Supplemental Table 1: Deactivation kinetic parameters. Mean ± SEM, from n experiments, for deactivation kinetics of WT and mutant Kv11.1 channels. The decay of current at -120 mV was fitted with a double exponential function to obtain fast (τfast,-120mV) and slow (τslow,-120mV) time constants for deactivation. The relative amplitudes of the fast and slow components are also shown (Af/(Af+As). Note that deactivation is dominated by the fast component at -120mV. Natural log of the fast time constant (ln.τfast,-120mV) for each mutant was compared to that of WT channels to provide Δln.τfast,-120mV.

Mutant	$\tau_{fast,-120mV}$ (ms)	ln.$\tau_{fast,-120mV}$	Δln.$\tau_{fast,-120mV}$	$\tau_{slow,-120mV}$ (ms)	$A_f / (A_f + A_s)_{,-120mV}$	n
WT Kv11.1	33.6 ± 0.7	3.51 ± 0.02		199.0 ± 7.1	0.83 ± 0.00	16
Δ2-137	8.6 ± 0.2	2.15 ± 0.03	1.36 ± 0.03	111.7 ± 10.1	0.87 ± 0.00	5
N-Cap+PAS Kv10.1-Kv11.1	11.3 ± 0.3	2.42 ± 0.03	1.09 ± 0.03	125.3 ± 15.3	0.90 ± 0.00	5
PAS Kv10.1-Kv11.1	16.2 ± 0.4	2.78 ± 0.02	0.73 ± 0.02	126.9 ± 10.2	0.93 ± 0.01	5
R4D	13.5 ± 0.2	2.60 ± 0.01	0.91 ± 0.01	154.9 ± 6.9	0.88 ± 0.01	13
R4E	14.4 ± 0.4	2.66 ± 0.02	0.85 ± 0.02	167.4 ± 9.11	0.89 ± 0.01	11
R4K	26.0 ± 0.4	3.26 ± 0.02	0.25 ± 0.02	202.6 ± 12.7	0.91 ± 0.01	13
R5D	13.4 ± 0.5	2.58 ± 0.04	0.93 ± 0.04	160.8 ± 10.6	0.87 ± 0.01	14
R5E	13.9 ± 0.7	2.61 ± 0.06	0.90 ± 0.06	155.9 ± 12.0	0.88 ± 0.01	11
R5K	22.8 ± 0.3	3.13 ± 0.01	0.38 ± 0.01	180.7 ± 8.5	0.85 ± 0.01	12
R20D	7.7 ± 0.2	2.03 ± 0.03	1.48 ± 0.03	110.7 ± 8.5	0.89 ± 0.02	11
R20E	17.5 ± 0.6	2.85 ± 0.04	0.66 ± 0.04	140.9 ± 20.3	0.88 ± 0.01	12
R20K	29.9 ± 0.9	3.39 ± 0.03	0.12 ± 0.03	206.5 ± 10.5	0.86 ± 0.01	15
R56D	9.7 ± 0.3	2.27 ± 0.03	1.24 ± 0.03	121.8 ± 23.0	0.91 ± 0.02	5
R56E	8.6 ± 0.2	2.14 ± 0.02	1.37 ± 0.02	116.7 ± 9.1	0.91 ± 0.01	14
R56K	42.6 ± 1.8	3.75 ± 0.04	-0.24 ± 0.04	275.8 ± 9.5	0.84 ± 0.01	8
D712R	30.1 ± 0.8	3.40 ± 0.02	0.11 ± 0.02	150.5 ± 7.1	0.88 ± 0.0	6
E722R	29.1 ± 1.0	3.37 ± 0.03	0.14 ± 0.03	162.4 ± 7.5	0.88 ± 0.01	4
D727R	41.3 ± 0.9	3.72 ± 0.02	-0.21 ± 0.02	170.7 ± 3.7	0.70 ± 0.00	15
D767R	28.8 ± 0.5	3.36 ± 0.02	0.15 ± 0.02	147.9 ± 8.2	0.88 ± 0.01	5
D774R	15.0 ± 0.7	2.71 ± 0.05	0.80 ± 0.05	228.5 ± 21.2	0.88 ± 0.03	6
E788R	14.8 ± 0.3	2.69 ± 0.02	0.82 ± 0.02	135.4 ± 9.2	0.87 ± 0.01	21
D793R	29.4 ± 0.8	3.38 ± 0.03	0.13 ± 0.03	219.2 ± 12.8	0.86 ± 0.01	15
D803R	17.3 ± 0.6	2.85 ± 0.04	0.66 ± 0.04	189.6 ± 12.3	0.94 ± 0.00	8
E807R	NE					
D821R	27.7 ± 0.6	3.32 ± 0.02	0.19 ± 0.02	226.8 ± 10.2	0.87 ± 0.01	21
D829R	45.8 ± 0.6	3.82 ± 0.01	-0.31 ± 0.01	275.3 ± 12.1	0.87 ± 0.01	13
D836R	31.1 ± 0.6	3.44 ± 0.02	0.07 ± 0.02	168.1 ± 1.8	0.88 ± 0.01	4
D837R	28.4 ± 0.5	3.35 ± 0.02	0.16 ± 0.02	172.8 ± 7.4	0.85 ± 0.02	5
E840R	30.3 ± 0.8	3.41 ± 0.03	0.10 ± 0.03	170.3 ± 5.5	0.88 ± 0.01	5
D843R	32.6 ± 0.7	3.48 ± 0.02	0.03 ± 0.02	194.2 ± 6.5	0.85 ± 0.01	11
E847R	28.2 ± 0.6	3.34 ± 0.02	0.17 ± 0.02	199.3 ± 7.7	0.83 ± 0.02	12
D850R	33.3 ± 0.8	3.50 ± 0.02	0.01 ± 0.02	218.6 ± 10.2	0.84 ± 0.01	16
E857R	25.8 ± 0.8	3.25 ± 0.03	0.26 ± 0.03	163.4 ± 9.4	0.88 ± 0.01	13
R4D + D803R	8.6 ± 0.3	2.15 ± 0.03	1.36 ± 0.03	123.8 ± 14.6	0.93 ± 0.01	10
R5D + D803R	8.1 ± 0.2	2.09 ± 0.02	1.42 ± 0.02	146.1 ± 9.8	0.92 ± 0.01	10
R20D + D803R	11.0 ± 0.3	2.40 ± 0.03	1.11 ± 0.03	139.3 ± 23.6	0.89 ± 0.01	7
R56D + D803R	29.3 ± 0.7	3.37 ± 0.02	0.14 ± 0.02	229.3 ± 16.7	0.90 ± 0.00	16
R4E + E788R	11.9 ± 0.4	2.47 ± 0.03	1.04 ± 0.03	127.6 ± 12.4	0.87 ± 0.01	12
R5E + E788R	11.6 ± 0.3	2.44 ± 0.03	1.07 ± 0.03	116.8 ± 9.2	0.93 ± 0.00	16
R20E + E788R	10.0 ± 0.4	2.29 ± 0.04	1.22 ± 0.04	139.0 ± 14.4	0.93 ± 0.01	12
R56E + E788R	12.6 ± 0.5	2.52 ± 0.04	0.99 ± 0.04	130.0 ± 7.4	0.91 ± 0.01	17
N12E	13.0 ± 0.2	2.57 ± 0.02	0.94 ± 0.02	128.0 ± 9.7	0.92 ± 0.00	5
N12E + E788R	20.0 ± 0.7	2.99 ± 0.03	0.52 ± 0.03	121.6 ± 5.8	0.90 ± 0.01	8

R4E + R5E	10.3 ± 0.2	2.33 ± 0.02	1.18 ± 0.02	92.0 ± 7.4	0.87 ± 0.00	5
E698R + E699R	21.4 ± 0.3	3.06 ± 0.01	0.45 ± 0.01	175.6 ± 8.5	0.88 ± 0.01	5
R4E + R5E + E698R + E699R	20.2 ± 0.8	3.00 ± 0.04	0.51 ± 0.04	156.8 ± 25.8	0.85 ± 0.02	5
R4D + R5D	10.6 ± 0.2	2.36 ± 0.02	1.15 ± 0.02	97.4 ± 7.7	0.87 ± 0.01	5
R4D + R5D + R56D	9.5 ± 0.3	2.25 ± 0.03	1.26 ± 0.03	145.3 ± 12.7	0.88 ± 0.01	6
R56A	20.0 ± 1.0	2.99 ± 0.05	0.52 ± 0.05	247.0 ± 19.2	0.96 ± 0.00	5
D803A	25.8 ± 1.1	3.24 ± 0.04	0.27 ± 0.04	185.7 ± 22.0	0.93 ± 0.01	11
R56A + D803A	28.5 ± 0.79	3.35 ± 0.03	0.16 ± 0.03	168.0 ± 9.9	0.91 ± 0.00	12
N12A	18.4 ± 1.0	2.91 ± 0.05	0.60 ± 0.05	190.6 ± 15.0	0.85 ± 0.01	4
E788A	17.7 ± 0.8	2.87 ± 0.04	0.64 ± 0.04	153.4 ± 11.7	0.92 ± 0.00	6
N12A + E788A	16.2 ± 0.2	2.79 ± 0.01	0.72 ± 0.01	157.9 ± 7.9	0.92 ± 0.00	7
R4A + R5A	14.8 ± 0.3	2.69 ± 0.02	0.82 ± 0.02	228.6 ± 18.4	0.88 ± 0.02	5
E698A + E699A	24.3 ± 0.7	3.19 ± 0.03	0.32 ± 0.03	245.1 ± 25.2	0.91 ± 0.00	6
R4A + R5A + E698A + E699A	18.2 ± 0.4	2.90 ± 0.02	0.61 ± 0.02	198.6 ± 10.4	0.85 ± 0.01	7

Supplemental Table 2. Chemical potential energy parameters. Mean ± SEM, from n experiments, obtained from Boltzmann fits of steady-state deactivation g-V curves (see Methods for details). The voltage required for half-maximal deactivation (V0.5), as well as the slope factors, are given for each mutant and WT Kv11.1 channels. Fitting with a thermodynamic form of the Boltzmann function provides a chemical potential energy at 0 mV (ΔG0) for each mutant, which can then be compared to that of WT Kv11.1 channels to give ΔΔG0.

Mutant	$V_{0.5}$ (mV)	Slope (mV)	ΔG0 (kj.mol^{-1})	ΔΔG0 (kj.mol^{-1})	n
WT Kv11.1	-61.2 ± 0.4	6.7 ± 0.2	-23.7 ± 0.5		5
Δ2-137	-29.4 ± 0.3	6.1 ± 0.2	-23.7 ± 0.5	0.0 ± 0.5	5
N-Cap/PAS Kv10.1-Kv11.1	-24.9 ± 1.1	8.2 ± 0.3	-7.7 ± 0.7	16.0 ± 0.7	5
PAS Kv10.1-Kv11.1	-40.9 ± 0.7	5.9 ± 0.1	-17.1 ± 0.3	6.6 ± 0.3	5
R4D	-37.2 ± 1.0	5.3 ± 0.1	-18.8 ± 1.0	4.9 ± 1.0	5
R4E	-36.6 ± 0.2	5.8 ± 0.1	-16.3 ± 0.5	7.4 ± 0.5	3
R4K	-56.0 ± 0.7	6.5 ± 0.6	-23.0 ± 1.7	0.7 ± 1.7	5
R5D	-34.5 ± 0.76	6.0 ± 0.1	-14.6 ± 0.5	9.2 ± 0.5	5
R5E	-38.4 ± 0.5	4.9 ± 0.1	-21.1 ± 0.7	2.6 ± 0.7	3
R5K	-55.4 ± 0.3	6.1 ± 0.1	-24.2 ± 0.4	-0.4 ± 0.4	4
R20D	-30.1 ± 1.0	6.7 ± 0.3	-11.8 ± 0.9	11.9 ± 0.9	5
R20E	-48.5 ± 0.4	5.5 ± 0.1	-22.9 ± 0.5	0.8 ± 0.5	12
R20K	-60.8 ± 1.0	6.4 ± 0.3	-25.5 ± 0.7	-1.8 ± 0.7	7
R56D	-25.4 ± 1.2	6.0 ± 0.2	-10.8 ± 0.9	12.9 ± 0.9	6
R56E	-32.3 ± 1.2	6.1 ± 0.2	-13.6 ± 1.0	10.2 ± 1.0	4
R56K	-64.6 ± 0.8	7.5 ± 0.6	-24.1 ± 1.7	-0.4 ± 1.7	3
D712R	-58.2 ± 0.6	6.8 ± 0.3	-22.2 ± 0.8	1.6 ± 0.8	6
E722R	-59.8 ± 0.1	6.4 ± 0.3	-23.6 ± 1.1	0.1 ± 1.1	4
D727R	-56.4 ± 0.8	6.7 ± 0.3	-22.0 ± 1.4	1.7 ± 1.4	5
D767R	-53.6 ± 0.8	6.9 ± 0.3	-19.8 ± 0.9	3.9 ± 0.9	5
D774R	-45.0 ± 0.4	5.9 ± 0.3	-20.9 ± 1.3	2.8 ± 1.3	6
E788R	-39.4 ± 0.3	6.2 ± 0.2	-17.0 ± 0.7	6.7 ± 0.7	7
D793R	-57.6 ± 0.3	6.1 ± 0.1	-25.4 ± 0.8	-1.7 ± 0.8	6
D803R	-29.9 ± 0.7	6.2 ± 0.2	-12.5 ± 0.7	11.3 ± 0.7	8
E807R	NE				
D821R	-58.7 ± 0.9	7.5 ± 1.0	-22.6 ± 2.7	1.1 ± 2.7	5
D829R	-64.9 ± 0.5	7.3 ± 0.2	-23.2 ± 0.7	0.1 ± 2.7	12
D836R	-60.2 ± 0.8	6.9 ± 0.5	-22.4 ± 1.6	0.5 ± 0.7	4
D837R	-59.2 ± 1.6	6.4 ± 0.2	-24.4 ± 1.6	-0.6 ± 1.6	4
E840R	-57.3 ± 0.7	6.8 ± 0.3	-21.5 ± 1.2	2.2 ± 1.2	4
D843R	-60.1 ± 0.8	6.2 ± 0.3	-24.8 ± 1.6	-1.0 ± 1.6	5
E847R	-58.8 ± 0.3	5.5 ± 0.1	-27.2 ± 0.4	-3.5 ± 0.4	6
D850R	-61.6 ± 0.3	5.9 ± 0.1	-26.4 ± 0.7	-2.7 ± 0.7	6
E857R	-48.5 ± 1.0	6.6 ± 0.6	-20.6 ± 1.8	3.2 ± 1.8	7
R4D + D803R	-17.3 ± 1.2	8.5 ± 0.5	-5.2 ± 0.6	18.5 ± 0.6	8

R5D + D803R	-17.8 ± 0.7	6.5 ± 0.2	-6.7 ± 0.4	17.0 ± 0.4	10
R20D + D803R	-28.4 ± 1.0	5.1 ± 0.1	-14.0 ± 0.5	9.8 ± 0.5	7
R56D + D803R	-51.4 ± 0.5	7.2 ± 0.1	-17.9 ± 0.4	5.8 ± 0.4	17
R4E + E788R	-34.3 ± 1.2	6.8 ± 0.3	-14.4 ± 0.9	9.3 ± 0.9	4
R5E + E788R	-21.4 ± 1.9	8.4 ± 1.0	-6.8 ± 1.1	16.9 ± 1.1	4
R20E + E788R	-34.1 ± 0.7	6.7 ± 0.4	-13.8 ± 1.0	9.9 ± 1.0	4
R56E + E788R	-28.7 ± 1.7	7.1 ± 0.3	-10.2 ± 0.8	13.5 ± 0.8	4
N12E	-31.3 ± 1.1	7.0 ± 0.5	-12.6 ± 1.1	11.1 ± 1.1	5
N12E + E788R	-41.3 ± 0.7	6.8 ± 0.5	-16.1 ± 1.0	7.6 ± 1.0	8
R4E + R5E	-28.1 ± 2.3	5.9 ± 0.2	-12.0 ± 1.2	11.7 ± 1.2	5
E698R + E699R	-34.8 ± 1.0	6.6 ± 0.3	-13.8 ± 0.8	9.9 ± 0.8	5
R4E + R5E + E698R + E699R	-29.5 ± 1.8	6.4 ± 0.4	-12.3 ± 1.2	11.5 ± 1.2	10
R4D + R5D	-25.3 ± 0.7	6.5 ± 0.1	-9.8 ± 0.3	13.9 ± 0.3	5
R4D + R5D + R56D	-26.1 ± 0.8	6.5 ± 0.1	-10.1 ± 0.5	13.6 ± 0.5	6
R56A	-35.1 ± 0.7	6.4 ± 0.3	-14.1 ± 0.8	9.7 ± 0.8	5
D803A	-46.6 ± 0.6	6.9 ± 0.4	-17.9 ± 1.0	5.8 ± 1.0	11
R56A + D803A	-51.4 ± 0.6	7.6 ± 0.5	-18.5 ± 0.9	5.2 ± 0.9	12
N12A	-45.3 ± 1.2	5.3 ± 0.2	-22.7 ± 0.9	1.1 ± 0.9	4
E788A	-35.2 ± 1.2	7.4 ± 0.6	-13.2 ± 1.3	10.6 ± 1.3	6
N12A + E788A	-38.6 ± 1.2	7.6 ± 0.6	-13.4 ± 1.1	10.3 ± 1.1	7
R4A + R5A	-28.4 ± 0.5	5.0 ± 0.1	-14.7 ± 0.3	9.0 ± 0.3	5
E698A + E699A	-47.8 ± 0.5	6.5 ± 0.2	-18.8 ± 0.6	5.0 ± 0.6	6
R4A + R5A + E698A + E699A	-43.1 ± 0.6	6.2 ± 0.1	-18.0 ± 0.4	5.7 ± 0.4	7