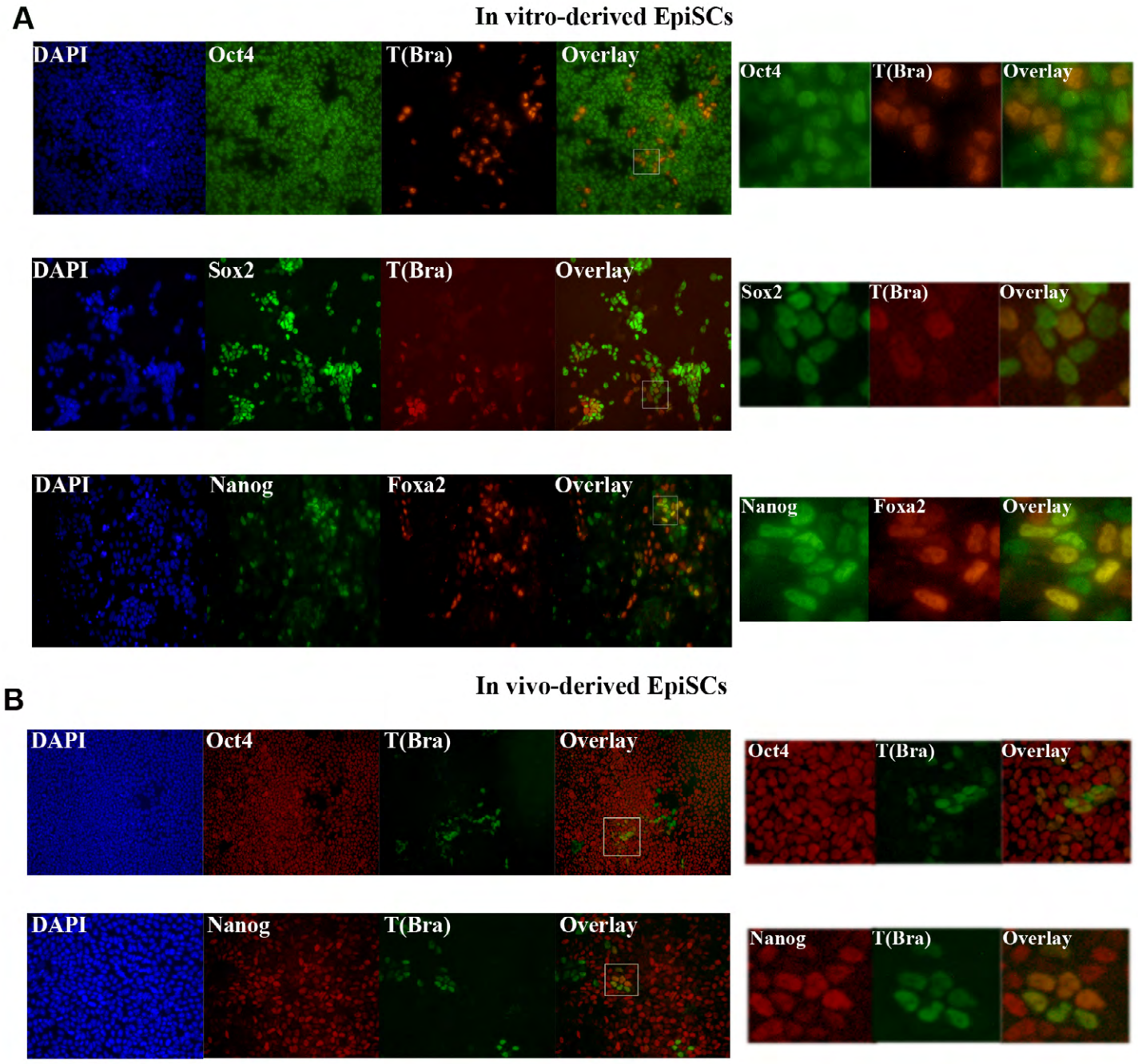

Figure S1. Lineage marker expression in EpiSCs. Immunocytochemistry analysis of T(Bra)/Foxa2 expression and various pluripotency markers in undifferentiated in vitro (**A**) and in vivo (**B**) derived EpiSCs. Magnified regions are also shown corresponding to the areas within the white box.

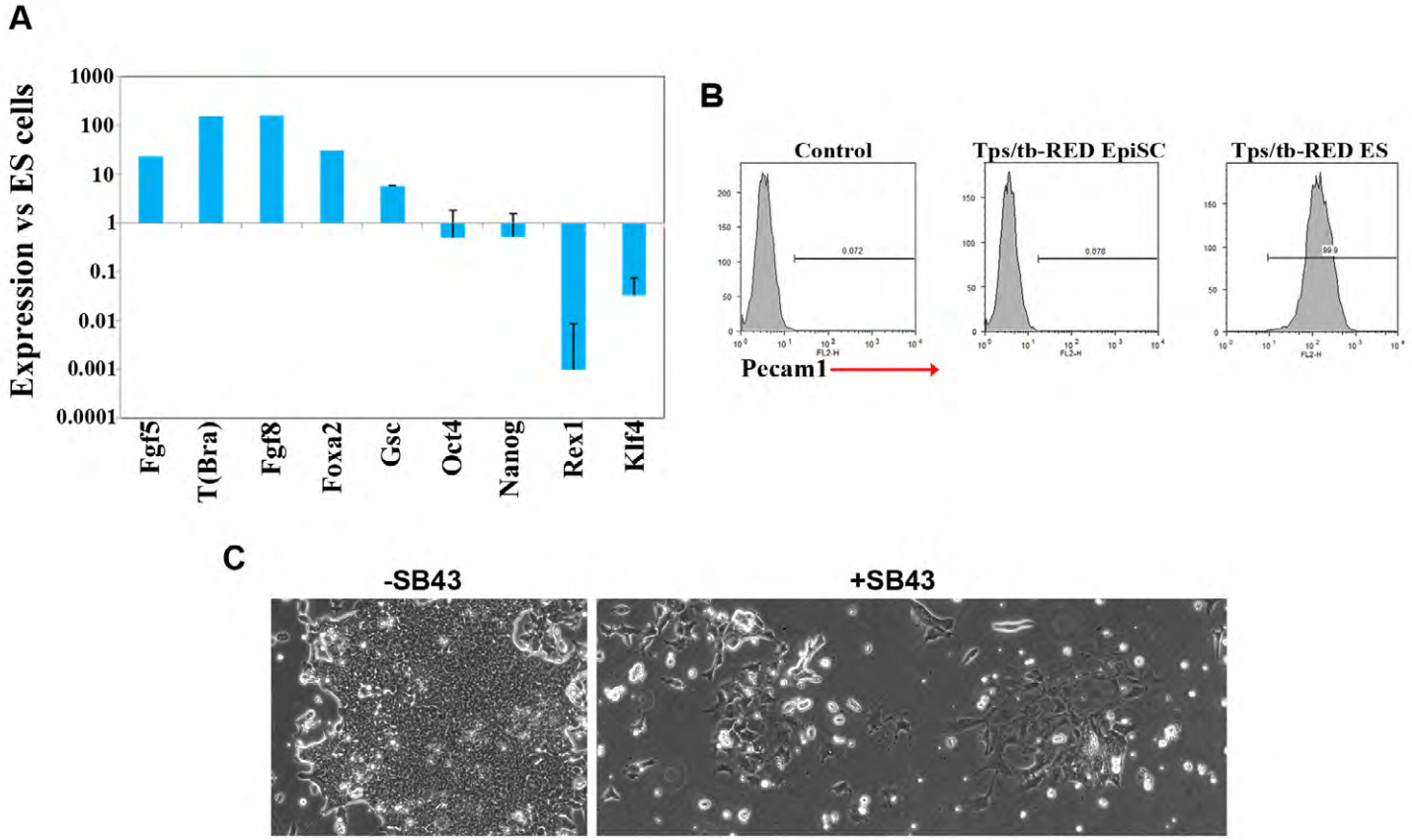

Figure S2. Tps/tb-RED EpiSCs exhibit hallmarks of primed pluripotency. (**A**) qPCR analysis of expression of representative EpiSC, pluripotency and ES cell-specific markers in established Tps/tb-RED EpiSCs. (**B**) Flow cytometry expression analysis of the ES cell-specific surface marker Pecam1 in established Tps/tb-RED EpiSCs. ES cells were also stained as a positive control. (**C**) Morphology of Tps/tb-RED EpiSC cultured at low density for 3 days in the presence or absence of the Alk4/5/7 activin/nodal receptor inhibitor SB431542 (10 μM).

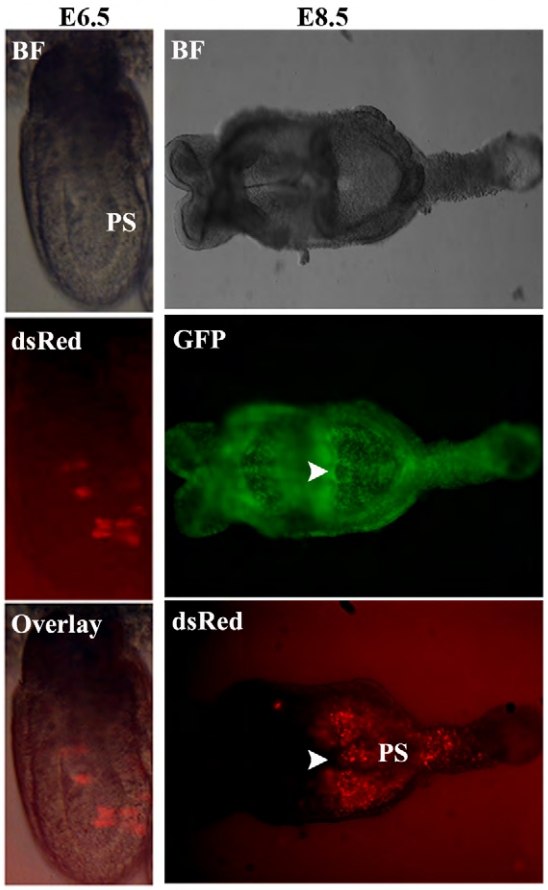

Figure S3. Tps/tb promoter activity in vivo. Tps/tb-dsRed2 expression in E6.5 and E8.5 chimeric embryos generated by morula aggregation of Tps/tb-RED ES cells (E6.5 embryo) or C2 ES cells (E8.5 embryo) which also contain a constitutively active GFP reporter to mark the extent of chimerism (see main text for details). The position of the node is marked by the arrowhead. BF, brightfield.

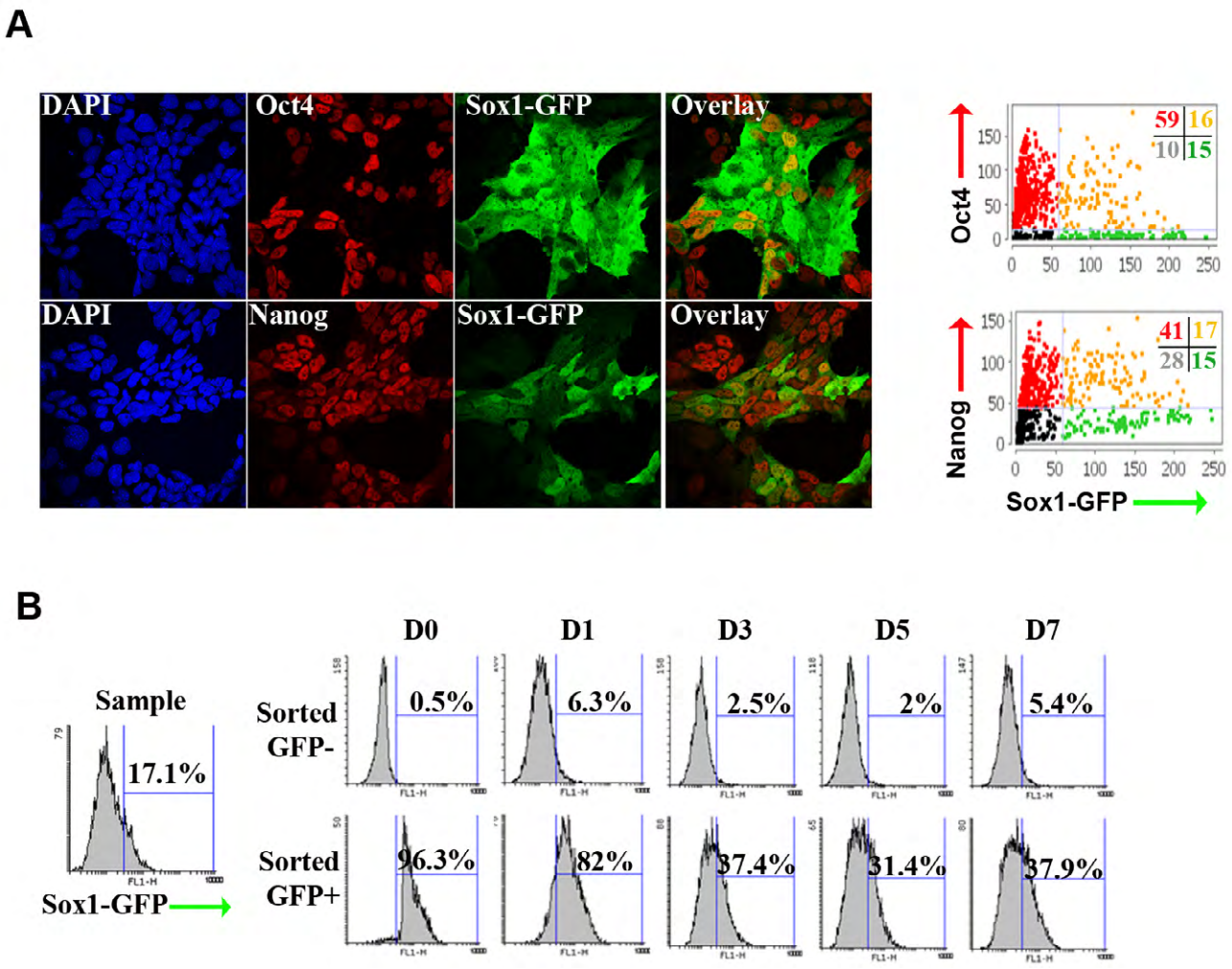

Figure S4. Characterisation of Sox1-GFP EpiSCs. (**A**) Immunocytochemical analysis of Oct4/Nanog and Sox1-GFP expression in undifferentiated 46C EpiSCs. Right, correlation and quantification of Oct4/Nanog (red) and Sox1-GFP (green) expression domains. (**B**) Flow cytometry-based time course analysis of Sox1-GFP expressing cells in separated GFP^+ and GFP^- populations after sorting and plating in EpiSC conditions. Steady state Sox1-GFP expression in the starting sample prior to sorting is shown on the left.

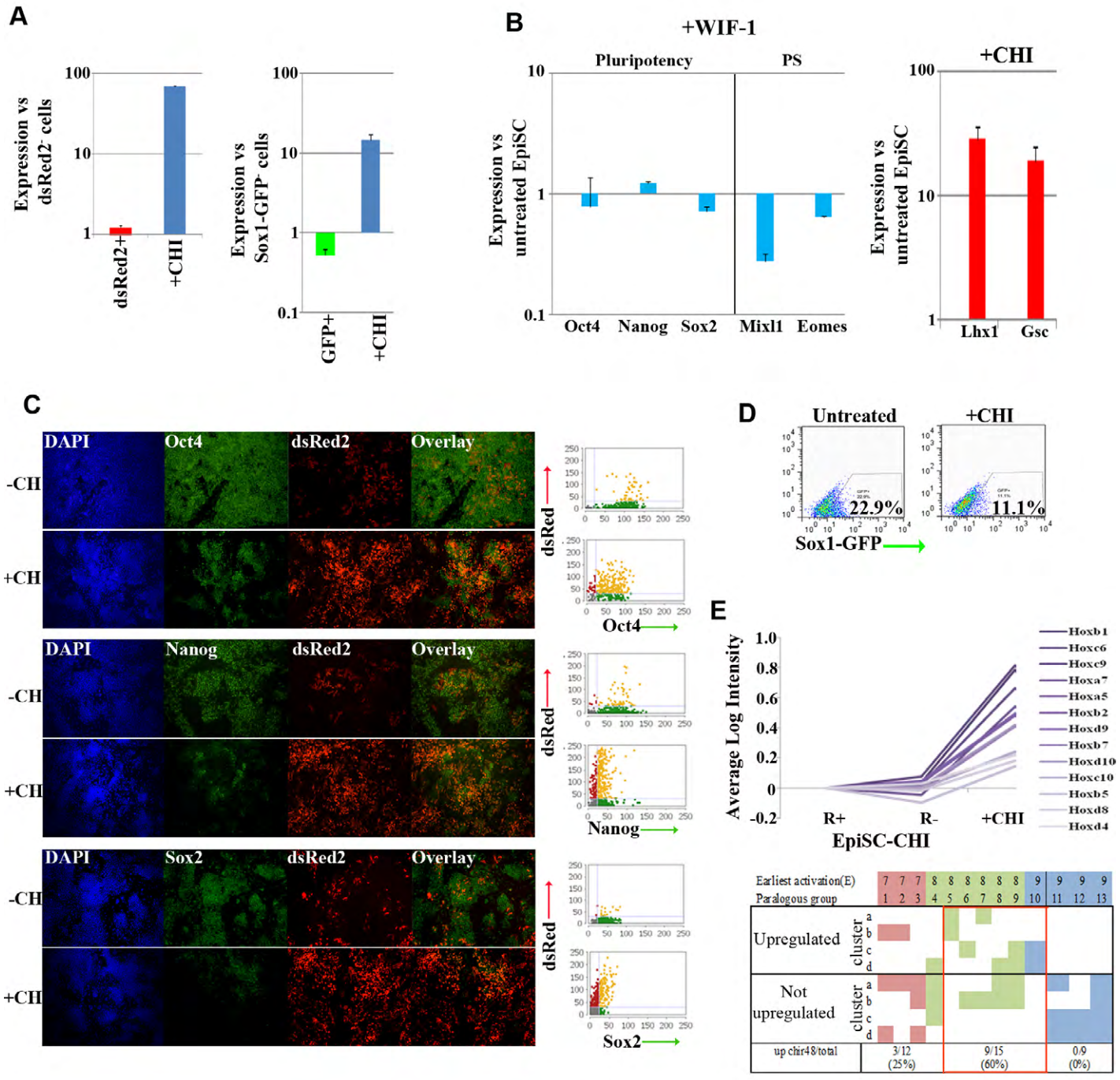

Figure S5. Effects of Wnt activity manipulation on EpiSCs. (**A**) qPCR expression analysis of the Wnt target *Axin2* in sorted dsRed2$^+$ (left) and Sox1-GFP$^+$ EpiSCs (right). The expression levels of *Axin2* in the respective unsorted CHIRON treated populations are also shown. Error bars represent s.e.m. (n=3). (**B**) Left: qPCR expression analysis of pluripotency markers (*Oct4* and *Nanog*) and additional PS markers (*Mixl1, Eomes*) in EpiSCs treated with the Wnt antagonist WIF-1 (5 days). Error bars represent s.d. (n=2). Right: qPCR expression analysis of additional PS markers *Lhx1* and *Gsc* in EpiSCs treated with CHI (48 hrs). Error bars represent s.e.m. (n=3) ($p<0.05$). (**C**) Quantitation of dsRed2 fluorescence and immunofluorescence of pluripotency markers in EpiSC cultures with or without addition of CHI. (**D**) FACS analysis of Sox1-GFP expression in 46C EpiSC in the absence or presence (48 hr) of CHI. (**E**) Microarray-based $\log_2$ intensity of Hox transcripts in dsRed2$^+$ (R+) and negative (R-) populations as well as in CHI-treated (48 hrs) EpiSC cultures. The table below summarises the expression data by correlating in vitro activation of the different Hox clusters with the timing of their up-regulation in vivo. The red box highlights the most-upregulated group of Hox genes after CHI treatment.

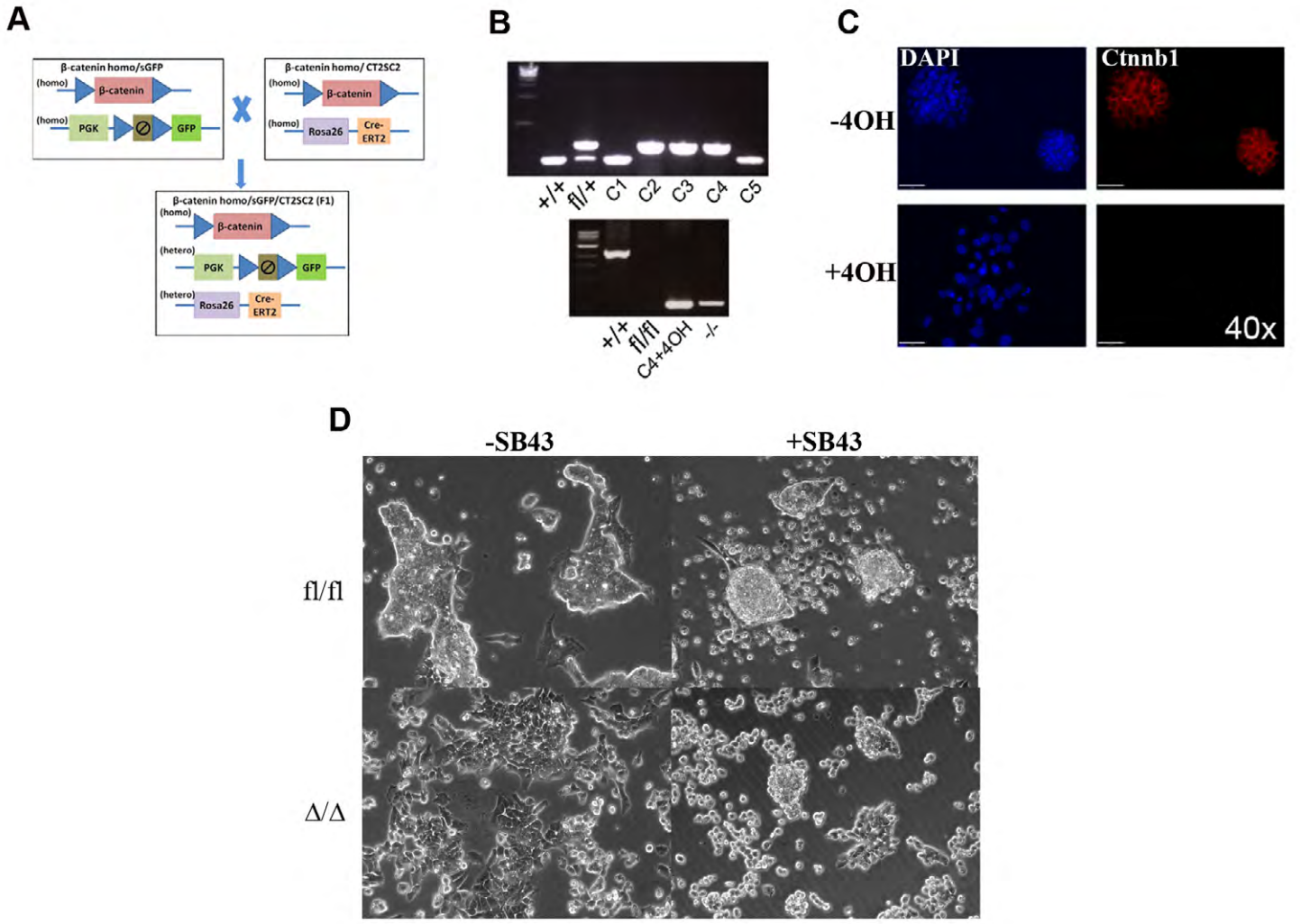

Figure S6. Generation of Ctnnb1-null EpiSCs. (A) Schematic representation of the strategy used to derive the floxed Ctnnb1 mouse line. (**B**) Confirmation of Ctnnb1 deletion by PCR-based genotyping of isolated clones (C1-5). (**C**) Confirmation of absence of Ctnnb1 protein (in red) by immunostaining of cells from the same clone in the presence (+4OH) or absence (-OH) of 4-hydroxytamoxifen (4OH). Cell nuclei were stained with DAPI (blue). (**D**) Morphology of fl/fl- (top) and Δ/Δcat (bottom) EpiSCs in the absence (left) or presence (right) of the Alk4/5/7 activin/nodal receptor inhibitor SB431542 (10 μM)

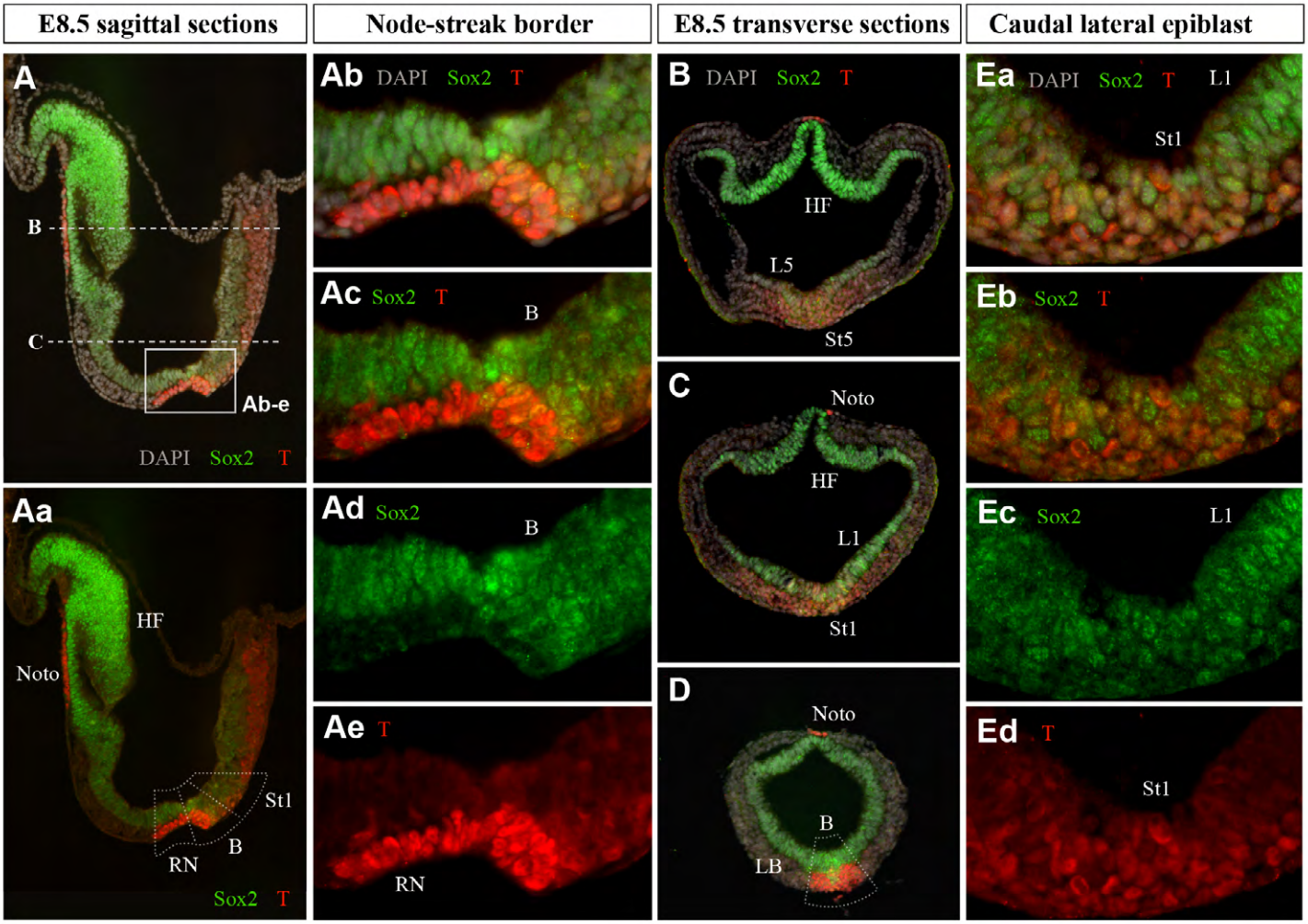

Figure S7. Sox2 and T(bra) coexpression marks NM progenitors in the node-streak border and the caudal lateral epiblast. Immunohistochemical detection of Sox2 (green) and T(bra) (red) at E8.5 (2-somite stage) in areas containing NM progenitors, the node-streak border and caudal lateral epiblast. (A, Aa) Detection of Sox2 and T protein on a midsagittal section. (Ab-e) High magnification image of the node-streak border region. (B,C) Transverse sections through the embryo at positions indicated on the embryo in (A) show double-positive cells in the caudal lateral epiblast and the primitive streak. (D) Double positive cells can be found in the node-streak border, but not in more lateral cells. (Ea-d) The caudal lateral epiblast contains Sox2-T positive cells. The regions and terminology are as in Cambray and Wilson (2007). Node-streak border (B), head folds (HF), rostral part of the caudal lateral epiblast (L1), caudal part of the caudal lateral epiblast (L5), cells lateral to the node-streak border (LB), notochord (Noto), rostral node (RN), rostral part of the streak (St1), caudal part of the streak (St5).

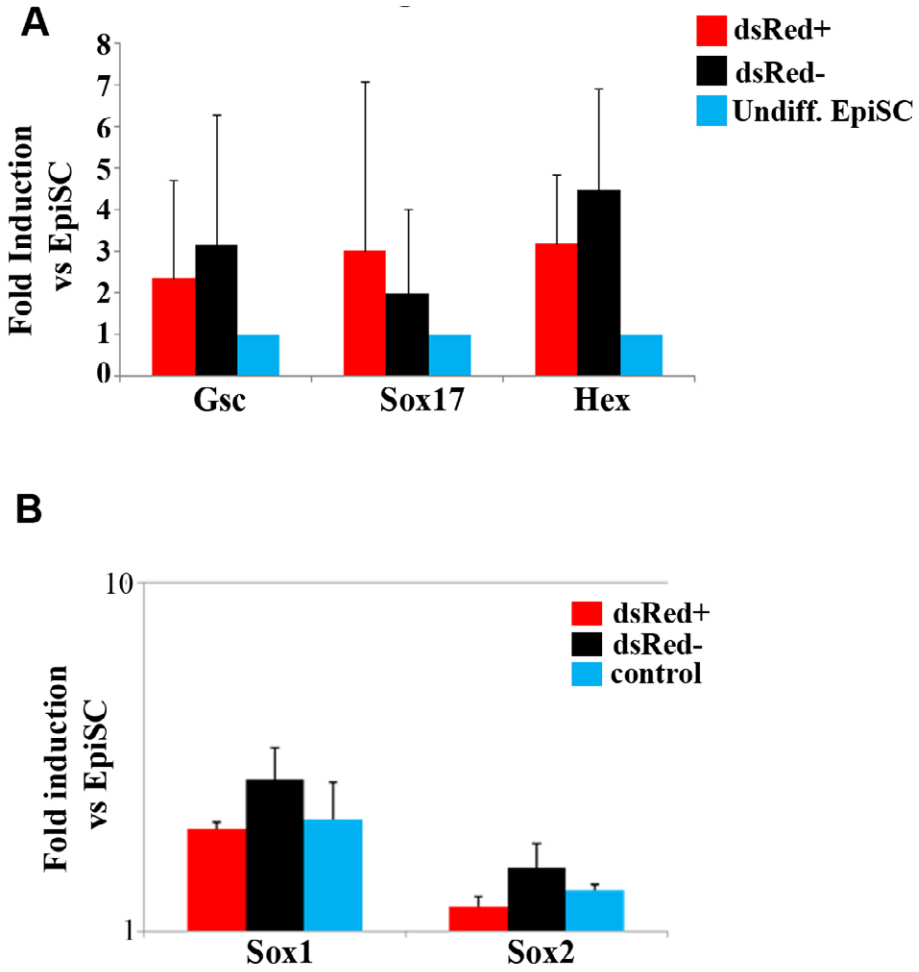

Figure S8. Neural and endoderm differentiation of PS-like EpiSCs. (**A**) qPCR expression analysis of indicated endodermal markers in day three EBs generated from sorted dsRed2^{+} and dsRed2^{-} EpiSC. Expression is represented relative to undifferentiated EpiSCs (set to one). Error bars represent s.d. (n=2). (**B**) qPCR analysis of expression of the neural specific markers Sox1 and Sox2 in sorted dsRed2 positive and negative cells plated for 48 hrs in N2B27 in the presence of the MEK/Erk and Activin/Nodal inhibitors PD03 and SB43. An unsorted control is also included. Expression is represented relative to undifferentiated EpiSCs. Error bars represent s.d. (n=2).

Table S1. List and analysis of differentially expressed genes following CHI treatment. (1) Reported in vivo expression patterns of most-induced genes following CHI treatment. The top 100 transcripts most significantly upregulated by CHI were examined for in vivo expression between E7.5-8.5 and published information could be obtained for 59 of them. Note that some genes are expressed in more than one tissue. (2) Reported links between the 100 most upregulated genes after CHIRON treatment with the main signalling pathways. Published information could be obtained for 52 of them. Note that some genes are linked to more than one signalling pathway. (3) GO term enrichment analysis of unique genes significantly upregulated in CHIRON-treated EpiSCs relative to untreated controls. (4) List of unique genes significantly upregulated in CHIRON-treated EpiSCs relative to untreated controls. (5) GO term enrichment analysis of unique genes significantly downregulated in CHIRON-treated EpiSCs relative to untreated controls. (6) List of unique genes significantly downregulated in CHIRON-treated EpiSCs relative to untreated controls.

Download Table S1

Table S2. Scoring of donor cell distribution in different tissues within host embryo sections

Embryo ID	Stage	Grafting Site	No. sections scored	NP	AXM	PXM/LM	EN	PS	AL	Non Integrated
dsRed-E1.1	M-LS	MP	22	0	0	8	14	6	0	1
dsRed-E1.2	M-LS	MP	13	4	0	8	2	0	0	5
dsRed-E9	M-LS	MP	20	0	0	16	0	11	0	3
dsRed-E10	M-LS	MP	30	0	1	16	2	13	0	8
dsRed+ No1	M-LS	MP	8	0	0	6	2	1	0	3
dsRed+ No5	M-LS	MP	4	0	1	3	0	0	0	0
dsRed+ E6	M-LS	MP	18	0	5	6	13	0	0	0
dsRed+ E4	M-LS	MP	39	0	2	13	24	0	0	0
UNSORTED C2 9.1	M-LS	MP	28	0	5	24	4	8	4	1
UNSORTED C2 10.2	M-LS	MP	40	0	1	20	17	15	2	0
UNSORTED C2 9.2	M-LS	MP	31	0	0	22	7	13	6	1
UNSORTED C2 10.1	M-LS	MP	27	0	2	21	8	2	9	4
dsRed-E4.2	M-LS	Ant	10	0	0	5	0	5	0	0
dsRed-E1.2	M-LS	Ant	15	0	5	12	0	0	0	0
dsRed-E2.2	M-LS	Ant	20	0	0	1	1	6	0	10
dsRed-E7	M-LS	Ant	42	38	4	10	5	3	0	9
dsRed-E8	M-LS	Ant	14	12	2	6	2	0	0	6
dsRed+ E3	M-LS	Ant	36	28	1	7	0	0	0	0
dsRed+ E5.1	M-LS	Ant	6	0	0	3	3	0	0	0

Embryo ID	Stage	Grafting Site	No. sections scored	NP	AXM	PXM/LM	EN	PS	AL	Non Integrated
dsRed+ E4.1	M-LS	Ant	35	0	4	12	22	1	2	9
dsRed+ E1.1	M-LS	Ant	9	2	0	5	7	0	0	0
UNSORTED C2 4.1	M-LS	Ant	37	11	12	10	22	3	0	5
UNSORTED C2 4.2	M-LS	Ant	36	16	6	13	0	0	0	0
+CHIR – W2E2	M-LS	Ant	39	0	0	31*	8	0	0	0
+CHIR – W2E1	M-LS	Ant	19	0	0	17*	7	0	0	0
+CHIR – W1E2	M-LS	Ant	29	0	0	30*	0	0	0	8
+CHIR – W2E2	M-LS	Ant	25	0	0	9*	17	0	0	10

*Includes ventral mesoderm.

NP, neural plate; AXM, axial mesoderm; PXM/LM, paraxial/lateral mesoderm; EN, endoderm; PS, primitive streak; AL, allantois; Non Integrated, unincorporated clump.