

SUPPLEMENTAL MATERIAL

Reantragoon et al., <http://www.jem.org/cgi/content/full/jem.20112095/DC1>

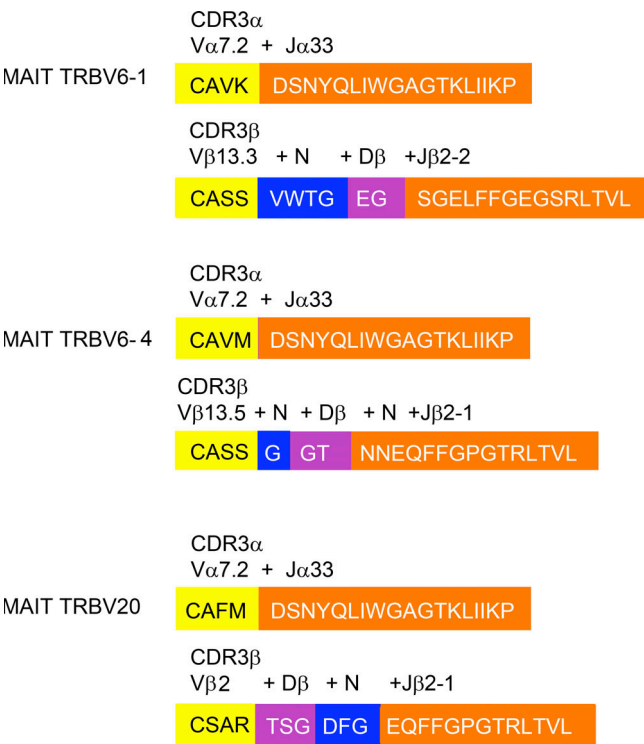


Figure S1. Comparison of the CDR3 amino acid sequences of MAIT TCRs using the β chains TRBV6-1, TRBV6-4, and TRBV20. Amino acid sequences of the Vα7.2 and Vβ (yellow), Jα and Jβ (orange), Dβ (purple), and N-region (blue) gene segments of the three MAIT TCR α and β chains are shown.

		CDR1	CDR2
Human	Vb13.3	MNHNS	YSASEGT
Human	Vb13.5	MRHNA	YSNTAGT
Human	Vb2	DFQATT	TSNEGSKA
Murine	Vb6	FNHDT	YSITEND
Murine	Vb8.1	NNHDY	YSYVADS

Figure S2. Comparison of the CDR1 and CDR2 regions of Vβ chains of human and mouse MAIT TCRs. The amino acid sequences of the CDR1 and CDR2 loops of human TRBV6-1 (Vβ13.3), TRBV6-4 (Vβ13.5), and TRBV20 (Vβ2), as well as murine TRBV19 (Vβ6) and TRBV13-3 (Vβ8.1), are indicated. The murine CDR1β consensus sequence Asn-His-Asp (NHD), and the human and murine CDR2β junctional consensus sequence Tyr/Thr-Ser (YS or TS) are indicated by light blue boxes.

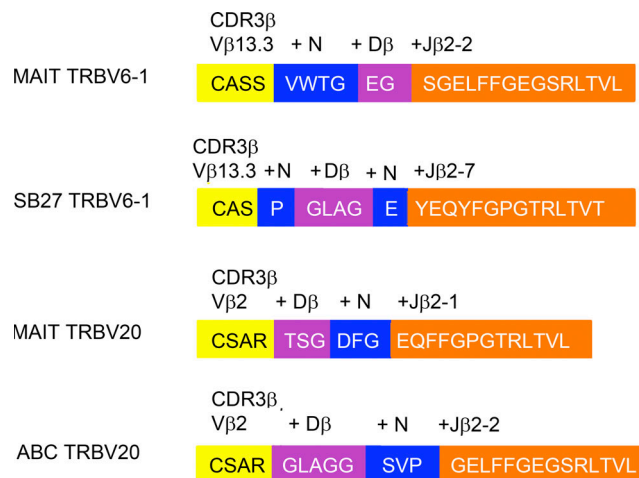


Figure S3. Comparison of the CDR3 β amino acid sequences of four TCR β chains. Amino acid sequences of the V β (yellow), J β (orange), D β (purple), and N-region (blue) gene segments of two MAIT TCR β chains: MAIT TRBV6-1 (V β 13.3) and TRBV20 (V β 2); and two conventional, non-MAIT TCR β chains: SB27 TRBV6-1 (V β 13.3) and ABC TRBV20 (V β 2) are shown.

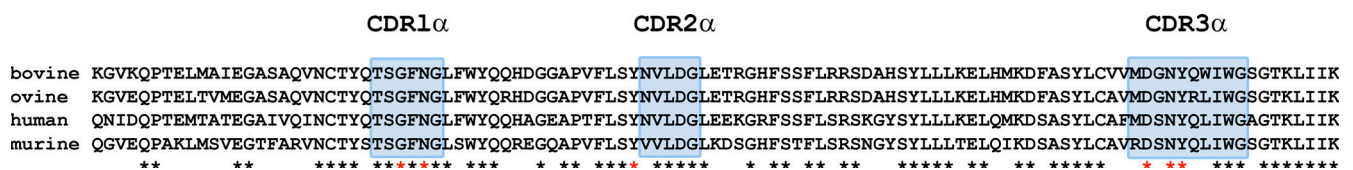


Figure S4. Alignment of MAIT V α 7.2/J α 33 amino acid sequences from four species. Bovine, ovine, human, and murine MAIT V α 7.2/J α 33 amino acid sequences have been aligned. The CDR1 α , CDR2 α , and CDR3 α regions have been highlighted with light blue boxes. Residues conserved across species are indicated with an asterisk (*). Residues that are conserved across species and that have an impact on recognition of bacterial Ags are indicated by a red asterisk. Adapted from Golfinch et al. (2010).

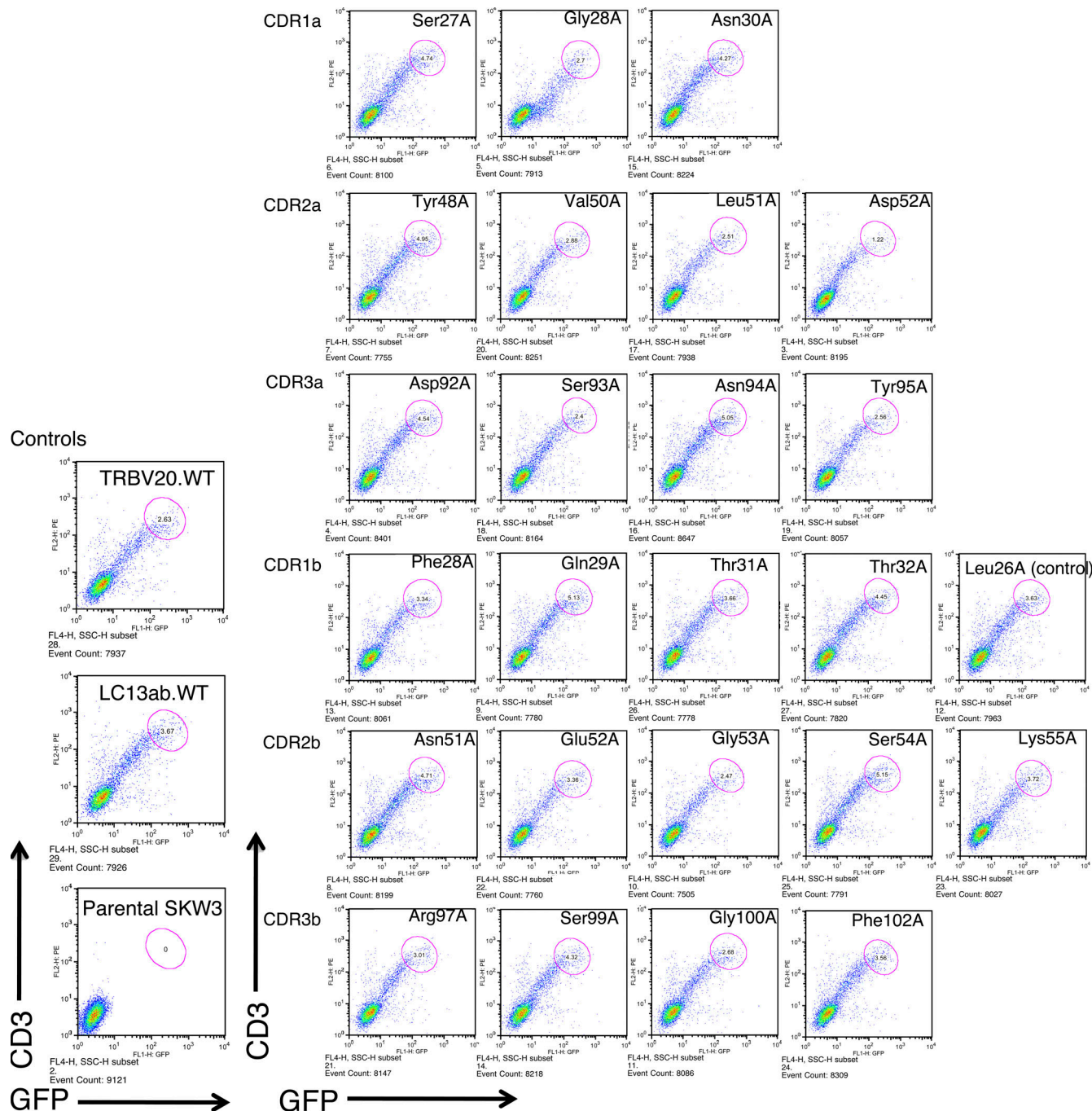


Figure S5. Gating strategy for CD69 measurement. Flow histograms of SKW3 cells transduced with genes encoding (left) wild-type MAIT TRBV20 (MAIT.TRBV20.WT), control LC13 (LC13 $\alpha\beta$), or untransduced (Parental SKW3); or (right) MAIT TRBV20 mutants within the CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β loops as indicated. Flow histograms show staining of CD3-PE on the y axis, and coexpressed GFP on the x axis. Shown are primary transduced cell lines, with gates placed to indicate cells that are matched for CD3-PE and coexpressed GFP, for which CD69 up-regulation was measured in activation experiments. Activation experiments were also performed using SKW3-transduced cell lines sorted by flow cytometry for high CD3- and GFP-expression where similar gating strategies were used in activation experiments.

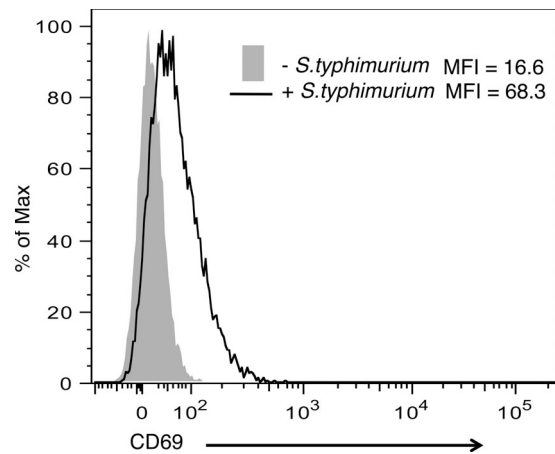


Figure S6. CD69 measurement of gated SKW3 cells. Flow histogram indicating CD69 staining of SKW3 MAIT cells gated for high CD3 and GFP expression after co-incubation with C1R cells either uninfected ($-S. typhimurium$) or infected ($+S. typhimurium$).

REFERENCE

Goldfinch, N., P. Reinink, T. Connelley, A. Koets, I. Morrison, and I. Van Rhijn. 2010. Conservation of mucosal associated invariant T (MAIT) cells and the MR1 restriction element in ruminants, and abundance of MAIT cells in spleen. *Vet. Res.* 41:41.