

Fig. S1 Immunofluorescence of WT and *Sall4* cKO embryos

(A) Confocal images of caudal part of E8.5 embryos stained on sections.

(B, C) Quantitation of SALL4+ cells (B) and cells with different combinations of T and SOX2

(C) in whole mount-stained embryos. n=5 for WT and *Sall4* cKO

(D) Confocal images of caudal part of E9.5 embryos stained on sections.

(E) Confocal images of caudal part of E10.5 embryos stained on sections.

Two right columns in A, D, E show closeup images of the boxed areas. Single channel images are shown in black/white. Merged images are shown with green (T) and magenta (SOX2).

(F) Quantitation of cells with different combinations of T and SOX2 in sections in the posterior.

n=5 for WT and n=4 for *Sall4* cKO

Scale bar = 100 μ m.

Fig. S2

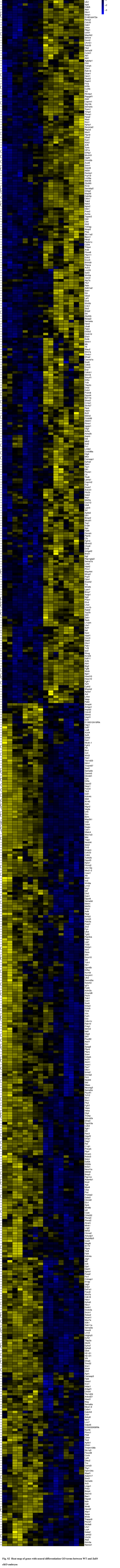


Fig. S2 Heat map of genes with neural differentiation GO terms between WT and *Sall4* cKO embryos

Heat map of genes with neural differentiation GO terms in WT vs *Sall4* mutants

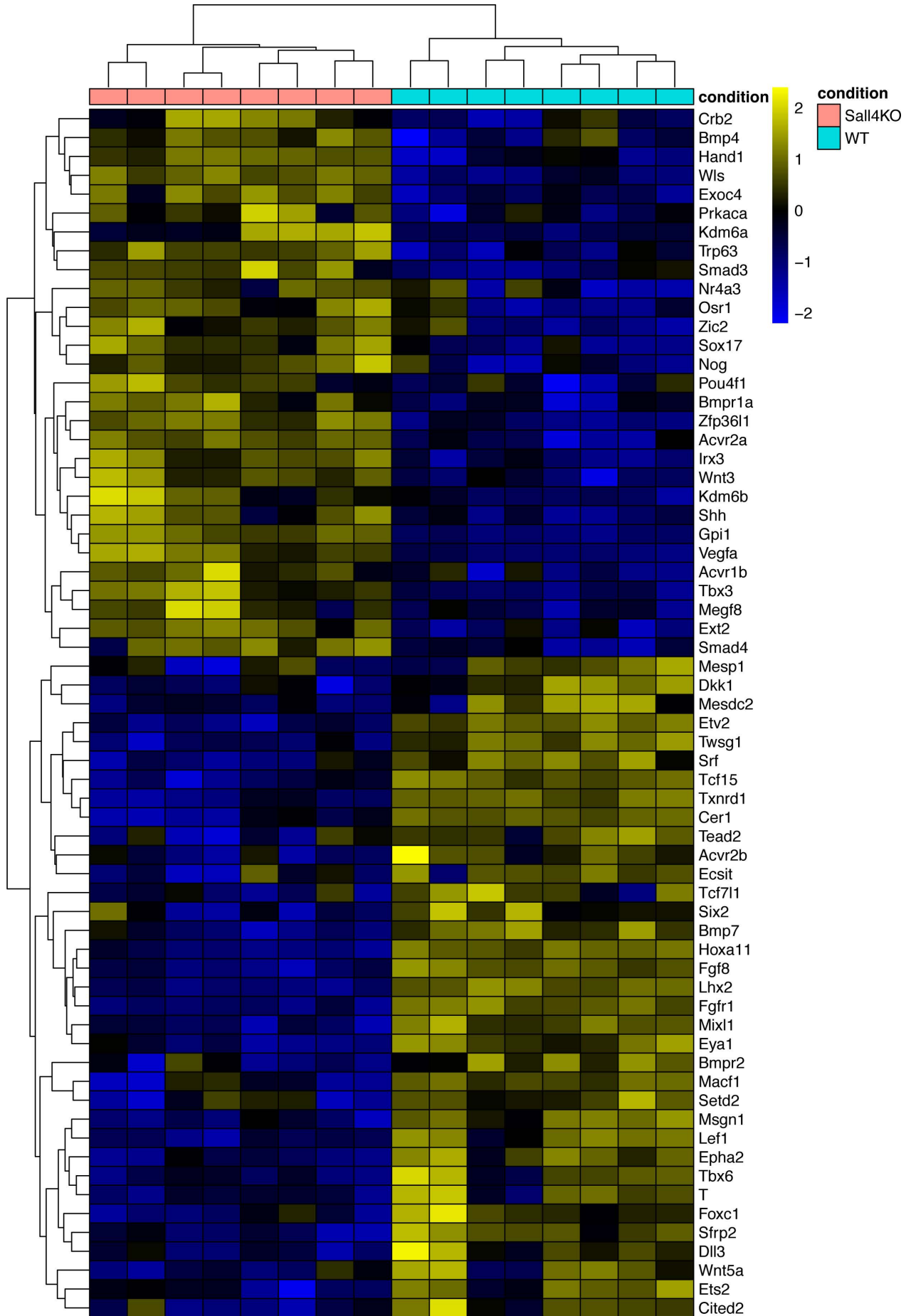


Fig. S3 Heat map of genes with mesoderm development GO terms between WT and *Sall4* cKO embryos

Heat map of genes with mesoderm development GO terms in WT vs *Sall4* mutants.

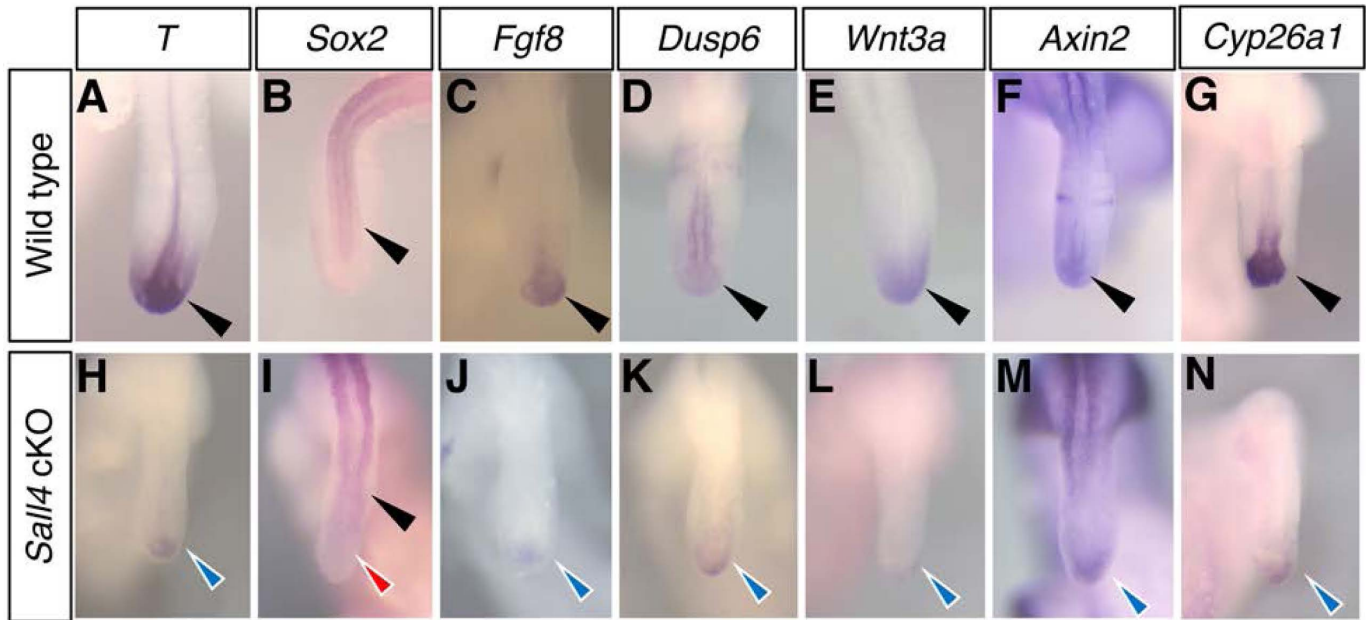


Fig. S4 Expression pattern of genes at E10.5 in WT and *Sall4* cKO embryos

Whole mount in situ hybridization of indicated genes in WT (A-G) and *Sall4* cKO (H-N) at E10.5. Black arrowheads point to normal expression in the posterior tip of the body in WT embryos (A-G) and *Sox2* expression in the *Sall4* cKO embryo (I). Blue arrowheads point to reduced expression in *Sall4* cKO embryos (H, J-N). Red arrowhead in I points to upregulated *Sox2* expression in the *Sall4* cKO embryo.

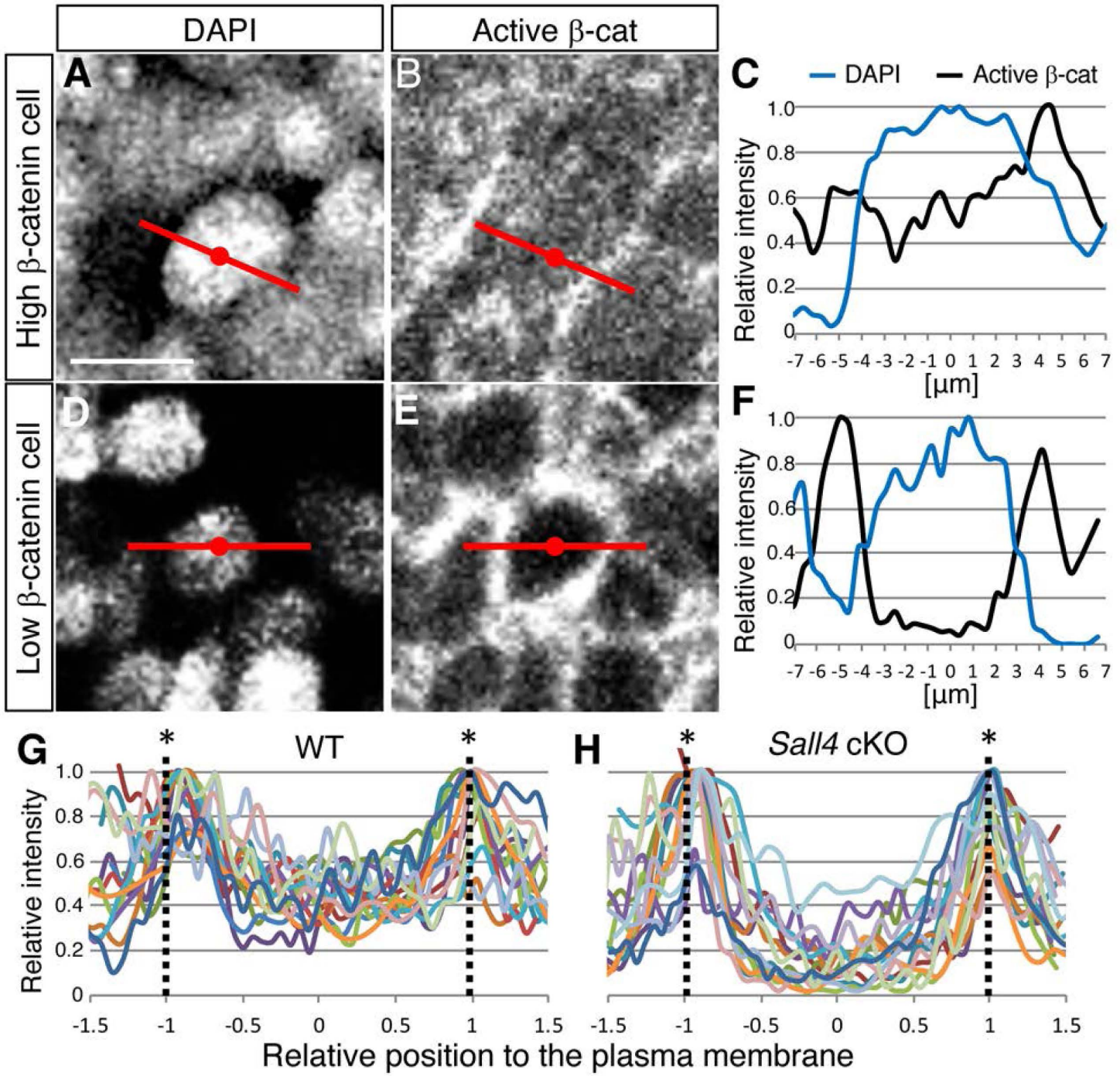


Fig. S5 Reduced levels of nuclear β -catenin in *Sall4* cKO embryos

(A-F) Typical single plane images of cells with high nuclear active β -catenin (A-C) and cells with low nuclear active β -catenin (D-F). Red dots positions at the center of the nucleus. Red lines indicate the line along which β -catenin signal intensity shown in C and F was measured. Scale bar = 10 μ m. In C and F, the highest β -catenin signal intensity at the plasma membrane is set as 1.0, and the highest nuclear DAPI signal in the nucleus is set as 1.0. Relative intensities of β -catenin and DAPI signals to the highest signal are shown. Scale bar = 10 μ m.

(G, H) Relative intensities of β -catenin signals of 15 cells randomly selected from the posterior of WT (G) and *Sall4* cKO (H) embryos. Because each cell size is different, the positions of plasma membrane of each cell are normalized to be -1 and 1 along the x axis, and the highest β -catenin signal intensity of each cell is normalized to be 1.0 along the y axis. WT cells exhibit higher levels of nuclear β -catenin than *Sall4* cKO cells.

The Posterior Tissue Motifs

Rank	Motif	Frequency	Position	% of Targets	% of Background	ENTPD5	Gene Match/Score
1	ATTTCGG	1e-121	2,485,402	34.33%	371.17%	142,869 (148,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
2	TCCCGGACAA	1e-98	2,485,402	32.26%	303.7%	126,292 (129,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
3	TACAGCG	1e-73	1,976,402	31.24%	377.14%	141,869 (151,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
4	CATCACTCCAC	1e-64	1,976,402	32.26%	303.7%	123,192 (127,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
5	TACAGCG	1e-62	1,976,402	32.26%	303.7%	126,292 (130,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
6	CAAAATTTCAT	1e-61	1,976,402	32.26%	303.7%	129,892 (134,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
7	CAATTCAT	1e-61	1,976,402	32.26%	303.7%	141,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
8	TTCATTCCAC	1e-57	1,976,402	32.26%	303.7%	128,292 (133,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
9	AAACCC	1e-76	1,976,402	35.28%	283.83%	141,869 (154,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
10	ACTTCTTC	1e-76	1,976,402	32.7%	303.7%	131,869 (146,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
11	CAATTCAT	1e-76	1,976,402	32.26%	303.7%	131,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
12	AAATTCAT	1e-74	1,976,402	32.7%	303.7%	131,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
13	TTCATTCC	1e-67	1,976,402	37.86%	31.78%	141,869 (157,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
14	TTCATTCC	1e-74	1,976,402	32.26%	303.7%	142,869 (157,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
15	TTCATTCC	1e-73	1,976,402	32.26%	303.7%	128,292 (143,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
16	TTCATTCC	1e-71	1,976,402	37.76%	34.2%	139,869 (158,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
17	GCTTACCAAT	1e-66	1,976,402	31.9%	303.7%	142,869 (160,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
18	ACACGACCAAT	1e-66	1,976,402	31.9%	303.7%	141,869 (160,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
19	ATTTTC	1e-64	4,984,402	32.26%	303.7%	142,869 (155,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
20	CTGTCC	1e-64	4,984,402	31.7%	303.7%	139,869 (158,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)

mESC Motifs

Rank	Motif	Frequency	Position	% of Targets	% of Background	ENTPD5	Gene Match/Score
1	TTCATTCCAA	1e-108	2,126,402	22.77%	3e-10%	136,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
2	TTACCTTCA	1e-102	2,126,402	24.23%	3e-10%	136,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
3	ACAAAGCG	1e-102	2,126,402	24.11%	3e-10%	133,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
4	CATCAACAC	1e-110	2,126,402	30.9%	3e-10%	86,786 (97,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
5	CATTCAAAATCC	1e-109	2,126,402	27%	3e-10%	87,286 (98,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
6	CTATTGGTTC	1e-108	2,126,402	24%	3e-10%	86,786 (97,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
7	GGGATCTGTG	1e-102	2,126,402	33.7%	3e-10%	136,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
8	TTCTTCAAAATCC	1e-102	2,126,402	24%	3e-10%	86,786 (97,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
9	ATCTTCACT	1e-110	2,126,402	32.2%	3e-10%	102,869 (118,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
10	AACTGATG	1e-110	2,126,402	32%	3e-10%	87,286 (98,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
11	CCTCAAAATCC	1e-106	2,126,402	30.9%	3e-10%	86,286 (97,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
12	AACTGATG	1e-110	2,126,402	32%	3e-10%	87,286 (98,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
13	CTTTTCCAAAT	1e-110	2,126,402	32%	3e-10%	71,286 (82,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
14	AAACTTTTAT	1e-106	2,126,402	33.9%	3e-10%	149,869 (170,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
15	ACAAACCCAC	1e-110	2,126,402	32%	3e-10%	87,286 (98,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
16	AACTGATG	1e-110	2,126,402	32%	3e-10%	87,286 (98,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
17	TTCTCTTTC	1e-114	2,126,402	30.9%	3e-10%	102,869 (118,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
18	CCAAACAAAT	1e-113	2,126,402	32.7%	3e-10%	136,869 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
19	CTCTCAAAAT	1e-113	2,126,402	30.9%	3e-10%	146,869 (167,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
20	ATCTCTTTC	1e-112	2,126,402	32%	3e-10%	86,786 (97,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)

Motifs in both the posterior tissue and mESC

Rank	Motif	Frequency	Position	% of Targets	% of Background	ENTPD5	Gene Match/Score
1	CAGCGGGATG	1e-19	4,484,402	3.52%	3e-05%	145,786 (147,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
2	CCGAAAAA	1e-19	4,484,402	20.7%	3e-05%	146,786 (177,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
3	AGTATTTCG	1e-17	4,176,402	1.04%	3e-05%	138,786 (172,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
4	ATTCAT	1e-14	3,324,402	7.26%	3e-05%	139,286 (161,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
5	CCATCCGA	1e-12	2,834,402	9.17%	3e-05%	145,786 (178,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
6	CACTCAAGCA	1e-11	2,732,402	9.1%	3e-05%	138,786 (177,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
7	CTCAGTCA	1e-11	2,674,402	2.35%	3e-05%	143,786 (158,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
8	AACCTCTG	1e-11	2,424,402	3.49%	3e-05%	140,786 (154,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
9	ATTCAGC	1e-9	2,274,402	7.44%	3e-05%	140,786 (158,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
10	CTCAGTCA	1e-9	2,126,402	1.04%	3e-05%	138,786 (162,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
11	TTCTCTG	1e-8	2,026,402	1.87%	3e-05%	144,786 (158,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
12	CTCAGTCA	1e-8	2,014,402	14.9%	3e-05%	142,786 (162,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
13	CTCAGTCA	1e-8	1,976,402	3.4%	3e-05%	122,786 (136,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
14	ATTCCTCA	1e-8	1,984,402	8.5%	3e-05%	142,786 (162,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
15	ATTCCTCA	1e-7	1,884,402	3.2%	3e-05%	143,786 (146,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
16	CTCAGTCA	1e-7	1,876,402	3.2%	3e-05%	140,786 (161,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
17	CTCAGTCA	1e-7	1,814,402	3.2%	3e-05%	141,786 (151,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
18	CTCAGTCA	1e-6	1,264,402	31.02%	3e-05%	144,786 (167,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)
19	CTCAGTCA	1e-6	1,174,402	8.7%	3e-05%	131,786 (142,786)	ENTPD5 (ENTPD5) ENTPD5 (ENTPD5) ENTPD5 (ENTPD5)

Fig. S6 Transcription factor motifs in the SALL4 enriched sequences

Top 20 de-novo motifs found in SALL4-enriched sequences in the posterior tissue, mESCs, and peaks in both cell/tissue types by Homer analysis.

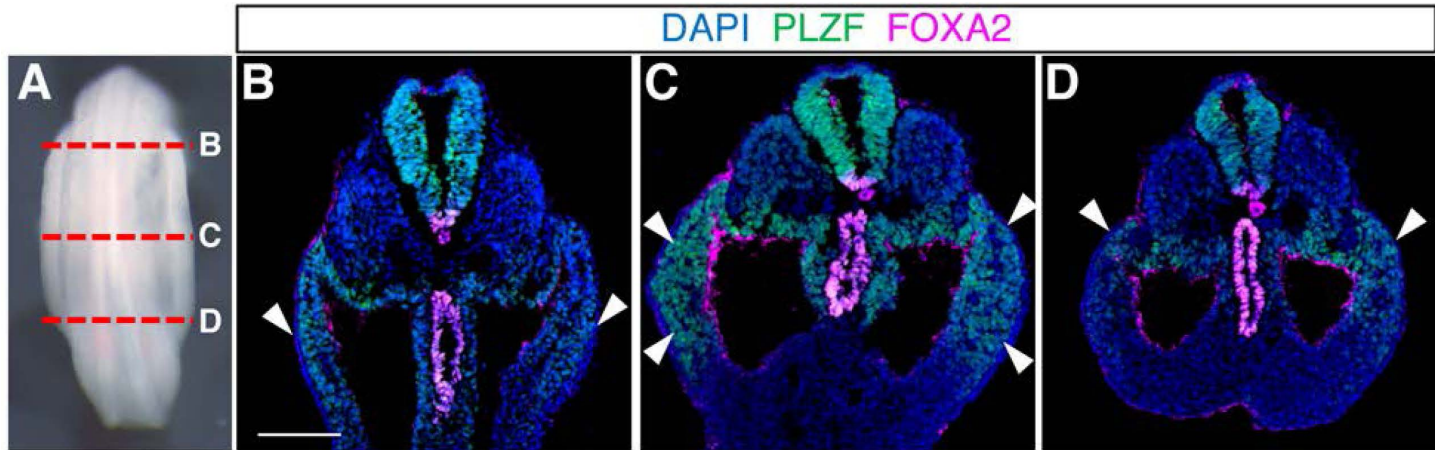


Fig. S7 Examples of PLZF immunofluorescence of E9.75 embryos at the hindlimb level

(A) Dorsal view of an E9.75 embryo at the hindlimb level.

(B-D) Immunofluorescence of PLZF (green) and FOXA2 (magenta) at the anterior (B), middle (C) and posterior (D) hindlimb levels. Arrowheads point to PLZF expression in the hindlimb mesenchyme. Scale bar = 100 μ m.

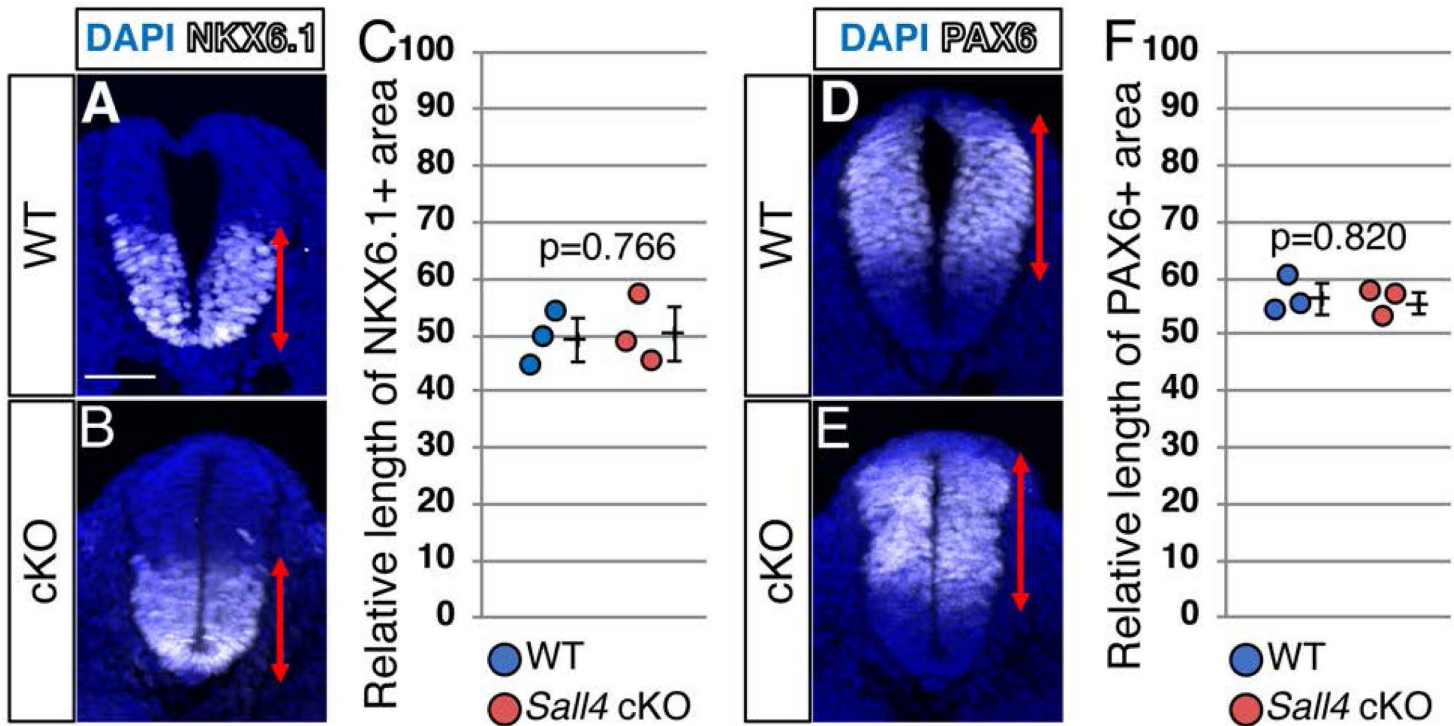


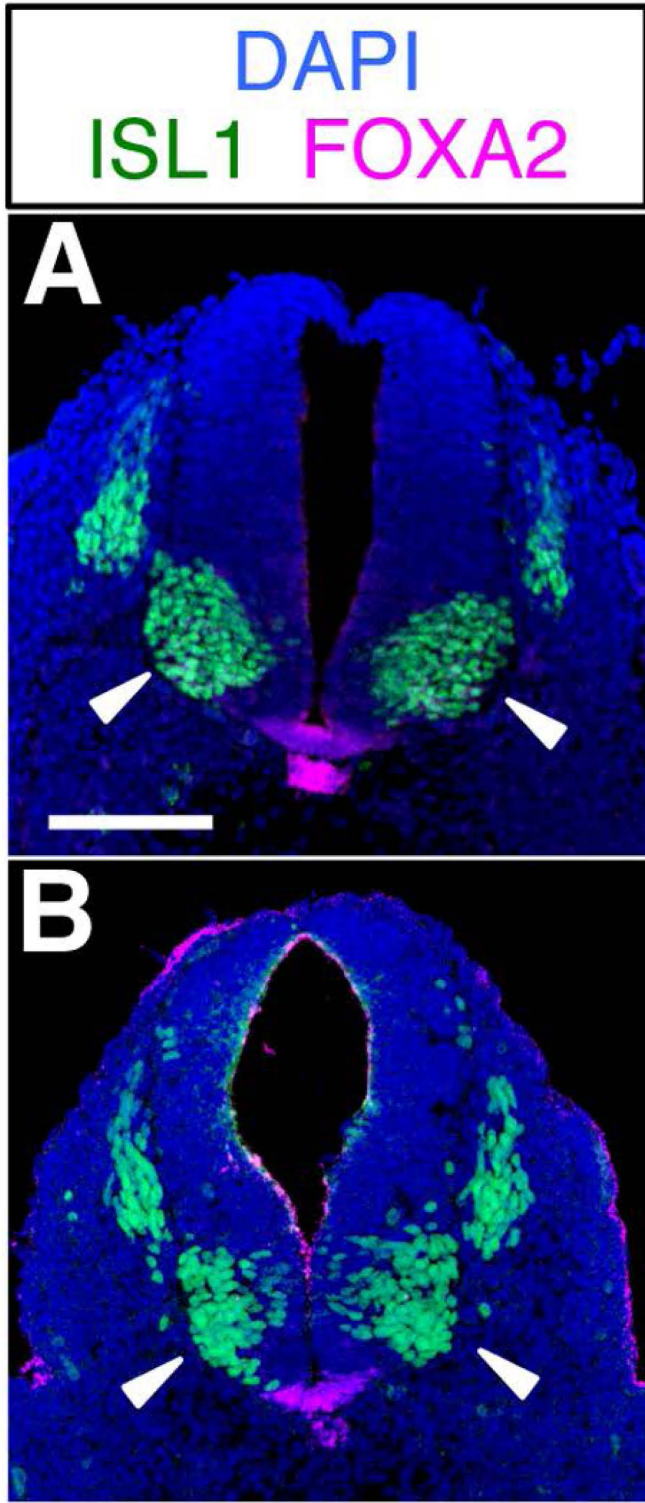
Fig. S8 NKX6.1 and PAX6 expression in the neural tube

(A, B) Immunofluorescence of NKX6.1 in WT (A) and *Sall4* cKO (B) embryos at the posterior hindlimb level at E10.25 (33/34 somite stage). Red double arrowhead line shows the length of the NKX6.1 expressing domain along the dorsal-ventral axis of the neural tube.

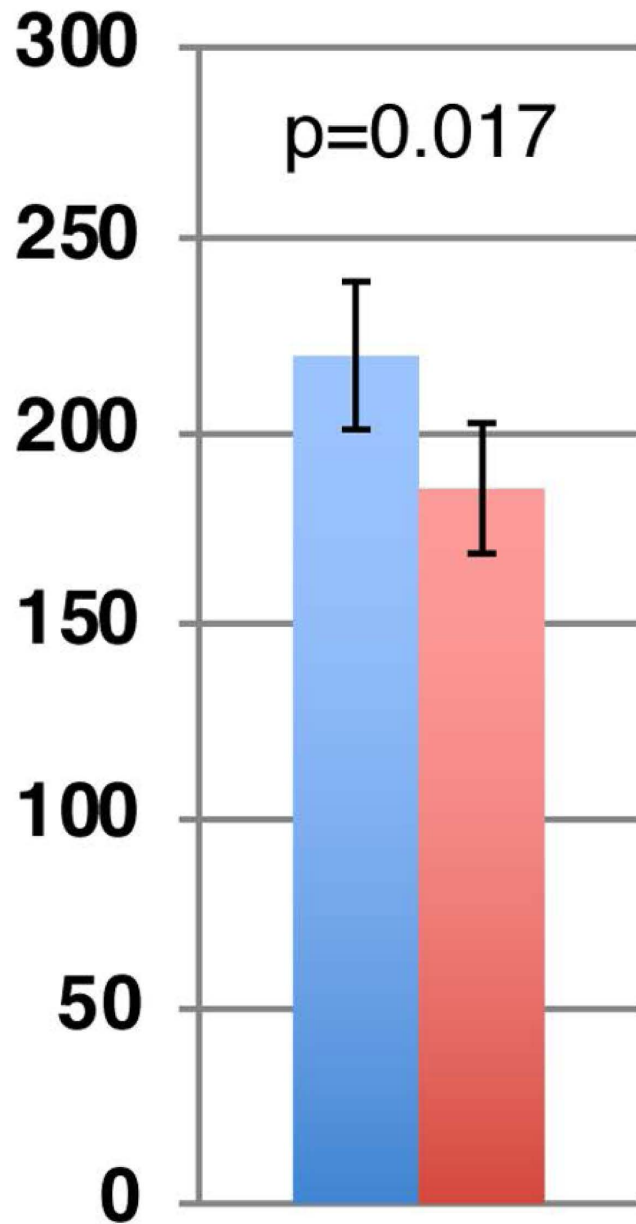
(C) Quantification of the length of the NKX6.1 expressing domain along the dorsal-ventral axis of the neural tube. P values by unpaired *t*-test are shown within the panel. $n=3$ for both WT and *Sall4* cKO.

(D, E) Immunofluorescence of PAX6 in WT (D) and *Sall4* cKO (E) embryos at the posterior hindlimb level at E10.25 (33/34 somite stage). Red double arrowhead line shows the length of the PAX6 expressing domain along the dorsal-ventral axis of the neural tube.

(F) Quantification of the length of the NKX6.1 expressing domain along the dorsal-ventral axis of the neural tube. P values by unpaired *t*-test are shown within the panel. $n=3$ for both WT and *Sall4* cKO.



C ISL1⁺ cell number



E10.25 FL

Fig. S9 Immunofluorescence analysis of neural tube in WT and *Sall4* cKO embryos at the forelimb bud level

(A, B) Immunofluorescence of ISL1 (green) and FOXA2 (magenta) in WT (A) and *Sall4* cKO (B) embryos at the forelimb level at E10.25. White arrowheads point to ISL1 positive cells. Scale bar = 100 μ m.

(C) Quantification of ISL1 positive cells at the forelimb level at E10.25. p value is shown within the panel. n=5 for both WT and *Sall4* cKO.

Table S1. Statistical examination of differences of cell type populations between WT and *Sall4* cKO posterior tissue

Tested hypothesis: T+/SOX2+ ratio is different between WT and *Sall4* cKO

Stage	P value
E8.5	< 2.2e-16
E9.5	6.139e-15
E10.5	< 2.2e-16

Two proportion Z test was used to examine differences.

At E8.5 and E9.5, whole mount stained embryos were analyzed. At E8.5, n=5 (WT and *Sall4* cKO). At E9.5, n=4 (WT) and n=3 (*Sall4* cKO).

At E10.5, n=5 (WT) and n=4 (*Sall4* cKO). A section that includes the chord-neural hinge region was analyzed from each embryo.

Table S2: SALL4-enrichment near the genes known to participate in NMP renewal

SALL4-enrichment near *Sox2*, *T*, *Cdx2*, *Fgf8*, *Wnt3a*, *Cyp26a1* and genes belonging to FGF signaling (GO:0008543) and WNT signaling (GO:0016055) are shown.

[Click here to download Table S2](#)

Table S3. List of antibodies and their dilution conditions for immunofluorescence

Primary antibodies

Active- β -catenin: Millipore/Upstate, #05-665, #2377065, mouse, 1/250
active caspase 3: BD Bioscience, 559565, rabbit, 1/200
Brachyury: Santa Cruz Biotechnology, sc-17743, goat, 1/100
DBX1: Vue TY, *et al.* (2007), rabbit, 1/100
FOXA2: Santa Cruz Biotechnology, sc-6554, goat, 1/200
ISL1: Developmental Studies Hybridoma Bank, 39.4D5-c, mouse, 4 μ g/ml
LEF1: Cell Signaling Technology, 2230P, C12AS, rabbit, 1/100
NKX2.2: Abcam, ab191077, rabbit, 1/1000
NKX6.1: R&D Systems, AF5858, goat, 15 μ g/ml
OLIG2: R&D Systems, AF2418, goat, 1/200
phospho Histone H3: Millipore, 06-570, rabbit, 1/100
PAX6: BD Bioscience, 561462, mouse, 1/500
PLZF: Santa Cruz Biotechnology, sc-22839, rabbit, 1/100
SALL4: Santa Cruz Biotechnology, sc-101147 (EE30), mouse, 1/300
SHH: R&D Systems, AF445, goat, 1/20
SOX1: R&D Systems, AF3361, goat, 1/100
SOX2: Millipore, AB5603, rabbit, 1/500
SOX2: R&D, MAB2018, mouse, 1/400

Secondary antibodies

Donkey anti-Mouse IgG Alexa 488, Invitrogen, A-21202, 1/500
Donkey anti-Rabbit IgG Alexa 647, Invitrogen, A-31573, 1/500
Donkey anti-Goat IgG Alexa 488, Invitrogen, A-11055, 1/500
Donkey anti-Goat IgG Alexa 594, Invitrogen, A-11058, 1/500
Goat anti-Mouse IgG Alexa 488, Invitrogen, A-11001, 1/500
Goat anti mouse IgG Alexa 594 Invitrogen, A-11005, 1/500
Goat anti-Rabbit IgG Alexa 488, Invitrogen, A-11008, 1/500
Goat anti-Rabbit IgG Alexa 594, Invitrogen, A-11012, 1/500
Goat anti-rabbit IgG Alexa 555, Invitrogen, A-21428, 1/500

Vue TY, *et al.* (2007) Characterization of progenitor domains in the developing mouse thalamus. *J Comp Neurol* 505(1):73-91.