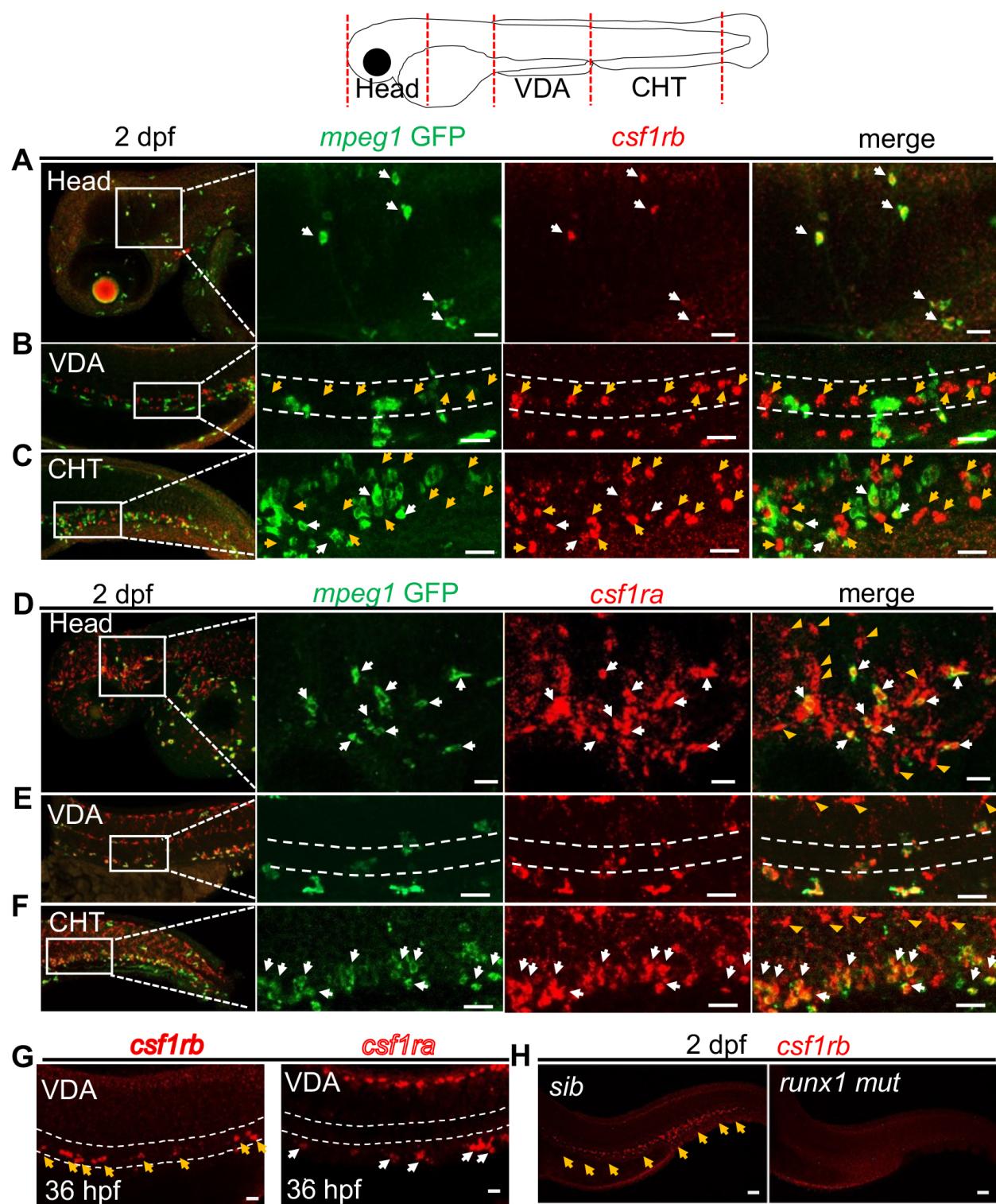
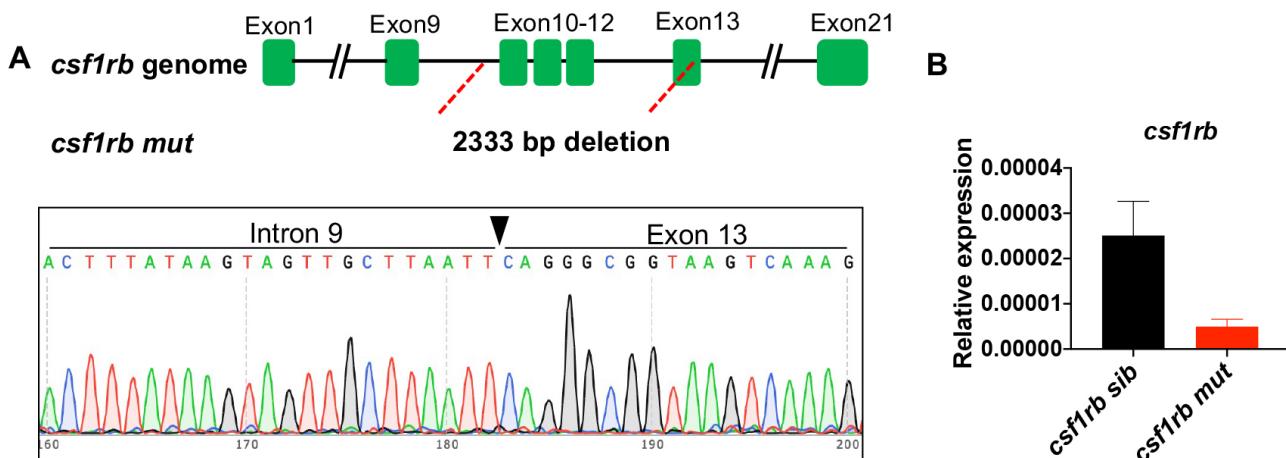


**Fig. S1**



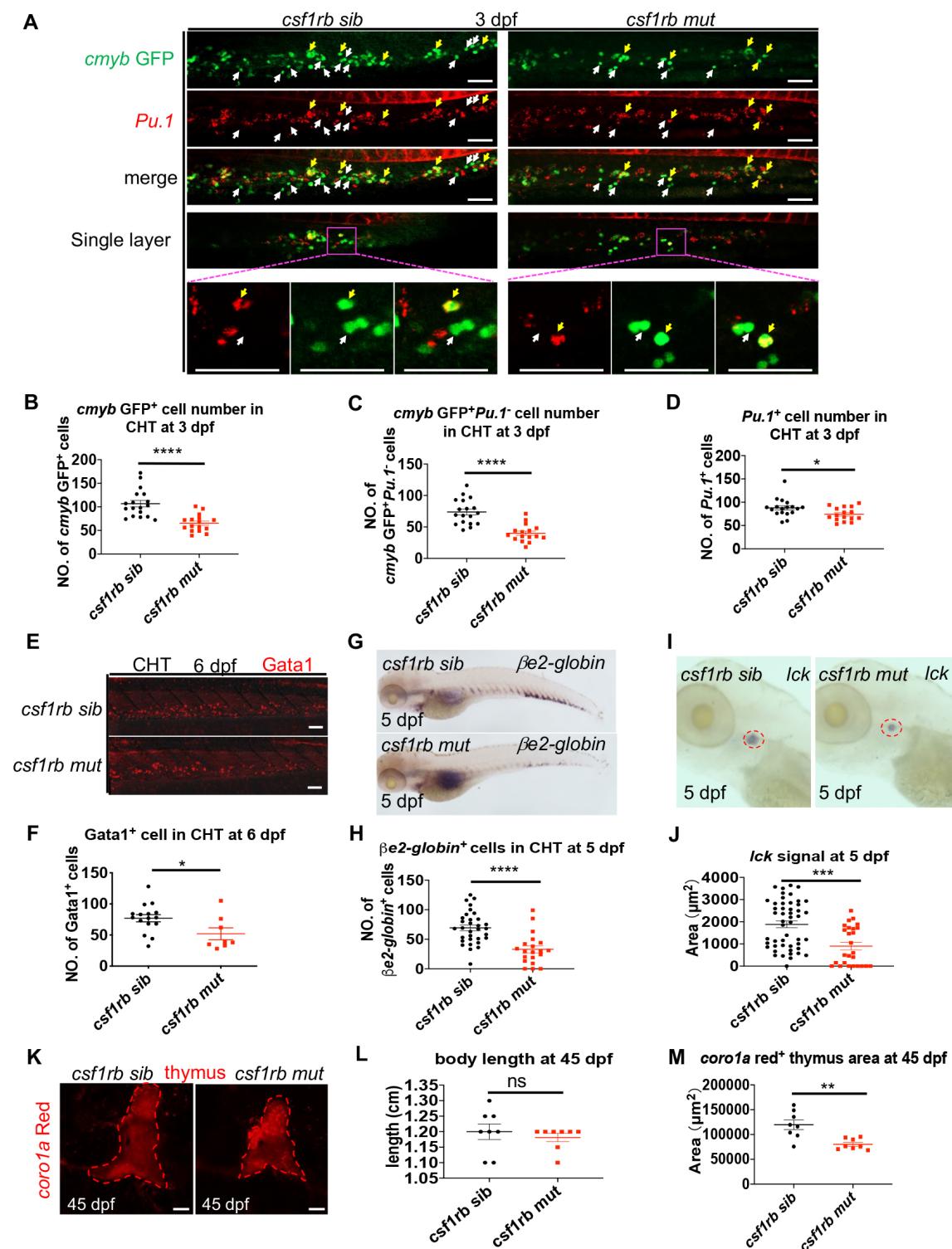
**Fig. S1. Expression of *csfIra*/*csfIrb* in zebrafish embryos.**

Diagram indicates the imaging region: the head, the VDA, and the CHT. **A-C.** WISH of *csfIrb* in the *Tg(mpeg1:GFP)* at 2 dpf. **A**, Head region; **B**, the VDA region; **C**, the CHT region. The white rectangle indicates the position zoomed in; the White dashed line indicates the VDA region. The white arrow indicates co-localization of *csfIrb* (red) and GFP<sup>+</sup> macrophages (green). The yellow arrow indicates *csfIrb* single positive signal. Scale bars, 20 μm. **D-F.** WISH of *csfIra* in the *Tg(mpeg1:GFP)* at 2 dpf. **D**, Head region; **E**, the VDA region; **F**, the CHT region. The white rectangle indicates the position zoomed in; the White dashed line indicates the VDA region. The white arrow indicates co-localization of *csfIra* (red) and GFP<sup>+</sup> macrophages (green). The yellow triangle indicates *csfIra* single positive xanthophore cells. Scale bars, 20 μm. **G.** WISH of *csfIrb* and *csfIra* at 36 hpf, White dashed line indicates the VDA region; Yellow arrow indicates *csfIrb*<sup>+</sup> HSPCs signal. White arrow indicates *csfIra*<sup>+</sup> macrophage signal; Scale bars, 20 μm. **H.** WISH of *csfIrb* at 2 dpf in *runx1* mutant, n (*sib*) = 52, n (*mut*) = 18; The yellow arrow indicates HSPCs signal. Scale bars, 50 μm.

**Fig. S2**

**Fig. S2. characterization of *csf1rb* mutant.** **A.** Diagram of *csf1rb* mutation. Mutant *csf1rb* genome harbors a 2333 bp deletion from intron 9 to exon 13, creating a pre-stop codon at exon14 in mutant *csf1rb* mRNA. The black triangle indicates the joint position of intron 9 and exon13 in mutant *csf1rb* genome. **B.** Relative expression level of *csf1rb* in *csf1rb* sibling and mutant embryos. Bars represent mean  $\pm$  s.e.m. of 3 biological replicates.

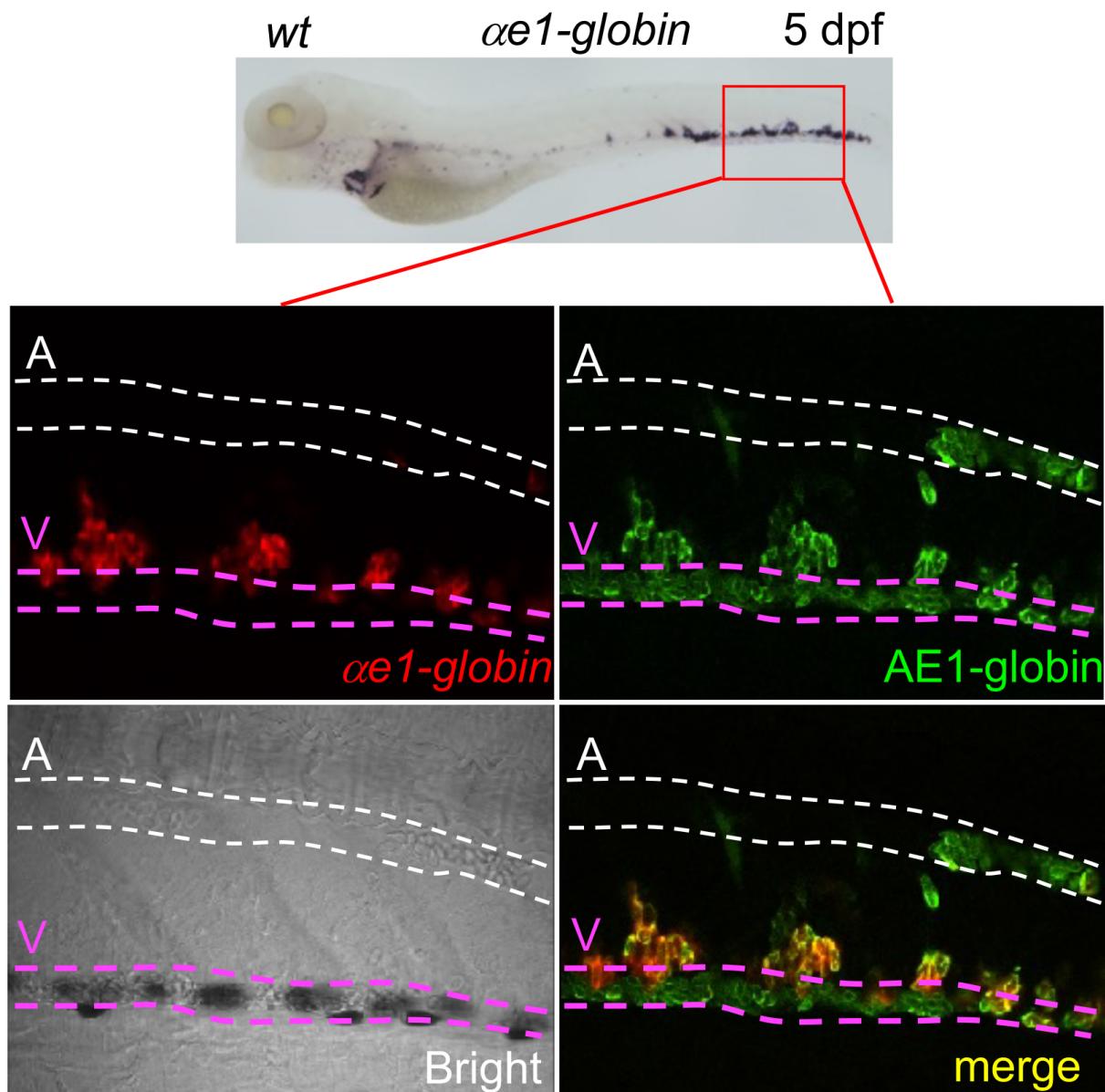
**Fig. S3**



**Fig. S3. HSPC and mature blood lineages defects in *csf1rb* mutant**

**A.** WISH of *pu.1* in the *Tg(cmyb:GFP)* at 3 dpf in the CHT region. The yellow arrow indicates co-localization of *pu.1* (red) and GFP (green). The white arrow indicates GFP single positive HSPC. The magenta square shows the enlarged region of a single Z layer, demonstrating *pu.1*/GFP double positive cells and GFP single positive cells. Scale bars, 50 µm. **B.** Quantification of *cmyb*-GFP positive total cells in the CHT region of *csf1rb* siblings (n=18) and *csf1rb* mutants (n=16). **C.** Quantification of *cmyb*-GFP<sup>+</sup>*pu.1*<sup>-</sup> cells in the CHT region of *csf1rb* siblings (n=18) and *csf1rb* mutants (n=16). **D.** Quantification of *pu.1* positive cell in the CHT region of *csf1rb* siblings (n=18) and *csf1rb* mutants (n=16). **E.** Immunostaining of Gata1 in the CHT at 6 dpf in siblings and *csf1rb* mutants. Scale bars, 50 µm. **F.** Quantification of the Gata1<sup>+</sup> erythroid progenitor cells in siblings (n=16) and *csf1rb* mutant embryos (n=8). **G.** WISH of  $\beta$  *e2-globin* at 5 dpf in siblings and *csf1rb* mutants. **H.** Quantification of  $\beta$ *e2-globin*<sup>+</sup> cells in the CHT at 5 dpf in siblings (n=32) and *csf1rb* mutants (n=20). **I.** WISH of *lck* at 5 dpf in siblings and *csf1rb* mutants. **J.** Quantification of *lck*<sup>+</sup> thymus area at 5 dpf in siblings (n=49) and *csf1rb* mutants (n=26). **K.** *Coro1a*-DsRedx<sup>+</sup> T cell signal in the thymus at 45 dpf in siblings and *csf1rb* mutants. Scale bars, 100 µm. **L.** Body length of siblings (n=8) and *csf1rb* mutants (n=8) at 45 dpf. **M.** Quantification of *coro1a*<sup>+</sup> thymus area at 45 dpf in siblings (n=8) and *csf1rb* mutants (n=8). Data are presented as mean ± s.e.m. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001; ns, not significant.

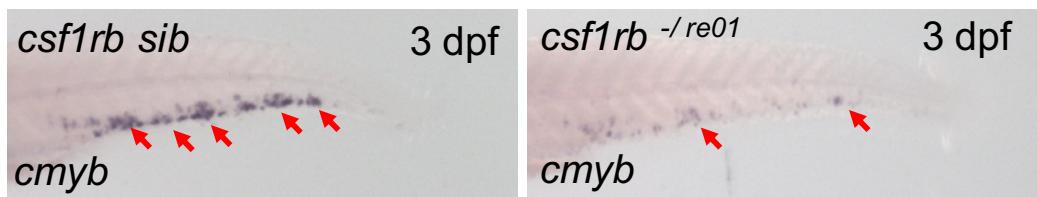
**Fig. S4**



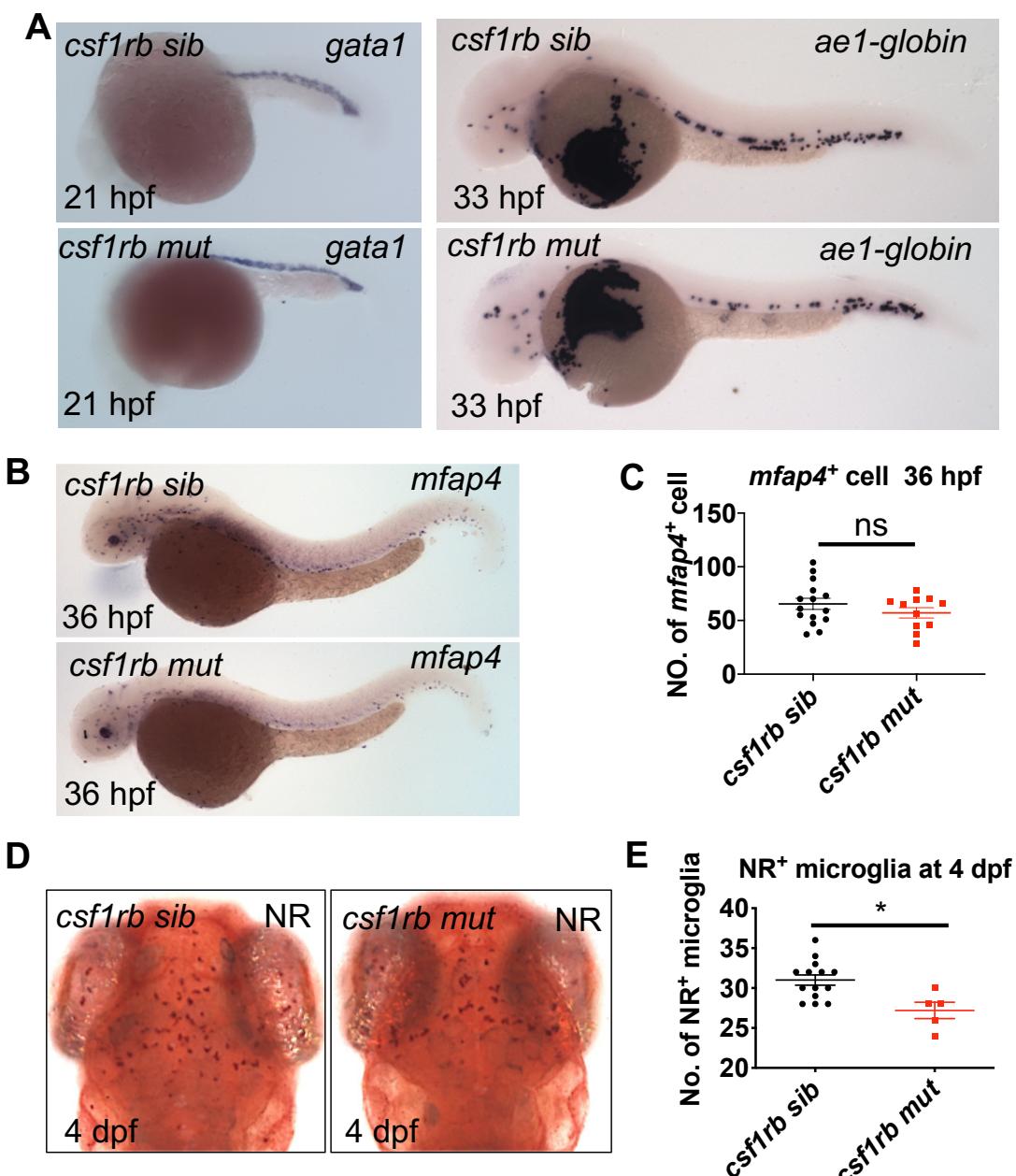
**Fig. S4. Immunostaining of  $\alpha e1\text{-globin}$  RNA and AE1-globin protein at 5 dpf.**

$\alpha e1\text{-globin}$  RNA (red) was mainly detected in the CHT with occasionally occurrence in the circulation. AE1-globin protein (green) retained in erythroid cells from both the circulation and the CHT. A, artery, white dashed line; V, vein, magenta dashed line.

**Fig. S5**

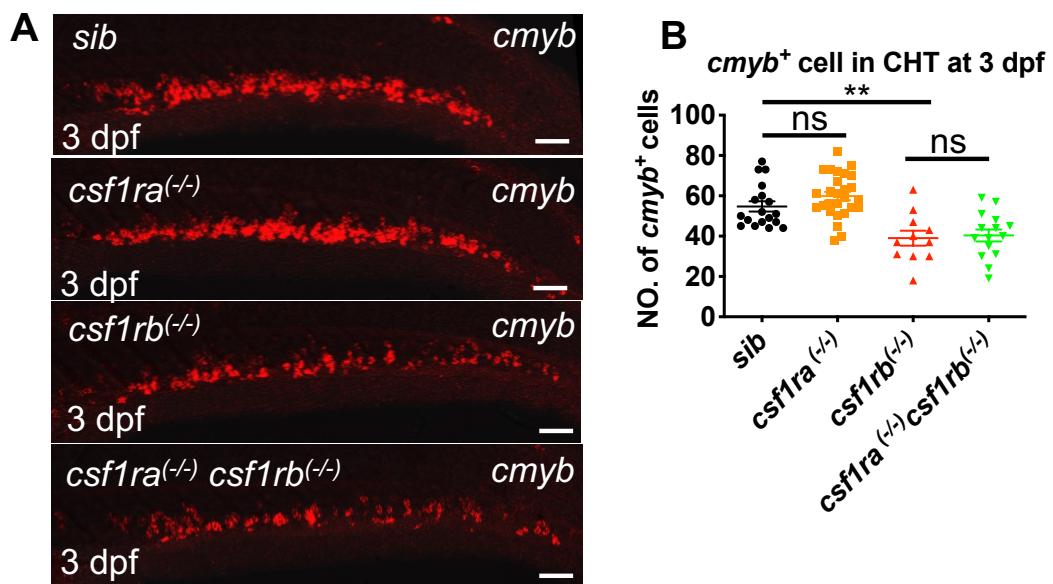


**Fig. S5. Hematopoietic defects in  $csf1rb^{-/-}$  mutants.** WISH of *cmyb* at 3 dpf in siblings ( $n = 21$ ) and  $csf1rb^{-/-}$  mutants ( $n = 18$ ). Red arrows indicate  $cmyb^+$  HSPCs.

**Fig. S6****Fig. S6. Primitive erythroid and myeloid development in siblings and *csf1rb* mutants.**

**A.** WISH of *gata1* at 21 hpf and *ae1-globin* at 33 hpf in siblings ( $n_{gata1} = 13$ ,  $n_{ae1} = 24$ ) and *csf1rb* mutants ( $n_{gata1} = 13$ ,  $n_{ae1} = 8$ ). **B.** WISH of *mfap4* at 36 hpf in siblings and *csf1rb* mutants. **C.** Quantification of the *mfap4<sup>+</sup>* myeloid cells of siblings ( $n=15$ ) and *csf1rb* mutant embryos ( $n=11$ ). **D.** Neutral red (NR) staining at 4 dpf in siblings and *csf1rb* mutants. **E.** Quantification of the NR<sup>+</sup> microglia in the brain of siblings ( $n=14$ ) and *csf1rb* mutant embryos ( $n=5$ ). Data are presented as mean  $\pm$  s.e.m. \* $p < 0.05$ , ns, not significant.

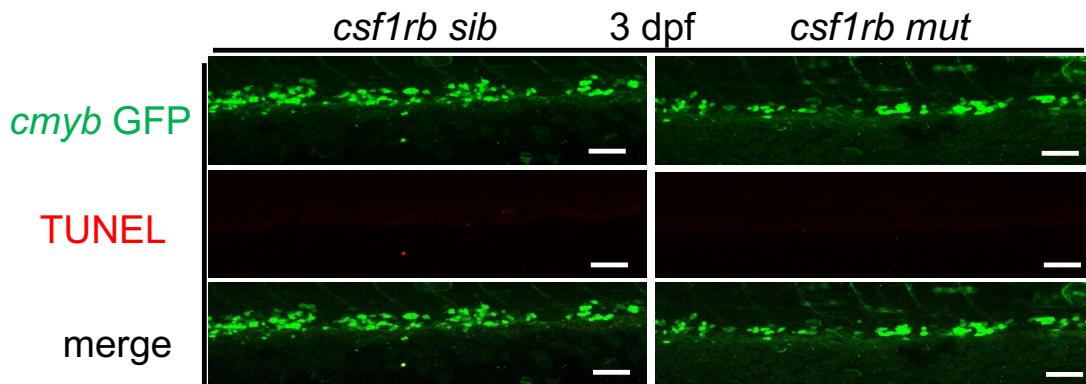
**Fig. S7**



**Fig. S7. HSPCs defects in *csf1ra* and *csf1rb* mutants.**

**A.** WISH of *cmyb* in siblings, *csf1ra*/*csf1rb* single or double mutants at 3 dpf. Scale bars, 50  $\mu$ m. **B.** Quantification of *cmyb*<sup>+</sup> HSPCs in the CHT at 3 dpf in siblings and *csf1ra*/*csf1rb* single or double mutants. n (*csf1rb*<sub>sib</sub> + *csf1ra*<sub>mut</sub> + *csf1rb*<sub>mut</sub> + *csf1ra*<sub>mut</sub>*csf1rb*<sub>mut</sub>) = n (18 + 27 + 11 + 15). Data are presented as mean  $\pm$  s.e.m. \*\* $p < 0.01$ . ns, not significant.

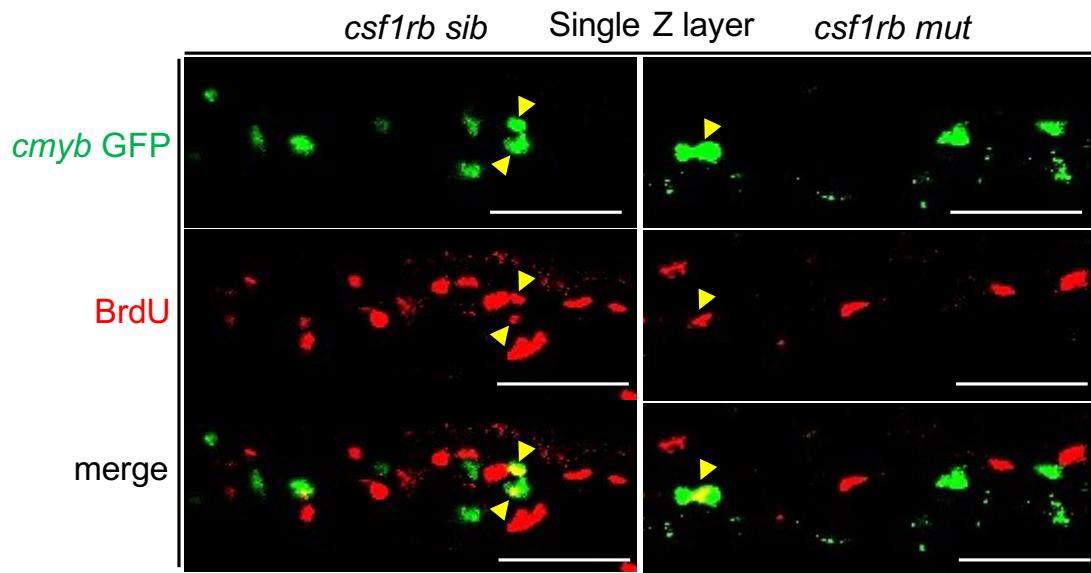
**Fig. S8**



**Fig. S8. No cell death of HSPCs is detected in *csf1rb* mutants.**

TUNEL assay in the CHT at 3 dpf in siblings (n=53) and *csf1rb* mutants (n=16). TUNEL is shown with red color. *cmyb*-GFP<sup>+</sup> HSPCs are indicated with green color. Scale bars, 50  $\mu$ m.

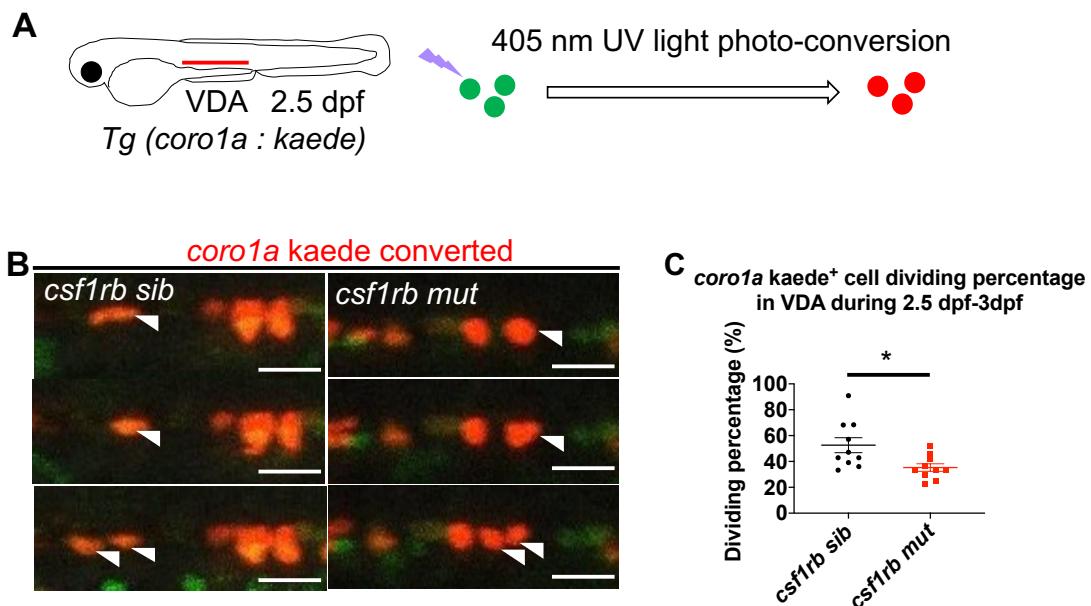
**Fig. S9**



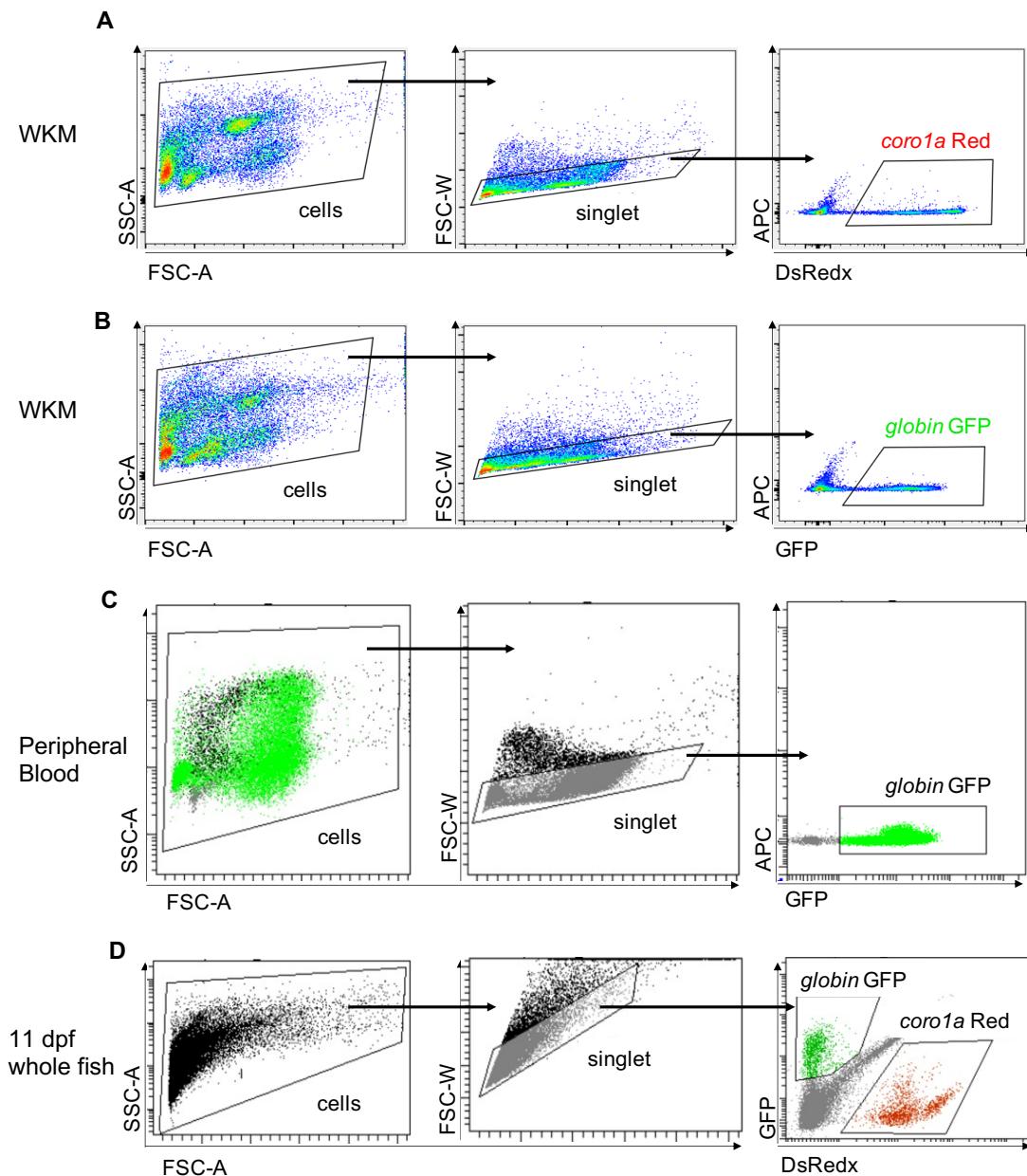
**Fig. S9. Demonstration of co-localization of BrdU and *cmyb*-GFP in *csf1rb* siblings and mutants within single layer images.**

Yellow arrow heads indicate Brdu (red) and GFP (green) double positive HSPCs. Scale bars, 50  $\mu$ m.

**Fig. S10**

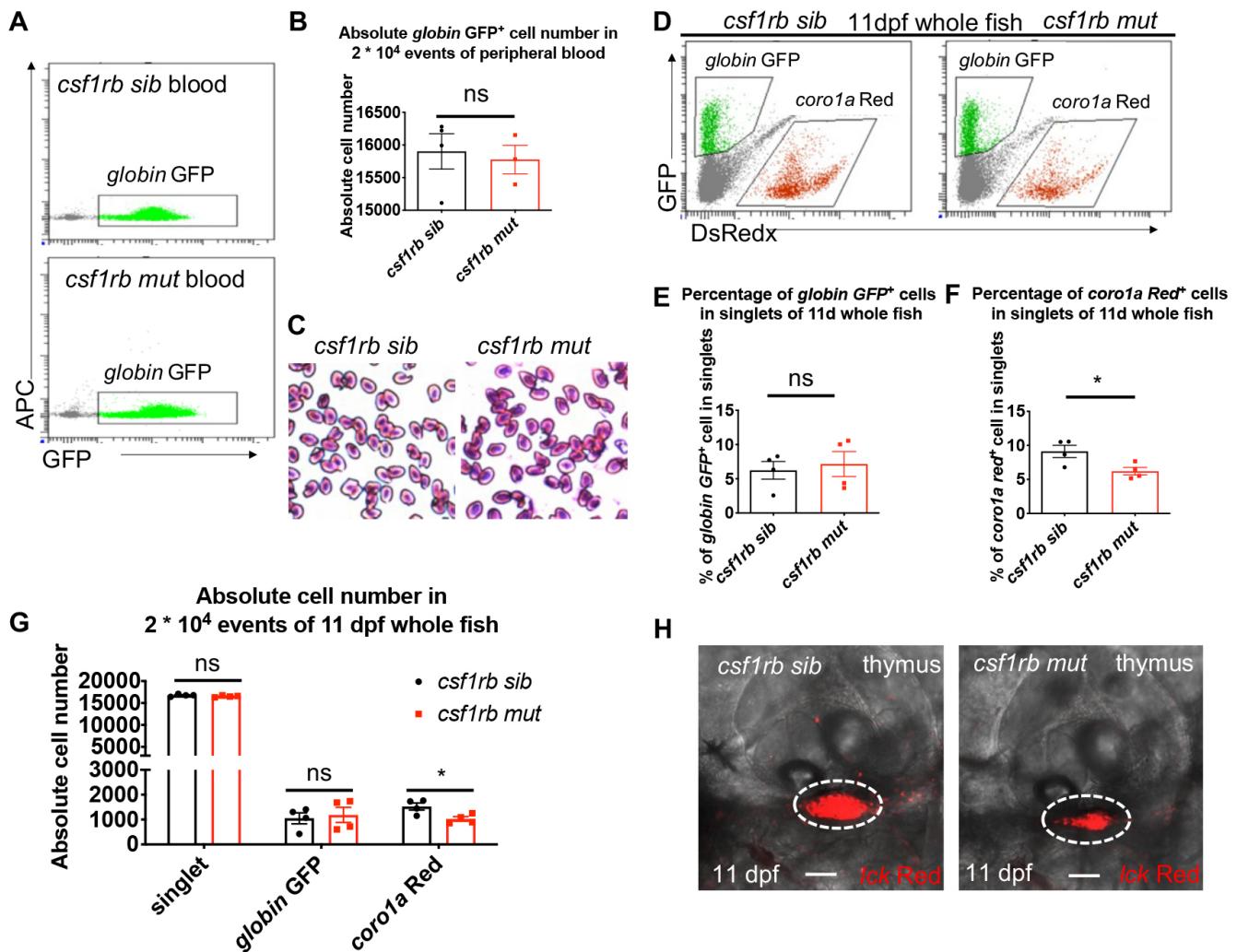


**Fig. S10. *Csf1rb* deficiency impairs the proliferation capacity of *coro1a*<sup>+</sup> HSPC/hematopoietic cells in the VDA.** **A.** Diagram of photo-conversion of *coro1a*- kaede<sup>+</sup> cells in the VDA. The Red line indicates the VDA region. Kaede<sup>+</sup> cells are shown in green color, after UV light conversion, cells are labeled in red color in the VDA. **B.** Conversion of *coro1a*-Kaede<sup>+</sup> cells in the VDA at 2.5 dpf in both siblings and *csf1rb* mutants. The Left and right panels show the dividing *coro1a*<sup>+</sup> cells in sibling and mutant, respectively. White triangles indicate the cell undergoes division. Scale bars, 20  $\mu$ m. **C.** Dividing percentage of converted *coro1a*<sup>+</sup> cells in the VDA from 2.5 dpf to 3dpf in siblings (n=10) and *csf1rb* mutants (n=10). Data are presented as mean  $\pm$  s.e.m. \* $p < 0.05$

**Fig. S11****Fig. S11. Gating strategies of flow cytometry.**

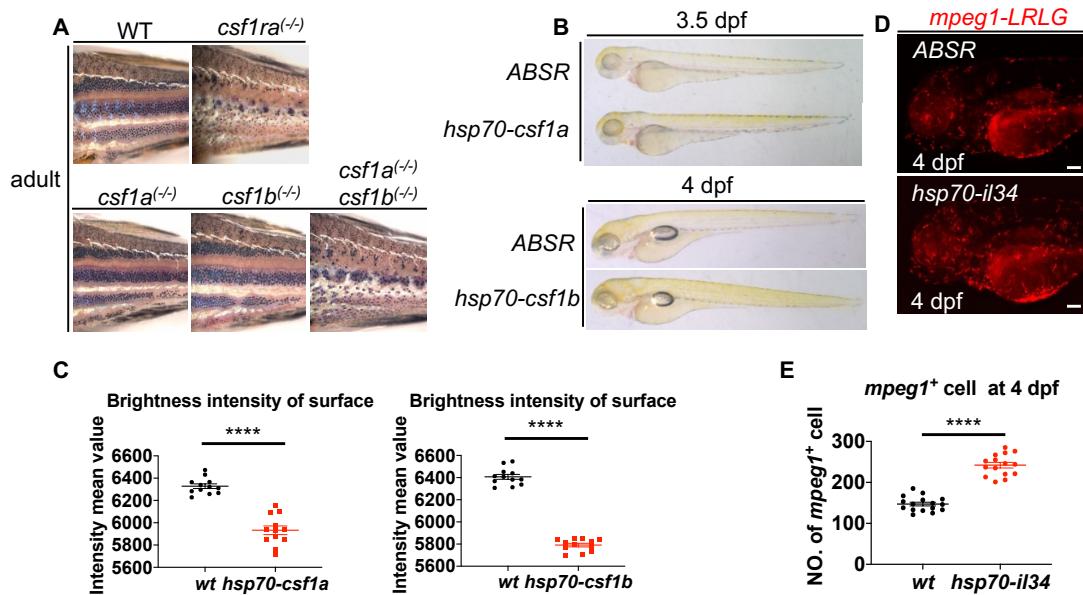
**A.** Gating strategies of flow cytometry of the adult whole kidney marrow cells (WKM) with *corola*-DsRedx<sup>+</sup> cells. **B.** Gating strategies of flow cytometry of the adult WKM with *globin*-GFP<sup>+</sup> cells. **C.** Gating strategies of flow cytometry of adult peripheral blood with *globin*-GFP<sup>+</sup> cells. **D.** Gating strategies of flow cytometry of 11 dpf whole fishes with *corola*-DsRedx<sup>+</sup> cells and *globin*-GFP<sup>+</sup> cells.

**Fig. S12**

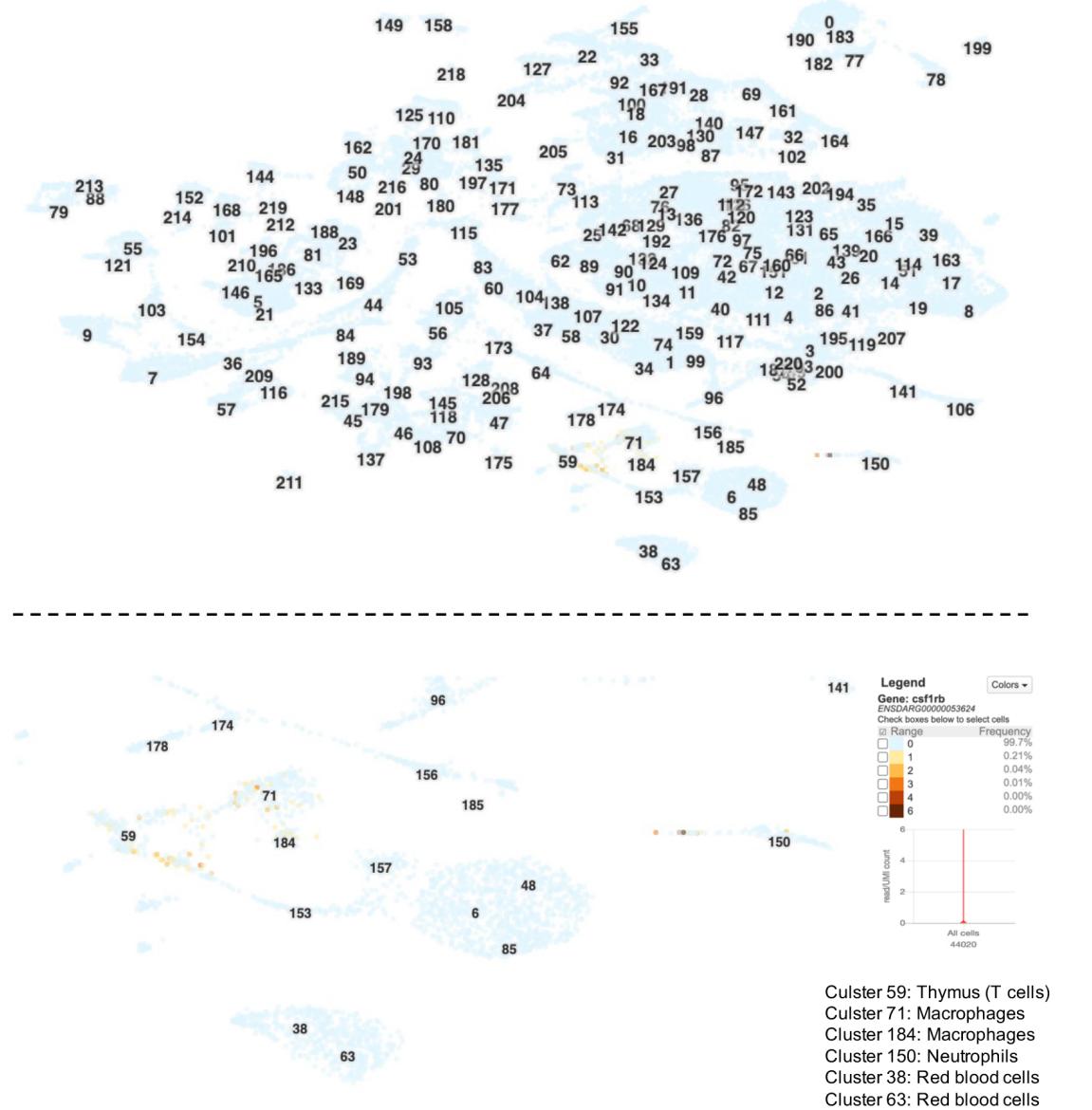


**Fig. S12. Normal Erythroid population in peripheral blood of adult *csf1rb* mutant and erythroid defects recover in juvenile *csf1rb* mutant.**

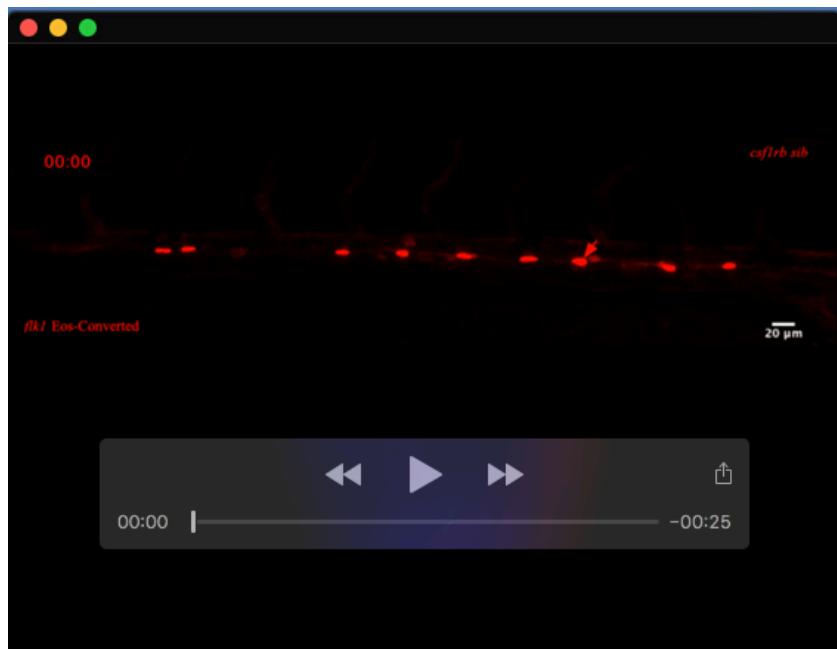
**A.** Flow cytometry file of peripheral blood of adult siblings and *csf1rb* mutants within the *Tg(globin:GFP)*, labeling mature erythroid cells. **B.** Absolute cell number of *globin*-GFP<sup>+</sup> erythroid cells in 2\*10<sup>4</sup> cell events of adult peripheral blood in siblings (n=4) and *csf1rb* mutants (n=3). **C.** May-Grunwald and Giemsa staining of adult peripheral blood in siblings and *csf1rb* mutants, top, siblings, bottom, mutants. **D.** Flow cytometry file of 11 dpf whole fish of siblings and *csf1rb* mutants within the *Tg(globin:GFP)* and *Tg(coro1a:DsRedx)*, labeling mature erythroid cells and leukocytes/progenitors, respectively. **E.** Percentage of *globin*-GFP<sup>+</sup> erythroid cells in singlets of whole fish in 11 dpf siblings (n=4) and *csf1rb* mutants (n=4). **F.** Percentage of *coro1a*-DsRedx<sup>+</sup> progenitors/leukocytes in singlets of whole fish in 11 dpf siblings (n=4) and *csf1rb* mutants (n=4). **G.** Absolute cell number of *coro1a*-DsRedx<sup>+</sup> cells and *globin*-GFP<sup>+</sup> erythroid cells in 2\*10<sup>4</sup> events of whole fish in 11 dpf siblings (n=4) and *csf1rb* mutants (n=4). **E-G**, each dot represents 2 juveniles pooled. **H.** *lck*-DsRedx<sup>+</sup> T cells in the thymus in 11 dpf siblings (n=11) and *csf1rb* mutants (n=10), white dashed line indicates the thymus region. Scale bars, 50 µm. Data are presented as mean ± s.e.m. \*p < 0.05. ns, not significant.

**Fig. S13****Fig. S13. Loss of function and gain of function of ligands.**

**A.** Double deficiency of *csf1a* and *csf1b* recapitulates pigment defects of *csf1ra* mutant fish at adulthood, suggesting that mutations of Csfl ligands lead to complete loss of protein functions. **B.** Ectopic expression of *csf1a* or *csf1b* increases xanthophore cells (yellow), suggesting functional overexpression of ligands. **C.** Quantification of surface brightness intensity of *wt* fish and fish with ectopic expression of *csf1a* or *csf1b*. **D.** Ectopic expression of *il34* increases *mpeg1*<sup>+</sup> macrophages, suggesting functional overexpression of ligand. Scale bars, 100  $\mu$ m. **E.** Quantification of *mpeg1*<sup>+</sup> macrophages in the imaged region of *wt* fish and fish with ectopic expression of *il34*. Data are presented as mean  $\pm$  s.e.m. \*\*\*\* $p < 0.0001$ . ns, not significant.

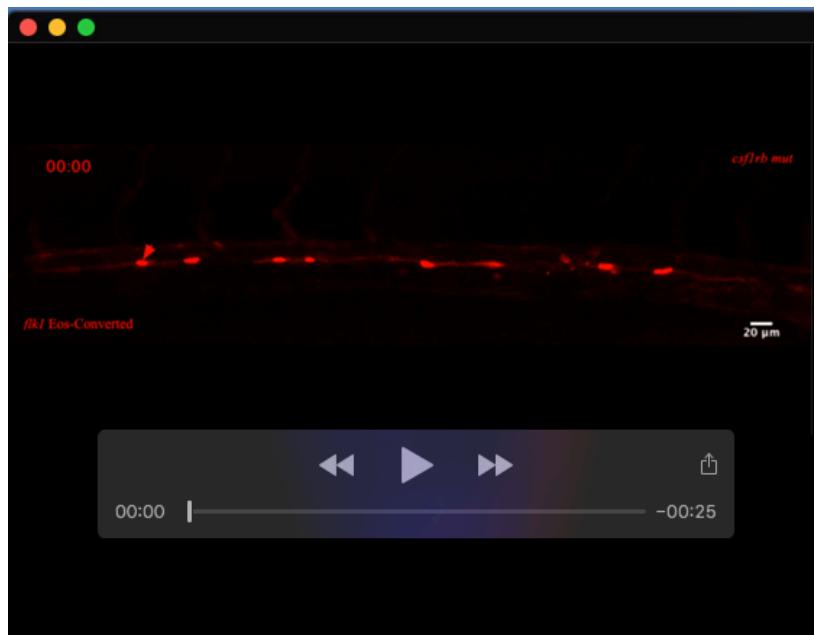
**Fig. S14****Fig. S14. Relative expression of *csf1rb* in the UCSC Cell Browser with Atlas for Zebrafish Development Dataset.**

Upper panel, cell type clusters from Single-cell RNA-seq data of 1 dpf, 2 dpf, and 5 dpf zebrafish embryos are defined and numbered in the UCSC Cell Browser. Bottom panel, clusters of T cells, myeloid cells, and erythrocytes are enlarged to show the relative expression of *csf1rb*. Expression of *csf1rb* were detected in T cells of the thymus (cluster 59) and myeloid cells (cluster 71, cluster 184, and cluster 150), but not in erythrocytes (cluster 38 and cluster 63).



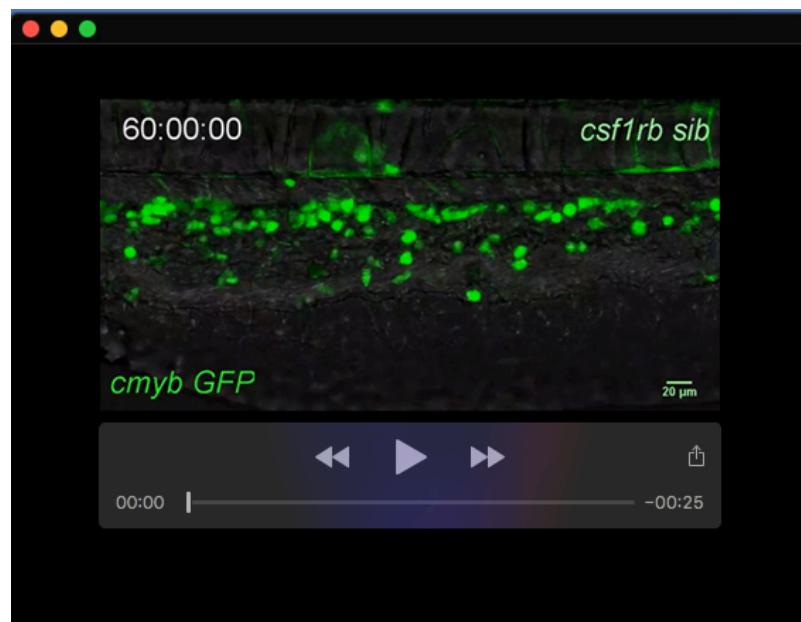
### Movie 1. EHT process in *csf1rb* sibling.

Time-Lapse imaging of converted *flk1*-Eos<sup>+</sup> endothelial cells (Red<sup>+</sup>) in the VDA region from 30 hpf right after conversion in *csf1rb* siblings. The red arrow indicates the cell undergo EHT during imaging. Time scale (min: sec) indicates the time starting from live imaging.



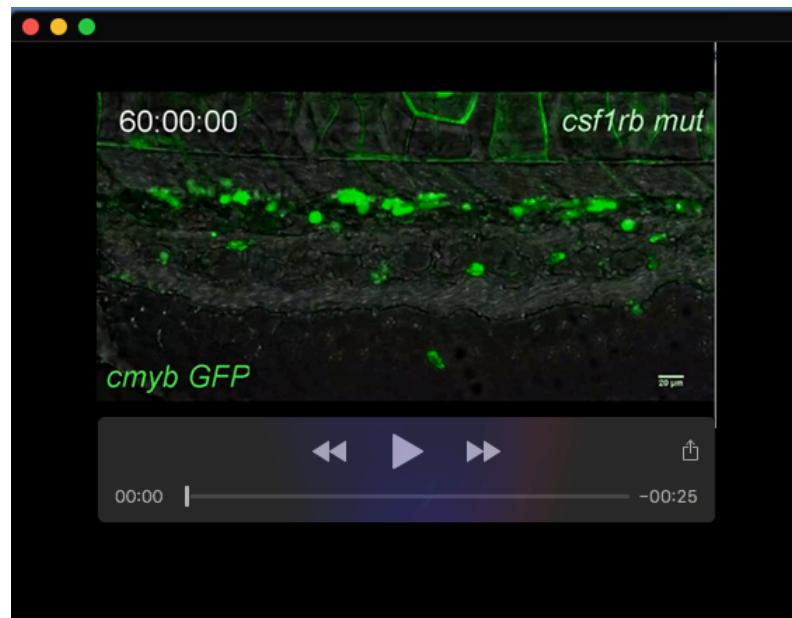
### Movie 2. EHT process in *csf1rb* mutant.

Time-Lapse imaging of converted *flk1*-Eos<sup>+</sup> endothelial cells (Red<sup>+</sup>) in the VDA region from 30 hpf right after conversion in *csf1rb* mutants. The red arrow indicates the cell undergo EHT during imaging. Time scale (min: sec) indicates the time starting from live imaging.



**Movie 3. Time-Lapse imaging of HSPCs in *csf1rb* sibling.**

Time-Lapse imaging of *cmyb*-GFP<sup>+</sup> HSPCs in the CHT region from 60-86 hpf in *csf1rb* siblings. Time scale (hr: min: sec) indicates the time starting from the development stage.



**Movie 4. Time-Lapse imaging of HSPCs in *csf1rb* mutant.**

Time-Lapse imaging of *cmyb*-GFP<sup>+</sup> HSPCs in the CHT region from 60-86 hpf in *csf1rb* mutants. Time scale (hr: min: sec) indicates the time starting from the development stage.