Long non-coding RNA expression profile in cytogenetically normal acute myeloid leukemia identifies a distinct signature and a new biomarker in NPM1-mutated patients

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SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

AML samples

Cohort 1 was composed of forty CN-AML samples collected from patients registered at the HIMIP (Hémopathies INSERM Midi-Pyrénées) collection. For the validation cohort (Cohort 2, n=134), thirty-four CN-AML samples were obtained from the HIMIP collection, 7 CN-AML samples from the Hematology Institute of Perugia University and 93 CN-AML samples from the FILOtheque AML, Paris. Fresh and thawed samples were obtained after informed consent and the study was conducted in accordance with the Declaration of Helsinki and was approved by a local ethics committee.

Seventy-four AML samples (40 samples for Cohort 1 and 34 samples for Cohort 2) were collected from patients registered at the HIMIP. In accordance with French law, the HIMIP collection has been declared to the Ministry of Higher Education and Research (DC 2008-307 collection 1) and a transfer agreement has been obtained (AC 2008-129) after approbation by local ethical committees (the Comité de Protection des Personnes Sud-Ouest et Outremer II and the APHP ethical committee). Clinical and biological annotations have also been declared to the CNIL (Comité National Informatique et Libertés).

Seven AML samples were obtained from the Hematology Institute of Perugia University. These patients had received standard induction regimens (i.e. an anthracycline and cytarabine "3+7" combination). Patients who had achieved complete remission received one or two cycles of consolidation therapy and/or allogeneic stem cell transplantation.

Ninety-three AML samples (included in Cohort 2) were collected from patients registered at the FILOtheque. The handling, conditioning and storing of patient samples was performed by the FILOtheque AML (N° BB-0033-00073) tumor bank of the FILO group, Cochin Hospital, Paris. Fifty-four patients were enrolled in the AML 2006 IR trial and thirty-nine

patients in the AML 2007 SA trial. AML 2006 IR was a multicenter, randomized, open-label, phase III trial assessing the efficacy of gemtuzumab ozogamycin (GO, or MYLOTARG) in association with intensive chemotherapy in intermediate risk AML patients aged 18 to 60 years, in which patients received standard daunorubicin/cytarabine induction therapy with or without GO at a dose of 6 mg/m^2 on day 4. AML 2007 SA was a multicenter randomized trial comparing induction therapy with IC (idarubicin and cytarabine, "5+7") to ICL (the same drugs plus lomustine (CCNU), 200 mg/m^2 administered orally at day 1) in patients older than 60 years. Patients in complete remission (CR) received a post-remission schedule with or without lomustine, according to randomization.

For prognosis analysis, we discarded patients that had not received intensive chemotherapy, who were older than 75 years old and for whom no clinical information was available.

RNA isolation and cDNA synthesis

Total RNAs were extracted with the TRIzol reagent (Ambion, Austin, TX, USA). RNA integrity was evaluated using the RNA 6000 Nano Chip kit (Agilent Technologies, Massy, France). Only RNA extracts with RNA integrity values ≥7 underwent further reverse transcription. All samples were reverse transcribed using the Superscript II reverse transcription kit (Invitrogen, Beijing, China), according to the manufacturer's protocol.

RNA sequencing library preparation, read generation and mapping

RNA sequencing was performed at the BGI (Hong Kong) for samples from Cohort 1 (n=40) and two CD33+ bone marrow cells from healthy samples (Stemcell; ABM032F). rRNA depletion was performed from total RNA with Ribo-Zero™ rRNA Removal Kits (Epicentre, Madison, WI, USA). Paired-end, strand-specific reads of 91 nt were generated on an Illumina HiSeqTM2000. Alignment and mapping were performed using Tophat[1] against the hg19

genome and the mapped reads were assembled by Cufflinks 2.0.2[2]. The Cuffcompare program was used to merge the RefSeq, ENCODE and UCSC human known genes freeze January 2013 into one gene annotation set for comparison with the assembled transcripts[3]. For novel lncRNA prediction, transcripts with a length >200 nt were extracted, according to the definition of lncRNA. Multi-exon (≥2 exons) transcripts and single-exon transcripts which recurred in multiple samples (≥3 samples) were then extracted. Assemblies that had long (≥300 nt) putative open reading frames (ORFs) were excluded. In order to filter out the known lncRNAs, the filtered assembly transcripts were BLASTed to find known lncRNAs from the ENCODE, Refseq and UCSC databases, according to the thresholds: identity >0.9 and coverage >0.8. Transcripts which did not match known lncRNAs were then used for protein annotation. The transcripts were aligned to four protein databases: kegg, nr, cog and swissprot, according to the thresholds: identity >0.9 and coverage >0.8. The coding potential of extracted transcripts which did not align with the protein databases was assessed using CPC based on six biologically meaningful sequence features such as ORF score, coverage, integrity, number of hits, hit score and frame score[4]. Known lncRNAs and predicted novel lncRNAs were then combined as the reference, and reads were aligned to this reference by SOAP2[5]. Among the 35046 putative lncRNA transcripts expressed in our samples, we selected only lncRNAs that were at least 90% identical to the reference transcript in at least two samples. Gene expression levels were calculated using the RPKM (Reads Per Kb per Million) method[6]. The raw and processed RNA-sequencing data have been deposited at the National Center for Biotechnology Information Gene Expression Omnibus (repository number GSE62852).

Statistical analysis

All unsupervised clustering and heatmap generations were performed using the « hclust » function in R and the Ward's agglomeration method. The partial least squares discriminant analysis (PLS-DA) and the sparse PLS-DA methods were used using the R mixOmics package. The “Perf” method, from the “Mixomics” package, was used to estimate the error rate of the model using leave-one-out cross-validation. Bootstrapping cluster analysis was carried out using the R ClassDiscovery package (1000-fold re-sampling).

Expression correlation between RNAseq and Fluidigm data was assessed using the Pearson correlation.

Event-Free Survival (EFS) was calculated from the date of diagnosis until relapse, death, treatment failure or the last received news. Overall survival (OS) time was calculated from the date of diagnosis until death or last received news. Disease-Free Survival (DFS) was calculated from the date of Complete Remission (CR) until relapse, death or last news. Surviving patients were censored at the date of last follow-up. For patients who underwent allogeneic transplantation, EFS, OS and DFS times were censored at the time of transplantation.

ROC curve analysis was performed to determine a suitable $-\Delta Ct$ cut-off value for each lncRNA. ROC curve analysis for EFS was performed 500 times on 13 randomly selected patients (5 *NPM1*+ patients and 8 *NPM1*-wild type patients) from Cohort 1. The final cutoff was taken as the mean of the 500 cutoffs. This cutoff was applied to Cohorts 1 and 2 to define the patient subgroups with high and low expression of each lncRNA.

The Kaplan-Meier method was used to generate survival curves, and these curves were compared using a log-rank test.

The Fisher exact test and the Mann Whitney test were used to assess the statistical significance of the differences between groups.

Cox proportional hazards model was used for EFS, OS and DFS to calculate univariable HRs. Multivariate Cox proportional hazards regression models were constructed for EFS, OS and DFS using a limited forward selection procedure. The following variables were first evaluated in univariate models: age; sex; leucocyte count; hemoglobin; platelet count; bone marrow blast percentage; treatment; ELN classification; presence of *NPM1*, *FLT3*-ITD, *DNMT3a*, *CEBPa*, *IDH1*R132 or *IDH2*R140 mutations; and the expression level of XLOC_109948. Variables with a univariate $p < 0.1$ were included in the initial multivariate models, and a stepwise backward variable selection procedure was applied.

For all analyses, p-values < 0.05 were considered significant.

All analyses were performed using the R 3.0.0 software package (available at www.r-project.org).

Quantitative real-time reverse transcriptase polymerase chain reaction analysis

Total RNA was isolated 48h post-transfection using the Trizol extraction protocol according to the manufacturer's instructions. Reverse transcription was performed with Superscript III reverse transcriptase (Invitrogen). Quantitative polymerase chain reaction (PCR) was performed with SYBR Premix Ex Taq (TAKARA) on 384-well plates using the Lightcycler 480 (Roche). The data presented correspond to the mean of $2^{-\Delta\Delta Ct}$ from at least three independent experiments, normalized to the ABL1 and TBP reference genes.

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SUPPLEMENTARY FIGURES AND TABLES

Figure S1: Specific LncRNA expression profile in *NPM1*-mutated AML patients with normal cytogenetics. A Hierarchical clustering and associated heatmap of RNAseq data from the first cohort of CN-AML patients (n=40) with 33 lncRNAs differentially-expressed between *NPM1*-mutated (n=14) and *NPM1*-wild type patients (n=26). B Hierarchical clustering and associated heatmap of RNAseq data from the first cohort of CN-AML patients (n=40) with 12 lncRNAs differentially-expressed between *NPM1*-mutated (n=14) and *NPM1*-wild type patients (n=26). The heatmap depicts high expression (red, +1) and low expression (blue, −1).

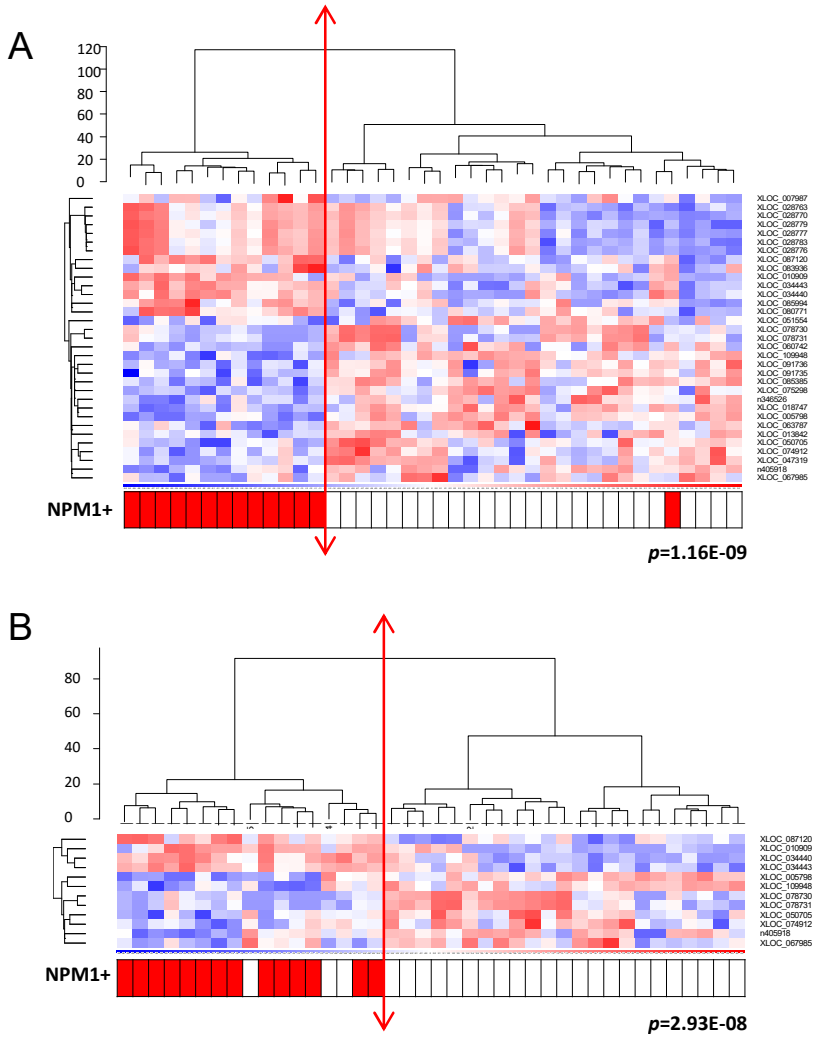

Figure S2: Bootstrapping cluster analysis showing the stability of the groups based on the 12 lncRNA signature. Bootstrapping analysis results using the 12 lncRNA signature with Cohorts 1 (**A**) and 2 (**B**) (1000-fold re-sampling). Hierarchical clustering was used, with Euclidean distance and Ward's method for agglomeration. The purity of the color corresponds to the stability of the samples in the specific groups. Analyses were carried out in R using the ClassDiscovery package.

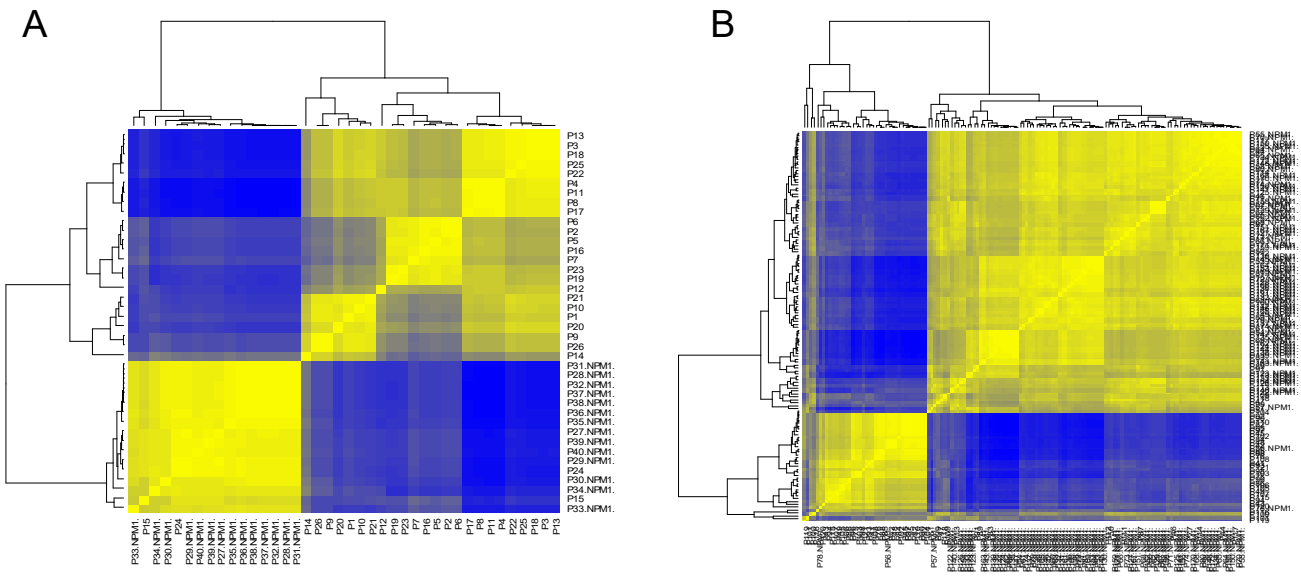

Figure S3: RNA-seq quantification of XLOC_109948 lncRNA expression level in normal CD33+ bone marrow (BM) cells compared to CN-AML samples. RPKM, Reads Per Kb per Million.

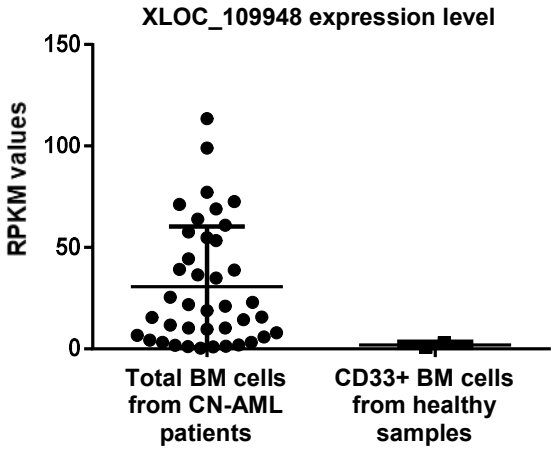

Table S1: Patient characteristics

Characteristics	Cohort 1 (n=40)	Cohort 2 (n=134)
Female Sex		
No.	19	67
%	47.5	50
Age at diagnosis		
Median	67.5	55.4
Range	16-87	20-75
French American British classification (FAB)		
M0	0 (0%)	4 (3%)
M1	14 (35%)	38 (28%)
M2	9 (22.5%)	46 (34%)
M4	13 (32.5%)	23 (17%)
M5	3 (7.5%)	19 (14%)
M6	1 (2.5%)	0 (0%)
NC	0 (0%)	5 (4%)
European Leukemia Net Classification (ELN)		
Favorable	8 (20%)	46 (34%)
Intermediate I	32 (80%)	88 (66%)
White Blood Cells Count (x10^9 cells/L)		
Median	30.5	34.5
Range	1.23-206	0.9-250
Hemoglobin level (g/dL)		
Median	10.1	9.35
Range	5.5-14.2	5.5-14.1
Platelet count (x10^9 cells/L)		
Median	79.5	73
Range	8-735	10-489
Bone Marrow Blast Count (%)		
Median	79.5	73
Range	19-99	10-100
Mutational Status		
NPM1 mutation	14/40 (35%)	80/134 (60%)
FLT3-ITD mutation	13/40 (32.5%)	58/134 (43%)
CEBPa	6/18 (33.5%)	16/119 (12%)
DNMT3a	11/40 (27.5%)	19/86 (22%)
*IDH1*R132	4/40 (10%)	11/83 (13%)
*IDH2*R140	8/40 (20%)	17/83 (20%)
Chemotherapy		
Intensive	25 (62.5%)	134 (100%)
NC	4 (10%)	0 (0%)

NC: Not Communicated

Table S2: Primer list.

lncRNAs	Forward Primer	Reverse Primer
n346526	GGAGAGCTTGGGTGAGGACCAC	AAGGGGGCTCAGCAGGTGATAA
n405918	AGCTTCGGCTATGGGACTGGTT	CGCATGATCTCTCAGTCCAGGTC
XLOC_005798	GGCAGTCAGGAAGGAAAACTGGA	CGGTGGCTGTTTCTCCACTGTC
XLOC_007987	AAACCCACCAACGTGACCAGAA	GCATCCTGGCAAATGAGGACAT
XLOC_010909	GACAAGGGGGAGACTACAAGAAGCA	CTGCGCTCAAGCTCGAAGAGTAA
XLOC_013842	GGTTTGGAGTGAAAGGCACATGA	CCAGGCAGCACTATCTGGGAAA
XLOC_018747	GCCTGGACAGATTACCAGATCATCA	AGCAAGCTGGAAGCTGAGCTCTT
XLOC_028763	CAGGCACCAGGAGAGGAGACAC	CACGGGGACCCCTGAACTTAAA
XLOC_028770	TCCGGTCATTGGAAAAGTGCAT	AGGGTTTCCTGGCCAACTTGAG
XLOC_028776	CTTTTGGTGGCCAAATCCTGTG	ACGGTTTCCACCCCTAGTGCAT
XLOC_028777	TTTGCTGCTCACCTGCTGTGAC	TCTAGGCTGGAGGCAGTGAGGA
XLOC_028779	TGAGCTTCCCCACTGCATGAAT	AGGCCGAAATGATTGTGATGTGA
XLOC_028783	CCTGGGCCAATCACTGATGTTC	GGGCAGGAAAGCTGTCAGTCAC
XLOC_034440	TGCTTAGTGAAAGATGCCAGACAAAA	TCCCCAGGCAGTAACCAAACTG
XLOC_034443	TGCACCGACTGACAGAATGAGG	TCCAGGGTTCCAAGATAGCGAAA
XLOC_047319	TCATCAGCCTCCTGCTCCACTC	TGATTACCACGCAAGGCTGACA
XLOC_050705	TTCCCGGTGGGTGTCATATCAG	CTAGCAGCCCCTCCCTCCATC
XLOC_060742	GGGAATGGAGAATTACCCTCTGGA	AAACAGGCCATTGACAGGTAGACAA
XLOC_063787	GCCAATGCCAACTTCACTCCAG	ACGCAGCCTCATACACCCATTG
XLOC_067985	GCAAATGGCCTCCAAATCAAAA	AGCCACAGATCCATTGCTGACC
XLOC_074912	CACAGGTGAATCTCCGCTGTCA	ATCCTTTGCTGGGGTTGGAAAA
XLOC_075298	TTGGGACTACCACCCAAAGCAG	GGTGTCAGCCATTCTGCTGGAA
XLOC_078730	GCATGCTAAGAGCCCATGAAGC	AGAAGCTGGCCCAAGTGTGTGT
XLOC_078731	GGCCAGGTGCAGTAGTTCATGC	AAACAAGGTTCCCACGGCTGAT
XLOC_080771	TACATGTGCCATGGTGGTTTGC	TAGGGGGCTAGGGGAGGGATAG
XLOC_083936	GCAATCTCGGCTCACTGCAACT	TGTGGCAGGTGCCTGTCTGTAG
XLOC_085385	TCCATTTTGGAGGCTGGGAAGT	TAGGGAGTTGGCCATCTGCAAG
XLOC_085994	GACCGAGATGAGGCAAAGAGCA	GAGCAATGGGGATAGTGCCACA
XLOC_087120	CCATGCACCAGGCACTGTAATG	TGGGTAGCACCTCTCCATGAGC
XLOC_091735	GCTGGTGGAAGGTCTGTTCTGC	TGGCAGCAAGGATTTGCTAAGG
XLOC_091736	AAGAACCCGTGTCAGGCTTGTG	CCTCTGACAGCCAGCAAGGAAA
XLOC_109948	CCTGCCTTCTTGTCCTCCACCT	GGCCAGGAAACAAATTGCAACA
Housekeeping genes	Forward Primer	Reverse Primer
MLN51	TAATCCCAGTTACCCTTATGCTCCA	GTTATAGTAGGTCACTCCTCCATATACCTGT
5S	ACGCGCCCGATCTCGTCTGAT	GCCTACAGCACCCGGTATTCCC
GAPDH	CGGGAAGCTTGTCATCAATGG	GGCAGTGATGGCATGGACTG
TBP	GCCTCCCCCACCCCCTTCTTT	GCCACACCCTGCAACTCAACATCC
ABL1	TGGAGATAACACTCTAAGCATAACTAAAGGT	GATGTAGTTGCTTGGGACCCA
ACTIN	TCCCTGGAGAAGAGCTACGA	AGGAAGGAAGGCTGGAAGAG
S14	ATCAAACTCCGGGCCACAGGA	GTGCTGTCAGAGGGGATGGGG

Antisense LNA GapmeRs	Sequence 5'-3'	
XLOC_109948a	TCTGTGGAGCTGTAAC	
XLOC_109948b	CACTAGAGGCAGGAAC	
Negative control A	AACACGTCTATACGC	

Table S3: List of mRNAs differentially-expressed between *NPM1*-mutated and *NPM1*-wild type patients from RNA sequencing data (EdgeR analysis). Highlighted genes are those known to be deregulated between *NPM1*-mutated versus *NPM1*–wild type AML patients. logFC (log Fold Change), log CPM (log Count per million), FDR (False Discovery Rate).

GeneID	logFC	logCPM	PValue	FDR
Over-expressed in NPM1 mutated patients				
FOXC1	3,80	6,62	1,12E-07	2,49E-05
SERPINB2	3,76	7,24	1,89E-07	3,66E-05
HOXA5	3,26	5,57	8,13E-10	4,35E-07
HOXA6	3,07	4,56	9,94E-08	2,38E-05
PTPN14	2,92	5,29	8,63E-08	2,31E-05
PTX3	2,91	5,09	8,41E-09	3,07E-06
HOXB3	2,87	5,99	7,27E-10	4,14E-07
KRT18	2,85	4,45	2,07E-06	2,35E-04
CD300E	2,64	6,69	9,94E-05	3,71E-03
HOXB4	2,61	5,45	9,03E-10	4,57E-07
IFIT2	2,49	6,24	2,03E-05	1,24E-03
NEU4	2,44	4,85	4,57E-05	2,16E-03
TP53INP2	2,40	5,81	7,70E-11	9,70E-08
PHLDA1	2,27	5,60	7,67E-06	6,47E-04
HOXA7	2,26	3,60	3,46E-05	1,81E-03
ETV5	2,21	3,91	8,32E-09	3,07E-06
IRAK2	2,19	4,33	1,27E-05	9,31E-04
PBX3	2,14	6,04	2,35E-13	7,15E-10
MEIS1	2,11	6,82	7,39E-07	1,04E-04
HOXA9	2,00	6,99	4,75E-05	2,20E-03
Down-regulated in NPM1 mutated patients				
HGF	-2,03	5,91	3,09E-07	5,21E-05
FZD6	-2,03	3,64	9,55E-05	3,64E-03
FAM105A	-2,04	5,47	3,93E-06	3,73E-04
C18orf1	-2,08	5,28	2,28E-06	2,54E-04
CEACAM4	-2,12	4,69	1,54E-06	1,90E-04
MYEF2	-2,18	4,08	1,59E-05	1,07E-03
STK32B	-2,18	3,22	1,72E-05	1,15E-03
LY9	-2,19	3,59	1,79E-06	2,07E-04
GATM	-2,22	3,51	1,86E-05	1,20E-03
SLAIN1	-2,24	4,06	2,80E-06	2,93E-04
TRPS1	-2,27	6,50	3,57E-08	1,08E-05
TNXB	-2,29	5,50	1,01E-04	3,72E-03
GNAI1	-2,34	4,89	3,23E-04	8,04E-03
COBLL1	-2,34	4,75	4,49E-07	7,05E-05

NRP1	-2,41	3,99	1,51E-04	4,78E-03
SPRY1	-2,42	4,78	2,53E-07	4,44E-05
PFKFB2	-2,43	5,28	5,28E-09	2,30E-06
IGJ	-2,52	7,66	2,65E-05	1,50E-03
PROM1	-2,53	6,82	5,94E-05	2,57E-03
LTF	-2,57	9,21	1,05E-04	3,78E-03
HLA-DQA1	-2,60	5,94	3,85E-04	9,13E-03
HIST1H3G	-2,62	7,50	4,40E-08	1,25E-05
C10orf10	-2,62	4,43	2,90E-06	2,98E-04
MN1	-2,68	4,58	1,02E-05	7,83E-04
STARD9	-2,71	5,64	5,71E-09	2,36E-06
SPARC	-2,72	6,10	6,78E-12	1,54E-08
FAM111B	-2,79	3,92	1,13E-09	5,41E-07
AKT3	-2,82	5,86	6,18E-10	3,75E-07
SETBP1	-2,82	4,22	2,62E-07	4,50E-05
NAV1	-2,86	4,08	1,37E-08	4,46E-06
RASD1	-2,88	4,83	5,73E-06	5,28E-04
PRR5L	-2,91	4,98	2,42E-06	2,66E-04
KLHL13	-2,92	4,25	3,73E-08	1,10E-05
ADAMTS10	-3,03	4,61	1,74E-07	3,44E-05
CD200	-3,07	4,10	2,20E-06	2,47E-04
APP	-3,11	6,96	1,10E-07	2,49E-05
ACY3	-3,11	4,46	4,57E-05	2,16E-03
IKZF2	-3,16	5,30	5,29E-09	2,30E-06
TMIGD2	-3,25	3,92	3,44E-10	2,41E-07
SLCO5A1	-3,26	4,12	2,48E-06	2,66E-04
HLA-DOA	-3,30	5,61	1,80E-10	1,36E-07
MLLT3	-3,67	5,53	1,76E-20	1,61E-16
L3MBTL4	-3,68	3,83	9,27E-08	2,38E-05
CRHBP	-3,71	4,93	9,51E-08	2,38E-05
CD34	-3,80	5,23	2,31E-07	4,28E-05
ATP10A	-3,93	5,31	4,23E-10	2,75E-07
BAALC	-4,15	7,25	5,02E-07	7,62E-05
BEND4	-4,69	4,42	1,20E-10	9,93E-08
LRP6	-4,78	4,29	4,10E-11	7,47E-08
SDK2	-5,29	4,83	8,71E-18	3,97E-14
TRH	-6,04	4,54	8,84E-11	9,70E-08

Table S4: Sequences and localization of the 33 lncRNAs differentially-expressed between *NPM1*-mutated and *NPM1*-wild type patients

n346526; chr9:139,534,589-139,541,618
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XLOC_007987; chr1:151,915,378-151,918,279

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XLOC_010909; chr10:61,810,320-61,845,161

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XLOC_018747; chr11:58,756,982-58,767,516

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XLOC_028763; chr13:110,303,472-110,305,351

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XLOC_028776; chr13:110,347,864-110,349,260

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XLOC_028777; chr13:110,359,142-110,360,361

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XLOC_028779; chr13:110,364,295-110,365,134

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XLOC_028783; chr13:110,377,963-110,379,023

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XLOC_034440; chr15:50,078,505-50,080,055

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XLOC_034443; chr15:50,091,315-50,094,732

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XLOC_047319; chr19:42,119,856-42,121,317

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XLOC_050705; chr2:166,666,979-166,668,466

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XLOC_051554; chr2:201,659,264-201,659,916

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XLOC_060742; chr21:21,616,713-21,631,054

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XLOC_087120; chr6:26,112,403-26,114,712

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XLOC_091735; chr7:24,732,006-24,732,506

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XLOC_091736; chr7:24,735,012-24,736,269

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XLOC_109948; chrX:45,688,377-45,689,937

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Table S5: Validation of RNA-seq results by Fluidgm. Table shows Pearson Correlation r values for each of the 32 lncRNAs comparing RNA-seq and Fluidigm data, using the CN-AML Cohort 1 (n=40).

GeneID	Pearson correlation
n346526	0.89
n405918	0.94
XLOC_005798	0.92
XLOC_007987	0.94
XLOC_010909	0.96
XLOC_013842	0.83
XLOC_018747	0.80
XLOC_028763	0.84
XLOC_028770	0.91
XLOC_028776	0.93
XLOC_028777	0.92
XLOC_028779	0.90
XLOC_028783	0.83
XLOC_034440	0.87
XLOC_034443	0.94
XLOC_047319	0.81
XLOC_050705	0.82
XLOC_060742	0.64
XLOC_063787	0.92
XLOC_067985	0.96
XLOC_074912	0.86
XLOC_075298	0.90
XLOC_078730	0.92
XLOC_078731	0.87
XLOC_080771	0.93
XLOC_083936	0.89
XLOC_085385	0.17
XLOC_085994	0.48
XLOC_087120	0.94
XLOC_091735	0.88
XLOC_091736	0.75
XLOC_109948	0.90

Table S6: Patients selected for clinical analysis (CA)

Characteristics	Cohort 1 CA (n=25)	Cohort 2 (n=134)
Female Sex		
No.	9	67
%	36	50
Age at diagnosis		
Median	61	55.4
Range	16-75	20-75
French American British classification (FAB)		
M0	0 (0%)	4 (3%)
M1	10 (40%)	38 (28%)
M2	7 (28%)	46 (34%)
M4	4 (16%)	23 (17%)
M5	3 (12%)	19 (14%)
M6	1 (4%)	0 (0%)
NC	0 (0%)	5 (4%)
European Leukemia Net Classification (ELN)		
Favorable	4 (16%)	46 (34%)
Intermediate I	21 (84%)	88 (66%)
White Blood Cells Count (x10^9 cells/L)		
Median	32.1	34.5
Range	1.23-206	0.9-250
Hemoglobin level (g/dL)		
Median	9.9	9.35
Range	5.5-12.8	5.5-14.1
Platelet count (x10^9 cells/L)		
Median	78	73
Range	8-367	10-489
Bone Marrow Blast Count (%)		
Median	84.5	73
Range	19-94	10-100
Mutational Status		
NPM1 mutation	9/25 (36%)	80/134 (60%)
FLT3-ITD mutation	9/25 (36%)	58/134 (43%)
CEBPa	4/14 (28.5%)	16/119 (12%)
DNMT3a	6/25 (24%)	19/86 (22%)
*IDH1*R132	2/25 (8%)	11/83 (13%)
*IDH2*R140	6/25 (24%)	17/83 (20%)

NC: Not Communicated