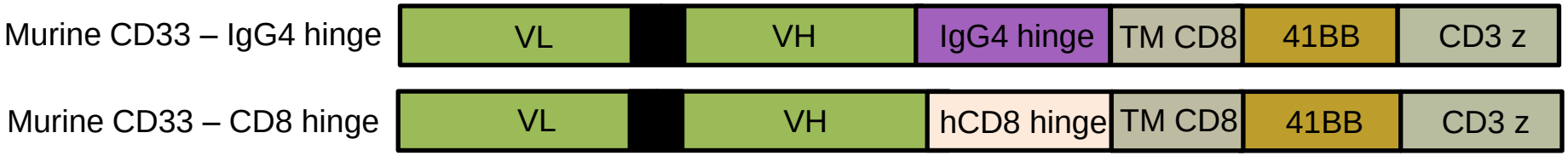

Figure S1. The murine CAR constructs used in this study. All are second generation CARs composed of: an extracellular domain (the light to heavy orientation of the scFV clone MY96 of gemtuzumab ozogamicin), a hinge derived from either human CD8 or IgG4 as indicated, a transmembrane domain (TM) derived from CD8, 41BB (CD137) costimulatory domain and CD3zeta intracellular signaling domain.

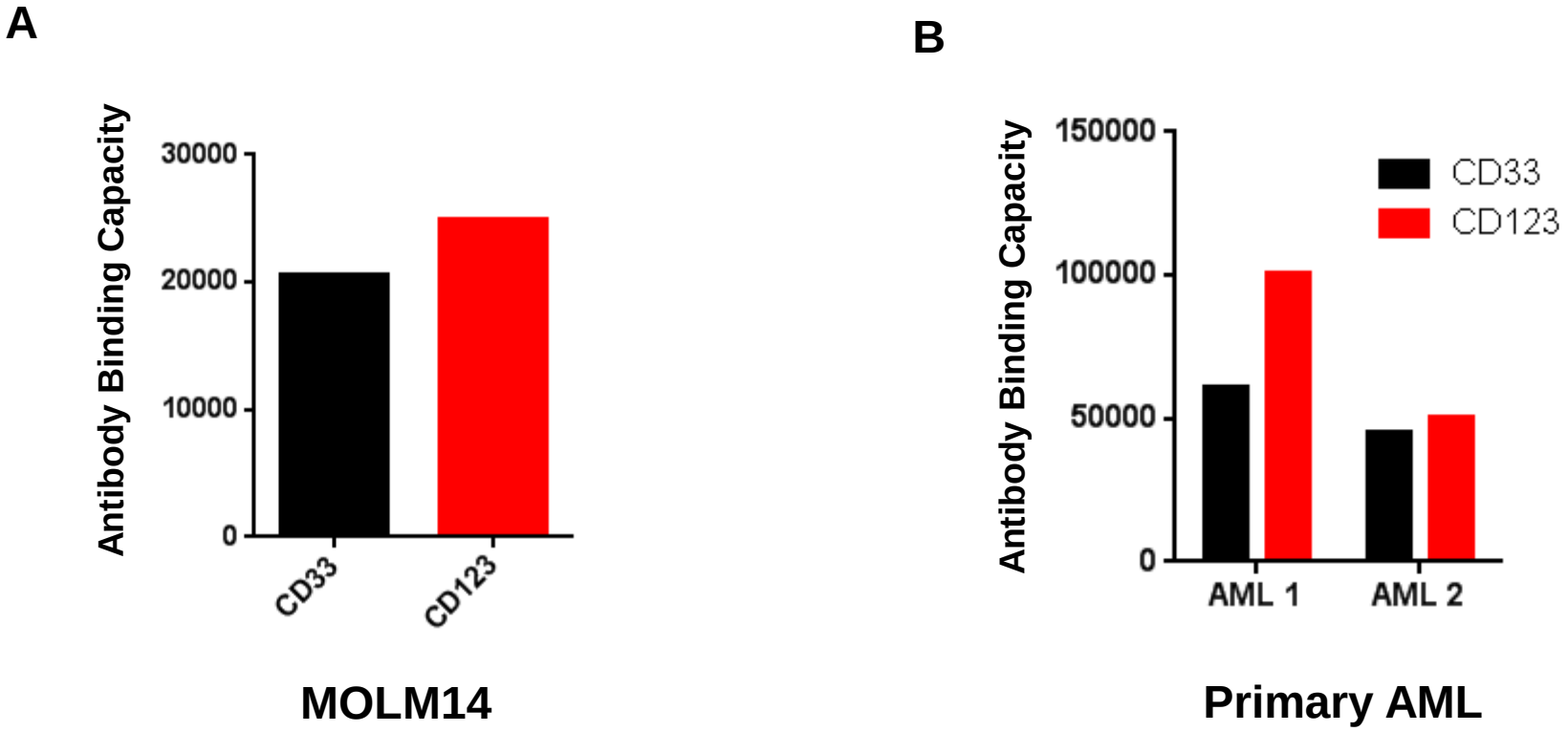

Figure S2. Antibody binding capacity of CD33 and CD123 on MOLM14 and Primary AML samples used for in vivo experiments. Assay was performed using QUANTUM SIMPLY CELLULAR kit (Bangs Laboratories, Inc). Samples were washed in flow buffer and then stain with the indicated antibody (CD33 or CD123) conjugated to PE. The five different microspheres provided in the kit were also stained with the same antibody. The mean fluorescence intensity of the target was compared to the that of the five microspheres and the value of antibody binding capacity was then calculated per the manufacture protocol. A) The antibody binding capacity of MOLM14 for CD33 and CD123. B) The antibody binding capacity of the primary samples used in these experiments for CD33 and CD123.

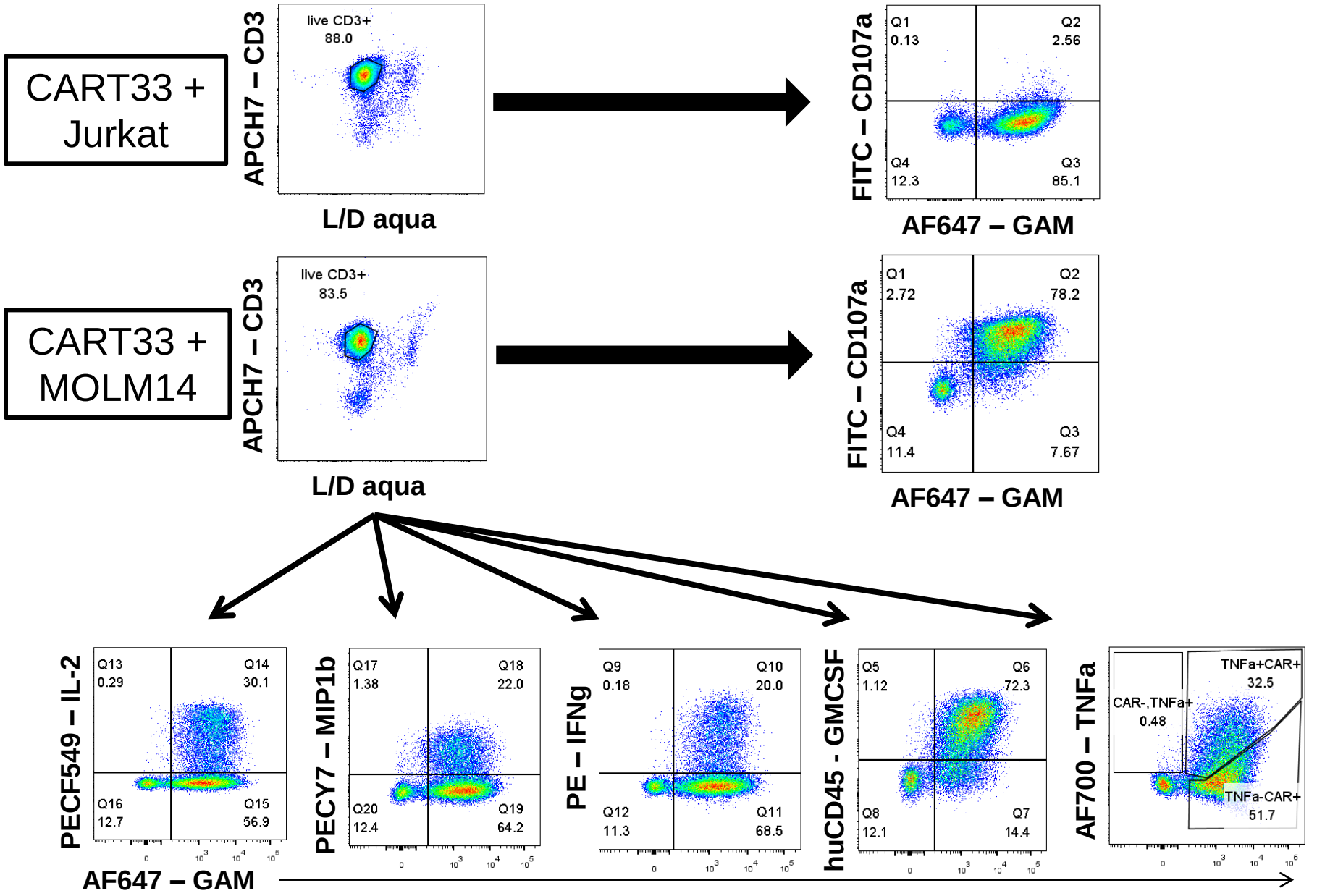

Figure S3. Gating strategies for degranulation and cytokine production assays. Cells were gated on a FSA vs SSA scale, duplets were excluded based on FSA vs FSH scale and then live CD3 cells were gated in. CARs were detected by goat anti-mouse conjugated to AF647.

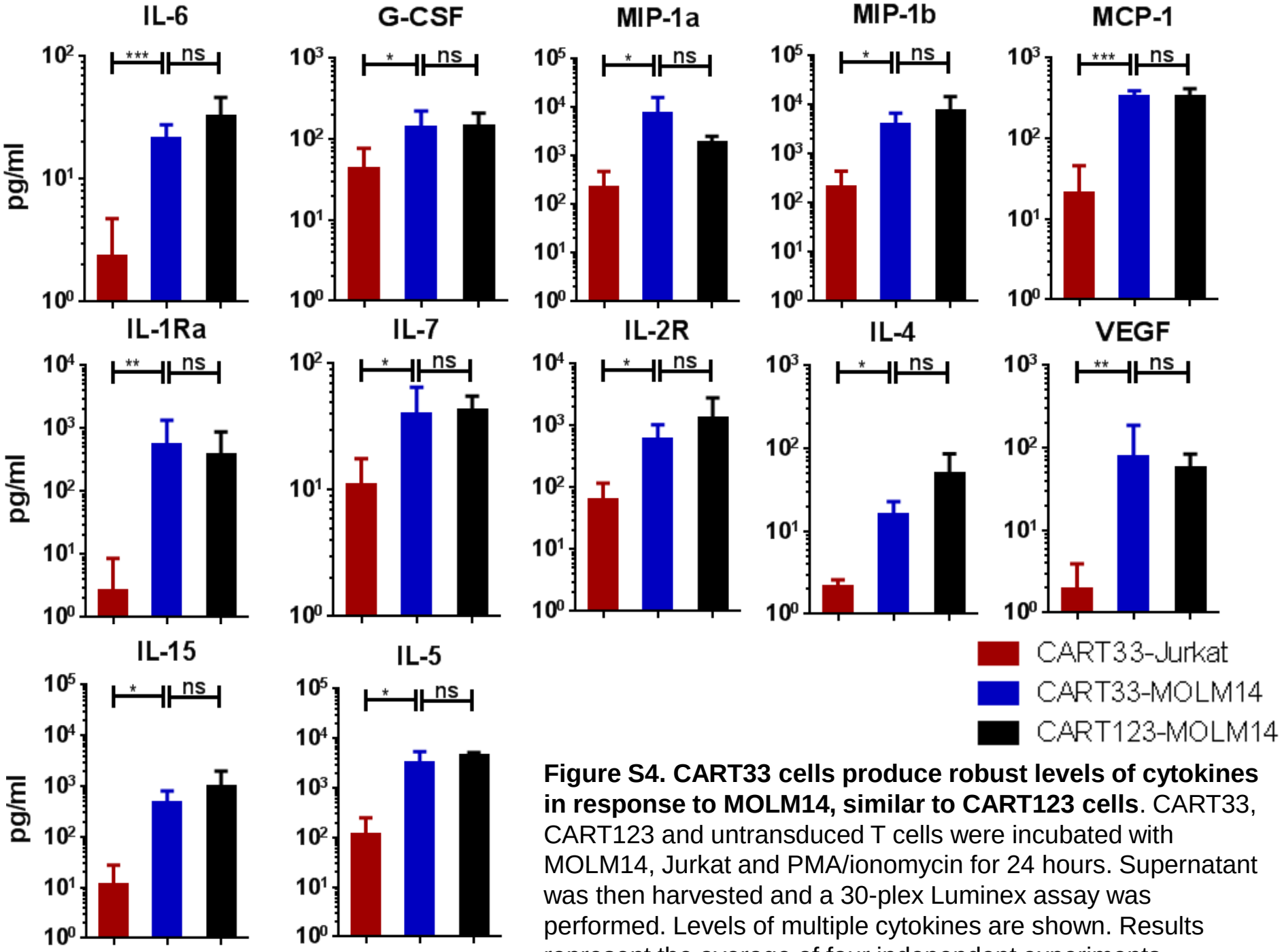

Figure S4. CART33 cells produce robust levels of cytokines in response to MOLM14, similar to CART123 cells. CART33, CART123 and untransduced T cells were incubated with MOLM14, Jurkat and PMA/ionomycin for 24 hours. Supernatant was then harvested and a 30-plex Luminex assay was performed. Levels of multiple cytokines are shown. Results represent the average of four independent experiments.

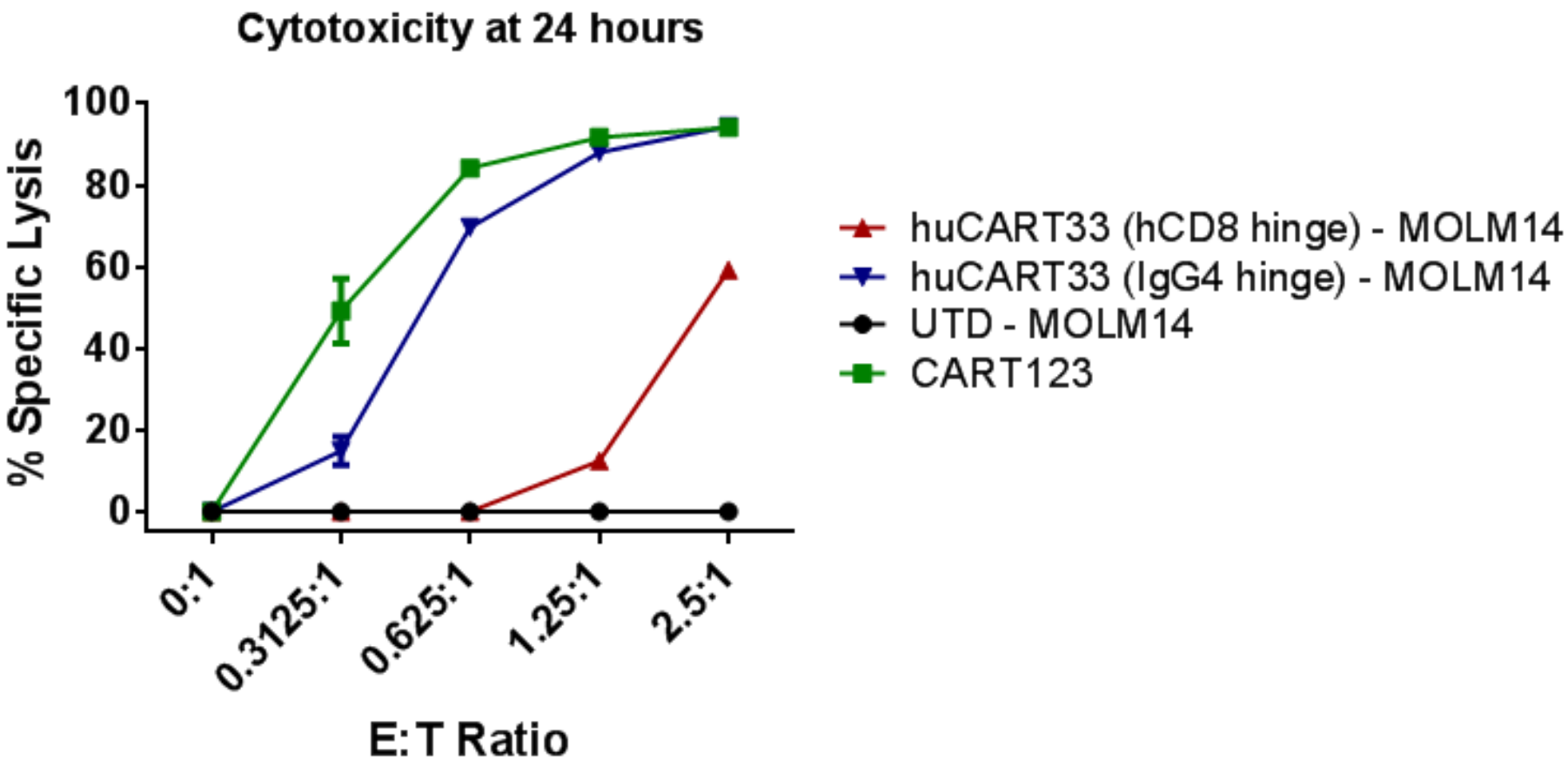

Figure S5. In-vitro killing assay of CART33 (IgG4 hinge) and CART33 (CD8 hinge): CART123, CART33 (CD8hinge) and CART33 (IgG4 hinge) were incubated with MOLM14 at the indicated E:T ratios and killing was assessed by bioluminescence imagine. CART33 (IgG4) resulted in more specific killing than CART33 (CD8) at lower E:T ratio.

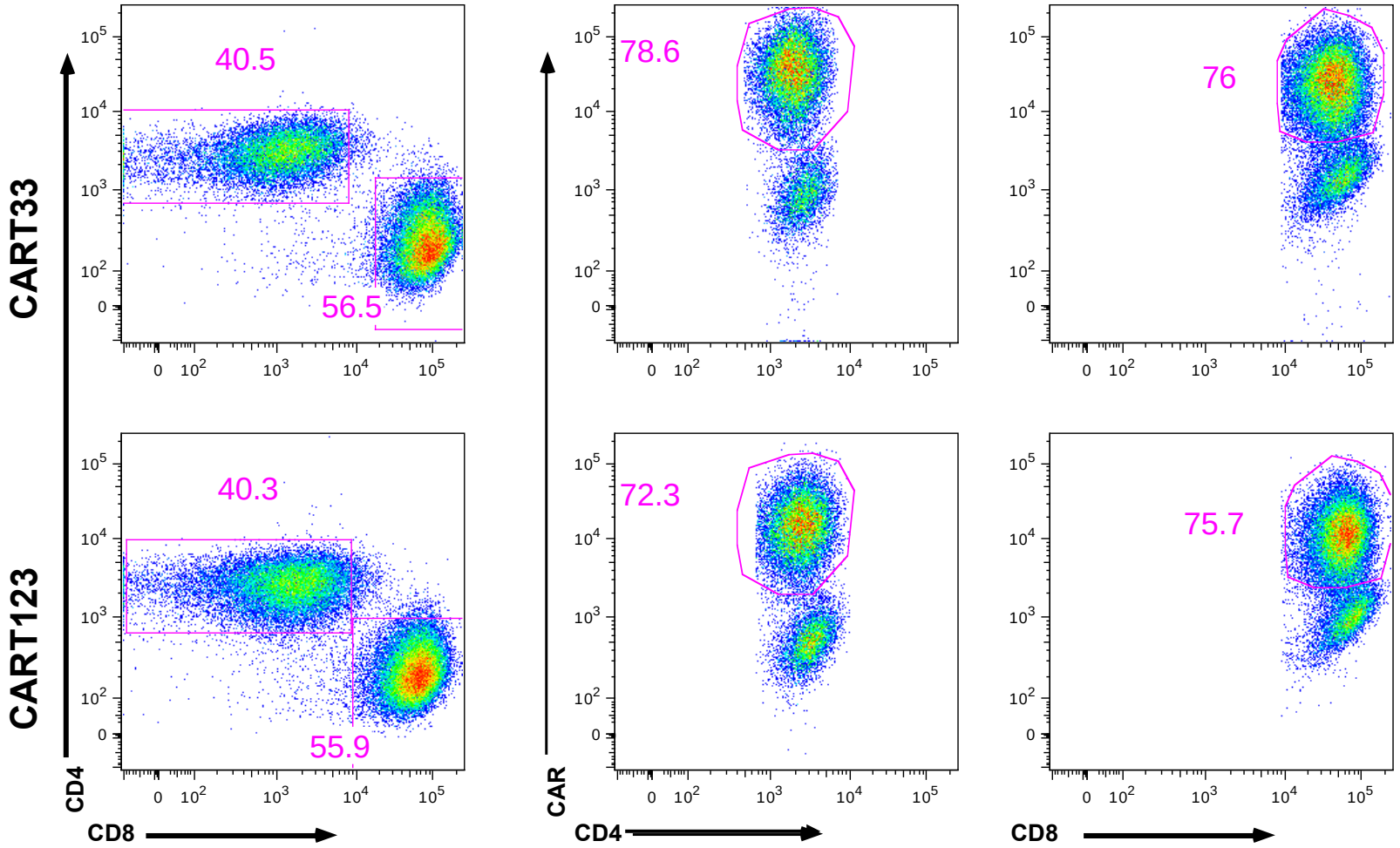

Figure S6 Phenotype of ND365 T cells as a representative of one the donors used in the in-vitro experiments performed in the study. The percentage of CAR+CD4+ and CAR+CD8+ was similar between CART33 and CART123

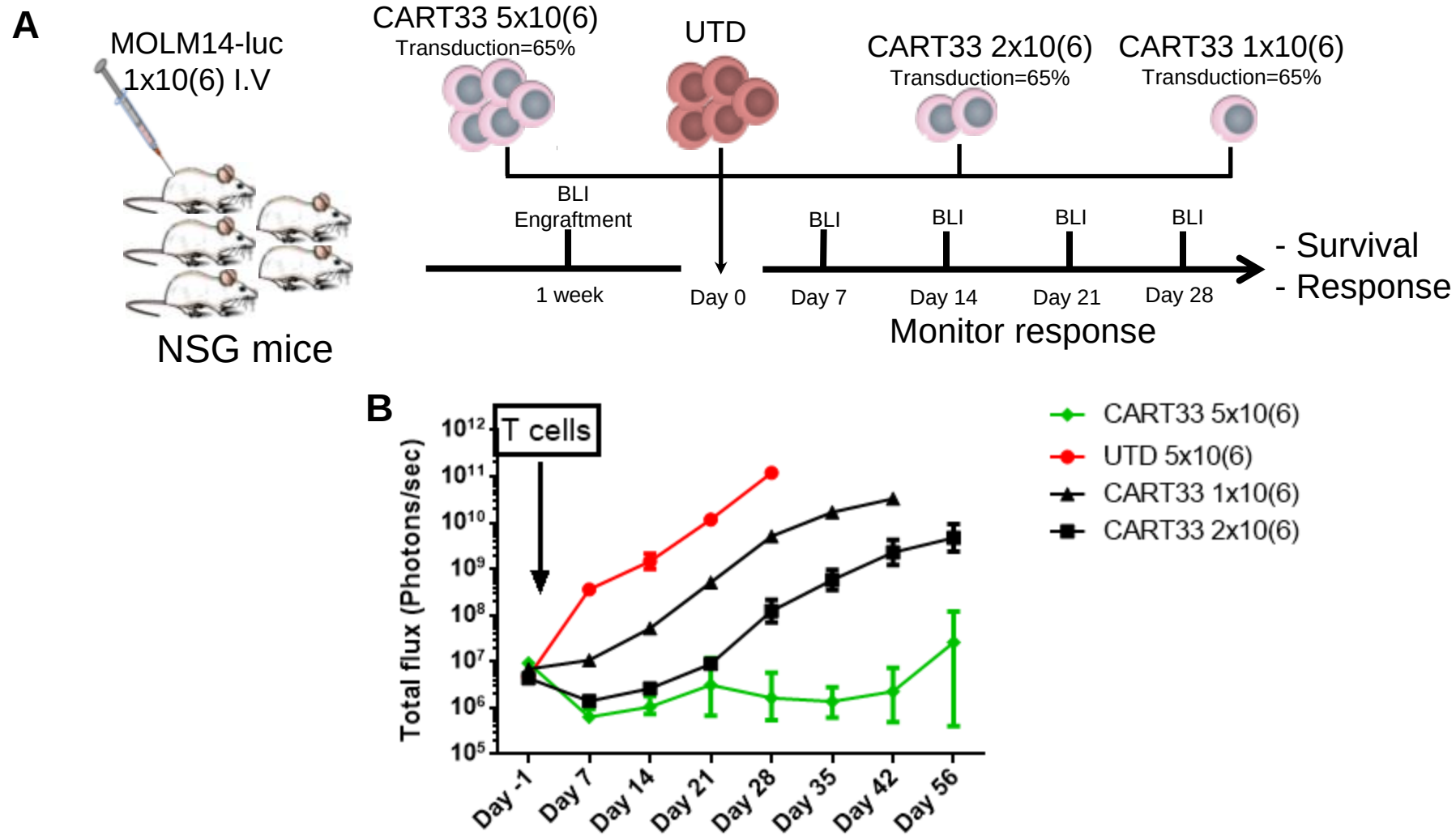

Figure S7. CART33 Treatment result in a dose dependent reduction of leukemia burden in MOLM14 engrafted xenografts. A) Experiment schema: NOD-SCID-gamma chain knockout (NSG) mice were injected with the AML cell line MOLM14 1x10(6) I.V and imaged for engraftment after 6 days. Mice were treated with CART33 5x10(6), CART33 2x10(6), CART33 1x10(6), or control untransduced T cells 5x10(6). The mice were followed with serial weekly imaging to assess the burden of AML. **B)** Tumor burden over time as measured by bioluminescent imaging (BLI) in different groups.

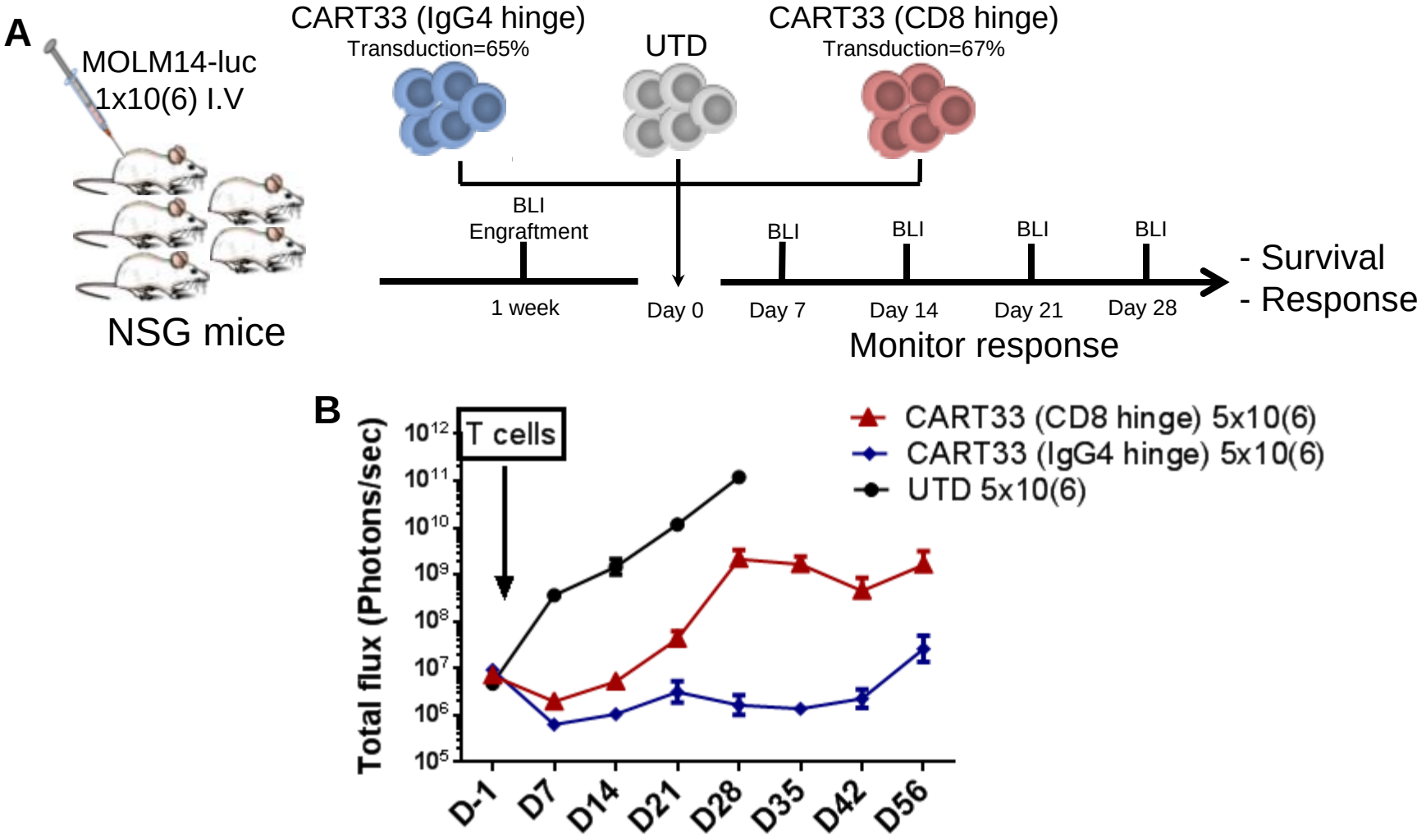

Figure S8. CART33 (IgG4 hinge) results in superior leukemia control compared to CART33 (CD8 hinge) in MOLM14 engrafted xenografts. A) Experiment schema: NOD-SCID-gamma chain knockout (NSG) mice were injected with the AML cell line MOLM14 1x10(6) I.V and imaged for engraftment after 6 days. Mice were then treated with a single injection of CART33 (IgG4 hinge) 5x10(6), CART33 (CD8 hinge) 5x10(6), or control untransduced T cells 5x10(6). The mice were followed with serial weekly imaging to assess the burden of AML. **B)** Tumor burden over time as measured by bioluminescent imaging (BLI) in the different groups.

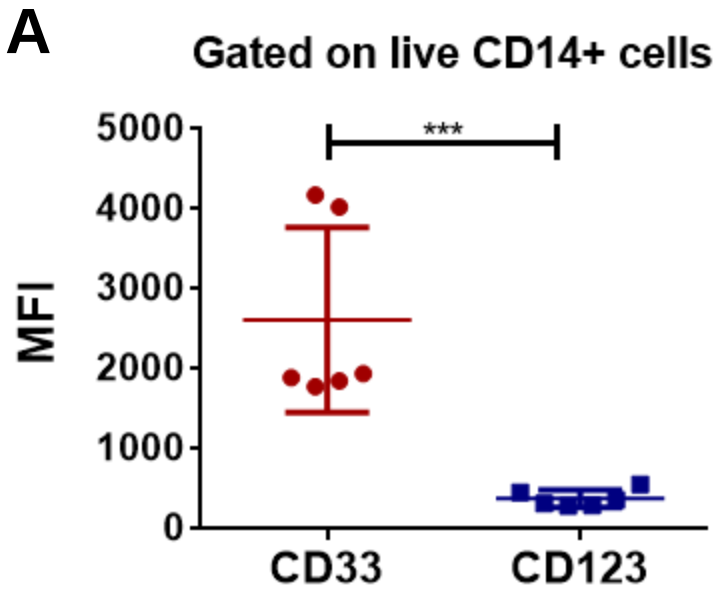

Figure S9. CD33 is brightly expressed on monocytes compared to CD123. A) CD33 is brightly expressed on monocytes from peripheral blood mononuclear cells of normal donors (monocytes were gated as live CD14+ cells, n=6) **B)** Histogram mean florescence intensity (MFI) of CD33 and CD123 expression from a normal donor peripheral blood mononuclear cells.