Supporting Information

Maksaev and Haswell 10.1073/pnas.1213931109

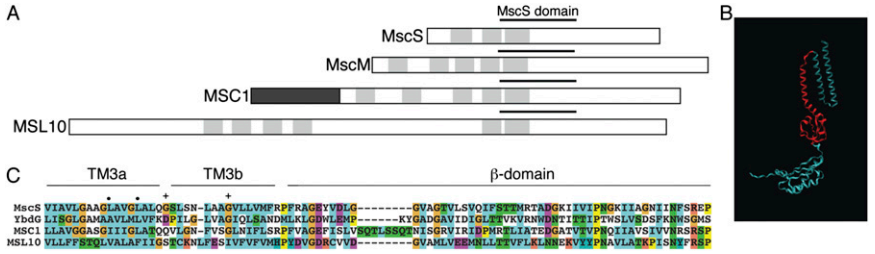

Fig. S1. Topology and conserved domains of select MscS homologs from bacteria, algae, and plants. (*A*) Topology of MscS (1) and predicted topology of MscS, MSC1, and MSL10. Dark gray box, chloroplast transit peptide; light gray boxes, transmembrane helices. (*B*) Ribbon diagram of a single subunit of MscS from the revised crystal structure (PDB ID code 20AU; refs. 2 and 3). The conserved MscS domain is indicated in red. (*C*) Alignment of "MscS domain" sequences from MscS and MscM (*Escherichia coli),* MSC1 (*Chlamydomonas reinhardtii),* and MSL10 (*Arabidopsis thaliana)*. Asterisks indicate conserved residues, + signs indicate residues in MscS that produce slow closure when mutated to alanine (4), and dots indicate the two hydrophobic seal residues. Sequences corresponding to TM3a, TM3b, and the β-domain of MscS are as in ref. 2.

1. Miller S, et al. (2003) Domain organization of the MscS mechanosensitive channel of Escherichia coli. *EMBO J* 22(1):36–46.
2. Bass RB, Strop P, Barclay M, Rees DC (2002) Crystal structure of Escherichia coli MscS, a voltage-modulated and mechanosensitive channel. *Science* 298(5598):1582–1587.
3. Steinbacher S, Bass RB, Strop P, Rees DC (2007) Structures of the Prokaryotic Mechanosensitive Channels MscL and MscS. *Mechanosensitive Ion Channels, Part A*, ed Hamill OP (Elsevier Academic Press, San Diego, CA), Vol 58.
4. Akitake B, Anishkin A, Liu N, Sukharev S (2007) Straightening and sequential buckling of the pore-lining helices define the gating cycle of MscS. *Nat Struct Mol Biol* 14(12):1141–1149.

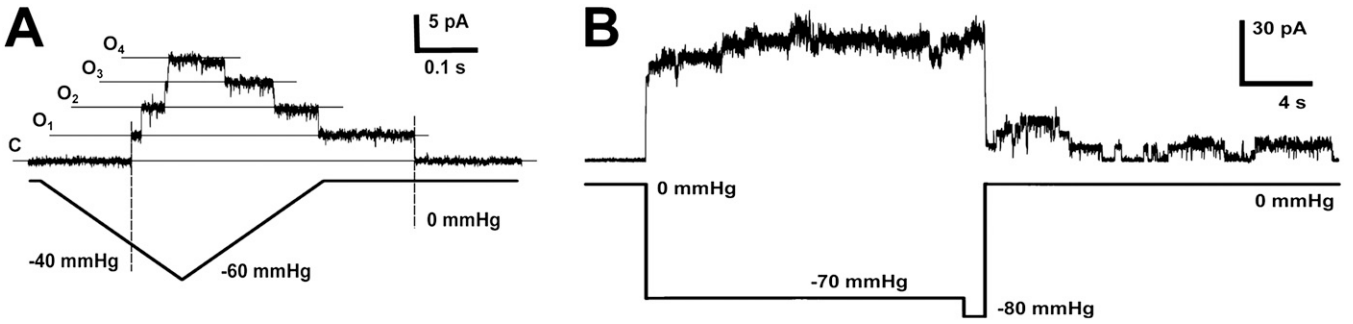

Fig. S2. MSL10 hysteresis and residual activity in high $MgCl_2$. (*A*) An example of slow closure of MSL10. Membrane potential −40 mV, bubble number (BN) 5. (*B*) Residual activity of MSL10 at zero applied tension. Membrane potential −60 mV, BN 4.5. Both *A* and *B* were performed in symmetric 60 mM $MgCl_2$ buffer.

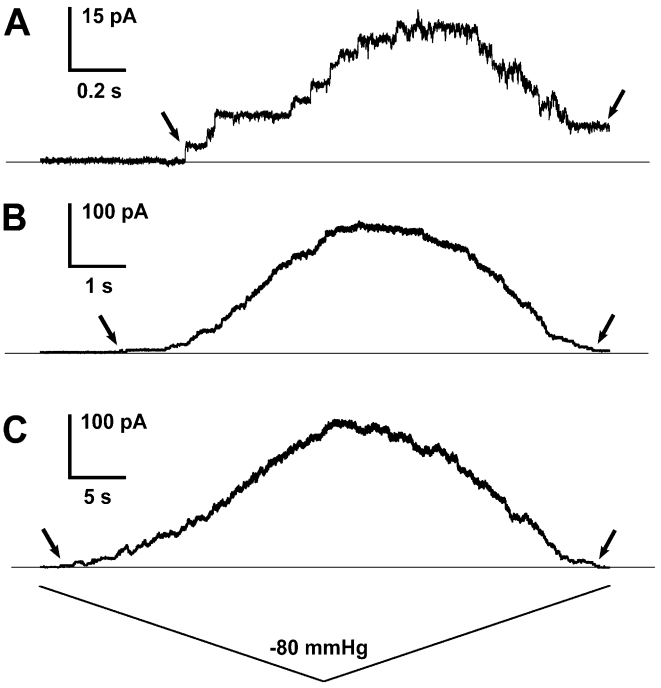

Fig. S3. Effect of ramp speed on the threshold pressure for MSL10 opening and closing. Traces were obtained from the same patch subject to pressure ramps of various length: 1 s (*A*), 5 s (*B*), and 25 s (*C*). Arrows indicate opening and closing pressure thresholds. Membrane potential −30 mV, pipette BN 5, symmetric ND96 buffer.

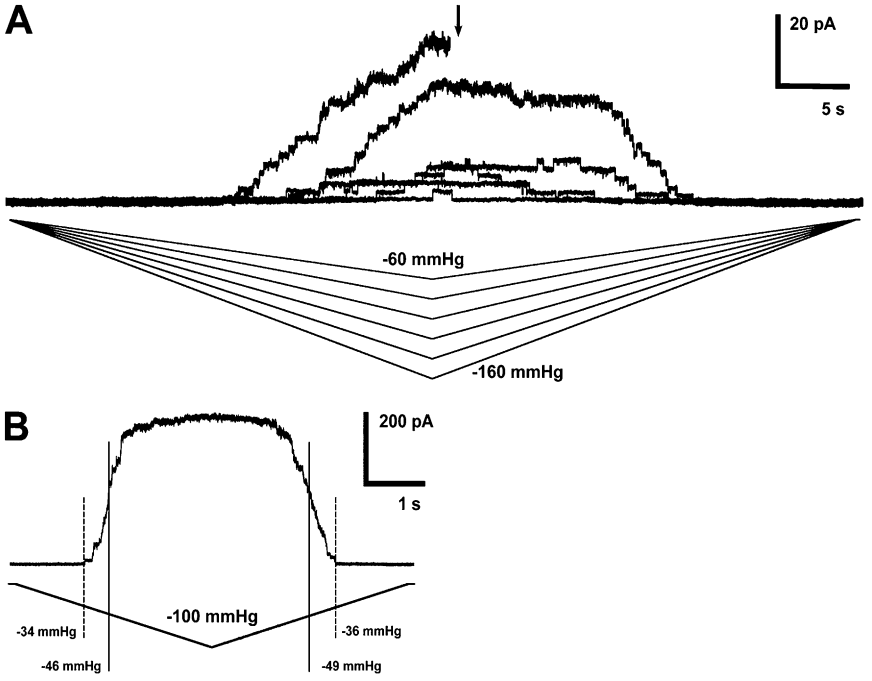

Fig. S4. (*A*) MSL10 dose–response traces from an excised inside-out patch. Arrow indicates the point where the patch collapsed. (*B*) Illustration of the easily achievable current saturation and lack of hysteresis in MscS expressed in *Xenopus* oocytes. Dotted lines indicate opening and closing pressure thresholds and dashed lines indicate midpoints of the activation curve. Both traces were recorded from excised inside-out patches from the same batch of oocytes, in symmetric ND96 buffer, pipette BN 4.5, at a membrane potential of −20 mV.