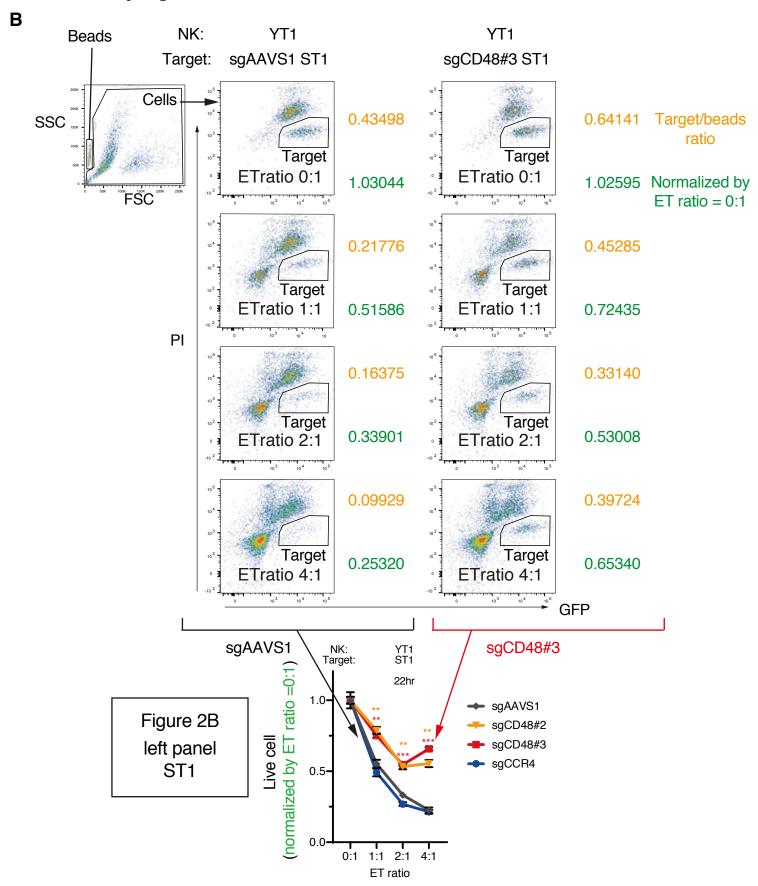
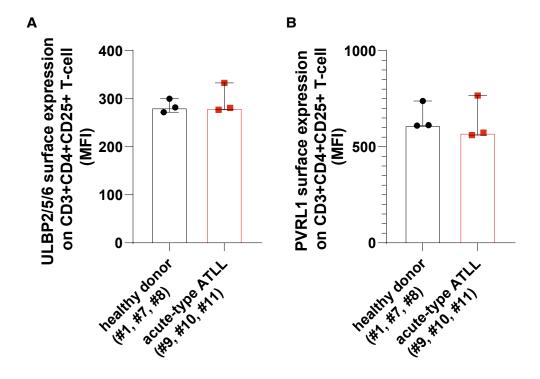


Example gating strategy for discreminating target cells from effector cells. First, the counting beads were excluded in the SSC/FSC plot. The cell population was further plotted using PI (Propidium iodide) and GFP. GFP-positive and PI-negative cell population represented target cells because the target cells were infected with sgRNA-expressing vectors co-expressing GFP. GFP-negative and PI-negative cell population represented effector NK cells. PI-positive cells represented debris and dead cells, and were excluded from our analysis.



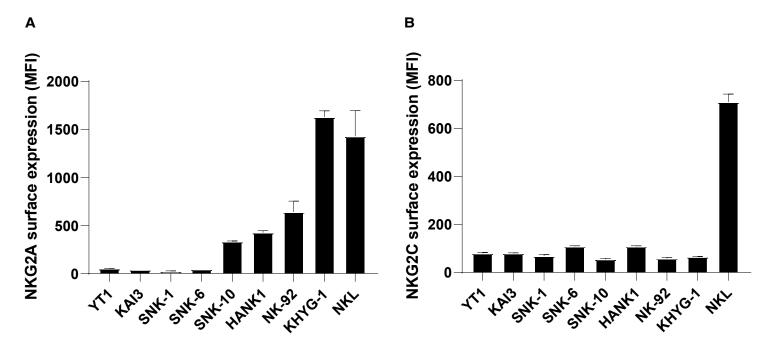
Gating strategy for discriminating ST1 target cells from effector NK cells in Figure 2B. Representative GFP/PI plots were indicated. Beads count normalized target cell count for each sample (target/beads ratio; colored in orange). Then, the target/beads ratio was normalized by the ratio of ET ratio = 0:1 (Normalized by ET ratio; colored in green). A duplicate in Figure 2B was represented as a dot plot in this Reviewer-only Figure 1B.



Cell surface expression of ULBP2/5/6 (A) and PVRL1 (B) on CD3+CD4+CD25+ cells in healthy donors and acute type ATLL were shown. Experiments were repeated at least 2 times. Error bars represent median with 95% CI .

The antibodies used were below.

Human ULBP-2/5/6 APC-conjugated Antibody; R&D # FAB1298A, APC anti-human PVRL1 Antibody; Sino Biological #11611-MM09-A, Pacific blue anti-Human CD3; BD Pharmingen #558117, PE anti-human CD25 Antibody; BioLegend #356104, PerCP/Cyanine5.5 anti-human CD4 Antibody; BioLegend #300530.



Cell surface expression of NKG2A and NKG2C NK cell lines (YT1, KAI3, SNK-1,SNK-6, SNK-10, HANK1, NK-92, KHYG-1 and NKL) were shown. MFI of NKG2A (A) and NKG2C (B) subtracted by MFI of isotype control were shown. Experiments were repeated at least 2 times. Error bars represent median with 95% CI.

Antibodies used were below. APC-CD159a, Beckman Coulter A60797; APC-CD159c (NKG2C) Antibody, Miltenyi Biotec 130-113-434