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; EP4^{ff} mice than in HS-treated EP4^{ff} mice (***P<0.001, n = 4). All values are means ± SEM. All P values were calculated by Student’s t test.

**Figure S4** Prostaglandin EP4 receptor tonically suppressed Th1 cytokine expression in cultured macrophages. (A) Murine macrophage RAW264.7 cells expressed COX-2, mPGES1 and VEGF-C. EP4 was the major EP receptors in RAW264.7 cells. COX-1 and EP1 receptor were undetectable. (B) Treatment of RAW264.7 cells with a selective EP4 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<0.01, n = 3). (C) PGE2 led to inhibition of iNOS expression, which was prevented by the selective EP4 receptor antagonist, L-161,982 (***P<0.001 vs. PGE2 alone, n = 4 in each group). All values are means ± SEM. All P values were calculated by Student’s t test.

**Figure S5** Macrophage EP4 receptor was effectively deleted in CD11b-Cre; EP4^{ff} mice. Peritoneal macrophages were isolated and EP4 mRNA was quantitated by qPCR. Macrophage EP4 mRNA levels were significantly lower in CD11b-Cre; EP4^{ff} mice than in EP4^{ff} 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 ENaC$\beta$ and ENaC$\gamma$ 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$_4^{ff}$ mice and CD11b-Cre; EP$_4^{ff}$ 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**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Na (mM)</th>
<th>K (mM)</th>
<th>Cl (mM)</th>
<th>TCO₂ (mM)</th>
<th>BUN (mg/dl)</th>
<th>HCT (%)</th>
<th>Hgb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>148 ± 1</td>
<td>5.5 ± 0.7</td>
<td>115 ± 2</td>
<td>21 ± 1</td>
<td>21 ± 4</td>
<td>52 ± 2</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>WT-WT</td>
<td>148 ± 1</td>
<td>5.6 ± 0.6</td>
<td>117 ± 2</td>
<td>22 ± 2</td>
<td>20 ± 1</td>
<td>50 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>COX-2⁻/⁻-WT</td>
<td>148 ± 2</td>
<td>5.6 ± 1.2</td>
<td>116 ± 2</td>
<td>25 ± 3</td>
<td>17 ± 3</td>
<td>48 ± 3</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

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

A

C57/Bl6 control
C57/Bl6 SC58236

B

C57/Bl6 wild type
C57/Bl6 COX-2−/−

Renal macrophage density (cells/field)

Vehicle SC-58236

***
Supplemental Figure 2
Supplemental Figure 3

A

Heart weight/body weight

WT-WT

COX-2^{−/−}-WT

B

Heart weight/body weight

EP_{4}^{fl}

CD11b-Cre; EP_{4}^{fl}

**

***
Supplemental Figure 4
Supplemental Figure 5
WT-WT  mPGES-1^{-/-}-WT

p-NCC immunostaining: original magnification: x 250.

Supplemental Figure 6
Supplemental Figure 7
Supplemental Figure 8

**A**

Skin Na content (%)

- WT-WT
- COX-2<sup>−/−</sup>-COX-2<sup>−/−</sup>

Skin K content (%)

- WT-WT
- COX-2<sup>−/−</sup>-COX-2<sup>−/−</sup>

**B**

Skin water content (%)

- WT-WT
- COX-2<sup>−/−</sup>-COX-2<sup>−/−</sup>
Supplemental Figure 9

Renal mRNA levels (fold of WT-WT)

- **COX-2^-/-WT**
- **mPGES-1^-/-WT**

ENaCβ

ENaCγ

* * *
Supplemental Figure 10
Supplemental Figure 11