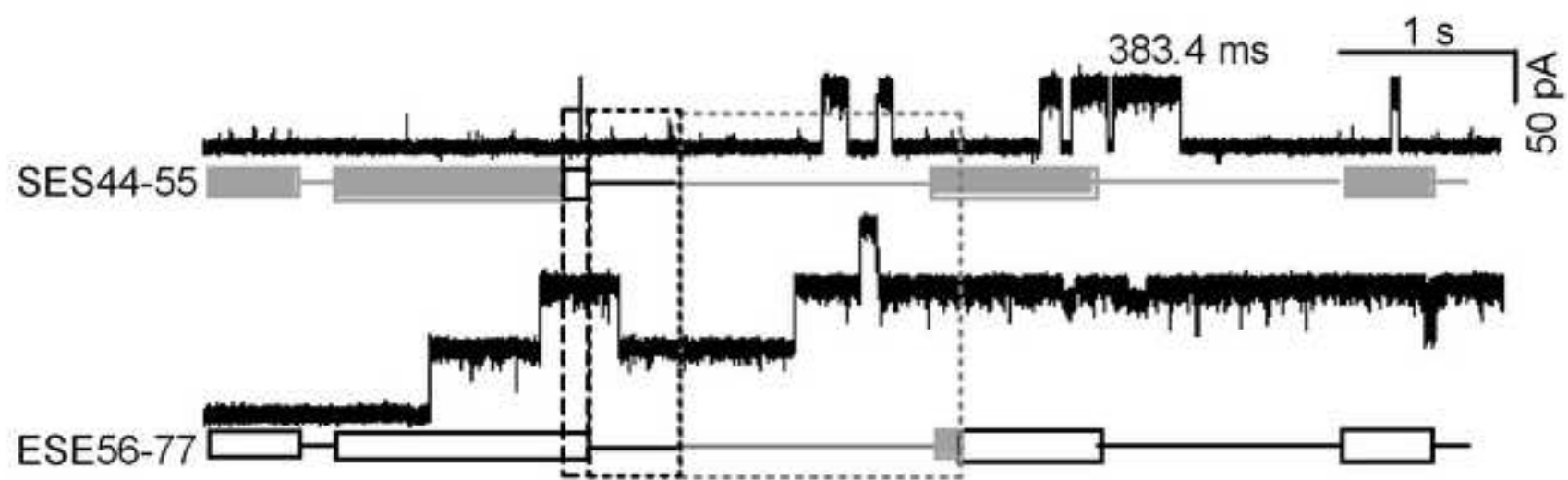


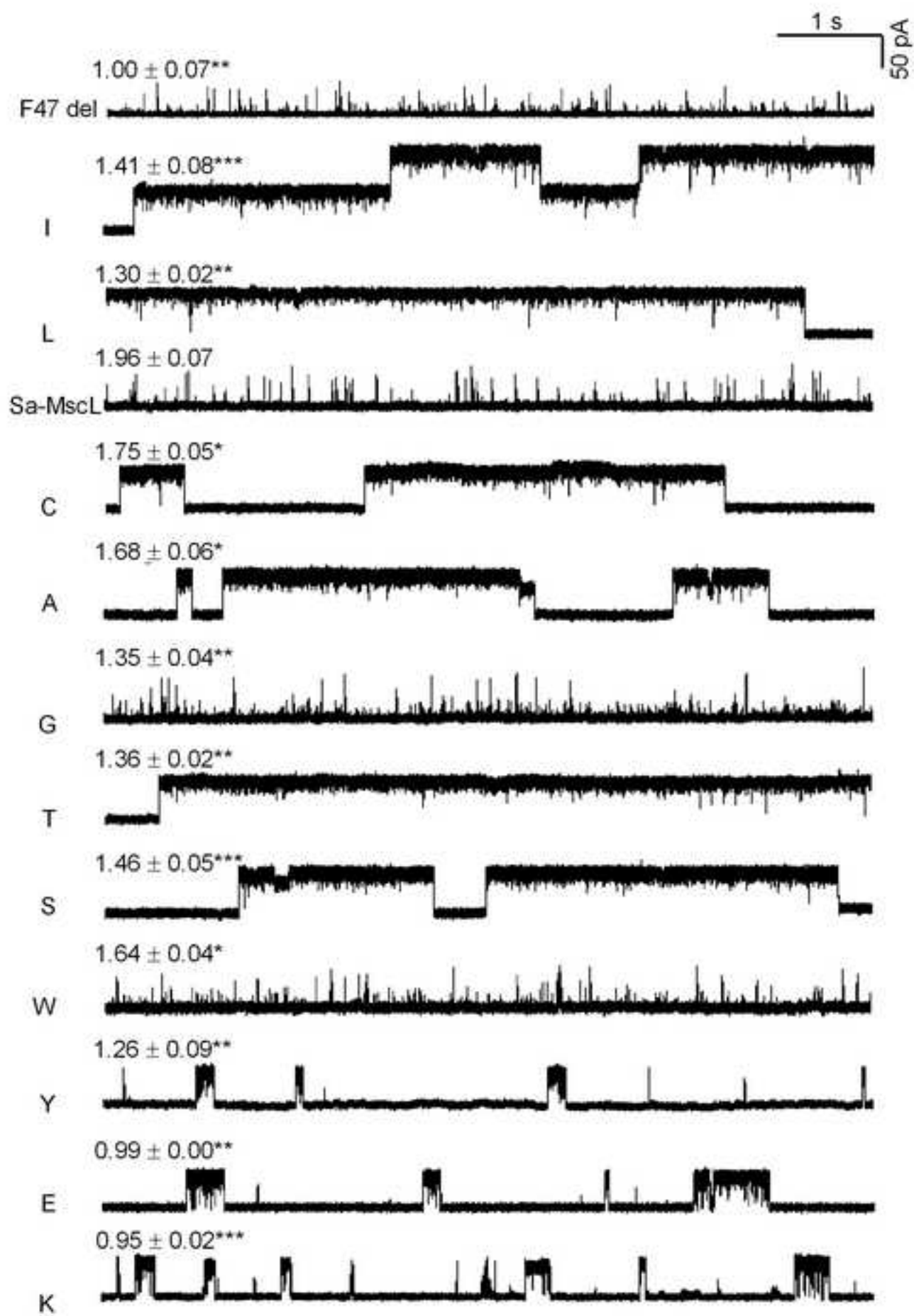
Figure S1. The periplasmic loop cannot be easily divided into functional subdomains. Shown are chimeras with the region aa 44-49 from Eco-MscL highlighted by the dashed black box, aa 50-55 from Eco-MscL highlighted by the dotted black box, and aa 56-77 from Sa-MscL highlighted by the dotted gray box. The scale bar is shown top right.

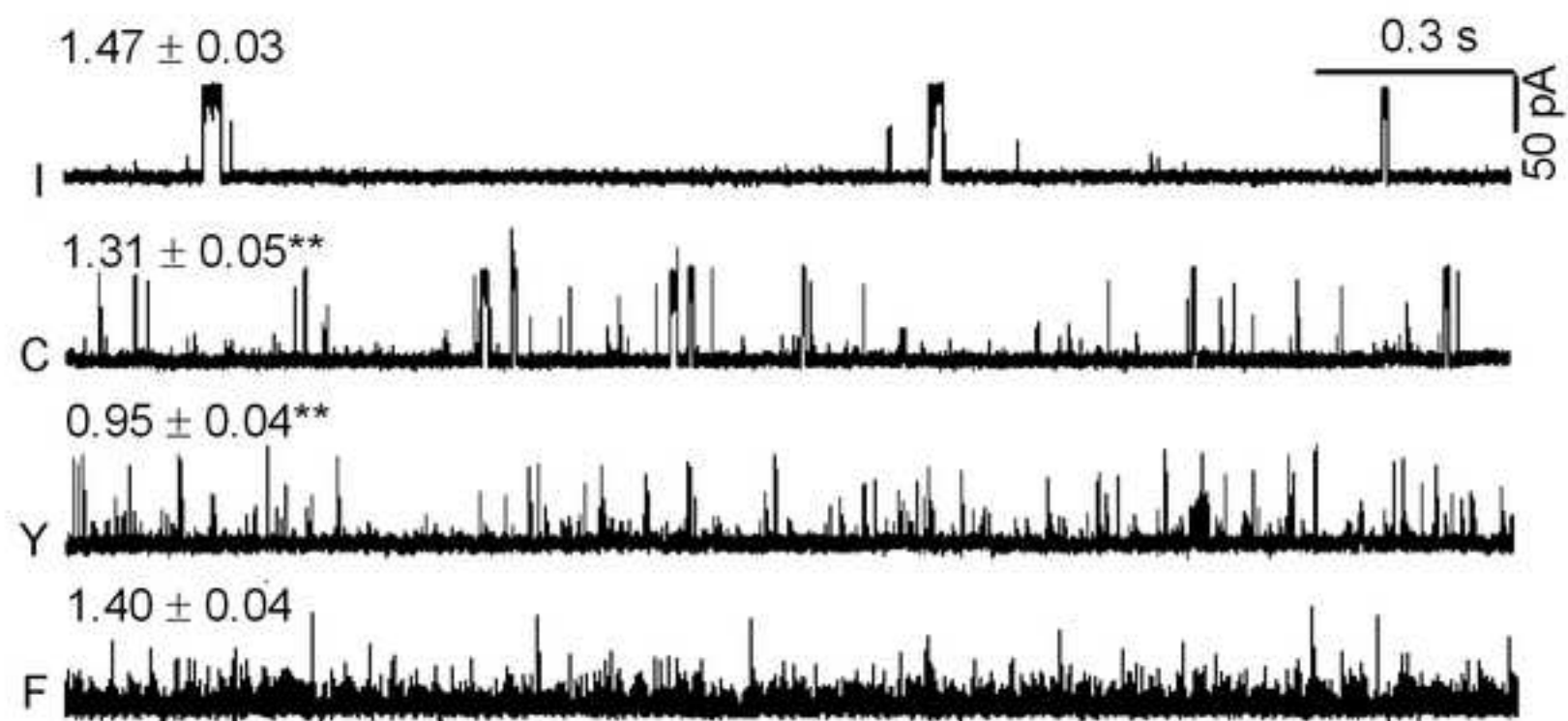
Figure S2. Mutation of F47 in *S. aureus* MscL drastically changes the channel open dwell time and mechanosensitivity. Representative traces of single-substituted mutants of F47 in *S. aureus* MscL. “F47 del” refers to F47 deletion. At top right of the traces is presented the scale bar. The pL/pS value of each mutant was shown in mean \pm s. e. m at top left of each trace.

Figure S3. Mutation of I49 in *E. coli* MscL causes changes in channel open dwell time and mechanosensitivity. Single-site substitutions of I49 in *E. coli* MscL were generated for patch clamp recording. Wild type *E. coli* MscL and I49C mutant are channels with open dwell times longer than I49Y and I49F mutants. pL/pS ratios expressed as mean \pm s.e.m are shown at top left of each trace, and the scale bar is shown at the top right.

Table S1. Channel open dwell time analyses of *S. aureus* MscL (Sa-MscL) wild type (WT) and site-directed mutants at position F47. Kinetic analysis of the open dwell time was fitted to a three-open state model. The two longest time constants (τ_2 and τ_3) are expressed as the mean \pm s.e.m, the short constant (τ_1) is below resolution (<1 ms) and is not shown.







Kyte Doolittle score	Mutant	Open dwell times (ms)	
		τ_2	τ_3
	F47 Δ	0.1 ± 0.0	0.4 ± 0.1
4.5	F47I	16 ± 4	227 ± 6
3.8	F47L	17 ± 7	307 ± 8
2.8	Sa-MscLWT	0.3 ± 0.0	0.9 ± 0.1
2.5	F47C	2.8 ± 0.1	117 ± 18
1.8	F47A	11 ± 1	118 ± 25
-0.4	F47G	0.6 ± 0.1	1.0 ± 0.0
-0.7	F47T	16 ± 6	254 ± 63
-0.8	F47S	3.3 ± 1.0	92 ± 12
-0.9	F47W	0.4 ± 0.1	1.6 ± 0.1
-1.3	F47Y	2.2 ± 0.2	11 ± 2
-3.5	F47E	1.0 ± 0.0	5.8 ± 0.3
-3.9	F47K	1.8 ± 0.5	23 ± 2