SUPPLEMENTAL FIGURE LEGENDS

Figure S1 **Genetic or pharmacologic COX-2 inhibition led to increased kidney macrophage infiltration.** Wild type or COX-$2^{-/-}$ mice (2 months old, C57/Bl6 background) were treated with a high salt diet (HS) for 4 weeks. **(A)** Kidney macrophage infiltration was markedly higher in HS plus SC58236-treated mice than in HS-treated mice, as indicated by immunostaining of F4/80, a marker of macrophages/dendritic cells (*** $P<0.001$, n = 4 in each group). **(B)** HS-treated COX-$2^{-/-}$ mice had more kidney macrophages compared to HS-treated wild type mice. Original magnification: x 160 in both **A** and **B**. All values are means ± SEM. All *P* values were calculated by Student's *t* test.

Figure S2 **COX-2 inhibition led to increased M1 but decreased M2 markers *in vivo* and *in vitro*.** (**A**) Kidney mRNA levels of M1/Th1 markers/cytokines including iNOS, CCL3, TNF-α, IL-1α and IL-1β were markedly higher but that of mannose receptor (MR) were markedly lower in HS plus SC58236-treated mice than in mice with HS alone (** $P<0.01$ and *** $P<0.001$, n = 4 in each group). **(B)** Freshly isolated peritoneal macrophages treated with 25 μM SC58236 for 24 h had decreased protein levels of MR and arginase 1 (M2 markers) but increased protein levels of TNF- α (M1 marker). All values are means ± SEM. All *P* values were calculated by Student's *t* test.

Figure S3 **Mice with a deficient hematopoietic cell COX-2 pathway had increased heart hypertrophy in response to chronic high salt intake.** (**A**) Heart weight vs. body weight ratios were higher in HS-treated COX-$2^{-/-}$-WT BMT mice than in HS-treated WT-WT BMT mice (** $P<0.01$, n = 4). (**B**) Heart weight vs. body weight ratios were also higher in HS-

treated CD11b-Cre; $EP_4^{f/f}$ mice than in HS-treated $EP_4^{f/f}$ mice (***P<0.001, n = 4). All values are means ± SEM. All P values were calculated by Student's t test.

Figure S4 **Prostaglandin EP_4 receptor tonically suppressed Th1 cytokine expression in cultured macrophages. (A)** Murine macrophage RAW264.7 cells expressed COX-2, mPGES1 and VEGF-C. EP_4 was the major EP receptors in RAW264.7 cells. COX-1 and EP_1 receptor were undetectable. **(B)** Treatment of RAW264.7 cells with a selective EP_4 receptor antagonist, L-161,982 (20 μM), led to increased mRNA levels of M1/Th1 markers/cytokines, including iNOS, IL-23α, CCL3, TNF-α, IL-1α and IL-6 (***P<0.001, n = 3). **(C)** PGE_2 led to inhibition of iNOS expression, which was prevented by the selective EP_4 receptor antagonist, L-161,982 (***P<0.001 vs. PGE_2 alone, n = 4 in each group). All values are means ± SEM. All P values were calculated by Student's t test.

Figure S5 **Macrophage EP_4 receptor was effectively deleted in CD11b-Cre; $EP_4^{f/f}$ mice**. Peritoneal macrophages were isolated and EP_4 mRNA was quantitated by qPCR. Macrophage EP_4 mRNA levels were significantly lower in CD11b-Cre; $EP_4^{f/f}$ mice than in $EP_4^{f/f}$ mice (**P<0.01, n = 4). All values are means ± SEM. All P values were calculated by Student's t test.

Figure S6 **The expression levels of p-NCC were increased in HS-treated mPGES-$1^{-/-}$-WT BMT mouse than in HS-treated WT-WT BMT control.**

Figure S7 **Increased medium NaCl elevated mRNA levels of COX-2 and NFAT5 and VEGF-C in cultured macrophage cells. (A)** Addition of 40 mM NaCl to the medium increased RAW264.7 cell COX-2 and NFAT5 mRNA levels at 2.5 h and also increased VEGF-C mRNA levels at 5 h (*P<0.05 and **P<0.01 vs. control, n = 4 in each group). **(B)**

PGE_2 (100 nM) stimulated RAW264.7 cell NFAT5 mRNA expression (**P<0.01, n = 4). All values are means ± SEM. All P values were calculated by Student's t test.

Figure S8 **COX-$2^{-/-}$-COX-$2^{-/-}$ BMT mice had increased skin Na and K content and increased water content in response to high salt intake. (A)** HS-treated COX-$2^{-/-}$-COX-$2^{-/-}$ BMT mice had higher skin sodium and potassium content, compared to HS-treated WT-WT BMT mice (*P<0.05, n = 4). **(B)** HS-treated COX-$2^{-/-}$-COX-$2^{-/-}$ BMT mice had higher skin water content, compared to HS-treated WT-WT BMT mice (*P<0.05, n = 4). All values are means ± SEM. All P values were calculated by Student's t test.

Figure S9 **Renal ENaC mRNA levels were higher in HS-treated COX-$2^{-/-}$-WT BMT and mPGES-$1^{-/-}$-WT BMT mice than in HS-treated WT-WT BMT mice.** Both ENaCβ and ENaCγ mRNA levels were significantly higher in COX-$2^{-/-}$-WT BMT and mPGES-$1^{-/-}$-WT BMT mice than in WT-WT BMT mice in response to a high salt diet. (*P<0.05, n = 5 in each group). All values are means ± SEM. All P values were calculated by Student's t test.

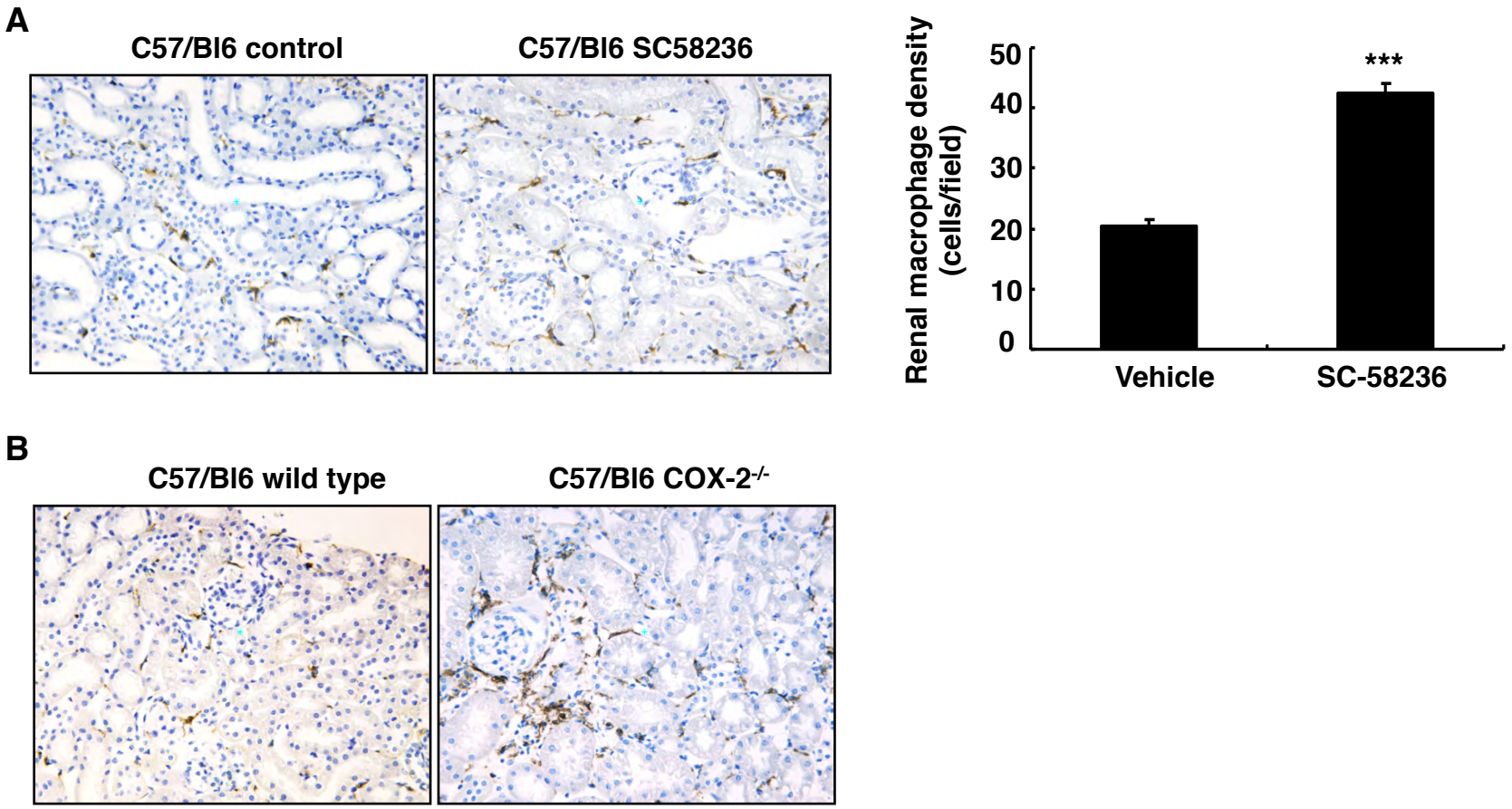
Figure S10. **Deficiency in hematopoietic cell COX-2 pathway had no effects on water and salt balance.** Trained mice were given 1 mEq of NaCl via gastric gavage, and urine was collected every 12 h for next 72 h. **(A and B)** Both urine volume and sodium excretion were comparable between WT-WT and COX-$2^{-/-}$-WT BMT mice (n = 4) (**A**) or between $EP_4^{f/f}$ mice and CD11b-Cre; $EP_4^{f/f}$ mice (n = 6) (**B**).

Figure S11 **Blood pressure was comparable between control COX-$2^{-/-}$-WT BMT and WT-WT BMT Mice measured by tail-cuff microphonic manometer or carotid catheterization** (n = 6 in each group).

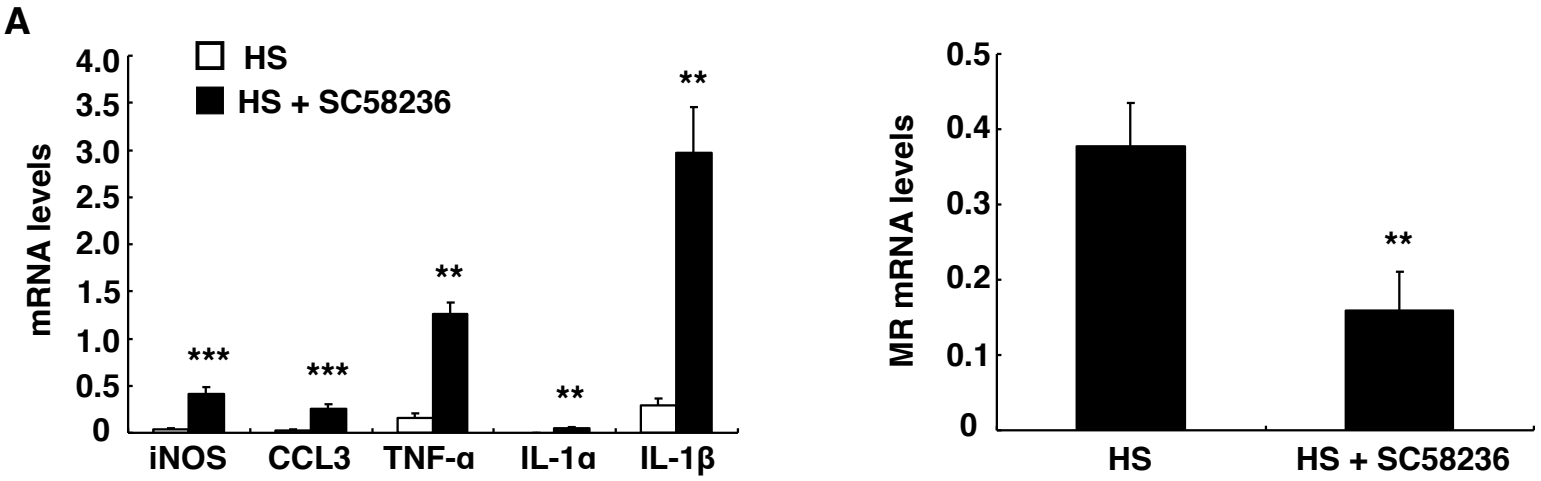
Supplemental Table 1. Renal and Hematologic Parameters After BMT

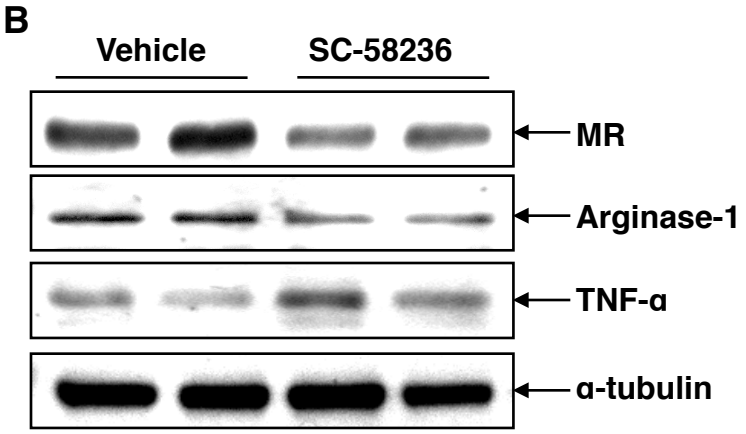
Parameters	Na (mM)	K (mM)	Cl (mM)	TCO_2 (mM)	BUN (mg/dl)	HCT (%)	Hgb (g/dl)
Control	148 ± 1	5.5 ± 0.7	115 ± 2	21 ± 1	21 ± 4	52 ± 2	17 ± 1
WT-WT	148 ± 1	5.6 ± 0.6	117 ± 2	22 ± 2	20 ± 1	50 ± 1	17 ± 1
COX-2$^{-/-}$-WT	148 ± 2	5.6 ± 1.2	116 ± 2	25 ± 3	17 ± 3	48 ± 3	16 ± 1

Six weeks after BMT, renal and hematologic parameters were measured. Data were presented as mean ± s.e.m (n = 3 in each group).

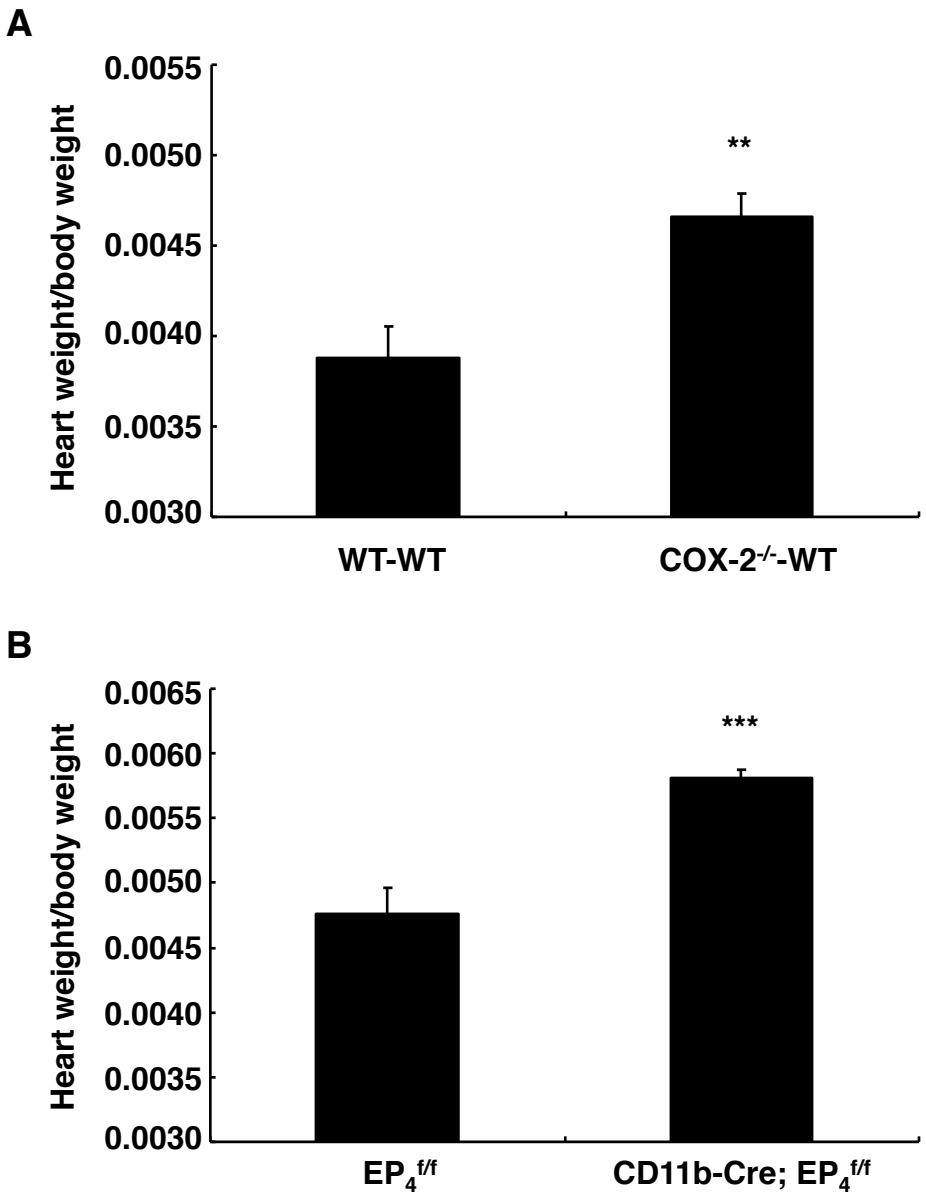


Supplemental Figure 1





Supplemental Figure 2



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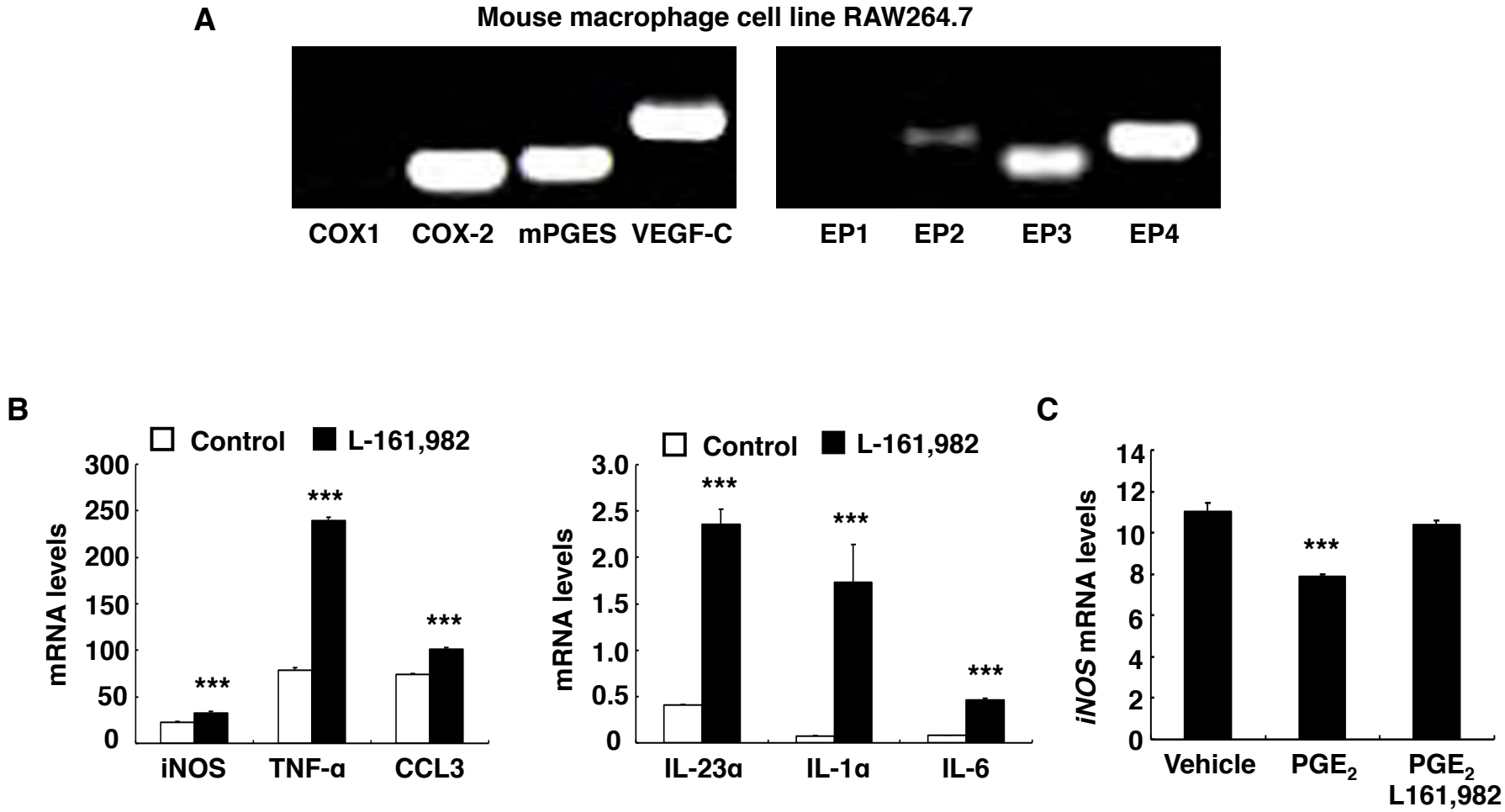

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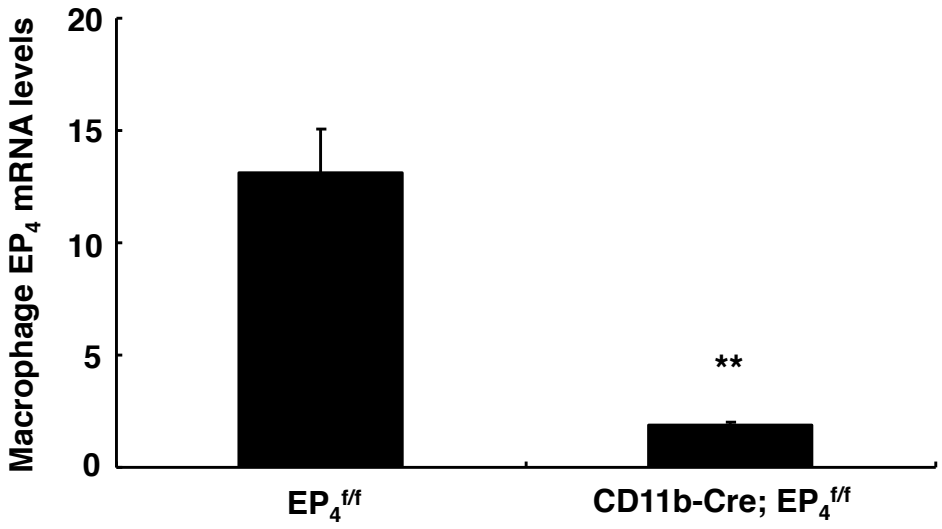

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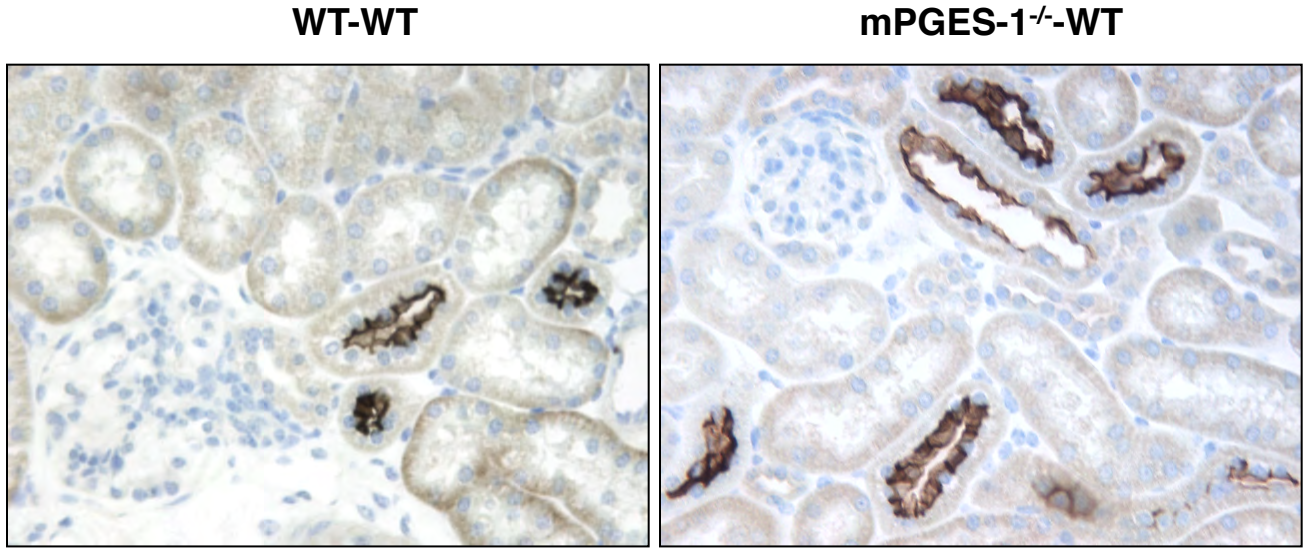
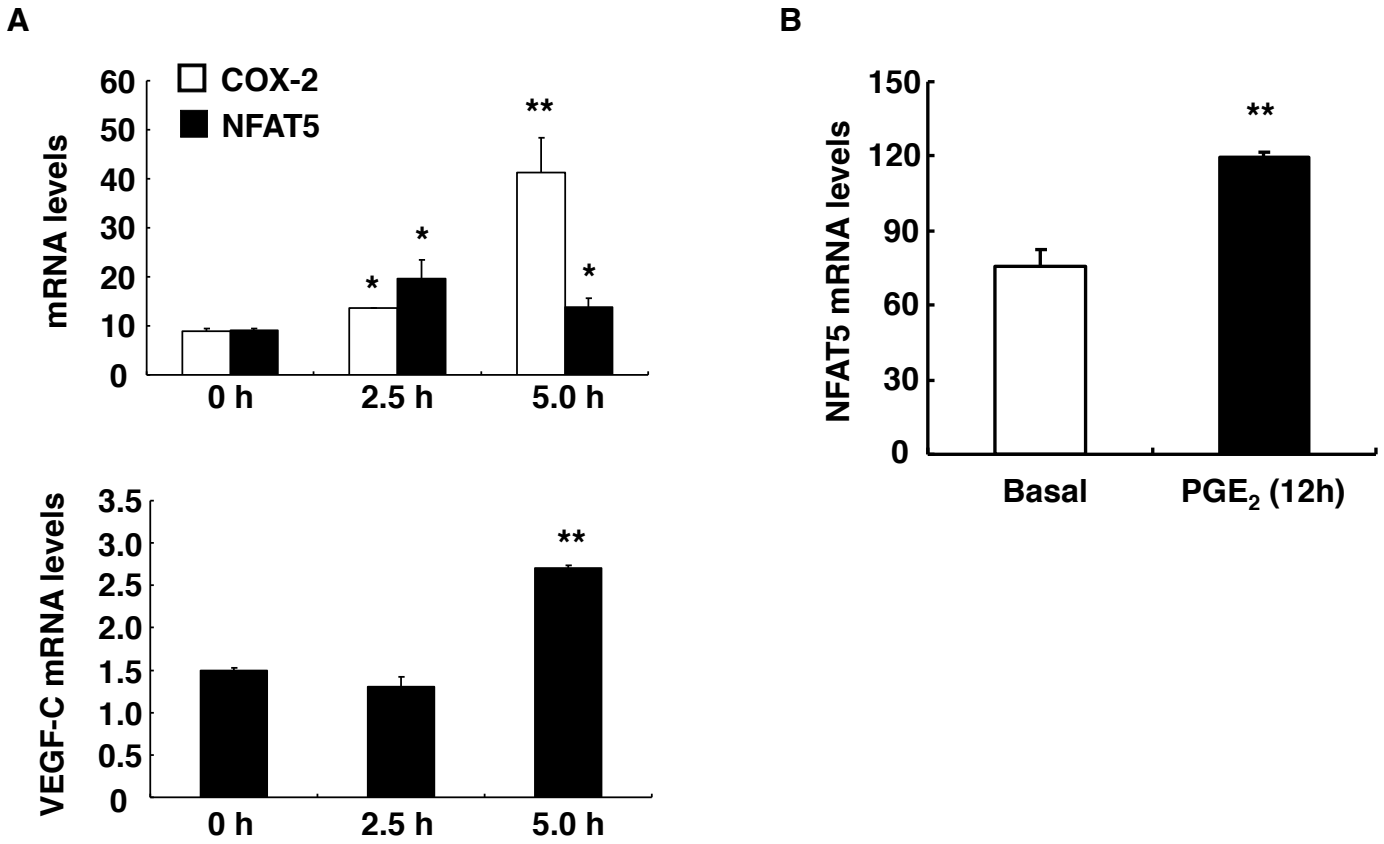


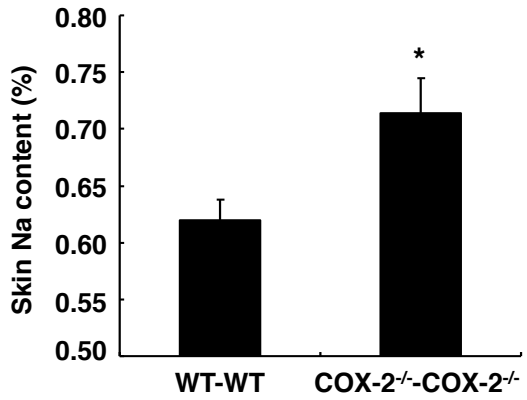
p-NCC immunostaining: original magnification: x 250.

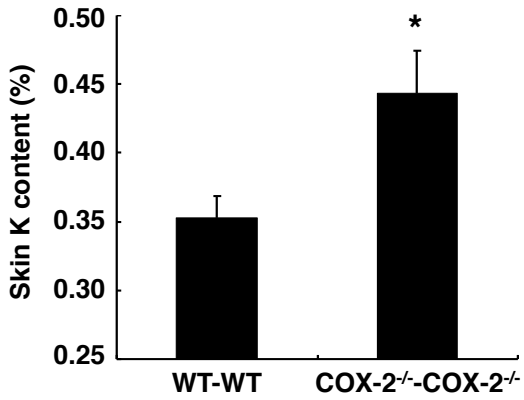
Supplemental Figure 6



Supplemental Figure 7

A





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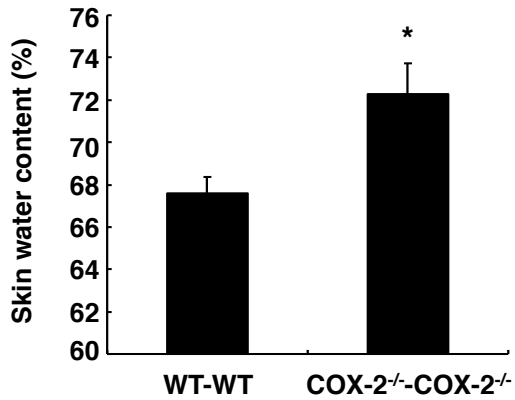
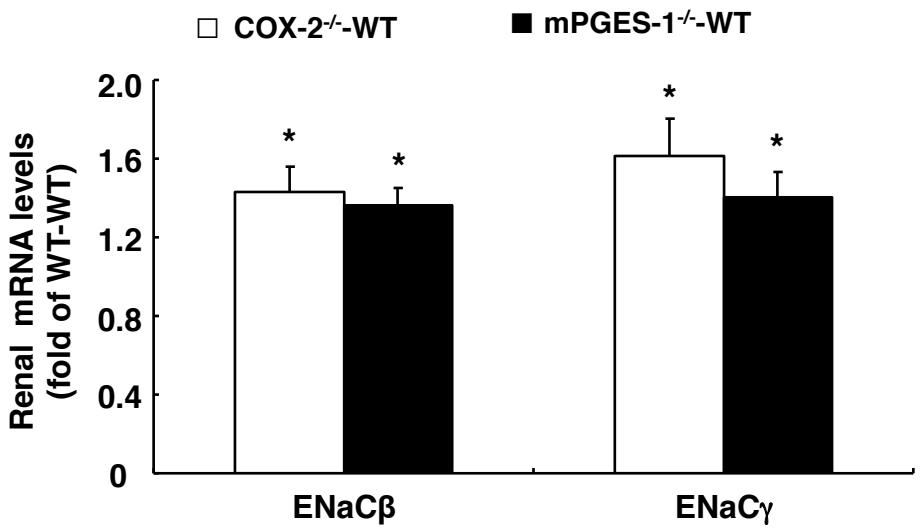


Supplemental Figure 8



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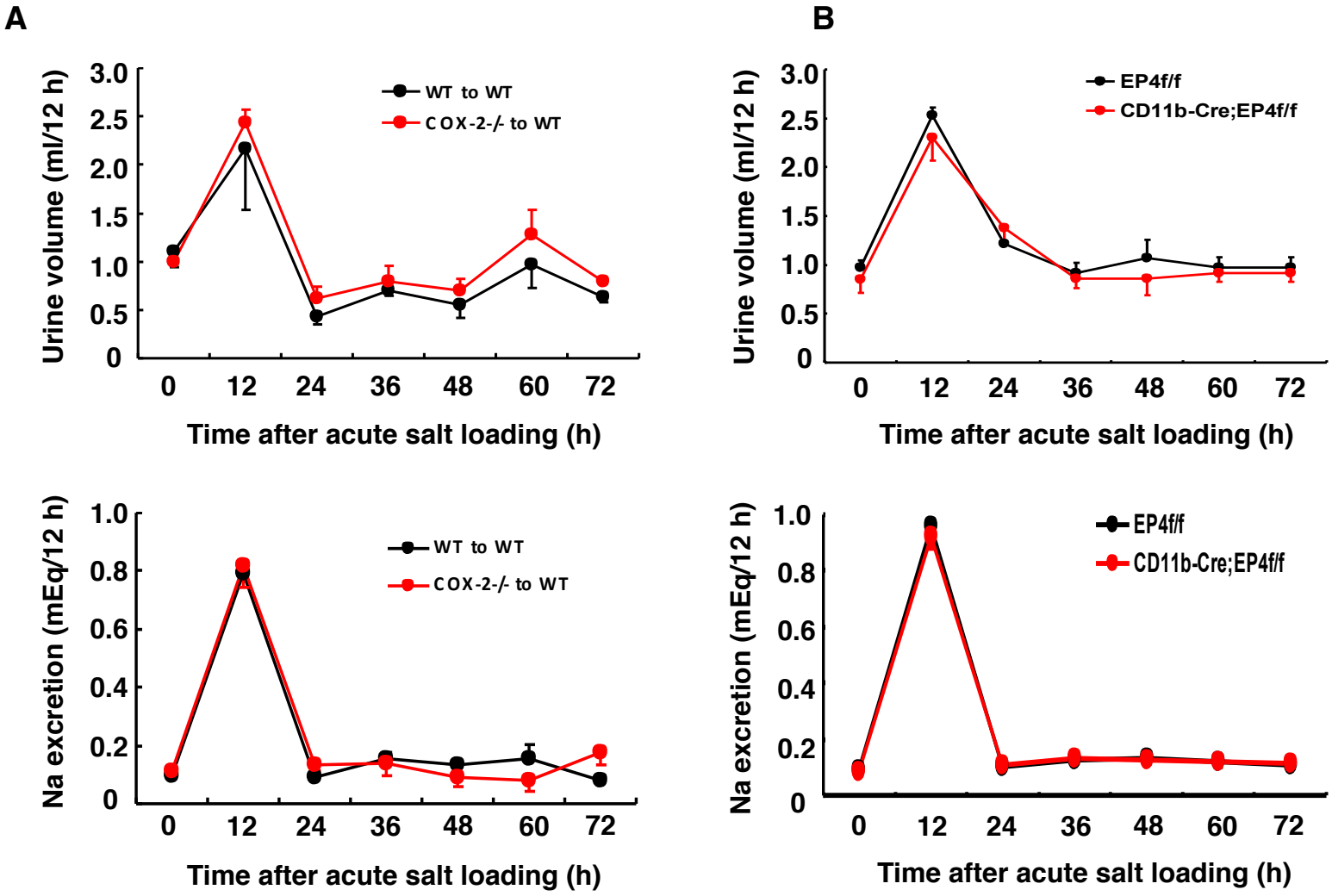
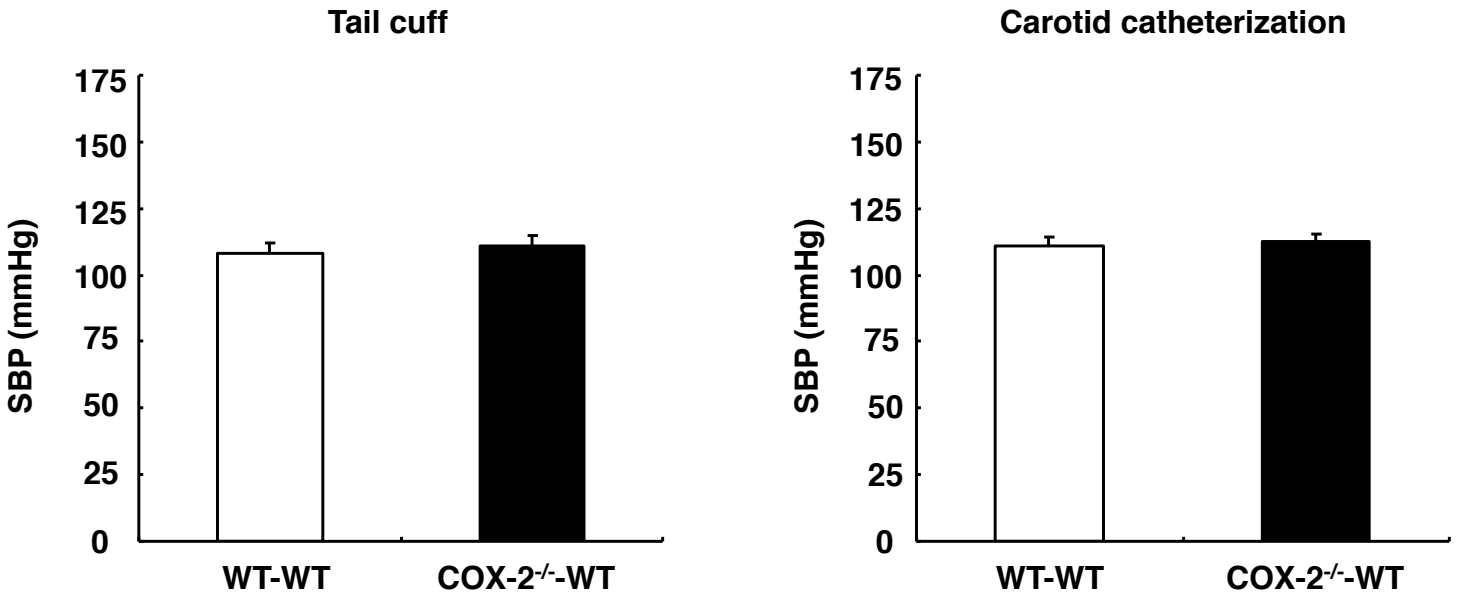


Supplemental Figure 10



Supplemental Figure 11