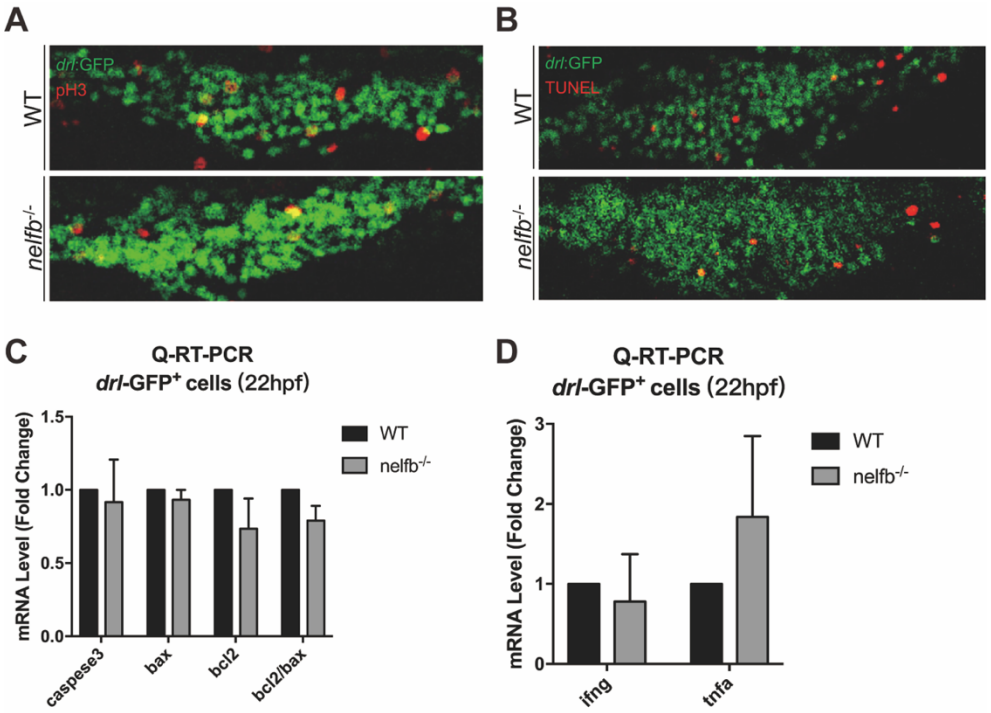
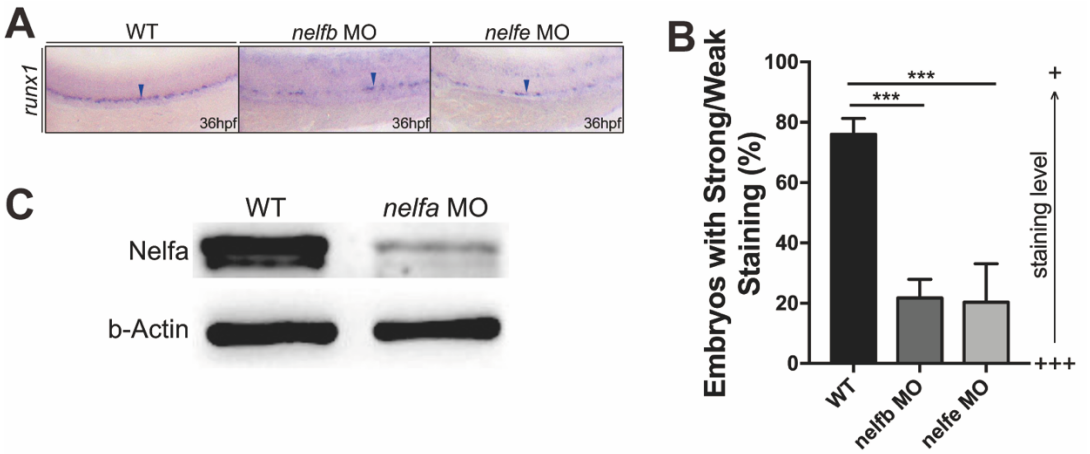
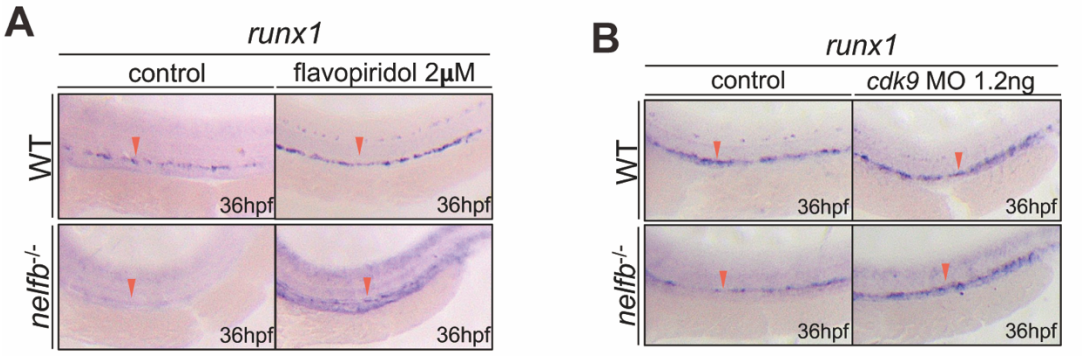



**Supplementary Figure S1. Proliferation, apoptosis and inflammatory cytokine expression of hematopoietic cells in *nelfb*$^{-/-}$ mutants are normal compared with WT siblings.**
**(A)** Double immunofluorescent staining for GFP (green) and phosphorylated histone H3 (pH3, red) in Tg(*drl:GFP*) embryos. **(B)** TUNEL staining in PBI of Tg(*drl:GFP*) embryos. **(C)** Q-RT-PCR analysis of apoptosis-related gene expression in sorted *drl-GFP*$^{+}$ cells in *nelfb*$^{-/-}$ and WT embryos at 22 hpf. **(D)** Q-RT-PCR analysis of inflammatory gene expression in sorted drl-GFP$^{+}$ cells in *nelfb*$^{-/-}$ and WT embryos at 22 hpf. PBI, posterior blood island.

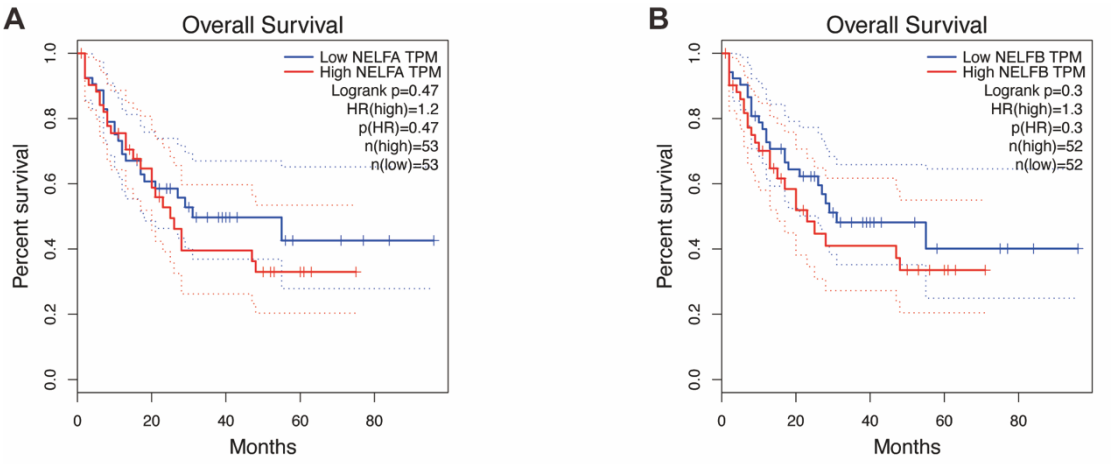



**Supplementary Figure S2. *nelfa*, *nelfb* and *nelfe* mopholino knock-down efficiency.**
**(A)** WISH for *runx1* in WT, *nelfb* or *nelfe* morphants at 36 hpf. **(B)** Quantification of WISH results in A (30-50 embryos per group). All results are presented as the mean ± SD from three independent experiments (t test, *** for p<0.001). **(C)** Western blot of Nelfa from WT and *nelfa* morphants at 22 hpf. B-Actin serves as a loading control.




**Supplementary Figure S3. CDK9 inhibition promotes HSC development.**

**(A, B)** WISH for *runx1* at 36 hpf in wildtype and *nelfb*$^{-/-}$ embryos treated with flavopiridol or injected with 1.2 ng of *cdk9* MO, respectively. Orange arrowheads indicate AGM region.




**Supplementary Figure S4. Low NELF expression elevate AML patients' overall survival rate**

**(A, B)** Overall survival (OS) analysis based on low- or high-expression of NELFA **(A)** or NELFB **(B)** by GEPIA (http://gepia.cancer-pku.cn) [1]

**Supplementary Table S1. Primers for *nelfb*$^{-/-}$ genotyping**

| primer | sequence (5'→3') |
|---|---|
| sense primer | ACAGACGGAAAATGGCATATTG |
| antisense primer | TTCACCTGTCCTCCTCCTTG |

**Supplementary Table S2. Primers for Q-RT-PCR**

| gene | sense primer (5'→3') | antisense primer (5'→3') |
|---|---|---|
| actb (5' end) | gatcttcactccccttgttca | ataccggagccgttgtca |
| actb (3' end) | cctctcttgctccttccacc | actcctgcttgctgatccac |
| scl (5' end) | cggtgacctcggatgagaag | tcagcggaaattgctcggat |
| scl (3' end) | tcagcggaaattgctcggat | ttgctaggatacatcccatactgtt |
| pu.1 (5' end) | ccactgtcagggcaggttac | actcacgcaccttatcacgt |
| pu.1 (3' end) | caacaggaggagagcatggta | tgttccttttctcgatccac |
| mpx (5' end) | agcacaacttactctgagcttca | cagcccaccacaaaaagaaaagt |
| mpx (3' end) | tcgagccttggaaagagact | cctgctacaccctgtggac |
| mfap4 (5' end) | gaaccgagaactgctgtcct | gagtctcgtgctctgtgtca |
| mfap4 (3' end) | tatcggccgtggagagacta | tgaacttcttgtggcgtgtc |
| gata1 (5' end) | gataagcaagcaaacaggcg | agaggagttctccatctgca |
| gata1 (3' end) | tgagactttcgccacctga | cagtccacaagcattacagagg |
| runx1 | cgtcttcacaaaccctcctcaa | gctttactgcttcatccggct |
| cmyb | tcgccagcttctaccaaa | cagggttgaggactttctgc |
| rag1 | tgagagctgggaatgaacaca | ccgtgcggtacatcttgtga |
| nelfa | tggatcacgagaaaaccctg | aaacactgtgtccaccagcat |
| nelfb | aaggtgctgccgacttacat | tccttcaccgactcactgc |
| nelfe | gaatcagcagaccaggctgt | gagaagcccagacagatttcc |

**Supplementary Table S3. Mopholino Sequences**

| MO | sequence (5'→3') | reference |
|---|---|---|
| nelfa-ATG | CGCTCTCCCTCATCGACGCCATCTT | this article |
| nelfb-ATG | CCGGCGAACATTTTCACACGCTCTC | [2] |
| nelfe-ATG | AACTGGGAAATATTGCCATTTTGC | [2] |
| cdk9-spl | ACACACAAACATCAAATACTCACCC | [2] |

**Supplementary Methods**

*TUNEL immunostaining*

The 22hpf Tg(*drl:GFP*) embryos were fixed in 4% PFA overnight at 4°C and dehydrated in 100% methanol at -20°C over 30 minutes. Further processing of embryos was conducted according to the previous article [3]. For analysis, the embryos were mounted in 1% low-melt agarose and imaged under a confocal microscope Leica SP8 microsystems (Leica, Heidelberg, DE, Germany).

*Phosphorylated Histone 3 Immunostaining*

The 22hpf Tg(*drl:GFP*) embryos were fixed in 4% PFA + 1% DMSO in 0.1M phosphate buffer (0.081M $Na_2HPO_4$, 0.014M $NaH_2PO_4$, pH7.4) overnight at 4°C. Embryos were dehydrated in 100% methanol at -20°C overnight after being washed with 0.1M phosphate buffer for 3 times. After digestion with pre-chilled acetone at -20°C for 15 minutes, the embryos were washed with incubation buffer (0.1M phosphate buffer + 0.2%BSA + 0.5%Triton-X) 15 minutes for 3 times and incubated with phosphorylated histone 3 primary antibody (#3377, CST) overnight at 4°C. The embryos were re-washed with incubation buffer 15 minutes for 3 times and incubated with coralite594-conjugated secondary antibody (SA00013-4, Proteintech) at room temperature for 4 hours.

The embryos were mounted in 1% low-melt agarose and imaged under a confocal microscope Leica SP8 microsystems (Leica, Heidelberg, DE, Germany) for analysis.

*Westernblot*

22 hpf embryos were dechorionated. Whole embryos were collected except yolk and treated with lysis buffer (RIPA lysis buffer (P0013B Beyotime Biotechnology, China) + 1mM PMSF (ST506 Beyotime Biotechnology, China)) on ice for 30 minutes to extract protein. Antibodies targeting b-Actin (66009-1, Proteintech, China) and NELFA (10456-1-AP, Proteintech, China) were used as the primary antibodies.

**Reference**


1. Wu, D., et al., *Pharmacological inhibition of dihydroorotate dehydrogenase induces apoptosis and differentiation in acute myeloid leukemia cells.* Haematologica, 2018. **103**(9): p. 1472-1483.
2. Yang, Q., et al., *RNA polymerase II pausing modulates hematopoietic stem cell emergence in zebrafish.* 2016. **128**(13): p. 1701.
3. Yu, S., et al., *BCAS2 is essential for hematopoietic stem and progenitor cell maintenance during zebrafish embryogenesis.* Blood, 2019. **133**(8): p. 805-815.