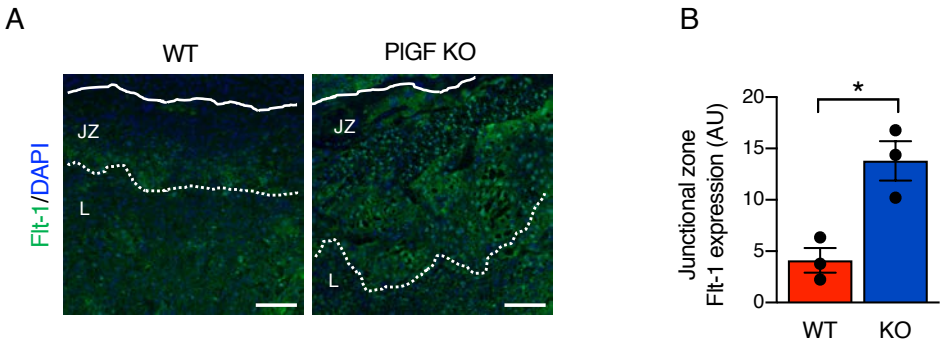
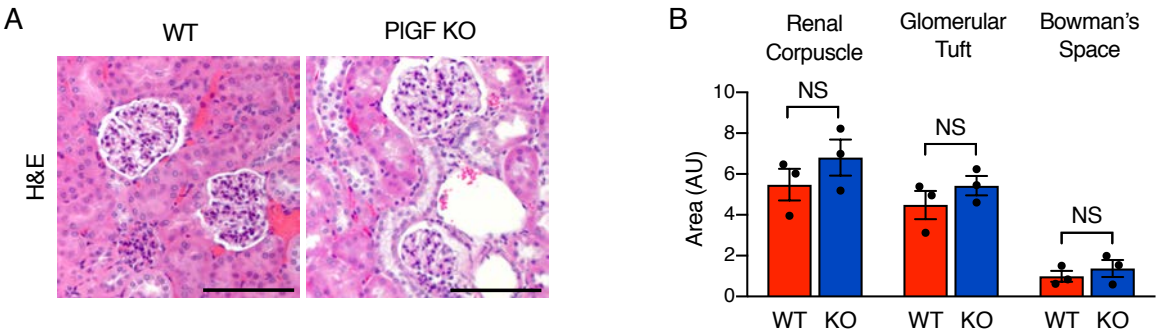
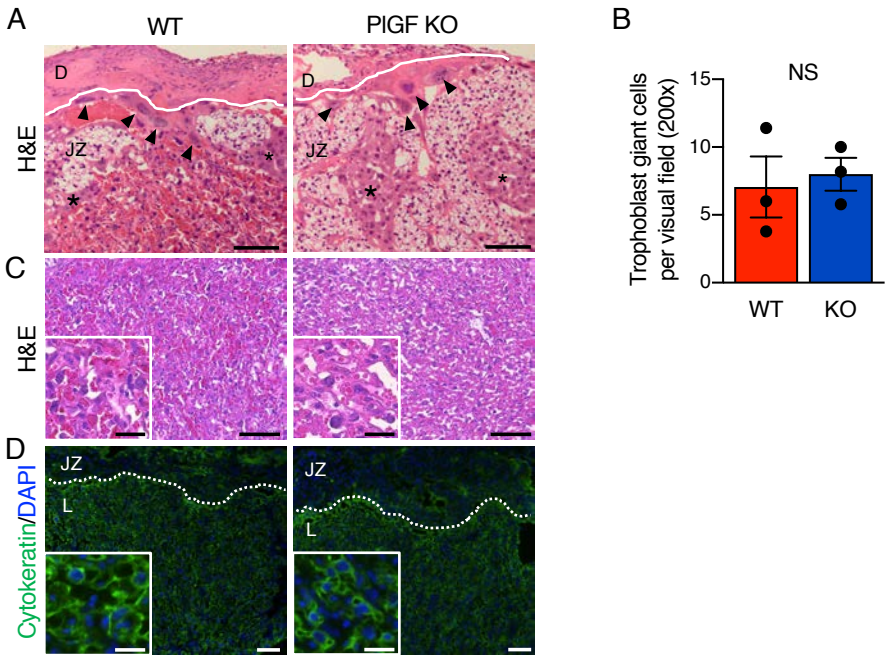


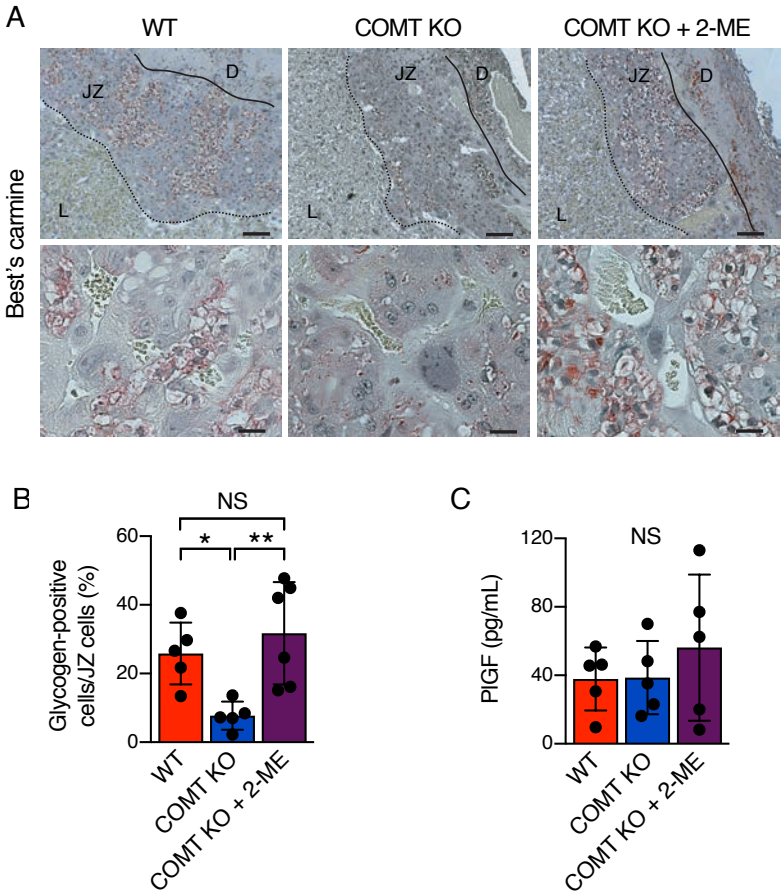
Supplemental Figure 1. Elevated placental Flt-1 expression in PlGF knockout mice.
(**A**) Immunofluorescence staining for Flt-1 and (**B**) quantification showing increased Flt-1 expression in the junctional zone of PlGF KO placentas (n=3 litters, each with 3 representative high-power fields examined per placenta). Scale bar: 100 μm. JZ, junctional zone; L, labyrinth. Results are shown as mean ± S.E.M. Two-tailed, unpaired t test; *$P < 0.05$.



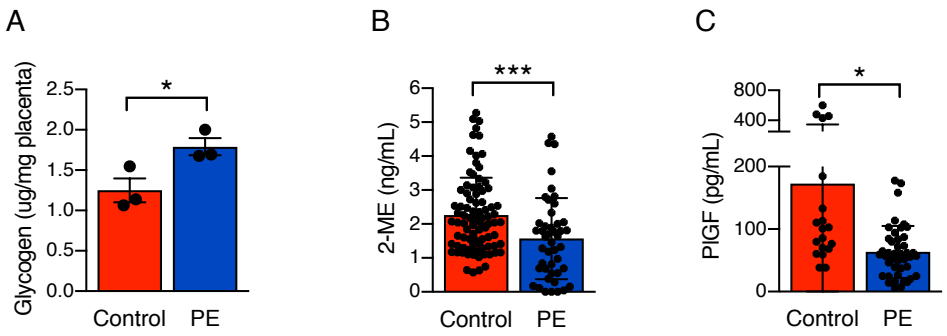
Supplemental Figure 2. Lack of renal and placental pathology in PlGF knockout.
(**A**) Representative high-magnification images of H&E-stained sections of maternal kidneys from WT and PlGF KO mice (E16-20). Scale bar: 100 μm. (**B**) Quantification of renal corpuscle, glomerular tuft, and Bowman's space area in the indicated groups (n=3, 6-10 glomeruli examined per mouse). AU, arbitrary units. Results are shown as mean ± S.E.M. Two-tailed, unpaired t test; NS, not significant.



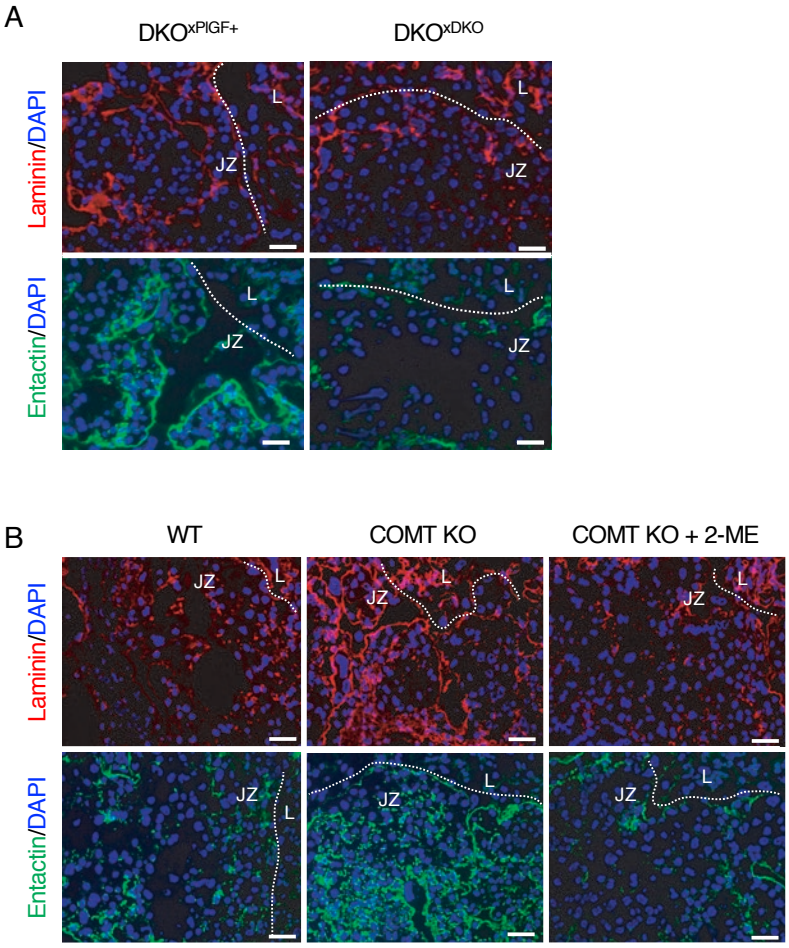
Supplemental Figure 3. Normal trophoblast giant cell morphology in PIGF knockout placentas. (**A-B**) Representative H&E-stained sections showing normal morphology and number of parietal trophoblast giant cells (black arrowheads) at the border of the decidua and junctional zone in WT and PIGF KO (n=3, 4 high-power fields per placenta). Groups of spongiotrophoblast cells marked with asterisks. Glycogen trophoblast cells appear as clusters of vacuolated cells between groups of spongiotrophoblast cells. Scale bar: 100 μm. (**C-D**) Representative images showing H&E staining (**C**) and immunofluorescence staining for pan-cytokeratin (**D**) showing similar labyrinth architecture and mononuclear sinusoidal trophoblast giant cell (cytokeratin positive) morphology between PIGF KO and WT. Sections from E19 placentas. Scale bar: 100 μm and 25 μm in low- and high (inset)-magnification images, respectively. Solid and dotted lines mark the border between the junctional zone and the decidua, and junctional zone and labyrinth, respectively. D, decidua; JZ, junctional zone; L, labyrinth. Results are shown as mean ± S.D. Two-tailed, unpaired t test; NS, not significant.



Supplemental Figure 4. Placental glycogen content and PlGF levels in the COMT knockout preeclampsia model. (**A**) Representative images of the placenta (low-magnification, top panels) and of the junctional zone (high-magnification, lower panels) showing glycogen staining (Best's carmine) of E17 placentas from the indicated groups. Solid and dotted lines mark the border between the junctional zone and the decidua, and junctional zone and labyrinth, respectively. Scale bar: 100 μm and 25 μm in low- and high-magnification images, respectively. (**B**) Ratio (%) of glycogen-positive cells to total junctional zone cells per visual field (n=2 litters, at least 2 placentas per group). (**C**) Circulating PlGF levels in the indicated groups measured by ELISA (n =5). 2-ME, 2-methoxyestradiol; COMT, catechol-O-methyltransferase; D, decidua; JZ, junctional zone; L, labyrinth. Results are shown as mean ± S.D. One-way ANOVA; NS, not significant, *P < 0.05, **P < 0.01.



Supplemental Figure 5. Levels of placental glycogen, 2-ME and PlGF in human preeclampsia.
(**A**) Quantification of tissue glycogen content measured from biopsies of human placenta (*n*=3). (**B**) 2-ME levels in normotensive pregnant controls (*n*=88) and preeclampsia cases (*n*=45). (**C**) Plasma PlGF levels in normotensive pregnant controls (*n*=19) vs. preeclampsia cases (*n*=43). PlGF samples here are also represented in Figure 4J. 2-ME, 2-methoxyestradiol; PE, preeclampsia. Results are shown as mean ± S.D. Two-tailed, unpaired *t* test (**A**) and (**C**) or Mann-Whitney test (**B**); $^{*}P < 0.05$, $^{***}P < 0.001$.



Supplemental Figure 6. Increased abundance of basement membrane proteins in COMT knockout, PlGF-positive placentas. (**A-B**) Representative images showing immunofluorescence staining of basement membrane proteins, laminin (red) and entactin (green) in placentas from DKO (**A**) and COMT KO (**B**) mice. Dotted lines mark the border between the junctional zone and labyrinth. Scale bar: 50 μm. 2-ME, 2-methoxyestradiol; COMT, catechol-O-methyltransferase; JZ, junctional zone; L, labyrinth.

Supplemental Table 1: Embryonic and placental weight comparisons among mouse cohorts.

Groups	PlGF			COMT[A]				DKO		
Genotypes	WT	KO	*P*	WT	KO	KO + 2-ME	*P*	DKO^{xDKO}	DKO^{xPlGF+}	*P*
Litters (no. of embryos), *n*	7 (63)	4 (40)	--	7 (48)	12 (86)	12 (88)	--	5 (52)	5 (38)	--
Placental weight, mg	85 ± 16	94 ± 16	NS	92 ± 14	85 ± 11[B]	93 ± 11[C]	<0.0001	87 ± 15	84 ± 10	NS
Embryo weight, g	0.96 ± 0.3	0.89 ± 0.3	NS	0.83 ± 0.1	0.85 ± 0.1	0.88 ± 0.1	NS	0.76 ± 0.2	0.88 ± 0.1	<0.001
Embryo/placenta weight ratio, g/g	11.6 ± 4.4	9.7 ± 3.6	<0.05	9.1 ± 1.6	10.1 ± 1.7[B]	9.6 ± 1.9	<0.01	8.9 ± 2.1	10.4 ± 1.5	<0.0001

Data are mean ± S.D. Comparisons made within each group: PlGF, Swiss/SV129 (E17-20); COMT, C57BL/6/SV129 (E17); DKO, C57BL/6/SV129/Swiss (E17). Statistical significance determined using two-tailed, unpaired *t* test for PlGF and DKO mice, one-way ANOVA with Tukey's multiple comparisons test for COMT mice. NS, not significant; 2-ME, 2-methoxyestradiol.

[A] Some of the data shown for the COMT model were published previously and shown here for comparative purposes only (from "Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia," by K. Kanasaki, et al., 2008, *Nature* 453, pp. 1117–1121. Copyright 2008 by Nature Publishing Group).

[B] Statistically significant difference when compared to WT.

[C] Statistically significant difference when compared to KO.

Supplemental Table 2: Comparison of blood pressure, proteinuria, and sFlt-1 among mouse cohorts.

Groups	PlGF			COMT[A]				DKO		
Genotypes	WT	KO	*P*	WT	KO	KO + 2-ME	*P*	DKO^{xDKO}	DKO^{xPlGF+}	*P*
Systolic BP, mmHg										
Non-pregnant	---	---	---	121 ± 7	117 ± 9		NS	142 ± 15		---
Pregnant	105 ± 8	111 ± 5	NS	108 ± 5	125 ± 9[B]	111 ± 7[C]	<0.001	121 ± 14[D]	151 ± 6	<0.01
Urine albumin/Cr										
Non-pregnant	2.2 ± 0.9	2.8 ± 0.6	NS	1.2 ± 0.2	1.2 ± 0.3		NS	1.7 ± 0.3		---
Pregnant	2.5 ± 1.1	2.0 ± 1.2	NS	1.0 ± 0.4	1.6 ± 0.3[B]	1.2 ± 0.4[C]	<0.05	0.9 ± 0.2[D]	1.3 ± 0.3	NS
sFlt-1, ng/mL										
Non-pregnant	1.6 ± 0.3	1.8 ± 0.9	NS	1.8 ± 1.4	1.4 ± 0.1		NS	0.8 ± 0.3		--
Pregnant	15.8 ± 4.4	38.4 ± 15.9	<0.05	11.6 ± 0.9	15.6 ± 1.5[B]	11.8 ± 1.8[C]	<0.05	28.3 ± 10.5[D]	19.9 ± 8.9	NS

Data are mean ± SD. Comparisons made within each strain: PlGF, Swiss/SV129 (E17-20); COMT, C57BL/6/SV129 (E17); DKO, C57BL/6/SV129/Swiss.

The following numbers of mice were used. Systolic BP: non-pregnant COMT WT, *n*=11; non-pregnant COMT KO, *n*=15; non-pregnant DKO, *n*=9; pregnant PlGF WT, *n*=5; pregnant PlGF KO, *n*=4; pregnant COMT WT, *n*=9; pregnant COMT KO, *n*=12; pregnant COMT KO + 2-ME, *n*=10; pregnant DKO^{xDKO}, *n*=9; pregnant DKO^{xPlGF+}, *n*=5. Urine albumin/Cr: non-pregnant PlGF WT, *n*=4; non-pregnant PlGF KO, *n*=14; non-pregnant COMT WT, *n*=7; non-pregnant COMT KO, *n*=7; non-pregnant DKO, *n*=6; pregnant PlGF WT, *n*=8; pregnant PlGF KO, *n*=11; pregnant COMT WT, *n*=6; pregnant COMT KO, *n*=11; pregnant COMT KO + 2-ME, *n*=9; pregnant DKO^{xDKO}, *n*=6; pregnant DKO^{xPlGF+}, *n*=6. sFlt-1 levels: non-pregnant PlGF WT, *n*=4; non-pregnant PlGF KO, *n*=5; non-pregnant COMT WT, *n*=6; non-pregnant COMT KO; *n*=7, non-pregnant DKO, *n*=3; pregnant PlGF WT, *n*=4; pregnant PlGF KO, *n*=5; pregnant COMT WT, *n*=8; pregnant COMT KO, *n*=13; pregnant COMT KO + 2-ME, *n*=13; pregnant DKO^{xDKO}, *n*=6; pregnant DKO^{xPlGF+}, *n*=5.

Statistical significance determined using two-tailed, unpaired *t* test for PlGF mice, one-way ANOVA with Tukey's multiple comparisons test for COMT and DKO mice. NS, not significant. 2-methoxyestradiol; BP, blood pressure; Cr, creatinine.

[A]Some of the data shown for the COMT model were published previously and shown here for comparative purposes only (from "Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia," by K. Kanasaki, et al., 2008, *Nature* 453, pp. 1117–1121. Copyright 2008 by Nature Publishing Group).

[B] Statistically significant difference when compared to WT.

[C] Statistically significant difference when compared to KO.

[D] Statistically significant difference when compared to non-pregnant DKO; $P<0.01$ for systolic BP and sFlt-1, $P<0.001$ for urine albumin/Cr.