**EXTENDED INFORMATION**
**Placental TRPV2 is indispensable for normal fetal development.**

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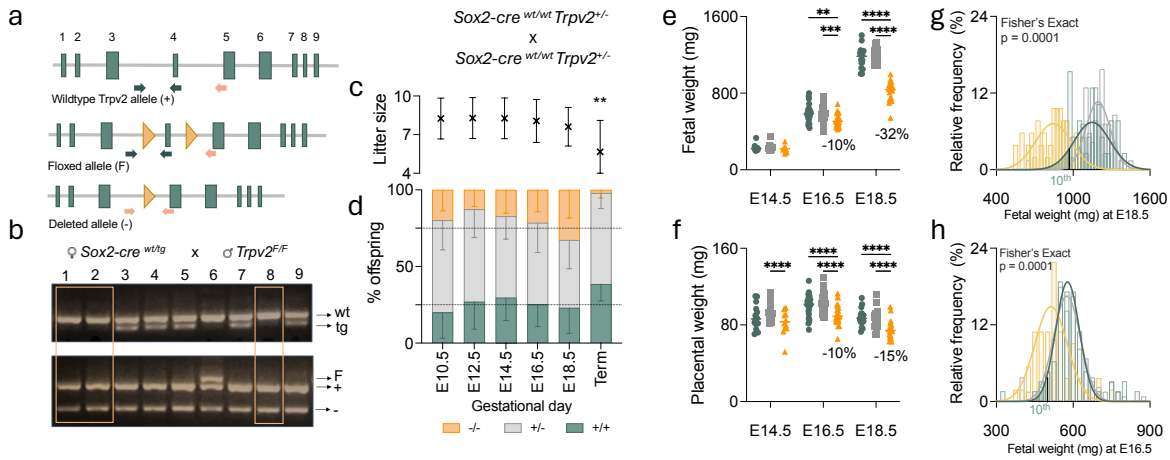
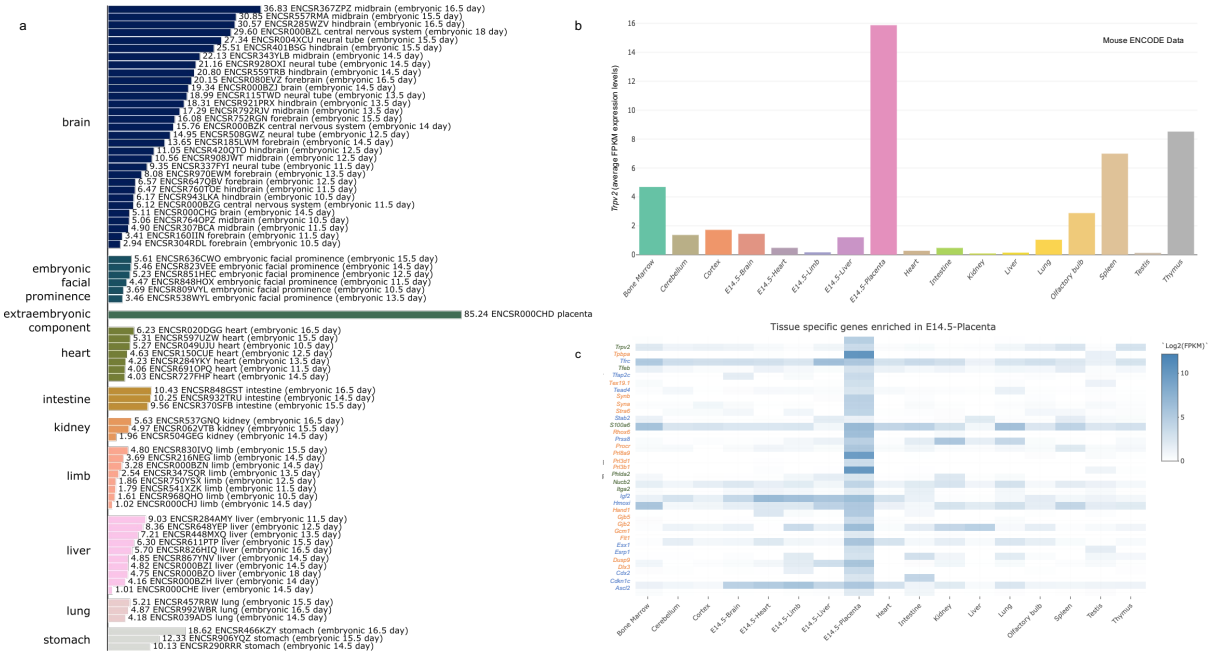
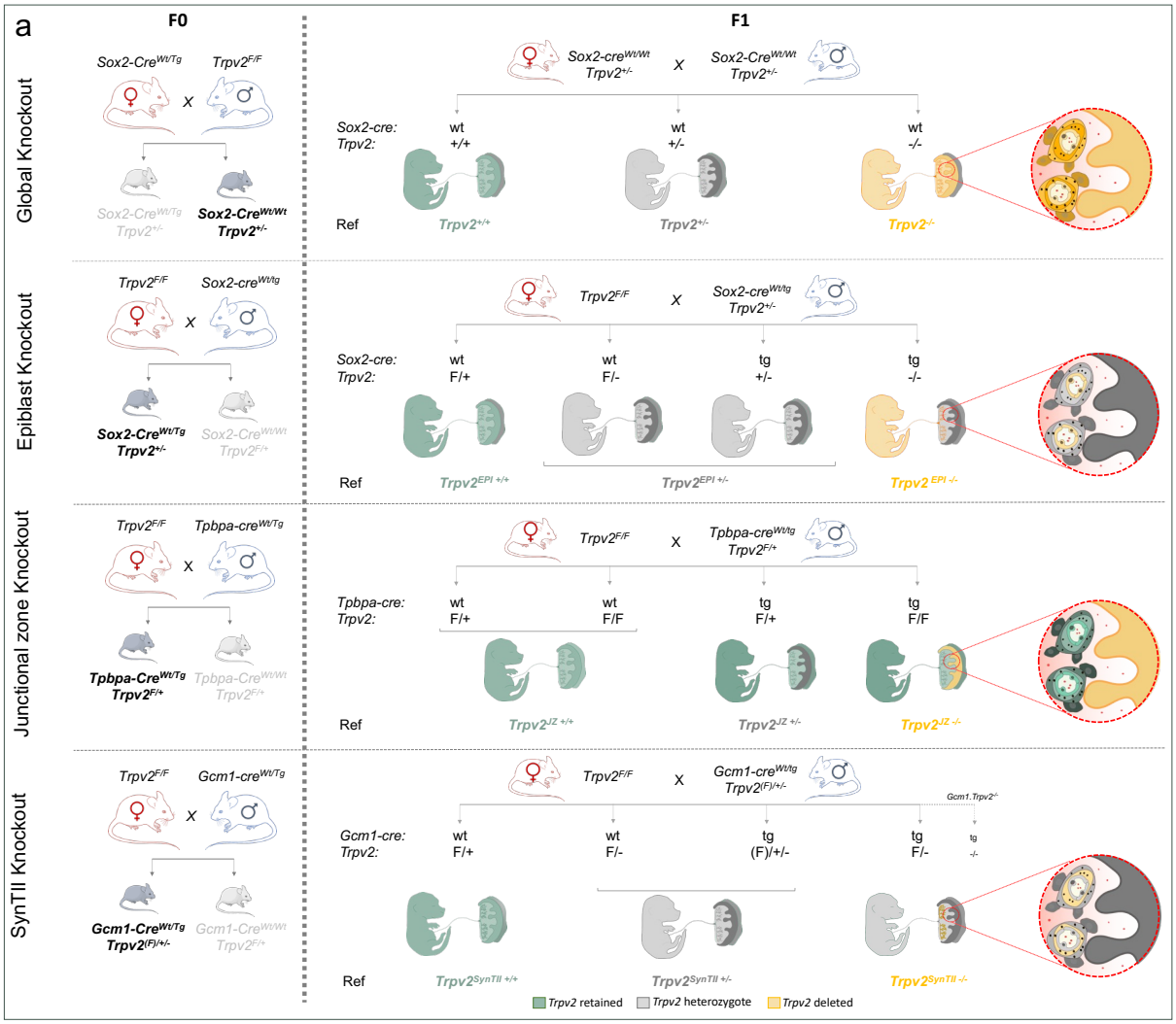
## FIGURES




*Extended Figure 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. ** 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 Fisher's exact test comparing proportion of animals below 10$^{th}$ 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

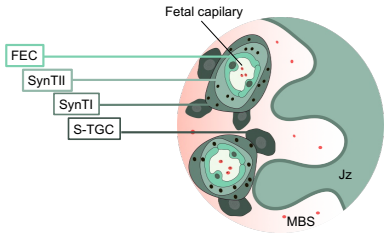



*Extended Figure 2*: *Trpv2 expression in developing embryo*. (a) Figure adapted from Encode/SCREEN database showing detailed expression of *Trpv2* in the developing embryo. (b) Figure adapted from TissueEnrich webtool showing *Trpv2* expression in embryo and adult organs. (c) Enrichment analysis by including *Trpv2* in the list of labyrinth specific genes obtained from the MGI database. 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).

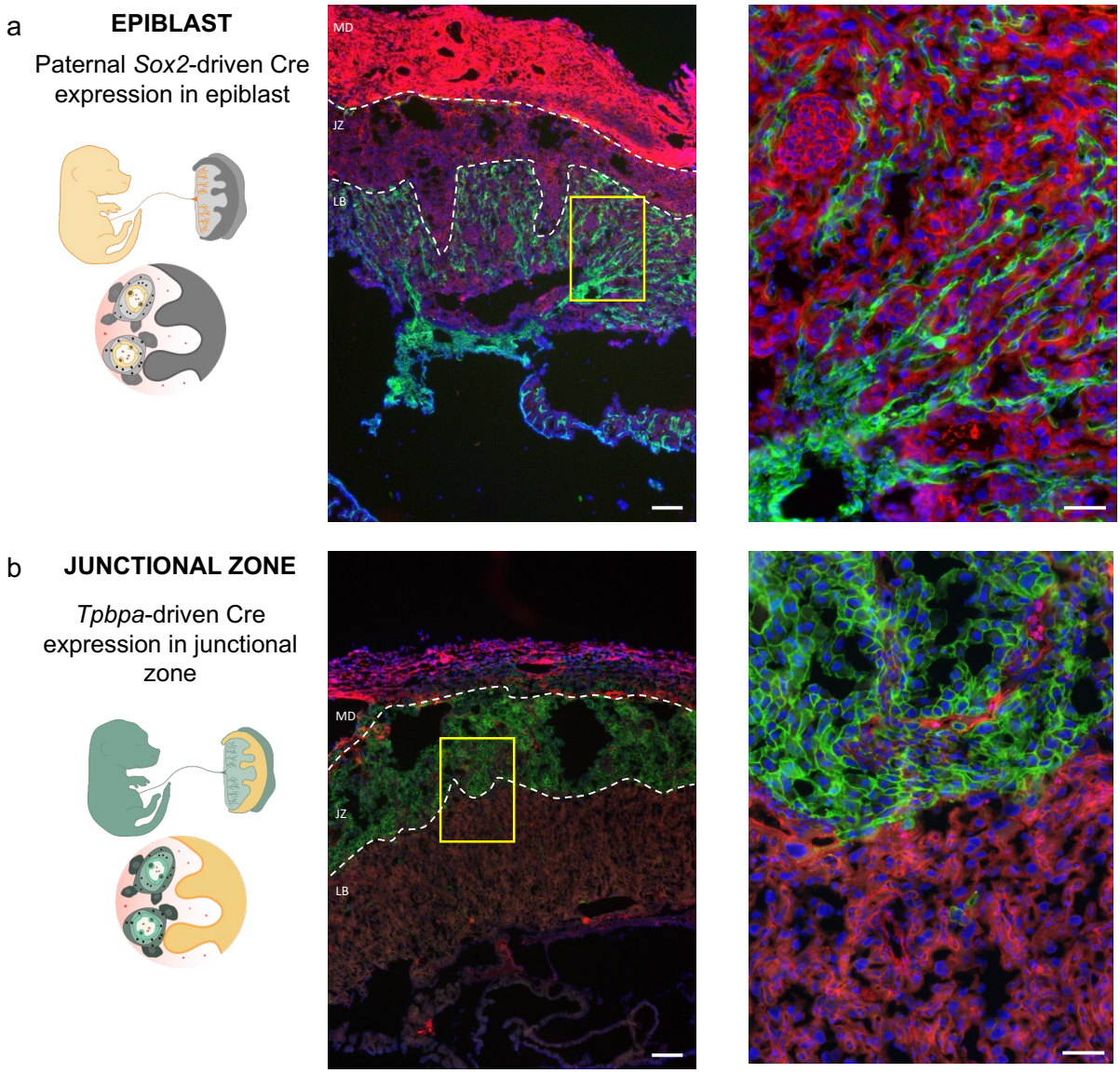



b

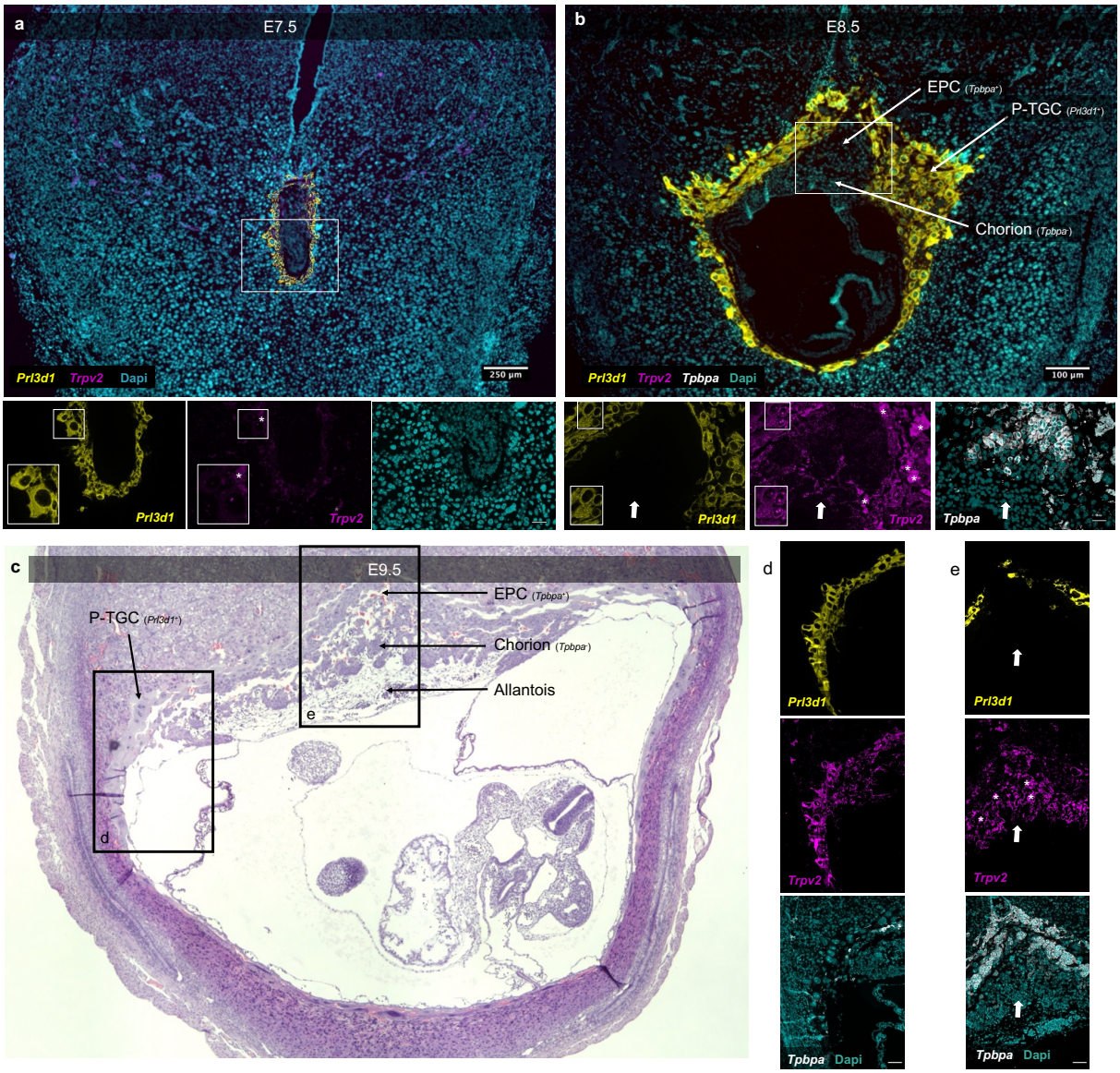
| | Epiblast | Trophectoderm | | | | |
|---|---|---|---|---|---|---|
| | Fetus | Placenta | | | | |
| | | FEC | SynTII | SynTI | S-TGC | JZ |
| Global knockout | -/- | -/- | -/- | -/- | -/- | -/- |
| Epiblast knockout | -/- | -/- | +/- | +/- | +/- | +/- |
| Junctional zone knockout | +/+ | +/+ | +/+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



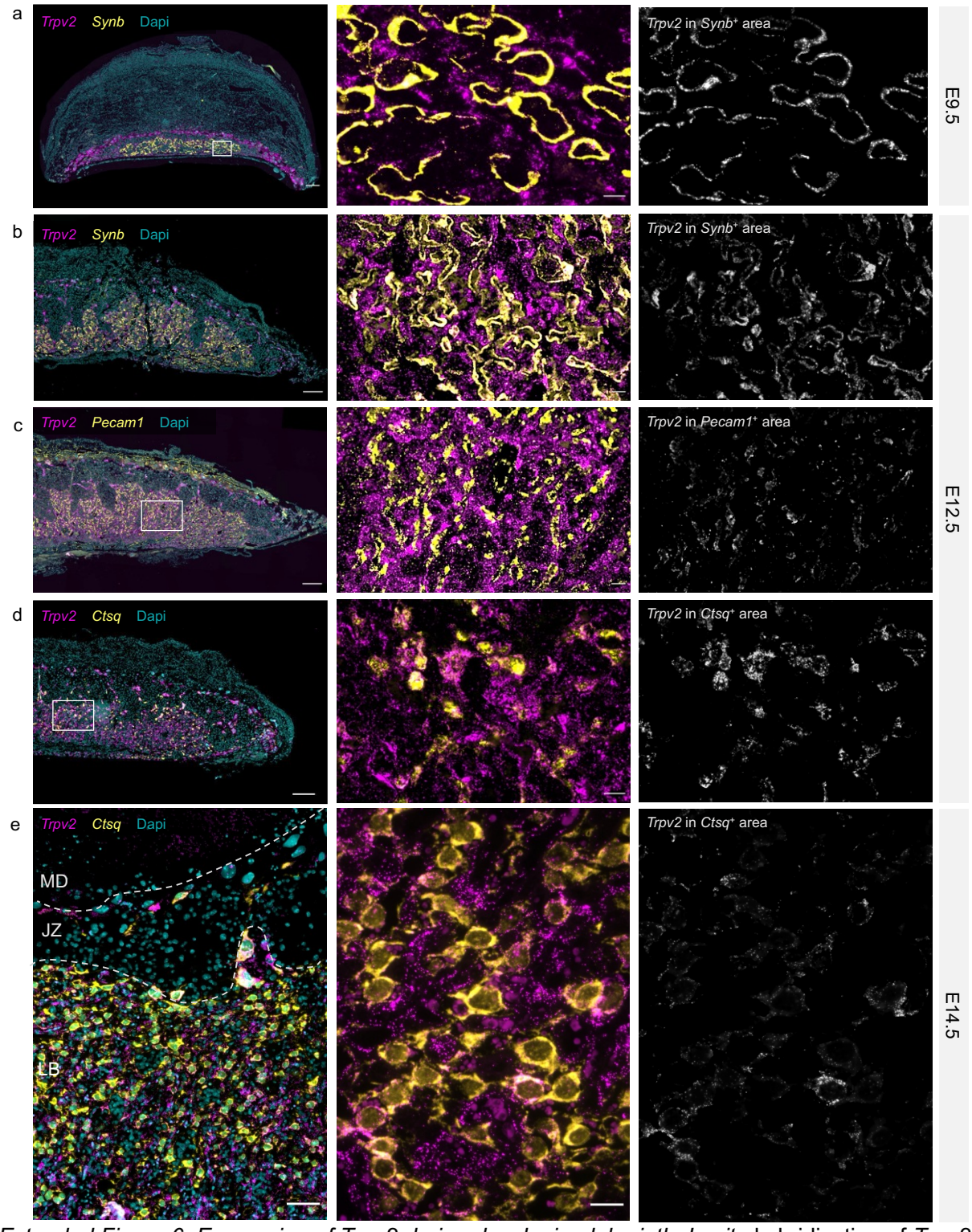

*Extended Figure 3: Overview of breeding strategy to obtain conditional Trpv2 knockouts.* (a) Breeding strategies. F0 represent the first breeding to obtain tissue specific heterozygotes, whereas F1 breeding were done to create tissue specific knockouts (yellow), and their respective wildtype (green) 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.



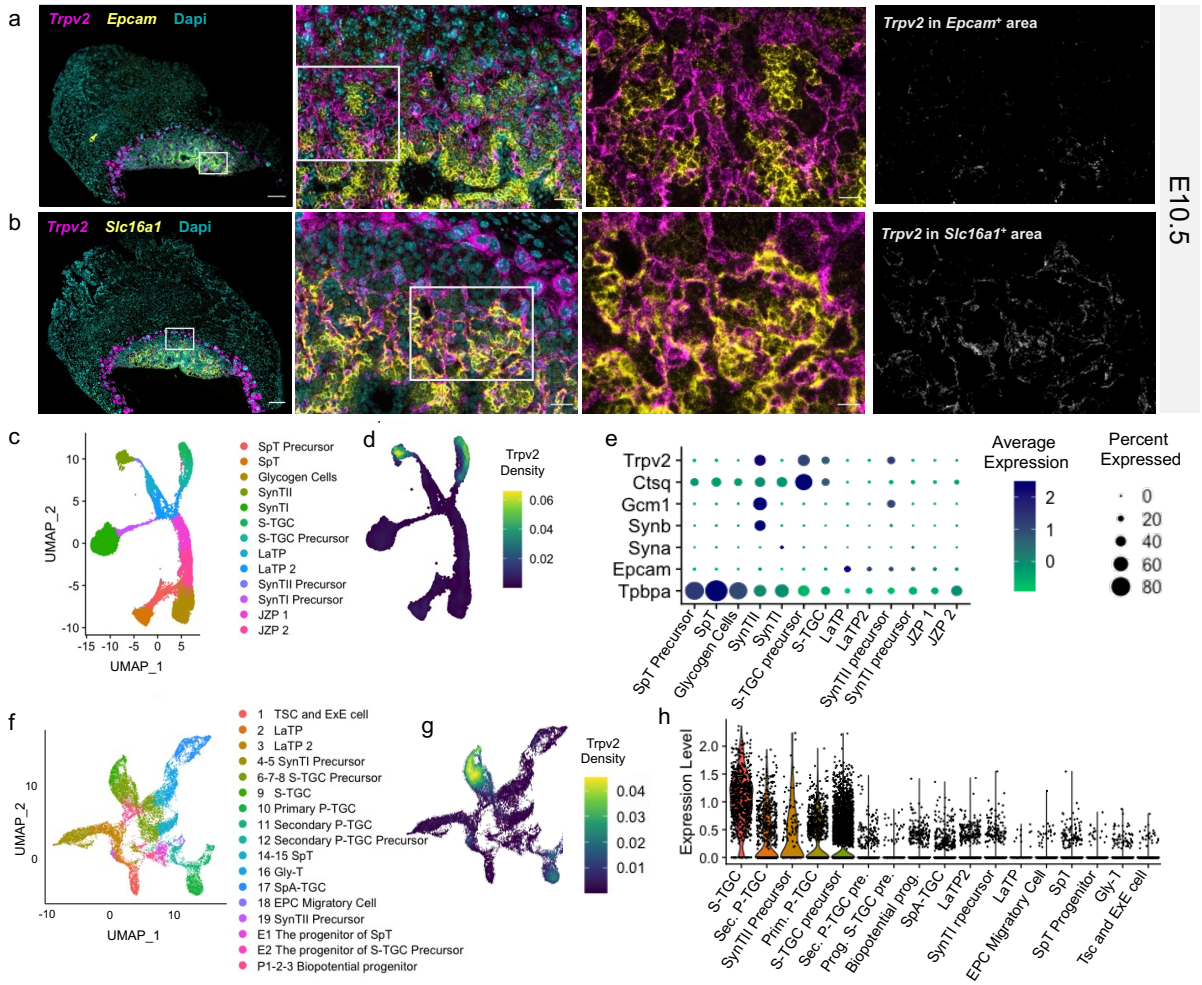

*Extended Figure 4: Detection of Cre activity in conditional knockout models*. (a) Confirmation of epiblast specific CRE expression. Placenta of *Sox2-cre*$^{wt/tg}$*; mTmG*$^{wt/tg}$ embryo at E14.5 showing Cre expression (green) in fetal capillaries, while trophoblast and decidua show no CRE expression (red). (b) 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). MD = maternal decidua, JZ = junctional zone, LB = labyrinth.



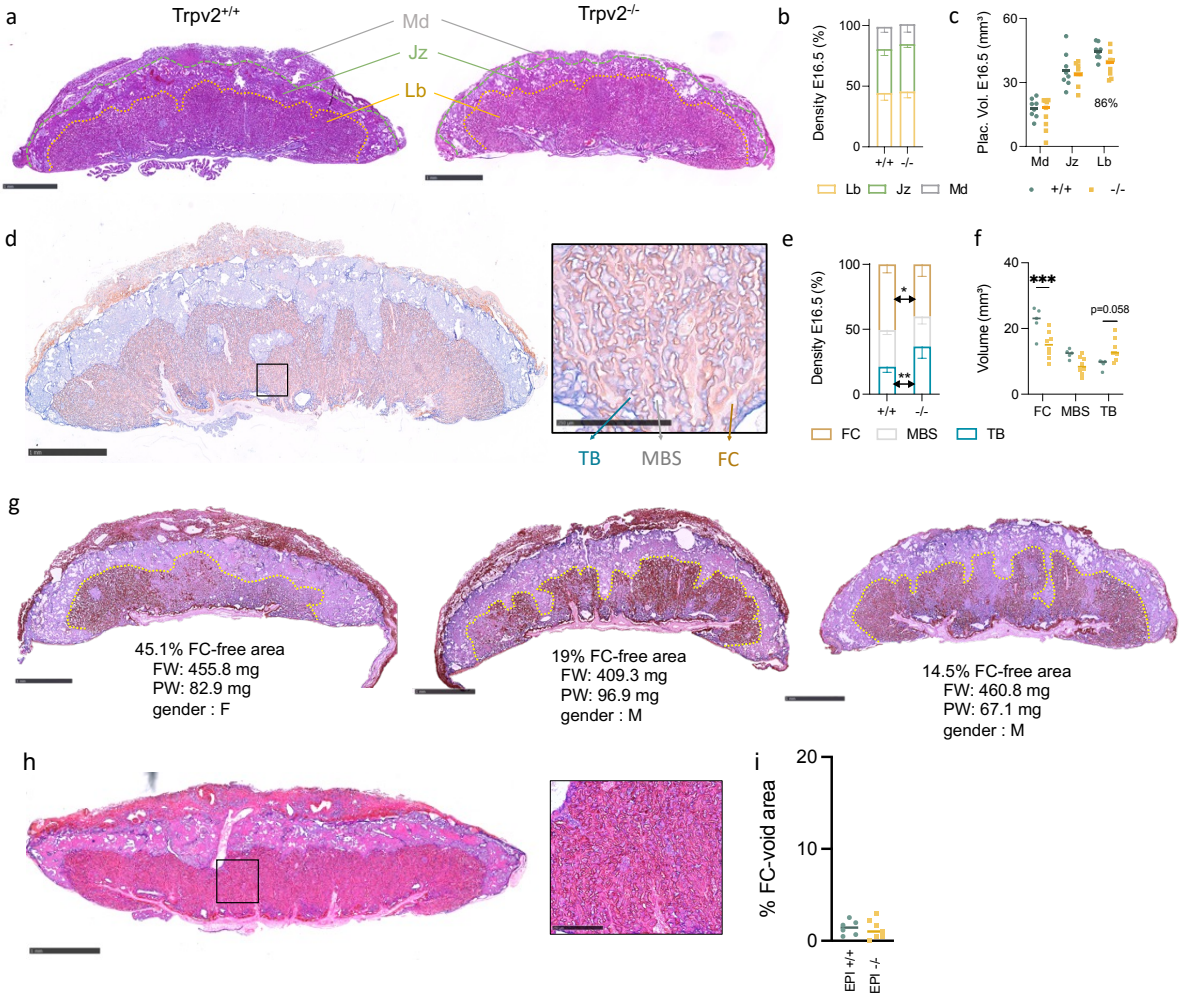

*Extended Figure 5: Expression of Trpv2 during early gestation.* (a) Fluorescent *In situ* hybridization (FISH) at E7.5 using *Prl3d1* to mark primary P-TGC. (b) *In situ* hybridization at E8.5 using *Prl3d1,* and *Tpbpa* which identifies ectoplacental cone lineages. (c) *In situ* hybridization of *Prl3d1* 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.



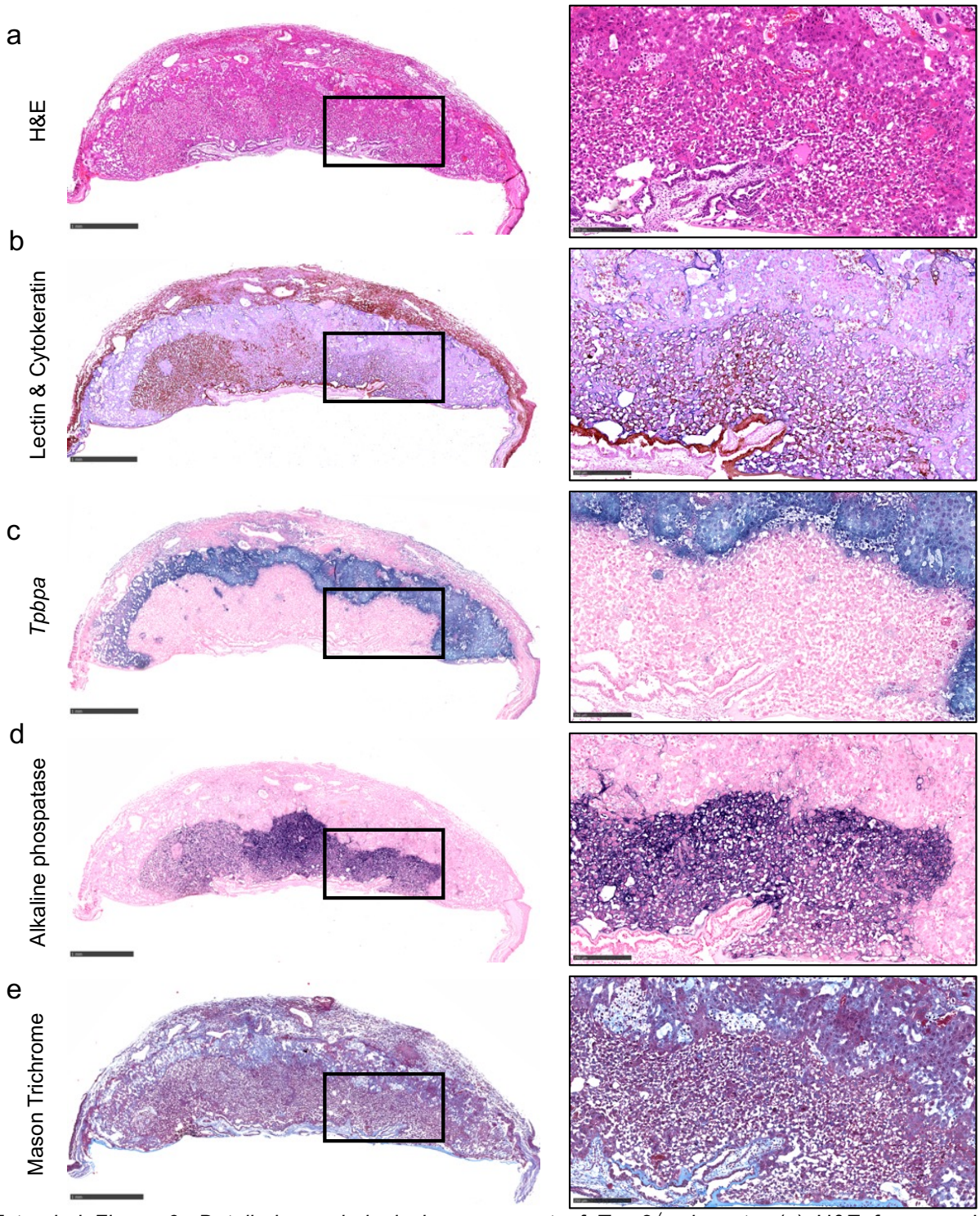

*Extended Figure 6: Expression of Trpv2 during developing labyrinth. In situ* hybridisation of *Trpv2* in SynTII cells using *Synb* at E9.5 and E12.5 (a, b), Fetal endothelial cells using *Pecam1* at E12.5 (c) and S-TGC cells using *Ctsq* at E12.5 and E14.5 (d,e). Scalebar = 250 µm (left row) and 50 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.



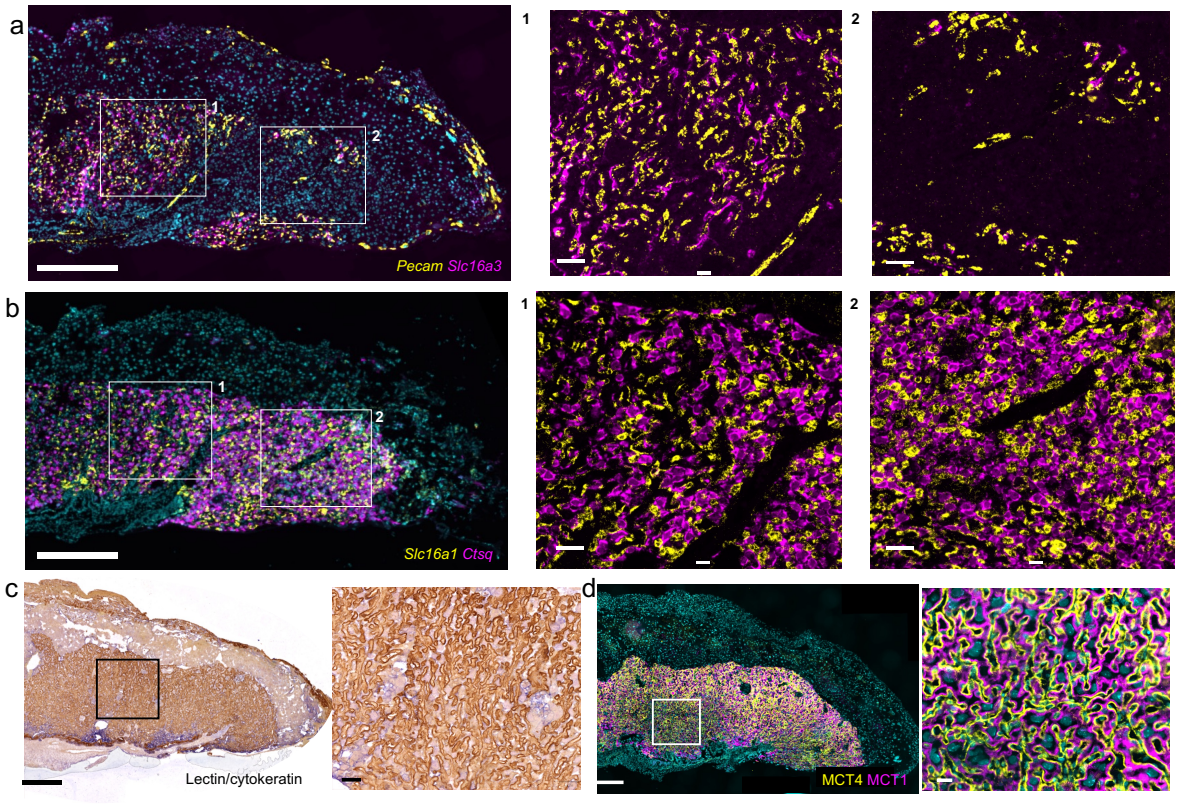

*Extended Figure 7: Trpv2 expression in the developing mouse placenta.* (a-b) FISH undifferentiated Labyrinth Progenitor cells using *Epcam* (a) and SynTI cells using *Slc16a1* (b) at gestational days E10.5. scalebar = 250 – 50 – 25 µm, from left to right. Image on most right shows *Trpv2* expression only in cells that were identified by the respective marker. (c) 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*[12]. (g) Nebulosa density expression of *Trpv2* in several trophoblast populations projected in UMAP space. (h) Dot plot showing average expression and percent of nuclei expressing labyrinth marker genes and *Trpv2*. (f) UMAP plotted according to transcriptome similarity of all trophoblasts, data adapted from Jiang *et al.*[14] (g) Nebulosa density expression of *Trpv2* in several trophoblast populations projected in UMAP space. (h) Violin Plot showing distribution of *Trpv2* expression in the different populations.



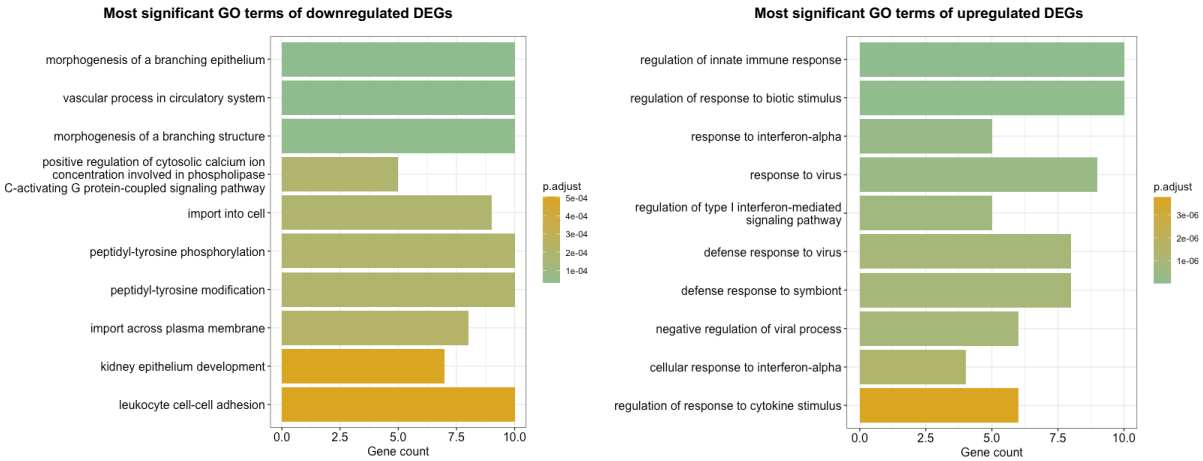

*Extended Figure 8: Details of labyrinth morphology*. (a) Representative H&E stained image and stereological analysis (b,c) of placental morphology at E16.5, scale bar = 1 mm. (d) Representative images of Lectin-cytokeratin double labeling of E16.5 *Trpv2*$^{+/+}$ placentas, scale bar = 1 mm and 250 µm in magnification. (e,f) Stereological analysis of labyrinth morphology in (c). Md = maternal decidua, Jz = junctional zone, Lb = labyrinth, TB = trophoblast, MBS = maternal blood space, FC = fetal capillaries. Data analysed by Two-Way ANOVA with Sidaks multiple comparison test for (b), (c), (e) and (f) with n = 8-9 from 8 litters. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (g) 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. (h) Lectin-cytokeratin double labeling of *Trpv2*$^{EPI\ -/-}$ placenta at E16.5, scale bar = 1mm, with magnification of the insert, scale bar = 200 µm (i) Quantification of FC-void areas in *Trpv2*$^{EPI\ -/-}$ and *Trpv2*$^{EPI\ +/+}$ placentas. n= 6-7 fetuses/genotype from 4 litters.



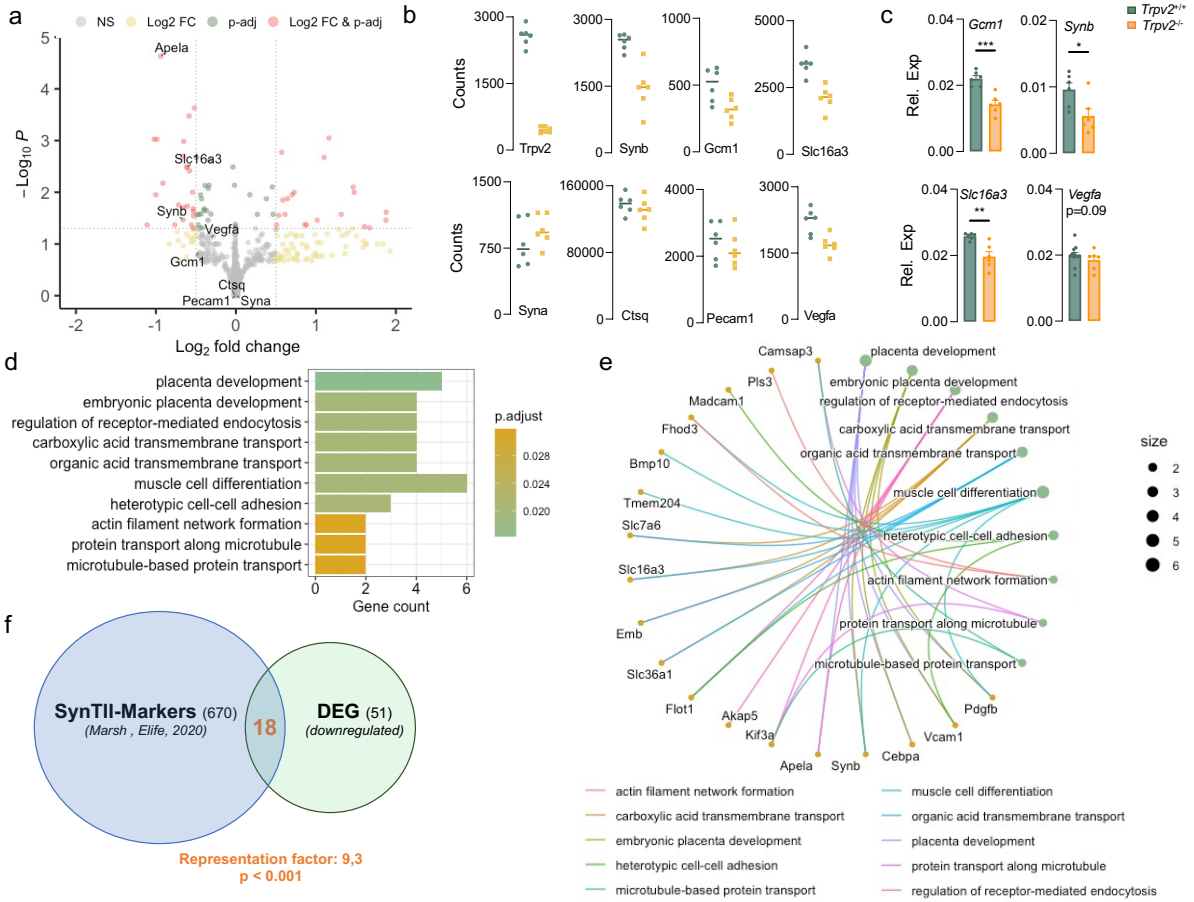

*Extended Figure 9: Detailed morphological assessment of Trpv2$^{-/-}$ placenta*. (a) H&E for normal morphology. (b) Lectin (brown) = basal membrane of fetal capillaries. Cytokeratin (blue) = all trophoblast cells. (c) *Tpbpa* stained with ISH = trophoblast cells of junctional zone. (d) Alkaline phosphatase = lining of maternal blood space. (e) Masson's Trichrome = collagen (fibrosis). Scale bar = 1 mm and 250 µm.



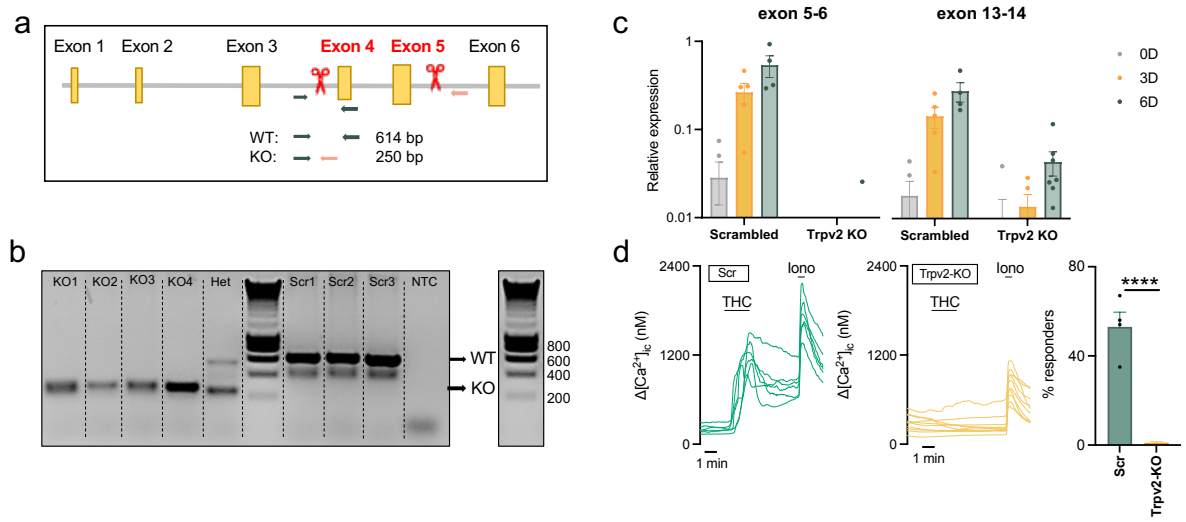

*Extended figure 10: Morphology of Trpv2$^{-/-}$ and Trpv2$^{+/+}$ littermates*. (a) FISH of *Pecam1* and *Slc16a3*, representative for fetal endothelial cells and SynTII cells, respectively, of E16.5 *of Trpv2$^{-/-}$ placentas* (b) FISH of *Slc16a1* and *Ctsq*, representative for SynTI cells and S-TGC, 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.



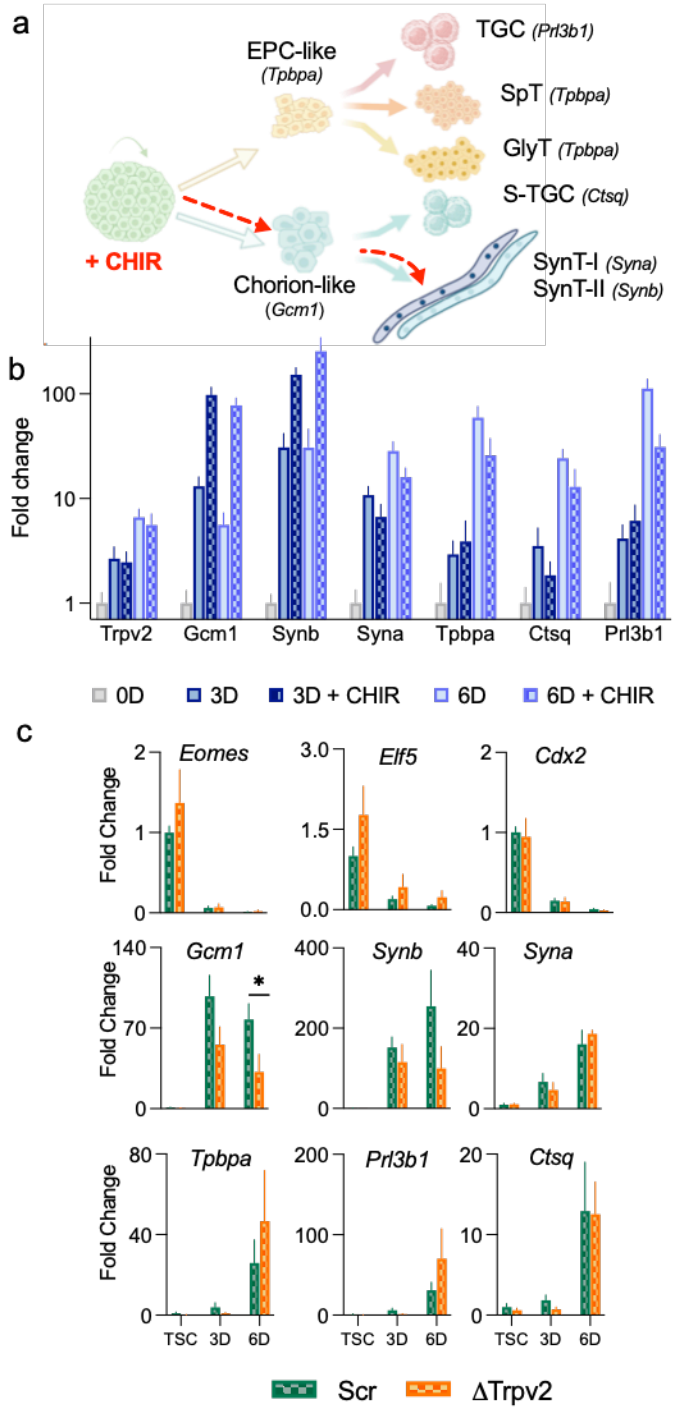

*Extended Figure 11: Enrichment in E14.5 placentas*. Most significant GO terms of downregulated and upregulated DEGs.



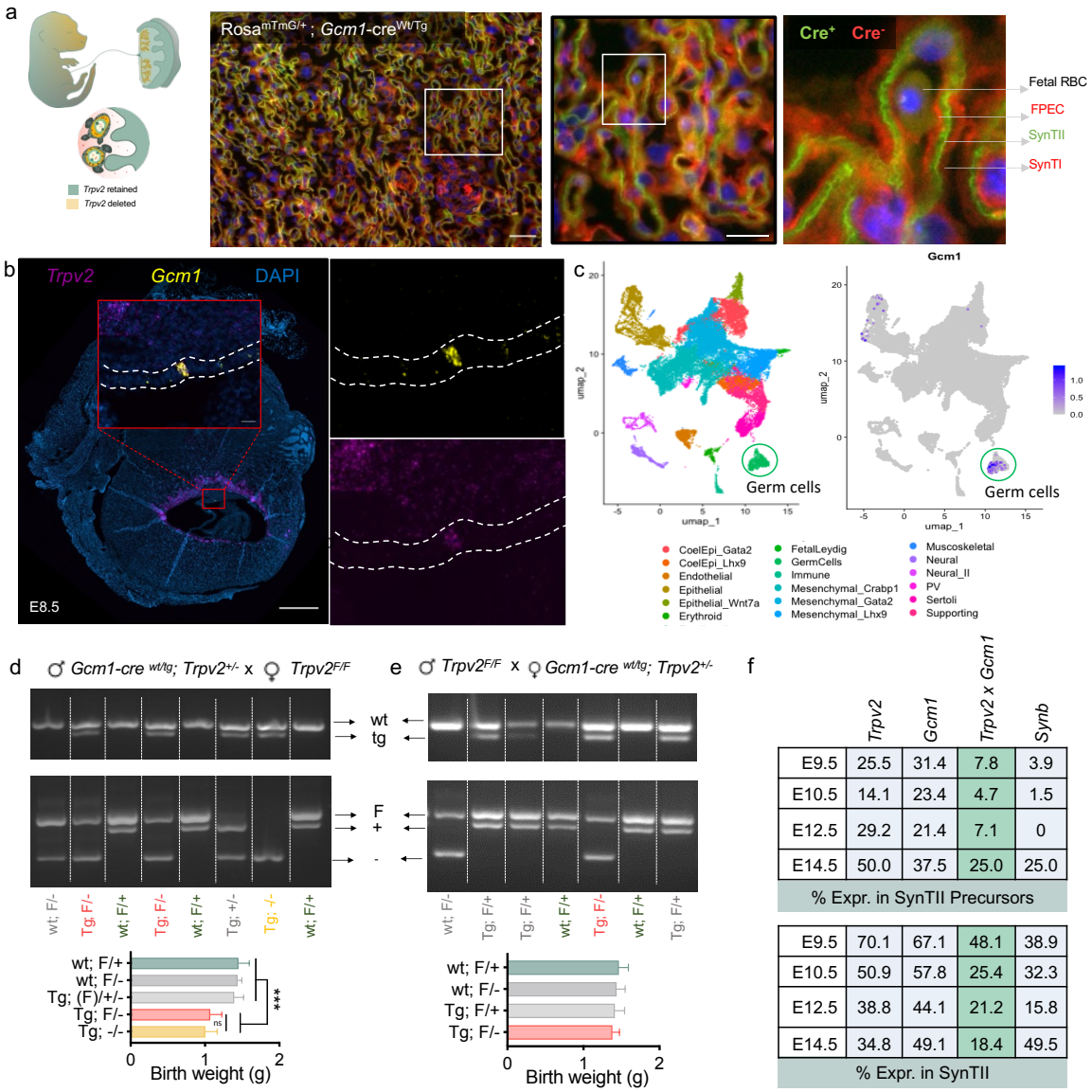

*Extended Figure 12: Impaired placental development in E16.5 Trpv2-KO labyrinth*. (a) Enhanced Volcano Plot representation of DEGs identified by RNAseq of E16.5 labyrinth (*Trpv2*$^{+/+}$ vs *Trpv2*$^{-/-}$), adjusted p-value (cut-off < 0.05) and $Log_2$ Fold Change (cut-off < 0.5) (n = 6/genotype, 3 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 (Student t-test). (d) Barplot of biological processes that are significantly enriched in downregulated DEGs and linked to placental functioning. (e) Cnetplot showing linkage between DEGs and enriched GO terms. (f) Significant overrepresentation of SynTII-markers in downregulated DEGs.




*Extended Figure 13: 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) qRT-PCR with primers spanning exon 5-6 (within the target region) and exon 13-14 (downstream of the target region), n = 5. (d) Examples traces of Fura-2AM based microfluorimetric calcium imaging in a scrambled (Scr) and a *Trpv2*-KO mTSC at day 6 of differentiation using THC (50 µM) as TRPV2 agonist and Ionomycin (Iono; 2 µM) as positive control. Percentage of THC-responding cells (263/493 Scr mTSC and 6/783 *Trpv2*-KO mTSC, from N = 4 measurements), Fisher's exact test; **** $p < 0.0001$.



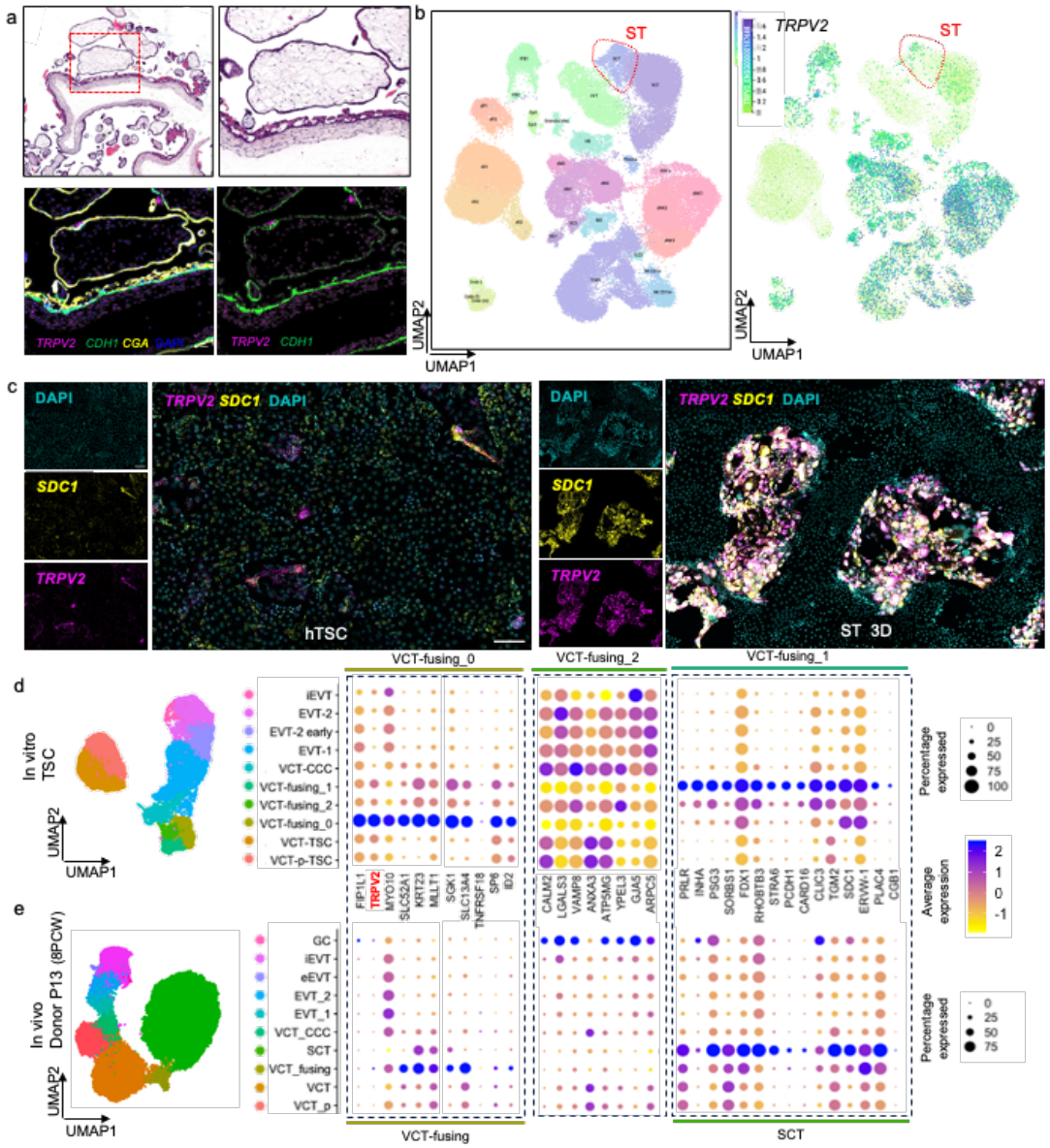

*Extended Figure 14: Impaired differentiation of SynTII-cells in vitro.* (a) Representation of mTSC differentiation pathway and marker genes. (b) Comparison of marker gene expression between normal, uncontrolled differentiation and SynTII differentiation. (c) Analysis of *Trpv2*-null mutants (orange) 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. * $p < 0.05$, Two-way ANOVA with Sidak's correction for multiple testing. n = 5-10 clones.




| | Trpv2 | Gcm1 | Trpv2 x Gcm1 | Synb |
|---|---|---|---|---|
| E9.5 | 25.5 | 31.4 | 7.8 | 3.9 |
| E10.5 | 14.1 | 23.4 | 4.7 | 1.5 |
| E12.5 | 29.2 | 21.4 | 7.1 | 0 |
| E14.5 | 50.0 | 37.5 | 25.0 | 25.0 |
| % Expr. in SynTII Precursors | | | | |

| | | | | |
|---|---|---|---|---|
| E9.5 | 70.1 | 67.1 | 48.1 | 38.9 |
| E10.5 | 50.9 | 57.8 | 25.4 | 32.3 |
| E12.5 | 38.8 | 44.1 | 21.2 | 15.8 |
| E14.5 | 34.8 | 49.1 | 18.4 | 49.5 |
| % Expr. in SynTII | | | | |

*Extended Figure 15: 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[1]. (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[2].




*Extended Figure 16: TRPV2 in the human placenta*. (a) H&E and subsequent FISH on placental tissue from first trimester showing stem villi. *CDH1* = marker for cytotrophoblast, *CGA* = marker for syncytiotrophoblasts. (b) UMAP projection and FeaturePlot of single cell RNAseq dataset of fetal-maternal interface of first trimester, with definitive ST population highlighted (www.reproductivecellatlas.com)[3]. (c) FISH on hTSC and on 3D differentiated STs. *SDC1* = marker for STs. Scale bar = 100 µm. (d) UMAP projection and DotPlot of scRNA-seq of *in vitro* hTSC, with subanalysis of VCT_fusing cells[4]. (e) UMAP projection and DotPlot of snRNA-seq donor P13 trophoblast nuclei in the maternal-fetal interface[4].

**TABLES**

| | | | 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* |

*Table S1: 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 |

*Table S2: 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 |

*Table S3: Taqman primers*

**References**


1 Garcia-Alonso, L. *et al.* Single-cell roadmap of human gonadal development. *Nature* **607**, 540-547 (2022). https://doi.org/10.1038/s41586-022-04918-4

2 Marsh, B. & Blelloch, R. Single nuclei RNA-seq of mouse placental labyrinth development. *Elife* **9** (2020). https://doi.org/10.7554/eLife.60266

3 Vento-Tormo, R. *et al.* Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* **563**, 347-353 (2018). https://doi.org/10.1038/s41586-018-0698-6

4 Arutyunyan, A. *et al.* Spatial multiomics map of trophoblast development in early pregnancy. *Nature* **616**, 143-151 (2023). https://doi.org/10.1038/s41586-023-05869-0