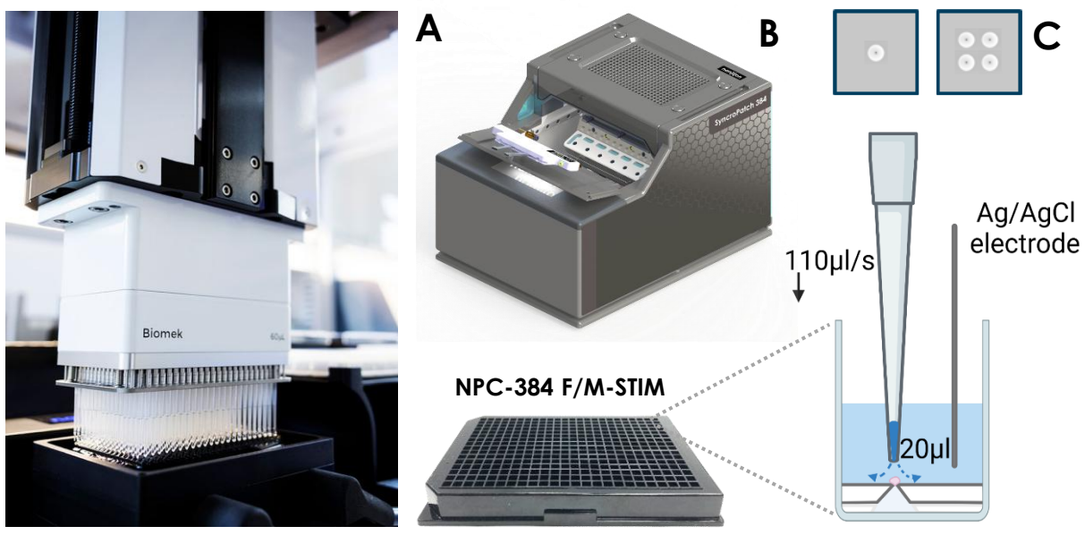
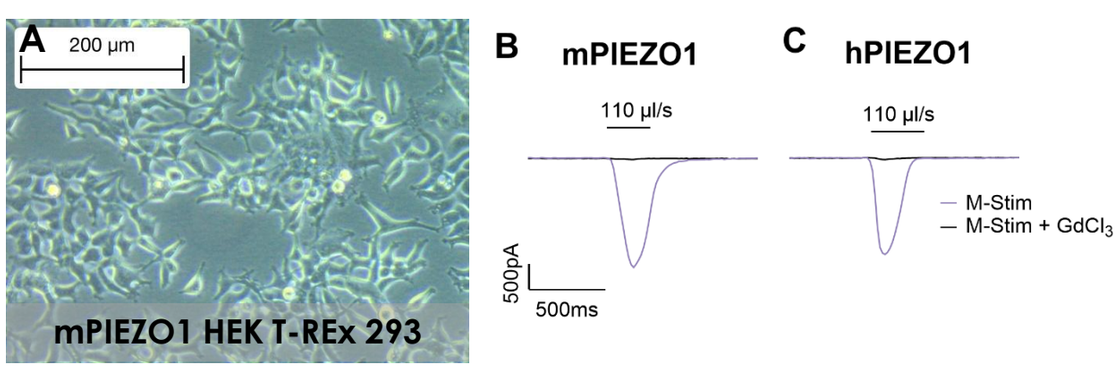


Figure 1 (**A**) SyncroPatch 384-pipetting head dispensing solution up to 110 µl/s. (**B**) The measurement chamber hosts a NPC-384 well chip, 384 independent external command electrodes and 12 internal ground Ag/AgCl electrodes. (**C**) Cross section of one NPC-384 chip well created with Biorender.com. M-Stim delivers 20 µl of solution locally to the cell and uses aspiration to recover the dispensed volume.



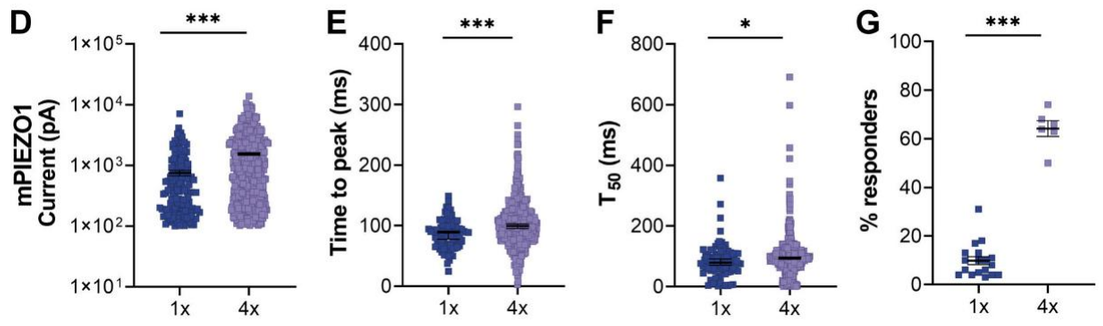


Figure 2 (**A**) M-Stim assay development was performed on HEK T-REx 293 cells overexpressing Piezo1 (kindly provided by University of Leeds). Representative mPIEZO1 (**B**) and hPIEZO1 (**C**) inward currents recorded from a four-hole chip elicited by M-Stim at 110 µl/s. Absolute current values (**D**), time to peak (**E**), T_{50} (**F**) and fraction of responding cells (**G**) from mPIEZO1 cells recorded using single hole chips (1×, blue) and four-hole chips (4×, purple).

Table 1 Compression or pressure (P [dyn/cm²]) was calculated via the Bernoulli's equation (impinging jet theory); ρ: fluid density [10^3 kg]/m³), v: flow velocity $4Q/\pi D^2$, D: orifice diameter [300µm], Q: flow rate [µl/s]. Pressure drops by 10 % towards the edge of the diameter. **Shear stress** (τ [dyn/cm²]) was calculated via the Hiemenz's analytic solution (of Navier-Stokes equations); η: viscosity 10^{-3} Pa*s, ρ: fluid density, r: radial position from the center of the pipette.

Q (µL/s)	P at center [dyn/cm²]	τ at r = 10 µm 1× [dyn/cm²]	τ at r = 70 µm 4× [dyn/cm²]	τ at r = 150 µm 4× [dyn/cm²]
40	1,600	7.8	55	120
60	3,600	14	100	210
110	12,000	35	250	530

$$P = \frac{\rho v^2}{2}$$

$$\tau = \frac{1.873}{2}\sqrt{\eta\rho}\left(\frac{\nu}{D}\right)^{3/2} r$$

3 Piezo1 pharmacology

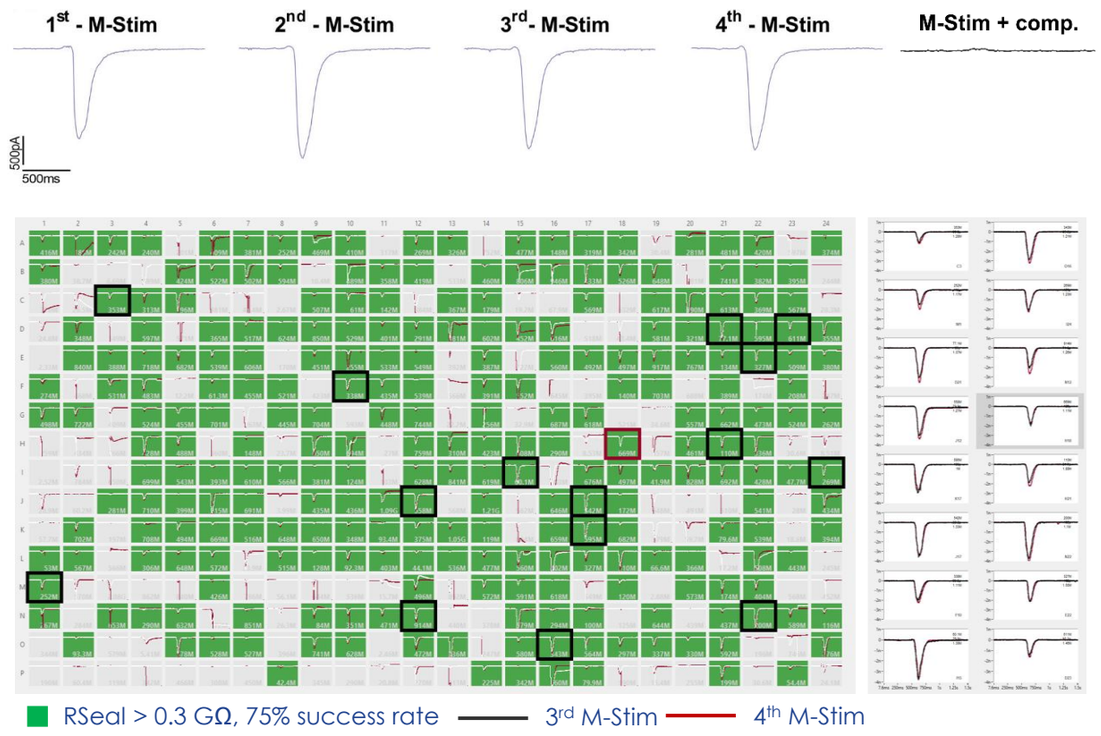


Figure 3 (**Top**) The experimental approach for pharmacological studies consisted of four to five sequential M-Stim additions, followed by a final addition in the presence of the test compound. The current amplitude stabilized within three M-Stim additions, only the last one was used for normalization. (**Bottom**) Screenshot from PatchControl 384 showing the 384-well panel with sixteen selected wells highlighted on the right. The third and fourth M-Stim additions are highlighted in black and red, respectively. Mechanically activated currents were observed in 257 of the 342 total cells (75% success rate).

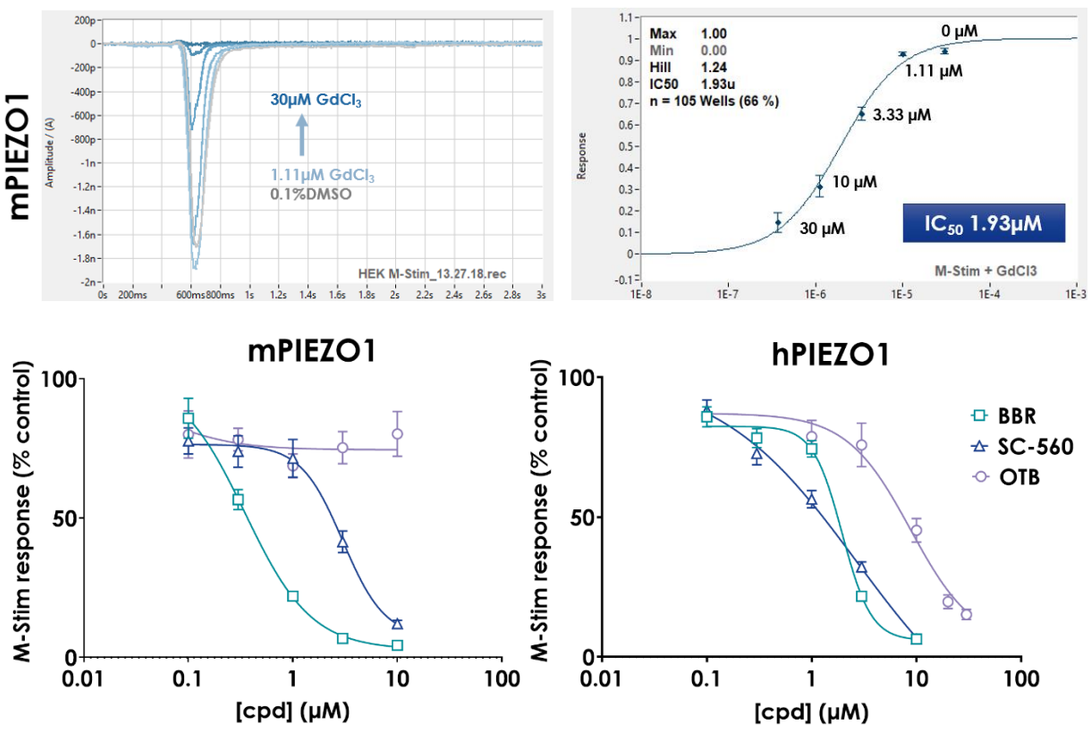


Figure 4 (**Top**) Traces and concentration-response graphs exported from DataControl 384, showing the inhibitory effect of gadolinium chloride ($GdCl_3$), a non-specific inhibitor of mechanically activated channels. (**Bottom**) Concentration-response analysis of test compounds benzbromarone (BBR), SC-560, and otenabant (OTB) on mPIEZO1 and hPIEZO1. OTB appeared to be specific for hPIEZO1.

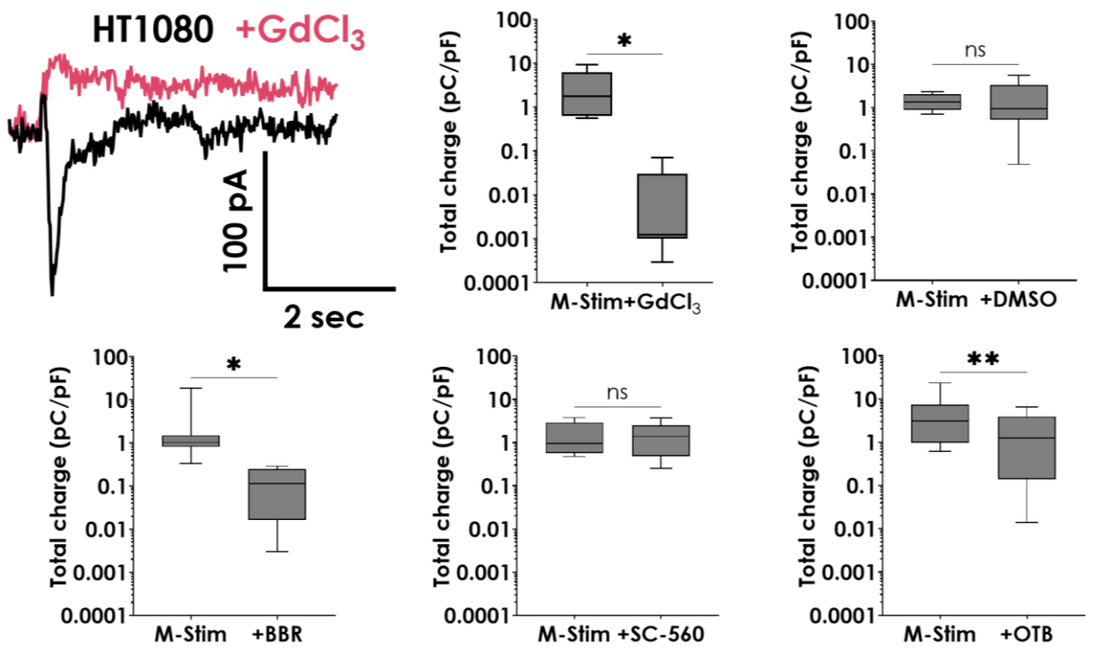


Figure 5 The effects of 30 µM $GdCl_3$, 0.1% DMSO and 5 µM of test compounds (BBR, SC-560, and OTB) were evaluated on mechanically evoked Piezo1 responses in HT1080 WT fibrosarcoma cells endogenously expressing human Piezo1 (kindly provided by the University of Geneva). OTB inhibitory effect was confirmed in the endogenous system.

Table 2 IC_{50} and percentage inhibition values of the test compounds on HEK T-REx 293 mPIEZO1, hPIEZO1, and HT1080 cells.

Compound	IC_{50} [µM]		Inhibition [%] ± SEM
	mPIEZO1	hPIEZO1	HT1080
$GdCl_3$	1.93		81.4 ± 14.6
BBR	0.35	1.99	93.1 ± 2.7
SC-560	3.01	2.71	-40.6 ± 46
OTB	-	8.78	69.8 ± 9.1

4 Summary

- M-Stim produced reproducible Piezo1 responses from overexpressing cells that scaled with flow rate and cell number per well.
- M-Stim and SyncroPatch 384 enable Piezo1 pharmacology studies across cellular contexts.
- BBR efficiently inhibited Piezo1 currents in overexpressing and endogenous systems.
- SC-560 inhibited Piezo1 currents in overexpressing systems.
- OTB selectively inhibited hPIEZO1, highlighting it as a promising scaffold for selective Piezo1 inhibitors.

References

Murciano N, Rotordam MG et al., *J. Gen. Physiol.*, 2023

Jaquet V, Penttinen R, et al. *bioRxiv*, 2026