SUPPLEMENTAL INFORMATION

Figure legends

Fig. S1. Cellular localization of SPAK and OSR1 in renal tubules

Double IF staining for SPAK (green) and NCC (red) in the cortex **(A)** and NKCC2 (red) in the medulla **(B)** of the kidneys in wild-type (WT) (left panels) and $SPAK^{-/-}$ (Ho) mice (right panels). The SPAK was expressed in both of NCC-positive (DCT) and NKCC2-positve (TAL) segments. The IF signal intensity in the cortex (A, left upper panel) was more strong than in medulla (B, left upper panel). **(C)** Double IF staining for OSR1 (red) and NCC (red) (left panel) in the cortex and NKCC2 (red) (right panel) in the medulla of the kidneys in WT mice. OSR1 was widely distributed in the cortex (right panels) and medulla (left panels) including the segments with NCC-positive (DCT) and NKCC2-positve (TAL). Scale = 50μm.

Fig. S2 IF staining of NKCC2 in kidney tissues.

(A) Representative IF micrographs of NKCC2 (upper panels) and p-NKCC2 (T96) (lower panels) in the medulla of wild-type (WT), $SPAK^{+/-}$ (He) and $SPAK^{-/-}$ (Ho) mice. Scale = 10μm.

Fig. S3. Assessment of p-NKCC2 (T96) antibody specificity.

Specificity of antibody to p-NKCC2 (T96) was verified by antigen absorption test using whole kidney tissues.

Fig. S4. Effect of chronic thiazide treatment on the expression of total NCC and p-NCC.

Representative immunoblotting of total NCC and p-NCC (T58) expression after

chronic hydrochlorothiazide (HCTZ) treatment (12.5 mg/kg/day ip for 3 days) in SPAK-null mice. HCTZ treatment enhanced total and p-NCC expression in wild-type (WT) and $SPAK^{+/-}$ (He) but not in $SPAK^{-/-}$ (Ho) mice.

Figure (S1)

(A)

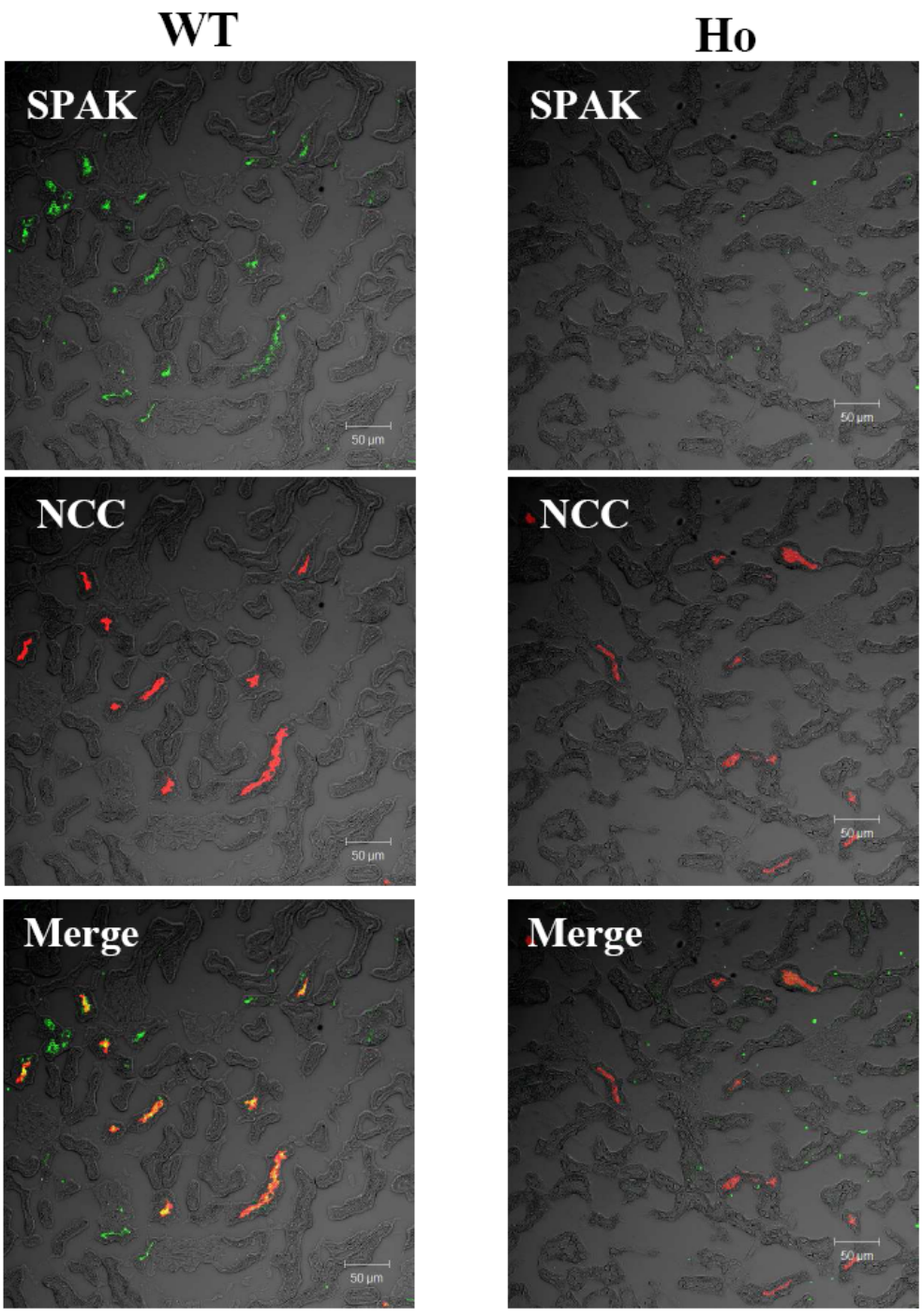
WT
Ho
SPAK
SPAK
NCC
NCC
Merge
Merge
50 µm
50 µm
50 µm
50 µm
50 µm
50 µm

Figure (S1)

(B)

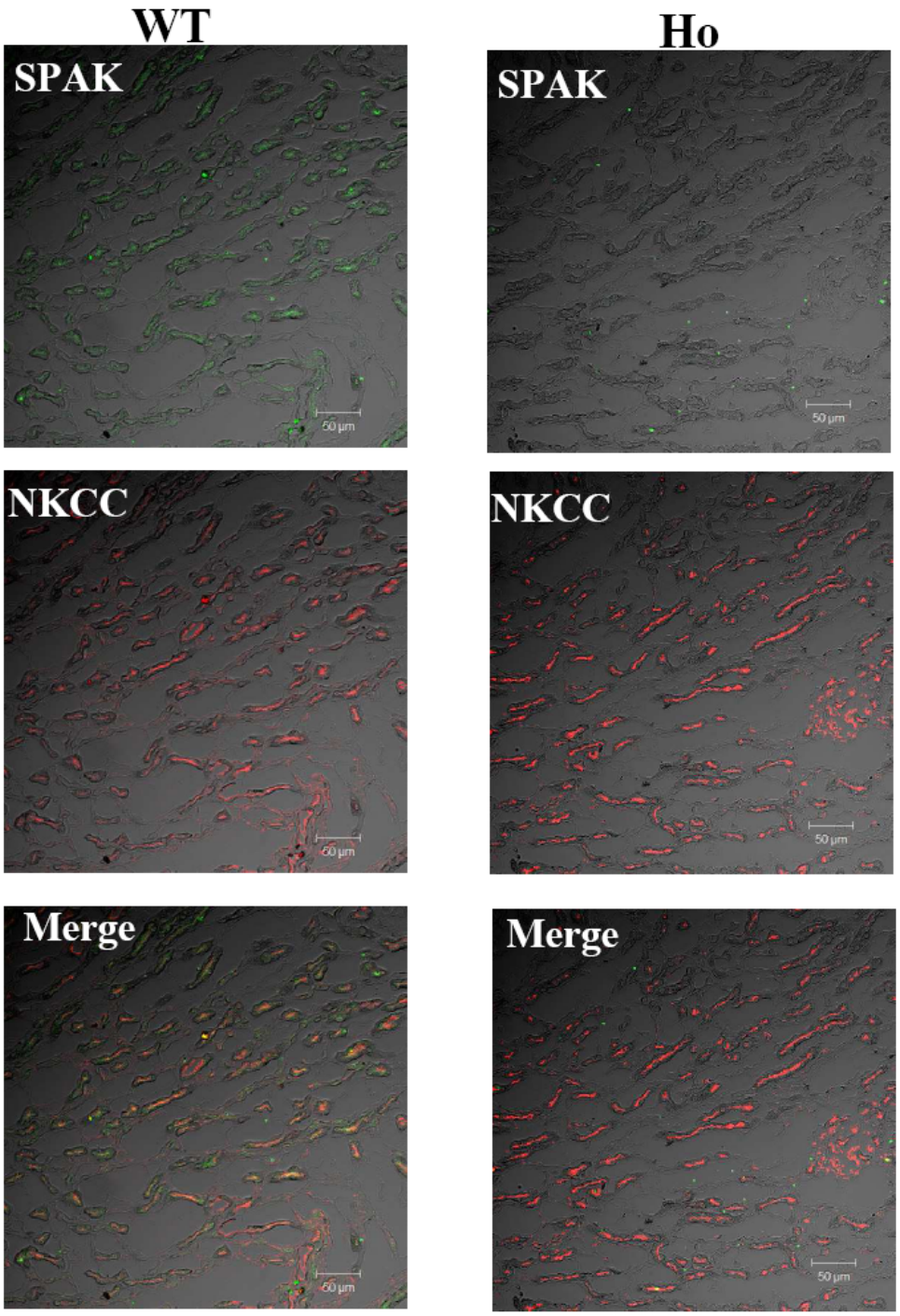
WT
Ho
SPAK
SPAK
NKCC
NKCC
Merge
Merge
50 µm
50 µm
50 µm
50 µm
50 µm
50 µm

Figure (S1)

(C)

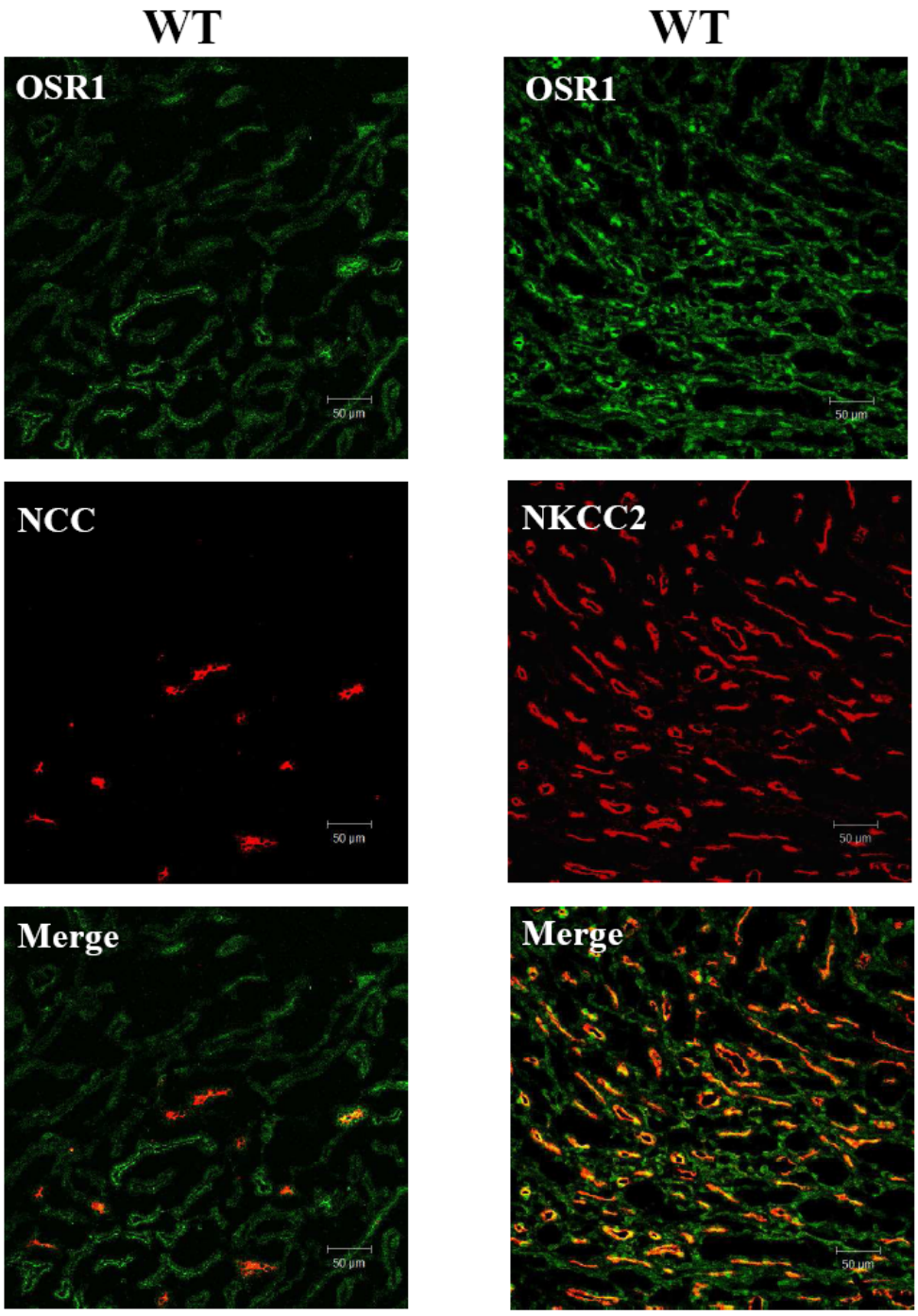
WT
WT
OSR1
OSR1
NCC
NKCC2
Merge
Merge
50 µm
50 µm
50 µm
50 µm
50 µm
50 µm

Figure (S2)

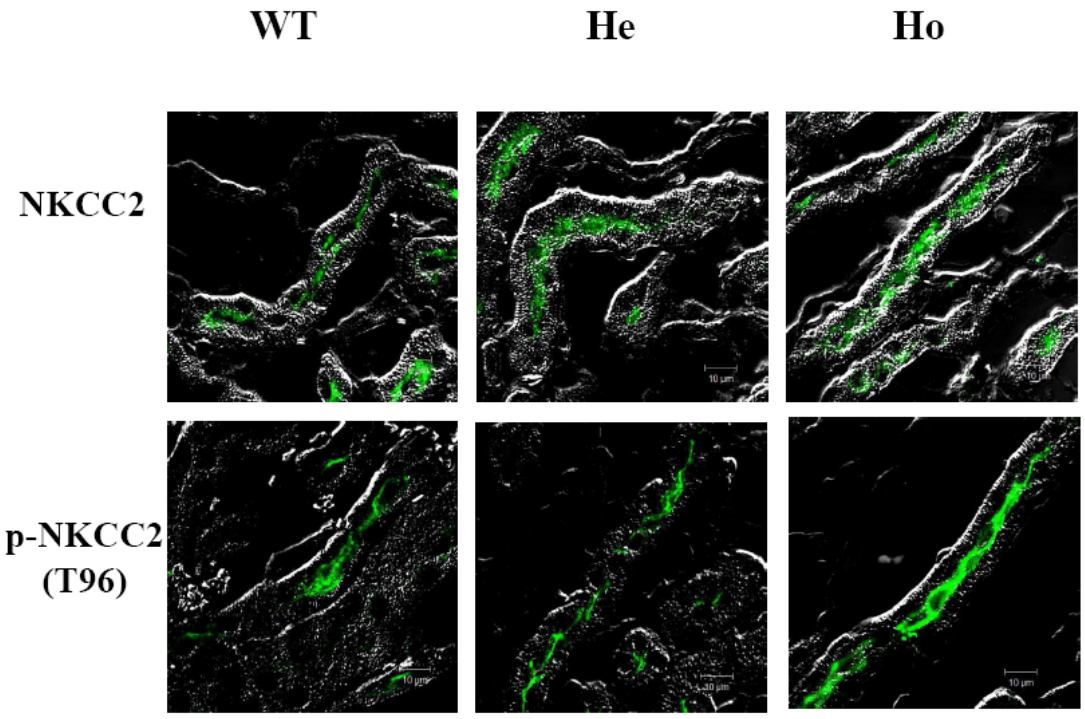

Figure (S3)

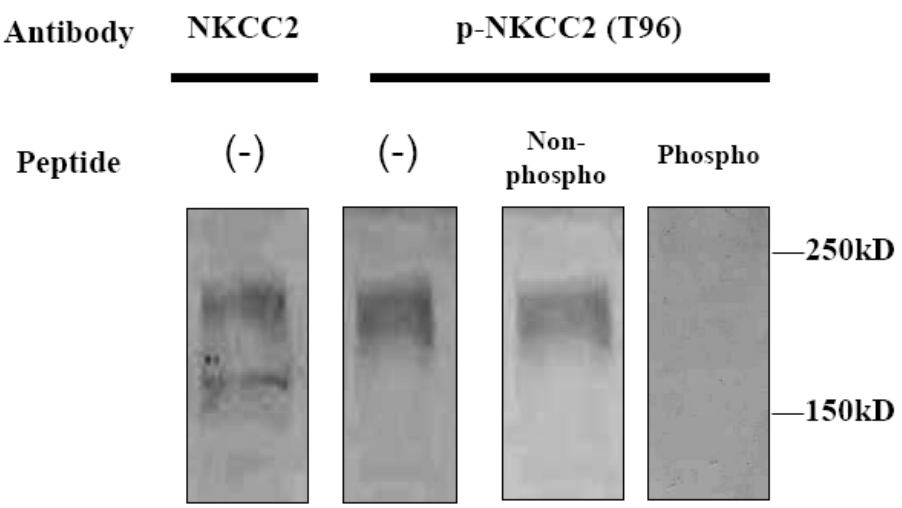
Antibody
NKCC2
p-NKCC2 (T96)
Peptide
(-)
(-)
Non-phospho
Phospho
250kD
150kD

Figure (S4)