Supplementary Figure S1: Efficient CRE recombination in endothelial cells in Cdh5.cre mice. Representative images of GFP (green) in Cdh5.cre Rosa26R.Tomato/GFP mice showing GFP in the endothelium. Cell membranes of non-expressing-CRE cells express Tomato (Red). (A, A’, A’’) Renal cortex showing GFP expression in glomerular capillaries, tubular interstitial capillaries and arterial endothelial cells. (B) Pancreas (C) Heart and (D) Liver. (E) Representative photomicrographs of EPAS1 expression (red) in renal arterioles stained for vWF (green) showing an increase of endothelial nuclear EPAS 1 staining after angiotensin II treatment in Epas1lox/lox mice which is absent in endothelial cells from Cdh5.cre Epas1lox/lox mice. Nuclei are counterstained by DAPI stain (blue). Scale bar 50μm.
Supplementary Figure S2: Endothelial EPAS1 abrogation aggravates angiotensin II induced albuminuria from 7 days of Ang II infusion. Urinary albumin excretion rate in 20 weeks-old Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice after AngII infusion for 7 or 14 days. Data represent mean ± SEM of n= 7 mice. # p<0.05, ** p<0.01, *** ### p<0.001, # different genotype same treatment, * same genotype, different treatment.
Supplementary Figure S3: Renal interstitial inflammatory infiltrate is not modified by endothelial EPAS1 deficiency during angiotensin II-induced hypertension. (A) Representative images of the expression of CD3 (upper panel) and F4/80 (lower panel) by immunohistochemistry in 20 weeks-old Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice. Nuclei were counterstained with hematoxylin (blue). Scale bar: 50 µm. (B) Quantification of the percentage of CD3- and F4/80- positive tissue area in renal cortex from 20 weeks-old Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice. Data represent means ± SEM of at least 5 mice. **p<0.01, * same genotype different treatment.
Supplementary Figure S4: CRE-induced off-target Epas1 deletion in macrophages do not altered EPAS1 expression nor macrophage differentiation. (A) Western blot analysis of EPAS1 expression in spleen from 20 weeks-old Epas1lox/lox and Cdh5.cre-Epas1lox/lox mice treated or not with angiotensin II for 42 days. βACTIN is used as loading control. For in vitro experiments (B-E) BMDM were isolated from non hypertensive or hypertensive WT and Cdh5.cre Epas1lox/lox mice. After one week of differentiation, cells were treated with LPS (50 ng/mL) or PBS as control for 24H. (B-C) Western blot analysis of EPAS1, IL1β and phospho-NFκB in BMDM from non hypertensive (B) and hypertensive (C) WT and Cdh5.cre Epas1lox/lox mice. (D) qPCR analysis of the mRNA expression of Vegfa, Il10, Arginase, I116 and Tnfa in BMDM from WT and Cdh5.cre Epas1lox/lox mice. (E) ELISA measurement of IL1β secretion in supernatants of BMDM from WT and Cdh5.cre Epas1lox/lox mice.
Supplementary Figure S5: Immune cell population profile by flow cytometry. Immune cell populations in blood and spleen from 20 weeks-old Epas1<sup>lox/lox</sup> and Cdh5.Cre-Epas1<sup>lox/lox</sup> mice after 6 weeks of chronic AngII infusion were analyzed by flow cytometry. (A) Gating strategy for blood. B cells (CD19<sup>+</sup>, population 3) and T cells (CD3<sup>+</sup>, population 2) were analyzed from CD45<sup>+</sup> alive cells (population 1). T CD4<sup>+</sup> (population 6) and T CD8<sup>+</sup> (population 5) were analyzed from population 3. Monocytes (Ly6C<sup>+</sup> Ly6G<sup>-</sup>, population 8) and neutrophils (Ly6C<sup>-</sup> Ly6G<sup>+</sup>, population 7) were analyzed from CD45<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> cells (population 4). (B) Corresponding quantification of leukocyte cell number in blood. (C) Gating strategy for splenocytes. B cells (CD19<sup>+</sup>, population 5) and T cells (CD3<sup>+</sup>, population 2) were analyzed from CD45<sup>+</sup> cells (population 1). T CD4<sup>+</sup> (population 3) and T CD8<sup>+</sup> (population 4) were analyzed from population 2. Monocytes (Ly6C<sup>+</sup> Ly6G<sup>-</sup>, population 7) and neutrophils (Ly6C<sup>-</sup> Ly6G<sup>+</sup>, population 8) were analyzed fromCD11B<sup>+</sup> CD11C<sup>-</sup> myeloid cells (population 6). (D) Corresponding quantification of leukocyte cell number in spleen. n=5 mice per group.
Supplementary Figure S6: Effect of chronic angiotensin II infusion on collagen deposition and arterial remodeling in kidney. (A) Representative images of collagen IV deposition (brown) by immunohistochemistry in kidney cortex (upper panel) and arteries (lower panel) from 20 weeks-old Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice. Nuclei were counterstained with hematoxylin (blue). Scale bar: 50 μm. Images are representative of at least 6 mice. (B) Quantification of wall-to-lumen ratio surface in renal arteries (diameter >100μm) from 20 weeks-old Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice showing similar arterial remodelling. n=4-6 mice per group.
**Supplementary Figure S7:** Effect of chronic angiotensin II infusion on glomerular mesangial cells. (A) Representative images of GATA-3 (red), PODXL (green) by immunofluorescence in kidney cortex from 20 weeks-old *Epas1^lox/lox* and *Cdh5.Cre-Epas1^lox/lox* mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated *Epas1^lox/lox* and *Cdh5.Cre-Epas1^lox/lox* mice. Nuclei were counterstained with Hoescht (blue). Scale bar: 50 µm. Images are representative of at least 6 mice. (B) Quantification of the number of GATA-3 positive cells per glomerulus from 20 weeks-old *Epas1^lox/lox* and *Cdh5.Cre-Epas1^lox/lox* mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated *Epas1^lox/lox* and *Cdh5.Cre-Epas1^lox/lox* mice. Data represent means ± SEM of at least 5 mice. No significant statistical difference was found.
Supplementary Figure S8: Effect of chronic angiotensin II infusion on renal tubular injury. (A) Representative images of KIM-1 expression (brown) by immunohistochemistry in kidney cortex from 20 weeks-old Epas1lox/lox and Cdh5.Cre-EPAS1lox/lox mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1lox/lox and Cdh5.Cre-EPAS1lox/lox mice. Nuclei were counterstained with hematoxylin (blue). Scale bar: 100 μm. Images are representative of at least 6 mice. (B) Quantification of the percentage of KIM-1 positive tissue area in renal cortex from 20 weeks-old Epas1lox/lox and Cdh5.Cre-EPAS1lox/lox mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1lox/lox and Cdh5.Cre-EPAS1lox/lox mice. Data represent means ± SEM of at least 5 mice. ***p<0.001, * same genotype different treatment. #p<0.05, # different genotype same treatment. qPCR analysis of the mRNA expression of *Kim-1* (C) and *Ngal* (D) in kidney cortex from from 20 weeks-old Epas1lox/lox and Cdh5.Cre-EPAS1lox/lox mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1lox/lox and Cdh5.Cre-EPAS1lox/lox mice. *p<0.05, * same genotype different treatment.
For Peer Review

WT1

Control

Angiotensin II

Epas1lox/lox

Cdh5.cre Epas1lox/lox

Epas1lox/lox

Cdh5.cre Epas1lox/lox

0

20

40

60

80

100

120

WT1+ cells / glomerular section

(% of control)

0

0.5

1.0

1.5

2.0

EPAS1/GAPDH

Supplementary Figure S9: Effect of chronic angiotensin II infusion on podocyte loss. (A) Representative images of WT1 expression (brown) by immunohistochemistry in glomeruli from 20 weeks-old Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice after 6 weeks of chronic AngII infusion and from 20 weeks-old untreated Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice. Nuclei were counterstained with hematoxylin (blue). Scale bar: 50 µm. Images are representative of 6 mice. (B) Quantification of the number of WT1 positive nuclei per glomerulus. Data are expressed as the percentage of variation compared to untreated Epas1lox/lox mice and represent means ± SEM of 6 mice. * p<0.05 **p<0.01, * same genotype different treatment.
Supplementary Figure S10: Effect of chronic angiotensin II infusion on PEC activation and differentiation. Representative images of claudin-1 (green) by immunofluorescence and CD44 (red) by immunohistochemistry in glomeruli from 20 weeks-old Epas1lox/lox and Cdh5.Cre-Epas1lox/lox mice after 6 weeks of chronic AngII infusion. Nuclei were stained with DAPI (blue). Scale bar: 20 µm. Images are representative of at least 6 mice.
Supplementary methods

Noninvasive ultrasound study of cardiac and renal hemodynamics

The same Cdh5.Cre-Epas\textsuperscript{lox/lox} and control mice were evaluated before Ang II treatment and at day 35 after Ang II infusion initiation. Ultrasound examination was carried out under light anesthesia, such that mice could be restrained without ligature. Isoflurane (2.5% isoflurane in ambient air) was administered through a vaporizer (model 100-F; Ohio Medical Instruments). Isoflurane induction was performed by over a 2-minute period in an isolation chamber and anesthesia was maintained by spontaneous breathing of the same mixture at a flow rate of 5 l/min through a small nose cone. We used an echocardiograph (Acuson S3000; Siemens®) equipped with a 14MHz linear transducer (14L5 SP). After sedation, the mice were shaved to improve probe contact. The mouse was placed in the left lateral decubitus position and the ultrasound device placed on the anterior part of the chest for cardiac output acquisition. The mouse was laid on a heating blanket (38°C) to avoid hypothermia induced by anesthesia affecting cardiac function and heart rate.

Measurement of cardiac parameters and cardiac output (CO).

A parasternal long-axis B-mode image of the chest allowed measurement of the pulmonary artery diameter. The imaging depth was set at 1.5 to 2 cm, and frame rate was 60 frames/s. Blood flow velocity (BFV) were measured with correction of the angle between the long axis of the pulmonary artery and the Doppler beam. The steering mode of the Doppler beam helped to avoid having to use an angle correction greater than 10°. CO was calculated with the following formula: \( CO = \left(\frac{V_{\text{mean}} \times 60 \times (Dpa/2)}{p} \right) \), where CO is expressed in ml/min, \( V_{\text{mean}} \) is the time-averaged mean BFV in cm/s, and \( Dpa \) is the pulmonary artery diameter in cm. Heart dimensions measured in both systole (s) and diastole (d) were left ventricular posterior wall thickness (LVPW), left ventricular internal diameter (LVD), and interventricular septum thickness (IVS). Heart rates were obtained with an electrocardiogram.

Measurements of kidney size and BFV in renal arteries.

Two-dimensional ultrasound imaging was used to measure kidney size. A left sided longitudinal B-mode image of the abdomen showed the aorta between the two renal arteries, allowing measurements of the width and the height of the right and left kidneys; the transducer was slightly displaced from one kidney to the other. Imaging depth was set at 2 to 3 cm. Color Doppler mode was activated and the renal arteries were drawn and localized on the screen by their color-coded blood flow. Time-averaged mean BFV was calculated by the
echograph after placing calipers at the beginning of a systolic peak to 5 to 10 following once.
All BFVs were measured with correction of the angle between the long axis of each vessel
and the Doppler beam.

**Blood count**

Blood count was performed on a Hemavet counter (Drew scientific) on fresh blood after
intracardiac puncture. EDTA was used as anticoagulant.

**Bone marrow derived macrophages (BMDM)**

Bone marrow cells were isolated from femurs of mice and cultured in RPMI 1640 + 10% FCS
+ 10% L929 media containing M-CSF + 1% Penicillin/Streptomycin (Lifetechnologies) for 6
days. BMDM were then treated with 50 ng/mL LPS for 24H or PBS as control.

**IL1β ELISA**

IL1β secretion was measured in BMDM supernatant by using IL1β ELISA kit (Abcam)
according to manufacturer’s recommendations.

**Flow cytometry**

After 6 weeks of AngII chronic infusion, mice were anesthetized, blood was collected on
EDTA by intra-cardiac puncture and spleen was harvested after cervical dislocation. Both
were immediately processed for flow cytometry staining.

Leukocytes populations in blood and spleen were analyzed using the following antibodies:
FITC-conjugated anti-CD45 (IBL-5/25, Immunotools), PE-conjugated anti-CD11b
(M1/70.15, Immunotools), PE-CF594 conjugated anti-Ly6C (AL21, BD Pharmingen),
PerCPCy5.5-conjugated anti-CD11c (HL3, BD Pharmingen), BUV737-conjugated anti-CD3
(145-2C11, BD Pharmingen), BV510-conjugated anti-CD4 (RM4-5, BD Pharmingen),
BV605-conjugated anti-CD8 (53-6.7, Bd Pharmingen), PE-Cy5-conjugated anti-CD19 (6D5,
Biolegend) and BV785-conjugated anti-Ly6G (1A8, Biolegend). Viability was assessed using
the LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit, an amine reactive dye
(ThermoFischer Scientific). After staining, blood was lysed and fixed using BD FACS™
Lysing Solution (BD Pharmingen) according to manufacturer’s instructions and splenic
leukocytes were fixed using PFA 4%. Cells were analyzed using a flow cytometer (FACSaria
IIu, BD Biosciences) and data were analyzed using FlowJo software.
### Supplemental Table 1: Effects on cardiac and renal hemodynamic parameters of AngII chronic infusion in Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice.

Noninvasive ultrasound studies of cardiac and renal hemodynamics were performed in untreated 10 weeks-old Epas1^{lox/lox} and Cdh5.Cre-Epas1^{lox/lox} mice and after 35 days of AngII infusion. Data represents means ± SEM of at least 7 mice from two independent experiments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control (n=9)</th>
<th>Control (n=7)</th>
<th>AngII (n=11)</th>
<th>AngII (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>24.8 ± 3.1</td>
<td>26.2 ± 2.5</td>
<td>27.3 ± 4.6</td>
<td>26.7 ± 5.4</td>
</tr>
<tr>
<td>Fractionnal shortening (%)</td>
<td>28.8 ± 6.2</td>
<td>31.3 ± 8.3</td>
<td>22.3 ± 2.8</td>
<td>25.3 ± 6.7</td>
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<tr>
<td>IVSd (mm)</td>
<td>0.73 ± 0.10</td>
<td>0.84 ± 0.14</td>
<td>0.93 ± 0.10</td>
<td>1.00 ± 0.12</td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>4.59 ± 0.55</td>
<td>4.30 ± 0.26</td>
<td>4.48 ± 0.49</td>
<td>4.44 ± 0.60</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.67 ± 0.13</td>
<td>0.67 ± 0.08</td>
<td>0.78 ± 0.09</td>
<td>0.88 ± 0.10</td>
</tr>
<tr>
<td>IVSs (mm)</td>
<td>1.03 ± 0.21</td>
<td>1.14 ± 0.19</td>
<td>1.22 ± 0.18</td>
<td>1.31 ± 0.16</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>3.36 ± 0.40</td>
<td>3.21 ± 0.29</td>
<td>3.49 ± 0.42</td>
<td>3.49 ± 0.64</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>0.97 ± 0.17</td>
<td>0.91 ± 0.12</td>
<td>1.04 ± 0.15</td>
<td>1.06 ± 0.16</td>
</tr>
<tr>
<td>Cardiac output (CO) (ml/min)</td>
<td>10.3 ± 0.7</td>
<td>10.2 ± 1.7</td>
<td>7.1 ± 1.1</td>
<td>7.3 ± 1.8</td>
</tr>
<tr>
<td>Renal mBFV /CO ratio</td>
<td>1.43 ± 0.3</td>
<td>1.44 ± 0.4</td>
<td>1.13 ± 0.7</td>
<td>0.84 ± 0.3</td>
</tr>
</tbody>
</table>

- p<0.05 vs. Epas1^{lox/lox} control
- p<0.05 vs. respective non hypertensive condition
- p<0.05 vs. Epas1^{lox/lox} AngII
Supplemental Table 2: Effects on blood cell count of AngII chronic infusion in Epas1<sup>lox/lox</sup> and Cdh5.Cre-Epas1<sup>lox/lox</sup> mice. Blood cell count in untreated 10 weeks-old Epas1<sup>lox/lox</sup> and Cdh5.Cre-Epas1<sup>lox/lox</sup> mice and after 42 days of AngII infusion. Data represents means ± SD of 5 mice. Any statistically significant variation is observed between groups.

<table>
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<tr>
<th>Mice</th>
<th>Epas1&lt;sup&gt;lox/lox&lt;/sup&gt;</th>
<th>Cdh5.cre Epas1&lt;sup&gt;lox/lox&lt;/sup&gt;</th>
<th>Epas1&lt;sup&gt;lox/lox&lt;/sup&gt;</th>
<th>Cdh5.cre Epas1&lt;sup&gt;lox/lox&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Control (n=5)</td>
<td>Control (n=5)</td>
<td>AngII (n=5)</td>
<td>AngII (n=5)</td>
</tr>
<tr>
<td>Leukocyte count (K/µL)</td>
<td>5.2 ± 0.7</td>
<td>3.7 ± 0.2</td>
<td>4.9 ± 0.6</td>
<td>5.5 ± 0.9</td>
</tr>
<tr>
<td>Neutrophils (K/µL)</td>
<td>2.3 ± 0.7</td>
<td>1.9 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Lymphocytes (K/µL)</td>
<td>2.7 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>3.1 ± 0.5</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>Monocytes (K/µL)</td>
<td>0.12 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.03</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Red blood cells (M/µL)</td>
<td>7.5 ± 0.3</td>
<td>7.5 ± 0.6</td>
<td>7.4 ± 0.6</td>
<td>8.5 ± 0.2</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.4 ± 0.2</td>
<td>12.2 ± 0.8</td>
<td>10.3 ± 1.0</td>
<td>12.1 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.5 ± 1.5</td>
<td>38.9 ± 2.5</td>
<td>32.6 ± 3.0</td>
<td>37.4 ± 0.9</td>
</tr>
<tr>
<td>Platelets (K/µL)</td>
<td>920 ± 27</td>
<td>734 ± 85</td>
<td>767 ± 74</td>
<td>835 ± 160</td>
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