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Supplemental Information

SMIT1 Modifies KCNQ Channel Function and Pharmacology by Physical Interaction with the Pore

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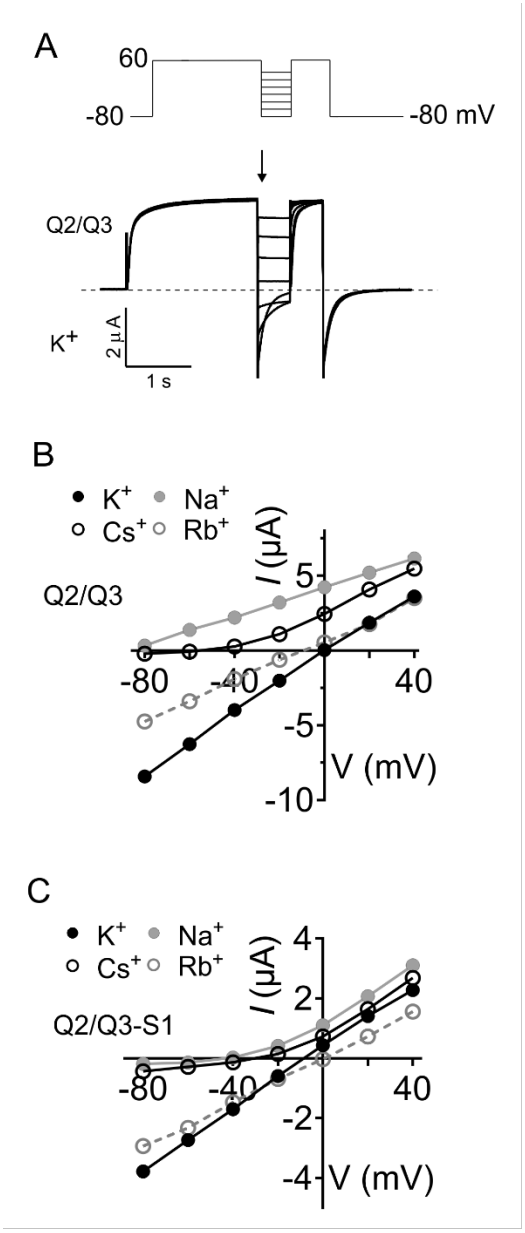
	Peak Current, +40 mV (μA)	τ act, -40 mV (ms)	τ deact, -30 mV (ms)	G/Gmax (mV)	Slope (mV)	TEA IC_{50} (mM)
Q1	1.79 ± 0.2 (*n* = 97)	1137.4 ± 20 (*n* = 97)	572.6 ± 39 (*n* = 97)	-25.4 ± 1.1 (*n* = 97)	14.1 ± 1.1 (*n* = 97)	29.3 ± 0.1 (*n* = 5)
Q1-S1	1.88 ± 0.2 (*n* = 102)	1189.1 ± 89 (*n* = 102)	550.6 ± 75 (*n* = 102)	-27.0 ± 1.5 (*n* = 102)	14.6 ± 1.4 (*n* = 102)	121 ± 0.09 (*n* = 5) ****
Q1/E1	11.40 ± 0.8 (*n* = 100)	4251.1 ± 347 (*n* = 12)	2006.5 ± 186 (*n* = 12)	-12.4 ± 3.7 (*n* = 12)	11.2 ± 3.3 (*n* = 12)	95 ± 0.25 (*n* = 4)
Q1/E1-S1	6.53 ± 0.8 (*n* = 90) ****	7166.7 ± 632 (*n* = 12) ***	1496.3 ± 83 (*n* = 12) *	5.1 ± 2.6 (*n* = 12) ****	14.8 ± 2.5 (*n* = 12)	103 ± 0.06 (*n* = 5) ****
Q1/E3	1.01 ± 0.1 (*n* = 13)	n.d.	n.d.	-40.8 ± 1.3 (*n* = 13)	9.57 ± 1.2 (*n* = 13)	n.d.
Q1/E3-S1	1.08 ± 0.2 (*n* = 13)	n.d.	n.d.	-47.8 ± 1.0 (*n* = 13) ***	10.1 ± 0.8 (*n* = 13)	n.d.
Q2	1.31 ± 0.2 (*n* = 32)	695.4 ± 25 (*n* = 32)	428 ± 17 (*n* = 32)	-37.5 ± 2.3 (*n* = 32)	17.9 ± 2.1 (*n* = 32)	n.d.
Q2-S1	1.05 ± 0.1 (*n* = 29)	638.9 ± 20 (*n* = 29)	507.3 ± 31 (*n* = 29)	-44.2 ± 3.3 (*n* = 29)	11.7 ± 2.8 (*n* = 29)	n.d.
Q2/Q3	4.08 ± 0.4 (*n* = 13)	1067.3 ± 117 (*n* = 13)	354.5 ± 32 (*n* = 13)	-30.7 ± 1.4 (*n* = 13)	9.4 ± 1.1 (*n* = 13)	6.9 ± 0.5 (*n* = 5)
Q2/Q3-S1	4.81 ± 0.9 (*n* = 10)	585.5 ± 36 (*n* = 10) **	331.7 ± 42 (*n* = 10)	-37.9 ± 0.7 (*n* = 10) ***	6.9 ± 0.9 (*n* = 10)	28.6 ± 0.4 (*n* = 5) ****
Q2/Q3 (100 mM K^+)	n.d.	n.d.	n.d.	-49.91 ± 2.9 (*n* = 11)	11.2 ± 2.3 (*n* = 11)	n.d.
Q2/Q3-S1 (100 mM K^+)	n.d.	n.d.	n.d.	-56.36 ± 1.08 (*n* = 11)	10.6 ± 0.8 (*n* = 11)	n.d.
Kv1.1	2.43 ± 0.28 (*n* = 13)	n.d.	n.d.	-16.9 ± 1.7 (*n* = 13)	15.5 ± 1.7 (*n* = 13)	n.d.
Kv1.1-S1	1.78 ± 0.3 (*n* = 13)	n.d.	n.d.	-13.5 ± 1.0 (*n* = 13)	17.6 ± 1.2 (*n* = 13)	n.d.

Table 1. KCNQ channel functional parameters ± KCNE1, KCNE3, SMIT1.
Statistics versus same channel in absence of SMIT1: ****p<0.0001, ***p=0.0008, **p=0.0015, *p=0.02, n.d., not determined. Bath solution contained 4 mM K^+ unless otherwise indicated. Abbreviations: E, KCNE; Q, KCNQ; S1, SMIT1; TEA, tetraethylammonium.

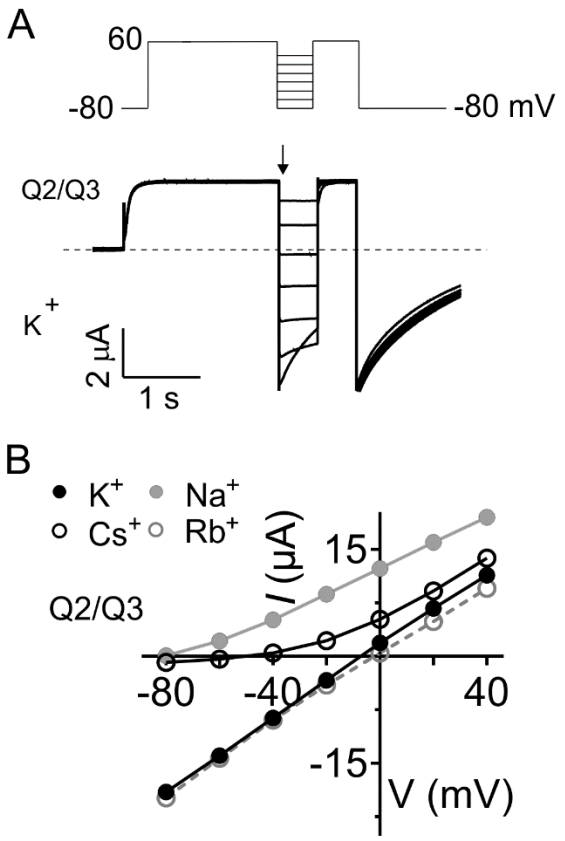


Supplementary Figure 1. The effect of SMIT1 on KCNQ2/Q3 ion selectivity is not removed by phlorizin

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/3 in 100 mM K^+ and 500 µM phlorizin (voltage protocol inset. 1 s pulse to 60 mV followed by a 500-ms inactivation recovery step to voltages between -80 and 40 mV, then a 500-ms -80 mV tail pulse).

B. Mean current-voltage relationship for KCNQ2/3 alone in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles) and 500 µM phlorizin, n = 13-14.

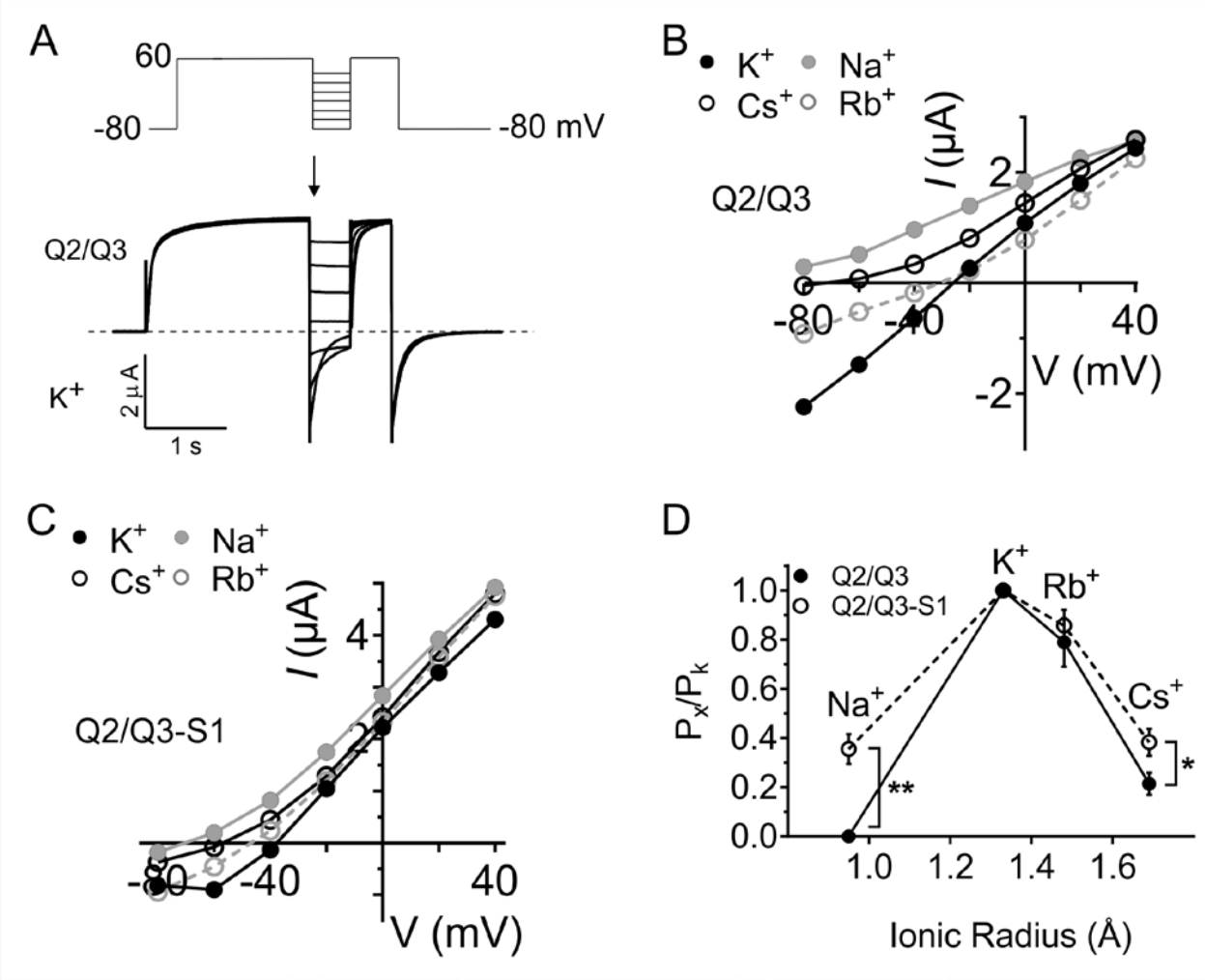
C. Mean current-voltage relationship for KCNQ2/3 in combination with SMIT1 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles) and 500 µM phlorizin, n = 13-14.



Supplementary Figure 2. The effect of SMIT1 on KCNQ2/Q3 ion selectivity is not mimicked by retigabine

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/3, in a bath solution containing 100 mM K^+ with 10 µM retigabine (voltage protocol inset. 1 s pulse to 60 mV followed by a 500-ms inactivation recovery step to voltages between -80 and 40 mV, then a 500-ms -80 mV tail pulse).

B. Mean current-voltage relationship for KCNQ2/3 alone in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles) and 10 µM retigabine, n = 8. See Fig. 3 F for corresponding selectivity series.



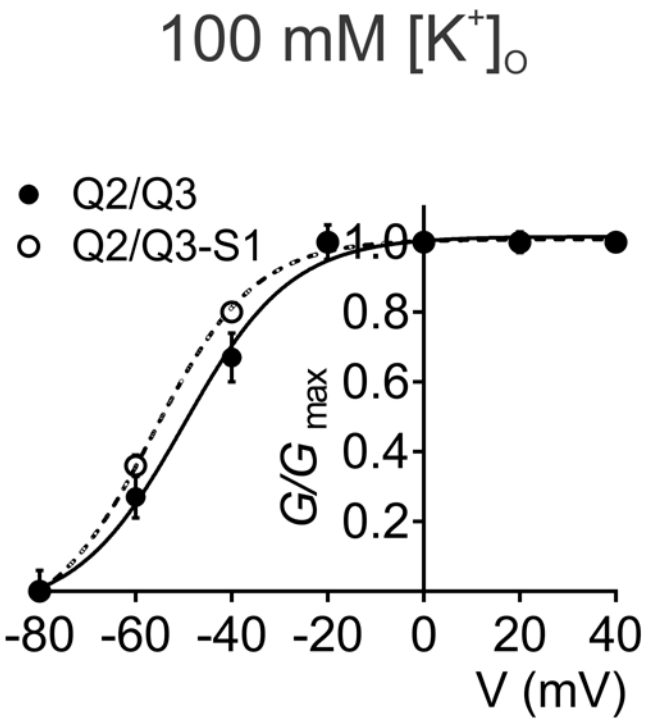
Supplementary Figure 3. SMIT1 alters KCNQ2/Q3 ion selectivity using 15 mM bath permeant ions

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/3 in 15 mM K^+ (voltage protocol inset. 1 s pulse to 60 mV followed by a 500-ms inactivation recovery step to voltages between -80 and 40 mV, then a 500-ms -80 mV tail pulse).

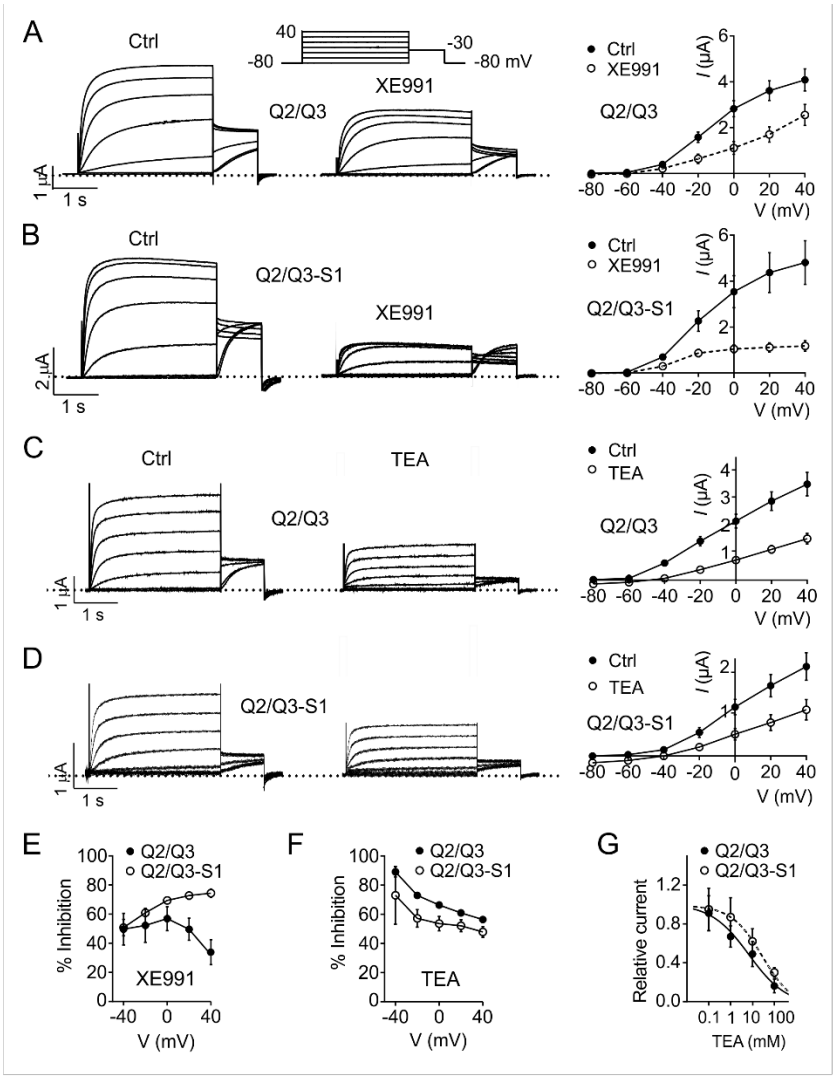
B. Mean current-voltage relationship for KCNQ2/3 alone in 15 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), $n = 6$.

C. Mean current-voltage relationship for KCNQ2/3 in combination with SMIT1 15 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), $n = 6$.

D. Estimated mean permeability relative to that of K^+ versus ionic radius (Pauling) for Na^+, Rb^+, and Cs^+ through KCNQ2/Q3 (solid circles) and KCNQ2/Q3+SMIT1 (open circles, dotted lines) channels, $n = 6$. Error bars indicate SEM. Statistical significance for comparison of relative permeability of Na^+: **$p<0.002$, Cs^+ *$p<0.03$. Values for relative permeability ratios, in the order Na^+, Rb^+, and Cs^+: KCNQ2/Q3, 0, 0.76, and 0.21; KCNQ2/Q3+SMIT1, 0.36, 0.85, and 0.38.



Supplementary Figure 4. SMIT1 negative-shifts KCNQ2/Q3 activation in 100 mM $[K^+]_O$
Mean normalized tail current (at -30 mV) versus prepulse potential for *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/Q3, alone or co-injected with cRNA encoding SMIT1, $n = 11$. Error bars indicate SEM.



Supplementary Figure 5. SMIT1 alters KCNQ2/Q3 pharmacology

A. *Left*, representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/3 in ND96 or 50 µM XE991. *Right*, mean current-voltage relationship for subunit combinations as in panel A, $n = 13$. Error bars indicate SEM.

B. *Left*, representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes co-injected with cRNA encoding KCNQ2/3 and SMIT1 in ND96 or 50 µM XE991. *Right*, mean current-voltage relationship for subunit combinations as in panel A, $n = 10$. Error bars indicate SEM.

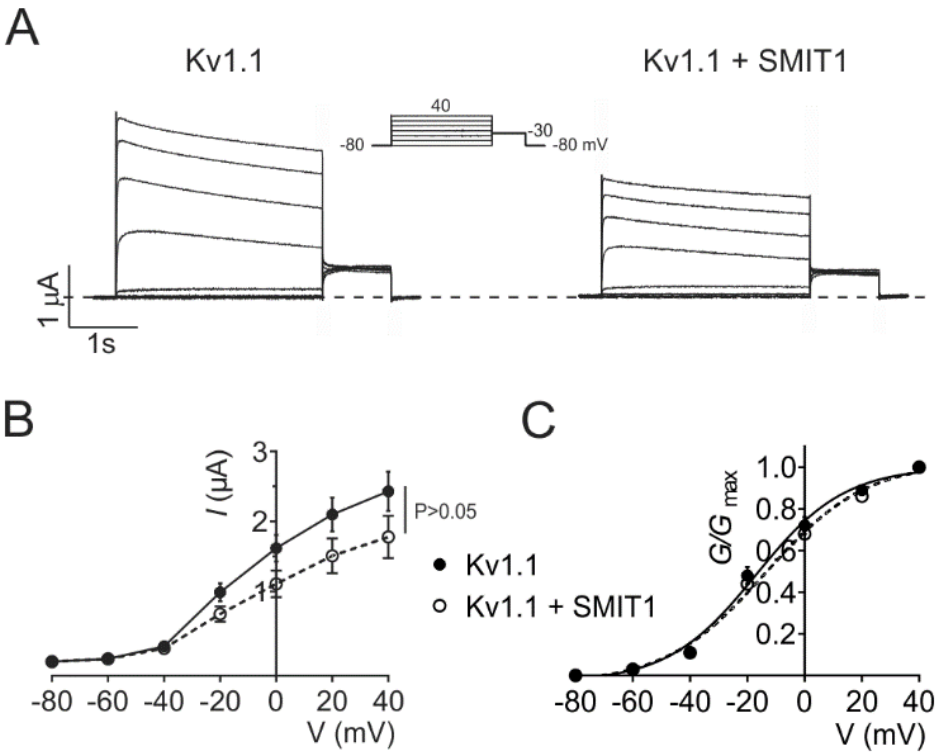
C. *Left*, representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/3 in ND96 or 100 mM TEA. *Right*, mean current-voltage relationship for subunit combinations as in panel A, $n = 13$. Error bars indicate SEM.

D. *Left*, representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes co-injected with cRNA encoding KCNQ2/3 and SMIT1 in ND96 or 100 mM TEA. *Right*, mean current-voltage relationship for subunit combinations as in panel A, $n = 6$. Error bars indicate SEM.

E. Mean % inhibition (50 µM XE991) versus voltage for KCNQ2/3 with or without SMIT1; $n = 10$-13. Error bars indicate SEM.

F. Mean % inhibition (100 mM TEA) versus voltage for KCNQ2/3 with or without SMIT1; $n = 6$. Error bars indicate SEM.

G. TEA dose response for KCNQ2/3 with or without SMIT1; $n = 5$. Error bars indicate SEM.

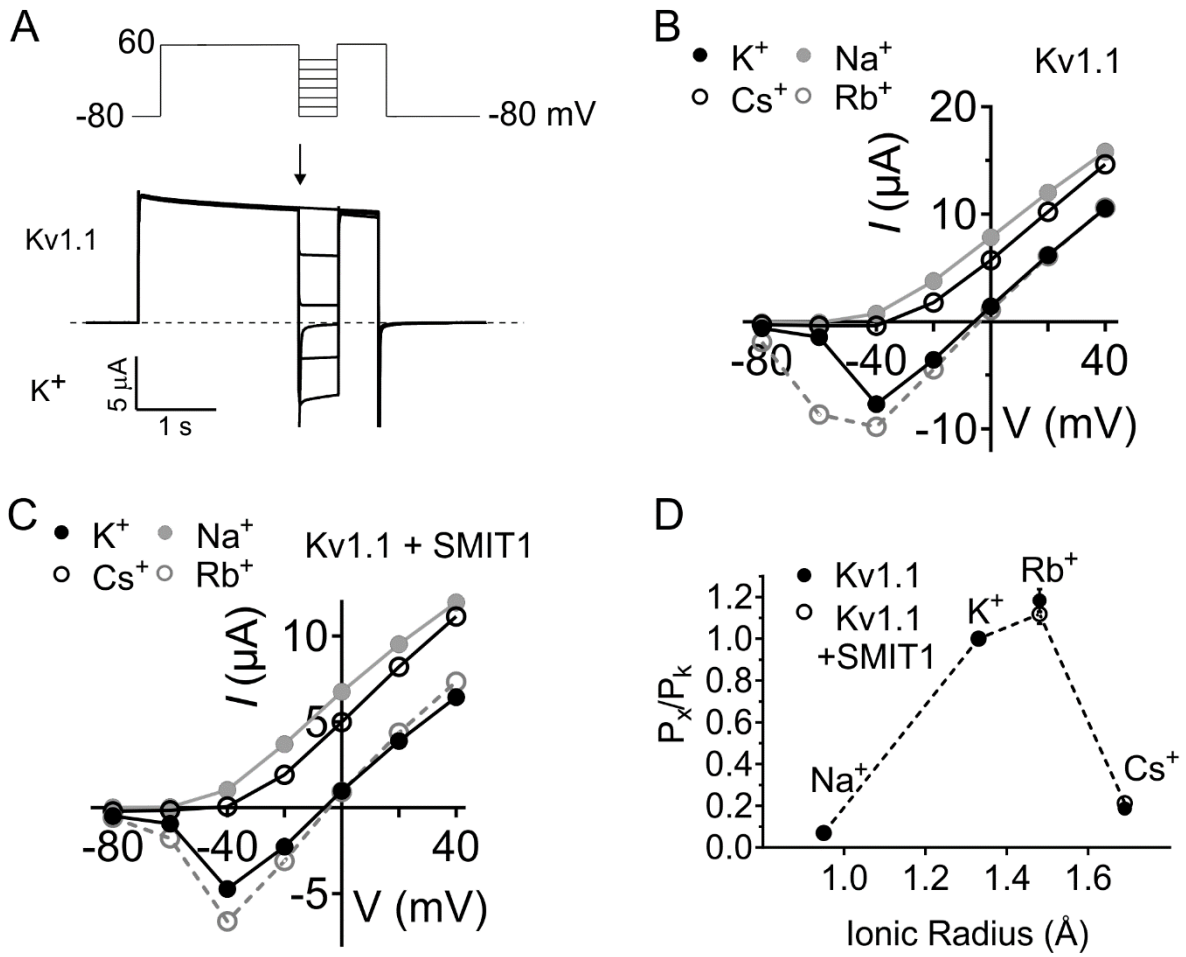


Supplementary Figure 6. SMIT1 does not alter Kv1.1 activity

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding Kv1.1, alone or co-injected with cRNA encoding SMIT1, *n* = 8-13.

B. Mean current-voltage relationship for subunit combinations as in panel A, *n* = 8-13. Error bars indicate SEM.

C. Mean normalized tail current (at -30 mV) versus prepulse potential for oocytes as in panel A, *n* = 8-13. Error bars indicate SEM.



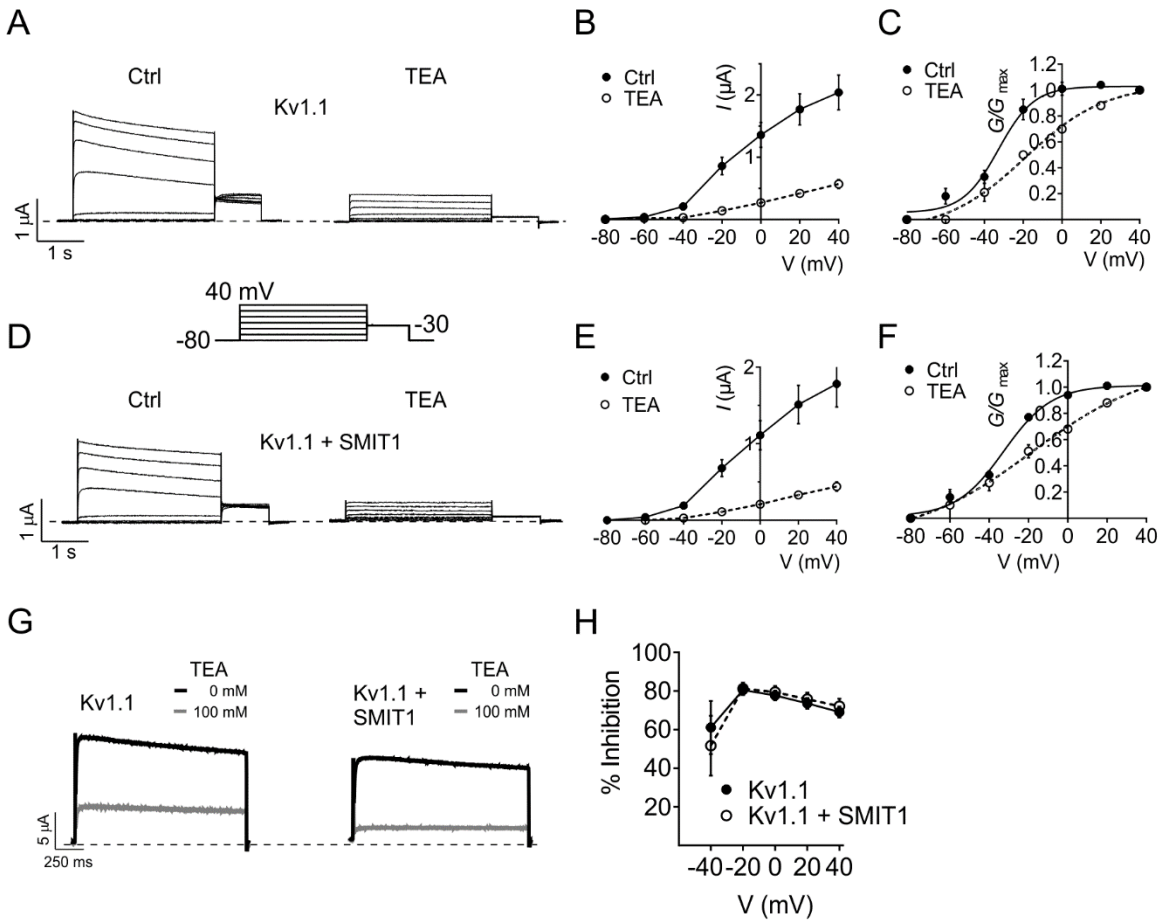
Supplementary Figure 7. SMIT1 does not alter Kv1.1 ion selectivity

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding Kv1.1 in 100 mM K^+ (voltage protocol inset. 1 s pulse to 60 mV followed by a 500-ms inactivation recovery step to voltages between -80 and 40 mV, then a 500-ms -80 mV tail pulse).

B. Mean current-voltage relationship for Kv1.1 alone in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), n = 12.

C. Mean current-voltage relationship for Kv1.1 in combination with SMIT1 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), n = 12.

D. Estimated mean permeability relative to that of K^+ versus ionic radius (Pauling) for Na^+, Rb^+, and Cs^+ through Kv1.1 alone (solid circles) or when co-expressed with SMIT1 (open circles, dotted lines) channels, n = 12-14. Error bars indicate SEM. Values for relative permeability ratios, in the order Na^+, Rb^+, and Cs^+: Kv1.1, 0.07, 1.18, and 0.18; Kv1.1+SMIT1, 0.07, 1.11, and 0.18.



Supplementary Figure 8. SMIT1 does not protect Kv1.1 from TEA block

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding Kv1.1, in bath ND96 or 100 mM TEA, *n* = 13. Voltage protocol inset: 3 s pulse to voltages between -80 and 40 mV followed by a 1 s -30 mV tail pulse. Error bars indicate SEM.

B. Mean current-voltage relationships for Kv1.1 recordings as in panel A, *n* =13. Error bars indicate SEM.

C. Mean normalized tail current (at -30 mV) versus prepulse potential for Kv1.1 recordings as in panel A, *n* = 13. Error bars indicate SEM.

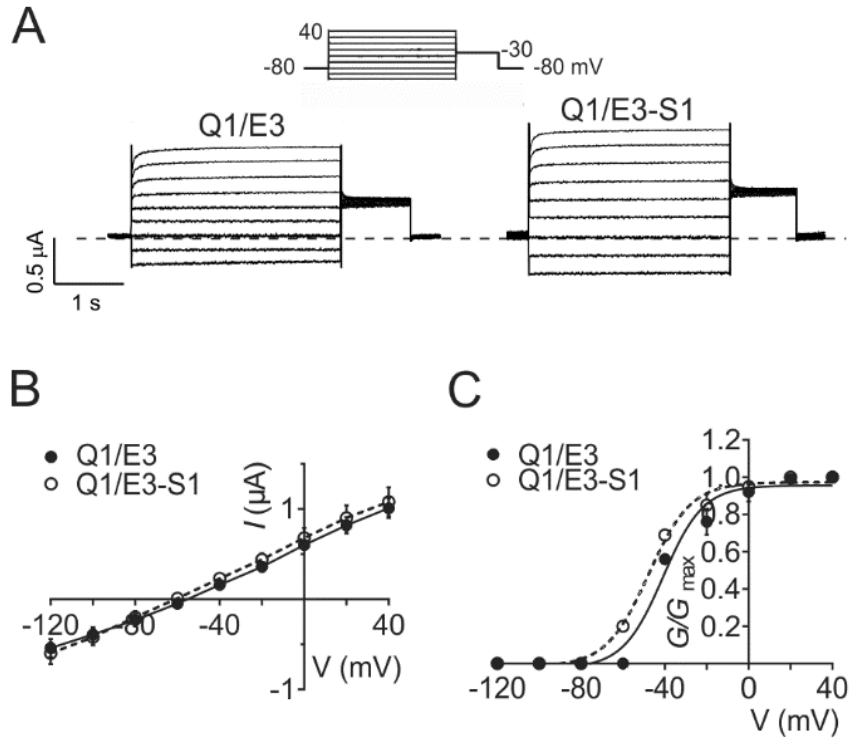
D. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNAs encoding Kv1.1 and SMIT1 in bath ND96 or 100 mM TEA, *n* = 8, using voltage protocol as in panel A. Error bars indicate SEM.

E. Mean current-voltage relationship for Kv1.1 and SMIT1 cRNA-injected oocytes as in panel D, *n* = 8. Error bars indicate SEM.

F. Mean normalized tail current (at -30 mV) versus prepulse potential for oocytes as in panel D, *n* = 8. Error bars indicate SEM.

G. Representative traces recorded at 0 mV for Kv1.1 with or without SMIT1 in 0 mM (black) versus 100 mM (gray) bath TEA, *n* = 8-13.

H. Mean % inhibition (100 mM bath TEA) versus voltage for Kv1.1 with or without SMIT1 (S1); *n* = 8-13. Error bars indicate SEM.

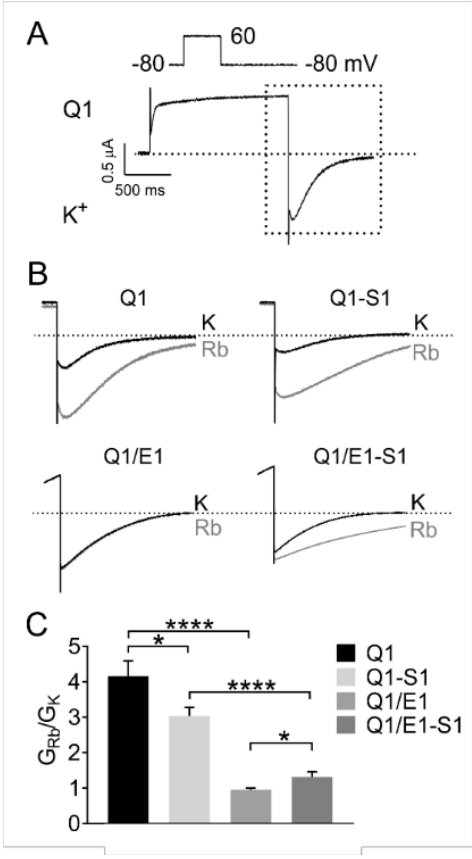


Supplementary Figure 9. SMIT1 negative-shifts KCNQ1/KCNE3 activation

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ1/KCNE3, alone or co-injected with cRNA encoding SMIT1, $n = 15$.

B. Mean current-voltage relationship for subunit combinations as in panel A, $n = 15$. Error bars indicate SEM.

C. Mean normalized tail current (at -30 mV) versus prepulse potential for oocytes as in panel A, $n = 15$. Error bars indicate SEM.

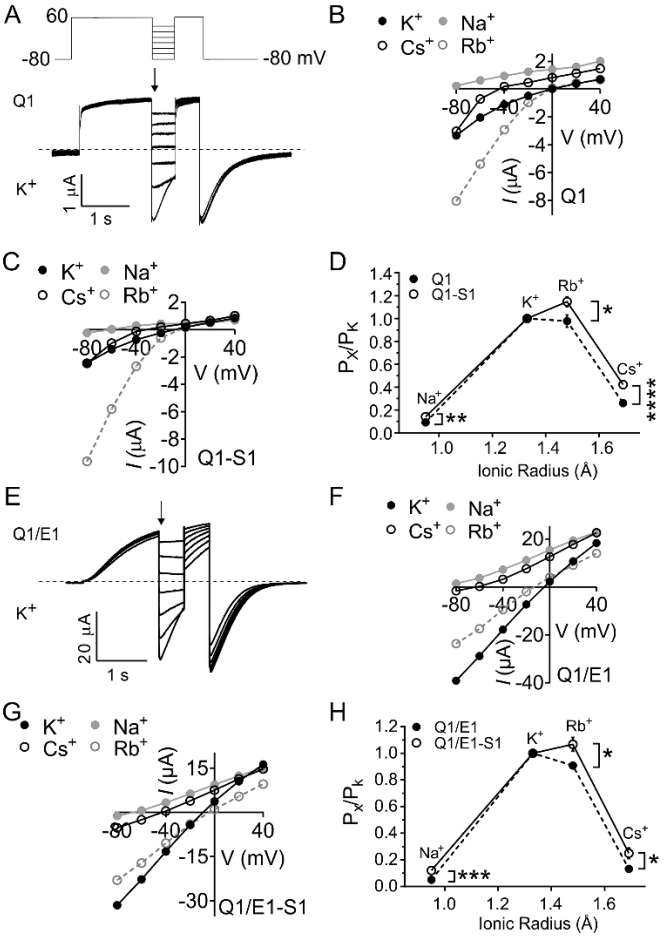


Supplementary Figure 10. SMIT1 alters KCNQ1 and KCNQ1/KCNE1 G_{Rb}/G_K permeability ratio

A. Representative trace from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ1 (Q1) in 100 mM K^+ (voltage protocol inset. 1 s pulse to 60 mV followed by a 500-ms -80 mV tail pulse).

B. Exemplar traces of tail currents recorded in 100 mM [K^+] followed by 100 mM [Rb^+] in the same oocyte for KCNQ1 (Q1) (n = 12), KCNQ1/SMIT1 (Q1-S1) (n = 18), KCNQ1/KCNE1 (Q1/E1) (n = 20), and KCNQ1/KCNE1/SMIT1 (Q1/E1-S1) (n = 16) (protocol inset). The G_{Rb}/G_K ratio was calculated by dividing the peak/plateau amplitude of the respective tail currents by the driving force (difference between –80 mV, and the equilibrium potential for K^+ or Rb^+ ions).

C. Mean G_{Rb}/G_K values calculated from traces as in panel B; *p<0.05; ****p<0.0001.



Supplementary Figure 11. SMIT1 alters KCNQ1 and KCNQ1/KCNE1 ion selectivity

A. Representative trace from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ1 (Q1) in 100 mM K^+ (voltage protocol inset. 1 s pulse to 60 mV followed by a 500-ms inactivation recovery step to voltages between -80 and 40 mV, then a 500-ms -80 mV tail pulse).

B. Mean current-voltage relationship for KCNQ1 (Q1) alone in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), n = 8.

C. Mean current-voltage relationship for KCNQ1-SMIT1 (Q1-S1) in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), n = 12.

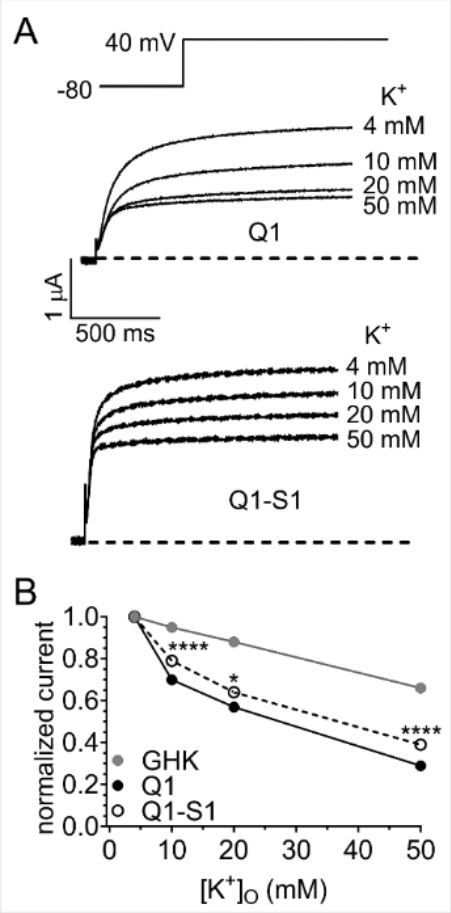
D. Estimated mean permeability relative to that of K^+ versus ionic radius (Pauling) for Na^+, Rb^+, and Cs^+ through KCNQ1 (solid circles) and KCNQ1/SMIT1 (open circles, dotted lines) channels, n = 8-12. Error bars indicate SEM. Statistical significance for comparison of relative permeability of Na^+: **p<0.05, Rb^+ *p<0.05, and Cs^+ ****p<0.0001. Values for relative permeability ratios, in the order Na^+, Rb^+, and Cs^+: KCNQ1, 0.11, 0.98, 0.3, and KCNQ1/SMIT1, 0.09, 1.15, and 0.42.

E. Representative trace from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ1/KCNE1 (Q1/E1) in 100 mM K^+ (voltage protocol as in panel D).

F. Mean current-voltage relationship for KCNQ1/KCNE1 (Q1/E1) in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), n = 6.

G. Mean current-voltage relationship for KCNQ1/KCNE1-SMIT1 (Q1/E1-S1) in 100 mM K^+ (black circles), Cs^+ (open circles) and Na^+ (grey circles), n = 10.

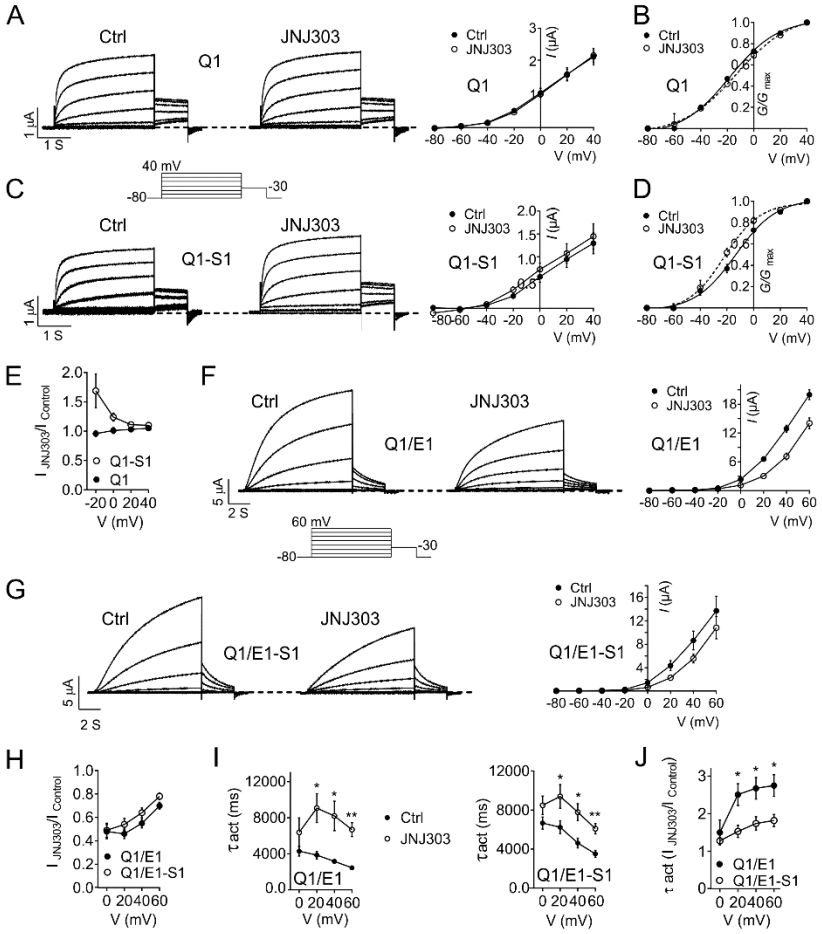
H. Estimated mean permeability relative to that of K^+ versus ionic radius (Pauling) for Na^+, Rb^+, and Cs^+ through KCNQ1/KCNE1 (solid circles) and KCNQ1/KCNE1/SMIT1 (open circles, dotted lines) channels, n = 6-10. Error bars indicate SEM. Statistical significance for comparison of relative permeability of Na^+: ***p<0.05, Rb^+ *p<0.05, and Cs^+ *p<0.05. Values for relative permeability ratios, in the order Na^+, Rb^+, and Cs^+: KCNQ1/KCNE1, 0.05, 0.91, and 0.13, and KCNQ1/KCNE1/SMIT1, 0.12, 1.1, and 0.25.



Supplementary Figure 12. SMIT1 partially protects KCNQ1 from the inhibitory effects of high extracellular K^+

A. Representative traces from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ1, +/- SMIT1 in 4, 10, 20 and 50 mM K^+ (voltage protocol inset. 1 s pulse to 60 mV).

B. Relative steady-state current amplitude as a function of potassium concentration as measured experimentally for KCNQ1 (filled circles, black line) $n = 8$, KCNQ1+SMIT1 (open circles, dotted line) $n = 8$, and theoretical values calculated from the GHK flux equation (filled circles, grey line). The currents are normalized to the current measured in 4 mM $[K^+]o$.****$p<0.0001$, *$p<0.05$.



Supplementary Figure 13. KCNQ1 and KCNQ1/KCNE1, +/- SMIT1 pharmacology: JNJ303

A. *Left*, Representative traces; *right*, Mean current-voltage relationship, from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes expressing KCNQ1 (Q1) in ND96 or 1 µM JNJ303, *n* = 7. Voltage protocol (inset), 3 s pulse to voltages between -80 and 40 mV followed by a 1 s -30 mV tail pulse. Error bars indicate SEM.

B. Mean normalized tail current (at -30 mV) versus prepulse potential for oocytes as in panel A, *n* = 7. Error bars indicate SEM.

C. *Left*, Representative traces; *right*, Mean current-voltage relationship, from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes expressing KCNQ1-SMIT1 (Q1-S1) in ND96 or 1 µM JNJ303, *n* = 7. Voltage protocol as in A. Error bars indicate SEM.

D. Mean normalized tail current (at -30 mV) versus prepulse potential for oocytes as in panel C, *n* = 7. Error bars indicate SEM.

E. Voltage dependence of mean effects, on Q1 versus Q1-S1 current, of 1 µM JNJ303, quantified from traces as in A and C, n = 7, error bars indicate SEM.

F. Left, representative traces; right, mean current-voltage relationship, from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes expressing KCNQ1/KCNE1 (Q1/E1) in ND96 or 1 µM JNJ303, *n* = 7. Voltage protocol inset. Error bars indicate SEM.

G. Left, representative traces; right, mean current-voltage relationship, from two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes expressing KCNQ1/KCNE1-SMIT1 (Q1/E1-S1) in ND96 or 1 µM JNJ303, *n* = 7. Voltage protocol as in panel F.

H. Voltage dependence of mean effects, on Q1/E1 versus Q1/E1-S1 current, of 1 µM JNJ303, quantified from traces as in F and G, *n* = 7, error bars indicate SEM.

I. Voltage dependence of mean effects, on Q1/E1 versus Q1/E1-S1 activation rate, of 1 µM JNJ303, quantified from traces as in F and G, *n* = 7, error bars indicate SEM.

J. Effect of SMIT1 (S1) on Q1/E1 activation slowing by 1 µM JNJ303, quantified from values in panel I, *n* = 7, error bars indicate SEM.