Supplemental Material

Table S1. Phenotypical characterization of diabetic (STZ) and control (VEH) WT and KO mice.

In experimental protocol 1, body weight is reported before diabetes induction (baseline) and after 3-4 weeks of diabetes. Mice were placed in metabolic cages to estimate 24-h urine output, food and water intake and feces after at least 3 weeks of diabetes. Weight of heart and both kidneys normalized to body weight are reported after terminating the experiments after 8 days ANGII infusion. ** $p < 0.01$, **** $p < 0.0001$ (compared within the same genotype). Electrolytes and creatinine were determined at termination of experiments.

Phenotypical characterization of mice				
	WT VEH	**WT STZ**	**KO VEH**	**KO STZ**
Body weight (g)				
- at baseline	22.4±1.0 (n=7)	21.6±0.5 (n=7)	17.8±0.7 (n=2)	20.7±1.1 (n=5)
- after 3 weeks	26.4±0.8 (n=7)	24.0±0.4 (n=7)	23.4±0.2 (n=2)	23.8±1.0 (n=5)
Water intake (mL)	1.0±0.1 (n=15)	8.0±1.4**** (n=11)	1.1±0.1 (n=5)	8.9±1.7** (n=8)
Urine output (mL)	2.3±0.2 (n=15)	13.5±1.5**** (n=11)	2.4±0.4 (n=5)	14.6±1.6**** (n=8)
Food intake (g)	10.1±0.7 (n=15)	13.5±0.6** (n=11)	11.5±0.3 (n=5)	13.8±0.8 (n=8)
Feces (g)	3.5±0.4 (n=15)	4.6±0.3 (n=11)	4.1±0.4 (n=5)	5.6±0.9 (n=8)
Heart/body weight (mg/g)	4.5±0.1 (n=9)	4.3±0.1 (n=8)	4.7±0.2 (n=7)	4.0±0.5 (n=6)
Kidneys/body	17.3±0.5 (n=9)	21.4±0.9**	17.8±0.5 (n=7)	19.2±1.1 (n=6)

weight (mg/g)		(n=8)		
P-Na^+ (mmol/L)	143±0.3 (n=10)	136±2.1** (n=7)	141±1.1 (n=8)	138±1.8 (n=5)
P-K^+ (mmol/L)	7.0±0.4 (n=10)	6.7±0.2 (n=7)	7.3±0.8 (n=8)	7.0±0.7 (n=5)
P-creatinine (µmol/L)	22±0.7 (n=10)	27±5.3 (n=5)	21±0.6 (n=7)	24±2.2 (n=6)

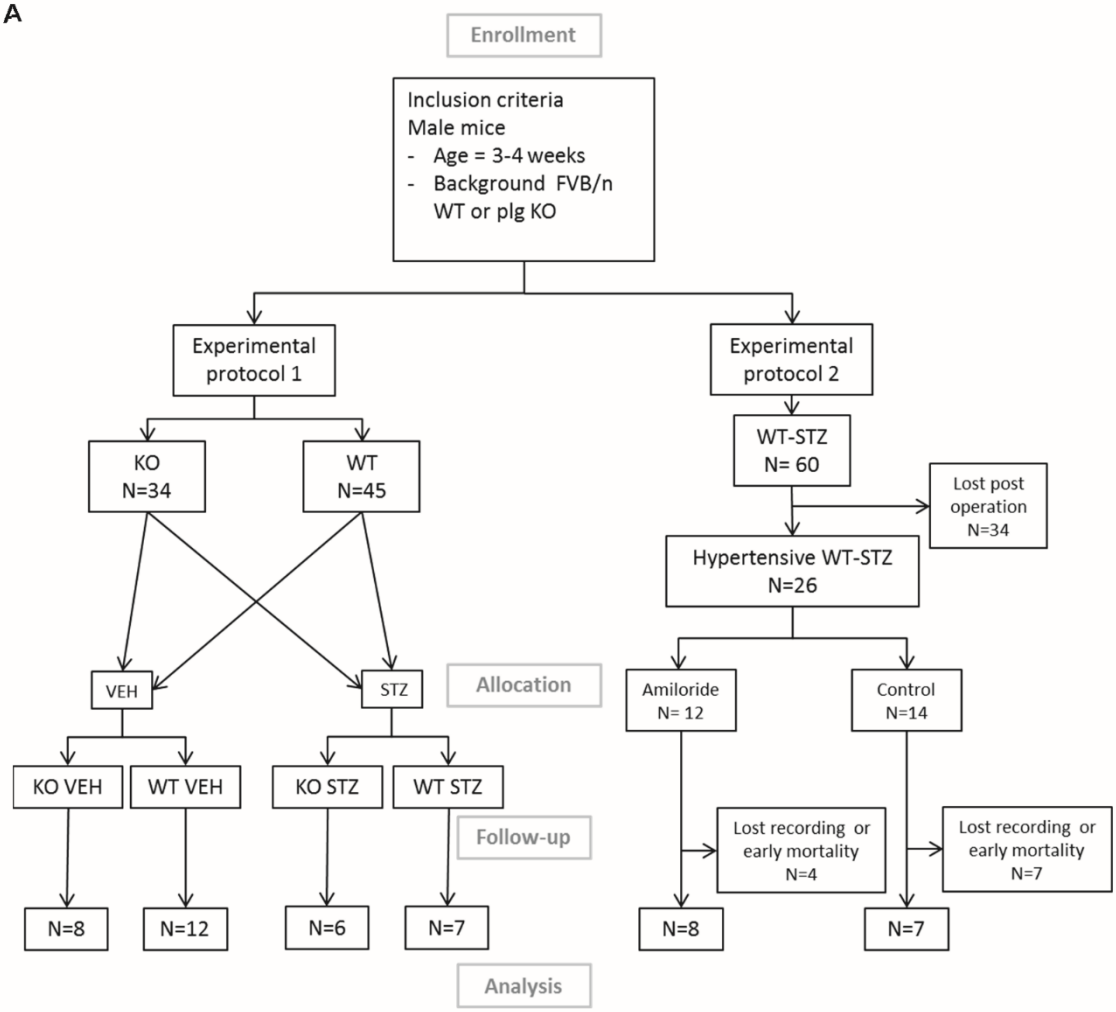
Table S2. Phenotypical characterization of diabetic (STZ) WT mice after 8 days of ANGII infusion (ANGII) with (w) or without (w/o) an addition of amiloride (Amil) for 5 days.

In Experimental protocol 2, body weight was reported before diabetes (baseline) and after 3 weeks of diabetes. Weight of heart and both kidneys normalized to body weight was after 8 days of ANGII with (w) or without (w/o) an addition of amiloride (Amil) for 4 days. There was no significant difference between ANGII w or w/o Amil.

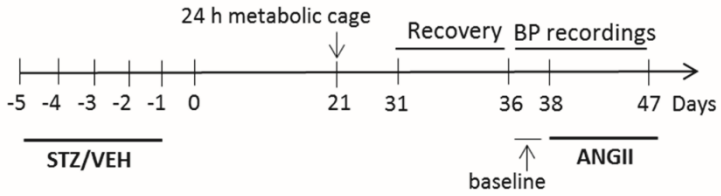
	WT-STZ ANGII w/o	**WT-STZ ANGII w Amil**
Body weight (g)		
- at baseline	20.17±0.4 (n=7)	20.6±0.7 (n=8)
- after 3 weeks	24.9±0.4 (n=7)	26.7±0.8 (n=8)
Heart/body weight (mg/g)	4.5±0.2 (n=7)	4.5±0.2 (n=8)
Kidneys/body weight (mg/g)	21.0±1.2 (n=7)	19.47±0.6 (n=8)

Figure S1. (A) Flow chart depicting use of mice from experimental protocol 1 and 2 **.** (B+C) Time line of protocol 1 and 2, respectively: mice (FVB, aged 3-4 weeks) received injections with streptozotocin (STZ) or vehicle for five consecutive days. Three weeks after last injection, mice were placed in metabolic cages for one 24 h urine collection. One week later, chronic indwelling catheters were placed in the femoral artery and vein for continuous blood pressure (BP) recordings, infusion of ANGII (30 ng/kg/min) or ANGII (60 ng/kg/min) with and without (controls) amiloride (±Amil, 2mg/kg/day) and blood sampling. Mice were allowed 5 days of recovery before BP measurements and 2 days of baseline recordings before initiation of ANGII infusion. When mice were lost it was either during surgery or shortly after implantation of catheters since both Plg-/- genotype and diabetes were associated with impaired survival.

A



B **Experimental protocol 1**



C **Experimental protocol 2**

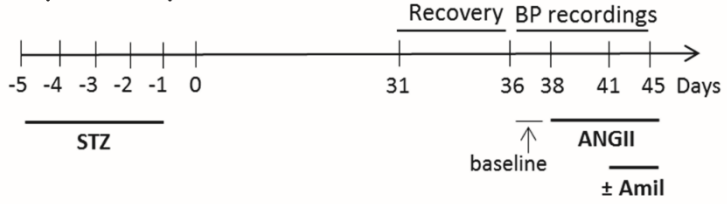


Figure S2.

(A) Fasting blood glucose was significantly higher in diabetic WT (WT STZ, n=5) and Plg -/- (KO STZ, n=5) mice compared to non-diabetic controls (WT VEH, n=8, and KO VEH, n=2, respectively) beginning at 7 days after last STZ injection. **** $p < 0.0001$ (compared within the same genotype).

(B-C) Fasting blood glucose in mice from series 2 during STZ injection (day -4 to 0) and four weeks past injections. Measurements are from diabetic wildtype mice before infusion with ANGII with (WT STZ Amiloride, n=8) or without (WT STZ controls, n=7) amiloride. Four weeks after streptozotocin treatment (STZ, day 28), fasting blood glucose was significantly increased compared to baseline (day -4). Mice assigned to either WT STZ Control or WT STZ Amiloride did not differ at baseline or in diabetes development after 4 weeks. *** $p<0.001$.

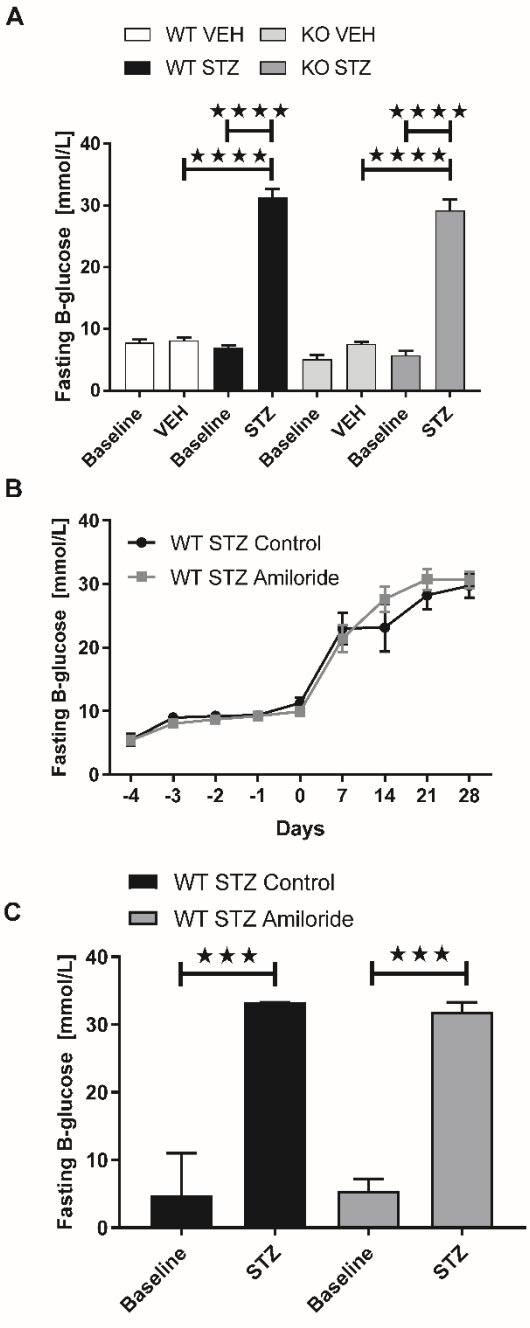
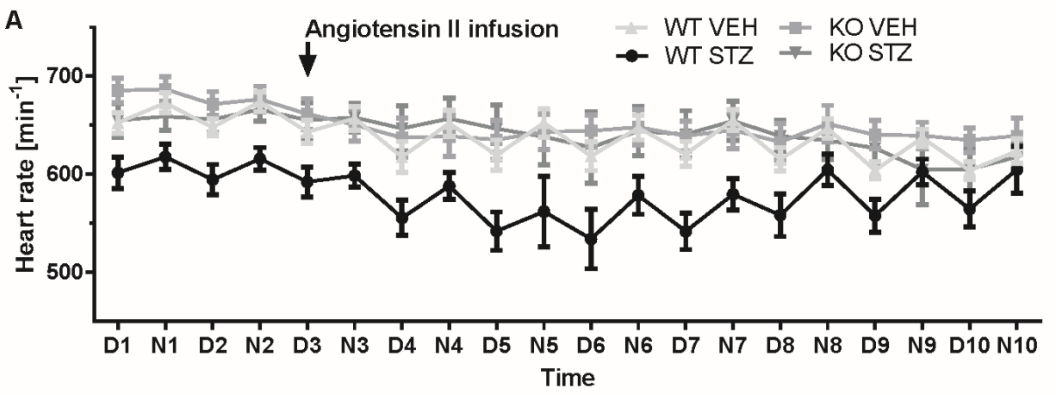

A
WT VEH
KO VEH
WT STZ
KO STZ
Fasting B-glucose [mmol/L]
0
10
20
30
40
Baseline
VEH
Baseline
STZ
Baseline
VEH
Baseline
STZ
B
WT STZ Control
WT STZ Amiloride
Fasting B-glucose [mmol/L]
0
10
20
30
40
-4
-3
-2
-1
0
7
14
21
28
Days
C
WT STZ Control
WT STZ Amiloride
Fasting B-glucose [mmol/L]
0
10
20
30
40
Baseline
STZ
Baseline
STZ

Figure S3.

(A) Traces show mean heart rate (HR) at baseline (day (D) and night (N) 1-2) and in response to angiotensin II (ANGII) infused intravenously (30 ng/kg/min) for 8 days in streptozotocin treated diabetic wildtype mice (WT STZ, n=7) and Plg -/- mice (KO STZ, n=6) and vehicle treated wildtype mice (WT VEH, n=12) and Plg -/- (KO VEH, n=8). (B) HR was significantly lower in WT STZ compared within both treatment and genotype, before and after ANGII. Baseline corresponded to days 1-2 and ANGII to days 6-9 (when response in MAP to ANGII reached a plateau) in (A). * $p < 0.05$, D1 = day 1 (6 a.m. to 6 p.m.), N1 = night 1 etc.



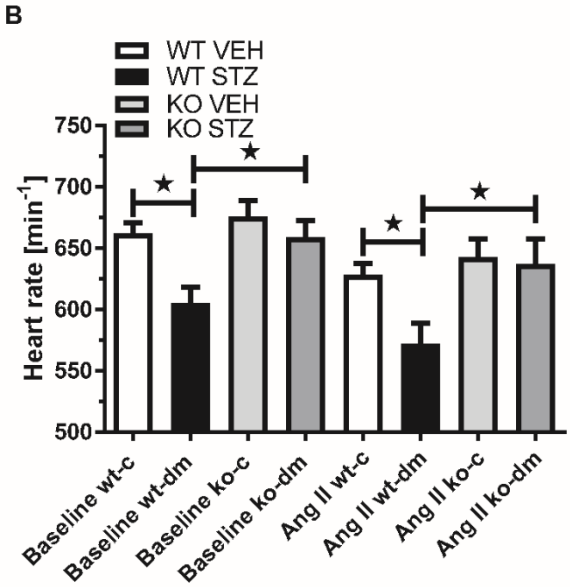


Figure S4.

(A) Traces show mean heart rate (HR) at baseline (day (D) and night (N) 1-2) and in response to angiotensin II (ANGII, D and N 3-9) infused intravenously (60 ng/kg/min) for 7 days with (WT STZ Amiloride, n=8) or without (STZ WT Control, n=7) addition of amiloride (2mg/kg/day) for 4 days (D and N 6-9), in diabetic wildtype mice (WT STZ). (B) HR tended to decrease after ANGII but remained statistically unaltered by ANGII and amiloride. Baseline corresponded to HR of days 1-2, ANGII to days 4-5 and with amiloride (AngII w Amil) or controls without (AngII w/o Amil) corresponded to days 8-9. D1 = day 1 (6 a.m. to 6 p.m.), N1 = night 1 etc.

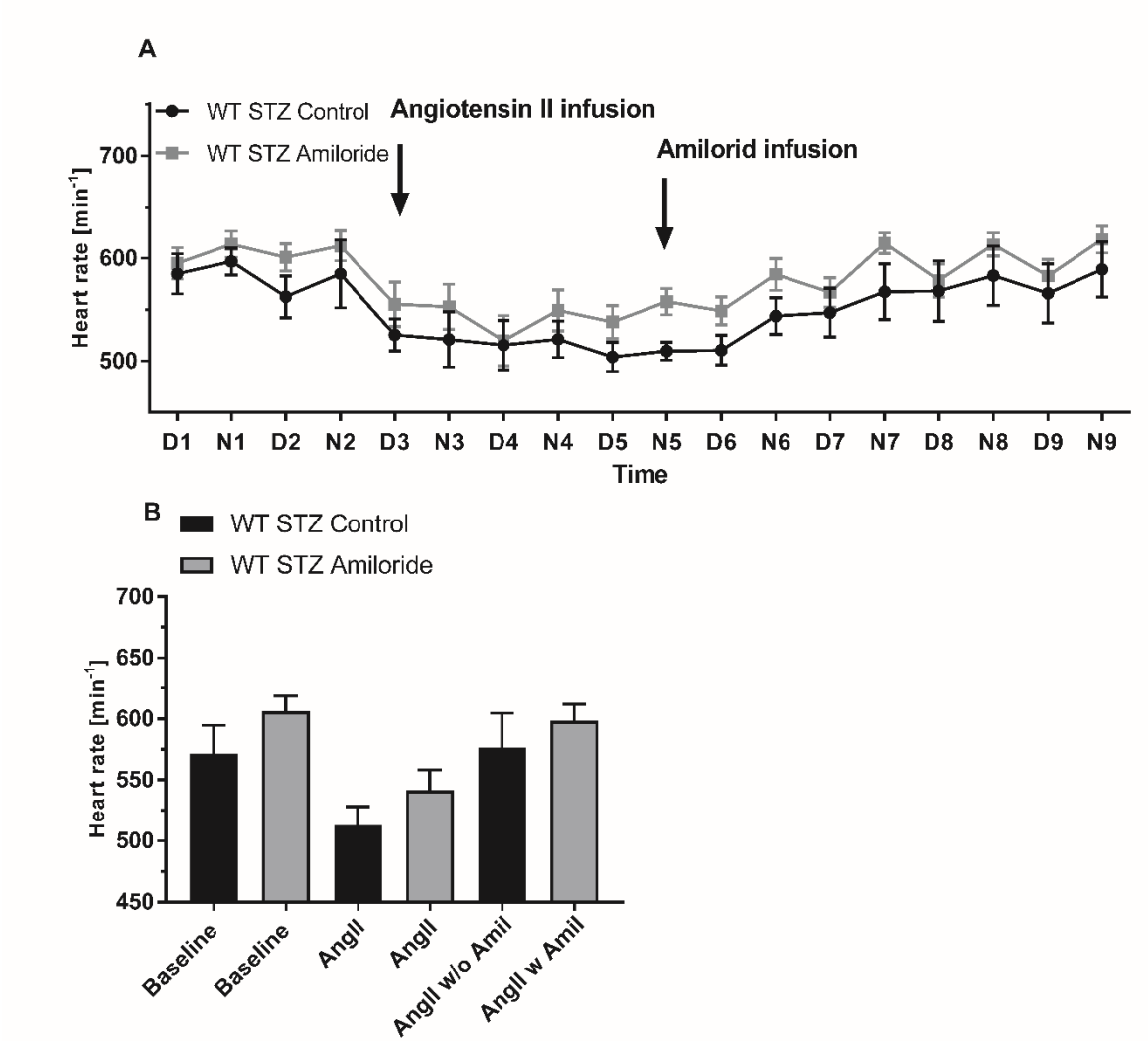


Figure S5.

(A) Immunoblot analysis of γENaC in kidney tissue homogenates from vehicle (VEH) and diabetic (STZ) wildtype (WT) and Plg $^{-/-}$ (KO) mice. Size markers are shown in kilodaltons (kDa). B shows densitometric evaluation of the faint 50 kDa cleavage product (n=5 in all groups). There were no significant differences between groups. C Shows western blotting for beta actin using homogenates as above in A. There were no differences between groups.

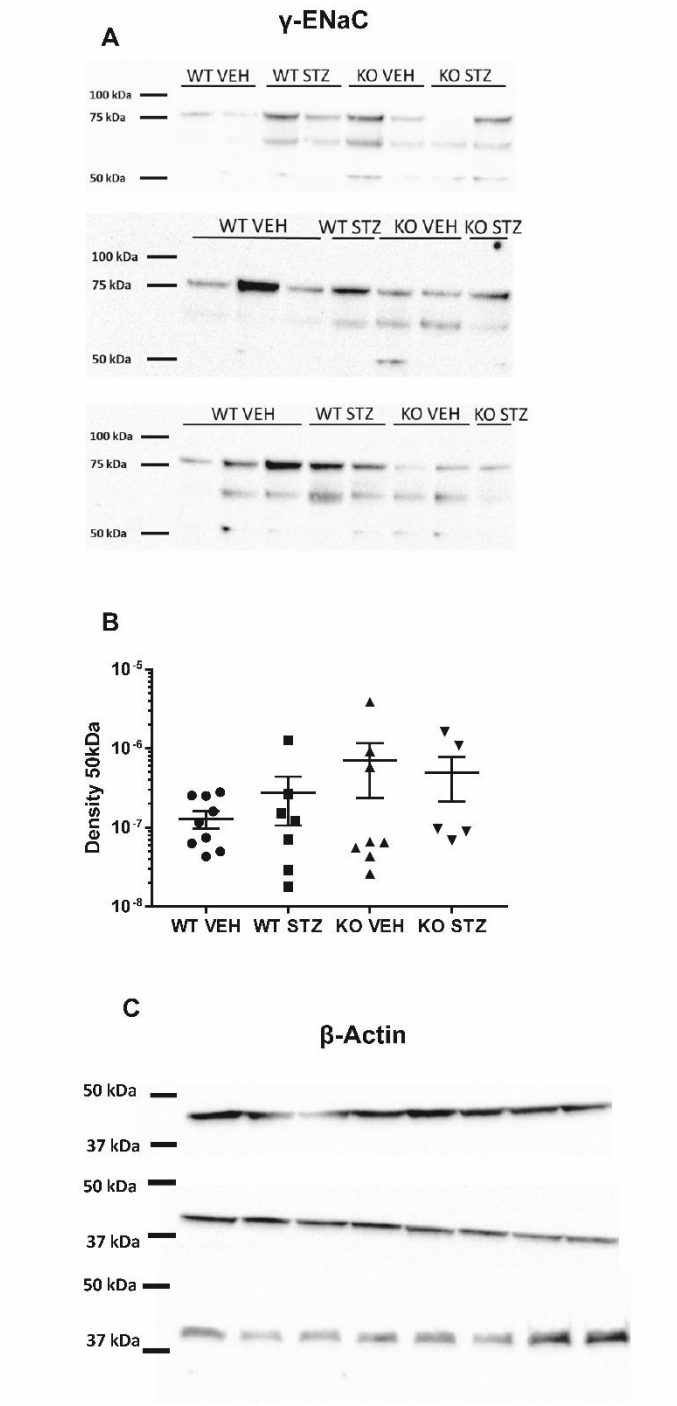
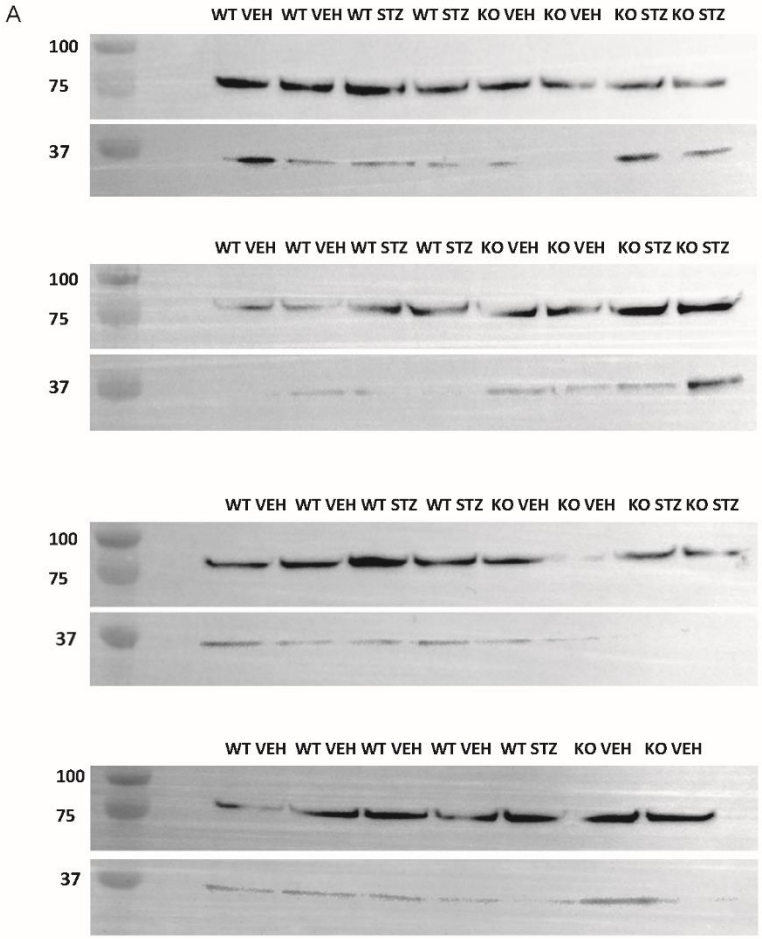

A
γ-ENaC
WT VEH
WT STZ
KO VEH
KO STZ
100 kDa
75 kDa
50 kDa
B
Density 50kDa
10-5
10-6
10-7
10-8
C
β-Actin
50 kDa
37 kDa

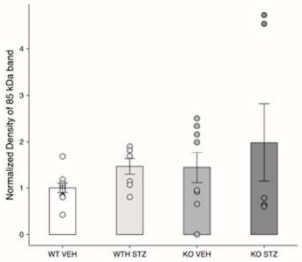
Figure S6.

(A) Immunoblot analysis of α-ENaC in kidney tissue homogenates from vehicle (VEH) and diabetic (STZ) wildtype (WT) and Plg $^{-/-}$ (KO) mice. At short exposure time, the dominant band migrated corresponding to predicted full length at 85-90 kDa while longer exposures revealed a weaker band migrating at ca 30 kDa corresponding to furin-cleaved protein detected at the N-terminal by the antibody. Size markers are shown in kilodaltons (kDa). B shows densitometric evaluation of the faint 85 and 30 kDa cleavage product (n=6 in all groups). There were no significant differences between groups.

A



B Normalized density of 85 kDa band



C Normalized density of 37 kDa band

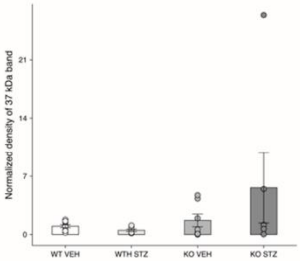


Figure S7. Plasma renin concentration was measured in plasma drawn from resting conscious mice before termination of experiments after 8 days of ANGII infusion with and without amiloride in diabetic wildtype mice. There were no differences between groups.

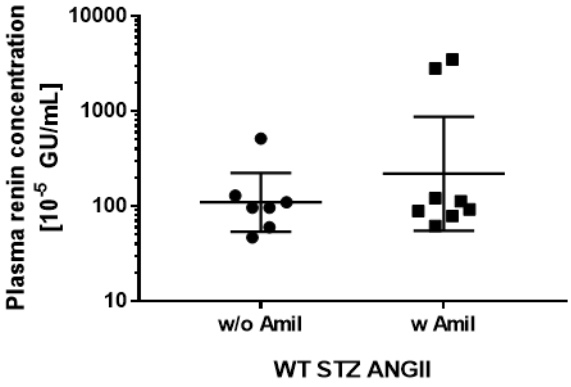


Figure S8.

(A-C) mRNA abundances of (A) “housekeeping” gene product ribosomal protein L41 (RPL41) in kidney tissue from the 4 experimental groups, (B) angiotensin II type 1 receptor A and B (AT1A/BR) and (C) angiotensin II type 2 receptor (AT2r) in aorta from diabetic wildtype mice (WT STZ, n=7) and Plg -/- mice (KO STZ, n=5) and vehicle treated wildtype mice (WT VEH, n=10) and Plg -/- (KO VEH, n=8). There were no differences between groups.

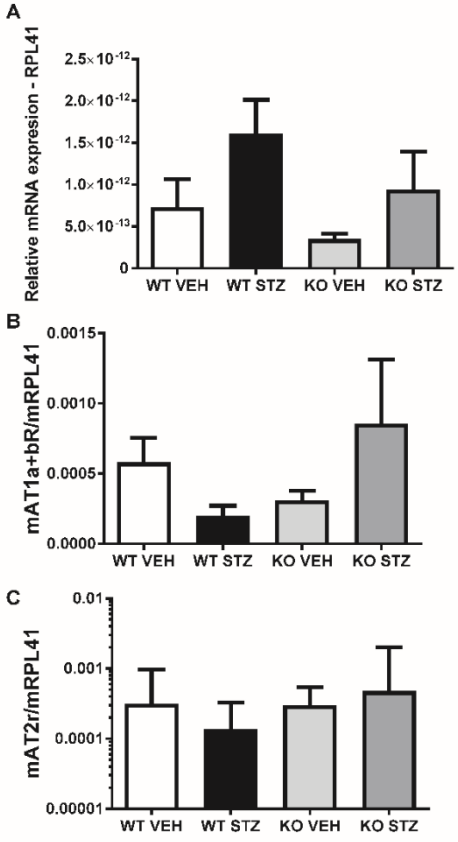


Figure S9.

A) Immunofluorescent staining of noradrenalin (red) in kidney tissue of wildtype and Plg -/- mice 4 weeks after vehicle or STZ treatment. Pictures are representative of the tendency seen in n=2 KO STZ and n=3 from WT STZ, WT VEH and KO VEH. From left to right: negative control, norepinephrine, 16x in silico magnification of dotted square insert from previous picture. White scale bar in lower right corner: 100 μm. Norepinephrine staining resulted in distinct signal from perivascular and peritubular structures particularly in cortex of WT VEH and WT STZ. There was a tendency to a less widespread signal in KO VEH that was further aggravated in KO STZ (lowest row). B) Diagram shows norepinephrine concentration in kidney tissue expressed per mg protein. N=7-10 in each group. Norepinephrine content was elevated in Plg-/- mice irrespective of diabetes. * $p < 0.05$; ** $p < 0.01$

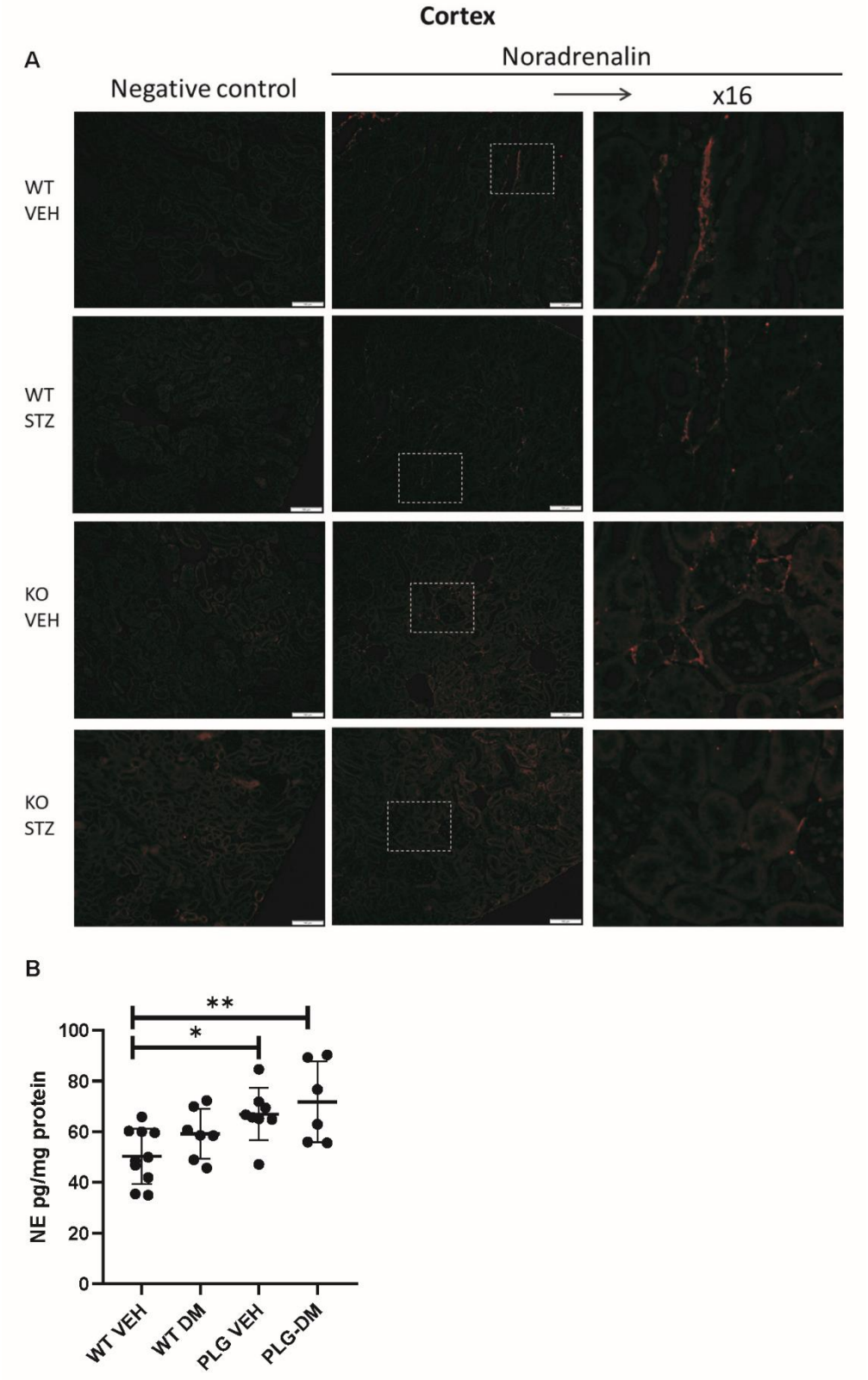
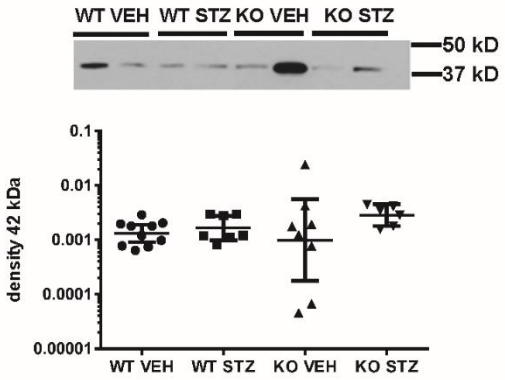

Cortex
A
Noradrenalin
Negative control
x16
WT
VEH
WT
STZ
KO
VEH
KO
STZ
B
**
*
NE pg/mg protein
100
80
60
40
20
0
WT VEH
WT DM
PLG VEH
PLG-DM

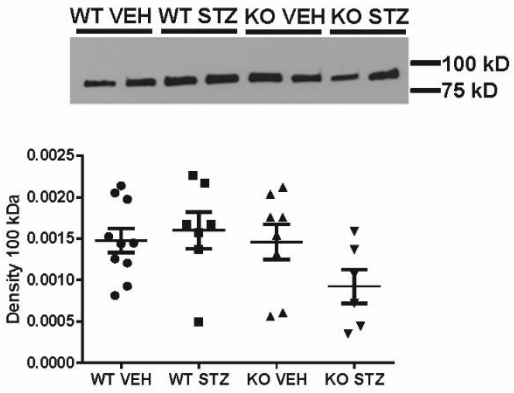
Figure S10.

(A) Immunoblot and densitometry analysis for the glomerular filtration barrier protein nephrin. (B) Immunoblot and densitometry analysis of the mesenchymal marker α-smooth muscle cell actin (αSMA) showed no difference between groups. (C) Trichrome Masson staining of kidney tissue from wildtype and Plg-/- mice 4 weeks after vehicle or STZ treatment. Pictures are representative of the tendency seen in n=3 mice from each group. Glomeruli are marked with G (left) and vessels are shown at the right side. Staining showed collagen accumulation (blue) in some of the glomeruli in the WT STZ mice, whereas no collagen was observed around glomeruli in either of the remaining groups. No differences between groups were observed in collagen accumulation in other areas of the kidney Thus perivascular connective tissue was not altered (4 pictures in right panel). Black scalebar in lower right corners: 50 μm.

A αSMA



B Nephrin



C

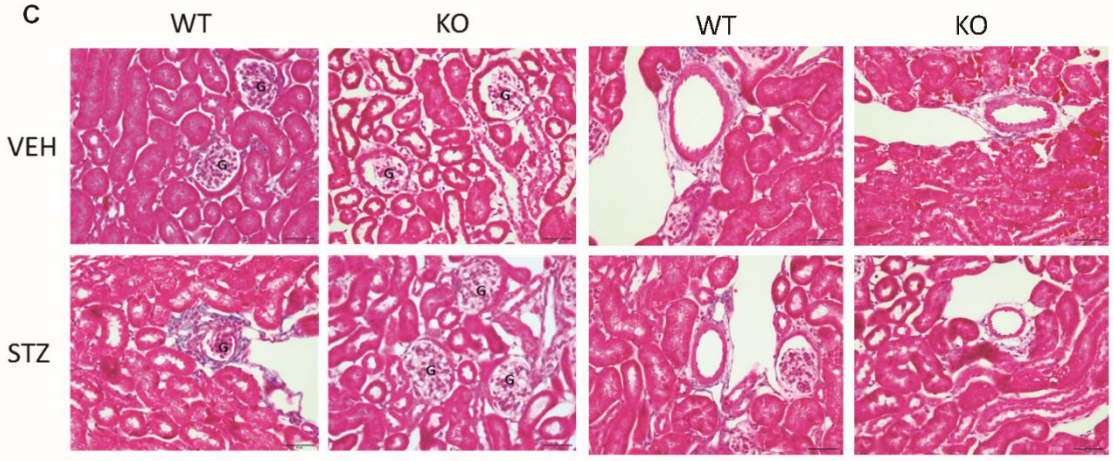


Figure S11.

Inflammatory cells and mediators in kidney tissue of vehicle and STZ-treated wildtype and Plg-/- mice 4 weeks after vehicle or STZ treatment (A) Immunohistochemistry staining of tissue sections for the leukocyte marker, receptor CD45. Black scale bar in lower right corners: 50 μm. Bottom: 16x magnification of dotted square in picture above. black arrows: CD45 positive cells. n=3 pr. group. Regardless of STZ/VEH treatment WT mice showed a tendency to have multiple clusters of CD45 positive cells in perivascular areas, whereas KO mice had only very few, scattered CD45 positive cells. (B-F) mRNA abundances of (B) "housekeeping" gene product ribosomal protein L41(RPL41), (C) qPCR analysis of the leukocyte marker CD45, there was no difference in abundance; (D) transcription factor T_H17 cell marker RORγ, (E) receptor of the innate immune system TLR4 and (F) pro-inflammatory cytokine TNFα. TNF mRNA abundance was significantly increased in KO STZ compared to WT STZ. No other significant difference was found in mRNA abundance. (G-H) Immunoblot analysis: (G) immunoblot and (H) densitometry analysis of immunoblot for cytokine TGFβ1. Human cortex pool (HCP) was used as positive control and shows active (13 kDa) and inactive (44 kDa) TGFβ1. No active TGFβ1 was revealed in either

group. No significant difference was found in inactive TGFβ1. * p<0.05