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## Supplementary Information for

**Title:** Intersection of Regulatory Pathways Controlling Hemostasis and Hemochorial Placentation

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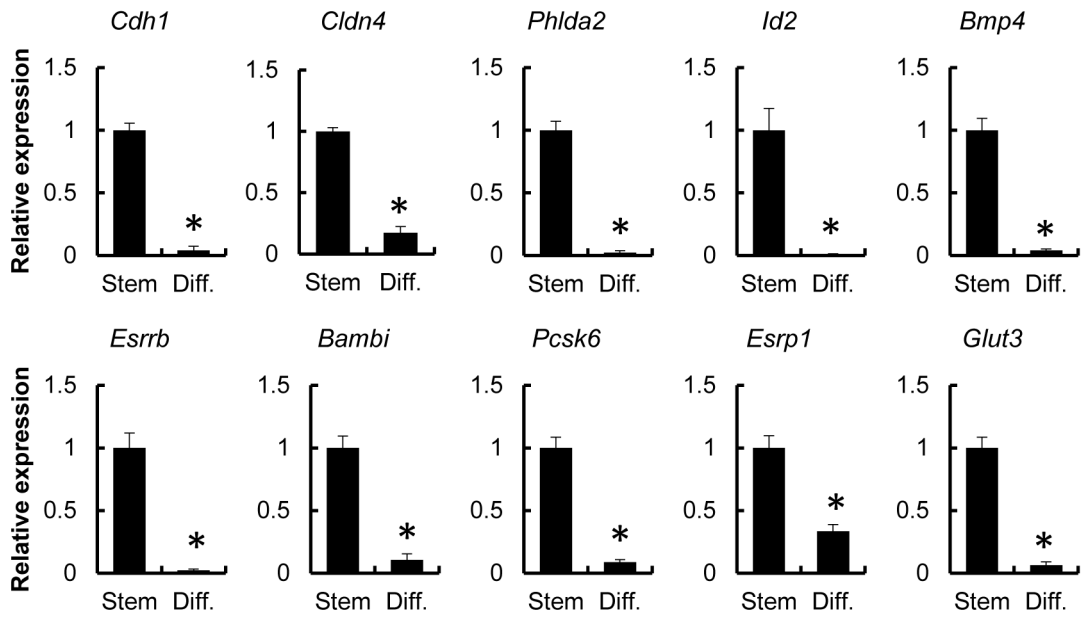
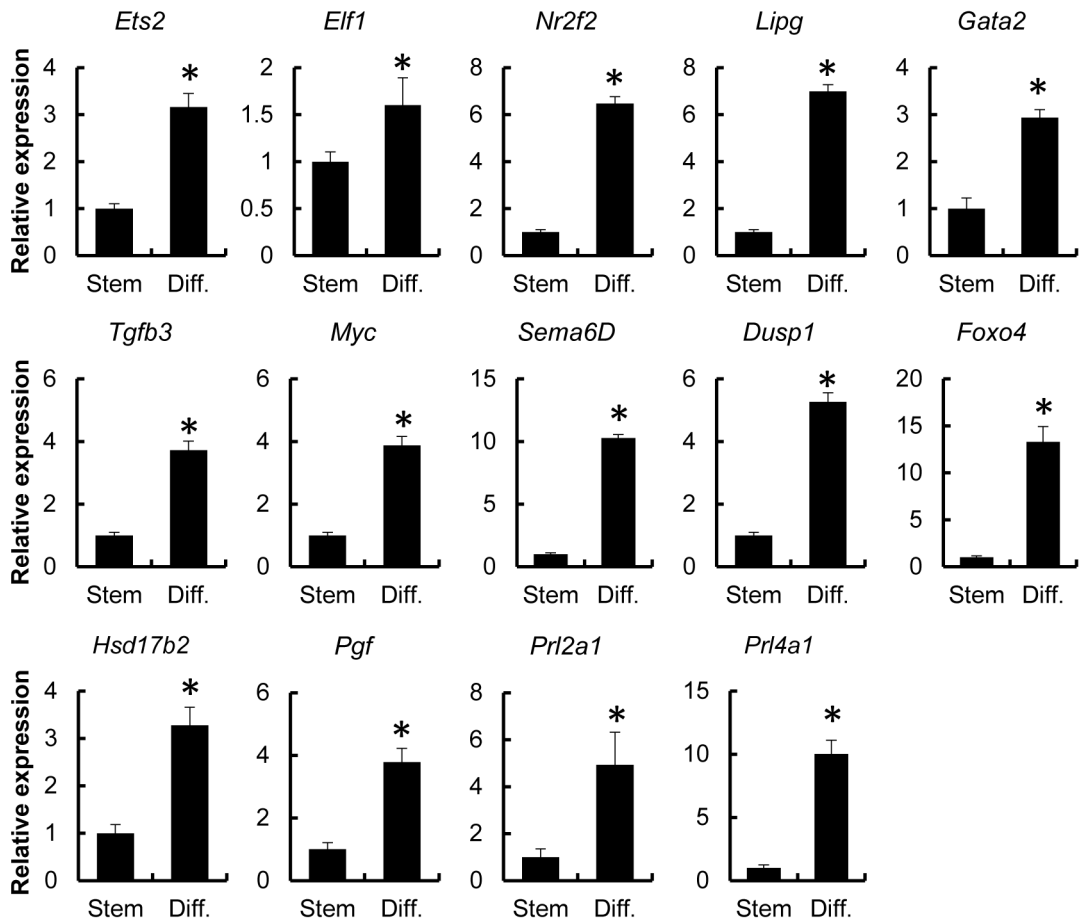
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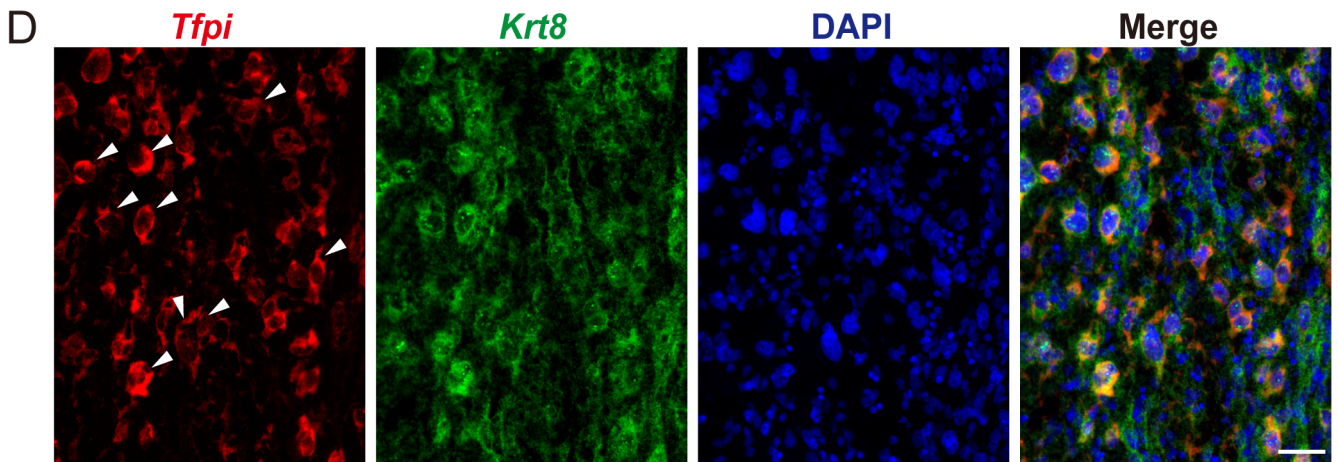
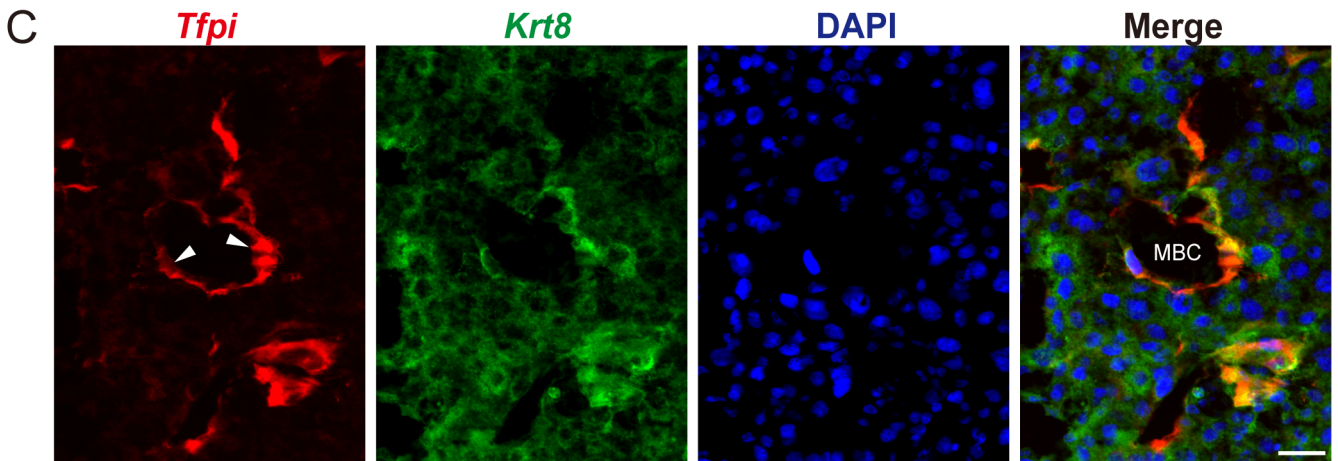
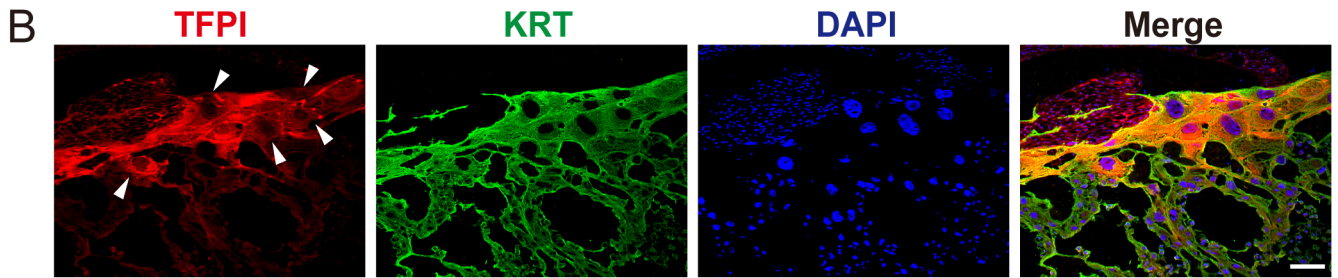
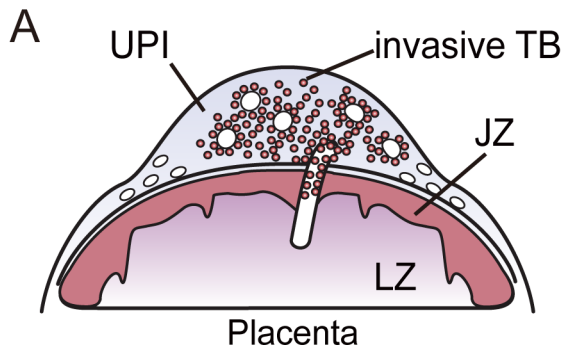
Figures S1 to S5  
Tables S1 to S2  
Legends for Datasets S1 to S3

### Other supplementary materials for this manuscript include the following:

Datasets S1 to S3 (separate files)

**A****B**

**Fig. S1 RT-qPCR validation of transcripts expressed in rat TS cells in stem and differentiated states. A,** Stem state was characterized by expression of transcripts contributing TS cell self-renewal. **B,** Differentiation was characterized by transcript signatures consistent with up-regulation of known differentiation-associated trophoblast cell transcripts. Asterisks denote  $P < 0.05$ .



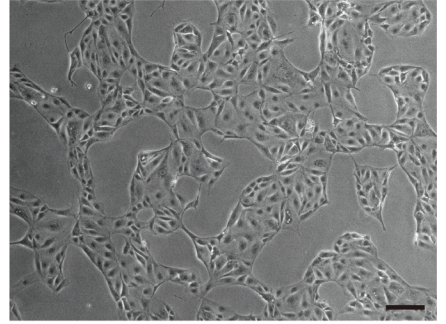
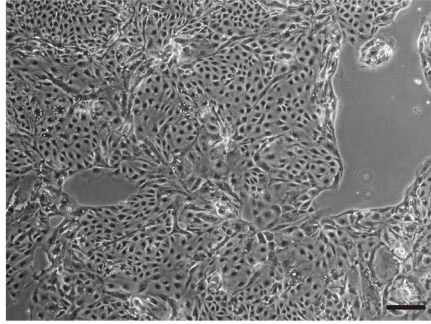
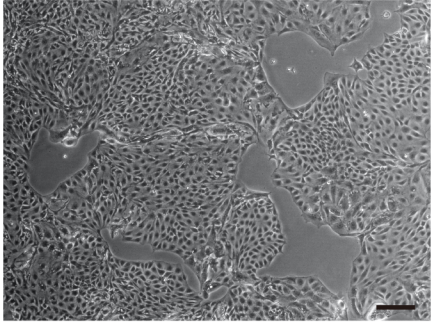
**Fig. S2 Distribution of TFPI within the rat placentation site.** **A**, Schematic representation of a late gestation placentation site, consisting of the uterine-placental interface (**UPI**), the junctional zone (**JZ**), the labyrinth zone (**LZ**), and invasive trophoblast cells (**TB**). Trophoblast giant cells are located at the boundary between UPI and the JZ. **B**, Immunohistochemical localization of TFPI (red) and KRT (green) within trophoblast giant cells of gd 15.5 placentation site (scale bar: 100  $\mu\text{m}$ ). **C**, *Tfpi* (red) and *Krt8* (green) transcripts detected using in situ hybridization of the gd 18.5 JZ (scale bar: 50  $\mu\text{m}$ ). *Tfpi* transcripts were restricted to trophoblast cells lining maternal blood channels (**MBC**) within the JZ. **D**, *Tfpi* (red) and *Krt8* (green) transcripts detected using in situ hybridization of the gd 18.5 LZ (scale bar: 50  $\mu\text{m}$ ). *Tfpi* transcripts were restricted to trophoblast cells lining maternal blood spaces within the LZ. DAPI marks cell nuclei (blue). Arrowheads in the TFPI panels show localization to trophoblast giant cells (**B**), trophoblast cells lining the MBCs (**C**) and trophoblast cells lining the maternal blood spaces in the labyrinth zone (**D**).

control

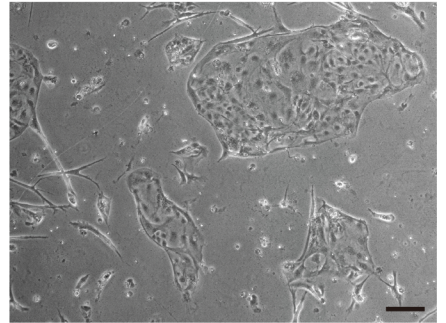
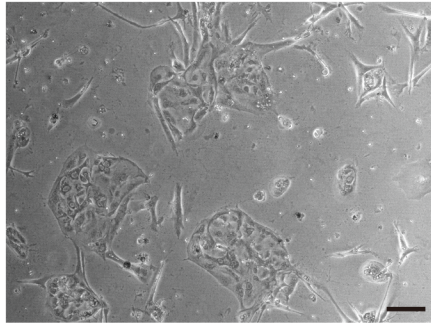
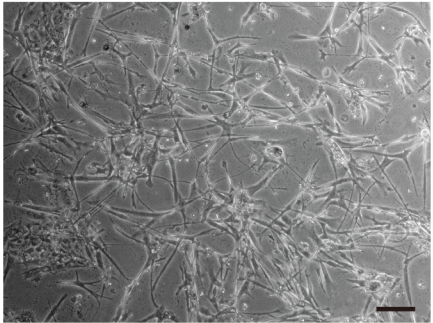
*TFPI* shRNA #2

*TFPI* shRNA #3

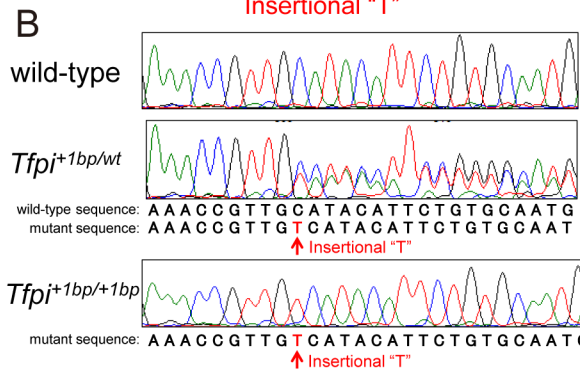
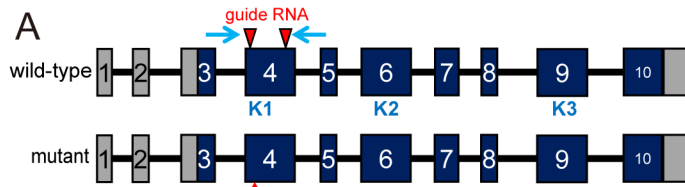
Stem cell condition



EVT differentiation



**Fig. S3 Examination of the effects of control and *TFPI* shRNAs (#2 and #3) on human TS cells.** Representative phase contrast images of transduced human TS cells in the stem state and following EVT cell differentiation (scale bar: 200  $\mu$ m).

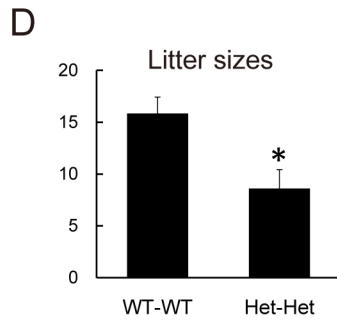


**C**

**TFPI (WT)**  
MTNKLKDHAFWAAVCLLSIVPELLNALPEEDDDTINTDSELRPMKPLHTFCAMKA  
EDGPCKAMIRSYFNMNSHQCEEFYGGCRGNKNRFDLTLEECRKTICIPGYKTTIKTT  
SGAEKPDFCFLEEDPGICRGMTRYFYNNQSKQCEQFKYGGCLGNSNNFETLEECRN  
TCEDPVNEVQKGDYVTNQITVDRRTVNNVVIPQATKAPSWDYDGPWCLEPADS  
GLCKASEKRFYYPNPAIGKCRQFNVTGCGGNNNNFTTKQDCNRACKKDSKSSKRAK  
TQRRRSFVKVMYENIH

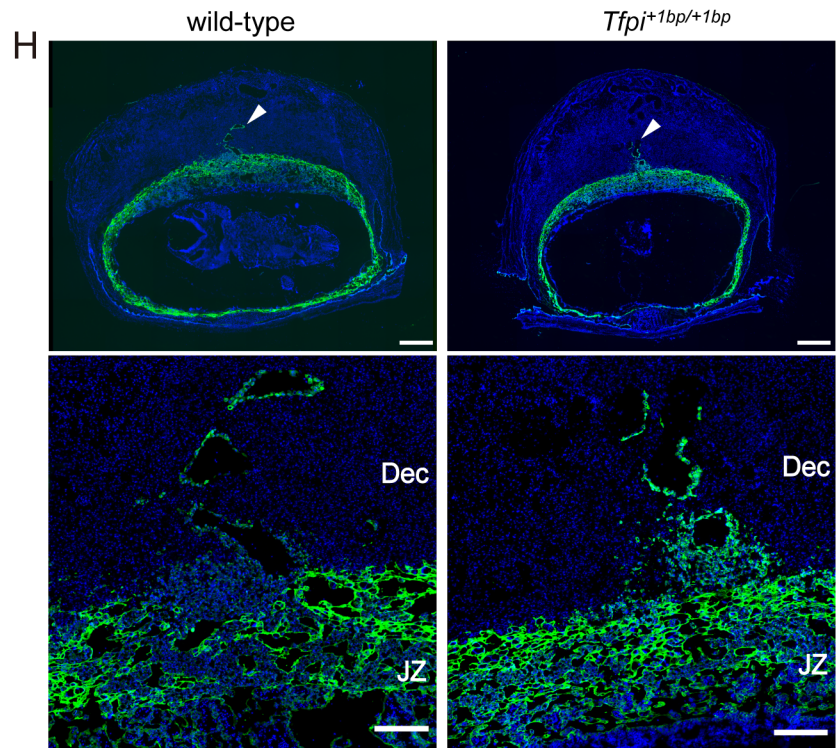
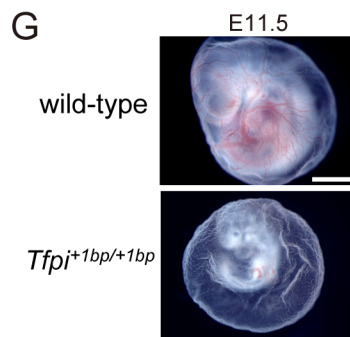
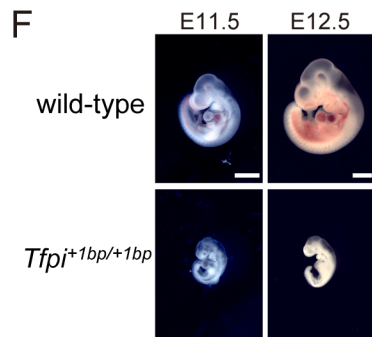
**TFPI (+1bp)**  
MTNKLKDHAFWAAVCLLSIVPELLNALPEEDDDTINTDSELRPMKPLSYILCNEGG  
RRALQSNDETELLFQYEQSPVstop

Under line : Kunitz domain  
Red : mutated region



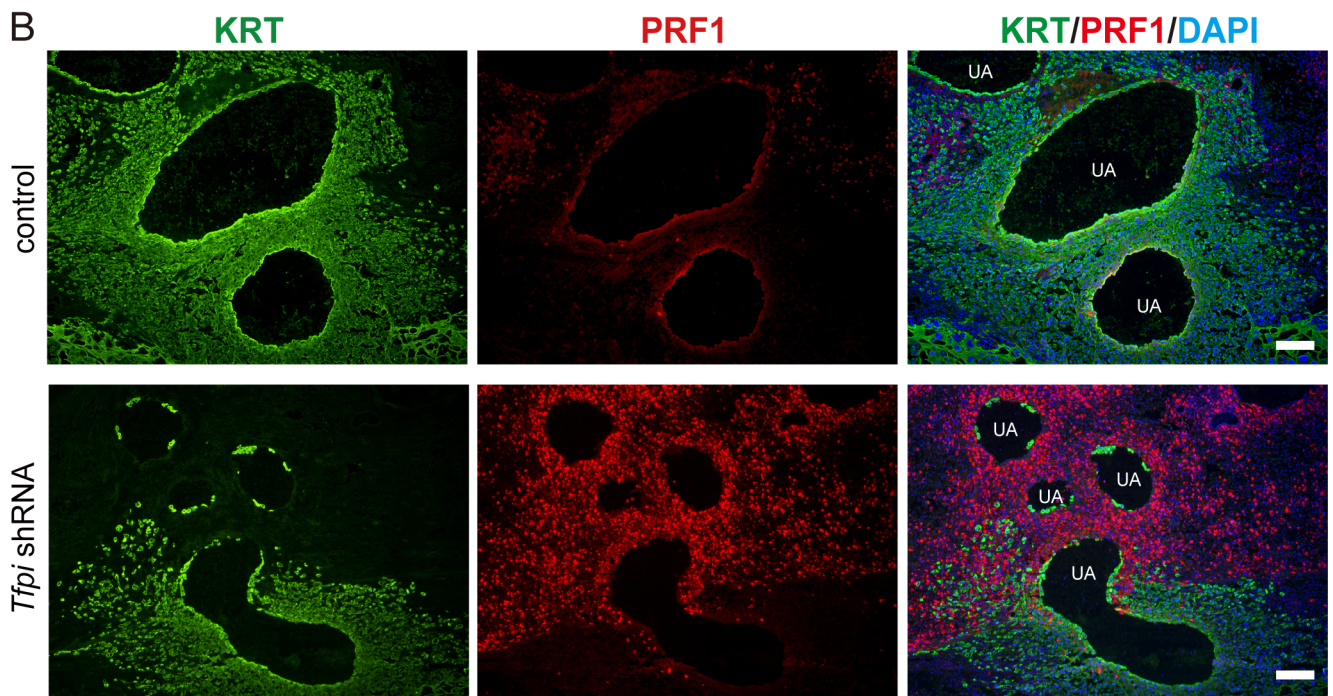
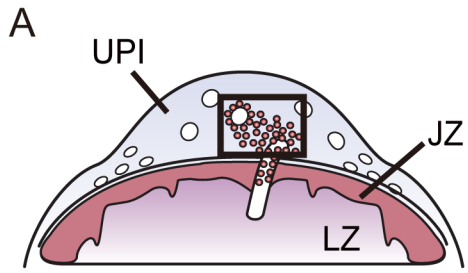
**E**

Day of gestation	No. of animal	Genotype			No. of Resorption	p-value
		wt/wt	+1bp/wt	+1bp/+1bp		
gd11.5	12	2	7	3	0	0.779
gd12.5	35	6	21	7	1	0.384
gd13.5	43	9	22	0	12	0.004
gd14.5	29	2	12	0	15	0.003
Born	142	53	89	0		2.7E-11





**Fig. S4 Phenotypic analysis of a rat model possessing a 1-base pair insertion within Exon 4 of the *Tfpi* gene.** **A**, Schematic representation of the rat *Tfpi* gene and the disruption of all Kunitz domains using CRISPR/Cas9 system. The red arrowheads indicate the target site of guide RNAs used in genome editing. The blue arrows indicate the location of the primer set used to amplify the *Tfpi* K1 domain. The insertion of a single thymidine nucleotide was found within Exon 4 (*Tfpi* K1 domain). K1, Kunitz domain 1; K2, Kunitz domain 2; K3; Kunitz domain 3. **B**, DNA sequence analysis showing a 1-bp insertion within Exon 4 of the *Tfpi* gene. **C**, The predicted amino acid sequences encoded by wild type (WT) and *Tfpi*<sup>+1bp</sup> alleles. Underlined sequences indicate the locations of the Kunitz domains within wild type TFPI alpha form. The 1bp insertion within Exon 4 altered the amino acid sequence prior to first Kunitz domain, resulting in out-of-frame sequence (red) and a premature stop codon and a null mutation. **D**, Litter sizes from *Tfpi*<sup>+1bp</sup> heterozygous intercrosses, P<0.05. **E**, Table showing the Mendelian ratios for gd 11.5 through gd 14.5 and post-delivery. **F,G**, Macroscopic analysis of wild type and *Tfpi*<sup>+1bp</sup> homozygous mutant embryos (**F**) and yolk sacs (**G**) at E11.5 (scale bar: 1 mm). **H**, Cytokeratin (**KRT**, green) immunohistochemical analysis for wild type and *Tfpi*<sup>+1bp</sup> mutant placentas (gd 12.5). Arrowheads demarcate the depth of endovascular trophoblast cell invasion. DAPI marks cell nuclei (blue). The lower panels are high magnification images of the upper panels (scale bar of upper panels: 500 μm, lower panels: 200 μm).



**Fig. S5 Distribution of uterine natural killer cells from control and trophoblast-specific *Tfpi* knockdown placentation sites.** **A**, Schematic representation of a gestation day (**gd**) 15.5 placentation site, consisting of the uterine-placental interface (**UPI**), the junctional zone (**JZ**), and the labyrinth zone (**LZ**). **B**, Immunohistochemical analysis for cytokeratin (**KRT**, green) and perforin 1 (**PRF1**, red) in the UPI of control and *Tfpi* knockdown gd 15.5 placentation sites (scale bar: 200  $\mu$ m). DAPI marks cell nuclei (blue). **UA**, uterine arterioles.

**Table S1. Primer sequences used for RT-qPCR**

Symbol	Accession Number	Forward primer	Reverse primer
<b>Rat</b>			
<i>Tfpi a</i>	NM_017200.1	AACACCTGTGAGGATCCAGTG	TCCAATGGCTGGGTTGTAG
<i>Tfpi b</i>	NM_001177321.1	CCTTGGAGGAGTGCAGGAAC	GGAAGTCAGAGAGCCTTGGT
<i>Cdh5</i>	NM_001107407.1	GGCGAGTTCACCTTCTGTGA	GTCGTAGCTTGTGGTGTCCA
<i>Esam</i>	NM_001004245.1	TGTGGGGACCAATGTGACTC	CTGTTTTGGGCCCTTGCAGAC
<i>Cd47</i>	NM_019195.2	CGCCCAGTTTTCGTGGTTTTTC	AAAAGGATGGCGCCAACAAC
<i>Faslg</i>	NM_012908.1	TCTGGTTGGAATGGGGTTAG	CTTGGCTTTTTGTTTTAGAG
<i>Adm</i>	NM_012715.1	ACGTCTCGGACTTTCTGCTT	GCTGCTGGACGCTTGTAGTT
<i>Vegfa</i>	NM_031836	GGTCTGGCAAAGAGAAG	TTAGTGGTTTTCAATGGTCTG
<i>Procr</i>	NM_001025733.2	TCAGCTCCAGGATGTTGACG	CTGATGCCTCCCCTGATAGG
<i>Plau</i>	NM_013085.3	TTGGTTCAGACTGTGAGAT	GCACTGTTCTGTGAGAAAT
<i>Fn1</i>	NM_019143.2	GGATCCCCTCCCAGAGAAGT	GGGTGTGGAAGGGTAACCAG
<i>Thbd</i>	NM_031771.2	TGAGTGCAGTCAAGGCGAAT	GGATGGGGTCACAGTCTTG
<i>Cdh1</i>	NM_031334.1	CGTCATCACAGTCAAACGGC	GAGCAGCTCTGGGTTGGATT
<i>Cldn4</i>	NM_001012022.1	CAAGGCTCCAGCGAGGATAG	GTAACGTGGAGGCGAGAGAG
<i>Phlda2</i>	NM_001100521.1	AGAGACCCACGGGAGTAG	AGCAGGTAACCAATATAAATACG
<i>Id2</i>	NM_013060.3	GGACATCAGCATCCTGTCTT	AAAAAGGAAAAAGTCCCCAAA
<i>Bmp4</i>	NM_012827.2	CGCAGCTTCTCTGAGCCTTT	ACGACCATCAGCATTTCGGTT
<i>Esrrb</i>	NM_001008516.2	TGCCTGAAGGGGATATCAAG	ACTCTGCAGCAGGCTCATCT
<i>Bambi</i>	NM_139082.3	ATGGATCGCCACTCCAGCTA	CCAGAGTGGTTTTGGGCCTG
<i>Pcsk6</i>	NM_012999.1	GCTCACGGCTACCTCAACTT	CTGTCTCTTGACCCTGCGTT
<i>Esrp1</i>	NM_001127564.2	GGGCACTTTAAATCGAAATGGCT	CTATTAGGCGAACCTGGGGG
<i>Glut3</i>	NM_017102.2	TGGCTACAACACCGGAGTCATCAA	CTGCAAAAGCGGTTGACAAAGAGT
<i>Ets2</i>	NM_001107107.1	GCCCAGAGAGAAATGCTTTG	AGCTGACAAAACCTCGGGCTA
<i>Elf1</i>	NM_053520.2	AGACAGTGCCACTCACAACG	GGACCTGCTGTACTGGGGTA
<i>Nr2f2</i>	NM_080778.2	TCAAAGTGGGCATGAGACGG	GACAGGTACGAGTGGCAGTT
<i>Lipg</i>	NM_001012741.1	TCCGCACTTCTAAGGACCCA	GCAGGGCTGACACGAGTTTA
<i>Gata2</i>	NM_033442.1	TAAGCAGCGAAGCAAGGCTC	AGCTTCAGATTCAATTTTCAGAGCA
<i>Tgfb3</i>	NM_013174.2	CCACGTCCCCTATCAGGTCC	AGAGGTAATTCCTTGGGGC
<i>Myc</i>	NM_012603.2	CCCCTGAAAAGAGCTCCTCG	CGCAGATGAAATAGGGCTGC
<i>Sema6D</i>	NM_001107768.1	GTCTTCTCCAGGCGGCAAG	TCCCTCCTCAGAAGTCCAC
<i>Dusp1</i>	NM_053769.3	CGCGCTCCACTCAAGTCTTC	CCCAGGTACAGGAAGGACAG
<i>Foxo4</i>	NM_001106943.1	TGCCTAGAAGAGGGTGTCTGT	TTTCAGGCTTTGGATGCTCT
<i>Cd93</i>	NM_053383.2	GCTCCCAATGGGGTCTCTTG	AGCCATTGAGAGAACCAGGC
<i>Mdk</i>	NM_030859.2	GCAGCACCGAAGTTTCTTCC	CAGGTCCACTCCGAACACTC
<i>Lgals9</i>	NM_012977.1	GAGCGTTGGTCTCCCGAAA	CTGAAGTCCGTTCTGCAACC
<i>Prl5a1</i>	NM_138527	TCCACACCAGACATTCCAGA	TTTCCAGGAAGCCAACATTC
<i>Prl7b1</i>	NM_153738	AACAATGCCTCTGGCCACTGC	AGGCCATTGATGTGCTGAGACAGT
<b>Human</b>			
<i>TFPI a</i>	NM_006287.6	ATGGAACCCAGCTCAATGCT	GGCAGACACAATCCTCTGT
<i>TFPI b</i>	NM_001032281.3	ATGGAACCCAGCTCAATGCT	ATGCATGAATGCAGAAGGCG
<i>THBD</i>	NM_000361.2	ACATCCTGGACGACGGTTTT	CGCAGATGCACTCGAAGGTA
<i>ESAM</i>	NM_138961.3	GACCAACTTGCTGCGGTTTT	GATGAAGACACCTCCCCTG
<i>FLT4</i>	NM_182925.5	GACTGTGGCTCTGCCTGG	GGTGTGATGACGTGTGACT
<i>FN1</i>	NM_001306131.1	CCGCCGAATGTAGGACAAGA	GGCACTGGTAGAAGTTCCAGG
<i>HLA-G</i>	NM_002127.5	CCACCACCCTGTCTTTGACTAT	ACGTCCTGGGTCTGGTCCT
<i>MMP2</i>	NM_001127891.2	TGGCACCCATTTACACCTACAC	ATGTCAGGAGAGGCCCATAGA
<i>LRP2</i>	NM_004525.3	CTGCTCCTGGCTCTCGTC	TCCCATCACACCTCCAGTCT
<i>EPCAM</i>	NM_002354.2	CTCTGAGCGAGTGAGAACCT	AACGCGTTGTGATCTCCTTCT
<i>F3</i>	NM_001993.5	AGACAGCCCGGTAGAGTGTA	AGCCAGGATGATGACAAGGAT
<i>F2RL1</i>	NM_005242.6	GCTAGCAGCCTCTCTCTCT	TTCCAGTGACGTGGGATGTG
<i>ASCL2</i>	NM_005170.2	GCACCAACACTTGGAGATTTT	AATGGATTCTGTGCCCTTAG
<i>CCR1</i>	NM_001295.3	AGTACCTGCGGCAGTTGTT	AAGGGGAGCCATTTAACCAG

<b>Table S2. shRNA sequences used for knockdown experiments</b>	
<b>TFPI shRNA</b>	<b>Sequence</b>
<b>Rat</b>	
shRNA No. 1	GCAGTGTGAACAATTCAAGTA
shRNA No. 2	GCCAATGAAACCGTTGCATAC
<b>Human</b>	
shRNA No. 1	ACAGTGTGAACGTTTCAAGTA
shRNA No. 2	GGAGTTGCCACCACTGAAACT
shRNA No. 3	GCGGATGATGGCCCATGTAAA

**Dataset S1. Transcript profiles in rat trophoblast stem and differentiated states.**

**Dataset S2. Effects of TFPI on the rat differentiated trophoblast cell transcriptome.**

**Dataset S3. Effects of TFPI on the development of the human EVT cell transcriptome.**