Supporting Information

Werry et al. 10.1073/pnas.1214875110

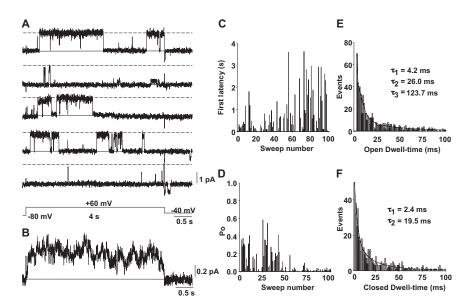


Fig. 51. Endogenous cell-attached single-channel activity in mouse *ltk*- cells. Recordings were performed on untransfected cells. (*A*) Five successive sweeps from the same patch of 4-s pulses to +60 mV from a holding potential of -80 mV. The protocol is shown at the bottom. Pulses were delivered every 10 s. The solid and short dashed lines denote the zero current and single-channel opening level, respectively. Note the long-lived openings during depolarizations and the inward channel openings at -40 mV repolarization potential. (*B*) The ensemble-averaged currents of 100 successive recordings from the patch in *A*. (*C*) Diary plot of the single-channel first latency in the recording. Latency values of 0 s correspond to the recording in which no channel opens. (*D*) Diary plot of the time-averaged open probability of the channel. (*E*) Open dwell-time histogram of endogenous channels. The superimposed smooth curve is a fitting of a triple-exponential function with the time constants indicated. (*F*) Closed dwell-time histogram of endogenous channels. A double-exponential fit is superimposed with the time constants indicated.

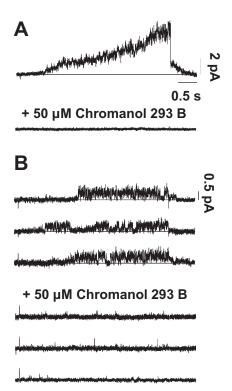


Fig. 52. I_{KS} single-channel activity is sensitive to Chromanol 293B. (A) Current from a multichannel cell-attached patch showing clear I_{KS} kinetics was inhibited completely after application of 50 μ M chromanol 293B to the extracellular bath solution (lower trace). (B) Chromanol (50 μ M) blocked single-channel activity from a patch different from that shown in A. The top three tracings were obtained in control solution during depolarizations from -80 to +60 mV for 4 s. The start of depolarization is marked by the small transient current near the start of each tracing.

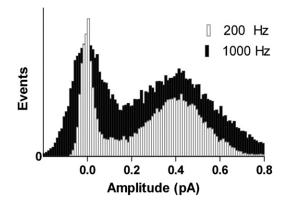


Fig. S3. An all-points histogram was generated from a single 4-s current record of IKs during a voltage step from -80 to +60 mV, filtered at 1,000 Hz (black), with a superimposed histogram constructed from the same sweep filtered at 200 Hz (white). The peaks of each histogram are normalized to the maximum number of closed events.

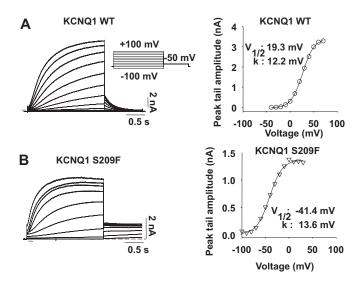


Fig. S4. Whole-cell data showing gain-of-function in the S3 mutant, I_{Ks} -S209F. (A) WT I_{Ks} data. (B) S209F I_{Ks} data. The half-activation voltage is shifted from + 19 to -41 mV in S209F, and tail currents on repolarization to -50 mV fail to deactivate in the mutant over the time scale shown.

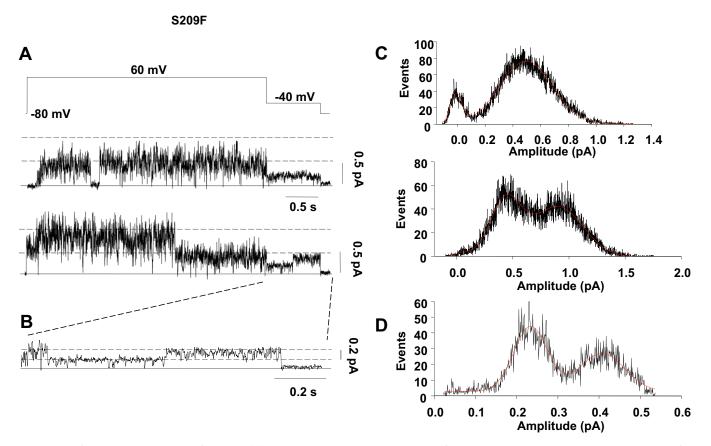


Fig. S5. Data from a two-channel patch of I_{KS} -S209F. (*A*) Four-s sweeps to +60 mV show opening of a single channel or both channels during the pulse, after short latencies. The solid line denotes zero current; dotted lines denote opening levels. (*B*) The tail current record at -40 mV from the lower record in *A* on an enlarged scale. (*C*) All-points histograms of event amplitudes during depolarization for current sweeps in *A*. Peaks of Gaussian fits to distributions are at 0.43 pA (single opening distribution) (*Upper*) and 0.45/0.912 pA (both channels opening) (*Lower*). (*D*) All-points histograms of event amplitudes during tits to distributions are at 0.223 and 0.412 pA (single opening distribution, and double opening level, respectively). Bin width for histograms was 0.001 pA.

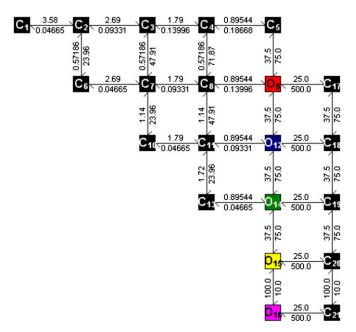


Fig. S6. Markov state model showing forward and reverse transition rates. All the closed transition rates in the activation pathway are from Silva and Rudy (1) calculated for a step to +60 mV. Rates are s⁻¹. The Markov model was used to represent the state diagram in Fig. 7A mathematically. Open states 9, 12, 14, 15, and 16 are shown in color.

1. Silva J, Rudy Y (2005) Subunit interaction determines IKs participation in cardiac repolarization and repolarization reserve. Circulation 112(10):1384–1391.

Level	n	Total dwell time (ms)	Probability	Mean dwell time (ms)	SE	<i>P</i> < 0.05
Closed	296	8,071.48 (346,108)	0.021 (0.896)	27.27	9.80	vs. 1, 2, 3, 4, 5
1	720	4,316.01	0.011	5.99	0.39	vs. closed, 4, 5
2	969	4,002.69	0.010	4.13	0.18	vs. closed, 4,5
3	1,231	8,413.02	0.022	6.52	0.22	vs. closed, 4, 5
4	1,258	1,7245.29	0.045	13.71	0.48	vs. closed 1, 2, 3
5	423	6,173.78	0.016	14.59	1.24	vs. closed, 1, 2, 3

Table S1. Sublevel total and mean dwell times

Results are from analysis of one single-channel patch run of 97 sweeps, of which 23 sweeps were active. Numbers in parentheses for the closed state include closed time of inactive sweeps and first latency periods of active sweeps. One-way ANOVA with a post hoc multiple pairwise comparison was used to test for significance.