

The co-receptor Neuropilin-1 enhances proliferation in inv(16) acute myeloid leukemia via VEGF signaling

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Supplementary information and materials

Cell assays

Annexin-V/7AAD assay: Cells were washed with 1X PBS, resuspended with 1X binding buffer (BD Biosciences, cat. # 559763), incubated with Annexin-V and 7AAD for 20 min at room temperature and analyzed through FACS.

Cell cycle analysis: After Puromycin selection, ME-1 cells were resuspended in complete media with 20 µg/mL Hoechst 33342, incubated at 37°C for 30 min, and the cell cycle profile was analyzed by flow cytometry (BD LSRII).

Giemsa cell staining: The ME-1 cells were harvested (10,000 cell/sample) at specified time points, washed with 1X PBS, and cytospun onto glass-slides. Cells were stained with Giemsa solution (Millipore Sigma, cat. # 620G-75) for 40 sec and rinsed thoroughly with water. Images were capture at 10X and 40X magnification, utilizing Zeiss Axioskop-40 microscope (Carl Zeiss).

Cell treatment with inhibitors: *CBFbeta inhibitors*: 20 x10⁴ ME-1 and THP-1 cells were seeded in 48-well plates in triplicate, and treated with DMSO (vehicle), AI-4-88 (inactive)

or AI-10-104 (active) CBFbeta inhibitor (27) for 24 hrs, utilizing concentrations indicated in the text. Cells were harvested, and RNA was isolated for estimation of *NRP1* expression levels by RT-PCR.

NRP1 blocking-antibody: 2.5×10^4 to 5×10^4 human AML cell lines were cultured for 14 days in media supplemented with anti-NRP1^A (for CUB domain) or anti-NRP1^B (for FV/VIII domain) blocking antibodies at a concentration range of 0 to 20 µg/mL (Genentech; ref. (33)). Cells were replated with fresh media supplemented with NRP1 antibodies every 24 hrs and cell number was estimated using trypan blue assay.

Axitinib treatment: Primary human samples (25×10^4) or mouse *Cbfb*^{MYH11/+} leukemic cells were incubated with 0.1, 0.5, 1, 2.5, 5, 10 and 25 µM of Axitinib (MedChemExpress, cat. #HY-10065) or DMSO (vehicle) in 96-well plates for 48 hrs. 20 µl of MTS (CellTiter 96 AQueous One Solution, Promega) was added and incubated for 4 hrs followed by reading in a spectrophotometer at 490 nm. IC₅₀ values were calculated using dose response curve using GraphPad prism software (v8.1.2).

Surface staining for flow cytometry analysis: Cells were harvested, washed twice with 1X PBS and incubated with respective antibodies in FACS buffer (0.1% BSA in 1X PBS). Cells were then washed with 1X PBS and acquired by flow cytometry (BD LSRII). The percentage of positive cells or MFI was calculated with respect to unstained cells. Antibodies used: CD11b-PE (BD Biosciences, cat. # 555388), CD15-APC (BD Biosciences, cat. # 561716), NRP1-PE (Bio Legend, cat. # 354504), NRP1-APC (Bio Legend, cat. # 354506), TGFBR2-PE (Bio Legend, cat. # 399703), CD117-APC (BD Biosciences, cat. # 553356), Ly-6A/E APC-Cy7 (BD Biosciences, cat. # 560654), Nrp1-

PE (Bio Legend, cat. # 145204), Mouse CD117-FITC (BD Biosciences, cat. # 561680).

Immunoblotting

Cells were lysed in modified RIPA buffer (50 mM Tris pH7.5, 150 mM NaCl, 1% NP40, 0.25% sodium deoxycholate and 1 mM EDTA) supplemented with phosphatase inhibitor (Millipore Sigma, cat. # P0044-1ML and P5726-1ML) and protease inhibitors (Thermo Fisher Scientific, cat. # 778429) for 30 min on ice, followed by centrifugation. Protein lysate concentrations were determined with the Bradford reagent (Bio-Rad, cat. # 5000006). Protein lysates were separated on 7.5%–12% Mini Protean TGX pre-casted gels (Bio-Rad, cat. # 456-1043) using the Mini Protean electrophoresis system and blotted onto PVDF membrane. The primary antibodies used in this study were anti-NRP1 (cat. # 3725S), anti-ERK1/2 (p44/42 MAPK; cat. # 9102S), anti-cleaved Caspase-3 (cat. # 9661) and anti- β -Actin (cat. # 5125), all from Cell Signaling Technologies; and anti-MYH11 (Novus Biologicals, cat. # 21370002). Membranes were incubated with secondary antibody (Cell Signaling Technology, cat. # 7074S). Images were captured using ChemiDoc Imaging System and bands quantified using Image Lab Software (Bio-Rad, Version 6.1).

DNA sequences

The *NRP1* locus was analyzed using the human genome browser hg19, with the *NRP1* gene the RefSeq NM_001024628; and the mouse genome browser mm10.

Chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) analysis

The ME-1 cells were transduced with shRNA lentiviruses (scramble or *MYH11-sh4*), and cultured in media supplemented with Puromycin. On day 7, Viable cells were purified by Ficoll gradient, protein/DNA cross-linked by the addition of 1% formaldehyde for 10 min at room temperature, and neutralized with Glycine. The fixed cells were incubated in lysis buffer supplemented with protease inhibitors and sonicated to an average DNA length of 200–400 bp with a Bioruptor sonicator (Diagenode). The chromatin fragments were immunoprecipitated with 5-10 µg of anti-RUNX1 (Abcam, cat. # ab23980) and anti-ERG (Novus Biologicals, cat. # SP06-04) antibody at 4°C overnight. Anti-GATA2 rabbit polyclonal antibody (provided by Emery Bresnick, University of Wisconsin, Madison) was used at 1/90 dilution. The antibody-bound complexes were isolated with Dynabeads (Thermo Fisher Scientific, cat. # 10003D), and the DNA was purified using ChIP DNA Clean & Concentrator kit (Zymo Research, cat. # D5201). The immunoprecipitated DNA was analyzed by qPCR amplification utilizing a StepOnePlus System (Applied Biosystems) with AzuraView™ GreenFast qPCR Blue Mix HR (Azura Genomics, cat. # AZ-2405) and calculated as percent of input.

CRISPR/Cas9 ribonucleoprotein nucleofection

50 pmols of purified 3xNLS-SpCas9 (41) and 100 pmol *sgRNA* (Integrated DNA Technology; Table S1) were mixed in buffer R (10 µL final volume) using Neon™ Transfection System Kit (Thermo Fisher Scientific, cat. # MPK1025), and incubated for 20 min at room temperature to form the ribonucleoprotein complex. 2x10⁵ cells/sample

were washed with 1X PBS, resuspended in solution with the ribonucleoprotein complex, and transfected with Neon™ Transfection System 10 µL Kit (Thermo Fisher Scientific, cat. # MPK1025) using settings: 1500 voltage, 10 pulse-width and 3 pulse-number. The transfected samples were immediately resuspended in complete media and seeded in 48-well plates.

MYH11 editing in inv(16) primary AML cells

Primary inv(16) AML cells were transfected with spCas9/sgRNA ribonucleoproteins using *MYH11-sg1* and *MYH11-sg2* or *sgAAVS1* (control) guide RNAs (Table S1), using the transfection program described above. Expression of NRP1 was tested by flow cytometry for cell surface-NRP1 48 hrs post-transfection.

NRP1 editing ME-1 cells

ME-1 cells were transfected with spCas9/sgRNA ribonucleoproteins using (*sg9NRP1+sg11NRP1*) and (*sg10NRP1+sg12NRP1*), or *sgAAVS1* (control) guide RNAs (Table S1), using the transfection program described above. Expression of NRP1 was tested by flowcytometry on day 7. Annexin assay was carried out on day 14 and trypan blue cell viability assays were carried out every 4 days until 28 days.

Nrp1 editing in mouse leukemic cells

Primary murine *Cbfb*^{+/MYH11}; *Nras*^{+/G12D} and *MLL::AF9* myeloid leukemia cells (14, 40) were transfected with spCas9/sgRNA ribonucleoproteins using *Nrp1-set1* (*sg1Nrp1+sg4Nrp1*) and *Nrp1-set2* (*sg2Nrp1+sg3Nrp1*), or *sgRosa* (control) guide RNAs

(Supplementary Table 1), using the transfection program described above. The editing efficiency was estimated by flow cytometry for cell surface-Nrp1 48 hrs post-transfection.

Deletion of RUNX1 peaks at NRP1 locus by CRISPR/Cas9 in AML cells

Each of the sequences encompassing RUNX1 peaks (RE+1, RE-30 and RE-174) were deleted using CRISPR/Cas9 techniques utilizing two specific sgRNAs for each sequence with the deletion size ranging between 511 and 685 bp. The selected negative control (NC) maps to an intronic region of the *NRP1* gene with no evident RUNX1 binding. The sgRNAs were designed using *CRISPick* webtool, and the efficiency of deletion was tested using quantitative PCR amplification of genomic DNA.

Transcriptome profiling (RNA-sequencing)

Transcription profiling preparation: 2×10^6 ME-1 cells were transduced with lentiviruses expressing *Scramble*- or *MYH11sh4-RNA* in triplicates, cultured for 2 days, and replaced with media supplemented with 5 μ g/mL Puromycin between days 3 and 6. At day 7, cells were processed to prepare total RNA. Day 7 (corresponding to day 1 post selection) live cells were isolated using Ficoll-gradient centrifugation, and total RNA was isolated with Monarch Total RNA Miniprep kit (New England Biolabs, cat. # T2010S). The RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and the RNA integrity was evaluated with RNA 6000 kit (Agilent Technologies) using a 2100 Bioanalyzer. The sequencing libraries were prepared and sequenced utilizing DNBseq™ Technology (BGI Genomics). The transcriptome profiling of *NRP1*-

shRNA treated ME-1 cells was performed using a similar procedure on day 7 of culture, using *Scramble*- or *NRP1sh3-RNA* transduced ME-1 cells, in triplicates.

Transcription profiling analysis: The RNA-seq analysis was performed with OneStopRNASeq (42). Paired-end reads were aligned to human primary genome hg38, with star_2.5.3a (43), annotated with GENCODE GRCh38.p12 annotation release 29 (44). Aligned exon fragments with mapping quality higher than 20 were counted toward gene expression with featureCounts_1.5.2 (45). Differential expression (DE) analysis was performed with DESeq2_1.20.0 (46) modeling all samples. Within DE analysis, 'ashr' was used to create log2 Fold Change (LFC) shrinkage for each comparison (47). Significant DE genes (DEG) were filtered with the criteria $FDR < 0.05$ and $|L2FC| > 1$. Zscore heatmap of DEG was created with ComplexHeatmap. Gene ontology (GO) analysis was performed using <http://geneontology.org/> server. Gene set enrichment analysis were performed with GSEA (48).

The RNA-seq analysis was performed for *NRP1*-shRNA treated samples using $FDR < 0.05$ and $L_2FC > 0.5$. To compare the number of commonly deregulated genes between *MYH11*- and *NRP1*-shRNA treated samples, $FDR < 0.05$ and $L_2FC > 0.5$ was used.

The analysis of *Nrp1* transcript levels were calculated from the following published RNA-seq datasets: *Cbfb*^{+/56M} preleukemic bone marrow c-kit⁺ cells (GSE102388), *Cbfb*^{+/56M} and *Cbfb*^{+/56M}, *Runx1*^{fl/fl} preleukemic bone marrow c-kit⁺ cells (GSE152573), and *Cbfb*^{+/56M} and *Cbfb*^{+/56M}, *Gata2*^{+/f} leukemic cells (GSE130343). Briefly, Paired-end reads were aligned to mouse genome browser mm10 using HISAT2 (49). Aligned exon fragments were counted toward gene expression with *featureCounts*_1.5.2 (45) and differential expression (DE) analysis was performed with DESeq2_1.20.0 (46) modeling all samples.

Quantitative RT-PCR analysis

Total RNA was extracted utilizing Monarch Total RNA Miniprep kit (NEB, cat. # T2010S) according to manufacturer's instruction. The RNA concentration was quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific), and the complementary DNA was synthesized utilizing Verso cDNA Synthesis kit (Thermo Fisher Scientific, cat. # AB1453B) according to the manufacturer's instruction. The RT-PCR amplification was carried out using gene specific primers (Table S1) on StepOnePlus System (Applied Biosystems) with AzuraView™ GreenFast qPCR Blue Mix HR (Azura Genomics). The expression levels were determined with the ΔC_t method and normalized to murine or human *ACTB* or *ERK* mRNA.

ChIP-sequencing and ATAC-sequencing data analysis from AML patient samples

Data used in this study have been previously made available at EGA (accession code EGAS00001004684; ref. (29)). For ChIP-seq, reads were aligned to the reference genome build hg19 with bowtie, and bigwig files were generated for visualization with deepTools bamCoverage using RPKM normalization (50). Peak calling was performed with MACS2 (51) (v 2.1.2) using default settings. For ATAC-seq, reads were aligned to the reference genome build hg19 with bowtie2 (v2.3.4.1) and mitochondrial and duplicate reads were excluded. Bigwig files were generated as described above. Peak calling was also performed with MACS2 (v 2.1.2) with the following settings: --nomodel --shift 100 --extsize 200.

Quantification of peak signal was carried out with the DiffBind R package (52) as follows. First, all peaks were combined in a single master list using the default settings, keeping only peaks present in at least 2 samples. Overlapping peaks across multiple samples were combined into a single entry. For each sample, reads mapping to this master list were counted, subtracting reads mapping to an input DNA.

For differential analysis, data were normalized using the trimmed mean of the M-values (TMM) with the sum of reads in consensus peaks as the library size. These normalization factors and the raw counts were passed to DESeq2 with the `dba.analyze` command.

ChIP-sequencing data analysis from human normal HSPC's and ME-1 cells

All reads were aligned to hg19 with bowtie2 without duplicate reads. The bigwig files were created via `bamCoverage` script in deepTools (normalized using-CPM), for paired-end reads parameter `-e 150` was used. Published ChIP-seq datasets retrieved for the study include: GSE231486 for normal HSPCs (53); GSE158794 for LMO2 and LYL1 (54); GSE46044 for TAL1 and FLI1 (18), and GSE117138 for RUNX1 (28).

Drug sensitivity data analysis

The gene expression and drug sensitivity data from 671 AML samples were retrieved from BeatAML2 dataset [vizome.org (34)]. Samples were categorized into four groups based on the expression levels of NRP1 (in RPKM) for the analysis of drug dependency: High (n=38, mean_{expression}: 4.4), Medium (n=70, Mean_{expression}: 3.64), Low (n=179, mean_{expression}: 1.11) and Negative (n=384; mean_{expression}: -2.26).

Mouse Studies

The mice utilized in this study were maintained at the University of Massachusetts Chan Medical School (UMCMS) animal facility, which is accredited by the American Association for Laboratory Animal Care. The experiments in mice here described have been approved by the UMCMS Institutional Animal Care and Use Committee (protocol #202100194; principal investigator: L.H.C.). All animal procedures adhere to the U.S. Public Health Service Policy on Humanae Care and Use of Laboratory animals.

For the transplantation studies, 2×10^3 mouse *Cbfb*^{MYH11/+} leukemic cells previously created (14, 15) were transfected with spCas9/sgRNA RNPs and transplanted into each of 5 sub-lethally (550 Gy) irradiated eight- to ten-week old wild-type C57BL/6 female mice (Taconic Farms) immediately after transfection. Transplanted mice were kept under observation to estimate the median latency of leukemia. For the colony-forming unit (CFU) assays, 1×10^4 transfected leukemic cells were mixed with Methocult (STEMCELL Technologies, cat. # M3534) media, cultured in 3mm plates in triplicate, and the CFU number was estimated on day 10 of culture.

Axitinib treatment in mice: 2×10^3 mouse *Cbfb*^{MYH11/+} leukemic cells were transplanted into each of eight sub-lethally irradiated (550 cGy) eight- to ten-week-old wild-type C57BL/6 female mice. Five days after transplantation, Axitinib (30 mg/kg) or vehicle (0.5% carboxymethyl cellulose; MP Biomedicals, cat. #101278) was administered orally BID for 16 days. Four weeks after transplantation, leukemia burden was monitored by evaluating the percentage of c-kit positive white blood cells by flow cytometry. Transplanted mice were kept under observation to estimate the median latency of leukemia.

Supplementary figure legends

Fig. S1. *MYH11* shRNA treatment deregulates expression of signaling pathway genes and induces apoptosis in inv(16) AML cells. **A.** Time-course growth curve of ME-1 cells transduced with *MYH11* (sh4) or *Scr* control shRNAs and treated with Puromycin for 5 days. **B.** *CBFB-MYH11* transcript (top) and protein (bottom) expression levels of ME-1 cells, 7 days post shRNA transduction. **C** and **D.** Cell viability (left; Annexin-V/7AAD⁻) and differentiation (right; CD11b and CD15 staining) of ME-1 cells, 14 days post shRNA transduction. **E.** Microscopy images (10x and 40x) of Giemsa stained of ME-1 cell spreads, 7 and 14 days after transduction with *MYH11* shRNAs. **F.** Cell cycle analysis of ME-1 cells transduced with *MYH11sh4* or *Scr* shRNAs, on days 7, 14 and 21. Bar plots are represented with error bars with standard deviation (n=3). Significance was calculated using an unpaired t-test, *p < 0.05 or **p < 0.005.

Fig. S2. *NRP1* is expressed in inv(16) leukemia and is regulated by

CBFbeta::MYH11/RUNX1. **A.** TCGA analysis for different signaling genes and *MYH11* in different leukemic primary samples. **B** and **C.** *NRP1* transcript levels and *NRP1* expression (MFI) in different leukemic cells. **D.** Quantification of *NRP1* through FACS analysis (MFI) in ME-1 cells transduced with 3 *MYH11* shRNAs (sh3 - sh5) and *Scr* control. **E.** *NRP1* and *RUNX1* transcript level in THP-1 cells transduced with *RUNX1* shRNA (sh1 and sh2) and *Scr* shRNA. **F.** *NRP1* transcript level in THP-1 cells treated with different concentrations (0, 2 and 5 μ M) of CBFbeta inhibitor AI-4-88 (inactive) and AI-10-104 (active) for 24 h. **G.** *RUNX1* transcript level in ME-1 and THP-1 cells. **H.** *Nrp1* transcript level (fold change) in WT and Cbfb^{+/56M} (preleukemic cells) bone marrow c-kit⁺

cells (GSE102388). **I.** *Nrp1* transcript level (fold change) in *Cbfb*^{+/-56M} and *Cbfb*^{+/-56M}, *Runx1*^{+/f} preleukemic cells, bone marrow c-kit⁺ cells (GSE152573). Bar diagrams represented with standard deviation of triplicates and significance was calculated using an unpaired t test, *p < 0.05 or **p < 0.005 in **D**, **E**, **F** and **G**.

Fig. S3. Regulatory elements involved with the NRP1 expression in inv(16) AML. **A.** RUNX1 ChIP-sequencing data at *NRP1* locus in ME-1 cells with or without CBFbeta::MYH11 KD (GSE117138). Regulatory elements are marked along with the negative control (NC) used in the ChIP qPCR studies **B.** Graphic representation H3K27Ac peaks at *NRP1* locus using inv(16) AML (blue) and non-inv(16) AML (orange) patient samples (n=8). **C.** Graphic representation ATAC sequencing peaks at *NRP1* locus using inv(16) AML (blue) and non-inv(16) AML (orange) patient samples (n=7). **D.** Agarose gel image depicting the efficiency of NC, RE+1, RE-30 and RE-174 deletion in ME-1 cells 48 hrs after sgRNA/SpCas9 electroporation.

Fig. S4. Heptad transcription factors regulate NRP1 expression in inv(16) leukemia. **A.** Transcript levels of *CBFB::MYH11*, *GATA2*, *ERG* and *RUNX1* in ME-1 cells transduced with 3 *MYH11* shRNAs (sh3 - sh5) and Scr control (Day 7). **B.** *Nrp1* transcript level (fold change) in *Cbfb*^{+/-56M} and *Cbfb*^{+/-56M}, *Gata2*^{+/f} bone marrow c-kit⁺ cells (GSE130343). **C.** Volcano plot representation of RNA-sequencing results highlighting the expression levels for *Gata2* and *Nrp1* in *Gata2*-deficient fetal liver hematopoietic cells (18). **D.** Diagrammatic illustration of the RE+1 enhancer, RUNX1 consensus binding sites (stars), and the P1 and P2 promoter region (gray boxes), the transcription start site (TSS),

and exons 1 and 2 (black boxes). Distances are not to scale and represented in base pairs. **E.** IGV ChIP seq track images showing NRP1 locus bound with transcription factors in human normal HSPC's (top) and ME-1 cells (bottom).

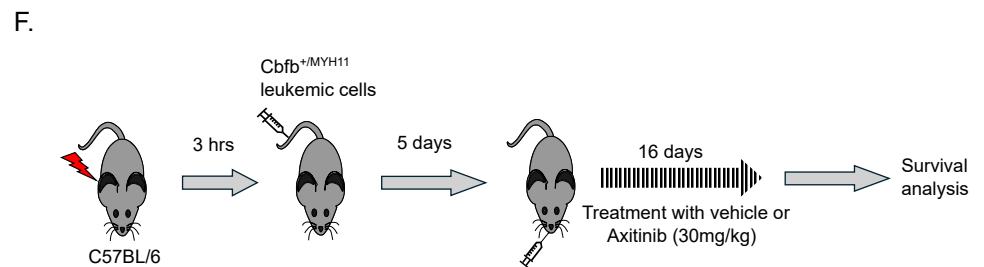
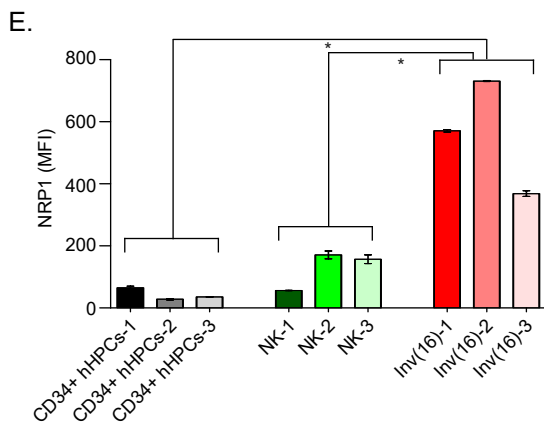
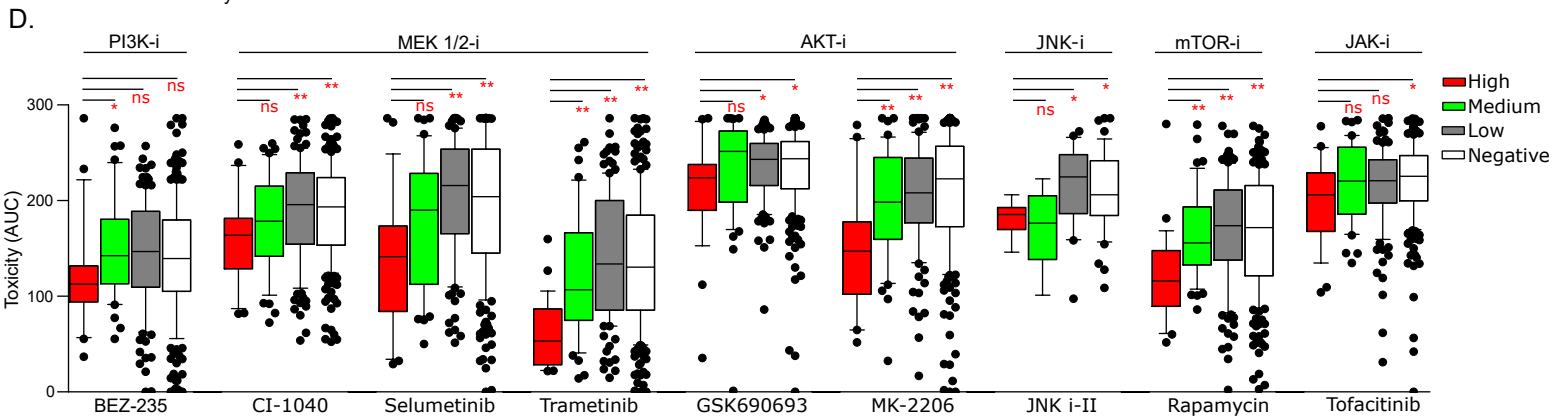
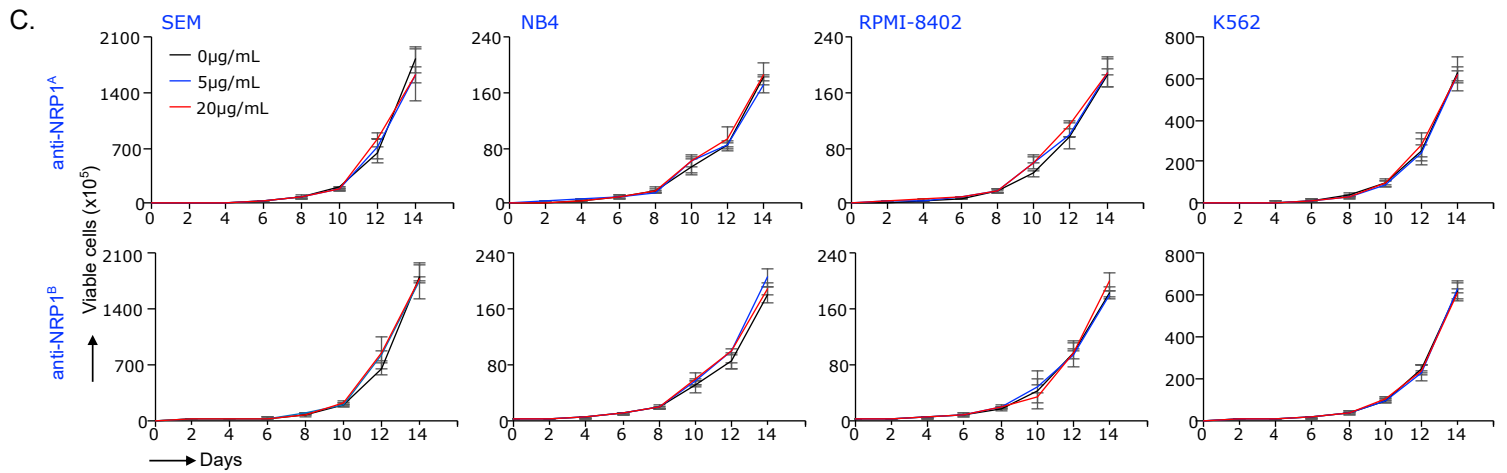
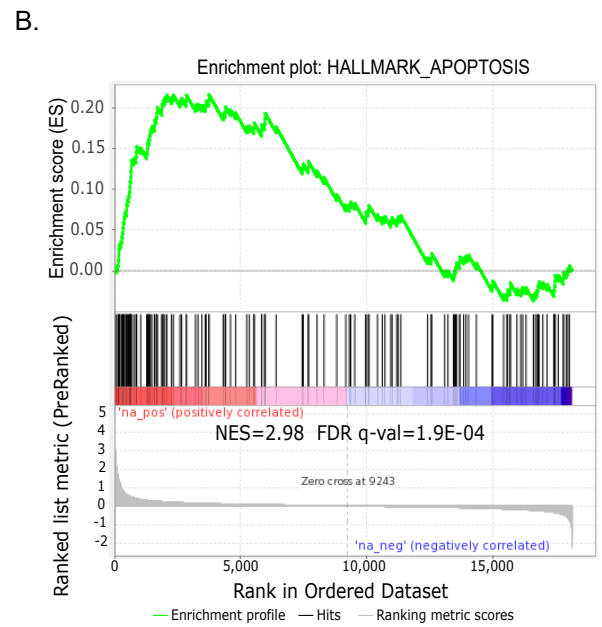
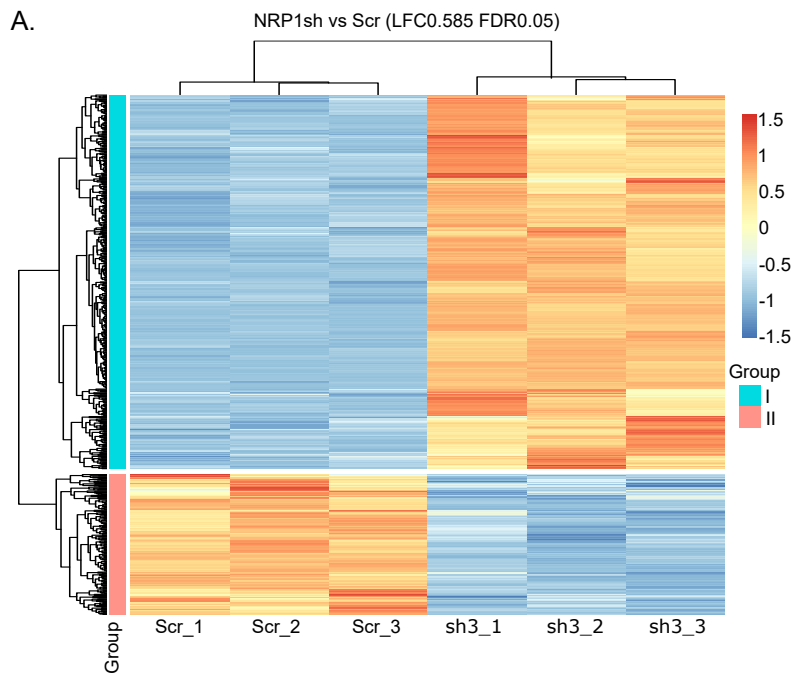
Fig. S5. NRP1 expression is essential for the survival of inv(16) leukemic cells. A.

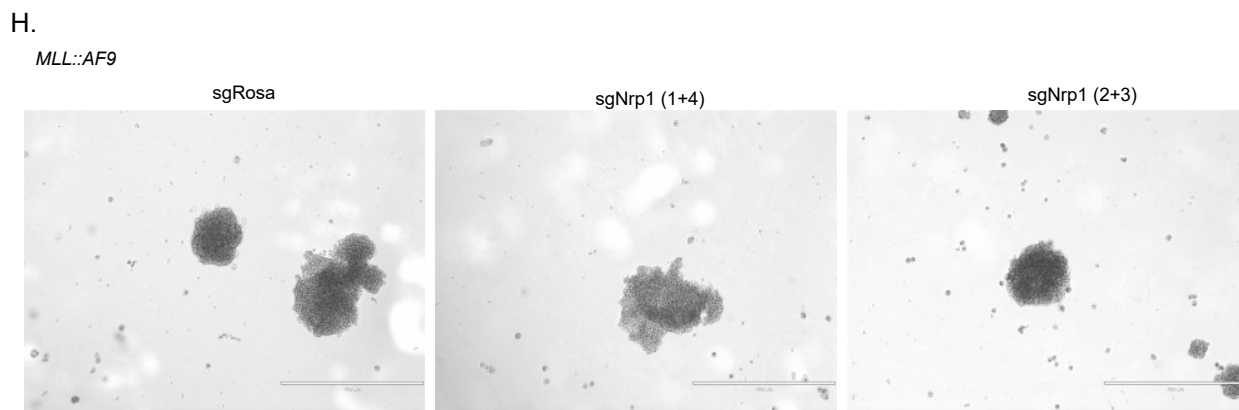
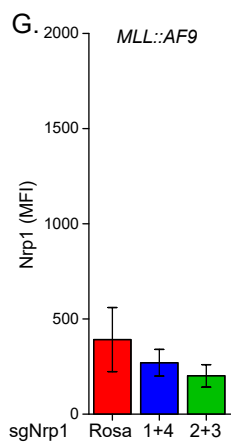
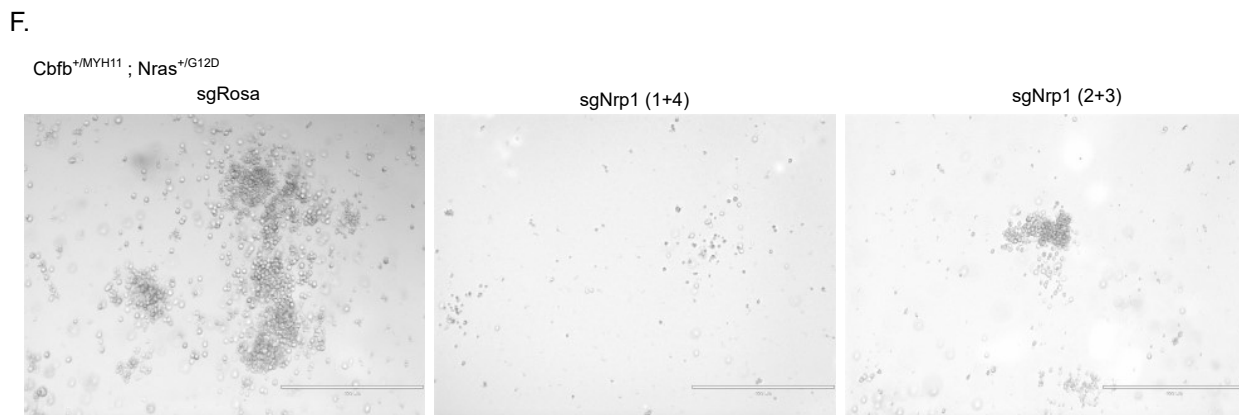
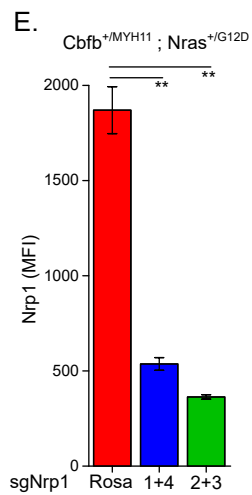
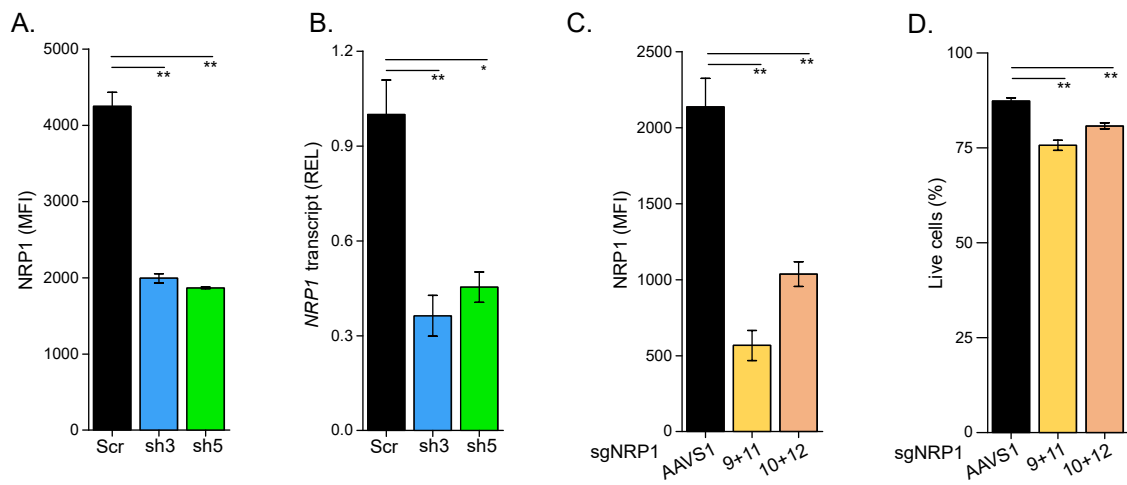
Expression of NRP1 (MFI) in *NRP1*-sh3, *NRP1*-sh5 and Scr-RNA, 7 days post transduction. **B.** Transcript levels of *NRP1* in ME-1 cells transduced with *NRP1* sh3, *NRP1* sh5 and Scr control, 7 days post shRNA transduction. **C.** Expression of NRP1 (MFI) in ME-1 cells transfected with *AAVS1* or *NRP1* sgRNA/SpCas9 electroporation 7 days post transduction. **D.** Estimation of cell viability (Annexin V⁻, 7AAD⁻) by flow cytometry in ME-1 cells transfected with *AAVS1* or *NRP1* sgRNA/SpCas9 electroporation 14 days post transduction. **E.** Nrp1 surface expression (MFI) in *Cbfb*^{+/MYH11}; *Nras*^{+/G12D} mouse leukemic cells edited with SpCas9 at the Nrp1 locus (48 h). **F.** Microscopy images (10x) of CFUs from *Nrp1* edited *Cbfb*^{+/MYH11}; *Nras*^{+/G12D} mouse leukemic cells (day 10). **G.** Nrp1 surface expression (MFI) in *MLL::AF9* leukemic cells edited with SpCas9 at the *Nrp1* locus (48 h). **H.** Microscopy images (10x) of CFUs from *Nrp1* edited *MLL::AF9* mouse leukemic cells (day 10). Bar diagrams represented with standard deviation of triplicates and significance was calculated using an unpaired t test, *p < 0.05 or **p < 0.005 in **A - E** and **G**.

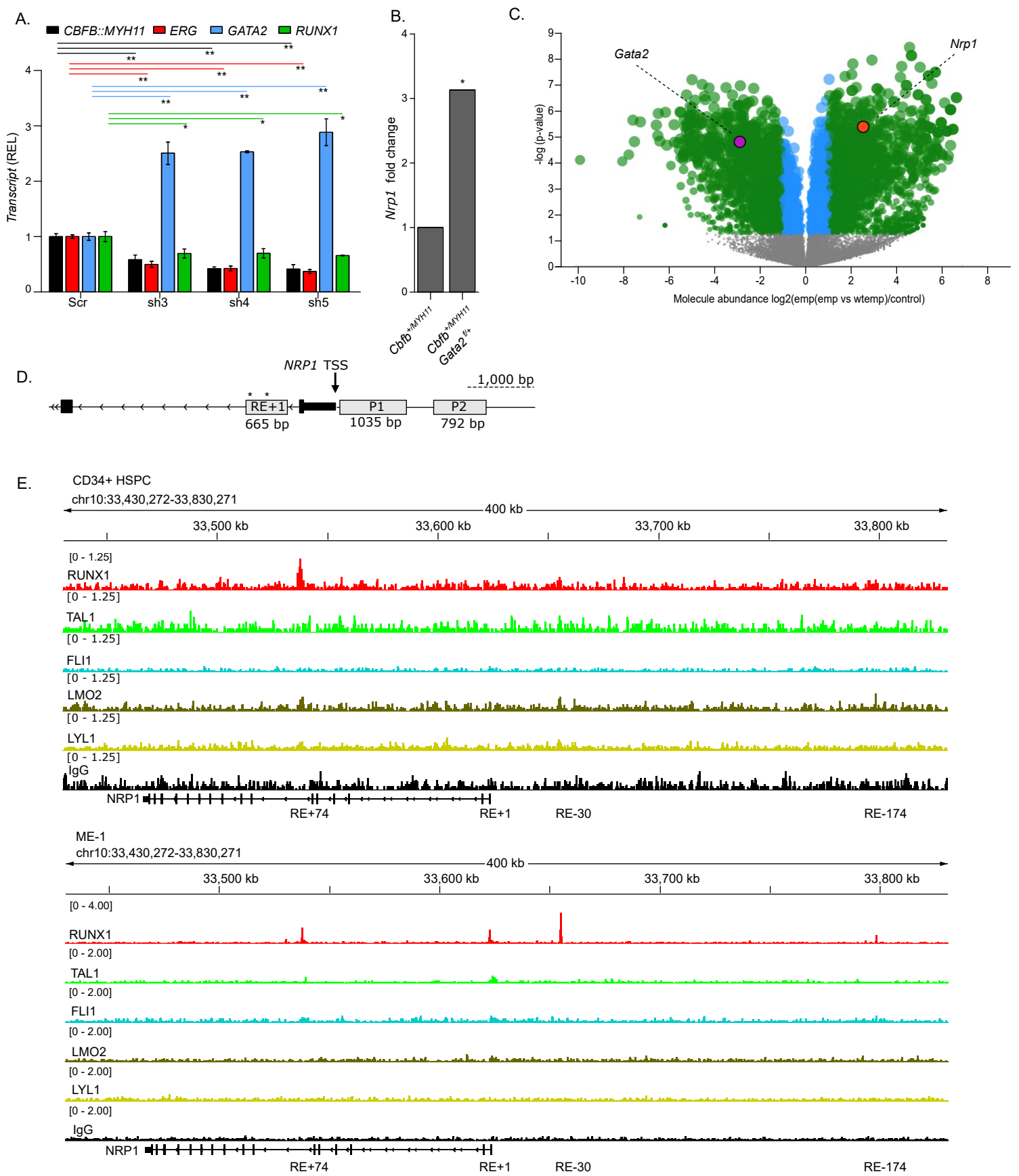
Fig. S6. NRP1 expressing inv(16) AML is sensitive for VEGF inhibitors A.

Heatmap, representing intensity of DEGs on day 7 in Scramble (Scr) and *NRP1*-sh3 (sh3) shRNAs, experiments are shown in biological triplicates. **B.** Gene set enrichment analysis showing the correlation of day 7 *NRP1*-sh3RNA DEGs with HALLMARK-Apoptosis gene-set. **C.**

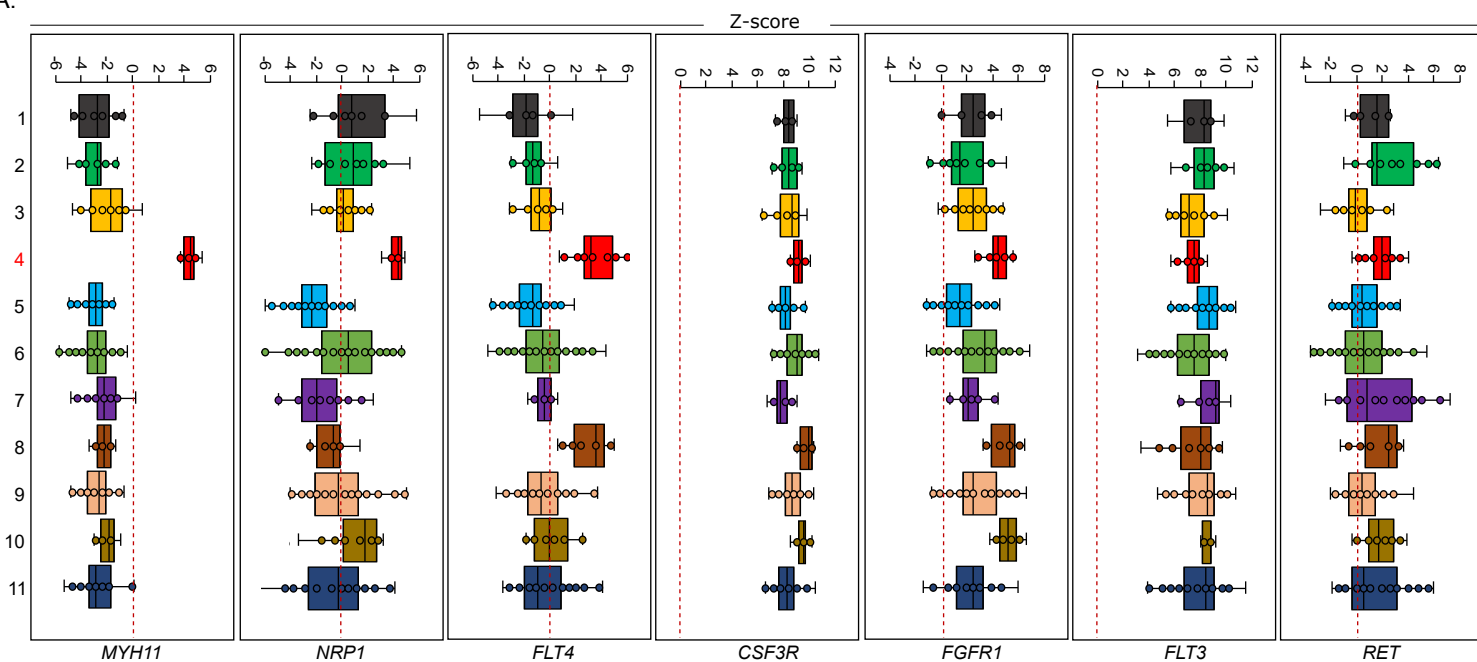
Dose response viability analysis of leukemic cell lines over 14 days treated with blocking antibodies for the extracellular CUB (anti-NRP1^A) or FV/VIII (anti-NRP1^B) domains (0, 5 and 20 µg/mL). Line chart represented with standard deviation of three experiments. **D.** Toxicity(AUC) levels of different inhibitors on primary AML cells expressing different levels of NRP1. **E.** NRP1 levels assessed by FACS analysis (MFI) in normal human CD34+ cord blood cells, normal karyotype AML and inv(16) primary patient samples used in toxicity studies (n=3). **F.** Representation of the experimental design for Axitinib treatment in vivo using *Cbfb*^{+/MYH11}; *Nras*^{+/G12D} leukemic cells. Bar diagrams represented with standard deviation. Significance was calculated using an unpaired t test, *p < 0.05 or **p < 0.005 in Figures **D** and **E**.





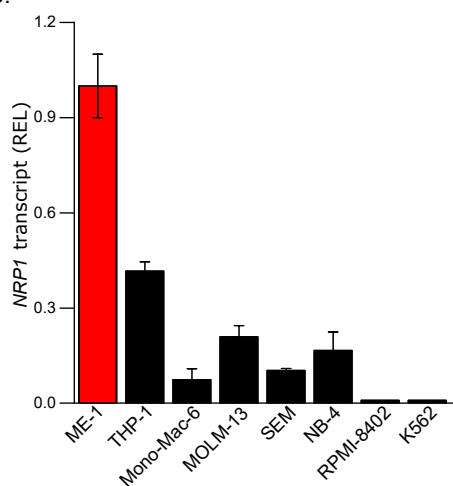


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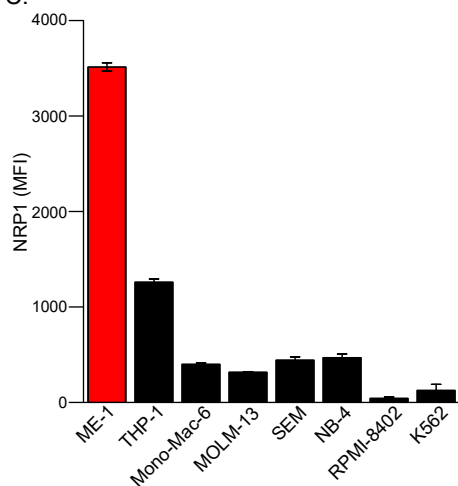


1) Acute Monocytic Leukemia. 2) Acute Myeloid Leukemia. 3) AML with Biallelic Mutations of CEBPA. 4) AML with *inv(16)(p13.1q22)*. 5) AML with Mutated NPM1. 6) AML with Myelodysplasia-Related Changes. 7) AML with Recurrent Genetic Abnormalities. 8) AML with *t(8;21)(q22;q22.1)*. 9) AML, NOS. 10) APL with PML::RARA. 11) Therapy-Related Myeloid Neoplasms.

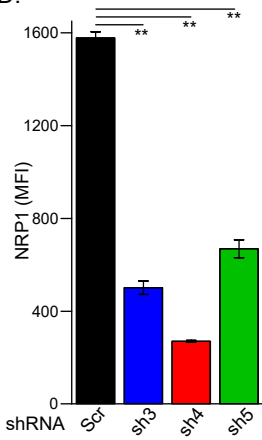
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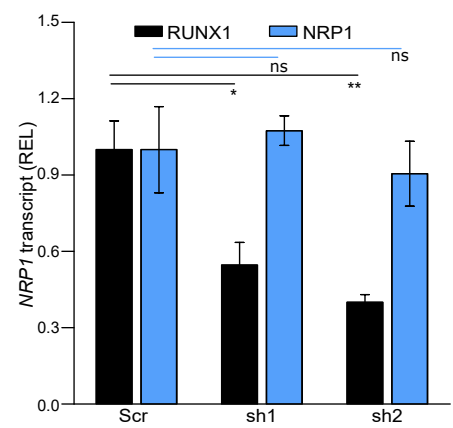
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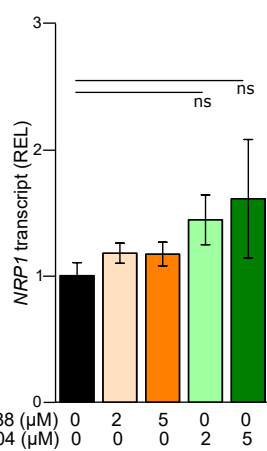
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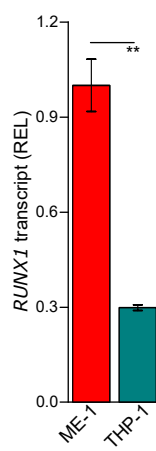
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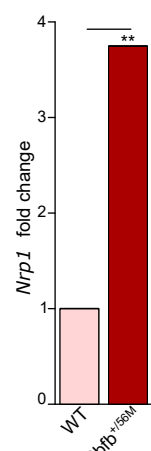
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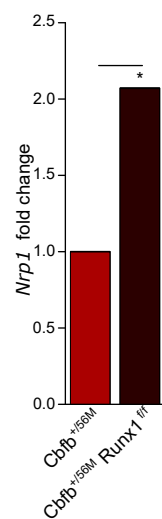
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H.



I.



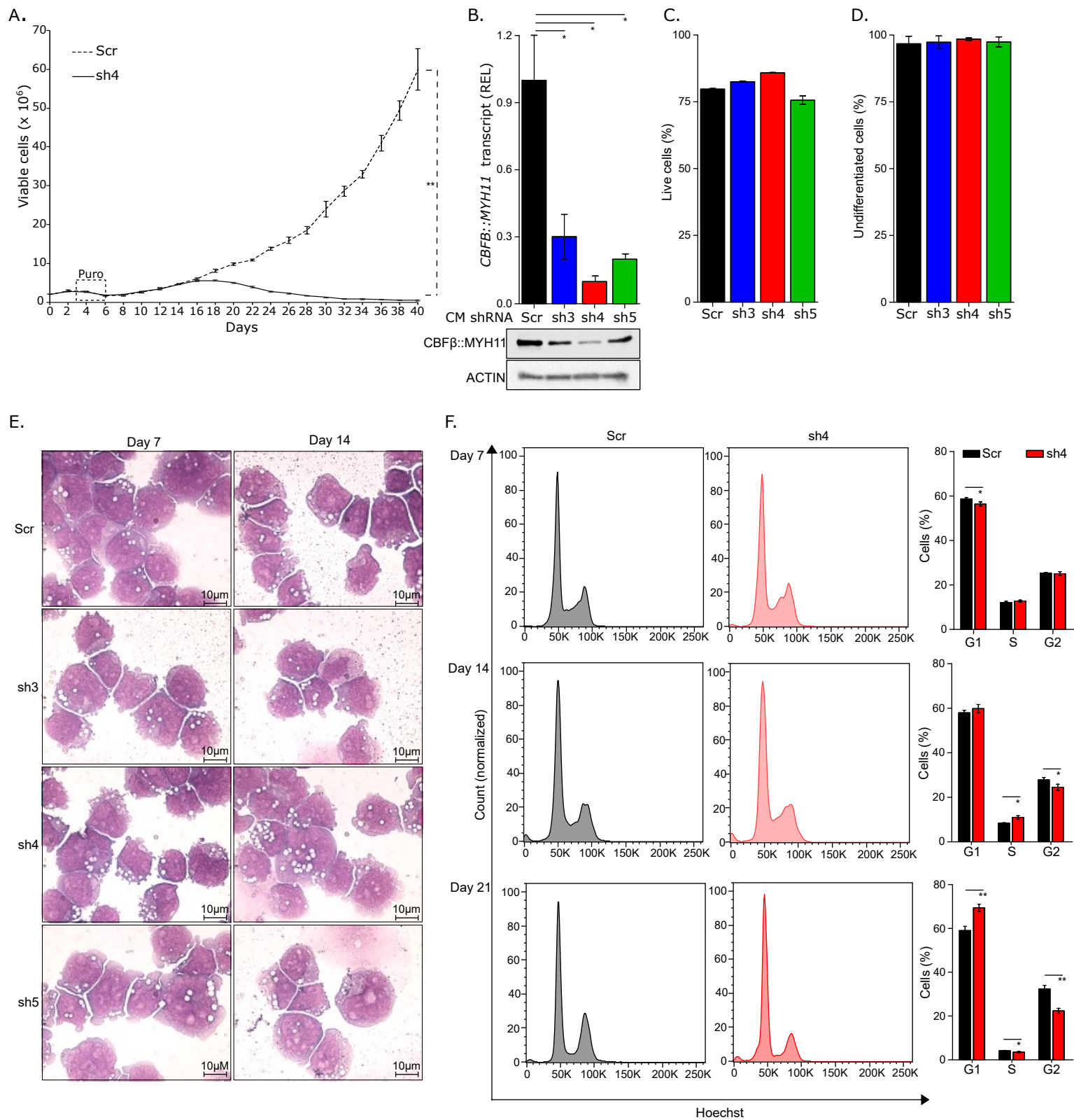


Table S1 - Oligo Sequences used in this study

shRNA hairpin		
Name	Forward	Reverse
Scramble sh	CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGGACTTAACCTTAGGTTTTTG	AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGGACTTAACCTTAGG
MYH11 sh3	CCGGGTCTGGAGGACGTAGAGTTATCTCGAGATAACTCTACGTCCTCCAGACTTTTTG	AATTCAAAAAGTCTGGAGGACGTAGAGTTATCTCGAGATAACTCTACGTCCTCCAGAC
MYH11 sh4	CCGGAGAGACAGCTTCACGAGTAGTCTCGAGCATACTCGTGAAGCTGTCTCTTTTTTG	AATTCAAAAAGAGACAGCTTCACGAGTAGTCTCGAGCATACTCGTGAAGCTGTCTCT
MYH11 sh5	CCGGGACAAAGAGCTGAAGGAAATCCTCGAGGATTTCTCTCAGCTTCTGTCTTTTTG	AATTCAAAAAGACAAAGAGCTGAAGGAAATCCTCGAGGATTTCTCTCAGCTTCTGTCT
NRP1 sh3	CCGGCAGCCTTGAATGCACCTTATATCTCGAGATAAAGTGCATTCAAGGCTGTTTTTG	AATTCAAAAACAGCCTTGAATGCACCTTATATCTCGAGATAAAGTGCATTCAAGGCTG
NRP1 sh5	CCGGGTATGGTGTCTGGACTTATTTCTCGAGAAATAAGTCCAGACACCATACTTTTTG	AATTCAAAAAGTATGGTGTCTGGACTTATTTCTCGAGAAATAAGTCCAGACACCATAC
ERG sh1	CCGGCGTCTCAGTTAGATCCTTATCTCGAGATAAGGATCTAACTGAGGACGTTTTTG	AATTCAAAAACGTCTCAGTTAGATCCTTATCTCGAGATAAGGATCTAACTGAGGACG
ERG sh2	CCGGATCTGGGCACCTTACTACTAAACTCGAGTTTAGTAGTAAGTGCCCAAGATTTTTG	AATTCAAAAATCTGGGCACCTTACTACTAAACTCGAGTTTAGTAGTAAGTGCCCAGAT
RUNX1 sh1	CCGGGAACCAAGTTGCAAGATTTAACTCGAGTTAAATCTTGCAACCTGGTTCTTTTTG	AATTCAAAAAGAACCAAGTTGCAAGATTTAACTCGAGTTAAATCTTGCAACCTGGTTT
RUNX1 sh2	CCGGGAACCACTCCACTGCCTTTAACTCGAGTTAAAGGCAGTGGAGTGTTCTTTTTG	AATTCAAAAAGAACCACTCCACTGCCTTTAACTCGAGTTAAAGGCAGTGGAGTGTTT
GATA2 sh1	CCGGCCGGCACCTGTTGTGCAAAATCTCGAGAAATTCACAAACAGGTGCCGTTTTTG	AATTCAAAAACCGGCACCTGTTGTGCAAAATCTCGAGAAATTCACAAACAGGTGCCG
GATA2 sh2	CCGGCTACAAGCTGCACAAATGTTAACTCGAGTTAACATTGTGCAGCTTGAGTTTTTG	AATTCAAAAACACAAGCTGCACAAATGTTAACTCGAGTTAACATTGTGCAGCTTGTA
RT-PCR primers		
Name	Forward	Reverse
NRP1	GGGAAGACTGGATCACCATAAA	GGAATACTGCAACCACAACATC
CBFB-MYH11	GTATGGGCTGTCTGGAGTTT	CGCTTTCATCTCCTCCATCTG
CSF3R	AGAACATCAGGCCCTTTCAG	GGGAGCCATTCTTGAGAGTAG
FGFR1	GAGGCTACAAGTCCGTTATG	GATGCTGCCGTACTCATTCT
FLT3	GTCCTGTTTCTCGGATGGATAC	TCCAGACTCCTTCTGTGATCT
FLT4	TGGAAGGCTCCGAGGATAA	TACAGAAAGATGAGGAGGAGGAG
RET	CATCAGCAAAGACCTGGAGAA	GAGAGGCCGTCGTCATAAAATC
KIT	GTCATCAGCCACCATCCTATT	AGTCCATACCTCCCTCTCTTT
IGF1R	TTCTCCCTTTCTCTCTCTCTC	GACAGCCACTTCCTCAAATC
RUNX1	CTGCTCCGTGCTGCCTAC	AGCCATCACAGTGACCAGAGT
ERG	GCTGCTCAACCATCTCCTTCC	AAGGCGGCTACTTGTGGTC
ERK	AGAGAACCCTGAGGGAGATAAA	CGATGGTTGGTGCTCGAATA
ACTIN	AGAAAACTCGGCACCACACC	AGAGGCGTACAGGGATAGCA
GATA2	CGACAACCAACCACTTAT	GTGGCCTGTTAACATTGTG
Nrp1	TAACAATGGCTCTGACTGGA	CGAAGCTCAGGTGTGTCATAG
Actin	CGAGGCCACAGCAAGAGAG	CGGTTGGCCTTAGGGTTACG
ChIP-q-PCR primers		
Name	Forward	Reverse
NC	GCAGCAAGAGGTTGTGTTTG	ACGTGTGCACAGTGAGAAA
RE+74	TCCACAAGAGGGCTGATTTAC	GCCTTCACTTATTCTGGAGGAG
RE+1	CAGTCATACTCCAGACAGATTACG	GGTTTGTGGTTGATGAAACTC
RE-30	TGAAGCACACTCTGCATTAGTC	CAGGCAGCAAAACCACTATGA
RE-174	CCACAGTTTAGTGCTCCTTAC	ACCTAGCACATTGGATCAAGAC
P1	GCTTCTCGCAACTTCAAATCC	CAGGATGGCGCGATGAG
P2	CGAGCGGCATCATCAGATTTA	TGCCAGGTCTCTGAGAGG
CRISPR/Cas9 guide RNAs for gene editing		
Name	Sequence	
sgAAVS1	GTCCCTCCACCCACAGTG	
sg9NRP1	GGACGGGGAGAGCACTCGGA	
sg10NRP1	GCTCGCTCTGCACCCCTAGA	
sg11NRP1	AAGTTACATAGTATCAATAC	
sg12NRP1	TAATAAGAGACCGCAAGTGG	
sg1MYH11	CCACGGAGGACGCCAACTG	
sg2MYH11	CAGCTACGCAAACTGCAGGT	
sgRosa	CTCCAGTCTTTCTAGAAGAT	
sg1Nrp1	TGAATGGCTAATCCAAGCTC	
sg2Nrp1	AGCCATTCACACTTCTCACT	
sg3Nrp1	GTCTCTGACTATGAGACACA	
sg4Nrp1	ATGAAGGCGGCCGCTGTGG	
Regulatory elements related oligos		
CRISPR/Cas9 guide RNAs for enhancer deletion		
Name	5' end	3' end
NC	CAACCTCTTGCTGCCCACTG	GATTATGGTGCAAGTTGAGTG
RE+1	GGTATGAGGCCCTCCAATTG	ACAGCTCTGTCTGTAAACAG
RE-30	GGCAGCAGAGCTTCTGAAAG	CCTTCAAAACCATCTCCCT
RE-174	ACAGTATTCACTATTGACGT	ATGAAAACATTCTACCCCA
Primers to detect the efficiency of enhancer deltion		
Name	Forward	Reverse
NC	CTTCTCTAACACCTCACCTGATT	TCCCAAAGTGCTGGGATTAC
RE+1	ATATTAGAGAGATTAACTTCCATC	TCGCAACGGTAAGTGAC
RE-30	TCAGTAGCAGAAGCAGAGAG	GCCTCCTTGATGTTGCATTA
RE-174	GAAGTCCTCAGGGAAGATACAG	CCTTTCCCACTCTCACTTAG

Table S2 - AML Patient information

Patient sample information related to Figure 2C - NRP1 expression in AML samples

ID	Karyotype	Mutations	sex	fab_class	age
3876	inv(16)	FLT3-835, EZH2	male	ni	51
5296	inv(16)	NF1, SF3B1, NOTCH1	female	ni	54
1520	inv(16)	-	male	ni	67.5
A-401	NK	ni	ni	ni	ni
A-215	NK	ni	ni	ni	ni
A-074	NK	ni	ni	ni	ni

Patient sample information related to Figure 2F - NRP1 expression in AML samples after MYH11 editing

ID	Karyotype	Mutations	sex	fab_class	age
C0018791	inv(16)	ni	ni	ni	ni
B0013014	inv(16)	ni	ni	ni	ni

Patient sample information related to Figure 3C and 3D - H3K27ac and ATAC-seq analysis

ID	Karyotype	Mutations	sex	fab_class	age
1595	NK	FLT3-ITD, ASXL1	female	M1	43
1747	NK	NPM1, FLT3-ITD, DNMT3A	male	M2	59
2239	NK	NPM1, FLT3-TKD, DNMT3A, NRAS	female	M5	48
2257	NK	FLT3-TKD, DNMT3A, NRAS, PTPN1	male	M1	40
3320	NK	FLT3-ITD	male	M1	51
3327	NK	CEBPA-DM	female	M1	59
3492	NK	FLT3-ITD, FLT3-TKD, IDH2, NRAS, PTPN1	female	M2	67
4333	NK	FLT3-ITD, WT1	male	M5	31
2172	inv(16)	FLT3-TKD, KIT, NRAS	male	M4	18
2274	inv(16)	-	female	M4	46
2287	inv(16)	FLT3-TKD, NRAS, KRAS	male	M4	16
3309	inv(16), del7q, complex	FLT3-TKD, NRAS, KRAS	male	M4	32
3324	inv(16)	KIT	male	M5	26
3487	inv(16)	NRAS, KRAS, RAD21	female	RAEB-t	72
5353	inv(16)	NRAS	female	M4	74
7315	inv(16)	NRAS	male	M4	45

Patient samples related with Figure 6E - Axitinib treatment

ID	Karyotype	Mutations	sex	fab_class	age
4721	NK	-	female	M1	40.8
4891	NK	-	male	M5	59.8
5768	NK	FLT3-ITD	male	ni	69.8
6767	inv(16)	-	female	ni	61
3340	inv(16)	IDH2, KIT, NRAS	male	ni	31
3876	inv(16)	FLT3-835, EZH2	male	ni	51

NK: normal karyotype; ni: no information.

[illegible]

Table S4 - Estimation of ATAC-seq and H3K27Ac ChIP-seq peaks at NRP1 locus, in AML patient samples

ATAC-seq

Element	chr	start	end	Fold	inv16	non_inv16	SD_inv16	SD_noninv16	p.value
1	chr10	33482227	33482652	1.01358694	31.2600857	29.1965429	7.49128362	10.4200092	0.76797887
2	chr10	33537449	33538004	2.14863515	51.6578429	21.5292714	10.2502297	6.61399926	5.0368E-07
3	chr10	33562236	33562769	1.1118072	31.7534714	22.9600714	9.66084265	11.5096154	0.19140973
4	chr10	33623591	33624559	2.27677083	156.858371	60.2112286	32.457985	33.1561727	9.8547E-07
5	chr10	33644192	33644686	1.42541437	38.0459	23.2549571	7.08665108	12.4556357	0.01063306
6	chr10	33654330	33655297	1.78911728	164.949314	83.1524571	42.315519	35.6831361	0.0001466
7	chr10	33701321	33702299	2.68950257	58.2371857	17.6971	22.2725155	13.7789664	2.8735E-06
8	chr10	33798080	33798645	1.51625601	79.6210857	42.4650714	7.15669105	25.0454513	0.0067919

ChIP-seq

1	chr10	33537179	33539659	3.47359862	102.524613	26.7203625	37.0953377	11.5867932	1.7268E-10
2	chr10	33622211	33625966	7.67386233	448.417113	51.1015125	203.40437	20.8616829	4.5841E-28
3	chr10	33653786	33656174	1.88474015	312.279413	138.646863	107.909678	101.187114	0.00157984
4	chr10	33701718	33702794	4.27738905	58.99045	11.4494875	29.4631716	4.54967313	2.7293E-10
5	chr10	33795185	33799392	3.60231743	559.012388	131.032338	206.87578	87.1908565	1.2894E-09

					inv16				
FDR	width	feature	insideFeature	symbol	2172	2274	2287	3309	3324
0.90942764	426	NRP1	inside	NRP1	22.0537	31.3879	23.2373	42.5328	38.8775
5.1737E-05	556	NRP1	inside	NRP1	60.0963	42.9519	52.7308	48.3328	69.4832
0.4500385	534	NRP1	inside	NRP1	36.94	42.9519	17.8749	32.8663	40.5319
8.4649E-05	969	NRP1	inside	NRP1	179.7377	122.2477	185.8985	119.8652	197.6964
0.07281093	495	NRP1	upstream	NRP1	41.3507	37.9959	37.5372	27.0663	31.4329
0.00366607	968	NRP1	upstream	NRP1	187.4565	145.3757	227.9044	104.3988	124.9044
0.00019229	979	NRP1	upstream	NRP1	77.188	41.2999	60.7745	44.4661	34.7416
0.05377619	566	NRP1	upstream	NRP1	82.1501	80.9478	75.9681	75.3991	70.3104

1.1073E-08	2481	NRP1	inside	NRP1	111.7006	166.6227	108.7397	101.702	126.4339
1.2046E-24	3756	NRP1	overlapStart	NRP1	478.6158	357.2856	504.9943	237.8262	302.5009
0.00753082	2389	NRP1	upstream	NRP1	458.8208	348.9959	442.3308	209.6625	185.4712
1.63E-08	1077	NRP1	upstream	NRP1	82.008	76.2651	55.2913	10.9525	30.3023
6.1708E-08	4208	NRP1	upstream	NRP1	534.4661	572.8174	429.4295	256.6019	387.1384

NORMALIZED COUNTS									
		non-inv16							
3487	5353	1595	1747	2257	3320	3327	3492	4333	X2172
29.7031	31.0283	18.7337	38.4742	20.3536	19.3235	35.6891	44.5087	27.293	40
39.7436	48.2663	31.6132	25.6495	15.6566	22.544	11.1528	22.2544	21.8344	109
20.081	31.0283	19.9046	24.2245	20.3536	46.1616	7.807	22.2544	20.0148	67
130.5263	162.0368	29.2715	99.0354	36.0102	61.191	30.1127	111.2719	54.5859	326
42.672	48.2663	17.5629	18.5246	31.3132	13.9558	7.807	44.5087	29.1125	75
185.3306	179.2748	36.2966	96.1855	97.071	46.1616	60.2253	122.3991	123.7281	340
97.4763	51.7139	10.5377	10.6873	15.6566	10.7353	4.4611	44.5087	27.293	140
79.4871	93.085	14.0503	71.961	42.2728	42.941	8.9223	73.4394	43.6687	149

97.4177	48.6642	58.9161	38.2965	23.8007	10.5096	18.6584	36.6822	34.7944	37.8009
498.8458	320.0608	887.2075	20.7895	62.7933	33.1881	45.9283	55.0233	48.3256	91.3522
248.5831	228.3475	376.0235	42.6733	105.837	93.4797	307.1455	50.9475	56.0577	248.8559
80.6215	41.1774	95.3055	10.9419	7.596	6.6376	14.3526	20.379	13.5312	7.8752
693.6812	681.299	916.6656	39.3907	169.6431	64.1636	279.8756	89.6677	69.5889	233.1055

RAW COUNTS									
inv16						non-inv16			
X2274	X2287	X3309	X3324	X3487	X5353	X1595	X1747	X2257	X3320
19	26	22	47	71	9	16	54	13	18
26	59	25	84	95	14	27	36	10	21
26	20	17	49	48	9	17	34	13	43
74	208	62	239	312	47	25	139	23	57
23	42	14	38	102	14	15	26	20	13
88	255	54	151	443	52	31	135	62	43
25	68	23	42	233	15	9	15	10	10
49	85	39	85	190	27	12	101	27	40

13.2202	131	191	41	49	226	35	14	5	16
51.4118	644	406	253	127	543	267	156	483	2
204.1783	624	413	219	109	336	123	101	199	14
10.2824	108	81	24	1	49	40	10	43	0
102.8236	720	672	208	128	704	385	338	502	10

X3327	X3492	X4333
32	20	15
10	10	12
7	10	11
27	50	30
7	20	16
54	55	68
4	20	15
8	33	24

17	1	18	9	0	17	0
92	40	56	14	0	44	17
171	158	413	11	1	147	128
3	7	15	5	0	2	0
295	99	371	33	0	134	34

Table S5 - p-values for figures and supplementary figures

Fig.1A

test samples

p-value

Scr vs sh3

6.28E-08

Scr vs sh4

1.43E-08

Scr vs sh5

0.000144

Sup.Fig.1A

test samples

p-value

Scr vs sh4

0.00004

Fig.1B

test samples

p-value

Scr vs sh3

0.000789

Scr vs sh4

4.4E-06

Scr vs sh5

0.000791

Sup.Fig.1B

test samples

p-value

Scr vs sh3

0.025578

Scr vs sh4

0.018623

Scr vs sh5

0.022371

Sup.Fig.1F

test samples

p-value

D7(G1)

0.0299

D14(S)

0.022583

D14(G2)

0.030492

D21(G1)

0.002117

D21(S)

0.033391

D21(G2)

0.001472

Fig.2A

test samples

p-value

CBFB-MYH11

0.000149

CSF3R

0.001248

FGFR1

0.021556

FLT3

7.14E-05

FLT4

0.000219

NRP1

0.000603

RET

0.00131

Fig.2C

test samples

p-value

Human (MF)

0.030315

Human (RT-PCR)

0.019628

Fig.2D

test samples

p-value

Mouse: WT vs CHN (MF)

0.003897

Mouse: MLL-AF9 vs CHN (MF)

0.002074

Mouse: WT vs CHN (RT-PCR)

4.83E-05

Mouse: MLL-AF9 vs CHN (RT-PCR)

0.000173

Fig.2E

test samples

p-value

Scr vs sh3

0.001138

Scr vs sh4

0.003547

Scr vs sh5

0.003327

Fig.2F

test samples

p-value

sgAAVS1 vs sg1 (P1)

0.000235

sgAAVS1 vs sg2 (P1)

6.4E-06

sgAAVS1 vs sg1 (P2)

4.26E-05

sgAAVS1 vs sg2 (P2)

0.00021

Fig.2G

test samples

p-value

RUNX1: Scr vs sh1

0.010976

RUNX1: Scr vs sh2

0.004827

NRP1: Scr vs sh1

0.009309

NRP1: Scr vs sh2

0.021185

Fig.2H

test samples

p-value

DMSO vs AI-10-104 (2uM)

0.03926

DMSO vs AI-10-104 (5uM)

0.003359

Fig.2I

test samples

p-value

DMSO vs AI-10-104 (Scr)

0.001179

DMSO vs AI-10-104 (sh4)

0.005475

Scr vs sh4 (DMSO)

0.00324

Sup.Fig.2D

test samples

p-value

Scr vs sh3

1.3E-06

Scr vs sh4

0.000102

Scr vs sh5

1.6E-06

Sup.Fig.2E

test samples

p-value

RUNX1: Scr vs sh1

0.008095

RUNX1: Scr vs sh2

0.004922

Sup.Fig.2G

test samples

p-value

ME-1 vs TFP-1 (RUNX1)

0.004165

Sup.Fig.2H

test samples

p-value

WT vs Cbfb+56M (Nrp1)

1.86E-70

Sup.Fig.2I

test samples

p-value

Cbfb+56M vs Cbfb+56M, Runx1 fl (Nrp1)

0.017468

Fig.3A

test samples

p-value

RE+1

0.011584

RE-30

0.032086

RE-174

3.18E-05

Fig.3C

test samples

p-value

RE+74

1.73E-10

RE+1

4.58E-08

RE-30

0.000147

RE-174

1.29E-09

Fig.3D

test samples

p-value

RE+74

5.04E-07

RE+1

9.85E-07

RE-30

0.000147

RE-174

0.006792

Fig.3E

test samples

p-value

Control vs RE+1

0.000287

Control vs RE-30

5.64E-07

Control vs RE-174

2.55E-07

Fig.3F

test samples

p-value

Control vs RE+1

0.000577

Control vs RE-30

0.001493

Control vs RE-174

0.007107

Fig.4A

test samples

p-value

LY1

0.000132

TAL1

7.19E-31

PLT1

4.76E-28

ERG

4.8E-114

GATA2

<0.00001

Fig.4B

test samples

p-value

ERG: Scr vs sh1

0.040599

ERG: Scr vs sh2

0.005147

NRP1: Scr vs sh1

0.001545

NRP1: Scr vs sh2

0.000229

Fig.4C

test samples

p-value

GATA2: Scr vs sh1

0.0002

GATA2: Scr vs sh2

0.00014

NRP1: Scr vs sh1

0.000649

NRP1: Scr vs sh2

0.00119

Fig.4D

test samples

p-value

RE+74

0.008649

RE+1

0.001235

RE-30

0.017343

RE-174

0.001339

Fig.4E

test samples

p-value

RE+1

0.021963

RE-30

0.042356

RE-174

0.004618

Fig.4F

test samples

p-value

RE+1

0.000447

P2

0.015923

Fig.4G

test samples

p-value

RE+1

0.002588

P1

0.001865

P2

0.049815

Fig.4H

test samples

p-value

RE+1

0.008458

P1

0.005248

Sup.Fig.4A

test samples

p-value

CBFB-MYH11 (Scr vs sh3)

0.003719

CBFB-MYH11 (Scr vs sh4)

0.000259

CBFB-MYH11 (Scr vs sh5)

0.001064

ERG (Scr vs sh3)

0.000746

ERG (Scr vs sh4)

0.000129

ERG (Scr vs sh5)

2.23E-05

GATA2 (Scr vs sh3)

0.003133

GATA2 (Scr vs sh4)

0.000258

GATA2 (Scr vs sh5)

0.003483

RUNX1 (Scr vs sh3)

0.012085

RUNX1 (Scr vs sh4)

0.012459

RUNX1 (Scr vs sh5)

0.021519

Sup.Fig.4B

test samples

p-value

Cbfb+56M vs Cbfb+56M, Gata2

0.007

Fig.5A

test samples

p-value

Scr vs sh3

3.5E-07

Scr vs sh5

2.88E-05

Fig.5B

test samples

p-value

Scr vs sh3

8.76E-08

Scr vs sh5

4.43E-07

Fig.5C

test samples

p-value

AAVS1 vs set1

0.000829

AAVS1 vs set2

0.00564

Fig.5D

test samples

p-value

sgRosa vs set1

0.0002

sgRosa vs set2

0.000299

Fig.5E

test samples

p-value

sgRosa vs set1

0.002

sgRosa vs set2

0.002

Fig.5F

test samples

p-value

sgRosa vs set1

0.016267

sgRosa vs set2

0.023828

Sup.Fig.5A

test samples

p-value

Scr vs sh3

0.000974

Scr vs sh5

0.001964

Sup.Fig.5B

test samples

p-value

Scr vs sh3

0.002319

Scr vs sh5

0.005858

Sup.Fig.5C

test samples

p-value

AAVS1 vs set1

0.000987

AAVS1 vs set2

0.00386

Sup.Fig.5D

test samples

p-value

AAVS1 vs set1

0.000537

AAVS1 vs set2

0.000559

Sup.Fig.5E

test samples

p-value

sgRosa vs set1

0.001561

sgRosa vs set2

0.002034

Fig.6B

test samples

p-value

0 vs 20uM

0.000952

Fig.6C

test samples

p-value

High vs medium

7.73E-05

High vs low

2.27E-27

High vs negative

1.02E-49

Fig.6D

test samples

p-value

Axitinib

0.044295

Levamisole

0.001185

Motesanib

0.004843

Cediranib

0.002074

High vs Medium

0.044295

High vs Low

0.001185

High vs Negative

0.004843

Medium vs Low

0.274282

Medium vs Negative

0.621645

Low vs Negative

0.258554

Fig.6F

test samples

p-value

CD34+ hNPCs vs Inv(16)

0.003625

Fig.6H

test samples

p-value

Vehicle vs Axitinib

0.0002

Fig.6I

test samples

p-value

Vehicle vs Axitinib

0.015

Sup.Fig.6D

test samples

p-value

BEZ-235

0.028

Cs-1040

0.065

Selumetinib

0.0001

Tametinib

0.0001

GSK6906

0.0005

ME-2208

0.0001

INK-4

0.0001

Rapamycin

0.0001

Tofacitinib

0.0001

Sup.Fig.6E

test samples

p-value

Inv(16) vs NK

0.043038

Inv(16) vs CD34+ hNPCs

0.03788