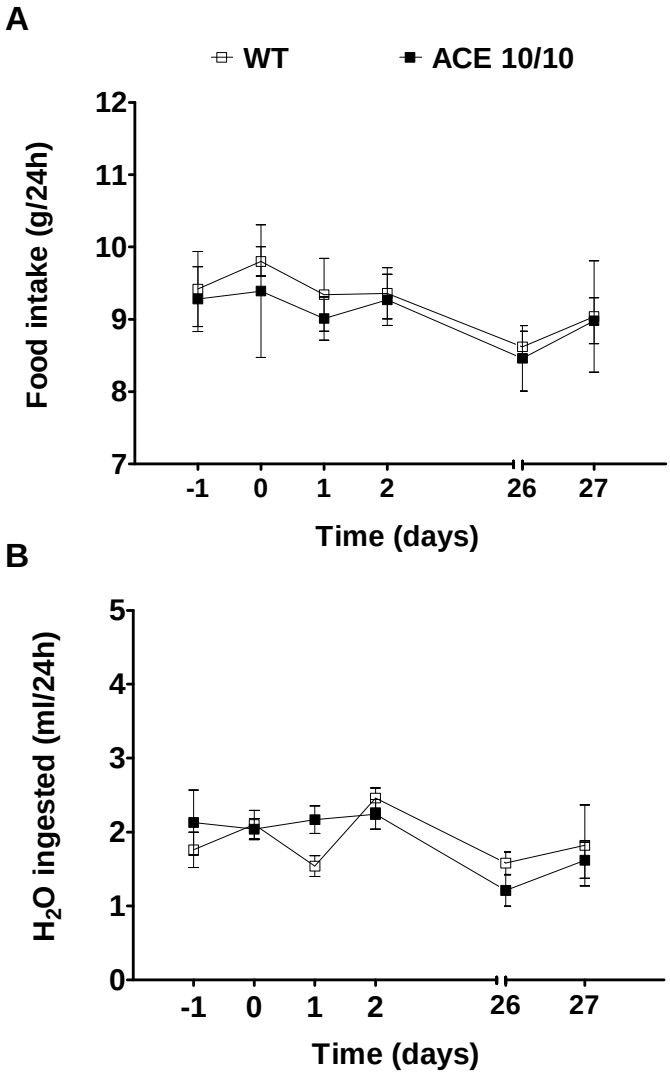
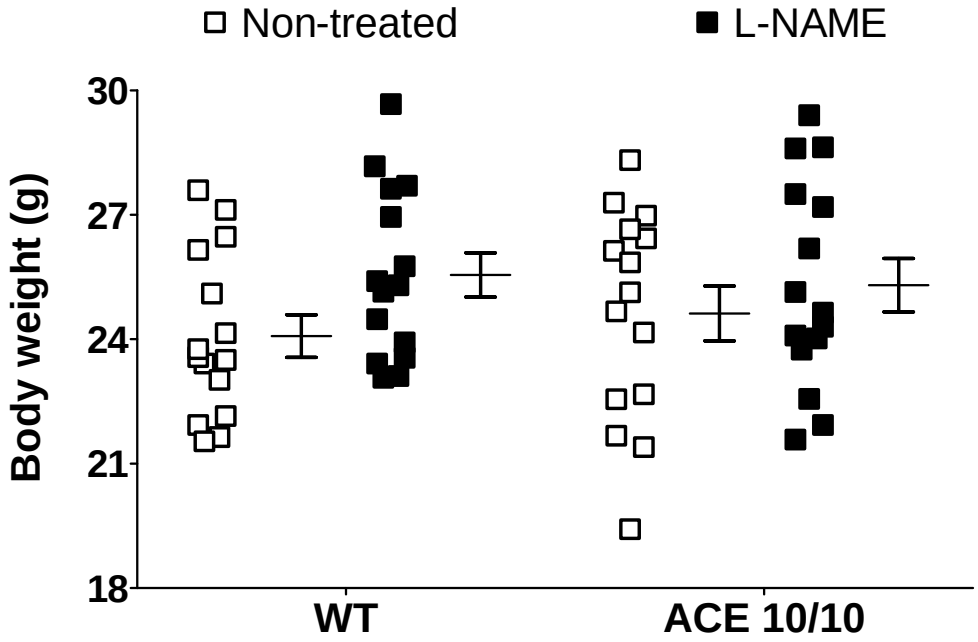
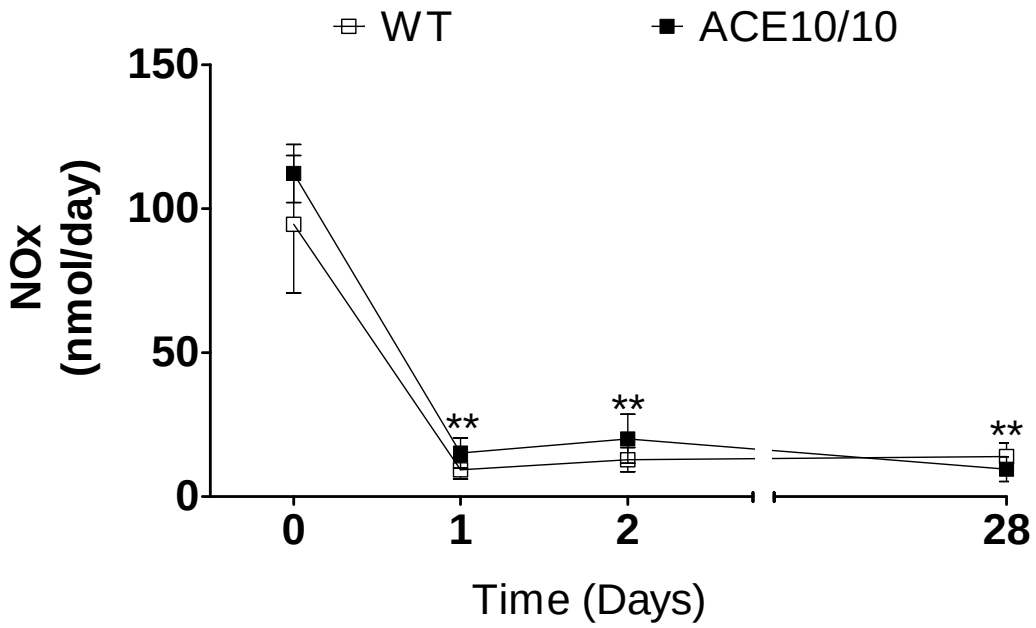
SUPPLEMENTAL DATA



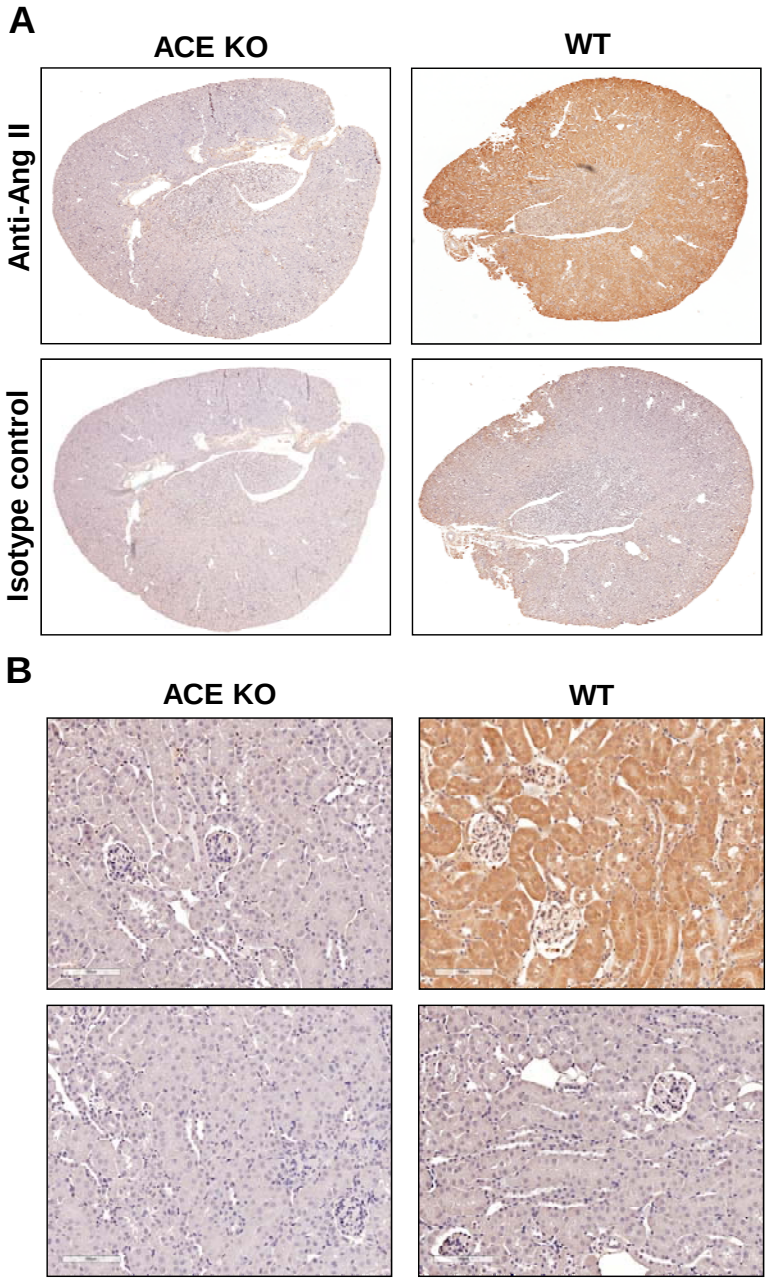
Supplemental Figure 1. Lack of changes in food and water consumption during L-NAME induced hypertension. Wild-type (WT) and ACE 10/10 mice were housed individually in metabolic cages with free access to food and water before and during L-NAME treatment (5 mg/10 mL in the drinking water for 4 weeks). n = 8–10. Values represent mean ± SEM. At baseline, food and water consumption were similar between wild-type and ACE 10/10 mice.



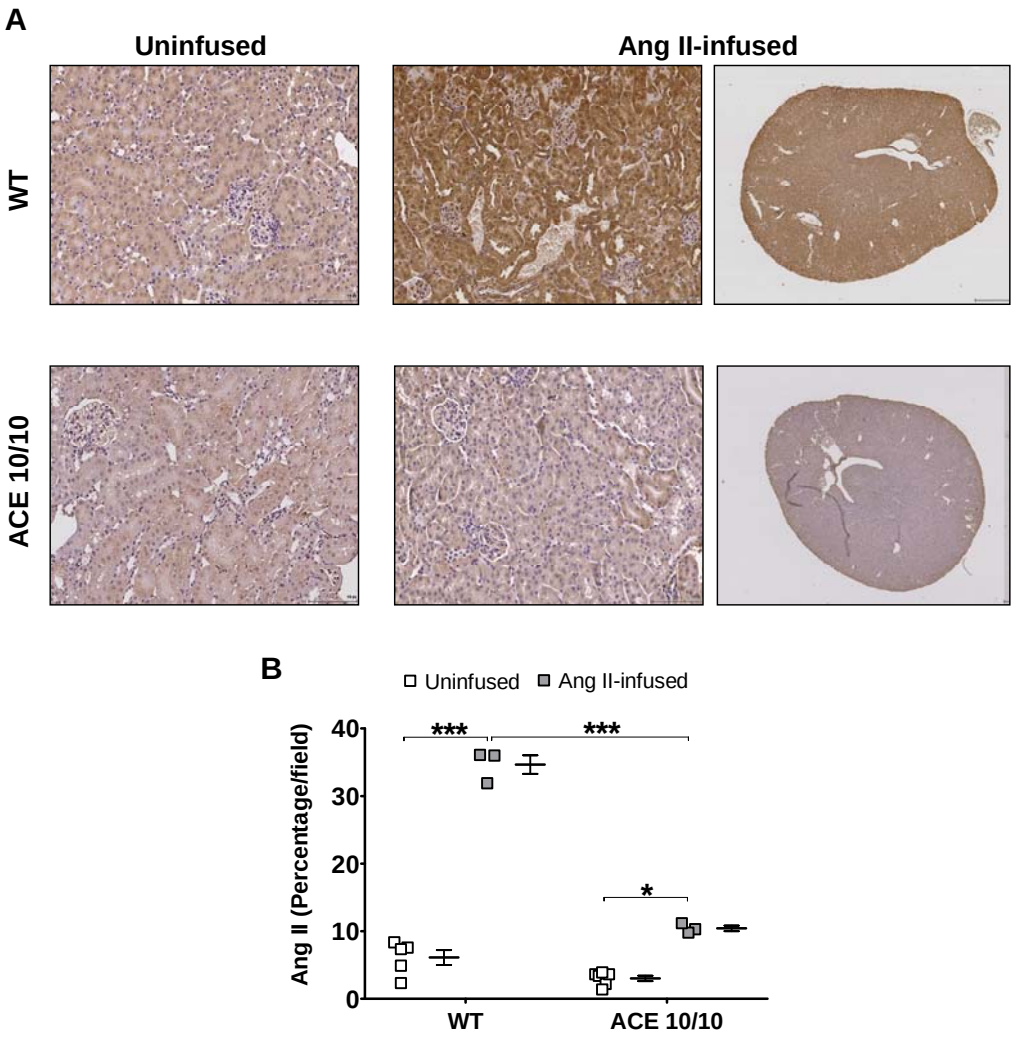
Supplemental Figure 2. Lack of body weight changes during L-NAME induced hypertension. Body weight was measured before and after L-NAME treatment (5 mg/10mL in the drinking water for 4 weeks). n=15. Data are presented as individual values and mean ± SEM.



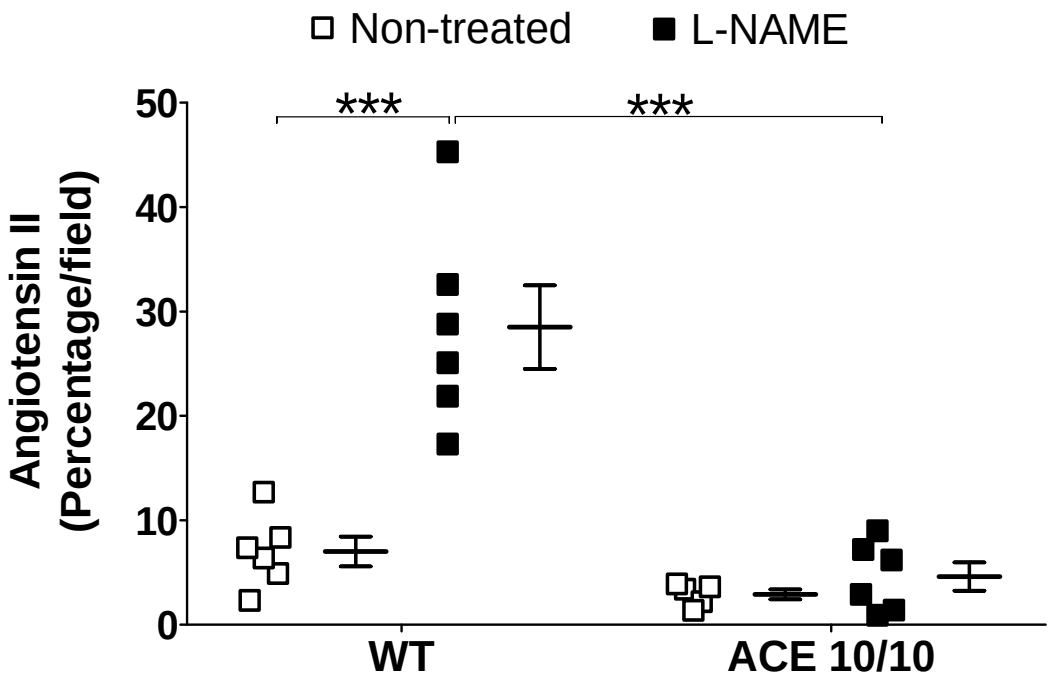
Supplemental Figure 3. Wild-type and ACE 10/10 mice showed consistently reduced urinary nitrite and nitrate (NOx) excretion during L-NAME induced hypertension. Urine from wild-type (WT) and ACE 10/10 mice was analyzed for NOx levels before and after 1, 2 and 28 days of L-NAME treatment (5 mg/10 mL in the drinking water for 4 weeks). Values were adjusted to urine volume to calculate daily excretion and expressed as mean ± SEM. n=10 per group, ***p*< 0.01 vs. non-treated mice. At baseline, NOx excretion was similar between wild-type and ACE 10/10 mice.



Supplemental Figure 4. Absence of renal angiotensin II staining in systemic ACE knockout mice. Renal sections from ACE KO mice and wild-type littermates were stained with an anti-angiotensin II antibody (Anti-Ang II) or with an isotype control (IgG) for background staining (A=30x, B=400x). Brown staining indicates angiotensin II positive areas. When compared to wild-type mice, renal sections from ACE KO mice show minimal amounts of angiotensin II staining.



Supplemental Figure 5. Renal angiotensin II content determination by immunohistochemistry in Ang II-infused mice. Renal sections from uninfused and angiotensin II-infused wild-type and ACE 10/10 mice were stained for angiotensin II. Brown staining indicates angiotensin II positive areas. (A) Representative stained sections (B) Semi-quantification of immunohistochemical analysis. Renal angiotensin II levels in the contralateral kidney, determined by radioimmunoassay and published elsewhere,[8] are similar. n=3-10, *p<0.05, ***p<0.001. Data are presented as individual values and mean ± SEM.



Supplemental Figure 6. Semi-quantification of renal angiotensin II by immunohistochemistry in L-NAME treated mice. Renal sections from non-treated and L-NAME treated wild-type and ACE 10/10 mice were stained for angiotensin II and semi-quantified. n=6, ***p<0.001. Data are presented as individual values and mean ± SEM.