Impaired sphingosine-1-phosphate synthesis induces preeclampsia by deactivating trophoblastic YAP (yes-associated protein) through S1PR2 (sphingosine-1-phosphate receptor-2)-induced actin polymerizations

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Supplemental Material

S1P regulates trophoblast mobility via S1PR2 mediated YAP activation: Implications for Preeclampsia

Running title: Impaired S1P synthesis causes preeclampsia

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Supplementary methods

Subject recruitment and sampling

25 PE pregnant women and 25 normotensive pregnant women admitted to the Department of Obstetrics at The First Affiliated Hospital of Chongqing Medical University for elective caesarian delivery were randomly selected and enrolled in the study. Clinical characteristics of those recruited are listed in table S1. The American College of Obstetrics and Gynecology (ACOG) guideline for PE was used for the diagnosis of PE: in brief, newly onset hypertension (≥140/90 mmHg) after 20 weeks of gestation with proteinuria or other organ damage. Women with other systematic chronic disorders, including preexisting hypertension, cardiovascular diseases, renal disease, immune disease, or other gestational complications and metabolic disorders were excluded. Placentas and decidua were collected immediately after the elective caesarian, then rinsed in pre-chilled 0.9% saline twice to remove blood clots, then allotted and snap-frozen in liquid-nitrogen and stored at -80°C for further use or immersed in 4% formaldehyde. All villus tissues were obtained from legal, elective abortions at 5-10 weeks of gestation. All sample collection procedures described in this experiment were carried out with informed consent from the pregnant women and in accordance with the principles stated in the Declaration of Helsinki and were approved by the Institutional Ethics Committee of Chongqing Medical University.

RNA extraction and quantitative real-time PCR analysis

RNA extraction from tissues or cultured cell was achieved using Trizol reagent (Invitrogen). RNA samples were quantified by NanoDrop 2000 (Thermo Fisher) and reverse-transcribed to complementary DNA (cDNA) using EvoScript Universal cDNA Master (07912439001, Roche).

RT-qPCR was performed using SYBR Green Master Mix (Roche) with Bio-Rad CFX Connect™ Real-Time System (Bio-Rad). Ct values were used for quantification, and *ACTB* was used as an internal control. All primer sequences for RT-qPCR were listed in supplementary table 2.

Immunoblotting

Placenta and villous tissues were homogenized with RIPA lysis buffer (Beyotime) supplemented with both PMSF (Beyotime) and phosphatase inhibitor cocktails (Selleckchem). For cultured cells, cells were lysed and scraped with RIPA lysis buffer. The extracted protein concentrations were quantified using BCA assay kit (Beyotime). 20 μg of total protein from each sample were subjected to SDS-PAGE (Bio-Rad) and transferred onto Poly Vinylidene Fluoride (PVDF) membranes (Roche). After blocking with 5% non-fat milk for 1h at RT, membranes were incubated with a corresponding primary antibody overnight at 4℃, followed by incubation with anti-rabbit (1:5000, SA00001-2, Proteintech) or anti-mouse (1:5000, SA00001-1, Proteintech) IgG secondary antibody conjugated with horseradish peroxidase for 1h at RT. Membranes were washed three times between each step using Tris-buffered saline. 0.1% Tween 20 (TBST) was applied for 5 min. Immunoreactive bands were detected using WesternBright reagent (Advansta). Images were captured and quantified with a ChemiDoc XRS+ system (Bio-Rad). Primary antibodies against SPHK1 (1:500, sc-365401), S1PR1 (1:500, sc-48356), S1PR2 (1:500, sc-365589), RhoA (1:500 , sc-418), YAP (1:500, sc-376830), and CTGF (1:500, sc-365970) were purchased from Santa Cruz Biotechnology. anti-S1PR3 (1:500, DF4869), anti-S1PR4 (1:500, DF4872), anti-S1PR5 (1:500, DF2714) and anti-p-LATS1 Thr1079 (1:500, AF7169) were purchased from Affinity Biosciences. anti-MYPT1 (1:1000, #8574), anti-p-MYPT1 Thr696 (1:1000, #5163), anti-p-YAP Ser 127 (1:1000, #4911), anti-MST1 (1:1000, #3682), and anti-LATS1 (1:1000, #3477) were purchased

from Cell Signaling Technology. anti-F-actin (1:1000, bs-1571R) and anti-YAP (1:500, bs-3605R) were purchased from Bioss. anti-α-tubulin (1:1000, 11224-1-AP) and anti-β-actin (1:1000, 66009-1-Ig) were purchased from Proteintech.

Immunofluorescence

Tissues were fixed with 4% paraformaldehyde (Bioservice) for 2h, then transferred to 30% sucrose overnight at 4℃. Fixed tissues were then embedded with O.C.T. Compound (Sakura) and snap-frozen in liquid-nitrogen. A 10 μm-thick cryosection was acquired. For HTR8/SVneo cells, cells were seeded onto coverslips, cultured and treated. When they reached 60-70% confluency, cells were harvested and fixed with 4% paraformaldehyde (Bioservice) for 10 min at RT. Sections or coverslips were brought to RT and permeabilized with 0.5% Triton X-100 (Sigma-Aldrich). After blocking with 5% bovine serum albumin (Servicebio), slices were incubated with primary antibody overnight at 4℃, followed by incubation with desired fluorescence-labeled secondary antibodies (Bioservice) for 1h at 37℃. 488-labeled Phalloidin (1:500, ab176753, Abcam) was used together with secondary antibodies. Nuclei were then stained with DAPI (Vector Laboratories, USA). Slides were washed 3 times with PBS after each step. Images were acquired using an Evos Fl Color Imaging System (Thermo Fisher Scientific, USA) and/or confocal microscope (Zeiss, Germany). Immunofluorescence calculation of YAP subcellular distribution was achieved by quantifying the nuclear fluorescence intensity to total fluorescence intensity.

Cell culture

HTR8/SVneo cells were obtained from American Type Culture Collection (ATCC,); human choriocarcinoma cell lines JAR and JEG3 were obtained from the Cell Bank of Chinese Academy

of Sciences. HTR8/SVneo cells and JAR cells were cultured with RPMI 1640 medium (Gibco), supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Beyotime). JEG3 was cultured with DMEM/F12 medium (Gibco) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All cells were cultured at 37°C with 5% CO_2. PF543 (S7177, Selleckchem) and S1P (S9666, Sigma-Aldrich) were dissolved in methanol and CCG-1423 (S7719, Selleckchem) and Latrunculin B (10010631, Cayman) were dissolved in dimethyl sulfoxide (DMSO, Sigma). Before all treatments, cells were serum starved for two hours.

S1P measurement

Sphingosine 1-phosphate ELISA kit (K-1900, Echelon) was used to measure S1P in placentas, cells and cell culture media. In brief, tissues were homogenized and protein levels were quantified with BCA. Lysates were then subjected to the assay according to the manufacturer's instructions. S1P concentrations were presented as molar quantities.

Animals

CD1 mice (8-10 weeks old) were obtained from the Experimental Animal Center of Chongqing Medical University and bred in a pathogen-free facility. For nonpregnant female mice, PF543 (1mg/kg) or saline was injected daily via tail vein for six consecutive days. Female mice were paired with age-matched males. Gestational day (GD) 0.5 was determined upon appearance of the vaginal plug. From GD 7.5 to GD 12.5, PF543 (1mg/kg) or saline was administrated daily by intravenous injection. Mice were sacrificed at GD 13.5 and/or GD 18.5 to collect samples and examine placental development and fetal growth. All animal experiments referred to in this study were carried out in accord with the National Institutes of Health guidelines for the use and care of

animals and approved by the Institutional Animal Care and Use Committee of Chongqing Medical University.

Mice blood pressure monitoring

Blood pressure was monitored by tail-cuff plethysmography BP-2000 Series II (Visitech Systems, USA) as previously reported[34]. In brief, mice were adapted five days before the test began. Blood pressure was measured every two days from GD 1.5-7.5 and every day from GD 7.5-18.5. For each recorded measurement, at least five effective measurements were obtained.

High frequency ultrasound examination

To monitor blood flow in the placenta-utero unit, high frequency ultrasound examinations were performed at GD 18.5 using a Vevo 3100 System (FujiFilm Visualsonics Inc). In brief, mice were anesthetized using isoflurane in an airtight box and placed on a heating platform in dorsal position. Abdominal hair was removed, the placental area was measured in B-Mode, and the blood flow of uterine and umbilical arteries was assessed in Color Doppler Mode and the Pulse-wave (PW) Doppler Mode. Peak systolic velocity (PSV), end diastolic velocity (EDV), the resistance index (RI) and pulsatility index (PI) were calculated using Vevo lab software (FujiFilm Visualsonics Inc).

Hematoxylin and Eosin (H&E) staining

Mouse kidneys were harvested at GD 13.5 and/or GD 18.5. Kidneys were fixed with 4% paraformaldehyde and embedded in paraffin, then cut into 3-μm sections. Sections were deparaffinized and rehydrated, then stained with hematoxylin for 5 min, followed by staining with Eosin for 2 min. Between each step, sections were washed in tap water. 5 random images of each section were captured with an EVOS microscope (Life Technologies). Glomerular cavities were

measured as the percentage of Bowman's capsule to renal corpuscle using ImageJ 1.50i software (https:// imagej.en.softonic.com/).

Immunohistochemistry (IHC)

Mouse placenta-utero units were harvested at GD 13.5 and/or GD 18.5. After fixation and embedding, tissues were sliced into 3-μm sections. For immunohistochemistry (IHC), sections were deparaffinized and rehydrated, and a PV-9000 IHC kit (ZSGB) was used according to manufacturer's instructions. Images were captured with an EVOS microscope (Life Technologies).

Cell proliferation assay

The HTR8/SVneo cells were seeded onto a 96-well plate (5000 cells/well) and treated with PF543 or S1P. Cell proliferation was detected after 24h treatment using Cell Counting Kit-8 (CCK-8) (MedChemExpress). Absorbance was measured at 450 nm using a Multiskan Go microplate reader (Thermo Fisher Scientific).

Cell viability assay

The HTR8/SVneo cells were seeded onto a 96-well plate and cultured to 50-60% confluency, then treated with PF543 or S1P for 24h. After treatment, cell death dye Ethd-II (1:1000, E3599, Thermo Fisher Scientific) was added to the medium and incubated for 15 min. The nucleus was stained with Hoechst (Boster Bio). Five random images were captured by an EVOS fluorescence microscope (Life Technologies). Cell death rates were calculated by Ethd-II positive cells to total cell nucleus counts using ImageJ 1.50i software.

Matrigel invasion assay

Invasion chamber (8 μm, Corning) and Matrigel (Corning) were used for the invasion assay following the previously report[35]. In brief, the upper invasion chamber was coated with previously diluted Matrigel and solidified overnight in the cell culture chamber. 6×10^4 HTR8/SVneo cells were resuspended in 200μl RPMI 1640 medium (Gibco) containing corresponding compounds and seeded into the pre-coated upper chamber, while 600μl RPMI 1640 medium supplement with 10% FBS containing corresponding compounds was added to the lower chamber. After 24h incubation, the upper invasion chambers were washed and fixed with 4% paraformaldehyde, then stained with crystal violet boric acid. The non-invaded cells were scrubbed off the chamber with a cotton swab, then photographed with an EVOS microscope (Life Technologies). Five 100x magnified images of each sample were randomly chosen, and stained cells were counted to quantify the number of invaded cells.

Cell migration assay

Migration of HTR8/SVneo cells were assessed by wound-healing test. Briefly, HTR8/SVneo cells were seeded onto a 6-well plate and cultured to 70% confluency, then treated with PF543 or S1P. After 24h, a cross-shaped scratch lesion was created with a 200μl pipette tip. Images were captured at 0h and 24h with an EVOS microscope (Life Technologies). Cell migration was assessed by the wound healing area with ImageJ 1.50i software.

GTP-RhoA assay

Active GTP-RhoA was assessed with a commercially available RhoA Pull-Down Activation Assay Biochem Kit (#BK036, Cytoskeleton) following manufacturer's instructions. In brief, tissue and

cell lysates were incubated with Rhotekin-RBD beads. Products were then subjected to western blotting. The amount of activated RhoA is determined using a RhoA specific antibody.

Coimmunoprecipitation (Co-IP)

anti-F-actin (Bioss), anti-G-actin (F49668, NSJ Bioreagents), anti-YAP (Santa Cruz Biotechnology), anti-LATS1 (Cell Signaling Technology) or anti-IgG (Santa Cruz Biotechnology) antibodies were incubated with Protein A/G Magnetic Beads (B23202, Bimake) for 4h at 4℃. After incubation, unbonded antibodies were separated and discarded with magnetic separation. Protein samples were prepared with Thermo Scientific™ Pierce™ IP Lysis Buffer (Thermo Fisher Scientific) supplemented with protease inhibitor (Selleckchem). Protein concentrations were determined by BCA assay. An equal concentration of total proteins was added to the antibody-beads complex and incubated overnight at 4℃. Immunoprecipitation products were magnetic-separated and washed, then analyzed by western blotting.

YAP-5SA plasmid

The construction of YAP-5SA plasmid was reported previously[36], and pQCXIH-Myc-YAP-5SA was a kind gift from Kunliang Guan (Addgene plasmid # 33093; http://n2t.net/addgene:33093 ; RRID:Addgene_33093). DH5alpha bacteria was added into LB medium supplemented with 1% Ampicillin and shake-cultured for 12h. Plasmids were extracted using Endo-Free Plasmid Mini Kit (D6950 , Omega) and concentrations quantified by NanoDrop 2000 (Thermo Fisher).

Transfection

HTR8-S/Vneo cells were seeded onto a 6-well plate and grown to 60-70% confluency. siRNAs (si-S1PR1, si-S1PR2, si-S1PR3, si-S1PR4, si-S1PR5, si-YAP, si-NC) or YAP1-5SA plasmid with

Lipofectamine 3000 (Thermo Fisher Scientific) were incubated for 20 min. 50 nM siRNA/plasmid-Lipo mix was then added to the serum free RPMI 1640 medium and incubated with cells. Media was replaced after 6h transfection. All cells were cultured for 48h after transfection before other treatments and detections. All siRNAs were synthesized by GenePharma (Shanghai). The sequence of siRNAs is listed in Supplementary Table 3.

RNA sequencing

HTR8-S/Vneo cells were cultured to 60-70% confluency and then treated with vehicle or S1P for 24h. Total RNA was extracted using TRIzol (Invitrogen). A total amount of 1 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB) following manufacturer's recommendations, and index codes were added to attribute sequences to each sample. FeatureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene. FPKM of each gene was calculated based on the length of the gene, and the reads count was mapped to this gene. Differential expression analysis of two conditions/groups (two biological replicates per condition) was performed using the DESeq2 R package (1.16.1). Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by clusterProfiler R package. GO terms with corrected P value less than 0.05 were considered significantly enriched by differential expressed genes.

Nuclear protein extraction

Cytosolic and nuclear fractions were separated with a commercial Minute™ Cytosolic and Nuclear Extraction Kit (NT-032, Invent Biotechnologies) according to the manufacturer's instructions. The

isolated nuclear proteins were denatured in detergent-containing buffer (Invent Biotechnologies) and analyzed by immunoblotting.

Transcription factor binding profiling

Transcription factor binding analysis was achieved using an online prediction tool - JASPAR (http://jaspar.genereg.net). The prediction procedure was reported previously[37]; in brief, TEAD4 binding motif was searched on the website, the upstream 2k bp sequence of desired genes was selected for scanning, and the scan was then run on JASPAR.

Supplementary figures

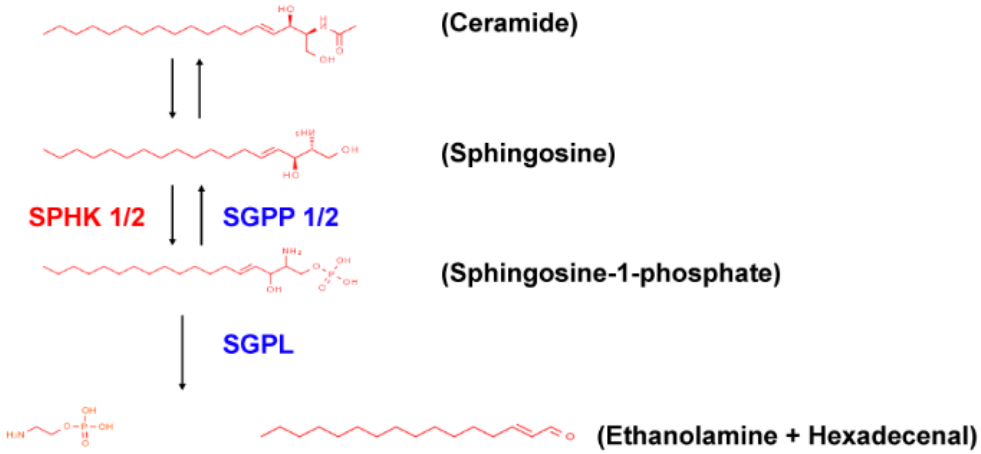


Figure S1. **Metabolic pathways of S1P.**

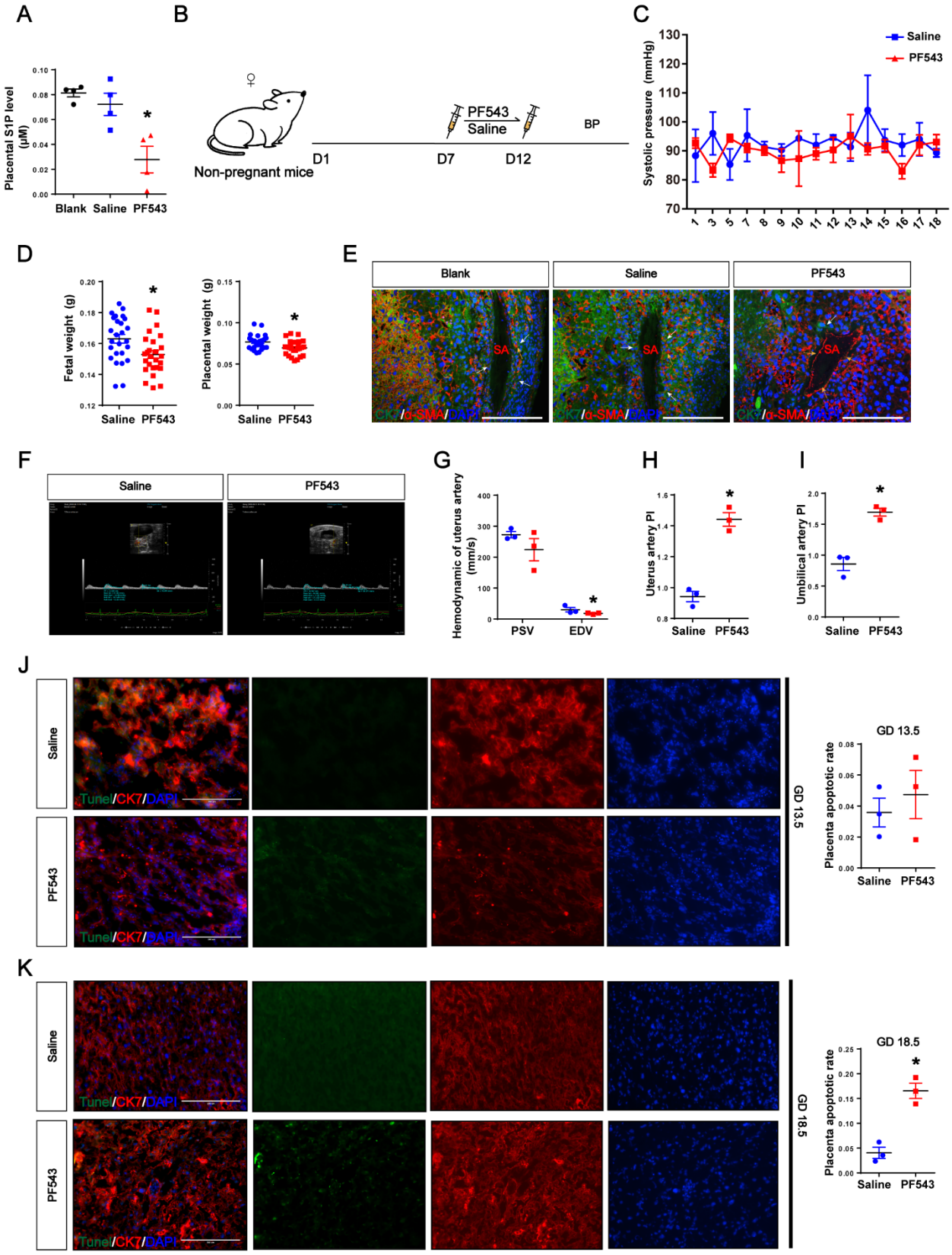


Figure S2. **SPHK1 inhibition during placentation exhibits PE phenotype in mice and impairs**

placentation.

(A) Placental S1P levels in mice treated with blank, saline or PF543 at GD 13.5, n=4, one-way ANOVA and Tukey's multiple comparison test,*$p<0.05$ vs. saline; (B) Schematic illustration of experimental design for non-pregnant mice; (C) Systolic blood pressure of non-pregnant mice treated with PF543 (1mg/kg) or saline from day 7 to day 12, n=3, two-tailed t-test; (D) Fetal and placental weight of mice treated with PF543 (1mg/kg) or saline (from GD7.5 – GD12.5) on GD 13.5, n=26, two-tailed t-test, *$p<0.05$; (E) IF staining of CK7 (green) and α-SMA (red) in mouse uteroplacental units collected on GD18.5, white arrows show trophoblast invasion , yellow arrows show spiral artery (SA) remodeling, scale bar 200 μm; (F - I) Hemodynamics of uterine and umbilical artery on GD18.5, representative figure (F) , PSV and EDV (G), and PI (H) of uterus artery, PI (I) of umbilical artery n=3, two-tailed t-test, *$p<0.05$; (J and K) Tunel staining of placenta on GD 13.5 (J) and GD 18.5 (K), n=3, two-tailed t-test, *$p<0.05$, scale bar 200 μm. Data presented as means ± SEM.

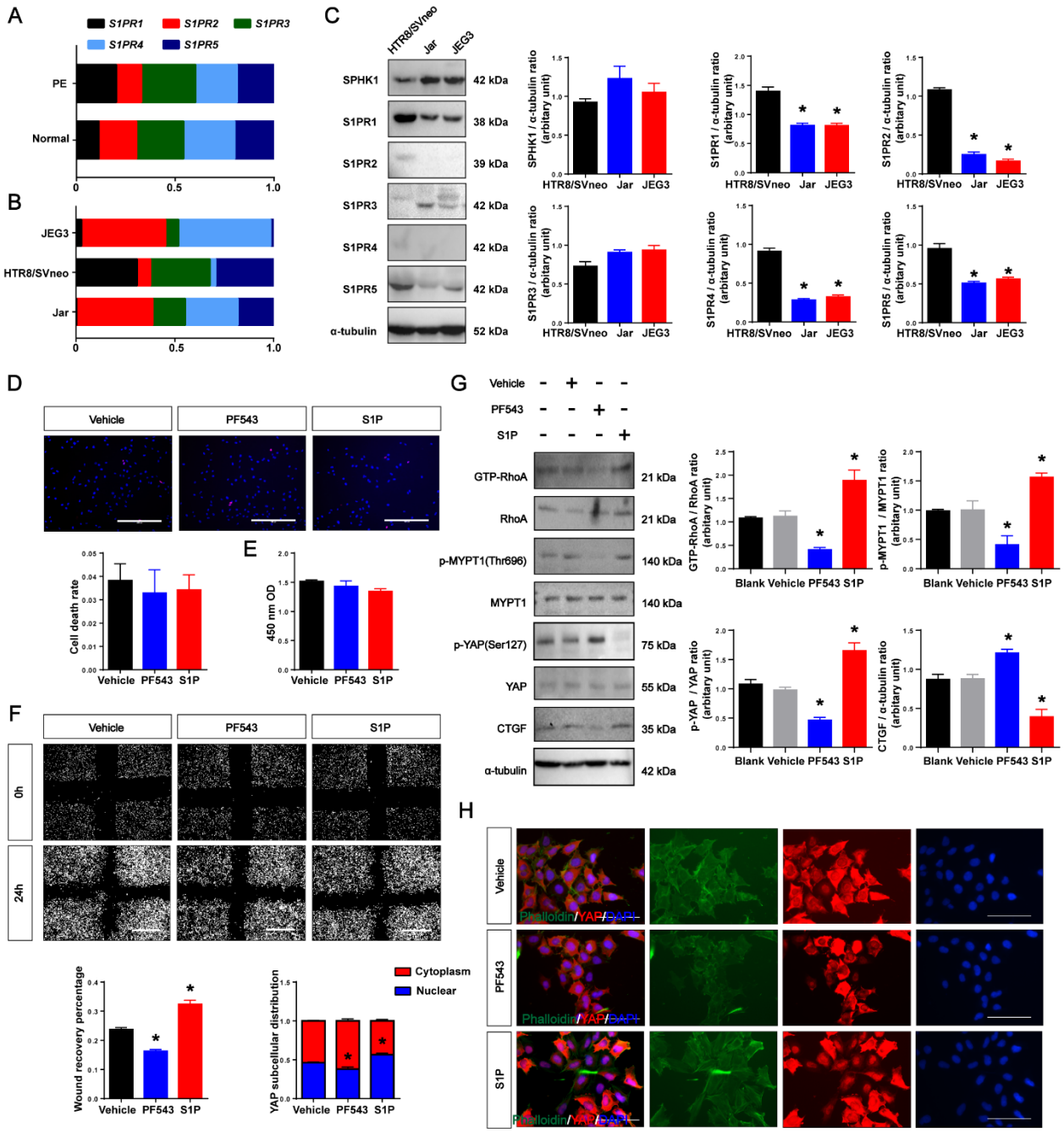
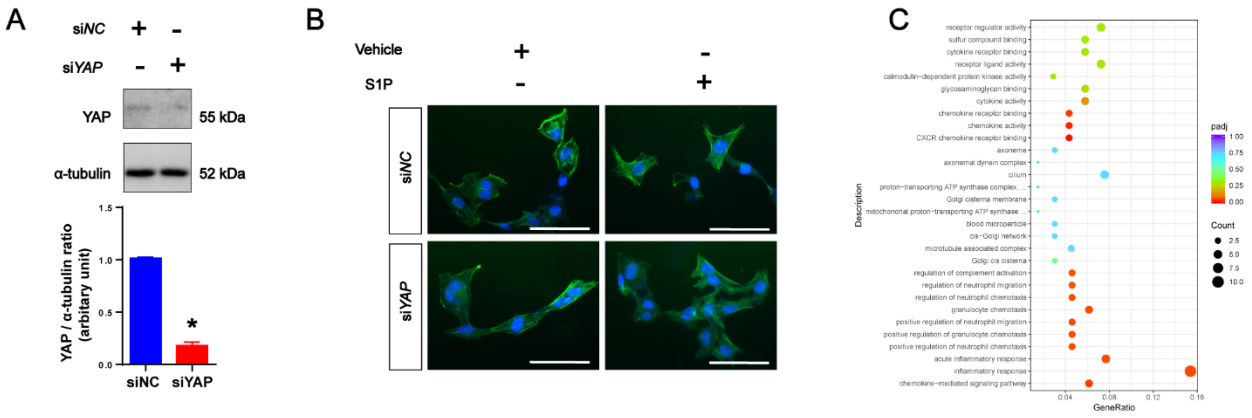


Figure S3. **S1P increases trophoblasts mobility through RhoA activation**

RT-qPCR analysis of *S1PRs* expression pattern in (A) human placentas and (B) trophoblast cell lines (HTR8/SVneo, Jar and JEG3); (C) Western blotting of SPHK1-5 in trophoblast cell lines (HTR8/SVneo, Jar and JEG3), n=3, one-way ANOVA and Tukey's multiple comparison test, *p<0.05 vs. HTR8/SVneo; (D) ETHD II (red) staining of HTR8/SVneo cells, nuclei were stained

with Hoechst (blue), n=3, one-way ANOVA and Tukey's multiple comparison test, scale bar 400 μm; (E) CCK8 assays on HTR8/SVneo cells treated with methanol, PF543 or S1P (250 nM), n=6, one-way ANOVA and Tukey's multiple comparison test; (F) The representative images and statistics of wound healing assay, n=3, one-way ANOVA and Tukey's multiple comparison test, *$p<0.05$ vs. vehicle, scale bar 1000 μm; (G) Western blotting of GTP-RhoA, RhoA, p-MYPT1(Thr696), MYPT1, p-YAP(Ser127), YAP and CTGF after 1 hour of incubation of HTR8/SVneo cells with 100 μM PF543 or 250 nM S1P, n=3, one-way ANOVA and Tukey's multiple comparison test, *$p<0.05$ vs. vehicle (methanol); (H) IF staining of YAP (red) and phalloidin (green) of HTR8/SVneo cells treated with PF543 and S1P, n=3, one-way ANOVA and Tukey's multiple comparison test, *$p<0.05$ vs. vehicle (methanol), scale bar 100 μm;. Data presented as means ± SEM.



D

Motif(TEAD4)	Name	Predictied site	Relative score	Predictied sequence
	COL9A3	1	0.834167858	AAGATTCCTGGG
	COL9A3	2	0.860407566	TTAATTTTTGGT
	CTSD	1	0.875925461	GACATTTGCCAT
	CTSD	2	0.83638564	GTGATTTCAGGG
	EMILIN1	1	0.839901653	AACATGCCTGGC
	EMILIN1	2	0.822162954	TTCATTTATCCC

Figure S4. **S1P induced HTR8/SVneo invasion is dependent on YAP**

(A) Western blotting of YAP in HTR8/SVneo cells transfected with si*YAP* or si*NC* for 6 hours, followed by culturing in fresh medium for another 48 hours, n=3, two-tailed t-test, *p<0.05; (B) Phalloidin staining of HTR8/SVneo cells pre-transfected with si*YAP* or si*NC* for 6 hours, then cultured in fresh medium for another 48 hours, followed by 1 hour incubation of 250 nM S1P or methanol, scale bar 100 μm; (C) Enriched gene ontology (GO) analysis of biological process of genes downregulated in S1P treatment group; (D) Transcription factor binding profiling, top two scored predicted sequences listed for each gene. Data presented as means ± SEM.

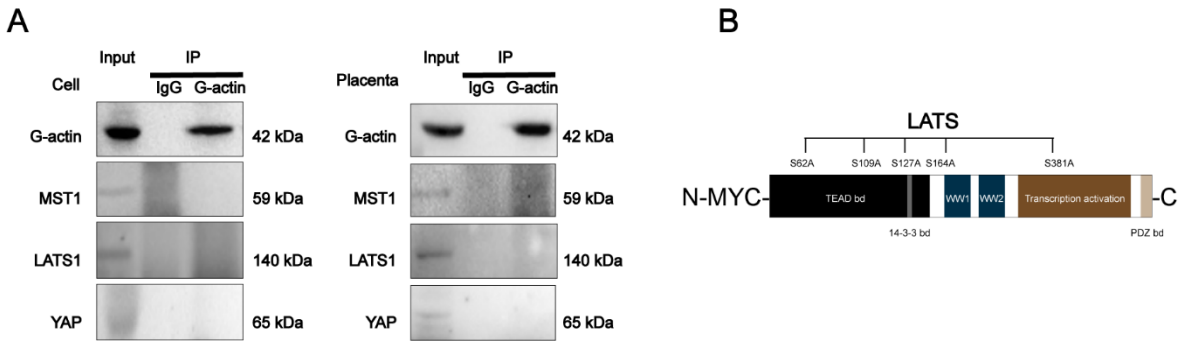


Figure S5. **S1P activates YAP in both Hippo-dependent and -independent ways.**

(A) Co-IP of G-actin with MST1, LATS1 and YAP in HTR8/SVneo cells and placenta tissue; (B) illustration of YAP-5SA.

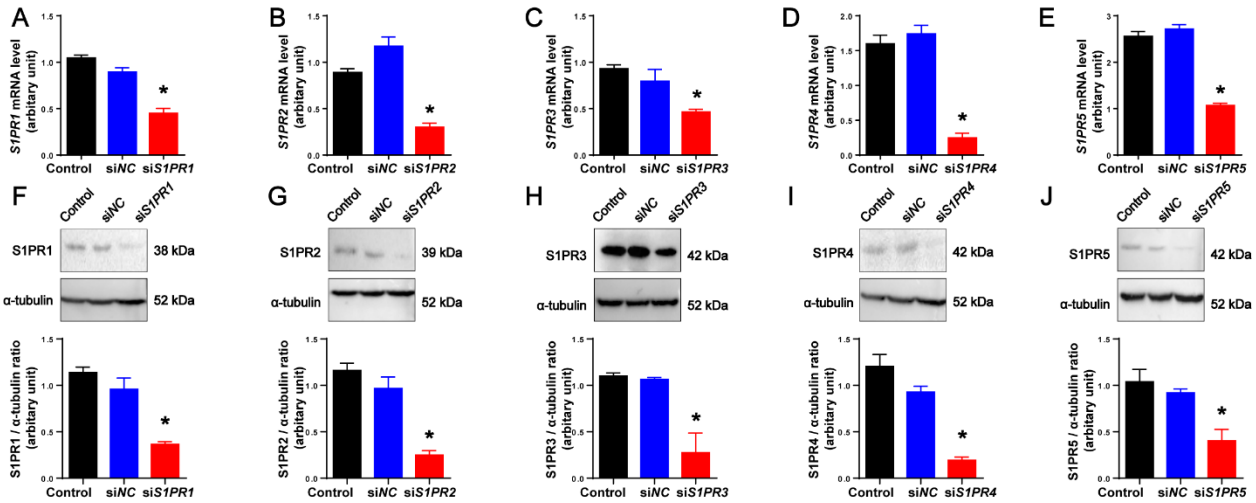


Figure S6. **Establishment of S1PR-deficient HTR8/SVneo cells**

(A - E) mRNA levels of *S1PR1-5* determined by RT-qPCR, n=3, one-way ANOVA and Tukey's multiple comparison test, *p<0.05 vs. si*NC*; (F - J) Western blotting of S1PR1-5 in si*S1PRs* or si*NC* cells, n=3, one-way ANOVA and Tukey's multiple comparison test, *p<0.05 vs. si*NC*. Data presented as means ± SEM.

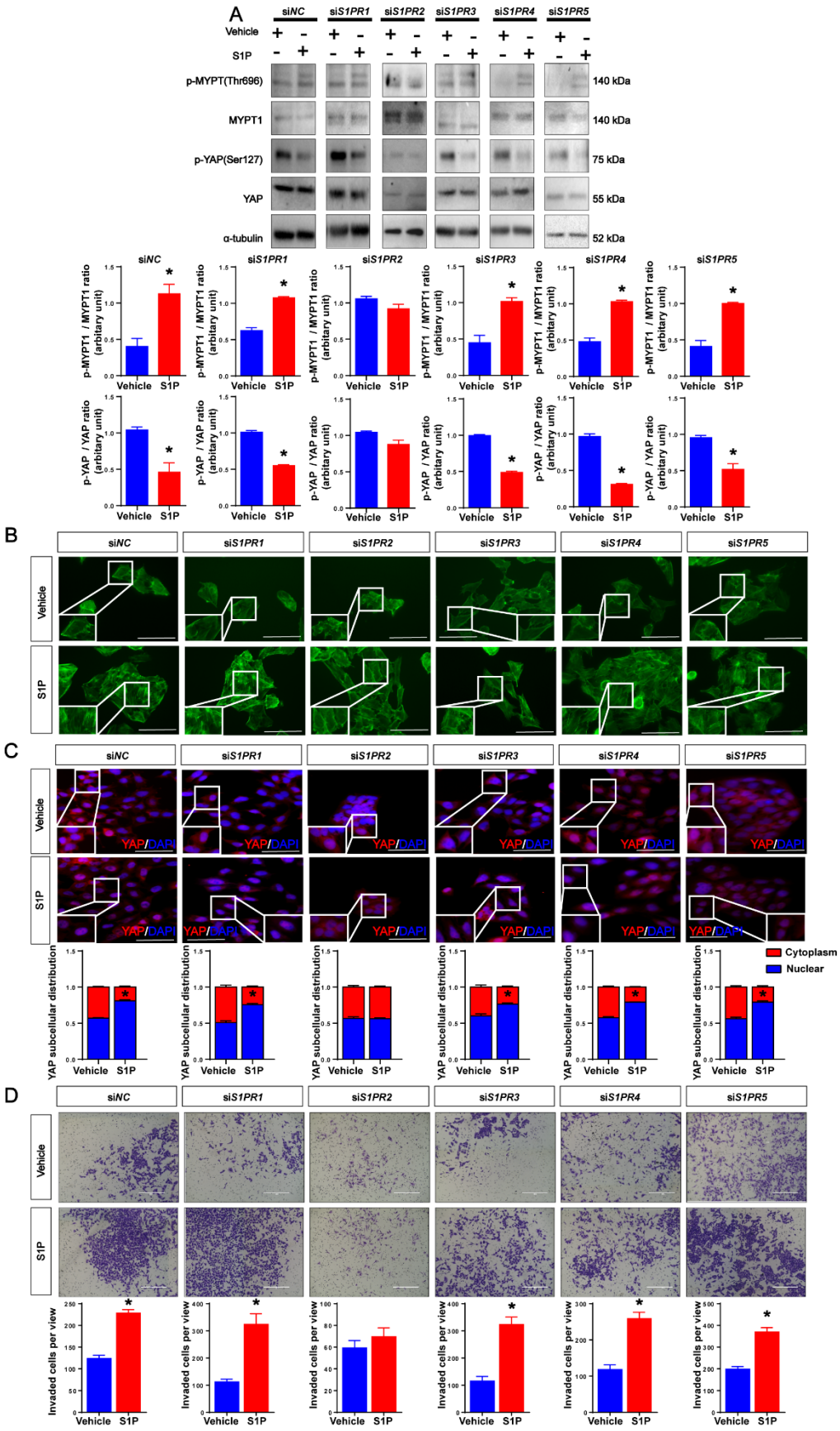

A
siNC
siS1PR1
siS1PR2
siS1PR3
siS1PR4
siS1PR5
Vehicle
S1P
p-MYPT(Thr696)
MYPT1
p-YAP(Ser127)
YAP
α-tubulin
140 kDa
140 kDa
75 kDa
55 kDa
52 kDa
p-MYPT1 / MYPT1 ratio (arbitary unit)
p-YAP / YAP ratio (arbitary unit)
B
C
D
YAP/DAPI
YAP subcellular distribution
Cytoplasm
Nuclear
Invaded cells per view

Figure S7. **S1P regulates actin polymerization and YAP activation through S1PR2.**

(A) HTR8/SVneo cells transfected with si*S1PRs* or si*NC* for 6 hours, followed by culturing in fresh medium for 48 hours before any treatments and measurements. Western blotting of p-MYPT1(Thr696), MYPT1, p-YAP(Ser127), YAP in *S1PRs* knockdown or si*NC* cells after 1 hour of incubation with methanol or 250 nM S1P, n=3, two-tailed t-test, *p<0.05; (B) IF staining of phalloidin (green) in *S1PRs* knockdown or si*NC* cells been treated with methanol or 250 nM S1P for 1h, scale bar 100 μm; (C) IF staining of YAP (red) in si*NC* or si*S1PRs* cells after 1 hour of 250 nM S1P or methanol treatment, representative images and nuclear YAP fluorescence quantification, n=3, two-tailed t-test, *p<0.05, scale bar 100 μm; (D) si*NC* or si*S1PRs* transfected HTR8/SVneo cells and WT were subjected to Matrigel transwell assay in the presence of vehicle or 250 nM S1P, invaded cells were stained and counted, n=3, two-tailed t-test, *p<0.05, scale bar 400 μm. Data presented as means ± SEM.

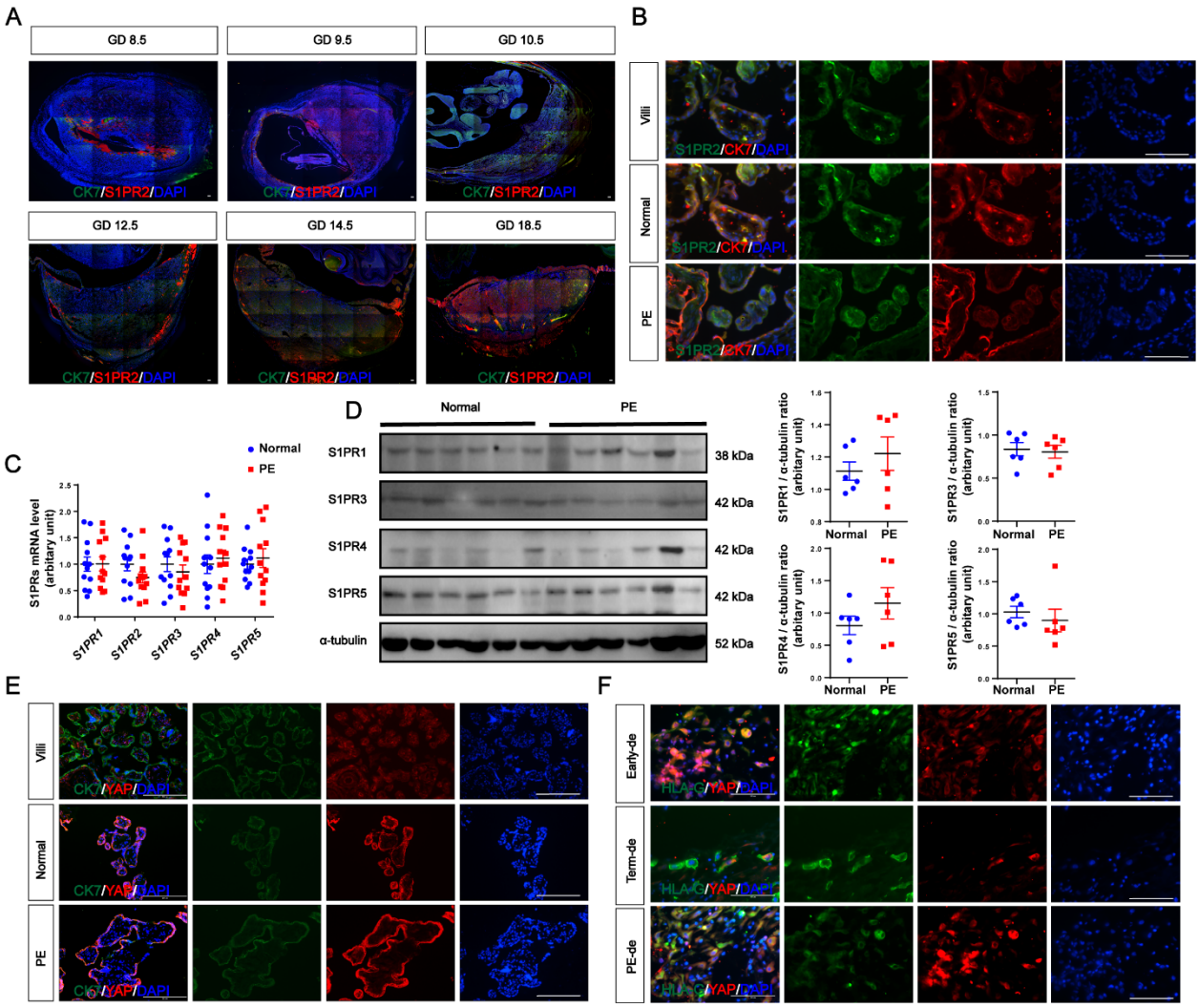


Figure S8. **S1PRs expression in mice and human placentas and YAP expression in human placentas and decidua.**

(A) IF staining of S1PR2 (red) and CK7 (green) in mouse uteroplacental units collected across gestation, scale bar 20000 μm; (B) IF staining of S1PR2 (red) in human placentas at different gestational ages. Trophoblasts were stained with CK7 (green), nuclei were counterstained with DAPI (blue), scale bar 200 μm; (C) mRNA levels of *S1PR1-5* in normal and PE placentas determined by RT-qPCR, n=12, two-tailed t-test; (D) Western blotting of S1PR1, 3, 4, 5 in normal and PE placentas, n=6, two-tailed t-test. (E) IF staining of YAP (red) in first-trimester villi, normal and PE term placentas, trophoblasts stained with CK7 (green), nuclei counterstained with DAPI (blue), scale bar 200 μm; (F) IF staining of YAP (red) in human decidua collected from normal (Term-de) and preeclamptic (PE-de) pregnancies at term, as well as decidua collected at early gestation (Early-de). EVTs stained with HLA-G (green), nuclei counterstained with DAPI (blue),

scale bar 200 μm. Data presented as means ± SEM.

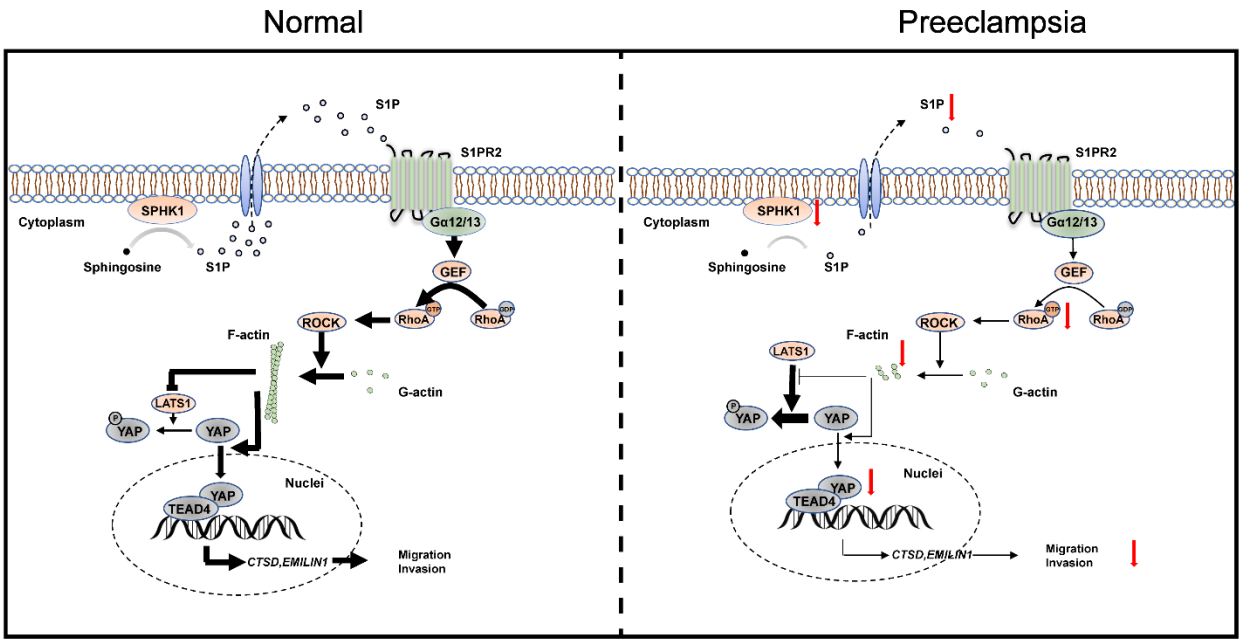


Figure S9. **Proposed working model of S1P-regulated YAP activation through S1PR2-RhoA-ROCK pathway in normal and PE placental trophoblasts.**

In trophoblasts from normal placenta, S1P produced by SPHK1 interacts with S1PR2 to activate downstream RhoA-ROCK signaling, which promotes F-actin formation. F-actin increases YAP activity and downstream gene (*CTSD, EMILIN1*) transcription to promote trophoblast invasion to maintain proper placental function and successful pregnancy. S1P synthesis is impaired in preeclampsia placentas; due to SPHK1 deficiency, RhoA-ROCK pathway and F-actin formation are downregulated, leading to the sequestering of YAP in the cytoplasm and inhibited *CTSD, EMILIN1* expression. Trophoblast invasion is thus inhibited, resulting in inadequate spiral artery remodeling and PE.

Table S1. Clinical characteristics of the recruits.

Parameters	normal	PE	P value
Age (years old)	29.44 ± 0.9146	32.33 ± 1.247	0.0802
Gestational age (weeks)	39.32 ± 0.3582	36.56 ± 0.7059	0.0030
Systolic pressure (mmHg)	111.4 ± 2.422	150.7 ± 3.155	<0.0001
Diastolic pressure (mmHg)	72.78 ± 2.966	95 ± 1.903	<0.0001
Fetal birth weight (g)	3300 ± 115.4	2594 ± 221.8	0.0159

Table S2. Primers for RT-qPCR.

Genes	Forward 5'-3'	Reverse 5'-3'
SPHK1	GGCAGGCATATGGAGTATGAA	CCACTGCAAACACACCTTTC
SPHK2	CTTTGCCCTCACCCTTACAT	CCACAGACAGGAAGGAGAAAC
SGPP1	CACCTGGAGCACATCCCGAG	ATGGCTTTTCCAAACAGAGTCAC
SGPP2	CAACCTTTGTGCCGATGCTT	CCTATGTCTTGGCTTTCATGTCC
SGPL	ATCTTCCCAGGACTACGCAAG	AGAAGTCACACATCCACACGAA
S1PR1	CGCGACAAGGAGAACAGCATTA	CCAAATGGTCAGCAAGACAAAG
S1PR2	ATCGTGCTAGGCGTCTTTATC	AAAGTAGTGGGCTTTGTAGAGG
S1PR3	CTCTGCGGCTGTGTTCTTAT	AGGCTGTTGGTCAAAGTAAGG
S1PR4	GGTCTACTATTGCCTGGTGAAC	GATGTAGCGCTTGGAGTAGAG
S1PR5	CCTCGTGGAATTGACGTTCT	TCCTTGTCTCCTCCCATTCT
CTSD	CGCTGCACAAGTTCACGTCCA	CCCGATGCCAATCTCCCCGT
COL9A3	ACCTTCAGTGCCCAAGTATCTGC	CTGCTCGCCTTTGTAGCCAGT
EMILIN1	TGTCCCCAAAGCATCATGTACCG	ACAGCACCTCCACTCCATGTCG
ACTB	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA

Table S3. The sequences of siRNA.

siRNA names	Sense sequence 5'-3'
si*S1PR1*	CCACCGACCCAUGUACUAUTT
si*S1PR2*	CCUUCGAGCCAAUACCUUTT
si*S1PR3*	UGAAAUUUAUUGUUUUUCCAG
si*S1PR4*	AAUAGUAGACCCAGCGUCGCG
si*S1PR5*	AAGAACAAGUCUGUAAAACUU
si*YAP*	GGUGAUACUAUCAACCAAA
si*NC*	UUCUCCGAACGUGUCACGUTT