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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: No code was used for data collection.

Data analysis	Western blot signals were visualized using Image Lab 6.0 software. Immunofluorescence was digitally photographed using CellP (Olympus). Single-cell multiome sequencing reads were processed with Cellranger Arc in Cumulus on the Terra platform (https://app.terra.bio/) using the Human GRCh38 sequences (GENCODE v32/Ensembl 98), cellranger arc reference 2.0.0. For the integrated analysis of single-cell ATAC-seq and RNA-seq data, the ArchR package was employed. To address batch effects, the Harmony algorithm (version 0.1.1) was applied to Latent Semantic Indexing (LSI) reduced-dimensionality datasets for both RNA and ATAC-seq. For subclustering cells, they were filtered and included in new ArchR objects using the BiocGenerics package. To distinguish cells with maternal or fetal origin, we used Freemuxlet, a genotype-free demultiplexing pipeline. For GWAS analysis, we used Sc-linker with Roadmap Epigenomics and Activity-By-Contact, as well as stratified LD score regression using ENCODE. Secondary analyses were performed with MAGMA. Slide-tags analysis was performed with Cell Ranger-arc v2.0.2 mkfastq to generate demultiplexed FASTQ files from the raw sequencing reads. Each Cell Ranger called cell was mapped to a spatial coordinate by using DBSCAN. SCTransform (version 0.3.5) was used to facilitate normalization. Chromatin accessibility peaks were initially discerned using MACS2 (version 2.2.7.1). Transcription factor motifs and their activities were visualized and assessed at the cellular level using ChromVAR. Lineage drivers and transition probabilities were discerned using CellRank. For STARmap, images for individual tiles were deconvoluted using Huygens (23.04). Image registration was performed using 3D Fourier Transform through numpy.fft and Scipy. Starfish v0.2.2 was used to process sequencing signals. ClusterMap (v0.0.1) was employed for 2D cell segmentation of identified transcripts. Segmented cells from individual tiles were integrated for subsequent single-cell analysis using Scanpy (version 1.9.4). Label transfer and imputation was performed using Seurat v4. We employed CellChat (version 1.5.0) to infer cell-cell interactions. Code for comprehensive analysis and figure generation as described here can be found at https://github.com/jian-shu-lab/hPlacenta-architecture.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data related to this study are available at the Broad Institute Single Cell Portal https://singlecell.broadinstitute.org/ under the accession number: SCP2601 (snRNA-seq, snATAC-sesq, Slide-Tags), and Zenodo https://zenodo.org/ under the accession number: 10981713 (STARmap). Unprocessed and uncropped western blots are available as source data. Public datasets used in this study include: Human GRCh38 sequences (https://www.gencodegenes.org/human/release_32.html), 1000 Genome Project (https://www.internationalgenome.org/data), CISBP database (https://cisbp.ccbr.utoronto.ca/), UK-Biobank (http://www.ukbiobank.ac.uk/register-apply; other utilized placental GWAS studies can be found in citations 78-80), Roadmap Epigenomics (http://www.roadmapepigenomics.org), Activity-By-Contact (https://www.engreitzlab.org/resources), and ENCODE (https://www.encodeproject.org/help/project-overview/).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	First trimester placental tissue was obtained from legal pregnancy terminations by participants of female sex with approval from ethics boards and informed consent. Sex of fetal cells from placental tissue was determined via Y chromosome gene expression; further sex-based analyses were not undertaken as the primary focus of the study was to serve as a resource for single-cell omics characterization of the human placenta.
Reporting on race, ethnicity, or other socially relevant groupings	No race, ethnicity, or other socially relevant groupings were used for this study.
Population characteristics	No population characteristics were relevant for this study.
Recruitment	First trimester placental tissue was obtained from legal pregnancy terminations. The recruitment process includes the following procedure: participants were fully informed by the clinicians. If they were willing to donate the placental material, written informed consent was signed by the participants. Self-selection was only based on the gestational weeks to provide a range alongside the first trimester; this selection restricts the study to the first trimester of pregnancy. Other forms of selection were not conducted. Placental tissue was then processed within 2 hours after collection.
Ethics oversight	Utilization of tissues and experimental procedures were approved by the ethics boards of the Medical University of Vienna (no. 084/2009). Written informed consent was required from donating women.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We sought to obtain a representative collection of placental samples representing the first trimester of pregnancy after major structures have been formed (weeks 6-11), as well as across multiple different individuals (n=9 donors); we reasoned that this amount of donors would be sufficient given the scarce and precious nature of first trimester tissue. For follow-up experiments including immuno-fluorescence, western blot, siRNA, and qPCR experiments of placental tissue, isolated placental cells, trophoblast organoids, and trophoblast stem cells, we analyzed at least three independent biological sample sets. Exact numbers are given in the respective figure legends.
Data exclusions	Single cells during single-cell analysis were excluded based on stringent filtering criteria (e.g., high mitochondrial reads, high/low gene/UMI counts).
Replication	For each assay performed, we utilized a minimum of 3 donors to ensure that results were replicable across samples from different donors.
Randomization	Samples were not allocated into groups, thus randomization was not relevant to this study.
Blinding	This study did not include any clinical trials, therapeutic evaluation, or quantification of immunodetection-based technologies. (The herein demonstrated immunofluorescence analyses for FOXP1 and SMARCC1 were not quantified but aimed to depict cell-type specific expression that were additionally supported by further experiments such as western blot analysis, STARmap-ISH, and snRNA/ATAC-seq results). As a result of these points and the fact that this was a study aiming to uncover broad genomic underpinnings of the first trimester placenta, blinding researchers would not have had any productive effect on this study; thus, blinding was not utilized and is not relevant to this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary Antibodies: Anti-human FOXP1 (837016), R&D Systems, cat# MAB45341; dilutions 1:200 IF(P), 1:1000 WB. Anti-p63-alpha (D2K8X), Cell Signaling, cat#13109; 1:1000 WB. Anti-HLA-G (4H84), Santa Cruz Biotechnology, cat# sc-21799; dilutions 1:200 IF, 1:1000 WB. Anti-CGB (polyclonal), DAKO, cat# A0231, 1:1000 WB. Anti-GAPDH (14C10), Cell Signaling, cat#2118S; dilution: 1:1000 WB. Anti-ENDOU (polyclonal), Sigma Prestige Antibodies, HPA012388; dilution 1:250 IF(P). Anti-SMARCC1 (named BAF155) (DXD7), Santa Cruz Biotechnology, cat# sc-32763, dilution 1:500 IF (P). Anti-Vimentin (SP20), Abcam, cat# ab16700, dilution 1:200 IF (P). Secondary Antibodies: Anti-mouse 488, Alexa Molecular Probes, cat# A11011, dilution 1:1000 IF(P). Anti-rabbit 568, Alexa Molecular Probes, cat# A21069, dilution 1:1000 IF(P). Anti-mouse HRP, Cell signaling, cat# 7076, 1:10000 WB. Anti-rabbit HRP, Cell signaling, cat# 7074, dilution 1:10000 WB. Antibodies for magnetic bead-based sorting: PE-labelled Anti CD49f/ITGA6 Antibody (GoH3), Biolegend, cat# 313612, dilution 1:20 for magnetic bead-based cell sorting. PE-labeled Anti-HLA-G antibody (MEM-G/9), Exbio, cat# PE1P-292-C100, dilution 1:20 for magnetic bead-based cell sorting.
Validation	Anti-human FOXP1 (R&D, MAB45341) used for IF(P) and WB: The manufacturer provides validation of the antibody for WB, immunocytochemistry, flow cytometry, and immunohistochemistry on paraffin sections (resources.rndsystems.com/pdfs/datasheets/mab45341.pdf?v=20240509). Anti-human SMARCC1 (Santa Cruz Biotechnology) used for IHC(P): The manufacturer provides validation of the antibody for WB, IP, IF, and IHC(P) on human samples. (www.scbt.com/de/p/baf155-antibody-dxd7) Anti-human P63-a (Cell signaling, cat#13109), used for WB: The manufacturer provides validation of the antibody for WB, IF, IP, F,

and ChIP on human samples (www.cellsignal.com/products/primary-antibodies/p63-a-d2k8x-xp-rabbit-mab/13109)
Anti-HLA-G (Santa Cruz Biotechnology, sc-21799) used for WB: The manufacturer provides validation of the antibody for WB, IP, IF, and IHC(P) on human samples (www.scbt.com/de/p/hla-g-antibody-4h84)
CGB (DAKO, A0231) used for WB: Labome (the world of laboratories) provides validation of the antibody for WB and IHC on human samples (www.labome.com/product/Dako/A0231.html)
Anti-ENDOU (Sigma Pestige Antibodies, HPA012388) used if IF(P): This antibody belongs to the enhanced validation antibodies (www.sigmaaldrich.com/AT/de/product/sigma/hpa012388)
Anti-Vimentin (Abcam, ab16700) used for IF(P): The manufacturer provides validation of the antibody for flow cytometry, WB, IHC(P), and ICC/IF on human samples (www.abcam.com/en-at/products/primary-antibodies/vimentin-antibody-sp20-ab16700)
Anti-GAPDH (Cell signaling, 2118) used for WB: The manufacturer provides validation of the antibody for WB, IHC, IF, and F on human samples (www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118)
Anti-CD49f Antibody (Biolegend, 313612) used for magnetic bead-based sorting: The manufacturer validates the FC reactivity with CD49f/ITGA6 on human samples (www.biolegend.com/de-at/products/pe-anti-human-mouse-cd49f-antibody-4108?GroupID=BLG10711). The enrichment of vCTBs (ITGA6-expressing trophoblast) by using this antibody is verified by WB (Figure 2A), by the enriched expression of P63 (vCTB marker), and the absence of HLA-G and CGB (markers for EVTs and STBs, respectively).
Anti-HLA-G-PE (Exbio, PE1P-292-C100) used for magnetic bead-based sorting: The manufacturer provides validation of the antibody for flow cytometry on human samples (www.exbio.cz/research-product/antibodies/mhc-antigens/anti-hla-g-pe-2?q=0.1+mg). The enrichment of EVTs (HLA-G-expressing trophoblast) by using this antibody is verified by WB (Figure 2A), by the enriched expression of HLA-G (EVT marker), and the absence of P63 and CGB (markers for vCTBs and STBs, respectively).
Trophoblast and other placental markers P63 (vCTB), HLA-G (EVT), CGB (STB), VIM (Stroma) as well as magnetic bead-based cell sorting of trophoblasts are cited multiple times and detected by above described antibodies in previous publications, e.g. doi: 10.1073/pnas.2120667119; doi: 10.1016/j.stemcr.2018.07.004; doi: 10.1073/pnas.2002630117; doi: 10.1073/pnas.1612335113, doi: 10.1016/j.placenta.2023.01.005. Routinely, in IF(P), primary antibodies were either replaced with appropriate isotype controls from Cell Signaling (rabbit mAb IgG isotype control cat#2729, dilution 1:100; mouse mAb IgG1 isotype control cat# 5415, 1:100; mouse mAb IgG2a isotype control, cat# 61656; and mouse mAb IgG2b isotype control cat#53484, 1:100), or with antibody dilution buffer to exclude unspecific expression signals provoked by the secondary antibodies.

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A