

**Table 1.** Functional characterization of key Kv2.1 phosphorylation sites.  $G_{1/2}$  is the half-maximal conductance calculated from the conductance-voltage ( $G$ - $V$  curve).  $V_{1/2}$  is the half-maximal steady-state inactivation potential calculated from the current-voltage ( $I$ - $V$ ) curve. ND, not determined. Values in bold are significantly different ( $P < 0.05$ ) from the respective values for wild-type Kv2.1. Mutation of Ser/Thr to Ala at constitutive phosphorylation sites S492, S499, S516, S586, S795, and T832 did not alter functional phenotype under control, ionomycin-, or AP-treated conditions.

Phosphorylation sites identified by LC-MS/MS	Dephosphorylation by ionomycin (SILAC)	Mutations studied	Voltage-dependent activation and steady-state inactivation parameters of channels					
			Control		Ionomycin-treated		AP-treated	
			$G_{1/2}$	$V_{1/2}$	$G_{1/2}$	$V_{1/2}$	$G_{1/2}$	$V_{1/2}$
Wild type			+16.4 ± 0.6	-26.2 ± 0.4	-10.1 ± 0.6	-58.3 ± 0.5	-19.8 ± 0.7	-60.2 ± 0.8
S563	Yes	S563A	<b>+0.2 ± 0.5</b>	<b>-43.5 ± 0.4</b>	-9.6 ± 0.4	-59.5 ± 0.7	-18.5 ± 0.8	-56.8 ± 0.8
		S563D	+15.8 ± 0.8	-28.1 ± 0.7	<b>+5.4 ± 0.3</b>	<b>-43.1 ± 0.4</b>	<b>-5.7 ± 0.4</b>	<b>-48.3 ± 0.5</b>
S603	Yes	S603A	<b>+4.3 ± 0.7</b>	<b>-34.8 ± 0.4</b>	-9.3 ± 0.3	<b>-55.4 ± 0.6</b>	-18.9 ± 0.5	-58.4 ± 0.4
		S603D	+16.7 ± 0.6	<b>-32.4 ± 0.4</b>	<b>-4.3 ± 0.6</b>	<b>-53.3 ± 0.8</b>	<b>-15.3 ± 0.7</b>	<b>-54.1 ± 0.5</b>
S537	Yes	S537A	<b>+5.7 ± 0.4</b>	<b>-36.2 ± 0.5</b>	-9.3 ± 0.6	-58.2 ± 0.6	-19.7 ± 0.3	-59.4 ± 0.7
		S537D	+16.1 ± 0.7	-29.7 ± 1.0	<b>-3.1 ± 0.5</b>	<b>-51.9 ± 0.4</b>	<b>-14.1 ± 0.8</b>	<b>-53.2 ± 0.4</b>
S715	Yes	S715A	<b>+5.9 ± 0.4</b>	<b>-35.4 ± 0.5</b>	-10.1 ± 0.5	-60.3 ± 0.8	-20.1 ± 0.7	-58.8 ± 0.6
		S715D	+15.8 ± 0.4	-26.3 ± 0.3	<b>-2.7 ± 0.5</b>	<b>-54.6 ± 0.7</b>	<b>-12.1 ± 0.7</b>	<b>-51.7 ± 0.6</b>
S651	Yes	S651A	+15.9 ± 0.6	-26.2 ± 0.4	-9.7 ± 0.5	-59.3 ± 0.7	-20.4 ± 1.2	-59.7 ± 0.8
S453	Yes	S453A	<b>+7.2 ± 0.7</b>	-28.0 ± 0.3	-10.2 ± 0.3	-58.9 ± 0.4	-19.6 ± 0.4	-59.2 ± 0.5
		S453D	+15.9 ± 0.4	-26.2 ± 0.4	<b>-3.1 ± 0.6</b>	<b>-49.6 ± 0.8</b>	<b>-9.2 ± 0.6</b>	<b>-52.4 ± 0.5</b>
S11	Yes	S11A	+16.7 ± 0.5	<b>-42.1 ± 0.7</b>	-9.7 ± 0.4	-59.1 ± 0.6	-20.3 ± 0.6	-61.1 ± 0.7
		S11D	+16.5 ± 0.6	-27.4 ± 0.5	<b>-8.7 ± 0.9</b>	<b>-47.3 ± 0.4</b>	-19.2 ± 0.5	<b>-48.2 ± 0.5</b>
	S11A + S453A		<b>+4.6 ± 0.5</b>	<b>-39.8 ± 0.7</b>	-10.4 ± 0.6	-59.3 ± 0.5	-19.8 ± 0.5	-60.1 ± 0.4
	S563A + S603A		<b>-4.3 ± 0.5</b>	<b>-48.2 ± 0.6</b>	-10.6 ± 0.6	-59.7 ± 0.6	-19.9 ± 0.5	-60.1 ± 0.7
	S453A + S563A + S603A		<b>-6.9 ± 0.4</b>	<b>-47.3 ± 0.4</b>	-11.3 ± 0.6	-58.9 ± 0.5	-20.7 ± 0.6	-59.5 ± 0.6
S480	ND	S480A	<b>-0.8 ± 0.5</b>	<b>-41.1 ± 0.8</b>	<b>-15.7 ± 0.9</b>	-59.8 ± 0.4	-20.1 ± 0.8	-60.1 ± 0.5
		S480D	+16.2 ± 0.4	-25.9 ± 0.5	<b>-2.4 ± 1.0</b>	<b>-42.7 ± 0.5</b>	<b>-4.1 ± 0.7</b>	<b>-44.6 ± 0.6</b>
S767	ND	S767A	<b>+8.1 ± 0.7</b>	<b>-33.1 ± 0.8</b>	-7.3 ± 1.1	<b>-54.7 ± 0.8</b>	-17.6 ± 0.5	-58.9 ± 0.8
		S767D	+15.4 ± 0.8	-26.8 ± 0.4	<b>+0.7 ± 0.8</b>	<b>-48.4 ± 0.6</b>	<b>-12.2 ± 0.8</b>	<b>-52.1 ± 0.5</b>
S800	No	S800A	16.7 ± 0.8	-27.4 ± 0.5	-9.8 ± 0.5	-59.1 ± 0.7	-20.1 ± 0.6	-59.9 ± 0.4