NIPBL: a new player in myeloid cell differentiation

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Statement of equal authors' contribution: M.M. and G.D.F. equally contributed to this work

Running head: *NIPBL*/*NPMc+* interplay in myeloid differentiation

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Supplementary Methods

Patients

Patients were previously characterized for specific molecular aberrancies, such as mutations for *NPM1* and *FLT3-ITD*, in addition to translocations t(9;22), t(8;21) and inv(16), in accordance to specific clinical protocol requirements. Patients enrolled belong to different French–American–British (FAB) classification systems (FABs), excluding M3, therefore all patients were negative for translocation t(15;17). Bone marrow of healthy individuals were collected as controls for gene expression assays, upon appropriate Informed Consent ASG-MA-052A approved on May 8th 2012 by Azienda San Gerardo (ASG).

Animals

The fish were maintained under standard conditions in the fish facilities of Bioscience Dept, University of Milan, Via Celoria 26 - 20133 Milan, Italy (Aut. Prot, n. 295/2012-A - December 20 2012); and Cogentech s.c.a.r.l. (Aut. Prot. n. 007894 – May 29 2018), via Adamello 16 - 20139 Milan, Italy).

We express the embryonic ages in hours post fertilization (hpf) and days post fertilization (dpf). Zebrafish AB strains obtained from the Wilson lab (University College London, London, UK) were maintained at 28°C on a 14 h light/10 h dark cycle. The zebrafish transgenic *TOPdGFP* line was described previously.[1] The zebrafish transgenic *CD41:GFP/kdrl:dsRED* line was described previously.[2] Embryos were collected by natural spawning, staged according to Kimmel and colleagues,[3] and raised at 28°C in fish water (Instant Ocean, 0,1% Methylene Blue) in Petri dishes, according to established techniques. After 24 hpf, to prevent pigmentation 0,003% 1-phenyl-2-thiourea (PTU, Sigma-Aldrich, Saint Louis, MO, USA) was added to the fish water. Embryos were washed, dechorionated and anaesthetized, with 0.016% tricaine (Ethyl 3-aminobenzoate methanesulfonate salt; Sigma-Aldrich), before observations and picture acquisitions.

Reverse transcription-PCR and real-time quantitative-PCR assays (RT-qPCR)

For human sample RT-qPCR experiments, Superscript II enzyme (Life Technologies) was used for cDNA synthesis. For this set of experiments, a Light Cycler 480II (Roche Diagnostics, Basel, Swiss) was used. Probes were selected according to the Software Probe Finder (Roche Diagnostics) and were reported in Supplementary Table S2. *hGUS* gene was used as reference and healthy patients cells as standard control. For zebrafish samples, after DNase I RNase-free (Roche Diagnostics) treatment to avoid possible genomic contamination, 1µg of RNA was reverse-transcribed using the "ImProm-II™ Reverse Transcription System" (Promega, Madison, Wisconsin USA) and a mixture of oligo(dT) and random primers according to manufacturer's instructions. RT-qPCRs were carried out in a total volume of 20 µl containing 1X iQ SYBR Green Super Mix (Promega), using proper amount of the RT reaction. RT-qPCRs were performed using the BioRad iCycler iQ Real Time Detection System (BioRad, Hercules, CA, USA). For normalization purposes, *rpl8* expression levels[4] were tested in parallel with the gene of interest. Primers were reported in Supplementary Table S3. Expression levels in the Y axis were relative to the control.

Western Blotting

At least 30 zebrafish embryos were used for protein preparation and from which the yolk was previously removed to avoid yolk protein contamination. Protein extracts were classically prepared in RIPA buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 150 mM NaCl, 0.25% sodium deoxycholate, 1mM EDTA, 1mM PMSF, protease inhibitors Roche) (2 µl/embryo). The protein concentration was determined using a Quantum Micro BCA protein assay kit according to the manufacturer's instructions (Euroclone, Pero, MI, Italy). 30-40 µg of each sample were loaded onto a 7.5% polyacrylamide gels and subjected to electrophoresis. The proteins were then transferred onto PVDF membranes which were blocked using a blocking solution at room temperature for 1 hour prior to

incubation with the primary antibodies: Nipbl (anti rabbit 1:200, Novus Biologicals Littleton, Colorado, USA), Gfp (anti rabbit 1:5000,Torrey Pines Biolabs Inc. Secaucus, NJ, USA) and Vinculin (anti mouse 1:6000, Sigma-Aldrich). After incubation with the HRP-conjugated secondary antibodies for 1 h at room temperature (mouse Santa Cruz Biotechnology, Dallas, TX, USA, rabbit Thermofisher, Waltham, MS, USA), the protein bands were detected using ECL detection systems (Cyangen, Bologna, Italy). Imaging acquisition has been done with the Alliance MINI HD9 AUTO Western Blot Imaging System (UVItec Limited, Cambridge, UK) and analyzed with the related software. Images were processed using the Adobe Photoshop software and when necessary, different parts of the same image have been took separately and later merged in a single image.

In situ *hybridization and immunofluorescent analyses.*

For quantification of the observed phenotypes, WISH experiments were done at least in 3 batches of embryos (minimum 15-20 embryos for each category). Embryos were fixed overnight in 4% paraformaldehyde (Sigma-Aldrich) in Phosphate Buffer Saline (PBS) at 4 °C, then dehydrated stepwise to methanol and stored at −20 °C. Antisense riboprobes were previously *in vitro* labelled with modified nucleotides (*i.e.* digoxigenin, Roche Diagnostics). *cmyb* and *spi1b* probes have been previously described.[5,6] Primary antibodies were anti-GFP (1:1000, Torrey Pines Biolab, Houston, TX, USA), anti Active-βcat (Clone 8E7, 1:50 Merck, Darmstadt, Germany) and anti PU.1 (1:100, Merck); the secondary antibodies were Alexa 488-conjugated goat anti-rabbit IgG and Alexa 546-conjugated goat anti-mouse IgG (1:400, Invitrogen Life Technologies, Carlsbad, CA, USA). Images were acquired using a microscope equipped with a digital camera with LAS Leica imaging software (Leica, Wetzlar, Germany). Images were processed using the Adobe Photoshop software and when necessary, different focal images planes of the same image have been took separately and later merged in a single image.

Sudan Black staining

Embryos were fixed with 4% PFA-PBS for 2 hours at room temperature, rinsed in PBS, incubated in Sudan Black (Sigma-Aldrich) for 20 minutes, washed in 70% ethanol in water, then rehydrated to PBS+0.1% Tween 20 (PBT).

FACS analyses

Embryos were anesthetized with 1X Tricaine/E3 medium (34.8 g NaCl. 1.6 g KCl. 5.8 g $CaCl_2 \cdot 2H_2O$. 9.78 g $MgCl_2 \cdot 6H_2O$) and dissociated with 0.25% trypsin-EDTA (Ethylenediaminetetraacetic acid) and Collagenase from Clostridium (Sigma-Aldrich C9891) 100mg/ml (500 µl dissociation mix/tube of embryos) by pipetting. 1 ml of DMEM (Dulbecco's modified of Eagle medium)-10% FBS (fetal bovine serum) was added and centrifuged 5' at 3000 rpm. Dissociated cells were washed twice in PBS 1X, filtered through 70 µm nylon mesh and transferred into a FACS (Fluorescence-activated cell sorting) tube. For PU.1 staining, cells were harvested as described above and incubated for 20' on ice in PBS 1X and formaldehyde 2%, washed in PBS 1X + 1% BSA (bovine serum albumin), resuspended in 100 µl TritonX100 0.1 % in PBS 1X and incubated 10' at room temperature. Then, cells were washed once in PBS 1X + 1% BSA and incubated for 45' at 4°C in BSA 5% in PBS 1X. Cells were spinned-down and the pellet was resuspended in 100 µl of anti PU.1 (1:100, Merck) diluted in PBS 1X + 1% BSA and incubated for 1 hour at room temperature, washed once in PBS 1X + 1% BSA; the secondary antibody was Alexa 488-conjugated goat anti-rabbit IgG (1:400, Invitrogen Life Technologies) diluted in PBS 1X + 1% BSA and incubated for 1 hour at room temperature. The pellet was washed and kept stained at 4°C until FACS analysis.

FACS sorting, RNA extraction and cDNA synthesis of GFP low positive cells

To sort the low and high GFP positive cells, 40 embryos for control, *nipblb*-MO and *NPMc*+ mRNA were collected and dissociated as in[7], suspended in 0.4 ml of PBS and sorted with the FACSAria cell sorter. GFP low positive cells were subjected to TRIZOL extraction following manufacturer instruction and resuspended in 5 uL of Nuclease free water. Half of the RNA obtained was used for

retro-transcription using Superscript IV VILO (ThermoFisher Scientific). In retro-transcription procedure, EzDNAse digestion step has been included, and the time of reverse transcription was increased to 30 minutes.

Injections

Two different morpholino against *nipblb*: the ATG-*nipblb*-MO (*nipblb*-MO) and the 5'UTR-*nipblb*-MO (5UTR*nipblb*-MO) were used (Gene Tools LLC, Philomath, OR, USA) both previously described and validated.[8] *nipblb*-MO was injected at the concentration of 1 pmol/embryo in 1x Danieau buffer (pH 7,6) for full dose experiments, and at a concentration of 0,6 pmol/embryo for subcritical doses. 5UTR*nipblb*-MO was injected at the concentration of 0,5 pmol/embryo in 1x Danieau buffer (pH 7,6) for full dose experiments, and at a concentration of 0,3 pmol/embryo for subcritical doses. As a control, we injected a standard control morpholino (ctrl-MO, Gene Tools). Human *NPM1* and *NPMc+* and zebrafish *dkk1b* mRNAs were injected at the following concentrations: 120 pg/embryo of *NPM1* mRNA, 100 pg/embryo of *NPMc+* mRNA and 50 pg/embryo of *dkk1b* mRNA. For dose-depended assays, *NPMc+* mRNA was injected at 25-75 and 125 pg/embryo. For subcritical doses, *NPMc+* was injected at 50 pg/embryo.

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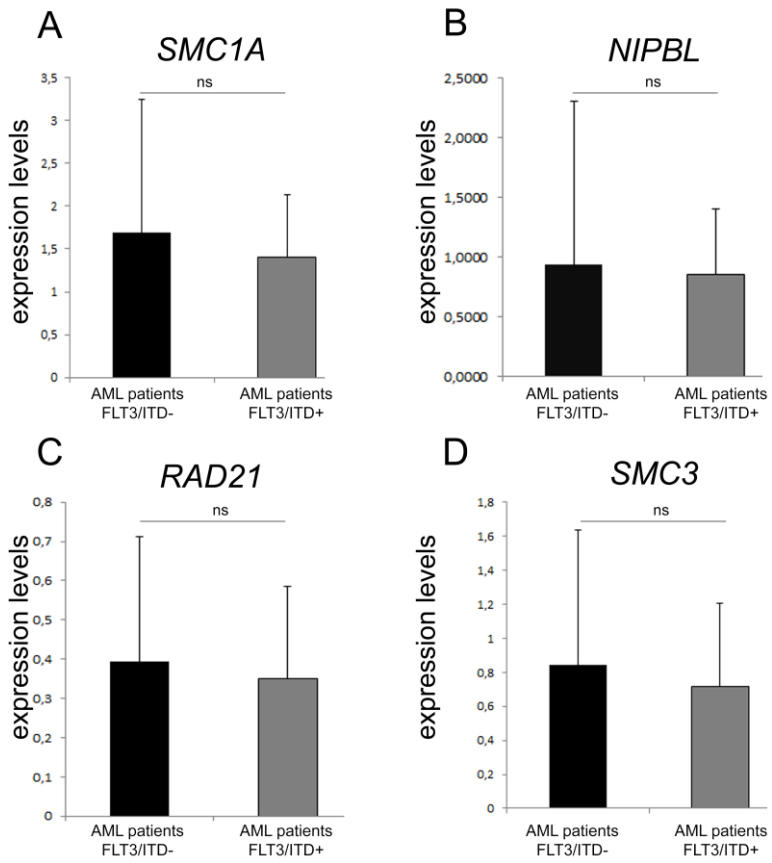
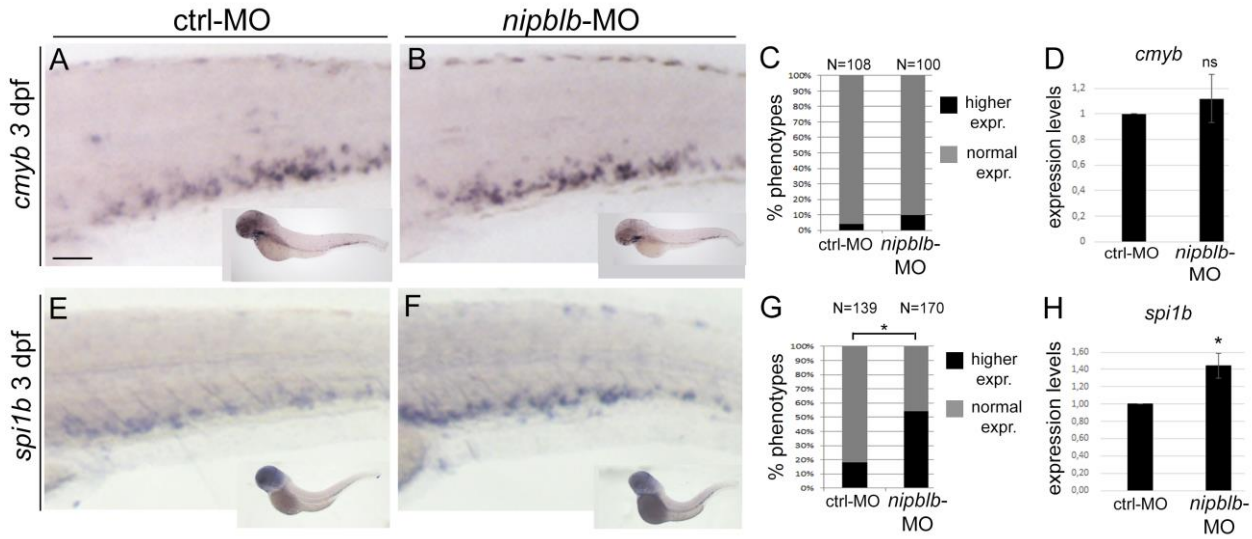
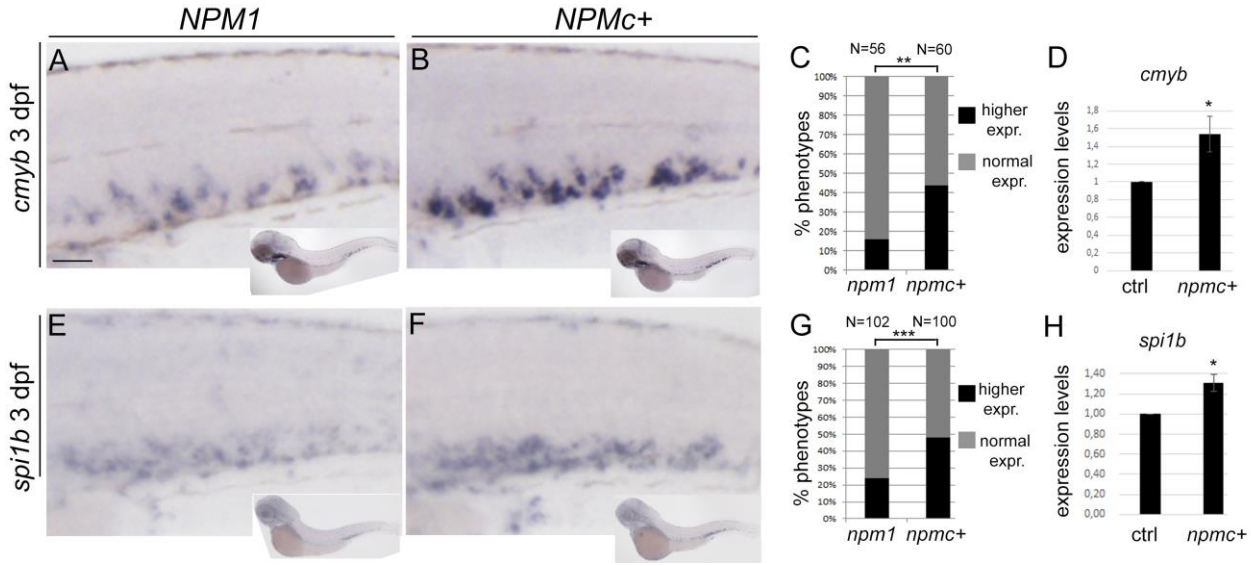
Supplementary Figure Legends and Tables



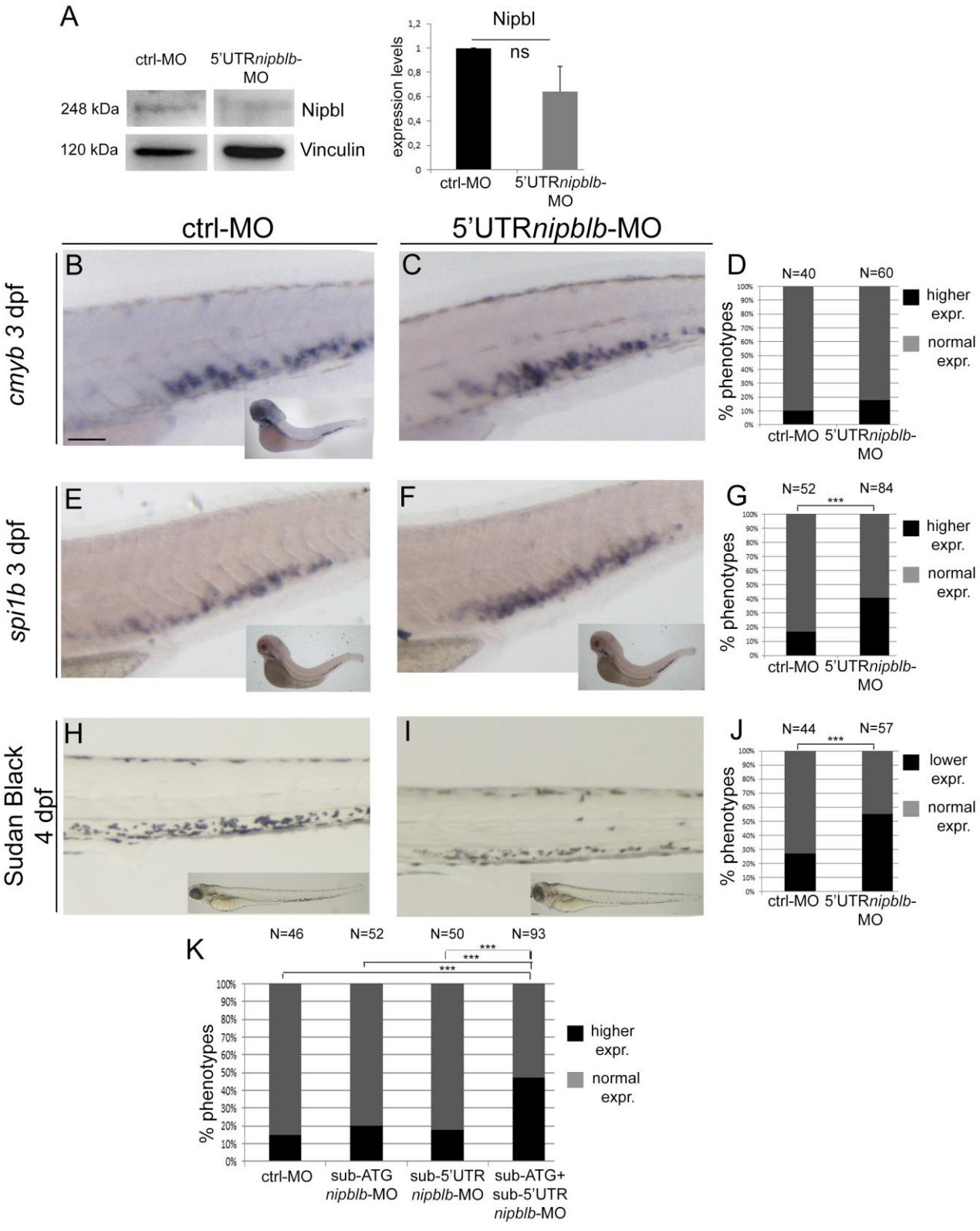
Suppl. Figure S1: Expression analyses of cohesin genes in Bone Marrow samples of AML adult patient divided in two subgroups for the absence/presence of *FLT3/ITD* mutations. *(A-D)* RT-qPCR analyses in AML adult patients indicated that the expression level of cohesin genes analyzed was not modified with *FLT3/ITD* mutation. ns: non-significant; AML: Acute Myeloid Leukemia, RT: Retro Transcription.



Suppl. Figure S2: Myeloid cell differentiation is affected in *nipblb*-loss-of-function embryos. *(A-B, E-F)* WISH analyses of *cmyb*, marker of HSCs and *spi1b*, marker of myeloid precursors at 3 dpf. *(A-D)* The expression of *cmyb* was comparable in *nipblb*-MO-injected embryos *(B)* and controls *(A);* quantification of the observed phenotypes in *(C).* RT-qPCR analyses of *cmyb* expression were performed on the tails of the embryos at 3 dpf *(D). (E-H)* The expression of *spi1b* was increased in *nipblb*-MO-injected embryos *(F)* in comparison to controls *(E);* quantification of the observed phenotypes in *(G).* RT-qPCR analyses of *spi1b* expression were performed on the tails of the embryos at 3 dpf *(H).* Scale bar represents 100 μm. Asterisks in *(G* and *H)* represent *=p<0.05. ns: non-significant; WISH: Whole Mount In Situ Hybridization; MO: morpholino; RT: Retro Transcription.



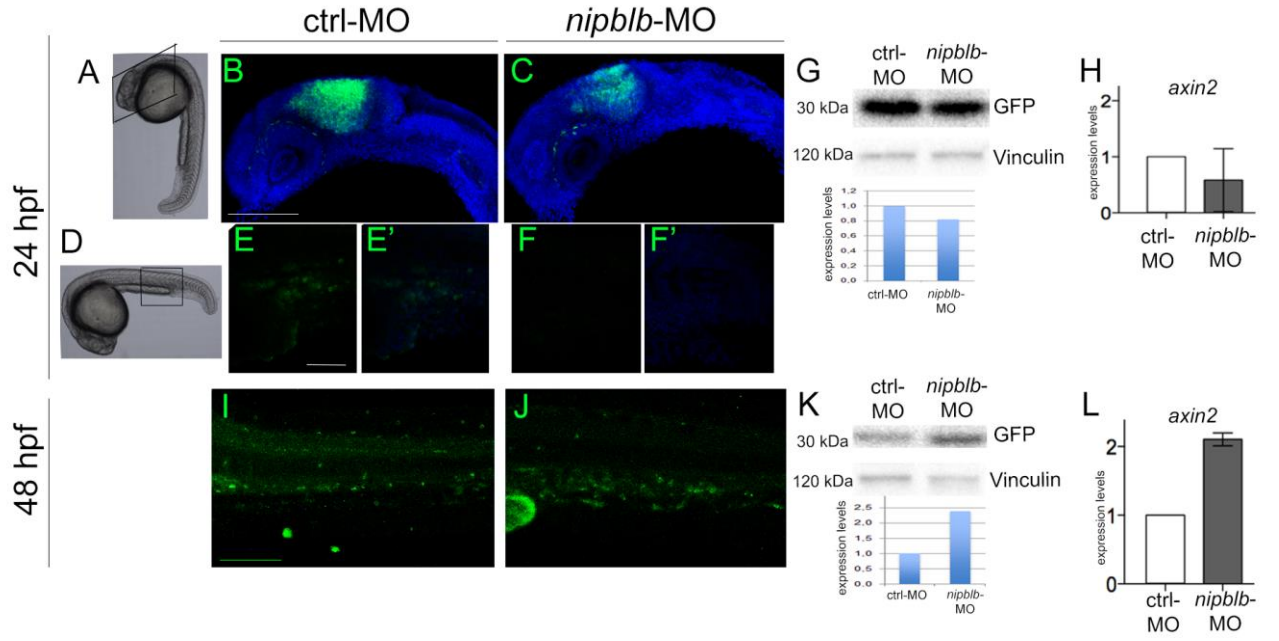
Suppl. Figure S3: HSCs and myeloid cell differentiation are affected in *NPMc+* injected embryos. *(A-B)* WISH analyses of *cmyb*, marker of HSCs, *(E-F) spi1b*, marker of myeloid precursors. The expression of *cmyb* and *spi1b* were increased in *NPMc+* injected embryos *(B)* and *(F)* in comparison to controls *(A)* and *(E);* quantification of the observed phenotypes in *(C-G).* RT-qPCR analyses of *cmyb* expression *(D),* and *spi1b* expression *(H),* were performed on the tails of the embryos. Scale bar represents 100 μm. Asterisks in *(D-H)* represents *=$p<0.05$, in *(C)* **=$p<0.01$ and in *(G)* ***=$p<0.001$. WISH: Whole Mount In Situ Hybridization; MO: morpholino; RT: Retro Transcription.



Suppl. Figure S4: Myeloid cell differentiation is affected in 5'UTR*nipblb*-loss-of-function embryos. *(A)* Western blot analyses, repeated twice, of Nipbl protein expression in embryos at 24 hpf injected with 5'UTR*nipblb*-MO. Vinculin marker was used for normalization. *(B, C, E, F)* WISH analyses of *cmyb*, marker of HSCs *(B-C)* and *spi1b*, marker of myeloid precursors *(E-F)*. The

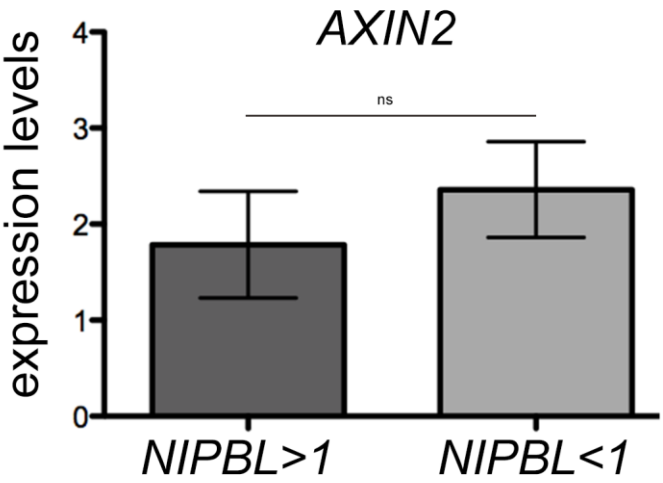
expression of *cmyb* was comparable in 5'UTR*nipblb*-MO-injected embryos *(C)* and controls *(B);* quantification of the observed phenotypes in *(D)*. The expression of *spi1b* was increased in 5'UTR*nipblb*-MO-injected embryos *(F)* in comparison to controls *(E);* quantification of the observed phenotypes in *(G). (H-I)* Sudan black staining of mature myeloid cells. The mature myeloid cells were diminished in 5'UTR*nipblb*-MO injected embryos *(I)* in comparison to controls *(H);* quantification of the observed phenotypes in *(J). (K)* Quantification of embryos with increased number of myeloid precursor cells positive for *spi1b*. The co-injection of subcritical doses of the two morpholinos against *nipblb*, but not that of the single morpholino, recapitulated the myeloid phenotype observed with the injection of full doses morpholino. Scale bar represents 100 μm. Asterisks in *(G, J, K)* represent ***=$p<0.001$. ns: non-significant; UTR: UnTranslated Region; WISH: Whole Mount In Situ Hybridization; MO: morpholino; HSCs: Hematopoietic Stem Cells; RT: Retro Transcription.

Suppl. Figure S5: Validation of inhibition of canonical Wnt pathway by means of *dkk1b* mRNA injection in the zebrafish Wnt reporter line *TOPdGFP*. *(A-D)* Confocal images of TOPdGFP zebrafish embryos at 3 dpf. The GFP+ cells in the hindbrain (h, arrow) and in the eye of controls *(A)*, were not present in *dkk1b* mRNA injected embryos *(B)*. *(C-D)* Also in the CHT region, the GPF+ cells were absent in *dkk1b* injected embryos *(D)* in comparison to controls *(C)* (arrows). Scale bar represents 100 μm. GFP: Green Fluorescent Protein; CHT: Caudal Hematopoietic Tissue.



Suppl Figure S6: Different canonical Wnt pathway modulation during zebrafish development following *nipblb* downregulation. *(A-H)* analysis of canonical Wnt pathway activation at 24 hpf in *nipblb*-loss-of-function zebrafish embryos. *(A)* Image of a 24 hpf embryo, the head region was selected for confocal images in *(B-C)*. The GFP expression in the Central Nervous System of TOPdGFP *nipblb*-MO injected embryos *(C)* was decreased in comparison to controls *(B)*. *(D)* Image of a 24 hpf embryo, the Aorta Gonad Mesonephric (AGM) tissue was selected for confocal images in *(E-F')*. The GFP expression of Wnt positive cells in AGM of controls *(E-E')* and *nipblb*-MO injected embryos *(F-F')* was indistinguishable from blood cells autofluorescence at this developmental stages. *(G)* Western blot analyses confirmed the GFP protein reduction in 24 hpf *nipblb*-MO injected embryos in comparison to controls. Vinculin was used as normalizer. *(H)* RT-

qPCR analyses of *axin2* expression, a marker of the status of canonical Wnt pathway. A representative experiment was shown. *(I-L)* Analyses of canonical Wnt pathway activation at 48 hpf. *(I-J)* The GFP expression in the CHT of TOPdGFP *nipblb*-MO injected embryos *(J)* was increased in comparison to controls *(I). (K)* Western blot analyses confirmed the GFP protein increase in 48 hpf *nipblb*-MO injected embryos in comparison to controls. Vinculin was used as normalizer. *(L)* RT-qPCR analyses of *axin2* expression, a marker of the status of canonical Wnt pathway. DAPI staining was used in *B, C, E', F'*. A representative experiment was shown. Scale bars represent 100 μm. RT-qPCR and Western blot experiment were replicated twice and the data in the figure are the average of the two different experiments. MO: morpholino; GFP: Green Fluorescent Protein; CHT: Caudal Hematopoietic Tissue; RT: Retro Transcription.



Suppl. Figure S7: *AXIN2* increased expression in AML patients with *NIPBL* downregulation. RT-qPCR analyses of *AXIN2* expression in N=40 AML adult patients divided in two subgroups depending on *NIPBL* expression: *NIPBL*>1 and *NIPBL*<1 in comparison to healthy donors. We did not observe a significant increase in *AXIN2* expression when *NIPBL* was downregulated. ns: non-significant; RT: Retro Transcription.

Suppl. Table S1

Supplementary Table S1. Clinical Features of patients' cohort.								
	AGE AT ONSET	KARYOTYPE	FAB CLASSIFICATION	NPM	FLT3-ITD	t(9;22)	t(8;21)	inv(16)
1	47	46,XX,t(10;11)(p11;p15)[20]	M0	NEG	NEG	NEG	NEG	NEG
2	49	46,XY[20]	M0/M1	NEG	NEG	NEG	NEG	NEG
3	48	46,XX[20]	M1	NEG	NEG	NEG	NEG	NEG
4	70	45,X,-Y,t(8;21)(q22;q22)[5]/46,XY[5]	M2	NEG	NEG	NEG	POS	NEG
5	72	47,XY,+mar[10]/46,XY[10]	M2	NEG	NEG	NEG	NEG	NEG
6	58	46,XX,t(3;5)(q25;q34)[20]	M2	NEG	NEG	NEG	NEG	NEG
7	70	44~45,XX,?+X,t(1;22)(q12;q11),+1,del(5)(q13q34),?inv(7)(q14q22),tas(8;15)(q24;p13),-17,-21,del(22)(q11)der(22)t(1;22)(q12;q11),+mar[cp18]/45,XX,t(1;22)(q12;q11),+1,del(5)(q13q34),-7,der(17)t(7;17)(q11;q25),-21,del(22)(q11)der(22)t(1;22)(q12;q11)[7]	M4	NEG	NEG	NEG	NEG	NEG
8	48	45,X,-X,t(8;21)(q22;q22)[20]/45,X,-X[3]	M2	NEG	NEG	NEG	POS	NEG
9	47	45-46,XY,del(3)(q?22q?26),der(4)t(?1;4)(p36;p16),add(11)(p14),-12,del(12)(p11),add(21)(q22)[cp13]/46,XY[7]	nk	NEG	NEG	nk	NEG	NEG
10	37	43,XY,?del(2)(q?33),-4,der(6)t(?4;6)(q?22;q21),i(11)(q10),-17,-18[19]/46,XY[2]	M1	NEG	NEG	NEG	nk	nk
11	59	46,XY[20]		NEG	POS	NEG	NEG	NEG
12	33	46,XY[15]	M1	NEG	POS	NEG	NEG	NEG
13	30	46,XY[20]	M5	NEG	POS	nk	NEG	NEG
14	20	46,XY,t(8;21)(q22;q22)[21]/46,XY[1]	nk	NEG	POS	nk	POS	NEG
15	58	46,XY,inv(16)(p13q22)[20]	M4	NEG	POS	nk	NEG	POS
16	76	nk	M5	NEG	POS	nk	NEG	NEG
17	78	46,XX[27]	M4	NEG	POS	nk	NEG	NEG
18	53	46,XY[22]	M4	NEG	POS	nk	NEG	NEG
19	64	46,XX[20]	M5	NEG	POS	nk	NEG	NEG
20	75	46,XY[26]	M4	NEG	POS	nk	NEG	NEG
21	39	46,XY[20]	M1	POS (A)	NEG	NEG	NEG	NEG
22	47	46,XX[20]	M5	POS (A)	NEG	NEG	NEG	NEG
23	63	46,XY,t(8;14)(q24;q32),add(13q34)[18]/46,XY[9]	nk	POS (D)	NEG	nk	NEG	NEG
24	58	46,XY/47,XY,+8[7/10]	nk	POS (QM)	NEG	nk	NEG	NEG
25	50	46,XX[20]	M4	POS (A)	NEG	nk	NEG	NEG
26	77	46,XY[20]	nk	POS (A)	NEG	nk	NEG	NEG
27	54	46,XX,t(9;22)(q34;q11)[14]/46,XX[6]	M4	POS (A)	NEG	POS	NEG	NEG
28	60	46,XX[6]	nk	POS	NEG	nk	NEG	NEG
29	62	46,XX[25]	M5	POS (A)	NEG ITD/POS D835/D836	nk	NEG	NEG
30	58	46,XX[20]	nk	POS (A)	NEG	nk	NEG	NEG
31	48	46,XX[20]	M4	POS (A)	POS	NEG	NEG	NEG
32	51	46,XX[20]	M5	POS (A)	POS	NEG	NEG	NEG
33	68	46,XX[20]	M4	POS (A)	POS ITD/POS D835/D836	NEG	NEG	NEG
34	46	46,XY[20]	M2	POS	POS	NEG	NEG	NEG
35	39	46,XX[22]	M1	POS (A)	POS	nk	NEG	NEG
36	58	46,XY	M5	POS (A)	POS	nk	NEG	NEG
37	35	46,XY,?r(18)(?)[16]/47,idem,+8[3]/46,XY[1]	nk	POS (B)	POS	nk	NEG	NEG
38	58	46,XY[24]	M1	POS (A)	POS	nk	NEG	NEG
39	70	46,XY[20]	M5	POS (A)	POS	nk	NEG	NEG
40	12	46,XY[24]	nk	POS (A)	POS	NEG	NEG	NEG

Suppl. Table S2

Human primers sequence and probe number used in qPCR experiments.			
PRIMER	**lenght**	**sequence**	**PROBE**
hGUS-L	20	CGCCCTGCCTATCTGTATTC	**57**
hGUS-R	20	TCCCCACAGGGAGTGTGTAG	
hNIPBL-L	19	CTATGCGAACAGCCCAAAA	**55**
hNIPBL-R	24	TTCACCTTGCTTACTACCACATTT	
hSMC1A-L	21	CGACATCTAGCCCTGAATCTG	**78**
hSMC1A-R	20	ATTAATGCGAGGCCCAAAGT	
hRAD21-L	20	ATTGACCCAGAGCCTGTGAT	**62**
hRAD21-R	20	GGGGAAGCTCTACAGGTGGT	
hSMC3-L	22	TGCACTGAATGATGAGATTCGT	**18**
hSMC3-L	27	TTAATTCTTTCATTTAGCAACTGTCTG	
hAXIN2-L	19	CCACACCCTTCTCCAATCC	**36**
hAXIN2-R	20	TGCCAGTTTCTTTGGCTCTT	

Suppl. Table S3

Zebrafish primers sequence used in qPCR experiments.		
PRIMER	**lenght**	**sequence**
***zrpl8*-L**	21	CTCCGTCTTCAAAGACCATGT
***zrpl8*-R**	21	TCCTTCACGATCCCCTTGATG
***znipblb*-L**	20	TGGAAGAAAAGACTCCTGGG
***znipblb*-R**	20	ACGTCCGTGGCTTCCACGGT
***zsmc1a*-L**	20	AAATGTGGAGGACGCTCGTA
***zsmc1a*-R**	20	AGGTCACTAGCTCCTCCAGA
***zrad21*-L**	20	AGGAAGGACAGGGAGGAGAT
***zrad21*-R**	20	GTTGTTTCTGCACAGCTCCA
***zsmc3*-L**	20	CCCTTCGGCTCAAAACACAA
***zsmc3*-R**	20	TGGCCGAAGATGACTGAACT
***zspi1b*-L**	19	GCCATTTCATGGACCCAGG
***zspi1b*-R**	19	ACACCGATGTCCGGGGCAA
***zcmyb*-L**	20	GACACAAAGCTGCCCAGTTG
***zcmyb*-R**	20	GCTCTTCCGTCTTCCCACAA

***zaxin2*-F**	20	GGCCACTGTAGTGGGTCTGT
***zaxin2*-R**	20	ATTAGGATTTCCGGGGTCAC
***zgfp*-F**	22	CTGGTCTAGCTGGACGGCGACG
***zgfp*-R**	22	CACGAACTCCAGCAGGACCATG

Suppl. Table S4

Antibodies	Specie	Concentration	Technique
Vinculin	mouse	1:6000	WB
NIPBL	rabbit	1:200	WB
Active-βcat	mouse	1:50	IF
PU.1	rabbit	1:100	IF/FACS
GFP	rabbit	1:5000	WB
Alexa anti rabbit 488	goat	1:400	IF/FACS
Alexa anti mouse 546	goat	1:400	IF
Anti-rabbit IgG HRP	goat		WB
Anti-mouse IgG HRP	goat		WB