Supplemental information (Methods, Tables and Figures)

Supplemental Methods

Cell lines

HeLa, 293T, NB4 and U937 cells were obtained from American Type Culture Collection (ATCC) or DSMZ. HeLa, NB4 and U937 were maintained in RPMI-1640 medium, whereas 293T was maintained in Dulbecco's Modified Eagle's Medium (DMEM), both supplemented with 10% fetal bovine serum.

Fusion transcript identification

The Illumina TruSight RNA Pan-Cancer Panel (targeting 1,385 cancer genes) was used to identify the *NUP98-JADE2* fusion in the patient. Libraries were sized on QIAxcel Advanced using the QIAxcel DNA screening kit (Qiagen) and quantified by real-time PCR. Sequencing was performed using the MiSeq Reagent Kit v3 at 76 bp paired-end on an Illumina MiSeq system. Data were analyzed by the RNA fusion analysis module (version 1.0.0.351) on Local Run Manager v2.0 (Illumina).

Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative RT-PCR

Total RNA was reverse transcribed into cDNA using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific). *NUP98-JADE2* fusion transcript was amplified with primers NUP98-F: 5'-GTTCTCCAGCAGCACATCAA-3' and JADE2-R: 5'-GGCTTCTTTTCGTTCTGTCG-3' (287-bp product). The reciprocal fusion was detected with primers JADE2-F: 5'-GGAGAGAGATTGCGCATGTT-3' and NUP98-R: 5'-CCAGCCCATCAAAGAGATGT-3' (237-bp product). *GAPDH* was amplified with primers GAPDH-F: 5'-CCACCCATGGCAAATTCCATGGCA-3' and GAPDH-R: 5'-ATCTAGACGGCAGGTCAGGTCCACC-3' (598-bp product). The GeneAmp Fast PCR Master Mix (Thermo Fisher Scientific) was used for the amplification.

Analysis of *HOXA* and *CDK6* mRNA expression was performed using the TB Green Premix Ex Taq II (Takara Bio) with the following primers: HOXA5-F: 5'-CAGATCTACCCCTGGATGCG-3' and HOXA5-R: 5'-TTCAATCCTCCTTCTGCGGG-3'; HOXA6-F: 5'-CTTGGATGCAGCGGATGAAC-3' and HOXA6-R: 5'-TGTCAGGTAGCGGTTGAAGT-3'; HOXA9-F: 5'-GCGCCTTCTCTGAAAACAAT-3' and HOXA9-R: 5'-CAGTTCCAGGGTCTGGTGTT-3'; HOXA10-F: 5'-CCTTCCGAGAGCAGCAAAG-3' and HOXA10-R: 5'-AAACTCCTTCTCCAGCTCCA-3'; CDK6-F: 5'-GCCTTGCCCGCATCTATAGT-3' and CDK6-R: 5'-TATGCAGCCAACACTCCAGA-3'. Each sample was measured in triplicate and expression levels were determined by 2^-ΔΔCt. *GAPDH* was used for normalization.

Targeted next-generation sequencing

Amplicon sequencing using paired diagnostic and remission bone marrow samples from the patient was performed to identify somatic mutations. Libraries were prepared from genomic DNA using the unique molecular identifier (UMI)-based QIAseq Targeted Human Myeloid Neoplasms Panel (Qiagen) and sequenced on an Illumina NextSeq 500 system. The panel covers the complete coding region of 141 myeloid-related genes (Table S1). The read processing, alignment (hg19 as the reference), calling and annotation of single nucleotide variants/small indels were performed with the UMI-based caller smCounter2 run on the GeneGlobe (Qiagen).[1] Variant filtering was performed based on the method previously described by the German AML Cooperative Group.[2] As our routine testing on a commercial myeloid DNA reference standard (Horizon) containing 22 variants in 19 genes with this method consistently identified variants with a variant allele frequency (VAF) of 5%, this was chosen as a cut-off for variant filtering. Variants with a population frequency of ≥0.1% in the 1000 Genomes Project (Phase 3), Genome Aggregation Database (v2.1.1) or dbSNP (Build 154) were excluded from the analysis.

Targeted copy number variation (CNV) analysis

The CNVkit[3]and quandico[4] tools were used to identify CNVs in the 141 genes from the amplicon sequencing data. Twelve DNA samples from healthy individuals (6 males and 6 females) were used as controls to build the reference data for comparison. CNVkit analysis (v0.9.6) was run with DNAnexus using the amplicon sequencing setting, whereas quandico was run with the GeneGlobe (Qiagen). Genomic regions with a $\log_2$ ratio of <-0.3 or >0.3 detected by CNVkit (these ratios are roughly equivalent to single-copy loss and single-copy gain in diploid leukemic cells accounting for ~40-50% of total cell population in the samples) and a Q score of ≥50 in quandico analysis were considered CNVs.

Plasmid constructs

Full-length cDNAs of *NUP98-JADE2* and *JADE2* were cloned into the pCMV-Myc (Takara Bio), pCMV-HA (Takara Bio) and LEGO-iG2[5] expression vectors using the In-Fusion HD Cloning Kit (Takara Bio). For pCMV-Myc and pCMV-HA, the C-terminal Tag vectors were used. The identity of the cDNAs was confirmed by direct sequencing and restriction analysis.

Immunofluorescence studies

HeLa cells were transfected with pCMV-HA and/or pCMV-Myc vectors as indicated using Lipofectamine 2000 (Thermo Fisher Scientific). After 48 hours of transfection, cells were fixed with

3.7% formaldehyde and permeabilized with 0.5% Triton X-100. Samples were then blocked with 5% bovine serum albumin and incubated with a rabbit anti-HA (C29F4) or a mouse anti-Myc (9B11) monoclonal antibody (Cell Signaling Technology) at room temperature for 1 hour. After successive washing, samples were incubated with an Alexa Fluor 488- (A21206) or Alexa Fluor 594-conjugated (A11005) secondary antibody (Thermo Fisher Scientific) for another 1 hour. Cells were counterstained with DAPI and examined by the Zeiss Imager M1 fluorescence microscope.

Co-immunoprecipitation (Co-IP) studies

Co-IP assays were performed with the Pierce MS-Compatible Magnetic IP Kit (Thermo Fisher Scientific) following the manufacturer's protocol. Briefly, after 48 hours of transfection, cell lysates were prepared and incubated with an anti-Myc monoclonal antibody (9E10, Thermo Fisher Scientific) at 4°C overnight. After repeated washing, samples were eluted and analyzed by immunoblotting with anti-Myc (9B11), anti-HA (C29F4) and anti-GAPDH (Ab9485, Abcam) antibodies. Twenty micrograms of cell lysates were analyzed in the input.

Luciferase reporter assays

The Cignal RARE Reporter Assay Kit (Qiagen) was used to study the transcriptional changes in response to *JADE2* perturbation. The RARE reporter contains a mixture of RARE-responsive firefly luciferase construct (tandem repeats of the DR5 retinoic acid-response element) and constitutively expressing *Renilla* luciferase construct for normalization. Briefly, cells were transiently co-transfected with the RARE reporter and expression plasmids/small interfering RNAs as indicated by Lipofectamine 2000. Luciferase activities were measured after 48 hours of the transfection using the Dual-Glo Luciferase Assay System (Promega). To study the effect of ATRA, cells were treated with 1μM of ATRA or DMSO (as vehicle control) for 6 hours before luciferase measurement. Firefly luciferase activity in cell lysates was normalized to *Renilla* luciferase activity for calculations. In all studies, parallel experiments using the negative control reporter were performed to adjust for any non-specific effects.

Lentiviral packaging and transduction

The Lenti-X Packaging System (Takara Bio) was used to prepare NUP98-JADE2 and control lentiviruses with the LEGO-iG2 constructs (co-expressed green fluorescent protein (GFP)) according to the manufacturer's protocol. U937 and NB4 cells were transduced in 6-well plates coated with 45μg/ml of RetroNectin (Takara Bio) with a multiplicity of infection (MOI) of 20 and 40, respectively. Ninety-

six hours post transduction, cells were treated with ATRA or DMSO as indicated and CD11b expression on GFP-positive cells was measured by a BD FACSCalibur flow cytometry.

RNA interference

5×10^6 NB4 cells were transiently transfected with 500nM siRNA by electroporation using the Bio-Rad Gene Pulser and 0.4cm cuvettes (300V and 950μF). The *JADE2* siRNAs (siRNA#1:ID_s23596, siRNA#2:ID_s23597) and negative control siRNA were obtained from Ambion. The ON-TARGETplus Smartpool *JADE2* siRNAs (Dharmcon) were used to independently validate *JADE2* target genes identified by RNA-seq and study the effects of *JADE2* knockdown on RARE-mediated transcriptional activity.

Whole transcriptome sequencing

Whole transcriptome sequencing was performed to identify gene expression changes upon *JADE2* knockdown. Total RNA was extracted from NB4 cells transfected with negative control siRNA, *JADE2* siRNA#1 and *JADE2* siRNA#2 for 24 hours. Messenger RNA was purified from the total RNA and libraries were prepared using the NEBNext RNA Library preparation kit for Illumina sequencing. After removal of low-quality reads, paired-end clean reads were aligned to the reference genome (hg19) using STAR (v2.5). HTSeq (v0.6.1) was then used to count the read numbers mapped of each gene. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) of each gene was calculated based on the length of the gene and reads count mapped to this gene. All the library preparation and data analysis steps were carried out by Novogene.

Validation of selected *JADE2* target genes was done by quantitative RT-PCR using TaqMan Gene Expression Assays (*CEBPE*: Hs00357657_m1; *BTG2*: Hs00198887_m1; *FOS*: Hs99999140_m1; *IL1B*: Hs01555410_m1; *S100A9*: Hs00610058_m1; *NCF2*: Hs01084940_m1; *TOMM20*: Hs03276810_g1) (Thermo Fisher Scientific). *GAPDH* was used for normalization.

References

1. Xu C, Gu X, Padmanabhan R, et al. smCounter2: an accurate low-frequency variant caller for targeted sequencing data with unique molecular identifiers. *Bioinformatics*. 2019;35(8):1299-1309.
2. Metzeler KH, Herold T, Rothenberg-Thurley M, et al; AMLCG Study Group. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128(5):686-698.
3. Talevich E, Shain AH, Botton T, et al. CNVkit: genome-wide copy number detection and visualization from targeted DNA sequencing. *PLoS Comput Biol*. 2016;12(4):e1004873.
4. Reinecke F, Satya RV, DiCarlo J. Quantitative analysis of differences in copy numbers using read depth obtained from PCR-enriched samples and controls. *BMC Bioinformatics*. 2015;16:17.
5. Weber K, Bartsch U, Stocking C, et al. A multicolor panel of novel lentiviral "gene ontology" (LeGO) vectors for functional gene analysis. *Mol Ther*. 2008;16(4):698-706.

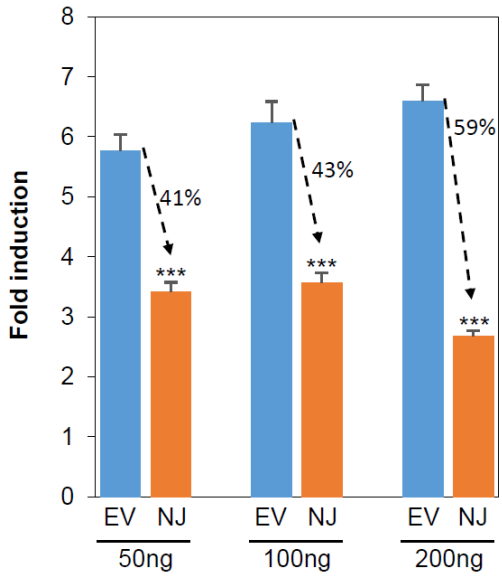
Supplemental Table 1. The 141 genes covered in the panel.

ABL1	*BRCA2*	*CTCF*	*FAS*	*JAK1*	*LUC7L2*	*NTRK3*	*PTPN11*	*SRP72*	*U2AF2*
ADA	*BRINP3*	*CUX1*	*FBXW7*	*JAK2*	*MAP2K1*	*OR13H1*	*RAD21*	*SRSF2*	*WAS*
ANKRD26	*C17orf97*	*DAXX*	*FLRT2*	*JAK3*	*MLH1*	*OR8B12*	*RB1*	*STAG2*	*WRN*
ASXL1	*CALR*	*DDX41*	*FLT3*	*KAT6A*	*MPL*	*P2RY2*	*RELN*	*STAT3*	*WT1*
ASXL2	*CARD11*	*DNM2*	*GATA1*	*KCNA4*	*MSH2*	*PAX5*	*RUNX1*	*STXBP2*	*XPO1*
ATM	*CBL*	*DNMT1*	*GATA2*	*KCNK13*	*MSH6*	*PCDHB1*	*SAXO2*	*SUZ12*	*ZRSR2*
ATRX	*CBLB*	*DNMT3A*	*GJB3*	*KDM6A*	*MYC*	*PDGFRA*	*SETBP1*	*TAL1*	
BCL6	*CBLC*	*EED*	*GNAS*	*KDR*	*MYD88*	*PHF6*	*SF1*	*TERC*	
BCOR	*CDKN2A*	*EGFR*	*HNRNPK*	*KIT*	*NBN*	*PML*	*SF3A1*	*TERT*	
BCORL1	*CEBPA*	*ELANE*	*HRAS*	*KLHDC8B*	*NF1*	*PMS2*	*SF3B1*	*TET2*	
BCR	*CHEK2*	*EP300*	*IDH1*	*KLHL6*	*NOTCH1*	*PRAMEF2*	*SH2B3*	*TNFRSF13B*	
BIRC3	*CREBBP*	*ETNK1*	*IDH2*	*KMT2A*	*NPAT*	*PRF1*	*SH2D1A*	*TP53*	
BLM	*CRLF2*	*ETV6*	*IKZF1*	*KMT2C*	*NPM1*	*PRPF40B*	*SMARCB1*	*TPMT*	
BRAF	*CSF1R*	*EZH2*	*IKZF3*	*KRAS*	*NRAS*	*PRPF8*	*SMC1A*	*TUBA3C*	
BRCA1	*CSF3R*	*FAM47A*	*IL7R*	*LRRC4*	*NSD1*	*PTEN*	*SMC3*	*U2AF1*	

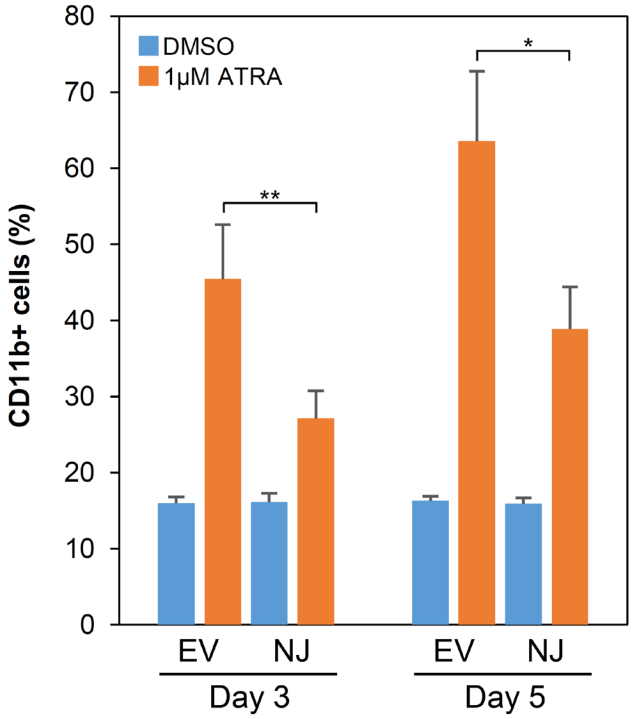
Supplemental Table 2. The list of genes consistently altered in *JADE2*-knocked down NB4 cells.

Downregulated genes			Upregulated genes	
Gene	**Mean fc***		**Gene**	**Mean fc***
NCF2	0.309		*NDUFA7*	2.176
IL1B	0.332		*AL158147.2*	1.869
S100A9	0.379		*MSRB3*	1.817
BTG2	0.384		*RPL36A*	1.720
SERPINE1	0.398		*TOMM20*	1.678
C5AR1	0.399		*CCND3*	1.631
NLRP12	0.428		*RDM1*	1.592
NEK7	0.442		*H1FX*	1.589
OSGIN1	0.461		*BOD1*	1.586
HMOX1	0.462		*SPG20OS*	1.577
ITGAM	0.479		*ISOC1*	1.564
PIK3R5	0.483		*CIT*	1.534
SH3TC1	0.488		*FBXO43*	1.524
ICAM1	0.498		*BANF1*	1.519
KLF4	0.520		*ERCC5*	1.512
NCF1	0.523		*GOPC*	1.512
SIGLEC7	0.526		*ACBD7*	1.509
ALOX5AP	0.531		*MEGF6*	1.501
GLIPR1	0.533		*RFXAP*	1.487
RAB8B	0.549		*CCDC136*	1.480
IRF8	0.551		*CCDC78*	1.447
IL1RAP	0.551		*PRR22*	1.441
CRISPLD2	0.554		*AP3S2*	1.425
TLR1	0.562		*GSE1*	1.410
FOS	0.566		*TAPT1*	1.404
IL13RA1	0.568		*PPP3CB*	1.397
SMIM14	0.571		*WDR90*	1.387
SLC43A2	0.573		*POLR3F*	1.379
IRAK2	0.575		*SMIM19*	1.374
GPR84	0.582		*MAMDC4*	1.373
CSF1R	0.586		*SSFA2*	1.369
PERP	0.586		*PDCD6*	1.363
MS4A6A	0.589		*FAM156B*	1.350
C10orf54	0.594			
MMP9	0.602			
KIAA0513	0.605			
TGFBR2	0.607			
FCGR2A	0.609			
EMR1	0.627			
DRAM1	0.635			
SLC12A6	0.638			
MMP14	0.638			
FOSL2	0.648			
THBD	0.649			
GALNT3	0.659			
GCNT2	0.664			
MILR1	0.665			
CREG1	0.672			
LAMC1	0.678			
DNAJB9	0.679			
PCMTD1	0.696			
EVI5	0.701			
CYTL1	0.717			
RNF125	0.723			
GRN	0.724			
PIK3AP1	0.760			

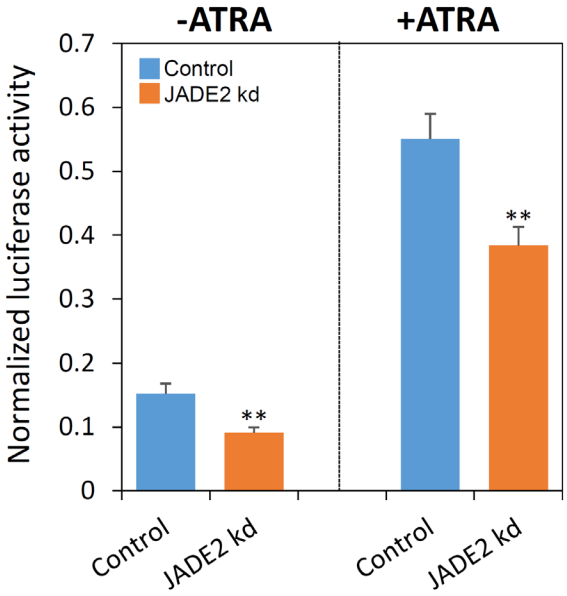
*Fold change (fc) was calculated by comparing the FPKM of each gene in the *JADE2* and negative siRNA groups. Mean fc was then calculated from the results of the two *JADE2* siRNAs (si#1 and si#2) in two independent experiments.



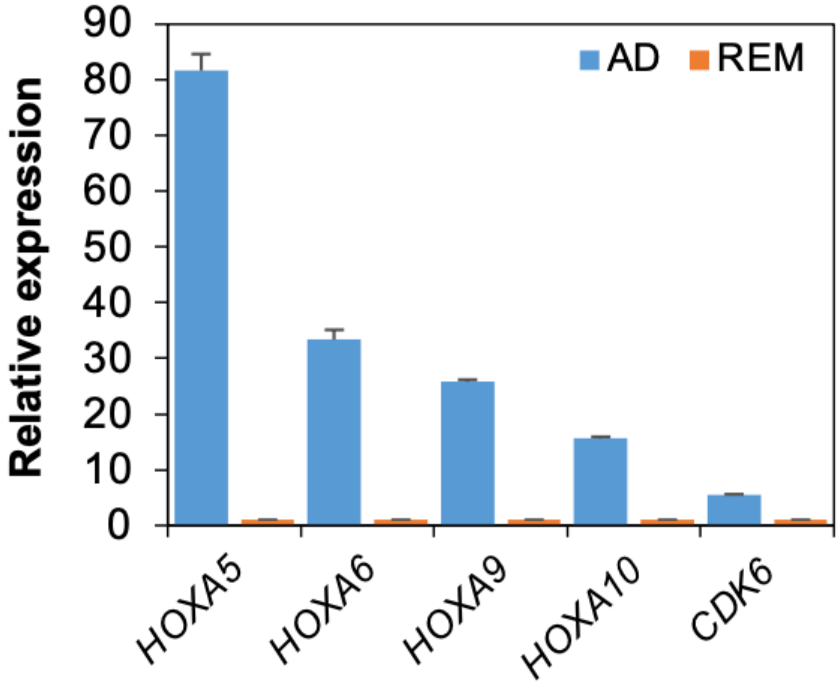
Supplemental Figure 1. NUP98-JADE2 inhibited ATRA-mediated transcriptional activation. 293T cells were co-transfected with the RARE reporter and an increasing amount of pCMV-HA-NUP98-JADE2 (NJ) or the empty pCMV-HA (EV). After 48 hours of the transfection, cells were treated with 1μM of ATRA or DMSO for 6 hours before luciferase measurement. Parallel experiments using the negative control reporter were performed to adjust for any non-specific effects. Firefly luciferase activity in cell lysates was normalized to *Renilla* luciferase activity, and results are presented as fold induction by comparing to DMSO treatment. Results are expressed as mean plus standard error from 3 independent experiments each performed in triplicate. *** indicates $P<0.001$ by the Mann-Whitney U test. A dose-dependent reduction of fold activation is indicated.



Supplemental Figure 2. NUP98-JADE2 inhibited ATRA-induced U937 cell differentiation. After 96 hours of transduction with NUP98-JADE2 (NJ) or control (EV) lentiviruses, U937 cells were treated with 1μM of ATRA or DMSO (vehicle control) for 3 and 5 days. CD11b expression on GFP-positive cells was measured by flow cytometry. Results are expressed as mean plus standard error from three independent experiments. * and ** indicates $P<0.05$ and $P<0.01$, respectively by paired t test.



Supplemental Figure 3. *JADE2* knockdown repressed RARE-mediated gene transcription. HeLa cells were transfected with the RARE reporter in the presence of the ON-TARGETplus Smartpool *JADE2* siRNAs (JADE2 kd) or negative control siRNA (Control). After 48 hours of the transfection, cells were treated with (+) or without (-) 1μM of ATRA for 6 hours before luciferase measurement. Parallel experiments using the negative control reporter were performed to adjust for any non-specific effects. Firefly luciferase activity in cell lysates was normalized to *Renilla* luciferase activity. Results are presented as normalized luciferase activity and expressed as mean plus standard error from 3 independent experiments each performed in triplicate. ** indicates $P<0.01$ by the Mann-Whitney U test.



Supplemental Figure 4. NUP98-JADE2 is associated with aberrant *HOXA* and *CDK6* expression. Quantitative RT-PCR analysis of *HOXA* genes and *CDK6* in the diagnostic (AD) and remission (REM) bone marrow (BM) samples from the patient. Whole BM cells were used for the analysis. Fold change (vs REM, ranged 5.5- to 81.7-fold) was calculated using 2^-ΔΔCt. Results are expressed as mean plus standard error from triplicate measurements.