Perry et al., http://www.jgp.org/cgi/content/full/jgp.201310975/DC1



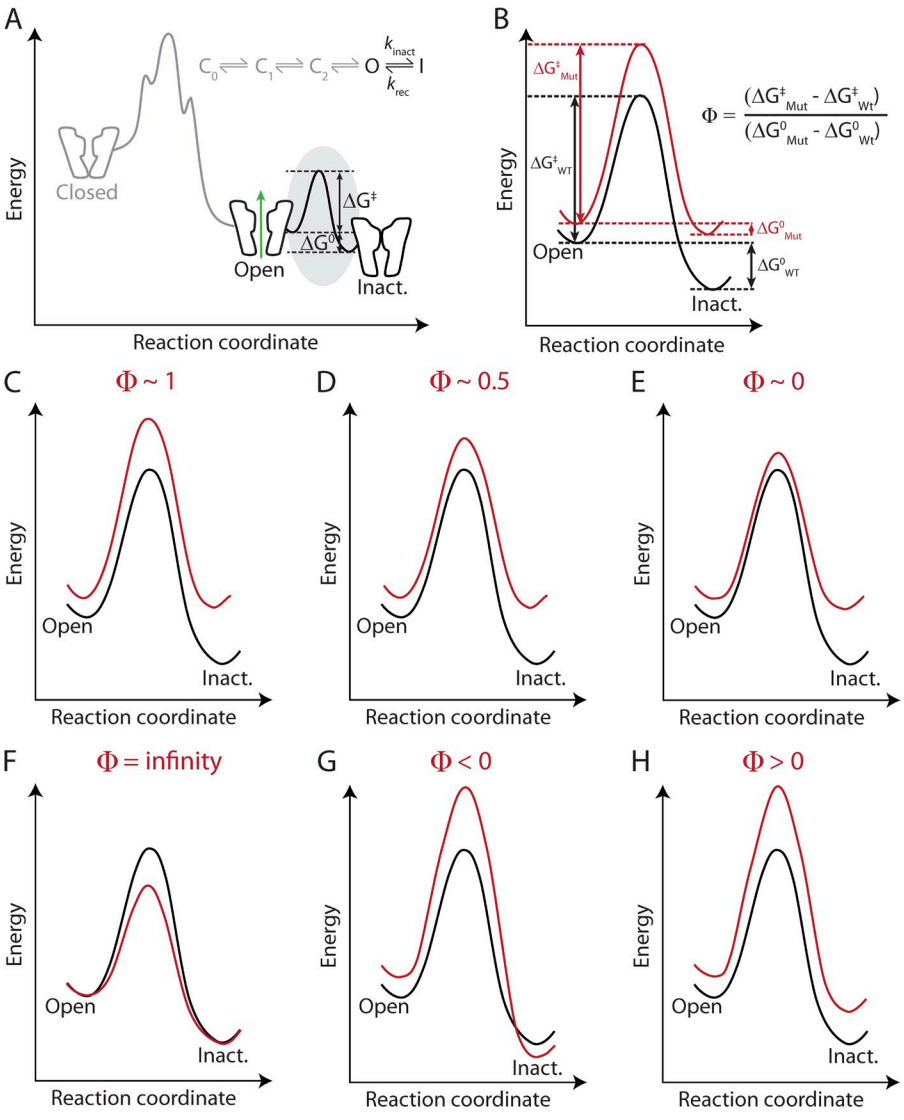


**Figure S1.** Theory of REFER analysis. (A) Gating scheme for Kv11.1 channels including the open (O)-to-inactivated (I) gating transition. (B–H) Energy diagrams of isolated open-to-inactivated gating transitions for WT (black) and theoretical mutant (red) channels. (B) REFER analysis calculates to what degree a mutation-induced perturbation in the equilibrium ($\Delta\Delta G^0$), which represents the energy difference between the ground states, is caused by a change in energy of the forward transition ($\Delta\Delta G^{\ddagger}$). The Φ-value reflects at what stage during the native reaction pathway the mutated residue experienced a change in environment, where Φ of 1 indicates the earliest step (C), fractional Φ-values indicate intermediate steps (D), and Φ of 0 indicates the final step (E). There are three scenarios that would result in an invalid Φ-value. Mutations affecting the transition state but not the equilibrium between ground states (i.e., a catalytic mutation) result in an infinite Φ-value (F). Mutations that have opposite effects on the transition state compared with the equilibrium between the two stable ground states result in a negative Φ-value (G), whereas mutations that have a greater effect on the transition state than on the equilibrium between the ground state result in a Φ-value >1 (H).

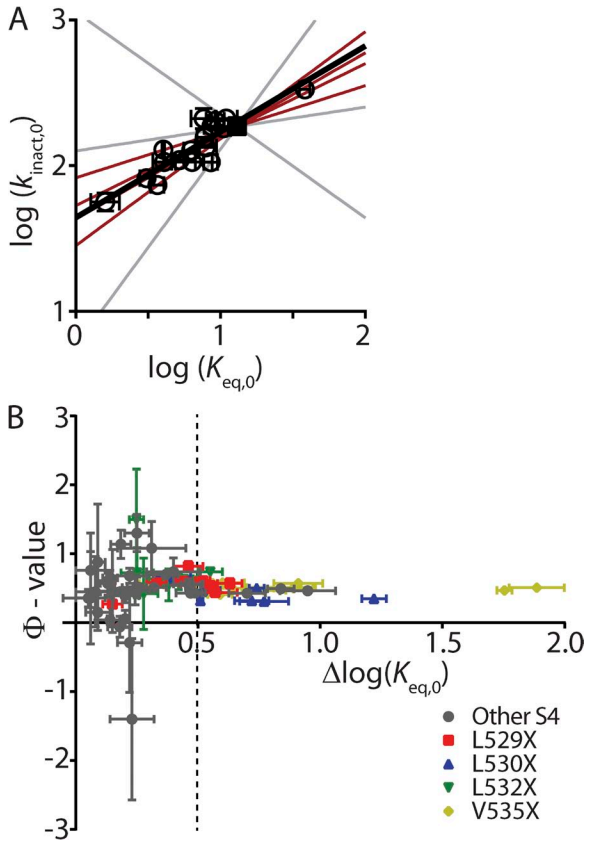


**Figure S2.** REFER variability between mutants. (A) REFER plot of the forward unidirectional rate constant, log($k_{inact,0}$), against equilibrium constant, log($K_{eq,0}$), for all individual S4 residues mutated to serine. Data are presented as means ± SEM for three to nine cells. The slope of the linear regression analysis for each mutant (vs. WT) represents the Φ-value. For mutation that causes a $\Delta\log(K_{eq,0}) < \pm 0.5$ log units (gray lines), the REFER slopes are highly variable (only three shown for clarity), but for mutations with a $\Delta\log(K_{eq,0}) > \pm 0.5$ log units (red lines), the slopes are far more consistent and better represent the overall REFER slope (black line). (B) Plot of Φ-value against $\Delta\log(K_{eq,0})$ for all S4 mutations. Φ-values are highly variable for mutations that cause a $\Delta\log(K_{eq,0}) < \pm 0.5$ log units but are more consistent for mutations that cause $\Delta\log(K_{eq,0}) > \pm 0.5$ log units. For this reason, we take a perturbation of greater than ±0.5 log units to be the minimum criteria to derive an accurate Φ-value.


**Figure S3.** Sequence alignment of Kv11.1 and Kv1.2/2.1 channels. Alignment spans from the S4 helix to the S6 helix, with the transmembrane regions of Kv1.2/2.1 boxed according to the secondary structure of 2R9R from the Protein Data Bank (Long et al., 2007). The S5P linker of Kv11.1 (residues 584–606) is excluded from the alignment, indicated by break, because of its much longer length compared with that of the Kv1.2/2.1 S5P linker.

*Table S1*

*The kinetic parameters for each S4 mutant investigated*

| Mutant | *n* | $\log(k_{inact,0})$ | $\log(K_{eq,0})$ | $\Delta\log(K_{eq,0})$ | Φ-value |
|---|---|---|---|---|---|
| G522A | 5 | 2.19 ± 0.03 | 0.98 ± 0.03 | −0.13 ± 0.03 | |
| G522W | 20 | 2.06 ± 0.02 | 0.63 ± 0.03 | −0.49 ± 0.03 | |
| G522S | 3 | 2.03 ± 0.06 | 0.80 ± 0.14 | −0.31 ± 0.14 | |
| L523A | 8 | 2.33 ± 0.03 | 1.06 ± 0.06 | −0.06 ± 0.06 | |
| L523W | [a] | | | | |
| L523S | 9 | 2.12 ± 0.05 | 0.90 ± 0.07 | −0.22 ± 0.07 | |
| L524A | 7 | 2.36 ± 0.03 | 1.06 ± 0.06 | −0.06 ± 0.06 | |
| L524W | 10 | 2.31 ± 0.04 | 1.17 ± 0.10 | 0.05 ± 0.10 | |
| L524S | 7 | 2.26 ± 0.03 | 1.02 ± 0.05 | −0.09 ± 0.05 | |
| K525A | [a] | | | | |
| K525W | 7 | 2.10 ± 0.02 | 0.93 ± 0.04 | −0.18 ± 0.04 | |
| K525S | 4 | 2.52 ± 0.02 | 1.58 ± 0.04 | 0.47 ± 0.04 | |
| T526A | 4 | 2.19 ± 0.06 | 1.02 ± 0.03 | −0.09 ± 0.03 | |
| T526W | 7 | 2.18 ± 0.02 | 0.97 ± 0.03 | −0.14 ± 0.03 | |
| T526S | 5 | 2.32 ± 0.07 | 0.88 ± 0.09 | −0.23 ± 0.09 | |
| A527W | 9 | 2.19 ± 0.05 | 0.87 ± 0.05 | −0.24 ± 0.05 | |
| A527S | 4 | 2.31 ± 0.02 | 0.96 ± 0.06 | −0.15 ± 0.06 | |
| R528A[b] | 5 | 2.50 ± 0.04 | 1.19 ± 0.07 | 0.07 ± 0.07 | |
| R528W | 4 | 2.02 ± 0.03 | 0.75 ± 0.05 | −0.37 ± 0.05 | |
| R528S | 7 | 2.19 ± 0.04 | 0.89 ± 0.05 | −0.22 ± 0.05 | |
| L529A | 5 | 1.95 ± 0.04 | 0.59 ± 0.03 | −0.52 ± 0.03 | 0.61 ± 0.06 |
| L529W | 8 | 1.91 ± 0.05 | 0.66 ± 0.06 | −0.46 ± 0.06 | |
| L529S | 8 | 1.91 ± 0.05 | 0.49 ± 0.05 | −0.63 ± 0.05 | 0.57 ± 0.07 |
| L529T | 5 | 1.97 ± 0.01 | 0.56 ± 0.02 | −0.55 ± 0.02 | 0.54 ± 0.03 |
| L529N | 7 | 2.00 ± 0.03 | 0.67 ± 0.03 | −0.44 ± 0.03 | |
| L529H | 5 | 2.08 ± 0.03 | 0.78 ± 0.04 | −0.33 ± 0.04 | |
| L529P | 5 | 2.02 ± 0.02 | 0.46 ± 0.09 | −0.57 ± 0.09 | 0.44 ± 0.05 |
| L529Q | 9 | 2.16 ± 0.02 | 0.96 ± 0.04 | −0.15 ± 0.04 | |
| L530A | 4 | 2.02 ± 0.03 | 0.71 ± 0.07 | −0.40 ± 0.07 | |
| L530W | 7 | 2.03 ± 0.02 | 0.59 ± 0.07 | −0.52 ± 0.07 | 0.47 ± 0.03 |
| L530S | 6 | 2.11 ± 0.01 | 0.60 ± 0.01 | −0.51 ± 0.01 | 0.32 ± 0.01 |
| L530T | 8 | 2.03 ± 0.05 | 0.40 ± 0.07 | −0.72 ± 0.07 | 0.32 ± 0.04 |
| L530N | 12 | 1.84 ± 0.03 | −0.11 ± 0.05 | −1.22 ± 0.05 | 0.35 ± 0.01 |
| L530H | [a] | | | | |
| L530P | 9 | 1.90 ± 0.02 | 0.37 ± 0.03 | −0.74 ± 0.03 | 0.50 ± 0.02 |
| L530Q | 7 | 2.04 ± 0.03 | 0.34 ± 0.10 | −0.77 ± 0.10 | 0.31 ± 0.03 |
| R531A | 7 | 1.97 ± 0.04 | 0.41 ± 0.08 | −0.70 ± 0.08 | 0.43 ± 0.03 |
| R531W | 4 | 2.07 ± 0.03 | 0.64 ± 0.06 | −0.47 ± 0.06 | |
| R531S | 4 | 1.82 ± 0.08 | 0.16 ± 0.11 | −0.95 ± 0.11 | 0.46 ± 0.04 |
| L532A | 8 | 2.03 ± 0.05 | 0.69 ± 0.07 | −0.42 ± 0.07 | |
| L532W | 4 | 2.02 ± 0.05 | 0.72 ± 0.09 | −0.39 ± 0.09 | |
| L532S | 7 | 1.87 ± 0.03 | 0.56 ± 0.05 | −0.55 ± 0.05 | 0.74 ± 0.02 |
| L532N | 10 | 1.96 ± 0.04 | 0.73 ± 0.07 | −0.38 ± 0.07 | |
| L532H | 14 | 2.06 ± 0.03 | 0.87 ± 0.03 | −0.25 ± 0.03 | |
| L532P | 13 | 1.98 ± 0.04 | 0.67 ± 0.05 | −0.44 ± 0.05 | |
| L532T | 11 | 2.09 ± 0.04 | 0.86 ± 0.07 | −0.25 ± 0.07 | |
| L532Q | 11 | 2.11 ± 0.10 | 0.84 ± 0.06 | −0.28 ± 0.06 | |
| V533A | 10 | 2.25 ± 0.02 | 0.91 ± 0.02 | −0.20 ± 0.02 | |
| V533W | 4 | 2.15 ± 0.02 | 0.86 ± 0.04 | −0.25 ± 0.04 | |
| V533S | 4 | 2.27 ± 0.02 | 0.94 ± 0.05 | −0.18 ± 0.05 | |

$\text{Log}(k_{inact,0})$, $\log(K_{eq,0})$, and $\Delta\log(K_{eq,0})$ (relative to WT) for each mutant channel. Φ-values are given for mutant channels that exhibit a $\Delta\log(K_{eq,0}) > \pm 0.5$ log units, which is the minimum requirement to derive an accurate Φ-value. *n* is the number of cells analyzed for each mutant, and data are presented as means ± SEM.

[a]Mutant channels that failed to express or expressed poorly.

[b]Mutants previously investigated by Wang et al. (2011. *Nat. Struct. Mol. Biol.* 18:35–41) and listed in their supplementary data.

| Mutant | $n$ | $\log(k_{inact,0})$ | $\log(K_{eq,0})$ | $\Delta\log(K_{eq,0})$ | Φ-value |
|---|---|---|---|---|---|
| R534A[b] | 4 | 1.99 ± 0.03 | 0.87 ± 0.05 | −0.25 ± 0.05 | |
| R534W | 4 | 2.01 ± 0.05 | 0.66 ± 0.06 | −0.46 ± 0.06 | |
| R534S | 5 | 2.02 ± 0.01 | 0.93 ± 0.05 | −0.18 ± 0.05 | |
| V535A | 4 | 1.92 ± 0.05 | 0.51 ± 0.09 | −0.60 ± 0.09 | 0.58 ± 0.03 |
| V535W | 7 | 1.92 ± 0.03 | 0.35 ± 0.09 | −0.77 ± 0.09 | 0.47 ± 0.03 |
| V535S | 7 | 1.75 ± 0.06 | 0.20 ± 0.10 | −0.91 ± 0.10 | 0.57 ± 0.01 |
| V535G | 6 | 2.02 ± 0.02 | 0.52 ± 0.05 | −0.59 ± 0.05 | 0.41 ± 0.02 |
| V535I | 4 | 2.07 ± 0.04 | 0.74 ± 0.11 | −0.37 ± 0.11 | |
| V535H | 5 | 1.95 ± 0.04 | 0.47 ± 0.06 | −0.64 ± 0.06 | 0.50 ± 0.04 |
| V535L | 5 | 2.06 ± 0.04 | 0.75 ± 0.06 | −0.36 ± 0.06 | |
| V535M | 5 | 1.83 ± 0.09 | 0.28 ± 0.14 | −0.84 ± 0.14 | 0.51 ± 0.03 |
| V535N | 6 | 1.30 ± 0.11 | −0.77 ± 0.11 | −1.89 ± 0.11 | 0.51 ± 0.03 |
| V535P | 6 | 1.95 ± 0.03 | 0.55 ± 0.07 | −0.56 ± 0.07 | 0.57 ± 0.02 |
| V535T | 5 | 1.82 ± 0.03 | 0.22 ± 0.08 | −0.89 ± 0.08 | 0.50 ± 0.01 |
| V535Y | 12 | 1.44 ± 0.03 | −0.64 ± 0.03 | −1.75 ± 0.03 | 0.47 ± 0.01 |
| A536W | 5 | 2.40 ± 0.03 | 1.36 ± 0.05 | 0.25 ± 0.05 | |
| A536S | 4 | 2.04 ± 0.04 | 0.72 ± 0.11 | −0.40 ± 0.11 | |
| R537A[b] | 3 | 2.14 ± 0.03 | 0.85 ± 0.07 | −0.26 ± 0.07 | |
| R537W | 14 | 1.84 ± 0.04 | 0.27 ± 0.05 | −0.84 ± 0.05 | 0.49 ± 0.02 |
| R537S[b] | 5 | 2.10 ± 0.03 | 0.80 ± 0.05 | −0.32 ± 0.05 | |
| K538A[b] | 14 | 2.14 ± 0.03 | 0.97 ± 0.06 | −0.14 ± 0.06 | |
| K538W | 8 | 2.18 ± 0.03 | 0.91 ± 0.06 | −0.20 ± 0.06 | |
| K538S[b] | 8 | 2.32 ± 0.04 | 1.04 ± 0.08 | −0.08 ± 0.08 | |
| L529S + V535S | 8 | 1.74 ± 0.03 | −0.20 ± 0.06 | −1.31 ± 0.06 | 0.40 ± 0.01 |
| L532S + V535S | 18 | 1.87 ± 0.05 | 0.30 ± 0.08 | −0.81 ± 0.08 | 0.50 ± 0.04 |
| L529S + L532S + V535S | 5 | 1.97 ± 0.02 | 0.57 ± 0.06 | −0.54 ± 0.06 | 0.58 ± 0.04 |
| **S5/S5P mutants** | | | | | |
| I560A | 7 | 1.79 ± 0.04 | 0.54 ± 0.06 | −0.58 ± 0.06 | 0.85 ± 0.03 |
| L564A | 7 | 1.77 ± 0.04 | 0.44 ± 0.07 | −0.67 ± 0.07 | 0.76 ± 0.03 |
| I567A | 6 | 2.11 ± 0.02 | 0.59 ± 0.04 | −0.52 ± 0.04 | 0.31 ± 0.03 |
| D591K | 13 | 1.56 ± 0.04 | 0.12 ± 0.06 | −1.00 ± 0.06 | 0.71 ± 0.03 |
| **Double mutants** | | | | | |
| L529S + I560A | 7 | 1.63 ± 0.07 | 0.27 ± 0.11 | −0.84 ± 0.11 | 0.77 ± 0.02 |
| L529S + L564A | 7 | 1.58 ± 0.06 | −0.12 ± 0.07 | −1.24 ± 0.07 | 0.55 ± 0.02 |
| L529S + I567A | 9 | 1.87 ± 0.03 | 0.29 ± 0.06 | −0.82 ± 0.06 | 0.48 ± 0.01 |
| L529S + D591K | 9 | 1.33 ± 0.03 | −0.39 ± 0.03 | −1.50 ± 0.03 | 0.62 ± 0.01 |
| L530S + I560A | 7 | 1.79 ± 0.02 | 0.56 ± 0.03 | −0.56 ± 0.03 | 0.86 ± 0.04 |
| L530S + L564A | 8 | 1.49 ± 0.03 | −0.29 ± 0.05 | −1.41 ± 0.05 | 0.55 ± 0.01 |
| L530S + I567A | 11 | 1.98 ± 0.03 | 0.35 ± 0.06 | −0.77 ± 0.06 | 0.38 ± 0.01 |
| L530S + D591K | 6 | 1.33 ± 0.06 | −0.52 ± 0.06 | −1.63 ± 0.06 | 0.57 ± 0.02 |
| L530N + I560A | 7 | 1.65 ± 0.03 | −0.11 ± 0.04 | −1.23 ± 0.04 | 0.50 ± 0.01 |
| L530N + L564A | 8 | 1.61 ± 0.02 | −0.26 ± 0.03 | −1.37 ± 0.03 | 0.48 ± 0.01 |
| L530N + I567A | 6 | 1.87 ± 0.04 | −0.12 ± 0.05 | −1.24 ± 0.05 | 0.32 ± 0.02 |
| L530N + D591K | 7 | 1.31 ± 0.04 | −0.61 ± 0.05 | −1.73 ± 0.05 | 0.55 ± 0.01 |
| L532S + I560A | 14 | 1.71 ± 0.02 | 0.48 ± 0.03 | −0.64 ± 0.03 | 0.88 ± 0.02 |
| L532S + L564A | 8 | 1.46 ± 0.06 | 0.04 ± 0.08 | −1.08 ± 0.08 | 0.75 ± 0.01 |
| L532S + I567A | 11 | 1.83 ± 0.02 | 0.32 ± 0.03 | −0.79 ± 0.03 | 0.55 ± 0.02 |
| L532S + D591K | 10 | 1.63 ± 0.03 | 0.25 ± 0.06 | −0.86 ± 0.06 | 0.75 ± 0.01 |
| V535S + I560A | 7 | 1.50 ± 0.04 | 0.08 ± 0.07 | −1.04 ± 0.07 | 0.74 ± 0.01 |
| V535S + L564A | 12 | 1.38 ± 0.06 | −0.40 ± 0.1 | −1.51 ± 0.1 | 0.59 ± 0.02 |
| V535S + I567A | 8 | 1.84 ± 0.05 | 0.14 ± 0.09 | −0.97 ± 0.09 | 0.43 ± 0.02 |
| V535S + D591K | 8 | 1.16 ± 0.05 | −0.68 ± 0.06 | −1.79 ± 0.06 | 0.63 ± 0.02 |

$\log(k_{inact,0})$, $\log(K_{eq,0})$, and $\Delta\log(K_{eq,0})$ (relative to WT) for each mutant channel. Φ-values are given for mutant channels that exhibit a $\Delta\log(K_{eq,0}) > \pm 0.5$ log units, which is the minimum requirement to derive an accurate Φ-value. $n$ is the number of cells analyzed for each mutant, and data are presented as means ± SEM.

[a]Mutant channels that failed to express or expressed poorly.

[b]Mutants previously investigated by Wang et al. (2011. *Nat. Struct. Mol. Biol.* 18:35–41) and listed in their supplementary data.

*Table S2*

*The activation parameters for each S4 mutant investigated*

| Mutant | *n* | Act $V_{0.5}$ | Slope factor | Prepulse |
|---|---|---|---|---|
| | | *mV* | | *mV* |
| WT | 11 | −25.01 ± 0.33 | 8.55 ± 0.26 | 40 |
| G522A | 6 | −44.17 ± 0.78 | 8.71 ± 0.45 | 40 |
| G522W | 7 | −54.31 ± 1.05 | 8.74 ± 0.26 | 40 |
| G522S | 3 | −27.61 ± 1.44 | 7.99 ± 0.31 | 40 |
| L523A | 7 | −57.64 ± 0.59 | 9.27 ± 0.14 | 40 |
| L523W | [a] | | | |
| L523S | 7 | −52.26 ± 1.22 | 10.35 ± 0.51 | 40 |
| L524A | 7 | −11.64 ± 1.56 | 7.21 ± 0.26 | 40 |
| L524W | 4 | −21.40 ± 1.15 | 7.82 ± 0.30 | 40 |
| L524S | 7 | −20.88 ± 0.39 | 7.24 ± 0.07 | 40 |
| K525A | [a] | | | |
| K525W | 7 | −66.46 ± 1.65 | 11.91 ± 0.14 | 40 |
| K525S | 6 | −40.90 ± 0.70 | 8.37 ± 0.16 | 40 |
| T526A | 4 | −28.91 ± 0.82 | 7.62 ± 0.18 | 40 |
| T526W | 7 | −63.28 ± 0.55 | 6.49 ± 0.20 | 40 |
| T526S | 5 | −27.34 ± 0.74 | 6.46 ± 0.25 | 40 |
| A527W | 6 | −15.50 ± 0.73 | 7.32 ± 0.21 | 40 |
| A527S | 4 | −23.84 ± 0.85 | 8.17 ± 0.08 | 40 |
| R528A | 10 | −16.94 ± 0.67 | 8.32 ± 0.26 | 40 |
| R528W | 4 | −36.40 ± 0.65 | 8.04 ± 0.18 | 40 |
| R528S | 8 | 1.88 ± 0.91 | 8.15 ± 0.09 | 40 |
| L529A | 5 | −9.36 ± 1.35 | 7.57 ± 0.25 | 40 |
| L529W | 8 | −24.44 ± 1.50 | 8.17 ± 0.50 | 40 |
| L529S | 6 | −19.77 ± 1.01 | 10.40 ± 0.66 | 40 |
| L529T | 5 | −25.05 ± 0.81 | 7.93 ± 0.33 | 40 |
| L529N | 7 | −59.17 ± 1.54 | 8.37 ± 0.56 | 40 |
| L529H | 5 | −55.59 ± 2.55 | 7.18 ± 0.20 | 40 |
| L529P | 5 | −13.07 ± 0.84 | 14.25 ± 0.35 | 40 |
| L529Q | 9 | −38.91 ± 0.53 | 6.77 ± 0.22 | 40 |
| L530A | 4 | −8.19 ± 0.34 | 7.89 ± 0.42 | 40 |
| L530W | 8 | 7.01 ± 1.16 | 6.08 ± 0.13 | 40 |
| L530S | 6 | −2.13 ± 0.84 | 6.53 ± 0.09 | 40 |
| L530T | 8 | −6.25 ± 0.52 | 6.03 ± 0.14 | 40 |
| L530N | 12 | 45.31 ± 0.61 | 7.16 ± 0.10 | 80 |
| L530H | [a] | | | |
| L530P | 9 | 4.63 ± 0.84 | 6.99 ± 0.11 | 40 |
| L530Q | 7 | 19.06 ± 0.40 | 5.90 ± 0.14 | 40 |
| R531A | 8 | 35.01 ± 1.04 | 7.19 ± 0.12 | 80 |
| R531W | 4 | 13.09 ± 0.87 | 6.46 ± 0.06 | 40 |
| R531S | 10 | 30.46 ± 1.24 | 7.44 ± 0.15 | 80 |
| L532A | 6 | −29.84 ± 0.87 | 7.10 ± 0.34 | 40 |
| L532W | 2 | −10.22 | 9.99 | 40 |
| L532S | 7 | −28.76 ± 1.20 | 8.70 ± 0.26 | 40 |
| L532N | 3 | −51.13 ± 0.75 | 5.22 ± 0.34 | 40 |
| L532H | 9 | −36.07 ± 0.43 | 7.78 ± 0.40 | 40 |
| L532P | 9 | 5.35 ± 0.45 | 11.67 ± 0.21 | 40 |
| L532T | 10 | −20.91 ± 0.87 | 8.07 ± 0.22 | 40 |
| L532Q | 4 | −43.47 ± 0.70 | 6.02 ± 0.43 | 40 |
| V533A | 6 | −35.17 ± 0.39 | 7.31 ± 0.30 | 40 |
| V533W | 4 | −11.99 ± 0.97 | 6.58 ± 0.21 | 40 |
| V533S | 4 | −30.77 ± 0.48 | 6.97 ± 0.25 | 40 |

The half-maximal voltage ($V_{0.5}$) and voltage-dependent slope for each mutant channel. Also shown is the prepulse potential used for inactivation protocols shown in Fig. 1. *n* is the number of cells analyzed for each mutant, and data are presented as means ± SEM.

[a]Mutant channels that failed to express or expressed poorly.

| Mutant | *n* | Act $V_{0.5}$ | Slope factor | Prepulse |
|---|---|---|---|---|
| R534A | 4 | −29.03 ± 0.68 | 6.23 ± 0.09 | 40 |
| R534W | 4 | −9.57 ± 0.53 | 6.43 ± 0.33 | 40 |
| R534S | 5 | −32.64 ± 0.67 | 5.80 ± 0.19 | 40 |
| V535A | 4 | 50.53 ± 0.37 | 5.04 ± 0.18 | 40 |
| V535W | 6 | 9.84 ± 0.58 | 11.24 ± 0.14 | 40 |
| V535S | 6 | −5.41 ± 1.05 | 12.12 ± 0.09 | 40 |
| V535G | 6 | −54.14 ± 1.24 | 7.53 ± 0.30 | 40 |
| V535I | 4 | −48.15 ± 1.12 | 5.71 ± 0.30 | 40 |
| V535H | 5 | 8.63 ± 1.15 | 14.79 ± 0.51 | 40 |
| V535L | 5 | −44.45 ± 2.11 | 9.12 ± 0.36 | 40 |
| V535M | 5 | −24.23 ± 1.11 | 16.21 ± 0.87 | 40 |
| V535N | 7 | 33.34 ± 1.35 | 12.05 ± 0.17 | 80 |
| V535P | 6 | −18.93 ± 0.18 | 7.71 ± 0.12 | 40 |
| V535T | 5 | −29.65 ± 1.04 | 7.29 ± 0.17 | 40 |
| V535Y | 12 | 27.49 ± 0.95 | 11.63 ± 0.44 | 80 |
| A536W | 6 | −88.19 ± 0.48 | 8.20 ± 0.40 | 40 |
| A536S | 4 | −40.02 ± 0.65 | 7.29 ± 0.26 | 40 |
| R537A | 7 | −20.77 ± 0.48 | 7.64 ± 0.13 | 40 |
| R537W | 14 | −16.44 ± 0.73 | 7.84 ± 0.29 | 40 |
| R537S | 8 | −25.75 ± 0.46 | 6.54 ± 0.10 | 40 |
| K538A | 13 | −49.79 ± 0.62 | 5.64 ± 0.14 | 40 |
| K538W | 10 | −62.29 ± 0.68 | 4.87 ± 0.11 | 40 |
| K538S | 10 | −63.47 ± 0.64 | 5.56 ± 0.10 | 40 |
| L529S + V535S | 8 | 34.23 ± 0.66 | 12.94 ± 0.16 | 60 |
| L532S + V535S | 12 | −2.53 ± 0.87 | 13.85 ± 0.20 | 40 |
| L529S + L532S + V535S | 13 | 26.03 ± 0.99 | 11.73 ± 0.55 | 80 |

The half-maximal voltage ($V_{0.5}$) and voltage-dependent slope for each mutant channel. Also shown is the prepulse potential used for inactivation protocols shown in Fig. 1. *n* is the number of cells analyzed for each mutant, and data are presented as means ± SEM.
[a]Mutant channels that failed to express or expressed poorly.