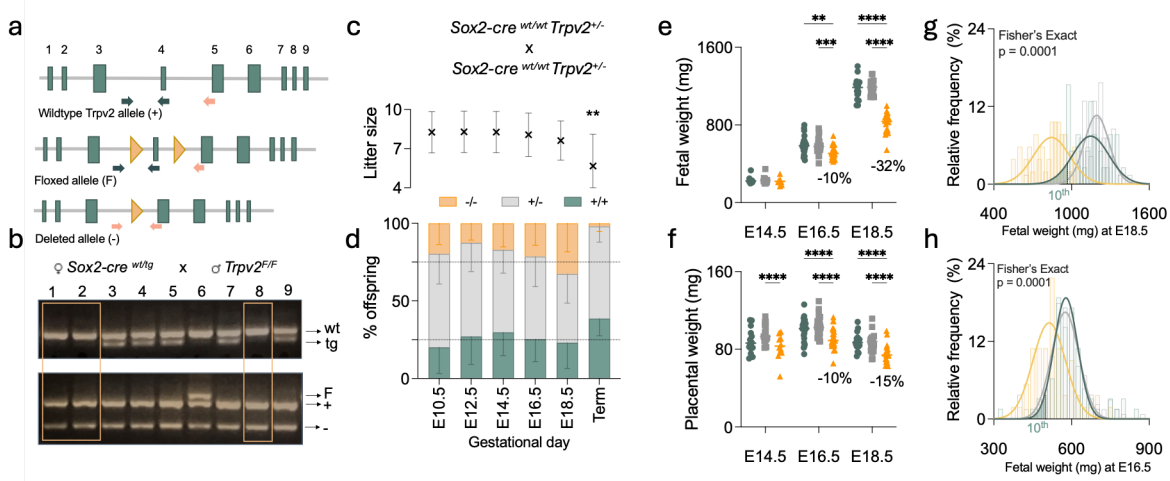
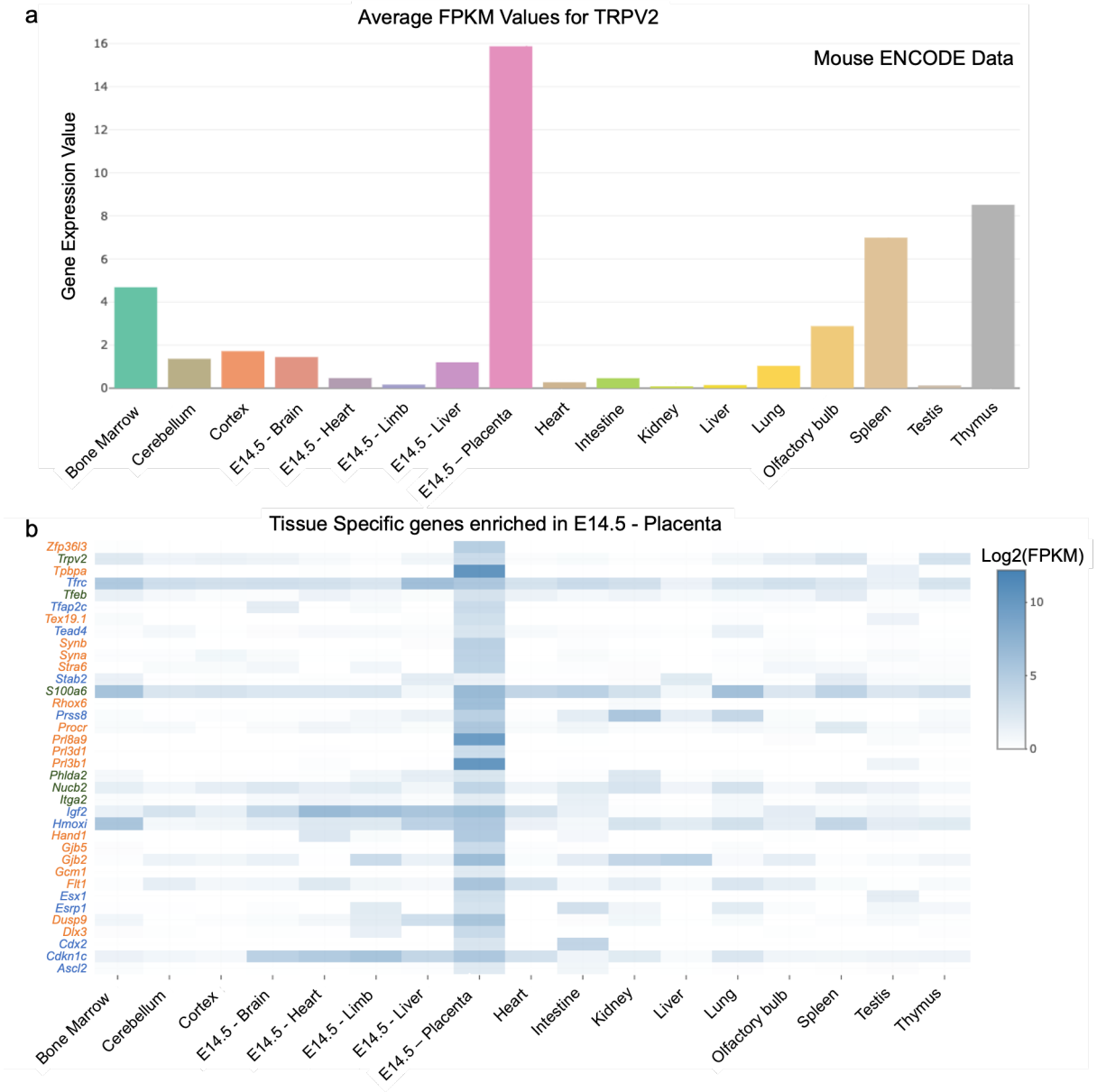
SUPPLEMENTARY INFORMATION
Placental TRPV2 supports trophoblast function and normal fetal development.

Katrien De Clercq[1,2,*], Vicente Pérez-García[3], Amitesh Panda[4], Seppe Gevaert[1], Jill Witvrouwen[1], Pilar Van Mechelen[1], Jorge Lopez-Tello[5,6,7], Katarzyna Maksym[8], Anna L David[8], Evy Van Den Broeck[2], Katrien Luyten[1], Andrei Segal[2], Suresh Poovathingal[9], Marta Wojno[9], Yuki Katanosaka[10,11], Rudi Vennekens[2], Vincent Pasque[4], Amanda N. Sferruzzi-Perri[6], Thomas Voets[2], Joris Vriens[1,2]

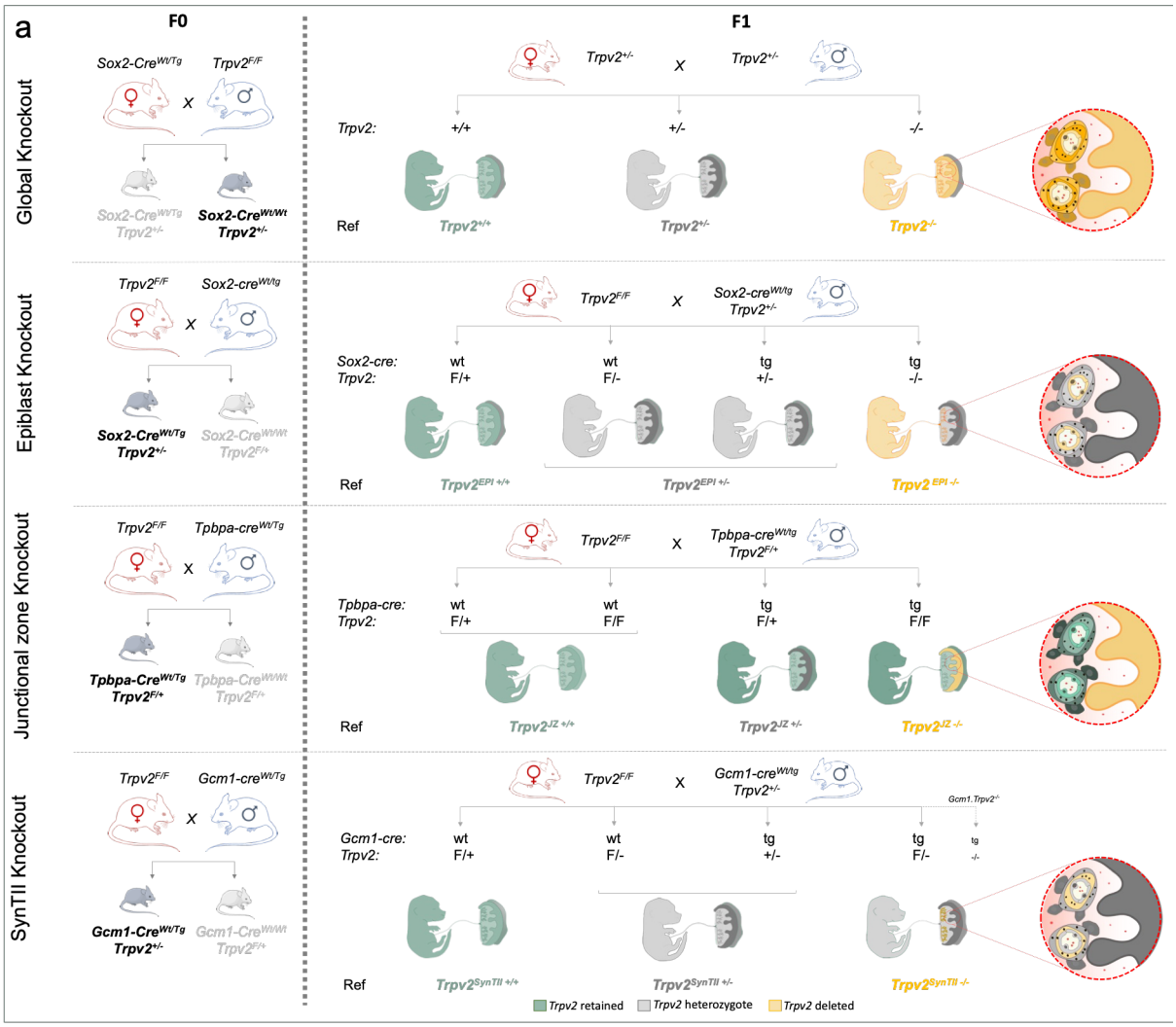
SUPPLEMENTARY FIGURES



Supplementary Fig. 1: Validation and characterization of global Trpv2$^{-/-}$ mice strain. (a) Representation of wild-type, floxed and cre-LoxP mediated deleted *Trpv2* allele. Arrows represent primers for genotyping PCR on mice genomic DNA obtained from the tail. Green arrows show the Lox-site primers, pink arrows show primers for the confirmation of successful Cre recombination, resulting in a deletion band. (b) Genotype of offspring obtained from F0 matings between *Sox2*-cre females x *Trpv2*$^{F/F}$ males resulting in recombination in all embryos (cfr. deletion band (-)), independent of inheritance of the maternal *Sox2*-cre transgene, exemplified in embryos 1, 2 and 8. These *Sox2*$^{wt/wt}$*,Trpv2*$^{+/-}$ were used for F1 matings. Of note, incomplete recombination, resulting in F/+/- was sporadically observed (embryo 6) but was not used in further crosses. (c) Litter size and (d) Mendelian inheritance pattern of *Trpv2*$^{+/-}$ x *Trpv2*$^{+/-}$ breedings at different developmental stages, data shown as mean ± SD, ** $p < 0.01$ Kruskal-Wallis with Dunn's Multiple comparisons test. n = 8 (E10.5), 11 (E12.5), 15 (E14.5), 32 (E16.5), 21 (E18,5) and 32 (term). (e) Fetal weight of *Trpv2*$^{+/+}$, *Trpv2*$^{+/-}$ and *Trpv2*$^{-/-}$ littermates during pregnancy. (f) Placental weight dynamics during pregnancy. (g) Frequency distribution of fetal weights at E16.5 of all genotypes. (h) Frequency distribution of fetal weights at E18.5 of all genotypes. Data analyzed by two-sided Fisher's exact test comparing proportion of animals below 10th percentile, compared to wild type. Repeated measures (mixed-model) Anova with Sidak's multiple comparisons test on litter average. n= 14 litters at E14.5, n=29 litters at E16.5 and n= 17 at E18.5. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Exact p-values are provided in the Source Data file.

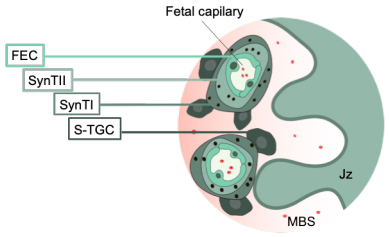


Supplementary Fig. 2*: *Trpv2 expression in developing embryo. (a) Figure adapted from TissueEnrich webtool showing *Trpv2* expression in embryo and adult organs. (b) Enrichment analysis by including *Trpv2* in the list of labyrinth specific genes obtained from the MGI database. Only genes meeting the TissueEnrich enrichment criteria are displayed in the heatmap. Green = Tissue Enhanced (e.g. genes with an expression level greater than or equal to 1 (TPM or FPKM) that also have at least five-fold higher expression levels in a particular tissue compared to the average levels in all other tissues), Orange = Tissue Enriched (e.g. genes with an expression level greater than or equal to 1 (TPM or FPKM) that also have at least five-fold higher expression levels in a particular tissue compared to all other tissues), Blue = Group Enriched (e.g. genes with an expression level greater than or equal to 1 (TPM or FPKM) that also have at least five-fold higher expression levels in a group of 2-7 tissues compared to all other tissues).

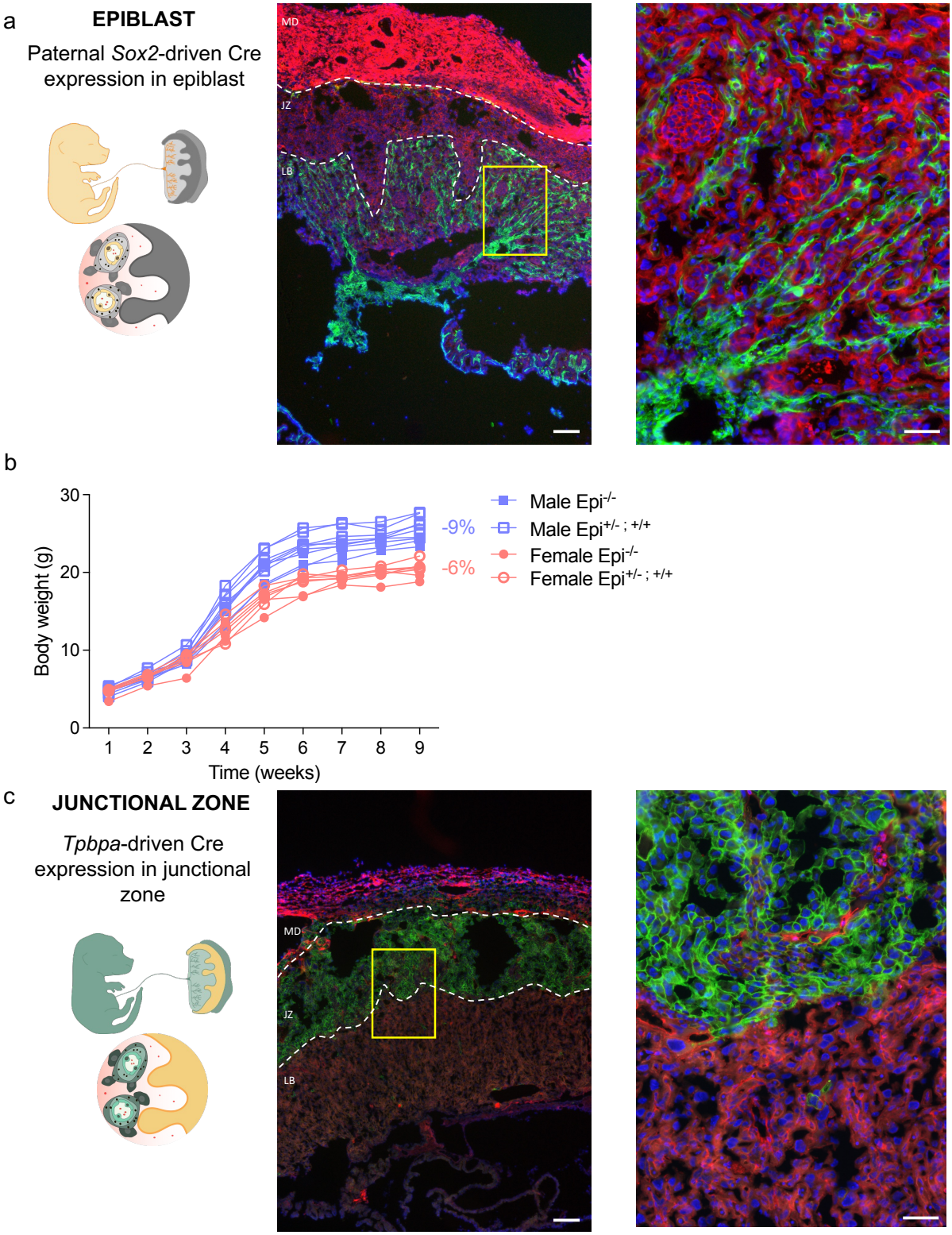


b

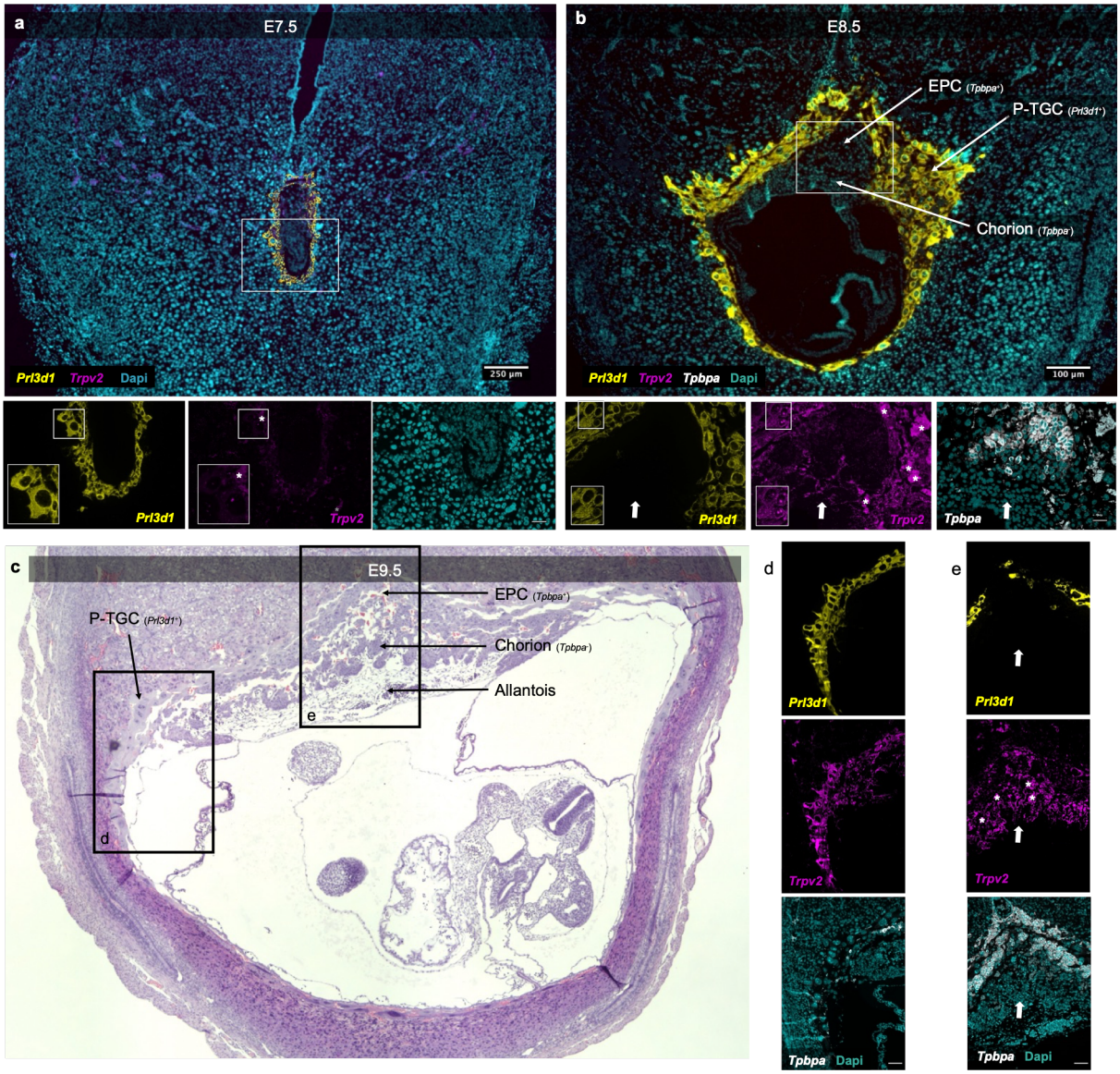
	Epiblast	Trophectoderm				
	Fetus	Placenta				
		FEC	SynTII	SynTI	S-TGC	JZ
Global knockout	-/-	-/-	-/-	-/-	-/-	-/-
Epiblast knockout	-/-	-/-	+/-	+/-	+/-	+/-
Junctional zone knockout	+/+	+/+	+/+	+/+	+/+	-/-
SynTII knockout	+/-	+/-	-/-	+/-	+/-	+/-



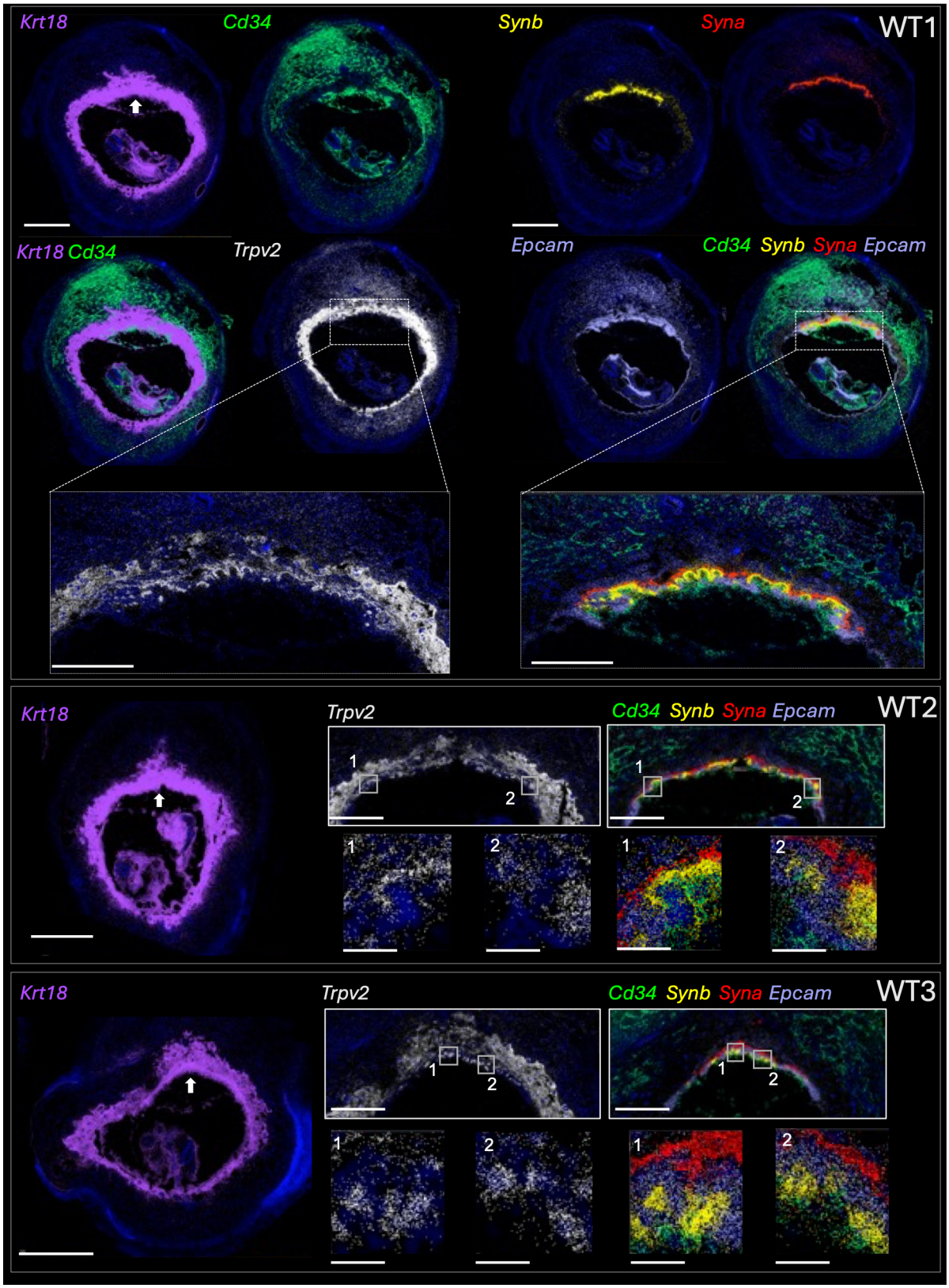
Supplementary Fig. 3: Overview of breeding strategy to obtain conditional Trpv2 knockouts. (a) Breeding strategies. F0 represent the first breeding to obtain tissue specific heterozygotes, whereas F1 breeding were done to create tissue specific knockouts (yellow), and their respective wildtype (green) littermates. Detailed overview of conditional deletion in placental tissue is shown in magnification. Genotypes are always determined in tail biopsies from offspring. *Sox2*-cre transgenic females induce recombination independent of transgene inheritance (global knockout), whereas *Sox2*-cre transgenic males generate epiblast-specific deletion, leaving extraembryonic tissues intact (Epiblast knockout). *Tpbpa*-cre specifically target the junctional zone of the placenta. *Gcm1*-cre mediates SynTII-specific deletion in placental tissue. Occasionally (*8-12%*), constitutive deletion was observed and represents full *Trpv2*$^{-/-}$ mice, that were not assessed. (b) Schematic table to simplify where *Trpv2* is deleted in the specific conditional knockout models. Yellow represents homozygous deletion of *Trpv2*. FEC = fetal endothelial cells, SynTII = Syncytiotrophoblast layer II, SynTI = syncytiotrophoblast layer I, S-TGC = sinusoidal trophoblast giant cells, JZ = junctional zone, MBS = maternal blood space.



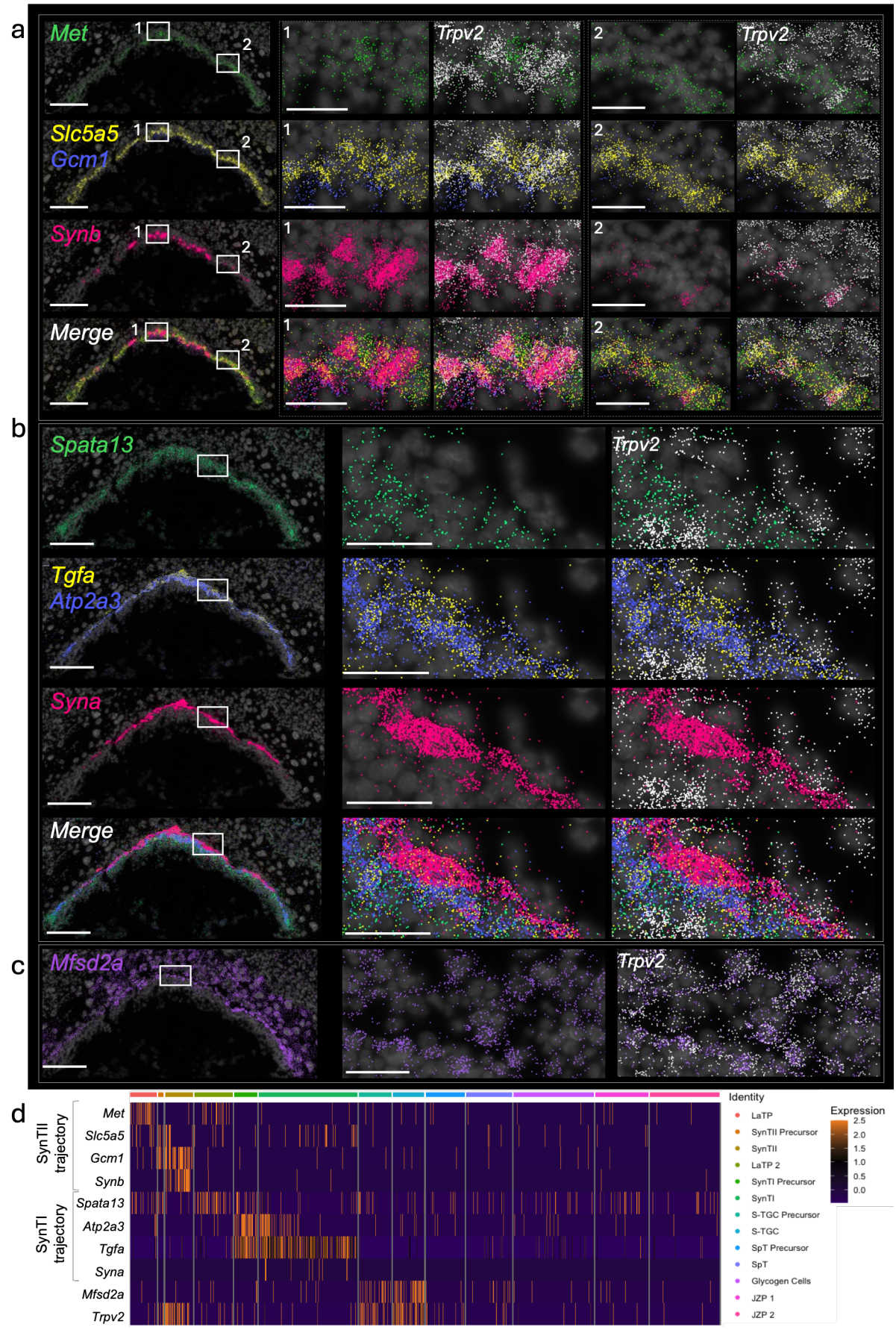
Supplementary Fig. 4: Detection of Cre activity in conditional knockout models. (a) Confirmation of epiblast specific CRE expression. Placenta of *Sox2-cre*$^{wt/tg}$*; mTmG*$^{wt/tg}$ embryo at E14.5 showing Cre expression (green) in fetal capillaries, while trophoblast and decidua show no CRE expression (red). Scale bar = 100 µm and 50 µm. (b) Body weight of *Trpv2*$^{EPI\ -/-}$ and *Trpv2*$^{EPI\ +/+;+/-}$ mice until 9 weeks after birth. Each datapoint represents an individual animal, n = 7-9 animals per genotype, Mixed-effect analysis with Sidak's multiple comparisons test. (c) Confirmation of Junctional zone specific Cre expression. Placenta of *Tpbpa-cre*$^{wt/tg}$*; mTmG*$^{wt/tg}$ embryo at E14.5 showing Cre expression (green) in the junctional zone, while labyrinth and decidua show no Cre expression (red). Scale bar = 100 µm and 50 µm. MD = maternal decidua, JZ = junctional zone, LB = labyrinth.



Supplementary Fig. 5: Expression of Trpv2 during early gestation. (a) Fluorescent *In situ* hybridization (FISH) at E7.5 using *Prl3d1* to mark primary P-TGC. (b) *In situ* hybridization at E8.5 using *Prl3d1,* and *Tpbpa* which identifies ectoplacental cone lineages. (c) *In situ* hybridization of *Prl3d1* and *Tpbpa* at E9.5. insert d = primary P-TGC, insert e = developing placenta. Arrow indicates expression in the *Tpbpa*$^{-}$ developing chorion. Asterisk shows autofluorescence in red blood cells. Scalebar of magnifications = 50 µm. EPC = ectoplacental cone; TGC = trophoblast giant cells. Representative images from n = 3 samples/stage with similar results.

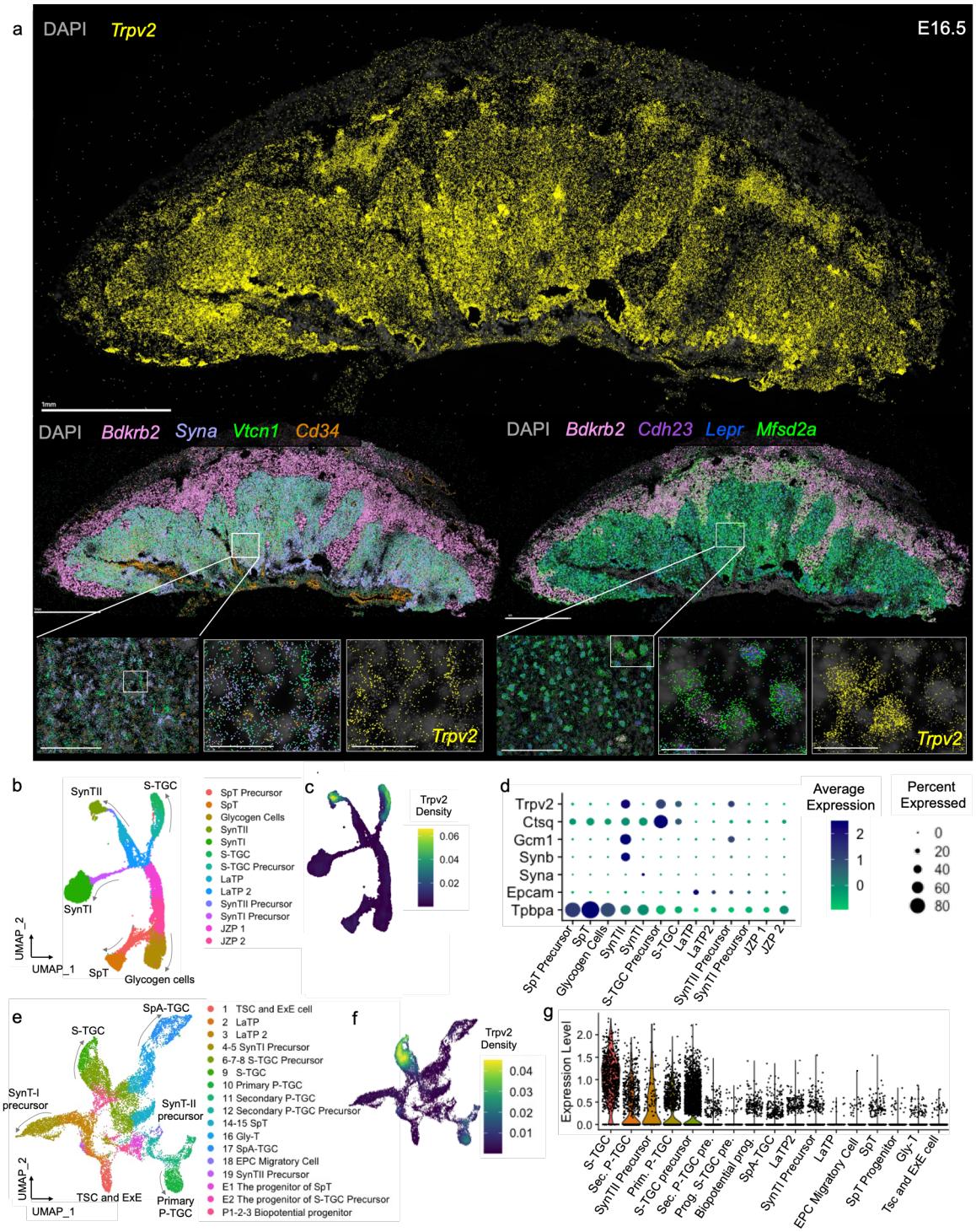


Supplementary Fig. 6: MERscope-based spatial transcriptomic analysis of E9.5 mouse placental section. MERscope spatial transcriptomic analysis of E9.5 mouse placental sections from three biological replicates (WT1–WT3). Shown are *Krt18* (trophoblast), *Cd34* (fetal endothelial cells), *Synb* (Syncytiotrophoblast-II), *Syna* (Syncytiotrophoblast-I), *Epcam* (trophoblast progenitors), and *Trpv2*. Higher-magnification views illustrate regional heterogeneity of *Trpv2* expression relative to *Synb* and other trophoblast markers within the developing labyrinth. Scale bars 1mm for overview and 500 µm for first magnifications and 50 µm for second magnification.

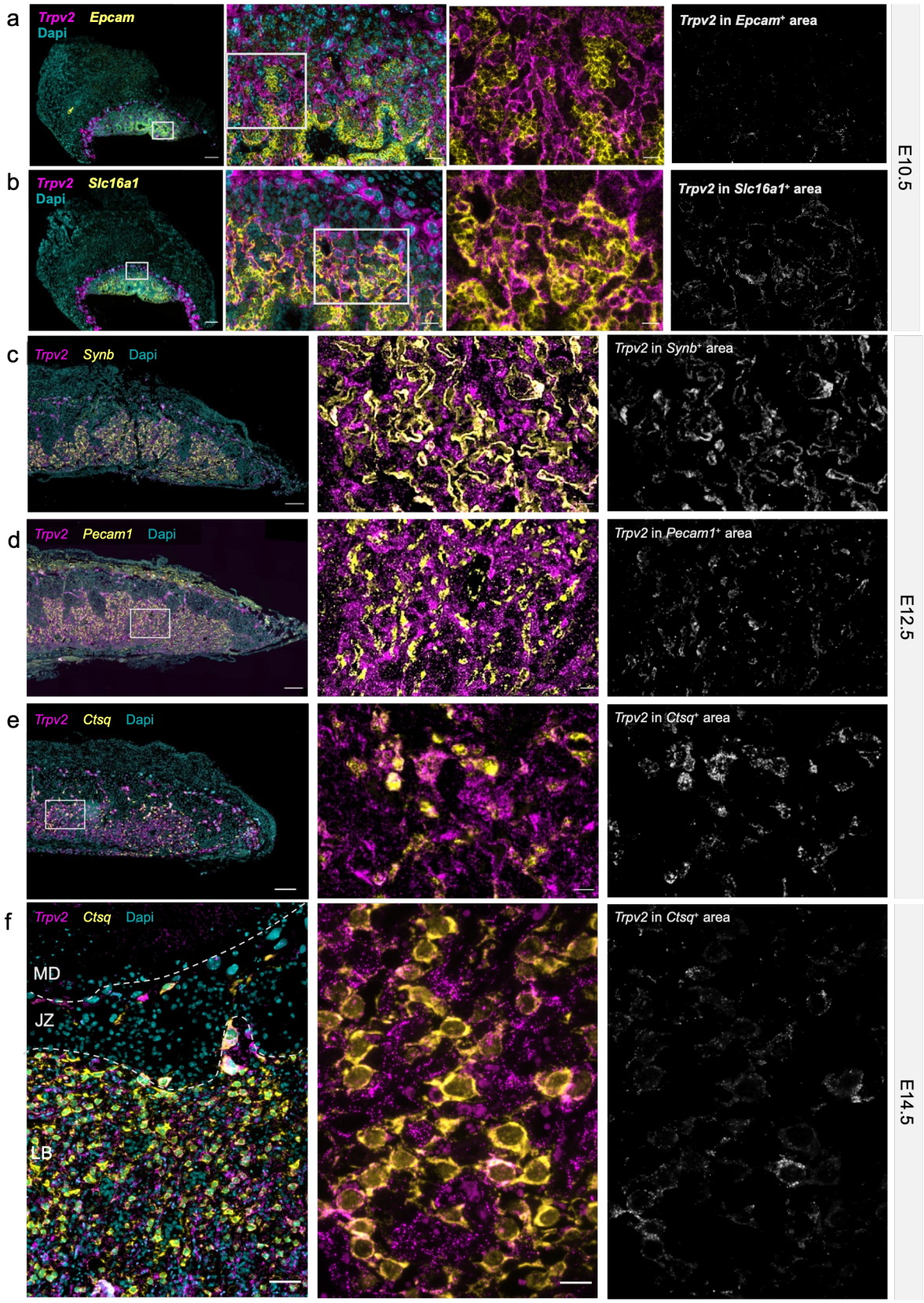


Supplementary Fig. 7: MERscope analysis of Trpv2 expression relative to early labyrinth trophoblast markers. MERscope spatial transcriptomic analysis of E9.5 placental sections showing

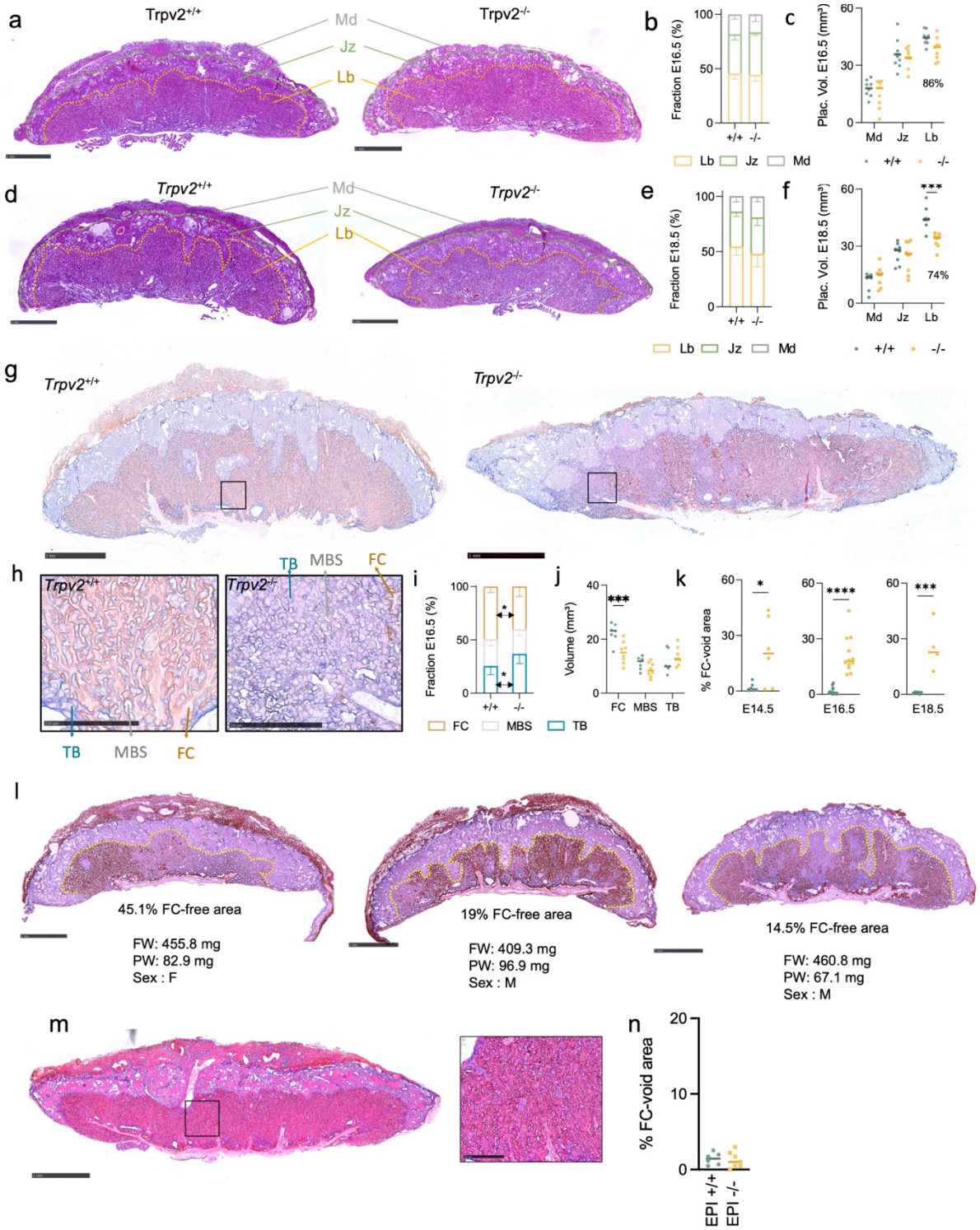
Trpv2 expression in relation to markers defining early labyrinth trophoblast populations. (a) SynTII trajectory with *Met* (labyrinth trophoblast progenitors, LaTP), *Slc5a5* and *Gcm1* (SynTII precursors), and *Synb* (SynTII), with boxed regions (1 and 2) shown at higher magnification to illustrate regional variation in *Trpv2* overlap. (b) SynTI trajectory with *Spata13* (LaTP2), *Tgfa* and *Atp2a3* (SynTI precursors), and *Syna* (SynTI) together with *Trpv2*. (c) S-TGC precursor using marker *Mfsd2a* alongside *Trpv2*. (d) heatmap summarizes expression of the indicated markers across trophoblast subsets in a published single-nucleus RNA-sequencing dataset[1] used to validate marker identity. Colour scale indicates relative expression (scaled z-score). Scale bars 250 μm for overview, 50 μm for magnification.



Supplementary Fig. 8: Trpv2 expression in the developing mouse placenta. (a) MERscope spatial transcriptomic analysis of E16.5 WT placental sections showing *Trpv2* expression in relation to markers defining trophoblast populations. Scale bars 1mm for overview, 250 µm and 100 µm for magnifications, respectively. (b) Uniform manifold approximation and projection (UMAP), plotted according to transcriptome similarity of all trophoblasts. Each dot represents one nucleus colored according to assignment by clustering analysis. Data adapted from Marsh *et al*[1]. (c) Nebulosa density expression of *Trpv2* in several trophoblast populations projected in UMAP space. (d) Dot plot showing average expression and percent of nuclei expressing labyrinth marker genes and *Trpv2*. Colour scale indicates average expression. (e) UMAP plotted according to transcriptome similarity of all trophoblasts, data adapted from Jiang *et al.*[14] (f) Nebulosa density expression of *Trpv2* in several trophoblast populations projected in UMAP space. Colour scale indicates Kernel density estimate (probability and intensity of gene expression across cell clusters). (g) Violin Plot showing distribution of *Trpv2* expression in the different populations.

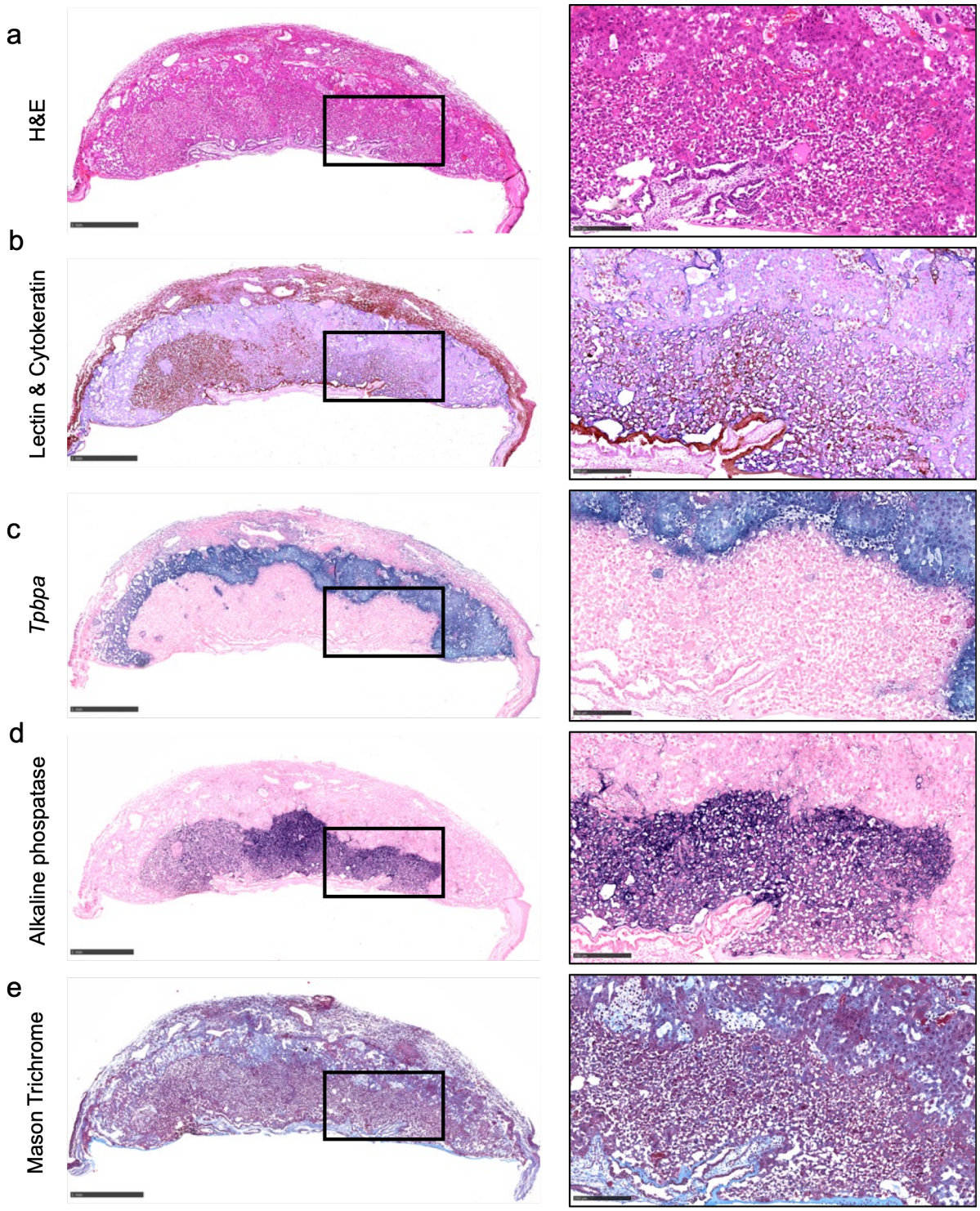


Supplementary Fig. 9: Expression of Trpv2 during developing labyrinth using FISH. RNAscope analysis of *Trpv2* expression at E10.5–E14.5 showing undifferentiated trophoblasts (*Epcam*), SynTII (*Synb*), SynTI (*Slc16a1*), S-TGC (*Ctsq*) and endothelial cells (*Pecam1*) at different time points. Scalebar = 250 µm (left row) and 50 µm (middle row). Images in right row shows *Trpv2* expression only in cells that were identified by the respective marker. MD = maternal decidua, JZ = junctional zone, LB = labyrinth. Representative images from n = 2 samples/stage with similar results.

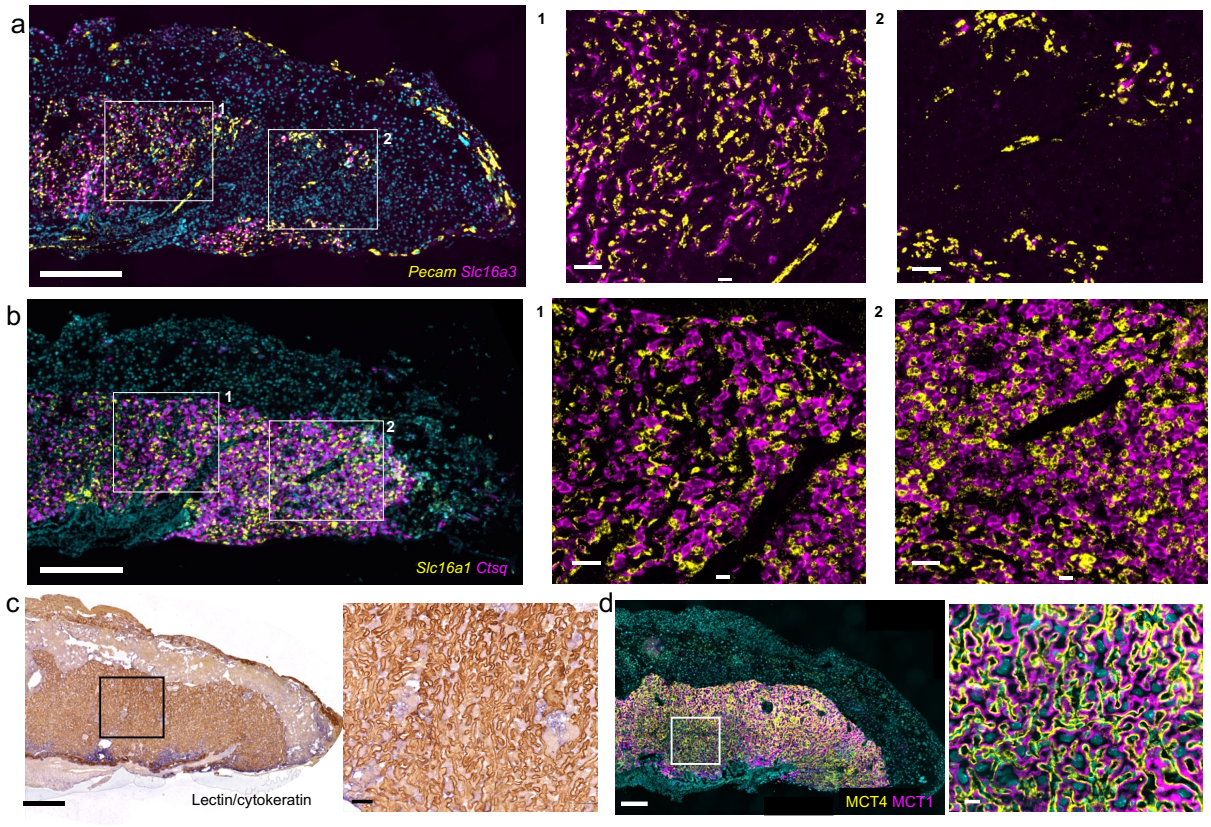


Supplementary Fig. 10: Details of labyrinth morphology. (a) Representative H&E stained image and stereological analysis (b,c) of placental morphology at E16.5, scale bar = 1 mm. Data shown as mean ± SD. (d) Representative H&E stained image and stereological analysis (e,f) of placental morphology at E18.5, scale bar = 1mm. Data shown as mean ± SD. (g) Representative images of Lectin-cytokeratin double labeling of E16.5 Trpv2$^{+/+}$ and Trpv2$^{-/-}$ placentas, scale bar = 1 mm, (h) respective magnification of (g), scale bar 250 µm. (i,j) Stereological analysis of labyrinth morphology in (h). Data shown as mean ± SD. (k) Quantification of FC-void areas within the labyrinth of Trpv2$^{+/+}$ and Trpv2$^{-/-}$ placentas. Data analysed by Two-Way ANOVA with Sidaks multiple comparison test for (b), (c), (e), (f) (i) and (j) with n = 8-9 from 5-8 litters. Data analysed by unpaired t-test (E14.5, E18.5) or nonparametric Mann Whitney test (E16.5) for (f) with n = 6 fetuses/genotype from 6 different litters at E14.5, n = 12-13 fetuses/genotype from 8 different litters at E16.5 and n = 5-6 fetuses/genotype from 5 different litters at E18.5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact p-values are provided in the soure data file. (l) Lectin-

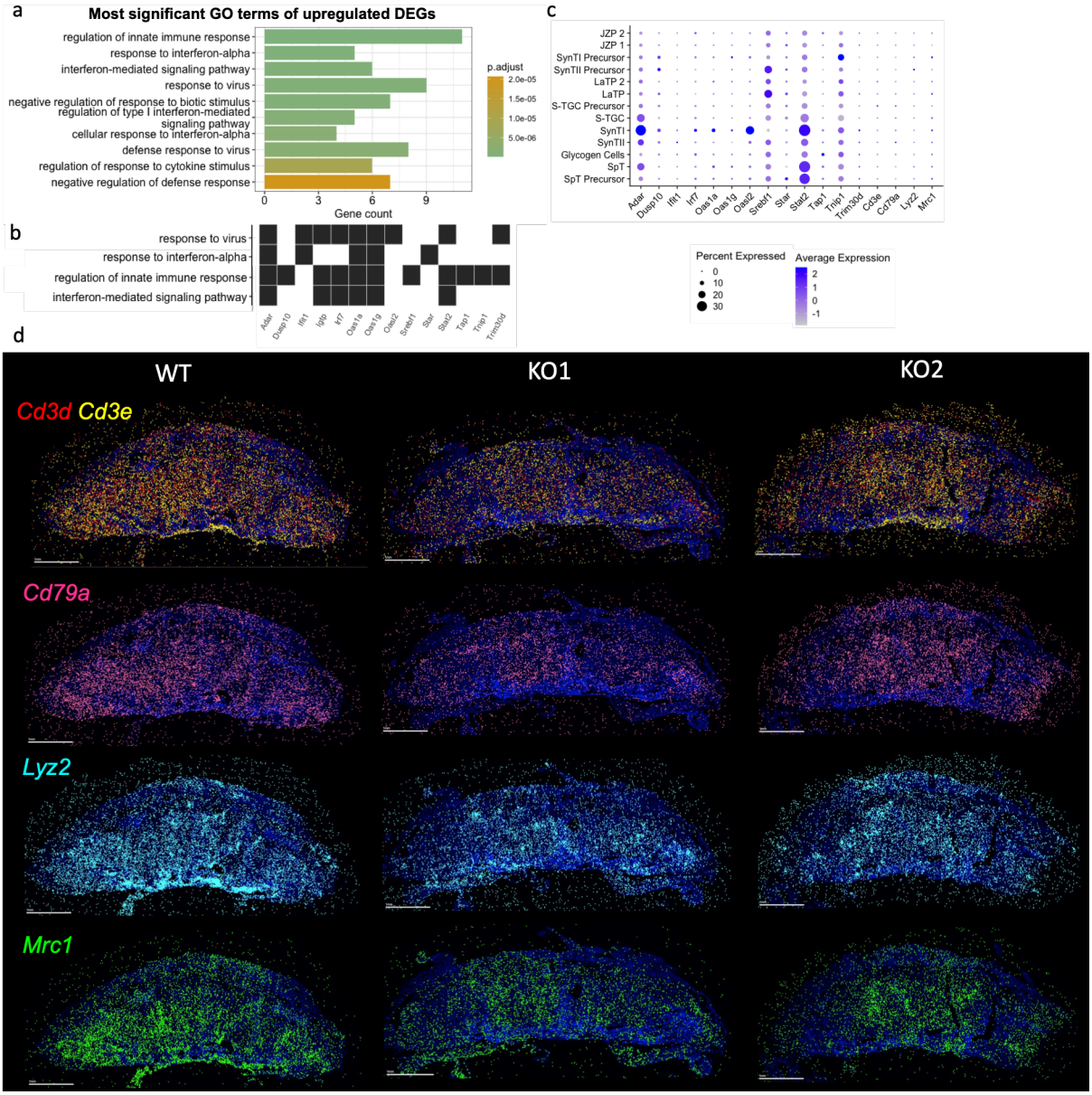
cytokeratin double labeling of different Trpv2$^{-/-}$ placentas within 1 litter at E16.5, with fetal characteristics, FW = fetal weight, PW = placental weight, F = female, M = male. (m) Lectin-cytokeratin double labeling of Trpv2$^{EPI\ -/-}$ placenta at E16.5, scale bar = 1mm, with magnification of the insert, scale bar = 200 µm (n) Quantification of FC-void areas in Trpv2$^{EPI\ -/-}$ and Trpv2$^{EPI\ +/+}$ placentas. n= 6-7 fetuses/genotype from 4 litters. Md = maternal decidua, Jz = junctional zone, Lb = labyrinth, TB = trophoblast, MBS = maternal blood space, FC = fetal capillaries.



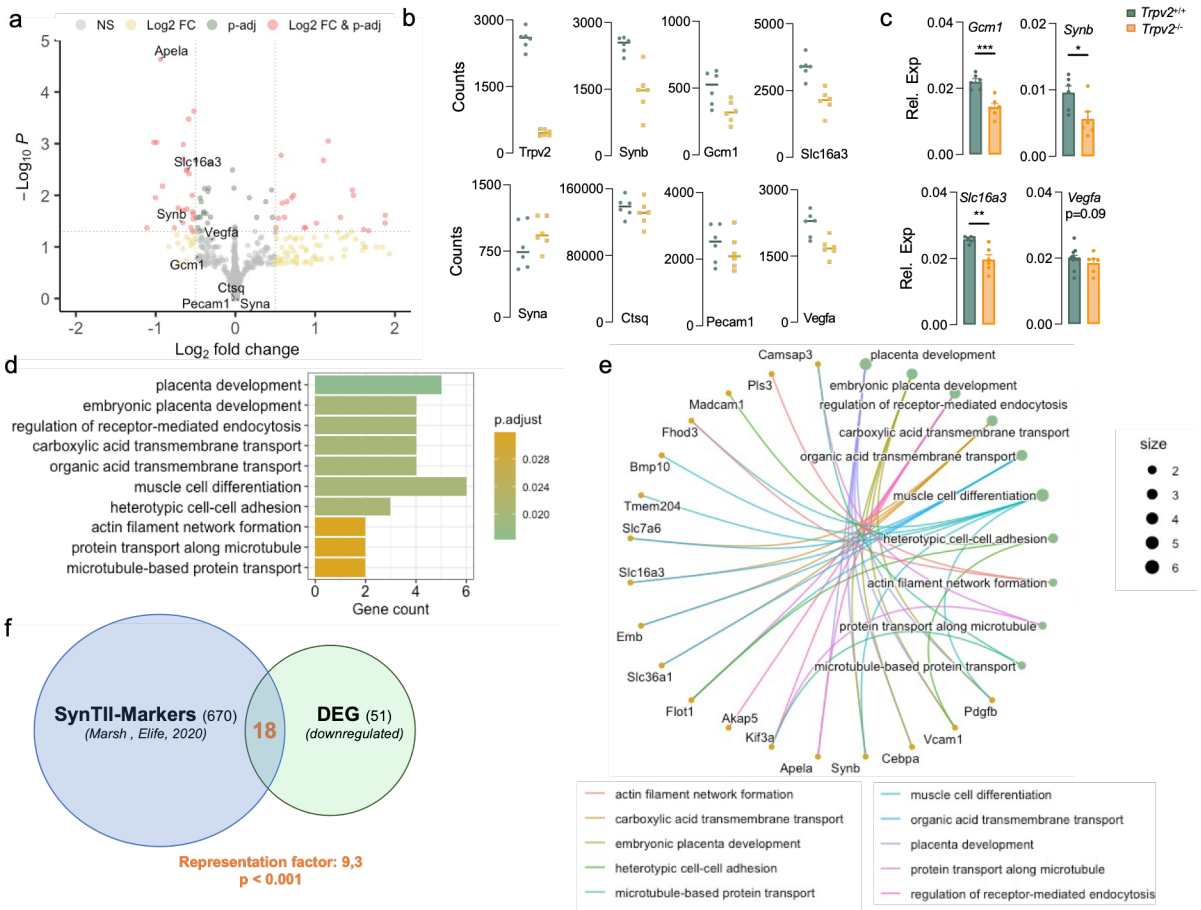
Supplementary Fig. 11: Detailed morphological assessment of Trpv2$^{-/-}$ placenta. (a) H&E for normal morphology. (b) Lectin (brown) = basal membrane of fetal capillaries. Cytokeratin (blue) = all trophoblast cells. (c) *Tpbpa* stained with ISH = trophoblast cells of junctional zone. (d) Alkaline phosphatase = lining of maternal blood space. (e) Masson's Trichrome = collagen (fibrosis). Scale bar = 1 mm and 250 µm. Representative images from n = 2 samples/stage with similar results.



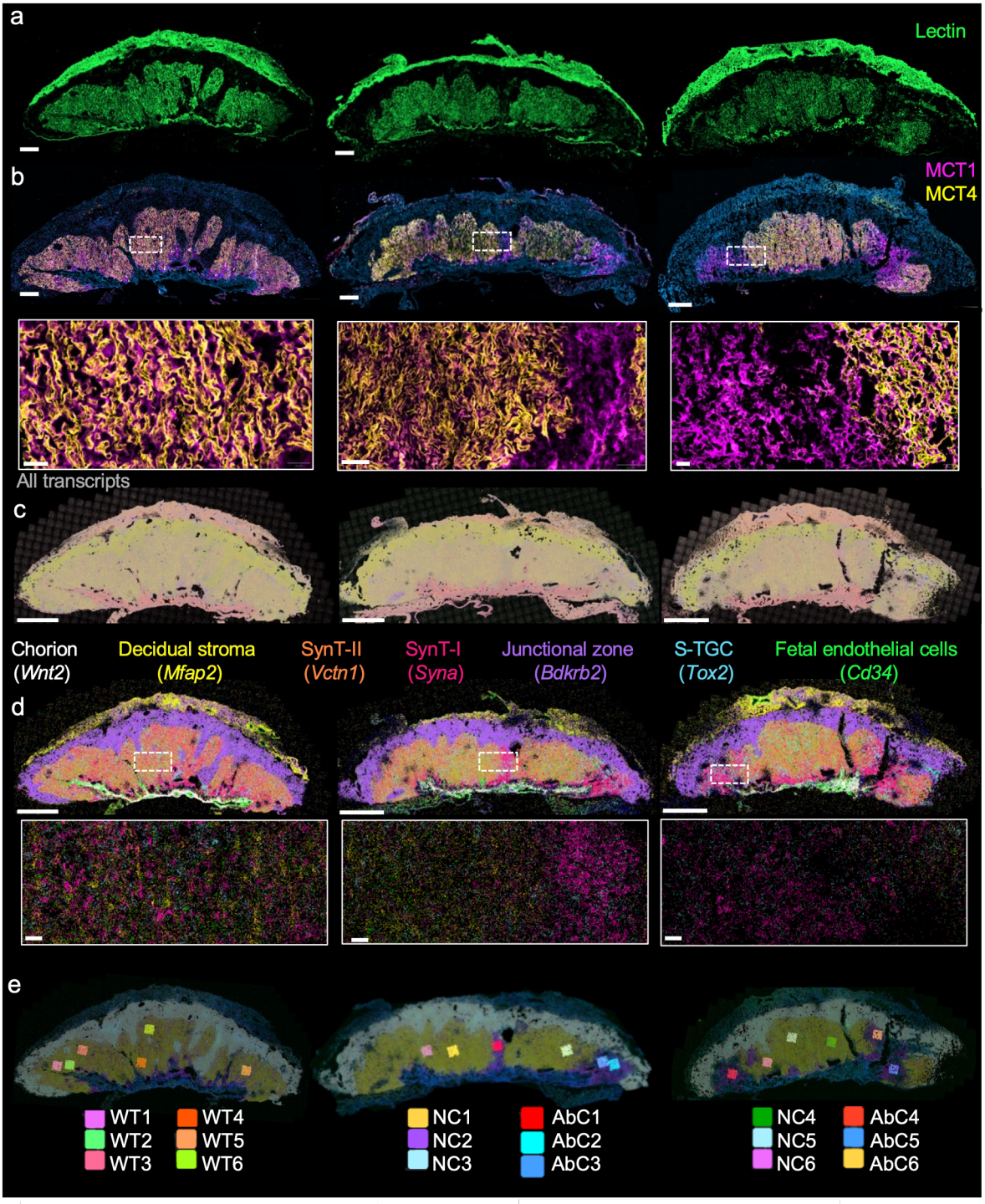
Supplementary Fig. 12: Morphology of Trpv2$^{-/-}$ and Trpv2$^{+/+}$ littermates. (a) FISH of Pecam1 and Slc16a3, representative for fetal endothelial cells and SynTII cells, respectively, of E16.5 of Trpv2$^{-/-}$ placentas (b) FISH of Slc16a1 and Ctsq, representative for SynTI cells and S-TGC, respectively, on consecutive slide of (a). Scale bar = 250 µm and 50 µm (c) Lectin/Cytokeratin double staining at E16.5 of Trpv2$^{+/+}$ placentas. Scale bar = 250 µm and 50 µm. Lectin (brown) = basal membrane of fetal capillaries. Cytokeratin (blue) = all trophoblast cells. (d) Immmuhistochemistry of MCT1 and MCT4, representing differentiated SynT-I and SynT-II, respectively, on consecutive slide of (c). Scale bar = 250 µm and 20 µm for magnification. Representative images from n = 2 samples/stage with similar results.

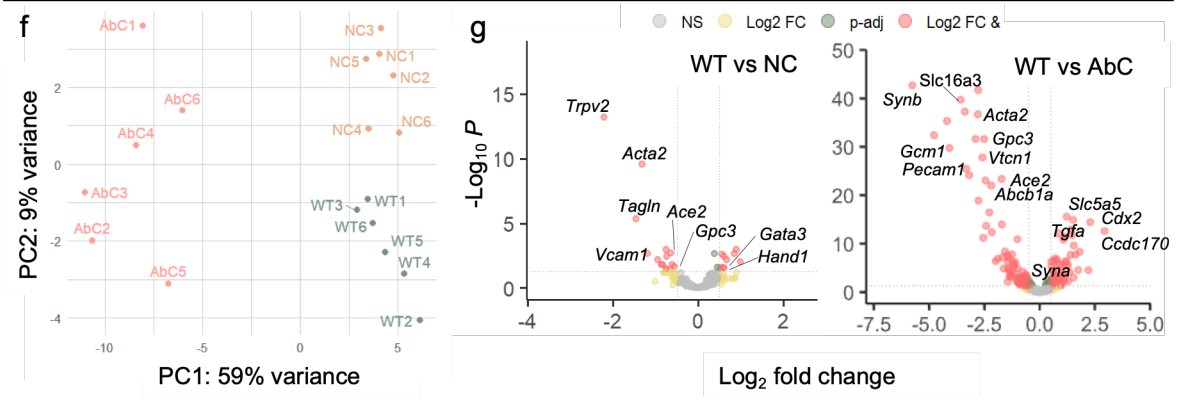


Supplementary Fig. 13: Immune-associated GO terms reflect trophoblast-intrinsic transcriptional programs rather than immune cell infiltration. (a) Most significantly enriched gene ontology terms among upregulated differentially expressed genes identified by bulk RNA-sequencing of E14.5 placentas. Colour scale showing adjusted p-values. (b) Heatplot illustrating genes contributing to immune- and interferon-associated GO terms, highlighting interferon-responsive and stress-associated regulators rather than canonical immune cell markers. (c) Dotplot showing expression of representative GO-driving genes across placental cell states in a published single-nucleus atlas[1]. Colour scale indicates averages expression. (d) MERscope localization of canonical immune cell markers (Cd3d/Cd3e for T cells, Cd79a for B cells, Lyz2 and Mrc1 for myeloid cells) in wild-type and Trpv2-deficient placentas, showing sparse and non-clustered expression without enrichment in abnormal labyrinth regions. Scale bar = 1 mm

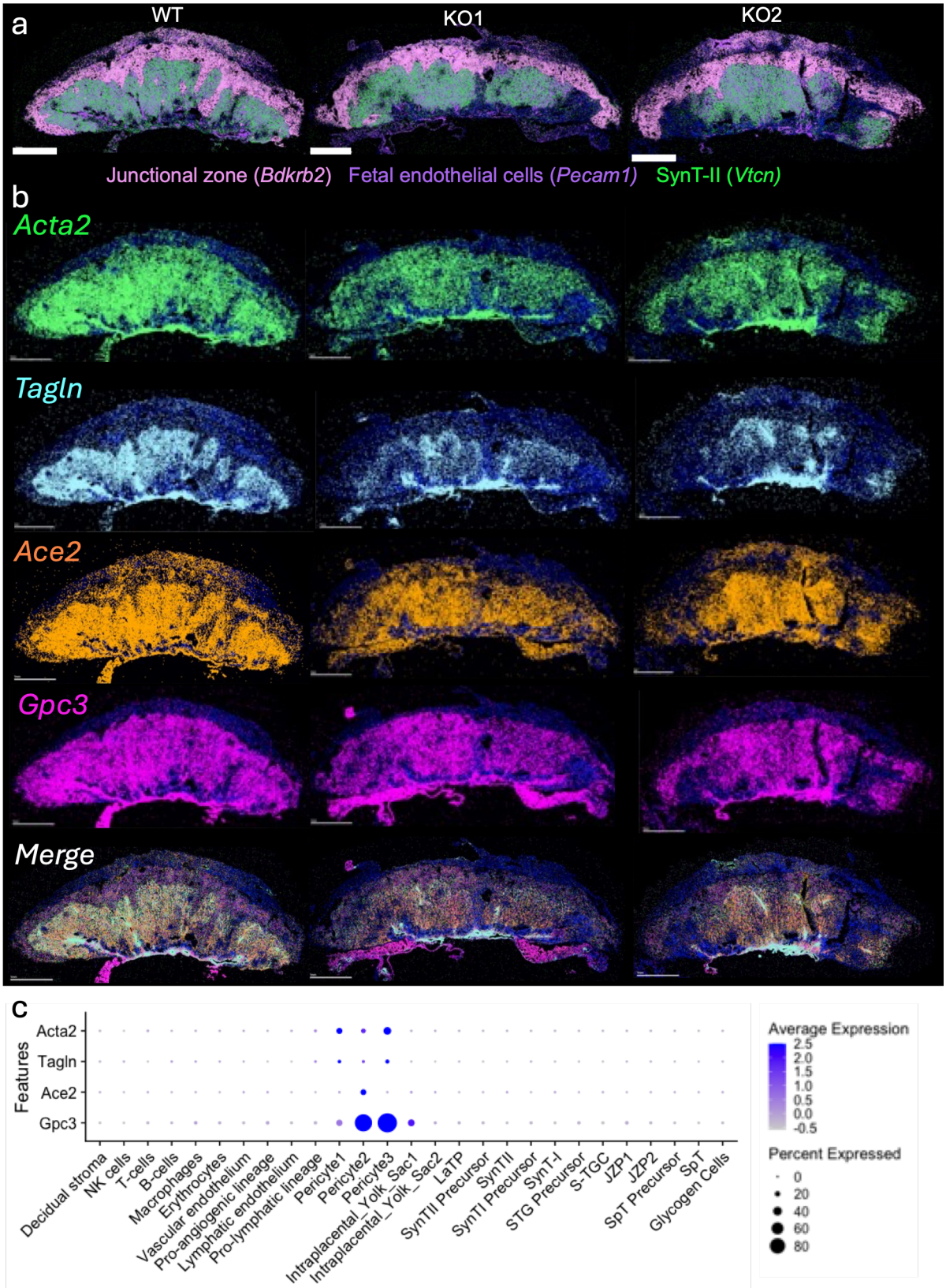


Supplementary Fig. 14: Impaired placental development in E16.5 Trpv2-KO labyrinth. (a) Enhanced Volcano Plot representation of DEGs identified by RNAseq of E16.5 labyrinth (*Trpv2*$^{+/+}$ vs *Trpv2*$^{-/-}$), adjusted p-value (cut-off < 0.05) and Log_2 Fold Change (cut-off < 0.5) (n = 6/genotype, 3 male/3 female from 7 different litters). Marker genes for different labyrinth trophoblasts are labeled. (b) gene counts of labyrinth marker genes. (c) qRT-PCR validation of most informative DEGs (two-sided Student t-test), n = 6/genotype, 3 male/3 female from 7 different litters. Exact p-values are provided in the source data file. (d) Barplot of biological processes that are significantly enriched in downregulated DEGs and linked to placental functioning. Colour scale showing adjusted p-values. (e) Cnetplot showing linkage between DEGs and enriched GO terms. (f) Significant overrepresentation of SynTII-markers in downregulated DEGs.

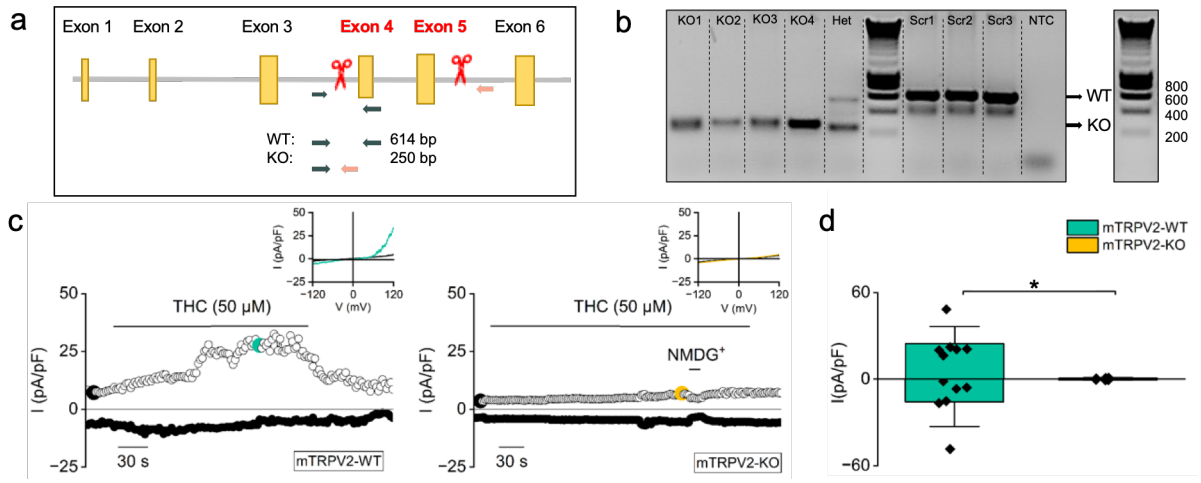

a
Lectin
b
MCT1
MCT4
All transcripts
c
Chorion
(Wnt2)
Decidual stroma
(Mfap2)
SynT-II
(Vctn1)
SynT-I
(Syna)
Junctional zone
(Bdkrb2)
S-TGC
(Tox2)
Fetal endothelial cells
(Cd34)
d
e
WT1
WT2
WT3
WT4
WT5
WT6
NC1
NC2
NC3
AbC1
AbC2
AbC3
NC4
NC5
NC6
AbC4
AbC5
AbC6


f
PC2: 9% variance
PC1: 59% variance
g
NS
Log2 FC
p-adj
Log2 FC &
WT vs NC
WT vs AbC
-Log10 P
Trpv2
Acta2
Tagln
Ace2
Gpc3
Gata3
Hand1
Vcam1
Slc16a3
Synb
Acta2
Gpc3
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Pecam1
Ace2
Abcb1a
Slc5a5
Cdx2
Tgfa
Ccdc170
Syna
Log2 fold change

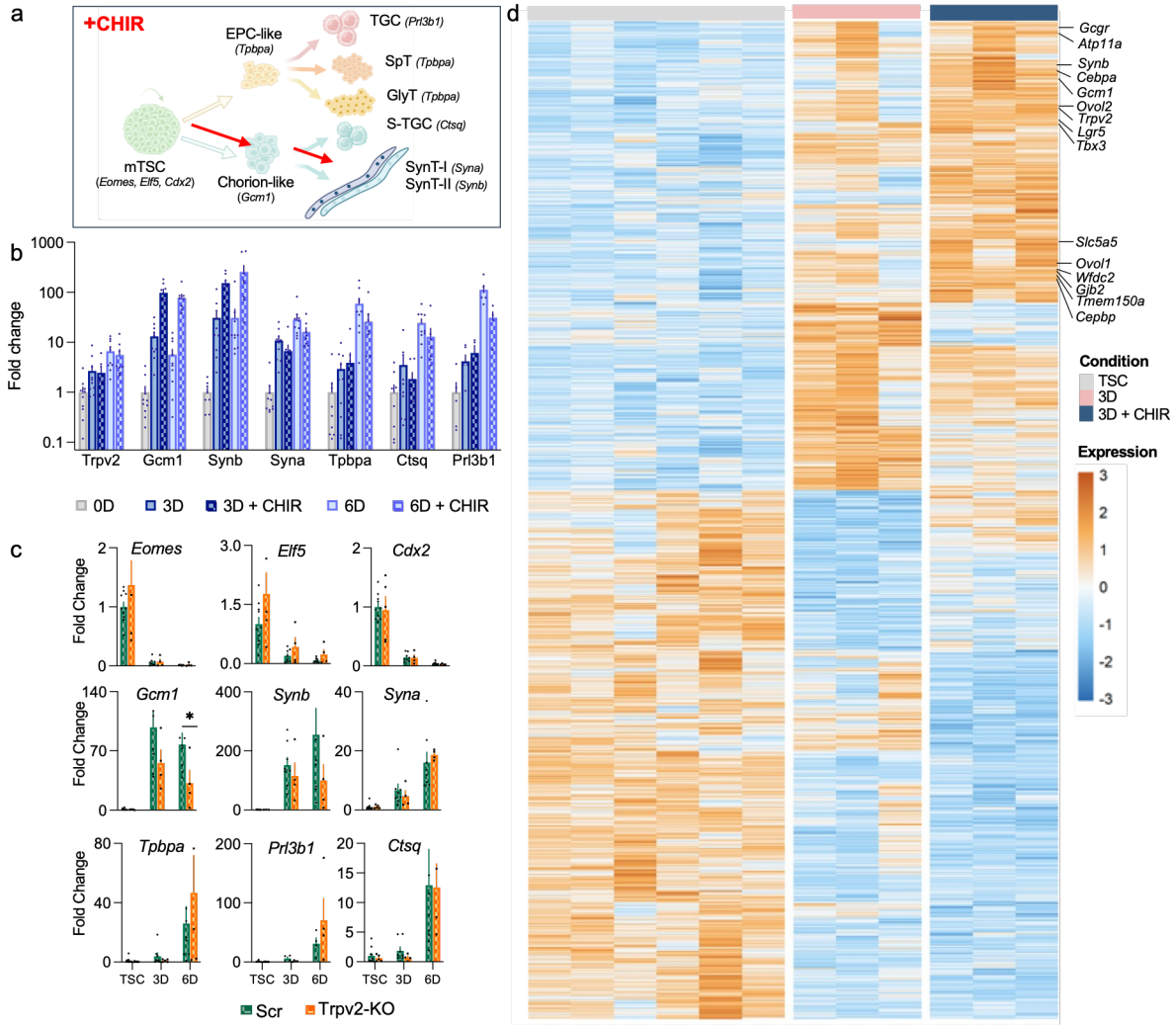
Supplementary Fig. 15: Validation and characterization of MERscope spatial transcriptomic analysis at E16.5. (a) Lectin staining of one wild-type and two Trpv2-deficient placentas at E16.5, illustrating normal and abnormal labyrinth morphology used for spatial transcriptomic analyses. (b) Immunohistochemical staining for MCT1 and MCT4 on consecutive sections corresponding to the MERscope samples, confirming altered SynTI and SynTII organization in Trpv2-deficient placentas. Scale bar = 400 µm and 50 µm insert (c) Overview of MERscope detection across consecutive placental sections, showing uniform transcript detection throughout the tissue and excluding region-specific assay failure. (d) MERscope expression of canonical markers identifying major placental compartments, including chorion, decidua, junctional zone, S-TGC, SynTI, SynTII, and fetal endothelial cells, confirming correct spatial annotation. Scale bar = 1 mm and 50 µm insert. (e) Schematic overview of spatial regions selected for region-based pseudobulk analysis in each sample, distinguishing wild-type (WT), morphologically normal knockout (NC), and abnormal knockout labyrinth regions (AbC). (f) Principal component analysis of region-based pseudobulk expression profiles, demonstrating clear separation between WT, NC and AbC regions. (g) Volcano plots showing differentially expressed genes between WT and NC regions (left) and between WT and AbC (right), highlighting genes associated with trophoblast differentiation and vascular organization.



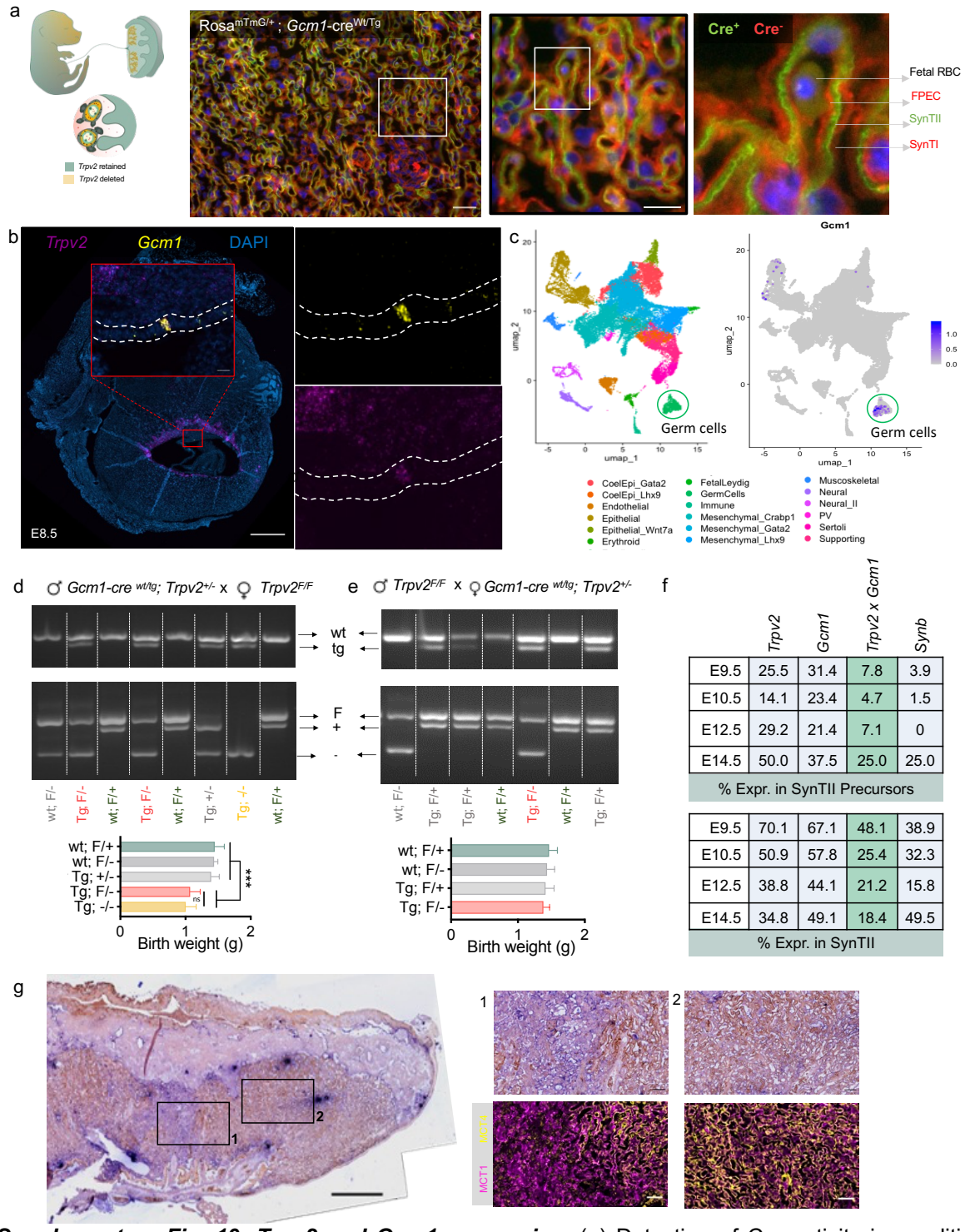
Supplementary Fig. 16: Decreased expression of vascular support markers in Trpv2-KO placentas. (a) overview of wildtype and knockout placentas using markers genes Bdkrb2, Pecam1 and Vtcn1 to label junctional zone, fetal endothelial cells and SynT-II cells, respectively. (b) Expression of genes thar were significantly downregulated in normal regions of knockout placentas compared to wildtype using MERscope. Scalebar = 1 mm. (c) DotPlot of the genes in a single-nucleus dataset[1] containing trophoblast and fetal-derived placental cells. Colour scale indicate average expression.



Supplementary Fig. 17: Design and validation of Trpv2-KO mTSC. (a) Guide RNA was designed to delete exon 4 and exon 5 in order to create a frameshift deletion. Arrows = primers designed to genotype the mTSC clones, with estimated size of PCR product. (b) Representative genotyping of clones transfected with gRNA (KO) or scrambled gRNA (Scr), compared to SmartLadder (Eurogentec). NTC = non template control. (c) Time course of whole-cell currents at ±120mV recorded in mouse trophoblast cells WT and TRPV2 KO upon application of THC (50µM). Inset: corresponding Current (I)–Voltage (V)-plot. Colors represent similar colored time points in the time course. (d) Current density change for WT and TRPV2 ko mouse trophoblast cells upon application of THC as shown in previous panels, two-sided Mann-Whitney U test, n = 3-6 cells (at +120 mV), p = 0.0238.

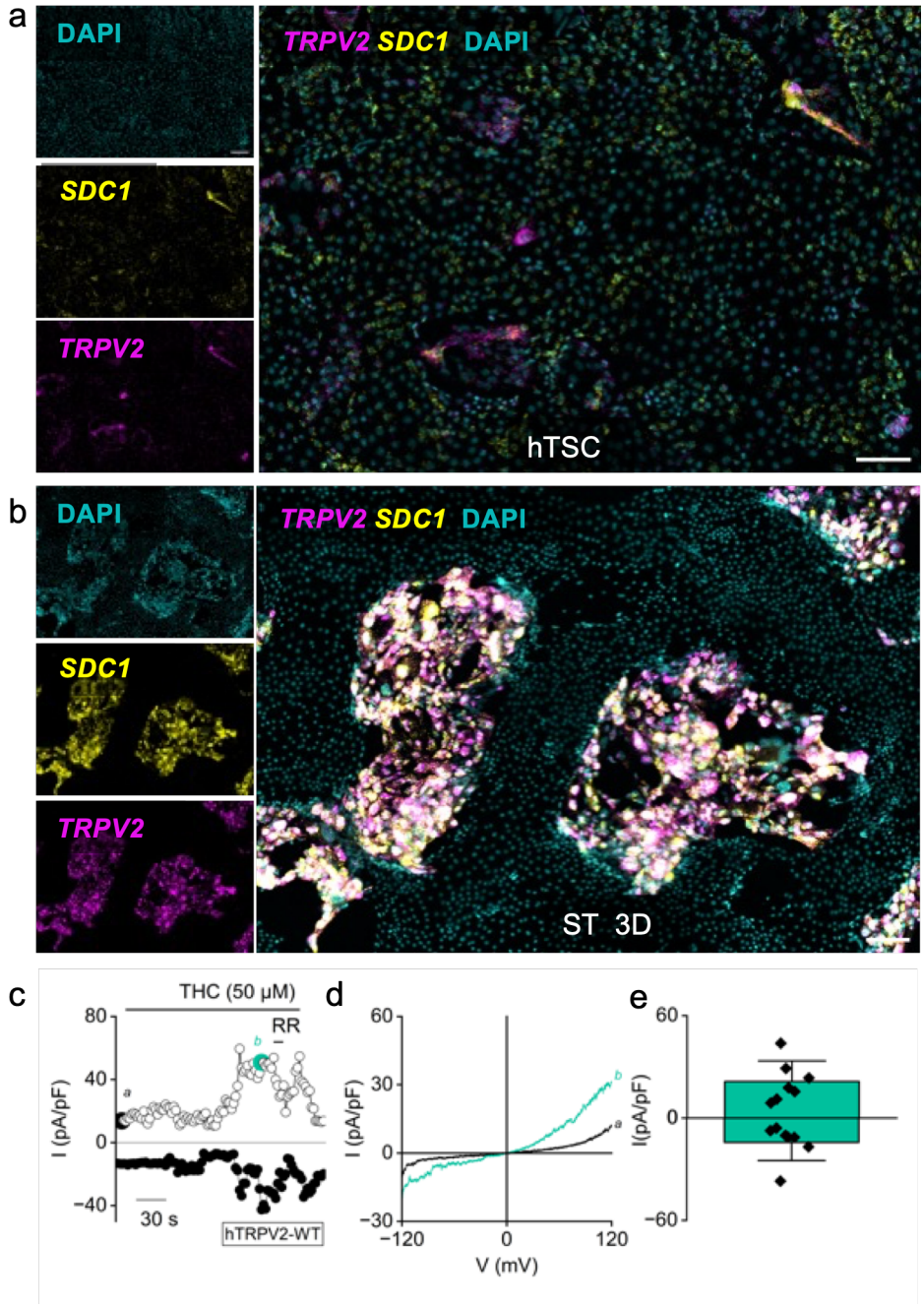


Supplementary Fig. 18: Impaired differentiation of SynTII-cells in vitro. (a) Representation of mTSC differentiation pathway and marker genes. (b) Comparison of marker gene expression between normal, uncontrolled differentiation and SynTII differentiation. (c) Analysis of *Trpv2*-null mutants (orange) and control (Scr, green) in self-renewal conditions (TSC) or after differentiation towards SynTII cells using CHIR99021 for 3 (3D) or 6 (6D) days. Data are shown as mean ± SEM. * p = 0.0276, Two-way ANOVA with Sidak's correction for multiple testing. n = 5-10 clones. (d) Heatmap of DEGs of scrambled stem conditions versus 3D differentiation and 3D differentiation + CHIR, annotated for SynTII precursor markers. Colour scale indicates relative expression (scaled z-score). DEGs were unsupervised clustered, n = 3-6 clones.

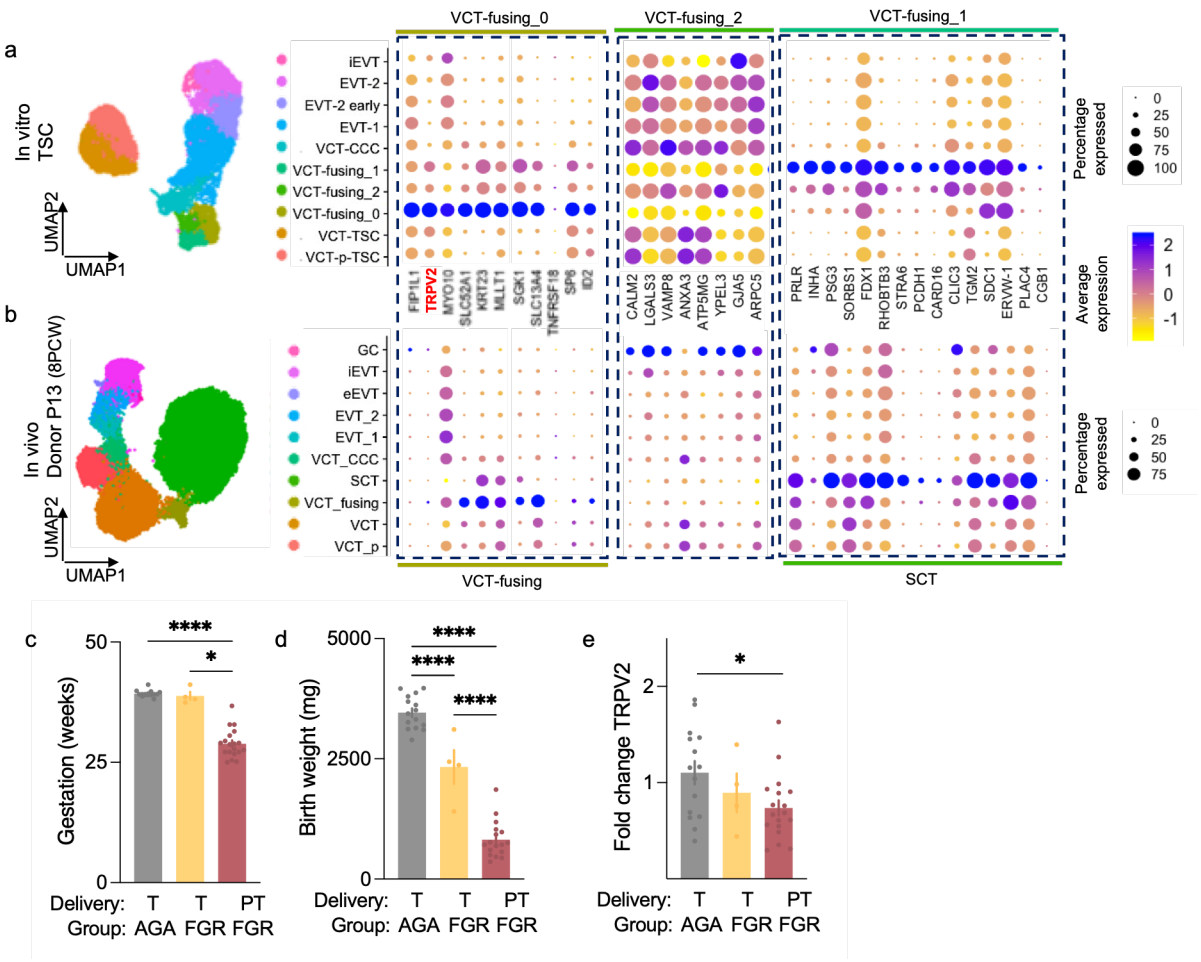


Supplementary Fig. 19: Trpv2 and Gcm1 expression. (a) Detection of Cre activity in conditional knockout model. Placenta of *Gcm1*-cre$^{wt/tg}$; mTmG$^{wt/tg}$ embryo at E14.5 showing Cre expression (green) in SynTII cell, while SynTI, S-TGC and fetal endothelial cells showed no Cre expression (red). Scalebar = 50 µm and 25 µm. (b) *In Situ* hybridization of *Trpv2* in SynTII cells using *Gcm1* at E8.5 of pregnancy; scalebar = 500 µm. (c) Expression of *Gcm1* in germ cells of male mouse, analysed from publicly available single cell RNAseq dataset[2]. Colour scale indicates average expression. (d) Genotype of offspring obtained from the cross between *Gcm1*-cre$^{wt/tg}$;*Trpv2*$^{+/-}$ males x Trpv2$^{F/F}$ females. Birth weight of all different genotypes; One-Way ANOVA with Tukey's multiple comparisons test. (e) Genotype of offspring obtained from the reciprocal cross between *Trpv2*$^{F/F}$ males x *Gcm1*-cre$^{wt/tg}$;*Trpv2*$^{+/-}$ females, in which *Gcm1*-cre is assumed to be inactive. Birth weight of all different genotypes; One-Way ANOVA with Tukey's multiple comparisons test. (f) Percentage of SynTII precursor or SynTII cells expressing

Trpv2, Gcm1, Trpv2 and *Gcm1,* and *Synb*, through analysis of publicly available snRNAseq dataset[1]. (g) Lectin-cytokeratin double labeling of E18.5 Trpv2$^{SynTII-/-}$ placentas, scale bar = 500μm and 50μm in magnification. Immunohistochemistry of MCT1 and MCT4 of serial sections.



Supplementary Fig. 20 : TRPV2 in the human trophoblast stem cells. (a-b) FISH on hTSC and on 3D differentiated STs. *SDC1* = marker for STs. Scale bar = 100 µm. Representative images from N = 3 independent differentiations. (c) Time course of whole-cell currents at ±120mV recorded in human trophoblast cells on day 1 of STB differentiation upon application of THC (50µM) and ruthenium red (RR; 5 µM). (d) Current (I)–Voltage (V)-plot. Colors represent similar colored time points as indicate in panel (c). (e) Current density change for human trophoblast cells upon application of THC (50µM) as shown in panel (c); n = 7 cells.



Supplementary Fig. 21: TRPV2 in the human placenta in health and disease. (a) UMAP projection and DotPlot of scRNA-seq of *in vitro* hTSC, with subanalysis of VCT_fusing cells[3]. (b) UMAP projection and DotPlot of snRNA-seq donor P13 trophoblast nuclei in the maternal-fetal interface[3]. Colour scale indicates average expression.Gestational age (c), birth weight (d), and TRPV2 expression (e) of appropriate for gestational age (AGA) and fetal growth restriction (FGR) pregnancies, diagnosed <32 week's gestation and delivered at term (≥37 week's gestation) or diagnosed <32 week's gestation and delivered preterm (<37 week's gestation). One-way ANOVA with Tukey's multiple comparisons test. n = 15 AGA, n=4 Term early-onset FGR and n= 18 Preterm early-onset FGR. Data is shown as mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Exact p-values are provided in the source data.

SUPPLEMENTARY TABLES

Target	Forward primer	Reverse primer	Detection
Trpv2	5'-TTA AAT GAC TTG TGA GGG AGA TAG C- 3'	5' -CAA GTA ACA CAA TCT ACC CAA GGT C- 3' 5' -TTC CGA TCA TAT TCA ATA ACC CTT A- 3'.	Wt: 322 bp Flox: 368 bp KO: 207 bp
Sox2-Cre	5' -CTT GTG TAG AGT GAT GGC TTG A- 3' 5' -CCA GTG CAG TGA AGC AAA TC- 3'	5' -TAG TGC CCC ATT TTT GAA GG-3'	Wt: 207 bp Tg: 165 bp
Tpbpa-Cre	5'-TCC AGT GAC AGT CTT GAT CCT TAA T-3'	5'-AAT TTT GGT GTA CGG TCA GTA AAT-3'	Tg: 220 bp
Gcm1-Cre	5' CTA GGC CAC AGA ATT GAA AGA TCT 3' 5' CCT TCA GCT GCT CTT GTG G 3'	5' GTA GGT GGA AAT TCT AGC ATC ATC C 3' 5' ACA CCG GCC TTA TTC CAA G 3'	Wt: 324 bp Tg: 260 bp

Supplementary Table 1: primers for genotyping different mouse strains.

			Genotype in target tissue			
Gestational age	Sum (litters)	Litter size (median)	+/+ *(% ± SD) (exp: 25%)*	+/- *(% ± SD) (exp: 50%)*	-/- *(% ± SD) (exp: 25%)*	p-value
			Epiblast knockout (Sox2-cre$^{wt/tg}$; Trpv2$^{+/-}$ x Trpv2$^{F/F}$)			
E16.5	56 (7)	7	16 (26 ± 23)	23 (42 ± 16)	17 (31 ± 18)	*ns*
E18.5	43 (5)	9	8 (22 ± 23)	23 (50 ± 17)	12 (27 ± 9)	*ns*
Term	42 (5)	9	13 (33 ± 14)	15 (33 ± 19)	14 (34 ± 15)	*ns*
			+/+ *(% ± SD) (exp: 50%)*	+/- *(% ± SD) (exp: 25%)*	-/- *(% ± SD) (exp: 25%)*	
			Junctional zone knockout (Tpbpa-cre$^{wt/tg}$; Trpv2$^{F/+}$ x Trpv2$^{F/F}$)			
E18.5	72 (8)	9	29 (39 ± 17)	19 (27 ± 13)	24 (34 ± 21)	*ns*
Term	72 (9)	8	23 (34 ± 14)	21 (27 ± 13)	27 (39 ± 19)	*ns*

Supplementary Table 2: Genotype distribution in mixed litters from Epiblast knockouts and Junctional zone knockouts at different developmental stages. *P-value of mendelian ratios compared to expected numbers analyzed with Chi-square test.*

For Epiblast knockouts:

"+/+" = Sox2-cre$^{wt/wt}$; Trpv2$^{F/+}$,

"+/-" = Sox2-cre$^{wt/wt}$; Trpv2$^{F/-}$ and Sox2-cre$^{wt/tg}$; Trpv2$^{+/-}$,

"-/-" = Sox2-cre$^{wt/tg}$; Trpv2$^{-/-}$.

For Junctional zone knockouts:

"+/+" = Tpbpa-cre$^{wt/wt}$; Trpv2$^{F/+}$ and Tpbpa-cre$^{wt/wt}$; Trpv2$^{F/F}$,

"+/-" = Tpbpa-cre$^{wt/tg}$; Trpv2$^{F/+}$,

"-/-" = Tpbpa-cre$^{wt/tg}$; Trpv2$^{F/F}$.

Name	exon	gRNA sequence
Trpv2_1a	Upstream exon 4	AAAGACTCGGTATCCCACGG
Trpv2_1b	Upstream exon 4	CAGCCTGAAATGACCCCCGT
Trpv2_2a	Downstream exon 5	TGAGCTTCCCTGGCTAATAG
Trpv2_2b	Downstream exon 5	GCTTAATCCTCTATTAGCCA
Scrambled_1	Non-targeting	GCTTTCACGGAGGTTCGACG
Scrambled_2	Non-targeting	ATGTTGCAGTTCGGCTCGAT

Supplementary Table 3: gRNA sequences for CRISPR-mediated Trpv2 knockout.

Gene	Taqman assay
Mouse	
Gcm1	Mm00492310_m1
Vegfa	Mm00437306_m1
Pecam1	Mm01242576_m1
Synb	Mm04212067_m1
Syna	Mm02744887_s1
Ctsq	Mm01349948_m1
Prl3d1	Mm04213281_g1
Tpbpa	Mm01352548_g1
Elf5	Mm00468732_m1
Eomes	Mm01351984_m1
Cdx2	Mm01212280_m1
Trpv2	Mm00449223_m1
Sdha	Mm01352366_m1
Gapdh	Mm99999915_g1
Actb	Mm02619580_g1
Slc16a3	Mm00446102_m1
Human	
TRPV2	Hs00901640_m1
CGB3	Hs00361224_gH
ERVW-1	Hs01926764_u1
ERVFRD-1	Hs01942443_s1
GCMa	Hs00172692_m1
TRPM7	Hs00918956_m1
GAPDH	Hs99999905_m1
TBP	Hs00427620_m1
YWHAZ	Hs01122445_g1
B2M	Hs00187842_m1

Supplementary Table 4: Taqman primers

SUPPLEMENTARY REFERENCES

1. Marsh, B., and Blelloch, R. (2020). Single nuclei RNA-seq of mouse placental labyrinth development. Elife *9*. 10.7554/eLife.60266.
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3. Arutyunyan, A., Roberts, K., Troulé, K., Wong, F.C.K., Sheridan, M.A., Kats, I., Garcia-Alonso, L., Velten, B., Hoo, R., Ruiz-Morales, E.R., et al. (2023). Spatial multiomics map of trophoblast development in early pregnancy. Nature *616*, 143-151. 10.1038/s41586-023-05869-0.