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SUPPLEMENTAL MATERIALS AND METHODS

Computer Homology Modeling

A computer homology model of the hERG channel based on KcsA was constructed based on available K+ channel crystal structure (Doyle, D.A., J. Morais Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. *Science.* 280:69–77; Long, S.B., E.B. Campbell, and R. Mackinnon. 2005. *Science.* 309:897–903; Long, S.B., E.B. Campbell, and R. Mackinnon. 2005. *Science.* 309:903–908; Clayton, G.M., S. Altieri, L. Heginbotham, V.M. Unger, and J.H. Morais-Cabral. 2008. *Proc. Natl. Acad. Sci. USA.* 105:1511–1515). Coordinates KcsA (PDB ID 1BL8) were downloaded and loaded into SYBYL (Tripos). Residues were mutated manually in SYBYL according to the following alignment.

KcsA residue 23 was matched with hERG residue 550. The sequence continues linearly in the C-terminal direction until reaching the hERG "turret" region. hERG residues 588 through 610 were omitted. hERG residue 611 then aligns with KcsA residue 62 and continues linearly to the end of the modeled region.

After mutation in SYBYL, side chain orientations were checked manually, keeping backbone fixed and torsioning side chain orientations to relieve clashes with other residues. After this, Gasteiger-Hückel charges were added and energy minimization was performed using the Tripos force field in SYBYL, first using the Simplex minimization. At this point, the model was checked manually for gross distortions. If none were seen, energy minimization using the Powell conjugate gradient method (Powell, M. 1977. *Math. Program.* 12:241–254) was performed until either the gradient change reached 0.05 kcal/Å·mol or 1,000 iterations were complete. A final manual check of the energy-minimized model was performed, and the resultant PDB file was loaded into the PyMol viewer (DeLano Scientific), where figures were generated.

Table S1.

Channel	Avg Deact τ in WT	Avg Deact τ in S620T
Ctrl	169.8 ± 43.9	123.9 ± 6.1
I655C	589.9 ± 8.4[a]	504.8 ± 21.8[b]
G657C	56.02 ± 6.7[a]	40.5 ± 4.3[b]
V659C	2501.6 ± 329.4[a,c]	798.6 ± 62.9[b,c]
S660C	198.8 ± 68.2	142.7 ± 8.7

Values reported were obtained by a weighted average of double exponential fits to deactivating current traces (see Materials and methods) and are represented ± SEM.

[a]$P < 0.05$ compared to Ctrl deactivation τ in WT background.
[b]$P < 0.05$ compared to Ctrl deactivation τ in S620T background.
[c]$P < 0.05$ compared to equivalent channel in different background.