Supplementary Information:

Controlled delivery of bioactive molecules into live cells using the bacterial mechanosensitive channel MscL

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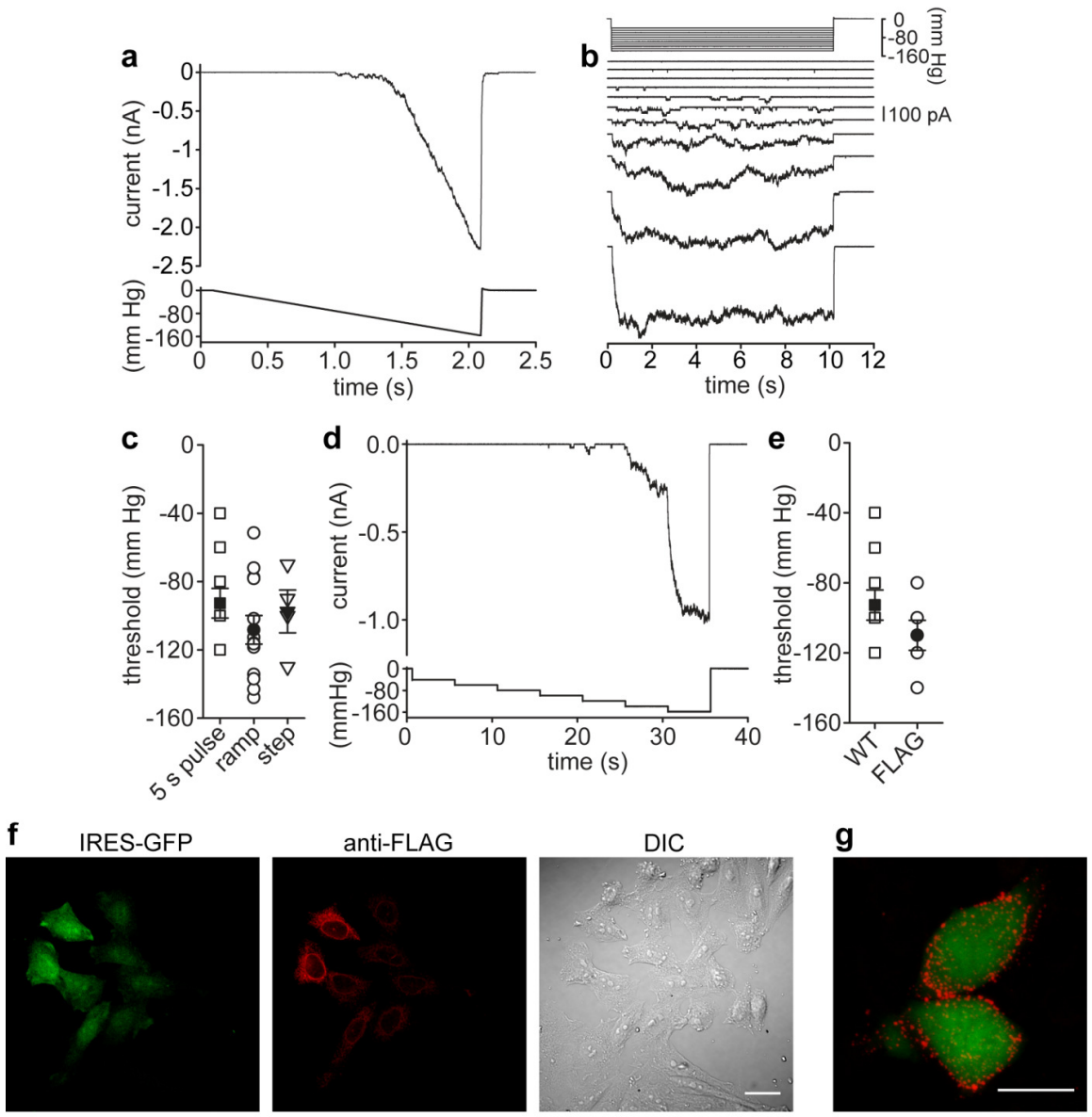
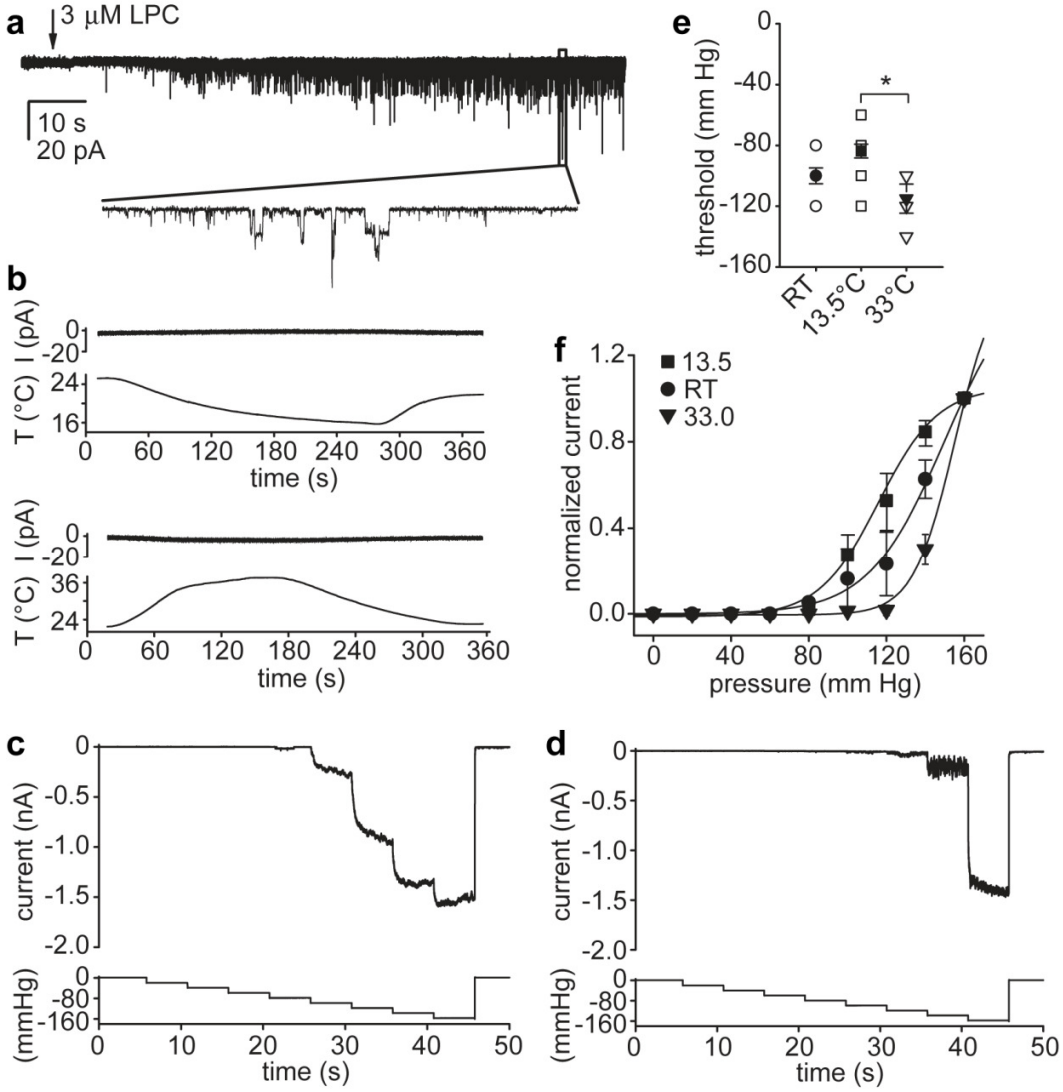
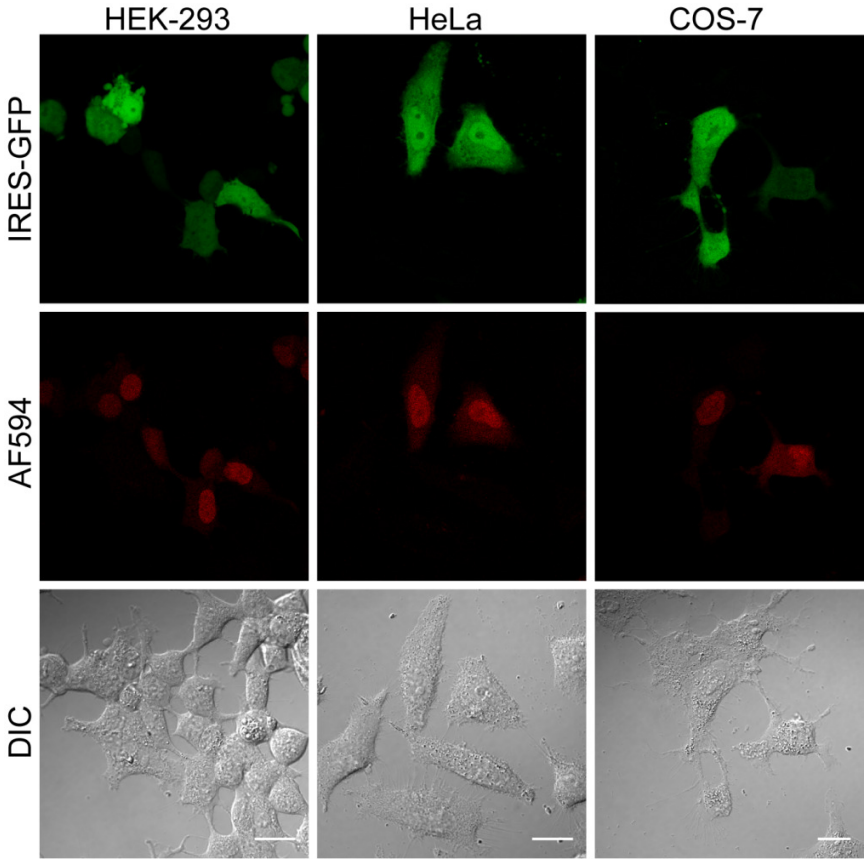
Supplementary Figures S1-S5



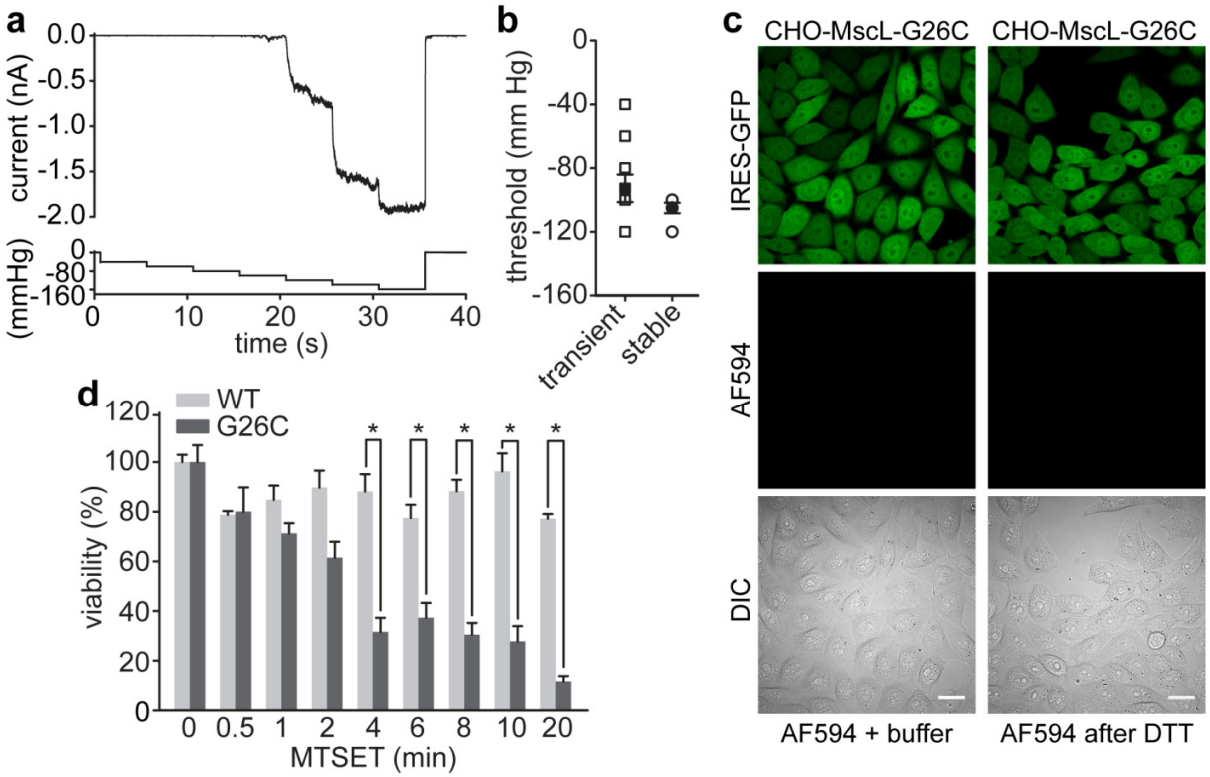
Supplementary Figure S1. Functional expression of *E.coli* MscL in mammalian cell lines. (a, b) Representative currents recorded from inside-out patches of MscL expressing CHO cells in response to a 2 s pressure ramp (a) or successively (10 mm Hg) increasing pressure steps (10 s) (b) at V_m = -10 mV. (c) MscL mean pressure threshold (filled symbols) in CHO cell membrane patches is consistent irrespective of the stimulation protocol (5 s pulse, -92.7 ± 8.6 mm Hg, *n* = 11; ramp, -108.3 ± 8.4 mm Hg, *n* = 13; step, -97.5 ± 12.5 mm Hg, *n* = 4). (d) Representative current recorded from a membrane patch expressing FLAG-tagged MscL at V_m = -10 mV. (e) MscL WT and FLAG-tagged channels display a comparable mean pressure threshold (filled symbols). Channels typically activate when the negative pressure exceeds -92.7 ± 8.6 mm Hg (WT, *n* = 11) and -110 ± 8.6 mm Hg (MscL-FLAG, *n* = 6), respectively. (f) Staining of fixed and permeabilized CHO cells expressing FLAG-tagged MscL in a bicistronic IRES-GFP vector. (g) MscL distribution on the cell surface. Average intensity z-projection of a stack of 14 confocal sections of anti-FLAG stained live CHO cells. Error bars in panel c and e represent SEM; note, several individual data points (open symbols) in c and e have the same value and thus are hidden; DIC, differential interference contrast. Scale bars, 20 µm.



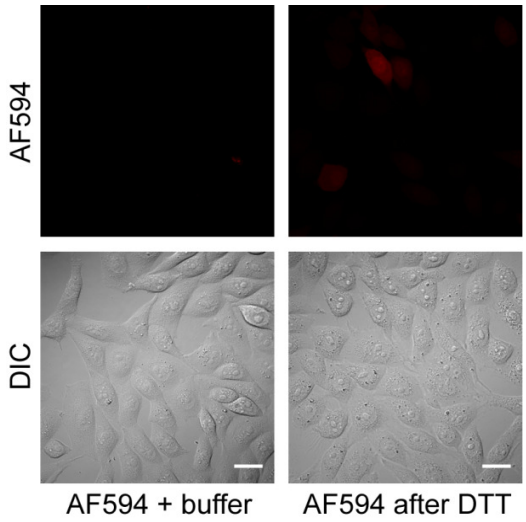
Supplementary Figure S2. Changes in the transbilayer lateral pressure gradient or membrane fluidity affect MscL pressure sensitivity. (a) Addition of 3 µM LPC to the cytoplasmic side of a CHO cell membrane patch expressing MscL activates the channel in the absence of negative pressure (V_m = -10 mV). The magnified inset illustrates MscL single channel openings. Mean calculated NPo after 1 min treatment with 3 µM LPC was 0.067 ± 0.03, n = 5. (b) MscL expressing inside-out patches (CHO cells) are unresponsive to cooling (upper panel, 25 to 16°C) or heating (lower panel, 22 to 36 °C) of the bath solution (V_m = -10 mV). (c, d) Representative currents (upper panel) recorded at 13.5 °C (c) or 33 °C (d) in response to 5 s pulses of gradually increasing negative pressure (lower panel) at V_m = -10 mV. (e) Mean pressure threshold at the respective temperatures (13.5 °C, 83.8 ± 4.6 mm Hg, n = 16; RT, 100 ± 5.2 mm Hg, n = 6; 33 °C, 115 ± 9.6 mm Hg, n = 4; the shift in pressure thresholds at 13.5 °C and 33 °C is statistically significant, P = 0.007, Student's non-paired t-test). (f) Normalized current-pressure relation at different temperatures. Solid lines represent fits to a Boltzmann equation (13.5 °C, $P_{0.5}$ = 115.9 ± 2.9, n = 7; RT, $P_{0.5}$ = 149.6 ± 11.5, n = 6; 33 °C, $P_{0.5}$ = 155.0 ± 0.9, n = 4; significant difference in half maximal activation pressure from 13.5 °C to 33 °C, P = 0.019, Student's non-paired t-test). Error bars in e and f represent SEM.



Supplementary Figure S3. MscL G26C mediated delivery of Alexa Fluor 594 into HEK-293, HeLa and COS-7 cells. Upper panel, transiently transfected MscL G26C being expressed from a bicistronic IRES-GFP vector; middle panel, Alexa Fluor 594 uptake (AF594); lower panel, differential interference contrast (DIC). HEK-293 cells were treated for 1 min with MTSET (1 mM) in the presence of 5 µM Alexa Fluor 594 followed by 5 min exposure to DTT (1 mM) to mediate channel inactivation. HeLa and COS-7 cells were incubated with MTSET (1 mM) for 2 min in the presence of 5 µM Alexa Fluor 594 and subsequently 10 min DTT (1 mM) to facilitate channel inactivation. Scale bars, 20 µm.



Supplementary Figure S4. Characterization of CHO-MscL-WT and CHO-MscL-G26C cell lines. (a) Representative current trace recorded from an excised inside-out patch of the polyclonal CHO-MscL-WT cell line in response to 5 s pulses of gradually increasing negative pressure (lower panel) at V_m = -10 mV. (b) Mean pressure threshold for MscL WT comparing transient transfected CHO cells (*n* = 11) with CHO-MscL-WT cells (*n* = 8). (c) The monoclonal CHO-MscL-G26C cell line does not take up dye under control conditions: incubation with dye (Alexa Fluor 594, 10 µM, 2 min) in K-aspartate based delivery solution with no added MTSET (left column); incubation with dye (Alexa Fluor 594, 10 µM, 2 min) after DTT treatment (1 mM, 10 min) and channel inactivation (right column). AF594, Alexa Fluor 594; DIC, differential interference contrast. Scale bars, 20 µm. (d) Cell-viability as a function of MscL activation time in Ringer solution. The monoclonal CHO-MscL-G26C cell line and the polyclonal CHO-MscL-WT cell line (serving as a control) were treated for the indicated time with MTSET (1 mM) followed by DTT exposure (1 mM, 10 min) to facilitate MscL inactivation. Viability was assessed using a MTT assay. Data were collected at 2 independent times in quadruplicate. Error bars represent SEM; *P* values < 0.05 vs. WT control after 4 - 20 min MTSET treatment by Student's non-paired *t*-test.



Supplementary Figure S5. Weak uptake of model cargo under control conditions following extended MscL G26C activation by MTSET. The monoclonal CHO-MscL-G26C cell line shows no, or only weak uptake of model cargo (Alexa Fluor 594) after extended MscL activation (8 min) under control conditions: incubation with the dye (Alexa Fluor 594, 20 µM, 8 min) in K-aspartate based delivery solution with no added MTSET (left column), or after DTT treatment (1 mM, 10 min) and channel inactivation (right column). AF594, Alexa Fluor 594; DIC, differential interference contrast. Scale bars, 20 µm.