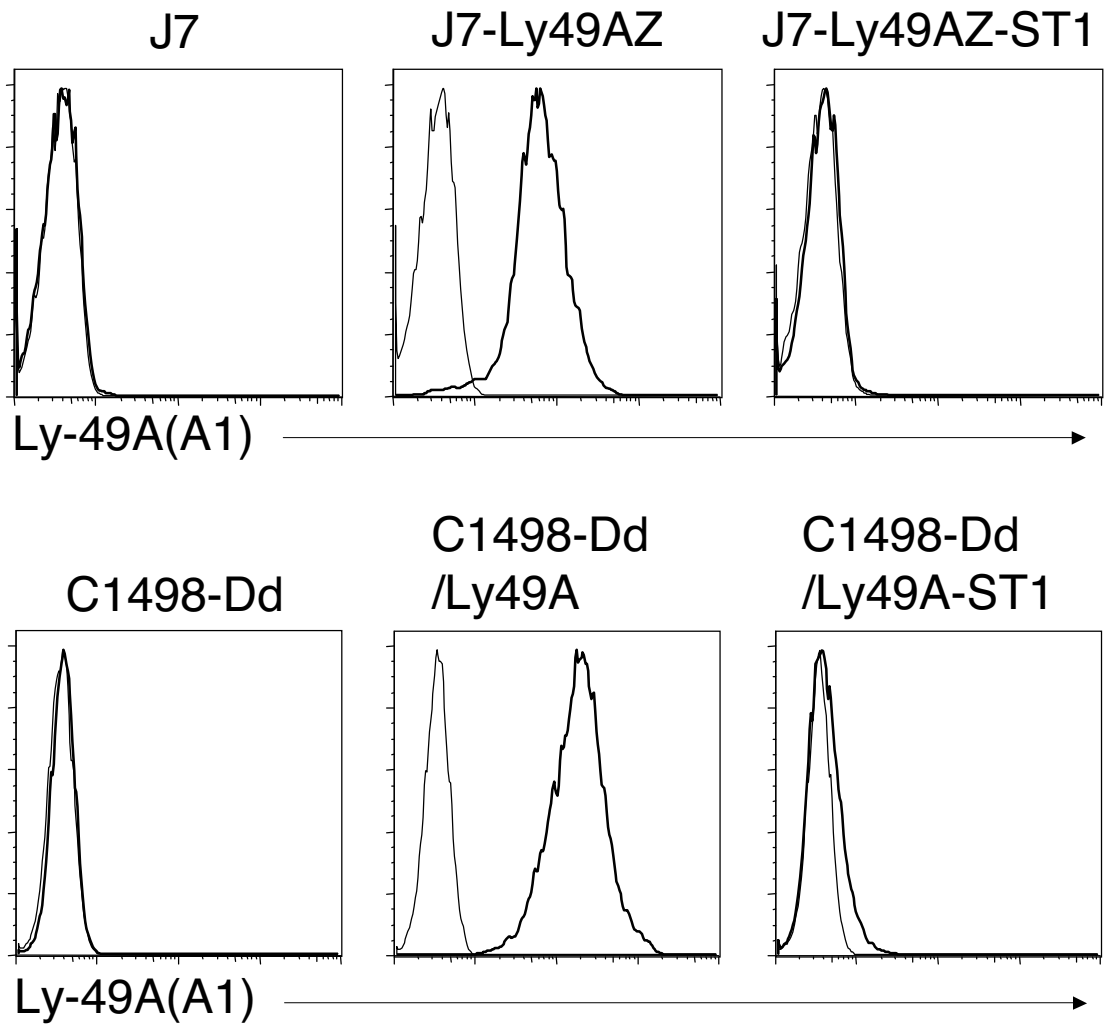
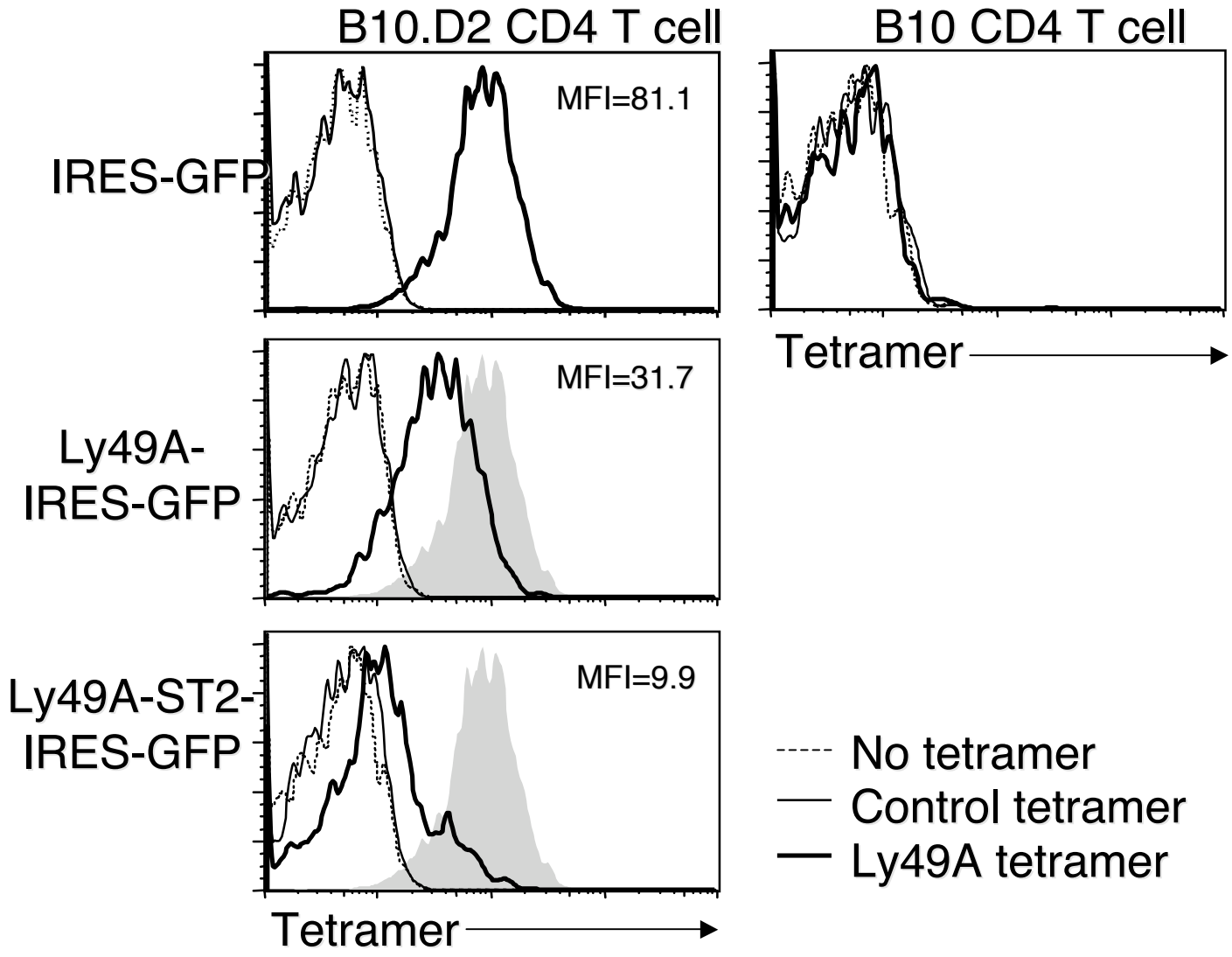
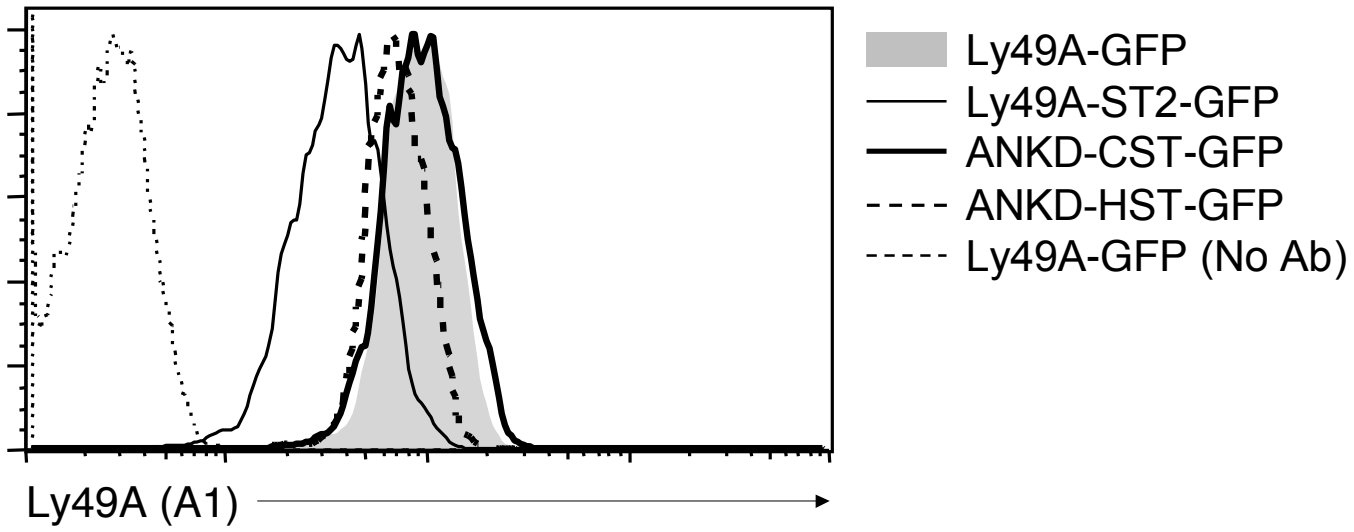
SI Appendix



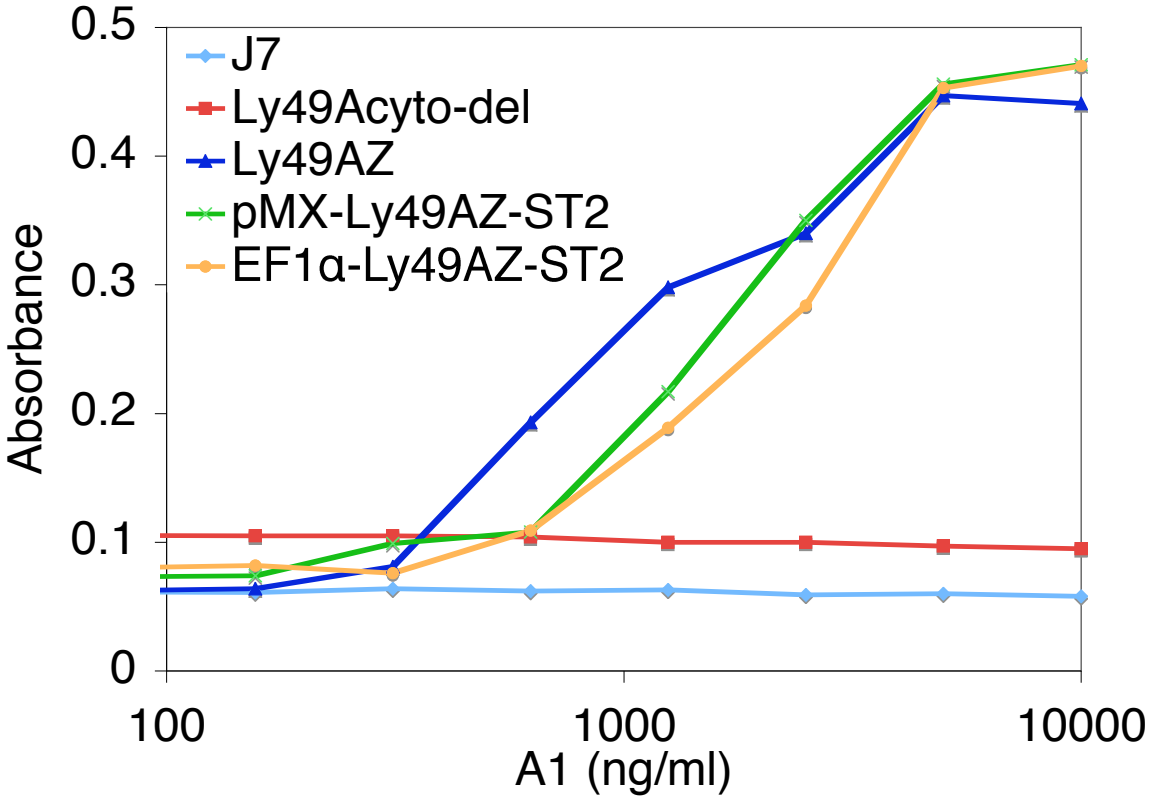
SI Appendix Image 1. No surface expression of Ly49A-ST1 on J7 reporter or C1498-D^d target cells: FACS analyses using an anti-Ly49A mAb (A1) are shown.



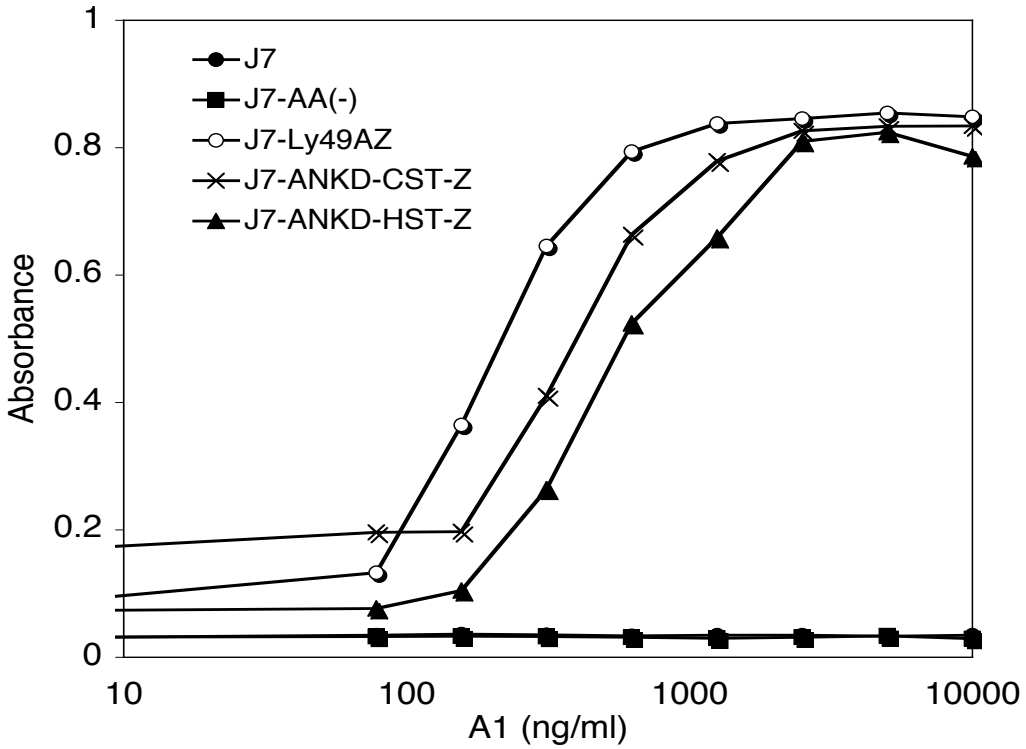
SI Appendix Image 2. Reduced Ly49A tetramer binding to primary CD4 T cells expressing $H2^d$ and Ly49A**.** Indicated retrovirus constructs were transduced into CD4 T cells from B10.D2, respectively. GFP-positive cells were gated and stained with streptavidin-phycoerythrin (SA-PE)-conjugated Ly49A or control ($H2D^d$/hβ2m) tetramers (left panel) and with biotinylated A1 mAb followed by SA-PE (right panel). Grey shades represent the overlaid histogram of the tetramer binding to the pMX-IRES-GFP-transduced cells.



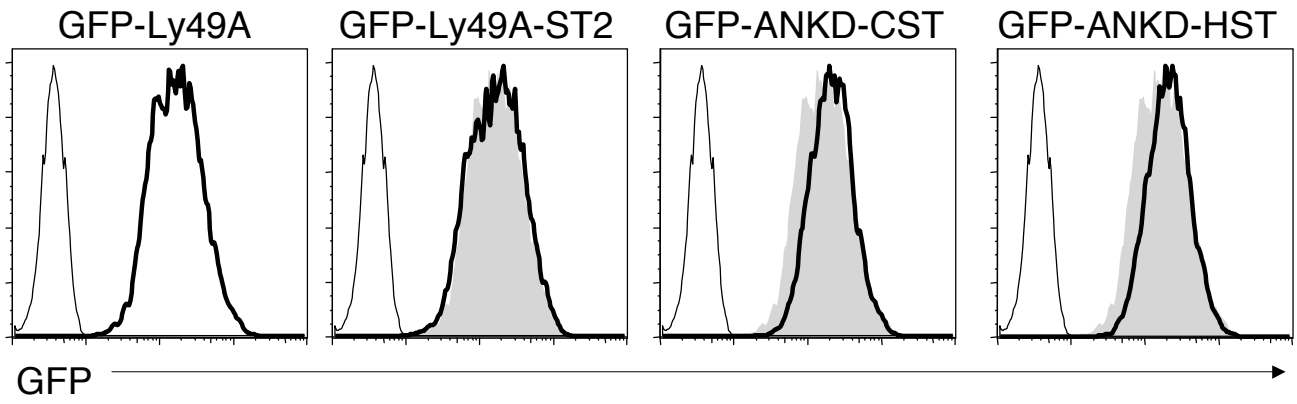
SI Appendix Image 3. Reduced anti-Ly49A mAb binding to Ly49A-ST2. Indicated retrovirus constructs of GFP-fusion protein were transduced into J7 cells, respectively. GFP-positive cells were gated and stained with A1 mAb.



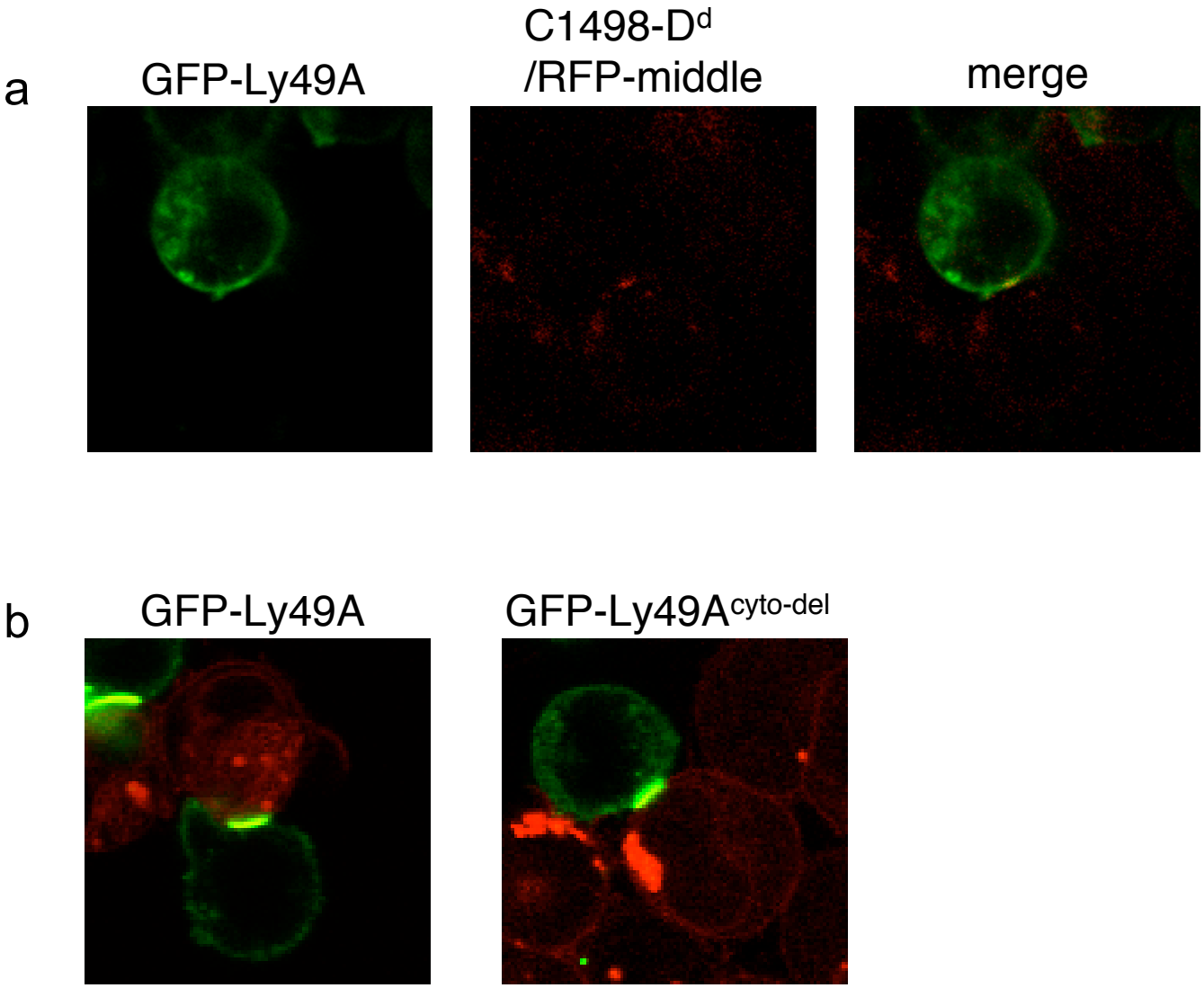
SI Appendix Image 4. Equivalent signaling ability of Ly49A-ST2 by mAb crosslinking regardless of the expression vector used. Ly49A-ST2 was transduced in J7 cells with lentivirus or retrovirus, and named as EF1α-Ly49A-ST2 and pMX-Ly49A-ST2, respectively. Indicated doses of A1 mAb (100μl) were immobilized in a 96-well plate. Cells ($1x10^5$) of each reporter cell line were plated and incubated over night. CPRG assays were then performed. J7 cells transduced with the lentivirus are referred to as J7-Ly49A-ST2 in the text.



SI Appendix Image 5. Signaling ability of stalk-chimeric Ly49A receptors by mAb crosslinking. Indicated reporter cells were crosslinked by A1 mAb as in Image 4.



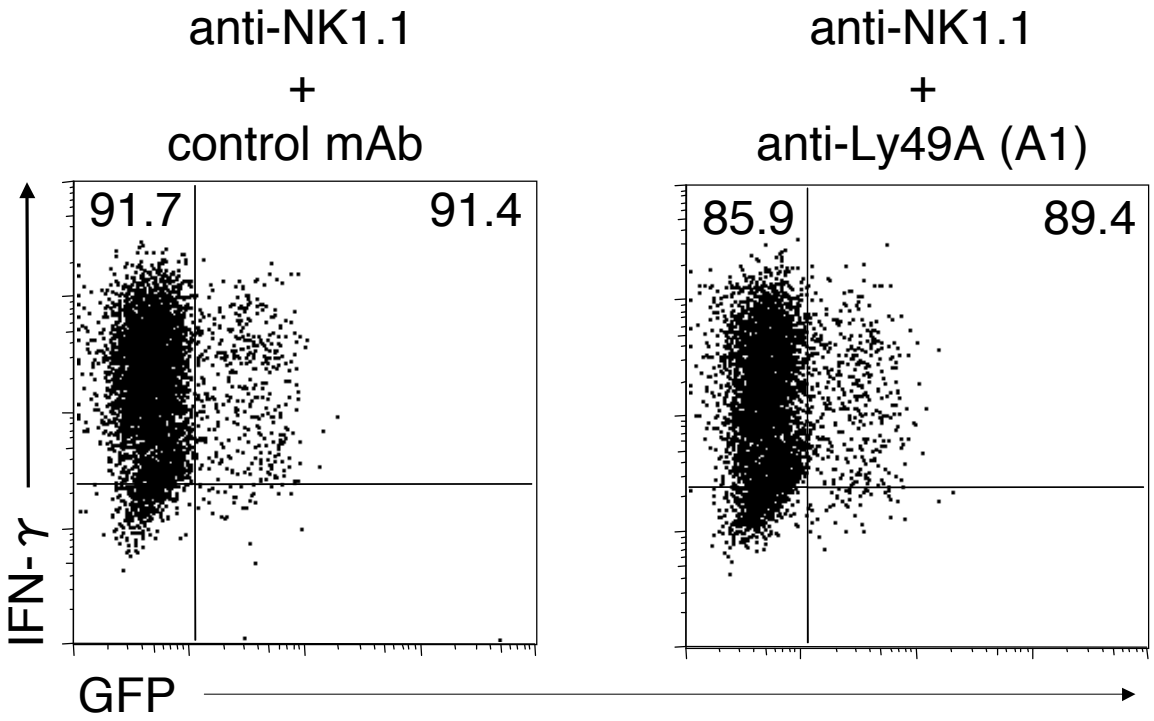
SI Appendix Image 6. Expression of stalk-chimeric Ly49A receptors fused to GFP. Indicated retrovirus constructs were transduced into J7 cells and GFP expression was analyzed by FACS. Grey shades represent the overlaid histogram of the GFP-Ly49A data.



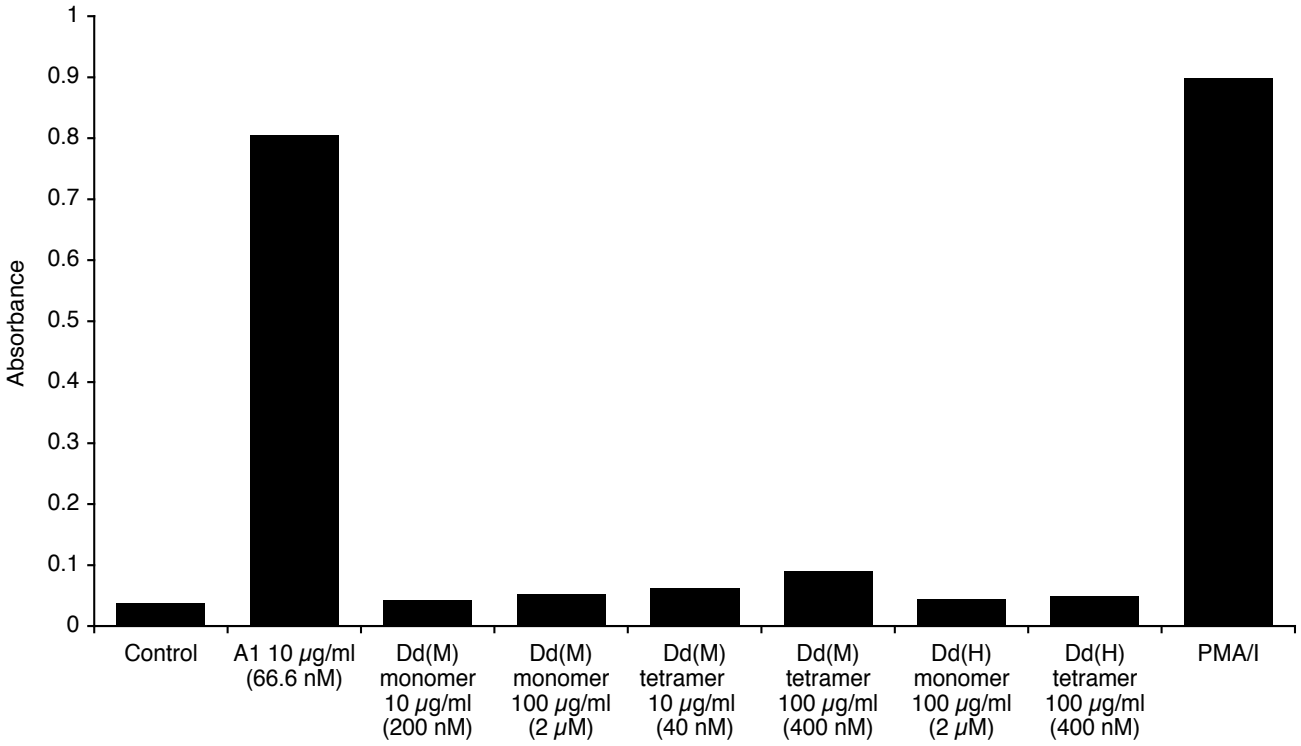
SI Appendix Image 7. IS formation. (*a*) Lower expression of D^d-RFP results in failure to form clear IS formation with J7 cells expressing GFP-Ly49A. (*b*) The cytoplasmic ITIM is not required for IS formation. J7 cells expressing the cytoplasmic-deleted Ly49A fused to GFP (GFP-Ly49A$^{cyto\text{-}del}$) formed ISs with C1498-D^d/RFP.

	Synapse size	GFP intensity
Ly-49A	0.33±0.07	5.36±1.31
ANKD-HST	0.33±0.10	5.37±0.83

SI Appendix Image 8. Similar clustering of stalk-chimeric ANKD-HST to wild-type Ly49A receptors. Synapse size was evaluated as the ratio of synapse length/diameter of cells. The integrated intensity of GFP was measured for each synapse. Averaged values from 20 synapses are shown. P-values (t-test) for size and intensity were 0.87 and 1.00, respectively.



SI Appendix Image 9. GFP-chimeric Ly49A receptors fail to inhibit cytokine production by mAb crosslinking. Ly49A-negative LAK cells were transduced with lentivirus encoding GFP-chimeric Ly49A. Three days after the infection, LAK cells were crosslinked with the anti-NK1.1 and anti-Ly49A mAb or the anti-NK1.1 and isotype control mAb. Numbers represent the percentages of IFN-γ cells among the GFP^+ or GFP^- cell populations. 100 μg/ml control mAb or A1 were immobilized. Representative data from three independent experiments are shown.



SI Appendix Image 10. Plate-bound D^d/mβ 2 monomer and tetramers fail to stimulate Ly49A reporter cells. Indicated doses of protein were coated on a 96-well plate. Ly49A reporter assays were performed. Dd(M): D^d/mβ2; Dd(H): D^d/hβ2; Control: no protein coated.