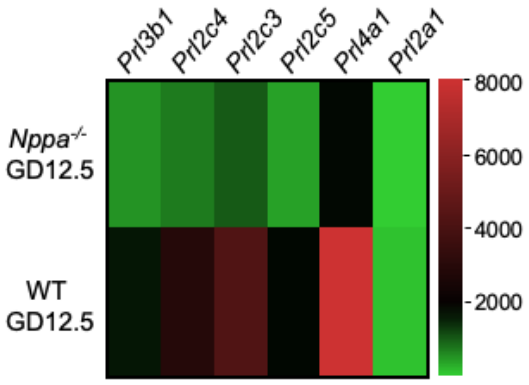
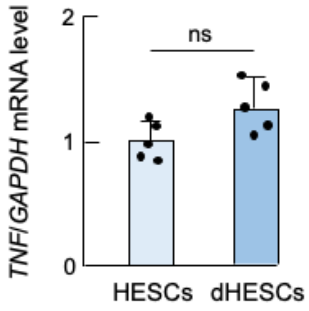
Atrial natriuretic peptide promotes uterine decidualization and a TRAIL-dependent mechanism in spiral artery remodeling

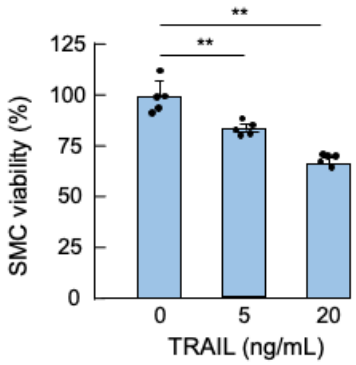
Supplemental Figures and Tables



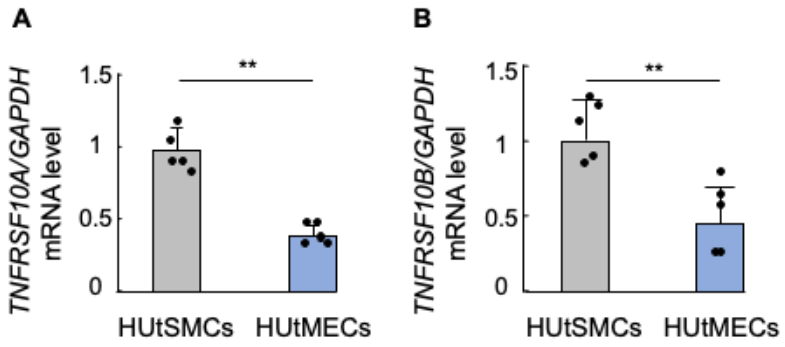
Supplemental Figure 1. Reduced expression of prolactin gene family members in uterine tissues from pregnant *Nppa*$^{-/-}$ mice. Total RNAs isolated from pregnant WT and *Nppa*$^{-/-}$ mouse uteruses were used for gene expression analysis using MouseRef-8 v2.0 BeadChip (Illumina, BD-202-0202). Expression levels of prolactin family genes were analyzed with the Illumina GenomeStudio software. A heat map is shown (right). Data are representative of two replicates.



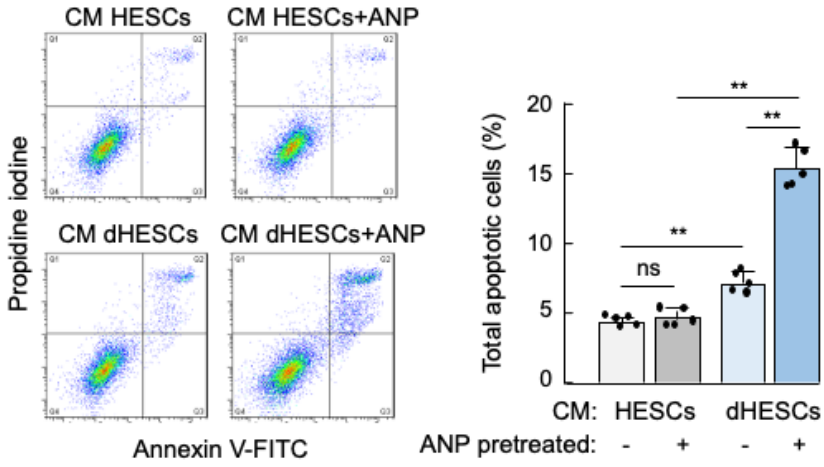
Supplemental Figure 2. *TNF* mRNA levels in HESCs and dHESCs. HESCs were cultured in non-differentiation or differentiation medium. mRNAs from HESCs and decidualized HESCs (dHESCs) were used for quantitative RT-PCR to examine *TNF* mRNA levels with *GAPDH* mRNA as a control. Data (mean ± S.D.) from five experiments were analyzed by Student's *t* test. ns: not significant.



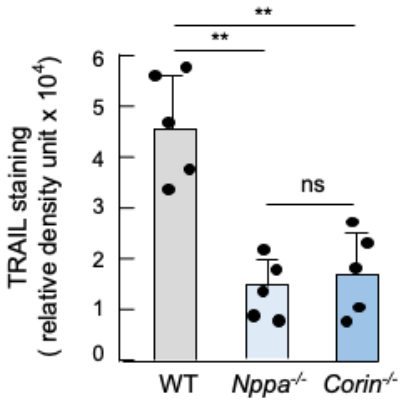
Supplemental Figure 3. TRAIL induces uterine SMC death. Human uterine SMCs were cultured with increasing concentrations of recombinant TRAIL. SMC viability was measured. Data (mean ± S.D.) from five experiments were analyzed by one-way ANOVA. ***P* < 0.01.



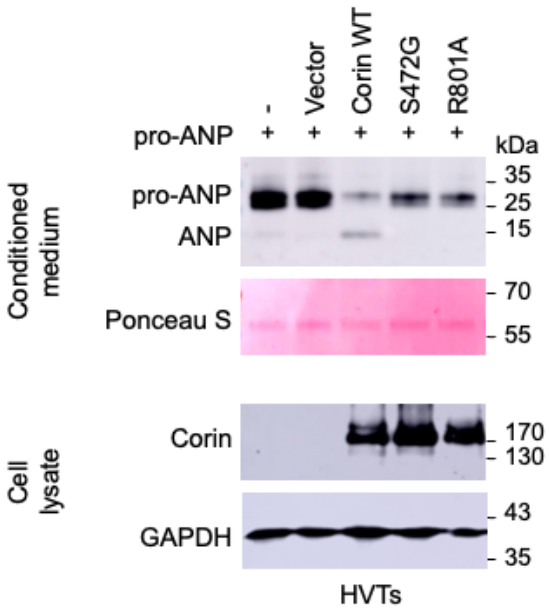
Supplemental Figure 4. *TNPRSF10A* and *TNFRSF10B* mRNA levels in human uterine SMCs and ECs. (**A** and **B**) mRNAs were isolated from human uterine SMCs (HUtSMCs) and microvascular ECs (HUtMECs) and used for quantitative RT-PCR to examine *TNPRSF10A* (**A**) and *TNFRSF10B* (**B**) mRNA levels with *GAPDH* mRNA as a control. Data (mean ± S.D.) from five experiments were analyzed by Student's *t* test. ***P* < 0.01.



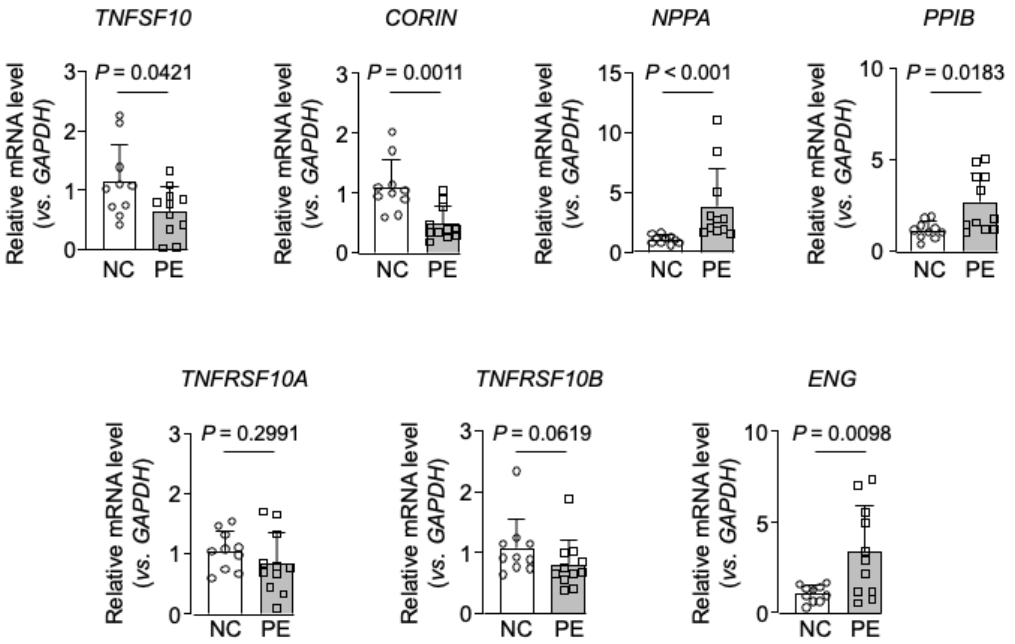
Supplemental Figure 5. Apoptosis in human uterine SMCs. Human uterine SMCs were incubated with CM from HESCs or dHESCs without (-) or with (+) recombinant ANP pretreatment. Apoptosis was analyzed by flow cytometry with an FITC-conjugated anti-annexin V antibody. Data (mean ± S.D.) from five experiments were analyzed by one-way ANOVA. ***P* <0.01. ns: not significant.



Supplemental Figure 6. Immunostaining of TRAIL in uteruses from pregnant mice. Uterine sections from pregnant (GD12.5) WT, *Nppa*$^{-/-}$ and *Corin*$^{-/-}$ mice were stained using an anti-TRAIL antibody. Stained sections were examined under a light microscope. Representative images are presented in Figure 6F. Relative TRAIL staining signals were quantified using ImageJ software. Data (mean ± S.D.) from five experiments were analyzed by one-way ANOVA. ***P* <0.01. ns: not significant.



Supplemental Figure 7. Corin-mediated pro-ANP processing in human villus trophoblasts (HVTs). Plasmids expressing human pro-ANP and corin WT and mutants S472G (identified in preeclamptic women) and R801A (an inactive control) or a control vector were transfected in cultured HVTs. After 24 h at 37°C, the conditioned medium was collected. Pro-ANP and ANP fragments were analyzed by western blotting (top panel). A Ponceau S-stained non-specific band in the conditioned medium was used as a protein loading control (second panel). Corin proteins in the transfected HVTs were verified by western blotting with GAPDH as a control (bottom two panels). Data are representative of three experiments.



Supplemental Figure 8. Gene expression analysis in normal and preeclamptic placentas. Placental tissues from normal pregnant controls (NC) (n=10) and preeclamptic (PE) women (n=11) were used to isolated mRNAs. *TNFSF10* (encoding TRAIL), *CORIN*, *NPPA* (encoding pro-ANP), *PPIB* (encoding cyclophilin B), *TNFRSF10A* (encoding TRAIL receptor 1), *TNFRSF10B* (encoding TRAIL receptor 2), and *ENG* (encoding endoglin) mRNA levels were analyzed by quantitative RT-PCR. *GAPDH* mRNA levels were used to normalize the data. Statistical analysis was done with Student's *t* and Mann-Whitney tests.

Supplemental Table 1. Antibodies used in this study

Antibody	Source	Identifier	Dilution or concentration
Caspase-3 (rabbit monoclonal)	Cell Signaling	14220	1:1000
Human FAS ligand (goat polyclonal)	R&D Systems	AF126-SP	1:1000
Mouse Fas ligand (rat monoclonal)	R&D Systems	MAB526-SP	1:1000
Human TRAIL (rabbit polyclonal)	R&D Systems	AF375-SP	1:800
Mouse TRAIL (rabbit polyclonal)	LSBio	LS-C331936-50	1:1000
Mouse TRAIL (goat polyclonal)	R&D Systems	AF1121	1:150
Human TRAIL R1 (goat polyclonal)	R&D Systems	AF347-SP	1:800
Human TRAIL R2 (goat polyclonal)	R&D Systems	AF631-SP	1:800
Mouse TRAIL R (rat monoclonal)	R&D Systems	MAB1540-SP	1:800
Cyclophilin B (mouse monoclonal)	R&D Systems	MAB5410-SP	1:1000
Mouse Prolactin (goat polyclonal)	Thermo Fisher	PA547140	1:500
Mouse IGFBP1 (rabbit polyclonal)	Thermo Fisher	PA579447	1:1000
SMA (rabbit monoclonal)	Cell Signaling	19245T	1:200
vWF (rabbit polyclonal)	Thermo Fisher	PA5-16634	1:100
Cytokeratin (mouse monoclonal)	Cell Signaling	4545S	1:100
Cleaved caspase-3 (rabbit polyclonal)	Cell Signaling	9661T	1:200
Cyclophilin B (mouse monoclonal)	Thermo Fisher	MAB5410-SP	1:200
AP-anti-mouse secondary antibody (goat polyclonal)	Thermo Fisher	31320	1:200
HRP-anti-rat secondary antibody (goat polyclonal)	GenScript	A00167	1:8000
HRP-anti-mouse secondary antibody (donkey polyclonal)	Fisher Scientific	OB641005	1:8000
HRP-anti-rabbit secondary antibody (goat polyclonal)	Cell Signaling	7074S	1:1000
V5 (mouse monoclonal)	Thermo Fisher	R96025	1:1000

HRP-V5 (mouse monoclonal)	Thermo Fisher	R96125	1:5000
HRP-anti-goat secondary antibody (donkey polyclonal)	Abcam	Ab97110	1:5000
Alexa Fluor 488-anti-rabbit secondary antibody (goat polyclonal)	Thermo Fisher	A-11008	1:1000
Alexa Fluor 594-anti-mouse secondary antibody (donkey polyclonal)	Thermo Fisher	A-21203	1:1000
Normal goat isotype control immunoglobulin Biotin (goat polyclonal)	Fisher Scientific	BAF108	5 µg/mL
Normal rabbit isotype control immunoglobulin Biotin (rabbit polyclonal)	Fisher Scientific	AB105C	5 µg/mL
Normal mouse isotype control immunoglobulin Biotin (mouse polyclonal)	Santa Cruz	Sc-2025	5 µg/mL
p44/42 MAPK (ERK1/2) (rabbit monoclonal)	Cell Signaling	4695S	1:1000
Phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204) (rabbit polyclonal)	Cell Signaling	9101S	1:1000

Supplemental Table 2. Primers used in qRT-PCR

Gene	Locus	Primer	Sequence
CORIN	NM_016869.3	forward reverse	CAAGTCTGAGGTCAACTGC TGTCCACTTCTGCATTCCAC
ENG (endoglin)	NM_000118	forward reverse	CGGTGGTCAATATCCTGTCGAG AGGAAGTGTGGGCTGAGGTAGA
FASLG (FAS ligand)	NM_000639.3	forward reverse	GCAGCCCTTCAATTACCCAT CAGAGGTTGGACAGGGAAGAA
GAPDH	NM_003842.5	forward reverse	GTCTCCTCTGACTTCAACAGCG ACCACCCTGTTGCTGTAGCCAA
PPIB (cyclophilin B)	NM_000942	forward reverse	AACGCAGGCAAAGACACCAACG TCTGTCTTGGTGCTCTCCACCT
TNF	NM_000594.4	forward reverse	CCGAGGCAGTCAGATCATCTT AGCTGCCCCTCAGCTTGA
TNFRSF10A (TRAIL receptor 1)	NM_003844.4	forward reverse	ACCTTCAAGTTTGTCGTCGTC AACTCTCCCAAAGGGCTATGT
TNFRSF10B (TRAIL receptor 2)	NM_003842.5	forward reverse	AAGACCCTTGTGCTCGTTGT AGGTGGACACAATCCCTCTG
TNFSF10 (TRAIL)	NM_003810.4	forward reverse	AGTCAAGTGGCAACTCCGTC GAGCTGCTACTCTCTGAGGAC

Supplemental Table 3. Characteristics of pregnant women

Characteristics	NC (n=10)	PE (n=11)	*P* value
Age (y)	29.1 ± 3.3	29.3 ± 6.3	0.847
BMI (kg/m^2)	26.6 ± 3.6	31.1 ± 6.5	0.046
Smoking	0	0	1
Pre-existing diabetes	2	4	0.6351
Parity	1.1 ± 0.1	1.5 ± 0.2	0.0789
Blood pressure			
Systolic (mmHg)	124.0 ± 8.5	149.0 ± 10.1	<0.0001
Diastolic (mmHg)	72.0 ± 10.4	99.0 ± 9.5	<0.0001
Proteinuria	0	6	0.0124
Gestational age (w)	39.3 ± 0.9	36.5 ± 2.1	0.0018
Abnormal Doppler	0	0	1
Mode of delivery			
Vaginal	10	1	<0.0001
Cesarean	0	10	<0.0001
Fetal birth weight (g)	3433.3 ± 446.5	2860.0 ± 671.2	0.0256

BMI: body mass index; NC: normal pregnant control; PE: preeclampsia; y: year; w: week; g: gram. Mean ± SD for continuous variables and numbers for categorical variables are presented. *P* values between NC and PE groups were analyzed by Student's *t* test for continuous data or Fisher's exact test for categorical data.