# Supporting Information

## Jiang et al. 10.1073/pnas.1217519109

### SI Materials and Methods

**Leukemic Samples and Treatment Protocols.** All of the acute myeloid leukemia (AML) patient samples were obtained at the time of diagnosis or relapse and with informed consent at the University of Chicago Hospital or other collaborative hospitals and were approved by the institutional review board of the institutes/hospitals. All patients were treated according to the protocols of the corresponding institutes/hospitals. The samples were stored in liquid nitrogen until used. Blasts and mononuclear cells (MNCs) were purified by use of NycoPrep 1.077A (Axis-Shield) according to the manufacturer's manual. All cell lines were maintained in the laboratory.

**Normal Control Samples.** The 15 normal bone marrow (BM) controls included 6 $CD34^+$ hematopoietic stem/progenitor cell, 5 $CD33^+$ myeloid progenitor cell, and 4 MNC samples. Three $CD34^+$ samples were purchased from AllCells directly. The remaining three $CD34^+$, five $CD33^+$, and four MNC normal control cell samples were isolated from normal BM cells purchased from AllCells. MNCs were isolated using NycoPrep 1.077A (Axis-Shield) according to the manufacturer's manual. The $CD34^+$ and $CD33^+$ cell samples were sorted by flow cytometry using corresponding monoclonal antibodies (BD Biosciences). The purity of the sorted $CD34^+$ or $CD33^+$ cell samples was assessed by flow cytometry as being over 97%.

**Cell Culture and Transfection.** THP-1 and KOCL-48 cells were grown in RPMI medium 1640 (Invitrogen) containing 10% (vol/vol) FBS, 1% Hepes, and 1% penicillin-streptomycin. MONOMAC-6 cells were maintained in RPMI 1640 supplemented with 10% (vol/vol) FBS, 1% Hepes, 2 mM L-glutamine, 100× nonessential amino acid, 1 mM sodium pyruvate, 9 μg/mL insulin, and 1% penicillin-streptomycin. siRNAs (Thermo Scientific) and/or plasmids were transfected into MONOMAC-6 cells with Cell Line Nucleofector Kit V following program T-037, and the other cells (THP-1 and KOCL-48) following program U-001, using the Amaxa Nucleofector Technology (Amaxa Biosystems). Experiments were performed 48 h after transfection.

The *MLL-ENL*-ERtm cell line was kept in RPMI 1640 supplemented with IL-3, IL-6, and GM-CSF, 10 ng/mL; stem cell factor (SCF), 100 ng/mL; and 10% (vol/vol) FBS and 1% penicillin-streptomycin. 4-Hydroxytamoxifen (Sigma-Aldrich) was added at a 100-nM final concentration as a 1-mM stock solution in ethanol. Cells were collected for experiments at the indicated days after drug withdrawal.

**Cell Apoptosis, Viability, and Proliferation Assays.** For apoptosis and viability assays, 48 h after transfection, cells were collected and seeded at requested concentration. Cell apoptosis and viability were assessed using the ApoLive-Glo Multiplex Assay Kit (Promega) according to the corresponding manufacturer's manuals.

For cell proliferation assays, one million cells were electroporated with 100 μM siRNA, or 1.0 μg plasmid. Twenty-four hours after transfection, cells were seeded in 96-well plates at the concentration of 10, 000 cells per well. Cell numbers were counted for the indicated number of days.

**Target Gene Analysis.** Four major miRNA–target prediction programs/databases, including TragetScan (www.targetscan.org) (1), PITA (http://genie.weizmann.ac.il/pubs/mir07/) (2), miRanda (www.microrna.org) (3), and miRBase Targets (http://microrna.sanger.ac.uk) (4), were used for the identification of putative miRNA–target pairs.

**Plasmid Construction.** The expression vector of miR-495 was amplified by PCR using the following primers: forward 5′-AAT CTC GAG TGT CAG CCC AGC CCT-3′ and reverse 5′-ACA GAA TTC GGC CTC GCC AAC TGT-3′, and was subsequently cloned into the XhoI and EcoRI sites of the retrovirus vector MSCV-PIG (MSCV- Puro-IRES-GFP vector; bearing *GFP* gene), a kind gift from Gregory Hannon (Cold Spring Harbour Laboratory, Cold Spring Harbour, NY), Scott Hammond (University of North Carolina, Chapel Hill, NC), and Lin He (Cold Spring Harbour Laboratory, Cold Spring Harbour, NY) (5, 6). The MSCVneo-*MLL-AF9* plasmid was a provided by Scott Armstrong. The 3′ UTRs of *PBX3* and *MEIS1* containing putative binding sites for miR-495 were amplified by PCR from human normal BM MNCs using the following primers: PBX3 3′ UTR: forward 5′-AAT AAT ACT AGT TTG CTA CGT CCT CTG GG-3′ and reverse 5′-GGC AAG CTT TCC TGT GTT GAA TTA AGA CC-3′; MEIS1 3′ UTR: forward 5′-AAT AAT ACT AGT CAT CGG TCA TGT GTG TAT-3′ and reverse 5′-AAT AAT AAG CTT CAA CTG GGC TTG GCG TT-3′, and then were cloned into pMIR-REPORT Luciferase miRNA Expression Reporter Vector (Ambion). Site mutations were induced by PCR according to the sequence shown previously for the miR-495 binding site mutants of 3′ UTR of *PBX3* and *MEIS1*. All of the insertions were confirmed by DNA sequencing.

**RNA Extraction and Quantitative RT-PCR.** Total RNA was extracted with the miRNeasy extraction kit (Qiagen) and was used as template to synthesize cDNA for quantitative RT-PCR (qPCR) analysis in a 7900HT real-time PCR system (Applied Biosystems). TaqMan qPCR assay was performed to validate the differential expression patterns of miRNAs using kits from Applied Biosystems. qPCR with SYBR Green dye (Qiagen) was used to determine expression of mRNA genes. *snoRNA202*, *GAPDH*, or *Gapdh* were used as endogenous controls for qPCR of miRNA and mRNA, respectively. Each sample was run in triplicate. qPCR primers are available upon request.

**Immunoblotting.** Transiently transfected THP-1 cells were harvested and lysed with RIPA buffer (Thermo Scientific). Proteins from the lysate were fractionated by electrophoresis through 4–15% polyacrylamide gels (Bio-Rad) and transferred to polyvinylidene fluoride membranes using Tris-Glycine Transfer buffer (Thermo Scientific). Blots were incubated with IRDye 800CW-conjugated or 700CW-conjugated antibody, and infrared fluorescence images were obtained with the Odyssey infrared imaging system (Li-Cor Bioscience). Anti-PBX3, anti-MEIS1 (Abcam), and anti-ACTIN (Santa Cruz Biotechnology) antibodies were used to detect corresponding proteins.

**Exiqon microRNA Array Assay.** Our microRNA (miRNA) expression profiling assay of 85 (10 *MLL*-rearranged and 75 non*MLL*-rearranged) AML samples and 15 human normal BM samples was performed by Exiqon using the miRCURY LNA arrays (v10.0; covering 757 human miRNAs). Briefly, after passing sample quality control (QC) on the Bioanalyser2100 and RNA measurement on the Nanodrop instrument, the samples were labeled using the miRCURY Hy3/Hy5 power labeling kit and hybridized on the miRCURY LNA Array (v.10.0; containing 757 human miRNAs). Each chip/slide contained two arrays. One individual sample (0.5 μg) labeled with Hy3 and an aliquot

(0.5 μg) of the common reference pool (a mixture of individual samples, allowing normalization across a set of arrays and direct comparison of all samples) labeled with Hy5 were put into the two arrays of a given chip. The quantified signals were then normalized using the global Lowess (LOcally WEighted Scatterplot Smoothing) regression algorithm (7). When calling a particular miRNA failed on an array, its expression value was indicated by the acronym "NA." The criterion for deciding that a miRNA had failed on a particular array was that three or more of the four replicated measures of this miRNA were flagged (i.e., the signal is below background) by the image analysis software. In addition, if the SD of signals of the replicated probes of a given miRNA in a particular array (i.e., an individual sample) was greater than 0.4, this miRNA also failed on this array. The expression values are log2 (Hy3/Hy5) ratios, which were obtained on the basis of the normalized data for which replicated measurements on the same slide have been averaged. Median-centering genes for each array and median-centering each gene across all arrays were conducted then for heatmap illustration.

**Agilent Custom-Design Gene Arrays of Human Samples.** Gene expression profiling of 79 human samples (9 *MLL*-rearranged AML, 61 non–*MLL*-rearranged AML, and 9 normal controls) were analyzed by use of Agilent's custom-design microarrays (Agilent Technologies) as described previously (8). RNA quality control, cRNA amplification, hybridization, and image scan were conducted in the Functional Genomics Facility of University of Chicago. Briefly, RNA quantity and integrity was assessed using NanoDrop ND-1000 spectrophotometers and followed by analysis using an Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA (0.5 μg per sample) was reverse transcribed, amplified, and labeled with Cy3 using Agilent one-color amplification protocol (Agilent Quick-Amp Labeling Kit, One-color; One-Color Microarray-Based Gene Expression Analysis). The amplified cRNA samples were hybridized overnight and then washed according to the protocol of Agilent Oligo Microarray (One-Color Microarray-Based Gene Expression Analysis V5.7, GE 8x15K, Gene Expression Hybridization Kit). Array slides were scanned on a GenePix 4000B scanner at 570 PMT and 100% Power standard setting according to the manufacture's instruction (Molecular Devices). Array slide images were then analyzed using Agilent Feature Extraction (9.5.1.1) to obtain gene expression signals and array QC reports. The Partek Genomics Suite was used for the data normalization. Briefly, background adjustment, quantile normalization, and log transformation were used to normalize and treat gene expression intensities, and then median-centering genes for each array and median-centering each gene across all arrays were conducted for heatmap illustration. Pearson correlation was used to assess expression correlation between miR-150 and predicted target genes by use of the Partek Genomics Suite. The complete microarray data set have been deposited in the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/, accession no. GSE30258).

**Affymetrix Gene Arrays of Mouse Samples.** As described previously (8), a total of 15 mouse BM samples, including 6 primary (including 3 each of negative control and *MLL-AF9*) and 9 secondary (including 3 negative control and 6 *MLL-AF9*) obtained from the in vivo mouse BM reconstitution assays were analyzed by use of Affymetrix GeneChip Mouse Gene 1.0 ST Array. Each array contains 28,853 mouse genes being represented on the array by ~27 probes spread across the full length of a given gene, providing a more complete and more accurate picture of gene expression than 3′-based expression array designs. The RNA quality control, cDNA amplification, hybridization, and image scan were conducted in the Functional Genomics Facility of University of Chicago. After hybridization and background correction according to the standard protocols, the quantified signals were then normalized using Robust Multiarray Average, which is a robust algorithm of background adjustment, quantile normalization, and log transformation (9). Then median-centering genes for each array and median-centering each gene across all arrays were also conducted for heatmap illustration. The complete-microarray data set is already available at the GEO database, and the accession number is GSE34185.

**Luciferase Reporter and Mutagenesis Assays.** Luciferase reporter and mutagenesis assays were conducted as described previously, with some modifications (10, 11). Briefly, for transfection, HEK293T cells were plated in 96-well plates at a concentration of 6,000 cells per well in triplicate for each condition. After overnight incubation, cells were transfected with 20 ng of the pMIR-REPORT bearing the *PBX3* or *MEIS1* 3′ UTR or the 3′ UTR with miR-495 binding site mutations, and 20 ng of MSCV-miR-495 or an empty MSCV vector using Effectene Transfection Reagent (Qiagen) according to the manufacturer's protocol. pMIR-REPORT Beta-galactosidase Reporter Control Vector (Ambion) (1 ng) was cotransfected for transfection efficiency control in all transfections. Cells were lysed, and firefly luciferase and β-galactosidase activities were detected using the Dual-Light Combined Reporter Gene Assay System (Applied Biosystems) 48 h after transfection. Firefly luciferase activity was normalized to β-galactosidase activity for each transfected well. Each experiment was performed in triplicate and repeated three times.

**Packaging of Recombinant Retroviruses, Transduction of Cells, and in Vitro Colony-Forming/Replating Assays.** Those experiments were conducted as described previously (10, 12), with some modifications. Briefly, retrovirus vectors were cotransfected with pCL-Eco packaging vector (IMGENEX) into 293T cells using Effectene Transfection Reagent (Qiagen) to produce the retroviruses. BM cells were harvested from a cohort of 4- to 6-wk-old B6.SJL (CD45.1) donor mice after 5 d of 5-fluorouracil treatment, and primitive hematopoietic progenitor cells were enriched with the Mouse Lineage Cell Depletion Kit (Miltenyi Biotec). An aliquot of enriched hematopoietic progenitor cells was added to retroviral supernatant together with polybrene in a plate, which were centrifuged at 2,000 × *g* for 2 h at 32 °C [i.e., "spinoculation" (8, 10, 13)], and then the media was replaced with fresh media and incubated for 20 h at 37 °C. Next day, the same procedure was repeated once.

Then, on the day after the second spinoculation, an equivalent of $2.0 \times 10^4$ cells were plated onto a 35-mm Petri dish in 1.1 mL of Methocult M3230 methylcellulose medium (Stem Cell Technologies) containing 10 ng/mL each of murine recombinant IL-3, IL-6, and GM-CSF, and 30 ng/mL of murine recombinant SCF (R&D Systems), along with 1.0 mg/mL of G418 and/or 2 μg/mL of puromycin. For each transduction, there were two duplicate dishes. Cultures were incubated at 37 °C in a humidified atmosphere of 5% $CO_2$ in air. The colonies were replated every 7 d under the same conditions. The colony-forming/replating assays were repeated three times.

**BM Transplantation Assays.** C57BL/6 (CD45.2) and B6.SJL (CD45.1) were purchased from Jackson Laboratories or Harlan Laboratories. Both male and female mice were used for the experiments. All laboratory mice were maintained in the animal facility at the University of Chicago. All experiments on mice in our research protocol were approved by Institutional Animal Care and Use Committee of the University of Chicago.

Normal BM cells of B6.SJL (CD45.1) mice were retrovirally transduced with MSCVneo + MSCV-PIG (as control), MSCVneo-*MLL-AF9* + MSCV-PIG (i.e., MLL-AF9), and MSCVneo-*MLL-AF9* + MSCV-PIG-miR-495 (i.e., MLL-AF9+miR-495), respectively, through two rounds of "spinoculation." Then, retrovirally transduced donor cells were injected by tail vein into lethally irradiated (960 rads) 8- to 10-wk-old C57BL/6 (CD45.2) recipient mice

with $3 \times 10^5$ donor cells plus a radioprotective dose of whole BM cells ($1 \times 10^6$; freshly harvested from a C57BL/6 mouse) per recipient mouse.

**Statistical Software.** The miRNA and gene/exon array data analyses were conducted by use of the Partek Genomics Suite, TIGR Mutiple Array Viewer software package (TMeV version 4.6; TIGR) (14), and/or Bioconductor R packages. The miRNA–gene expression correlation was analyzed by use of the Partek Genomics Suite. The heatmaps were constructed by use of TIGR Mutiple Array Viewer software package. The *t* test, Kaplan-Meier method, and log–rank test, etc. were performed with WinSTAT (R. Fitch Software) and/or the Partek Genomics Suite. Gene Ontology analysis was done with the R package (15).


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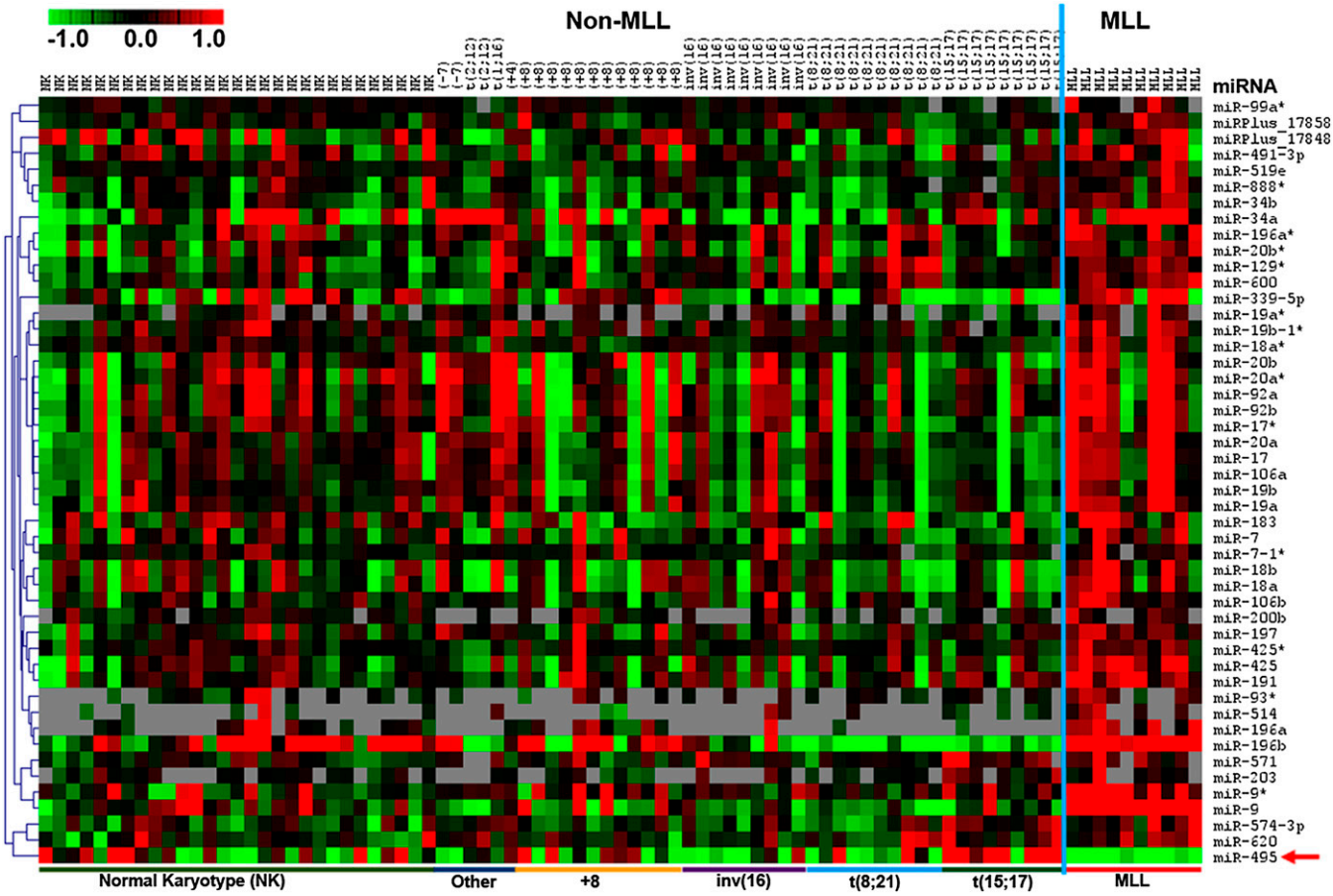


**Fig. S1.** Expression profiles of the 48 miRNAs that are significantly differentially expressed [$q < 0.05$, false discovery rate (FDR) $< 0.05$] between *MLL*-rearranged ($n = 10$) and non–*MLL*-rearranged ($n = 75$) AML groups. Expression data were mean centered, and the relative value for each sample is represented by a color, with red representing a high expression and green representing a low expression (scale shown at upper left).

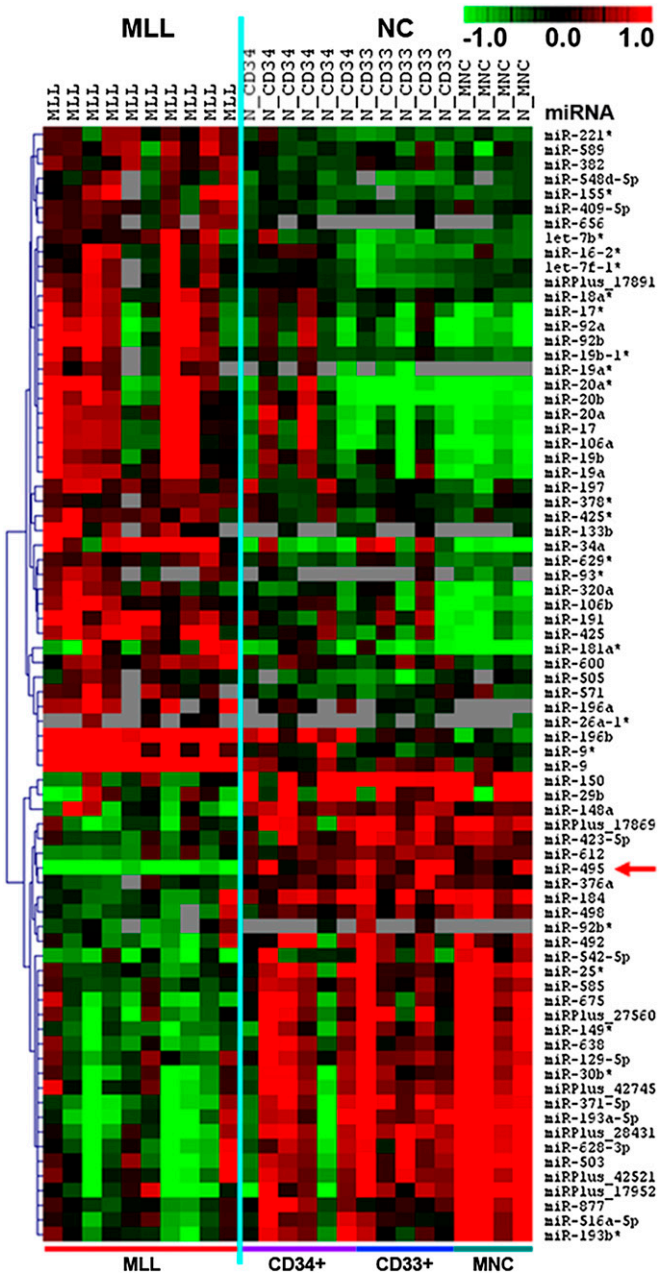


**Fig. S2.** Expression profiles of the 76 miRNAs that are significantly differentially expressed ($q < 0.05$, FDR < 0.05) between *MLL*-rearranged AML ($n = 10$) and normal controls ($n = 15$). Expression data were mean centered, and the relative value for each sample is represented by a color, with red representing a high expression and green representing a low expression (scale shown at upper right).

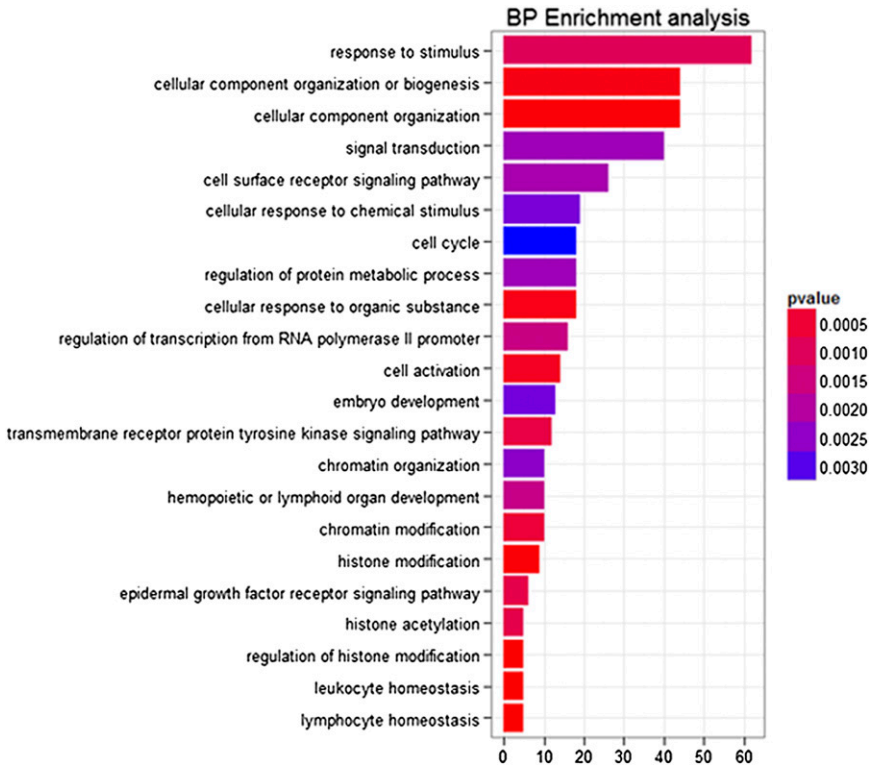


**Fig. S3.** Gene Ontology analysis of the 128 candidate target genes of miR-495. The number of genes among the 128 candidate targets enriched in each Biological Process (BP) category is shown. The *P* value scale is shown as a bar on the middle right.

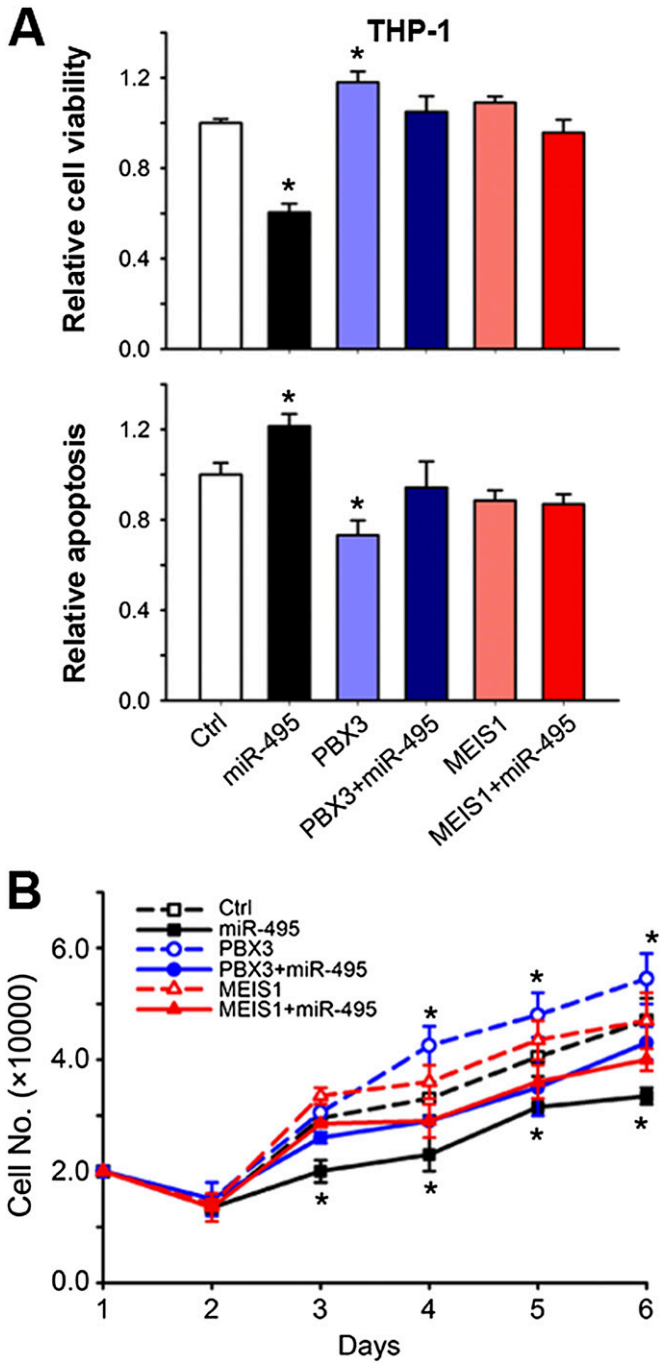


**Fig. S4.** Both *PBX3* and *MEIS1* are functionally important target genes of miR-495 in THP-1 cells. The coding regions (CDS) of *PBX3* and *MEIS1* were cloned into MSCVneo, and thus their ectopic expression would not be repressed by endogenous or cotransfected miR-495. $*P < 0.05$; $**P < 0.01$, two-tailed *t* test. The cells transfected with MSCV-PIG+MSCVneo (Ctrl) were used as controls. (*A*) Analysis of the effects of forced expression of miR-495 (MSCV-PIG-miR-495+MSCVneo), *PBX3* (MSCV-PIG+MSCVneo-*PBX3*), *PBX3*+miR-495 (MSCV-PIG-miR-495+MSCVneo-*PBX3*), *MEIS1* (MSCV-PIG+MSCVneo-*MEIS1*), and *MEIS1*+miR-495 (MSCV-PIG-miR-495+MSCVneo-*MEIS1*), respectively, on cell viability (*Upper*) and apoptosis (*Lower*) of THP-1 cells. Cell viability and apoptosis were detected 48 h after transfection. (*B*) Analysis of their effects on cell growth/proliferation of THP-1 cells. Cell numbers were counted every day after transfection for 6 d.

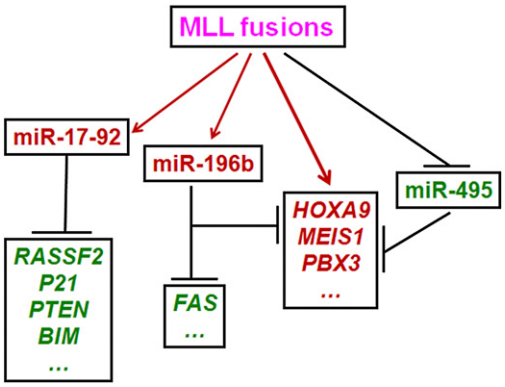


**Fig. S5.** Model to illustrate the miRNAs deregulated by MLL fusions and the genes targeted by those miRNAs. The miRNAs and genes shown in red are oncogenic miRNAs or genes, whereas those shown in green are tumor-suppressor ones. The arrows in red represent positive regulation, whereas the lines in black represent negative regulation.

**Table S1. List of the 128 candidate target genes of miR-495 that exhibited a significantly inverse correlation of expression with miR-495 in 79 human samples (including 70 AML and 9 normal controls)**

| Gene | Correlation coefficient (*r*) | *P* value |
|---|---|---|
| *miR-495* | | |
| *ABHD2* | −0.26765 | 0.017094 |
| *ACTR1A* | −0.24607 | 0.028816 |
| *AHR* | −0.23622 | 0.036092 |
| *AKAP7* | −0.51152 | 1.45E-06 |
| *ALDH3A2* | −0.40285 | 0.000233 |
| *ALKBH5* | −0.30947 | 0.005515 |
| *ANGEL1* | −0.29698 | 0.007866 |
| *ANXA11* | −0.23755 | 0.035027 |
| *AP1S2* | −0.30901 | 0.005589 |
| *APBA2* | −0.23906 | 0.033854 |
| *APLP2* | −0.26956 | 0.016289 |
| *ARRB1* | −0.32746 | 0.003221 |
| *ATP6V1C1* | −0.22219 | 0.049064 |
| *ATPAF1* | −0.27972 | 0.012539 |
| *BAK1* | −0.26694 | 0.017402 |
| *BCL2L11* | −0.24781 | 0.027672 |
| *BMI1* | −0.46538 | 1.55E-05 |
| *C10ORF140* | −0.33238 | 0.002765 |
| *C5ORF23* | −0.26311 | 0.019142 |
| *CAB39L* | −0.2973 | 0.007797 |
| *CCDC6* | −0.38472 | 0.000464 |
| *CDC73* | −0.26336 | 0.019024 |
| *CFL2* | −0.26472 | 0.018392 |
| *CIAPIN1* | −0.24401 | 0.030225 |
| *CLTA* | −0.23047 | 0.041009 |
| *CNOT7* | −0.27898 | 0.012784 |
| *COTL1* | −0.35564 | 0.001298 |
| *CRADD* | −0.27054 | 0.015891 |
| *CYB5R4* | −0.31509 | 0.004678 |
| *DACH1* | −0.2476 | 0.027806 |
| *DAZAP2* | −0.25771 | 0.021854 |
| *DDEF1* | −0.2549 | 0.023388 |
| *DDX19A* | −0.31718 | 0.004398 |
| *DUSP4* | −0.25966 | 0.020838 |
| *EHD3* | −0.22495 | 0.046247 |
| *EID1* | −0.35876 | 0.001167 |
| *EIF2C4* | −0.22565 | 0.045556 |
| *ENPP4* | −0.23504 | 0.037061 |
| *EPB41L2* | −0.46987 | 1.25E-05 |

**Table S1. Cont.**

| Gene | Correlation coefficient (*r*) | *P* value |
|---|---|---|
| *EPN2* | −0.28371 | 0.011284 |
| *EREG* | −0.268 | 0.016944 |
| *EVI1* | −0.25219 | 0.024949 |
| *FLNB* | −0.27969 | 0.012547 |
| *FOXP1* | −0.23032 | 0.041145 |
| *G3BP2* | −0.25485 | 0.023415 |
| *GLTP* | −0.24343 | 0.030633 |
| *GNA12* | −0.25679 | 0.022346 |
| *GNAI1* | −0.27485 | 0.014232 |
| *GNPDA1* | −0.2576 | 0.02191 |
| *GPR126* | −0.2598 | 0.020768 |
| *GPR137B* | −0.22211 | 0.049144 |
| *HDAC9* | −0.26425 | 0.01861 |
| *HMGA1* | −0.27719 | 0.013396 |
| *HMGCS1* | −0.24637 | 0.028617 |
| *HOMER2* | −0.22659 | 0.044635 |
| *HOXA10* | −0.40966 | 0.000178 |
| *HSP90AB1* | −0.24709 | 0.028137 |
| *IDH3A* | −0.30913 | 0.00557 |
| *IGF2BP2* | −0.32196 | 0.00381 |
| *IVNS1ABP* | −0.30768 | 0.005808 |
| *KCTD12* | −0.28054 | 0.012271 |
| *KLHL5* | −0.30584 | 0.006124 |
| *KPNB1* | −0.23394 | 0.037985 |
| *LETMD1* | −0.23781 | 0.034826 |
| *LIPA* | −0.50012 | 2.69E-06 |
| *LY75* | −0.3245 | 0.003527 |
| *MEF2C* | −0.46323 | 1.72E-05 |
| *MEIS1* | −0.46814 | 1.36E-05 |
| *MMRN1* | −0.34965 | 0.001585 |
| *MN1* | −0.23757 | 0.035011 |
| *MR1* | −0.29895 | 0.007444 |
| *MSI1* | −0.26646 | 0.017611 |
| *MTMR12* | −0.44545 | 3.89E-05 |
| *MTPN* | −0.27378 | 0.014628 |
| *MYO6* | −0.27701 | 0.013458 |
| *NLK* | −0.23245 | 0.039262 |
| *NMD3* | −0.24437 | 0.029977 |
| *NR5A2* | −0.22677 | 0.044462 |
| *NUFIP2* | −0.22673 | 0.044499 |
| *NUP210* | −0.24378 | 0.030385 |
| *NUP93* | −0.33318 | 0.002696 |
| *ODC1* | −0.28595 | 0.010628 |
| *PBX3* | −0.24042 | 0.032822 |
| *PECAM1* | −0.22558 | 0.045619 |
| *PGPEP1* | −0.35048 | 0.001542 |
| *PLCB2* | −0.37436 | 0.000677 |
| *PPM1M* | −0.30533 | 0.006214 |
| *PRKAR2B* | −0.24055 | 0.032722 |
| *PTDSS1* | −0.34673 | 0.001746 |
| *PTEN* | −0.27933 | 0.012668 |
| *PTER* | −0.22694 | 0.044298 |
| *PTGER2* | −0.32108 | 0.003912 |
| *PTP4A3* | −0.28292 | 0.011524 |
| *RALBP1* | −0.22178 | 0.049495 |
| *RBPMS* | −0.23519 | 0.036939 |
| *REEP3* | −0.26478 | 0.018365 |
| *REPS2* | −0.27363 | 0.014686 |
| *RNF145* | −0.45187 | 2.91E-05 |
| *RNF2* | −0.26651 | 0.017588 |
| *RPL6* | −0.22241 | 0.048834 |
| *RPS6* | −0.22378 | 0.047422 |

**Table S1. Cont.**

| Gene | Correlation coefficient (*r*) | *P* value |
|---|---|---|
| *SENP6* | −0.25638 | 0.022568 |
| *SERBP1* | −0.26525 | 0.018153 |
| *SET* | −0.26115 | 0.020089 |
| *SF3A1* | −0.23716 | 0.035337 |
| *SLC38A1* | −0.3019 | 0.006851 |
| *SLC4A7* | −0.2738 | 0.014622 |
| *SMCR7L* | −0.37904 | 0.000572 |
| *SNRPB2* | −0.44207 | 4.53E-05 |
| *SPG3A* | −0.28195 | 0.011822 |
| *SPRYD4* | −0.22294 | 0.048287 |
| *ST7* | −0.25162 | 0.025294 |
| *STARD7* | −0.27449 | 0.014364 |
| *SYNJ2BP* | −0.37574 | 0.000644 |
| *SYT17* | −0.28839 | 0.009955 |
| *TAF7* | −0.36384 | 0.00098 |
| *TAPBP* | −0.30783 | 0.005783 |
| *TMEM97* | −0.35269 | 0.001433 |
| *TNFRSF1B* | −0.25316 | 0.02438 |
| *TP53* | −0.26541 | 0.01808 |
| *TPP1* | −0.23088 | 0.040641 |
| *TRPS1* | −0.36027 | 0.001109 |
| *TXNDC1* | −0.23763 | 0.034967 |
| *UBE2Z* | −0.22407 | 0.047128 |
| *VCL* | −0.3222 | 0.003783 |
| *VDAC2* | −0.36292 | 0.001012 |
| *WDR68* | −0.30636 | 0.006033 |
| *WTAP* | −0.23662 | 0.035771 |

**Table S2. Enrichment of the 128 candidate target genes of miR-495 in gene sets in MSigDB**

| Gene set name | Description | No. genes in overlap | *P* value |
|---|---|---|---|
| Enriched in genes up-regulated in leukemic or normal hematopoietic stem cells | | | |
| DIAZ_CHRONIC_MEYLOGENOUS_LEU.K.EMIA_UP | Genes up-regulated in CD34+ [Gene ID = 947] cells isolated from BM of CML (chronic myelogenous leukemia) patients, compared with those from normal donors. | 24 (*ACTR1A, AHR, APBA2, ARRB1, BMI1, CCDC6, CNOT7, CRADD, EID1, G3BP2, HMGCS1, HOXA10, HSP90AB1, MEIS1, NLK, NMD3, ODC1, PBX3, PRKAR2B, RALBP1, SF3A1, SNRPB2, TAF7, VCL*) | 2.57E-09 |
| TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_3D_UP | Genes up-regulated in CD34+ [Gene ID = 947] hematopoetic cells by expression of NUP98-HOXA9 fusion [Gene ID = 4928, 3205] off a retroviral vector at 3 d after transduction. | 10 (*AHR, BCL2L11, DUSP4, EREG, FLNB, GNAI1, HOMER2, MEIS1, PRKAR2B, SLC38A1*) | 3.33E-09 |
| BYSTRYKH_HEMATOPOIESIS_STEM_CELL_QTL_TRANS | Transcripts in hematopoietic stem cells (HSC) which are transregulated (i.e., modulated by a QTL (quantitative trait locus) not in a close proximity to the gene). | 17 (*FLNB, EREG, CRADD, BMI1, NLK, VCL, CCDC6, ST7, RBPMS, SET, ALDH3A2, MYO6, NR5A2, GLTP, TPP1, SYNJ2BP, UBE2Z*) | 6.37E-07 |
| Enriched in genes up-regulated in *MLL*-associated leukemia | | | |
| MULLIGHAN_MLL_SIGNATURE_1_UP | The 'MLL signature 1': genes up-regulated in pediatric AML (acute myeloid leukemia) with rearranged MLL [Gene ID = 4297] compared with all AML cases with the intact gene. | 11 (*ABHD2, ALDH3A2, DACH1, GNA12, HOXA10, MEIS1, MR1, NUP210, PBX3, PECAM1, TPP1*) | 6.08E-07 |
| MULLIGHAN_MLL_SIGNATURE_2_UP | The 'MLL signature 2': genes up-regulated in pediatric AML (acute myeloid leukemia) with rearranged MLL [Gene ID = 4297] compared with the AML cases with intact MLL and NPM1 [Gene ID = 4869]. | 13 (*ABHD2, ALDH3A2, AP1S2, BMI1, DACH1, GNA12, HOXA10, MEIS1, MR1, NUP210, PBX3, PECAM1, TPP1*) | 2.41E-08 |
| YAGI_AML_WITH_11Q23_REARRANGED | Genes specifically expressed in samples from patients with pediatric acute myeloid leukemia (AML) bearing 11q23 rearrangements. | 17 (*ALDH3A2, ANGEL1, AP1S2, BMI1, CLTA, CRADD, DACH1, ENPP4, GNAI1, HDAC9, IDH3A, MEF2C, MEIS1, MYO6, PBX3, PTP4A3, RBPMS*) | 1.21E-13 |
| YAGI_AML_WITH_T_9_11_TRANSLOCATION | Genes specifically expressed in samples from patients with pediatric acute myeloid leukemia (AML) bearing *t*(9;11) translocation. | 9 (*BMI1, DACH1, FLNB, IDH3A, MEF2C, MEIS1, MR1, MYO6, PBX3*) | 4.84E-09 |
| ROSS_LEU.K.EMIA_WITH_MLL_FUSIONS | Top 100 probe sets associated with MLL fusions [Gene ID = 4297] irrespective of the lineage of the pediatric acute leukemia. | 8 (*ALDH3A2, BMI1, DACH1, HOXA10, MEIS1, MYO6, PBX3, SENP6*) | 1.35E-09 |
| Enriched in potential targets of MAZ, LEF1 and SP1 | | | |
| GGGAGGRR_V$MAZ_Q6 | Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif GGGAGGRR which matches annotation for MAZ: MYC-associated zinc finger protein (purine-binding transcription factor) | 28 (*ACTR1A, AP1S2, CCDC6, DACH1, DUSP4, EPN2, G3BP2, HMGA1, HOMER2, HOXA10, IVNS1ABP, KPNB1, MEIS1, MSI1, NLK, PBX3, PLCB2, PRKAR2B, PTGER2, RBPMS, REPS2, RNF2, SET, SLC38A1, ST7, TP53, TRPS1, VCL*) | 3.98E-10 |
| CTTTGT_V$LEF1_Q2 | Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif CTTTGT which matches annotation for LEF1: lymphoid enhancer-binding factor 1 | 32 (*ABHD2, ACTR1A, AKAP7, BCL2L11, BMI1, CAB39L, CFL2, CNOT7, CRADD, DACH1, DUSP4, FOXP1, HMGA1, HMGCS1, HOXA10, IVNS1ABP, KCTD12, KLHL5, KPNB1, MEF2C, MEIS1, MN1, NLK, ODC1, PBX3, PGPEP1, PRKAR2B, RBPMS, SET, SLC38A1, ST7, TP53*) | 7.99E-15 |
| GGGCGGR_V$SP1_Q6 | Genes with promoter regions [-2kb,2kb] around transcription start site containing the motif GGGCGGR which matches annotation for SP1: Sp1 transcription factor | 32 (*ABHD2, AHR, AP1S2, APLP2, CCDC6, CFL2, CNOT7, DACH1, DAZAP2, EPN2, G3BP2, GNA12, HMGA1, HMGCS1, IDH3A, IVNS1ABP, KPNB1, MEF2C, MEIS1, MN1, NLK, PTER, REPS2, RNF2, SET, SF3A1, SLC38A1, STARD7, TP53, TRPS1, VCL, VDAC2*) | 1.74E-10 |