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Gene expression profiling in kidney, heart and brain tissue

Tissue samples were stored in RNAlater and total RNA was isolated from homogenised tissues (Tissue Rupture) using RNA Micro Kit. 750 ng of isolated RNA from each sample were reverse transcribed into cDNA (QuantiTect Reverse Transcription Kit). Semi-quantitative real-time PCR was performed with an ABI PRISM 7300 (Applied Biosystems, Waltham, USA) and the SYBR Green master mix (all reagents from Qiagen, Venlo, The Netherlands). The relative expression of target genes was normalized to GAPDH housekeeping gene expression.

The following QuantiTect Primer Assays (Qiagen, Venlo, The Netherlands) were used:

mGAPDH	QT01658692
mα2A-adrenoceptor	QT00287063
mα2B-adrenoceptor	QT00311675
mα2C-adrenoceptor	QT01543458

The following primers (designed with Primer 3 (Whitehead Institute for Biomedical Research, Cambridge, USA) and synthesized by Eurofins Genomics (Ebersberg, Germany)) were used:

	Forward Primer	Reverse Primer
mNephrin	CGGGGGCGGGGTTAGCAGAC	CCCAGAGCGCCCCATTCAAAG
mKIM1	GGAGATTCCTGGATGGTTTAATG	AGCTGTGGGCCTTGTAGTTGT
mNGAL	GCCCAGGACTCAACTCAGAAC	CAGGCCCACAACGTACCAC
mColl1	CTGGTCCACAAGGTTTCCAAG	AGCTTCCCCATCATCTCCATT
mRenin	ACAGTATCCCAACAGGAGAGACAAG	GCACCCAGGACCCAGACA
mANP	CTGGGCTTCTTCCTCGTCTT	CCTCATCTTCTACCGGCATCT
mBNP	AAGGTGCTGTCCCAGATGATT	CCATTTCCTCCGACTTTTCTC
mbMHC	CTATGCTGGAGCTGATGCCCC	AAGTGAGGGTGCGTGGAGCG

FACS analysis of mouse kidneys to determine immune cell infiltration

Kidney portions were chopped, digested (1.2mg/ml Collagenase A [10103578001, Sigma-Aldrich, St. Louis, USA] in 0.5% human serum albumin [Grifols, Barcelona, Spain]) for 40 minutes at 37°C while shaking and then passed through a 100µm filter (Greiner Bio-One, Frickenhausen, Germany) followed by centrifugation at 300g for 10 minutes. The pellet was resuspended in PBS with 0.5% human serum albumin and incubated with FcR blocking agent (Miltenyi Biotec, Bergisch Gladbach, Germany) and respective antibodies anti-CD45-PE-Cy7 1:400 [552848] and anti-CD45-APC 1:200 [559864] from BD Bioscience, Franklin Lakes, USA; anti-CD3-APC 1:200 [100312] and anti-F4/80-PE 1:200 [123110] from BioLegend, San Diego, USA and anti-CD11b-PE-Cy7 1:200 [25-0112-82] from eBioScience, San Diego, USA for 20 minutes at 4°C. After washing, samples were stained with DAPI (1mg/ml, Sigma-Aldrich, Saint Louis, USA). BD FACSCanto II flow cytometry (BD Bioscience, Franklin Lakes, USA) was used for measurement and FlowJo software (BD Bioscience, Franklin Lakes, USA) was used for evaluation.

Determination of plasma Cystatin C concentration

Murine plasma cystatin C concentration was measured by using mouse/rat cystatin C Quantikine® (R&D Systems, Minneapolis, USA) ELISA Kit in accordance to manufacturer's protocol.

Determination of plasma lipids and lipoprotein

Plasma total cholesterol and triglyceride levels were measured using an automated clinical method.

Determination of albuminuria

Urinary albumin was determined by ELISA (CellTrend GmbH, Luckenwalde, Germany) and creatinine was measured using an automated clinical method. Proteinuria was normalized to urinary creatinine.

Determination of urinary components by ELISA

Urinary norepinephrine concentration was determined by ELISA KA3836 (Abnova, Taipei City, Taiwan), urinary plasminogen concentration by ab198511 ELISA kit

(abcam, Cambridge, UK), 8-isoprostane content by 516351 ELISA (Cayman Chemicals, Ann Arbor, USA) and urinary aldosterone levels by ELISA 501090 (Cayman Chemicals, Ann Arbor, USA) according to the manufacturer's protocol.

Haematocrit and haemoglobin content

Haematocrit and haemoglobin content were measured in heparinized blood (20U/ml) using Sysmex KX-21N (Sysmex Corporation, Bellport, USA).

Kidney WT1, CD3 and F4/80 staining

Wilms' tumor (WT) 1, a key regulator of podocyte function, was stained in wild-type and α2A-AR-KO kidneys. After deparaffinization and re-hydration, the slides were incubated with pH 6 citrate buffer (Agilent Dako, Santa Clara, USA) at 98°C for 20 minutes followed by further incubation at room temperature for 30 minutes. After washing with distilled water and immersing in 3% H_2O_2 (Merck Schuchardt, Hohenbrunn, Germany), each sample was incubated with 2.5% normal horse serum (MP-7401, Vector Laboratories, Burlingame, USA) for 20 minutes at room temperature. WT1 (C-19) (sc-192, Santa Cruz Biotechnology, Dallas, USA) primary antibody was added (1:800) and samples were incubated overnight at 4°C. Detection was performed using ImmPress^{TM} reagent anti-rabbit IgG (Vector Laboratories, Burlingame, USA) for 30 minutes at room temperature followed by application of 3,3'Diaminobenzidine chromogen (Agilent Dako, Santa Clara, USA) for 2-2.5 minutes at room temperature. 20x light microscopy (Olympus Microscope BX 40, Tokyo, Japan; Nikon Camera D7000, Tokyo, Japan) was used to take 15-20 pictures from randomly selected areas of the stained renal cortex. WT1 positive cells within the glomerulus were counted using multi point tool (Image J, National Institute of Health, USA) and glomerular tuft area was defined as described before. Number of WT1 positive cells per 1000µm^2 glomerular tuft were defined as [(number of WT1 positive cells / area of glomerular tuft) *1000].

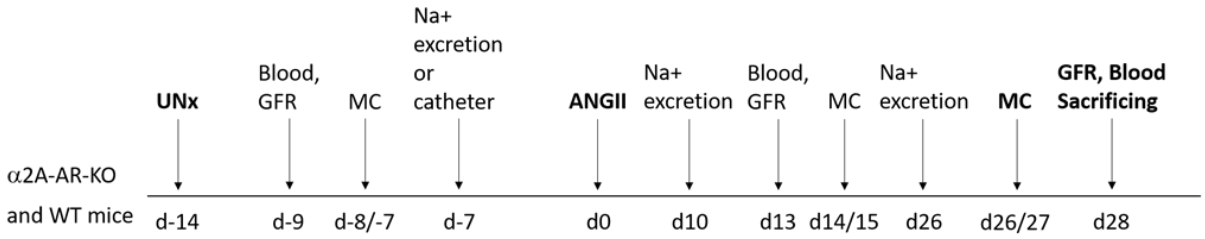
Cluster of differentiation (CD3) protein was stained to identify T cells. For antigen unmasking, samples were incubated with TRIS/EDTA (TE) buffer (pH 9) at 98°C for 20 minutes followed by 30 minutes incubation at room temperature. Endogenous peroxidase activity was blocked by immersing the samples in 3% H_2O_2 (Merck Schuchardt, Hohenbrunn, Germany) for 10 minutes and normal horse serum (2.5%, Vector Laboratories, Burlingame, USA) was applied for 20 minutes at room

temperature to block non-specific binding. CD3 primary antibody (IS503, Agilent Dako, Santa Clara, USA) was added for 1 hour followed by ImmPRESS™ reagent Anti-rabbit IgG (Vector Laboratories, Burlingame, USA) for 30 minutes at room temperature. For visualisation, 3,3'Diaminobenzidine chromogen (Agilent Dako, Santa Clara, USA) was used for 3.5 to 4 minutes at room temperature. Samples were shortly immersed in Mayer's hemalum (Merck Schuchardt, Hohenbrunn, Germany) to stain the nuclei. 20x light microscopy (Olympus Microscope BX 40, Tokyo, Japan; Nikon Camera D7000, Tokyo, Japan) was used to take 15-20 pictures from randomly selected areas of the stained renal cortex.

Kidney samples were stained for F4/80 antigen to detect murine macrophages. Unmasking was performed with proteinase K (Agilent Dako, Santa Clara, USA) for 3 minutes at room temperature and blocking of endogenous peroxidase activity was done with 10 minutes immersion in 3% H_2O_2 (Merck Schuchardt, Hohenbrunn, Germany). Normal goat serum (2.5%, Vector Laboratories, Burlingame, USA) was applied for 20 minutes to avoid unspecific binding. Each sample was covered with F4/80 primary antibody (1:100, MCA497, Bio-Rad, Hercules, USA) and incubated overnight at 4°C followed by incubation for 30 minutes at room temperature with ImmPRESS™ reagent Anti-rat IgG (mouse adsorbed) (Vector Laboratories, Burlingame, USA). Visualisation and microscopic inspection were performed as described for CD3 staining.

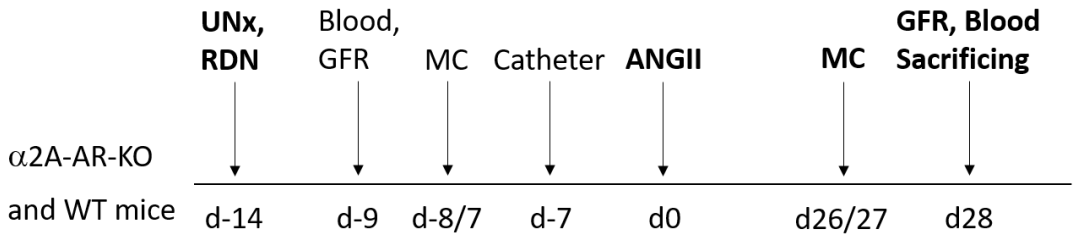
Supplemental Tables

Supplemental Table 1



Supplemental Table 1: Project outline for animal experiments without renal denervation. Na+ excretion and telemetry catheter implantation were performed in some mice according to experimental protocol and always in different subsets of mice; MC experiments and GFR measurements were performed in all mice at day 26/27 and day 28 respectively and only in subsets of mice at earlier time points. Bold fonts = integral part of study, Normal fonts = variable components of study protocol, UNx = unilateral nephrectomy, RDN = renal denervation, Catheter = telemetry catheter implantation, Na+ excretion = determination of sodium excretion, Blood = blood sampling, GFR = determination of glomerular filtration rate, MC = metabolic cage experiment, ANGII = chronic angiotensin II infusion (1µg/kg/min).

Supplemental Table 2



Supplemental Table 2: Project outline for animal experiments with renal denervation (RDN). Telemetry catheter implantation was performed in subsets of mice; MC experiments and GFR measurements were performed in all mice at day 26/27 and day 28 respectively and only in subsets of mice at earlier time points. Bold fonts = integral part of study, Normal fonts = variable components of study protocol, UNx = unilateral nephrectomy, RDN = renal denervation, Catheter = telemetry catheter implantation, Na+ excretion = determination of sodium excretion, Blood = blood sampling, GFR = determination of glomerular filtration rate, MC = metabolic cage experiment, ANGII = chronic angiotensin II infusion (1µg/kg/min).

Supplemental Table 3

Antibody Target	~kDa	μg/lane kidney	Primary Ab supplier	Ab host	Dilution	Time	Secondary Ab supplier	Host & target	Dilution	Time
ENaC-α	100 30	40, 20	Loffing (Zurich)	Rb	1:5000	O/N	Invitrogen	GAR 680	1:5000	1 hr
ENaC-β	100	40, 20	Loffing (Zurich)	Rb	1:15000	2 hr	Invitrogen	GAR 680	1:5000	1 hr
ENaC-γ	80 60	40, 20	Loffing (Zurich)	Rb	1:15000	O/N	Invitrogen	GAR 680	1:5000	1 hr
NCC	150	40, 20	McDonough	Rb	1:5000	O/N	Invitrogen	GAR 680	1:5000	1 hr
NCCpS71	150	40, 20	Loffing (Zurich)	Rb	1:5000	2 hr	Invitrogen	GAR 680	1:5000	O/N
NHE3	83	40, 20	McDonough	Rb	1:2000	O/N	Invitrogen	GAR 680	1:5000	1 hr
NKCC2	160	20, 10	DSHB (U. Iowa)	Mu	1:6000	O/N	LI-COR	GAM 800	1:5000	1 hr
NKCC2-pT96T101	160	20, 10	Forbush (Yale)	Rb	1:2000	2 hr	Invitrogen	GAR 680	1:5000	1 hr

Supplemental Table 3. Mouse immunoblot protocol and antibody details. ~kDa refers to apparent molecular weight determined by SDS-PAGE molecular weight markers. μg protein per lane: to ensure linearity of the detection system, 1 and 1/2 amounts of each sample were assessed. Only one amount is shown in figures, although both amounts are used to calculate the changes in abundance. Ab = antibody, Mu = mouse, Rb = rabbit, Sh = sheep, O/N = overnight, GAR = goat anti-rabbit, GAM = goat anti-mouse, DAS = donkey anti-sheep.

Supplemental Table 4

	Hb (g/dl)	Hct (%)
WT	11±1	36±4
α2A-AR-KO	10±1	34±3

Supplemental Table 4. Hemoglobin (Hb) and hematocrit (Hct) levels in angiotensin II-infused WT and α2A-AR-KO mice. After 28 days of chronic angiotensin II infusion, no differences in Hb and Hct levels were detected between WT and α2A-AR-KO mice.

Supplemental Figures

Supplemental Figure 1. Kidney renin expression and urinary aldosterone excretion levels in untreated and in angiotensin II-infused α2-AR-KO and WT mice. Chronic angiotensin II infusion decreased (A) renin mRNA significantly and increases (B) urinary aldosterone excretion levels in α2-AR-KO and WT mice. No differences were seen between α2-AR-KO and WT mice. Unpaired two-tailed Student's t-test or one-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

Supplemental Figure 2. Renal sodium transporter profile in normotensive and angiotensin II-infused WT and α2-AR-KO mice. (A) Representative immunoblots of NHE3, NKCC2, NKCC2p, NCC, NCCp, αENaC Fl, αENaC clvd, ßENaC, γENaC Fl and γENaC clvd. (B) Under normotensive conditions, abundance of renal sodium transporter did not differ between WT and α2-AR-KO mice (n=5-6). (C) Chronic angiotensin II treatment did not differentially modulate abundance or phosphorylation of NCC in WT vs. α2-AR-KO mice (n=5-6). Density values are expressed as relative abundance normalized to normotensive WT mice, defined as 1.0. Unpaired two-tailed student's t-test was used to compare differences between normotensive groups. One-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to test differences between the groups.

Supplemental Figure 3. Accumulated urinary sodium and volume excretion after sodium challenge in α2A-AR-KO mice at baseline and 10 days after start of angiotensin II infusion. At baseline, no differences in the accumulated urinary (A) Na^+ (n=9-10) and (B) volume (n=9-10) excretion between α2A-AR-KO and WT mice were observed. After 10 days of angiotensin II infusion, accumulated urinary (C) Na^+ (n=9-10) and (D) volume (*$p<0.05$, n=9-10) excretion was reduced in α2A-AR-KO mice compared to WT mice over a 5-hour collection period. Two-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

Supplemental Figure 4. Representative pictures and specific analysis of tubular damage in angiotensin II-infused WT and α2A-AR-KO. (A) PAS stained kidney samples were evaluated to detect tubular damage. Representative pictures for analysis of tubular damage in angiotensin II-infused α2A-AR-KO and WT mice. (B-C) After angiotensin II infusion, tubular dilation (α2A-AR-KO vs. WT: 3.2±0.6 vs. 2.1±0.2,

*p=0.52, n=9-10), brush border loss (2.8±0.6 vs. 1.5±0.1, *p<0.05, n=9-10), tubule degeneration (1.0±0.1 vs. 0.8±0.1, p>0.05, n=9-10), tubule necrosis or apoptosis (0.30±0.11 vs. 0.08±0.03, *p<0.05, n=9-10) and tubular cast formation (0.9±0.1 vs. 0.7±0.2, p>0.05, n=9-10) was increased in α2A-AR-KO compared to WT mice. (D and E) Representative pictures for analysis of glomerular injury and WT1 positive cells in angiotensin II-infused α2A-AR-KO and WT mice. Unpaired two-tailed student's t-test was used to compare differences between the groups.

Supplemental Figure 5. WT1 positive cells and average glomerular tuft area in WT, α2-AR-KO and α2-AR-KO + RDN at baseline and after angiotensin II infusion. (A) Numbers of WT1 positive cells did not differ in normotensive α2A-AR-KO and WT mice (15.0±0.4 vs. 14±0.5, p>0.05, n=4-5). Compared to normotensive α2A-AR-KO (15.0±0.04 vs. 11.4±0.8, p<0.05, n=5-10) and angiotensin II-infused WT mice (14.0±0.6 vs. 11.4±0.8, p<0.05, n=9-10) WT1 positive cells were significantly lower in angiotensin II-infused α2A-AR-KO mice. (B) Compared to their normotensive controls, the average glomerular tuft area was significantly increased in angiotensin II-infused WT (1876±57 vs. 2772±87μm^2, p<0.01, n=4-9) and angiotensin II-infused α2A-AR-KO (2209±179μm^2 vs. 3254±201μm^2, p<0.001, n=5-10). (C) WT1 positive cells were increased in α2A-AR-KO + RDN compared to α2A-AR-KO mice (14.0±1.0 vs. 11.4±1.3, p=0.07, n=5). (D) After angiotensin II infusion, the average glomerular tuft area did not differ between α2A-AR-KO + RDN and α2A-AR-KO mice (3302±287 vs. 3256±344μm^2, p>0.05, n=5). One-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups. Unpaired one-tailed student's t-test was used to compare differences between two groups

Supplemental Figure 6. No differences in CD3 positive and F4/80 positive cells within the kidney of angiotensin II-infused α2-AR-KO and WT mice. Immune cell infiltration into the kidney was measured by (A+C) immunohistochemistry and (B+D) flow cytometry. After chronic angiotensin II infusion, the amount of (A+B) CD3 positive and (C+D) F4/80 positive cells did not differ between α2-AR-KO and WT mice. Unpaired two-tailed Student's t-test or one-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

Supplemental Figure 7. No differences in hypertensive cardiac damage between α2A-AR-KO and WT mice. (A) Compared to normotensive conditions, chronic angiotensin II infusion induced heart hypertrophy in α2A-AR-KO (4.3 vs. 6.4mg/g, ***<0.001, n=5-10) and WT (3.7 vs. 6.1mg/g, ***p<0.001, n=5-12) mice. No differences were seen between both groups (6.1 vs. 6.4mg/g, n=10-12) infused chronically with angiotensin II. Compared to normotensive conditions, relative cardiac (B) BNP (WT: 1.0±0.1 vs. 2.5±0.3, n=5-10, p<0.01; α2A-AR-KO: 1.4±0.1 vs. 2.8±0.4, n=5-7, p<0.05), (C) ANP (WT: 1.0±0.1 vs. 2.2±0.1, n=5-10, p<0.05) and (E) collagen-1 (α2A-AR-KO: 1.0±0.1 vs. 1.5±0.3, n=5-7, p<0.05) was significantly increased in angiotensin II infused mice. Relative cardiac (B) BNP (n=7-10), (C) ANP (n=7-10), (D) ß-MHC (n=7-10) and (E) collagen 1 (n=7-10) mRNA expression did not differ between chronic angiotensin II-infused α2A-AR-KO and WT mice. One-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the groups

Supplemental Figure 8. Effect of renal denervation on renal norepinephrine content. Renal denervation reduces renal norepinephrine content significantly compared to innervated kidneys (19±3 vs. 56±10pg/mg kidney, *p<0.05, n=4). Unpaired two-tailed Mann Whitney test.

Supplemental Figure 9. Effects of renal denervation on diastolic blood pressure and heart rate in α2A-AR-KO mice. Renal denervation attenuates angiotensin II-dependent hypertension in α2A-AR-KO mice. (A) Renal nerve denervation did not affect baseline diastolic blood pressure (α2A-AR-KO+RDN: 105±5; α2A-AR-KO: 109±7mmHg, n=6). However, diastolic blood pressure response to chronic angiotensin II infusion was significantly reduced in renal denervated α2A-AR-KO compared to sham-innervated α2A-AR-KO mice (week 1: 126±6 vs. 136±6mmHg *p>0.05, n=6*; week 2: 135±4 vs. 151±6mmHg *p>0.05, n=6*; week 3: 126±3 vs. 152±6mmHg ***p<0.01, n=6*; week 4: 129±5 vs. 156±5mmHg **p<0.05,* n=5). (B) Renal denervation did not affect baseline heart rate (α2A-AR-KO+RDN: 632±11; α2A-AR-KO: 606±22bpm, n=6). During angiotensin II infusion, heart rate tended to be lower in renal denervated compared to sham-denervated α2A-AR-KO+RDN mice (week 1: 577±10 vs. 598±18bpm *p>0.05, n=6*; week 2: 628±14 vs. 653±12bpm *p>0.05, n=6*; week 3: 621±21 vs. 651±12bpm *p>0.05, n=6*; week 4: 627±15 vs. 663±17bpm *p>0.05,* n=5). Two-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

Supplemental Figure 10. Representative pictures and specific analysis of tubular damage in angiotensin II-infused α2A-AR-KO and α2A-AR-KO + RDN. (A) PAS stained kidney samples were evaluated to detect tubular damage. Representative pictures for analysis of tubular damage in renal denervated or sham-operated α2A-AR-KO and α2A-AR-KO + RDN infused with angiotensin II. (B) Renal denervation attenuated tubular dilation (α2A-AR-KO+RDN vs. α2A-AR-KO: 1.6±0.3 vs. 2.0±0.3, *p<0.05, n=7-9), brush border loss (0.8±0.1 vs. 1.2±0.2, *p<0.05, n=7-9), tubule degeneration (0.6±0.1 vs. 1.2±0.2, *p<0.05, n=7-9), tubule necrosis or apoptosis (0.12±0.06 vs. 0.08±0.02, p>0.05, n=7-9) and tubular cast formation (0.48±0.13 vs. 0.84±0.13, p>0.05, n=7-9) in angiotensin II-infused α2A-AR-KO mice. (C and D) Representative pictures for analysis of glomerular injury and WT1 positive cells in angiotensin II-infused α2A-AR-KO and α2A-AR-KO + RDN mice. Unpaired two-tailed t-test was used.

Supplemental Figure 11. Effects of renal denervation on diastolic blood pressure and heart rate in WT mice. Renal denervation had no significant effect on angiotensin II-dependent hypertension in WT mice. (A) Renal denervation did not affect diastolic blood pressure at baseline and during angiotensin II infusion (baseline WT+RDN: 108±2; WT: 96±8mmHg, week 1: 121±5 vs. 125±3mmHg *p>0.05*; week 2: 135±4 vs. 140±5mmHg *p>0.05*; week 3: 135±5 vs. 133±2mmHg *p>0.051*; week 4: 133±4 vs. 131±3mmHg **p>0.05,* n=5). (B) Renal denervation did not affect heart rate at baseline and during angiotensin II treatment in WT mice (baseline WT+RDN: 636±12; WT: 604±30bpm, week 1: 581±18 vs. 597±13bpm *p>0.05*; week 2: 569±25 vs. 622±14bpm *p>0.05*; week 3: 581±24 vs. 613±16bpm *p>0.05*; week 4: 583±18 vs. 593±16bpm *p>0.05;* n=5). (D) In WT mice, renal denervation did not affect urinary norepinephrine excretion at baseline (WT+RDN: 2.6±0.2 vs. WT: 3.4±0.8µg/mg creatinine) and during angiotensin II infusion (WT+RDN: 3.6±0.7 vs. WT: 4.0±0.9 µg/mg creatinine). (E) Renal denervation did not affect albuminuria at baseline (WT+RDN: 0.4±0.2 vs. WT: 0.5±0.4mg/mg creatinine, n=5) but tended to reduce albuminuria during angiotensin II infusion (WT+RDN: 45.5±9.2 vs. WT: 68.1±17.5 mg/mg creatinine, n=5). **p<0.01 and ***p<0.001 vs. baseline. Two-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

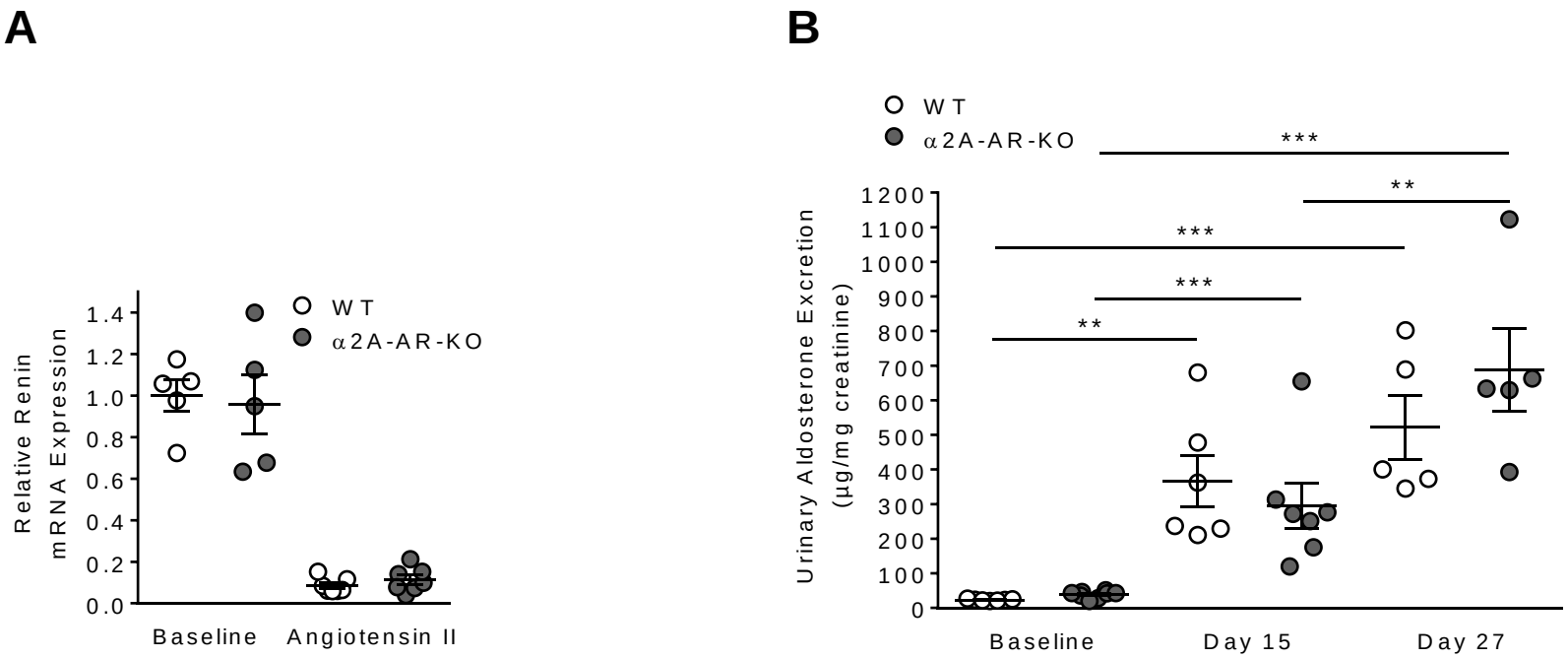
Supplemental Figure 12. Relative mRNA expression levels of α2-adrenoceptor subtypes in central nervous system (CNS), heart and kidney. No significant

differences in mRNA expression levels of α2A-, α2B- and α2C-adrenoceptor subtypes in (A-C) CNS, (D-F) heart and (G-I) kidney at baseline and after chronic angiotensin II infusion of WT and α2A-AR-KO mice. Two-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

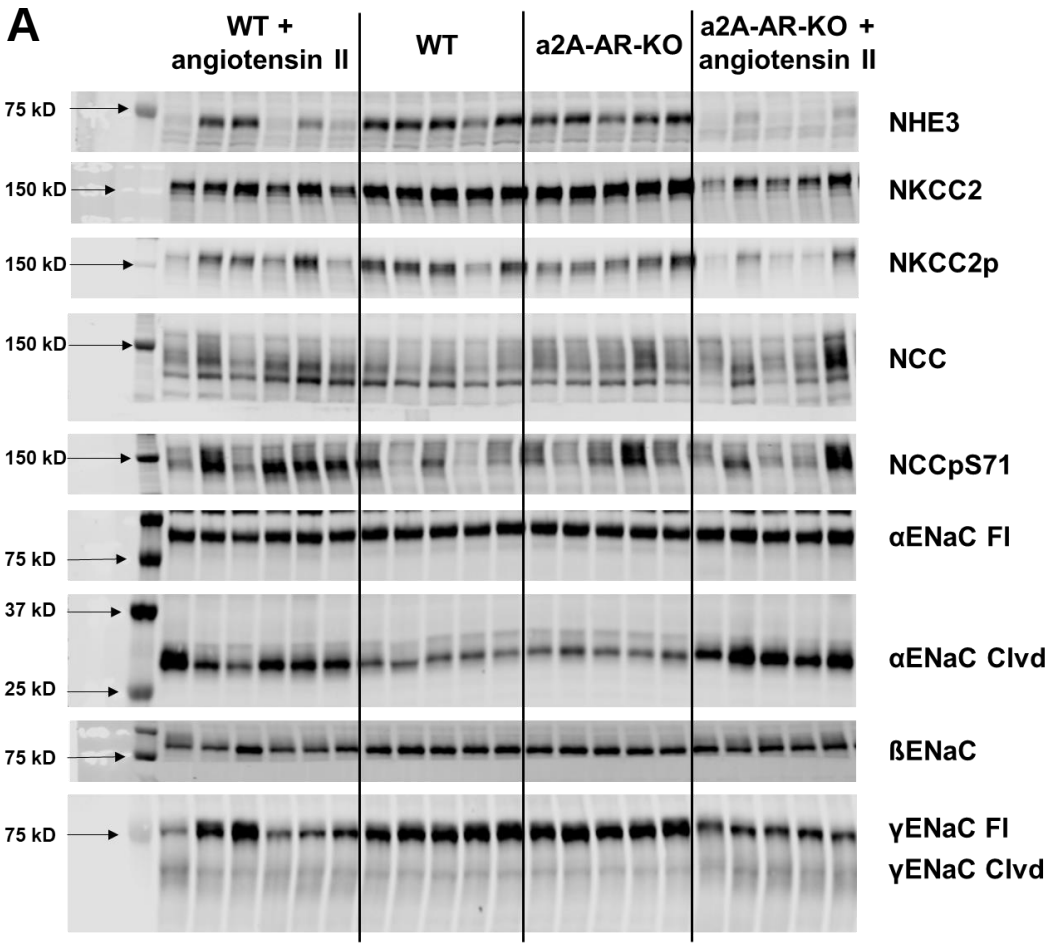
Supplemental Figure 13. GFR, albuminuria and urinary levels of plasminogen, 8-Isoprostane and norepinephrine at baseline as well as 15 and 27 days after start of angiotensin II infusion. (A-E) At baseline, no differences in GFR, urinary plasminogen, 8-Isoprostane and norepinephrine were detected between WT and α2A-AR-KO mice. (A) GFR was significantly decreased in α2A-AR-KO compared to WT after 15 and 27 days of chronic angiotensin II infusion. (B-C) Albuminuria and urinary norepinephrine excretion was significantly elevated in α2A-AR-KO compared to WT mice after 27 days of chronic angiotensin II infusion. (D-E): No differences in urinary plasminogen and 8-Isoprostane levels between α2A-AR-KO and WT mice during angiotensin II infusion. *p<0.05, **p<0.01, ***<0.001. One-way ANOVA followed by Bonferroni's multiple comparison post-hoc test was used to compare the differences between the two groups.

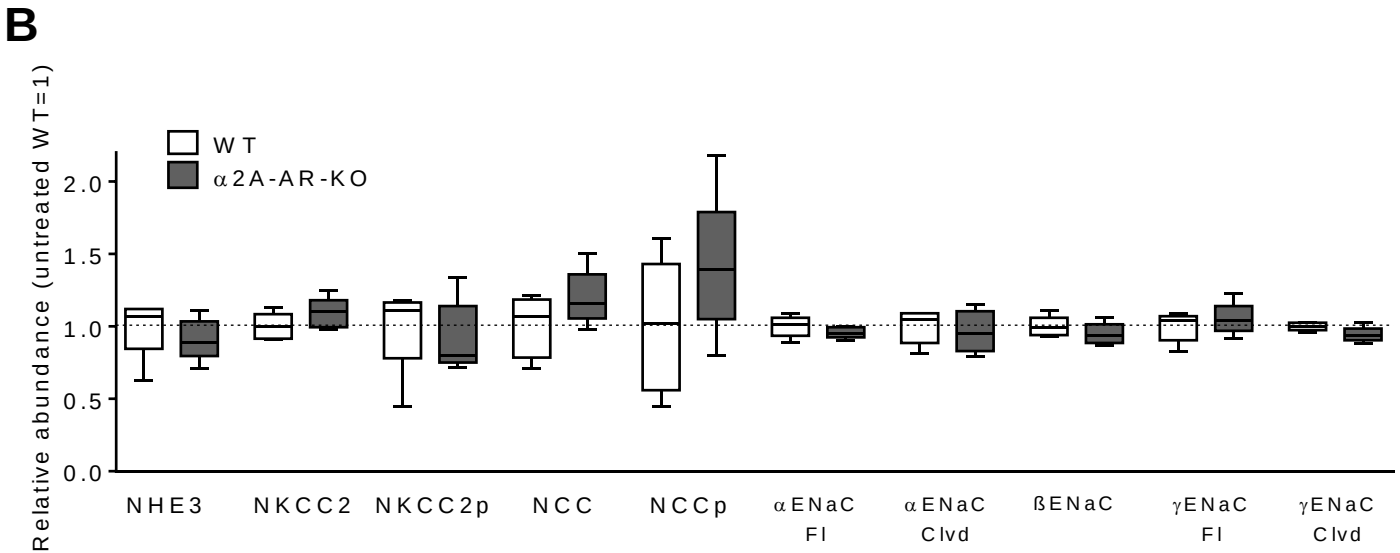
Supplemental Figure 14. Direct protein staining of the gel was implemented to assess equivalent protein loading per lane. (A) Image of a Coomassie stained SDS-PAGE gel (not blot) of renal cortex homogenate samples loaded at 12 µg/lane. (B) three unidentified protein bands were chosen arbitrarily and quantified by the LI-COR Odyssey system. (C) summarizes the density of each of the three arbitrary bands (color coded) in each sample. The sample to sample variation was less than 5%, viewed as acceptable.

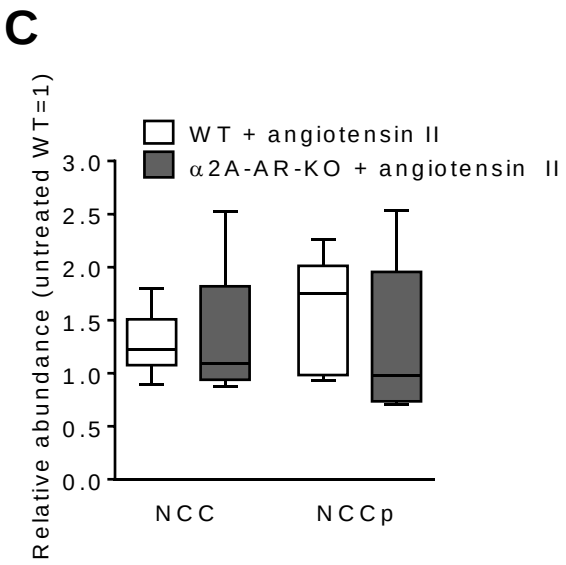
Supplemental Figure 1



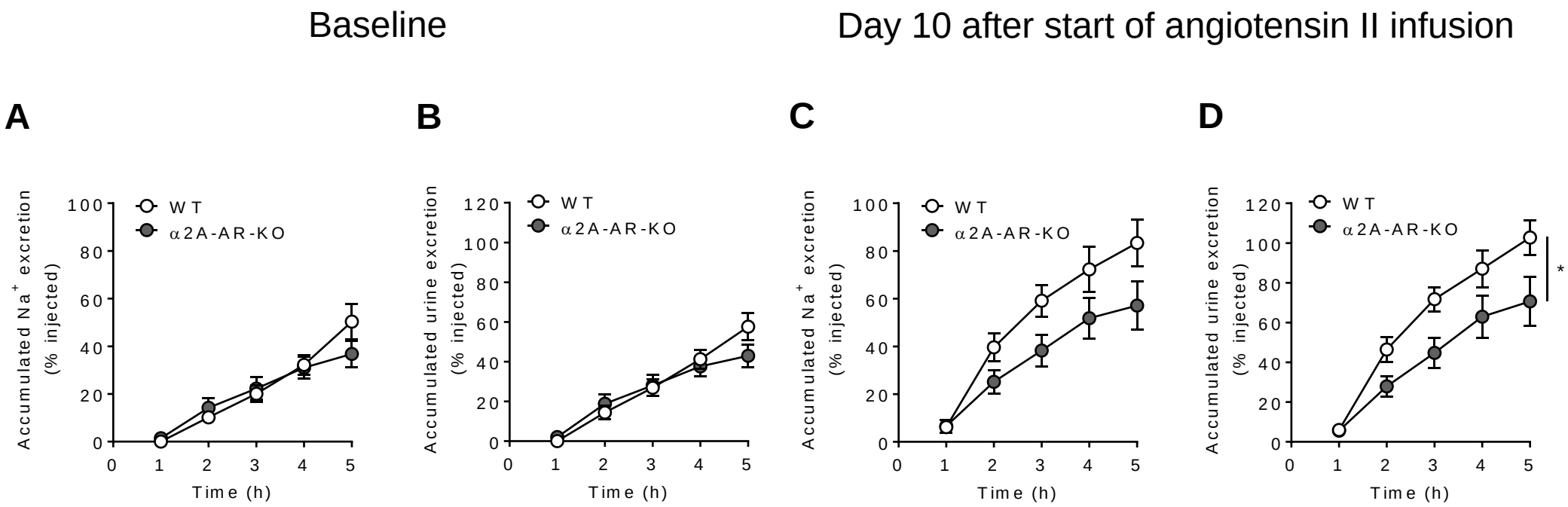
Supplemental Figure 2



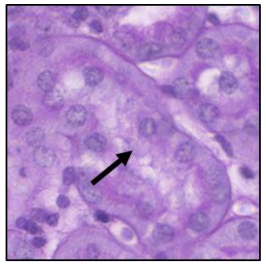


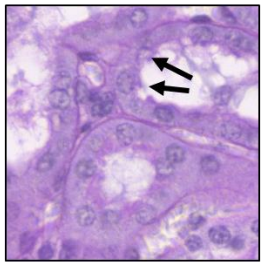


Supplemental Figure 3

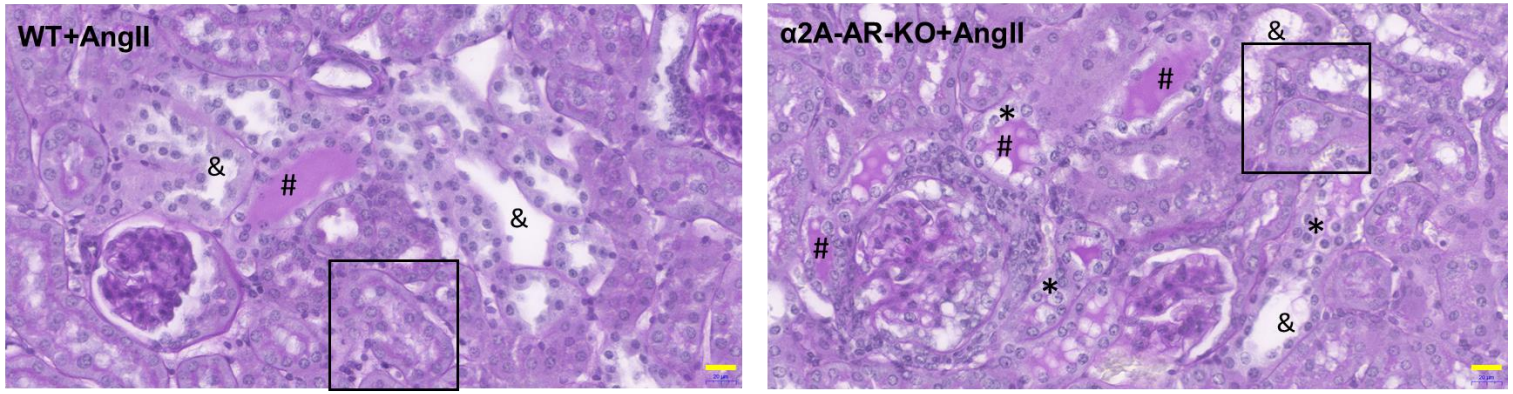


Supplemental Figure 4



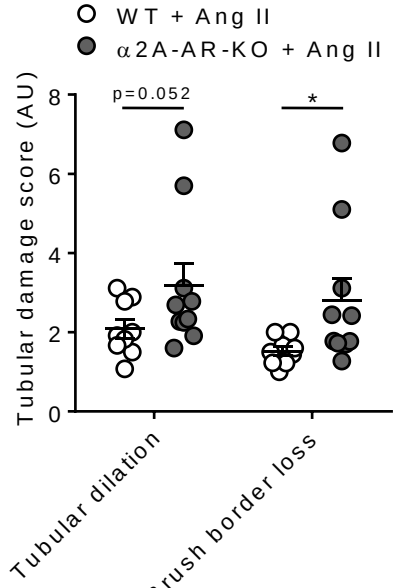


A

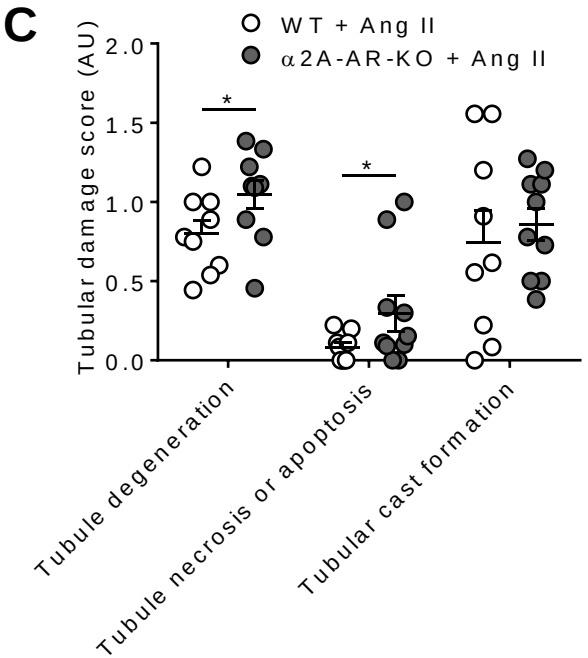


& tubular dilatation, <-brush border loss, *tubular degeneration, # tubular cast

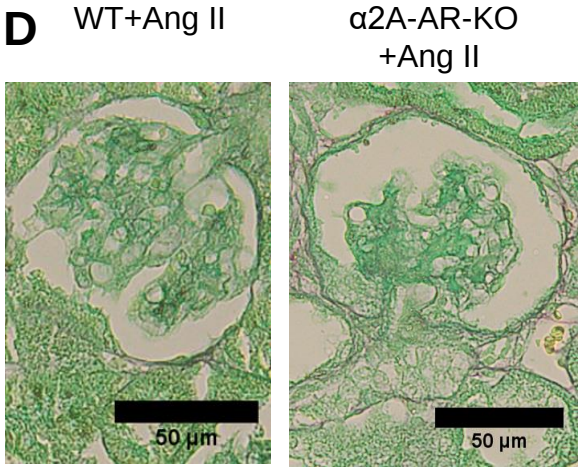
B



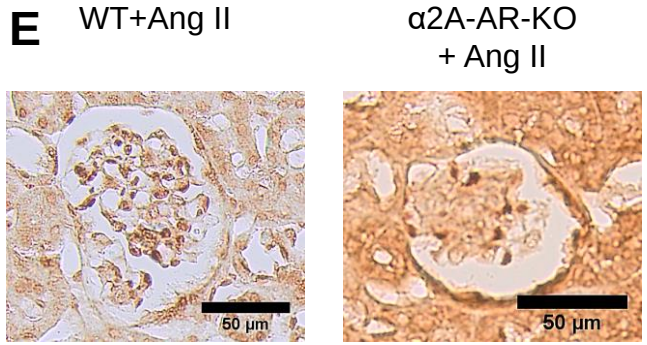
C



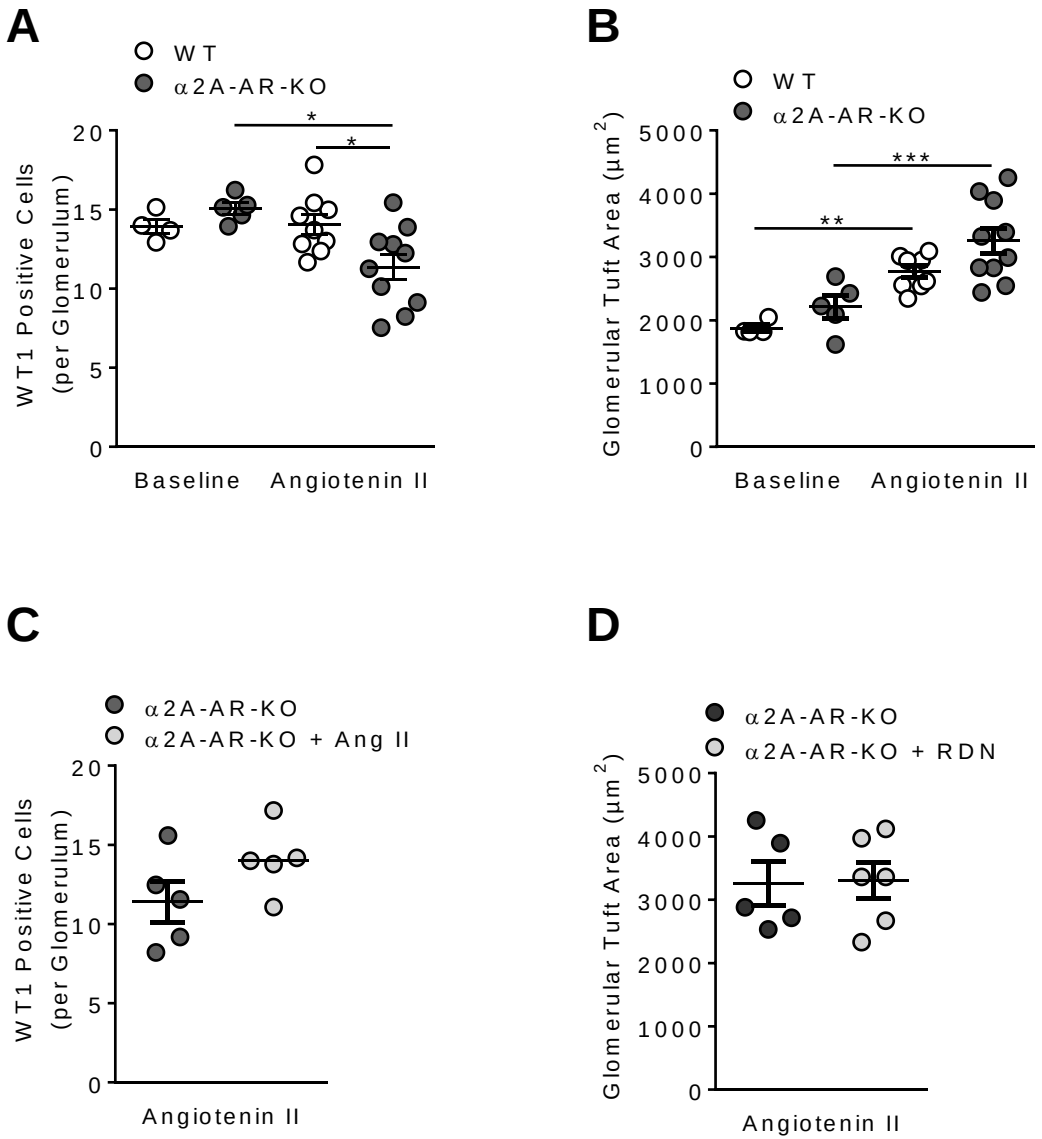
D



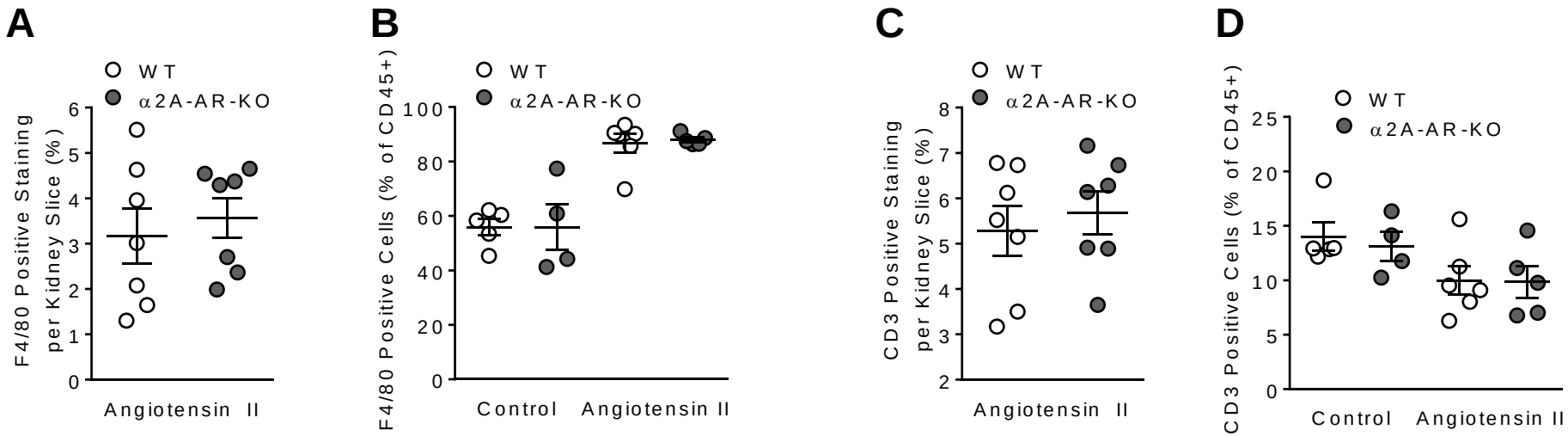
E



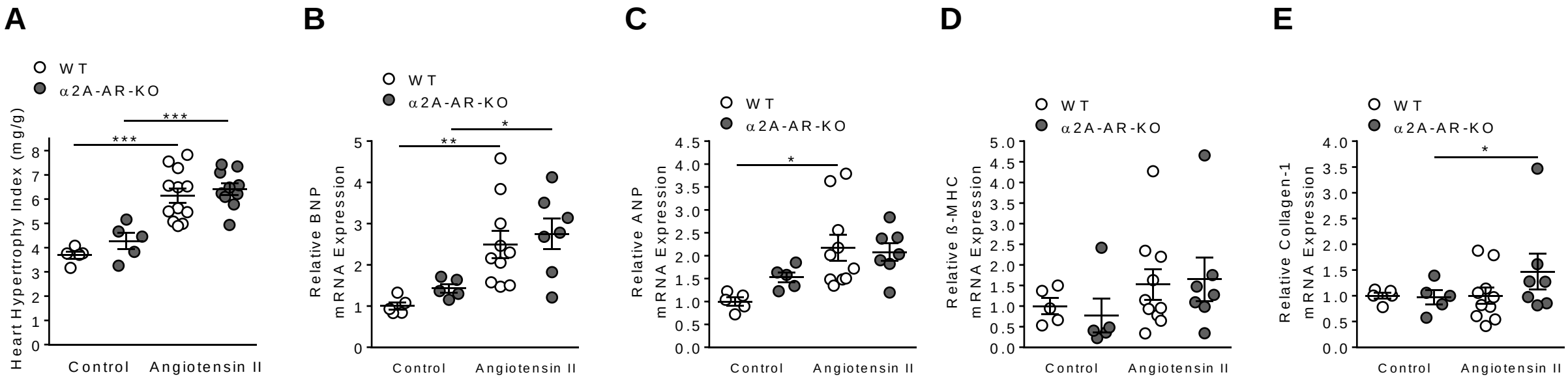
Supplemental Figure 5



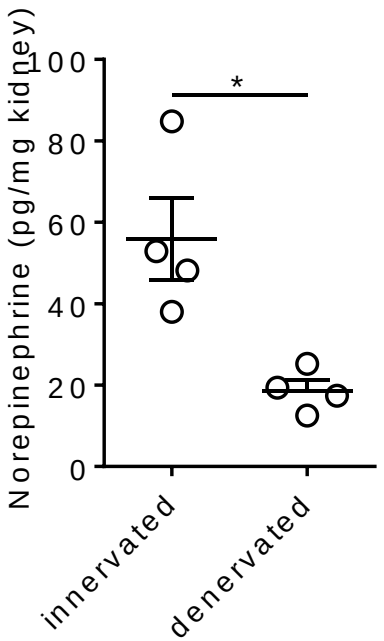
Supplemental Figure 6



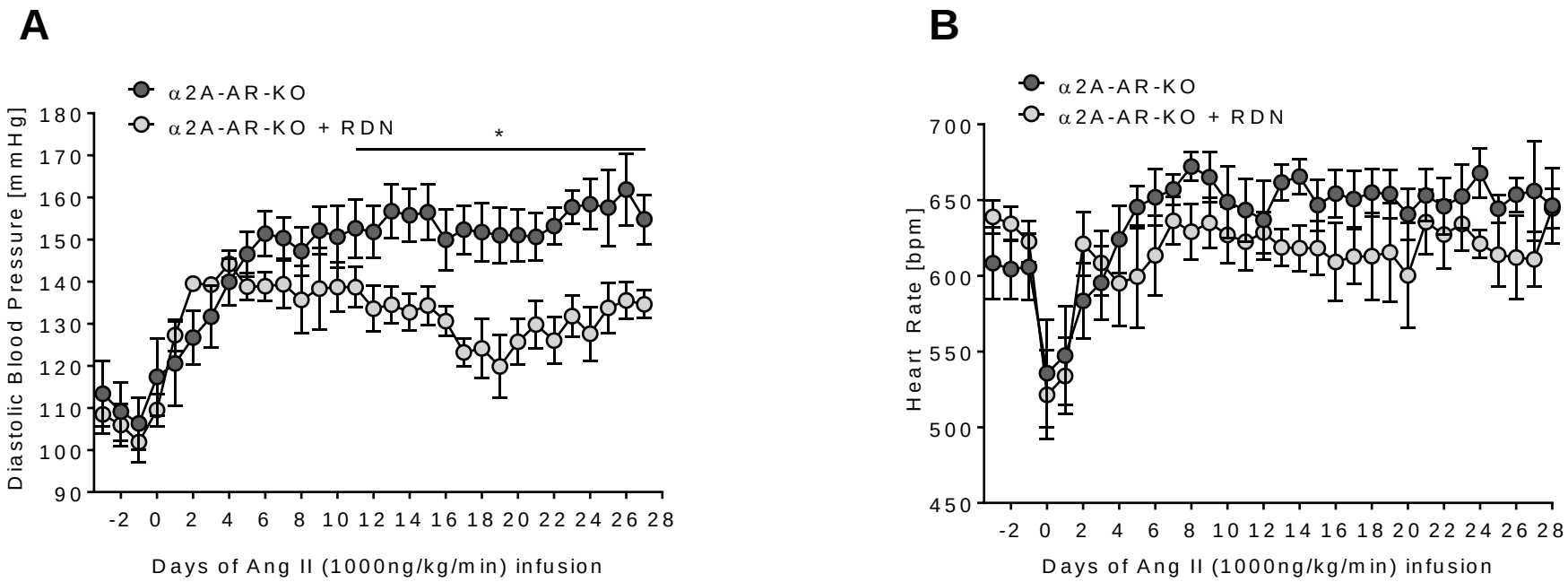
Supplemental Figure 7



Supplemental Figure 8

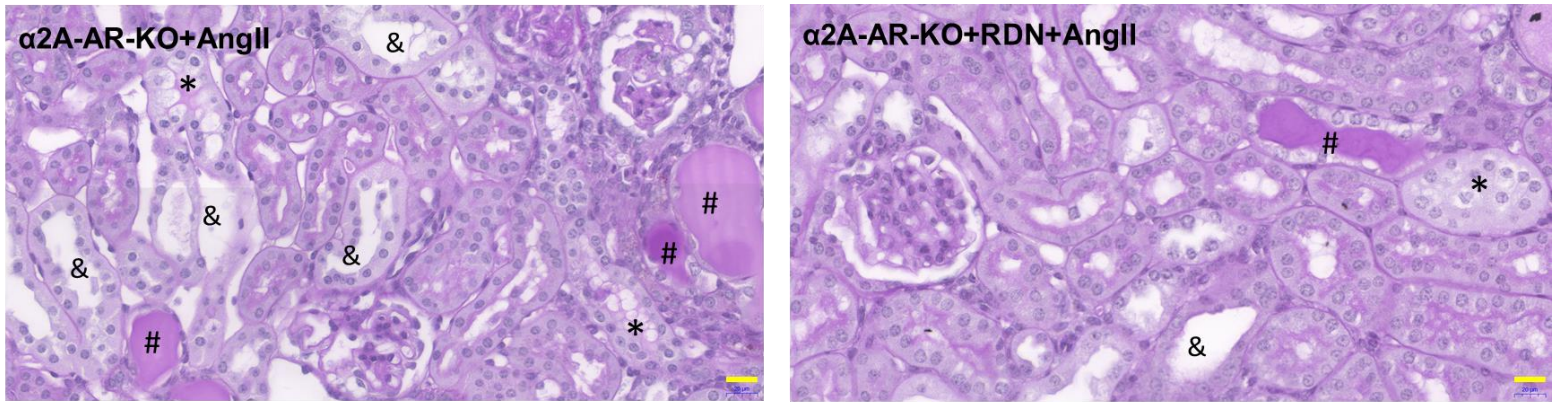


Supplemental Figure 9



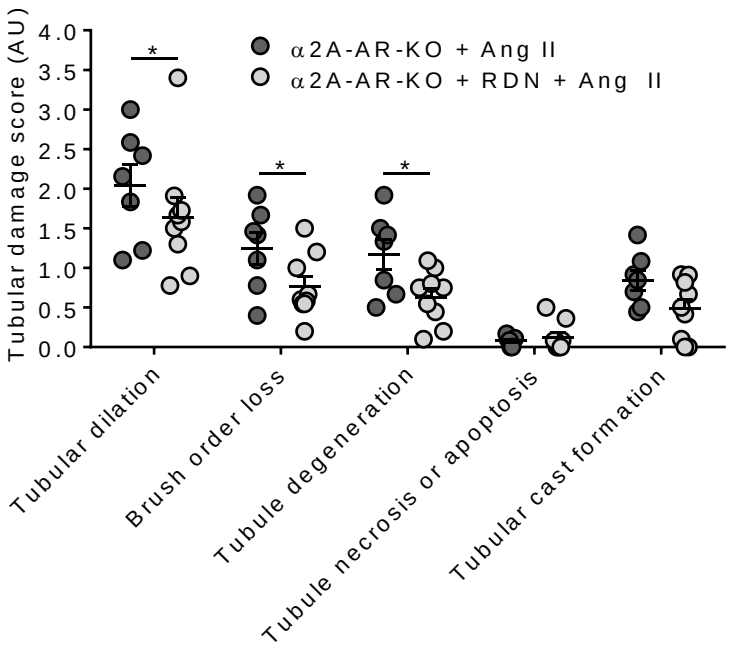
Supplemental Figure 10

A

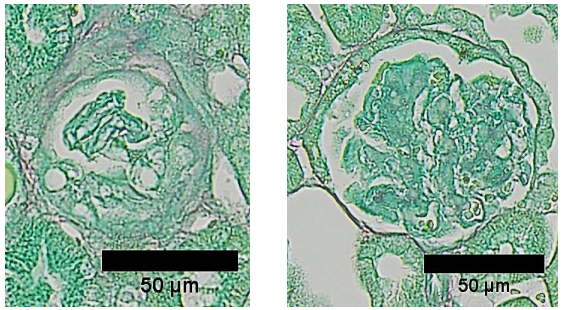


& tubular dilatation, *tubular degeneration, # tubular cast

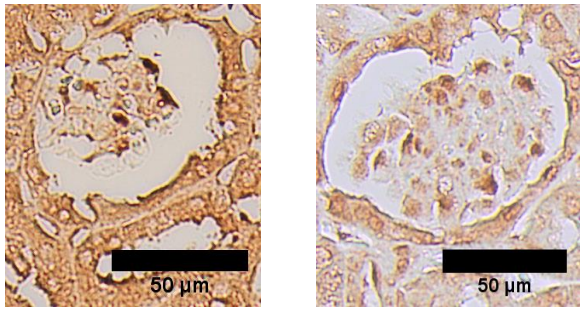
B



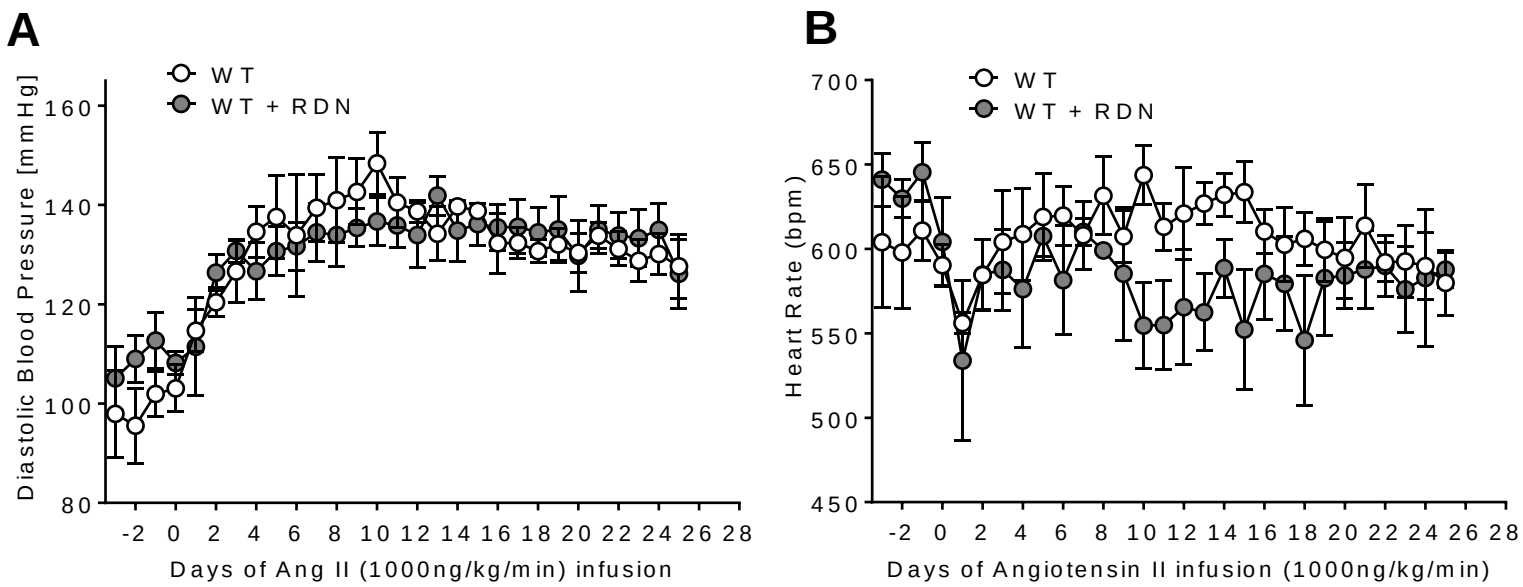
C α2A-AR-KO | α2A-AR-KO +RDN



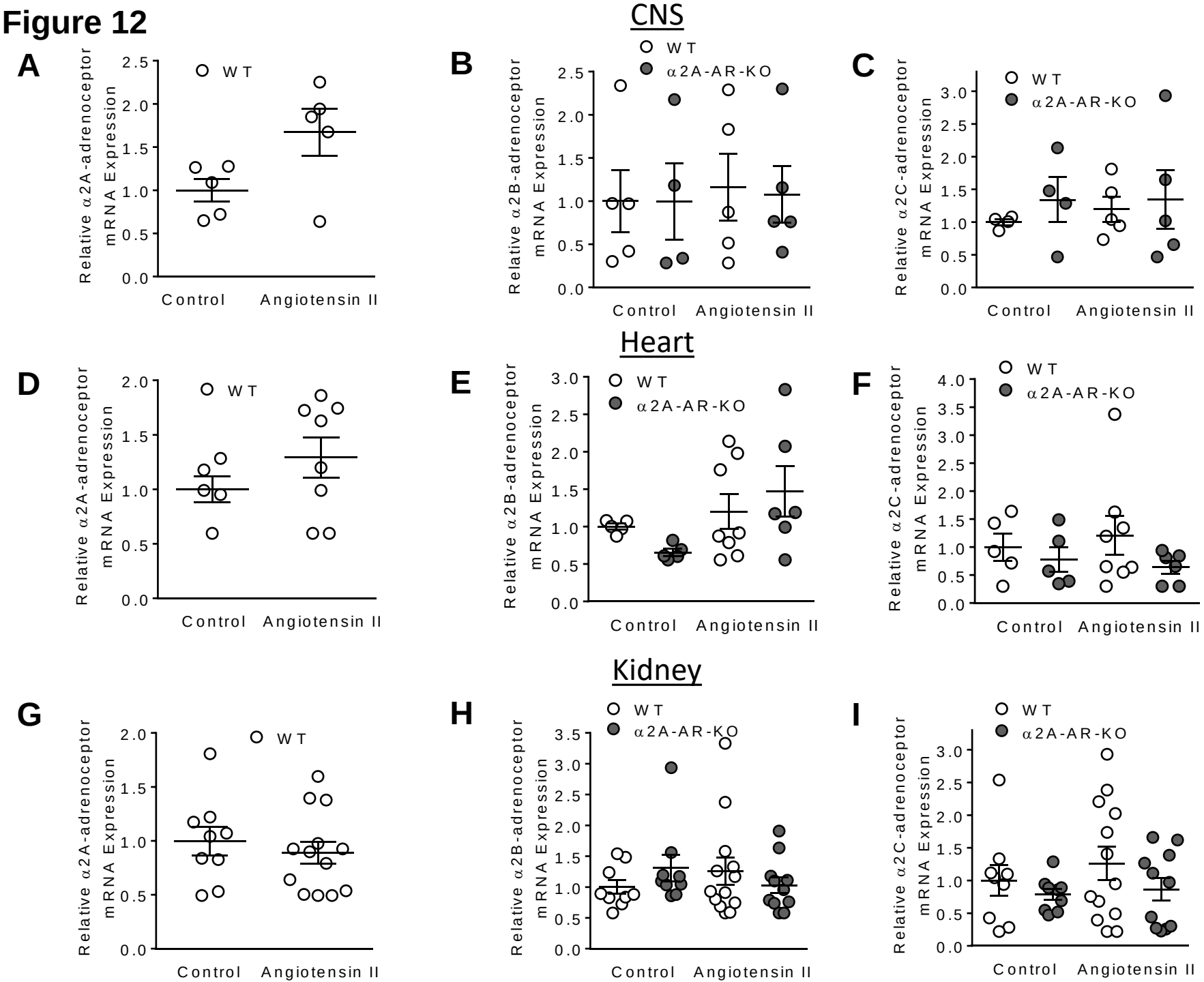
D α2A-AR-KO | α2A-AR-KO +RDN



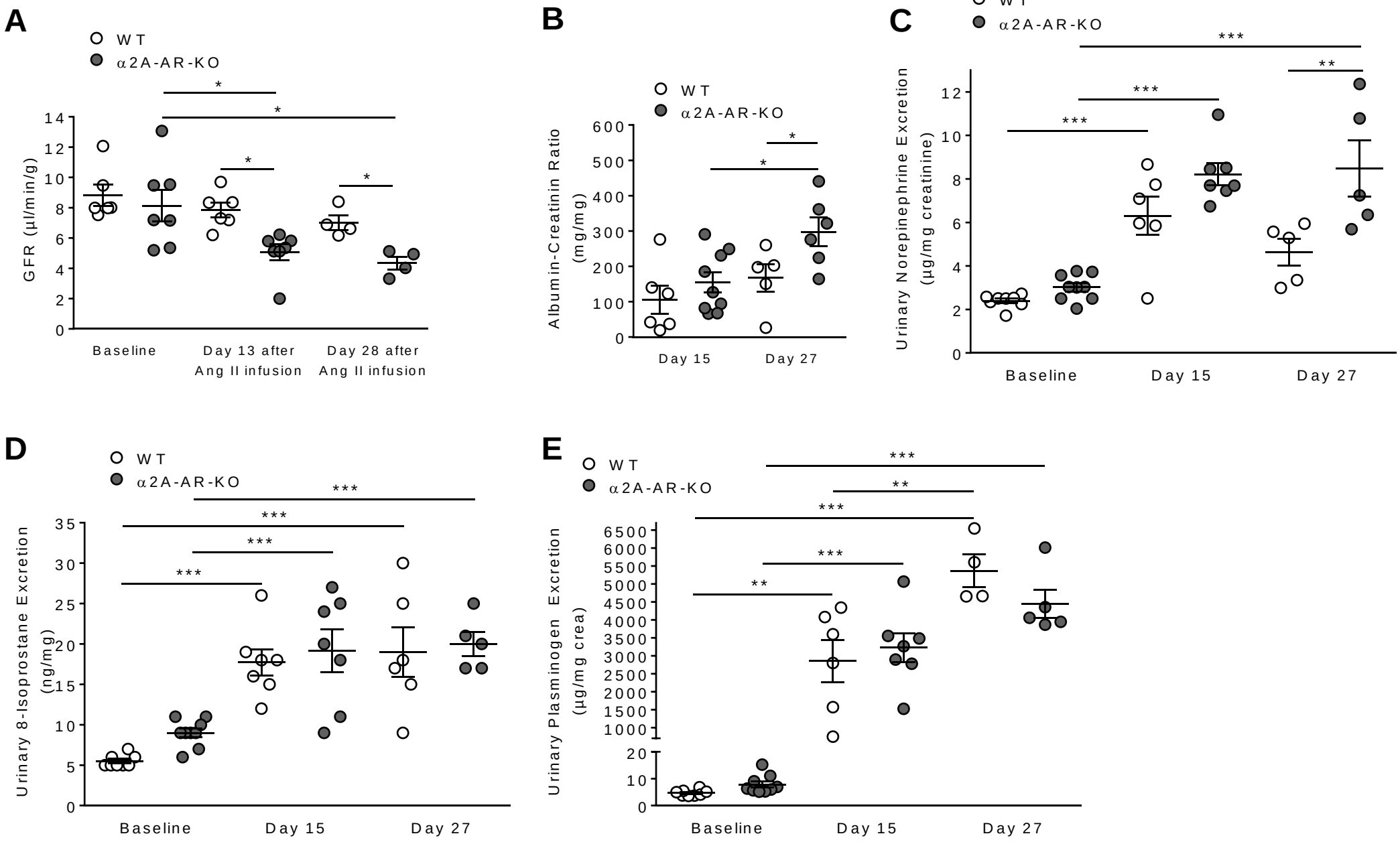
Supplemental Figure 11



Supplemental Figure 12

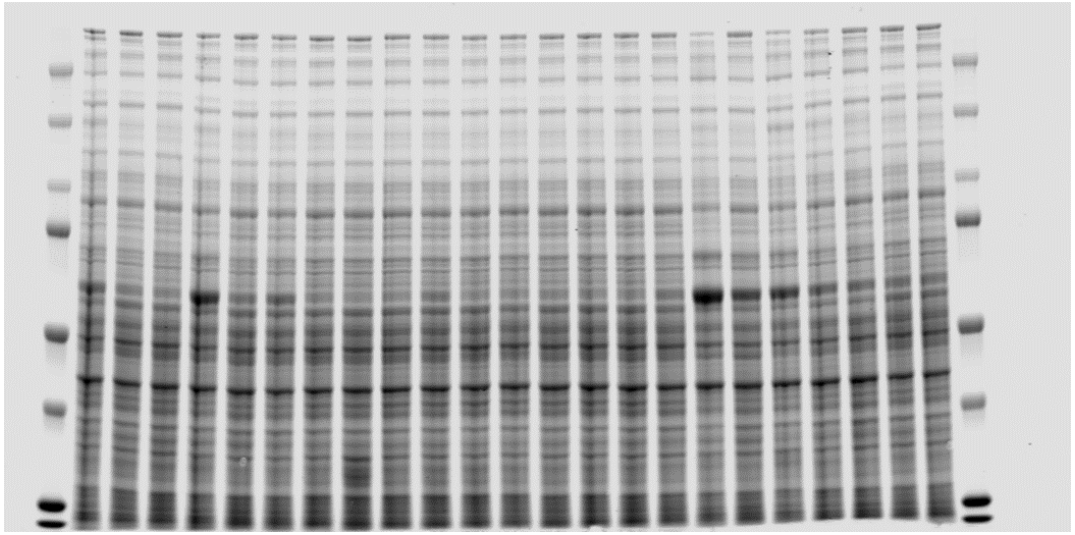


Supplemental Figure 13

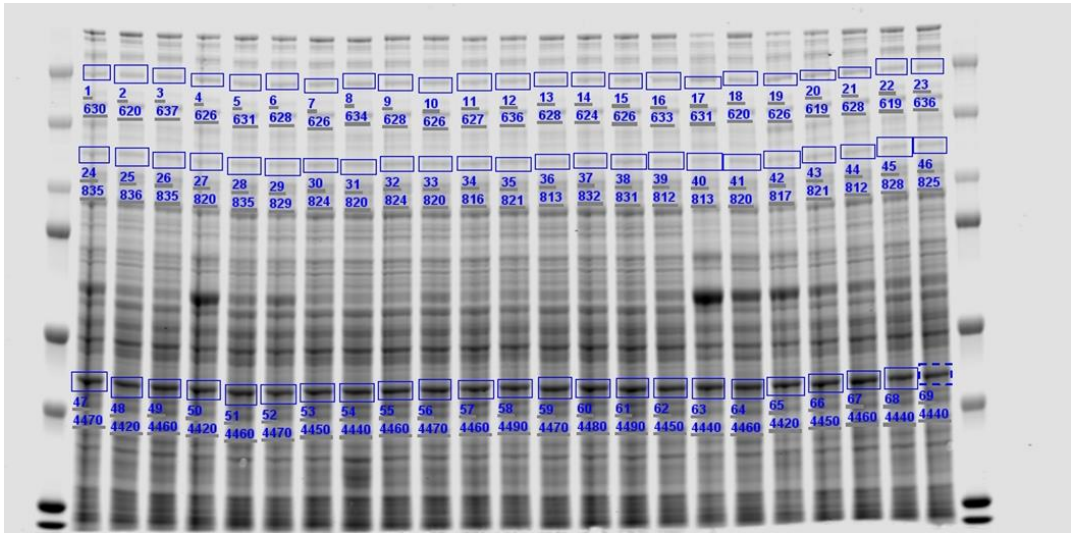


Supplemental Figure 14

A



B



C