# SUPPLEMENTAL MATERIAL

## Online Methods

*Patients*

Human placenta, decidua and blood sampling was approved by the Regional Committee of Medical Research Ethics in Eastern Norway and the Medical Faculty of Charité Berlin. Placental biopsies (from a centrally located placental cotyledon, avoiding maternal decidua) were obtained following cesarean sections from 50 preeclamptic women (PE) and 28 women with normotensive and uncomplicated pregnancies. Decidual tissue was collected through vacuum suctioning of the placental bed.[1] The PE group was divided into early onset PE (<34 gestational week, n=26) and late onset PE (≥34 gestational week, n=24). The uncomplicated pregnancy group consisted of healthy, normotensive women undergoing cesarean section due to breech presentation or other reasons. Patient characteristics are shown in Online Table I.

*Animals*

Local authorities (LaGeSo, Berlin, Germany) approved all experiments. Primary cultures of macrophages were generated from the bone marrow of male 10-12 week old wild-type (C57Bl/6JOlaHsd) mice; Harlan Laboratories, Rossdorf, Germany) or CD74 knockout (*B6-(Cd74)tm*) mice. Mice were fed normal chow diets (#V1124-300, Ssniff, Soest, Germany) and housed under standardized light-dark cycles and SPF conditions. Mice were observed daily in order to minimize harm. To evaluate the preeclamptic phenotype, wild-type (*C57Bl/6JOlaHsd*) and CD74 knockout (*B6-(Cd74)tm*) mice were bred (plug-recognition day was assigned as day 1) and transferred to metabolic cage on day 16 of pregnancy for 24 h. Blood pressure was monitored by telemetric bloodpressure measurement. All experimental animals were sacrificed on day 18. Fetuses and placentas were counted and weighted. Urinary mouse albumin was measured with a commercially available ELISA (CellTrend, Germany).

Doppler ultrasound studies were performed on day 15/17 as described previously.[2] The animals were anesthetized with 1.5% isoflurane via an oxygen mask. Maternal heart rates and rectal temperatures were monitored (Model THM100, Indus Instruments). Rectal temperature was maintained at 36°C to 38°C. The hair was removed from the abdomen, and prewarmed gel was used as an ultrasound-coupling medium. The pregnant mice were imaged with an ultrasound biomicroscope and a 30-MHz or 40-MHz transducer at 30 frames per second (Model Vevo 660, VisualSonics Inc). The Doppler waveforms were obtained in the proximal uterine artery, the distal uterine artery (distal of the main branch of the uterine artery between 2 embryonic implantation sites) and the embryonic umbilical artery. Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured from 3 consecutive cardiac cycles that were not affected by motion caused by maternal breathing, and the results were averaged. The resistance index (RI=(PSV−EDV)/PSV) was calculated. For biometric measurements, abdominal and head transversal and longitudinal diameters were measured, and the circumference was calculated.

For Two-cell stage embryo transfer, 9-12 weeks old female CD74 knockout (B6-(Cd74)tm) mice or wild-type C57Bl/6JOlaHsd mice were treated (i.p.) by 5 IU Pregnant-Mare-Serum-Gonadotropin (Intergonan 240 I.E./ml, Intervet). After 48 hours, mice were injected (i.p.) with 5 IU human chorionic gonadotropin (Ovogest

1000 I.E./ml, Intervet) and mated by male CD74 knockout (B6-(Cd74)tm) mice or wild-type C57Bl/6JOlaHsd. Two-cell stage embryos were flushed with EmbryoMax M2 Medium (MR-015-D, Merck Milipore) from the oviduct after cervical dislocation of the female donor mice 1.5 days after mating. 8-12 two-cell stage embryos were transferred by embryo transfer Pipette under stereomicroscope into the oviduct of an anesthetized pseudopregnant wild-type recipient (day 0.5 of pseudopregnancy). Mice were sacrificed on day 18 of pregnancy. Fetuses and placentas were counted and weighted.

*Isolation of primary cells from human placenta*

All placentas were processed within 2 h after delivery. Whole placentas were placed on ice in 0.9% (w/v) NaCl buffer, dissected/removed from fetal membranes, basal plate, umbilical cord and fibrotic tissue, and villous tissue was minced and washed several times, to minimize blood contamination. Then, various trypsin (Sigma) and DNase I (Roche) digestion steps were used and supernatants were filtered and collected in New Born Calf Serum (Biochrome). For Hofbauer cell isolation supernatant was discarded and undigested tissue was digested further with collagenase A (Roche) and DNase I. Cell suspensions were loaded on Percoll (GE Healthcare) gradual gradients. Cells were collected (Hofbauer cells: 35-45% gradient; trophoblasts: 40-50% gradient; general cells: 70% gradient) and washed. For the general cell population protocol, cells were directly processed for flow cytometry. For primary trophoblasts, cells were negatively immunopurified using HLA-ABC (DakoCytomation) and magnetic dynabeads (Dynal Biotech), for Hofbauer cell isolation, cells were purified by an adhesion step (culturing on a plastic dish for 30min).

*Macrophage preparation and cell culture*

Macrophages were generated from two different origins: Human peripheral blood mononuclear cells (PBMCs) and mouse bone marrow-derived (BMD). Nomenclature and experimental design of *in vitro* differentiated and activated macrophages follows the guidelines described by Murray et al.[3]

PBMCs derived macrophages: 80 ml of blood were drawn from human healthy donors. The study was approved by the Regional Committee of Medical Research Ethics (Charité). PBMCs were purified by Ficoll gradient centrifugation. To enrich monocyte yield, an adhesion step was performed by seeding the cells ($3.5*10^5$ cells/$cm^2$) in serum-free RPMI1640 medium (Sigma) containing 10mM HEPES (Gibco), 50 µM β-mercaptoethanol (β-ME) (Sigma) and 1% (v/v) penicillin/streptomycin (P/S) (Gibco) for 2 h. Cells were washed and cultivated in basal RPMI containing 20% fetal calf serum (FCS) (v/v) (Biochrom), 10 mM HEPES, 50 µM β-ME, 1% (v/v) P/S media with M- colony stimulating factor (CSF) (100ng/ml) for 6 days with media change every 3 days to reach the M(-)status (also known as M0). For further activation of M(-) into M(IL-4) (also known as M2) cells were cultured in basal medium with IL-4 (20 ng/ml) for 18 h. All cytokines were bought from Active Bioscience (Germany).

BMD macrophages: Cells were isolated from the femur and tibia of freshly euthanized mice, by flushing with ~10 ml of activation media: RPMI1640 containing L-glutamine (Gibco), 10% (v/v) FCS, 10 mM HEPES, 50 µM β-ME, 1% (v/v) P/S, without CSF-1. Cells then were pelleted, and resuspended into monocyte differentiation media: DMEM (Gibco), 10 % (v/v) FCS, 5% (v/v) adult horse serum (Cell Concepts), 1:100 non-essential amino acids (Sigma), 50 µM β-ME (Sigma), with 20% (v/v) L929 conditioned media containing CSF-1 /RPMI media. Conditioned

media containing CSF-1 was generated by collecting the media from L929 cells (ATCC) cultured for 14 d in DMEM containing 10 % (v/v) FCS, 1:100 non-essential amino acids, 10 mM HEPES and 1% (v/v) P/S. For macrophage differentiation, 10 x $10^6$ bone-marrow derived cells were cultivated in 50 ml of differentiation media for 7 d in sealed, hydrophobic Teflon® bags (FT FEP 100 C (DuPont), American Durafilm) at 37ºC and 10% $CO_2$. The yield of BMD M(-) macrophages (also known as M0) from one bag was consistently ~70-100 x $10^6$ cells with a purity of >95% (determined as F4/80+ CD11b+ cells by flow cytometry, data not shown). For activation of M(-) into M(IL-4+IL-13) (also known as M2) or M(LPS) (also known as M1), BMD M(-) cells were harvested from Teflon bags, pelleted and resuspended into activation media containing recombinant mouse IL-4 (10 ng/mL) and IL-13 (10 ng/ml) or LPS (100ng/ml). For analysis by real-time RT-PCR and Western blotting, 2 x $10^6$ BMD M(-) and BMD M(IL-4+IL-13) cells were plated per well in 6-well plates. In all cases, the macrophages were first allowed to rest and adhere for 2 h. Unless otherwise noted, cells were activated for 24 h at 37ºC and 5% $CO_2$.

SGHPL-4 cells derived from primary human first trimester extravillous trophoblasts (EVT) transfected with the early region of SV40, known previously as MC418, were a kind gift from Judith E. Cartwright (St. George's University of London, London, United Kingdom). These cells show similar invasive capabilities to primary EVTs and retain features of normal EVTs.[4] SGHPL-4 cells were cultivated in HAM's F10 (Biochrom) media containing 10% (v/v) FCS and 1% (v/v) P/S.

1 x $10^6$ BMD M(IL-4+IL-13) and 2 x $10^5$ SGHPL-4 cells were cultivated separately or together in HAM's F10 media containing 1% (v/v) FCS and 1% (v/v) P/S for 48 h. Media was collected and stored.

Endotoxin-free recombinant human MIF was purified as previously described.[5, 6] Prior to stimulation protocol SGHPL-4, Hofbauer cells and Blood-derived macrophages were cultivated in starvation media (basal media supplemented with 0.1% FCS) for 18 hrs. Cells were treated with varying concentrations (10, 50, 200, 200 ng/ml) of MIF. After indicated time (5, 10, 15, 30 min) cells were washed with cold PBS and proteins were isolated on ice using RIPA buffer supplemented with Complete protease inhibitor cocktail (Roche Diagnostics) and phosphatase inhibitory cocktail (Sigma). 100 µg of protein lysates were loaded on SDS-PAGE. Cells treated with EGF in concentration of 10 ng/ml for 10 min (Hofbauer cells) and 15 min (SGHPL-4) were used as a positive control. For expression analysis, starved SGHPL-4 cells were stimulated with 50 ng/ml MCP-1, 200 ng/ml CCL5 (Miltenyi Biotec) and 10ng/ml TNF$\alpha$ (Sigma-Aldrich) or in combination of all three cytokines for 6h or 24h as indicated.

*mRNA isolation, real-time RT-PCR and microarray analysis*

Total mRNA was isolated from tissues (homogenized by ceramic balls) and cells using QIAzol lysis reagent and Qiagen RNeasy mini kit (Qiagen) with on-column deoxyribonuclease I step (Qiagen) according to manufacturer's protocol. mRNA quality and concentration was measured by NanoDrop-1000 spectrophotometer (PeqLab). 2 µg of mRNA was reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time polymerase chain reaction (PCR) was detected on ABI 7500 Fast Sequence Detection System (Applied Biosystems) and analyzed by 7500 Fast System Software (Applied Biosystems). Primers and probes (Online Table II) were designed with PrimerExpress 3.0 (Applied Biosystems) and synthesized by Biotez (Germany). Expression of target genes was normalized to 18s expression.

Microarray analysis of human placenta and decidua from the Oslo Pregnancy Biobank was described earlier.[7] Microarray analysis of M(-) and M(IL-4) was done with the Illumina HumanHT-12_V3_0_R2 according the Minimum Information About a Microarray Experiment (MIAME) criteria. Data is provided at ArrayExpress under accession number E-MTAB-3309. Differentially expressed genes (65% FDR score) were investigated through the use of QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN).

*Western blot*

Placental tissue or cells were lysed with RIPA buffer. Protein concentration was measured using Bradford reagent (ROTH) and certain amounts of denaturized protein samples were separated on SDS-PAGE. Further, proteins were transferred to nitrocellulose membrane using semi-dry blotting. Unspecific binding was reduced by 3% w/v BSA, while hCD74- (abcam), β-actin- (CellSignaling), eIF4- (CellSignaling) and mCD74- (R&D) specific antibody incubations were carried out overnight at 4$^{o}$C. Secondary antibodies were conjugated with fluorochrome (IRDye800CW), excited and documented on ODYSSEY infrared scanner (LI-COR Biosciences). For loading control, membranes were stripped and re-incubated.

*Flow cytometry and high-throughput sampler (HTS)*

Placental cells were kept on ice in washing-buffer (PBS containing 0.1% FCS). 1 x $10^6$ cells were stained for extracellular markers (CD14-PE-Cy7, CD74-PE, BD Bioscience), fixed and cell membrane was permeabilized using Fix/Perm buffer (BD Biosciences). Then, Fc receptor blocking was performed (eBioscience) and cells were incubated with antibodies against intracellularly expressed proteins (cytokeratin 7, Abcam). Incubation with secondary antibody was performed (anti-rabbit-APC, ImmunoResearch). 1 x $10^5$ events were recorded on BD FACSCanto II system using BD FACSDiva software (BD Biosciences). Data was analyzed using FlowJo software (TreeStar Inc.). Positive gating coordinates were set according to isotype controls.

Trypsinized cells after co-culture experiments were loaded onto 96-well plates and analyzed on high throughput sampler. 100 µl of cell suspension was sampled and the amount of cells was recorded and counted on BD FACSCanto II system using BD FACSDiva software. Data was analyzed using FlowJo software and is presented as absolute number of cells in SGHPL-4 cell gate (coordinates set according to forward scatter (FSC) and side scatter (SSC) of SGHPL-4 single culture).

*Immunostaining*

Human control placental sections were deparaffinized in xylene followed by rehydration through graded ethanol. Antigen was retrieved by heat-induced epitope retrieval (HIER) method in 0.01 M citrate buffer pH 7. Non-specific antibody binding was blocked with Ultra V Block (Lab Vision). Double immunostaining of mouse anti-CD74 (abcam) and rabbit anti-cytokeratin 7 (abcam) or rabbit anti-CD163 (DB Biotech) was performed and secondary antibody goat anti-mouse Alexa555 and goat anti-rabbit Alexa488 was applied. After extensive washes with PBS/T cell nuclei were stained with DAPI.

Paraffin embedded mouse placental tissue was cut into 4 µm histological sections at the mid sagittal plane using a microtome (Leica). Tissue sections were deparaffinized, rinsed in distilled water, and dehydrated twice in ethanol 70 %. Masson-Goldner trichrome staining kit (VWR international) was used to visualize the morphologically different areas of placental tissues.[8] Briefly, tissue sections were

stepwise stained with Weigert's iron hematoxylin, Azophloxine staining solution and phosphotungstic acid Orange G, and Light green SF solution following manufacturer´s instructions. Finally, the tissue was dehydrated and mounted. Image acquisition was performed using a slide scanner (Mirax Midi, Zeiss). The size of placental labyrinth and junctional zone was assessed based on morphological criteria and quantified using the program MiraxViewer. The ratio between the labyrinth and junctional zone (L/Jz ratio), which is considered as a marker for placental function[9], was calculated by dividing the size of the labyrinth by the size of the junctional zone. Additionally, parallel sections were stained with Periodic Acid Schiff (PAS; for general morphological evaluation and detection of fibrinoid) and α-actin (marker for vascular smooth muscle cells, VSMC) (DAKO M851) with MOM-Kit (Vector Laboratories) for investigating spiral artery remodeling. The Protocol was described before.[10] Briefly, we used Depex mounting medium (VWR international GmbH), Aqua-Poly/Mount (Polysciences, Inc.), and non-conjugated goat anti-mouse immunglobulins (Acris Antibodies) at a final dilution 1:50. Image acquisition was performed using Zeiss microscope Axio Imager M2 with AxioVision 4.1.

Spiral artery cross sections within the mesometrium were investigated for remodeling process. The loss of smooth muscle cells (α-actin) was detected and evaluated by % of circumference. We interpreted 100% (representing total loss of smooth muscle cells) as vascular remodeling of the spiral artery. Area and perimeter of spiral arteries were also measured using AxioVision 4.1.

*Soluble CD74-, MIF-ELISA, sFlt1-, PLGF- and cytokine/chemokine analysis*

Determination of MIF levels were performed with an ELISA technique as previously described, using capture antibody MAB289 and detection antibody BAF289 (R&D, Germany).[11]

The measurement of circulating CD74 was performed using a competitive sandwich ELISA as previously described.[12] As capture antibody anti-CD74 (clone C-16, Santa Cruz, SC-5438, 250ng/ml) was used and for detection clone LN-2 (Santa Cruz, sc-6262, 400ng/ml). The rhCD74 protein standard used for the ELISA was purchased from R&D systems (3590-CD). The inter-assay variability was 25% and the assay sensitivity was 1.56 ng/ml.

For cytokine/chemokine analysis, supernatants were loaded on 96-well plates supplied with Mouse Luminex Screening Assay and procedure was carried out according to manufacturer's protocol (R&D). Cytokines included in assay were: CCL2, CCL4, CCL5, CXCL1, GDF-15, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-10, IL-17A, Il-2, IL-4, IL-6, TNF-α and VEGF. Data was recorded and analyzed on Luminex 200 System using Bio-Plex Manager software 6.0 (Bio-Rad).

Serum PLGF and sFlt1 were analyzed on Elecsys (Roche Diagnostics). sFlt1/PLGF ratios were calculated.

*Silencing RNA (siRNA)*

For silencing procedure, human M(-) and M(IL-4) were cultivated in RPMI1640 media w/o P/S and w/o M-CSF. Transfection was carried out according to manufacturer's protocol using DharmaFECT 1 transfection reagent and ON-TARGETplus Non-targeting Pool siRNA (NT) as control and SMARTpool: ON-TARGETplus CD74 siRNA (siCD74) (Thermo Scientific) all in a final concentration of 50 nM. After 24 h, media was changed to RPMI1640 containing 20% (v/v) FCS, 10mM HEPES, 50 µM β-ME, 1% (v/v) P/S to reduce cytotoxicity. Experiments were terminated after 48h with mRNA or protein extraction.

*Adhesion assay*

Human M(IL-4) were stained with CellTracker Green CMFDA Dye (Life technologies) for 45min in 37$^{o}$C in RPMI1640 containing 10mM HEPES, 50 µM β-ME, 1% (v/v) P/S. After additional 30min in the same media without CellTracker, cells were detached from culture dish using Trypsin-EDTA (Sigma). Cells were suspended to cell concentration of 5 x $10^5$ / 200 µl in serum-free media. Cells were then added onto SGHPL-4 cell layer formed by overnight culture of 5 x $10^5$ cells in 96-well plate format. After 8 h non-adherent macrophages were washed out and adherent cells were documented with fluorescence microscopy and AxioVision software (Carl Zeiss MicroImaging). Afterwards, cells were lysed using 0.1M NaOH for 30 min in 37$^{o}$C and fluorescence was measured on microplate reader (TECAN).

*Statistics*

Data are presented as mean ± SEM when normally distributed or as median with interquartile range when non-normally distributed (or as indicated). Normal distribution was assessed by Kolmogorov-Smirnov test. Groups were compared using the unpaired t test, Mann–Whitney U test, 1-way ANOVA, or Kruskal-Wallis test as appropriate and indicated in the figure legends. Multigroup comparisons were followed by post hoc testing, including the Scheffe test, Dunnett T3, and Mann–Whitney U tests with a Bonferroni correction. $p<0.05$ was considered as statistically significant.

## Online Tables

## Online Table I. Clinical characteristics of expression studies subjects.

| Characteristics at delivery | Normotensive controls (n=28) | Early onset Preeclampsia: Delivery <34 GW | Late onset Preeclampsia: Delivery ≥ 34 GW | P value (Control vs early onset PE and control vs late onset PE) |
|---|---|---|---|---|
| Maternal age (years) | 31.2 ± 4.2 | 30.7 ± 5.2 | 32.5 ± 6.0 | 0.7 and 0.4 |
| BMI ($kg/m^2$) | 28.6 ± 3.4 | 30.5 ± 4.0 | 32.6 ± 6.2 | 0.06 and 0.007 |
| Gestational weeks | 39.0 ± 0.9 | 30.7 ± 2.3 | 36.9 ± 1.6 | <0.001 and <0.001 |
| Blood pressure Systolic (mm Hg) | 119 ± 11.6 | 168 ± 17.1 | 160 ± 14.5 | <0.001 and <0.001 |
| Blood pressure Diastolic (mm Hg) | 72.1 ± 10.9 | 102.3 ± 7.4 | 99.9 ± 5.8 | <0.001 and <0.001 |
| Baby weight (g) | 3492 ± 402 | 1372 ± 415 | 3035 ± 681 | <0.001 and 0.007 |

Clinical characteristics of the preeclamptic cases and controls from the Oslo study population. Data are presented as mean ± standard deviation, BMI: body mass index.

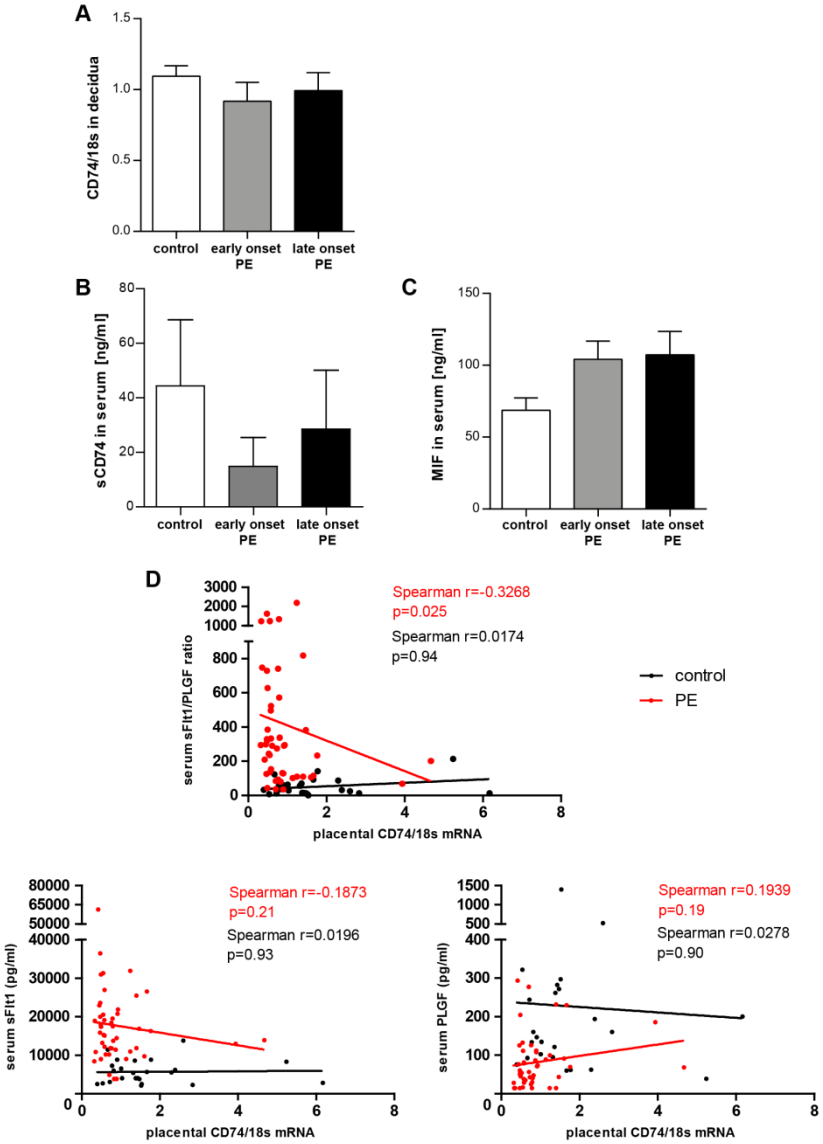
## Online Table II. Primer and probes

| Gene | Primer | Sequence |
|---|---|---|
| **18S** | Forward | 5'-ACATCCAAGGAAGGCAGCAG-3' |
| | Reverse | 5'-TTTTCGTCACTACCTCCCCG-3' |
| | Probe | 5'-FAM-CGCGCAAATTACCCACTCCCGAC-TAMRA-3' |
| **hCD74** | Forward | 5'-ACCAAGTATGGCAACATGACAGA-3' |
| | Reverse | 5'-AGTGGCGGGTACACCTTCAG-3' |
| | Probe | 5'-FAM-TGATGCACCTGCTCCAGAATGCTGAC-TAMRA-3' |
| **hALCAM** | Forward | 5'TCTGCTCTTCTGCCTCTTGATCT-3' |
| | Reverse | 5'TGGTATCTCCATATGCTGAATTTACAG-3' |
| | Probe | 5'-FAM-CGCCACCGTCTTCAGGCCAGG-TAMRA-3' |
| **hICAM4** | Forward | 5'-TGGGCCACCTCCAGGAT-3' |
| | Reverse | 5'-TTTAAGACCGGAGGCTCCAA-3' |
| | Probe | 5'- FAM-ACCGCCTACAAACCGCCCCACA-TAMRA-3' |
| **hSDS2** | Forward | 5'-GAAGCTTCAGGAGTGTATCCTATTGA-3' |
| | Reverse | 5'-CTCTGGACTCTCTACATCCTCATCAG-3' |
| | Probe | 5'-FAM-ACGATGACTACGCTTCTGCGTCTGGC-TAMRA-3' |
| **hsFlt1** | Forward | 5´-AATCAGAGGTGAGCACTGCAAC-3´ |
| | Reverse | 5´-TGGTACAATCATTCCTTGTGCTTT-3´ |
| | Probe | 5´-FAM-AAAAGGCTGTTTTCTCTCGGATCTCCAAATTT-TAMRA-3´ |
| **hPLGF** | Forward | 5‘-CCTACGTGGAGCTGACGTTCT- 3‘ |
| | Reverse | 5‘-CCTTTCCGGCTTCATCTTCTC-3‘ |
| | Probe | 5‘-FAM-TCGCTGCGAATGCCGGCC-TAMRA-3‘ |
| **hTNFα** | Forward | 5´-TGGCCCAGGCAGTCAGA-3´ |
| | Reverse | 5´-GGTTTGCTACAACATGGGCTACA-3´ |
| | Probe | 5´- FAM-CATCTTCTCGAACCCCGAGTGACAAGC-TAMRA-3´ |
| **hIL-8** | Forward | 5’-CTGGCCGTGGCTCTCTTG-3’ |
| | Reverse | 5’-CCTTGGCAAAACTGCACCTT-3’ |
| | Probe | 5’-FAM- CAGCCTTCCTGATTTCTGCAGCTCTGTGT-TAMRA-3’ |

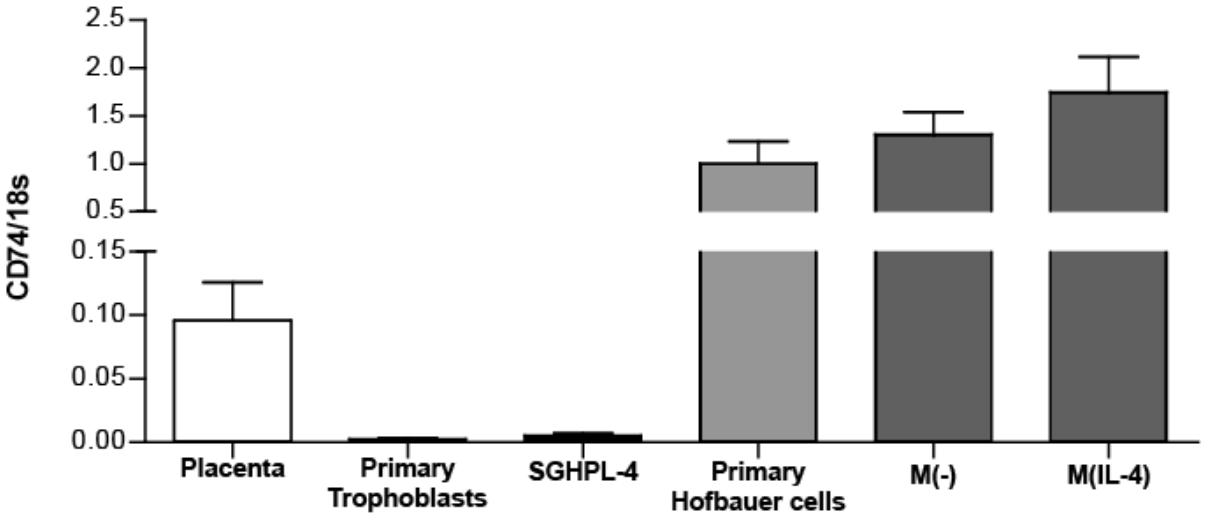
| | | |
|---|---|---|
| **hCYP2J2** | Forward | 5´-AGAGCTTAGAGGAACGCATTCAG-3´ |
| | Reverse | 5´-GGTCAAAAGGCTGTCCGTTCT-3´ |
| | Probe | 5´-FAM-AGGCCCAACACCTCACTGAAGCAATAAAAG-TAMRA-3´ |
| **mCD74** | Forward | 5'-ATGACCCAGGACCATGTGATG-3' |
| | Reverse | 5'-CCCTTCAGCTGCGGGTACT-3' |
| | Probe | 5'-FAM-CTGCTCACGAGGTCTGGACCCCTG-TAMRA-3' |
| **mMRC-1** | Forward | 5'-AATACCTTGAACCCATTTATCATTCC-3' |
| | Reverse | 5'-GCATAGGGCCACCACTGATT-3' |
| | Probe | 5'-FAM-CGATGTGCCTACCGGCTGCCC-TAMRA-3' |
| **mArg1** | Forward | 5'-CTCCCTGCATATCTGCCAAAG-3' |
| | Reverse | 5'-TAGTGTTCCCCAGGGTCTACGT-3' |
| | Probe | 5'-FAM-CATCGTGTACATTGGCTTGCG-TAMRA-3' |
| **mFizz1** | Forward | 5'-CGTGGAGAATAAGGTCAAGGAACT-3' |
| | Reverse | 5'-CACTAGTGCAAGAGAGAGTCTTCGTT-3' |
| | Probe | 5'-FAM-TTGCCAATCCAGCTAACTATCCCTCCACTG-TAMRA-3' |
| **mNOS2** | Forward | 5'-GGGCAGCCTGTGAGACCTT-3' |
| | Reverse | 5'-TGCATTGGAAGTGAAGCGTTT-3' |
| | Probe | 5' FAM-TCCGAAGCAAACATCACATTCAGATCCC-TAMRA-3' |
| **mCCL5** | Forward | 5'-GCAGTCGTGTTTGTCACTCGAA-3' |
| | Reverse | 5'-GATGTATTCTTGAACCCACTTCTTCTC-3' |
| | Probe | 5'-FAM-AACCGCCAAGTGTGTGCCAACCC-TAMRA-3' |
| **mMCP-1** | Forward | 5'-GGCTCAGCCAGATGCAGTTAA-3' |
| | Reverse | 5'-CCTACTCATTGGGATCATCTTGCT-3' |
| | Probe | 5'-FAM-CCCCACTCACCTGCTGCTACTCATTCA-TAMRA-3' |
| **mTNFa** | Forward | 5'-GGTCCCCAAAGGGATGAGAA-3' |
| | Reverse | 5'-TGAGGGTCTGGGCCATAGAA-3' |
| | Probe | 5'-FAM-TTCCCAAATGGCCTCCCTCTCATCA-TAMRA-3' |

| | | |
|---|---|---|
| **mIL-6** | Forward | 5'-GTTGCCTTCTTGGGACTGATG-3' |
| | Reverse | 5'-GGGAGTGGTATCCTCTGTGAAGTCT-3' |
| | Probe | 5'-FAM-TGGTGACAACCACGGCCTTCCC-TAMRA-3' |
| **mPD-L2** | Forward | 5'-GAGCCAGTTTGCAGAAGGTAG-3' |
| | Reverse | 5'- ATCCGACTCAGAGGGTCAATG -3' |

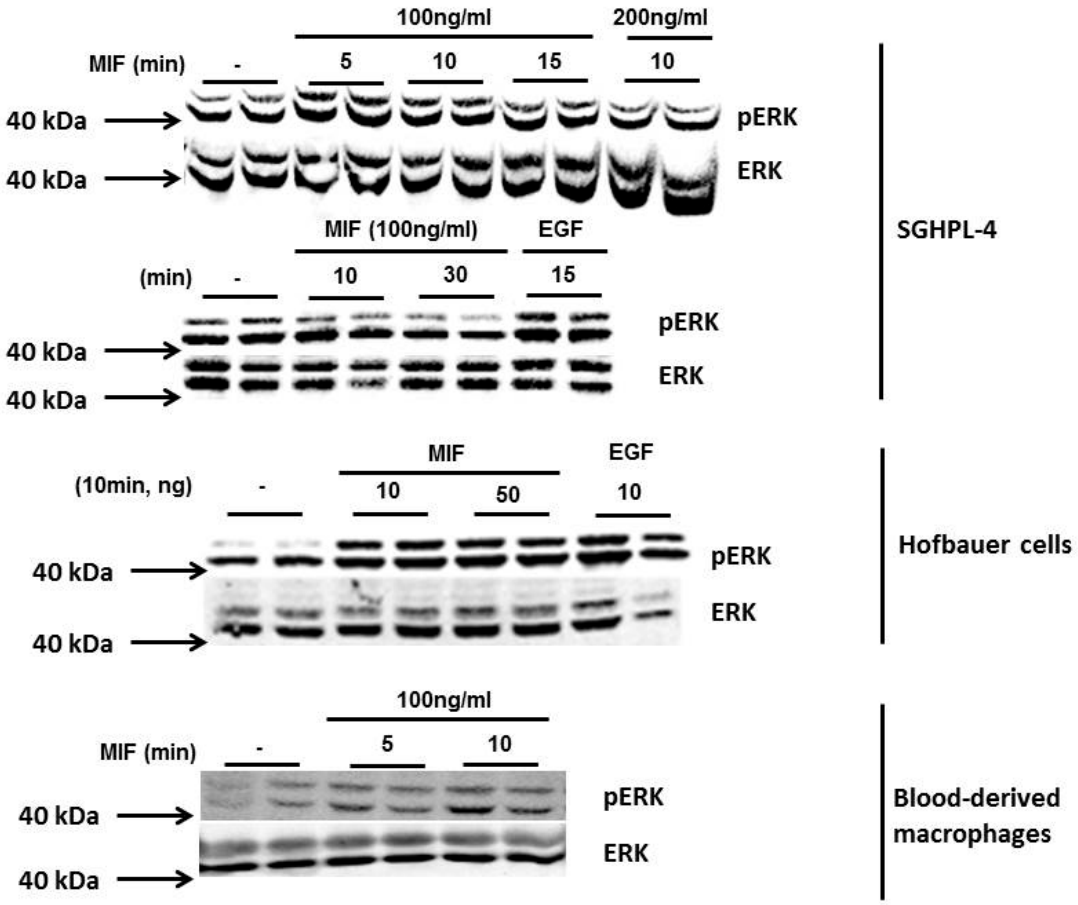
## Online Figures



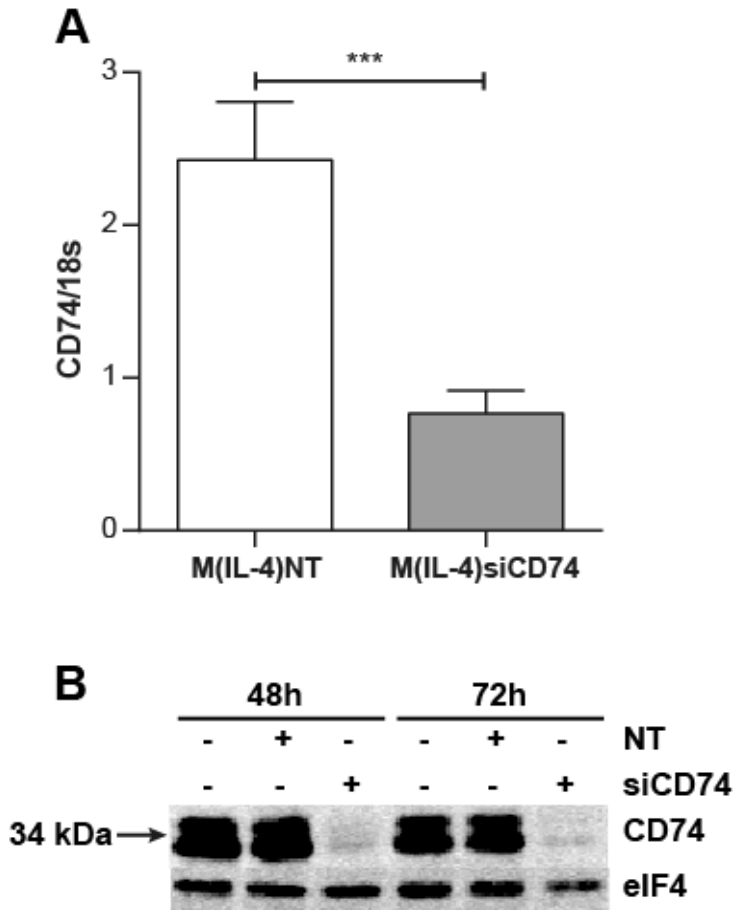

**Online Figure I. CD74 in decidua, soluble CD74 (sCD74), macrophage migration inhibitory factor (MIF) in circulation of preeclamptic women (PE) and correlation of CD74 expression and (anti)-angiogenic factors. A)** CD74 expression was not altered in deciduas of PE compared to healthy women (control; n=28). PE is subdivided in early onset PE (delivery <34 week of gestation; grey bar; n=26) and late onset PE (delivery ≥34 week of gestation; black bar; n=24). **B)** sCD74 was lowered in early PE and late PE compared to control, although not statistically significant. **C)** Accordingly, MIF showed a trend to be enhanced both in early PE and late PE, although not statistically significant. **D)** Placental CD74 expression significantly correlated to the serum sFlt1/PLGF ratio in the PE group but not in controls. Correlation of placental CD74 expression to serum sFlt1 and PLGF is also shown.



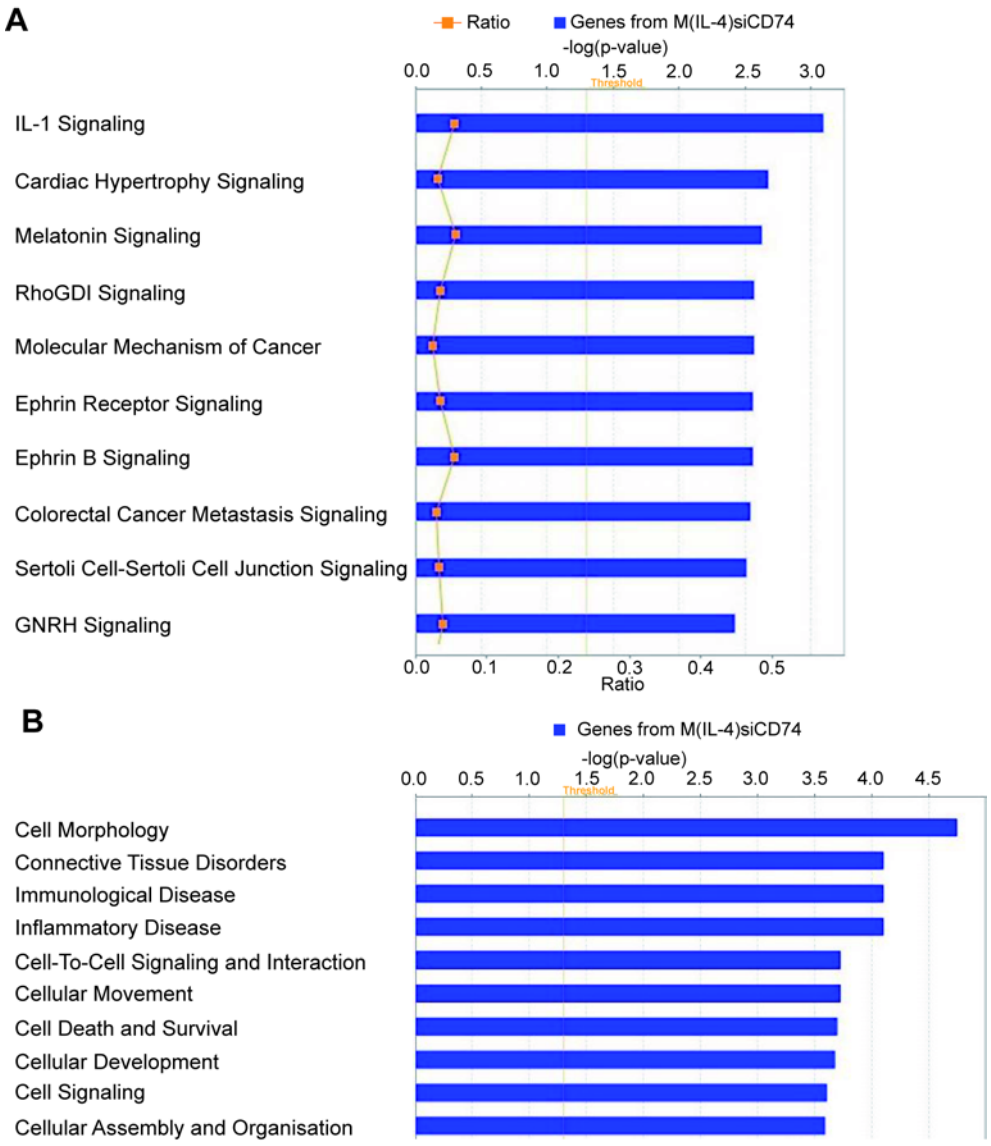

**Online Figure II. CD74 expression in human placenta and different cell types.** CD74 expression normalized to 18s was high in primary Hofbauer cells isolated from placenta (n=5), and in *in vitro* differentiated and activated human PBMC-derived macrophages (M(-) and M(IL-4)), intermediate in placenta (n=4) and low in primary trophoblasts isolated from placenta (n=5) and in a first trimester derived trophoblast cell line (SGHPL-4) (n=3).



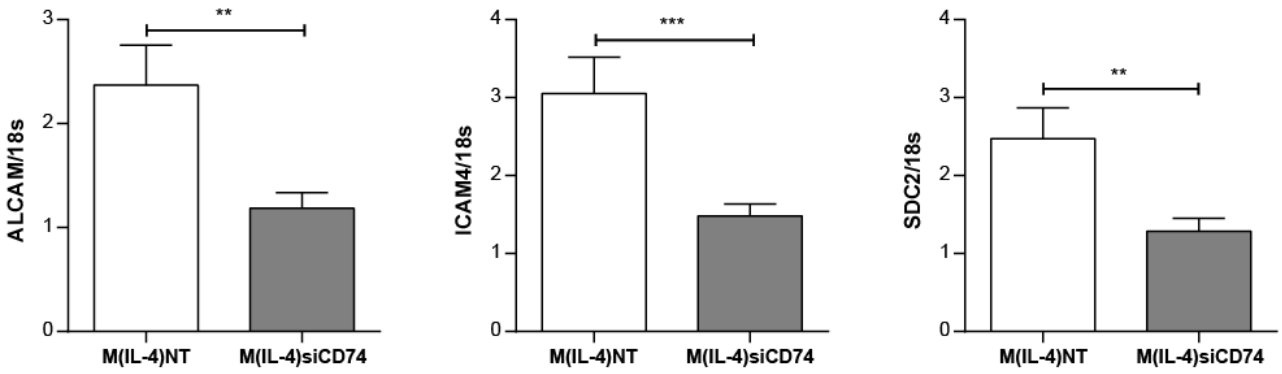

**Online Figure III. Phospho-ERK1/2 (pERK) signaling in SGHPL-4, Hofbauer cells and blood derived macrophages.** Stimulation by macrophage migration inhibitor factor (MIF) of Hofbauer cells and blood-derived macrophages led to an activation of pERK (phosphorylation of p44 and p42). SGHPL-4 cells did not show an activation by MIF stimulation. Epidermal growth factor (EGF) served as positive control.



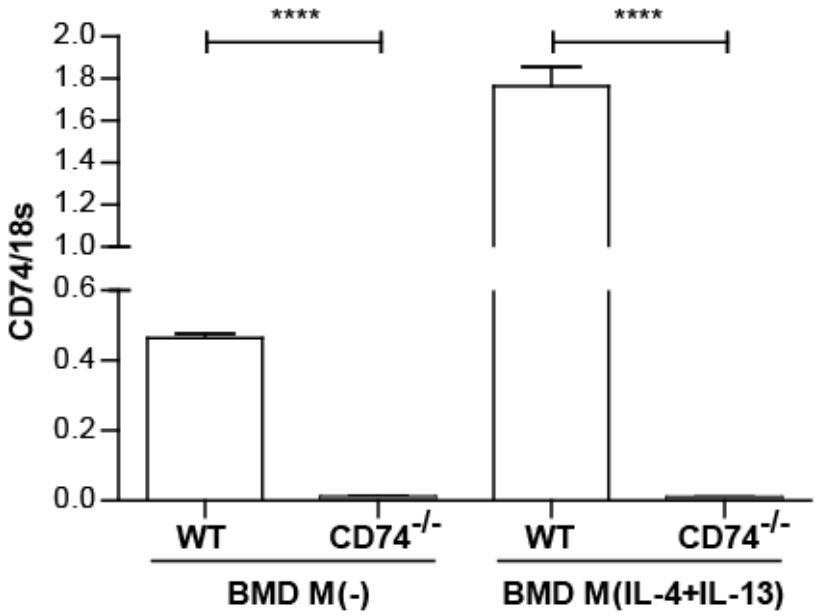

**Online Figure IV. Characterization of CD74 in CD74 silenced macrophages. A)** CD74 expression normalized to 18s was lowered by CD74 silencing (siCD74) in *in vitro* differentiated and activated human PBMC derived macrophages (M(IL-4)) compared to control (NT) (n=27 each; ***p<0.001; Mann Whitney test). **B)** CD74 protein was lowered after 48h and 72h in M(IL-4)siCD74 vs. M(IL-4)NT. eIF4 was used as a loading control.



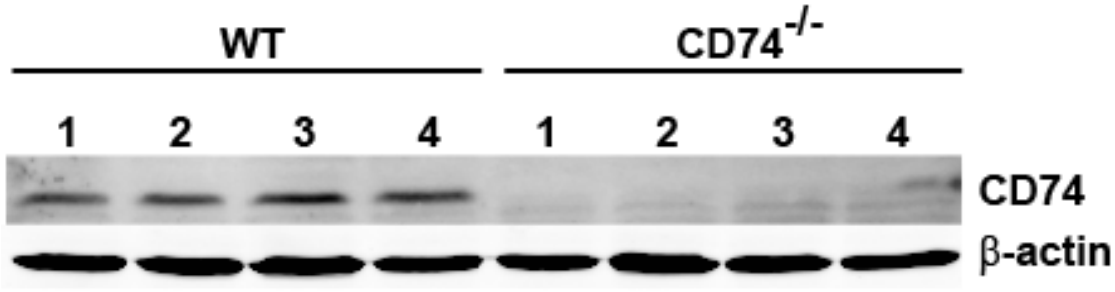

**Online Figure V. Ingenuity Pathways Analysis (IPA) summary. A)** Top ten canonical pathways and **(B)** top ten diseases and biological functions influenced by CD74 down-regulation in M(IL-4) macrophages are shown. The ratio is calculated as a number of genes from the dataset that map to the pathway divided by the number of total genes included into the pathway. The yellow line represents the threshold of $p<0.05$ as calculated by Fischer's test.



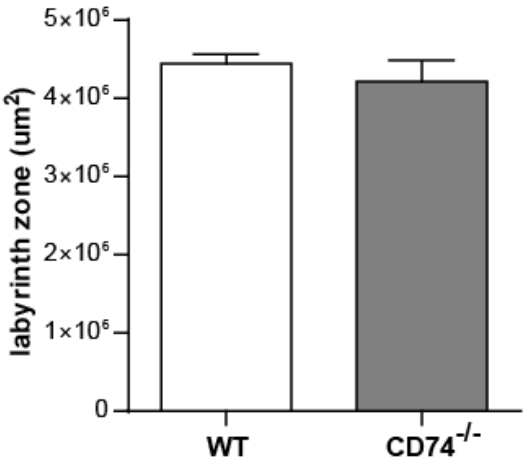

**Online Figure VI. Adhesion molecule expression in CD74 silenced macrophages.** In *in vitro* differentiated and activated human PBMC derived macrophages (M(IL-4)) CD74 silencing (siCD74) let to a downregulation of ALCAM, ICAM and SDC2 expression (normalized to 18s) when compared to control (NT) (n=27 each; **p<0.01, ***p<0.001; Mann Whitney test).



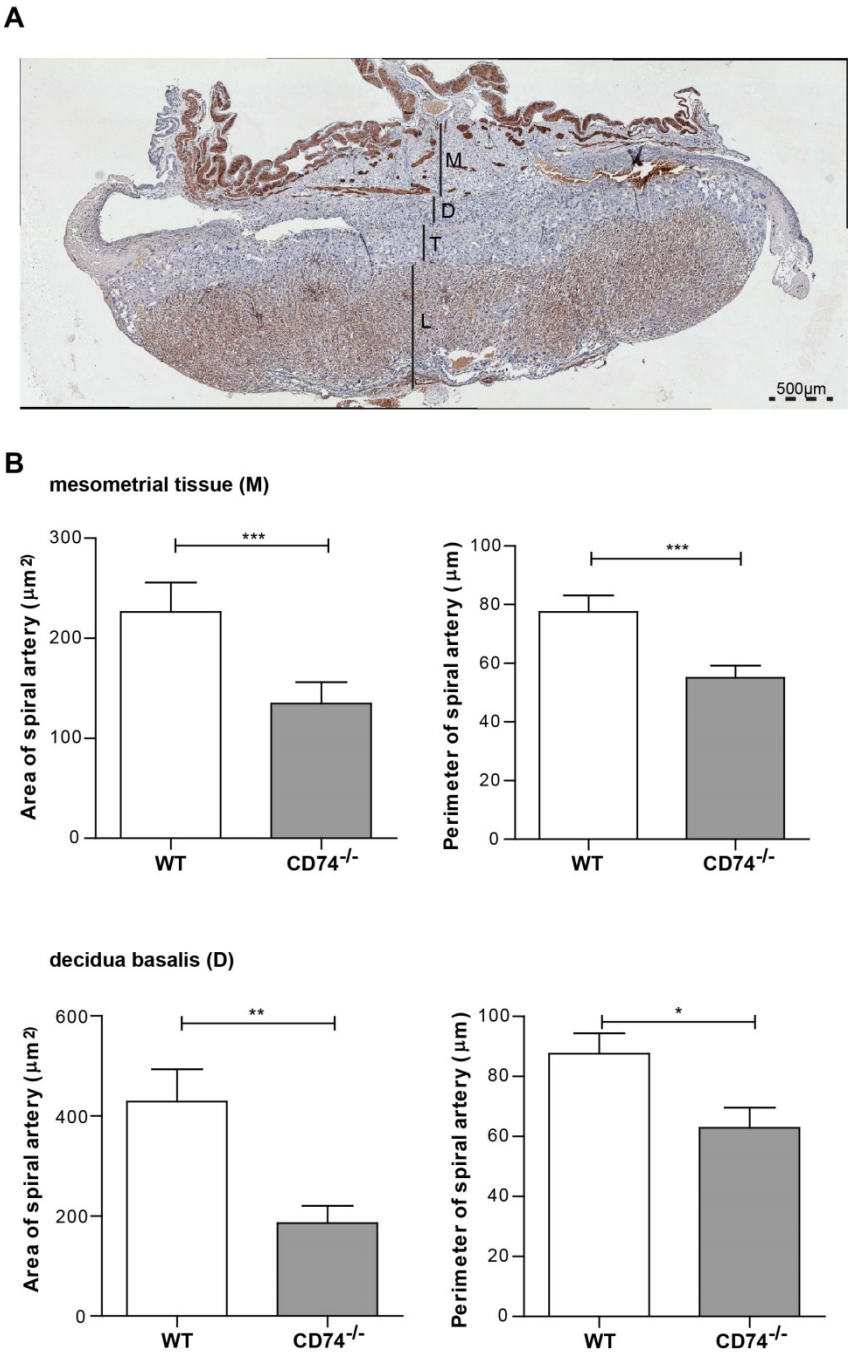

**Online Figure VII. Characterization of CD74 in bone-marrow derived (BMD) macrophages of CD74-knockout (CD74-KO) mouse.** *In vitro* differentiated and activated BMD macrophages (M(IL-4+IL-13)) showed high CD74 expression (normalized to 18s) when derived from WT but no expression when derived from CD74-KO mice (n=6 each; ****p<0.0001; Unpaired t test).




**Online Figure VIII. Characterization of CD74 in placenta of CD74-knockout (CD74-KO) mouse.** CD74 was absent in placentas of CD74-KO mice. Wild-type (WT) mice showed robust CD74 protein level.

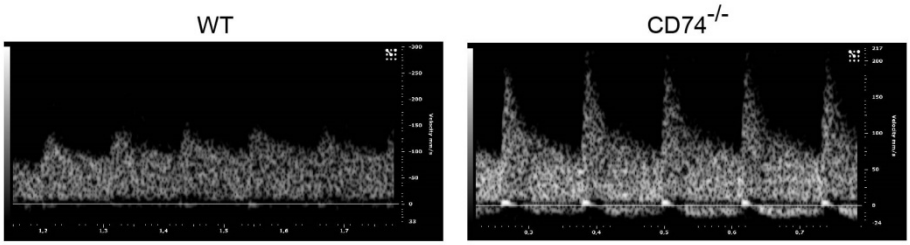



**Online Figure IX. Labyrinth zone CD74$^{-/-}$ mouse.** Size of labyrinth zone was not changed in CD74$^{-/-}$ (n=13) in comparison to WT (n=17).

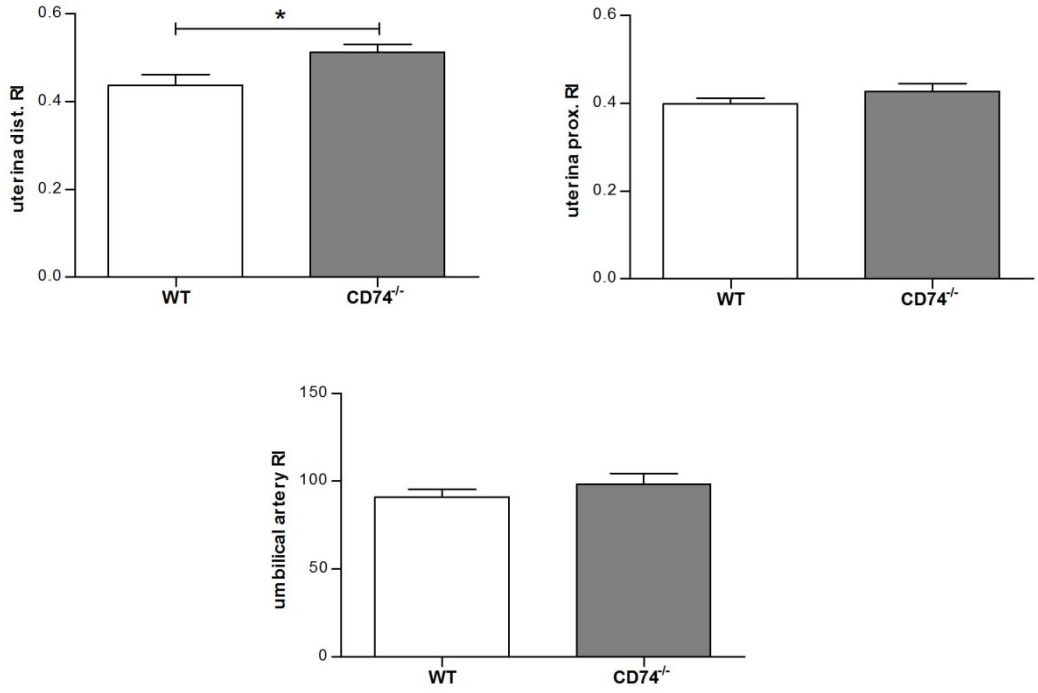



**Online Figure X. Spiral arteries in CD74$^{-/-}$ mouse. A)** Representative picture of a placenta stained with α-actin. M=mesometrial tissue; D=decidua basalis; T=trophospongium; L=labyrinth. **B)** Area and perimeter of spiral arteries localized in mesometrial tissue and decidua basalis were lowered in CD74$^{-/-}$ (n=24) compared to WT (n=37). M: CD74$^{-/-}$ (n=86); WT (n=95). D: CD74$^{-/-}$ (n=24); WT (n=37). *p<0.05, **p<0.01, ***p<0.001; T test).

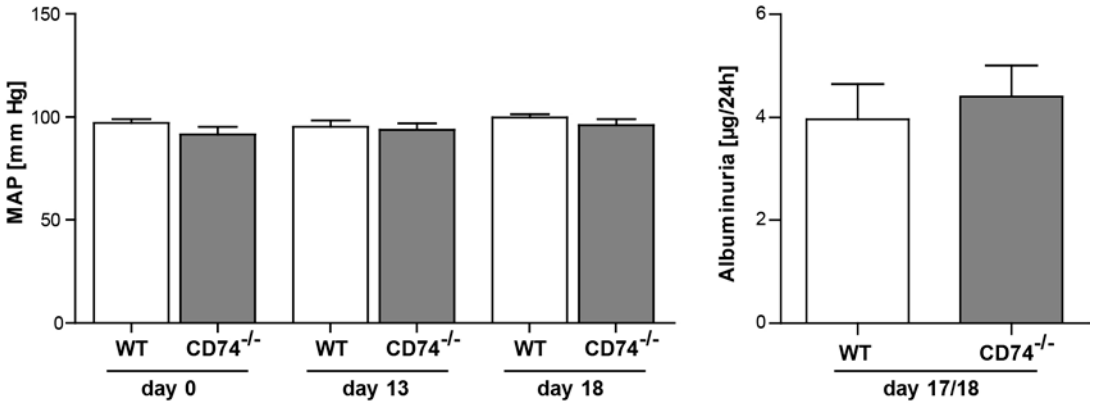
A



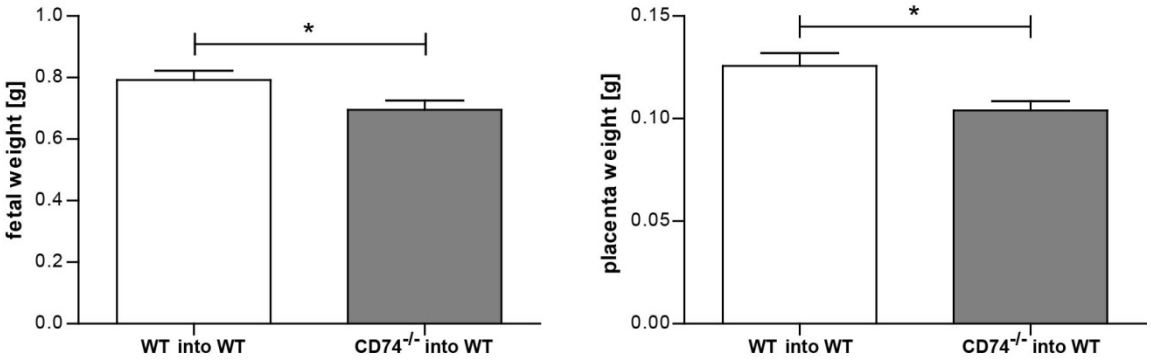

B




**Online Figure XI. Resistance Index (RI) of Uterine arteries and umbilical artery in CD74$^{-/-}$ mice. A)** Representative pictures of the flow in the distal uterine artery in WT and CD74$^{-/-}$ mice measured by Doppler ultrasound. **B)** Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured in proximal uterine artery, distal uterine artery and embryonic umbilical artery and the resistance index (RI = (PSV−EDV)/PSV) was calculated. RI was increased in uterine distalis of CD74$^{-/-}$ mice compared to wild type (WT) on day 15/17 of pregnancy. RI of the proximal uterine artery and umbilical artery was unchanged (n=10 each; *p<0.05; Mann Whitney test).




**Online Figure XII. Characterization of maternal syndrome in CD74$^{-/-}$ mice.** Mean arterial pressure (MAP) (n=6 each) and albuminuria (n=4 each) did not change in CD74$^{-/-}$ vs. wild type (WT).




**Online Figure XIII. Fetal growth restriction and lowered placenta weights of CD74$^{-/-}$ fetuses developed in WT foster mothers.** Pup and placental weights resulting of CD74$^{-/-}$ derived two-cell stage embryos transferred into WT mice (n=14) were lower on day 18 of pregnancy when compared to WT derived embryos (n=15). *p<0.05; unpaired t-test.

## Online References


**1.** Staff AC, Ranheim T, Khoury J, Henriksen T. Increased contents of phospholipids, cholesterol, and lipid peroxides in decidua basalis in women with preeclampsia. *Am J Obstet Gynecol.* 1999;180:587-592.

**2.** Verlohren S, Niehoff M, Hering L, Geusens N, Herse F, Tintu AN, Plagemann A, LeNoble F, Pijnenborg R, Muller DN, Luft FC, Dudenhausen JW, Gollasch M, Dechend R. Uterine vascular function in a transgenic preeclampsia rat model. *Hypertension.* 2008;51:547-553.

**3.** Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity.* 2014;41:14-20.

**4.** McCormick J, Whitley GS, Le Bouteiller P, Cartwright JE. Soluble HLA-G regulates motility and invasion of the trophoblast-derived cell line SGHPL-4. *Hum Reprod.* 2009;24:1339-1345.

**5.** Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W, Manogue KR, Cerami A, Bucala R. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. *Nature.* 1993;365:756-759.

**6.** Bernhagen J, Mitchell RA, Calandra T, Voelter W, Cerami A, Bucala R. Purification, bioactivity, and secondary structure analysis of mouse and human macrophage migration inhibitory factor (MIF). *Biochemistry.* 1994;33:14144-14155.

**7.** Herse F, Lamarca B, Hubel CA, Kaartokallio T, Lokki AI, Ekholm E, Laivuori H, Gauster M, Huppertz B, Sugulle M, Ryan MJ, Novotny S, Brewer J, Park JK, Kacik M, Hoyer J, Verlohren S, Wallukat G, Rothe M, Luft FC, Muller DN, Schunck WH, Staff AC, Dechend R. Cytochrome P450 subfamily 2J polypeptide 2 expression and circulating epoxyeicosatrienoic metabolites in preeclampsia. *Circulation.* 2012;126:2990-2999.

**8.** Stelzer IA, Thiele K, Solano ME. Maternal microchimerism: lessons learned from murine models. *Journal of reproductive immunology.* 2015; 108:12-25

**9.** Sferruzzi-Perri AN, Macpherson AM, Roberts CT, Robertson SA. Csf2 null mutation alters placental gene expression and trophoblast glycogen cell and giant cell abundance in mice. *Biology of reproduction.* 2009;81:207-221.

**10.** Geusens N, Verlohren S, Luyten C, Taube M, Hering L, Vercruysse L, Hanssens M, Dudenhausen JW, Dechend R, Pijnenborg R. Endovascular trophoblast invasion, spiral artery remodelling and uteroplacental haemodynamics in a transgenic rat model of pre-eclampsia. *Placenta.* 2008;29:614-623.

**11.** Flieger O, Engling A, Bucala R, Lue H, Nickel W, Bernhagen J. Regulated secretion of macrophage migration inhibitory factor is mediated by a non-classical pathway involving an ABC transporter. *FEBS letters.* 2003;551:78-86.

**12.** Assis DN, Leng L, Du X, Zhang CK, Grieb G, Merk M, Garcia AB, McCrann C, Chapiro J, Meinhardt A, Mizue Y, Nikolic-Paterson DJ, Bernhagen J, Kaplan MM, Zhao H, Boyer JL, Bucala R. The role of macrophage migration inhibitory factor in autoimmune liver disease. *Hepatology.* 2014;59:580-591.