

## Supplemental Figure Legends

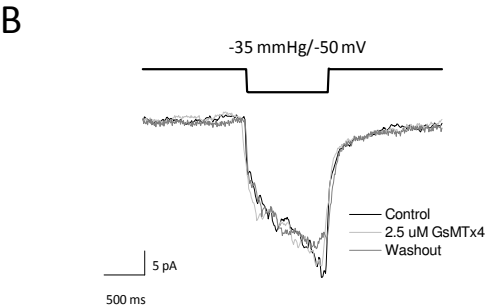
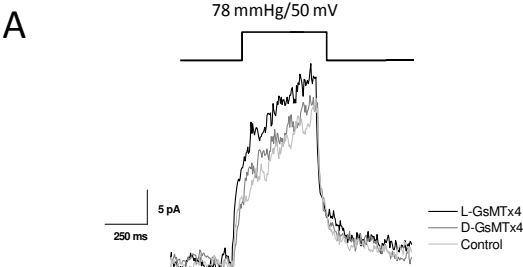
**Figure S1 Panel A.** Mechanically activated currents in O-O patches expressing the TREK-1 channel are not inhibited by GsMTx4. **Panel B** GsMTx4 applied to the intracellular side of inside-out patches does not inhibit Piezo1 activity..

**Figure S2.** GsMTx4 is a closed channel blocker and streptomycin is an open channel blocker in O-O patches. **Panel A** shows the effect of application of saturating GsMTx4 prior to mechanical stimulation. If GsMTx4 functions as a closed channel blocker, subsequent mechanical stimulation will show no channel activity and there was none. The black trace is the response of the O-O patch to the indicated pressure pulses prior to GsMTx4 perfusion (+50 mV holding potential to minimize inactivation). While that patch was at rest, GsMTx4 was applied for 6 s and then it was mechanically stimulated. There were no currents except at the highest pressures where the mechanical stimulus surpassed the prestress inhibition. The figure shows the inhibitory shift in the gating curve (red trace). The currents returned to control levels with washout [Note: a few channels remained active during washout]. **Panel B** shows that streptomycin, a non-specific inhibitor of cationic MSCs, is an open channel blocker displaying a classic use-dependent response. The upper trace indicates Piezo1 activity at +25 mmHg and +60 mV. Streptomycin was applied for 90 s prior to a series of 25 mmHg pulses. **Panel C**) The simulation and the response are shown in the left panel). Fitting that data with a simple three state model (closed-open-open/blocked), right panel) produced the rate constants indicated in the reaction diagram (MAC routine in QUB (20) ([www.qub.buffalo.edu](http://www.qub.buffalo.edu)). The rates are in units of  $s^{-1}$  and the states are: C=rest, O=open, I=open-inhibited. The rates are in the format  $k_0 \cdot \exp(q \cdot \text{pressure})$  where  $k_0$

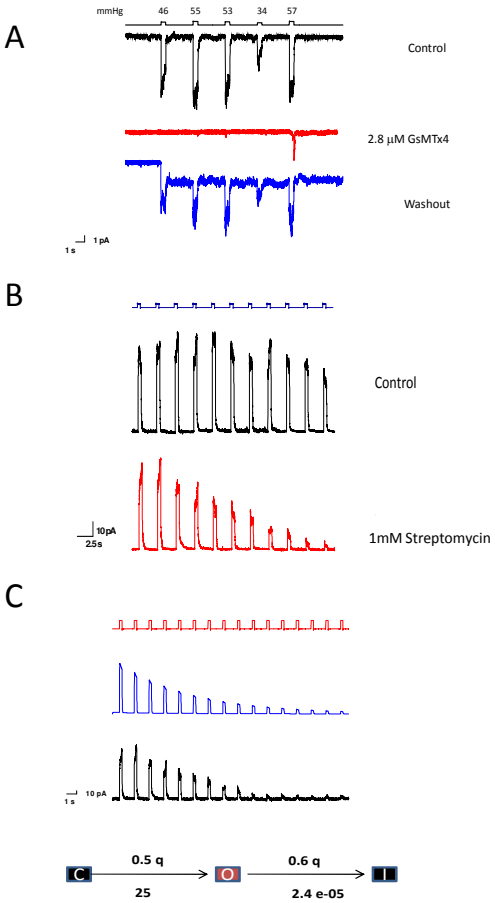
is the pre exponential shown in the diagram,  $q$  is the pressure-sensitivity of these rates, and  $q$  values were fixed to  $10 \text{ mmHg}^{-1}$  for  $k_{12}$  and  $-1$  for  $k_{23}$  after some trial and error fitting. The other rates were optimized. The unevenness of the full amplitude heights reflects sequential changes in patch mechanics with stimulation. For the opening rate  $k_{12}$ ,  $q$  reflects an increased rate of opening with increasing membrane tension (pressure) while for  $k_{23}$ , the value of  $q$  reflects a slower rate of block at higher tension.

**Figure S3** Panel A shows that endogenous stretch activated currents do not inactivate. Outside-out patches ( $n=3$ , HEK293 cells) produced currents between 70 to 100 mmHg. The data shown are averaged for 10 Pressure pulses each 500 ms long. Panel B shows that the unitary conductance is insensitive to pressure (membrane tension).

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

