Integrative nucleophosmin mutation-associated microRNA and gene expression pattern analysis identifies novel microRNA - target gene interactions in acute myeloid leukemia

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Supplementary Design and Methods

Patients' samples

Samples from 43 adult patients (median age 46 years; range, 23-60) with cytogenetically normal acute myeloid leukemia (CN-AML) were provided by the German-Austrian AML Study Group (AMLSG) with informed consent of the patients and institutional review board approval from all participating centers. Samples were enriched for mononuclear cells by FicoII gradient purification from diagnostic (i.e. pre-treatment) peripheral blood (n=24) or bone marrow (n=19) samples. Following enrichment, the percentage of leukemic cells was at least 80%. All patients were enrolled into the AMLSG treatment protocol AML HD98A (ClinicalTrials.gov Identifier: NCT00146120). Twenty-three of the 43 patients (53%) had mutated *NPM1*. Details of the clinical characteristics of patients, as well as the results of molecular genetic analyses are provided in *Online Supplementary Table S1*.

Cytogenetic and molecular genetic analyses

Conventional cytogenetic (chromosome banding), interphase cytogenetic (fluorescence *in situ* hybridization, FISH), and molecular genetic analyses [screening for *FLT3* internal tandemduplications (ITD) and tyrosine kinase domain (TKD) mutations, *CEPBA* mutations and *NPM1* mutations (*NPM1*^{mut})] were performed as previously described at the central reference laboratory of the AMLSG at our institution.¹⁻⁵

Cell lines and cell culture

Leukemic cell lines (CMK, HEL, K-562, Kasumi-1, KG-1, M-07e, ME-1, MONO-MAC-1, MV4-11, NB-4, OCI-AML2, OCI-AML5, THP-1, UT-7) and HeLa cells were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) and cultured according to their guidelines (www.dsmz.de/human_and_animal_cell_lines/).

miRNA expression profiling

To screen miRNA expression in AML and leukemic cell lines, we set up a microarray platform using a commercially available oligonucleotide probe set based on version 6.0 of the Sanger miRNA database (*mir*Vana miRNA Probe Set, Ambion). In brief, this probe set, representing 281 human miRNAs, was spotted

onto GAPS-coated glass slides (Corning). Total RNA was isolated from frozen mononuclear AML samples and leukemic cell lines using the *mir*Vana miRNA Isolation Kit (Ambion). Pooled RNA from nine different tumor cell lines served as a common reference for both miRNA and gene expression analyses (see below).⁶⁻⁷ Size-fractionated small RNA (flashPAGE Fractionator System, Ambion) containing only the mature miRNA fraction was labeled for microarray analysis using an end-labeling strategy (*mir*Vana miRNA Labeling Kit, Ambion). Following purification, the Cy5-labeled AML and Cy3-labeled common reference samples were mixed, co-hybridized for 14 hours onto the miRNA microarrays and washed according to the manufacturer's protocol (Ambion).

Microarrays were imaged, and log² transformed fluorescence ratios were normalized and filtered as previously described.⁷ The complete filtered miRNA microarray dataset is provided as in *Online Supplementary Table S2* (arrays from 3 patients were excluded because of borderline quality). For hierarchical cluster analysis, we applied average linkage clustering (distance measure: correlation uncentered) and visualized results using TreeView (Eisen Lab; *http://rana.lbl.gov/eisen/*).⁸

Validation of microRNA findings

Northern blot analysis and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) of selected miRNAs using the TaqMan MicroRNA Reverse Transcription Kit and MicroRNA Assays (Applied Biosystems) were performed as previously described,⁹ and as described below.

Northern blot analysis of microRNAs

For technical validation of miRNA microarray findings, we performed northern blot analyses for miR-20a and let-7a. Briefly, 10 µg of total RNA were separated on denaturing polyacrylamide gels and transferred to positively charged nylon membranes. Membranes were incubated with ³²P-labeled probes, complementary to the respective miRNAs, which were designed and processed according to the *mir*Vana miRNA probe construction kit (Ambion). Next, the membranes were washed and subjected to autoradiography. As a loading control, total RNA was detected by ethidium bromide staining of the gels before transfer to the membrane.

Quantitative reverse transcriptase polymerase chain reaction for microRNA detection

Microarray based miRNA expression findings were also validated by qRT-PCR of selected miRNAs using the TaqMan MicroRNA Reverse Transcription Kit and MicroRNA Assays (Applied Biosystems) according to the manufacturer's protocols (Applied Biosystems). miR-374 was used for data normalization, as this miRNA, according to our analysis, was expressed at a well detectable level with only slight variation among the samples, and therefore served as an endogenous control. The qPCR analysis was run on a 7900HT Fast Real-Time PCR System (Applied Biosystems).

Gene expression profiling

Gene expression profiling (GEP) had been previously performed on all 43 samples using TRIzol (Invitrogen) prepared RNA from identical pre-treatment samples and cDNA microarrays provided by the Stanford Functional Genomics Facility.^{7,10} The complete gene expression microarray dataset is available at the Gene Expression Omnibus data repository (accession number: GSE31644).

Data analysis of microarray based expression data sets

Supervised analyses were performed on data sets of varying filter criteria stringency. To correlate miRNA and/or mRNA expression findings with molecular genetics, the Significance Analysis of Microarrays (SAM) method with adjustment for multiple testing was used (false discovery rate < 10%).¹¹ Analyses were performed using BRB-Array Tools Version 3.6.1 (developed by Dr. Richard Simon and Amy Peng Lam, available at *http://linus.nci.nih.gov/BRB-ArrayTools.html*) and R Version 2.6.0 (available at www.r-project.org).

Immunoblot analysis

Total cell extracts were fractionated on 12% or 4-12% sodium dodecylsulfate polyacrylamide gels (NuPAGE Bis-Tris Gels, Invitrogen) and electroblotted to polyvinylidene fluoride membranes (Immobilon-P, Millipore). Membranes were reacted with anti-N-Ras (F155, sc-31; Santa Cruz Biotechnology), anti-E2F1 (clones KH20&KH95; Upstate), anti-APP (A 8717; Sigma), anti-cyclin-D1 (H-295, sc-753; Santa Cruz Biotechnology), anti-IRF2 (C-19, sc-498; Santa Cruz Biotechnology), anti-SPARC (H-90, sc-25574; Santa Cruz Biotechnology), anti-β-actin (ab8227; Abcam) or anti- α -tubulin (ab7291; Abcam), followed by incubation with secondary horse radish peroxidase-linked antibodies (GE Healthcare). Immunoreactivity was determined using ECL Western Blotting detection reagents (GE Healthcare). Blots were quantified using ImageJ (available at *http://rsbweb.nih.gov/ij/*).

Luciferase reporter assays

Large parts of the 3'UTR (containing one or two putative miRNA binding sites) or, if less than 1500 bp in length, the fulllength 3'UTR of predicted target genes were cloned into the 3'UTR region of the firefly luciferase reporter gene in the pMIR-REPORT vector (Ambion), which was cleaved with SpeI or SpeI/ HindIII. The SpeI restriction site is marked in green. The Hind III restriction site is marked in blue. The 3'UTR was amplified by PCR from cDNA of cell lines using the following primers (all 5' to 3'):

SPARC:

fwd: GCATACTAGTCCTTCCACAGTACCGGATTC rev: GCATAAGCTTCCGGTGTGTGTGTGACAGGTG

CLCN3:

fwd: GCTAACTAGTGAGAGAAGAAGAAGAGAGAGA rev: GCTAACTAGTCAGAATACTTCCTTCTCTTTACTGTCA

CRKL:

fwd: GCATACTAGTCCCTTTACGCACGTCAAAAT rev: CTGAACTAGTTGGTCTCCCTAGTCACTAAAACA

IRF2:

fwd: GCATACTAGTACTCTCCGCGGTGGTTGT rev: GCTAACTAGTTCATTTATTATCAATCCACAGGAAAA

KIT:

fwd: GCATACTAGTCTTGCATCCAACTCCAGGAT rev: GCTAACTAGTTGAAGTGCCCCTGAAGTACC

MN1:

fwd: GTCAACTAGTTGACAAGAAAGATCCCCTCCT rev: GCATAAGCTTCGTTTTAGTAAGACACGCTCGTT

SERPINB9:

fwd: GTACACTAGTAATTGCAGTCCAAATCCCATA rev: GCATAAGCTTTCCTAGATATATACCCAAGAGAATGC

As a control, the predicted miRNA binding sites were mutated by introducing three base pair changes using the QuikChange Lightning site-directed mutagenesis kit (Agilent Technologies Stratagene Products) according to the manufacturer's protocol. Primers were designed using the QuikChange Primer Design Program on the manufacturer's website (*http://www.genomics.agilent.com/*). For constructs with two predicted miRNA binding sites (BS), BS1 is located closer to the 5' end and BS2 closer to the 3'end of the gene.

The sequences of primers used for site-directed mutagenesis were as follows (the mutated miRNA binding sites are shaded in gray):

SPARC:

miR-29a BS1_sense: 5'-ggtttgttgttgttgttgtgggggggggggggggggg
miR-29a BS1_antisense: 5'-gttaatgtattcacttaaatctatgttcgaaacttgtctccaggcagaacaacaaacc-3'
miR-29a BS2_sense: 5'-acatagatttaagtgaatacattaacgtttegaaaaatgaaaattetaacceaagacatgaca-3
miR-29a BS2_antisense: 5'-tgtcatgtcttgggttagaattttcattttttcgaaaegttaatgtattcacttaaatctatgt-3'

CLCN3:

miR-15a BS1_sense: 5'-tgatatacaagtgctgttgagcataattaaataaaattegggtgctttgacagtaaagaga-3' miR-15a BS1_antisense: 5'-tctctttactgtcaaagcaecegaattttatttaattatgctcaacgcacttgtatatca-3'

CRKL:

miR-15a BS1_sense: 5'-getgttgecetgttteettegggtttgttgttetgeetgte-3' miR-15a BS1_antisense: 5'-gacaggcagaacaacaaacecganggaaacagggcaacage-3' miR-15a BS2_sense: 5'-tcaggetgecagagaaagttggttegggtcatactggtete-3' miR-15a BS2_antisense: 5'-gagaccagtatgaccegaaccaactttetetggcagectga-3'

IRF2:

miR-20a BS1_sense: 5'-tgcttctgcaccttatcttaaaggagtgacagataggccttcttgtg-3' miR-20a BS1_antisense: 5'-cacaagaaggcctatctgtcactcctttaagataaggtgcagaagca-3'

KIT:

miR-19a BS1_sense: 5'-ggccgttatctggaagtaaccatgttetetggagttetatgetet-3' miR-19a BS1_antisense: 5'-agagcatagaactecagagaacatggttacttecagataacggce-3' miR-20a BS1_sense: 5'-caatectgtetttetgagcactegtgagtggccgatgatttttgtca-3' miR-20a BS1_antisense: 5'-tgacaaaaatcateggccacteacgagtgeteagaagacaggattg-3'

MN1:

SERPINB9:

miR-29a BS1_sense: 5'-ettaaceegetgeeteagtggtaataaaltggggtagatattgetaetattittataga-3' miR-29a BS1_antisense: 5'-tetataaaatagtageaatatetaeeeeaatttattaeeaetgaggeagegggtaag-3' miR-29a BS2_sense: 5'-gatattgetaetattittatagattteettggggtageettaaaaaaaggtigtaaaatgtae-3' miR-29a BS2_antis.: 5'-gataettttaeaaettittataaggetaaeeeeaaggaaatetataaaatagtageaaatge-3' For the reporter assays, HeLa cells were cultured in 24-well plates and transfected with a firefly luciferase-reporter construct (with wild-type or mutated miRNA binding sites), plus a renilla containing vector (CMV-Renilla) in a ratio of 6:1, using Lipofectamine 2000 transfection reagent (Invitrogen; 300 ng DNA, 0.9 μ L LF2000 per well). Cells were co-transfected with either a Pre-miR or a negative control RNA (Ambion) at a final concentration of 50 nM. Firefly and renilla luciferase activities were measured 30-38 h after transfection using the Dual-Luciferase Reporter Assay System (Promega).

Nucleofection of myeloid cell lines

For target gene investigations and functional analyses, HEL and K-562 cells were transfected with Pre-miRs at a final concentration of 100 nM using the Nucleofector II Device and Cell Line Nucleofector Kit V (Lonza). For both HEL and K-562 cells, 2x10⁶ cells per nucleofection were used (volume per well: 2.1 mL; HEL: nucleofector program X-005; K-562: program T-016).

Transfection efficiencies, monitored by green fluorescent protein (GFP) plasmid transfection, were as follows:

HEL, 48 h post-transfection	% viable cells	% GFP positive cells in viable cells	% GFP positive cells in total cells
Non-transfected cells	84%	0%	0%
GFP-transfected cells	66%	89%	58%
K-562, 48 h post-transfection	% viable cells	% GFP positive cells in viable cells	% GFP positive cells in total cells
Non-transfected cells	87%	0%	0%
GFP-transfected cells	80%	86%	68%

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Online Supplementary Table S1. Clinicopathological characteristics of sample sets.

Clinicopathological parameter		CN-AML samples (n=43)
Sex	Male Female	n=21/43 (49%) n=22/43 (51%)
Age (in years)	Median (range)	46 (23-60)
White blood cell count $(x10^{9}/L)$	Median (range)	46 (2-192)
Lactate dehydrogenase (in U/L)	Median (range)	559 (139-2800)
Preceding malignancy		n=0/43 (0%)
French-American-British classification	FAB M0 FAB M1 FAB M2 FAB M3 FAB M4 FAB M5 FAB M6	n=3/43 (7%) n=13/43 (30%) n=8/43 (19%) n=0/43 (0%) n=13/43 (30%) n=6/43 (14%) n=0/43 (0%)
Molecular markers	<i>FLT3</i> -ITD mutation <i>FLT3</i> -TKD mutation <i>NPM1</i> mutation <i>CEBPA</i> mutation	n=19/43 (44%) n=8/43 (19%) n=23/43 (53%) n=6/40 (15%)
Follow-up (days)	Median (range) All patients Survivors	322 (19-2780) 1654 (1033-2780)
Deaths		n=31/43 (72%)

Online Supplementary Table S2. See attached Excel file: ARuss_SupplemTableS2 Data have been normalized and log₂ transformed.

Online Supplementary Table S3. Quantitative RT-PCR for mRNA detection: primer sequences and cycling program.

Primer sequences:		Sequence (5' to 3')
Predicted target genes:		
ABCC5	fwd rev	AGAACTCGACCGTTGGAATGC CATGATGGTACTTTCCCTTGGG
AKAP13	fwd rev	GCCCGCGAGAGACATTGAT TTCTCCTCGGTTAGAAGCTGG
APP	fwd rev	CATCCCCACTTTGTGATTCC GTTTCGCAAACATCCATCCT
BCL2L1	fwd rev	CATGGCAGCAGTAAAGCAAG TGCTGCATTGTTCCCATAGA
CCND1	fwd rev	GAACAAACAGATCATCCGCAAAC GCGGTAGTAGGACAGGAAGTTG
CLCN3	fwd rev	GCATAGACGGATCAACAGCA TGGTTGTACCACAACGCACT
CRKL	fwd rev	CCAGGAATTTGACCATTTGC ATTGGTGGGCTTGGATACCT
DUSP16	fwd rev	TGACTTTATCCCCGAGTCTCAT GAGATCCCAGCTAAACAGTGC
FOXA1	fwd rev	GAAGATGGAAGGGCATGAAA CGCTCGTAGTCATGGTGTTC
FOXO1	fwd rev	AAGAGCGTGCCCTACTTCAA TTCCTTCATTCTGCACACGA
FOXO3	fwd rev	CTTCAAGGATAAGGGCGACA TCTTGCCAGTTCCCTCATTC
FOXO4	fwd rev	TTGCCCAGATCTACGAGTGG TCGAGTTCTTCCATCCTGCT
GAB1	fwd rev	ACTACCTGTTGCTCATCAACTG GGGACGTTATCATTGGAGTCTG
GSPT1	fwd rev	GGCAAAGACAGCAGGTGTAAA GCTCCAGTAAGTCCTGAGCAG
ID2	fwd rev	CTGGACTCGCATCCCACTAT AATTCAGAAGCCTGCAAGGA
IRF2	fwd rev	CTACCGAATGCTGCCCCTATC CCAGAGATGACTCAACTGGTTC
ITGA6	fwd rev	GTTGCTGTTGGTTCCCTCTC TGGAGGCATATCCCACTAGG
KAT2B	fwd rev	CGAATCGCCGTGAAGAAAGC GAGGGGTTAGGGTTTTTCCAG
KIT	fwd rev	GTTCTGCTCCTACTGCTTCGC CCACGCGGACTATTAAGTCTGA
LPP	fwd rev	AACCCCAGCATTTCAGTGTC ACCCTCACCTCCAGGTTGTT
LRP6	fwd rev	GAGTTGGATCAACCCAGAGC CGACTTGAACCATCCATTCC
MAP4K4	fwd rev	AAGAACCCACACTCTATGATCCA GCTACTGATGTAGGCATCTCTCC
MLL	fwd rev	TCTGTCACGTTTGTGGAAGG CTTTGCCTGGAGTTGTGGAT
MN1	fwd rev	GAGCACCATTGACCTGGACT GGATGCTGAGGCCTTGTTT
MXI1	fwd rev	GCCAAAGCACACATCAAGAA ACCCTGCAGCTGTTCCAGT
NRP1	fwd rev	CAAGATCGACGTTAGCTCCA AACATCTGTGGGGGTTGGTGT
OTX2	fwd rev	AAGCACTGTTTTGCCAAGACC CATACCTGCACCCTCGACTC
PAWR	fwd rev	AAGCACAACCAGTGTCTCTGAA CTGAAACATTTGCATCCCTGT
PIK3CA	fwd rev	CCCAGGTGGAATGAATGGCT AGCACCCTTTCGGCCTTTAAC

PRKCE	fwd	GGAAAAAGCTCATTGCTGGT
	rev	AGGTGGGTGCTGACTTGGAT
PROM1	fwd	GCAATCTCCCTGTTGGTGAT
	rev	CCAGTTTCCGACTCCTTTTG
RALA	fwd	AAGTCATCATGGTGGGGCAGT
	rev	AGTCCTCCACAAACTCATCG
SERPINB9	fwd	TCAACACCTGGGTCTCAAAA
	rev	CATCATCTGCACTGGCCTTT
SIRT1	fwd	CAGCATCTTGCCTGATTTGT
	rev	CATCGAGGAACTACCTGATTAAAAA
SP100	fwd	GGACAAGACCACGACTTITTCA
00140	rev	
SP140	fwd	AACITCAGGATGGTCGCAGAG
CDADO	feed	
SPARC	IWO	
ጥለ፤ 1	fund	
IALI	IWO	
TD52IND	fud	
TEDDINE	rev	AGTAGGTGACTCATCACTGATGT
TRIR9	fwd	СТСААССТСССССАААТТСАТ
TRID2	rev	TTGTCGGAGAGGGGAATCATC
TRPS1	fwd	CTTGGCCCTTCATAACATGG
int of	rev	CCTTGGCAATCTGGTGTTTT
UBE2D3	fwd	CAGGTCCAGTTGGGGATGATA
	rev	AAGAATACACCGCCTTGATATGG
Positive control genes		
F9F1	fwd	TGCCAAGAAGTCCAAGAACC
E21 1	rev	CAGCTGTCGGAGGTCCTG
NRAS	fwd	GCTTCCTCTCTCTCTCTCCCA
	rev	GCACCATAGGTACATCATCCG
Househooping many (and the		
Housekeeping genes (endoge	nous control	
ACIB	twd	AGAGCTACGAGCTGCCTGAC
L MND 1	rev	
TWINR I	fwd	CIGGAAAIGITIGCAICGAAGA
	rev	OUTCULATIONTOATU
PGK1	fwd	AAGTGAAGCTCGGAAAGCTTCTAT
	rev	TGGGAAAAGATGCTTCTGGG

Cycling program for qRT-PCR:

Analyses were carried out using the Fast SYBR Green Master Mix (Applied Biosystems) with a 7900HT Fast Real-Time PCR System (Applied Biosystems) in the fast mode:

Step	Temperature (°C)	Duration	
AmpliTaq® Fast DNA Polymerase, UP Activation	95	20 sec	hold
Denaturation	95	5 sec	١
Annealing/extension	60	20 sec	40 cycles
Measurement (temperature suitable			[
for all primers used)	69	20 sec	/
	95	15 sec	hold
Dissociation curve	65	15 sec	(dissociation
ramp rate 1%	90	15 sec	step)

Online Supplementary Table S4. SAM analysis for the identification of NPM1^{mut}-associated gene signature.

See attached Excel file: ARuss_Supplementary Table S4 for the SAM list of significant genes *NPM1^{mut} versus NPM1^{wt}*, which was used for further integrative analysis. The 307 genes overlapping between the lists of predicted target genes and the *NPM1^{mut}* associated GEP are highlighted in blue (genes were down-regulated and the putative targeting miRNA was up-regulated).

Online Supplementary Table S5. SAM list of 66 significant miRNAs differentially expressed between $NPM1^{\text{mut}}$ and $NPM1^{\text{wt}}$ as determined by BRB Array Tools. The 66 miRNAs were determined by SAM $NPM1^{\text{mut}}$ versus $NPM1^{\text{wt}}$; false discovery rate = 0.09631. The geometric means of ratios (red signal/green signal) in each class and the fold difference of geometric means $(NPM1^{\text{mut}}/NPM1^{\text{wt}})$ are given.

ratios redigreen ratios redigreen nclass 1: NPM inclass 2: NPM (NPM1 MUT / NPM1 wt) / 1212 0.857 2.436 hsa-mR-10a X 0.558 0.2266 2.051 hsa-4er.70 X 0.498 0.266 1.071 hsa-mR-10b X 0.498 0.266 1.071 hsa-mR-20b X 0.035 0.167 2.200 hsa-4er.70 X 0.036 0.266 2.031 hsa-4er.70 X 0.036 0.266 2.031 hsa-4er.70 X 0.036 0.0107 2.200 hsa-4er.70 X 0.0370 0.033 2.118 hsa-mR-20a X 0.0370 0.0234 1.158 hsa-mR-20a X 0.0370 0.0234 1.158 hsa-mR-20a X 0.0370 0.224 1.188 hsa-mR-20a X 0.0371 0.0255 1.206 hsa-mR-20a X 0.0370 0.234 1.581 hsa-mR-20a X 0.0371 0.0255 1.206 hsa-mR-20a X 0.0371 0.0255 1.206 hsa-mR-20a X 0.0370 0.234 1.551 hsa-4er.70 X 0.0371 0.0255 1.206 hsa-mR-20a X 0.0371 0.0255 1.206 hsa-mR-20a X 0.0370 0.234 1.554 hsa-mR-20a X 0.0371 0.0255 1.206 hsa-mR-20a X 0.0391 0.207 1.1271 hsa-mR-20a X 0.0391 0.207 1.1271 hsa-mR-20a X 0.0391 0.224 1.581 hsa-mR-20a X 0.0391 0.224 1.581 hsa-mR-20a X 0.0391 0.224 1.581 hsa-mR-20a X 0.0391 0.224 1.156 hsa-mR-20a X 0.0391 0.224 1.156 hsa-mR-20a X 0.0469 0.515 1.1060 hsa-mR-20a X 0.0591 0.059 1.226 hsa-mR-20a X 0.0593 0.059 1.127 hsa-mR-15a X 0.0390 0.211 1.466 hsa-mR-20a X 0.0390 0.511 1.151 hsa-mR-20a X 0.0390 0.511 1.151 hsa-mR-20a X 0.0390 0.511 1.151 hsa-mR-20a X 0.0391 0.511 1.151 hsa-mR-20a X 0.0591 0.201 1.202 hsa mR-215 X 0.0591 0.201 1.202 hsa mR-215 X 0.0591 0.201 hsa mR-225 X 0.0595 0.1400 hsa mR-225 x 0.0595 1.100 hsa mR-225 x	Geom mean of	Geom mean of	Fold difference of			
n class 1: NPM in class 2: NPM ii (NPM MUT) mitNA mane of malue Grazon et al., MPM iii class 2: NPM iii mitNA PAAS 2008 X X X X X X X X X X X X X X X X X X	ratios red/green	ratios red/green	geom means			
MU1 IV IPPAN W01 IPPAS 2008 1 1218 0.763 1.121 InsamiR-10a X 0.0505 0.246 2.051 InsamiR-20a X 0.0498 0.266 1.071 InsamiR-20a X 0.0515 1.122 1.386 InsamiR-20a X 0.0510 0.646 0.266 1.071 InsamiR-20a X 0.0510 0.033 2.121 InsamiR-20a X X 0.0464 0.159 2.130 InsamiR-20a X X 0.0464 0.251 1.021 InsamiR-20a X X 0.0461 0.251 1.020 InsamiR-20a X X 0.0510 0.231 1.227 InsamiR-20a X X 0.0511 1.260 InsamiR-20a X X X 0.0540 0.577 1.310 InsamiR-20a X X 0.0540 0.578 1.260 InsamiR-20a X X X X X X X X X <td>in class 1 : NPM1</td> <td>in class 2 : NPM1</td> <td>(NPM1 MUT /</td> <td>name of mature</td> <td>Garzon et al.,</td> <td></td>	in class 1 : NPM1	in class 2 : NPM1	(NPM1 MUT /	name of mature	Garzon et al.,	
2112 0.897 2430 InsamiR-100 X 1278 0.793 1011 InsamiR-100 X 0.696 0.266 2051 InsamiR-100 X 0.698 0.266 1071 InsamiR-208 X 0.591 0.426 1.331 InsamiR-208 X 0.691 0.426 1.331 InsamiR-208 X 0.646 0.272 1.700 InsamiR-208 X 0.616 0.666 1.420 InsamiR-208 X 0.616 0.666 1.420 InsamiR-208 X 0.636 0.666 1.420 InsamiR-208 X 0.636 0.666 1.420 InsamiR-208 X 0.636 0.637 1.620 InsamiR-208 X 0.636 0.637 1.620 InsamiR-208 X 0.646 0.257 1.620 InsamiR-208 X 0.646 0.515 1.620 InsamiR-208 X 0.646 0.515 1.620 InsamiR-208 X 0.64	MUT	wt	NPM1 wt)	miRNA	PNAS 2008	
1208 0.733 101 101 marker.top x 0.498 0.286 1071 marker.top x 0.385 0.167 2.300 marker.top x 0.498 0.286 1.671 marker.top x 0.591 0.426 1.81 marker.top x 0.591 0.426 1.81 marker.top x 0.406 0.592 2.121 marker.top x 0.406 0.592 2.121 marker.top x 0.406 0.131 1.671 marker.top x 0.406 0.237 1.420 marker.top x 0.510 0.177 1.951 marker.top x 0.511 0.237 1.320 marker.top x 0.519 0.334 1.551 marker.top x 0.519 0.334 1.551 marker.top x 0.526 0.198 1.464 marker.top x 0.332 0.235 1.320 marker.top x 0	2.112	0.867	2.436	hsa-miR-10a	÷	1
0.383 0.243 2.033 marrier108 X 0.158 0.117 1.386 marrier108 X 0.385 0.117 2.030 marrier108 X 0.0591 0.428 1.361 marrier108 X 0.0464 0.152 2.128 marrier108 X 0.0464 0.152 2.128 marrier108 X 0.0464 0.152 1.128 marrier108 X 0.0464 0.152 1.128 marrier108 X 0.050 0.150 1.027 marrier108 X 0.051 0.207 1.028 marrier208 X 0.052 0.205 1.208 marrier208 X 0.052 0.255 1.208 marrier208 X 0.202 1.056 1.228 marrier208 X 0.202 <td>1.278</td> <td>0.793</td> <td>1.012</td> <td>hea-lot-7b</td> <td><u> </u></td> <td>1</td>	1.278	0.793	1.012	hea-lot-7b	<u> </u>	1
0.285 0.285 1.22 1.280 1.280 name and the state of the	0.505	0.240	2.053	hea miP 20a	×	
0.205 0.1107 2.205 x 0.205 0.228 1.381 Name MR 20 X 0.300 0.233 2.121 Name MR 20 X 0.440 0.159 2.130 Name MR 20 X 0.444 0.272 1.700 Name MR 20a X 0.316 0.193 1.837 Issam R 20a X 0.331 0.193 1.837 Issam R 20a X 0.331 0.234 1.621 Issam R 20a X 0.331 0.234 1.621 Issam R 20a X 0.331 0.234 1.625 Issam R 30a X 0.341 0.237 1.439 Issam R 49a X 1.027 1.039 1.221 Issam R 49a X 1.029 0.757 1.310 Issam R 49a X 0.436 0.515 1.201 Issam R 49a X 0.362 0.255 1.226 Issam R 49a X 0.362 0.255 1.326 Issam R 47a X 0.363 0.578<	1.555	1 122	1 386	hsa-miR-196h	<u>^</u>	
0.591 0.428 1.381 issamiR.30c n 0.070 0.033 2.121 issamiR.21 X 0.444 0.272 1.705 issamiR.20a X 0.316 0.159 1.211 issamiR.20a X 0.316 0.153 1.627 issamiR.20a X 0.351 0.516 0.153 1.627 issamiR.20a X 0.351 0.177 1.683 issamiR.20a X X 0.361 0.177 1.683 issamiR.20a X X 0.364 0.625 1.629 issamiR.20a X X 0.464 0.751 1.626 issamiR.20a X X 0.452 0.625 1.629 issamiR.20a X X 0.454 0.515 1.205 issamiR.20a X X 0.454 0.516 1.206 issamiR.20a X X 0.454 0.451 1.426 issamiR.30a X X 0.454 0.451 1.426 issamiR.30a X X 0.454 0.265 <t< td=""><td>0.385</td><td>0.167</td><td>2 305</td><td>hsa-let-7c</td><td>x</td><td></td></t<>	0.385	0.167	2 305	hsa-let-7c	x	
0.000 0.033 2.121 issemiR-21 X 0.040 0.159 2.136 issemiR-20 X 0.016 0.150 1.026 issemiR-20 X 0.0351 0.177 1.156 issemiR-20 X 0.0361 0.237 1.439 issemiR-20 X 0.0361 0.237 1.439 issemiR-20 X 0.0361 0.344 1.556 issemiR-20 X 0.040 0.515 1.206 issemiR-22 X 0.0519 0.334 1.556 issemiR-22 X 0.0520 0.577 1.310 issemiR-22 X 0.0532 0.578 1.226 issemiR-22 X 0.332 0.285 1.236 issemiR-276 X 0.332 0.285 1.326 issemiR-276 X 0.332	0.503	0.428	1.381	hsa-miR-30c	^	
0.340 0.159 2.138 bas-let.7a X 0.316 0.173 1.637 bas-mR.20b X 0.316 0.193 1.637 bas-mR.20b X 0.351 0.637 0.234 1.551 bas-mR.20b X 0.341 0.237 1.435 bas-mR.20b X X 0.440 0.736 1.271 bas-mR.198 X X 1.097 0.897 1.225 bas-mR.20a X X 0.456 1.020 bas-mR.491-5p X X X 0.456 0.551 1.26b bas-mR.20a X X X 0.326 0.981 1.64b bas-let.77 X X Y Y 0.326 0.186 1.47b bas-mR.20a Y X X X X X X X X X X X X X X X X X X	0.070	0.033	2,121	hsa-miR-21	x	
0.464 0.272 1.706 InsamR-23b 0.867 0.608 1.426 InsamR-23b 0.351 0.177 1.931 InsamR-23b 0.351 0.177 1.931 InsamR-23b 0.351 0.177 1.931 InsamR-23a 0.351 0.177 1.931 InsamR-23b 0.791 0.625 1.266 InsamR-23a 1.077 1.939 1.221 InsamR-33a 0.519 0.334 1.554 InsamR-32a 1.277 1.039 1.226 InsamR-23a X 0.991 0.809 1.226 InsamR-18-3a X 0.991 0.809 1.226 InsamR-24b X 0.991 0.809 1.236 InsamR-195a X 0.320 0.198 1.466 InsamR-195a X 0.320 0.285 1.230 InsamR-27b X 0.320 0.285 1.230 InsamR-27b X 0.320 0.811 1.146 InsamR-27b X 0.332 0.285	0.340	0.159	2.138	hsa-let-7a	X	
0.316 0.193 1.637 hsa-mR-23b 0.370 0.234 1.626 hsa-mR-30b 0.351 0.177 1.935 hsa-mR-23a 0.351 0.177 1.935 hsa-mR-23a 0.941 0.237 1.436 hsa-mR-23a 0.940 0.736 1.227 hsa-mR-23a 0.940 0.736 1.227 hsa-mR-24a 1.967 0.697 1.223 hsa-mR-24a 1.277 1.039 1.226 hsa-mR-24a 1.277 1.039 1.226 hsa-mR-26a 1.269 1.055 1.220 hsa-mR-27b 0.992 0.757 1.310 hsa-mR-27b 0.326 0.198 1.646 hsa-mR-125a 0.326 0.285 1.236 hsa-mR-27b 0.326 0.285 1.236 hsa-mR-27a 1.310 1.138 1.151 hsa-mR-27b 0.326 0.285 1.236 hsa-mR-27b 0.326 0.285 1.236 hsa-mR-27b 0.332 0.611 1.276 hsa-mR	0.464	0.272	1.706	hsa-miR-20a	X	
0.867 0.606 1.426 hsamR.30b 0.351 0.177 1.981 hsamR.20b 0.351 0.177 1.983 hsamR.20b 0.791 0.625 1.230 hsamR.20b 0.791 0.625 1.266 hsamR.20b 0.940 0.735 1.277 hsamR.19a X 1.077 1.039 1.226 hsamR.92a X 1.277 1.039 1.226 hsamR.19a X 0.991 0.809 1.226 hsamR.19a X 0.992 0.757 1.266 hsamR.242 X 0.725 0.576 1.266 hsamR.242 X 0.326 0.198 1.646 hsamR.27a X 1.297 1.120 1.156 hsamR.1636 X 0.362 0.285 1.266 hsamR.27a X 0.362 0.263 1.466 hsamR.17a X 0.354 0.261 1.356 hsamR.30a X 1.309 0.910 1.142 hsamR.433 X 1	0.316	0.193	1.637	hsa-miR-23b	-	
0.370 0.224 1.981 hsa-mR-23a 0.351 0.177 1.983 hsa-mR-200 X 0.940 0.735 1.271 hsa-mR-200 X 0.940 0.735 1.271 hsa-mR-200 X 1.097 0.692 1.285 hsa-mR-29a X 1.1097 0.697 1.220 hsa-mR-29a X 1.277 1.039 1.220 hsa-mR-36a X 0.992 0.757 1.300 hsa-mR-26a X 0.992 0.757 1.300 hsa-mR-27a X 0.326 0.757 1.326 hsa-mR-19a X 0.326 0.285 1.326 hsa-mR-27a X 0.326 0.285 1.326 hsa-mR-36a X 0.326 0.285 1.326 hsa-mR-36a X 0.326 0.281 1.366 hsa-mR-37a X 1.310 1.138 htsa-mR-17 X X 0.823 1.681 hsa-mR-17 X X 0.830 0.911 <	0.867	0.608	1.426	hsa-miR-30b		
0.351 0.177 1.93 hsa-let-7d X 0.341 0.237 1.439 hsa-mR-206 X 0.040 0.736 1.277 hsa-mR-19a X 1.097 0.897 1.221 hsa-mR-19a-3p X 1.1277 1.038 1.220 hsa-mR-19a-3p X 1.277 1.039 1.220 hsa-mR-19a-3p X 0.649 0.515 1.200 hsa-mR-226 X 0.0449 0.515 1.200 hsa-mR-226 X 0.026 0.757 1.10 hsa-mR-226 X 0.026 0.757 1.254 hsa-mR-226 X 0.026 0.757 1.254 hsa-mR-275 X 0.038 0.689 1.226 hsa-mR-275 X 0.038 0.285 1.236 hsa-mR-275 X 0.038 0.818 1.147 hsa-mR-155 X 0.039 0.211 1.468 hsa-mR-266 X 0.039 0.211 1.426 hsa-mR-275 X 0.030 0.613 1.627 hsa-mR-165 X 0.033 0.218 1.147 hsa-mR-1824 X 0.0501 0.	0.370	0.234	1.581	hsa-miR-23a		
0.341 0.237 1.439 has-miR-30b 0.791 0.625 1.266 has-miR-30 X 1097 0.897 1.221 has-miR-19a X 1.277 1.039 1.222 has-miR-373 A 1.271 1.039 1.220 has-miR-19a X 0.519 0.334 1.554 has-miR-22a X 1.260 1.056 1.20 has-miR-32a X 0.0992 0.757 1.310 has-miR-29c X 0.649 0.515 1.260 has-miR-29c X 0.236 0.188 1.646 has-let.71 X 0.236 0.285 1.260 has-miR-395.0 X 0.326 0.281 1.316 has-miR-395.0 X 0.335 0.211 1.461 has-miR-396.0 X 0.335 0.211 1.421 has-miR-195.0 X 0.333 <td< td=""><td>0.351</td><td>0.177</td><td>1.983</td><td>hsa-let-7d</td><td>X</td><td></td></td<>	0.351	0.177	1.983	hsa-let-7d	X	
0.791 0.625 1.266 hsa-miR-198 X 0.991 0.897 1.221 hsa-miR-198 X 1.097 0.897 1.223 hsa-miR-198-3p 1 1.277 1.039 1.220 hsa-miR-198-3p 1 1.289 1.056 1.220 hsa-miR-198-3p X 0.992 0.757 1.310 hsa-miR-292 X 0.0449 0.515 1.260 hsa-miR-292 X 0.0226 0.198 1.646 hsa-miR-293-3p X 1.039 0.211 1.464 hsa-miR-196-3p X 0.326 0.198 1.646 hsa-miR-27b X 0.0382 0.285 1.236 hsa-miR-27a X 1.1310 1.138 1.151 hsa-miR-17 X 0.0354 0.261 1.565 hsa-miR-17 X 0.622 0.693 1.186 hsa-miR-155 X 0.0572 0.434 1.316 hsa-miR-155 X 0.561 0.432 1.299 hsa-miR-165 X <td>0.341</td> <td>0.237</td> <td>1.439</td> <td>hsa-miR-200b</td> <td></td> <td></td>	0.341	0.237	1.439	hsa-miR-200b		
0.940 0.736 1.271 hsamR-13a X 1.097 0.897 1.221 hsamR-373 X 0.519 0.334 1.554 hsamR-198a X 1.277 1.039 1.229 hsamR-198a X 0.649 0.515 1.200 hsamR-295 X X 0.0991 0.809 1.225 hsamR-215.5p X 0.226 0.578 1.24 hsamR-125.5p X 0.309 0.211 1.464 hsamR-126 X 0.309 0.211 1.464 hsamR-126 X 0.309 0.211 1.464 hsamR-126 X 0.309 0.211 1.464 hsamR-127 X 0.302 0.685 1.300 hsamR-155.5p X 0.310 1.38 1.115 hsamR-368.5b Y 0.323 0.6818 1.147 hsamR-155.5p X 0.354 0.261 1.356 hsamR-155 X 0.333 0.218 1.529 hsamR-155 X 0.333 0.218 1.529 hsamR-165 X 0.503 0.376 1.338 hsamR-165	0.791	0.625	1.266	hsa-miR-98	X	
1.097 0.087 1.223 hsa-miR-373 0.519 0.334 1.554 hsa-miR-92a 1.277 1.039 1.22b hsa-miR-136 0.092 0.757 1.310 hsa-miR-29c X 0.0449 0.515 1.260 hsa-miR-29c X 0.0449 0.615 1.260 hsa-miR-29c X 0.0226 0.198 1.664 hsa-miR-29c X 0.1207 1.100 1.158 hsa-miR-29c X 0.326 0.198 1.664 hsa-miR-105a X X 0.332 0.285 1.236 hsa-let.77 X 0.321 0.585 1.236 hsa-miR-27a X 0.414 0.293 1.413 hsa-miR-380 Y 0.333 0.818 1.147 hsa-miR-326 X 0.334 0.818 1.147 hsa-miR-155 X 0.332 0.693 1.241 hsa-miR-155 X 0.333 0.218 1.529 hsa-miR-150 X 0.561 0.432 1.299 hsa-miR-155 X 0.561 0.432 1.299 hsa-miR-302 X 0.566 0.460 1.315 hsa-miR-325 </td <td>0.940</td> <td>0.736</td> <td>1.277</td> <td>hsa-miR-19a</td> <td>X</td> <td></td>	0.940	0.736	1.277	hsa-miR-19a	X	
0.519 0.334 1.554 hsamR-192a 1.277 1.039 1.220 hsamR-138 0.992 0.757 1.310 hsamR-29c X 0.649 0.515 1.220 hsamR-29c X 0.091 0.809 1.225 hsamR-29c X 0.0326 0.198 1.646 hsamR-125-5p X 0.326 0.198 1.646 hsamR-106a X 0.309 0.211 1.464 hsamR-106a X 0.309 0.211 1.464 hsamR-106a X 0.309 0.211 1.464 hsamR-106a X 0.414 0.293 1.413 hsamR-27a upregulated 0.414 0.293 1.413 hsamR-105a X 0.414 0.293 1.413 hsamR-177 X 0.623 0.693 1.186 hsamR-17 X 0.624 0.261 1.356 hsamR-152c in NPM1mut 1.350 1.181 hsamR-165 X X 0.623 0.697 1.151 hsamR-165 X 0.501 0.432 1.291 hsamR-165 X 0.503 0.376 1.338 hsamR-185 h </td <td>1.097</td> <td>0.897</td> <td>1.223</td> <td>hsa-miR-373</td> <td></td> <td></td>	1.097	0.897	1.223	hsa-miR-373		
1.277 1.039 1.229 haa-mR.199a-30 1.229 1.056 1.200 haa-mR.136 0.992 0.757 1.310 haa-mR.29c X 0.649 0.515 1.260 haa-mR.29c X 0.0991 0.809 1.251 haa-mR.29c X 0.026 0.198 1.664 haa-mR.256-5p X 0.309 0.211 1.464 haa-mR.1256-5p X 0.309 0.221 1.464 haa-mR.27b X 0.302 0.285 1.236 haa-let.77 X 0.302 0.285 1.236 haa-mR.27a X 0.414 0.293 1.413 haa-mR.230 Y 1.468 1.271 1.156 haa-mR.230 Y 1.352 1.176 1.150 haa-mR.230 X 1.039 0.910 1.142 haa-mR.155 X 0.037 0.674 1.371 haa-mR.155 X 1.039 0.910 1.142 haa-mR.230 X 0.503 0.376 1.33 haa-mR.230 X 0.503 0.376 1.38 haa-mR.230 X 0.551 0.432 1.29 haa-mR.195<	0.519	0.334	1.554	hsa-miR-92a		
1269 1.056 1.202 hsa-miR-136 0.992 0.757 1310 hsa-miR-29c X 0.725 0.578 1.226 hsa-miR-224 X 0.725 0.578 1.224 hsa-miR-226 X 0.326 0.198 1.646 hsa-miR-226 X 0.326 0.198 1.646 hsa-miR-226 X 0.339 0.211 1.464 hsa-miR-105a X 0.339 0.211 1.464 hsa-miR-27a X 0.414 0.293 1.413 hsa-miR-360 X 1.310 1.138 1.151 hsa-miR-360 X 0.414 0.293 1.413 hsa-miR-360 X 0.334 0.261 1.356 hsa-miR-360 X 0.334 0.261 1.356 hsa-miR-320 X 0.433 0.671 1.157 hsa-miR-320 X 0.561 0.432 1.299 hsa-miR-155 X 0.561 0.432 1.299 hsa-miR-166 X 0.561 0.432 1.299 hsa-miR-195 X 0.561 0.432 1.299 hsa-miR-195 X 0.561 0.432	1.277	1.039	1.229	hsa-miR-199a-3p		
0.992 0.757 1.310 hsamiR-29c x 0.991 0.809 1.225 hsamiR-291-5p x 0.725 0.578 1.254 hsamiR-125s-5p x 0.326 0.198 1.646 hsa-iet-7f X 1.297 1.120 1.158 hsamiR-369-3p X 0.382 0.285 1.340 hsamit-105a X 0.0382 0.285 1.236 hsa-iet-7e x 0.414 0.293 1.413 hsamit-27a x 1.310 1.138 1.151 hsamit-27a x 0.338 0.818 1.147 hsamit-151-5p x 1.332 1.176 1.156 hsamit-151-5p x 0.354 0.261 1.356 hsamit-155 x 0.0572 0.434 1.318 hsamit-155 x 1.039 0.910 1.121 hsamit-155 x 0.0503 0.376 1.338 hsamit-155 x 0.0503 0.376 1.338 hsamit-155 x 0.0513 0.508 1.271 hsamit-16 x 0.552 0.599 1.137 hsamit-155 x 0.566	1.269	1.056	1.202	hsa-miR-136		
0.649 0.515 1.260 has-mR-491-50 0.991 0.285 has-mR-224 0.725 0.578 1.264 has-mR-125a-5p 0.326 0.188 1.646 has-det/T X 0.309 0.211 1.464 has-mR-105a-5p X 0.309 0.211 1.464 has-mR-105a X 0.414 0.293 1.413 has-mR-27b miRNAs 0.414 0.293 1.413 has-mR-27b x 0.332 0.131 1.150 has-mR-320 x 0.354 0.261 1.356 has-mR-15b X 0.572 0.434 1.318 has-mR-155 X 0.503 0.376 1.388 has-mR-15b X 0.503 0.376 1.388 has-mR-30° X 0.561 0.432 1.207 has-mR-325 p X 0.564 0.460 1.230 has-mR-221 X	0.992	0.757	1.310	hsa-miR-29c	X	
0.991 0.809 1.225 hsa-miR-224 0.725 0.578 1.297 1.260 x 1.297 1.120 1.156 hsa-ret-7f X 0.302 0.225 1.340 hsa-ret-7e X 0.322 0.225 1.340 hsa-ret-7e X 0.414 0.293 1.413 hsa-ret-7e X 0.332 0.211 1.150 hsa-ret-7e X 0.352 1.176 1.150 hsa-ret-7f X 0.823 0.693 1.88 hsa-ret-7f X 0.561 0.432 1.291 hsa-ret-7f X 0.561 0.432 1.291 hsa-ret-7f X 0.562 0.991 1.137 <td>0.649</td> <td>0.515</td> <td>1.260</td> <td>hsa-miR-491-5p</td> <td></td> <td></td>	0.649	0.515	1.260	hsa-miR-491-5p		
0.725 0.578 1.254 hsa-miR-125a-5p x 1.297 1.120 1.158 hsa-miR-256b x 0.382 0.285 1.340 hsa-miR-27b x 0.723 0.585 1.236 hsa-let.7c x 0.414 0.293 1.413 hsa-miR-27b x 0.414 0.293 1.413 hsa-miR-27b x 1.310 1.138 1.151 hsa-miR-380 y 1.486 1.271 1.155 hsa-miR-526b x 1.352 1.176 1.150 hsa-miR-526b x 0.354 0.261 1.366 hsa-miR-15c x 0.354 0.261 1.366 hsa-miR-15c x 1.039 0.837 1.241 hsa-miR-155 x 1.039 0.910 1.142 hsa-miR-155 x 0.551 0.432 1.207 hsa-miR-300 x 0.551 0.432 1.207 hsa-miR-304 x 0.551 0.432 1.209 hsa-miR-304 x 0.566 0.460 1.207 hsa-miR-305 x 0.544 0.477 1.160 hsa-miR-305 x 0.566 0.460 1.220 hsa-miR-329 x 0.566 0.460 1.230 hsa-miR-329 x 0.566 0.460 1.231 hsa-miR-329 x 0.566 0.460 1.231 hsa-miR-329 x 0.566 0.460 1.231 hsa-miR-329 x 0.566 0.460 1.231 hsa-miR-329 x 0.566 0.460 1.230 hsa-miR-329 x 0.566 0.460 1.230 hsa-miR-329 x 0.566 0.460 1.230 hsa-miR-329 x 0.566 0.460 1.230 hsa-miR-320 x 0.565 1.009 hsa-miR-320 x 0.566 0.460 1.230 hsa-miR-320 x 0.565 1.009 hsa-miR-320 x 0.565 1.009 hsa-miR-320 x 0.565 1.009 hsa-miR-320 x 0.565 1.009 hsa-miR-320 x 0.645 0.777 1.088 hsa-miR-320 x 0.545 0.491 1.110 hsa-miR-321 5 0.0455 0.0491 1.110 hsa-miR-325 5 0.718 0.655 1.009 hsa-miR-325 5 0.718 0.655 1.009 hsa-miR-320 x 0.545 0.491 1.110 hsa-miR-320 x 0.545 0.491 1.110 hsa-miR-320 x 0.545 0.491 1.110 hsa-miR-320 x 0.545 0.491 1.110 hsa-miR-321 5 0.545 0.491 1.110 hsa-miR-325 x 0.545 0.491 1.110 hsa-miR-325 x 0.545 0.491 1.110 hsa-miR-325 x 0.545 0.491 1.110 hsa-miR-325 x 0.545 0.491 1.110 hsa-miR-326 x 0.545 0.491 1.110 hsa	0.991	0.809	1.225	hsa-miR-224		
0.326 0.198 1.646 hsa-miR-369-3p X 1.297 1.120 1.158 hsa-miR-369-3p X 0.309 0.211 1.464 hsa-miR-105a X 0.382 0.285 1.240 hsa-miR-27b miRN-369-3p 0.723 0.585 1.236 hsa-ter/7e miRNAs 0.414 0.293 1.413 hsa-miR-57a miRNAs 1.310 1.138 1.151 hsa-miR-526b* in NPM1mut 0.354 0.261 1.356 hsa-miR-516c* in NPM1mut 0.354 0.261 1.356 hsa-miR-17 X 0.354 0.261 1.356 hsa-miR-156 X 1.039 0.837 1.241 hsa-miR-155 X 1.039 0.910 1.142 hsa-miR-165 X 0.572 0.434 1.318 hsa-miR-165 X 0.303 0.216 hsa-miR-1724 X 0.503 0.376 1.328 hsa-miR-165 X 0.561 0.432 1.299 hsa-miR-165 X 0.503 0.376 1.38 hsa-miR-295 X 0.544 0.477 1.40 hsa-miR-30a X <	0.725	0.578	1.254	hsa-miR-125a-5p		
1297 1.120 1.158 hsamiR-369-3p X 0.399 0.211 1.464 hsamiR-105a X 0.723 0.685 1.236 hsa-miR-27b x 0.414 0.293 1.413 hsa-miR-27a x 1.310 1.138 1.151 hsa-miR-27a x 0.938 0.818 1.147 hsa-miR-27a x 0.938 0.818 1.147 hsa-miR-27a x 0.938 0.818 1.147 hsa-miR-27a x 0.354 0.261 1.356 hsa-miR-27a x 0.354 0.261 1.356 hsa-miR-15c x 0.354 0.263 1.158 hsa-miR-15c x 0.572 0.434 1.318 hsa-miR-155 X 1.039 0.910 1.142 hsa-miR-155 X 0.503 0.276 1.338 hsa-miR-165 X 0.561 0.432 1.299 hsa-miR-30a X 0.561 0.432 1.297 hsa-miR-165 X 0.566 0.430 1.207 hsa-miR-30a X 0.566 0.430 1.207 hsa-miR-30a X 0.566	0.326	0.198	1.646	hsa-let-7f	X	
0.309 0.211 1.464 hsamik-106a X 0.309 0.285 1.340 hsamik-27b miRNAs 0.723 0.885 1.236 hsamik-27b miRNAs 0.414 0.293 1.413 hsamik-27a miRNAs 1.310 1.138 1.151 hsamik-380 upregulated 0.938 0.818 1.147 hsamik-526b* in NPM1mut 1.352 1.176 1.150 hsamik-518c* miRNAs upregulated 0.823 0.693 1.188 hsamik-520 in NPM1mut 1.039 0.837 1.241 hsamik-155 X 0.672 0.434 1.318 hsamik-16 X 0.807 0.674 1.197 hsamik-124 miR-124 0.807 0.674 1.197 hsamik-16 X 0.561 0.432 1.299 hsamik-16 X 0.563 0.333 0.218 hsamik-16 X 0.563 0.376 1.338 hsamik-16 X X 0.566 0.432 1.207 hsamik-205 X X 0.544 0.4777 1.108 hsamiR-255 X X	1.297	1.120	1.158	hsa-miR-369-3p	X	
0.382 0.285 1.340 Insa-mik-276 0.0414 0.293 1.413 hsa-mik-27a 1.310 1.138 1.151 hsa-mik-27a 0.393 0.818 1.147 hsa-mik-26b* 0.393 0.818 1.147 hsa-mik-151-5p 1.352 1.176 1.150 hsa-mik-152 0.423 0.693 1.188 hsa-mik-155 X 0.423 0.693 1.188 hsa-mik-165 X 0.507 0.434 1.318 hsa-mik-165 X 0.503 0.376 1.338 hsa-miR-165 X 0.503 0.376 1.338 hsa-miR-30a X 0.504 0.431 1.299 hsa-miR-30a X 0.505 0.201 1.137 hsa-miR-30a X 0.504 0.432 1.299 hsa-miR-329 X <td>0.309</td> <td>0.211</td> <td>1.464</td> <td>hsa-miR-106a</td> <td>×</td> <td></td>	0.309	0.211	1.464	hsa-miR-106a	×	
0.123 0.585 1.236 Insarter/# 0.1414 0.293 1.413 Issartific.276 1.310 1.138 1.151 Issartific.276 0.938 0.818 1.147 Issartific.266* 0.938 0.818 1.147 Issartific.266* 0.354 0.261 1.366 Issartific.276 0.0354 0.261 1.366 Issartific.276 1.039 0.837 1.241 Issartific.200 1.039 0.837 1.241 Issartific.266 0.672 0.434 1.318 Issartific.266 X 0.572 0.434 1.318 Issartific.26 X 0.807 0.674 1.197 Issartific.26 X 0.561 0.432 1.299 Issartific.26 X 0.561 0.432 1.299 Issartific.26 X 0.561 0.432 1.299 Issartific.26 X 0.564 0.477 1.108 Issartific.26 X 0.566 0.460 1.201 Issartific.255 X	0.382	0.285	1.340	hsa-miR-2/b		
0.414 0.293 1.413 Insamik-27a 1.310 1.138 1.151 Insamik-380 1.468 1.271 1.155 Insamik-380 0.938 0.818 1.147 Insamik-516; 0.354 0.261 1.356 Insamik-516; 0.354 0.261 1.356 Insamik-516; 1.039 0.837 1.241 Insamik-516; 0.623 0.693 1.188 Insamik-155 0.0572 0.434 1.318 Insamik-155 1.039 0.910 1.142 Insamik-155 0.677 0.674 1.197 Insamik-155 0.333 0.218 1.528 Insamik-195 0.0501 0.432 1.299 Insamik-195 0.0503 0.376 1.338 Insamik-195 0.0503 0.376 1.338 Insamik-395 0.0513 0.508 1.207 Insamik-195 0.052 0.990 1.157 Insamik-320 1.300 1.173 I.108 Insamik-195 0.554 0.440 1.477 1.224 1.092 1.121 Insamik-182 0.355 1.251 1.091 Insamik-322 0.551 0.661 0	0.723	0.585	1.236	hsa-let-7e		mi DNIA o
1.310 1.138 1.151 Insa-miR-3800 wpregulated 0.938 0.818 1.147 Insa-miR-526b* in NPM1mut 1.352 1.176 1.150 Insa-miR-516c* in NPM1mut 0.354 0.261 1.356 Insa-miR-151c* x 0.823 0.693 1.188 Insa-miR-155 x 0.522 0.434 1.318 Insa-miR-155 x 1.039 0.910 1.142 Insa-miR-155 x 0.807 0.674 1.197 Insa-miR-303* 1.115 0.967 1.153 Insa-miR-304* 0.333 0.218 1.528 Insa-miR-165 x 0.501 0.432 1.299 Insa-miR-165 x 0.503 0.376 1.338 Insa-miR-304 x 1.300 1.173 1.088 Insa-miR-165 x 0.551 0.432 1.299 Insa-miR-304 x 0.564 0.477 1.140 Insa-miR-165 x 0.566 0.460 1.230 Insa-miR-265 x <td>0.414</td> <td>0.293</td> <td>1.413</td> <td>nsa-miR-2/a</td> <td></td> <td>Inircinas</td>	0.414	0.293	1.413	nsa-miR-2/a		Inircinas
1.488 1.2/1 1.155 [hsa-miR-3205] 0.938 0.818 1.147 [hsa-miR-151-5p 1 1.352 1.176 1.150 [hsa-miR-1515c] X 0.833 0.693 1.188 [hsa-miR-155 X 0.1039 0.837 1.241 [hsa-miR-155 X 0.572 0.434 1.318 [hsa-miR-155 X 1.039 0.910 1.142 [hsa-miR-165 X 0.807 0.674 1.197 [hsa-miR-493] X 0.501 0.432 1.299 [hsa-miR-165 X 0.503 0.376 1.338 [hsa-miR-165 X 0.503 0.376 1.338 [hsa-miR-30a X 0.503 0.376 1.338 [hsa-miR-30a X 0.504 0.477 1.140 [hsa-miR-30a X 0.544 0.477 1.140 [hsa-miR-32b X 0.544 0.477 1.131 [hsa-miR-105 X 1.224 1.092 1.137 [hsa-miR-325p X 0.566 0.460 1.230 [hsa-miR-381 1 1.318 1.202 1.097 [hsa-miR-382 - 0.54	1.310	1.138	1.151	hsa-miR-380		> upregulated
0.938 0.816 1.147 Isa-miR-151-50 1.352 1.176 1.150 Isa-miR-518c* 0.354 0.261 1.356 hsa-miR-17 X 0.823 0.693 1.188 hsa-miR-155 X 1.039 0.837 1.241 hsa-miR-155 X 0.572 0.434 1.318 hsa-miR-15a X 1.039 0.910 1.142 hsa-miR-15a X 0.807 0.674 1.197 hsa-miR-15a X 0.561 0.432 1.299 hsa-miR-300 1.300 1.173 1.108 hsa-miR-30a 1.300 1.173 1.108 hsa-miR-30a 1.300 1.173 1.108 hsa-miR-30a 1.300 1.173 1.108 hsa-miR-20b X 0.544 0.477 1.140 hsa-miR-18a X 0.556 0.460 1.230 hsa-miR-25p X 0.566 0.460 1.230 hsa-miR-382 X 0.565 1.092 1.131 hsa-miR-382 X	1.408	1.2/1	1.155	nsa-miR-5260		in NPM1mut
1.352 1.176 1.150 1.150 1.150 0.823 0.693 1.188 hsa-miR-17 X 0.823 0.693 1.188 hsa-miR-155 X 0.572 0.434 1.318 hsa-miR-155 X 1.039 0.910 1.142 hsa-miR-155 X 1.039 0.910 1.142 hsa-miR-165 X 0.807 0.674 1.197 hsa-miR-183 X 0.333 0.218 1.528 hsa-miR-30* 0.561 0.432 1.299 hsa-miR-165 X 0.561 0.432 1.299 hsa-miR-30a 1.300 1.100 1.173 1.08 hsa-miR-30a X 0.564 0.477 1.131 hsa-miR-29b X 0.566 0.460 1.230 hsa-miR-225 X 0.566 0.460 1.230 hsa-miR-381 1.318 1.202 1.097 hsa-miR-381 1.318 1.202 1.097 1.318 1.202 1.097 hsa-miR-381 1.318 1	0.938	0.818	1.14/	haa miR-101-0p		Intervention
0.334 0.201 1.330 InsamiR-17 X 0.823 0.693 1.188 hsa-miR-155 X 1.039 0.837 1.241 hsa-miR-155 X 1.039 0.837 1.241 hsa-miR-155 X 1.039 0.910 1.142 hsa-miR-163 X 1.039 0.910 1.142 hsa-miR-164 X 0.807 0.674 1.197 hsa-miR-493* X 1.115 0.967 1.153 hsa-miR-164 X 0.561 0.432 1.299 hsa-miR-165 X 0.561 0.432 1.299 hsa-miR-185 X 0.503 0.376 1.338 hsa-miR-30a 1.300 1.173 1.108 hsa-miR-30a 1.300 1.173 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-18a X 0.544 0.477 1.140 hsa-miR-28-5p X 0.566 0.460 1.230 hsa-miR-326 hsa-miR-326 0.652 0.591 <td< td=""><td>1.352</td><td>1.1/0</td><td>1.150</td><td>haa miR-516C</td><td>~</td><td></td></td<>	1.352	1.1/0	1.150	haa miR-516C	~	
0.020 0.0337 1.100 Insa-miR-155 X 0.0572 0.434 1.318 hsa-miR-155 X 1.039 0.910 1.142 hsa-miR-15a X 0.807 0.674 1.197 hsa-miR-124 0.807 0.674 1.197 hsa-miR-124 0.333 0.218 1.528 hsa-miR-16 X 0.561 0.432 1.299 hsa-miR-18b X 0.561 0.432 1.299 hsa-miR-19b X 0.561 0.432 1.299 hsa-miR-19b X 0.561 0.432 1.299 hsa-miR-19b X 0.561 0.432 1.299 hsa-miR-30a X 1.300 1.173 1.108 hsa-miR-30a X 0.543 0.831 1.135 hsa-miR-32b X 0.544 0.477 1.140 hsa-miR-18a X 0.544 0.477 1.140 hsa-miR-25p X 0.566 0.460 1.230 hsa-miR-329 X 0.652 0.591 <td>0.354</td> <td>0.201</td> <td>1.330</td> <td>hea.miP.320</td> <td><u> </u></td> <td></td>	0.354	0.201	1.330	hea.miP.320	<u> </u>	
1.039 0.037 1.241 InsamiR-135 X 0.0572 0.434 1.318 Insa-miR-155 X 1.039 0.910 1.142 hsa-miR-493* 1.115 0.807 0.674 1.197 hsa-miR-493* 1.115 1.115 0.967 1.153 hsa-miR-340* 1.115 0.333 0.218 1.528 hsa-miR-16 X 0.561 0.432 1.299 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-19b X 0.503 0.508 1.207 hsa-miR-30a 1.157 hsa-miR-30a 1.300 1.173 1.08 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-18a X 0.552 0.591 1.37 hsa-miR-105 X 0.552 0.591 1.03 hsa-miR-325 0.0552 0.552 0.591 1.03 hsa-miR-306 0 0.544 0.777 1.088 hsa-miR-306 0	1.030	0.093	1.100	hea.miP.155	×	
0.012 0.400 1.670 Instamin-124 0.030 0.674 1.197 hsa-miR-124 0.807 0.674 1.197 hsa-miR-340* 0.333 0.218 1.528 hsa-miR-340* 0.501 0.432 1.299 hsa-miR-16 X 0.503 0.376 1.338 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-30a X 1.300 1.173 1.108 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-28b X 0.449 0.659 1.137 hsa-miR-28-5p X 0.656 0.460 1.230 hsa-miR-30e X 0.656 0.460 1.230 hsa-miR-30e X 0.655 1.091 hsa-miR-30e X	0.572	0.434	1 318	hsa-miR-15a	x	
0.807 0.674 1.197 hsa-miR-493* 1.115 0.967 1.153 hsa-miR-340* 0.333 0.218 1.528 hsa-miR-16 X 0.561 0.432 1.299 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-19b X 0.613 0.508 1.207 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-225 X 0.566 0.460 1.230 hsa-miR-28-5p X 0.566 0.460 1.230 hsa-miR-28-5p X 0.566 0.460 1.230 hsa-miR-382 X 0.565 1.091 hsa-miR-382 X X 0.649 0.573	1 039	0.910	1 142	hsa-miR-124	- ^	
1.115 0.967 1.153 hsa-miR-340* 0.333 0.218 1.528 hsa-miR-16 X 0.503 0.432 1.299 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-19b X 0.613 0.508 1.207 hsa-miR-30a 1 1.300 1.173 1.108 hsa-miR-379 X 1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-18a X 0.749 0.659 1.137 hsa-miR-105 X 1.224 1.092 1.121 hsa-miR-285 X 0.566 0.460 1.230 hsa-miR-285 X 0.652 0.591 1.101 hsa-miR-382 X 1.318 1.202 1.097 hsa-miR-326 X 0.649 0.573 1.133 hsa-miR-326 X 0.649 0.573 1.133 hsa-miR-325 X 0.9456	0.807	0.674	1,197	hsa-miR-493*		
0.333 0.218 1.528 hsa-miR-16 X 0.561 0.432 1.299 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-19b X 0.613 0.508 1.207 hsa-miR-30a 1 1.300 1.173 1.108 hsa-miR-30a 1 1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-195 X 0.566 0.460 1.230 hsa-miR-195 X 0.566 0.460 1.230 hsa-miR-142-5p X 0.566 0.460 1.230 hsa-miR-182 X 0.566 0.460 1.230 hsa-miR-325-p X 0.566 0.460 1.230 hsa-miR-326-p X 0.652 0.591 1.091 hsa-miR-326-p X </td <td>1 115</td> <td>0.967</td> <td>1 153</td> <td>hsa-miR-340*</td> <td></td> <td></td>	1 115	0.967	1 153	hsa-miR-340*		
0.561 0.432 1.299 hsa-miR-19b X 0.503 0.376 1.338 hsa-miR-15b 0 0.613 0.508 1.207 hsa-miR-30a 0 1.300 1.173 1.108 hsa-miR-30a 0 1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-195 X 0.566 0.460 1.230 hsa-miR-125 X 0.566 0.460 1.230 hsa-miR-221 1 1.212 1.102 1.100 hsa-miR-28-5p X 0.652 0.591 1.031 hsa-miR-381 1 1.318 1.202 1.097 hsa-miR-30e X 0.649 0.573 1.133 hsa-miR-315-5p X	0.333	0.218	1.528	hsa-miR-16	X	
0.503 0.376 1.338 hsa-miR-15b 0.613 0.508 1.207 hsa-miR-30a 1.300 1.173 1.108 hsa-miR-379 1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.566 0.460 1.230 hsa-miR-18a X 0.566 0.460 1.230 hsa-miR-221 1 1.212 1.102 1.001 hsa-miR-28-5p 0 0.652 0.591 1.103 hsa-miR-381 1 1.318 1.202 1.097 hsa-miR-381 1 1.318 1.202 1.097 hsa-miR-39-5p 0 0.718 0.655 1.096 hsa-miR-39-5p 0 0.718 0.655 1.096 hsa-miR-395 0 0.945 0.777 0.	0.561	0.432	1.299	hsa-miR-19b	X	
0.613 0.508 1.207 hsa-miR-30a 1.300 1.173 1.108 hsa-miR-379 1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-195 X 0.544 0.477 1.140 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.566 0.460 1.230 hsa-miR-125 X 0.566 0.460 1.230 hsa-miR-28-5p X 0.652 0.591 1.101 hsa-miR-28-5p X 0.655 0.591 1.031 hsa-miR-28-5p X 0.655 1.091 hsa-miR-381 X X 1.318 1.202 1.097 hsa-miR-30e X 0.649 0.573 1.133 hsa-miR-329-5p X 0.718 0.655 1.096 hsa-miR-323 X 0.545 0.491 </td <td>0.503</td> <td>0.376</td> <td>1.338</td> <td>hsa-miR-15b</td> <td></td> <td></td>	0.503	0.376	1.338	hsa-miR-15b		
1.300 1.173 1.108 hsa-miR-379 1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-18a X 0.749 0.659 1.137 hsa-miR-105 X 1.224 1.092 1.121 hsa-miR-125p X 0.566 0.460 1.230 hsa-miR-221 X 1.212 1.102 1.100 hsa-miR-28-5p X 0.652 0.591 1.131 hsa-miR-125b X 0.652 0.591 1.131 hsa-miR-381 X 1.318 1.202 1.097 hsa-miR-382 X 0.649 0.573 1.133 hsa-miR-30e X 0.649 0.573 1.133 hsa-miR-30e X 0.718 0.655 1.096 hsa-miR-39-5p X 0.718 0.655 1.096 hsa-miR-321-5p X 0.545 0.491 1.110 hsa-miR-146a X 0.956 1.149 0.832 hsa-miR-495 X 0.606 0.770 0.787 hsa-miR-192 X 0.606 0.7	0.613	0.508	1.207	hsa-miR-30a		
1.052 0.909 1.157 hsa-miR-195 X 0.943 0.831 1.135 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-29b X 0.749 0.659 1.137 hsa-miR-18a X 0.749 0.659 1.137 hsa-miR-18a X 0.749 0.659 1.137 hsa-miR-125p X 0.566 0.460 1.230 hsa-miR-142-5p X 0.566 0.460 1.230 hsa-miR-21 1 1.212 1.102 1.100 hsa-miR-25b 0 0.652 0.591 1.131 hsa-miR-381 1 1.365 1.251 1.091 hsa-miR-382 0 0.649 0.573 1.133 hsa-miR-39-5p 0 0.845 0.777 1.088 hsa-miR-39-5p 0 0.913 0.815 1.120 hsa-miR-315p 0 1.040 0.805 1.292 hsa-miR-146a 0 0.956 1.149 0.832 hsa-miR-192 X 0	1.300	1.173	1.108	hsa-miR-379		
0.943 0.831 1.135 hsa-miR-29b X 0.544 0.477 1.140 hsa-miR-18a X 0.749 0.659 1.137 hsa-miR-105 1.224 1.092 1.121 hsa-miR-142-5p X 0.566 0.460 1.230 hsa-miR-221 1 1.212 1.102 1.100 hsa-miR-28-5p 0 0.652 0.591 1.103 hsa-miR-494 0 0.406 0.359 1.131 hsa-miR-381 1 1.365 1.251 1.091 hsa-miR-382 0 0.649 0.573 1.133 hsa-miR-39-5p 0 0.718 0.655 1.096 hsa-miR-39-5p 0 0.718 0.655 1.096 hsa-miR-310-5p 0 1.040 0.805 1.292 hsa-miR-146a 0 0.956 1.149 0.832 hsa-miR-495 0 0.606 0.770 0.787 hsa-miR-192 X in NPM1mut	1.052	0.909	1.157	hsa-miR-195	X	
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0.749 0.659 1.137 hsa-miR-105 1.224 1.092 1.121 hsa-miR-142-5p X 0.566 0.460 1.230 hsa-miR-221 1 1.212 1.102 1.100 hsa-miR-28-5p 0 0.652 0.591 1.103 hsa-miR-294 0 0.406 0.359 1.131 hsa-miR-125b 1 1.365 1.251 1.091 hsa-miR-381 1 1.318 1.202 1.097 hsa-miR-302 0 0.649 0.573 1.133 hsa-miR-30e 0 0.649 0.573 1.133 hsa-miR-30e 0 0.718 0.655 1.096 hsa-miR-7 0 0.945 0.777 1.088 hsa-miR-395-5p 0 0.718 0.655 1.096 hsa-miR-7 0 0.913 0.815 1.120 hsa-miR-361-5p 0 1.040 0.805 1.292 hsa-miR-495 0 0.545 0.491 1.110 hsa-miR-146a 0 0.956 1.149 0.832 hsa-miR-495 0 0.606 0.770 0.787 hsa-miR-192 X in NPM1mut <td>0.544</td> <td>0.477</td> <td>1.140</td> <td>hsa-miR-18a</td> <td>X</td> <td></td>	0.544	0.477	1.140	hsa-miR-18a	X	
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0.606 0.770 0.787 hsa-miR-192 X in NPM1mut	0.040	1 140	0,833	hsa-miR-405		
overlan 24 miRNAs in NPM1mut	0.556	0.770	0.032	hsa-miR-192	x	⊢ downregulated
	0.000	0.70	0.107	overlan	24 miRNAs	in NPM1mut

33 core miRNAs

Online Supplementary Table S6. Overview of qRT-PCR target gene validation results. miRNA-target gene relations (n=42 genes; 177 different miRNA-target gene pairs) were investigated by analyzing target gene mRNA levels by qRT-PCR in HeLa cells transfected with either of one of 11 synthetic miRNA mimics or a negative control RNA. In accordance with previous reports of no significant effects of this particular miRNA on mRNA level, we also found no mRNA level changes for the known miR-19a-CCND1 and miR-155-FOXO3 interaction.¹²⁻¹³

let-7a	miR-15a	miR-19a	miR-20a	miR-23a	miR-29a	miR-30c	miR-106a	miR-142-5p	miR-155	miR-369-3
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mRNA reduction more than 30% mRNA reduction 10-30% no mRNA reduction gene described as target in literature with reference (ref.)

Colors were assigned according to mean expression of the gene (mean of 2 independent transfection and qRT-PCR experiments (measured in duplicates): harvest at 2 different time points, normalization by 3 different housekeeping genes).

References (genes described as targets in literature):

ref. 2: Patel et al., Mol Neurodegener. 2008 ref. 3: Bonci et al., Nat Med. 2008 ref. 5: Yu et al., J Cell Biol. 2008 ref. 6: Kong et al., J Bi ref. 8: Gironella et al., Proc Natl Acad Sci U S A. 2007 ref. 6: Kong et al., J Biol Chem. 2010

ref. 10: Johnson et al., Cell. 2005

ref. 1: Hébert et al., Neurobiol Dis. 2009 ref. 4: Qin et al., Proc Natl Acad Sci U S A. 2010 ref. 7: Kapinas et al., J Cell Biochem. 2009 ref. 9: O'Donnell et al., Nature. 2005

For detailed references see Supplementary Information.

ref. 11: Lin and Fu, Dis Model Mech. 2009

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Online Supplementary Table S7. *NPM1*^{met}-associated, downregulated genes and core miRNAs predicted to target the respective genes. See attached Excel file: ARuss Supplem Table S7 Online Supplementary Table S8. Validation of predicted miRNA targets by qRT-PCR. Normalized qRT-PCR analyses of selected miRNA target genes in HeLa cells which were transfected with the indicated miRNA mimic or negative control and harvested 24 or 48 h post-transfection. The values of normalization by three different housekeeping genes, as depicted in Figure 3 in the main manuscript, are given. The values given are the mean values of two independent transfections and qRT-PCR experiments.

[normalization by				
	ACTB	LMNB1	PGK1		
SPARC_29a_24h	0.59	0.88	0.51		
SPARC_29a_48h	0.42	0.57	0.43		
SPARC_23a_24h	0.87	1.61	0.87		
SPARC_23a_48h	0.63	0.99	0.70		
CCND1_15a_24h	0.61	0.66	0.52		
CCND1_15a_48h	0.81	1.00	0.69		
CCND1_20a_24h	0.69	0.55	0.58		
CCND1_20a_48h	0.97	0.94	0.83		
CCND1_let-7a_24h	0.81	0.80	0.82		
CCND1_let-7a_48h	0.79	0.63	0.59		
CCND1_106a_24h	0.44	0.49	0.51		
CCND1_106a_48h	0.65	0.73	0.69		
CCND1_142-5p_24h	0.72	0.68	0.76		
CCND1_142-5p_48h	0.83	0.76	0.81		
CCND1_155_24h	0.69	0.66	0.92		
CCND1_155_48h	0.57	0.58	0.59		
CRKL_15a_24h	0.65	0.71	0.62		
CRKL_15a_48h	0.61	0.75	0.59		
CRKL_30c_24h	0.89	0.73	0.90		
CRKL_30c_48h	0.70	0.66	0.84		
IRF2_20a_24h	0.67	0.72	0.61		
IRF2_20a_48h	0.56	0.74	0.58		
IRF2_23a_24h	0.58	1.07	0.58		
IRF2_23a_48h	0.61	0.95	0.67		
KIT_19a_24h	0.55	0.50	0.55		
KIT_19a_48h	0.25	0.30	0.32		
KIT_20a_24h	0.51	0.57	0.47		
KIT_20a_48h	0.46	0.64	0.51		
MN1_15a_24h	0.59	0.64	0.58		
MN1_15a_48h	0.73	0.90	0.70		
SERPINB9_let-7a_24h	0.52	0.46	0.44		
SERPINB9_let-7a_48h	0.63	0.57	0.43		
SERPINB9_29a_24h	0.36	0.56	0.32		
SERPINB9_29a_48h	0.26	0.37	0.27		
SERPINB9_369-3p_24h	1.17	1.12	1.10		
SERPINB9_369-3p_48h	0.55	0.55	0.75		

Online Supplementary Figure S1. Agarose gel picture of primer testing by conventional PCR. Prior to qRT-PCR, all primers were tested for sensitivity and specificity by conventional PCR using HeLa and HEL cDNA. The annealing temperature was 60 °C. Only primers resulting in one amplicon of the correct size were included in further analyses. Exemplary amplicons are shown below.



Online Supplementary Figure S2. Dissociation curve graphs (qRT-PCR) of exemplary amplicons. All primers were tested for sensitivity and specificity by conventional PCR and qRT-PCR (annealing temperature 60°C), resulting in one amplicon and one clear peak in the dissociation curve. Dissociation curves of exemplary amplicons are shown for the three housekeeping genes ACTB, LMNB1 and PGK1 as well as of investigated target genes MN1, *IRF2* and *KIT*.



Online Supplementary Figure S3A. Validation of miRNA microarray results by miRNA northern blot analysis. For northern blot analyses, 10 μ g of total RNA were loaded. RNA originated from the same isolation as the sample previously hybridized to the microarray. "High" and "low" refer to expression of the miRNA on the array. mut = *NPM1* mutated; wt = *NPM1* wild-type. Upper panels: autoradiography films; lower panels: loading control, ethidium bromide stained gels. The numbers given show quantitation of mature miRNA bands, normalized by loading control (the sum of all bands was used). Quantitation was performed with ImageJ (available at *http://rsbweb.nih.gov/ij/*).



Online Supplementary Figure S3B. Validation of miRNA microarray results by miRNA qRT-PCR analysis. Three patients' samples with either high or low expression of the respective miRNA according to the microarray were analyzed by TaqMan based qRT-PCR (Applied Biosystems). qRT-PCR data were normalized by expression of miR-374, which in the microarray was expressed at a well detectable level with only slight variation among the samples. Heatmaps: log. transformed, mean-centered microarray and corresponding qRT-PCR data (transformed in the same way) are shown. Array data are sorted (low to high) in the plots. Green color = low expression; red color = high expression of the miRNA. Expression data are depicted by color scales, as indicated. Correlation coefficients were as follows: let7a: 0.83; miR-155: 0.82; miR-20a: 0.79. For let-7a, patients "low 1" and "low 3" have wild-type *NPM1*, whereas patients "high 1" and "high 3" have mutated *NPM1*. Normalized, untransformed let-7a qRT-PCR expression data were as follows: "low 1" = 0.57 and "low 3" = 0.84 (mean 0.705), and "high 1" = 2.00 and "high 3" = 3.01 (mean 2.505). This results in a ratio "high 3" / "low 1" = 5.28, and a mean ratio *NPM1*^{mit} = 0.55. [Note: Patient "low2" had a t(8;21) and patient "high2" a t(11q23), as these patients were part of an originally larger cohort of 91 AML cases including all cytogenetic subgroups.]



Online Supplementary Figure S4. Hypothetical expression values to determine the significance levels of qRT-PCR data (as depicted in Online Supplementary Table S6 and Figure 3). Hypothetical normalized expression values compared to the negative control, which is set to 1 are shown. The six data points of each reduction stage show the same standard deviation of 0.1138, which is the average standard deviation of the qRT-PCR data shown in Figure 3 (for the genes depicted in Figure 3, the average standard deviation for the six data points was 0.118, as seen for example for CCND1-miR-106a or MN1-miR-15a). The expression cut-offs underlying the color coding of Online Supplementary Table S6 (dark green: >30% reduction of mRNA compared to negative control transfected cells, and yellow: 10-30% reduction) are based on the significance levels, as shown in the table below. In general, a reduction of mRNA of more than 30% results in highly significant P values (P<0.001; one-sample t test, compared to 1.0 = negative control), whereas a 10-30% reduction with average SD results in significant P values (P=0.001 - 0.05).



5nline Supplementary Figure S5. Validation of predicted miRNA targets by qRT-PCR in myeloid cells and comparison to effects observed in HeLa cells. Selected miRNA-target gene interactions were validated in two myeloid cell lines (HEL, transfected with miR-15a, -20a, -23a, -29a, -106a and miR-155; K-562, transfected with miR-15a, -29a, -106a and miR-142-5p). While these suspension cells displayed poorer transfectability than HeLa cells (see *Online Supplementary Design and Methods*), we nevertheless could reproduce all previous findings in these cell lines (the relations *CCND1*-miR-106a and *MN1*-miR-15a were seen in K-562 cells only), although with a lower but consistent reduction of target mRNA levels. Of note, transfection of K-562 cells (dark gray bars) resulted in greater effects as compared to HEL cells (light gray bars), which was possibly due to the higher transfection on target mRNA levels of SPARC were higher in both myeloid cell lines and *SERPINB9* higher in K-562 cells compared to HeLa cells. Normalized qRT-PCR results of selected miRNA target genes in HEL and K-562 cells which were transfected with the indicated miRNA mimic or negative control and harvested 24 h post-transfection are depicted. Each bar represents the mean effect plus standard deviation by normalization with the housekeeping genes *ACTB* and *PGK1* of two independent transfection and qRT-PCR experiments. Each value is calculated as percentage expression of target gene mRNA of miRNA-of-interest transfected cells compared to negative control (1.0 = 100%) transfected cells.



Online Supplementary Figure S6. Validation of miRNA targets by luciferase reporter experiments. Modified presentation of data contained in Figures 4C and 4D. Here, only the first column (reporter construct with wt binding site and co-transfection of negative control RNA) of each miRNA-target gene pair analyzed was set to 1 and the other conditions were set in relation to it. Differences in basal luciferase expression levels might be due to formation of different secondary structures of RNA of the reporter constructs with wild-type or mutant binding sites. Variations in plasmid DNA preparations should also be taken into account. (C) and (D) Normalized ratios firefly/renilla luciferase as determined by reporter assays in HeLa cells which were transfected with the indicated miRNA mimics or a negative control (neg.ctr. and wt construct; ratio set to 1) and co-transfected with the pMIR-REPORT vector containing the 3'UTR of candidate target genes downstream of the firefly luciferase reporter gene. (C) 3'UTRs containing one putative miRNA binding site, (D) 3'UTRs containing two putative miRNA binding sites. MiRNA binding sites mutated; light gray bars). Bars represent the mean of two independent experiments with transfections performed in triplicate, error bars depict the standard deviation (SD). All effects observed were highly significant (P<0.001; unpaired t test).

