Fig. S1

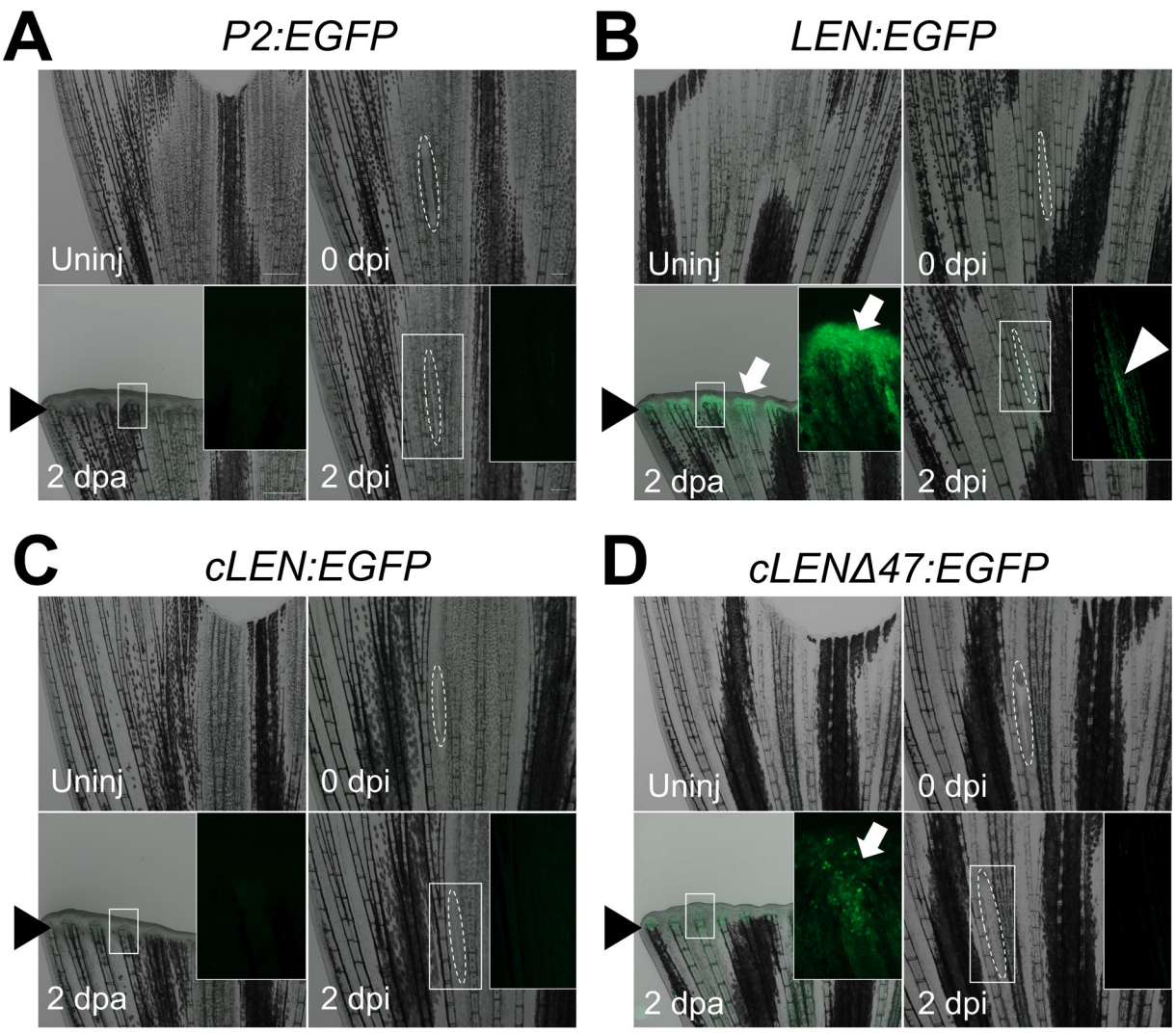

Figure S1. *LEN* is an injury-responsive regeneration enhancer. (A-D) Whole-mount images of uninjured (Uninj.), freshly interray incised (0 day post-incision, dpi), 2 days post-amputation (dpa), and 2 dpi fins. Uninjured or freshly injured fins do not have directed EGFP expression (A-D). EGFP is barely detectable in the *P2:EGFP* and *cLEN:EGFP* injured fins (2 dpa and 2 dpi; A and C, respectively). Fin amputation drives robust blastema expression in the *LEN:EGFP* fin (B, white arrow). Incision injury directs *LEN:EGFP* expression surrounding wound area (B, arrowhead), but the expression level

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is noticeably less robust than that after fin amputation (B). In contrast to *cLEN:EGFP*, *cLENΔ47* drives ectopic EGFP induction in some epidermal cells in 2 dpa fins (D, arrow). The boxed area is enlarged in the right panel. EGFP signal in enlarged view is enhanced to improve the visibility of the weak EGFP signal. The black arrowheads in 2 dpa fins indicate amputation plane.Incision injuries are outlined with white dotted lines.

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Fig. S2

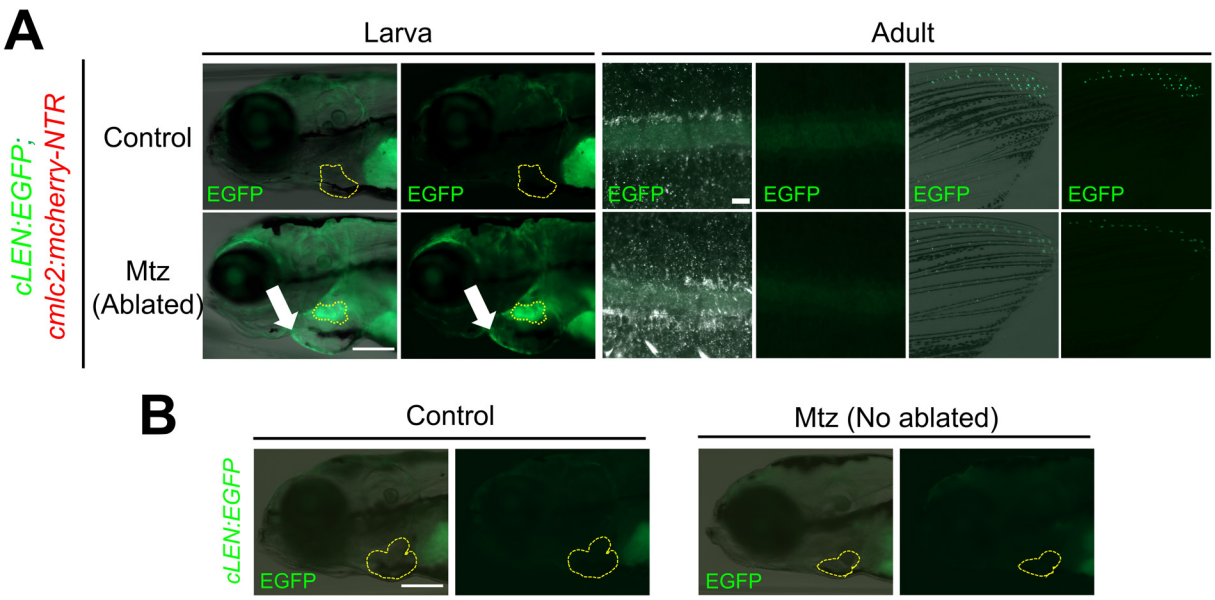

Figure S2. *cLEN* directs cardiac injury-dependent gene expression. **(A)** Cardiac muscle cell ablation induced by Mtz administration in *cmlc2:mCherry-NTR* directs epidermal expression (arrows) in 5 dpf larvae. However, cardiac muscle cell ablation does not induce *cLEN:EGFP* expression in epidermis of the body trunk and caudal fin at adult stages. **(B)** Mtz administration in *cLEN:EGFP* does not direct the EGFP expression in the hearts, indicating that *cLEN* is specifically activated upon cardiac injury. Scale bar, 200 μm in A and B.

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Fig. S3

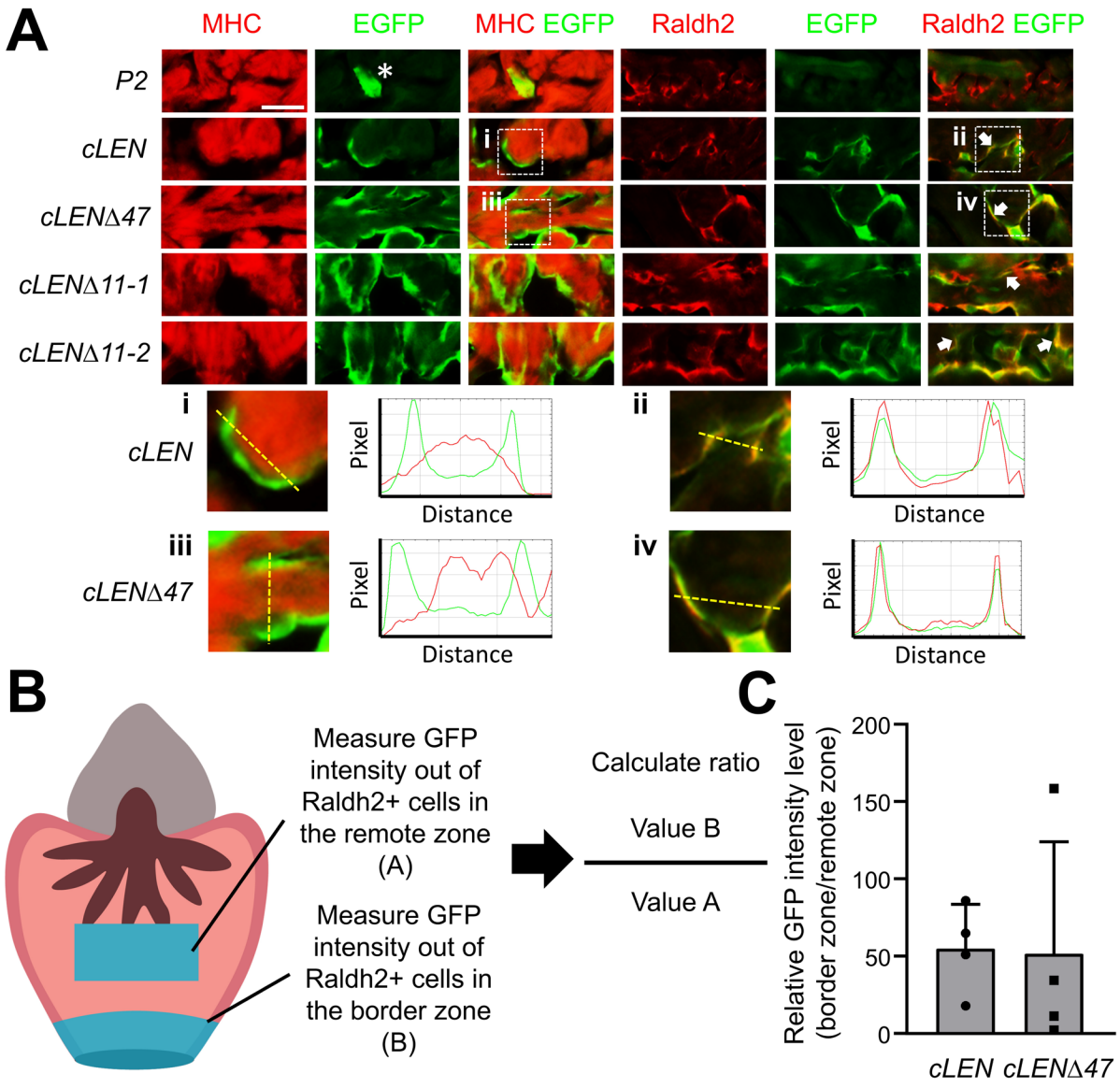

Figure S3. *cLEN* is active primarily in endocardial cells. (A) Immunostained section images of transgenic fish carrying P2 (minimal promoter), *cLEN*, *cLENΔ47, cLENΔ11-1* and *cLENΔ11-2.* MHC and Raldh2 are used to label CMs and endocardial cells, respectively. The P2 minimal promoter directs injury-induced expression in a few MHC^{+} CMs at the wound area (asterisk), but not in Raldh2^{+} endocardial cells. In addition to CMs

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at the wound area, *cLEN, cLENΔ47, cLENΔ11-1* and *cLENΔ11-2* drive primarily endocardial expression in the injured hearts. Ectopic CM expression is observed only in the border zone of the injured hearts, but there is no expression in the uninjured hearts. Enlarged views of the boxed areas are shown at the bottom. Line plots demonstrate that $EGFP^+$ cells are colocalized with $Raldh2^+$ cells. The arrows indicate endocardial EGFP expression. **(B)** Schematic of quantification of EGFP intensity of $Raldh2^+$ cells between border and remote zones. **(C)** Quantification graphs of relative EGFP intensity ratio between border and remote zones in 3 dpa *cLEN* and *cLENΔ47* hearts. EGFP level is higher in the border zone compared to that in the remote zone. The data are presented as mean ± SD, n = 4. Scale bar, 20 μm in A.

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Fig. S4

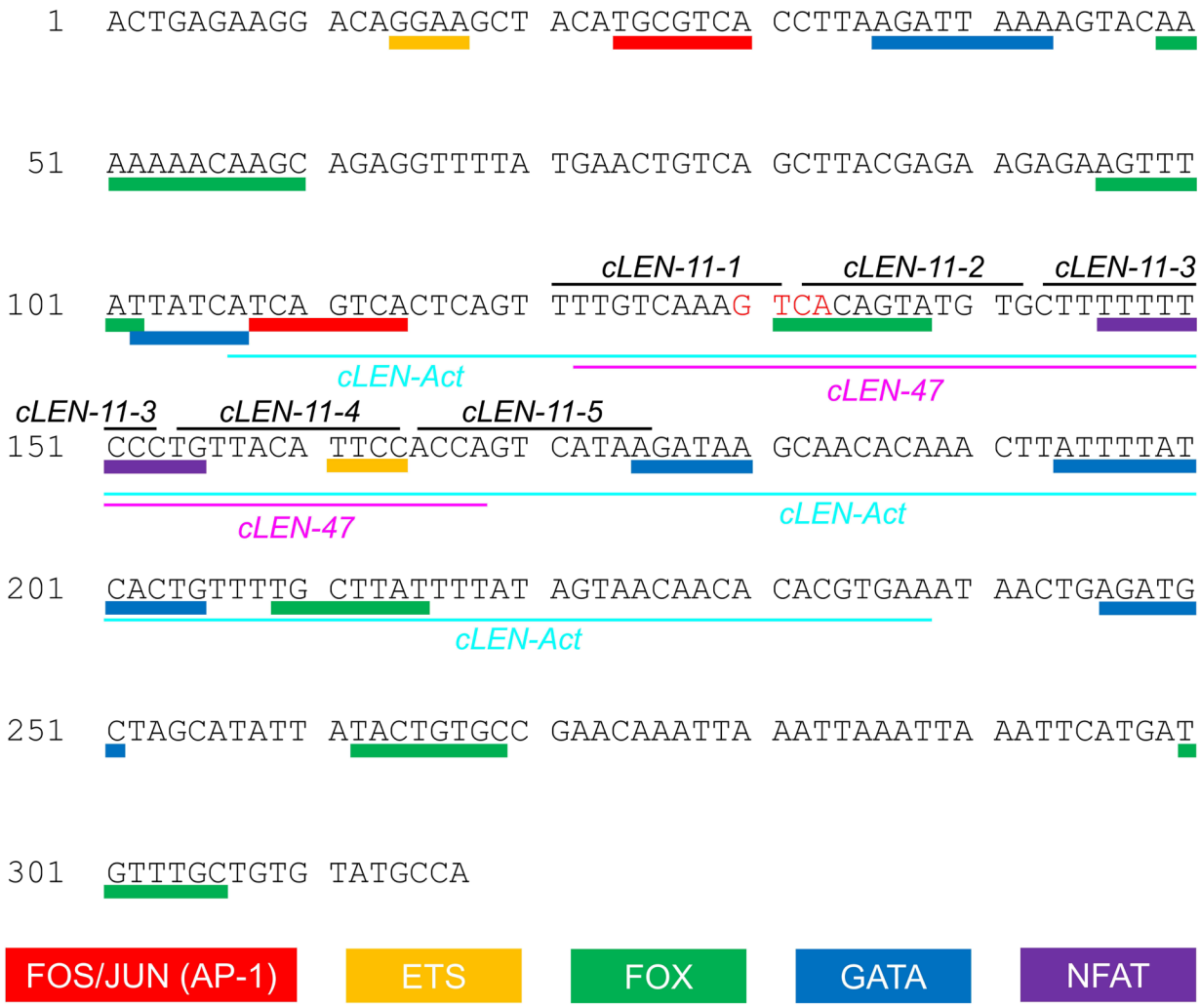

Figure S4. *cLEN* sequence with annotation of transcription factor (TF) binding motifs. TF binding sites are indicated by colored lines. Deleted sequences are marked with black and magenta lines. Conserved GTCA sequence spanning *cLEN-11-1* and *cLEN-11-2* is shown in red.

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Fig. S5

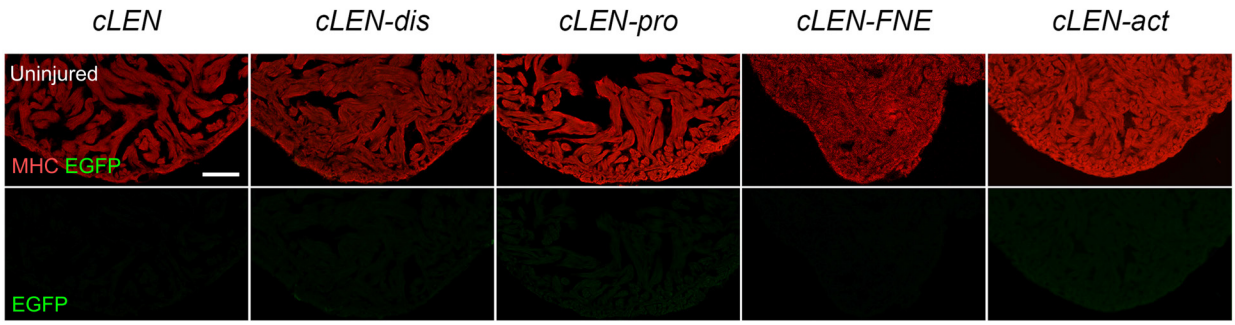

Figure S5. Uninjured cardiac section images. EGFP signal is undetectable in the uninjured hearts of *cLEN, cLEN-dis, cLEN-pro, cLEN-FNE,* and *cLEN-act*. MHC, myosin heavy chain, a CM marker. Scale bar, 100 μm.

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Fig. S6

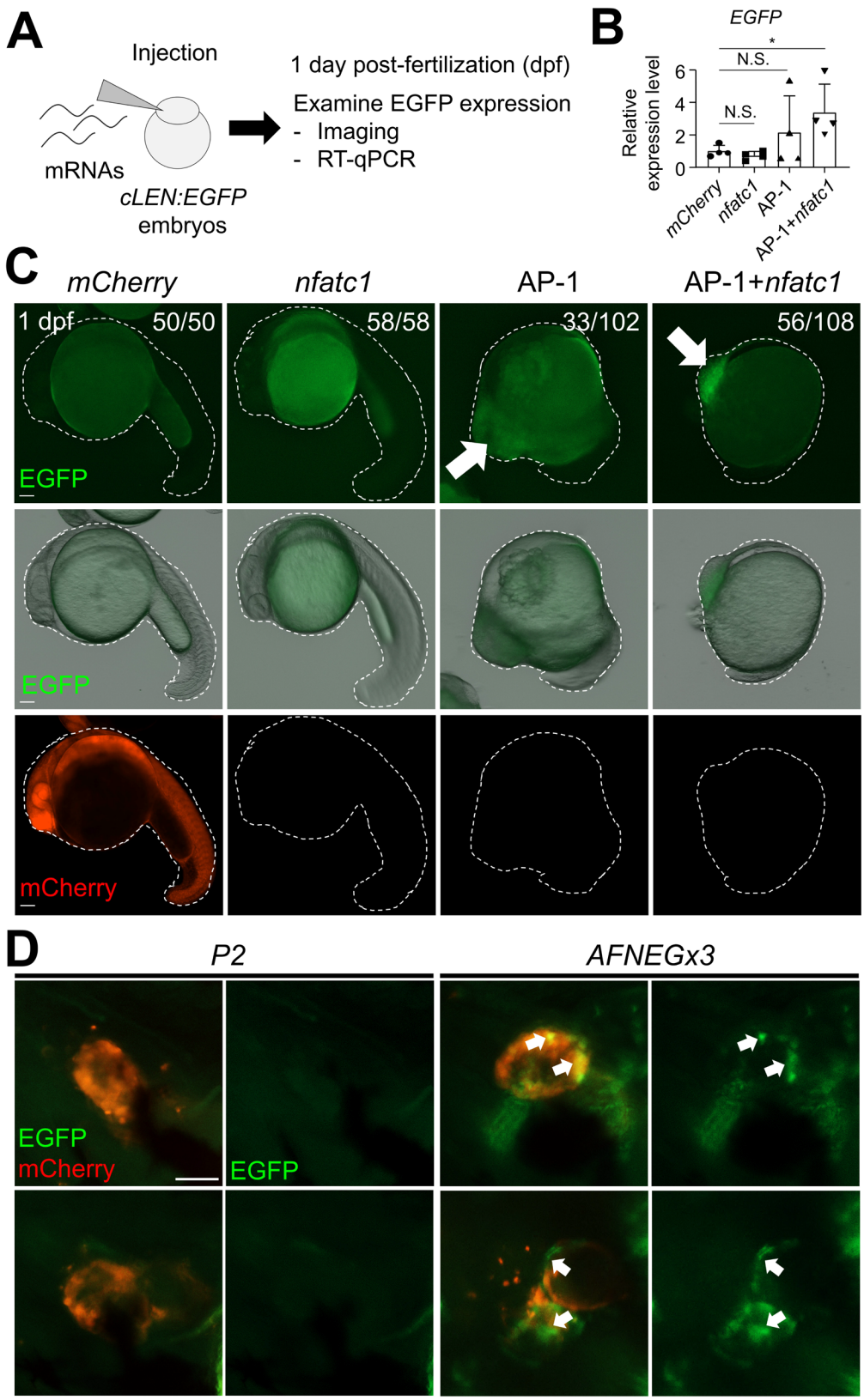

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Figure S6. *cLEN* activation by AP-1 mRNA injection. (A) Schematic of mRNA injection experiments. *cLEN:EGFP* heterozygote embryos were obtained by mating *cLEN:EGFP* homozygote and EK wild-type fish. mCherry was used as a control. A total of 60 pg of mRNA was injected, and embryos were imaged and harvested at 1 day post-fertilization. **(B)** RT-qPCR assays of the *EGFP* mRNA levels. EGFP expression is not induced in the *mCherry* and *nfatc1* mRNA injected embryos. In contrast, AP-1 mRNA injection likely, but not significantly, increases EGFP expression. Co-injection of AP-1 and *nfatc1* mRNAs induces EGFP expression significantly, compared to that in *mCherry*. The data are presented as the mean ± SD, n = 4. *, $p < 0.05$; N.S., Not significant. One-way ANOVA with Tukey post-analysis. **(C)** Representative whole-mount images of 1 dpf embryos. mCherry signal is detectable only in the *mCherry* mRNA injected embryos. Note that AP-1 mRNA injection results in abnormal development, which is noticeable at 1 dpf; thus, EGFP induction in hearts cannot be tested. Embryos are outlined with white dotted lines. **(D)** Injured heart images of *cLEN:EGFP* larvae injected with *P2* and *AFNEGx3*. Although the majority of *AFNEGx3* injected larvae did not induce EGFP (See Fig. 2E), some larvae showed significant EGFP expression, which is distinct to that in *P2* injected larvae. Scale bar, 100 and 50 μm in C and D, respectively.

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Fig. S7

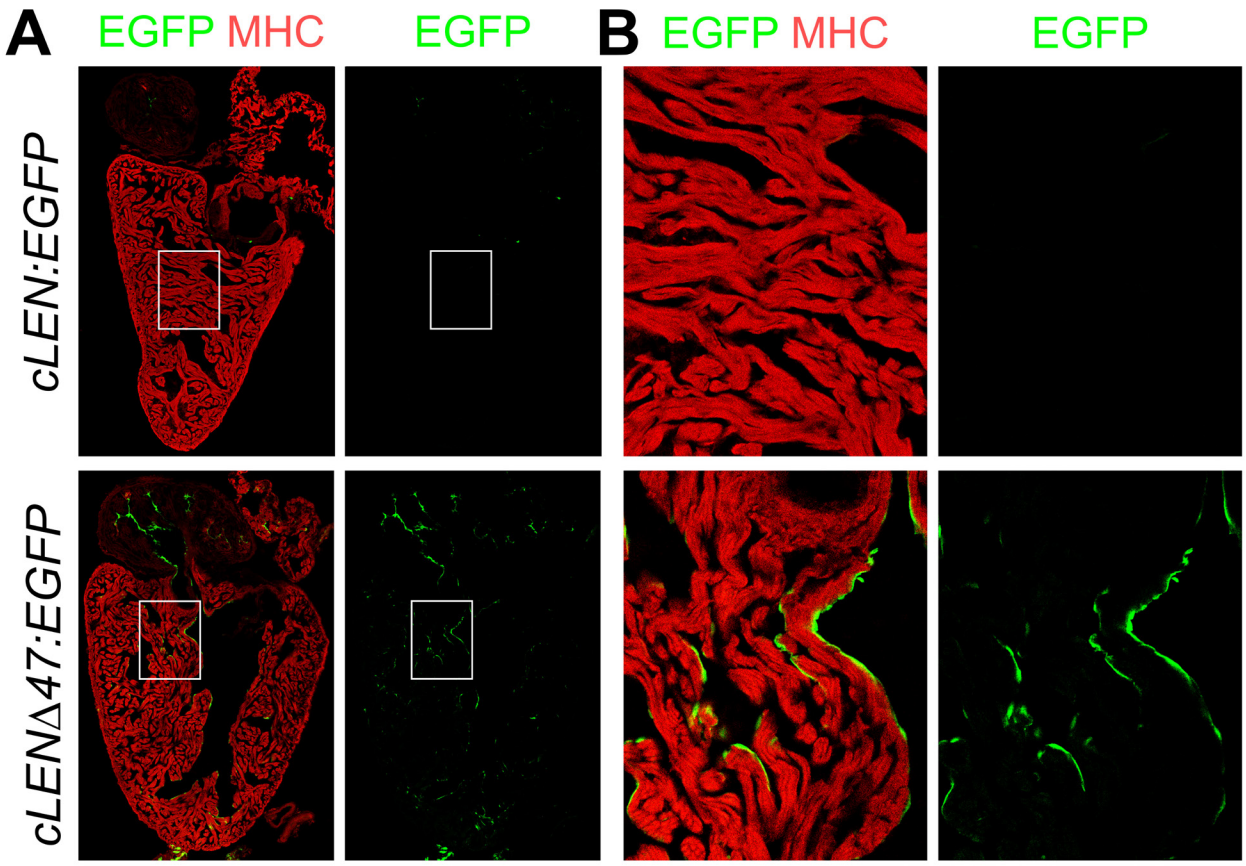

Figure S7. Uninjured cardiac section images of *cLEN* and *cLENΔ47*. (A) Confocal imaging of uninjured ventricles of *cLEN* (top) and *cLENΔ47* (bottom). **(B)** Enlarged view of the boxed areas in (A). EGFP is detectable in uninjured hearts of *cLENΔ47* but not in *cLEN.*

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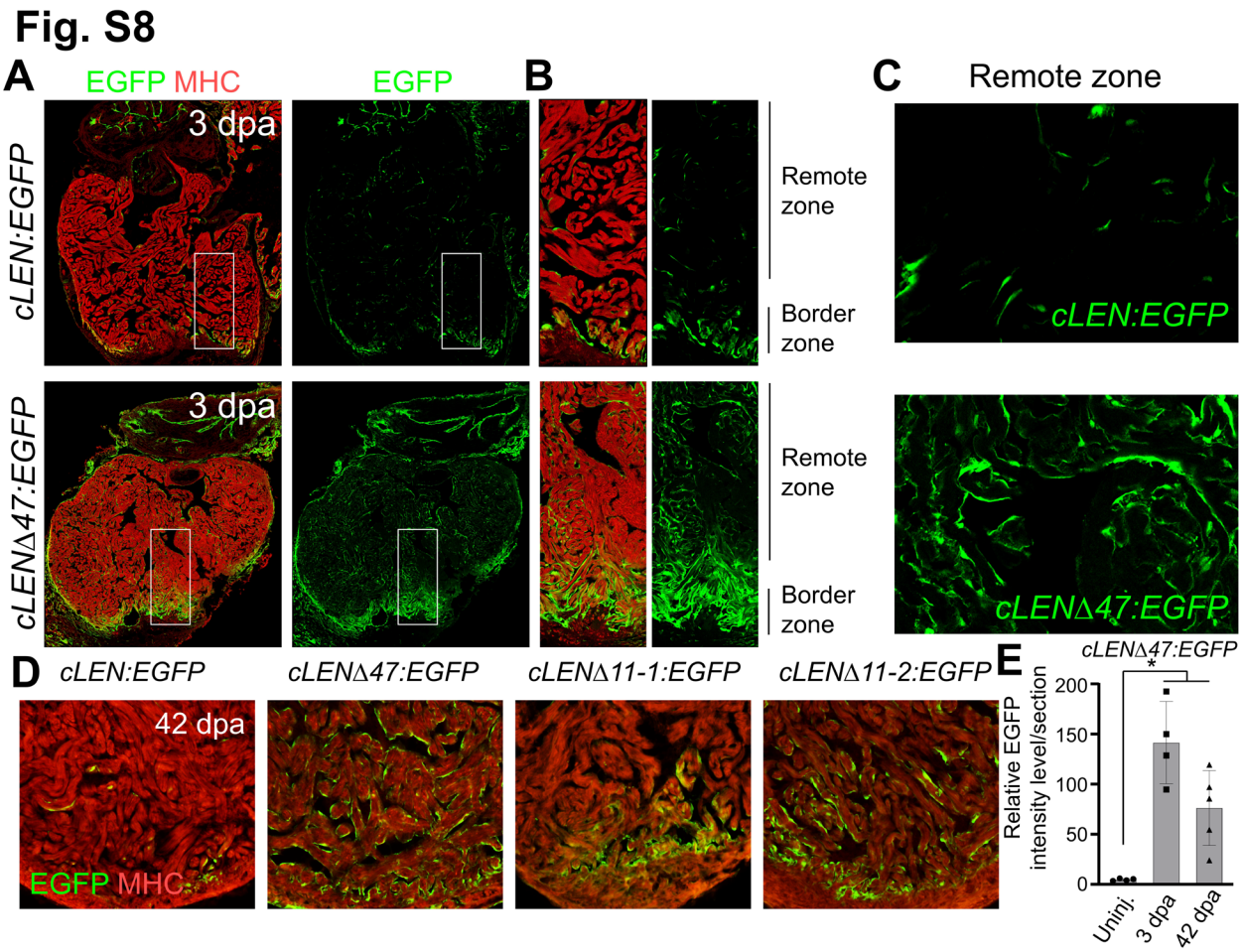

Figure S8. Repression is functional in regenerating hearts. (A) Confocal imaging of 3dpa ventricles of *cLEN* (top) and *cLENΔ47* (bottom). **(B)** Enlarged view of the boxed areas in (A). **(C)** Zoomed view of the remote zone. While EGFP^{+} endocardial cells are limited in *cLEN* (top), almost all endocardial cells are EGFP^{+} in *cLENΔ47* (bottom). **(D)** Cardiac section images of 42 dpa hearts of *cLEN*, *cLENΔ47, cLENΔ11-1* and *cLENΔ11-2*. EGFP expression level in 42 dpa hearts is returned to the level detected in uninjured *cLEN* hearts (also see **Fig. 1G-I**). In contrast, EGFP levels are not decreased in *cLENΔ47, cLENΔ11-1* and *cLENΔ11-2* hearts at 42 dpa. **(E)** Quantification of EGFP expression intensity normalized to that in the wild-type uninjured images in the wound area. The data are presented as the mean ± SD. Animal numbers are shown in Table S3. *, $p < 0.05$. One-way ANOVA with Tukey post-analysis.

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Fig. S9

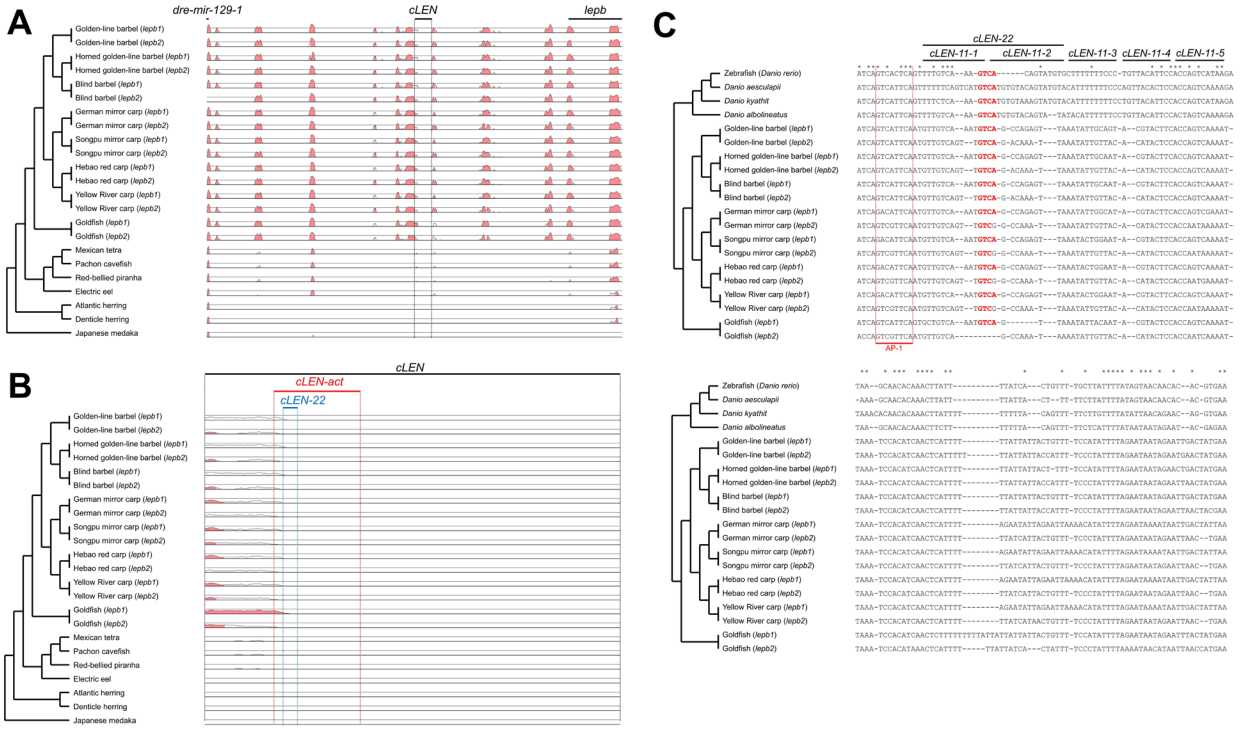

Figure S9. Sequence comparison of genomic regions from *mir129* to *lepb* across teleost fish species (A) VISTA plot of genomic regions from *mir129* to *lepb* based on the LAGAN alignment with reference zebrafish sequence. **(B)** Zoomed-in view of *cLEN* in (A). Sequence comparison indicates that *cLEN* is not present in non-Cyprinidae fish species. The red box indicates the *cLEN-act* region. The blue box indicates *cLEN-22*, where a repressive element resides. **(C)** Alignment of *cLEN-act* in *D. rerio*, *D. aesculapii*, *D. kyathit*, *D. albolineatus*, barbels, carps, and goldfish. Conserved GTCA sequence spanning *cLEN-11-1* and *cLEN-11-2* is marked in red. The dendrogram indicates phylogenetic relationship.

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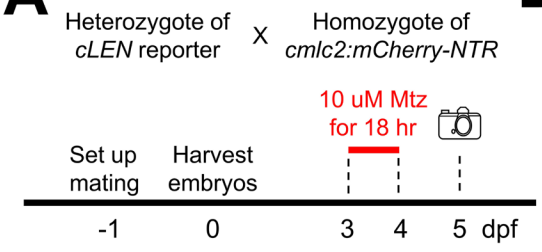
Fig. S10

A



B

Construct	Control		Ablation (Mtz)	
	EGFP$^+$ in hearts	Total	EGFP$^+$ in hearts	Total
cLEN	0	101	57 (42.5%)	134
cLENΔ47	0	16	17 (44.7%)	38
cLENΔ11-1	0	65	54 (55.7%)	97
cLENΔ11-2	0	32	20 (46.5%)	43
cLENΔ11-3	0	35	21 (50.0%)	42
cLENΔ11-4	0	30	19 (39.6%)	48
cLENΔ11-5	0	15	18 (47.4%)	38

C

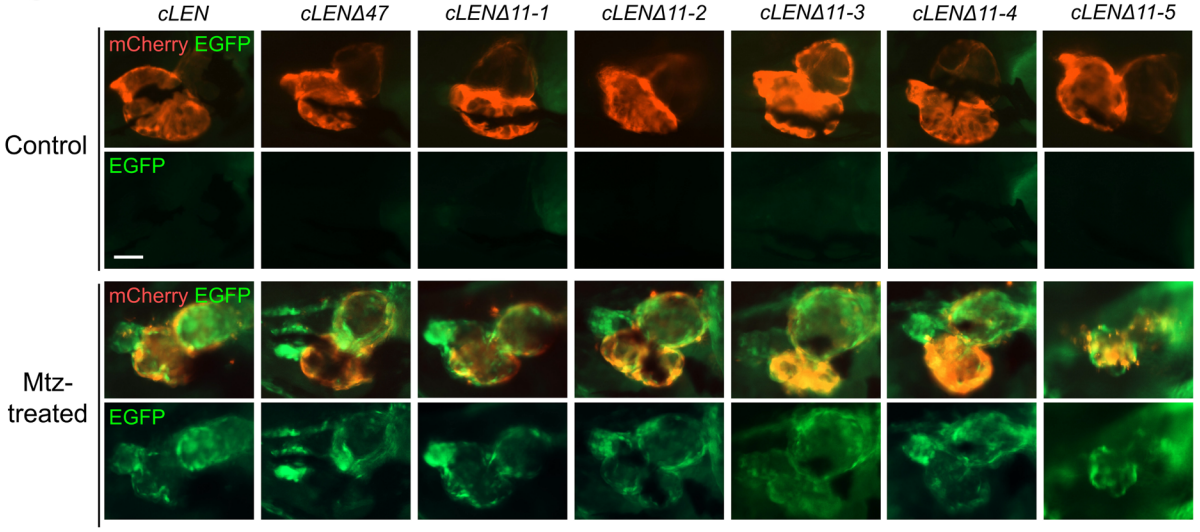


Figure S10. *cLEN, cLENΔ47* and *cLENΔ11* deletion constructs can direct EGFP expression upon cardiac injury. (A) Zebrafish strains and experimental strategy employed to examine injury-responsive activity of various *cLEN* constructs. **(B)** The results of EGFP expression in the heart of 5 dpf larvae. **(C)** Representative whole-mount hearts images of the control and Mtz-treated fish. Scale bar, 50 μm in C.

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Table S1. Sequences of primers used in this study

Click here to Download Table S1

Table S2. Transgenic lines used in this study

Click here to Download Table S2

Table S3. Animal number used in this study

Click here to Download Table S3

Table S4. *lepb* coordinates used for VISTA analysis

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