Science Advances
AAAS

Supplementary Materials for

Force- and cell state–dependent recruitment of Piezo1 drives focal adhesion dynamics and calcium entry

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Sci. Adv. **8**, eabo1461 (2022)
DOI: 10.1126/sciadv.abo1461

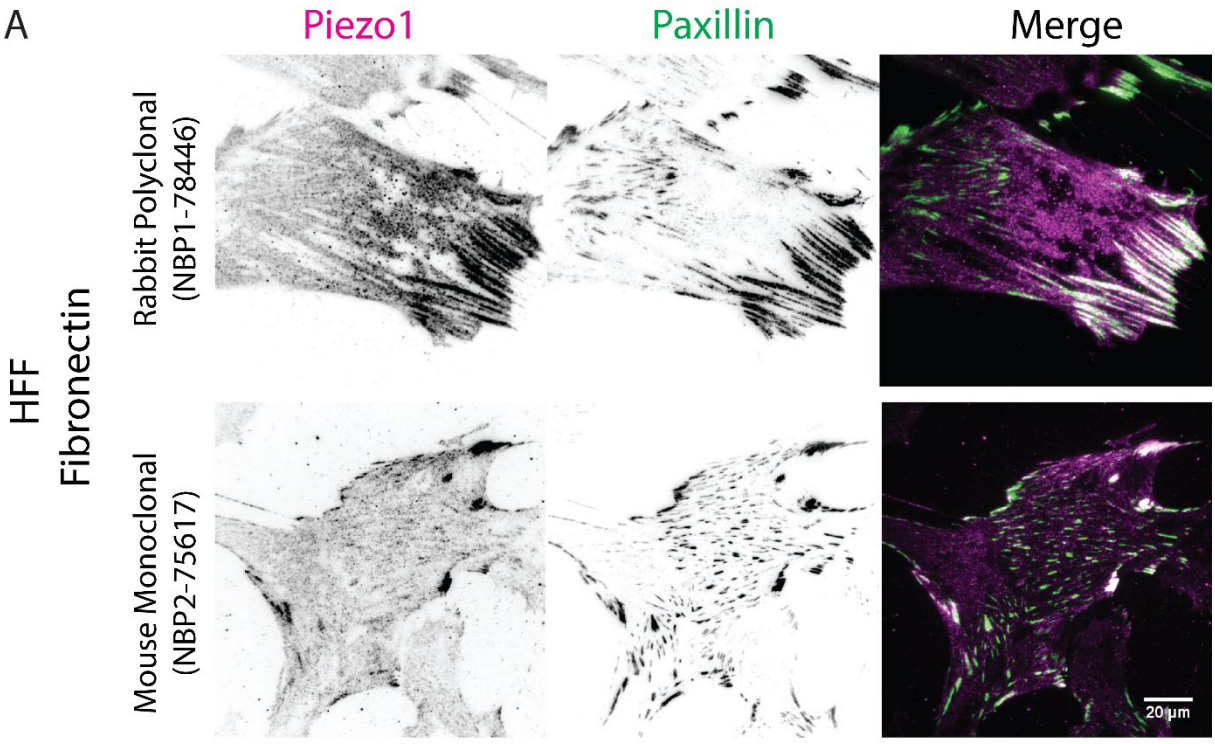
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Figures

Figure S1

A



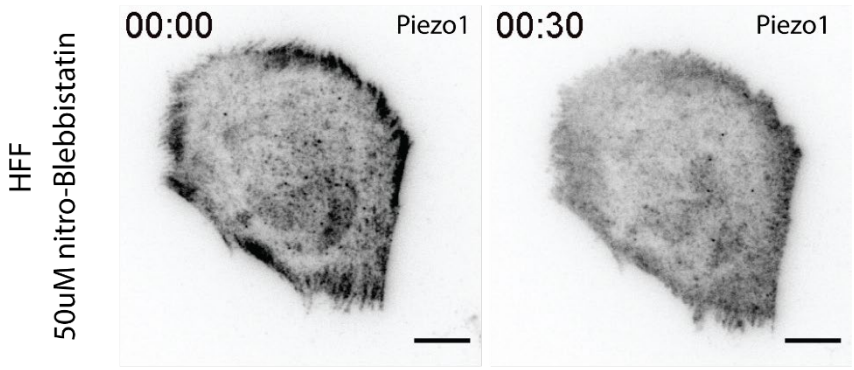
B

Target Sequence :	GCAAGTTCGTGCGCGGATT
siRNA sense sequence :	GCAAGUUCGUGCGCGGAUUdTdT
siRNA antisense sequence :	AAUCCGCGCACGAACUUGCdTdT

Figure S1, Immunostaining of Piezo1 localization of HFF cells. (A) HFF cells were seeded on fibronectin surfaces overnight, fixed with paraformaldehyde and stained for Piezo1 with two validated Piezo1 antibody and paxillin. (B) Sequence information of *Piezo1* siRNA used in the study (SASI_HS01_00208585 from Merck Sigma-Aldrich).

Figure S2

A



B

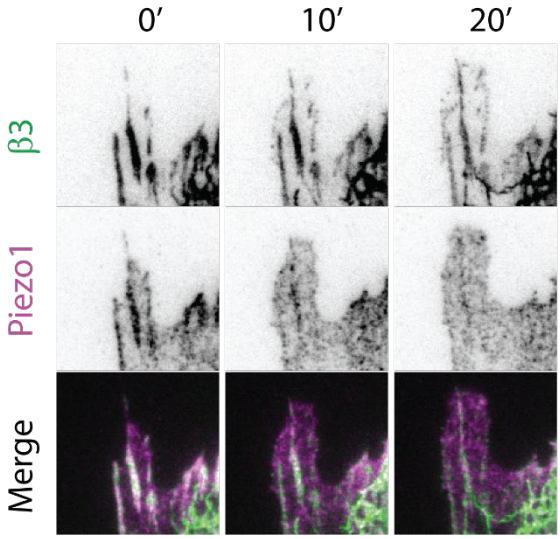


Figure S2. (A) HFF cells transiently transfected with Piezo1-mruby3 were treated with 50 µM nitro-Blebbistatin at time 0. After 30 min incubation, the localization of Piezo1 at focal adhesions were lost. Scale bars denote 10 µm. (B) HFF cells transiently transfected with mEmerald-integrin β3 and Piezo1-mruby3 were pre-treated with 2.5 µM PTP-PEST inhibitor that inhibits adhesion turnover by phosphatases before addition of 20 µM Y-27632. In this condition, Piezo1 dissociated from adhesions before integrin β3 upon tension release.

Figure S3

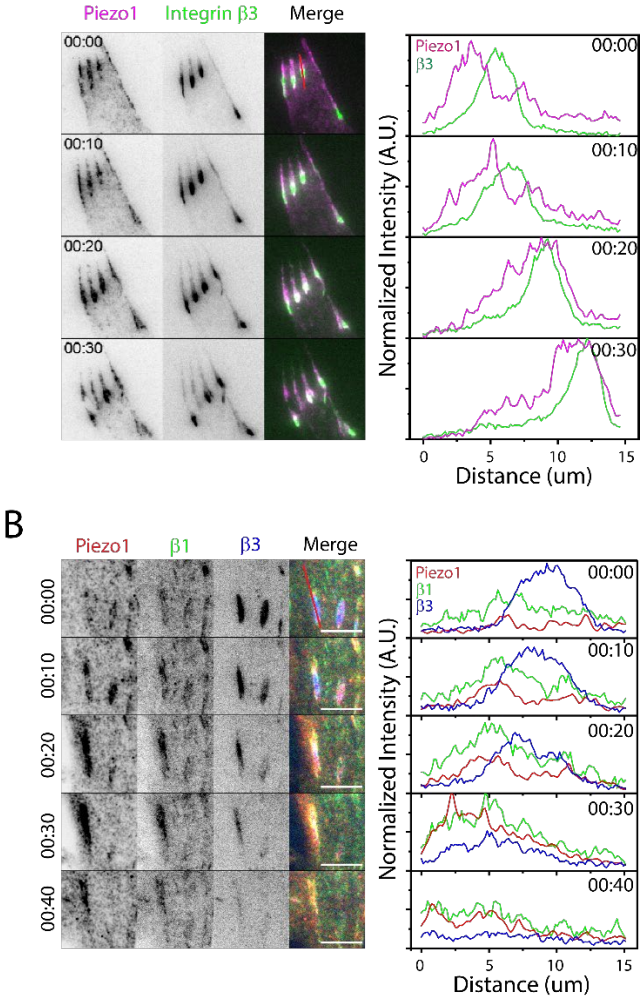


Figure S3. Dynamics of Piezo1 localization in relation to integrins. (A) Adhesion time lapse HFF cells transiently transfected with mEmerald-integrin β3 and Piezo1-mruby3. The right panel showed normalized intensity profiles of the adhesions marked by red line over time. (B) Adhesion time lapse of HFF cells transfected with BFP-integrin β3, mEmerald-integrin β1 and Piezo1-mruby3. The right panel showed normalized intensity profiles of the adhesions marked by red line over time. The normalizations were done by dividing the intensity profiles by the maximum intensity value observed for each protein in the whole time series. Scale Bars denote 10 µm.

Figure S4

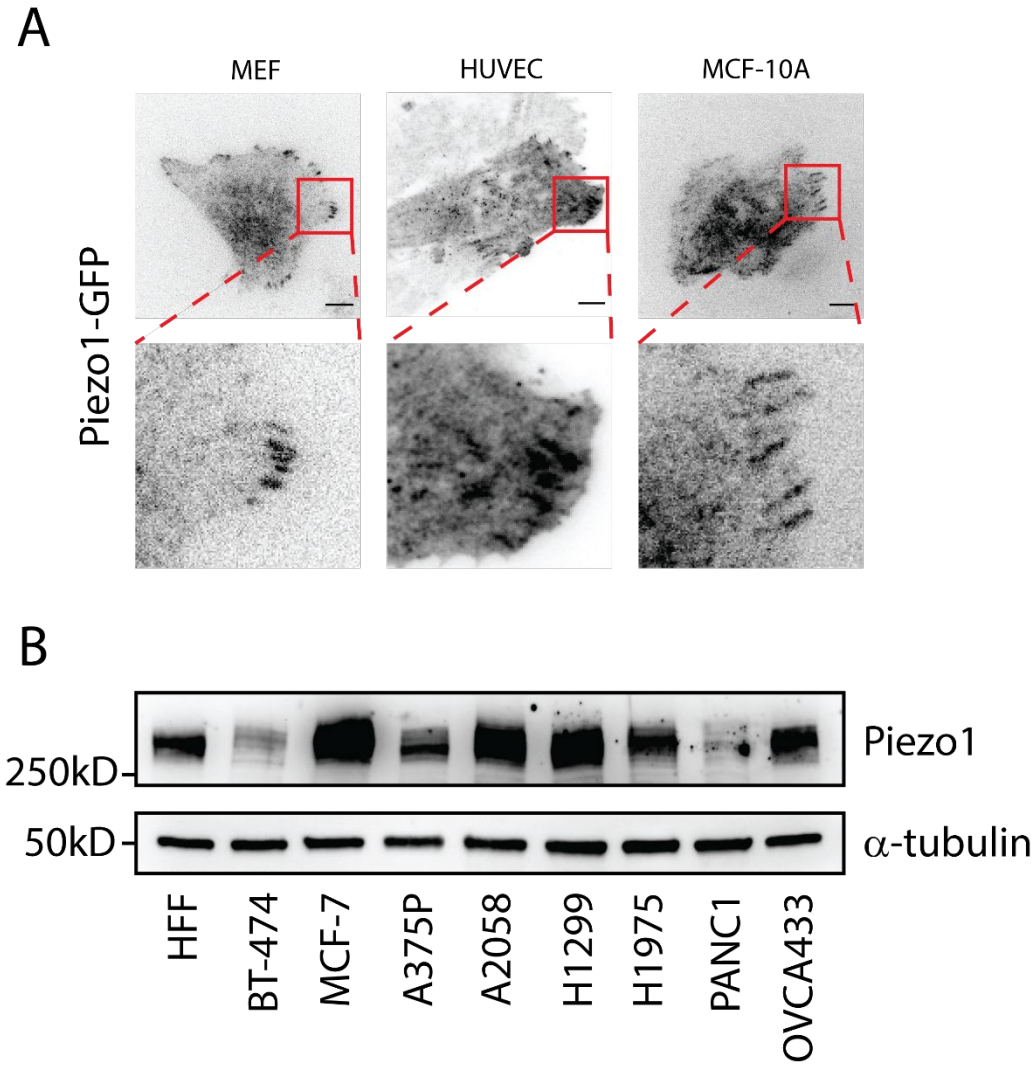


Figure S4 (A) TIRF live images of Piezo1-GFP localization in more normal cell lines tested. (B) Western blot images of Piezo1 expression in various cancer cell lines compared against HFF cells.

Figure S5

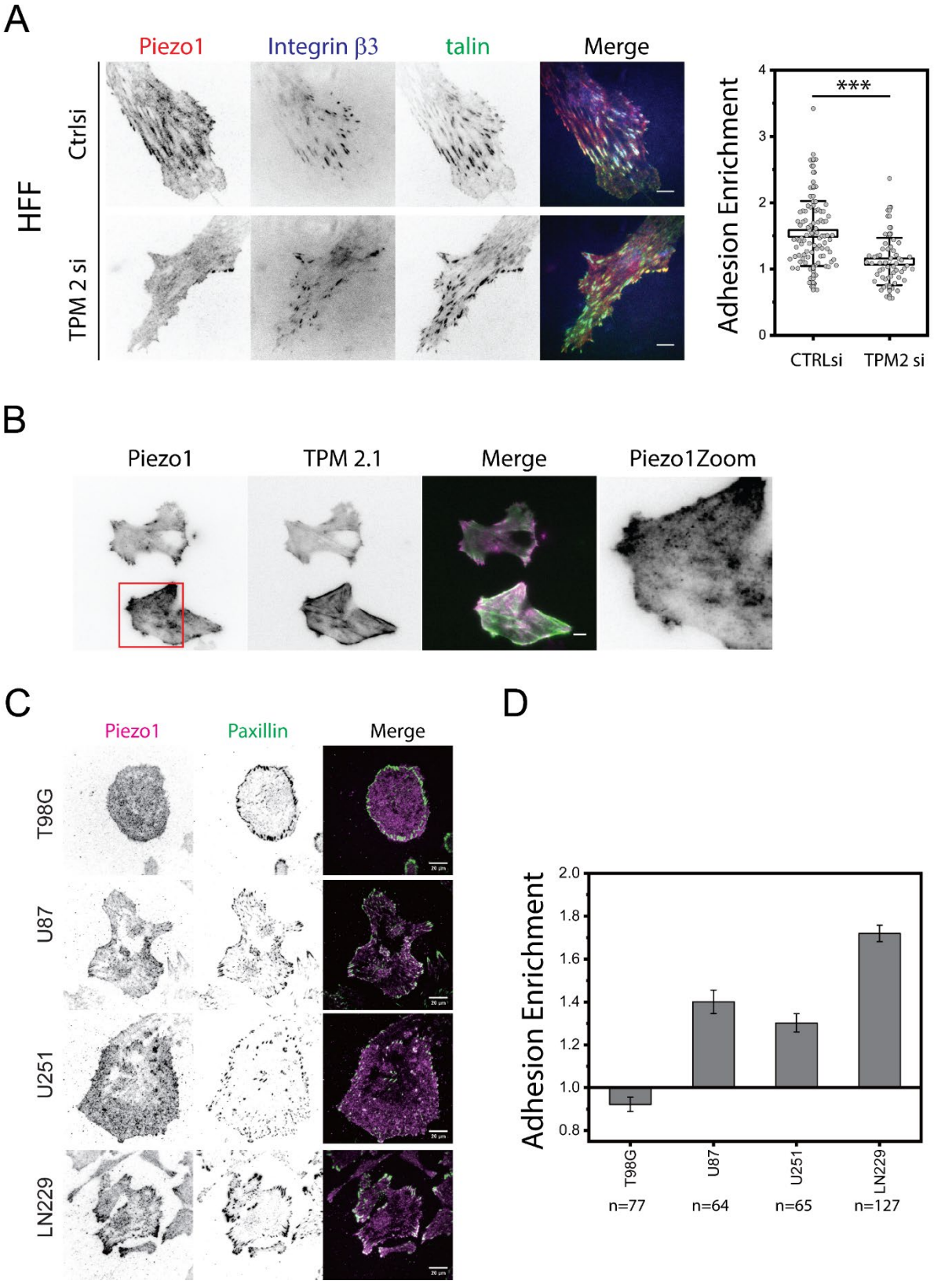

A
Piezo1
Integrin β3
talin
Merge
HFF
CtrlSi
TPM 2 si
Adhesion Enrichment
***
CTRLsi
TPM2 si
B
Piezo1
TPM 2.1
Merge
Piezo1Zoom
C
Piezo1
Paxillin
Merge
T98G
U87
U251
LN229
D
Adhesion Enrichment
T98G
U87
U251
LN229
n=77
n=64
n=65
n=127

Figure S5. Piezo1 localization in cells with altered transformation state. (A) Representative HFF cells with Control siRNA and TPM 2.1 siRNA knock down were transfected with Piezo1-mruby3, Integrin β3-BFP and talin-GFP. The cells were seeded on fibronectin surfaces overnight and imaged by TIRFM. Right Panel: quantification of adhesion enrichment with Ctrlsi and TPM 2.1 si. (B) Transient expression of TPM 2.1 - YFP in MDA-MB-231 cells led to the recruitment of Piezo1 to the end of stress fibres decorated by TPM 2.1 (TOP panel). The images present representative cells out of > 20 imaged. Scale Bars denote 10 μm. (C) Immunostaining of Piezo1 and Paxillin localization in several glioblastoma cell lines. (D) Quantification of Piezo1 adhesion enrichment for cells in panel (C). In several glioblastoma lines there are significant Piezo1 recruitment to focal adhesions.

Figure S6

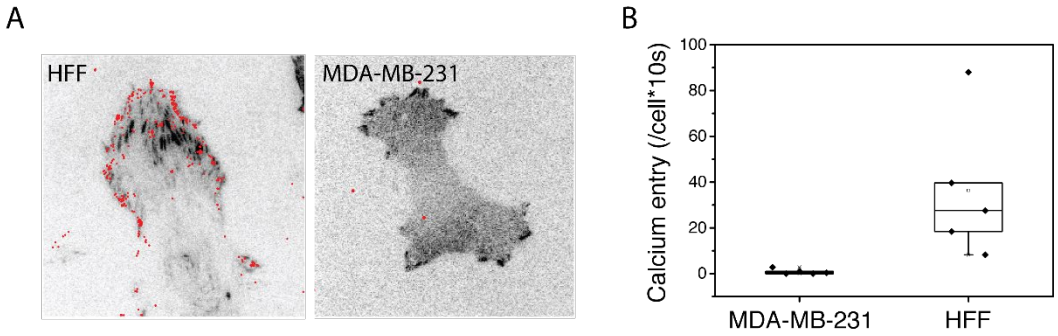


Figure S6 HFF and MDA-MB-231 cells showed distinct local calcium entry pattern. (A) Locations of local calcium entry events (marked by red dots) in HFF cells and MDA-MB-231 cells measured at 100 frames/s for 50s, overlaid with focal adhesions marked by paxillin. Significantly more local calcium signal is observed in HFF cells. (B). Box plot of the rate of local calcium events per cell for HFF and MDA-MB-231 cells. Each point denotes an independent cell.

Figure S7

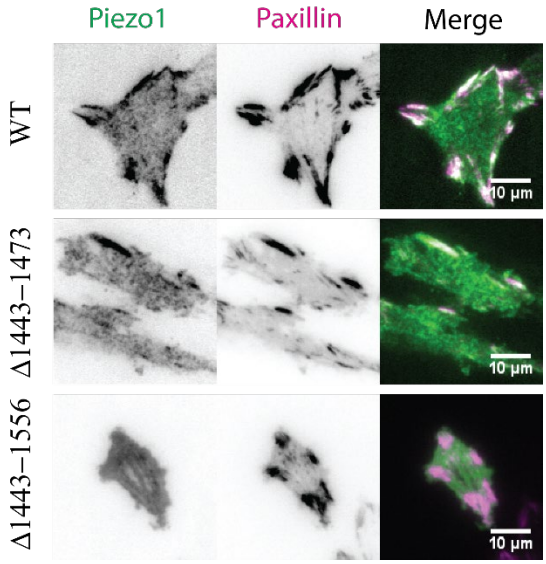


Figure S7 Linker domain mutants of Piezo1. Representative images of FAK-/- MEF cells transfected with paxillin-mapple and wild type/linker deletion mutant of Piezo1 with GFP-tag. Similar behaviors were observed in 3 biological repeats with > 10 cells for each condition.

Figure S8

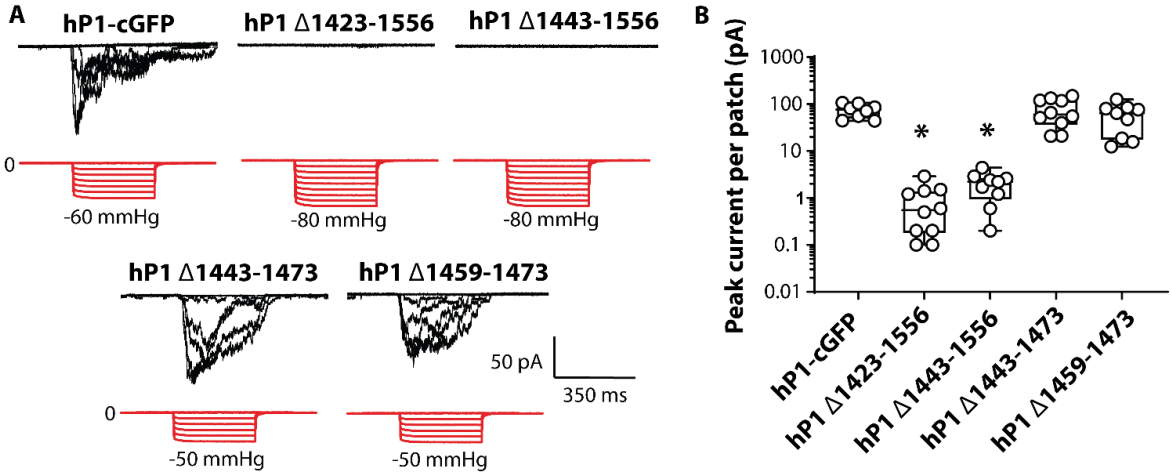


Figure S8. Determination of stretch activation of Piezo1 deletion mutants. (A) Representative patch-clamp traces from human Piezo1 c-terminal EGFP fusion (hP1-cGFP) in cell-attached patches at -60 mV holding potential expressed in Piezo1 KO HEK293T cells compared to four deletion mutants. Current traces are shown in black and square wave negative pressure pulses increasing from 0 mmHg in increments of -10 mmHg are shown in red with the peak negative pressure documented for clarity. (B) Box and whiskers plots indicating the peak current elicited per patch from negative pressure application with all mutant Piezo1 channels. * represents statistical significance from hP1-cGFP using one-way ANOVA with Dunnett's post hoc test.

Supplementary movie captions

Supplementary Movie 1. Timelapse of HFF cell initial spreading. The HFF cell was transiently labelled with paxillin-BFP (green) and Piezo1-mRuby3 (magenta).

Supplementary Movie 2. Timelapse of HFF cell transiently labelled with paxillin-BFP, integrin β3-mEmerald and Piezo1-mRuby3, treated with 30uM Y-27632 for 15 minutes followed by wash-out.

Supplementary Movie 3. Timelapse of HFF cell transiently labelled with integrin β3-BFP, IPA-GFP and Piezo1-mRuby3 before and after treatment with 30uM Y-27632.

Supplementary Movie 4-6. Timelapse of the focal adhesion dynamics of HFF cells transiently labelled with integrinβ3-mEmerald/ integrinβ1-mEmerald and Piezo1-mRuby3.

Supplementary Movie 7-10. Timelapse of HFF WT, HFF Piezo1 KD, HT1080 WT and MDA-MB-231 WT cells transfected with GCAMP7s-paxillin-mScarleti construct under identical imaging conditions. The Normalized ratios were obtained by taking the ratio image between the GCAMP7s and mScarleti channels.

Supplementary Movie 11-12. Timelapse of FAK -/- MEF cells co-expressing Δ1443-1473 Piezo1-GFP/ Δ1443-1556 Piezo1-GFP and paxillin-mapple.