Supplementary Information

Structural insights into the potency of SK channel positive modulators

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Running title: Positive modulation of SK channels

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Table 1. Crystallographic statistics

Data Collection[a]		
	CaM-CaMBD2-a with SKS-11	**CaM-CaMBD2-a with SKS-14**
Space Group Unit Cell Dimensions	C2 a=77.3 Å, b=66.1 Å, c=65.4 Å α=90.0°, β=93.8°, γ=90.0°	C2 a=77.0 Å, b=66.0 Å, c=65.3 Å α=90.0°, β=93.8°, γ=90.0°
Wavelength (Å)	1.3148	1.3424
Resolution range (Å)	26.84–1.9 (1.95-1.88)	27.86-2.30 (2.38-2.30)
Completeness (%)	98.4 (84.4)	99.6 (99.9)
Total Observations	165,310 (4,536)	95,275 (8,569)
Unique Observations	25,882 (1,568)	14,551 (1,431)
Mean Redundancy	6.4 (2.9)	6.5 (6.0)
Mean I/σ(I)	14.9 (3.2)	19.9 (8.7)
R_{merge}[b]	0.073 (0.368)	0.057 (0.156)
R_{pim}[c]	0.029 (0.245)	0.024 (0.068)
Model Refinement[a]		
Resolution Range (Å)	25.79-1.9 (1.97-1.9)	25.69-2.3 (2.38-2.3)
No. reflections	24,969 (2,043)	14,509 (1,424)
R_{work}[d]	0.188 (0.307)	0.196 (0.28)
R_{free}[d]	0.242 (0.398)	0.248 (0.36)
No. atoms / Avg. B (Å^2)	2,197/32.7	2,157/34.8
protein	1,931/31.4	1,931/34.0
calcium ions	2/35.2	2/47.9
solvent	218/38.7	175/34.6
ligand	48/56.1	51/64.9
Phi/Psi angles favored (%) / outliers (#)	99.6/0.4	99.6/0.4
r.m.s.d. bond angles (°)	1.1	1.5
r.m.s.d. bond lengths (Å)	0.007	0.008

[a] Values in parentheses refer to data in the highest resolution shell.

[b] $R_{merge} = \sum_{hkl}\sum_j | I_j - \langle I \rangle | \ / \ \sum_{hkl}\sum_j I_j$. $\langle I \rangle$ is the mean intensity of *j* observations of reflection *hkl* and its symmetry equivalents.

[c] R_{pim} (precision-indicating merge) $= \sum_{hkl} (1/n_{hkl} - 1)^{1/2} \sum_j | I_j - \langle I \rangle | \ / \ \sum_{hkl}\sum_j I_j$. *n* is the number of observations of reflection hkl.

[d] $R_{cryst} = \sum_{hkl} | F_{obs} - kF_{calc} | \ / \ \sum_{hkl} | F_{obs} |$. $R_{free} = R_{cryst}$ for 5% of reflections excluded from crystallographic refinement.

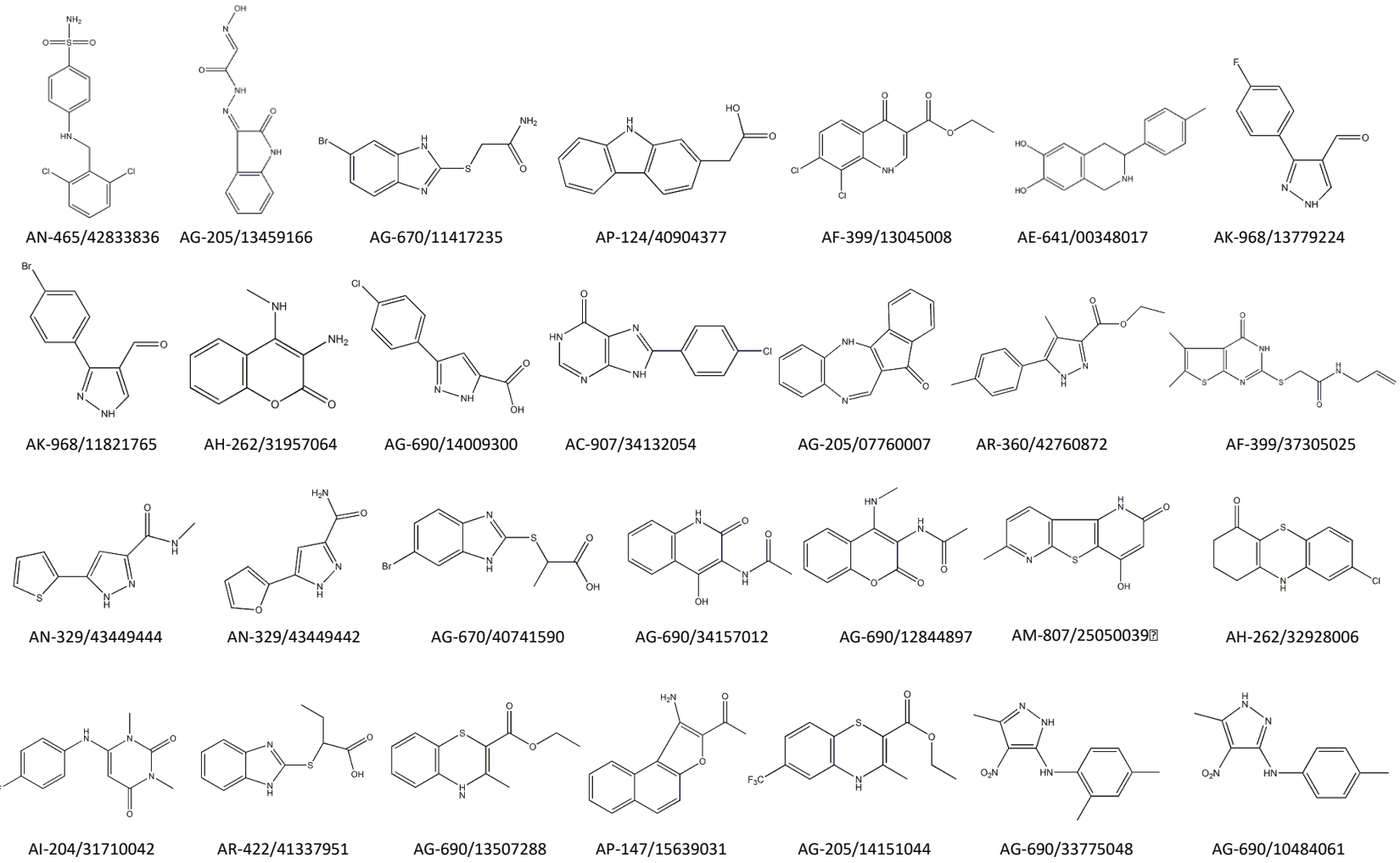

Figure S1. The inactive compounds tested experimentally. Chemical structures and Specs ID (www.specs.net) of the 28 compounds tested inactive are shown.

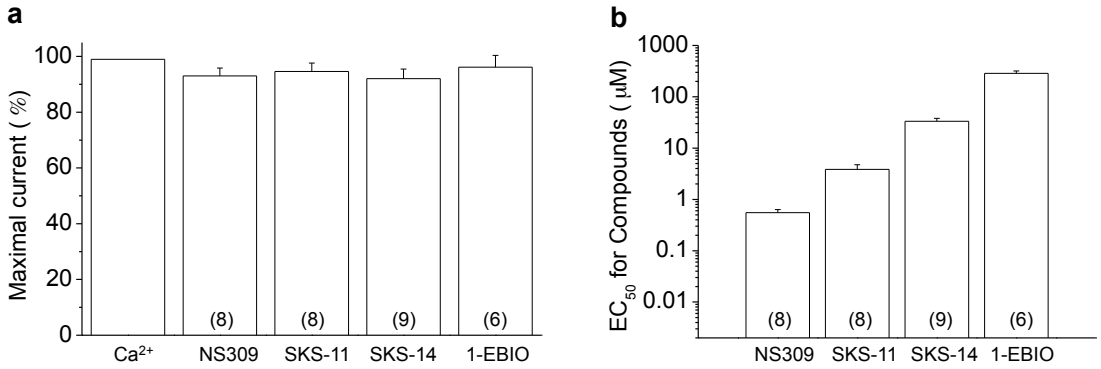

Figure S2. The efficacy and potency of NS309, SKS-11, SKS-14 and 1-EBIO. (a) The maximal responses to compounds are normalized by the SK2 current induced by saturating 10 μM Ca^{2+}. (b) The potency (EC_{50}) of the four compounds in potentiating the SK2 current is different from each other. All data are presented in mean ± s.e.m. The numbers of experiments are shown in the parentheses.

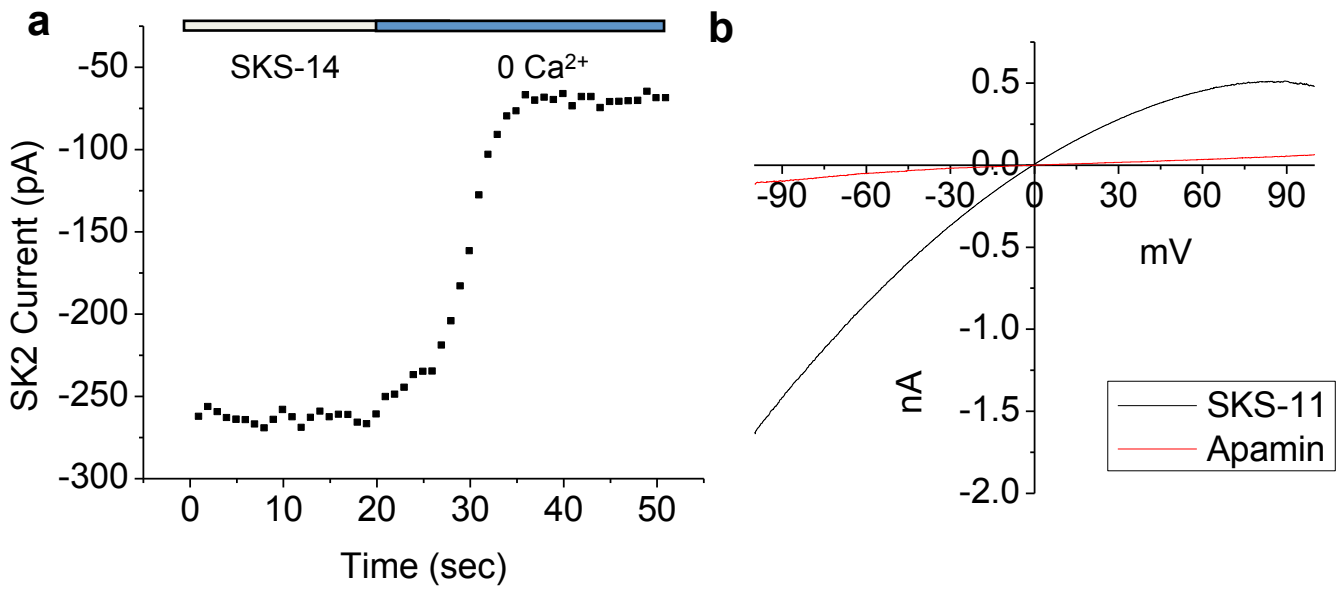

Figure S3. Control experiments of SK2 current. (a) In inside-out patch recordings, 0 Ca^{2+} inhibited the current induced by SKS-14 (100 μM) in the presence of 0.1 μM Ca^{2+}. (b) In whole-cell recordings, apamin inhibited the current induced by SKS-11 (30 μM).