Microsomal Prostaglandin E Synthase 1 Deletion Retards Renal Disease Progression But Exacerbates Anemia in Mice With Renal Mass Reduction

Zhanjun Jia, Haiping Wang, Tianxin Yang

See Editorial Commentary, pp 12–13

Abstract—Microsomal prostaglandin E synthase 1 (mPGES-1) is a cytokine-inducible enzyme responsible for generation of prostaglandin E₂ (PGE₂) during the inflammatory response. In the present study, we investigated the role of mPGES-1 in the development of chronic renal failure in mice with 5/6 nephrectomy (Nx). After 4 weeks of Nx, wild-type mice with renal mass reduction exhibited increased blood urea nitrogen, plasma creatinine and phosphorus concentrations, and defective urine concentrating capability, all of which were significantly attenuated by mPGES-1 deletion. The Nx wild-type mice developed a 2.6-fold increase in urinary albumin excretion, accompanied by glomerulosclerosis and reduction of nephrin and wild-type 1 expression in the remnant kidney. In contrast, the Nx KO mice had normal albuminuria with improvement of glomerular injury. Nx-induced increases in circulating and renal tumor necrosis factor 1α and renal interleukin 1β and monocyte chemoattractant protein 1 expressions were all remarkably attenuated or abolished by mPGES-1 deletion. Paradoxically, the Nx knockout mice developed worsened anemia, accompanied by impaired erythropoietin synthesis. The coinduction of mPGES-1 and cyclooxygenase 2 but not cyclooxygenase 1 mRNA expressions, along with increased PGE₂ synthesis, was demonstrated in the remnant kidney of wild-type mice. 

mPGES-1 deletion remarkably reduced renal PGE₂ content and urinary PGE₂ excretion after renal ablation but had a limited effect on the baseline PGE₂ production. We conclude that mPGES-1 deletion ameliorates chronic renal failure in the mouse model of renal mass reduction, and mPGES-1 deletion paradoxically exacerbates anemia in this model likely via suppression of erythropoietin synthesis. (Hypertension. 2012;59:122-128.) ● Online Data Supplement

Key Words: prostaglandin E₂ ▪ chronic renal failure ▪ microsomal prostaglandin E synthase 1 ▪ cyclooxygenase 2 ▪ proteinuria

Chronic kidney disease (CKD) is a progressive loss of renal function, eventuating in renal replacement therapy—dialysis or kidney transplantation.¹ The number of patients experiencing CKD is growing, as reflected by the increasing cases of renal replacement therapy. There are 26 million American adults with CKD, and millions of others are at increased risk. Unfortunately, there is no specific treatment unequivocally shown to slow the progression of CKD. Renal mass reduction in the rodents leads to systemic hypertension, chronic renal failure, proteinuria, and glomerulosclerosis, mimicking the major features of human CKD. This model in recent years contributes to the elucidation of the pathophysiology and the development of new therapies for this disease.

Prostaglandin E₂ (PGE₂) is a major product of arachidonic acid metabolism that is sequentially catalyzed by cyclooxygenase (COX) and prostaglandin E synthase (PGES). PGE₂ is a pleiotropic hormone involved in multiple physiopathological processes, particularly in the inflammatory response. In recent years, the investigation of PGE₂ function has been facilitated by the identification of PGES. To date, ≥3 major forms of PGES have been cloned and characterized: membrane-associated PGES (mPGES) 1, mPGES-2, and cytosolic PGES, with mPGES-1 being the best-characterized PGES.²³ Like COX-2, mPGES-1 expression in inflammatory cells is highly inducible in response to proinflammatory stimuli. Moreover, mPGES-1–deficient mice exhibit blunted pain and inflammatory responses.⁴ In light of the proinflammatory properties of mPGES-1, this enzyme has been viably viewed as a alternative target for the development of analgesics.⁵ Emerging evidence supports a potential physiological role of mPGES-1 in renal control of fluid metabolism and blood pressure.⁶⁻⁷ However, there is scant information regarding a possible role for mPGES-1 in renal inflammatory diseases. The goal of
the present study was to examine the role of mPGES-1 in a mouse model of chronic renal failure (CRF).

Methods

Animals

mPGES-1 null mice were originally generated by Trebino et al. This mouse colony was propagated at the University of Utah and maintained on a mixed DBA/1lacJ × C57/B6 × 129/SV background. The −/− mice were bred using homozygous females to homozygous males. Nonlittermate wild-type (WT) mice, which were derived from the same mPGES-1 colony after ~20 intercrosses, were used as controls. The use of nonlittermate controls was because of the ease of breeding and maintaining the colonies. Male mice (3–4 months old) were used for all of the experiments. All of the protocols using mice were conducted in accordance with the principles and guidance of the University of Utah Institutional Animal Care and Use Committee.

Generation of the Nephrectomy Mouse Model

Under the isoflurane anesthesia, mPGES-1 +/- and −/− mice underwent a surgery to remove five sixths of total renal mass by resecting the right kidney and cauterizing the upper and lower poles of the left kidney, as described previously. The sham-operated animals were used as controls.

Specific Methods

The methods of measurements of blood pressure and hematocrit (Hct), histological analysis, quantitative RT-PCR (qRT-PCR), immunoblotting, enzyme immunoassay, and statistical analysis are shown in the online Data Supplement at http://hyper.ahajournals.org.

Results

Assessment of Renal Function

mPGES-1 WT and knockout (KO) mice were subjected to 5/6 nephrectomy (Nx) (termed “Nx WT” and “Nx KO,” respectively). Renal function was significantly deteriorated in both groups 4 weeks after Nx. The Nx WT mice exhibited renal dysfunction as evidenced by parallel increases in blood urea nitrogen, plasma creatinine, and decreased creatinine clearance, as compared with the sham control group (Figure 1A through 1C). As compared with the Nx WT mice, the indices of renal dysfunction were significantly attenuated in the Nx KO mice (Figure 1A through 1C). Similar results were obtained for plasma phosphorus concentration, another index of renal dysfunction (Figure S1A, available in the online Data Supplement). Plasma calcium concentration remained constant among all of the groups (data not shown). In parallel with the overall improvement of renal function, body weight loss was also less in the Nx KO mice (Figure S1B). Urinary albumin excretion was increased 2.6-fold in the Nx WT mice, and this increase was completely blocked in the Nx KO mice (Figure 1D).

Assessment of Glomerular Damage

Considering the important role of the podocyte in the development of albuminuria in various forms of CKD, we assessed renal expression of podocyte markers, including the Wilms tumor 1 gene (WT1) and nephrin, by qRT-PCR. The Nx WT mice exhibited a marked reduction of mRNA expressions of both WT1 and nephrin in the remnant kidney, a sign of podocyte injury (Figure 2A and 2B). In contrast, the reduction of both podocyte markers was completely prevented in the Nx KO mice (Figure 2A and 2B). The changes in WT1 expression were confirmed by immunohistochemistry (Figure 2C). The podocyte number as assessed by counting WT1-positive cells was significantly reduced in the Nx WT mice but was nearly normalized in the Nx KO mice (Figure 2D).

The remnant kidney weight was not different between the genotypes (Figure 3A and 3B). The glomerular morphology was evaluated by periodic acid-Schiff staining and quantified by the semiquantitative scoring. The Nx WT mice had increased matrix deposition in the glomerulus, a sign of glomerulosclerosis, which was less in the Nx KO mice (Figure 3C and 3D).
Assessment of Urine Concentrating Capability and Blood Pressure

Disturbance of fluid metabolism is an important feature of CRF. We, therefore, performed metabolic studies to evaluate the status of fluid metabolism in the 2 genotypes after renal ablation. Significant increases in water intake and urine output were observed in the Nx WT mice, and these increases were less in the Nx KO mice (Figure S2A and S2B). Water balance estimated by subtracting water intake by urine output was not different between the 2 genotypes (Figure S2C). We then measured urine osmolality as an index of urine concentrating capability. A significant decrease in urine osmolality was observed in the Nx WT mice, and this decrease was partially attenuated in the Nx KO mice (Figure S2D). These results suggest improvement of urine concentrating capability by mPGES-1 deletion after ablation. The levels of key Na\(^+/\)/H\(^+/\) and water transporters, such as aquaporin-2 (AQP2), and Na\(^+/\)/K\(^+/\)/2Cl cotransporter, are an important determinant of urine concentrating capability. We, therefore, examined the changes of expression of key water and Na\(^+/\)/H\(^+/\) transporters in the remnant kidney. AQP2 mRNA and protein levels were upregulated in parallel in the remnant kidney, and there was no difference between the genotypes (Figure S3A and S3B). A similar pattern was observed for AQP1 mRNA (Figure S3C). Interestingly, increases in AQP3 and Na\(^+/\)/K\(^+/\)/2Cl cotransporter mRNA levels in the remnant kidney were observed in the KO but not the WT mice (Figure S3D and S3E). Renal mass ablation produced a modest increase in systolic blood pressure, as determined by tail-cuff plethysmography, and there was no difference between the genotypes (Figure S4).

![Figure 2. Assessment of expression of podocyte markers in microsomal prostaglandin E synthase 1 (mPGES-1) +/- and +/- mice after renal ablation. mRNA expressions of Wilms tumor 1 gene (WT1; A) and nephrin (B) were determined by quantitative RT-PCR and normalized by 18S rRNA. WT1 positive cells as evaluated by immunostaining were used to reflect the number of podocytes (C, representative micrographs of WT1 immunostaining; D, the number of WT1 positive cells per glomerulus). N=6 to 9 per group. Data are mean±SE.](image)

![Figure 3. Kidney morphological analysis. A, The gross appearance of representative remnant kidneys in microsomal prostaglandin E synthase 1 (mPGES-1) +/- and +/- mice. B, The remnant kidney weight between the genotypes. C, Representative micrographs of periodic acid-Schiff–stained paraffin sections. D, Glomerulosclerosis index. N=6 to 9 per group. Data are mean±SE.](image)
Assessment of Inflammation
In light of the well-established role of mPGES-1 in the inflammatory response, we tested whether mPGES-1 deletion affected the expression of proinflammatory cytokines or chemokines, such as tumor necrosis factor (TNF)-α, interleukin 1β, and monocyte chemoattractant protein 1 in the remnant kidney. ELISA detected a significant increase in TNF-α protein expression in the remnant kidney of WT mice as compared with the sham control. This increase was completely abolished in the Nx KO mice (Figure S5A).

Similarly, qRT-PCR demonstrated parallel increases in mRNA expression of TNF-α, interleukin-1β, and monocyte chemoattractant protein 1 in the remnant kidney of WT mice as compared with the sham control; the increases of the proinflammatory indices were all completely blocked or significantly suppressed in the absence of mPGES-1 (Figure S5B through S5D).

Assessment of Anemia
Anemia is an important symptom of CRF. Therefore, we assessed the extent of anemia by examining Hct and spleen weight. The Nx WT mice exhibited small but significant decreases in Hct and increases in spleen weight (Figure 4A and 4B), suggesting modest anemia. An accelerated anemia was demonstrated in the Nx KO mice as compared to WT mice, as evidenced by a greater fall in Hct and more severe splenomegaly (Figure 4A and 4B). To gain insight into this observation, we analyzed erythropoietin (EPO) mRNA expression in the remnant kidney by qRT-PCR and plasma EPO concentration by ELISA. EPO mRNA in the remnant kidney of WT mice increased 19-fold, which was reduced by 50% in the absence of mPGES-1 (Figure 4C). Plasma EPO exhibited a similar pattern of changes, except for a trend of difference between the genotypes (Figure 4D).

The Activation of mPGES-1 in the Remnant Kidney
To gain further evidence for involvement of mPGES-1 in kidney disease progression, we tested whether mPGES-1 expression or activity was stimulated in the remnant kidney. Despite the significant reduction of the kidney mass, the Nx WT mice generated PGE2 output almost comparable to the sham control group (Figure S6A), suggesting increased PGE2 synthesis after renal ablation. Indeed, PGE2 content was increased 3-fold in the remnant kidney of WT mice as compared with the sham control (Figure S6B). mPGES-1 deletion led to a 37.0% and 67.8% reduction of urinary PGE2 excretion at baseline and after ablation, respectively, and a 77.6% reduction of PGE2 content in the remnant kidney (Figure S6A and S6B). In light of the potential role of thromboxane A2 (TXA2) in the pathogenesis of CKD, we determined the level of thromboxane B2, a stable product of TXA2, in the remnant kidney. Unexpectedly, thromboxane B2 level tended to be lower than the sham group irrespective of the genotype (Figure S6C). As expected, however, thromboxane B2 level was unaffected by mPGES-1 deletion (Figure S6C). qRT-PCR detected parallel increases in COX-2 and mPGES-1 mRNA expressions in the remnant kidney contrasting to unaltered COX-1 expression (Figure S6D through S6F).

Discussion
A large body of evidence consistently demonstrates a pathogenic role of COX-2 in mediating kidney injury in animal models of 5/6 Nx, as well as other glomerular diseases.11–14 COX-2 activity generates 5 biologically active prostanoids, including PGE2, prostaglandin D2, prostaglandin I2, TXA2, and prostaglandin F2α. The contribution of a specific prostanooid to COX-2–mediated kidney injury still remains to be
determined. The availability of mPGES-1 null mice offers a novel tool to assess the involvement of PGE₂ in progressive kidney disease in vivo. We demonstrated that mPGES-1 deletion almost abolished proteinuria and also remarkably improved renal dysfunction induced by renal ablation, similar to COX-2 inhibition. Moreover, mPGES-1 expression was induced in parallel with COX-2 in the remnant kidney. These results strongly suggest a specific coupling between COX-2 and mPGES-1 in mediating kidney injury after renal ablation. Consistent with the present study, Stitt-Cavanagh et al.¹⁰ elegantly demonstrate a maladaptive role of the EP4 receptor in podocyte injury by analyzing the phenotype of mice with either podocyte-specific overexpression or deletion of this EP subtype after renal ablation. However, podocyte-specific EP4 deletion produces a partial attenuation of proteinuria without an effect of WT1 expression, contrasting to a more robust effect of mPGES-1 deletion on these parameters. The difference in the magnitude of changes in proteinuria and podocyte marker expression with the 2 different approaches may suggest involvement of other EP subtypes as well. In support of this possibility, Bek et al.¹⁵ demonstrate that cultured podocytes express the EP1 receptors that may be responsible for PGE₂-elicited intracellular calcium response. Other than PGE₂, TXA₂ is shown to mediate kidney injury induced by renal ablation with variable reports. For example, treatment with 2 distinct thromboxane synthase inhibitors, OKY1581¹⁶ and FCE 22178,¹⁷ decrease the excessive renal TXA₂ synthesis, reduce proteinuria, and ameliorate the renal disease progression and hypertension in rats with subtotal renal mass reduction. However, treatment with the selective TXA₂ receptor antagonist GR 32191 in the renal ablation model fails to influence progressive kidney disease.¹⁸ Interestingly, thromboxane receptor deletion but not podocyte-specific EP4 KO in COX-2–expressing mice reduces proteinuria and improves renal pathology in a mouse model of Adriamycin-induced kidney disease.¹⁹ It seems possible that COX-2 may differentially couple with thromboxane or PGE₂/EP4 to mediate renal disease progression, depending on the type of injurious insults.

Chronic renal insufficiency is often associated with polyuria as a result of impaired urine concentrating capability. We demonstrated that mPGES-1 deletion significantly improved renal ablation-induced impairment of urine-concentrating capability, as evidenced by reduced water intake and urine volume, and elevated urine osmolality. Our results agree with the study of Sanchez et al.¹⁸ who report improvement of urine-concentrating capability in the subtotal Nx rats after treatment with COX-2 inhibitors. The mechanism by which prostanoid synthesis inhibition improves urine-concentrating capability after renal ablation is unclear. The similar compensatory upregulation of AQP2 expression in the remnant kidney between mPGES-1 WT and KO mice has ruled out a primary role of AQP2. On the other hand, AQP3 and Na⁺-K⁺-2Cl cotransporter mRNA expressions in the remnant kidney of mPGES-1 KO but not WT mice were significantly elevated as compared with their sham controls. It seems possible that the upregulation of renal AQP3 and Na⁺-K⁺-2Cl cotransporter expression may in part account for the improved urine-concentrating capability in mPGES-1 KO mice with renal mass reduction.

Inflammation plays an important role in progressive kidney disease in both human and animal models of renal failure.²⁰,²¹ This is particularly evidenced by the increased levels of proinflammatory cytokines, such as TNF-α, in patients with CKD²² and in 5/6 nephrectomized rats.²³ TNF-α is also responsible for production of other cytokines and chemokines, such as interleukin 1β; regulated upon activation, normal T-cell expressed, and secreted; macrophage inflammatory protein 2; monocyte chemoattractant protein 1; and TGF-β1, leading to inflammatory injury in the kidney. Consistent with previous reports,²³ we observed significant increases of circulating TNF-α and renal TNF-α mRNA in WT mice after renal ablation. In contrast, the increases of TNF-α, along with interleukin 1β and monocyte chemoattractant protein 1, were significantly ameliorated or completely abolished by mPGES-1 deletion. These results are compatible with the well-known proinflammatory properties of mPGES-1. It seems conceivable that mPGES-1 may influence progressive kidney disease via mediating inflammation. Another explanation is that mPGES-1–derived PGE₂ may mediate hyperfiltration in the remnant nephron, a maladaptive response to renal mass reduction.²⁴ This possibility is suggested by the observation that prostaglandin synthesis inhibition nearly normalized glomerular and tubular function in remnant nephrons but did not affect function of control nephrons.²⁵ However, because the remnant kidney weight was not different between the genotypes, our results do not support a role for mPGES-1 in mediating the structural hypertrophy induced by renal ablation. Assuming that the structural hypertrophy is the consequence of the functional hypertrophy, one can speculate that mPGES-1 may not be a critical determinant of hyperfiltration in the remnant nephron. Functionally, in the present study, mPGES-1 deletion leads to an increase but not a decrease of glomerular filtration rate, again arguing against the role of mPGES-1–derived PGE₂ in mediating hyperfiltration induced by renal ablation. This finding reinforces the pathogenic role of PGE₂ in ablation-induced renal injury.

In support of the pathogenic role of mPGES-1 in kidney injury induced by renal ablation, mPGES-1 expression in the remnant kidney is induced in parallel with COX-2 but not COX-1, supporting a specific coupling between mPGES-1 and COX-2 in progressive kidney disease. Despite the 75% renal mass reduction, urinary PGE₂ excretion in nephrectomized mice was comparable to that in the sham group, suggesting increased PGE₂ synthesis in the remnant kidney. This was subsequently confirmed by measurement of tissue PGE₂ content. mPGES-1 deletion produces only a partial attenuation of urinary and renal PGE₂ levels at basal condition but remarkably reduces these parameters after renal ablation. These results strongly suggest that mPGES-1 becomes a dominant contributor of renal PGE₂ synthesis in the remnant kidney.

In contrast to the overall improvement of renal function in nephrectomized mPGES-1 KO mice, these animals exhibited exacerbated anemia as evidenced by a greater fall of Hct and more severe splenomegaly. In light of the critical role of EPO in regulation of erythropoiesis, we examined renal and circulating EPO levels. Increased EPO levels were demon-
strated after renal ablation, possibly reflecting a compensatory response to anemia; the increases were significantly ameliorated by mPGES-1 deletion. Together, these results support an important role of mPGES-1–derived PGE$_2$ in pathological regulation of erythropoiesis after renal ablation. Consistent with this notion, a significant number of in vitro and in vivo studies report a stimulatory effect of PGE$_2$ on erythropoiesis and/or EPO synthesis. Thus, the impaired EPO synthesis may in part account for the worsened anemia associated with CRF in the absence of mPGES-1. An alternative explanation is that PGE$_2$ may act directly on the bone marrow independent of an effect on EPO production. In support of this possibility, COX-2 KO mice are shown to exhibit enhanced anemia associated with decreased bone marrow cell counts and reduced numbers of erythroid and colony-forming cells in the absence of changes in EPO levels. However, our results disagree with the study of Zhang et al, who report suppressed EPO synthesis in a mouse model of subtotal Nx-induced CRF. This study is limited in that the analysis of EPO expression is solely dependent on conventional RT-PCR, which is not a quantitative method. The discrepancy may also arise from the differences in the stages of renal failure or genetic background. It has been reported that the EPO synthesis response to hemodilution is well preserved in mild CRF but was impaired in advanced CRF. Indeed, the plasma EPO level is not low or even increased in the patients with mild or modest CRF.

In summary, mPGES-1 deletion improves renal function, urine concentrating capability, and albuminuria, accompanied by a marked attenuation of renal inflammation, in an Nx mouse model of CRF. Paradoxically, the null mice exhibit worsened anemia and suppressed EPO synthesis after renal mass reduction. These results suggest a complex role of mPGES-1–derived PGE$_2$ in different pathological processes in progressive kidney disease.

**Perspectives**

Inflammation plays a major role in the pathogenesis of CKD. The present study for the first time demonstrates that mPGES-1 contributes to kidney disease progression, likely because of its proinflammatory properties. Conversely, this enzyme partially mediates the compensatory EPO synthesis, thereby mitigating the extent of anemia. These results suggest that mPGES-1 inhibition may offer a new therapeutic intervention for CKD, but caution should be paid to worsened anemia. On the other hand, the adverse effect of mPGES-1 inhibition may indicate erythropoiesis-stimulating potential of PGE$_2$ that may have a therapeutic implication for anemia management for patients with or without CRF.

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**Disclosures**

None.

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Running Head: mPGES-1 and chronic renal failure

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Methods

Measurement blood pressure. Systolic blood pressure was measured by tail-cuff plethysmography using a Visitech BP2000 Blood Pressure Analysis System (Apex, NC). All animals were habituated to the blood pressure measurement device for 7 days. They all underwent two cycles of 20 measurements reordered per day for a minimum of three days.

Measurement of hematocrit (Hct). The sphenous vein was punctured using a #23 gauge needle and one drop of blood (~5-10 µl) was collected using a 10 µl capillary glass (Idaho Technology). One side of the tube was sealed with Hemato-Seal and then centrifuged for 4 minutes in a Thermo IEC microcentrifuge machine.

Histology and immunohistochemical staining. Under anesthesia, kidneys are removed and fixed with 4% paraformaldehyde. The tissues were subsequently embedded in paraffin and 3-µm sections were cut and stained with periodic acid Schiff (PAS). The scoring of mesangial matrix accumulation and sclerosis of glomerular tuft was performed on a 0-4 scale with 0 indicating normal glomerulus and 4 global sclerosis >70%, as reported previously. Immunohistochemical staining was performed using a routine protocol. Anti-WT1 antibody was purchased from Dako (Mob437, Dako).

qRT-PCR. Total RNA isolation and reverse transcription were performed as previously described. Oligonucleotides were designed using Primer3 software (available at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). qPCR amplification was performed using the SYBR Green Master Mix (Applied Biosystems) and the Prism 7500 Real time PCR Detection System (Applied Biosystems). Cycling conditions were 95°C for 10 min followed by 40 repeats of 95°C for 15 s and 60°C for 1 min.

Immunoblotting. Renal tissue were lysed and subsequently sonicated in PBS that contained 1% Triton x-100, 250 µM phenylmethanesulfonyl fluoride (PMSF), 2 mM EDTA, and 5 mM dithiothrietol (DTT) (pH 7.5). Protein concentrations were determined by the use of Coomassie reagent. 40 µg of protein for each sample was denatured in boiling water for 10 min, then separated by SDS-PAGE, and transferred onto nitrocellulose membranes. The blots were blocked overnight with 5% nonfat dry milk in Tris-buffered saline (TBS), followed by incubation for 1 h with anti-AQP2 antibody (gift from Mark A. Knepper, NIH/NHLBI). After washing with TBS, blots were incubated with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody and visualized using Enhanced Chemiluminescence (ECL).

Enzyme immunoassay. Enzyme immunoassay was performed to measure the concentrations of PGE₂ (Cayman Chemicals, Ann Arbor, MI), albumin (EXOCELL), EPO (Biomerica), and TNF-α (BD Bioscience,) in the biological fluid or tissue homogenates according to the manufacturers’ instructions.

Statistical analysis. All values are presented as mean ± SE. Statistical significance was assessed by unpaired Student’s t test for single comparisons or analysis of variance (ANOVA) with the Bonferroni correction for multiple comparisons. Differences were considered to be significant when the P value was less than 0.05.
References


Fig. S1.  Plasma phosphorus concentration (A) and body weight loss (B) in mPGES-1 +/+ and +/- mice after renal ablation. N = 6-9 per group.
Fig. S2. Fluid metabolism in mPGES-1 +/+ and -/- mice after renal ablation. (A) Water intake. (B) Urine volume. (C) Water balance estimated by the difference between water intake and urine volume. (D) Urine osmolality. N = 6-9 per group.
Fig. S3. Assessment of renal expression of key water and Na+ transporter proteins in mPGES-1 +/+ and -/- mice after renal ablation. AQP2 expression was determined by qRT-PCR (A) and immunoblotting (B). AQP2 protein was detected as 35-50 kDa and 29 kDa bands. Densitometric values were shown underneath the immunoblot. *, P <0.05 vs. sham. The expression of AQP1 (C), AQP3 (D), and NKCC2 (E) were determined by qRT-PCR. N = 4-9 per group. Data are mean ± SE.
Fig. S4. Systolic blood pressure (SBP) in mPGES-1 +/+ and +/- mice after renal ablation. SBP was measured by using tail-cuff plethysmography. N = 6-9 per group.
Fig. S5

The levels of pro-inflammatory cytokines in mPGES-1 +/+ and +/- mice after renal ablation. Renal TNF-content was determined by immunoblotting (A) and renal mRNA expressions of TNF-α (B), IL-1β (C), and MCP1 (D) by qRT-PCR. N = 6-9 per group.
Fig. S6. Regulation of PGE2 and thromboxane B2 production and prostaglandin synthesis enzymes by renal mass reduction. Urinary PGE2 excretion (A) and renal PGE2 content (B) and renal thromboxane B2 (TXB2) content (C) in sham and nephrectomized mPGES-1 +/+ and -/- mice were measured by ELISA. Renal mRNA expressions of mPGES-1 (D), COX-2 (E), and COX-1 (F) in sham and nephrectomized mPGES-1 +/- mice were determined by qRT-PCR. N = 6-9 per group.