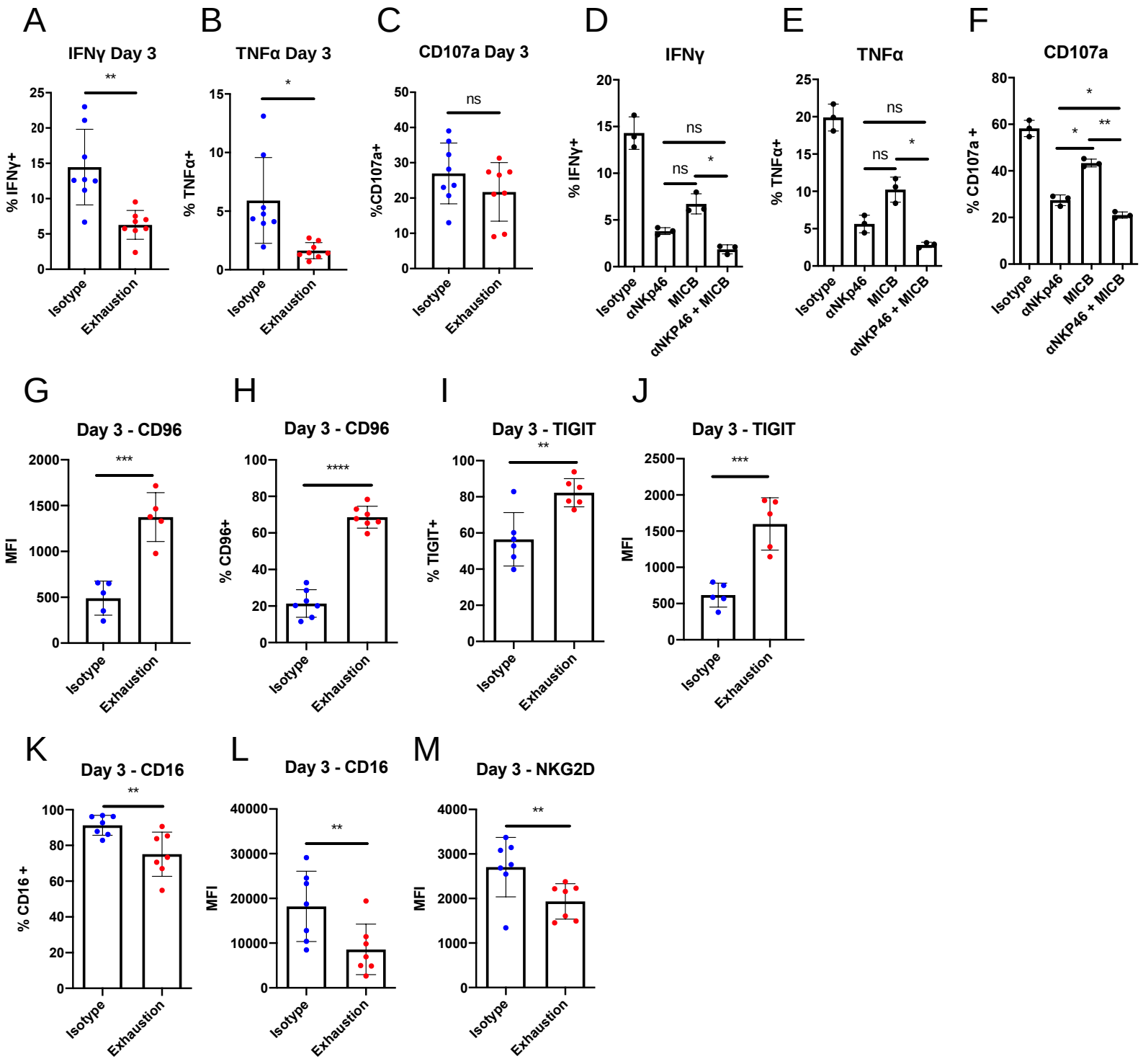
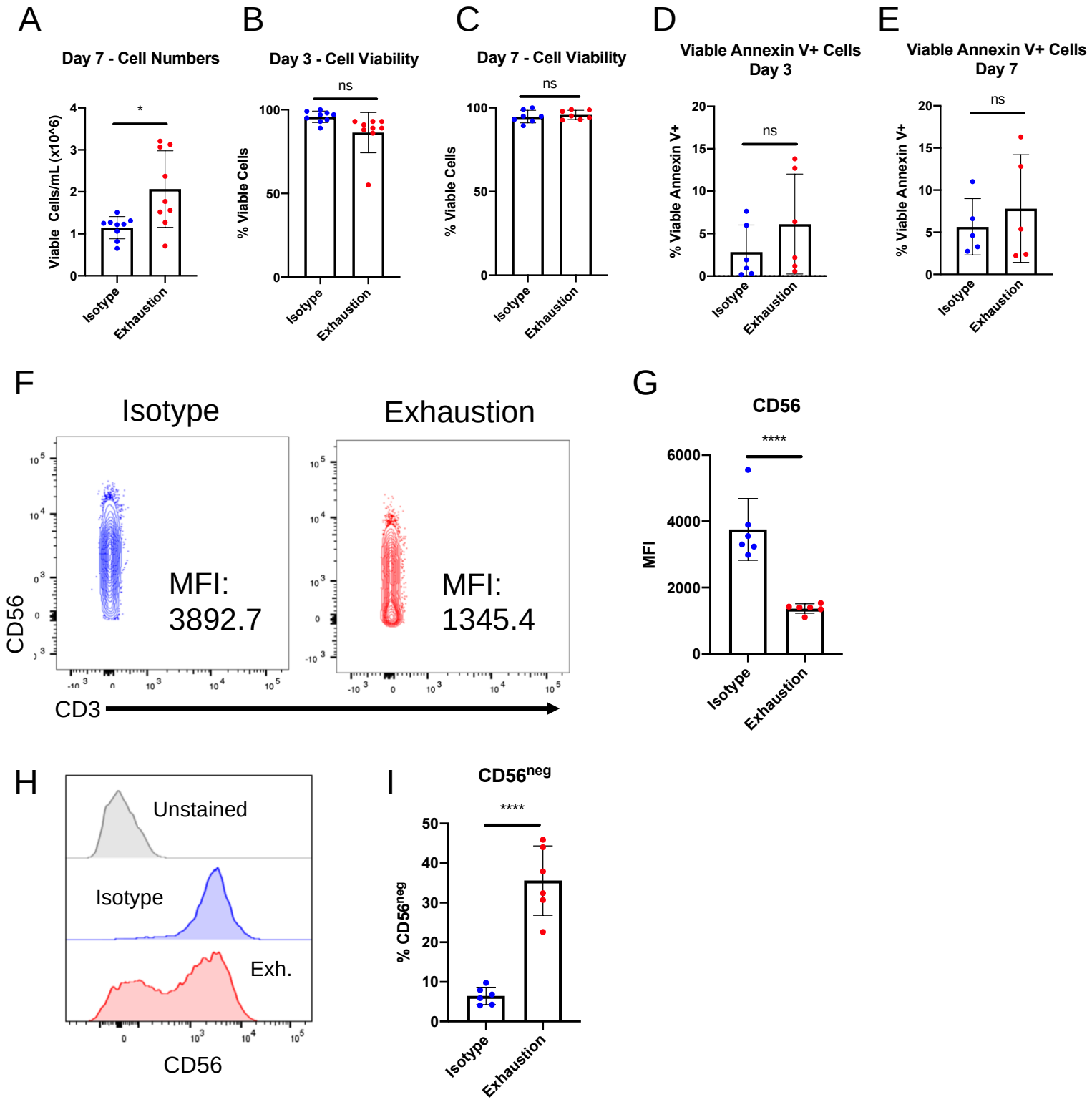
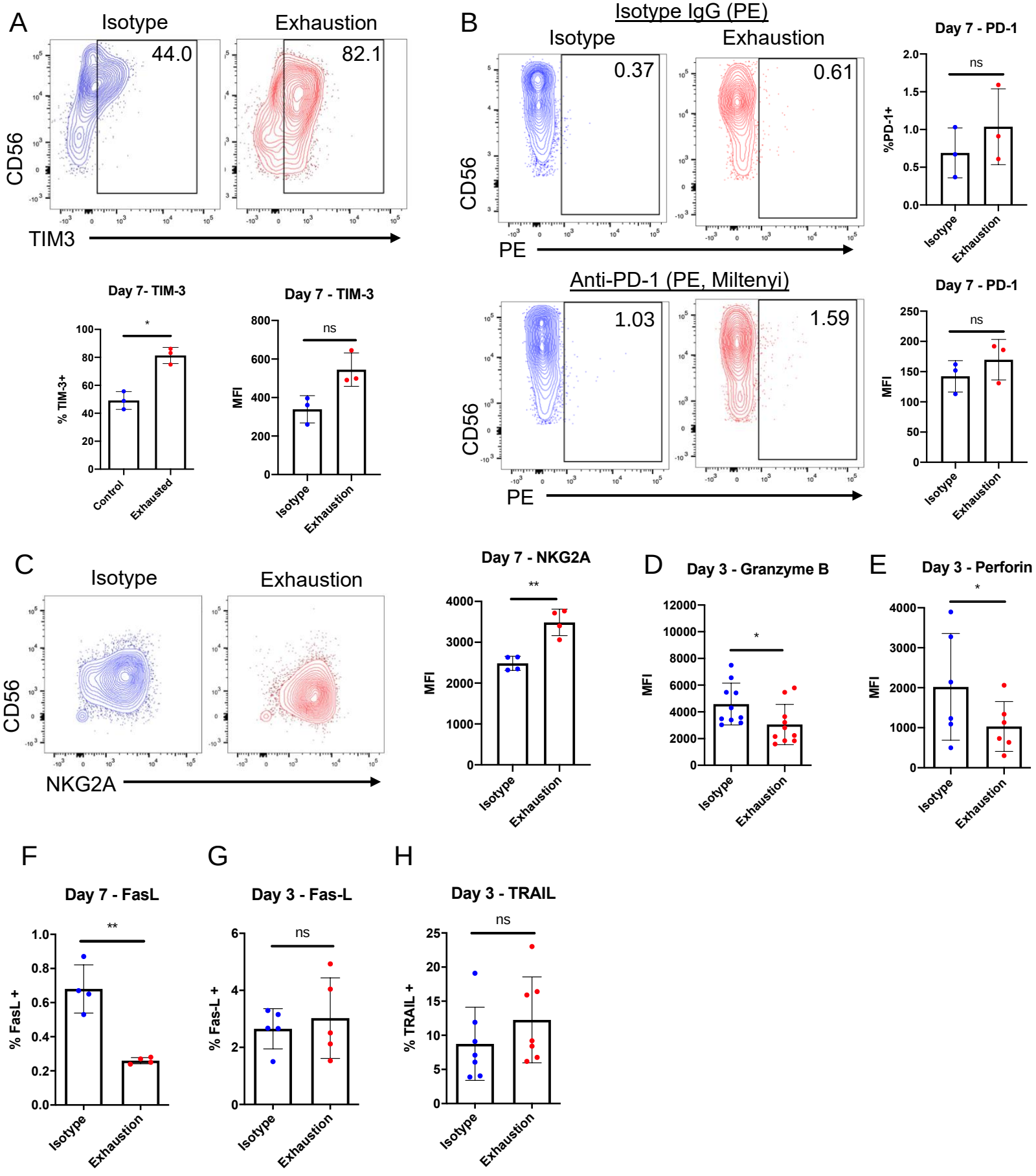



**SUPPLEMENTARY FIGURE 1.** (**A**-**C**) NK cells harvested from isotype-coated and exhaustion plates (day 3) were incubated with K562 targets for four hours (E/T: 2:1). Production of IFNγ (A) (*n*=8) and TNFα (B) (*n*=8) and degranulation (CD107a [C]) (*n*=8) were measured via flow cytometry. (**D**-**F**) NK cells were stimulated through NKp46 and NKG2D independently and simultaneously for 7 days. Production of IFNγ (D) (*n*=3) and TNFα (E) (*n*=3) and degranulation (CD107a [F]) (*n*=3) were measured via flow cytometry. (**G-M**) On day 3 of stimulation, flow cytometry was used to stain for several receptors. Percent of parent and MFI are included where indicated. CD96 (G-H), TIGIT (I-J), CD16 (K-L), NKG2D (M); (*n*=7); **P* ≤ 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001 ns, not significant.

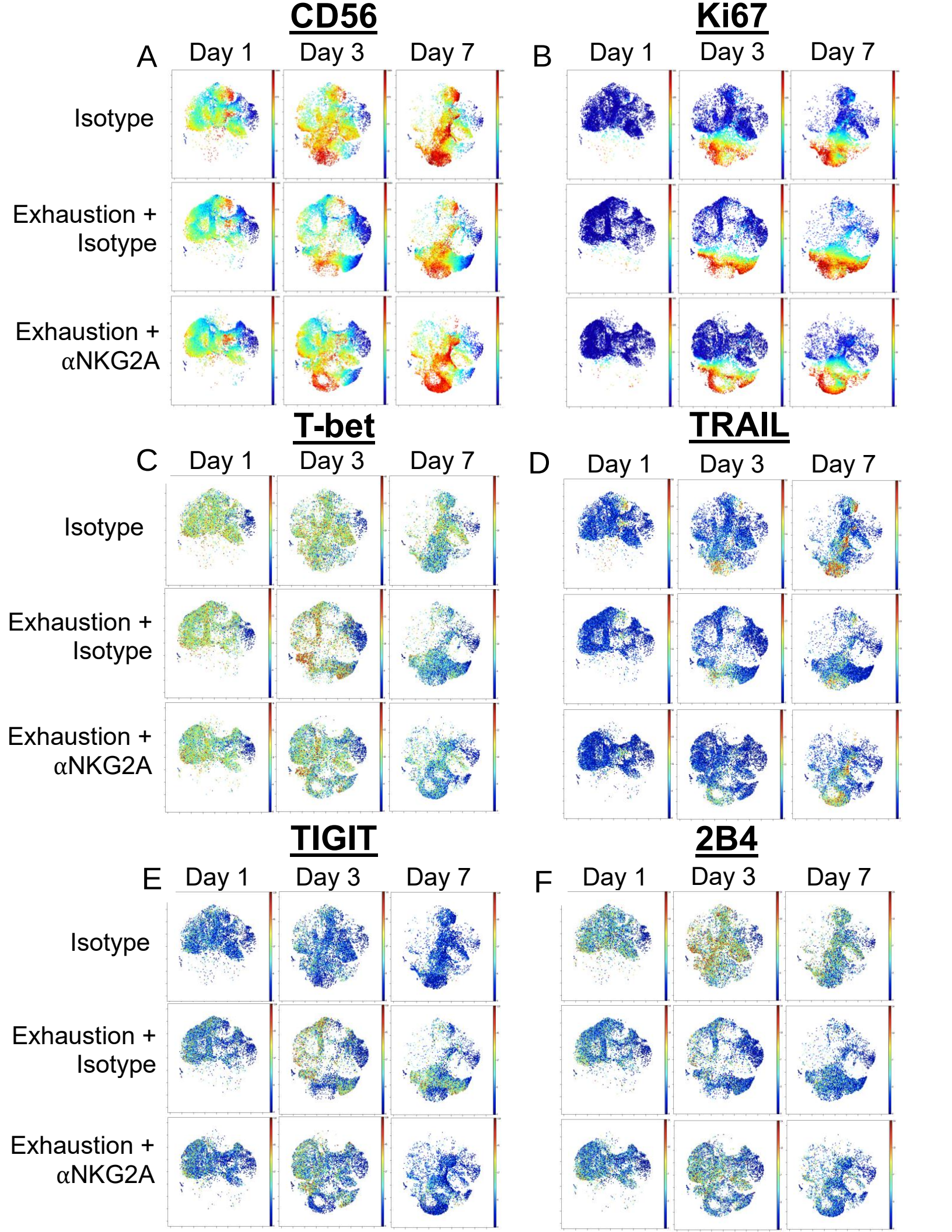


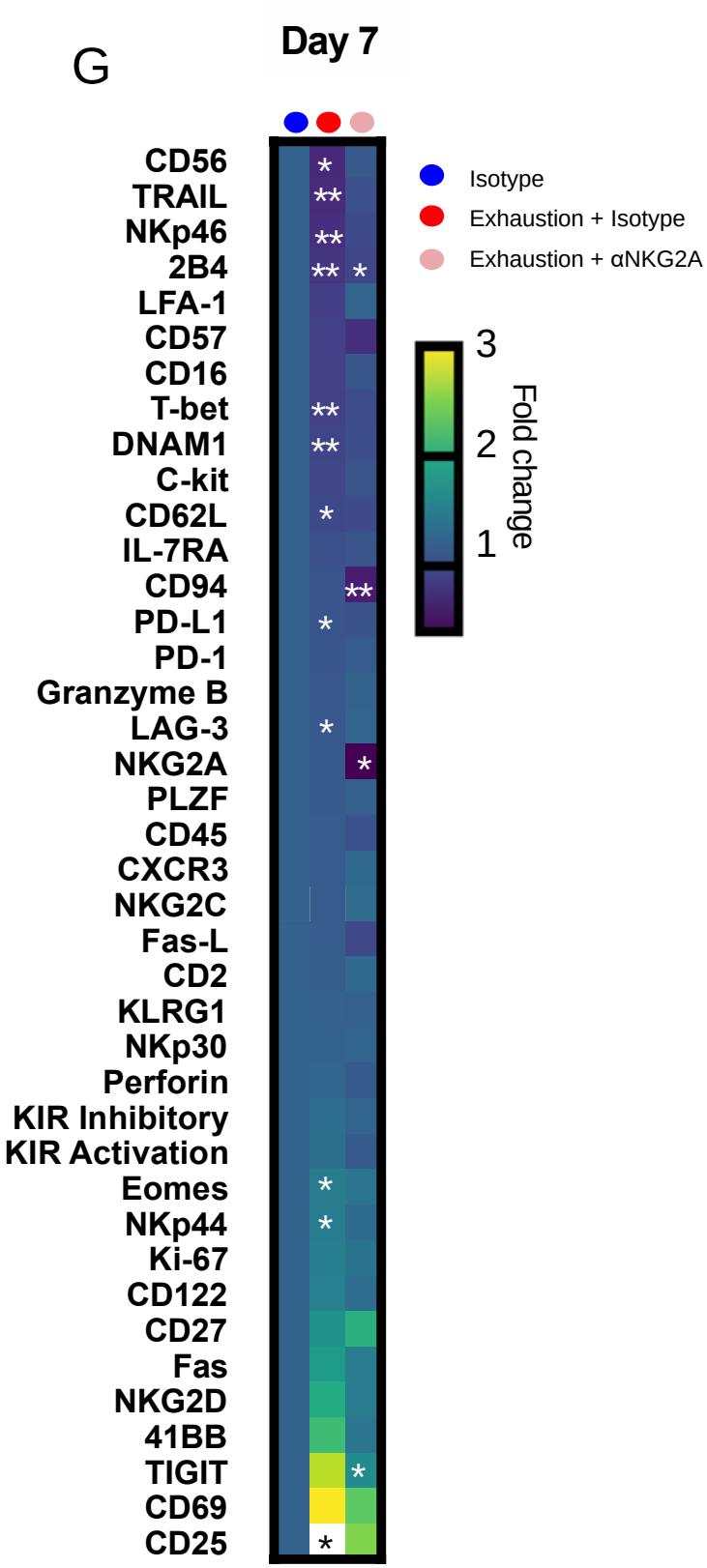

**SUPPLEMENTARY FIGURE 2.** (**A**) Viable cells were quantified at day 7 using the Beckman Coulter ViCell counter. (Starting cell density = 1x10^6 cells/mL, *n*=9.) Paired *t* tests were used for comparisons. **P* ≤ 0.05. (**B**-**C**) Viability of NK cells harvested from isotype-coated and exhaustion plates (harvested at days 3 [B] [*n*=9] and 7 [C] [*n*=7]) was assessed via Trypan blue uptake. (**D-E**) NK cells harvested from isotype-coated

and exhaustion plates (days 3 [D] [*n*=6] and 7 [E] [*n*=5]) were stained with Annexin V to assess apoptosis. (Analysis was performed via flow cytometry.) (**F**) Representative contour plots of CD56 expression for isotype (blue) and exhausted (red) cells. Analysis was performed via flow cytometry: NK cells were live, CD3- CD56+. (**G**) Quantification of pooled results (*n*=7). (F) Histogram of CD56 expression. (I) Quantification of pooled results (*n*=*6*) ***$P < 0.001$; ****$P < 0.0001$ ns, not significant.

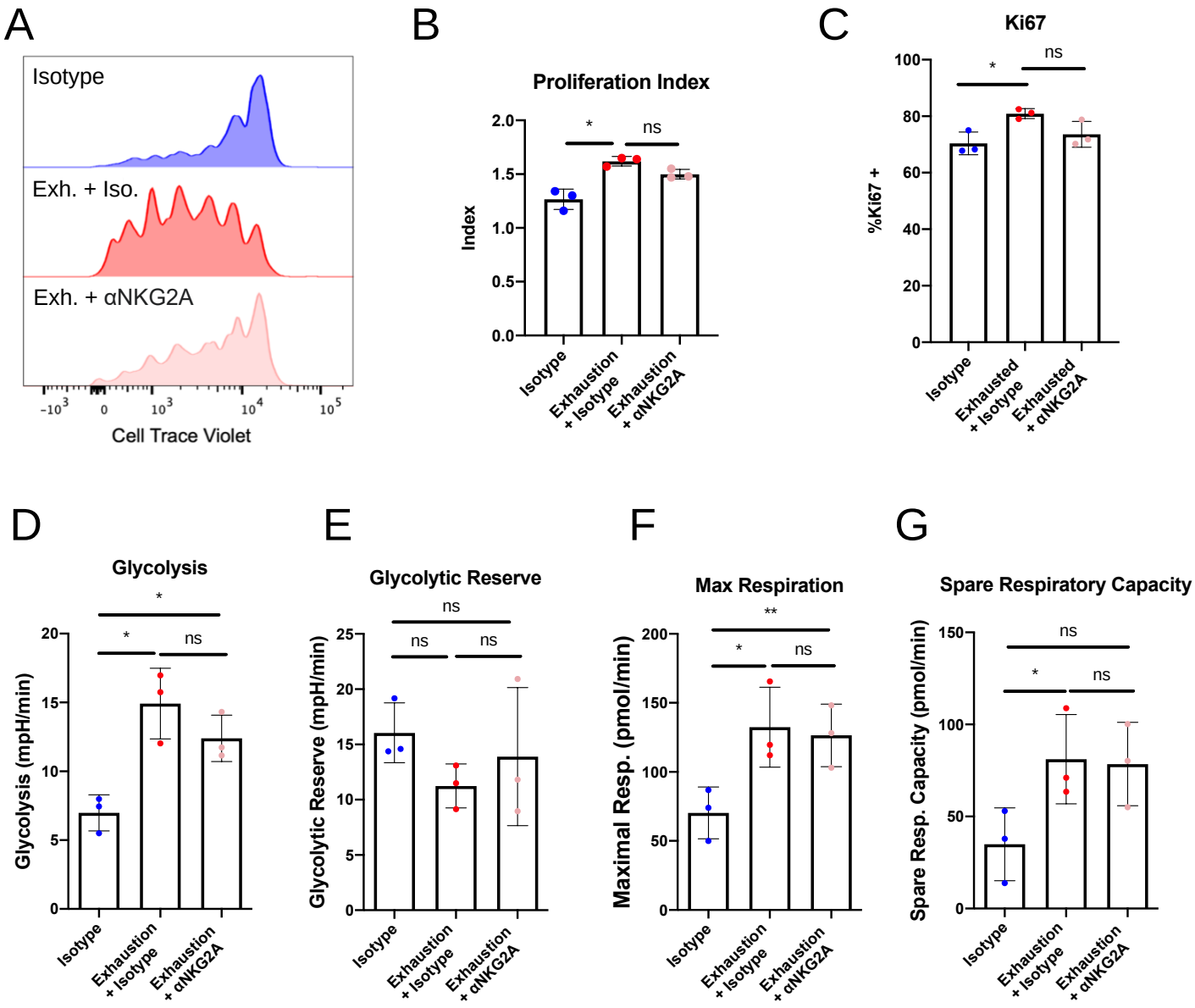

A
Isotype
Exhaustion
44.0
82.1
CD56
TIM3
Day 7- TIM-3
% TIM-3+
Control
Exhausted
Day 7 - TIM-3
MFI
ns
Isotype
Exhaustion
B
Isotype IgG (PE)
Isotype
Exhaustion
0.37
0.61
CD56
PE
Day 7 - PD-1
%PD-1+
ns
Anti-PD-1 (PE, Miltenyi)
1.03
1.59
Day 7 - PD-1
MFI
C
Isotype
Exhaustion
CD56
NKG2A
Day 7 - NKG2A
D
Day 3 - Granzyme B
E
Day 3 - Perforin
F
Day 7 - FasL
% FasL +
G
Day 3 - Fas-L
% Fas-L +
H
Day 3 - TRAIL
% TRAIL +

**SUPPLEMENTARY FIGURE 3.** (**A-H**) NK cells were harvested from isotype-coated and exhaustion plates and the expression of various receptors was analyzed via flow cytometry: TIM3, Day 7 (A) (*n*=3) representative dot plots, MFI data, and % of parent included; PD-1, Day 7 (B) (*n*=3) PE-conjugated isotype control, representative dot plots, MFI data, and % of parent included; NKG2A (C) (*n*=4) representative dot plots and MFI data included; Granzyme B, Day 3 (D) (*n*=10); Perforin, Day 3 (E) (*n*=6); Fas-L, Day 7 and 3 (F-G) (*n*=4, *n*=5); TRAIL, Day 3 (H) (*n*=*8*). $*P \leq 0.05$; $**P < 0.01$; ns, not significant.

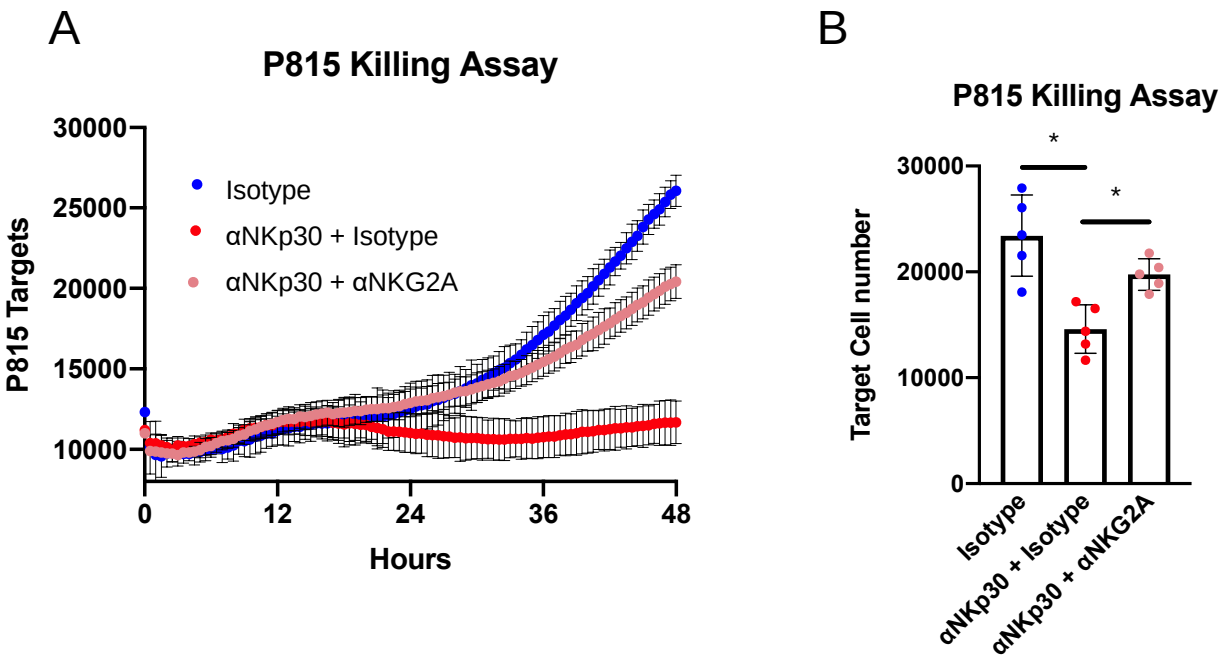

CD56
A
Day 1
Day 3
Day 7
Isotype
Exhaustion + Isotype
Exhaustion + αNKG2A
Ki67
B
Day 1
Day 3
Day 7
T-bet
C
Day 1
Day 3
Day 7
Isotype
Exhaustion + Isotype
Exhaustion + αNKG2A
TRAIL
D
Day 1
Day 3
Day 7
TIGIT
E
Day 1
Day 3
Day 7
Isotype
Exhaustion + Isotype
Exhaustion + αNKG2A
2B4
F
Day 1
Day 3
Day 7




**SUPPLEMENTAL FIGURE 4.** (**A-G**) At day 7 of stimulation, cells from three donors were analyzed via mass cytometry (CyTOF). viSNE analysis was performed for several markers: CD56 (A), Ki67 (B), T-bet (C), TRAIL (D), TIGIT (E), and 2B4 (F). Heatmap (G) indicates fold change relative to isotype mean metal intensity (MMI). * $P \leq 0.05$; ** $P < 0.01$; data that is not significant was left unlabeled ($n$=3).

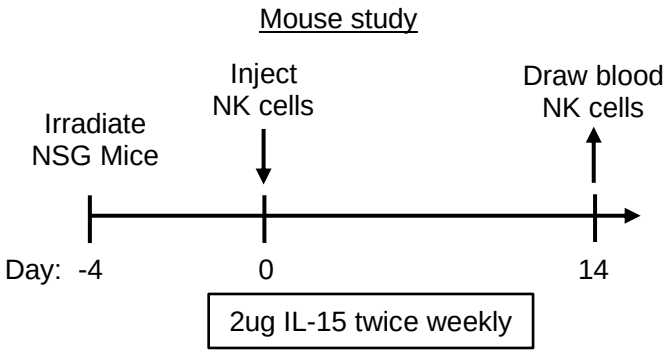



**SUPPLEMENTAL FIGURE 5.** (**A**) NK cells were stained with Cell Trace Violet and cultured on stimulatory plates for 7 days, as previously described. Cells were analyzed via flow cytometry 7 days later. (**B**) Proliferative indices were calculated using FlowJo V10 (**C**) NK cells were stained with Ki67 and analyzed via flow cytometry (*n*=3). (**C**) NK cells were harvested at day 7 and stained for Ki67 (*n*=3). (**D-G**) NK cells were immobilized on tissue culture plates and subjected to live-cell metabolic assays using Agilent's Seahorse XFe24 Analyzer. Extracellular acidification rates (ECAR) were measured following injection of glucose (G), oligomycin (O), FCCP (F), and Antimycin A + Rotenone (A+R). Oxygen consumption rates were measured following injection of glucose (G), oligomycin (O), FCCP (F), and Antimycin A + Rotenone (A+R). Graphical representations of glycolysis (D), glycolytic reserve (E), maximal respiration (F), and spare respiratory capacity (G) are pictured (*n*=3) ONE way ANOVA was used for comparisons. *$P \leq 0.05$; **$P < 0.01$; ns, not significant.

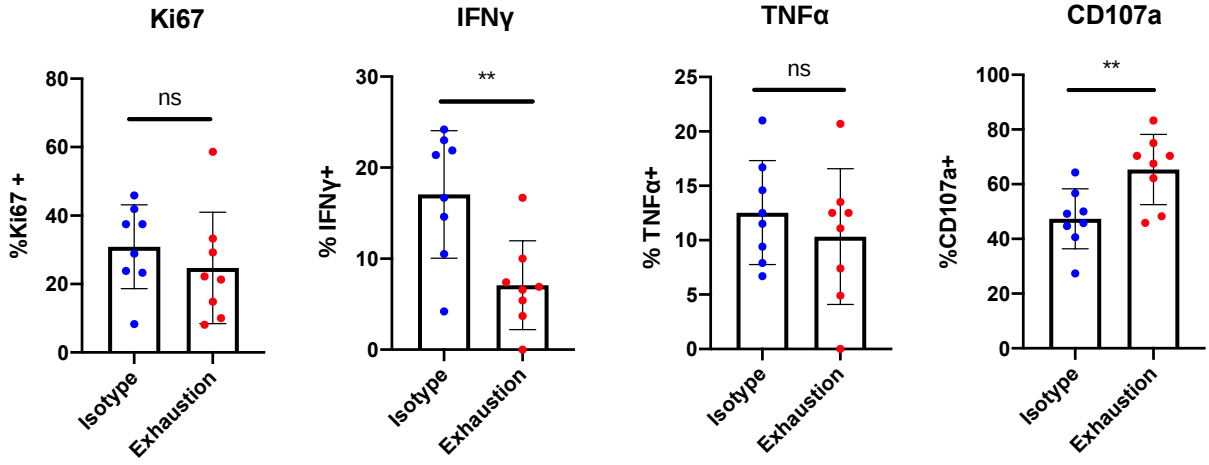



**SUPPLEMENTAL FIGURE 6.** (**A**) P815 targets were labelled with Cell trace Far Red and coated with either anti-NKp30 and anti-NKG2A or anti-NKp30 and isotype IgG. P815 cells coated in isotype alone served as a negative control. NK cells were incubated with P815 target cells (E/T 2:1) and target cell growth was tracked using the Incucyte live cell imaging platform. (**B**) The number of target cells remaining after 48 hours was quantified for every condition and E/T ratio ($n$=5). *$P \leq 0.05$.

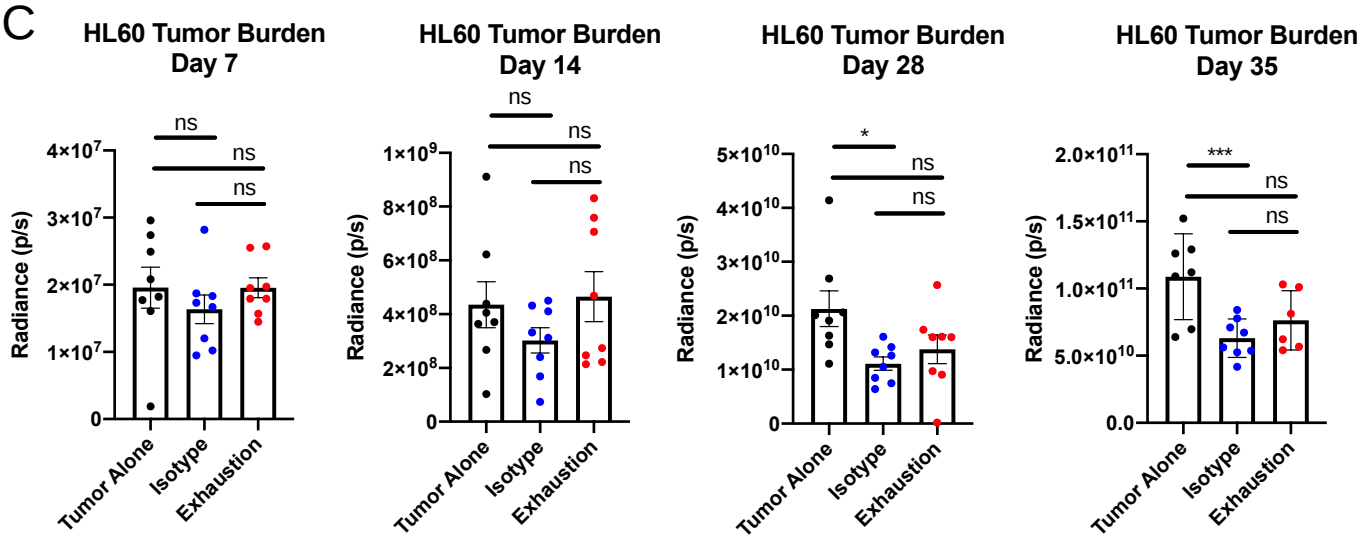
A


Mouse study
Inject
NK cells
Draw blood
NK cells
Irradiate
NSG Mice
Day: -4
0
14
2ug IL-15 twice weekly

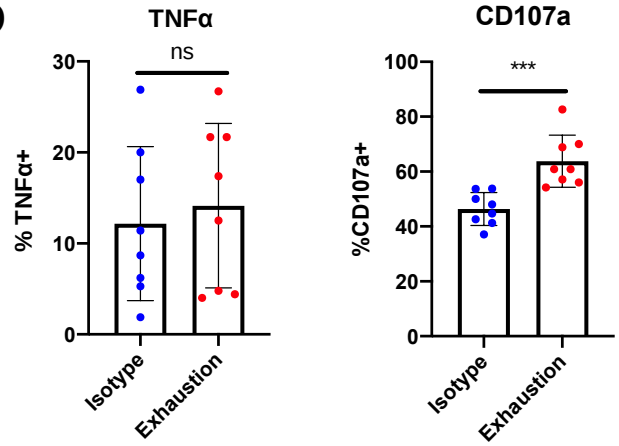
B


Ki67
%Ki67 +
ns
IFNγ
% IFNγ+
**
TNFα
% TNFα+
ns
CD107a
%CD107a+
**
Isotype
Exhaustion

C

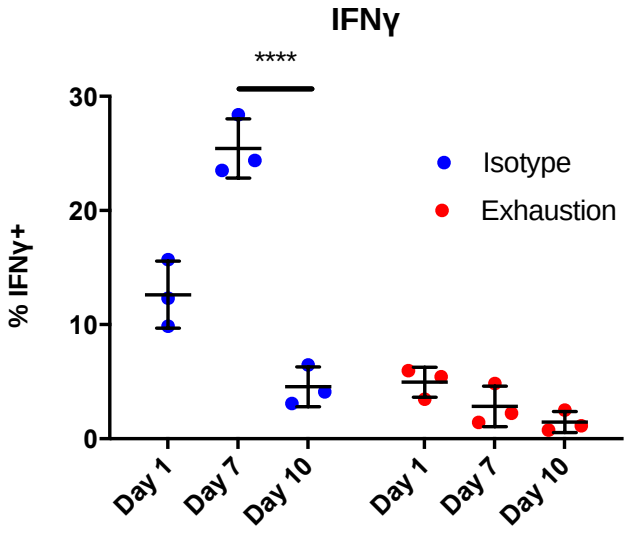

HL60 Tumor Burden
Day 7
HL60 Tumor Burden
Day 14
HL60 Tumor Burden
Day 28
HL60 Tumor Burden
Day 35
Radiance (p/s)
ns
*
***
Tumor Alone
Isotype
Exhaustion

D


TNFα
% TNFα+
ns
CD107a
%CD107a+
***
Isotype
Exhaustion

**SUPPLEMENTAL FIGURE 7.** (**A**) Schematic representing experimental design: sublethally irradiated NSG mice were injected (i.v.) with $1x10^6$ NK cells. NK cells had been incubated for 7 days on plates with either isotype IgG (control) or anti-NKp46 and MICA/B as previously described. (**B**) 14 days post NK cell injection in the non-tumor model, blood was drawn, and NK cells were restimulated with K562 leukemia cells for 4 hours as previously described. Ki67, IFNγ, TNFα, and CD107a expression was assessed via flow cytometry. NK cells were CD45h+ CD56+ CD3-. Paired *t* tests were used for comparisons (*n*=8). ***P* < 0.01; ns, not significant. (**C**) Tumor burden at several time points was measured via BLI. One way ANOVA was used for comparisons. **P* < 0.05; ****P* < 0.001; ns, not significant (*n*=8). (**D**) 14 days post NK cell injection in the HL60 tumor model, blood was drawn, and NK cells were restimulated with K562 leukemia cells for 4 hours as previously described. TNFα and CD107a expression was assessed via flow cytometry. NK cells were CD45h+ CD56+ CD3-. Paired *t* tests were used for comparisons (*n*=8). ****P* < 0.001; ns, not significant.

A




**SUPPLEMENTARY FIGURE 8.** (**A**) NK cells harvested from isotype-coated and exhaustion plates (days 1, 7, and 10) were incubated with K562 targets for four hours (E/T: 2:1). IFNγ production was measured via flow cytometry. Paired *t* tests were used for comparisons ($n$=3). ****$P < 0.0001$.

| Marker | Metal | Catalog no. | Clone |
|---|---|---|---|
| CD3 | 141Pr | 3141019B | UCHT1 |
| KIR Activating Pool | 142Nd | 201142A | KIR2DL1(143211) |
| C-Kit | 143Nd | 3143001B | 104D2 |
| CD69 | 144Nd | 3144018B | FN50 |
| KIR Inhibitory Pool | 145Nd | 201145A | KIR2DS1 (1127B) |
| CD8a | 146Nd | 3146001B | RPA-T8 |
| Fas Ligand | 147Sm | 201147A | NOK-1 |
| CD274 (PD-L1) | 148Nd | 3148017B | 29E.2A3 |
| CD25 (IL-2R) | 149Sm | 3149010B | 2A3 |
| PLZF | 150Nd | 201150A | 6318100 |
| CD2 | 151Eu | 3151003B | TS1/8 |
| CD95/Fas | 152Sm | 3152017B | DX2 |
| CD62L (L-selectin) | 153Eu | 3153004B | DREG-56 |
| CD16 | 154Sm | 3154016B | MBSA43 |
| CD27 | 155Gd | 3155001B | L128 |
| CXCr3 | 156Gd | 3156004B | G025H7 |
| CD137/4-1BB | 158Gd | 3158013B | 4B4-1 |
| CD337 (NKp30) | 159Tb | 3159017B | Z25 |
| NKG2C | 160Gd | 201160A | 2098A |
| Eomes | 161Dy | 201161A | 644730 |
| CD335 (NKp46) | 162Dy | 3162021B | BAB281 |
| CD56 (NCAM) | 163Dy | 3163007B | NCAM 16.2 |
| TRAIL | 164Dy | 201164A | RIK2 |
| CD223/LAG-3 | 165Ho | 3165037B | 11C3C65 |
| CD314 (NKG2D) | 166Er | 3166016B | ON72 |
| NKp44 | 167Er | 201167A | P-448 |
| Ki-67 | 168Er | 3168007B | B56 |
| CD159a (NKG2A) | 169Tm | 3169013B | Z199 |
| CD122 | 170Er | 3170004B | Tu27 |
| CD226 DNAM-1 | 171Yb | 3171013B | DX11 |
| CD57 | 172Yb | 3172009B | HCD57 |
| Granzyme B | 173Yb | 3173006B | GB11 |
| CD279 (PD-1) | 174Yb | 3174020B | EH12.2H7 |

| | | | |
|---|---|---|---|
| Perforin | 175Lu | 3175004B | B-D48 |
| CD127 (IL-7Ra) | 176Yb | 3176004B | A019D5 |
| TIGIT | 209Bi | 3209002B | MBSA43 |
| CD45 | 89Y | 3089003B | HI30 |
| CD94 | 111CD | 201111A | DX22 |
| LFA-1 | 112CD | 201112A | M24 |
| KLRG-1 | 113CD | 201113A | 14C2A07 |
| 2B4 | 114CD | 201114A | C1.7 |
| T-bet | 116CD | 201116A | 4B10 |

**SUPPLEMENTARY TABLE 1.** Antibodies used for Mass Cytometry by time-of-flight (CyTOF). Marker, metal tag, catalog number, and clone ID for each antibody are listed.