SUPPLEMENTARY FIGURES

Figure S1

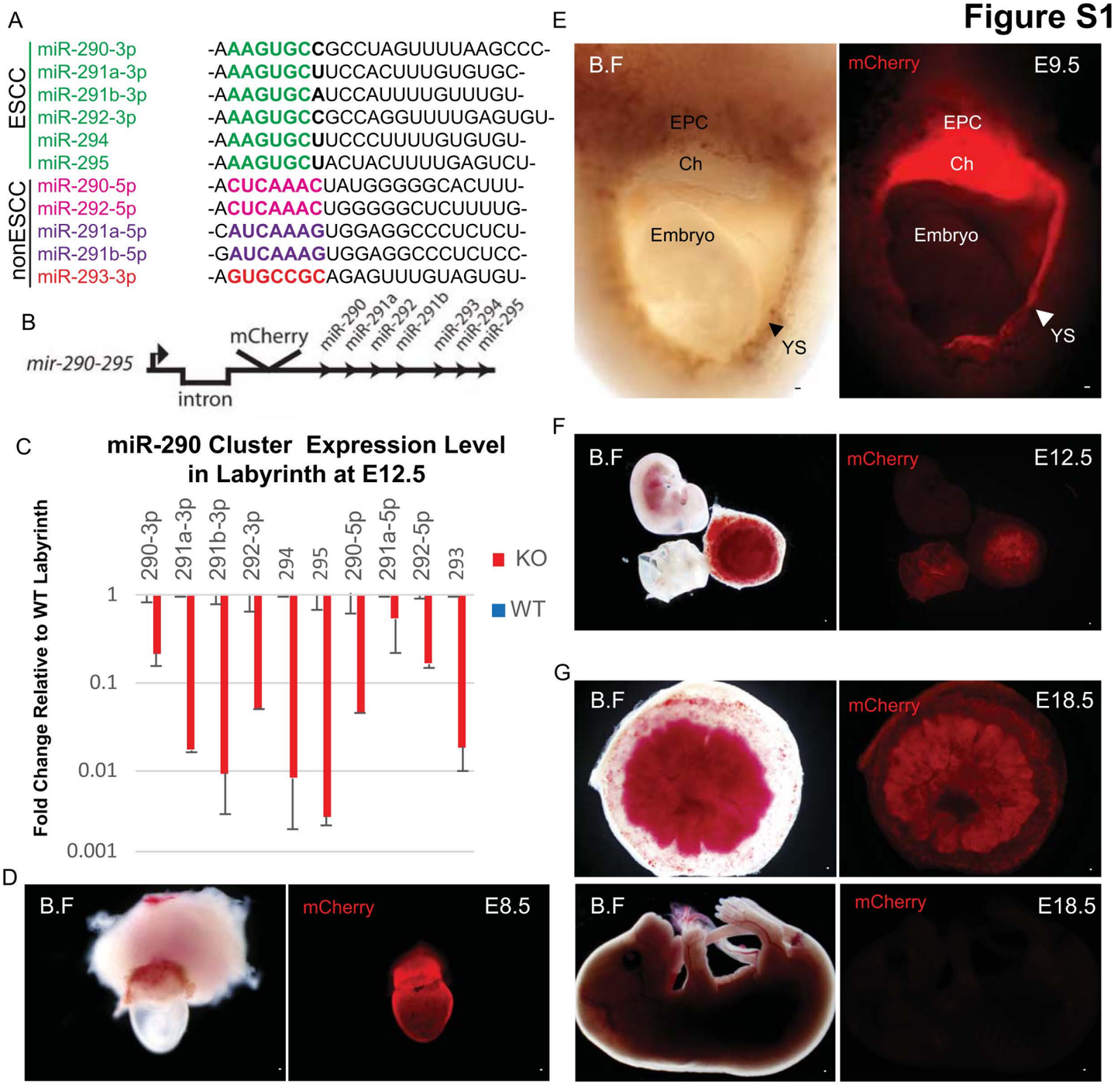

Figure S1 (Related to Figure 1): Whole-mount expression of miR-290 during pregnancy.

(A) The four families of mature miRNAs produced by the miR-290 cluster. (B) Schematic of miR-290-mCherry reporter (Parchem et al., 2014). (C) qRT-PCR results showing expression of different mature miRNAs arising from the miR-290 cluster in wild-type and miR-290 cluster knockout placental labyrinths at E12.5, normalized to Sno202 (three biological replicates). (D-G) Whole mounts showing miR-290 cluster is strongly expressed in yolk sac, chorion, and ectoplacental cone, but not in embryo from E8.5 to the birth. (H, I) qRT-PCR results showing expression of the mature miRNAs arising from the miR-290 cluster in wild-type yolk sac and labyrinth at different timepoints normalized to Sno202 (*n*=3 for each timepoint). BF, bright field; mCherry, fluorescent image of mCherry fluorescent reporter (red signal) co-expressed with miR-290 cluster; EPC, ectoplacental cone; Ch, chorion; YS, yolk sac. Error bars represent SD. The scale bar represents 100 μm.

Figure S2

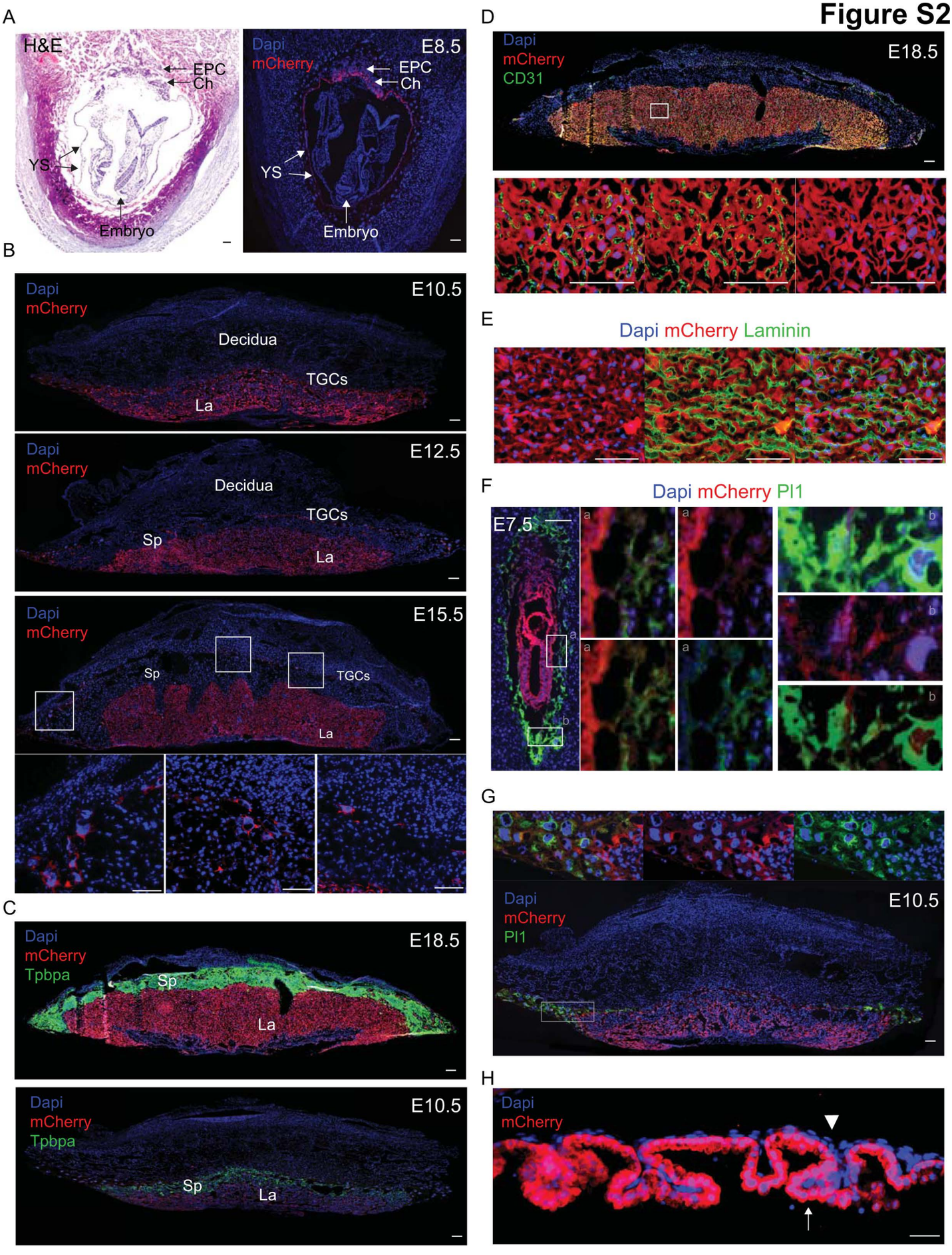

Figure S2 (Related to Figure 2): Expression of miR-290 cluster in different cellular compartments of the conceptus.

(A) Sections showing miR-290 cluster expression in ectoplacental cone, chorion, and yolk sac placenta, but not in embryo at E8.5. Blue, Dapi; Red, mCherry. (B, C) Sections at later timepoints showing miR-290 cluster expression in labyrinth and parietal trophoblast giant cells, but not in spongiotrophoblast cell layer. Trophoblast specific protein alpha (Tpbpa) is a marker of spongiotrophoblast cells. Blue, Dapi; Red, mCherry; Green, Tpbpa. (D) MiR-290 is positive in trophoblastic cells of the labyrinth, but allantois-derived endothelial cells (CD31 positive) are miR-290 negative. Blue, Dapi; Red, mCherry; Green, CD31. (E) Co-staining for mCherry and laminin confirms miR-290 cluster is not expressed in endothelial cells which are surrounded by basement membrane. Blue, Dapi; Red, mCherry; Green, Laminin. (F, G) Co-staining for Pl-1 shows cells that are double positive for this parietal TGC marker and miR-290 cluster, both at E7.5 and E10.5. Pl-1 is no longer expressed after E10.5. Blue, Dapi; Red, mCherry; Green, Pl1. (H) MiR-290 cluster is expressed in endoderm-derived cell layer of yolk sac (arrow) but not in mesoderm-derived cells (arrowhead) Blue, Dapi; Red, mCherry. EPC, ectoplacental cone; Ch, chorion; YS, yolk sac placenta; La, labyrinth; Sp, spongiotrophoblast layer, TGCs, trophoblast giant cells. The scale bar represents 100 μm.

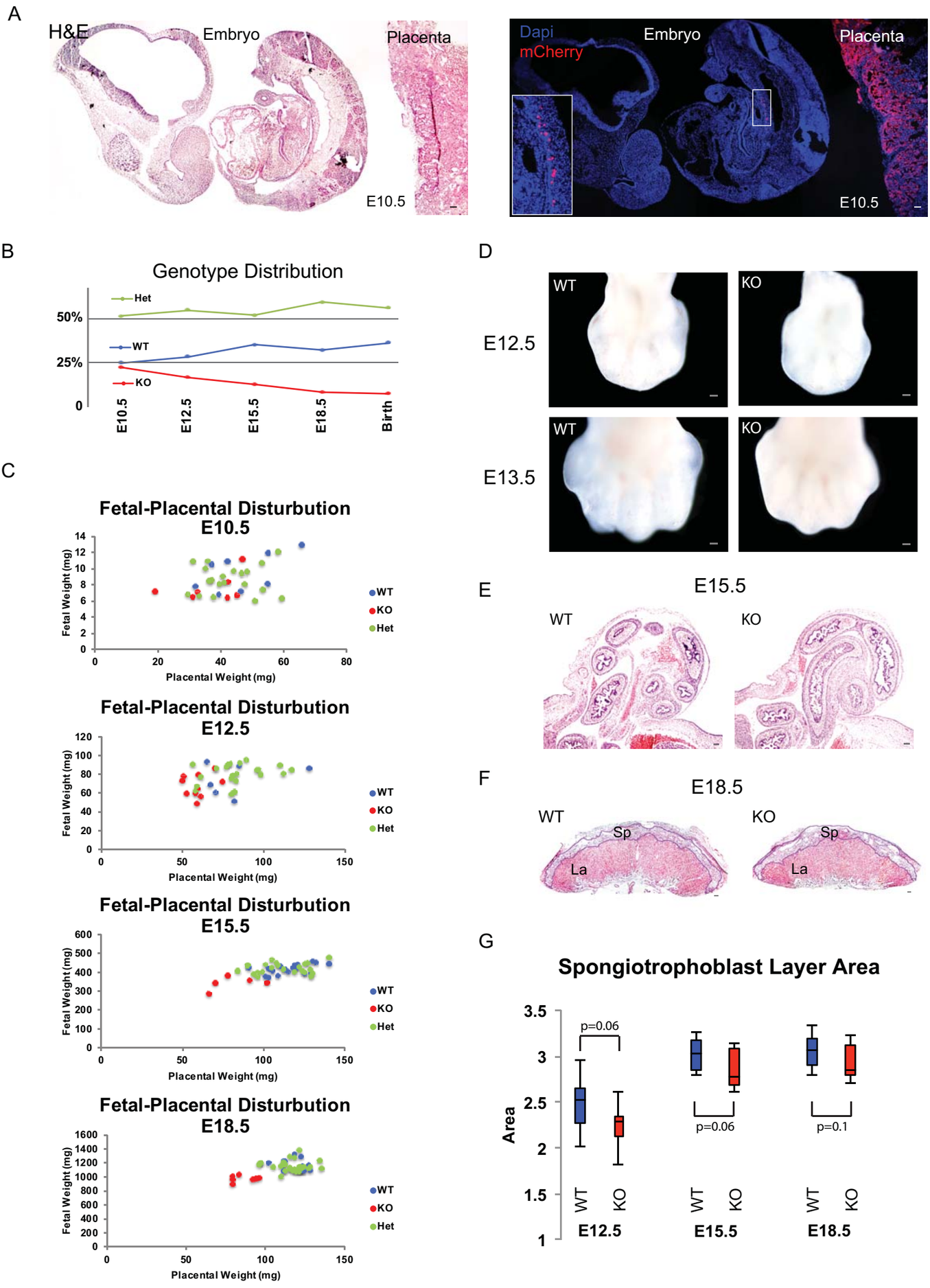

Figure S3 (Related to Figure 3): Phenotypic analysis of miR-290 cluster knockout. (A) E10.5, expression pattern of miR-290 cluster in placenta and primordial germ cells. Blue, Dapi; Red, mCherry. (B) Genotype distribution of wild-type, heterozygous and miR-290 cluster knockout mice in different time points for Het to Het breeding (C) Fetal and placental weight distribution of wild-type, heterozygous, and miR-290 cluster knockout conceptuses at different time points. (D) Hand plate. At E12.5, shows angular contours corresponding to the future digits. At E13.5, distal border of hand plate is indented and the definitive location of digits is clearly seen. (E) E15.5, H&E stained sections of wild-type and miR-290 cluster knockout embryos showing normal timing of midline closure in knockout embryos. (F) E18.5, Section of miR-290 cluster knockout and wild-type placentas at same depth as defined by umbilical vessels attachment site, H&E stained. (G) Quantification of spongiotrophoblast layer area in miR-290 cluster knockout and wild-type placentas. (n > 3 placentas for each time point, at least three sections per placenta). upper and lower whiskers represent max and min. middle line denotes median. The scale bar represents 100 μm.

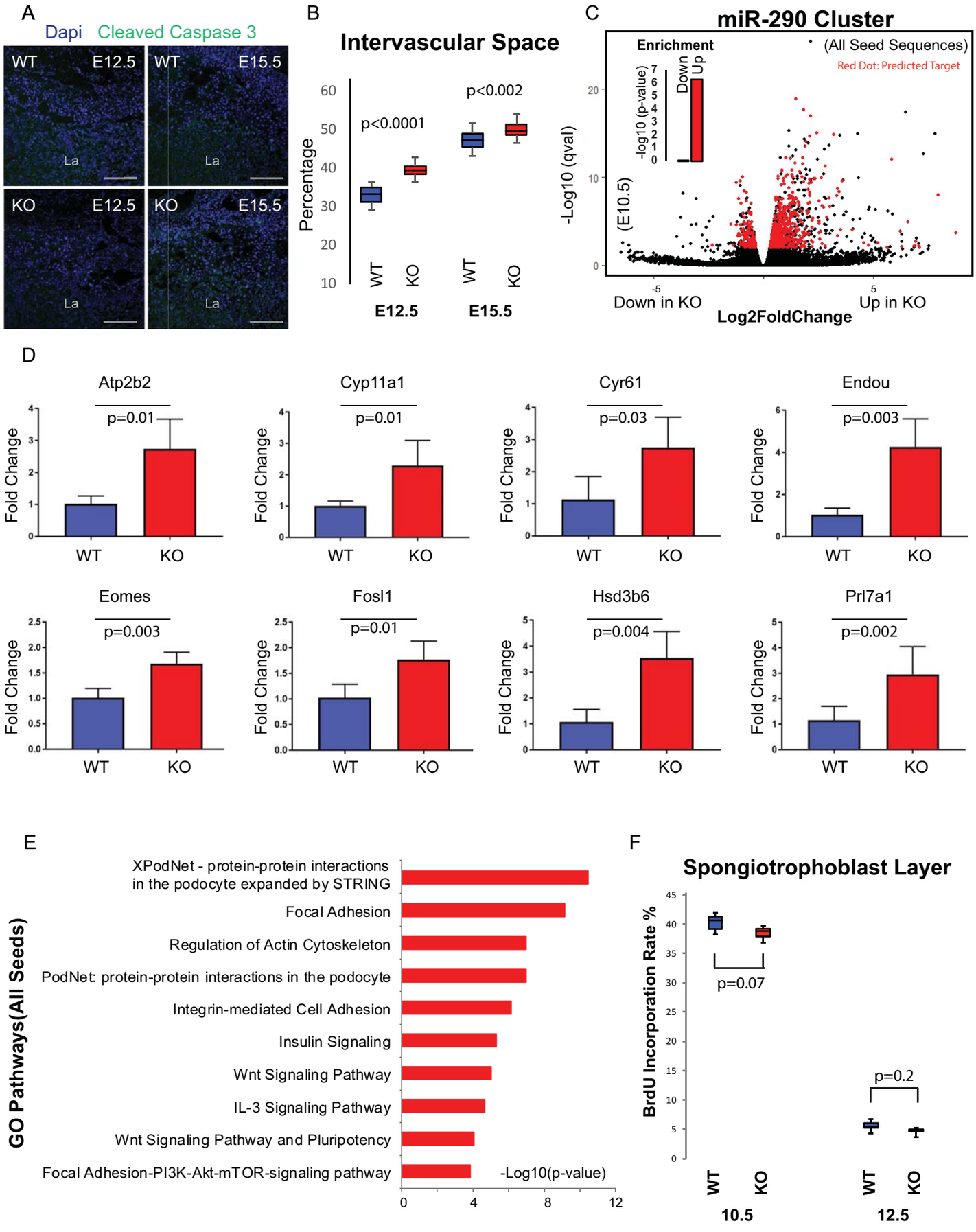

Figure S4 (Related to Figure 4&5&6): Phenotype and molecular analysis.
(A) E12.5 and E15.5, Cleaved Caspase 3 staining showed very few apoptotic cells in labyrinth of both wild-type and miR-290 cluster knockout placental labyrinth. Blue, Dapi; Green, Cleaved Caspase 3. (B) Quantification of intervascular area in labyrinth of E12.5 and E15.5 placentas. (*n* >3 placentas for each timepoint, more than five sections per placenta). Upper and lower whiskers represent max and min. middle line denotes median. (C) RNA-Seq results, placental labyrinth at E10.5, volcano plots showing fold change and significance values for combined targets of all seed families produced by the miR-290 cluster. Targets predicted by TargetScan, are highlighted in red. Inset bar graphs show enrichment p values for targets among the significantly up and down regulated genes. Up, up regulated; Down, down regulated. (D) qRT-PCR of eight predicted targets that were upregulated in RNA-Seq analysis. Expression was normalized to GAPDH (four biological replicates). Error bars represent SD. (E) Top ten GO terms identified by gene ontology analysis using Enrichr for genes that are upregulated and have a seed match to all miR-290 cluster families. (F) Percentage of BrdU positive cells of spongiotrophoblast layer in wild-type and miR-290 cluster knockout placentas (*n* > 3 placentas for each timepoint, more than three sections per placenta, cells are counted in more than ten HPFs, randomly selected, in each section). Upper and lower whiskers represent max and min, middle line denotes median. The scale bar represents 100 μm.

SUPPLEMENTARY TABLES

Table S1: List of qRT-PCR primer sequences

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Table S2: List of all genes that are differentially expressed in wild-type versus miR-290 cluster knockout placental labyrinths at E10.5, E12.5, and E15.5. Each tab represents a time point. Target ID, gene name, q value, and log fold change are provided for all genes.

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Table S3 (Related to Figures 6, S4): List of target genes for each miRNA family within the miR-290 that are differentially expressed in wild-type versus miR-290 cluster knockout E10.5 placental labyrinth. Each tab represents all or distinct miRNA family within the miR-290 cluster. Target ID, gene name, q value, and log fold change are provided for all and each TargetScan predicted target that has a q value for fold change <0.01.

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