SUPPLEMENTAL MATERIAL

Elevated placental adenosine signaling contributes to the pathogenesis of preeclampsia

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Supplemental Methods

Generation and characterization of fetal liver rescued ADA-deficient mice. *Construction of transgene.* The use of an ADA minigene under control of α-fetoprotein gene regulatory elements was attempted based on the assumption that expression of ADA in the fetal liver would also protect ADA-deficient fetuses from perinatal lethality. For this purpose the 3.2-kb mouse alpha-fetoprotein promoter (AFP), containing enhancer I, which confers tissue- and developmental stage-specific expression of alpha-fetoprotein, was excised from pAFP-CAT [1] by AccI/PstI digestion. This restriction fragment was ligated to SmaI and PstI sites of pBluescript II KS. The 1.5-kb human wild-type ADA cDNA was removed from pADA211 [2] by EcoRI digestion, and ligated downstream from the AFP promoter at the EcoRI site of pBluescript II KS (**Supplemental Figure 1A**).

Generation of fetal liver rescued ADA-*deficient mice by a two stage genetic approach.* First stage, the recombinant plasmid was purified by equilibrium centrifugation on a cesium chloride density gradient. The SacI/ApaI fragment containing the transgene was fractionated on an agarose gel, electroeluted and purified using a Qiagen (Valencia, CA) mini-plasmid kit. The DNA fragment was injected into (C57BL/6J x C3H/HeJ) F1 eggs for transgenic mouse production using standard procedures. Ten founder mice, designated as *fLi-Tg*$^{+}$, were derived. The mouse showing highest fetal liver expression of human ADA was selected. At the second stage, the selected *fLi-Tg*$^{+}$ mice were mated with *Ada*$^{+/-}$ mice, which are heterozygous for the null *Ada* allele and transgene-positive heterozygous mice were backcrossed to the *Ada*$^{+/-}$ mice or intercrossed to derive *Ada*$^{-/-}$*/fLi-Tg*$^{+}$ offspring (**Supplemental Figure 1B**). Under control of α-fetoprotein gene regulatory elements, the hepatic production of ADA subsides during the first week following birth and the resulting ADA-deficient mice die by three weeks of age if not

treated by adenosine deaminase enzyme replacement therapy as described below.

Polymerase chain reaction for genotyping. DNA was extracted from tail biopsies by the proteinase K/phenol/chloroform method. Primers for polymerase chain reaction (PCR) for detection of the transgene were 5'GAGCGGCATTCACCGTACTG and 5'TGACTGCATGACTCCGTGTCC, and for positive identification of the null and wild-type *Ada* alleles were 5'ACTAGTGAGACGTGCTACTT and 5'AGATCCACAACGTCATCAGG, and 5'AAGTGCGCTATAGCCCACAC and 5'AGATCCACAACGTCATCAGG, respectively. PCR reactions were carried out using the GeneAmp kit from PE BioSystems (Foster City, CA), according to the manufacturer's instructions. PCR products were analyzed on a 2% NuSieve 3:1 gel (Schleicher and Schuell, Keene, NH).

Western blot analysis for ADA proteins and zymogram for ADA activities. The presence or absence of ADA in the fetal organs was readily determined by immunoblotting to detect ADA protein or zymogram analysis to detect ADA enzymatic activity. As shown in Supplemental Figure. 1C-D, ADA protein (**Supplemental Figure 1C**) and enzymatic activity **(Supplemental Figure 1D**) were only detected in fetal livers and not in any other fetal organs tested, including brain, kidney and skin (genotype $Ada^{-/-}/fLi\text{-}Tg^{+}$). Significantly, production of ADA exclusively in fetal liver was sufficient to rescue the fetuses from perinatal lethality and allowed viable fetuses to be delivered at term. Thus, we conclude that the AFP-driven human ADA transgene was able to provide sufficient ADA activity in fetal liver to prevent the perinatal lethal phenotype of the ADA-deficient mice. More importantly, fetal liver rescued ADA-deficient mice provided us a genetic investigative tool to produce pregnancies with ADA-deficient placentas allowing us to assess the impact of elevated placental adenosine throughout pregnancy *in vivo*.

Placental rescued ADA-deficient mice. ADA-deficient mice equipped with an *Ada* minigene that is expressed exclusively in the trophoblast cell lineage were generated and genotyped as previously described [3, 4]. Mice homozygous for the null *Ada* allele were designated as $Ada^{-/-}$/*Pl-Tg*$^{+}$ mice. The rationale for placenta specific ADA expression to rescue ADA-deficient mice from prenatal lethality is based on the fact that the highest levels of ADA during prenatal development are found in the trophoblast cells of the placenta [4]. The perinatal lethality and associated liver impairment of the global *Ada* knockout is prevented by genetically restoring ADA production to the trophoblast cell lineage of the placenta [3]. Following birth, and removal of the placenta, the resulting mice are completely ADA deficient and die within three weeks of age from severe pulmonary impairment due to excessive accumulation of adenosine. However, it is possible to keep the mice alive indefinitely by the use of ADA enzyme replacement therapy that prevents the cytotoxic accumulation of adenosine [5] (for details see in following section).

Maintenance of ADA-deficient mice by ADA enzyme replacement therapy. To prevent postnatal lethality ADA-deficient mice (both placental rescued and fetal liver rescued $Ada^{-/-}$ mice) were provided ADA enzyme replacement therapy in the form of polyethyleneglycol-modified ADA (PEG-ADA) based on a previous report [5]. $Ada^{-/-}$ mice were identified at birth by screening for ADA enzymatic activity in the blood by zymogram analysis and were maintained on i.m. injections of PEG-ADA on postnatal days 1, 5, 9, 13, and 17 (1.25, 2, 2.5, 3.75, and 5 U, respectively) as previously described [6]. Beginning with postnatal day 21 $Ada^{-/-}$ mice were treated with 7.5 U PEG-ADA weekly by intraperitoneal injection.

Generation of ADA and ADORA2B double-deficient mice. $Adora2b^{-/-}$ mice were generated and genotyped as previously described [7]. And then, mice deficient in ADORA2B on the background of fetal liver rescued ADA-deficient mice were generated by mating $Adora2b^{-/-}$ mice

with *Ada*$^{-/-}$*/fLi-Tg*$^{+}$ mice.

Mating strategy of fetal liver rescued ADA-deficient mice, ADA and ADORA2B double-deficient mice, and placental rescued ADA-deficient mice. To generate pregnant mice with elevated placental adenosine, we crossed 8-10 week-old *Ada*$^{+/-}$*/fLi-Tg*$^{+}$ females with *Ada*$^{-/-}$*/fLi-Tg*$^{+}$ males. By this mating strategy, we generated pregnant mice in which half of the placentas were ADA-deficient and half were ADA-positive. All fetuses expressed the ADA transgene in the fetal liver. In control crosses of *Ada*$^{+/-}$*/fLi-Tg*$^{+}$ females with *Ada*$^{+/+}$ males, all placentas were ADA-positive. Similarly, *Ada*$^{+/-}$*/fLi-Tg*$^{+}$*/Adora2b*$^{-/-}$ female mice and *Ada*$^{+/-}$*/PL-Tg*$^{+}$ female mice were mated with *Ada*$^{-/-}$*/fLi-Tg*$^{+}$*/Adora2b*$^{-/-}$ male mice and *Ada*$^{-/-}$*/PL-Tg*$^{+}$ male mice, respectively. The day when the copulation plug was detected was designated as E0.5. DNA was extracted from tail biopsies of fetuses by the proteinase K/phenol/chloroform method and PCR was conducted with primers for the genotyping of each mice. The mice were housed in the animal care facility of the University of Texas, Houston and had access to food and water ad libitum. All the protocols involving animal studies were reviewed and approved by the Institutional Animal Welfare Committee of the University of Texas Houston Health Science Center.

Introduction of human autoantibody (AT1-AA) into pregnant mice

PE mouse models induced by AT1-AA were conducted as previously described[8]. Briefly, purified IgGs were isolated from PE patients or NT pregnant women sera (PE-IgG, NT-IgG respectively). And then, 8 to 10 week-old timed-pregnant wild type dams mated with wild type males or CD73-deficient (*Cd73*$^{-/-}$) dams mated with *Cd73*$^{-/-}$ males which were generated and genotyped as previously reported [9] or *Adora2b*$^{-/-}$ dams mated with *Adora2b*$^{-/-}$ males were treated

with NT-IgG or PE-IgG (0.8mg) on E13.5 and E14.5 by retro-orbital sinus injection. All mice were sacrificed on E18.5 prior to delivery, and their blood and organs were collected.

The measurement of blood pressure and proteinuria in mice

The systolic blood pressure of all mice was measured at the same time daily by a carotid catheter-calibrated tail-cuff system (CODA, Kent Scientific), and the mice were kept warm using a warming pad (AD Instruments Co). Mice were trained several times for at least one week prior to the actual measurement in the non-pregnant state. The intracarotid mean arterial blood pressure (MAP) was also measured on E18.5 as previously descried [10]. MAP was monitored from the right carotid artery with a mouse jugular catheter connected to a pressure transducer and an amplifier unit. The amplifier was linked to a data acquisition module and MAP was recorded on a personal computer by Chart 5 Software (AD Instruments Inc). Blood pressure was recorded and averaged over a 10-min period. For the measurement of proteinuria, urine was collected for analysis using metabolic cages (Nalgene). Total microalbumin and creatinine in the urine were determined by using ELISA kit (Exocell) and the ratio of urinary albumin to creatinine was calculated as an index of proteinuria as previously described [8, 10].

Measurement of adenosine levels

Samples were rapidly removed and collected in liquid nitrogen. Adenosine was extracted from frozen placenta tissues or plasma using 0.6 N perchloric acid, separated and quantified using reversed phase HPLC as described previously [10, 11].

Zymogram analysis of tissue ADA activity

ADA activity detection in mouse tissue samples were performed by zymogram analysis as

previously described [3, 12] with several modifications. Briefly, tissue samples were lysed in lysis buffer containing 20 mM Tris, 1mM EDTA, 0.03% 2-mercaptoethanol and adjusted the protein concentration. 2 µl lysate was loaded to the G493 thin agarose gel films (Authentifilm thin agarose gel films, Innovative Chemistry, Marshfield, Massachusetts, USA) and subjected to electrophoresis at 70 V for 30 min in the running buffer (0.5 M Tris, 16 mM EDTA, 0.65 M boric acid, pH8) in a temperature-controlled electrophoresis chamber (Innovative Chemistry, Marshfield, Massachusetts, USA). Then, gel films were overlaid with a 10 ml solution containing adenosine (42.5 mg, Sigma), sodium arsenate (38 mg, Sigma), nitro blue tetrazolium (13 mg), Tris-HC1 (pH 8, 0.68 ml of 1 M stock), purine nucleoside phosphorylase (2.4 units, CalBiochem), xanthine oxidase (0.2 unit, Sigma), phenazine methosulfate (0.8 mg, Sigma), and melted agar (9.2 ml of a 1% solution, Sigma) in water. The gels were allowed 30 min at 37ºC to develop intense purple bands signaling the presence of ADA activity.

Spectrophotometric assay for the measurement of placental ADA activity

Small pieces of mouse and human placentas were homogenized in ice-cold PBS with proteinase inhibitor cocktails (Roche Diagnostics) by sonication. Then, ADA activity was measured in the supernatant obtained from the high-speed centrifugation under saturating substrate conditions using a spectrophotometric assay as previously described [3]. Briefly, approximate 990 µl assay buffer (12.5ml 0.1M K_2HPO_4, 5ml 0.1M KH_2PO_4, added distilled water to 50 ml) was added to 3 µl supernatant (30µg protein) and 10 µl 10mM adenosine, and was mixed immediately. The decrease in absorbance at 265 nm resulting from deamination of adenosine to inosine was continuously monitored in a Beckman DU-50 spectrophotometer and the rate of inosine production was calculated at linearity. Specific activities are presented as nanomoles of adenosine converted to inosine per min per mg of protein.

Real-time RT-PCR analysis

RNA isolation and real-time RT-PCR were conducted as previously described [13]. Syber green was used for the analysis of all genes measured using the following primers: Mouse *Adora1*: forward; 5'-TGTGCCCGGAAATGTACTGG-3' and reverse; 5'-TCTGTGGCCCAATGTTGATAAG-3', Mouse *Adora2a*: forward; 5'-GCCATCCCATTCGCCATCA-3' and reverse; 5'-GCAATAGCCAAGAGGCTGAAGA-3', Mouse *Adora2b*: forward; 5'-GCGAGAGGGATCATTGCTG-3' and reverse; 5'-CAGGAACGGAGTCAATCCAA-3', Mouse *Adora3*: forward; 5'-ATACCAGATGTCGCAATGTGC-3' and reverse; 5'-GCAGGCGTAGACAATAGGGTT-3', Mouse *Cd73*: forward; 5'-CAGATCCGCAAGGAAGAACC-3' and reverse; 5'-ATGGTGCCCTGGTACTGGTC-3', Mouse *Cd39*: forward; 5'-GCAAGCAGAGACAGCAAAAAC-3' and reverse; 5'-GCAAAATCTCTTCACCTTAGAATCC-3', Mouse *Ent-1*: forward; 5'-CAGCCTCAGGACAGGTATAAGG-3' and reverse; 5'-GTTTGTGAAATACTTGGTTGCGG-3', Mouse *Ent-2*: forward; 5'-TCATTACCGCCATCCCGTACT-3' and reverse; 5'-CCCAGTTGTTGAAGTTGAAAGTG-3', Mouse *Ada*: forward; 5'-ACCCGCATTCAACAAACCCA-3' and reverse; 5'-AGGGCGATGCCTCTCTTCT-3', Mouse *Flt-1*: forward; 5'-CCACCTCTCTATCCGCTGG-3' and reverse; 5'-ACCAATGTGCTAACCGTCTTATT-3', Mouse *Gapdh*: forward; 5'-TGACCTCAACTACATGGTCTACA-3' and reverse; 5'-CTTCCCATTCTCGGCCTTG-3', Human *ADORA2B*: forward; 5'-TGCACTGACTTCTACGGCTG-3' and reverse; 5'-GGTCCCCGTGACCAAACTT-3', Human *CD73*: forward; 5'-ACCACGTATCCATGTGCATTT-3' and reverse; 5'-AAAGGGCAATACAGCAGCCAG-3',

Human *CD39*: forward; 5'- AGCAGCTGAAATATGCTGGC-3' and reverse; 5'-GAGACAGTATCTGCCGAAGTCC-3', Human *GAPDH*: forward; 5'-TGCACCACCAACTGCTTAGC-3' and reverse; 5'-ACAGTCTTCTGGGTCGCAGTG-3'.

Measurement of circulating sFlt-1 levels

sFlt-1 levels in mouse plasma and supernatants from human villous explants were quantitatively determined by using ELISA kits which are commercially available (Qantikine ELISA) (R&D Systems) as previously described [13].

Electron Microscopy of glomeruli

Cortical tissue from the mouse kidneys was harvested for electron microscopy in order to assess glomerular ultrastructure. The tissue was fixed in glutaraldehyde and processed for plastic block embedding. Semi-thin sections were performed and stained with toluidine blue fuchsin in order to assess adequacy of the cortical renal tissue for electron microscopic examination. Thin sections were then performed and stained with lead citrate and uranyl acetate and transmission electron microscopy was performed to assess glomerular ultrastructure. Digital images were taken at standard magnifications (1000x and 4000x) and qualitative comparisons were made among the experimental groups by an American Board of Pathology certified anatomic pathologist.

Assessment of histopathologic changes in kidneys by hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) staining

Kidneys were dissected from the mice on E18.5, fixed in 4% formaldehyde and embedded in paraffin. Tissue blocks were cut into 4 μm and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) by standard procedures. The extent of renal damage was assessed by

quantifying the glomeruli that showed characteristic features of damage in PE such as decreased Bowman's space and occlusion of capillary loop spaces. To examine those features, the glomeruli were counted in 5 fields of randomized and blinded slides (10x magnification), with each field having at least 10 glomeruli. A highest score of 5 was accorded to glomeruli with a normal amount of capillary space within Bowman's capsule. A score of 1 was assigned to the glomeruli that showed complete loss of capillary space and an intermediate score of 3 was assigned to the glomeruli that displayed reduced, but not completely obliterated, capillary space.

Immunohistochemistry

Formalin fixed tissue blocks were cut into 4-µm thick sections and subjected to immunohistochemistry. Briefly, endogenous peroxidase activity was quenched by 10 min of incubation in a 3% hydrogen peroxide/methanol buffer. Antigen retrieval was conducted by incubating slides in sodium citrate buffer (pH 6.0) at 89°C for 15 min. After blocking with the normal goat serum, the slides were then incubated with antibody against CD31 (1:200, ab124432, abcam) or human CD73 (1:30, 2B6, Lifespan Biosciences) or mouse CD73 (1:50, H-300, Santa Cruz) in a humidified chamber at 4°C overnight. After the primary antibody incubation, ABC staining system kit (VEACTASTAIN ABC-AP, VECTOR LAB) was used according to the protocol. Antigen-antibody reactions were visualized with alkaline phosphatase substrate kit (VECTOR Red Substrate Kit, VECTORLAB) and then nuclei were counterstained with Mayer's hematoxylin.

Immunoblotting

Tissues were homogenized and lysed with RIPA lysis buffer (Santa Cruz) in the presence of proteinase inhibitor cocktail (Roche Diagonistics). Lysates were resolved on SDS–PAGE and

electroblotted onto polyvinylidene difluoride membranes. After blocking with Odyssey Blocking Buffer (LI-COR), the membranes were probed with an antibody against ADA which was purified from sheep anti-ADA antiserum [12] or human ADORA2B (1:1000, ab40002, abcam) and then probed with secondary antibodies labeled with IRDye fluorophores (LI-COR). The antibody/antigen complexes were scanned and detected using the ODYSSEY infrared imaging system and software (LI-COR).

Human Placental Villous Explant Culture

Human placentas were obtained from normotensive patients who delivered at term at Memorial Hermann Hospital in Houston. The explant culture system was conducted as described previously [13]. On delivery, the placentas were placed on ice and submerged in phenol red–free DMEM containing 10% BSA and antibiotics. Villous explant fragments weighing 50 mg were dissected from the placenta and transferred to 12-well plates at 37°C under 5% CO_2. The explants were incubated for 24 hours and then pretreated with or without ADORA2B antagonist (10 nM MRS1754) (Tocris Biosciences) for 30 min and then treated with a non-metabolized adenosine analogue (1μM NECA) (Sigma-Aldrich) or an ADORA2B agonist (1 μM BAY60-6583) (Tocris Biosciences) for 24 hours, followed by the collection of medium and cells.

Table.S1 Patient characteristics

	NT (n=13)	PE (n=13)
Age - yr	27.2±1.7	27.6±2.2
Primigravida - no. (%)	5 (38.4%)	6 (46.2%)
Race - no. (%) Caucasian	4 (30.8%)	4 (30.8%)
African American	5 (38.5%)	6 (46.2%)
Hispanic	1 (7.7%)	2 (15.4%)
Other or unknown	3 (23.1%)	1 (7.7%)
Body-mass index	30.5±1.3	35.8±2.0*
Systolic blood pressure - mmHg	117.8±1.9	171.5±4.6**
Diastolic blood pressure - mmHg	72.4±1.2	107.9±3.8**
Proteinuria - mg/24h	N/A	1784±420.9
Gestational age at delivery - week	37.8±1.2	36.1±2.7
Infant's birth weight - g	3157±111.7	2277±230.5**
Small-for gestational age infant - no. (%)	0 (0%)	4 (30.8%)

Data presented as mean ± SEM were statistically analyzed by the Mann-Whitney's *U* test.

Data shown as number of cases and percentage were statistically analyzed by the Fisher's exact test.

(*P<0.05, **P<0.01 vs NT)

Table.S2 Summary for the weights of fetuses and placentas

Mating pair and Placental adenosine	*Ada*$^{+/-}$/*fLi-Tg*$^{+}$ ♀ X *Ada*$^{+/+}$ ♂ (Control dam with normal placental adenosine)		*Ada*$^{+/-}$/*fLi-Tg*$^{+}$ ♀ X *Ada*$^{-/-}$/*fLi-Tg*$^{+}$ ♂ (Dam with elevated placental adenosine)		*Ada*$^{+/-}$/*fLi-Tg*$^{+}$/*Adora2b*$^{-/-}$ ♀ X *Ada*$^{-/-}$/*fLi-Tg*$^{+}$/*Adora2b*$^{-/-}$ ♂ (*Adora2b*$^{-/-}$ dam with elevated placental adenosine)		*Ada*$^{+/-}$/*PL-Tg*$^{+}$ ♀ X *Ada*$^{-/-}$/*PL-Tg*$^{+}$ ♂ (dam with normal adenosine by placental rescue)	
No. of moms	5		7		3		5	
Average No. of fetuses / mom	8.0±0.71		8.3±0.47		8.0±1.1		7.4±0.93	
Embryonic genotype	*Ada*$^{+/-}$	*Ada*$^{+/+}$	*Ada*$^{-/-}$	*Ada*$^{+/-}$	*Ada*$^{-/-}$/ *Adora2b*$^{-/-}$	*Ada*$^{+/-}$/ *Adora2b*$^{-/-}$	*Ada*$^{-/-}$	*Ada*$^{+/-}$
No. of fetuses	21 (52.5%)	19 (47.5%)	27 (46.5%)	31 (53.4%)	12 (50.0%)	12 (50.0%)	18 (48.6%)	19 (51.3%)
Fetal weight (g)	0.95±0.02	0.96±0.02	0.72± 0.02**	0.89±0.05	1.01±0.02	0.99±0.03	1.03±0.03	1.08±0.04
Placental weight (g)	0.095± 0.003	0.094± 0.003	0.076± 0.002**	0.093± 0.004	0.098±0.003	0.099±0.004	0.097±0.004	0.103±0.004

Data of weights in fetuses and placentas are expressed as mean ± SEM.
(**P<0.01 vs other groups)

Supplemental References

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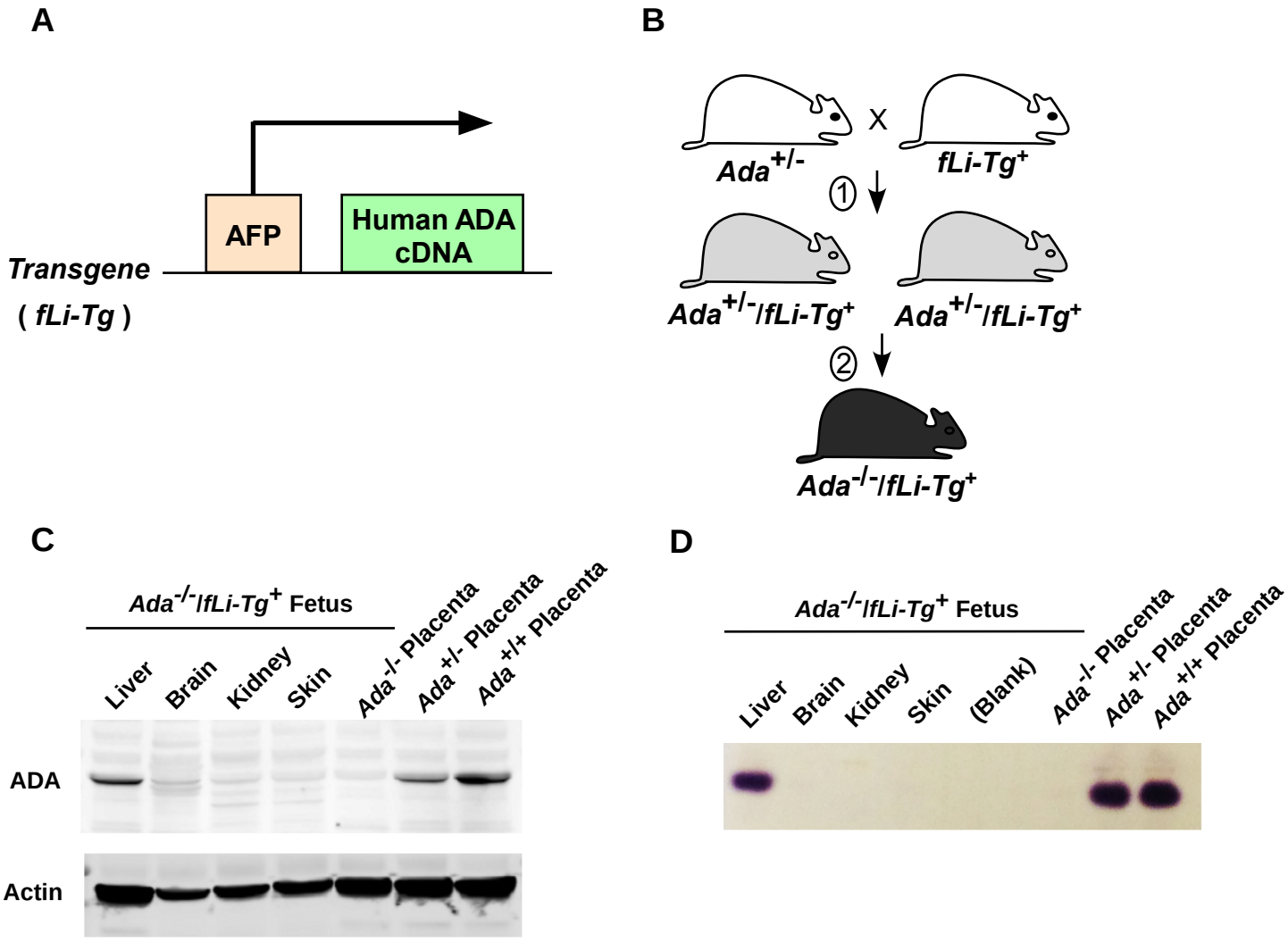
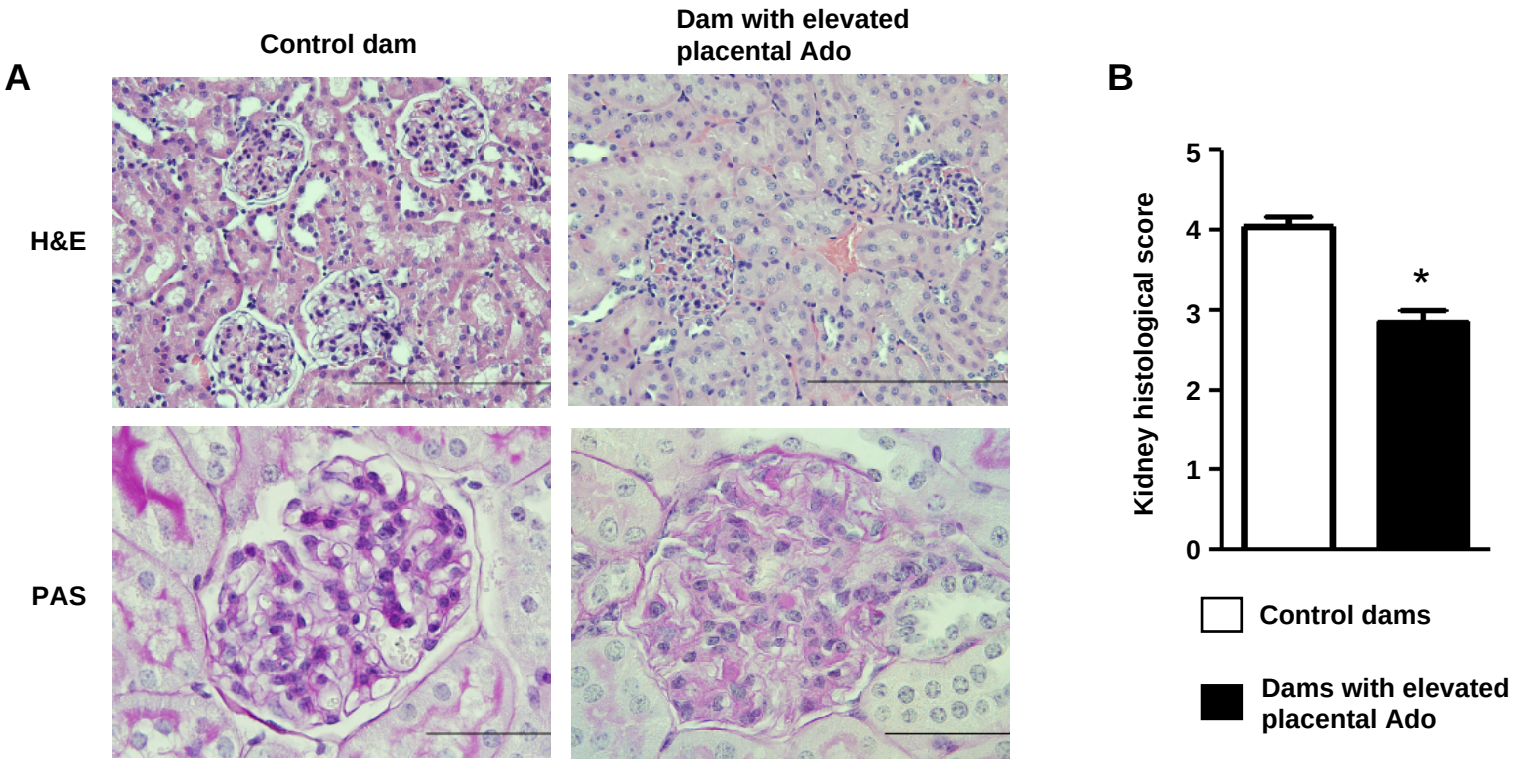
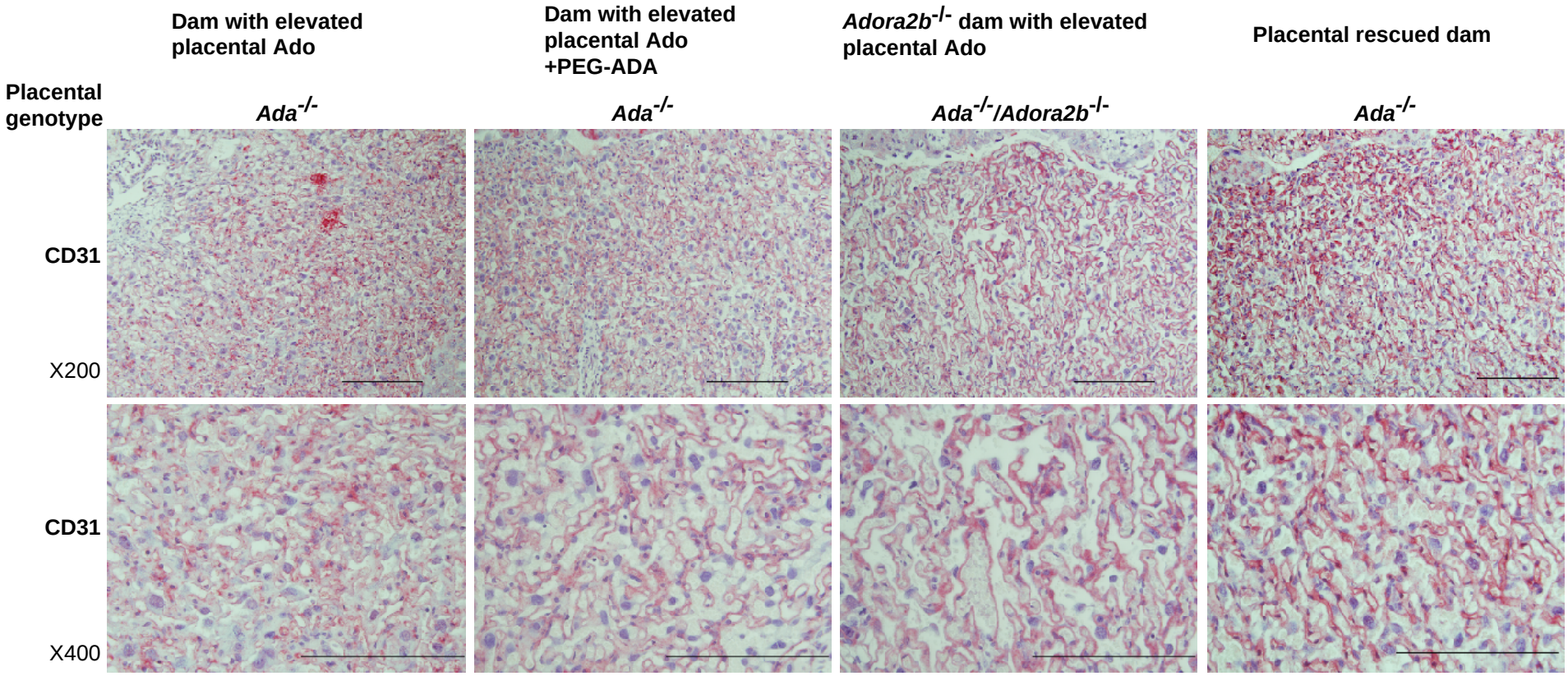


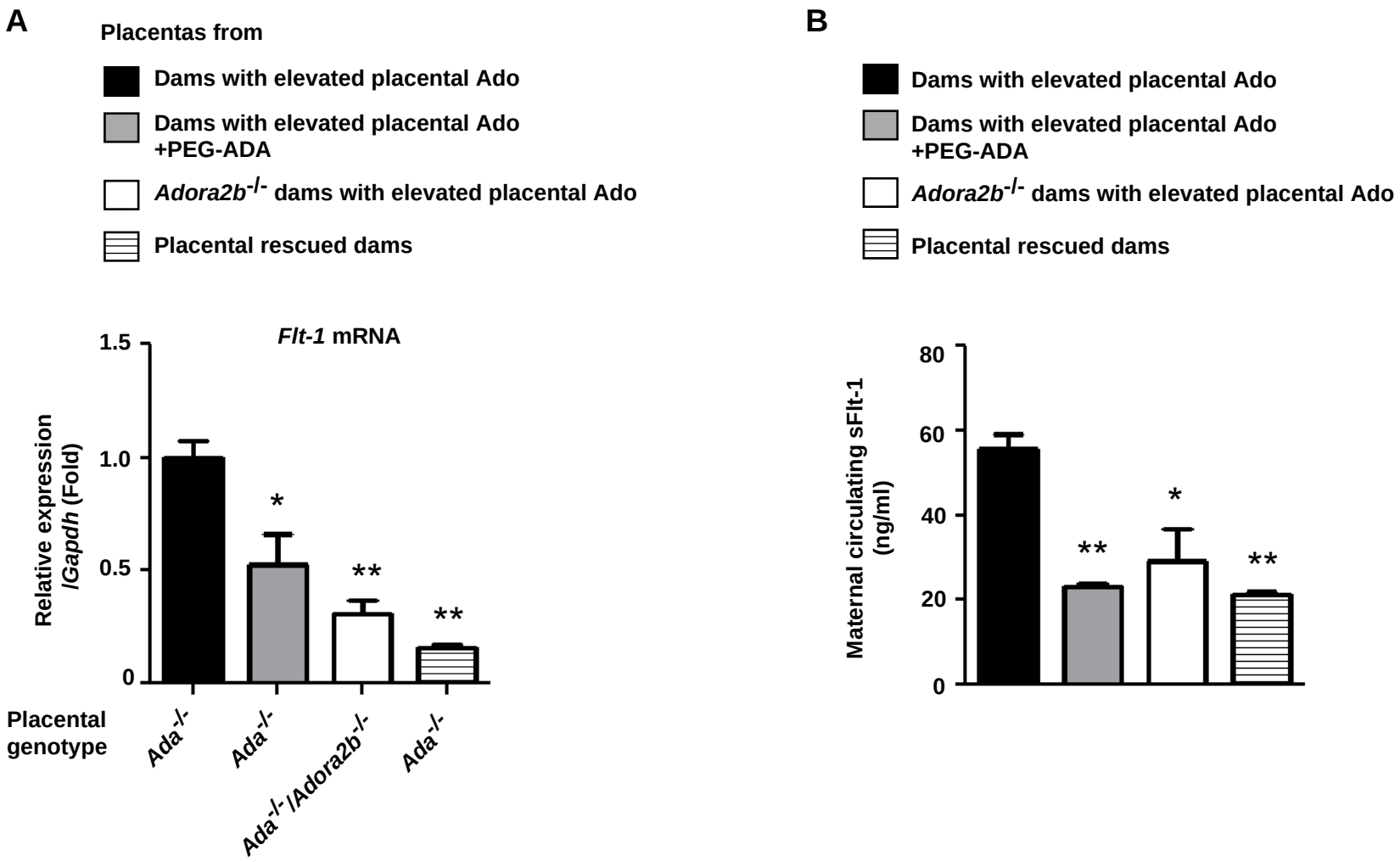
Supplemental Figure 1 Generation of ADA-deficient mice harboring the transgene to produce ADA only in the liver during the fetal period (*Ada*$^{-/-}$/*fLi-Tg*$^{+}$) (**A**) Schema of transgene harboring the human ADA cDNA and alpha-fetoprotein (AFP) promoter and enhancer elements to induce the transcription of ADA minigene exclusively in the fetal liver (*fLi-Tg*). (**B**) Schematic drawing of generation of fetal liver rescued ADA-deficient mice (*Ada*$^{-/-}$/*fLi-Tg*$^{+}$) by two stage genetic approaches. 1) generation of transgenic mice containing a transgene expressing human ADA cDNA only in fetal liver by alpha fetal protein (AFP) promoter (*fLi-Tg*$^{+}$); 2) generation of fetal liver rescued Ada-deficient mice (*Ada*$^{-/-}$/*fLi-Tg*$^{+}$) by mating *Ada*$^{+/-}$/*fLi-Tg*$^{+}$ to each other. (**C**) Expression levels of ADA protein in the liver, brain, kidney, skin, and placenta from fetal liver rescued ADA-deficient fetus (*Ada*$^{-/-}$/*fLi-Tg*$^{+}$), and the placentas from *Ada*$^{+/+}$, *Ada*$^{+/-}$/*fLi-Tg*$^{+}$ fetuses were detected by immunoblotting (20 μg of protein from each tissue was loaded). (**D**) ADA enzymatic activity in tissues determined by ADA zymogram analysis. ADA zymogram analysis was performed on 8 μg of protein from each tissue.



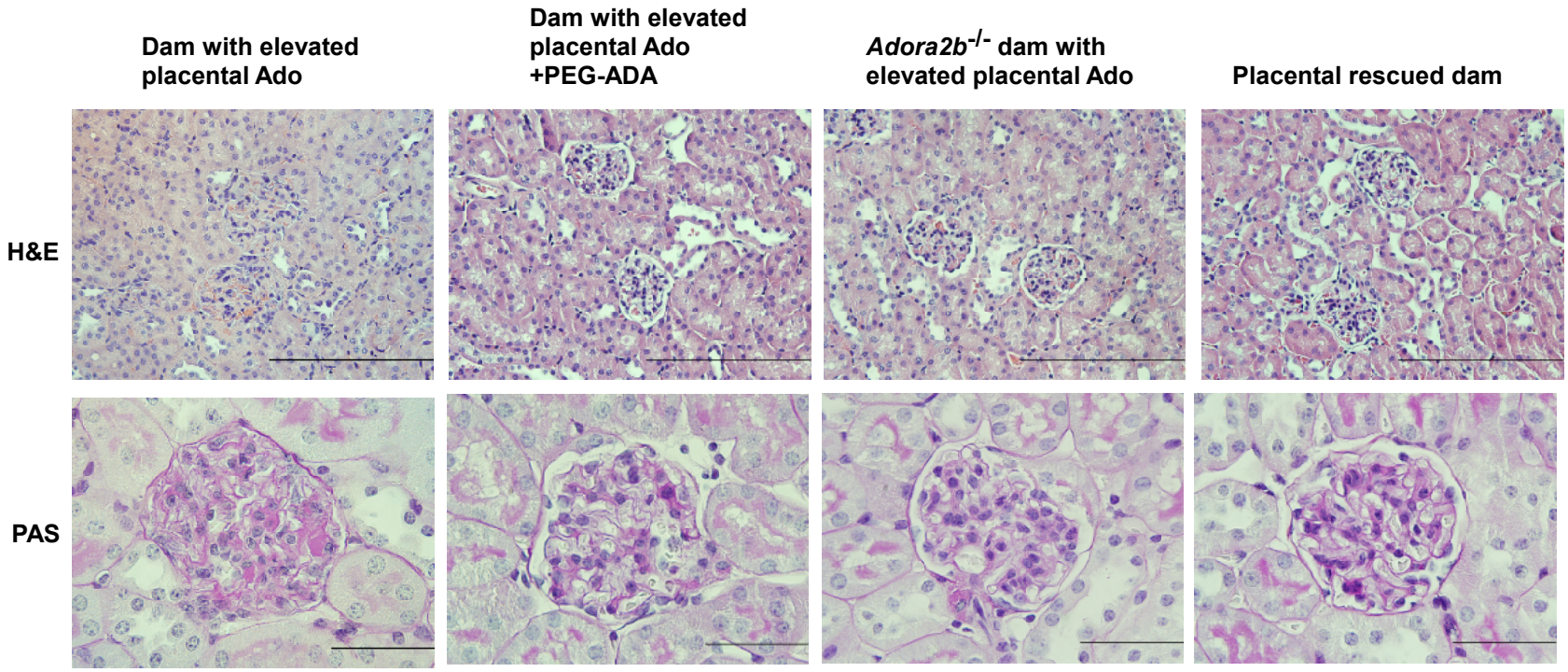
Supplemental Figure 2 Renal histology was assessed by hematoxylin and eosin staining (H&E) and periodic acid-Schiff staining (PAS) (**A**) Pathologic changes in kidneys (swollen glomeruli with narrowed capillary loops and Bowman's spaces) were observed in dams with elevated placental adenosine ($Ada^{+/-}$/$fLi\text{-}Tg^{+}$ females mated with $Ada^{-/-}$/$fLi\text{-}Tg^{+}$ males). Scale bar; 200μm (upper panel), 50μm (lower panel). (**B**) The glomerular damage among each group is quantified. (n=4 each), (*P<0.05 vs control dams).

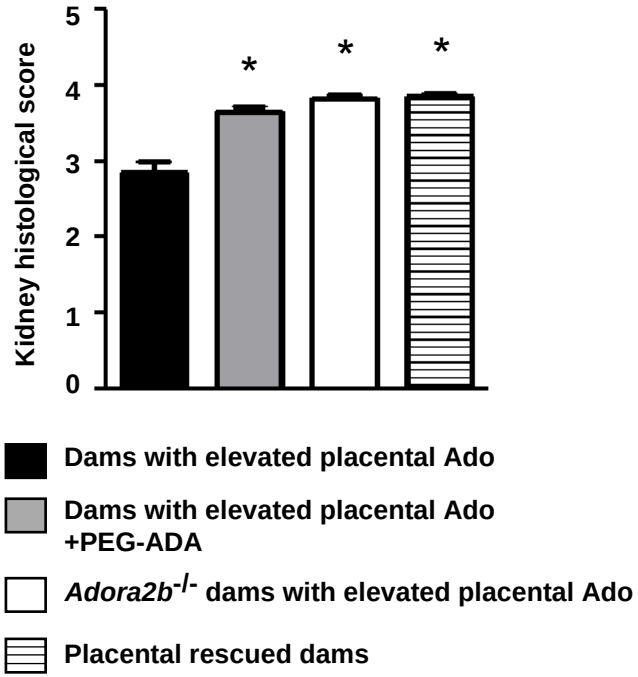


Supplemental Figure 3 Placental vasculature was assessed by CD31 immunostaining In the ADA-deficient placentas from dams with elevated placental adenosine, positive CD31 staining (red) was found to be disorganized and did not localize along vascular structures in the labyrinth zone compared with the ADA-deficient placentas with PEG-ADA treatment, ADA and ADORA2B double-deficient placentas, and placentas from placental rescued ADA-deficient mice. Scale bar; 200 μm.

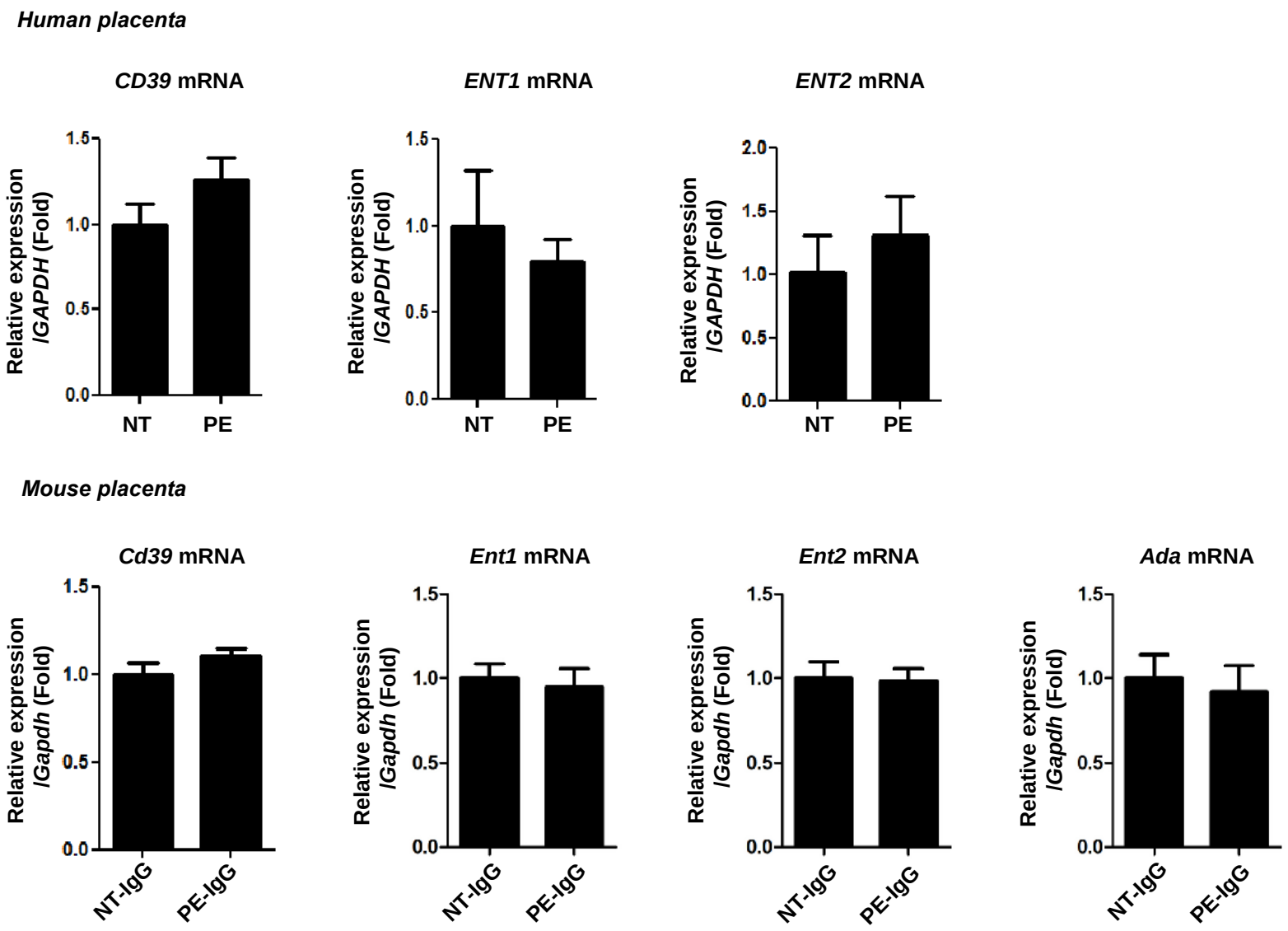


Supplemental Figure 4 Expression levels of *Flt-1* gene in the mouse placentas and maternal circulating levels of sFlt-1 (**A**) *Flt-1* mRNA levels in the mouse placentas of indicated genotypes from dams described above were determined by real-time RT-PCR. (n=5-6 per group), (**P*<0.05, ***P*<0.01 vs $Ada^{-/-}$ placentas from dams with elevated placental adenosine). (**B**) Maternal circulating sFlt-1 levels were determined by ELISA (n=4 per group), (**P*<0.05, ***P*<0.01 vs Dams with elevated placental adenosine).

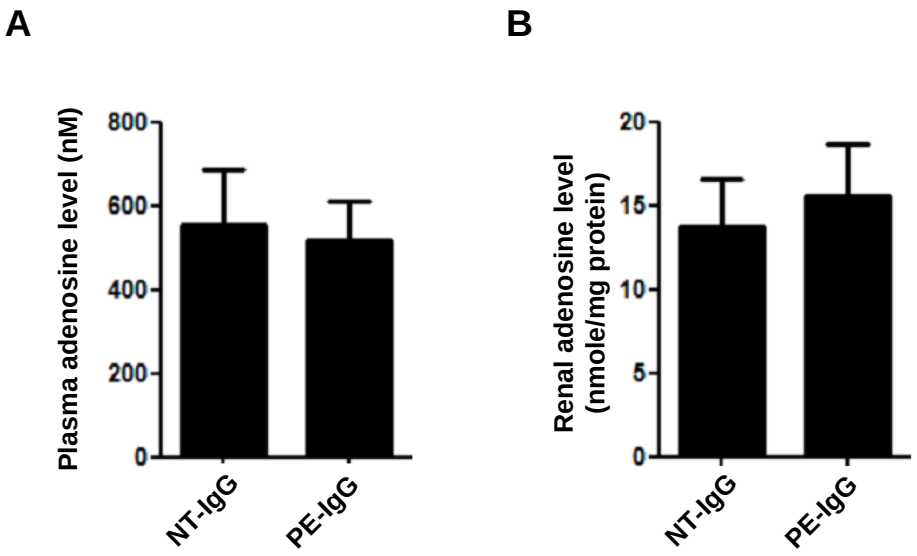




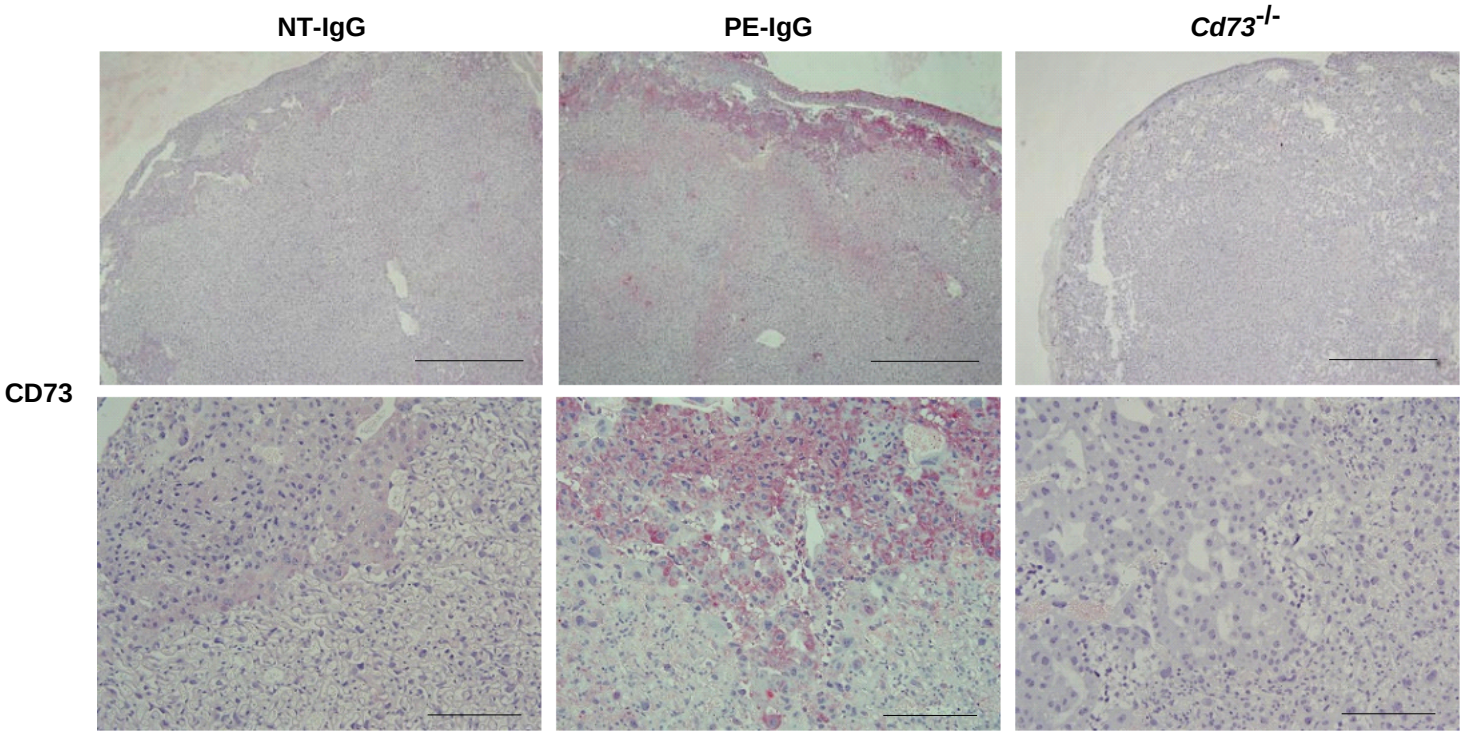
Supplemental Figure 5 Renal histology was assessed by hematoxylin and eosin staining (H&E) and periodic acid-Schiff staining (PAS) Scale bar; 200μm (upper panel), 50μm (lower panel). The glomerular damage among each group was quantified. (n=4 each), (**P*<0.05 vs Dams with elevated placental adenosine).



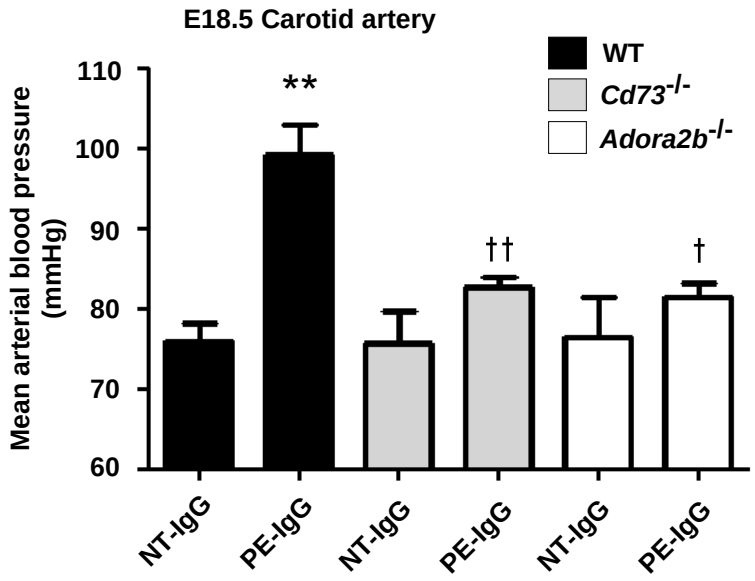
Supplemental Figure 6 Placental expression profiling of purinergic molecules in human and mouse placentas mRNA expression levels of purinergic molecules in human placentas from normotensive pregnant women (NT) or preeclampsia patients (PE) (n=10 per group), or placentas of mice injected with NT- or PE-IgG (NT-IgG:n=6, PE-IgG:n=6) were determined by real-time RT-PCR. mRNA expression level was determined as a relative value to GAPDH, and each value was expressed as fold induction relative to placentas of NT or NT-IgG-treated mice. No significant difference was observed between groups.



Supplemental Figure 7 Adenosine levels in the circulation and the kidneys of pregnant mice treated with PE patient-derived IgG (PE-IgG) or normotensive pregnant women-derived IgG (NT-IgG) (**A** and **B**) Adenosine levels in the plasma (A) or kidneys (B) of pregnant mice treated with NT- or PE-IgG were determined by HPLC on E18.5. (n=4 each), No significant difference was detected.



Supplemental Figure 8 Expression of CD73 protein in mouse placentas detected by immunohistochemistry. PE-IgG treatment induced the elevation of placental CD73 expression. The staining in the placenta from PE-IgG-injected *Cd73*$^{-/-}$ dam was displayed as a negative control. Scale bar, upper panels: 1.0mm, lower panels: 200μm



Supplemental Figure 9 Intra carotid mean arterial blood pressure of WT, *Cd73*$^{-/-}$, and *Adora2b*$^{-/-}$ dams treated with NT- or PE-IgG was measured on E18.5 Increase of intra carotid mean arterial blood pressure (MAP) seen in PE-IgG-treated WT dams was significantly suppressed in *Cd73*$^{-/-}$ or *Adora2b*$^{-/-}$ dams. (n=3-5 per group), (**P*<0.05 vs NT-IgG-treated WT dams, † *P*<0.05, †† *P*<0.01 vs PE-IgG-treated WT dams)