Meis1 supports leukemogenesis through stimulation of ribosomal biogenesis and Myc

Maria-Paz Garcia-Cuellar, Andreas Prinz and Robert K. Slany

Department of Genetics, Friedrich-Alexander-University Erlangen-Nürnberg, Germany

Supplemental material for Garcia-Cuellar et al.
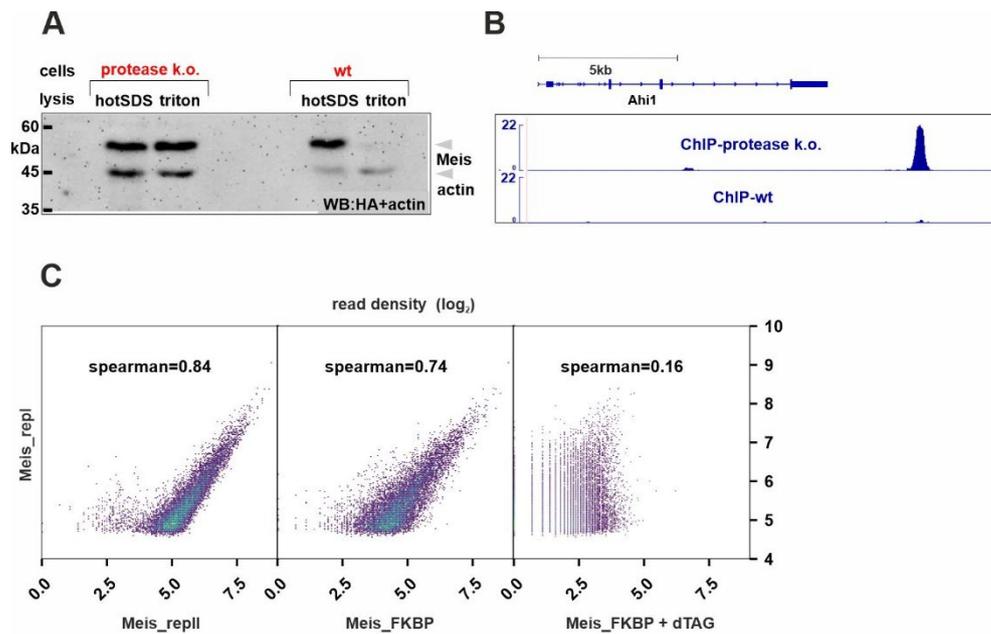
supplemental figure 1 (supplement to fig. 1)

Supplemental figure 1: Meis1 is degraded by myeloid granule proteases and can be reproducibly precipitated from protease negative cells.

A: Meis1 is rapidly degraded in wt myeloid precursors upon cell lysis. HA (Meis1) and actin specific western blot of extracts either from wt or protease triple-k.o. HSPCs transduced with HoxA9 and HA-Meis1. Extracts were generated by lysis with hot SDS and by a triton based method (including a full complement of protease inhibitors) and probed by immunoblot for presence of HA-Meis1.

B: Meis1 can not be efficiently precipitated from wt cells. Exemplary IGV plot comparing anti-HA ChIP results generated either from wt- or granule protease knock-out cells as indicated. The exemplary Meis1 peak at the major Myb enhancer is shown.

C: Meis1 ChIP patterns are highly reproducible. Global comparison of the 10000 top-scoring Meis1 peaks comprising a defined population with high reproducibily across replicates and a Meis1-FKBP$_{F36V}$ sample. Correlation breaks down after degradation of Meis1.
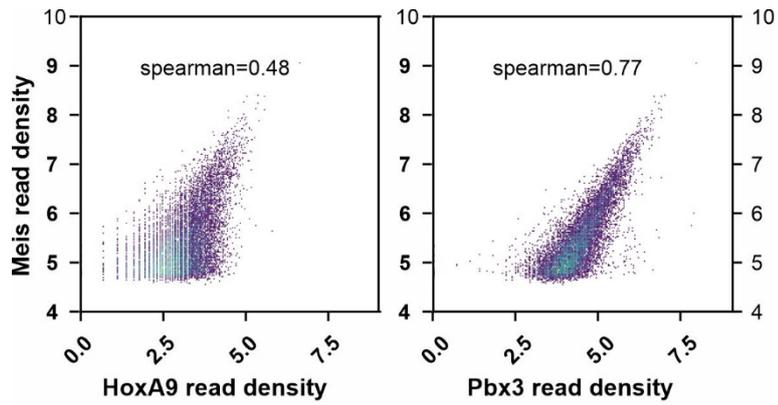
supplemental figure 2 (supplement to fig. 3)

Supplemental figure 2: Meis1 binds chromatin together with HoxA9 and Pbx3

Comparsion of Meis1, HoxA9 and Pbx3 read densities for the top scoring 10000 Meis1 peaks detected by ChIP.

A

B Enrichment plot: HALLMARK_MYC_TARGETS_V2

FDR = 0.0
p = 0.0
NES=2.6

Enrichment plot: DANG_MYC_TARGETS_UP

FDR = 0.0
p = 0.0
NES=2.2

C

known Myc enhancer

D Enrichment plot: REACTOME_RRNA_PROCESSING

FDR = 0.0
p = 0.0
NES=3.0

Enrichment plot: KEGG_RIBOSOME

FDR = 0.0
p = 0.0
NES=2.9
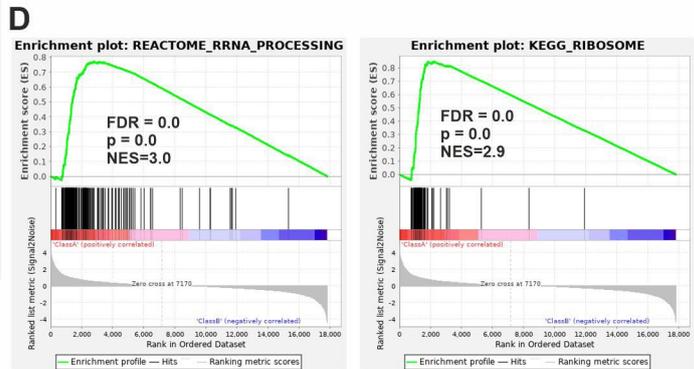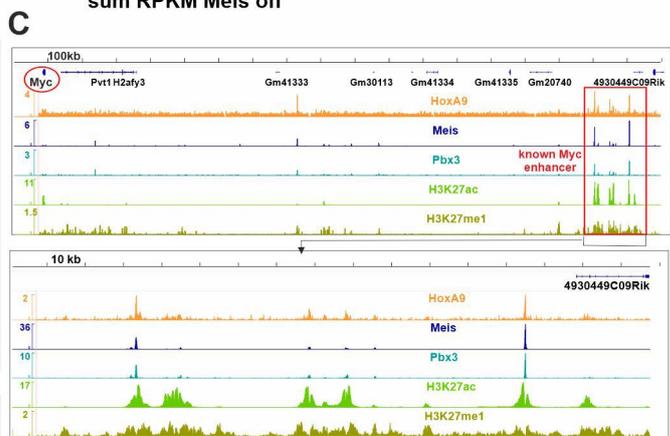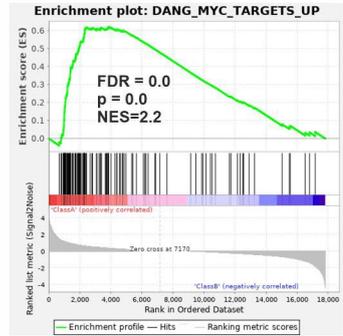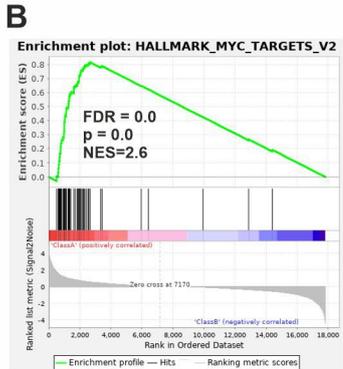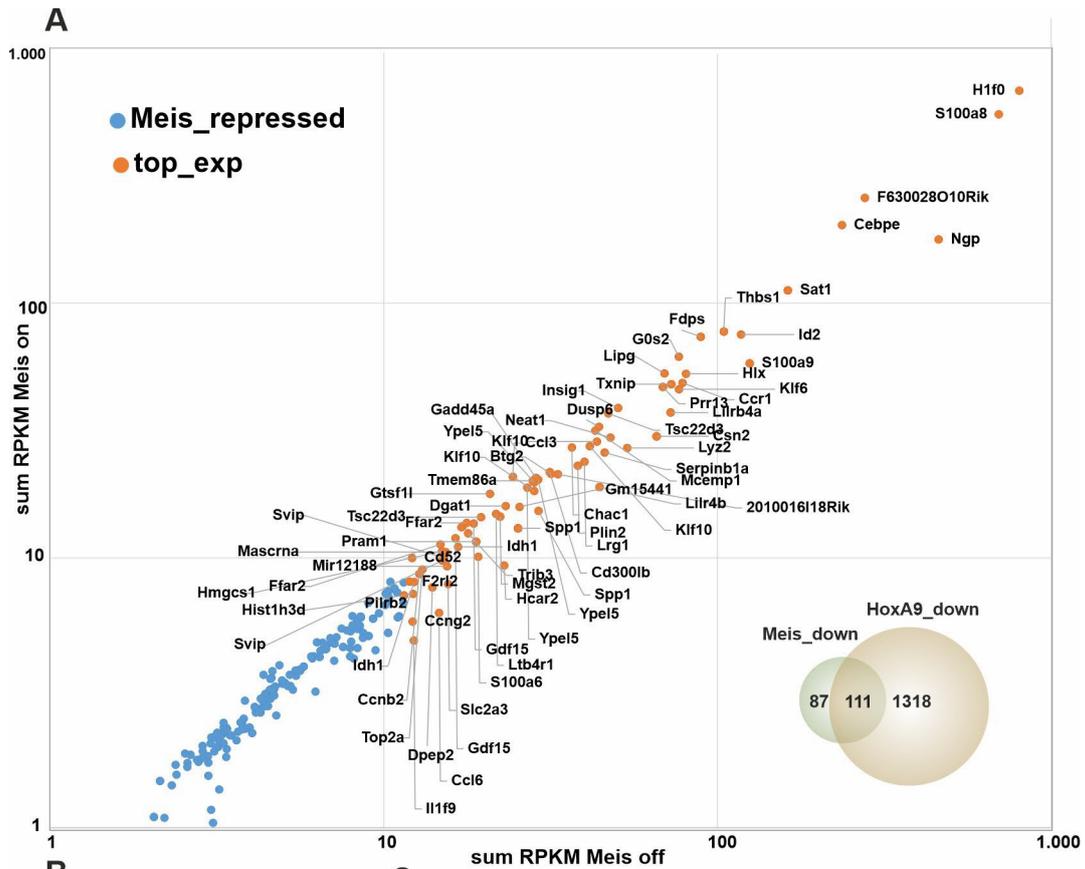
**supplemental figure 3
(supplement to fig. 4)**

Supplemental figure 3: Genes downregulated after Meis1 induction, Meis1 strongly binds the known Myc enhancer

A: Genes under negative control of Meis1. Dot plot of transcripts with reduced expression after induction of Meis1 expression. Transcript rates were determined as described for main figure 4D. For plotting and labeling individual transcripts (accession numbers) were collapsed to genes. Orange dots denote the top 100 transcripts with highest expression. The inset shows a Venn diagram detailing numbers of genes whose transcript rates were reduced by Meis1 and HoxA9 as determined previously.

B: GSEA analysis corroborates a strong Myc signature in the Meis1 controlled gene expression program.

C: Meis1 and Pbx3 bind to the known Myc enhancer.

Top panel: IGV plot showing the genetic environment of Myc and its major enhancer a 2MB distance that has been shown to be active in hematopoietic cells.

Lower panel: Zoom in on enhacer regions. Tracks correspond to binding of Meis1, HoxA9, and Pbx3, as well as enhancer modifications H3K27ac and H3K27me1 as indicated.

D: A ribosomal signature is overrepresented in the Meis1 induced gene expression program.
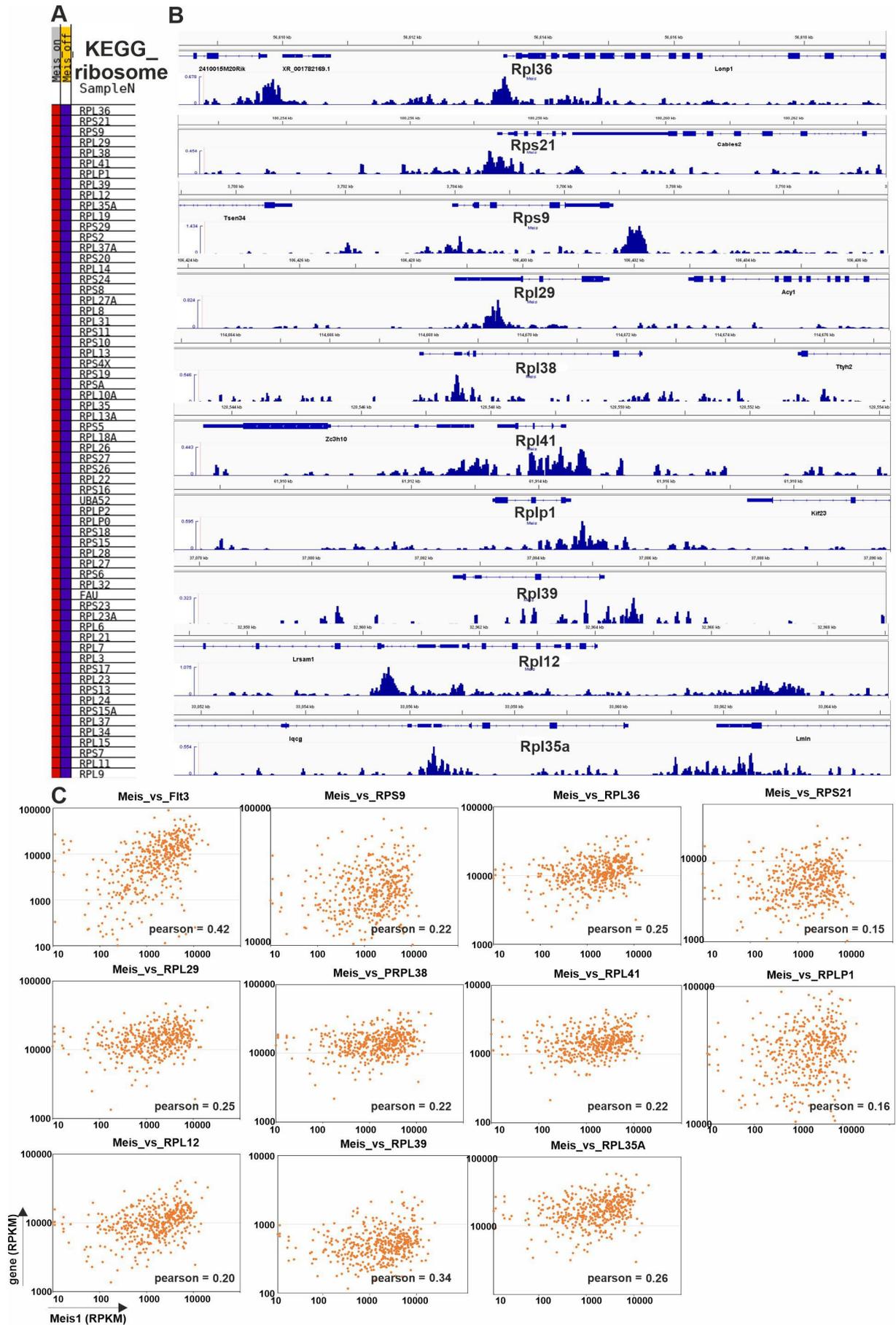
supplemental figure 4 (supplement to fig. 4)

Supplemental figure 4: Meis1 regulates ribosomal genes

A: Gene constituents of the "KEGG_ribosome" molecular signature.

B: Exemplary Meis1 binding in the vicinity of the leading ribosome-signature genes. Shown are IGV plots.

C: Correlation of MEIS1 and ribosomal genes in human AML. Plotted are expression values of MEIS1 (X-axis) and ribosomal genes as indicated (Y-axis) from a data cohort of 562 primary AML patients [33].
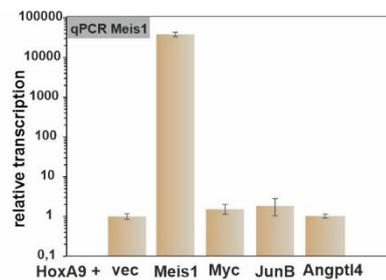
supplemental figure 5 (supplement to fig. 5)

Supplemental figure 5: Myc does not induce Meis1 expression.

RNA was isolated from primary cells transduced as indicated and used as input for RT-qPCR with Meis1 specific primers.

Supplemental table 1: Meis1 regulated gene expression program

The table lists gene expression values obtained by nascent-RNAseq in cells before and after induction or degradation of Meis1, respectively.

Detailed Material and Methods

DNA, cells, inhibitors, antibodies

Retroviral plasmids were constructed in pMSCV (Clontech, Palo Alto, CA) vectors. All insert sequences were either derived from laboratory stocks or amplified from cDNA isolated from murine cells and confirmed by sequencing. The FKBP$_{F36V}$ mutation was introduced by PCR. Retroviral packaging was performed in Phoenix-E cells. HPSCs were isolated either from wt or C57/BL6 mice with a triple-ko for *Elane*, *Prtn3*, and *Ctsg* [22]. Transduction was done with CD117 (Kit) selected cells enriched with magnetic beads (Miltenyi, Bergisch-Gladbach, Germany) essentially as recommended by the manufacturer. To generate transformed lines, cells were cultivated in methylcellulose (M3534, StemCellTechnologies, Cologne, Germany) for two rounds under antibiotics selection, then explanted and maintained in RPMI1640 (Thermo-Scientifc, Germany) supplemented with 10% FCS, penicillin-streptomycin, 5ng/ml recombinant murine IL-3, IL-6, GM-CSF, and 50ng/ml recombinant murine SCF (Miltenyi, Bergisch-Gladbach, Germany). 293T cells were from DSMZ (ACC-635 Braunschweig, Germany) and cultivated in DMEM + 10% FCS without cytokines.

CX5461 and CX4945 were purchased from SelleckChem (AbSourceDiagnostics, Munich, Germany). dTAG13 was from Tocris (NobleParkNorth, Australia). All other chemicals were provided either by Sigma (Taufkirchen, Germany) or Roth (Karlsruhe, Germany).

For western blot anti-flag M2 from Sigma (#F1804) and anti-beta actin as well as anti-HA from Cell Signaling Technologies (#3700, #3724) was applied. FACS antibody (PE-antiCD117/kit) was from ThermoScientific (eBioscience #12-1171-82). Protein synthesis rate were determined by O-propargyl-puromycin incorporation with the Click-iT™ Plus OPP Alexa Fluor™ 488 Protein Synthesis Assay Kit from Invitrogen (Waltham, MA, # C10456) according to the manufacturer's protocol. MTT for proliferation and viability assays was purchased from Sigma

and used according to standard procedures (0.33mg/ml MTT final concentration for 4h-8h cell labeling, followed by lysis in 10% SDS and readout at 550nm wave-length).

ChIP-Seq, cell lysis, nascent-RNA isolation

ChIP was performed as described in [23] applying a 10 min crosslink in 1% formaldehyde at RT followed by lysis in deoxycholate buffer (50mM Tris/HCl pH8.0, 10mM EDTA, 100mM NaCl, 1mM EGTA, 0.1% sodium-deoxycholate, 0.5% N-lauroylsarcosine 1mM PMSF and 1% HALT complete protease inhibitor cocktail (Pierce, Thermo-Fisher, Germany). Precipitation for all samples was performed with protein G coupled paramagnetic beads (Cell Signaling Technologies #9006). Antibodies used for ChIP: anti-HA rabbit monoclonal, Cell Signaling Technologies (#3724) 5µl per 5x10^6 cells; anti-H3K27ac and anti-H3K4me rabbit monoclonals, Cell Signaling Technologies (#8173, #5326) each 5µl per 5x10^6 cells.

Cell lysis for western was done in 20mM HEPES pH 7.5, 10mM KCl, 0.5mM EDTA, 0.1% triton-X100 and 10% glycerol supplemented with 1mM PMSF and 1% HALT complete protease inhibitor (triton lysis) or in hot (95°C) 50mM TrisHCl pH6.8, 0.2% SDS followed by a 2min nucleic acid digestion at RT with 10 units of benzonase after supplementation with 2 mM MgCl$_2$ (SDS lysis). Nascent-RNA isolation was done exactly as described in [24].

NGS and bioinformatics

ChIP sequencing libraries were prepared using NEBNext® Ultra™ II DNA Library Prep Kit reagents (NEB, Ipswitch, MA) according to the procedure recommended by the manufacturer. Size selection was done after final PCR amplification with Illumina index primers for 14 cycles. Nascent RNA was converted into Illumina compatible libraries with NEBNext® Single Cell/Low Input RNA Library Prep reagents according to the standard protocol. Sequencing was done at the in house core facility on a HiSeq2500 instrument yielding 100bp single- or paired-end reads.

Data were mapped with BWA mem (0.7.17) [25] to the *Mus musculus* mm10 genome. Reads mapping more than once were excluded by filtering for sequences with a mapping quality score > 4. For visualization BAM files were normalized and converted to TDF format with IGV-tools of the IGV browser package [26]. Peak finding, motif analysis and peak annotation was done with Homer (4.9.1) [27]. BAM files were converted to bigwig by Deeptools (3.0.0, bamCoverage) [28]. Metagene plots were created with Deeptools (3.0.0). Matrices were calculated with calculateMatrix and plotted with plotHeatmap from the Deeptools suite. RNA derived reads were aligned with STAR (v020201) [29] to the reference genome mm10 and reads derived from repetitive sequences were excluded by samtools (view)1.8 [30]. Transcripts were quantified by Homer analyzeRNA routines and further analyzed with standard spreadsheet tools.

Transplantation experiments

For transplantation experiments C57/BL6 mice were sublethally irradiated with 6Gy gamma-radiation. Syngenic cells for transplant were prepared by retroviral transduction followed by antibiotic selection during two rounds of methocel culture as above. Animals were injected 24h after irradiation intravenously with 0.5×10^6 transduced cells and 0.5×10^6 total bone marrow cells for radiation protection. Drinking water was supplemented with antibiotics for one week after transplant (1.1g/L Neomycin, 10^6 units/L PolymixinB), thereafter recipients were kept with regular water and chow *ad libitum*. Daily monitoring for signs of disease (reduced activity and body hygiene, weight loss, hunched posture, altered breathing frequency) in combination with a standard institutional scoring system guided humane endpoint decisions. Post-mortem analysis confirmed leukemia development (enlarged spleen and liver, organ infiltration). All procedures were approved by the proper institutional and state authorities and license numbers are available on request.

Data availability

Raw NGS reads were submitted to the European Nucleotide Archive under accession number

ERP134562

Statistics

Where appropriate two-tailed T-test statistics were applied.