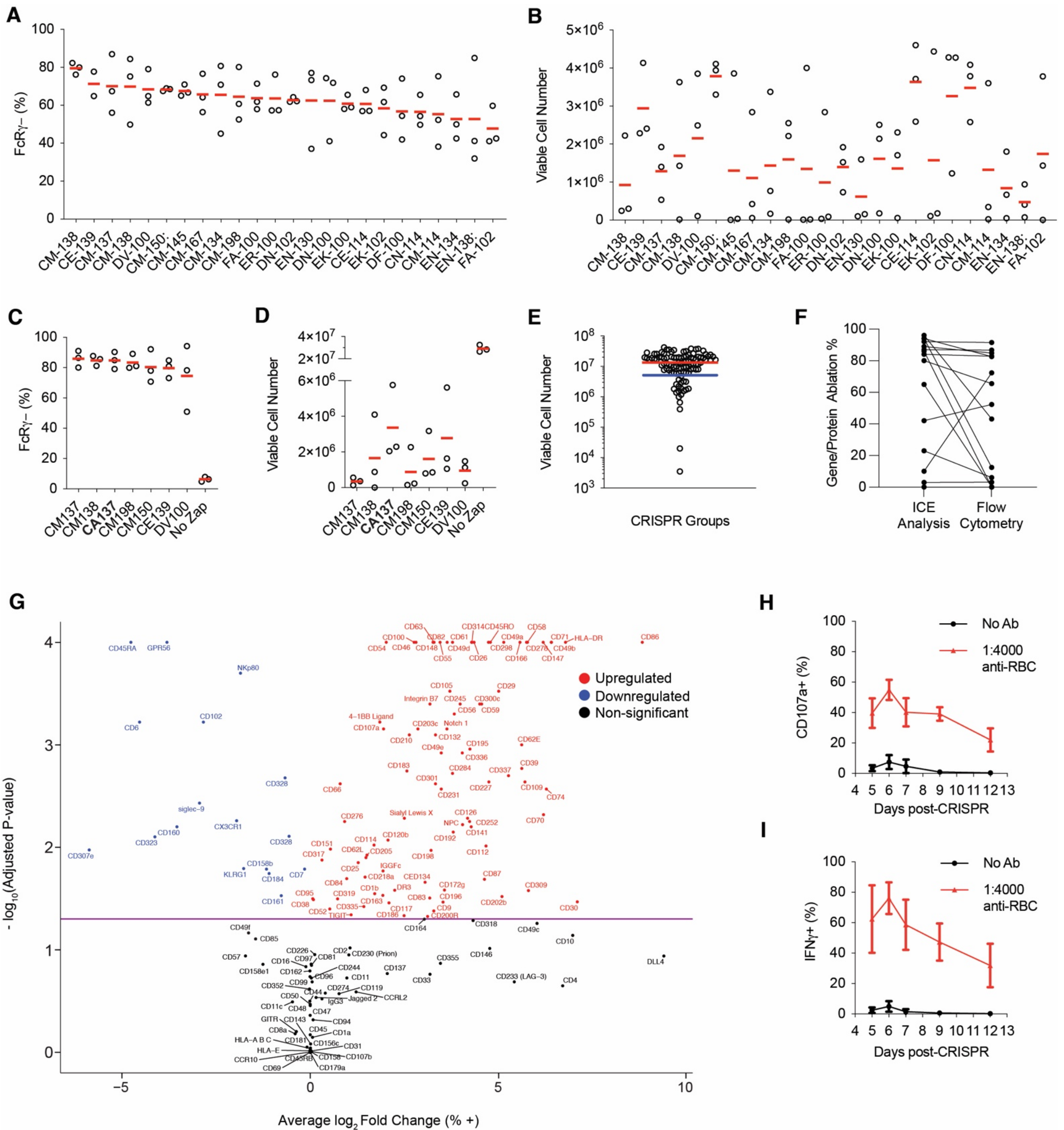
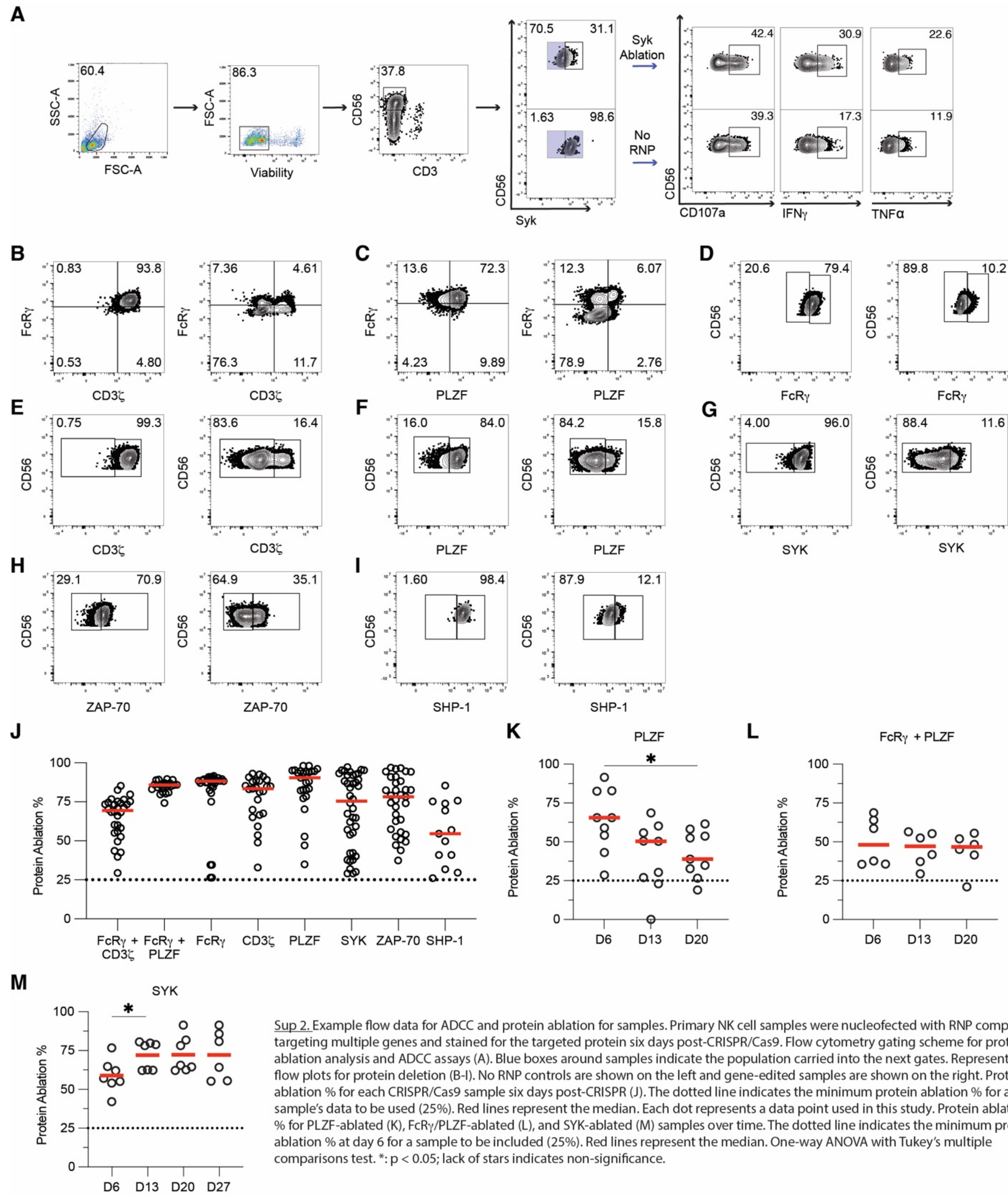
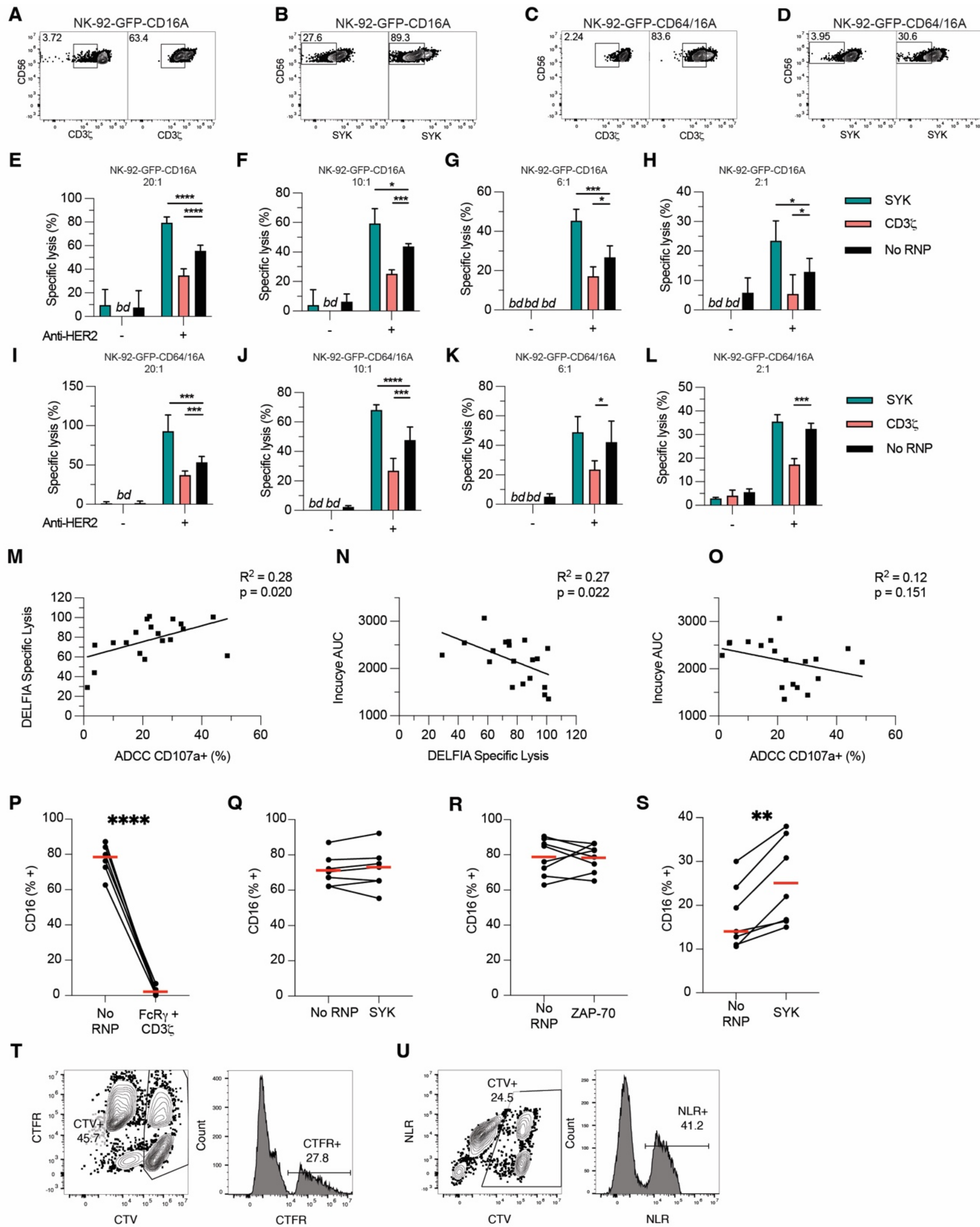


Sup 1. Optimization and validation of the CRISPR/Cas9 gene-editing protocol. Primary NK cell samples (n = 3) were nucleofected with an RNP complex targeting FcRγ. 24 Amaxa-4D Nucleofector codes were used. Six days following CRISPR/Cas9, the samples were stained for FcRγ expression (A) and cell recovery (B). The experiment was repeated with six codes from A-B and CA137, which is in bold (C-D). Each dot is one subject/donor. Red lines represent the median. Primary NK cell samples were nucleofected with a RNP complexes targeting various genes and stained for viability six days post-CRISPR/Cas9 (E). Dots represent individual subject + RNP combinations. The blue line shows the number of cells nucleofected ($5x10^6$), whereas the red line shows the average viable cell count six days post-CRISPR/Cas9 ($10.7x10^6$). Data from three independent experiments. DNA was isolated from a subset of FcRγ/PLZF-ablated primary NK cell samples six days post-CRISPR/Cas9 and sequenced for ICE analysis alongside staining for FcRγ and PLZF expression (F). ICE analysis values indicate the gene ablation %, whereas flow cytometry values represent the protein ablation % for a sample. Each dot is one subject/donor. Lines connect samples from the same donor + RNP combination. Data from three independent experiments. No RNP cells from days 0 and 7 in the CRISPR/Cas9 protocol were thawed and phenotyped using Biolegend's LEGENDScreen PE Kit (n = 4, G). Volcano plots show change in expression of all markers from day 0 to day 7. Red dots indicate markers that were significantly upregulated, black dots indicate non-significant changes in expression, and blue dots indicate proteins that were downregulated. Data from one experiment. Two-way ANOVA with Tukey's multiple comparisons test. No RNP NK cell samples (n = 3) were nucleofected with CA137 and used in anti-RBC ADCC assays at days 5, 6, 7, 9, and 12 post-CRISPR/Cas9. CD107a (H) and IFNγ (I) expression was measured by flow cytometry. Data shown is the average +/- SD of the three subjects at each timepoint for the ADCC (red line) and negative control (black line) groups. Data from one experiment.



Sup 2. Example flow data for ADCC and protein ablation for samples. Primary NK cell samples were nucleofected with RNP complexes targeting multiple genes and stained for the targeted protein six days post-CRISPR/Cas9. Flow cytometry gating scheme for protein ablation analysis and ADCC assays (A). Blue boxes around samples indicate the population carried into the next gates. Representative flow plots for protein deletion (B-I). No RNP controls are shown on the left and gene-edited samples are shown on the right. Protein ablation % for each CRISPR/Cas9 sample six days post-CRISPR (J). The dotted line indicates the minimum protein ablation % for a sample's data to be used (25%). Red lines represent the median. Each dot represents a data point used in this study. Protein ablation % for PLZF-ablated (K), FcRγ/PLZF-ablated (L), and SYK-ablated (M) samples over time. The dotted line indicates the minimum protein ablation % at day 6 for a sample to be included (25%). Red lines represent the median. One-way ANOVA with Tukey's multiple comparisons test. *: $p < 0.05$; lack of stars indicates non-significance.



Sup 3. NK-92 ADCC function and CD16A expression enhanced by SYK-ablation. CD3ζ (A, C) and SYK (B, D) were ablated from NK-92-GFP cells expressing CD16A (A-B, E-H) and CD64/16A (C-D, I-L) via CRISPR/Cas9. Following CRISPR/Cas9, the gene-ablated NK-92 cells were used in DELFIA assays at E:T ratios of 20:1 (E, I), 10:1 (F, J), 6:1 (G, K) and 2:1 (H, L). Technical triplicates were used in the DELFIA assay. Data shown is the specific lysis average + standard deviation (SD) in two independent experiments. Values of no lysis are shown as bd (below detection). Paired T-tests between the gene-ablated cell lines and no RNP cell lines. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$; ****: $p < 0.001$. Subject-matched ADCC, DELFIA, and Incucyte assays ran concurrently (n = 14) were used to correlate degranulation/CD107a expression (target cell: RBC), DELFIA (target cell: SKOV-3) specific lysis, and Incucyte (target cell: SKOV-3) area under the curve (AUC) results (M-O). Data from four independent experiments. Each dot represents an individual subject. R^2, p-value, and line of best fit from linear regression analysis shown. Six days post-CRISPR/Cas9, no RNP and gene-ablated unstimulated primary NK cell samples were stained for CD16A expression (P-R). Six, 13, and 20 days post-CRISPR, stimulated SYK-ablated and control primary NK cell samples were stained for CD16A expression (S). Dots indicate samples from one subject. Red lines indicate the mean. Data shown is from three independent experiments. Paired t test. **: $p < 0.01$; ****: $p < 0.001$; lack of stars indicates non-significance. Flow cytometry gating scheme for SKOV-3 (T) and Raji (U) conjugation assays.