Blood pressure-independent renoprotective effects of small interference RNA targeting liver angiotensinogen in experimental diabetes

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SUPPLEMENTAL MATERIAL

Table S1. List of qPCR primer sequences.

Gene		Sequence (5' to 3')
Agt	Forward primer	CCAGCACGACTTCCTGACTT
	Reverse primer	GCAGGTTGTAGGATCCCCGA
Renin	Forward primer	TGTGGTAACTGTGGGTGGAAT
	Reverse primer	GCATGAAGGGTATCAGGGGC
AT_1aR	Forward primer	ATCACCAGGTCAAGTGGATTTCG
	Reverse primer	TTCCCACCACAAAGATGATGC
AT_1bR	Forward primer	CTGAATCTTGCCCTGGCTGA
	Reverse primer	ACATAGGTGGTTGCCGAAGG
B2M	Forward primer	ATGGCTCGCTCGGTGACCG
	Reverse primer	TGGGGAGTTTTCTGAATGGCAAGCA
Actb	Forward primer	GGGAAATCGTGCGTGACATT
	Reverse primer	GCGGCAGTGGCCATCTC
Neph1	Forward primer	GGATGGCGGTAAGGTGGAGTG
	Reverse primer	CGTTATTGATGGTGAGAGTGGACAG
Nephrin	Forward primer	CGTCAGCATCAGCAGCAACC
	Reverse primer	AGCCACATCTTCCAGCCTCTC
Podocin	Forward primer	TGGACTCAGTGACCTGTGTTTGG
	Reverse primer	CAGCAATCACCCGCACTTTGG
WT-1	Forward primer	TGTGACTTCAAGGACTGCGAGAG
	Reverse primer	GGTGTGGGTCTTCAGGTGGTC

Agt, angiotensinogen; $AT_{1a}R$, angiotensin II type 1a receptor; $AT_{1b}R$, angiotensin II type 1b receptor; B2M, β_2-microglobulin; Actb, β-actin; WT-1, Wilms tumor-1.

Table S2. Angiotensin metabolites in blood, kidney and heart of diabetic Ren2 rats treated with either vehicle, valsartan, captopril, angiotensinogen (AGT) small interfering RNA (siRNA), AGT siRNA + valsartan, or captopril + valsartan for 3 weeks. Data are mean±SD of n=7-9. Numbers in combination with the symbol < denote the lower limit of quantification. *P<0.05 versus vehicle.

Angiotensin metabolite	vehicle	valsartan	captopril	captopril+ valsartan	siRNA	siRNA+ valsartan
Blood (pmol/L)						
Ang-(1-10) = Ang I	93±31	1,378±389*	1,675±806*	1,251±272*	94±40	42±39
Ang-(1-8) = Ang II	45±13	633±170*	39±35	22±13	45±13	24±15
Ang-(1-7)	8.2±0.6	38±19*	80±42*	44±5.7*	10±6.0	<8
Ang-(2-8) = Ang III	4.5±2.8	86±34*	8.0±11	<2.5	3.9±1.2	2.9±1.2
Ang-(3-8) = Ang IV	3.5±1.7	59±21*	6.4±8.8	2.3±0.6	3.9±1.7	2.1±0.3
Ang-(1-5)	10.4±3.0	136±59*	8.7±5.1	6.4±2.4	8.0±2.4	7.2±6.3
Ang II/I ratio	0.5±0.1	0.5±0.0	0.0±0.0*	0.0±0.0*	0.5±0.1	0.7±0.1*
Kidney (fmol/g)						
Ang-(1-10) = Ang I	176±195	195±128	388±237*	131±103	21±14*	<6*
Ang-(1-8) = Ang II	606±158	140±93*	232±109*	68±97*	396±165*	26±42*
Ang-(1-7)	32±28	92±101	195±123*	51±50	23±11	<15
Ang-(2-8) = Ang III	41±22	24±48	33±35	10±12	25±12	<6
Ang-(3-8) = Ang IV	<10	<10	<10	<10	<10	<10
Ang-(1-5)	12±6.1	58±39*	7.5±4.3	7.0±4.0	6.0±2.0	<5
Ang II/I ratio	8.0±2.5	1.0±0.3	0.9±0.2	0.5±0.2	36±13*	4.3±2.6
Heart (fmol/g)						
Ang-(1-10) = Ang I	18±14	99±42*	128±71*	67±26*	<10	<10
Ang-(1-8) = Ang II	30±17	31±27	20±20	9.7±4.5*	15±17	<8*
Ang-(1-7)	<15	25±28	55±56*	<15	<15	<15
Ang-(2-8) = Ang III	<10	<10	<10	<10	<10	<10
Ang-(3-8) = Ang IV	<10	<10	<10	<10	<10	<10
Ang-(1-5)	5.4±1.2	65±22*	7.8±5.3	6.0±2.7	5.1±0.3	<5
Ang II/I ratio	2.3±1.6	0.3±0.2*	0.1±0.1*	0.2±0.0*	1.5±1.7	0.8±0.0*

Table S3. Main characteristics of diabetic Ren2 rats treated with either vehicle, valsartan, captopril, angiotensinogen (AGT) small interfering RNA (siRNA), AGT siRNA + valsartan, or captopril + valsartan for 3 weeks. Data prior to the induction of diabetes mellitus (DM) and treatment are also provided. Data are mean±SD. #P<0.001 versus non-DM; *P<0.05 versus vehicle).

Parameters	0 weeks	15 weeks	18 weeks					
	non-DM	DM	vehicle	valsartan	captopril	captopril +valsartan	AGT siRNA	AGT siRNA +valsartan
N	47	47	8	8	8	7	9	7
General								
body weight (g)	395±38	401±44	448±37	435±29	411±31	388±15*	422±44	416±46
Δtreatment (g)			26±21	31±28	19±18	24±12	9.6±17	12±34
food intake (g/day)	25±6	35±12#	25±10	28±8.3	38±6.7*	28±11	30±8.1	29±5.5
water intake (mL/day)	38±8	136±52#	119±55	123±56	173±58	133±67	150±76	113±59
serum glucose (mmol/L)			39±11	38±21	52±14	55±10	46±6	50±13
insulin (U/day)			3.6±1.3	3.5±1.1	3.3±1.2	3.0±1.1	3.0±1.1	3.6±1.4
Blood								
plasma ALT (U/L)	56±14	181±130#	239±122	181±213	117±38	116±100	145±43	116±54
plasma AST (U/L)	85±30	280±267#	327±204	265±324	150±64	233±201	186±95	179±167
serum K^+ (mmol/L)			6.3±1.8	6.9±1.3	8.2±1.6	9.0±1.0*	7.2±2.3	7.7±1.3
serum Na^+ (mmol/L)			134±4.8	134±6.5	128±4.3	128±4.2	130±4.1	128±4.7
plasma urea (mmol/L)	5.1±0.9	8.9±1.6#	6.6±1.1	7.4±1.6	8.5±1.1	21±14*	8.7±1.5	14±4.5
plasma creatinine (µmol/L)	25±5	19±4#	19±2.1	23±4.8	19±3.8	41±24*	24±9.2	37±14*
Urine								
urine volume (mL/day)	22±6	132±53#	128±48	126±65	155±50	109±44	134±64	104±57
K^+ excretion (mmol/day)	3.1±0.6	4.8±1.2#	3.5±1.7	4.3±1.6	4.6±1.4	3.0±0.9	3.7±1.2	3.9±1.6
Na^+ excretion (mmol/day)	2.3±0.6	3.3±1.1#	2.5±1.4	2.8±1.3	3.3±1.0	2.1±0.6	2.3±1.1	2.3±0.7
urea excretion (mmol/day)	12±2.0	23±5.2#	15±8.2	20±8.4	20±6.7	13±3.7	18±5.1	17±7.9
creatinine excretion (µmol/day)	128±20	123±27	82±44	120±35	87±31	74±28	96±33	111±38
creatinine clearance (mL/min)	3.8±0.9	4.6±1.2#	3.2±2.1	3.6±1.1	3.2±1.0	1.4±0.6	2.9±1.0	2.6±1.5

Figure S1. Systolic and diastolic blood pressure (SBP, DBP) in diabetic Ren2 rats treated with either vehicle, valsartan, captopril, angiotensinogen (AGT) small interfering RNA (siRNA), AGT siRNA + valsartan, or captopril + valsartan for 3 weeks. Treatment was started 15 weeks after the induction of diabetes. Days -3 to 0 correspond to the period immediately before treatment. Data are mean±SEM of n=7-9. *P<0.05 versus vehicle and/or indicated group.

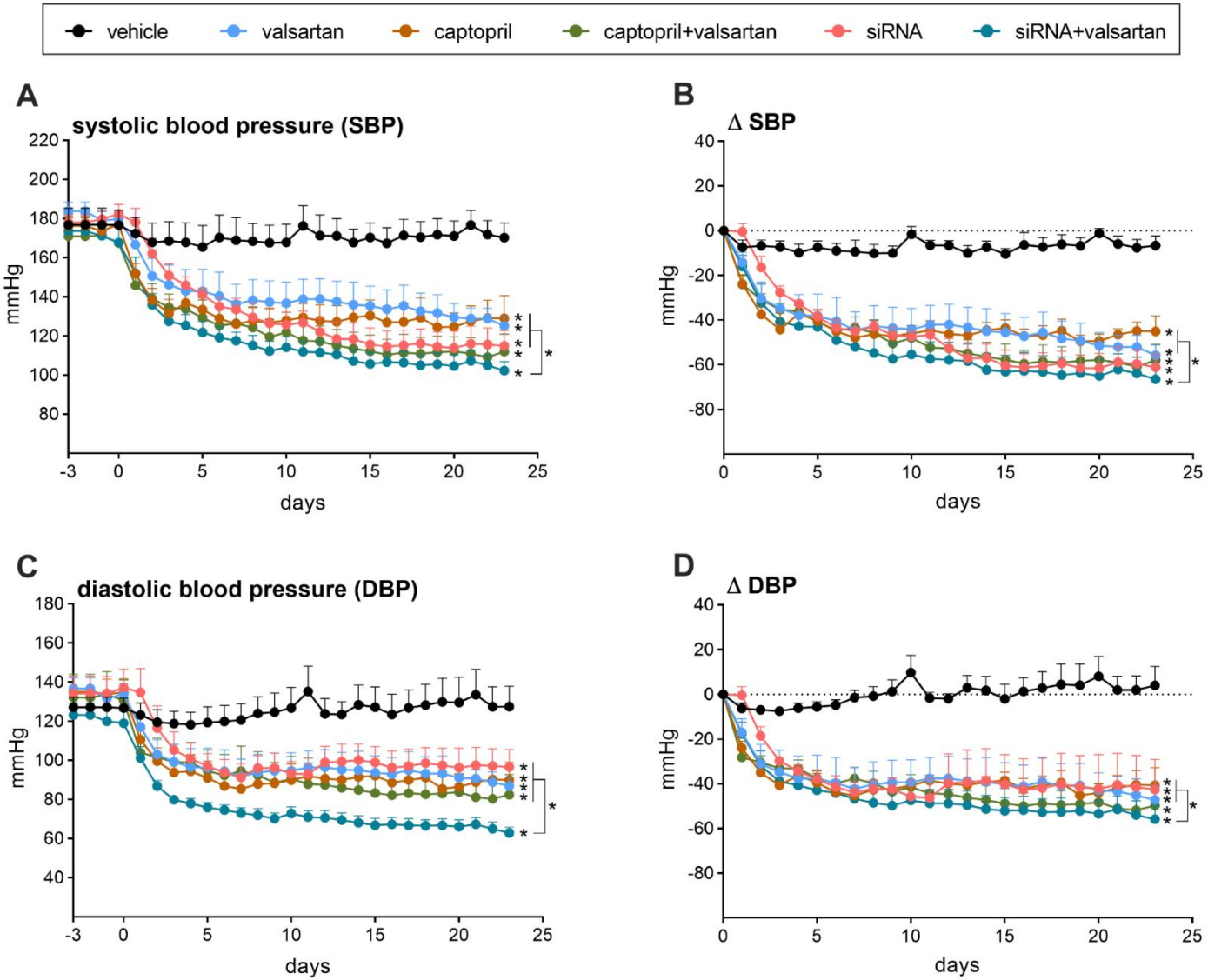

Figure S2. Effect of diabetes induction on the levels of angiotensinogen (A), renin, (B), and renin (C) in Ren2 rats after 15 weeks, and the relationship between renin (D) or prorenin (E) and angiotensinogen. Data are mean±SEM of n=47; *P<0.0001.

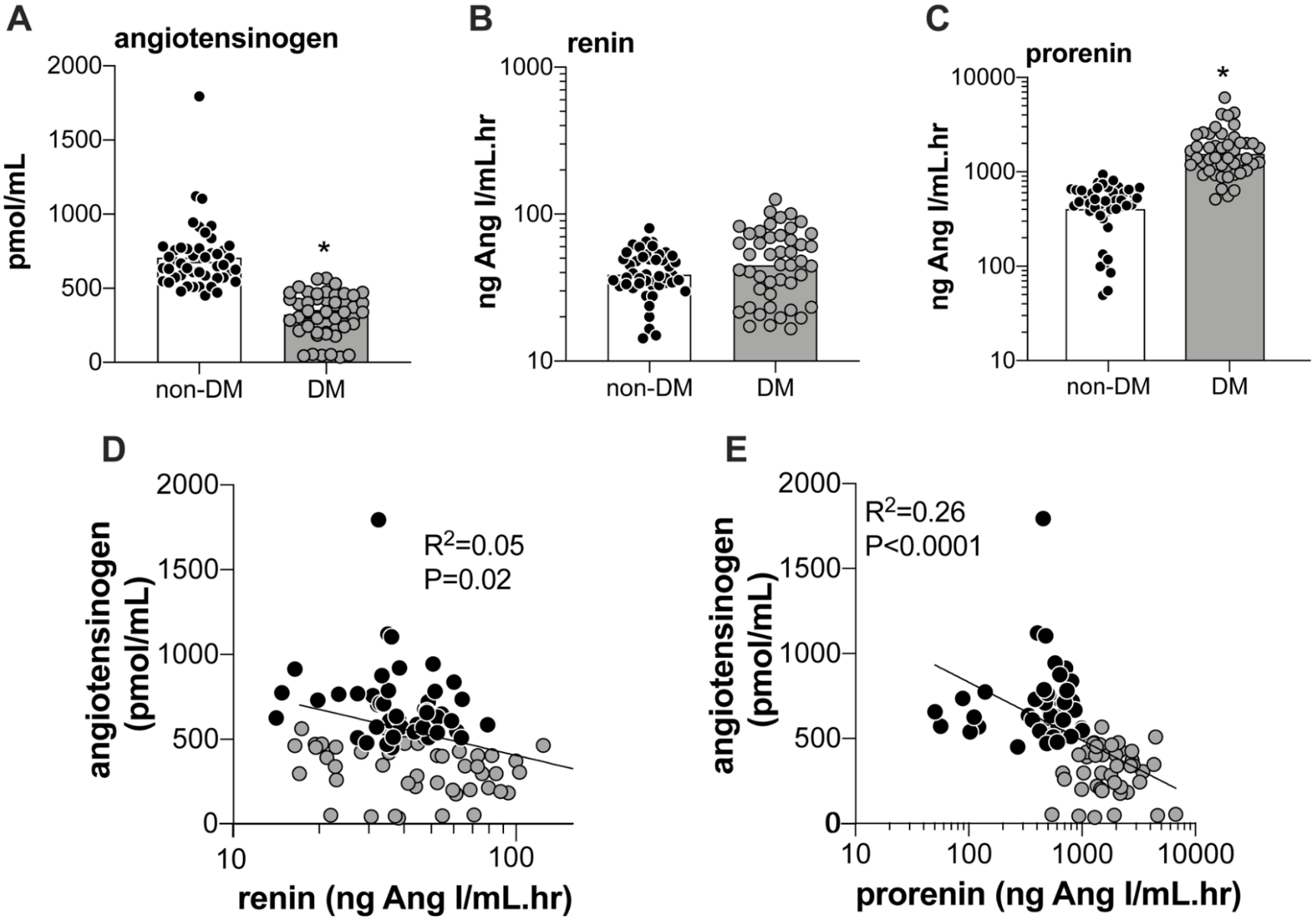

Figure S3. Hepatic (panel A) and renal (panel B) angiotensinogen levels in diabetic Ren2 rats treated with either vehicle, angiotensinogen (AGT) small interfering RNA (siRNA) or AGT siRNA + valsartan**.** Treatment was started 15 weeks after the induction of diabetes. Panel C shows the immunoblots. Data are mean±SEM of n=7-9. *P<0.05 versus vehicle.

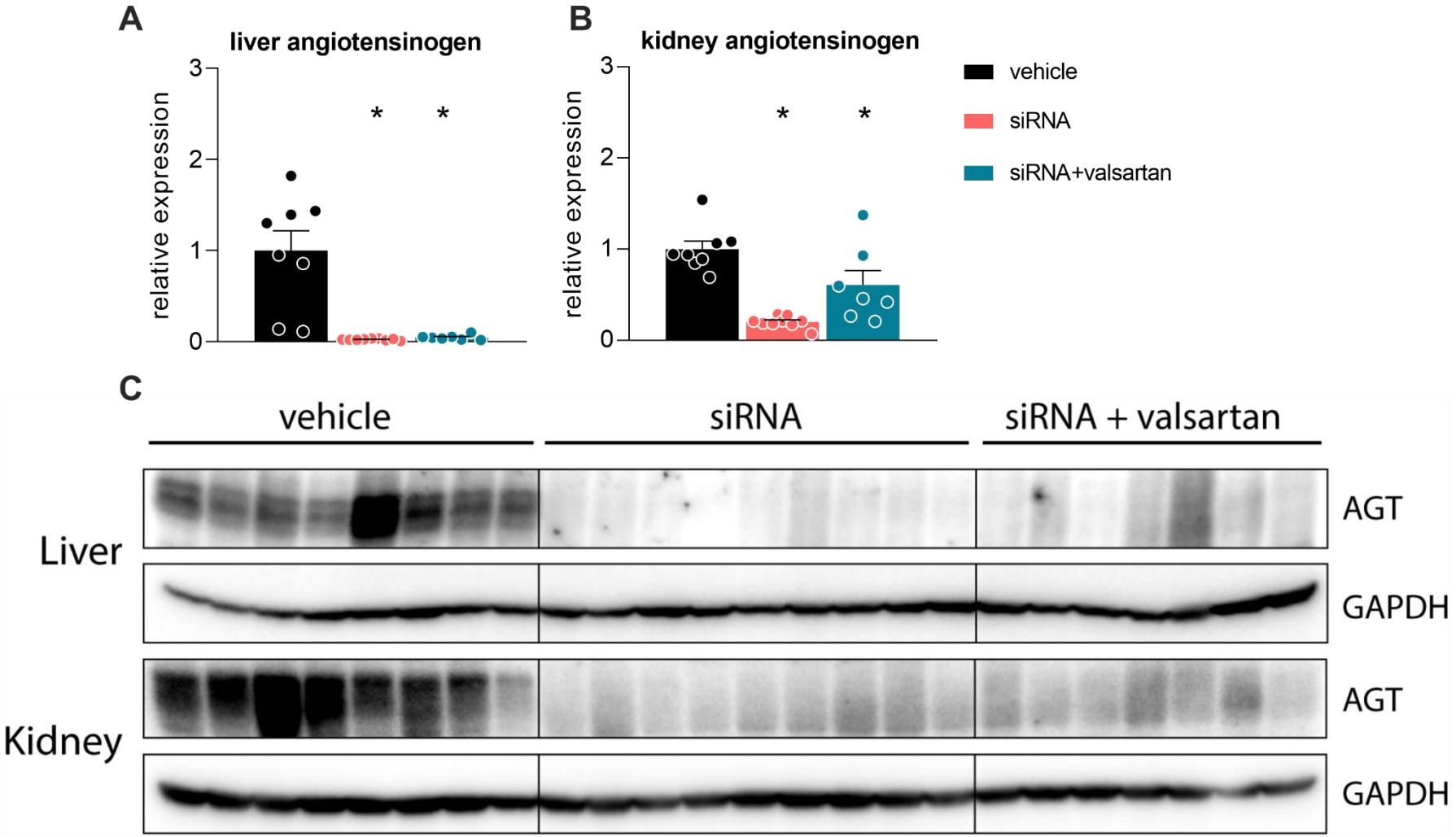

Figure S4. Renal expression of the angiotensin II type 1a and 1b (AT_{1a}, AT_{1b}) receptor (A and B), Neph1 (C), nephrin (D) and podocin (E) in diabetic Ren2 rats treated with either vehicle, valsartan, captopril, angiotensinogen (AGT) small interfering RNA (siRNA), AGT siRNA + valsartan, or captopril + valsartan for 3 weeks. Treatment was started 15 weeks after the induction of diabetes. Data are mean±SEM of n=7-9. NS, not significant.