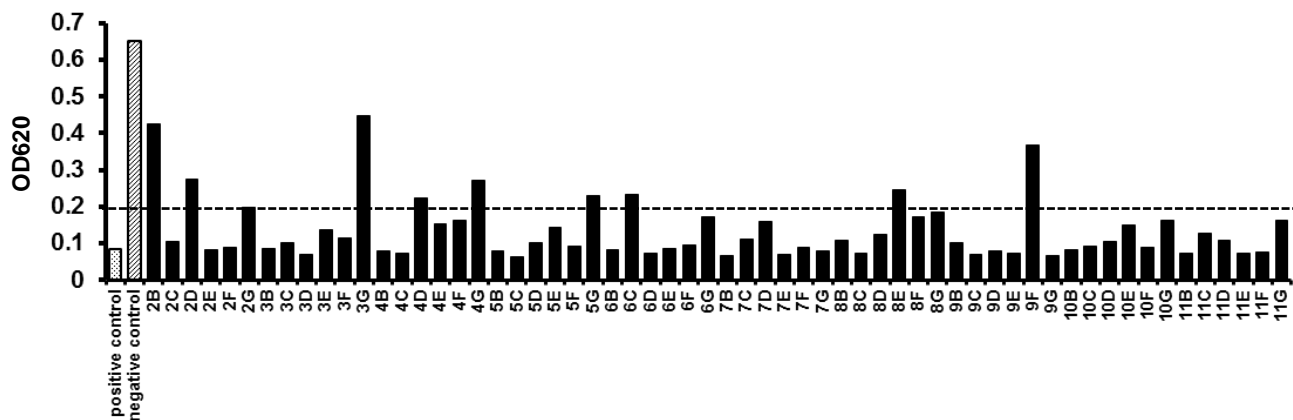


Supplementary Figure 1. The effect of increasing concentration of GST-NKCC2 and ATP on SPAK-regulated phosphorylation in the ELISA system.

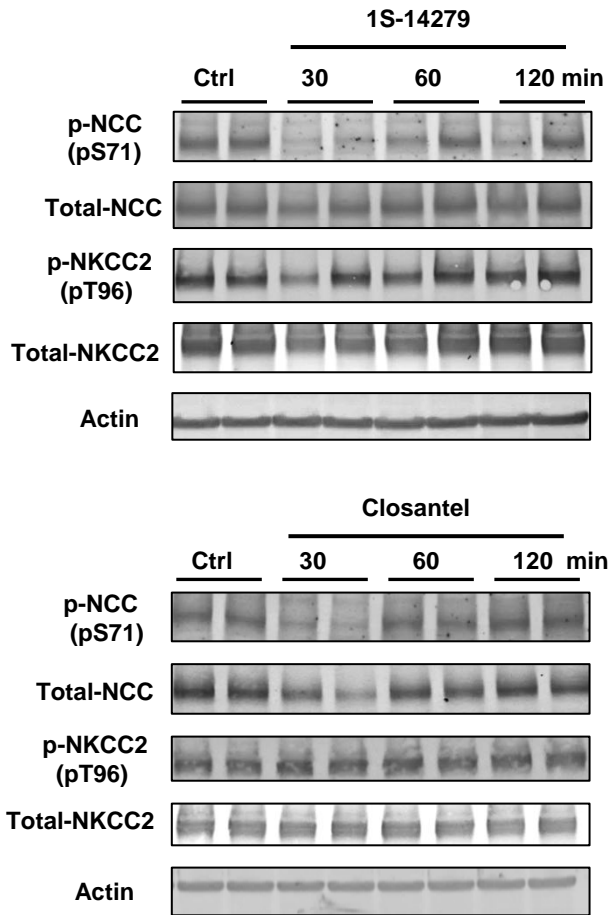
(A) NKCC2 dose-dependent (0 to 20 pmol/well) increase of phosphorylation signal was observed in the presence of ATP (n = 3, mean \pm SEM). (B) ATP dose-dependent (0 to 2 mM) phosphorylation of GST-NKCC2 (n = 3, mean \pm SEM). Maximal NKCC2 phosphorylation was observed between 1.0 and 2.0 mM of ATP.

Secondary assay of the primary positive compounds



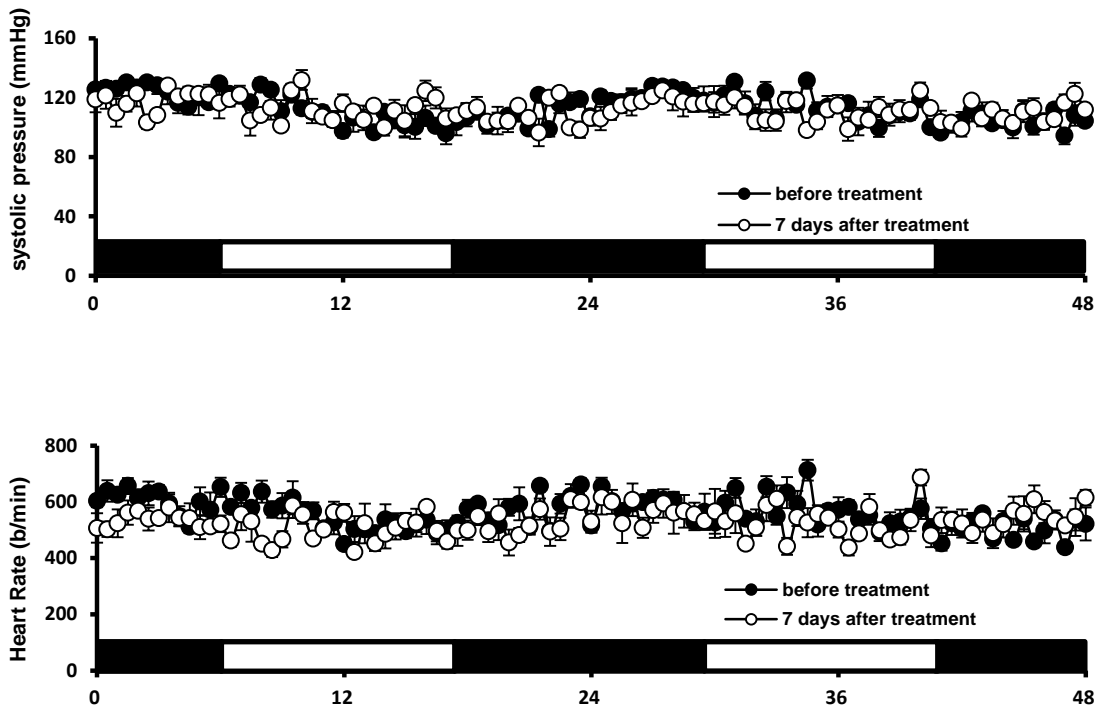
Supplementary Figure 2. Secondary assay to confirm the reproducibility of primary positive compounds under the same conditions as the initial screening (the concentration of compounds was 50 μ M each).

This panel shows reproducibility of our ELISA assay. The inhibitory effects of primary positive compounds were almost the same as those observed in the primary assays, indicating that our system showed high reproducibility.



Supplementary Figure 3. Inhibitory effect of acute injection of 1S-14279 and Closantel in mouse kidney were short acting and reversible.

Representative immunoblots of time course of the effects of 1S-14279 and Closantel on total- and p-NCC and NKCC2 (n = 4 in two separate experiments). After 30 min of acute injection of 1S-14279 and Closantel, NCC phosphorylation rapidly reduced but recovered after 120 min.



Supplementary Figure 4. Effect of chronic Closantel treatment on blood pressure.

The dosage of Closantel in the mouse chow was 300 mg/kg/d. The line graphs in the upper and lower panels represent the averaged hourly systolic pressure (mmHg) and heart rate (b/min) data during the dark (active time, black panels) and light (inactive time, white panel) periods.

Line graphs with closed and open circles show blood pressure before and during (on day 7) Closantel treatment ($n = 4$, mean \pm SEM), respectively. No significant differences were observed in blood pressure or heart rate after chronic Closantel treatment.

Supplementary Table 1. The effect of chronic Closantel treatment on blood and urine biochemistries

	Control (n = 4)	Closantel (n = 4)
Plasma		
Na ⁺ (mmol/l)	142.5 ± 0.3	143 ± 0.8
K ⁺ (mmol/l)	3.3 ± 0.03	3.4 ± 0.07
Cl ⁻ (mmol/l)	108.7 ± 0.48	109.2 ± 0.45
Cr (mg/dl)	0.46 ± 0.02	0.39 ± 0.05
Urine		
Na ⁺ /Cr (mmol/mg)	0.18 ± 0.03	0.25 ± 0.05
K ⁺ /Cr (mmol/mg)	0.14 ± 0.02	0.16 ± 0.03
Cl ⁺ /Cr (mmol/mg)	0.15 ± 0.03	0.23 ± 0.05
FENa (%)	0.59 ± 0.07	0.66 ± 0.05
FEk (%)	19.1 ± 1.3	18.2 ± 2.6
FECl (%)	0.62 ± 0.1	0.78 ± 0.04

Cr, creatinine. Values are mean ± SEM.

No significant difference in any parameters between control and Closantel treated-mice.