Supplementary information

PKA phosphorylation reshapes the pharmacological kinetics of BmK AS, a unique site-4 sodium channel-specific modulator

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1. Supplementary figures and figure legends:

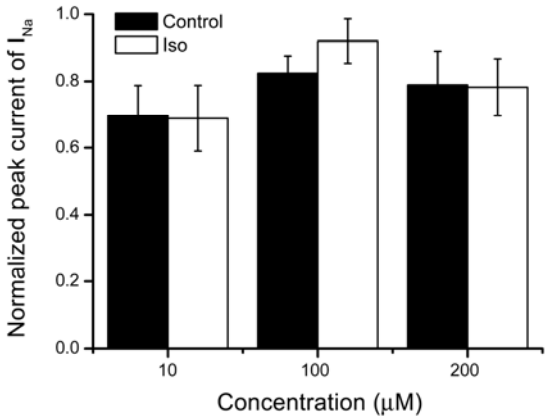

Figure S1. Effects of isoproterenol on peak Na^+ current of $Na_v1.2\alpha$ alone. Peak Na^+ currents of $Na_v1.2\alpha$ were normalized to the mean amplitudes in each group of oocytes tested.

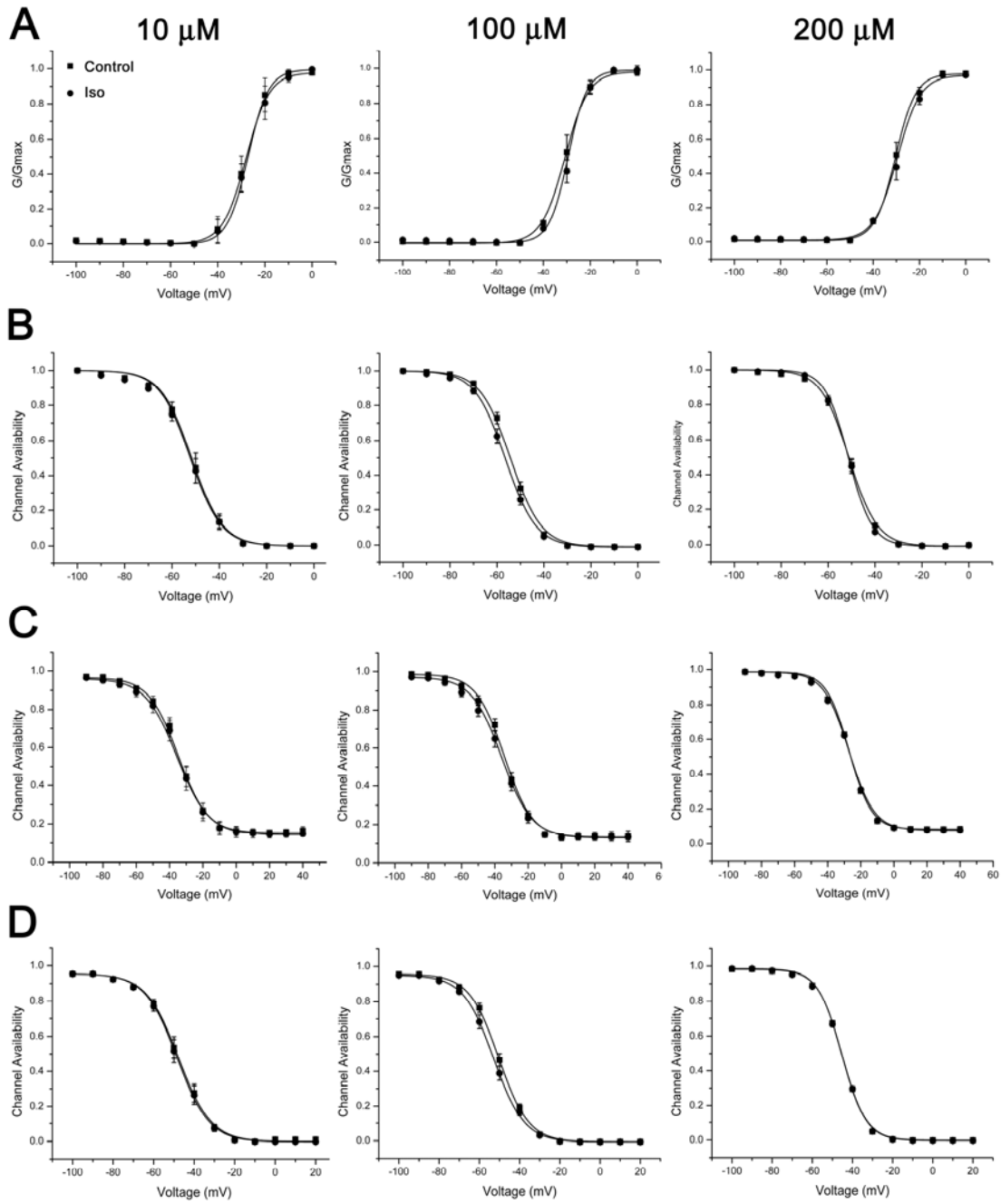

Figure S2. Effect of isoproterenol on the voltage-dependent activation and inactivation of $Na_v1.2\alpha$. Statistical plots of the G-V relationship for activation (A) and I–V relationship for steady-state, fast and slow inactivation (B-D) of phosphorylated (solid circle) and non-phosphorylated (solid box) $Na_v1.2$.

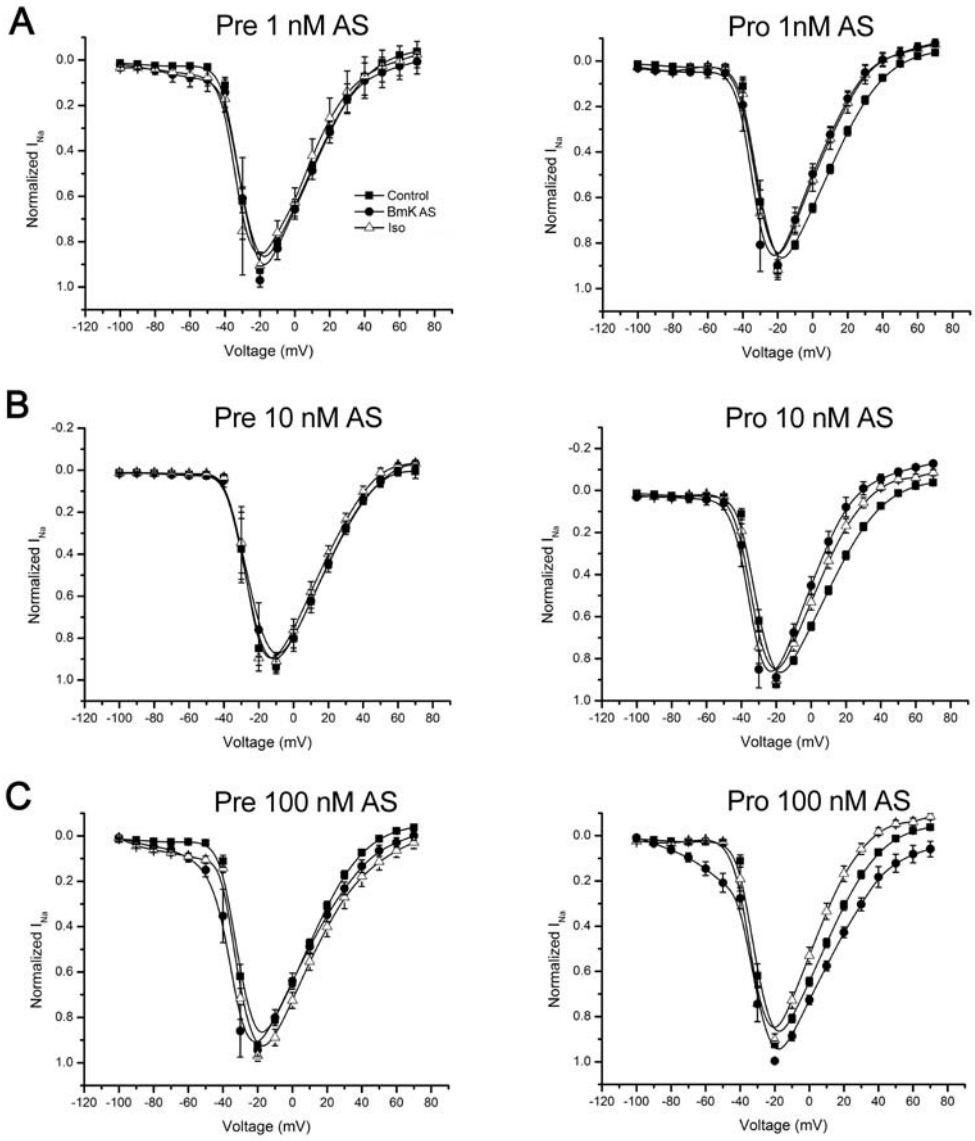

Figure S3. Effect of isoproterenol on the voltage-dependent activation of $Na_v1.2\alpha$ expressed in *Xenopus* oocytes. Statistical plots of the I–V relationship of phosphorylated /non-phosphorylated rNav1.2 before (A-C, left panel) and after (A-C, right panel) application of 1, 10 and 100 nM BmK AS. Each oocyte was subjected to one concentration of BmK AS for 20 min before or after treatment of Iso. n=4, 6, 8, 9 , 8 and 7 respectively.

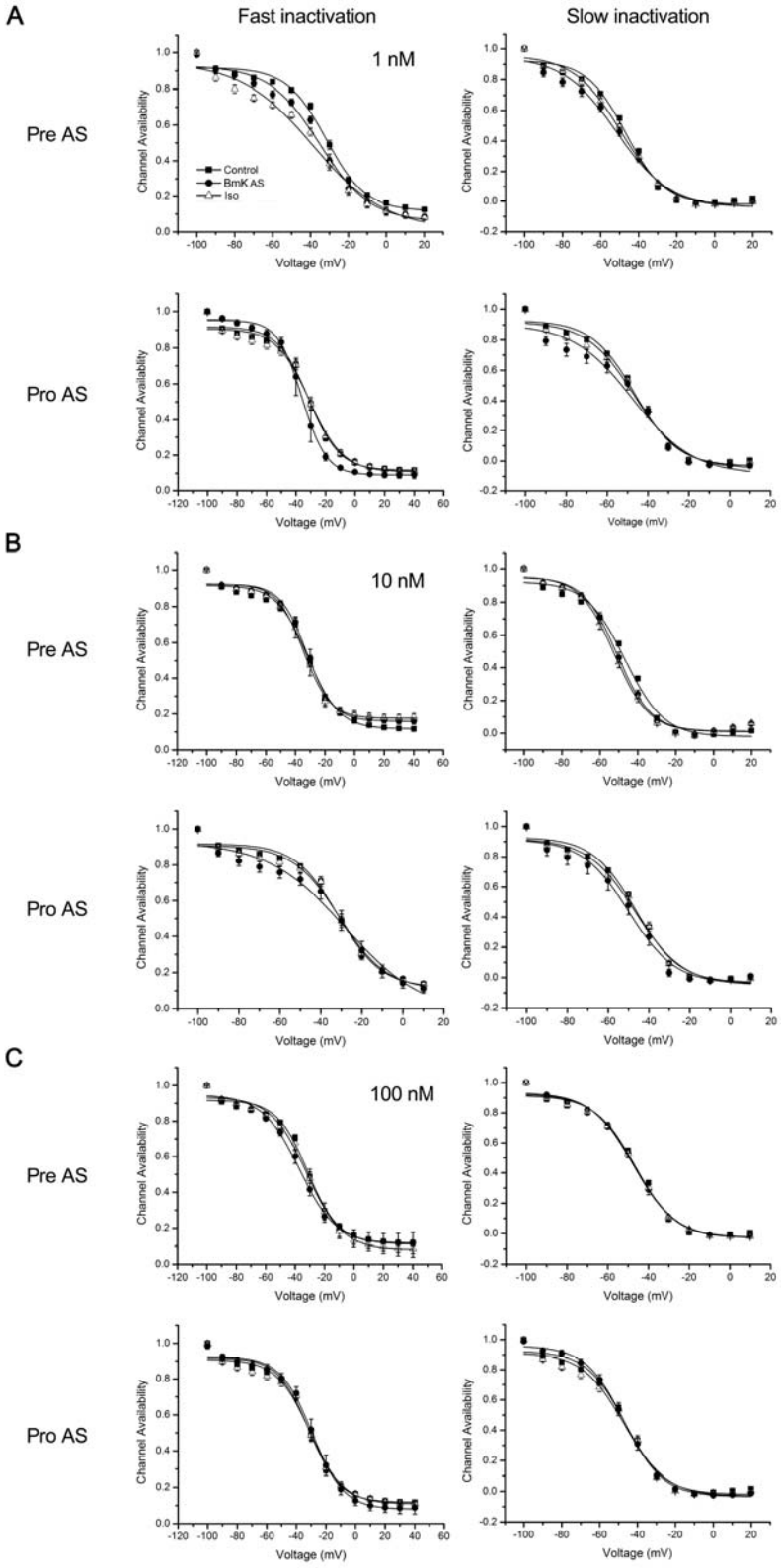

Figure S4. Modulation of BmK AS on voltage-dependent fast and slow inactivation of phosphorylated/unphosphorylated $Na_v1.2\alpha$. All the currents were recorded before

(Up-panel,Pre-AS) or after (Bottom-panel,Pro-AS) treatment of Iso for PKA activation. Each cell was subjected to one concentration of BmK AS for 20 min 1, 10 and 100 nM (A-C). The plots of channel availability versus voltage fit in well with Boltzmann functions for either fast inactivation (Left) or slow inactivation.The fitting parameters are indicated in Table 1. ■, control conditions; △，100 μM Iso treatment; ●, BmK AS administration.

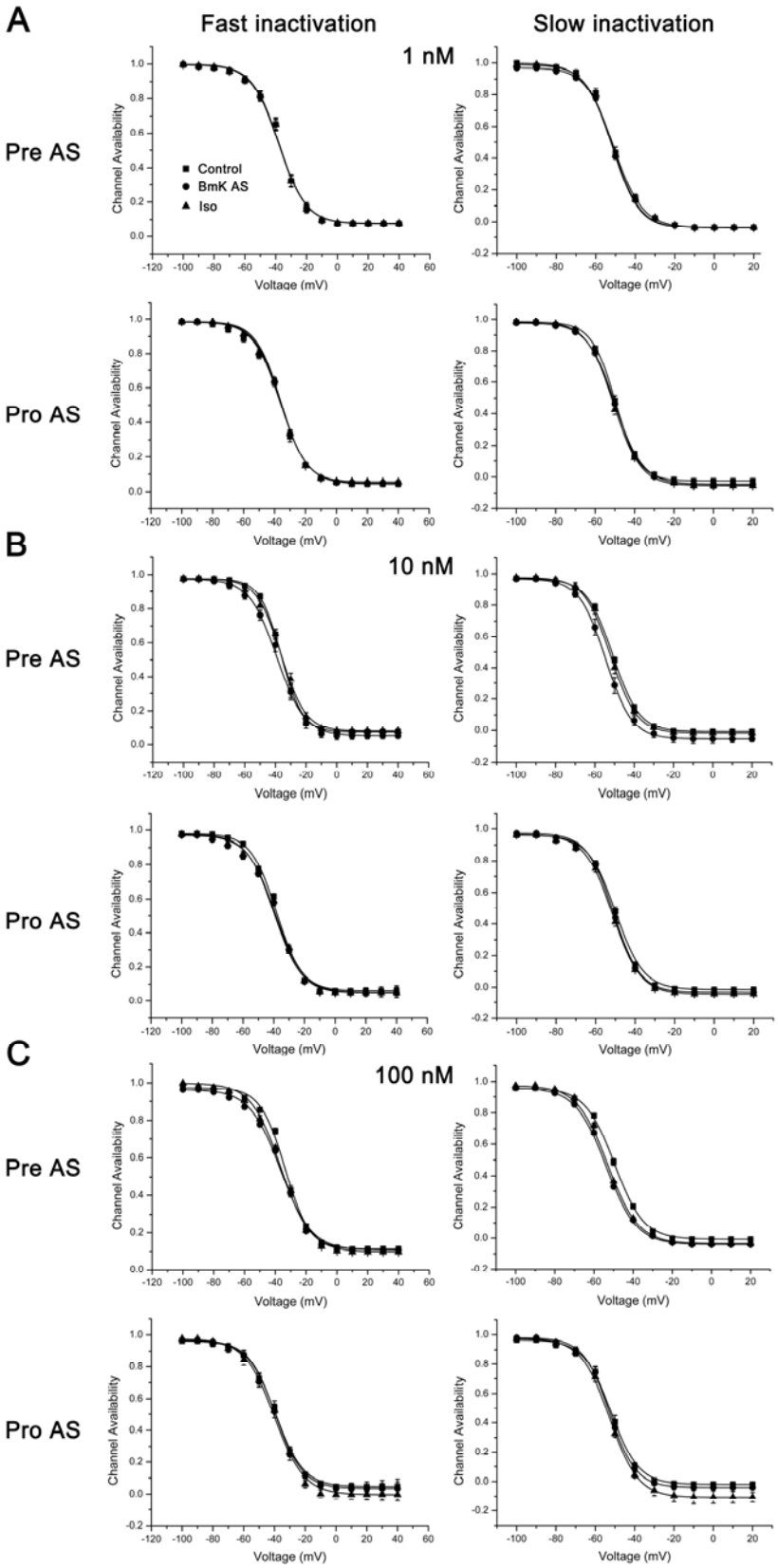

Figure S5. Modulation of BmK AS on voltage-dependent fast and slow inactivation of phosphorylated/unphophorylated $Na_v1.2$ (α+β1). All the currents were recorded

before (Up-panel,Pre-AS) or after (Bottom-panel,Pro-AS) treatment of Iso for PKA activation. Each cell was subjected to one concentration of BmK AS for 20 min 1, 10 and 100 nM (A-C). The plots of channel availability versus voltage fit in well with Boltzmann functions for either fast inactivation (Left) or slow inactivation.The fitting parameters are indicated in Table 2. ■, control conditions; ▲，100 μM Iso treatment; ●, BmK AS administration.

2. Supplementary table:

Table S1: Parameters for voltage-dependence activation and inactivation of $Na_v1.2\alpha$ after applying with different concentrations of isoproterenol

Iso[1]	**Control**		**1 μM**		**Control**		**10 μM**		**Control**		**200 μM**	
Activation		**N**[2]		**N**		**N**		**N**		**N**		**N**
$V_{1/2}$ (mV)	-28.05±0.42	6	-27.25±0.67	6	-30.67±2.34	5	-29.48±0.89	5	-30.48±1.23	5	-29.65±0.98	6
k_m (mV)	4.93±0.35		4.36±1.03		5.00±0.73		3.99±0.36		4.40±0.29		5.01±0.24	
Steady-state inactivation												
$V_{1/2}$ (mV)	-51.30±0.34	5	-52.27±0.21	6	-54.22±0.34	6	-56.44±0.35	5	-51.11±0.25	6	-51.32±0.35	6
k_m (mV)	6.54±0.30		6.52±0.34		6.53±0.56		6.57±0.32		6.23±0.36		5.24±0.48	
Fast inactivation												
$V_{1/2}$ (mV)	-34.93±0.65	6	-35.62±0.69	6	-34.38±0.75	6	-35.92±0.73	6	-27.91±0.53	6	-27.95±0.58	6
k_m (mV)	8.76±0.47		9.44±0.50		8.39±0.51		8.92±0.48		6.78±0.35		7.56±0.46	
Slow inactivation												
$V_{1/2}$ (mV)	-47.68±0.32	6	-48.66±0.65	6	-50.32±0.54	6	-52.82±0.78	6	-45.18±0.21	5	-45.26±0.27	5
k_m (mV)	8.02±0.38		7.91±0.35		7.36±0.32		7.57±0.35		6.03±0.28		6.01±0.33	

[1] Kinetic parameters were listed as Mean±SEM separately.

[2] N indicates the number of samples tested.