

**Supplementary information for:**

**Synthetic mycobacterial diacyl trehaloses reveal differential recognition by human T cell receptors and the C-type lectin Mincle**

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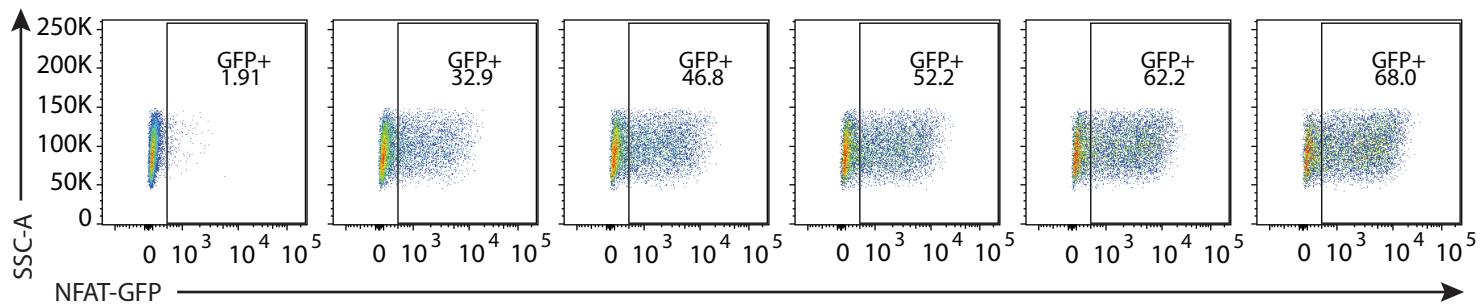
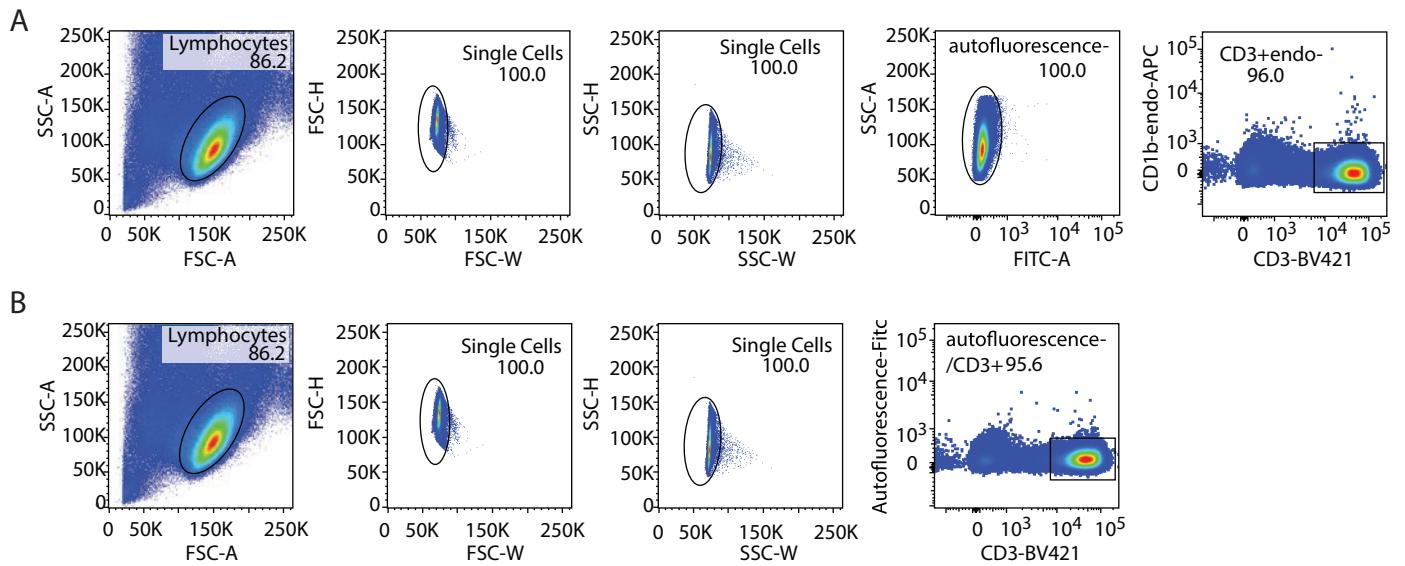


Figure S1. Gating strategy for Figure 1d. NFAT-GFP expression in reporter cells expressing murine Mincle and it signaling subunit Fc $\epsilon$ R $\gamma$  chain, after stimulation with DAT $_3$ .



**Figure S2.** Gating strategy of T cells enriched from PBMC by column purification from healthy donors. (A) Pre-gating on  $CD3^+CD1b\text{-endo}^-$  T cells. (B) Pre-gating on  $CD3^+$  cells.

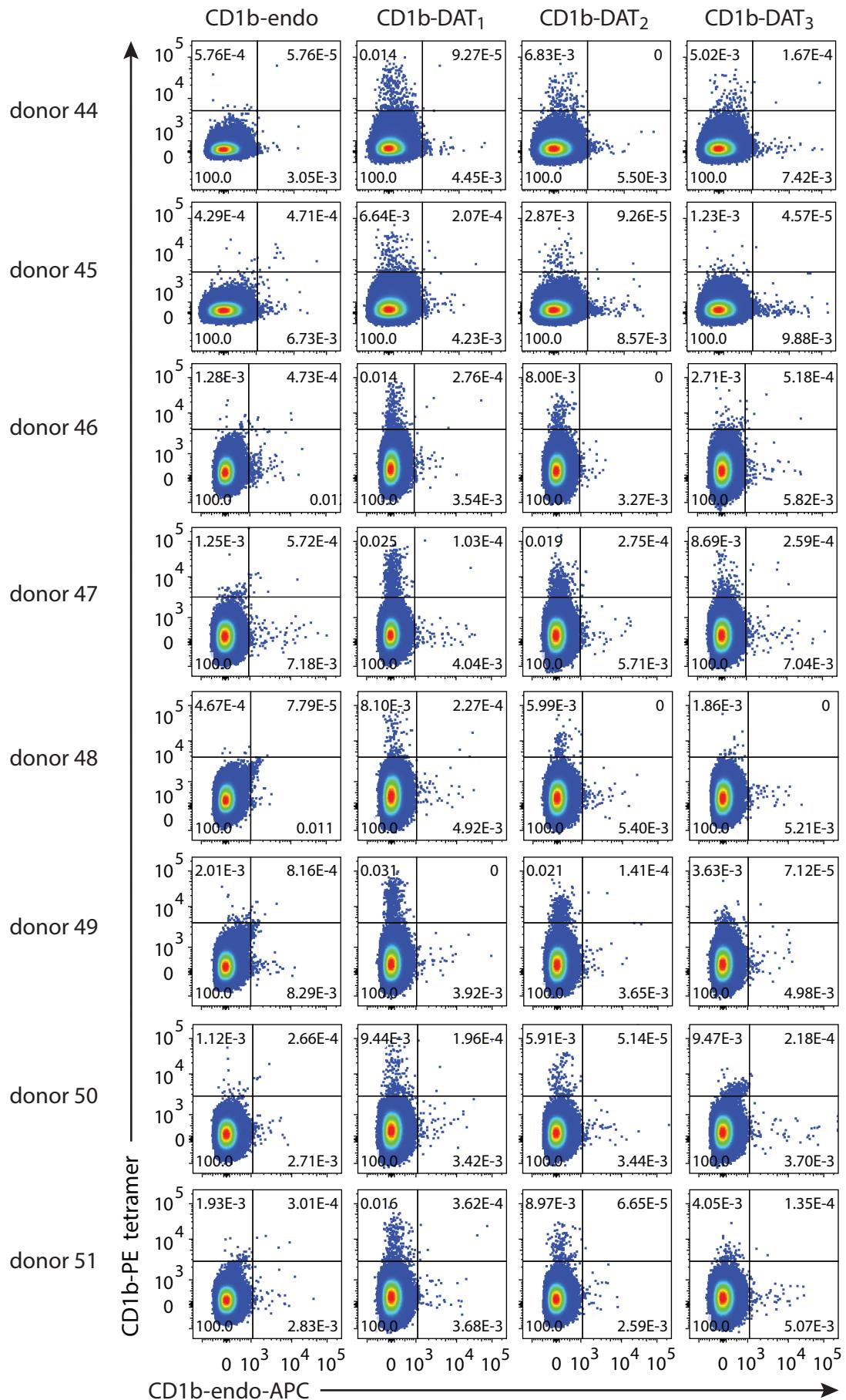


Figure S3. Flow cytometry dot plots of T cells enriched from PBMC by column purification from 8 healthy donors stained with CD1b-endo, -DAT<sub>1</sub>, -DAT<sub>2</sub>, and -DAT<sub>3</sub> tetramers after pre-gating on CD3<sup>+</sup> T cells.

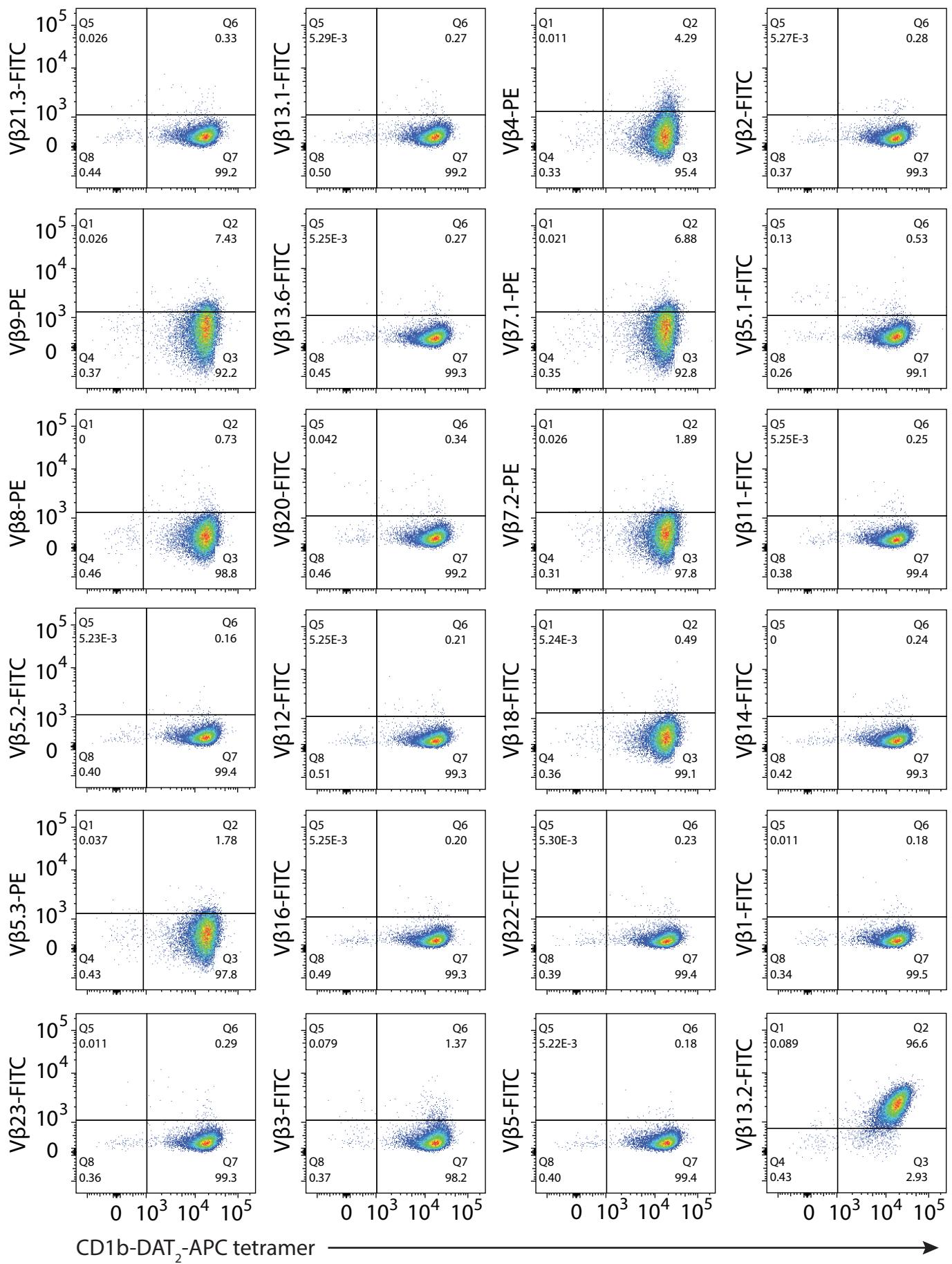


Figure S4. Flow cytometry dot plots of line HD1A stained with CD1b-DAT<sub>2</sub> tetramer and 24 different anti-V $\beta$  antibodies after pre-gating for CD3 $^{+}$  T cells.