

Supplemental Materials

Table supplement 1

Candidate list of Wnt regulated genes

Genes	Wnt3a ^{-/-} embryo (Fold change)	ESC Lef1 overexpression (Fold change)	ESC Sp5 overexpression (Fold change)
CDX4	0.22	-	6.97
SP5	0.33	24.14	164.27
CDX1	0.39	-	12.89
MIXL1	0.43	2.89	2.74
CDKN1A	0.52	1.72	2.53
PDLIM4	0.54	2.54	1.70
GNA14	0.57	2.58	3.10
TNFRSF19	0.6	7.37	6.14
ZFP703	0.61	1.95	1.67
GBX2	0.62	-	3.65
ALPL	0.64	1.60	1.52
CDX2	0.69	-	14.67
SP8	0.7	3.65	-
APCDD1	0.74	2.13	2.54
NGFR	0.75	4.81	2.08
ACOT7	0.75	1.57	-
ZFP428	0.75	2.30	2.08
PHLDA3	0.76	1.61	1.22
SLC27A4	0.76	1.55	1.26
EFNA4	0.77	1.62	1.44
T	*	12.48	138.09
MCC**	0.67	-	3.57
EVX1**	0.52	9.79	75.91
EFNA3**	0.76	-	5.94
AXIN2	0.51	4.80	6.13
SEMA4F	0.36	-	1.88
CITED1	0.48	1.73	-
ITGA8	0.49	-	3.62
CLDN26	0.5	2.30	-
2510009E07RIK	0.5	-	4.20
GM6792	0.52	2.83	-
5730457N03RIK	0.52	-	113.02
MLLT3	0.52	-	1.77

H2-BL	0.52	-	1.49
ATP1A2	0.56	-	4.07
ETV4	0.59	1.88	-
ADAMTS20	0.59	-	2.13
CRISPLD1	0.6	-	1.61
UBL5	0.63	1.58	-
SHF	0.63	-	1.57
TMEM132C	0.63	-	5.63
LMO1	0.64	-	2.24
S1PR5	0.64	-	3.10
GFRA3	0.64	-	8.41
GAD1	0.64	-	5.60
RBP1	0.65	1.56	-
SH3GL3	0.66	-	1.42
FAM118A	0.66	1.56	-
MRPL55	0.67	1.61	-
RNF213	0.68	-	1.78
NTN1	0.68	-	1.52
RFTN1	0.68	-	1.35
MFGE8	0.69	1.51	-
RAI14	0.69	-	1.87
SCML2	0.69	-	2.73
PIM1	0.69	2.18	-
TTC28	0.69	-	2.84
MSX1	0.69	-	5.68
SELM	0.7	2.16	-
HDDC3	0.7	1.57	-
PLAGL1	0.7	-	2.78
ZFP697	0.7	-	1.37
CUEDC1	0.71	1.88	1.93
4933402E13RIK	0.71	-	2.24
CALM3	0.71	1.42	-
GPC6	0.72	-	1.33
TSPAN13	0.72	-	2.18
OSBPL6	0.72	-	3.00
FREM2	0.73	2.65	2.89
MMGT2	0.73	-	1.36
KCNK3	0.73	-	1.97
KLHL24	0.73	-	1.37
ROR2	0.74	2.86	3.54
IGDCC3	0.74	1.76	3.01

CMTM7	0.74	1.67	-
RICTOR	0.74	-	1.21
ZBTB39	0.74	-	1.17
LEMD1	0.74	-	2.10
MRPS10	0.74	1.49	-
CACHD1	0.74	-	1.75
1190002N15RIK	0.74	-	1.20
NRARP	0.75	1.85	-
IFITM1	0.75	-	2.74
EPB4.1L2	0.75	-	1.29
HOXB2	0.75	-	2.93
NRP2	0.75	-	2.04
CHCHD5	0.76	1.57	-
PRTG	0.76	1.39	1.39
CCND2	0.76	1.51	-
EMID2	0.76	-	4.89
ADCY2	0.76	-	1.88
MAPK8IP1	0.76	1.43	-
EXOC6B	0.76	-	1.47
PLCL2	0.77	1.53	2.03
PCDH18	0.77	-	1.86
DPYSL4	0.77	-	1.93
A730017C20RIK	0.77	9.77	-
NEFL	0.77	3.02	1.56
PLEKHH1	0.77	-	1.29
NR5A2	0.77	-	3.98
SLC19A3	0.77	2.25	-
TRAPPC6A	0.78	1.90	-
STRA13	0.78	1.79	-
COL27A1	0.78	2.05	-
GALNT10	0.78	-	1.23
S100A16	0.78	-	1.60
RALGPS2	0.78	-	1.15
LHX9	0.78	-	5.81
SPATA24	0.79	-	1.61
LRRC45	0.79	1.72	-
CAMKK2	0.79	-	1.84
SGCE	0.79	-	1.34
SNRPB	0.8	1.70	-
MRPS5	0.81	1.72	-

- Not detected as a significantly regulated gene
- * Not present in Wnt3a microarray
- ** Unable to clone

Figure supplement 1

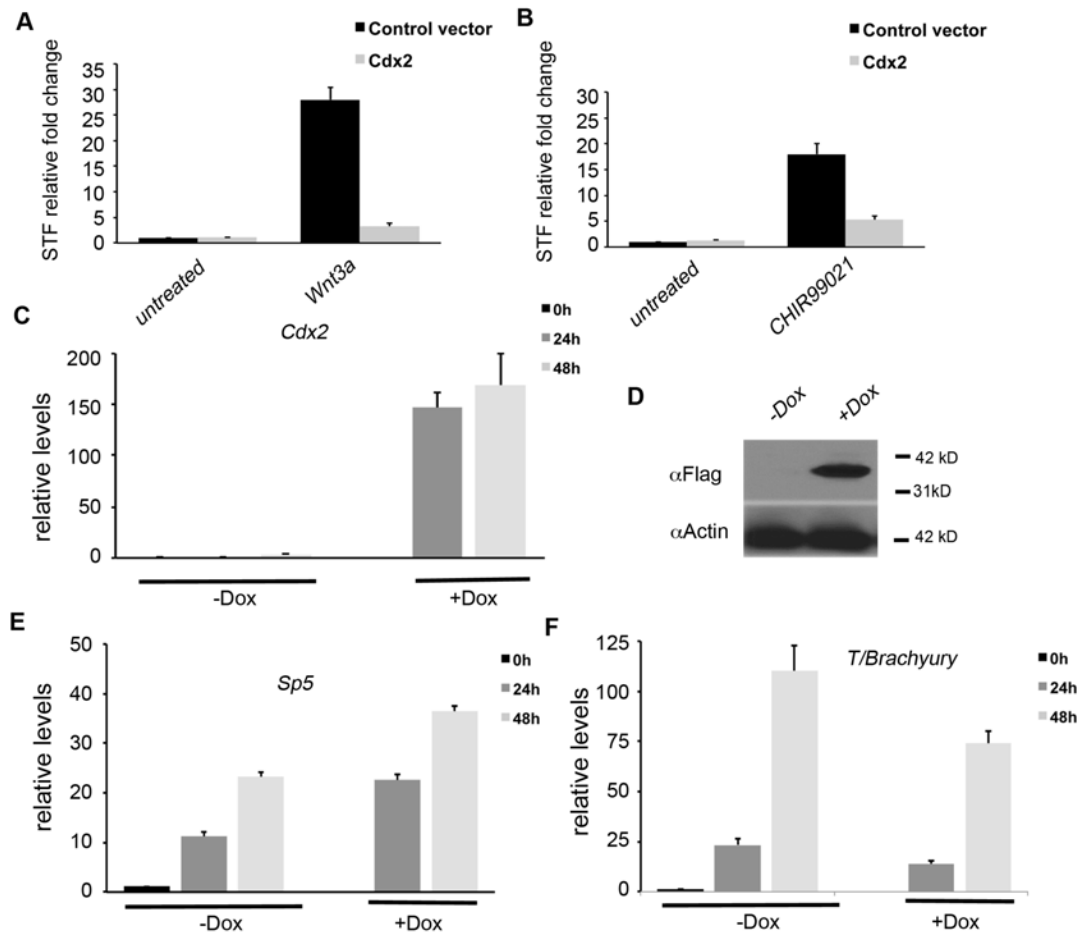


Fig. S1. Characterization of Wnt pathway inhibitors. (A) Luciferase assay of super TOPFlash (STF) TCF/LEF reporter in untreated and Wnt3a treated 293T cells comparing control (empty vector) and Cdx2 transfected. (B) Luciferase assay of STF in untreated and CHIR99021 treated 293T cells comparing control (empty vector) and Cdx2 transfected. (C) qPCR analysis of Cdx2 expression in

A2lox.3F-Cdx2 mouse ESCs (-/+ Dox). (D) Immunoblot of A2lox.3F-Cdx2 mouse ESCs (-/+ Dox) to detect induced protein expression. (E) qPCR of Wnt target gene *Sp5* in differentiating A2lox.3Flag-Cdx2 mouse ESCs (-/+ Dox). Note, that *Sp5* is induced rather than repressed. (F) qPCR of Wnt target gene *T/Brachury* in differentiating A2lox.3F-Cdx2 mouse ESCs (-/+ Dox). Note, that *T* is repressed.

Figure supplement 2

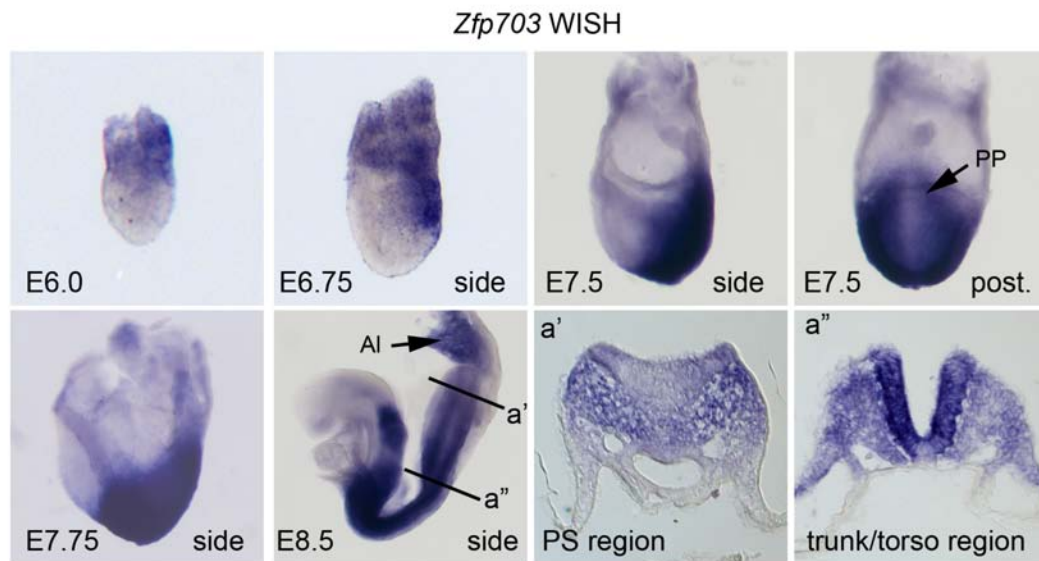
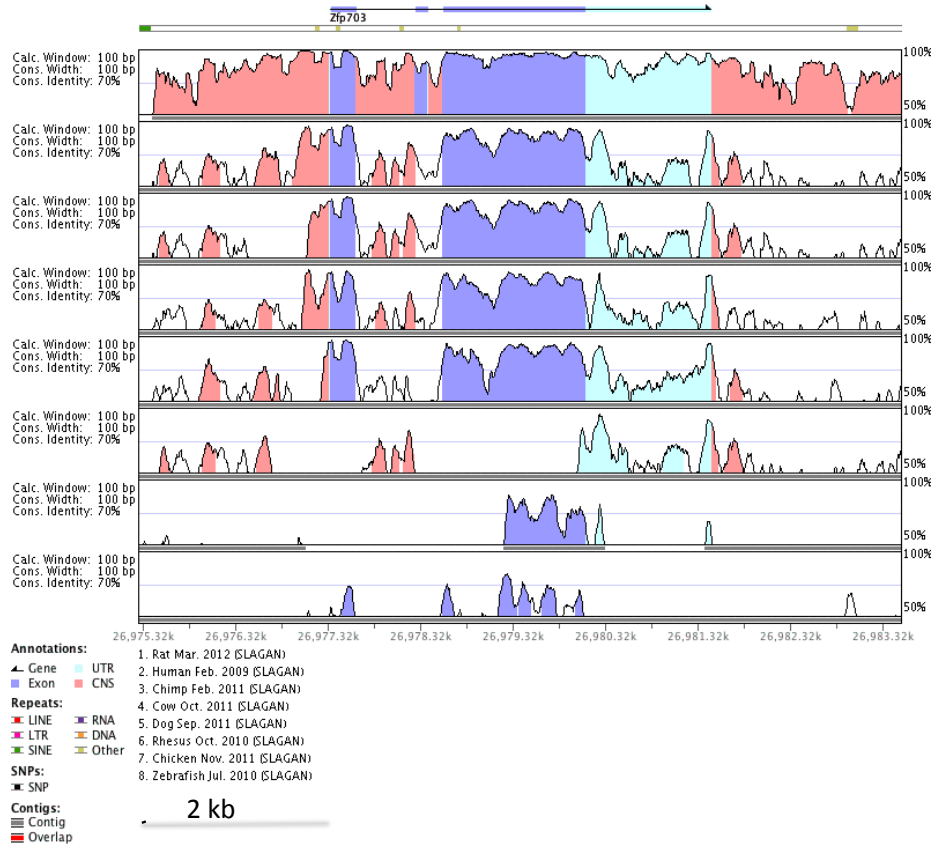


Fig. S2. Whole-mount In situ detection of *Zfp703* in mouse embryos. At E6.0 *Zfp703* was detected in extraembryonic tissue. At E6.75 *Zfp703* began to be detected in the primitive streak (PS). E7.5 embryos showed *Zfp703* widely expressed in the PS region. Posterior view of E7.5 embryo showed *Zfp703* detection in posterior progenitors (PP). At E7.75 *Zfp703* was detected in the PS region and in the allantois. At E8.5 stage its expression is predominantly present in the anterior trunk/torso region. The same could be confirmed in cross-sections through the PS at E8.5 stage revealing that *Zfp703* expression is less intense in PS domain compared to its expression in the trunk/torso domain. It is predominantly expressed in the neural tube of trunk/torso region and rhombomeres 3 and 5.

Figure supplement 3 Comparative genomics of *Zfp703*

A



B

>mm10_refGene_NM_001110508 range=chr8: 28086234-28087828

Non-coding conserved regions between mouse and human: Yellow highlight

TCF/LEF binding site: Red highlight

Start codon: Green highlight

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ttcatgattggttctatgattcgcctccttccgctcactttgacgaccctttggaatctcttgctctcacgt
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ccgccccccagTGGCACTGCCTCCTCCAAATG
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Fig. S3. Sequence and annotation of TCF/LEF binding sites in the β -catenin peak at the *Zfp703* locus. (A) Comparative genomics vistaplot analysis of *Zfp703* with respect to other species. Blocks of highly conserved non-coding regions are shown in orange color. (B) Sequence and annotation of putative TCF/LEF binding sites within a region of the mouse *Zfp703* locus identified in a genome-wide survey of β -catenin binding. The β -catenin ChIP-seq peak is centered at -839, relative to the transcriptional start site of *Zfp703*. Red highlighted regions indicate TCF consensus binding sites CTTTG,A/T,A/T. Yellow highlights indicate the 3 most proximal sequence blocks conserved between mouse and human, taken from vista analysis depicted in (A).

Figure supplement 4

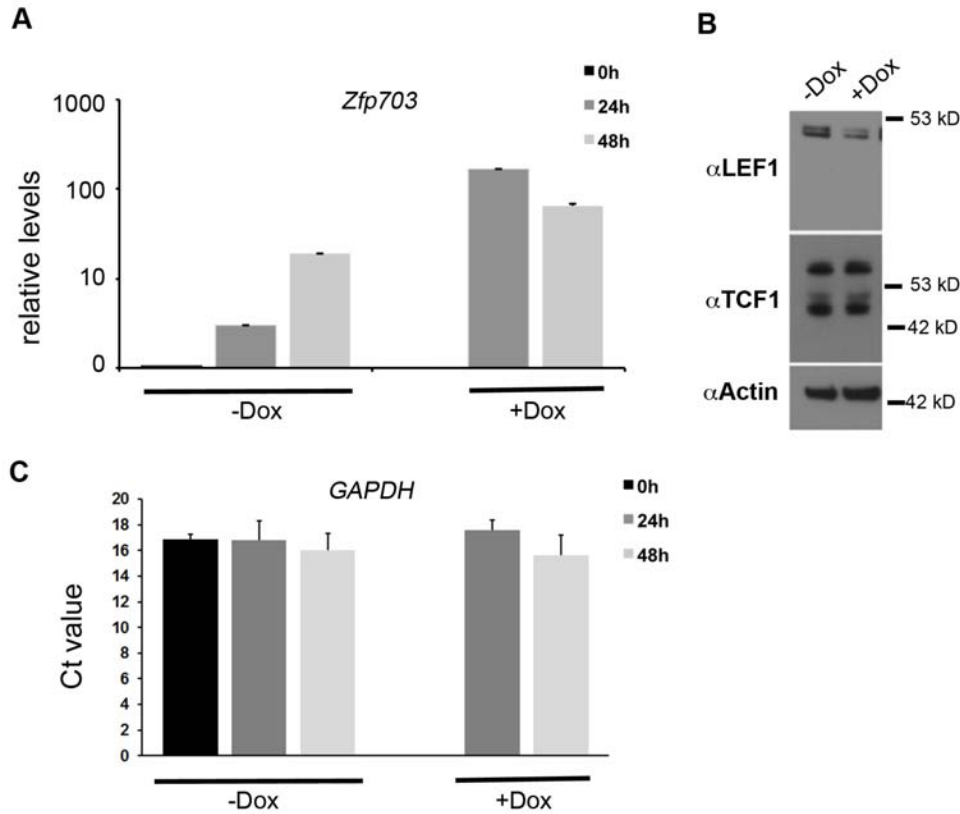


Fig. S4. ZFP703 regulation and activity in 293T human embryonic kidney cells and mouse embryonic stem cells. (A) qPCR of *Zfp703* in A2lox.3F-*Zfp703* mouse ESCs (-/+ Dox). (B) Immunoblot of Lef1 and Tcf1 in A2lox.3F-*Zfp703* mouse ESCs (-/+ Dox), Actin was used as a loading control. (C) Raw Ct values for *Gapdh* (Average+SD from six independent experiments) by qPCR from cDNA derived from equal amounts of RNA from A2lox.3F-*Zfp703* mouse ESCs (-/+ Dox).

Figure supplement 5

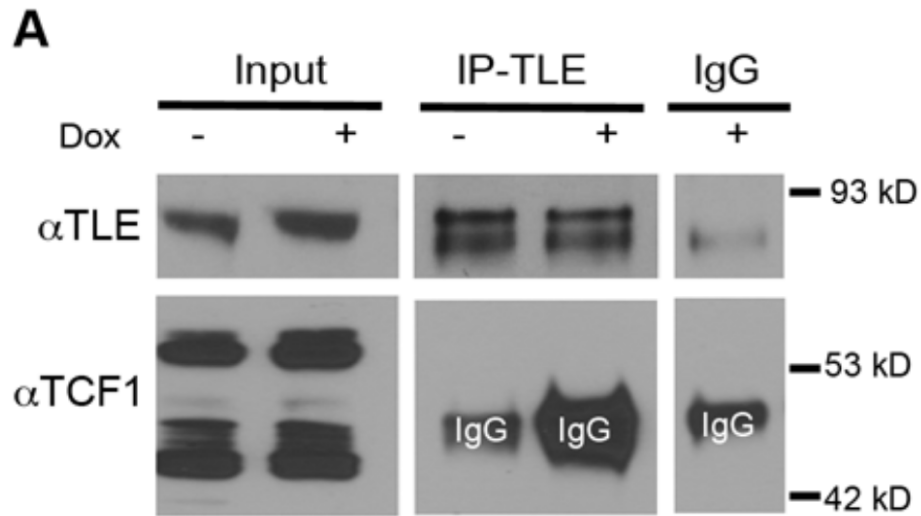


Fig. S5. Failure of detection of Tcf1 and Tle by Co-IP. (A) Co-IP experiment of endogenous Tle using a pan-Tle antibody and endogenous Tcf1 in A2lox 3F-Zfp703 mouse ESCs (-/+ Dox). Equal amounts of Tle and Tcf1 were observed in the input; however, Tcf1 was not co-immunoprecipitated with Tle. IgG precipitates were used as control.

Supplementary Table 2

Primers for PCR

qPCR oligos	Oligo sequence		Oligo sequence
Gapdh-fw	AGGTCGGTGTGAACGGATTG	Gapdh-rv	TGTAGACCATGTAGTTGAGGTCA
Zfp703-fw	AGTACCTGCAACCGCTGTC	Zfp703-rv	TACGGAGTTGAGCTTGGATGAG
Lef1-fw	AGCTGAGTGCACGCTAAAGG	Lef1-rv	GTATTTGGCCTGCTCTTCCC
Sp5-fw	CGAAGTCTTGCTGTCCAGCCCA	Sp5-rv	AGCAGCGACTCCCACAAGCAAG
ZFP703-fw	AGTACCTGCAACCGCTGTC	ZFP703-rv	CACCGAGTTGAGTTTGGAGGAG
Axin2-fw	AGAGGGACAGGAACCACTCG	Axin2-rv	CACTTGCCAGTTTCTTTGGC
Tcf1-fw	GCGGACATCAGCCAGAAG	Tcf1-rv	TCACAGTATGGGGGAGCTGT
Brachyury-fw	CTCGGATTCACATCGTGAGAG	Brachyury-rv	AAGGCTTTAGCAAATGGGTTGTA
Mesogenin-fw	AATTACCTGCCGCCTGTCTA	Mesogenin-rv	GATTCTCCAGAGGGCACAAC
Tbx6-fw	CCGAGAAAATGGCAGAAACT	Tbx6-rv	GTGTATCCCCACTCCACAG
Sox1-fw	TGTAATCCGGGTGTTCTTC	Sox1-RV	AGTGAAGGTCATGTCCGAG
Pax6-fw	GCAGATGCAAAAGTCCAGGTG	Pax6-rv	CAGGTTGCGAAGAACTCTGTTT
Sox2-fw	CGAGATAAACATGGCAATCAAATG	Sox2-rv	AACGTTTGCCTTAAACAAGACCAC
Cloning oligos			
p2-Lox-Zfp703-fw	TCCGACGCGTATGAGCGATTGCCCCGCTGG	P2-Lox-Zfp703-rv	TCCGAGCTCTTACTGGTATCCAAGC GCAGAGGC
Zfp703-en-fw	CCAAACCTTCAACCACGCTTC	Zfp703-en-rv	TGGCAGGAGGCAGTGCC
p2-Lox-CdX2-fw	TCCGACGCGTATGTACGTGAGCTACCTTC	p2-Lox-CdX2-rv	AGACGCTTAAGTCACTGGGTGACAG TGGAG
ChIP-qPCR oligos			
Cdx1-WRE-fw	AAGGAGACAAATTGCCGCCCT	Cdx1-WRE-rv	CCCGTTTGAAGTCAGCCTTGC
Axin2-WRE-fw	GAGCGCCTCTGTGATTGG	Axin2-WRE-rv	GACCCACCTTTTACAGCAA
Msgn1-WRE-fw	GTGGAGGGAGTTCAAAGGAAG	Msgn1-WRE-rv	CAAAGAGGGGAAAGTGACACC GGATTCTCTCGGCTTCAGACAGACT TT
control-fw	GGAGTCCCCTAGGAAGGCATTAATAGTTT	control-rv	
Brachyury-WRE-fw	CTTTGATGGAGGTGCAAACA	Brachyury-WRE-rv	CCCTTTGAAGTACCGAGCAG