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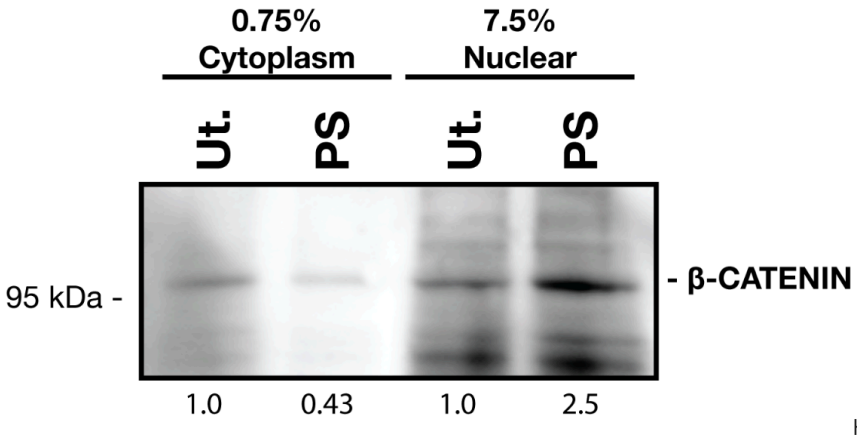

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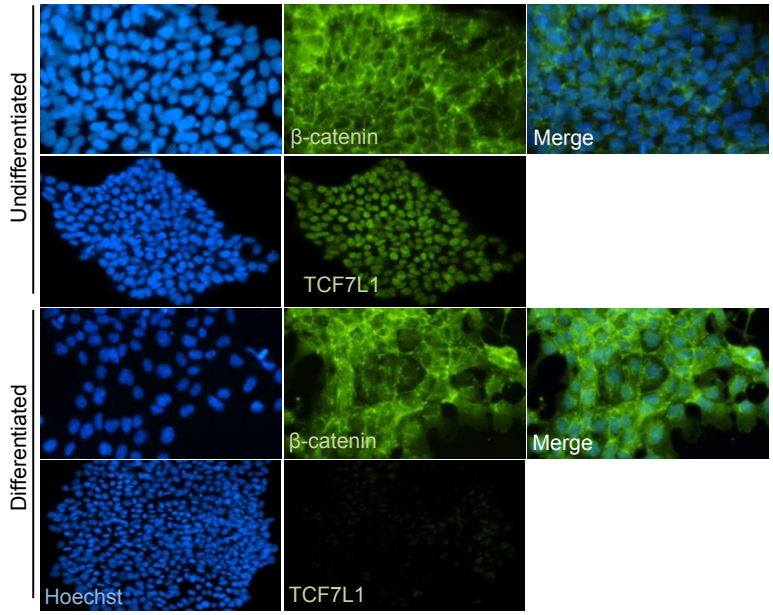

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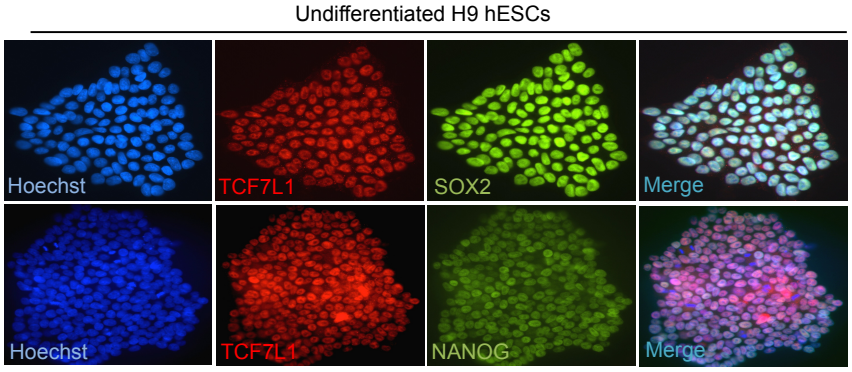
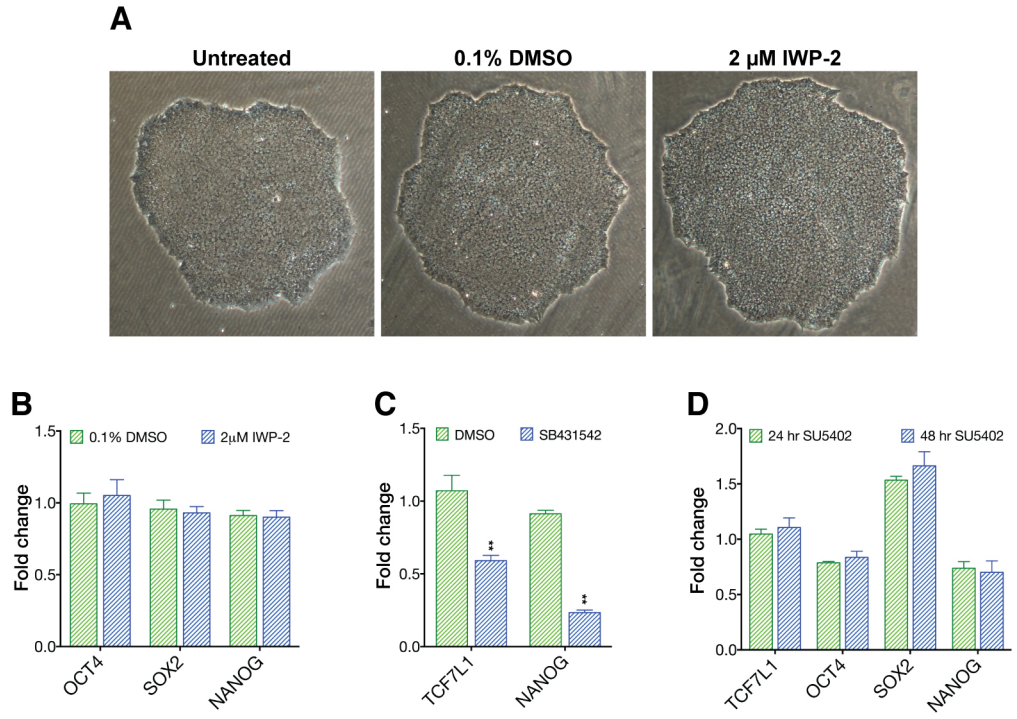


Supplemental Figure 1. Analysis of TCF7L1, SOX2, NANOG and β-CATENIN expression in human embryonic stem cells (hESCs).

A. Immunoblot analysis of β-CATENIN in cytoplasm and nuclear extracts. Nuclear/cytoplasmic fractionation analysis of β-CATENIN distribution after 48 hrs of PS differentiation. Equal lysate percentages were loaded for each fraction and the band intensities were quantitated relative to the untreated β-CATENIN band for each condition. N=1.

B. Immunofluorescence analyses of the active form of β-CATENIN using a non-phosphorylated anti-β-CATENIN antibody and an anti-TCF7L1 antibody in undifferentiated and differentiated hESCs. Cells (1 x 10^5/well) were grown on a 12-well multiwall glass bottom plate (MatTek) coated with Matrigel and cultured in mTeSR plus ROCKi to increase single cell survival. Cells were cultured in mTeSR for another 24 hrs before treatment with 50 ng/mL BMP4 and ACTIVIN A in E5/E6 basal medium for 48 hrs. Undifferentiated H9 cells showed membrane bound β-CATENIN and expression of TCF7L1, while differentiated cells showed an increase of the active form of β-CATENIN and a loss of TCF7L1 in the nucleus (stained with Hoechst dye). N=1.

C. Immunofluorescence analyses of NANOG, SOX2, and TCF7L1 in undifferentiated hESCs. Cells were cultured at a 1:24 ratio on 35 mm dish coated with Matrigel for 48 h in TeSR E8 medium. Note the co-expression of NANOG and TCF7L1, as well as SOX2 and TCF7L1 in the nuclei (Hoechst) of hESCs. N=1.



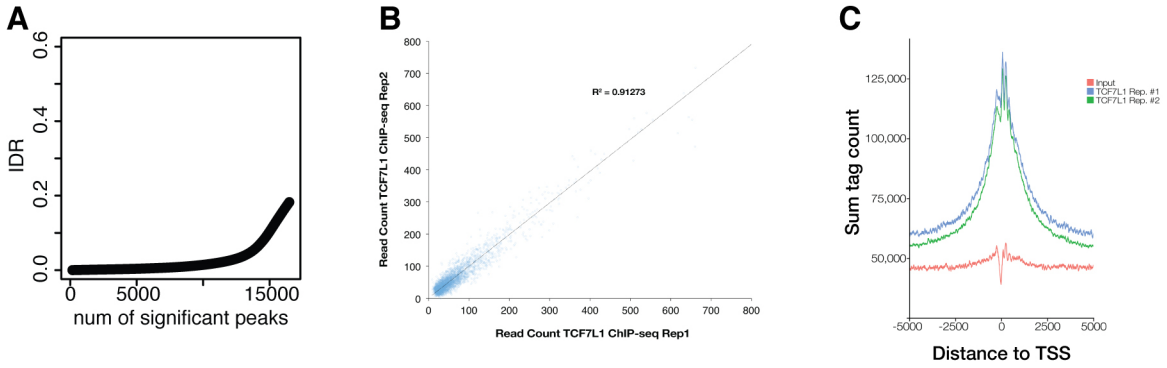
Supplemental Figure 2. Effects of Signaling Pathway Manipulation on *TCF7L1* Expression.

A. Phase contrast images (10X) after 7 days of inhibition of WNT signaling with IWP-2 treatment under feeder-free conditions. In all three conditions hESCs were morphologically indistinguishable from each other.

B. qPCR analysis of pluripotent marker genes - *OCT4*, *SOX2* and *NANOG* - was performed 7 days after treating hESCs, under feeder-free conditions, with IWP-2 (2 μM) (n=3). Treatment had no affect as compared to DMSO vehicle control.

C. TC*F7L1* and *NANOG* mRNA expression were down-regulated following 24 h treatment with the ALK5 inhibitor SB431542 (10 μM) under feeder-free conditions (n=3 two-tailed *t*-test **P≤0.01).

D. *TCF7L1*, *OCT4*, *SOX2* and *NANOG* mRNA expression were assayed by qPCR following 24 and 48 hrs of treatment with the FGFR inhibitor SU5402 (10 μM) under feeder-free conditions (n=3). There was a slight down-regulation of *OCT4* and *NANOG*, no effect on *TCF7L1* and slight up-regulation of *SOX2*.



Supplemental Figure 3. Analysis of TCF7L1 ChIP-seq Replicates.

A. Irreproducibility discovery rate (IDR) plot showing a high degree of reproducibility in the two ChIP-seq biological replicates. The IDR is plotted as an increasing number of reproducible ChIP-seq peaks are found. IDR analysis reveals the appropriate cutoff for the number of ChIP-seq peaks to be included in all subsequent analyses. An IDR cutoff of 0.013249 (top 9760) were used for the analysis, which is well within the range suggested by ENCODE.

B. Scatter plot illustrating the high degree of reproducibility of the two TCF7L1 ChIP-seq replicates. The number of reads that mapped to the top 3518 peaks (IDR <0.002406) were determined for each TCF7L1 ChIP-seq biological replicate. The replicate with a greater number of mapped reads was scaled to the replicate with less mapped reads.

C. coverageBed (Quinlan & Hall, 2010) was used to determine the number of reads that map to each base-pair position +/-5 kb of the TSS of all human hg19 RefSeq genes. The number of mapped reads used for this analysis was the same for each sample (approximately 54 million). R was used to calculate and plot the mean tag count at each position, which represents the total number of reads mapped to each base divided by the number of RefSeq genes.

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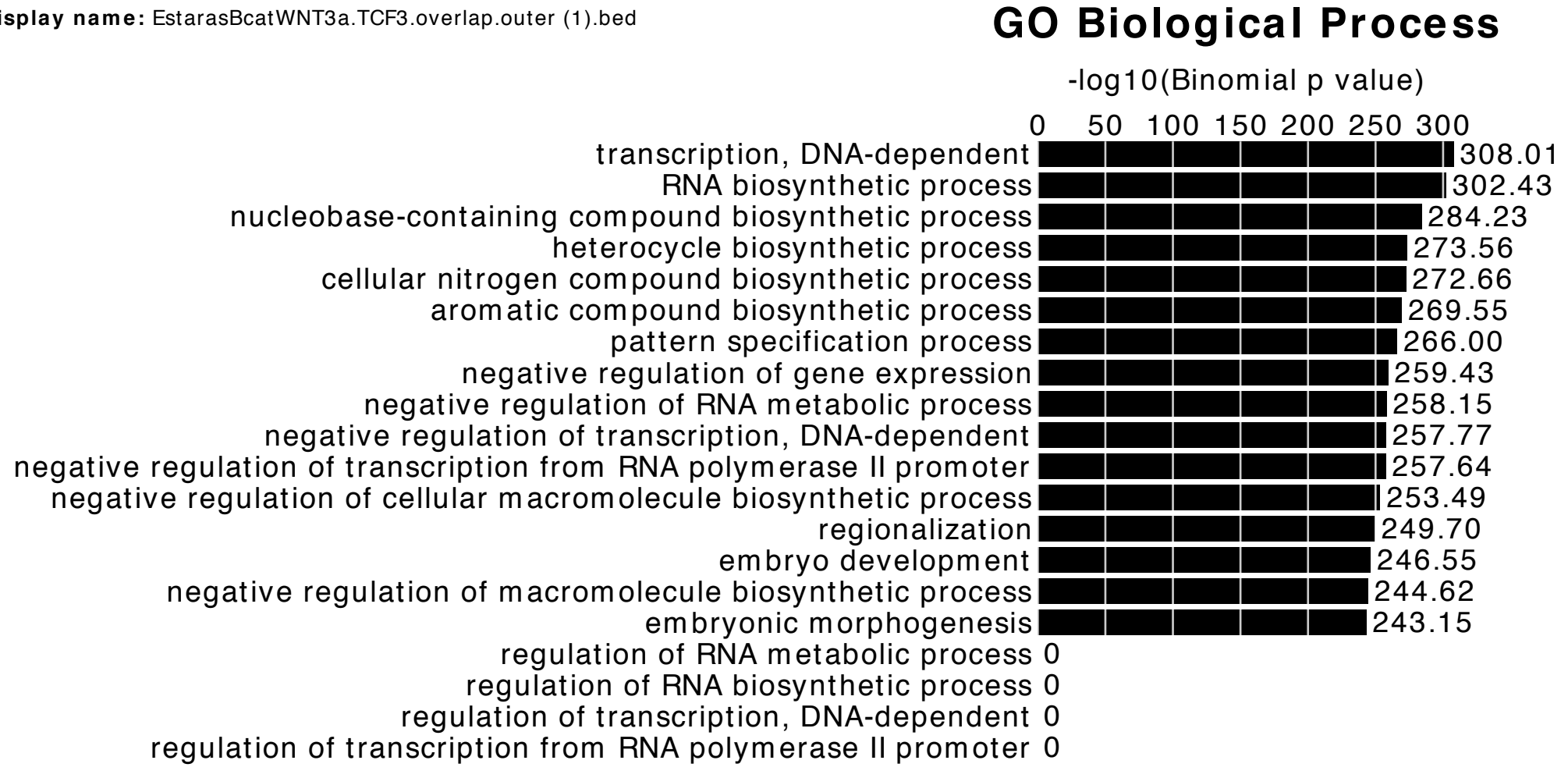

Supplemental Figure 4. Analysis of non-overlapping TCF7L1 peaks with β-Catenin after 4 hours of WNT treatment.

GO analysis showing biological processes of the TCF7L1 peaks not overlapping with β-CATENIN. The most prominent categories were: transcription, DNA-dependent RNA biosynthetic process nucleobase-containing compound biosynthetic process and heterocycle biosynthetic process. Regionalization, embryo development, negative regulation of macromolecule biosynthetic process, embryonic morphogenesis were represented in these non-overlapping peaks but with a lower degree of confidence.

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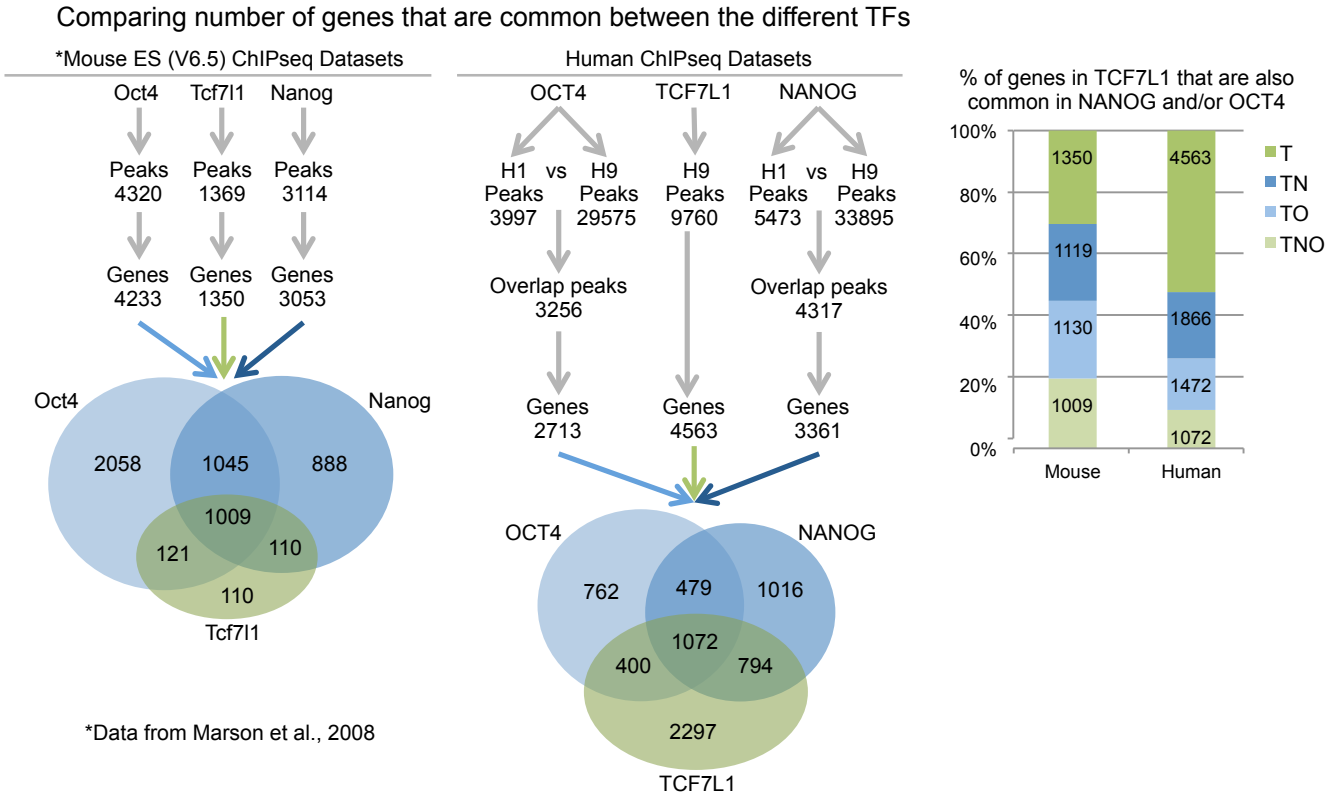

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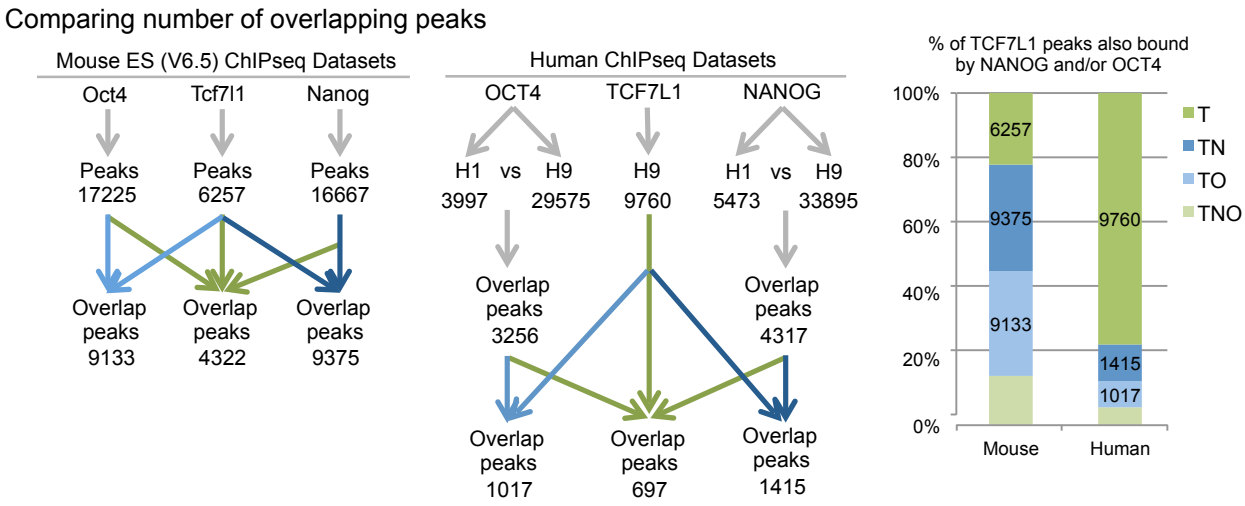
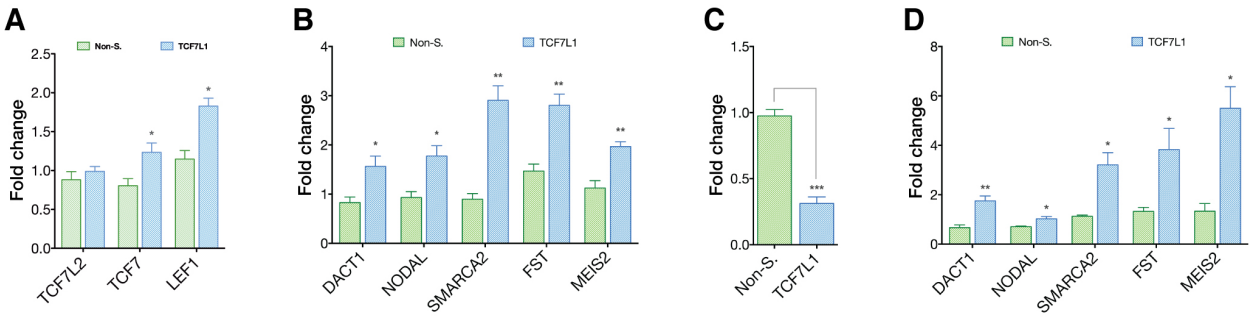


Supplemental Figure 5. Analyses of TCF7L1 overlap with OCT4 (POU5F1) and NANOG in mouse and human ESCs.

A. Flow diagram of the analyses of mouse (LEFT) and human (CENTER) ESCs ChIPseq datasets to determine common genes that are regulated by OCT4, NANOG, and TCF7L1. Annotation for neighboring genes near (+/- 5-8 kb) peaks or overlapping peaks and compared those genes that are associated with OCT4, NANOG, and TCF7L1 in a venn diagram. Table S2 from Marson et al supplemental data was used for the mouse analysis (Marson et al., 2008). Mouse genes are from RefSeq while human genes are from Ensembl (ENSG ID) genes. (FAR RIGHT) Stock graph showing the percentage of genes in N – NANOG and/or O – OCT4 that are also common in T – TCF7L1.

B. Flow diagram of the analyses of mouse (LEFT) and human (CENTER) ESCs ChIPseq datasets to determine overlapping peaks that are enrich for OCT4, NANOG, and TCF7L1. Identification of total overlapping peaks for TN, TO, and TNO in human ESCs were determined using ChIPpeakAnno. Table S3 from Marson et al supplemental data was used for the mouse analysis (Marson et al., 2008). (FAR RIGHT) Stock graph showing the percentage of overlapping peaks between TN, TO, and TNO over the total TCF7L1 peaks.



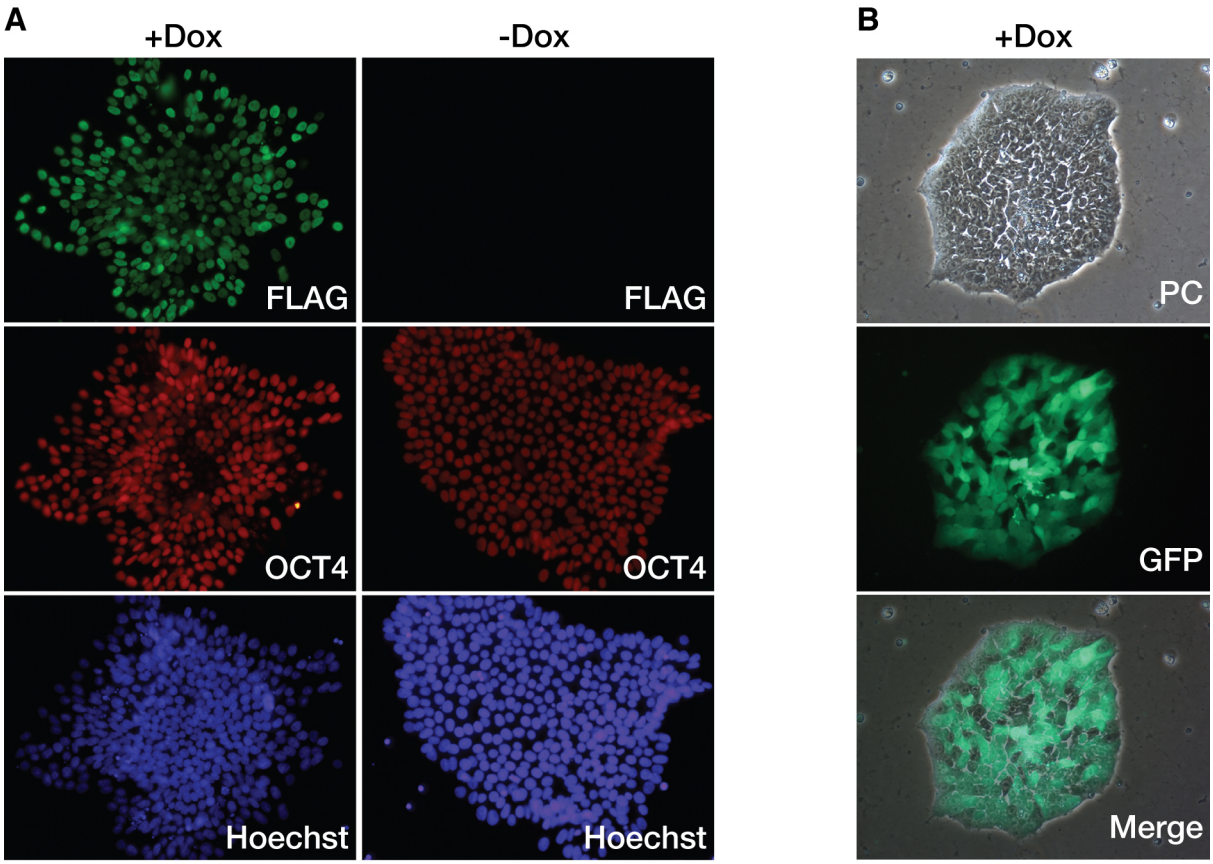
Supplemental Figure 6. Analysis of TCF7L1 knockdown in H9 hESCs.

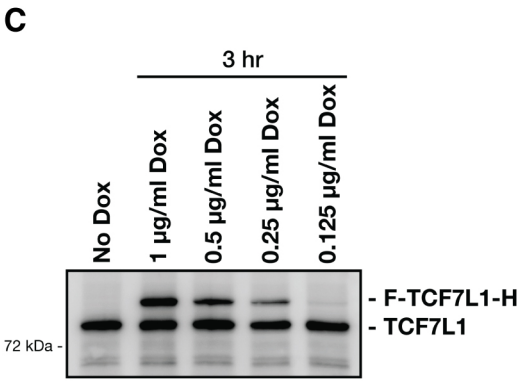
A. qPCR analysis of LEF/TCF mRNAs 3 days post-siRNA KD of TCF7L1 show minimal up-regulation of *LEF1* (Avg. Ct = 34.1) and *TCF7* (Avg. Ct = 29.7) (n=3 two-tailed *t*-test *P≤0.05).

B. Confirmation of *TCF7L1* siRNA KD up-regulation of target genes in hESC line H14 (n=3 two-tailed *t*-test *P≤0.05, **P≤0.01).

C. *TCF7L1* knockdown using a second siRNA (siRNA #2) after 3 days. Non-silencing (Non-S.) and *TCF7L1* siRNA were used at 50 nM (n=3 two-tailed *t*-test ***P≤0.001).

D. Confirmation of *TCF7L1*-regulated genes identified in the microarray study using siRNA #2 by qPCR (n=3 two-tailed *t*-test *P≤0.05, **P≤0.01).





Supplemental Figure 7. Characterization of TCF7L1 H9 Over-Expression Cell Line.

A. FLAG-TCF7L1-HTBH is localized to the nucleus in H9-TCF7L1 line. Immunofluorescence analysis of OCT4 (red) and FLAG (green) in H9-TCF7L1 under feeder-free conditions with or without 24 hrs of doxycycline induction (1 μg/ml). Nuclei were stained with Hoechst.

B. The H9-GFP inducible cell line robustly and uniformly expresses GFP in the presence of doxycycline. Live cells were imaged for GFP expression after 24 hours of doxycycline (1 μg/ml) induction.

C. FLAG-TCF7L1-HTBH (F-TCF7L1-H) over-expression is robustly controlled by doxycycline. H9-TCF7L1 cell line was induced for 3 hrs with the indicated concentrations of doxycycline and then whole cell extracts were harvested for Western blot analysis. The higher molecular weight band is FLAG-TCF7L1-HTBH and the second, slightly lower, band is endogenous TCF7L1 that serves as a loading control. The data show rapid responses to doxycycline and no observable leaky expression in the absence of doxycycline.

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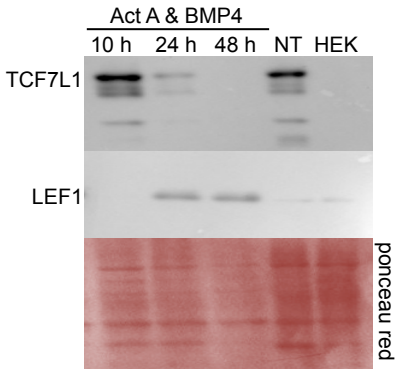

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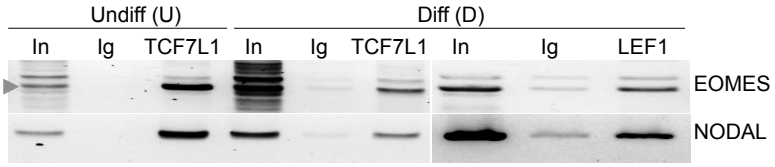
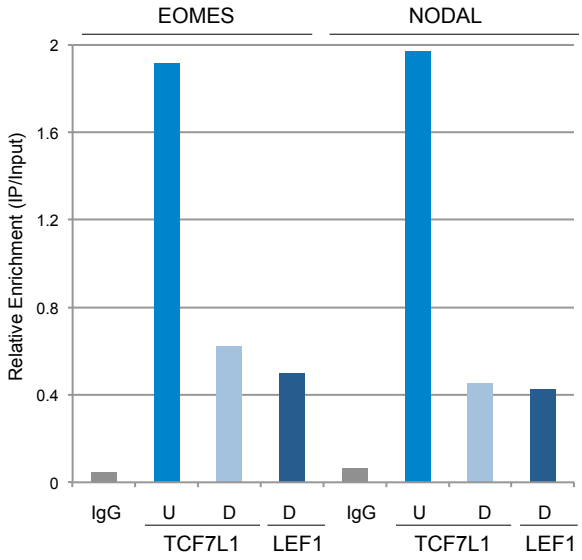
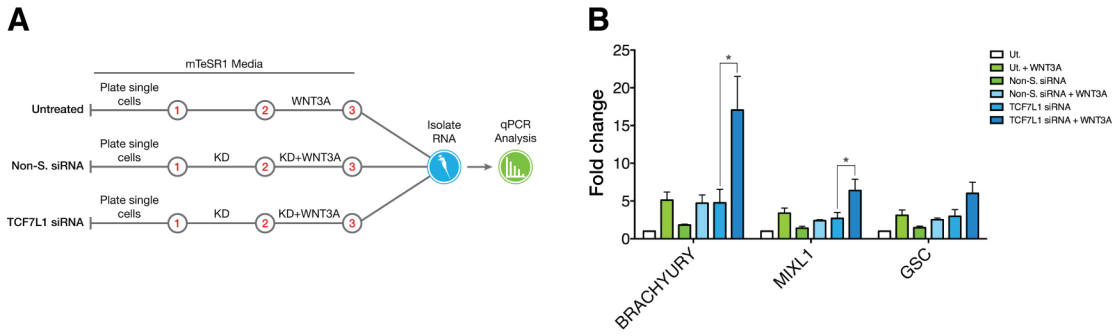




Supplemental Figure 8. Analyses of TCF7L1 and LEF1 protein expression and chromatin binding following mesoderm induction.

A. Western blot analyses of TCF7L1 and LEF1 protein levels in H9 hESCs following differentiation into mesendoderm. Twenty microgram of total protein was loaded per lane. NT2D and HEK293 were used as positive and negative controls respectively. Ponceau red staining was used to verify equal loading and transfer of protein across samples. Note that TCF7l1 protein levels decline following induction of differentiation while levels of LEF1 protein increase.

B. Analyses of TCF7L1 and LEF1 binding at the EOMES and NODAL loci in undifferentiated (U) and differentiated (D) H9 hESCs. PCR analyses with primers that amplified regions near the promoters of EOMES and NODAL. Bar graphs show the average quantification of the band intensities from two replicates. Percentage of enrichment (IP/input) was measured using ImageJ.



Supplemental Figure 9. Loss of TCF7L1 Sensitizes hESCs to WNT3A-induced Gene Expression.

A. Schematic of experiment. hESCs were treated with WNT3A (10 ng/ml) while performing *TCF7L1* siRNA (50 nM) knockdown under feeder-free conditions. Cells were harvested after 48 hrs of siRNA knockdown and 24 hrs of WNT3A treatment. Although absent from schematic, we performed control experiments without WNT3A treatment for each condition. Red numbers indicate days of procedure.

B. qPCR analysis showing increased up-regulation of PS markers when WNT3A treatment is combined with *TCF7L1* knockdown (n=4 one-tailed *t*-test *P≤0.05).

Table S1. qPCR Probes and Primers.

Taqman Assays		SYBR Green Assays		
GENE SYMBOL	**Cat#**		**Fwd. Primer**	**Rev. Primer**
TCF7L1	Hs01064111_m1	AXIN2	CTGGGGGCAGCGAGTATTAC	GCCTTTCCCATTGCGTTTGG
TCF7L2	Hs01009038_m1	WNT3	AGGGCACCTCCACCATTTG	GACACTAACACGCCGAAGTCA
TCF7	Hs01556515_m1	NODAL	TACAGGCAGAAGATGTGGCAGTGGAT	ATCCTCTTGTTGGCTCAGGAAGGA
LEF1	Hs01547250_m1	GAPDH	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT
POU5F1	Hs00999632_g1	DACT1	TGGAGGAGAAGTTCTTGGAGGAGA	TCCAGTCTCAGGTCACTTATCTGC
SOX2	Hs01053049_s1	BMP4	ATGATTCCTGGTAACCGAATGC	CCCCGTCTCAGGTATCAAACT
NANOG	Hs02387400_g1	DGKK	ATTGATCTGTCTCAAGCCACTG	TCTTCCATGTCTTTCCGGTTG
BRACHYURY	Hs00610080_m1	TMEM130	TCCATCATCGGGACCTTCAC	CCCGGTCTTCTGCTTCACAG
MIXL1	Hs00430824_g1	RUNDC34	CGGGGCTTTTGGGACTATATC	GCAGAGCCGTGGTGATGTATT
GSC	Hs00418279_m1	SYT4	ATGGGATACCCTACACCCAAAT	TCCCGAGAGAGGAATTAGAACTT
SMARCA2	Hs00268234_m1	TCF7L1 (PCR)	TCTCCCTCACCACCAAACCAGAAA	TGTCTTCTCACATGGTGATGGCCT
FST	Hs00246256_m1	B-ACTIN PCR	TGACGGGGTCACCCACACTGTGCCCATCTA	CTAGAAGCATTTGCGGTGGACGATGGAGGG
MEIS2	Hs00542638_m1			
18S	Hs99999901_s1			
GAPDH	Hs99999905_m1			

Table S2 Antibodies.

Antibody	Company	Cat #	Species	Western Blot	ICC	ChIP
FLAG M2	Sigma	F3165	Mouse	1.2 ug/ml (1:2500)	8 ug/ml (1:375)	
TCF7L1	Cell Signaling	2883	Rabbit mAB	1:1000	1:100	
OCT4	R&D	AF1759	Goat	1:1000	1:100	
ACTIN	Santa Cruz	sc-1616	Goat	1:1000		
B-CATENIN	Cell Signaling	2677	Mouse mAb		1:200	
LEF1	Cell Signaling	76010	Rabbit mAb	1:1000		1:50
TCF7L1	Santa Cruz	sc-166411	Mouse mAb		1:100	
TCF7L1	Santa Cruz	sc-8635	Goat pAb		1:100	5.0 ug
B-CATENIN (non-phospho)	Cell Signaling	8814	Rabbit mAb		1:100	
NANOG	Santa Cruz	sc293121	Mouse mAb		1:100	
SOX2	Santa Cruz	sc365964	Mouse mAb		1:100	
TCF7L1	Active Motif	61125	Rabbit pAb			10 uL
Normal Rabbit IgG	Millipore	12-370	Rabbit IgG			2.5 ug

Table S3 Small Molecules and siRNAs.

Small Molecule	**Vendor**	**Concentrations**
IWP-2	Stemgent	2 µM
CHIR-99021	Selleckchem	3 µM
SB431542	Tocris	10 µM
SU5402	EMD Millipore	10 µM

siRNA	**Company**	**Cat. No.**	**Conc.**
TCF7L1 #1	Dharmacon	J-014703-05	50 nM
TCF7L1 #2	Dharmacon	J-014703-06	50 nM
Non-targeting	Dharmacon	D-001810-02-05	50 nM

Table S4 ChIP-qPCR Primers.

	Fwd. Primer (5' > 3')	Rev. Primer (5' > 3")	Coordinants
NODAL	CCCAGTGATTTCAGGAGGAAAG	GCCCAGAGATCAAAGTGAGTG	chr10:72202124+72202246
WNT3	GTGAATGTGTGGGACCTTAGAC	GTGGAGCCTCACTGAATACAC	chr17:44899079+44899218
TDGF1	ACGTCCGCCTGGAATTTG	GACTAGGAAGCTTGAAACTGAGATAG	chr3:46618663+46618753
BMP4	GCCTGTGACCAGCTTCTT	CCTATGGTGAGCAAGGCTAC	chr14:54423188+54423273
EOMES	GGAGTCAGTCAGAACCAAAGAG	CGGGAGGGCACTTGATTT	chr3:27769942+27770027
GSC	GTTGTCGATGCTGAACATGC	CGCTCTCTTTCGGTTTGGT	chr14:95236323+95236461
OTX2	TGCAAAGTCGGCCCAAAT	CCTTAGTTCCACTGCTCCAAAC	chr14:57272193+57272299
MIXL1	CAGGCTGTAAAGCTGCAAATC	AGCAACTGTCTGGTTCACTATC	chr1:226398694+226398828
FOXD3	GTGCGCTGCTCTTACTCTTTA	CGAGGTTCCCATATCGTGTTT	chr1:63787728+63787822
BMPR2	GCACTACACAAATCCTTGGAAAC	GAGTTAGAGTTGTGTCGGGATAG	chr2:203240062+203240160
Neg. Control	CCGAATTTGGGCCTCTACAA	CATGGTGGCTACGGTGAATAA	