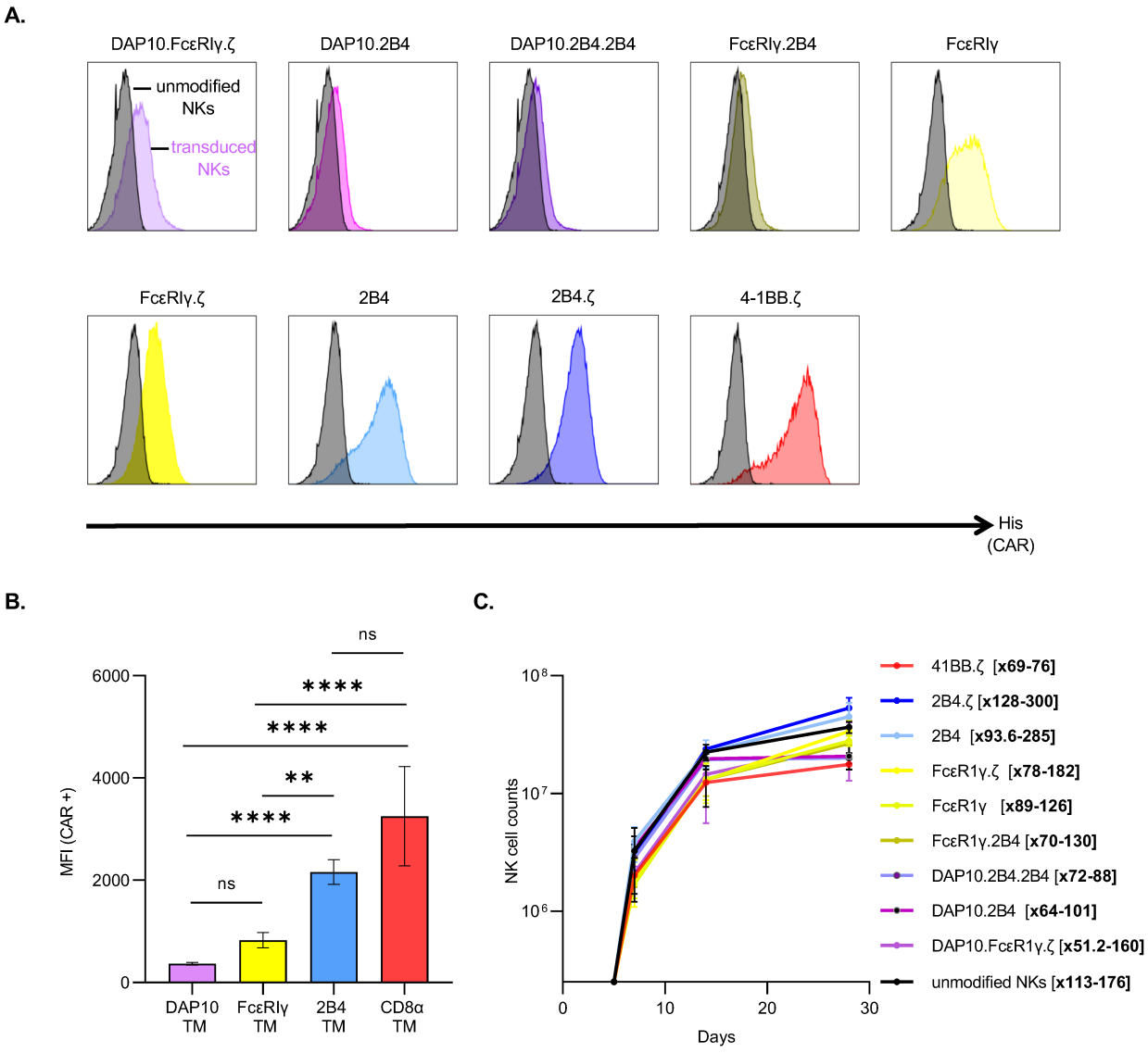
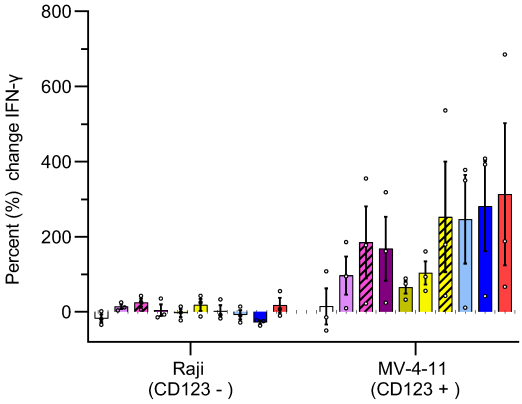
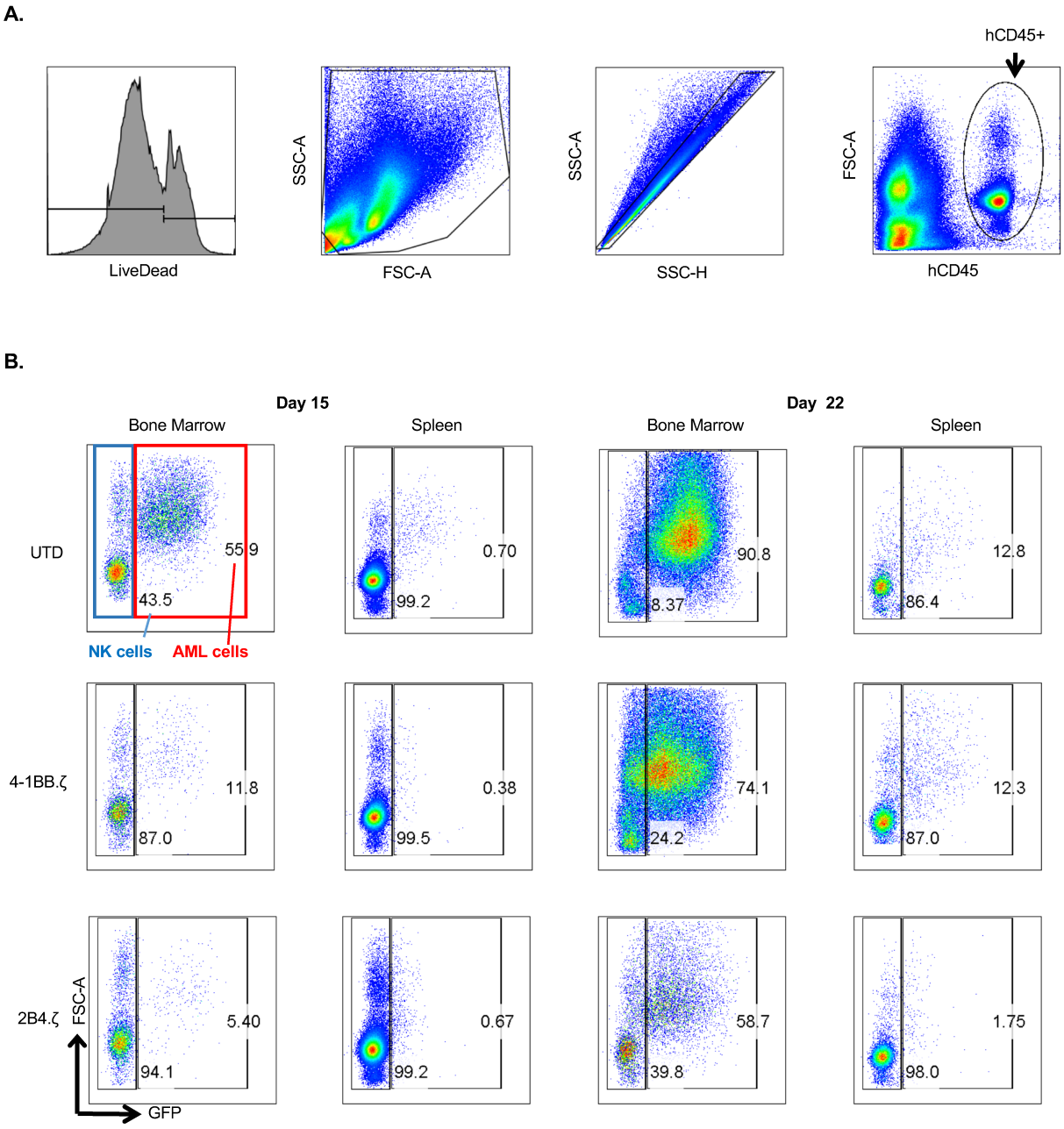


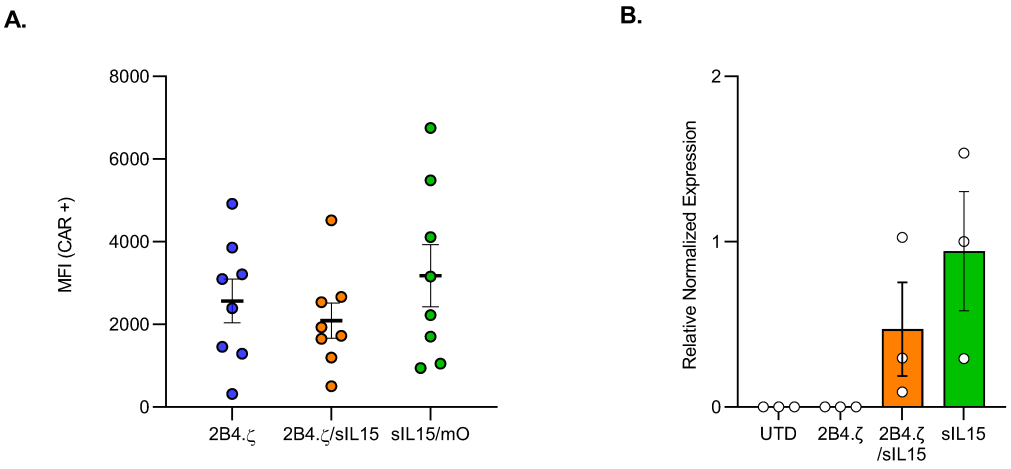
Supplemental Figure 1. CAR expression and proliferative rate of CAR-NK cells. (**A**) FACS plots representing the gating strategy used to identify CAR+ NK cells. (**B**) Bar plot comparing the mean fluorescence intensities +/- SEM of CARs with indicated transmembrane (TM) domains. (**C**) Rate of expansion of our CAR-NK cells calculated with manual counting of absolute number. Initial seeding count was 250,000 cells. Fold expansion indicated in [ ] to the right of legend. Mean +/- SEM (n=3 donors).



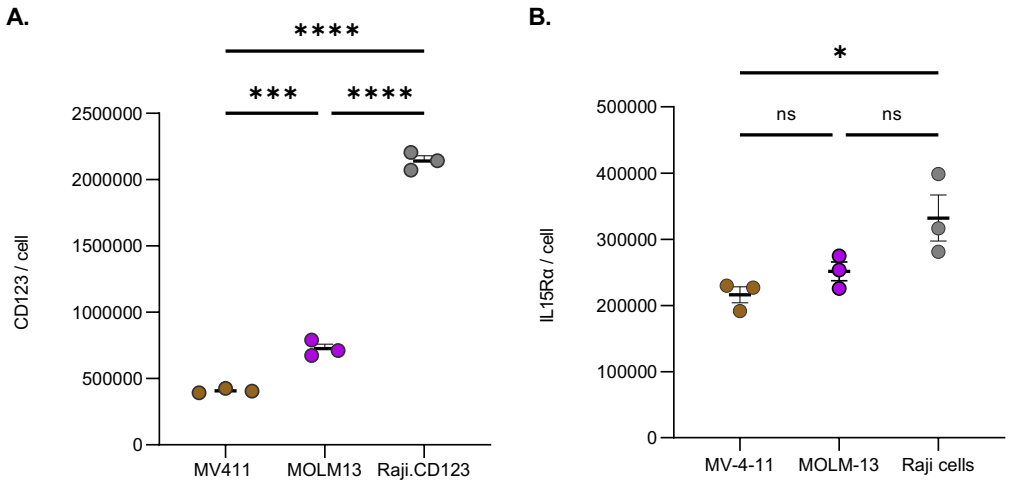
Supplemental Figure 2. Anti-CD123 CAR-NK cells are activated by CD123+ cells. Percent (%) change of IFNγ secretion from baseline measured at 24-hours in co-culture assays of indicated CAR-NKs with Raji [CD123(-)] and MV-4-11 [CD123 (+)] cancer cell lines. IFNγ secretion was measured with ELISA. Each bar representative of the mean plus or minus the standard error of mean (+/- SEM); each dot is representative of an individual NK cell donor.



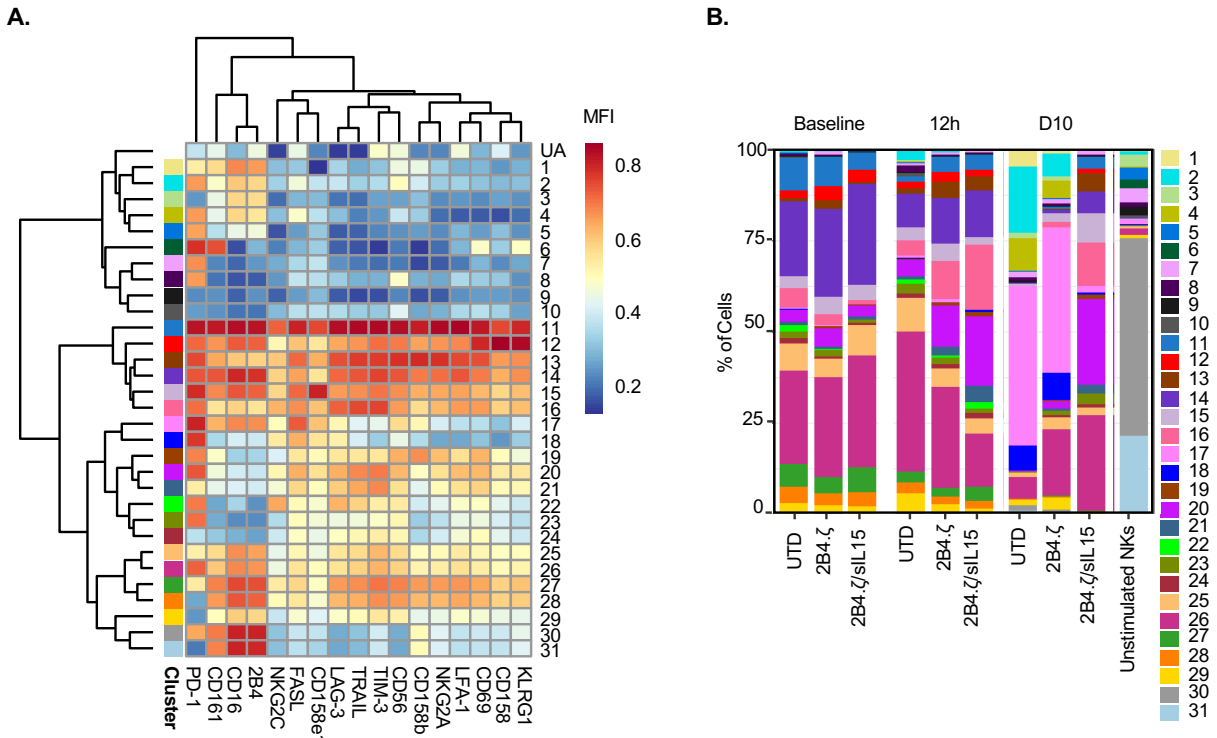
Supplemental Figure 3. NK and leukemia cell percentages in the bone marrow and spleen of MV-4-11 engrafted mice on experimental days 15 and 22. A. Gating strategy used to identify hCD45+ cells in all *in vivo* experiments. LiveDead negative cells (alive) → excluding cellular debris → Single cells→ human CD45 positive (+) cells (human cells). **B.** FACS plots of hCD45(+) cells in the bone marrow and spleen of mice on day 15 (8 days after NK cell injection) and on day 22 (15 days after NK cell injection). MV-4-11.ffLuc cells (red rectangle) are GFP positive. Numbers represent percentages of NK or AML cells. One mouse analyzed per condition per day.



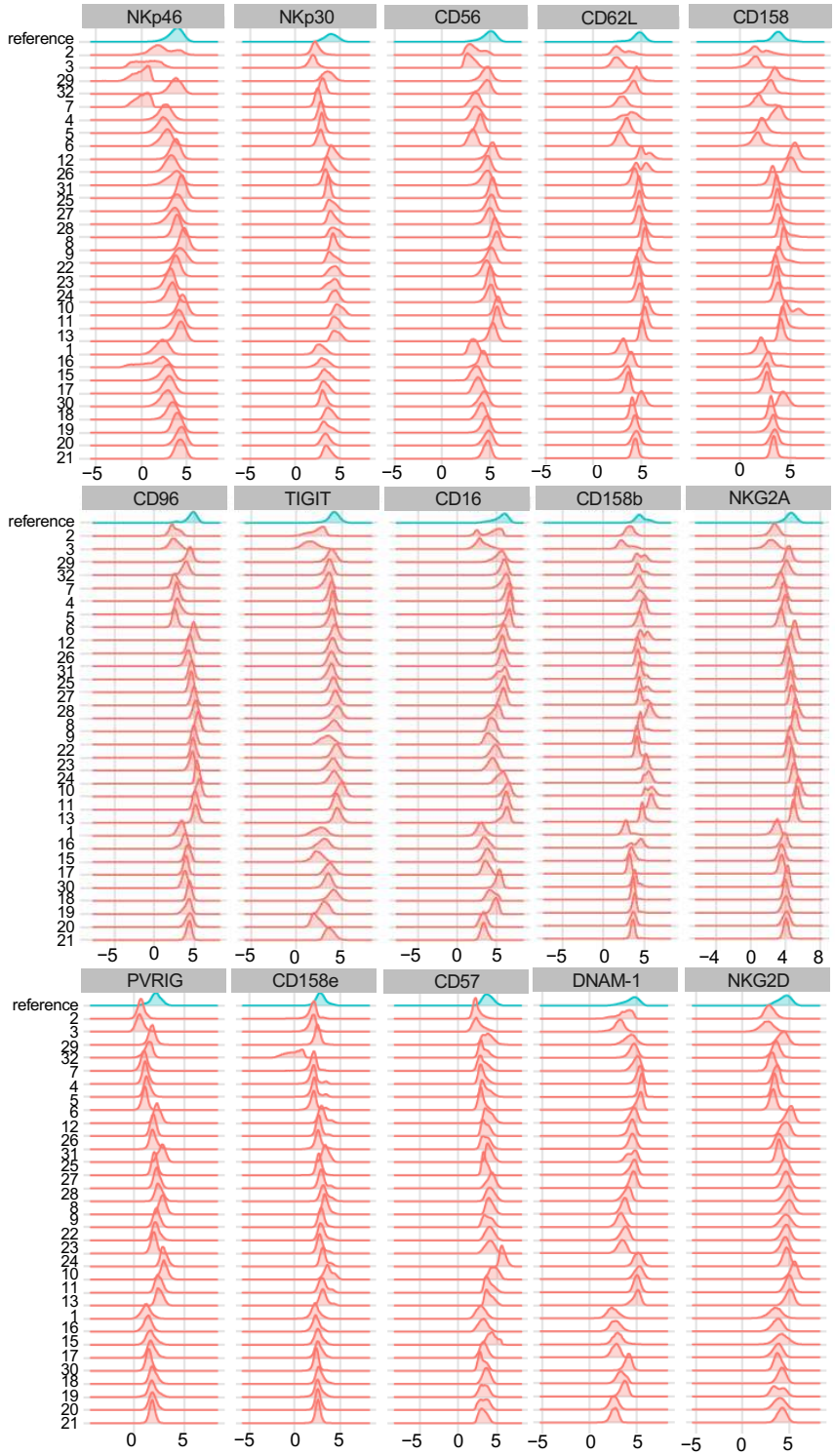
Supplemental Figure 4. Expression of CARs and IL15 in NK cells. (**A**) Mean fluorescence intensity (MFI) of CAR (2B4.ζ and 2B4.ζ/sIL15) or mOrange (sIL15/mO) expression on transduced NK cells (n=4 donors measured at 2 time points). (**B**) Transgenic IL15 expression measured with Real-Time quantitative PCR. Normalized gene expression plotted relative to donor #3 sIL15 condition. GAPDH used as reference gene for normalization of gene expression (n=3 donors).



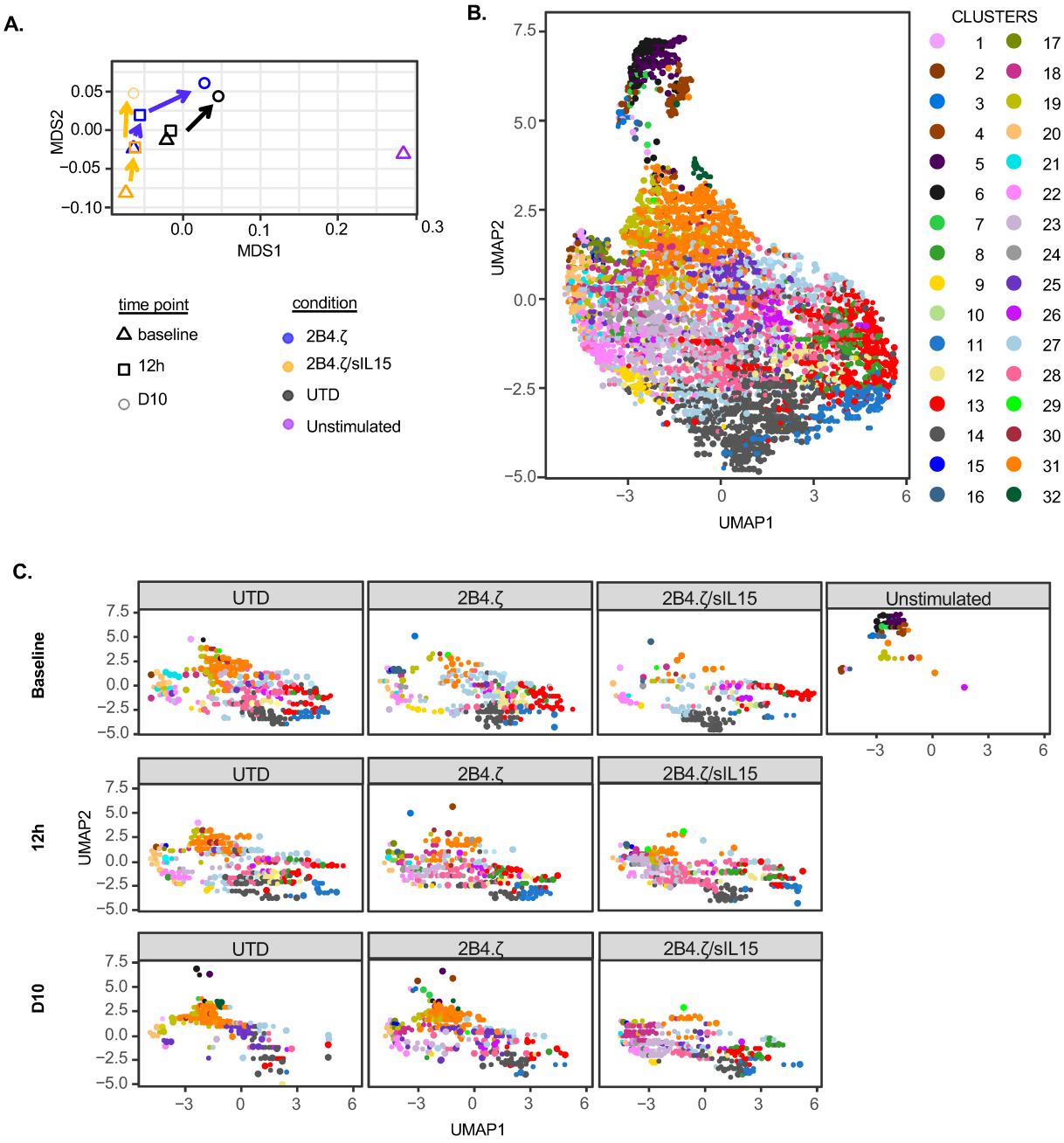
Supplemental Figure 5. Surface antigen quantification on target cells. Quantification of (**A**) CD123 and (**B**) IL15Rα per cell (*p<0.05; ***p<0.001; ****p<0.0001).



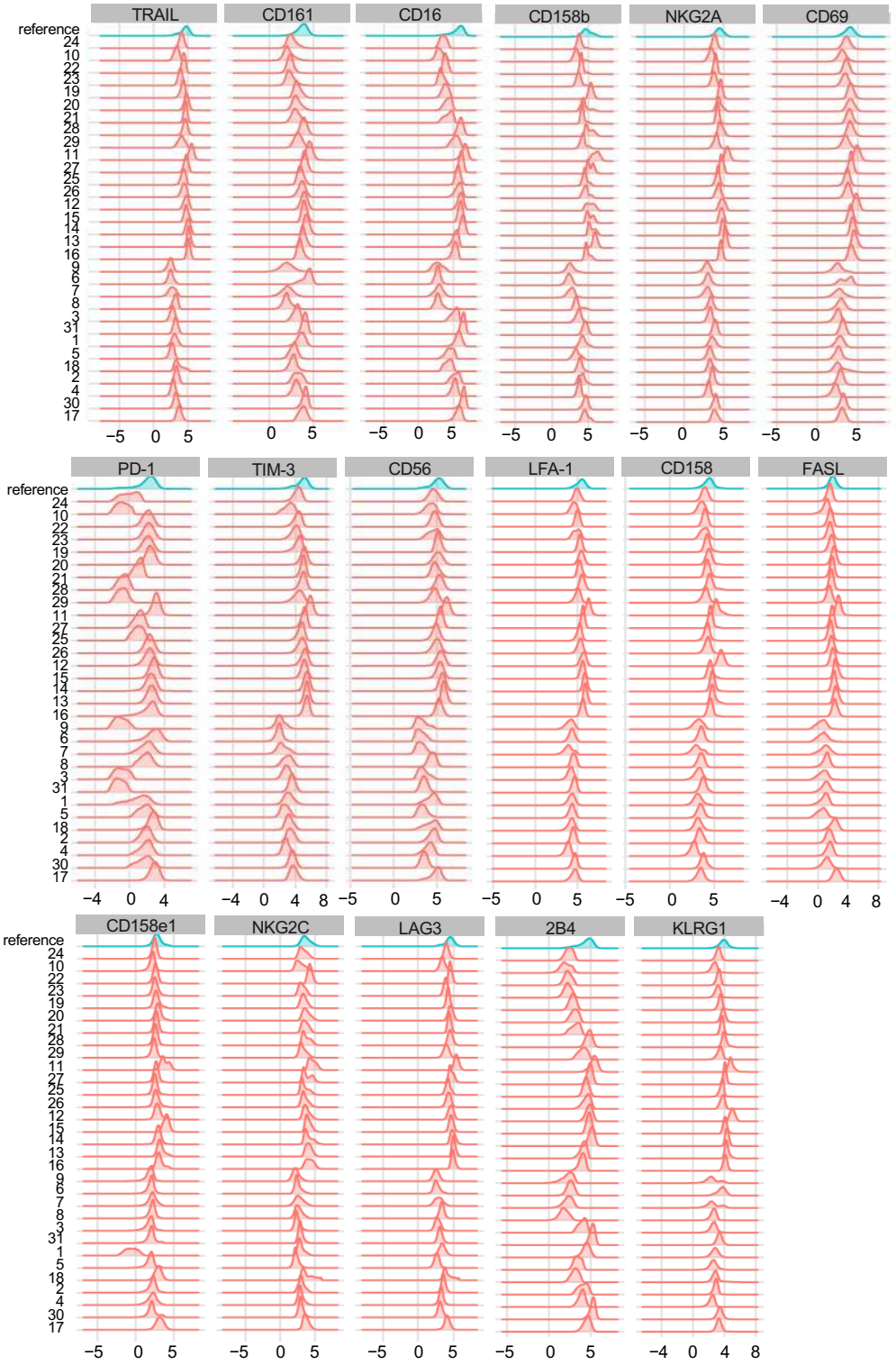
Supplemental Figure 6. IL15-secreting CAR-NK cells demonstrate a distinct phenotype after chronic antigen stimulation. (**A**) Heatmap of flow cytometry data showing expression of 17 different NK cell surface markers. Heatmap coloring represents arcsinh transformed median marker intensities. (**B**) Bar plots of relative abundance of the 31 population subsets found in each sample. *The population clusters of the first panel (shown in **Fig. 4**) are not the same (numbers/colors) as the ones of the second panel, shown here.* Unstimulated, freshly isolated NK cells are used as controls.



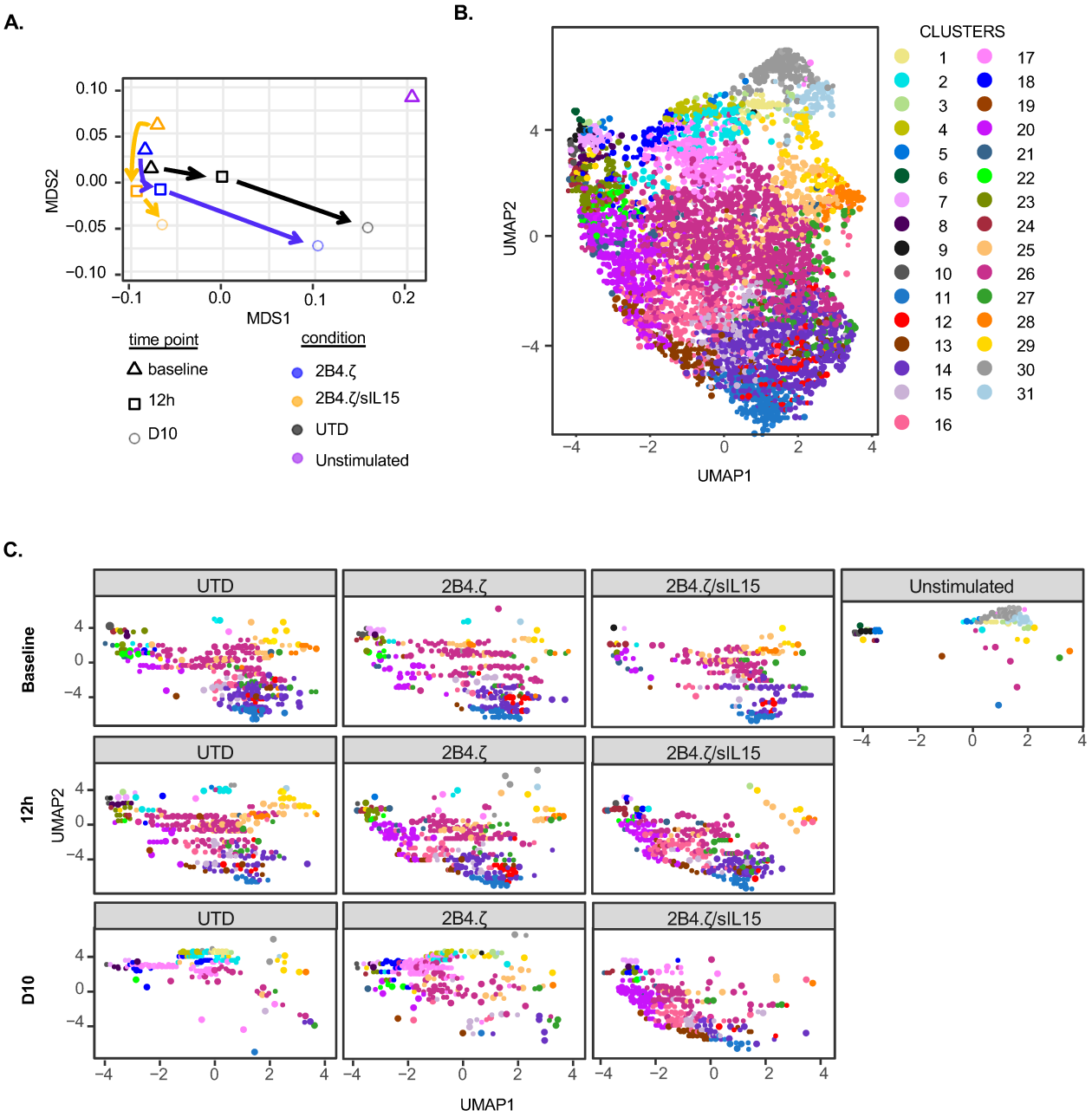
Supplemental Figure 7. Distribution of marker intensities of indicated "Panel A" receptors in the identified 32 NK cell clusters. The NK cell clusters (on the left side) are named as in **Figure 4**. Red histograms represent the respective marker in each cluster. Blue histograms are a reference calculated from all cells.



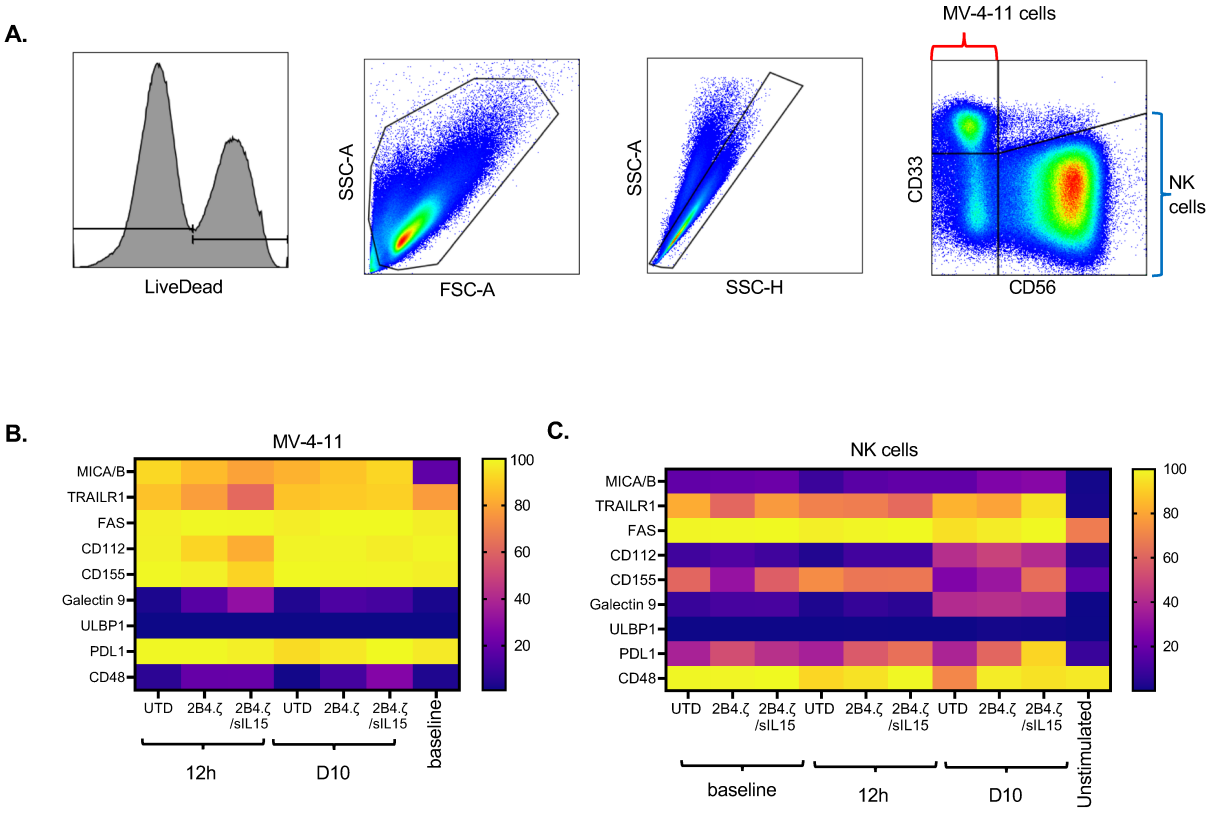
Supplemental Figure 8. Visual representation of "Panel A" immunophenotype data (**A**) Multidimensional scaling (MDS) plot. Unstimulated, freshly isolated NK cells are used as controls. Arrows indicate the transitions across timepoints of experiment. (**B**) Uniform Manifold Approximation and Projection (UMAP) plot was generated based on the arcsinh-transformed expression of the 15 expression markers in the NK cells from the whole dataset. Cells are colored according to the 32 clusters generated after manually merging the 40 meta-clusters obtained with FlowSOM. (**C**) Individual UMAP plots of different NK cell conditions. Different time points indicated on the left side (baseline, 12h, D10).



Supplemental Figure 9. Distribution of marker intensities of the indicated "Panel B" receptors in the 31 NK cell clusters. The NK cell clusters (on the left side) are specific for **Supplemental Fig. 6.** Red histograms represent the respective marker in each cluster. Blue histograms are a reference calculated from all cells.



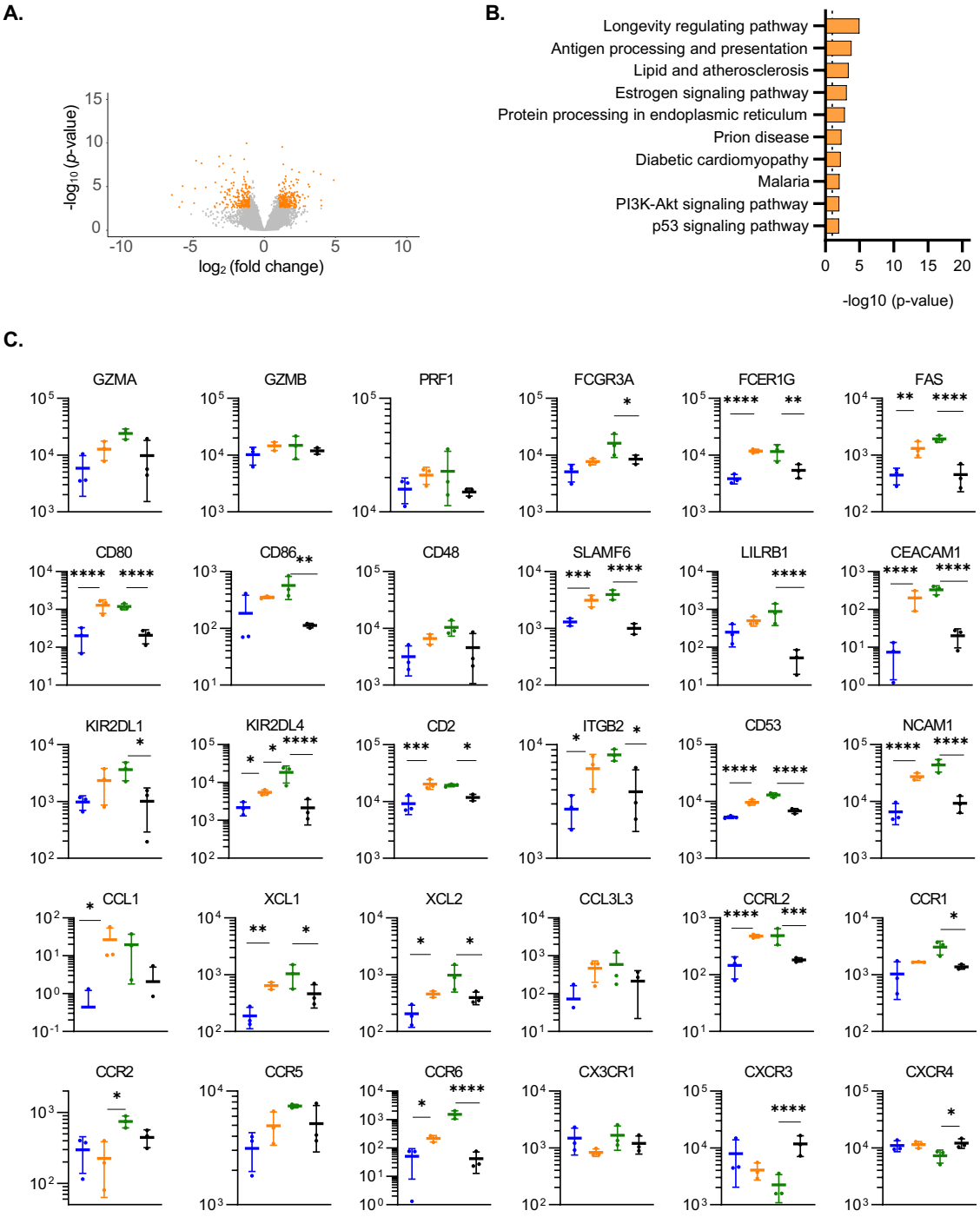
Supplemental Figure 10. Visual representation of "Panel B" immunophenotype data (**A**) Multidimensional scaling (MDS) plot. Unstimulated, freshly isolated NK cells are used as controls. Arrows indicate the transitions across timepoints of experiment. (**B**) Uniform Manifold Approximation and Projection (UMAP) plot was generated based on the arcsinh-transformed expression of the 17 expression markers in the NK cells from the whole dataset. Cells are colored according to the 31 clusters generated after manually merging the 40 meta-clusters obtained with FlowSOM. (**C**) Individual UMAP plots of different NK cell conditions. Different time points indicated on the left side (baseline, 12h, D10).



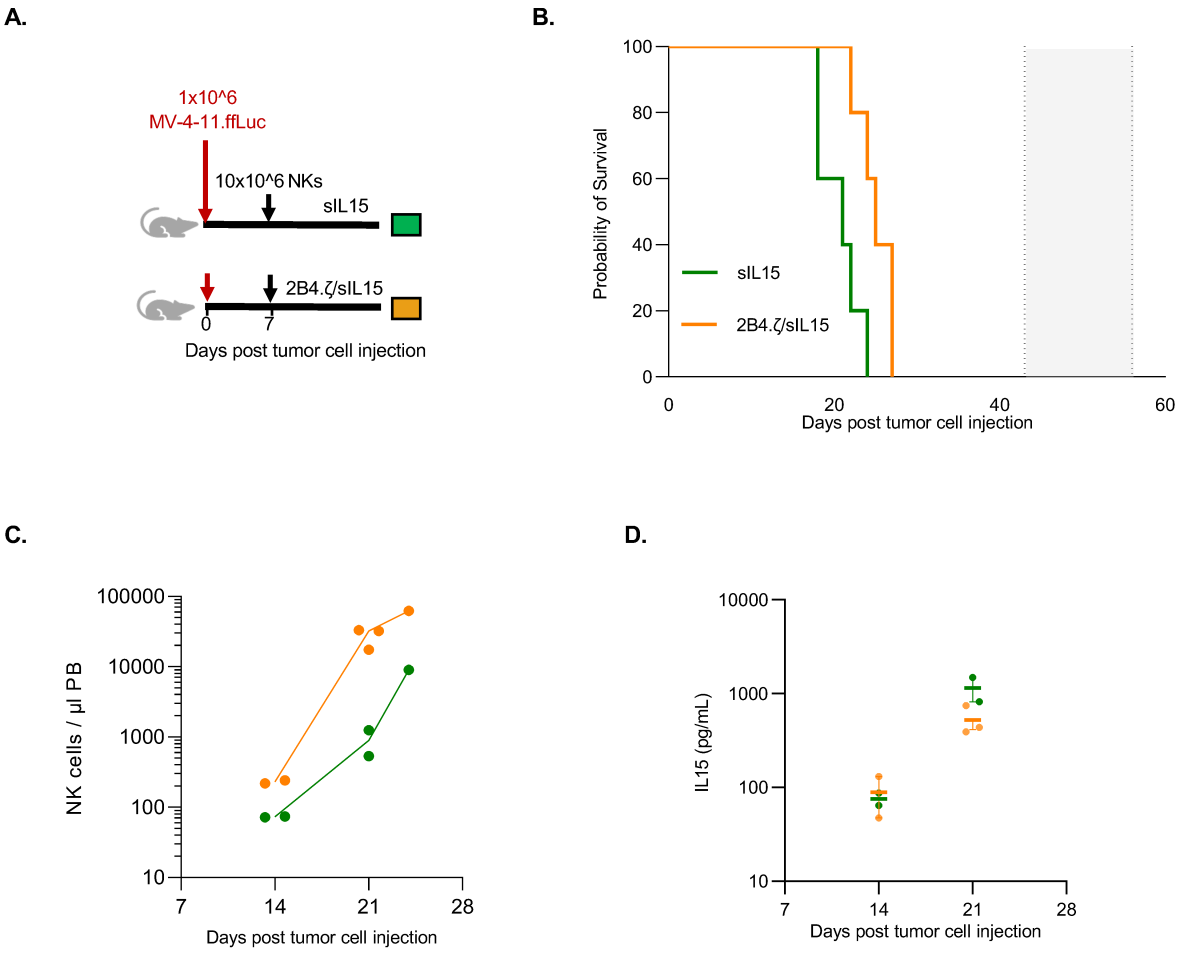
Supplemental Figure 11. Expression of NK cell receptor ligands on target and NK cells. (**A**) Gating strategy. (**B**) Heatmap of the percent (%) expression of indicated ligands on MV-4-11 cells or (**C**) NK cells at each experimental time point.

D1	D2	D3	2B4.ζ			sIL15			2B4.ζ /sIL15		
D4	D5	D6									
D7	D8	D9									
D10											
UTD			< 0.0001	<0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
			0.053	>0.99	>0.99	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
			>0.99	>0.99	>0.99	0.0002	0.0023	0.98	< 0.0001	< 0.0001	< 0.0001
			>0.99			>0.99			0.0016		
2B4.ζ						>0.99	0.0054	< 0.0001	>0.99	0.0024	< 0.0001
						< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
						< 0.0001	0.0023	0.98	< 0.0001	< 0.0001	< 0.0001
						>0.99			0.0016		
sIL15									>0.99	>0.99	>0.99
									0.504	0.846	0.032
									< 0.0001	< 0.0001	< 0.0001
									0.0016		

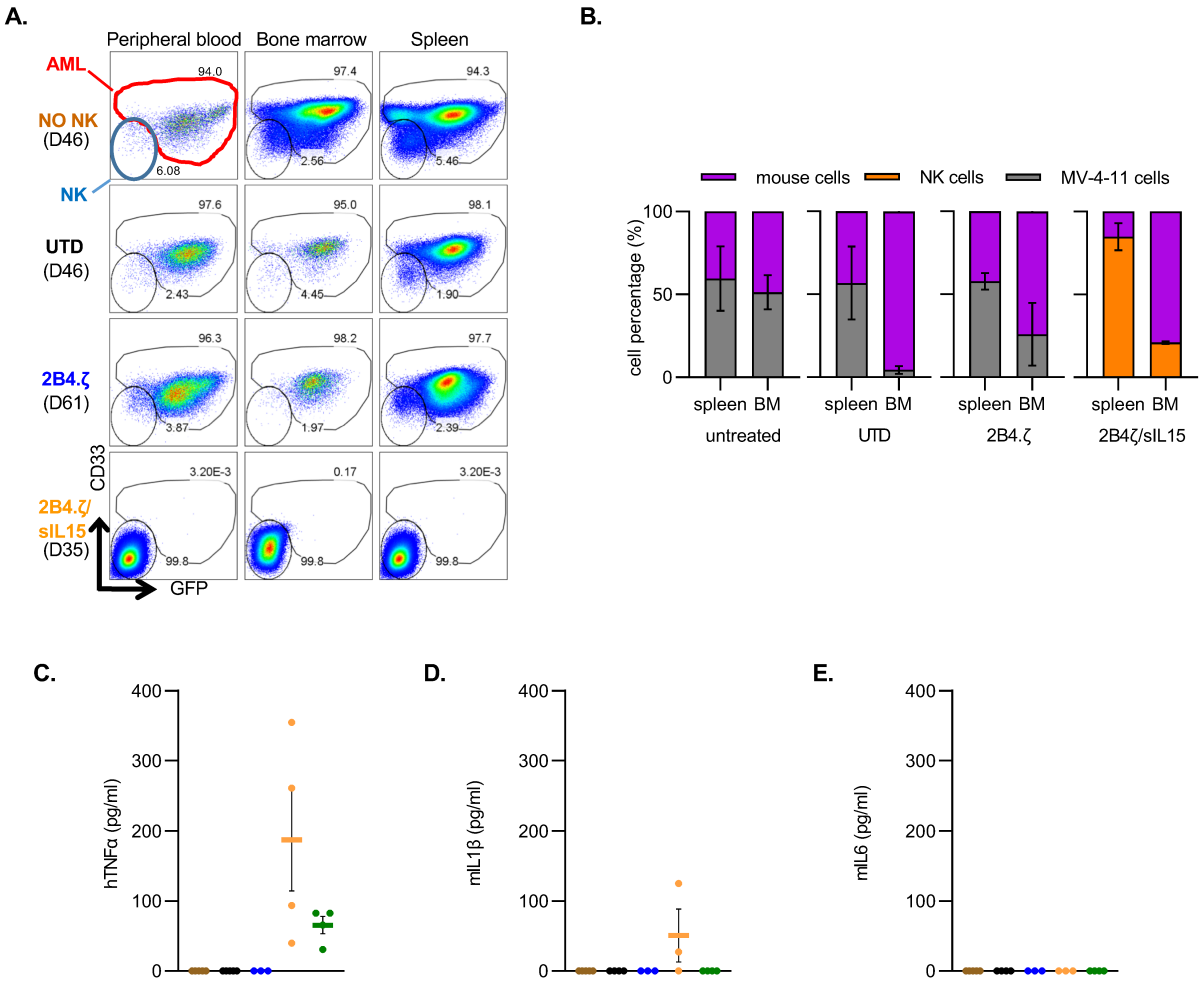
Supplemental Figure 12. Comparisons of mean percent (%) cytotoxicity between different NK cell conditions in the serial stimulation assay shown in Fig. 4E. Each cell is subdivided to represent days 1-10 as indicated in upper left corner. Every cell's value signifies the p-value of each comparison for every day. P values generated with ordinary 2-way ANOVA corrected for multiple comparisons using the method of Bonferroni.



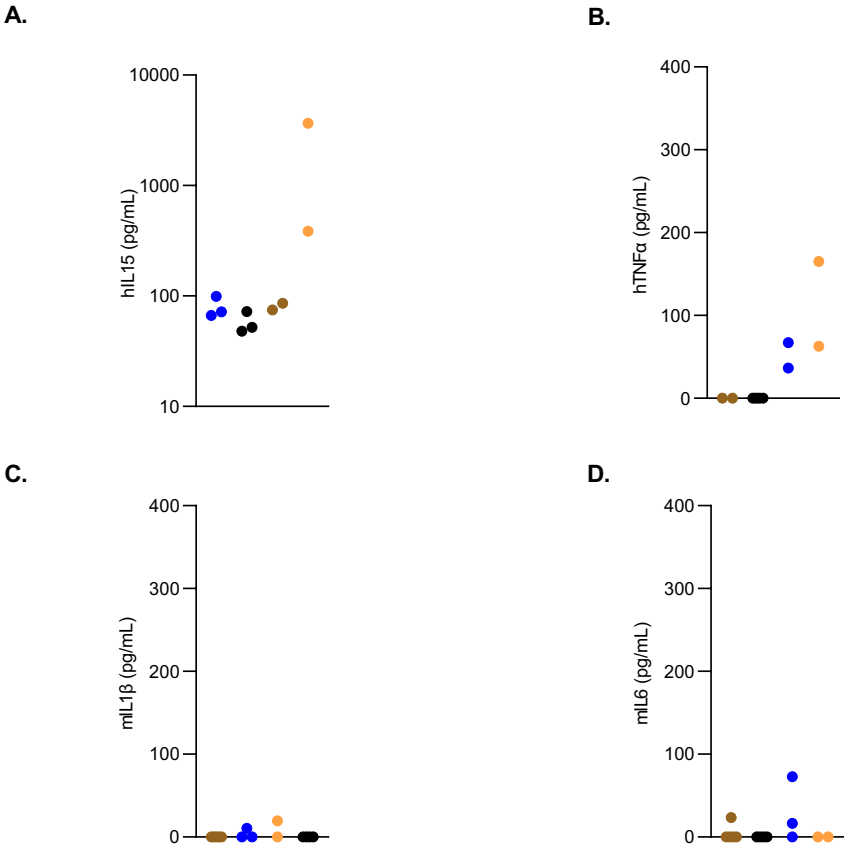
Supplemental Figure 13. Transcriptomic evaluation of individual genes and differential expression analysis of IL15 secreting NKs. (**A**) Volcano plot representing upregulated and downregulated genes in 2B4.ζ/sIL15 compared to sIL15 NK cells. Orange dots on the left represent downregulated and dots on the right represent upregulated genes. Location of each data point is calculated as log2(FC) × −log10(p-value). Color cutoffs: p-value < 0.05; fold change cutoff >2 or <1/2. (**B**) Bar plot depicting the top 10 significant pathways enriched in the differentially expressed genes. KEGG 2021 Human gene Set Enrichment Analysis was used. Dotted line represents the cutoff p-value of 0.05.(**C**) Dot plots displaying the normalized expression of individual genes (n=3 independent donors). Statistical significance: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.



Supplemental Figure 14. IL15 stimulated NK cells promote lethal toxicity of MV-4-11 engrafted mice. (**A**) Schematic of MV-4-11 xenograft treated with IL15-secreting NK cells. On day 0, NSG mice were injected via tail vein with $1x10^6$ CD123(+) MV-4-11 cells. In treatment groups (2B4.z/sIL15 or sIL15), $10x10^6$ NK cells were administered on day 7. (**B**) Kaplan–Meier survival analysis of MV-4-11 xenografts (n=5 mice each group). Dotted lines border grey shading representing usual survival window of MV-4-11 xenograft model. (**C**) Mouse peripheral blood (PB) was collected at indicated time points and analyzed via flow cytometry. NK cell numbers per microliter of mouse PB were tracked starting on day 14 of the experiment. Each dot represents cell numbers from a single mouse. (**D**) Human IL15 from peripheral blood of MV-4-11 engrafted mice at the indicated time points was quantified (pg/mL) with ELISA (mean +/- SEM, each dot representative of a single mouse).



Supplemental Figure 15. IL15 stimulation promotes NK cell expansion and inflammation *in vivo.* (**A**) FACS plots of hCD45(+) cells in the peripheral blood, bone marrow, and spleen of mice at necropsy. hCD45(+)CD33(-)GFP(-) cells: NK cells (blue). Percentage of cells populating NK or AML gates indicated. (**B**) Percentage (%) cell populations in spleen and bone marrow at necropsy (n=3 mice). Mean +/- SEM. (**C**) Human TNFα, (**D**) mouse IL1β and (**E**) mouse IL6 from peripheral blood of MV-4-11 engrafted mice drawn at necropsy and quantified with ELISA. Each dot derived from a single mouse. Mean +/- SEM.



Supplemental Figure 16. IL15-secreting CAR-NK cell treatment promotes inflammation in MOLM-13 engrafted mice. (**A**) Human IL15, (**B**) human TNFα, (**C**) mouse IL1β and (**D**) mouse IL6 analysis from the peripheral blood drawn at necropsy from MOLM-13 engrafted mice. Cytokine measurement (pg/mL) with ELISA. Each dot represents data from a single mouse. n=2-5 mice.