ARTICLE - Hematopoiesis

The Znf711-Phf8 complex functions as a transcriptional rheostat essential for neutrophil development

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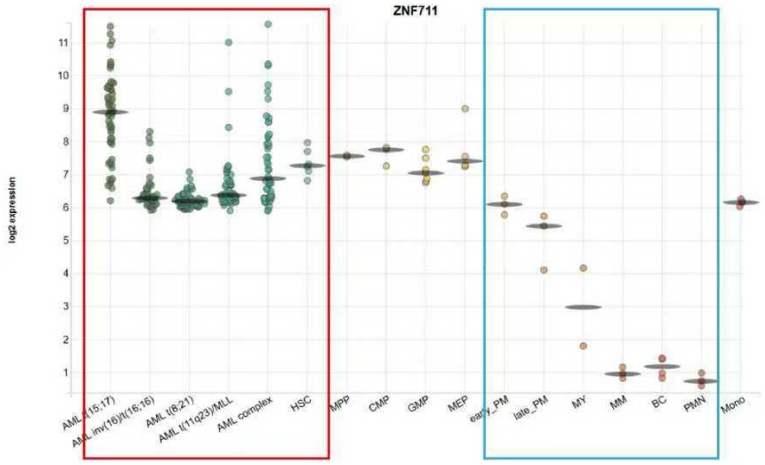
Supplemental Information

Supplemental Figures and Figure legends

Figure S1. Expression and mutation landscape of *ZNF711* in normal granulopoiesis and AML. (A) Expression of *ZNF711* during normal human granulopoiesis and in AML subtypes (the BloodSpot database). *ZNF711* expression peaks in early neutrophils and declines with terminal differentiation (blue box). In most AML subtypes, *ZNF711* expression is lower compared to normal controls, except in t(15;17) acute promyelocytic leukemia (red box). (B) Spectrum of *ZNF711* mutations in five AML patients (the BeatAML database).

A



Abbreviation	Name	Immunophenotype
AML t(15;17)	AML with t(15;17)	Whole BM unsorted
AML inv(16)/t(16;16)	AML with inv(16)/t(16;16)	Whole BM unsorted
AML t(8;21)	AML with t(8;21)	Whole BM unsorted
AML t(11q23)/MLL	AML with t(11q23)/MLL	Whole BM unsorted
AML complex	AML with complex aberrant karyotype	Whole BM unsorted
HSC	Hematopoietic stem cell	Lin- CD34+ CD38- CD90+ CD45RA-
MPP	Multipotential progenitors	Lin- CD34+ CD38- CD90- 45RA-
CMP	Common myeloid progenitor cell	Lin- CD34+ CD38+ CD45RA- CD123+
GMP	Granulocyte monocyte progenitors	Lin- CD34+ CD38+ CD45RA+ CD123+
MEP	Megakaryocyte-erythroid progenitor cell	Lin- CD34+ CD38+ CD45RA- CD123-
early_PM	Early Promyelocyte	Lin- FSChi SSCint CD34- CD15int CD49dhi CD33hi CD11b- CD16-
late_PM	Late Promyelocyte	Lin- FSChi SSChi CD34- CD15hi CD49dhi CD33hi CD11b- CD16-
MY	Myelocyte	Lin- FSChi SSChi CD34- CD15hi CD49dhi CD33hi CD11bhi CD16-
MM	Metamyelocytes	Lin- FSChi SSChi CD34- CD15hi CD49d- CD33- CD11bhi CD16-
BC	Band cell	Lin- FSChi SSChi CD34- CD15hi CD49d- CD33- CD11bhi CD16int
PMN	Polymorphonuclear cells	Lin- FSChi SSChi CD34- CD15hi CD49d- CD33- CD11bhi CD16hi
Mono	Monocytes	CD14+ CD16-

B

Patients with variants in ZNF711 (Chr X: 84523336-84523336)

Export

Patient ▲	Sample	Transcript	Variant classification	Amino acids	Protein position
2094	BA2728	ENST00000373165	missense_variant	C/F	318/761
2408	BA2193	ENST00000373165	missense_variant	C/F	318/761
2408	BA2193	ENST00000373165	missense_variant	A/S	320/761
2430	BA2652	ENST00000373165	missense_variant	C/F	318/761
2467	BA2047	ENST00000373165	start_stop_gain_loss_variant	E/*	316/761
2555	BA2284	ENST00000373165	missense_variant	C/F	318/761

Figure S2. Sequence alignment analysis of human ZNF711 and zebrafish Znf711. (A) Their Zfx/Zfy transcription activation regions and consecutive zinc finger domains are highly conserved. Hs: Homo sapiens, Dr: Danio rerio.

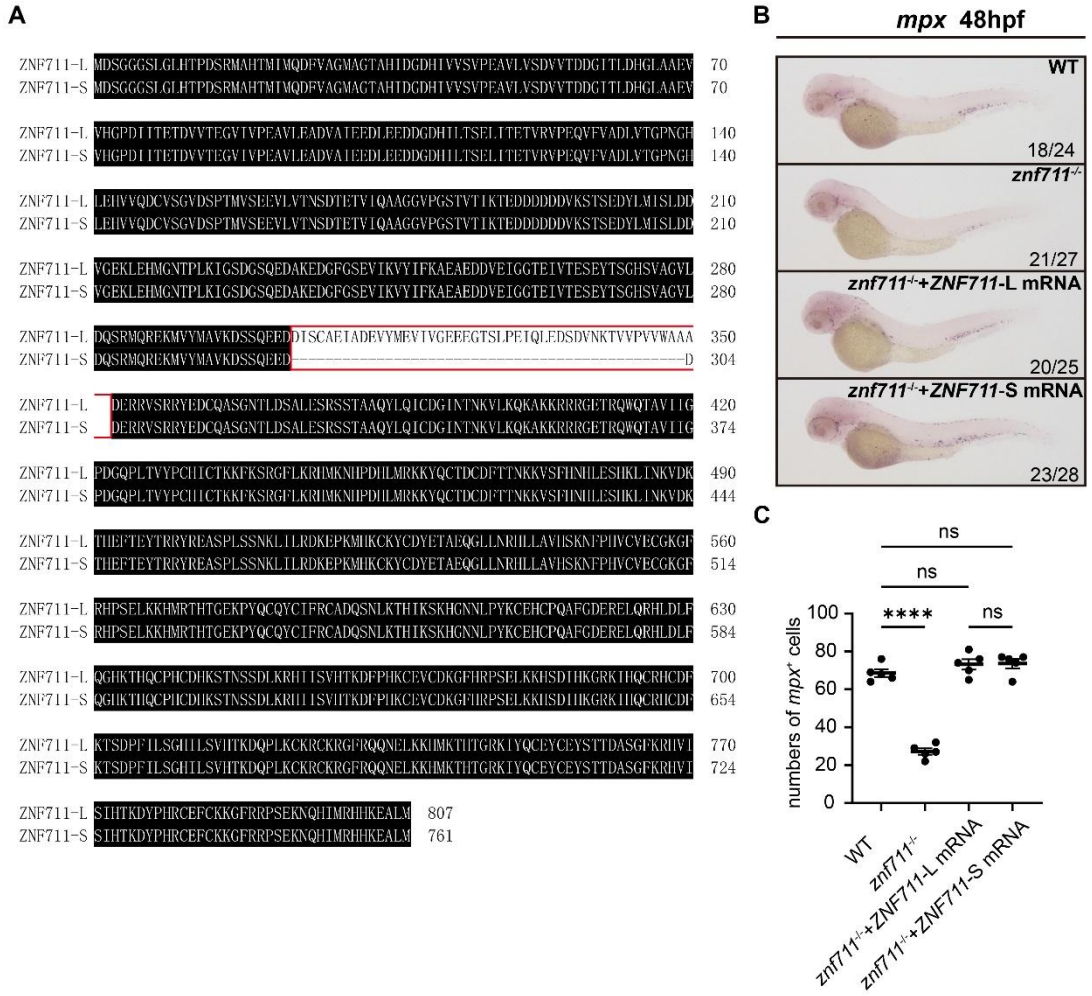


Figure S3. Generation of a *znf711*-deficient zebrafish line. (A, B) Schematic representation of the Cas9 target site in the fourth exon of the *znf711* gene. The deleted nucleotides in the mutant gene are marked by hyphens. Schematic representation of the wild-type (761 aa) and mutant Znf711 proteins (29 aa). Dr: Danio rerio. (C) RT-qPCR data confirmed the significant reduction of *znf711* transcripts in the homozygous mutants. To determine the relative expression rate, data were normalized to the expression level of WT groups (which were set to 1.0) after normalized to the internal control of *β-actin* (Student's *t*-test, N = 3. Error bars represent mean ± SEM. ****$P < 0.0001$).

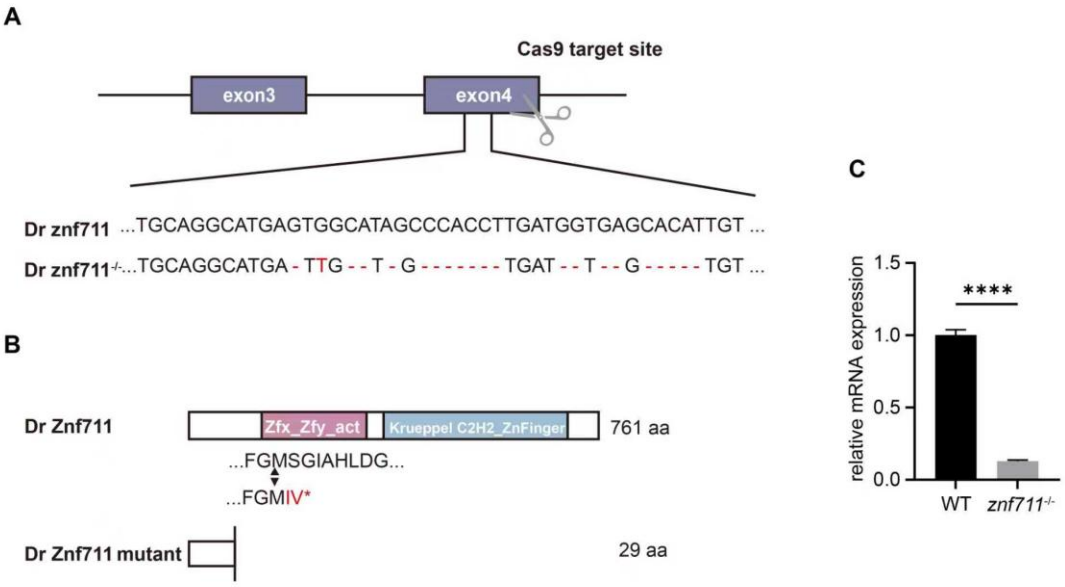


Figure S4. Expression of lineage-specific markers during primitive and definitive hematopoiesis stages in *znf711*-deficient embryos and larvae. (A-D') WISH analyses of hematopoietic progenitor marker *scl*, erythroid markers *gata1*, *hbae1*, macrophage marker *mfap4* in ICM and RBI at 22 hpf. (E-I') WISH analyses of HSPCs marker *c-myb*, macrophage markers *mfap4* and *csf1ra*, erythroid marker *hbae1*, lymphoid marker *rag1* in VDA and CHT during definitive stage of hematopoiesis. (J) Neutral red staining (NR) to detect macrophages at 72 hpf. (K) O-diansidine staining to detect hemoglobin-containing cells at 72 hpf. (L) Statistical results for A-K' (Student's *t*-test, N = 5, 18-35 embryos/larvae were used for each probe. Each dot represents the mean value of one experiment, which was obtained from the counts of all of the embryos/larvae in the same group. ns: not statistically significant).

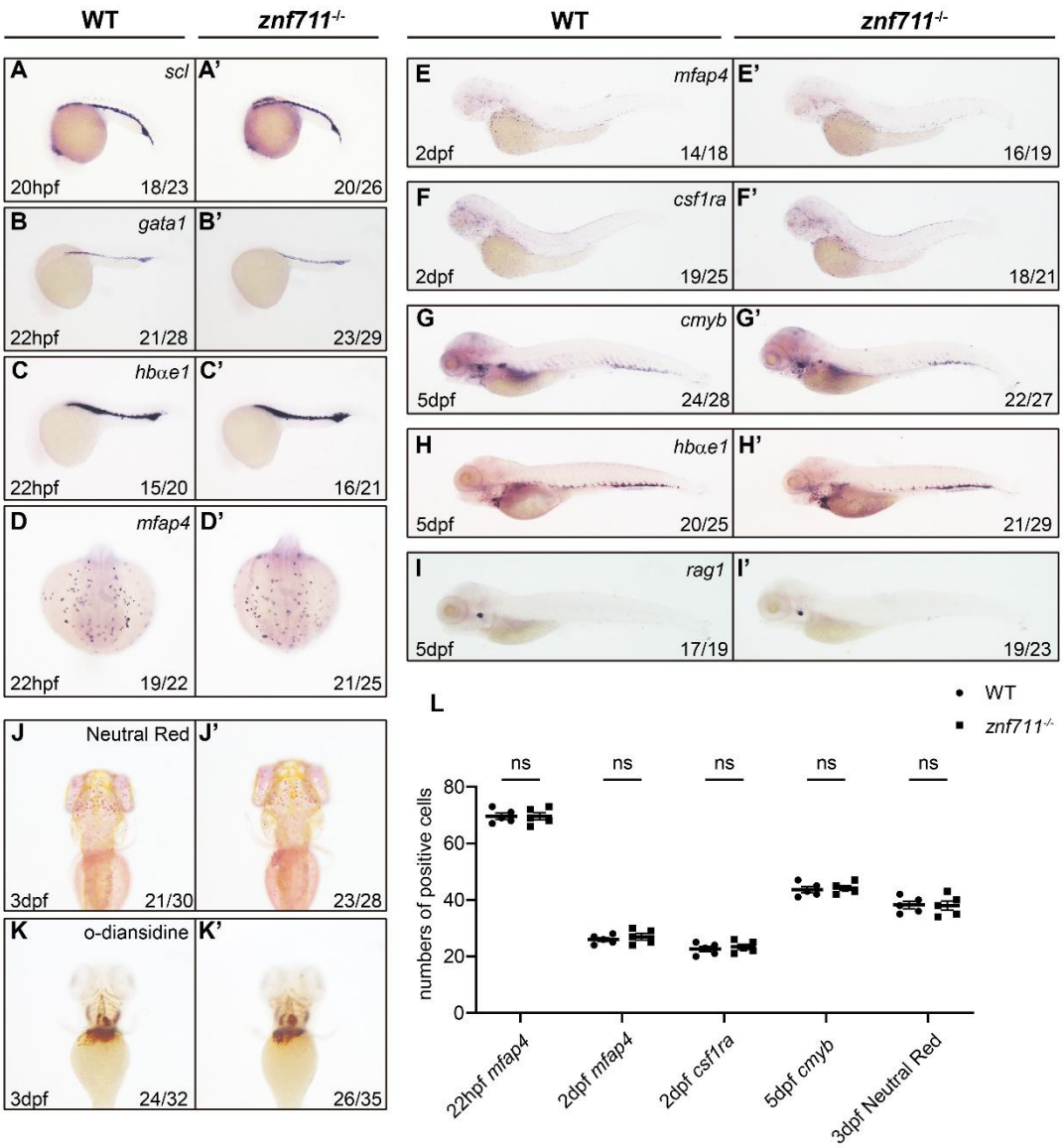

WT
znf711-/-
A scl
A'
20hpf 18/23
20/26
B gata1
B'
22hpf 21/28
23/29
C hbαe1
C'
22hpf 15/20
16/21
D mfap4
D'
22hpf 19/22
21/25
J Neutral Red
J'
3dpf 21/30
23/28
K o-diansidine
K'
3dpf 24/32
26/35
WT
znf711-/-
E mfap4
E'
2dpf 14/18
16/19
F csf1ra
F'
2dpf 19/25
18/21
G cmyb
G'
5dpf 24/28
22/27
H hbαe1
H'
5dpf 20/25
21/29
I rag1
I'
5dpf 17/19
19/23
L
WT
znf711-/-
ns
numbers of positive cells
80
60
40
20
0
22hpf mfap4
2dpf mfap4
2dpf csf1ra
5dpf cmyb
3dpf Neutral Red

Figure S5. The percentages of cells in the erythrocyte and lymphocyte gates were comparable between WT and *znf711*-deficient mutants. FACS analysis of the erythrocyte and lymphocyte gates of WKMs from one-year-old WT and *znf711*$^{-/-}$ zebrafish. Light scatter profiles of WT and mutant groups in one representative experiment were shown. FSC: forward light scatter, SSC: side light scatter. (B) Statistical results for A. The statistical significance was calculated by using Student's *t*-test, N = 5, each time two WKMs were used in the WT and mutant groups, respectively. Error bars represent mean ± SEM. ns: not statistically significant).

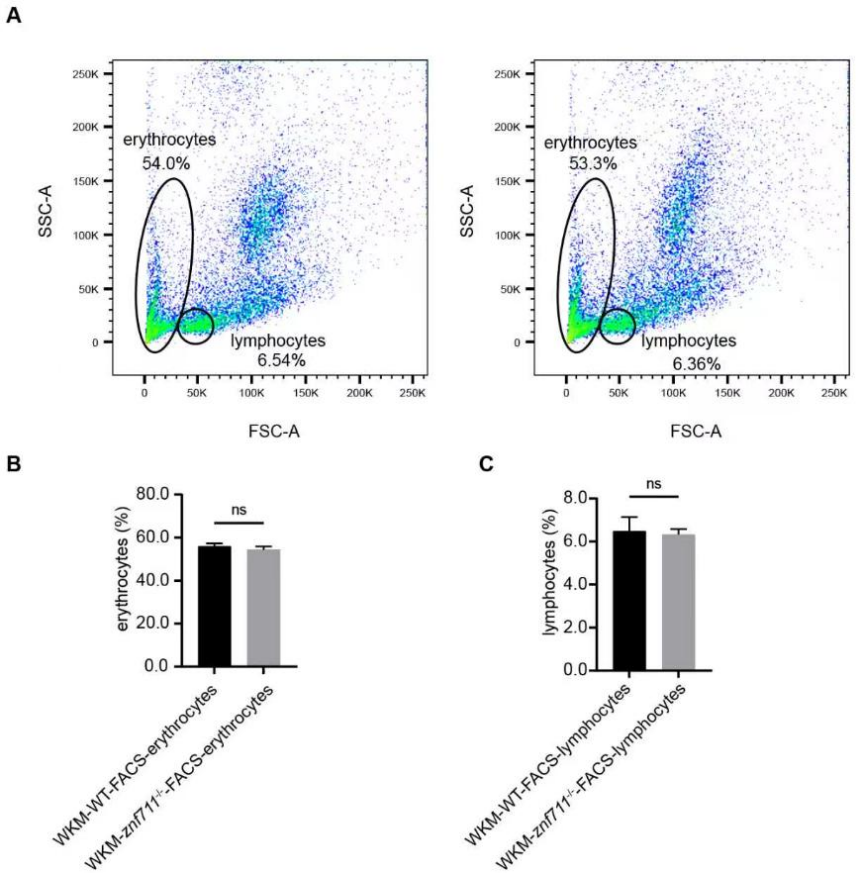
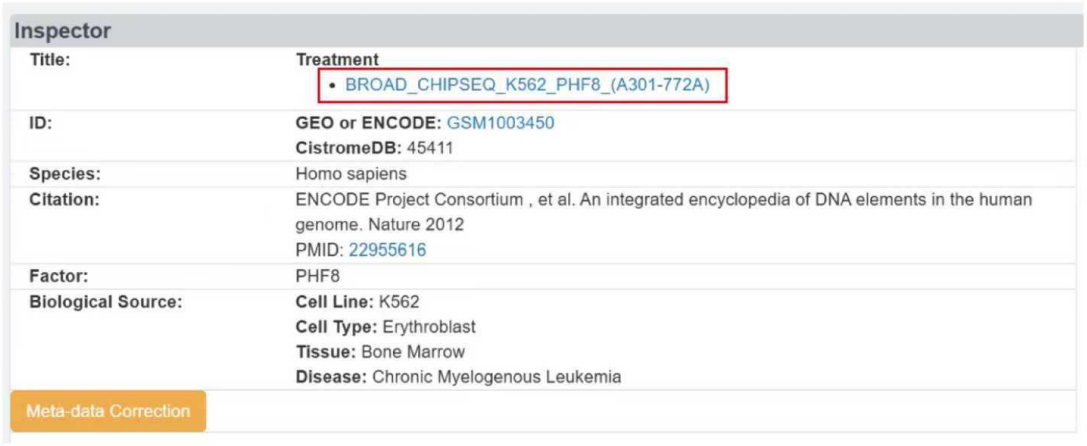


Figure S6. PHF8 occupancy at the *C/EBPα* promoter in human myeloid cell line K562 (data from the Cistrome website).

A



B

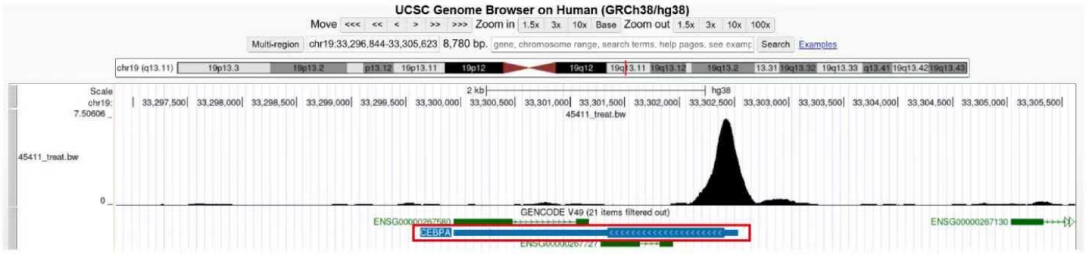


Figure S7. Generation of a *phf8*-deficient zebrafish line. (A, B) Schematic representation of the Cas9 target site in the exon 5 of the *phf8* gene. The deleted nucleotides in the mutant gene are marked by hyphens. Schematic representation of the wild-type (1032 aa) and mutant Phf8 proteins (155 aa). Dr: Danio rerio. (C) RT-qPCR data confirmed the significant reduction of *phf8* transcripts in the homozygous mutants. To determine the relative expression rate, data were normalized to the expression level of WT groups (which were set to 1.0) after normalized to the internal control of *β-actin* (Student's *t*-test, N = 3. Error bars represent mean ± SEM. ****$P < 0.0001$).

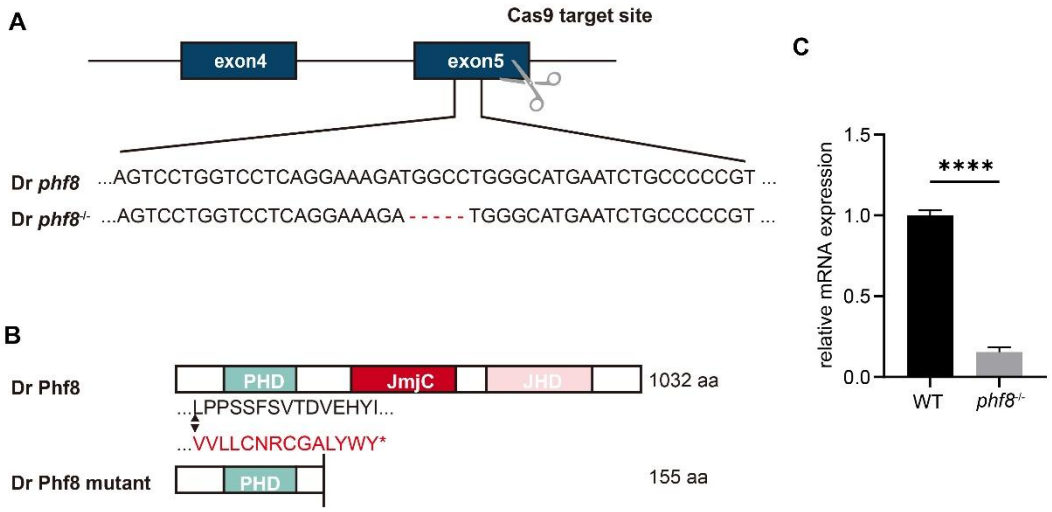


Figure S8. Quantification of indicated transcripts. (A) RT-qPCR analyses of *c/ebpα* in the *mpx*$^{+}$ cells enriched from WT and *phf8*$^{-/-}$//Tg(*mpx*:eGFP) zebrafish larvae at 48 dpf. To determine the relative expression rate, data were normalized to the expression level of WT groups (which were set to 1.0) after normalized to the internal control of *β-actin* (Student's *t*-test, N = 3. Error bars represent mean ± SEM. ****$P < 0.0001$). (B) RT-qPCR analyses of *gfi1aa* transcripts in WT and *gfi1aa*$^{-/-}$ zebrafish larvae at 48 dpf. Data were normalized to the expression level of WT groups (which were set to 1.0) after normalized to the internal control of *β-actin* (Student's *t*-test, N = 3. Error bars represent mean ± SEM. ****$P < 0.0001$). To determine the relative expression rate, data were normalized to the expression level of WT groups (which were set to 1.0) after normalized to the internal control of *β-actin* (Student's *t*-test, N = 3. Error bars represent mean ± SEM. **$P < 0.01$).

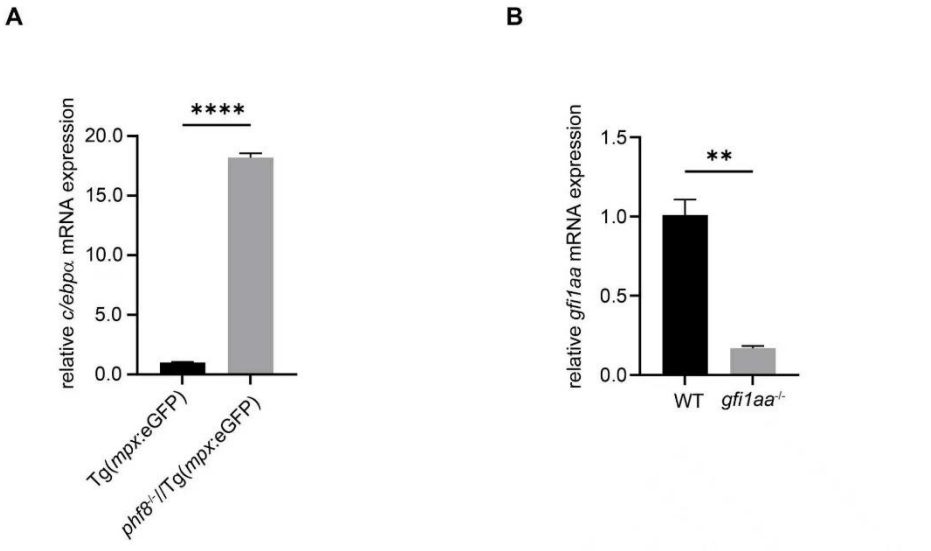


Figure S9. Co-IP assays of the components forming the GFI1-LSD1-RCOR1 complexes in the presence and absence of PHF8. (A, B) Co-IP assays indicated that HA-GFI1 could interact with FLAG-LSD1 and FLAG-RCOR1 in HEK293T cells. Upon silencing of endogenous *PHF8*, the interactions between GFI1 and the other two components were not affected.

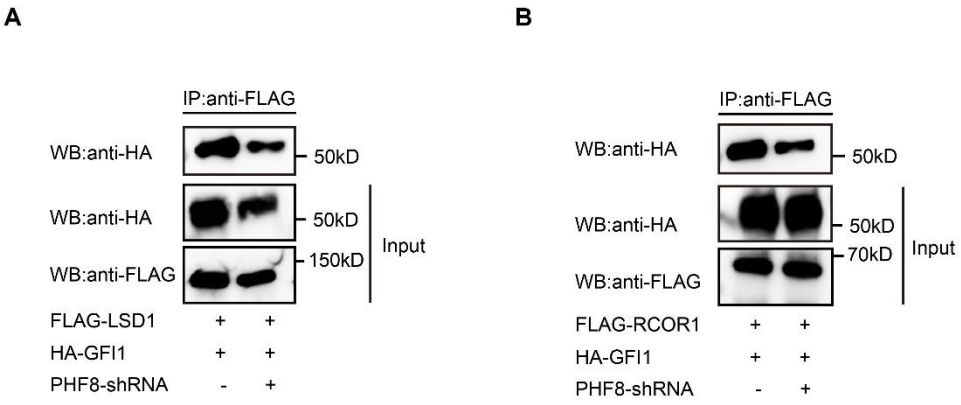


Figure S10. Prediction of C/EBPα binding sites in the *znf711* promoter. (A) Four putative C/EBPα binding sites were predicted in the -2.0 kb upstream region using JASPAR. Two sites within the core promoter (red boxes) were validated by ChIP-qPCR.

Fig S10

A

Analysis results

Scan results

Total **4** putative site(s) were predicted with relative profile score threshold 90%.

Show FASTA Sequence

Display 10 profiles Filter:

Matrix ID	Name	Score	Relative score	Sequence ID		Start	End	Strand	Predicted sequence
MA0102.3	MA0102.3.CEBPA	12.594508	0.9488727	1	-0.3kb	1691	1701	-	ATTGCCTAACA
MA0102.3	MA0102.3.CEBPA	11.274717	0.93306154	1		1159	1169	-	GTTTCACAAGC
MA0102.3	MA0102.3.CEBPA	9.760794	0.91492456	1		188	198	-	GTTTCAAAAGC
MA0102.5	MA0102.5.CEBPA	11.5849	0.90014446	1	-0.36kb	1634	1643	-	ATTGCACATT

Showing 1 to 4 of 4 entries Previous 1 Next

Figure S11. Both *znf711*- and *phf8*-deficient zebrafish displayed brain and craniofacial developmental abnormalities. (A, B) Brain and craniofacial development were delayed at 24 hpf and 7 dpf in both lines.

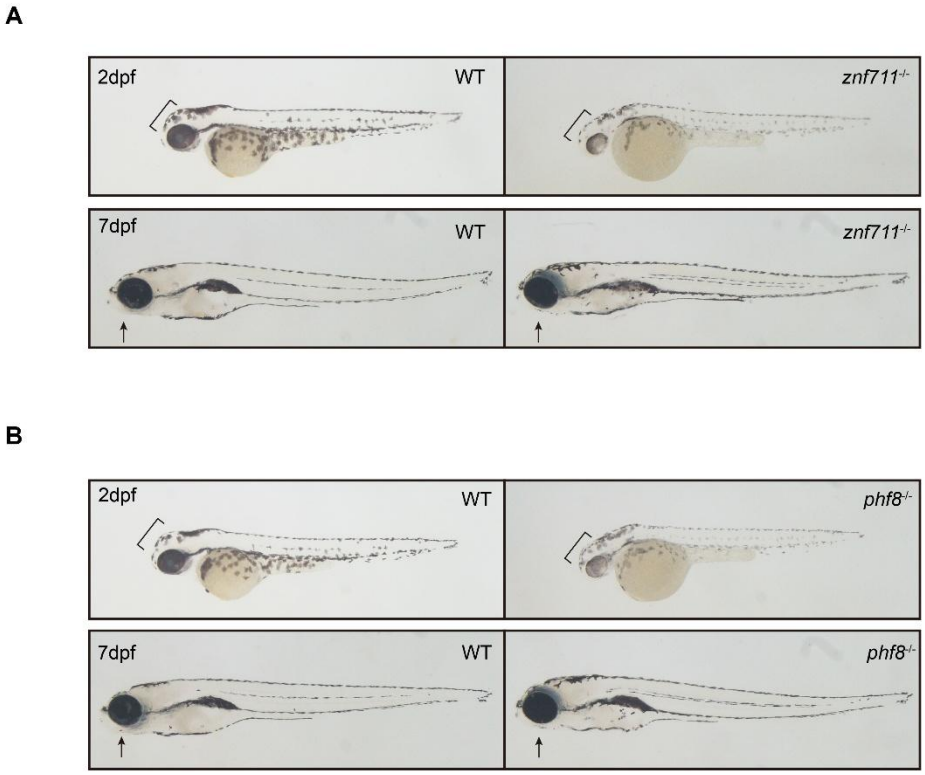


Figure S12. *in vivo* mRNA rescue assays with the long and short isoforms of human *ZNF711*. (A) Human ZNF711 has two isoforms generated through alternative splicing. They differ in 46 amino acid residues within the Zfx/Zfy region (the boxed area). ZNF711-L: the long isoform of ZNF711, ZNF711-S: the short isoform of ZNF711. (B) WISH assays of *mpx* conducted in *znf711*$^{-/-}$ mutants injected with the long or short isoforms of human *ZNF711* mRNA at 48 hpf. (C) Statistic results for B. The statistical significance was calculated by using one-way ANOVA. The asterisk indicates a statistical difference (N = 5, 25-30 larvae were used for each experiment. Each dot represents the mean value of one experiment. Error bars represent mean ± SEM. ns: not statistically significant, ****$P < 0.0001$).

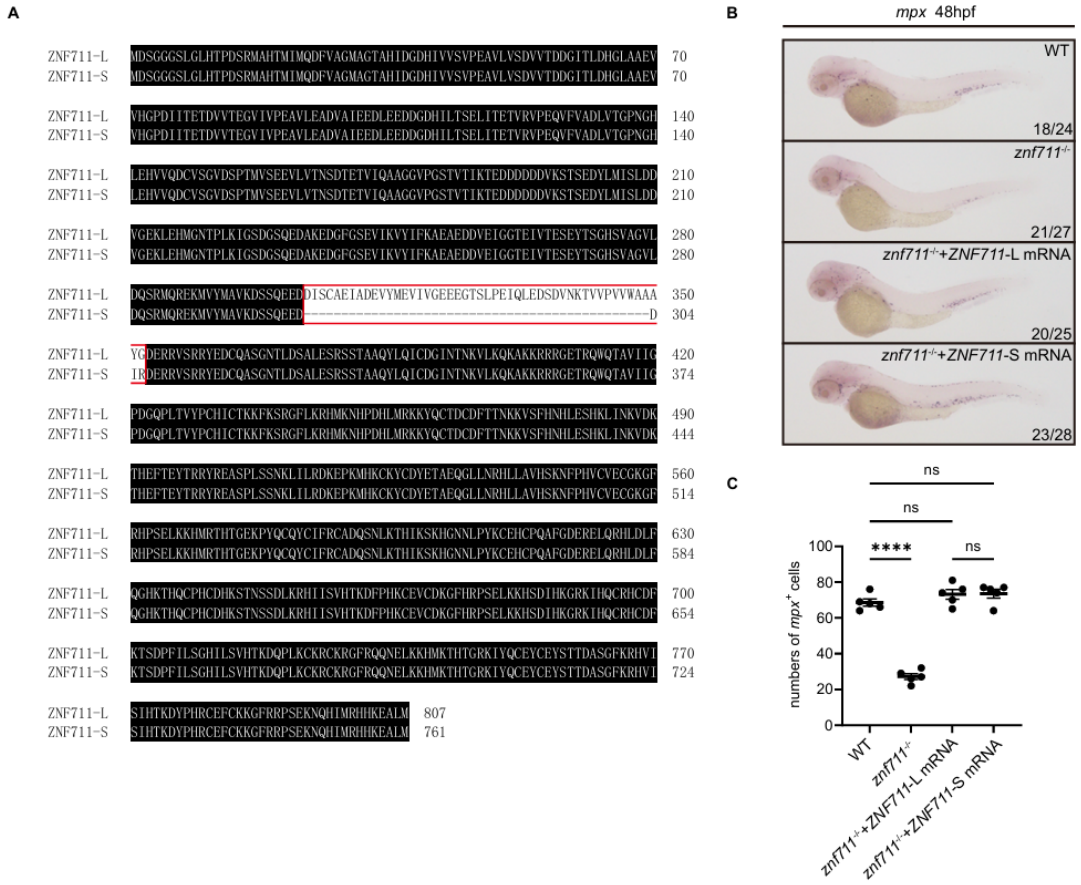


Table S1. Primers used in this study.

Primers for real-time RT-qPCR

Species	**Gene**	**Forward (5'-3')**	**Reverse (5'-3')**
Danio rerio	*β-actin*	TGCTGTTTTCCCCTCCATTG	TTCTGTCCCATGCCAACCA
Danio rerio	*c/ebpα*	TGAAGATTGGCGATCGAGGG	ATTTTCGCCTTGTCCCGACT
Danio rerio	*c/ebp1*	TCTGCACATACACAGGATTTTGC	AGCTGCAGCGCCCTCT
Danio rerio	*csf3r*	CCTCCGGTGTGACTGGAATC	ACCTTTTGTGACTTGGCCCTAA
Danio rerio	*mpx*	GAGGTTCCTTGCTTCATTG	GCACATGAAGGGCGCGAGCC
Danio rerio	*lyz*	GCAGGTTTAAGACCCACCGA	TTCAGCCCGTCCATTTTCAC
Mus musculus	*Gapdh*	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Mus musculus	*Phf8*	AGGTTCCTGTCAGTGTGTGT	TCTCTAGACGATGGCTGACA
Mus musculus	*C/ebp α*	GCAAAGCCAAGAAGTCGGTG	TCTCCACGTTGCGTTGTTTG
Mus musculus	*Znf711*	CTTGAAGCCGATGTTGCCATT	TCAGCCACAAAAACCTGCTCA
Homo sapiens	*GAPDH*	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC
Homo sapiens	*GFI1*	CCGCGCTCATTTCTCGTCA	ACGGAGGGAATAGTCTGGTCC
Homo sapiens	*ZNF711*	ATGGATTCAGGCGGTGGAAG	CAGCCATTCCAGCCACAAAA
Homo sapiens	*PHF8*	GCTTCTCGAAGCCCCTG	TGGACGATAGCGCGGC

Primers for ChIP-qPCR

Species	**Gene**	**Forward (5'-3')**	**Reverse (5'-3')**
Danio rerio	*c/ebpα*-NP	AGGAAAGCATTAGGACCGCC	GCCACACGGGATCTCCTTTA
Danio rerio	*c/ebpα*-PP	GTCCGCGCTTACCTCTTCTC	GGTGCAAGTGGGAAATGTGC
Danio rerio	*znf711*-NP	CTCCCAAGGTTGTGAATCGGT	GTCACTCTCAGCGTCCCAAT
Danio rerio	*znf711*-PP	CGGAGTAGTGGACAAGCTGTT	CCTGTAAAGATCGGCCCACA

Primers for plasmid construction

Species	Plasmid	Forward (5'-3')	Reverse (5'-3')
Danio rerio	HA-*znf711*	CCATCGATATGTACCCATACGATGTTCCAGATTACGCTATGGATCAAGGTGGAGGGATCCTG	CCCTCGAGTTATAACAGGGTTTCCTTGTGATG
Danio rerio	HA-*znf711*-Δ DBD	GTTCCAGATTACGCTGAATTCATGGATCAAGGTGGAGGGATCC	ACGACTCACTATAGTTCTAGATTACAGTGGCTGCCCATCAGG
Danio rerio	HA-*znf711*-Δ Zfx/y	GTTCCAGATTACGCTGAATTCACTGTATACCCCTGTCACATCTGTGG	ACGACTCACTATAGTTCTAGATTATAACAGGGTTTCCTTGTGATGTC
Danio rerio	HA-*gfi1aa*	GTTCCAGATTACGCTGAATTCATGCCGAGGTCATTTTTGGTG	ACGACTCACTATAGTTCTAGATTATTTTAGCCCGTGCTGTGTTT
Danio rerio	Flag-*phf8*	GACGATGACGACAAGGAATTCATGGCATCTGTTCCGGTTTACT	ACGACTCACTATAGTTCTAGACTACAGCAGCAGCTTGCCG
Danio rerio	Flag-*phf8*-Δ PhD	GCATCTGTTCCGGGACCATCTGTC	GACAGATGGTCCCGGAACAGATGC
Danio rerio	Flag-*phf8*-Δ JmjC	TTCTCAGACACCGAAAAGATCTTT	AAAGATCTTTTCGGTGTCTGAGAA
Danio rerio	TOL2(*mpx:c/ebpα*)	AATATGTGTTTTTAGGGATCCATGGAGCAAGCAAACCTCTACG	CCTCGCCCTTGCTCACCATGGTTAAGCGCAGTTGCCCATG
Danio rerio	Tol2(*mpx:c/ebpα*-bZIP)	AATATGTGTTTTTAGGGATCCAAGAACAGCACCGAGTACAGGC	CCTCGCCCTTGCTCACCATGGGCCCCGTAACGTCTCCAGTT

Danio rerio	Tol2(*mpx*:*PHF8*)	AATATGTGTTTTTAGGGATCCATGGCCTCGGTGCCGGTG	CCTCGCCCTTGCTCACCATGGTCACAGAAGTAGTTTGCCATTTCTG
Danio rerio	Tol2(*mpx*:*PHF8*^{K840R})	CCTCCTGAGCCTAGACAAGAGGCCCTG	CAGGGCCTCTTGTCTAGGCTCAGGAGG
Danio rerio	Tol2(*mpx*:*PHF8*^{K840R}-*SUMO1*)	GCAAACTACTTCTGACCATGGATGTCAGACACGGAGACCAAGC	CCTCGCCCTTGCTCACCATGGCTAGTCGTTCCGACAGCCTCC
Danio rerio	HA-*sumo1*	GTTCCAGATTACGCTGAATTCATGTCAGACACGGAGACCAAGC	ACGACTCACTATAGTTCTAGACTAGTCGTTCCGACAGCCTCC
Homo sapiens	HA-*ZNF711*-S	CGGGATCCATGTACCCATACGATGTTCCAGATTACGCTATGGATTCAGGCGGTGGAAGTCTT	CCCTCGAGTTACATAAGAGCCTCTTTGTGGTG
Homo sapiens	HA-*ZNF711*-L	GATATCAGTTGCGCTGAAATAGCAGATGAAGTTTACATGGAAGTCATTGTAGGGGAAGAGGAAGGAACTTCTCTCCCTGAGATTCAGCTTGAGGACTCTGATGTTAATAAAACAGTTGTCCCTGTTGTCTGGGCTGCGGCATATGGAGATGAAAGAAGAGTTTCCCG	ATCTTCTTCTTGAGAAGAAT
Homo sapiens	FLAG-*PHF8*	GACGATGACGACAAGGAATTCATGGCCTCGGTGCCGGTG	ACGACTCACTATAGTTCTAGATCACAGAAGTAGTTTGCCATTTCTG

Homo sapiens	FLAG-*PHF8*^{K840R}	CCTCCTGAGCCTAGA CAAGAGGCCCTG	CAGGGCCTCTTGTCTAGGC TCAGGAGG

Primers for PLVX-shRNA generation

Species	**Gene**	**Forward (5'-3')**	**Reverse (5'-3')**
Homo sapiens	*ZNF711*	GATCCGCCAATGTGAGTATT GTGAATATTCAAGAGATATTC ACAATACTCACATTGGTTTTT TG	AATTCAAAAAACCAATGTGAGT ATTGTGAATATCTCTTGAATATT CACAATACTCACATTGG CG
Homo sapiens	*PHF8*	GATCCGCTGGCCAGTTGAGC TATAATTTCAAGAGAATTATA GCTCAACTGGCCAGCTTTTT TG	AATTCAAAAAAGCTGGCCAGTT GAGCTATAATTCTCTTGAAATTA TAGCTCAACTGGCCAGC G

Primers for pLKO.1-shRNA generation

Homo sapiens	*GFI1*	CCGGCATCAAGTGCAGCAA GGTGTTCTCGAGAACACCTT GCTGCACTTGATGTTTTTT	AATTAAAAAACATCAAGTGCAG CAAGGTGTTCTCGAGAACACCT TGCTGCACTTGATG

Primers for luciferase assays

Species	**Gene**	**Forward (5'-3')**	**Reverse (5'-3')**
Danio rerio	*c/ebpα* promoter (-0.6~-0.9k)	GGGGTACCCCAA TGTTTATTTGTGT GTGAATAAAT	CCCTCGAGGGTGTTTCTTAAC GTTTTGATGCTCA
Danio rerio	*znf711* promoter (-2.0k)	GCGTGCTAGCCC GGGCTCGAGTTT TAATCGTATTCAA AAACTTGTTTGA	ACTTAGATCGCAGATCTCGAG TCCGTTTCAGCCAACCAGAC

Primers for CRISPR/Cas9 genotyping

Species	Gene	Forward (5'-3')	Reverse (5'-3')
Danio rerio	*znf711*	CTCTCAGAACAAAGGCCTGA	TGTCACCACATCTGACACCA
Danio rerio	*phf8*	CGAGTTTCTTCGCAGGTGTT	GTCTGCTTGGCGACTGACAT
Danio rerio	*gfi1aa*	CCGGACAGGCTCCTGAAAGCC	TGAGCGGTCACACACACTGCC

Supplemental Methods

Zebrafish maintenance and mutant generation

Zebrafish were raised, bred, and staged according to standard protocols [1]. The following strains were used: AB, *Tg(mpx:eGFP)rj30* (ZFIN database). For CRISPR9 mediated *znf711* and *phf8* knockout zebrafish generation, guide RNAs (gRNA) targeting the exon 4 of *znf711*, and the exon 5 of *phf8*, were designed using an online tool ZiFiT Targeter software (http://zifit.partners.org/ZiFiT), which were synthesized by cloning the annealed oligonucleotides into the gRNA transcription vector. Cas9 mRNA and gRNA were co-injected into one-cell stage zebrafish embryos. The injected F0 founder embryos were raised to adulthood and then outcrossed with wild-type zebrafish. F1 embryos carrying potential indel mutations were raised to adulthood. Then PCR amplification and sequencing were performed on genomic DNA isolated from tail clips of F1 zebrafish to identify mutants. Both *znf711* and *phf8* lines produce truncated proteins that lack functional domains.

Whole-mount *in situ* hybridization (WISH)

Digoxigenin-labeled RNA probes were transcribed with T7, T3 or SP6 polymerase (Ambion, Life Technologies, Carlsbad, USA). WISH was performed as described previously [2]. The probes labeled by digoxigenin were detected using alkaline phosphatase coupled anti-digoxigenin Fab fragment antibody (Roche, Basel, Switzerland) with 5-bromo-4-chloro-3-indolyl-phosphate nitro blue tetrazolium staining (Vector Laboratories, Burlingame, CA, USA). At least 15 ~ 30 embryos or

larvae were used for each probe. The positive signals were counted under a microscope, and the mean value was obtained from the counts of all of the embryos/larvae in the same group.

Sudan Black staining

The zebrafish larvae treated with 4% paraformaldehyde (PFA) overnight at 4˚C were incubated with a Sudan Black (Sigma-Aldrich, St. Louis, MO, USA) solution for about 30 minutes to detect the granules of granulocytes. The detailed method was described previously [3]. Staining was then observed under a microscope.

FACS analysis and Cell collection

FACS analysis and cell collection were performed as previously described [4]. Wild-type *Tg(mpx:eGFP)* and *znf711*$^{-/-}$//*Tg(mpx:eGFP)* larvae were dissociated into single cells using 0.05% trypsin (Sigma-Aldrich, St. Louis, MO, USA) as previously described [5]. These dissociated cells were passed through a 40-μm mesh, centrifuged at 450g, and suspended in 5% FBS/PBS before addition of propidium iodide to a final concentration of 1 μg/ml for exclusion of dead cells. wild-type zebrafish (without GFP) were used as blank to determine the background values in GFP-controls. The GFP^{+} cells of each group were collected from a total of ~ 1000 larvae using a FACS Vantage flow cytometer (Beckton Dickenson) (~ 300 larvae once, performed 3 times). For the whole kidney marrow (WKM) samples, FACS analysis was based on forward and side scatter characteristics, propidium iodide exclusion and GFP fluorescence. The GFP^{+} cells in the myeloid gate was enriched from WKM samples of wild-type *Tg(mpx:eGFP)* and *znf711*$^{-/-}$//*Tg(mpx:eGFP)* zebrafish (one-year-old, each time one male and one female were used in the WT and mutant groups, respectively).

RNA sequencing and Quantitative RT-PCR

At 48 hpf, GFP positive cells were isolated from either wild-type *Tg(mpx:eGFP)* or *znf711* MO injected *Tg(mpx:eGFP)* larvae by FACS. mRNA was extracted from

sorted cells using RNeasy Micro (Qiagen, Manchester, UK) and mRNA libraries were constructed using the VAHTS Universal V8 RNA-seq Library Prep Kit (vazyme Technology Co., Ltd., Nanjing, China). Then libraries with different indexs were multiplexed and loaded on a Navoseq6000 instrument for sequencing using a 2 x 150 paired-end (PE) configuration according to manufacturer's instructions.

The quantitative PCR was carried out with SYBR Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan) with ABI 7900HT real-time PCR machine and analyzed with Prism software. *β-actin* was served as the internal control. The primers used are listed in Table S1. Each time a different batch of samples was used. The expression levels of each interested gene were normalized to internal control *β-actin* by real-time qPCR and compared with WT group which was set to 1.0. Real time qPCR was performed with gene specific primers and gene expression levels were analyzed by comparative CT method.

Plasmid construction

Zebrafish *znf711* and its serial mutants were cloned into pCS2^{+} vector. For the luciferase reporter, the promoter (-600 bp ~ -960 bp) of zebrafish *c/ebpα* was cloned into the PGL3 basic vector (Promega, Madison, WI, USA). Primers used were listed in Table S1.

Morpholino and mRNA synthesis for microinjection

Morpholinos for *znf711* (5'-AGATTATGGATCAAGGTGGAGGGAT-3'), *phf8* (5'-ATGGCATCTGTTCCGGTTTACTGCC-3'), and *gfi1aa* (5'-GTAAACATGCCGAGGTCATTTTTGG-3') were designed and purchased from Gene Tools. Full-length capped mRNA samples were all synthesized from linearized plasmids using the mMessage mMachine SP6 kit (Invitrogen, Thermo Fisher, Waltham, USA). Microinjection concentration of mRNA was between 50 ~ 200 ng/μl and 2 nl of mRNA was injected at one-cell stage embryos. All injections were performed with a Harvard Apparatus micro-injector.

Cell culture and dual-luciferase reporter assay

HEK293T cells were maintained in DMEM (Gibco, Life technologies, Carlsbad, USA) with 10% Fetal Bovine Serum (Gibco, Life technologies, Carlsbad, USA). Plasmid transfection was carried out with Effectene Transfection Reagent (Qiagen, Manchester, UK) according to manufacturer's instruction. For the luciferase reporter assay, cells were harvested 48 hours after transfection and analyzed using the Dual Luciferase Reporter Assay Kit (Beyotime, Shanghai, China), according to the manufacturer's protocols.

Co-immunoprecipitation and western blot assay

HEK293T cells, which had been transfected with plasmids for 48 hours, were washed with phosphate-buffered saline (PBS) buffer for 1 minute 3 times. Lysates were prepared using RIPA lysis buffer (Beyotime, Shanghai, China) with proteinase inhibitor (Roche, Basel, Switzerland), after shaking on ice for 30 minutes, the cells were harvested and centrifuged at 15,000 × g for 30 min. Antibody indicated in the results (Cell Signaling Technology) was mixed with the protein-G-agarose beads (30 μl) in the supernatant at 4˚C overnight. The beads were prepared by centrifugation and washed three times with RIPA lysis buffer. Proteins binding to the beads were eluted by adding 30 μl of 2× SDS sample buffer and analyzed by immunoblotting using antibody indicated (Cell Signaling Technology).

Chromatin immunoprecipitation qPCR (ChIP-qPCR)

For ChIP analysis, GFP and GFP-Znf711 expressing larvae were harvested at 48 hpf for brief fixation. Cross-linked chromatin was immunoprecipitated with anti-GFP antibody according to the procedure described (Cell Signaling Technology) [6]. The resultant immunoprecipitated samples were subjected to quantitative PCR using primer pairs (Table S1).

The genomic regions targeted by ChIP-qPCR were selected as follows: putative

binding sites for sequence-specific transcription factor C/EBPα were predicted at JASPAR website; regions for the chromatin-associated co-regulator PHF8, which lacks a defined DNA-binding motif, were chosen based on its occupancy peaks in public ChIP-seq datasets (Cistrome Database).

Cell line and treatment

32Dcl3 cells were maintained in 1640 (Life technologies, Grand Island, NY, USA) with 10% Fetal Bovine Serum (Life technologies, Grand Island, NY, USA) and 1 ng/ml murine IL-3 (PeproTech, USA). 32Dcl3 cells were treated with 1 μM of ATRA (Sigma Aldrich, St. Louis, MO, USA) for 72 hours.

KEY RESOURCES TABLE

REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Antibodies		
ZNF711 Polyclonal Antibody	Abcam	Cat#AB254776
GFI1 Polyclonal Antibody	Santa Cruz Biotechnology	Cat#sc-373960
PHF8 Polyclonal Antibody	Bethyl Laboratories	Cat# A301-772A
Normal Rabbit IgG	Cell Signaling Technology	Cat#2729P
Anti-HA tag ChIP-Grade	Abcam	Cat#ab9110
Rabbit monoclonal anti-HA	Cell Signaling Technology	Cat#3724
Rabbit monoclonal anti-FLAG	Cell Signaling Technology	Cat#14793
Mouse monoclonal anti-GAPDH	Proteintech	Cat#60004-1-Ig
Chemicals, peptides, and recombinant proteins		
ATRA	Sigma-Aldrich	Cat#R2625
Recombinant Murine IL-3	PeproTech	Cat#213-13
Critical Commercial Assays		
SimpleChIP® Plus Sonication Chromatin IP Kit	Cell Signaling Technology	Cat#56383S

Dual-Lumi™ Luciferase Reporter Gene Assay Kit	Beyotime	Cat#RG088S
Experimental Models: Cell Lines		
Human: HEK293T	ATCC	CRL-11268
Mouse: 32D Clone3	ATCC	CRL-3594
Software and algorithms		
ImageJ	NIH	N/A
GraphPad Prism 9.0	Graphpad, Inc	N/A

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