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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Software used include: R, Python 3, SQLAlchemy, PostgreSQL, HISAT2, 10X Genomics' Cell Ranger, Seurat, Monocle 2, Cytoscape, Demuxlet, HTSeq-count, Samtools, GATK, BWA, Picard, bcftools, TraCeR, Kallisto, MMSEQ, BD FACS DIVA software, Axiovision and ZEN software (zeiss), Graphpad, Photoshop, Illustrator. Detailed parameters of each of the methods are mentioned in relevant sections in Methods.
Data analysis	Software used include: R, Python 3, SQLAlchemy, PostgreSQL, HISAT2, 10X Genomics' Cell Ranger, Seurat, Monocle 2, Cytoscape, Demuxlet, HTSeq-count, Samtools, GATK, BWA, Picard, bcftools, TraCeR, Kallisto, MMSEQ, Graphpad, Axiovision and ZEN software (zeiss), FlowJo, Photoshop, Illustrator. Detailed parameters of each of the methods are mentioned in relevant sections in Methods.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data is uploaded to ArrayExpress:
Experiment: E-MTAB-6701, E-MTAB-6678, E-MTAB-7304

Field-specific reporting

Please select 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

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical methods were used to predetermine sample size. We followed standards in the field and Human Cell Atlas criteria.
Data exclusions	No exclusion was applied to the uploaded raw data in ArrayExpress. For the final count matrix, we excluded cells based on pre-established criteria for single-cells: we excluded low quality samples and contaminating cells (i.e. - cells with low number of detected genes and high mitochondria content) - exclusion criteria for each case are comprehensively detailed in the relevant Methods section.
Replication	We did scRNAseq and flow cytometry analysis on 11 deciduas, 5 placentas and 6 matched peripheral blood from 6-14 gestational weeks. Additional samples were used for the validation experiments: (a) Immunohistochemistry: staining of perivascular populations was performed in two independent individuals (Fig. 3b); ACTA2 staining of decidual stromal cells was performed in three independent individuals (Fig. 3c; Extended Data Figure 7a), staining of PDL1 was performed in five independent individuals (Extended Data Figure 9c); (b) smFISH: staining of stromal populations was performed in two independent individuals (Fig. 3d, Extended Data Figure 6b, Extended Data Figure 7b), staining of stromal population in Extended Data Figure 7c was performed in one individual; (c) Flow cytometry: flow cytometry for Figure 4d was performed in 6 of the deciduas sequenced and dNK for two of them were isolated and stained with Giemsa-Wright stain after cytospin 8c ; seven to ten independent individuals were additionally used for flow cytometry analysis on Figure 4f. Any replication experiment was excluded.
Randomization	Only healthy individuals were considered in our analysis. Therefore, any further randomization protocol was required.
Blinding	Only healthy individuals were considered in our analysis. Therefore, no blinding was performed

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

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

Antibodies

Antibodies used	Antibody Company Catalog number Clone Use Dilution (Supplementary table 10) CD45-BUV395 BD Bioscience 563791 Clone HI30 (RUO) Flow cytometry - index data; Fig. 5d 2.5ul:100ul CD94-BV421 BD Bioscience 743948 HP-3D9 (RUO) Flow cytometry - index data 2.5ul:100ul

CD16-BV500 BD Bioscience 561394 3GH Flow cytometry - index data; Fig. 5d 2.5ul:100ul
CD3-BV605 Biolegend 344836 SK7 Flow cytometry - index data; Fig. 5d 2.5ul:100ul
CD4-BV711 Biolegend 300558 RPA-T4 Flow cytometry - index data 2.5ul:100ul
HLA-DR BV786 Biolegend 307642 L243 Flow cytometry - index data 2.5ul:100ul
HLA-G FITC BioRad MCA2044 MEM-G/9 Flow cytometry - index data 2.5ul:100ul
CD56-PE Miltenyi 130-100-622 REA196 Flow cytometry - index data; Fig 5d 2.5ul:100ul
CD14-PECF594 BD Bioscience 562334 PE-CF594 Flow cytometry - index data 2.5ul:100ul
CD9-APC BD Bioscience 341638 M-L13 Flow cytometry - index data 2.5ul:100ul
CD8-AF700 Biolegend 300920 HIT8a Flow cytometry - index data 2.5ul:100ul
CD19-APCCy7 BIoLegend 302217 HIB19 Flow cytometry - index data 2.5ul:100ul
CD20-APCCy7 BIoLegend 302313 2H7 Flow cytometry - index data 2.5ul:100ul
Cytokeratin 7 Dako MA5-11986 OV-TL 12/30 Immunochemistry Dilution:1:200; antigen retrieval buffer:Citrate
PD-L1 Cell Signaling Technology 13684 E13LN Immunochemistry Dilution:1:400; antigen retrieval buffer:TRIS/EDTA
HLA-G Biorad MCA2043 MEM-G1 Immunochemistry Dilution:1:25; antigen retrieval buffer:Citrate
CD146 / MCAM Abcam ab75769 EPR3208 Immunochemistry Dilution:1:2500; antigen retrieval buffer:Citrate
Smooth Muscle Actin Dako M0851 1A4 Immunochemistry Dilution:1:100 antigen retrieval buffer:TRIS/EDTA
NKG2A - Viobright Miltenyi 130-105-646 REA110 Flow cytometry - Fig 5f Dilution: 1:100 in cocktail
KIR2DL2/3/S2 - PE-Cy5.5 Beckman Coulter A66900 GL183 Flow cytometry - Fig 5f Dilution: 1:50 in cocktail
KIR2DS4 - APC Miltenyi 130-114-773 REA860 Flow cytometry - Fig 5f Dilution: 1:50 in cocktail
GzmB - AF700 BD Biosciences 560213 GB11 Flow cytometry - Fig 5f Dilution: 1:100 in intracellular cocktail
KIR2DL1 - APC-Vio770 Miltenyi 130-103-937 REA284 Flow cytometry - Fig 5f Dilution: 1:25 in cocktail
Perforin - BV421 Biolegend 308122 DG9 Flow cytometry - Fig 5f Dilution: 1:100 in intracellular cocktail
CD3 - BV510 BioLegend 317332 OKT3 Flow cytometry - Fig 5f Dilution: 1:100 in cocktail
CD14 - BV510 BioLegend 301842 M5E2 Flow cytometry - Fig 5f Dilution: 1:100 in cocktail
CD19 – BV510 BioLegend 302242 HIB19 Flow cytometry - Fig 5f Dilution: 1:100 in cocktail
LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit Thermofisher L34957 N/A Flow cytometry - Fig 5f Dilution: 1:100 in secondary cocktail (from 200ul dilution of stock powder)
KIR2DL3 biotin - biotin Miltenyi 130-100-126 REA147 Flow cytometry - Fig 5f Dilution: 1:100 in cocktail
streptavidin Qdot605 (detection of KIR2DL3)- Q605 Thermofisher Q10103MP N/A Flow cytometry - Fig 5f Dilution: 1:250 in secondary cocktail
Granulysin - PE Biolegend 348003 DH2 Flow cytometry - Fig 5f Dilution: 1:200 in intracellular cocktail
CD56-PE-Dazzle BioLegend 318348 HCD56 Flow cytometry - Fig 5f Dilution: 1:200 in intracellular cocktail
GzmA - PE-Cy7 ebioscience/Thermo Fischer 25-9177-42 CB9 Flow cytometry - Fig 5f Dilution: 1:100 in intracellular cocktail
CD49a-BV421 Bd Bioscience 742357 SR84 (RUO) Flow cytometry - Fig 5d 2.5ul:100ul
CD14- BV605 Bd Bioscience 564054 M5E2 (RUO) Flow cytometry - Fig 5d 2.5ul:100ul
CD19-BV605 Bd Bioscience 740394 HIB19 (RUO) Flow cytometry - Fig 5d 2.5ul:100ul
CD20-BV605 Bd Bioscience 747736 2H7 (RUO) Flow cytometry - Fig 5d 2.5ul:100ul
CD103-PerCP-Cy5.5 Biolegend 350225 Ber-ACT8 Flow cytometry - Fig 5d 2.5ul:100ul
KIR2DL1 - PeVio770 MACS - Miltenyi Biotec 130-103-936 REA284 Flow cytometry - Fig 5d 2.5ul:100ul
CD18 (ITGB2)- APC BioLegend 366307 CBR LFA-1/2 Flow cytometry - Fig 5d 2.5ul:100ul
CD39 -APC Cy7 BioLegend 328225 A1 Flow cytometry - Fig 5d 2.5ul:100ul

Validation

Antibody Validation (Supplementary table 10)
CD45-BUV395 Flow cytometry (Routinely Tested) . Flow cytometric analysis of CD45 expression on human peripheral blood lymphocytes (website).
CD94-BV421 Flow cytometry (Qualified) (website)
CD16-BV500 Flow cytometry (Routinely Tested). Flow cytometric analysis of CD16 expression on human peripheral blood lymphocytes. (Website)
CD3-BV605 Flow cytometry. Human peripheral blood lymphocytes were stained with CD3 (clone SK7) Brilliant Violet 605™ (website)
CD4-BV711 Flow cytometry. Human peripheral blood lymphocytes were stained with CD4 (clone RPA-T4) Brilliant Violet 711™ (filled histogram) (website)
HLA-DR BV786 Flow cytometry. Human peripheral blood lymphocytes were stained with HLA-DR (clone L243) Brilliant Violet 785™. (website)
HLA-G FITC Evaluation of HLA-G expression on transfected cells by flow cytometry (website)
CD56-PE REAfinity clone is tested against different known clones, in a blocking experiment, to identify whether they recognize completely overlapping (++), partially overlapping (+), or completely different epitopes (-). In a blocking experiment, cells are incubated with an excess of purified unconjugated REAfinity clone followed by staining with conjugated form of the other known clones (website).
CD14-PECF594 Flow cytometry (Routinely Tested). Flow cytometric analysis of CD14 expression on human peripheral blood monocytes (webpage)
CD9-APC Flow cytometry (Routinely Tested) (website). ImmunogenHuman C-ALL Cells.
CD8-AF700 quality control tested by immunofluorescent staining with flow cytometric analysis (website)
CD19-APCCy7 quality tested by flow cytometry (website)
CD20-APCCy7 quality tested by flow cytometry (website)
Cytokeratin 7 Tested in Immunocytochemistry (ICC), Immunofluorescence (IF), Immunohistochemistry (Paraffin) (IHC (P)) and Western Blot (WB)
PD-L1 Tested in Western Blotting, Immunoprecipitation, IHC-Leica® Bond™, Immunohistochemistry (Paraffin), Flow Cytometry (website)
HLA-G Tested in Immunohistochemistry (Paraffin), Western Blotting (website)
CD146 / MCAM Tested in immunofluorescence, immunohistochemestry, Western Blotting, Flow Cytometry (website)
Smooth Muscle Actin Tested in Western Blotting, Immunofluorescence, ELISA, Immunohistochemestry (website)
NKG2A - Viobright Flow cytometry. Human peripheral blood and decidual NK cells used for titration.
KIR2DL2/3/S2 - PE-Cy5.5 Tested by FACS of cell line transfected with gene target
KIR2DS4 - APC Flow cytometry. Human peripheral blood and decidual NK cells used for titration.

GzmB - AF700 Flow cytometry. Human peripheral blood and decidual NK cells used for titration.
KIR2DL1 - APC-Vio770 Tested by FACS of cell line transfected with gene target
Perforin - BV421 Flow cytometry. Human peripheral blood and decidual NK cells used for titration.
CD3 - BV510 Flow cytometry. Human peripheral blood lymphocytes used for titration.
CD14 - BV510 Flow cytometry. Human peripheral blood lymphocytes used for titration.
CD19 – BV510 Flow cytometry. Human peripheral blood lymphocytes used for titration.
Amine reactive dye Flow cytometry. Human peripheral blood lymphocytes used for titration of each batch since diluted aliquotes are stored frozen.
KIR2DL3 biotin - biotin REAfinity clone is tested against different known clones, in a blocking experiment, to identify whether they recognize completely overlapping (++), partially overlapping (+), or completely different epitopes (-). In a blocking experiment, cells are incubated with an excess of purified unconjugated REAfinity clone followed by staining with conjugated form of the other known clones (website).
streptavidin Qdot605 (detection of KIR2DL3)- Q605 Flow cytometry. Human peripheral blood lymphocytes used for titration.
Granulysin - PE Flow cytometry. Human peripheral blood and decidual NK cells used for titration.
CD56-PE-Dazzle Flow cytometry. Human peripheral blood and decidual NK cells used for titration.
GzmA - PE-Cy7 Flow cytometry. Human peripheral blood and decidual NK cells used for titration.
CD49a-BV421 Flow cytometry (Qualified) (website)
CD14- BV605 Human (QC Testing). Flow cytometric analysis of CD14 expression on human peripheral blood monocytes (website)
CD19-BV605 Human (Tested in Development) (website)
CD20-BV605 Human (Tested in Development) (website)
CD103-PerCP-Cy5.5 Flow cytometry (Quality tested) (website)
KIR2DL1 - PeVio770 REAfinity clone is tested against different known clones, in a blocking experiment, to identify whether they recognize completely overlapping (++), partially overlapping (+), or completely different epitopes (-). In a blocking experiment, cells are incubated with an excess of purified unconjugated REAfinity clone followed by staining with conjugated form of the other known clones (website).
CD18 (ITGB2)- APC Flow cytometry (Quality tested) (website)
CD39 -APC Cy7 Flow cytometry (Quality tested) (website)

Human research participants

Policy information about studies involving human research participants

Population characteristics	Fetal, decidual samples and peripheral blood samples were collected from woman undergoing elective terminations. All participants in the study were healthy pregnant woman (6-14 gestational weeks) without any exclusion.
Recruitment	Samples were taken in UK. Ethnicity was not recorded but is expected to be primary caucasian. This is not expected to affect the composition of cells in the maternal–fetal interface during early pregnancy. All tissue samples used for this study were obtained with written informed consent from all participants in accordance with the guidelines in The Declaration of Helsinki 2000 from multiple centres. Human embryo, fetal and decidual samples were obtained from the MRC/WellcomeTrust funded Human Developmental Biology Resource (HDBR36, http://www.hdbr.org) with appropriate maternal written consent and approval from the Newcastle and North Tyneside NHS Health Authority Joint Ethics Committee (08/H0906/21+5). HDBR is regulated by the UK Human Tissue Authority (HTA; www.hta.gov.uk) and operates in accordance with the relevant HTA Codes of Practice. Decidual tissue for smFISH (Extended Data Figure 7c-d) was also covered by this ethics. Peripheral blood from woman undergoing elective terminations were under appropriate maternal written consent and approvals from the Newcastle Academic Health Partners (reference NAHPB-093) and HRA NHS Research Ethics committee North-East-Newcastle North Tyneside 1 (REC reference 12/NE/0395) Decidual tissue for immunohistochemistry (Figure 3b,c and Extended Data Figure 9c) were obtained from elective terminations of normal pregnancies at Addenbrooke's Hospital between 6 and 12 weeks gestation, under ethical approval from the Cambridge Local Research Ethics Committee (04/Q0108/23). Decidual tissue for smFISH (Figure 3d and Extended Data Figure 6b and Extended Data Figure 7b) were obtained from the Newcastle Uteroplacental Tissue Bank and Ethics numbers are Newcastle and North Tyneside Research Ethics Committee 1 Ref:10/H0906/71 and 16/NE/0167.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Decidual and blood cells were incubated at 4°C with 2.5ul of antibodies in 1% FBS in DPBS without Calcium and Magnesium (ThermoFisher Scientific, 14190136). DAPI was used for live/dead discrimination. For intracellular staining of granule proteins, dNK were surface stained for 30 mins in FACS buffer with antibodies. Cells were

	washed with FACS buffer followed by staining with dead cell marker (DCM Aqua) and strepavidinQ605. dNK were then treated with FIX & PERM (Thermofisher) and stained for granule proteins.
Instrument	Becton Dickinson (BD) FACS Aria Fusion. For granule experiment we used LSRFortessa FACS analyser (BD Biosciences)
Software	Becton Dickinson (BD) FACS Aria Fusion was controlled using BD FACS DIVA software (version 7) and FlowJo v10.3 was used for analysis.
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Gating strategy for a panel of 14 antibodies to analyse immune cells in decidual samples by SS2 (CD3, CD4, CD8, CD9, CD14, CD16, CD19, CD20, CD34, CD45, CD56, CD94, DAPI, HLA-DR, HLA-G). Cells isolated for SS2 data were gated on: live; CD19/20-ve, singlets, and the following cell types sorted: i) CD45+, HLA-DR++; ii) CD45+, HLA-DR+; iii) CD45+, HLA-DR-, CD56-, CD3+, CD4+; CD8- iv) CD45+, HLA-DR-, CD56-, CD3+, CD8+; v) CD45+, HLA-DR-, CD56-, CD3+, CD4-, CD8-; vi) CD45+, HLA-DR-, CD3-, CD56-, CD94- (all -); vii) CD45+, HLA-DR-, CD3-, CD56+, CD94-; viii) CD45+, HLA-DR-, CD3, CD56+, CD94+, CD9-; ix) CD45+, HLA-DR-, CD3, CD56+, CD94+, CD9+; x) CD45-, HLA-G+; xi) CD45-, HLA-G-. For the isolation of the three dNK subsets, dNK cells were gated on live, single-cell, LIN- (CD3, CD14, CD19, CD20), CD56+, CD16-, CD49a+. dNK3 were additionally gated on CD103 and ITGB2. dNK1 were additionally gated on CD39 and ITGB2. dNK2 were additionally gated on CD103 and ITGB2. For the detection of granule molecules dNK were gated as CD3-CD14-CD19-, live cells then CD56+NKG2A+ and then KIR+ and KIR- subsets generated using Boolean functions with the gates for all the different KIRs stained (KIR+), and its inverse gate (KIR-).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.