A genome-wide CRISPR-Cas9 knockout screen identifies essential and growth-restricting genes in human trophoblast stem cells

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The recent derivation of human trophoblast stem cells (hTSCs) provides a scalable in vitro model system of human placental development, but the molecular regulators of hTSC identity have not been systematically explored thus far. Here, we utilize a genome-wide CRISPR-Cas9 knockout screen to comprehensively identify essential and growth-restricting genes in hTSCs. By cross-referencing our data to those from similar genetic screens performed in other cell types, as well as gene expression data from early human embryos, we define hTSC-specific and -enriched regulators. These include both well-established and previously uncharacterized trophoblast regulators, such as ARID3A, GATA2, and TEAD1 (essential), and GCM1, PTPN14, and TET2 (growth-restricting). Integrated analysis of chromatin accessibility, gene expression, and genome-wide location data reveals that the transcription factor TEAD1 regulates the expression of many trophoblast regulators in hTSCs. In the absence of TEAD1, hTSCs fail to complete faithful differentiation into extravillous trophoblast (EVT) cells and instead show a bias towards syncytiotrophoblast (STB) differentiation, thus indicating that this transcription factor safeguards the bipotent lineage potential of hTSCs. Overall, our study provides a valuable resource for dissecting the molecular regulation of human placental development and diseases.
The placenta is a transient organ containing trophoblast cells derived from the trophoderm (TE) of the early embryo. It performs several critical functions throughout gestation, including facilitating maternal-fetal exchanges, remodeling maternal spiral arteries, secreting pregnancy-related hormones, and acting as a protective barrier. Indeed, trophoblast defects can lead to pathologies such as miscarriage, preeclampsia, and intrauterine growth restrictions. However, the placenta also remains one of the least understood organs. Recently, a culture condition that allows for the derivation and maintenance of bona fide hTSCs from the blastocyst or first trimester placenta was developed, which provides an invaluable tool to study human trophoblast development. Subsequent studies from our laboratory and others showed that hTSCs with similar biological and molecular properties can also be derived from naïve hPSCs or somatic cells via direct reprogramming. Nevertheless, efforts to systematically identify crucial regulators of hTSCs have so far been lacking.

Here, we employ a pooled genome-wide CRISPR-Cas9 knockout screen, an increasingly powerful tool to uncover and characterize genes controlling a variety of biological processes, to comprehensively determine the essential genes (EGs) and growth-restricting genes (GRGs) of hTSCs. Using a combination of chromatin accessibility, gene expression, and genome-wide location analyses, we further show that one such regulator, the transcription factor TEAD1, plays major role during progressive stages of human trophoblast development. Overall, this work provides important insights into the regulation of hTSCs and will be a valuable resource for future studies of trophoblast biology.

Results and discussion
A genome-wide CRISPR-Cas9 knockout screen identifies hTSC EGs. We transduced the blastocyst-derived BT5 hTSC line with the Brunello human CRISPR knockout pooled lentiviral library, which contains 76,441 single guide RNAs (sgRNAs) targeting 19,114 genes. Following puromycin selection, the cells were cultured and passaged for 18 days, with genomic DNA samples harvested for deep sequencing at days 0, 6, 12, and 18. Two replicates with independent transductions showed good reproducibility (Supplementary Fig. 1a) and the identity of the hTSCs at day 18 was confirmed by homogeneous expression of the hTSC-specific surface markers EGFR and ITGA6 (Fig. 1b and Supplementary Fig. 1b). We analyzed the enrichment of sgRNAs targeting reference essential and non-essential genes and calculated a precision-recall curve, which indicated that the screen showed excellent performance (Fig. 1c and Supplementary Fig. 1c, d). Furthermore, analysis at intermediate timepoints revealed the lack of a bottlenecks effect (Supplementary Fig. 1a and Supplementary Data 1). In total, the screen identified 2139 EGs (Fig. 1d, Supplementary Fig. 1e, and Supplementary Data 1).

To confirm the specificity of our screen, we plotted the mean normalized sgRNA read counts of all EGs over time together with Bayesian Factor (BF), a classifier of gene essentiality. As expected, the mean normalized sgRNA reads of all EGs steadily declined while their BF scores increased. In contrast, the gRNA enrichment and BF scores of neighboring genes remained unchanged (Fig. 1e). Amongst the hTSC EGs were several well-established trophoblast regulators, such as TAFAP2C, USP47, and TSC22D2 (Fig. 1e and Supplementary Fig. 3a). Biological processes and pathways enriched among the hTSC EGs included WNT, EGF, and histone acetylation (Fig. 1f and Supplementary Data 2), all of which are modulated by inhibitors or growth factors contained within hTSC medium. We also detected enrichment in components of mTOR signaling and the pentose phosphate pathway (Supplementary Fig. 3b and Supplementary Data 2), which were previously implicated in mouse TE specification.

Consistent with their role in promoting hTSC fitness, EGs identified in our screen were expressed much more abundantly than nonessential genes in hTSCs (Supplementary Fig. 3c). We performed principal component analysis (PCA) using published RNA-sequencing (RNA-seq) data from naïve and primed hPSCs and various trophoblast cell types and found that hTSC EGs (n = 2139) and essential TFs (n = 124) accurately demarcated pluripotent and trophoblast identities, despite representing only minor fractions of all genes (Fig. 1g, Supplementary Fig. 3d, e, and Supplementary Data 1). We also compared the expression of hTSC EGs in different trophoblast cell types at 10 and 12 days post fertilization (d.p.f.) in 3D human embryos cultured through implantation stages. According to our prior analysis, these are the stages of early trophoblast development to which hTSCs most closely correspond. At both timepoints, the hTSC EGs were statistically significantly upregulated in the cytotrophoblast (CTB) relative to the terminally differentiated extravillous trophoblasts (EVT) and syncytiotrophoblasts (STB) (Supplementary Fig. 3f, g). Similar expression patterns of hTSC EGs could be observed in trophoblast cells derived in vitro (Supplementary Fig. 3h). These transcriptional data further support the notion that hTSC EGs are central to the identity of hTSCs.

Identification and characterization of hTSC-specific EGs. To better define regulators specific to hTSCs, we referenced our hTSC EGs to core EGs that largely contain common housekeeping genes, as well as EGs identified through genetic screens in primed hPSCs (Fig. 2a). Since hTSCs and primed hPSCs correspond to post-implantation CTB and epiblast (EPI), respectively, we reasoned that such a comparison may help identify essential regulators unique to these extraembryonic stem cells. While the hTSC EGs overlapped considerably with the core and especially primed hPSC EGs, we also identified 872 EGs specific to hTSCs (Fig. 2a and Supplementary Data 1), which were mainly localized to the nucleus and cytosol (Supplementary Fig. 4a). Pathways enriched in EGs shared between hTSCs and other cell types included housekeeping functions such as ribosome and spliceosome (Fig. 2b and Supplementary Data 2), while those unique to hTSC-specific EGs included HDAC and TNF signaling pathways (Fig. 2c and Supplementary Data 2). We then sought to further distinguish hTSC-specific regulators based on gene expression in the 10 and 12 d.p.f. human embryo, since genes with increased expression in CTBs relative to other embryonic and extraembryonic lineages more likely represent critical regulators of the trophoblast lineage in vivo (Fig. 2d, Supplementary Fig. 4b, and Supplementary Data 1). These genes included known trophoblast regulators such as ARID3A, CTNNBI, GATA2, SKP2, as well as genes like ARID5B, TCAF1, and TEAD1, which have not previously been associated with trophoblast biology (Fig. 2e and Supplementary Fig. 4c). Screening data from intermediate timepoints showed that while core EGs became essential early in the screen, EGs more relevant to hTSC biology achieved essentiality at later time points (Supplementary Fig. 4d and Supplementary Data 1). We then validated the essentiality of SKP2, TEAD1, and TCAF1 via proliferation assays in hTSCs derived from H9 naïve hPSCs to ensure reproducibility in a different genetic background than the one used for the screen. hTSCs transduced with targeting sgRNAs displayed significantly fewer cells compared to cells transduced with non-targeting sgRNAs (Fig. 2f–h and Supplementary Fig. 4e, f). For most of these targets, the fraction of sgRNA-transduced cells that contain frameshift mutations declined over time, which...
indicates that they were outcompeted by cells that are wild-type or contain in-frame mutations. These results demonstrate that our screen has identified EGs that are functionally relevant for the maintenance of hTSCs.

Investigating the role of TEAD1 in hTSC specification and maintenance. We were particularly intrigued by the hTSC-specific essential regulator TEAD1. While its paralog TEAD4 has been shown to be instrumental for mouse and human TE specification, TEAD1 was reportedly dispensable for mouse placentation. However, TEAD1 ranked more highly (#129) in our screen for hTSC EGs compared to TEAD4 (#1407), and its downregulation has been associated with recurrent spontaneous abortion, suggesting that it may have a human-specific role in trophoblast regulation. To explore this hypothesis, we utilized CRISPR-Cas9-mediated genome editing to generate stable TEAD1 homozygous knockout (KO) cell lines in the H9 primed hPSC background (Fig. 2i). We then reset wildtype (WT) control and TEAD1 KO primed hPSCs to the naïve state using our chemically defined conditions (Fig. 2i). The removal of TEAD1 did not impair the ability to generate naïve hPSCs as evidenced by flow cytometry analysis for the naïve-specific cell surface markers CD75 and SUSD2 (Supplementary Fig. 5a). However, when we assayed the ability of these different genotypes to give rise to hTSCs (Fig. 2i), TEAD1 KO cells displayed significantly impaired proliferation (Supplementary Fig. 5b–c). Analysis of hTSC specification dynamics by flow cytometry revealed that TEAD1 KO cells adopt an hTSC identity with reduced kinetics compared to WT cells early in the derivation process (Supplementary Fig. 5d), although stable TEAD1 KO hTSC cell lines can still be obtained (Fig. 2i, k and Supplementary Fig. 5d). These findings indicate that TEAD1 promotes the efficient derivation of hTSCs from the naïve state.

Fig. 1 A genome-wide CRISPR-Cas9 knockout screen identifies hTSC EGs. a The experimental scheme of the CRISPR screen. b Flow cytometry analysis for hTSC markers ITGA6 and EGFR in B7 hTSCs following the CRISPR screen endpoint. c Fold change distribution of sgRNAs targeting core essential and nonessential genes at day 18 of the screen. Note that the Log2 fold change (L2FC) of the core EGs gradually decreased relative to day 6 and day 12, while the L2FC of the nonessential genes largely remained unchanged across different timepoints. d Percentage of essential genes (EGs) identified among all targeted genes. The mean of sgRNA normalized read counts and Bayes Factors (BFs) of all or selected hTSC EGs and their neighboring up- and downstream genes over time. The results are representative of two independently transduced screening experiments. f Selected GO Biological Processes terms that are significantly enriched among all hTSC EGs. g PCA featuring published AN and H9 primed hPSCs, naïve hPSCs, naïve hTSCs, EVT, and STB RNA-seq samples using the gene expression data of hTSC essential transcription factors.
We proceeded to investigate the consequences of the loss of TEAD1 in hTSCs. TEAD1 KO hTSCs had slightly increased G0/G1 phase and decreased S phase populations compared to WT hTSCs based on cell cycle analysis (Supplementary Fig. 5e), although levels of Annexin V-positive apoptotic cells were similar between the two genotypes (Supplementary Fig. 5f). We then performed RNA-seq on WT and TEAD1 KO hTSCs to characterize their global gene expression changes. There were 162 genes significantly downregulated in TEAD1 KO hTSCs (p-adjust < 0.05) (Fig. 2l), including many trophoblast regulators such as FOS, FN1, ITGB8, MET, and LOXL2. Genes downregulated in TEAD1 KO hTSCs were enriched for GO terms such as integrin-mediated signaling pathway, regulation of TGF-β production, and regulation of cell motility (Fig. 2l, m).
Interestingly, a large number of genes downregulated in TEAD1 KO hTSCs are known to promote EVT differentiation; in fact, the EVT marker HLA-G was the most downregulated gene (Fig. 2l). On the other hand, there were 251 genes significantly upregulated in TEAD1 KO hTSCs (p-adjust < 0.05), including the trophoblast regulators AMOTL2, TET2, and CITED2 (Fig. 2l). Many STB marker genes such as CGB2, CGB7, and PSG11 were also upregulated in TEAD1 KO hTSCs (Fig. 2l). GO analysis revealed that genes upregulated in KO were enriched for terms such as response to hormone, cell adhesion, and apoptosis (Fig. 2m). We also used the Assay for Transposable Accessible Chromatin with high-throughput sequencing (ATAC-seq) to compare the chromatin accessibility landscape in TEAD1 WT and KO hTSCs. There were 3988 and 4116 peaks that significantly differed between WT and KO hTSCs (Supplementary Fig. 6f, g), suggesting that TEAD1 helps to specify and regulate hTSCs by activating CTB-specific transcriptional programs. However, TEAD1 target genes were not biased toward up- or downregulation in EVT and WT hTSCs (Fig. 3l and Supplementary Fig. 6h), which may point to an additional role for TEAD1 during differentiation into the EVT lineage. Finally, to uncover the direct regulation of gene expression and chromatin accessibility by TEAD1, we referenced group 1 and 2 TEAD1 CUT&Tag targets to differentially expressed genes (DEGs) and differentially accessible regions (DARs) between WT and TEAD1 KO hTSCs. Integration with RNA-seq data indicated that 26 and 34 TEAD1 target genes were down- and upregulated in TEAD1 KO hTSCs, respectively (Supplementary Fig. 6i). For example, the direct TEAD1 targets FOXG, MYLK3, and SERPINB7 were downregulated in TEAD1 KO hTSCs, while AMOTL2 and DDIT3 were upregulated in TEAD1 KO hTSCs (Fig. 3m). Meanwhile, integration with ATAC-seq data indicated that 141 and 14 TEAD1 targets displayed reduced or increased chromatin accessibility in TEAD1 KO hTSCs, respectively (Supplementary Fig. 6i). For example, chromatin accessibility was reduced at TEAD1 binding sites within the POL4, MYLK3, and SERPINB7 loci, and increased at a TEAD1 binding site within the NARRP locus (Fig. 3n). Collectively, these data indicate that TEAD1 maintains hTSC identity by regulating proper cell cycling, modulating key trophoblast regulators, and suppressing the STB program via direct or indirect mechanisms.

Investigating the role of TEAD1 in EVT and STB differentiation. Given the differential RNA-seq and ATAC-seq signals of EVT- and STB-associated genes in WT and TEAD1 KO hTSCs (Fig. 2l–o) and the expression levels of TEAD1 and its target genes in different trophoblast cell types (Fig. 3a, j–l, and...
Supplementary Fig. 6f–h), we hypothesized that TEAD1 may promote the differentiation of hTSCs into the EVT lineage, but present a barrier to STB differentiation (Fig. 4a). To test this hypothesis, we first performed lineage-directed EVT differentiation on WT and TEAD1 KO hTSCs by treating the cells with Neuregulin (NRG1) and the TGF-β inhibitor A83-01 in the presence of Matrigel. Unlike WT cells, TEAD1 KO cells subjected to this EVT differentiation protocol failed to acquire the typical spindle-like EVT morphology and were far less invasive in a Matrigel-coated transwell assay (Fig. 4b, c).

We profiled the transcriptomes of WT and TEAD1 KO samples by RNA-seq and all samples were clearly separated by cell type and genotype in the expression of TEAD1 (Fig. 4d, e). We then performed Cut&Tag to identify the genomic regions occupied by TEAD1 in WT and TEAD1 KO hTSCs (Fig. 4f, g). We found that TEAD1 binding sites were located closer to neighboring genes in WT hTSCs compared to TEAD1 KO hTSCs (Fig. 4h).

We also identified TEAD1 target genes by analyzing the expression of genes that were differentially expressed between WT and TEAD1 KO hTSCs (Fig. 4i). We found that many of these genes were related to placenta development, Wnt signaling, and establishment or maintenance of cell polarity (Fig. 4j, k, l). These findings suggest that TEAD1 may play a key role in regulating the differentiation of hTSCs into the EVT lineage.
Fig. 3 Investigation of TEAD1 targets in hTSCs. a TEAD1 gene expression in published AN and H9 pluripotent and trophoblast cell types10. β Immunofluorescence staining for TEAD1 in H9 hTSCs. The scale bars indicate 75 μm. The experiment was performed once. c Selected TF binding motifs significantly enriched among TEAD1 CUT&Tag peaks and their p-values according to HOMER Motif Analysis. d TEAD1 CUT&Tag peaks overlapping with naïve hPSC or hTSC ATAC-seq peaks were categorized into three groups: those overlapping with hTSC-specific open chromatin regions (OCRs) (group 1), shared OCRs (group 2), or naïve hPSC-specific OCRs (group 3). e Top GO biological processes significantly enriched among group 1 (blue) and group 2 (gray) TEAD1 CUT&Tag peaks and their p-values. f The AN and H9 hTSC ATAC-seq signal over group 1-3 TEAD1 CUT&Tag peaks50. g The AN and H9 naïve hPSC ATAC-seq signal over groups 1-3 TEAD1 CUT&Tag peaks10. h CUT&Tag and hTSC ATAC-seq signal of group 1-3 TEAD1 CUT&Tag peaks. i Expression of genes in H9 hTSCs10, binned by the distance of TEAD1 CUT&Tag peaks to TSS. Two independent samples were used for analysis. Boxplot presents the 25th, median, and 75th quartiles, the whiskers extend 1.5 of interquartile ranges, and the dots are outside values >1.5 times and <3 times the interquartile range beyond either end of the box. j-l Percentage of all genes, all TEAD1 target genes, group 1 TEAD1 target genes, and group 2 (excluding those already in group 1) TEAD1 target genes that are significantly up- or downregulated in AN and H9 hTSCs vs. naïve hPSCs (j), hTSCs vs. STBs (k), hTSCs vs. EVTs (l)10. m TEAD1 CUT&Tag and WT and TEAD1 KO hTSC RNA-seq data shown in the vicinity of selected genes. n TEAD1 CUT&Tag and WT and TEAD1 KO hTSC ATAC-seq data shown in the vicinity of selected genes.

PCA (Fig. 4d). TEAD1 KO “EVTs” displayed significant down-regulation of 1,715 genes and upregulation of 1,328 genes compared to WT EVTs (p-adjust < 0.05) (Fig. 4e). Downregulated DEGs included key EVT markers such as ASCL2, ITGA5, and HLA-G (Fig. 4e), and were enriched for GO terms like cytoskeleton organization, cell projection organization, and cell migration (Fig. 4f). The expression of EVT-specific genes reported by the human embryo scRNA-seq data37 was also significantly lower in TEAD1 KO compared to WT EVTs (Fig. 4g). On the contrary, DEGs that were upregulated in TEAD1 KO EVTs consisted of a large number of STB regulators such as CGA, CGB, and ERVW-J, and were enriched for GO terms such as organic acid transport, neutral amino acid transport, and secretion, which are typically associated with the function of STBs in mediating maternal-fetal exchange (Fig. 4e, f). We also profiled the chromatin accessibility landscapes of WT and TEAD1 KO EVTs via ATAC-seq. All samples were again clearly separated by cell type and genotype in the PCA (Fig. 4b). There were 24,675 and 10,026 loci with decreased or increased ATAC-seq signal in KO hTSC and WT and KO hTSC ATAC-seq data shown in the vicinity of selected genes. These findings highlight the crucial role of Hippo signaling in regulating hTSCs.

Identification and characterization of hTSC GRGs. Since the failure to restrict cellular growth often leads to diseases such as cancer, identification of GRGs is equally important for understanding regulatory mechanisms in hTSCs, especially given their potential relevance to chorioangiocarcinoma74. Our screen identified 619 GRGs in hTSCs (Fig. 5a, Supplementary Fig. 8a, and Supplementary Data 3), which mainly showed nuclear and cytosolic localizations (Supplementary Fig. 8b). The mean normalized read counts and log2 fold changes of all sgRNAs targeting GRGs steadily increased throughout the screen, while those targeting neighboring genes remained unchanged (Fig. 5b). Among the GRGs in hTSCs were GCM1, which encodes a transcription factor involved in STB differentiation73,74, and TGFBI, which is induced by TGFβ signaling and encodes a secreted extracellular matrix protein75. Consistent with the growth-restricting function of TGFβ in hTSCs, pharmacological inhibition of TGFβ signaling is integral to hTSC derivation and maintenance74. Other GRGs included the genes encoding the phosphatase PTPN14, the 5-methylcytosine hydroxylase TET2, and the orphan receptor NR6A1 (Supplementary Fig. 8c). hTSC GRGs were the most enriched in pathways including negative regulation of cyclin-dependent kinases and negative regulation of Hippo signaling (that prevent YAP/TAZ nuclear translocation), such as SAV1, LAT51, AMOTL2, and AJUBA (Fig. 5c and Supplementary Data 2). These findings highlight the crucial role of Hippo signaling in regulating hTSCs. We validated the growth-restricting bisphosphate biosynthetic process, response to organic cyclic compound, and in utero embryonic development (Supplementary Fig. 7c). Conversely, genes significantly downregulated in TEAD1 KO STBs were enriched for GO terms related to cytokine response and regulation of cell death (Supplementary Fig. 7c). We also performed ATAC-seq on these cells, which revealed 19,629 and 29,025 DARs that became significantly more closed or open in TEAD1 KO STBs, respectively. Open DARs were enriched for glucose transport, and closed DARs were enriched for regulation of gonadotropin secretion (Supplementary Fig. 7d). We again found that DARs were correlated to gene expression changes for a number of key trophoblast regulators (Supplementary Fig. 7e). Genes that gained ATAC-seq and RNA-seq signals in TEAD1 KO STBs included STB-specific genes such as FIBCD137, while those that lost ATAC-seq and RNA-seq signals included hTSC-specific genes like ITGA234, KRT1733, and IFITM233 (Supplementary Fig. 7e). These data suggest that TEAD1 indeed acts as a barrier for STB differentiation and that TEAD1 KO STBs are potentially more mature than WT STBs. Taken together, we postulate that TEAD1 performs a variety of functions during the induction, maintenance, and differentiation of hTSCs by modulating the expression and chromatin accessibility of target trophoblast regulators.
Fig. 4 Investigating the role of TEAD1 in EVT differentiation. a Experimental scheme to assess the requirement of TEAD1 during EVT and STB differentiation. b Phase contrast images of H9 WT EVTs and TEAD1 KO hTSCs that have undergone EVT differentiation. The scale bars indicate 75 μm. The images are representative of four independent experiments. c The relative number of invading cells following Matrigel invasion assay of H9 WT EVTs and TEAD1 KO hTSCs that have undergone EVT differentiation. Error bars indicate ±1 standard error of ve technical replicates. The center of the error bar indicates the mean. One-tailed student’s t test was used for statistical analysis. Significance is indicated by *p < 0.05. The exact p-values were 0.028856521 (#1), 0.020949253 (#2), and 0.029918938 (#3). d Principal component analysis (PCA) of WT and TEAD1 KO hTSCs, EVTs, and STBs based on RNA-seq data. e Scatter plot showing the differential gene expression analysis between H9 WT and EVTs KO. WT contains two RNA-seq samples each from three independent clones. Two-tailed Wilcoxon Rank Sum Test was used for statistical analysis. Boxplot presents the 25th, median, and 75th quartiles, the whiskers extend 1.5 of interquartile ranges. f Genes that were significantly upregulated or downregulated in TEAD1 KO “EVTS” relative to WT EVTs. g The expression of EVT-specific genes (n = 462) in H9 WT and TEAD1 KO EVTs. WT contains two RNA-seq samples, and TEAD1 KO contains two RNA-seq samples each from three independent clones. Two-tailed Wilcoxon Rank Sum Test was used for statistical analysis. h Scatter plot showing the differential gene expression analysis between H9 WT and EVTs KO. WT contains two RNA-seq samples each from three independent clones. Two-tailed Wilcoxon Rank Sum Test was used for statistical analysis. i Selected GO biological processes that are enriched among DEGs significantly upregulated or downregulated in TEAD1 KO “EVTS” relative to WT EVTs. j WD and TEAD1 KO EVTs. WT contains two RNA-seq samples, and TEAD1 KO contains two RNA-seq samples each from three independent clones. Two-tailed Wilcoxon Rank Sum Test was used for statistical analysis. k Principal component analysis (PCA) of WT and TEAD1 KO hTSCs, EVTs, and STBs based on ATAC-seq data. l Selected GO biological processes that are enriched among ATAC-seq data shown in the vicinity of selected genes. m Levels of WT hTSC and WT and TEAD1 KO EVT ATAC-seq signal over regions that are specifically open in WT EVTs. Each condition contains two ATAC-seq samples.

function of two of the GRGs, PTPN14 and TET2, via proliferation assays in H9 hTSCs following sgRNA transduction (Fig. 5d and Supplementary Fig. 8d, e). As expected, the fraction of sgRNA-transduced cells bearing frameshift mutations in these target genes increased over time. It has been reported that PTPN14 directly inhibits YAP in cancer cells in a density-dependent manner79–81. Interestingly, TET2 reportedly cooperates with TET1 to maintain mTSC self-renewal82, suggesting a potentially disparate role in mouse versus human trophoblast.

We proceeded to uncover GRGs that have unique expression patterns in CTBs relative to other lineages in human embryos cultured through implantation stages37. First, we identified GRGs uniquely upregulated in CTBs at 10 and 12 d.p.f., which represents the stage of in vivo trophoblast development most closely aligned with hTSCs (Fig. 5e, Supplementary Fig. 8f, and Supplementary Data 3). These included the genes encoding GCM1,75,76, the mTSC cell cycle modulators TET2 and E2F8,56,82, the negative Hippo regulator AJUBA, and the negative WNT regulator AXIN2 (Fig. 5f and Supplementary Fig. 8g). We also identified GRGs uniquely depleted in CTBs at 10 or 12 d.p.f. (Fig. 5g, Supplementary Fig. 8h, and Supplementary Data 3), such as NODAL, TBX3, and MAPKAPK3 (Fig. 5h). The protein products of these genes include important components of the pluripotency network83–85, although TBX3 has also been
implicated in human STB development\textsuperscript{86}. CTB-depleted GRGs also included genes encoding the negative Hippo regulators SAV1 and NF2 (Fig. 5h), which ensure ICM specification and prevent ectopic TE formation\textsuperscript{87}. We found that hTSC GRGs mostly belonged to three different categories. The first were primarily housekeeping genes, exemplified by the enrichment of cell-cycle related pathways (Fig. 5c). The second category of GRGs included genes such as AJUBA and AXIN2 that antagonize pathways essential for hTSCs. The third included GRGs that promote other cellular identities, for instance, GCM1 (STB) and NODAL (EPI). Hence, two key functions of hTSC GRGs are to prevent the over-proliferation of hTSCs and the ectopic activation of hTSC-specific

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**Fig. 5 Identification and characterization of hTSC GRGs.** a Growth-restricting genes (GRGs) identified among all targeted genes. b The mean of sgRNA normalized read counts and Log2 fold changes of all or selected hTSC GRGs and their neighboring up- and downstream genes over time. The results are representative of two independently transduced screening experiments. c Top 5 most enriched GO Biological Processes terms among all hTSC GRGs. d Top left, live cell counts relative to the mean of control sgRNA transduced H9 hTSCs 6 days after seeding. Error bar represents the standard error of three biological replicates. Two-tailed student’s t test was used for statistical analysis. "**" indicates a p-value < 0.05, "***" indicates a p-value < 0.01, and "****" indicates a p-value < 0.001. The exact p-values from left to right are 0.000870208, 0.005413584, 0.018757677, and 0.000646371. Rest of the panel, phase contrast images of H9 naïve hTSCs transduced with control or targeting sgRNAs at 6 days following seeding. The scale bars indicate 75 μm. The images are representative of three independent replicates in an experiment. e Heatmap showing the expression of 50 CTB-enriched GRGs in the CTB, EPI, and PrE of published human embryo scRNA-seq data\textsuperscript{37}. f Dot plot showing the expression of selected CTB-enriched GRGs in the CTB, EPI, and PrE of published human embryo scRNA-seq data\textsuperscript{37}. g Heatmap showing the expression of 33 CTB-depleted GRGs in the CTB, EPI, and PrE of published 10 d.p.f. human embryo scRNA-seq data\textsuperscript{37}. h Dot plot showing the expression of selected CTB-depleted GRGs in the CTB, EPI, and PrE of published human embryo scRNA-seq data\textsuperscript{37}.
transcriptional programs in other cell types. Overall, our analysis revealed that hTSC GRGs are a diverse group of genes that together ensure the balanced development of the early human embryo.

Analysis of hTSC EGs and GRGs implicated in mouse placentation. It is well-established that given the evolutionary diversity of the mammalian placenta, animal models inadequately recapitulate human placental development. Therefore, we anticipated that few hTSC EGs and GRGs would be conserved regulators of mouse placentation. To address this question, we compared hTSC EGs and GRGs with regulators of mouse placentation identified by a large-scale analysis of mouse gene knockouts that cause intrauterine lethality. Of the 61 genes that led to mouse placentation defects, 18 were identified as EGs and 2 as GRGs in hTSCs in this study. We found that these 20 conserved placental regulators were expressed more highly in villous cytotrophoblast (VCT or CTB) than in other placental, decidual, or immune cell types in scRNA-seq data sampled from the human maternal-fetal interface. These 20 conserved regulators include TRAF2, which encodes a member of the TNF signaling pathway that promotes the activation of NF-κB and MAPK pathways downstream, as well as multiple mitochondrial genes such as TRUB2, TIMMDC1, CRLS1, DHCP7, and DHODH.

Fig. 6 Comparison of hTSC EGs and GRGs with genes required for mouse placentation. a Overlap of hTSC EGs and GRGs with a published list of embryonic lethal genes known to cause placental defects in mouse. b Expression of genes that lead to mouse placental defects and overlap with hTSC EG/GRG lists in different cell types found in published human maternal-fetal interface scRNA-seq dataset. Cell types were ranked based on mean gene expression. Boxplot presents the 25th, median, and 75th quartiles, the whiskers extend 1.5 of interquartile ranges, and the dots are outside values >1.5 times and <3 times the interquartile range beyond either end of the box. c Expression visualization of selected mouse-human conserved regulators in the human maternal-fetal interface scRNA-seq dataset. d Expression of genes required for mouse placentation that do not and do overlap with hTSC EGs and GRGs in the VCTs (CTBs), EVTs, and SCTs (STBs) of published human maternal-fetal interface scRNA-seq dataset. Two-tailed Wilcoxon Rank Sum Test was used for statistical analysis. * indicates a p-value < 0.05 and ** indicates a p-value < 0.01. The exact p-values are 0.011 (VCT), 0.047 (EVT), and 0.0033 (SCT). VCT: 9479 cells; EVT: 3626 cells; SCT: 1261 cells.
Supplementary Fig. 9a), highlighting the importance of mitochondrial function and oxidative phosphorylation in trophoblast development.\textsuperscript{19} Several other conserved regulators with strong enrichment in trophoblast cells include SQUAL, SMG9, VIRMA, WRAP53, NHLRC2, and GPATCH1 (Fig. 6c and Supplementary Fig. 9a). We also confirmed our findings by comparing our hTSC regulators to an independent list of mouse placenta regulators curated by the Jackson Laboratory (Supplementary Fig. 9c–e).\textsuperscript{100} Lastly, we observed a sizable overlap between hTSC EGs/GRGs and genes associated with pregnancy-related diseases (Supplementary Fig. 9f, g),\textsuperscript{101,102} which suggests that hTSCs could provide a powerful cellular model system to study trophoblast-related pathologies.

In conclusion, we present the findings from a genome-wide CRISPR-Cas9 knockout screen in hTSCs. By identifying and characterizing hTSC EGs and GRGs, we systematically uncovered potential regulators of the hTSC state. Genetic deletion studies indicated that the removal of one such regulator, TEAD1, impaired the generation and maintenance of hTSCs and prevented the faithful differentiation of hTSCs into the invasive EVT lineage. Furthermore, integrated analysis of ATAC-seq, CUT&Tag, and RNA-seq data demonstrated that TEAD1 regulates the gene expression and chromatin accessibility of many trophoblast regulators in hTSCs, suggesting that this transcription factor plays an important role during progressive stages of human trophoblast development. The hTSC EGs and GRGs also overlapped with pregnancy disease-associated genes and regulators of placentation during mouse development in vivo, which provides a framework for elucidating conserved and species-specific regulators of trophoblast development. Overall, our study provides a valuable resource for future research into the genetic circuitry controlling placental development and disease.

Methods

Ethics statement. All human embryonic stem cell experiments performed in this study have been approved by Embyronic Stem Cell Research Oversight Committee at Washington University School of Medicine.

Culture of hTSCs. hTSCs were cultured as previously described.\textsuperscript{8,10} Prior to seeding, 6-well plates were coated with 5 μg/mL Collagen IV (Corning, 354233) at 37 °C overnight. Cells were cultured in 2 mL hTSC medium [DMEM/F12 ( Gibco, 11320) supplemented with 0.1 mM 2-mercaptoethanol (Millipore, S85740), 0.2% FBS (Millipore Sigma, ES-009-B), 0.5% Penicillin-Streptomycin (Gibco, 15140-045) and 0.03% β-mercaptoethanol] in a humidified CO2 incubator (5% CO2 and 20% O2) at 37 °C. On the following day, the cells were cultured in hTSC medium in T150 flasks (Corning, 51800) in 10% CO2 and 5% O2. The cell culture is regularly tested and confirmed negative for mycoplasma contamination.

To increase the growth rate, control and TEAD1 KO naïve hPSCs were expanded in PXGLY medium [N2B27 supplemented with 1 μM PD0325901, 2 μM XAV939 (Selleckchem, S1180), 2 μM SB590885 (Tocris, 2352), 10 μM Y-27632, and 10 ng/mL human LIF]\textsuperscript{104} prior to hTSC derivation. Naïve hPSCs were cultured in 5% O2, 5% CO2. For primed-to-naïve hPSC conversion, 2.5 × 10⁵ single primed cells were seeded on a six-well plate with MEF feeder layer in 2 mL mTeSR1 supplemented with 10 μM Y-27632. Two days later, medium was switched to 5i/L/A. After 6 to 10 days of differentiation, the cells were expanded polyclonally using TrypLE Express on a MEF feeder layer. Tissue culture media were filtered using a 0.22 μm filter. Media were changed every 1–2 days. Naïve hPSCs before passage 10 were used for experiments. The cell culture is regularly tested and confirmed negative for mycoplasma contamination.

Derivation of hTSCs from naïve hPSCs. hTSCs were derived from naïve hPSCs according to our recently described methodology.\textsuperscript{8} Briefly, naïve hPSCs were single-cell dissociated using TrypLE Express. For EVT differentiation, six-well plates were coated with 1 μg/mL Collagen IV overnight. On day 0, 0.75 × 10⁶ hTSCs were seeded per well in 2 mL EVT basal medium [DMEM/F12 supplemented with 0.1 mM β-mercaptoethanol, 0.5% penicillin-streptomycin, 0.3% BSA, 1% ITS-X, 7.5 μM A83-01, 2.5 μM Y27632] supplemented with 4% KSR (Gibco, 10828) and 100 ng/mL NRG2 (Cell signaling, 52185C). Matrigel was added to a 2% final concentration shortly after resuspension in the hTSC medium. At day 3, the media were replaced with 2 mL EVT basal medium supplemented with 4% KSR, and Matrigel was added to a 0.5% final concentration. At day 6, the media were replaced with 2 mL EVT basal medium, and Matrigel was added to a 0.5% final concentration. At day 8, the cells were ready for downstream analysis.

For STB differentiation, 2.5 × 10⁵ hTSCs were seeded per well in 3 mL 3D STB medium [DMEM/F12 supplemented with 0.1 mM β-mercaptoethanol, 0.5% penicillin-streptomycin, 0.3% BSA, 1% ITS-X, 2.5 μM Y-27632, 50 ng/mL EGF, 2 μM Forskolin (Sigma-Aldrich, F9317), and 4% KSR] in an ultra-low attachment six-well plate. At day 3, another 3 mL of 3D STB medium was added per well. At day 6, the cells were transferred to a 15 mL tube and gravity sedimented for 15 min, and the cells at the bottom of the tube were collected and used for downstream analysis.

TEAD1 CRISPR targeting. Guide RNAs (gRNAs) aimed at introducing out-of-frame indels to trigger nonsense-mediated decay of TEAD1 were designed and validated in K562 cells by the Genome Engineering and iPSC center (GEIC) at Washington University in St. Louis. gRNAs were cloned into an AAV vector and delivered to N2B27 differentiated hPSCs. hPSCs were cultured in mTeSR1 supplemented with 5 μM A83-01, 2.5 μM Y-27632, 100 ng/mL EGF, 2 μM Forskolin (Sigma-Aldrich, F9317), and 4% KSR) for 10 days in 3D STB medium. For primed-to-naïve hPSC conversion, 2.5 × 10⁵ single primed cells were co-transfected with 5 μg/mL Collagen IV and cotransfected with 0.25 × 10⁶ cells were seeded on a six-well plate with MEF feeder layer and cultured in 2 mL naïve media for 1 day in 5% CO2 and 5% O2. On the following day, the cells were cultured in hTSC medium in 10% CO2 and 20% O2 and media were replaced every 2 days. Upon reaching 80–100% confluence, the cells were passed at a ratio of 1:2 to 1:4 to six-well plates pre-coated with 5 μg/mL Collagen IV, and cultured from this point on as regular hTSCs. Cell numbers were quantified at each passage to obtain the cumulative cell count for the TEAD1 knockout experiment. Trypan Blue (Invi trogen, T10282) was used to exclude dead cells.

EVT and STB differentiation from hTSCs. Differentiation of hTSCs into terminal cell types was performed as previously described,\textsuperscript{8} with minor modifications. Prior to differentiation, hTSCs were grown to about 80% confluence, and then single-cell dissociated using TrypLE Express. For EVT differentiation, six-well plates were coated with 1 μg/mL Collagen IV overnight. On day 0, 0.75 × 10⁶ hTSCs were seeded per well in 2 mL EVT basal medium [DMEM/F12 supplemented with 0.1 mM β-mercaptoethanol, 0.5% penicillin-streptomycin, 0.3% BSA, 1% ITS-X, 2.5 μM A83-01, 2.5 μM Y27632] supplemented with 4% KSR (Gibco, 10828) and 100 ng/mL NRG2 (Cell signaling, 52185C). Matrigel was added to a 2% final concentration shortly after resuspension in the hTSC medium. At day 3, the media were replaced with 2 mL EVT basal medium supplemented with 4% KSR, and Matrigel was added to a 0.5% final concentration. At day 6, the media were replaced with 2 mL EVT basal medium, and Matrigel was added to a 0.5% final concentration. At day 8, the cells were ready for downstream analysis.

For STB differentiation, 2.5 × 10⁵ hTSCs were seeded per well in 3 mL 3D STB medium [DMEM/F12 supplemented with 0.1 mM β-mercaptoethanol, 0.5% penicillin-streptomycin, 0.3% BSA, 1% ITS-X, 2.5 μM Y-27632, 50 ng/mL EGF, 2 μM Forskolin (Sigma-Aldrich, F9317), and 4% KSR] in an ultra-low attachment six-well plate. At day 3, another 3 mL of 3D STB medium was added per well. At day 6, the cells were transferred to a 15 mL tube and gravity sedimented for 15 min, and the cells at the bottom of the tube were collected and used for downstream analysis.

Cloning. Individual sgRNA lentiviral expression vector cloning was performed as previously described.\textsuperscript{11} Briefly, LentiTIRISPR v2 (Addgene, #52961) plasmid was digested with EcoRI restriction enzyme (Thermo Fisher, FD0454), dephosphorylated with FastAP (Thermo Fisher, EF0651), and gel purified. sgRNA oligos (sequence taken from the Brunello library and listed below) were phosphorylated with T4 PNK (NEB, M0010S) and annealed. The digested plasmid and annealed oligos were then ligated using Quick Ligase (NEB, M1801S) and transformed into One Shot STbl3 Chemically Competent cells (Thermo Scientific, C37330). Single colonies were then picked, and the identities of the clones were verified using Sanger sequencing.

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TEAD1 CRISPR KO gRNA 1 F
TEAD1 CRISPR KO gRNA 2 F
SKP2 CRISPR KO gRNA 1 F
SKP2 CRISPR KO gRNA 2 F
PTPN14 CRISPR KO gRNA 1 F
PTPN14 CRISPR KO gRNA 2 R
TET2 CRISPR KO gRNA 1 F
TET2 CRISPR KO gRNA 2 R
TEAD1 CRISPR KO gRNA 2 R
NKX4.2 CRISPR KO gRNA 1 R
NKX4.2 CRISPR KO gRNA 2 F
CRISPR screen analysis. Reads of every sgRNA in the Human CRISPR Knockout Pooled library (Brunello) were calculated by using the MAEGeCK count module (v0.2) [107]. The sgRNA reads were inputted into the BAGEL package (v9.1)[25] and “BAGEL-calc_foldchange” script was used to calculate the fold change (FC) at day 6, day 12, and day 18, against the baseline at day 0. Mean of the log2 Bayes Factors (BF), recall, precision, and FDR were further calculated following the manual instruction of the “BAGEL-py” package of the BAGEL2 package[99]. The core essential and nonessential gene lists required for BAGEL were obtained from previous studies[24,26]. EGs in CRISPR screening were defined as the depleted genes at day 18 compared to the day 0 baseline, with FDR < 0.05. 2159 genes with reliable levels of expression (RPMK > 0) in BT5 hTSCs were considered as GRGs in this study. GENCODE human gene annotation (v27, hg38) was used to determine the genomic coordinates of EGs and GRGs in the human genome, and to identify their upstream and downstream neighboring genes.

Individual candidate gene validation. A puromycin kill curve was performed on hTSCs derived from H9 naive hPSCs, and it was determined that 1 μg/mL was the minimal concentration required to kill all the cells. hTSCs were transduced with lentivirus expressing Cas9 and a single targeting (cloned as described above) or control (Addgene, #107402) sgRNA. One day after transduction, the media were replaced with puromycin-containing fresh hTSC medium. Two days after puromycin selection, the cells were dissociated and counted. For each condition, 50,000 cells were seeded per well in 6 wells. This is considered day 0 of the validation experiment, and media were replaced every 2 days. The total number of live cells in each well were counted on day 4 and/or day 6 of the experiment, with 3 wells (biological triplicates) counted at each time point. Trypan Blue was used to exclude dead cells. Two-tailed student’s t-test was used for statistical analysis. Additionally, cells were analyzed by NGs for presence of indels around the cut site at day 0, 6, 12, and 18 of the validation experiments.

Gene Ontology, pathway, and cellular compartment analysis. The Gene Ontology (GO) analysis and pathway analysis of EGs and GRGs were performed by using ToppFun[110]. Protein localization data from Subcellular Localization Database (http://compartment.senselab.org/About) [111] were used to determine the subcellular localization/compartments of identified EGs and GRGs. Gene-compartment assignment was determined by using the maximal confidence score method as described previously[25]. Genes that have the same maximal confidence scores in multiple compartments were assigned to each compartment independently. Hypergeometric test was further used to calculate the statistical significance of the enrichment/depletion of EGs and GRGs in each cellular compartment.

Re-analysis of published transcriptomic data. Gene-level expression data (FPKM) of the CTB, EVT, PrE, EPI, and STB were obtained from a published study (GEO accession: GSE36447) [37]. The expression of select EGs and GRGs was obtained to generate the expression heatmaps and dot-plots in distinct embryonic and extraembryonic cell types at different developmental stages.

Human-level RNA-seq expression data (reads counts) of cultured STB, EVT, and naive hPSC were downloaded from our prior study (accession: GSE138688)[20]. DEGs (hTSC vs naive hPSC, hTSC vs STB, hTSC vs EVT) were identified using DESeq2 with criteria as absolute log2FC > 1, p < 0.01, and FDR > 0.1 of libraries of either condition. To generate PCA plots, the genes with low expression (CPM < 1) in all libraries were removed to ensure high specificity. The transcription factors in EGs were confirmed by using the human TF list retrieved from Animal TFDB 3.0 (http://bioinfo.life.hust.edu.cn/AnimalTFDB)[112].

Gene-level single-cell RNA-seq data (FPKM) of human first trimester maternal-fetal interface were obtained through EMBL-EBI ArrayExpress (accession: EMTAB6678 and EMTAB6701)[91]. UMAP visualization of processed scRNA-seq data was generated as previously reported[26]. The expression of selected hTSC EGs and GRGs was obtained to generate boxplots and violin plots across distinct cell types.

Flow cytometry. Cells were single-cell dissociated using TrypLE Express and washed once in FACs buffer (PBS supplemented with 5% FBS). The cells were then resuspended in 100 μL fresh FACs buffer, and incubated with antibodies for 30 min on ice. The following antibodies were used: anti-SSUD2-PE, 1:100 (BioLegend, 500-0121-82); anti-CD75-eFluor 660, 1:100 (Thermo Fisher, 50-0759-42); anti-ITGA6-Alexa 488, 1:100 (Thermo Fisher, 50-0369-82); anti-CD117-PECy7, 1:100 (BioLegend, 553916). 

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incubation, the cells were washed once with FACS buffer, resuspended in fresh FACS buffer, and passed through a cell strainer. Unstained cells that have undetected gene expression were removed using RUVr function of RUVseq normalization. Cells were balanced and subject to SDS-PAGE analysis. Primary antibodies used were TEAD1 (1:100, Cell Signaling Technology, 12292S); guinea pig-anti-rabbit-ActA (1:400, Invitrogen, A-31573). The nuclei were stained with Hoechst 33258 (Thermo Fisher, H3569). Cells were washed 3 times in PBS and imaged with a Leica DMi-8 fluorescence microscope. Some images were globally adjusted for brightness and/or contrast.

**Western blotting.** Cells were harvested and resuspended in radioimmunoprecipitation assay (RIPA) buffer, then incubated on ice for 20 min. Whole cell extract concentration was measured by Bradford assay (Pierce, 23236). Proteins were balanced and subject to SDS-PAGE analysis. Primary antibodies used were TEAD1 (1:1000, Cell Signaling Technology, 12292S) and beta-actin (1:1000, Cell Signaling Technology, 4970). The membrane was imaged using the Thermo Fisher iBright Imaging System.

**Quantitative real-time PCR.** Total RNA was isolated using the E.Z.N.A. total RNA kit I (Omega, D6834), and cDNA synthesis was performed from total RNA using the high capacity cDNA reverse transcription kit (Applied Biosystems, 4388814). Real-time PCR was performed using PowerUp SYBR green master mix (Applied Biosystems, A25743) on the StepOnePlus Real-Time PCR System (Applied Biosystems). All analyses were done in triplicate. Gene expression was normalized to RPL9. Error bars represent the standard error (SE) of the mean of triplicate reactions. Two-tailed student’s t-test was performed for statistical analysis. Primer sequences are included in the following primer table:

| Gene | Primer sequence (5' → 3') |
|------|--------------------------|
| RPL9-F | GCTTCCGTGAGGCTTGCTCC |
| RPL9-R | CGTGGTTGAGAGGTGGTGA |
| SKP2-F | ATGCCAGAATCTGCTCCATCT |
| SKP2-R | CACGGACTGAGTAATGAGTT |
| TEAD1-F | CTCCTGATCTATCCACCATGGT |
| TEAD1-R | ATGGCAAGAACGAACACGTA |
| TCAF1-F | ATGAGGGCCAGCCTGTTAG |
| TCAF1-R | GGACCTTTGCAGGATGTTG |
| TPTN1-F | AAGGATCTGACCAAGT |
| TPTN1-R | TGTTCTTTGGTTCGTCCAC |
| TET2-F | CAAGCATCTGTTGAGTAC |
| TET2-R | TTTTCTTCTGTTGGCAAAGT |

**Matrigel invasion assay.** In vitro invasion assay was performed in Matrigel-coated transwell inserts with 8.0 μm pores (Corning, 354480). EVTs were single cell dissociated, and seeded at a density of 2 × 10³ cells per well into the upper chamber of Matrigel-coated transwells in 200 μL EVT basal medium. The lower chamber was filled with 800 μL of the same type of medium containing 20% FBS. Cells were cultured at 37 °C in 5% CO₂ and 20% O₂. After 36 h, cells in the upper chamber were carefully removed with a cotton swab. The lower chamber was fixed with 4% paraformaldehyde, washed with PBS, and then stained with crystal violet. Invaded cells were imaged on a Leica DMi8 microscope. Thereafter, the stained cells from five random fields were counted to calculate the relative fold change in the number of invading cells. One-tailed student’s t-test was performed for statistical analysis.

**RNA-seq.** Total RNA was isolated using the E.Z.N.A. total RNA kit I. Library construction was performed using the SMARTer Directional cDNA Library Construction Kit (Clontech, 634933). Libraries were sequenced on an Illumina NovaSeq 54 × 150 platform. The data were processed as previously described119. Raw reads of RNA-seq libraries were processed using the RNA-seq pipeline, which consists of data processing, quality control, integrative analysis, and data visualization. In this pipeline, RNA-seq reads were aligned to the human genome hg38 with STAR version 2.5.4h112. Gene counts were derived from the number of uniquely aligned unambiguous reads by Subread featureCount (version 1.4.6)114, with hg38 gene annotation ENCODE V2712. Unwanted variables among gene counts were removed using RUVseq normalization with the estimated number of factors of unwanted variation k as 1. Then the normalized gene counts were imported into the R/Bioconductor package DESeq2115. Genes with CPM > 1.0 were converted into a DESeq2 dataset and then regularized log-transformed using the log function from the DESeq2 package. Adjusted p-values for DEGs were determined by DESeq2 using the p.adj() function with the Benjamini and Hochberg correction to determine the false discovery rate. Absolute log2(fold change of expression change) > 0.5 and adjusted p-value < 0.05 were considered to require a gene as differentially expressed. Lists of WT vs. TEAD1 KO DEGs could be found in Supplementary Data 4.

**ATAC-seq.** ATAC-seq was performed as previously described with minor modifications116. Cells were harvested by TrypLE Express dissociation and centrifuged at 500 × g for 5 min at 4 °C. The supernatant was aspirated. Cells were washed once with cold PBS containing 0.04% BSA. Cell pellets of hTSCs and EVT were resuspended in 300 μL DNase I (Thermo Fisher, EN0521) solution [20 mM Tris pH 7.4, 150 mM NaCl, 1X reaction buffer with MgCl₂, 0.1 μL DNase I] on ice for 3 min. After DNase I treatment, 1 mL PBS containing 0.04% BSA was added and cells were centrifuged at 500 × g for 5 min at 4 °C. Another two washes were performed. Cell pellets of DNase I treated hTSC, EVT, and non-DNase I treated STB were then lyzed in 100 μL ATAC-seq RSB [10 mM Tris pH 7.4, 10 mM NaCl, 3 mM MgCl₂ containing 0.1% NPO, 0.1% Tween-20, and 0.01% Ditiontyn by protease inhibitors] and incubated on ice for 3 min at 4 °C, then washed with PBS 3 times. The nuclei were resuspended in 20 μL 2× TD buffer ([20 mM Tris pH 7.4, 0.5 mM MgCl₂, 0.01% Tween-20]) and then washed with PBS 3 times. The nuclei were counted, and 50,000 counted nuclei were then transferred to a tube with 2× TD buffer filled up to 25 μL. 25 μL of transposition mix [2.5 μL Transposase (100 nM final) (Illumina, 20034197), 16.5 μL PBS, 0.5 μL 1% Ditiontyn, 0.5 μL 10% Tween-20, 5 μL H2O] was then added. Transposition reactions were mixed and incubated at 37 °C for 30 min. After 37 °C centrifuged eq, every 0.5 mL of nuclear lysate were cleaned up with the Zymo DNA Clean and Concentrator-5 kit (Zymo Research, D4014). The ATAC-seq library was prepared by amplifying by 9 cycles on a PCR machine. The PCR reaction was purified with Ampure XP beads using double size selection following the manufacturer’s protocol, in which 2.5 μL beads (0.5XX sample volume) and 50 μL beads (0.1X sample volume) were selected by 0.5 μL PCR reaction. The ATAC-seq libraries were quantitated by Qubit assays and sequenced on an Illumina NexSeq 500 platform. Data analysis was performed on data generated in this study and ATAC-seq data that we previously generated from hTSCs derived from naïve hPSCs (GSE138761). The data were processed as previously described119. Raw reads of ATAC-seq libraries were aligned to human reference genome (hg38) and further processed by using upA119, which consists of four steps: data processing, quality control, integrative analysis, and data visualization. The raw peak files of all ATAC-seq libraries were further merged by using the merge function of BEDTools suite130, and the read counts of each ATAC-seq peak were quantified by using bamCoverage R package. Replicated peak of unique-specific and hPSC-specific DARs were identified by using DESeq2117 with the cutoff as p<5e−5 and absolute log2(fold change)>2 as previously described110. Batch effects among read counts under ATAC-seq peaks of the WT and TEAD1 KO hTSC, EVT, and STB samples were removed using RUVr function of R package, with the adapted mean value of unmerged reads on each sample k as 1. Cell type-specific DARs were identified by DESeq2 with the cutoff as p<0.01 and absolute log2(fold change)>0.1. TEAD1 KO-specific and WT-specific DARs were identified among hTSCs, EVTs and STBs using DESeq2 with the cutoff as p<0.05 and absolute log2(fold change)>0.5. GREAT tool (v4.04) was used to identify the enriched GO terms and pathways of the genes around TEAD1 KO-specific and WT-specific DARs.
an hour. The reaction was stopped and the DNA solubilized by adding 10 μl 0.5 M EDTA (Research Organics, 3002E), 3 μl 10% SDS (Sigma Aldrich, L4509), and 2.5 μl Protease K (Thermo Fisher, EO0492) per sample, and incubated at 50°C for an hour. The DNA was then extracted, excess RNA digested with RNase A (Thermo Fisher, EN0531), and PCR amplified with i5 and i7 indexing primers. The PCR product was cleaned up with AMPure XP beads, and the size distribution and concentration were confirmed using Tapestation. The libraries were then sequenced on an Illumina NovaSeq S4 2 x 150 platform.

TEAD1 CUT&Tag raw data of each biological replicate were processed with the human reference genome hg38 and further processed by using AIAP(v1.1)119, which consists of four steps: data processing, quality control, integrative analysis, and data visualization. The enriched TEAD1 peaks were identified by using MACS2 peak calling function with the q-value cutoff 1e−5. The peak files of all replicates were merged using the merge function of BEDTools suite. Only the TEAD1 peaks identified in at least 2 replicates were considered as high-quality binding sites and were further assigned to their nearest neighboring genes for the downstream analysis.

HOMER (v4.11.1)121 was used to calculate the motif enrichment (Supplementary Data 5) and genomic enrichment under the high-quality TEAD1 binding regions. GREAT (tool v4.0.4)122 was used to identify the enriched GO terms and pathways of the genes around TEAD1 binding peaks in human hTSCs. ATAC-seq signals on TEAD1 binding regions were visualized using deepTools123 with parameters detailed as “computeMatrix reference-point—referencePointCenter -a 5000 --b 5000 –bs 100 --missingDataAsZero”, and the averaged ATAC-seq signals were subsequently plotted by using plotHeatmap in deepTools package.

References

1. Shahbazi, M. N. & Zernicka-Goetz, M. Deconstructing and reconstructing the mouse and human early embryo. Nat. Cell Biol. 20, 878–887 (2018).
2. James, L. J., Carter, A. M. & Chamley, L. W. Human placentation from nidation to 5 weeks of gestation. Part I: What do we know about formative transient organ. Philos. Trans. R. Soc. B: Biol. Sci. 370, 20140066 (2015).
3. Burton Graham, J., & Fowden Abigail, L. The placenta: A multifaceted, transient organ. Philos. Trans. R. Soc. B: Biol. Sci. 370, 20140066 (2015).
4. Burton, G. J., Woods, A. W., Jauniaux, E. & Kingdom, J. C. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental flow during human pregnancy. Placenta 30, 473–482 (2009).
5. Norwitz, E. R. Defective implantation and placenta: Laying the blueprint for pregnancy complications. Reprod. BioMed. Online 13, 591–599 (2006).
6. Moffett, A. & Loke, C. Immunology of placentation in eutherian mammals. Nat. Rev. Immunol. 6, 584–594 (2006).
7. Koot, Y. E., Teklenburg, G., Salker, M. S., Brosens, J. J. & Macklon, N. S. Molecular aspects of implantation failure. Biochim. Biophys. Acta 1822, 1943–1950 (2012).
8. Okae, H. et al. Derivation of human trophoblast stem cells. Cell Stem Cell 22, 50–63.e6 (2018).
9. Io, S. et al. Capturing human trophoblast development with naive pluripotent stem cells in vitro. Cell Stem Cell 28, 1023–1039.e13 (2021).
10. Dong, C. et al. Derivation of trophoblast stem cells from naive human pluripotent stem cells. Elife 9, e52504 (2020).
11. Liu, X. et al. Reprogramming roadmap reveals route to human induced trophoblast stem cells. Nature 586, 101–107 (2020).
12. Cinkorppumin, J. K. et al. Naive human embryonic stem cells can give rise to cells with a trophoblast-like transcriptome and methylome. Stem Cell Rep. 15, 198–213 (2020).
13. Castel, G. et al. Induction of human trophoblast stem cells from somatic cells and pluripotent stem cells. Cell Rep. 33, 108419 (2020).
14. Koike-Yusa, H., Li, Y., Tan, E. P., Velasco-Herrera, M. D. C. & Yusa, K. Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. Nat. Biotechnol. 32, 267–273 (2014).
15. Chen, S. et al. Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. Cell 160, 1246–1260 (2015).
16. Hart, T. et al. High-resolution CRISPR screens reveal fitness genes and genotype-specific cancer liabilities. Cell 163, 1515–1526 (2015).
17. Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343, 84–87 (2014).
18. Wang, T., Wei, J. I., Sabatini, D. M. & Lander, E. S. Genetic screens in human cells using the CRISPR-Cas9 system. Science 343, 80–84 (2014).
19. Ihry, R. J. et al. Genome-scale CRISPR screens identify human pluripotency-specific genes. Cell Rep. 27, 616–630. e6 (2019).
20. Maor, B. et al. Essential gene profiles for human pluripotent stem cells identify uncharacterized genes and substrate dependencies. Cell Rep. 27, 599–615.e12 (2019).
21. Yilmaz, A., Peretz, M., Aharony, A., Sagi, I. & Benvenisty, N. Defining essential genes for human pluripotent stem cells by CRISPR-Cas9 screening in haploid cells. Nat. Cell Biol. 20, 610–619 (2018).
22. Yilmaz, A., Braverman-Cross, G., Biler-Tuvpin, A., Peretz, M. & Benvenisty, N. Mapping gene circuits essential for germ layer differentiation via loss-of-function screens in haploid human embryonic stem cells. Cell Stem Cell 27, 679–691.e6 (2020).
23. Doench, J. G. et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat. Biotechnol. 34, 184–191 (2016).
24. Hart, T., Brown, K. B., Sircoulomb, F., Rottapel, R. & Moffat, J. Measuring error rates in genomic perturbation screens: gold standards for human functional genomics. Mol. Syst. Biol. 10, 733 (2014).
25. Hart, T. & Moffat, J. BAGEL: A computational framework for identifying essential genes from pooled library screens. Mol. Cell. Biol. 37, 398–410 (2017).
26. Hart, T. et al. Evaluation and design of genome-wide CRISPR/SaCas9 knockout screens. G3: Genes|Genomes|Genet. 7, 2719–2727 (2017).
27. Auman, H. J. et al. Transcription factor AP-2δ is essential in the extra-embryonic lineages for early postimplantation development. Development 129, 2733–2747 (2002).
28. Werling, U. & Schorle, H. Transcription factor gene AP-2δ essential for early murine development. Mol. Cell. Biol. 22, 3149–3156 (2002).
29. Biadasiwicz, K. et al. Transcription factor AP-2a promotes EGF-dependent invasion of human trophoblast. Endocrinology 152, 1458–1469 (2011).
30. Kuchenberg, P., Kubackza, C. & Schorle, H. The role of transcription factor Tcfap2c/TFAP2C in trophoderm development. Reprod. BioMed. Online 25, 12–20 (2012).
31. Kreidl, C. et al. GATA2/3-TFAP2A/C transcription factor network couples human pluripotent stem cell differentiation to trophoblast with repression of pluripotency. Proc. Natl Acad. Sci. USA 114, E9579–E9588 (2017).
32. Yagi, R. et al. Transcription factor TEAD4 specifies the trophoderm lineage at the beginning of mammalian development. Development 134, 3827–3836 (2007).
33. Nishio, N. et al. The Hippo signaling pathway components Lat8 and Yap pattern Tead4 activity to distinguish mouse trophoblast from inner cell mass. Dev. Cell 16, 398–410 (2009).
34. Nishio, N. et al. Tead4 is required for specification of trophoderm in pre-implantation mouse embryos. Mol. Embryol. 23, 270–283 (2008).
35. Saha, B. et al. TEAD4 ensures postimplantation development by promoting trophoblast self-renewal: An implication in early human pregnancy loss. Proc. Natl Acad. Sci. USA 117, 17864–17875 (2020).
36. Chi, F., Sharpley, M. S., Nagaraj, R., Roy, S. S. & Banerjee, U. Glycylglycine-independent glucose metabolism distinguishes TE from ICM fate during mammalian embryogenesis. Dev. Cell 53, 9–26.e4 (2020).
37. Xiang, L. et al. A developmental landscape of 3D-cultural human pre-gastrulation embryos. Nature 577, 537–542 (2020).
38. Nichols, J. & Smith, A. Naive and primed pluripotent states. Cell Stem Cell 4, 487–492 (2009).
39. Wolfiler, M., Moid, B., Bauer, S., Griesinger, G. & Husslein, P. TNF-α/TNFRI in primary and immortalized first trimester cytotrophoblasts. Placenta 21, 525–535 (2000).
40. Rhee, C. et al. ARID3A is required for mammalian placenta development. Dev. Biol. 422, 83–91 (2017).
41. Home, P. et al. Reduced redundancy of GATA factors in the extraembryonic trophectoderm lineage ensures the progression of preimplantation and postimplantation mammalian development. Development 144, 876–888 (2017).
42. Yamauchi, Y. et al. Skp2 contributes to cell cycle progression in trophoblast lineage of human, cow, and mouse embryos. Nature 587, 443–447 (2020).
43. Wang, Y. et al. [Expression of lethal gene mRNA on placenta villi in patients with spontaneous abortion]. Zhonghua Fu Chan Ke Za Zhi 37, 342–354 (2002).
44. Thuneissen, T. W. et al. Molecular criteria for defining the naive human pluripotent state. Cell Stem Cell 15, 502–515 (2016).
45. Thuneissen, T. W. et al. Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. Cell Stem Cell 15, 471–487 (2014).
46. Collier, A. J. et al. Comprehensive cell surface protein profiling identifies specific markers of human naive and primed pluripotent states. Cell Stem Cell 20, 874–890, e7 (2017).
47. Bredenkamp, N., Stirparo, G. G., Nichols, J., Smith, A. & Guo, G. The cell-surface marker Sushi Containing Domain 2 facilitates establishment of human naive pluripotent stem cells. Stem Cell Rep. 12, 1212–1222 (2019).
48. Donesda, L., Bulut, A., Grimaldi, G., Grimaldi, L. & Laroni, L. Localization of fos, jun, kit and SCF mRNA in human placenta throughout gestation using in situ RT-PCR. Early Pregnancy 3, 265–271 (1997).
49. Renaud, S. J., Kubota, K., Rumi, M. A. & Soares, M. J. The FOS transcription factor family differentially controls trophoblast migration and invasion. J. Biol. Chem. 289, 5025–5039 (2014).
50. Ji, J. et al. Fibronectin 1 inhibits the apoptosis of human trophoblasts by activating the PI3K/Akt signaling pathway. Int. J. Mol. Med. 46, 1908–1922 (2020).
51. Tilburgs, T. et al. Human HLA-G+ extravillous trophoblasts: Immune-activating cells that interact with decidual leukocytes. Proc. Natl Acad. Sci. USA 112, 7219–7224 (2015).
52. Ueno, M. et al. Cell-dependent multipotent labyrinth trophectoderm progenitors establish placental exchange interface. Dev. Cell 27, 373–386 (2013).
53. Xu, X.-H. et al. Downregulation of lysyl oxidase and lysyl oxidase-like protein 2 suppressed the migration and invasion of trophoblasts by activating the TGF-ß/collagen pathway in preeclampsia. Exp. Mol. Med. 51, 1–12 (2019).
54. Zhao, B. et al. Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. Genes Dev. 25, 51–63 (2011).
55. Chrysanthou, S. et al. A critical role of TET1/2 proteins in cell-cycle regulation of imprints in mammals. DNA Cell Biol. 37, 438–468 (2018).
56. Cockburn, K., Biechele, S., Garner, J. & Rossant, J. The Hippo pathway member Nf2 is required for inner cell mass specification. Curr. Biol. 23, 1193–1201 (2013).
57. Carter, A. M. Animal models of human placentaation—a review. Placenta 28, 541–547 (2007).
58. Roberts, R. M., Green, J. A. & Schulz, L. C. The evolution of the placenta. Reproduction 152, R179–R189 (2016).
59. Perez-Garcia, V. et al. Placentation defects are highly prevalent in embryonic lethal mouse mutants. Nature 555, 463–468 (2018).
60. Vento-Tormo, R. et al. Single-cell reconstruction of the early maternal–fetal interface in humans. Nature 563, 347–353 (2018).
61. Marsh, B. & Bliebloc, R. Single nuclei RNA-seq of mouse placental development. Elife 9, e06266 (2020).
62. Shi, J.-H. & Sun, S.-C. Tumor necrosis factor receptor-associated factor regulation of nuclear factor-kB and mitogen-activated protein kinase pathways. Front. Immunol. 9, 1849 (2018).
63. Antonicka, H. et al. A pseudouridine synthase module is essential for mitochondrial protein synthesis and cell viability. EMBO Rep. 18, 28–38 (2017).
64. Guarani, V. et al. TIMMDC1/C3orf1 functions as a membrane-embedded mitochondrial complex I assembly factor through association with the MCLA complex. Mol. Cell Biol. 35, 3163–3177 (2015).
65. Lee, R. G. et al. Cardiolipin is required for membrane docking of mitochondrial ribosomes and protein synthesis. J. Cell Sci. 133, jcs240374 (2020).
66. Kasahara, T. et al. Cardiolipin is essential for early embryonic viability and mitochondrial integrity of neurons in mammals. Faseb J. 34, 1465–1480 (2020).
67. Boukalova, S. et al. Dihydrolipoate dehydrogenase in oxidative phosphorylation and cancer. Biochim. Biophys. Acta Mol. Basis Dis. 1866, 165759 (2020).
68. Houghton, F. D. Energy metabolism of the inner cell mass and trophoderm of the mouse blastocyst. Differentiation 74, 11–18 (2006).
69. Eppig, J. T. & Eppig, T. J. The mammalian phenotype ontology: Enabling robust annotation and comparative analysis. Wiley Interdiscip. Rev. Syst. Biol. Med. 1, 390–399 (2009).
101. Köhler, S. et al. Expansion of the human phenotype ontology (HPO) knowledge base and resources. Nucleic Acids Res. 47, D1018–D1027 (2018).

102. Wu, G., Bazer, F. W., Wallace, J. M. & Spencer, T. E. BOARD-INVITED REVIEW: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84, 2316–2337 (2006).

103. Fischer, I. A., Khan, S. A. & Theunissen, T. W. Induction of human naive pluripotency using S/1/LA medium. Methods Mol. Biol. 2416, 13–28 (2022).

104. Doedenkamp, N. et al. Wnt inhibition facilitates RNA-mediated reprogramming of human somatic cells to naive pluripotency. Stem Cell Rep. 13, 1083–1098 (2019).

105. Dong, C. & Theunissen, T. W. Generating trophoblast stem cells from human naive pluripotent stem cells. Methods Mol. Biol. 2416, 91–104 (2022).

106. Sanson, K. R. et al. Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nat. Commun. 9, 416 (2018).

107. Li, W. et al. MAGECK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biol. 15, 554 (2014).

108. Li, W. et al. Quality control, modeling, and visualization of CRISPR screens with MAEGeCK-VISPR. Genome Biol. 16, 218 (2015).

109. Kim, E. & Hart, T. Improved analysis of CRISPR fitness screens and reduced off-target effects with the BAGEL2 gene essentiality classifier. Genome Med. 13, 2 (2021).

110. Chen, J., Bardes, E. E., Aronow, B. J. & Jegga, A. G. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. 37, W305–W311 (2009).

111. Binder, J. X. et al. COMPARTMENTS: UniProtKB and protein subcellular localization evidence. Database 2014, bau012 (2014).

112. Hu, H. et al. AnimalTFDB 3.0: A comprehensive resource for annotation and prediction of animal transcription factors. Nucleic Acids Res. 47, D33–D38 (2019).

113. Dobin, A. et al. STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21 (2012).

114. Liao, Y., Smyth, G. K. & Shi, W. The Subread aligner: Fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 41, e108 (2013).

115. Harrow, J. et al. GENCODE: The reference human genome annotation for The ENCODE Project. Genome Res. 22, 1760–1774 (2012).

116. Rissolo, D., Ngai, J., Speed, T. P. & Duduit, S. Normalization of RNA-seq data using factor analysis of control genes or samples. Nat. Biotechnol. 32, 896–902 (2014).

117. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).

118. Corces, M. R. et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat. Methods 14, 959–962 (2017).

119. Liu, S. et al. AIAAP: A quality control and integrative analysis package to improve ATAC-seq data analysis. Genomics, Proteomics & Bioinformatics 19, 641–651 (2021).

120. Quinlan, A. R. & Hall, I. M. BEETools: A flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841–842 (2010).

121. Heinz, S. et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell 38, 576–589 (2010).

122. McLean, C. Y. et al. GREAT improves functional interpretation of cis-regulatory regions. Nat. Biotechnol. 28, 495–501 (2010).

123. Ramirez, G. et al. deepTools2: A next generation web server for deep-sequencing data analysis. Nucleic Acids Res. 44, W160–W165 (2016).

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Author contributions
C.D. and T.W.T conceived of and designed the study. C.D. performed the experiments and collected the data. R.M.K., B.C., L.A.F., X.X., J.K.H., and P.P. contributed to experiments and data analysis. C.D. and S.F. performed the data analysis. C.D. wrote the paper with input from T.W.T, T.W.T., B.Z., T.W., and R.K. supervised the study. T.W.T. and B.Z. acquired funding for the study.

Competing interests
The authors declare no competing interests.

Additional information
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