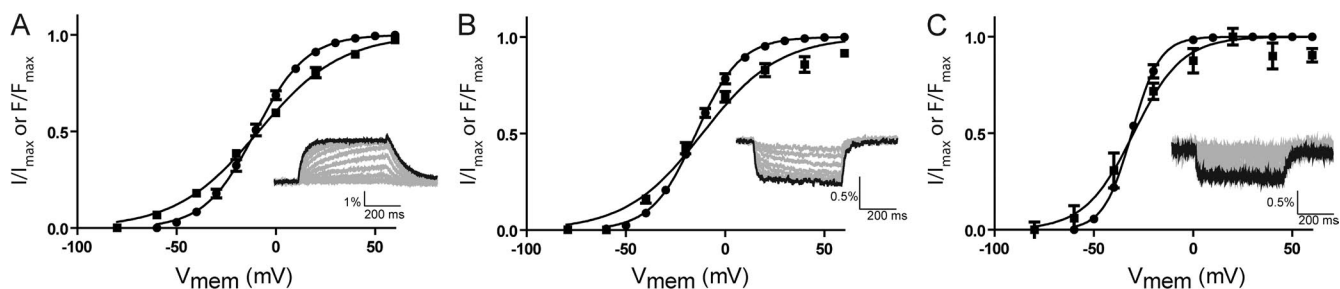
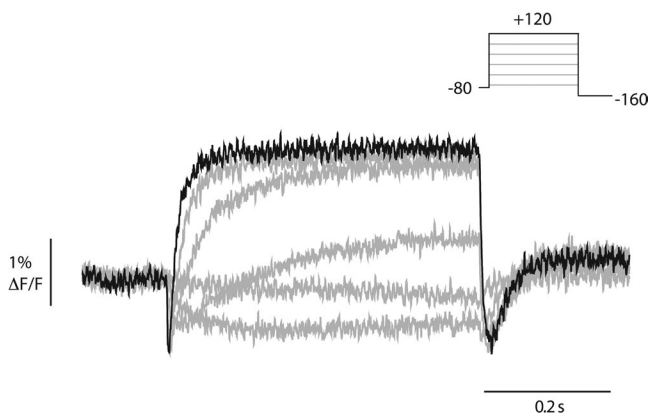


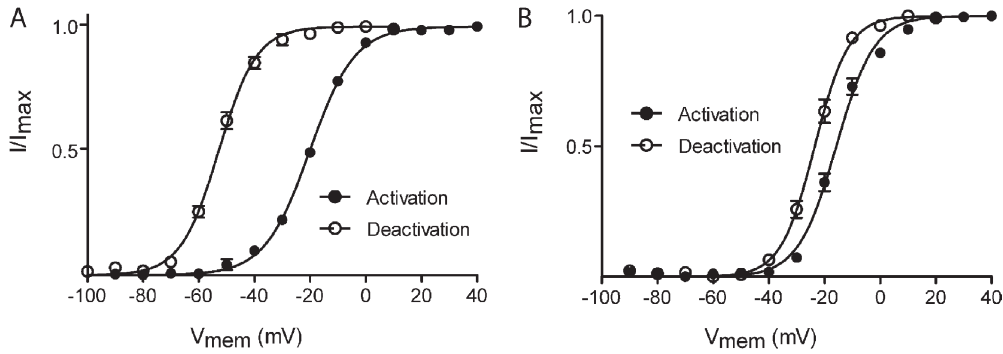
Tan et al., <http://www.jgp.org/cgi/content/full/jgp.201110761/DC1>



**Figure S1.** Comparison of MTSR labeling of positions 518, 519, and 520. (A–C) Voltage-dependent equilibria of current activation (I) and VSD upward motion (F) for MTSR-labeled E518C (A), E519C (B), and L520C (C) hERG channels. Error bars are SEM;  $n = 5$ . Insets are typical fluorescent traces recorded in response to a series of depolarizing steps from  $-80$  mV. A previous study using voltage-clamp fluorometry to examine VSD movement in hERG channels has labeled positions 518, 519, and 520 with TMRM (Smith and Yellen, 2002). We have tested each of these positions and found that for the MTSR probe, although all positions gave signals with comparable characteristics, position 518 gave the most reproducible and consistent labeling and so was chosen for this study.



**Figure S2.** Fluorescent record from TMRM-labeled 518C hERG. Unlike MTSR-labeled 518C, the fluorescence profile in response to depolarization is biphasic as previously reported for this position (Smith and Yellen, 2002).



**Figure S3.** Effect of N-terminal truncation on mode shift of ionic current in wild-type hERG. (A and B) Voltage-dependent equilibria of activation and deactivation were measured for wild-type (A) and  $\Delta 2-25$  (B) hERG using the same protocols illustrated in Fig. 1. Solid lines are fits of the Boltzmann function to the data.  $V_{0.5}$  values for activation and deactivation, respectively, were measured as  $-20 \pm 1$  mV and  $-56 \pm 1$  mV (SEM;  $n = 6$ ) for wild-type and  $-16 \pm 1$  mV and  $-23.5 \pm 2$  mV (SEM;  $n = 6$ ) for  $\Delta 2-25$  hERG.

TABLE S1

Summary of parameters for Boltzmann fits to the voltage-dependent equilibria of ionic current activation and deactivation for the alanine scan of the hERG N terminus

Mutant	$V_{0.5, \text{act}}$	$V_{0.5, \text{deact}}$	$\Delta G_{0, \text{act}}$	$\Delta G_{0, \text{deact}}$
	mV	mV	kcal/mol	kcal/mol
Wild type	$-19.97 \pm 0.42$	$-53.7 \pm 1$	$-1.28 \pm 0.08$	$-5.48 \pm 0.16$
P2A	$-21.4 \pm 0.44$	$-40.2 \pm 0.6$	$-2.0 \pm 0.13$	$-4.58 \pm 0.15$
V3A	$-19.5 \pm 0.33$	$-31.1 \pm 0.6$	$-2.0 \pm 0.08$	$-4.46 \pm 0.16$
R4A	$-19.5 \pm 0.32$	$-30.7 \pm 1.2$	$-1.5 \pm 0.05$	$-2.94 \pm 0.41$
R5A	$-12.2 \pm 0.8$	$-22.6 \pm 0.4$	$-0.96 \pm 0.07$	$-1.9 \pm 0.13$
G6A	$-15.8 \pm 0.36$	$-24.2 \pm 0.99$	$-1.4 \pm 0.1$	$-2.41 \pm 0.17$
H7A	$-18.6 \pm 0.37$	$-38.4 \pm 0.5$	$-1.8 \pm 0.05$	$-4.31 \pm 0.21$
V8A	$-18.2 \pm 0.38$	$-47.6 \pm 0.8$	$-1.5 \pm 0.12$	$-5.20 \pm 0.35$
P10A	$-19.4 \pm 0.64$	$-53.6 \pm 0.3$	$-1.6 \pm 0.07$	$-5.34 \pm 0.2$
Q11A	$-20.3 \pm 0.55$	$-55.0 \pm 0.7$	$-1.6 \pm 0.06$	$-5.4 \pm 0.20$
N12A	$-18.1 \pm 0.2$	$-38.5 \pm 0.8$	$-1.76 \pm 0.02$	$-4.96 \pm 0.12$
T13A	$-24 \pm 1.6$	$-61.9 \pm 0.6$	$-1.79 \pm 0.17$	$-6.06 \pm 0.46$
F14A	$-21.1 \pm 0.3$	$-45.0 \pm 0.6$	$-1.93 \pm 0.07$	$-4.56 \pm 0.07$
L15A	$-18.5 \pm 1.3$	$-42.7 \pm 1.5$	$-1.74 \pm 0.14$	$-4.89 \pm 0.33$
D16A	$-18.2 \pm 0.34$	$-56.4 \pm 1.0$	$-1.59 \pm 0.06$	$-6.56 \pm 0.68$
T17A	$-19.7 \pm 0.56$	$-47.7 \pm 0.7$	$-1.83 \pm 0.08$	$-5.39 \pm 0.38$
I18A	$-22.9 \pm 0.5$	$-41.2 \pm 0.3$	$-2.35 \pm 0.09$	$-5.29 \pm 0.33$
I19A	$-22.9 \pm 0.34$	$-39.9 \pm 0.4$	$-2.34 \pm 0.11$	$-4.91 \pm 0.39$
R20A	$-26.8 \pm 0.66$	$-44.8 \pm 0.5$	$-2.33 \pm 0.09$	$-5.77 \pm 0.46$
K21A	$-27.1 \pm 0.81$	$-52.8 \pm 0.5$	$-2.49 \pm 0.09$	$-6.33 \pm 0.21$
F22A	$-26.6 \pm 0.9$	$-50.9 \pm 1.2$	$-2.25 \pm 0.07$	$-5.37 \pm 0.59$
E23A	$-24.7 \pm 0.31$	$-48.3 \pm 0.6$	$-2.11 \pm 0.03$	$-5.16 \pm 0.50$

## REFERENCE

Smith, P.L., and G. Yellen. 2002. Fast and slow voltage sensor movements in HERG potassium channels. *J. Gen. Physiol.* 119:275–293. <http://dx.doi.org/10.1085/jgp.119.3.275>