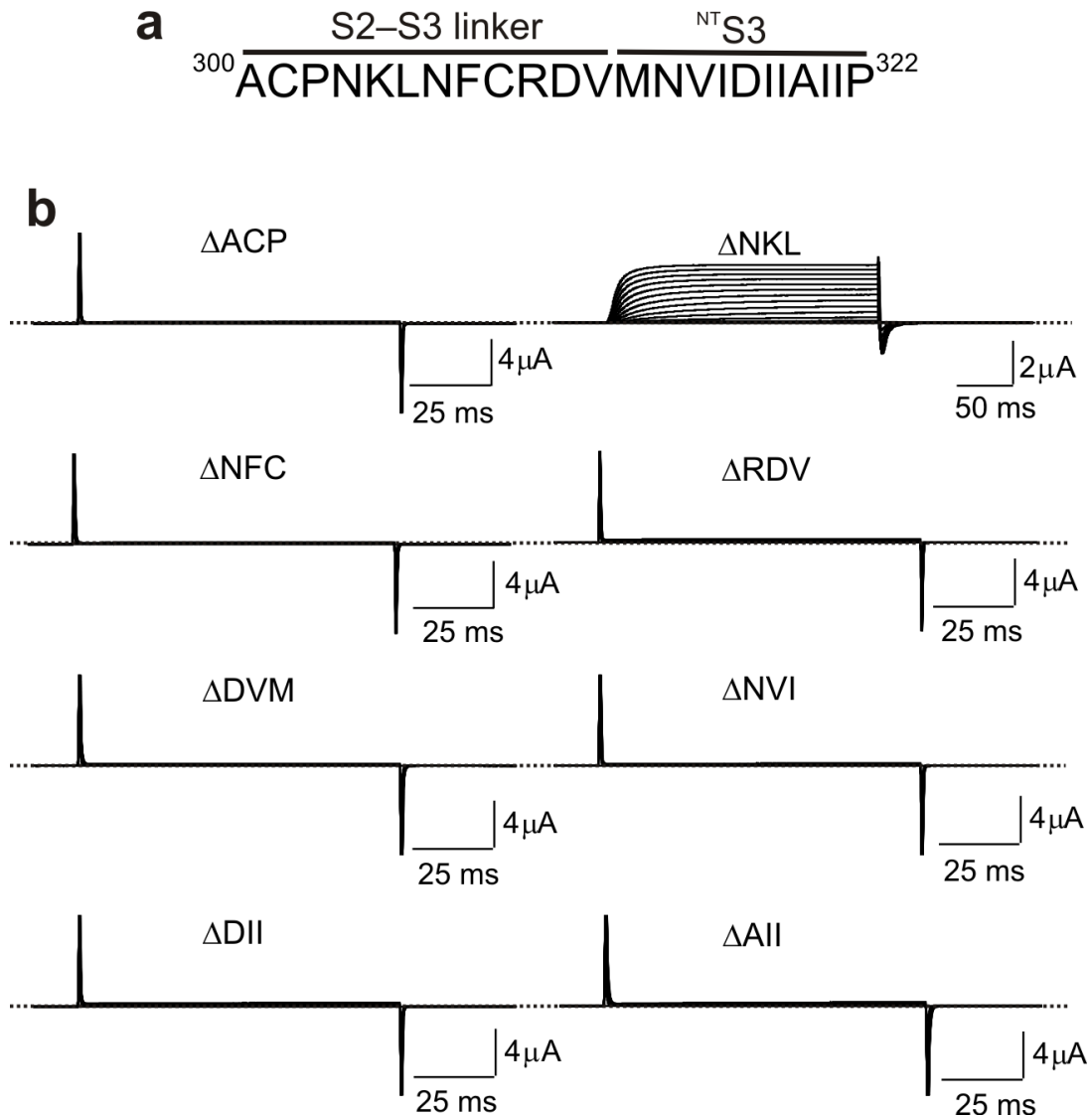
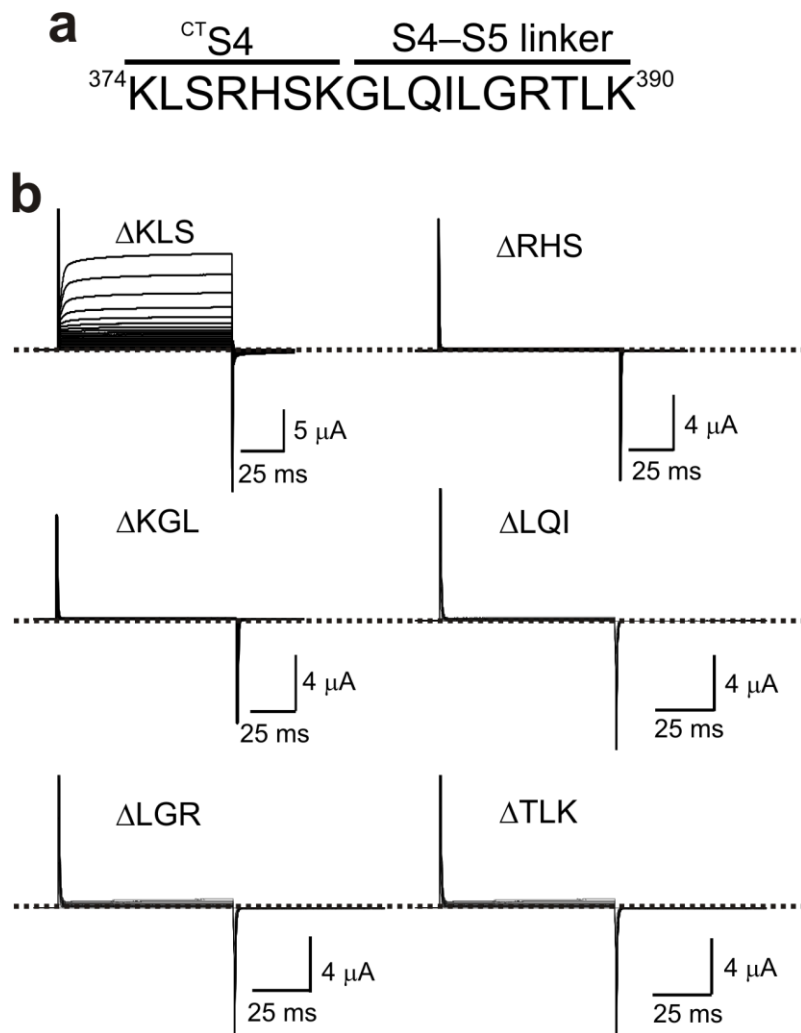


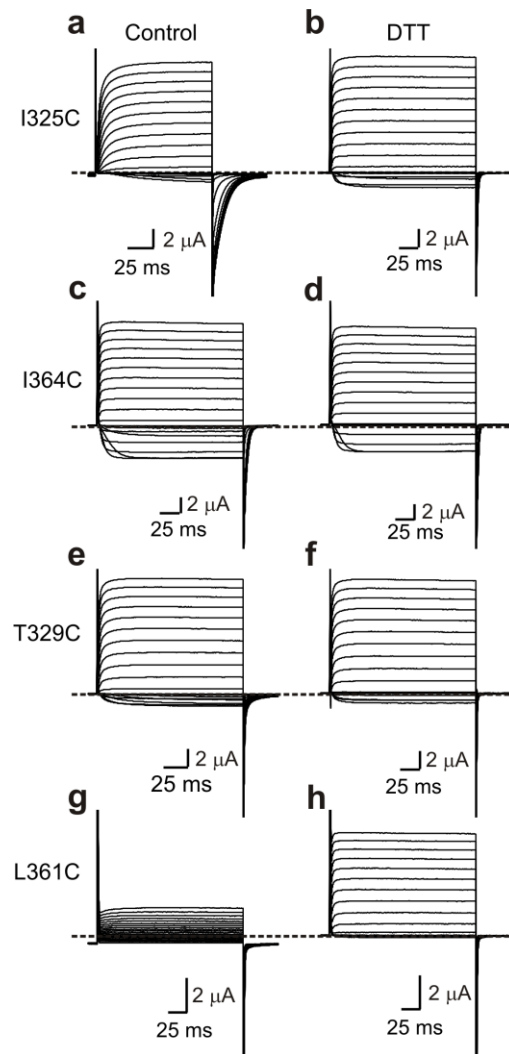
Supplementary figures



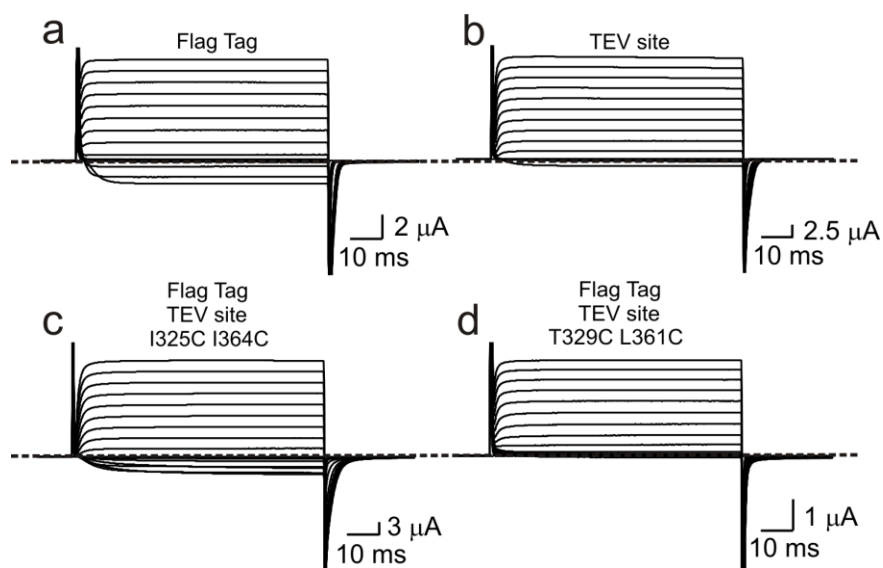
Supplementary Figure 1. Deletion analysis of the S2–S3 linker and ^{NT}S3. **(a)** Sequence of the S2–S3 linker through ^{NT}S3. **(b)** Mutant channel currents elicited by stepping from the -100 mV holding potential to 120 mV in 10 mV increments. In each of the eight mutants, three residues were deleted at a time within the S2–S3 linker or ^{NT}S3, as indicated.



Supplementary Figure 2. Deletion analysis of ^{CT}S4 and the S4–S5 linker helix. **(a)** Sequence of ^{CT}S4 through the S4–S5 linker helix. **(b)** Current of mutant channels elicited by stepping from the -100 mV holding potential to 120 mV in 10 mV increments. In each of the six mutants, three residues were deleted at a time within ^{CT}S4 or the S4–S5 linker helix, as indicated.



Supplementary Figure 3. Effect of DTT on channels containing a single cysteine mutation in the paddle motif. (a–h) Currents of mutants in 100 mM extracellular K^+ , without (control; a, c, e and g) or with (b, d, f and h) 1mM DTT treatment, elicited by stepping membrane voltage from the -80 mV (-120 mV for g) holding potential to between -80 mV (-120 mV for g) and 80 mV, in 10-mV increments.



Supplementary Figure 4. Currents of mutant channels with a Flag epitope, TEV site and double cysteine mutation. **(a–d)** Currents of mutants elicited by stepping membrane voltage from the -80 mV holding potential to between -80 mV and 80 mV, in 10-mV increments. Mutant channels contained an N-terminal Flag epitope **(a)**, a TEV site in the S3–S4 linker **(b)**, or both an N-terminal Flag epitope and a TEV site, in addition to one of two pairs of cysteine mutations (after exposure to DTT) **(c and d)**, as indicated.