Supporting Information

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SI Text

Q - V Curve Fits. The equation used to fit the Q - V curves was the following sum of two Boltzmann distributions:

$$Q/Q_{\max} = \frac{Z_0}{Z_0 + Z_1} \frac{1}{1 + \exp(-Z_0 F(V - V_0)/RT)} + \frac{Z_1}{Z_0 + Z_1} \frac{1}{1 + \exp(-Z_1 F(V - V_1)/RT)}.$$

With Z_0 and Z_1 , the apparent valences of the first and the second transition, respectively, V_0 and V_1 , the midpoints of the first and the second transition, respectively, and V, F, R, and T the membrane potential, the Faraday constant, the gas constant, and absolute temperature, respectively.

The goodness of the fit and values of the fitted parameters are shown in Table S1.



Fig. S1. Time constants (τ , in ms) of Shaker gating currents as a function of the pulse voltage (V, in mV) and of the residue present at position 290 (indicated on each graph). Gating currents were measured during activation (black squares) and deactivation (open circles). Wild-type gating kinetics are shown at Phe (*Top Left*). The data represent at least six independent experiments for each mutant.



Fig. 52. The weighted time constant of the activation gating currents is plotted as a function of the voltage and of the indicated amino acid present at position 290. The wild-type Phe residue is shown as pink squares. The arrow indicates the appearance of a slow component between +50 mV and +100 mV for the mutants. The data are representative of at least five independent experiments.



Fig. S3. Q – V curves for F290A (open squares), R4K (open circles), and for the double mutant R4K/F290A (full up triangles). The data are representative of four to six independent experiments for each mutant.

Table S1.	. Values	of the f	fitted pa	arameters	for the (Q – V	curves of	f the inc	licated	F290
mutants										

Residue at position 290	V ₀ (mV)	V ₁ (mV)	Z ₀	Z ₁	R ²
Тгр	-81 ± 0.2	-63 ± 0.8	1.72 ± 0.23	4.20 ± 0.22	0.999
Phe	-55 ± 0.5	-48 ± 0.3	2.30 ± 0.10	5.00 ± 0.10	0.999
Tyr	-58 ± 0.3	-45 ± 0.7	1.75 ± 0.18	6.02 ± 0.50	0.999
Asn	-32 ± 0.3	-14 ± 0.6	3.16 ± 0.04	1.62 ± 0.05	0.999
Thr	-36 ± 0.2	-12 ± 0.6	2.80 ± 0.02	1.10 ± 0.03	0.999
Leu	-43 ± 0.4	-12 ± 0.5	2.78 ± 0.09	2.52 ± 0.09	0.999
lle	-34 ± 0.5	10 ± 0.2	2.60 ± 0.08	0.87 ± 0.04	0.999
Met	-43 ± 1.0	14 ± 1.1	2.56 ± 0.10	1.53 ± 0.06	0.999
Gly	-36 ± 0.7	17 ± 2.8	2.60 ± 0.10	0.96 ± 0.07	0.999
Val	-39 ± 0.6	25 ± 2.2	2.66 ± 0.10	1.07 ± 0.06	0.999
Ala	-43 ± 0.4	46 ± 1.4	2.72 ± 0.09	1.26 ± 0.05	0.999
Ser	-38 ± 1.0	68 ± 0.4	1.71 ± 0.10	0.73 ± 0.05	0.997
Gln	-53 ± 0.6	92 ± 3.8	2.13 ± 0.08	0.66 ± 0.03	0.998