The Thesis Committee for Nadima Uprety
Certifies that this is the approved version of the following thesis:

The role of Fosl1 in transdifferentiation of embryonic stem (ES) cells to trophoblast giant-like cells

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor: _____
Jonghwan Kim

_____
Tanya Paull

The role of Fosl1 in transdifferentiation of embryonic stem (ES) cells to trophoblast giant-like cells

by

Nadima Uprety, B.S.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Arts

The University of Texas at Austin

December 2016

Acknowledgements

I would like to express my sincere gratitude to my advisor Dr. Jonghwan Kim for his continuous support, understanding and patience throughout my graduate experience. From him, I have learned to always hold my work and myself to a higher standard. I also want to thank my fellow labmates in Kim lab for their support. I want to thank my friends that have been there for me through ups and downs of graduate school. I also want to express deepest gratitude to my parents and my brother for their unconditional love and continuous encouragement. Most of all, I want to thank my loving husband for being my "rock" and always supporting me in my decisions.

Abstract

The role of Fosl1 in transdifferentiation of embryonic stem (ES) cells to trophoblast giant-like cells

Nadima Uprety, M.A.

The University of Texas at Austin, 2016

Supervisor: Jonghwan Kim

During mammalian embryonic development, the first lineage commitment event gives rise to two distinct cell populations: the trophectoderm (TE) and the inner cell mass (ICM). The TE consists of outer cells of the blastocyst and ultimately forms the placenta while the ICM gives rise to all the embryonic tissues. Numerous transcription factors (TFs) guiding ICM differentiation into different embryonic tissues have been characterized. However, only a few TFs that are required for TE specification and differentiation have been identified, and much less is understood as to how these TFs interact with other TFs or with their chromosomal targets in order to drive cell fate towards TE lineage. Understanding TE development is crucial because cells in this lineage are required for proper embryo implantation in the uterus. Defects in TE lineage can cause early failure of pregnancy as well as other pregnancy related disorders such as preeclampsia and intrauterine growth restriction (IUGR). Here, we characterize the function of one of TE-specific TFs, Fosl1, which was previously suggested as having

some roles in placental development. We utilized mouse embryonic stem (ES) cells (derived from ICM) and showed that ectopic expression of Fosl1 can transdifferentiate ES cells to trophoblast giant-like cells. We show that Fosl1 does so by directly binding and activating TE-specific genes and genes associated with epithelial-mesenchymal transition (EMT). Using mouse trophoblast stem (TS) cells, we also establish that Fosl1 is required for specification of TS cells to trophoblast giant cells (TGCs) subtype. Therefore, our results suggest that Fosl1 serves as an important mediator of cell fate conversion from ES cells to trophoblast giant-like cells and that Fosl1 is a critical regulator of TS cell differentiation.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Early mouse development

1.1.1 The first cell fate decision: Inner Cell Mass (ICM) versus Trophectoderm (TE)

During early embryonic development, a totipotent cell undergoes a series of cell divisions. In the first stages of division, all of these cells are identical. However, from 8 cells to 32 cells stage, blastomeres compact and divide either symmetrically or asymmetrically to the cleavage plane and produce two distinct cell population for the first time during embryogenesis: apolar inner cells and polar outer cells (Senner and Hemberger, 2010). Apolar inner cells develop into the inner cell mass (ICM) while polar outer cells form the outer trophectoderm (TE) layer. Prior to this, blastomeres are capable of adopting either of the two fates. The ICM differentiates into the three germ layers, known as the endoderm, mesoderm, and ectoderm, which form all of the tissues of the fetus. The TE gives rise to all of the specialized cells of the placenta, a transient yet crucial organ that nourishes and protects the developing embryo during pregnancy. The lineage determination of these two cell populations is not only determined spatially, but also by the interplay of various transcription factors (TFs), which activate or repress cell type-specific transcriptional programs.

The first cell fate decision is controlled by multiple TFs, mainly Oct4 and Cdx2. During development, all blastomeres express Oct4 and Cdx2. However, after the first cell fate decision, Oct4-expressing cells are restricted to the ICM while Cdx2-expressing cells are restricted to TE (Ralston and Rossant, 2008). Studies have shown that $Oct4^{-/-}$ conceptuses give rise to blastomere that is committed to trophoblast fate, independent of polarity cues

(Nichols et al., 1998). Cdx2$^{-/-}$ embryos fail to maintain epithelial integrity despite undergoing lineage commitment, and they do not implant. These Cdx2$^{-/-}$ cells are also devoid of potential to differentiate further into trophoblast subtypes (Strumpf et al., 2005). These data suggest that Oct4 and Cdx2 play critical roles in ICM and TE development, respectively. Interestingly, using mouse embryonic stem (ES) cells, it has been shown that Oct4 and Cdx2 also negatively regulate each other and that Oct4 repression or Cdx2 induction can transdifferentiate ES cells to trophoblast stem (TS)-like cells (Niwa et al., 2005).

In addition to Oct4 and Cdx2, the TF Tead4 also plays an important role in establishing TE population. The Hippo pathway, which is activated in response to cell-to-cell contacts, spatially controls Tead4 expression (Nishioka et al., 2009). Yap1 is a co-factor of Tead4 and required for activation of TE-specific genes. In inner cells, where this pathway is active, Yap1 (co-factor of Tead4) is phosphorylated by Lats kinase, excluded from the nucleus and degraded. Due to absence of Yap1 in the nucleus, Tead4 cannot stimulate expression of Cdx2. Thereby, Oct4 becomes dominant and inner cells cannot differentiate into TE lineages. In outer cells, where Hippo pathway is inactive, nuclear localized Yap1 can form Tead4-Yap1 complex and promote expression of Cdx2 (Nishioka et al., 2009), which further activates TE marker genes, while repressing Oct4 activity (Niwa et al., 2005). Mouse studies have shown that Tead4 knockout (KO) mutants die due to failure to form blastocoel (fluid filled cavity of blastocyst). Interestingly, Tead4 KO cells also lack Cdx2 expression (Nishioka et al., 2008), suggesting that Tead4 remains at the top of the transcriptional network hierarchy

determining TE segregation from ICM. Gata3, another important TF for TE development, has been proposed to work downstream of Tead4 and parallel to Cdx2 to promote TE specification by activating genes critical in TE development (Home et al. 2009; Ralston et al. 2010). Gata3 was also shown to be able to induce trophoblast fate in ES cells when overexpressed and plays important roles in the differentiation of TS cells towards more specialized subtypes (discussed below) (Ralston et al. 2010).

1.1.2 The TE lineage and the placenta

Similar to the ICM which can give rise to cells of various lineages that comprise the fetus (ectoderm, mesoderm and endoderm), the TE lineage is capable of giving rise to various trophoblast cell subtypes: trophoblast giant cells (TGCs), spongiotrophoblasts, glycogen cells, and syncytialtrophoblasts (Ain et al., 2003; Rossant and Cross, 2001; Soares et al., 1996). TS cells represent the precursors of these differentiated cells of the placenta. Each of the subtypes possesses unique functions necessary for development of a healthy placenta. However, our understanding of how these different subtypes arise during development from a single layered TE is still incomplete.

TGCs are the first subtype to arise and do so during two phases of development: 1) at the blastocyst stage, and 2) after implantation. Primary TGCs arise at the blastocyst stage from mural TE, which is TE that is not in contact with ICM due to formation of blastocoel (Cross et al., 1994). These cells go through endoreplication, through which they enlarge and undergo rounds of DNA replication without entering mitosis (MacAuley et al., 1998). Secondary TGCs arise after implantation where cells in the ectoplacental

cone, which is the thickened trophoblast of the blastocyst that becomes the fetal portion of the placenta, differentiate into TGCs (Cross et al., 1994). These cells have invasive and endocrine functions (Riley et al., 1998). They facilitate the process of implantation of the embryo into the uterus and also produce several hormones and cytokines that promote physiological adaptations in the mother such as regulation of maternal blood flow to the implantation site and production of progesterone from the ovary (Cross et al., 2002; Linzer and Fisher, 1999).

Another important subtype of cells making up the placenta are the spongiotrophoblasts, which arise from the ectoplacental cone. Even though the function of this cell type is not completely known, it is speculated that spongiotrophoblasts perform endocrine functions by producing factors such as anti-angiogenic factors that may prevent the growth of maternal blood vessels into the fetal placenta (Cross et al., 2002). Spongiotrophoblasts also give rise to secondary TGCs. Glycogen cells appear within the spongiotrophoblast layer and also give rise to invasive trophoblast cells that penetrate the maternal uterus, which increases blood flow to the placenta (Adamson et al., 2002). Syncytialtrophoblasts arise from the fusion of multiple trophoblast cells that have left the cell cycle and these cells help transport nutrients and waste between maternal and fetal tissues (Cross, 2000). Together, all of these differentiated cell subtypes mediate separate yet crucial functions of maintaining a healthy placenta.

The differentiation of cells in TE lineage towards more specialized TGCs is regulated by various basic helix-loop- helix (bHLH) TFs like Mash2 and Hand1. Expression of Mash2 and Hand1 overlaps in the ectoplacental cone and spongiotrophoblasts, which are the

precursors of secondary TGCs. However, Mash2 suppresses differentiation to TGCs and maintains proliferation of such precursors, i.e. spongiotrophoblast, whereas Hand1 promotes the formation of TGCs suggesting that these two TFs antagonize one another (Scott et al., 2000). TFs like Flt1 and Tpbqa are also expressed within the spongiotrophoblasts. Initiation of differentiation towards syncytialtrophoblasts is under the control of another TF Gcm1. Ectopic expression of Gcm1 is sufficient to promote differentiation of TS cells towards the syncytialtrophoblast fate and block their ability to differentiate into TGCs (Hughes et al., 2004). TS cells cultured *in vitro* (discussed in later sections) also readily differentiate into TGCs after withdrawal of Fgf4 and heparin from the culture medium (Simmons and Cross, 2005).

These various cellular subtypes comprise the placenta, the first organ to form during mammalian embryogenesis and is vital for the survival of the embryo (Cross et al. 2001). Due to these cells, placenta becomes an interface between fetal and maternal environment and is responsible for exchange of gases, nutrients and waste products. Placenta is an important source of pregnancy-associated hormones and growth factors and therefore, any genetic or environmental factor that affects the placenta leads to fetal growth retardation and even death (Rossant and Cross, 2001). Therefore, furthering our understanding of trophoblast development will provide novel insights into placental function.

1.1.3 The second cell fate decision: Epiblast (Epi) vs Primitive Endoderm (PrE)

During the second cell fate decision, the ICM gives rise to the epiblast (Epi) and primitive endoderm (PrE). This separation occurs from around embryonic day (E) 3.5 to 4.5 and is marked by the expression of TFs, Nanog and Gata6 in Epi and PrE, respectively (Chambers et al., 2003; Mitsui et al., 2003). Nanog and Gata6 are initially co-expressed in ICM and gradually localize in a 'salt and pepper pattern' (Rossant et al., 2003). Nanog expression is followed by expression of Oct4 and Sox2 in Epi (Avilion et al., 2003; Rosner et al., 1990; Schöler et al., 1990), while in PrE, the expression of Gata6 is followed by the expression of Sox17, Gata4, Sox7, and Hnf4 (Arceci et al., 1993; Duncan et al., 1994; Masui et al., 2007; Morris et al., 2010; Niakan et al., 2013; Plusa et al., 2008). Together, these groups of TFs guide different populations within the ICM toward Epi and PrE lineages. Studies have implicated the Fgf signaling pathway in this mutually exclusive expression pattern of Nanog and Gata6 and the specification of Epi and PrE. While Fgf4 is upregulated in Epi precursor cells, its receptor Fgfr2 is downregulated. In PrE precursor cells however, Fgfr2 expression is maintained while Fgf4 is downregulated (Guo et al., 2010). This distribution is taken as the first sign of lineage commitment within ICM.

1.2 Mouse Stem Cells as Model System

The use of *in vitro* model systems represents a valuable tool for understanding cellular development. Mouse ES cells, derived from the ICM, and mouse TS cells, derived from

the TE, have become invaluable resources for investigating molecular mechanisms and pathways underlying early mammalian development of both lineages.

1.2.1 Embryonic Stem (ES) cells

ES cells were established by isolating the mouse blastocyst and cultivating them on feeder cells (mitotically inactive mouse embryonic fibroblasts-MEFs) (Evans and Kaufman, 1981). The cells were then expanded and once established, had the potential to self-renew and retained the ability to contribute to all embryonic germ lineages. Since ES cells required layer of feeder cells to grow, it suggested that these cells provided ES cells with some critical factors that allowed them to proliferate or to suppress differentiation. Later, two groups independently identified the factor as leukemia inhibitory factor (LIF) (Smith et al., 1988; Williams et al., 1988). This discovery allowed for the replacement of the feeder layers with cytokine LIF in the media. One of the signaling pathways regulated by LIF is the JAK/STAT3 pathway. Inactivation of STAT3 has been shown to promote spontaneous differentiation of ES cells *in vitro* (Boeuf et al., 1997). LIF also activates other wide range of downstream effector molecules such as extracellular signal-regulated kinases (ERK), mitogen-activated protein kinases (MAPK) and phosphatydilinositol-3 kinase (PI3K). It was shown that inhibition of ERK pathway favors ES cells self-renewal in culture and impairs differentiation (Burdon et al., 1999). PI3K and Wnt pathway both inhibit Gsk3, which allows ES cells to promote self-renewal (Reya and Clevers, 2005; Watanabe et al., 2006). This suggested that there is a fine balance between positive and negative regulator of self-renewal under the control of LIF. Now, combination of

inhibitors of Erk and Gsk3 has allowed us to use the 2 inhibitors (2i) condition for derivation of ES cells, which is suggested to mimic the environment in mouse ICM and allows ES cells to enter naïve ground state where ES cells are more homogeneous and "ICM-like" (Guo et al., 2010; Ying et al., 2008).

Along with the signaling pathways, transcriptional regulators also affect self-renewal and differentiation of ES cells. At the top of the hierarchy of the transcriptional network is Oct4, one of the master regulators of pluripotency. The precise amount of Oct4 is critical for self-renewal of ES cells as upregulation or downregulation of Oct4 causes ES cells to transdifferentiate towards PrE and TE lineage, respectively (Niwa et al. 2000). Another critical TF involved in maintenance of ES cell self-renewal is Sox2, with which Oct4 interacts. ES cells deficient in Sox2 also transdifferentiate towards TE lineage (Masui et al., 2007). Nanog also plays an important role in maintaining ES cell pluripotency as ES cells cannot be derived from $Nanog^{-/-}$ conceptuses (Silva et al., 2009). However, it has also been shown that Esrrb can slightly compensate for Nanog deficiency (Festuccia et al., 2012). The three core TFs Oct4, Nanog and Sox2 share similar chromosomal targets in an interconnected autoregulatory loop (Boyer et al., 2005) and are key in sustaining self-renewal in ES cells.

1.2.2 Trophoblast Stem (TS) cells

TS cells are derived from either TE of blastocyst or extra-embryonic ectoderm (ExE) and are cultured in the presence of MEF cells (Tanaka et al., 1998). However, it was found that conditioned medium supplemented with Fgf4 and its co-activator heparin was able to

replace the need for feeder cells. Conditioned medium contained factors released by MEF cells, which later was found to be TGF-β and activin-A (Erlebacher et al. 2004). In the presence of these factors, TS cells maintain self-renewal, i.e. they proliferate without undergoing differentiation. However, removal of Fgf4 results in differentiation of TS cells. TS cells can differentiate into all trophoblast cell types, *in vitro* and when injected into the blastocyst, they become part of extra-embryonic tissues and placenta (Tanaka et al., 1998). Apart from Hippo pathway and Fgf4 mentioned earlier, the Ras-Mapk pathway is involved in TE specification as well, where consecutive activation of H-Ras in ES cells has been shown to transdifferentiate ES cells into self-renewing TS-like cells (Lu et al., 2008).

The key TFs that promote self-renewal of TS cells comprise of Cdx2, Tead4, Tcfapc2, Esrrb, and Eomes. These TFs have the ability to convert ES cells to TS-like cells when overexpressed in ES cells (Senner and Hemberger, 2010). Another group of TFs, including Gata3, Ets2 and Elf5, not only possess the ability to transdifferentiate ES cells but also trigger trophoblast differentiation (Senner and Hemberger, 2010). Therefore, the functions of TFs involved in TS cell network are divided into two groups: one that allows for self-renewal of TS cells and other that promotes differentiation. Like ES cell core factors, these TFs also positively regulate each other as seen for Cdx2 and Gata3 as well as Elf5, Eomes, and Tcfapc2c (Latos et al. 2015; Ralston et al. 2010).

1.2.3 Extra-embryonic Endoderm (XEN) cells

Extra-embryonic endoderm (XEN) cells are derived from PrE of blastocyst-stage embryos and cultured in layer of MEF cells. These cells can contribute specifically to extra-embryonic endoderm layer when injected into blastocysts (Kunath et al., 2005). Very little is known about mechanism sustaining self-renewal of these cells. Recently, XEN cells have been established from ES cells by overexpressing Gata6 as well as by using retinoic acid and activin to convert ES cells to XEN-like cells (Niakan et al., 2013). As mentioned earlier, XEN cells also express Sox17, Gata4, and Sox7, which promote self-renewal of these cells.

1.3 Transdifferentiation of ES cells to TS-like cells

Early reprogramming methods such as somatic cell nuclear transfer (SCNT) and cell fusion demonstrated the flexibility of cellular identity. These experiments showed that it is possible to convert somatic cells back to an embryonic pluripotent state. This idea became the basis of the concept of lineage reprogramming, which is direct conversion of cells of one lineage to a different lineage (Graf and Enver, 2009). The feasibility of this was first demonstrated by a study showing that overexpression (OE) of single myogenic TF, MyoD, alone can covert fibroblasts into myoblasts (Davis et al., 1987). This led to variety of studies showing TF-mediated lineage reprogramming, including the conversion of monocytic precursors to erythroid-megakaryocytic cells using Gata1 and B cells to macrophages using C/EBPα or by ablation of Pax5 (Kulessa et al., 1995; Laiosa et al., 2006; Nutt et al., 1999; Xie et al., 2004) and exocrine cells to islet β- cells using Pdx1,

Ngn3, and Mafa (Yechoor et al., 2009; Zhou et al., 2008). In recent years, pioneering studies have shown that somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells through ectopic expression of four TFs: Oct4, Sox2, Klf4 and Myc (Takahashi and Yamanaka, 2006). Since then, several terminally differentiated cells such as cardiomyocytes, neuronal cells, hepatocytes and so on have been produced by reprogramming of other terminally differentiated cells, using combinations of various TFs (Heinrich et al., 2010; Huang et al., 2011, 2014; Ieda et al., 2010). Recently, various groups have also shown that ectopic expression of TFs, such as Tfap2c, Gata3, Eomes, Ets2 and Myc could generate induced TS-like (iTS) cells from MEFs (Benchetrit et al., 2015; Kubaczka et al., 2015). These studies suggest that TFs can overcome lineage restrictions and facilitate lineage conversions.

The transdifferentiation of ES cells to TS-like cells is an informative *in vitro* model to study mechanisms of the first cell fate decision and TE lineage development. The study of transdifferentiation has allowed us to understand what kind of factors affect the cell fate choice between two lineages that need to exist during the same developmental window. Studies have shown that controlled Oct4 repression, Cdx2 induction (Niwa et al., 2005), or forced activation of Ras/Erk signaling pathway in ES cells (Lu et al., 2008) lead to conversion of ES cells to TS-like cells and that ES cells deficient in DNA methylation can give rise to terminally differentiated trophoblast giant-like cells (Ng et al., 2008). In summary, transdifferentiation of ES cells to TS-like cells is an interesting tool that allows us to look at whether perturbation of one key factor is enough to induce TE lineage conversion programs in ES cells. Since human TS cells are not yet available,

it may be a valuable way to produce induced TS cells by transdifferentiation of human ES cells, which would be an excellent resource for cell therapy to treat pregnancy-related disorders.

1.4 Role of Fosl1 in trophoblast lineage

There are several TFs that regulate self-renewal and differentiation of TS cells, although their exact functions and molecular mechanisms were previously unknown. In particular, we decided to study Fosl1 (or Fra1). Fosl1 is a TF known to be upregulated in differentiated TS cells, but its detailed role in regulating differentiation has not been well characterized.

1.4.1 PI3K/AKT signaling pathway

The PI3K/AKT pathway is implicated in trophoblast cell development. Activation of PI3K upon trophoblast cell differentiation results in phosphorylation and activation of AKT. This signaling pathway in turn regulates the production of PRL family of genes, such as Prl3d1, Prl3b1, and Prl4a1 (Kamei et al., 2002). Fosl1 is one of the downstream mediators of PI3K/AKT pathway that controls the pro-invasive and pro-vascular remodeling phenotype of TS cells (Kent et al., 2011). Among the three isoforms of AKT (AKT1, AKT2, and AKT3), AKT1 is predominantly expressed in developing TGCs (Kamei et al., 2002; Yang et al., 2003). However, studies have suggested that each AKT isoform might play different roles in regulation of trophoblast cell phenotype as knockdown (KD) of individual AKT isoforms produce isoform-specific effects on the

genes related to invasion and vascular remodeling (Kent et al., 2011). Specifically, inhibition of PI3K/AKT results in the decrease in nuclear accumulation of Fosl1 while KD of Fosl1 leads to decreased expression of the genes associated with invasive-vascular remodeling and trophoblast invasion (Kent et al., 2011). However, what the precise mechanism behind Fosl1 regulation by PI3K/AKT pathway is unclear. In addition, PI3K/AKT pathway is implicated in placental disorders such as preeclampsia and intrauterine growth restriction (IUGR). Preeclampsia is characterized by poor trophoblast invasions leading to insufficient oxygen availability and fetal hypoxia (Goldman-Wohl and Yagel, 2002). IUGR is characterized by reduced fetal growth and some of the causes include inadequate placental development and trophoblast invasion (Scifres and Nelson, 2009). In a preeclamptic placenta, the levels of AKT activity is increased due to upregulation of PI3K (Park et al., 2010), while in IUGR, expression of the AKT1 pathway is downregulated (Yung et al., 2008).

1.4.2 Fosl1

Fosl1 is a component of activator-protein 1 complex (AP-1), consisting of Fos-Jun family dimerization, which is implicated in regulating cell proliferation and differentiation (Shaulian and Karin, 2002). The Fos family consists of c-Fos, FosB, Fosl1, and Fosl2, while the Jun family consists of c-Jun, Junb, and JunD. The AP-1 complex can consist either of Fos-Jun heterodimers or Jun-Jun homodimers (Eferl and Wagner, 2003). The composition of these dimers affects the activity of AP-1 and has been noted be cell type-specific. In addition to differentiation of trophoblast cells, Fosl1 has also been implicated

in tumorigenesis, cancer progression, cell invasion (Verde et al., 2007), and bone development (Wagner, 2002). Additionally, Fosl1 null mice die due to placental defects at approximately E10.5 (Schreiber et al., 2000). However, the mechanism through which Fosl1 regulates its actions in trophoblast cells is not known. Therefore, Fosl1 is an excellent candidate to study the potential of a single TF to transdifferentiate ES cells to TS-like cells in order to gain insight into its mechanism.

Chapter 2: Materials and Methods

2.1 Cell culture

Mouse J1 ES cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 18% fetal bovine serum (FBS), 50U/ml penicillin/streptomycin (Gibco), 2mM L-glutamine (Gibco), 100μM MEM nonessential amino acids (Gibco), nucleosides (Millipore), 100μM β-mercaptoethanol (Sigma) and 1000U/ml recombinant leukemia inhibitory factor (LIF, Millipore). ES cells were cultured on 0.1% gelatin-coated plates. Mouse TS cells (a gift from Dr. Janet Rossant (University of Toronto) and Dr. Guang Hu (National Institutes of Health [NIH] / National Institutes of Environmental Health Sciences [NIEHS]) were maintained at a ratio of 3:7 of TS medium to MEF-conditioned TS medium. The TS medium consisted of RPMI 1640 (Gibco) supplemented with 20% FBS, 100μM β-mercaptoethanol, 2mM L-glutamine, 1mM sodium pyruvate, 50U/mL penicillin, and 50mg/mL streptomycin. To acquire MEF-conditioned TS medium, mitomycin-treated MEF cells were cultured in TS medium for 3 days and the medium was collected every 3 days three times. The 3:7 ratio medium was supplemented with 25ng/mL Fgf4 and 1μg/mL heparin to maintain TS cells in self-renewing state and removed for differentiation of TS cells. HEK293T cells were maintained in DMEM supplemented with 10% FBS, 2mM L-glutamine, and 50U/mL penicillin/streptomycin. All cells were incubated in 37°C and 5% CO2.

2.2 Stable cell line generation

Full length Fosl1 cDNA was cloned into pEF1a-FLBIO (FB) vector (Kim et al., 2009). The primer sequences used for cloning are listed in Table 1. Fosl1-containing vector (FB-Fosl1) was introduced into BirA-expressing ES (BirA ES) cells by electroporation. These cells were allowed to grow under puromycin and geneticin selection for 9 days and colonies were picked and maintained under ES media conditions described above along with continuation of puromycin and geneticin selection. OE of Fosl1 was confirmed by RT-qPCR and Western blotting. Western blots were performed using anti-streptavidin-HRP (1:2000, RPN1231V, GE Healthcare Life Sciences) and anti-Fosl1 (Fra-1 (N-17)) antibody (1:500, sc-183, Santa Cruz).

2.3 Western blotting

For whole cell lysate preparation, cells were washed with PBS, and lysed in 2X Laemelli sample buffer (Bio-rad). The cells lysates were boiled at 100°C for 15 min and centrifuged prior to loading. Proteins were separated on 4-20% gradient acrylamide gels (Bio-rad) and transferred onto PDVF membrane using Trans-Blot® Turbo™ Transfer Starter System (Bio-rad). After protein transfer, membranes were blocked with 5% BSA (Sigma) in TBST (20mM Tris-HCl, pH 7.6, 13 mM NaCl, and 0.1% Tween-20) for 1 hour and incubated with primary antibody at 4°C overnight. The membranes were then washed with TBST and incubated with secondary antibody for 1 hour at room temperature. The proteins were detected using ECL reagents (GE Healthcare Amersham ECL prime) with Bio-rad Molecular Imager® ChemiDoc™ XRS+ system. The

antibodies used are anti-Fra-1 (N-17) (1:500, sc-183, Santa Cruz) and anti-β-actin (1:20000, ab20272, Abcam).

2.4 Alkaline Phosphatase (AP) staining

Alkaline Phosphatase (AP) staining was performed according to manufacturer's protocol using Alkaline Phosphatase Detection Kit (Millipore). Cells were imaged using an inverted microscope.

2.5 Real time-quantitative PCR (RT-qPCR)

Total RNA was isolated using RNeasy plus Mini Kit (Qiagen). 500ng of total RNA was used for cDNA synthesis with ReadyScript® cDNA Synthesis Mix (Sigma). The cDNA generated was diluted 20X and RT-qPCRs were performed using 2μl of diluted cDNA with PerfeCTa SYBR® Green FastMix, Low ROX™ (Quanta). RT-qPCR primers were designed to amplify exon junctions and their sequences are listed in Table 1. Gapdh was used as an internal control to normalize variability in expression levels.

2.6 Bio-ChIP-sequencing (Bio-ChIP-seq)

Bio-ChIP assays were performed using BirA ES cell lines- expressing BirA only (control) or BirA and biotin-tagged proteins (sample) as previously described (Kim et al., 2009) using streptavidin magnetic particles. Briefly, cells were cross-linked in 1% formaldehyde for 7 min at room temperature and the reaction was quenched for 5 minutes by adding glycine to final concentration of 125mM. Fixed cells were rinsed with PBS

twice and centrifuged. The fixed cell pellets were used immediately for experiments or stored at -80°C.

Cells were re-suspended in ChIP buffer (1% TritonX-100, 2mM EDTA, 20mM TrisCl, pH 8.1, 150mM NaCl, 0.1% SDS, and protease inhibitor), and then sonicated for 30 min (30 sec on / 1 min off) and centrifuged at maximum speed for 10 min. The supernatant was transferred to new tubes and pre-cleared with Protein A beads for 4 hours, rotating in 4°C. The samples were then centrifuged and the supernatant was incubated in 10μg streptavidin beads overnight (Roche). The beads were washed for 8 minutes, twice with 2% SDS, once with high salt buffer (0.1% Deoxycholate, 1% Triton X-100, 1mM EDTA, 50mM HEPES (pH 7.5), and 500mM NaCl), once with LiCl wash buffer (250mM LiCl, 0.5% NP40, 0.5% Deoxycholate, 1mM EDTA, and10mM TrisCl pH 8.1) and twice with TE buffer. Samples were eluted by incubating the beads in 150μl of SDS Elution buffer (1% SDS, 10mM EDTA, and 50mM TrisCl pH 8.1) overnight in 65°C water bath, then for 30 min in 65°C water bath with 50ul of SDS Elution buffer. 200μl of TE buffer was added to the eluted samples and were treated with 1μg RNase A for 30 minutes in 37°C and 1μg Protease K for at least 2 hours in 37°C.

ChIP-seq library prep kits (New England BioLabs) were used to generate ChIP-seq libraries and samples were sequenced using Illumina HiSeq 2500 sequencer at the Genomic Sequencing and Analysis Facility (GSAF) of The University of Texas at Austin.

2.7 RNA-sequencing (RNA-seq)

Total RNA was extracted using RNeasy® Plus Mini Kit (Qiagen). 1μg of RNA was used to prepare libraries for RNA-seq using NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England BioLabs). RNA-seq libraries were sequenced using Illumina HiSeq 2500 sequencer at the Genomic Sequencing and Analysis Facility (GSAF) of The University of Texas at Austin.

2.8 shRNA-mediated knockdown (KD)

HEK293T cells were plated in 6-well plate and incubated overnight. Cells were transfected with 1200ng of pLKO-neo empty or pLKO-neo Fosl1 shRNA with 800ng of Δ8.9 and 400ng of VSVG helper plasmids using Fugene (Promega), according to manufacturer's instruction. After 24 hours, HEK293T medium was replaced with TS media (without Fgf4 and heparin). 48 hours after transfection, supernatants containing viral particles were collected and filtered through 0.45μm pore size filters. For infection, 1.5 X 10^5 TS cells were plated in 24-well plate with virus-containing supernatant supplemented with polybrene (Millipore). To observe the effect of Fosl1 KD during differentiation, TS cells were expanded to 6-well plates under differentiation condition (media without Fgf4 and heparin).

Chapter 3: Results

3.1 Overexpression (OE) of Fosl1 in ES cells leads to differentiation of ES cells to TS-like cells

In order to investigate the functions of Fosl1, we monitored the mRNA expression levels of Fosl1 in TS cells and differentiating TS cells. We found that the level of Fosl1is higher in differentiating TS cells (**Figure 1A**), indicating that Fosl1 expression increases over the course of differentiation of TS cells. To examine whether Fosl1 can promote transdifferentiation of ES cells to TS-like cells, we generated stable cell line of Fosl1 overexpressing BirA ES cells. BirA ES cells serve as a control. Using RT-qPCR and Western blot, we validated ectopic expression of Fosl1 (**Figure 1B**). Compared to the typical round-shaped colony morphology of control ES cells, Fosl1 OE cells displayed differentiated (flattened) morphology along with weak AP activity, indicative of the exit of ES cell pluripotency (**Figure 1B**). We also investigated how OE of Fosl1 affects the expression of various lineage markers using RT-qPCR. We found that the expression levels of ES pluripotency-associated genes, such as Oct4, Nanog and Sox2, remained similar to that of control ES cells while the expression levels of marker genes representing mesoendoderm, ectoderm, and mesoderm lineages were either similar to or lower than the control ES cells (**Figure 1C**). However, RT-qPCR revealed that markers associated with differentiated TS cells such as Gata3, Hand1, Krt8, Plf and Tead3 were significantly upregulated (**Figure 1C**). This suggests that Fosl1 activates genes associated with differentiated TE lineage. Even though some TS self-renewal markers such as Tead4, Id2 and Arid3a showed slight upregulation, levels of other TS self-renewal

marker such as Cdx2, Eomes, Elf5 and Esrrb did not show significant change compared to control (**Figure 1C**), which further suggest that Fosl1's role in TS cells is related to differentiation rather than self-renewal. These results prompted us to look at the expression level of Fosl1 is in Fosl1 OE cells, compared to TS cells and differentiating TS cells. We found that the expression of Fosl1 was almost 40 fold higher in Fosl1 OE cells compared to TS cells, confirming that OE was successful. In contrast, Fosl1 levels in Fosl1 OE cells were comparable to that of differentiated (Day 3) TS cells (**Figure 2**). This suggested that Fosl1 OE cells are physiologically similar to differentiated TS cells, with regards to Fosl1 expression level. Collectively, these results indicated that Fosl1 might possess potential to direct ES cells to differentiated TS-like cells.

3.2 Fosl1 OE leads to global induction of TS differentiation markers as well as Prolactin (Prl) family genes

To get a more comprehensive idea of how Fosl1 OE impacts the global transcriptional program, we performed RNA-seq. The expression analysis confirmed that the levels of Oct4, Sox2 and Nanog (ES pluripotency factors) as well as markers of various lineages, such as Gsc, Cxcr4 (mesoendoderm), Fgf5, Otx2, Nes, Hoxb1 (ectoderm), Gata4, Gata6, Sox17, Isl1 (endoderm) and Bmp2 (mesoderm) were similar between Fosl1 OE cells and control ES cells (**Figure 3A, 3B**). Consistent with our RT-qPCR data, we were also able to confirm that the levels of TS self-renewal markers such as Cdx2, Elf5, Esrrb, and Tcfap2c have not changed significantly in Fosl1 OE cells compared to control ES cells (**Figure 3C**). On the other hand, TS differentiation markers, such as Hand1, Krt8, and

H19 were upregulated upon OE of Fosl1 in ES cells (**Figure 3D**). It is possible that we did not observe decrease in the expression of TS self-renewal markers since it has been noted that TS cells derived from Cdx2-/-, Eomes-/-, and Tead4-/- embryos fail to differentiate or form TGCs, when cultured *in vitro* (Strumpf et al. 2005; Nishioka et al. 2008). Among the genes that were induced upon Fosl1 OE, we found that H19 was one of the very highly upregulated genes (**Figure 3D**). It has been shown that induction of H19, a noncoding RNA, leads to commitment of mouse ES cells to trophoblast lineages (Fujimori et al. 2013). We also found that expression of members of Prolactin (Prl) family, such as Prl2c2, Prl2c5, Prl2a1, and Prl3d1 is also elevated (**Figure 3E**). Given that TGCs have been characterized by expression of the Prl gene family (Hamlin et al., 1994; Sahgal et al., 2005), increased level of such genes suggests that Fosl1 OE cells have similar transcriptional landscape to differentiated TS cells, and perhaps have been converted into trophoblast giant-like cells.

3.3 GSEA analysis reveals that Fosl1 OE cells possess characteristics of differentiated TS cells

We further performed Gene Set enrichment analysis (GSEA) to compare the gene expression profile of Fosl1 OE cells to those of ES cells (GSE3766, Ralston et al. 2010) and TS cells (GSE12985, Ralston et al. 2010). As seen in **Figure 4A**, genes enriched upon Fosl1 OE were found to have negative correlation with ES cell-specific gene set, while the analysis revealed that there was no significant correlation with TS cell-specific gene set. Since upregulation of differentiated TS cell markers was observed in **Figure**

1C, we wanted to investigate which stage of differentiating TS cells are similar to Fosl1 OE cells. To do this, we used a published dataset obtained from time-course differentiation of TS cells for 6 days (GSE12985, Ralston et al. 2010) (**Figure 4B**). Remarkably, we found that correlation between genes upregulated upon Fosl1 OE increased as the differentiation of TS cells progressed, with the highest correlation seen at differentiation day 6. This implied that genes that are upregulated upon differentiation of TS cells are also upregulated upon Fosl1 OE in ES cells and thus, transdifferentiate ES cells to differentiated TS-like cells.

Since it has been noted that TS cells undergo epithelial-mesenchymal transition (EMT) as they differentiate (Sutherland, 2003), we examined the correlation between EMT gene set and gene expression pattern obtained from Fosl1 OE in ES cells and found that there was a high correlation with genes enriched upon Fosl1 OE (**Figure 4C**). Significant enrichment was observed for genes, such as Serpine2, Col4a1, Col4a2, Col5a1, Col7a1, Itga5, Itgb5, Ecm1, and Mmp14 upon Fosl1 OE.

There was also a noticeable negative correlation with genes specific for G2 to M checkpoint of cell cycle (**Figure 4C**). We found that levels of genes, such as AurkA, Chek1, Cdkn1b, Cdc6, and Hus1 are lower in Fosl1 OE cells. These data support our observations in **Figure 1B**, in which Fosl1 OE cells are larger in size and show prominent nuclei, suggesting that Fosl1 OE cells might undergo endoreplication, which is a hallmark of non-proliferating TGCs (DePamphilis et al., 2012). These results suggest that Fosl1 OE cells are indeed biased towards differentiated TE lineage, particularly the TGC subtype.

3.4 Chromosomal targets of Fosl1 reflects its function in ES cells to TS-like cells transdifferentiation

In order to map the global target loci of Fosl1, we used *in vivo* biotinylation-mediated ChIP followed by massive parallel sequencing (Bio-ChIP-seq) We found that Fosl1 occupies primarily intergenic (~50%) and intron (~41%) regions of the genome (**Figure 5A**). Genomic Regions Enrichment of Annotations Tool (GREAT) analysis revealed that Fosl1 binding occurs mostly within 50 to 500 kb from the transcription start sites (TSS) of well annotated genes (**Figure 5A**). Interestingly, we found that Fosl1 binds to its own promoter and distal enhancer regions (**Figure 5B**), indicating that Fosl1 may form an auto-regulatory loop and activate its own transcription. Examples of such loop have been known for master regulators of ES cells, such as Oct4, Sox2 and Nanog, which form "interconnected auto-regulatory loops" (Boyer et al., 2005). This suggests that Fosl1 might also possess properties of a key regulator for TS cell differentiation. We also found that Fosl1 occupies a larger region of Junb gene (one of the components of AP-1 dimer complex) (**Figure 5B**), which suggests that Fosl1 predominantly works with Junb as part of its action mechanism.

In addition, Fosl1 also occupied large set of genes upregulated upon differentiation of TS cells, including Krt8, Bhlhe40, Gata2, and Ovol2 (**Figure 5C**). This shows that Fosl1 might bind to and regulate these genes for transition of ES cells to differentiated TS-like cells. When we tested Fosl1 occupancy for the genes associated with self-renewal of TS cells, we found that it occupies regions of genes such as Esrrb, Eomes, Tead4, and Elf5 (**Figure 5D**). This is unexpected since we did not see dramatic changes in expression

levels of these genes upon OE of Fosl1 in ES cells by RT-qPCR (**Figure 1C**) and RNA-seq (**Figure 3C)**. What this suggests is that Fosl1 not only orchestrates the differentiation of TS cells, but also sustains the TS-specific gene expression.

Furthermore, we also found Fosl1 occupancy for Oct4 but not Nanog or Sox2 (**Figure 5E**), which is also surprising since we do not see significant downregulation of Oct4 upon Fosl1 OE. This could suggest that Fosl1 is the downstream executor of Oct4-depletion mediated TE differentiation or that Fosl1 acts through a pathway independent of Oct4-depletion mediated TE differentiation.

We also found that Fosl1 also occupies regulatory regions of EMT-associated genes such as Epcam, Vim, and various matrix metalloproteinase including Mmp9, Mmp14, and Mmp28 (**Figure 5F**). This implies that Fosl1 directly regulates these genes and modulates differentiation of TS cells by activating EMT.

3.5 Gene Ontology (GO) analysis reveals role of Fosl1 related to placental development

Gene ontology (GO) analysis of Fosl1 targets showed significant enrichment in processes associated with placental development, trophectodermal cell differentiation, cell junction organization/assembly, and adherens junction organization (**Figure 6A**). GREAT analysis also revealed that dysregulation of Fosl1 targets leads to mouse phenotype associated with abnormal trophoblast layer morphology, abnormal TGCs, and abnormal cell adhesion, implicating the roles of Fosl1 in these processes *in vivo* (**Figure 6B**). In addition, GO molecular processes showed that targets of Fosl1 are enriched in the

processes of phosphatidylinositol 3-kinase (PI3K) binding (**Figure 6C**). This confirms the results of previous studies showing that Fosl1 is a downstream effector of PI3K-AKT pathway (Kent et al., 2011). This further emphasizes the important role of Fosl1 in transdifferentiation of ES cells to giant-like cells as an active PI3K pathway favors TGCs and survival of TGCs are influenced by PI3K signaling (Kent et al., 2010). Mouse Genome Informatics (MGI) expression patterns of Fosl1 targets also show that normal trophectoderm development is affected at an early time point upon dysregulation of Fosl1 targets (**Figure 6D**). Therefore, these results reinforce our hypothesis that Fosl1 is crucial for TE development and that Fosl1 OE can initiate TE differentiation program in ES cells.

3.6 ChIP-seq data reveals that Fosl1 acts as an activator of genes associated with TE lineage

In order to understand how Fosl1 regulates transdifferentiation of ES cells to TS-like cells, we compared the gene expression profile of Fosl1 OE cells with target occupancy of Fosl1, using moving window average (**Figure 7A**). We found that most of the genes that were upregulated upon Fosl1 OE were direct targets of Fosl1. However, genes downregulated upon OE of Fosl1 were not the direct targets of Fosl1. This demonstrates that Fosl1 acts an activator and not a repressor of target gene transcription. This is similar to Cdx2, one of the key regulators of TE lineage, for which upregulation of its target genes upon OE in ES cells is accomplished by direct binding, but not for downregulated genes (Nishiyama et al., 2009).

In addition, motif analysis of Fosl1 targets identified the known Fosl1 binding motif (TGAGTCA) as well as Junb binding motif (predicted by GREAT) (**Figure 7B**). This result implies that Fosl1 may regulate its targets with Junb which supports previous studies suggesting that Fosl1 and Junb interaction might be the critical determinant of regulation of the target genes by Fosl1 (Seldeen et al., 2009).

3.7 Fosl1 knockdown (KD) impedes differentiation of mouse TS cells to TGCs

In order to validate the role of Fosl1 in differentiation of TS cells, we used shRNA-mediated KD to downregulate the levels of Fosl1 in mouse TS cells. We cultured control TS cells (pLKO-neo empty) and Fosl1 KD cells in differentiation condition (without Fgf4 and heparin) and collected samples at Day 3 and Day 7 of differentiation. We observed that at Day 3, while control TS cells started to acquire differentiation morphology, Fosl1 KD cells maintained undifferentiated morphology (**Figure 8A**). By Day 7, control TS cells had differentiated into giant cells and formed clumps (signs of TS cell differentiation), Fosl1 KD cells showed a smaller number of giant cells and majority of undifferentiated morphology was still prevalent. Fosl1 KD was verified using RT-qPCR (**Figure 8B**). Upon differentiation, TS self-renewal marker, Esrrb was highly maintained in Fosl1 KD cells, compared to control TS cells (**Figure 8C**). RT-qPCR also showed that genes upregulated upon differentiation of TS cells, such as Hand1, Prl3da, and Prl15a, were not activated in Fosl1 KD cells upon differentiation, compared to control TS cells (**Figure 8D**). These data show that Fosl1 is necessary for proper and timely upregulation of TS cell differentiation markers. Interestingly, marker of spongiotrophoblast, (a subtype

of differentiated TS cells), Flt1, had begun to increase by Day 7 of differentiation, specifically Flt1 (**Figure 8E**). This suggests that Fosl1 is critical for TS cell differentiation to TGCs, and that in its absence, TS cells may differentiate into other subtype of TS cells, specifically spongiotrophoblast. This highlights the importance of studying the roles of TS cell-specific TFs in placental development, as improper differentiation can lead to serious health risks during pregnancy, including preeclampsia.

Chapter 4: Discussion

In this study, we have established that the ectopic expression of Fosl1 is sufficient to transdifferentiate ES cells to differentiated TS-like cells. Our results reveal that Fosl1 acts as a transcriptional activator of its target genes, and most of the genes that are upregulated upon Fosl1 OE are involved in biological processes involved in placental development, trophectodermal cell differentiation, adherens junction organization and other TE-related processes. Surprisingly, we did not observe drastic downregulation of ES cell core factors such as Oct4, Sox2, and Nanog upon Fosl1 OE in ES cells. It is possible that Fosl1 acts independently of Oct4 depletion-mediated differentiation or that Fosl1 is a downstream executor of Oct4 depletion-mediated differentiation. Since Junb is known to act as a negative regulator of cell proliferation in fibroblasts, it is possible that Junb, which dimerizes with Fosl1, acts as a repressor during transdifferentiation of ES cells to TS-like cells (Shaulian and Karin, 2002). In addition, we also did not see downregulation of TS self-renewal markers such as Cdx2, Eomes, and Tead4 upon OE of Fosl1 in ES cells. Studies have shown that when cultured *in vitro*, $Cdx2^{-/-}$, $Eomes^{-/-}$, and $Tead4^{-/-}$ embryos fail to differentiate or form TGCs (Nishioka et al., 2008; Strumpf et al., 2005). Therefore, it is plausible that sustained expression of these markers is important for Fosl1-mediated ES cells to TS-like cells transdifferentiation, which leads to cells with properties of differentiated TS cells.

We also observed a strong positive correlation of genes upregulated upon Fosl1 OE and genes upregulated upon TS cell differentiation. In addition, we found upregulation of Prl family genes, which are associated with TS cell differentiation, upon Fosl1 OE.

Crucially, we observed that KD of Fosl1 in TS cells led to delay in differentiation of TS cells compared to control cells. Additionally, gene involved in differentiation of spongiotrophoblast subtype was upregulated by Day 7 in Fosl1 KD TS cells, while genes involved in differentiation towards TGCs were not, indicating that Fosl1 is required for differentiation of TS cells to TGCs. GREAT analysis revealed that dysregulation of Fosl1 leads to mouse phenotype associated with abnormal trophoblast layer morphology and abnormal TGCs. All these indicate that Fosl1 is an important regulator of TGC development.

We also found that Fosl1 binds to its own regulatory elements, in addition to TE-specific gene. Such autoregulatory loops are properties of master regulators as shown in ES cells (Boyer et al., 2005). This indicates that Fosl1 might be a key regulator of differentiation of TS cells. In lieu of these findings, it is important to test closely the role of Fosl1 in placental development. Differentiated cell types in TE lineages have numerous functions, including invasion into maternal uterine vasculature (Adamson et al., 2002; Hemberger et al., 2003), endocrine functions, regulation of maternal-fetal nutrient/gas exchange, and excretion of fetal waste (Rossant and Cross, 2001; Soares et al., 1996). Therefore, it is essential to gain better understanding of TE lineage differentiation in order to understand diseases associated with dysregulation of such cell types. As a key downstream mediator of PI3K/AKT pathway, which is involved in preeclampsia and IUGR, Fosl1 can give us insight into these diseases, and may be even used as a therapeutic target in the future.

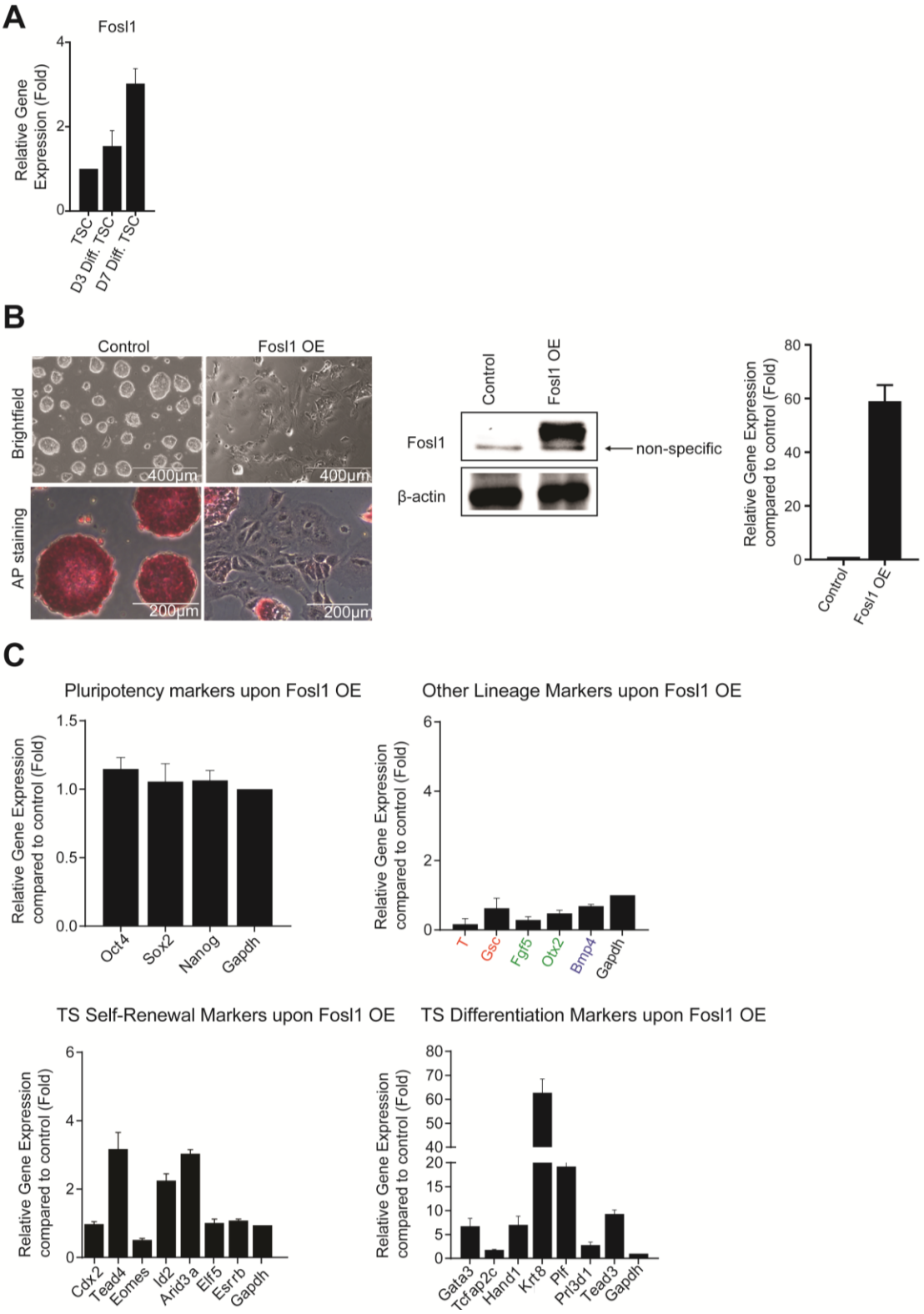

Figure 1, continued on next page

Figure 1: Expression levels of various lineage markers in Fosl1 OE cells l. (A) Expression analysis of Fosl1 in TS cells, and D3, D4 differentiating TS cells. (B) ES cells morphology upon overexpression of Fosl1. Fosl1 OE cells were cultured for 3 days and stained for Alkaline Phosphatase (AP). Protein and mRNA levels of Fosl1 following Fosl1 OE as measured by Western Blot and RT-qPCR. B-actin was used as loading control. (C) Expression analysis of ES pluripotency-associated genes, lineage markers (mesoendoderm (orange); ectoderm (green); mesoderm (blue)) and TS self-renewal and differentiation markers by RT-qPCR, compared to control.

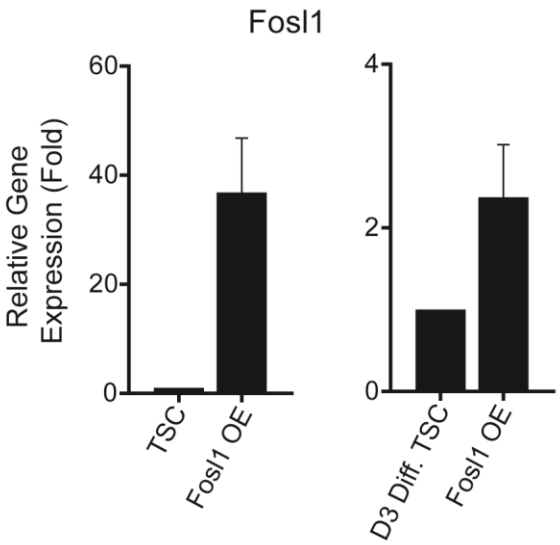

Figure 2: Fosl1 levels in Fosl1 OE, TS, and differentiating TS cells. Expression analysis of Fosl1 in Fosl1 OE cells, compared to TS cells and D3 differentiating TS cells.

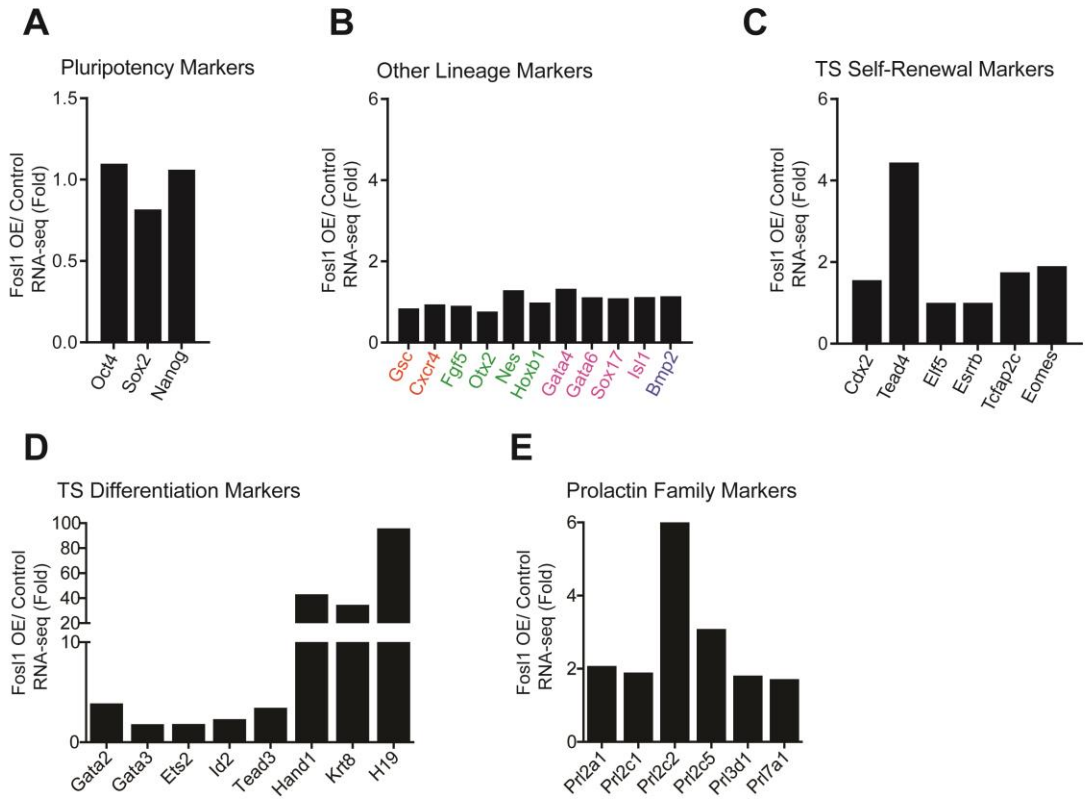

Figure 3: Fosl1 OE leads to global induction of TS differentiation markers. (A, B, C, D, E) RNA-seq analysis confirming expression of (A) ES pluripotency-associated genes in Fosl1 OE cells, (B) Lineage-associated genes (mesoendoderm (orange); ectoderm (green); endoderm (pink), mesoderm (blue)) in Fosl1 OE cells. (C) TS self-renewal markers, (D) TS differentiation markers (E) Prolactin (Prl) family markers. RNA-seq data with RPKM values is available in supplementary material.

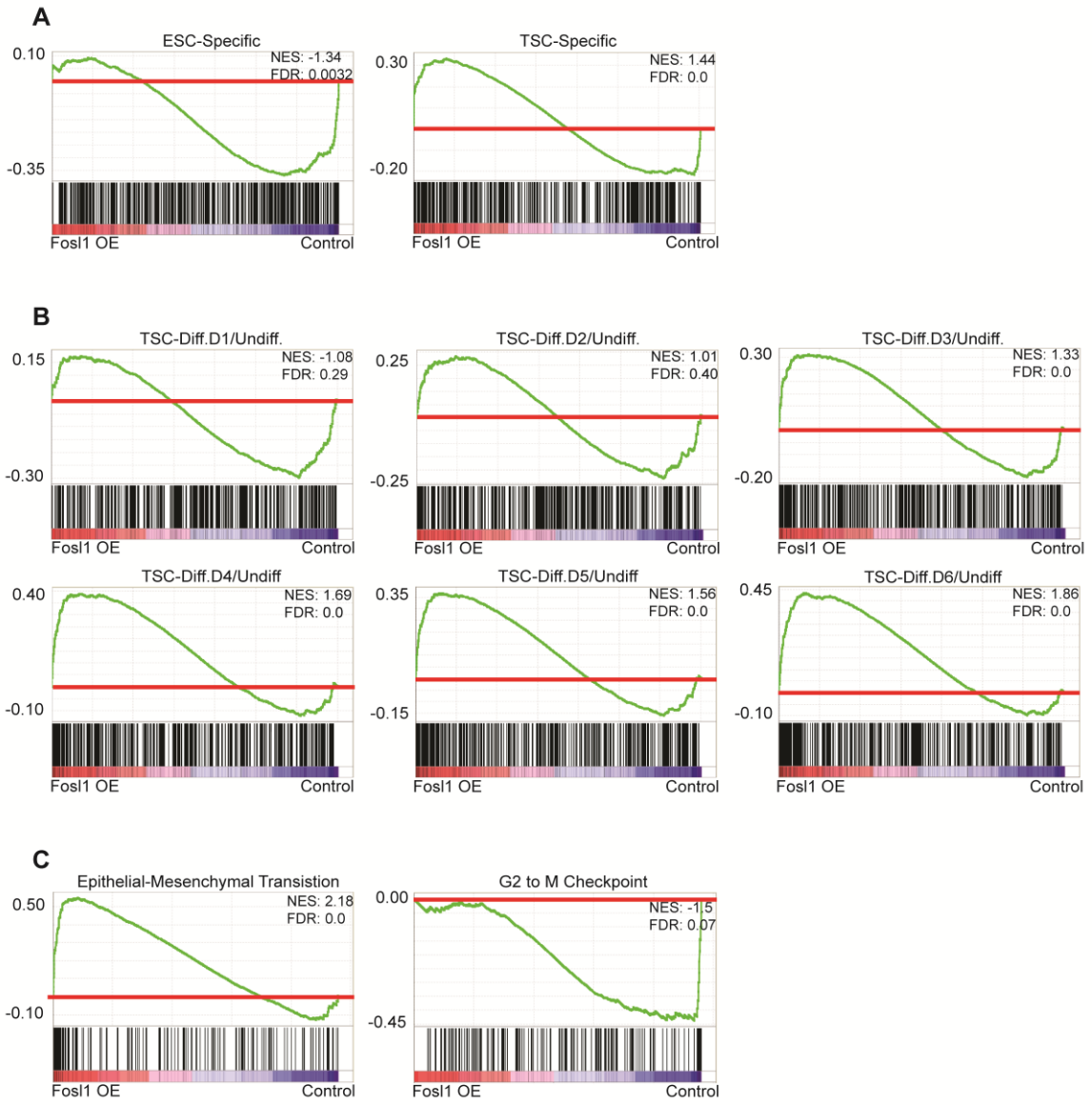

Figure 4: Fosl1 OE cells possess properties of differentiated TS cells. (A, B, C) GSEA analysis using ordered gene expression levels from Fosl1 overexpressing cells and control ES cells. Normalized enrichment score (ES) and false discovery rate (FDR) are shown. (A) GSEA using top 500 highly expressed in genes in ES cells (GSE3766), and TS cells (GSE12985). (B) GSEA using top 500 genes upregulated at different time points of TS cells differentiation (GSE12985). (C) GSEA analysis using GSEA dataset using genes correlated upon epithelial-mesenchymal transition (EMT) and G2 to M checkpoint of cell cycle. The gene sets used for GSEA analysis is available in supplementary material.

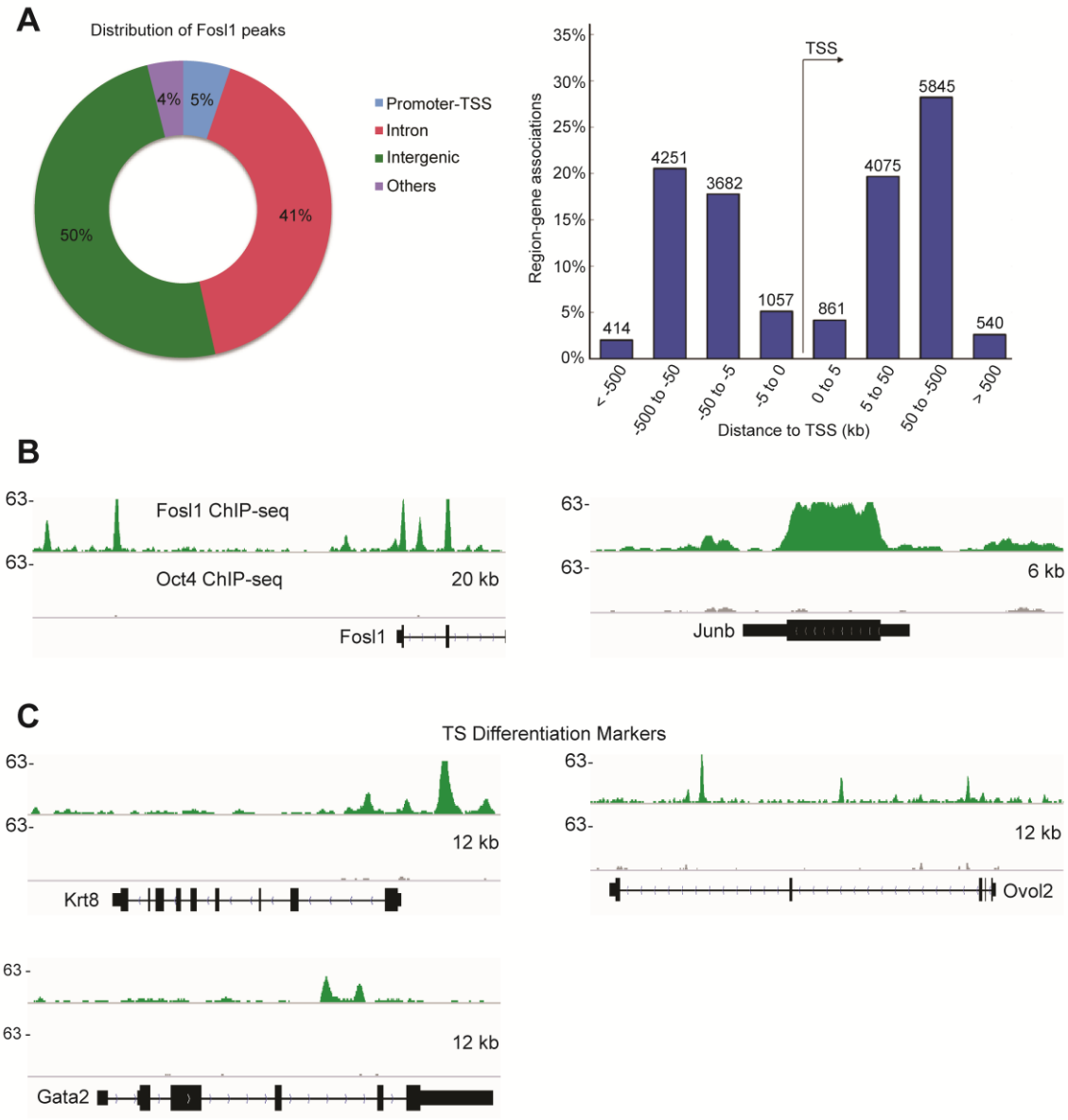

Figure 5, continued on next page

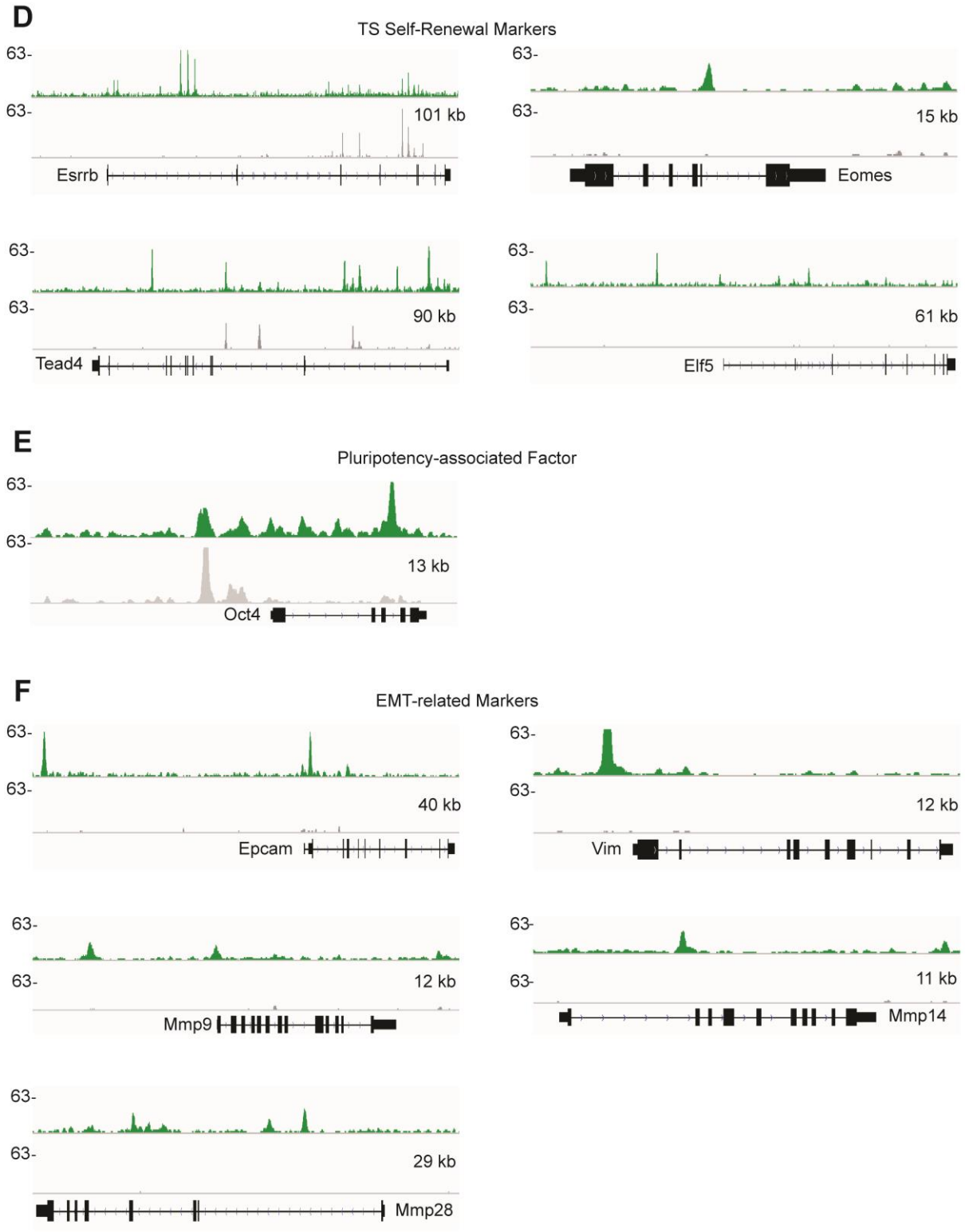

Figure 5, continued on next page

Figure 5: Chromosomal targets of Fosl1 reveals its function in ES cells to TS-like cells transdifferentiation. (A) Pie chart representing distribution of Fosl1 peaks. Bar graph showing pattern of Fosl1 binding at various regions with respect to distance to TSS. (B, C, D, E, F) Snapshots of ChIP-seq signal tracks of Fosl1 and Oct4 at regulatory regions of (B) Fosl1 and Junb (C) TS differentiation markers (D) TS self-renewal markers (E) Oct4 and (F) EMT-associated genes.

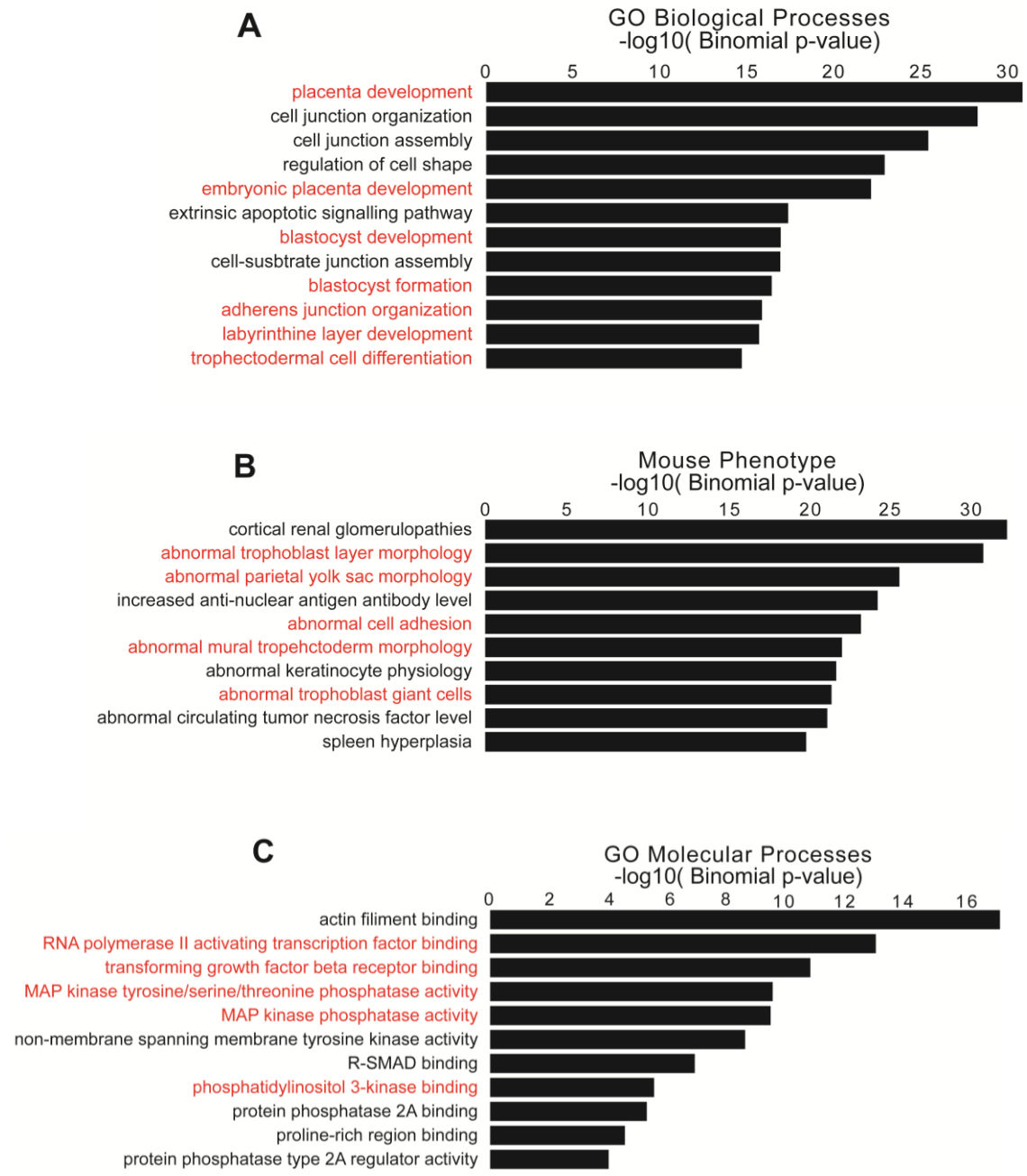

Figure 6, continued on next page

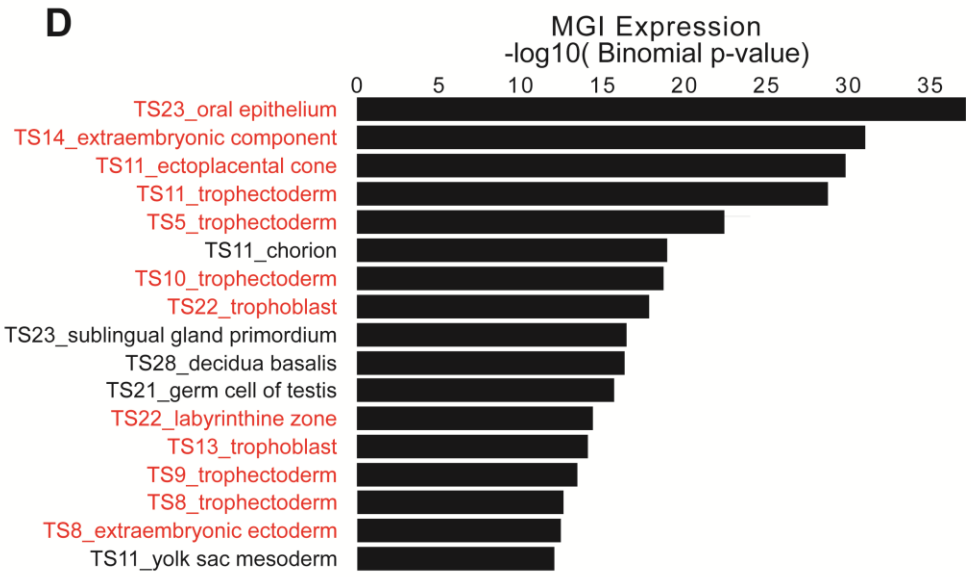

Figure 6: Gene Ontology analysis reveals Fosl1's role in placental development. (A) Significantly enriched terms related to biological processes upon upregulated genes upon Fosl1 OE by Gene ontology (GO). (B) Mouse phenotype affected upon dysregulation of genes regulated by Fosl1. (C) Significantly enriched terms related to molecular processes upon upregulated genes upon Fosl1 OE by Gene ontology (GO). (D) Endogenous gene expression of genes affected by Fosl1 OE during mouse development by Mouse Genome Informatics (MGI).

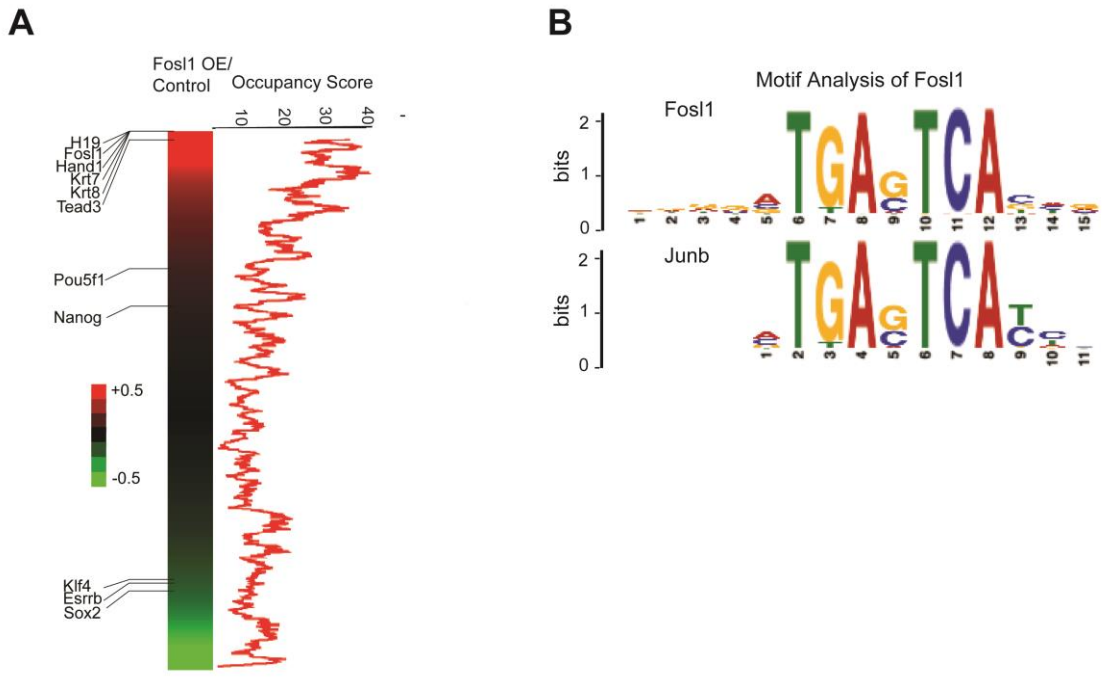

Figure 7: Fosl1 acts as an activator of genes associated with TE lineage. (A) A heat map representation of expression profile of Fosl1 overexpressing cells denoting TE-specific genes and pluripotency-associated genes. Genes were ordered according to gene expression levels in Fosl1 overexpressing cells relative to control ES cells. Moving average with window size of 250 was plotted to corresponding Fosl1 occupancy signals. (B) Motif analysis of Fosl1 target loci indicates high overlap with target motif of Junb. ChIP-seq occupancy scores are available in supplementary material.

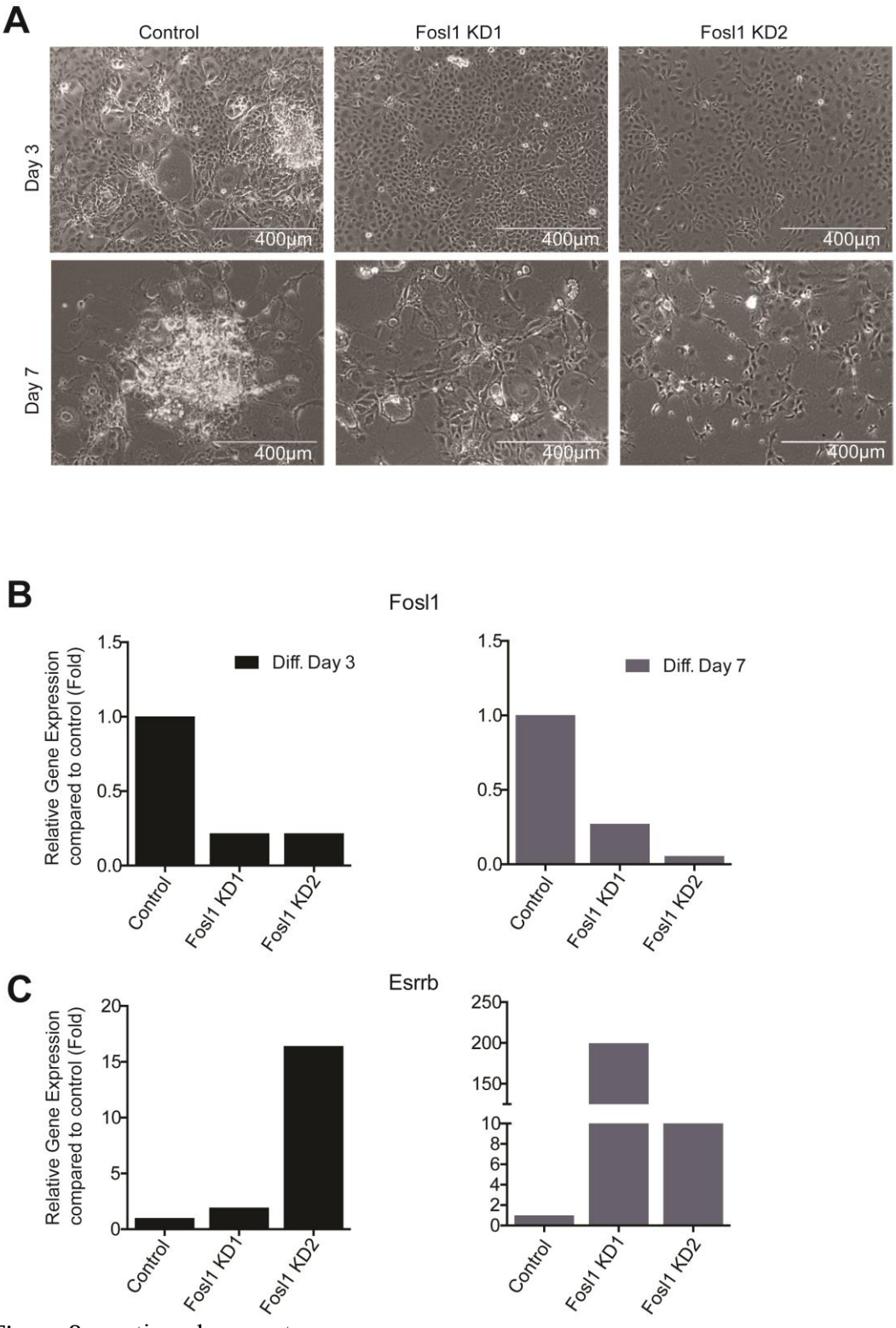

Figure 8, continued on next page

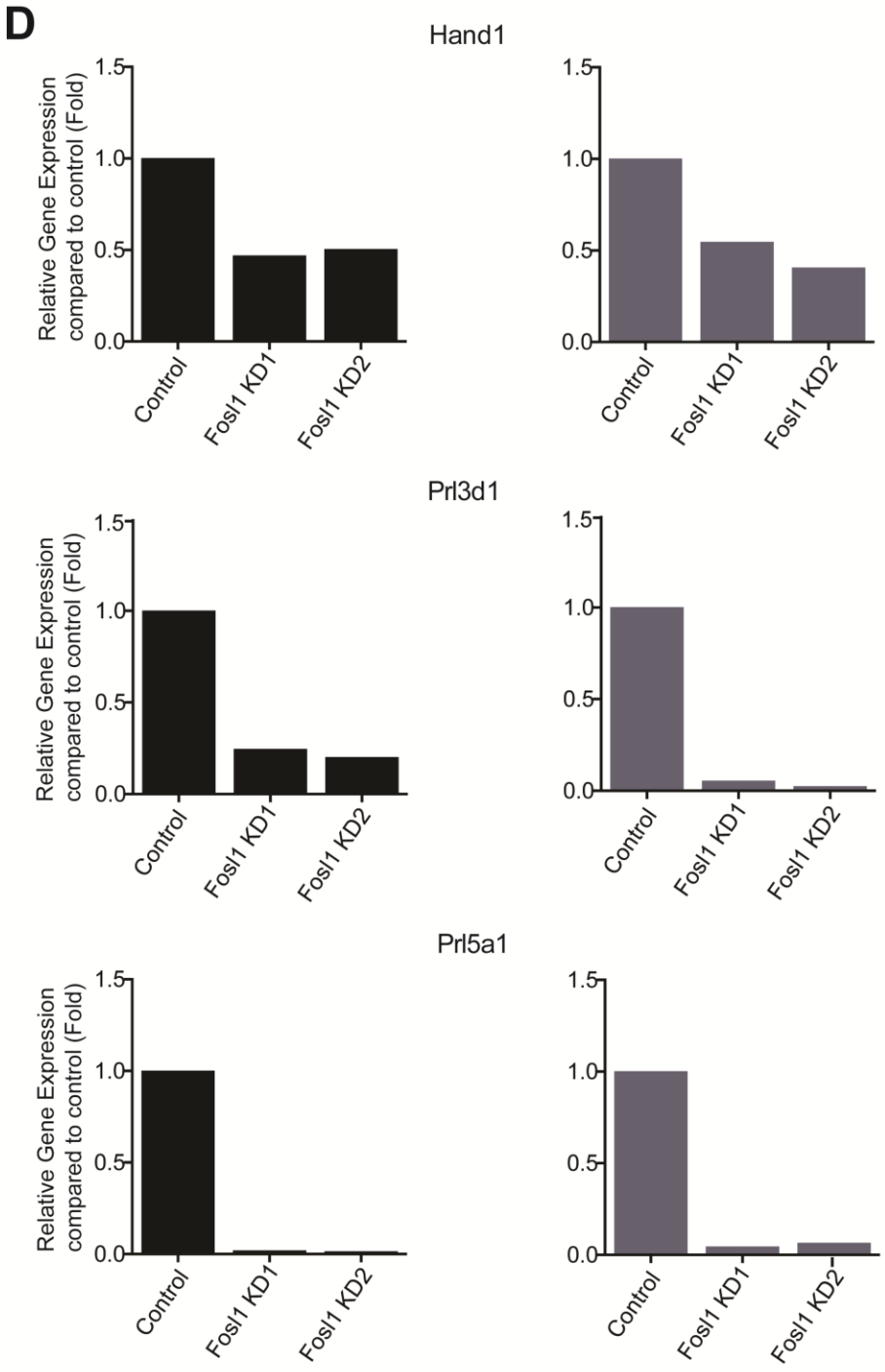

Figure 8, continued on next page

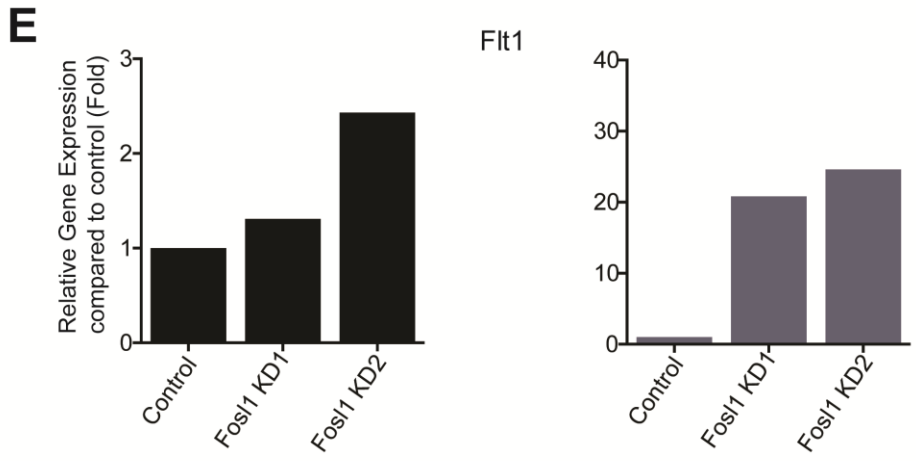

Figure 8: Fosl1 KD in TS cells hinders formation of TGCs. (A) Morphology of control TS cells and TSC with Fosl1 knockdown (KD) at various time points (D3 and D7) of differentiation. (B) mRNA level of Fosl1 in Fosl1 KD TS cells compared to control TS cells. Expression analysis of various (C) TS cell self-renewal markers (D, E) TS cell differentiation markers, by RT-qPCR.

Table 1: Primers and shRNA sequences

RT-qPCR primers		
	Forward	Reverse
Arid3a	AGGTTATCAACAAGAAACTGTGGAG	TACTTCATGTACTGTGTCCGAAGTG
Bmp4	GAGTTTCCATCACGAAGAACATCT	AGGAGATCACCTCATTCTCTGG
Cdx2	GCGAAACCTGTGCGAGTGGATG	CGGTATTTGTCTTTTGTCCTGGTTTTCA
Elf5	CAAGGTTACTCCTTTTTCAATGATG	GCTGTGACAGTCTTGACTCTTGAT
Eomes	ATAAGATGTACGTTCACCCAGAATC	GCACCTTTGTTATTGGTGAGTTTTA
Esrrb	TAAAAAGCCATTGACTAAGATCGTC	CAATTCACAGAGAGTGGTCAGG
Fgf5	GGATTGTAGGAATACGAGGAGTTTT	AACTTACAGTCATCCGTAAATTTGG
Flt1	TCTACCAAATCATGTTGGATTGCTG	ATGTAATCTTTCCCATCCTGTTGGA
Fosl1	CTAAGTGCAGAAACCGAAGAAAG	CTTCTGCAGCTCTTCAATCTCTC
Gapdh	AAATTCAACGGCACAGTCAAG	CACCCCATTTGATGTTAGTGG
Gata3	TGGGCTGTACTACAAGCTTCATAA	CTTTTTCGATTTGCTAGACATCTTC
Gsc	AGAAGGTGGAGGTCTGGTTTAAG	GAGGACGTCTTGTTCCACTTCT
Hand1	CCTTCAAGGCTGAACTCAAAAA	GCGCCCTTTAATCCTCTTCT
Id2	ATCACCAGAGACCTGGACAGAAC	GCTATCATTCGACATAAGCTCAGA
Krt8	AGAATGAATTTGTCCTCATCAAGAA	GAAGTTGATCTCGTCGGTCAGTC
Nanog	AGGGTCTGCTACTGAGATGCTCTG	CAACCACTGGTTTTTCTGCCACCG
Oct4	TCTGGAGACCATGTTTCTGAAGT	TACAGAACCATACTCGAACCACAT
Otx2	AAGTGAGTTCAGAGAGTGGAACAAG	CTCCAGATAGACACTGGAGCACT
Prl3d1	ACTGAAGACCTGTATACTCGTTTGG	GGAAGTGTTCTGTCTGTTATCCAAG
Prl5a1	GATCTCGATGGAACTCCTTATGTTA	TCAGGATTAACCTGGCTGAGTATAG
Sox2	GCGGAGTGGAAACTTTTGTC	TATTTATAATCCGGGTGCTCCT
T	CTTCAAGGAGCTAACTAACGAGATG	GTCCAGCAAGAAAGAGTACATGG
Tcfap2c	CCTAGTAAAGCGGTGGCTGA	GAACTCCTTGCACACCTGCT
Tead3	AAAACCAGGACAAGAAAACAGGT	GAGAGCTTTGTCCTTGGAGACTT
Tead4	ATGACAAGTTCCCAGAGAAGAAG	GTCATCGATGTTGGTATTGAGGT

Cloning primers		
	Forward	Reverse
FB-Fosl1	CCGAGCTCGAGGATCCTCAT GTACCGAGACTACGGGGA	TAGAACTAGTGGATCCTCACA AAGCCAGGAGTGTAG

Table 1, continued on next page

	Forward	Reverse
shFosl1-1	CCGGCGACAAATTGGAGGATGA GAACTCGAGTTCTCATCCTCCAA TTTGTCGTTTTTG	AATTCAAAAACGACAAATTGGAG GATGAGAACTCGAGTTCTCATCCT CCAATTTGTCG
shFosl1-2	CCGGCCAGTGCCTTGCATCTCC CTTCTCGAGAAGGGAGATGCAA GGCACTGGTTTTTG	AATTCAAAAACCAGTGCCTTGCAT CTCCCTTCTCGAGAAGGGAGATGC AAGGCACTGG

References

Adamson, S.L., Lu, Y., Whiteley, K.J., Holmyard, D., Hemberger, M., Pfarrer, C., and Cross, J.C. (2002). Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. Dev. Biol. *250*, 358–373.

Ain, R., Canham, L.N., and Soares, M.J. (2003). Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: Novel endocrine phenotype and regulation. Dev. Biol. *260*, 176–190.

Arceci, R.J., King, A.A., Simon, M.C., Orkin, S.H., and Wilson, D.B. (1993). Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. Mol. Cell. Biol. *13*, 2235–2246.

Avilion, A.A., Nicolis, S.K., Pevny, L.H., Perez, L., Vivian, N., and Lovell-Badge, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. *17*, 126–140.

Benchetrit, H., Herman, S., van Wietmarschen, N., Wu, T., Makedonski, K., Maoz, N., Yom Tov, N., Stave, D., Lasry, R., Zayat, V., et al. (2015). Extensive nuclear reprogramming underlies lineage conversion into functional trophoblast stem-like cells. Cell Stem Cell *17*, 543–556.

Boeuf, H., Hauss, C., Graeve, F. De, Baran, N., and Kedinger, C. (1997). Leukemia Inhibitory Factor–dependent Transcriptional Activation in Embryonic Stem Cells. J. Cell Biol. *138*, 1207–1217.

Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G., et al. (2005). Core Transcriptional Regulatory Circuitry in Human Embryonic Stem Cells. Cell *122*, 947–956.

Burdon, T., Stracey, C., Chambers, I., Nichols, J., and Smith, A. (1999). Suppression of SHP-2 and ERK Signalling Promotes Self-Renewal of Mouse Embryonic Stem Cells. Dev. Biol. *210*, 30–43.

Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S., and Smith, A. (2003). Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell *113*, 643–655.

Cross, J.C. (2000). Genetic insights into trophoblast differentiation and placental morphogenesis. Semin. Cell Dev. Biol. *11*, 105–113.

Cross, J.C., Werb, Z., and Fisher, S.J. (1994). Implantation and the placenta: key pieces of the development puzzle. Science *266*, 1508–1518.

Cross, J.C., Hemberger, M., Lu, Y., Nozaki, T., Whiteley, K., Masutani, M., and Adamson, S.L. (2002). Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. Mol. Cell. Endocrinol. *187*, 207–212.

Davis, R.L., Weintraub, H., and Lassar, A.B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell *51*, 987–1000.

DePamphilis, M.L., de Renty, C.M., Ullah, Z., and Lee, C.Y. (2012). "The octet": eight protein kinases that control mammalian DNA replication. Front. Physiol. *3*, 1–20.

Duncan, S.A., Manova, K., Chen, W.S., Hoodless, P., Weinstein, D.C., Bachvarova, R.F., and Darnell, J.E. (1994). Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. Proc. Natl. Acad. Sci. U. S. A. *91*, 7598–7602.

Eferl, R., and Wagner, E.F. (2003). AP-1: a double-edged sword in tumorigenesis. Nat. Rev. Cancer *3*, 859–868.

Erlebacher, A., Price, K.A., and Glimcher, L.H. (2004). Maintenance of mouse trophoblast stem cell proliferation by TGF-β/activin. Dev. Biol. *275*, 158–169.

Evans, M.J., and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. Nature *292*, 154–156.

Festuccia, N., Osorno, R., Halbritter, F., Karwacki-Neisius, V., Navarro, P., Colby, D., Wong, F., Yates, A., Tomlinson, S.R., and Chambers, I. (2012). Esrrb is a direct Nanog target gene that can substitute for Nanog function in pluripotent cells. Cell Stem Cell *11*, 477–490.

Goldman-Wohl, D., and Yagel, S. (2002). Regulation of trophoblast invasion: from normal implantation to pre-eclampsia. Mol. Cell. Endocrinol. *187*, 233–238.

Graf, T., and Enver, T. (2009). Forcing cells to change lineages. Nature *462*, 587–594.

Guo, G., Huss, M., Tong, G.Q., Wang, C., Li Sun, L., Clarke, N.D., and Robson, P. (2010). Resolution of cell fate decisions revealed by single-cell gene expression analysis from zygote to blastocyst. Dev. Cell *18*, 675–685.

Hamlin, G.P., Lu, X.J., Roby, K.F., and Soares, M.J. (1994). Recapitulation of the pathway for trophoblast giant cell differentiation in vitro: stage-specific expression of members of the prolactin gene family. Endocrinology *134*, 2390–2396.

Heinrich, C., Blum, R., Gascón, S., Masserdotti, G., Tripathi, P., Sánchez, R., Tiedt, S., Schroeder, T., Götz, M., and Berninger, B. (2010). Directing astroglia from the cerebral cortex into subtype specific functional neurons. PLoS Biol. *8*, e1000373.

Hemberger, M., Nozaki, T., Masutani, M., and Cross, J.C. (2003). Differential expression of angiogenic and vasodilatory factors by invasive trophoblast giant cells depending on depth of invasion. Dev. Dyn. *227*, 185–191.

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.

Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X., and Hui, L. (2011). Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature *475*, 386–389.

Huang, P., Zhang, L., Gao, Y., He, Z., Yao, D., Wu, Z., Cen, J., Chen, X., Liu, C., Hu, Y., et al. (2014). Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell *14*, 370–384.

Hughes, M., Dobric, N., Scott, I.C., Su, L., Starovic, M., St-Pierre, B., Egan, S.E., Kingdom, J.C.P., and Cross, J.C. (2004). The Hand1, Stra13 and Gcm1 transcription factors override FGF signaling to promote terminal differentiation of trophoblast stem cells. Dev. Biol. *271*, 26–37.

Ieda, M., Fu, J., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell *142*, 375–386.

Kamei, T., Jones, S.R., Chapman, B.M., McGonigle, K.L., Dai, G., and Soares, M.J. (2002). The phosphatidylinositol 3-kinase/Akt signaling pathway modulates the endocrine differentiation of trophoblast cells. Mol. Endocrinol. *16*, 1469–1481.

Kent, L.N., Konno, T., and Soares, M.J. (2010). Phosphatidylinositol 3 kinase modulation of trophoblast cell differentiation. BMC Dev. Biol. *10*, 97.

Kent, L.N., Rumi, M.A.K., Kubota, K., Lee, D.-S., and Soares, M.J. (2011). FOSL1 is integral to establishing the maternal-fetal interface. Mol. Cell. Biol. *31*, 4801–4813.

Kim, J., Cantor, A.B., Orkin, S.H., and Wang, J. (2009). Use of in vivo biotinylation to study protein-protein and protein-DNA interactions in mouse embryonic stem cells. Nat. Protoc. *4*, 506–517.

Kubaczka, C., Senner, C.E., Cierlitza, M., Araúzo-Bravo, M.J., Kuckenberg, P., Peitz, M., Hemberger, M., and Schorle, H. (2015). Direct induction of trophoblast stem cells from murine fibroblasts. Cell Stem Cell 1–12.

Kulessa, H., Frampton, J., and Graf, T. (1995). GATA-1 reprograms avian myelomonocytic cell lines into eosinophils, thromboblasts, and erythroblasts. Genes Dev. *9*, 1250–1262.

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.

Laiosa, C. V, Stadtfeld, M., Xie, H., de Andres-Aguayo, L., and Graf, T. (2006). Reprogramming of committed T cell progenitors to macrophages and dendritic cells by C/EBPα and PU. 1 transcription factors. Immunity *25*, 731–744.

Latos, P.A., Goncalves, A., Oxley, D., Mohammed, H., Turro, E., and Hemberger, M. (2015). Fgf and Esrrb integrate epigenetic and transcriptional networks that regulate self-renewal of trophoblast stem cells. Nat. Commun. *6*, 7776.

Linzer, D.I., and Fisher, S.J. (1999). The placenta and the prolactin family of hormones: regulation of the physiology of pregnancy. Mol. Endocrinol. *13*, 837–840.

Lu, C., 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. *40*, 921–926.

MacAuley, A., Cross, J.C., and Werb, Z. (1998). Reprogramming the cell cycle for endoreduplication in rodent trophoblast cells. Mol. Biol. Cell *9*, 795–807.

Masui, S., Nakatake, Y., Toyooka, Y., Shimosato, D., Yagi, R., Takahashi, K., Okochi, H., Okuda, A., Matoba, R., Sharov, A.A., et al. (2007). Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat. Cell Biol. *9*, 625–635.

Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M., and Yamanaka, S. (2003). The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell *113*, 631–642.

Morris, S.A., Teo, R.T.Y., Li, H., Robson, P., Glover, D.M., and Zernicka-Goetz, M. (2010). Origin and formation of the first two distinct cell types of the inner cell mass in the mouse embryo. Proc. Natl. Acad. Sci. *107*, 6364–6369.

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.

Niakan, K.K., Schrode, N., Cho, L.T.Y., and Hadjantonakis, A.-K. (2013). Derivation of extraembryonic endoderm stem (XEN) cells from mouse embryos and embryonic stem cells. Nat. Protoc. *8*, 1028–1041.

Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Schöler, 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.

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*, 372–376.

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.

Nutt, S.L., Heavey, B., Rolink, A.G., and Busslinger, M. (1999). Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. Nature *401*, 556–562.

Park, J.K., Jeong, J.W., Kang, M.Y., Baek, J.C., Shin, J.K., Lee, S.A., Choi, W.S., Lee, J.H., and Paik, W.Y. (2010). Inhibition of the PI3K-Akt pathway suppresses sFlt1 expression in human placental hypoxia models in vitro. Placenta *31*, 621–629.

Plusa, B., Piliszek, A., Frankenberg, S., Artus, J., and Hadjantonakis, A.-K. (2008). Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. Development *135*, 3081–3091.

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.

Ralston, A., Cox, B.J., Nishioka, N., Sasaki, H., Chea, E., Rugg-Gunn, P., Guo, G., Robson, P., Draper, J.S., and Rossant, J. (2010a). Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. Development *137*, 395–403.

Ralston, A., Cox, B.J., Nishioka, N., Sasaki, H., Chea, E., Rugg-Gunn, P., Guo, G., Robson, P., Draper, J.S., and Rossant, J. (2010b). Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. Development *137*, 395–403.

Reya, T., and Clevers, H. (2005). Wnt signalling in stem cells and cancer. Nature *434*, 843–850.

Riley, P., Anaon-Cartwight, L., and Cross, J.C. (1998). The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. Nat. Genet. *18*, 271–275.

Rosner, M.H., Vigano, M.A., Ozato, K., Timmons, P.M., Poirie, F., Rigby, P.W.J., and Staudt, L.M. (1990). A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature *345*, 686–692.

Rossant, J., and Cross, J.C. (2001). Placental development: lessons from mouse mutants. Nat. Rev. Genet. *2*, 538–548.

Rossant, J., Chazaud, C., and Yamanaka, Y. (2003). Lineage allocation and asymmetries in the early mouse embryo. Philos. Trans. R. Soc. B Biol. Sci. *358*, 1341–1349.

Sahgal, N., Canham, L.N., Konno, T., Wolfe, M.W., and Soares, M.J. (2005). Modulation of trophoblast stem cell and giant cell phenotypes: analyses using the Rcho-1 cell model. Differentiation *73*, 452–462.

Schöler, H.R., Dressler, G.R., Balling, R., Rohdewohld, H., and Gruss, P. (1990). Oct-4: a germline-specific transcription factor mapping to the mouse t-complex. Trends Genet. *6*, 314.

Schreiber, M., Wang, Z.Q., Jochum, W., Fetka, I., Elliott, C., and Wagner, E.F. (2000). Placental vascularisation requires the AP-1 component fra1. Development *127*, 4937–4948.

Scifres, C.M., and Nelson, D.M. (2009). Intrauterine growth restriction, human placental development and trophoblast cell death. J. Physiol. *587*, 3453–3458.

Scott, I.C., Anson-Cartwright, L., Riley, P., Reda, D., and Cross, J.C. (2000). The HAND1 basic helix-loop-helix transcription factor regulates trophoblast differentiation via multiple mechanisms. Mol. Cell. Biol. *20*, 530–541.

Seldeen, K.L., McDonald, C.B., Deegan, B.J., and Farooq, A. (2009). Single Nucleotide Variants of the TGACTCA Motif Modulate Energetics and Orientation of Binding of the Jun-Fos Heterodimeric Transcription Factor †. Biochemistry *48*, 1975–1983.

Senner, C.E., and Hemberger, M. (2010). Regulation of early trophoblast differentiation – Lessons from the mouse. Placenta *31*, 944–950.

Shaulian, E., and Karin, M. (2002). AP-1 as a regulator of cell life and death. Nat. Cell Biol. *4*, E131–E136.

Silva, J., Nichols, J., Theunissen, T.W., Guo, G., van Oosten, A.L., Barrandon, O., Wray, J., Yamanaka, S., Chambers, I., and Smith, A. (2009). Nanog is the gateway to the pluripotent ground state. Cell *138*, 722–737.

Simmons, D.G., and Cross, J.C. (2005). Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. Dev. Biol. *284*, 12–24.

Smith, A.G., Heath, J.K., Donaldson, D.D., Wong, G.G., Moreau, J., Stahl, M., and Rogers, D. (1988). Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature *336*, 688–690.

Soares, M.J., Chapman, B.M., Rasmussen, C.A., Dai, G., Kamei, T., and Orwig, K.E. (1996). Differentiation of trophoblast endocrine cells. Placenta *17*, 277–289.

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.

Sutherland, A. (2003). Mechanisms of implantation in the mouse: Differentiation and functional importance of trophoblast giant cell behavior. Dev. Biol. *258*, 241–251.

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.

Verde, P., Casalino, L., Talotta, F., Yaniv, M., and Weitzman, J.B. (2007). Deciphering AP-1 Function in Tumorigenesis: Fra-ternizing on Target Promoters. Cell Cycle *6*, 2633–2639.

Wagner, E.F. (2002). Functions of AP1 (Fos/Jun) in bone development. Ann. Rheum. Dis. *61 Suppl 2*, ii40–ii42.

Watanabe, S., Umehara, H., Murayama, K., Okabe, M., Kimura, T., and Nakano, T. (2006). Activation of Akt signaling is sufficient to maintain pluripotency in mouse and primate embryonic stem cells. Oncogene *25*, 2697–2707.

Williams, R.L., Hilton, D.J., Pease, S., Willson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N.A., and Gough, N.M. (1988). Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature *336*, 684–687.

Xie, H., Ye, M., Feng, R., and Graf, T. (2004). Stepwise reprogramming of B cells into macrophages. Cell *117*, 663–676.

Yang, Z.-Z., Tschopp, O., Hemmings-Mieszczak, M., Feng, J., Brodbeck, D., Perentes, E., and Hemmings, B.A. (2003). Protein kinase Bα/Akt1 regulates placental development and fetal growth. J. Biol. Chem. *278*, 32124–32131.

Yechoor, V., Liu, V., Espiritu, C., Paul, A., Oka, K., Kojima, H., and Chan, L. (2009). Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes. Dev. Cell *16*, 358–373.

Ying, Q., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P., and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. Nature *453*, 519–523.

Yung, H., Calabrese, S., Hynx, D., Hemmings, B.A., Cetin, I., Charnock-Jones, D.S., and Burton, G.J. (2008). Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. Am. J. Pathol. *173*, 451–462.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature *455*, 627–632.