Supporting Information

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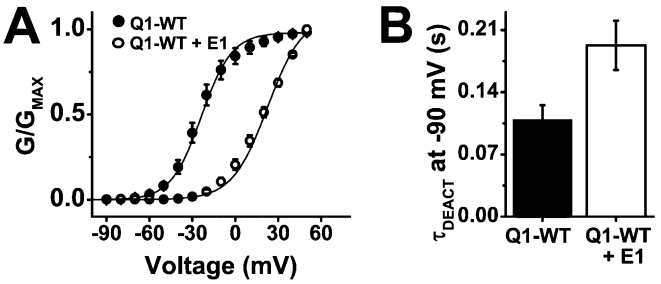

Fig. S1. Voltage-dependence of activation and deactivation kinetics of Q1-WT channels and Q1-WT + E1 channels. Ionic current from cells expressing Q1-WT or Q1-WT + E1 was studied using cut-open oocyte voltage clamp (COVC) at 28 °C, as in Fig. 1. Normalized conductance-voltage relationships are fit as in Fig. 2. Data are means ± SEM for six to seven cells in each group. (*A*) Normalized conductance, G/G_{max}, for Q1-WT (●) and Q1-WT+ E1 (○). (*B*) Deactivation time-constant, τ_{DEACT}, for Q1-WT (black bar), and Q1-WT + E1 (white bar) at −90 mV from a 40-mV test pulse.

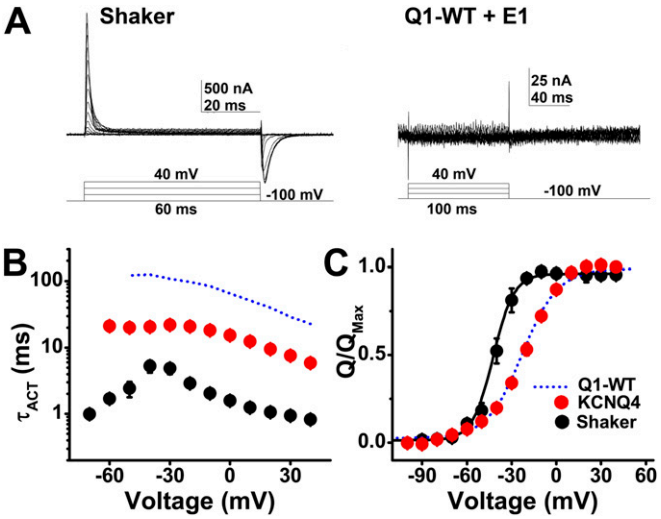

Fig. S2. Gating currents for Shaker, KCNQ4, KCNQ1, and Q1-WT + E1 channels. Gating currents in cells expressing Shaker W434F, KCNQ4, Q1-WT or Q1-WT + E1 channels as described in Fig. 2 using the voltage protocol indicated. Test-pulse durations were 60 ms for Shaker, 70 ms for KCNQ4, 300 ms for Q1-WT, and 100 ms for Q1-WT + E1. Activation time-constant and normalized charge-voltage relationships were determined as described in Fig. 2. Data are mean ± SEM for $n = 6–7$ cells. (*A*) Family of gating currents from a representative cells expressing (*Left*) Shaker W434F or (*Right*) Q1-WT + E1 channels. Note that gating currents recorded from cells expressing Q1-WT + E1 are below the level of resolution and cannot be analyzed. (*B*) Activation time-constant, τ_{ACT}, for Shaker (black circles), KCNQ4 (red circles), and Q1-WT from Fig. 2*F* (dotted blue line) plotted on a $\log_{10}$ scale against test potential. (*C*) Normalized Q_{ON}-*V* relationships for Shaker (black circles), KCNQ4 (red circles), and Q1-WT from Fig. 2*G* (dotted blue line).

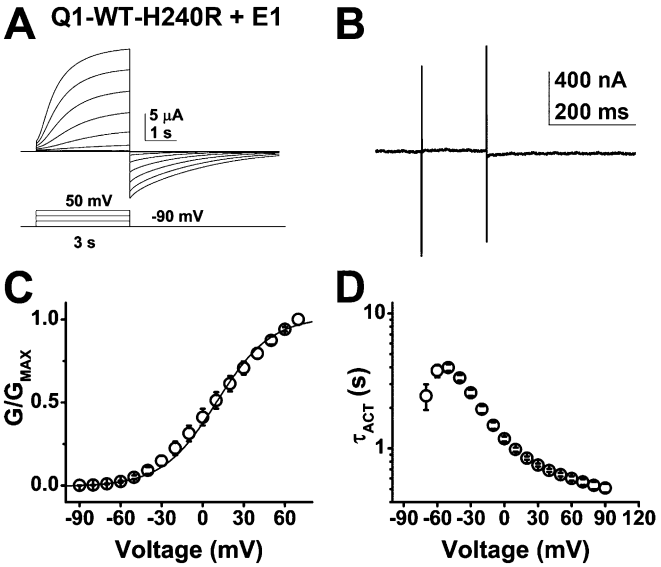

Fig. S3. Ionic and gating currents for Q1-H240R + E1 channels. Ionic and gating currents studied in cells expressing Q1-H240R + E1 using COVC at 28 °C and the protocol indicated. Data are mean ± SEM for four cells. (*A*) A representative family of ionic currents shows the hallmarks of I_{Ks} current, including slow activation and deactivation kinetics and no inactivation. Channels studied using the voltage protocol indicated, shown in 20 mV intervals for clarity. (*B*) Gating currents cannot be resolved from cells expressing Q1-H240R +E1 channels. A representative trace at 40 mV is shown. (*C*) A normalized conductance-voltage (*G-V*) relationship for Q1-H240R + E1 channels. The data are fitted as described in Fig. 2. The $V_{1/2}$ is depolarized by >30 mV with respect to Q1-H240R alone (Fig. 3*D* and Table 1), and is left-shifted with respect to wild-type I_{Ks} channels by 12 ± 3 mV (Fig. S1 and Table 1). (*D*) Mean τ_{ACT} for ionic current, determined for each test potential as in Fig. 1.

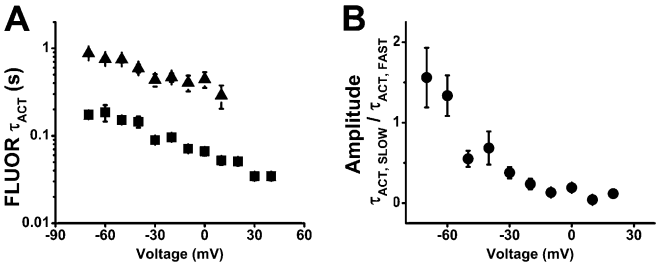

Fig. S4. Fluorescence signals from Q1-F channels have two components of activation. Double-exponential fits of the fluorescent signals recorded from Q1-F channels labeled with tetramethylrhodamine-5-maleimide (TMRM), as in Fig. 4, indicate the signals develop with two distinct time constants. Data are mean ± SEM for $n = 7$ cells. (*A*) Faster (▲) and slower (■) activation time constants (τ_{ACT}) are plotted against test potential. The slower component becomes more prominent at more hyperpolarized potentials. (*B*) Relative ratio of the amplitude of the faster and slower components of the fluorescence signal with respect to the test voltage.

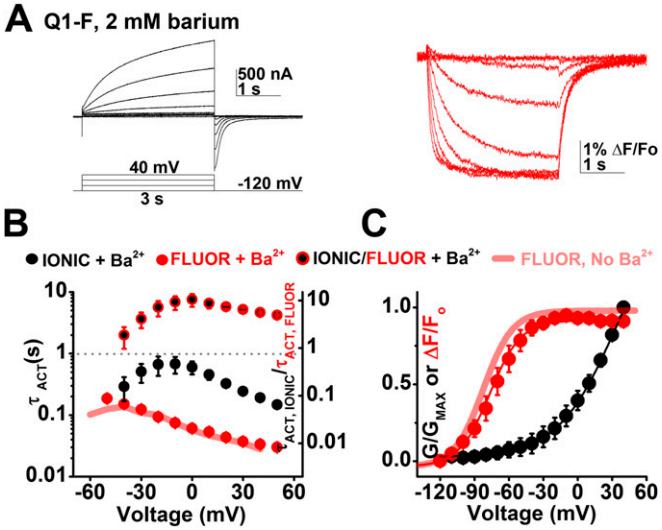

Fig. S5. Barium blocks the pore without impacting voltage sensor movement. Ionic current and fluorescence signals were recorded simultaneously from cells expressing Q1-F or Q1-F + E1 channels using the protocols indicated in the presence of 2 mM external barium. Data are mean ± SEM for three to six cells. (*A*, *Left*) A representative family of ionic currents recorded from a cell expressing Q1-F channels using the same protocol as in Fig. 4 *A* and *B*. Shown in 20-mV intervals for clarity. (*Right*) A representative family of fluorescence traces recorded from the same cell. (*B*) The left axis shows mean activation time-constants, τ_{ACT} for ionic current (black circles), and the fast-component of the fluorescent signal (red circles). Fluorescence signals recorded without barium (Fig. 4*D*) are shown for reference (pink). The right axis shows mean ratios of τ^{I}_{ACT} to τ^{F}_{ACT} (black/red circles). The ratio is close to 1 (dotted gray line; right axis) over the voltage range studied. (*C*) Mean, normalized *F-V* (red) and *G-V* (black) relationships for Q1-F-H240R channels in the presence of 2 mM external barium, measured after 3 s and fit to the Boltzmann equation, as described in Fig. 2. The *F-V* relationship without barium (Fig. 4*E*) is included for reference (pink line).

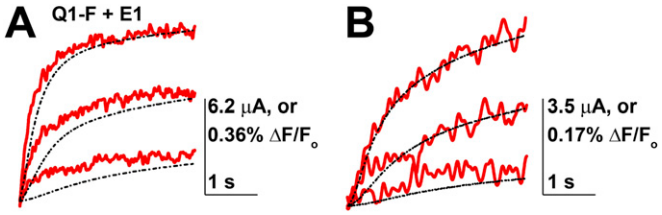

Fig. S6. Ionic currents and fluorescence signals from Q1-F + E1 channels develop with closely matching kinetics. Cells expressing Q1-F + E1 channels were studied as described in Fig. 5. (*A* and *B*) Examples of normalized ionic current and fluorescence signals superimposed at 70, 30, and −10 mV recorded from the same cells. The kinetics of fluorescence signals always correlated with the kinetics of the ionic current, where fast/highly expressing ionic currents also had fast voltage sensor activation kinetics, and slow/lower-expressing ionic currents had correspondingly slower voltage sensor activation kinetics.

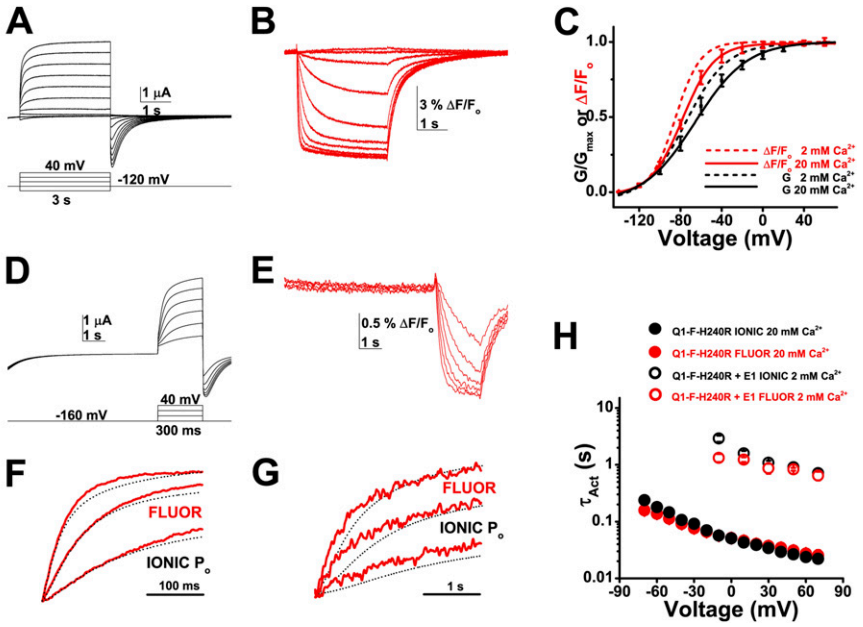

Fig. S7. Activation Q1-F-H240R channels and Q1-F-H240R + E1 channels studied in the presence of 20 mM external calcium. The voltage-dependent activation of Q1-F-H240R channel ionic current is hyperpolarized by 17 mV with respect to Q1-F channels (Fig. 4 and Table 2). To right-shift voltage-dependent activation, Q1-F-H240R channels were studied in the presence of 20 mM external Ca^{2+} unless otherwise indicated. Data are means ± SEM for three to six cells. (*A*) A representative family of ionic current from cells expressing Q1-F-H240R. Protocol as in Fig. 6*A*, shown from −120 mV to 40 mV in 20 mV intervals. (*B*) A representative family of fluorescence signals evoked in the same cell studied in *A*, recorded simultaneously. (*C*) Normalized fluorescence and conductance-voltage relationships for Q1-F-H240R channels studied in the presence of 2 mM (dotted red and black lines) or 20 mM (solid red and black lines, respectively) external Ca^{2+}. (*D*) Representative family of ionic currents studied following a 1-s prepulse to −160 mV. Shown from 40 to −60 mV in 20 mV intervals. (*E*) Fluorescence signals recorded in the same cells studied in *D*, simultaneously. (*F*) Normalized superposition of fluorescence signals and open probability for Q1-F-H240R channels, recorded in the presence of 20 external mM Ca^{2+} using the protocol in *D*. Shown at 10, −30, and −70 mV. (*G*) Normalized superposition of fluorescence signals and open probability of Q1-F-H240R + E1, recorded in the presence of 2 mM Ca^{2+}, shown at 70, 30, and −10 mV. (*H*) Mean activation time-constants for ionic current and fluorescence signals evoked from cells expressing Q1-F-H240R or Q1-F-H240R + E1 channels in the presence of 2 or 20 mM external Ca^{2+}, as indicated.