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Supplemental Material to:

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Long intergenic non-coding RNA HOTAIRM1 regulates cell cycle progression during myeloid maturation in NB4 human promyelocytic leukemia cells

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Supplemental Material

1. 2013RNABIOL0282R1-SupTable1-6.xlsx

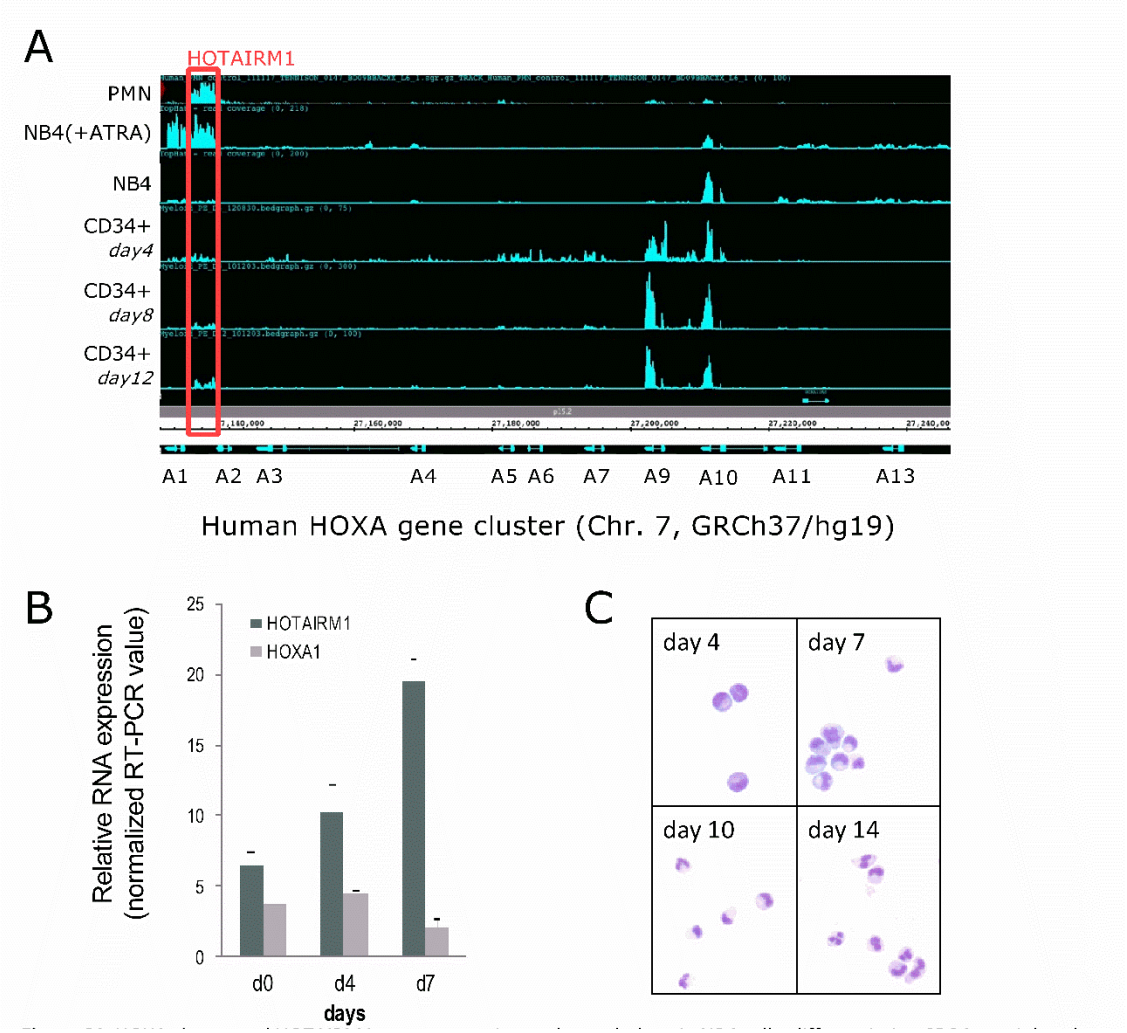

Figure S1. HOXA cluster and HOTAIRM1 gene expression and morphology in NB4 cells, differentiating CD34+ peripheral blood stem cells, and human neutrophils.

A. Transcription mapping of the human HOXA cluster in NB4 cells +/- ATRA, differentiating G-CSF-mobilized human CD34+ peripheral blood cells at the indicated days of culture, and primary circulating human neutrophils (PMN) by deep sequencing (RNA-Seq). HOXA cluster genes are mapped in the lower margin and HOTAIRM1 is indicated by the red box.

B. qRT-PCR measurement of HOTAIRM1 expression in differentiating CD34+ cells.

C. Morphological maturation of CD34+ at the indicated days of culture by light microscopy (Wright-Giemsa staining).

Mobilized peripheral blood CD34+ cells were expanded *in vitro* in serum free medium (StemSpan® SFEM supplemented with StemSpan® CC100 Cytokine Cocktail; STEMCELL Technologies), and differentiated by further culture with G-CSF.[1] Total RNA was isolated from cells lysed in Tri-Reagents (Molecular Research Center, Inc. Cincinnati, OH) according to the manufacturer's instructions and treated with TurboDNAse (Ambion). The integrity of the RNA was evaluated on an Agilent Bioanalyzer, and samples with sufficient RNA integrity were used to create libraries for deep sequencing. Samples were sequenced by paired-end reads on the Genome Analyzer Iix (Illumina, San Diego, CA). Base calling and paired-end short reads (FASTQ format) were generated by Genome Analyzer Pipeline software. The resulting sequence reads were aligned to the human reference genome Hg19 February 2009 GRCh37 build using short read aligner Bowtie.[2]

[1] Haylock DN, To LB, Dowse TL, Juttner CA, Simmons PJ. Ex vivo expansion and maturation of peripheral blood cd34+ cells into the myeloid lineage. Blood. 1992;80(6):1405–12

[2] Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009;10(3):R25

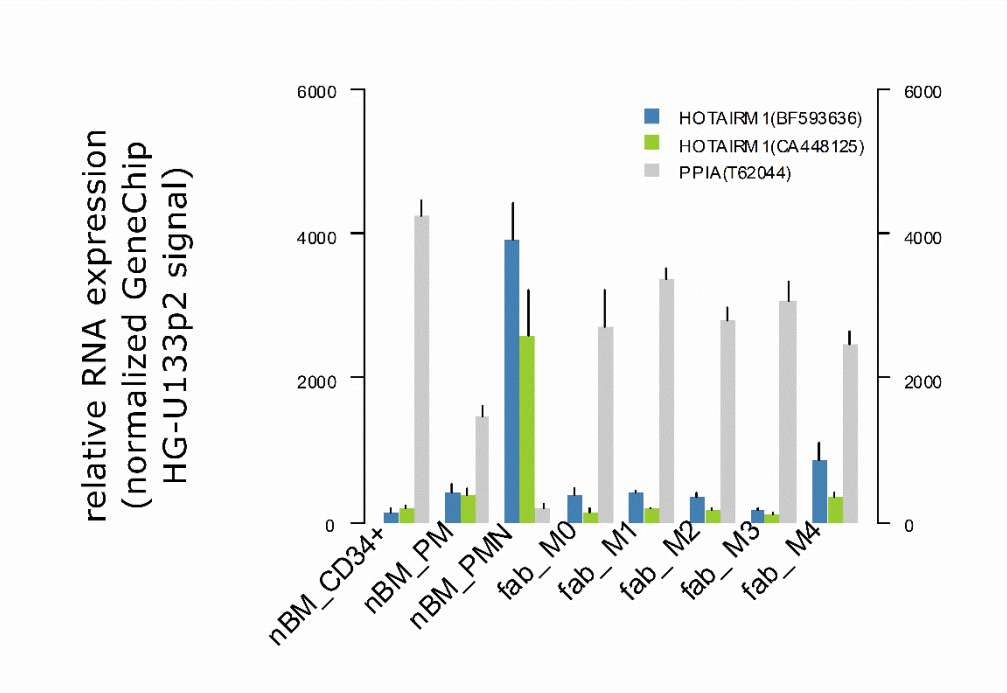

Figure S2. Expression of HOTAIRM1 in normal and acute myeloid leukemia (AML) human bone marrow cells

Expression of HOTAIRM1 was examined in human bone marrow cells sorted into the indicated fractions and in bone marrow samples collected at diagnosis from human AML patients with the indicated French-American-British (fab) subtypes of AML (M0 to M4). Data were derived from data set GSE12662 available from GEO (Gene Expression Omnibus, NIH).

Abbreviations: **nBM**, normal bone marrow; **PM**, promylocyte; **PMN**, polymorphonuclear (mature) neutrophils

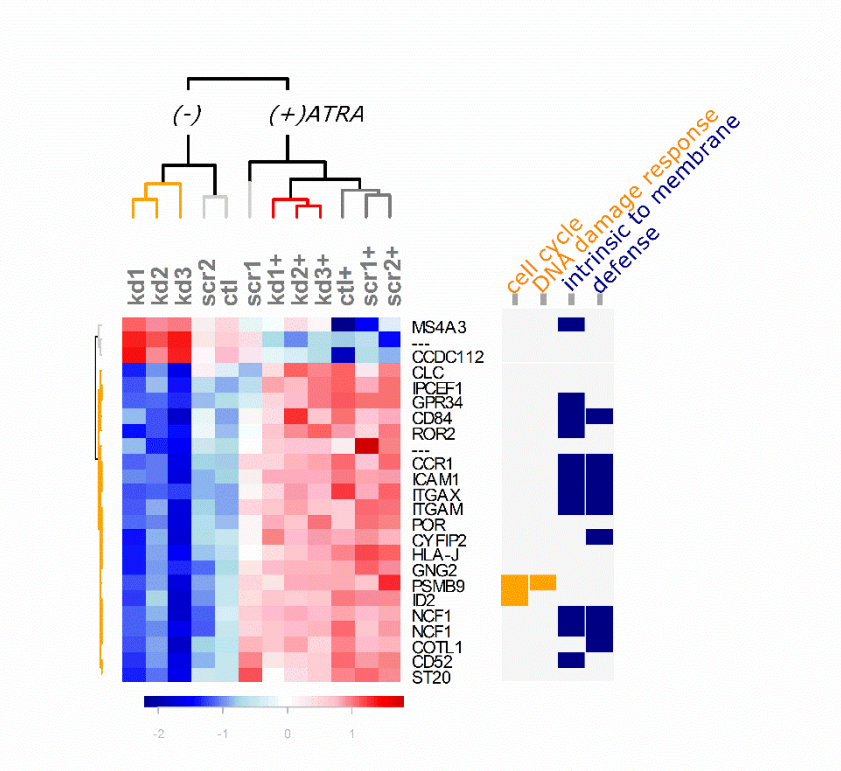

Figure S3. Heatmap representation of gene expression perturbation by HOTAIRM1 knockdown in NB4 cells +/- ATRA induction.

Cell culture and gene expression by microarray analysis were performed as described in Methods. Statistical significance was evaluated by moderated t-tests comparing knockdown and scramble control NB4 cells without ATRA induction.

Abbreviations: **ctl**, non-transduced control; **scr**, scramble control; **kd**, HOTAIRM1 knockdown cells

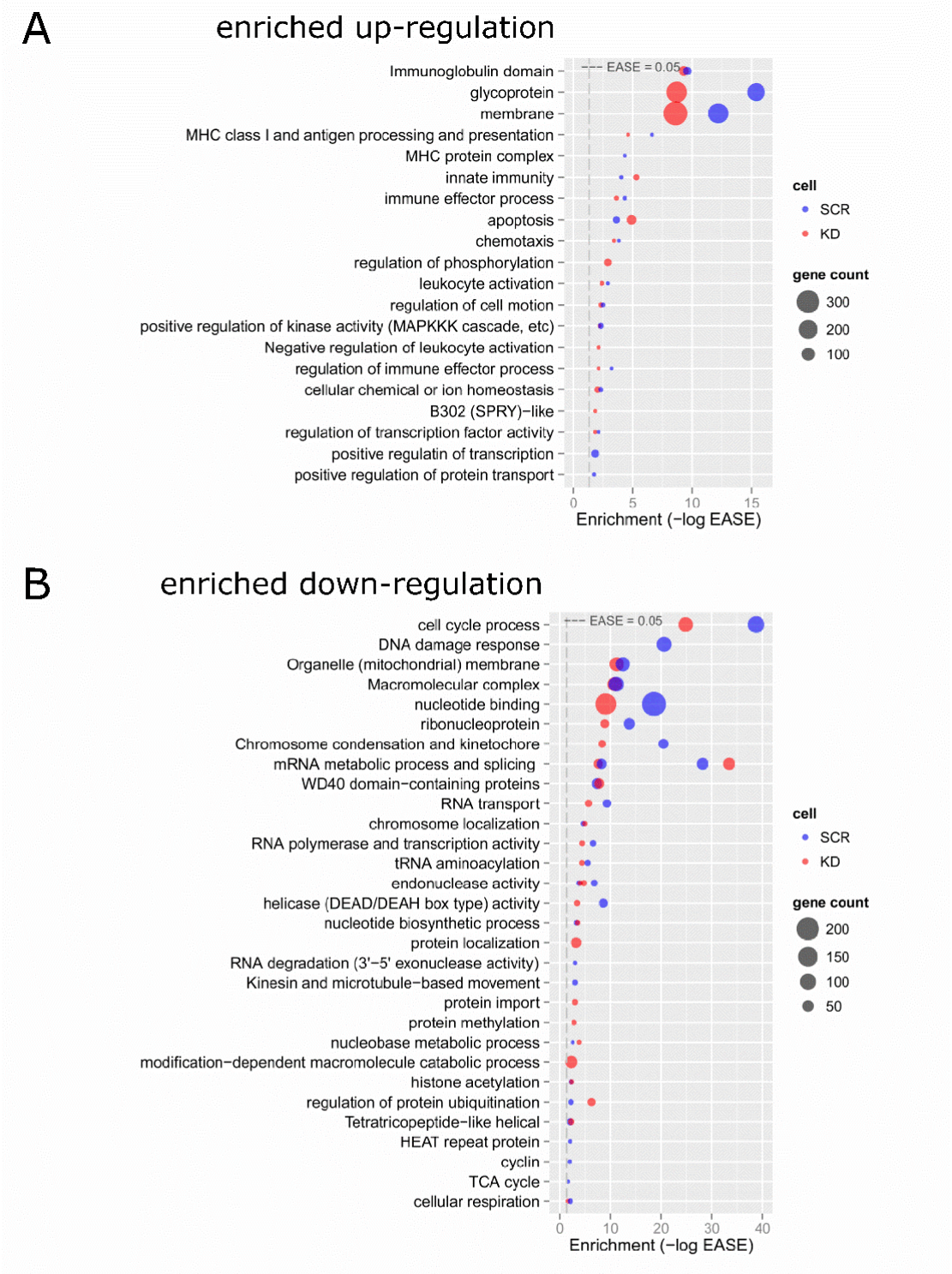

Figure S4. Functional enrichment analysis of profiles of ATRA-induced gene expression in HOTAIRM1 knockdown and scramble control NB4 cells.

The graphs present enrichment analyses of genes that significantly responded to ATRA treatment in either KD or controls or both, and are significantly different in a direct statistical comparison between separately analyzed KD and scramble controls.

A. In up-regulated genes, the impact of HOTAIRM1 knockdown showed more enrichment of apoptosis genes in the KD profiles and enrichment of the "negative regulation of leukocyte activation" group only in the KD profile

B. In down-regulated genes, HOTAIRM1 knockdown resulted in less down-regulation of cell cycle-related functional categories, notably the loss of significant enrichment of the "DNA damage response" and "cyclin" groups, and reduced enrichment of cell membrane glycoproteins, MHC class I and antigen presentation genes.

Abbreviations: **SCR**, scramble control; **KD**, HOTAIRM1 knockdown cells

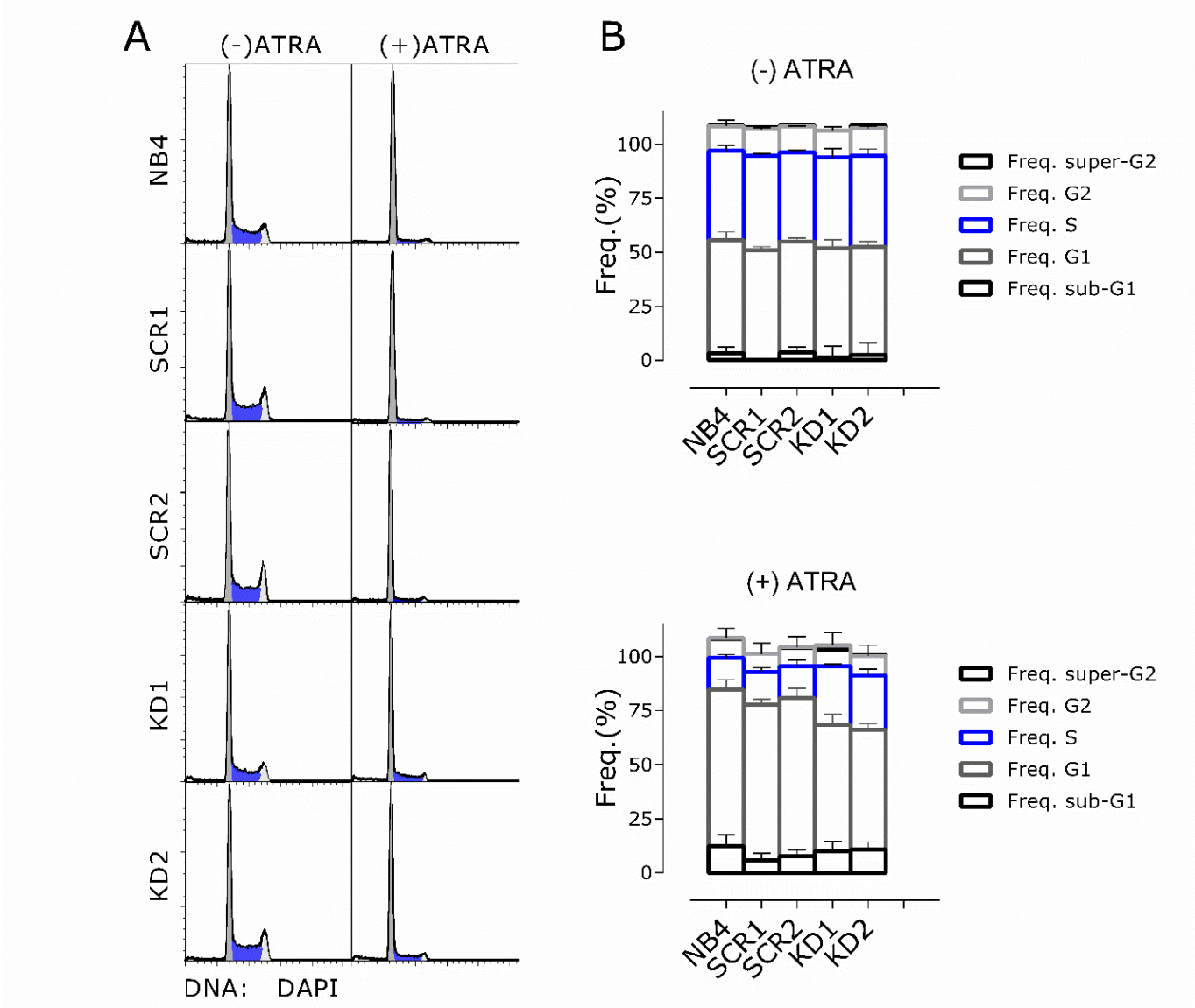

Figure S5. Preservation of S phase progression by HOTAIRM1 knockdown during ATRA-induced granulocytic differentiation of NB4 cells.

A. Representative cell cycle histograms of DNA content, measured by DAPI staining of fixed knockdown and control cells. Shaded regions represents the G1 (grey), S (blue), and G2 (light grey) phases.

B: Cell cycle phase distribution of knockdown and control NB4 cells, before ["(-)ATRA"] and 4 days after ATRA induction ["(+)ATRA"], determined as proportions of cells in cycling populations by the Flowjo cell cycle analysis module.

Plotted values represent means of at least 3 representative experiments

Abbreviations: **NB4**, non-transduced control; **scr**, scramble control clones; **KD1/2**, HOTAIRM1 knockdown clones

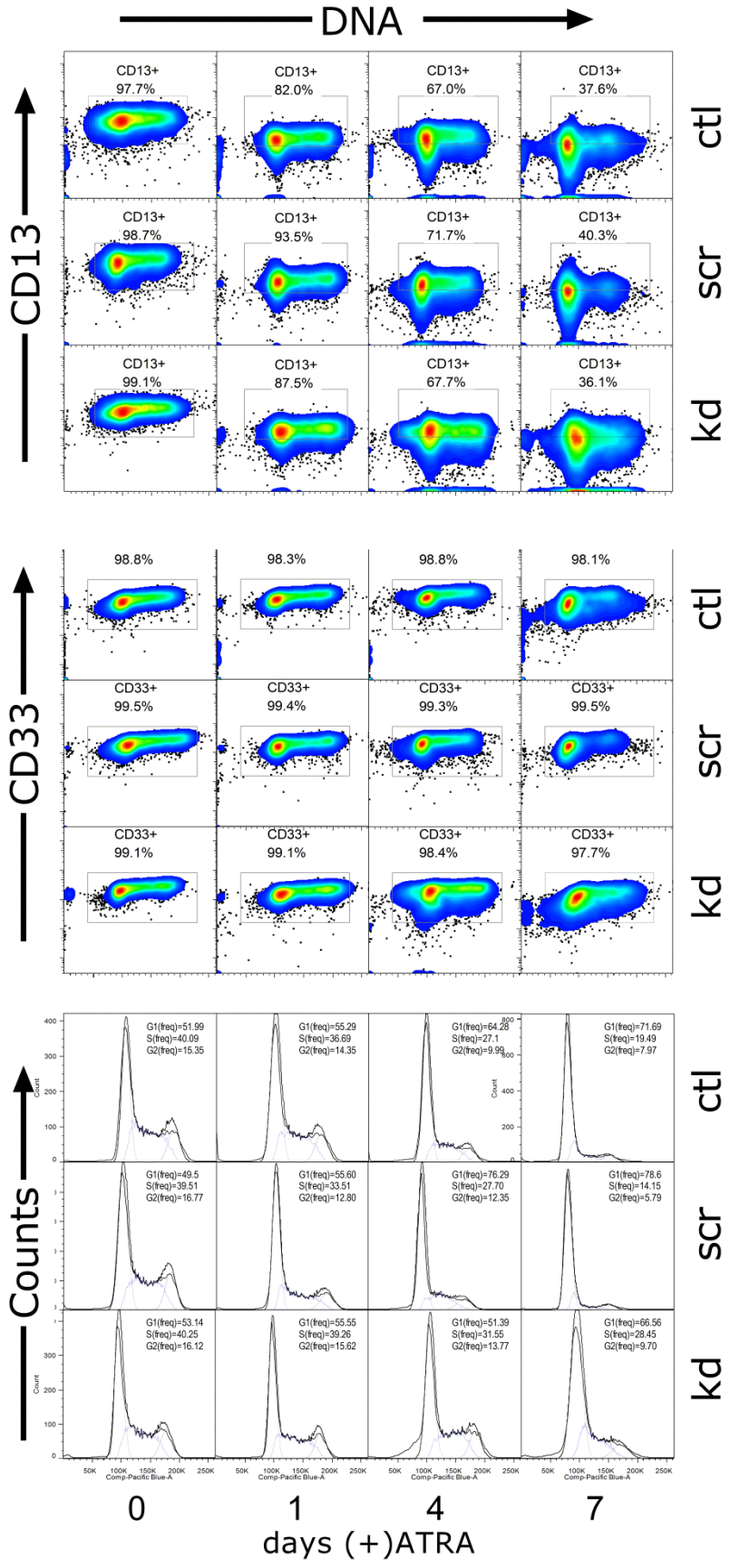

Figure S6. Multicolor flow cytometric analysis of cell surface expression of CD13 and CD33 and of cell cycle during ATRA-induced myeloid differentiation in HOTAIRM1 knockdown NB4 cells

Upper panel: HOTAIRMl knockdown did not significantly change the down-regulation of CD13, which was not associated with differential S phase progression.

Middle panel: HOTAIRMl knockdown did not significantly change cell surface CD33 expression, which was not associated with differential S phase progression.

Lower panel: Cell cycle phase distribution by simultaneous DNA staining.

Abbreviations: **ctl**, non-transduced control; **scr**, scramble control; **kd**, HOTAIRMl knockdown cells