Supplementary file for

Circulating maternal sFLT1 is sufficient to impair spiral arterial remodeling in a preeclampsia mouse model

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This supplementary file includes:

Materials and Methods – Supplement

Animals

Detailed information for mouse line generation and underlying genetics of hsFLT1/rtTA mice is previously described in Vogtmann et al. 2019[1]. For experimental set-up in this study hsFLT1/rtTA mice (6-9-month-old) were mated overnight, and the following day was counted as 0.5-day post conception (dpc). From mid-pregnancy (10.5 dpc) until the end of the second trimester (14.5. dpc) or the end of the pregnancy (18.5 dpc), dams were treated with either 2 mg/ml doxycycline (Dox) (0.2% [w/v]; Merck, Darmstadt, Germany) and 30 mg/ml sucrose (3% [w/v]; Carl Roth, Karlsruhe, Germany) in the PE and Dox Ctrl group or with sucrose only in the Ctrl group. The experimental “PE” group is defined by double-transgenic dams having both transgenic alleles (hsFLT1 and rtTA) and receiving 2 mg/ml Dox and 30 mg/ml sucrose in the drinking water, which induced the systemic hsFLT1 overexpression. The first control group named “Ctrl”, is defined by double-transgenic dams having both transgenic alleles (hsFLT1 and rtTA) like dams of the PE group but receiving only 30 mg/ml sucrose in the drinking water without doxycycline. This control group was chosen to test for effects of the two transgenic alleles without hsFLT1 overexpression. Furthermore, a second control group was performed to test for doxycycline side effects, named “Dox Ctrl”. The Dox Ctrl group is defined by single-transgenic hsFLT1 mice lacking the rtTA allele and receiving (like the PE group) 2 mg/ml doxycycline (Dox) and 30 mg/ml sucrose in the drinking water, and thus not expressing hsFLT1 but revealing possible Dox effects. Sampling was done at 14.5 (Ctrl: n=6, Dox Ctrl: n=4, PE: n=6) or 18.5 dpc (Ctrl: n=11, Dox Ctrl: n=3, PE: n=11). Mice were housed in a specific-pathogen–free environment at the animal facility of the University Hospital Essen or Berlin, were exposed to cycles of 12 h of light/dark and were provided with food and water ad libitum. In the parental generation of double-transgenic hsFLT1/rtTA mice, systemically expressed rtTA induces hsFLT1 expression upon Dox treatment. When Dox is added, rtTA can bind to the TetO promoter of the hsFLT1 transgene, leading to hsFLT1 expression (PE); without Dox, hsFLT1 is not expressed (Ctrl). Single transgenic hsFLT1 mice (lacking the rtTA allele) treated with Dox do not express hsFLT1 and served as a control for Dox side effects (Dox Ctrl). Since Dox passes the placental barrier, double transgenic hsFLT1/rtTA fetuses/placentas (PE hom:

homozygous for hsFLT1 and rtTA or PE het: homozygous for hsFLT1 and heterozygous for rtTA) in the first filial generation of the PE group can also express hsFLT1, whereas single transgenic fetuses/placentas (PE wt: lacking the rtTA allele) cannot. According to this, different variation of hsFLT1 expression was achieved in the PE group according to the fetal rtTA-genotype. Figure 1 A-B illustrates the distribution of hsFLT1 expression in PE wt (exclusive maternal) compared to PE het or hom group (maternal and feto-placental).

In vivo blood pressure measurements by telemetry

A set of mice (Ctrl n=6, PE n=6) were treated with Carprofen (5 mg/kg s.c.) prior procedure. After anesthesia by a mixture of air and 2-2,5% [v/v] Isoflurane, a vertical midline skin incision along the neck was made and the submaxillary glands were gently separated. The left common carotid artery was carefully isolated. Then the catheter was introduced into the carotid artery through a small incision in the vessel wall and the body of the transducer [PA-C10 model, Data Systems International (DSI), Overland Park, KS] was secured in a subcutaneous pouch along the animal's right flank through the same ventral neck incision. The neck incision was closed with 3.0 silk. Mice were kept warm on a heating pad and monitored closely until full recovery from anesthesia and treated for 3 days by Metamizole (1.33 mg/ml in drinking water). Recording of blood pressure was continuously monitored a few days prior mating and during whole pregnancy by use of RLA 1020 telemetry receivers (DSI), BCM consolidation matrix (DSI), and an adapter, where the signal was demultiplexed. This output subsequently was band-pass filtered and amplified. The information was fed to data acquisition and recording system, Dataquest software (version 4.31 (DSI)). Acquired data was normalized to mean prior pregnancy for each value respectively (systolic and diastolic blood pressure, as well as mean arterial pressure) for the PE and Ctrl group. Here we did not analyze the Dox-Ctrl group since it has already been confirmed that Dox alone does not influence the blood pressure in a Dox-inducible transgenic rat model of type 2 diabetes mellitus[6].

Tissue Preparation

Tissue preparation was done as previously described[1].At 14.5 (Ctrl n=6, Dox Ctrl n=4, PE n=6) or 18.5 dpc (Ctrl n=5, Dox Ctrl n=3, PE n=5), a distinct set of pregnant mice (compared to telemetry) were anesthetized via Ketamine 100 mg/kg body weight (BW) and Xylazine 10 mg/kg BW for an injection volume of 50µl/10g BW and killed by cervical dislocation or perfused with phosphate-buffered saline to obtain blood free organs (one dam per experimental group at 18.5 dpc). Maternal blood was collected; maternal kidneys and thoracic aortas, as well as whole implantation sites (14.5 dpc for histology) or fetuses, placentas and mesometrial triangle (MT) (14.5 dpc for mRNA; 18.5 dpc for histology and mRNA) were dissected in sterile phosphate-buffered saline (PBS) and weighed with an ALJ 220-4NM analytical balance (Kern, Ebingen, Germany) with a linearity of ±0.2mg. Fetal and placental weight were measured after fetuses, placentas and MT were separated. Organs were either frozen and stored at −80°C (for RNA, DNA) or immediately fixed in 4% [w/v] paraformaldehyde (PFA) for 24 h at 4°C and stored in 70% [v/v] ethanol at 4°C until being embedded in paraffin standard procedures (for morphology).

Genomic DNA Isolation, Genotyping, and Sex Determination

Genomic DNA was isolated from ear punch or fetal tail tissue samples as previously described[1, 2], with the REDExtract-N-AmpTM Tissue PCR Kit (#XNAT; Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. Genotyping and sex

determination of mice were performed with a standard PCR program (hsFLT1: initial denaturation 95◦C, 5 min; 40 cycles 94°C, 45 s, 60◦C, 45 s, 72◦C, 1 min, final extension 72°C, 5min; rtTA: initial denaturation 94°C, 3 min; 35 cycles 94°C, 45 s, 65°C, 1 min., 72°C, 1 min, final extension 72°C, 2 min; Syr/IL-3: initial denaturation 95°C, 4 min 30s; 35 cycles 95°C, 35 s, 50°C, 1 min, 72°C, 1 min, final extension 72°C, 5 min) and the appropriate primers (Table S1).

Serum hsFLT1 measurements

Serum samples were prepared as previously described[1]. Briefly, after centrifuging clotted blood for 15 min at 3000 g and 4°C; undiluted serum sample was used to measure the concentration of hsFLT1 with a BRAHMS KRYPTOR compact PLUS analyzer, according to the manufacturer's protocol. The following dams were tested: At 14.5 dpc: Ctrl n = 6, Dox Ctrl n = 4 and PE n = 6, at 18.5 dpc Ctrl n = 11, Dox Ctrl n = 3 and PE n = 11.

Serum Cytokine Array

111 murine cytokines were simultaneously detected in serum of dams with a Proteome Profiler Mouse XL Cytokine Array according to the manufacturer's protocol (ARY028; R&D Systems, Minneapolis, MN USA). In principle, selected capture antibodies for each of 111 different angiogenesis proteins have been spotted in duplicate on nitrocellulose membranes. For cytokine detection, a total of 200 µl serum of a pooled sample of each condition (Ctrl n = 5, Dox Ctrl n = 5, PE low sFLT1 n = 5 and PE high sFLT1 n = 5; each 40 µl) was diluted and mixed with a cocktail of biotinylated detection antibodies. The sample/antibody mixture was incubated with the array membrane at 4°C overnight. Streptavidin-horseradish peroxidase and chemiluminescent detection reagents were added, and chemiluminescence was detected with ChemiDoc™ XRS+ System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Pixel intensity for each spot was measured with Fiji/ImageJ[3] and normalized to negative and reference spots. Normalized intensities of the pair of duplicate spots representing each cytokine were determined and the most relevant proteins were presented.

Quantification of urinary albumin/creatinine ratio

Random, single-void urine specimens gained on 18.5 dpc, were used to determine albumin-to-creatinine ratio for monitoring kidney function. Albumin concentration was determined by Mouse Albumin ELISA kit (ICL/Dunn Labortechnik, Asbach, Germany) according to manufacturer's instructions. Creatinine levels were measured by colorimetric creatinine assay kit (Cayman chemical, Michigan, USA). The units for albumin/creatinine are mg/mg. The following dams were tested: 18.5 dpc: Ctrl, Dox-Ctrl and PE n = 5.

Histological analysis of kidneys

For conventional light microscopy, paraffin sections (2 µm) were rehydrated via decreasing concentrations of ethanol and stained with two different staining methods: periodic acid Schiff (PAS) and acid fuchsin-orange G (AFOG). For PAS staining, tissues were immersed in 0.9% periodic acid (Carl Roth, Germany) for 10 min, followed by 10 min incubation with Schiff reagent (Merck Millipore, Germany) and 2 min incubation in Mayer's hematoxylin solution (Sigma Aldrich, United States). For AFOG staining, sections were fixed via Bouin solution at 70°C for 1 h. Fixed tissues were submersed in Weigert's hematoxylin (Carl Roth, Germany) for 5 minutes, followed by incubation in a solution of 4% HCl, 63% ethanol for 10 sec and incubation in 1% phosphomolybdic acid for 5 min. At last, the sections were incubated in AFOG solution

for 10 min. For both staining protocols after dehydration, kidney sections were covered with histomount using coverslips. Imaging was done via the Leica SCN400 Slide Scanner on a 40x magnification.

Immunostaining of mouse kidney

For immunostaining, the rehydrated paraffin-embedded kidney sections (3 µm) were demasked using Tris-EDTA pH=9.0 at 110°C for 10 minutes and blocked with 3% [w/v] fetal bovine serum for 5 minutes. Sections were incubated with the primary antibodies overnight at 4°C; anti-Cd31 polyclonal goat antibody (1:1000; R&D systems, Minneapolis, USA), anti-Nephrin polyclonal Guinea pig antibody (1:200; Fitzgerald, Acton, USA), and anti-WT1 monoclonal rabbit antibody (1:1000; Abcam, Berlin, Germany). Consequently, tissues were incubated with the corresponding secondary antibody. Afterwards, the coverslips were mounted with Prolong Gold antifade DAPI (Invitrogen, Carlsbad, CA). Images were obtained using confocal immunofluorescence microscopy; SP8 confocal microscope, Leica Microsystems in 63x magnification.

Quantitative image analysis of mouse placenta and aorta

For all placental analyses, formalin-fixed and paraffin-embedded samples were sectioned parallel to the mesometrial-fetal axis and aortas were sectioned at 5 µm and mounted on Superfrost Plus Slides (R. Langenbrinck, Emmendingen, Germany). Stained slides were scanned with the Aperio CS2 ScanScope slide scanner (Leica, Wetzlar, Germany) at 20× or 40× magnification (Westdeutsche Biobank, University Hospital Essen, Germany), and images were converted to TIFFs via Image Scope (Version 12.3.2.8013; Leica). Scanned slides were opened (plugin "bioformats_package.jar.") and analyzed using Fiji/ImageJ[3]. Histological and immunohistochemical characterization of the spiral artery (SpA) remodeling was performed on two serial sections (Masson trichrome staining and PAS reaction), or on one serial section (for immunohistochemistry) at three different parts (100 µm interval) in the proximity of the umbilical cord, from each experimental group respectively (at 14.5 and 18.5 dpc Ctrl n = 12, Dox Ctrl n = 10, PE wt n = 4, PE het n = 6 and PE hom n = 6; and at 18.5 dpc Ctrl n = 8, Dox Ctrl n = 6, PE wt n = 12, PE het n = 6 and PE hom n = 5). Histological characterization of maternal aortas was performed on two serial sections (Masson trichrome staining) at two different parts (100 µm interval) from each experimental group (at 18.5 dpc Ctrl n=5, Dox Ctrl n=3, PE n=5).

Masson Goldner Trichrome staining of mouse placenta and aorta

For morphometric analysis of SpA-remodeling and aorta histology, sections were stained with Masson-Goldner's trichrome (MGT) staining kit (#3459; Carl Roth GmbH, Karlsruhe, Germany) according to manufacturer's protocol. In short, sections were deparaffinized, rehydrated, 3 min incubated with iron hematoxylin solution according to Weigert and 15 min blued in flowing tap water; followed by 5 min Goldner's stain I, 20 min Goldner's stain II and 10 min Goldner's stain III, plus rinsing with acetic acid solution 1% [v/v] in between and followed by a standard dehydration procedure and mounting in xylene mountant. MGT-staining visualizes nuclei in black, muscle cells in red/brown, erythrocytes in orange, and connective tissue in green. MGT-staining was used to quantify the lumen sizes of the mesometrial triangle and decidual arteries. The luminal and total vessel area was measured, and the luminal to total vessel area ratio was calculated as a marker for spiral arterial remodeling. A ratio of almost 1 represents a fully remodeled SpA, whereas a ratio below 1 represents worse SpA-remodeling. MGT-staining of aortas was used to assess aortic lumen and tunica media area.

Verhoeff's Van Gieson staining of mouse aorta

For analysis of aortic elastin fibers, sections were stained with Verhoeff's Van Gieson (VVG) stain kit (ab150667; Abcam, Cambridge, UK) according to manufacturer's protocol. In short, sections were deparaffinized, rehydrated, 15 min incubated with working elastic stain solution (30 ml hematoxylin solution (5%), 12 ml ferric chloride solution (10%) and 12 ml Lugol's iodine solution), followed by rinsing in tap water, short differentiation (Ferric Chloride (2%)) and 1 min incubation in sodium thiosulfate solution. Counterstain was performed by using Van Gieson's solution for 3 min, followed by a standard dehydration procedure and mounting in xylene mountant. VVG-staining visualizes nuclei in blue, elastic fibers in black, collagen in red and muscle cells in yellow. VVG-staining was used to analyze breaks, thinning and loss of elastic fibers of the thoracic aorta.

Periodic Acid Schiff (PAS) reaction of mouse placenta

Murine uterine natural killer (uNK) cells are recognized histochemically as containing glycoprotein-rich, cytoplasmic granules which react with Periodic Acid Schiff's (PAS) reagent. For visualization of uNKs in the mouse placenta PAS reaction was used. Therefore, sections were deparaffinized, rehydrated, incubated 10 min with 1% periodic acid (#HP00.1; Carl Roth), washed in tap water, incubated 20 min with Schiff's reagent (#X900.1; Carl Roth) and treated 3 x 2 min with sulfite water (18 ml 10% sodium-bisulfite solution + 300 ml distilled water + 15 ml 1M HCl) to reduce pseudo-PAS reaction. Quantification of PAS-positive area of total decidua and MT compartment was performed without counterstain of the nuclei.

Immunostaining of mouse placenta

SpA-remodeling was specifically assessed by counting the percentage of cells with immunoreactivity on α-smooth muscle actin (α -SMA) and Pan-Cytokeratin (Cyto), as reported previously[4]. Lymphatic mimicry of endothelial cells was visualized by immunoreactivity on cluster of differentiation 31 (Cd31) and Fms-related tyrosine kinase 4 (Flt-4). Deparaffinized and rehydrated sections were used for immunostaining. Antigens were retrieved by boiling sections with citrate buffer (pH 7,0) for 20 min with 20 min cool-down following. Endogenous peroxidase was blocked 10 min with H_2O_2 in methanol (1 ml methanol per 25 µl H_2O_2). After blocking 20 min with 0,5% [w/v] bovine serum albumin (BSA) in PBS, sections were incubated separately overnight at 4°C with rabbit anti-α-SMA (1:500; RB-9010-80, Thermo-Scientific (Pittsburgh, USA)), rabbit anti-Cyto (1:500; Z0622, Dako, Glostruck, Denmark), rat anti-Cd31 (1:20; DIA310; Dianova, Hamburg, Germany), or rat anti-Flt4 (1:10; 552857, BD PharMingen, Carlifornia, USA) primary antibody. Bound primary antibody was visualized by 1 h incubation with goat anti-rabbit immunoglobulin G horseradish peroxidase secondary antibody (1:100; P0448, Dako) for α-SMA and Cyto at room temperature, followed by signal detection using the liquid DAB+ substrate chromogen system (#K3468; Dako, Carpinteria, CA, USA) or with rabbit anti-rat immunoglobulin G biotinylated (1:100; 31834, Thermo Scientific), followed by the vectastain elite ABC HRP Kit (PK-6100, Vector Laboratories, Burlingame, USA) according to manufacturer's protocol and the liquid DAB+ substrate chromogen system. After washing, nuclei were counterstained 20 sec with hematoxylin followed by a standard dehydration procedure and mounting in xylene mountant.

RNA Extraction, cDNA Synthesis, and Quantitative PCR of mouse placenta and mesometrial triangle tissue

Total RNA was extracted from ~20mg of frozen tissue samples respectively with the RNeasy Mini Plus Kit (#74134, Qiagen, Hilden, Germany) according to the manufacturer's protocol. The quality and quantity of DNA were verified with µCuvette G1.0 and BioPhotometer Plus (Eppendorf, Hamburg, Germany). Only RNA with 260/280 = ~2.0 and 260/230 = 2.0-2.2 was used for complementary DNA (cDNA) synthesis. cDNA was synthesized with 2 µg RNA as previously described[1, 2]. Gene expression was measured from 1 µl cDNA with 19 µl of the PowerUP SYBR Green Master Mix (#A25742; Applied Biosystems, Foster City, CA, USA) and the ABI Prism 7300 Sequence Detection System (Applied Biosystems) with a standard PCR program. The quantitative PCR (qPCR) analyses were carried out in triplicate. The amount of cDNA in each sample was normalized to glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and β-Actin (Actb) as housekeeping genes and final gene expression analysis was done by standard curve method. Relative mRNA level was calculated by normalization to mean of the Ctrl group. Primer design was done with Primer3 and primer sequences are listed in Table S1. The following experimental groups were tested: At 14.5 dpc Ctrl n = 9, Dox Ctrl n = 9, PE wt n = 3 and PE het n = 7, and at 18.5 dpc Ctrl n = 8, Dox Ctrl n = 6, PE wt n = 8 and PE het n = 6 for placental or MT tissue, respectively.

Immunoblot analysis

One half (~20mg) of frozen MT tissue was homogenized in radioimmunoprecipitation assay (RIPA) protein extraction buffer as described previously[5] [50 mM Tris/HCl, 150 mM NaCl, 1% [w/v] NP-40, 0.25% [w/v] Na-deoxycholate, 1 mM ethylenediaminetetraacetic acid (EDTA)]. The protein content was determined with the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). 20 µg of total protein lysates were separated on 4%–15% polyacrylamide gels (BioRad, USA). Following electrophoresis, proteins were transferred onto nitrocellulose membranes via semi-dry blotting for 1 h. Non-specific binding sites were blocked with 5% [w/v] milk powder solved in TBST (Tris-buffered saline with 0.1% [v/v] Tween20) for 1 h. Nitrocellulose membranes were incubated with antibodies specific for VEGFR-2/Flk-1 (1:1000; #2479, Cell Signaling), VEGFR3/Flt-4 (1:200; AF743, R&D Systems), or β-Actin Peroxidase (1:200.000; A3854, Sigma) in 0.5% [w/v] bovine serum albumin or 0.5% [w/v] milk powder solved in TBS-T at 4°C overnight. Membranes were washed in TBST for three times and incubated for 1 h at room temperature with 1:5000 diluted anti-rabbit (p0448, DAKO), or donkey anti-goat HRP (sc2033, Santa Cruz Biotechnology) in 0.5% [w/v] milk powder in TBST. Detection was achieved with the SuperSignal West Dura Extended Duration Substrate Kit (Thermo Fisher Scientific) according to the protocol and detected using the Chemidoc XRS+ imaging system (BioRad, Feldkirchen, Germany). Protein level was determined by densitometric analyses of specific bands using Fiji/ImageJ[3] and normalized against β-Actin. For normalization purposes, the same reference-sample ran on each blot as an internal control, together with the PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific, Pittsburgh, USA) to determine protein size.

References – Supplement

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323 **Tables – Supplement**

324

325 **Table S1:** Oligonucleotide primer for quantitative real time PCR, genotyping and sex
326 determination.

Gene	NCBI number	Primer sequence (5′ → 3′)	Product length (bp)
		Housekeeping genes	
β-Actin	NM_007393.5	for: CCTCTATGCCAACACAGTGC	206
		rev: CCTGCTTGCTGATCCACATC	
Gapdh	XM_011241214.1	for: ACAACTCACTCAAGATTGTCAGCA	121
		rev: ATGGCATGGACTGTGGTCAT	
		Trophoblast invasion – Figure 3	
Plf	NM_011954.3	for: AGGAGCCATGATTTTGGATG	203
		rev: ACCAGGCAGGGTTCTTCTTT	
Mmp2	NM_008610.3	for: CGATGTCGCCCCTAAAACAG	176
		rev: GCATGGTCTCGATGGTGTTC	
Mmp9	NM_013599.4	for: AAAACCTCCAACCTCACGGA	190
		rev: GTGGTGTTCGAATGGCCTTT	
Tnfα	NM_001278601.1	for: CGTCAGCCGATTTGCTATCT	206
		rev: CGGACTCCGCAAAGTCTAAG	
Tgfβ	NM_011577.2	for: TGCTTCAGCTCCACAGAGAA	182
		rev: TGGTTGTAGAGGGCAAGGAC	
Infγ	NM_008337.4	for: CGCTACACACTGCATCTTGG	181
		rev: GTCACCATCCTTTTGCCAGT	
		uterine natural killer (uNK) cell marker – Figure 4	
Cd56	NM_001311065.1	for: TTGTTCAAGCAGACACACCG	226
		rev: TCAGGTTTCAGGCCCATGAT	
Cd49a	NM_001033228.3	for: CCTTTCAAACTGAGCCCACC	170
		rev: AATGTTGACGTCCCCATCCT	
Prf1	NM_011073.3	for: TCTTGGTGGGACTTCAGCTTTC	150
		rev: TCTGCTTGCATTCTGACCGA	
Gzma	NM_010370.3	for: CCTGAAGGAGGCTGTGAAAGAA	106
		rev: CGCCAGCACAGATGGTATTTG	
Klrg1	NM_016970.1	for: AGGGAGTGAAGCTGTTTGGAG	77
		rev: CCTCCAGCCATCAATGTTCCT	
		Angiogenesis – Figure 5	
hsFLT1	XM_017020485.1	for: AATCATTCCGAAGCAAGGTG	221
		rev: TTTCTTCCCACAGTCCCAAC	
Flk-1	NM_001363216.1	for: GGCGGTGGTGACAGTATCTT	162
		rev: GTCACTGACAGAGGCGATGA	

Flt-4	NM_008029.3	for: GTGGCTGTGAAGATGCTGAA	199
		rev: TGACACGCAAGAAGTTGGAG	
Plgf	XM_011244016.1	for: CGTCCTGTGTCCTTCTGAGT	200
		rev: CCTCTTCCTCTTCCCCTTGG	
Vegfa	NM_001025257.3	for: CAGGCTGCTGTAACGATGAA	140
		rev: GCATTCACATCTGCTGTGCT	
Vegfb	NM_011697.3	for: AACACAGCCAATGTGAATGC	157
		rev: GGAGTGGGATGGATGATGTC	
Vegfc	NM_009506.2	for: CAAGGCTTTTGAAGGCAAAG	159
		rev: TCCCCTGTCCTGGTATTGAG	
Vegfd	NM_001308489.1	for: CAACAGATCCGAGCAGCTTC	155
		rev: AAAGTTGCCGCAAATCTGGT	
		Hypoxia – Figure S3	
Hif1α	NM_001313920.1	for: TCAAGTCAGCAACGTGGAAG	198
		rev: TATCGAGGCTGTGTCGACTG	
Hif2α	NM_010137.3	for: GCAAGCCTTCCAAGACACAA	228
		rev: CCTGAGCTCCTGGTAGATGG	
Phd1	NM_001357767.1	for: TGCATCAAGTGGAGGAGGAG	246
		rev: GATGCTCCTGGACGGTGATA	
Phd2	NM_001363475.1	for: AGGTGAGAAAGGTGTGAGGC	228
		rev: CACAGTACAGTCCAGCAGA	
		Genotyping	
hsFLT1	NM_001159920.2	for: CAAGGACGTAACTGAAGAGG	465
		rev: TTTCTTCCCACAGTCCCAAC	
Col1a1		for: CCATCCCAACAATACATCACA	200
		rev: TGGTTTCTTTGGGCTAGAGG	
rtTA		for: AAAGTCGCTCTGAGTTGTTAT	
		rev-wt: GGAGCGGGAGAAATGGATATG	650
		rev-mut: GCGAAGAGTTTGTCCTCAACC	340
		Sex determination	
IL-3	NM_010556.4	for: GGGACTCCAAGCTTCAATCA	544
		rev: TGGAGGAGGAAGAAAAGCAA	
Sry	NM_011564.1	for: TGGGACTGGTGACAATTGTC	402
		rev: GAGTACAGGTGTGCAGCTCT	

Figures – Supplement

Figure S1

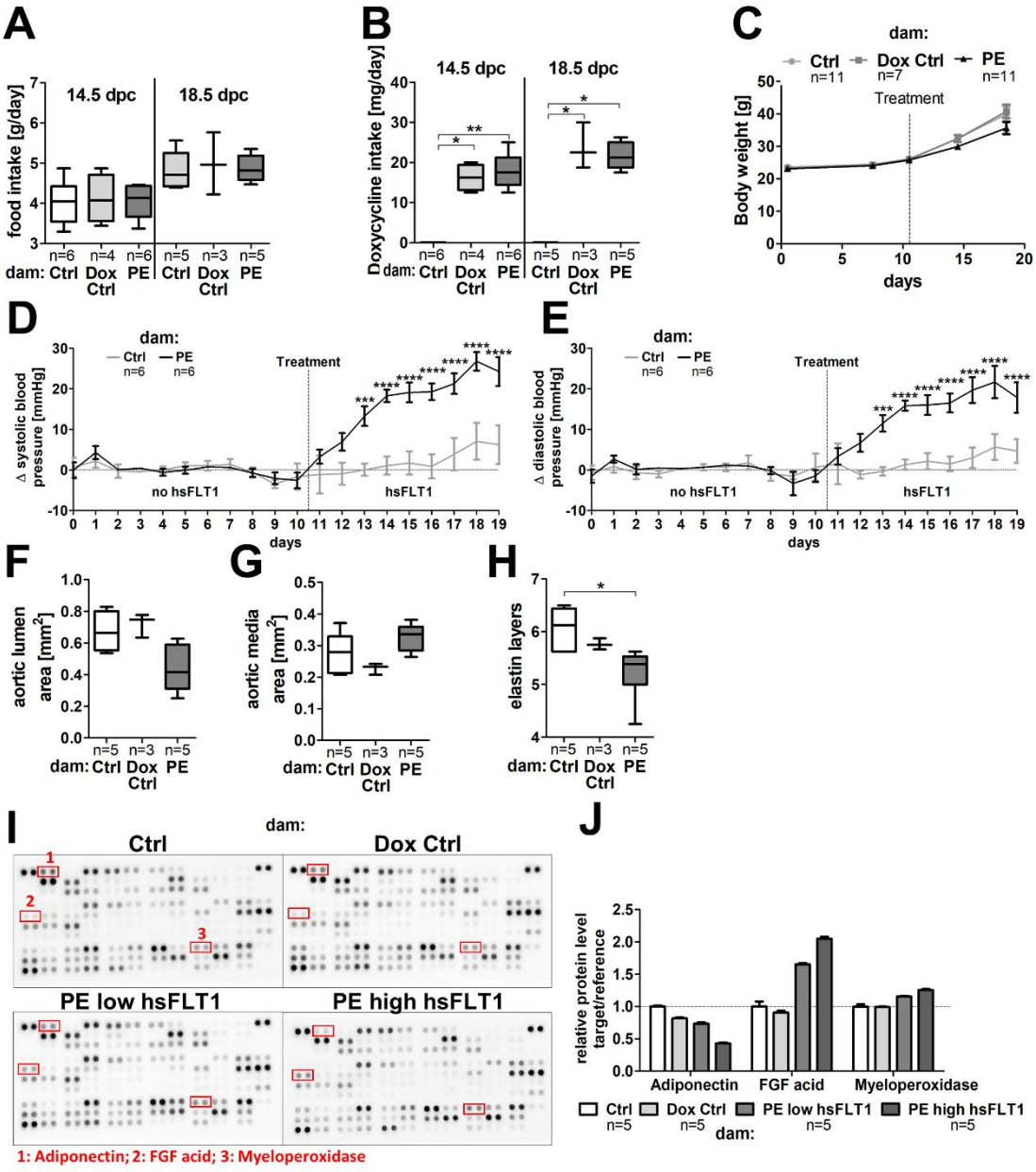

Figure S1: Maternal characteristics during experimental sFLT1-related preeclampsia.

[A] Food and [B] doxycycline intake per day and [C] weight gain of dams during (Ctrl, Dox Ctrl and PE), showed no differences between experimental groups. [D] Systolic and [E] diastolic blood pressure during whole pregnancy of dams of Ctrl and PE group indicating hypertension exclusively upon hsFLT1 overexpression. [F] Thoracic aortas of dams at 18.5 dpc exhibited reduced aortic lumen, [G] combined with increase in aortic tunica media area and [H] decrease in elastin layers upon hsFLT1 expression (PE) compared to controls. [I] Mouse XL Cytokine Arrays incubated with 200 µl of pooled serum samples (n=5 dams) for each experimental group. [J] Most prominently changed cytokines are listed, showing downregulation of Adiponectin and upregulation of FGF acid and Myeloperoxidase upon hsFLT1 overexpression compared to controls (Ctrl and Dox Ctrl), depending on maternal hsFLT1 serum level. Data are presented as box plot with median, interquartile range ± upper/lower extreme, or as mean ±

standard error of the mean; sample size n is listed under each graph respectively; Kruskal-Wallis combined with Dunn's multiple comparisons test, or with Mann Whitney U test was used to set *p<0.05; **p<0.01; ***p<0.001 and ****p<0.0001.

Figure S2

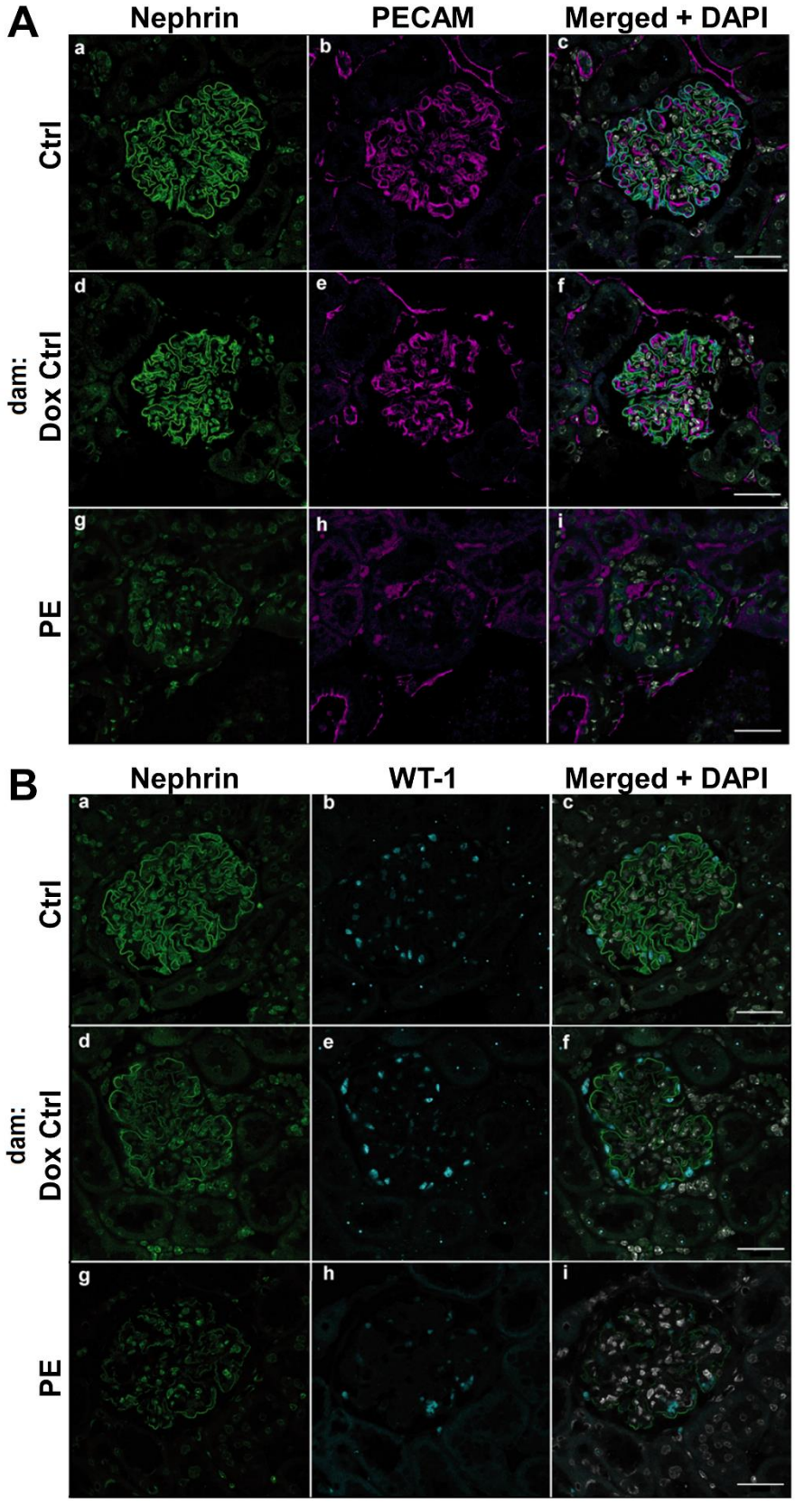

Figure S2: Immunofluorescence staining of glomeruli. [A] Double staining of glomeruli with slit diaphragm marker Nephrin (a, d, g) and endothelial cell marker Cd31 (b, e, h). Compared to control mice (Ctrl, Dox Ctrl), systemically hsFLT1 expressing mice (PE) showed reduced Nephrin (g) and Cd31 staining (h). [B] Double staining of glomeruli with Nephrin antibody (a, d, g) and for podocyte specific transcription factor WT-1 (b, e, h) showing reduced Nephrin and WT-1 staining upon hsFLT1 expression (PE) compared to both controls (Ctrl and Dox Ctrl). Scale bar: 200µm.

Figure S3

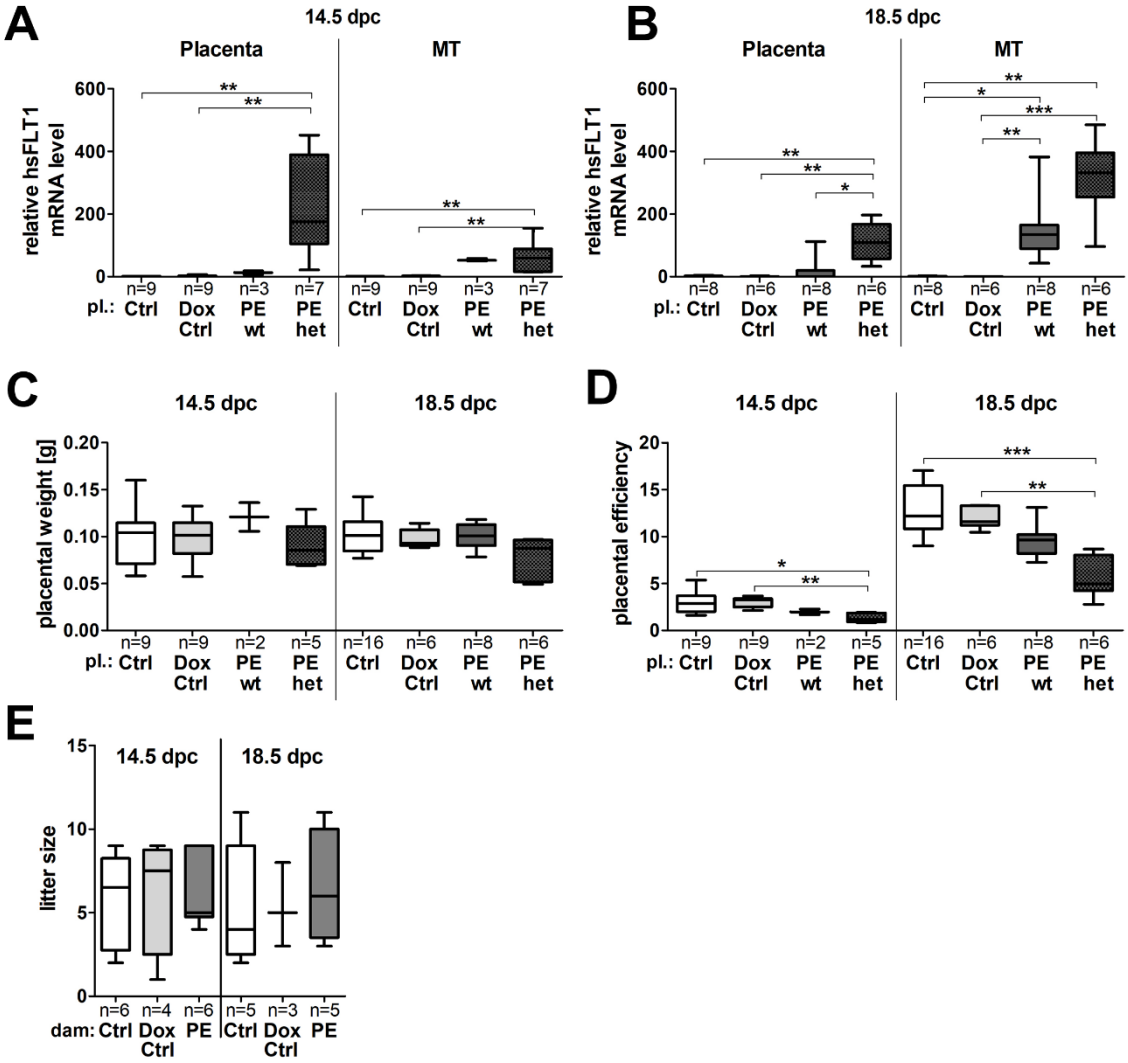

Figure S3: Placental hsFLT1 mRNA expression, placental weight, placental efficiency (placental weight/fetal weight) and litter size. [A] hsFLT1 level of placental and MT tissue at 14.5 and [B] 18.5 dpc. [C] Placental weight, [D] placental efficiency (placental/fetal weight) and [E] litter size in the PE, Ctrl and Dox-Ctrl group at 14,5 and 18.5 dpc. The placental weight and the litter size did not change significantly among groups at both timepoints, but to a reduced placental efficiency upon hsFLT1 overexpression. Data are presented as box plot with median, interquartile range ± upper/lower extreme, or as mean ± standard error of the mean; sample size n is listed under each graph respectively; Kruskal-Wallis combined with Dunn's multiple comparisons test/Mann Whitney U test; *p<0.05, **p<0.01 and ***p<0.001.

Figure S4

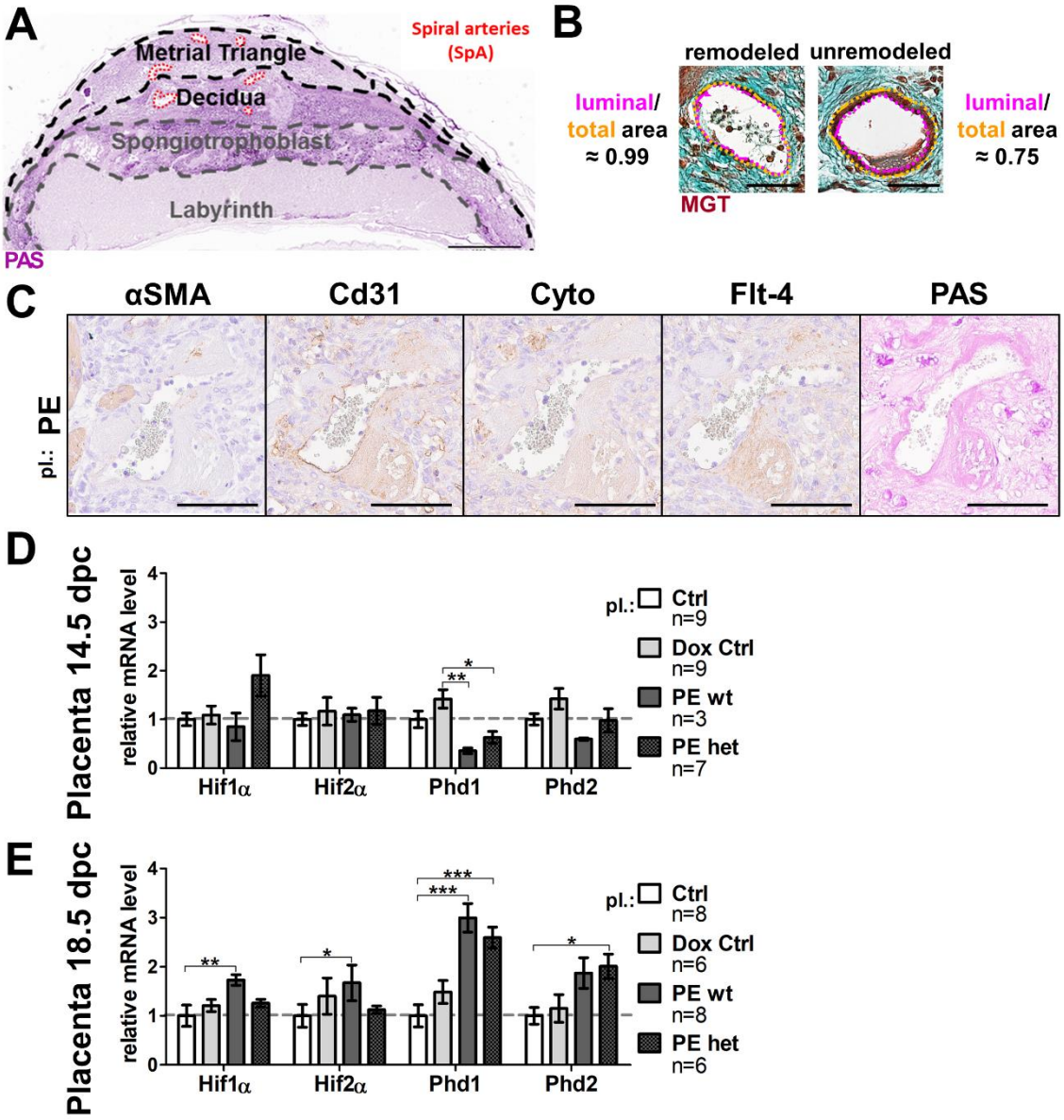

Figure S4: Mesometrial triangle markers. [A] Example image of the location of spiral arteries (SpA) in the two maternal implantation compartments decidua and mesometrial triangle (MT), scale bar: 1000 µm. [B] Example images of remodeled and unremodeled SpAs in the MT stained with Masson Goldner trichrome and illustration of luminal (pink line) to total vessel (orange line) area ratio calculation, scale bar: 100 µm. [C] Representative images of the same decidual spiral artery (SpA) at 18.5 dpc of the PE group in different staining indicating many Cd31-, Flt-4 and Cytokeratin-positive cells and only a few αSMA-positive cells in the PAS-positive fibrinoid deposition surrounding the SpA. [D/E] mRNA expression analysis of *Hypoxia-inducible factor* (*Hif1α*, *Hif2α*) and *Prolyl hydroxylases* (*Phd1*, *Phd2*) at 14.5 dpc and at 18.5 dpc in placental tissue. [D] mRNA level of *Hif1α* and *Hif2α* were unaffected at 14.5 dpc but [E] upregulated upon maternal hsFLT1 overexpression (PE wt) at 18.5 dpc, whereas mRNA level of Phd1 and Phd2 were downregulated upon maternal hsFLT1 overexpression (PE wt) at 14.5 dpc [F] and upregulated at 18.5 dpc [G]. Data are presented as box plot with median, interquartile range ± upper/lower extreme, or as mean ± standard error of the mean; sample size n is listed under each graph

393 respectively; Kruskal-Wallis combined with Dunn's multiple comparisons test, or with
394 Mann Whitney U test was used to set *p<0.05; **p<0.01; ***p<0.001 and ****p<0.0001.