

**Supplementary Table 1. Activation and steady-state inactivation parameters**

**Activation**

Constructs	0.1 mM			0.25 mM			0.8 mM			2.4 mM		
	$V_{1/2}$ (mV)	$k$ (mV)	n	$V_{1/2}$ (mV)	$k$ (mV)	n	$V_{1/2}$ (mV)	$k$ (mV)	n	$V_{1/2}$ (mV)	$k$ (mV)	n
WT	14.7 ± 0.6 <sup>b</sup>	-14.8 ± 0.6 <sup>b</sup>	20	2.3 ± 0.6	-12.2 ± 0.5	23	2.6 ± 0.3	-10.6 ± 0.2	64	2.1 ± 0.7	-12.4 ± 0.6	16
D1546A	ND	ND		7.9 ± 0.6 <sup>b</sup>	-12.9 ± 0.5	13	1.7 ± 0.5	-10.2 ± 0.4	28	-3.8 ± 0.4 <sup>b</sup>	-9.6 ± 0.4	16
D1546N	9.7 ± 0.7	-13.5 ± 0.6	6	7.0 ± 0.8 <sup>b</sup>	-13.7 ± 0.8	13	0.2 ± 0.7	-12.1 ± 0.7	44	-3.8 ± 0.4 <sup>b</sup>	-9.5 ± 0.4	16
D1546S	7.9 ± 0.8 <sup>b</sup>	-13.8 ± 0.8	10	0.1 ± 0.8	-12.2 ± 0.7	15	0.9 ± 0.5	-10.7 ± 0.5	35	-8.7 ± 0.4 <sup>b</sup>	-7.5 ± 0.3	12
D1546K	6.1 ± 0.6 <sup>b</sup>	-12.6 ± 0.6	11	-1.7 ± 0.9 <sup>b</sup>	-12.0 ± 0.9	8	-8.2 ± 0.6 <sup>a</sup>	-9.8 ± 0.6	49	-0.4 ± 0.7 <sup>b</sup>	-11.7 ± 0.6	18
K1539D	23.4 ± 0.4 <sup>b</sup>	-15.2 ± 0.4	10	20.5 ± 0.7 <sup>b</sup>	-16.0 ± 0.6	17	9.7 ± 0.6 <sup>a</sup>	-14.1 ± 0.6 <sup>a</sup>	71	6.8 ± 0.9	-13.1 ± 0.9	21
K1543D	6.1 ± 0.5 <sup>b</sup>	-12.5 ± 0.5	18	3.8 ± 0.3 <sup>b</sup>	-11.5 ± 0.3	21	-3.8 ± 0.3 <sup>a</sup>	-9.2 ± 0.3	47	-2.5 ± 0.4	-9.7 ± 0.3	9

**Inactivation**

Constructs	0.1 mM			0.25 mM			0.8 mM			2.4 mM		
	$V_{1/2}$ (mV)	$k$ (mV)	n	$V_{1/2}$ (mV)	$k$ (mV)	n	$V_{1/2}$ (mV)	$k$ (mV)	n	$V_{1/2}$ (mV)	$k$ (mV)	n
WT	-21.9 ± 1.2	13.2 ± 1.2	9	-21.5 ± 1.1	9.1 ± 1.0	13	-24.5 ± 0.7	9.9 ± 0.7	23	-29.7 ± 0.6 <sup>b</sup>	8.6 ± 0.5	8
D1546A	ND	ND		-21.0 ± 0.8	8.0 ± 0.7	10	-21.1 ± 1.0	8.9 ± 0.9	12	-31.2 ± 0.6 <sup>b</sup>	8.4 ± 0.6	10
D1546N	-20.1 ± 2.2	10.6 ± 2.0	4	-19.7 ± 1.0	8.3 ± 0.9	11	-24.2 ± 0.7	8.1 ± 0.6	15	-31.8 ± 0.6 <sup>b</sup>	8.5 ± 0.5	10
D1546S	-20.1 ± 1.3	12.5 ± 1.2	5	-25.2 ± 0.7	7.8 ± 0.6	9	-22.1 ± 0.8	9.2 ± 0.8	29	-29.9 ± 0.9 <sup>b</sup>	9.0 ± 0.6	9
D1546K	-13.8 ± 1.5	11.9 ± 1.4	6	-18.4 ± 1.3	10.6 ± 1.2	6	-16.7 ± 1.8	15.7 ± 1.8	16	-20.6 ± 1.4	13.6 ± 1.4	10
K1539D	-33.3 ± 3.9	17.2 ± 4.7	5	-26.9 ± 2.4	11.0 ± 2.4	10	-23.0 ± 1.2	11.0 ± 1.1	29	-33.9 ± 0.7 <sup>b</sup>	8.7 ± 0.7	10
K1543D	-25.1 ± 3.6	13.8 ± 3.8	9	-27.5 ± 2.8	9.3 ± 2.6	13	-25.8 ± 1.0	10.2 ± 1.0	21	-32.5 ± 0.9 <sup>b</sup>	8.7 ± 1.0	11

<sup>a</sup> Values are significantly (p < 0.001) different than WT parameters;

<sup>b</sup> Values are significantly (p < 0.001) different than 0.8mM Mg.

ND, Not determined