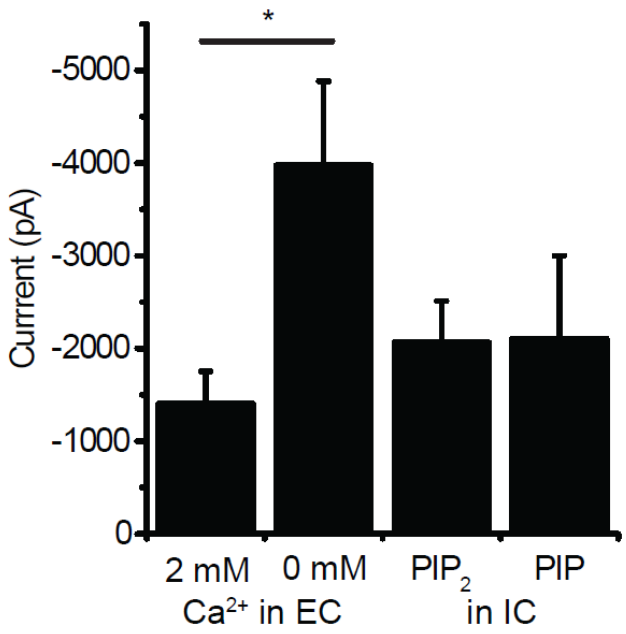

Figure S1: Capsaicin-induced inward (TRPV1) current amplitudes in HEK293 cells transfected with Piezo1 and TRPV1. Data were summarized from Figure 3 for control measurements in 2 mM Ca^{2+} containing extracellular solution, Ca^{2+} free extracellular solution, and for measurements with 40 µM $PI(4,5)P_2$ or 40 µM PI(4)P in the patch pipette. Data are shown and mean ± SEM, *p<0.05, ANOVA

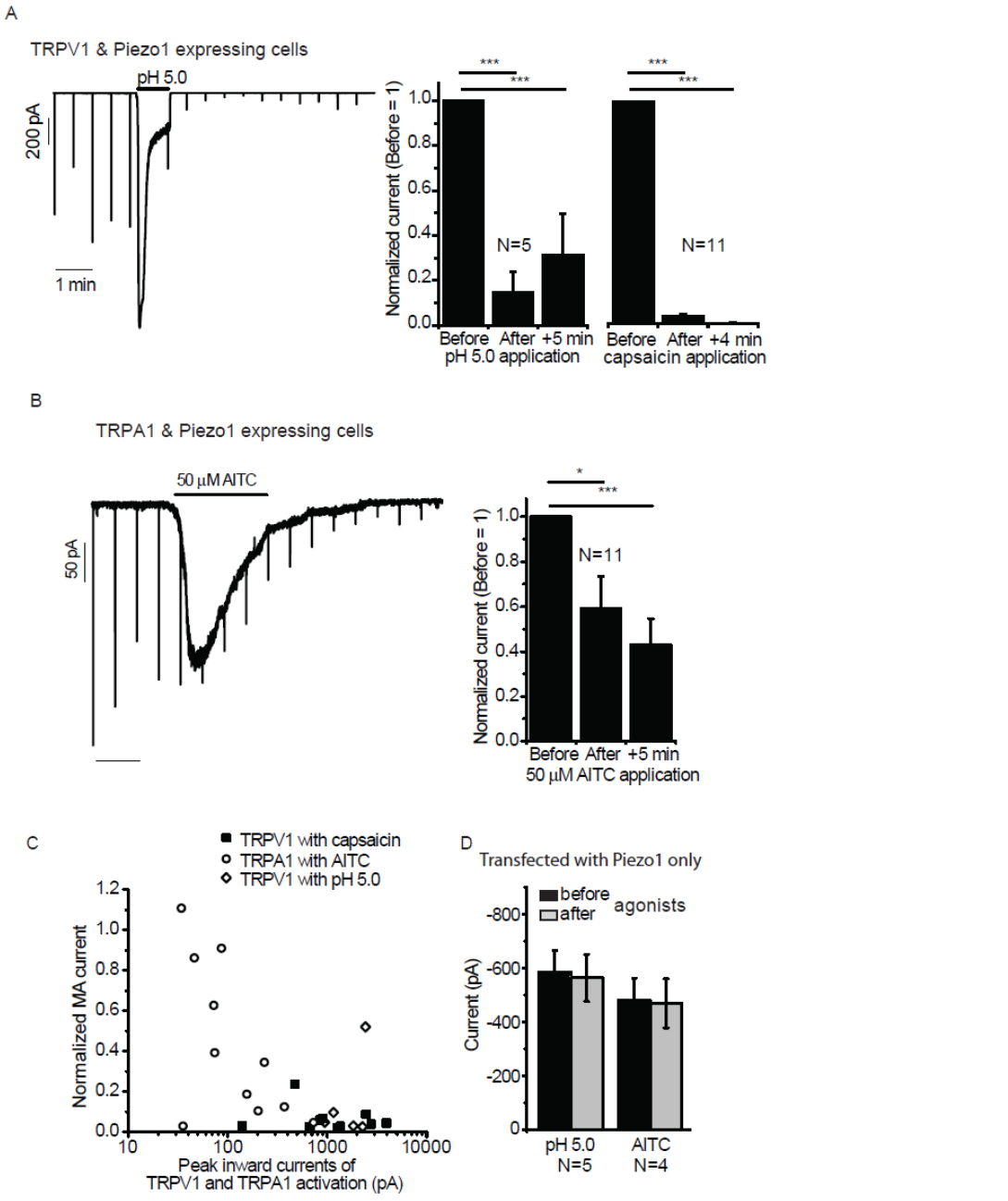

Figure S2. The effects of TRPV1 activation by low pH and TRPA1 activation by Allyl isothiocyanate (AITC) on Piezo1 activity. (**A**) Whole-cell patch clamp experiment in HEK293 cells cotransfected with TRPV1 and Piezo1. The application of low pH solution is shown by the horizontal line, middle panel shows statistical summary for the effect of low pH, right panel for the effect of capsaicin, re-plotted from Figure 3. (**B**) Whole-cell patch clamp experiment in HEK293 cells cotransfected with TRPA1 and Piezo1. The application of 50 µM AITC is shown by the horizontal line, right panel shows statistical summary. (**C**) Summary of the correlation between inward currents evoked by TRPA1 activation by AITC or TRPV1 activation by capsaicin or low pH (X axis), and the extent of inhibition of Piezo1 currents: MA current amplitudes after the application of the agonists were normalized to the initial current amplitudes (y axis). The symbols correspond to individual measurements. (**D**) Summary for the effect of low pH and 50 µM AITC on peak MA currents in cells transfected with Piezo1 alone. Data shown as mean ± SEM, *p≤0.05, ***p≤0.001; ANOVA.

Figure preparation guidance

Add the normalized capsaicin data to the bar graph so the reader can see this comparison. It is not possible to evaluate this easily by comparing the supplementary data with the data in Figure 3. Update the legend as needed to explain the addition of that data.

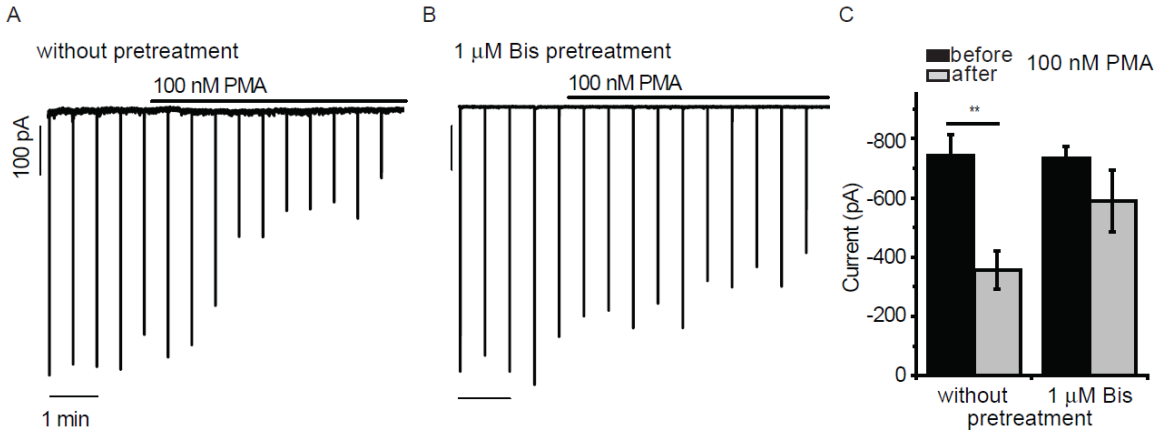

Figure S3. Effects of PKC activation on Piezo1-mediated MA currents. (**A**, **B**) Representative traces of the PKC activator phorbol 12-myristate 13-acetate (PMA, 100 nM) application without or with pretreatment for 10 min with the PKC inhibitor bisindolylmaleimide (Bis). (**C**) Statistical analysis of the peak current before and after application of PMA. Data shown as mean ± SEM, **p≤0.01; ANOVA.

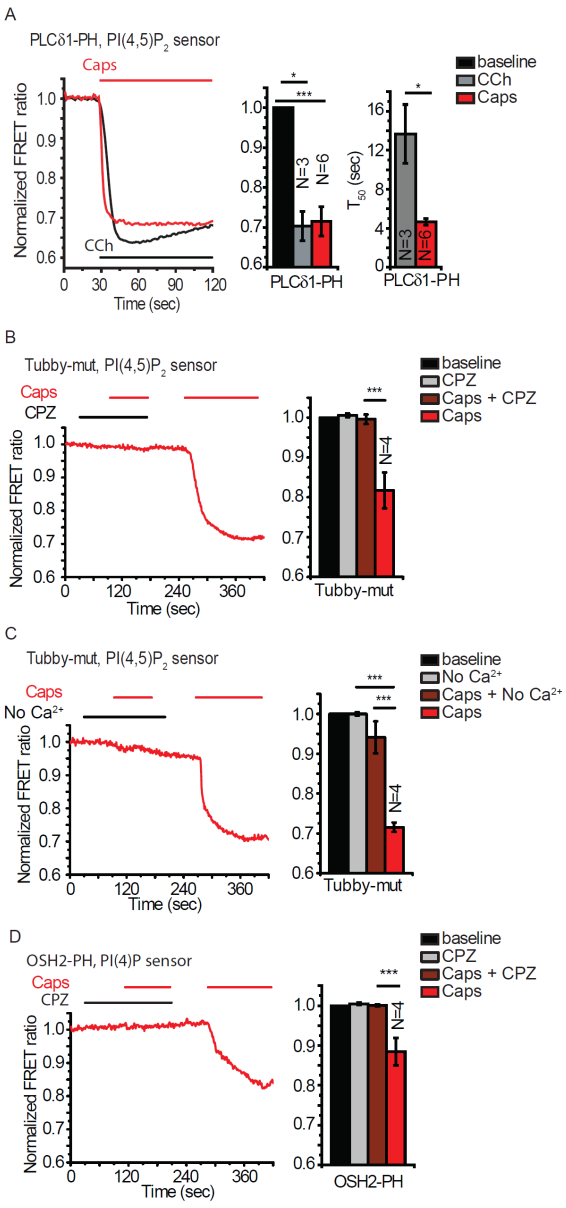

Figure S4. Fluorescence-based PI(4,5)P_2 and PI(4)P measurements in HEK293 cells cotransfected with hM1 or TRPV1 and various phosphoinositide sensors. (**A**) FRET measurements in cells expressing the PLCδ1-PH-domain PI(4,5)P_2 sensor. (A, left) Individual traces in response to 100 µM carbachol (CCh) in an hM1-cotransfected cell and in response to 1 µM capsaicin (Caps) in a TRPV1-cotransfected cell. (A, middle) Statistical analysis of FRET ratios. (A, right) FRET decay half times for cells exposed to carbachol or capsaicin. (**B**-**C**) Measurements in HEK293 cells transfected with the Tubby-mut PI(4,5)P_2 sensor and TRPV1. The applications of 10 µM capsazepine (CPZ), Ca^{2+} -free EC solution, and 1 µM capsaicin are shown by the horizontal lines. The right panel shows the statistical summary. (**D**) Measurements in HEK293 cells transfected with the OSH2-PH PI(4)P sensor and TRPV1. The application of 10 µM capsazepine and 1 µM capsaicin are shown by the horizontal lines. The right panel shows the statistical summary. Data shown as mean ± SEM, ***p≤0.001; ANOVA.

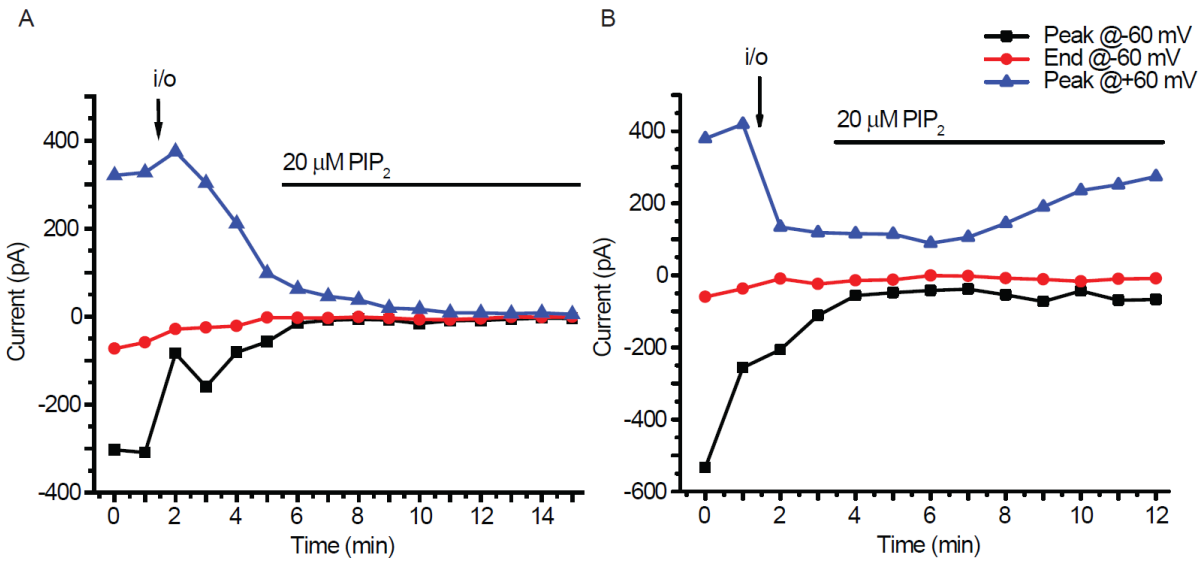

Figure S5: Attempts to reactivate Piezo1 currents in excised inside out patches in HEK293 cells. Measurements were performed as in Figure 7, blue triangle show currents at +60 mV, black squares at -60 mV at the peak of the MA current, and red circles show currents at -60 mV at the end of the -20 mmHg pressure step. (A) Representative trace for 3 almost identical measurements, where $PI(4,5)P_2$ did not reactivate Piezo1 currents after rundown; (B) a single measurement, with a slow and partial reactivation.

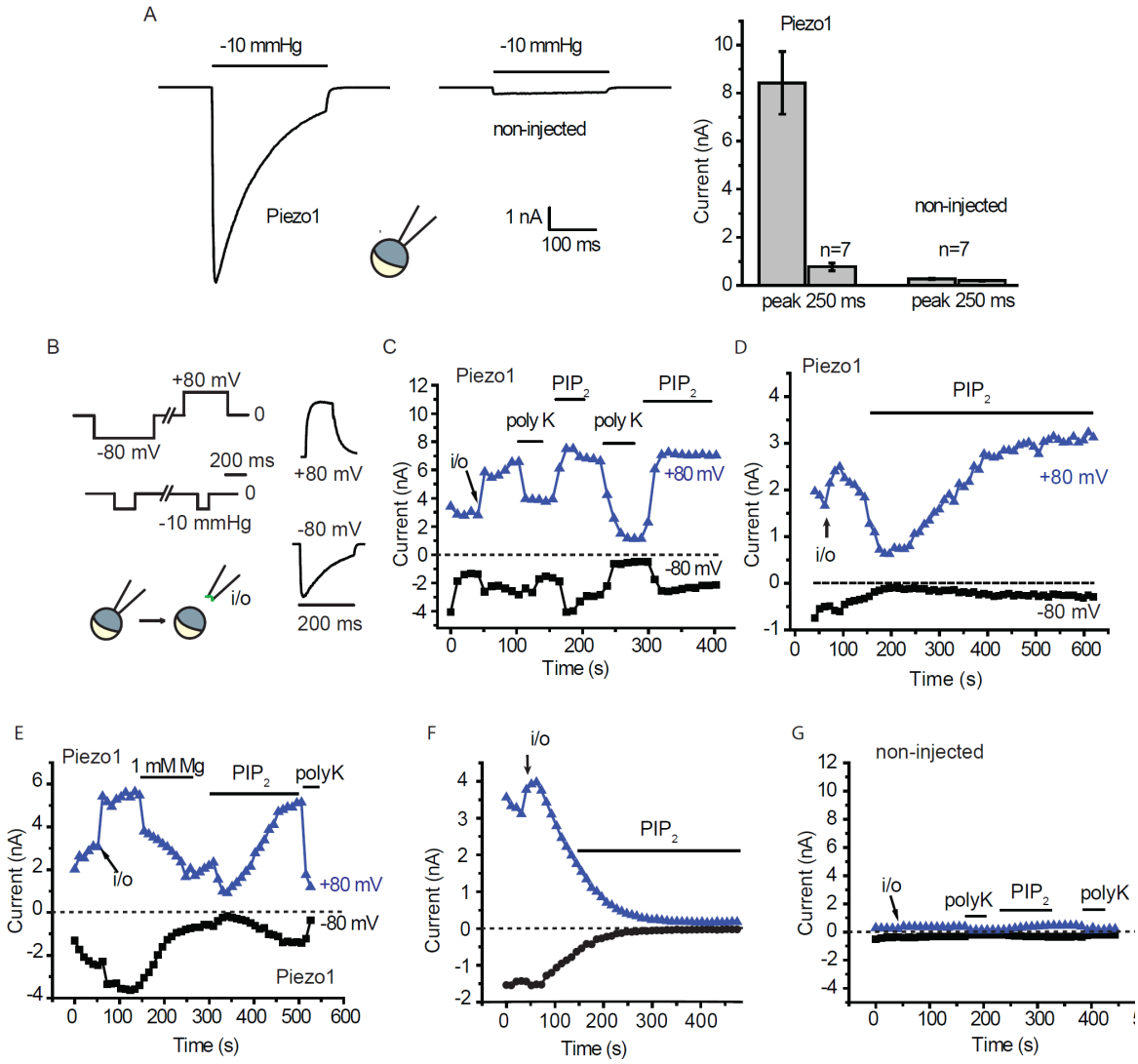

Figure S6. Cell-attached and excised inside-out and patch clamp measurements of MA currents in *Xenopus* oocytes. (**A**) Representative traces and summary for measurements in the cell-attached configuration for Piezo1-injected and noninjected oocytes. (**B**) The voltage and pressure protocol and representative traces for -80 mV and +80 mV. (**C**-**F**) Representative traces in Piezo1-injected oocytes and (**G**) in a noninjected oocyte. Repetitive mechanical stimuli of -10 mmHg were applied and currents were plotted at +80 and -80 mV. The applications of 30 µM poly-Lys (Poly K) and 10 µM AASt PI(4,5)P_2 (PIP_2) are indicated by the horizontal lines. The establishment of the inside-out (i/o) configuration is marked by arrows. Mg^{2+} (1 mM) is applied in (E) to stimulate lipid phosphatases to accelerate rundown.