

Supplemental Material and Methods

RNA isolation and reverse transcription. Total RNA was isolated from the peripheral blood mononuclear cells (PBMCs) of healthy donors (HDs) or AML patients (upon depletion of red blood cells using the ACK lysing buffer, from Thermo Fisher Scientific) as well as from isolated CD33⁺ malignant blasts, using the RNeasy Mini Kit (Qiagen) as per the manufacturer's recommendations. RNA concentration and purity were determined using a NanoDrop 2000c (Thermo Scientific), while RNA integrity was assessed using an Agilent 2000 Bioanalyzer (Agilent). Purified RNA samples were stored at -80°C, and only samples with an integrity index ≥ 7 were subsequently used for reverse transcription and RNAseq. The cDNA for the quantification by RT-qPCR was synthesized from total RNA using the iScript cDNA Synthesis Kit (Bio-Rad).

Bulk RNA sequencing and analysis. Sequencing libraries were created using NEBNext Poly(A) mRNA Magnetic isolation Module and NEBNext Ultra II Directional RNA Library Prep kits (both New England BioLabs) and were sequenced by NovaSeq 6000 SP (Illumina). Raw FASTQ sequencing files were aligned to human reference genome (build h19) with bowtie2 (version 2.3.2) and tophat2 (version 2.1). Expression levels as raw “counts” were calculated from aligned reads with mapping quality ≥ 10 using htseq-count (version 0.6.0). Differential gene expression analyses were performed using DESeq2 (version 1.24.0) in R. Heatmaps were assembled using the ComplexHeatmap package in R.

The Cancer Genome Atlas program. The normalized gene expression data for 152 AML patients (after M3 and M7 AML subtypes exclusion) were retrieved from the public database (TCGA) and were used in correlation analyses.

Cell lines. KASUMI-1 and MOLM13 cells were grown in RPMI 1640 Medium containing 4 mM L-glutamine and 4500 mg/L glucose (Gibco), and supplemented with 20% fetal bovine serum (FBS,

from PAA) and 100 U/mL penicillin plus 100 µg/mL streptomycin (Gibco). MV4-11 and K562 cells were cultured in IMDM containing 4 mM *L*-glutamine and 4500 mg/L glucose (Gibco) and supplemented with 10% FBS and 100 U/mL penicillin plus 100 µg/mL streptomycin. PBMCs and CD33⁺ blasts were cultured in RPMI 1640 Medium containing 4 mM *L*-glutamine and 4500 mg/L glucose and supplemented with 10% plasma derived human pooled serum (PHS, from Thermo Fisher) and 100 U/mL penicillin plus 100 µg/mL streptomycin. All cell lines were regularly tested for *Mycoplasma* contamination using the MycoAlert[®] Mycoplasma Detection Kit (Lonza) and Spark reader (Tecan) to detect luminescence. Cell lines were used for experiments 2 to 5 passages from thawing and were maintained at 30-70% confluency.

Multiplex assay. Concentration of IFN-α2 protein in the sera of AML patients was assessed by MAGPIX system (Luminex) using Magnetic Bead Panel HCYTOMAG-60K, 6-plex (Merck) according to manufacturer's protocol.

ELISA. Concentration of IFN-β in cell culture supernatant was determined using High sensitivity human IFN Beta TCM ELISA (PBL) or by Human IFN-beta DuoSet ELISA (R&D) according to manufacturer's protocol. Sample absorbance was measured by Sunrise Microplate reader (Tecan).

[³H]-thymidine incorporation assay. Inhibition of cell proliferation was determined using the [³H]-thymidine incorporation assay. The cells (2 x 10⁴/well) were seeded into a 96-well flat-bottom tissue culture plate (Nunc) and were cultivated in growth medium in final volume of 250 µL. The plates were incubated in a 5% CO₂ atmosphere at 37°C. Triplicates to pentaplets were used for each test condition. At the beginning of the assay cells were incubated with rIFN-α and rIFN-β (500 pg/mL) for 7 days and each well was pulsed with 1 µCi (37 kBq) of [³H]-thymidine for the last 6–8 h of total incubation time. The cells were then harvested on glass fiber filters (Perkin Elmer) using a cell

harvester (Tomtec) and the radioactive emission was measured in a scintillation counter (1450 Microbeta TriLux, Wallac).

Colony-forming assays. For colony-forming assays, 5×10^3 CD34⁺ enriched cells were cultured in 6-well plates in a mixture of 300 μ L IMDM plus 2700 μ L of the semi-solid methylcellulose-based media MethoCult™ H4534 Classic Without EPO (StemCell Technologies). Colonies were quantified according to the manufacturer's guidelines. LSCs were cultured and maintained using a specific medium that preserves the LSC phenotype: StemSpan™ Leukemic Cell Culture Kit (StemCell Technologies).

Preparation of knockout cell line. KASUMI-1^{IFNAR2^{-/-}} cell lines was prepared by Crispr-Cas9 guided gene targeting technology. Two gRNA were designed with aim to delete exon three, specifically g353 targeting exon two and g1716 targeting 3' end of exon three (**Supplemental Table 4**). KASUMI-1 cells were electroporated with mixture of gRNAs incorporated in pSpCas9(BB)-2A-GFP (PX458) expression vector for dual expression of Cas9 and gRNA. Two days after electroporation, single GFP⁺ cells were single cell sorted into 96wp coated with bone marrow cells of NSG mice and expanded. Thereafter, genomic DNA from single clones was extracted and PCR amplification of the genomic gRNA target sites was performed. Of all 14 clones analyzed, none exhibited deletion of exon three that would otherwise result in the presence of 1.4kb PCR fragment, the only PCR fragment detected was wt-like 2.2kb (**Supplemental Fig. 2A**). Next, we chose several clones and performed sequencing of gRNA target regions. In contrast to parental KASUMI-1 cells, some clones provided consistent sequence reads only to the expected break site (DSB) indicating presence of multiple sequences downstream of DSB caused by presence of indels (**Supplemental Fig. 2B**). Several clones were analyzed for expression of IFNAR2 by FACS using APC-labelled anti IFNAR2 antibody (**Supplemental Table 3**). Several clones do not express membrane bound IFNAR2 compared to KASUMI-1 cells (**Supplemental Fig. 2 C**). For further studies, clone D was

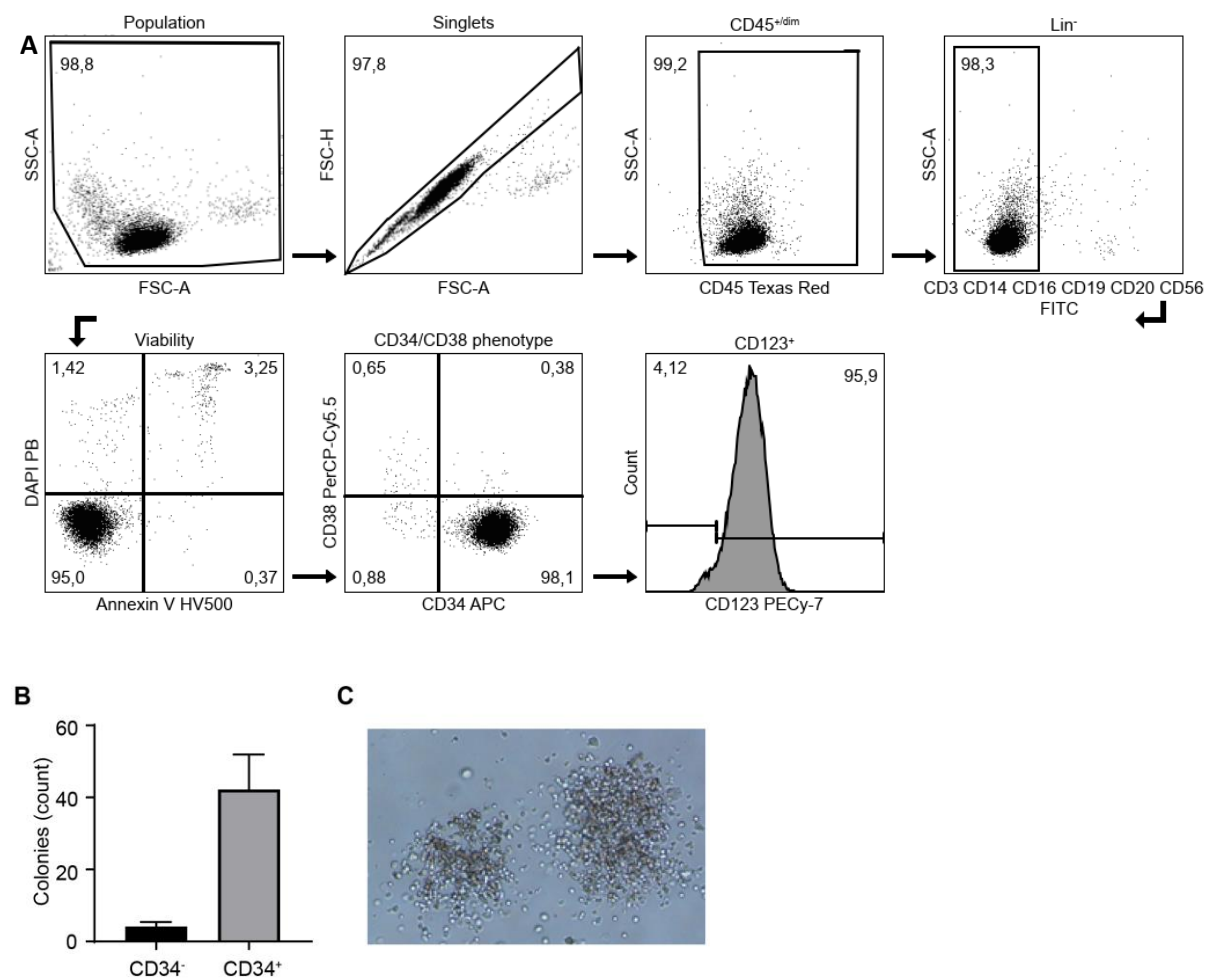
selected based on similarity in response to daunorubicin (**Supplemental Fig. 2D**), proliferation rates (data not shown) to parental cell line and no expression of IFNAR2 protein as shown by flow cytometry. Confirmation that both IFNAR2 alleles were successfully targeted, we performed TA cloning (pGEM®-T Easy, Promega) followed by sequencing of gRNA target regions of clone D. In total we analysed 13 clones and identified two different DNA repair mechanisms for both g352 and g1716: for g352 insertion of GG and indel (deletion of six nucleotides combined with insertion of CTC), for g1716 insertions, CGTACAAC and GCCA in DSB site were found. Moreover, sequencing revealed that all four mutations were combined with each other resulting in 4 different clones. We therefore presumed that established culture originates from two clones, however none of them has DNA sequence reads at gRNA target region corresponding to parental WT cells. All insertions have changed reading frame or have removed splice donor site of exon 3 (CGTACAAC insertion in g1716) resulting in creation of premature stop codon downstream, indel in g352 keeps the reading frame, however, it was combined with stop-codon creating mutations in g1716. We can conclude that, that in clone D, IFNAR2 gene was homozygously targeted, resulting in complete gene knock-out.

Preparation of cell lysates and immunoblotting analysis

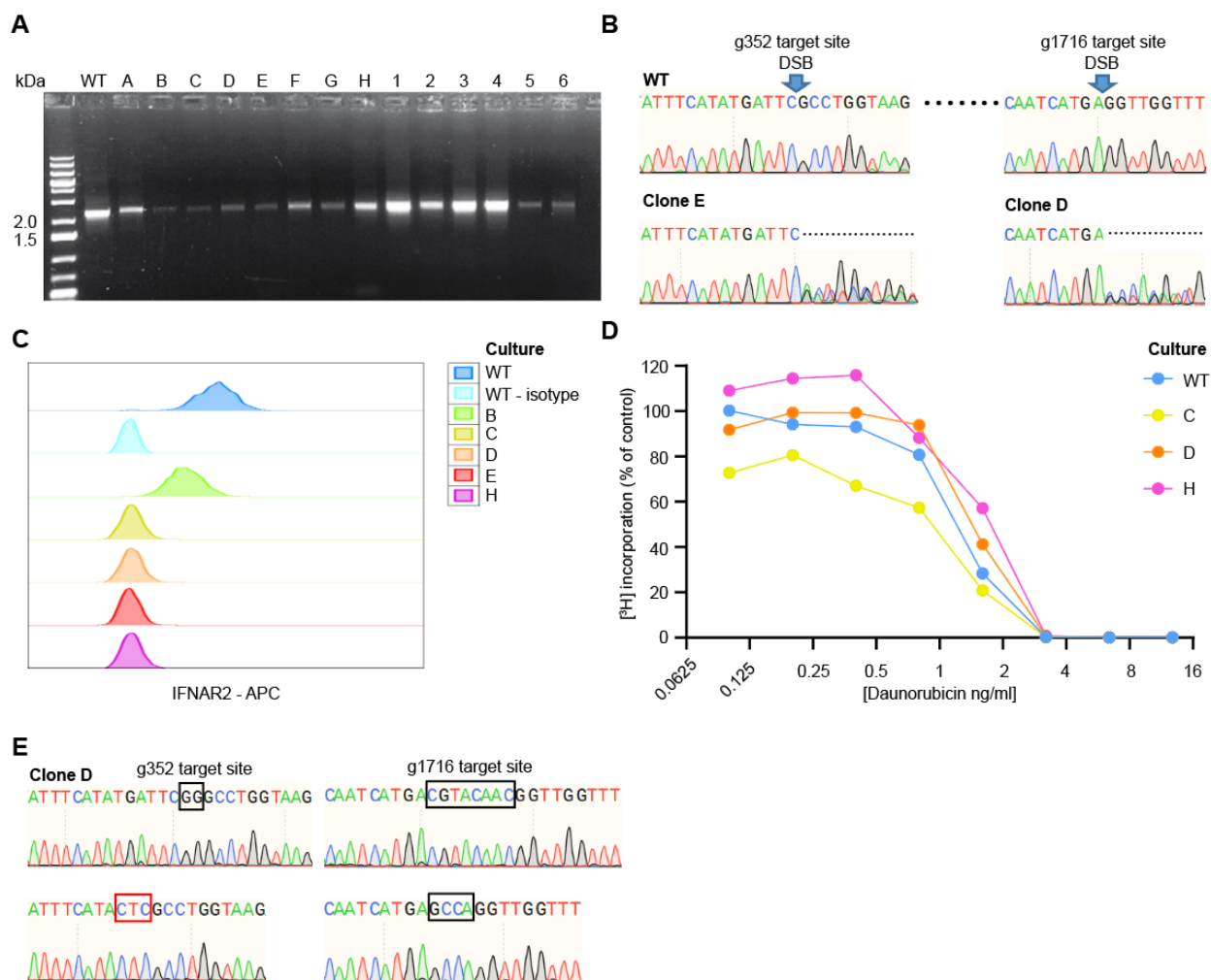
Cells were harvested and washed with ice-cold PBS and subsequently lysed in sample buffer (300 mM Tris pH 6.8, 5% SDS, 50% Glycerol, 360 mM β -Mercaptoethanol, 0.05% Bromophenol blue). Proteins were separated by 11% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to 0.45 μ m nitrocellulose membranes (Biorad). Efficiency of a protein transfer was checked by Ponceau-S staining (Abcam). The membranes were blocked in 5% nonfat dry milk in TBST buffer (50 mM Tris, 150 mM NaCl, 0.05% Tween 20) for 1h at room temperature and incubated with primary antibody overnight at 4°C. Membranes were washed in TBST buffer and incubated for 1h at room temperature with relevant horseradish peroxidase-conjugated secondary antibodies. SuperSignal West Dura Extended duration detection system (ThermoFisher) was used for a protein visualization, performed by imaging system ChemiDoc (Biorad).

Mice and in vivo experiments. Rag2^{-/-} mice on BALB/c background (*H-2^d*) used for *in vivo* experiments were 8-20 weeks old and with body weight at least 19 g. Mice were obtained from the Institute of Microbiology of the Czech Academy of Sciences (Prague, Czech Republic) breeding colony. Food and water were given *ad libitum*. All animal work strictly followed the protocol approved by the Institutional Animal Care and Use Committee of the Czech Academy of Sciences, with all experiments conducted in compliance with local and European guidelines. Rag2^{-/-} mice were i.v. inoculated with 2.5 x 10⁶ KASUMI-1^{WT} or KASUMI-1^{IFNAR2^{-/-}} cells in 300 µL PBS on day 0. Human rIFN-β was i.p. injected on days 13, 14, 15 and 16 twice daily (early morning and late afternoon) in 250 µL PBS using 1.5 µg/dose, i.e. 3 ug per mouse each day. Daunorubicin was administered via tail vein injection on days 15, 17 and 19 in 300 µL PBS using 4 mg/kg per one dose. Body weight of experimental mice was recorded 2-3 times a week throughout the whole experiment.

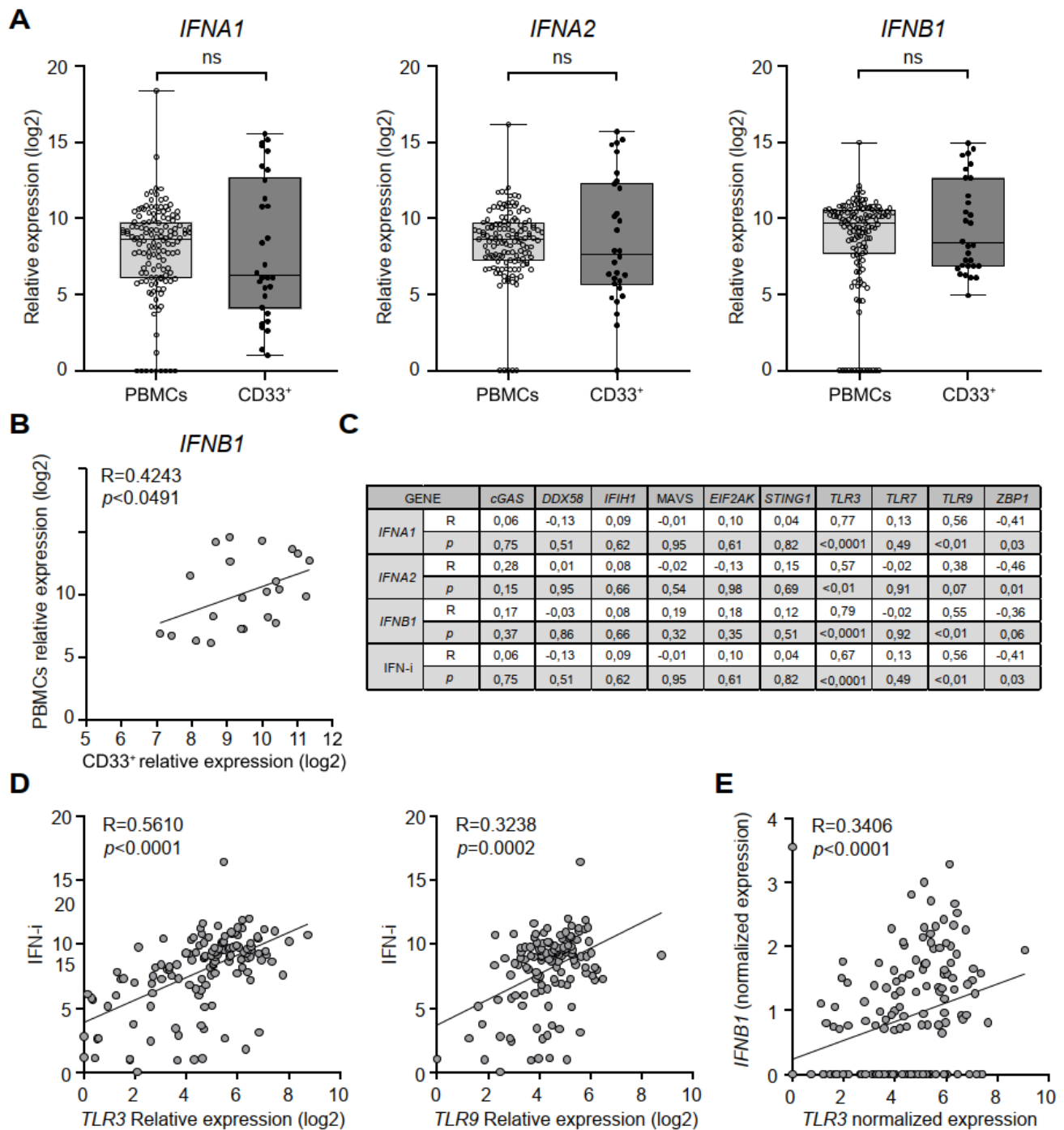
Supplemental Figures



Supplemental Figure 1. Isolation of leukemic stem cells (LSCs). (A) Gating strategy for CD45^{dim}, Lin⁻ (CD3⁻CD14⁻CD16⁻CD19⁻CD20⁻CD56⁻), CD34⁺, CD38^{+/-} and CD123^{+/-dim} LSCs phenotype on isolated CD34⁺ cells of AML patients. (B) Number of colonies formed by CD34⁺ versus CD34⁻ fraction obtained by CD34⁺ magnetic purification from 10 AML patients (study cohort 1) assessed by bright field microscopy (C) The representative image of LSC colonies.

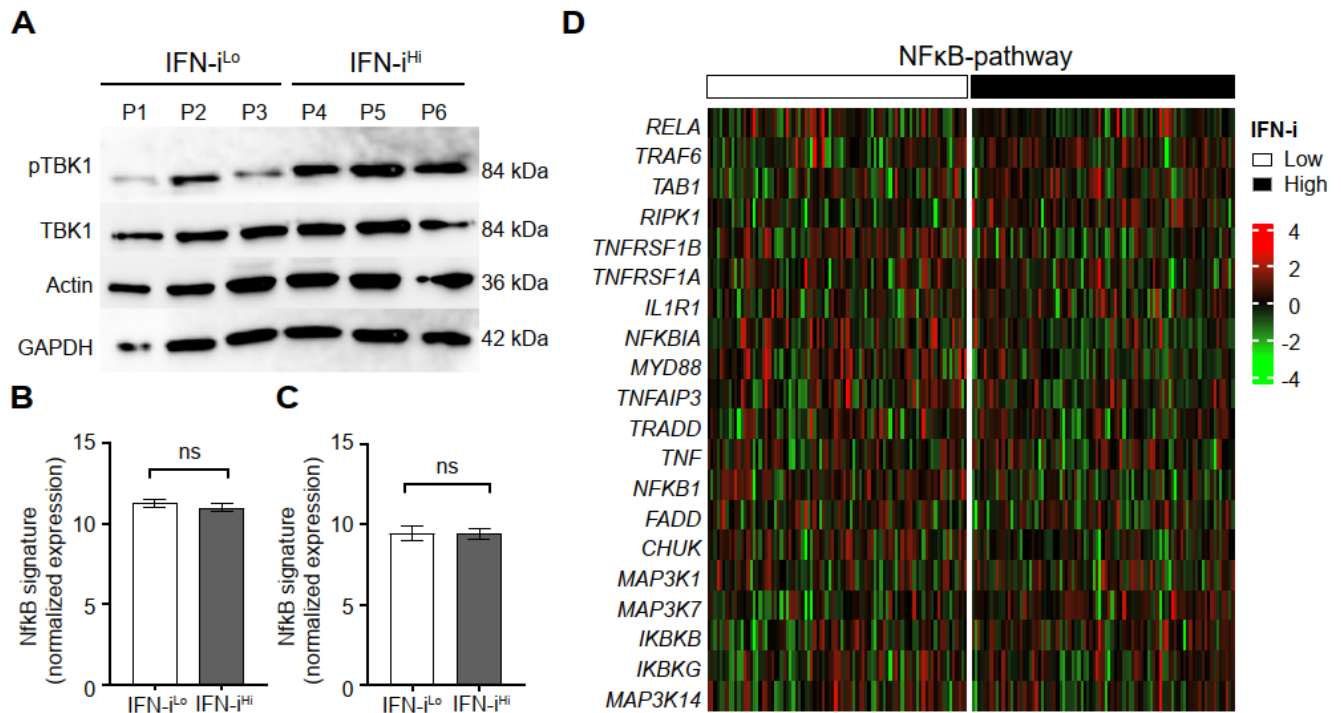


Supplemental Figure 2. Generation of KASUMI-1^{IFNAR2} cell line. (A) PCR products of a target region of *IFNAR2* gene using mixture of g352 and g1716. (B) Sequencing of genomic gRNA target sites of several clones and WT KASUMI-1 cells. (C) Expression of *IFNAR2* determined by flow cytometry. (D) Response of selected clones and WT culture to daunorubicin as analyzed by [³H]-thymidine incorporation. (E) Sequencing of gRNA target site of clone D.

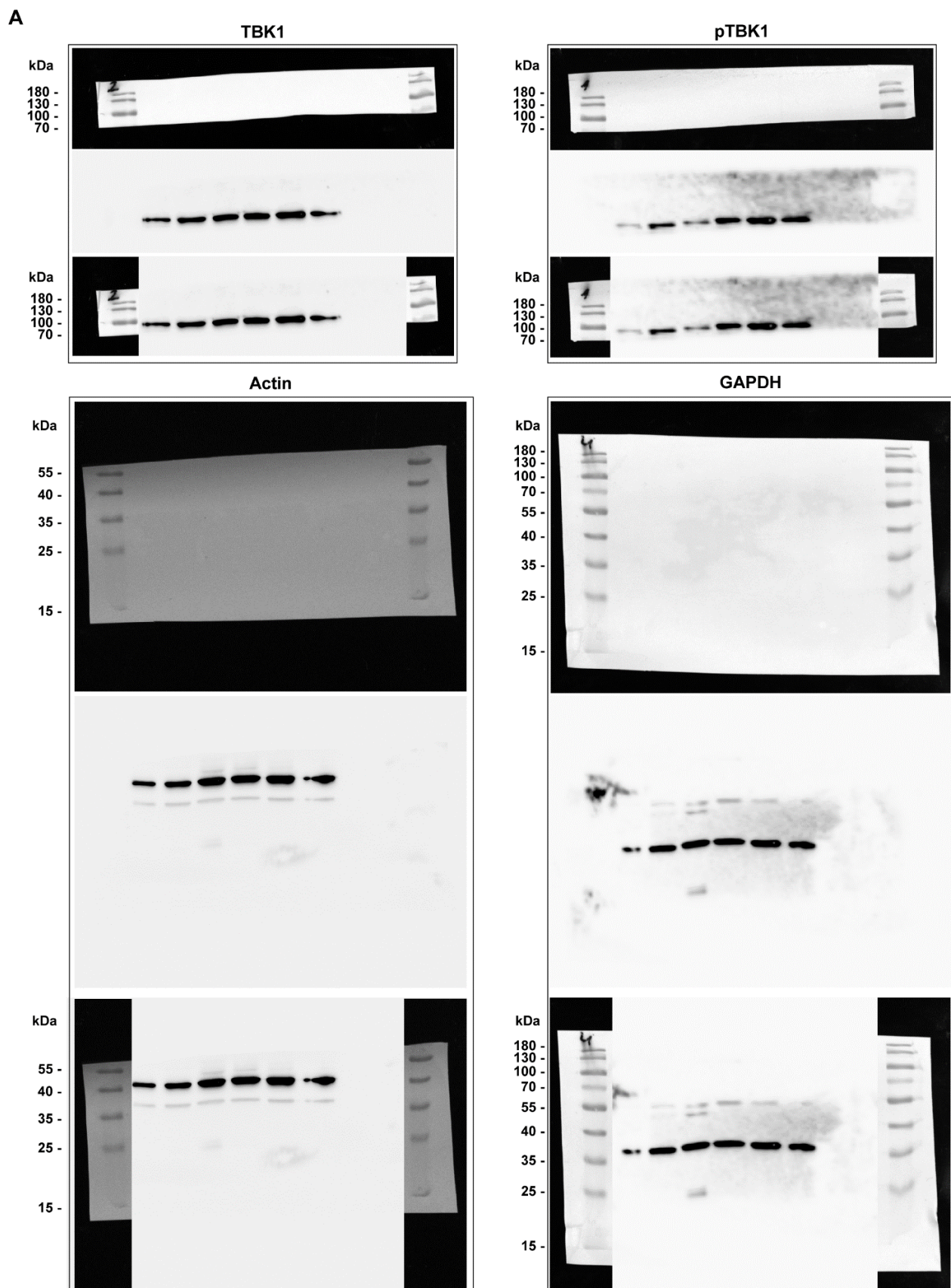


Supplemental Figure 3. TLR3 drives type I IFN secretion from AML blasts. (A) Relative expression level of *IFNA1*, *IFNA2*, and *IFNB1* in peripheral blood of AML patients (n=132) and in isolated CD33⁺ malignant blasts (n=30) (study cohort 1), as determined by RT-qPCR. Statistical significance was calculated by the Mann-Whitney test. ns: not significant. plots: lower quartile, median, upper quartile; whiskers, minimum, maximum. (B) Correlation between *IFNB1* expression

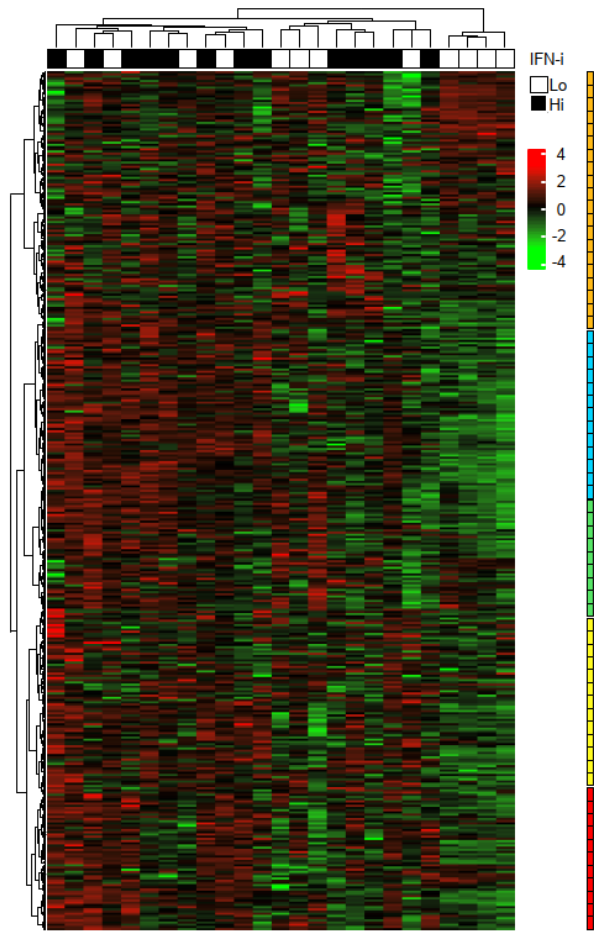
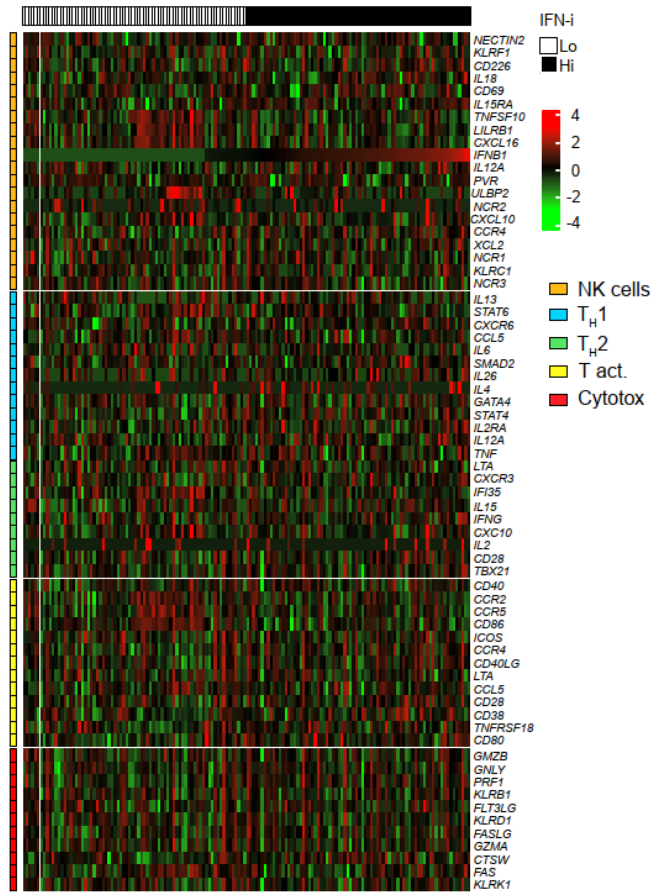
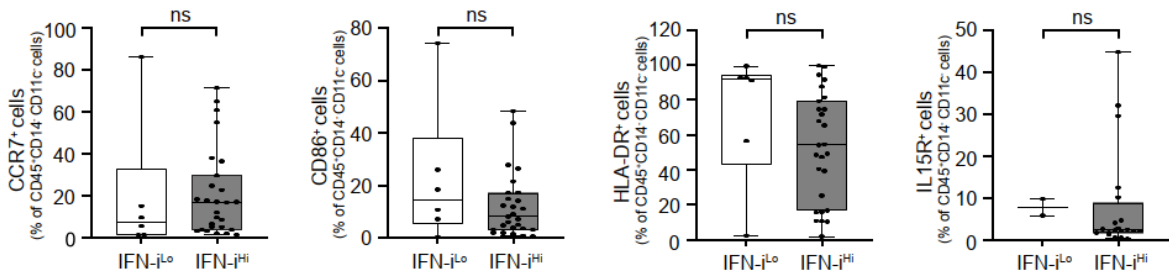
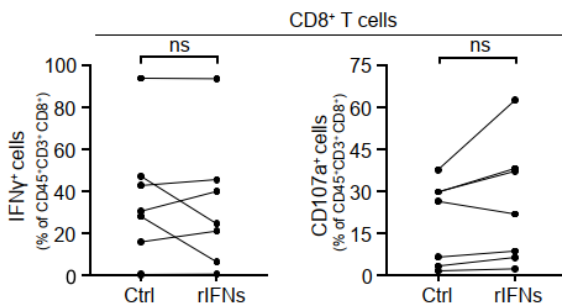
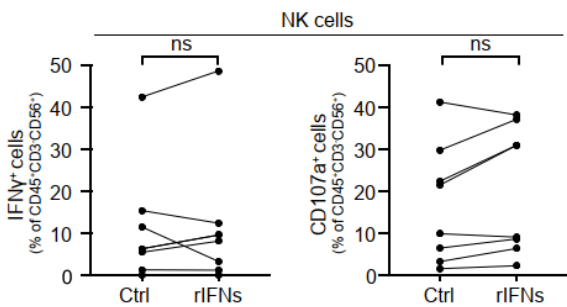
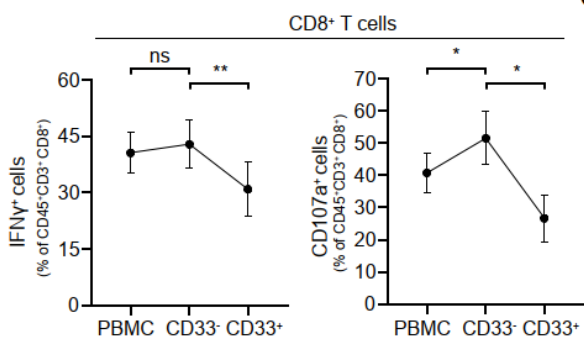
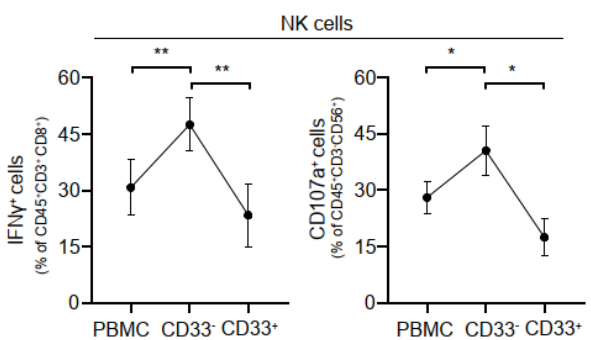
levels in peripheral blood and isolated CD33⁺ malignant blasts in 22 paired AML patient's samples; R, Pearson's correlation coefficient. (C) Correlation between type I IFN genes (*IFNA1*, *IFNA2*, *IFNB1*), IFN index (IFN-i) and *CGAS*, *DDX58*, *IFIH1*, *MAVS*, *EIF2AK*, *STING1*, *TLR3*, *TLR7*, *TLR9* and *ZBP1* in isolated CD33⁺ malignant blasts (n=30) from AML patients. Pearson's correlation coefficient or Spearman's correlation coefficient, R and *p* (also see Figure 1E). (D) Correlation between IFN-i and *TLR3* and *TLR9* expression levels in peripheral blood of 132 AML patients (study cohort 1). R, Spearman's correlation coefficient. (E) Correlation between *IFNB1* and *TLR3* expression levels of 152 AML patients retrieved from TCGA public database (study cohort 2). R, Spearman's correlation coefficient.



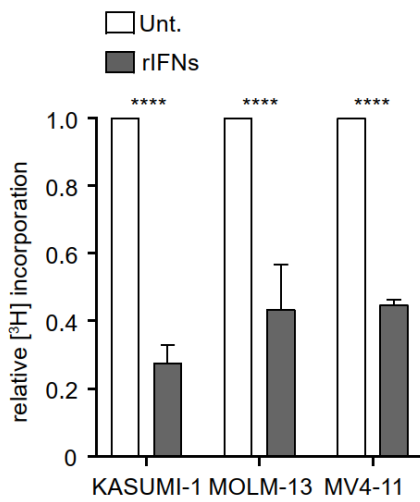
Supplemental Figure 4. Activation of TBK1 signaling pathway downstream of TLR3 in IFN- i^{Hi} AML patients. Phosphorylation of TBK1 in CD33⁺ malignant blasts of 3 IFN- i^{Lo} and 3 IFN- i^{Hi} AML patients from study cohort 1 determined by immunoblot detection of phosphorylated TBK1, TBK1 irrespective of its phosphorylation state, using actin and GAPDH as loading control **(A)**. **(B-D)** Relative expression levels and heatmap of gene signature associated with NFκB signaling pathway in 12 type I IFN i^{Lo} (n=12) and IFN- i^{Hi} (n=12) patients from study cohort 1 **(B)** and in type I IFN- i^{Lo} (n=76) and IFN- i^{Hi} (n=76) AML patients from TCGA public database determined by RNAseq **(C, D)**. Data are presented as median. Significant *p* values are reported; ns, not significant (Mann-Whitney test).



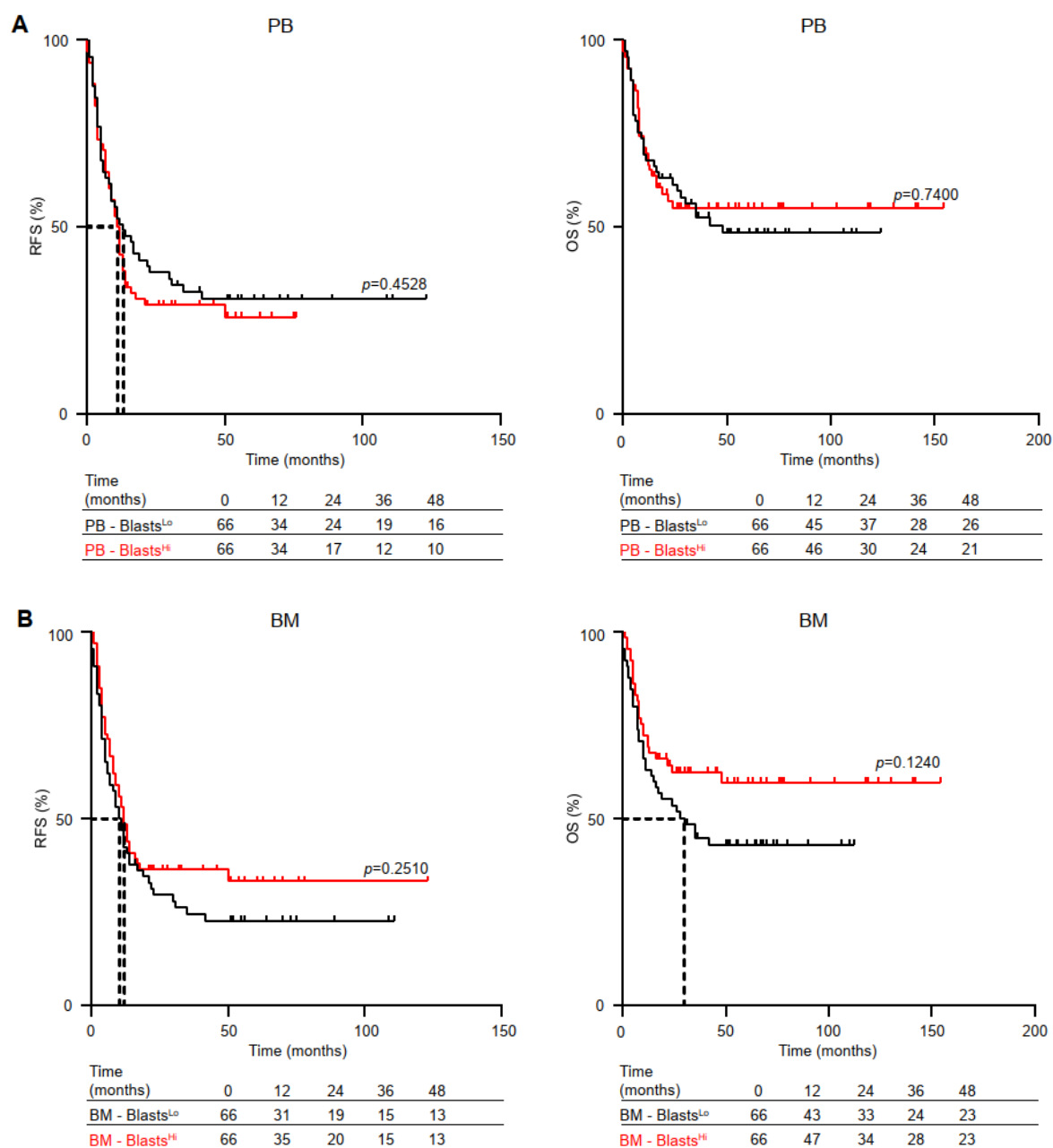
Supplemental Figure 5. (A) Uncropped full-length images of western blot membranes (see Supplementary Figure 4A)

A**B****C****D****E****F****G**

Supplemental Figure 6. Type I IFN-driven immunostimulation is suppressed by malignant blasts. (A) Hierarchical clustering of 433 transcripts that were significantly changed in IFN- i^{Lo} vs IFN- i^{Hi} peripheral blood samples from study cohort 1, as determined by RNA sequencing. (B) Expression levels of selected genes associated with NK cells, T_H1 and T_H2 response, T cell activation and cytotoxicity in 76 *IFNB1*^{Lo} and 76 *IFNB1*^{Hi} AML patients from study cohort 2. (C) Percentage of circulating CCR7⁺, CD86⁺, HLA-DR⁺ CD14⁺CD11c⁺ cells in 6 IFN- i^{Lo} versus 27 IFN- i^{Hi} AML patients from study cohort 1 prior induction chemotherapy. Statistical significance was calculated by the Mann-Whitney test. Box plots: lower quartile, median, upper quartile; whiskers, minimum value, maximum value; ns: p value > 0,05. A paired comparison of the percentage of IFN- γ ⁺ and CD107a⁺ CD8⁺ T cells (D) and NK cells (E) upon PMA/Ionomycin stimulation in peripheral blood of AML patients (study cohort 1; n=7) with/without pre-incubation with rIFN, as determined by flow cytometry. A paired comparison of IFN- γ ⁺ and degranulating CD107a⁺ CD8⁺ T cells (F) and NK cells (G) after PMA/Ionomycin stimulation of 10 AML patient PBMCs before, after CD33⁺ malignant blasts separation (CD33⁻) and after re-addition of viable autologous CD33⁺ malignant blasts using PMA/Ionomycin for stimulation. Statistical significance was calculated by the Wilcoxon test. p values are indicated.

A

Supplemental Figure 7. Recombinant type I IFN mediates direct cytostatic activity on AML cell lines. (A) Proliferation capacity of KASUMI-1, MOLM-13 and MV4-11 cells after 24h treatment with recombinant IFNs (rIFNs) determined by incorporation of $[^3\text{H}]$ -thymidine. Statistical significance was calculated by the paired T-test; Data are reported as a fold change relative to control cells; bars represent the means of three independent experiment; whiskers: standard deviation. *** $p < 0.001$ (paired t-test).



Supplemental Figure 8. The clinical relevance of malignant blasts frequency in peripheral blood. (A, B) Relapse-free (RFS) and overall survival (OS) of 132 AML patients from study cohort 1 stratified into two groups based on median value of frequency of malignant blasts in peripheral blood (A) or in bone marrow (B). Survival curves were estimated by the Kaplan-Meier method, and differences between groups were evaluated using log-rank test. Number of patients at risk and p values are reported.

Supplemental tables

Supplemental Table 1. The main clinical and biological characteristics of 16 AML patients from Study Cohort 3.

Variable	Study cohort n=16
Age at diagnosis	
< 50 years	6 (37%)
≥ 50 years	10 (63%)
Median (years)	51
Range (years)	39-68
Sex	
Male	3 (19%)
Female	13 (81%)
Peripheral-blood white cell count	
< 30.000/mm ³	10 (63%)
≥ 30.000/mm ³	6 (37%)
Median (10 ⁹ cells/l)	27,4
Range (10 ⁹ cells/l)	2,16-375,8
Blasts peripheral blood	
Median (%)	42
Range (%)	0,9-96
Blasts bone marrow	
Median (%)	54
Range (%)	23-95
De novo AML	16 (100%)
Secondary AML	
MDS/MPN, <i>n</i>	0 (0%)
Therapy related, <i>n</i>	0 (0%)
Not specified, <i>n</i>	0 (0%)
FAB classification	
M0	0 (0%)
M1	3 (19%)
M2	6 (38%)
M4	7 (43%)
M5	0 (0%)
M6	0 (0%)
Cytogenetic profile	
Favorable	2 (12,5%)
Intermediate	12 (75%)
Adverse	2 (12,5%)
Missing data	0 (0%)

<i>Molecular characteristics</i>	
<i>DNMT3A</i>	3 (19%)
<i>FLT3</i> -ITD	3 (19%)
<i>FLT3</i> -TKD	3 (19%)
CBF β ::MYH11	1 (6%)
<i>NPM1</i>	5 (31%)
<i>IDH2</i>	4 (25%)
<i>Induction chemotherapy</i>	
Daunorubicin + Ara-C (3+7)	3 (19%)
Idarubicin + Ara-C (3+7)	13 (81%)
BIDFA	0 (0%)
FLA-IDA	0 (0%)
HAM	0 (0%)
Complete remission	15 (94%)
<i>Consolidation</i>	
Chemotherapy only	5 (31%)
HSCT	11 (69%)

Abbreviations: AML, acute myeloid leukemia; Ara-C, cytarabine (well known as cytosine arabinoside); BIDFA, combination of twice daily fludarabine and cytarabine; CBF β ::MYH11, Core binding factor β - Myosin Heavy Chain 11 fusion protein ; DNMT3A, DNA (cytosine-5)-methyltransferase 3A; FAB, French-American-British; FLA-IDA, fludarabine-idarubicin; FLT3-ITD, Fms Related Receptor Tyrosine Kinase - internal tandem duplication; FLT3-TKD, Fms Related Receptor Tyrosine Kinase – tyrosine kinase domain mutation; HAM, high-dose cytosine arabinoside and mitoxantrone; HSCT, hematopoietic stem cell transplantation; IDH2, Isocitrate dehydrogenase 2 (NADP⁺); MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NPM1, nucleophosmin 1.

Supplemental Table 2. Primers and probes with sequences used for RT-qPCR.

Gene Name	Sequence 5' - 3'	Company
CGAS_fw	GACTGAAGTGCGACTCCG	Generi Biotech
CGAS_probe	6FAM-TGCTGAACACCGGGAGCTACTATGA--BBQ	Generi Biotech
CGAS_rev	TGACATCAAATTCATTAGGTGCAG	Generi Biotech
DDX58_probe	6FAM-AGAACACAGGAATGACCTCCCG--BBQ	Generi Biotech
DDX58_fw	CCACTGGCTTTGAATGCATC	Generi Biotech
DDX58_rev	TGGCATATTGACTGGACGTG	Generi Biotech
EIF2AK2_fw	GCGAACAAGGAGTAAGGGAAC	Generi Biotech
EIF2AK2_probe	6FAM-TGCGATACATGAGCCCAGAACAGATTT--BBQ	Generi Biotech
EIF2AK2_rev	AATTAGCCCCAAAGCGTAGAG	Generi Biotech
IFIH1_probe	6FAM-TGAGAGCACCTACGTCCTGGTTG--BBQ	Generi Biotech
IFIH1_fw	CGTCACCAATGAAATAGCCATG	Generi Biotech
IFIH1_rev	TTCGATAACTCCTGAACCACTG	Generi Biotech
IFNA1_fw	CATCTggTCCAACATgAAAACAA	TIB MOLBIOL
IFNA1_probe	6FAM-CATgAAAAGCgTgACCTggTgTATgAgTC--BBQ	TIB MOLBIOL
IFNA1_rev	gggTgAgAgTCTTTgAAATgACAg	TIB MOLBIOL
IFNA2_fw	TGACTTGCAGCTGAGCAC	Generi Biotech
IFNA2_probe	6FAM-ACCAGTAAAGCAAAGGTCAAGGCCA--BBQ	Generi Biotech
IFNA2_rev	CCCATTTCaACCAGTCTAGCAG	Generi Biotech
IFNB1_fw	gATgCTCCAgAACATCTTTgC	TIB MOLBIOL
IFNB1_probe	6FAM-CCAgCCAgTgCTAgATgAATCTTgTCTgAABBq--BBQ	TIB MOLBIOL
IFNB1_rev	TAgCCAggAggTTCTCAACAATAgTC	TIB MOLBIOL
MAVS_fw	GATTCTGCCTTACCTGCCC	Generi Biotech
MAVS_probe	6FAM-TCACAGCAAGAGACCAGGATCGACT--BBQ	Generi Biotech
MAVS_rev	GAAGAGATGCCAGAGGGTG	Generi Biotech
STING1_fw	AAAAGGGAATTTCaACGTGGC	Generi Biotech
STING1_probe	TCCGATGTAATATGACCATGCCAGCC	Generi Biotech
STING1_rev	GTAAGTTCTGAATCCGGGCC	Generi Biotech
SURF1_fw	AgTACATCgTgACCTggTATgg	TIB MOLBIOL
SURF1_probe	6FAM-AgAAATTCCTACgTgggACACCTggT--BBQ	TIB MOLBIOL
SURF1_rev	gTAgCACATgATCCAgCATAAAg	TIB MOLBIOL
TLR3_fw	AACATCCCTGAGCTGTCAAG	Generi Biotech
TLR3_probe	CCACTACCTTTGCAACACTCCACCT	Generi Biotech
TLR3_rev	AAAAGTCTCACTGGGAACCC	Generi Biotech
TLR7_fw	gTTTCCAATgTggACACTgAAgAgA	Generi Biotech
TLR7_probe	F-TTAggAAACCATCTAgCCCCAaggA--Q	Generi Biotech
TLR7_rev	CTTgTCTgTgCagTCCACgAT	Generi Biotech
TLR9_fw	CTATAACCGGAACCTTCTGCCAG	Generi Biotech
TLR9_probe	6FAM-AATAGCCGTGAGCCGGAATCCTG--BBQ	Generi Biotech
TLR9_rev	TGAGGTGAGTGTGGAGGTG	Generi Biotech
ZBP1_fw	AAACTCCGAAGCCATCCAG	Generi Biotech
ZBP1_probe	6FAM-TCTGTCTTGTGAATGATGTTCCCGTGTC--BBQ	Generi Biotech
ZBP1_rev	CATTGAAGGGAGGTGGCG	Generi Biotech

Supplemental Table 3. Antibodies used for flow cytometry.

Parameter	Source	Producer	Clone	Fluorochrome	Dilution
Annexin V	na	EXBIO	na	APC	1:100
Annexin V	na	BD Biosciences	na	HV500	1:100
IFN- γ	mouse	eBioscience	4S.B3	PE-Cy7	1:100
CD3	mouse	eBioscience	OKT3	FITC	4:100
CD34	mouse	eBioscience	4H11	APC	6:100
CD123	mouse	Biolegend	6H6	PE-Cy7	2:100
Granzyme B	mouse	BD Biosciences	GB11	Brilliant Violet 421	5:100
Granzyme B	mouse	eBioscience	GB11	PE	5:100
CD38	mouse	Biolegend	HIT2	PerCP-Cy5.5	5:100
CD33	mouse	Biolegend	WM53	PE	4:100
CD3	mouse	EXBIO	MEM-57	AF700	6:100
CD4	mouse	eBioscience	RPA-T4	PE-Cy7	5:100
CD4	mouse	Biolegend	OKT4	BV421	5:100
CD8	mouse	BD Biosciences	RPA-T8	HV500	5:100
CD14	mouse	EXBIO	MEM-15	FITC	6:100
CD11c	mouse	EXBIO	BU-15	APC	6:100
CD16	mouse	EXBIO	LNK16	FITC	6:100
CD19	mouse	EXBIO	LT19	FITC	6:100
CD20	mouse	EXBIO	LT20	FITC	6:100
CD45	mouse	Life Technologies	HI30	PE-Texas Red	6:100
CD45	mouse	EXBIO	MEM-28	PerCP	6:100
CD56	mouse	Beckman Coulter	N901	ECD	6:100
CD56	mouse	BioLegend	MEM-188	PerCP-Cy5.5	6:100
CD56	mouse	EXBIO	MEM-188	FITC	6:100
CCR7	mouse	Biolegend	G043H7	PerCP-Cy5.5	6:100
CD86	mouse	BD Biosciences	2331(FUN-1)	AF700	6:100
IL15Ralpha	mouse	Biolegend	JM-7A4	PE	6:100
HLA-DR	mouse	BD Biosciences	L243	PE-Cy7	6:100
IFNAR2	Rabbit	Sinobiological	#122	APC	6:100
CD107a	mouse	BioLegend	H4A3	FITC	8:100

Supplemental Table 4. gRNA targets and primers used in CRISP/Cas9 guided gene therapy.

Target/Primer	Sequence
<i>gRNA352 target</i>	GGTATTTTCATATGATTCGCC <u>TGG</u>
<i>g352 sense (5'-3')</i>	CACCGGTATTTTCATATGATTCGCC
<i>g352 antisense (5'-3')</i>	AAACGGCGAATCATATGAAATACC
<i>gRNA1716 target</i>	GCTGTATACAATCATGAGGT <u>TGG</u>
<i>g1716 sense (5'-3')</i>	CACCGCTGTATACAATCATGAGGT
<i>g1716 antisense (5'-3')</i>	AAACACCTCATGATTGTATACAGC

Supplemental Table 5. Antibodies used for immunoblotting analyses.

Parameter	Source	Producer	Clone	Diluation	Secondary antibody
TBK1/NAK	rabbit	Cell Signaling	D1B4	1:1000	Peroxidase AffiniPure F(ab') ₂ Fragment Donkey Anti-Rabbit IgG (H+L)
p-TBK1/NAK	rabbit	Cell Signaling	D52C2	1:1000	Peroxidase AffiniPure F(ab') ₂ Fragment Donkey Anti-Rabbit IgG (H+L)
GAPDH	mouse	GeneTex	1D4	1:1000	Peroxidase AffiniPure F(ab') ₂ Fragment Donkey Anti-Mouse IgG (H+L)
Actin	rabbit	Sigma Aldrich	na	1:1000	Peroxidase AffiniPure F(ab') ₂ Fragment Donkey Anti-Rabbit IgG (H+L)

Supplemental Table 6. Genes differentially represented in IFN- i^{Lo} and IFN- i^{Hi} AML patients (study cohort 1) as determined by RNA-sequencing.

Gene Symbol	Fold change (log2)	adj. p-value
<i>COL14A1</i>	4,55	0,0003
<i>POU4F1</i>	3,94	0,0001
<i>ROBO1</i>	3,41	0,0307
<i>HCAR1</i>	3,12	0,0022
<i>MYO18B</i>	2,78	0,0000
<i>PCDHB13</i>	2,54	0,0003
<i>CSRP2</i>	2,33	0,0018
<i>PCDHB14</i>	2,30	0,0017
<i>CFAP47</i>	2,23	0,0031
<i>PPL</i>	2,22	0,0017
<i>PNMT</i>	2,07	0,0015
<i>FGF13</i>	1,99	0,0046
<i>PCDHGC3</i>	1,98	0,0044
<i>PCDHGA12</i>	1,97	0,0042
<i>KCNJ12</i>	1,93	0,0110
<i>PCDHGB6</i>	1,91	0,0044
<i>PCDHGB3</i>	1,88	0,0010
<i>SLC9A2</i>	1,87	0,0093
<i>PCDHGC5</i>	1,82	0,0106
<i>DLL3</i>	1,81	0,0269
<i>SVOPL</i>	1,79	0,0273
<i>PCDHGA1</i>	1,78	0,0052
<i>GPC6</i>	1,78	0,0463
<i>PSMA8</i>	1,74	0,0423
<i>PCDHGA2</i>	1,73	0,0156
<i>SELP</i>	1,73	0,0204
<i>PCDHGA3</i>	1,71	0,0175
<i>PCDHGC4</i>	1,71	0,0157
<i>LIMS2</i>	1,69	0,0150
<i>PCDHGB2</i>	1,66	0,0353
<i>AEBP1</i>	1,65	0,0295
<i>KLRF2</i>	1,64	0,0372
<i>REN</i>	1,64	0,0292
<i>ACKR2</i>	1,64	0,0398
<i>LTK</i>	1,63	0,0172
<i>IRX6</i>	1,62	0,0108
<i>PCDHGA6</i>	1,58	0,0090
<i>PCDHGA9</i>	1,58	0,0267
<i>APBA2</i>	1,55	0,0185
<i>PCDHGA7</i>	1,49	0,0409

TRIM6	1,49	0,0305
APBA2	1,49	0,0244
PCDHGA8	1,48	0,0310
KCNH8	1,45	0,0494
TDRD9	1,44	0,0066
TMEM44	1,43	0,0066
ITPKA	1,43	0,0305
TMEM184A	1,42	0,0440
FBXL13	1,40	0,0240
SCN1B	1,37	0,0403
ADPRHL1	1,33	0,0134
NEK10	1,24	0,0227
DLGAP2	1,24	0,0260
TGFA	1,23	0,0461
RPS26	1,21	0,0007
NT5M	1,21	0,0110
TRH	1,16	0,0076
SCUBE3	1,13	0,0391
EPDR1	1,11	0,0192
SHD	1,02	0,0018
HSD17B1	1,01	0,0389
STMN3	0,98	0,0281
TSGA13	0,94	0,0377
FTO	0,82	0,0003
SLPI	0,80	0,0304
DDAH2	0,79	0,0075
LRWD1	0,78	0,0241
CCDC189	0,77	0,0106
PTPN7	0,75	0,0232
RAB37	0,74	0,0397
DDAH2	0,73	0,0156
TRIM24	0,72	0,0265
DDAH2	0,72	0,0162
DDAH2	0,72	0,0162
DDAH2	0,72	0,0162
MFRP	0,71	0,0198
DDAH2	0,64	0,0371
DDAH2	0,64	0,0371
SMYD5	0,61	0,0426
C1QTNF5	0,59	0,0167
CDAN1	0,57	0,0353
ESYT2	0,56	0,0293
DNAAF2	0,53	0,0157
SOCS2	0,34	0,0090
APBB2	0,30	0,0444
KCNIP3	0,29	0,0245

<i>SLITRK5</i>	0,28	0,0003
<i>DEFB1</i>	0,28	0,0397
<i>KHDRBS3</i>	0,28	0,0439
<i>CDSN</i>	0,27	0,0433
<i>HS3ST1</i>	-0,08	0,0178
<i>CACNG4</i>	-0,20	0,0061
<i>ENHO</i>	-0,27	0,0110
<i>FAM120AOS</i>	-0,33	0,0200
<i>CSMD1</i>	-0,34	0,0408
<i>CLEC10A</i>	-0,46	0,0114
<i>NMNAT1</i>	-0,56	0,0272
<i>NAGS</i>	-0,56	0,0350
<i>RBMS3</i>	-0,56	0,0427
<i>FAM156A</i>	-0,57	0,0377
<i>NBPF20</i>	-0,57	0,0447
<i>FAM156B</i>	-0,58	0,0370
<i>TTC13</i>	-0,60	0,0491
<i>RAPGEF1</i>	-0,60	0,0160
<i>ZYG11B</i>	-0,62	0,0230
<i>AKIRIN2</i>	-0,70	0,0371
<i>ATP6V1A</i>	-0,71	0,0369
<i>SLCO2B1</i>	-0,71	0,0211
<i>SAMD8</i>	-0,72	0,0420
<i>SLC38A6</i>	-0,75	0,0345
<i>LRRC8C</i>	-0,76	0,0197
<i>C1QA</i>	-0,79	0,0419
<i>TRIM3</i>	-0,80	0,0118
<i>CLEC20A</i>	-0,81	0,0059
<i>ST18</i>	-0,84	0,0042
<i>ABR</i>	-0,87	0,0466
<i>ITPRIPL2</i>	-0,87	0,0341
<i>ZFAND5</i>	-0,89	0,0308
<i>GLTP</i>	-0,90	0,0136
<i>PLOD1</i>	-0,90	0,0480
<i>HOOK3</i>	-0,92	0,0167
<i>BMP2</i>	-0,92	0,0000
<i>TCF7L2</i>	-0,92	0,0403
<i>FKBP15</i>	-0,94	0,0345
<i>RPH3A</i>	-0,95	0,0022
<i>AC253572.1</i>	-0,95	0,0389
<i>SOAT1</i>	-0,99	0,0397
<i>ZSWIM6</i>	-1,01	0,0494
<i>PRKACA</i>	-1,01	0,0235
<i>PRKACA</i>	-1,01	0,0235
<i>CPPED1</i>	-1,04	0,0452
<i>SIDT2</i>	-1,04	0,0213

<i>CPNE3</i>	-1,05	0,0348
<i>RCBTB2</i>	-1,06	0,0466
<i>EVI5</i>	-1,07	0,0270
<i>CEMIP2</i>	-1,07	0,0244
<i>RAB24</i>	-1,07	0,0397
<i>SGTB</i>	-1,09	0,0247
<i>FTL</i>	-1,09	0,0303
<i>CHST2</i>	-1,10	0,0422
<i>FTH1</i>	-1,11	0,0135
<i>PLAUR</i>	-1,12	0,0370
<i>KIF1C</i>	-1,13	0,0391
<i>IFNGR2</i>	-1,13	0,0247
<i>IFNGR2</i>	-1,14	0,0272
<i>PLXNA1</i>	-1,14	0,0453
<i>SNTB2</i>	-1,16	0,0136
<i>OSBPL11</i>	-1,16	0,0148
<i>GAA</i>	-1,17	0,0458
<i>UBXN11</i>	-1,18	0,0363
<i>PLPPR2</i>	-1,19	0,0039
<i>SIMC1</i>	-1,19	0,0125
<i>HEXB</i>	-1,19	0,0118
<i>PILRA</i>	-1,20	0,0277
<i>PPM1N</i>	-1,20	0,0340
<i>RWDD2A</i>	-1,21	0,0070
<i>MILR1</i>	-1,22	0,0399
<i>TAGLN</i>	-1,22	0,0110
<i>ID2</i>	-1,23	0,0203
<i>C1orf54</i>	-1,24	0,0403
<i>RMDN2</i>	-1,24	0,0180
<i>ZFP36L1</i>	-1,24	0,0466
<i>IQSEC1</i>	-1,25	0,0222
<i>HEG1</i>	-1,25	0,0374
<i>MCUB</i>	-1,25	0,0281
<i>BCL2L11</i>	-1,25	0,0175
<i>AP1S2</i>	-1,25	0,0180
<i>IL4I1</i>	-1,26	0,0094
<i>PELI1</i>	-1,28	0,0243
<i>SLC35F6</i>	-1,29	0,0041
<i>HLA-B</i>	-1,30	0,0397
<i>SETBP1</i>	-1,30	0,0381
<i>TFEB</i>	-1,30	0,0214
<i>RIN1</i>	-1,31	0,0221
<i>NACC2</i>	-1,35	0,0295
<i>KCTD17</i>	-1,35	0,0110
<i>NAV2</i>	-1,35	0,0447
<i>NFKBIZ</i>	-1,35	0,0260

<i>PPEF2</i>	-1,35	0,0122
<i>TNFRSF8</i>	-1,35	0,0322
<i>ASPHD2</i>	-1,35	0,0098
<i>ARHGEF11</i>	-1,35	0,0244
<i>TBC1D8</i>	-1,36	0,0071
<i>PPFIA4</i>	-1,36	0,0466
<i>AC068580.4</i>	-1,37	0,0099
<i>ANKRD37</i>	-1,37	0,0216
<i>NIBAN2</i>	-1,38	0,0482
<i>BMERB1</i>	-1,38	0,0478
<i>CTSB</i>	-1,38	0,0304
<i>MEGF9</i>	-1,38	0,0338
<i>SLC31A1</i>	-1,38	0,0044
<i>SLC26A11</i>	-1,40	0,0460
<i>IFITM10</i>	-1,41	0,0483
<i>OTOA</i>	-1,41	0,0225
<i>POU2F2</i>	-1,41	0,0241
<i>PEAK3</i>	-1,42	0,0292
<i>CD93</i>	-1,42	0,0481
<i>RIN2</i>	-1,42	0,0309
<i>CMTM4</i>	-1,43	0,0180
<i>SLC18B1</i>	-1,43	0,0066
<i>AC007192.1</i>	-1,44	0,0277
<i>TNFAIP2</i>	-1,46	0,0045
<i>SNED1</i>	-1,46	0,0185
<i>SLC9A9</i>	-1,46	0,0164
<i>TGM2</i>	-1,46	0,0423
<i>SLC22A1</i>	-1,46	0,0106
<i>FGR</i>	-1,48	0,0162
<i>HORMAD1</i>	-1,48	0,0114
<i>NR4A1</i>	-1,48	0,0449
<i>VMO1</i>	-1,49	0,0322
<i>CDKL5</i>	-1,49	0,0048
<i>TNFRSF1B</i>	-1,49	0,0208
<i>SMCO4</i>	-1,50	0,0094
<i>PSAP</i>	-1,51	0,0090
<i>SCO2</i>	-1,51	0,0334
<i>MUC4</i>	-1,51	0,0441
<i>NOD2</i>	-1,51	0,0402
<i>MUC4</i>	-1,51	0,0412
<i>PLD3</i>	-1,52	0,0058
<i>SETD7</i>	-1,52	0,0050
<i>JAZF1</i>	-1,52	0,0150
<i>CHST15</i>	-1,52	0,0452
<i>AL049634.2</i>	-1,52	0,0353
<i>LRRC25</i>	-1,53	0,0373

<i>ZNF385A</i>	-1,53	0,0134
<i>CES4A</i>	-1,54	0,0453
<i>PLD2</i>	-1,54	0,0166
<i>CTSS</i>	-1,54	0,0156
<i>SLC43A2</i>	-1,54	0,0048
<i>MFSD2A</i>	-1,54	0,0222
<i>CTSD</i>	-1,54	0,0050
<i>AL355315.1</i>	-1,55	0,0010
<i>LILRB2</i>	-1,56	0,0272
<i>CACNB1</i>	-1,56	0,0010
<i>CD4</i>	-1,56	0,0090
<i>GALNT18</i>	-1,56	0,0454
<i>CPVL</i>	-1,56	0,0345
<i>SIRPB1</i>	-1,57	0,0299
<i>LILRA5</i>	-1,57	0,0482
<i>TICAM2</i>	-1,58	0,0230
<i>LILRA5</i>	-1,58	0,0454
<i>LILRA5</i>	-1,58	0,0454
<i>LILRA5</i>	-1,58	0,0454
<i>LILRA5</i>	-1,58	0,0454
<i>SLC43A2</i>	-1,58	0,0037
<i>TYMP</i>	-1,58	0,0341
<i>APOBEC3A</i>	-1,58	0,0247
<i>LILRB2</i>	-1,58	0,0304
<i>MARCKS</i>	-1,59	0,0213
<i>LILRA3</i>	-1,59	0,0475
<i>GRN</i>	-1,60	0,0070
<i>PCDH12</i>	-1,60	0,0353
<i>GABBR1</i>	-1,60	0,0370
<i>AC241409.1</i>	-1,60	0,0170
<i>IGF2R</i>	-1,60	0,0010
<i>RXRA</i>	-1,60	0,0034
<i>CXCL16</i>	-1,62	0,0320
<i>BAG3</i>	-1,62	0,0074
<i>RASSF4</i>	-1,62	0,0059
<i>ZNF703</i>	-1,62	0,0347
<i>FAM20A</i>	-1,63	0,0006
<i>TMEM170B</i>	-1,63	0,0125
<i>ADAP2</i>	-1,63	0,0106
<i>C9orf72</i>	-1,63	0,0027
<i>MS4A6E</i>	-1,63	0,0226
<i>LSAMP</i>	-1,64	0,0094
<i>CYB5R2</i>	-1,64	0,0492
<i>LILRA6</i>	-1,64	0,0328
<i>CYBB</i>	-1,65	0,0263
<i>RGL1</i>	-1,65	0,0325

<i>SCIMP</i>	-1,65	0,0393
<i>NINJ1</i>	-1,66	0,0043
<i>SGSH</i>	-1,66	0,0110
<i>ARHGAP29</i>	-1,66	0,0474
<i>LILRA1</i>	-1,67	0,0096
<i>FAM151B</i>	-1,67	0,0049
<i>NHS</i>	-1,67	0,0389
<i>TPRG1</i>	-1,67	0,0340
<i>CD14</i>	-1,67	0,0481
<i>GCNT2</i>	-1,67	0,0015
<i>GCNT2</i>	-1,68	0,0014
<i>PDE4A</i>	-1,69	0,0088
<i>C3</i>	-1,69	0,0238
<i>LILRB4</i>	-1,69	0,0420
<i>LYNX1</i>	-1,69	0,0244
<i>FOLR2</i>	-1,70	0,0296
<i>LILRA1</i>	-1,71	0,0076
<i>SLAMF8</i>	-1,71	0,0062
<i>LILRA1</i>	-1,71	0,0072
<i>LILRB4</i>	-1,72	0,0397
<i>SPON1</i>	-1,72	0,0461
<i>LILRA1</i>	-1,72	0,0071
<i>SGK1</i>	-1,72	0,0157
<i>PI4K2A</i>	-1,72	0,0001
<i>DNAJB5</i>	-1,73	0,0045
<i>SEPTIN10</i>	-1,73	0,0277
<i>LILRB5</i>	-1,73	0,0241
<i>HFE</i>	-1,73	0,0169
<i>SLC15A3</i>	-1,74	0,0094
<i>LILRB2</i>	-1,74	0,0094
<i>PHLDA2</i>	-1,75	0,0157
<i>PHLDA2</i>	-1,75	0,0157
<i>LILRB3</i>	-1,75	0,0181
<i>CAMK2D</i>	-1,75	0,0007
<i>TBC1D12</i>	-1,76	0,0240
<i>GASK1B</i>	-1,76	0,0207
<i>PPFIBP2</i>	-1,76	0,0008
<i>RUFY4</i>	-1,76	0,0226
<i>PTPRM</i>	-1,77	0,0331
<i>MMP17</i>	-1,77	0,0166
<i>CHIT1</i>	-1,78	0,0272
<i>IL15</i>	-1,78	0,0205
<i>COPZ2</i>	-1,79	0,0162
<i>LILRB2</i>	-1,79	0,0059
<i>CTSH</i>	-1,79	0,0066
<i>LIMCH1</i>	-1,80	0,0322

<i>MS4A14</i>	-1,80	0,0070
<i>LILRB5</i>	-1,80	0,0157
<i>SHROOM1</i>	-1,81	0,0106
<i>NWD1</i>	-1,81	0,0331
<i>CTSL</i>	-1,81	0,0156
<i>WNK2</i>	-1,82	0,0022
<i>CD300LB</i>	-1,83	0,0097
<i>SNX21</i>	-1,83	0,0009
<i>SLC37A2</i>	-1,84	0,0150
<i>LILRB2</i>	-1,85	0,0043
<i>CD1D</i>	-1,85	0,0122
<i>KLF4</i>	-1,85	0,0075
<i>S1PR3</i>	-1,85	0,0188
<i>PELI3</i>	-1,85	0,0050
<i>CHI3L1</i>	-1,85	0,0240
<i>IL19</i>	-1,85	0,0122
<i>HMOX1</i>	-1,86	0,0101
<i>VDR</i>	-1,86	0,0027
<i>PAPLN</i>	-1,86	0,0136
<i>SIRPD</i>	-1,86	0,0121
<i>PCSK5</i>	-1,86	0,0024
<i>ITGA7</i>	-1,86	0,0039
<i>NPL</i>	-1,87	0,0051
<i>CHRD1</i>	-1,87	0,0211
<i>NMNAT2</i>	-1,87	0,0267
<i>CFAP99</i>	-1,88	0,0199
<i>EXT1</i>	-1,89	0,0027
<i>MS4A7</i>	-1,89	0,0027
<i>RGMA</i>	-1,90	0,0178
<i>LILRA6</i>	-1,90	0,0104
<i>MEIS3</i>	-1,90	0,0185
<i>CAMK1</i>	-1,90	0,0135
<i>GPBAR1</i>	-1,90	0,0113
<i>SHISA4</i>	-1,91	0,0122
<i>MT1H</i>	-1,91	0,0086
<i>CAVIN3</i>	-1,91	0,0175
<i>LGALS3</i>	-1,92	0,0070
<i>NLRP12</i>	-1,92	0,0059
<i>ACSM5</i>	-1,92	0,0103
<i>RAB11FIP5</i>	-1,92	0,0044
<i>CD300E</i>	-1,93	0,0156
<i>FGD5</i>	-1,94	0,0045
<i>SOWAHC</i>	-1,94	0,0071
<i>LILRB5</i>	-1,95	0,0076
<i>ACVR2A</i>	-1,95	0,0001
<i>LILRB5</i>	-1,95	0,0076

<i>FXYD6-FXYD2</i>	-1,95	0,0022
<i>CX3CR1</i>	-1,96	0,0050
<i>TSHZ3</i>	-1,96	0,0024
<i>LILRB5</i>	-1,96	0,0073
<i>LRP1</i>	-1,97	0,0114
<i>LILRB1</i>	-2,00	0,0031
<i>SDC3</i>	-2,00	0,0094
<i>AC113554.1</i>	-2,03	0,0070
<i>SLC16A2</i>	-2,03	0,0094
<i>LILRB1</i>	-2,06	0,0020
<i>CARD14</i>	-2,07	0,0066
<i>HMGA2</i>	-2,07	0,0022
<i>TMEM150B</i>	-2,07	0,0027
<i>MYCT1</i>	-2,07	0,0050
<i>CDKN2B</i>	-2,07	0,0048
<i>OR52K2</i>	-2,08	0,0043
<i>CCDC151</i>	-2,08	0,0033
<i>ASGR2</i>	-2,08	0,0039
<i>RAB39A</i>	-2,08	0,0031
<i>C8orf88</i>	-2,10	0,0050
<i>LILRB1</i>	-2,10	0,0030
<i>ISL2</i>	-2,10	0,0051
<i>LILRB1</i>	-2,11	0,0018
<i>SERINC2</i>	-2,11	0,0027
<i>FCN2</i>	-2,12	0,0034
<i>LILRB1</i>	-2,12	0,0020
<i>TWIST2</i>	-2,14	0,0037
<i>TWIST2</i>	-2,14	0,0037
<i>CHST1</i>	-2,15	0,0053
<i>MYO7A</i>	-2,15	0,0048
<i>CLEC4G</i>	-2,17	0,0045
<i>PKD4</i>	-2,17	0,0034
<i>KLK1</i>	-2,17	0,0018
<i>MAFB</i>	-2,18	0,0037
<i>CADM1</i>	-2,18	0,0017
<i>VCAN</i>	-2,20	0,0039
<i>ACE</i>	-2,22	0,0017
<i>PLAAT3</i>	-2,24	0,0003
<i>PKD2L1</i>	-2,25	0,0005
<i>HTR7</i>	-2,27	0,0010
<i>MT1E</i>	-2,29	0,0010
<i>RAPH1</i>	-2,29	0,0007
<i>CYP27A1</i>	-2,32	0,0007
<i>TMEM176B</i>	-2,33	0,0017
<i>RGL3</i>	-2,35	0,0016
<i>FXYD6</i>	-2,36	0,0003

<i>IL31RA</i>	-2,37	0,0005
<i>TRIM7</i>	-2,38	0,0003
<i>CES1</i>	-2,40	0,0009
<i>CACNB2</i>	-2,44	0,0118
<i>TMEM176A</i>	-2,45	0,0007
<i>SLC8A1</i>	-2,46	0,0001
<i>CES1</i>	-2,46	0,0006
<i>PID1</i>	-2,49	0,0004
<i>ZNF462</i>	-2,50	0,0198
<i>SDC2</i>	-2,53	0,0004
<i>EPB41L3</i>	-2,53	0,0003
<i>TNNT1</i>	-2,54	0,0003
<i>KNDC1</i>	-2,59	0,0002
<i>CDC42EP1</i>	-2,60	0,0002
<i>SLC46A2</i>	-2,61	0,0002
<i>KAZALD1</i>	-2,64	0,0002
<i>MLXIPL</i>	-2,64	0,0227
<i>ASGR1</i>	-2,66	0,0000
<i>FCN1</i>	-2,66	0,0001
<i>PLA2G2C</i>	-2,73	0,0001
<i>SMPDL3A</i>	-2,74	0,0001
<i>CYP1B1</i>	-2,77	0,0001
<i>TRPV4</i>	-2,78	0,0001
<i>CACNG8</i>	-2,84	0,0094
<i>SASH1</i>	-2,87	0,0000