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Supplementary information

Expanding the cytokine receptor alphabet reprograms T cells into diverse states

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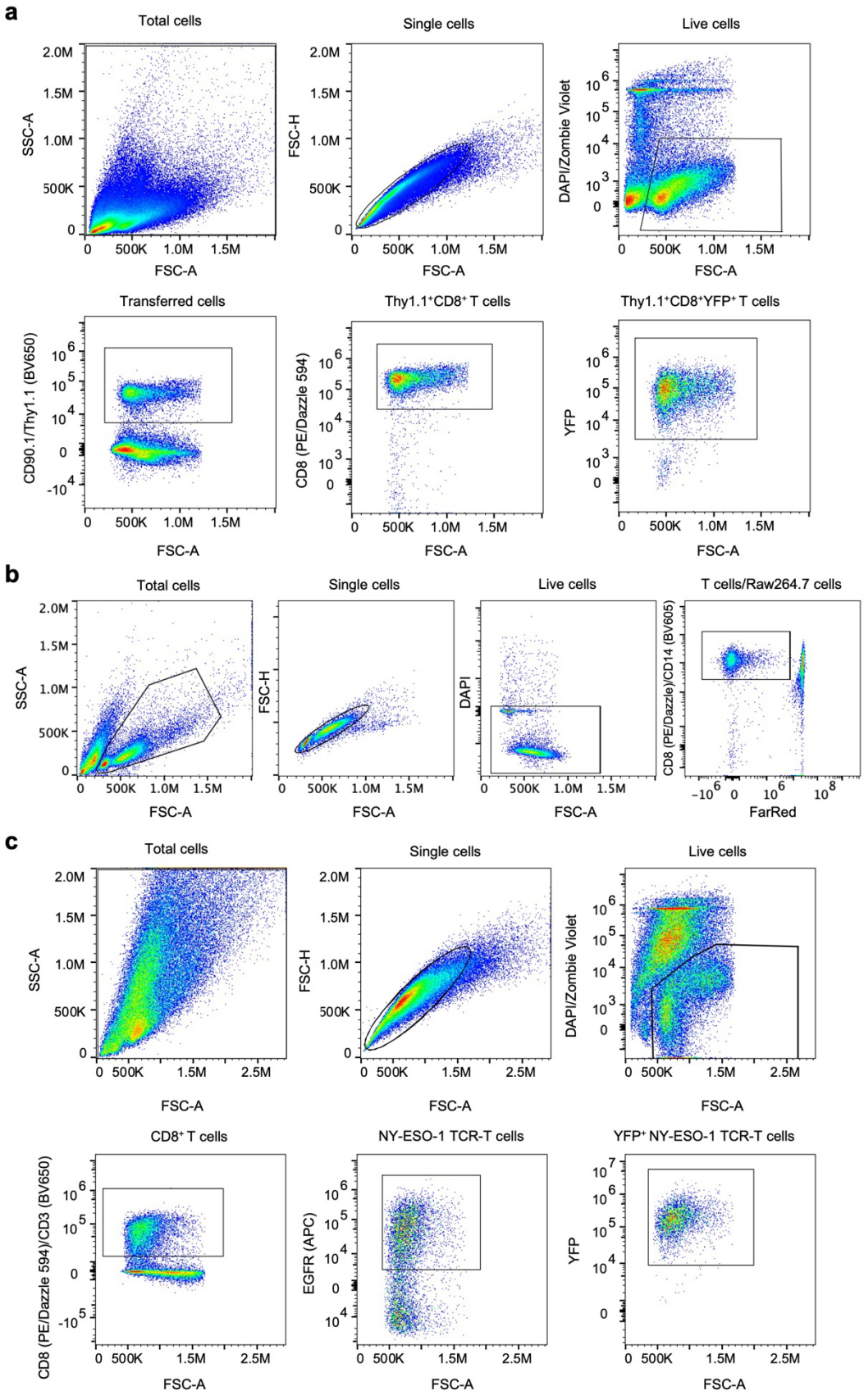
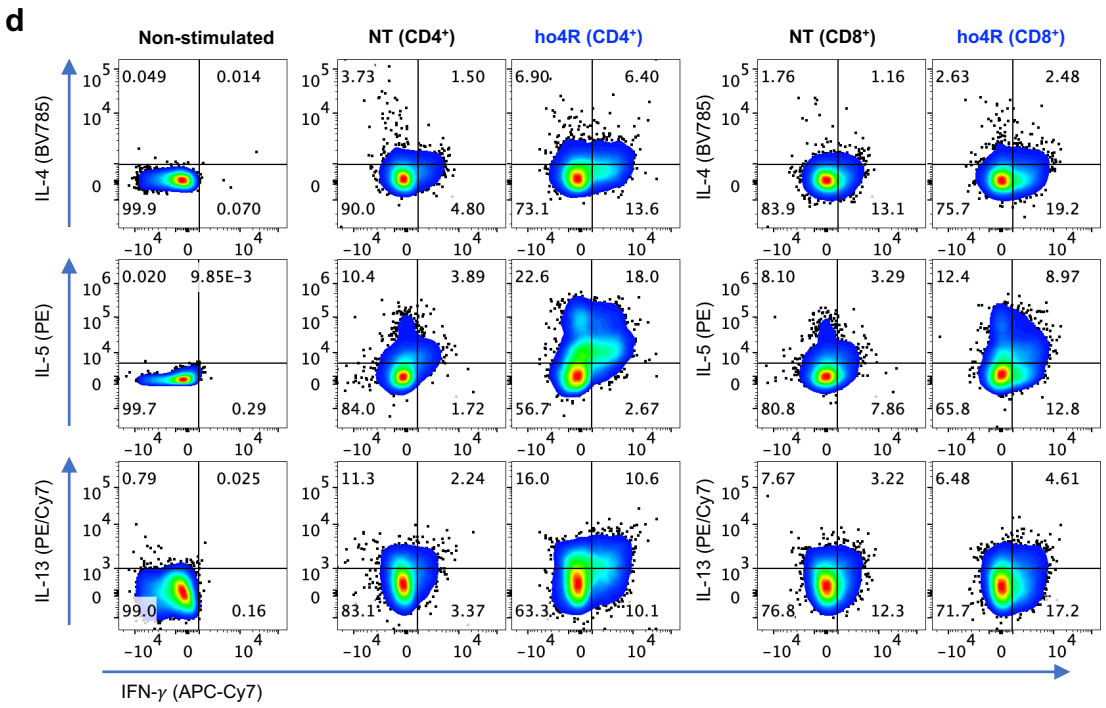

a
Total cells
SSC-A
FSC-A
Single cells
FSC-H
FSC-A
Live cells
DAPI/Zombie Violet
FSC-A
Transferred cells
CD90.1/Thy1.1 (BV650)
FSC-A
Thy1.1+CD8+ T cells
CD8 (PE/Dazzle 594)
FSC-A
Thy1.1+CD8+YFP+ T cells
YFP
FSC-A
b
Total cells
SSC-A
FSC-A
Single cells
FSC-H
FSC-A
Live cells
DAPI
FSC-A
T cells/Raw264.7 cells
CD8 (PE/Dazzle)/CD14 (BV605)
FarRed
c
Total cells
SSC-A
FSC-A
Single cells
FSC-H
FSC-A
Live cells
DAPI/Zombie Violet
FSC-A
CD8+ T cells
CD8 (PE/Dazzle 594)/CD3 (BV650)
FSC-A
NY-ESO-1 TCR-T cells
EGFR (APC)
FSC-A
YFP+ NY-ESO-1 TCR-T cells
YFP
FSC-A



Supplementary Figure 1. Gating strategy.

a. Gating Strategy for in vivo pmel T cell phenotyping in Fig. 3j, Fig. 4g–l, Fig. 4p, Extended Data Fig. 4, and Extended Data Fig. 5h–k. Pmel T cells were gated using Thy1.1$^+$CD8$^+$, and orthogonal-receptor-expressing pmel T cells were gated using Thy1.1$^+$CD8$^+$YFP$^+$.

b. Gating Strategy for antibody-dependent cellular phagocytosis assay in Fig. 4n,o and Extended Data Fig. 6k. Phagocytic activity was measured as FarRed$^+$ cells within CD8$^+$ pmel T cells and CD14$^+$ RAW264.7 cells after gating for single, live cells and excluding FarRed$^+$ target cells.

c. Gating Strategy for in vivo NY-ESO-1 TCR-T cell phenotyping in Fig. 5k, Fig. 6h,i and Extended Data Fig. 11d. NY-ESO-1 TCR-T cells were gated using CD3$^+$EGFR$^+$, or CD8$^+$EGFR$^+$ for exhaustion marker and T_{SCM} analysis. Orthogonal-receptor-expressing NY-ESO-1 TCR-T cells were gated using CD3$^+$EGFR$^+$YFP$^+$, or CD8$^+$EGFR$^+$YFP$^+$ for exhaustion marker and T_{SCM} analysis. For the in vitro assays shown in Fig. 5c–h, Extended Data Fig. 7b,c,g, Extended Data Fig. 8e–j, Extended Data Fig. 10e,f, and Extended Data Fig. 11j–m, the same gating strategy was used to identify NY-ESO-1 TCR-T cells.

d. Gating Strategy for cytokine profiles of ho4R NY-ESO-1 TCR-T cells in Fig. 5c–g, and Extended Data Fig. 7b.