Electronic Supplementary Information:

Analysis of Sphingosine Kinase Activity in Single Natural Killer Cells from Peripheral Blood

Alexandra J. Dickinson,[a] Megan Meyer,[b] Erica A. Pawlak,[d] Shawn Gomez,[c] Ilona Jaspers,[b,d,e] Nancy L. Allbritton[a,c]

[a] Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599, United States

[b] Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599, United States

[c] Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27599, United States and North Carolina State University, Raleigh, NC 27695, United States

[d] Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina, Chapel Hill, NC 27599, United States

[e] Department of Pediatrics, University of North Carolina, Chapel Hill, NC 27599, United States

*Corresponding author e-mail: nlallbri@unc.edu and fax 919-962-2388

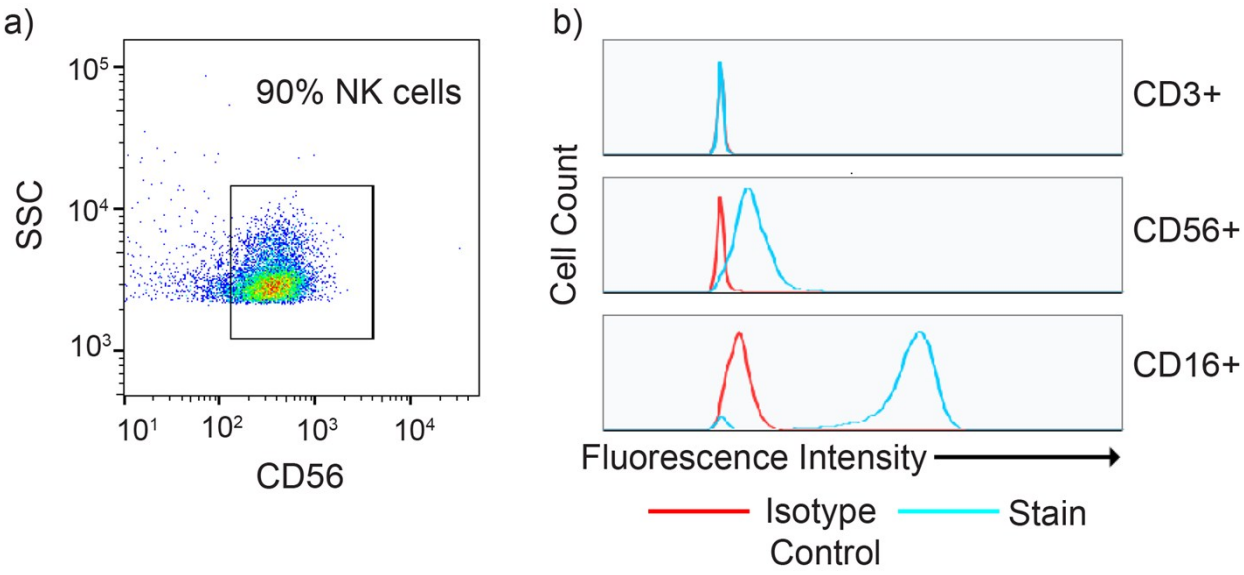


Fig. S1 Representative flow cytometry traces characterizing the enrichment of PBMCs for a representative sample of NK cells from one subject. a) Side scatter (SSC) plotted against the fluorescence intensity of cells immunostained for CD56, a marker for NK cells. b) Histograms showing cell count plotted against the fluorescence intensity of cells immunostained for CD3, CD56, and CD16, which are markers for T cells, NK cells, and cytotoxic NK cells, respectively. Also shown is the isotype or negative control in which cells were incubated with the fluorescent secondary antibody but not the primary antibody against the surface protein.

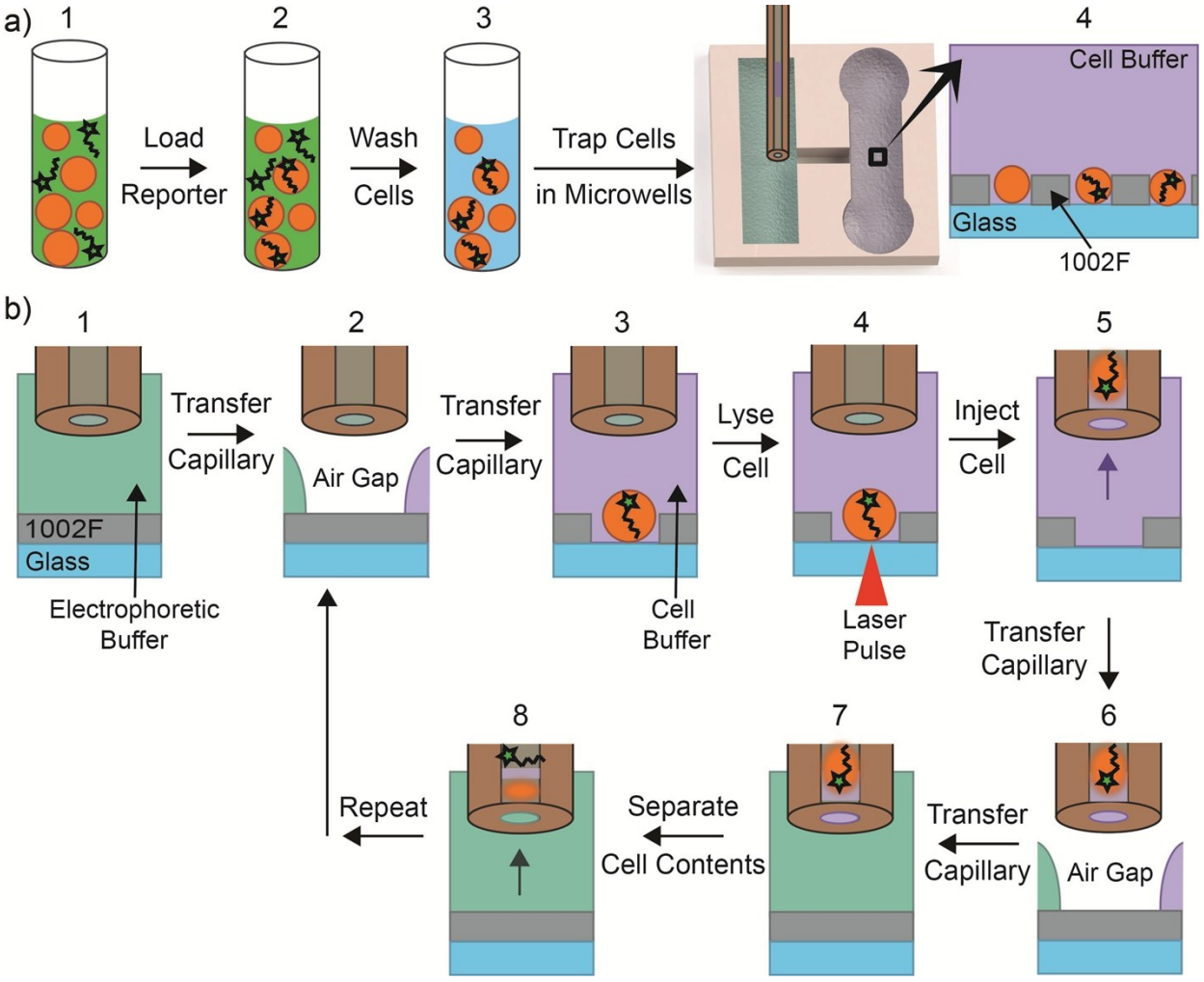


Fig. S2 Schematic of the experimental protocol. a) Off-chip loading of NK cells with the fluorescent SF reporter. Steps 1-2: cells were incubated with the fluorescent reporter for 30 min. Step 3: cells were washed in a physiologic buffer to remove reporter from the extracellular fluid. Step 4: cells were captured on-chip in microwell traps and continuously bathed in physiologic buffer. b) Single-cell CE assay. Steps 1-2: the capillary was transferred from the electrophoretic buffer through the air gap and into the cellular buffer. Step 3: the capillary was aligned directly over a single NK cell positioned in a cell trap. Steps 4-5: the cell was lysed and its contents immediately loaded into the capillary. Steps 6-7: the capillary was transferred through the air gap and into the electrophoretic buffer. Step 8: a high voltage was applied across the capillary, and the contents of the cell was separated. This entire process was then repeated for each cell in the microwell array. The "reporter exposure time" for the reporter was the time between step a-1 and step b-5.

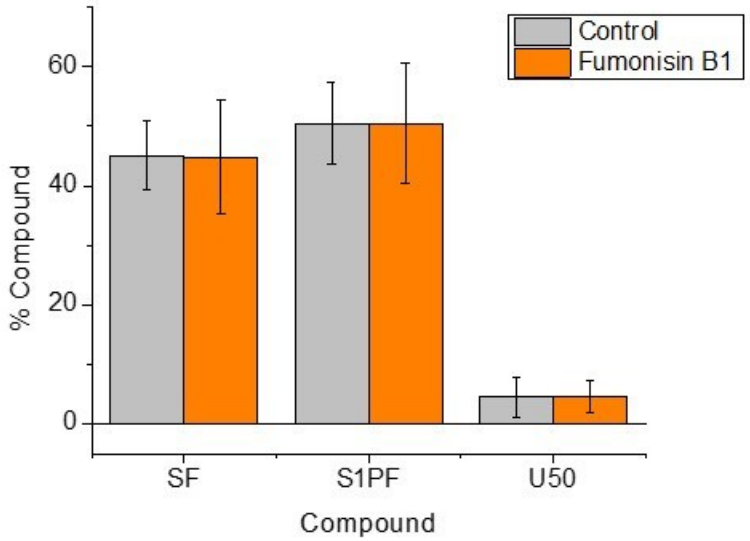


Fig. S3 The percent of SF, S1PF, and U_{50} (relative to total reporter loaded) in cell lysates incubated with a phosphate-buffered saline vehicle control or 10 μM fumonisin B_1, a ceramide synthase inhibitor, for 3 h. There was no statistically significant difference between the control cells and the fumonisin-treated cells (n=3).

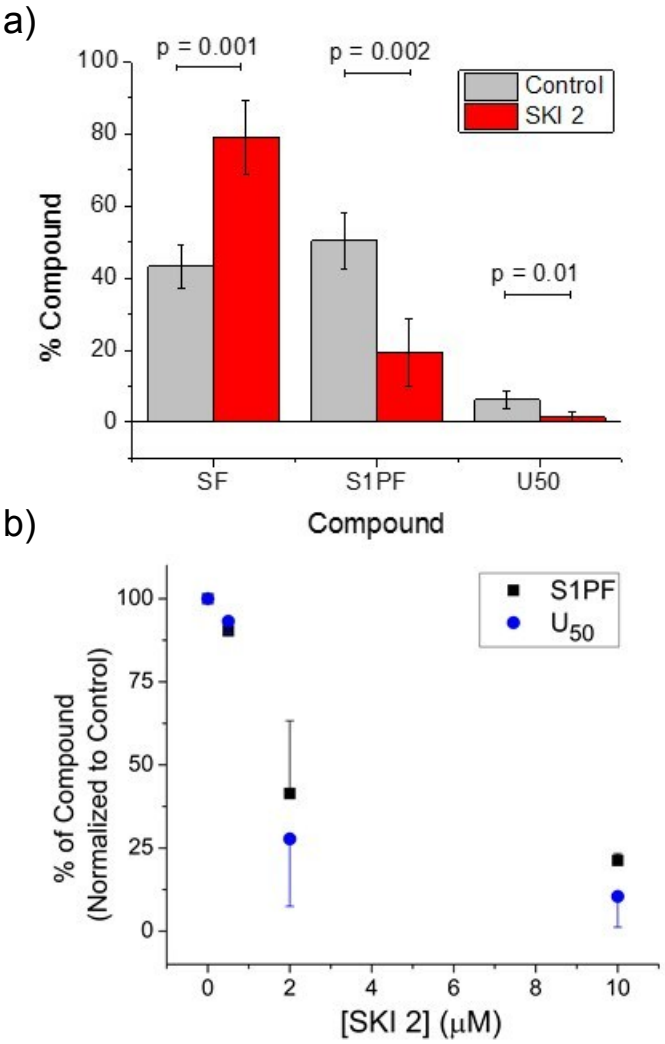


Fig. S4 SK inhibition decreases formation of U_{50}. a) The percent of SF, S1PF, and U_{50} formed in cell lysates treated with a DMSO control or with 2 μM SK inhibitor 2 (SKI 2) for 1.5 h (n = 3). b) The percent of S1PF and U_{50} formed in cell lysates was plotted against the concentration of SKI 2 (n = 3).

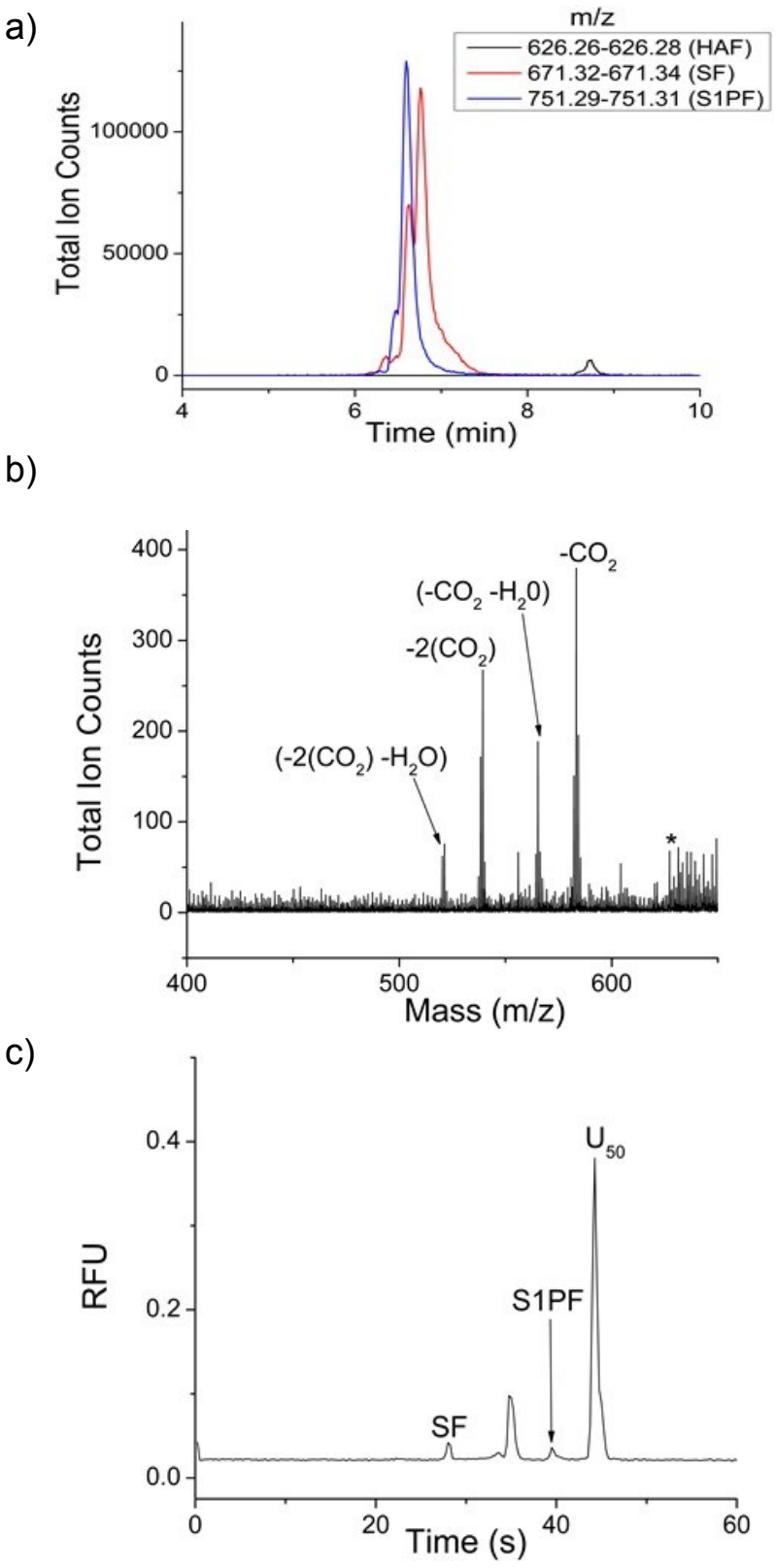


Fig. S5 HPLC-MS analysis of K562 cells loaded with SF and then lysed. a) The total ion counts (y axis) recorded for each of three molecular-weight species eluting over time from the HPLC of

the cell lysate. The molecular weight of 627.29 is consistent with that of hexadecanoic acid plus a proton. The molecular weights of 671.33 and 751.29 correspond to SF and S1PF, respectively b) Collision induced dissociation spectrum of the molecule with the molecular weight of 627.29. The observed fragments at molecular weight so of 583.28, 565.28, 539.30, and 521.28 are consistent with hexadecanoic acid with loss of CO_2, CO_2 plus H_2O, two CO_2, and two CO_2 plus H_2O, respectively. c) CE separation of the HPLC fraction possessing the species of molecular weight 627.29. This species migrates at 50 s suggesting that it is the U_{50} peak.

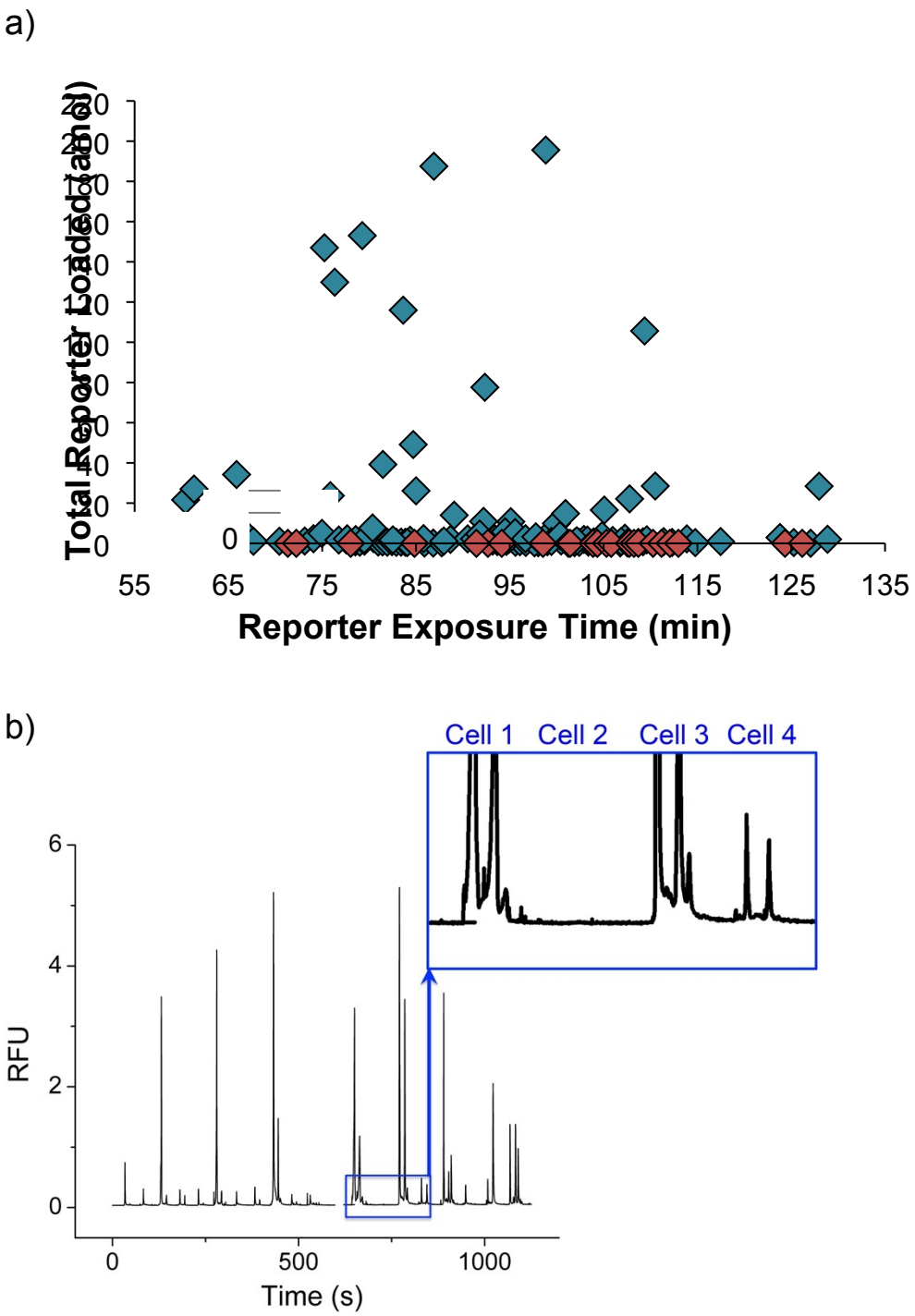


Fig. S6 Loading of Reporter into Cells. a) The amount of total reporter loaded *vs* the reporter exposure time for all NK cells measured using single-cell CE. The 14% of cells that did not contain fluorescent signal above the limit of detection are highlighted in red and were placed on the x axis. b) An example electropherogram highlighting a cell without sufficient signal to detect fluorescent reporter is shown. Cell 1, 3, and 4 all contain sufficient fluorescent signal to detect SF and S1PF. Cell 2 does not contain fluorescent signal above the limits of detection of the system.

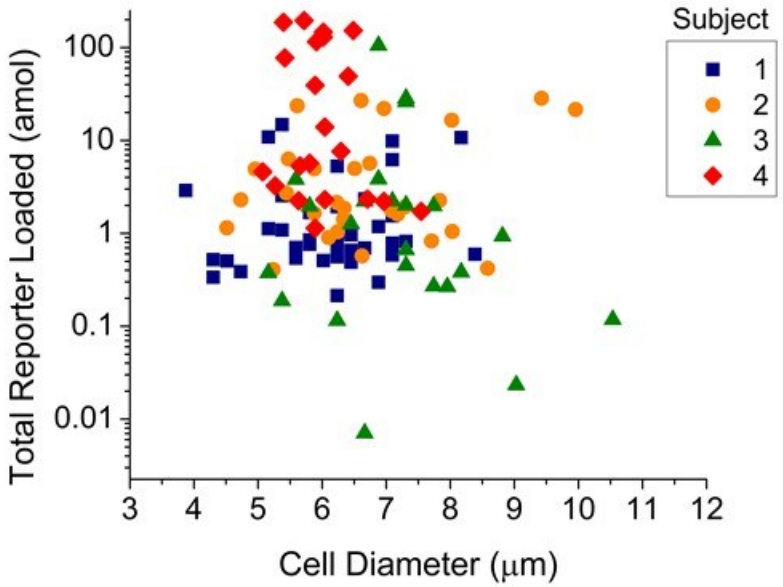


Fig. S7 The total reporter loaded into cells (SF_{mol} + $S1PF_{mol}$ + HAF_{mol}) was plotted against the diameter of the cell (n = 111).

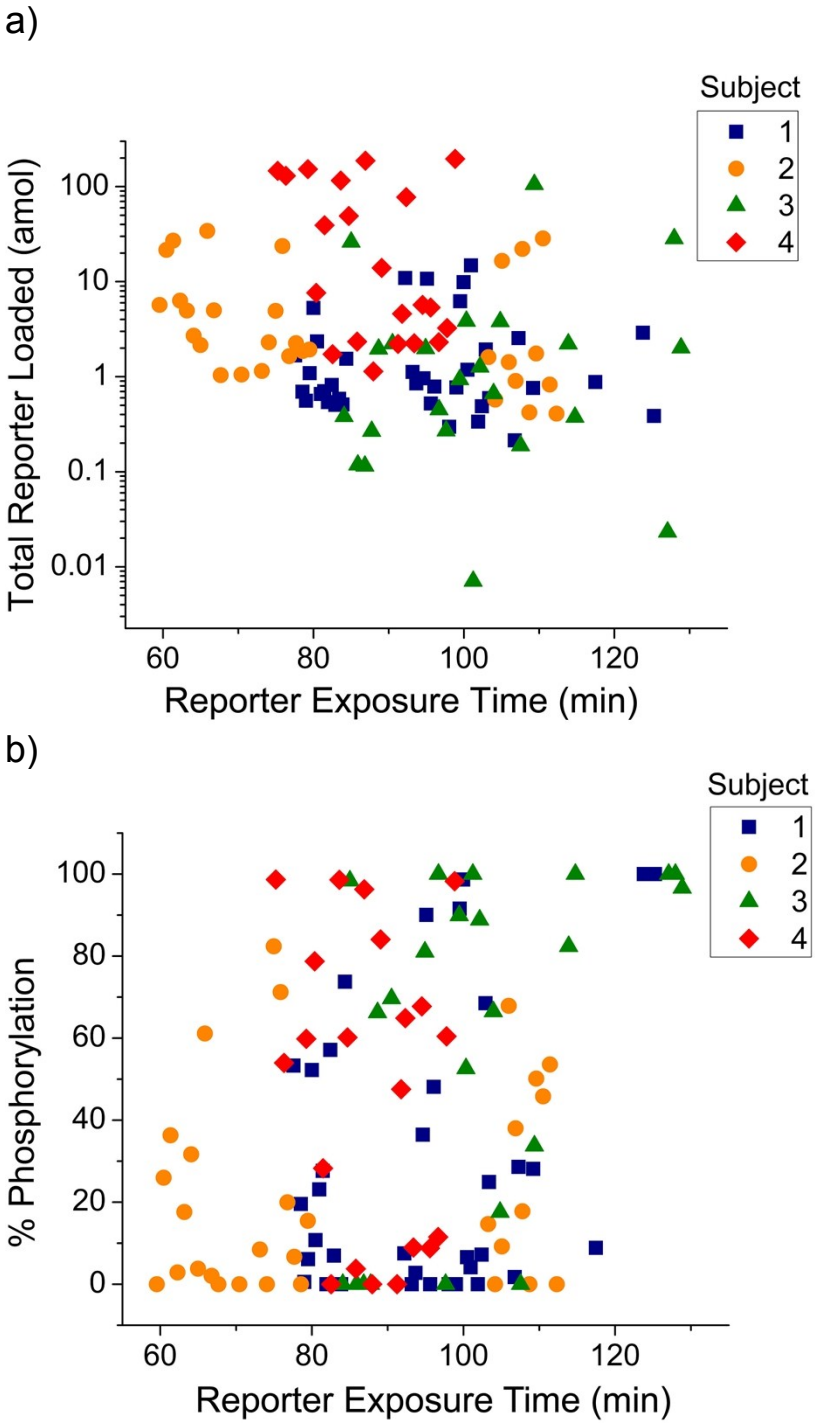


Fig. S8 Effect of reporter exposure time on the amount of reporter loaded and the percentage converted to S1PF. a) The total reporter loaded into cells ((SF_{mol} + $S1PF_{mol}$ + HAF_{mol}) and b) the percent phosphorylation of the reporter ($S1PF/(SF_{mol} + S1PF_{mol} + HAF_{mol})$) were plotted against the reporter exposure time in the cells.

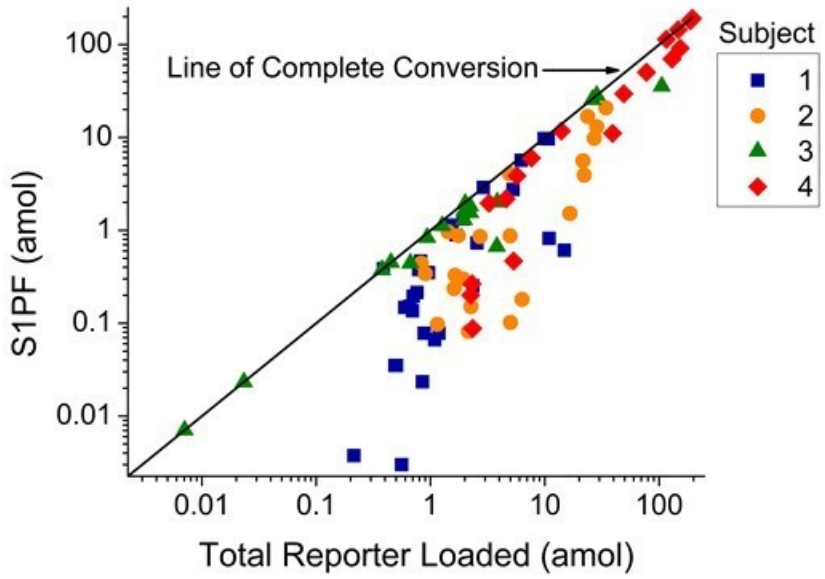
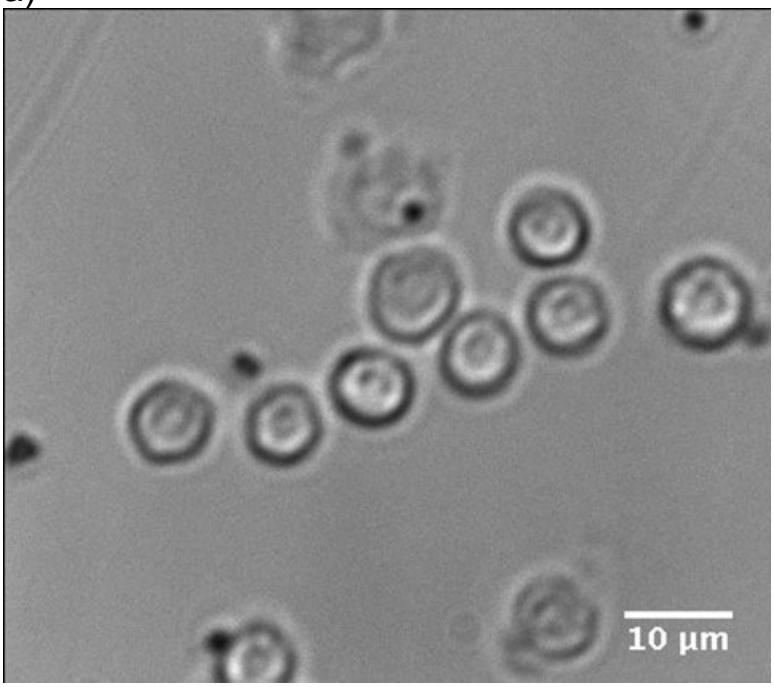


Fig. S9 The total amount of S1PF formed per cell was plotted against the total reporter loaded into that cell (SF_{mol} + $S1PF_{mol}$ + HAF_{mol}). The line of complete conversion highlights cells that converted 100% of the loaded reporter to S1PF.

a)



b)

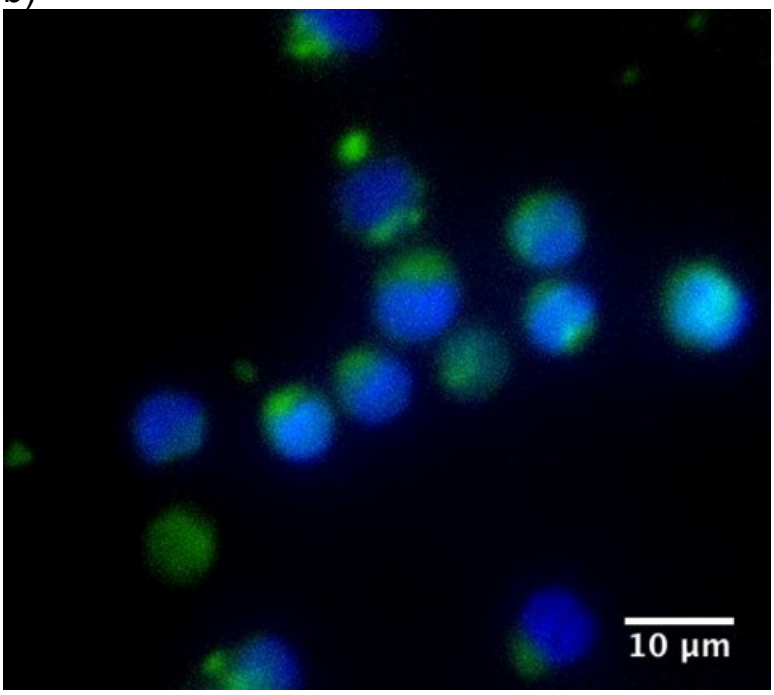


Fig. S10 Microscopy images of enriched NK cells loaded with S1PF. a) Brightfield image of NK cells. b) Fluorescence image of S1PF reporter (green) and Hoechst dye (blue).

Table S1 Demographic information for six subjects

Subject	Gender	Race	Age	BMI*
1	Male	White	33	33.1
2	Male	White	39	28.2
3	Male	White	25	-
4	Female	Asian	24	19.3
5	Male	White	31	22.7
6	Female	White	23	23.1

* Body Mass Index (BMI)