Development 137, 395-403 (2010) doi:10.1242/dev.038828
© 2010. Published by The Company of Biologists Ltd

Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2

Amy Ralston[1,2], Brian J. Cox[1], Noriyuki Nishioka[3], Hiroshi Sasaki[3], Evelyn Chea[1], Peter Rugg-Gunn[1], Guoji Guo[4,5], Paul Robson[4,5], Jonathan S. Draper[6,*] and Janet Rossant[1,*]

SUMMARY

The mouse blastocyst and stem cells derived from its tissue lineages provide a unique genetic system for examining the establishment and loss of pluripotency. The transcription factor Cdx2 plays a central role by repressing pluripotency genes, such as *Oct4*, and promoting extraembryonic trophoblast fate at the blastocyst stage. However, genetic evidence has suggested that Cdx2 does not work alone in the trophoblast lineage. We have used bioinformatic and functional genomic strategies to identify the transcription factor Gata3 as a trophoblast factor. We show Gata3 to be capable of inducing trophoblast fate in embryonic stem cells and driving trophoblast differentiation in trophoblast stem cells. In addition, *Cdx2* is not required for Gata3-induced expression of a subset of trophoblast genes in embryonic stem cells. We show that Gata3 is coexpressed with Cdx2 in the blastocyst, but this does not depend on *Cdx2*. In the embryo, expression of Gata3, like that of Cdx2, depends on *Tead4*, and the expression of both factors becomes restricted to trophoblast by a mechanism that does not initially rely on *Oct4*. These observations suggest that Gata3 and Cdx2 can act in parallel pathways downstream of Tead4 to induce the expression of common and independent targets in the trophoblast lineage, whereas Oct4 is required for continued repression of trophoblast fate in the embryonic lineage.

KEY WORDS: Trophectoderm, Placenta, Implantation, Pluripotency, Lineage restriction, Embryogenesis, Mouse

INTRODUCTION

The first developmental decisions during mouse development lead to the establishment of the embryonic and extraembryonic tissue lineages. Stem cell lines have been isolated from these early lineages, including embryonic stem (ES) and trophoblast stem (TS) cells (Evans and Kaufman, 1981; Martin, 1981; Tanaka et al., 1998). Both stem cell types are self-renewing and capable of lineage-appropriate differentiation. For example, ES cells can differentiate into a wide range of fetal cell types, but fail to form trophoblast (Beddington and Robertson, 1989). Conversely, TS cells differentiate along the trophoblast/placenta lineage, and fail to form fetal cell types (Tanaka et al., 1998). To create a placenta, the trophoblast lineage must achieve several distinct goals simultaneously at the blastocyst stage. Trophoblast cells must override the pluripotency program of the embryonic lineage, they must establish the ability to self-renew, and they must maintain the ability to differentiate into mature trophoblast cell types. *Cdx2* and *Eomes* are required for trophoblast survival and maturation starting around the blastocyst stage (Russ et al., 2000; Strumpf et al., 2005). These genes are also important for TS cell establishment (Strumpf et al., 2005), suggesting roles in proliferation. However, not all cells of the trophectoderm are proliferative, as some trophectoderm cells visibly differentiate as early as implantation. This suggests that programs that promote proliferation and differentiation might coexist at the blastocyst stage.

Besides *Cdx2* and *Eomes*, genetic evidence suggests that other genes participate in trophoblast formation in the blastocyst. For example, loss of *Tead4*, which is required for expression of *Cdx2* in the trophectoderm (Yagi et al., 2007; Nishioka et al., 2008), leads to a more severe phenotype than loss of *Cdx2*. Thus, Tead4 must have multiple trophoblast targets acting at the blastocyst stage to regulate trophoblast development. Consistent with this proposal, constitutively active Tead4 is sufficient to induce trophoblast formation even in the absence of *Cdx2* in ES cells (Nishioka et al., 2009). Other factors capable of overriding the pluripotency pathway and promoting trophoblast fate must therefore exist.

To identify new factors involved in early lineage decisions in the mouse, we used a bioinformatic strategy to compare expression profiles of stem cells from the blastocyst. Transcripts encoding the transcription factor Gata3 were specifically enriched in TS cells and in the trophoblast lineage, consistent with recent reports (Home et al., 2009; Ray et al., 2009). Although Gata3 expression is restricted to the trophectoderm at the blastocyst stage, we found that this expression does not depend on *Cdx2*. Rather, expression of Gata3, like that of Cdx2, depends on *Tead4*. We show that *Oct4* (*Pou5f1*) is not initially involved in restricting expression of either Cdx2 or Gata3 to the trophectoderm, but Oct4 maintains repression of these genes in the epiblast. In ES cells, Gata3 is capable of overriding pluripotency and directing the expression of a multitude of *Cdx2*-independent trophoblast genes, whereas in TS cells Gata3 promotes differentiation.

MATERIALS AND METHODS

Bioinformatic analysis

For comparison of ES, TS and XEN cell expression profiles, MGU74v2A microarray.CEL files for ES (GSE3766), XEN (GSE2204) and TS (GSE3766) cells were downloaded from the GEO website

[1]Program in Developmental and Stem Cell Biology, Hospital for Sick Children Research Institute, MARS Building, Toronto Medical Discovery Tower, 101 College St, Toronto, ON M5G 1L7, Canada. [2]Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA 95064, USA. [3]Laboratory for Embryonic Induction, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan. [4]Department of Biological Sciences, National University of Singapore, Singapore 117543. [5]Stem Cell and Developmental Biology, Genome Institute of Singapore, 138672 Singapore. [6]McMaster Stem Cell and Cancer Research Institute, 1200 Main Street West, MDCL 5032, Hamilton, ON L8N 3Z5, Canada.

*Authors for correspondence (draperj@mcmaster.ca; janet.rossant@sickkids.ca)

Accepted 24 November 2009

DEVELOPMENT

(http://www.ncbi.nlm.nih.gov/projects/geo/) and processed using GCOS software (Affymetrix) with the 'statistical method' algorithm to generate signal intensities and absent/present calls. Log_2 ratios and fold-change calls for probe sets in TS and XEN cell samples versus ES cells were determined. Probe sets with more than a 2-fold difference in expression in TS cells ($P<0.003$) versus ES cells and with GO annotation as a transcription factor (GO term ID: 000635) were selected for hierarchical clustering. GO annotation was obtained from the Affymetrix annotation file for the gene chip. Hierarchical clustering was performed with Cluster 3.0 (de Hoon et al., 2004) using the 'uncentered correlation similarity' metric with average linkage, and resulting clusters were visualized with Java TreeView.

For comparison of Gata3 and Cdx2 activity in wild-type and *Cdx2* null cells, Affymetrix MOE430 2.0 microarrays were performed on four independent Gata3-expressing lines, two Cdx2-expressing lines, and three Gata3-expressing *Cdx2* null lines. Data for these and TS cells were batch processed using Expression Console software (Affymetrix) to normalize arrays. Probe intensities were calculated using the PLIER algorithm. To reduce sample complexity, redundant probes matching to a single gene were filtered for a representative probe that had the largest number of signals greater than three times the global median signal across all samples. Ratios of gene expression for *Gata3*-expressing and *Cdx2*-expressing cells were calculated relative to tamoxifen-treated R1 cells and were log_2 transformed. Ratios for *Gata3*-expressing *Cdx2* null cells were calculated relative to tamoxifen-treated *Cdx2* null cells. Expression ratios for TS cells and their differentiated samples were calculated relative to the median expression level of the probe set across all the TS samples. To facilitate mining of the data set, all expression and probe set annotation was placed into a custom database using MySQL and queried using command line scripts. Raw data are available at GEO with accession numbers GSE12985 and GSE12986.

Cell culture

The ES cell lines R1 and dKO23-5 (Niwa et al., 2005) were maintained on gelatin using standard culture conditions. To create stably transformed lines, $1\text{-}3\times10^7$ ES cells were electroporated with 20-30 μg plasmid (pCAG-hCdx2ERT2-ires-puro^r, or pCAG-hGata3ERT2-ires-puro^r), and were then seeded on two 10-cm gelatinized plates. Cells were fed with medium to select for expression of the plasmid (ES medium + 1.2 μg/ml puromycin) and individual colonies expanded. To induce transgene activity, cells were treated with induction medium [TS medium + 1 μg/ml tamoxifen (Sigma)]. For microarray analysis, 2×10^5 cells were seeded on gelatinized 35-mm wells in ES medium, and then switched to TS cell medium with tamoxifen the following day, and fed daily for 6 days. To derive TS-like cell lines, cells were treated with tamoxifen on mouse embryonic feeder layers for 6 days in TS cell medium, and then passaged onto fresh feeders in TS cell medium.

TS cell lines included TS3.5 and TS6.5, which were isolated from blastocyst and E6.5 embryos, respectively, and were maintained or differentiated as described (Tanaka et al., 1998), and TS_{WT}, which was isolated from ICR blastocysts. For overexpression of Gata3, TS_{WT} cells were electroporated with Gata3ER, followed by selection for plasmid expression (with 1.2 μg/ml puromycin in TS cell medium) and tamoxifen treatment (1 μg/ml) for 5 days.

Gene expression analysis

RNA was harvested from plated cells using Trizol (Invitrogen). For real-time PCR analysis, cDNA was synthesized using the Quantitect Kit (Qiagen). Real-time PCR analyses were performed using SYBR Green and a LightCycler 480 (Roche). All reactions (12 μl) were performed in triplicate, with 100-200 ng cDNA and 300 nM primers (shown 5′ to 3′) per reaction: *Hprt1*, AAACAATGCAAACTTTGCTTTCC and GGTCCTTTTCACCAGCAAGCT; *Gata3*, GGGTTCGGATGTAAGTCGAG and CCACAGTGGGGTAGAGGTTG; *Cdx2*, AGACAAATACCGGGTGGTGTA and CCAGCTCACTTTTCCTCCTGA; *Prl2c2*, AGCCCCATGAGATGCAATAC and CATCCAAAATCATGGCTCCT; *Bmp4*, AGGAGGAGGAGGAAGAGCAG and ACTGGTCCCTGGGATGTTCT; *Pdgfra*, ACGTTCAAGACCAGCGAGTT and CGATCGTTTCTCCTGCCTTA; *Ascl2*, TTTTCGAGGACGCAATAAGC and CACTGCTGCAGGACTCCCTA; *Eomes*, GTGACAGAGACGGTGTGGAGG and AGAGGAGGCCGTTGGTCTGTGG; *Elf5*, TGCCTTTGAGCATCAGACAG and TACTGGTCGCAGCAGAATTG; *Tead4*, ACGGAGGAAGGCAAGATGTA and CTGGAGACCTGCTTCCTTGT. A standard primer efficiency curve for each primer pair was generated using TS3.5 cDNA. Levels of *Hprt1* served as an internal reference for all reactions. Amplification of a single PCR product for each reaction was confirmed by melting curve analysis, and all primers spanned exon junctions. RNA samples used for microarray hybridization were collected using Trizol, and then further purified using the RNeasy Mini Kit (Qiagen). Samples were analyzed by Affymetrix mouse MOE 430 2.0 cDNA microarrays (The Center for Applied Genomics, Toronto, Canada).

For single-blastocyst qPCR, total RNA was extracted from individual blastocysts using the PicoPure RNA Isolation Kit (Arcturus Bioscience), and cDNA synthesized at 37°C for 2 hours using the high-capacity cDNA Archive Kit (Applied Biosystems). One eighth of each cDNA preparation was preamplified for 16 cycles (95°C for 15 seconds and 60°C for 4 minutes) using the TaqMan PreAmp Master Mix Kit (Applied Biosystems) and gene-specific primers. Products were then diluted 5-fold for PCR (Applied Biosystems) in 48.48 Dynamic Arrays on a BioMark System (Fluidigm). Threshold cycle (Ct) values were calculated using the system's software (BioMark Real-time PCR Analysis) and were normalized to *Actb* Ct values.

Immunofluorescence and in situ hybridization

Preimplantation embryos were harvested, stained and examined by confocal microscopy as described previously (Ralston and Rossant, 2008). Primary antibodies included mouse anti-Cdx2 (1:200, Biogenex CDX2-88), rabbit anti-Cdx2 (1:200) (Chawengsaksophak et al., 1997), mouse anti-Gata3 (1:20, Santa Cruz H-48) and mouse anti-Oct4 (1:10, Santa Cruz C-10). Secondary antibodies included Alexa488- or Alexa546-conjugated goat anti-mouse, rabbit or rat IgG (Molecular Probes) and Cy3-conjugated anti-mouse, rabbit or rat IgG (Jackson). Secondary antibody-only controls were performed in parallel (not shown). Whole-mount embryo in situ hybridization was performed as described (Yamanaka et al., 2007).

Mouse strains

Mouse strains used in this study included wild-type (ICR) mice and mice heterozygous for null alleles of *Cdx2* ($Cdx2^{tm1Fbe}$) (Chawengsaksophak et al., 1997), *Oct4* ($Pou5f1^{tm1Scho}$) (Kehler et al., 2004) and *Tead4* ($Tead4^{tm1Hssk}$) (Nishioka et al., 2008). All mice were treated in accordance with institutional guidelines. For genotyping, blastocysts were individually recovered following confocal microscopy and lysed using the Extract-N-Amp Tissue PCR Kit (Sigma) in a total of 10 μl per embryo, of which 2 μl was used for 10 μl PCR genotyping reactions, with 5 μl PCR Red mix and 0.5 μl each 10 μM primer (Kehler et al., 2004; Strumpf et al., 2005; Nishioka et al., 2008).

RESULTS

Gata3 is enriched in trophoblast stem cells

To identify potential trophoblast-inducing factors, we compared microarray expression profiles of stem cells derived from the blastocyst lineages: ES, TS and extraembryonic endoderm stem (XEN) cells (Kunath et al., 2005) (see Materials and methods). Probe sets that were significantly increased in TS and XEN cells relative to ES cells ($P<0.003$) were retained, and analysis of corresponding gene ontology (GO) terms yielded 122 transcription factors specifically enriched in TS cells (see Fig. S1 in the supplementary material), as represented by 138 probe sets. Importantly, this list included genes known to be essential for early trophoblast development, such as *Cdx2* (Strumpf et al., 2005), *Eomes* (Russ et al., 2000; Strumpf et al., 2005) and *Tead4* (Yagi et al., 2007; Nishioka et al., 2008). We therefore reasoned that other transcription factors enriched in this list could likewise be important for trophoblast development.

We focused on the zinc-finger transcription factor Gata3, the expression of which was specifically highly enriched in TS cells. Consistent with the microarray data, quantitative RT-PCR (qPCR)

DEVELOPMENT

analysis of *Gata3* levels indicated a greater than 100-fold enrichment of *Gata3* in TS cells compared with ES cells (Fig. 1A). *Gata3* is known to be expressed in, and required for the function of, the trophoblast lineage at later stages of development of the placenta (Ma et al., 1997). However, its high level of expression in TS cells suggested a previously unrecognized role for Gata3 in regulating stem cells of the trophoblast lineage. Examination of differentiating TS cells revealed that *Gata3* levels increase during TS cell differentiation (Fig. 1B). These observations suggested that Gata3 might promote TS cell differentiation, consistent with its requirement in directing the formation of giant cells in the placenta (Ma et al., 1997).

Global comparison of Gata3 and Cdx2 trophoblast-inducing activity

ES cells are normally restricted in developmental potential to embryonic fates, having lost or suppressed the ability to generate trophoblast cell types (Beddington and Robertson, 1989). Overexpression of key trophoblast factors has been shown to lead to an increase in trophoblast gene expression in ES cells within a 6-day time frame (Niwa et al., 2005; Lu et al., 2008; Ng et al., 2008; Nishioka et al., 2009; Nishiyama et al., 2009). Subsequent passage of these cells in TS cell medium can lead to the establishment of self-renewing TS-like cells in some cases (Niwa et al., 2005; Lu et al., 2008; Nishioka et al., 2009). We examined the ability of Gata3 to induce the formation of TS-like cells by overexpressing *Gata3ER*, which encodes a fusion between Gata3 and the ligand-binding domain of the estrogen receptor (ER). Gata3ER was activated by addition of tamoxifen and, under TS cell derivation conditions, TS-like colonies were detected among cultures within 6 days (Fig. 1C; 4/5 lines examined), but not in control ES cells grown under the same conditions (Fig. 1E). However, endoderm-like cells were also present in all Gata3 cultures (Fig. 1C′), and these were not present in ES cells overexpressing Cdx2 (Fig. 1D). Continued passage of *Cdx2*-overexpressing ES cells led to the establishment of TS-like cell lines (4/5 lines examined). TS-like colonies were continuously detected among Gata3-overexpressing cells. However, cultures were consistently heterogeneous, and the TS cell phenotype could not be enriched under the conditions examined (5/5 lines examined). Thus, although both genes appear capable of inducing trophoblast differentiation in ES cells, only Cdx2 produced stable TS cell lines when overexpressed in ES cells.

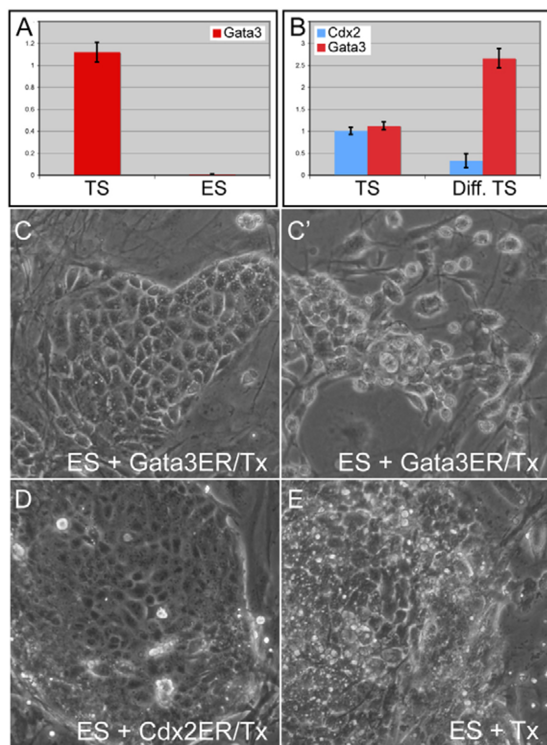


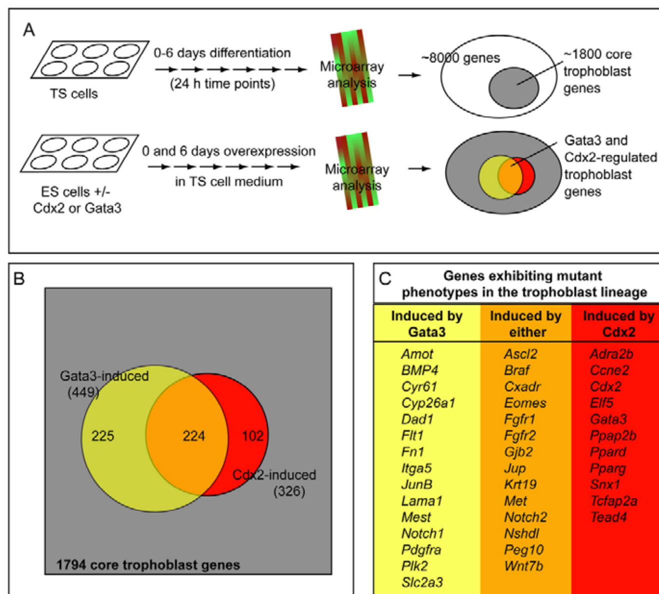
Fig. 1. *Gata3* is sufficient to induce trophoblast morphology in mouse ES cells. (**A**) qPCR analysis of *Gata3* levels in embryonic stem (ES) and trophoblast stem (TS) cells. For this and all subsequent qPCR analyses, expression levels have been normalized to those in TS cells. Error bars, variation in technical replicates. (**B**) qPCR analysis of *Gata3* levels in self-renewing and TS cells differentiated for 6 days. (**C**) TS-like morphology resulting from overexpression of Gata3 in ES cells for 6 days and subsequent passage on feeders. (**C′**) Endoderm-like morphology present among ES cells overexpressing Gata3. (**D**) TS-like cells derived from ES cells overexpressing Cdx2. (**E**) Control ES cells treated in parallel. Tx, tamoxifen.

We next compared the ability of Gata3 and Cdx2 to induce trophoblast at the gene expression level, comparing global gene expression profiles of ES cells overexpressing either gene. To restrict our analysis to trophoblast-specific genes, we began by defining a set of ~1800 core trophoblast genes, using TS cells as a reference (Fig. 2A; see Table S1 in the supplementary material; see Materials and methods). We then used this set to filter data sets from *Cdx2*-expressing and *Gata3*-expressing ES cell lines. This led to lists of genes induced by Gata3 (449/1794 core trophoblast genes) or Cdx2 (326/1794 core trophoblast genes) (see Table S2 in the supplementary material). Gata3 was therefore capable of inducing more trophoblast genes than Cdx2.

To examine qualitative similarities and differences in trophoblast genes induced by the overexpression of these two genes, we examined the overlap between the two lists. This revealed trophoblast genes induced by *Gata3* (225 genes) or *Cdx2* (102 genes) alone, as well as common genes induced by either factor (224 genes) (Fig. 2B; see Table S2 in the supplementary material). Thus, although around half of the trophoblast genes induced by *Gata3* were also induced by Cdx2, each factor also induced the expression of a unique set of trophoblast genes. These differences were validated by qPCR for a subset of the genes (see Fig. S2 in the supplementary material). This analysis suggested that Gata3 expression is induced by ectopic *Cdx2*, although Gata3 targets were not in turn detected in the *Cdx2*-overexpression array. This disparity could be due to differences in the levels of overexpressed Gata3 in these two conditions. Alternatively, Cdx2 might directly or indirectly repress the expression of a subset of Gata3 target genes. This possibility is further addressed below.

These observations predict that Gata3 and Cdx2 will have both shared and distinct roles during trophoblast development. Among the genes induced by either factor, an examination of phenotypes for those that have been knocked out (Fig. 2C) revealed defects in multiple trophoblast subtypes and at multiple developmental stages (see Table S3 in the supplementary material). No single trophoblast phenotype was predominant in any of the lists, suggesting that Cdx2 and Gata3 targets are likely to play diverse, and possibly overlapping, roles in trophoblast development.

This analysis, which was designed to focus on the trophoblast roles of these genes, excluded genes that were not included among the core trophoblast gene list. We noted 347 'non-trophoblast' genes induced by *Gata3* and 72 induced by *Cdx2* (see Table S4A,B in the supplementary material). Interestingly, among the genes induced by



Induced by Gata3	Induced by either	Induced by Cdx2
Amot	*Ascl2*	*Adra2b*
BMP4	*Braf*	*Ccne2*
Cyr61	*Cxadr*	*Cdx2*
Cyp26a1	*Eomes*	*Elf5*
Dad1	*Fgfr1*	*Gata3*
Flt1	*Fgfr2*	*Ppap2b*
Fn1	*Gjb2*	*Ppard*
Itga5	*Jup*	*Pparg*
JunB	*Krt19*	*Snx1*
Lama1	*Met*	*Tcfap2a*
Mest	*Notch2*	*Tead4*
Notch1	*Nshdl*	
Pdgfra	*Peg10*	
Plk2	*Wnt7b*	
Slc2a3		

Fig. 2. Gata3 is sufficient to induce trophoblast gene expression in ES cells. (**A**) Data mining strategy for examining trophoblast gene expression in ES cells overexpressing *Cdx2* or *Gata3*. TS cells were differentiated for 6 days, and samples harvested daily during this period to generate a 6-day differentiation profile. Around 1800/8000 genes (core trophoblast genes, gray) exhibited a 2-fold or greater change in absolute expression level over the course of the experiment and were retained as likely to be important for trophoblast development. Changes in the expression of core trophoblast genes were then examined in ES cells overexpressing *Gata3* or *Cdx2* for 0 and 6 days. Genes exhibiting a greater than 2-fold increase in expression level in at least one of the cell lines examined were retained. (**B**) Venn diagram showing overlap between the lists of core trophoblast genes (gray) exhibiting a 2-fold or greater enrichment in lists from *Gata3*-expressing (yellow) or *Cdx2*-expressing (red) cells. The number of genes within each subset is indicated. (**C**) Subset of genes represented in B with Mouse Genome Informatics (MGI)-archived mutant phenotypes that affect the trophoblast lineage.

Gata3 were many known endodermal genes, including *Foxa2*, *Sox17* and *Sox7*. Thus, both Cdx2 and Gata3 are capable of inducing non-trophoblast targets in ES cells, consistent with the diverse developmental roles played by these genes and the plasticity of ES cells to respond to inductive cues.

Gata3 exhibits both *Cdx2*-dependent and -independent induction of trophoblast gene expression

In ES cells, downregulation of *Oct4* leads to upregulation of *Cdx2* and the adoption of trophoblast fate (Niwa et al., 2000), raising the possibility that *Gata3* overexpression could induce trophoblast gene expression by simply altering Oct4/Cdx2 levels. To address this possibility, we examined trophoblast gene expression following *Gata3* overexpression in the dKO23-5 ES cell line that is *Cdx2* null and expresses *Oct4* constitutively (Niwa et al., 2005). Gata3 overexpression in dKO23-5 cells led to changes in cell morphology, and TS cell lines could not be established in this genetic background, as expected (5/5 lines examined). A microarray comparison of differences in the induction of trophoblast genes following overexpression of *Gata3* in wild-type and dKO23-5 ES cells revealed that *Gata3* was still able to induce a large number of core trophoblast genes (284/1794, compared with 449/1794 in wild-type cells). *Gata3* is therefore sufficient to induce trophoblast gene expression in a *Cdx2*-independent manner.

However, the expression of many trophoblast genes was lost in this genetic background. Examining the intersection between the lists of core trophoblast genes induced by *Gata3* in either wild-type or dKO23-5 cells (Fig. 3A) revealed that the expression of 172/449 Gata3 targets was unchanged, whereas 277/449 targets were no longer induced by Gata3 in dKO23-5 cells. Therefore, the expression of some Gata3 targets relied on the Oct4/Cdx2 pathway, whereas the expression of others, such as *Eomes* and *Ascl2*, did not (Fig. 3B). The genes that were dependent on *Cdx2*, however, did not necessarily overlap with those induced by Cdx2, suggesting differences in the necessity and sufficiency of *Cdx2* for trophoblast gene expression.

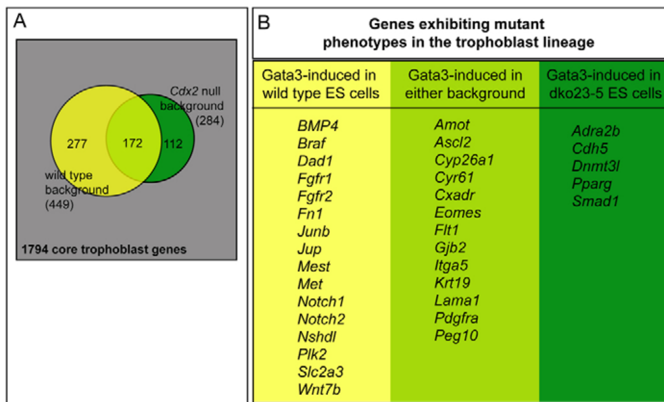
This analysis also identified 112 genes that were induced by Gata3 in dKO23-5 and not wild-type ES cells (Fig. 3A; see Table S5 in the supplementary material). This suggests that Cdx2 might repress the Gata3-mediated induction of some trophoblast genes. Taken together, these observations suggest that Gata3 can act via Cdx2, and in parallel to Cdx2, to induce trophoblast gene expression. In addition, these observations suggest that Gata3 might play a unique role in regulating trophoblast development independently of Cdx2.

Gata3 is expressed in the trophoblast lineage in vivo

The findings that *Gata3* is enriched in TS cells and is sufficient to induce trophoblast gene expression in ES cells suggested that Gata3 might be expressed in the trophoblast during lineage establishment in vivo. We examined the expression of Gata3 during trophoblast development at preimplantation stages (Fig. 4A-E). Gata3 protein was detectable within the nuclei of the trophectoderm at the blastocyst stage, where it colocalized with Cdx2 (Fig. 4D) (n=10). In fact, Gata3 colocalized with Cdx2 at earlier preimplantation stages as well (Fig. 4A-C) (n=31 embryos, 8- to 32-cell stages). Prior to becoming restricted to outside cells of the nascent trophectoderm, Cdx2 is expressed in an unpatterned, mosaic manner beginning around the late 8-cell stage (Dietrich and Hiiragi, 2007; Ralston and Rossant, 2008). Gata3 colocalized with Cdx2 in nuclei on a cell-by-cell basis (723/730 cells) in embryos examined at the 8- to 32-cell stages (31 embryos). Among embryos in which Gata3 and Cdx2 expression did not perfectly correlate (5/31 embryos), Gata3-positive/Cdx2-negative and Cdx2-positive/Gata3-negative nuclei were detected at equivalent frequency (four and three nuclei, respectively). Thus, Gata3 is coexpressed with Cdx2 from the earliest developmental stages.

Since TS cells have also been derived from post-implantation embryos, around the time of gastrulation (Tanaka et al., 1998; Uy et al., 2002), we next examined *Gata3* expression around gastrula stages by in situ hybridization. *Gata3* expression was detected throughout the trophoblast lineage from embryonic day (E) 6.5 to 8.5 (Fig. 4E) (n=12). This expression was consistent with previous reports (George et al., 1994) and included the extraembryonic ectoderm (EXE)/chorion and ectoplacental cone (EPC). However, we noted that higher levels of *Gata3* were detected in the EPC than in the EXE. By contrast, *Cdx2* levels appeared higher in the EXE than in the EPC (Fig. 4F). These differences were confirmed by qPCR, following microdissection of these regions (Fig. 4G). Since the EPC is thought to be more differentiated than the EXE, these observations suggest that *Gata3* levels increase during trophoblast differentiation.

DEVELOPMENT



Genes exhibiting mutant phenotypes in the trophoblast lineage

Gata3-induced in wild type ES cells	Gata3-induced in either background	Gata3-induced in dko23-5 ES cells
BMP4	*Amot*	*Adra2b*
Braf	*Ascl2*	*Cdh5*
Dad1	*Cyp26a1*	*Dnmt3l*
Fgfr1	*Cyr61*	*Pparg*
Fgfr2	*Cxadr*	*Smad1*
Fn1	*Eomes*	
Junb	*Flt1*	
Jup	*Gjb2*	
Mest	*Itga5*	
Met	*Krt19*	
Notch1	*Lama1*	
Notch2	*Pdgfra*	
Nshdl	*Peg10*	
Plk2		
Slc2a3		
Wnt7b		

Fig. 3. Gata3 induces trophoblast though *Cdx2*-dependent and -independent mechanisms. (**A**) Venn diagram showing overlap between lists of core trophoblast genes (gray) upregulated by Gata3 in wild-type (yellow) or *Cdx2* null (green) ES cells, with the number of genes indicated. (**B**) Subset of genes represented in A with MGI-archived mutant phenotypes that affect the trophoblast lineage.

Notably, *Gata3* and *Cdx2* were also detected within the embryo proper around the gastrula stage and later, with *Gata3* in a restricted anterior region (Fig. 4E and data not shown), consistent with previous reports (Manaia et al., 2000), and *Cdx2* in posterior regions (Fig. 4F and data not shown) (Beck et al., 1995). This pattern is consistent with the proposal that both genes can also induce non-trophoblast targets in ES cells.

Gata3 is sufficient to induce differentiation of TS cells

Increasing levels of *Gata3* during trophoblast differentiation, both in TS cells and in the post-implantation embryo, suggested that Gata3 promotes differentiation. We therefore examined whether Gata3 is sufficient to induce differentiation in TS cells. We introduced the Gata3ER fusion construct into a TS cell line and examined changes in morphology and gene expression following treatment with tamoxifen for 5 days. As in previous experiments, cells expressing the Gata3ER fusion protein were selected by drug resistance. Control TS cells treated with tamoxifen maintained a generally undifferentiated state (Fig. 5A). However, *Gata3*-overexpressing TS cells appeared largely differentiated, with numerous giant cells present throughout the culture (Fig. 5B), despite the continued presence of TS cell medium. Plasmid-electroporated cells treated with lower doses of tamoxifen did not appear differentiated, arguing that the differentiation effect was Gata3 dependent. We therefore conclude that Gata3 overexpression is sufficient to induce differentiation of TS cells. This proposal was confirmed by an examination of TS cell and giant cell markers by qPCR (Fig. 5C).

Common mechanisms of regulation of Cdx2 and Gata3 in the blastocyst

Coexpression of Cdx2 and Gata3 at the blastocyst stage led us to investigate whether these genes are regulated by a common mechanism in vivo. The transcription factor Tead4 is required for Cdx2 expression prior to the blastocyst stage (Yagi et al., 2007; Nishioka et al., 2008). In *Tead4* mutants, Cdx2 is initially detected around the 16-cell stage (Nishioka et al., 2008), but this expression is lost and embryos die prior to blastocyst formation around the 32-cell stage (Yagi et al., 2007; Nishioka et al., 2008). We hypothesized that Tead4 could play a role in the regulation of Gata3 prior to blastocyst formation, and examined expression of Gata3 in *Tead4* mutants at E3.5. Nuclear levels of Gata3 were greatly reduced in *Tead4* mutants (n=5) compared with non-mutants (n=17) (Fig. 6A,B). Similar to Cdx2, however, low levels of Gata3 could be detected in the nuclei of some cells (not shown), consistent with Tead4 regulating the maintenance, rather than initiation, of *Gata3* expression.

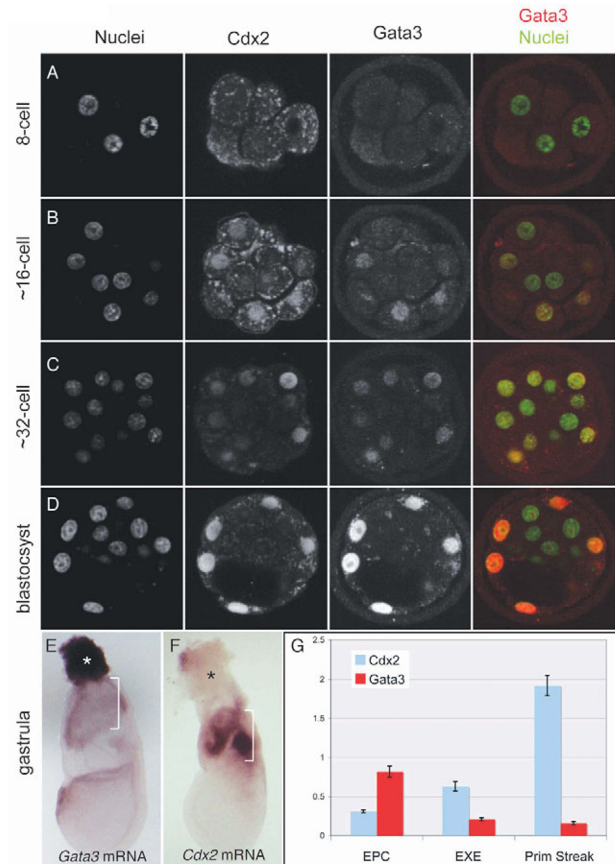


Fig. 4. Gata3 is expressed in the trophoblast lineage. (**A-D**) Representative confocal sections of preimplantation mouse embryos (stages indicated) showing simultaneous localization of Cdx2 and Gata3. Merged images show Gata3 and nuclei, emphasizing the localization of Gata3 in nuclei in outside cells of the embryo (yellow), as previously shown for Cdx2. Since the expression levels of Cdx2 and Gata3 appear to increase steadily during preimplantation stages, confocal settings were changed between embryos so as to optimize the signal-to-noise ratio for each developmental stage examined. Note that neither Cdx2 nor Gata3 is detectable in early 8-cell embryos (shown), but they become detectable during the 8- to 16-cell transition. Background fluorescence from the zona pellucida (zp) can be detected in some channels. The apparent cytoplasmic staining detectable in the Cdx2 channel is likely to be background as it is still present in *Cdx2* mutants stained with this polyclonal antibody (not shown). (**E**) Representative image of *Gata3* whole-mount in situ hybridization at ~E7.5. Note expression in the extraembryonic ectoderm (EXE) (bracket) and apparently higher levels of expression in the ectoplacental cone (EPC) (asterisk). (**F**) Representative image of *Cdx2* in situ hybridization at ~E7.5, with expression in EXE bracketed. (**G**) Quantification of *Cdx2* and *Gata3* levels in EXE, EPC and primitive streak regions from a pool of ten E7.5 embryos. Results are representative of experiments performed in triplicate.

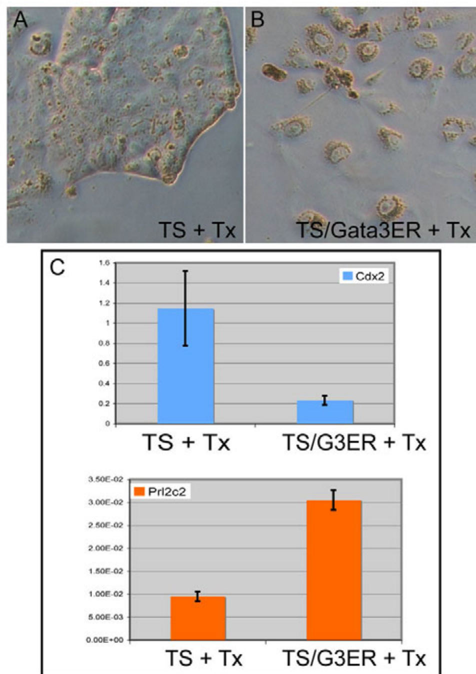


Fig. 5. Gata3 is sufficient to induce differentiation in TS cells. (**A**) Control TS cells treated with tamoxifen (Tx) in TS cell medium for 5 days. (**B**) Giant cells have formed after 5 days of overexpression of Gata3ER in TS cells in the presence of tamoxifen and TS cell medium. (**C**) qPCR analysis of stem cell (*Cdx2*) and giant cell (*Prl2c2*) markers in control and Gata3-expressing TS cells. Results are representative of experiments performed in duplicate.

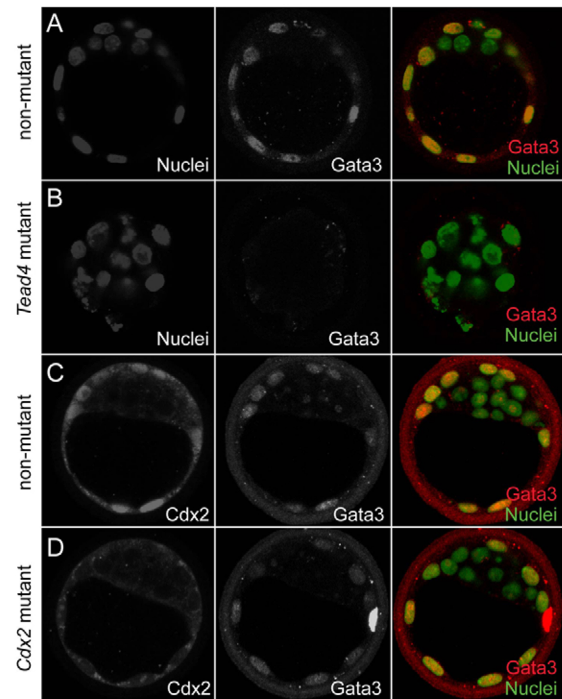


Fig. 6. Parallel regulation of Cdx2 and Gata3 during blastocyst formation. (**A**) Confocal section of a non-mutant blastocyst from a *Tead4* heterozygous intercross, showing Gata3 protein and nuclear stain. Merged red and green signals appear yellow. (**B**) Confocal section of a *Tead4* mutant at the same time point as in A, showing greatly reduced levels of Gata3 in outside cells of the embryo. *Tead4* mutants contain roughly the same number of cells as non-mutants at this stage. (**C**) Confocal section of non-mutant blastocyst from a *Cdx2* heterozygous intercross, following immunofluorescent staining to detect Cdx2 and Gata3 proteins and nuclear stain. Note that the zona pellucida (zp) and polar body (pb) can also be detected in some channels/planes. (**D**) Confocal section of *Cdx2* mutant blastocyst showing Cdx2 and Gata3 protein within the trophectoderm. For each mutation examined, images were collected within a single confocal session and with identical settings.

Cdx2 expression is also lost in *Tead4* mutants (Yagi et al., 2007; Nishioka et al., 2008), suggesting that Cdx2 could be required for *Gata3* expression during preimplantation. We therefore examined the requirement for *Cdx2* in Gata3 expression in the trophectoderm by examining Gata3 expression in *Cdx2* null embryos at E3.5. By confocal analysis, Gata3 expression was unaffected by loss of *Cdx2* (Fig. 6C,D) (n=6) at the blastocyst stage. This was validated at the mRNA level by qPCR (see Fig. S3 in the supplementary material). We conclude that *Cdx2* is not required for the expression of Gata3 during trophectoderm formation, consistent with the similar timing of their expression at earlier stages. Rather, *Gata3* appears to be regulated by Tead4 in parallel to *Cdx2* during blastocyst formation.

Oct4 does not restrict trophectoderm gene expression during early blastocyst formation

We have shown that, like Cdx2, Gata3 is initially expressed throughout the preimplantation embryo, suggesting that both genes become patterned by a process of repression within inside cells during blastocyst formation. *Oct4* is required for repression of *Cdx2* in ES cells (Niwa et al., 2000), consistent with a possible role for *Oct4* in repressing trophectoderm fates in the embryonic lineage. However, whether *Oct4* is required for repression of *Cdx2* in the inner cell mass during blastocyst formation has not been examined. Likewise, the role of Oct4 in regulating *Gata3* expression during blastocyst formation remains unknown.

To examine the requirement for *Oct4* in repressing *Cdx2* and *Gata3* in vivo, we examined the expression of these markers in embryos lacking zygotic *Oct4* (Kehler et al., 2004). At the blastocyst stage (E3.5), Oct4 protein was undetectable in *Oct4* mutants (Fig. 7B) (n=3), whereas Oct4 was detected throughout the blastocyst at this stage in non-mutants (Fig. 7A). However, Cdx2 and Gata3 expression patterns were largely unaffected in *Oct4* mutants (n=4/5 and n=2/2, respectively) (Fig. 7C). Although weak Cdx2 expression was detected in the inner cell mass of one *Oct4* mutant embryo, Cdx2 expression levels in the trophectoderm of this mutant embryo were also weaker than in non-mutant littermates (not shown). This pattern is normally observed in early blastocysts (Ralston and Rossant, 2008), suggesting that *Oct4* mutants can exhibit a slight developmental delay relative to non-mutant littermates. Indeed, this proposal is consistent with the previous observation that the trophectoderm marker keratin 8 (Krt8, detected by TROMA1 antibody) is detected in the inner cell mass of some, but not all, *Oct4* null embryos (Nichols et al., 1998). Since Krt8 is also expressed in the inner cell mass of early blastocysts (Ralston and Rossant, 2008), a developmental delay of *Oct4* mutants could explain this phenotype. Nonetheless, the majority of *Oct4* mutants exhibited the normal trophoblast-restricted expression of Cdx2 at the blastocyst stage. These results therefore suggest that zygotic *Oct4*

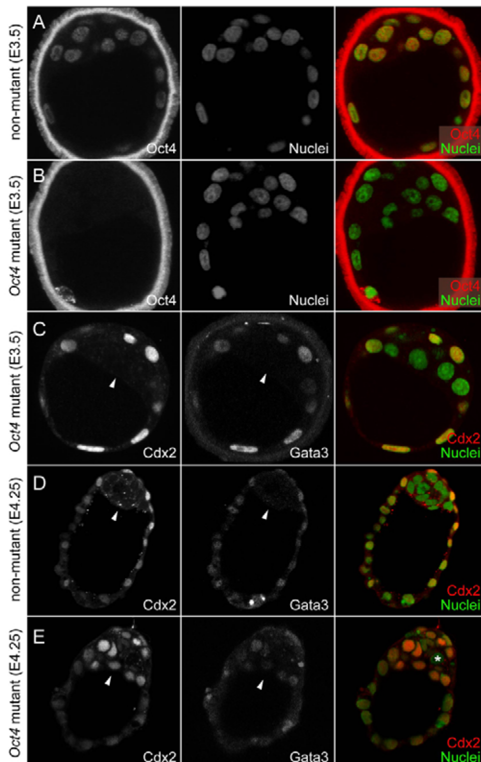


Fig. 7. Oct4 maintains, but does not initiate, repression of trophectoderm genes in the inner cell mass. (**A**) Confocal section showing Oct4 protein and nuclear stain in a non-mutant blastocyst resulting from intercrossing *Oct4* heterozygous mice. (**B**) Confocal section showing nuclear stain and lack of detectable Oct4 protein in *Oct4* mutant blastocyst. (**C**) Confocal sections showing Gata3 and Cdx2 protein in *Oct4* mutant blastocysts. Note the absence of detectable Gata3 or Cdx2 in cells of the inner cell mass (arrowheads). (**D**) Confocal sections showing Gata3 and Cdx2 protein and nuclei in a non-mutant implanting blastocyst from an *Oct4* heterozygous intercross. Note the lack of detectable nuclear Gata3 and Cdx2 in epiblast and primitive endoderm cells (arrowheads). (**E**) Confocal sections showing nuclear Gata3 and Cdx2 in cells occupying epiblast and primitive endoderm regions (arrowheads), and a cell that lacks expression of either (asterisk). For each litter examined, images were collected within a single confocal session and with identical settings.

is not required for initial repression of trophectoderm genes *Cdx2* or *Gata3*, indicating that other mechanisms lead to the restriction of both of these genes to the trophectoderm.

To examine whether *Oct4* is required for maintaining restricted expression of trophectoderm genes at later developmental stages, we attempted to examine the expression of Cdx2 and Gata3 in *Oct4* mutants after implantation, at ~E4.25. At this stage, *Oct4* mutants were extremely rare (1 mutant/27 non-mutant embryos), consistent with a requirement for *Oct4* for embryo survival. However, in a rare mutant recovered at this stage, Cdx2 and Gata3 were clearly upregulated in cells occupying epiblast and primitive endoderm territories (Fig. 7E). By contrast, both Cdx2 and Gata3 were always excluded from nuclei in epiblast and primitive endoderm populations in non-mutants (Fig. 7D) (n=26). Thus, *Oct4* is required for continued repression of *Cdx2* and *Gata3* in the late blastocyst, similar to its proposed role in repressing trophoblast fate in ES cells (Niwa et al., 2005). These observations indicate that the establishment of trophoblast and embryonic lineages proceeds by a mechanism that is distinct from the program that regulates this lineage restriction in established ES cells or the epiblast.

DISCUSSION

Here, we have used a combination of bioinformatic and functional genomic approaches to address fundamental questions about the first lineage restriction in the mouse. Specifically, what other factors act downstream of *Tead4*, are these sufficient or necessary to induce trophoblast fate, and are trophoblast factors themselves regulated in the embryo through mechanisms similar to those used in ES cells? Through genetic analyses performed in stem cells and in the mouse embryo, we provide evidence that *Gata3* acts downstream of *Tead4* and in parallel to *Cdx2*. A fundamental challenge in the field of stem cell biology is the paucity of truly trophoblast-specific markers. To overcome this challenge, we used TS cells as a reference tissue to define a set of core trophoblast genes. This enabled a deeper molecular comparison of trophoblast phenotypes resulting from the overexpression of *Gata3* or *Cdx2*, and provides a reference for future studies of this type.

We have shown that *Gata3* is sufficient to induce trophoblast genes in ES cells, consistent with another study (Nishiyama et al., 2009). Our analysis, however, revealed differences between *Gata3* and *Cdx2*. First, although expression of *Gata3* can induce trophoblast differentiation in ES cells, stable TS cell lines could not be maintained, unlike the situation with Cdx2. Rather, Gata3 appears to act as a pro-differentiation factor in TS cells. Second, unlike *Cdx2*, *Gata3* is probably not required for the early lineage decision in the embryo. Whereas shRNA-mediated knockdown of *Gata3* leads to developmental delay during the morula-to-blastocyst transition (Home et al., 2009), *Gata3* null embryos survive until E10.5, whereupon they exhibit defects in the placenta and numerous fetal tissues (Ma et al., 1997). Thus, *Gata3* is both necessary and sufficient to promote trophoblast maturation, but is not sufficient to stabilize the stem cell state. Other studies have shown that other factors, including Eomes, Elf5 and activated Ras, can also destabilize the pluripotent state of ES cells and drive trophoblast differentiation (Niwa et al., 2005; Lu et al., 2008; Ng et al., 2008; Nishiyama et al., 2009). Together, these observations suggest that there are multiple pathways capable of overriding the pluripotency program to induce trophoblast fate in ES cells.

Another intriguing difference between *Cdx2* and *Gata3* lies in their expression patterns at later stages of trophoblast development. Whereas *Cdx2* and *Gata3* were coexpressed in the EXE, *Gata3* was expressed at much higher levels within the EPC around the time of gastrulation. These observations suggest where Cdx2/Gata3 targets might be expressed. For instance, common trophoblast targets would be expected to be expressed in the EXE. Consistent with this proposal, many genes involved in EXE development, such as *Fgfr2*, *Wnt7b* and *Bmp4* (Orr-Urtreger et al., 1993; Coucouvanis and Martin, 1999; Kemp et al., 2005), were induced by either *Cdx2* or *Gata3* overexpression in ES cells. In addition, EXE genes such as *Eomes* and *Ascl2* (Guillemot et al., 1994; Ciruna and Rossant, 1999; Russ et al., 2000) were induced by Gata3 even in the absence of *Cdx2*, suggesting that Gata3 can reinforce trophoblast fate through a *Cdx2*-independent mechanism. Intriguingly, *Gata3* was expressed at higher levels in the EPC than in the EXE, whereas *Cdx2* was not. This provides potential biological relevance for the set of trophoblast genes that were induced by *Gata3* only in the absence of *Cdx2*. Genes in this list included *Pparg* and *Dnmt3l*, loss of which lead to defects in trophoblast differentiation (Barak et al., 1999; Bourc'his et al., 2001). Thus, Gata3 may promote a program of trophoblast

differentiation in the EPC where *Cdx2* expression is low or lacking. When overexpressed in TS cells, Gata3 induced differentiation of the cells towards more differentiated cell fates, consistent with this role. In this way, Gata3 could play a dual role, either promoting stem cell (EXE) fates or differentiation (EPC/giant cell fates) depending on the presence of other factors such as Cdx2. This proposal is consistent with evidence that *Gata3* is required for self-renewal of TS cells (Home et al., 2009). Moreover, Gata2 has been proposed to promote self-renewal versus differentiation of hematopoietic progenitor cells in a level-dependent manner (Heyworth et al., 1999), arguing that Gata3 might play a similar role in the trophoblast lineage.

We have also examined whether trophoblast factors are regulated in the embryo through mechanisms similar to those used in ES cells. In ES cells, Oct4 normally represses trophoblast fate (Niwa et al., 2000). However, it has not been clear whether this relationship applies to the embryo. Since Cdx2 and Gata3 are initially expressed in both inside and outside cells, Oct4 could repress the expression of these factors in inside cells during blastocyst formation. However, we show that Oct4 is not involved in the repression of trophoblast fate in the embryo until around the time of implantation. Indeed, trophoblast cells can coexpress Cdx2 and Oct4 in a variety of contexts (Niwa et al., 2005; Strumpf et al., 2005; Lu et al., 2008; Ng et al., 2008), arguing that Oct4 cannot be providing the initial patterning information along the inside/outside axis of the embryo. Rather, it was recently shown that the absence of Hippo signaling promotes Cdx2 expression in outside cells during blastocyst formation (Nishioka et al., 2009). Thus, the maintenance of ES cell fate might reflect molecular interactions that are relevant to stages of development following the initial lineage decisions. This proposal could help to explain why *Cdx2* is not required for Gata3-mediated induction of *Eomes* in ES cells, even though *Cdx2* is initially required for expression of Eomes in the blastocyst (Ralston and Rossant, 2008). Studies conducted in ES cells may therefore be viewed as reflecting a lineage maintenance, rather than establishment, program.

Finally, our study suggests that culture conditions influence cell fate changes induced by transcription factor overexpression in ES cells. Cdx2, and not Gata3, was sufficient to induce the formation of TS-like cells from ES cells. However, the isolation of stable TS-like cells is only possible following continued passage in TS cell medium after an initial period of transient transcription factor overexpression, a process that takes at least 6 days. This is reminiscent of the process involved in reprogramming mature cell types to pluripotency (Takahashi and Yamanaka, 2006), and suggests that similar mechanisms might be involved in the generation of stable TS cell lines. Given that many non-trophoblast genes were induced by the overexpression of either *Cdx2* or *Gata3*, altering culture and selection conditions could therefore lead to an enrichment of different cell fate outcomes. For instance, the use of different cell culture medium, growth factors or small molecules might enable the enrichment of TS-like cells or endoderm from *Gata3*-expressing ES cells. Given that multiple pathways can override the pluripotent state, the manipulation of intrinsic and extrinsic factors could facilitate the selection of other lineage-specific stem cell types during this process.

Acknowledgements
We thank Owen Tamplin for in situ help; Jorge Cabezas, Colm Rea and Andres Nieto for animal help; Malgosia Kownacka, Jodi Garner and Alison Hirukawa for cell culture help; Dr Yojiro Yamanaka, Dr Michael A. Halbisen and members of the J.R. laboratory for helpful suggestions; Dr Alexey Tomilin for the *Oct4* mutant mice; and Brian De Veale, the laboratory of Dr Derek van der Kooy and the Toronto Centre for Phenogenomics for help with mouse import. P.R.-G. was funded by a Canadian Institutes of Health Research Bisby Fellowship. J.S.D. was funded by the Biotechnology and Biological Sciences Research Council, UK. This work was funded by Grant #FRN13426 from the Canadian Institutes of Health Research.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material for this article is available at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.038828/-/DC1

References

Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A. and Evans, R. M. (1999). PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol. Cell* **4**, 585-595.
Beck, F., Erler, T., Russell, A. and James, R. (1995). Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. *Dev. Dyn.* **204**, 219-227.
Beddington, R. S. and Robertson, E. J. (1989). An assessment of the developmental potential of embryonic stem cells in the midgestation mouse embryo. *Development* **105**, 733-737.
Bourc'his, D., Xu, G. L., Lin, C. S., Bollman, B. and Bestor, T. H. (2001). Dnmt3L and the establishment of maternal genomic imprints. *Science* **294**, 2536-2539.
Chawengsaksophak, K., James, R., Hammond, V. E., Kontgen, F. and Beck. F (1997). Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* **386**, 84-87.
Ciruna, B. G. and Rossant, J. (1999). Expression of the T-box gene Eomesodermin during early mouse development. *Mech. Dev.* **81**, 199-203.
Coucouvanis, E. and Martin, G. R. (1999). BMP signaling plays a role in visceral endoderm differentiation and cavitation in the early mouse embryo. *Development* **126**, 535-546.
de Hoon, M. J., Imoto, S., Nolan, J. and Miyano, S. (2004). Open source clustering software. *Bioinformatics* **20**, 1453-1454.
Dietrich, J. E. and Hiiragi, T. (2007). Stochastic patterning in the mouse pre-implantation embryo. *Development* **134**, 4219-4231.
Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154-156.
George, K. M., Leonard, M. W., Roth, M. E., Lieuw, K. H., Kioussis, D., Grosveld, F. and Engel, J. D. (1994). Embryonic expression and cloning of the murine GATA-3 gene. *Development* **120**, 2673-2686.
Guillemot, F., Nagy, A., Auerbach, A., Rossant, J. and Joyner, A. L. (1994). Essential role of Mash-2 in extraembryonic development. *Nature* **371**, 333-336.
Heyworth, C., Gale, K., Dexter, M., May, G. and Enver, T. (1999). A GATA-2/estrogen receptor chimera functions as a ligand-dependent negative regulator of self-renewal. *Genes Dev.* **13**, 1847-1860.
Home, P., Ray, S., Dutta, D., Bronshteyn, I., Larson, M. and Paul, S. (2009). GATA3 is selectively expressed in the trophectoderm of peri-implantation embryo and directly regulates Cdx2 gene expression. *J. Biol. Chem.* **284**, 28729-28737.
Kehler, J., Tolkunova, E., Koschorz, B., Pesce, M., Gentile, L., Boiani, M., Lomeli, H., Nagy, A., McLaughlin, K. J., Scholer, H. R. et al. (2004). Oct4 is required for primordial germ cell survival. *EMBO Rep.* **5**, 1078-1083.
Kemp, C., Willems, E., Abdo, S., Lambiv, L. and Leyns, L. (2005). Expression of all Wnt genes and their secreted antagonists during mouse blastocyst and postimplantation development. *Dev. Dyn.* **233**, 1064-1075.
Kunath, T., Arnaud, D., Uy, G. D., Okamoto, I., Chureau, C., Yamanaka, Y., Heard, E., Gardner, R. L., Avner, P. and Rossant, J. (2005). Imprinted X-inactivation in extra-embryonic endoderm cell lines from mouse blastocysts. *Development* **132**, 1649-1661.
Lu, C. W., Yabuuchi, A., Chen, L., Viswanathan, S., Kim, K. and Daley, G. Q. (2008). Ras-MAPK signaling promotes trophectoderm formation from embryonic stem cells and mouse embryos. *Nat. Genet.* **40**, 921-926.
Ma, G. T., Roth, M. E., Groskopf, J. C., Tsai, F. Y., Orkin, S. H., Grosveld, F., Engel, J. D. and Linzer, D. I. (1997). GATA-2 and GATA-3 regulate trophoblast-specific gene expression in vivo. *Development* **124**, 907-914.
Manaia, A., Lemarchandel, V., Klaine, M., Max-Audit, I., Romeo, P., Dieterlen-Lievre, F. and Godin, I. (2000). Lmo2 and GATA-3 associated expression in intraembryonic hemogenic sites. *Development* **127**, 643-653.
Martin, G. R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. USA* **78**, 7634-7638.
Ng, R. K., Dean, W., Dawson, C., Lucifero, D., Madeja, Z., Reik, W. and Hemberger, M. (2008). Epigenetic restriction of embryonic cell lineage fate by methylation of Elf5. *Nat. Cell Biol.* **10**, 1280-1290.
Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H. and Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379-391.

DEVELOPMENT

Nishioka, N., Yamamoto, S., Kiyonari, H., Sato, H., Sawada, A., Ota, M., Nakao, K. and Sasaki, H. (2008). Tead4 is required for specification of trophectoderm in pre-implantation mouse embryos. *Mech. Dev.* **125**, 270-283.

Nishioka, N., Inoue, K., Adachi, K., Kiyonari, H., Ota, M., Ralston, A., Yabuta, N., Hirahara, S., Stephenson, R. O., Ogonuki, N. et al. (2009). The hippo signaling pathway components lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev. Cell* **16**, 398-410.

Nishiyama, A., Xin, L., Sharov, A. A., Thomas, M., Mowrer, G., Meyers, E., Piao, Y., Mehta, S., Yee, S., Nakatake, Y. et al. (2009). Uncovering early response of gene regulatory networks in ESCs by systematic induction of transcription factors. *Cell Stem Cell* **5**, 420-433.

Niwa, H., Miyazaki, J. and Smith, A. G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* **24**, 372-326.

Niwa, H., Toyooka, Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R. and Rossant, J. (2005). Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* **123**, 917-929.

Orr-Urtreger, A., Bedford, M. T., Burakova, T., Arman, E., Zimmer, Y., Yayon, A., Givol, D. and Lonai, P. (1993). Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2). *Dev. Biol.* **158**, 475-486.

Ralston, A. and Rossant, J. (2008). Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. *Dev. Biol.* **313**, 614-629.

Ray, S., Dutta, D., Rumi, M. A., Kent, L. N., Soares, M. J. and Paul, S. (2009). Context-dependent function of regulatory elements and a switch in chromatin occupancy between GATA3 and GATA2 regulate Gata2 transcription during trophoblast differentiation. *J. Biol. Chem.* **284**, 4978-4988.

Russ, A. P., Wattler, S., Colledge, W. H., Aparicio, S. A., Carlton, M. B., Pearce, J. J., Barton, S. C., Surani, M. A., Ryan, K., Nehls, M. C. et al. (2000). Eomesodermin is required for mouse trophoblast development and mesoderm formation. *Nature* **404**, 95-99.

Strumpf, D., Mao, C. A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F. and Rossant, J. (2005). Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. *Development* **132**, 2093-2102.

Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.

Tanaka, S., Kunath, T., Hadjantonakis, A. K., Nagy, A. and Rossant, J. (1998). Promotion of trophoblast stem cell proliferation by FGF4. *Science* **282**, 2072-2075.

Uy, G. D., Downs, K. M. and Gardner, R. L. (2002). Inhibition of trophoblast stem cell potential in chorionic ectoderm coincides with occlusion of the ectoplacental cavity in the mouse. *Development* **129**, 3913-3924.

Yagi, R., Kohn, M. J., Karavanova, I., Kaneko, K. J., Vullhorst, D., DePamphilis, M. L. and Buonanno, A. (2007). Transcription factor TEAD4 specifies the trophectoderm lineage at the beginning of mammalian development. *Development* **134**, 3827-3836.

Yamanaka, Y., Tamplin, O. J., Beckers, A., Gossler, A. and Rossant, J. (2007). Live imaging and genetic analysis of mouse notochord formation reveals regional morphogenetic mechanisms. *Dev. Cell* **13**, 884-896.

DEVELOPMENT

Table S1. Core trophoblast gene

Gene symbol
0610010B08Rik /// LOC627901 /// LOC628084
0610010K14Rik
0610012G03Rik /// LOC638521
0910001L09Rik
1100001H23Rik /// LOC100045163
1110001A07Rik
1110004E09Rik
1110007M04Rik
1110012J17Rik
1110020G09Rik
1110029I05Rik /// LOC100044848
1110034A24Rik
1110061A14Rik
1110065P19Rik /// 2310040A07Rik /// LOC100042420
1190002H23Rik
1190007I07Rik /// 1810014B01Rik
1190017O12Rik
1200009I06Rik
1200011I18Rik
1500005K14Rik
1500011B03Rik
1500011H22Rik
1500035H01Rik
1600014K23Rik
1600021P15Rik
1600029D21Rik
1700001E04Rik
1700007K13Rik
1700011M02Rik
1700037H04Rik
1700052K11Rik
1700086L19Rik
1810013L24Rik
1810020D17Rik
2010002N04Rik
2010106G01Rik
2010109K11Rik
2010204K13Rik
2200002D01Rik
2200002K05Rik
2210010L05Rik
2210010N04Rik
2210412D01Rik
2310008H09Rik
2310016E02Rik
2310026E23Rik
2310031A18Rik /// LOC100047808
2310033E01Rik
2310040A07Rik /// LOC100042420
2310040C09Rik
2310047D13Rik
2310057J16Rik
2310066E14Rik
2410015N17Rik
2410018C20Rik
2410022L05Rik
2410085M17Rik
2410116G06Rik
2600005O03Rik
2600010E01Rik
2610021A01Rik
2610027C15Rik
2610028L16Rik
2610029G23Rik
2610036L11Rik
2610201A13Rik
2610207I05Rik
2610528E23Rik /// Frag1
2700007P21Rik
2700094F01Rik
2700094K13Rik
2810003C17Rik
2810004N23Rik
2810021G02Rik
2810025M15Rik
2810026P18Rik
2810410L24Rik
2810410M20Rik
2810417H13Rik
2810433K01Rik
2810457I06Rik /// LOC677224
2810474O19Rik
2900002H16Rik
2900026A02Rik
2900053A13Rik
3000004C01Rik
3110001A13Rik
3110009E18Rik
3110048E14Rik
3222402P14Rik
3830408D24Rik
3830417A13Rik
3830431G21Rik
3830612M24
4631422O05Rik
4632432E15Rik
4732435N03Rik
4832420A03Rik
4833408C14Rik
4922503N01Rik
4930428B01Rik
4930515G01Rik
4930519N13Rik
4930547N16Rik
4930579G24Rik
4930583H14Rik
4931406C07Rik
4932417I16Rik
4932442L08Rik
4933404O12Rik
4933413G19Rik /// Foxm1 /// Pebp1
5033421C21Rik
5230400G24Rik /// LOC637796
5430416N02Rik
5730408K05Rik
5730446C15Rik
5730601F06Rik
5830404H04Rik
5830483C08Rik
5930416I19Rik
6030408C04Rik
6030426L16Rik /// LOC100043371
6230416J20Rik
6230424C14Rik
6230427J02Rik
6330442E10Rik
6330503K22Rik
6330505N24Rik
6330509M23Rik
6430510B20Rik
6530401C20Rik
6720460F02Rik
6720463M24Rik
9030409G11Rik
9230111E07Rik
9330112F22Rik
9430029L20Rik
9430079B08Rik
9530018I07Rik
9630050M13Rik
9830001H06Rik
9930012K11Rik
A030007L17Rik
A130022J15Rik
A230083H22Rik
A830080D01Rik
A930008G19Rik
A930041I02Rik
AA408865
AA467197
Aard
Abca1
Abcb7
Abcc4
Abcd4
Abhd12
Abhd14a
Abhd2
Abhd3
Abhd4
Abhd5
Abhd6
Acaa1a
Acaa1a /// Acaa1b
Acaa1b
Acot1 /// Acot2 /// LOC100044830
Acot1 /// LOC100044830
Acox1
Acp5
Acpl2
Acpp
Ada
Adamts1
Adamts4
Adcy7
Adcy9
Adk
Adra2a /// LOC100044679
Adra2b
Afap1l2
Ahcy
AI314976
AI413582
AI425999
AI465270
AI480535
AI481772
AI661453
AI851523
Ak1
AK220484
Ak3
Ak3l1 /// LOC100047616
Akap11
Akap13
Akap2
Akp2
Akr1b3
Akt1 /// LOC100047666
AL118220
Alas1
Alas1 /// LOC100045674
Aldh18a1
Aldh2
Aldh3b2
Aldh5a1
Alg10b
Alg13
Als2cr4
Amd1 /// Amd2 /// LOC100040814 /// LOC100041585 //
Amfr /// LOC100046262

Amot
Ampd2
Ankrd12
Ankrd13a
Ankrd25
Ankrd28
Ankrd47
Ankrd50
Anln
Anp32a
Anp32e
Anxa1
Anxa11 /// LOC100039484 /// LOC100039503
Anxa4
Aof1
Ap1s3
Ap2b1
Ap3m2
Apba1
Apcdd1
Apex1
Apob48r
Apoc1
Apoe
App
Aqp3
Ard1
Arf3
Arhgap18
Arhgap19
Arhgap29
Arhgap30
Arhgap8
Arhgdib
Arhgef3
Arhgef6
Arih2
Arl2
Arl3
Arl5b
Arl6ip2
Arpp19
Arrb2
Asahl
Ascl2
Asns
Asph
Aspm
Asxl1
Ate1
Atf6
Atf7
Atf7ip2
Atg16l2
Atg4a /// LOC100038961 /// LOC100045570
Athl1
Atm
Atp1b1
Atp2a2
Atp2a3
Atp2c2
Atp5k
Atp6v0a1
Atrx
Atxn3
AU020206
AU023006
Auh
Aurka
Aurkb

Auts2
AW061234
AW124722
AW214353
AW494418
AW544981
AW550831
B230120H23Rik
B3gnt3
B3gnt8
B430119L13Rik
B4galt6 /// LOC675709
B830017H08Rik
B930006L02Rik
Bag4
Baiap2
Bat5
BB001228
Bbx
BC004044
BC016495
BC017612
BC023744
BC027344
BC031748
BC033915 /// LOC100048082
BC038156
BC043118
BC043476
BC046404 /// LOC100045343
BC049349
BC050078
BC055324
Bcam
Bcas2
Bcl2l11
Bcl7a
Bcl9
Bclaf1
Bdh1
Bex4
BF642829
Bhlhb2
Bicd2
Birc5
Blcap
Blmh
Bmp1
Bmp2k
Bmp4
Bmp8b
Bok
Bola2
Braf
Brca2
Brd3
Brd8
Brp44l /// LOC100041649 /// LOC100047509
Brpf3
Bspry
Btbd1
Btbd11
Btbd14b
Btd
Bub1
Bub1b
Bub3
Bzw1 /// LOC382722 /// LOC667737
C030014M07Rik
C030033M12Rik
C130069I09Rik /// LOC100043772 ///

LOC100048150
C1qtnf6
C230013L11Rik
C230091D08Rik
C330027C09Rik
C530028O21Rik
C730049O14Rik
C77370
C79267
C79407
C920025E04Rik /// H2-T23 /// LOC100046736
Cad
Cadm1
Camk2d
Camkk2
Camta2
Canx
Capn1
Caprin1
Car5b
Card10
Casc4
Casc5
Caskin1
Casp3
Casp7
Cbfa2t3h
Cbs
Cbx4
Cbx5
Cbx6
Ccdc34
Ccdc43
Ccdc64
Ccdc69
Ccdc77
Ccdc93
Ccdc98
Ccna2
Ccnb1
Ccnb1 /// Ccnb1-rs1
Ccnb1 /// Ccnb1-rs1 /// EG434175 /// LOC667005
Ccnb2
Ccnd1
Ccne2
Ccng1
Ccni
Ccnj
Ccny /// LOC100044842
Ccrl2
Cd276
Cd300a
Cd40
Cd55
Cd81
Cd82
Cdc14b
Cdc20
Cdc25b
Cdc25c
Cdc2l6
Cdc42ep3
Cdca2
Cdca3
Cdca5
Cdca7
Cdca7l
Cdca8
Cdh10
Cdh3

Cdh5
Cdk5r1
Cdkn3
Cdon
Cds1
Cds2
Cdx2
Cdyl2
Ceacam9
Cebpa
Cebpg
Ceecam1
Celsr1
Cenpa
Cenpe
Cenpf
Cenpk
Cenpl
Cenpm
Cenpn
Cenpp
Centa1
Centb1 /// LOC100045877
Centg2
Cep27
Cep55
Cgn
Cgref1
Chac1
Chd1
Chd7
Chdh
Chek1
Chek2
Chpt1
Chst11 /// Phactr1
Chst12
Chst2
Cit
Cited2
Ckap2
Cks1b
Cks2 /// LOC100039474 /// LOC100044750 /// LOC1000
Cks2 /// LOC100039474 /// LOC100044764
Clcn3
Clcn5
Cldn23
Cldn3
Cldn6
Clic5
Clip2
Clip3
Cln3
Clstn1
Clu /// LOC100046120
Cmtm7
Cnih4
Cnn3
Cnn3 /// LOC100047856
Cnnm2
Cnp
Col18a1
Col3a1
Copg2
Cops7a
Cox6b2
Cox7a2
Cpd /// LOC100046781
Cpm
Cpne3

Cpne8
Cpsf2
Cpsf6
Cpt1a
Crebzf
Crispld2
Csnk2a1
Ctbp2
Ctdspl2
Ctla2a
Ctla2a /// Ctla2b
Ctla2b
Ctnnal1
Cts7
Cts8
Ctsa
Ctsb
Ctsj
Ctsq
Ctsr
Cttn
Cuedc2
Cul5
Cutl1
Cxadr
Cxcr7
Cxxc6
Cyb5r3
Cyp17a1
Cyp26a1
Cyp51
Cyr61
D10627
D10Ertd322e
D11Ertd497e
D16Bwg1494e
D16Wsu65e
D1Bwg0212e
D230012E17Rik
D2Ertd750e
D330027G24Rik
D3Ertd300e
D3Ertd508e
D4Wsu114e
D5Ertd40e
D5Wsu178e
D630045J12Rik
D6Wsu163e
D7Ertd143e
D8Ertd82e
Daam1
Dad1
Dalrd3
Dap
Dbt
Dci
Dcxr
Ddah1
Ddc
Ddit3
Ddit4
Ddr1
Ddx5
Ddx58
Decr1
Def8
Dennd1a /// LOC100047738
Dennd2a
Denr
Depdc1a
Depdc1b
Dfna5h

Dgka
Dgke
Dhcr24
Dhodh
Dhrs3
Dhx36
Diap2
Dicer1
Dkc1
Dlc1
Dlg3
Dmrta2
Dmrtc1a
Dmxl2
Dnajb1
Dnajc9
Dnali1
Dnmt3a
Dnmt3b
Dnmt3l
Dock11
Dock8
Dok2
Dolk
Dpp7
Dppa2
Dppa4
Dst
Dtl
Dtnb
Dtx1
Dusp4
Dusp5
Dusp6
Dusp7 /// LOC674944
Dut
DXBay18 /// EG434795 /// EG434797 /// EG546325 ///
Dyrk3
E130203B14Rik
E2f5
E2f7 /// LOC639365
E2f8
E330009J07Rik
Ect2
Eda2r
Edg1
Edg4
Ednrb
Eef2k
Efna3 /// LOC100046031
Efna4
EG434858 /// EG665646 /// Hnrpa1 /// LOC100043855
EG546265 /// Ube2i
EG665407 /// EG668032 /// LOC100043295 /// LOC1000
EG665903 /// LOC100044729 /// LOC100046579 /// Lsm
EG665989 /// EG667598 /// LOC433064 /// LOC624822
EG665989 /// LOC433064 /// LOC629952 /// LOC666411
EG666684 /// EG666772 /// EG666890 /// ENSMUSG0000
EG666684 /// EG666772 /// ENSMUSG00000057445 /// E
EG668339
Egfl7
Egln3
Ehbp1
Ehhadh

Eid2
Eif1ay
Eif2c1
Eif2s1
Eif2s2
Eif4e
Eif4e /// LOC630527
Eif4e3
Elf5
Elk3
Elmo1
Elovl5
Elovl6
Emid1
Emilin1
Enc1
Eng
ENSMUSG00000074630
Entpd1
Eomes
Epas1 /// LOC100048537
Epb4.1l2
Epb4.1l3
Epha2
Epha4
Ephb3
Eps8
Eps8 /// LOC632638
Eras
Erbb2
Erbb3
Erf
Ergic1
Ern1
Errfi1
Esam1
Esco2
Espl1
Esrrb
Etohi1 /// LOC100043126 /// LOC100043914 /// LOC62
Etv4
Etv5
Exoc4
Exod1
Ext1
F2rl1
F630043A04Rik
F630110N24Rik
Fabp5
Fabp5 /// LOC547041 /// LOC620603
Fads1
Fads3
Fancd2
Fasn
Fblim1
Fbxo10
Fbxo32
Fbxo39
Fbxo5
Fchsd2
Fdps
Fdxr
Fgd1
Fgd5
Fgd6
Fgfbp1
Fgfr1
Fgfr1op
Fgfr2
Fgfr4
Fgfrl1 /// LOC100046239

Fhl1
Fhl2
Fhod1
Fkbp3
Flt1
Flvcr2
Fn1
Fnbp1
Fnip1
Folr1
Fosl1
Foxh1
Foxj2
Foxo1
Fpgs
Frrs1 /// LOC100046401
Fscn1
Fstl3
Fxc1
Fxr1h
Fyn
Fzd1
G3bp2
Gab2
Gale
Galnt10
Galnt11
Gas2l3
Gas2l3 /// LOC100047967
Gas5
Gata3
Gatad2a
Gba
Gbe1
Gbp2
Gbp3
Gbp6
Gcat
Gclm
Gcnt1 /// LOC635918
Gcnt2
Gcsh /// LOC674321
Gdpd1
Gdpd5
Gemin6
Gfpt1
Ghrh
Gins1
Gipc2
Git2
Gjb2
Gkap1
Glb1
Gldc
Glis2
Glo1
Gm1752
Gm237
Gm2a
Gm9
Gmfb
Gmip
Gmppb
Gmps
Gnb4
Gng10
Gng2
Gnpnat1 /// LOC665402 /// LOC676647
Golga2
Golga4
Got1l1

Gp1bb /// LOC100044138 /// Sept5
Gpc1
Gpkow
Gpr116
Gpr126
Gpr137b
Gpr137b /// Gpr137b-ps
Gpr137b /// Gpr137b-ps /// LOC100044979
Gpr137b /// LOC100044979
Gpr137b-ps
Gpr153
Gpr50
Gpr56
Gprasp1
Gprc5a
Gpx3
Gramd1b
Grcc10
Grhl3
Grina
Grk6
Grlf1
Gsg2
Gspt1
Gstcd
Gstk1
Gsto1
Gtf2i
Gtf2ird1
Gtl2
Gtpbp1
Gtpbp10
Gtse1
Gyltl1b
H2afv
H2afy2 /// H2afy3
H2afz
H2afz /// LOC626950
Hat1
Hbxip
Hcn2
Hdac2
Hdac5
Hdlbp
Herpud1
Hexb
Hibch
Hic1
Hif1a
Hip1
Hirip3
Hivep2
Hk1
Hmga2
Hmgb2
Hmgb2l1 /// LOC100045749
Hmgb3
Hmgcl
Hmgcr
Hmgcs1 /// LOC100040592
Hmmr
Hnrpa0
Hnrpa1
Hnrpa3
Hnrpab
Hnrpc
Hnrpdl
Hnrph2
Homer1
Hpse
Hrb

Hrc /// Tcfap2a
Hs2st1
Hs3st1
Hs3st3b1
Hs6st1 /// LOC100047260
Hsbp1
Hsd11b2
Hsd17b11
Hsd17b2
Hspa4
Htatip2
Htatsf1
Htra1
Htra2
Icam1
Icosl
Id1
Id2
Id3
Ide
Idh3a
Idi1
Ier2
Ier3
Ier5l /// LOC100047268
Ifitm2
Igf1r
Igsf3
Il28ra
Il6st
Impad1
Ing1
Ing1 /// LOC100045040
Ing2
Inhbb /// LOC100046802
Inpp1
Inpp5a
Insig1
Insm1
Ints8
Ippk
Iqgap3
Irak2
Irf1
Irs3
Irx1
Irx2 /// LOC100045612
Irx5
Isg20
Isg20l1
Islr2
Isoc1
Itga1
Itga5
Itga6
Itgb3
Itgb5
Itm2b
Itm2c
Itpr1
Jak1
Jmjd3
Junb
Jup
Kbtbd11
Kcnk1
Kctd1
Kctd12
Kctd17
Kctd6
Kif11
Kif18a

Kif20a
Kif21b
Kif22
Kif26a
Kif2c
Kif2c /// LOC631653
Kifc1 /// LOC100042970 /// LOC100044006 /// LOC100
Kit
Kitl
Klc4
Klf13
Klf4
Klf5
Klhdc2
Klhdc5
Klhl24
Kntc1
Kpnb1
Krt15
Krt19
L1cam
L2hgdh
Lama1
Lamp2
Laptm4b
Las1l
Las1l /// LOC100044857
Lasp1
Lbr
Lcor
Lcp1
Ldlr
Ldlrad3
Ldlrap1
Ldoc1
Lgals3bp
Lgals8
Lgals9
Lhfp
Lhfpl2
Lhx2
Lif
Lima1
Limk1
Lims2
Lin28
Lipg
Liph
Lmbr1
Lmnb1
LOC100039155 /// Snx9
LOC100039343 /// LOC100045062 /// Timm8a1
LOC100040525 /// LOC100040596 /// Tmem181
LOC100041484 /// LOC100044056 /// LOC100046623 ///
LOC100042000
LOC100042253 /// LOC100044607 /// LOC100046670 ///
LOC100044101 /// Txnrd2
LOC100044162 /// Sema3e
LOC100044232 /// Rasgef1b
LOC100044313 /// Rhox4a /// Rhox4b /// Rhox4c ///
LOC100044395 /// Rbpms
LOC100044683
LOC100044735 /// Zxda
LOC100045440 /// Rg9mtd2
LOC100045503 /// Tmem108
LOC100045567 /// LOC667034 ///

Pnp
LOC100045567 /// Pnp
LOC100045629
LOC100045707 /// Pou3f1
LOC100045887
LOC100045971 /// Tm7sf3
LOC100045988
LOC100045988 /// Zic5
LOC100046143
LOC100046333 /// Zfp423
LOC100046393 /// LOC545704 /// LOC666386 /// Ppp2r
LOC100046586
LOC100046589 /// Pafah1b1
LOC100046698
LOC100046988 /// LOC624275 /// Paox
LOC100047199 /// Odf2
LOC100047200 /// Tbx3
LOC100047202 /// Mmp1a
LOC100047268
LOC100047292
LOC100047324 /// Sesn1
LOC100047506 /// Pbx3
LOC100047539
LOC100047579 /// Tmem20
LOC100047592 /// Tmem63b
LOC100047651 /// Zfpm1
LOC100047749 /// Pde8a
LOC100047896 /// Zfand5
LOC100047936 /// Sorl1
LOC100048307 /// Slc35f2
LOC100048460 /// Lzts2
LOC100048636 /// Pla2g4d
LOC100048654 /// Pabpc4
LOC245350 /// LOC634012
LOC545261
LOC630539 /// Trim59
LOC630776 /// Nid1
LOC631639 /// Lonrf1
LOC632664 /// Ptprg
LOC666793 /// Rps24
LOC671878 /// Sms
LOC672215 /// Ntn1
LOC674944
LOC675709
LOC677224
Lonp2
Lonrf3
Lphn1
Lpl
Lrig2
Lrrc40
Lrrc8c
Lrrfip1
Lrrfip2
Lsm3
Lsm6
Lsm8
Ltb4dh
Luc7l
Ly6a
Lyst
Mad2l1
Maged1
Magi3
Map3k8
Mapk1
Mapk13
Mapkapk3
Mapre2
Marcks

Matr3
Mbnl3
Mcm7
Mdm4
Med13l
Med14
Meis1
Melk
Mertk
Mest
Met
Metrnl
Mfge8
Mfsd2
Mgat4a
Mgll
Micall2
Midn
Minpp1
Mki67
Mll1
Mll3
Mmp14
Mnd1
Mobkl2c
Mogat2
Morc4
Mospd1
Mpa2l
Mphosph1
Mphosph9
Mpped2
Mreg
Mrg1
Mrpl33
Mrpl50
Mrps24
Msh2
Msh6
Msi2
Msl31
Msx2
Mt1
Mtap7
Mtdh
Mtf2
Mthfd1
Mtm1
Mtmr4
Mtss1
Muc1
Mvd
Mvp
Mxd4
Myb
Mybl2
Mycbpap
Myef2
Myh10
Myl4
Myo1b
Myo1c
Myo1d
Nagpa
Nap1l1
Nasp
Nat8l
Nat9
Ncam1
Ncapd2
Ncapg
Ncapg2

Ncaph
Ncl
Ncoa3
Nde1
Ndrg2
Ndst1
Ndufa12l
Nek2
Nfat5
Nfatc2ip
Nfkb1
Nfya
Nfyb
Ngly1
Niban
Nin
Nipsnap1
Nkrf
Nme4
Nmral1
Nmt2
Nnt
Nola3
Nolc1
Nos2
Nostrin
Notch1
Notch2
Notch3
Npcd /// Nptxr
Nppb
Npr2
Nr0b1
Nr2f2
Nr4a1
Nrarp
Nrip1
Nrk
Nrm
Nsbp1
Nsdhl
Nt5e
Nuak2
Nucb2
Nucks1
Nudcd2
Nudt21
Nuf2
Nup155
Nup43
Nup50
Nupr1
Nusap1
Oaf
Oas1c
Oip5
Oprs1
Optn
Osbpl8
OTTMUSG00000000724 /// Serpinb9e /// Serpinb9f ///
Oxct1
P2rx4
Pa2g4
Paics
Paip1
Palb2
Palm
Pam
Paqr4
Park7
Parp1

Parp4
Pask
Patz1
Pbef1
Pbk
Pcbp2
Pcbp4
Pcca
Pccb
Pcdh12
Pck2
Pcmtd1
Pcnp
Pcsk1n
Pcx
Pdcd10
Pdcd6ip
Pde10a
Pde1c
Pde4dip
Pdgfra
Pdia4
Pdia5
Pdk1
Pdk3
Pdlim2
Pds5b
Pdx1
Pdxp
Pea15a
Peg10
Perp
Pfas
Pfn2
Pftk1
Pgls
Pgs1
Phactr1
Phf19
Phip
Phlda1
Phpt1
Pi4kb
Pif1
Pigf
Pik3cb
Pitpna
Pkd2l1
Pklr
Pkn1 /// Ptger1
Pkp2
Pkp4
Pla2g4a
Pla2g4f
Plaa
Plac1
Plac8
Plagl1
Plat
Plcb4
Plcg1
Pld1
Plekha3
Plekha6
Plekhg1
Plekhg3
Plekhg5
Plekhg6
Plekhh3
Plekhm1
Plk1
Plk2

Plod1
Plxnb1
Pmaip1
Pmm1
Pmvk
Pnkd
Pnma1
Pnpla3
Pofut1
Pold2
Pold3
Pole
Polh
Polr3g
Pomp
Ppap2a
Ppap2b
Ppap2c
Ppard
Pparg
Ppat
Ppfibp2
Ppm1a
Ppm1f
Ppp1r15b
Ppp1r3g
Ppp2ca
Ppp2r2c
Ppp2r5c
Ppp3r1
Ppp5c
Praf2
Prc1
Prdm1
Prkar1a
Prkar2a
Prkch
Prl2b1
Prl3b1
Prl4a1
Prl5a1
Prl6a1
Prl7c1
Prl7d1
Prmt2
Prmt7
Prodh
Prpf39
Prpf40a
Prps1
Prrc1
Prtg
Psat1
Pscd1
Psd4
Psmc3ip
Psmc6
Psrc1
Pstk
Pstpip1
Ptbp2
Ptch1
Ptger3
Ptgfrn
Pthlh
Pthr1
Ptk2b
Ptk7
Ptp4a3
Ptpn14
Ptpn21
Ptprg

Pttg1
Pvt1
Pwwp2
Pxmp4
Pxn
Pycr1
Qpct
Qsox1
Rab31
Rab34
Rab43
Rab6b
Racgap1
Rad51ap1
Rad54l
Ralgds
Ran /// Rasl2-9
Rangrf
Rapgef1
Rapgef3
Rasa3
Rassf4
Rassf6
Rassf8
Rbm20
Rbm38
Rbms2
Rbmx
Rbpj
Rbpms
Rbpms2
Rdh12
Reep5
Rfc4
Rgs19
Rgs2
Rhebl1
Rhobtb1
Rif1
Ripk4
Rnaseh2c
Rnd2
Rnf12
Rnf125
Rnf141
Rnf149
Rnf157
Rnf187
Rnf24
Rnf38
Rpl15
Rpl22
Rpl23
Rpl31
Rpo1-3
Rpp21
Rps3
Rps6ka6
Rps6kb1
Rrbp1
Rrp1b
Rsad2
Rsf1
Rsrc1
Rtn1
Rusc2
Rwdd4a
S100a13
Saal1
Sall1
Sall2
Samd9l

Samhd1
Sap30
Saps3
Satb1
Sbk1
Sbsn
Sc4mol
Scarb2
Schip1
Scmh1
Sct
Sdc1
Sdhd
Sec14l1
Seh1l
Selm
Sema3f
Sema4c
Sema4d
Sema6a
Sema6d
Sema7a
Senp7
Sept8
Sept9
Serbp1
Serpinb6b
Serpinb9
Serpinb9b
Serpinb9c
Serpinb9g
Serpinh1
Sesn2
Setd1b
Sf3b1
Sf3b5
Sfrs1
Sfrs17b
Sfrs18
Sfrs3
Sfrs6
Sfrs7
Sft2d2
Sgk2
Sgms1
Sgol1
Sgol2
Sgpl1
Sgpp1
Sh2b2
Sh3bgrl2
Sh3bp1
Sh3bp4
Sh3kbp1
Shcbp1
Shmt1
Shprh
Shroom1
Sin3b
Sipa1l3
Siva1
Six4
Skap2
Ski
Skil
Skp2
Slc13a4
Slc15a2
Slc16a13
Slc16a3
Slc16a9
Slc1a4

Slc22a5
Slc25a10
Slc25a20
Slc25a36
Slc25a37
Slc25a39
Slc26a2
Slc2a12
Slc2a3
Slc30a1
Slc31a2
Slc35e4
Slc38a1
Slc39a14
Slc40a1
Slc44a1
Slc4a7
Slc4a9
Slc5a3
Slc6a2
Slc7a6
Slc9a6
Slco2a1
Slco4a1
Slk
Smad1
Smad3
Smad6
Smarca2
Smarca5
Smc2
Smo
Smpd1
Smtn
Smtnl2
Smyd3
Snai1
Snai3
Snap91
Snf1lk
Snhg6
Snhg7
Snord22
Snrk
Snrpd1
Snrpd3
Snx1
Snx10
Snx16
Snx22
Snx5
Snx9
Soat1
Socs2
Socs3
Socs5
Solh
Sord
Sos1
Sox2
Sox21
Spag5
Spata13
Spc24
Spc25
Speg
Spred1
Spred2
Spry2
Spry4
Spsb1
Spsb4

Sptlc2
Srd5a1
Srebf2
Srpk1
Srr
Srxn1
Ss18
Ssbp3
Ssbp4
Ssh3
St13
St3gal1
St3gal4
St6galnac2
St6galnac4
St6galnac6
Stambp
Stard10
Stard4
Stard8
Stk10
Stk35
Stk39
Stmn3
Stra13
Stra6
Sub1
Sugt1
Suhw3
Sulf2
Suox
Suv39h2
symb
Tacstd1
Tacstd2
Taf9b
Tanc2
Tbc1d10b
Tbc1d2b
Tbc1d4
Tbc1d8
Tbpl1
Tbx20
Tbx3
Tcea1
Tceb1
Tceb3
Tcf4
Tcf7
Tcfap2a
Tcfcp2l1
Tcfl5
Tchp
Tcn2
Tdp1
Tdrd7
Tead4
Tec
Tenc1
Terf1
Tesk2
Tfrc
Tgfbr3
Tgif1
Tgm1
Tgm2
Thoc6
Thyn1
Timm17b
Timp2
Tiparp
Tipin

Tlr3
Tm6sf1
Tm7sf3
Tm9sf3
Tmc4
Tmem104
Tmem106a
Tmem140
Tmem144
Tmem166
Tmem180
Tmem181
Tmem183a
Tmem2
Tmem34
Tmem37
Tmem4
Tmem48
Tmem50b
Tmem55a
Tmem58
Tmem64
Tmem86a
Tmem9b
Tmpo
Tmprss2
Tnfaip2
Tnfrsf9
Tnk2
Tnks2
Tnrc18 /// Zfp469
Tns1
Tns4
Tomm70a
Top1mt
Top2a
Top3b
Tor1aip1
Tpbg
Tpbpa
Tpbpb
Tpd52l2
Tpm2
Tpp1
Tpst1
Tpx2
Traf3ip1
Traf4
Traf5
Tram2
Trib1
Trib3
Trim24
Trim33
Trim37
Trim44
Trim47
Trim59
Trim68
Trim7
Trim8
Trio
Trnt1
Trp53
Trp53inp1
Trpv2
Tsc22d3
Tspan9
Tst
Ttc21b
Ttk
Ttll4

Ttll5
Tulp3
Twsg1
Txndc10
Txndc11
Txnl1
Uaca
Ube2c
Ube2d2
Ube2d3
Ube2i
Ube2o
Ube2t
Ubn1
Ubqln4
Ubr1
Uck2
Ugdh
Ugp2
Umps
Unc119
Unc5b
Upf3b
Uqcrh
Usp14
Usp15
Usp25
Usp27x
Utf1
Utp14a
Vamp5
Vangl2
Vash2
Vat1
Vav3
Vbp1
Vegfa
Vezf1
Vgf
Vgll3
Vhlh
Vim
Vps24
Vps36
Was
Wasl
Wbscr27
Wdr12
Wdr35
Wdr40b
Wdr62
Wdr77
Wdr8
Wfdc2
Wfs1
Whsc1l1
Wipf3
Wipi1
Wnk1
Wnt6
Wnt7b
Wrnip1
X99384
Xdh
Xpo5
Xpot
Xylb
Yme1l1
Ythdf3
Zbed3
Zbtb7c
Zc3h12c

Zc3h7b
Zcchc3
Zdhhc14
Zfand2a
Zfand5
Zfhx3
Zfp110
Zfp219
Zfp26
Zfp28
Zfp281
Zfp335
Zfp367
Zfp37
Zfp462
Zfp568
Zfp608
Zfp7
Zfp90
Zim1
Zmat3
Zmynd11
Zmynd19
Znhit1
Zranb3
Zwilch
Zxdb
Zxdc
Genes exhibiting a 2-fold change or greater during the course of differentiation of TS3.5 and TS6.5 cells were retained for further analyses

Table S2. Trophoblast genes induced by *Gata3* and *Cdx2*

Gata3 induced	Common	Cdx2 induced
Gene symbol	Gene symbol	Gene symbol
0610010B08Rik /// LOC627901 /// LOC628084	1100001H23Rik /// LOC100045163	1190002H23Rik
1110012J17Rik	1200009I06Rik	2200002K05Rik
1190017O12Rik	1500005K14Rik	3830612M24
1500011H22Rik	1600014K23Rik	4932442L08Rik
1700052K11Rik	1600021P15Rik	6030426L16Rik /// LOC100043371
2010002N04Rik	2010109K11Rik	AA408865
2010204K13Rik	2200002D01Rik	Acpp
2310016E02Rik	2310040C09Rik	Adra2b
2310026E23Rik	2310057J16Rik	AI425999
2310047D13Rik	2600010E01Rik	AI465270
2810417H13Rik	2610027C15Rik	Alas1 /// LOC100045674
2810457I06Rik /// LOC677224	2810003C17Rik	Anxa11 /// LOC100039484 /// LOC100039503
2900053A13Rik	2900026A02Rik	Aof1
3110001A13Rik	3830417A13Rik	Arhgef3
4631422O05Rik	6330505N24Rik	Atg16l2
4922503N01Rik	9530018I07Rik	Bat5
4933413G19Rik /// Foxm1 /// Pebp1	9830001H06Rik	BC023744
6720460F02Rik	Abcd4	BC038156
9930012K11Rik	Abhd5	BC046404 /// LOC100045343
Abhd6	Acot1 /// Acot2 /// LOC100044830	Bdh1
Acaa1a /// Acaa1b	Acot1 /// LOC100044830	C920025E04Rik /// H2-T23 /// LOC100046736
Acox1	Adcy7	Cbfa2t3h
Acpl2	Afap1l2	Ccne2
Adamts1	AI481772	Cdx2
Adamts4	AI661453	Cln3
Ak3	AK220484	Dmxl2
Akap2	Akap13	Dock8
Aldh5a1	Alas1	Dusp7 /// LOC674944
Ankrd50	Amfr /// LOC100046262	Eif4e3
Anxa1	Ampd2	Elf5
Arhgap18	Anxa4	Fgd6
Arhgdib	Apob48r	Fpgs
Asahl	App	Fstl3
Asph	Arhgap29	Gata3
Athl1	Arhgap8	Gkap1
Atp1b1	Ascl2	Gm9
Atp6v0a1	Atp2c2	Gp1bb /// LOC100044138 /// Sept5
AW550831	B230120H23Rik	Gpkow
BC017612	B430119L13Rik	Gpr56
BC031748	B4galt6 /// LOC675709	Grhl3
Bcam	Bbx	Gyltl1b
Bcl2l11	BF642829	Hsd11b2
Bcl9	Bicd2	Icosl
Bhlhb2	Blcap	Irak2
Bmp1	Bmp8b	Irx2 /// LOC100045612
Bmp4	Bspry	Kif26a
Bok	C79267	Krt15
C230013L11Rik	Camk2d	Las1l /// LOC100044857
Ccdc64	Camta2	Lcp1
Ccnd1	Capn1	Lgals3bp
Cd276	Card10	Lgals8
Ceecam1	Ccdc93	Lims2
Cgref1	Cd55	LOC100044313 /// Rhox4a /// Rhox4b /// Rhox4c ///
Clip3	Cdon	LOC100044683
Clstn1	Cebpa	LOC100048307 /// Slc35f2
Clu /// LOC100046120	Cgn	LOC674944
Cmtm7	Chst12	Lonrf3
Col18a1	Cited2	Metrnl

Col3a1	Cldn6	Mpped2
Cpne8	Cpm	Niban
Ctsa	Cxcr7	Nsbp1
Cyp26a1	D330027G24Rik	Pcsk1n
Cyp51	D8Ertd82e	Pdxp
Cyr61	Dap	Ppap2b
Daam1	Ddr1	Ppard
Dad1	Dgka	Pparg
Dcxr	Dhrs3	Ppfibp2
Ddah1	Dlg3	Prkch
Dennd2a	Dnmt3b	Pscd1
Dhcr24	E2f7 /// LOC639365	Rassf6
Dock11	Edg4	Rbm38
Dok2	Eomes	Rsad2
Dst	Erbb2	Sema4d
Dusp4	Erbb3	Serpinb9g
Dusp5	F630110N24Rik	Setd1b
E2f8	Fdps	Sfrs17b
Efna3 /// LOC100046031	Fgfbp1	Sgk2
Efna4	Fgfr1	Sgpp1
Egfl7	Fgfr2	Ski
Elk3	Flvcr2	Slc15a2
ENSMUSG00000074630	Folr1	Slc22a5
Epas1 /// LOC100048537	Foxo1	Slc25a37
Epb4.1l3	Gab2	Slc2a12
Ephb3	Gale	Slc31a2
Eps8	Gcnt1 /// LOC635918	Slco4a1
Eps8 /// LOC632638	Git2	Smad6
Esam1	Gjb2	Snx1
Ext1	Glis2	Stk10
Fabp5	Gm2a	Tacstd1
Fabp5 /// LOC547041 /// LOC620603	Golga2	Tcfap2a
Fads1	Gpc1	Tead4
Fasn	Gpr137b	Tesk2
Fgfrl1 /// LOC100046239	Gpr137b /// Gpr137b-ps /// LOC100044979	Ube2o
Fhl1	Gpr137b /// LOC100044979	Ugp2
Fhl2	Gpr137b-ps	Vav3
Flt1	Gprc5a	Vhlh
Galnt10	Gpx3	Wbscr27
Gba	Grina	Wdr62
Gbp2	Hip1	Wfs1
Gbp3	Hk1	Zfand2a
Gdpd5	Hmga2	Zfp335
Gipc2	Hs6st1 /// LOC100047260	Zxdc
Gmppb	Id2	
Gng10	Ier3	
Gng2	Ing2	
Gstk1	Inpp5a	
Gsto1	Itgb5	
Hdac5	Itm2c	
Hmgb3	Jmjd3	
Hmgcr	Jup	
Hmgcs1 /// LOC100040592	Kcnk1	
Hs2st1	Kctd17	
Hs3st1	Kitl	
Htra1	Klhdc5	
Id1	Krt19	
Id3	Lcor	
Ier5l /// LOC100047268	Ldlrap1	
Igsf3	Ldoc1	
Inpp1	Lgals9	
Insig1	Lif	
Itga5	Lima1	
Itga6	Lipg	
Junb	LOC100039155 /// Snx9	
Kctd6	LOC100040525 /// LOC100040596 /// Tmem181	

Kit	LOC100044162 /// Sema3e
Lama1	LOC100047506 /// Pbx3
Lhfp	LOC100047592 /// Tmem63b
Lin28	LOC100047651 /// Zfpm1
LOC100042253 /// LOC100044607 /// LOC100046670 ///	LOC100048460 /// Lzts2
LOC100045707 /// Pou3f1	Lrrfip1
LOC100046333 /// Zfp423	Ltb4dh
LOC100046586	Ly6a
LOC100046988 /// LOC624275 /// Paox	Maged1
LOC100047268	Mapk13
LOC100047324 /// Sesn1	Mbnl3
LOC100047579 /// Tmem20	Med14
LOC245350 /// LOC634012	Met
LOC632664 /// Ptprg	Mgll
LOC677224	Morc4
Lpl	Msl31
Lsm6	Msx2
Mapre2	Mtmr4
Meis1	Mvd
Mest	Mvp
Mfge8	Myh10
Midn	Myl4
Mll3	Myo1d
Mmp14	Ndst1
Mogat2	Notch2
Mospd1	Notch3
Mrg1	Nrm
Mxd4	Nsdhl
Myo1b	OTTMUSG00000000724 /// Serpinb9e /// Serpinb9f ///
Nat9	Palm
Nde1	Pdlim2
Nostrin	Peg10
Notch1	Perp
Npr2	Pik3cb
Nuak2	Pkp2
Pam	Plac1
Pcbp4	Plagl1
Pde10a	Plekhg3
Pdgfra	Plekhg6
Pdia4	Ppap2c
Pdia5	Prtg
Pfn2	Ptk2b
Phlda1	Ptpn14
Phpt1	Pwwp2
Plat	Ralgds
Plk2	Rapgef1
Plod1	Rbms2
Pmvk	Rbmx
Ppap2a	Ripk4
Praf2	Rnd2
Pstpip1	Rnf24
Pthr1	Rrbp1
Ptk7	Satb1
Ptpn21	Sbsn
Pxmp4	Sct
Qsox1	Sema4c
Rab34	Senp7
Rab43	Sept10
Rgs19	Serpinb6b
Rnaseh2c	Serpinb9b
Rusc2	Sh3bp1
Sall2	Slc16a13
Scmh1	Slc25a10
Selm	Slc40a1
Serpinb9	Slco2a1
Serpinh1	Smad3

Sgms1	Smpd1
Sgpl1	Snrk
Sh3bp4	Snx9
Slc16a3	Sptlc2
Slc26a2	Srebf2
Slc4a7	Ssbp3
Slc7a6	Stambp
Slc9a6	Stard10
Smarca2	Suox
Smo	Tacstd2
Smtn	Tanc2
Snai1	Tdrd7
Soat1	Tec
St3gal1	Tgfbr3
Stard4	Tgm1
Stard8	Tmc4
Tbc1d2b	Tmem106a
Tbx20	Tmem37
Timp2	Tmprss2
Tmem166	Tnrc18 /// Zfp469
Tmem58	Tns4
Top1mt	Tor1aip1
Tpbg	Tpm2
Traf4	Trio
Tram2	Tspan9
Uqcrh	Uaca
Usp27x	Ugdh
Vangl2	Unc119
Vgf	Unc5b
Vim	Usp25
Zc3h12c	Vgll3
Zfp462	Wfdc2
Zfp608	Wnt7b
Zmat3	Zxdb
Zmynd11	

Lists summarizing trophoblast genes upregulated by *Gata3* alone, both *Gata3* and *Cdx2*, and *Cdx2* alone. Members of the core trophoblast gene set exhibiting a 2-fold or greater change in at least one biological replicate were retained.

Table S3. MGI-archived phenotypes associated with *Cdx2*- or *Gata3*-induced genes

Gene symbol	Phenotype				
Cyr61	Abnormal	Chorioallantoic	Fusion		
Dad1	Abnormal	Chorioallantoic	Fusion		
Pdgfra	Abnormal	Chorioallantoic	Fusion		
Fgfr2	Abnormal	Chorioallantoic	Fusion		
Wnt7b	Abnormal	Chorioallantoic	Fusion		
Cdx2	Abnormal	Chorioallantoic	Fusion		
Ppap2b	Abnormal	Chorioallantoic	Fusion		
Snx1	Abnormal	Chorioallantoic	Fusion		
Bmp4	Abnormal	Chorion	Morphology		
Ascl2	Abnormal	Chorion	Morphology		
Fgfr1	Abnormal	Chorion	Morphology		
Wnt7b	Abnormal	Chorion	Morphology		
Elf5	Abnormal	Chorion	Morphology		
Cyr61	Abnormal	Chorionic	Plate	Morphology	
Notch2	Abnormal	Chorionic	Plate	Morphology	
Pparg	Abnormal	Chorionic	Plate	Morphology	
Lama1	Abnormal	Ectoplacental	Cone		
Elf5	Absent	Extraembryonic	Ectoderm		
Dad1	Abnormal	Extraembryonic	Endoderm	Formation	
Lama1	Abnormal	Extraembryonic	Endoderm	Formation	
Bmp4	Abnormal	Extraembryonic	Tissue	Morphology	
Dad1	Abnormal	Extraembryonic	Tissue	Morphology	
Flt1	Abnormal	Extraembryonic	Tissue	Morphology	
Itga5	Abnormal	Extraembryonic	Tissue	Morphology	
Junb	Abnormal	Extraembryonic	Tissue	Morphology	
Fgfr1	Abnormal	Extraembryonic	Tissue	Morphology	
Krt19	Abnormal	Extraembryonic	Tissue	Morphology	
Ccne2	Abnormal	Extraembryonic	Tissue	Morphology	
Ppard	Abnormal	Extraembryonic	Tissue	Morphology	
Snx1	Abnormal	Extraembryonic	Tissue	Morphology	
Tcfap2a	Abnormal	Extraembryonic	Tissue	Morphology	
Fgfr2	Abnormal	Membranous	Labyrinth		
Cyr61	Abnormal	Placenta	Morphology		
Fgfr2	Abnormal	Placenta	Morphology		

Notch2	Abnormal	Placenta	Morphology		
Ccne2	Abnormal	Placenta	Morphology		
Cdx2	Abnormal	Placenta	Morphology		
Ppap2b	Abnormal	Placenta	Morphology		
Pparg	Abnormal	Placenta	Morphology		
Notch2	Abnormal	Placenta	Size		
Mest	Decreased	Placenta	Weight		
Cyr61	Pale	Placenta			
Mest	Small	Placenta			
Pdgfra	Enlarged	Placenta			
Jup	Small	Placenta			
Krt19	Small	Placenta			
Met	Pale	Placenta			
Met	Small	Placenta			
Nsdhl	Small	Placenta			
Peg10	Small	Placenta			
Pdgfra	Abnormal	Placental	Development		
Plk2	Abnormal	Placental	Development		
Ascl2	Abnormal	Placental	Development		
Fgfr2	Abnormal	Placental	Development		
Gjb2	Abnormal	Placental	Development		
Met	Abnormal	Placental	Development		
Wnt7b	Abnormal	Placental	Development		
Adra2b	Abnormal	Placental	Development		
Ppard	Abnormal	Placental	Development		
Pparg	Abnormal	Placental	Development		
Plk2	Abnormal	Placental	Labyrinth	Morphology	
Gjb2	Abnormal	Placental	Labyrinth	Morphology	
Met	Abnormal	Placental	Labyrinth	Morphology	
Notch2	Abnormal	Placental	Labyrinth	Morphology	
Nsdhl	Abnormal	Placental	Labyrinth	Morphology	
Cdx2	Abnormal	Placental	Labyrinth	Morphology	
Ppard	Abnormal	Placental	Labyrinth	Morphology	
Pparg	Abnormal	Placental	Labyrinth	Morphology	
Cyr61	Abnormal	Placental	Labyrinth	Vasculature	Morphology
Notch1	Abnormal	Placental	Labyrinth	Vasculature	Morphology
Ascl2	Abnormal	Placental	Labyrinth	Vasculature	Morphology

Gjb2	Abnormal	Placental	Labyrinth	Vasculature	Morphology
Pparg	Abnormal	Placental	Labyrinth	Vasculature	Morphology
Fgfr2	Absent	Placental	Labyrinth		
Peg10	Absent	Placental	Labyrinth		
Gjb2	Abnormal	Placental	Transport		
Notch1	Abnormal	Placental	Vasculature		
Pdgfra	Abnormal	Placental	Vasculature		
Fgfr2	Abnormal	Placental	Vasculature		
Notch2	Abnormal	Placental	Vasculature		
Cdx2	Abnormal	Placental	Vasculature		
Dad1	Abnormal	Reichert's	Membrane		
Lama1	Abnormal	Reichert's	Membrane		
Ascl2	Abnormal	Spongiotrophoblast	Layer	Morphology	
Fgfr2	Abnormal	Spongiotrophoblast	Layer	Morphology	
Jup	Abnormal	Spongiotrophoblast	Layer	Morphology	
Peg10	Abnormal	Spongiotrophoblast	Layer	Morphology	
Eomes	Abnormal	Trophectoderm	Morphology		
Fgfr2	Abnormal	Trophectoderm	Morphology		
Cdx2	Abnormal	Trophectoderm	Morphology		
Tead4	Abnormal	Trophectoderm	Morphology		
Met	Decreased	Trophoblast	Giant	Cell	Number
Eomes	Absent	Trophoblast	Giant	Cells	
Fgfr2	Abnormal	Trophoblast	Giant	Cells	
Ccne2	Abnormal	Trophoblast	Giant	Cells	
Cdx2	Absent	Trophoblast	Giant	Cells	
Elf5	Abnormal	Trophoblast	Giant	Cells	
Pparg	Abnormal	Trophoblast	Giant	Cells	
Tead4	Absent	Trophoblast	Giant	Cells	
Pdgfra	Abnormal	Trophoblast	Layer	Morphology	
Eomes	Abnormal	Trophoblast	Layer	Morphology	
Fgfr2	Abnormal	Trophoblast	Layer	Morphology	
Krt19	Abnormal	Trophoblast	Layer	Morphology	
Ccne2	Abnormal	Trophoblast	Layer	Morphology	
Cdx2	Abnormal	Trophoblast	Layer	Morphology	
Pparg	Abnormal	Trophoblast	Layer	Morphology	

Batch analysis of trophoblast phenotypes. Lists of trophoblast genes expressed by Cdx2- and Gata3-expressing cells (see Table S2) were uploaded to MGI, where the batch query tool was used to recover mammalian phenotype (MP) terms. Genes were then sorted according to trophoblast subtype affected and color coded as follows: genes upregulated by *Cdx2* (red), genes upregulated by *Gata3* (yellow), and genes upregulated by both (orange). No obvious difference in the distribution of red and yellow blocks is apparent, indicating gene phenotypes affect multiple trophoblast subtypes and developmental stages.

Table S4. Non-trophoblast genes induced by *Cdx2* or *Gata3* in ES cells

A. List of genes not included among core trophoblast list upregulated by 2-fold or greater following overexpression of *Cdx2*

Gene symbol
1500002K03Rik
2010001K21Rik
2010305C02Rik
2810022L02Rik
2810051F02Rik
5133401H06Rik
5830461L22Rik
6330403M23Rik
9930021J17Rik
A430060F13Rik
AI585793
AI843639
Ankrd56
Ankrd57
Anxa9
Arhgap6
Atrnl1
AW046287
B4galnt2
BC100530///Stfa1
Bcl6
Blnk
C130073F10Rik
Casp8
Ccdc28b

Gene symbol
Chrnb1
Cntnap2
Ctso
Cxcl12
D330050I23Rik
Eif2ak4
ENSMUSG00000073738
Evpl
Fbp2
Fmnl2
Fosl2///LOC634417
Foxc1
Fras1
Frk
Fxyd3
Gats
Hspa1a
Ifit3
Kcne3
Lass4
Mal
Nab2
Npnt
Ocln
Olfml3

Gene symbol
Pdpn
Pdzk1
Plagl2
Psd3
Ptges
Ptplad2
Ror2
Saa3
Scnn1a
Sema3b
Sema3c
Serping1
Sh3rf2
Slc1a1
Slc39a8
Slc5a6
Sox6
Spin2
Tm7sf2
Upk1a
Wipf1
Zfp353

B. List of non-trophoblast genes upregulated by 2-fold or greater following overexpression of *Gata3*

1110006O17Rik
1110032E23Rik
1200009F10Rik
1700012H17Rik
1810011O10Rik
2010305C02Rik
2210011C24Rik
2210016H18Rik
2310035K24Rik
2610018G03Rik
2810022L02Rik
2810051F02Rik
2810451A06Rik
2810459M11Rik
3321401G04Rik
4631426J05Rik
4732466D17Rik
4732473B16Rik
4833411O04Rik
4833412E19Rik
4930431H11Rik
5033414K04Rik
6720475J19Rik
7420416P09Rik
9030625A04Rik
9130008F23Rik
9230114K14Rik

9530006C21Rik
A230001M10Rik
A530088I07Rik
A730062M13Rik
AA407331
AA415038
Acta1
Adamts15
Adamts5///LOC100048332
Adrb2
Aebp1
Afp
Agbl5
Ahnak2
AI585793
Air
Akr1c14
Aldh1l1///LOC100047937
Amn
Ang
Ankrd56
Apoa1
Apoa2
Apoc2
Aqp8
Arhgap6
Armcx3

Atp6v0e2
Atxn1
AW146242
AW742931
Axin2
Axl
B130021B11Rik
B230343A10Rik
B230380D07Rik
B4galnt2
B4galt4
B630019K06Rik
Bach2
BC025446
BC030046
BC068157
Bgn
Bicc1
Bmp2
C030019F02Rik
C130006E23
C130073F10Rik
C430045I18Rik
C530014P21Rik
Car7
Casp8
Cav1

Table S4, cont.

Cav2
Ccnd2
Cd200
Cd44
Cdk6
Cdkn2a
Cdkn2b
Cflar
Clcn5///LOC100045272
Cnksr2
Col11a1
Col1a1
Col1a2
Col4a5
Col5a2
Colec12
Cpe///LOC100046434
Crabp2
Ctsc
Ctsh
Ctso
Cubn
Cxcl10///LOC100045000
Cxcl11
Cxcl12
Cyp1b1
Cyp2s1
D0H4S114
D16H22S680E
D330050I23Rik
D3Ertd452e
D3Wsu106e
D5Wsu152e
D830012I24Rik
Dab2
Dact1
Dcbld1
Dkk1
Dlk1
Dnajb4
Dpp4
Dpysl3
E030004N02Rik
E230025E14Rik
Edg7
Edn1
Efemp1
EG622782///EG625349///EG666200///EG666464///LOC100041709///LOC544983///LOC545175///LOC619711///LOC624831
Elmod1
Emp1
Emp3
Eno3
ENSMUSG00000073237
ENSMUSG00000074917
Ets1
Etv6
F5
Fbxo25
Fgf13

Fgf5
Fkbp7
Flrt3///LOC100048721
Fmnl2
Fosl2///LOC634417
Foxa2
Foxq1
Fras1
Frem2
Frzb
Fst
Fxyd3
Fyco1
Gabrb3
Gap43
Gata4
Gata6
Gats
Gbp1
Ggcx
Ghr
Gkn1
Gkn2
Glipr1
Gm784///LOC676436
Gna12///LOC100048021
Gnai1
Gpc3
Gpc6///LOC100045283
Gpr124
H6pd
Has2
Hhex
Hkdc1
Hmgcll1
Hmgn3
Hnf4a
Hpn
Hs6st2
Hspa2
Hspb2
Igfbp6
Igsf11
Il24
Il33
Irs1
Jph2
Kif1a
Kif5c
Klb
Klhl23
Lamb2
Lass4
Limch1
Lipa
LOC100044194///Mcc
LOC100044198///Ppnr
LOC100044927///Tnfaip6
LOC100045359
LOC100045628///Slc16a2
LOC100047339///Loxl2
LOC100047419///Maf
LOC100048391///Prcp

LOC100048879///Pacs2
LOC640441
LOC640441///Thbs1
LOC676546///Mmd
Loxl1
Lrig3
Lrp12
Lrp2
Lrrc1
Lrrc8a///Phyhd1
Ltbp1
Lypd6
Mal
Map4k1
Mast4
Mgst1
Mmp2
Moxd1
Msln
Mta1
Mtap6
Mttp
Myo6
Nab2
Naglu
Nebl
Nefl
Nipa1
Nnat
Nog
Nox4
Npl
Npnt
Npr3
Nr2c1
Nrp1
Nudt11
Ocln
Odz3
Pard3b
Parp3
Pcdh18
Pde1b
Pdgfc
Pdgfrb
Pdgfrl
Pdpn
Pfkfb3
Pga5
Pik3ip1
Pitx2
Pla2g12b
Plagl2
Plekho1
Plscr1
Plxdc2
Postn
Ppbp
Ppp1r14a
Ppp2r5b
Prkaa2
Prkag2
Prrx2

Table S4, cont.

Prss23
Ptges
Ptgs2
Ptk6
Ptplad2
Ptprm
Ptx3
Rgma
Rhobtb3
Rnd3
Ror1
Ror2
Rutbc1
S100g
Samd4
Scel
Sdc2
Sema3b
Sema3c
Sema3e
Sepp1
Serpina3m
Serping1
Sertad4
Sesn3
Sfxn3
Sh3bgrl
Sh3rf1
Sh3rf2
Sh3yl1
Slc12a5
Slc39a8
Slc7a9
Smarca1
Soat2
Sorbs2
Sox11
Sox17
Sox7
Spg3a
Spin2
Spink3
Spns2
Srgn
Stard13
Steap2
Strn3
Stxbp6
Sulf1
Synpo2l
Tagln
Tceal1
Tcf2
Tcfec
Tgfbi
Thra
Tm7sf2
Tmc7
Tmcc2
Tmem130
Tmem88
Tnc
Tnfaip3

Tnfrsf19
Tnfsf9
Tnni2
Tspan2
Tspan5
Tspan8
Ttll1
Ttr
Ube2e2
Ugt2b34
Wisp1
Zdbf2
Zdhhc2
Zfp385
Zfp503
Zfp52
Zfpm2

Table S5. Subsets of trophoblast genes induced by *Gata3* in wild-type and *Cdx2* null ES cells

Cdx2 dependent	Cdx2 independent	Cdx2 repressed
Gene symbol	Gene symbol	Gene symbol
0610010B08Rik /// LOC627901 /// LOC628084	1100001H23Rik /// LOC100045163	1700086L19Rik
1190017O12Rik	1110012J17Rik	2310031A18Rik /// LOC100047808
1500005K14Rik	1200009I06Rik	3222402P14Rik
1500011H22Rik	1700052K11Rik	4732435N03Rik
1600014K23Rik	2310026E23Rik	4932442L08Rik
1600021P15Rik	2310040C09Rik	9430079B08Rik
2010002N04Rik	2310047D13Rik	Acp5
2010109K11Rik	2810457I06Rik /// LOC677224	Acpp
2010204K13Rik	4631422O05Rik	Adra2a /// LOC100044679
2200002D01Rik	4922503N01Rik	Adra2b
2310016E02Rik	6330505N24Rik	Ankrd12
2310057J16Rik	9530018I07Rik	Aof1
2600010E01Rik	9930012K11Rik	Apoe
2610027C15Rik	Abhd5	Arhgef6
2810003C17Rik	Abhd6	AW061234
2810417H13Rik	Acox1	Bat5
2900026A02Rik	Adamts1	Bmp2k
2900053A13Rik	Adcy7	C1qtnf6
3110001A13Rik	AI481772	C920025E04Rik /// H2-T23 /// LOC100046736
3830417A13Rik	Akap13	Cdh10
4933413G19Rik /// Foxm1 /// Pebp1	Akap2	Cdh5
6720460F02Rik	Anxa4	Cds1
9830001H06Rik	Apob48r	Chd7
Abcd4	App	Chdh
Acaa1a /// Acaa1b	Arhgap18	Chst11 /// Phactr1
Acot1 /// Acot2 /// LOC100044830	Asahl	Chst2
Acot1 /// LOC100044830	Ascl2	Cldn23
Acpl2	Asph	Clic5
Adamts4	Atp1b1	Clip2
Afap1l2	Atp6v0a1	Cts8
AI661453	AW550831	Cxadr
AK220484	B230120H23Rik	D16Wsu65e
Ak3	BC017612	D230012E17Rik
Alas1	BF642829	Ddit3
Aldh5a1	Camk2d	Dennd1a /// LOC100047738
Amfr /// LOC100046262	Ccdc93	Dmrtc1a
Ampd2	Cd55	Dnmt3l
Ankrd50	Cited2	Dock8
Anxa1	Cldn6	Dpp7
Arhgap29	Clu /// LOC100046120	Fbxo32
Arhgap8	Cpm	Fstl3
Arhgdib	Ctsa	Gkap1
Athl1	Cyp26a1	Gp1bb /// LOC100044138 /// Sept5
Atp2c2	Cyp51	Gpr116
B430119L13Rik	Cyr61	Gpr137b /// Gpr137b-ps
B4galt6 /// LOC675709	Dap	Gpr50
Bbx	Ddah1	Gpr56
BC031748	Dlg3	Gramd1b
Bcam	Dok2	Gsg2
Bcl2l11	Dusp5	Herpud1
Bcl9	E2f8	Hs3st3b1
Bhlhb2	Edg4	Hsd11b2
Bicd2	Efna3 /// LOC100046031	Hsd17b2
Blcap	Efna4	Icosl
Bmp1	Egfl7	Il28ra
Bmp4	Eomes	Irs3
Bmp8b	Epas1 /// LOC100048537	Irx1
Bok	Epb4.1l3	Irx2 /// LOC100045612
Bspry	Ephb3	Isg20
C230013L11Rik	Eps8	Kctd12
C79267	Eps8 /// LOC632638	Kif26a

Camta2	Esam1	Klf13
Capn1	Ext1	Klf4
Card10	Fgfbp1	Lamp2
Ccdc64	Fhl2	Las1l /// LOC100044857
Ccnd1	Flt1	Lcp1
Cd276	Flvcr2	Lgals3bp
Cdon	Gab2	Lgals8
Cebpa	Galnt10	Lhx2
Ceecam1	Gba	Lims2
Cgn	Gdpd5	LOC100042000
Cgref1	Git2	LOC100044735 /// Zxda
Chst12	Gjb2	LOC630776 /// Nid1
Clip3	Gpr137b	Lonrf3
Clstn1	Gpr137b /// LOC100044979	Mertk
Cmtm7	Gpr137b-ps	Metrnl
Col18a1	Gpx3	Mfsd2
Col3a1	Grina	Mreg
Cpne8	Hip1	Nagpa
Cxcr7	Hmgb3	Nppb
D330027G24Rik	Hs3st1	Nsbp1
D8Ertd82e	Id1	Nucb2
Daam1	Id3	Oaf
Dad1	Insig1	Pde4dip
Dcxr	Itga5	Phactr1
Ddr1	Kit	Plac8
Dennd2a	Kitl	Plekha6
Dgka	Krt19	Pnpla3
Dhcr24	Lama1	Pparg
Dhrs3	Lcor	Ppfibp2
Dnmt3b	Ldlrap1	Prkch
Dock11	Ldoc1	Prl5a1
Dst	Lgals9	Prl7d1
Dusp4	Lima1	Ptger3
E2f7 /// LOC639365	Lin28	Rab6b
Elk3	Lipg	Rassf6
ENSMUSG00000074630	LOC100039155 /// Snx9	Rbm20
Erbb2	LOC100040525 /// LOC100040596 /// Tmem181	Rsad2
Erbb3	LOC100046586	Sema6d
F630110N24Rik	LOC100047651 /// Zfpm1	Serpinb9c
Fabp5	LOC632664 /// Ptprg	Serpinb9g
Fabp5 /// LOC547041 /// LOC620603	LOC677224	Shroom1
Fads1	Mbnl3	Slco4a1
Fasn	Mgll	Snap91
Fdps	Mll3	Ssh3
Fgfr1	Mogat2	Stra6
Fgfr2	Mospd1	Tbc1d4
Fgfrl1 /// LOC100046239	Msl31	Tnfaip2
Fhl1	Mtmr4	Trpv2
Folr1	Mvp	Ubn1
Foxo1	Mxd4	Uck2
Gale	Myl4	Vash2
Gbp2	Myo1d	
Gbp3	Nostrin	
Gcnt1 /// LOC635918		
Gipc2	Pde10a	
Glis2	Pdgfra	
Gm2a	Pdia5	
Gmppb	Peg10	
Gng10	Pkp2	
Gng2	Plac1	
Golga2	Plat	
Gpc1	Plod1	
Gpr137b /// Gpr137b-ps /// LOC100044979	Pthr1	
Gprc5a	Ptpn21	
Gstk1	Pwwp2	
Gsto1	Qsox1	

Hdac5	Rbmx
Hk1	Rnf24
Hmga2	Rrbp1
Hmgcr	Sall2
Hmgcs1 /// LOC100040592	Sbsn
Hs2st1	Sct
Hs6st1 /// LOC100047260	Sema4c
Htra1	Senp7
Id2	Serpinb6b
Ier3	Serpinb9
Ier5l /// LOC100047268	Serpinb9b
Igsf3	Serpinh1
Ing2	Sgpl1
Inpp1	Slc26a2
Inpp5a	Slc7a6
Itga6	Slc9a6
Itgb5	Slco2a1
Itm2c	Smarca2
Jmjd3	Snai1
Junb	Snrk
Jup	Snx9
Kcnk1	Soat1
Kctd17	St3gal1
Kctd6	Stard10
Klhdc5	Stard4
Lhfp	Stard8
Lif	Tanc2
LOC100042253 /// LOC100044607 /// LOC100046670 ///	Tbc1d2b
LOC100044162 /// Sema3e	Tbx20
LOC100045707 /// Pou3f1	Tdrd7
LOC100046333 /// Zfp423	Tec
LOC100046988 /// LOC624275 /// Paox	Tgfbr3
LOC100047268	Tmc4
LOC100047324 /// Sesn1	Tmem106a
LOC100047506 /// Pbx3	Tmem166
LOC100047579 /// Tmem20	Tnrc18 /// Zfp469
LOC100047592 /// Tmem63b	Tns4
LOC100048460 /// Lzts2	Top1mt
LOC245350 /// LOC634012	Tpbg
Lpl	Tram2
Lrrfip1	Ugdh
Lsm6	Usp27x
Ltb4dh	Vgf
Ly6a	Zc3h12c
Maged1	Zxdb
Mapk13	
Mapre2	
Med14	
Meis1	
Mest	
Met	
Mfge8	
Midn	
Mmp14	
Morc4	
Mrg1	
Msx2	
Mvd	
Myh10	
Myo1b	
Nat9	
Nde1	
Ndst1	
Notch1	
Notch2	
Notch3	

Npr2
Nrm
Nsdhl
Nuak2
Palm
Pam
Pcbp4
Pdia4
Pdlim2
Perp
Pfn2
Phlda1
Phpt1
Pik3cb
Plagl1
Plekhg3
Plekhg6
Plk2
Pmvk
Ppap2a
Ppap2c
Praf2
Prtg
Pstpip1
Ptk2b
Ptk7
Ptpn14
Pxmp4
Rab34
Rab43
Ralgds
Rapgef1
Rbms2
Rgs19
Ripk4
Rnaseh2c
Rnd2
Rusc2
Satb1
Scmh1
Selm
Sept9
Sgms1
Sh3bp1
Sh3bp4
Slc16a13
Slc16a3
Slc25a10
Slc40a1
Slc4a7
Smad3
Smo
Smpd1
Smtn
Sptlc2
Srebf2
Ssbp3
Stambp
Suox
Tacstd2
Tgm1
Timp2
Tmem37
Tmem58
Tmprss2
Tor1aip1
Tpm2

Traf4
Trio
Tspan9
Uaca
Unc119
Unc5b
Uqcrh
Usp25
Vangl2
Vgll3
Vim
Wfdc2
Wnt7b
Zfp462
Zfp608
Zmat3
Zmynd11

Lists of trophoblast genes upregulated by more than 2-fold following expression of *Gata3* in wild-type cells alone, in either wild-type or *Cdx2* null cells, and in *Cdx2* null cells alone.