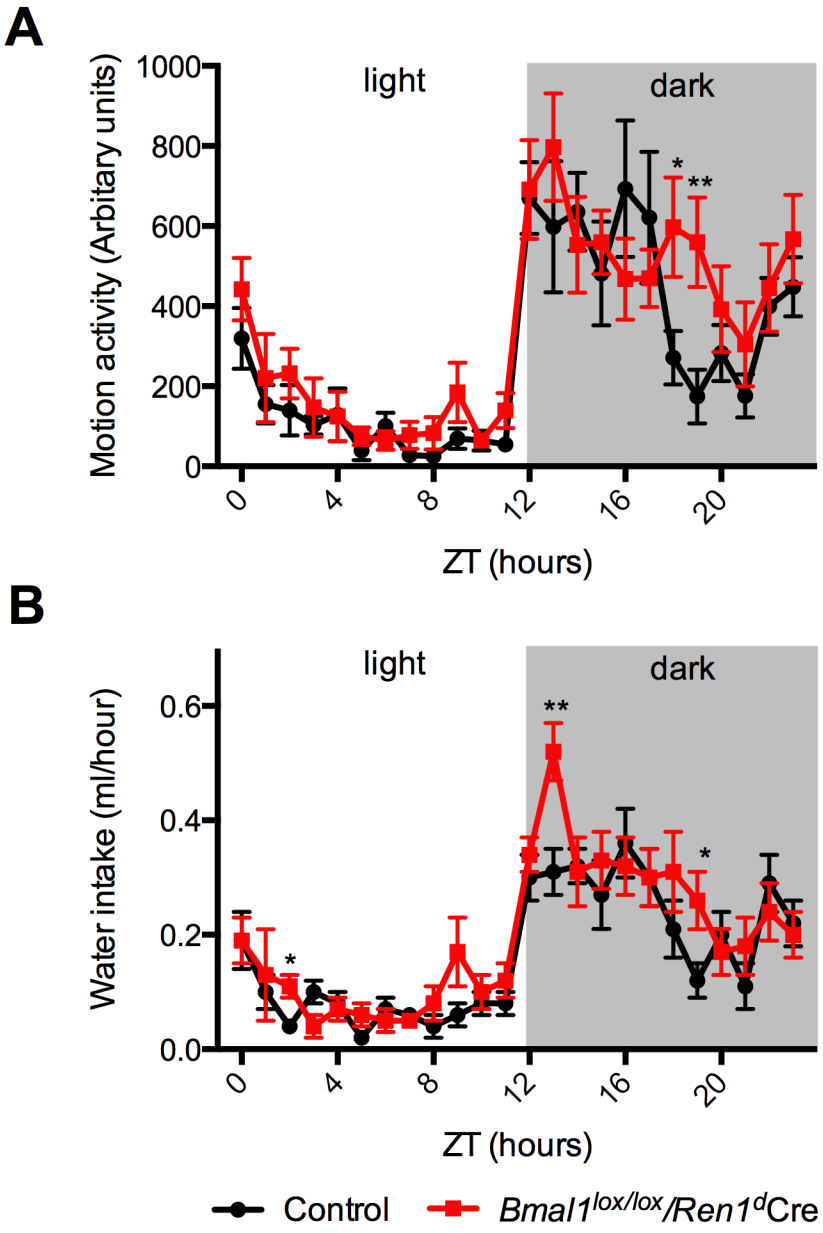
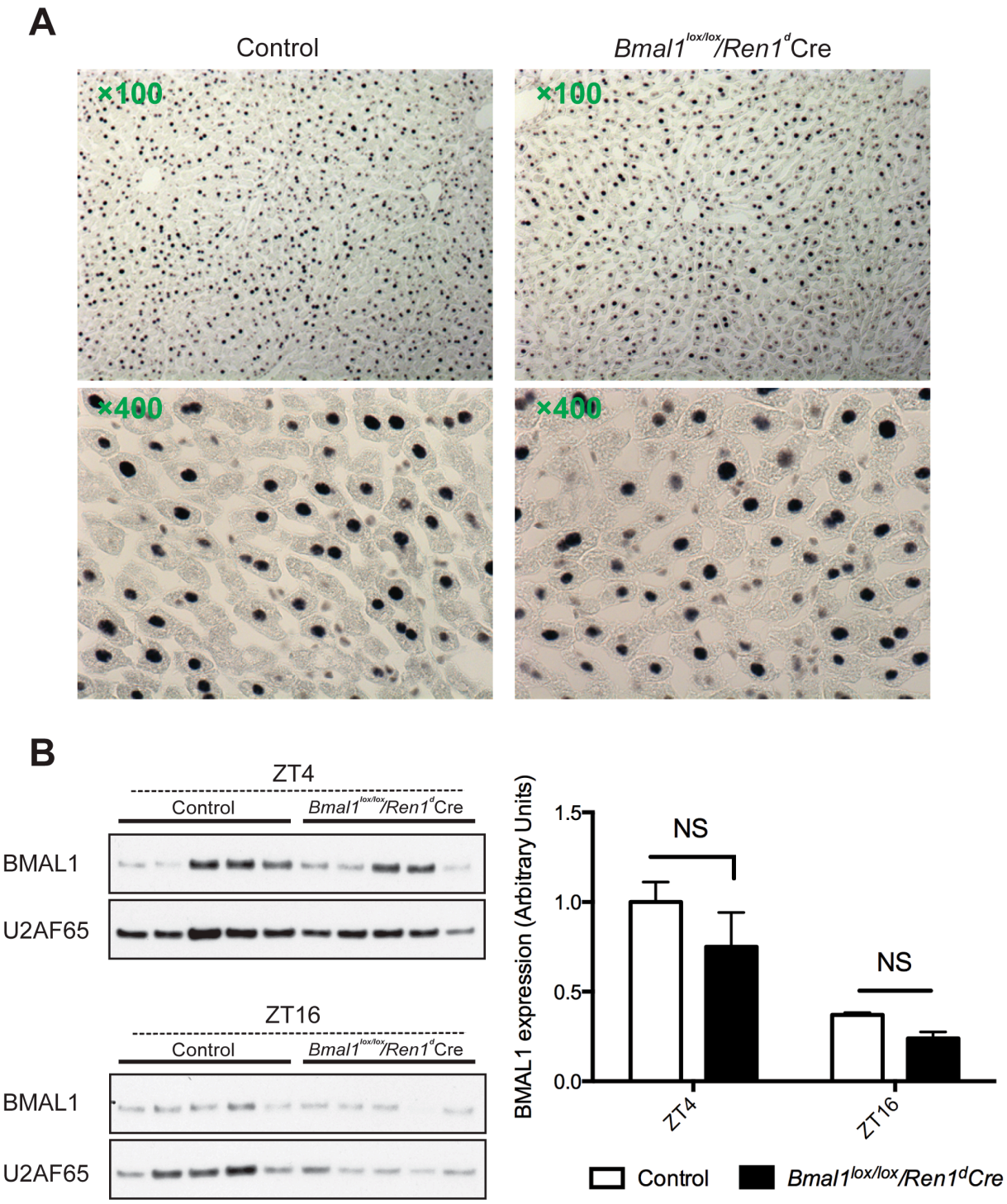
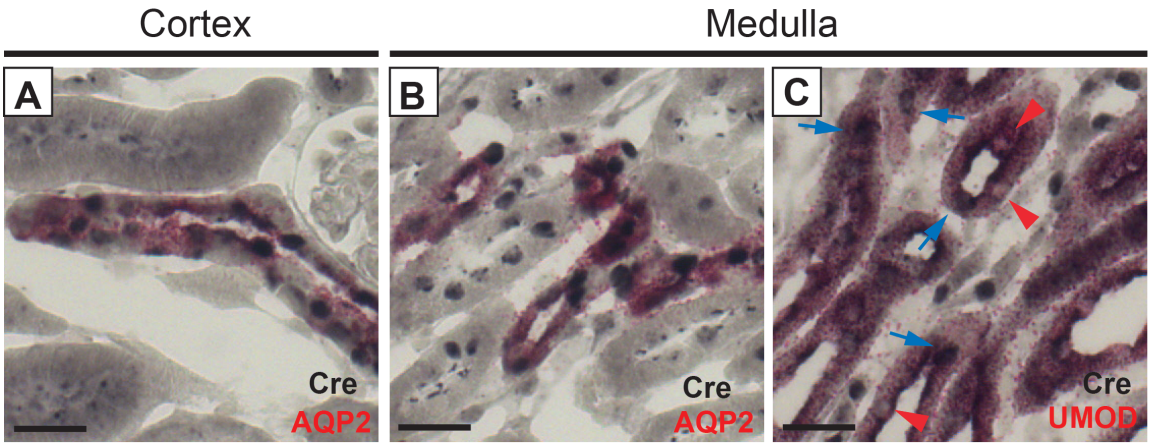


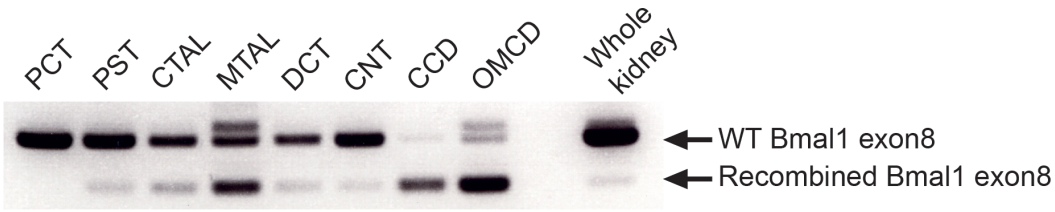
Supplementary Figure 1. Water intake and general motion activity in control and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice. **A**. Discontinuous 24-h measurement of general motion activity in control mice (black-line) or *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (red-line) integrated on an hourly basis. **B**. Discontinuous 24-h measurement of water intake in control mice (black-line) or *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (red-line) integrated on an hourly basis. Values are means from 9 individual mice for each genotype. Error bars show SEM. *, P<0.05, **, P<0.01, Statistical significance was calculated using unpaired *t*-test.



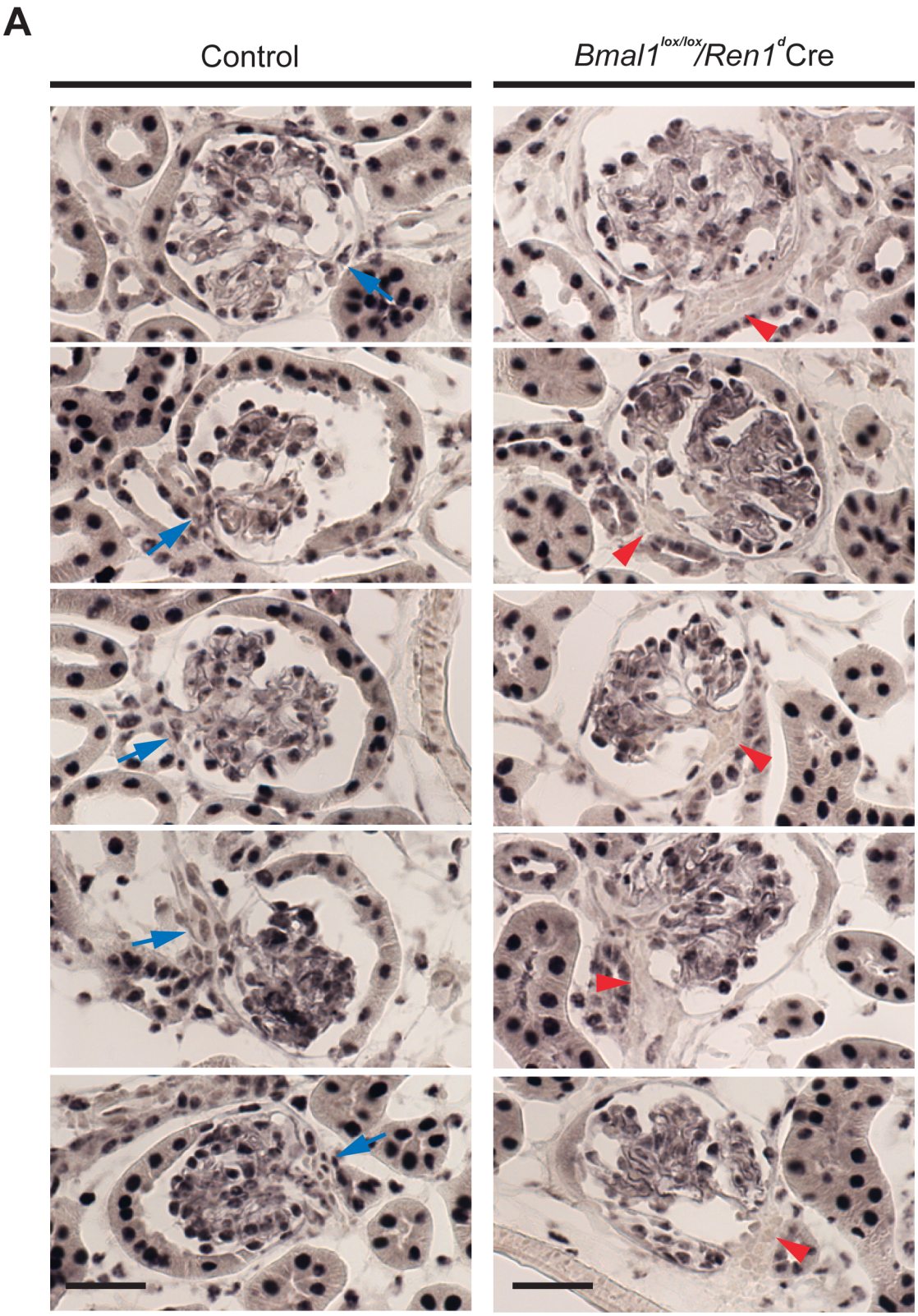
Supplementary Figure 2. Bmal1 protein expression in the liver of control and activity in control and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice. **A**. Immunohistochemical localization shows that a vast majority of liver cells express Bmal, both in control and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice (ZT4). **B**. Western blot performed with an anti-Bmal1 antibody on nuclear extracts prepared from the liver of control and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice (ZT4 and ZT16). The densitometry analysis of Western blots (**B**, right panel) did not reveal significant difference in Bmal1 protein expression levels between control and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice (*t*-test). The U2AF65 protein was used as control for experimental variations. Error bars show SEM.



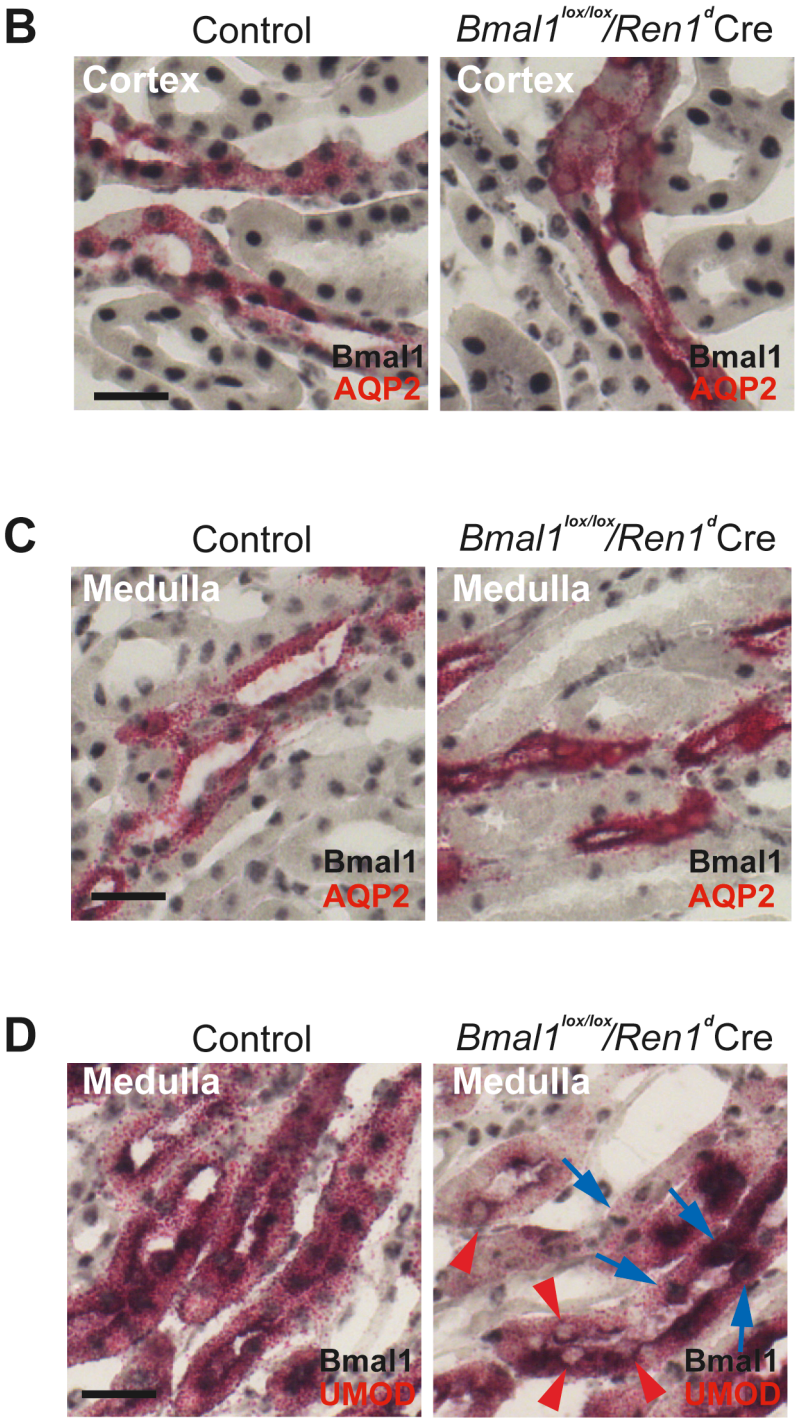
Supplementary Figure 3. Immunohistochemical localization of Cre recombinase in the renal cortex and outer medulla (ZT4)**.** Strong nuclear Cre staining (black) is present in the cortical collecting duct (**A**) and outer medullary collecting duct (**B**) as evidenced by co-staining with aquaporin-2 water channel (red). **C**. Cre recombinase expression in outer medullary thick ascending limb co-stained with uromodulin (red) is mosaic. Nucleus with or without Cre staining are shown by blue arrow or read arrow head respectively.



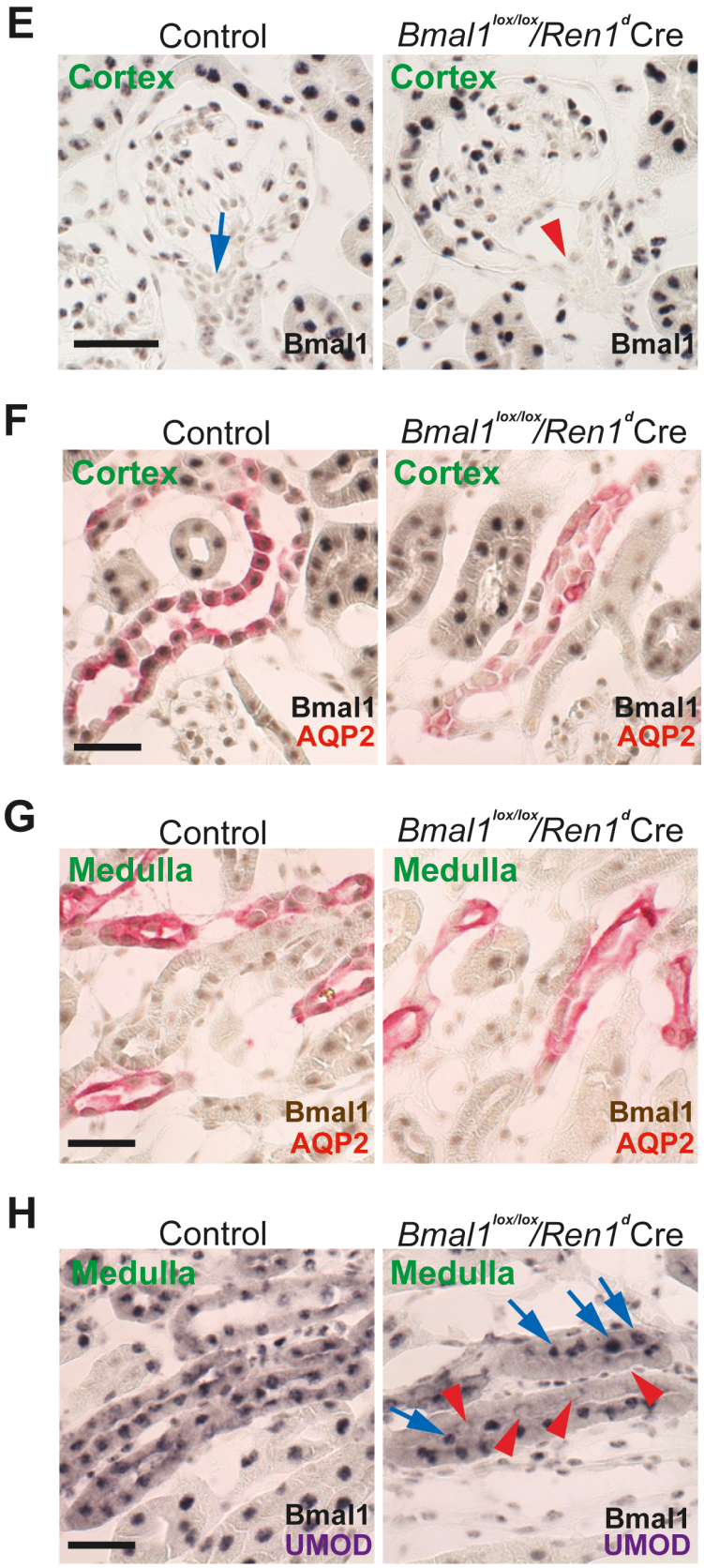
Supplementary Figure 4. RT-PCR analysis of Bmal1 mRNA expression in *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice performed with primers flanking the excised region on whole kidney or microdissected tubules of conditional Bmal1 knockout mice (ZT0).



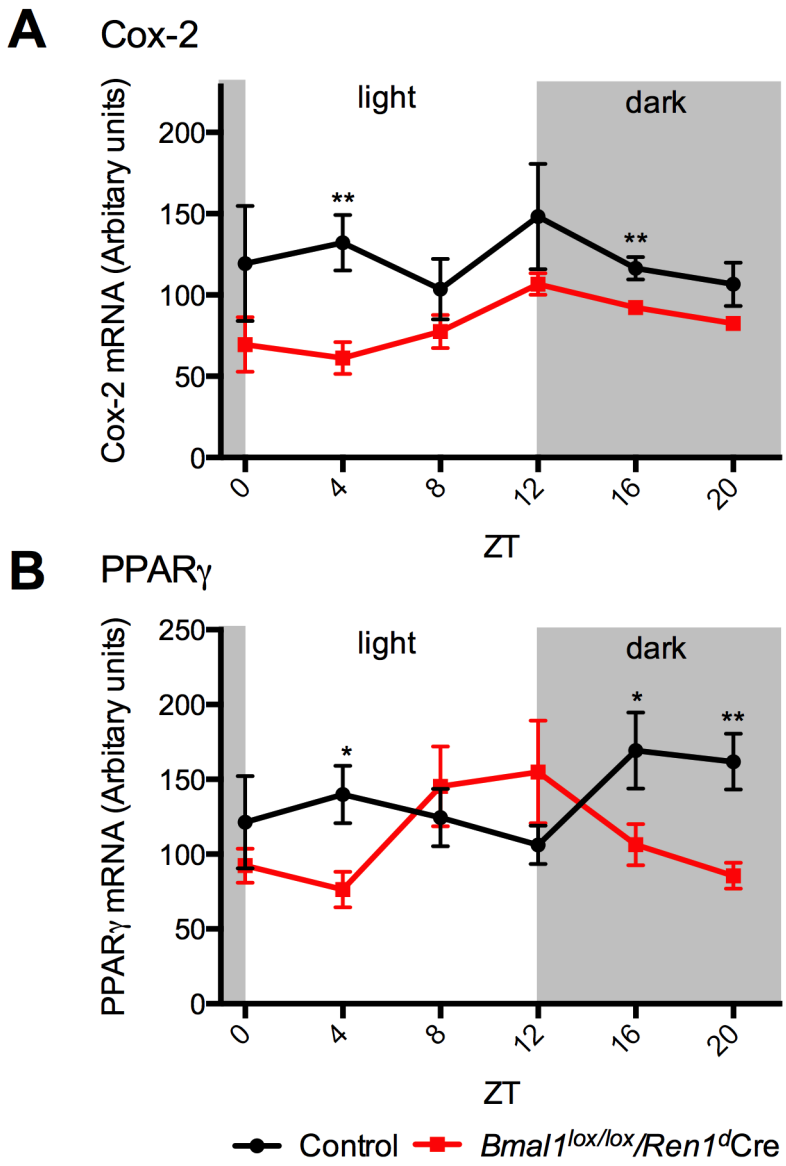
Supplementary Figure 5-1. **A.** Immunohistochemical localization of Bmal1 in the renal cortex (ZT4). Strong nuclear Bmal1 staining (black) is present in the arterioles of juxtaglomerular apparatus in Control mice (blue arrow) and is absent in *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice (red arrow head).



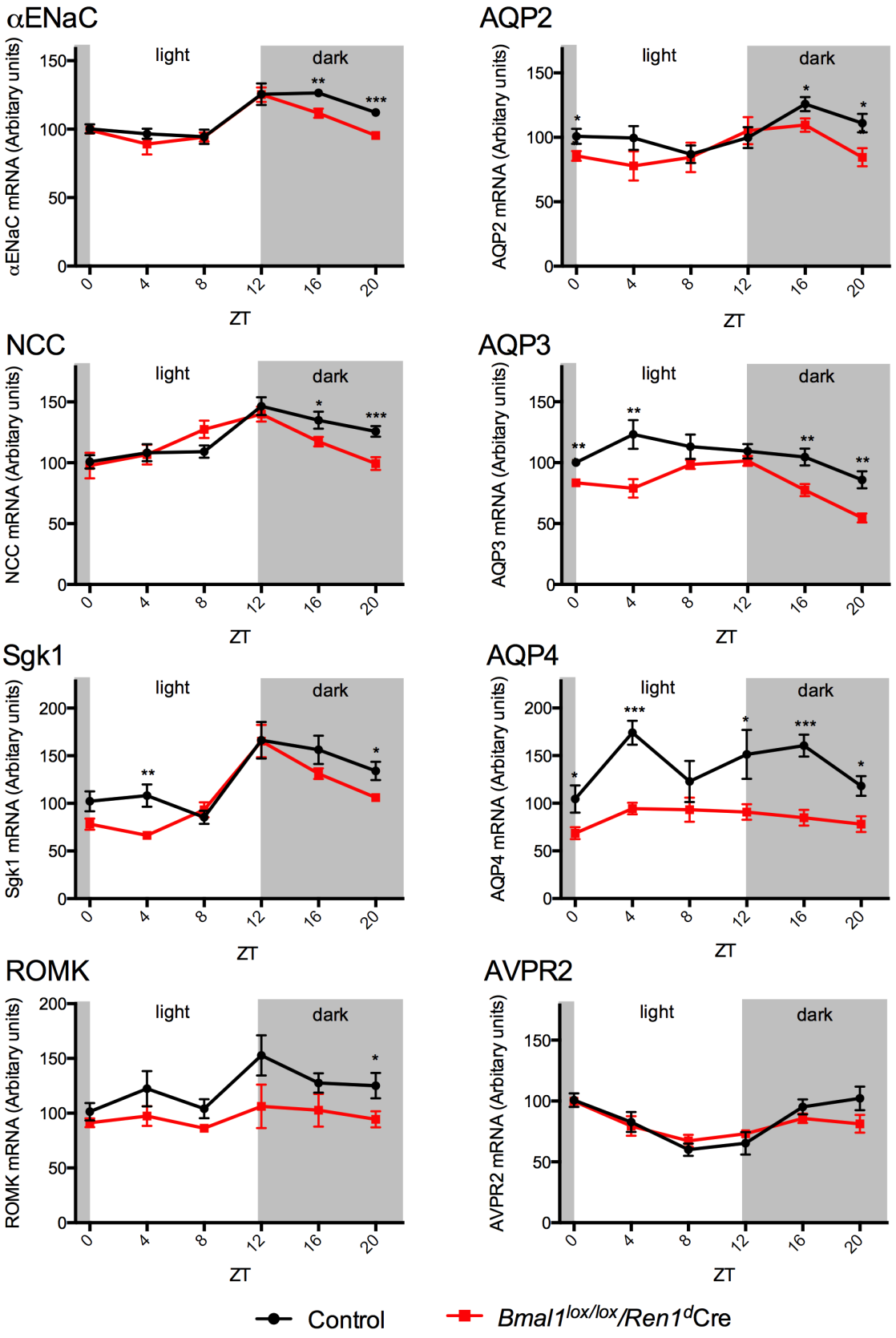
Supplementary Figure 5-2. Immunohistochemical localization of Bmal1 in the renal cortex and outer medulla (ZT4)**.** Strong nuclear Bmal1 staining (black) is ubiquitously present in control mice (left panels of **B,C,D**), whereas in *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice, the Bmal1 staining is absent in the cortical collecting duct (**B**, right panel) and outer medullary collecting duct (**C**, right panel), as evidenced by co-staining with aquaporin-2 water channel (red). **D**. partial decrease in Bmal1 expression in outer medullary thick ascending limb of *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice. Medullary thick ascending limb is co-stained with uromodulin (red). Nucleus positive or negative for Bmal1 are pointed by blue arrow or red arrow head, respectively.



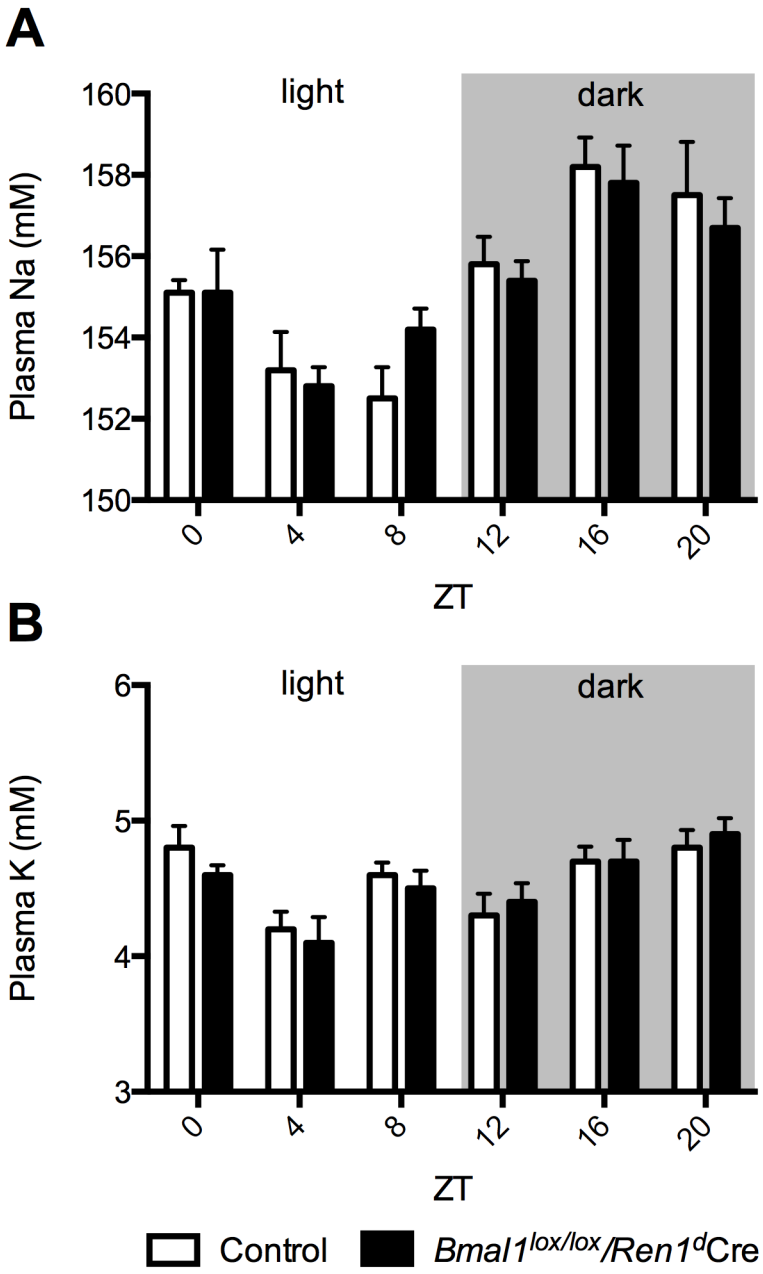
Supplementary Figure 5-3. Immunohistochemical localization of Bmal1 in the juxtaglomerular apparatus (**E**), the cortical collecting duct (CCD) (**F**), the outer medullary collecting duct (**G**) and the medullary thick ascending limb (MTAL) (**H**) at ZT16**.** Strong nuclear Bmal1 staining (black) is ubiquitously present in control mice (left panels of **E** (the juxtaglomerular cells are marked with the blue arrow head)**, F, G and H**), whereas in *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre mice, the Bmal1 staining is absent in juxtaglomerular apparatus (**E,** righ panel, the juxtaglomerular cells are marked with the red arrow head), the CCD (**F**, right panel), the OMCD (**G**, right panel) and in a subpopulation of the MTAL cells (**H**, right panel; the Bmal1-positive cells are marked with blue arrow heads whereas the Bmal1-negative cells are marked with red arrow heads). The CCD and the OMCD were recognized by co-staining with aquaporin-2 water channel (red) and the MTAL was recognized by co-staining with uromodulin.



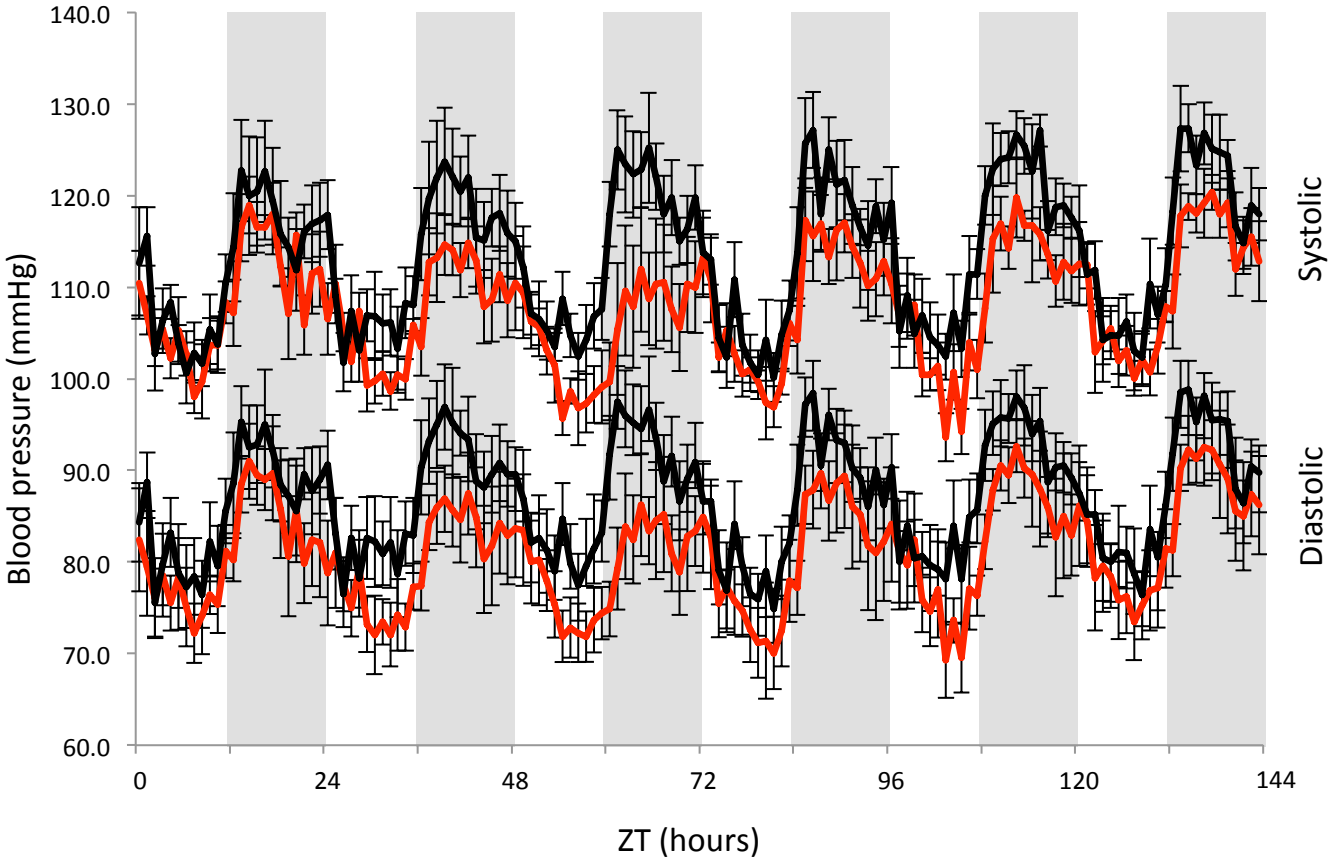
Supplementary Figure 6. **A.** Circadian pattern of Cox-2 mRNA expression in kidneys of Control (black line) and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (red line) mice (n=6/time-point). **B.** Circadian pattern of PPARγ mRNA expression in kidneys of Control (black line) and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (red line) mice (n=6/time-point). Kidneys were extracted from mice sacrificed at indicated circadian time-points. Data are means ± SEM. *, P<0.05, **, P<0.01, Statistical significance was calculated using unpaired *t*-test.



Supplementary Figure 7. Circadian pattern of αENaC, NCC, Sgk1, ROMK, AQP2, AQP3, AQP4, and AVPR2 mRNA expression in kidneys of Control (black line) and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (red line) mice (n=6/time-point) Kidneys were extracted from mice sacrificed at indicated circadian time-points. Data are means ± SEM. *, P<0.05, **, P<0.01, ***, P<0.005 Statistical significance was calculated using unpaired *t*-test



Supplementary Figure 8. Circadian patterns of plasma sodium (**A**) and potassium (**B**) concentrations in Control (white bars) and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (black bars) mice (n=6/time point). Data are means ± SEM.



Supplementary Figure 9. 6-days recordings of blood pressure in Control (black traces) and *Bmal1*$^{lox/lox}$/*Ren1*^{d}Cre (red traces) mice. Data are means ± SEM (n=7).

Supplementary Table 1. Analysis of Variance

Measure	Reference	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Ren1 mRNA	Figure 4A	Genotype	1	7112	7112.1	9.6399	**0.002775**
Ren1 mRNA	Figure 4A	Time	5	11483	2296.6	3.1128	**0.013719**
Ren1 mRNA	Figure 4A	Interaction Genotype:Time	5	1236	247.2	0.335	0.890036
Renin protein	Figure 4D	Genotype	1	1.0527	1.05265	4.4786	**0.03886**
Renin protein	Figure 4D	Time	5	0.8417	0.16833	0.7162	0.61399
Renin protein	Figure 4D	Interaction Genotype:Time	5	0.9107	0.18214	0.7749	0.57197
PRC	Figure 5A	Genotype	1	55	55.4	0.0712	0.790167
PRC	Figure 5A	Time	5	29467	5893.4	7.5859	**6.08E-06**
PRC	Figure 5A	Mouse to mouse variability	22	44654	2029.7	2.6126	**0.0008468**
PRC	Figure 5A	Interaction Genotype:Time	5	4498	899.5	1.1578	0.3366006
Plasma aldosterone	Figure 5B	Genotype	1	423505	423505	30.1296	**4.30E-07**
Plasma aldosterone	Figure 5B	Time	5	718615	143723	10.2249	**1.17E-07**
Plasma aldosterone	Figure 5B	Mouse to mouse variability	18	332602	18478	1.3146	0.2005
Plasma aldosterone	Figure 5B	Interaction Genotype:Time	5	376656	75331	5.36E+00	**0.0002557**
Plasma angiotensinogen	Figure 5C	Genotype	1	0.8164	0.81645	6.8591	**0.01021**
Plasma angiotensinogen	Figure 5C	Time	5	10.8235	2.16471	18.186	**9.17E-13**
Plasma angiotensinogen	Figure 5C	Mouse to mouse variability	22	3.0155	0.13707	1.1515	0.30915
Plasma angiotensinogen	Figure 5C	Interaction Genotype:Time	5	0.5335	0.1067	0.8964	0.48667
Urine Na/Creatinine	Figure 6B	Genotype	1	8.1	8.09	0.5662	0.4525
Urine Na/Creatinine	Figure 6B	Time	1	2521.8	2521.79	176.3791	**<2E-16**
Urine Na/Creatinine	Figure 6B	Mouse to mouse variability	40	5188.2	129.71	9.0719	**<2E-16**
Urine Na/Creatinine	Figure 6B	Interaction Genotype:Time	1	481.2	481.2	33.6561	**1.99E-08**
Urine K/Creatinine	Figure 6C	Genotype	1	64.8	64.79	1.2576	0.26318
Urine K/Creatinine	Figure 6C	Time	1	519.7	519.66	10.0868	**0.001682**
Urine K/Creatinine	Figure 6C	Mouse to mouse variability	40	28895.1	722.38	14.0216	**<2E-16**
Urine K/Creatinine	Figure 6C	Interaction Genotype:Time	1	102	102.02	1.9802	0.160617
Urine Na/K	Figure 6D	Genotype	1	0.01406	0.01406	3.3634	0.06784
Urine Na/K	Figure 6D	Time	1	1.45183	1.45183	347.4199	**<2E-16**
Urine Na/K	Figure 6D	Mouse to mouse variability	40	2.06581	0.05165	12.3586	**<2E-16**
Urine Na/K	Figure 6D	Interaction Genotype:Time	1	0.21856	0.21856	52.3007	**5.65E-12**
Systolic blood pressure	Figure 7A	Genotype	1	4790.5	4790.5	196.8897	**<2e-16**

Systolic blood pressure	Figure 7A	Time	23	15267.8	663.8	27.283	**<2e-16**
Systolic blood pressure	Figure 7A	Mouse to mouse variability	12	6138	511.5	21.0228	**<2e-16**
Systolic blood pressure	Figure 7A	Interaction Genotype:Time	23	487.1	21.2	0.8703	0.6388
Diastolic blood pressure	Figure 7B	Genotype	1	2617.5	2617.47	127.5658	**<2e-16**
Diastolic blood pressure	Figure 7B	Time	23	10771	468.31	22.8235	**<2e-16**
Diastolic blood pressure	Figure 7B	Mouse to mouse variability	12	22968	1914	93.2814	**<2e-16**
Diastolic blood pressure	Figure 7B	Interaction Genotype:Time	23	359.7	15.64	0.7622	0.777
Plasma Na	Suppl. Figure 7A	Genotype	1	0.08	0.081	0.0177	0.89451
Plasma Na	Suppl. Figure 7A	Time	5	305.92	61.183	13.3224	**4.01E-09**
Plasma Na	Suppl. Figure 7A	Mouse to mouse variability	17	141.93	8.349	1.818	**0.0426**
Plasma Na	Suppl. Figure 7A	Interaction Genotype:Time	5	21.22	4.243	9.24E-01	0.4708
Plasma K	Suppl. Figure 7B	Genotype	1	0.0088	0.00884	0.0731	0.78774
Plasma K	Suppl. Figure 7B	Time	5	4.9424	0.98848	8.1672	**4.08E-06**
Plasma K	Suppl. Figure 7B	Mouse to mouse variability	17	3.7335	0.21962	1.8146	**0.04314**
Plasma K	Suppl. Figure 7B	Interaction Genotype:Time	5	0.6399	0.12797	1.0574	0.39145

Supplementary Table 2. Circadian periodicity analysis

Measure	Genotype	Acrophase (ZT hours)	P value
Plasma Na	WT	17.70	3.71E-07
Plasma Na	KO	17.15	2.30E-04
Plasma K	WT	20.01	3.98E-02
Plasma K	KO	18.31	1.60E-03
PRC	WT	8.74	1.47E-05
PRC	KO	10.72	1.97E-02
Plasma Aldosterone	WT	1.34	3.06E-04
Plasma Aldosterone	KO	22.10	7.66E-03
Plasma Angiotensinogen	WT	7.70	1.22E-02
Plasma Angiotensinogen	KO	7.27	4.81E-06

Supplementary methods

1. Immunohistochemistry

Mice were anesthetized with ketamine/xylazine and 20 ml of 2% paraformaldehyde in PBS was perfused from the renal artery at ZT4. Kidneys were removed, decapsulated and transferred to 30% sucrose in PBS. Then, kidneys were embedded in OCT compound (Tissue-Tek) and were frozen. 8μm-thick frozen sections were microwaved for 15 minutes for the antigen retrieval in the buffer containing 1mM EDTA and 10mM Na-Citrate (pH 6.0). After washing steps, sections were blocked with 4% Block Ace (DS Pharma) in 0.05% Tween 20, PBS and avidin/biotin blocking solution (Vector Laboratories) for 1 hour at room temperature. Sections were then incubated with primary antibodies: anti-Bmal1 rabbit IgG (1/400), anti-Cre-recombinase rabbit IgG (1/200, Novagen), anti-AQP2 goat IgG (1/200, SantaCruz), anti-UMOD sheep IgG (1/20, LS-Bio), over night at room temperature. After washing steps with PBS, sections were incubated with 0.3% H_2O_2 in PBS for 30 minutes in order to quench the endogenous peroxidase. Then, sections were incubated with secondary antibodies: anti-rabbit biotinylated antibody (1/200, Vector Laboratories), anti-goat alkaline phosphatase conjugated antibody (1/500, Vector Laboratories), and anti-sheep alkaline phosphatase conjugated antibody (1/500, SantaCruz) for 30 minutes at room temperature. Avidin/biotin complex was enhanced by ABC elite kit (Vector Laboratories) and detected by DAB-Nickel solution supplemented with hydrogen peroxidase. Alkaline phosphatase conjugated signal was detected by VectorRED (Vector Laboratories). Then, sections were dehydrated and mounted with Histkitt (Roti), and images were acquired with Axovision (Zeiss).

2. Microdissection

Mice were anesthetized with ketamine/xylazine and perfused with 10 ml of DMEM supplemented with 40 μg/ml liberase (Roche) at ZT0. Then, left kidneys were decapsuled and cut into small pieces followed by the incubation for 30 minutes at 37°C in DMEM with 40 μg/ml liberase. Kidney pieces were washed 2 times with DMEM and kidney segments were microdissected in ice-cold 0.05% BSA/DMEM.

3. Blood and tissue collection

For plasma renin concentration (PRC) and plasma angiotensinogen concentration, ~ 10 μl of blood was collected into ice-cold glass capillaries containing Na-EDTA from the tail vein of conscious mice to avoid stress-induced renin secretion due to the anesthesia. Blood collection was performed on the same mice at ZT0, 4 and 8 on mice adapted to the normal circadian cycle and at ZT12, 16 and 20 on mice adapted to the inverted circadian cycle with at least one week of interval between blood collections at different time-points. For plasma aldosterone and electrolytes measurement, mice were anesthetized with ketamine/xylazine and heparinized arterial and venous mix blood was collected on ice from retro-orbital sinus. Plasma was isolated after the centrifugation for 10 min at 6000 rpm, 4°C and stored at -80°C. For RNA and protein extraction, organs were collected after the blood collection described above. Mice were sacrificed by cervical dislocation and kidneys, brain, lung, heart, liver and adrenal gland were dissected and snap frozen in liquid nitrogen.

4. RNA extraction and RT-PCR for microdissected tubules and whole organs

Microdissected tubules were collected into 200 μl Trizol and RNA was extracted as manufacturer protocol. Extracted RNA was purified with RNeasy Micro kit (QIAGEN). 5 μl RNA was used for the reverse transcription with PrimeScript RT Reagent kit (TAKARA) and cDNA was diluted 30 times. For whole organ RNA, half frozen kidneys and whole other organs were homogenized with the aid of Polytron homogenizer in D-buffer (4M Guanidium thiocyanate, 25mM Na-citrate, 0.5% Na-lauroylsarcosyl, 0.1M β-mercaptoethanol), NaOAC (pH 4.0), saturated phenol (pH 4.0) and chloroform-isomylalcohol solution followed by centrifuging at 10’000 g for 20 min. RNA pellet was obtained from the aqueous phase following isopropanol addition and centrifugation. Pellet was washed with 70% ethanol and eluted with 300μl RNase-free water. Genomic DNA was digested with DNase I (QIAGEN), and RNA was purified with RNeasy Micro kit (QIAGEN) as manufacture protocol. Reverse transcription was performed with 1μg RNA by using PrimeScript RT Reagent kit (TAKARA) and cDNA was diluted 30 times.

5. Quantitative PCR

4μl of cDNA was used for quantitative real-time PCR. Assays were performed with Taqman probes and master mix (Applied Biosystems). mRNA expression was normalized with Actin expression for each assay.

6. Qualitative PCR for recombined Bmal1 gene

Recombined Bmal1 transcript was detected by PCR with cDNA described as above and primers designed as following; forward: 5'-TGGACACAGACAAAGATGACCCTCA-3' and reverse: 5'-TCCCTCGGTCACATCCTACGACA-3'.

7. Western blot

Whole decapsulated kidneys were homogenized with a polytron in 3 ml RIPA buffer containing 50mM Tris-HCl (pH 7.2), 150mM NaCl, 0.1% SDS, 0.5% Na-deoxycholate, 1% NP40, 1mM PMSF and protease inhibitors. Protein extracts were sonicated and centrifuged for 5 minute at 1500rpm. The supernatant was recovered and the protein concentration was measured with Pierce BCA protein assay reagent (Thermo), then the concentration was adjusted to 8mg/ml with RIPA buffer. Samples were mixed with Laemmli sample buffer (BIO-RAD) supplemented with 2-mercaptoethanol (SIGMA), and were heated for 15 minutes at 56℃. 40μg of protein samples were migrated in the Mini- PROTEAN TGX gels, 4-20% (BIO-RAD), then transferred to the nitrocellulose membrane in the transfer buffer containing 20% Methanol. Membranes were stained with Ponseau S and then washed in TBS containing 0.2% NP40. Then, membranes were incubated with 5% skim milk in 0.2% NP40/TBS for 1 hour at the room temperature for the blocking, followed by the incubation with primary antibodies in the blocking solution, overnight (cold room). Membranes were washed and blocked as above, and incubated with anti-rabbit horseradish peroxidase conjugated IgG in 5% skim milk in 0.2% NP40/TBS for 1 hour at the room temperature. After the washing steps, SuperSignal west dura extended duration substrate (Thermo) was used and signals were visualized on Kodak Biomax XAR film (Kodak). Bands were digitalized by Epson Expression 1680 (EPSON) and were quantified with Quantity One 1-D Analysis software (BIO-RAD).

8. Metabolic cage experiments and urine, blood electrolyte analysis

Mice were housed in the individual metabolic cage (Tecniplast) and were habituated for 4-5 days before urine collection. Every hour urine collection was performed with 12-channel peristaltic pump (IPC, Ismatec) connected to a fraction collector (FC204, Gilson). The peristaltic pump and the fraction collector were automatically switched on every hour for two minutes. For urinary chemical analysis, sodium and potassium concentration was determined by flame photometry (Instrumentation laboratory). Urinary pH was measured by using pH meter (Metrohm). Creatinine was quantified with creatinine (urinary) assay kit (Cayman chemicals). Other electrolyte components in blood and urine were measured in the Laboratoire Central de Chimie Clinique, Centre Hospitalier Universitaire Vaudoise (CHUV) University Hospital (Lausanne, Switzerland).

9. Measurement for drinking and activity patterns

Drinking and activity patterns were measured by using Mouse-E-Motion system (Infra-E-Motion Gmbh). This system allows simultaneous real-time measurements of water intake and general motion activity in mice housed in normal laboratory cages.

10. GFR measurement

GFR was measured by measuring and calculating FITC coupled inulin clearance on anesthetized mice as described previously (Sturgeon et al, 1998, Zhonghua et al, 2004). FITC-inulin (5% in 0.85% NaCl) was dialyzed over night and 50µl of dialyzed FITC-inulin was injected retro-orbitally. Venous blood was collected from the saphenous vein at 3, 7, 10, 15, 20, 40 and 60 minutes after the injection. Plasma was recovered after the centrifugation and diluted 5 times with 0.5M HEPES and measured for the fluorescence intensity by Nanodrop 3300 (Thermo).

11. Arterial blood pressure measurement

Arterial blood pressure was measured by the telemetry system (Data Science International) in conscious unrestrained mice. Measurement was performed at least 1 week after the operation to allow mice to recover from the telemetric device implantation. The mean of data from 6 measurements was calculated for a single value of diastolic and systolic blood pressure for individual mouse.

PCR primers used in this study

Gene	Taqman probe ref.
Actb	4352341E
Aqp2	Mm00437575_m1
Aqp3	Mm01208559_m1
Aqp4	Mm00802131_m1
Arntl_exon8	Mm00500222_m1
Avpr2	Mm01193534_g1
Ptgs2	Mm01137144_m1
Ren1	Mm02342889_g1
ROMK	Mm01173990_m1
Slc12a3	Mm00490213_m1
aENaC	(probe) AGAGGATCTGGAAGAGCTGGACCGCA
	(Fw) GCACCCTTAATCCTTACAGATACACTG
	(Rv) CAAAAAGCGTCTGTTCCGTG

	SYBR green primers	
	(forward)	(reverse)
Cre	CCTGGAAAATGCTTCTGTCCG	CAGGGTGTTATAAGCAATCCC
Pparg	CAAGCCCTTTACCACAGT	ATAATAAGGTGGAGATGCAGGTTCTA

	primers for genotyping
Bmal1 L1	ACTGGAAGTAACTTTATCAAACTG
Bmal1 L2	CTGACCAACTTGCTAACAATTA
Bmal1 R4	CTCCTAACTTGGTTTTTGTCTGT
RenCre Fw	GAAGGAGAGCAAAAGGTAAGAG
RenCre Rv	TTGGTGTACGGTCAGTAAATTGGAC

	Primers flanking the exon8 of Bmal1	
	(forward)	(reverse)
Bmal1	TGGACACAGACAAAGATGACCCTCA	TCCCTCGGTCACATCCTACGACA