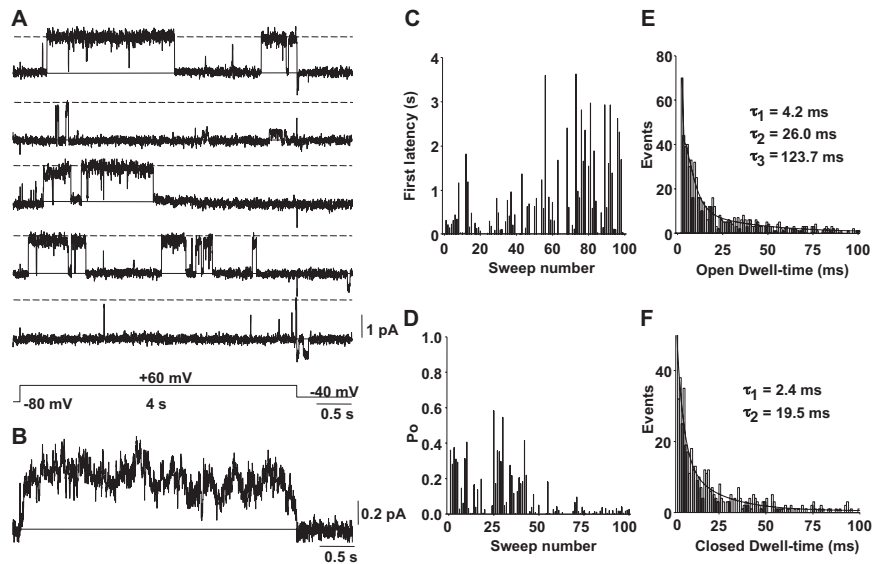


# Supporting Information

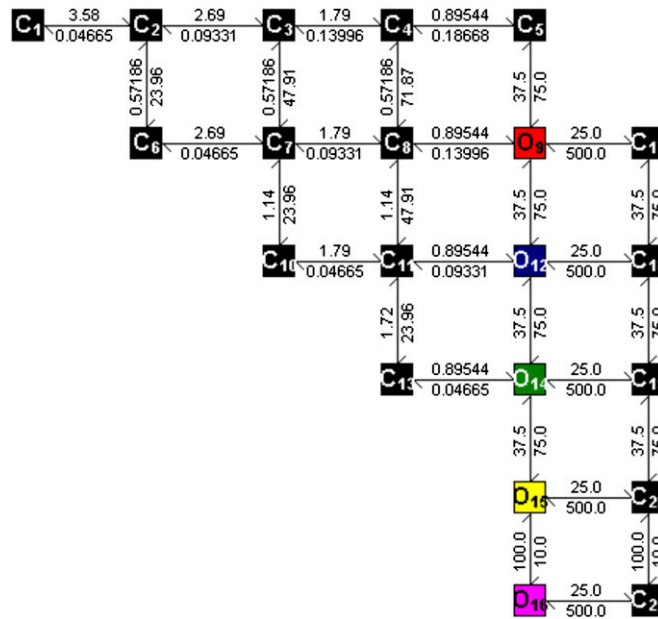
Werry et al. 10.1073/pnas.1214875110



**Fig. S1.** Endogenous cell-attached single-channel activity in mouse *Itk*- 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. 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.

**Table S1. Sublevel total and mean dwell times**

Level	<i>n</i>	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

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.