PNAS
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Supplementary Information for

Human γδ T cells recognize CD1b by two distinct mechanisms

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Table S1

www.pnas.org/cgi/doi/10.1073/pnas.2010545117

Fig. S1

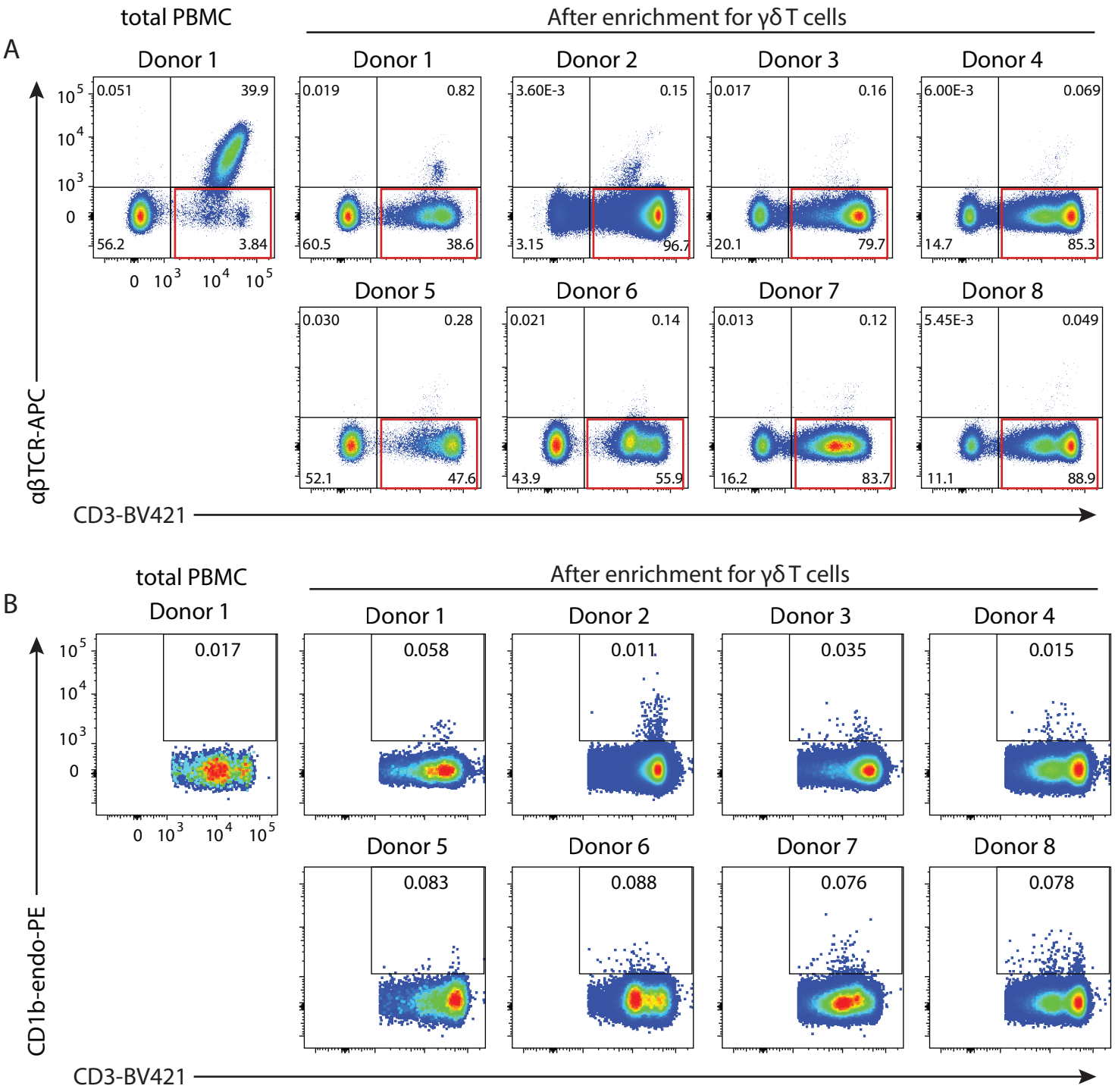

Figure S1. ***Ex vivo*** **staining of γδ T cells. a** Flow cytometry dot plots showing gating on γδ T cells in red rectangle ($CD3^{+}\alpha\beta TCR^{-}$ cells) **b** Flow cytometry dot plots showing CD1b-endo tetramer staining on γδ T cells enriched from PBMC from 8 donors by column purification (gated on $CD3^{+}\alpha\beta TCR^{-}$ cells).

Fig. S2

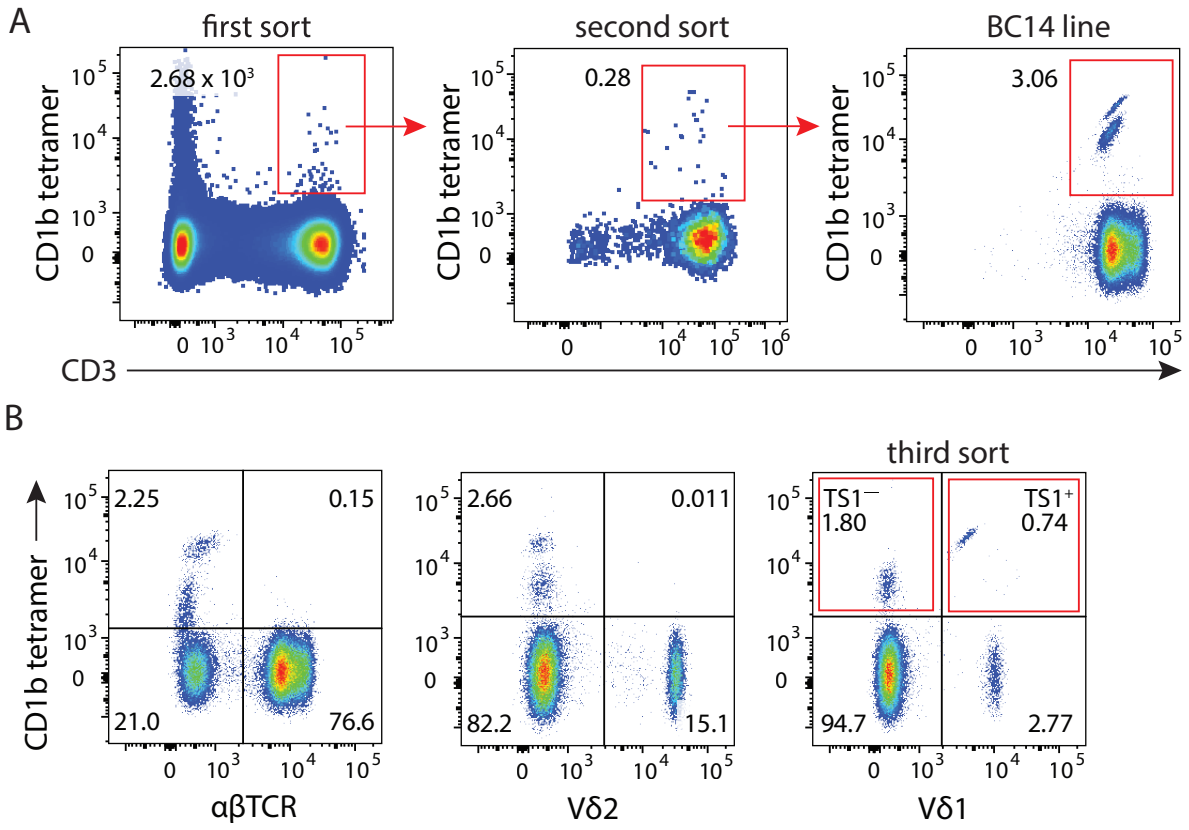

Figure S2. Generation of CD1b-endo recognizing γδ T cell lines. a CD1b-tetramer^{+}CD3^{+} PBMCs from Buffy Coat 14 (BC14) were sorted, followed by expansion in vitro for 14 days and another round of sorting and expansion. The resulting line was called "BC14". Staining of CD3^{-} cells in PBMC is thought to reflect aspecific binding to dead or dying cells or binding to an unknown receptor other than the TCR. **b** Flow cytometry dot plots of line BC14 stained with CD1b tetramer and antibodies against the αβ TCR, Vδ1, or Vδ2. Populations in outlined red areas were sorted generating lines TS1^{+} and TS1^{-}.

Fig. S3

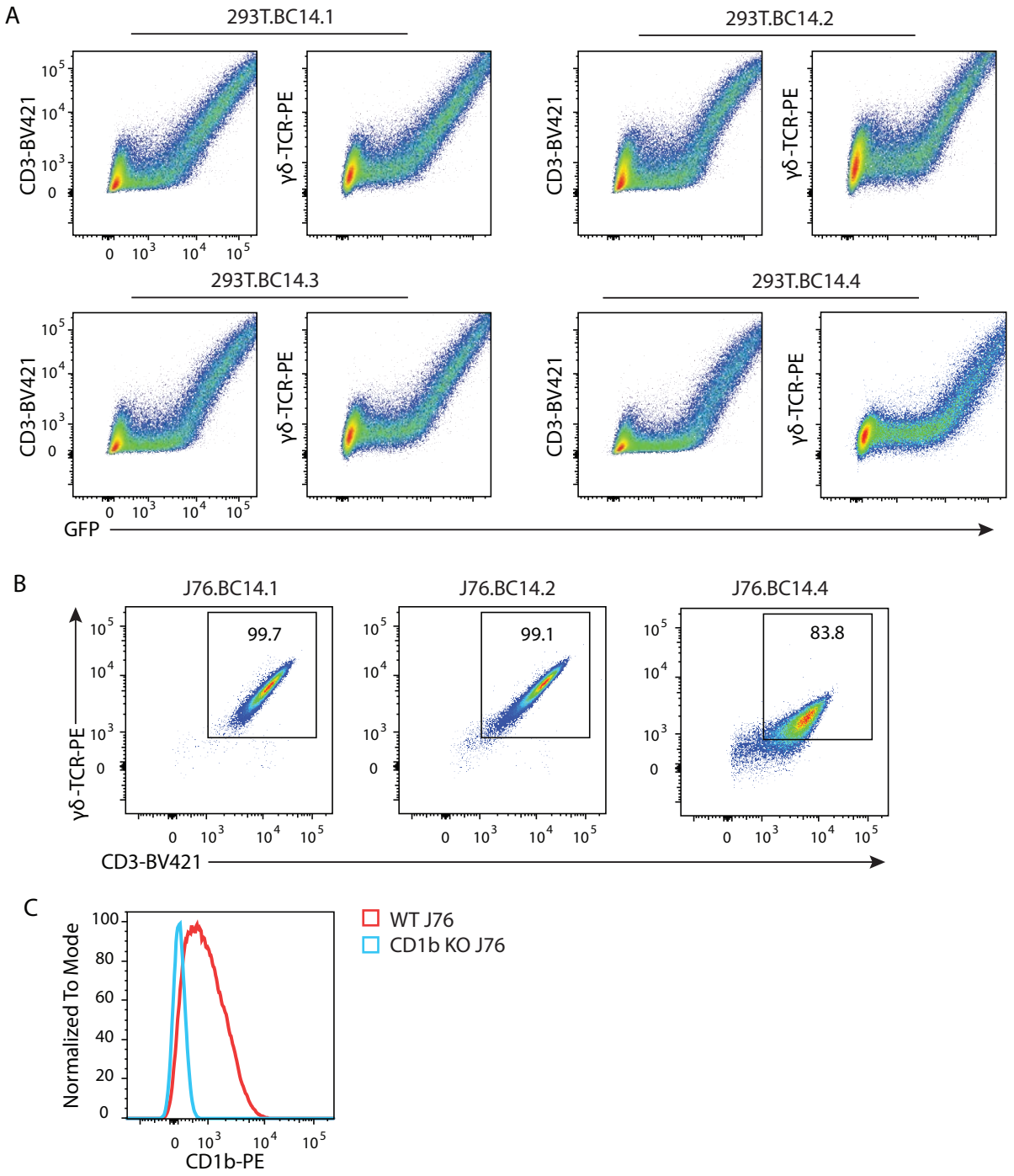

Figure S3. TCR transfer and CD1b knock out. a Flow cytometry dot plots showing TCR and CD3 expression versus GFP expression by 293T cells transfected with γδ TCRs and CD3 subunits. **b** Flow cytometry dot plots showing cell surface expression of CD3 and TCR (using a "pan γδ TCR" antibody) of the stable J76.BC14.1, J76.BC14.2, and J76.BC14.4 cell line. **c** Flow cytometry histograms showing CD1b expression of WT Jurkat76 and Jurkat76 cells where CD1b was knocked out (KO).

Fig. S4

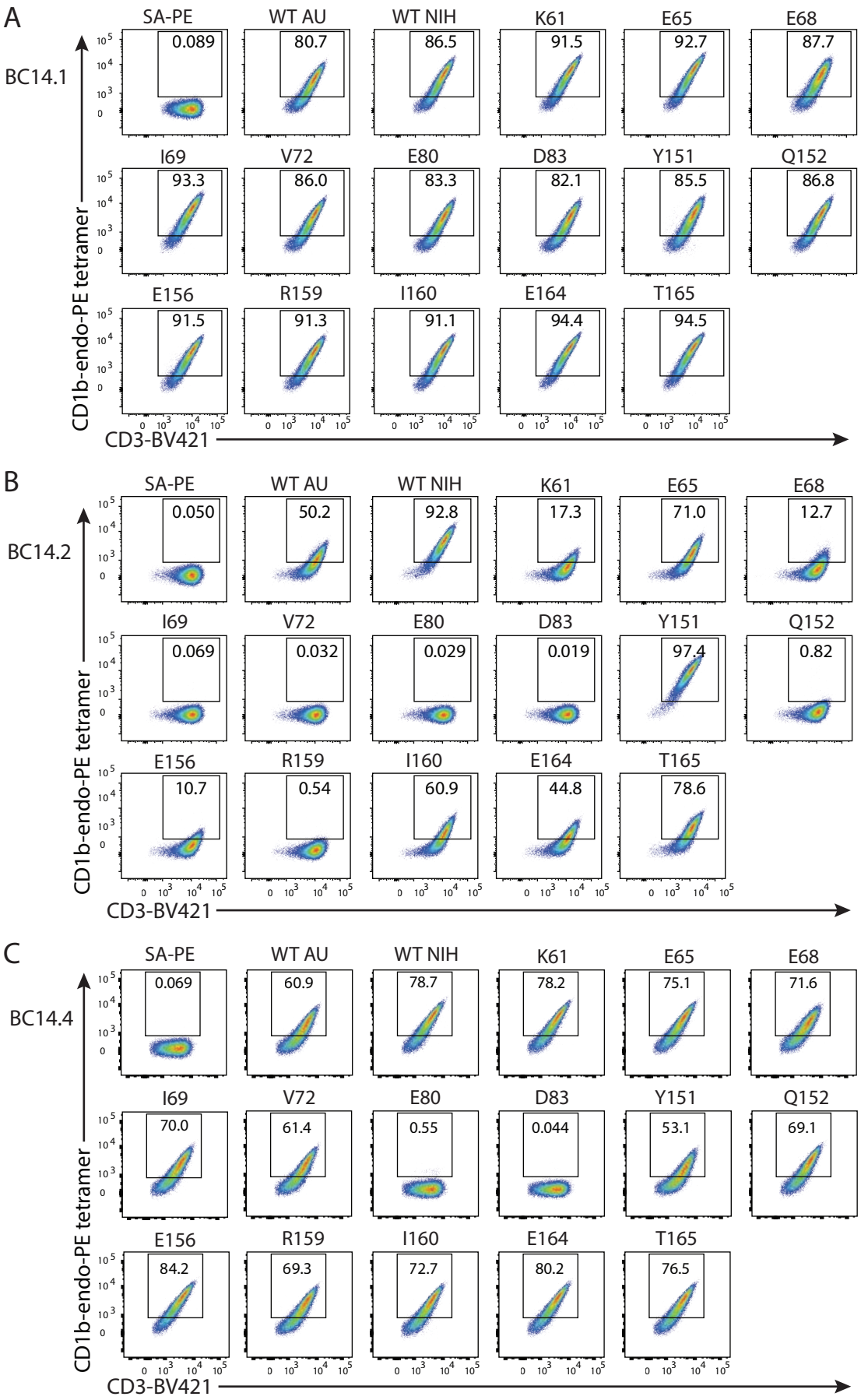

Figure S4. Staining patterns transduced Jurkat76 lines. a-c Flow cytometry dot plots of J76.BC14.1 (**a**), J76.BC14.2 (**b**), and J76.BC14.4 (**c**) showing binding of CD1b-endo tetramers with the indicated point mutations and WT CD1b-endo tetramers. WT AU: WT tetramers created in parallel with the mutant tetramers, using the same expression platform. WT NIH: WT tetramers obtained from the NIH tetramer facility.

Fig. S5

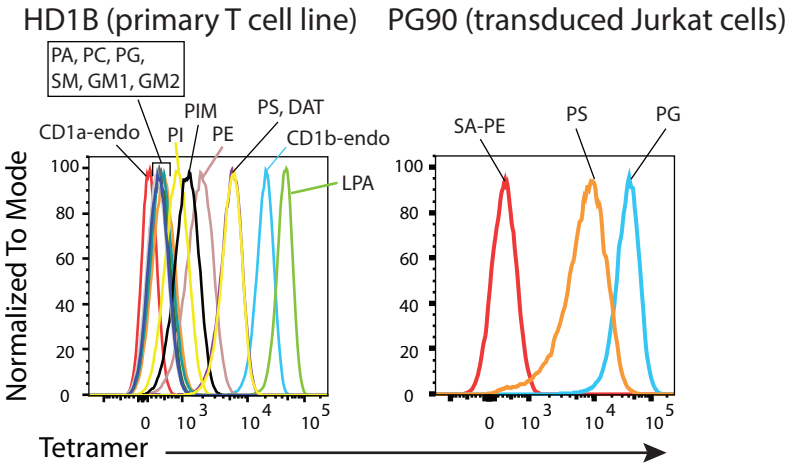

Figure S5. Control staining for tetramer panel treated with self and bacterial lipids. a Flow cytometry histograms showing binding of tested tetramers by cell lines HD1B (T cell line) and PG90 (Jurkat cell line), two αβ TCRs with known lipid reactivity pattern.

Fig. S6

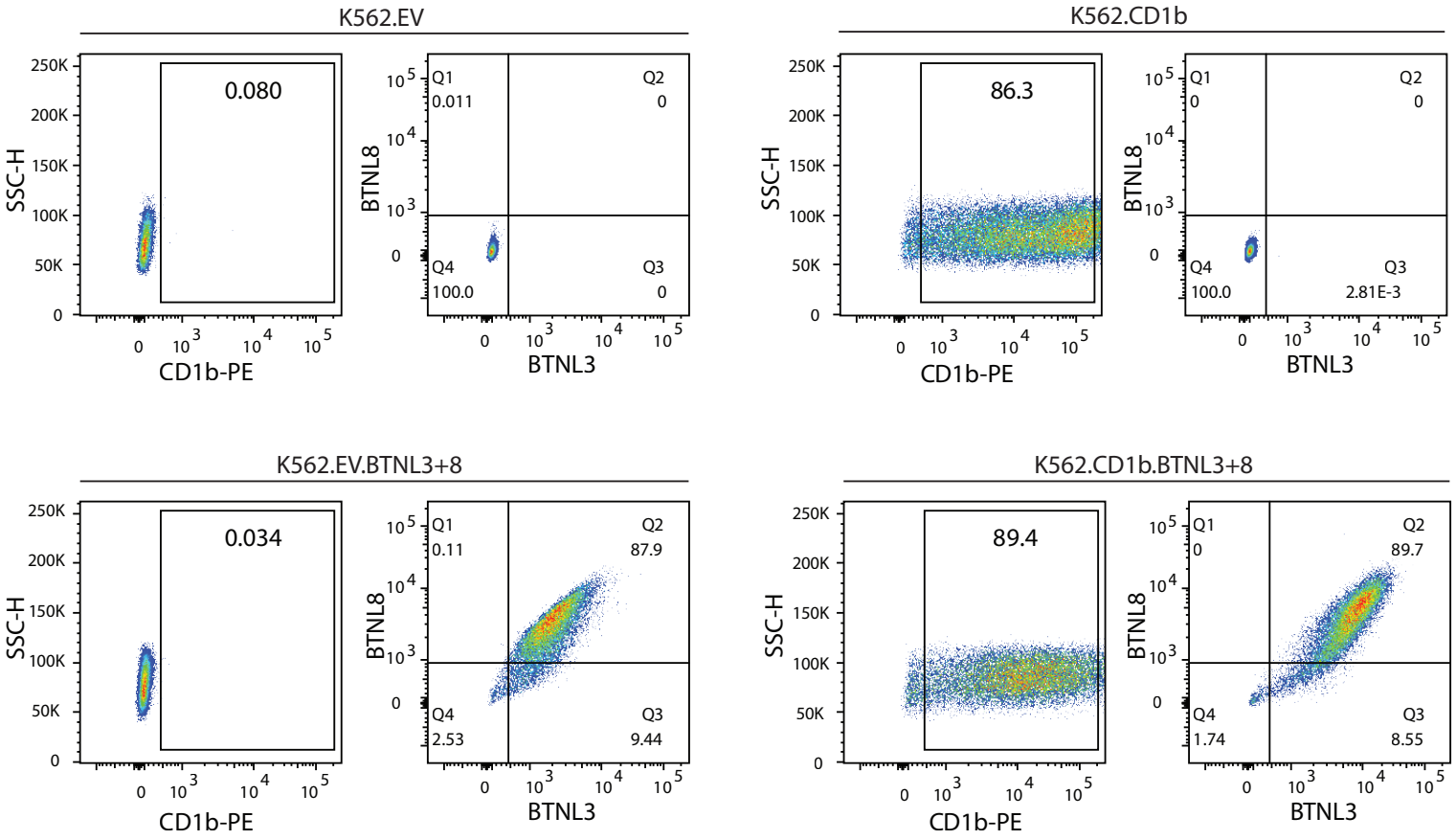

Figure S6. BTNL expression by transduced K652 cells. Flow cytometry dot plots showing expression of CD1b, BTNL3, and BTNL8 by the transduced K562 cell lines.

Fig. S7

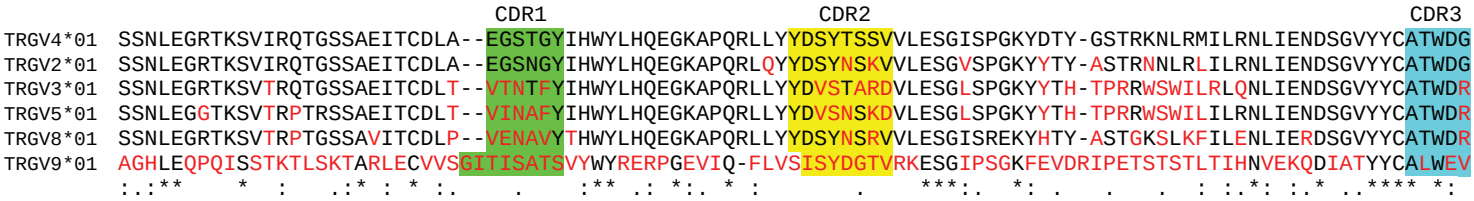

Figure S7. TRGV sequence alignment. Alignment of the amino-acid sequences of human TCR Vγ chains. Red font: divergence from Vγ4; asterisks (*): identical residues; periods (.): semi-conserved residues; colons (:): conserved residues (following the standard ClustalW designations for amino acid comparisons). Colored highlights indicate variable regions (CDR1: green; CDR2: yellow; CDR3: blue).

Supplementary table

Publication	Clone	Restriction + antigen	TCRγ TRGV	TRGJ	CDR3γ
Bai 2012 EJI	DP10.7	CD1d-sulfatide	GV4	GJ1	CATWDEKYYKKLF
Roy 2016 JI	22.4	CD1c-PM	GV2	GJ1	CATWDLIKKLFGSG
Roy 2016 JI	12.9-2	CD1c-PM	GV8	GJ2	CATWDVESYKKLFGSG
Roy 2016 JI	12.16-3	CD1c-PM	GV3	GJP2	CATWDRRSDWIKTFAKG
Uldrich 2013 NI	9C2	CD1d-a-GalCer	GV5	GJ1	CATWDRGNPKTHYYKKLF
Uldrich 2013 NI	6	CD1d-a-GalCer	GV9	GJ1	CALWEARPFYYKKLF

Table S1. Published Vγ sequences from CD1-specific TCRs used for designing modified BC14.1 TCRs