**Supplementary Material**

**Maternal platelet-derived factors induce trophoblastic LAIR2 expression to promote trophoblast invasion and inhibit platelet activation at the foetal-maternal interface**

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**Supplementary Materials and Methods**

*Platelet isolation and preparation of platelet releasate*

The study was approved by the ethical committee of the Medical University of Graz (31-019 ex 18/19). About 15 ml citrated whole blood per patient were collected from healthy donors before caesarean section and prior any labour activity. Patients signed written informed consents. Characteristics of the different study populations are shown in Supplemental Table 1. Blood samples were centrifuged at 100 x g for 15 min at room temperature (RT) before platelet rich plasma (PRP) was gently mixed in equal volume with a platelet wash buffer consisting of distilled water containing with 128 mM NaCl (Supelco®, Merck; Darmstadt, Germany), 11 mM glucose (Sigma) 7.5 mM Na2HPO4 (Merck), 4.8 mM sodium citrate (Sigma-Aldrich), 4.3 mM NaH2PO4 (Lactan; Graz, Austria), 2.4 mM citric acid (Merck) and 0.35% bovine serum albumin (Biowest; Nuaillé, France) with addition of 2.5 ng/µl prostaglandin (Cayman Chemical Company; Ann Arbor, MI, USA). After centrifugation at 1962 x g for 15 min at RT, the pellet was resuspended in 10 ml wash buffer. After repeated centrifugation at 1962 x g for 15 min at RT, platelets were resuspended in DMEM/F12 (1:1, Gibco) supplemented with 0.1 U/ml penicillin and 0.1 µg/ml streptomycin (Gibco) and 1% (v/v) L‑glutamine (Gibco; 20 mM 100X) to physiological platelet concentrations. Platelets were counted by the Sysmex KX-21NTM system (Sysmex; Horgen, Switzerland). Platelets were either directly used for co-incubation with ACH-3P spheroids, or aggregometry measurements, or further processed.

*Culture of trophoblast cell line ACH-3P and spheroid formation*

The human first trimester trophoblast cell line ACH-3P was kindly provided by Gernot Desoye (Department of Obstetrics and Gynecology, Medical University Graz, Austria). ACH-3P cells were cultured in DMEM/F12 (1:1, Gibco, life technologies; Paisley, UK) supplemented with 10% FCS (Gibco), 0.1 U/ml penicillin and 0.1 µg/ml streptomycin (Gibco) and 1% (v/v) L-glutamine (Gibco; 20 mM 100X) in a humidified atmosphere of 5% CO2 at 37°C. Cells were incubated with selection medium containing 5.7 µM azaserin (Sigma-Aldrich) and 100 µM hypoxanthine (Sigma-Aldrich) every 10th passage. Cells were mycoplasma free and short tandem repeat analysis was performed regularly.

*RNA Sequencing and Bioinformatics analysis*

Total RNA from matched PR-treated and control ACH-3P spheroids (5 per condition) was isolated with SV Total RNA Isolation System (Promega, Madison, Wi, USA) according to the manufacturer’s protocol. RNA samples were then sent to Genewiz from Azenta Life Sciences and subjected to RNA quantification using Qubit 4.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA integrity was checked with RNA Kit on Agilent 5300 Fragment Analyzer, RIN > 9 (Agilent Technologies, Palo Alto, CA, USA). rRNA depletion was achieved using the NEBNext® rRNA Depletion Kit (Human/Mouse/Rat Cat. No. E6310). RNA sequencing library preparation was performed using the NEBNext Ultra RNA Library Prep Kit for Illumina following the manufacturer’s recommendations (NEB, Ipswich, MA, USA). Samples were sequenced on the Illumina NovaSeq 6000 instrument in a paired-end configuration resulting in 2x250bp read pairs.

RNA-seq analysis of the 10 samples was done within Galaxy [1]: The provided FASTQ files were subjected to quality control using FASTQC (version: Galaxy Version 0.73+galaxy0 ) [2] as well as MultiQC (version: Galaxy Version 1,7) [3], and remaining adapter content was trimmed using Trimmomatic (version: Galaxy Version 0.38.1) [4] with recommended parameters. Reads were aligned to the basic human reference annotation available on GENCODE (GRCh38, v42) [5] using STAR (version: Galaxy Version 2.7.7a) [6] with --twopassMode Basic, --sjdbOverhang 149 (read length -1) and otherwise default parameters. The resulting BAM files were inspected using Qualimap (version: 2.2.2d+galaxy1) [7] and quantified with htseq (version: Galaxy Version 0.9.1) [8]. Resulting count data was analyzed and visualized in R (version: 4.4.1) [9]: The DESeq2 (version: 1.44.0) [10] package was used for differential expression analysis, and a gene was considered to be significantly deregulated if its absolute log2 fold change was ≥ log2(1.5) and its adjusted p-value was ≤ 0.05. Plots were generated utilizing the R packages ggplot2 (version: 3.5.1) [11], EnhancedVolcano (version: 1.22.0) [12] and pheatmap (version: 1.0.12, based on relative rlog values [13]) [14]. Next, we performed gene set enrichment analysis (GSEA), applying the R packages clusterProfiler (version: 4.12.3) [15], DOSE (version: 3.30.2) [16] and org.Hs.eg.db (version: 3.19.1) [17] and used an adjusted p-value of 0.05 as significance cutoff for GSEA. All raw RNA-seq files are provided through the NCBI Short Read Archive, accession number PRJNA1194512.

*qPCR analysis*

Total RNA was isolated from pooled spheroid or organoid samples using the SV Total RNA Isolation System (Promega; Madison, WI, USA) according to the manufacturer´s protocol. In addition to spheroids and organoids, 100 µl of fresh culture medium supplemented with platelet releasates (PR) and 100 µl conditioned culture medium collected after cell culture were subjected to RNA isolation and subsequent qPCR analysis. NanoDrop (ND-1000, Peqlab Biotechnology GmbH; Erlangen, Germany) was used for quality check and calculation of concentrations before reverse transcription of 1 µg total RNA per reaction using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's manual. qPCR was performed with SYBR Green (Biozym, Vienna, Austria) using the Bio-Rad CFX384 Touch Real-Time PCR Detection System (Bio-Rad; Hercules, CA, USA). Primers used are shown in Supplemental Table 2. Cq values and normalised expression (ΔΔCq analysis) were automatically generated by the CFX Manager 3.1 software (Bio-Rad). The expression of YWHAZ and TBP was used as reference.

*Analysis of single-nucleus RNA sequencing data*

Single-nucleus RNA-sequencing (snRNA-seq) human first trimester villi datasets were accessed from Zenodo (10.5281/zenodo.8159511) and used without additional preprocessing. Matrices were loaded into R (v4.1.2) and LAIR2 expression visualized using Seurat (v4.1.0). Trophoblast nuclei (n = 58, 198 nuclei) were subsetted and used to model the differentiation trajectory of the trophoblast in-silico. Trophoblast datasets were harmonised with the ‘sctransform’ package (v0.3.3). Linear dimensionality reduction was performed on the 4,000 most variable genes and principal components (PCs) calculated. Using the first 30 PCs, high dimensional latent space was reduced and visualized by UMAP. Louvain clusters were computed including a random seed for reproducibility. A resolution of 0.4, yielding 11 clusters was chosen. Count matrices and associated metadata from Seurat were used to create a SingleCellExperiment object (v.1.22.0). The trajectory was modelled on the subsetted UMAP graph using the slingshot package (v2.2.1) with the starting cluster set to cluster 5 based on expression patterns of proliferation markers *TOP2A* and *MKI67*. The package identifies global structure with a cluster-based minimum spanning tree and fits simultaneous principal curves to describe each inferred lineage.

Transition genes were calculated as described in Chen et al. [18]. Genes with a log2FC ± 0.25 between the first 20% and last 20% of nuclei in the inferred pseudotime were scaled between 0 and 1. A gene‑wise Spearman correlation against pseudotime values was fitted using the stats package (v4.1.2). Genes with a correlation coefficient of ± 0.4 were identified as transition genes. Endpoint DEGs per lineage were empirically calculated using a generalized linear model likelihood ratio test via the edgeR package (v3.36.0), controlling for potential outliers and variability across samples. Genes with an FDR (Benjamini-Hochberg) < 0.01 and logFC ± 0.25 were identified to demarcate start and end phenotypes.

*In situ Padlock probe analysis*

Sequences for padlock probe design were obtained from the National Center for Biotechnology Information (NCBI) with the GenBank accession number NM\_002288.6 (LAIR2). In brief, cDNA was produced using specific reverse transcription primers. Afterwards, padlock probes were hybridized to the cDNA. After ligation, circularized padlock probes were subjected to amplification through rolling circle amplification, and visualization with fluorescently labelled detection probes in the channel TexasRed (LAIR2). As internal positive control served human actin beta (ACTB), which was detected with atto488 fluorescent detection probes. All oligonucleotide sequences are available in Supplemental Table 3. Images were analysed using Visiopharm (Version 2021.9. Visiopharm A/S, Hørsholm, Denmark). Single spheroid sections were analysed by splitting each spheroid cross section into an outer ring area with a thickness of 60 µm and an inner area covering the rest of the spheroid cross section leaving out holes without any detected nuclei. The acquired outer spheroid area was used to assess nuclei and signal counts. Nuclei were detected using the “Nuclei Detection, AI” app within Visiopharm based on a DAPI stain. Atto488 (ACTB) and TexasRed (LAIR2) signals as well as anchor signals in Cy7 were enhanced using a polynomial blob filter after which a threshold was applied on theses filtered images in order to get single signal objects. Signals below an area of 0.3 µm² were excluded. Atto488 (ACTB) and TexasRed (LAIR2) signals that did not align with an anchor signal in Cy7 were excluded as false positive signals.

*Primary trophoblast isolation and organoid formation*

First trimester placental tissue was obtained from Auckland Medical Aid Centre, Auckland, New Zealand, following ethical approval by the Northern X Ethics Committee (NTX/12/06/057/AM012) and written informed consent. Primary mononuclear villous trophoblasts were isolated and cultured as organoids as previously described with minor changes [19]. In brief, 1 g of placental villi from first trimester placental tissue (6+0 - 8+4 weeks of gestation, n=3) was cut into small pieces and enzymatically digested with 10 ml of Digestion Solution containing 1.5 mg/ml DNase I (Sigma-Aldrich, Burlington, MA, USA) and 0.25% Trypsin (Gibco, Auckland, New Zealand) in PBS for 10 min at 37°C. Supernatant was discarded and villi were washed with PBS until the supernatant remained clear. A second enzymatic digest was performed with 10 ml of the same Digestion Solution as before overnight at 4°C. The following day supernatant was filtered through a 70 µm mesh filter and washed 10 times with 20 ml PBS. After centrifugation at 450 x g for 8 minutes at RT cell pellets were resuspended in Trophoblast Organoid Medium (TOM) consisting of Advanced DMEM/F12 (1X, Gibco) containing B-27 supplement (50X without vitamin A, Gibco), N‑2 supplement (100X, Gibco), 10 mM HEPES (1 M, Gibco), 2 mM L-glutamine (200 mM, Gibco), 0.5% penicillin-streptomycin (Gibco), 3 µM CHIR99021 (Sigma Aldrich), 100 ng/ml rhEGF (Abcam) and 1 µM A83-01 (Sigma Aldrich). After repeated centrifugation 450 x g for 8 minutes at RT, CTBs were collected in fresh TOM and counted. Cells were resuspended in the appropriate amount of TOM (40% v/v) and Cultrex® Reduced Growth Factor Basement Membrane Extract, Type R1 (60% v/v) (R&D Systems, Minneapolis, MN, USA). In each well of a 24-well plate (Multiwell 24 well, Falcon®, Corning Incorporated, Corning, NY, USA) 1.0 x 105 cells in 40 µl of cell/cultrex mix were placed and incubated for 1 min before turning the plate upside down to evenly distribute cells in forming domes. After 15 min plates were turned back to the right side and domes were overlaid with TOM and incubated at 37°C and 5% CO2. Organoids formed over the next 20-30 days depending on the patient sample. When most of the organoids reached a size of 200‑500 µm, organoids were either stimulated to undergo differentiation towards the EVT lineage or maintained in Trophoblast Organoid Media (TOM). To stimulate EVT differentiation TOM was exchanged for extravillous trophoblast medium 1 (EVTM1, see Supplementary Material) consisting of Advanced DMEM/F-12 supplemented with 4% KnockOut™ SR (1x, Gibco), B-27 supplement (1x, Gibco), N-2 supplement (1X, Gibco), 10 mM HEPES (1 M, Gibco), 2 mM L-glutamine (200 mM, Gibco), 0.5% penicillin-streptomycin (Gibco), 7.5 µM A83-01 (Sigma Aldrich) and 100 ng/ml NRG-1 (Abcam).

*ELISA*

LAIR2 levels were measured from pooled protein lysates of ACH-3P spheroids treated in presence or absence of PR. Twelve spheroids were pooled and lysed in RIPA buffer (Sigma-Aldrich) including protease inhibitor cocktail (Roche Diagnostics; Mannheim, Germany) and phosSTOP (Roche Diagnostics). Cell lysates were centrifuged at 8000 rpm at 4°C for 10 min. Total protein concentration was determined in clear supernatants using the Lowry method. Samples were diluted 1:4 and measured in duplicates using a LAIR2 ELISA kit (ELH-LAIR2, RayBiotech Life Inc., USA), according to manufacturer’s manual. Absorbance was measured using a Spark 10M Multimode Microplate Reader (Tecan; Maennedorf, Switzerland) at 450 nm wavelength. Mean absorbance was calculated and concentrations were determined via standard calibration curve and normalized to total protein concentration.

*Hematoxylin and eosin staining*

Placenta tissue sections were deparaffinized and incubated in acid hemalaun solution for 10 min according to Mayer (Hematoxylin monohydrate, Merck). Afterwards, sections were incubated shortly in 1 % ammonia water (Ammonia solution 25 %, Merck). Slides were transferred to 1 % aqueous eosin (Eosin Y (yellowish), Sigma-Aldrich) for 1 min and permanently mounted with Cytoseal (Glas™ Tissue Mount™, Tissue-Tek®, Sakura Finetek Germany GmbH, Umkirch, Germany) and a cover slip. Images were obtained with an Evident Olympus VS200 Slide Scanner (Evident Corporation, Tokyo, Japan).

*Immunofluorescence staining*

For immunofluorescence double staining, 5 μm sections were mounted on Superfrost Plus slides (Thermo Fisher Scientific), and subjected to antigen retrieval. Afterwards, slides were blocked with Ultra V block (Thermo Fisher Scientific) for 10 min at RT. Primary antibodies (Supplemental Table 4) were diluted in antibody diluent (Dako) and incubated on slides for 45 min at RT. Slides were washed three times in PBS. Secondary fluorescence-labelled antibodies (Supplemental Table 4) were diluted in PBS (1:200) and incubated on slides for 30 min at RT. After washing three times with PBS, DAPI was incubated (1:2000 in PBS) on the slides for 5 min. Slides were washed three times with PBS, left to dry and mounted with ProLong Gold Antifade reagent (Invitrogen). Images were obtained with an Evident Olympus VS200 Slide Scanner or an Evident FV3000 Confocal Laser Scanning Microscope (Evident).

*Platelet aggregometry*

Aggregometry of isolated platelets from pregnant women was assessed using impedance measurements with Chrono-log Model 700 Aggregometer (Chrono-Log Corporation; Havertown, PA, USA). 1 ml platelet suspension was pre-warmed to 37°C before probe with electrode was inserted. First, the baseline was established until a stable electrical current was measured. For activation either Thrombin Receptor Activator Peptide 6 (TRAP-6; HY-P0078, MedChemExpress; Monmouth Junction, NJ, USA) with a final concentration of 50 µM, or collagen type I (Col1; Chrono-Log Corporation) with a final concentration of 1 µg/ml were added to the platelet suspension. To measure the influence of LAIR2 on platelet activation, agonists were pre-incubated with or without 1 µg/ml recombinant human (rh)LAIR2 protein (ab182705, abcam) for 30 minutes at RT before measurements. The aggregation was measured by monitoring the increasing electrical resistance (ohm) in the electrodes over a period of 6 min. Experiments were repeated with platelets from five individual donors (see Supplemental Table 1).

*Invasion assay*

To measure the invasion ability of the ACH-3P cells through an extracellular matrix (ECM), aQCM*™* 96-Well Cell Invasion Assay (ECM555, Merck) was performed according to manufacturer’s protocol. In short, 1x105 cells were seeded per chamber with or without 1 µg/ml rhLAIR2 protein (ab182705, abcam). Additionally, we used cells which underwent siRNA-mediated knockdown of LAIR2 expression prior to this assay. For positive controls the same number of cells was seeded and incubated simultaneously without the invasion insert. After 24 hours, cells were detached from the bottom of the chamber, lysed and stained with CyQuant GR Dye provided in the kit. Finally, samples were measured with a fluorescence plate reader CLARIOStar (BMG LABTECH GmbH, Ortenberg, Germany) 15 minutes after adding Lysis Buffer/Dye Solution to each well, preceded by shaking at 500 rpm. A settling time of 0.2 seconds and a flash mode of 40 flashes per well were used, along with top optic settings and a focal height of 5.2 mm. All measurements were performed with an emission spectrum ranging from 505‑535 nm, using a gain of 1100.

**Supplementary Table S1**

*Characteristics of the study groups*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristic | | PR pool  (n=13) | PR pool  (n=11) | PR  (n=3) | aggregometry  (n=5) | co-culture  (n=5) |
| Maternal age | [years] | 33.5 (6.2) | 32.4 (5.5) | 25.0 (1.4) | 31,0 (5.1) | 32.2 (2.7) |
| Maternal BMI | [kg/m2] | 23.1 (4.2) | 21.9 (2.5) | 24.1 (2.1) | 23.4 (1.6) | 24.1 (2.0) |
| Gestational age | [days] | 270 (8) | 270 (5) | 270 (2) | 275 (5) | 273 (6) |
| Platelet count | [x103 platelets/µl] | 163 (44) |  | 138 (62) | 179 (36) | 188 (32) |

Data are presented as mean with SD.

**Supplementary Table S2**

*qPCR Primer sequences*

|  |  |  |
| --- | --- | --- |
| **Gene** | **forward (5’🡪3’)** | **reverse (5’🡪3’)** |
| *HAND1* | AACTCAAGAAGGCGGATGGC | CAGGGCAGGAGGAAAACCT |
| *ITGA2* | TTAGCGCTCAGTCAAGGCAT | CGGTTCTCAGGAAAGCCACT |
| *ITGA3* | GTGCTTACAACTGGAAAGGAAACA | AAGCTGCCTACCTGCATCGT |
| *LAIR2* | AGACCATCCACACGCAGG | CCTCTCCAGGCGGAATGTTT |
| *MEST* | GTTGTGCTTTTACACGGTTTT | AGTGATGTGGTCTCGGTTTG |
| *MMP15* | TACGAGTGAAAGCCAACCTG | CCGTGTAGTTCTGGATGCTAA |
| *NLRP2* | CAAGGCAATGACCAGGATGA | AACAAGACACCAACCCGAGA |
| *NLRP7* | GCTCGTGGATTGTGGATTCTC | TTCATAGGTCTTCAACCGTAGG |
| *TBP* | TGACCCAGCATCACTGTTTC | CCAGCACACTCTTCTCAGCA |
| *TRPV6* | TCAGAATGGGGGTCACTCG | GAAGGCATAGGTGATGATGAGGA |
| *YWHAZ* | GGTGGCCAATATGGGGATGT | TCCCTTTTTTCCCCGCCAG |

**Supplementary Table S3**

*Materials used for in-situ padlock probe hybridization.*

The + symbol indicates that the following base is LNA (locked nucleic acid) modified. The padlock probe was 5´-phosphorylated and underlined sequences indicate the targeted complement sequence. Atto488 and CY7 are fluorescent labels.

|  |  |  |
| --- | --- | --- |
|  |  | Sequence 5´🡪3´ |
| **Primer** | ACTB\_LNA | C+GG+GC+GG+CG+GATCGGCAAAG |
|  | RV\_LAIR2\_1 | GAATGTTTGAACCCC |
|  | RV\_LAIR2\_2 | ATCCTCCCTCTCCAG |
|  | RV\_LAIR2\_3 | GAGGCAGCGATAAAG |
|  | RV\_LAIR2\_4 | TTCACCAGCAGCTCC |
|  | RV\_LAIR2\_5 | GGCCTCCAGAGCTTT |
|  | RV\_LAIR2\_6 | TCTCCCCATTGAAGT |
|  | RV\_LAIR2\_7 | GCATCTGTGCATTTCT |
| **Padlock Probe** | plp\_ACTB | /5Phos/AGCCTCGCCTTTGCC**TCTACGAGTTTGCAGTCACGTGCGTCTATTTAGTGGAGCC**GGTTGCTACGATGACTCACGCCCCGCGAGCACAG |
|  | plp\_LAIR2\_1 | /5Phos/CGGGGCCCGGTTGGG**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**CACCTTACACGAAGCAATGGTGACTTTCATGTGC |
|  | plp\_LAIR2\_2 | /5Phos/AGGAAATGCCGGGCT**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**CACCTTACACGAAGCAATGTGACTCAGTAAGTGA |
|  | plp\_LAIR2\_3 | /5Phos/CTGAGCACAGTGACT**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**CACCTTACACGAAGCAATGAGCCCCCTGGATGGT |
|  | plp\_LAIR2\_4 | /5Phos/CCGTCTTGTGAACTT**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**CACCTTACACGAAGCAATGAGGAGAAATGGCCTC |
| **Detection Oligos** | D1 | Atto488-TCTACGAGTTTGCAGTCACG |
|  | D2 | CY7-UGCGUCUAUUUAGUGGAGCC |
|  | D3 | TexasRed-CAGTGAATGCGAGTCCGTCT |

**Supplementary Table S4**

*Antibodies used for staining*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| antibody | clone / order # | company | species | AG retrieval | Dilution IHC | Dilution IF |
| CK7 | DB 051 | DB Biotech | rabbit | pH9 | - | 1:100 |
| HLA-G | 4H84 | BD Pharmingen | mouse | pH9 | 1:2000 | 1:1000 |
| LAIR2 | 319701 | Invitrogen | mouse | pH9 | 1:500 | 1:200 |
| CD42b GP1BA | 12860-1-AP | Proteintech Europe | rabbit | pH9 | 1:1000 | - |
| CD31 | ab28364 | abcam | rabbit | pH9 | - | 1:50 |
| COL1A1 | 72026 | Cell Signaling | rabbit | pH6 | 1:200 | - |
| IgG1 |  | Mouse | Dako | pH9 | 1:1000 | - |
| Alexa Fluor 555 |  | Invitrogen | mouse | - | - | 1:200 |
| Alexa Fluor 633 |  | Invitrogen | rabbit | - | - | 1:200 |
| DAPI |  | Invitrogen | - | - | - | 1:2000 |

**Supplementary figure legends**

**Supplementary Figure S1**

Dot plot, visualizing selected GO terms from a gene set enrichment analysis comparing PR treated to control ACH-3P spheroids. The term, gene ratio signifies the number of core enriched genes compared to the total amount of genes associated with a GO term. The size of dots and their color depend on the number of core enriched genes and the adjusted p-value respectively.

**Supplementary Figure S2**

Immunohistochemistry for CD42b confirmed the platelet origin of the present cells (**a**). Platelets seemed to be negative for LAIR2, showing no to very faint staining (**b**). Mouse IgG served as a negative control (**c**). Scale bars represent 20 µm

**References**

[1] E. Afgan, D. Baker, B. Batut, M. van den Beek, D. Bouvier, M. Čech, J. Chilton, D. Clements, N. Coraor, B.A. Grüning, A. Guerler, J. Hillman-Jackson, S. Hiltemann, V. Jalili, H. Rasche, N. Soranzo, J. Goecks, J. Taylor, A. Nekrutenko, D. Blankenberg, The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update, Nucleic Acids Res. 46 (2018) W537–W544. https://doi.org/10.1093/nar/gky379.

[2] S. Andrews, Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data, (2010). https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

[3] P. Ewels, M. Magnusson, S. Lundin, M. Käller, MultiQC: summarize analysis results for multiple tools and samples in a single report, Bioinformatics 32 (2016) 3047–3048. https://doi.org/10.1093/bioinformatics/btw354.

[4] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinforma. Oxf. Engl. 30 (2014) 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

[5] A. Frankish, M. Diekhans, A.-M. Ferreira, R. Johnson, I. Jungreis, J. Loveland, J.M. Mudge, C. Sisu, J. Wright, J. Armstrong, I. Barnes, A. Berry, A. Bignell, S. Carbonell Sala, J. Chrast, F. Cunningham, T. Di Domenico, S. Donaldson, I.T. Fiddes, C. García Girón, J.M. Gonzalez, T. Grego, M. Hardy, T. Hourlier, T. Hunt, O.G. Izuogu, J. Lagarde, F.J. Martin, L. Martínez, S. Mohanan, P. Muir, F.C.P. Navarro, A. Parker, B. Pei, F. Pozo, M. Ruffier, B.M. Schmitt, E. Stapleton, M.-M. Suner, I. Sycheva, B. Uszczynska-Ratajczak, J. Xu, A. Yates, D. Zerbino, Y. Zhang, B. Aken, J.S. Choudhary, M. Gerstein, R. Guigó, T.J.P. Hubbard, M. Kellis, B. Paten, A. Reymond, M.L. Tress, P. Flicek, GENCODE reference annotation for the human and mouse genomes, Nucleic Acids Res. 47 (2019) D766–D773. https://doi.org/10.1093/nar/gky955.

[6] A. Dobin, C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, T.R. Gingeras, STAR: ultrafast universal RNA-seq aligner., Bioinforma. Oxf. Engl. 29 (2013) 15–21. https://doi.org/10.1093/bioinformatics/bts635.

[7] F. García-Alcalde, K. Okonechnikov, J. Carbonell, L.M. Cruz, S. Götz, S. Tarazona, J. Dopazo, T.F. Meyer, A. Conesa, Qualimap: evaluating next-generation sequencing alignment data, Bioinforma. Oxf. Engl. 28 (2012) 2678–2679. https://doi.org/10.1093/bioinformatics/bts503.

[8] S. Anders, P.T. Pyl, W. Huber, HTSeq--a Python framework to work with high-throughput sequencing data, Bioinforma. Oxf. Engl. 31 (2015) 166–169. https://doi.org/10.1093/bioinformatics/btu638.

[9] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2018.

[10] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 550. https://doi.org/10.1186/s13059-014-0550-8.

[11] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, 1st ed., Springer-Verlag New York, New York, 2016.

[12] K. Blighe, S. Rana, M. Lewis, EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling., R Package Version 1140 (2022). https://github.com/kevinblighe/EnhancedVolcano.

[13] M.I. Love, S. Anders, V. Kim, W. Huber, RNA-Seq workflow: gene-level exploratory analysis and differential expression, F1000Research 4 (2016) 1070. https://doi.org/10.12688/f1000research.7035.2.

[14] R. Kolde, pheatmap, (2019). https://cran.r-project.org/web/packages/pheatmap/index.html.

[15] T. Wu, E. Hu, S. Xu, M. Chen, P. Guo, Z. Dai, T. Feng, L. Zhou, W. Tang, L. Zhan, X. Fu, S. Liu, X. Bo, G. Yu, clusterProfiler 4.0: A universal enrichment tool for interpreting omics data, The Innovation 2 (2021) 100141. https://doi.org/10.1016/j.xinn.2021.100141.

[16] G. Yu, L.-G. Wang, G.-R. Yan, Q.-Y. He, DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis, Bioinformatics 31 (2015) 608–609. https://doi.org/10.1093/bioinformatics/btu684.

[17] M. Carlson, org.Hs.eg.db: Genome wide annotation for Human. R package version 3.19.1., (2024). https://bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html.

[18] H. Chen, L. Albergante, J.Y. Hsu, C.A. Lareau, G. Lo Bosco, J. Guan, S. Zhou, A.N. Gorban, D.E. Bauer, M.J. Aryee, D.M. Langenau, A. Zinovyev, J.D. Buenrostro, G.-C. Yuan, L. Pinello, Single-cell trajectories reconstruction, exploration and mapping of omics data with STREAM, Nat. Commun. 10 (2019) 1903. https://doi.org/10.1038/s41467-019-09670-4.

[19] C. Sun, L.W. Chamley, J.L. James, Organoid generation from trophoblast stem cells highlights distinct roles for cytotrophoblasts and stem cells in organoid formation and expansion., Placenta (2024) S0143400424007914. https://doi.org/10.1016/j.placenta.2024.12.003.