SUPPLEMENTAL INFORMATION

Altered Subcellular Localization of Transcription Factor TEAD4 Regulates First Mammalian Cell Lineage Commitment.

Pratik Home, Biswarup Saha, Debasree Dutta, Soma Ray, Sumedha Gunewardena, Byunggil Yoo, Arindam Pal, Jay L. Vivian, Melissa Larson, Margaret Petroff, Patrick G. Gallagher, Vincent Schulz. Kenneth L. White, Thaddeus G. Golos, Barry Behr, and Soumen Paul.

Supplemental Information Inventory:

Items	Related to	General Description
Table S1		Describes Genome-wide TEAD4 binding regions, identified by ChIP-Seq analysis in mouse TS cells.
Table S2		Describes core mouse TS-cell-specific TEAD4 target genes.
Figure S1	**Related to Figure 1**	
Figure S2	**Related to Figure 1**	
Figure S3	**Related to Figure 1**	
Figure S4	**Related to Figure 2**	
Figure S5	**Related to Figure 3**	
Figure S6	**Related to Figure 3 and 4**	
Figure S7	**Related to Figure 5**	
Figure S8	**Related to Figure 5**	
Figure S9	**Related to Figure 5**	
Figure S10	**Related to Figure 5**	
SI Experimental Procedures with References		Describes experimental procedures, details of bioinformatics analysis of ChiP-seq data and lists oligonucleotides and antibodies used for the study.

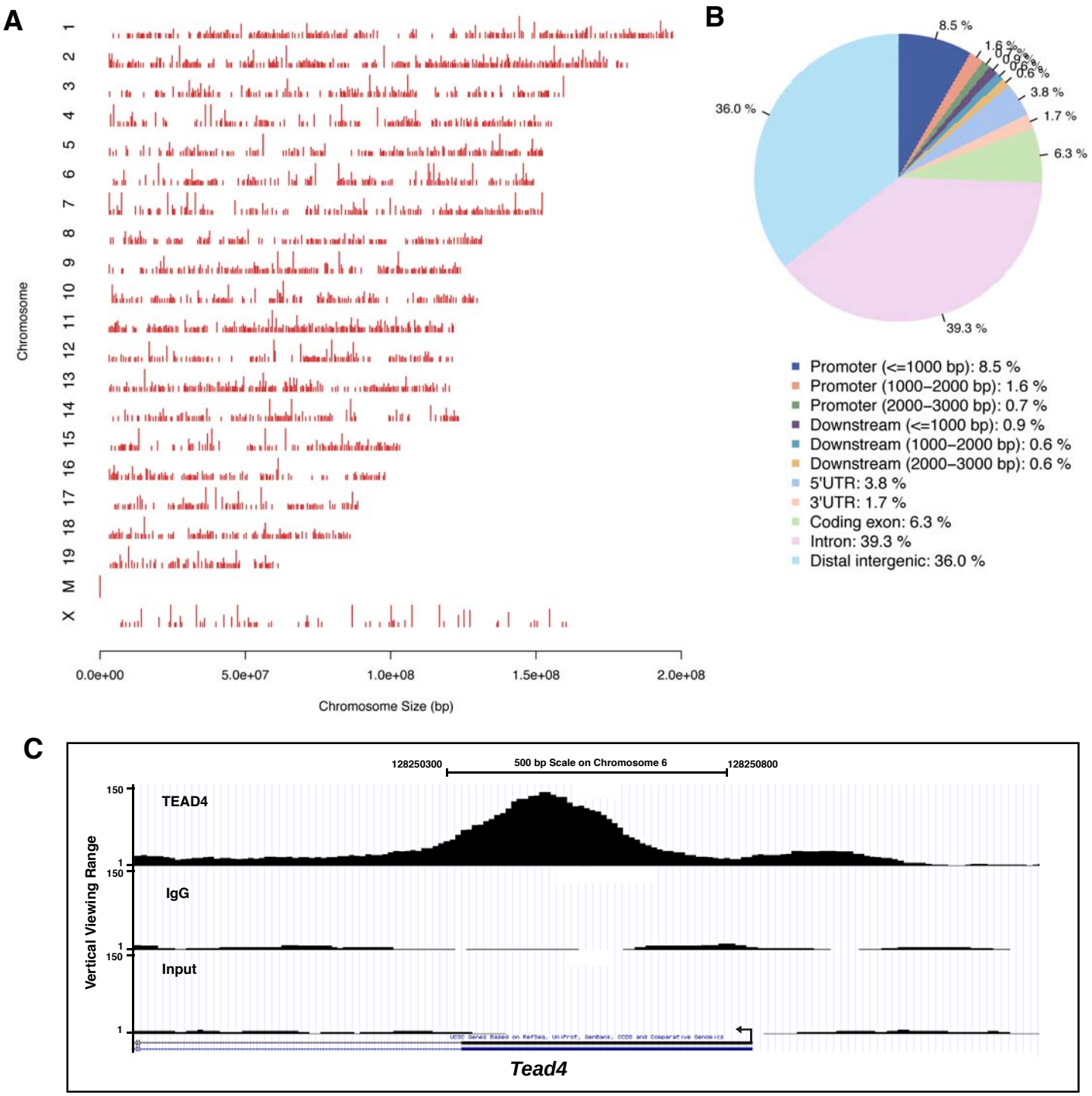


Figure S1 (related to Figure 1): A, The figure shows the distribution of TEAD4 ChIP-peak regions over the mouse genome as obtained by Cis-Regulatory Element Annotation System (CEAS) analysis. B, The figure shows how TEAD4 ChIP-peak regions are distributed over important genomic features. C, The figure shows the TEAD4 binding peak (determined by MACS analysis of the ChIP-Seq data) at the *Tead4* 5'UTR region when compared with the IgG and Input samples. A 500 bp scale bar with actual base positions in mouse chromosome 6 is indicated above the binding peak.

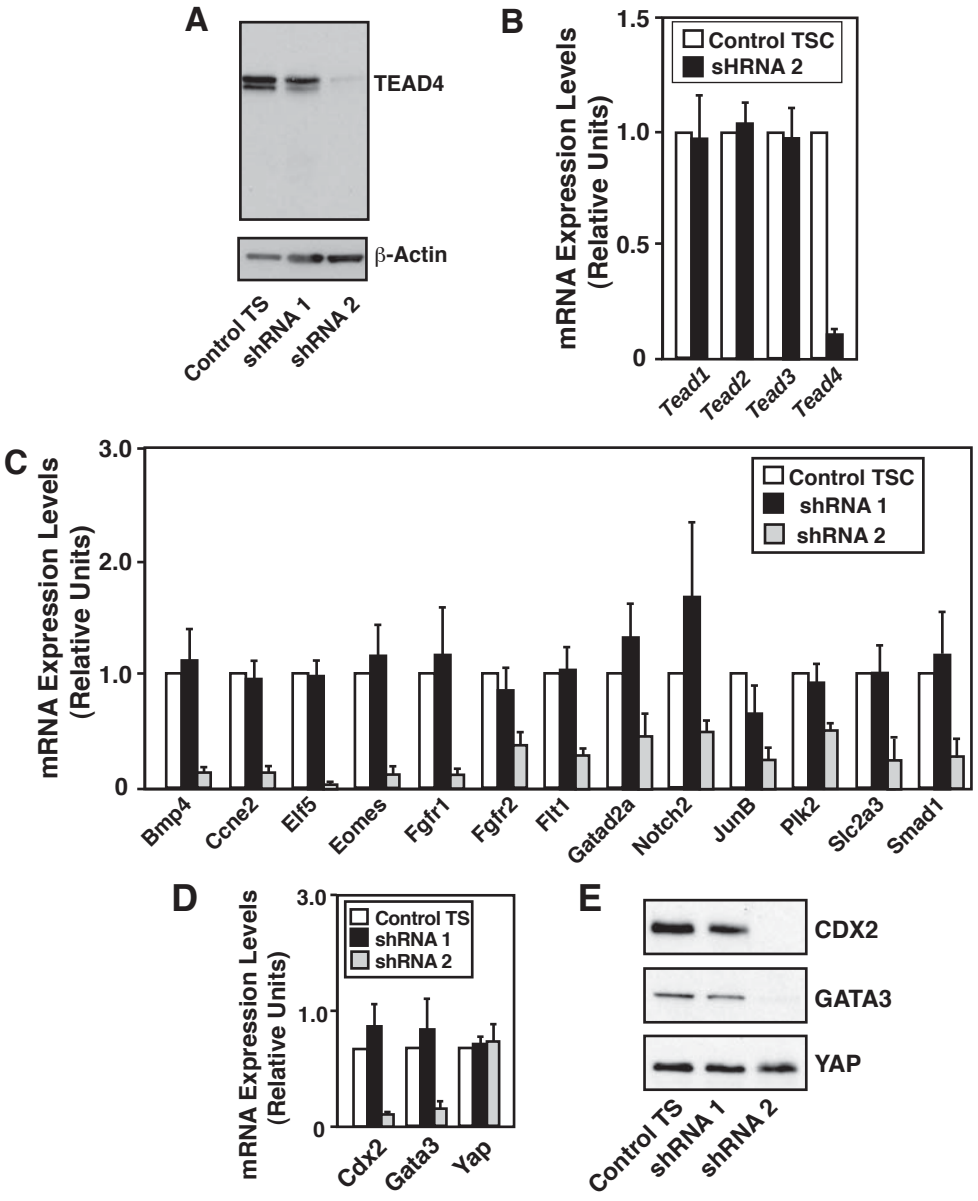
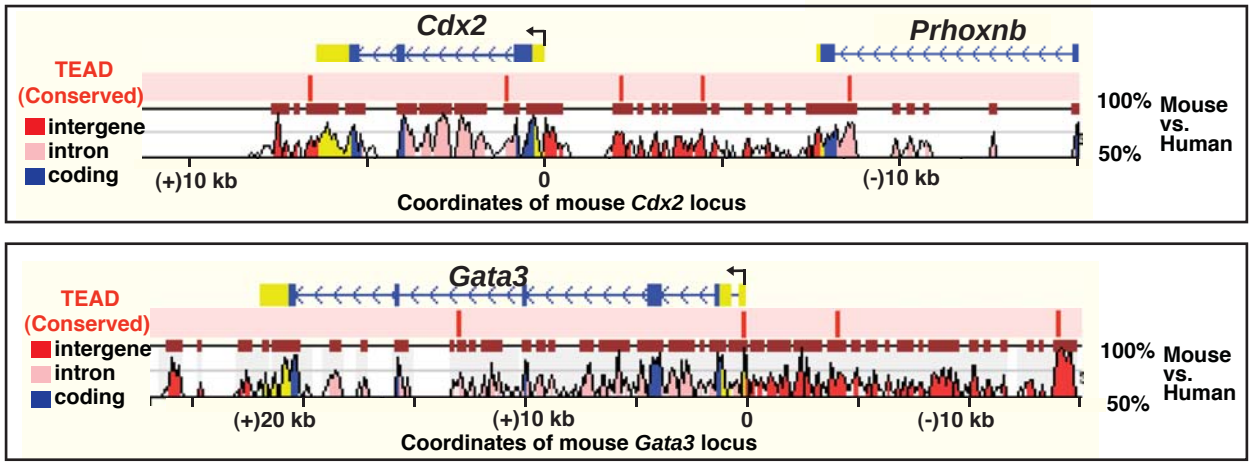


Figure S2 (related to Figure 1): A, Western blot analysis in mTSCs expressing shRNAs against TEAD4 or empty vector (control TS). The blot shows efficient knockdown of TEAD4 with shRNA2. B, RT-PCR analyses (mean± SE of triplicate experiments) showing maintenance of mRNA expression level of other TEAD family transcription factors in TEAD4 knocked-down mTSCs. C, RT-PCR analyses (mean± SE of triplicate experiments) of TEAD4 target genes in TEAD4 knocked-down mTSCs. D, and E, quantitative RT-PCR (qRT-PCR, mean± SE of triplicate experiments) and western blot analyses, respectively, showing loss of CDX2 and GATA3 expression in mTSCs upon TEAD4 knockdown. TEAD4 knockdown did not alter TEAD4 cofactor YAP expression.

A



B

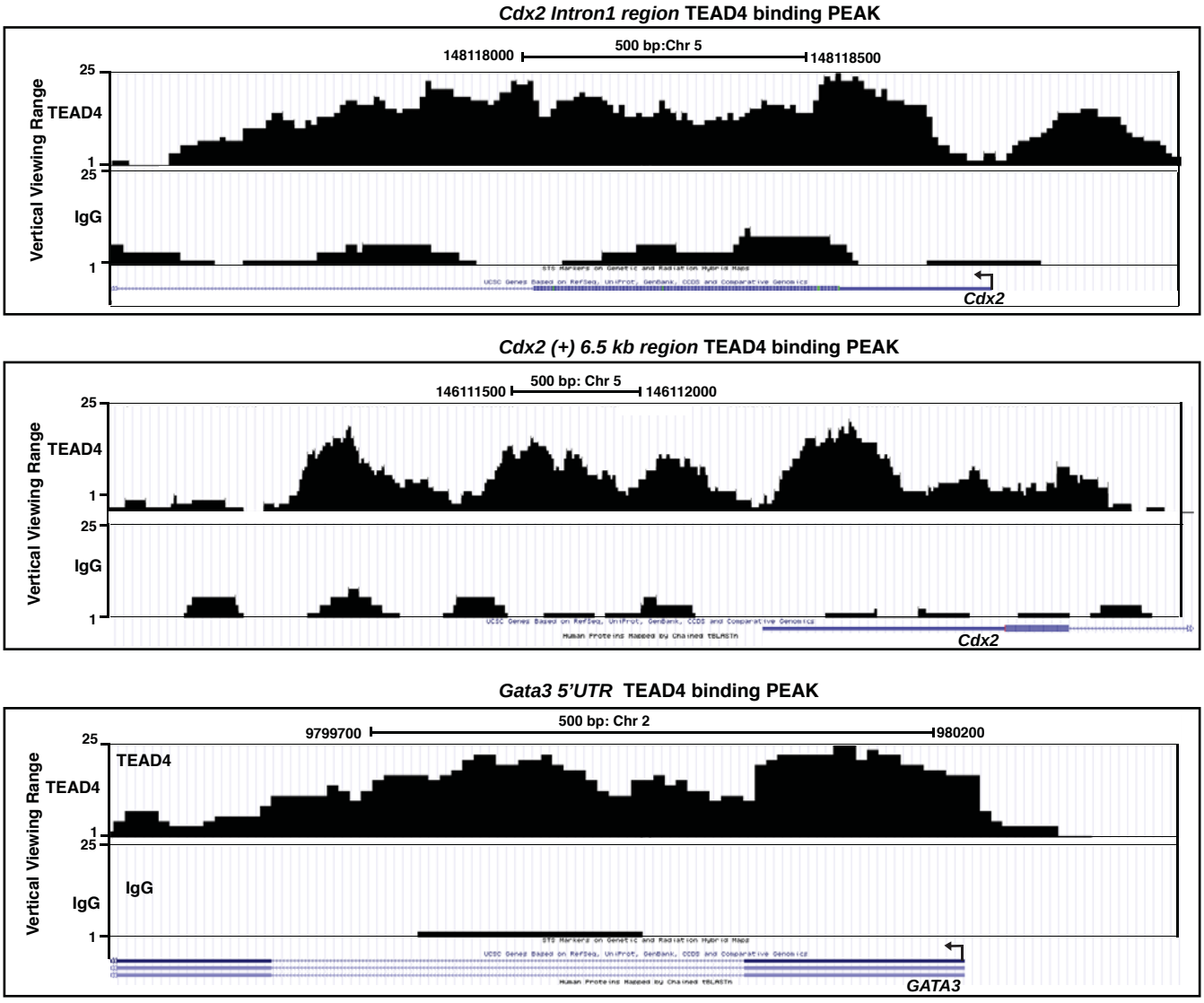


Figure S3 (Related to Figure 1): A, Alignment of mouse and human *Gata3* and *Cdx2* loci showing regions with high (50-100%) sequence conservation. The red vertical bars indicate positions of conserved TEAD motifs in the mouse loci. B, C, The panel shows the MACS analysis plots of the ChIP-Seq data for TEAD4 and IgG samples at the *Cdx2* intron 1, *Cdx2* (+) 6.5 kb, and *Gata3* 5' UTR regions . 500 bp scale bars with actual base positions of mouse chromosome 5 and 2 are also shown.

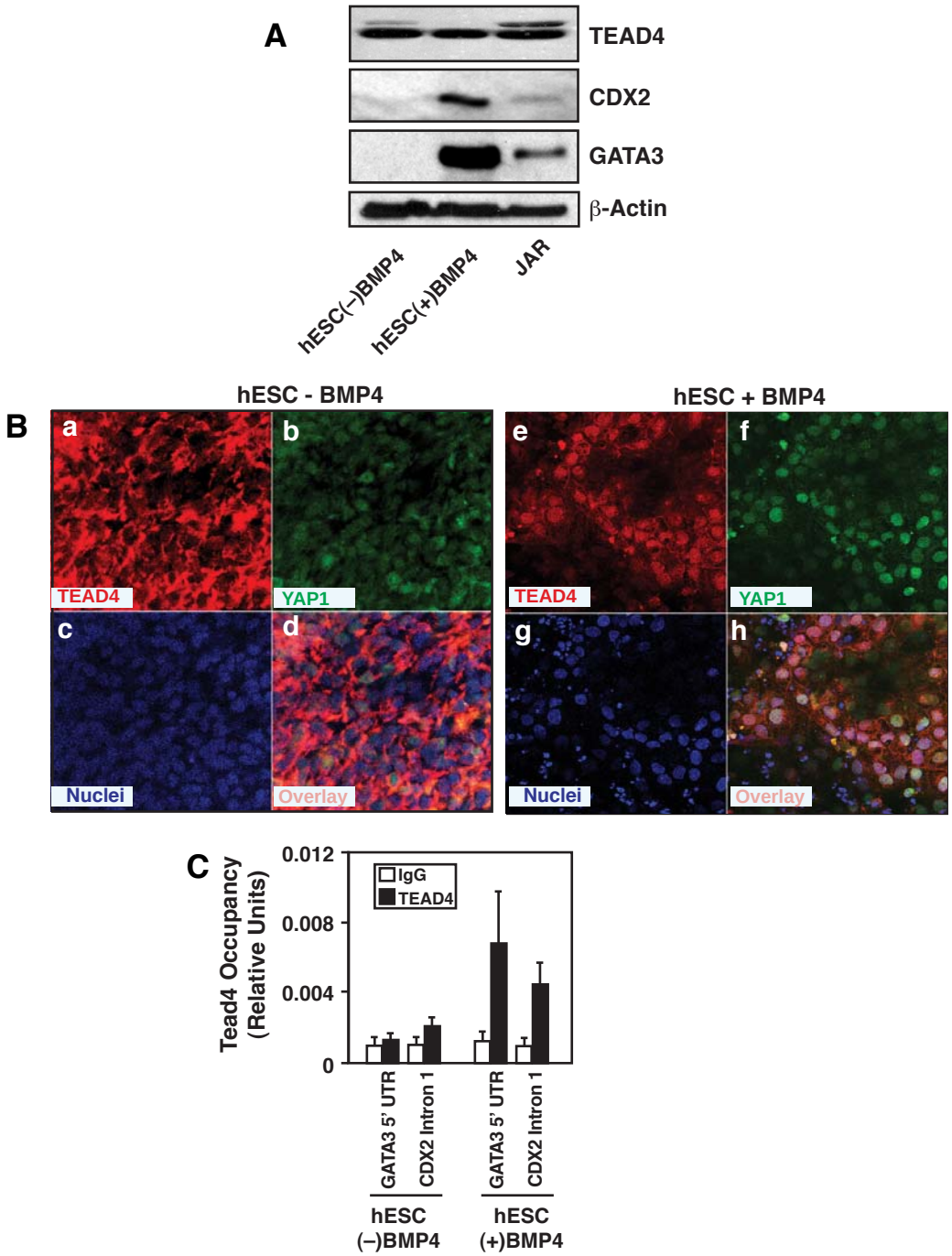


Figure S4 (related to Figure 2): A, Western blot analysis in H9 hESCs, with or without BMP4 treatment for 5 days, showing TEAD4, CDX2 and GATA3 expression. A human choriocarcinoma-derived JAR cells were used as a positive control for trophoblast marker expression. B, H9 hESCs were treated with or without BMP4 for 5 days and were analyzed for TEAD4 (red) and YAP1 (green) expression, and their intracellular localization. Images show that, in undifferentiated hESCs, TEAD4 is mainly localized within cytoplasm (panel a) and BMP4 treatment induced TEAD4 nuclear localization (panel e). However, YAP1 is nuclear in both control (panel b) and BMP4-treated (panel f) hESCs. D, ChIP analysis in cells, analyzed in "A" and "B", showing TEAD4 occupancy at the *Gata3* locus only in BMP4-treated hESCs.

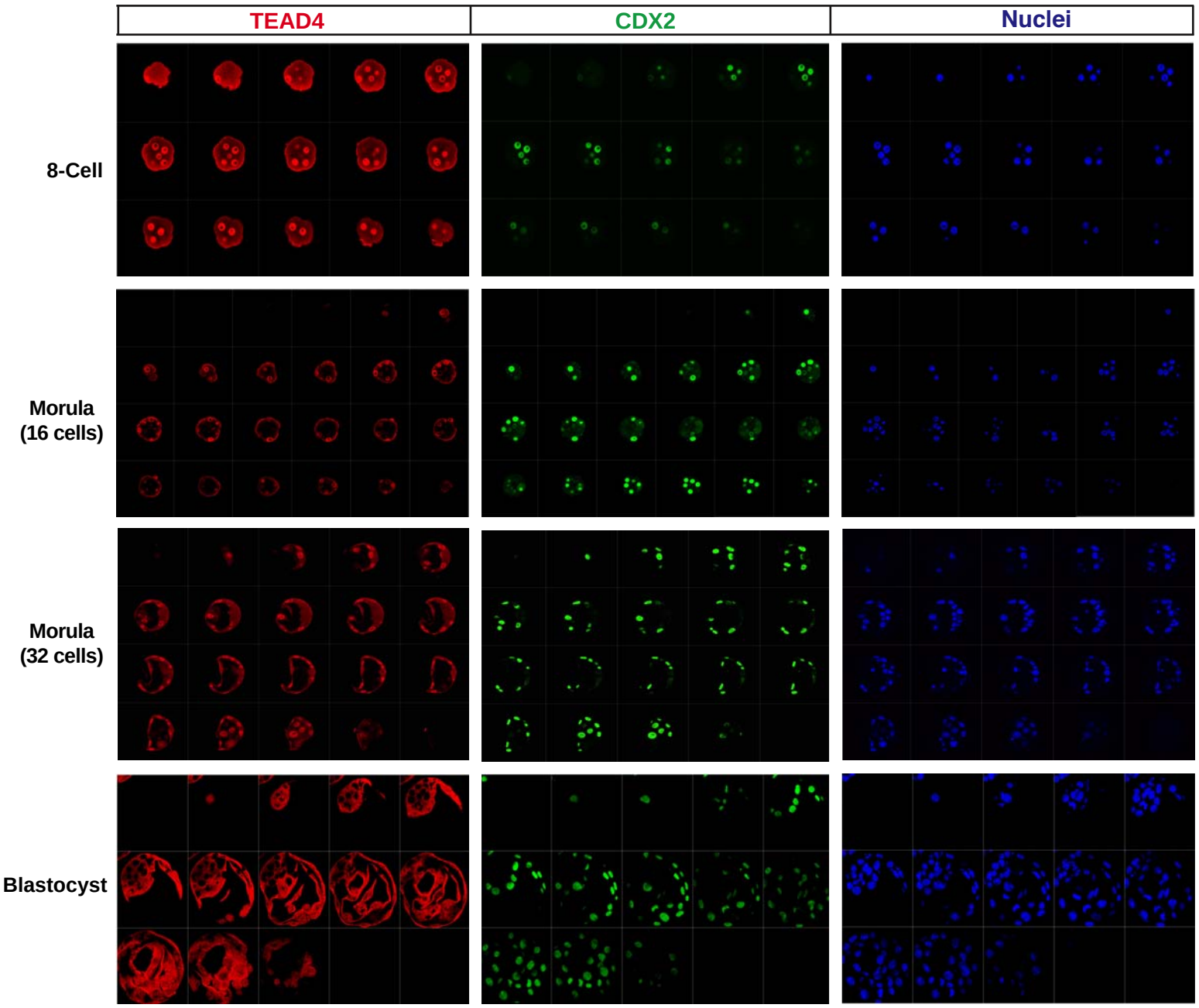


Figure S5 (Related to Figure 3). Z-Series confocal images showing TEAD4, CDX2 and Nuclei in embryos at different developmental stages as shown in Figure 5A. Nuclei of inner cells at the morula stage and in the ICM in blastocyst lack TEAD4 protein and CDX2 expression.

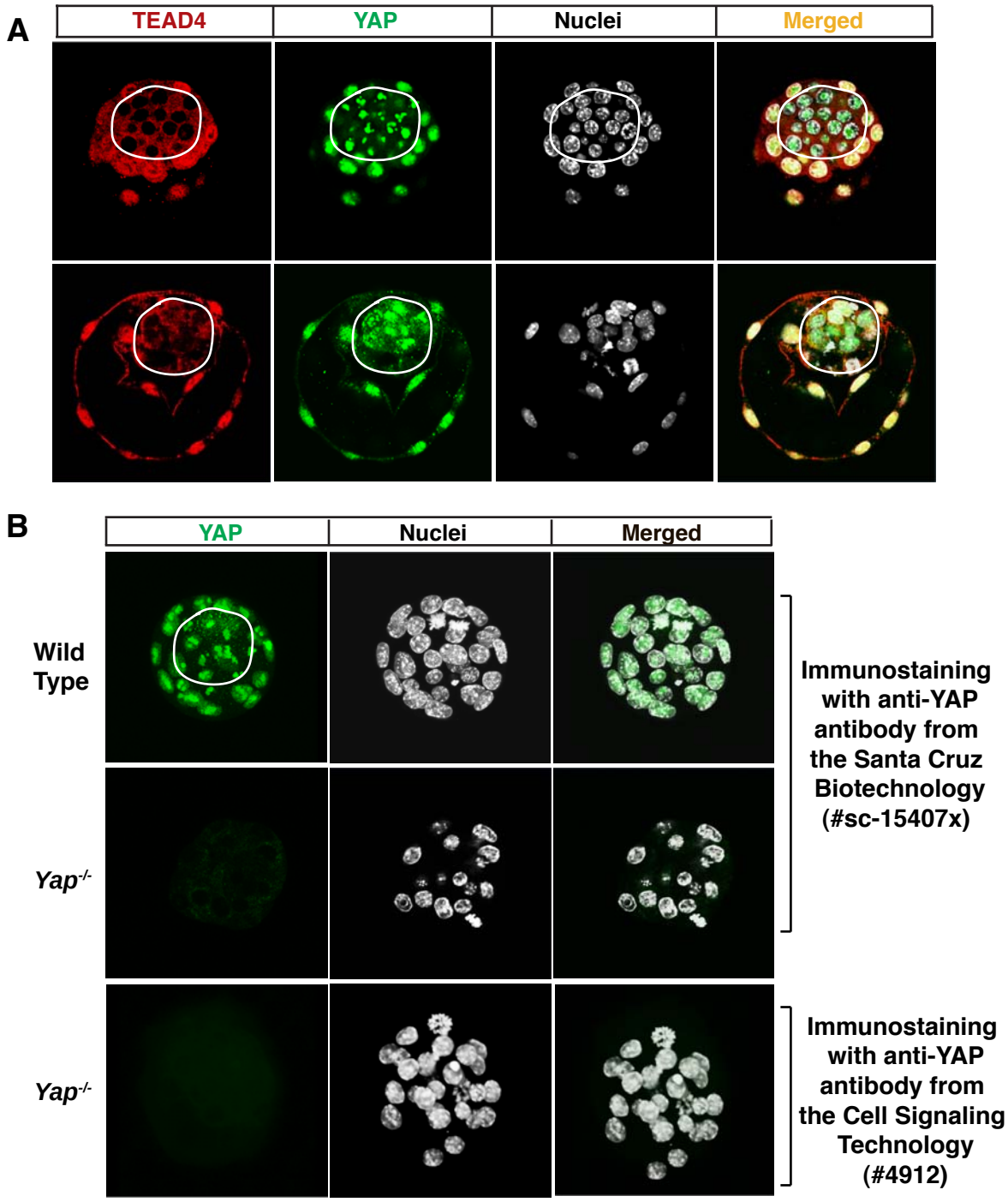


Figure S6 (related to Figure 3): A, Immunofluorescence studies of two mouse blastocysts showing absence of TEAD4 and presence of YAP in the ICM nuclei. We used anti-YAP antibody from the Cell Signaling Technology (#4912) for these studies. B, Morula/Early blastocyst stage embryos (wild type or *Yap*$^{-/-}$) were immunostained with two different anti-YAP antibodies, one from the Santa Cruz Biotechnology (#sc-15407X) and the other one from the Cell Signaling technology (used for studies in panel A). Negative staining in *Yap*$^{-/-}$ embryos confirms specificity of both of the anti-YAP antibodies.

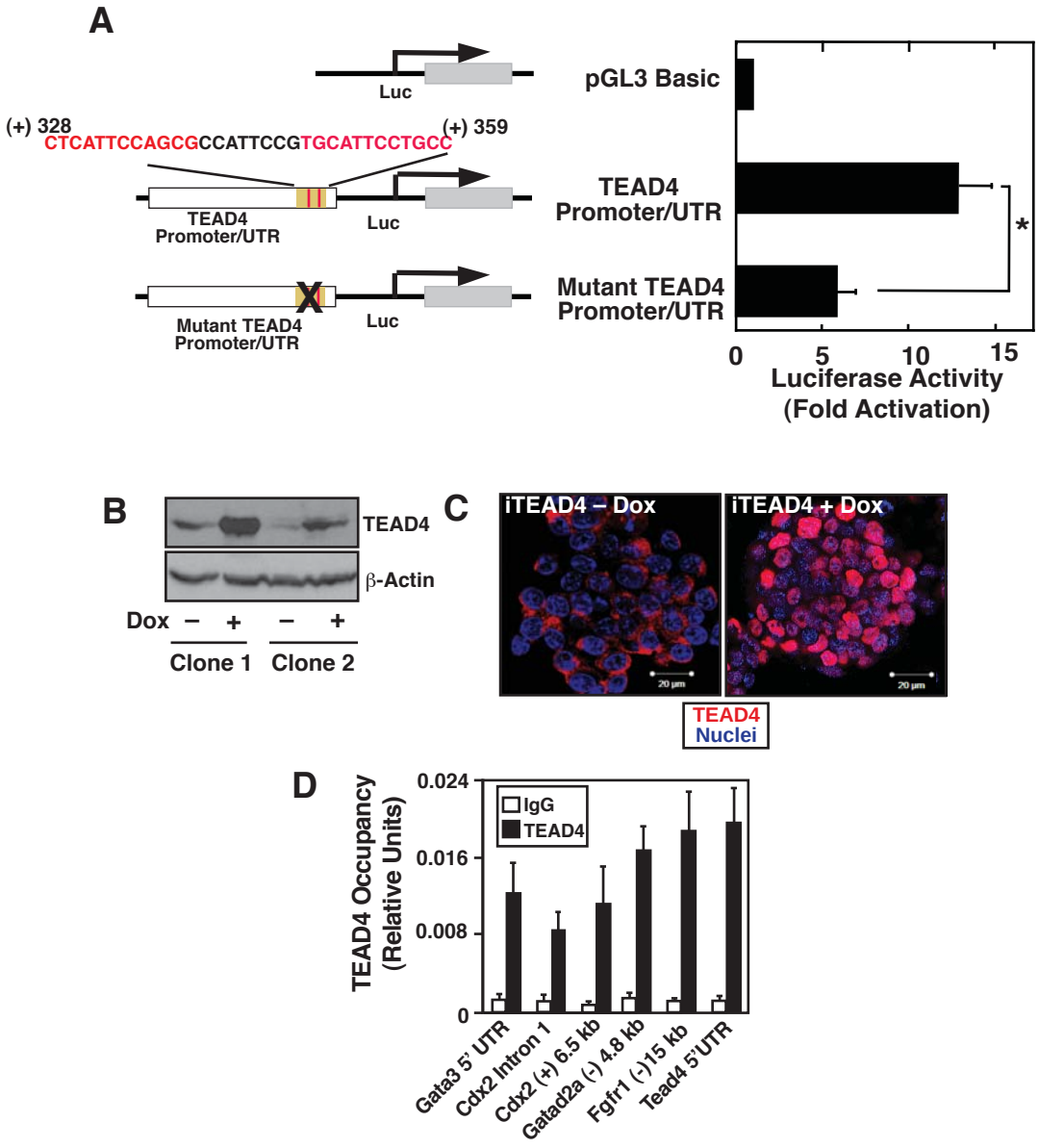


Figure S7 (related to Figure 5): A, Mouse TSCs were transiently transfected with plasmids in which TEAD4 promoter/5'UTR element was fused in front of a luciferase (Luc) reporter gene. The sequence with two conserved TEAD motifs (shown in red with their coordinates with respect to the *Tead4* transcription start site) were deleted in the mutant construct. The plots depict luciferase activity of the cell lysates normalized by the protein concentration of the lysates (mean ± S.E, n=4). B, Western blot analyses of TEAD4 expression in two different clones of iTEAD4 cells with or without doxicyclin (Dox) treatment (for 72 h). C, iTEAD4 cells (clone 1 of panel "B") were treated with (right panel) or without (left panel) Dox for 72 h and confocal images were taken to determine cellular localization of TEAD4 (red) with respect to nuclei (blue). D, TEAD4 ChIP analyses (mean ± SE, n=3) in iTEAD4 + Dox cells, from panel "C".

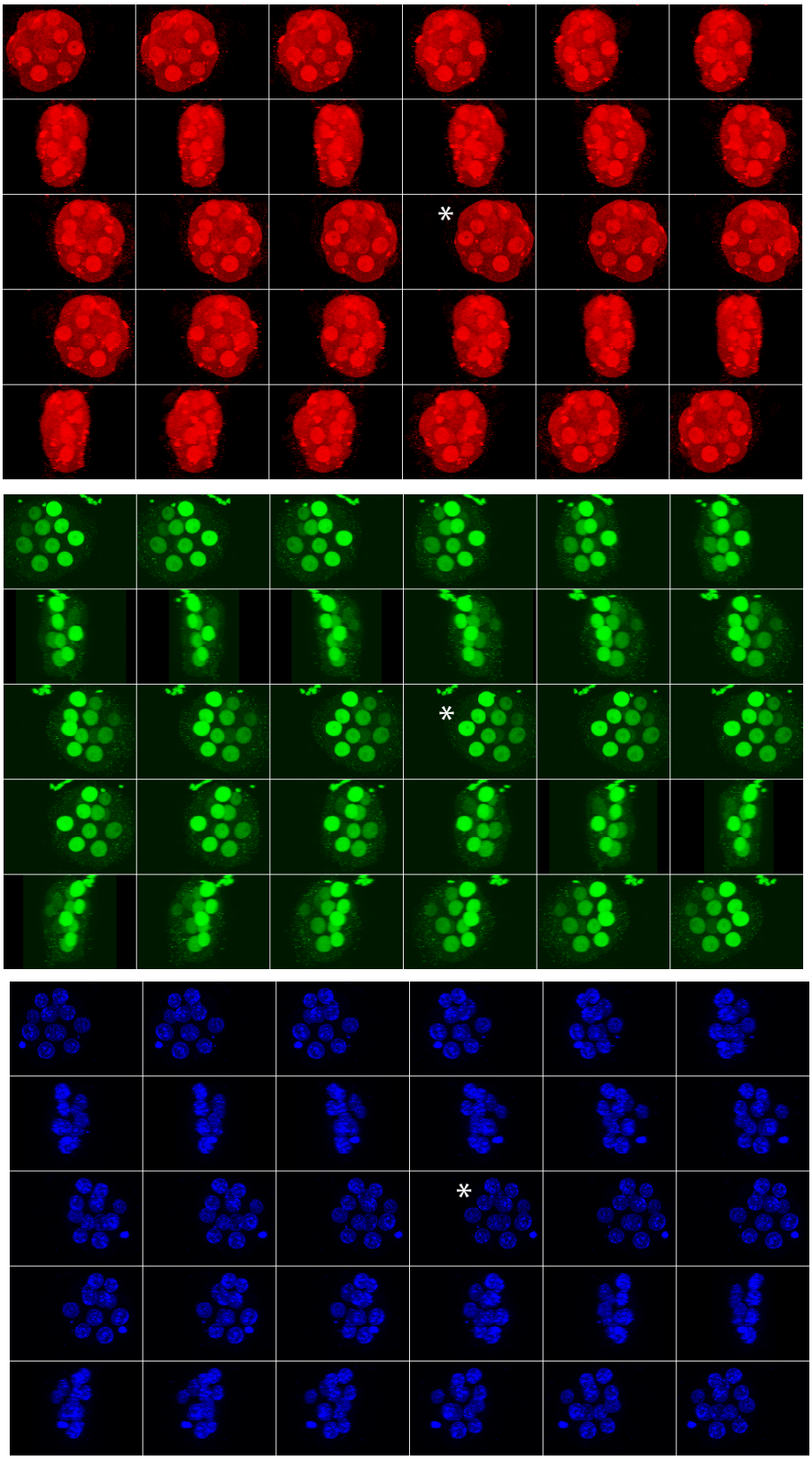


Figure S8 (related to Figure 5): The panels show different angels of three dimensional projection of the BMP4-treated embryo (Experiment 1) shown in Figure 5G of the main manuscript. The panels show presence of TEAD4 (red) and CDX2 (green) in both outer and inner cell nuclei (blue) of the embryo. The asterixes denote the panels that were used to generate Figure 5G.

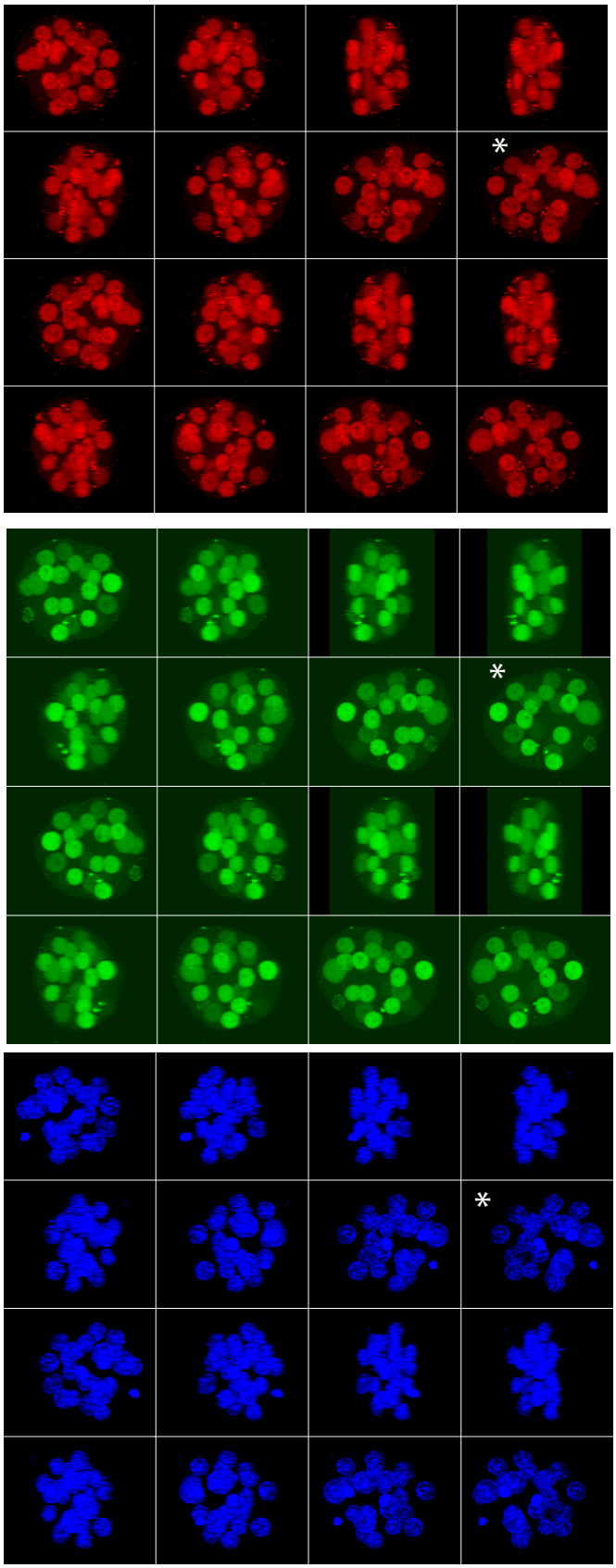


Figure S9 (related to Figure 5): The panels show different angels of three dimensional projection of the BMP4-treated embryo (Experiment 2) shown in Figure 5G of the main manuscript. The panels show presence of TEAD4 (red) and CDX2 (green) in all outer and inner cell nuclei (blue) of the embryo. The asterixes denote the panels that were used to generate Figure 5G.

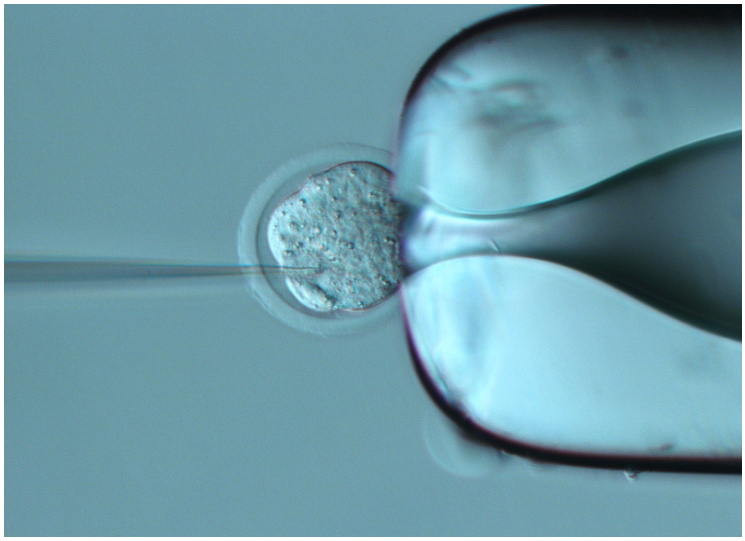


Figure S10 (**related to Figure 5**): The micrograph shows microinjection of BMP4 at the blastocoel of a very early stage mouse blastocyst. We injected either BMP4 or vehicle as control.

SI EXPERIMENTAL PROCEDURES

Cell culture and reagents

mTS, E14Tg2a (E14) and iTEAD4 mES cells were cultured as described earlier (1, 2). TEAD4 expression was induced in iTEAD4 cells by treating with 1μg/ml Dox for 3 days. H9 hESCs were cultured in mTeSR medium (Stem Cell Technologies, Vancouver, Canada) in six-well plates precoated with Matrigel (BD Biosciences, San Jose, CA). After they form well-shaped colonies, mTeSR was replaced by MEF conditioned medium, supplemented with 4 ng/ml recombinant human bFGF (R&D Systems, Mckinley Place, MN). For differentiation, cells were treated with 100 ng/ml BMP4 (R&D Systems) for different time intervals (1-7 days) for different experiments. JAR cells were cultured in DMEM/F12 with 10% FBS.

Quantitative RT-PCR

Total RNA from cells was extracted with TRIzol reagent (Invitrogen) and analyzed for qRT-PCR following procedures, described earlier (3, 4). For expression analysis in preimplantation embryos, total RNA was isolated using PicoPure RNA isolation kit (MDS Analytical Technology, Sunnyvale, CA) and processed as described earlier (1). Oligonucleotudes, used for qRT-PCR analyses are described below.

Western blot analysis.

Western blot analysis was performed as mentioned earlier (1). Antibodies are described below.

ChIP and ChIP-Seq analyses

Quantitative ChIP analyses with cultured cells were performed following published protocols (1, 4, 5). For ChIP analysis with preimplantation embryos, ~200 embryos were subjected to immunosurgery, cross-linked with 1% formaldehyde, and ChIP analysis performed with recovered blastomeres

according to a protocol described earlier (1). For ChIP analyses with first-trimester (8-9 weeks) human placental tissue, collected tissues were crosslinked with 1% formaldehyde for 15 min, homozenized, sonicated, and cross-linked chromatin fragments were immunoprecipitated with anti-TEAD4 antibody. Primers used for quantitative ChIP analyses are described below.

For TEAD4 ChIP-seq in mTSCs, Immunoprecipitated chromatin fragments from three independent experiments were pooled and genomic libraries were sequenced in Illumina Genome Analyzer II and in Illumina HiSeq platforms to generate 35 bp single end reads. Two control samples, input and IgG, were used to eliminate false positive peak calls of TEAD4 binding sites. Input control reads were comprised of sonicated reverse-cross-linked chromatin not subjected to any antibody treatment. IgG control reads were comprised of sonicated chromatin immunoprecipitated DNA using mouse IgG that does not specifically bind to any DNA binding protein. Detailed bioinformatic analysis is described below.

ChIP-seq data analysis

ChIP-seq samples were generated as described above from mTS cell-chromatin fragments by precipitating with anti-TEAD4 antibody. Libraries were sequenced in Illumina Genome Analyzer II using TruSeq SBS kit v5-GA chemistry and in Illumina HiSeq using TruSeq SBS v2-HS chemistry (Illumina, San Diego, CA) to generate 35 bp single-end reads. Sequence reads were extended in-silico at their 3' end to 200 bps, approximating the average fragment length in the size-selected library. Two different but standard control samples, input and IgG, were used to eliminate false positive peak calls of TEAD4 binding sites. Input control reads were comprised of sonicated reverse-cross-linked chromatin not subjected to any antibody treatment. IgG control reads were comprised of immunoprecipitated chromatin using IgG antibody that does not specifically bind to any DNA binding protein.

Sequences were aligned using ELANDv2 (CASAVA 1.7) to the mouse reference genome (NCBI37/mm9) using default parameters. Peak detection was performed using the Model-based

Analysis of ChIP-Seq (MACS) algorithm (6, 7). The TEAD4 ChIP-peak regions were further analyzed with Cis-Regulatory Element Annotation System (CEAS) (8) to determine chromosomal distribution (Figure S1). Peak detection by MACS analysis was performed separately with Input and IgG-ChIP control samples using a peak detection p-value cutoff set at 1e-5 (default). The results were integrated by selecting only those ChIP regions that were significantly enriched over both Input and IgG. This resulted in a set of 4959 TEAD4 binding sites highly enriched against both Input and IgG control samples.

We searched for the TEAD4 consensus sequence within a 250 bp region from either side of the called peaks using a weight-matrix match with at least 80% similarity. The TEAD1 weight matrix obtained from the JASPAR database (9) was used as a surrogate to model TEAD4 binding sites. A substantial proportion (60.5%) of the highly enriched TEAD4 binding sites contained at least one TEAD4 consensus site. *De-novo* motif detection in the vicinity of TEAD4 binding sites was carried out using the Hypergeometric Optimization of Motif Enrichment tools (HOMER) (10) with default settings.

All identified TEAD4 binding sites were annotated with their closest up-stream, down-stream and overlapping genes from the UCSC Known Genes dataset for mouse (11). We calculated the significance of the number of core TSC-specific genes within the annotated genes up-stream, down-stream, and overlapping TEAD4 binding peaks using a right tailed Fisher's exact test.

RNA Interference

pLKO.1 lentiviral vectors (Open Biosystems, Huntsville, Al) expressing short-hairpin RNAs (shRNAs) targeting mouse *Tead4* mRNA were used for Tead4 knock down in mTSCs. Lentiviral supernatants were produced in HEK-293T cells. Undifferentiated mTSCs were grown at ~70% confluence without MEF feeder layer and infected with lentiviral supernatants, and selected with puromycin (Sigma, St. Louis, MO). After 3 days, samples were prepared for mRNA and protein analysis. Results with two *Tead4* target sequences 5′-GACAATGATGCAGAGGGTGTA -3" (shRNA1), and ′5′-

GCTGAAACACTTACCCGAGAA-3′ (shRNA 2) are presented. sHRNA2 construct efficiently knocked down TEAD4 expression, validating an earlier report (12)

Immunostaining of cells

Cells were cultured on glass cover slip, washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde followed by permeabilization with 0.25% Triton X (TX)-100. Blocking was done with PBS containing 10% FBS and 0.1% TX-100. Primary antibody incubations were performed in blocking solution at 1:100 dilution over night at 4°C followed by wash with 0.1% TX-100 in PBS. Cells were incubated with Alexa fluor-conjugated secondary antibodies at 1:400 dilutions at room temperature for 1 h. Washed cells were mounted on slides with Slowfade anti-fade (Invitrogen) reagent containing DAPI as nuclear stain.

Direct labeling of primary antibody

Mouse anti-GATA3 antibody was labeled with CF488A fluorophore using Mix-n-Stain CF488A antibody labeling kit (Biotium Inc. Hayward, CA) while mouse anti-TEAD4 antibody (Abcam) was labeled with CF568 fluorophore with Mix-n-Stain CF568 antibody labeling kit (Biotium Inc.) as per manufacturer's protocol. Directly labeled primary antibodies were used at 1:2000 dilutions for immunostaining in hES cells (Data shown in Figure 4G).

Collection and culture of preimplantation embryos

All procedures were performed after obtaining IACUC approvals at University of Kansas Medical Center, Utah State University and University of Wisconsin University of Wisconsin-Madison. Embryos isolated from individual species are mentioned below.

Mouse embryos: 3–4-week-old CD-1 females were superovulated by intraperitoneal injection of 5 international units P.G. 600 (Intervet, Millsboro, DE), followed by 5 international units hCG (Sigma) 48 h later. Females were mated with C57BL/6 males and euthanized the following morning (d

0.5). Oviducts were removed, 1-cell embryos were harvested in M2 medium (Millipore, Billerica, MA) after treatment with hyaluronidase (300 µg/ml M2, Sigma) and cultured in KSOM (Millipore) at 37 °C in a humidified chamber at 5% CO_2. Preimplantation embryo developments were also evaluated following BMP4 treatment (100ng/ml) in KSOM until the blastocyst stage. Embryos were visualized daily, photographed, and sampled for immunostaining.

For observation of BMP4 induced TEAD4 nuclear localization in the ICM lineage cells, BMP4 (200 ng/ ml, ~50 pl) was microinjected in the blastocoel of early mouse blastocysts (Fig. 5H). The injection buffer (Millipore #MR-095-F) was used as vehicle control. Embryos were cultured in KSOM for another 6-8 hours and analyzed for TEAD4 nuclear localization and CDX2 expression.

Rat embryos: 6-week old female Dark Agouti rats (DA/OlaHsd strain, Harlan Laboratories) were mated overnight to DA males (8 week old) and euthanized by cervical dislocation on day 4.5 post coitum. Uteri were collected after euthanasia and flushed with M2 medium (Millipore) for the collection of blastocysts.

Cattle embryos: Bovine oocytes from follicles 3-8 mm in size were aspirated into 50-ml centrifuge tubes using an 18-gauge needle attached to a vacuum pump. Oocytes with uniform cytoplasm and intact multiple layers of cumulus cells were selected and washed with PB1+ (PBS with Ca2+ and Mg2+ plus 5.55 mM glucose, 0.32 mM sodium pyruvate, 3 mg/ml BSA). Oocytes were transferred into 500 mL of maturation medium, M199, containing 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT), 0.5 mg/ml FSH (Sioux Biochemicals, Sioux City, IA), 5 mg/ml LH (Sioux Biochemicals), 100 units/ml penicillin (Life Technologies, Grand Island, NY), and 100 mg/ml streptomycin (Life Technologies) in four-well culture dishes (Nunc, Milwaukee, WI) and cultured at 39^0C in a humidified atmosphere of 5% CO_2 and air for 24 h. 24 h *in vitro* matured bovine oocytes were subjected to *in vitro* fertilization following a standardized protocol (13) for 18-20 h at 39^0C in 5% CO2 and air. After fertilization, oocytes were vortexed for 2 min 40 sec in a 15-ml conical centrifuge tube containing 1 ml PB1+ medium to completely remove unbound sperm. Presumptive embryos were co-cultured on a monolayer of bovine cumulus cells in CR1aa medium containing 3% FBS at

39^0C in 5% CO_2 and cleavage was determined 24 h after removal of sperm. Medium was changed every 2 days during the *in vitro* culture period until day 7 at which time blastocysts were placed into 4% paraformaldehyde in PBS in order to fix the embryos.

Monkey embryo: Rhesus monkeys were from the colony maintained at the Wisconsin National Primate Research Center (Madison, WI). All surgical procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and under the approval of the University of Wisconsin Graduate School Animal Care and Use Committee. Rhesus monkey oocytes were obtained from the oocyte donor after treatment with 30 or 45 IUof recombinant human FSH administered twice daily for 7 days followed by one injection of 1000 IU of recombinant hCG. Oocytes were retrieved from visible follicles by laparoscopic surgery. Oocytes were matured *in vitro* followed by *in vitro* fertilization (IVF) with sperm collected the same day. IVF-derived zygotes were cultured to the blastocyst stage (7 days).

Human embryo: The human preimplantation embryos were collected at the Stanford University according to all the institutional rules, a two-stage consent process (14), and approval from institutional committees at both Stanford University and the University of Kansas Medical Center. Fixed, discarded, and de-identified human blastocysts were analyzed for TEAD4 expression pattern by immunofluorescence study.

Ectopic expression of TEAD4 in a preimplantation mouse embryo:

Lentiviral vector *pLKO.Tead4-T2A-EGFP* (Fig. 5A) was used to ectopically express TEAD4 in preimplantation mouse embryos. Lentiviral supernatants were collected 48 h post-transfection and viral particles were concentrated at 50,000g and resuspended in PBS. Lentiviral vector titers were determined by measurement of p24 gag antigen by ELISA (Advanced Bioscience Laboratories, Kensington, MD). $5X10^9$ viral particles were used to microinject in the perivitelline space of zygotic mouse embryos. Injected embryos were incubated in KSOM in 5% CO_2 in a 37°C incubator. Embryos

were visualized and photographed every day and were subjected to immunostaining or immunosurgery followed by RT-PCR or ChIP analyses.

Immunostaining of preimplantation embryos:

For immunostaining, preimplantation embryos were fixed with 4% paraformaldehyde, permeabilized in 0.25% Triton X-100, and blocked with 10% fetal bovine serum and 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 1 h at room temperature. Embryos were incubated with antibodies (1:100 dilution) overnight at 4 °C, washed in 0.1% Triton X-100 in PBS. After incubation (1:400, 1 h, room temperature) with conjugated-secondary antibodies, embryos were washed, and mounted using anti-fade mounting medium (Invitrogen, Crlsbad, CA) containing DAPI and viewed in LSM 5 Laser Scanning Microscope (Carl Zeiss Microimaging, Thornwood, NY).

YAP immunostaining

YAP immunostaining was done using two different commercially available antibodies: YAP Antibody (#4912) from Cell Signaling Technology and YAP (H-125) X antibody (sc-15407 X) from Santa Cruz Biotechnology. Both of these antibodies were extensively used for studying YAP [For example references (15-22) used the antibody from Cell Signaling Technology and references (23-26) used the Santa CruZ antibody]. To validate specificities of YAP antibodies, *Yap*$^{-/-}$ embryos were used. The *Yap*$^{-/-}$ embryos were recovered after immunostaining, washed with PBS, lysed in genomic lysis buffer (20mM Tris, pH 8.0, 100 mM KCl, 4 mM MgCl2, 0.9% Nonidet P40, 0.9% Triton X-100 and 300 ng/ml proteinsase K) followed by 1 hour heat inactivation of proteinase K at 98°C which also serves the purpose of reverse cross-linking. The lysate was used for genotyping using primers 5'-GAAGCTGTGGCACAAAGA-3' (forward), 5'-CGACGTTAACGGTACCAA-3' (mutant), 5'-ATGCAAAGGCCACACTGT-3' (wild type).

Generation of mESC lines with Inducible TEAD4 expression system

Cre-mediated recombination was used to generate a mESC line with doxycycline inducible TEAD4 following procedures described previously (27). In brief, a cDNA encoding mouse TEAD4 (Open Biosystems, Clone ID 40142595, GenBank: BC130257.1) was cloned into the engineered PALP targeting vector, and the resulting construct was co-electroporated with a Cre-expressing plasmid in Ainv15 cells (28). After G418-selection, clones with appropriate recombination were confirmed via PCR genotyping, and tested for Dox-inducible expression.

Mouse *Tead4* promoter/5'UTR cloning, TEAD motif mutation and reporter assay

Mouse Tead4 promoter region (-699 bp from transcription start site to +401 bp) was cloned into Mlu I/ Bgl II in pGL3 Basic luciferase reporter vector (Promega) to produce pGL3 –Tead4 promoter construct. For the deletion mutation, -699 bp to + 330 bp (two close TEAD binding motifs CTCATTCCAGCG and TGCATTCCTGCC, are located within +328 to +359bp) was cloned into Mlu I/ Xho I in pGL3 Basic to generate clone 1. Next +362 bp to +401 bp was cloned into Xho I/ Bgl II in clone 1 to generate pGL3-DTead construct in which the two successive TEAD binding motifs were deleted and were replaced by a Xho I restriction site. These constructs were used to transfect TS cells and transfected cells were used to measure luciferase activity as described previously (1).

Generation of TEAD4-T2A-GFP construct

To generate the TEAD4-T2A-GFP construct, oligos corresponding to the T2A sequence were annealed and cloned into BamH1/Kpn1 sites of the TOPO-TA vector (Invitrogen) to generate TOPO-T2A. PCR purified EGFP from pLKO.3G (Addgene #14748) was cloned into Kpn1/EcoR1 sites downstream of T2A. Tead4 cDNA was PCR amplified from a *Tead4* cDNA clone (GenBank: BC130257.1, Open Biosystems) and ligated into the BamH1/Sal1 of TOPO-T2A. Tead4-T2A-EGFP fragment was finally cloned into pLKO.3G in BamH1/EcoRV restriction sites to construct pLKO.Tead4-T2A-EGFP.

Embryo immunosurgery

Immunosurgery of mouse morula/early blastocyst was performed according to the method described earlier (1). Zonae pellucidae were removed from morula/early blastocysts using 0.5% Pronase (Sigma) for 5 min. The zona-free embryos were then treated with rabbit anti-mouse serum (Sigma) at 1:100 dilution for 30 min, washed with PBS, and incubated with guinea pig complement (Innovative Research, Novi, MI) at 37 °C in 5% CO_2 for 30 min. After washing, inner cells were collected for RNA preparation or ChIP analyses. mRNAs were isolated by the PicoPure RNA isolation kit, and subsequent RT-PCR analysis was performed as mentioned earlier (1) .

Oligonucleotides used

Primers for quantitative ChIP analysis:

Species	Location	Forward (5'-3')	Reverse (5'-3')
mouse	Gata3 5' UTR	TCTTTCTCCTCTCCCTCTCTCA	GGCGCCGTCTTGATAGTTT
mouse	Gata3 -4 kb	CGACCCAAGGACTAGGGAAA	ATCAATCGCATGGAAGGAAG
mouse	Gata3 Intron 4	CCTGCCTTTGTGAAATGTGA	GGCGTTGGTTTTCAGTAGGA
mouse	Gata3 -13.5 kb	TATGGCGTTTTCTTGCCTCT	TCCTGGATATGTGAGCAGGAC
mouse	Cdx2 +6.5 kb	ACCCCCAGGAAAAGCACA	GGAGCAAGTCCTGAGCCTTC
mouse	Cdx2 Intron 1	ACCCTCTCGGCCAGGAAC	CTGAATCGTGCCCTTGAGTT
mouse	Cdx2 -2 kb	TAAACCTGAGGGTGGGTGAG	CCTTCCTGGTCCTTCCTTCT
mouse	Cdx2 -4.2 kb	AGCTCCCTCACCATTGTCTC	AACCTGTAGGCTTTCCTTGG
mouse	Cdx2 -8.6 kb	GAAGACTTCCGCCAAAAGC	CCCCTGTCTTTTCACCTTTG
mouse	BMP4 -22 kb	TCTCTCTTCCGTCTCCCTGA	TGTGCATTGTTTTGGACTTCA
mouse	BMP4 +49.9 kb	TGCCCTTGGTCTGTTTCTCT	AGCTTCCTGCTGCACACAGT
mouse	Flt1 +88.2 kb	GGGGGAAATGCTATTGTTTACTC	TGTCTTTCACGTGGGAATTG
mouse	Slc2a3 -30 kb	AATACCAAGCAACCCAGCAG	AGCACACACGTCCTCCAAG
mouse	Notch2 +0.6 kb	CTCTGCTGCGGTTCCAGT	AGGAGTTGGGGATCGTCAC
mouse	Gatad2a –4.8 kb	GGGGAATCAACTTGACGAAC	CCAGCTCCCACTTCCCTAT
mouse	Plk2 +50.3 kb	GGTTGAGGAGAGCCTTTCAG	CCAGTTCATCCTGACTTGGAA
mouse	Fgfr1 -15 kb	GGCTCTGGAATTTACCACCA	GGCCCTTCCAGGAATGAATA
mouse	Fgfr2 +74.2 kb	TCATCTGTCGCCAGGTAAGG	GGAGGCCTGCAGATAGGTTA
mouse	Junb +1.6 kb	TCCCCTCCCTGTTAAATACAC	TTCCAGCGTATTTTGTATGTTTT
mouse	Tead4 5' UTR	GGACTGGAGTTTGCAGAGGA	GGTCTCCTCCCAAAGGTG
mouse	Ccne2 -0.1 kb	GCTCCCACGTACCTCGTC	GCCGCAGAATGTAAACACG
mouse	Tcfap2c -34.7 kb	TCCTGTCCTTTACCCTGCTG	TATAGGCGACCCCAGTACCA
mouse	Tcfap2c -48.6 kb	TCTGAGTCCGCAGGGTATTC	GCGTGAAGGGAGGTATGACT
mouse	Elf5 -17 kb	CCATTTTCCTGGTTGCAAAA	GGACTTCAGAGGGCTTTTGAG
mouse	Smad1 -36 bp	CGCTTAGGGATCCTCTCCA	GCAGCTAGGCAGGGACTAGA
mouse	Eomes -88.3 kb	CACCTCTTCCAAAGCTATTGTT	CGGAGAGGAGGTTAGGGAAG
human	Gata3 5' UTR	GGCGCCGTCTTGATACTTT	TCTCTCTCTCTCCCTCTCTCAG
human	Cdx2 Intron 1	CGAGCCTCCCTCTCTCCT	ATGTCGGCGTGAGCACTG

Primers for quantitative RT-PCR analysis:

Species	Location	Forward (5'-3')	Reverse (5'-3')
mouse	Gata3	CGGGTTCGGATGTAAGTCGA	GTAGAGGTTGCCCCGCAGT
mouse	Cdx2	GCAGTCCCTAGGAAGCCAAGTGA	CTCTCGGAGAGCCCGAGTGTG
mouse	Tead4	ATCCTGACGGAGGAAGGCA	GCTTGATATGGCGTGCGAT
mouse	Tead1	CTGGCCTGGGATGATACAGAC	GCTGACGTAGGCTCAAACCC
mouse	Tead2	CCAGACGTGAAGCCCTTCTC	TACCCTGGTAGGTCAGAGGC
mouse	Tead3	GCATTAAGGCTATGAACCTGGAC	CAGAGACGATTTGGGCAGAC
mouse	Eomes	ACCAATAACAAAGGTGCAAACAAC	TGGTATTTGTGCAGAGACTGCAA
mouse	Yap1	TGGCCAAGACATCTTCTGGT	GCCATGTTGTTGTCTGATCG
mouse	BMP4	GACTTCGAGGCGACACTTCTA	GAATGACGGCGCTCTTGCTA
mouse	Ccne2	AGCCGTTTACAAGCTAAGCAA	TGGCCTGAATTATCTGGGTTTC
mouse	Notch2	GACTGCCAATACTCCACCTCT	CCATTTTCGCAGGGATGAGAT
mouse	Gata2d2a	TCCCTCGCCTGATGTGATTG	GTGCTGGTGTCTTTCAAGGCT
mouse	Slc2a3	ATGGGGACAACGAAGGTGAC	CAGGTGCATTGATGACTCCAG
mouse	Junb	CTATCGGGGTCTCAAGGGTC	CTGTTGGGGACGATCAAGC
mouse	Plk2	AGCCAGAAGTCCGATACTACC	TCCCTAGCTTGAGATCCCTGT
mouse	Fgfr1	ATGAGTATGGGAGCATCAACCA	GCGGAGATCGTTCCACGAC
mouse	Fgfr2	CGGCCCTCCTTCAGTTTAGT	CTTCTCGGTGTTGGTCCAGT
mouse	Flt1	CGAAGAAAAAGAAAGCGGAAT	TGGGTATGTCAGTGTGCATCTCTA
mouse	Tcfap2c	ATCCCTCACCTCTCCTCTCC	CCAGATGCGAGTAATGGTCGG
mouse	Smad1	GACGCTTTGGTGAAGAAACTGA	CACACGGCAATAAATGACATGAG
mouse	Elf5	ATGTTGGACTCCGTAACCCAT	GCAGGGTAGTAGTCTTCATTGCT
mouse	Gapdh	TGCCCCCATGTTTGTGATG	TGTGGTCATGAGCCCTTCC
human	Tead4	ACGGCCTTCCACAGTAGCAT	ACGGCCTTCCACAGTAGCAT
human	Gata3	ATGCAAGTCCAGGCCCAAG	TTGCCCCACAGTTCACACAC
human	Cdx2	AGCAGTCCCTCGGCAGCC	TCCAGCCGCTGGTGGT
human	HPRT	ATTGGTGGAGATGATCTCTCAACTTT	CCAGTGTCAATTATATCTTCCACAA

Primers for cloning (5'-3'):

For mouse Tead4 cDNA cloning into PALP vector
forward: CGGGATCCGCCACCATGACCTCCAACGAGTGGAGCTCTCCCGA
reverse: CCGCTCGAGCCTATTCTTTCACAAGTCGGTAGATGTGG

For T2A cloning into TOPO vector
Sense:
GGATCCACGCGTGCTAGCGTCGACGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAG
GAGAATCCTGGCCCATGTACAGGTACCG
Antisense:
AATTCGGTACCTGTACATGGGCCAGGATTCTCCTCGACGTCACCGCATGTTAGCAGACTTCCTCT
GCCCTCGTCGACGCTAGCACGCGTGGATCCGTAC

For EGFP cloning into TOPO vector
forward: GGGGTACCATGGTGAGCAAGGGCGAGGAG
reverse: CGGAATTCCTTACTTGTACAGCTCGTCCATGCCGA

For mouse Tead4 cDNA cloning into TOPO-T2A-EGFP
forward: CGGGATCCGCCACCATGACCTCCAACGAGTGGAGCTCTCCCGA
reverse: GAATTAAGTCGACTTCTTTCACAAGTCGGTAGATGTGGT

For cloning Luciferase reporter construct with wild-type Tead4 promoter/5'UTR region
forward: CGCACGCGTCAGCGTCTCCCCGTGCTACCC
reverse: CGCAGATCTCCGGTCCCCACCCCCGGGACT

Additional primers For cloning TEAD motif mutated Luciferase reporter construct
forward: GTGCTCGAGTGCCGGGCTCCTCTGCAAACT
reverse: CGCCTCGAGAGACGCGCGCGGCTGGGG

Antibodies Used:

Primary Antibody	**Species raised in**	**Vendor**	**Cat. No.**
anti-TEAD4	Mouse	Abcam	ab58310
anti-GATA3	Mouse	BD Biosciences	558686
anti-CDX2	Rabbit	Epitomics	2475-1
anti-b-actin	Mouse	Sigma	A5441
anti-YAP1 (antibody 1)	Rabbit	Cell signaling	4912
anti-YAP1 (antibody 2)	Rabbit	Santa Cruz Biotechnology	sc-15407x
Purified IgG1 k isotype control	Mouse	BD Biosciences	554121
Purified IgG	Rabbit	BD Biosciences	550875

Secondary Antibody	**Species raised in**	**Vendor**	**Cat. No.**
Alexa Fluor 568 anti-mouse	Goat	Invitrogen	A11031
Alexa Fluor 488 anti-rabbit IgG	Goat	Invitrogen	A11008
anti-Mouse IgG-HRP	Goat	Santa Cruz Biotechnology	sc-2005
anti-Rabbit IgG-HRP	Goat	Santa Cruz Biotechnology	sc-2004
Biotinylated anti-mouse IgG	Goat	Vector Labs	BA-9200
Biotinylated anti-rabbit IgG	Goat	Vector Labs	BA-1000

SI REFERENCES

1. Home P*, et al.* (2009) GATA3 is selectively expressed in the trophectoderm of peri-implantation embryo and directly regulates Cdx2 gene expression. *J Biol Chem* 284(42):28729-28737 .

2. Dutta D*, et al.* (2011) Self-renewal versus lineage commitment of embryonic stem cells: protein kinase C signaling shifts the balance. *Stem Cells* 29(4):618-628 .

3. Dutta D, Ray S, Vivian JL, & Paul S (2008) Activation of the VEGFR1 chromatin domain: an angiogenic signal-ETS1/HIF-2alpha regulatory axis. *J Biol Chem* 283(37):25404-25413 .

4. Ray S*, et al.* (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(8):4978-4988 .

5. Dutta D*, et al.* (2010) Regulation of angiogenesis by histone chaperone HIRA-mediated incorporation of lysine 56-acetylated histone H3.3 at chromatin domains of endothelial genes. *J Biol Chem* 285(53):41567-41577 .

6. Zhang Y*, et al.* (2008) Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 9(9):R137 .

7. Feng J, Liu T, & Zhang Y (2011) Using MACS to identify peaks from ChIP-Seq data. *Curr Protoc Bioinformatics* Chapter 2:Unit 2 14 .

8. Shin H, Liu T, Manrai AK, & Liu XS (2009) CEAS: cis-regulatory element annotation system. *Bioinformatics* 25(19):2605-2606 .

9. Vlieghe D*, et al.* (2006) A new generation of JASPAR, the open-access repository for transcription factor binding site profiles. *Nucleic Acids Res* 34(Database issue):D95-97 .

10. Heinz S*, et al.* (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38(4):576-589 .

11. Fujita PA*, et al.* (2011) The UCSC Genome Browser database: update 2011. *Nucleic Acids Res* 39(Database issue):D876-882 .

12. Benhaddou A*, et al.* (2012) Transcription factor TEAD4 regulates expression of Myogenin and the unfolded protein response genes during C2C12 cell differentiation. *Cell Death Differ* 19(2):220-231 .

13. Reed WA, Suh TK, Bunch TD, & White KL (1996) Culture of in vitro fertilized bovine embryos with bovine oviductal epithelial cells, Buffalo rat liver (BRL) cells, or BRL-cell-conditioned medium. *Theriogenology* 45(2):439-449 .

14. Kalista T, Freeman HA, Behr B, Pera RR, & Scott CT (2011) Donation of embryos for human development and stem cell research. *Cell Stem Cell* 8(4):360-362 .

15. Benhamouche S*, et al.* (2010) Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. *Genes & development* 24(16):1718-1730 .

16. Happe H*, et al.* (2011) Altered Hippo signalling in polycystic kidney disease. *The Journal of pathology* 224(1):133-142 .

17. Zhang N*, et al.* (2010) The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Developmental cell* 19(1):27-38 .

18. Schlegelmilch K*, et al.* (2011) Yap1 acts downstream of alpha-catenin to control epidermal proliferation. *Cell* 144(5):782-795 .

19. Tufail R, Jorda M, Zhao W, Reis I, & Nawaz Z (2012) Loss of Yes-associated protein (YAP) expression is associated with estrogen and progesterone receptors negativity in invasive breast carcinomas. *Breast cancer research and treatment* 131(3):743-750 .

20. Bennett HL, Brummer T, Jeanes A, Yap AS, & Daly RJ (2008) Gab2 and Src co-operate in human mammary epithelial cells to promote growth factor independence and disruption of acinar morphogenesis. *Oncogene* 27(19):2693-2704 .

21. Levin VA*, et al.* (2010) Different changes in protein and phosphoprotein levels result from serum starvation of high-grade glioma and adenocarcinoma cell lines. *Journal of proteome research* 9(1):179-191 .

22. Striedinger K*, et al.* (2008) The neurofibromatosis 2 tumor suppressor gene product, merlin, regulates human meningioma cell growth by signaling through YAP. *Neoplasia* 10(11):1204-1212 .

23. Zender L*, et al.* (2006) Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* 125(7):1253-1267 .

24. Dong A, Gupta A, Pai RK, Tun M, & Lowe AW (2011) The human adenocarcinoma-associated gene, AGR2, induces expression of amphiregulin through Hippo pathway co-activator YAP1 activation. *The Journal of biological chemistry* 286(20):18301-18310 .

25. Zhang L*, et al.* (2011) Yes-associated protein promotes cell proliferation by activating Fos Related Activator-1 in oral squamous cell carcinoma. *Oral oncology* 47(8):693-697 .

26. Imanaka Y*, et al.* (2011) MicroRNA-141 confers resistance to cisplatin-induced apoptosis by targeting YAP1 in human esophageal squamous cell carcinoma. *Journal of human genetics* 56(4):270-276 .

27. Galvin KE, Travis ED, Yee D, Magnuson T, & Vivian JL (2010) Nodal signaling regulates the bone morphogenic protein pluripotency pathway in mouse embryonic stem cells. *J Biol Chem* 285(26):19747-19756 .

28. Kyba M, Perlingeiro RC, & Daley GQ (2002) HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. *Cell* 109(1):29-37 .