Supporting Information

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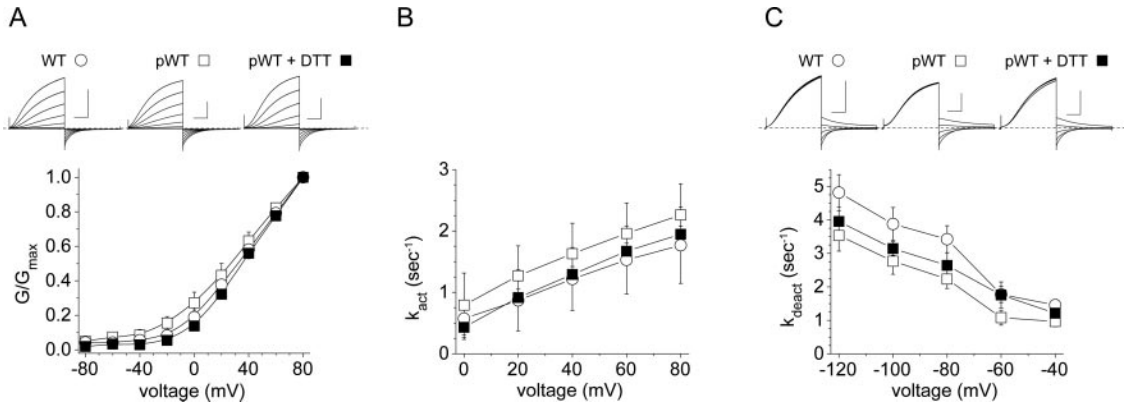

Fig. S1. Comparison of the functions of WT I_{KS} and pWT I_{KS}. (*A*) Isochronal (2-s conditioning pulses) G/G_{max} as a function of voltage for WT ($n = 4$, open circles), pWT ($n = 5$, open squares), and pWT + DTT ($n = 4$, closed squares). (*B*) Rate constants of activation (k_{act}) for WT, pWT, and pWT + DTT. (*C*) Rate constants of deactivation (k_{deact}) for WT ($n = 5$), pWT ($n = 6$), and pWT + DTT ($n = 4$). For current traces, vertical scale is 200 pA/pF and horizontal scale is 0.5 s. Dotted line indicates zero current.

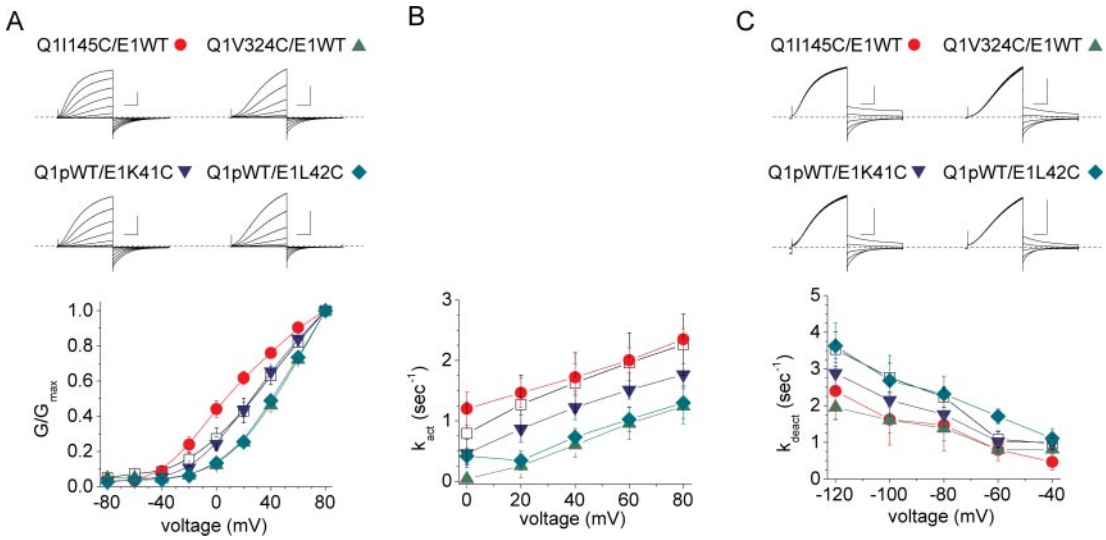

Fig. S2. Single-sided Cys mutant I_{KS}. (*A*) Dependence of isochronal G/G_{max} on voltage and (*B*) k_{act} for pWT (open squares), Q1 I145C/E1 WT ($n = 4$, red circles), Q1 V324C/E1WT ($n = 5$, green triangles), Q1 pWT/E1 K41C ($n = 4$, blue triangles), and Q1 pWT/E1 L42C ($n = 5$, blue-green diamonds). (*C*) k_{deact} for pWT, Q1 I145C/E1 WT ($n = 6$), Q1 V324C/E1WT ($n = 4$), Q1 pWT/E1 K41C ($n = 4$), and Q1 pWT/E1 L42C ($n = 5$). For current traces, vertical scale is 200 pA/pF and horizontal scale is 0.5 s. Dotted line indicates zero current.

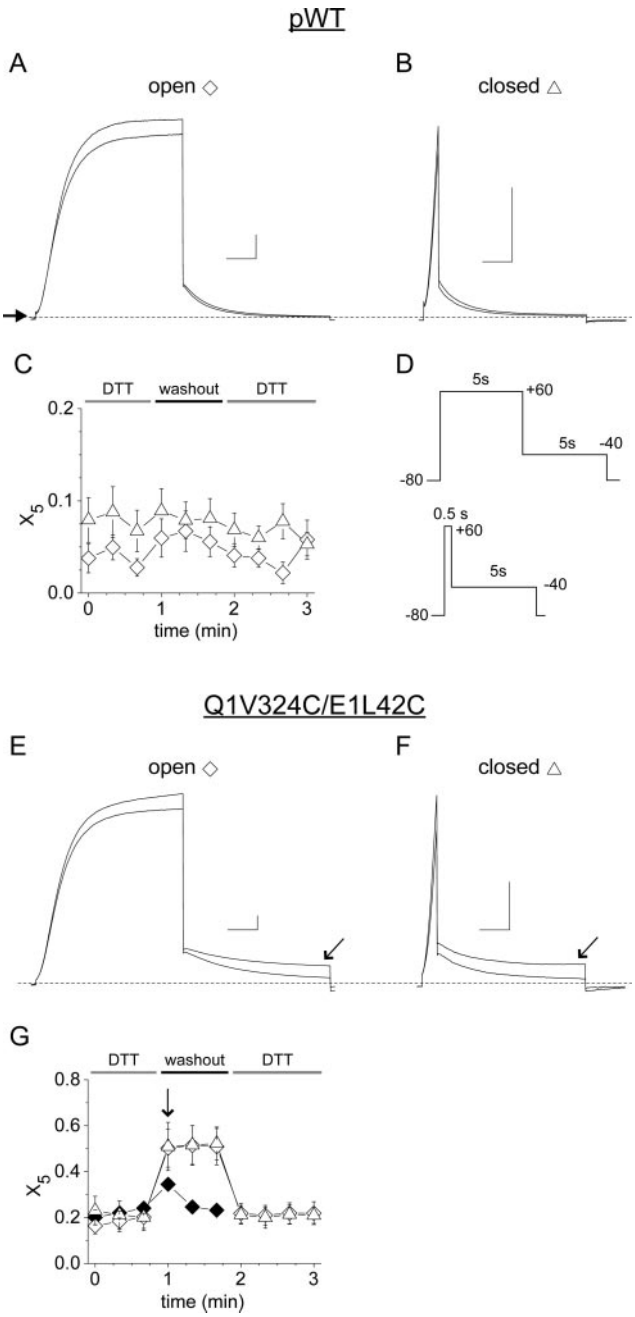

Fig. S3. State-dependence of Q1 V324C/E1 L42C cross-link. (*A–C*): pWT; (*E–G*): Q1 V324C/E1 L42C. (*D*): Pulse protocol; upper, open; lower, closed. (*A* and *E*): Open protocol current traces. (*B* and *F*): closed protocol current traces. The first and fourth current traces (arrows) are shown. (*C* and *G*): fraction of channels open after 5 seconds of repolarization at -40 mV (designated X_5) vs. time. Cells were preincubated in 10 mM DTT for a minimum of 15 min. Washout and re-application of DTT solution were added at the beginning of the depolarization for the open state protocol or 5 sec before the subsequent depolarization for the closed state protocol. Start-to-start pulse interval was 20 sec. (*G*) Filled diamonds indicate the open state protocol with 2 mM NEM in the washout solution. Solution change at arrow. For current traces, vertical scale is 50 pA/pF and horizontal scale is 0.5 s. Dotted line indicates zero current.

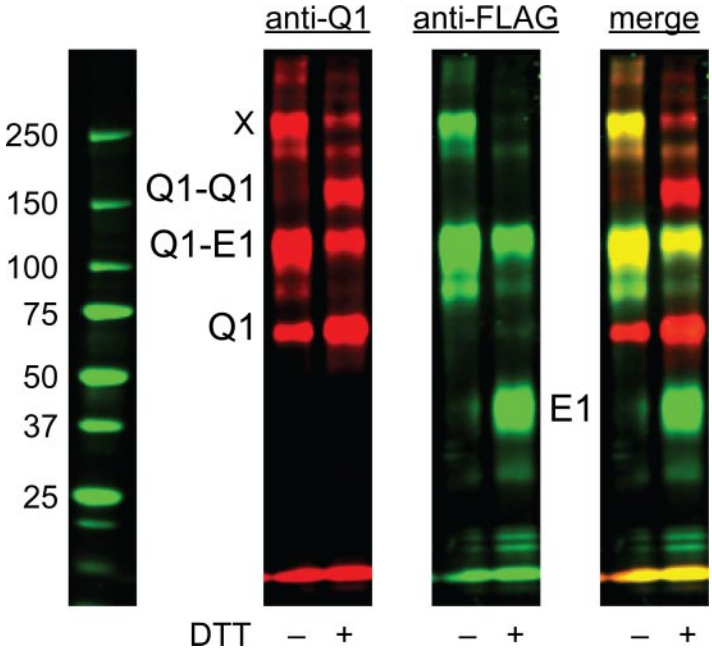

Fig. S4. Full-length blots of Q1 T144C/E1 G40C. Q1 (Q1) is represented as a red signal and E1 as a green signal. The merged red and green in the cross-linked Q1-E1 (Q1E1) band is yellow. The samples in the right lanes were reduced with DTT in sample buffer. X indicates a band of molecular weight greater than 250 kDa, possibly containing approximately two Q1s per E1. The molecular weights of the markers are in kDa.

Table S1. Activation and deactivation parameters for I_{KS} channels used in study

	$V_{1/2}$		k_{act} at + 60 mV		k_{deact} at −40 mV	
	−DTT, mV	+DTT, mV	−DTT, s^{-1}	+DTT, s^{-1}	−DTT, s^{-1}	+DTT, s^{-1}
WT	29 ± 8 (4)		1.4 ± 0.5 (4)		1.4 ± 0.4 (5)	
pWT	23 ± 13 (6)	34 ± 4 (4)	1.7 ± 0.5 (5)	1.7 ± 0.1 (4)	0.9 ± 0.3 (6)	1.2 ± 0.2 (4)
Q1I145C/E1K41C	8 ± 8 (5)	1 ± 12 (5)	2.2 ± 1.0 (5)	2.2 ± 0.6 (5)	0.03 ± 0.04 (6)	0.8 ± 0.4 (6)
Q1I145C/E1L42C	26 ± 6 (7)	23 ± 7 (4)	1.3 ± 0.2 (7)	1.5 ± 0.3 (4)	3.1 ± 0.7 (6)	0.7 ± 0.2 (6)
Q1V324C/E1K41C	38 ± 3 (5)	24 ± 7 (4)	1.0 ± 0.2 (5)	1.7 ± 0.3 (4)	1.1 ± 0.2 (6)	0.9 ± 0.2 (6)
Q1V324C/E1L42C	n/a	39 ± 4 (4)	n/a	1.4 ± 0.2 (4)	0.000 ± 0.007 (3)	0.7 ± 0.1 (5)
Q1I145C/E1WT	8 ± 8 (4)		2.0 ± 0.2 (4)		0.5 ± 0.2 (6)	
Q1V324C/E1WT	40 ± 4 (5)		1.0 ± 0.3 (5)		0.81 ± 0.06 (4)	
Q1pWT/E1K41C	24 ± 6 (4)		1.5 ± 0.2 (4)		1.0 ± 0.2 (4)	
Q1pWT/E1L42C	38 ± 4 (4)		1.0 ± 0.2 (4)		1.1 ± 0.3 (5)	

−DTT: channel properties of cells that were not treated with 10 mM DTT. +DTT: channel properties of cells pretreated with 10 mM DTT for at least 15 min and in the continued presence of DTT.