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Supplemental Information

A Genetically Engineered Primary Human Natural Killer Cell Platform for Cancer Immunotherapy

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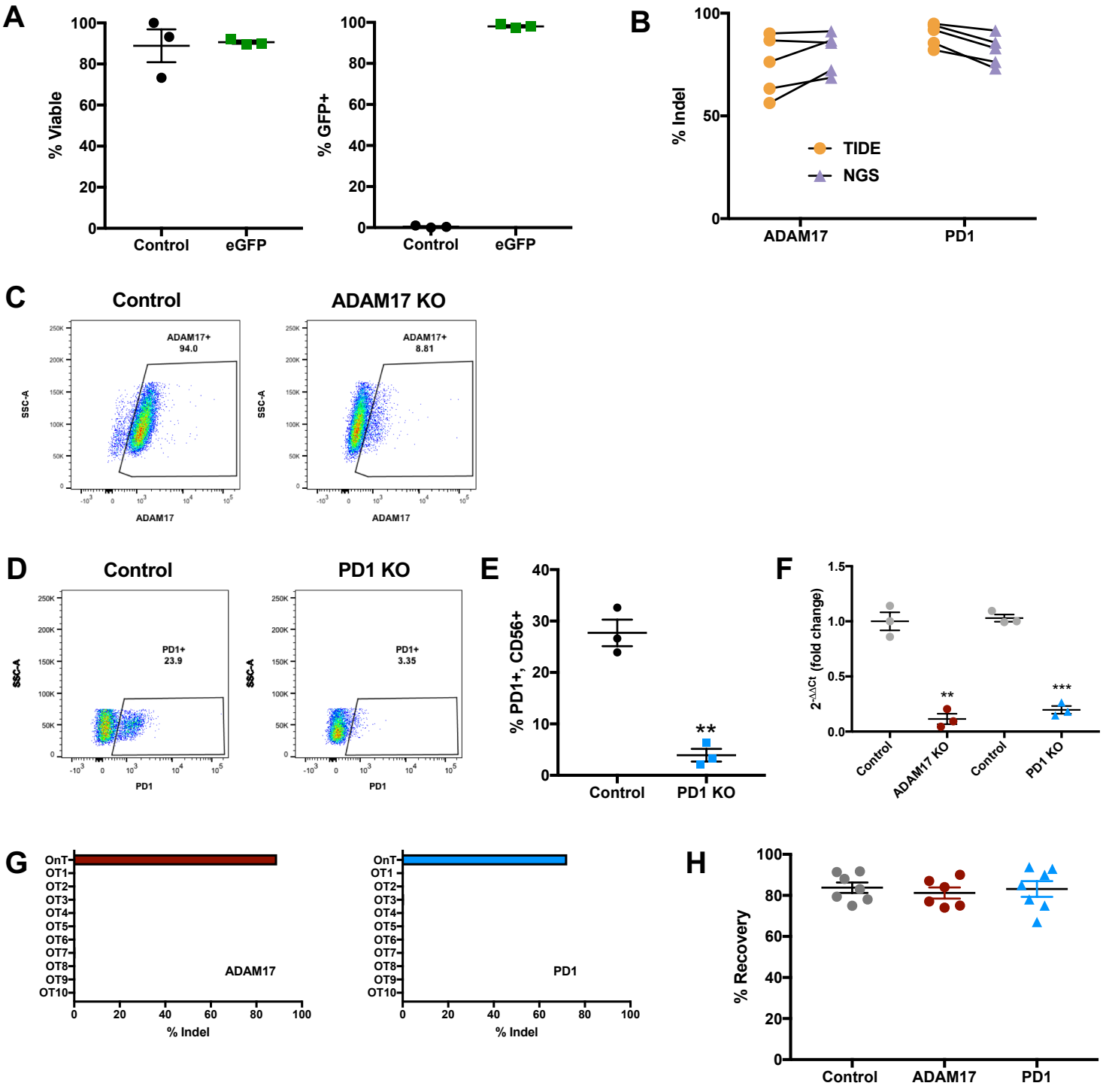
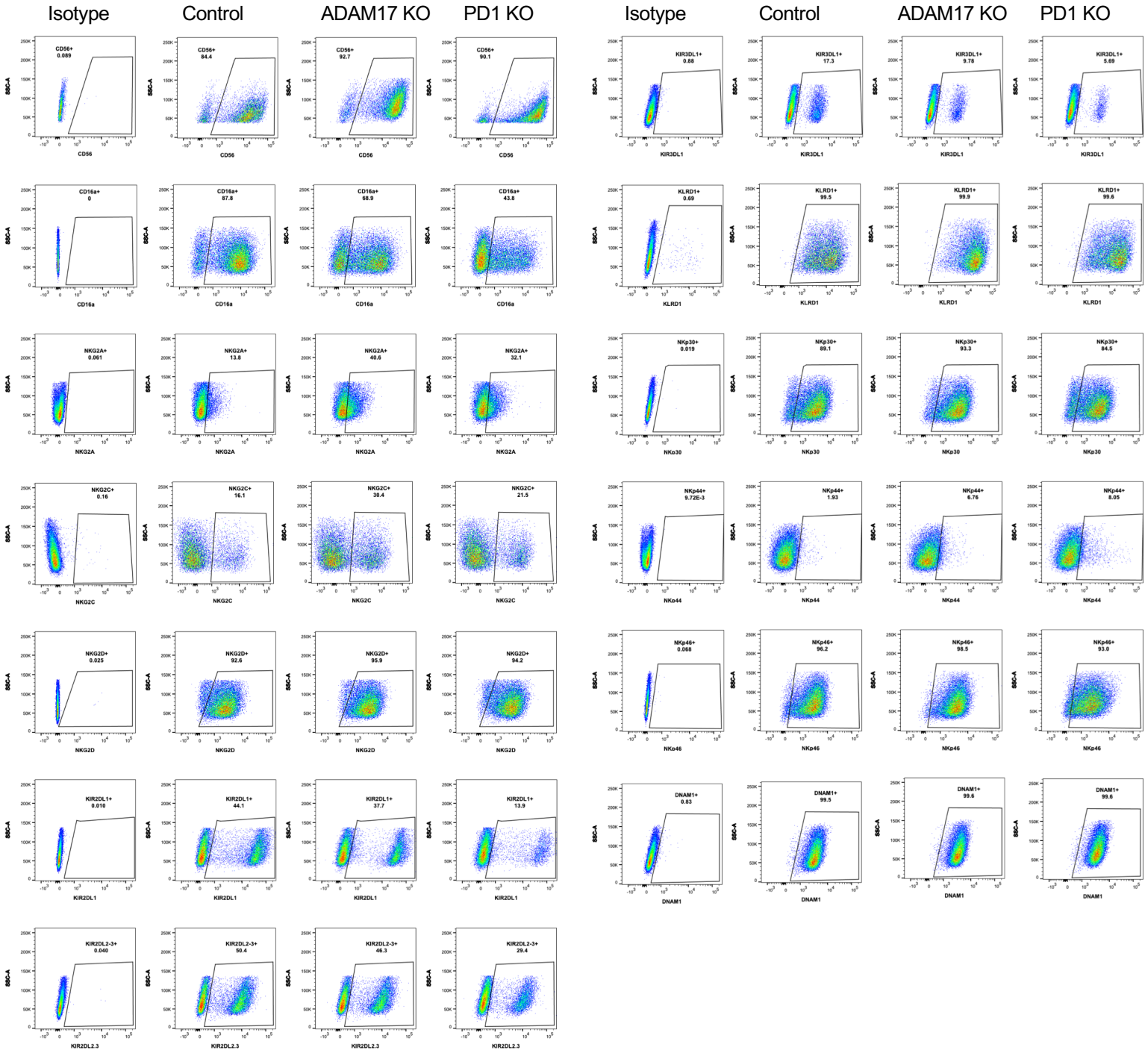
Supplemental Figure 1



Supplemental Figure 1. Analysis of gene knockout in activated NK cells. (**A**) Activated NK cells were electroporated with PBS (gray) or mRNA encoding eGFP (green). Cell viability (left) was measured using Trypan blue exclusion and transfection efficiency (right) was measured by eGFP expression 48-hours after electroporation. (**B**) Indel formation in ADAM17 and PD1 measured by NGS and compared to TIDE (n=5 independent donors). (**C-E**) Representative flow cytometry plots for loss of ADAM17 and PD1. (**F**) mRNA loss after gene knockout was confirmed by RT-PCR. Target RNA expression was normalized to expression of *ACTB* (**G**) Percent of cells with on-target (OnT) and off-target (OT#) amplicons after PCR using primers designed to amplify the top 10 predicted off-target sites for each gRNA. Percentage based on Next Generation Sequencing of amplicons. Note: PD1 OT8 did not amplify. (**H**) Control or gene-edited NK cells were frozen in CryoStor medium at 1x10^7 cells/mL. Percent recovery was calculated by counting viable cells using Trypan blue exclusion 1 hour after thawing cells, after culture in 1 ng/mL IL15

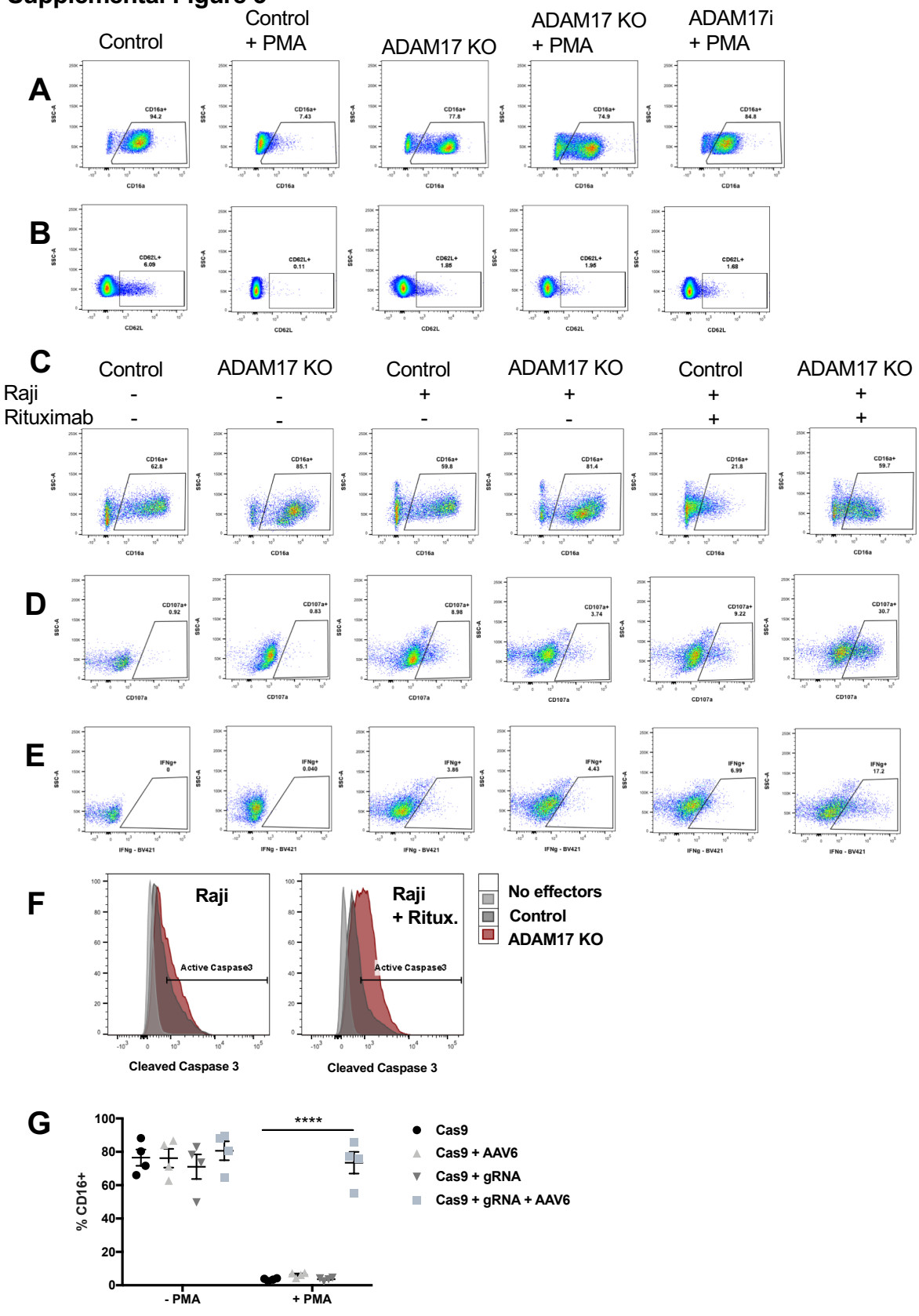
Supplemental Figure 2

A



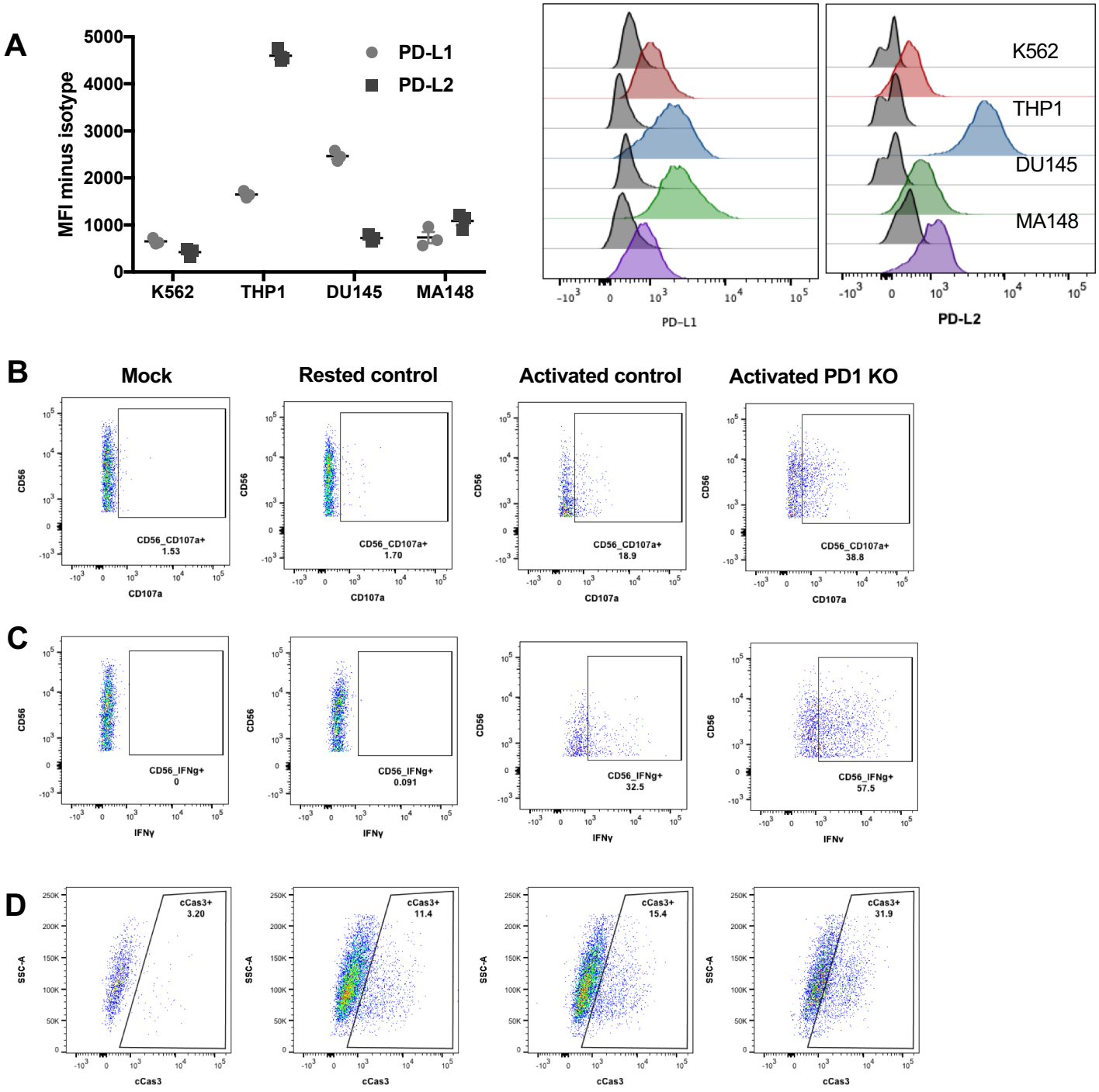
Supplemental Figure 2. CRISPR-edited NK cells maintain expression of common NK cell receptors. (**A**). Representative flow cytometry plots of expression of NK cell receptors in control or genetically modified NK cells. Gates were defined using fluorescently-labeled isotype controls for each marker.

Supplemental Figure 3



Supplemental Figure 3. ADAM17 KO NK cells display enhanced ADCC. (A-B) Representative flow cytometry plots of NK cell CD16a **(A)** and CD62L **(B)** expression after stimulation with 1 ug/mL PMA. **(C-F)** Representative flow cytometry plots of NK cell CD16a expression **(C)**, CD107a expression **(D)**, IFNγ production **(E)**, and target cell cleaved caspase 3 expression **(F)** after 6 hour co-culture of NK cells with Rituximab-coated Raji cells (E:T=2:1). **(G)** CD16a (S197P)-KI NK cells or controls were stimulated for 1 hour with PMA/ionomycin (1 μg/mL) and CD16a expression was analyzed by flow cytometry (n=4 independent donors, ****P=<0.0001, two-way ANOVA with Tukey's *post-hoc* test).

Supplemental Figure 4



Supplemental Figure 4. PD1 KO NK cells display enhanced antitumor activity. (A) Target cell lines were incubated overnight with 500 IU/mL IFNγ and PD-L1 and PD-L2 expression was measured by flow cytometry (n=3 biological replicates). **(B-D)** Representative flow cytometry plots of NK cell CD107a expression **(B)**, NK cell IFNγ production **(C)**, and target cell cleaved caspase 3 expression **(D)** after 6 hour co-culture of NK cells with MA148 target cells (E:T=1:1).