Supplemental Material and Methods

Western blot

Protein was extracted from 3-dpf whole embryos, and was quantified, and assessed by western blot analysis as described previously[1]. Protein lysates were probed with rabbit anti-zebrafish-c-Myb (1:500 dilution, Global Biotech, ABIN1977902). Mouse antibody against human actin (1:5000 dilution, ABclonal, AC004) was included as an internal control.

Quantitative RT-PCR

All assays were performed in duplicate. The relative quantity of gene expression was calculated by the 2(-ΔΔCt) method with normalization to the level of elongation factor 1α (*ef1α*). Primers were designed using Primer5 software (Supplemental Table 1).

Dual-Luciferase Reporter Activity Assay

HKE293T cells in a 124-well plate were transfected with plasmids for expressing *c-myb*-WT, *c-myb*-T1 targeting *lect2l* promoter and the luciferase reporter pGL4.1-Luc and pRL-CMV for 24 h. The cell lysates were harvested for the Dual-Luciferase assay according to the instructions of the manufacturer. Three independent experiments were performed. Cells were regularly authenticated and tested for mycoplasma.

5'and 3'-RACE

RACE was performed using the SMARTer® RACE 5'/3' Kit (Clontech, CA, USA; 634858) in 2 dpf *c-myb-gfp/c-myb*$^{hkz3/hkz3}$ embryos[2], according to the manufacturer's instruction. *c-myb* gene-specific primer for 5'-RACE PCR was 5'-GATTACGCCAAGCTTTCCATGATCGAACGCCTCAGGTTGGGA-3', for 3'-RACE PCR

was 5’-GATTACGCCAAGCTTCGAGGCGGCACAGACACAGTGTTTACAG-3’. PCR products were electrophoresed for recovery, and were inserted into a pRACE vector for sub-cloning. More than 12 different individual clones were selected for sequencing.

Bromodeoxyuridine labeling

Bromodeoxyuridine (BrdU) labeling was performed as described previously.[3] Embryos were incubated with 10 mM BrdU (Sigma-Aldrich; B5002) for 2 h, and stained with mouse anti-BrdU (Roche, Germany; 10875400) and rabbit anti-Lcp, followed by Alexa Fluor anti-mouse 555 and anti-rabbit 488 (Invitrogen, CA, USA; A31570 and A21206) for fluorescent visualization.

Terminal deoxynucleotidyl transferase dUTP nick end labeling

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was carried out using the In Situ Cell Death Detection Kit TMR red (Roche; 12156792910) as described, followed by rabbit anti-Lcp and Alexa anti-rabbit 488 (Invitrogen; A21206). Images were captured using an Olympus fluoview 1000 confocal microscope.

Flow cytometry

Hematopoietic cells isolated from PB or KM in siblings or *c-myb*^{hyper} fish were processed as described above, washed and resuspended in 0.9× PBS plus 5% FBS, and then passed through a filter with a 40-μm pore size. Flow cytometry analysis and sorting were based on forward scatter and side scatter using a BD FACS Aria 1 flow cytometer (CA, USA).

Histology

Leukemic fish and age-matched controls were fixed for 3 days at 4˚C in 4% paraformaldehyde, then dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Tissues were sectioned at 5 μm and stained with hematoxylin and eosin (H&E).

Supplemental Figure legends

Supplemental Figure 1. Duplication sequences and different *c-myb* transcripts in *c-myb-gfp* transgenic fish. (A) *c-myb* mini promoter duplication, including a 315-bp core promoter (short purple bar) and 170-bp exon 1 sequence (black bar), and its surrounding sequence is indicated. **(B)** Sequences around the breakpoint of intron 10 for the second duplication (red box). Exons of *c-myb* (black bar); pWSMK-T (light blue bar) including GFP (green bar), SV40 polyA signal (red bar) and the reversed ampicillin resistance gene (AmpR, yellow bar); 77-bp additional unknown sequence are indicated (blue bar). **(C)** The 5'and 3'-RACE PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The 5'and 3'-RACE PCR products of endogenous *c-myb* and *c-myb*-WT is 1.5-kb and 3-kb, respectively. The 5'and 3'-RACE PCR products of *c-myb*-T1 is 2.8-kb and 4.2-kb, respectively. **(D)** Immunoblot detection of c-Myb proteins in sibling (left) and *c-myb*^{hyper} (right). **(E)** HEK293T cells were transfected with c-Myb-WT (blue bar) or c-Myb-T1 (red bar) targeting *lect2l* and Pgl4.1- Luc together with pRL-CMV as an internal control. (*t*-test, n=3; mean ±SD; **P*<0.05, ***P*<0.01).

Supplemental Figure 2. Granulocytes increased in *c-myb-gfp* embryos and myeloid expansion by *c-myb*-WT and *c-myb*-T1 overexpression. (A and B) Myeloid cells were incressed in *c-myb*^{hyper} embryos. WISH of *c/ebpa* (A) and *lcp* (B) expression at 3 dpf in *c-myb-gfp* fish and non-transgenic siblings. **(C)** Accumulation of neutrophils in *c-myb-gfp* embryos. WISH of *mpx* (C) expression at 3 dpf in *c-myb-gfp* fish and non-transgenic siblings. **(D and E)** Macrophages were not increased in *c-myb*^{hyper} embryos. WISH of *mfap4* (D) and

c-fms (E) expression at 3 dpf in *c-myb-gfp* fish and non-transgenic siblings. The CHT regions were enlarged in red box (20×). Numbers in each panel indicate the number of embryos with elevated expression of markers out of total number of fish (Fisher's exact tests, $P<0.01$). (F-H) Overexpression of *c-myb*-WT and *c-myb*-T1 under the *ef1a* promoter. cDNAs of *c-myb*-WT-*gfp*, *c-myb*-T1-*gfp* and *gfp* control driven by *ef1a* promoter were injected into one-cell stage WT embryos, respectively. Representative pictures of SB positive cell counting in 3 dpf embryos (F). SB^+ myeloid cell counts (G) and relative *c-myb* transcript levels (H) after injection were calculated and compared at 3 dpf (*t*-test; *gfp*, *c-myb*-WT-*gfp*, *c-myb*-T1-*gfp*, n=24, n=21 and n=20, respectively; mean±SD; **P*<0.05, ***P*<0.01).

Supplemental Figure 3. *c-myb*^{hyper} adult fish display MDS phenotype. (A-D) Abnormal myeloid cells are increased in 3-month *c-myb*^{hyper} kidney. Peripheral blood (PB) cells (A) and whole kidney marrow (KM) blood cells (C) in 3-month-old *c-myb*^{hyper} and sibling fish stained with May-Grunwald/Giemsa. Blood cell counts of PB (B) and KM (D) in siblings (blue bars) and *c-myb*^{hyper} (red bars) fish were calculated manually based on their morphology. The black asterisks indicate statistical differences (*t*-test) (PB in 3-month siblings and *c-myb*^{hyper}, n=6 and n=9, respectively; KM in 3-month siblings and *c-myb*^{hyper}, n=8 and n=8, respectively; mean±SEM; **P*<0.05, ***P*<0.01). **(E and F)** Analysis of *c-myb*^{hyper} fish blood components by flow cytometry. FSC was directly proportional to cell size and SSC was indicative of cellular granularity. Relative means of each scatter population were obtained from nine adult zebrafish. **(G-J)** Aberrant *c-myb* activity did not affect apoptosis of myeloid cells in embryos (G and H) and KM of adults (I and J). Double staining of TUNEL/Lcp show TUNEL

incorporation of CHT/KM Lcp$^+$ cells in 3 dpf (G) and 3 month (I) *c-myb*^{hyper} and siblings. Arrows indicate Lcp/TUNEL double-positive cells. Percentage of the CHT and KM localized Lcp$^+$ myeloid cells that incorporate TUNEL (H and J) in Lcp$^+$ myeloid cells (*t*-test, sibling and *c-myb*^{hyper}, n=13 and n=14, respectively; mean ±SEM; **P*<0.05, ***P*<0.01).

Supplemental Figure 4. Pathologic external features in *c-myb*^{hyper} fish. (A-J) External features in siblings (A) and *c-myb*^{hyper} fish (B-J). All siblings (A) and most *c-myb*^{hyper} fish (B) exhibit normal external features. Some of *c-myb*^{hyper} fish with AML (C-F) or ALL (G-J) show pathologic external features, such as cachexia (C and G), exophthalmos (D and H), bleeding (E and I), abdominal mass (F), and curvature (J). Arrows indicate diseased sites. Numbers in each panel indicate the number of fish with pathologic external features out of total number of fish.

Supplemental Figure 5. *c-myb*^{hyper} zebrafish develop AML and ALL in adulthood. (A) HE staining show neoplastic infiltration of gill, muscle, KM and liver in *c-myb*^{hyper} fish. Blue arrows indicate infiltration of neutrophils in MDS-like fish and red arrows indicate infiltration of myeloid blasts in AML-like fish. (B) HE staining shows invasion of gill, muscle, kidney, liver and nervous system components such as eye and brain in ALL-like fish. Yellow arrows indicate lymphoid infiltration in ALL-like fish. (C) Fish with lymphoid leukemic cell diffusing were identified from *c-myb*^{hyper} in *rag2-dsRed* background. *rag2-dsRed* siblings (upper panels) and diseased *rag2-dsRed*/*c-myb*^{hyper} (lower panels) fish. Numbers in each panel indicate the number of fish with leukemia infiltration out of total

number of fish (Fisher's exact tests, $P<0.05$).

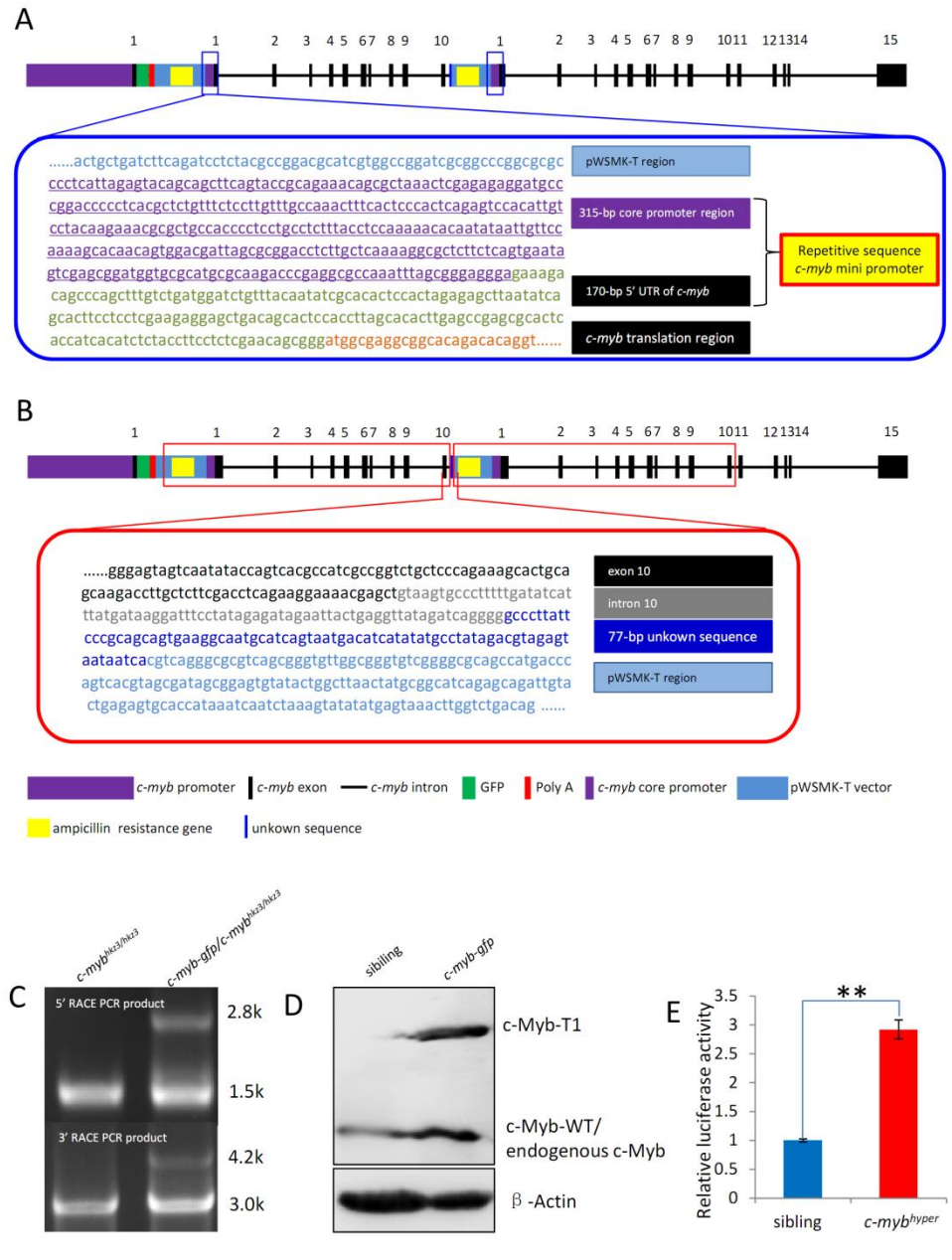
Supplemental Figure 6. Wild type fish transplant with MDS-like leukemic cells show myeloid expansion in the early stage. (A-C) dsRed positive cells repopulated in recipients within 2 weeks after transplantation of MDS-like *c-myb*^{hyper}*/lyz-dsRed* or *lyz-dsRed* control KM cells (A). KM cells in fish transplanted with *c-myb*^{hyper}*/lyz-dsRed* MDS cells (left panel) and *lyz-dsRed* KM cells (right panel) were stained by May-Grunwald/Giemsa (B). Blood cell counts of KM were calculated manually based on their morphology (C). (KM in recipients transplanted with *lyz-dsRed* and *c-myb*^{hyper}*/lyz-dsRed* KM cells. n=10 and n=10, respectively).

Supplemental Figure 7. The effects of chemotherapy on zebrafish development. (A) The percentages of the embryos that developed to normal or deformed depend on the chemotherapeutics concentration. Black, purple, green, blue and red lines indicate PBS, DMSO, Ara-C, flavopiridol and quizartinib, respectively (n=50). **(B)** Drug treatments in adult fish. Blood cell counts of 1 year siblings and *c-myb*^{hyper} KM after intraperitoneal injection with cytarabine (600 mg/kg/d or 2000mg/kg/d once daily for 4 days) (*t*-test, n=10; mean ±SD; **P*<0.05, ***P*<0.01).

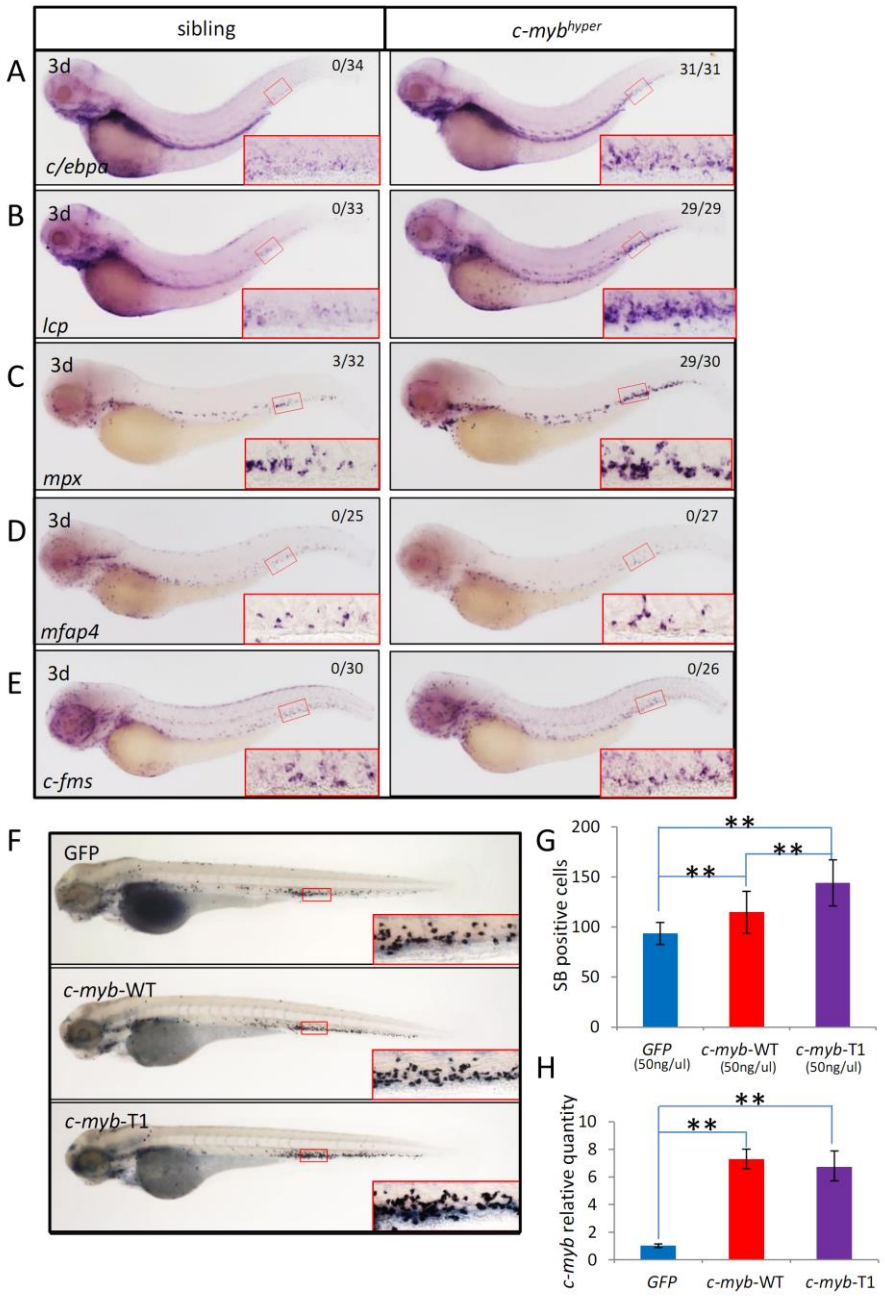
Supplemental table 1 qPCR primers

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
ef1a	GAGAAGTTCGAGAAGGAAGC	CGTAGTATTTGCTGGTCTCG
c-myb 5'primer	CTGCTGCTATCCAGAGACAC	AGCCGTTCATGGAGATTGGTAG
c-myb 3'primer	CCTTTGCAGCAGTTGAACAC	TCATATGACCAGAGCTCGC
c-myb-T1	GGAAAACGAGCTTGTTTACAGTAGC	TCGATTCGGTAGAAAGCTGGC
tcra	GAAGCCGAATATTTACCAAGTG	AACAAACGCCTGTCTCCT
igu	GTTTCCTCAGCTCAACCA	AGTATAATCTCCTTCCTTCCC
Iglc3	AAGGAACTAAACCCATTGTGACGGA	TCGCTGCATTCAGATTTCCTGATG
ccna1	TGGCTCAGGGTCATTTATGGA	ATTCTTCGCCAACTTCCACC
ccnb1	GGCGTTAAGGTTGTGTCTGAG	TTCACTGCAAAGCATGGGA
ccne	CTGGCTAATGGAGGTTTGTGAG	GGCAGCTATAAAGAGACAGGAG
ccnd1	CGGCGAATTATTGCAAATGGA	AGAGGGCCACAAAGGTCTG
ccnh	TTGATCTGAAGACCAGGTACCC	ATCCATCTTGAGGCCAGCAC
cdk1	GTTGTACGCCTGCTAGATGTG	CCCTCCAGGATCTGATAAAGGT
cdk2	GAATCTCCTCATCAACGCTCAG	GCCCTCCGAGTAATCATTTCAG
cdk7	AGTTGTCACAAGATGGTATCGG	GATCTCATCTGTTGGTGTTCCC
cdc20	TACGCACCAGAGGGTTATCAG	TGTCTCCTTCACCAGCATCC
pcna	GCATTCCAGAGCAGGAATACAG	TGATGTTGCCTGTGCCCA
cdkn1c	CGGTAGCTCAAGAATCCGA	GGAAGAAGTCTGTAATTTGCGG
cdkn2d	AGGACCACCGAGTAGAACC	ATACTCCACCACGTCCCAG

Supplemental Fig 1



Supplemental Fig 2



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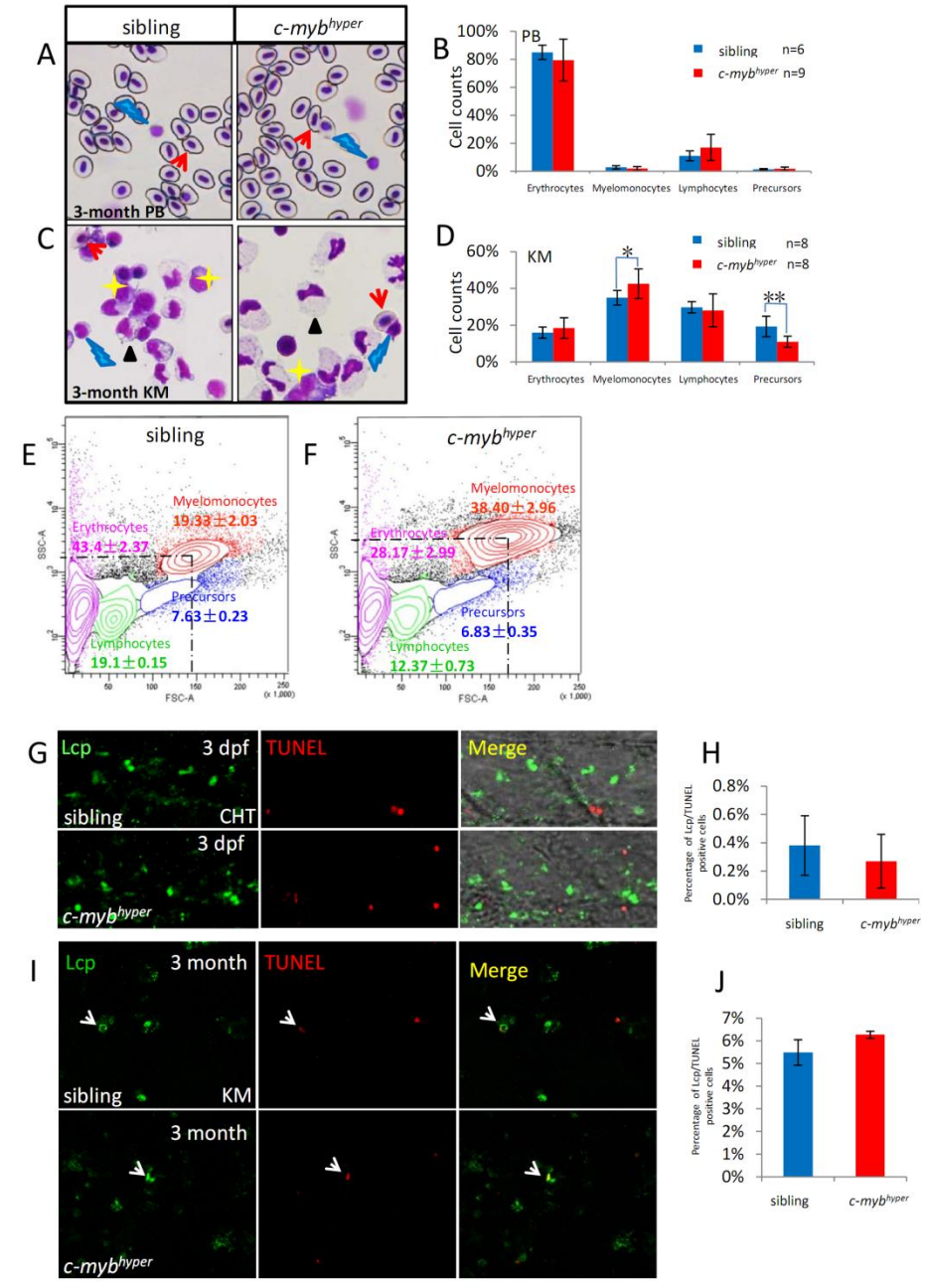

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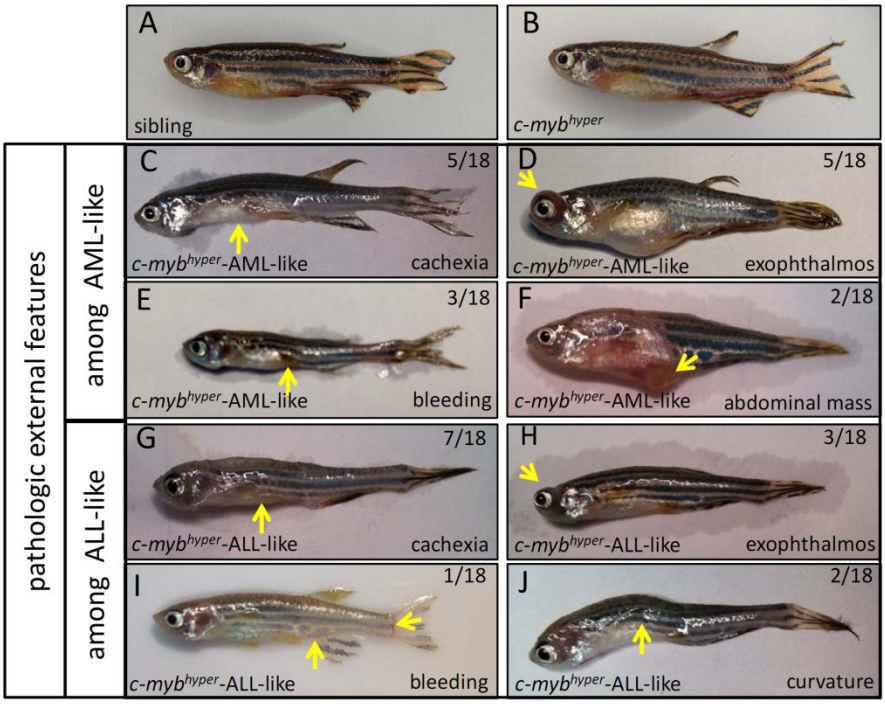

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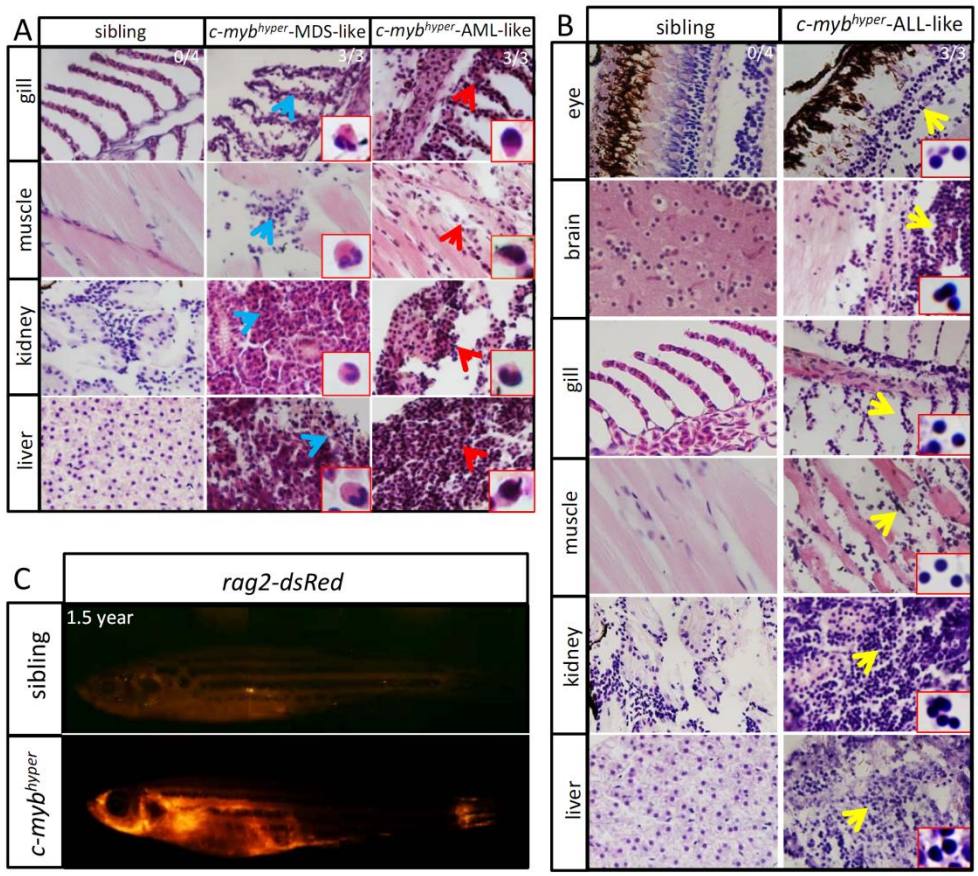

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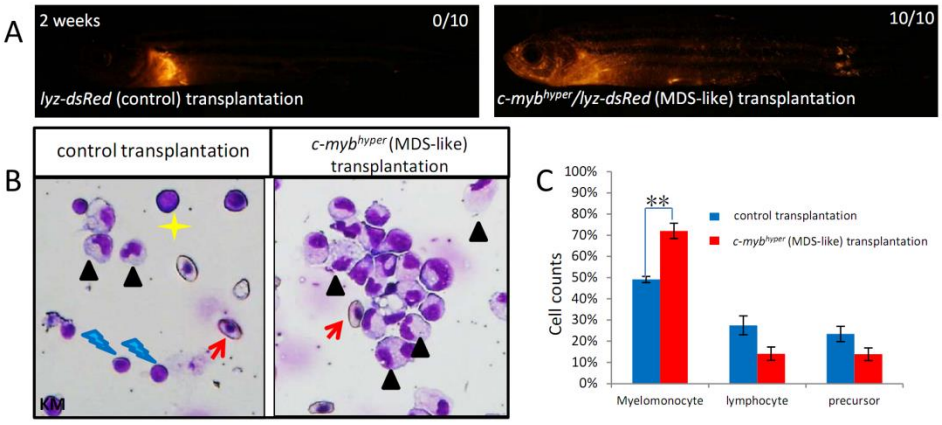

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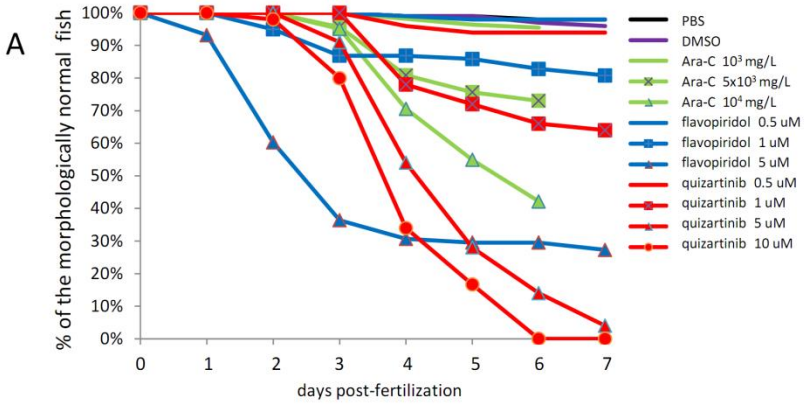

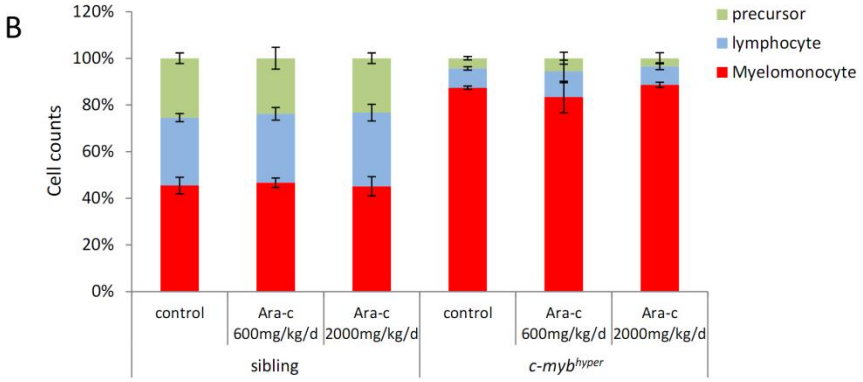

Reference

1. Westerfield M. The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio). *Zebrafish Book A Guide for the Laboratory Use of Zebrafish* 2000.

2. Zhang Y, Jin H, Li L, Qin FX, Wen Z. cMyb regulates hematopoietic stem/progenitor cell mobilization during zebrafish hematopoiesis. *Blood* 2011 Oct 13; **118**(15)**:** 4093-4101.

3. Jin H, Li L, Xu J, Zhen F, Zhu L, Liu PP*, et al.* Runx1 regulates embryonic myeloid fate choice in zebrafish through a negative feedback loop inhibiting Pu.1 expression. *Blood* 2012 May 31; **119**(22)**:** 5239-5249.