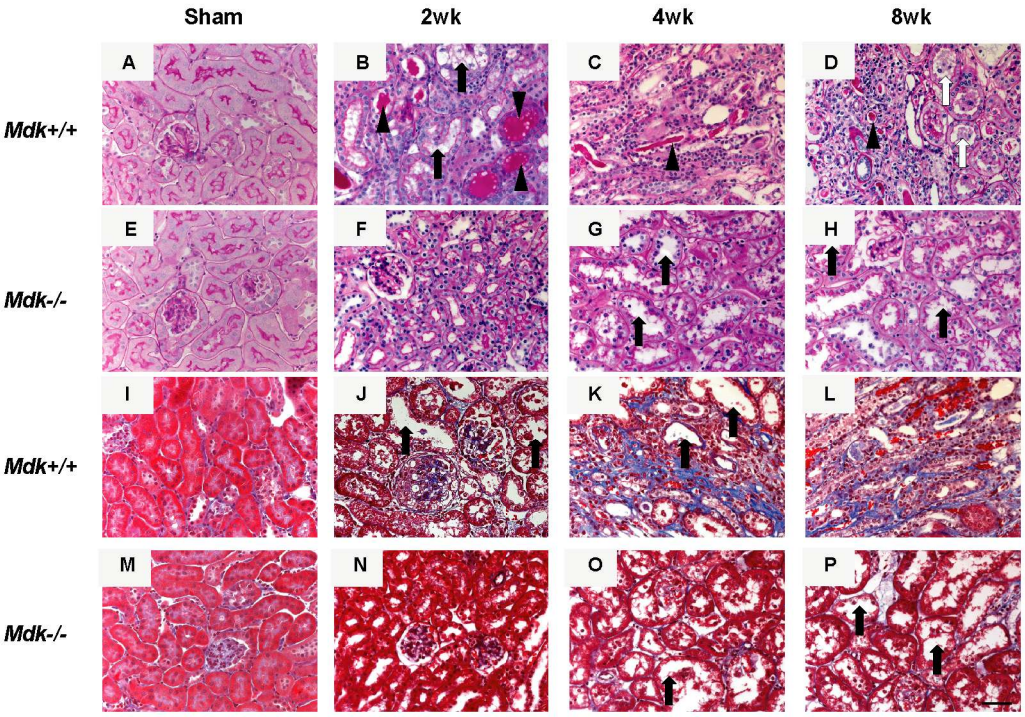
Figure S1.

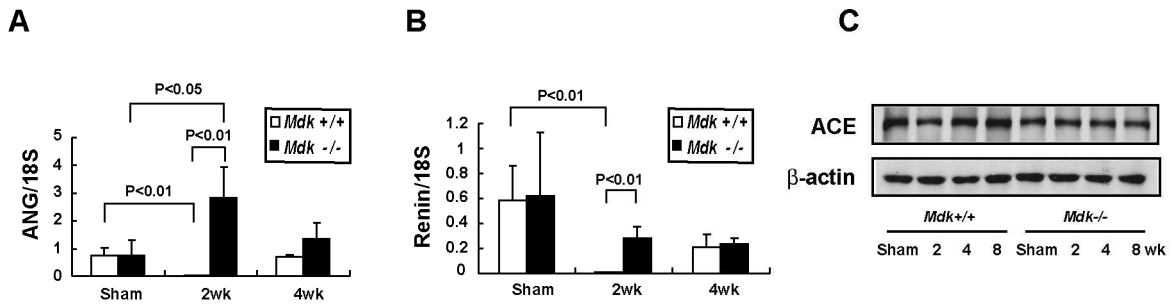
The tubulointerstitial damage induced by 5/6 nephrectomy.



(A-H) PAS staining. Tubular dilatation (arrows), cast formation in the tubular lumen (arrowheads) and tubular epithelial degeneration (open arrows) are indicated. (I-P) Masson's trichrome staining. Collagen deposition is shown by blue staining. Bar = 50 μm.

Figure S2.

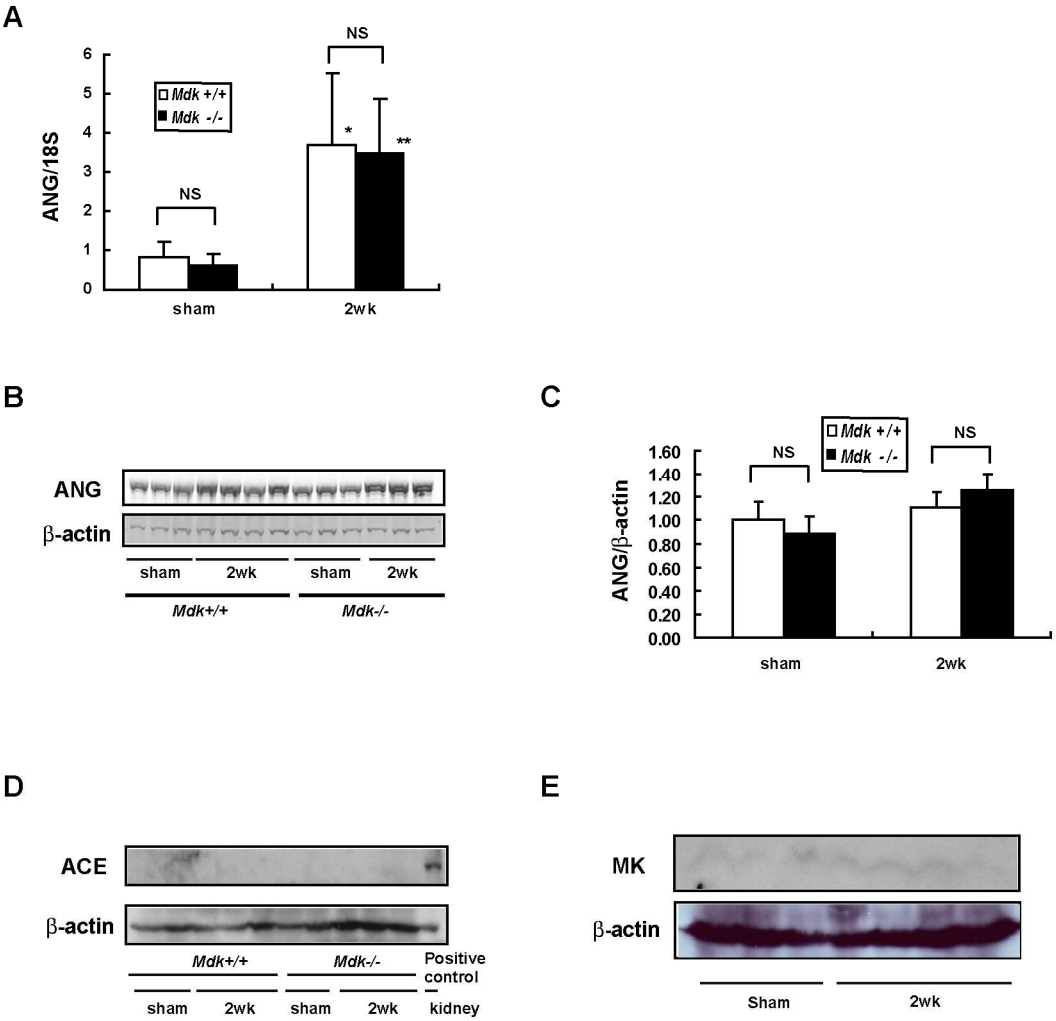
Angiotensinogen, renin and ACE expression in renal cortex from *Mdk*+/+ mice and *Mdk*-/- mice after 5/6 nephrectomy.



(A) Angiotensinogen mRNA was determined by real-time PCR. Angiotensinogen mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). (*Mdk*+/+: sham, n=5; 2 weeks, n=5; 4 weeks, n=3. *Mdk*-/-: sham, n=3; 2 weeks, n=3; 4 weeks, n=3). ANG, angiotensinogen. (B) Renin mRNA was determined by real-time PCR. Renin mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). (*Mdk*+/+: sham, n=5; 2 weeks, n=5; 4 weeks, n=3. *Mdk*-/-: sham, n=3; 2 weeks, n=3; 4 weeks, n=3). (C) Time course of ACE expression in renal cortex of *Mdk*+/+ mice and *Mdk*-/- mice after 5/6 nephrectomy. ACE protein was determined by Western blotting.

Figure S3.

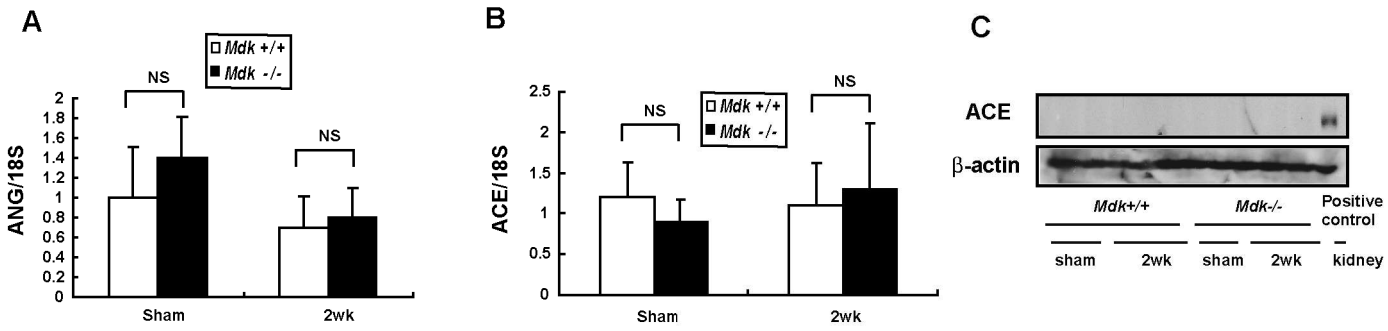
Angiotensinogen, ACE and MK expression in the liver after 5/6 nephrectomy.



(A) Angiotensinogen mRNA was determined by real-time PCR. Angiotensinogen mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). n=4. * $P < 0.05$ vs. sham *Mdk*+/+. ** $P < 0.05$ vs. sham *Mdk*-/-. ANG, angiotensinogen. (B) Angiotensinogen expression in the liver after 5/6 nephrectomy. Angiotensinogen protein was determined by Western blotting, and a representative result is shown. The liver tissues were obtained at the indicated time points. ANG, angiotensinogen. (C) Quantitative analysis for angiotensinogen protein expression using densitometoric densities. Data are presented as means (columns) and SD (bars). (*Mdk*+/+: sham, n=3; 2 weeks, n=4; *Mdk*-/-: sham, n=3; 2 weeks, n=3). ANG, angiotensinogen. (D) ACE expression in the liver after 5/6 nephrectomy. ACE protein was determined by Western blotting, and a representative result is shown. The liver tissues were obtained at the indicated time points. The kidney protein was positive control of ACE. (E) MK expression in the liver after 5/6 nephrectomy. MK protein was determined by Western blotting, and a representative result is shown. The liver tissues were obtained at the indicated time points.

Figure S4.

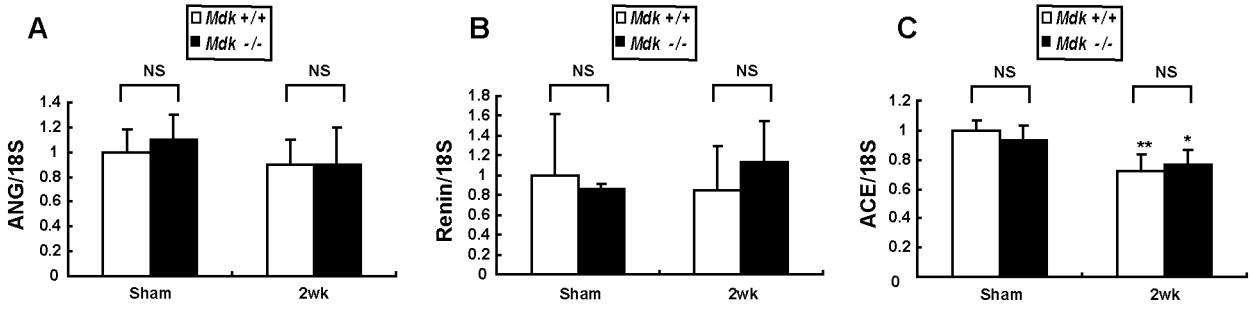
Angiotensinogen and ACE expression in the brain after 5/6 nephrectomy.



(A) Angiotensinogen expression in the brain after 5/6 nephrectomy. Angiotensinogen mRNA was determined by real-time PCR. Angiotensinogen mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). n=5. ANG, angiotensinogen. (B) ACE expression in the brain after 5/6 nephrectomy. ACE mRNA was determined by real-time PCR. ACE mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). n=5. (C) ACE expression in the brain after 5/6 nephrectomy. ACE protein was determined by Western blotting, and a representative result is shown. The brain tissues were obtained at the indicated time points. The kidney protein was positive control of ACE.

Figure S5.

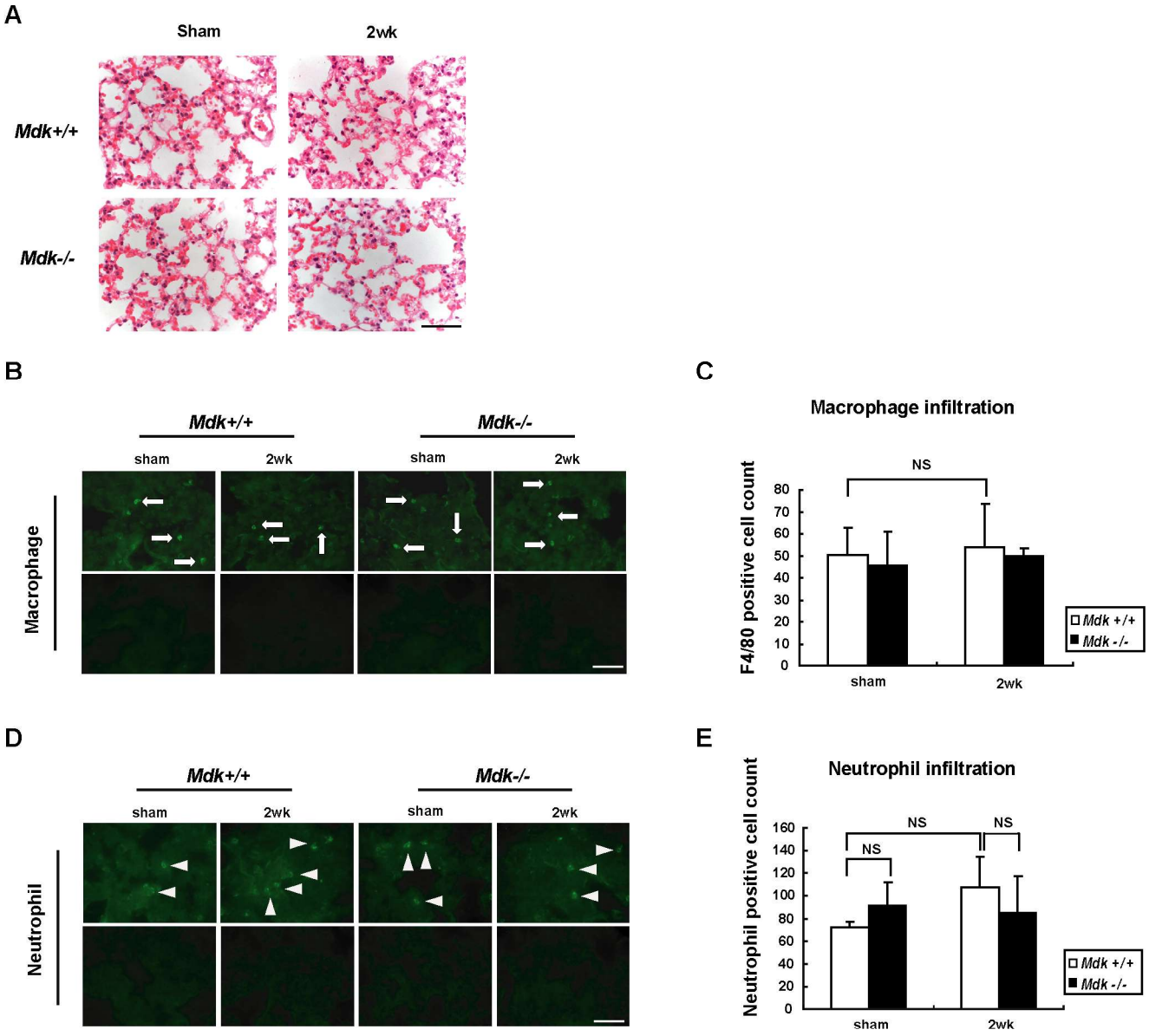
Angiotensinogen, Renin and ACE expression in the heart after 5/6 nephrectomy.



(A) Angiotensinogen expression in the heart after 5/6 nephrectomy. Angiotensinogen mRNA was determined by real-time PCR. Angiotensinogen mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). n=5. ANG, angiotensinogen. (B) Renin expression in the heart after 5/6 nephrectomy. Renin mRNA was determined by real-time PCR. Renin mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). n=5. (C) ACE expression in the heart after 5/6 nephrectomy. ACE mRNA was determined by real-time PCR. ACE mRNA was normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). n=5. * $P < 0.05$ vs. *Mdk-/-* sham; ** $P < 0.01$ vs. *Mdk+/+* sham.

Figure S6.

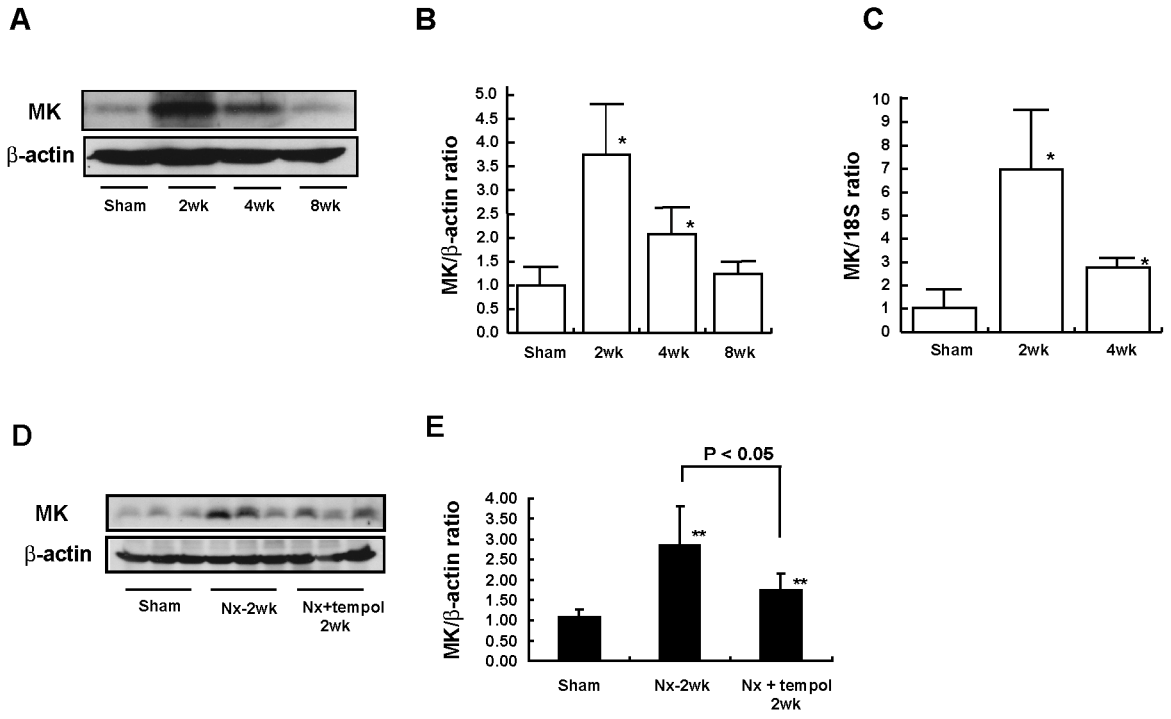
Lung histology after 5/6 nephrectomy in *Mdk* +/+ and *Mdk* -/- mice.



(A) Lung histology at 0 and 2 weeks after 5/6 nephrectomy in *Mdk*+/+ and *Mdk*-/- mice, respectively, is shown by H-E staining. Bar=50 μm. (B) Immunofluorescence staining of macrophage in the lung at 0 and 2 weeks after 5/6 nephrectomy. Lower panels are negative controls using isotype-matched IgG as the first antibodies. Open arrow, macrophages. Bar=50 μm. (C) The number of infiltrated macrophages were counted at indicated weeks as described in the Methods section. Data are presented as means (columns) and SD (bars). n=5. (D) Immunofluorescence staining of neutrophil in the lung at 0 and 2 weeks after 5/6 nephrectomy. Lower panels are negative controls using isotype-matched IgG as the first antibodies. Arrowhead, neutrophils. Bar=50 μm. (E) The number of infiltrated neutrophils were counted at indicated weeks as described in the Methods section. Data are presented as means (columns) and SD (bars). n=5.

Figure S7.

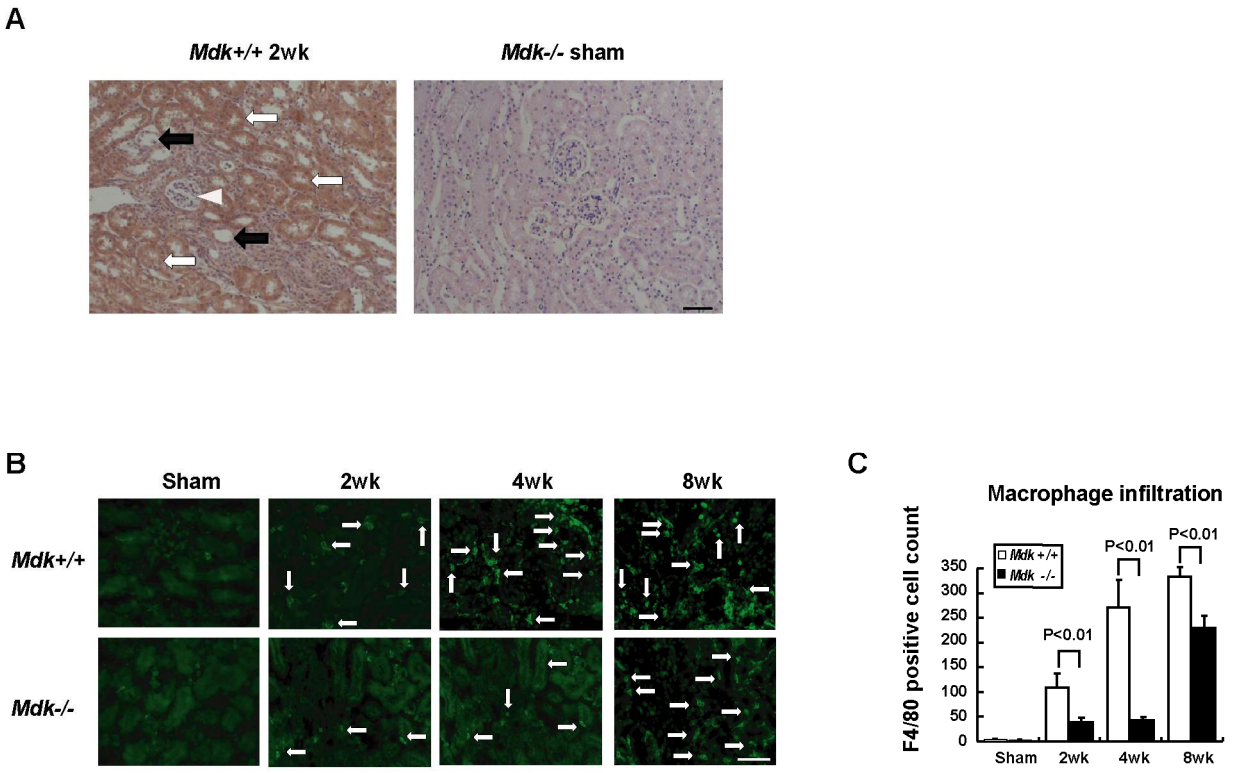
MK expression in renal cortex from *Mdk*+/+ mice after 5/6 nephrectomy.



(A) Time course of MK expression in renal cortex of *Mdk*+/+ mice after 5/6 nephrectomy. MK protein was determined by Western blotting. (B) The intensity of MK bands relative to that of control (sham) in A was normalized as to β-actin. Data are presented as means (columns) and SD (bars). (Sham, n=6; 2 weeks, n=5; 4 weeks, n=6; 8 weeks, n=3). * $P < 0.05$ vs. sham. (C) Time course of MK expression in renal cortex of *Mdk*+/+ mice after 5/6 nephrectomy. MK mRNA was determined by real-time PCR. MK mRNA normalized to 18S mRNA. Data are presented as means (columns) and SD (bars). (Sham, n=5; 2 weeks, n=5; 4 weeks, n=3). *$P < 0.05$ vs. sham. (D) Effect of Tempol on MK expression in the renal cortex. Tempol mixed in drinking water was administered to *Mdk*+/+ mice after 5/6 nephrectomy. MK protein expression was determined by Western blotting. (E) The intensity of MK bands on Western blotting was normalized as to that of β-actin. Data are presented as means (columns) and SD (bars). n=6. Nx, nephrectomy. **$P < 0.01$ vs. sham.

Figure S8.

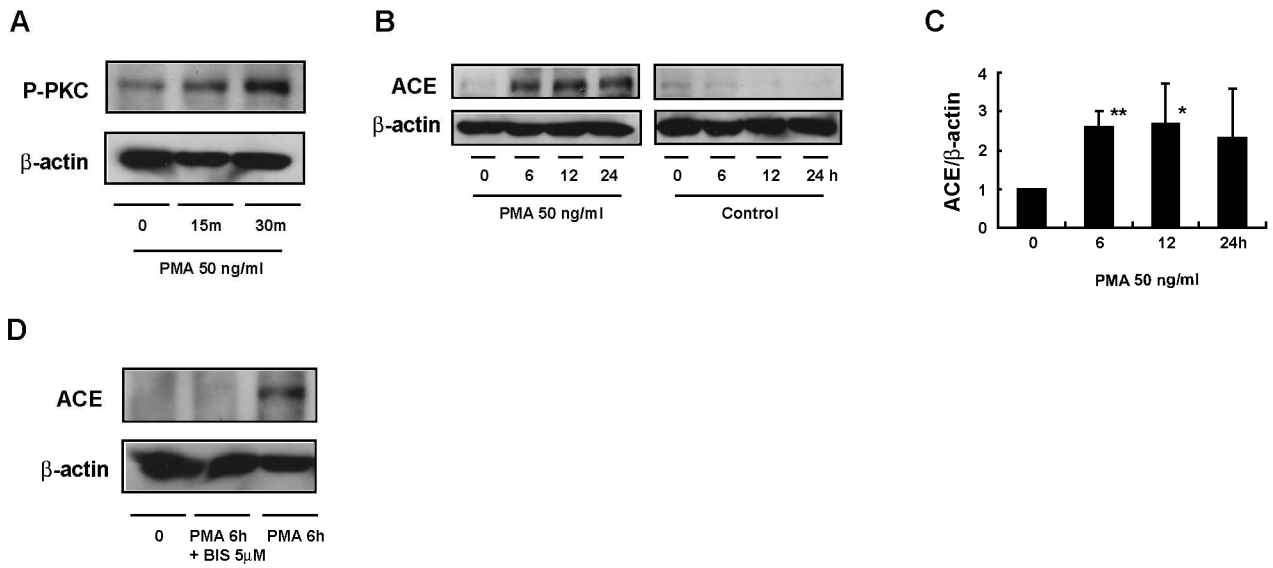
Macrophage infiltration in the kidney after 5/6 nephrectomy.



(A) Immunohistochemical staining of MK expression in the kidney after 5/6 nephrectomy. Left panel; *Mdk*+/+ mice 2weeks after 5/6 nephrectomy. Right panel; *Mdk-/-* mice. Open arrow, proximal tublues. Closed arrow, distal tubules. Arrowhead, glomerulus. Bar=50 μm. (B) Immunofluorescence staining of macrophage in the kidney at 0, 2, 4 and 8 weeks after 5/6 nephrectomy. Upper panels; *Mdk*+/+ mice. Lower panels; *Mdk-/-* mice. Open arrow, macrophages. Bar=50 μm. (C) The number of infiltrated macrophages were counted at indicated weeks as described in the Methods section. Data are presented as means (columns) and SD (bars). (*Mdk*+/+: sham, n=5; 2 weeks, n=4; 4 weeks, n=5; 8 weeks, n=5. *Mdk-/-*: sham, n=5; 2 weeks, n=4; 4 weeks, n=3; 8 weeks, n=3).

Figure S9.

Induction of ACE transcription by a PKC-dependent mechanism in HMVEC-L.



(A) Phospho-PKC expression in HMVEC-L treated with exogenous PMA (50 ng/ml). n=3. Western blot data are shown. (B) ACE expression in HMVEC-L treated with PMA (50 ng/ml) and control. Western blot data are shown. (C) Data in B (left panel) was quantified using densitometric densities, and presented as means (columns) and SD (bars). n=3. * $P < 0.05$ vs. 0 time point; ** $P < 0.01$ vs. 0 time point. (D) ACE expression in HMVEC-L treated with exogenous PMA (50 ng/ml). For PKC inhibition, Bisindolylmaleimide I (BIS, 5 μM) was added 1h before a PMA treatment. n=3. Western blot data are shown.