

Fig. S1. SALL4 immunofluorescence on WT and Sall4 cKO embryos

Confocal images after SALL4 immunofluorescence on cross sections of the posterior trunk of WT (A-B', n=17) and *Sall4* cKO (C-D', n=23) embryos at E9.5. DAPI signals (A, C) and SALL4 signals (B, B', D, D') are shown in a black/white mode. B' and D' show the boxed areas in B and D, respectively. With the settings to detect residual SALL4 immunoreactivity in *Sall4* cKO sections, the SALL4 signals in the WT embryos were essentially saturated. nt: neural tube, pm: paraxial mesoderm. Scale bar, 100  $\mu$ m.





SALL4 ChIP-seq tracks of *Mesp2* (A), *Ripply2* (B), *Epha4* (C), *Efnb2* (D), *Notch2* (E), *Dll1* (F), *Tcf15* (G), *Meox1* (H) and *Aldh1a2* (I). Black bars under the track indicate statistically significant SALL4 enrichment.



**Fig. S3. Gene expression in E8.5 trunk between WT and** *Sall4* **cKO** Relative expression of *Sall4* (A), *Mesp2* (B), *Ripply2* (C), *Epha4* (D) and *Efnb2* (F) in the trunk of E8.5 WT and *Sall4* cKO embryos.

Each dot represents an embryo. Eight to ten somite stage embryos were used. P values by unpaired t-test are shown in each panel.





Dorsal viewed images were imported into FIJI, and the width of the most posterior expression domain was measured. Each dot represents a somite, and p values by unpaired t-test are shown in each panel.



## Fig. S5. Analysis of gene expression involved in trunk-tail transition

(A-F") Dorsal views of the posterior part of WT and *Sall4* cKO embryos hybridized with *Gdf11* (A, A', C, C'), *Lin28a* (B, B', D, D'), *Hoxb13* (E, E') and *Hoxc13* (F, F') probes. E9.5 (A-B') and E10.5 (C-F') embryos are shown. Black arrows and blue arrows point to normal and reduced expression, respectively. Asterisks indicate lack of expression.

(A"-F") Relative expression levels of *Gdf11* (A", C"), *Lin28a* (B", D"), *Hoxb13* (E") and *Hoxc13* (F") at E9.5 (A", B") and E10.5 (C"-F") are shown. Each dot represents an embryo, and p values by unpaired t-test are shown in each panel.



**Fig. S6. Expression of** *Axin2* and *Lef1* in the E10.5 tail culture experiments Relative expression levels of *Axin2* (A) and *Lef1* (B) after culturing the tail for indicated times (2, 4, or 6 hours) with CHIR, compared to DMSO.

Each dot represents a tail, and p values by unpaired t-test are shown in each panel.



## Fig. S7. Heatmap of ATAC-seq data after TOBAIS analysis

Heat map of the CTCF motifs in the *Sall4* cHet posterior trunk, *Sall4* cKO posterior trunk, WT mouse embryonic stem cells in two independent studies (mESC #1 and #2) and WT mouse embryonic fibroblasts (MEF). The x-axis corresponds to  $\pm$  100 base pairs from the center of accessible regions (shown as 0). The *Sall4* cHet and *Sall4* cKO heatmaps are the same as Fig. 6C.

Table S1. Genomic regions with enriched SALL4 binding in the posterior trunk mesoderm

Available for download at https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.202649#supplementary-data

Table S2. Regions with enriched SALL4 binding in and around select genes related to this study

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 Table S3. Differentially accessible regions between Sall4 cHet and Sall4 cKO posterior trunk

 mesoderm

Available for download at https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.202649#supplementary-data

**Table S4.** Results of gene ontology analysis of genes nearest to differentially accessible regionsbetween Sall4 cHet and Sall4 cKO posterior trunk mesoderm

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**Table S5.** Results of gene ontology analysis of differentially expressed genes between WT andSall4 cKO posterior trunk tissue

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**Table S6.** CTCF occupancy scores of CTCF binding motifs in *Sall4* cKO and *Sall4* cHet samples

 from footprinting analysis of ATAC-seq data

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**Table S7.** Results of gene ontology analysis of genes closest to preferentially occupied CTCF sitesin Sall4 cHet relative to Sall4 cKO posterior trunk mesoderm

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Table S8. Primers used for qPCR

Gene	Reference	Primers (5'->3')
	sequence	
Actb	NM_007393	TGTTACCAACTGGGACGACA
		CTGGGTCATCTTTTCACGGT
Axin2	NM_015732	TGACTCTCCTTCCAGATCCCA
		TGCCCACACTAGGCTGACA
Efnb2	NM_010111	ATTATTTGCCCCAAAGTGGACTC
-		GCAGCGGGGTATTCTCCTTC
Epha4	NM_007936	TGGAATTTGCGACGCTGTCA
		CACTTCCTCCCACCCTCCTT
Gdf11	NM_010272	CCGGCGTCACATCCGTATC
		ACTTGCTTGAAGTCGATGCTC
Hoxb13	NM_008267	TCTTGCCGAGTATCCAGGAG
		GGAGTTTCCGAAGGGTAGGTAG
Hoxc13	NM_010464	GCCGTCTACACGGACATCC
		CCCCAAATGGGTAACCATAGC
Lefl	NM_010703	GCCACCGATGAGATGATCCC
		TTGATGTCGGCTAAGTCGCC
Lin28a	NM_145833	TGGTGTGTTCTGTATTGGGAGT
		AGTTGTAGCACCTGTCTCCTTT
Mesp2	NM_008589	CGGCGTTCTCTCACCGATG
		CACCCCACTACTCATGGCTG
Msgnl	NM_019544	CTTCTGACACCGCTGGTCTG
		GTGACTGCCGTAGCCATCG
Ripply2	NM 001379243	CCTGGAGAGAAAGAGAAGCG
	—	ATAGCCTGACAGGGTGTTGG
Tbx6	NM 011538	ATGTACCATCCACGAGAGTTGT
	—	GGTAGCGGTAACCCTCTGTC
Tcf15	NM 009328	GTGTAAGGACCGGAGGACAA
J	—	GATGGCTAGATGGGTCCTTG