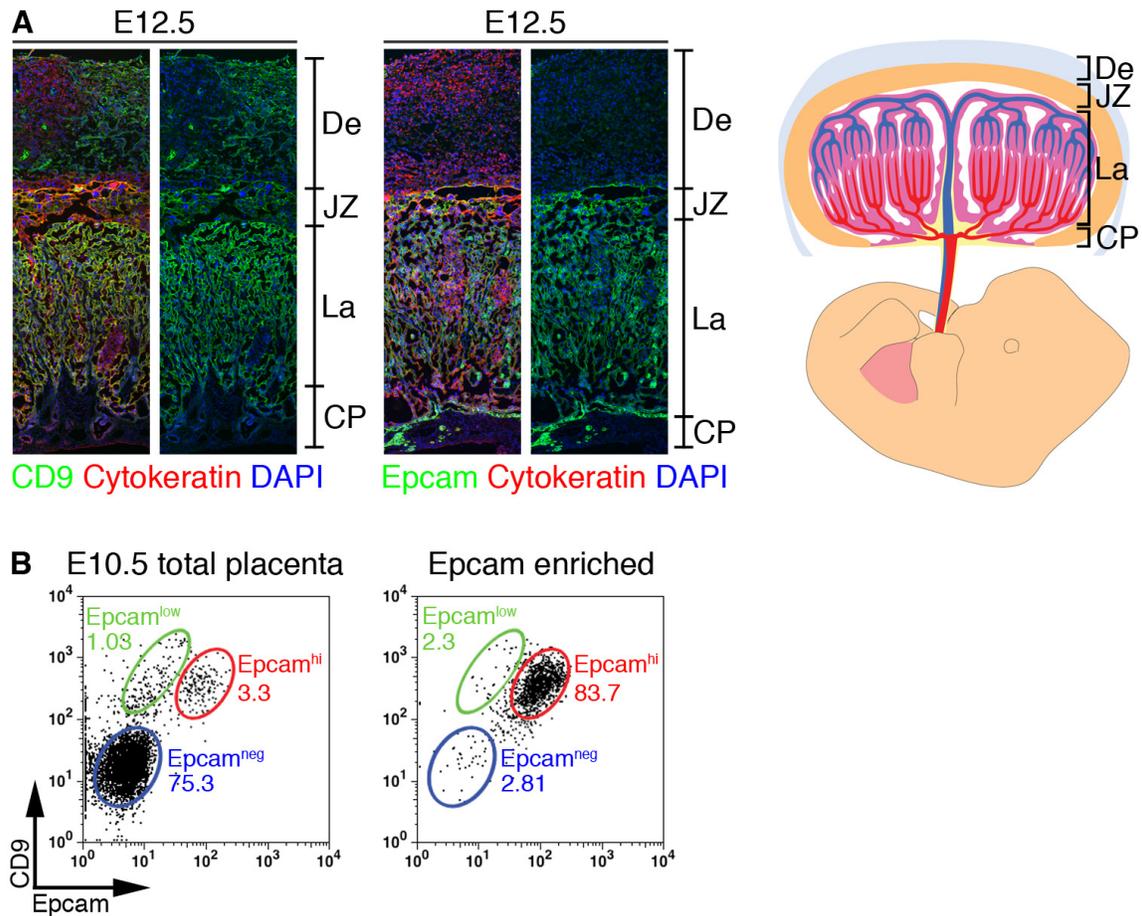


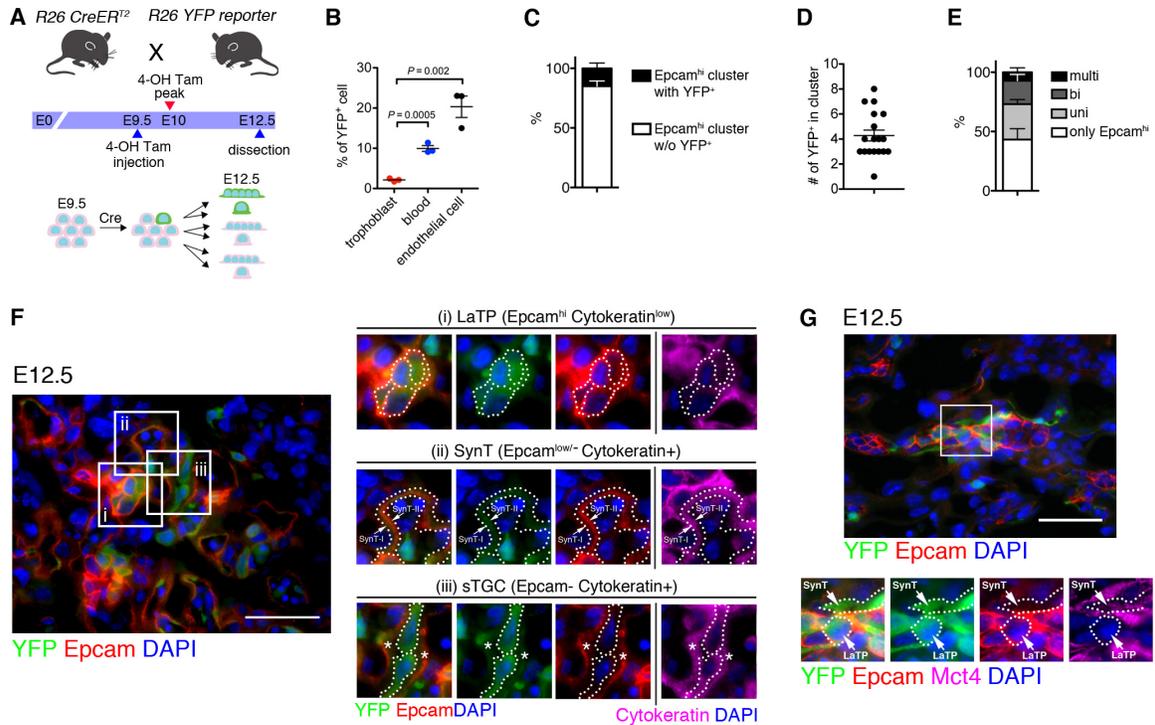
## Supplemental Figures



**Figure S1. Labyrinth trophoblasts express CD9 and Epcam.** Related to Figure 1.

(A) Immunofluorescence and schematic representation of mouse placental structure. Sections from E12.5 placenta were stained with antibodies for CD9 or Epcam (green), and Cytokeratin (red, trophoblasts). DAPI (blue) indicates nuclei. The fully developed placenta is composed of three major layers: a decidua layer (De), which includes decidua cells of the uterus; a junctional zone (JZ) which attaches the fetal placenta to the decidua and contains trophoblast giant cells (TGC) and spongiotrophoblasts (Sp); and a labyrinth layer (La), composed of fetal endothelial cells and labyrinth trophoblasts (SynT-I and -II, and sinusoidal trophoblast giant cells (sTGC)) that establish the substance exchange interface between fetal and maternal circulations. CP, chorionic plate.

(B) FACS analysis of cells enriched by anti-Epcam antibody documents effective isolation of Epcam<sup>hi</sup> cells without significant contamination of differentiated Epcam<sup>low</sup> cells.



**Figure S2. Epcam<sup>hi</sup> LaTP cells are clonally linked to terminally differentiated SynT.** Related to Figure 2.

(A) Schematic for *in vivo* clonality experiment. *Rosa26 (R26) YFP* reporter mice were mated with *R26 CreER<sup>T2</sup>* mice. At E9.5 Cre-mediated gene deletion was induced by injection with 4OH-tamoxifen and embryos were dissected at E12.5.

(B) Frequency of Cre-mediated gene-recombination in different cell types in the placenta documenting rare marking of trophoblasts. (C) Frequency of Epcam<sup>hi</sup> clusters containing YFP-labeled cells.

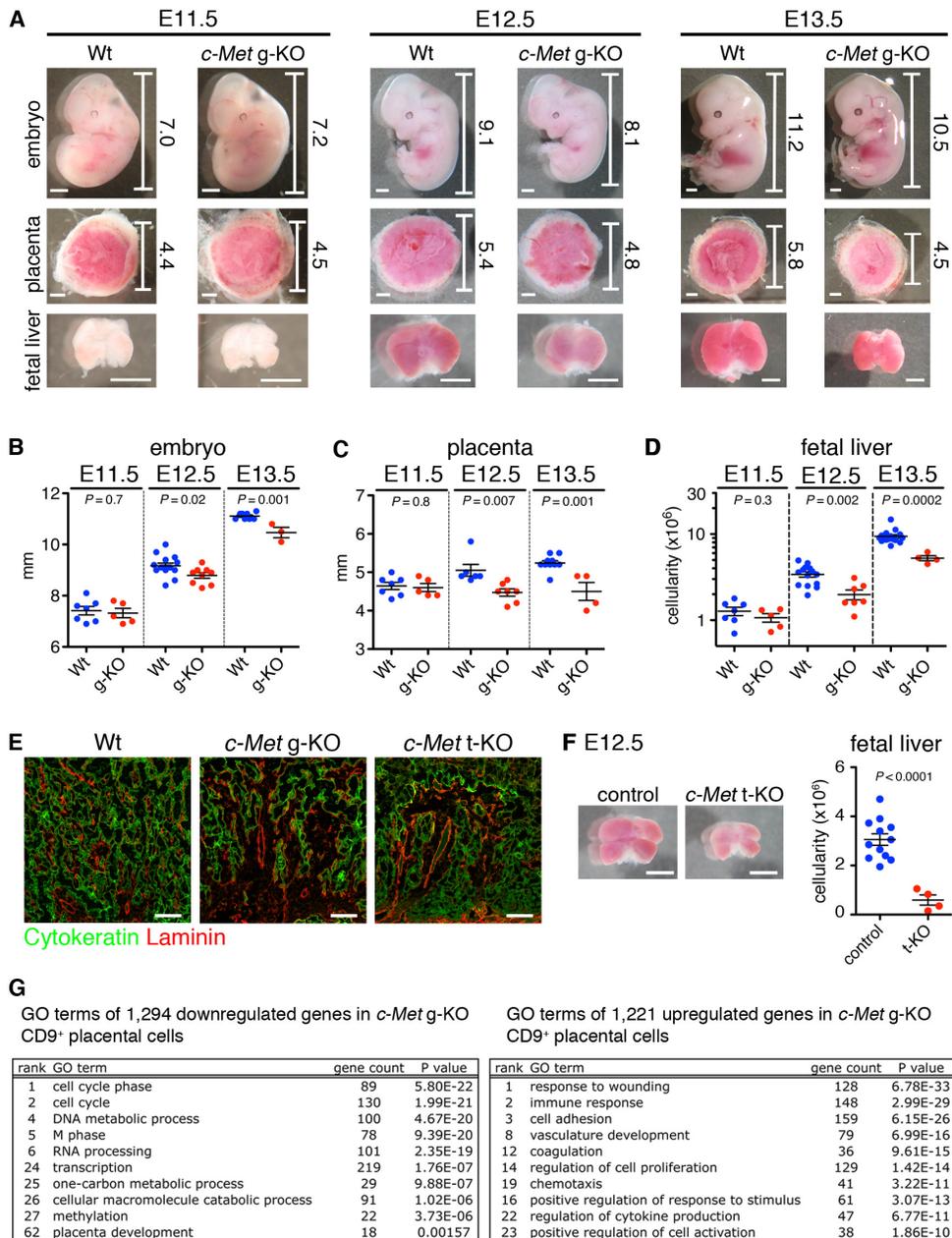
(D) Average number of YFP<sup>+</sup> cells in a labeled Epcam<sup>hi</sup> cell cluster.

(E) Differentiation potential of YFP labeled cells in individual clusters. Multi, Epcam<sup>hi</sup> LaTP with SynT-I, SynT-II and sTGC; bi, Epcam<sup>hi</sup> with SynT-I and SynT-II; uni, Epcam<sup>hi</sup> with sTGC, SynT-I or SynT-II.

(F) Documentation of multi-lineage differentiation in a cluster of YFP<sup>+</sup> labeled trophoblasts. (i) Epcam<sup>hi</sup> Cytokeratin<sup>low</sup> (LaTP), (ii) SynT-I (Epcam<sup>-</sup> Cytokeratin<sup>+</sup>) and SynT-II (Epcam<sup>low</sup> Cytokeratin<sup>+</sup>) and sTGC (iii) (Epcam<sup>-</sup> Cytokeratin<sup>+</sup>), \*, SynT layer.

(G) Documentation of terminally differentiated SynT cells (Epcam<sup>low</sup> Mct4<sup>+</sup>) in the same YFP<sup>+</sup> marked clusters as Epcam<sup>hi</sup> LaTP. Epcam (red), YFP (green), DAPI (blue), Mct4 (purple). Scale bar 100  $\mu$ m.

All error bars indicate SEM (Standard error of mean).



**Figure S3. *c-Met* regulates both placental and embryonic development.** Related to Figure 3.

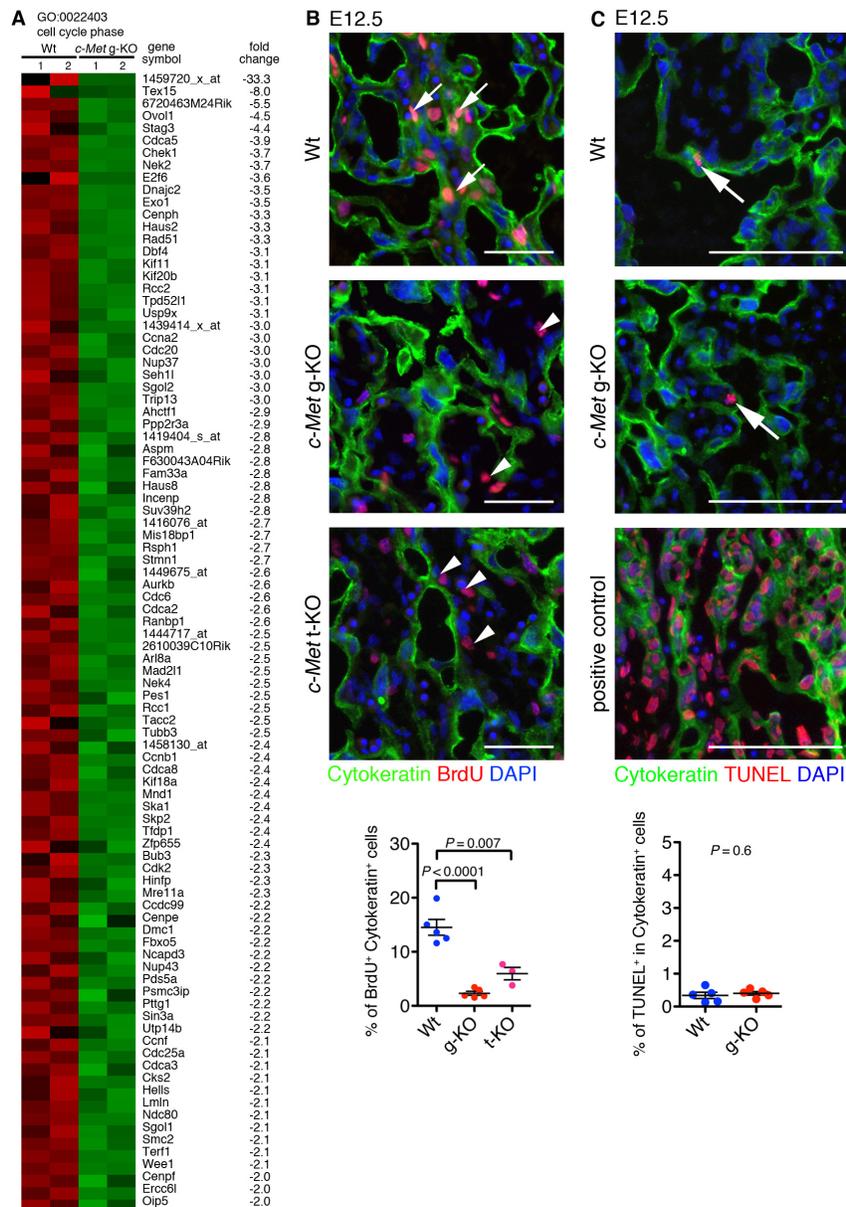
(A, B, C and D) Kinetic analysis documenting growth retardation of *c-Met* deficient embryos, fetal livers and placentas after E12.5. Scale bar 1 mm.

(E) IF for trophoblast specific marker (Cytokeratin) and ECM marker that marks blood vessels (Laminin) documenting poorly developed branching structure in *c-Met* g- and t-KO placentas.

(F) *c-Met* t-KO fetal livers show smaller size and low cellularity.

(G) Selected GO (gene ontology) categories representing genes down- or up-regulated in placental CD9<sup>+</sup> cells in the absence of *c-Met* signaling.

All error bars indicate SEM (Standard error of mean).



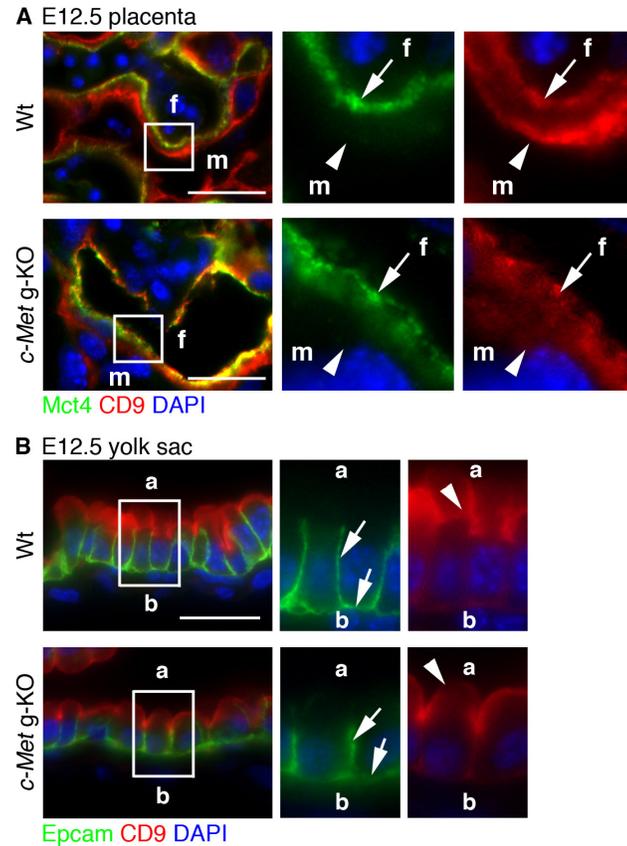
**Figure S4. c-Met directly regulates trophoblast proliferation.** Related to Figure 4.

(A) Microarray analysis of CD9<sup>+</sup> trophoblasts from Wt vs. *c-Met* g-KO placenta. The heat map presents expression of various genes that related to GO category “cell cycle phase” that are suppressed in CD9<sup>+</sup> trophoblasts of *c-Met* g-KO placenta.

(B) BrdU incorporation assay. Sections from E12.5 Wt, *c-Met* g-KO and t-KO placentas were stained for Cytokeratin (green), BrdU (red), and DAPI (blue). Although BrdU<sup>+</sup> Cytokeratin<sup>+</sup> trophoblasts (arrows) were observed in Wt placenta, only Cytokeratin<sup>-</sup> cells (arrowheads) showed mitotic activity in *c-Met* g- and t-KO placenta. Scale bar 100  $\mu$ m.

(C) Sections from Wt and *c-Met* g-KO placentas were stained for TUNEL (red), Cytokeratin (green) and DAPI (blue). Arrows indicate TUNEL and Cytokeratin double-positive cells. No significant difference was observed in apoptosis between Wt and *c-Met* g-KO trophoblasts. Scale bar 100  $\mu$ m.

All error bars indicate SEM (Standard error of mean).

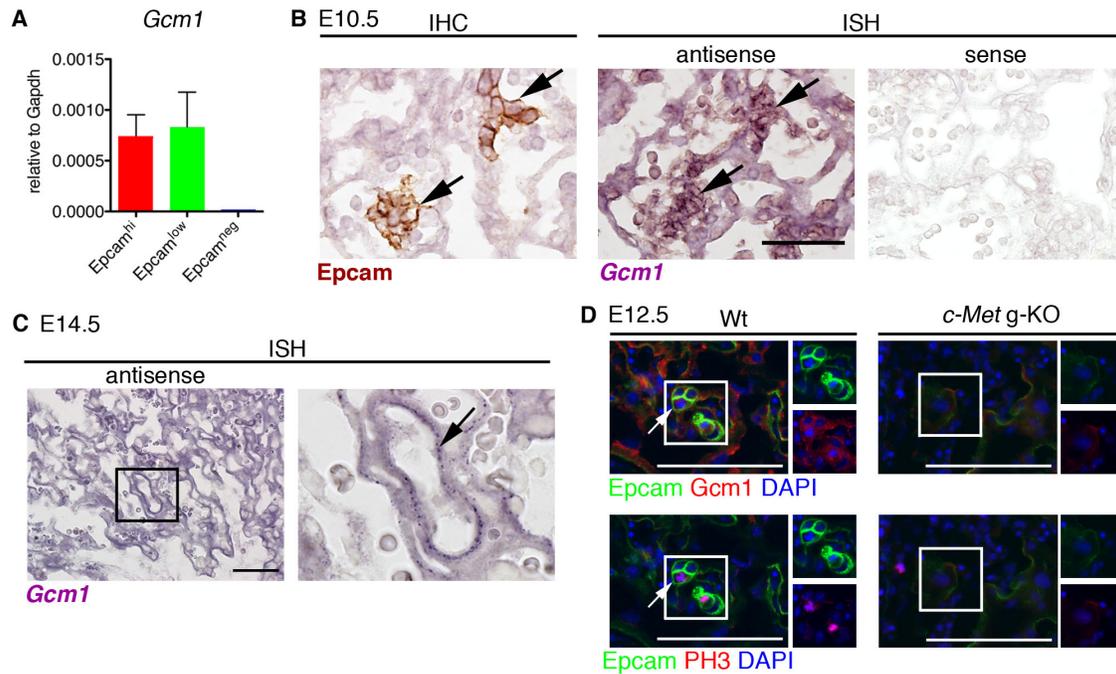


**Figure S5. Loss of *c-Met* compromises cell polarity specifically in the placenta.**

Related to Figure 5.

(A) IF for Mct4 (green), CD9 (red) and DAPI (blue) in Wt and *c-Met g-KO* placentas at E12.5. Presence of both Mct4<sup>-</sup> CD9<sup>+</sup> cells and Mct4<sup>+</sup> CD9<sup>+</sup> cells indicates that both SynT-I and II are present in Wt and *c-Met g-KO* placentas. Notably, in Wt placenta, Mct4 is specifically expressed on the fetal side in SynT-II and CD9 is expressed on both apical side of SynT-I and fetal side of SynT-II. However diffused localization of both CD9 and Mct4 was observed in *c-Met g-KO* placenta. Arrows indicate fetal side of SynT-II and arrowheads indicate apical membrane of SynT-I. f, fetal side. m, maternal side. Scale bar 25  $\mu$ m.

(B) IF for Epcam (green), CD9 (red), and DAPI (blue) on Wt and *c-Met g-KO* yolk sac. No abnormal localization of Epcam or CD9 and was observed in *c-Met g-KO* yolk sac. Arrows indicate basolateral localization of Epcam and arrowheads indicate apical staining of CD9; a, apical; b, basal. Scale bar 25  $\mu$ m.



**Figure S6. *Gcm1* is expressed in LaTP and SynT in the placenta.** Related to Figure 6.

(A) QRT-PCR documenting the enriched expression of *Gcm1* in both Epcam<sup>hi</sup> LaTP and Epcam<sup>low</sup> SynT in E 10.5 placenta. Error bars indicate SEM (Standard error of mean)

(B) IHC with anti-Epcam antibody and *in situ* hybridization (ISH) with *Gcm1* antisense probe indicates high expression of *Gcm1* in Epcam<sup>hi</sup> LaTP (arrows) in E10.5 placenta. Scale bar 100  $\mu$ m.

(C) ISH analysis showing high expression of *Gcm1* in SynT-II layer (arrow in the inset) adjacent to maternal blood spaces in E14.5 placenta. Scale bar 100  $\mu$ m.

(D) Sections from Wt and *c-Met* g-KO placentas at E12.5 were stained for Epcam, *Gcm1*, PH3, and DAPI. Arrows indicate Epcam<sup>hi</sup> and PH3<sup>+</sup> cells that also express *Gcm1*. Scale bar 100  $\mu$ m.

## **Supplemental Table Legends**

**Table S1. 1,294 genes down-regulated in *c-Met* g-KO CD9<sup>+</sup> placental cells with p value <0.05 and fold change cutoff  $\geq 2.0$ . Related to Figure 3.**

**Table S2. 1,221 genes up-regulated in *c-Met* g-KO CD9<sup>+</sup> placental cells with p value <0.05 and fold change cutoff  $\geq 2.0$ . Related to Figure 3.**

## Supplemental Extended Experimental Procedures

### Generation of germline *c-Met* KO and trophoblast specific *c-Met* KO embryos

The *c-Met* germline KO (g-KO) mice were generated by breeding *c-Met*<sup>fl/wt</sup> mice with *VavCre* transgenic mouse (kindly provided by Dr Thomas Graf, Center for Genomic Regulation, Barcelona, Spain), which occasionally results in Cre mediated gene deletion in germ cells. Subsequent breeding was carried out with germline deleted heterozygous animals.

To generate trophoblast specific *c-Met* KO (t-KO) mice, lentiviral Cre was used to delete conditionally targeted *c-Met* floxed alleles in the trophoblasts. FU-CreGFP-W (lentiviral vector expressing *Cre-Gfp* under the *UbiC* promoter) vector was kindly provided by Dr. Guoping Fan (UCLA, Los Angeles, CA, USA). *c-Met*<sup>fl/fl</sup> or *c-Met*<sup>fl/w</sup> female mice were superovulated by treatment with pregnant mare's serum gonadotropin followed by human chorionic gonadotropin 48 h later, and then mated with *c-Met*<sup>fl/fl</sup> male mice. Blastocysts were collected from uterus at 3.5 day after copulation. Embryos were maintained in M2 media (Sigma-Aldrich) during microinjection process. Lentiviral vector (titer 1-5 x 10<sup>8</sup>/ml) was injected using a Fematotips II glass needle and FematoJet microinjector (Eppendorf) with an injection pressure Pi=120 and constant flow pressure Pc=20. After lentiviral vector injection, embryos were transplanted into the uterus of 2.5-day

pseudopregnant recipients. After 10 days of transplantation, embryos (E12.5) were dissected and analyzed.

### **Immunofluorescence Staining**

IF was performed as previously reported (Rhodes et al., 2008). Briefly, for IHC and IF, tissues were fixed in 4% para-formaldehyde at 4°C overnight, and frozen in O.C.T. Compound (Sakura Finetek). For Bromodeoxyuridine (BrdU) incorporation assay, BrdU (Sigma-Aldrich, 100 µl of 10 mg/ml) was injected intraperitoneally into pregnant females. The females were sacrificed 1 hr after injection, and placentas were fixed and processed for IF (see below). For detection of Cytokeratin or Epcam, antigen retrieval was performed with Proteinase K treatment. For detection of incorporated BrdU, section was treated with 1 mg/ml DNase (Sigma-Aldrich) for 20 min. For amplification signal, Tyramide Signal Amplification kit (Invitrogen) was used for detection of Cre-Gfp and BrdU antigen. Following antibodies were used: FITC-conjugated anti-BrdU, Anti-CD31 (PECAM), FITC-conjugated and PE-conjugated anti-CD9, FITC-conjugated anti-CD71, and anti-Epcam antibody (CD326), (all from BD Pharmingen); Anti-phospho-histone H3 and anti-Mct4 antibody (Millipore); Biotinylated anti-FITC, Cy3-conjugated anti-Rat IgG , and biotinylated anti-rabbit IgG antibody (Jackson ImmunoResearch); Anti-Prkcz and anti-Gcm1 antibody (Santa Cruz Biotechnology); anti-Uvomorulin/ E-cadherin antibody (Sigma-Aldrich), anti-Cytokeratin antibody (Dako). For detection of YFP, Alexa Fluor 488 conjugated anti-GFP antibody (Invitrogen) was used. TUNEL assay was

performed using *in situ* cell death detection kit (Roche). End-labeled DNA with fluorescein-dUTP was detected by anti-FITC antibody. To stain cultured LaTP and their progeny, Epcam<sup>hi</sup> cells were first cultured on cover glass and then fixed with 4% PFA for 5 min. All stainings were performed as indicated for IF sections. For detection of BrdU, fixed cells were treated with 2N HCl for 5min before staining with anti-BrdU antibody. For detection of cell-cell boundaries, anti-uvomolulin/E-cadherin antibody (Sigma) was used. Contrast and levels of images were adjusted for display with Adobe Photoshop.

### **Microarray analysis**

To isolate trophoblasts for microarray analysis, single cell suspension of E12.5 placentas was stained with PE-conjugated anti-CD9 antibody, followed by coating with anti-PE antibody-microbeads (Miltenyi Biotec) and separation of CD9 positive cells with MACS LS column (Miltenyi Biotec) according to the manufacture's instructions. RNA from CD9<sup>+</sup> cells was purified by RNeasy mini kit (Qiagen). For comparison of gene expression in Wt and *c-Met* germline KO (g-KO) CD9<sup>+</sup> trophoblasts, Affymetrix MOE430\_2.0 microarrays (Affymetrix) were performed on CD9<sup>+</sup> cells from two Wt and two *c-Met* g-KO placentas. Raw data are available at GEO with accession numbers GSE38342. Differential expression assessment was performed using the LIMMA package (Smyth, 2004). Probes with a p value less than 0.05 and absolute fold change threshold of two were called as differentially expressed. Functional annotation (gene ontology terms

and pathway terms enrichment analysis) was performed using the online software DAVID (<http://david.abcc.ncifcrf.gov/>) on the list of differentially expressed genes for both up-regulated and down-regulated genes. The normalized expression values derived using RMA method (Irizarry et al., 2003) were converted to z-scores for drawing heatmaps.

### **Quantitative RT-PCR (QRT-PCR)**

CD9<sup>+</sup> cells from placenta were isolated and total RNA was extracted (see above). cDNA synthesis was carried out according to the manufacturer's protocol of Quantitect Reverse transcription kit (QIAGEN), and QRT-PCR was performed using a LightCycler 480 (Roche) with LightCycler 480 SYBR Green I Master (Roche). Primer sequences are shown in Table S3.

**Table S3. List of primers used for QRT-PCR. Related to Figures 1-6.**

gene name	forward primer sequence	reverse primer sequence
Ccna2	TGATAGATGCTGACCCGTACCTT	CTCTGGTGGGTTGAGAAGAGAAA
Ccne1	TTGAATTGGGGCAATAGAGAAGA	AGTCCTGTGCCAAGTAGAACGTC
Ccne2	GTGCATTCTAGCCATCGACTCTT	AGGCACCATCCAGTCTACACATT
Cdc45	CCTGAAGCAAGTCAAGCAGAAAT	AGTCTGTACACGCATGTCCTTCA
Cdh1	AGAGAACTCATTTACAGTGGCATTG	GTTTCTCTCCTTTTCTCTCCTCTGT
Cebpa	GCAGTGTGCACGTCTATGCT	AAGTCTTAGCCGGAGGAAGC
Chek1	CGCTTACTGAACAAGATGTGTGG	TTTATGAAGCAAAGCCAGAGGAG
Crb3	CCTTTCACAAATAGCACAACTCAAC	AGAAACAGTCCCCTGCTATAAAGG
Ctsq	TGGAAAGAGTGGATGGGAAG	CAAGTGCACGTTTCCAGAGA
Dlx3	AGTCACTGACCTGGGCTATTACA	CCTATAGGATCCCCCGTAGGTAT
Gapdh	GGAGAAACCTGCCAAGTATGATG	AAGAGTGGGAGTTGCTGTTGAAG
Gcm1	GCCTTACGAAGAGAAAGTATCTGTG	AGAACAGAAGTTTAGGAGCATCTCA
Gjb2	AAACTTCTAGACTCCCAATCCTGTT	TATTGTTACAAAATGGCTTTCCAAT
Gjb3	CTCCCCTTTGAATTCATAAGCTA	AGTTCTTTTTGCTGTTGCTATGTCT
Hand1	GTTCCCATTTCGTTGCTGAAT	CTGCGAGTGGTCACACTGAT
Limk1	CATGTCTTCACTCCGCTTCA	GTGGGGAGCACAGAATTGAT
Limk2	GTGGGCTCAGTCAAAAGCTC	CCACAAGGGTGCAAAGAAAT
Mct1	TTGCCCTTTGTCTACAACC	CAGCATTCCACAATGGTCAC
Mct4	GGATGGTCGTGCTTCATTTT	AATGGATCCAATCCAACCAA
Nodal	ACTTTGCTTTGGGAAGCTGA	ACCTGGAACCTTGACCCTCCT
Nr6a1	CTGTTTCCGTCCCAGATGAT	TGTTGCAAATGCTCCTCTTG
Ovol2	AACTCCAGAGCTTCACGACG	CTGGGTGAAGGCCTTGTTAC
Prkc3	GAACACTGAAGACTACTTTTCCAA	TGGGATCCTTATTTAAAAATCCTTT
Prl3b1	CCACACTGCTGCAATCCTTA	CTGACCATGCAGACCAGAAA
Prl3d1	GGGAGAATGTGTCCTCCAAA	ATCTGCGGCCAAGATAAATG
Syna	TACCCTGTCTGTGGACACCA	ACCAGAGGAGTTGAGGCAGA
Synb	ATCCCCATAAGGACCGTTTC	AGGCAGAGAGGTTGCCTACA
Tead3	CGAACGCTTTCTTCTTGTC	TACTTCTCGGGCAGATGCTT
Tppa	AAGTTAGGCAACGAGCGAAA	AGTGCAGGATCCCCTTGTC

## Supplemental references

Irizarry, R. A., Bolstad, B. M., Collin, F., Cope, L. M., Hobbs, B., & Speed, T. P. (2003). Summaries of Affymetrix GeneChip probe level data. *Nucleic acids research*, 31(4), e15.

Smyth, G. K. (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Statistical applications in genetics and molecular biology*, 3, Article3. 1544-6115.1027