PNAS
www.pnas.org

Supplementary Information for

Distinct human circulating NKp30^{+}FcεRIγ^{+}CD8^{+} T cell population exhibiting high natural killer-like anti-tumor potential

Margareta P. Correia, Ana Stojanovic, Katharina Bauer, Dilafruz Juraeva, Lars-Oliver Tykocinski, Hanns-Martin Lorenz, Benedikt Brors, Adelheid Cerwenka

Corresponding author: Margareta P. Correia
Email: m.correia@dkfz.de

This PDF file includes:

Supplementary text: SI Materials and Methods
Figs. S1 to S9
Table S1
References for SI reference citations

Other supplementary materials for this manuscript include the following:

Dataset S1: microarray data (differentially expressed genes list)

www.pnas.org/cgi/doi/10.1073/pnas.1720564115

Supplementary Information

SI Materials and Methods

Cell isolation and culture. Blood buffy coats obtained from healthy donors were provided by DRK-Blutspendedienst Baden-Württemberg-Hessen (Mannheim, Germany). Written informed consent from the blood donors was obtained and ethical approval 87/04 was granted by the Ethik Kommission II of the Medical Faculty Mannheim (Mannheim, Germany). Peripheral blood mononuclear cells (PBMCs) were separated by gradient centrifugation (Biocoll Separating Solution, Biochrom) and red blood cells were lysed. Highly pure $CD8^+CD56^-$ T cells were isolated first using the Human $CD8^+$ T Cell Isolation Kit (Miltenyi Biotec), followed by CD8 MicroBeads (Miltenyi Biotec) isolation. Purified $CD8^+$ T cells were cultured in RPMI 1640 medium supplemented with 10% human serum (PAA) and 1% Penicillin-Streptomycin. Recombinant human IL-15 (R&D Systems) was used as indicated in the Figures. NK cells were isolated using the Human NK Cell Isolation Kit (Miltenyi Biotec) and cultured under similar conditions. For proliferation studies, cells were labeled with 10μM CFSE (Sigma-Aldrich).

Antibodies. The following anti-human antibodies were used for flow cytometry: anti-CD3 (HIT3a or SK7, Biolegend), anti-CD8 (SK1, Biolegend), anti-CD56 (HCD56, Biolegend) anti-NKp30 (P30-15, Biolegend), anti-NKp46 (9E2, Biolegend), anti-NKp44 (P44-8, Biolegend), anti-NKG2D (1D11, Biolegend), anti-DNAM1 (TX25, Biolegend), anti-CD28 (CD28.2, Biolegend), anti-CD122 (IL-15Rβ) (TU27, Biolegend), anti-CD132 (γc) (TUGh4, Biolegend), anti-CD25 (BC96, Biolegend), anti-granzyme B (GB11, Biolegend), anti-perforin (B-D48, Biolegend), anti-CCR7 (G043H7, Biolegend), anti-CD45RA (HI100, Biolegend), anti-IL-7Rα (AO19D5, Biolegend), anti-PD-1 (EH12.2H7, Biolegend), anti-CTLA-4 (L3D10, Biolegend), anti-LAG-3 (11C3C65, Biolegend), anti-γδTCR (B1, Biolegend), anti-Vα24 (6B11, Biolegend), anti-T-bet (4B10, Biolegend), anti-CD107a (H4A3, Biolegend), anti-IFNγ (B27, Biolegend), anti-CD3ζ (6B10.2, eBiosciences), polyclonal anti-FcεRIγ (Millipore), anti-Syk (4D10.1, eBiosciences), anti-PLZF (Mags.21F7, eBiosciences), anti-CD45 (HI30, Biolegend). Corresponding isotype controls were used in parallel: mIgG1 (MOPC-21, Biolegend), mIgG2a (MOPC-173, Biolegend), mIgG2b (MPC-11, Biolegend), rat IgG2b (RTK4530, Biolegend), rabbit IgG-FITC (eBiosciences). Anti-B7-H6 clone 1.18 and corresponding isotype control were produced as previously described (1). The following recombinant human fusion proteins were also used for staining: NKp44-Fc and CD99-Fc (both from R&D) and NKp46-Fc (kindly provided by Dr. Frank Momburg, DKFZ, Heidelberg), followed by incubation with goat anti-human IgG (Jackson ImmunoResearch). PE-conjugated HLA-A2-peptide loaded multimers we used to detect cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza (Flu) and Mart-1- specific TCR (kindly provided by Prof. Dr. David Price and Prof. Dr. Robert Thimme). TCR repertoire studies were performed using a IOTest® Beta Mark TCR V beta Repertoire Kit (Beckman Coulter). For functional studies, the following anti-human functional-grade purified antibodies were used: anti-NKp30 (P30-15), anti-NKp46 (9E2), anti-NKp44 (P44-8), anti-NKG2D (1D11), anti-CD3 (UCHT1), anti-CD28 (37.51) or the corresponding isotype control, mIgG1(MOPC-21), all from Biolegend. For blocking experiments, anti-NKp30 (210845, R&D) or the corresponding isotype control mIgG2a (20102, R&D), anti-NKp46 (9E2), anti-NKp44 (P44-8), or the corresponding isotype control, mIgG1(MOPC-21), were used.

Flow cytometry analysis and sorting. Prior to surface staining with the antibodies, cells were blocked with human serum. For dead cell exclusion, 7AAD (Biolegend) was used. For intracellular staining, cells were fixed and permeabilized with the Foxp3/Transcription Factor Staining Buffer Set (eBioscience), washed, blocked with mouse or rabbit serum and incubated with the antibodies

for 30min at 4°C. Zombie Aqua™ Fixable Viability Kit (Biolegend) was used to label dead cells before staining, according to the manufacturer instructions. Stained cells were acquired using a FACS Calibur, FACS Canto or FACS Fortessa (all from BD) and data was analyzed using FlowJo software. t-SNE analysis was also performed using FlowJo software. FACSAria II or FACSAria Fusion (BD) were used for FACS-sorting.

Cell lines, B7-H6 and luciferase transduction. Human melanoma cell lines SK-Mel-28, SK-Mel-37 and the human embryonic kidney cell line HEK293T were cultured in D-MEM (Sigma-Aldrich) supplemented with 10% FCS and 1% Penicillin-Streptomycin. Cells were dissociated with trypsin-EDTA (Sigma-Aldrich) for regular splitting or with non-enzymatic dissociation solution (Sigma-Aldrich) for analysis of ligand expression. SK-Mel-28 cells were retrovirally transduced with pMXneo-B7-H6, as previously described (2). Stably B7-H6 overexpressing SK-Mel-28 cells were then retrovirally transduced with the pBabe-puro-eGFP-2A-CBGr99 luciferase plasmid (eGFP-2A-CBGr99 luciferase construct kindly provided by Prof. Dr. Günter Hämmerling, DKFZ, Heidelberg).

Functional assays. For plate-bound Ab stimulation assays, 96-well flat-bottom plates (Maxisorp, Nunc) were pre-coated with different antibodies. $1x10^5$ cells were added to the wells in complete medium containing anti-CD107a mAb. After 1h of incubation, GolgiStop (BD) was added and incubation followed for four additional hours at 37°C. Afterwards, cells were washed, fixed/permeabilized and stained with anti-IFNγ antibodies. In experiments with inhibitors, cells were pre-incubated for 45 min at 37°C with the described inhibitors or corresponding solvent control. Afterwards, anti-CD107a mAb was added, cells were distributed in the Ab-coated wells and the procedure continued as described above. *In vitro* killing studies were performed by a standard ^{51}Chromium release assay, for 4h at 37°C. In blocking experiments, effector cells were pre-incubated with the corresponding blocking antibodies for 30 min at RT.

RNA isolation, cDNA synthesis and quantitative RT-PCR. Cells were lysed and RNA was isolated using RNeasy Mini Kit (Qiagen). Genomic DNA contamination was removed using the TURBO DNase Kit (Ambion). For cDNA synthesis, ProtoScript M-MuLV First Strand Synthesis Kit (NEB) was used, all according to the manufacturers' instructions. Quantitative RT-PCR (qPCR) was performed using a LightCycler® 480 SYBR Green I Master kit (Roche), according to the manufacturer's instructions, measured using a LightCycler 480 Instrument (Roche) and relative expression calculated by the $2^{-\Delta\Delta CT}$ method. *B2M* was used as housekeeping gene. NKp30 isoforms were quantified by quantitative RT-PCR (qPCR) using TaqMan probes, as previously described (3).

Primers. The following primer sets were used:
NCR1: F, 5′AGCAGACTCTCCCAAAACCG3′, R, 5′CTCCCCAACCCGATAGATGC3′;
NCR2: F, 5′GCCAGGGAAATTATGGGGCT3′, R, 5′CAGGATGAACCGAGAGGGTG3′;
NCR3: F, 5′TCCAGGGTACGAATCTCAGG3′, R, 5′AACTGGGACATCTTCCGACA3′;
FCER1G: F, 5′ATTCCAGCAGTGGTCTTGCT3′, R, 5′TCCATACAGAAACAGGATGGC3′;
CD3Z: F, 5′AGAGTGAAGTTCAGCAGGAGC3′, R, 5′GGGTTCTTCCTTCTCTGCGG3′;
ZBTB16: F, 5′ATGAAGACGTACGGGTGCGAG3′, R, 5′CACACAGCAGACAGAAGACGG3′;
SYK: F, 5′CTCTGCCACTACCACTCCCA3′, R, 5′TCAAATCCTCAAAGGGCCCAG3′;
B2M: F, 5′GATGCCGCATTTGGATTGGA3′, R, 5′CTCTAAGTTGCCAGCCCTCC3′.
For FcεRIγ cloning into pMp71 vector, the following primers were used:
F, 5'GTGATGAGCGGCCGCATGATTCCAGCAGTG3',
R, 5'GCATGGACCGAATTCCTACTGTGGTGGTTTC3'.

Genome-wide expression profiling and microarray data analysis. Purified $CD8^+$ T cells cultured for 12 days with IL-15 (10ng/ml) from 6 different donors were FACS-sorted into $NKp30^+$ or $NKp30^-$ $CD8^+$ T cells and total RNA was isolated, as described above. RNA quality was confirmed using a Bioanalyzer (Nanodrop ND-1000, Agilent 2100 Bioanalyzer). Genome-wide expression profiling was performed using Illumina Human HT-12 v4 BeadChip Sentrix arrays by the Genomics and Proteomics Core facility from DKFZ. Data were quantile normalized, $\log_2$ transformed and analyzed using Chipster software (4). A cutoff of coefficient of variance (cv)< 0.5 was applied, and a paired two group Empirical Bayes statistical test was used to assess differential expression, P-value<0.05. Additional thresholds of the fold-change that were used for further filtering are described in the respective figure legends. Heatmap and volcano plot visualizations were generated using R (version 3.2.2). GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/) was also used for the generation of heatmaps, when described in the figure legends. For data visualization, the probe with highest fold-change (Fc) value per gene was displayed. For volcano plot visualizations, probes with a P-value<10^{-6} were arbitrarily displayed with a P-value=10^{-7}. For the Principal Component Analysis (PCA) of the gene expression data, our dataset from Illumina arrays was normalized using the function normalize.quantiles from preprocessCore (version 1.33.0). The previously published data from Affymetrix arrays (5) were preprocessed using the function threestep from affyPLM (version 1.46.0), and data were subsequently normalized using the method "RMA convolution model background". The average values of the expression in each probeset were assigned to genes using WGCNA (version 1.51). We accounted for the array platform-specific differences in the data in the following way: to reduce the platform specific differences in the data, we applied two approaches, quantile discretisation and z-score normalization. For the PCA analysis, we used the quantile discretisation method; the normalized gene expression values were grouped into 16 bins (6). For visualization of the expression patterns across five cell populations using heatmaps, the z-score normalization was applied to data from Illumina and Affymetrix separately, and then the two datasets were combined. For PCA, each dot represents a donor plotted in three dimensions using its projections onto the second-, third- and fourth-principal components (PC2, 3 and 4). PC1 was excluded in order to remove the residual platform specific differences. Pathway analysis was performed through the use of QIAGEN´s Ingenuity Pathway Analysis (IPA, www.qiagen.com/ingenuity).

Protein isolation, immunoprecipitation and immunoblotting. Cells were lysed with a 1% Digitonin lysis buffer solution containing 1% PMSF (Sigma) and 1% Proteinase and Phosphatase inhibitor (Thermo Scientific). Total protein concentrations in lysates were quantified using a Pierce BCA protein assay kit (Thermo Scientific). Immunoprecipitation (IP) was performed by incubating lysates with anti-NKp30 (goat polyclonal, R&D) and magnetic µMACS Protein G Microbeads (Miltenyi Biotec), followed by isolation using µColumns (Miltenyi Biotec), according to the manufacturer's instructions. Polyclonal rabbit anti-human FcεRIγ antibody (Millipore) was used for the Western blot.

Proximity ligation assay (PLA) and microscopy. Sorted $NKp30^+$ or $NKp30^-$ $CD8^+$ T cells were adhered to Poly-L-Lysine coated slides (Sigma-Aldrich) by centrifugation using cytospin (~1×10^4 cells/slide, 800 rpm for 10 min at RT). Cells were washed, fixed/permeabilized with ice-cold acetone, blocked and incubated with the antibodies (polyclonal rabbit anti-FcεRIγ (Millipore) and mouse anti-human NKp30 (P30-15, Biolegend)) and reagents, according to the Duolink (Sigma-Aldrich) kit instructions. PLA detects sub-40-nm co-localization with high sensitivity and specificity, which can be visualized by the presence of red foci. Images were acquired in a Leica TCS SP5 II (Leica) confocal microscope and analyzed with the Fiji software (http://fiji.sc/Fiji).

FcεRIγ cloning and CD8+ T cell transduction. Full length *FCER1G* was amplified from cDNA

derived from sorted NKp30+CD8+ T cells using the primers described above, and cloned into the retroviral vector pMP71Pre (kindly provided by Prof. Dr. Wolfgang Uckert, MDC, Berlin). Viral supernatants were obtained, filtered through 0.45µm PVDF filters (Millipore) and concentrated using Retro-Concentin Virus Precipitation Solution (System Biosciences). Sorted NKp30$^-$CD8$^+$T cells were pre-activated with plate-bound αCD3/αCD28 and transduced with the viral supernatants with addition of protamine sulfate (8µg/ml), IL-15 (25ng/ml) and the TLR inhibitor BX795 (6µM) (7), by spinoculation.

Genomic DNA isolation and pyrosequencing methylation analysis. FACS-sorted NKp30$^+$, NKp30$^-$ CD8$^+$ T cells or NK cells (from the same donors) were washed twice in PBS and genomic DNA isolated with the DNeasy Blood and tissue kit (Qiagen). CpG dinucleotide methylation analysis was determined by bisulphite treatment of RNase-treated genomic DNA, followed by PCR amplification and pyrosequencing (Pyro Q-CpG, Qiagen). Pyro Q-CpG was performed by EpigenDX. The following CpGs were covered in our study (from ATG): -418, -401, -384, -375, -367, -359, -321, -242, -237.

Mouse experiments and *in vivo* imaging. NSG mice were maintained in the animal facility of the DKFZ. All animal experiments were approved by the "Regierungspräsidium Karlsruhe". For tumor experiments, 2.5x10^5 luciferase-expressing SK-Mel-28.B7-H6.luci cells were injected *i.v* together with 1x10^6 of sorted NKp30$^+$ or NKp30$^-$ CD8$^+$ T cells or PBS control into γ-irradiated (3.5 Gy) NSG mice. In the therapeutic model, 2.5x10^5 SK-Mel-28.B7-H6.luci cells were injected *i.v.* 2 days before injection of 30x10^6 bulk IL-15-cultured-CD8$^+$ T cells or PBS control. Before effector cell injection, mice were distributed into groups with similar average bioluminescence intensity (BLI) measurements. 20000U IL-2 (NIH) were injected *i.p.* every 2 days. BLI was measured using an *in vivo* imaging IVIS-100 system (Xenogen). For the *in vivo* generation of NKp30$^+$CD8$^+$ T cells, 1x10^6 freshly purified CD8$^+$ T cells were injected into NSG mice. IL-15/IL-15Rα-Fc complexes were prepared by pre-incubating IL-15 (Peprotec) with recombinant IL-15Rα-Fc (R&D Systems) for 30 min at 37°C. Each mouse was injected *i.p.* with 200µl IL-15 (2.5µg) + IL-15Rα–Fc (15µg) complex every 3 days and sacrificed on day 12. Single cell suspensions from peripheral blood and spleen were collected after red blood cell lysis. Livers were perfused, cut and treated with digestion buffer (0.5 mg/ml hyaluronidase V and 0.5 mg/ml DNase (Sigma-Aldrich)). Viable cells were collected after centrifugation over a Lympholite-M gradient (Cedarlane).

SLE and healthy blood isolation, plasma preparation and ELISA. PBMCs and plasma were isolated from freshly collected heparinized peripheral blood from patients or healthy controls. PBMCs were used for FACS staining with the indicated antibodies. Plasma was centrifuged at 14000 rpm for 10 min at 4°C, incubated for 1h in Protein L coated plates (Pierce) with shaking at RT and then used for IL-15 measurement by a Human IL-15 Quantikine ELISA Kit (R&D, assay range 3.9 - 250 pg/ml), according to the manufacturer's protocol. Healthy controls were gender and age matched with SLE patients (**SI Appendix, Table S1**).

Statistics. Paired *t*-test was applied for the comparison between groups with observations from the same donor cells. Unpaired *t*-test was used to compare independent groups. For comparison between healthy donors and SLE patient's data, Mann-Whitney test was used. P≤0.05 was considered significant. All tests are indicated in each individual figure legend.

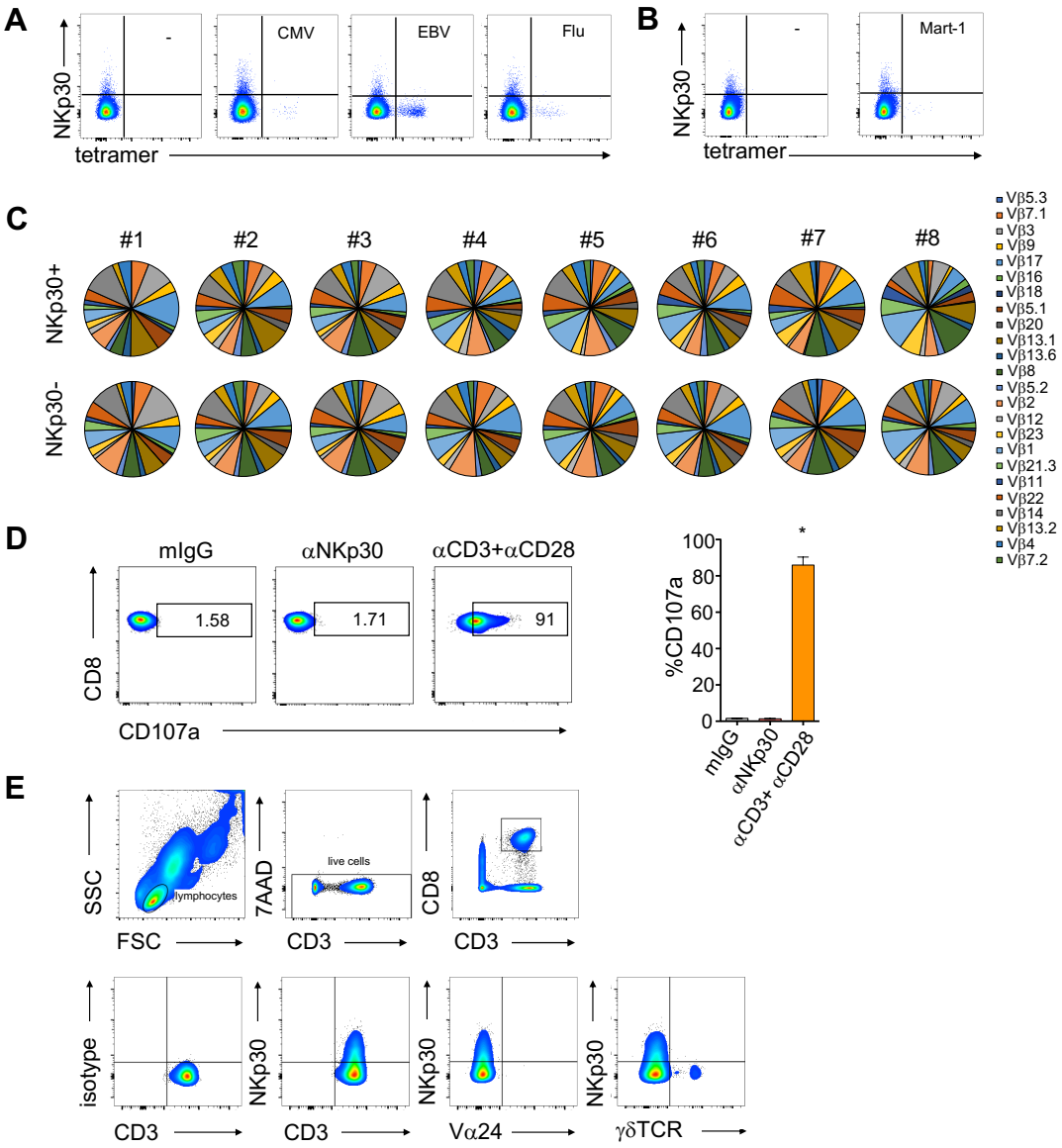

Fig. S1. Characterization of the NKp30^{+}CD8^{+} T cell population in healthy blood and blood from SLE patients. PBMCs from HLA-A2 typed healthy donors were stained for surface NKp30 and **(A)** virus-specific tetramers against CMV, EBV or Flu or **(B)** MART-1-specfic tetramer. Dot-plots are pre-gated on CD8^{+}CD3^{+}7AAD^{-} cells. **(C)** Naïve CD8^{+} T cell were MACS-sorted from healthy donor PBMCs (donor #1-8), stained with a combination of different monoclonal antibodies against individual Vβ TCRs and analyzed by flow cytometry (IOTest Beta Mark kit, Beckman Coulter). Cells were pre-gated on NKp30^{+} or NKp30^{-} CD3^{+}7AAD^{-} cells. **(D)** Naïve CD8^{+} T cell were MACS-sorted from healthy donor PBMCs and incubated with the indicated plate-bound antibodies for 4h, in the presence of anti-CD107a-FITC Abs. Representative plots show CD107a expression after stimulation. Graph showing the percentage of CD107a expressing cells, n=3, mean±SEM, *P≤0.05, paired Student´s *t*-test. **(E)** PBMCs from blood of SLE patients were isolated by gradient density centrifugation, stained and analyzed by flow cytometry. Lymphocytes were gated according to FSC/SSC parameters, dead cells were excluded by 7AAD labeling (7AAD^{-}), and CD3^{+}CD8^{+} cells were gated for phenotypical analysis. Representative plots showing NKp30 expression and absence of Vα24 or γδTCR markers on the NKp30^{+}CD8^{+} T cell gated population.

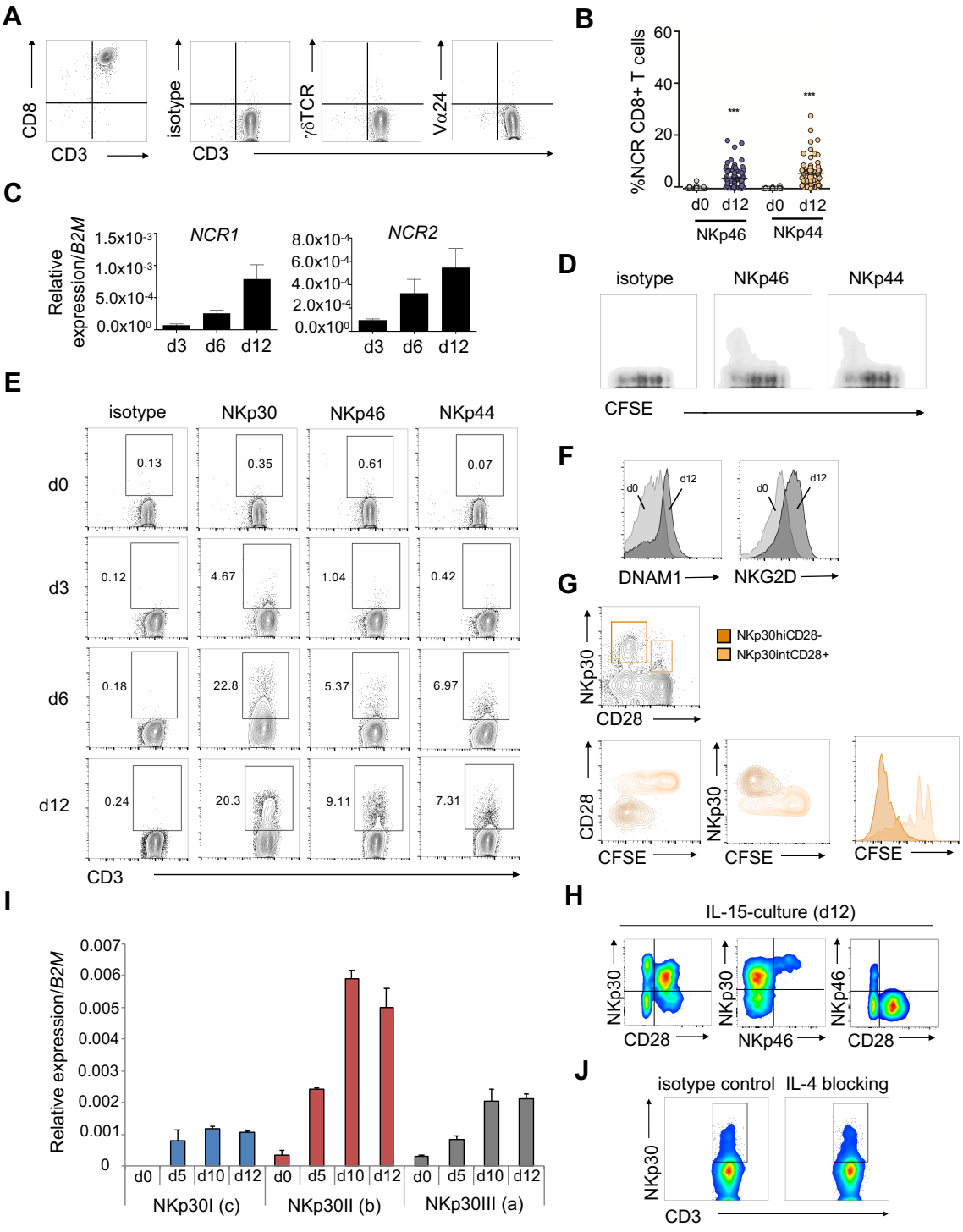

Fig. S2. Induction of NCRs on CD8$^+$ T cells, NKp30 isoform expression and IL-4 blocking during culture with IL-15. Highly purified human peripheral blood CD8$^+$ T cells were cultured for 12 days with IL-15. **(A)** Purity of human peripheral blood CD56$^-$CD8$^+$T cells that were isolated by a double-round of magnetic isolation (d0). (**B**) Percentage of NCR expressing CD8$^+$ T cells after isolation (d0) and on d12 was analyzed by flow cytometry. n=64-67, mean±SEM, ***P≤0.001, paired Student´s *t*-test. **(C)** qPCR analysis of NCR expression on d3, d6 and d12 (relative to *B2M*),

mean±SEM. (**D)** Representative plots showing NKp46 and NKp44 expression and CFSE dilution on d12. **(E)** Representative plots showing NKp30, NKp46 and NKp44 expression kinetics on d0, d3, d6 and d12. **(F)** Histograms showing NKG2D and DNAM1 expression on d0 and d12 after culture with IL-15. **(G)** FACS plots showing NKp30 and CD28 expression on d12 and CFSE dilution for gated $NKp30^{hi}CD28^{-}$ and $NKp30^{int}CD28^{+}$ populations. **(H)** $NKp30^{+}CD8^{+}$ T cells were FACS-sorted from resting peripheral blood $CD8^{+}$ T cells and cultured with IL-15 for 12 days. FACS plots show NKp30, NKp46 and CD28 expression. **(I)** Relative expression of different NKp30 isoforms (a, b and c) on d0, d5, d10 and d12 after culture with IL-15. *B2M* was used as housekeeping gene. **(J)** Flow cytometry plots showing NKp30 cell surface expression after 12 days with IL-15 in the presence of 10μg/ml anti-IL-4 blocking antibodies (8D4-8, Biolegend) or the corresponding isotype control (mIgG1, Biolegend).

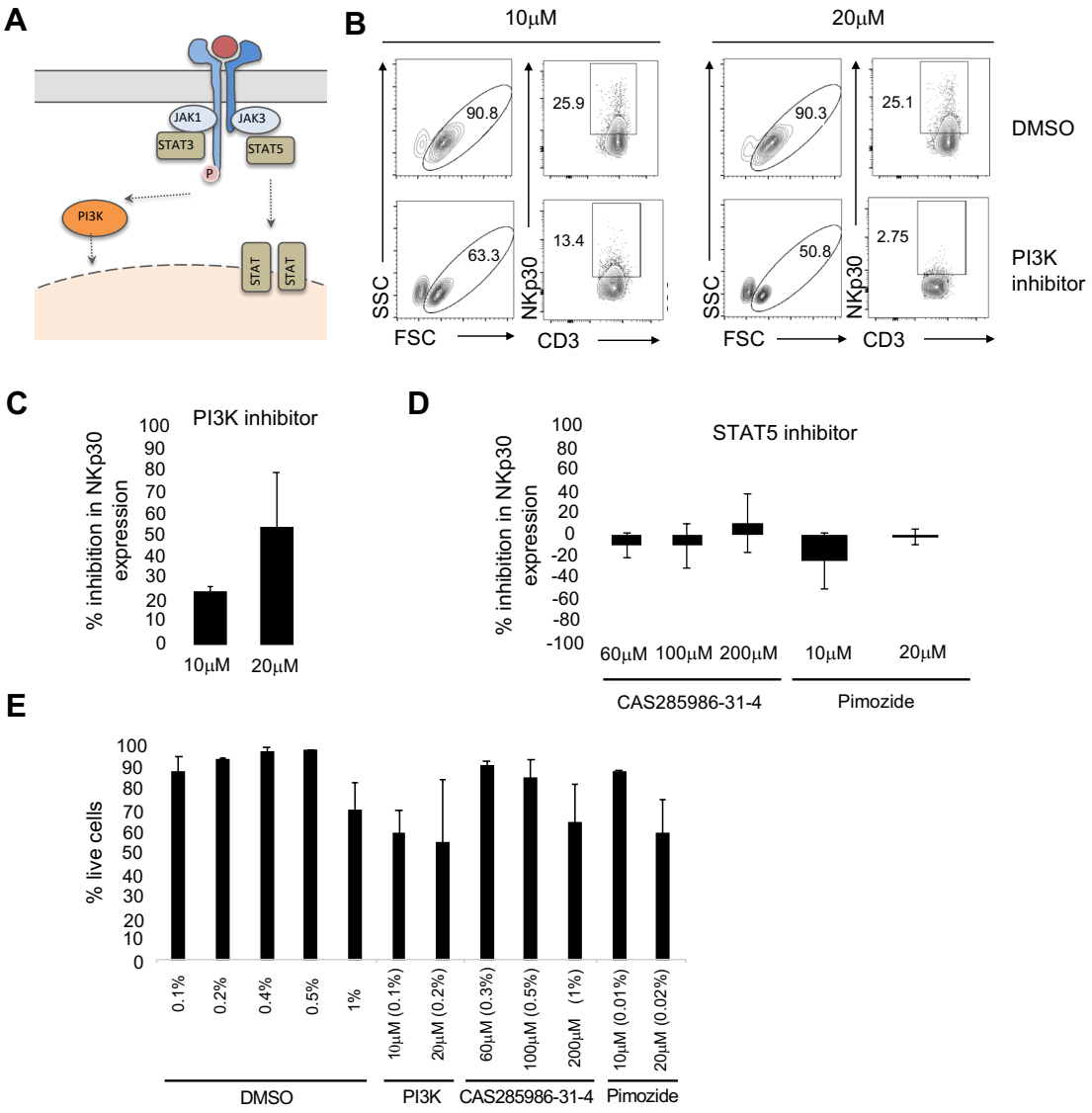

Fig. S3. PI3K signaling inhibition during culture with IL-15 impairs acquisition of NKp30 expression by CD8$^+$ T cells. (A) Scheme illustrating involvement of STAT5 and PI3K in IL-15 signaling. **(B-E)** Highly purified human peripheral blood CD56$^-$CD8$^+$T cells were cultured with IL-15 for 12 days in the presence of the indicated inhibitors or DMSO solvent control. **(B)** Representative plots showing NKp30 expression on live CD8$^+$ T cells cultured with IL-15 in the presence of a PI3K inhibitor (AS605240, Sigma) at the indicated concentrations. **(C)** Graph showing the % of inhibition of NKp30 expression on CD8$^+$ T cells (n=3-5 independent experiments, mean±SD). **(D)** Graph showing the % of inhibition of NKp30 expression in CD8$^+$ T cells in the presence of STAT5 inhibitors (CAS 285986-31-4, Calbiochem; or STAT5 Inhibitor III, Pimozide - CAS 2062-78-4, Calbiochem), at the indicated concentrations (n=2-5 independent experiments, mean±SD). For each concentration, % of inhibition of NKp30 expression was calculated as = 100 - ((%NKp30 expression on CD8$^+$ T cells cultured with inhibitor*100)/ (%NKp30 expression on CD8$^+$ T cells cultured with equivalent volume of DMSO)). **(E)** Graph showing the % live cells after the treatment with the different inhibitors or DMSO (n=3-5 independent experiments, mean±SD).

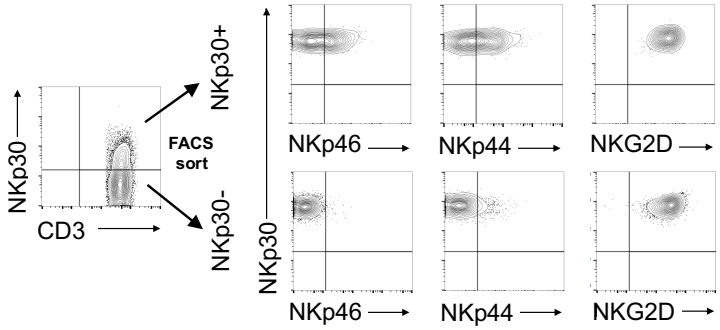

Fig. S4. FACS-sorting scheme and phenotype of sorted NKp30$^+$ and NKp30$^-$ CD8$^+$ T cell populations. Highly purified CD8$^+$ T cells were cultured for 12 days with IL-15 and then FACS-sorted into NKp30$^+$ or NKp30$^-$ CD8$^+$CD3$^+$7AAD$^-$ cells. Representative plots showing phenotype of NKp30$^+$ and NKp30$^-$ CD8$^+$ T cells after sorting are depicted.

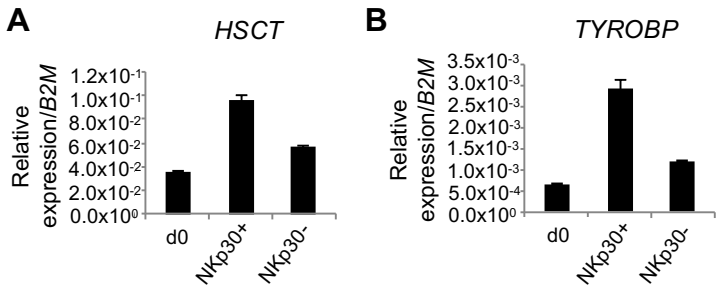

Fig. S5. NKp30^{+}CD8^{+} T cells express increased levels of *HSCT* and *TYROBP* compared with NKp30^{-}CD8^{+} T cells. (A-B) Highly purified human peripheral blood CD56^{-}CD8^{+}T cells were cultured for 12 days with IL-15, and then FACS-sorted into NKp30^{+} or NKp30^{-} CD8^{+} T cells. Relative expression of *HSCT* **(A)** and *TYROBP* **(B)** transcripts on d0 CD8^{+} T cells and on d12 FACS-sorted NKp30^{+} and NKp30^{-} CD8^{+} T cells (relative to *B2M*). Representative experiment out of >3, mean±SEM.

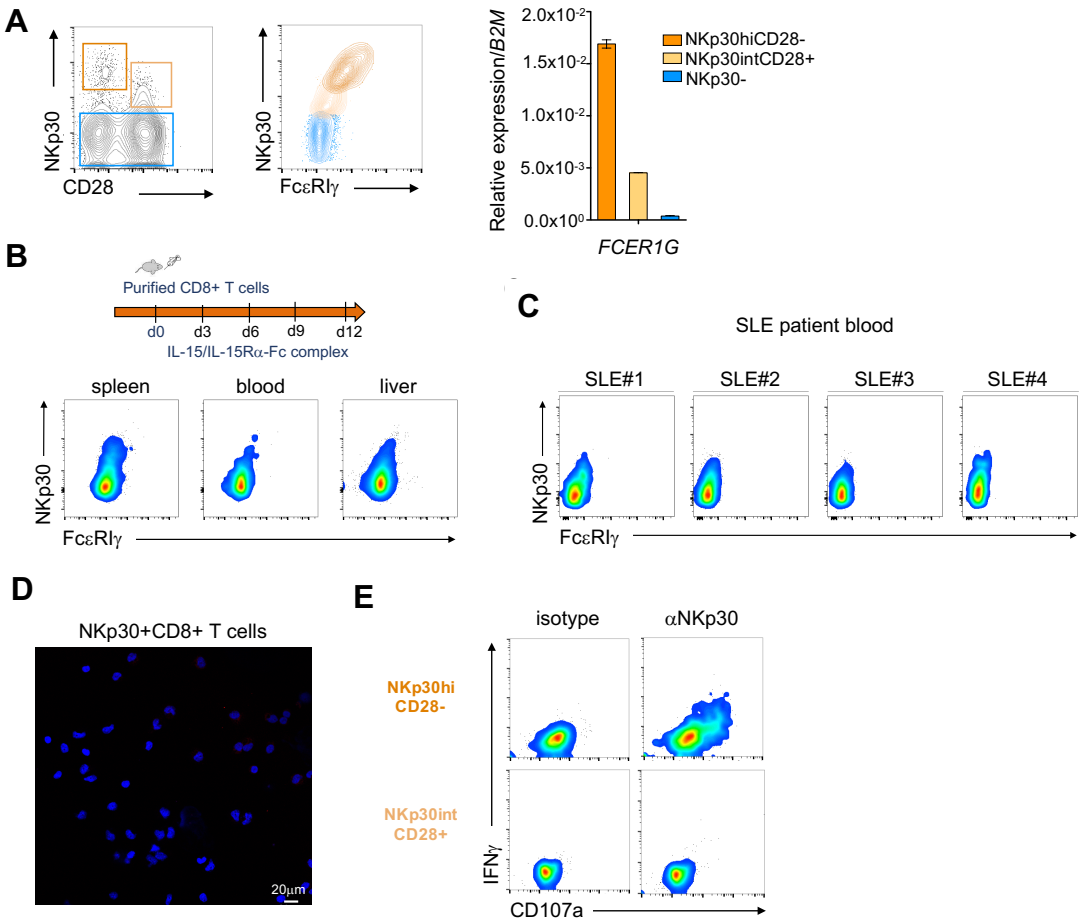

Fig. S6. FcεRIγ expression in the NKp30^{+}CD8^{+} T cell population. (A) Purified CD8^{+} T cells were cultured for 12 days with IL-15 (10ng/ml) and then FACS-sorted into NKp30^{-}, NKp30^{hi}CD28^{-} or NKp30^{int}CD28^{+} CD8^{+} T cells. FACS plots showing FcεRIγ expression and graph showing *FCER1G* transcript levels detected by qPCR (relative to *B2M*) for those subsets. **(B)** 1x10^{6} of highly purified peripheral blood human CD8^{+} T cells were injected in NSG mice. IL-15/IL-15Rα-Fc complexes were supplied every 3 days. On day12, CD8^{+} T cells were re-isolated from mice and analyzed by flow cytometry. Plots show NKp30 and FcεRIγ staining for human CD8^{+} T cells in spleen, blood and liver. Cells were pre-gated on live CD45^{+}CD3^{+}CD8^{+} cells. Representative data of one out of two independent experiments from different donors (each with 3 mice/ group) is shown. **(C)** Flow cytometry plots showing FcεRIγ and NKp30 expression on CD8^{+} T cells from 4 representative SLE patients (SLE#1-4). Cells were pre-gated on live CD45^{+}CD3^{+}CD8^{+} cells. **(D)** Proximity ligation assay (PLA) shows absence of protein-protein interaction between FcεRIγ and CD56 molecules. NKp30^{+}CD8^{+} T cells, sorted after 12 days of culture of purified CD8^{+} T cells with IL-15, were washed, fixed/permeabilized with ice-cold acetone, blocked and incubated with the antibodies (polyclonal rabbit anti-human FcεRIγ (Millipore) and mouse anti-human CD56 (ERIC-1, Thermo Scientific) and reagents, according to the Duolink (Sigma-Aldrich) kit instructions. PLA detects sub-40 nm co-localization, visualized by the presence of red foci. Nuclei labeled with DAPI, blue. Scale marker: 20μm. Images were acquired in a Leica TCS SP5 II (Leica) confocal microscope and analyzed with the Fiji software (http://fiji.sc/Fiji). **(E)** FACS-sorted NKp30^{hi}CD28^{-} or NKp30^{int}CD28^{+} CD8^{+} T cells were incubated with the indicated plate-bound mAbs for 4h in the presence of anti-CD107a-FITC Abs. Representative plots show CD107a and IFNγ expression.

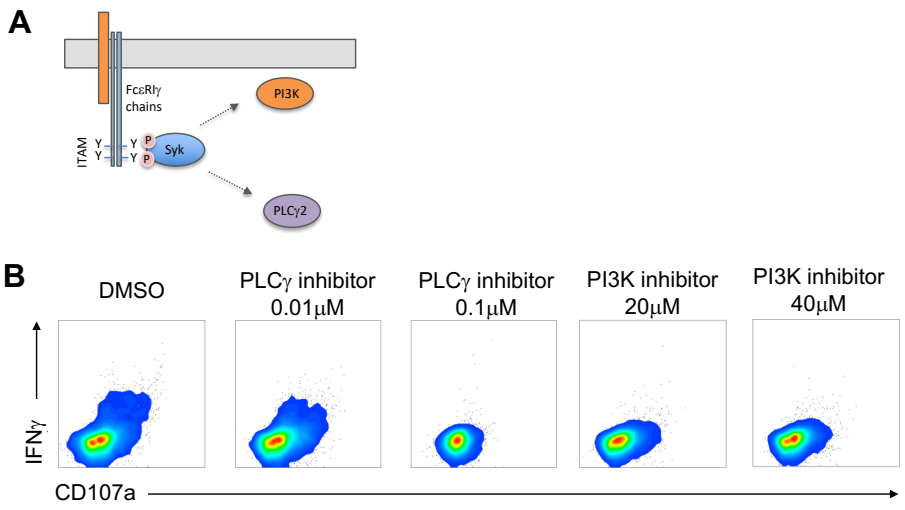

Fig. S7. Inhibition of PLCγ and PI3K signaling leads to an impairment in NKp30 function of NKp30^{+}CD8^{+} T cells. (A) Scheme representing participation of Syk, PI3K and PLCγ2 molecules downstream of FcεRIγ signaling. **(B)** FACS-sorted NKp30^{+}CD8^{+} T cells were incubated with plate-bound anti-NKp30 mAbs for 4h, in the presence of anti-CD107a-FITC Abs. Representative plots showing CD107a and IFNγ expression in the presence of PLCγ2 (U73122, EMD Millipore), or PI3K (AS605240, Sigma) inhibitors, at the indicated concentrations, or the solvent control (DMSO).

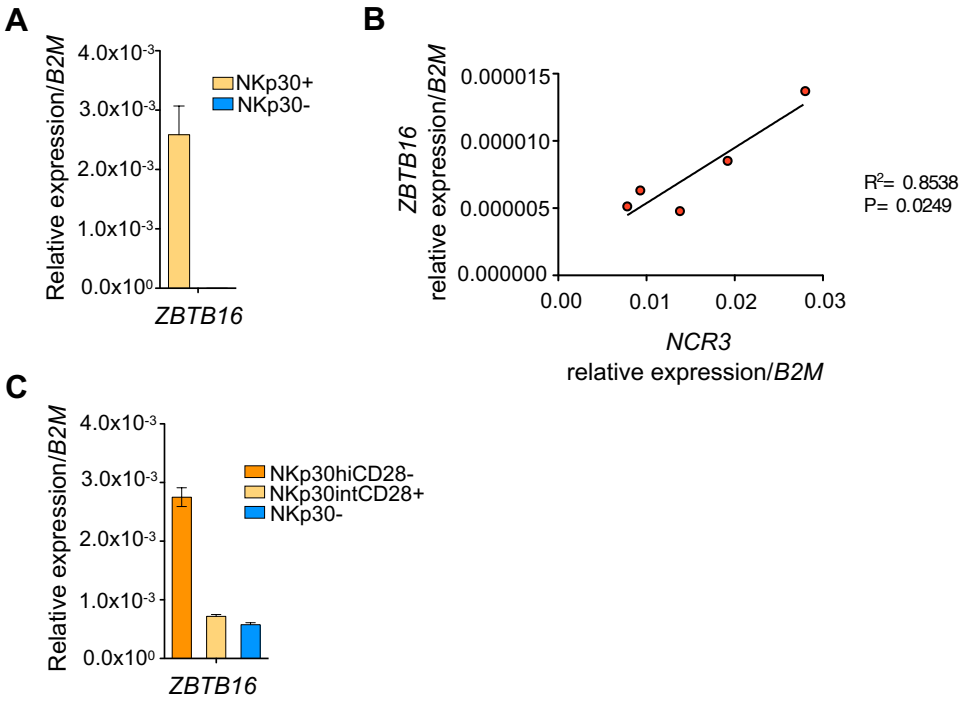

Fig. S8. *ZBTB16* expression in the NKp30^{+}CD8^{+} T cell population. (A) Peripheral blood CD8^{+}T cells were purified from healthy donors and FACS-sorted into NKp30^{+} and NKp30^{-} CD8^{+} T cells and relative expression of *ZBTB16* transcripts was determined by quantitative RT-PCR. **(B-C)** CD8^{+} T cells were cultured with IL-15 for 12 days. **(B)** Graph showing relative expression of *ZBTB16* and *NCR3* transcripts from sorted NKp30^{+}CD8^{+} T cells, determined by quantitative RT-PCR (n=5). Pearson correlation, R^2=0.8538, P=0.0249. **(C)** Graph showing *ZBTB16* transcript levels in FACS-sorted NKp30^{-}, NKp30^{hi}CD28^{-} or NKp30^{int}CD28^{+} CD8^{+} T cells, determined by quantitative RT-PCR. Relative expression was calculated relative to *B2M*.

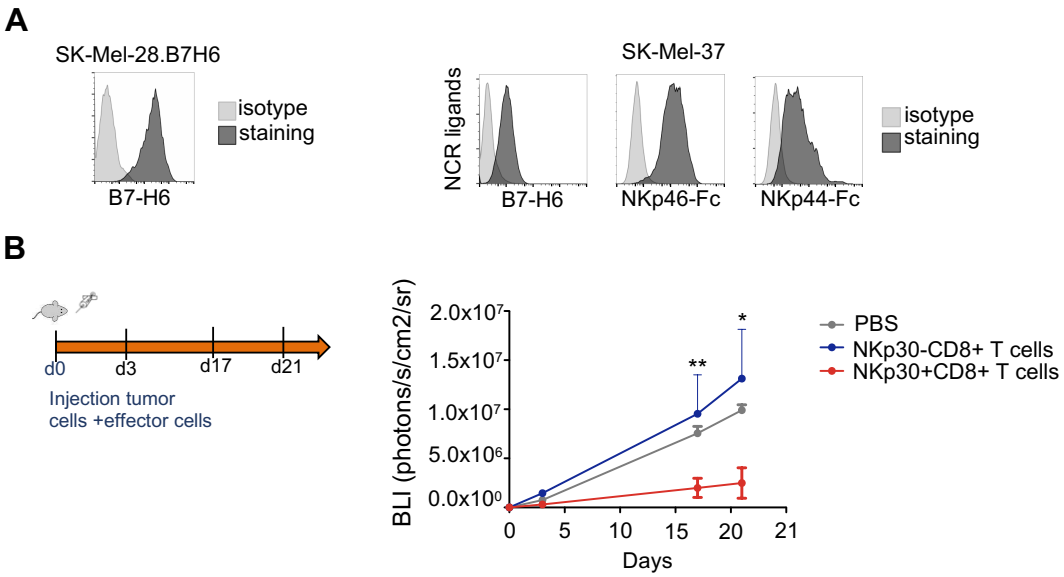

Fig. S9. IL-15-induced NKp30^{+}CD8^{+} T cells display *in vivo* activity against tumor cells. (A) Histograms showing expression of B7-H6 in the transduced cell line, SK-Mel-28.B7H6 and endogenous expression of B7-H6 and staining with NKp46-Fc and NKp44-Fc in the cell line SK-Mel-37. **(B)** Highly purified CD8^{+} T cells were cultured with IL-15 and on d12 were FACS-sorted into NKp30^{+} and NKp30^{-} CD8^{+} T cells. 1x10^{6} sorted NKp30^{+}, NKp30^{-} CD8^{+} T cells or PBS were *i.v.* injected in NSG mice together with 0.25x10^{6} SK-Mel-28.B7-H6.luci cells. BLI values are shown in the graph for each day of measurement. Graph shows mean±SEM for the representative experiment, n=3 mice/group for each specific time-point, **P≤0.01, *P≤0.05 (unpaired Student´s *t*-test, NKp30+ and NKp30- compared with PBS control).

Table S1. Systemic lupus erythematosus (SLE) patients and healthy controls.

	SLE patients			Controls
Age (years)	mean=46; median=48; range (19-67)			mean=44; median=46; range (20-60)
Sex (female/male)	29/7			10/5
SLEDAI	range (0-8) SLEDAI 0, n=20, SLEDAI 2, n=13, SLEDAI 4, n=1, SLEDAI 6, n=1, SLEDAI 8, n=1			-
Disease duration (years)	mean=16.4, median=17, SD=9.1, range (1-36)			-
SLE treatment	Total patients	%NKp30$^+$CD8$^+$ T cells		-
		≤6%	>6%	
Hydroxychloroquine	(33/36)	(16/18)	(17/18)	
Immunosuppressive drugs*	(17/36)	(8/18)	(9/18)	
Steroids*	(19/36)	(9/18)	(10/18)	
NSAID*	(2/36)	(0/18)	(2/18)	
Other treatments for associated problems				
Circulatory/ heart/ hypertension*	(18/36)	(7/18)	(11/18)	
Digestive/ gastrointestinal*	(8/36)	(4/18)	(4/18)	
Respiratory*	(3/36)	(1/18)	(2/18)	

*Immunosuppressive drugs (Methotrexate (MTX), Leflunomide, Mycophenolic acid or Azathioprine), *Steroids (Prednisone/ Prednisolone), *NSAID (Celecoxib), *Circulatory/heart/hypertension (Rivaroxaban, Amlodipine, Losartan, Ramipril, Metoprolol, Clonidine, Enalapril, Furosemide, Candesartan, Molsidomine, Atorvastatin, Ranolazine, Clopidogrel, Diltiazem, Bisoprolol, Verapamil or Triamterene), *Digestive/gastrointestinal (Pantoprazole or Esomeprazole), *Respiratory (Aclidinium bromide or Formoterol). Abbreviations: SLEDAI= Systemic Lupus Erythematosus Disease Activity Index, NSAID= Nonsteroidal anti-inflammatory drug).

References

1. Fiegler N, *et al.* (2013) Downregulation of the activating NKp30 ligand B7-H6 by HDAC inhibitors impairs tumor cell recognition by NK cells. *Blood* 122(5):684-693.
2. Schlecker E, *et al.* (2014) Metalloprotease-mediated tumor cell shedding of B7-H6, the ligand of the natural killer cell-activating receptor NKp30. *Cancer Res* 74(13):3429-3440.
3. Delahaye NF, *et al.* (2011) Alternatively spliced NKp30 isoforms affect the prognosis of gastrointestinal stromal tumors. *Nat Med* 17(6):700-707.
4. Kallio MA, *et al.* (2011) Chipster: user-friendly analysis software for microarray and other high-throughput data. *BMC Genomics* 12:507.
5. Chan WK, *et al.* (2013) Multiplex and genome-wide analyses reveal distinctive properties of KIR+ and CD56+ T cells in human blood. *J Immunol* 191(4):1625-1636.
6. Warnat P, Eils R, & Brors B (2005) Cross-platform analysis of cancer microarray data improves gene expression based classification of phenotypes. *BMC Bioinformatics* 6:265.
7. Sutlu T, *et al.* (2012) Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapy. *Hum Gene Ther* 23(10):1090-1100.