Supplemental Methods:

Blood Pressure Monitoring

Direct blood pressure monitoring was done using radiotelemetry (DataSciences International; DSI). Surgical implantation of TA11PA-C10 transmitters was performed via left common carotid artery occlusion. Briefly, 10-week-old female CD-1 mice (Charles River Laboratories) were anesthetized with inhaled isoflurane in O_2, with meloxicam (5mg/Kg, s.c.) as analgesic. Following surgical site preparation, a 1cm midline incision was made on the ventral surface from the sternal notch rostrally. The subcutaneous tissues and salivary glands within the neck were bluntly dissected; the carotid sheath on the left side was exposed and overlying tissue was dissected. The left common carotid artery is isolated from the carotid sheath to expose as much length as possible. A small vascular clamp was placed proximally and an occluding suture was tied distally. The artery was then punctured with a 25G needle, which guides the tip of the catheter. The needle and vascular clamp were removed. The catheter was carefully advanced into the artery; the tip was placed just within the arch of the aorta. The catheter was then sutured into place, permanently occluding the artery. The body of the transmitter was placed into a subcutaneous pocket on the right flank of the mouse. The incision is sutured and the mouse is monitored until recovered. Telemetry wearing mice were permitted a 7-10 day recovery period prior to entry into experimental protocols.

Data Acquisition was through DataQuest A.R.T. (DSI), which was set to record every four minutes for 10 seconds, 24h/day. For acute Ang II response,

data acquisition rate was increased to every 30 seconds for one hour. Data for each animal was exported into Excel for analysis.

Microultrasound

The uterine arteries were evaluated using ultrasound at gd16 (Vevo® 2100, VisualSonics). Briefly, mice were anesthetized lightly using 1.5% isoflurane in medical air (21% oxygen and 78% nitrogen) and secured on the warmed stage. Body temperature was maintained at 37°C, and respiratory rate and ECG were monitored to ensure appropriate plane of anesthesia. Fur was removed from the abdomen and pelvic region with depilatory, and then warmed sonography gel was applied as a coupling agent. The left and right uterine arteries were identified dorsal to the bladder using 40MHz transducer in Doppler mode. The angle of the beam was kept <60°. All images and waveforms were saved for later analysis. A blinded expert confirmed images of all waveforms.

Plasma and Urine Assays

Plasma was obtained by collection of whole blood into EDTA Microtainers™ or Vacutainers™ (BD Vacutainer™) and centrifuged immediately at 4°C for 10 minutes at 1100*g*. Plasma was aliquoted and stored at -80°C. Urine was collected by direct aspiration from the bladder during terminal surgery and stored at -80°C. Plasma and urine were assayed with kits for cGMP and creatinine (R&D Systems). Plasma samples were assayed by ELISA for murine sFLT1 (R&D Systems), renin (Sigma) and Ang II (Enzo Life Sciences). All samples,

controls and standards were run in duplicate, with R^2>0.90 for standard curve. Samples were diluted appropriately, without extrapolation beyond manufacturerôs standard curve.

Semi-quantitative Scoring of Phospho-eNOS and Nitrotyrosine Expression in Mouse Vessels

Scores were generated from brightfield microscopy images obtained using an Olympus EX-41 stereomicroscope linked to a camera. Representative images with an original magnification of 20X from mouse mesenteric resistance vessels (phospho-eNOS) and abdominal aorta (nitrotyrosine) were acquired. Twenty vessels were scored for phospho-eNOS per mouse as replicates (n=4-5/group). For nitrotyrosine staining, four sections were scored (n=4-6/group). Semi-quantitative scoring was based on relative staining intensity of arterial endothelium: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = intense staining. The operator was blinded to the treatment of each sample.

Morphometric Quantification by Fluorescence and Light Microscopy

Measurements were generated from fluorescence microscopy images for MitoSOXÊ Red and light microscopy for placental phospho-eNOS and sFLT1 with an original magnification of 40x. Representative digital images of mouse aortas (n=3/group) were acquired. Five images were obtained and quantified per sample as replicates. For human placental samples, four replicate images of phospho-eNOS and two replicates images of sFLT1 staining were quantified

(n=6/group). Morphometric measurements were performed using NIH ImageJ software. To determine staining intensity, the threshold was set to include the MitoSOXÊ Red fluorescence product or DAB staining and the mean intensity (optical density; OD) of reaction product was calculated per image. The mean intensity was divided by tissue area to calculate positivity per area.

RT-PCR

Fresh tissue was snap frozen in liquid nitrogen, and then placed into -80°C. Tissues were subjected to Trizol/chloroform extraction and mechanical disruption. RNA was quantified, diluted to 1εg/εl and converted to cDNA via High-Capacity RNA-to-cDNA kit (Invitrogen). qRT-PCR was run using 1εg cDNA and TaqMan Fast Universal PCR mastermix and primer/probes for mouse *At1ra*, *At1rb*, *At2r*, *Bdkrb2*, *Edn1* and *Actb* (Mm01957722_s1, Mm02620758_s1, Mm01341373_m1, Mm00437788_s1, Mm00438656_m1 and Mm00607939_s1) from Invitrogen. All cDNA samples were run in duplicate with internal controls run on the same plate.

Wire Myography

Second order mesenteric resistance arteries were harvested from gd17 pregnant CD-1 mice injected with adenovirus expressing CMV-null, *sFlt1* or *sFlt1* with sildenafil treatment. Arterial rings were mounted (Danysh MyoTechnology) for isometric tension recordings using PowerLab software (AD Instruments). Administration of 10εM phenylephrine was used to test arterial viability, and

presence of intact endothelium was verified by acetylcholine (Ach, 1εM)-induced relaxation of a half-maximal phenylephrine-induced contraction. Ang II was administered at 100nM, previously reported to induce significant contraction.

Supplemental Table 1: Live birth delivery day (by gestational day; gd) in CD-1 females by treatment group. Data obtained from pregnant mice permitted to deliver spontaneously.

	CMV-null	*sFlt1*
gd16	0	1
gd17	0	4
gd18	4	4
gd19	4	8

Supplemental Figure 1: (A) Representative renal histology from CMV-null-injected (left) and *sFlt1*-injected mice (right). H&E staining reveals normal renal morphology in CMV-null, with significantly enlarged glomeruli with endotheliosis lesions in *sFlt1* mice. Bar = 50εm.

(B) Mean arterial pressure (MAP) and (C) heart rate (HR) in a single mouse recorded every 5 minutes for 30 minutes following 30ng bolus IV Ang II is shown during non-pregnant state and at various gestational days during pregnancy. Each curve shows immediately prior (t=0) and following Ang II administration as a moving average of four acquired measures. (D) qHR immediately following bolus IV Ang II (30ng in 30εl saline) for animals shown in Figure 1E is depicted. qHR was calculated by subtracting individual datapoints for each animal every five minutes for 15 minutes following injection. Baseline was recorded as nonpregnant (NP), then at increasing gestational days and post-partum (PP). Presented as median (minimum to maximum, n=3-5). *P<0.01 compared to NP and *sFlt1* at gd14 via two-way repeated measures ANOVA. (E) Plasma sFLT1 levels in gd17 CMV-null (n=5) and sFlt1 mice (n=3) without exogenous Ang II or with chronic Ang II (CMV-null, n=3; sFlt1, n=4). sFLT1 protein is elevated in *sFlt1* injected mice relative to CMV-null (*P<0.01). Analysis by one-way ANOVA with Bonferroni's post-hoc test. Unless specified, data are mean ± SEM.

Supplemental Figure 2: Gene expression analysis of (A) uterine vessels and (B) uterus obtained from CMV-null and *sFlt1* mice as nonpregnant (NP), gd14, gd17 and post-partum (PP). mRNA analysis (qRT-PCR) was completed for the Ang II receptors (*At1ra*, *At1rb* and *At2r*). (C) Uterine and (D) mesenteric vessels were additionally assayed for gene expression of endothelin-1 (*Edn1*) and bradykinin receptor beta2 (*Bdkrb2*). All data were normalized to beta actin (*Actb*; qCT). Comparisons between groups of nonpregnant (NP), gd14, gd17 and post-partum (PP) mice revealed no differences. Nonpregnant mice were injected with adenovirus at least 7 days prior to collection. n=3-8 mice per time point were assayed per tissue. Mean ± SEM.

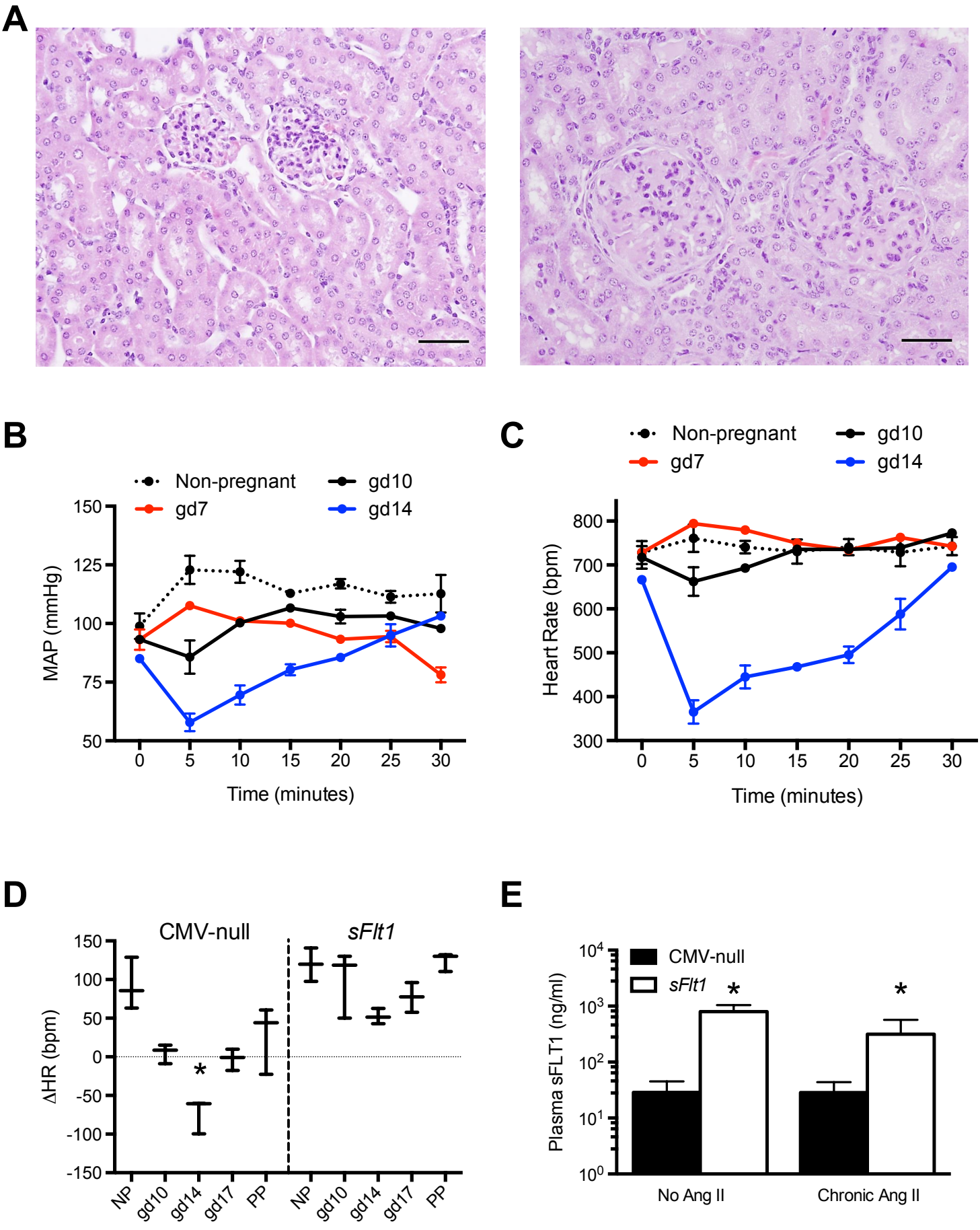
Supplemental Figure 3: (A) Semi-quantitative scores for phospho-eNOS staining intensity in mesenteric resistance vessels obtained from gd17 CMV-null (n=4) or *sFlt1* (n=5) mice. 20 vessels per animal were scored randomly: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = intense staining. *P<0.001 via two-way ANOVA.

(B) Quantitation of MitoSOX Red immunofluorescence in aortic tissue from gd17 CMV-null, *sFlt1* and L-NAME treated mice. Optical density (OD) per area (pixels^2) of tissue was calculated in five high power fields per sample (n=3 aortas/group). *P<0.001 vs. *sFlt1* and L-NAME by two-way ANOVA and Tukey's post-hoc test.

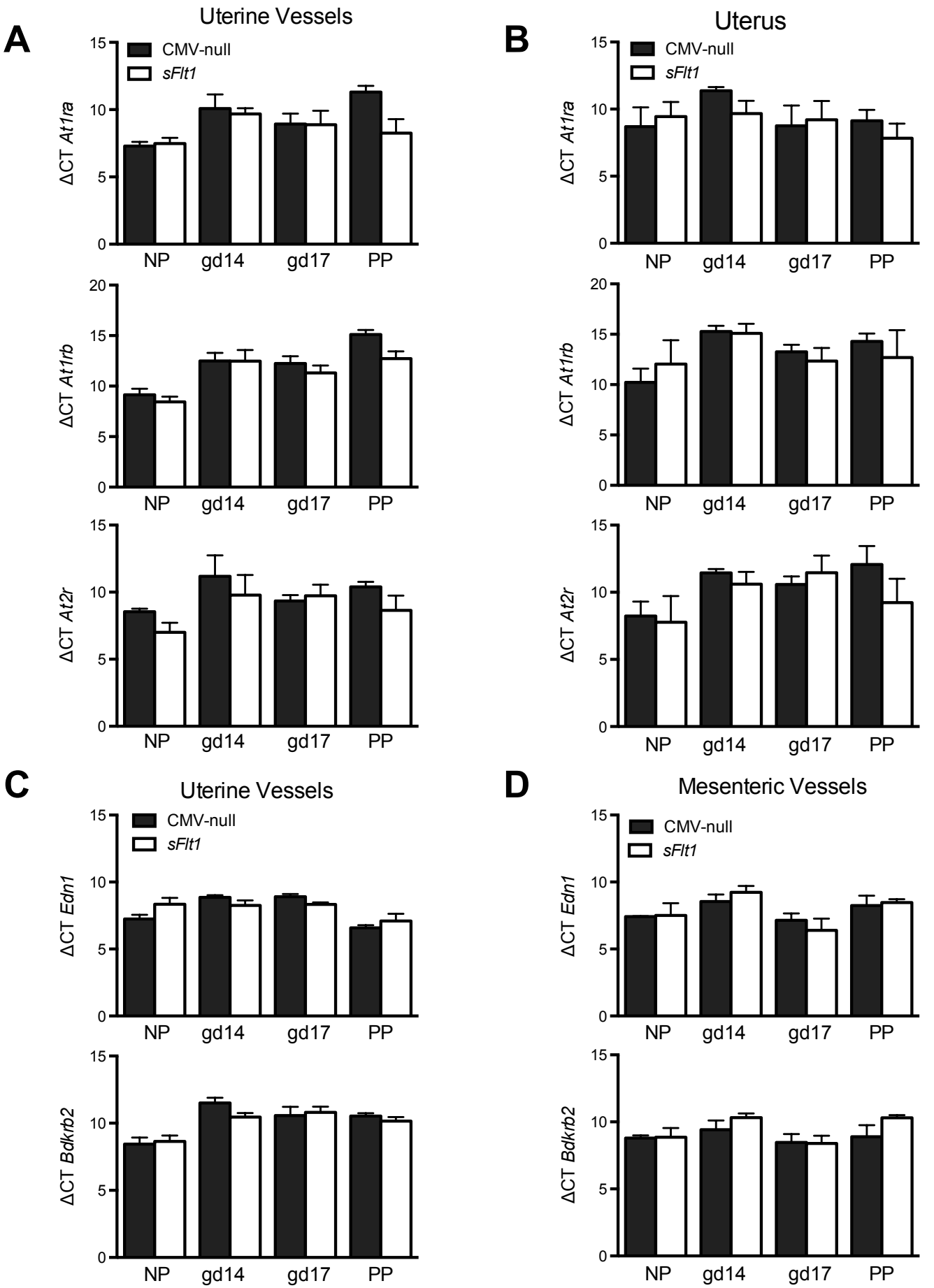
(C) Semi-quantitative scores for nitrotyrosine staining intensity in abdominal aorta obtained from gd17 CMV-null (n=4), *sFlt1* (n=5), and L-NAME (n=6) mice. Four

fields per animal were scored randomly: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = intense staining. *P<0.001 via two-way ANOVA. Presented as mean ± SEM.

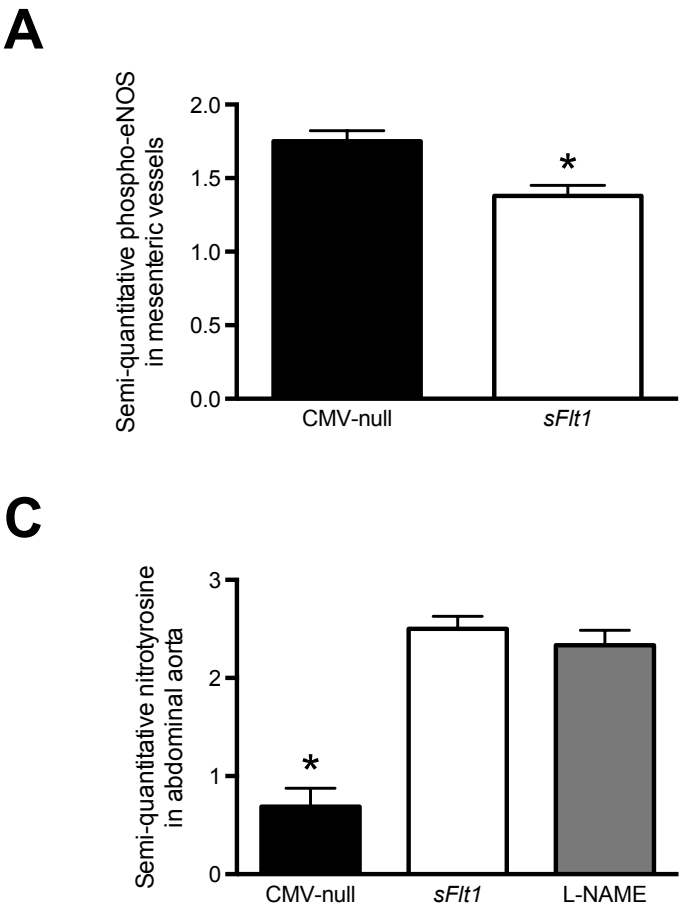
Supplemental Figure 4: (A) Fetal and (B) placental weights of *sFlt1* and *sFlt1* + sildenafil-treated pregnancies at gd17 (C) Ratio of placental weight to fetal weight as a proxy indicator of placental vascular insufficiency. *sFlt1* + SILD mice had an increase in this ratio; *P<0.05. (D) Litter sizes and (E) resorption rates were not different from gd17 *sFlt1* or *sFlt1* + SILD mice. (F) Plasma sFLT1 protein levels at gd11 and gd17 from paired mice randomized to treatment. There were no differences at gd11 or gd17; there was a significant increase between the two time points (two-way repeated measures ANOVA). All data presented from n=5/group as mean ± SEM. Unless specified, analysis was via one-way ANOVA with Tukey's post-test.



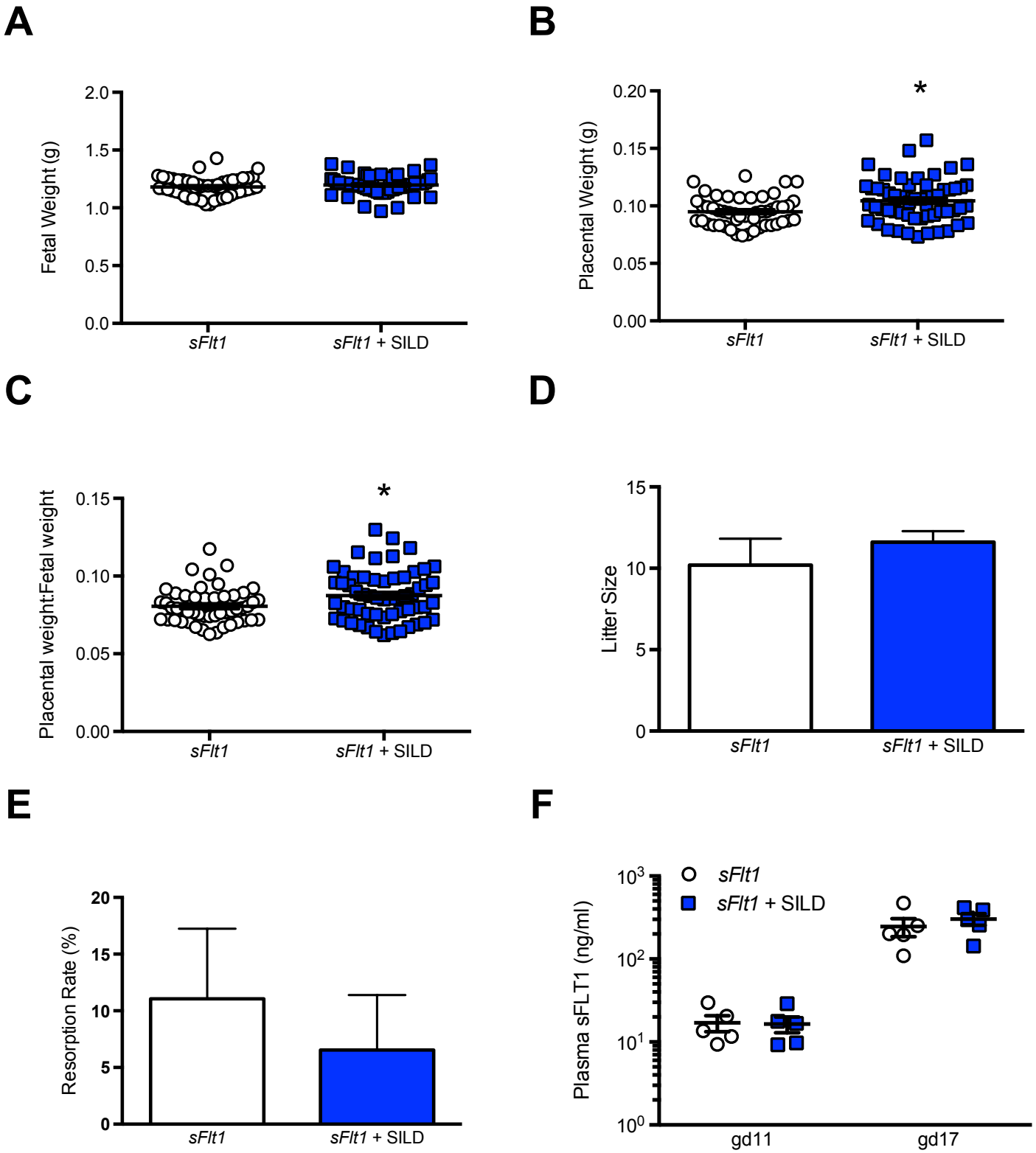
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4