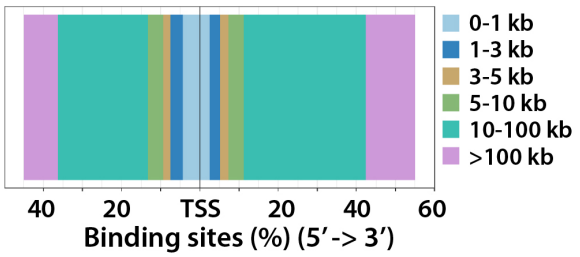
Figure S1 Comparison of heat shock transgenic lines. Refers to Figure 1.

Embryos were heat shocked at the 15s stage for 30 mins at 40°C and then left to develop at the standard temperature of 29°C. Whereas wildtype siblings show no phenotypic effects due to the heat shock, both transgenic lines produced the same posterior defects as described earlier (Ye and Kimelman, 2020), demonstrating that the FLAG tag does not alter Hoxa13b activity. Note that for both transgenic lines the fluorescent protein is produced separately from the Hoxa13b due to the 2A peptide that separates the two proteins.

A



B

		Rate (%)	E-value	Known motifs
MEME	1	78.8	1.2e-381	cad (MA0216.2), HOXC13 (MA0907.1), HOXD13 (MA0909.1)
	2	5.9	1.3e-030	BPC1 (MA1404.1), BPC6 (MA1402.1), RAMOSA1 (MA1416.1)
	3	11.9	1.8e-022	MSC (MA0665.1), Tcf12 (MA0521.1), hlh-1 (MA0545.1)
DREME	1	70.4	2.3e-225	HOXC10 (MA0905.1), Abd-B (MA0165.1), HOXD11 (MA0908.1)
	2	36.6	5.6e-083	HOXB13 (MA0901.1), HOXA13 (MA0650.1), HOXD13 (MA0909.1)
	3	20.8	2.8e-021	No

Figure S2 Hoxa13b binding targets genomic location and motif discovery. Refers to Figure 3.

A) Distribution of Hoxa13b targets according to their distance to the transcription start site (TSS) of the nearest gene. B) Top 3 motifs discovered by MEME and DREME. Rate shows the percentage of peaks that contributed to each motif. The 2nd ranked motif for MEME is composed of plant proteins, and the 3rd ranked motif has proteins are not expressed in the zebrafish tailbud (MSC), expressed at an extremely low level (Tcf12) or not homologous to any zebrafish protein (Hlh-1). Note that the DREME motif 2 is also partially included in motif 1, which is why the combined rate exceeds 100%. The first motif from MEME and the first and second motifs from DREME belong to the same cluster that has high similarity to known posterior Hox motifs.

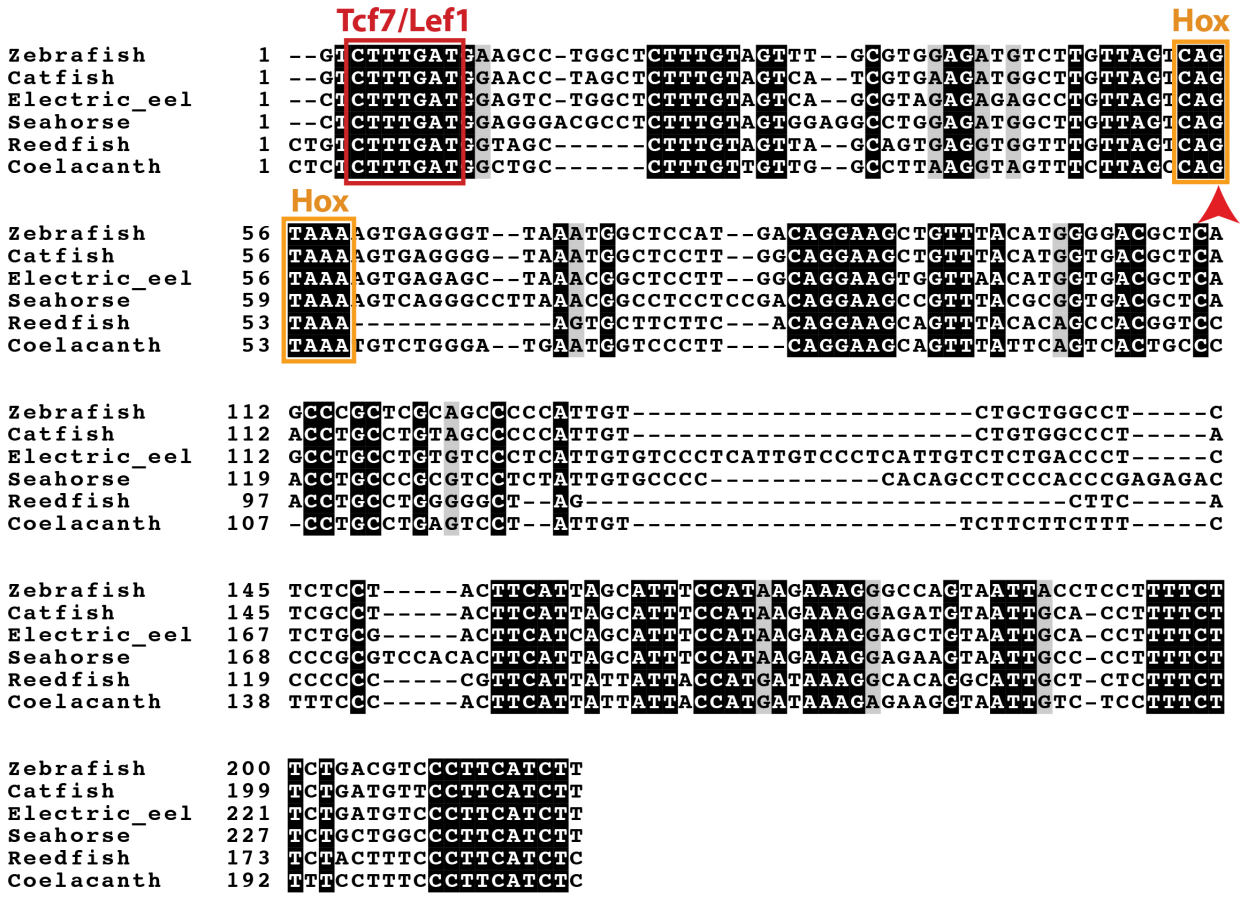


Figure S3 Hox element 2. Refers to Figure 4

The alignment of Hox element 2 in a variety of species is shown, with consensus sites for Hox and Tcf7/Lef1 binding shown. The Hox site has a C at the nucleotide position indicative of posterior Hox binding (arrowhead).

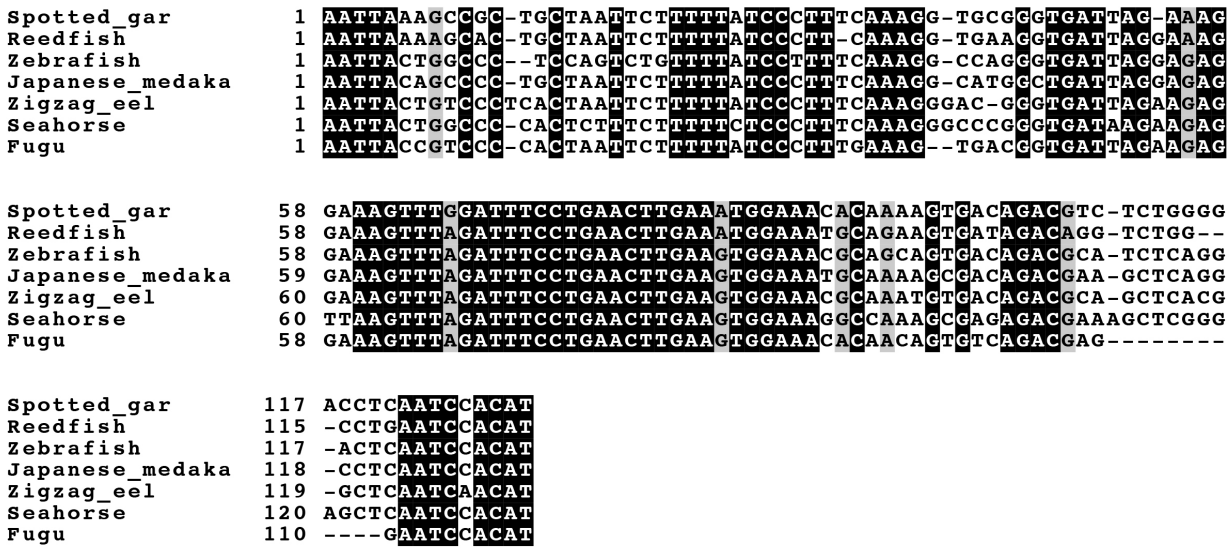


Figure S4 -5.4 kb element. Refers to Figure 5

The alignment of the conserved element located at approximately -5.4 kb in zebrafish relative to the same sequence in a variety of species is shown.

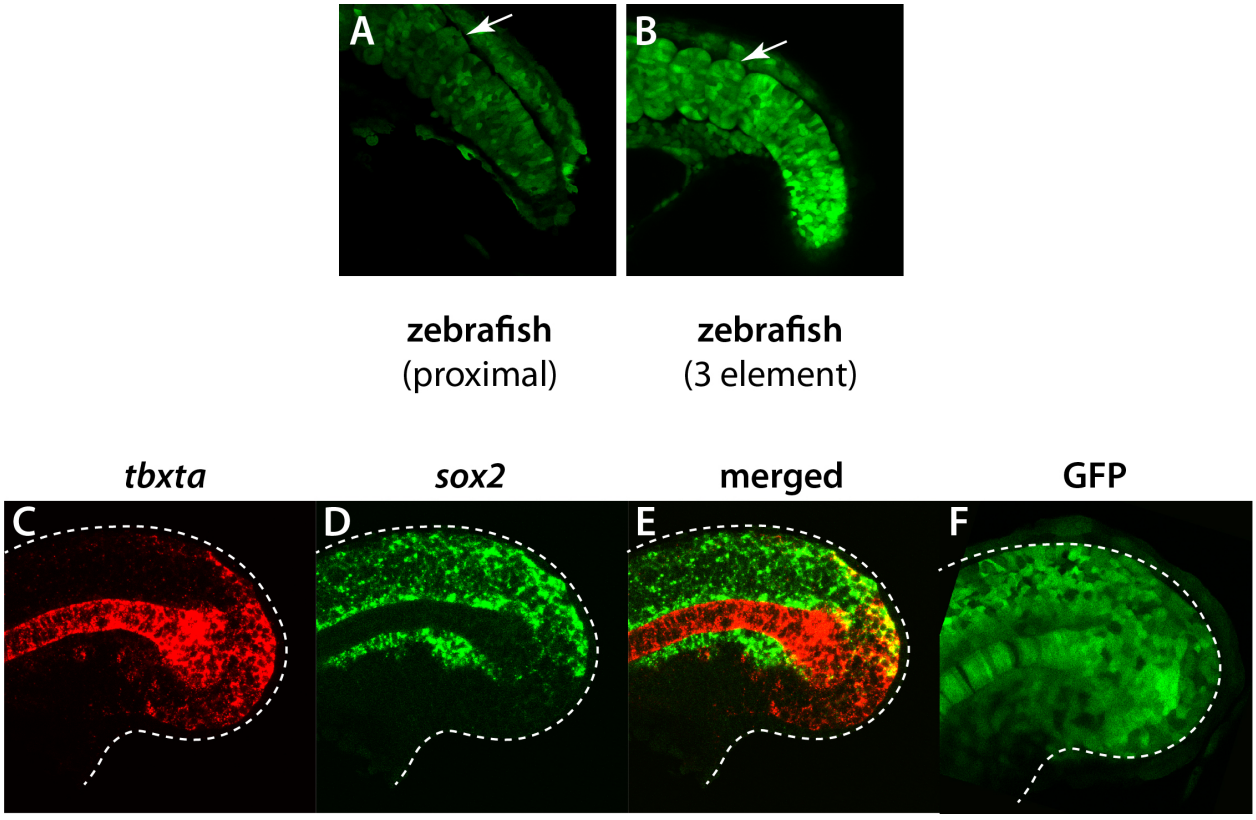


Figure S5 Hoxa13b binding elements regulate expression in the posterior somites and NMps. Refers to Figure 5.

A, B) GFP expression in the posterior somites of a 20s embryo from transgenic lines containing just the proximal *tbxta* promoter (A) or the proximal *tbxta* promoter with the three upstream elements (B). Note the strong somite GFP expression in panel B compared to A (arrows). (C-F) Comparison of GFP expression (F) from the transgenic line containing the *tbxta* promoter with the three elements to fluorescent in situ hybridization for *tbxta* (C) and *sox2* (D). The NMps are yellow in the merged panel (E).

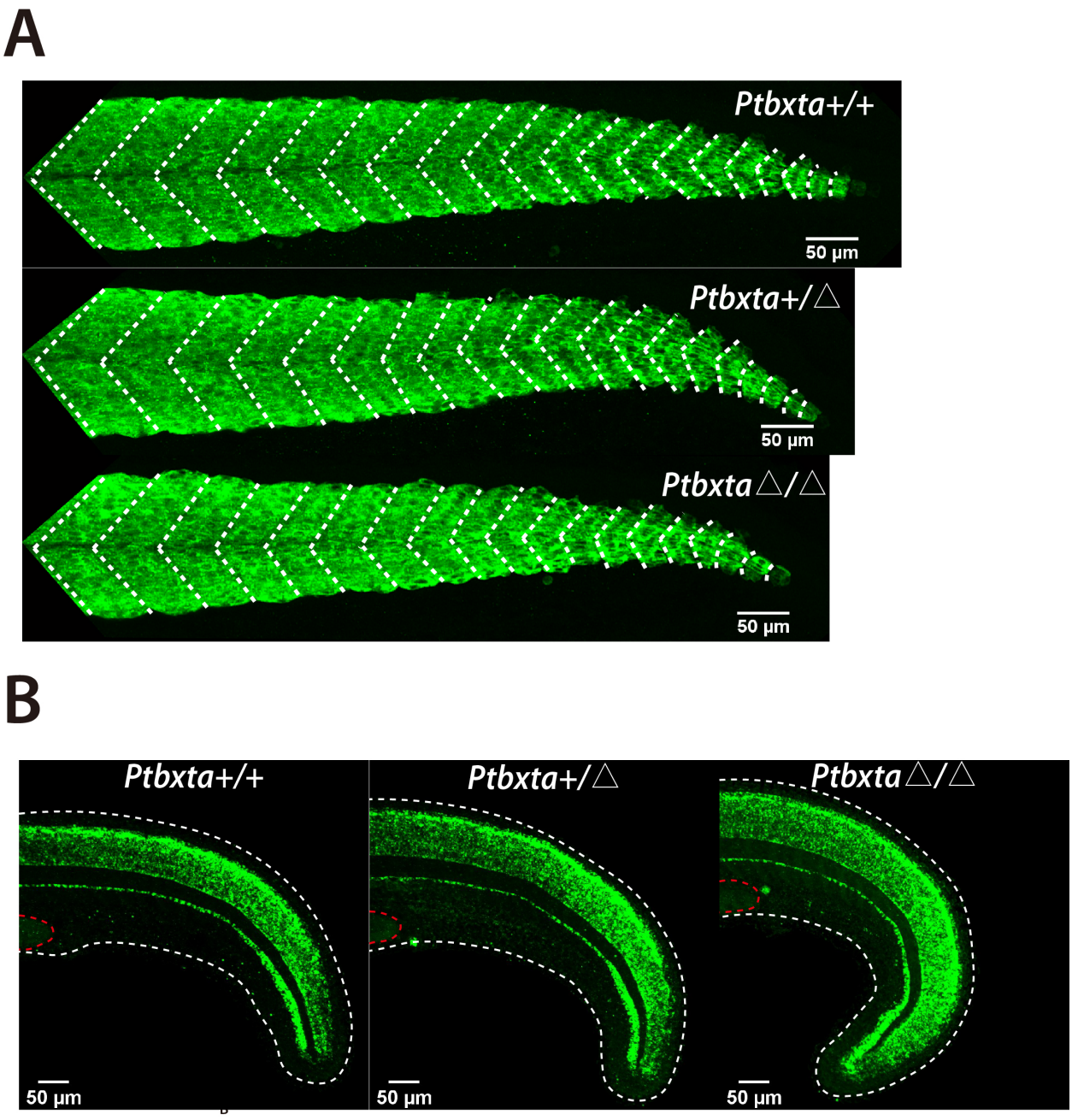


Figure S6 Hox element 1 deletion embryos have reduced posterior somites and an enlarged posterior neural tube. Refers to Figure 6.

A) Homozygous embryos show defects in the formation of posterior somites as shown by staining with the muscle antibody MF20 (for quantification of volume of the posterior somites see Figure 6H). Shown are snapshots of the 3D reconstructions in a lateral view, with dotted lines showing the somite borders. B) Homozygous embryos show an enlarged neural tube as

shown by *sox2* FISH. (for quantification of volume of the posterior neural tube see Figure 6I). Shown are snapshots of the 3D reconstructions in a lateral view, with white dotted lines showing the body border and red dotted lines showing the yolk protrusion.

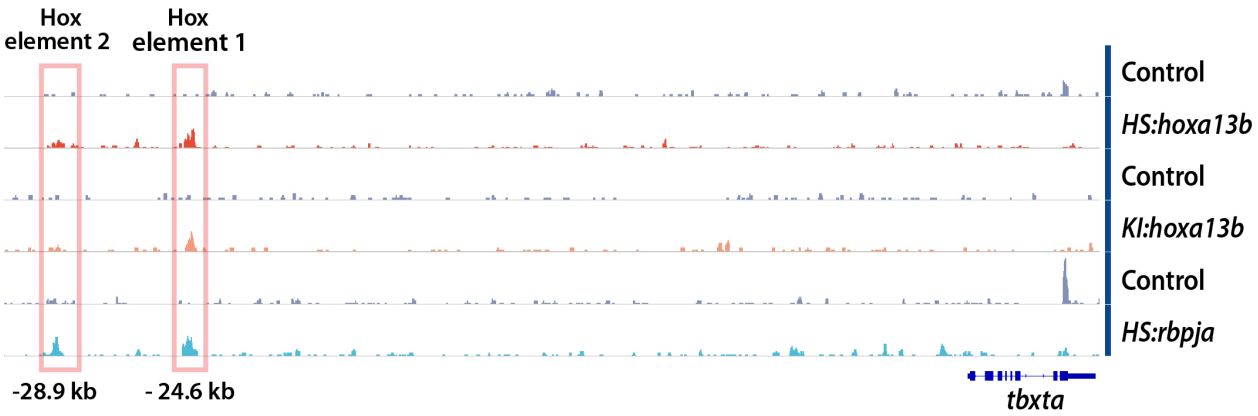


Figure S7 Rbpja binds to Hox element 1 and Hox element 2. Refers to Figure 7.

Rbpja CUT&RUN Peaks were found at Hox element 1 and element 2 sites, which also have the H3K4me1 and H3K27ac histone marks, as shown in Figure 4.

Table S1 H3K4me1 peak annotation.
Peaks were annotated to the nearest gene based on the org.Dr.eg.db annotation database.

Click here to download Table S1

Table S2 H3K27ac peak annotation.
Peaks were annotated to the nearest gene based on the org.Dr.eg.db annotation database.

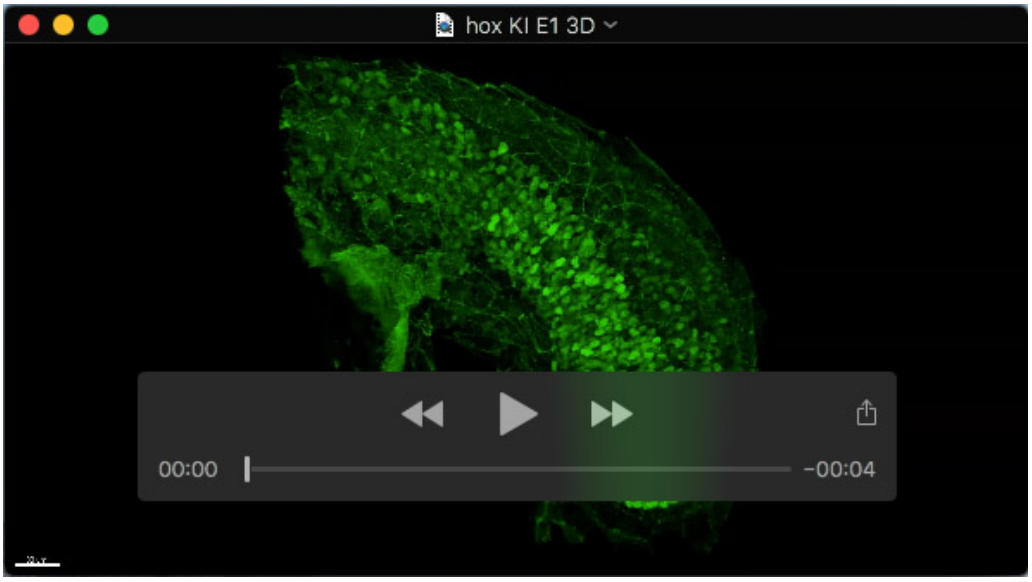
Click here to download Table S2

Table S3 Hoxa13b targets and annotation.
Shown are the CUT&RUN peaks from the *HS:hoxa13b-FLAG-GFP* line, with overlapping peaks from the *KI:hoxa13b-FLAG-GFP* line shown. Relevant Histone modification data from Tables S1 and S2 are included. Peaks were annotated to the nearest gene based on the org.Dr.eg.db annotation database.

Click here to download Table S3

Table S4 Rbpja targets and annotation.
Peaks were annotated to the nearest gene based on the org.Dr.eg.db annotation database.

Click here to download Table S4



Movie 1