SUPPLEMENTARY INFORMATION

Supplementary Figure S1. Derivation of CVS-TOs.

a-**c,** Isolation and single-cell transcriptional profiling of trophoblast populations from CVS tissue**. a**, CVS tissue obtained from first-trimester pregnancies (10-14 weeks of gestation). Inset shows magnification of villi. Arrows highlight the mesenchymal core and villous protrusions. **b**,**c**, Single-cell profiling of cells derived from CVS tissue (*n* = 6,481). **b**, UMAP plot showing 4 distinct clusters (Trophoblast, *n* = 5,759; Stromal cells, *n* = 437; Hofbauer cells, *n* = 158; Fetal blood cells, *n* = 127). **c**, Dotplot showing normalized expression and percentage of expressing cells for a range of marker genes. **d,** Outgrowth of EVT-like cells after depletion of Wnt stimulators. Brightfield images of EVT outgrowth in CVS-TOs cultured in trophoblast organoid medium deprived for Wnt stimulators. EVT cell population is highlighted in the magnification. Scale bars, 200 µm.

Supplementary Figure S2. Genotyping of CVS-TOs.

a, Genotyping of DS-TOs using SNP array analysis. Log2 Ratio and B-allele frequency plots of the whole genome confirmed trisomy 21. Altered percentages of B-allele frequencies observed for chromosome 21 in DS-TOs. **b**, Sanger sequencing confirmed *SMC1A*; c.532A>C mutation in CdLS-TOs.

Supplementary Figure S3. Clustering and cluster proportions of integrated single-cell transcriptional profiles of DS-TOs, CdLS-TOs and control CVS-TOs.

a, Table showing cluster proportions and corresponding cell numbers for the integrated single-cell transcriptional profiles of DS-TOs, CdLS-TOs and control CVS-TOs at day 14 of culture. **b**, Relative differences in cell proportions for each cluster between CdLS-TOs versus control CVS-TOs and CdLS-TOs versus DS-TOs. Red clusters have an FDR <0.05 and mean log2 fold enrichment >1 compared with control or DS-TOs (permutation test; *n* = 10,000). **c**, Relative differences in cell proportions for each cluster between DS-TOs versus control (permutation test; *n* = 10,000).

Supplementary Figure S4. Population of progenitor CTBs expressing stress markers.

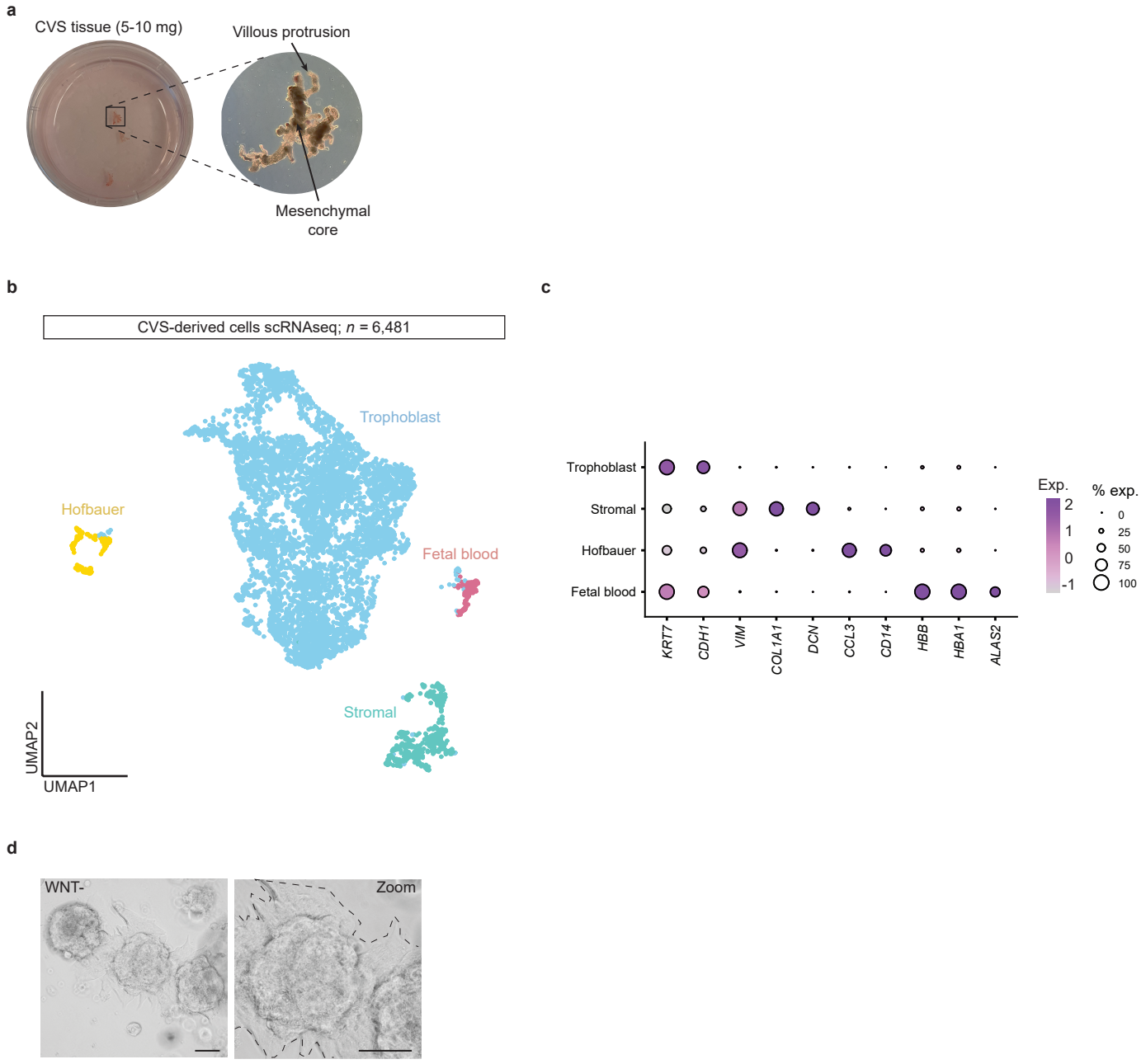
UMAP plots showing normalized expression of stress markers *FOS*, *FOSB, JUN, JUNB, ATF3* and *KLF6* in the integrated CVS-TO single-cell dataset.

Supplementary Table S1: Summary of CVS-TO derivation and characterization.

Supplementary Table S2: Differential gene expression analysis in CVS-TOs. Genes were identified using the following thresholds: minimum log fold change value > 0.25; adjusted p-value < 0.01; detected in a minimum of 25% cells in each cluster. First sheet shows differentially expressed genes per cluster in control CVS-TOs. Second sheet shows differentially expressed genes per cluster in the integrated CVS-TO single-cell dataset (DS-TOs, CdLS-TOs and control CVS-TOs).

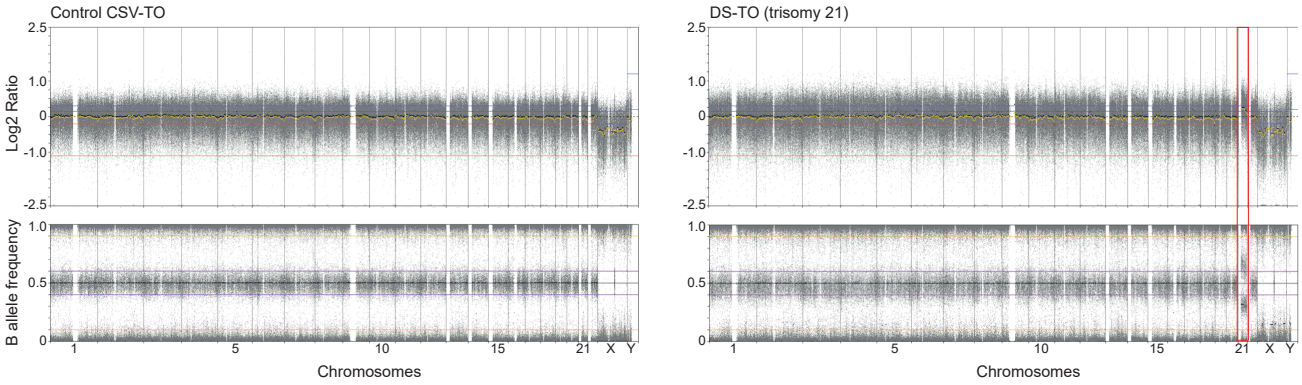
Supplementary Table S3: LC-MS/MS analysis of CVS-TO supernatants.

Supplementary Figure S1



Supplementary Figure S2

a



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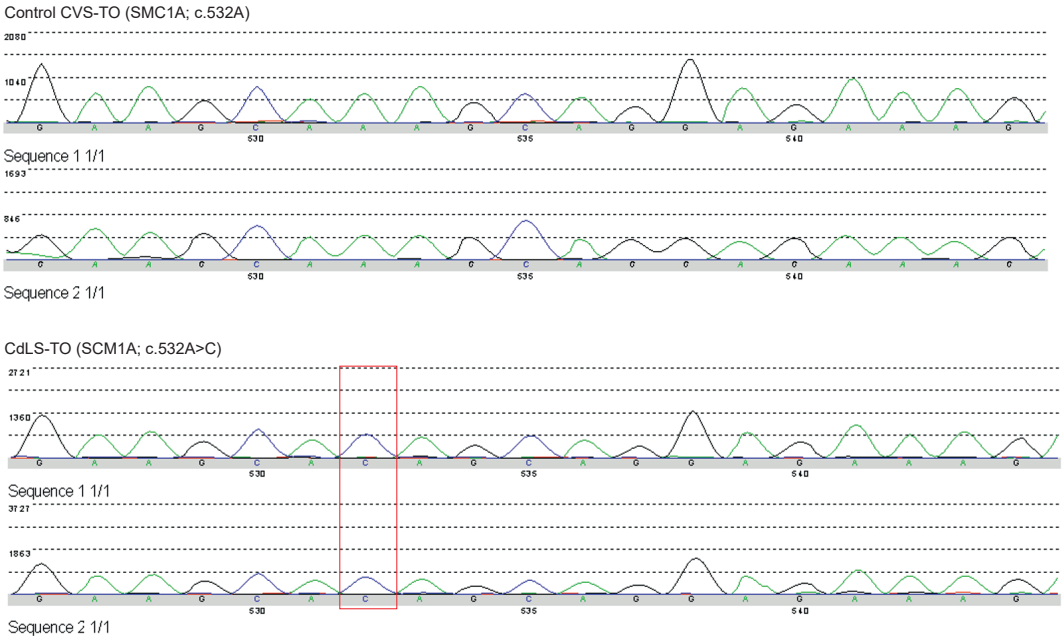
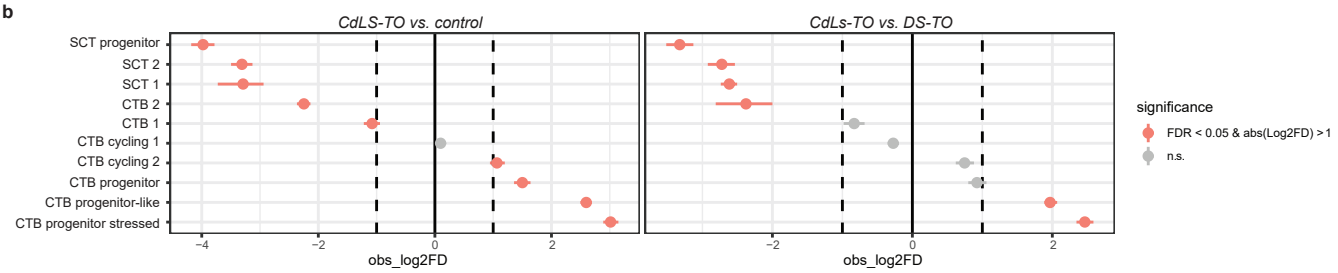


Supplementary Figure S3

a

Cluster	DS-TO (*n* = 12,816)	CdLS-TO (*n* = 15,509)	Control-TO (*n* = 15,901)
CTB 2	3335 (26%)	658 (4%)	3207 (20%)
CTB progenitor-like	1038 (8%)	4917 (32%)	833 (5%)
CTB cycling 1	2257 (18%)	2259 (15%)	2164 (14%)
SCT progenitor	1888 (15%)	228 (1%)	3682 (23%)
SCT 2	1386 (11%)	254 (1%)	2582 (16%)
CTB progenitor stressed	573 (4%)	3826 (25%)	485 (3%)
CTB cycling 2	674 (5%)	1370 (9%)	672 (4%)
CTB 1	816 (6%)	555 (4%)	1202 (8%)
CTB progenitor	604 (5%)	1385 (9%)	502 (3%)
SCT 1	245 (2%)	57 (0%)	572 (4%)

b



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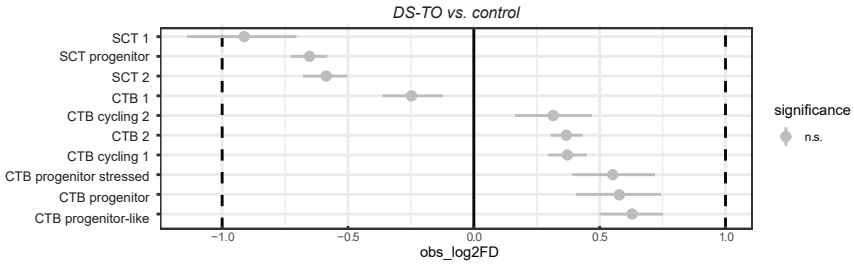
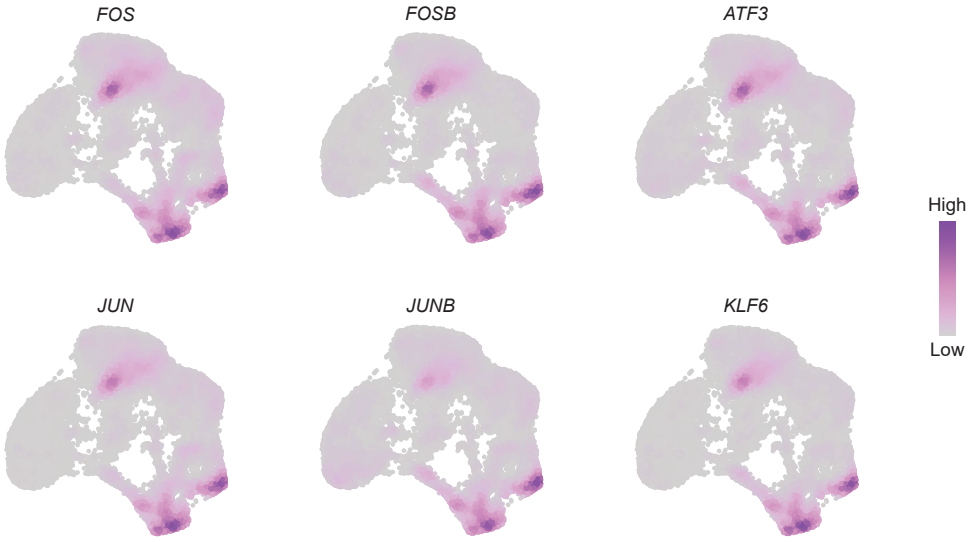
Supplementary Figure S4