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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Patient libraries were sequenced using the Ion PGM platform (version 5.0, Thermo Fisher). Flow cytometry data were collected using either FACS Diva (BD Biosciences, version 7.0) or CytExpert software (Beckman Coulter, version 2).
Data analysis	Quantification of immunoblots was performed using Fiji software (version 2.0.0). Statistical analysis was performed using Prism software (GraphPad, version 7). Flow cytometry data was analyzed using FloJo-Software (versions 10.0.8 or 10.2). Patient libraries were sequenced and analyzed using the Ion Reporter AML pipeline (version 5.0, Thermo Fisher). For the analysis of external datasets, calculations were performed with R version 3.3.1 in R-Studio (version 0.99.903). For gene expression arrays, fluorescence intensities were measured on the Agilent scan control software (version A.8.4.1, Agilent) on an Agilent DNA Microarray Scanner and extracted from images using Feature Extraction software (version 10.7.31, Agilent). After normalization, the microarray probes were filtered according to the flag information provided by Feature Extraction software (Version 10.7.3.1, Agilent). For RNAseq, sequences were aligned to hg19 reference genome using the STAR alignment software with Gencode version 19 as the transcriptome annotation. Bioconductor DESeq2 (version 3.8) was used to generate a list of differentially expressed genes. IBM SPSS Statistics Version 25 was used for biometrical analysis of patient data. For gene set enrichment analysis, GSEA (Broad Institute, version 2.2.2) was used. Complete blood counts were determined on an Advia120 Hematology Analyzer using Multispecies software (version 5.9.0-MS, Bayer).

For biometrical analyses, patient data were analyzed using IBM SPSS Statistics (version 25, IBM).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data shown in this study will be deposited in Gene Expression Omnibus prior to publication. All other relevant data are available from the corresponding author upon request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. For all in vitro experiments, at least three biological replicates were used or grouped analyses were carried out. For the in vivo experiments three or more mice per sorted fraction were used unless otherwise indicated; the number of injected cells and mice were chosen according to availability of cells therefore grouped analyses were carried out.
Data exclusions	No technically sound data were excluded from the analyses, except when technical issues were encountered, such as: - lot-dependent unspecific binding of the secondary antibody for Fc chimera protein leading to failure to correctly separate NKG2DLneg from NKG2DLpos AML cells (affected experiments: 4 experiments prepared to generate data as presented in Fig. 1g) - lack of engraftment of any human cell population, due to transplantation to NSG mice that due to unknown circumstances were not anymore immune suppressed (identified in each transplanted animal to carry, among others, mouse B-cells); affected experiments: 8 experiments prepared as additional replicate data to the results presented in Fig. 2e-f). - one experiment aimed to replicate the results in Figure 4c and Extended Data Figure 8d) with additional two AML patient samples showed for unknown reasons very low cell viability after culture, including the vehicle control, and was therefore excluded from the analysis. - one experiment aimed to replicate the results in Fig. 4e with additional five AML patient samples showed for unknown reasons very low cell viability after culture in all conditions including the vehicle control and was therefore excluded from analysis. - in Figure 4j, two experiments were excluded due to retrospectively confirmed T-cell contamination in the injected pNKCs. For FACS experiments, Propidium iodide, DAPI, or 7-AAD and/or physical parameters were used to exclude dead cells.
Replication	All attempts at replication, that did not encounter technical issues during the experiment, reliably reproduced the presented data sets. For all experiments, at least three biological replicates were performed. Additionally, some of the biological replicates were furthermore each performed in technical replicates.
Randomization	AML samples were chosen according to availability of cellular material and blast percentage (with a preference for samples with higher blast counts). Patients carrying active infections (hepatitis, HIV) were excluded to protect experimentators. Cells from individual AML samples were then distributed to all experimental groups for every sample. Mouse experiments were not randomised.
Blinding	Blinding was not routinely performed. Results including data from blinded experiments are shown in Fig. 1f-h, Fig. 3c-d, Fig. 4j and Extended Data Fig. 4e-g and j-l. Experiments with blinding have in all cases reproduced the data generated without blinding. Importantly, key experiments were independently replicated by different co-authors and in the three different involved laboratories.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials: Reagents targeting NKG2DL are commercially available, but can also be provided upon an scientific collaboration without charge

Antibodies

Antibodies used

For flow cytometry of human samples following mouse anti-human antibodies were used according to the data sheet:
From BioLegend, San Diego, CA, USA:

anti-human CD47-BV605 (clone CC2C6 ; dilution: 1:100)
anti-human CD3-APC (clone 17A2 ; dilution: 1:100)
anti-human GPR56-PE/Cy7 (clone CG4 ; dilution 1:50)
anti-human CD80-PE/Cy7 (clone 2D10 ; dilution: 1:50)
anti-human CD86-BV605 (clone IT2.2 ; dilution: 1:200)
anti-human CD112-PE/Cy7 (clone TX31 ; dilution: 1:50)
anti-human CD155-APC (clone SKII.4 ; dilution: 1:50)
anti-human CD33-BV421 (clone WM53 ; dilution: 1:200)
anti-human CD14-PE/Cy7 (clone HCD14 ; dilution: 1:50)

From BD Biosciences, Franklin Lakes, NJ, USA:
anti-human HLA-ABC-PE (clone G46-2.6 ; dilution: 1:25)
anti-human CD3-APC (clone UCHT1 ; dilution: 1:50)
anti-human CD56-FITC (clone NCAM16.2 ; dilution: 1:25)
anti-human CD69-PE (clone FN50 ; dilution: 1:25)
anti-human CD107a-PE (clone H4A3 ; dilution: 1:25)
anti-human CD33-PE (clone WM53 ; dilution: 1:50)
anti-human CD33-BV421 (clone WM53 ; dilution: 1:200)
anti-human CD34-APC (clone 581 ; dilution: 1:25)
anti-human CD34-FITC (clone 581 ; dilution: 1:50
anti-human CD38-FITC (clone HIT2 ; dilution: 1:25)
anti-human CD133 (clone W6B3C1 ; dilution: 1:25)
anti-human CD117-PE/Cy7 (clone TX31 ; dilution: 1:25)
anti-human CD45-FITC (clone HI30 ; dilution: 1:25)
anti-human CD45-PE (clone HI30 ; dilution: 1:50)
anti-human CD44-PE/Cy7 (clone G44-26 ; dilution: 1:100)
anti-human CD123-FITC (clone 7G3 ; dilution: 1:00
anti-human CD99-PE (clone Tü12 ; dilution: 1:50)
anti-human CD96-PE (clone 6F9 ; dilution: 1:50)
anti-human CD25-APC (clone M-A251 ; dilution 1:00)
anti-human PD-L1-APC (clone MIH1 ; dilution: 1:25)
anti-human TIM3-FITC (clone ; dilution: 1:50)

From eBiosciences, San Diego, CA, USA:
anti-human CD11c-APC (clone 3.9 ; dilution: 1:100)
anti-human CD14-APC (clone Sa2-8 ; dilution 1:100)
anti-human CD14-Pe/Cy (clone Sa2-8; dilution 1:100)
anti-human CD13-APC (clone WM15 ; dilution 1:100)
anti-human CD13-Pe/Cy7 (clone WM15; dilution 1:100)
anti-human Ly6A/E (Sca-1)

From R&D, Mineapolis, Minesota, USA
anti-human B7-H6-APC (clone 875001 ; dilution: 1:50)

The monoclonal antibodies targeting NKG2DL were previously designed and described by the authors Alexander Steinle and Helmut Salih: Anti-MICA (AMO1), anti-MICB (BMO1), anti-ULBP1 (AUMO3), anti-ULBP2 (BUMO1), anti-ULBP2/5/6 (R&D)

FITC-labeled Annexin V (BD Biosciences) and 7-AAD (1:100 dilution each, BD Biosciences) were used for apoptotic measurements

For flow cytometry of murine samples following antibodies from Biolegend were used:
Pacific Blue anti-mouse Lineage Cocktail (#133310, Components include anti-mouse CD3, clone 17A2; anti-mouse Ly-6G/Ly-6C, clone RB6-8C5; anti-mouse CD11b, clone M1/70; anti-mouse CD45R/B220, clone RA3-6B2; anti-mouse TER-119/Erythroid cells, clone Ter-119.)
PE/Cy7 anti-mouse Ly-6A/E (Sca-1) antibody (clone D7, #108113)
APC anti-mouse CD117 (c-kit) antibody (clone 2B8, #105811)
PerCP/Cy5.5 anti-mouse CD48 antibody (clone HM48-1, #103421)
PE anti-mouse CD150 (SLAM) antibody (clone TC15-12F12.2 , #115903)
PE anti-mouse CD16/32 antibody (clone 93, #101307)
For flow cytometry of murine samples the following antibody from eBioscience was used:
FITC anti-mouse CD34 antibody (clone RAM34, #11-0341)

250 µg InVivoMAb anti-mouse NK1.1 antibody (BioXcell, West-Lebanon, NH, USA) was used for NK cell depletion per mouse.

CD33 (Ventana, Tucson, Arizona, USA, Mouse monoclonal, Clone: QBEnd 10, RTU) and human CD34 (Ventana, Mouse monoclonal, Clone: QBEnd 10, RTU) were used for histophathological analyses.

For immunoblot, polyclonal rabbit anti-human PARP1 (#9542S, Cell Signaling, 1:1000) and mouse anti-β-Actin (8H10D10, #3700S, Cell Signaling, 1:3000) or anti-GAPDH (#5174P, 1:3000), Anti-rabbit IgG, HRP-linked Antibody (#7074, 1:3000) and HRP-linked antibody (#7076, 1:3000) were used according to the data sheet.

Validation

All Western Blot and FACS antibodies are commercially available and have been tested for the species used in this manuscript. Monoclonal antibodies against NKG2DL were validated in Hilpert et al, J Immunol 2012, Schmiedel et al, IJC 2010, Welte et al, Eur. J. Immunol. 2003, and Salih et al, Blood 2003.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

K562-41BBL-IL15 cells obtained from St. Jude's Children's Research Hospital were used as feeder to generate polyclonal natural killer cells from healthy PBMCs.

Authentication

Authenticity was determined by validating the respective immunophenotype described by the provider using FACS every 6 month.

Mycoplasma contamination

K562-41BBL-IL15 cells were routinely checked for mycoplasma contamination every 3 months. No contamination was observed.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell line was used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

NOD.Cg-Prkdcscid IL2rgtmWjl/Sz (also termed NOD/SCID/IL2Rγnull, NSG) mice purchased from Jackson Laboratory (Bar Harbor, ME, USA) were maintained under pathogen-free conditions and bred in house according to the Swiss and German federal and state regulations. 6-10 week old animals were used for this study (both males and females,but usually gender matched per experiment). Bone marrow cells from MLL-ENL and MLL-PTD/FLT3-ITD leukemia models were obtained from Juerg Schwaller (University Hospital Basel, Switzerland) and Robert Zeiser (University Hospital Freiburg, Germany).

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics

177 AML patient samples (PBMC isolated from peripheral blood) have been used in this study. 71 females, 106 males, mean age 60 years (21-89), mean blast count 72 % (3-100). These are further details regarding karyotype, molecular analysis (qRT-PCR, NGS) and CD marker profile see Supplementary Table 1.
Human cord blood samples of healthy newborn babies of both sexes at the University Hospital Basel upon availability.

Recruitment

Peripheral blood or bone marrow samples were obtained at diagnosis from patients with AML after informed consent in accordance with the Helsinki protocol. For the correlations with clinical and molecular characteristics shown in Fig. 1p and Extended Data Fig. 3, all patients admitted to the University Hospital Tuebingen between 2005 and 2017, that agreed to participate in the study after informed consent, were included if technically feasible. Patient samples were anonymised by the PIs (C.L., H.R.S., A.T.) using continuous case numbers. Human cord blood samples of healthy new-born babies of both sexes were collected at the University Hospital Basel upon avail-ability. All relevant ethical regulations were taken into account and the study was conducted according to the guidelines of the local Ethics committees (vote 13/2007V, S-112/2010, EKNZ2015/335).

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Peripheral blood from AML patients was collected and prepared as described in the Supplemental Methods Section. In brief, PBMCs were purified upon a Ficoll gradient followed by a lysis of remaining erythrocytes. Cells were counted, frozen in RPMI1640 supplemented with 20% FCS and 10% DMSO at a density of 10x10^6 or 20x10^6 depending of the available cell number and stored in liquid nitrogen. For analysis and sorting, cells were thawed freshly and stained immediately as described in the Supplemental Methods section.
Instrument	Flow cytometry analyses were performed on either a FACS CantoII or LSR II Fortessa (both BD Biosciences) or a BD cytoflex
Software	BD FACS Diva or CytExpert software were used for data collection. FlowJo versions 10.0.8 or 10.2 were used for data analysis.
Cell population abundance	Post-sort purity was always >95% and determined by re-analysis on a FACS Canto II or LSR II Fortessa for sorted populations.
Gating strategy	AML cells were identified from patient PBMCs by firstly gating on live cells based on their exclusion of the 7-AAD dye, then on single cells (SSC-H versus SSC-W) followed by gating on a forward scatter (FSC)/side scatter (SSC) plot. AML cells were gated on leukemic antigens according to the patient sample's phenotypic information and if applicable NKG2D-Fc chimaera, and by applying corresponding isotype and FMO (Fluorescence minus one) controls.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.