Supporting Information For

GATA-transcriptional program dictate cell fate equilibrium to establish the maternal-fetal exchange interface and fetal development.

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(iii) SI References

Other supporting materials for this manuscript include the following:

Datasets S1 to S4

Supporting Materials and Methods

Collection of mouse embryos and tissue isolation

Animals were euthanized on at desired day points, as indicated in the main text. Pregnant female animals were identified by presence of vaginal plug (gestational day 0.5), and embryos were harvested at various gestational days. Uterine horns from pregnant females were dissected out, and individual embryos were analyzed under microscope and photographed. Littermate control and *Gcm1*^{Cre}GATA DKO conceptuses were used for all comparative analyses. Conceptuses were dissected to isolate embryos, yolk sacs, and placentae. All embryos and placentae were photographed at equal magnification for comparison purposes. Uteri containing placentation sites were dissected from pregnant female mice on E8.5-E14.5., tissues for histological analysis were kept in dry ice cooled heptane and stored at −80 °C. Tissues were subsequently embedded in optimum cutting temperature (OCT) (Tissue-Tek, Torrance, CA) and were cryosectioned (10mm thick) for immunohistochemistry (IHC) studies using Leica CM-3050-S cryostat. Yolk sacs from each of the dissected embryos were collected, and genomic DNA preparation was done using Extract-N-Amp tissue PCR kit (Sigma, St. Louis, MO). Placenta tissues were collected in RLT buffer, and RNA was extracted using RNAeasy Mini Kit (Qiagen). RNA was eluted and concentration was estimated using Nanodrop ND1000 spectrophotometer. Placenta samples were carefully isolated, ensuring the decidual layer was peeled off. Individual samples were briefly digested in the presence of collagenase and were made into single-cell suspensions by passing them through a 40μm filter. These cell suspensions were further used for Flow analysis or FACS. Corresponding embryonic tissues were used to confirm genotypes.

Genotyping

Genomic DNA samples were prepared using tail tissues or embryonic tissues from the mice using the REDExtract-N-Amp Tissue PCR kit (Sigma-Aldrich). Genotyping was done using REDExtract-N-Amp PCR ReadyMix (Sigma-Aldrich) and respective primers. Respective primers are listed in the materials and methods section.

Immunofluorescence and Immunohistochemistry analyses

For immunostaining with mouse tissues, slides containing cryosections were dried, fixed with 4% PFA followed by permeabilization with 0.25% Triton X-100 and blocking with

10% fetal bovine serum and 0.1% Triton X-100 in PBS. Sections were incubated with primary antibodies overnight at 4°C, washed in 0.1% Triton X-100 in PBS. After incubation (1:400, one hour, room temperature) with conjugated secondary antibodies, sections were washed, mounted using an anti-fade mounting medium (Thermo Fisher Scientific) containing DAPI and visualized using Nikon Eclipse 80i fluorescent microscope. *mT/mG* positive embryos and cryosections were imaged directly under a Nikon Eclipse 80i fluorescent microscope. Immunohistochemistry was performed using paraffin sections of human placenta. The slides were deparaffinized by histoclear and subsequently with 100%, 90%, 80% and 70% ethanol. Antigen retrieval was done using Decloaking chamber at 80^{O}C for 15minutes. The slides were washed with 1X PBS and treated with 3% H_2O_2 to remove endogenous peroxidase followed by 3 times wash with 1X PBS. 10% goat serum was used as a blocking reagent for 1hour at RT followed by overnight incubation with 1:100 dilution of primary antibody or IgG at 4^{O}C. The slides were washed with 1X PBS and 1:200 dilution of secondary antibody was used for 1 hour at RT. The slides were washed again with 1X PBS followed by treatment with horseradish peroxidase streptavidin for 20 minutes at RT. The slides were washed again and proceeded to color development using DACO 1ml buffer and 1 drop of chromogen. The reaction was stopped in distilled water after sufficient color developed. The slides were counterstained with Mayer's hematoxylin for 5minutes and washed with warm tap water until sufficient bluish coloration observed. The slides were then dehydrated by sequential treatment using 70%, 80%, 90%, 100% ethanol and histoclear. The sections were completely dried and mounted using Toluene as mounting agent and imaged using Nikon TE2000 microscope.

Electron Microscopy

Placental samples were carefully isolated at E9.5. Each placenta was then placed in 2.5% glutaraldehyde fixing solution and chopped into smaller pieces to isolate regions of the labyrinth. Tissue samples were post-fixed in 1% osmium tetroxide, dehydrated in graded series of ethanol followed by dehydration with propylene oxide and infiltration with Embed 812 resin (Electron Microscopy Sciences, Hatfield, PA). Tissue polymerized in Embed 812 resin at 60ºC. Blocks trimmed with EMTrim2 (Leica Biosystems, Deer Park, IL). Finally, 75nm thick sections cut with a DiATOME diamond knife using Leica UC-7 ultramicrotome and placed on 200 mesh copper grids. Sections were contrasted

with uranyl acetate followed by lead citrate. Grids with sections air dried were viewed on JEOL JEM-1400 TEM at 100kV.

Single-cell RNA sequencing data analysis

The sequencing results were loaded using Read10X function of Seurat and processed through Cellranger pipeline from 10X genomics to obtain unique molecular identified (UMI) count matrix. The values in this matrix represent the number of molecules for each feature (i.e., gene; row) that are detected in each cell (column). In the next step we have created a Seurat object for individual samples using the CreateSeuratObject function and provided parameters to perform some initial filtering in order to exclude genes that are expressed in fewer than 3 cells, and to exclude cells that contain fewer than 500 expressed genes. Next, we start with filtration of cells based on QC metrics, which is followed by data normalization, scaling and finally detection of highly variable features. We excluded the cells having less than 500 and greater than 5000 unique genes. Along with it, we also excluded nuclei having mitochondrial counts 10 percentage for further downstream analysis. We put all samples into list and used NormalizeData() and ScaleData() function of Seurat with default settings for normalizing each of sample. Further we used FindVariableFeatures function and provided 2500 features to subset the samples for downstream analysis. SelectIntegrationFeatures function screens the features that will be used while performing integration and FindIntegrationAnchors was implemented to get anchors. Finally IntegrateData function was used which create integrated assay. We used DefaultAssay to make this integrated assay default, which will be used in downstream analysis. We used RunPCA function to perform PCA. Next characterization of the principal components and estimate the number of significant PCs that captures the signal of interest, while minimizing noise was accessed using ElbowPlot and DimHeatmap functions. Further functions like FindNeighbors and FindClusters were executed sequentially for clustering. In the final steps dimensional reduction methods UMAP and tSNE was performed using RunUMAP and RunTSNE function of Seurat. Differentially expressed genes were identified using FindAllMarkers and cell clusters were identified based on the expression of marker genes mentioned in Supplementary Table 1 below. To further analyze trophoblast cell cluster, data from initial clustering was further analyzed for trophoblast cell clusters using the function SubsetData based upon annotations from marker genes identified by FindAllMarkers. Integration of the trophoblast only dataset was performed using the same integration

methods above (using 20 dimensions for FindIntegrationAnchors and IntegrateData, and 20 PCs and a resolution of 0.5 for FindClusters and RunUMAP). Differentially expressed genes for each integrated dataset were identified using FindAllMarkers (Supplementary Table 2).

Supplementary Table 1: Marker Genes for Initial Cell Clustering

Trophoblast	2,4,13,14	Epcam, Ly6a, Prl3d1, Prl2c2, Tpbpa
Endothelial	3	Pecam1, Cldn5, Cd34, Kdr, Eng
Endodermal	6, 19	Procr, Cldn6, Rbp4, Afp Procr, S100g, Klf5
Blood cells	0,15,17- B-cell	Cd74, Igkc, Cd79a, Cd79b
	9 - T-cell	Trbc1, Lmo2
	8 - NK cells	Thy1, Klrg1, Cst7, Prf1
	1,20 - Erythroblasts	Blvrb, Hbb-y
	11 - Megakaryocytes	Itga2b, Tal1, Nfe2, Lmo2
	7 - Macrophages	Spp1, Adgre1, Cx3cr1, Apoe
	21 - Dendritic cells	Cd83, Cst3, H2-Aa, Cd209a
	12 - granulocytes	Neat1, Cd69
Stromal	18,10,16,5	Acta2, Dcn, Col3a1, Col1a2, Adm, Cryab

Supplementary Table 2: Marker Genes for Trophoblast Cell Clusterinng

Trophoblast sub-clusters	Cluster number	Marker genes
Multipotent progenitors	3	*Tcf7l1, mKi67, Tbx3, Ly6a, Egfr*

SynTI precursors	6	*Slc16a1, Slc40a1, Nr6a1, Dlx3*
SynTII precursors	5	*Slc16a3, Esx1, Fabp3, Wnt7b*
S-TGC precursors	8	*Podxl, Lifr, Hsd17b2*
JZ precursors 1 (JZP1)	1	*Hand1, Ctsd, Podxl, Prl2b1*
JZ precursors 2 (JZP2)	2	*Prl8a1, Slco2a1*
JZ precursors 3 (JZP3)	7	*Prune2, Prl2b1, Prl3b1, Prl8a9*
JZ precursors 4 (JZP4)	4	*Pla2g4d, Ncam1, Igfbp7, Pcdh12, Tpbpa*

RNA velocity study

RNA Velocity enables measuring the underlying kinetics of a gene's expression from each cell based on the single cell RNA sequencing dataset. The concept of RNA velocity utilizes the information about newly transcribed pre-mRNAs (unspliced) from mature mRNAs (spliced), which can be detected in standard single-cell RNA-seq protocols from the presence of introns. The RNA velocity function distinguishes the spliced from the unspliced mRNA for each gene in the cell and generates velocities across each gene based on their expression. The change in mRNA abundance is termed RNA velocity (1). Positive velocity indicates a recent increase in pre-mRNA transcripts (thus abundances being higher than expected in steady state) followed by up-regulation in spliced transcripts. Conversely, negative velocity indicates down-regulation. The combination of velocities across genes is then used to estimate the future state of an individual cell. For the study of RNA velocity in single cell transcriptomes data, we generated the loom files which contains both splices and un-spliced contents using the Space Ranger output along with the GTF file. The tool velocyto 0.17.16 has been utilized to generate loom file. Further, we have used scVelo tool to perform RNA velocity on our sample. The loading was done using scv.read function. In the next step, we perform the gene selection, normalizing and logarithmizing using scv.pp.filter_and_normalize function. In the next step Scanpy 1.9.1 was used to get the first and second order moments (means and uncentered variances) calculated using scv.pp.moments, which are required for velocity estimation and stochastic estimation, respectively. We have used stochastic model of

scVelo to calculate the RNA velocity for both samples. The tSNE cell embeddings were utilized to display the RNA velocity.

Single Cell Proportion test

The variability between biological samples used for scRNA-Seq can be high due to variability in the source of the samples, technical reasons like the dissociation protocol used or intrinsic properties of the sample, the single cell proportion test is a function of the R library (https://github.com/rpolicastro/scProportionTest) that analyses scRNA-Seq samples to account for variability in the proportions of cell clusters between samples. A permutation test first calculates a p-value for each cluster, then bootstrapping is used to calculate the confidence interval for the magnitude difference.

Flowcytometry analysis and cell sorting

For analyzing multipotent $SCA1^+$ trophoblast population, placental single-cell suspensions were stained with PerCP/Cy5.5-conjugated anti-mouse CD34 (BioLegend, San Diego, CA), PE-conjugated anti-mouse Ly-6A/E (SCA1) (BioLegend), PE/Cy7 anti-mouse CD45 (BioLegend) and Pacific, Blue-conjugated anti-mouse Lineage cocktail (BioLegend) monoclonal antibodies. Unstained, isotype and single-color controls were used for optimal gating strategy. Samples were run on either an LSRII flow cytometer or an LSRFortessa (BD Biosciences), and the data were analyzed using FACSDiva software.

Quantitative RT-PCR

Total RNA from either whole cell extract or placental tissues was isolated using RNeasy Mini Kit (Qiagen, 74104) according to manufacturer protocol. Purified RNA was used to prepare cDNA using a cDNA preparation kit and analyzed by qRT-PCR following procedures described earlier (2). The mouse or human specific primers used in qRT-PCR are listed below. 20ng equivalent of cDNA was used for amplification reaction using Power SYBR Green PCR master mix (Applied Biosystems, Waltham, MA).

Western Blot analysis

Cell pellets were washed once with PBS followed by addition of 1X SDS-PAGE buffer for protein extract preparation and western blot analyses were performed following earlier described protocol (2). Antibodies used for the study are mentioned below.

Organoid culture:

Human trophoblast cell (human TSC) lines that were derived from first trimester CTBs were used to generate the organoids. We followed earlier described protocols that were used to generate CTB-organoids (3, 4). Human TSCs were cultured and passaged in DMEM/F12 media that was supplemented with 0.1mM 2-mercaptoethanol, 0.2% FBS, 0.5% penicillin-streptomycin, 0.3% BSA, 1% ITS-X supplement, 1.5µg/ml L-ascorbic acid, 50ng/ml EGF, 2µMCHIR99021, 0.5µM A83-01, 1µM SB431542, 0.8mM VPA and 5µM Y27632. Cells were cultured at 37°C in 5% CO2. To generate GATA2 KD and GATA3 human TSCs, shRNA mediated RNAi was performed as described earlier. To obtain organoids from human TSCs (control/ GATA2 KD human TSCs/ GATA3 KD human TSCs) we modified the protocol described previously (7). The hTS cells were harvested and re-suspended in ice-cold basic trophoblast organoid medium (b-TOM) containing advanced DMEM/F12 supplemented with 10mM HEPES, B27 (Gibco, Waltham, MA), N2 (Gibco) and 2mM glutamine (Gibco). The cells were then centrifuged for 3 minutes at 1200rpm following which the cells were re-suspended in ice-cold advanced trophoblast organoid medium (aTOM) which is bTOM supplemented with 100ng/ml R-spondin (PeproTech, Rocky Hill, NJ), 1µM A83-01 (Sigma), 100ng/ml recombinant human epidermal growth factor (rhEGF, Wako), 50ng/ml recombinant murine hepatocyte growth factor (rmHGF, PeproTech), 2.5µM prostaglandin E2 (R&D System, Minneapolis, MN), 3µM CHIR99021 (Wako, Richmond, VA) and 100ng/ml Noggin (Invitrogen, Waltham, MA). Growth factor reduced Matrigel (Corning) was added to the a-TOM cell suspension to reach a final concentration of 60%. 35µl of the viscous cell solution containing 2.5x104 cells was plated in the center of a 24-well plate. The solution rests as a dome-shaped droplet in the center of the well. The plates are then turned upside down and kept at 37°C for 10-15 minutes to ensure proper spreading of the cells in the solidifying matrigel domes. Finally, the plates are returned to their upward position and the domes are overlaid with 500µl of room temperature a-TOM medium. The organoids are allowed to form for 10 days with fresh media being changed every 2 days. Brightfield images were taken to observe the growth of the organoids. For staining purposes, the organoid growth was stopped at day 10 by removing the a-tom media and fixing them in ice cold 4% paraformaldehyde solution. The fixed organoids were then embedded in 4-5% agarose followed by making a paraffin block. These paraffinized blocks were then sectioned into 10µm sections using a microtome and stained (staining protocol and mentioned above).

RNA-Seq analyses

RNA-seq analysis was performed according to published protocol (2, 5). Total RNA from the control human TSCs as well as GATA2-KD and GATA3-KD human TSCs were isolated using RNeasy Mini Kit (Qiagen, Germantown, MD) per the manufacturer's protocol with on-column deoxyribonuclease digestion. RNA concentrations were quantified using a NanoDrop Spectrophotometer at a wavelength of 260 nm. Integrity of the total RNA samples was evaluated using an Agilent Technologies 2100 Bioanalyzer. The total RNA fraction was processed by oligo dT bead capture of mRNA, fragmentation, and reverse transcription into cDNA. After ligation with the appropriate Unique Dual Index (UDI) adaptors, the cDNA library was prepared using the Universal Plus mRNA-seq +UDI library preparation kit (NuGEN; Marion, SD). The raw data for RNA-seq analyses have been submitted to the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds), with accession No.GSE214486.

CUT&RUN Analyses

Proliferating semiconfluent 200,000 live hTSC were used per sample for CUT&RUN following published protocol (6, 7). Trypsinized hTSC were washed twice with the wash buffer (Wash buffer was prepared by adding 1ml 1M HEPES pH7.5, 1.5ml 5M NaCl, 12.5µL 2M Spermidine and 1 Roche Complete Protease Inhibitor EDTA-Free tablet to final volume to 50ml with dH2O). Concanavalin A-coated beads (10µL/sample, from EpiCypher, Durham, NC) were washed with binding buffer (binding buffer was prepared by mixing 400µL 1M HEPES-KOH pH 7.9, 200µL 1M KCl, 20µL 1M $CaCl_2$ and 20µL 1M $MnCl_2$, and bring the final volume to 20 ml with dH_2O. Stored up to 6 months at 4°C.) and recovered by placing on the magnetic stand. Washed Concanavalin-A coated beads were resuspended in wash buffer and incubated with the hTSC under rotation at room temperature for 10minutes. Using a magnetic stand, the cells bound to the Concanavalin A-coated beads were separated from the suspension, the liquid was discarded, and the cells were then treated for permeabilization for antibody binding by resuspending them in 100µl of antibody buffer, which was prepared by adding EDTA (2mM final concentration) in wash buffer containing 0.5% (wt/vol) Digitonin (Dig-wash buffer). Efficient cell permeabilization was also confirmed by trypan bluse test. Primary antibody of interest (GATA2 or GATA3 or IgG antibody; 1:100 dilution) was added to the samples and incubated overnight at 4°C with intermittent shaking. Tri-Methyl-Histone H3 (Lys4) (C42D8) was used as a positive control antibody. The samples were washed

twice with 150µL of Dig-wash buffer while keeping the samples on ice, magnetic stand was used to remove the residual liquid. Next, these cells bound to the Concanavalin A-coated beads were resuspended in 50µl of Dig-wash buffer and incubated with 1.5µl . Protein AG-Micrococcal Nuclease (pA/G-MNase, EpiCypher) for 1 hour with intermittent shaking/rotation in cold room. Incubation was followed by vortexing, a quick spin and then placed on the magnet stand to separate out and discard the liquid. The samples were washed twice with Dig-wash buffer and resuspended in the 100µL of Dig-wash buffer and placed on the metal eppendorf rack equilibrated in an ice-water bath. pA/G-MNase reaction was activated by adding 2µL 100 mM CaCl2 to each sample, mixed well and incubated the tubes for 30 minutes on the metal rack placed in an ice-water bath. The MNase reaction was stopped by adding equal volume of 2X STOP buffer (stop buffer was prepared by adding 3.44ml dH2O, 136µl 5M NaCl, 400µL 0.2M EGTA, 40µL 5% Digitonin, 10µL RNase A, 20µL 20 mg/ml glycogen). These samples were mixed by vortexing and incubated for 10 minutes at 37°C to release CUT&RUN fragments from the insoluble nuclear chromatin. Release CUT&RUN fragments were recovered by centrifugation for 5 min in 4°C at 16,000g and placed on magnetic stand. Supernatant with CUT&RUN fragments were transferred into a fresh 1.5ml microcentrifuge tube containing 2µL of 10% SDS and 2.5µL of Proteinase K (Thermo Scientific). These samples were incubated for 10 minutes at 70°C. CUT&RUN DNA fragments were purified by using a phase-lock tube (Qiagen) and Phenol–chloroform–isoamyl alcohol. The purified CUT&RUN DNA fragments were transferred to a fresh tube and precipitated following ethanol precipitation. The dried CUT&RUN DNA pellet was dissolved in 20µL 1 mM Tris-HCl pH8 0.1 mM EDTA. The DNA was quantified using 1µL of the freshly dissolved pellet, for example using fluorescence detection with a Qubit instrument (Life Technologies, Waltham, MA). Evaluated the presence of cleaved fragments and the size distribution by capillary electrophoresis with fluorescence detection using a 4200 TapeStation System (Agilent). Samples from three individual CUT&RUN experiments for each experimental condition were used for sequencing. Sequencing libraries were prepared following protocol from Swift bioscience 1S Plus Combinatorial Dual Indexing Kit and Accel-NGS 1S Plus DNA Library Kit. We have used “nf-core” pipeline (3) for downstream analyses of CUT&RUN data. We have used Model-based Analysis of ChIP-seq-2 (MACS2) algorithm to call the candidate peaks/binding sites. The MACS2 algorithm uses Poisson distribution to effectively capture local biases and build a model for more robust prediction of peaks. A region was considered to have a significant

binding with a p value of <1e-5. Reproducible GATA2 and GATA3 associated peak coordinates were used to identify GATA2 and GATA3 regulated genes using GREAT tool (GREAT version 4.0.4)(8).

Heatmap for binding enrichment

We have used deepTools (9) to see the binding enrichment of GATA2 or GATA3 around the transcription start site (TSS). For this we first calculate the values for heatmaps by providing the parameters "-a 3000, -b 3000 --missingDataAsZero -p 20 –skipZeros" to the function "computeMatrix reference-point" of deepTools, along with the bigWig file and bed file containing peaks coordinates. The output of this matrix was supplied to the plotHeatmap function with parameter "--sortRegions descend" to generating the heatmap around TSS.

Collection and analysis of human placentae

First trimester and term placental samples were obtained from RCWIH biobank, Toronto, or collected at the University of Kansas Medical Center after IRB approval from both the universities. Only de-identified placental tissues were used for this study.

Primers used for genotyping:

Gata2$^{f/f}$ and *Gata2-KO*	GCCTGCGTCCTCCAACACCTCTAA	TCCGTGGGACCTGTTTCCTTAC
Gata3$^{f/f}$	CAGTCTCTGGTATTGATCTGCTTCTT	GTGCAGCAGAGCAGGAAACTCTCAC
Gata3-KO	TCAGGGCACTAAGGGTTGTTAACTT	GTGCAGCAGAGCAGGAAACTCTCAC
Cre	AAAATTTGCCTGCATTACCG	ATTCTCCCACCGTCAGTACG

List of RT-PCR primers

Human gene	Forward	Reverse
GATA2	*CCAGCTTCACCCCTAAGCAG*	*CCACAGTTGACACACTCCCG*
ERVW-	*CTACCCCAACTGCGGTTAAA*	*GGTTCCTTTGGCAGTATCCA*

1		
CGA	*TCTGGTCACATTGTCGGTGT*	*TTCCTGTAGCGTGCATTCTG*
CGB	*GTGTGCATCACCGTCAACAC*	*GGTAGTTGCACACCACCTGA*
PSG4	*CGATGGGACTGGAGGAGTAA*	*AGTTGCTGCTGGAGATGGAG*
TEAD4	*ACG GCC TTC CAC AGT AGC AT*	*CTT GCC AAA ACC CTG AGA CT*
VGLL1	*TCA GAG TGA AGG TGT GAT GCT*	*GCA CGG TTT GTG ACA GGT ACT*
TP63	*GTC ATT TGA TTC GAG TAG AGG GG*	*CTG GGG TGG CTC ATA AGG T*
PPARG	*ACC AAA GTG CAA TCA AAG TGG A*	*ATG AGG GAG TTG GAA GGC TCT*
ITGA6	*GGC GGT GTT ATG TCC TGA GTC*	*AAT CGC CCA TCA CAA AAG CTC*
TFAP2 C	*TAC TGG GAG GTG TTC TCA GAA G*	*GGC CGG AAG ATT CAA CCC AAT*
HPRT1	*ACCCTTTCCAAATCCTCAGC*	*GTTATGGCGACCCGCAG*
18S rRNA	*AACCCGTTGAACCCCATT*	*CCATCCAATCGGTAGTAGCG*
Mouse gene	**Forward**	**Reverse**
Gcm1	*AGAGATACTGAGCTGGGACATT*	*CTGTCGTCCGAGCTGTAGATG*
Dlx3	*CACTGACCTGGGCTATTACAGC*	*GAGATTGAACTGGTGGTGGTAG*
Pparg	*AGCTGTCATTATTCTCAGTGGAG*	*ATGTCCTCGATGGGCTTCAC*
SynA	*ATGGTTCGTCCTTGGGTTTTC*	*GTGTTGAGTGAGGTTTACCAGG*
SynB	*TGGGTCCTCTGTTTCGTCCTT*	*GGGAAGAGTTGGTATCACGTAGG*
Ctsq	*CATTGCCAGTTGACAACACCAG*	*ATAGCCTTCATTTCGCCAATCA*
Hand1	*CTACCAGTTACATCGCCTACTTG*	*ACCACCATCCGTCTTTTTGAG*
Prl2c2	*TCCTGGATACTGCTCCTACTACT*	*GACCATTCCTCATTGCACACA*
Tpbpa	*TCCGGTCAGCTAACTGATGA*	*TCCTCTTCAAACATTGGGTGT*
Prl3d1	*ACATTTATCTTGGCCGCAGATGTGT*	*TTTAGTTTCGTGGACTTCCTCTCGAT*
18S rRNA	*AGTTCCAGCACATTTTGCGAG*	*TCATCCTCCGTGAGTTCTCCA*

Antibodies used for Immunofluorescence, Immunohistochemistry and Western Blots

Primary Antibodies	company	Catalog number
GATA2	Abcam	ab109241
GATA3	BD Biosciences	558686

Pan-cytokeratin	Abcam	ab9377
β-actin	Sigma	A5441
HCGβ	Abcam	ab53087
Cytokeratin 7	Dako	M7018
E-cadherin	Abcam	ab1416
MCT1	EMD Millipore	AB1286-I
MCT4	EMD Millipore	AB3314P
Proliferin	Santa Cruz	sc-47347
Cre recombinase	Santa Cruz	sc-83398
Secondary Antibodies		
Alexa fluor 488 goat anti-rabbit IgG	Invitrogen	A11008
Alexa fluor 568 goat anti-mouse IgG	Invitrogen	A11031
Alexa fluor 488 donkey anti-mouse IgG	Invitrogen	A21202
Alexa fluor 568 donkey anti-rabbit IgG	Invitrogen	A10042
Alexa fluor 568 donkey anti-goat IgG	Invitrogen	A11057
Alexa fluor 488 goat anti-chicken IgY	Invitrogen	A32931
Goat anti-mouse IgG-HRP	Santa Cruz	sc2005
Goat anti-rabbit IgG-HRP	Santa Cruz	sc2004

Antibodies used in FACS	**Species raised in**	**Vendor**	**Catalog number**
APC anti-mouse CD117 (c-kit)	Rat	BioLegend	105812
PerCP/Cy5.5 anti-mouse CD34	American Hamster	BioLegend	128607
PE anti-mouse Ly-6A/E (Sca-1)	Rat	BioLegend	108108

PE/Cy7 anti-mouse CD45	Rat	BioLegend	103113
Pacific Blue anti-mouse Lineage Cocktail	Rat	BioLegend	133310

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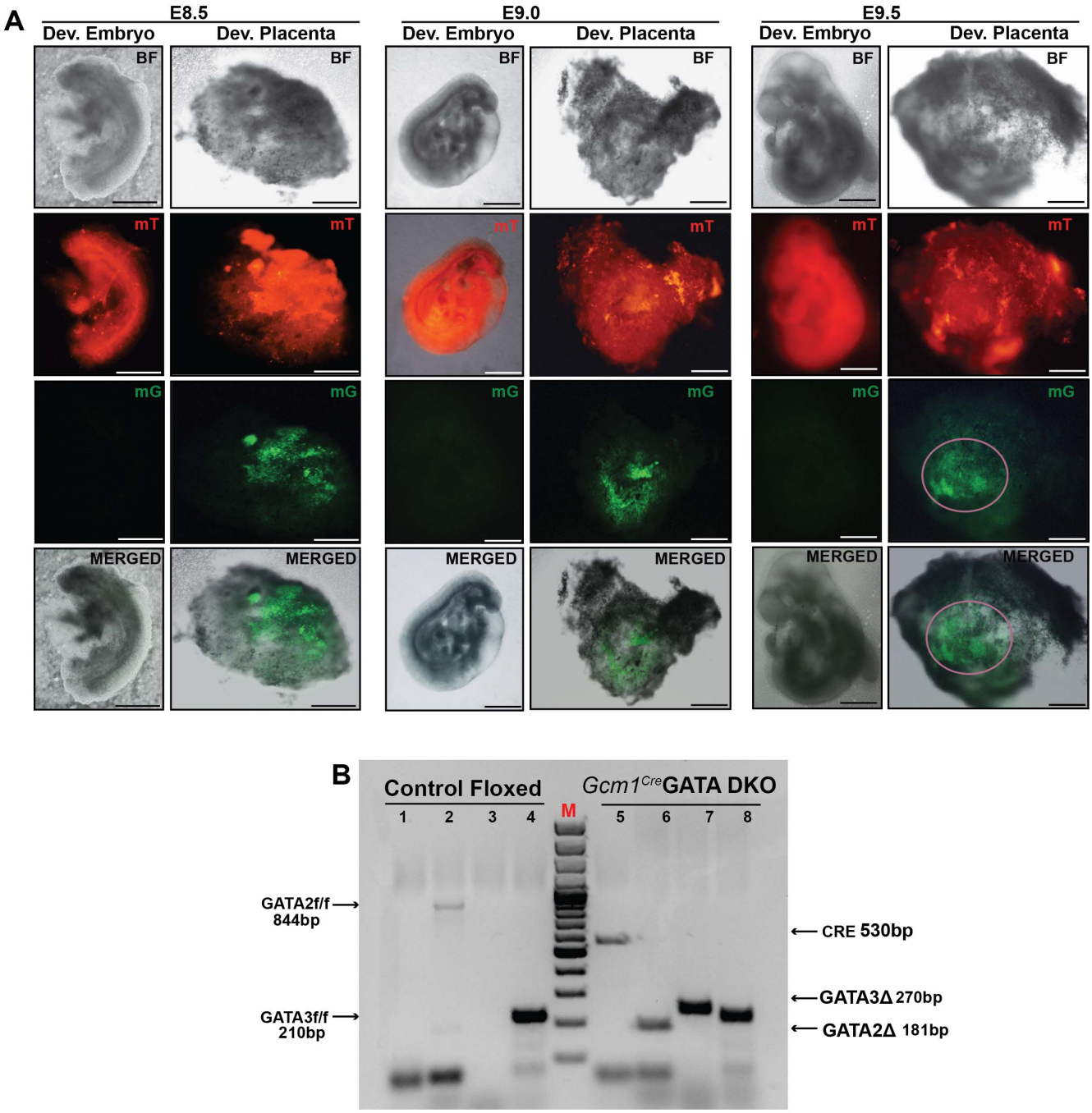
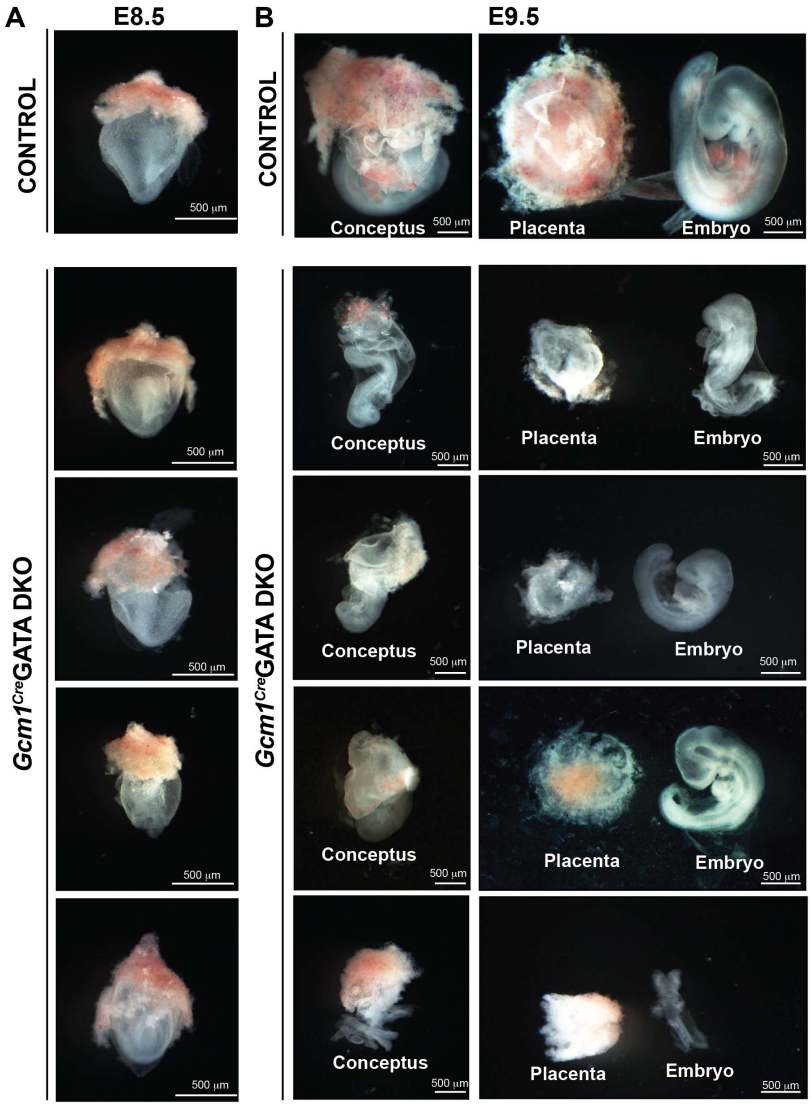
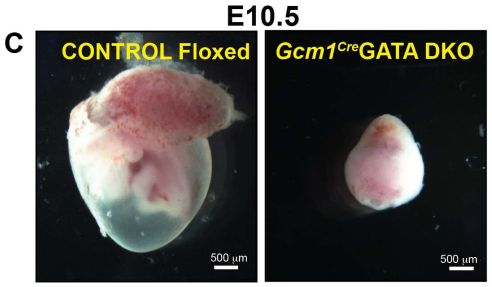


Figure S1. (A) mT/mG/*Gcm1*^{Cre} embryos and placentae were tested for the specificity of Cre expression. Images show that Cre-mediated recombination at the mT/mG locus induces EGFP expression within developing placentae at E8.5, E9.0 and E9.5. In contrast, EGFP expression is not detected within the embryo proper at these developmental stages. (scale bars 500 μm) (B) Agarose gel image representing the genotyping analysis. Homozygous *Gcm1* ^{Cre}GATA DKO (*Gata2*$^{f/f}$ *Gata3*$^{f/f}$ *Gcm1* ^{Cre}) embryos were confirmed by genotyping. The control samples have no Cre band (lane1), no GATA3 deletion band (lane 3), show floxed band for GATA2 (lane 2) and GATA3(lane4). The *Gcm1* ^{Cre}GATA DKO samples show prominent Cre band (lane 5), deletion band for GATA2 (lane 6) and GATA3 (lane 7) and GATA3 floxed band (lane 8). M, molecular weight marker.

Fig. S2





D

MOUSE PHENOTYPE ANALYSIS				
	Conceptuses analyzed		Embryos with Developmental Defect	
	Floxed Control	*Gcm1*^{Cre} GATA DKO	Control	*Gcm1*^{Cre} GATA DKO
E8.5	18	10	0	0
E9.5	40	28	0	27
E10.5	27	13	0	13

Figure S2. (A) Images show E8.5 control and *Gcm1* ^{Cre}GATA DKO conceptuses. (B) Images show control and *Gcm1* ^{Cre}GATA DKO conceptuses, placentae and Embryos at E9.5. Note that all *Gcm1* ^{Cre}GATA DKO placentae and embryos show developmental defects by E9.5 (C) E10.5 control and *Gcm1* ^{Cre}GATA DKO conceptuses. Most of the *Gcm1* ^{Cre}GATA DKO embryos are resorbed at E10.5 (scale bars 500 µm) . (D) Table showing number and phenotype of control and *Gcm1* ^{Cre}GATA DKO embryos, analyzed at E8.5 (n=28), E9.5 (n=68) and E10.5 (n=40), 'n' represents number of independent experiments conducted with multiple pregnant females.

Fig. S3

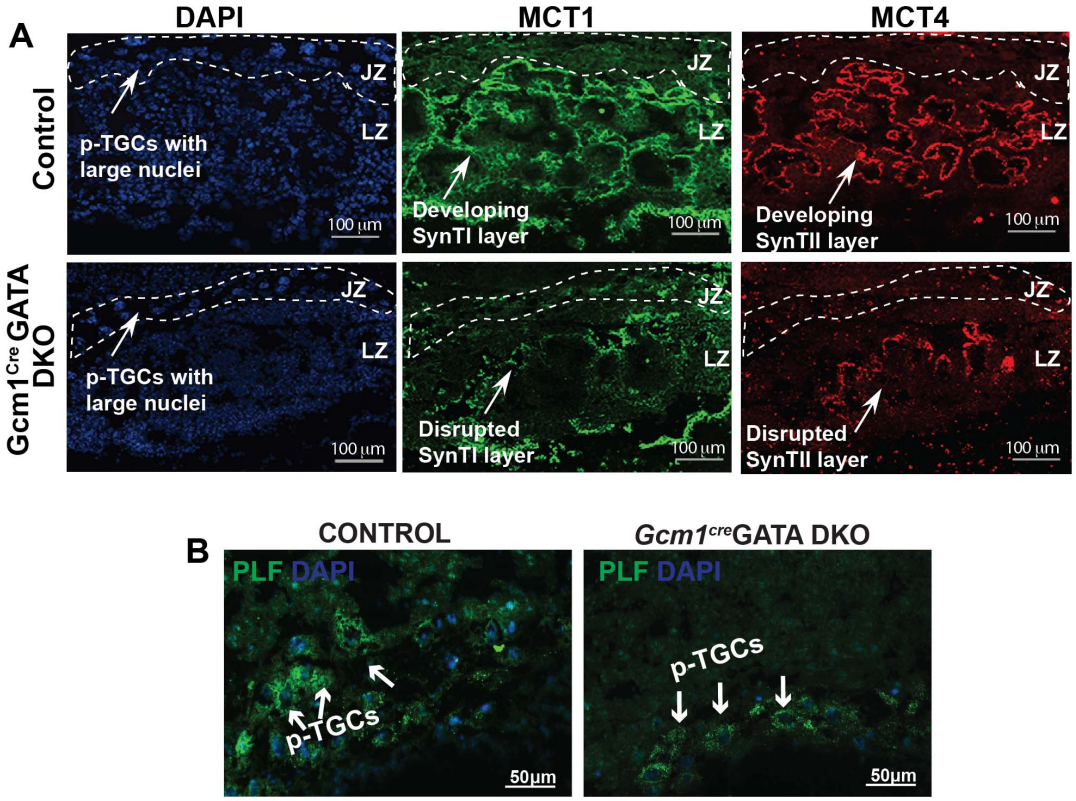


Figure S3. (A) Immunofluorescent analysis of the mature SynTI and SynTII layers was done in control and *Gcm1*^{Cre}GATA DKO placental labyrinth with MCT1 (green, marks SynTI layer) and MCT4 (red, marks SynTII layer) antibodies. The developing *Gcm1*^{Cre}GATA DKO placental labyrinth zone (LZ) contained defective SynTI and SynTI layers, they were disrupted as compared to juxtaposed layered organization observed in the control (Scale bars, 100 µm.). B, Immunofluorescent analysis of the pTGCs was done in control and *Gcm1* ^{Cre}GATA DKO placental labyrinth with PL1 (red, marks p-TGCs) antibody. The junctional zone (JZ) in both the control and the *Gcm1* ^{Cre}GATA DKO contained parietal TGCs (p-TGCs). (Scale bars, 50 µm.).

Fig. S4

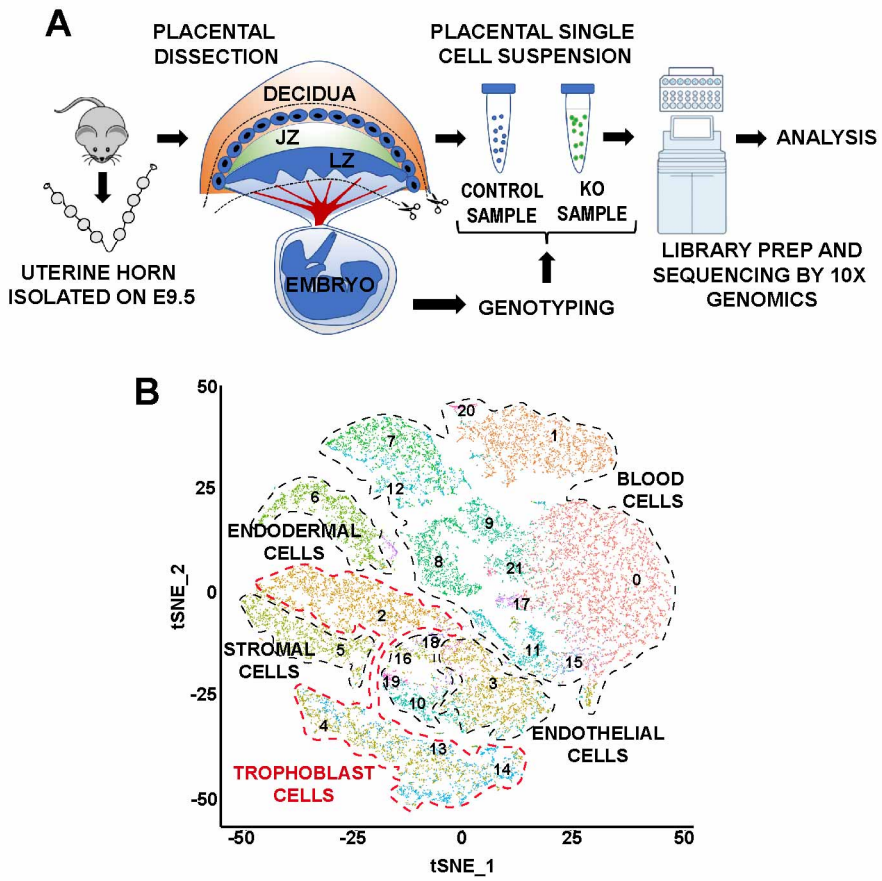


Figure S4. (A) Schematic showing the strategy of performing single cell RNA seq (scRNA-Seq) with mouse placenta. The uterine horn at day 9.5 post fertilization was isolated from pregnant mice (cross mentioned in fig 1A). Cartoon representation of the main regions of the placenta – Labyrinth (LZ), Junctional Zone (JZ), and Decidua are shown. Removal of the decidual stroma and the allantois is marked by scissors and cut lines. After removal of the decidua and the embryo proper, the placentae (5 control placentae and 6 $Gcm1^{Cre}$ GATA DKO placentae were pooled together to generate the control and knockout samples respectively) were used to make a single cell suspension for sequencing using the 10x genomics platform; the embryo proper was used for genotyping to segregate control and $Gcm1^{Cre}$ GATA DKO placental samples. (B) Visualization of the scRNA-Seq data with the analysis plotted in two dimensions by transcriptome similarity using t-distributed stochastic neighbor embedding (t-SNE). t-SNE plot represents all the different cell types observed in the placenta at E9.5, clustered, and plotted according to transcriptome similarity. Clusters were annotated according to canonical marker genes. Each dot represents one cell colored according to assignment by clustering analysis . Dotted lines encircle clusters with common properties segregated into five broad groups.

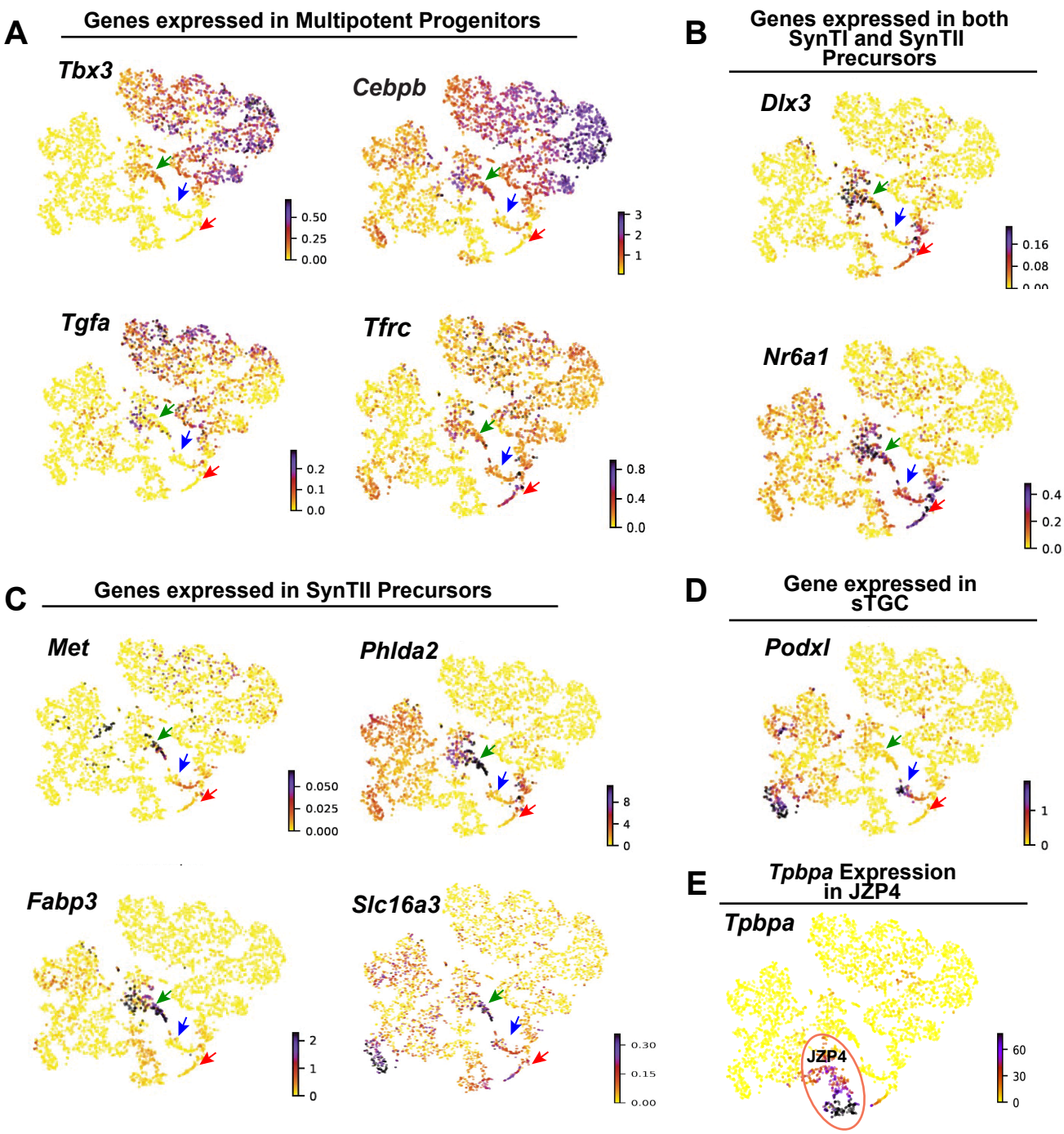


Figure S5. RNA velocity plots showing the expression of marker genes in specific clusters, the scale represents the level of expression- yellow (low), red (intermediate) and blue (high). The colored arrows mark specific populations- green=SynTII precursors, blue= S-TGC precursors and red= SynTI precursors. (A) *Tbx3, Cebpb, Tfrc*, Tgfa are highly expressed in the multipotent progenitor cluster. (B) *Dlx3* and *Nr6a1* are highly expressed in the SynTI precursors (red arrow), SynTII precursors (green arrow) but absent in S-TGC precursors (blue arrow). (C) *Met, Phlda2, Fabp3* and *Slc16a3* are highly expressed in the SynTII precursors. (D) *Podxl* is expressed by the S-TGC precursors (red arrow). (E) Tpbpa expression is observed only in JZP4 cluster.

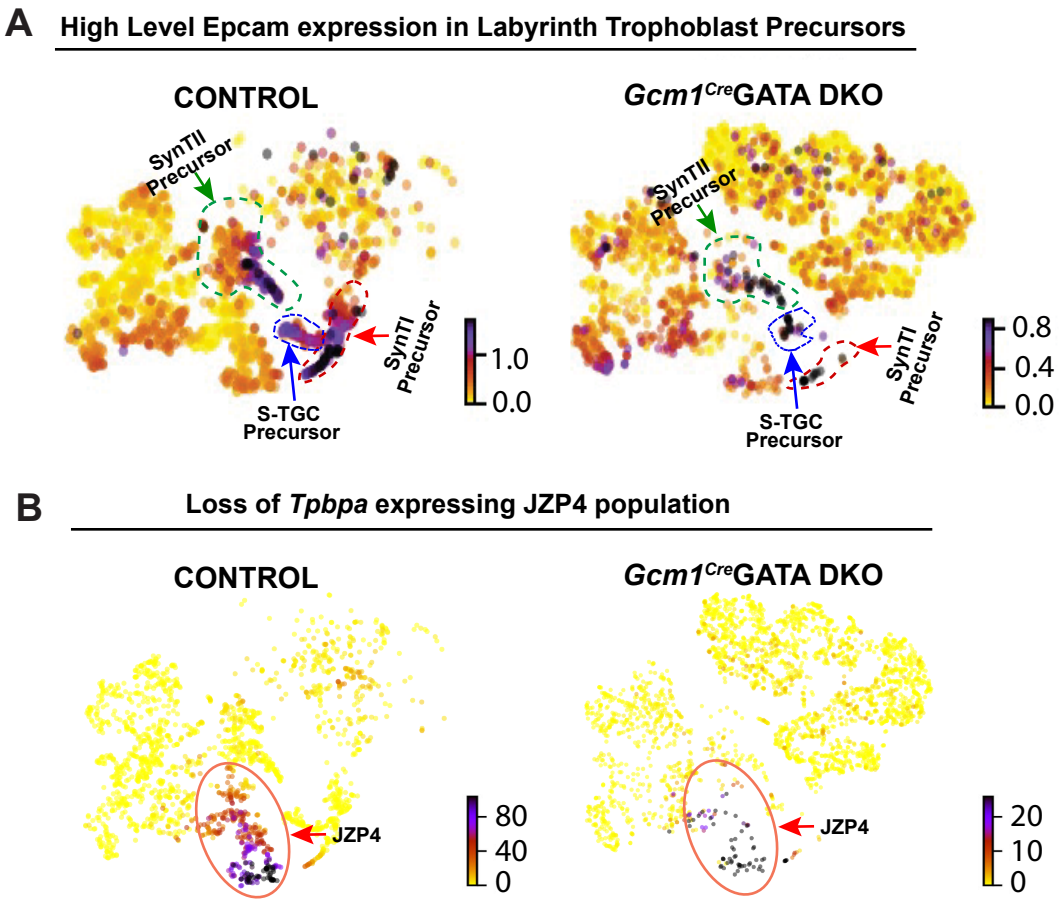


Figure S6. RNA velocity plots showing the expression of marker genes in specific clusters, the scale represents the level of expression- yellow (low), red (intermediate) and blue (high). The colored arrows mark specific populations- green=SynTII precursors, blue= S-TGC precursors and red= SynTI precursors. (top) Epcam (High) expression is observed in the SynTI precursors, SynTII precursors and S-TGC precursors in both control as well as *Gcm1*^{Cre} GATA DKO placentae. (bottom) Tpbpa expression is observed only in JZP4 cluster in the control but diminishes in the *Gcm1*^{Cre}GATA DKO.

Fig. S7

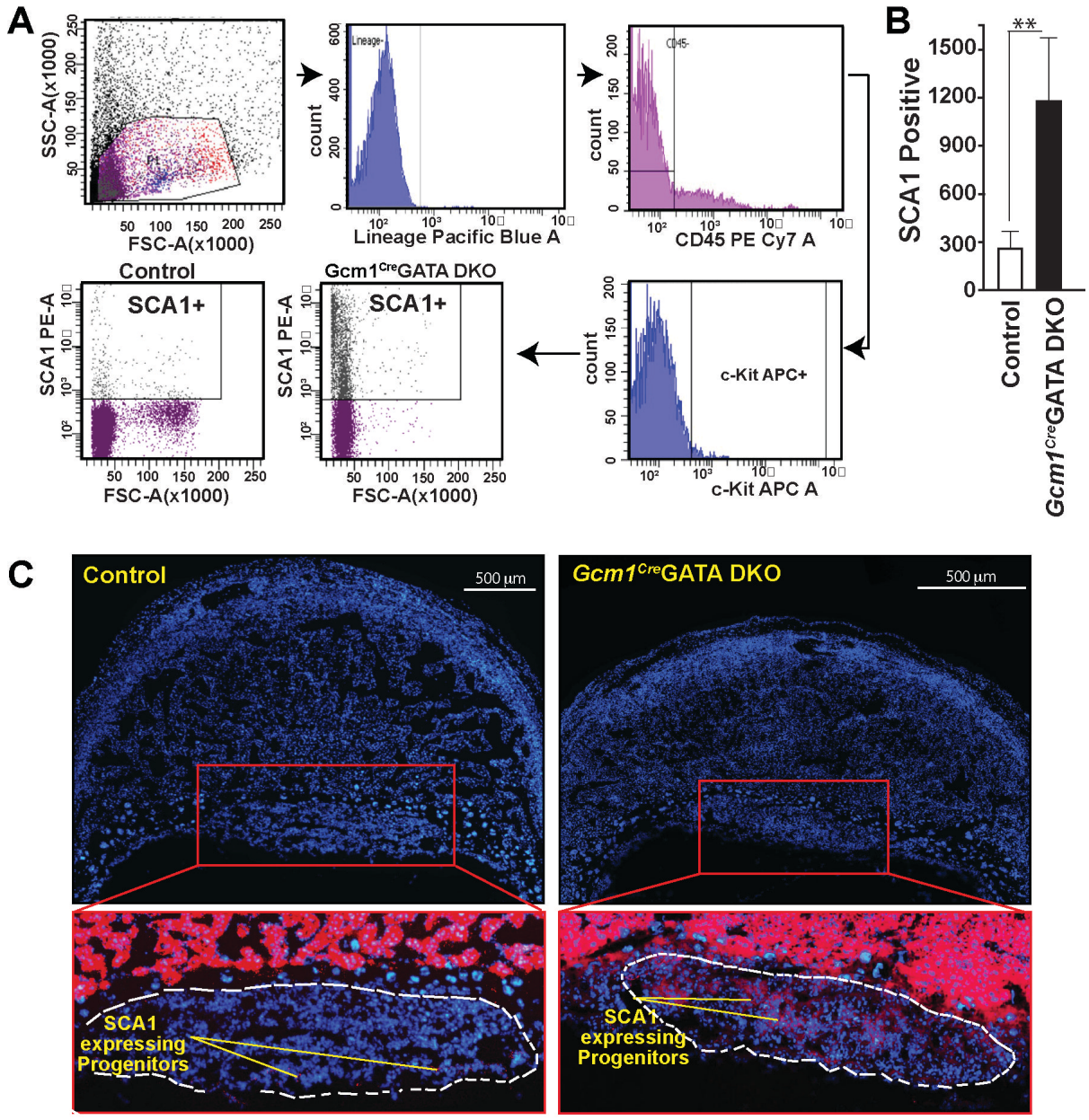


Figure S7. (A) FACS sorting of SCA1 (Ly6a) positive trophoblast cells from ~E9.0 control and *Gcm1*^{Cre}GATA DKO placentae confirmed the accumulation of these cells in the absence of the GATA factors. Single cell preparation from control and *Gcm1*^{Cre}GA-TA-DKO placentae were prepared followed by removal of all the hematopoietic and endothelial cells (Lineage negative, CD34 negative, CD45 negative), and tested percentage of cells that were SCA1 positive. (B) The quantitative plot shows relative numbers of SCA1 positive progenitors in control and *Gcm1*^{Cre}GATA DKO placentae (n=3, p=0.001). (C) Immunofluorescence images showing SCA1 expressing progeni-tors in E9.0 Control and *Gcm1*^{Cre}GATA DKO placentae. The labyrinth zone of the control placenta only have a few SCA1 positive cells. In contrast, Labyrinth zone of the *Gcm1*^{Cre}GATA DKO placenta is showing accumulation of many SCA1 positive cells. Note high levels of SCA1 expression within uterine cells in both sections.

Fig. S8

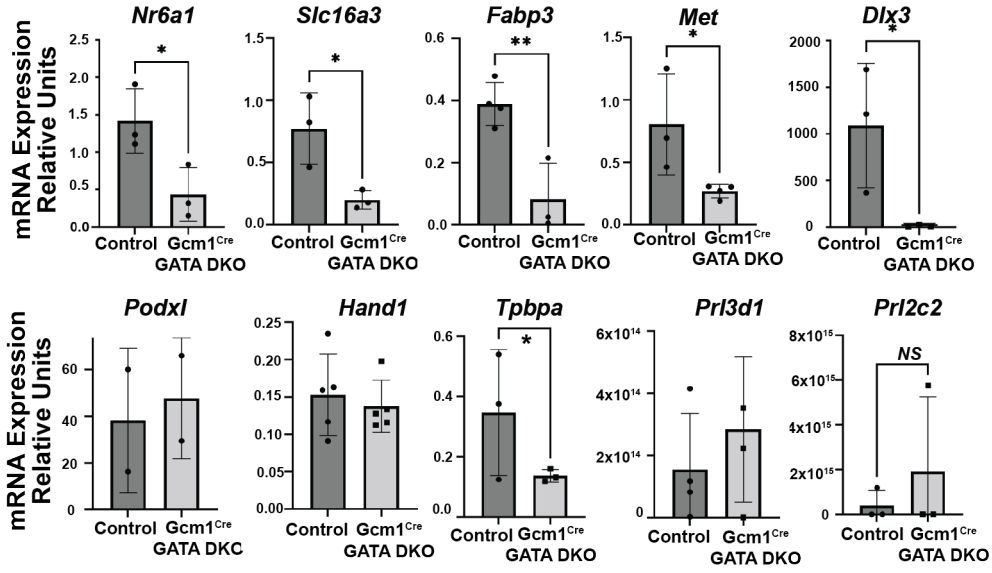


Figure S8. Quantitative RT-PCR analyses (mean ± SE; n = 3, P ≤ 0.001) reveal downregulation of SynT precursor specific markers like *Nr6a1*, *Slc16a3*, *Fabp3*, *Met* and *Dlx3* in *Gcm1*^{Cre}GATA DKO placentae compared to control at E9.5. While markers specific for S-TGC precursors like Hand1 and Podxl and junctional zone trophoblasts markers like *Tpbpa*, *Prl3d1* and *Prl2c2* remain unchanged.

Fig. S9

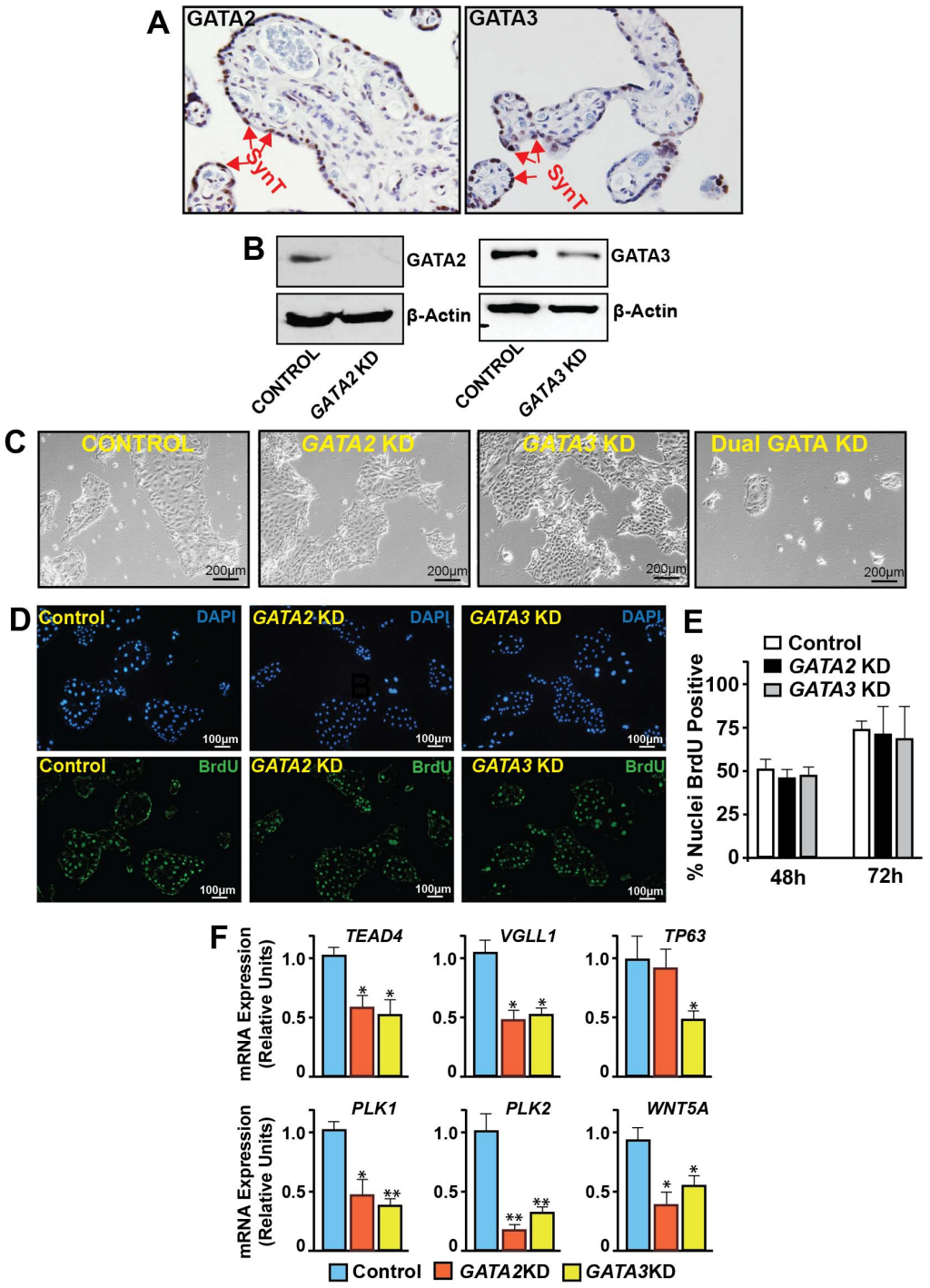


Figure S9. (A) Immunohistochemistry showing expression of GATA2 and GATA3 in term (35wk) human placenta. (B) Westernblot analyses showing shRNA-mediated depletion of GATA2 and GATA3 in human TSCs. (C) The stem state colony formation and proliferation of scrambled, *GATA2*KD and *GATA3*KD human TSCs was not severely affected after multiple passages (images represent passage 4, P4). But the dual knockdown of GATA2 and GATA3 in human TSCs affected stem state colony maintenance at P0 and could not be passaged further. (D) BrdU incorporation assay in control, *GATA2*KD and *GATA3*KD human TSCs in 2D culture. (E) Quantitation of BrdU assay. (F) Quantitative RT-PCR analyses (mean ± SE; n = 3, *,P ≤ 0.05, **,P ≤ 0.001) reveal impaired expression of genes, which are important to maintain Human TSC stem-state and proliferation, in *GATA2*KD and *GATA3*KD human TSCs.

A

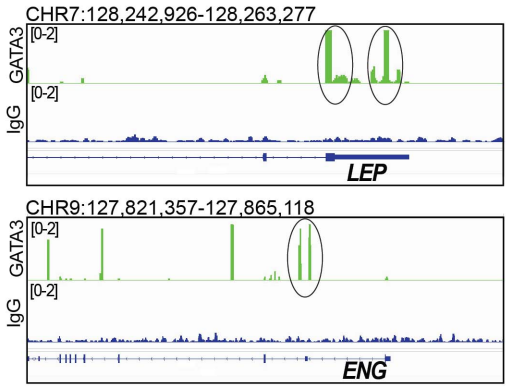
Motif	Name	P-Value
	GATA	$1e^{-52}$
	TEAD	$1e^{-38}$
	MYC	$1e^{-32}$
	SMAD2	$1e^{-30}$
	FOS	$1e^{-24}$
	FOXM1	$1e^{-19}$
	CEBP	$1e^{-17}$
	STAT6	$1e^{-15}$
	ZFX	$1e^{-14}$
	GATA	$1e^{-13}$

Motif	Name	P-Value
	GATA	$1e^{-16}$
	HINFP	$1e^{-14}$
	SMAD4	$1e^{-12}$
	TFAP2C	$1e^{-12}$
	c-JUN	$1e^{-12}$
	ZNF692	$1e^{-12}$
	TEAD	$1e^{-12}$
	GLI3	$1e^{-11}$
	ZFX	$1e^{-10}$
	NFY	$1e^{-10}$

B

GENE	Upregulated in PE/FGR	Fold chnage in *GATA2* KD hTSCs	Fold chnage in *GATA3* KD hTSCs	GATA2 Target	GATA3 Target
FSTL3	PE/FGR	Not significant	1.582	No	Yes
FLT1	PE	1.546	1.635	Yes	Yes
HTRA4	PE	1.823	1.867	No	Yes
LEP	PE	1.920	1.890	No	Yes
ENG	PE	(-) 1.880	(-) 1.798	No	Yes
DIO2	PE/FGR	(-) 4.134	(-) 4.380	Yes	Yes
TREM1	PE/FGR	(-) 3.227	(-) 3.040	Yes	No

C



D

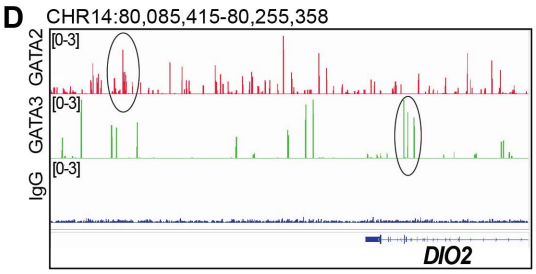


Figure S10. (A) HOMER analyses showing most enriched trnascription factor motifs within the GATA2 and GATA3 binding regions. (B) The table shows GATA2 and GATA3 target genes (in human TSCs), which are significantly upregulated in the placentae from pregnancies complicated with PE and/or FGR. These genes are also differentially regulated in *GATA2*KD and *GATA3*KD human TSCs. (C) IGV tracks showing GATA3 binding peaks at the *ENG* and *LEP* loci. (D) IGV tracks showing GATA2 and GATA3 binding peaks at the *DIO2* locus.

Fig. S11

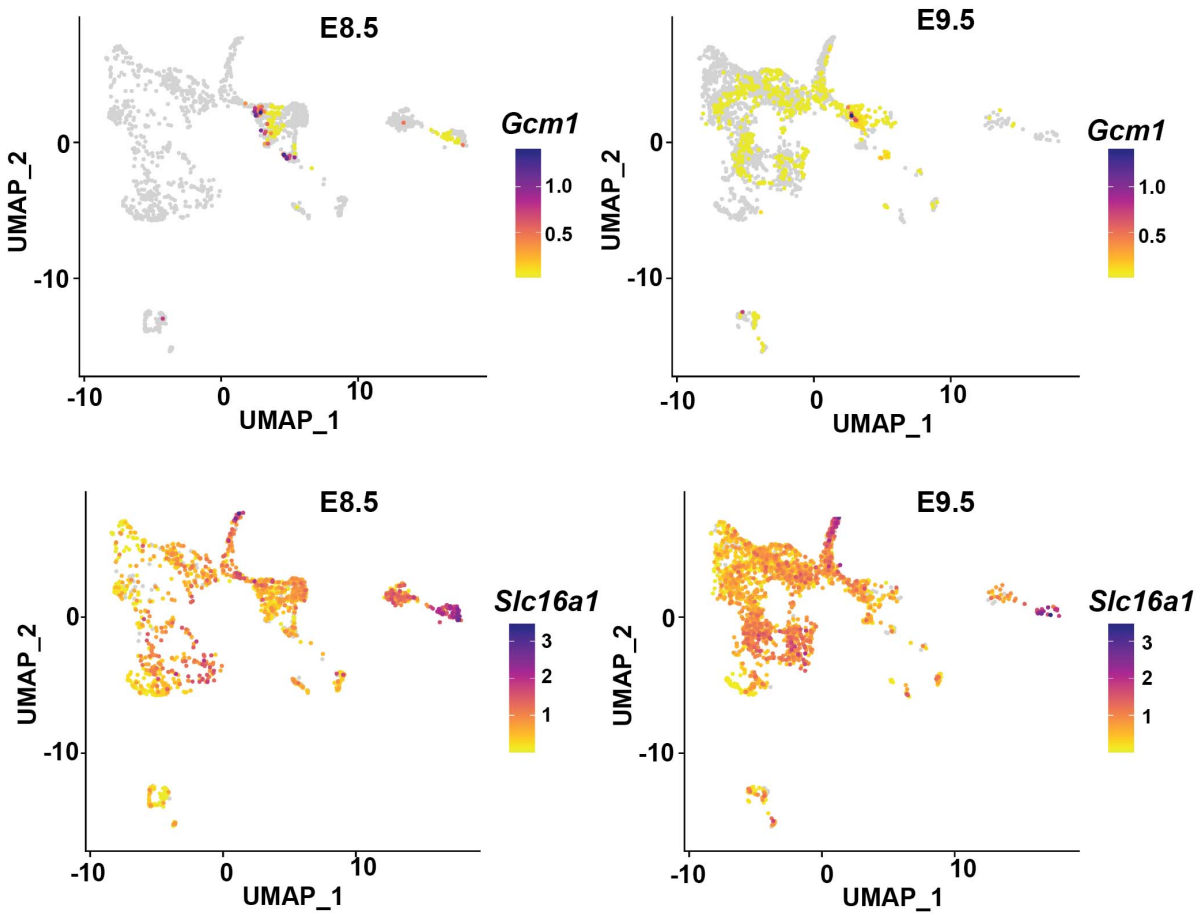


Figure S11: *Gcm1* mRNA is expressed in many trophoblast cells in an E9.5 mouse placenta. The UMAP plots were generated using the scRNA-seq data from Xiangxiang Jiang et. al., Cell Discovery, 2023. Plots show *Gcm1* and *Slc16a1* mRNA expression in trophoblast cells of developing mouse placentae at E8.5 and E9.5. Note that at E8.5 *Gcm1* mRNA expression is detected only in a few cells, including a few cells with low level expressions (Yellow dots). In contrast, at E9.5, *Gcm1* is expressed at a low level in many trophoblast cells, including cells, which also express *Slc16a1*.