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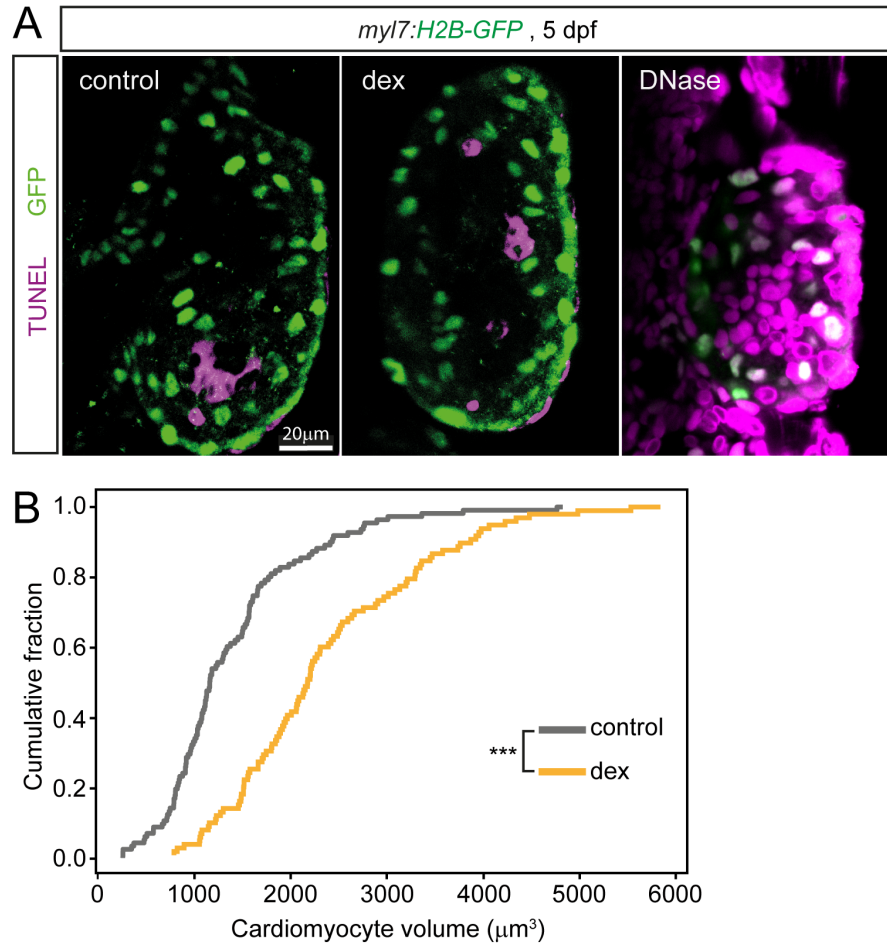
## **Supplemental Information**

### **Early-Life Stress Regulates Cardiac Development through an IL-4-Glucocorticoid Signaling Balance**

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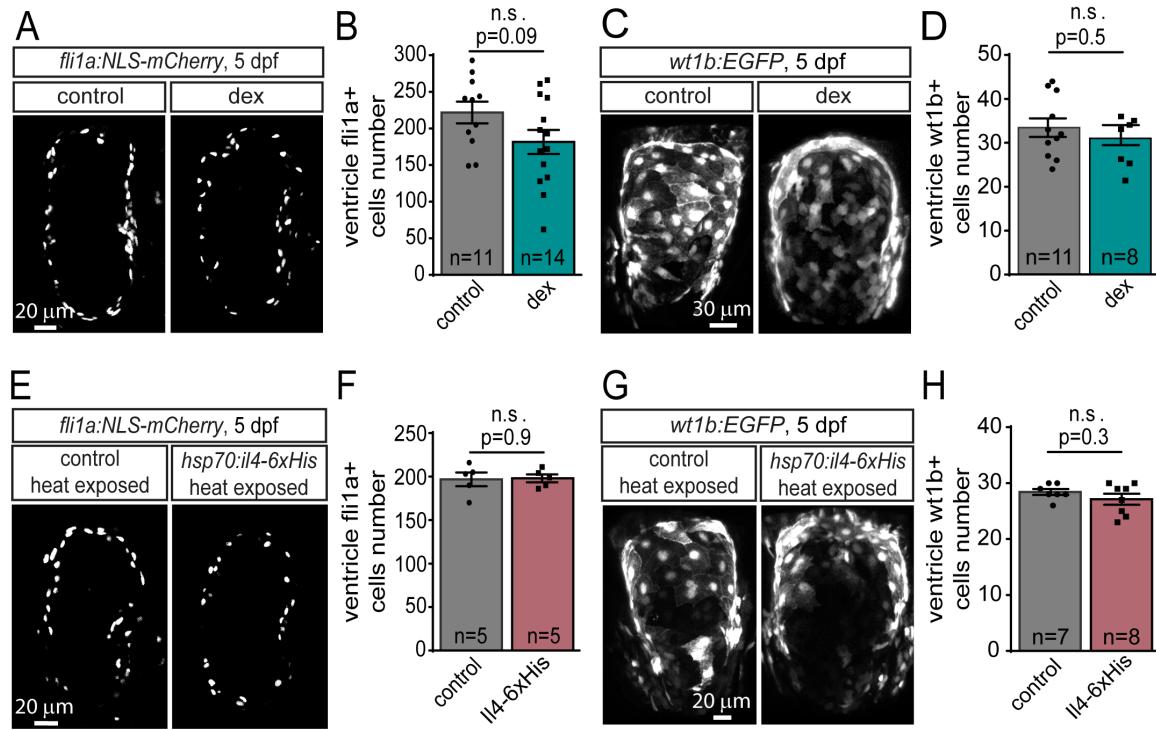
## Supplemental Information

### Supplemental Figures



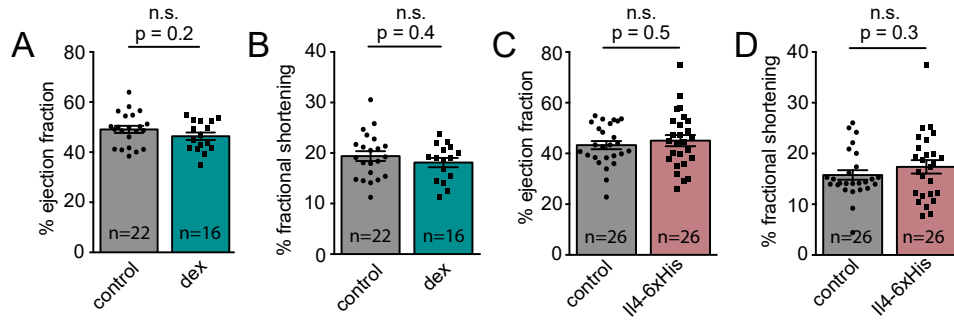
**Figure S1 (related to Figure 1).** GR activation does not induce apoptosis of cardiomyocytes, but causes their hypertrophic growth. **A.** Confocal images of cardiac ventricles of 5 dpf *Tg(myI7:H2B-GFP)<sup>zf521</sup>* larvae treated with dex or DMSO control solutions. Nuclei of cardiomyocytes are labeled by immunofluorescence staining of H2B-GFP. Cell death was detected with TUNEL assay. The image on the right was taken from a 5 dpf *Tg(myI7:H2B-GFP)<sup>zf521</sup>* larva treated with DNase post-fixation, as a positive control

for TUNEL staining. Negligible amounts of TUNEL+/H2B-GFP+ apoptotic cardiomyocytes were detected in all hearts analyzed. **B.** Graph depicting cumulative fractions of cardiomyocyte volumes of 5 dpf *Tg(myl7:H2B-GFP)<sup>zf521</sup>*; *Tg(myl7:mKate-CAAX)<sup>sd11</sup>* larvae treated with dexamethasone (dex) or control solution. \*\*\*  $p = 5.5 \times 10^{-13}$ , two-sample Kolmogorov-Smirnov test.  $n = 111$  cardiomyocytes from 9 larvae (control),  $n = 98$  cardiomyocytes from 7 larvae (dex).

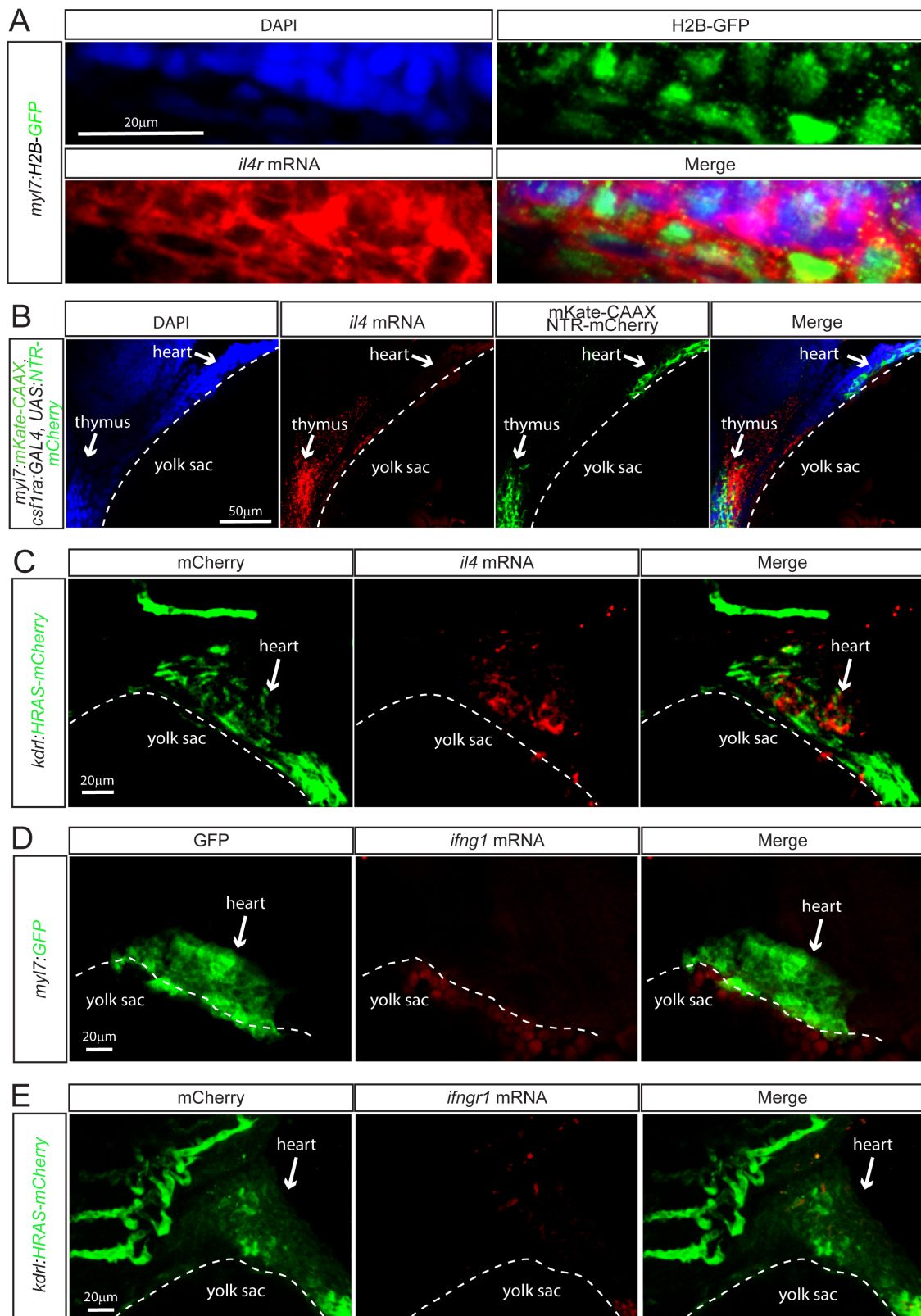


**Figure S2 (related to Figures 1 and 2).** GR activation and *il4-6xHis* overexpression do not alter gross morphology of endocardium and epicardium, nor endocardial and epicardial cell numbers. **A.** Images of ventricles of 5 dpf *Tg(fli1a:NLS-mCherry)<sup>ubs10</sup>* larvae following control and dex treatment. **B.** Graph showing average numbers of *fli1a*<sup>+</sup> endocardial cells in control and dex-treated larvae. **C.** 5 dpf control and dex-treated *Tg(wt1b:EGFP)<sup>li1</sup>* hearts. **D.** Chart depicting average numbers of *wt1b*<sup>+</sup> epicardial cells in control and dex-treated larvae. **E.** Ventricles of 5 dpf control *Tg(fli1a:NLS-mCherry)<sup>ubs10</sup>* and *il4-6xHis*-overexpressing *Tg(fli1a:NLS-mCherry)<sup>ubs10</sup>; Tg(hsp70:il4-6xHis)<sup>md74</sup>* larvae. **F.** Graph showing average amounts of *fli1a*<sup>+</sup> endocardial cells in control and *il4-6xHis*-overexpressing fish. **G.** hearts of 5 dpf control *Tg(wt1b:EGFP)<sup>li1</sup>* and *il4-6xHis*-overexpressing *Tg(wt1b:EGFP)<sup>li1</sup>; Tg(hsp70:il4-6xHis)<sup>md74</sup>* larvae. **H.** Chart depicting average numbers of *wt1b*<sup>+</sup> epicardial cells in control and *il4-6xHis*-overexpressing larvae. Data are presented as

mean  $\pm$  S.E.M. n.s. not significant, t-test. n indicates number of larvae used for experiments.

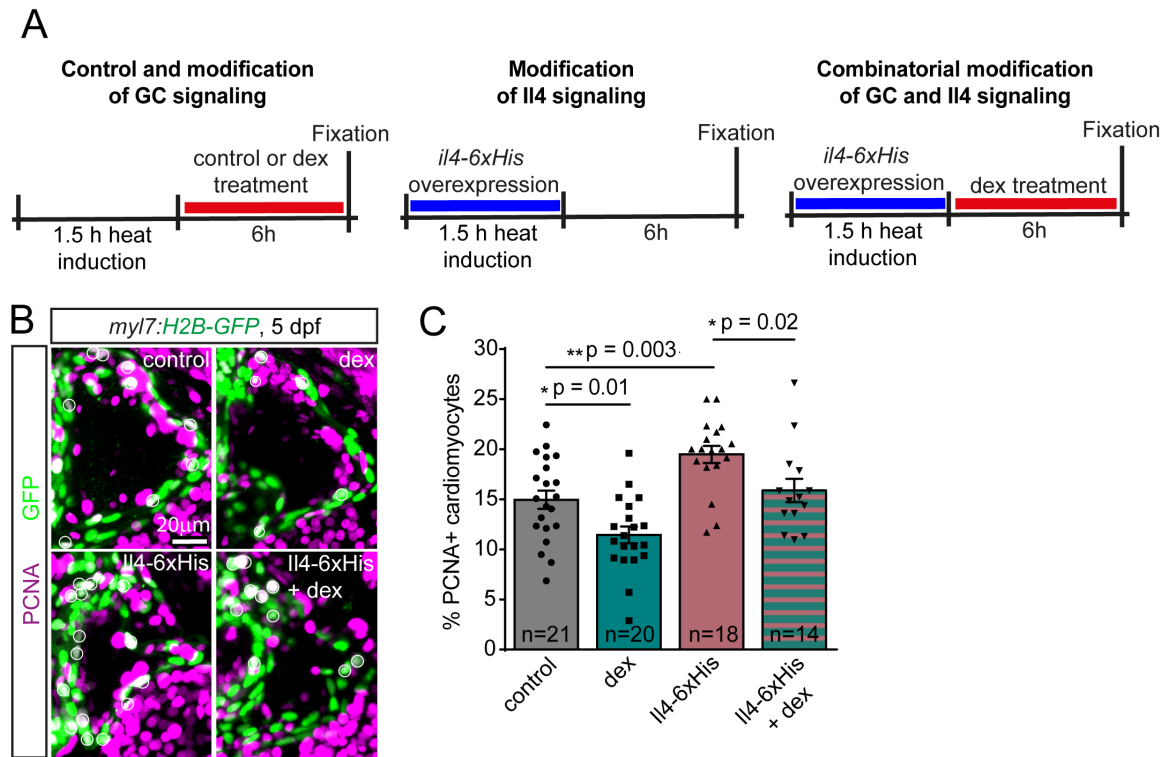


**Figure S3 (related to Figures 1 and 2).** GR activation and *il4-6xHis* overexpression do not alter percentage of ejection fraction and fractional shortening. Bar graphs showing percentage ejection fraction (A, C), and fractional shortening (B, D) of 5 dpf wild type larvae treated with dex or controls solution (A, B), or heat-exposed *Tg(myI7:H2B-GFP)<sup>zf521</sup>*; *Tg(myI7:mKate-CAAX)<sup>sd11</sup>* (control) and *Tg(hsp70:il4-6xHIS)<sup>md74</sup>*; *Tg(myI7:H2B-GFP)<sup>zf521</sup>*; *Tg(myI7:mKate-CAAX)<sup>sd11</sup>* (*il4-6xHIS*) larvae (C, D). Data are presented as mean  $\pm$  S.E.M. n.s. not significant, t-test. n indicates number of larvae used for experiments.

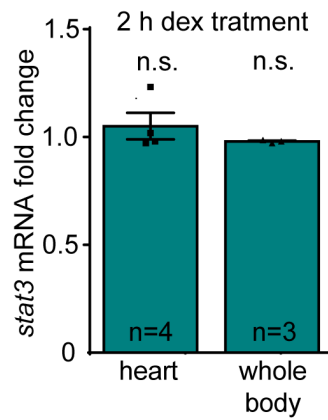


**Figure S4 (related to Figure 2).** *il4r*, but not *il4*, *ifng1* and *ifng1r*, is expressed in cardiomyocytes. **A.** Images showing *il4r* mRNA in situ hybridization on 3 dpf *Tg(myI7:H2B-GFP)<sup>zf521</sup>* larvae. *il4r* mRNA is present in the cytoplasm surrounding H2B-GFP positive cardiomyocyte nuclei. **B.** *il4* mRNA in situ hybridization on 3 dpf *Tg(myI7:mKate-CAAX)<sup>sd11</sup>*; *Tg(csf1ra:Gal4)<sup>i186</sup>*; *Tg(UAS:NTR-mCherry)<sup>c26</sup>* larvae. mKate-CAAX (labeling cardiomyocytes) and NTR-mCherry (labeling myeloid cells) were detected with an anti-RFP antibody. *il4* is present in the thymus, and is not expressed by cardiomyocytes. **C.** *il4* mRNA in situ hybridization on 3 dpf *Tg(kdrl:HRAS-mCherry)<sup>s896</sup>* larvae, showing that *il4* is not expressed by endocardial cells (labeled with HRAS-mCherry). The *il4* mRNA in the heart is localized in the cardiac lumen, most likely labeling erythrocytes. **D, E.** mRNA *in situ* hybridizations on *Tg(myI7:GFP)<sup>f1</sup>* (D) or *Tg(kdrl:HRAS-mCherry)<sup>s896</sup>* (E) fish, showing that interferon  $\gamma$  (*ifng1*) and its receptor *ifngr1* are not expressed in cardiomyocytes.

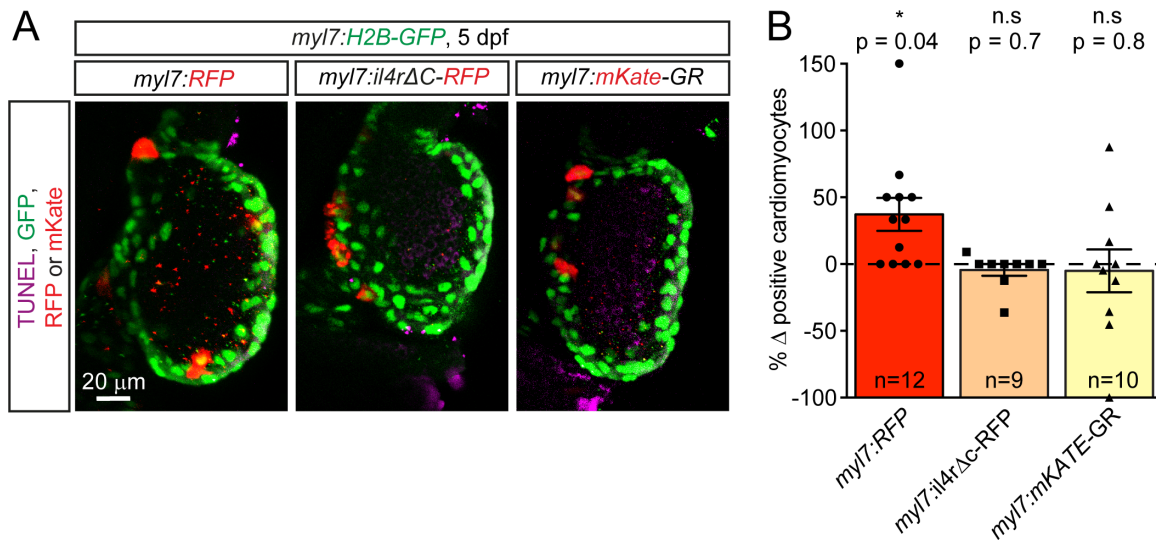




**Figure S5 (related to Figure 3).** GR and IL4 signaling regulates proliferation of cardiomyocytes in the atrium. **A.** Scheme summarizing timeline of dex treatment (red bars) and heat induction of *il4-6xHis* expression (blue bars). **B.** Images of 5 dpf *Tg(myI7:H2B-GFP)<sup>zf521</sup>* cardiac atria following control or dex treatment (dex), or *Tg(hsp70:il4-6xHis)<sup>md74</sup>*; *Tg(myI7:H2B-GFP)<sup>zf521</sup>* atria after *il4-6xHis* overexpression (IL4-6xHis) and combinatorial *il4-6xHis* overexpression and dex-treatment (IL4-6xHis + dex), stained with antibodies against PCNA and GFP. **C.** Bar chart depicting average percentage of PCNA-positive (PCNA+) atrial cardiomyocytes in 5 dpf larvae exposed to different combinations of GR signaling activation (dex) and *il4-6xHis* overexpression. Data are presented as mean  $\pm$  S.E.M. \*  $p < 0.05$ , \*\*  $p < 0.01$ , t-test. n indicates number of larvae used for experiments.



**Figure S6 (related to Figure 4).** GR activation does not alter expression of *stat3*. Graph showing average fold changes of *stat3* mRNA in the heart or whole body of 5 dpf larvae treated with dex for 2 hours. Data are presented as mean  $\pm$  S.E.M. n.s. not significant, t-test. n indicates biological replicates (number of samples of pooled hearts or larvae).



**Figure S7 (related to Figure 5).** Expression of transgenic *GR* or truncated *il4r* does not cause death of cardiomyocytes. **A.** Images of cardiac ventricles of 5 dpf *Tg(myl7:H2B-GFP)<sup>zf521</sup>* larvae injected at 1-2-cell-embryonic stage with plasmids containing *myl7:RFP*, *myl7:il4rΔC-RFP*, or *myl7:mKate-GR* transgenic constructs. Nuclei of cardiomyocytes are labeled by H2B-GFP. Cell death was detected with TUNEL assay. We did not detect TUNEL signal in cardiomyocytes expressing the transgenic constructs in all hearts analyzed. **B.** Graph showing average percentage change of amounts of cardiomyocytes expressing different transgenic constructs between 3 and 5 dpf. Numbers of cardiomyocytes containing the control *myl7:RFP* construct increased, while amounts of cells expressing *myl7:il4rΔC-RFP* or *myl7:mKate-GR* remained stable between the two developmental stages. These data suggest that the *myl7:il4rΔC-RFP* or *myl7:mKate-GR* inhibited proliferation, but did not induced cell death. Data are presented as mean  $\pm$  S.E.M. \*  $p < 0.05$ , n.s. not significant, t-test. n indicates number of larvae used for experiments.

Table S1		
Oligonucleotides	Source	Identifier
NheI-kozak-il4r.F: GATTGCTAGCGCCGCCACCATGAAGTTCAATGTTTCGTTT	Eurofins Genomics	N/A
NcoI-il4rdeltaC.R: GACACCATGGCCAAAAACAGATGAAGGTCAT	Eurofins Genomics	N/A
IL4.F: ATGAAGACCTGAAGATCTCAACATCTGGATACATC	Eurofins Genomics	N/A
IL4R.F: GTTTCGTTTGCGAATAGGGAAGCAG	Eurofins Genomics	N/A
T7IL4.R: TAATACGACTCACTATAGGGTTATGTCCTTTGAGCCGAG	Eurofins Genomics	N/A
T7IL4R.R: TAATACGACTCACTATAGGGGAGCAGTGGTGAATGAACTG	Eurofins Genomics	N/A
ifng1.F: ATGATTGCGCAACACATGATGGGCT	Eurofins Genomics	N/A
T7ifng1.R: TAATACGACTCACTATAGGGACCTCTATTTAGACTTTTGC	Eurofins Genomics	N/A
ifngr1.F:  GTTGGATACAACTCTGTGGTAATAATAATGCGGATATTGATCTGT C	Eurofins Genomics	N/A
T7ifngr1.R: TAATACGACTCACTATAGGGGAAAGCTCATGTACGCCTCG	Eurofins Genomics	N/A
<i>bcl2l1</i> forward: GCAGATTGTGTTATGGGTATGAGC	Eurofins Genomics	N/A
<i>bcl2l1</i> reverse: GGTTGCAGGGGTAGTTCCTC	Eurofins Genomics	N/A
<i>c-myc</i> forward: GGCAGCGATTGAGAAGATGAAG	Eurofins Genomics	N/A
<i>c-myc</i> reverse: CCGTCTCGTGCCTTTTCTGT	Eurofins Genomics	N/A
<i>cyclinD1</i> forward: GCCAAACTGCCTATACATCAG	Eurofins Genomics	N/A
<i>cyclinD1</i> reverse: TGTCGGTGCTTTTCAGGTAC	Eurofins Genomics	N/A
<i>gapdh</i> forward: GTGGAGTCTACTGGTGTCTTC	Eurofins Genomics	N/A
<i>gapdh</i> reverse: GTGCAGGAGGCATTGCTTACA	Eurofins Genomics	N/A
<i>il4</i> forward: CTGTTGGTACTTACATTGGTCCCC	Eurofins Genomics	N/A
<i>il4</i> reverse: AGTGTCTGTCTCATATATGTCAGGT	Eurofins Genomics	N/A
<i>il4r</i> forward: AGCAGCCAGCAGACTGAAAT	Eurofins Genomics	N/A
<i>il4r</i> reverse: ATGGGATCGTCACAAAGTGCT	Eurofins Genomics	N/A
<i>il13ra1</i> forward: GCATGTCAGAGCTTCCTCCG	Eurofins Genomics	N/A

<i>il13ra1</i> reverse: AGACCTTGTTGGTGGCAACT	Eurofins Genomics	N/A
<i>il13ra2</i> forward: GAGCGATGGAGGAGTGTTTCG	Eurofins Genomics	N/A
<i>il13ra2</i> reverse: ATTGGTACAGGCGCACTTCA	Eurofins Genomics	N/A
<i>socs1a</i> forward: GCGCTCTGAGGAAACCTCTA	Eurofins Genomics	N/A
<i>socs1a</i> reverse: GAGACTCATCGGTCGTTTTAGT	Eurofins Genomics	N/A
<i>stat3</i> forward: GGACTTCCCGGACAGTGAG	Eurofins Genomics	N/A
<i>stat3</i> reverse: ATCGCTTGTGTTGCCAGAG	Eurofins Genomics	N/A
Il4ra_wt: TGTGGGCTCAGAGTGACCAT	Eurofins Genomics	N/A
Il4ra_mut: CCAGACTGCCTTGGGAAAAG	Eurofins Genomics	N/A
Il4ra_common: CAGGGAACAGCCCAGAAAAG	Eurofins Genomics	N/A

**Table S1 (related to Key Resources Table).** List of oligonucleotides used for experiments.