SUPPLEMENTAL DATA

A High-throughput Assay to Identify Drugs that can Treat Long QT Syndrome Caused by Trafficking-deficient K_V11.1 (hERG) Variants

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Table 1 - Oligonucleotide primers used for identification of 3' Kv11.1 stop codon.

Oligonucleotide name	**Sequence (5' – 3')**	**Description**
3' anchor primer 1 (AP1)	ACAGCAGGTCAGTCAAGCAGTA	
3' anchor primer 2 (AP2)	AGCAGTAGCAGCAGTTCGATAA	Nested AP1
Random 7mer adapter	ACAGCAGGTCAGTCAAGCAGTAGCAGCAGTTCGATAA**GCGGCCGCCATGG**ANNNNNNN	
5' Kv11.1-GSP (1)	TCACCTTCAACCTGCGAGAT	Base pairs 2573-2592
5' Kv11.1-GSP (2)	TAGAGGGTGGCTTCAGTCGG	Base pairs 2630-2649
5' Kv11.1-GSP (3)	ACCTGCGAGATACCAACATGA	Nested 5' Kv11.1-GSP (1)
5' Kv11.1-GSP (4)	AACGCAAGCGCAAGTTGTC	Nested 5' Kv11.1-GSP (2) (Start at 2651)

Figure S1 – Western blot and sequencing reveal K_V11.1 truncation at glycine residue at amino acid 965 (G965). A) Western blot showing K_V11.1 and K_V11.1-G601S-G965*X variant with and without increased K_V11.1 protein trafficking with 24-hour E-4031 treatment. B) Sequencing revealed K_V11.1 cDNA stops at residue G965 and encodes an additional 17 amino acids before a stop codon. Black line represents end of gene sequence and blue line indicates end of 17 amino acid addition.

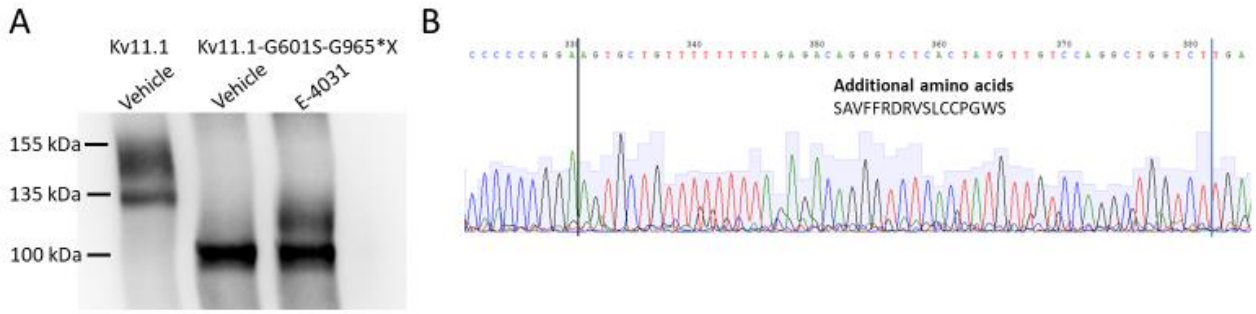

Figure S2 – Kv11.1-G601S-G965*X variant performs better than Kv11.1-G601S in optimized Tl^+-flux assay. **A**) Fluorescent traces from HEK-293 cell monolayers treated for 24 hours with positive or vehicle control after optimizing stimulus concentrations, Thallos loading, and cell density for the Tl^+ flux assay. Black arrow represents Tl^+ (4.5 mM) addition. All wells were washed out before experiments. **B**) Scatter plot showing Tl^+ flux slope values (ΔF/s) after 24-hour treatment with positive and vehicle control. Red line indicates mean slope (ΔF/s) for positive and vehicle control. Dotted black lines indicate ±3 SDs from the mean of each control. For all experiments, vehicle (0.1% DMSO) or positive (10 µM E-4031) were used (n=96 wells/treatment condition).

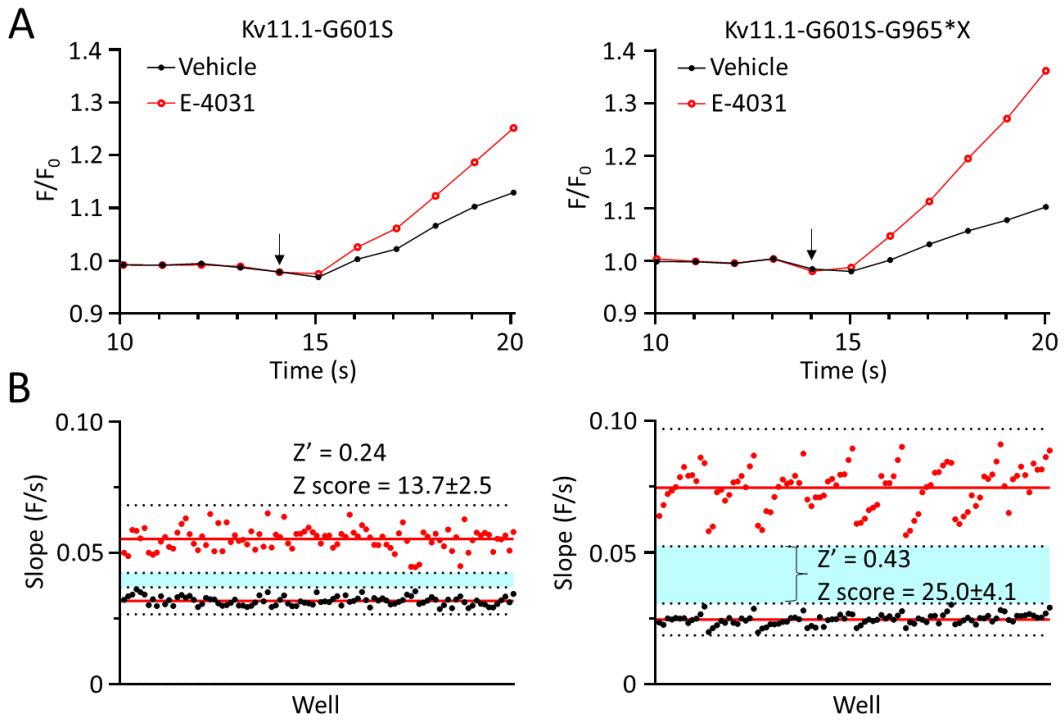

Figure S3 – Kv11.1 channel activator (VU0405601) increases steady-state activation current in wild-type Kv11.1 **A**) Example current traces of Kv11.1 in absence and presence of 10 µM VU0405601. Dotted black line represents 0 current. **B)** Current-voltage (I-V) relationship of Kv11.1 steady-state current in absence (n=6 cells) and presence (n=3 cells) of 10 µM VU0405601. **C)** Normalized tail current data from cells in absence (n=6 cells) and presence (n=3 cells) of 10 µM VU0405601. All data in C normalized to the average of maximum tail current (pA/pF) from cells expressing Kv11.1 treated with vehicle. All data reported as mean ± SD. Vehicle control = 0.1% DMSO treatment.

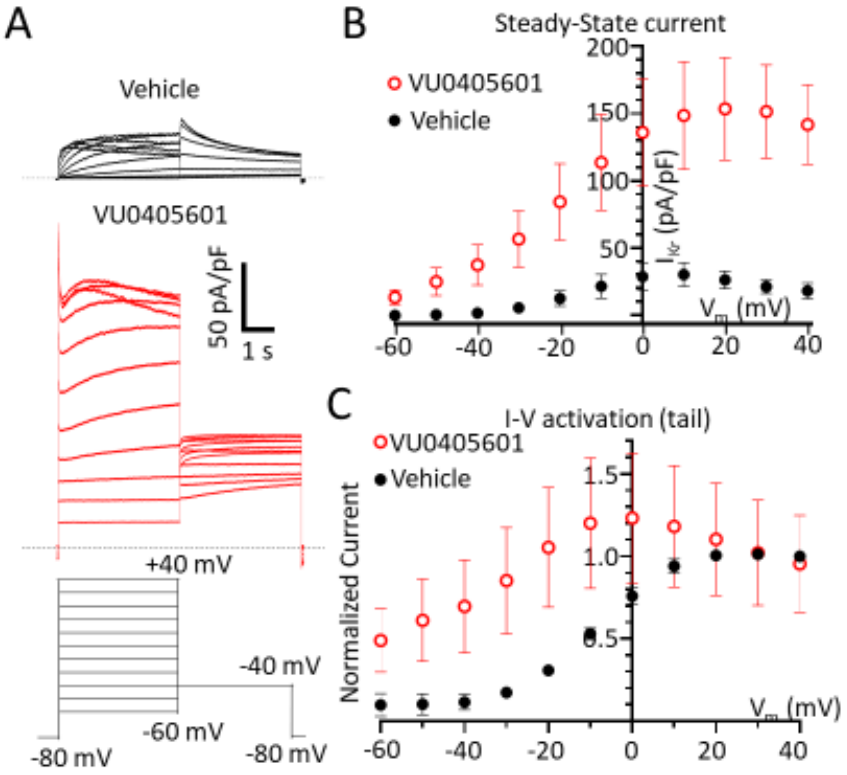

Figure S4 – Kv11.1 channel activator (VU0405601) decreases Kv11.1 channel inactivation in wild-type Kv11.1. **A)** Example Kv11.1 current traces in absence and presence of 10 µM VU0405601 in extracellular bath solution during experiments. Inset shows Kv11.1 tail currents used to analyze peak current. Dotted black line indicates 0 current. **B)** Normalized current-voltage (I-V) relationship of Kv11.1 channel inactivation in absence and presence (n=3 cells/treatment) of 10 µM VU0405601. Data normalized to the average of maximum tail current (pA/pF) in cells expressing Kv11.1 treated with vehicle. All data reported as mean ± SD. Vehicle control = 0.1% DMSO.

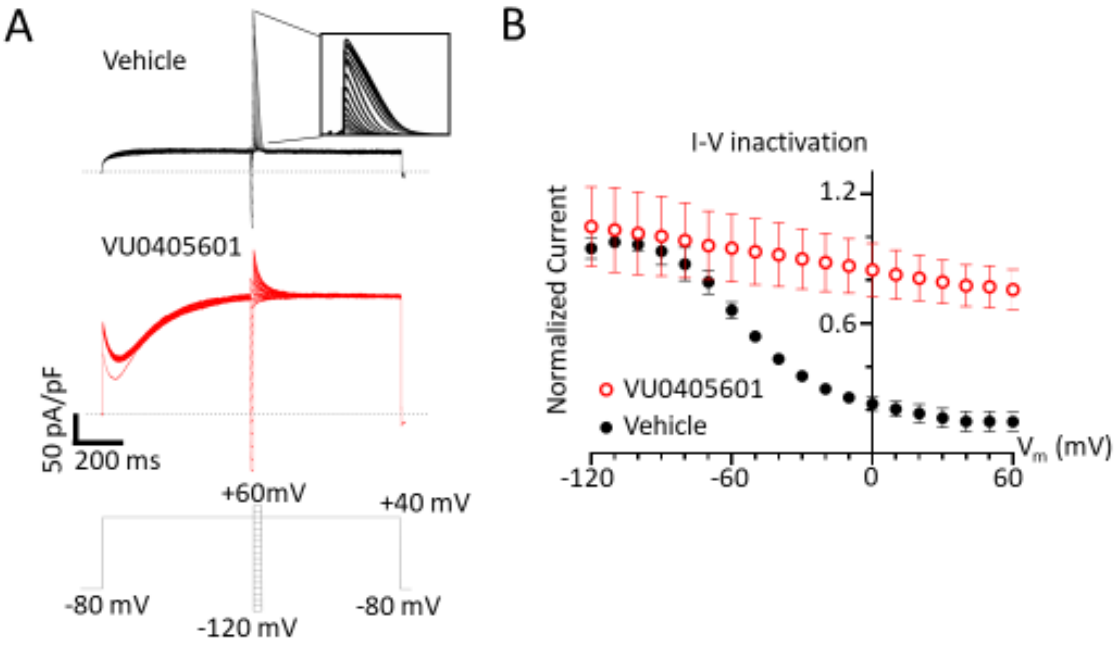

Figure S5 – Western blot from HEK-293 cells expressing Kv11.1-G601S showing increased fully glycosylated protein, representative of increased protein trafficking after 24-hour treatment with 10 µM of azelastine, azaperone, or ibutilide. All wells loaded with 20 µg of protein. Vehicle = 0.1% DMSO.

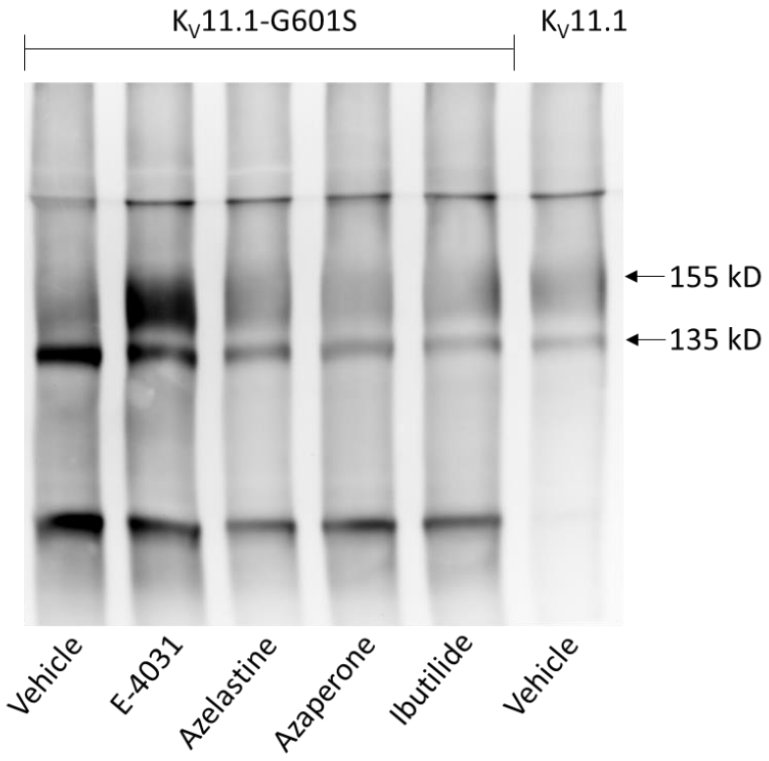

Figure S6 – Drugs that increase Kv11.1-G601-G965*X trafficking do not increase Kv11.1 (wild-type) trafficking or function. Concentration response comparing 24-hour treatment and washout with 10 µM positive control (E-4031) or azelastine, azaperone, and ibutilide in Kv11.1 (blue open symbols) and Kv11.1-G601S-G965X (black closed symbols). The graph shows slope (ΔF/s) with the average slope of 38 wells (0.1% DMSO control) subtracted. N=5 wells for each concentration tested. All data reported as mean ± SD.