Supplementary Information for

The mechanosensitive channel of small conductance (MscS) functions as a Jack-In-The-Box

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	Forward	Reverse
V29A	GCTGCTAAGTTATGCAGCAAACATCGTGGCGGCACTCGCGGTC	GACCGCGAGTGCCGCCACGATGTTTGCTGCATAACTTAGCAGC
V29L	TGCTGCTAAGTTATGCATTAAACATCGTGGCGGCACTCGCGGTC	GACCGCGAGTGCCGCCACGATGTTTAATGCATAACTTAGCAGCA
V32A	ATGCAGTAAACATCGCGGCGGCACTCGCGG	CCGCGAGTGCCGCCGCGATGTTTACTGCAT
V32L	AAGTTATGCAGTAAACATCTTGGCGGCACTCGCGG	CCGCGAGTGCCGCCAAGATGTTTACTGCATAACTT
A36L	GTAAACATCGTGGCGGCACTCCTAATCATCATCGTTGGTTTGATT	AATCAAACCAACGATGATGATTAGGAGTGCCGCCACGATGTTTAC
V40A	ACTCGCGGTCATCATCGCTGGTTTGATTATCGCGC	GCGCGATAATCAAACCAGCGATGATGACCGCGAGT
V40L	CACTCGCGGTCATCATCCTTGGTTTGATTATCGCG	CGCGATAATCAAACCAAGGATGATGACCGCGAGTG
I43L	GGTCATCATCGTTGGTTTGCTTATCGCGCGGATGATTTC	GAAATCATCCGCGCGATAAGCAAACCAACGATGATGACC
I44L	CGGTCATCATCGTTGGTTTGATTCTCGCGCGGATGA	TCATCCGCGCGAGAATCAAACCAACGATGATGACCG
M47A	GGTTTGATTATCGCGCGGGCGATATCCAACGCGGTGAA	TTCACCGCGTTGGATATCGCCCGCGCGATAATCAAACC
M47L	GTTTGATTATCGCGCGGTTGATATCCAACGCGGTG	CACCGCGTTGGATATCAACCGCGCGATAATCAAAC
A51L	CGCGCGGATGATATCCAACCTAGTGAATCGCCTGATGATCT	AGATCATCAGGCGATTCACTAGGTTGGATATCATCCGCGCG
N53A	TGATATCCAACGCGGTGGCTCGCCTGATGATCTCCC	GGGAGATCATCAGGCGAGCCACCGCGTTGGATATCA
N53L	GATGATATCCAACGCGGTGCTACGCCTGATGATCTCCCGTA	TACGGGAGATCATCAGGCGTAGCACCGCGTTGGATATCATC
L55A	CCAACGCGGTGAATCGCGCGATGATCTCCCGTAAAATA	TATTTTACGGGAGATCATCGCGCGATTCACCGCGTTGG
S58A	GTGAATCGCCTGATGATCGCCCGTAAAATAGATG	CATCTATTTTACGGGCGATCATCAGGCGATTCAC
S58L	GCGGTGAATCGCCTGATGATCTTACGTAAAATAGATGCC	GGCATCTATTTTACGTAAGATCATCAGGCGATTCACCGC
Outer cloning primers	CAATTTCACACAGGAAACAGGC	TACACGGAGGCATCAGTGACCAAA

Table S1: PCR primers for point mutations to alanine or leucine to MscS in the pB10b vector.

	Percent Recovery
MscS	106 ± 9 %
V29A	118 ± 9 %
V29L	100 ± 1 %
V32A	104 ± 8 %
V32L	103 ± 6 %
A36L	103 ± 4 %
V40A	107 ± 8 %
V40L	101 ± 9 %
I43A	75 ± 7 %
I43L	112 ± 12 %
I44A	91 ± 11 %
I44L	105 ± 3 %
M47A	98 ± 8 %
M47L	103 ± 7 %
A51L	95 ± 4 %
N53A	100 ± 5 %
N53L	92 ± 3 %
L55A	100 ± 5 %
S58A	93 ± 6 %
S58L	106 ± 6 %
Empty Plasmid	7±1 %

Table S2: Results from osmotic downshock analysis of wildtype MscS and MscS harboring mutations of lipid lining residues mutated to either alanine or leucine. Error bars represent the standard error of the mean for 6 independent trials.
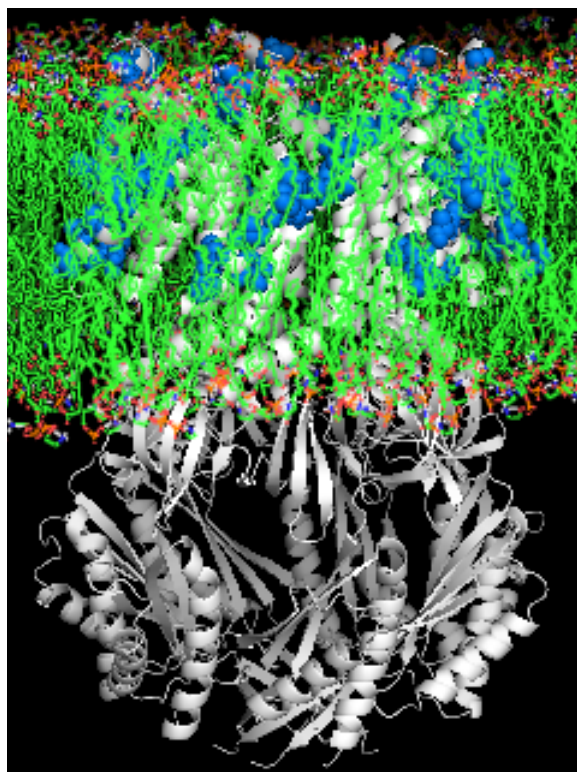
Figure S1: Open state lipid interactions. The open state crystal structure of MscS is shown embedded into a POPE membrane with the predicted lipid interacting residues (V29, V32, A36, V40, I43, I44, M47, A51, N53, L55, and S58) highlighted in blue [1].

	LB+Ap						Un-induced Score	LB+IPTG+Ap						Induced Score	Ratio	Steady State at OD$_{600}$
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}			
MscS							4.0±0.8							3.5±0.6	0.9±0.2	1.74±0.02
V29A							4.0±0.0							4.5±1.0	1.1±0.3	1.69±0.05
V29L							4.25±0.5							4.0±0.0	0.9±0.1	1.69±0.02
V32A							4.25±0.5							3.75±1.0	0.9±0.3	1.67±0.02
V32L							4.25±0.5							4.0±0.0	0.9±0.1	1.69±0.01
A36L							4.0±0.0							3.75±1.0	0.9±0.2	1.71±0.03
V40A							3.75±1.0							4.0±0.8	1.1±0.4	1.67±0.02
V40L							4.25±0.5							3.75±0.5	0.9±0.2	1.66±0.01
I43A							4.25±0.5							3.75±1.0	0.9±0.3	1.65±0.03
I43L							3.75±1.0							4.0±0.0	1.1±0.3	1.69±0.02
I44A							3.75±0.5							4.25±0.5	1.1±0.2	1.66±0.05
I44L							4.5±0.6							3.5±0.6	0.8±0.2	1.69±0.05
M47A							3.5±0.6							4.25±0.5	1.2±0.3	1.68±0.01
M47L							4.25±0.5							3.75±0.5	0.9±0.2	1.65±0.02
A51L							3.5±0.6							3.75±0.5	1.1±0.2	1.63±0.03
N53A							3.75±0.5							4.5±0.6	1.2±0.2	1.63±0.04
N53L							4.25±0.5							4.0±0.0	0.9±0.1	1.66±0.03
L55A							4.25±0.5							4.25±0.5	1.1±02	1.66±0.02
S58A							4.0±0.0							4.25±0.5	1.1±0.1	1.66±0.04
S58L							4.0±0.8							3.75±0.5	0.9±0.2	1.65±0.07

Figure S2: Gain of function analysis of MscS mutations. Representative bacteria growth on LB medium with and without IPTG is shown for all mutations. Plates were scored by counting the number of dilutions with bacterial growth. The ratio of induced to uninduced was determined by dividing the induced score by the uninduced score. The steady state optical density was measured at 600 nm. In all cases, error represents the standard deviation for four independent trials.

References
[1] W. Wang, S.S. Black, M.D. Edwards, S. Miller, E.L. Morrison, W. Bartlett, C. Dong, J.H. Naismith, I.R. Booth, The structure of an open form of an E. coli mechanosensitive channel at 3.45 A resolution, Science, 321 (2008) 1179-1183.