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Supplementary Information for

MAIT Cells Are Major Contributors to the Cytokine Response in Group A Streptococcal Toxic Shock Syndrome

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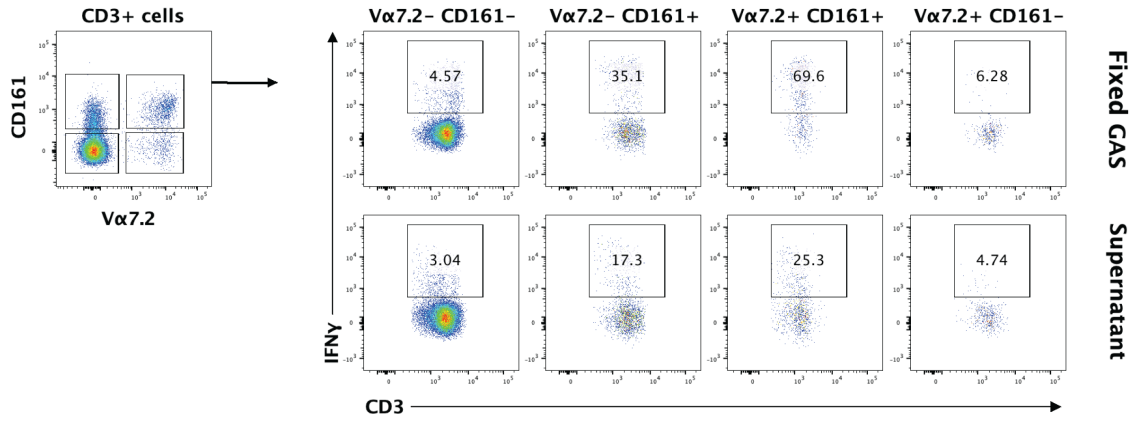


Fig. S1. MAIT cells are the main IFN γ producing subset in response to fixed GAS 2006 or GAS 2006 supernatant. PBMC were stimulated with GAS for 24 hours and the frequencies of IFN γ + cells among CD3+ subsets were assessed by flow cytometry. The figure shows one representative donor.

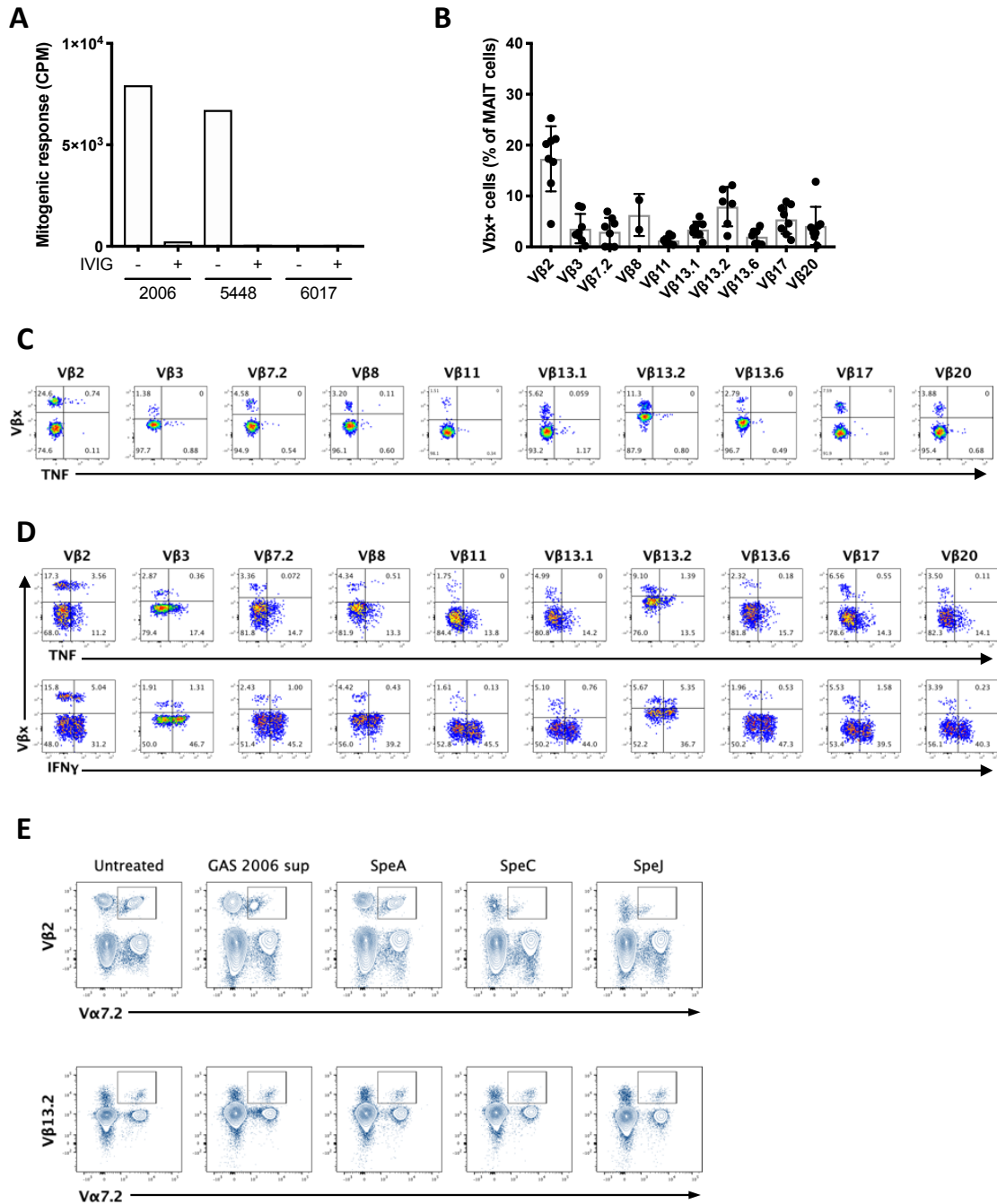


Fig. S2. Early TNF production by MAIT cells in response to GAS supernatant is Vβ2-specific. (A) Superantigenic activity of GAS (2006 and 5448) and GGS (6017) supernatants towards human PBMCs after 72 hours of stimulation in the presence or absence of 1 mg/ml IVIG. Mitogenic response of PBMCs measured as counts per minute (CPM). (B) Mean ± SD frequencies of Vβx+ MAIT cells in unstimulated cultures assessed by flow cytometry. (C-D) PBMC were stimulated with GAS 2006 supernatant for (C) 8 hours or (D) 24 hours. FACS plots show cytokine production in Vβx+ and Vβx- MAIT cells in one representative donor. (E) Vα7.2 expression among Vβ2 or Vβ13.2 MAIT cells after 8 hours of stimulation with superantigens or GAS supernatant.

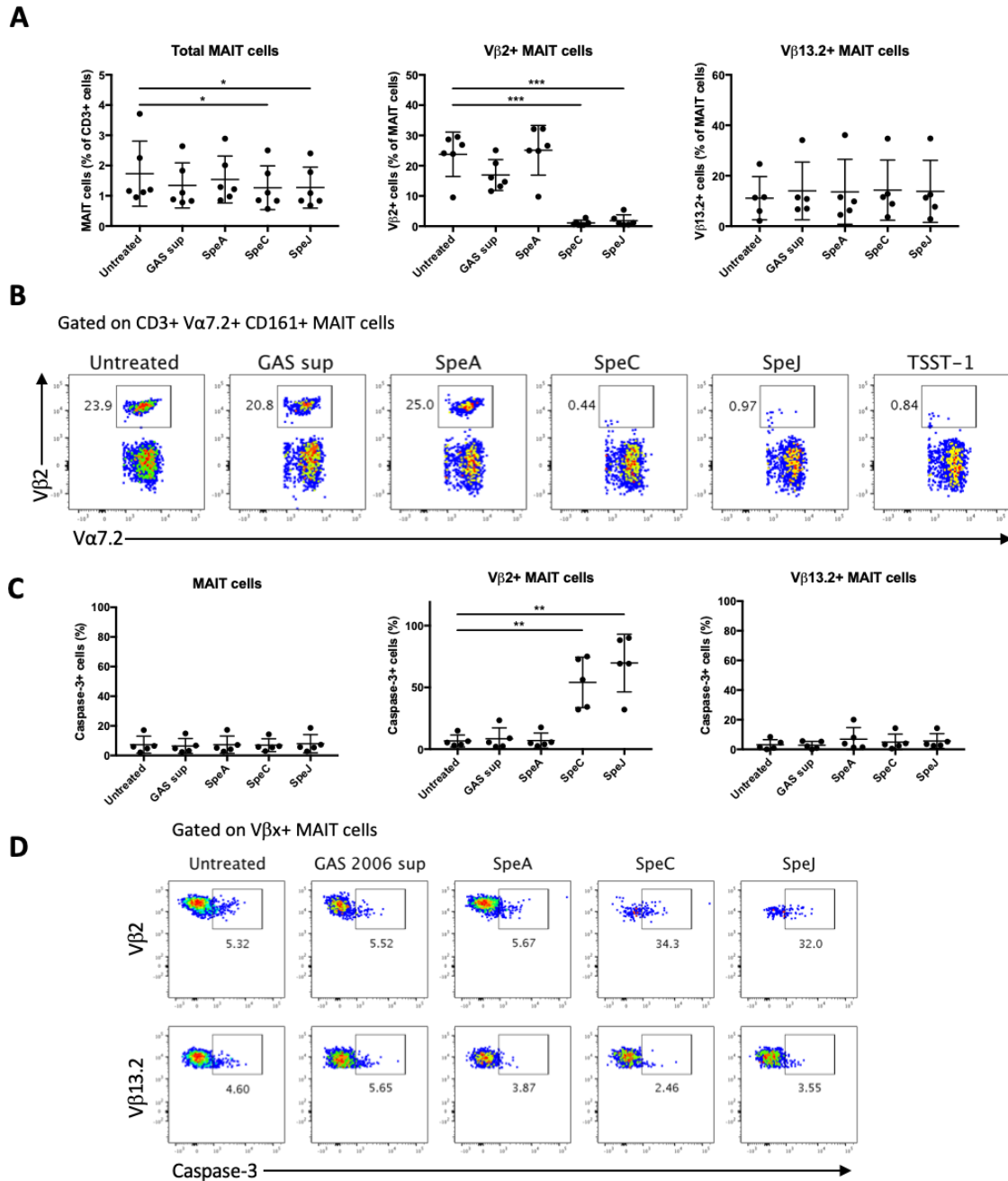


Fig S3. Vβ2+ MAIT cells are lost after stimulation with Vβ2-specific superantigens. (A-B) PBMC were stimulated with GAS 2006 supernatant or recombinant superantigens for 8 hours and total MAIT cell, Vβ2+ MAIT cell, and Vβ13.2+ MAIT cell frequencies were assessed by flow cytometry. (A) Mean frequencies \pm SD of 6 donors. (B) Representative plots of Vβ2+ MAIT cells in one donor. (C-D) PBMC were stimulated with GAS 2006 supernatant or recombinant superantigens for 8 hours and caspase-3 expression was assessed in total MAIT cells, Vβ2+ MAIT cells, and Vβ13.2+ MAIT cells by flow cytometry. (C) Mean frequency \pm SD of caspase-3+ cells in 5 donors. (D) Caspase-3 expression in one representative donor. (A and C) Paired *t* test was used to detect significant differences between samples. ****P* < 0.001; ***P* < 0.01; **P* < 0.05

Table S1. Antibodies used for flow cytometry.

Antibody name	Clone	Antibody source
anti-Caspase-3 BV650 anti-CD3 FITC anti-CD25 BUV395 anti-CD56 BUV737 anti-CD69 AF700 anti-CD161 PECy5 anti-HLA-DR APC-H7 anti-IFN γ APC anti-Ki67 BUV395 anti-TNF PECy7	C92-605 SK7 2A3 NCAM16.2 FN50 DX12 L243 25723.11 B56 MAb11	BD Biosciences
anti-CD3 BV650 anti-CD4 BV711 anti-CD8 BV570 anti-CD38 BV421 anti-CD45 AF700 anti-IFN γ BV785 anti-IL-17 BV421 anti-LAG-3 AF647 anti-PD-1 BV785 anti-V α 7.2 PE, APC, PECy7	OKT3 OKT4 RPA-T8 HIT2 HI30 4S.B3 BL168 11C3C65 EH12.2H7 3C10	BioLegend
anti-CD69 ECD anti-V β 2 PE anti-V β 3 FITC anti-V β 7.2 PE anti-V β 8 PE anti-V β 11 PE anti-V β 13.1 PE anti-V β 13.2 PE anti-V β 13.6 PE anti-V β 17 PE anti-V β 20 PE	TP1.55.3 MPB2D5 CH92 Zizou4 56C5.2 C21 IMMU 222 H132 JU74.3 E17.5F3.15.13 ELL1.4	Beckman Coulter
LIVE/DEAD® Fixable Aqua, Green, and Near-IR Dead Cell Stain Kits streptavidin Qdot 585		Invitrogen / Life Technologies
Anti-TIM-3 AF488	344823	R&D Systems