

Supp. Table S1. Post-hoc analysis of the ANOVA statistics for Figure 2: Immunoblot

| B. Total hERG Protein | | |
|--|---------------------------|----|
| ANOVA P value | p= 0.7548 | |
| Post-Hoc Analysis | WT hERG v. 50/50 Mix | NS |
| | WT hERG v. p. Asp219Val | NS |
| | 50/50 Mix v. p Asp.219Val | NS |
| | | |
| C. Mature/Immature hERG Protein Ratio | | |
| ANOVA P value | p= 0.8808 | |
| Post-Hoc Analysis | WT hERG v. 50/50 Mix | NS |
| | WT hERG v. p. Asp219Val | NS |
| | 50/50 Mix v. p Asp.219Val | NS |

These analyses demonstrate that none of the differences between the WT hERG, 50/50 mix or p. Asp219Val hERG protein on immunoblot are significant, either for **B)** total protein or **C)** ratio of mature to immature hERG.

Supp. Table S2. Post-hoc analysis of the ANOVA statistics for Figure 3: Activation

| B. Normalized Current Density | | |
|--|----------------------------|----------|
| ANOVA P value | p= 0.2078 | |
| Post-Hoc Analysis | WT hERG v. 50/50 Mix | NS |
| | WT hERG v. p. Asp219Val | NS |
| | 50/50 Mix v. p. Asp.219Val | NS |
| | | |
| D. Voltage Dependence of Activation | | |
| ANOVA P value | p <0.001 | |
| Post-Hoc Analysis | WT hERG v. 50/50 Mix | p <0.05 |
| | WT hERG v. p. Asp219Val | p <0.001 |
| | 50/50 Mix v. p. Asp.219Val | p <0.001 |

B) These analyses demonstrate that none of the differences between the current densities for WT hERG, 50/50 mix or p. Asp219Val hERG protein are significant. **D)** This post-hoc analysis demonstrates that the differences between the $V_{1/2}$ for the WT, 50/50 mix or p. Asp219Val hERG in their voltage dependence of activation are all significant.

Supp. Table S3. Post-hoc analysis of the ANOVA statistics for Figure 4: Inactivation

| A. Steady State Inactivation | | | C. Recovery from Inactivation | | |
|-------------------------------------|----------------------------|---------------|--------------------------------------|--------------|---------------|
| ANOVA P value | p <0.001 | | ANOVA | Voltage (mV) | ANOVA p Value |
| Post-Hoc Analysis | WT hERG v. 50/50 Mix | p <0.001 | | -60 | p <0.05 |
| | WT hERG v. p. Asp219Val | p <0.001 | | -50 | p <0.001 |
| | 50/50 Mix v. p. Asp.219Val | NS | | -40 | p <0.001 |
| | | | | -30 | p <0.001 |
| B. Onset of Inactivation | | | | | |
| ANOVA | Voltage (mV) | ANOVA p Value | WT hERG v. 50/50 Mix | Voltage (mV) | |
| | -20 | NS | | -60 | p <0.05 |
| | -10 | NS | | -50 | p <0.001 |
| | 0 | NS | | -40 | p <0.001 |
| | 10 | NS | | -30 | p <0.001 |
| | 20 | NS | | | |
| | 30 | NS | WT hERG v. p. Asp219Val | Voltage (mV) | |
| | 40 | NS | | -60 | p <0.05 |
| | 50 | p <0.05 | | -50 | p <0.001 |
| | 60 | p <0.05 | | -40 | p <0.001 |
| | 70 | NS | | -30 | NS |
| Post-Hoc Analysis | | | | | |
| | | | 50/50 Mix v. p. Asp.219Val | Voltage (mV) | |
| WT hERG v. 50/50 Mix | Voltage (mV) | | | -60 | NS |
| | -20 | NS | | -50 | NS |
| | -10 | NS | | -40 | p <0.001 |
| | 0 | NS | | -30 | p <0.001 |
| | 10 | NS | | | |
| | 20 | NS | | | |
| | 30 | NS | | | |
| | 40 | NS | | | |
| | 50 | p <0.05 | | | |
| | 60 | p <0.05 | | | |

| | | |
|-------------------------------|--------------|----|
| | 70 | NS |
| WT hERG v. p. Asp219Val | Voltage (mV) | |
| | -20 | NS |
| | -10 | NS |
| | 0 | NS |
| | 10 | NS |
| | 20 | NS |
| | 30 | NS |
| | 40 | NS |
| | 50 | NS |
| | 60 | NS |
| | 70 | NS |
| 50/50 Mix v. p. Asp.219Val | Voltage (mV) | |
| | -20 | NS |
| | -10 | NS |
| | 0 | NS |
| | 10 | NS |
| | 20 | NS |
| | 30 | NS |
| | 40 | NS |
| | 50 | NS |
| | 60 | NS |
| | 70 | NS |

A) These analyses demonstrate that the differences between the steady state inactivation $V_{1/2}$ for WT hERG and 50/50 mix, as well as WT hERG and p. Asp219Val hERG protein are significant, while the differences between 50/50 mix and p. Asp219Val hERG are not significant. **B)** This post-hoc analysis demonstrates that the differences between the onsets of inactivation for the WT and 50/50 mix as well as WT hERG and p. Asp219Val hERG are significant at voltages -50mV and -60 mV, while the differences between 50/50 mix and p. Asp219Val hERG are not significant. **C)** This post-hoc analysis demonstrates that the differences between the WT and 50/50 mix in recovery from inactivation are significant at all voltages tested. The differences in WT hERG and p. Asp219Val hERG in recovery from inactivation are significant at voltages -60mV- -40 mV, and the differences between 50/50 mix and p. Asp219Val hERG are significant at -40mV and -30 mV.

Supp. Table S4. Post-hoc analysis of the ANOVA statistics for Figure 5: Deactivation

| <i>B. Deactivation Time Constants</i> | | | <i>C. Ratio of Tau Fast to Total Deactivation Time</i> | | |
|---------------------------------------|--------------|---------------|--|--------------|---------------|
| <i>Tau Slow</i> | | | ANOVA | Voltage (mV) | ANOVA p Value |
| ANOVA | Voltage (mV) | ANOVA p Value | | -100 | NS |
| | -100 | NS | | -90 | NS |
| | -90 | NS | | -80 | NS |
| | -80 | NS | | -70 | p <0.001 |
| | -70 | NS | | -60 | p <0.05 |
| | -60 | NS | | -50 | p <0.001 |
| | -50 | NS | | -40 | p <0.001 |
| | -40 | NS | | | |
| | | | Post-Hoc Analysis | | |
| Post-Hoc Analysis | | | | | |
| | | | WT hERG v. 50/50 Mix | Voltage (mV) | |
| WT hERG v. 50/50 Mix | Voltage (mV) | | | -100 | NS |
| | -100 | NS | | -90 | NS |
| | -90 | NS | | -80 | NS |
| | -80 | NS | | -70 | p <0.001 |
| | -70 | NS | | -60 | NS |
| | -60 | NS | | -50 | p <0.001 |
| | -50 | NS | | -40 | p <0.001 |
| | -40 | NS | WT hERG v. p. Asp219Val | Voltage (mV) | |
| WT hERG v. p. Asp219Val | Voltage (mV) | | | -100 | NS |
| | -100 | NS | | -90 | NS |
| | -90 | NS | | -80 | NS |
| | -80 | NS | | -70 | p <0.001 |
| | -70 | NS | | -60 | p <0.001 |
| | -60 | NS | | -50 | p <0.001 |
| | -50 | NS | | -40 | p <0.001 |
| | -40 | NS | 50/50 Mix v. p Asp.219Val | Voltage (mV) | |

| | | | | | |
|------------------------------|-----------------|------------------|--|------|----------|
| 50/50 Mix v. p Asp.219Val | Voltage (mV) | | | | |
| | -100 | NS | | -100 | NS |
| | -90 | NS | | -90 | NS |
| | -80 | NS | | -80 | NS |
| | -70 | NS | | -70 | NS |
| | -60 | NS | | -60 | NS |
| | -50 | NS | | -50 | p <0.001 |
| | -40 | NS | | -40 | NS |
| | | | | | |
| <i>Tau Fast</i> | | | | | |
| ANOVA | Voltage (mV) | ANOVA p Value | | | |
| | -100 | NS | | | |
| | -90 | p <0.001 | | | |
| | -80 | p <0.001 | | | |
| | -70 | p <0.001 | | | |
| | -60 | p <0.001 | | | |
| | -50 | p <0.001 | | | |
| | -40 | p <0.001 | | | |
| | | | | | |
| Post-Hoc Analysis | | | | | |
| | | | | | |
| WT hERG v. 50/50 Mix | Voltage (mV) | | | | |
| | -100 | NS | | | |
| | -90 | p <0.001 | | | |
| | -80 | p <0.001 | | | |
| | -70 | p <0.001 | | | |
| | -60 | p <0.001 | | | |
| | -50 | p <0.001 | | | |
| | -40 | p <0.001 | | | |
| | | | | | |
| WT hERG v. p. Asp219Val | Voltage (mV) | | | | |
| | -100 | NS | | | |
| | -90 | p <0.001 | | | |
| | -80 | p <0.001 | | | |
| | -70 | p <0.001 | | | |
| | -60 | p <0.001 | | | |
| | -50 | p <0.001 | | | |
| | -40 | p <0.001 | | | |

| 50/50 Mix v. p Asp.219Val | Voltage (mV) | |
|------------------------------|-----------------|----|
| | -100 | NS |
| | -90 | NS |
| | -80 | NS |
| | -70 | NS |
| | -60 | NS |
| | -50 | NS |
| | -40 | NS |

B) These analyses demonstrate that none of the differences in the Tau Slow time constant for WT hERG, 50/50 mix, and p. Asp219Val hERG protein are significant at any voltage. This post-hoc testing does demonstrate significant differences in the Tau Fast time constant between WT hERG and 50/50 mix at voltages -90 mV - -40 mV, as well as significant differences between the WT hERG and p. Asp219Val hERG at voltages -90mV - -40mV. There is not a significant difference in Tau Fast time constants between 50/50 Mix and p. Asp219Val hERG. **C)** Post-hoc analysis demonstrates that the differences between ratios of Tau Fast to total deactivation time is significant at voltages -70- -40 mV. These differences are significant between WT hERG and 50/50 mix at -70 mV, -50 mV and -40 mV, between WT hERG and p. Asp219Val from -70 mV- -40mV and between 50/50 mix and p. Asp219Val at -50 mV.