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Supplementary Information for
Conservation at the uterine-placental interface.

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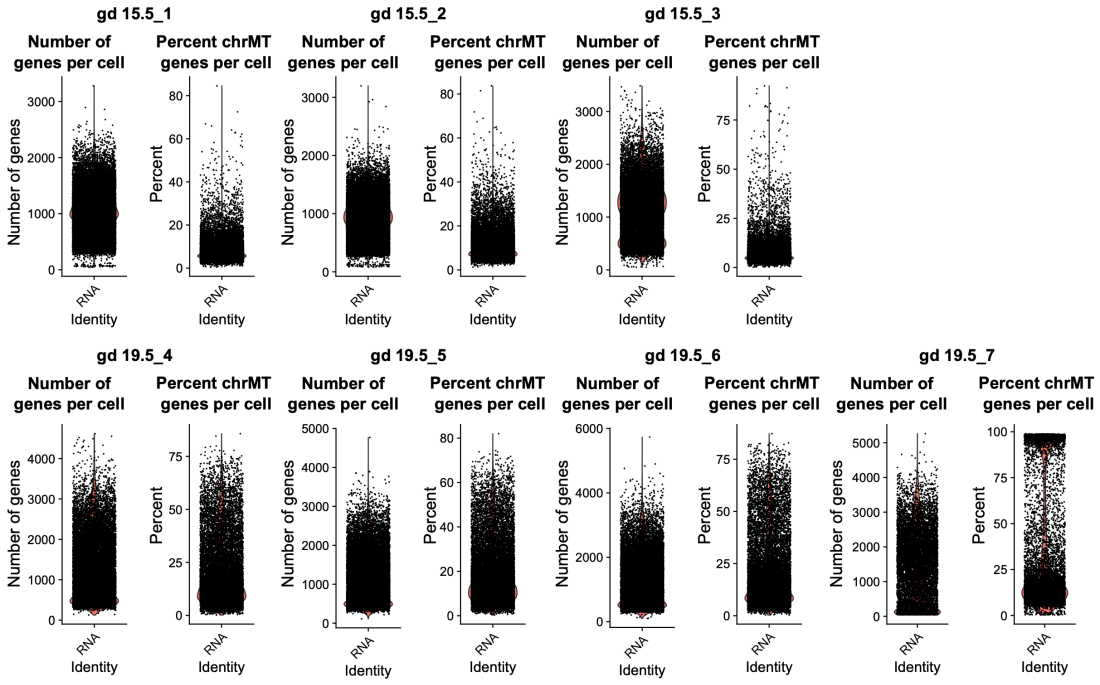
This PDF file includes:

Supplementary text
Figures S1 to S9

Other supplementary materials for this manuscript include the following:

Datasets S1 to S5

A



B

Sample	Before filtering	After filtering
gd 15.5_1	17769 genes across 29487 cells	17769 genes across 25384 cells
gd 15.5_2	17663 genes across 30757 cells	17663 genes across 24845 cells
gd 15.5_3	17649 genes across 18338 cells	17649 genes across 15613 cells
gd 19.5_4	18547 genes across 15401 cells	18547 genes across 8664 cells
gd 19.5_5	18283 genes across 18388 cells	18283 genes across 10641 cells
gd 19.5_6	18606 genes across 15444 cells	18606 genes across 8750 cells
gd 19.5_7	17727 genes across 10391 cells	17727 genes across 5562 cells

C

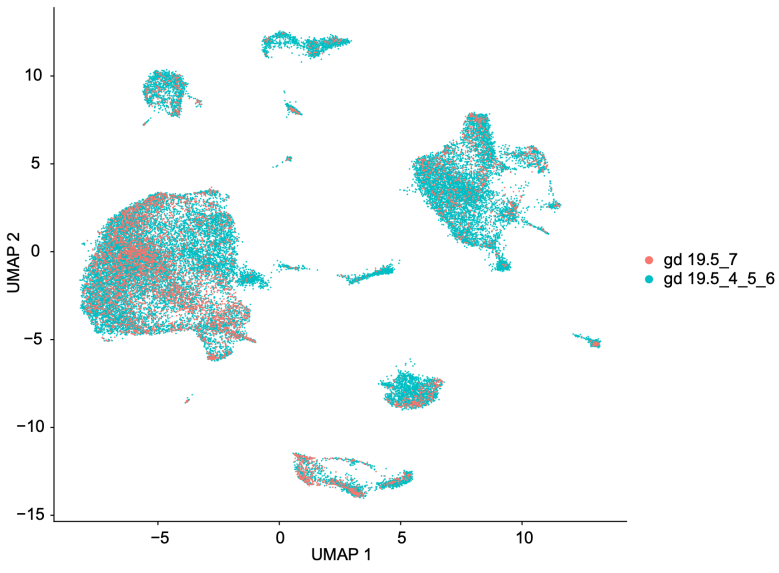
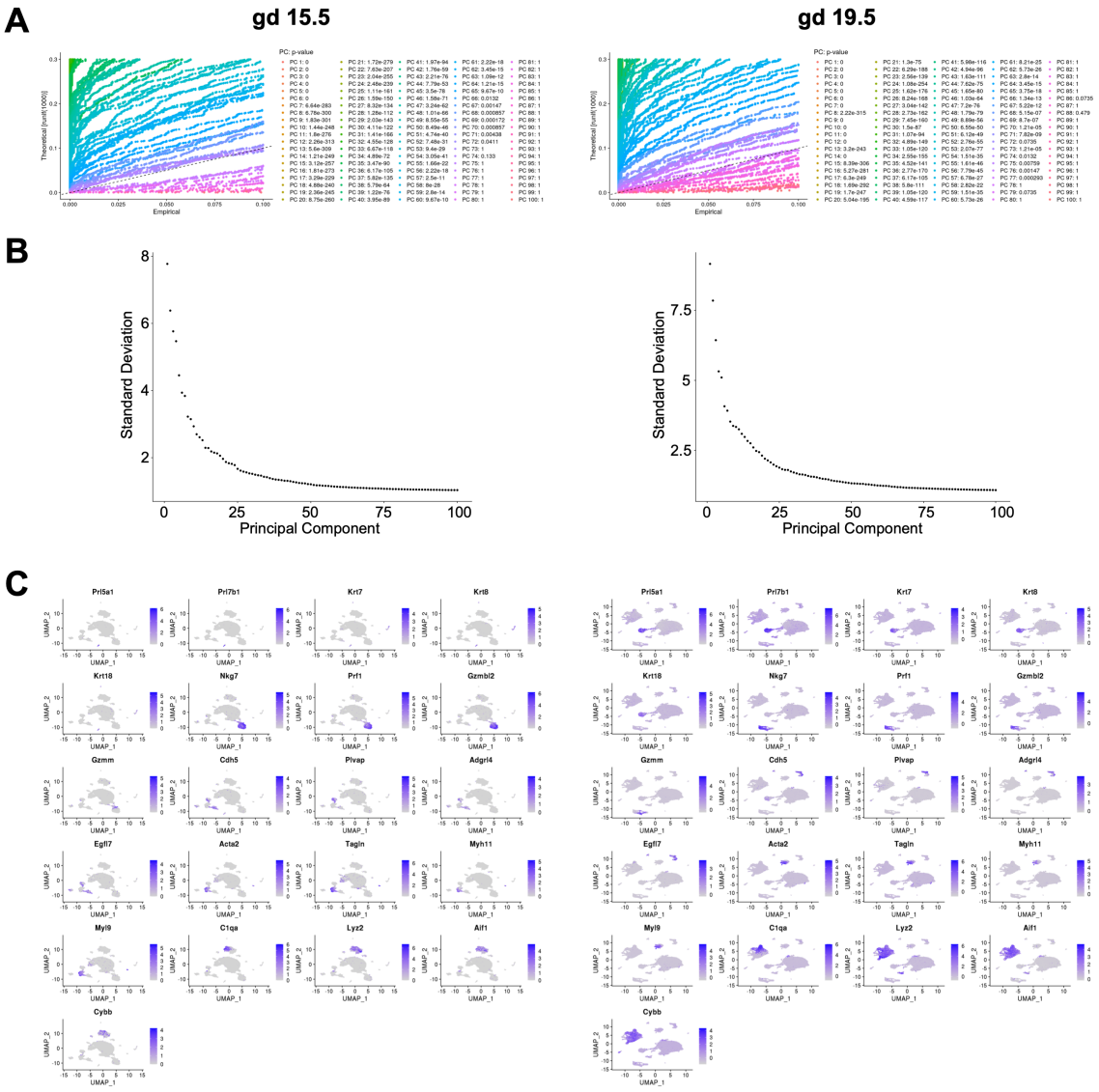


Fig. S1. Quality control and processing of single cell RNA sequencing (scRNA-seq) data.
A) Violin plots showing the distributions of number of genes and percentage of mitochondrial chromosome (chrMT) genes expressed per cell. Cells with the number of unique genes between 500 and 3500, and less than 20% of mitochondrial genes were retained. **B**) Summary table of the number of genes and cells retained in each sample after preprocessing. **C**) UMAP plot showing the cells from samples gd 19.5_4_ 5_6 and sample gd 19.5_7, which were generated in different batches, are integrated and well blended after the integration process.



D

	gd 15.5 cell counts	gd 15.5 clusters
Endothelial cells	4611	Cluster 10, 13, 15
Macrophages	7058	Cluster 7, 8, 11
Natural killer cells	5343	Cluster 6, 9, 19
Invasive trophoblast cells	320	Cluster 23
Smooth muscle cells	2611	Cluster 14, 17, 26
Other cells	45899	Cluster 0, 1, 2, 3, 4, 5, 12, 16, 18, 20, 21, 22, 24, 25, 27, 28
Total cells	65842	

	gd 19.5 cell counts	gd 19.5 clusters
Endothelial cells	1555	Cluster 10, 20
Macrophages	7447	Cluster 1, 7, 11, 17, 18
Natural killer cells	1270	Cluster 9
Invasive trophoblast cells	1820	Cluster 6
Smooth muscle cells	1311	Cluster 8, 26
Other cells	20214	Cluster 0, 2, 3, 4, 5, 12, 13, 14, 15, 16, 19, 21, 22, 23, 24, 25
Total cells	33617	

Fig. S2. Clustering and cluster annotations.

A) QQ plots showing the JackStraw analysis for principal component (**PC**) analysis significance. P-values indicating the significance of each PC were also shown. Based on the p-values, the first 72 PCs were used for the analyses on gd 15.5 samples, and the first 77 PCs were used for gd 19.5. **B**) Elbow plots showing the amount of standard deviation each PC represents. The first 72 PCs of gd 15.5 samples, and 77 PCs of gd 19.5 samples, captured the majority of the variation in the data. **C**) UMAP plots showing expression level of marker genes across cell clusters. **D**) Summary table of the cell cluster identities and number of cells in each cell group.

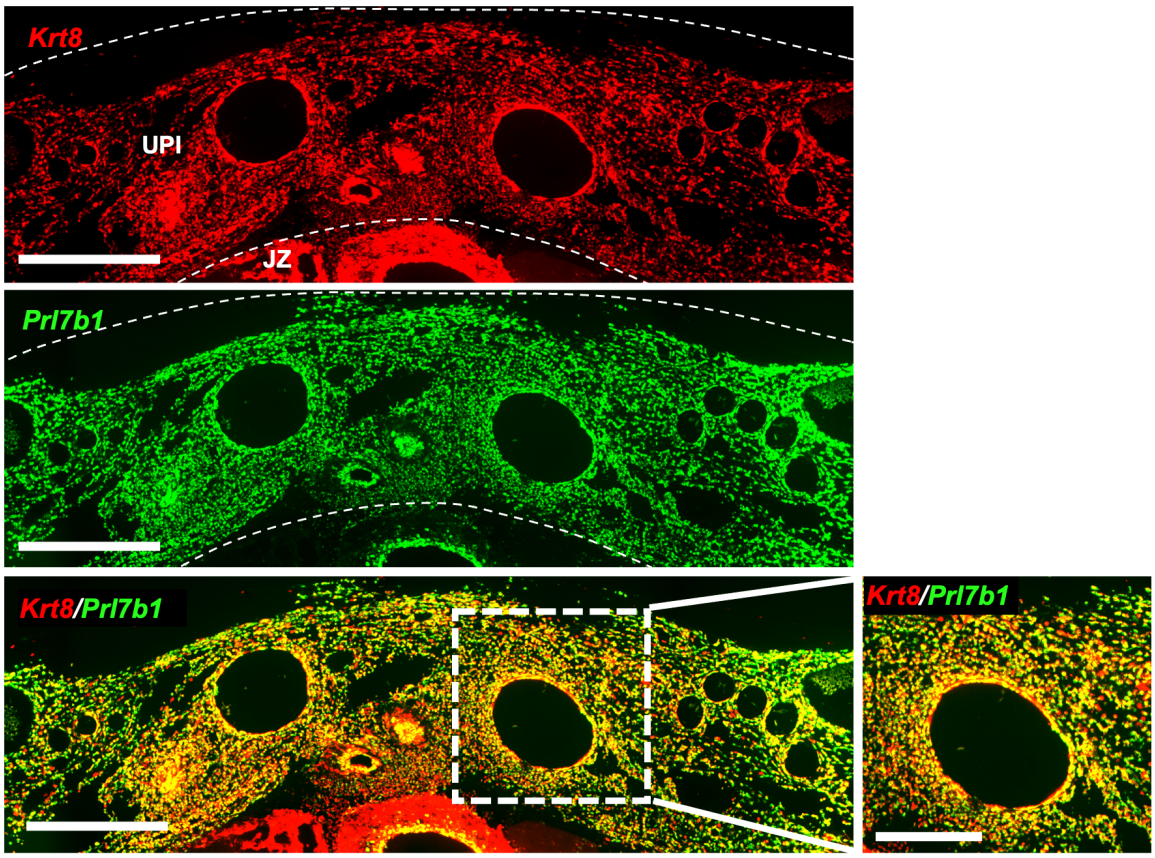


Figure S3. Colocalization of *Krt8* and *Prl7b1* in invasive trophoblast cells at the uterine-placental interface. In situ hybridization of gd 19.5 rat placentation site for *Krt8* (red; top panel), *Prl7b1* (green; center panel), and co-localization of *Krt8* and *Prl7b1* (bottom panels). Scale bar (left panels)=1000 μm, scale bar (right panel)=500 μm. Abbreviations: UPI, uterine-placental interface; JZ, junctional zone.

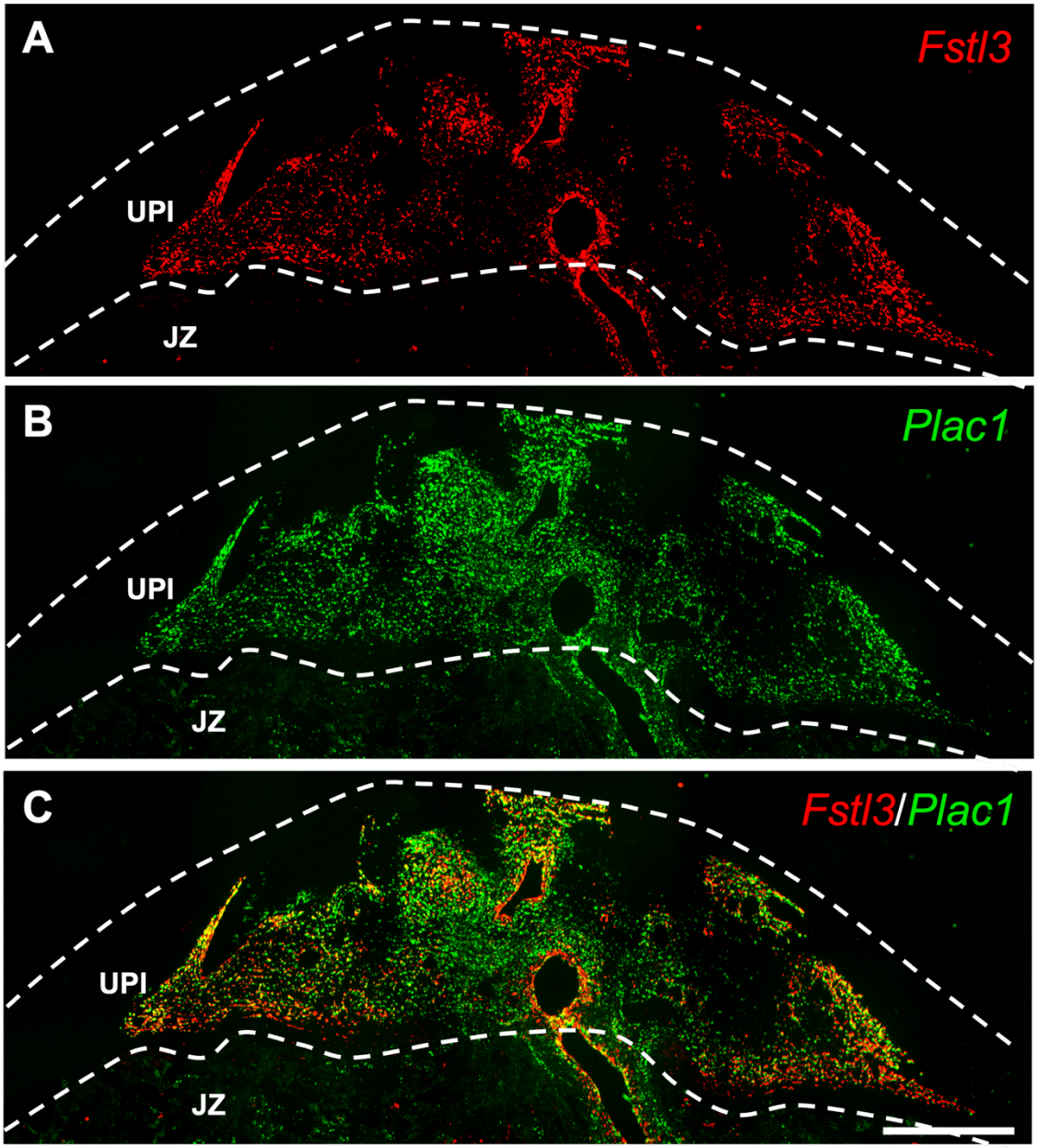


Figure S4. Colocalization of *Fstl3* and *Plac1* in invasive trophoblast cells at the uterine-placental interface. In situ hybridization of gd 19.5 rat placentation site for **A**) *Fstl3* (red)*, **B**) Plac1* (green) and **C**) colocalization of *Fstl3* (red) and *Plac1* (green) . Scale bar=1000 μm. Abbreviations: UPI, uterine-placental interface; JZ, junctional zone.

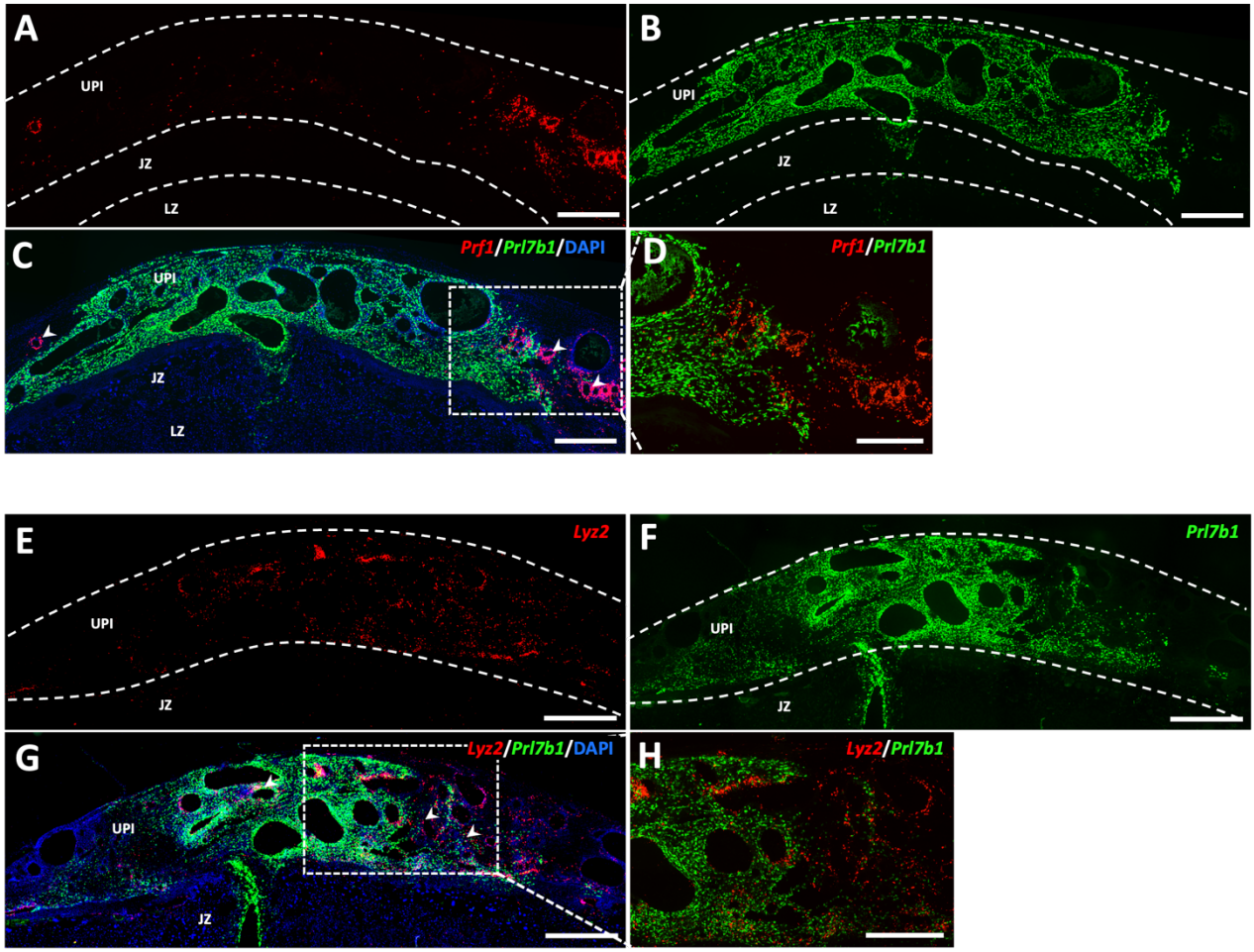


Figure S5. Distribution of NK cells and macrophages within the gestation day (gd) 19.5 rat uterine-placental interface. A-D) NK cell and invasive trophoblast cells were identified in gd 19.5 placentation sites using in situ hybridization for **A**) *Prf1* (red) and **B**) *Prl7b1* (green), respectively. **C**) Image from **Figure 3**, top right panel. **D**) High magnification image from **C**. **E-H**) Macrophages and invasive trophoblast cells were identified in gd 19.5 placentation sites using in situ hybridization for **E**) *Lyz2* (red) and **F**) *Prl7b1* (green), respectively. **G**) Image from **Figure 3**, bottom right panel. **H**) High magnification image from **G**. Scale bar (A-C, E-G)=1000 μm, Scale bar (D, H)=500 μm. Abbreviations: UPI, uterine-placental interface; JZ, junctional zone, LZ, labyrinth zone.

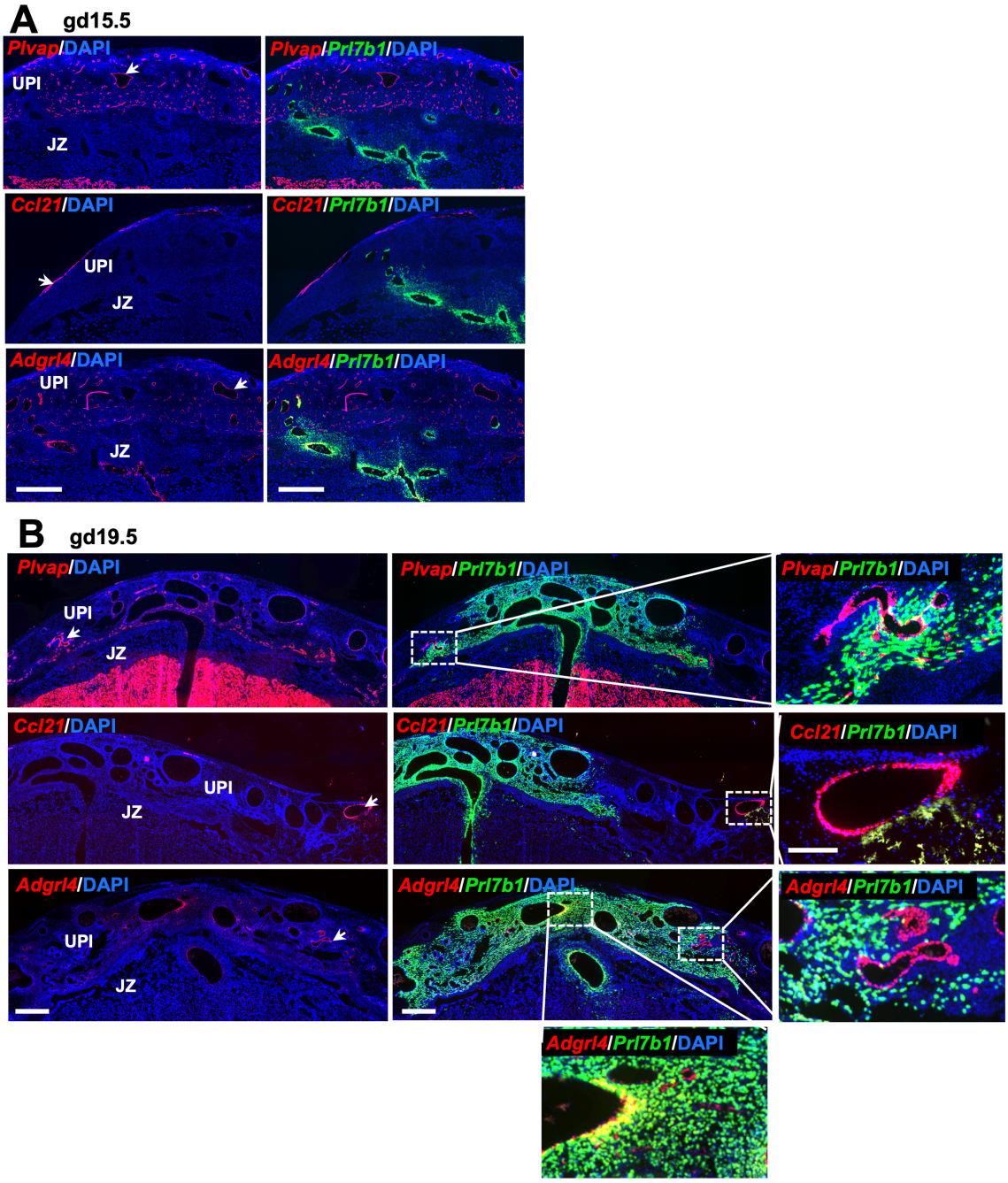


Figure S6. Endothelial cells at the uterine-placental interface. A) In situ hybridization of gd 15.5 rat placentation site for endothelial cell-specific transcripts *Plvap, Ccl21,* and *Adgrl4* (red; left panels) and localization of each endothelial cell marker with an invasive trophoblast cell-specific transcript (*Prl7b1*; right panels). Scale bar=1000 μm. **B**) In situ hybridization of gd 19.5 rat placentation sites for *Plvap, Ccl21, and Adgrl4,* and (red; left panels) and localization of each endothelial cell marker with an invasive trophoblast cell-specific transcript (*Prl7b1;* center, right, and bottom panels). Panels on the right and bottom are high magnification images of regions shown in the center panels. Scale bar (left and center panel)=1000 μm, scale bar (right and bottom panels)=500 μm. Abbreviations: UPI, uterine-placental interface; JZ, junctional zone.

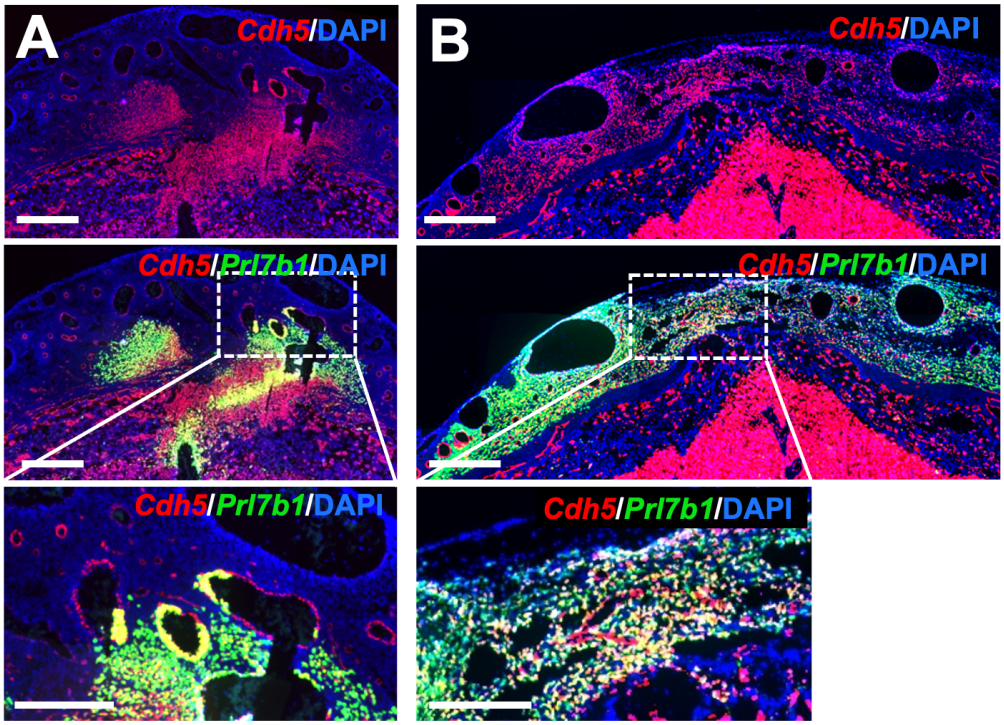


Figure S7. Localization of *Cdh5* in endothelial cells and invasive trophoblast cells. **A**) In situ hybridization localization of transcripts for *Cdh5* (upper) and *Cdh5* and *Prl7b1* (center and lower) within gd 15.5 placentation sites. **B**) In situ hybridization localization of transcripts for *Cdh5* (upper) and *Cdh5* and *Prl7b1* (center and lower) within gd 19.5 placentation sites. The lower panels are high magnification images of regions shown in the central panels. Scale bar=1000 μm.

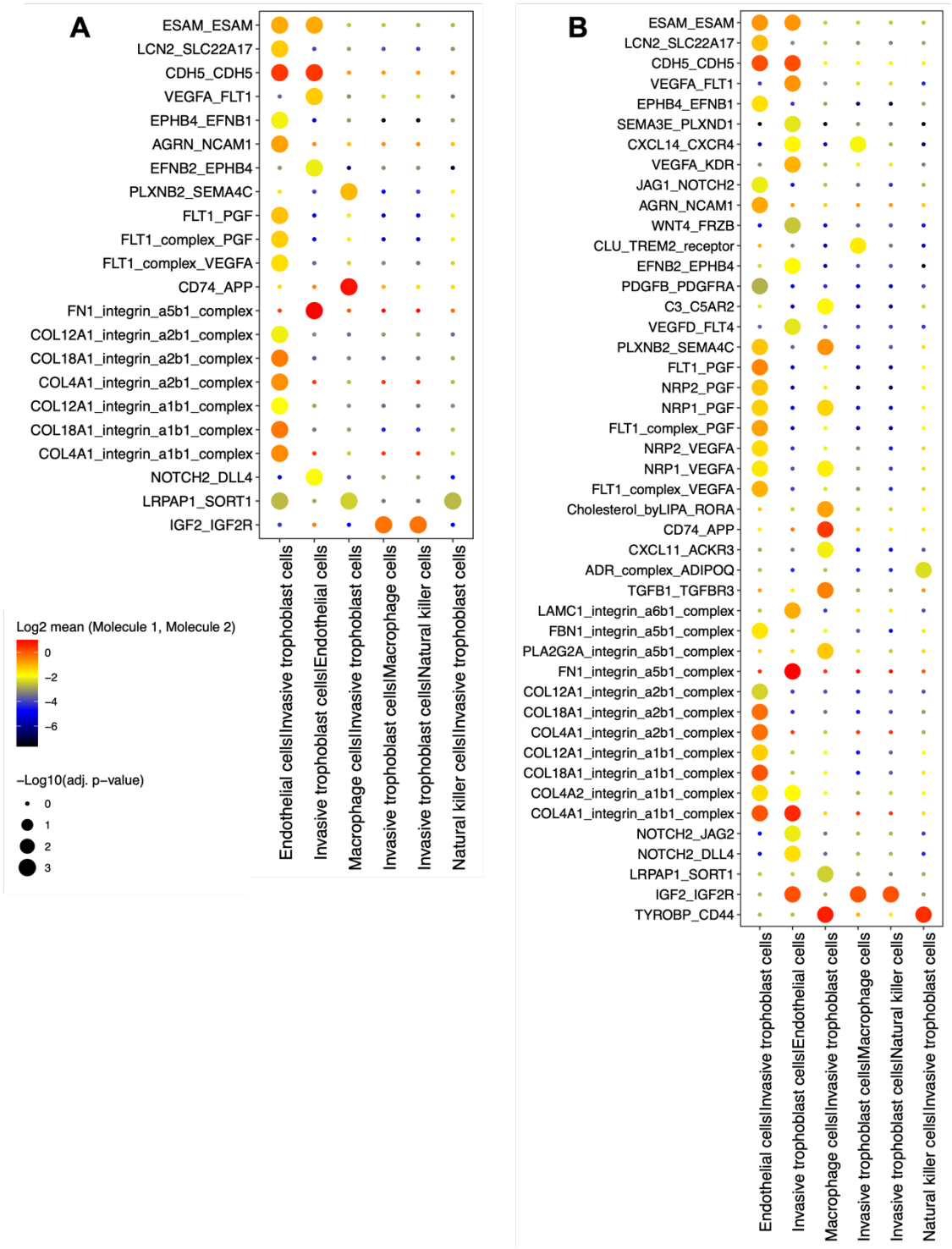


Figure S8. Ligand-receptor pair analysis in the uterine-placental interface using CellPhoneDB. Dot plots showing mean expression level of curated ligand – receptor pairs for invasive trophoblast cells with endothelial cells, macrophages, or natural killer cells from gestation day (**gd**) 15.5 (**A**) and gd 19.5 (**B**) uterine-placental interface. Only curated interactions are shown (adj. p-value ≤ 0.05). The x-axis shows the cell type pairs. The y-axis shows the ligand – receptor pairs. Dot size corresponds to -log10(adj. p-value) of the tests for interaction significance. Dot color corresponds to log2(mean expression) of the molecules in each pair.

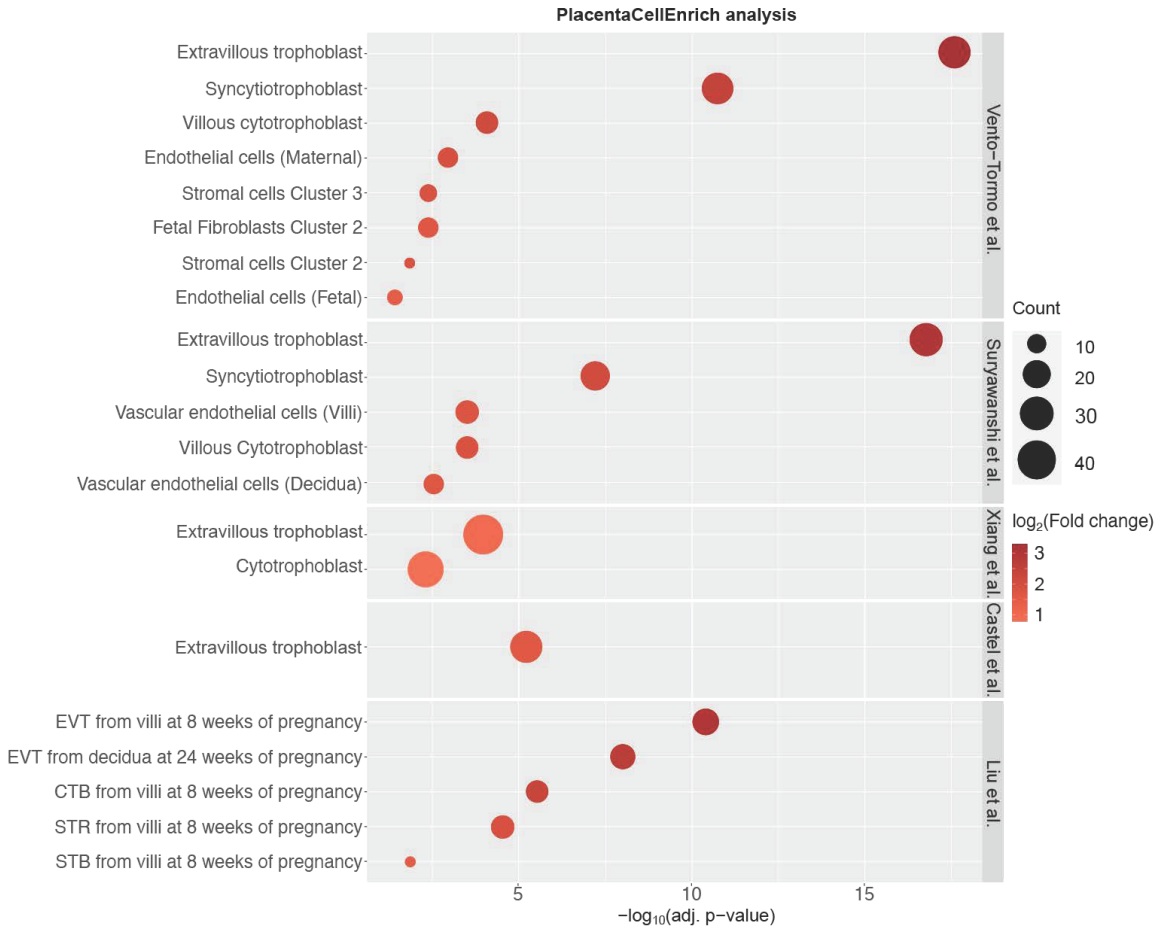


Figure S9. Full PlacentaCellEnrich results. Dot plots showing cell type enrichment when using marker genes from the invasive trophoblast cell gene clusters in rats as input to PlacentaCellEnrich. A significant enrichment has an adjusted p-value ≤ 0.05, fold change ≥ 1.5, and number of observed genes ≥ 5. Dot size represents the number of observed genes specific to a cell type; dot colors correspond to $\log_2$(Fold change) of the enrichment. Abbreviations: EVT, extravillous trophoblast; SCT, syncytiotrophoblast; CTB, cytotrophoblast; STR, villous stromal cells.

Dataset S1 (separate file).

Dataset S2 (separate file).

Dataset S3 (separate file).

Dataset S4 (separate file).

Dataset S5 (separate file).