SUPPLEMENTAL DATA

Supplemental Detailed Methods

Animals

The RUPP procedure is a well-established model for studying the link between placental ischemia and hypertension in the pregnant rat and has been described in detail previously.(1) First timed pregnant Sprague–Dawley rats purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN) were used in this study. Twenty-eight pregnant rats were randomly assigned to either the NP, NP *rAgt*-targeting siRNA, RUPP or the RUPP *rAgt*-targeting siRNA group. On day 12 of gestation (GD), siRNA was subcutaneously injected into rats (10 mg/kg). On gestational day 14, under isoflurane anesthesia, normal pregnant (NP) rats undergoing the RUPP procedure underwent a reduction in uterine perfusion pressure (RUPP) with the application of a constrictive silver clip (0.203 mm) to the aorta superior to the iliac bifurcation performed while ovarian collateral circulation to the uterus was reduced with restrictive clips (0.100 mm) to the bilateral uterine arcades at the ovarian end, whereas NP dams underwent a sham procedure. Rats were excluded from the study when the clipping procedure resulted in total reabsorption of the fetuses. Animals were instrumented, and arterial pressure was determined in all groups of rats at day 19 of gestation as described previously.(1) Briefly, on day 18 of gestation, rats were instrumented with carotid catheters of V-3 tubing (SCI) while under isoflurane anesthesia. Catheters were tunneled to the back of the neck and exteriorized after implantation. On day 19 of gestation, rat dams were placed in individual restraining cages for arterial pressure measurements using a pressure transducer (Cobe III Transducer CDX Sema). Mean arterial pressure (MAP) was recorded continuously for a 2-hour period after 1 hour of stabilization.

All experimental animals were sacrificed on day 19 (RUPP model) or 21 (transgenic model). The fetuses and organs were removed, weighed and collected. All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering.

Online Tables

Table S1. Primer sequences used for RT-PCR.

Gene		**Sequences (5'→3')**
hAgt	for	ATTCTGCACACCGAGCTGAA
	rev	TCAAGCTCAAAAAAAATGCTGTTC
	probe	CTGCAAAAATTGAGCAATGACCGCATC
rAgt	for	GCACGGACAGCACCCTATTT
	rev	AGAACTCATGGAGCCCAGTCA
	probe	TCAACACCTACGTTCACTTCCAAGGGAAGA
rNgal	for	CAGGGCAGGTGGTTCGTT
	rev	AGCGGCTTTGTCTTTCTTTCTG
	probe	TCGGCCTGGCAGCGAATGC
18S	for	ACATCCAAGGAAGGCAGCAG
	rev	TTTTCGTCACTACCTCCCCG
	probe	CGCGCAAATTACCCACTCCCGAC
rSix2	for	GGAAAGGGAGAACAGCGAGAA
	rev	ACTTGCCGCTGCCATTGA
	probe	TCCAGTAGCCACAACCCGCTGGC
rHoxb7	for	TTGGCGGCCGAGAGTAAC
	rev	GCCCCGCTTTCGTTCAGT
	probe	TCCGGATCTACCCCTGGATGCGA
rOct4	for	GGCTGGACACCTGGCTTCAGA
	rev	TGGTCCGATTCCAGGCCCA
	probe	SYBR

rPax2	for	AGCTGGAAGCTTTGGATCGA
	rev	TTGATGTGCTCTGATGCTTGGA
	probe	SYBR
rAdora1	for	TGGTGATTTGGGCTGTGAAG
	rev	AGTGACACGATGAAGCAGAAGGT
	probe	SYBR
rSyp	for	TCGTCGGCTGAATTCTTTGTC
	rev	CAGGGCCCCCATGGA
	probe	SYBR
rNrg1	for	GCCAAGCTCCCCTAAATCG
	rev	GGGCATTGACACCGTCATG
	probe	SYBR
rNrp1	for	GAACCCCGTGGACGAGTGT
	rev	CCTGTGAGCTGGAAGTCATCAC
	probe	SYBR

Online Figures

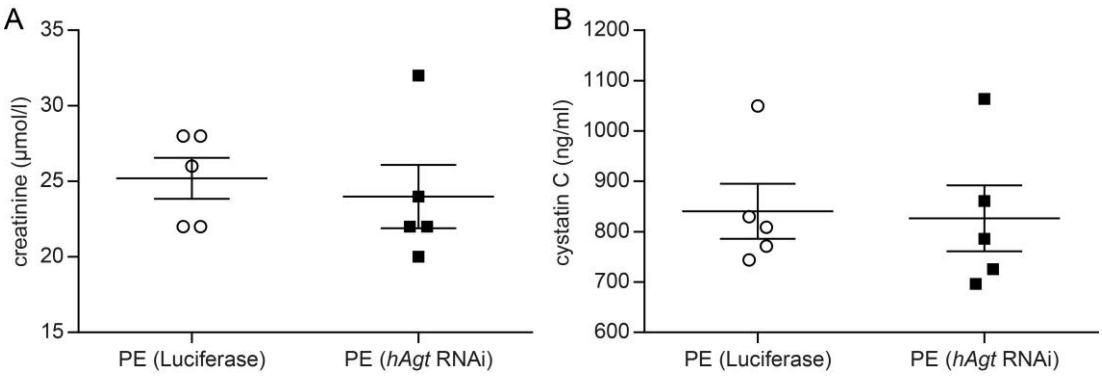

Figure S1. Effect of siRNA targeting *hAgt* on renal function (A) Serum creatinine and (B) cystatin C were not altered after administration of siRNA targeting *hAgt* (n=5 each; mean ± SEM).

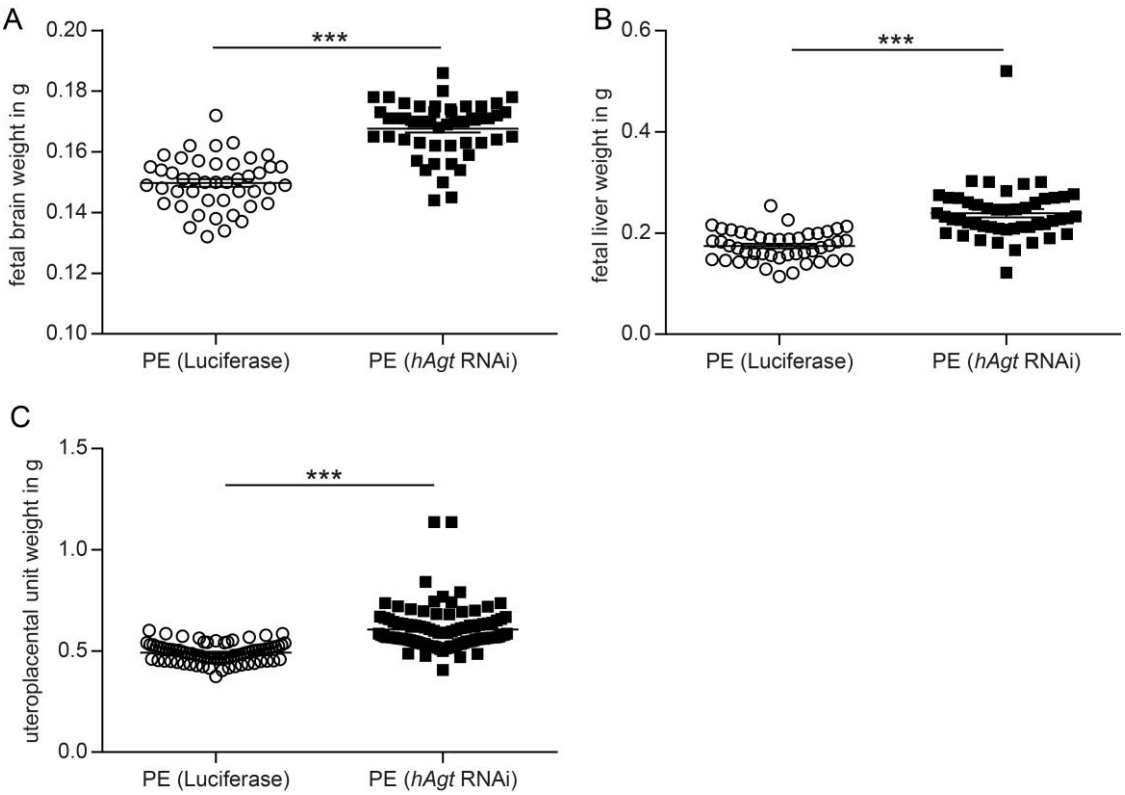

Figure S2. Effect of siRNA targeting *hAGT* on fetal phenotype

Fetal brain weight (A) and liver weight (B) were increased at gestational day 21 in *hAgt*-targeting siRNAtreated (n=49) compared to Luciferase-targeting siRNA-treated preeclamptic rats (n=44) (***p<0.001 Mann Whitney test; mean ± SEM). C) Uteroplacental unit weights were increased after application of *hAGT*-targeting siRNA (n=102) versus Luciferase-targeting siRNA (n=78) (***p<0.0001; Mann Whitney test; mean ± SEM).

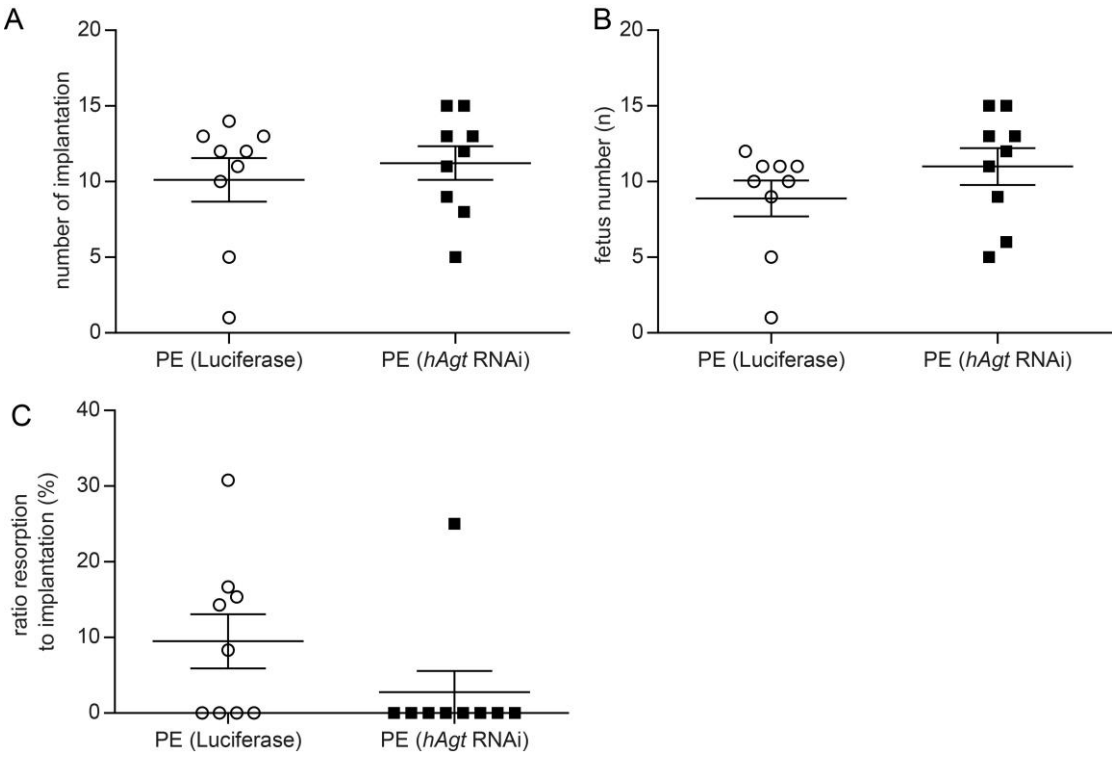

Figure S3. **Effect of siRNA against *hAgt* on pregnancy outcomes** Administration of siRNA had no impact on the number of implantation sites (A) or fetus number (B), although there was a trend (p=0.09) to a decrease in number of embryonic resorptions (C) in *hAgt*-targeting siRNA treated rats (n=9 each; Mann Whitney test; mean ± SEM).

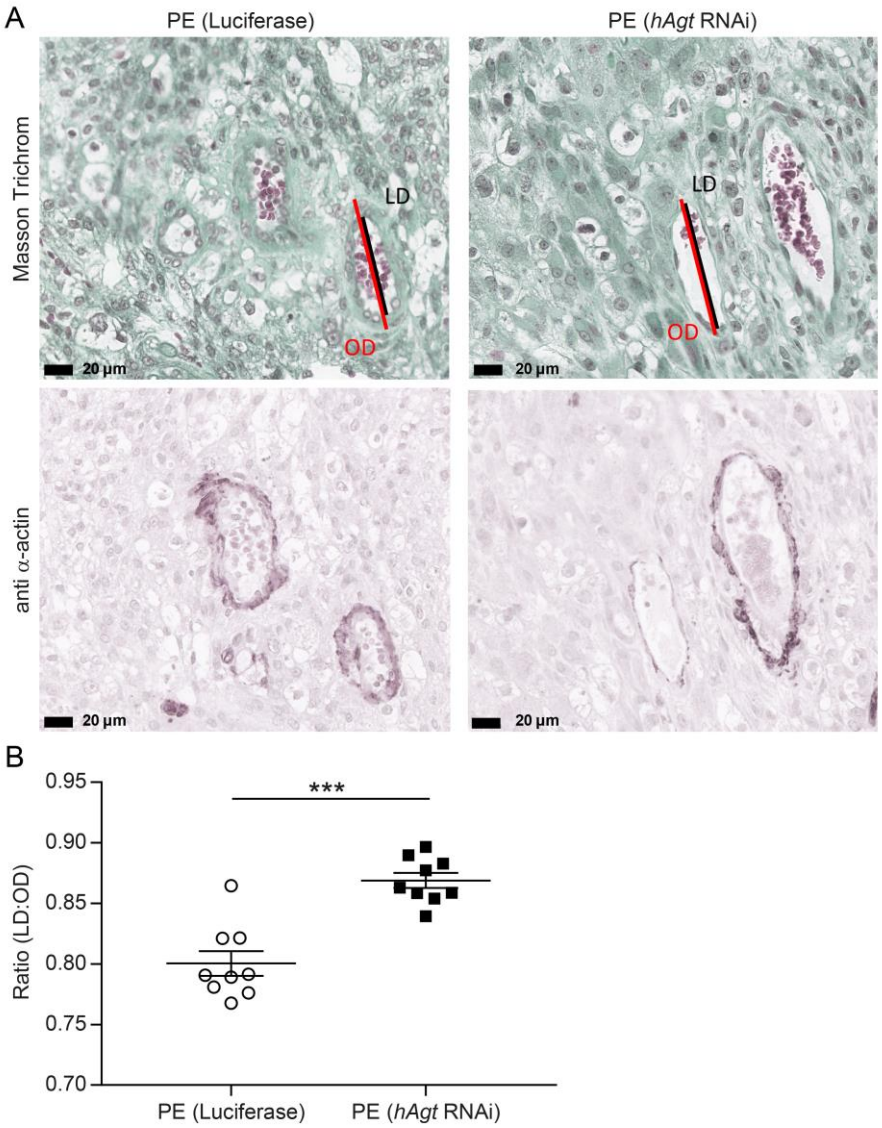

Figure S4. siRNA against *hAgt* improves spiral artery remodeling.

A) Representative images of Masson Trichome-stained (upper level) and anti α-actin stained (lower level) uteroplacental unit sections of Luciferase-targeting (n=9) and *hAgt*-targeting (n=9) siRNA-treated PE rats at gestational day 21. Maternal vessels in the mesometrial triangle of Luciferase-targeting siRNA-treated PE rats (upper left panel) showed thicker arterial walls. In contrast, vessels in this region of *hAgt*-targeting (n=9) siRNA-treated PE rats (upper right panel) showed thinner arterial wall. B) These differences are summarized as ratios of the inner lumen to-outer diameter ratios (LD/OD). The outer diameter (OD, red line) and luminal diameter (LD, black line) of

each vessel were measured at the point of the largest OD. (n=9 each; ***p<0.001; Mann Whitney test; mean ± SEM).

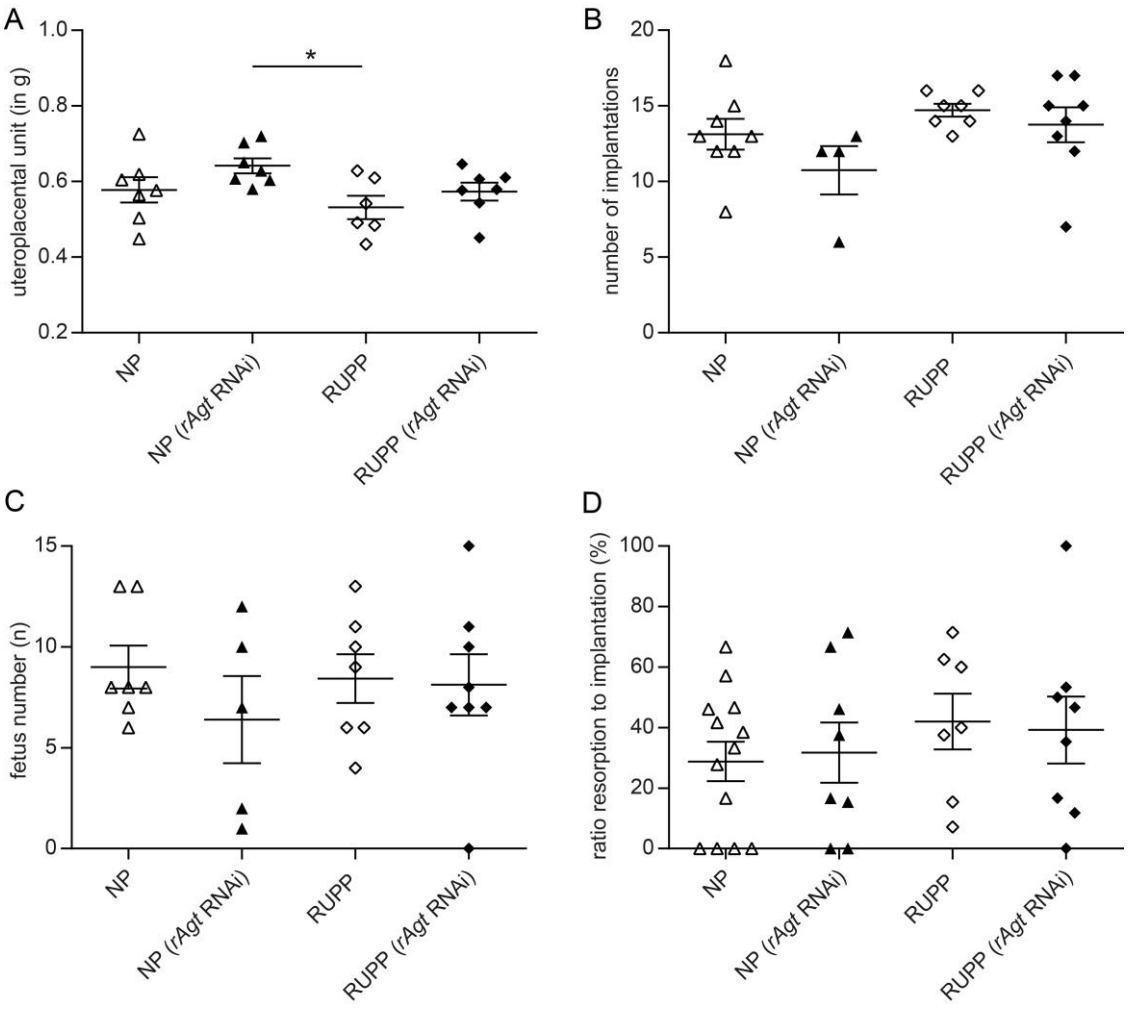

Figure S5 Pregnancy outcomes in the RUPP model

A) Uteroplacental unit weights were decreased in untreated RUPP (n=6) compared to NP treated with *rAgt*-targeting siRNA (n=7) but unchanged compared to NP rats (n=7). Administration of *rAgt*-targeting siRNA to RUPP rats (n=7) did not change uteroplacental weight. (*p<0.05; one-way ANOVA with Tukey's post hoc; mean ± SEM). B-D) Administration of siRNA has no negative effect on number of implantation (B), fetus number (C), and ratio of resorptions to implantations (D) in normal pregnant (NP) and RUPP rats. (NP n=7, NP(*rAgt* RNAi) n= 5, RUPP n=7, RUPP(*rAgt* RNAi) n=8; mean ± SEM)

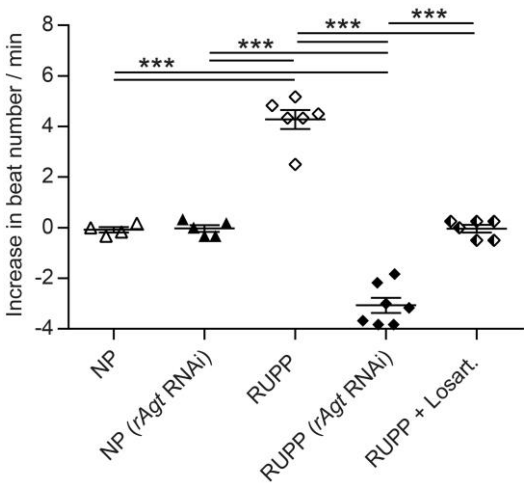

Figure S6. siRNA against *rAgt* decreases the concentration of angiotensin II receptor type 1 autoantibodies (AT1-AA) in the RUPP model.

Isolated rat neonatal cardiomyocytes were treated in vitro with IgG from *rAgt*-targeting siRNA-treated NP and RUPP rats as well as untreated NP and RUPP. Data are presented as an increase of beating rate of cardiomyocytes after treatment with IgG compared with baseline. Administration of AT1 receptor blocker (Losartan) was used to show that the effect is mediated via the AT1 receptor (n=5 each; ***p<0.001; one-way ANOVA with Tukey's post hoc; mean ± SEM).

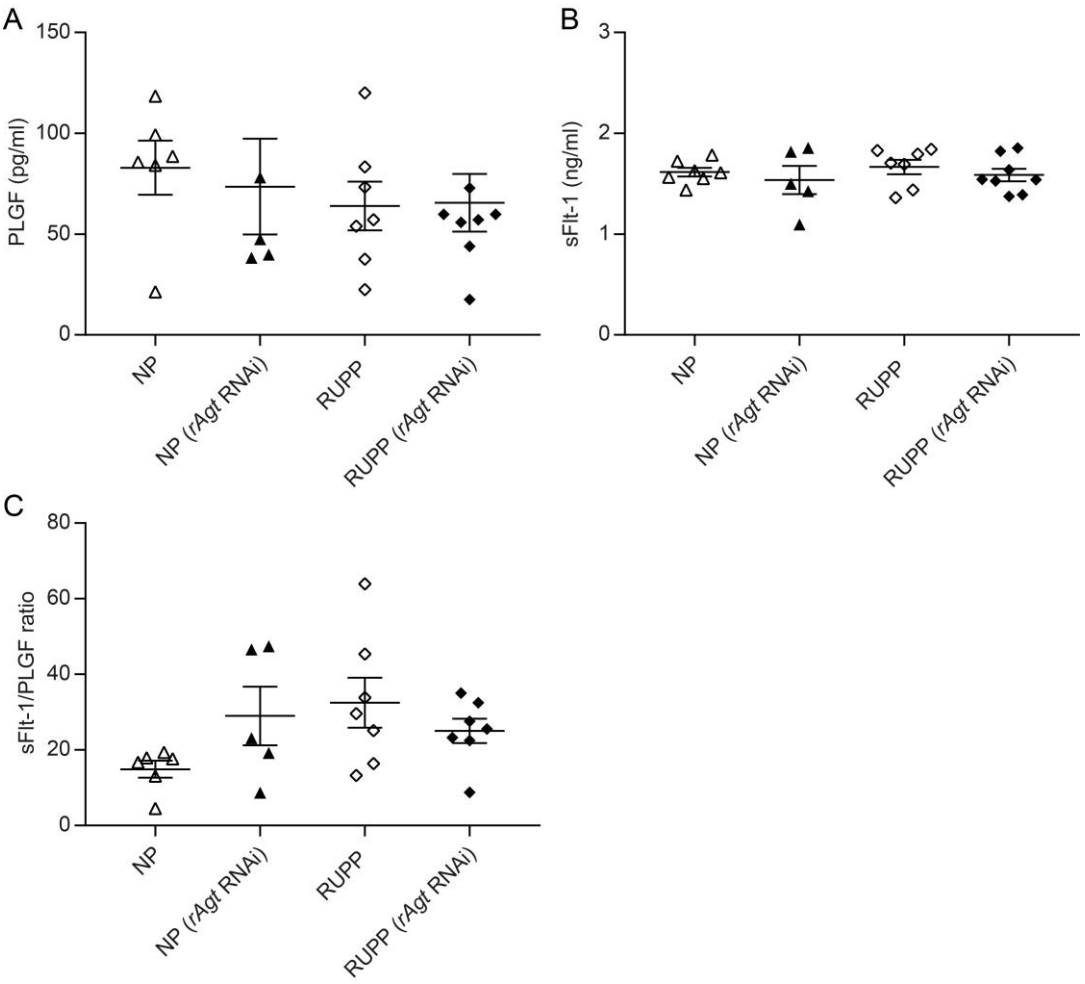

Figure S7 siRNA against *rAgt* has no impact on proangiogenic and antiangiogenic factors in the RUPP model.

A-B) Serum PLGF(A) and sFLT-1 (B) concentration was unchanged in *rAgt*-targeting siRNA-treated rats compared to untreated rats (NP n=6, NP(*rAgt* RNAi) n= 5, RUPP n=7, RUPP(*rAgt* RNAi) n=8; mean ± SEM) C) No changes in the sFLT-1:PLGF ratio was observed between the groups (NP n=6, NP(*rAgt* RNAi) n= 5, RUPP n=7, RUPP(*rAgt* RNAi) n=8; mean ± SEM).

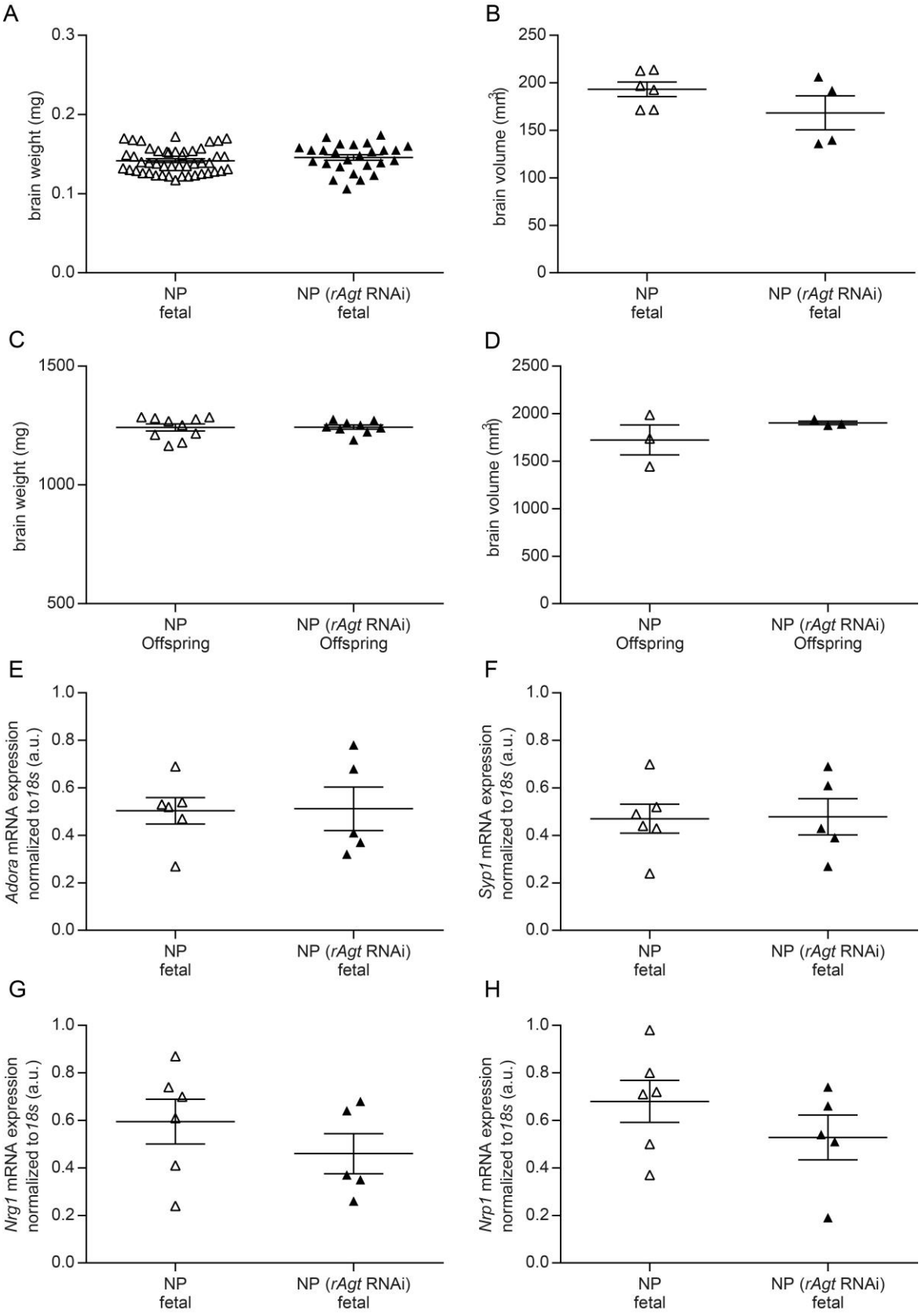

Figure S8 *rAgt* siRNA application to the mother during gestation did not affect brain weight and volume, or expression of many genes critical to brain development.

A) Brain weight was not affected by the siRNA treatment in fetuses at the end of pregnancy (NP n=47, NP (*rAgt* RNAi) n= 26; each; mean ± SEM). B) Volumetric analysis of fetal brain by histology showed no differences between untreated and siRNA treated groups (n=3; each; mean ± SEM). C) The weight of the brain of 3-week-old offspring was not different between untreated and siRNA treated groups (n=9-10; each; mean ± SEM). D) Volumetric analysis of fetal brain was not altered by the siRNA treatment in 3-week-old offspring (n=3; each; mean ± SEM). E-H) mRNA expression analysis of brain development genes for adenosine receptor1 (*Adora* – neuromodulation) (E), synaptophysin (*Syp1* - synaptic plasticity) (F), neuregulin-1 (*Nrg1* - neurogenesis) (G), and neuropilin-1 (*Nrp1* – axonal growth) (H) showed no effect of siRNA treatment (n=5-6; each; mean ± SEM)

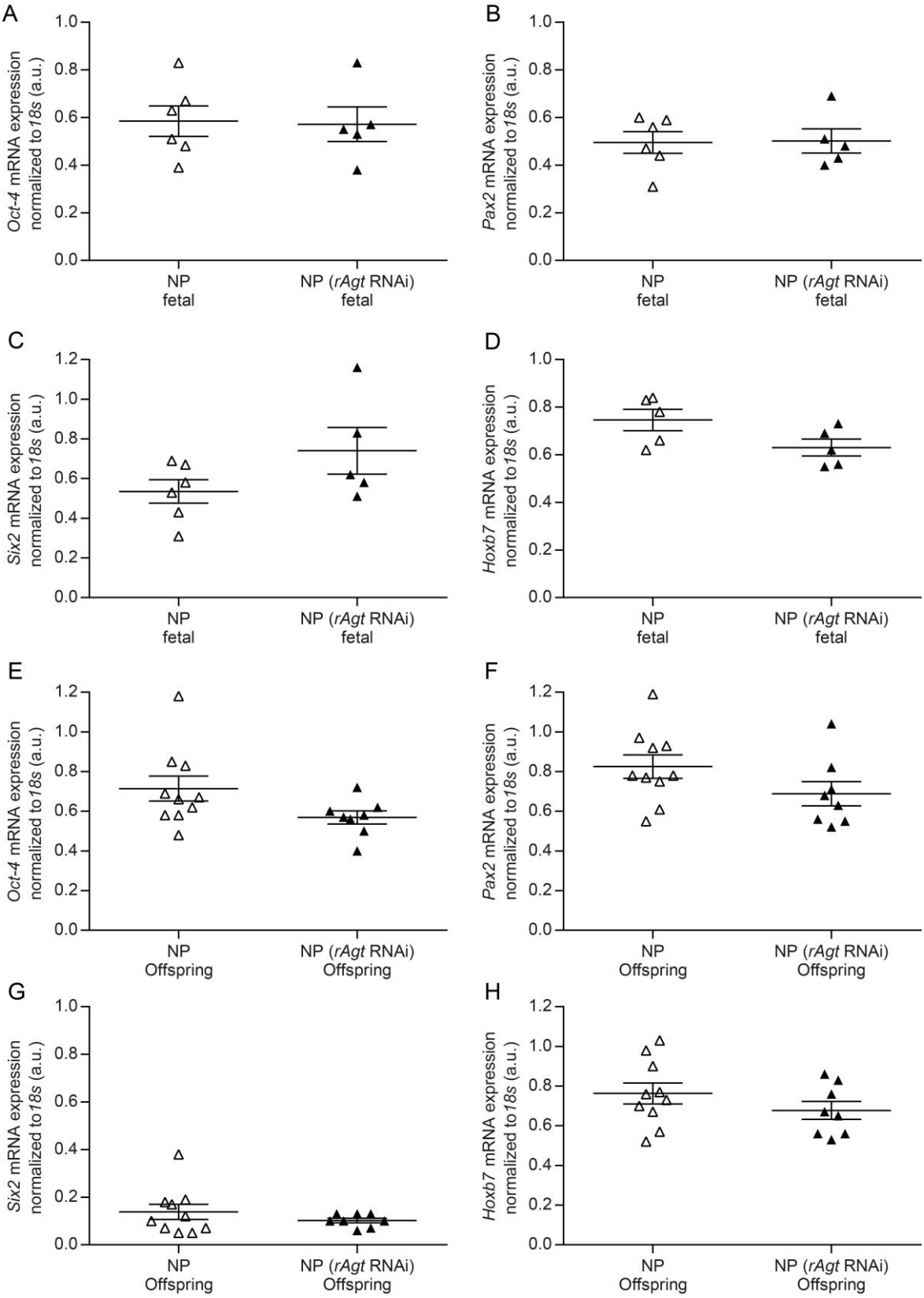

Figure S9 *rAgt* siRNA has no impact on kidney developmental genes.

A-D) mRNA expression analysis of kidney development gene octamer-binding transcription factor 4 (*Oct4* - renal stem/progenitor cells) (A), paired box gene 2 (*Pax2*

- intermediate mesoderm) (B), sine oculis-related homeobox 2 (*Six2* - nephron progenitor cells) (C), and homeobox protein B7 (*Hoxb7* - ureteric bud) (D) showed no regulation by the siRNA treatment in the fetus (n=5-6; each; mean ± SEM). E-H) mRNA expression analysis of kidney development genes for *Oct4* (E), *Pax2* (F), *Six2* (G), and *Hoxb7* (H) showed no regulation by the siRNA treatment in the offspring (n=7-8; each; mean ± SEM)

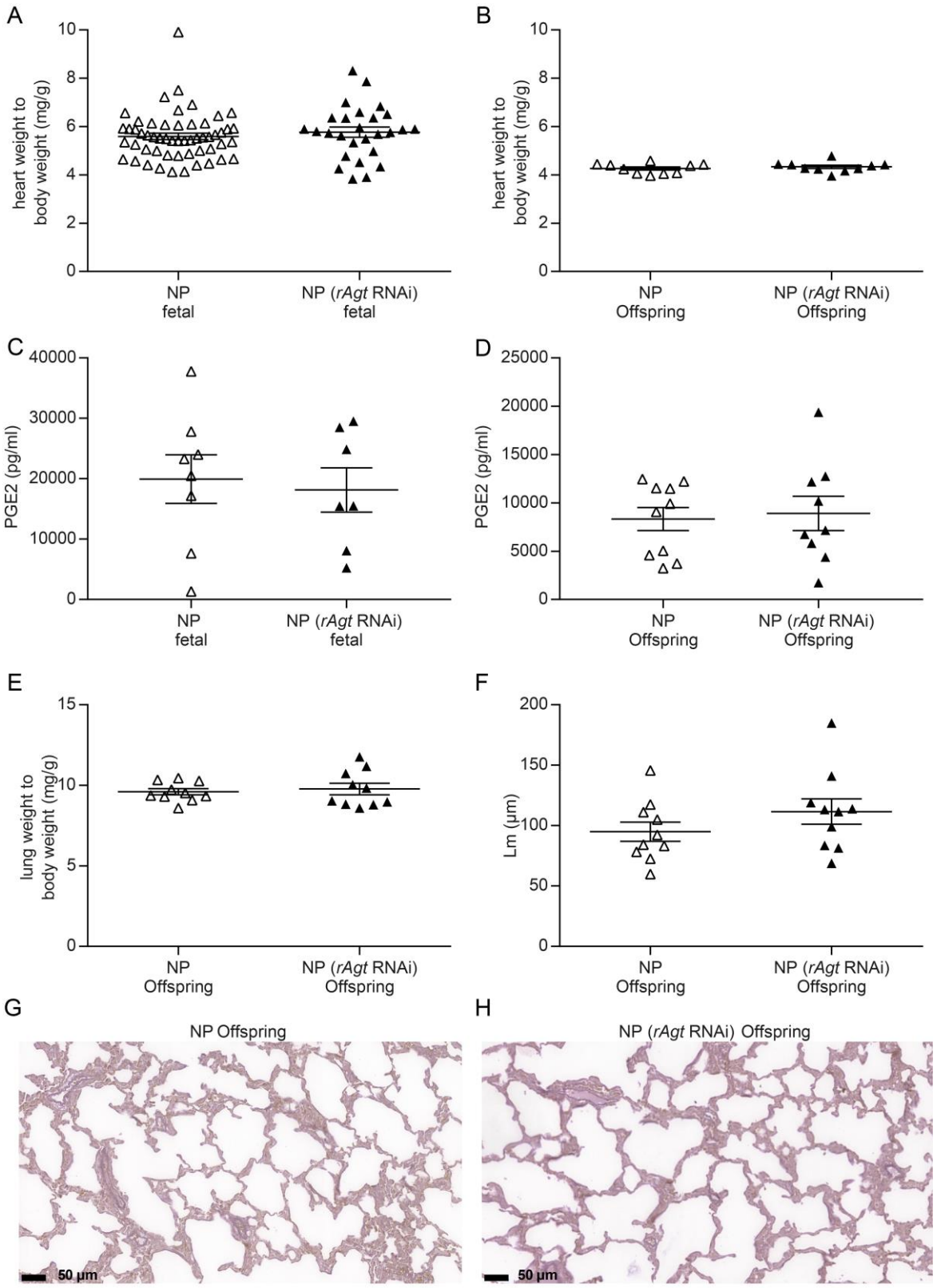

Figure S10 *rAgt* siRNA has no negative impact on the fetal heart and lung.

A-B) The heart weight to body weight ratio of the fetus (A) and 3-week-old offspring (B) was not changed between untreated and siRNA treated groups (NP fetal n=47,

NP(*rAgt* RNAi) fetal n= 26; each offspring n=10; each; mean ± SEM). C-D) Plasma PGE2 concentrations in the fetus (C) and 3-week-old offspring (D) were not altered by the administration of siRNA during gestation (n=7-10); each; mean ± SEM). E) The lung weight to body weight ratio of 3-week-old offspring was not changed between untreated and siRNA treated groups (n=10; each; mean ± SEM) F) The mean linear intercept in the offspring lung was not affected by the siRNA treatment (n=10; each; mean ± SEM). G) Representative images of Hematoxylin Eosin-stained lung sections of offspring from untreated and siRNA treated normal pregnant rats.

Online References

1. Granger JP, LaMarca BB, Cockrell K, Sedeek M, Balzi C, Chandler D, and Bennett W. Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia. *Methods Mol Med.* 2006;122(383-92.