Supplemental Materials

Title: Protein Phosphatase 2A Activation via ApoER2 in Trophoblasts Drives Preeclampsia in a Mouse Model of the Antiphospholipid Syndrome

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Expanded Materials and Methods

Animal models

In vivo studies were performed in wild-type C57BL/6J mice (Jackson Laboratory), in littermate control mice (apoER2$^{fl/fl}$) or in mice lacking trophoblast apoER2 (ApoER2$^{\Delta TR}$) on identical FVB× C57BL/6J background [24,26]. Mice were kept in temperature-controlled in a UTSW AAALAC-approved barrier facility. All mice were fed with chow diet (Teklad 2016 global protein rodent diets, irradiated) with corncob bedding. ApoER2$^{fl/fl}$ mice were generated as previously reported[24], and the mice lacking ApoER2 specifically in trophoblasts were generated by crossing them with mice expressing Cre recombinase under the regulation of the trophoblast-specific CYP19 promotor (Cyp19Cre)[26]. Female mice were studied at 8-10 weeks of age, and male mice of the same genotype were used for mating at 8-16 weeks of age. Following mating, the females were injected intraperitoneally with NHIgG or aPL (10 mg/mouse) on day 8 and 12 of pregnancy. The average gestation length of C57BL/6 mice is 19 days[71]. Recognizing that ApoER2$^{\Delta TR}$ pregnancies administered aPL are the key group for testing the role of the trophoblast receptor in the aPL-induced phenotype, we assigned 50% more mice to the ApoER2$^{\Delta TR}$-aPL group compared to the ApoER2$^{\Delta TR}$-NHIgG group. However, the treatment of the ApoER2$^{\Delta TR}$ mice with NHIgG versus aPL was otherwise done in a random manner. Among the mice who showed the vaginal plug, the ones that did not ultimately conceived were excluded from the study, except for the groups shown in Fig. 1C. ApoER2 gene expression in mouse placentas and in mouse endothelial cells was determined by qRT-PCR using TaqMan primer (Mm00474030_m1, catalog #4448892). The expression of ApoER2 in mouse placenta was evaluated by immunohistochemistry as previously described[25]. In experiments assessing the effect of PP2A inhibitor Cantharidin, pregnant C57BL/6 mice were injected with aPL (10 mg/mouse) on day 8 and 12 of pregnancy, and they

received either vehicle (1% DMSO in PBS) or Cantharidin (1mg/kg) intraperitoneal injections on day 8, 10, 12, and 14. Studies in a preliminary cohort of mice revealed that Cantharidin did not impact body weight (**Online Fig. VIII**), indicating that overall maternal well-being was minimally unaffected. For blood pressure measurements, Cantharidin was administered through osmotic minipump (ALZET model 1002, cat#0004317, 2.08mg/ml, 0.25μl/h). Mice were euthanized on day 15 of pregnancy, uteri were dissected, embryos were weighed, fetal resorption rates were calculated (number of resorptions/(number of live fetuses + number of resorptions)), and placentas were isolated for PP2A activity assay. Plasma and urine were also collected on day 15. The sample size and power calculations were performed in a small pilot study with C57BL/6J pregnant female mice. In the experiment, we observed differences of 9.4 mmHG in BP between NHIgG and aPL treatment groups with average standard deviations of 6.34. Using these predictions with a standardized difference of 0.78 with 80% power using a cutoff for statistical significance of 0.05, it was expected that 7 animals would be needed.

IgG preparation

Normal human IgG (NHIgG) and aPL were isolated from healthy individuals and four APS patients as previously described[24,25]. The relevant clinical and laboratory features of the APS patients are as follows: Patient #1, age 32, male, aCL (>80 LA PGA) positive, anti-β2GPI positive, with clinical features of recurrent arterial thrombosis, and catastrophic APS. Patient #2, age 57, male, aCL (>80 LA PGA) positive, anti-β2GPI positive, with clinical features of arterial thrombosis, recurrent pulmonary hemorrhage, and catastrophic APS. Patient #3, age 36, female, aCL (>80 LA PGA) positive, anti-β2GPI positive with clinical features of deep vein thrombosis, renal microthrombotic angiopathy, and stroke. Patient #4, age 43, male, aCL (>80

LA PGA) positive, anti-β2GPI positive, with clinical features of arterial thrombosis, and a digital infarct and leg ulcer. Written informed consent was provided before study participation. All protocols were approved by the Institutional Review Boards of the Hospital for Special Surgery (Study #00001338) and the University of Texas Southwestern Medical Center (#STU112017-042, #STU 1222013-075). Mouse monoclonal antibody directed against β2GPI (designated 3F8) and its isotype-matched control (designated BBG) were generated as previously described[25,72] .

Human placentas

The placentas were collected from the mothers with the clinical features as follows: Placenta #1, age 33, term pregnancy, diagnosed with APS, systemic lupus erythematosus (SLE), and Sjogren's, with clinical features of deep vein thrombosis, autoantibodies positive for antinuclear antibodies (ANA), anticardiolipin antibodies (aCL), β2GPI, and lupus anticoagulant (LAC). Placenta #2, age 34, term pregnancy affected by IUGR, diagnosed with SLE, autoantibodies positive for ANA, aCL, β2GPI, and LAC. Placenta #3, age 26, term pregnancy, diagnosed with APS and non-lupus connective tissue disorder, with clinical features of deep vein thrombosis and pulmonary embolism, autoantibodies positive for ANA, double stranded DNA, aCL, β2GPI, and LAC. Placenta #4, age 32, preterm pregnancy at 32 weeks gestation affected by severe pre-eclampsia, diagnosed with APS, with clinical features of multiple spontaneous abortions and fetal demise, autoantibodies positive for aCL, β2GPI, and LAC. The frozen near term placental explants (39 weeks) from normotensive or preeclamptic women were obtained, tissue lysates were prepared and PP2A activity, PHD2 phosphorylation and HIF1α expressions were evaluated. The average systolic blood pressure in the normotensive (N=6) and preeclamptic group (N=6) were 122.3±3.9 and 163.2±4.5, respectively ($P<0.0001$).

The average diastolic blood pressure was 79.0±2.1 and 98.8±2.3 (P<0.0001). There were no significant differences in age (24.5±3.5 vs. 26.0±3.4 years) or in gestational age at delivery (39.2±0.7 vs. 39.72±0.52 weeks) between the groups. N=6/group, Mean±SEM. All protocols were approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center (#STU072017-034, #STU 1222013-075).

Cell culture, siRNA and adenoviral transfection

In siRNA experiments, HTR8/SVeo cells or Sw.71 cells were transfected with the siRNAs shown below (ThermoFisher) using siPORT Amine transfection reagent as previously described[24,25]. Control siRNA was purchased from GE Dharmacon (ON-TARGETplus Non-targeting Control siRNA). For MKK3 (MAAP2K3) siRNA was purchased from Thermofisher (siRNA ID s11175, cat#4390824). The rest of the sequences for siRNA was the following:

Name	Sequence (sense)	Sequence (antisense)
PP2A-Aα	CCUAAUUACUUGCAUAGAAtt	UUCUAUGCAAGUAAUUAGGa
PP2A-Bδ	GCCGUGAUGUUGUCACUGAtt	UCAGUGACAACAUCACGGCta
PP2A-B'δ	AGUGCAACGUGUUCGUCUAtt	UAGACGAACACGUUGCACUgg
ApoER2	CAUCCCUAAUCUUCACCAAtt	UUGGUGAAGAUUAGGGAUGgg
Dab2	CGACACCAAUCAGAAUUCUtt	AGAAUUCUGAUUGGUGUCGat
JIP4	GGAUCUGACGGGUGACAAtt	UUUGUCACCCGUCAGAUCCgt
P38α	GAAGCUCUCCAGACCAUUUtt	AAAUGGUCUGGAGAGCUUCtt

Trophoblast proliferation and migration were assessed as described previously[25]. All findings in cell culture were replicated in 3 independent experiments. The trophoblast cell lines were used at the passage 15-20.

PP2A B subunit expression

In HTR8/SVneo or Sw.71 cells, expression of PP2A B subunits was assessed by RT-PCR using the following primers:

Gene name	Alternative name	Forward	Reverse
PPP2R1A	1A	tgagcttctgcctttccttac	atgggagagagacccatgat
PPP2R1B	1B	cctctggtgaaacgcttagcaag	caggcggaccctccatttggc
PPP2R2A	Bα, 2A	cttgaaagaggaggatggaagg	ggcttcagaactgtgcgaggc
PPP2R2B	Bβ, 2B	gtcagcgagcgtgataagagg	gcggaggtagtcatgaacctgg
PPP2R2C	Bγ, 2C	ccacatcaactccatctccgtc	ctgtcgctcccgttccaggcac
PPP2R2D	Bδ, 2D	ttcgagacccatttaggatcacggc	ccagactgtccacactgatctcg
PPP2R5A	B'α, 5A	cttggcctcacatacagttgg	caatggtcggattccagtgttc
PPP2R5B	B'β, 5B	cctgaatttgaccctgaagag	cctccagaccttgccataac
PPP2R5C	B'γ, 5C	ctaggcttgagagcttacatcag	atggtgctggcttgactatac
PPP2R5D	B'δ, 5D	ccagctcgtgtatgagttcttc	tctgcttctctgccttgtattg
PPP2R5E	B'ε, 5E	cagaagaagatgaacctaccc	ctcacgctgacgatctgacttg
PPP2R3A	B"α, 3A	tcctcccaggccaatttatcag	ctgcatagctcattcttctctc
PPP2R3B	B"β, 3B	gcagggacgagagtagttcag	ccagacaaagtcggcatagc
PPP2R3C	B"γ, 3C	caggagtgtctcacttatgatg	gtccagaagccattcaaatcg
PPP2A-Ca	Cα	gagagcagacagatcacacaag	ggtcatggcaccagttatatcc
PPP2A-Cb	Cβ	ggagactgtgactcttcttgtag	gcagcctggttcccacaacg

Immunoprecipitation and immunoblot analysis

HTR8/SVeo cells or Sw.71 cells were lysed with ice-cold lysis buffer (1% Triton X-100 in 1xTBS, pH 8.0 containing 1mM $CaCl_2$, 1mM $MgCl_2$, and protease inhibitors (Roche Diagnostics)). Cell lysates were centrifuged at 14,000rpm for 10min at 4°C, and supernatants were incubated with HA-agarose beads (for HA-tagged ApoER2 constructs shown in Fig. 4B) or protein A beads coated with appropriate antibodies (for endogenous proteins) for 3h at 4°C. Beads were washed three times and immunoprecipitated complexes were extracted and analyzed by immunoblot or by mass spectrometry. Immunoblot analyses were performed using antibodies shown below. Specificity of the antibodies were validated by comparing the cells treated with control siRNA versus siRNA targeting the protein(s) detected by the

antibodies. Specificity of the antibodies against post-translational modifications such as phosphorylation or methylation were validated by comparing non-treated cells with cells treated with known stimuli of the modification. Cell or tissue lysates extracted from cultured trophoblasts or mouse placenta were used for immunoblotting analysis. Total protein concentration was measured using the BCA protein kit (Thermo Fisher, USA) with bovine serum albumin that contained in the kit as the standard protein. Equal amounts of protein from each sample were subjected to 8-10% SDS PAGE gels electrophoresis, and were subsequently transferred to PVDF membranes (Millipore). The membranes were incubated with the blocking buffer (5% milk in Tris-Buffered Saline, 0.1% Tween® 20 Detergent (TBST), 1h at room temperature) followed by incubation with specific antibody or internal control antibody at 4°C overnight. After three washes with TBST for 30 min, the PVDF membranes were incubated with the horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit, rabbit anti-goat or goat anti-mouse lgG) for 1 h at room temperature. The protein bands were visualized using an enhanced chemiluminescence system (Thermo Fisher, USA) and the intensity of bands was quantified using ImageQuantTL software (General Electric Company). All findings in cell culture were replicated in 3 independent experiments.

Target antigen	Vendor or Source	Catalog #	Working concentration	RRID #	Persistent ID / URL
ApoER2 (for IF)	Abcam	ab108208	1:100	AB_10862841	
ApoER2 (for WB)	Abcam	Ab108208	1:1000	AB_10862841	
MMP14	Abcam	ab51074	1:2000	AB_881234	
GAPDH	Santa Cruz	sc-365062	1:1000	AB_10847862	
PP2A-Aα	Cell Signaling	2039	1:1000		
PP2A-C (Met-L-309)	Abcam	Ab66597	1:1000	AB_2169497	
PP2A-C (pY307)	Abcam	Ab59222	1:1000	AB_945023	
PP2A-C	Cell Signaling	2038	1:1000	AB_2169495	
pAkt-S473	Cell Signaling	4060	1:1000	AB_2305337	
Akt	Cell Signaling	9272	1:1000	AB_329827	
Dab2	BD	610464	1:1000		

	Biosciences				
JIP4	Santa Cruz	Sc-271492	1:1000	AB_10659098	
pJNK T183/Y185	Cell Signaling	9255	1:1000		
JNK	Cell Signaling	9252	1:1000		
MKK3	Cell Signaling	8535	1:1000	AB_11220233	
P-p38α	Cell Signaling	4511	1:1000	AB_2139682	
P38α	Cell Signaling	9212	1:1000	AB_330713	
HIF-1a	thermofisher	MA 1-16504	1:500		
pPHD2 (S125)	Millipore	MABC1612	1:1000		
PHD2	NOVUS	NB100-2219	1:500	AB_1522843	
PP2A-B'δ	Abcam	Ab188325	1:500		
Ki67	Abcam	Ab16667	1:100	AB_302459	
E-cadherin	Cell Signaling	3195	1:300	AB_2291471	
Dab1	Abcam	111684	1:1000	AB_10865153	
Mouse anti-Rabbit	Santa Cruz	sc-2357	1:1000	AB_628497	
Goat anti-Mouse	Invitrogen	31430	1:2500	AB_228307	
Mouse anti Goat	Santa Cruz	sc-2354	1:1000	AB_628490	

Quantitative RT-PCR, ELISA

Transcript abundance was evaluated in HTR8/SVneo cells, mouse placentas or mouse aortic endothelial cells by quantitative RT-PCR using TaqMan Gene Expression. Assays (ThermoFisher; human endoglin ENG Hs00923996_m1, human MMP14 Hs01037003_g1, human GAPDH Hs02758991_g1, mouse Mmp14 Mm00485054_m1 mouse endoglin Mm00468252_m1, mouse Gapdh Mm99999915_g1). Total RNA was extracted from the placenta of mice or human trophoblasts using Trizol (Thermo Fisher, USA). One microgram of total RNA was subjected to cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher, USA) according to the instructions of the manufacturer. The RT-PCR amplification was conducted using a TaqMan™ Gene Expression Master Mix (Thermo Fisher,USA) according to manufacturer's instructions. The reaction was carried out using ABI Prism 7900 Detector System (Applied Biosystems). RT-PCR conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 15s, 60°C for 40s. The data obtained from the

assays were analyzed with SDS 2.3 software (Applied Biosystems). The relative gene expression was calculated using the ΔΔCt method with GAPDH as the endogenous control. Mouse plasma albumin (Abcam, #ab108792), creatinine (Sigma #MAK080-1KT), endoglin/CD105 (R&D, MNDG00), MMP14 (MyBioSource, MBS2021894), mouse VEGFR1/Flt-1 (R&D, MVR100), human endoglin/CD105 (R&D, DNDG00) and human (Abcam, ab197747) were measured according to the manufacturers' instructions.

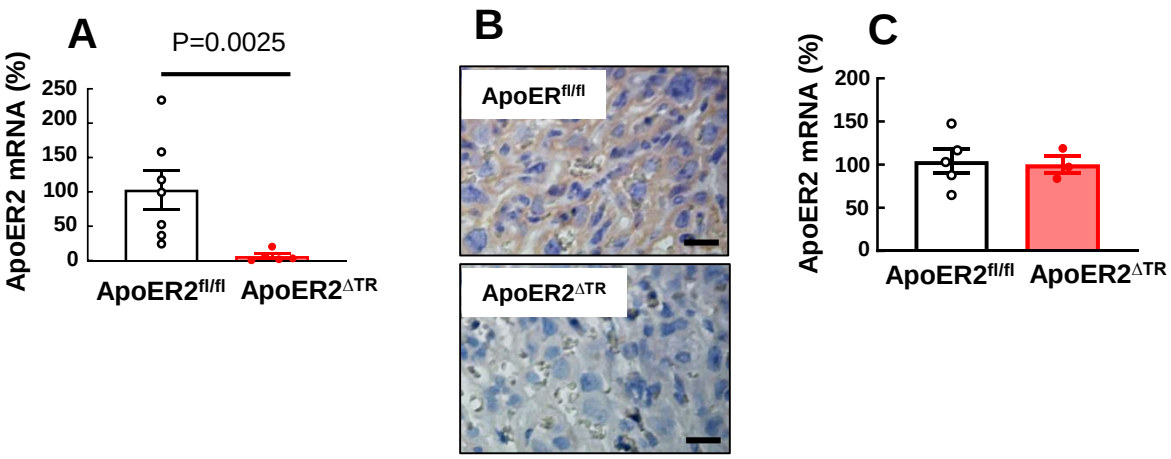
Isolation of mouse aortic endothelial cells

To evaluate the expression of ApoER2 in endothelial cells in mice, primary endothelial cells were isolated from mouse aortas as previously described[73].

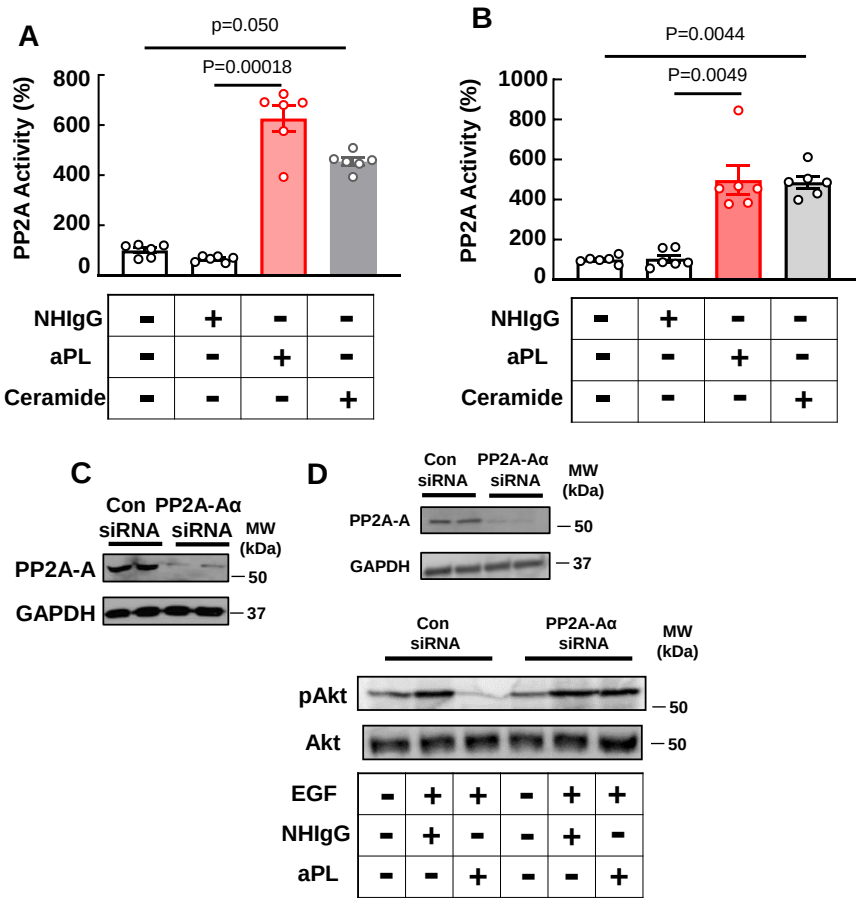
Mouse placenta histology

Pregnant C57BL/6 mice were intraperitoneally injected with NHIgG or aPL (10 mg/mouse) on day 8 of pregnancy, and 48 h after mice were euthanized, placentas were isolated and prepared for immunohistochemistry. Briefly, placental samples were washed with PBS three times, then they were fixed with 4% paraformaldehyde overnight and then paraffin embedded. Sections (5μm) were de-paraffinized and rehydrated followed by antigen retrieval in Buffer A (Electron Microscopy Sciences, USA) using a 2100-Retriever (Electron Microscopy Science, USA). The placenta sections were stained with antibodies against Ki67 (rabbit monoclonal, Abcam #ab16667) or E-cadherin (rabbit monoclonal, Cell Signaling Technology #3195) using the Impress HRP staining kit (Vector labs, Anti-Rabbit IgG Polymer Kit, #MP-7401) and Dako Liquid DAB+ Substrate Chromogen System (#K3468). Antibodies were used at 1:100 and 1:300, respectively following a previously published protocol[74-76]. Specificities of the antibodies were confirmed by including negative controls without the primary antibodies,

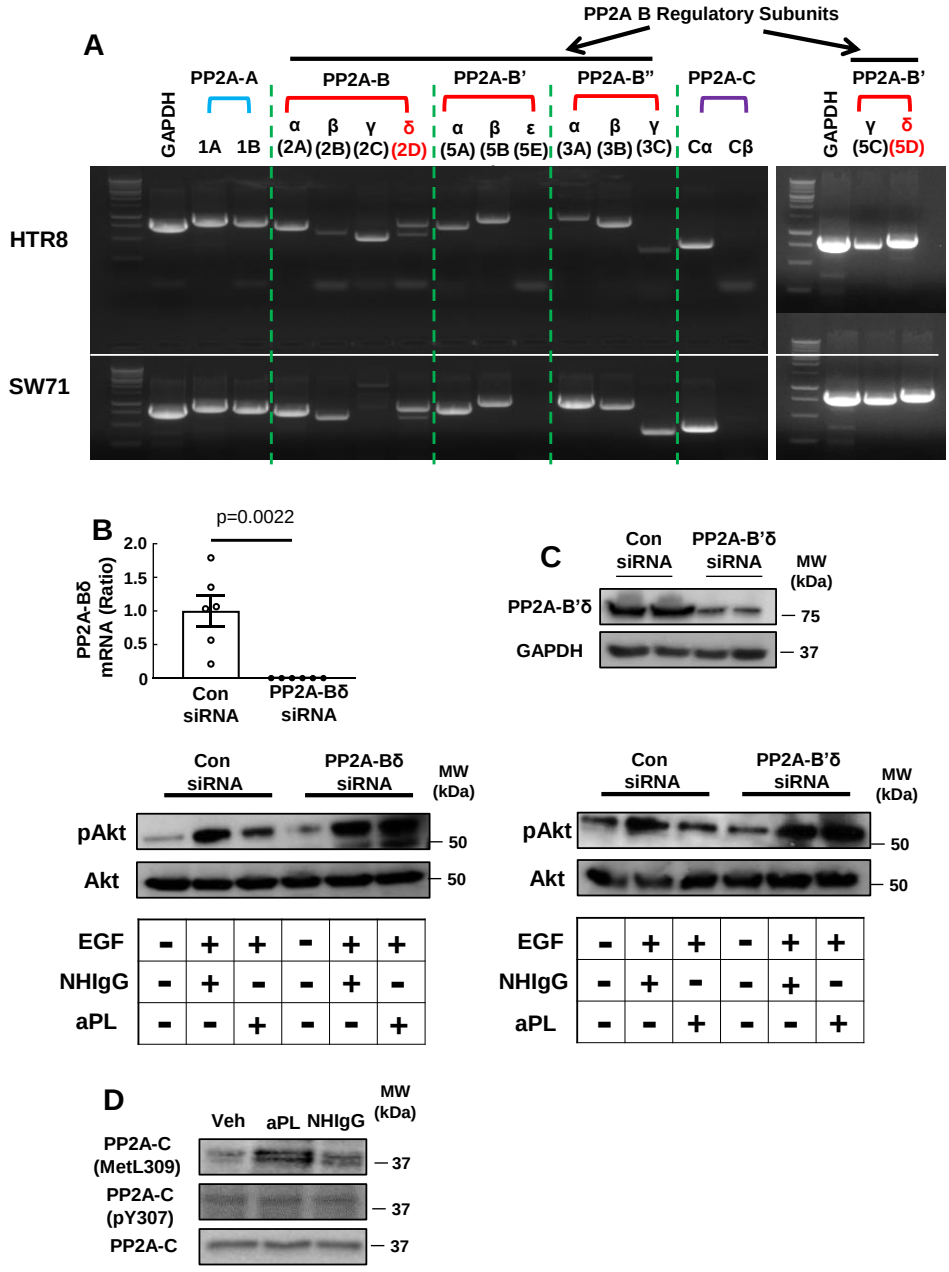
which showed negligible signals. The sections were also probed for Gcm-1 mRNA using in situ hybridization to identify syncytiotrophoblast in the labyrinth layer. The Gcm-1 cRNA probe (a kind gift from Dr. James C. Cross, Cumming School of Medicine, University of Calgary), which had been extensively characterized[77-79], was used in hybridization and detection as previously reported[76,80]. In addition to antisense probe, experiments included the complementary, sense control to account for non-specific binding and background staining, which was undetectable.



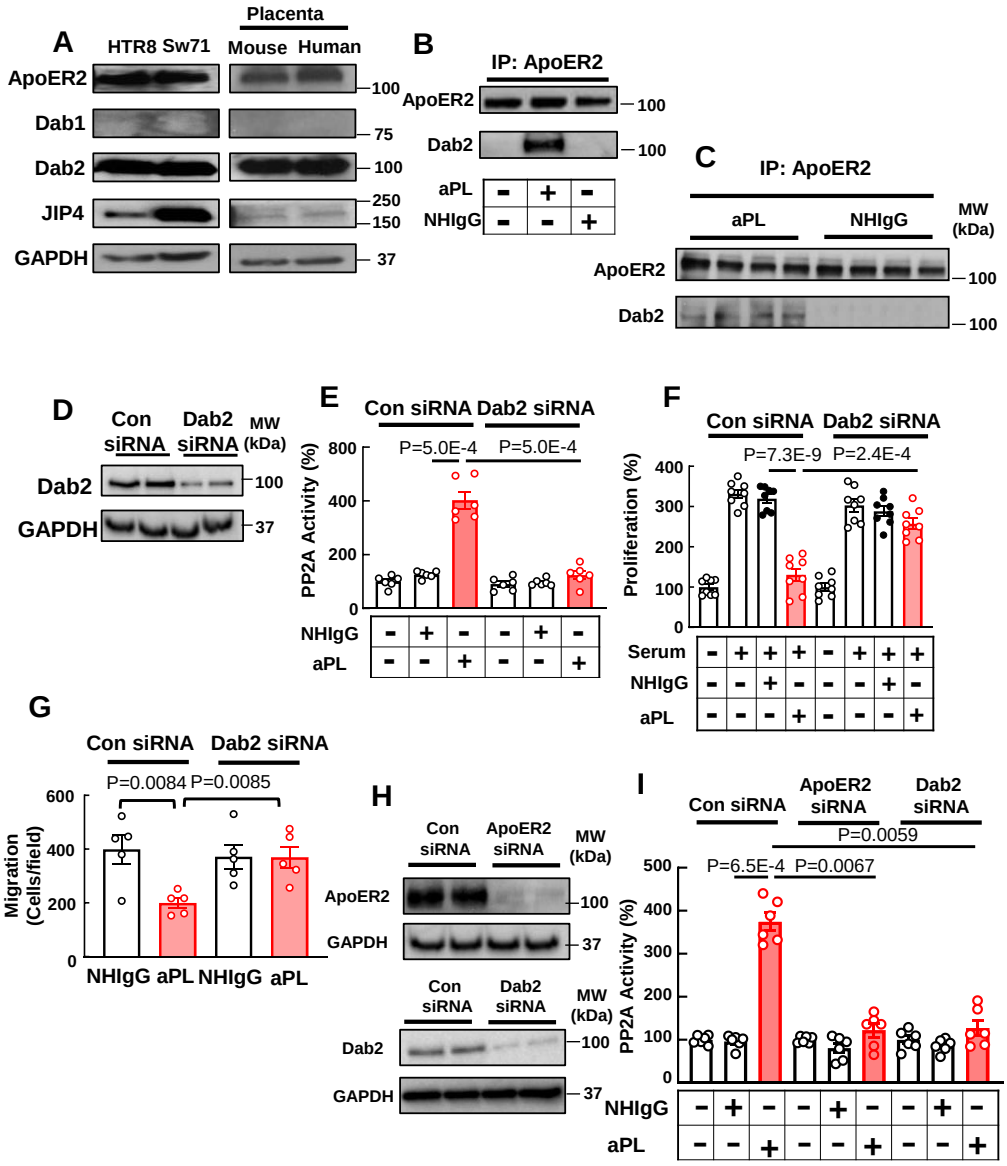
Online Figure I Selective deletion of ApoER2 in trophoblasts. **(A,B)** Deletion of ApoER2 in trophoblasts was confirmed in ApoER2$^{\Delta TR}$ mice compared to control ApoER2$^{fl/fl}$ mice at day 15 of pregnancy. ApoER2 mRNA expression was evaluated in the placenta (A, N=7,5), and relative ApoER2 protein abundance in trophoblasts was assessed by immunohistochemistry (brown staining) (B). Scale bars = 50 μm. **(C)** ApoER2 mRNA expression was evaluated in aortic endothelial cells (C, N=5,3). Values are Mean±SEM. Mann-Whitney U test was performed in A.



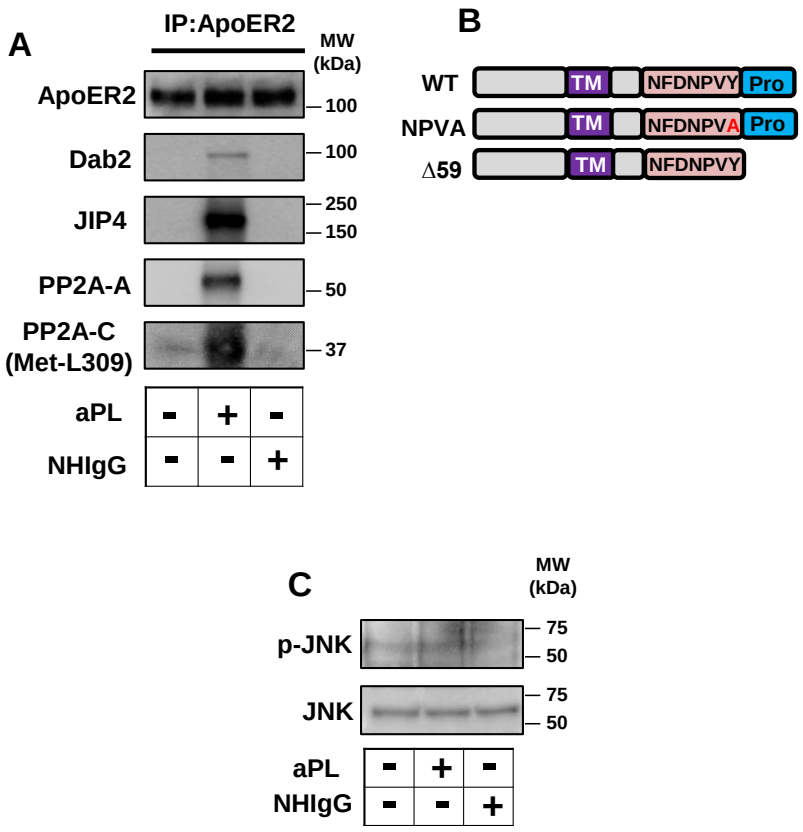
Online Figure II APL promote PP2A activation and resulting antagonism of EGF-stimulated Akt S473 phosphorylation in cultured trophoblasts. **(A, B)** HTR8/SVneo trophoblasts (A) or Sw.71 trophoblasts (B) were incubated with NHIgG, aPL or C2 ceramide and PP2A activity was measured. N=6, values are Mean±SEM. One-way ANOVA. **(C)** HTR8/SVneo trophoblasts were transfected with control siRNA or siRNA targeting PP2A-Aα subunit, and PP2A-Aα protein abundance was evaluated. **(D)** Sw.71 trophoblasts were transfected with control siRNA or siRNA targeting PP2A-Aα, and either PP2A-Aα abundance was evaluated (upper panel), or cells were treated with NHIgG or aPL, and EGF stimulation of Akt S473 phosphorylation was assessed (lower panel). Kruskal-Wallis with Dunn's post hoc test was performed (A, B).



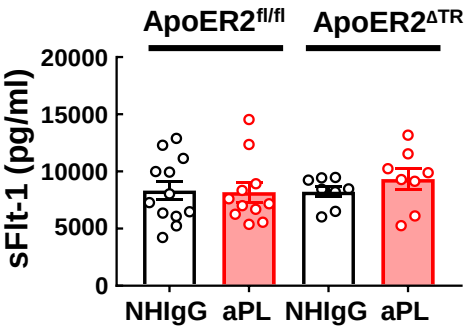
Online Figure III Trophoblasts express subsets of PP2A-B subunits, PP2A-Bδ and PP2A-B'δ are required for Akt dephosphorylation by aPL, and aPL induce PP2A-C L309 methylation but not Y307 phosphorylation. **(A)** PP2A-B subunit transcript expression in HTB8/SVneo or Sw.71 trophoblasts. **(B, C)** HTB8/SVneo cells were transfected with control siRNA, siRNA targeting PP2A-Bδ (B) or PP2A-B'δ (C), and either PP2A-Bδ transcript abundance (N=6, unpaired t-test) or PP2A-B'δ protein abundance was evaluated (upper panels), or cells were treated with NHIgG or aPL and EGF stimulation of Akt S473 phosphorylation was assessed (lower panels). **(D)** Effect of NHIgG versus aPL on PP2A-C L309 methylation in Sw.71 cells. Mann-Whitney U test was used in B.



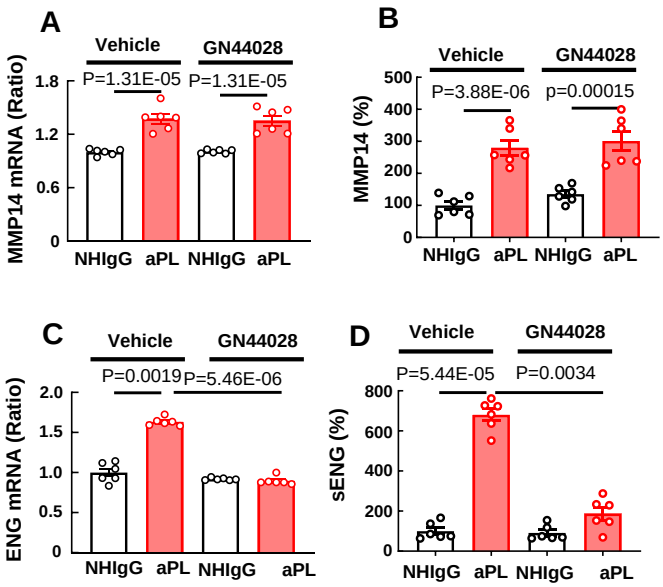
Online Figure IV Dab2 is expressed in cultured human trophoblasts and in mouse and human placenta, and required for aPL inhibition of cell proliferation and migration and aPL activation of PP2A in cultured trophoblasts. **(A)** ApoER2, Dab1, Dab2, JIP4 and GAPDH protein abundance was evaluated in HTR8/SVneo or Sw.71 trophoblasts, in mouse placenta (day 15 of pregnancy) and in human placenta (term). **(B)** HTR8/SVneo trophoblasts were treated with vehicle, NHIgG or aPL, ApoER2 was immunoprecipitated (IP), and ApoER2 and Dab2 were detected. **(C)** Pregnant C57BL/6 mice were injected with NHIgG or aPL on day 8 and 12 of pregnancy, and on day 15 ApoER2 was immunoprecipitated (IP) from the placenta, and ApoER2 and Dab2 were detected. N=4. **(D-G)** HTR8/SVneo trophoblasts were treated with control (Con) siRNA or siRNA targeting Dab2, and Dab2 abundance was evaluated (D), PP2A activation by NHIgG versus aPL was assessed (E, N=6), or effects of NHgG versus aPL on cell proliferation (F, N=8) or cell migration were determined (G, N=5). **(H, I)** Sw.71 trophoblasts were transfected with control siRNA or siRNA targeting ApoER2 or Dab2, and either ApoER2 or Dab2 abundance was evaluated (H), or PP2A activation by NHIgG versus aPL was assessed (I, N=6). Values are Mean±SEM. Aligned rank transform two-way ANOVA with Benjamini and Hochberg correction was performed (E, F, G, I).



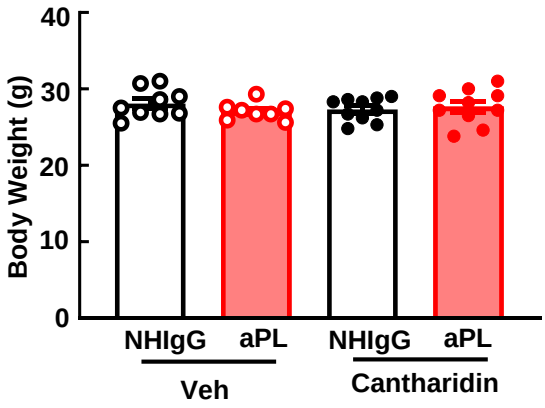
Online Figure V APL promote the association of JIP4 with ApoER2 in Sw.71 trophoblasts, but JNK T183/Y185 phosphorylation is not affected by aPL. **(A)** Sw.71 cells were treated with vehicle, NHIgG or aPL, ApoER2 was immunoprecipitated (IP), and ApoER2, Dab2, JIP4, PP2A-A, and PP2A-C L309 methylation were detected. **(B)** Schematics of ApoER2 constructs for wild-type ApoER2 (WT), mutant ApoER2 with a point mutation in the Dab binding motif (NPVA), and mutant ApoER2 with a C-terminal deletion (Δ59). **(C)** HTR7/SVneo cells were treated with vehicle, NHIgG or aPL, and phosphorylation of JNK T183/Y185 (p-JNK) and total JNK were detected.



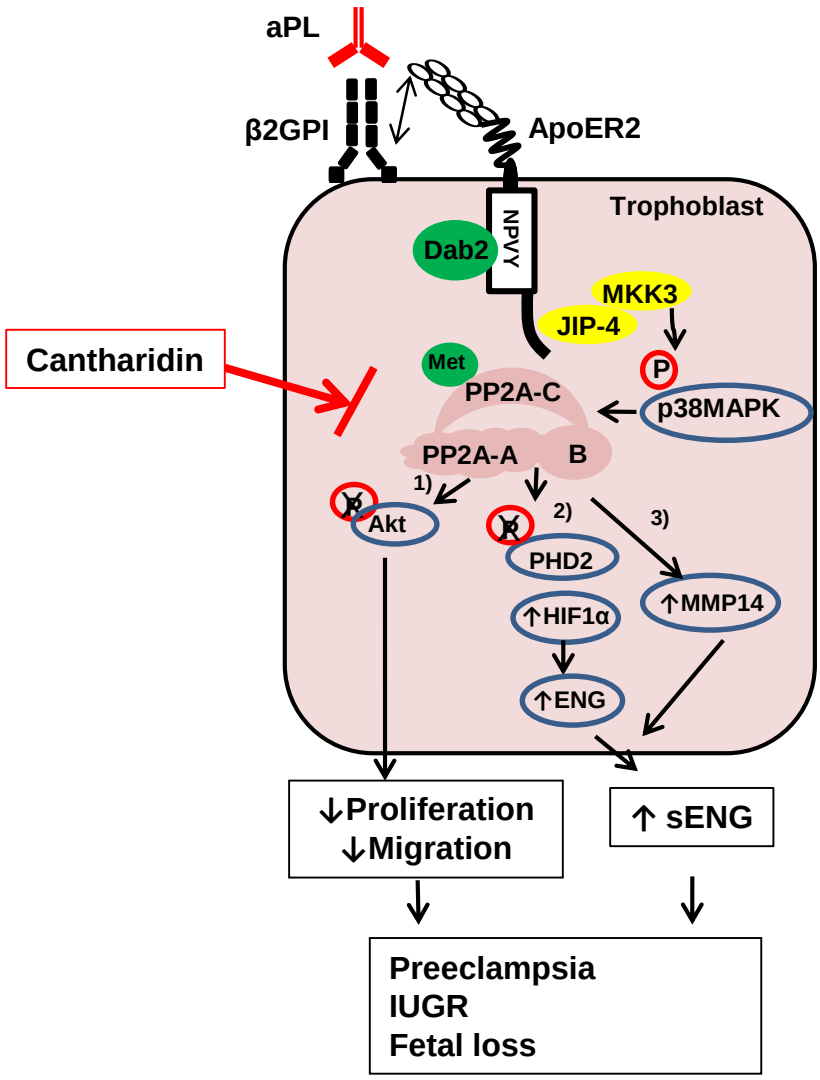
Online Figure VI Administration of aPL has no effect on levels of sFlt-1 in the maternal circulation. ApoER2$^{fl/fl}$ or ApoER2$^{\Delta TR}$ mice were injected with NHIgG or aPL on day 8 and 12 of pregnancy, and plasma sFLT-1 levels were determined on day 15. N=12,11, 8, 8.



Online Figure VII HIF1α inhibition prevents aPL-induced upregulation of ENG in cultured trophoblasts. Following pretreatment with the HIF-1α inhibitor GN44028, HTR8/SVneo cells were treated with NHIgG or aPL, and MMP14 or ENG mRNA abundance was evaluated (**A** and **C**, respectively), or MMP14 or sENG release was assessed (**B** and **D**, respectively). N=6, values are mean±SEM. Aligned rank transform two-way ANOVA with Benjamini and Hochberg correction was performed (A-D).



Online Figure VIII Administration of NHIgG, aPL or PP2A inhibitor Cantharidin does not affect maternal body weight. Pregnant C57BL/6 mice were injected with NHIgG or aPL on day 8 and 12 of pregnancy, and they received either vehicle (Veh) or Cantharidin by IP injection on day 8, 10, 12, and 14, and maternal body weight was measured on day 15. N= 9,8,10,10.



Online Figure IX Molecular basis of aPL-induced trophoblast dysfunction and preeclampsia. In response to aPL recognition of cell surface β2 glycoprotein (β2GPI) and resulting β2GPI interaction with ApoER2, an ApoER2-Dab2-JIP4 complex forms in trophoblasts to assemble and activate the heterotrimeric protein phosphatase PP2A. Dab2 recruitment to the ApoER2 NPXY motif enables activating L309 methylation of PP2A-C, and concurrently JIP4 recruitment to ApoER2 mediates interaction and activation of p38MAPK through MKK3. Resulting PP2A activation causes 1) Akt dephosphorylation and inactivation, which leads to decreased proliferation and migration of trophoblasts, 2) PHD2 dephosphorylation which increases HIF-1α leading to increases in sEng and 3) MMP14 upregulation, which also promotes an increase in sEng. In combination these processes lead to the development of preeclampsia and the fetal complications of IUGR and fetal loss.

Genotype	Treatment	Total number of pregnancies	Resorption (%)	Number of pregnancies without resorptions	Total number of fetuses	Fetal weight (g)
ApoER2$^{fl/fl}$	**NHIgG**	**28**	**7.246±1.616**	**14/28 (50%)**	**178**	**0.411±0.002**
ApoER2$^{fl/fl}$	**aPL**	**30**	**15.65±2.404****	**8/30 (26.6%)**	**184**	**0.395±0.003******
ApoER2$^{\Delta TR}$	**NHIgG**	**17**	**3.411±1.369**	**12/17 (70.5%)**	**97**	**0.404±0.004**
ApoER2$^{\Delta TR}$	**aPL**	**26**	**4.722±1.514*****	**18/26 (69.2%)**	**189**	**0.404±0.003**

Online Table I Summary of pregnancy outcomes in ApoER2$^{fl/fl}$ and ApoER2$^{\Delta TR}$ mice injected with NHIgG or aPL. Values are the mean ± SEM, **p=0.0098 vs. ApoER2$^{fl/fl}$ NHIgG, ***p= 5.1E-04 vs. ApoER2$^{fl/fl}$ aPL, and ****p=5.2E-05 vs ApoER2$^{fl/fl}$ NHIgG. Aligned rank transform two-way ANOVA with BH correction.

Gene ID	Gene Name	Function	Fold change with aPL
Q14114	LRP8 (ApoER2)	IP bait	-
P02749	β2GPI (APOH)	aPL antigen	aPL only
P62714	PP2AB (PP2A catalytic subunit C)	PP2A	5.6
P30153	2AAA (PP2A scaffolding subunit Aα)	PP2A	4.1
O60271	JIP4 (JNK-interacting protein 4)	Adaptor	aPL only

Online Table II Proteins detected in ApoER2 immunoprecipitates from HTR8/SVneo trophoblasts treated with NHIgG or aPL. The proteins that are undetectable in ApoER2-immunoprecipitates from NHIgG- treated cells are indicated as “aPL only”.

Gene ID	Gene Name	Function	Fold change with aPL
P67775	PPP2CA (catalytic C subunit)	IP bait	-
P30153	PPP2R1A (Scaffolding A subunit α)	PP2A	14.25
P30154	PPP2R1B (Scaffolding A subunit β)	PP2A	7.98
Q66LE6	PPP2R2D (regulatory B 'subunit δ (2D))	PP2A	14.58
Q14738	PPP2R5D (regulatory B subunit δ (5D))	PP2A	33.69

Online Table III PP2A subunits detected in PP2A-C immunoprecipitates from HTR8/Svneo trophoblasts treated with NHIgG or aPL.