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**Supplemental Information**

**Stabilization of the Activated hERG Channel Voltage Sensor by Depolarization Involves the S4-S5 Linker**

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## **Supplementary Material**

### **Stabilization of the activated hERG channel voltage sensor by depolarization involves the S4-S5 linker**

Running title: Stabilization of the activated hERG voltage sensor

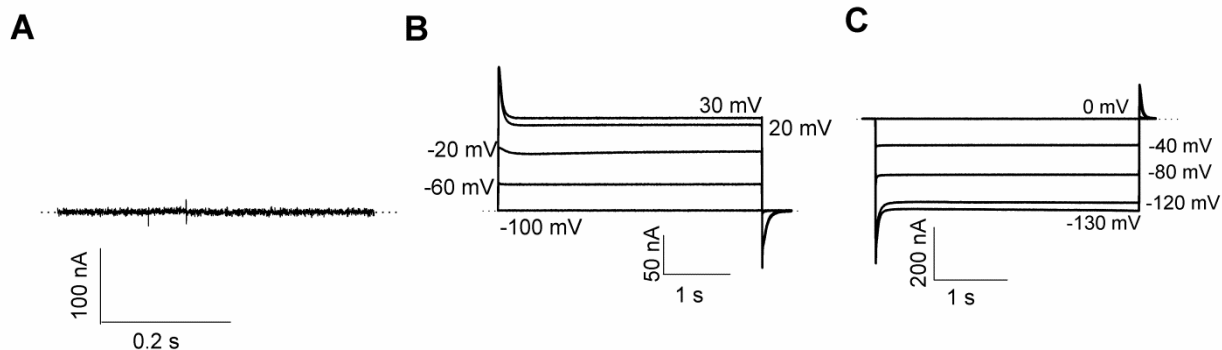
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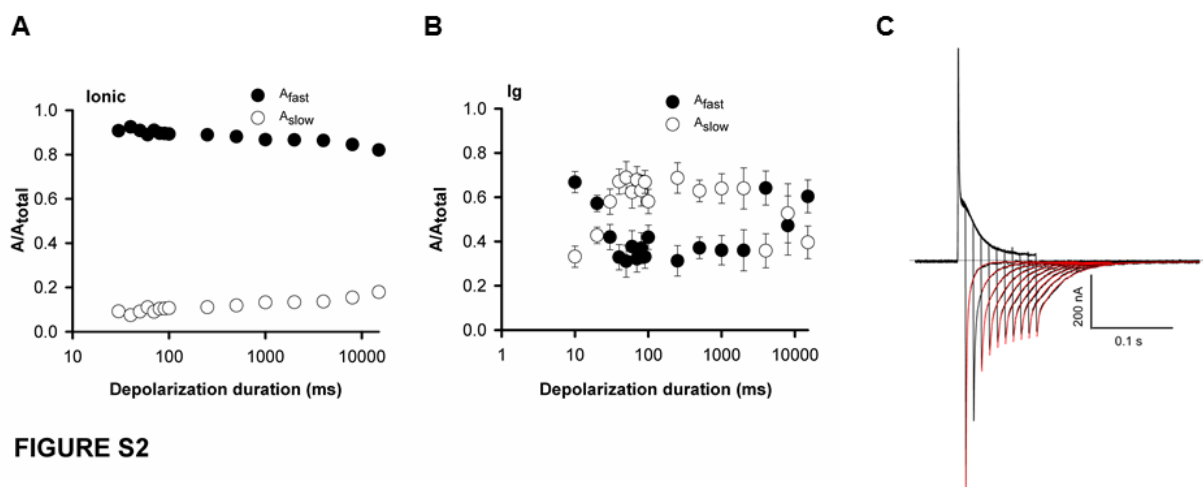
Key words: hERG, voltage sensor, deactivation, relaxation, mode-shift, S4-S5 linker

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**FIGURE S1**

**FIGURE S1. Gating current recordings from WT channels and un-injected oocytes (A)** Representative leak and capacity subtracted currents recorded from un-injected oocyte in response to a -100 mV step from a holding potential of 0 mV. **(B and C)** Non-leak subtracted WT hERG currents recorded in response to depolarization **(B)** and repolarization **(C)** to the indicated voltages from a holding potential of -100 mV and 0 mV respectively.



**FIGURE S2**

**FIGURE S2 Dependence of relative amplitudes of the fast and slow phases of current decay on pre-pulse duration. (A and B)** Plot of mean relative amplitudes of the phases of deactivating **(A)** and off-gating current decay **(B)** measured at -110 mV against depolarizing step duration. **(C)** Representative hERG WT gating current traces as in Figure 2B inset showing double-exponential fits (red lines) of off-gating current decays.

**TABLE S1 Activation and deactivation GV Boltzmann fit parameters for hERG  $\Delta$ 2-135 channels with different depolarizing step durations ( $\Delta t$ )**

$\Delta t$ (s)	Activation GV			Deactivation GV		
	$V_h$ (mV)	k (mV)	n	$V_h$ (mV)	k (mV)	n
0.25	$-6.7 \pm 2.1$	$10.6 \pm 0.6$	5	-	-	-
0.5	$-13.8 \pm 1.3$	$8.4 \pm 0.4$	5	$-34.3 \pm 1.2$	$16.2 \pm 1.5$	5
1	$-17.3 \pm 0.6$	$7.2 \pm 0.3$	5	$-29.7 \pm 1.0$	$12.7 \pm 0.5$	5
2	$-19.4 \pm 0.4$	$6.6 \pm 0.4$	5	$-24.7 \pm 0.9$	$9.1 \pm 0.8$	5
4	$-19.9 \pm 0.5$	$6.5 \pm 0.4$	5	$-23.2 \pm 0.5$	$9.4 \pm 0.9$	5
8	$-19.7 \pm 0.8$	$6.4 \pm 0.5$	5	$-24.9 \pm 1.3$	$9.4 \pm 0.5$	5

**TABLE S2 Activation and deactivation GV Boltzmann fit parameters for hERG S3-S4 and S1-S2 linker mutant channels**

	Activation GV			Deactivation GV			Mode-shift	
	$V_h$ (mV)	k (mV)	n	$V_h$ (mV)	k (mV)	n	$V_h$ (mV)	n
WT hERG	$-21.5 \pm 0.5$	$7.9 \pm 0.1$	5	$-52.8 \pm 1.7$	$8.1 \pm 0.3$	5	$-31.3 \pm 2.0$	5
hERG/Sh	$-34.1 \pm 0.7$	$8.0 \pm 0.2$	5	$-68.1 \pm 1.0$	$7.8 \pm 0.3$	5	$-34.5 \pm 1.2$	5
hERG 9G	$-33.3 \pm 2.2$	$11.2 \pm 0.3$	4	$-62.0 \pm 2.8$	$11.2 \pm 0.4$	4	$-28.8 \pm 1.1$	4
ETEE/QQQQ	$-35.8 \pm 2.6$	$8.9 \pm 0.9$	5	$-59.3 \pm 4.0$	$7.3 \pm 0.9$	5	$-24.5 \pm 2.9$	5

To compare the effect of mutations on the extent of mode-shift, activation and deactivation were measured using non-steady-state durations. Activation GV relations were collected from oocytes held at -80 mV and subjected to 2 s depolarizing steps to +50 mV in 10 mV increments followed by a 2 s step to -110 mV. Deactivation GV relations were collected from oocytes held at -80 mV and subjected to a 500 ms depolarizing step +60 mV followed by 8 s steps from -110 mV to +40 mV in 10 mV increments followed by a repolarizing step to -110 mV. Boltzmann fits were used to obtain the  $V_h$  and k values.