Supplementary material for: Evaluation of an *In-Silico* Cardiac Safety Assay: Using Ion Channel Screening Data to Predict QT Interval Changes in the Rabbit Ventricular Wedge

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S Supplementary Material

S.1 Downloading Datasets and Simulation Software

All of the datasets and simulation software used in this study are publically available to download from `www.cs.ox.ac.uk/chaste/download`.

S.2 Additional Results

S.2.1 Further Combinations of Ion Channel Data Use

Table S1: **Prolongation metric values calculated (with 95% confidence intervals indicated) for evaluation of the assay's predictivity of QT prolongation in the rabbit ventricular wedge experiments when considering the different uses of the available ion channel data described which were not included in the main text. Entries are ordered alphabetically by the data type used in the simulations.**

Case Number	Ion Channel Data Use Combination	Accuracy (%)	Kappa Value	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
S1	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value *(121 Compounds)*	**65.3** *(56.5-73.2)*	**0.35** *(0.19-0.51)*	**89.2** *(75.3-95.7)*	**54.8** *(44.1-65.0)*	**46.5** *(35.4-58.0)*	**92.0** *(81.2-96.8)*
S2	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(121 Compounds)*	**66.1** *(57.3-73.9)*	**0.36** *(0.20-0.52)*	**89.2** *(75.3-95.7)*	**56.0** *(45.3-66.1)*	**47.1** *(35.9-58.7)*	**92.2** *(81.5-96.9)*
S3	IonWorks/FLIPR Data, Multiple Channel Block Including KCNQ1 Interactions, Fitting For IC_{50} Value & Hill Coefficient *(121 Compounds)*	**75.2** *(66.8-82.0)*	**0.46** *(0.29-0.63)*	**73.0** *(57.0-84.6)*	**76.2** *(66.1-84.0)*	**57.4** *(43.3-70.5)*	**86.5** *(76.9-92.5)*
S4	PatchXpress Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(77 Compounds)*	**71.4** *(60.5-80.3)*	**0.47** *(0.28-0.66)*	**100** *(86.7-100)*	**57.7** *(44.2-70.1)*	**53.2** *(39.2-66.7)*	**100** *(88.6-100)*
S5	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient, One-Dimensional Simulation *(77 Compounds)*	**70.1** *(59.2-79.2)*	**0.41** *(0.21-0.61)*	**84.0** *(65.3-93.6)*	**63.5** *(49.9-75.2)*	**52.5** *(37.5-67.1)*	**89.2** *(75.3-95.7)*
S6	QSAR Data, hERG Block Only *(372 Compounds)*	**54.6** *(49.5-59.6)*	**0.16** *(0.07-0.26)*	**94.4** *(89.8-97.1)*	**23.8** *(18.6-30.0)*	**48.9** *(43.4-54.4)*	**84.7** *(73.5-91.8)*

Table S2: **Shortening metric values calculated (with 95% confidence intervals indicated) for evaluation of the assay's predictivity for QT shortening in the rabbit ventricular wedge experiments considering the different uses of available ion channel data described which were not included in the main text. Entries are ordered alphabetically by the data type used in the simulations. Missing entries are due to the inability to calculate the metrics due to there being no compounds in the relevant categories for their computation.**

Case Number	Ion Channel Data Use Combination	Accuracy (%)	Kappa Value	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
S1	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value *(121 Compounds)*	**74.4** *(65.9-81.3)*	**0.00** *(-0.30-0.30)*	**0.00** *(0.00-11.0)*	**100** *(95.9-100)*	**-**	**74.4** *(65.9-81.3)*
S2	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(121 Compounds)*	**74.4** *(65.9-81.3)*	**0.00** *(-0.30-0.30)*	**0.00** *(0.00-11.0)*	**100** *(95.9-100)*	**-**	**74.4** *(65.9-81.3)*
S3	IonWorks/FLIPR Data, Multiple Channel Block Including KCNQ1 Interactions, Fitting For IC_{50} Value & Hill Coefficient *(121 Compounds)*	**71.1** *(62.4-78.4)*	**0.22** *(0.00-0.44)*	**38.7** *(23.7-56.2)*	**82.2** *(73.1-88.8)*	**42.9** *(29.8-57.0)*	**79.6** *(70.3-86.5)*
S4	PatchXpress Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(77 Compounds)*	**76.6** *(66.0-84.7)*	**0.00** *(-0.40-0.40)*	**0.00** *(0.00-17.6)*	**100** *(93.9-100)*	**-**	**76.6** *(66.0-84.7)*
S5	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient, One-Dimensional Simulation *(77 Compounds)*	**74.0** *(63.3-82.5)*	**0.27** *(0.00-0.55)*	**44.4** *(24.6-66.3)*	**83.1** *(71.5-90.5)*	**44.4** *(30.2-59.6)*	**83.1** *(71.5-90.5)*
S6	QSAR Data, hERG Block Only *(372 Compounds)*	**82.8** *(78.6-86.3)*	**0.00** *(-0.22-0.22)*	**0.00** *(0.00-5.66)*	**100** *(98.8-100)*	**-**	**82.8** *(78.6-86.3)*

S.2.2 Performance-Related Metrics Calculated For 44 Compounds With Data from Each Ion Channel Assay

Table S3: **Prolongation metric values calculated (with 95% confidence intervals indicated) for evaluation of the assay's predictivity of QT prolongation in the rabbit ventricular wedge experiments when considering the different uses of the available ion channel data described for a subset of 44 compounds for which there were ion channel data available from all three assays. Entries are ordered alphabetically by the data type used in the simulations.**

Case Number	Ion Channel Data Use Combination	Accuracy (%)	Kappa Value	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
S1	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value *(44 Compounds)*	**72.7** *(58.2-83.7)*	**0.47** *(0.22-0.73)*	**92.9** *(68.5-98.7)*	**63.3** *(45.5-78.1)*	**54.2** *(35.1-72.1)*	**95.0** *(76.4-99.1)*
S2	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**72.7** *(58.2-83.7)*	**0.47** *(0.22-0.73)*	**92.9** *(68.5-98.7)*	**63.3** *(45.5-78.1)*	**54.2** *(35.1-72.1)*	**95.0** *(76.4-99.1)*
S3	IonWorks/FLIPR Data, Multiple Channel Block, Fitting For IC_{50} Value *(44 Compounds)*	**81.8** *(68.0-90.5)*	**0.60** *(0.34-0.85)*	**78.6** *(52.4-92.4)*	**83.3** *(66.4-92.7)*	**68.8** *(44.4-85.8)*	**89.3** *(72.8-96.3)*
S4	IonWorks/FLIPR Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.50** *(0.22-0.77)*	**71.4** *(45.4-88.3)*	**80.0** *(62.7-90.5)*	**62.5** *(38.6-81.5)*	**85.7** *(68.5-94.3)*
S5	IonWorks/FLIPR Data, Multiple Channel Block Including KCNQ1 Interactions, Fitting For IC_{50} Value *(44 Compounds)*	**79.5** *(65.5-88.8)*	**0.55** *(0.29-0.81)*	**78.6** *(52.4-92.4)*	**80.0** *(62.7-90.5)*	**64.7** *(41.3-82.7)*	**88.9** *(71.9-96.1)*
S6	IonWorks/FLIPR Data, Multiple Channel Fitting For IC_{50} Value & Hill Coefficient Including KCNQ1 Interactions *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.50** *(0.22-0.77)*	**71.4** *(45.4-88.3)*	**80.0** *(62.7-90.5)*	**62.5** *(38.6-81.5)*	**85.7** *(68.5-94.3)*
S7	PatchXpress Data, hERG Block Only, Fitting For IC_{50} Value *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.56** *(0.32-0.80)*	**100** *(78.5-100)*	**66.7** *(48.8-80.8)*	**58.3** (38.8-75.5)	**100** *(83.9-100)*

S8	PatchXpress Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**75.0** *(60.6-85.4)*	**0.52** *(0.28-0.77)*	**100** *(78.5-100)*	**63.3** *(45.5-78.1)*	**56.0** *(37.1-73.3)*	**100** *(83.2-100)*
S9	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value *(44 Compounds)*	**70.5** *(55.8-81.8)*	**0.33** *(0.03-0.64)*	**57.1** *(32.6-78.6)*	**76.7** *(59.1-88.2)*	**53.3** *(30.1-75.2)*	**79.3** *(61.6-90.2)*
S10	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**75.0** *(60.6-85.4)*	**0.45** *(0.18-0.73)*	**71.4** *(45.4-88.3)*	**76.7** *(59.1-88.2)*	**58.8** *(36.0-78.4)*	**85.2** *(67.5-94.1)*
S11	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value, One-Dimensional Simulation *(44 Compounds)*	**68.2** *(53.4-80.0)*	**0.38** *(0.12-0.65)*	**85.7** *(60.1-96.0)*	**60.0** *(42.3-75.4)*	**50.0** *(31.4-68.6)*	**90.0** *(69.9-97.2)*
S12	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient, One-Dimensional Simulation *(44 Compounds)*	**63.6** *(48.9-76.2)*	**0.32** *(0.05-0.58)*	**85.7** *(60.1-96.0)*	**53.3** *(36.1-69.8)*	**46.2** *(28.8-64.5)*	**88.9** *(67.2-96.9)*
S13	QSAR Data, hERG Block Only *(44 Compounds)*	**50.0** *(35.8-64.2)*	**0.19** *(-0.05-0.43)*	**100** *(78.5-100)*	**26.7** *(14.2-44.4)*	**38.9** *(24.8-55.1)*	**100** *(67.6-100)*
S14	QSAR Data, Multiple Channel Block *(44 Compounds)*	**54.5** *(40.1-68.3)*	**0.24** *(0.00-0.49)*	**100** *(78.5-100)*	**33.3** *(19.2-51.2)*	**41.2** *(26.4-57.8)*	**100** *(72.2-100)*

Table S4: **Shortening metric values calculated (with 95% confidence intervals indicated) for evaluation of the assay's predictivity for QT shortening in the rabbit ventricular wedge experiments considering the different uses of available ion channel data described for a subset of 44 compounds for which there were data available from all three assays. Entries are ordered alphabetically by the data type used in the simulations. Missing entries are due to the inability to calculate the metrics due to there being no compounds in the relevant categories for their computation.**

Case Number	Ion Channel Data Use Combination	Accuracy (%)	Kappa Value	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
S1	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.00** *(-0.54-0.54)*	**0.00** *(0.00-27.8)*	**100** *(89.8-100)*	**-**	**77.3** *(63.0-87.2)*
S2	IonWorks Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.00** *(-0.54-0.54)*	**0.00** *(0.00-27.8)*	**100** *(89.8-100)*	**-**	**77.3** *(63.0-87.2)*
S3	IonWorks/FLIPR Data, Multiple Channel Block, Fitting For IC_{50} Value *(44 Compounds)*	**70.5** *(55.8-81.8)*	**0.13** *(-0.27-0.53)*	**30.0** *(10.8-60.3)*	**82.4** *(66.5-91.7)*	**33.3** *(15.6-57.6)*	**80.0** *(64.1-90.0)*
S4	IonWorks/FLIPR Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**72.7** *(58.2-83.7)*	**0.27** *(-0.08-0.62)*	**50.0** *(23.7-76.3)*	**79.4** *(63.2-89.7)*	**41.7** *(21.5-65.0)*	**84.4** *(68.2-93.1)*
S5	IonWorks/FLIPR Data, Multiple Channel Block Including KCNQ1 Interactions, Fitting For IC_{50} Value *(44 Compounds)*	**70.5** *(55.8-81.8)*	**0.13** *(-0.27-0.53)*	**30.0** *(10.8-60.3)*	**82.4** *(66.5-91.7)*	**33.3** *(15.9-56.9)*	**80.0** *(64.1-90.0)*
S6	IonWorks/FLIPR Data, Multiple Channel Block Including KCNQ1 Interactions, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**70.5** *(55.8-81.8)*	**0.19** *(-0.18-0.56)*	**40.0** *(16.8-68.7)*	**79.4** *(63.2-89.7)*	**36.4** *(17.7-60.3)*	**81.8** *(65.6-91.4)*
S7	PatchXpress Data, hERG Block Only, Fitting For IC_{50} Value *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.00** *(-0.54-0.54)*	**0.00** *(0.00-27.8)*	**100** *(89.8-100)*	**-**	**77.3** *(63.0-87.2)*

S8	PatchXpress Data, hERG Block Only, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.00** *(-0.54-0.54)*	**0.00** *(0.00-27.8)*	**100** *(89.8-100)*	**-**	**77.3** *(63.0-87.2)*
S9	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value *(44 Compounds)*	**79.5** *(65.5-88.8)*	**0.23** *(-0.22-0.68)*	**20.0** *(5.67-51.0)*	**97.1** *(85.1-99.5)*	**66.7** *(41.7-84.8)*	**80.5** *(66.0-89.8)*
S10	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.18** *(-0.27-0.63)*	**20.0** *(5.67-51.0)*	**94.1** *(80.9-98.4)*	**50.0** *(28.5-71.5)*	**80.0** *(65.2-89.5)*
S11	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value, One-Dimensional Simulation *(44 Compounds)*	**72.7** *(58.2-83.7)*	**0.10** *(-0.34-0.53)*	**20.0** *(5.67-51.0)*	**88.2** *(73.4-95.3)*	**33.3** *(18.0-53.3)*	**78.9** *(63.7-88.9)*
S12	PatchXpress Data, Multiple Channel Block, Fitting For IC_{50} Value & Hill Coefficient, One-Dimensional Simulation *(44 Compounds)*	**75.0** *(60.6-85.4)*	**0.20** *(-0.20-0.61)*	**30.0** *(10.8-60.3)*	**88.2** *(73.4-95.3)*	**42.9** *(26.0-61.6)*	**81.1** *(65.8-90.5)*
S13	QSAR Data, hERG Block Only *(44 Compounds)*	**77.3** *(63.0-87.2)*	**0.00** *(-0.54-0.54)*	**0.00** *(0.00-27.8)*	**100** *(89.8-100)*	**-**	**77.3** *(63.0-87.2)*
S14	QSAR Data, Multiple Channel Block *(44 Compounds)*	**68.2** *(53.4-80.0)*	**-0.05** *(-0.51-0.40)*	**10.0** *(1.79-40.4)*	**85.3** *(69.9-93.6)*	**16.7** *(7.70-32.4)*	**76.3** *(60.8-87.0)*

S.3 Experimental Protocols Used For Ion Channel Screening

S.3.1 Screening using IonWorks

Cell Preparation

Human NaV1.5:

Human Embryonic Kidney-293 (HEK293) cells were stably transfected with human NaV1.5 expression vector (pCIN5-hNaV1.5). Cells were cultured in DMEM with F12, supplemented with 10% FBS, 1x NEAA, plus 400μg/ml geneticin. Cells were grown and maintained at 37℃ in a humidified environment containing 5% CO2. Media without geneticin was used for cell harvesting. Cells with less than 80% confluency were detached from the T75 culture flask for passage and harvesting using TrypLE or Versene. After media aspiration cells were washed with pre-warmed Ca^{2+}- and Mg^{2+}-free D-PBS. Then 3 ml pre-warmed TrypLE or Versene were added for 3-5 minutes, respectively, followed by addition of 10-12 ml pre-warmed Ca^{2+}- and Mg^{2+}-containing D-PBS. Finally cells were gently mixed 3-4 times. The suspension was centrifuged at 300 x G for 2 minutes, the pellet resuspended to a cell concentration of of 2-3 million cells/ml and that solution added to the IonWorks™ instrument.

hERG:

Chinese Hamster Ovary (CHO) cells stably expressing hERG were cultured in M1 DMEM Ham's with F12, supplemented with 10% FBS and 400μg/ml geneticin. Cells were thawed in T175 flasks at 6-8millions cells per T175 flask, maintained at 37℃ in a humidified environment containing 5% CO2 for 3-4 hours and transferred to a 30℃ incubator containing 5% CO2 and incubate for another 72 hours before assaying. On day of assay, cells which were over 80% confluence were used. Media were removed and cells were washed with warm PBS (without magnesium and calcium) two times. 5 ml pre-warmed Versene was added for 6 minutes, followed by addition of 10 ml of warm M1 media. The suspension was placed into a 15 ml centrifuge tube and spun for 2 minutes at 1K rpm. The supernatant was removed and cells were re-suspended in 5 ml of warm M1 media and incubated for 5 minutes for the cells to recover. After 5 minutes the cells the suspension was centrifuged at 1K rpm for 2 minutes, the pellet re-suspended to a cell concentration of 4-5 million cells/ml and that solution was added to the IonWorks™.

KCNQ1:

Chinese hamster ovary (CHO) cells were stably transfected with KCNQ1 (also known as Kv1.7 or KvLQT1) the pore forming unit of the cardiac potassium current inward rectifier -and KCNE1 (also known as minK) the auxiliary subunit in the cariac ion channel. Cells were cultured in IMDM ISCOVE media, supplemented with 10% FBS and 800μg/ml geneticin, 1 ml hygromycin, 5 ml Pen/Strep and filtered. Cells were thawed in T175 flasks at 6-8 millions cells per T175 flask, maintained at 37℃ in a humidified environment containing 5% CO2 for 24 hours and transferred to a 30℃ incubator containing 5% CO2 and incubated for another 48 hours before assaying. On day of assay, confluency of cells should be $< 40\%$ for screening. Media were removed, and cells were washed with warm KCNQ1 external solution (without magnesium and calcium). 3 ml pre-warmed TrypLE was added for 2-3 minutes, followed by addition of 10 ml of warm KCNQ1 external solution (without magnesium and calcium). The suspension was placed into a 15 ml centrifuge tube and spun for 2 minutes at 1K rpm. The supernatant was removed and cells re-suspended in 5 ml of warm KCNQ1 external solution (with magnesium and calcium). The pellet was re-suspended to a cell concentration of 3.5-4.5 million cells/ml and that solution added to the IonWorks™.

Experimental Protocols

All currents were recorded before and after the addition of compound using a Molecular Devices IonWorks Quattro automated electrophysiology instrument in Population Patch-Clamp mode.

Human NaV1.5:

The intracellular solution contained the following: 100 mM K-gluconate, 40 mM KCl, 3.2 mM $MgCl_2$, 5 mM HEPES, 3 mM EGTA, pH 7.3 with KOH. Amphotericin-B solution was prepared as 50 mg/ml stock solution in dimethylsulfoxide (DMSO) and diluted to a final working concentration of 0.1 mg/ml in intracellular solution. The external solution was D-PBS and contained the following: 0.90 mM $CaCl_2$, 2.67 mM KCl, 1.47 mM KH_2PO_4, 0.493 mM $MgCl_2$, 137.9 mM NaCl, 8.06 mM Na_2HPO_4, pH 7.4. All wells with a pre- and post-drug addition resistance of $> 20M\Omega$ and which yielded a > 200pA transient inward NaV current were included in the analysis.

The voltage pulse protocol applied pre- and post- compound addition was as follows: From a holding potential of -80 mV (30 seconds), a train of five 200 millisecond depolarising voltage pulses were applied at a frequency

of 2 Hz. The peak of the inward currents during the first and fifth 0 mV depolarisation were exported for the pre- and post-drug conditions. The amount of compound block observed at the fifth pulse determines the accumulated block observed and is expressed a percentage of the pre-compound current observed at the first pulse to give a measure of the "global" (tonic and use-dependent) block achieved by the compound.

hERG:

The KCl intracellular solution contained the following: 140 mM KCI, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 20 mM HEPES, pH 7.3 with KOH. Amphotericin-B solution was prepared as 50 mg/ml stock solution in dimethylsulfoxide (DMSO) and diluted to a final working concentration of 0.1 mg/ml in intracellular solution. The external solution was D-PBS (-). The voltage pulse protocol applied pre- and post- compound addition was as follows: hERG currents were activated by 4 seconds depolarising pulse to +40 mV from a holding potential of −80 mV. The cells were then repolarised to −50 mV to generate large outward tail currents for 5 seconds.

KCNQ1:

The KCNQ1 internal solution contained the following: 100 mM Potassium Gluconate, 54 mM Potassium Chloride, 3.2 mM $MgCl_2$, 5 mM HEPES, pH 7.3 with KOH. All solutions were filtered before use. Amphotericin-B solution was prepared as 50 mg/ml stock solution in dimethylsulfoxide (DMSO) and diluted to a final working concentration of 0.1 mg/ml in intracellular solution. The KCNQ1 external solution with Ca^{2+} and Mg^{2+} contained the following: 65 mM Sodium Gluconate, 70 mM Sodium Chloride, 5 mM Potassium Chloride, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.4 with NaOH. The KCNQl external solution without Ca^{2+} and Mg^{2+} contained the following: 65 mM Sodium Gluconate, 70 mM Sodium Chloride, 5 mM Potassium Chloride, 5 mM HEPES, pH 7.4 with NaOH. The voltage pulse protocol applied pre- and post- compound addition was as follows: From a holding potential of −80 mV (100 ms), test opener potential 0 mV for 4 secongs, step to −10mV for 2 seconds, holding potential −80 mV for 5 secongs, test blocker potential +50 mV for 4 seconds, step to −10 mV for 2 seconds. The amount of compound block observed at the end of the +50 mV pulse and is expressed a percentage of the pre-compound current observed at the beginning of the +50 mV pulse to give a measure of the tonic block achieved by the compound.

Data Analysis

IonWorks recordings are population patch measurements in which the average of the current across many cells is determined. Hence the word observation is used for a single concentration response curve. There could be several observations on a certain day.

Human NaV1.5:

Comparisons between pre-drug peak transient inward currents at the first pulse and post-drug peak transient inward currents fifth pulse were used to determine the global inhibitory effect of the compound. Data were normalised to the high and low controls. Low controls were wells in which 100 μM tetracaine was added for the NaV1.5 blocker assay. High controls were wells in which only 1% DMSO was added for the NaV1.5 blocker assay. The normalised data were analysed by using ActivityBase software. The amount of NaV1.5 current inhibition observed at the fifth depolarising pulse after compound addition was expressed as a percentage of the peak current observed at the first pulse before compound addition and used to generate a global block concentration dose response.

hERG:

Data were normalised to the high and low controls. Low controls were wells in which 50 μM Quinindine was added for the hERG blocker assay. High controls were wells in which only 1% DMSO was added for the hERG assay. The normalised data were analysed by using ActivityBase software. The amount of tonic block is calculated from peak (maximum tail current value). This value is amplitude of the peak tail current minus the steady state average value obtained at -50 mV holding potential before the first voltage step to $+40$ mV.

KCNQ1:

Data were normalised to the high and low controls. Low controls were wells in which an internal characterised full block compound was added for the KCNQ1 blocker assay. DMSO concentration in compound wells and high controls wells were only 0.25% for the KCNQ1 assay. The normalised data were analysed by using ActivityBase software. The current elicited at the end of the 50 mV pulse is normalised to that at the start of the pulse and normalised to control data.

For All Channels:

Concentration response data were derived using a four parameter concentration-effect curve fitting procedure. pIC50 values and Hill coefficients were deter-

mined from these inhibition curves.

S.3.2 CaV1.2 FLIPR Screen

Cell Preparation

Human Embryonic Kidney-293 (HEK293) cells were stably transfected with the β2a and the $\alpha 2\delta 1$ subunits of CaV1.2 (L-type calcium channel). Cells were cultured in DMEM HAMS-F12 +10% FBS. Cells were grown and maintained at 37℃ in a humidified environment containing 5% CO2. 24 hours prior to assay media was removed and replaced with DMEM-F12 media supplemented with 10% FBS and containing 2.5% hIK and 5% alpha1C BacMam virus transduction reagents to the cell suspension. Cells with less than 80% confluency were detached from the T75 culture flask for passage and harvesting using TrypLE or Versene. Cells were resuspended in DMEM-F12 media supplemented with 10% FBS and seeded at 15,000 cells per well in clear-bottom, black-walled 384 well plates.

Experimental Protocol

Human CaV1.2 fluorescence was measured before and after the addition of a depolarising addition of 400 mM KCl using a Molecular Devices 384 well fluorescent imaging plate reader (FLIPR).

Cell media was replaced with tyrodes salt solution supplemented with 20 mM HEPES, 11.9 mM NaHCO3, 2.5 mM probenecid, 0.01% Pluronic acid, 2.5 μM Fluo4-AM (a calcium sensitive fluophore) and 250 μM Brilliant Black (quenching solution) at pH 7.4 and the cells were incubated at 37℃ in a humidified environment containing 5% CO2 for 60 minutes. Compounds were prepared as serial dilution concentration response curves in DMSO before being diluted in tyrodes medium immediately prior to assay. Cells were incubated for 15 minutes in the presence of tyrodes buffer containing compounds of interest. Cell plates containing compounds were placed inside the FLIPR and changes in fluorescence measured (λ_{ex}=488 nm, λ_{EM}=54 nm) (Sullivan et al., 1999) before and after the addition of the depolarising solution.

Data Analysis

The timecourse of fluorescence across each well of the plate was measured before and after stimulation, and the maximum, minimum and basal values were extracted for further analysis.

Blockade of the transient increase in fluorescence caused by the influx of calcium through the activated calcium channel was used to determine the global inhibitory effect of the compound. Data (maximum minus minimum divided by basal fluorescence value) were normalised to the high and low controls. Low controls were wells in which 1 mM nimodipine was added for the CaV1.2 blocker assay. High controls were wells in which only 1% DMSO was added for the CaV1.2 blocker assay. The normalised data were analysed by using ActivityBase (IDBS) software. Concentration response data were derived using a four parameter concentration-effect fitting procedure. pIC50 values were determined from these inhibition curves.

S.3.3 Screening using PatchXpress

NaV1.5:

See (Donovan et al., 2011).

hERG:

See (Donovan et al., 2011).

CaV1.2:

Cell Preparation

Flasks of continuously cultured cells were grown (medium descriptions can be found in Tables S5 and S6 below) at 37℃, 5% CO2 and split when cells were between 35 and 75% confluency to retain surface channel expression. To harvest cells for experiments, all solutions were warmed to 37℃. Flasks to be harvested had their medium aspirated and 10 mL of no Ca^{2+}, no Mg^{2+} PBS (Invitrogen Corporation, Carlsbad, CA) added. The PBS was gently rolled over the cell layer then aspirated. 4 mL of a 1:5 solution of TrypLE (recombinant trypsin, Invitrogen Corporation, Carlsbad, CA) diluted in no Ca^{2+}, no Mg^{2+} PBS was added. The solution was gently rolled over the cell layer and allowed to sit for 90 seconds at room temperature. The flask was tapped to dislodge cells. Eight mL of medium was used to quench the harvesting agent. The resulting cell stock was pipetted up and down twice with a 5 mL pipette to break up any cell clumps. The cell stock was spun at 1000 rpm for 90 seconds, the supernate aspirated off and 4 mL of fresh medium was added. The cells mass was broken up gently with a 200 μL pipettor. Cells were placed in a 37℃ incubator to allow the cells to recover from the harvest. After 10 minutes, the cells were removed from

the incubator and spun down at 1000 rpm for 90 seconds. The aspirate was removed and 2 mL of external assay buffer (assay buffer detailed in Tables S5 & S6, depending on the assay) added. The cell stock was broken with a 200 μL pipettor and spun at 1000 rpm for 60 seconds. The supernate was removed and 150 to 225 μL of external buffer added. This cell stock was added to the PatchXpress for the experiment. This protocol was followed for all cell lines.

For experiments using frozen cell lines, thawing consisted of removing the cells from the -180℃ freezer and putting the vial in a 37℃ water bath for 15-20 seconds. The thawed cell stock was added to a vial containing 8 mL warmed growth medium. The rest of the procedure follows the procedure for constantly cultured cells at the point of the first centrifuging step.

Cells were re-suspended in 100 to 300 μL of external buffer (Table S5 and S6, depending on which assay is being run) and put into the PatchXpress. 3.5 μL of stock was aliquoted to each well.

Experimental Protocol

Two command protocols were used for the CaV1.2 assay. For both versions of the CaV1.2 assay, each cell was clamped at a -90 mV holding potential.

In the earlier version, every thirty seconds a command protocol was delivered consisting of ten depolarizing steps to +10 mV. Each step lasted ten milliseconds. The block was measured as the percent decrease in current measured at the tenth pulse. In the later version, a command pulse to zero millivolts was given every two seconds. The pulse lasted for 200 milliseconds with the peak inward current measured at each pulse.

In concentration-response experiments for all PatchXpress experiments, each solution was added three times (40 μL each addition for 120 μL total, 11 seconds apart). After the addition, the compound was given 5 minutes to reach steady-state block. If after 5 minutes, the difference in peaks between sweeps was less than 0.1%, the next concentration was given, otherwise, the cell was allowed up to three more minutes to reach steady-state block before the next concentration addition.

At the end of each dose-response series, a negative control of external buffer with an identical amount of DMSO (vehicle) was added to wash off the compound effect. After this, a positive control was given to completely block the current (50 μM cadmium).

Data Analysis

Data analysis for PatchXpress data was done using DataXpress, v2.0.4.5, raw data from this program was compiled using Microsoft Excel and fitted to a four parameter fit using GraphPad Prism v5.0. The four parameter fit used to curve fit for the voltage dependence of activation was,

$\mathrm{Y} = \mathrm{Ymin} + \frac{(Ymax - Ymin)}{(1+10\exp((\log(EC50) - X)Hillslope))}$.

Table S5: **Composition of culture medium for CaV1.2β2aα2δ1 expressing HEK293 cells used in PatchXpress screen of CaV1.2 channels.**

Chemical	**Catalog # (manufacturer)**	**[stock]**	**[final]**	**lot #**	**mL**
Dulbecco's modified eagle medium/F12 Ham's	11330-032 (Invitrogen)	100%	–	1344957	869
Fetal bovine serum	10437-028 (Invitrogen)	100%	10%	13558888	100
Glutamine	25030-149 (Invitrogen)	200 nM	2 mM	1318770	10
Puromycin	P9620-10ML (Sigma)	10 mg/mL	0.625 μg/mL	106K4072	0.0625
Non-essential amino acids	11140-050 (Invitrogen)	10 nM (100x)	1%	1382464	10
G418/geneticin	10131-027 (Invitrogen)	50 mg/mL	400 μg/mL	1387855	8
hygromycin B	10687-010 (Invitrogen)	50 mg/mL	100 μg/mL	A8269	2

10 μM nitrendipine needs to be added to the medium daily for passage of cells to maintain viability. We make a 10 mM stock solution and aliquot it into 200 mL batches to be added into a 200 mL medium batch for a final concentration of 10 μM. We currently use Tocris pharmaceuticals nitrendipine, catalog number 0601, lot # 1A/73728.

Table S6: **Composition of internal solution for CaV1.2 used in PatchXpress screen of CaV1.2 channels.**

Chemical	**molecular weight**	**catalog #**	**mM**	**grams /0.25L**
Cesium methane sulfonate	228.0	C1426	108	6.156
HEPES	238.31	H3375	24	1.430
EGTA	468.3	E8145	10	1.171
MgCl2	95.22	M8266	4.5	1125 μL of 1 M stock
CaCl2	147.0	C5080	0.1	250 μ L of 100 mM stock
ATP (disodium salt)	551.1	A2383	4	0.551
GTP (disodium salt)	523.2	G8877	0.3	0.039
creatine	131.14	C7080	5	0.164
phosphocreatine (disodium)	453.38	P1937	5	0.567
pyruvate (free acid)	88.06	15940 (Fluka)	5	86.68 μL
Oxalacetate	132.07	171255 (Aldrich)	5	0.165

Initial pH should be $\sim$ 4.89. Adjust to 7.30 using CsOH. Adjust osmolality to 295-300 mOsm.

References

Donovan, B., Bakshi, T., Galbraith, S., Nixon, C., Payne, L., & Martens, S. (2011). Utility of Frozen Cell Lines in Medium Throughput Electrophysiology Screening of hERG and NaV1.5 Blockade. *Journal of Pharmacological and Toxicological Methods*, *64*, 269–276.

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