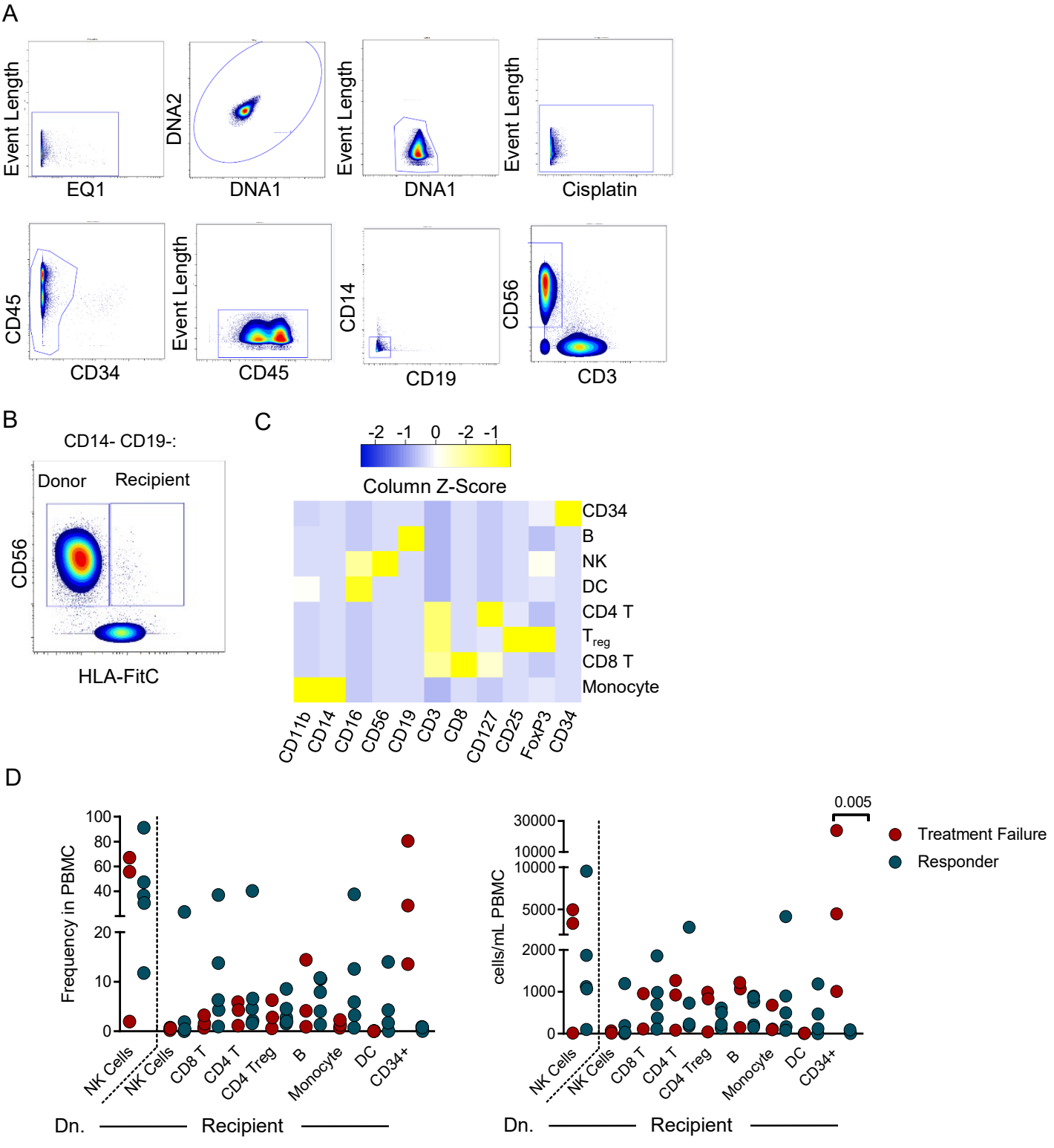

Fig. S1. Gating schema and lymphocyte subsets rel/ref AML patients treated with ML NK cell adoptive therapy. (**A**) NK Gating schema. NK cells are identified as Live (Cisplatin$^-$), CD34$^-$ CD45$^+$CD14$^-$CD19$^-$CD3$^-$ CD56$^+$. (**B**) Representative example of how HLA staining was utilized to distinguish donor versus recipient NK cells, CD34$^-$ CD45$^+$CD14$^-$CD19$^-$ cells are shown. In this example, donor cells are HLA-negative, while recipient (CD3$^+$) cells are HLA$^+$. (C) Heatmap displaying the expression of the indicated marker within the FlowSOM gated lymphocyte subsets. (D) Frequency and total numbers of each lymphocyte population by response, 7 days post-infusion. Dn. Donor NK cells, based on HLA expression.

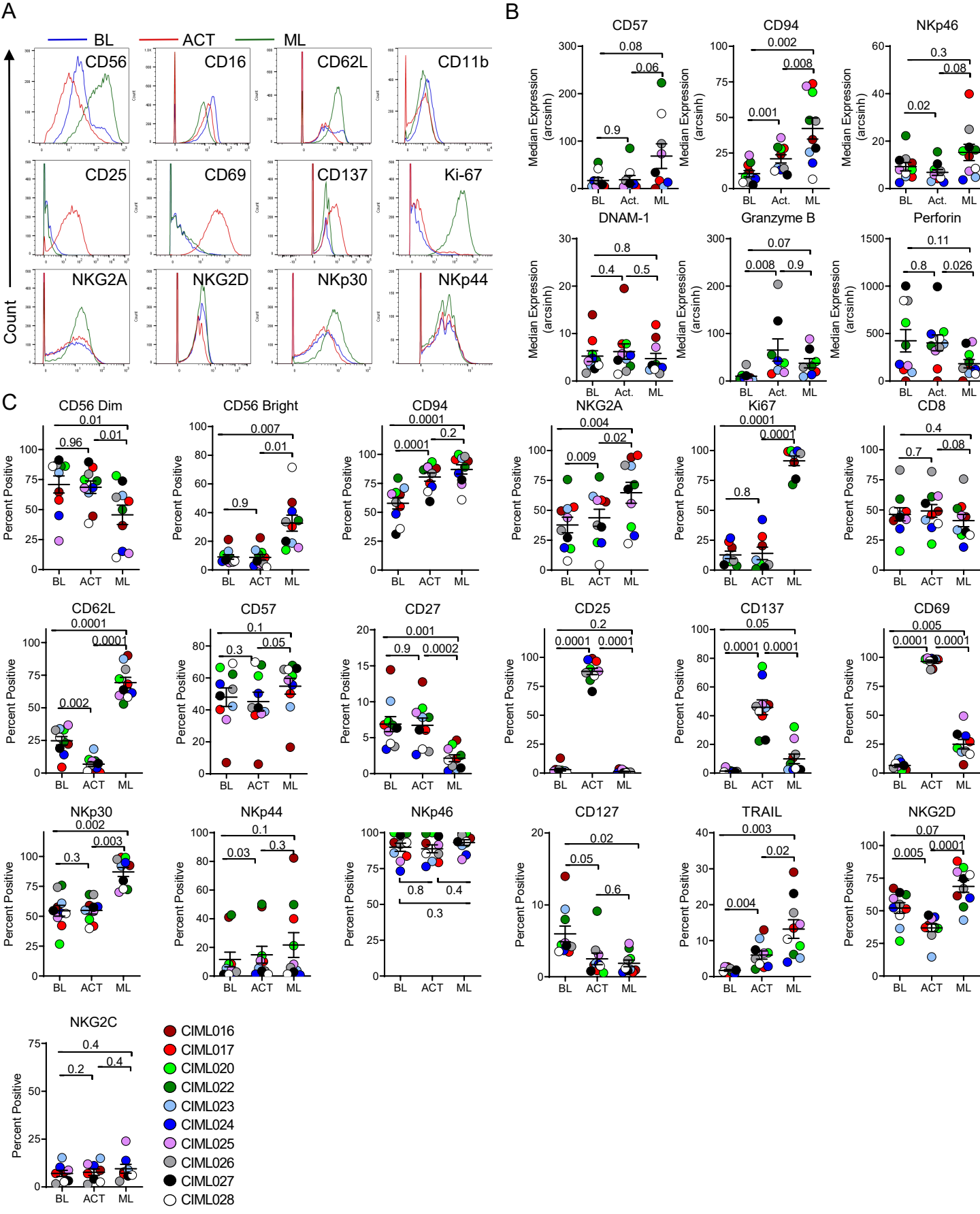

Fig. S2. Phenotypic differences in baseline, activated and ML NK cells. (**A**) Representative histograms of the indicated markers, from Fig. 2. (**B**) Summary data demonstrating median expression of the indicated markers. (**C**) Summary data of percent positive of each indicated marker.

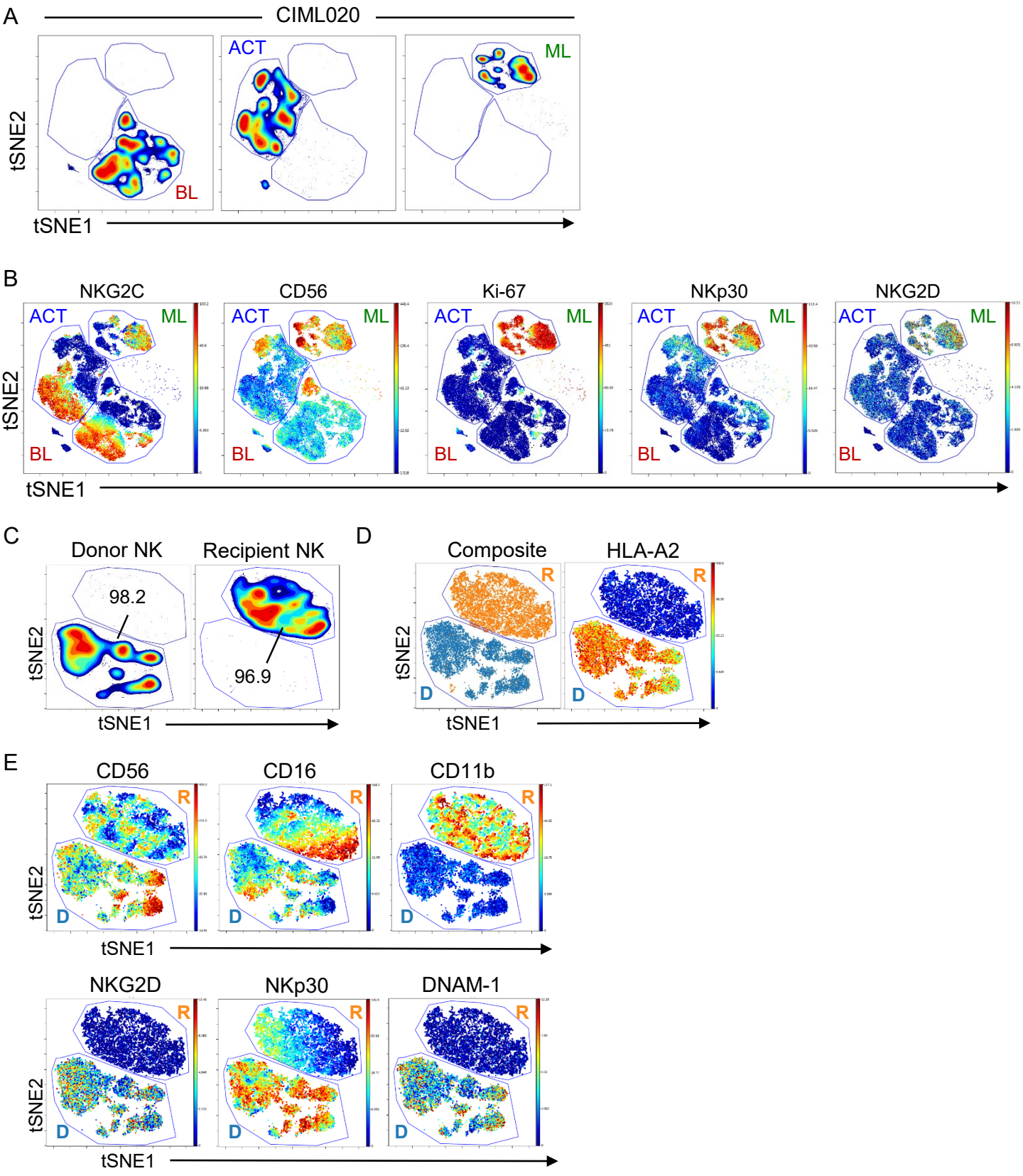

Fig. S3. Phenotypic differences in baseline, activated and ML NK cells for NKG2C+ donor. (**A**) viSNE map of one patient (CIML020) with robust NKG2C^{+} Population. (**B**) Median expression of the indicated markers in this patient, consistent with ML NK cell differentiation. (**C-E**) Recipient v Donor NK cells in the blood on D7, analyzed as in Figure 2. (**C**) viSNE density map of CIML020 donor and recipient NK cells. (**D**) Overlay viSNE plot of Donor (D) and Recipient (R) NK cells (left) and HLA-A2 staining (not included in the viSNE clustering). (**E**) Median expression of the indicated markers on D v R NK cells.

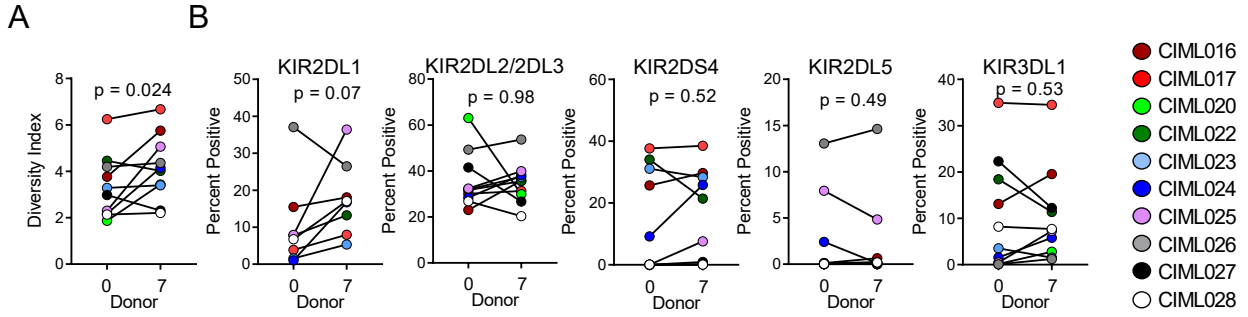

Fig. S4. KIR Diversity prior to infusion and after in vivo ML differentiation. (**A**) KIR diversity was examined on donor NK cells pre-infusion and from the PB on day 7. (**B**) Frequency of the indicated KIR on donor NK cells, pre-infusion and at D7.

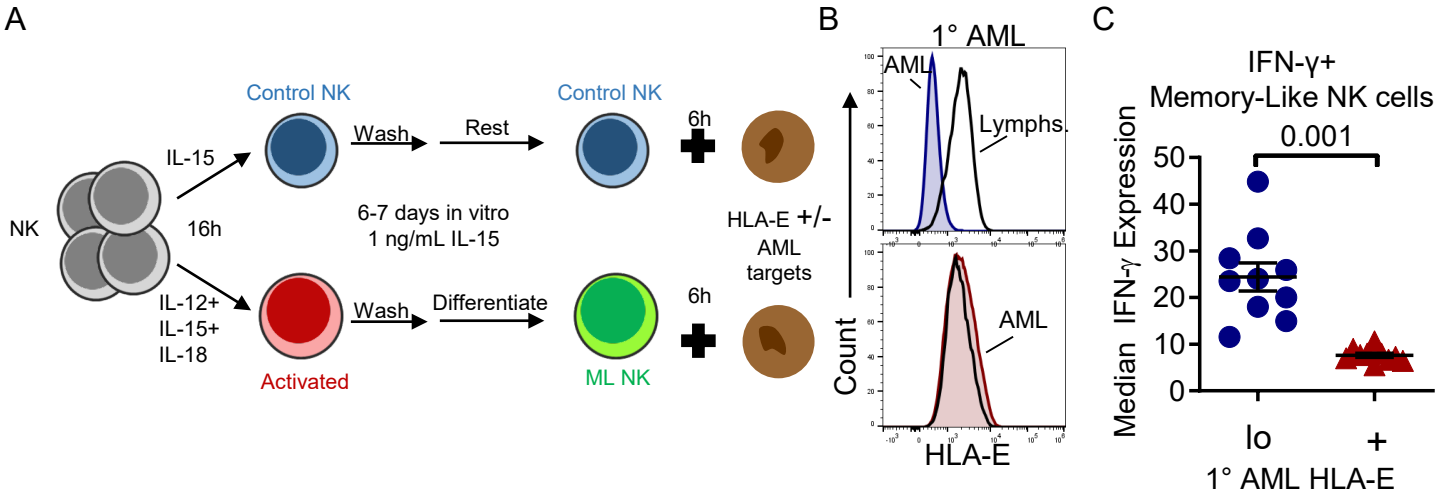

Fig. S5. HLA-E on tumor targets inhibits ML NK cell responses. (**A**) Assay Schema. (**B-C**) Memory-like NK cells were generated in vitro, and stimulated with HLA-E^{+} or HLA-E^{-} primary AML. (**B**) Representative histogram of two primary HLA-E^{+} (red, bottom) or HLA-E^{lo} (blue, top) AML samples. Black line indicates lymphocytes within the sample, color histogram represents AML blasts (CD45^{lo}CD34^{+}). (**C**) Summary data showing median IFN-γ expression on IFN-γ^{+} cells stimulated with HLA-E^{+} or HLA-E^{lo} primary AML. Mean and SEM are shown and compared using paired T-test from 5 normal donors, stimulated with 3 different primary AML over 3 independent experiments.

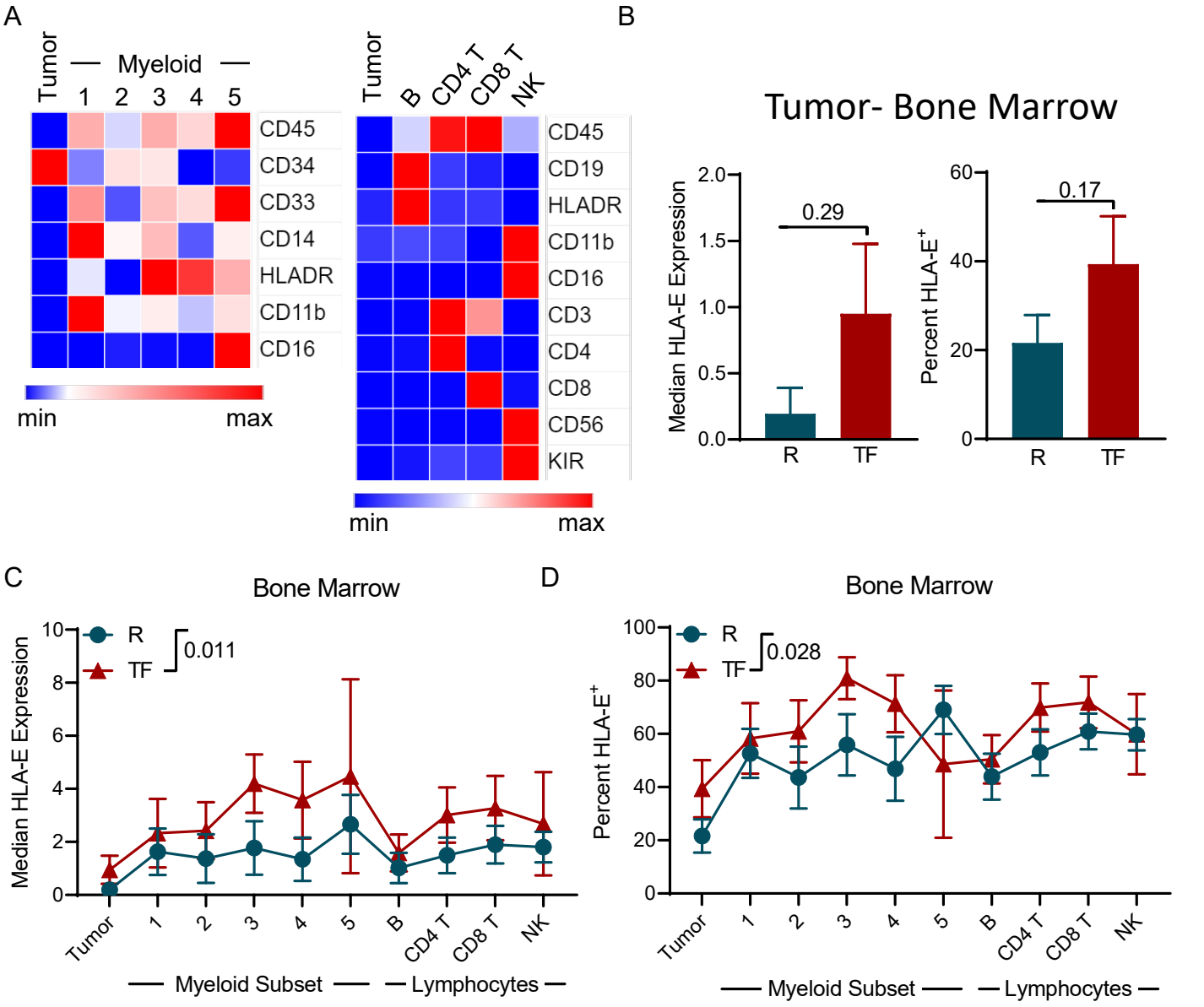

Fig. S6. HLA-E expression on tumor, myeloid and lymphoid populations within the bone marrow tumor microenvironment from patients prior to ML NK cell therapy. Patient BM aspirate obtained upon enrollment in the study, but prior to treatment was assessed using mass cytometry. FlowSOM was used to identify cell subsets within the BM and median HLA-E expression was compared between treatment failure patients and responders. (**A**) Heatmap demonstrating the phenotype of each FlowSOM identified meta-cluster. (**B**) Median HLA-E expression (left) and percent positive (right) on the tumor subset, as defined in A, for responders v TF. Data were compared using Mann-Whitney or unpaired t test. (**C**) Median HLA-E on the indicated cell subset. (**D**) Percent HLA-E positive on the indicated cell subset. Data are represented as mean and SEM, and were compared using 2way ANOVA.

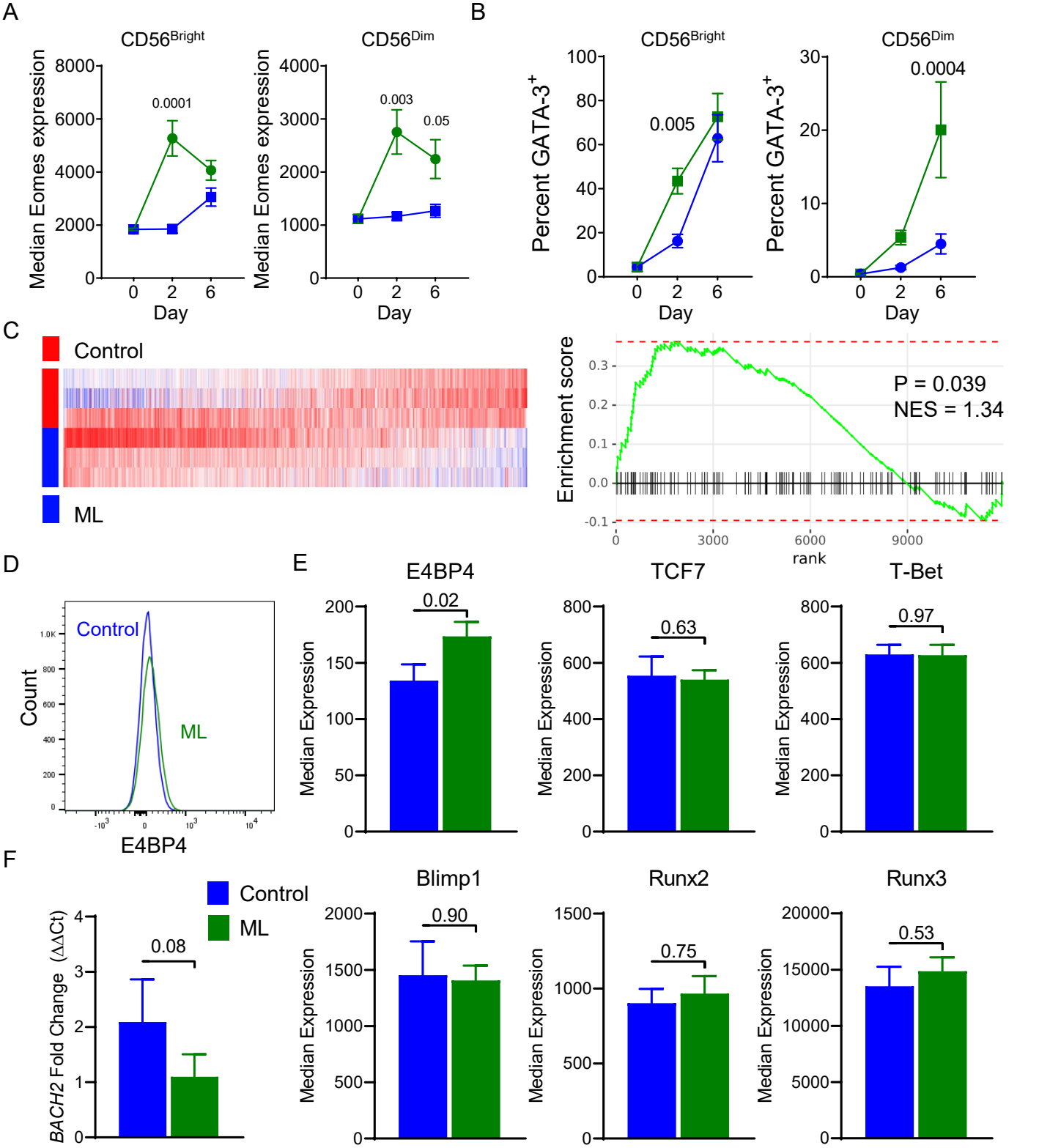

Fig. S7. Transcription factor expression in control and ML NK cells. Control and ML NK cells were generated in vitro and assessed. (**A-B**) Eomes (A) and GATA-3 (B) expression in CD56^{Bright} and CD56^{dim} subsets were assessed by intracellular flow cytometry at the indicated timepoints. Data are mean and SEM, compared using 2way ANOVA with Sidaks correction, from 6 normal donors, 3 independent experiments. C. RNA from control and ML NK cells after 6 days in vitro was sequenced. GSEA comparing the top 12,000 expressed genes to a GATA-3 target gene list. (D-F) Control and ML NK cells were generated in vitro and assessed after 7 days for the indicated transcription factors by flow cytometry (D-E), or by qPCR (F). Mean and SEM displayed. Data are from 5-6 normal donors in 2-3 independent experiments. Data were compared using Paired t tests.

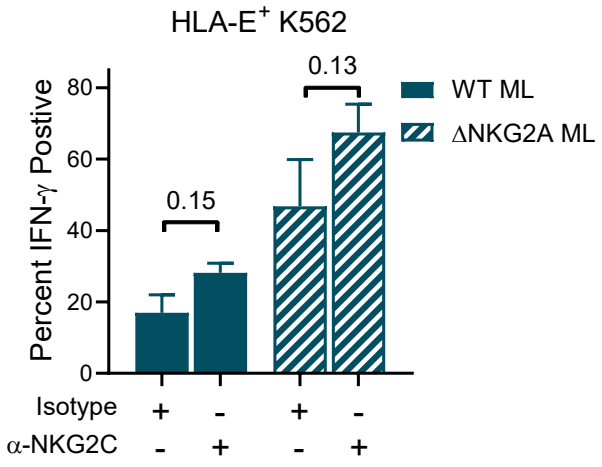

Fig. S8. HLA-E on tumor targets inhibits ML NK cell responses via NKG2A. WT and ΔNKG2A ML NK cells were stimulated with HLAE^{+} K562 leukemia targets in the presence of IgG isotype or anti-NKG2C blocking antibody. Summary data showing percent IFN-γ by flow (n=4, 2 independent experiments). Mean and SEM are shown and compared using RM-ANOVA. P-values are indicated in the graphs.

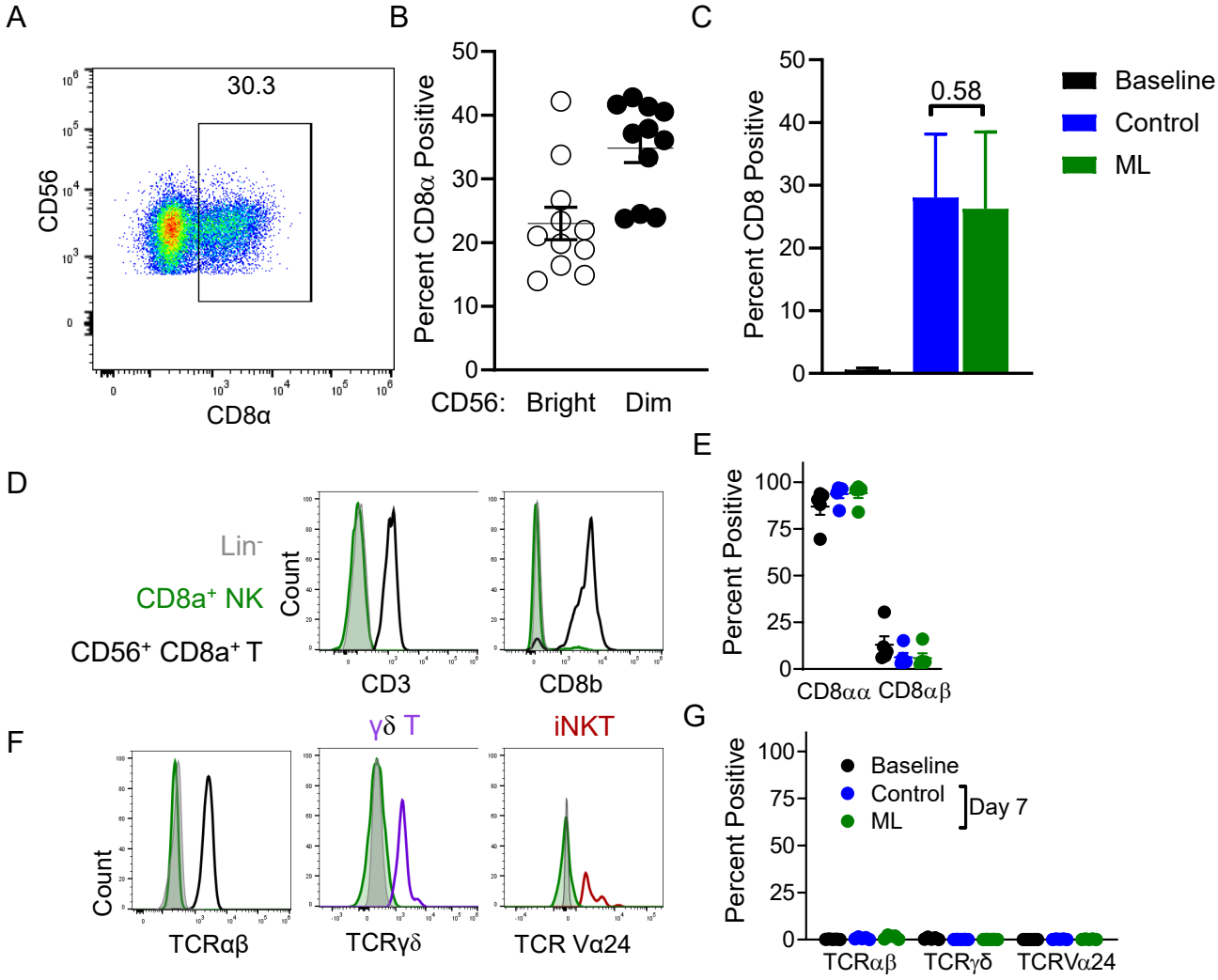

Fig. S9. CD8α expression on CD3$^-$ CD56$^+$ NK cells. (**A-B**) Freshly isolated NK cells were assessed for CD8 α expression by flow cytometry. (**A**) Representative flow plot depicting CD8α$^+$ NK cells. The number represents the frequency of cells within the gate. (**B**) Summary data showing CD8α expression on the indicated NK cell subsets. Data show mean and SEM. Data compared using paired t test. (**C-G**) Freshly isolated NK cells (baseline), control and ML NK cells were generated in vitro and assessed on Day 7. (**C**) CD8α expression on flow sorted CD8α-negative cells at baseline, and day 7 (control and ML). (**D-G**) Unsorted NK cells were assessed for the indicated markers and compared to T cells. (**D**) Representative histogram comparing the indicated markers on Lin- (CD45$^+$ CD3$^-$ CD56$^-$), CD8α$^+$ NK cells (CD45$^+$ CD3$^-$ CD56$^+$ CD8a$^+$), and T cells (CD45$^+$ CD3$^+$ CD56$^+$ CD8a$^+$). (**E**) Summary data from D. (**F**) Representative histogram comparing the indicate markers on T cells (black), γδ T cells (purple, CD45$^+$ CD3$^+$ γδ TCR$^+$), and iNKT cells (red, CD45$^+$ CD3$^+$ Vα24-Jα18 TCR$^+$). (**G**) Summary data from (F). Data are from 2 independent experiments, n=5 normal donors.

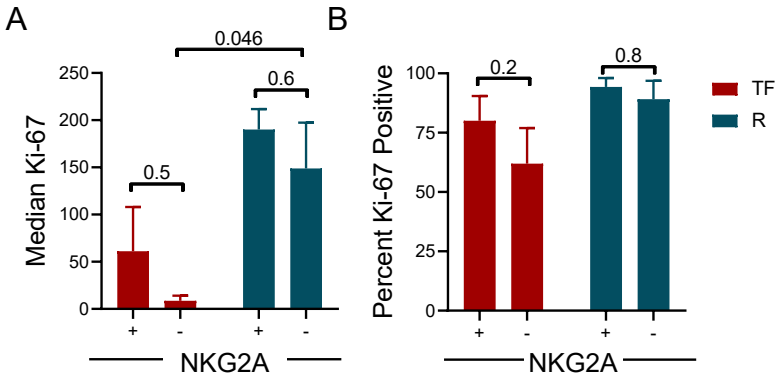

Fig. S10. NKG2A upregulation and proliferation during in vivo ML differentiation. (**A**) Median and (**B**) percent Ki-67+ cells on NKG2A^{+} and NKG2A^{-} in vivo differentiated donor ML NK cells from TF v R patient peripheral blood on day 7 post-NK cell infusion. Mean and SEM are depicted, data compared using 2-way ANOVA.

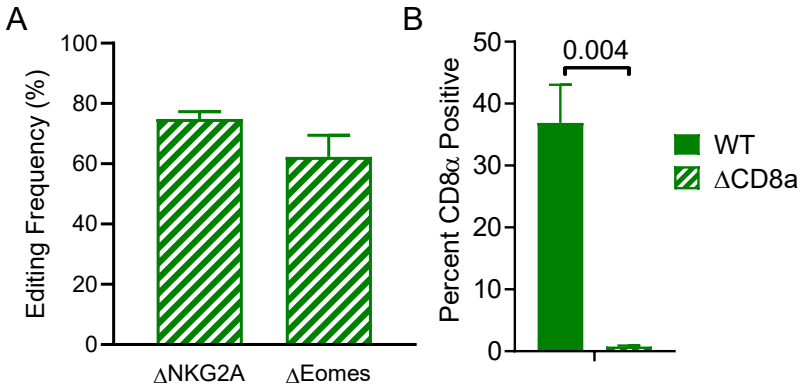

Fig. S11. CRISPR/ Cas9 efficiency. (**A**) DNA from ΔNKG2A and ΔEomes NK cells was isolated and NGS sequencing performed. Summary data from 4-5 donors from 2-3 independent experiments. (**B**) Summary flow cytometry data from WT and ΔCD8a NK cells indicate efficient CRISPR/Cas9 gene editing. Data from 5 normal donors in 2 independent experiments were compared using Paired t test. Mean and SEM are depicted.