

Figure S1

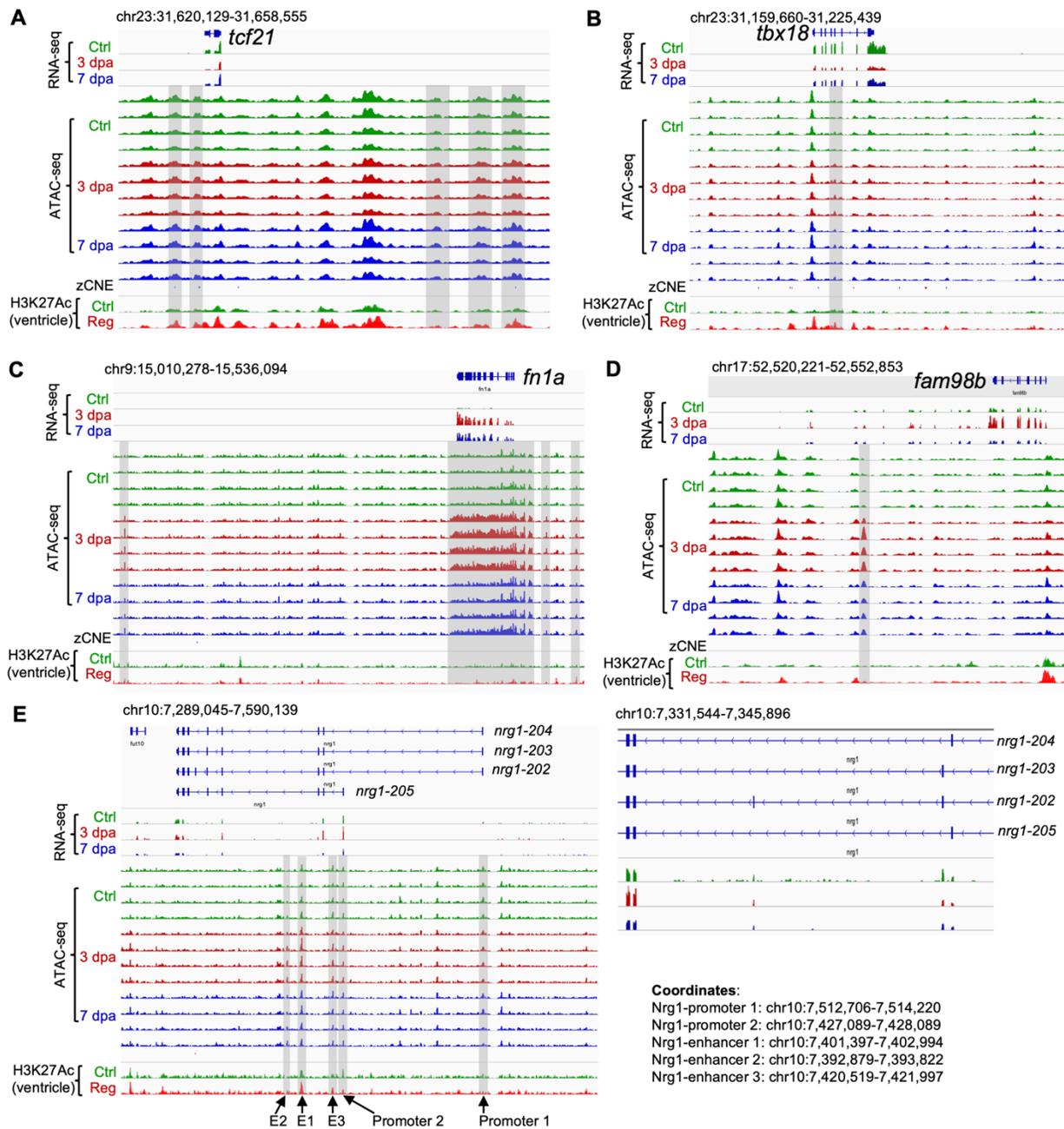


Fig. S1. Browser tracks of the genomic regions comprising *tcf21*, *tbx18*, *fn1a*, *fam98b*, or *nrg1*. Chromatin accessibility profiles in the epicardium, the zCNE sites, and the whole-ventricle H3K27Ac profile are shown. Selected putative enhancer regions or promoters are highlighted in gray boxes. Four *nrg1* isoforms are shown. Two promoters and three putative enhancers (E1-E3) region are marked with the coordinates shown on the bottom right.

Figure S2

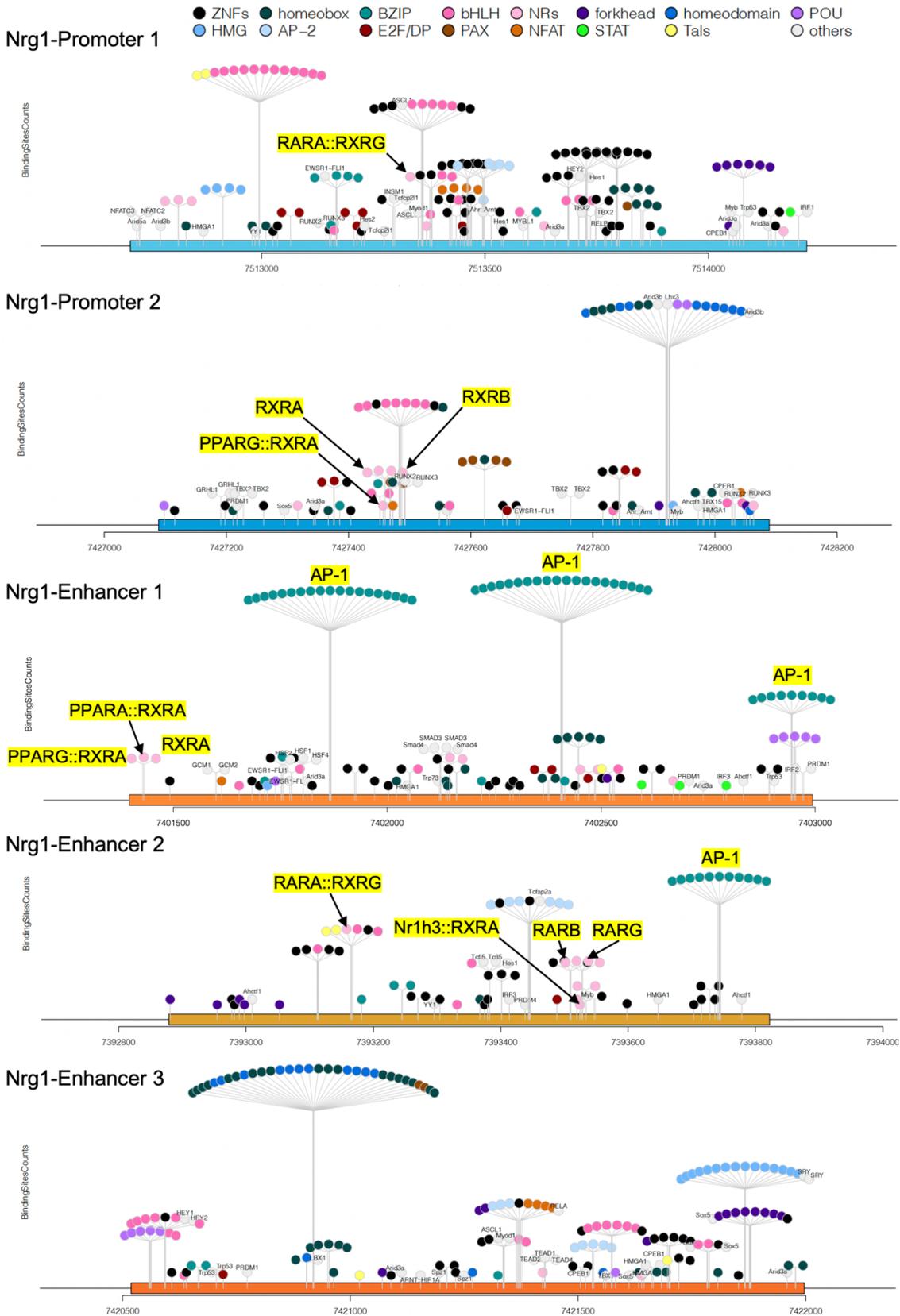


Fig. S2. Motif search results of *nrg1* promoters and putative enhancers. AP-1 complex, Retinoid X Receptor, and Retinoic Acid Receptor binding sites are highlighted. Refer to Table S3 for details.

Figure S3

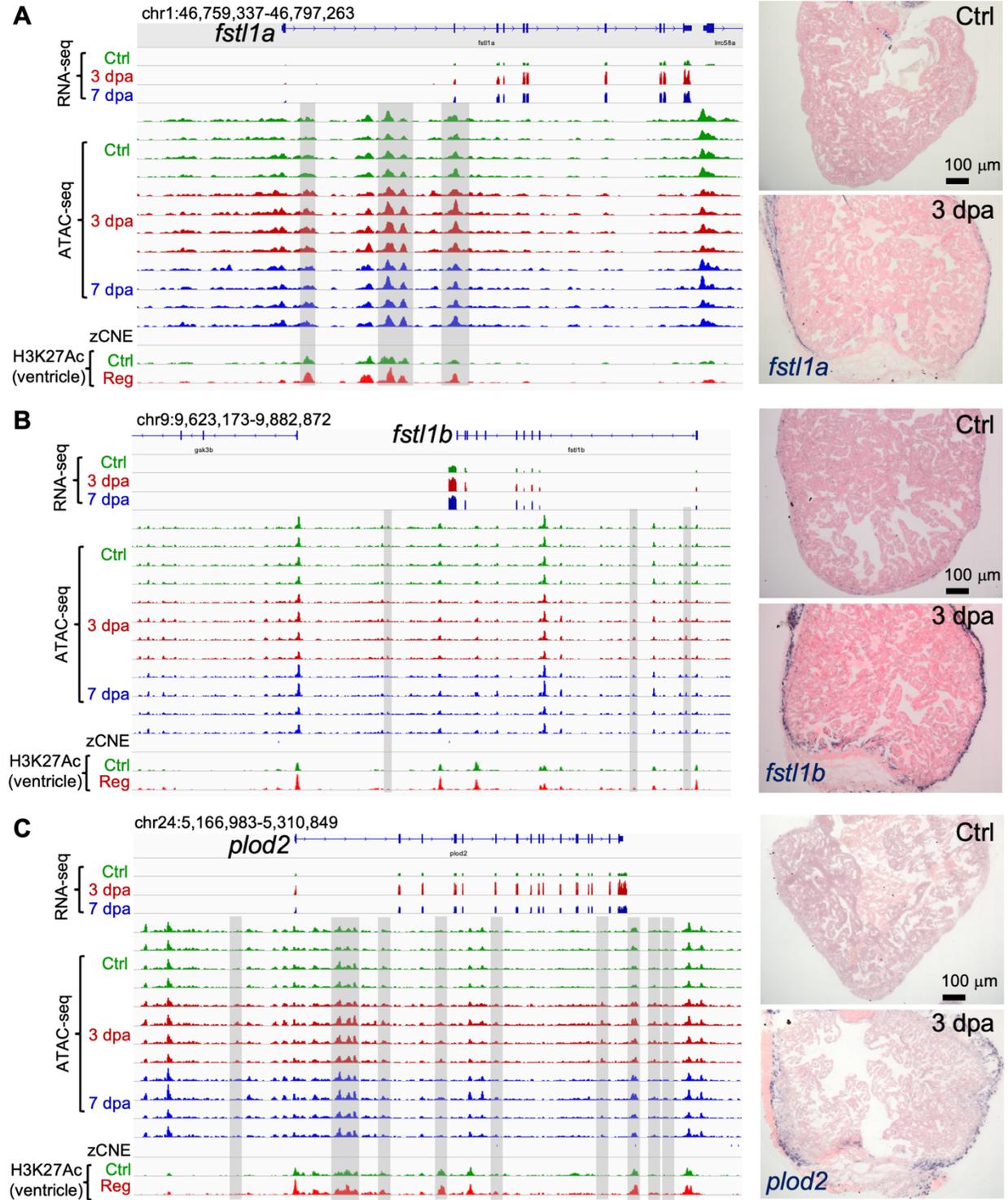


Figure S3-continued

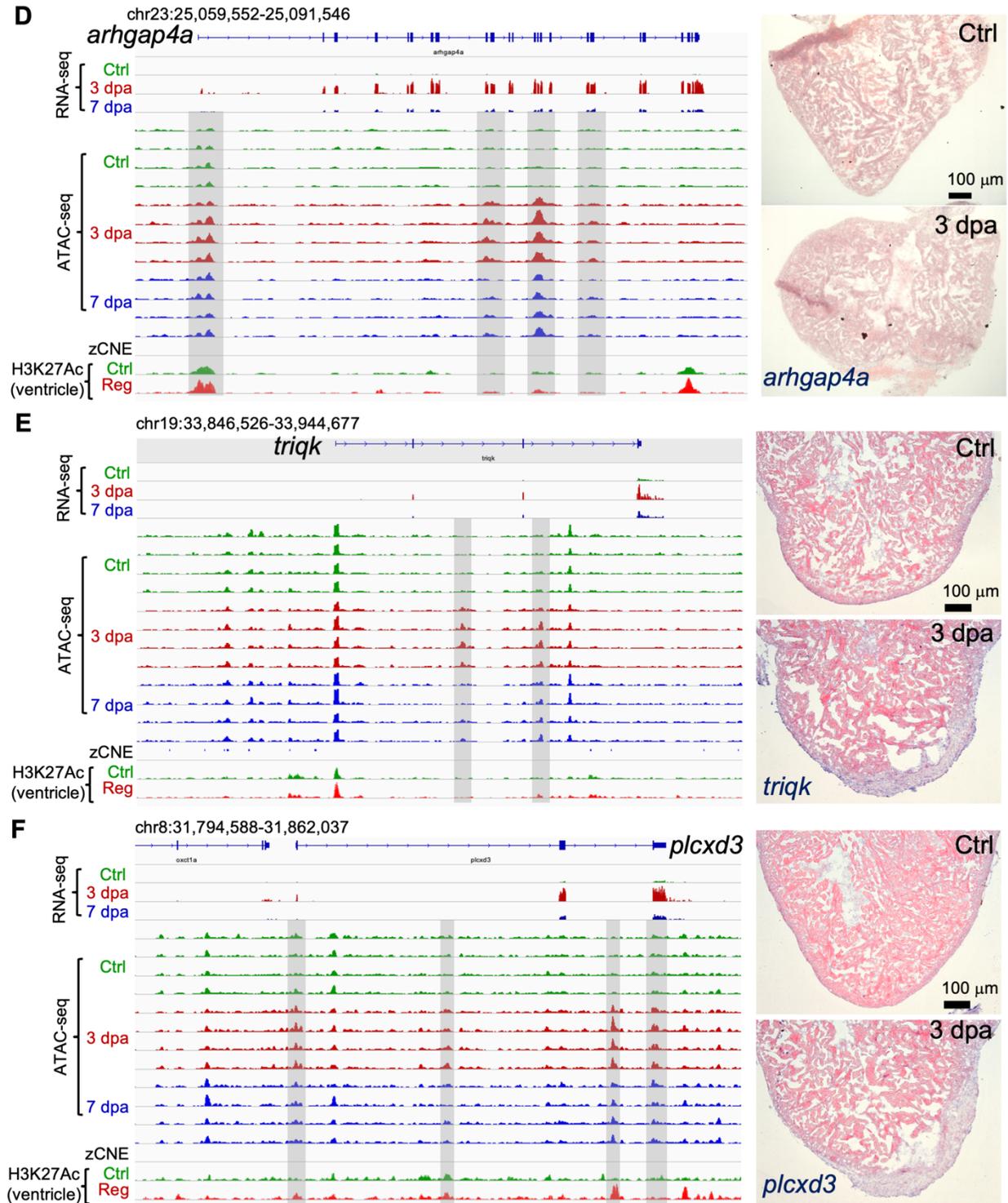


Fig. S3. Browser tracks of the genomic regions and *in situ* hybridization results.

Chromatin accessibility profiles in the epicardium, the zCNE sites, and the whole-ventricle H3K27Ac profile are shown. Selected putative enhancer regions are highlighted in gray boxes. *In situ* hybridizations were performed on ventricular sections of uninjured (Ctrl) adult hearts or hearts collected at 3 dpa. Gene expressions in the epicardium were detected upon injury for *fstl1a*, *fstl1b*, *plod2*, *trfqk*, and *plcxd3*. Scar bars, 100 μ m.

Figure S4

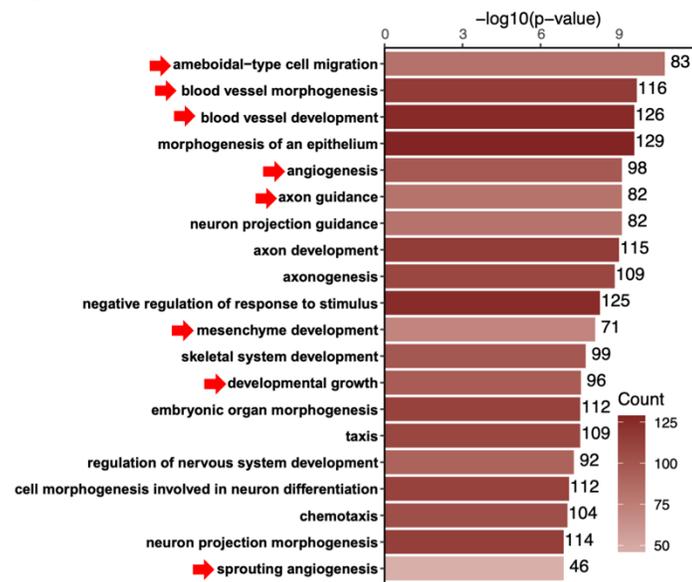


Fig. S4. Barplot showing the 20 most enriched GO biological function terms obtained from peaks with increased chromatin accessibility in 3 dpa samples (FDR < 0.01). The full list is shown in Table S5.

Figure S5

ncam1a

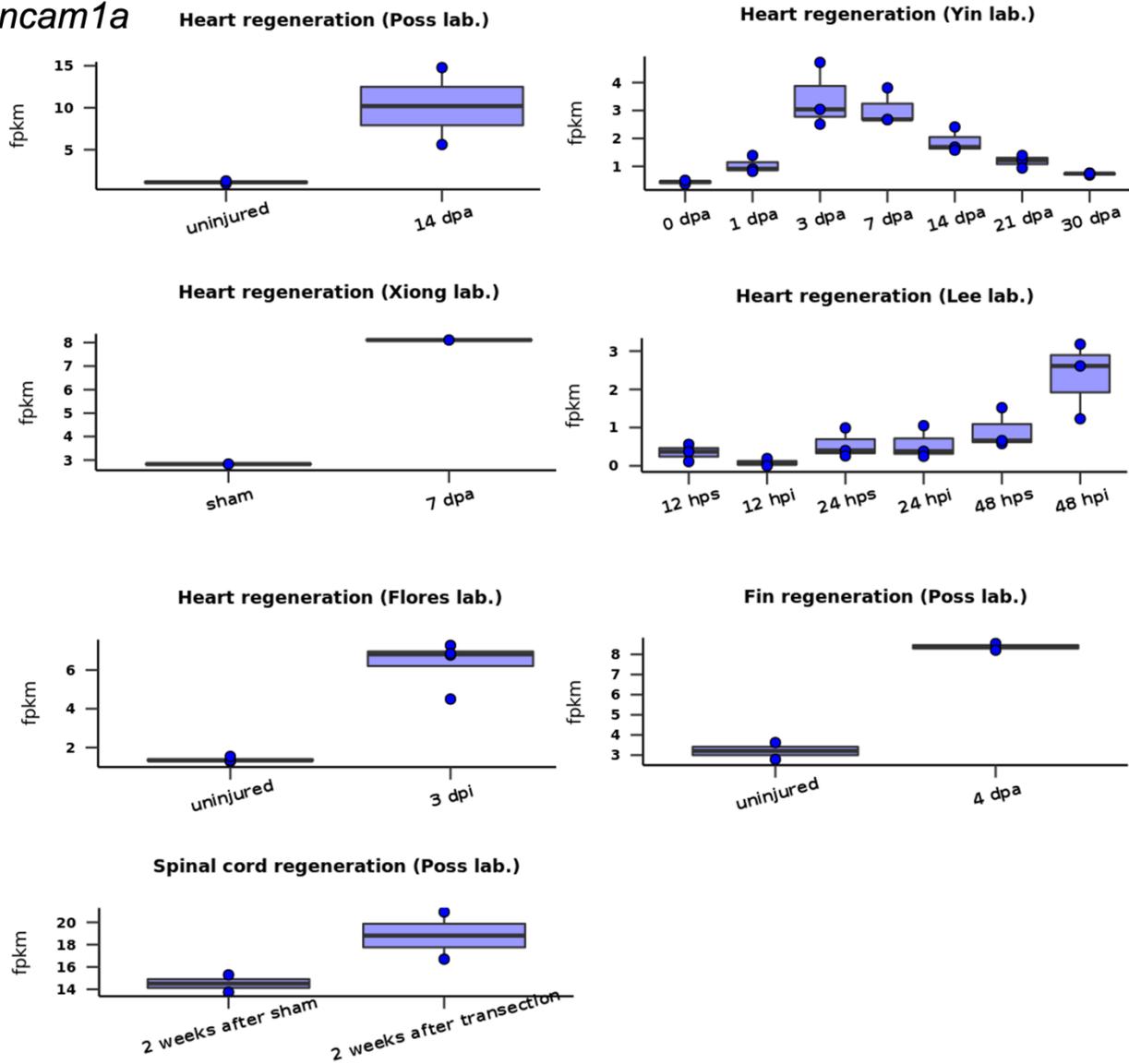


Fig. S5. *ncam1a* expression during zebrafish heart, fin, or spinal cord regeneration.

Plots were generated using the Zebrafish Regeneration Database.

Figure S6

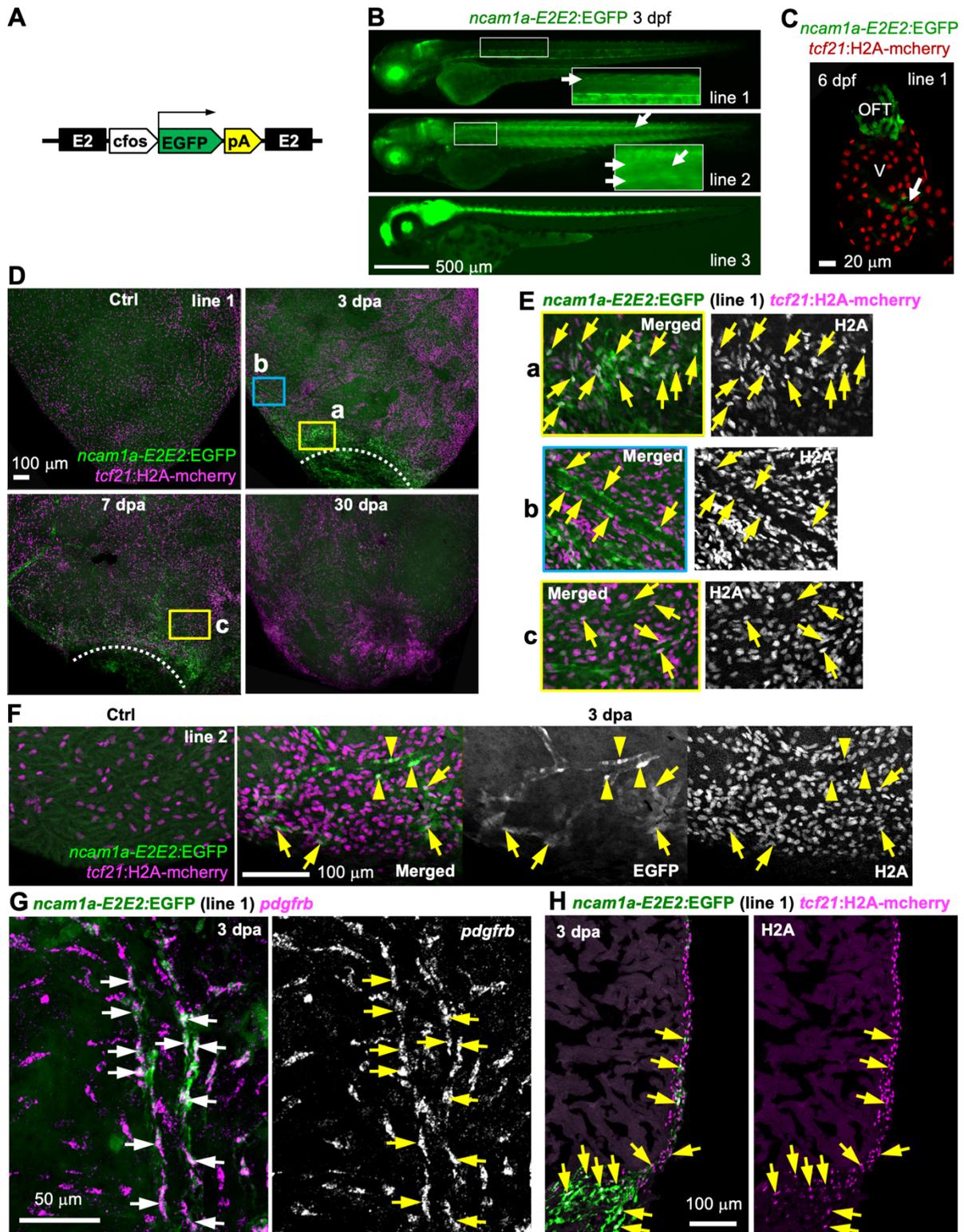


Fig. S6. *ncam1a-E2E2* directs injury-induced epicardial gene expression.

(A) The *ncam1a-E2E2:EGFP* enhancer reporter construct. A second *ncam1a-E2E2:EGFP* enhancer was placed after the poly-A sequence. **(B)** Larval expression of the *ncam1a-E2E2:EGFP* lines. Besides the eye, brain, spinal cord, and notochord expressions, additional EGFP signals are detected in the skeletal muscle for line 2. Scale bar, 500 μm . **(C)** Whole-mount images of a 6 dpf heart showing EGFP expression in the outflow tract (OFT) and the atrioventricular valves (arrows). *tcf21:H2A-mCherry* (red) labels the epicardial cells. V, ventricle. Scale bar, 20 μm . **(D)** Whole-mount images (maximum projections) of the ventricular surface showing expressions of *ncam1a-E2E2:EGFP* line 1 (green) in uninjured (Ctrl) and 3, 7, and 30 dpa samples. *tcf21:H2A-mCherry* (magenta) labels the epicardial cells. White dashed lines indicate the injury sites. The framed regions are enlarged to show details in (E). Scale bar, 100 μm . **(E)** Single optical section images of the framed regions in (D). Single-channel images are shown in grayscale. Arrows indicate representative GFP⁺mCherry⁺ cells. Double-positive perivascular cells are shown in (b) and (c). **(F)** Whole-mount images showing expressions of *ncam1a-E2E2:EGFP* line 2 (green) in uninjured (Ctrl) and 3 dpa samples. *tcf21:H2A-mCherry* (magenta) labels the epicardial cells. Single-channel images of the 3 dpa sample are shown in grayscale. Arrowheads (perivascular cells) and arrows indicate representative EGFP⁺mCherry⁺ cells. Scale bar, 100 μm . **(G)** HCR staining result of *pdgfrb* (magenta) on a whole-mounted heart carrying the *ncam1a-E2E2:EGFP* reporter (line 1, green). Single-channel image shows staining signals of *pdgfrb*. Arrows denote EGFP⁺*pdgfrb*⁺ cells. Scale bar, 50 μm . **(H)** Section images demonstrating expressions of *ncam1a-E2E2:EGFP* (green) in *tcf21:H2A-mCherry*⁺ (magenta) cells. Arrows denote representative double-positive cells. Scale bar, 100 μm .

Figure S7

rgmb

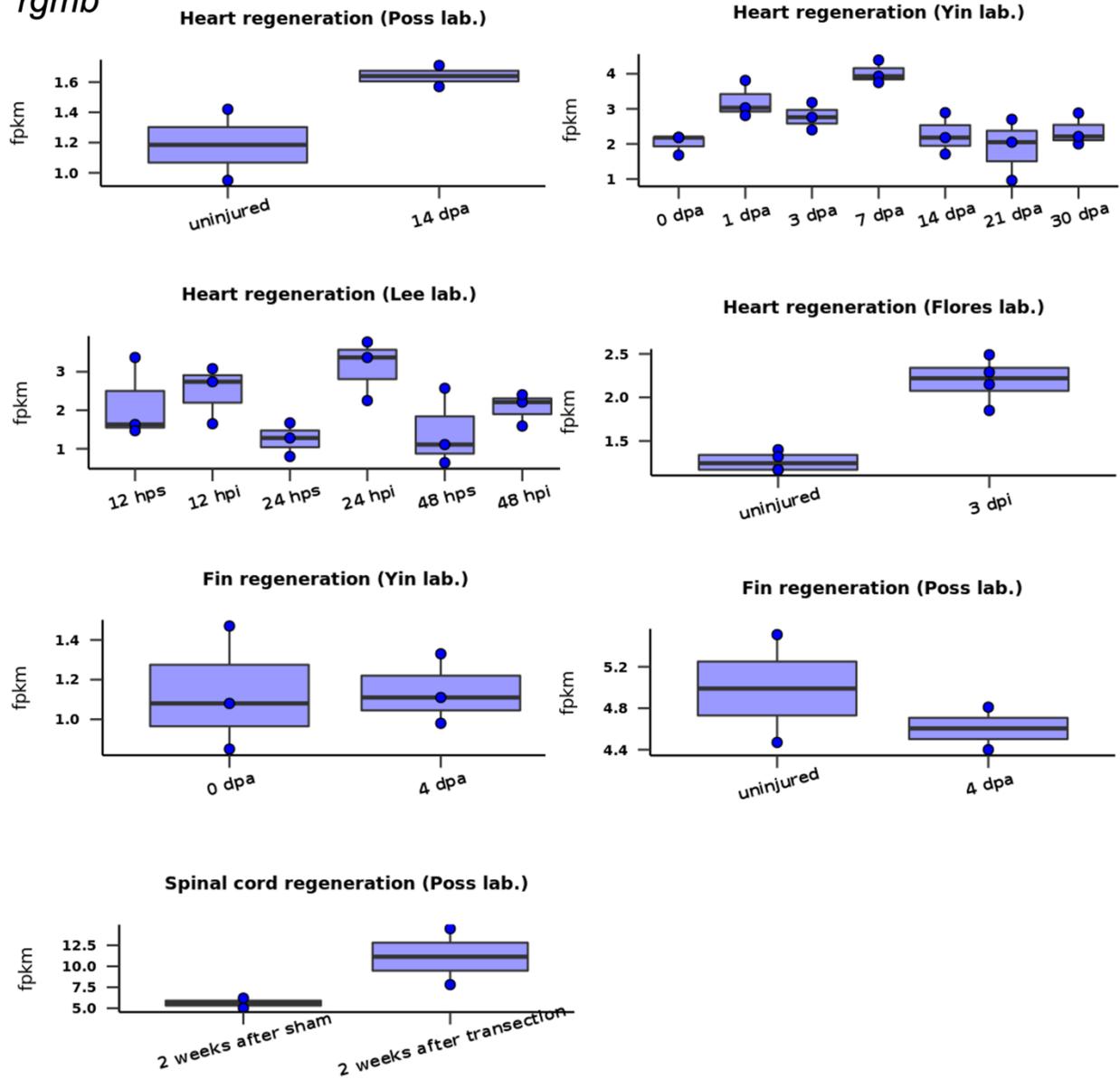


Fig. S7. *rgmb* expression during zebrafish heart, fin, or spinal cord regeneration.

Plots were generated using the Zebrafish Regeneration Database.

Figure S8
gnai3

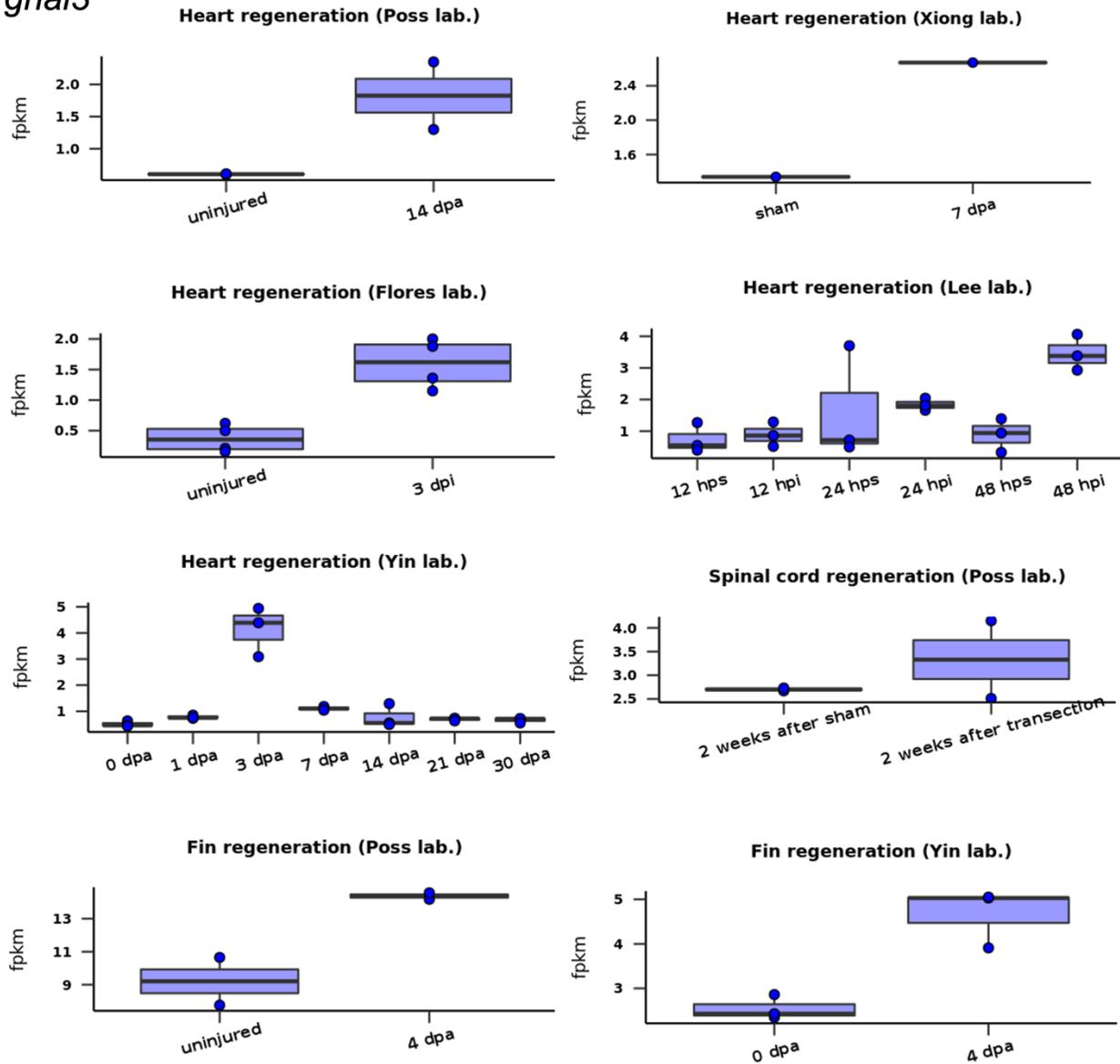


Fig. S8. *gnai3* expression during zebrafish heart, fin, or spinal cord regeneration.

Plots were generated using the Zebrafish Regeneration Database.

Table S1. Alignment information of the ATAC-seq datasets.

[Click here to download Table S1](#)

Table S2. List of differential ATAC-seq peaks derived from paired comparisons.

[Click here to download Table S2](#)

Table S3. Motif search results of nrg1 promoters and the linked putative enhancers.

[Click here to download Table S3](#)

Table S4. List of differential transcripts from paired comparisons.

[Click here to download Table S4](#)

Table S5. Enrichment annotation results of the ATAC-seq peaks with increased accessibility at 3 dpa.

[Click here to download Table S5](#)

Table S6. List of differential ATAC-seq peaks linked to nearby differential transcripts in 3 dpa versus uninjured (Ctrl) samples.

[Click here to download Table S6](#)

Table S7. List of differential ATAC-seq peaks (3 dpa vs. Ctrl) containing conserved sequences.

[Click here to download Table S7](#)

Table S8. Primer sequences.

<i>ncam1a-E2</i>	chr21:23,525,859-23,527,970 (-)	<u>TACGGATCCAAGGGT</u> GTGGAAGTATATCCGA	<u>CCGGAATTC</u> ACTGGTC TGTATAGAGCGTG
<i>ncam1a-E4</i>	chr21:23,598,190-23,599,475 (-)	<u>GGACTAGTCATGTGGT</u> CACTCATCCAGG	<u>CGGGAATTC</u> ACACAAA CTCTGCCAAGGAG
<i>rgmb-E1</i>	chr5:51,699,022-51,700,574 (-)	<u>GGACTAGTAGCGTGAT</u> ATAAAGGGTCCA	<u>CGGGAATTC</u> ATTACTGT GAACTACCTTACGG
<i>rgmb-E2</i>	chr5:51,702,457-51,704,115 (-)	<u>GGACTAGTGTCCTTG</u> GTCATTCCCAAGG	<u>GGACTAGTG</u> CAAAACA ACACTGCAAAGTGTG
<i>gnai3-E1</i>	chr8:25,179,294-25,180,454 (+)	<u>GGACTAGTACACCATG</u> GTCTCTTAAAGTCCAA	<u>CGGGAATTC</u> CAGCATG ACATGTCTAAAGCAGA G
<i>gnai3-E2</i>	chr8:25,192,321-25,193,867 (+)	<u>CCGGAATTC</u> GATGGTT TGTTCTGTAGACTATC G	<u>TAGGCTAGC</u> AGCTAATA ATTCAGCCAGC
Probes		Forward primers	Reverse primers
<i>ncam1a</i>		GGGAACCTAATCCTCC TAAACTG	GTCAAGGAGACTGTGC TAGAG
<i>rgmb</i>		AACCAGTGCTCATGTT TTGTGC	CTTTCCTTCATTGCTCC TCTGG
<i>gnai3</i>		ACTATCCAGTCCATCA TTGCC	AAGAGTTGCTGAATTC CTGTCC
<i>junba</i>		TAACGGCGTCATCACA TCAC	AAACAGTTAAAGGTCT GGCTC
<i>fstl1a</i>		CCTCTGTCTCTCATC CCCTGATC	CTCTTCAGACTTCTTTA TCACCCA
<i>fstl1b</i>		ATGATGTTTCGGTGTT TACCCGTG	TTAGACTTCCTTTGTGC TGGTCTT
<i>plod2</i>		AGAGAAATACATCCAT GCCA	AGCACAATTTGGGAAA TCAC
<i>arhgap4a</i>		CTCATCGTTTCCTCAT ATCCT	TTTCCTTTCCATCCCAG TCC
<i>trfq</i>		CGCATCCATTTGAAGT CCAC	ACAACATCCTTGAAATC CTGCT
<i>plcx3</i>		GTACAGAACCTCCATC ACGA	TCATACAAGTTACTACG ACCAC

The underlined base pairs denote restriction enzyme cutting sites.

Table S9. Enhancer lines used in this study.

#	Strain Name	Allele
1	<i>Tg(ncam1a-E2:EGFP) line 1</i>	<i>wcm103</i>
2	<i>Tg(ncam1a-E2:EGFP) line 2</i>	
3	<i>Tg(ncam1a-E2E2:EGFP) line 1</i>	<i>wcm104</i>
4	<i>Tg(ncam1a-E2E2:EGFP) line 2</i>	
5	<i>Tg(ncam1a-E2E2:EGFP) line 3</i>	
6	<i>Tg(ncam1a-E4:EGFP) line 1</i>	<i>wcm105</i>
7	<i>Tg(ncam1a-E4:EGFP) line 2</i>	
8	<i>Tg(ncam1a-E4:EGFP) line 3</i>	
9	<i>Tg(ncam1a-E4E4:EGFP) line 1</i>	<i>wcm106</i>
10	<i>Tg(rgmb-E1:EGFP) line 1</i>	<i>pd340</i>
11	<i>Tg(rgmb-E1:EGFP) line 2</i>	
12	<i>Tg(rgmb-E2:EGFP) line 1</i>	<i>pd341</i>
13	<i>Tg(rgmb-E2:EGFP) line 2</i>	
14	<i>Tg(rgmb-E2:EGFP) line 3</i>	
15	<i>Tg(gnai3-E1:EGFP) line 1</i>	<i>pd342</i>
16	<i>Tg(gnai3-E1:EGFP) line 2</i>	
17	<i>Tg(gnai3-E1:EGFP) line 3</i>	
18	<i>Tg(gnai3-E2:EGFP) line 1</i>	<i>pd343</i>
19	<i>Tg(gnai3-E2:EGFP) line 2</i>	
20	<i>Tg(gnai3-E2:EGFP) line 3</i>	
21	<i>Tg(gnai3-E2:EGFP) line 4</i>	