Supplemental Data

Polycombs and microRNA-223 regulate human granulopoiesis by transcriptional control of target gene expression.

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Supplemental Methods

Co-immunoprecipitation assay was performed on cell extracts (500μg of proteins/sample). Where indicated, samples were treated with 10μg/ml RNAse A for 3 h at 37°C before the addition of antibody. Samples were precleared with 60 μg of protein A agarose beads (Upstate) for 30 min on ice. Samples were then immunoprecipitated using 5 μg of anti-Dicer1 antibody (Abcam) for 1 h at 4°C. Rabbit control IgG antibody (Santa Cruz Biotechnology) was used as nonspecific antibody. Immuno-complexes were precipitated using 40 μg of Protein A agarose beads. Beads were washed 8 times with 1 ml of NET gel buffer: 200 mM NaCl, 50 mM Tris-HCl pH 7.8, 0.1 % NP40, 1 mM EDTA, 0.25% bovine gelatine. Loading sample buffer was added to the samples that were boiled for 10 min in order to detach proteins from the beads. Immunoprecipitated samples were fractionated by electrophoresis on 4-12% SDS polyacrylamide gradient gel, electroblotted to nitrocellulose membrane and probed with the anti-YY1 antibody (Santa Cruz Biotechnology).

Immunophenotypic analysis was carried out on HL60, HL60R, HL60-PGK, HL60 siSuz12#1, HL60 siSuz12#6, HL60-pGIPZ, HL60 siAGO1, HL60 siDicer1, HL60 miRZip-223 and miRZip-scr cell lines by direct immunofluorescence staining of cells using allophycocyanin (APC)–conjugated mouse anti–human CD11b (Becton Dickinson). A minimum of 50,000 events was recorded for each sample by a FACScan flow cytometer (Becton Dickinson) using CellFit software (Becton Dickinson) for data acquisition and analysis.

Plasmid constructs, lentiviral infection, site-directed mutagenesis, and luciferase reporter activity: siSuz12 shRNA was designed using siRNA advanced design tools from the MWG (http://www.mwg-biotech.com/) and Ambion (http://www.ambion.com/) websites: these tools emply various published criteria to obtain the most effective siRNA in terms of silencing efficiency and reduced off-targeting effects (including custom BLAST analysis). The siRNA encoding the siSuz12 sense 5'-CAAGCCTGGTTCAGTTAAA-3' was subcloned into the pSUPER.retro.neo+GFP plasmid, from which the shRNA expression cassette was then cloned into the EcoRV site of pRRLcPPT.hPGK.EGFP.WPRE lentiviral vector (pgk) to generate the siSuz12-vector. Following bacterial transformation and overnight grown on ampicillin (100μg/ml) LB-agar plates, several bacterial colonies were selected and grown separately in LB broth. Infective particles were produced and HL60 cells were infected by the spin inoculation method. Briefly, HL60 cells were plated at $1X10^5$/ml of virus, 4μg/ml polybrene, and centrifugated at 32°C for 45 min after which the cells were incubated at 37°C 5% CO_2 for 1h 15 min followed by plating in fresh media. After infection, cells were cultured and purified by fluorescence-activated cell sorting (FACS). After 48 hours, pGIPZ lentiviral HL60 infected cells were puromycin selected (0.4 μg/ml). The efficiency of Suz12, AGO1 and Dicer1 knockdown in different clones was evaluated by western blot using a specific antibody anti-Suz12, anti-Dicer1 (Abcam, Cambridge UK), anti-

AGO1 (Upstate). siSuz12#1 and #6, siAGO1 and siDicer1 clones were selected on the base of their interference efficiency.

NFI-A wild type promoter sequence from nt -1400 to +24 was cloned in a pGL4.20 luciferase-vector (luc2/puro) (Promega Madison, WI USA). Briefly, the DNA fragment ranging from nt -1400 to +24 was amplified from HL60 genomic DNA using the following oligonucleotides: Fwd 5'-TTTCCTTTTGGAATTTTGAG-3', Rev 5'-ACTGTACGCGGGTGTTAAAG-3' and cloned in the pCR2.1 vector. All the plasmids were verified by sequencing.

Site-directed mutagenesis was used to mutate two putative miR-223 DNA target sequences in the NFI-A promoter region by the Quick Change mutagenesis kit (Stratagene, La Jolla, CA). The following primer pairs:

Fwd 5' GAGGTTAAAAAAAAA***CCCGCATGC***AAGCAGGCTTACTGG-3'

Rev 5' CCAGTAAGCCTGCTT***GCATGCGGG***TTTTTTTTTAACCTC 3', and

Fwd 5' CCAGAGGTGTCCCAG***CCCGCATGC***CGGTAATGATAGCCT 3'

Rev: 5'-AGGCTATCATTACCG***GCATGCGGG***CTGGGACACCTCTGG-3'; were respectively used to mutagenize the sequences located between nt -1240 and -1231 (NF-mut-1) and nt -1055 and -1046 (NF-mut-2) of the NFI-A wild type promoter. Both primer pairs were used at once to originate the double mutant form of the NFI-A wild type promoter (NF-mut (1, 2)). Annealed oligonucleotides generated a SphI restriction site that allowed verifying the success of the mutagenesis. All the plasmids were verified by sequencing. The pGL4.20 empty vector, and vectors carrying the wild type, NF-mut-1, -2 and -mut (1, 2) forms of the NFI-A promoter region were introduced into HL60 cells by elettroporation. After 48 hours, the transfected cells were puromycin selected (0.4 μg/ml) and treated or not with RA (1 μM) for the indicated times. Cells were lysed and luciferase activity was quantified by Dual Luciferase Reporter Kit (Promega, Inc). Experimental values were normalized by the total amount of protein in each cell lysate.

Confocal microscopy. Sub-cellular localization of miR-223: HL60 cells and primary APL blasts were plated at $4x10^5$ cells/ml in a 24 well plates. miRIDIAN miR-223 Mimic-Cy5 or miRIDIAN miR-Let-7a3 Mimic-Cy5 (30 pmol/well) were transiently transfected following *Trans*IT-TKO® transfection reagent instructions (MIR7201-Mirus). After 4 h from cell transfection, HL60 cells and patient APL blasts were treated or not with 1μM RA at 24 and 96 h or 72 h, respectively. For each time point, cells were collected, PBS-BSA 1% washed (three times), spotted on glass slides by cytospin centrifugation (200 rpm/4 min) fixed and made permeable for primary mouse monoclonal anti-βtubulin (Sigma-Aldrich), dilution 1:2000 in PBS-BSA 1%. miRIDIAN miR-223 Inhibitor-Cy5 was used as a probe to detect endogenous miR-223 sub-cellular location in HL60 cells and APL blasts, while microRNA hairpin Inhibitor Negative Control-Cy5 (NC) was used as experimental negative control as suggested by Dharmacon. In this case, cells treated or not with 1μM RA at the reported time points, were made permeable and added of primary mouse monoclonal anti-βtubulin (diluted 1:2000 in PBS-BSA 1%) first, and after washes with PBS-BSA 1%, of miRIDIAN miR-223 Inhibitor-Cy5 (0.36 μg/μl in PBS-BSA 1%) for 45 min in the dark. Thus, glass slides were incubated with the secondary anti mouse FITC conjugated anti-IgG antibody (1:100 in PBS-BSA 1%) for 45 min in the dark. Glass slides were washed in PBS (three times) and analyzed by confocal microscopy. miRIDIAN miR-223 Inhibitor-Cy5 was used, as previously described except for the use of DAPI for nuclear staining to detect miR-223 localization also in peripheral blood cells from a consenting healthy donor. Mitotic chromosome spread slides were obtained from HL60 cells transiently transfected with miRIDIAN miR-223 Mimic-Cy5 as described above. Cells were treated for 48 hours with 1 μM RA, and then Colcemid (Invitrogen) 0.1 μg/ml was added to the cells for 50 min at 37°C. Cells were washed once in PBS, resuspended in KCl 75 mM and kept for 45' at 37°C. Then, cells were fixed by adding methanol/acetic acid 3:1

for 30 min at 4°C and dropped onto pre-warmed (50°C) slides. Slides were dried at 50°C and incubated twice in 50 ml 6X SSC containing 20 µg/ml Acridine Orange (Sigma-Aldrich) for 5 min at room temperature and successively air dried and mounted with glycerol containing 2.5g/100ml Dabco Antifade reagent (Sigma-Aldrich). Images were obtained using the Leica TCS-SP5 confocal laser scanning microscopy system (Leica Microsystems, Mannheim, Germany).

Sub-cellular localization and colocalization of YY1, Dicer1 and miR-223: HL60 cells were transiently transfected with miRIDIAN miR-223 Mimic-Cy5 or with miRIDIAN Negative Control Mimic-Cy5 (30 pmol/well) and treated or not with 1µM RA at 72 h as described above. Cells were collected, glass slides prepared and cells made permeable for primary rabbit polyclonal anti-YY1 (dilution 1:500 in PBS/BSA 2%) and mouse monoclonal Dicer1 (dilution 1:100 in PBS/BSA 2%) and incubated for 1 hour. Glass slides were thoroughly washed and incubated with AlexaFluo-488 anti-Rabbit (dilution 1:400 in PBS + BSA 2%) and AlexaFluor-555 anti-Mouse (dilution 1:400 in PBS + BSA 2%) for 45 min in the dark. Glass slides were washed in PBS, and Dapi solution (7µg/ml) added for 2 min. Glass slides were washed in PBS (three times). This quadruple labelled material was esamined under confocal laser scanning microscope (CLSM, Zeiss 700,) equipped with four solid states laser lines: violet diode emitting at 405 nm, blu diode emitting at 488 nm, green diode emitting at 543 nm and red diode emitting at 633 nm. Images were acquired in sequential scan mode by using the same acquisitions parameters (laser intensities, gain photomultipliers, pinhole aperture, objective 100X, zoom 2). For visualization purposes, channel colours were palette assigned and may not reflect the true fluorochrome colour. For production of figures and visualization purposes, brightness and contrast of images were adjusted by taking care to use the same values to help comparison between the different experimental groups. Nucleus cytoplasm boundaries of the cells were delineated according to DAPI staining.

Supplemental Tables

Table S1: Primer sequences used for semi-quantitative RT-PCRs.

NFI-A	F:5'-TGGCATACTTTGTGCATGCAGC-3' R:5'-ACCTGATGTGACAAAACTGTCC-3'
GAPDH	F:5'-GCATGGTCAACTGCAACGATG-3' R:5'-GGGCGCATCGTACTTGGTG-3'.
Tgase-II	F:5'-ATCAGCAATGCCTCTGCAC-3' R:5'-TGGCATGGACTGTGGTCATG-3'

Table S2: Primer sequences used for ChIPs.

NFI-A region 1	F:5'-ATCCGGTGAATCTTCTGTGG-3' R:5'-GAAATGCGGTAAGGCTCAAA-3'	-791/-616 nt
NFI-A Region 2	F:5'-TACAATCCAAAGCACGCAAG-3' R:5'-AATTGCAGGCTTCTTGGAGA-3'	-5230/-5027 nt
NFI-A Region 3	F:5'-AAAGCTTGCAGAGGTTTTGC-3' R:5'-AAAATTAGCCAGGCATGGTG-3'	-8016/-7797 nt
LMO2	F:5'-GAATCACCCGGGAGGAAG-3' R:5'-GCCTGGACCCTTCAGAGTAA-3'	+582/+401 nt

Table S3: Primer sequences used for bisulphite genomic sequencing.

CpG island 26	F:5'-GTGTGTAGATTTGATTAAAAGGTG-3' R:5'-TAACCAATAAAAACCCCTACATTC-3'	-5648/-5431 nt
CpGs cluster	F:5'-GTTTGTGTTTAAATTTATTAGTTTTATAG-3' R:5'-ACACATTTACATATATACAAATTTC-3'	-861/-549 nt
CpG island 99	F:5'-GATTGYGGAATAGTAGTGTTTT-3' R:5'-AAAACTAACCRCAACCTACC-3'	+615/+1012 nt

Table S4: ^{32}P-labeled oligonuclotide sequences used to detect NFI-A gene regions.

NFI-A Region 1	5'-ATGCCACAGGACCGAAGCTGC-3'	-741/-720 nt
NFI-A Region 2	5'-GAATCTGAGAACCAGCCAGCC-3'	-5195/-5174 nt
NFI-A Region 3	5'-CAGGAGATTGAGACCATCCTG-3'	-7873/-7852 nt

Table S5. Antibodies Used in this Study

ChIP experiments
α - H3ac, Upstate Biotechnology, Lake Placid, NY, cat#06-599
α - H3K4me3, Abcam, Cambridge, UK, cat#ab8580
α - H3K27me3, Abcam, cat#ab6002
α - Dicer1, Abcam, cat#ab14601
α - YY1, Santa Cruz Biotechnology, Santa Cruz, CA, cat#H-414X
α - Ago1, Upstate, cat#07-599
α - Ago2, Abnova, cat#h00027161-M01
α - Suz12, Abcam, cat#ab12073
α - RNApol-II, Abcam, cat#ab817
α - Cy5, Sigma-Aldrich, Clone Cy5-15 cat#C1117

Immunoprecipitation and Immunoblotting
α - NFI-A, Abcam, cat#ab11988
α - LMO2, Santa Cruz Biotechnology, cat#N-16, sc-10497
α - TGase-II, Abcam, cat#ab2972
α - β-tubulin, Sigma-Aldrich, cat#T5168
α - Ago1, Upstate, cat#04-083
α - Dicer1, Abcam, cat#ab14601
α - YY1, Santa Cruz Biotechnology, cat#H-414X
α - Suz12, Abcam, cat#ab12073

Confocal microscopy
α - YY1, Santa Cruz Biotechnology, cat#H-414X
α - Dicer1, Abcam, cat#ab14601
AlexaFluor-488 Donkey anti-Rabbit, Life Technologies-Molecular Probes, USA, A-21206
AlexaFluor-555 Goat anti-Mouse, Life Technologies-Molecular Probes, A-21422

Table S6. Target Sequences for siRNA Experiments

SUZ12
Clone 1 and 6, 5'-CAAGCCTGGTTCAGTTAAA-3

DICER1
5'-TGGTCTTTCTTACAATCAA-3 (Clone ID V3LHS_391373 OpenBiosystems)

AGO1
5'-CCAGAAATCTCTGATATCA-3' (Clone ID V2LHS_15322 OpenBiosystems)

Supplemental Figure Legends

Figure S1. Modulation of miR-223, NFI-A mRNA/protein expression levels by RA treatment in HL60 and HL60R cells, in NB4 and NB4MR4 cells and by Ara-C treatment in K562 cells. Cells were collected at the indicated times of RA treatment (1μM) and tested by: A) Upper panel: northern blot analysis of miR-223 expression levels. U6 detection was used as RNA loading control; Middle panel: relative qRT-PCR analysis of the NFI-A mRNA expression levels in control (□) or RA-treated (■) cells; Lower panel: immunoblot analysis of NFI-A and LMO2 protein levels. B) LMO2 mRNA relative qRT-PCR expression levels in control (□) and RA treated (■) HL60 cells. C) QRT-PCRs measuring the expression of NFI-A and miR-223 mRNAs in HL60 and K562 cell lines induced to granulocytic and erythroid differentiation by treatment with RA 1μM and Ara-C 0.5μM, respectively, for the indicated time points; D) HL60 cells were untreated (CTR) or treated with 1μM RA (RA) for 5 and 72 h. Actynomicin D (Act-D) was then added for 0, 0.5, 1.5 and 4 h. Upper panel: northern blot detecting miR-223 levels (U6 loading control). Lower panel: qRT-PCR measuring the NFI-A mRNA expression levels in untreated (□) and RA-treated (■) HL60 cells. Bars show the remaining NFI-A mRNA levels relative to the values measured in the absence of actinomycin-D (Act-D h 0 set as control value=1). Error bars are the mean ±SD of three independent evaluations. E) relative qRT-PCR measuring the NFI-A mRNA expression levels in HL60 cells untreated (□) and treated (■) with 1μM RA for 24 hours in absence (-) or presence (+) of protein synthesis inhibitor cycloheximide (25μg/ml), error bars are the SD of three independent evaluations. F) Histograms of the qRT-PCRs performed to amplify NFI-A region 1 in ChIP assays carried out using an anti-RNAPolII antibody on chromatins prepared from HL60 treated or not with 1 μM RA and in K562 cells treated or not with 0.5 μM Ara-C. Data are plotted as the ratio of values obtained in RA or Ara-C treated versus untreated cells (CTR). Error bars

represents SD of three independent evaluations. G) ChIP assays were performed, using the indicated antibody, to evaluate the recruitment of polycomb and RNA interference elements at Lmo2 proximal promoter in HL60 cells, treated (RA) or not (CTR) with RA 1μM. H) QRT-PCRs of the NFI-A mRNA levels in immature CD34+-hematopoietic stem/progenitor cells (CD34+), mature CD34- cell fraction (CD34-), HL60, HL60R, NB4, NB4-MR4 myeloid cell lines and in the K562 erythroleukemic cell line. I) ChIP assay performed using anti-H3K4me3 and H3K27me3 antibodies and qRT-PCR amplification of NFI-A region 1 in HL60, NB4 and NB4-MR4 myeloid cell lines. Error bars represent SD of three independent evaluations.

Figure S2. Bisulfite sequencing of NFI-A promoter and recruitment of Ago1 and Ago2 proteins at the NFI-A promoter regions in RA-treated HL60 cells.

A) DNA methylation status of the NFI-A promoter. Diagram of the distribution of CpG island-26, CpG island-99 (gray boxes) and clusters of CpG dinucleotides (○) present along the NFI-A gene (http://genome.ucsc.edu). Numbers are the nucleotides relative to the NFI-A transcription start site (+1). Bisulphite sequencing assay was performed on genomic DNA isolated from HL60 cells untreated (CTR) or treated with RA 1μM for the indicated times. The methylation level of each CpG is represented by a circle depicted by increasing grey intensities. The grey intensity scale defines the percentage of methylation of each CpG dinucleotide analyzed in 6-10 different clones for each sample. B) ChIP assays were performed in HL60 cells treated (RA) or not (CTR) with RA 1μM using antibodies anti-Ago1 and anti-Ago2, at the indicated time points. QRT-PCRs were performed to amplify the NFI-A region 1. Error bars represents SD of three independent evaluations.

Figure S3: Morphological analysis of HL60 cells with lentivirus-based stable knockdown of Suz12, Ago1 and Dicer1 upon RA treatment. Changes in morphology by light-field microscopy

of Wright-Giemsa stained untreated cells (CTR) or cells treated for 96 hours with 1μM RA. WT: wild type; Pgk and pGIPZ: control vectors. WT and vector-control cells treated with RA displayed chromatin condensation with nuclear segmentation, changes in nuclear/cytoplasmic ratio, reduced cytosolic basophilia, appearance of paranuclear Golgi region and specific granules (scale bar, 5μM).

Figure S4. Intracellular localization of miR-223. HL60 cells, APL blasts and PB cells were analysed by immunofluorescence confocal microscopy. A) A miR-223-Inhibitor-Cy5 was used as a probe to detect endogenous miR-223 sub-cellular localization in HL60 cells and APL blasts treated (RA) or not (CTR) at the indicated time points. βtubulin marks cell cytoplasm. Merge of the two channels. B) subcellular localization of microRNA hairpin Inhibitor Negative Control-Cy5 (NC), sequence based on *c. elegans* miR-239b (upper panel) and of mir-Let7-a3-Cy5 (Mimic-Let7-a3) (lower panel) in HL60 cells treated (RA) or not (CTR) for 96h. βtubulin marks cell cytoplasm. Merge of the two channels; C) βtubulin marks cell cytoplasm of PB cells (left), miR-223 localization in PB cells (right), lower panels merge of βtubulin and miR-223 (left), merge of miR-223 and DAPI staining (right).

Figure S5: Sub-cellular localization and colocalization of Mimic-223, YY1 and Dicer1 in HL60 cells. A) High magnification of quadruple labeling (DAPI, YY1, Dicer1, Mimic-NC) and double channels visualization of untreated (CTR) or treated (RA) HL60 cells transiently transfected with Mimic-NC. B, C) single channel and double channels visualization of the colocalization features of HL60 cells transiently transfected with Mimic-223 (B) or Mimic-NC (C) used as negative control. Channel colours were palette assigned for visualization purposes and to emphasize colocalization features. A mask landmark (blu line) delineate the nuclear boundaries.

Figure S6. Localization of miR-223 on mitotic chromosomes. Acridine Orange-stained mitotic chromosomes from HL60 cells (A) untransfected; B) transfected with the miR-223-Inhibitor-Cy5 (Inhibitor-223) to sequester miR-223 activity, before and after 48h RA treatment and the merge of the two channels.

Figure S7. Complementarities of mature miR-223 and pre-mi-R223 nucleotides to NFI-A promoter DNA sequence, and sequence conservation among vertebrates

Figure S8. A) qRT-PCR measuring LMO2 mRNA expression in HL60 wild type cells (wt), and infected with a lentiviral vector expressing miR-223 (Lenti-223) or an empty viral vector (pgk); B) ChIP assays were performed in HL60 cells infected with a lentiviral vector expressing miR-223 (Lenti-223) or not (pgk), using the indicated antibodies. Recovered DNA was PCR amplified using primer sets for NFI-A gene region 1, 2, and 3 or LMO2 proximal promoter.

Figure S9. Inhibition of miR-223 activity impairs the granulocytic differentiation potential of RA-treated HL60 cells. A) Percentage of CD11b positive HL60 cells infected or not (wt) with an anti-miR-223 microRNA (miRZip-223), or the scramble hairpin control (miRZip-scr) constructs and treated (RA) or not (CTR) with the indicated RA concentrations for 72 hours, as measured by Flow Cytometry. The results represent the average of three independent evaluations +/- SD; B) Changes in morphology by light-field microscopy of Wright-Giemsa stained cells (scale bar, 10μM).

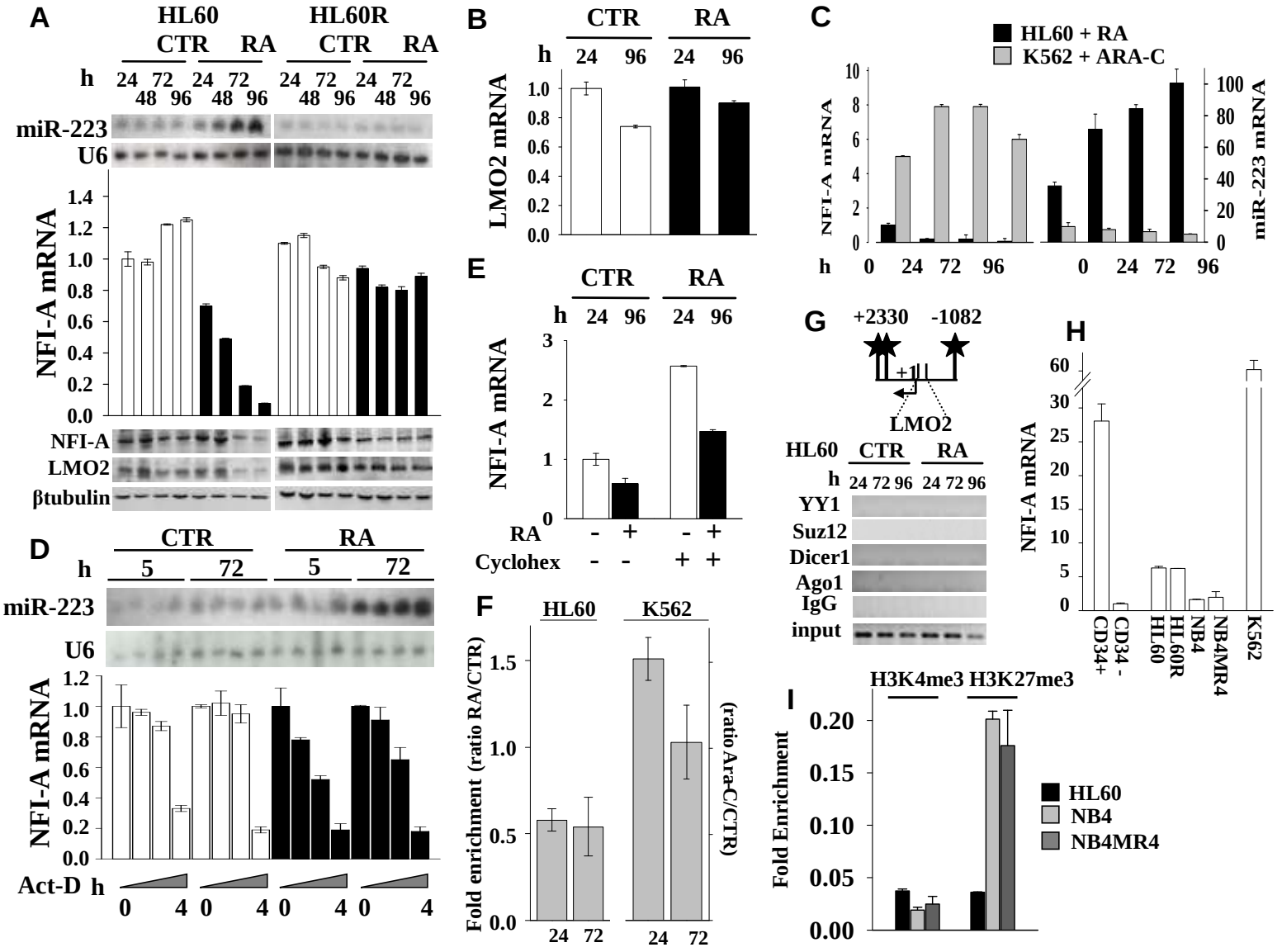
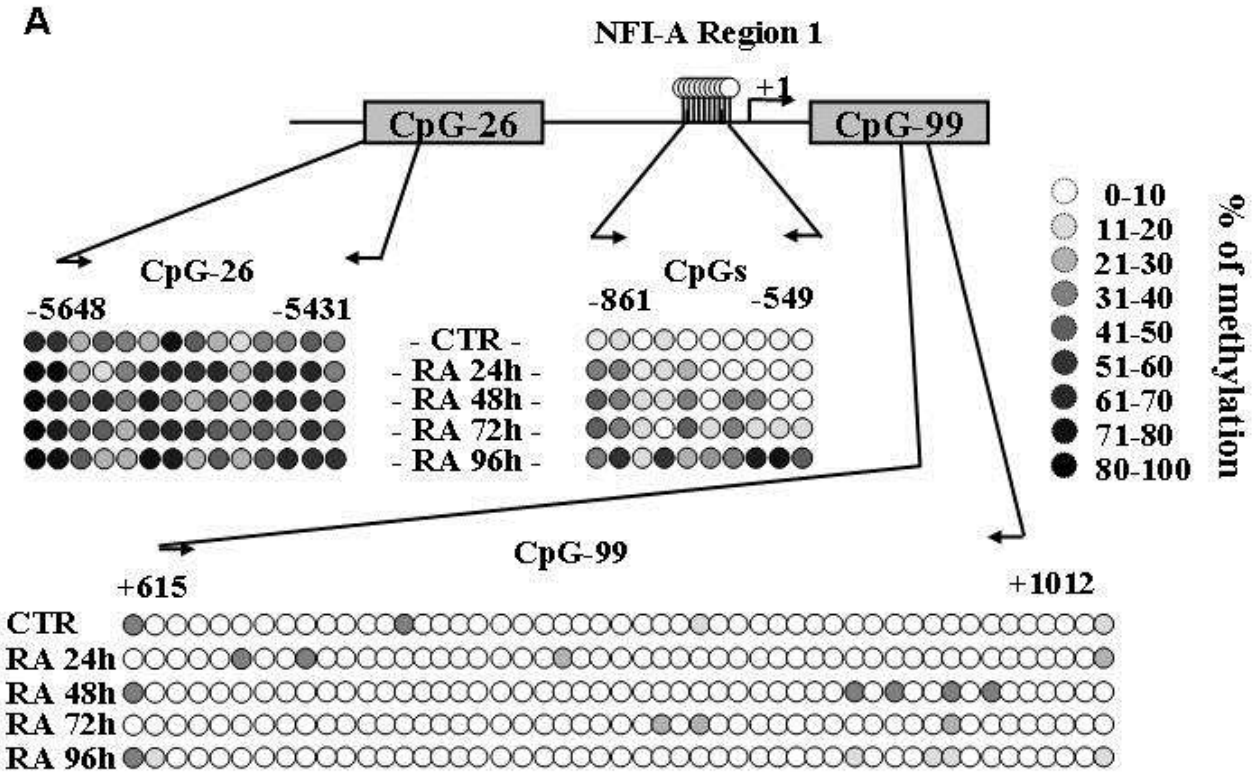

A
HL60
HL60R
CTR
RA
h
24 72 48 96
miR-223
U6
NFI-A mRNA
NFI-A
LMO2
βtubulin
B
LMO2 mRNA
C
HL60 + RA
K562 + ARA-C
NFI-A mRNA
miR-223 mRNA
D
Act-D h
0 4
E
RA
Cyclohex
F
HL60
K562
Fold enrichment (ratio RA/CTR)
(ratio Ara-C/CTR)
G
+2330
-1082
+1
LMO2
YY1
Suz12
Dicer1
Ago1
IgG
input
H
CD34+
CD34 -
HL60
HL60R
NB4
NB4MR4
K562
I
H3K4me3
H3K27me3
Fold Enrichment
HL60
NB4
NB4MR4

Figure S1


A
NFI-A Region 1
+1
CpG-26
CpG-99
CpG-26
-5648
-5431
CpGs
-861
-549
- CTR -
- RA 24h -
- RA 48h -
- RA 72h -
- RA 96h -
0-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
80-100
% of methylation
CpG-99
+615
+1012
CTR
RA 24h
RA 48h
RA 72h
RA 96h

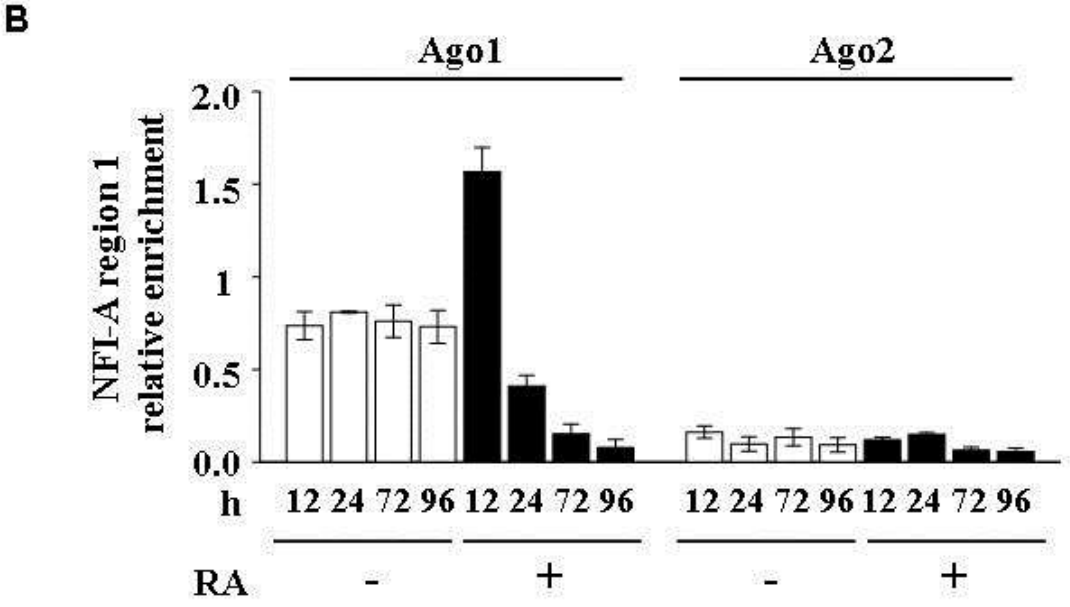

B
Ago1
Ago2
NFI-A region 1
relative enrichment
2.0
1.5
1
0.5
0.0
h
12 24 72 96 12 24 72 96
12 24 72 96 12 24 72 96
RA
-
+
-
+

Figure S2

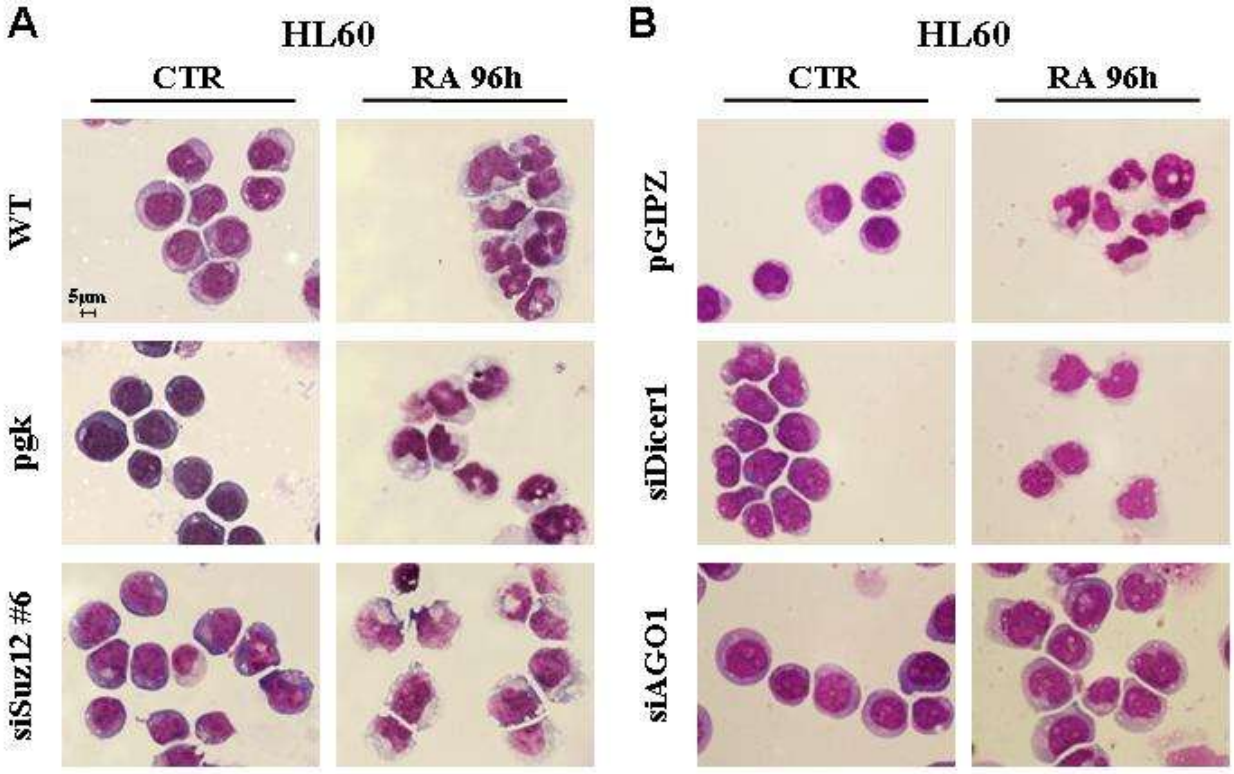

A
HL60
CTR
RA 96h
WT
pgk
siSuz12 #6
5µm
B
HL60
CTR
RA 96h
pGIPZ
siDicer1
siAGO1

Figure S3

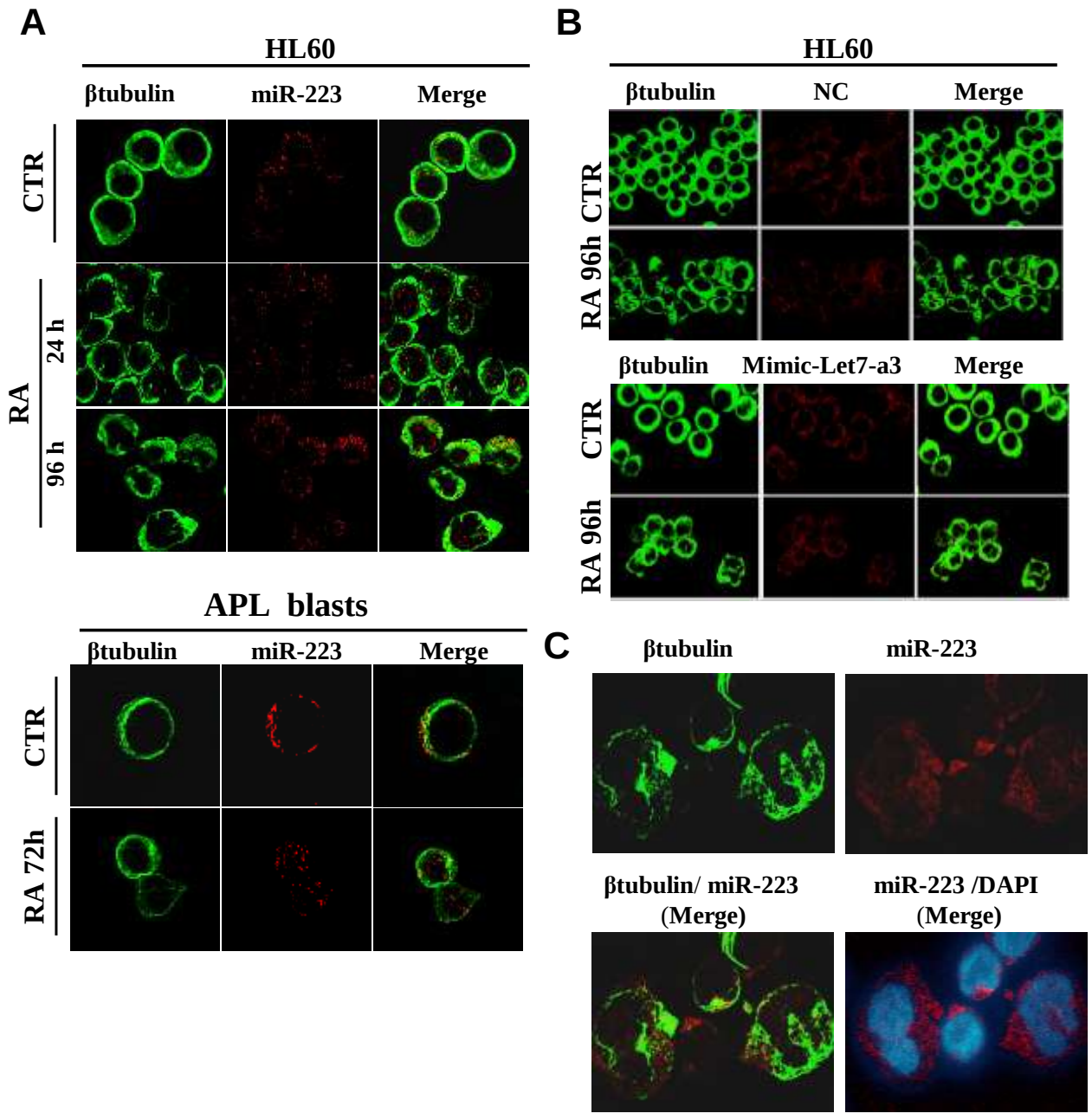
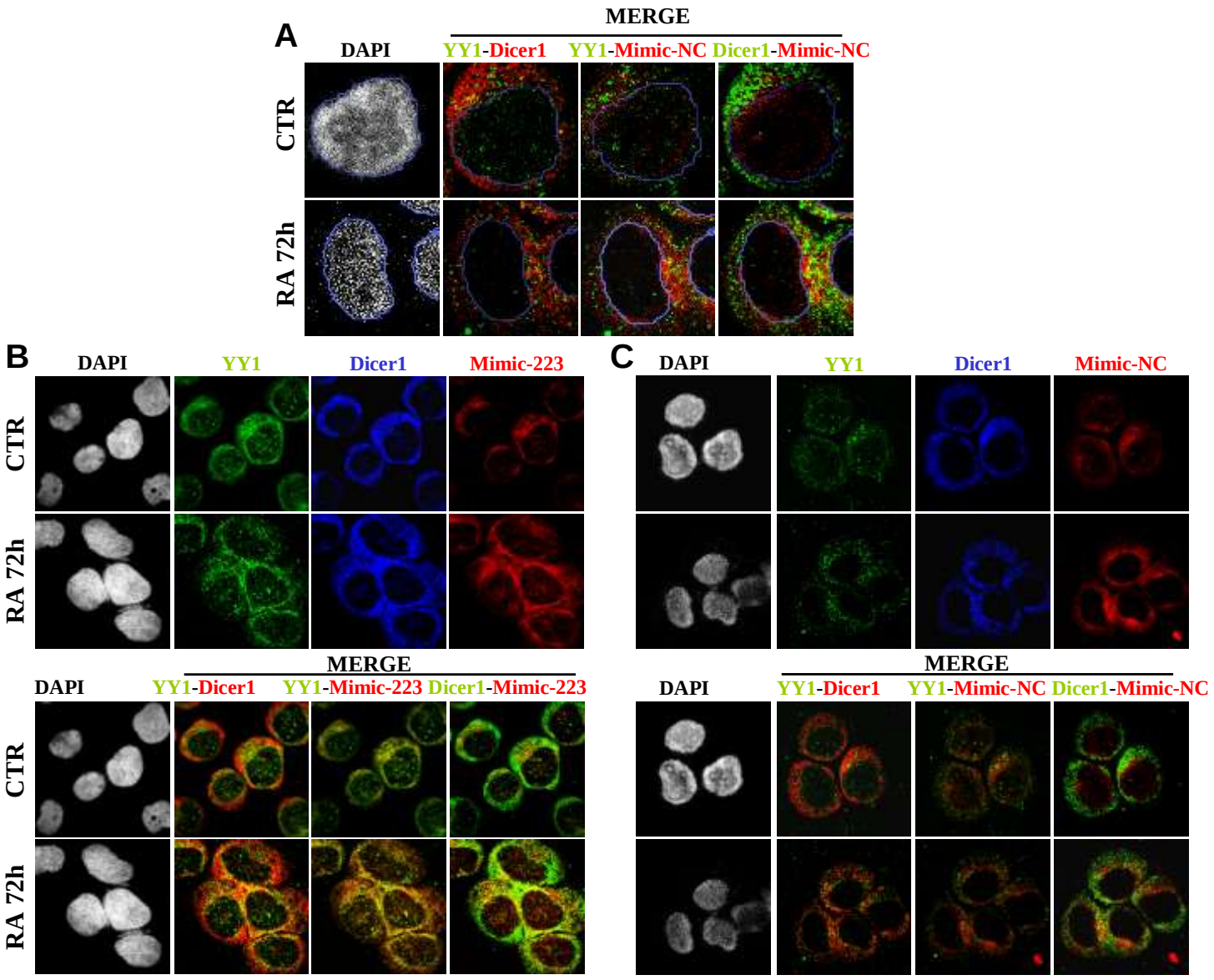

A
HL60
βtubulin
miR-223
Merge
CTR
RA
24 h
96 h
APL blasts
βtubulin
miR-223
Merge
CTR
RA 72h
B
HL60
βtubulin
NC
Merge
CTR
RA 96h
βtubulin
Mimic-Let7-a3
Merge
CTR
RA 96h
C
βtubulin
miR-223
βtubulin/ miR-223
(Merge)
miR-223 /DAPI
(Merge)

Figure S4


A
MERGE
DAPI
YY1-Dicer1
YY1-Mimic-NC
Dicer1-Mimic-NC
CTR
RA 72h
B
DAPI
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Mimic-223
CTR
RA 72h
MERGE
DAPI
YY1-Dicer1
YY1-Mimic-223
Dicer1-Mimic-223
CTR
RA 72h
C
DAPI
YY1
Dicer1
Mimic-NC
MERGE
DAPI
YY1-Dicer1
YY1-Mimic-NC
Dicer1-Mimic-NC

Figure S5

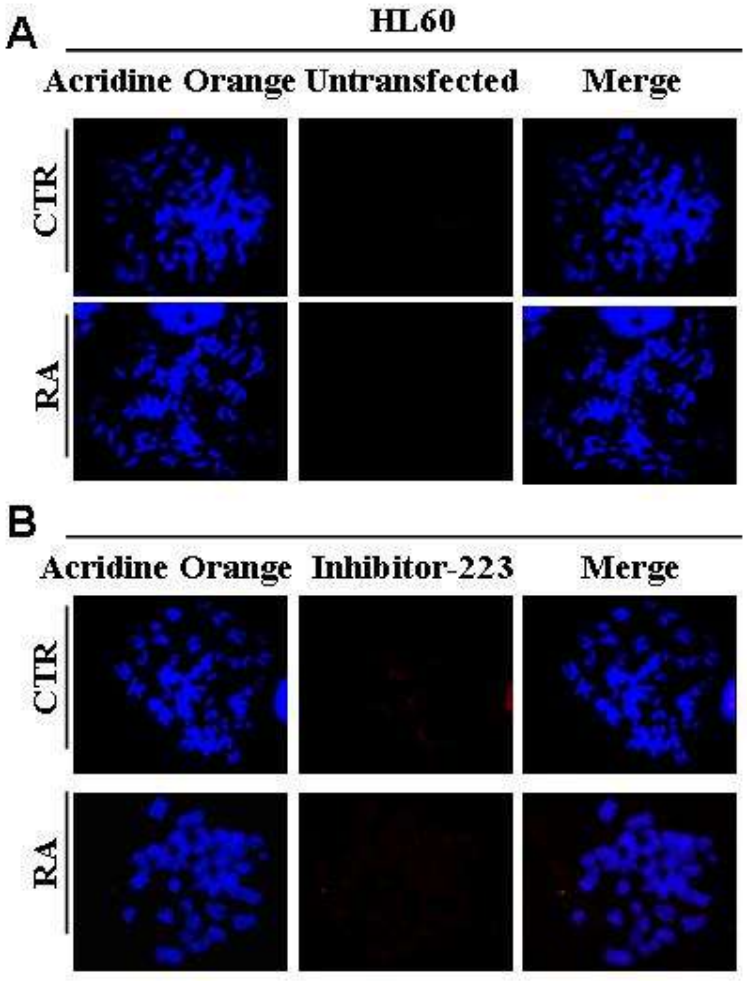
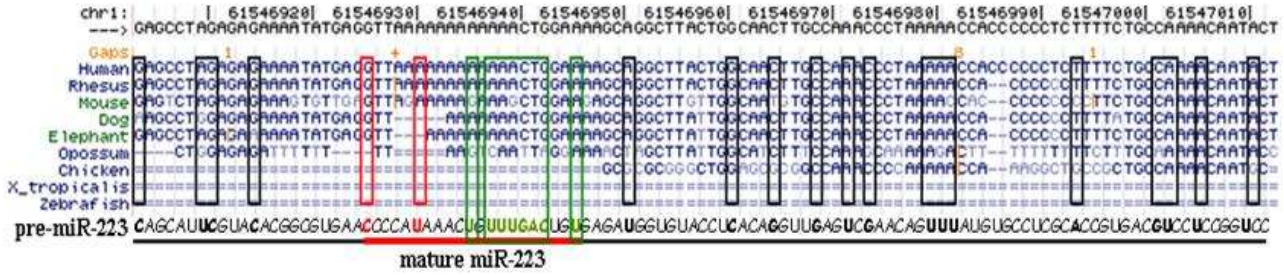

A
HL60
Acridine Orange
Untransfected
Merge
CTR
RA
B
Acridine Orange
Inhibitor-223
Merge
CTR
RA

Figure S6

DNA seq1



DNA seq2

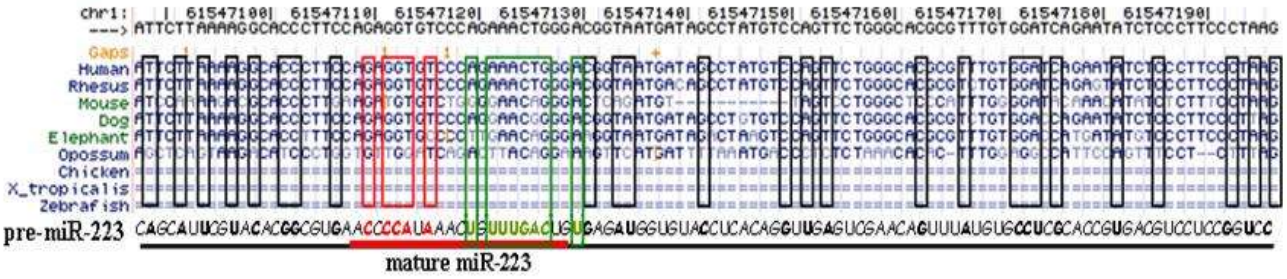


Figure S7

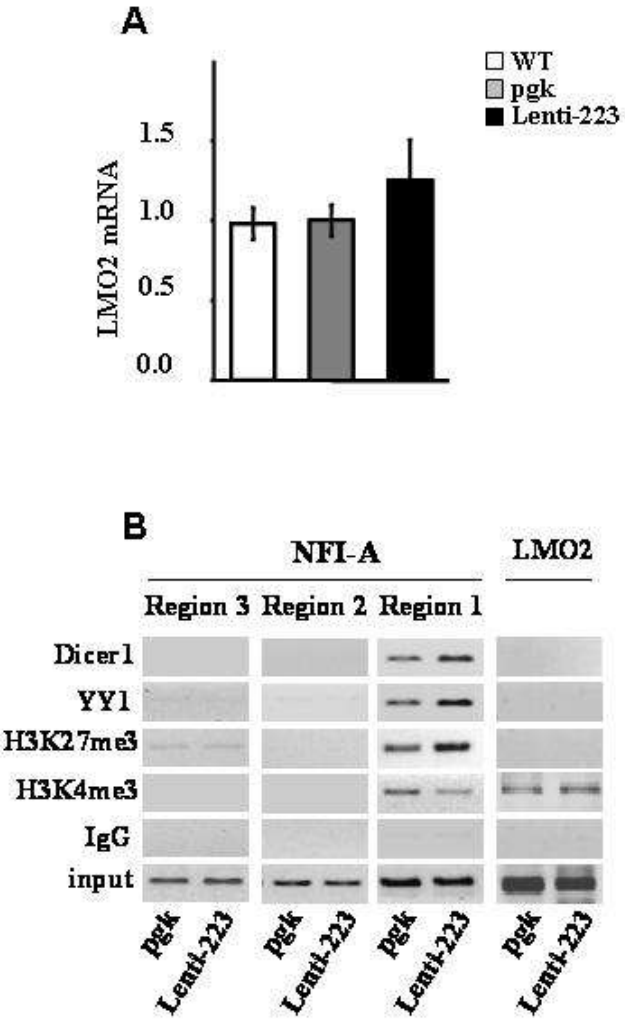

A
WT
pgk
Lenti-223
LMO2 mRNA
1.5
1.0
0.5
0.0
B
NFI-A
LMO2
Region 3
Region 2
Region 1
Dicer1
YY1
H3K27me3
H3K4me3
IgG
input
pgk
Lenti-223
pgk
Lenti-223
pgk
Lenti-223
pgk
Lenti-223

Figure S8

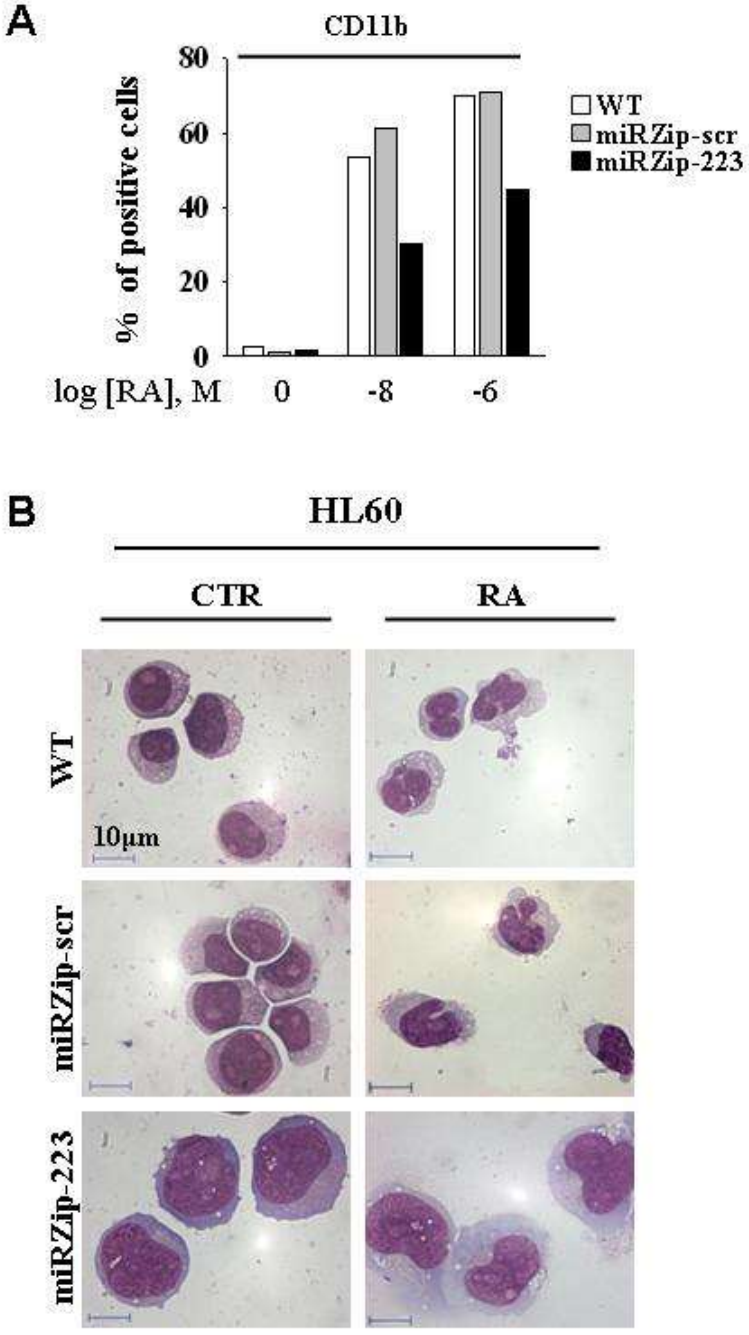

A
CD11b
80
60
40
20
0
% of positive cells
WT
miRZip-scr
miRZip-223
log [RA], M
0
-8
-6
B
HL60
CTR
RA
WT
10µm
miRZip-scr
miRZip-223

Figure S9