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Supplementary Information for

The CD4$^-$CD8$^-$ MAIT cell subpopulation is a functionally distinct subset developmentally related to the main CD8$^+$ MAIT cell pool

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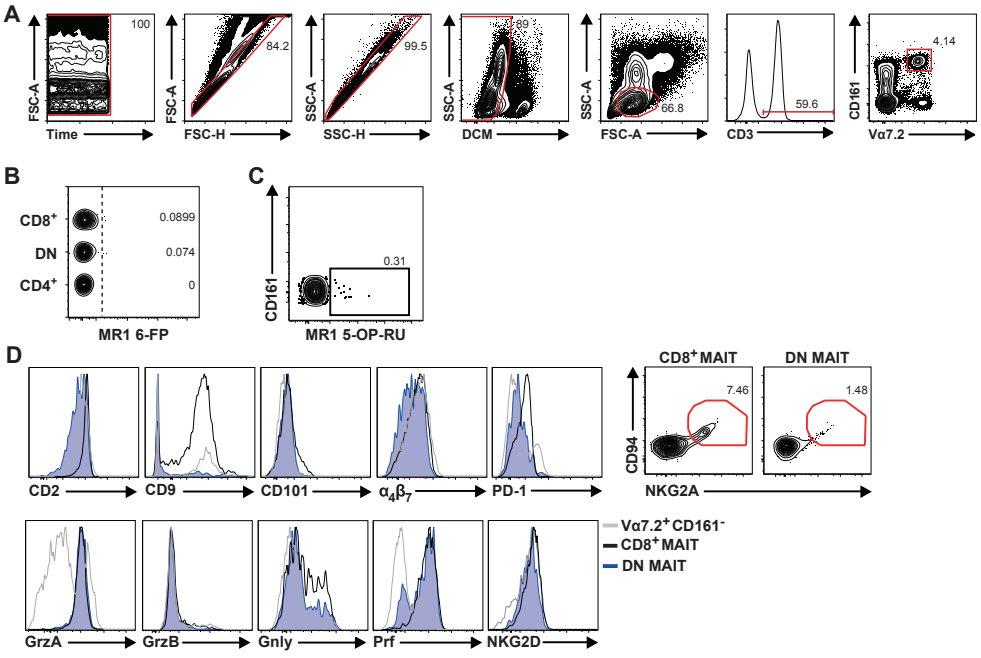

Fig. S1. Flow cytometry identification of MAIT cells. (A) Representative example of the gating strategy to identify CD161^{hi}Vα7.2^{+} MAIT cells. (B) Representative example of the MR1 6-FP tetramer staining in CD8^{+}, DN, and CD4^{+} CD161^{hi}Vα7.2^{+} MAIT cells. Data are representative from 10 donors. (C) Representative FACS plot of MR1 5-OP-RU tetramer staining on CD161^{-}Vα7.2^{+} non-MAIT cells. (D) Representative FACS plot of the expression of CD2, CD9, CD101, α4β7, PD-1, CD94, NKG2A, NKG2D, GrzA, GrzB, Gnly, and Prf on CD8^{+} and DN MAIT cells. CD161^{-}Vα7.2^{+} non-MAIT T cells were used as controls. Grz; Granzyme, Gnly; granulysin, Prf, perforin.

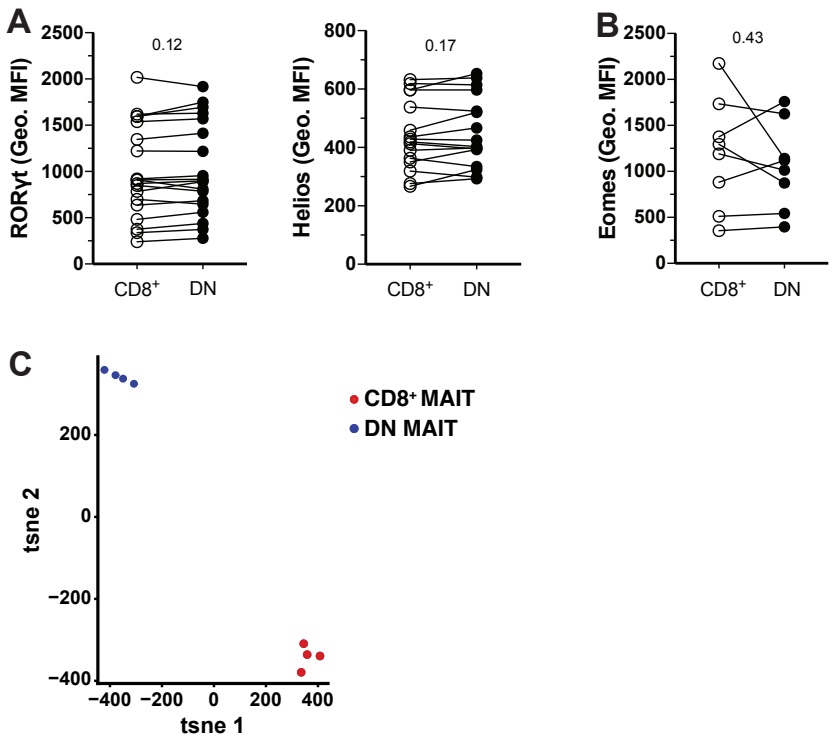

Fig. S2. T cell transcription factor expression. The expression of (A) RORγt and Helios on $CD8^+$ and DN MAIT cells from peripheral blood, and (B) Eomes on $CD8^+$ and DN MAIT cells from the endometrium. (C) t-SNE analysis of $CD8^+$ and DN MAIT cells based on their transcriptional profiles. Data are from 16-19 (A) and 8 (B) donors. Lines in the graphs represent individual donors. The paired *t*-test was performed to detect significant differences between paired samples.

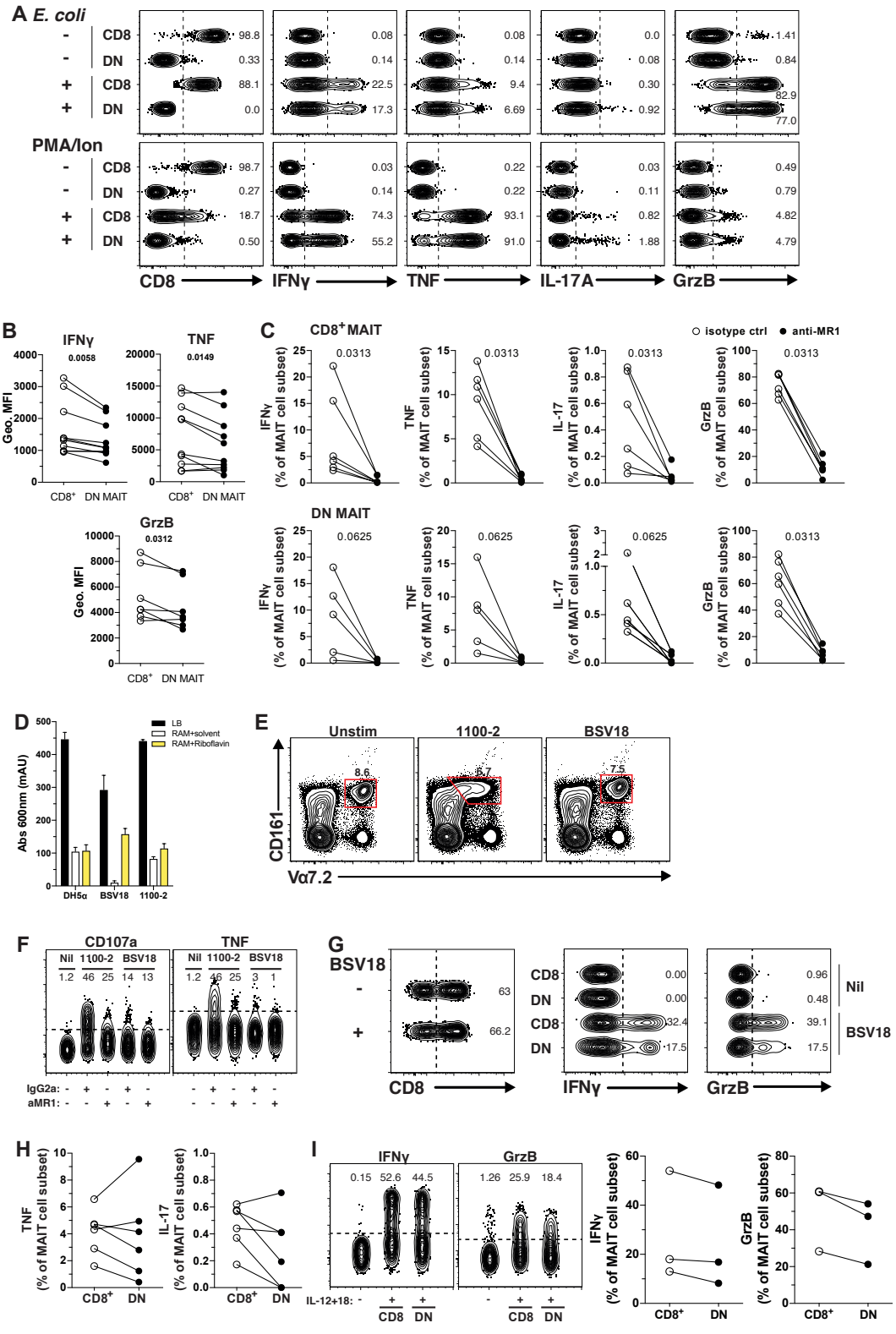
A E. coli
PMA/Ion
CD8
DN
IFNγ
TNF
IL-17A
GrzB
B
C
CD8+ MAIT
DN MAIT
isotype ctrl
anti-MR1
Geo. MFI
IFNγ (% of MAIT cell subset)
TNF (% of MAIT cell subset)
IL-17 (% of MAIT cell subset)
GrzB (% of MAIT cell subset)
D
LB
RAM+solvent
RAM+Riboflavin
Abs 600nm (mAU)
DH5α
BSV18
1100-2
E
Unstim
1100-2
BSV18
CD161
Vα7.2
F
CD107a
TNF
Nil
1100-2
BSV18
IgG2a:
aMR1:
G
BSV18
Nil
H
I

Fig. S3. Expression of CD8, cytokines and cytolytic molecules after MAIT cell stimulation. (A) Representative example of the expression of CD8, IFNγ, TNF, IL-17, and GrzB in FACS-sorted $CD8^+$ and DN MAIT cell populations stimulated with *E. coli* for 24 h (n=7) or PMA/ionomycin for 6 h (n=10). (B) Geometric mean fluorescence intensity (Geo. MFI) of IFNγ, TNF, and GrzB staining in FACS-sorted $CD8^+$ and DN MAIT cells positive for these molecules (IFNγ, TNF after PMA/ionomycin and GrzB after *E. coli* stimulation) (C) Percentages of FACS-sorted $CD8^+$ and DN MAIT cells expressing IFNγ, TNF, IL-17, and GrzB after *E. coli* stimulation and in the presence of MR1-blocking mAb or isotype ctrl. Data are from 7 (A), 7 to 10 (B), and 5 to 6 (C) donors. Lines represent individual donors. The Wilcoxon's signed-rank test was performed to detect significance. (D) The growth of *E. coli* strains DH5α, riboflavin-auxotroph BSV18, and its congenic riboflavin-autotroph 1100-2 in lysogeny broth (LB), riboflavin assay medium (RAM) with and without riboflavin supplementation. Growth was determined by turbidity measurement at 600 nm. Data are from at least 3 independent experiments. Bar graphs and error bars represent mean and SD. (E) The expression of Vα7.2 and CD161 on MAIT cells following stimulation with *E. coli* strains 1100-2 and BSV18. Representative FACS plots from 9 independent donors are shown. (F) MAIT cell degranulation (CD107a) and TNF production following stimulation with *E. coli* strains 1100-2 and BSV18 in the presence of isotype control (IgG2a) or anti-MR1. (G) CD8 expression on MAIT cells following stimulation with *E. coli* BSV18. Representative FACS plots from 6-9 individual donors are shown. (H) TNF and IL-17 production following stimulation with *E. coli* BSV18. Data are from at least 5 independent donors represented by individual lines. (I) IFNγ and GrzB expression by $CD8^+$ and DN MAIT cells following IL-12 and IL-18 stimulation. Data are from 3 independent donors represented by individual lines.

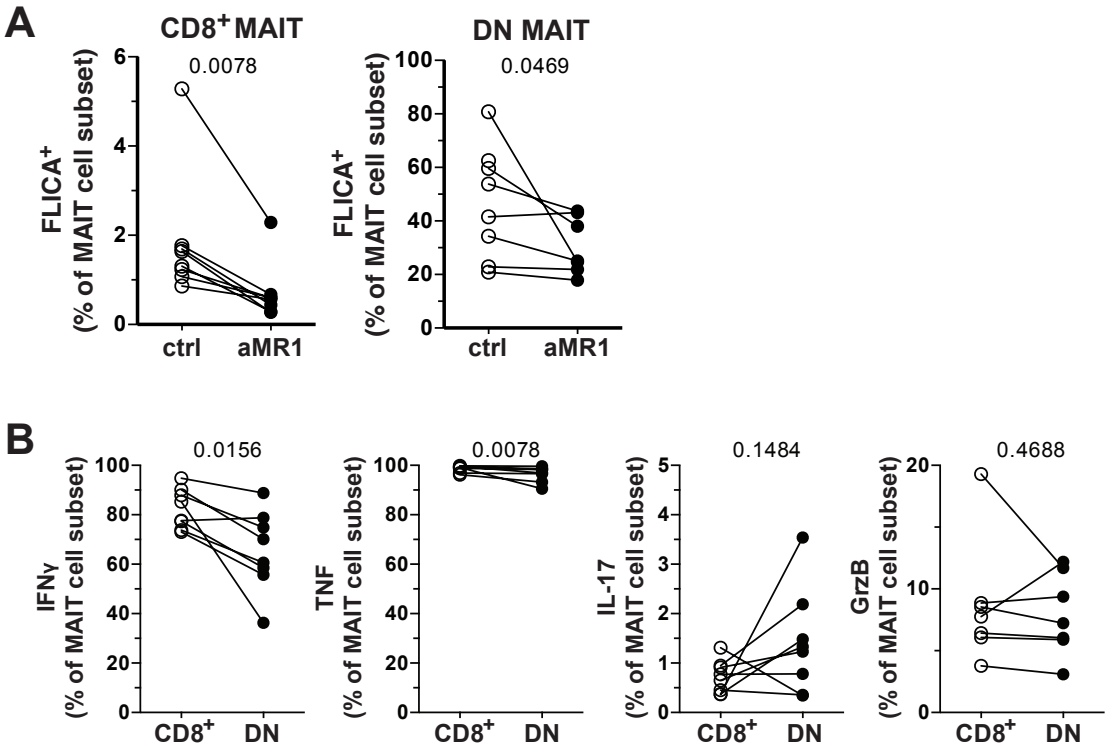

Fig. S4. MR1-dependency of CD8$^+$ and DN MAIT cell apoptosis and functionality of non-apoptotic CD8$^+$ and DN MAIT cells. (A) Percentage of FLICA$^+$ FACS-sorted CD8$^+$ and DN MAIT cells after *E. coli*-stimulation and in the presence of MR1-blocking mAb or isotype ctrl. (B) Percentages of DCM$^-$FLICA$^-$ FACS-sorted CD8$^+$ and DN MAIT cells expressing IFNγ, TNF, IL-17, and GrzB after 6 h PMA/ionomycin stimulation. Data are from 7 to 8 donors. Lines represent individual donors. The Wilcoxon's signed-rank test was used to detect significant differences between paired samples. aMR1, anti-MR1; ctrl, control.

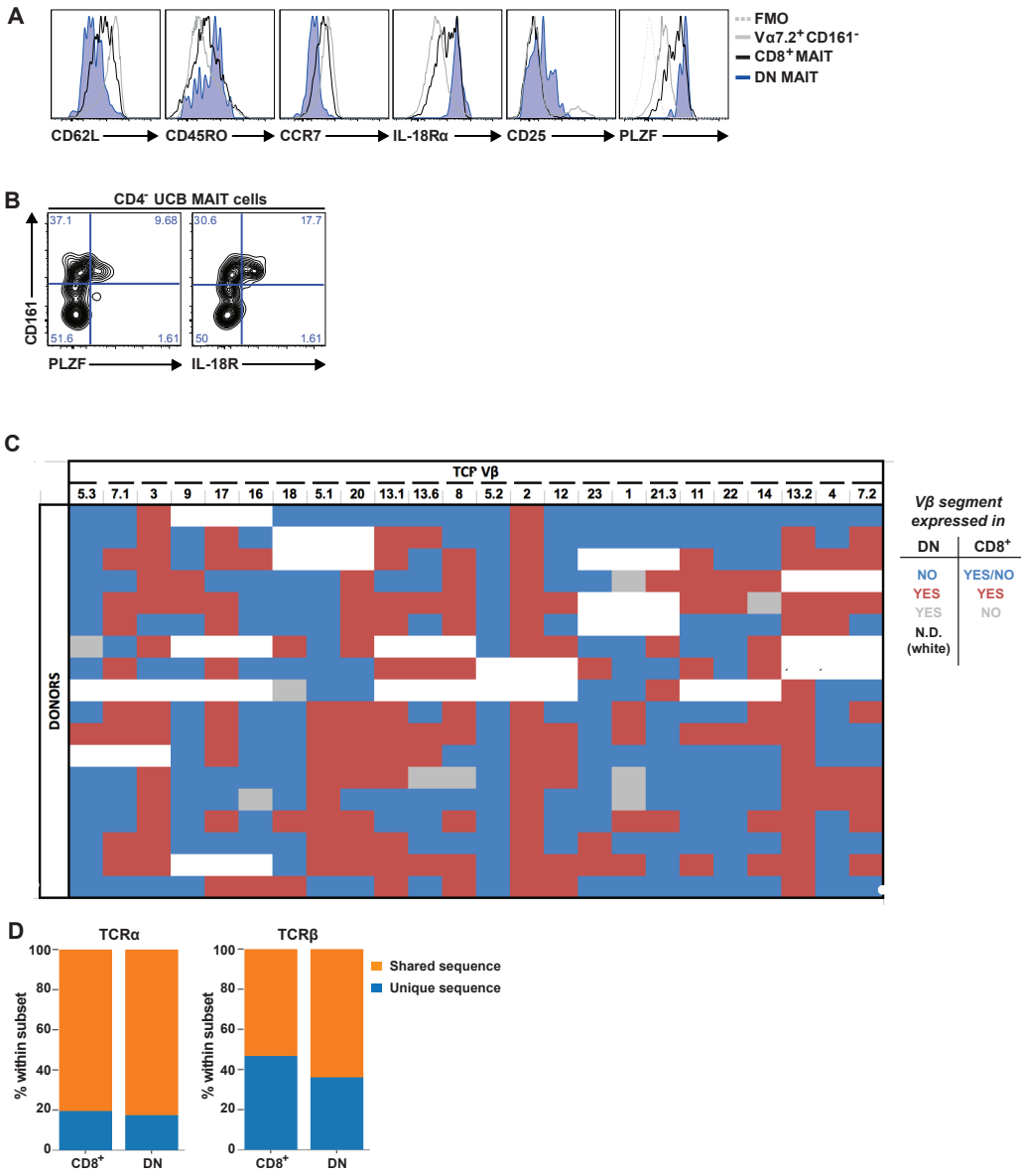

Fig. S5. PLZF and IL-18R expression in UCB MAIT cells, and individual TCR Vβ analysis. (A) Representative FACS plots of CD62L, CD45RO, CCR7, IL-18Rα, CD25, and PLZF in fetal splenic $CD8^+$ and DN MAIT cells. The expression in $CD161^-Vα7.2^+$ cells and the FMO control are also shown. Representative FACS plots from at least 5 donors are shown. (B) Representative FACS staining for CD161, PLZF, and IL-18R expression on umbilical cord blood (UCB) $CD4^-$ MAIT cells (n=6). (C) Heat map showing the TCR Vβ segments that are not expressed in DN MAIT cells independently of whether they are expressed or not in $CD8^+$ MAIT cells from the same donor (blue), that are expressed in DN and $CD8^+$ MAIT cells from the same donor (red), and that are expressed in DN but not in $CD8^+$ MAIT cells of the same donor (gray). N.D., not determined (blank). Data are from 18 donors. (D) The proportion of shared and unique

TCRα and TCRβ sequences obtained from the RNAseq analysis of FACS-sorted $CD8^+$ and DN MAIT cells. The data are an example from 4 donors.

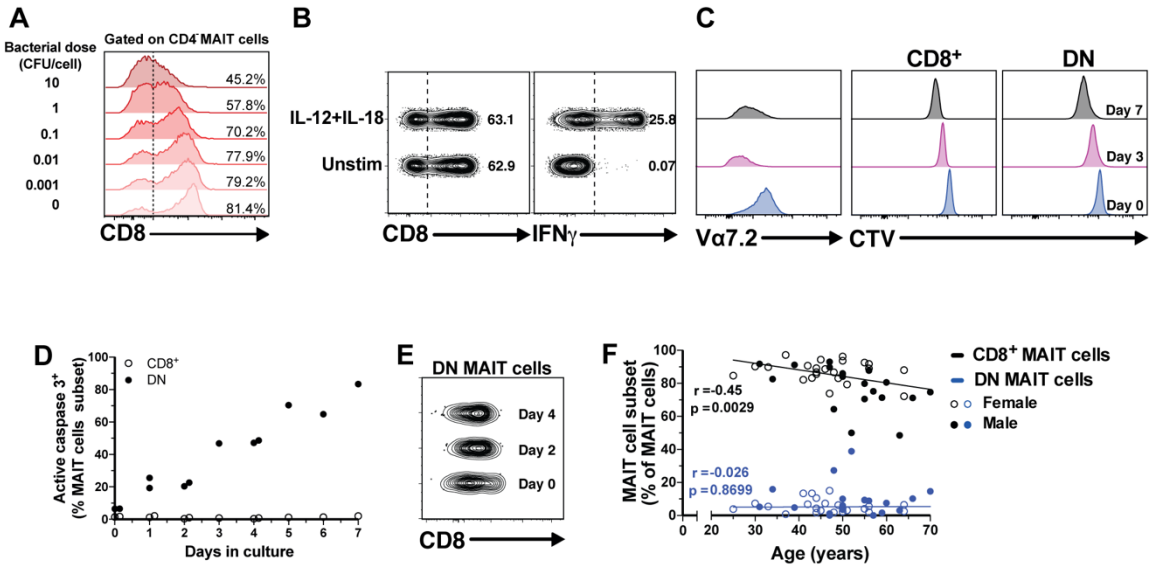

Fig. S6. CD8 expression on CD8$^+$ and DN MAIT cells. (A) Representative example of CD8 downregulation after stimulation of MAIT cells in the mixed PBMC culture with increasing doses of *E. coli*. (B) CD8 expression on MAIT cells following 24 h stimulation with IL-12 and IL-18 (*left*). IFNγ production by MAIT cells was used as a positive control for the IL-12 and IL-18 stimulation (*right*). (C) TCR Vα7.2 expression, and CellTrace Violet (CTV) levels on FACS-sorted CD8$^+$ and DN MAIT cells after 7 days in culture plates that had been pre-coated with Vα7.2 and CD28 mAbs. Representative plots/histograms from 3 individual donors are shown. All data are from 3 independent donors. (D) Percentage of active caspase 3 expression by CD8$^+$ and DN MAIT cells following stimulation of FACS-sorted CD8$^+$ MAIT cells with immobilised mAbs against the Vα7.2 TCR and CD28 over time (up to 2 independent donors). (E) CD8 expression on FACS-sorted DN MAIT cells following 4 days in culture plates that had been pre-coated with Vα7.2 and CD28 mAbs (n=3). (F) Correlation between the donor age and the levels of adult peripheral blood CD8$^+$ and DN MAIT cells as a proportion of T cells (n=42). Correlation was calculated using the Spearman's test.<insert page break here>

Table S1. Frequency of $CD4^{-}CD8^{+}$ and $CD4^{-}CD8^{-}$ MAIT cells expressing the proteins of the surface immuno-proteome dataset. Values are the average from three donors

Surface protein	$CD4^{-}CD8^{+}$ MAIT cells	$CD4^{-}CD8^{-}$ MAIT cells
CD1a	0.410	0.031
CD1b	0.463	0.047
CD1c	0.280	0.054
CD1d	0.712	0.099
CD2	96.033	80.433
CD5	97.500	98.900
CD6	99.967	99.967
CD9	83.167	68.800
CD10	0.485	0.183
CD11a	99.867	99.967
CD11b	4.707	3.478
CD11b (activated)	0.751	0.094
CD11c	0.863	0.120
CD13	0.777	0.199
CD15	0.673	0.074
CD18	99.967	99.933
CD21	0.693	0.113
CD22	0.758	0.023
CD23	0.388	0.027
CD24	0.623	0.055
CD25	0.282	0.000
CD26	99.867	99.967
CD27	81.267	77.000
CD28	99.767	99.800
CD29	99.967	99.800
CD30	0.635	0.098
CD31	44.000	40.387
CD32	1.405	0.395
CD33	1.261	0.259
CD34	1.071	0.266
CD35	12.867	8.683
CD36	2.077	1.523
CD38	3.607	1.967
CD39	1.665	0.603
CD40	0.630	0.028
CD41	0.771	0.416
CD42b	2.287	1.329
CD43	100.000	99.767

CD44	100.000	99.967
CD45RA	86.967	85.167
CD45RB	99.967	99.967
CD45R0	98.767	94.867
CD46	99.967	99.567
CD47	100.000	100.000
CD48	100.000	100.000
CD49a	1.234	0.290
CD49c	0.506	0.040
CD49d	99.500	96.867
CD49e*	100.000	100.000
CD49f*	100.000	100.000
CD50 (ICAM-3)	99.967	99.900
CD51	1.202	0.340
CD51/61	1.253	0.086
CD52	99.867	99.933
CD53	100.000	99.967
CD54*	100.000	100.000
CD55*	100.000	100.000
CD57	1.297	0.657
CD58	99.933	99.833
CD59*	100.000	99.967
CD61	0.641	0.501
CD62E	0.911	0.269
CD62L	7.323	2.350
CD62P (P-Selectin)	1.973	0.595
CD63	93.200	89.567
CD64	1.570	0.128
CD66a/c/e	1.159	0.090
CD66b	0.718	0.029
CD69	10.353	12.910
CD70	3.913	3.367
CD71	1.106	0.168
CD73	43.167	29.433
CD74	1.366	0.152
CD79b	0.774	0.117
CD80	1.189	0.140
CD81	99.900	99.833
CD82*	100.000	100.000
CD83	1.316	0.274
CD84	67.500	70.567
CD85a (ILT5)	1.746	0.982
CD85d (ILT4)	0.869	0.170

CD85g (ILT7)	1.038	0.175
CD85h (ILT1)	1.001	0.053
CD85j (ILT2)	1.940	0.186
CD85k (ILT3)	1.650	0.033
CD86	0.929	0.111
CD87	0.860	0.234
CD88	0.651	0.079
CD89	0.816	0.149
CD90 (Thy1)	9.097	4.947
CD93	0.768	0.229
CD94	19.533	11.543
CD95	93.367	90.100
CD96*	100.000	100.000
CD97*	99.967	100.000
CD99	100.000	99.833
CD100*	99.767	99.233
CD101 (BB27)	25.830	15.677
CD102*	100.000	99.967
CD103	2.004	1.443
CD104	0.724	0.076
CD105	0.665	0.105
CD106	0.386	0.000
CD107a (LAMP-1)	1.810	1.327
CD108	0.697	0.082
CD109	1.623	0.369
CD111	0.730	0.095
CD112 (Nectin-2)	0.982	0.434
CD114	0.692	0.095
CD115	0.785	0.273
CD116	0.848	0.200
CD117 (c-kit)	0.851	0.222
CD119 (IFN-γ R α chain)*	100.000	100.000
CD122	0.675	0.056
CD124	0.591	0.081
CD126 (IL-6Rα)*	0.133	0.050
CD129 (IL-9R)	1.021	0.220
CD131	0.801	0.124
CD132*	100.000	99.933
CD134	0.823	0.094
CD135	0.648	0.025
CD137 (4-1BB)	0.807	0.057
CD137L (4-1BB L)	0.790	0.056
CD138	0.684	0.305

CD140a	0.585	0.000
CD140b	0.760	0.190
CD141	0.775	0.117
CD143	0.660	0.135
CD144	0.799	0.067
CD146	1.603	0.453
CD148	2.117	1.828
CD150 (SLAM)	99.133	99.200
CD152	0.598	0.088
CD154	0.674	0.053
CD155 (PVR)	0.908	0.084
CD156c (ADAM-10)	99.933	99.933
CD158a/h	0.828	0.322
CD158b (NKAT2)	0.988	0.067
CD158d	0.906	0.051
CD158e1 (NKB1)	0.548	0.038
CD158f	0.726	0.137
CD162	99.967	100.000
CD163	1.123	0.352
CD164*	100.000	100.000
CD165	1.215	0.930
CD166	0.608	0.072
CD167a (DDR1)	0.782	0.075
CD169	0.592	0.045
CD170 (Siglec-5)	0.929	0.084
CD172a (SIRPa)	0.862	0.107
CD172b (SIRPb)	0.808	0.000
CD172g (SIRPg)	88.367	77.767
CD178 (Fas-L)	0.754	0.224
CD179a	0.801	0.175
CD179b	0.732	0.000
CD180 (RP105)	0.798	0.164
CD181 (CXCR1)	89.100	80.367
CD182 (CXCR2)	0.789	0.232
CD183	96.767	94.233
CD184 (CXCR4)	99.733	99.800
CD193 (CCR3)	97.467	95.800
CD195 (CCR5)	99.500	99.367
CD196	98.733	99.233
CD197 (CCR7)	1.486	1.154
CD200 (OX2)	1.038	0.381
CD200 R	0.949	0.250
CD201 (EPCR)	0.698	0.076

CD202b (Tie2/Tek)	0.481	0.144
CD203c (E-NPP3)	0.707	0.251
CD205 (DEC-205)	0.623	0.075
CD206 (MMR)	0.689	0.027
CD207 (Langerin)	0.653	0.207
CD209 (DC-SIGN)	0.697	0.148
CD210 (IL-10R)	0.749	0.198
CD213a2	0.990	0.404
CD215 (IL-15Rα)	0.895	0.564
CD218a (IL-18Rα)	98.600	97.167
CD220	1.301	0.720
CD221 (IGF-1R)	0.467	0.000
CD226 (DNAM-1)	73.200	69.450
CD229 (Ly-9)*	100.000	100.000
CD231 (TALLA)	1.110	0.128
CD235ab	0.750	0.248
CD243*	100.000	100.000
CD244 (2B4)	92.000	85.250
CD245 (p220/240)	0.861	0.259
CD252 (OX40L)	0.999	0.597
CD253 (Trail)	0.895	0.229
CD254	1.763	1.013
CD255 (TWEAK)	0.836	0.092
CD257 (BAFF)	0.772	0.217
CD258 (LIGHT)	0.748	0.202
CD261 (DR4)	0.715	0.304
CD262 (DR5)	0.553	0.120
CD263 (DcR1)	3.710	2.472
CD266 (Fn14)	0.441	0.078
CD267 (TACI)	0.508	0.032
CD268 (BAFF-R)	0.437	0.106
CD270 (HVEM)*	100.000	99.800
CD271	0.527	0.235
CD273 (B7-DC)	0.699	0.505
CD274 (B7-H1)	99.067	96.967
CD275 (B7-H2)	0.320	0.205
CD276	0.821	0.398
CD277*	100.000	99.900
CD278 (ICOS)*	100.000	100.000
CD279 (PD-1)	17.590	10.423
CD282 (TLR2)	0.672	0.139
CD284 (TLR4)	0.767	0.189
CD286 (TLR6)	0.811	0.000

CD290	0.544	0.086
CD294	0.521	0.115
CD298	99.800	99.667
CD300e (IREM-2)	0.420	0.421
CD300F	0.915	0.501
CD301	0.770	0.537
CD303	0.653	0.038
CD304	0.444	0.000
CD307	0.503	0.073
CD307d (FcRL4)	0.660	0.024
CD314 (NKG2D)*	100.000	100.000
CD317*	100.000	100.000
CD318 (CDCP1)	0.616	0.173
CD319 (CRACC)	91.967	85.367
CD324 (E-cadherin)	1.599	0.410
CD325	0.930	0.404
CD326 (Ep-CAM)	0.421	0.095
CD328 (Siglec-7)	33.467	27.533
CD334 (FGFR4)	0.591	0.036
CD335 (NKp46)	0.358	0.019
CD336 (NKp44)	0.405	0.031
CD337 (NKp30)	0.490	0.033
CD338 (ABCG2)	0.810	0.544
CD340 (erbB2)	0.742	0.000
CD344 (Frizzled-4)	0.913	0.380
CD351	0.540	0.079
CD352 (NTB-A)	99.933	99.933
CD354 (TREM-1)	0.767	0.140
CD355 (CRTAM)	0.786	0.353
CD357 (GITR)	0.889	0.587
CD360 (IL-21R)	0.965	0.173
β2-microglobulin	99.900	99.733
BTLA*	99.967	100.000
C3AR*	100.000	100.000
C5L2	1.401	0.448
CLEC12A	0.756	0.145
CLEC9A	0.685	0.096
CX3CR1*	100.000	100.000
CXCR7	2.873	1.344
delta-Opioid Receptor	1.319	0.220
DLL1	0.620	0.159
DLL4	0.580	0.039
DR3 (TRAMP)	0.738	0.220

EGFR	0.641	0.021
erbB3/HER-3	0.738	0.024
FcRL6	0.873	0.095
Galectin-9	0.926	0.433
GARP (LRRC32)	1.341	0.390
HLA-A,B,C	99.933	100.000
HLA-A2	1.082	0.189
HLA-DQ	0.564	0.115
HLA-DR	0.975	0.193
HLA-E*	100.000	99.833
HLA-G	0.971	0.187
IFN-γ R β chain	0.782	0.158
Ig light chain k	0.918	0.047
Ig light chain lambda	0.927	0.020
IgD	1.044	0.433
IgM	1.176	0.120
IL-28RA	1.045	0.117
Integrin α9β1	0.600	0.131
Integrin β5	0.469	0.064
Integrin β7*	100.000	100.000
Jagged 2	0.672	0.149
LAP	1.313	0.413
LT-beta R	0.981	0.402
Mac-2 (Galectin-3)	0.592	0.182
MAIR-II	0.671	0.221
MICA/MICB	0.561	0.165
MSC (W3D5)	0.575	0.454
MSC (W5C5)	0.663	0.372
MSC (W7C6)	0.883	0.257
MSC and NPC (W4A5)	1.204	0.524
MSCA-1 (MSC)	0.243	0.033
NKp80	32.433	34.533
Notch 1	0.892	0.046
Notch 2	0.862	0.092
Notch 3	0.522	0.000
Notch 4	0.766	0.052
NPC (57D2)	0.507	0.314
Podoplanin	0.768	0.000
Pre-BCR	0.814	0.118
PSMA	0.565	0.048
Siglec-10	0.714	0.212
Siglec-8	0.732	0.071
Siglec-9	11.330	6.177

SSEA-1	0.611	0.097
SSEA-3	0.542	0.000
SSEA-4	0.645	0.020
SSEA-5	0.702	0.381
TCR γ/δ	0.747	0.152
TCR Vβ13-2	10.513	3.993
TCR Vβ23	0.610	0.095
TCR Vβ8	0.568	0.299
TCR Vβ9	1.315	0.416
TCR Vδ2	0.174	0.034
TCR Vγ9	0.704	0.067
TCR Vα24-Jα18	0.835	0.035
TCR α/β	99.667	99.300
Tim-1	1.125	0.232
Tim-3	0.596	0.059
Tim-4	0.762	0.131
TLT-2	0.924	0.558
TRA-1-60-R	0.554	0.143
TRA-1-81	0.972	0.215
TSLPR (TSLP-R)	0.663	0.345
CD7	95.067	92.600
CD16	0.357	0.062
CD45	100.000	100.000
CD56	42.333	39.000
CD127*	100.000	100.000

* Staining pattern ambiguous for these markers.

Table S2. Gene expression analysis determined by Fluidigm Biomark

Gene Name	Description	Assay ID	- Log_{10} p-value	Log_2 Fold Change	FDR q-value
BCL2	Bcl-2, apoptosis regulator	Hs00608023_m1	0.602059991	-0.023626767	0.558139535
BCL2A1	Bcl-2 related protein A1, apoptosis regulator	Hs00187845_m1	0.134082116	-0.248145443	0.925324675
BIRC3	Baculoviral IAP repeat containing 3	Hs00985031_g1	1.26211193	-0.186091381	0.181034483
CASP1	Caspase 1	Hs00354836_m1	0.134082116	0.075364959	0.925324675
CASP3	Caspase 3	Hs00234387_m1	0.24388711	-0.001030547	0.842307692
CCL3	MIP-1a, C-C motif chemokine ligand 3	Hs00234142_m1	0.204119983	-1.481561099	0.882042254
CCL4	MIP-1b, C-C motif chemokine ligand 4	Hs99999148_m1	0.086020671	0.841855521	0.972222222
CCL5	RANTES, C-C motif chemokine ligand 5	Hs00174575_m1	2.10720997	0.429663658	0.046875
CCNA2	Cyclin A2	Hs00996788_m1	0	0.068297006	1
CCND2	Cyclin D2	Hs00153380_m1	0	-0.0203602	1
CCR1	CD191, C-C motif chemokine receptor 1	Hs00928897_s1	0.24388711	-0.218479003	0.842307692
CCR6	CD196, C-C motif chemokine receptor 6	Hs01890706_s1	0.040884044	-0.20127442	1
CCR7	CD197, C-C motif chemokine receptor 7	Hs01013469_m1	1.26211193	-0.75356261	0.181034483
CD16A	CD16b, Fc fragment of IgG receptor IIIb	Hs00275547_m1	0.204119983	-0.023168682	0.882042254
CD160	CD160 molecule	Hs00199894_m1	1.129486364	0.848337448	0.2375
CD161.KLRB1	Killer cell lectin like receptor B1	Hs00174469_m1	0.304436244	0.312935924	0.786885246
CD226	CD226 molecule, DNAM-1	Hs00170832_m1	0.692236622	-0.384724059	0.475609756
CD244	CD244 molecule, 2B4	Hs00900271_m1	0.370813467	0.850072728	0.743181818
CD274	CD274 molecule, PDL1	Hs01125301_m1	0	-0.359775013	1
CD3	CD3d molecule	Hs00174158_m1	0.52174924	-0.02851531	0.627717391
CD38	CD38 molecule	Hs01120071_m1	0	-0.968282729	1
CD4	CD4 molecule	Hs01058407_m1	0.903089987	-4.748462272	0.342857143
CD69	CD69 molecule, CLEC2C	Hs00934033_m1	0.24388711	-0.063442225	0.842307692
CD8	CD8a molecule, Leu2	Hs00233520_m1	2.408239965	2.697664787	0.026785714
CDK6	Cyclin dependent kinase 6	Hs01026371_m1	0.304436244	0.223045018	0.786885246
CRTAM (CD355)	Cytotoxic and regulatory T-cell molecule	Hs00219699_m1	1.010299957	1.096006519	0.275735294
ENTPD1	Ectonucleoside triphosphate Diphosphohydrolase 1, CD39	Hs00969559_m1	0	-1.44560206	1
EOMES	Eomesodermin	Hs00172872_m1	0.889726025	0.897555536	0.34375
FAS	Fas cell surface death receptor, CD95	Hs00531110_m1	1.709269961	-0.335296512	0.098684211
FASLG	Fas ligand, CD178	Hs00181225_m1	0.129486364	-0.05477507	0.925324675
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1	0.040884044	0.036930866	1
GNLY	Granulysin, LAG-2	Hs00246266_m1	0.304436244	0.084745999	0.786885246
GZMA	Granzyme A, CTLA3	Hs00989184_m1	1.26211193	0.403346859	0.181034483
GZMB	Granzyme B, CTLA1	Hs00188051_m1	0.301029996	4.998777502	0.786885246
GZMK	Granzyme K, TRYP2	Hs00157878_m1	1.563141925	0.30654727	0.114130435
HLADRA	Major histocompatibility complex, class II, DR alpha	Hs00219578_m1	1.010299957	-1.83575486	0.275735294
ICOS	Inducible T-cell costimulator, CD278	Hs00359999_m1	0.370813467	-0.311366657	0.743181818
IFNG	Interferon gamma	Hs00989291_m1	0.336357958	2.060832829	0.786885246
IFI16	Interferon gamma inducible protein 16	Hs00194261_m1	0.444452138	-0.05553129	0.704081633
IGF2R	Insulin like growth factor 2 receptor, CD222	Hs00974474_m1	0.185523494	0.111193734	0.882042254
IKZF1	IKAROS family zinc finger 1	Hs00958474_m1	0.444452138	0.027089644	0.704081633
IKZF2	IKAROS family zinc finger 2, HELIOS	Hs00212361_m1	0.784990675	0.46218061	0.403846154
IL12RB1	Interleukin 12 receptor subunit beta 1, CD212	Hs00538167_m1	0.086020671	-0.440078514	0.972222222
IL12RB2	Interleukin 12 receptor subunit beta 2	Hs01548202_m1	1.806179974	0.177127139	0.088235294
IL18R1	Interleukin 18 receptor 1, IL18RA	Hs00977691_m1	0.370813467	-0.101777681	0.743181818
IL18RAP	Interleukin 18 receptor accessory protein, IL-18Rbeta	Hs00977695_m1	1.26211193	0.967475377	0.181034483
IL21R	Interleukin 21 receptor, CD360	Hs00222310_m1	1.010299957	0.41740512	0.275735294
IL2RA (CD25)	Interleukin 2 receptor subunit alpha	Hs00907778_m1	0	-1.956521878	1
IL2RG	Interleukin 2 receptor subunit gamma, CD132	Hs00953624_m1	0.040884044	-0.04312786	1
IL7R	Interleukin 7 receptor, CD127	Hs00902334_m1	0.086020671	0.01681744	0.972222222
IRF4	Interferon regulatory factor 4	Hs01056533_m1	0	-1.215190249	1

IRF7	Interferon regulatory factor 7	Hs01014809_g1	0.134082116	0.380800315	0.925324675
KIR3DL1, KIR3DS1	Killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1	Hs00744448_s1	1.408239965	-0.841851849	0.15625
KLF10	Kruppel like factor 10	Hs00921811_m1	0.040884044	0.018617069	1
KLRC1	Killer cell lectin like receptor C1, NKG2A	Hs00970273_g1	1.26211193	1.31019038	0.181034483
KLRC2, KLRC3	Killer cell lectin like receptor C2, NKG2C	Hs04192492_gH	0	-1.888357108	1
KLRD1	Killer cell lectin like receptor D1, CD94	Hs00233844_m1	2.10720997	0.711815903	0.046875
KLRF1	Killer cell lectin like receptor F1	Hs00212979_m1	0.134082116	0.55688785	0.925324675
KLRG1	Killer cell lectin like receptor G1	Hs00929964_m1	0.370813467	0.266707715	0.743181818
KLRK1,KLRC4,KLRK1	Killer cell lectin like receptor K1, NKG2D	Hs00183683_m1	2.408239965	0.493858571	0.026785714
LAIR1	Leukocyte associated immunoglobulin like receptor 1, CD305	Hs00253790_m1	0.425968732	-0.352001914	0.72
LAMP1 (CD107a)	Lysosomal associated membrane protein 1	Hs00174766_m1	0.602059991	-0.17560898	0.558139535
MAP3K8	Mitogen-activated protein kinase kinase kinase 8	Hs00178297_m1	0.040884044	-0.11211791	1
MIR155, MIR155HG	MIR155 host gene	Hs01374569_m1	0.329058719	-1.226696612	0.786885246
MTHFD2	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	Hs00759197_s1	2.408239965	-0.435292714	0.026785714
NCAM (CD56)	Neural cell adhesion molecule 1	Hs00941830_m1	0.370813467	0.394010313	0.743181818
NCR1	Natural cytotoxicity triggering receptor 1, CD335	Hs00183118_m1	0.204119983	-1.352297494	0.882042254
NCR3	Natural cytotoxicity triggering receptor 3, CD337	Hs00394809_m1	1.010299957	0.148483517	0.275735294
NFKB1	Nuclear factor kappa B subunit 1, NF-kB1	Hs00765730_m1	0.086020671	-0.08315448	0.972222222
NFKBIA	NFKB inhibitor alpha	Hs00355671_g1	0.52174924	0.216397771	0.627717391
NFKBID	NFKB inhibitor delta	Hs01076336_m1	0.185523494	0.075867561	0.882042254
NKG7	Natural killer cell granule protein 7	Hs01120688_g1	1.709269961	0.444677702	0.098684211
PDCD1	Programmed cell death 1, PD-1, CD279	Hs01550088_m1	0.073786214	0.037546225	0.987804878
ZBTB16 (PLZF)	Zinc finger and BTB domain containing 16	Hs00957433_m1	0.52174924	0.115355429	0.627717391
SERPINB9	Serpin family B member 9	Hs00394497_m1	0.24388711	0.129690935	0.842307692
SLAMF5	CD84 molecule	Hs01547121_m1	0.185523494	0.277539808	0.882042254
SLAMF7	SLAM family member 7, CD319	Hs00900280_m1	1.563141925	0.469132473	0.114130435
STX11	Syntaxin 11	Hs01891623_s1	0.692236622	-0.171519622	0.475609756
TBX21	T-box 21, T-bet	Hs00203436_m1	0.444452138	0.06473791	0.704081633
TESPA1	Thymocyte expressed, positive selection associated 1	Hs00207702_m1	1.563141925	-0.564904512	0.114130435
TGFBR1	Transforming growth factor beta receptor 1	Hs00610318_m1	0.134082116	0.128596868	0.925324675
TIGIT	T-cell immunoreceptor with Ig and ITIM domains	Hs00545087_m1	0.784990675	-1.367715848	0.403846154
TNF	Tumor necrosis factor, TNF-alpha	Hs01113624_g1	0.784990675	-0.100012861	0.403846154
TNFRSF9	TNF receptor superfamily member 9, CD137	Hs00155512_m1	0	-0.34392676	1
TNFSF10	Tumor necrosis factor superfamily member 10, TRAIL	Hs00921974_m1	1.563141925	-0.310541877	0.114130435

Table S3. Gene expression analysis determined by RNA-seq

Gene name	logFC	AveExpr	t	P.Value	B
Genes upregulated in DN MAIT cells					
ZNF536	-4.373452875	-1.50887295	-15.26899811	2.54E-07	3.794794638
RRM2	-6.476059532	-0.807202866	-11.23889548	2.84E-06	2.973000439
ESR1	-4.600578185	-1.040832527	-9.287939032	1.23E-05	2.303301355
NBEAP1	-4.366224237	-1.59843468	-8.640337813	2.11E-05	2.018902096
RP3-331H24.6	-4.901041333	-1.32856425	-8.36972801	2.68E-05	1.888760118
PIH1D2	-4.689102645	-0.600133787	-7.707031223	4.93E-05	1.538397082
AC098973.1	-4.793037854	-1.500276345	-7.131759137	8.65E-05	1.193505044
SLITRK4	-4.310239578	-1.643311563	-7.026163456	9.62E-05	1.125670154
RP4-539M6.22	-5.834530925	1.66242543	-5.948642679	0.000308271	0.342867221
SCUBE1	-4.700267565	-1.434277838	-6.217516369	0.000227564	0.554591378
TCL6	-4.562751641	-1.518362113	-6.01432497	0.000285998	0.395658716
MASP2	-4.069433258	-1.253279571	-5.972721448	0.000299892	0.362301761
SUSD1	-4.421749095	-0.661408831	-5.622778845	0.000450928	0.070377296
MYEOV	-4.866015891	-0.354733313	-4.996174562	0.000975636	-0.50567707
WNT10A	-4.300553423	-1.137646235	-4.901207022	0.001102011	-0.599232634
ESAM	-4.396670279	-1.612871596	-4.845877787	0.001183765	-0.65451707
CBFA2T3	-4.789103101	-1.018881751	-4.475139271	0.001934296	-1.039883208
PDGFA	-4.482589136	0.23274816	-4.512557621	0.001839084	-0.999803943
RASD2	-4.718285713	0.029968889	-4.210523367	0.002780363	-1.330912354
OSM	-4.305702167	0.11741218	-4.150697383	0.003022365	-1.398545994
IRF8	-5.665055754	2.546695428	-3.763732284	0.005250655	-1.851987282
LRAT	-4.089590001	-1.259826928	-3.664799194	0.00606751	-1.972213943
NR4A3	-4.119295116	1.771073696	-3.572147673	0.00695573	-2.086314484
NLN	-4.417123321	-1.554366073	-3.347285598	0.009735349	-2.368964201

HSPA6	-5.435601767	1.034849953	-2.570399838	0.032394375	-3.392664347
Genes upregulated in CD8⁺ MAIT cells					
MB21D2	4.626181301	-0.879301368	16.14872846	1.63E-07	3.91269017
CD8B	7.97416245	2.431660886	8.577817244	2.23E-05	1.989458209
CD8A	4.320835812	6.919509211	10.61813472	4.41E-06	2.786092659
ZNF436-AS1	4.551666634	-0.79127475	9.924899132	7.40E-06	2.549894459
FAM167A	4.552117592	-1.342103967	9.577242721	9.72E-06	2.419092864
MZB1	4.708108775	-1.335105865	8.816261008	1.82E-05	2.099808202
CTD-2270P14.5	4.073707527	-1.105158538	7.37323442	6.80E-05	1.343227655
SDCBP2	4.426527991	-1.025346485	7.1571148	8.43E-05	1.209576328
SLC44A3	4.286174461	-1.420332694	7.030094798	9.58E-05	1.128222039
C3orf18	4.575961735	-0.531778496	6.857084136	0.000114486	1.013950662
TMC3-AS1	4.008901226	-1.60943888	6.121473584	0.000253362	0.48028521
BHLHB9	4.836152516	-1.840296269	5.759118441	0.000383939	0.186558055
ACTG1P1	4.175736878	-1.094960824	5.588572748	0.000469677	0.040729078
RP11-77P6.2	4.121861942	-0.514509226	5.491023781	0.000527995	-0.044937456
CCDC168	4.04451627	-0.452445264	4.973497115	0.001004311	-0.52786452
LINC01422	4.831042053	-0.378596777	4.388191971	0.002176739	-1.134042632
TKTL1	4.159719648	1.032085592	4.168855933	0.002946603	-1.377946539
TSPY26P	4.105405213	-0.062568043	3.713143623	0.005652641	-1.913253281
ZNF837	4.489382775	0.17814574	3.657925376	0.006129078	-1.980629597
ARSJ	4.054115349	0.463441538	3.269045491	0.010959429	-2.469087208
EHD3	4.182122054	0.333304573	3.037223883	0.015628831	-2.770467286

Table S4. Flow cytometry antibodies used in the study

Antibody name	Clone	Antibody source
anti-active Caspase 3 Brilliant Violet (BV) 650	C92-605	**BD Biosciences**
anti-CD3 FITC, Alexa Fluor (AF) 700, APC, BV510	UCHT1	
anti-CD4 APC-H7	SK3	
anti-CD4 APC-H7, V500	RPA-T4	
anti-CD8 PE	RPA-T8	
anti-CD8 PerCP-Cy5.5	SK1	
anti-CD14 AF700	M5E2	
anti-CD19 FITC, AF700	HIB19	
anti-CD25 PECy7	M-A251	
anti-CD62L V450	DREG-56	
anti-CD161 PECy5, FITC	DX12	
anti-GrzB AF700	GB11	
anti-TNF PECy7	MAb11	
anti-RORγt PE	Q21-559	
anti-Bax AF488	2D2	**BioLegend**
anti-Bcl-2 PE	100	
anti-CD2 FITC	RPA-2.10	
anti-CD3 BV650, BV785	OKT3	
anti-CD4 BV510, BV711	OKT4	
anti-CD8 PE, APC, BV570	RPA-T8	
anti-CD9 PE	HI9a	
anti-CD20 FITC	2H7	
anti-CD27 BV711	O323	
anti-CD45 AF700	HI30	
anti-CD45RO PECy7	UCHL1	
anti-CD94 FITC	DX22	
anti-CD95 FITC	DX2	
anti-CD101 APC	BB27	
anti-CD123 FITC	6H6	
anti-CD127 BV650	A019D5	
anti-CD161 BV605	HP-3G10	
anti-CCR7 BV421	G043H7	
anti-FcεRIα FITC	AER-37 (CRA-1)	
anti-Gnly PE	DH2	
anti-GrzA AF700	CB9	
anti-GrzB B FITC	GB11	
anti-IFNγ BV785	4S.B3	

anti-IL-17 BV711 anti-IL-18Rα (CD218) PE anti-NKG2D (CD314) BV421 anti-PD-1 BV421 anti-Prf BV421 anti-T-bet BV711 anti-Vα7.2 PE, APC, PECy7, and FITC LEGENDScreen™ Lyophilized Antibody Panel, Human Cell Screening (PE) Kit streptavidin BV421	BL168 H44 1D11 EH12.2H7 B-D48 4B10 3C10	
anti-Eomes FITC anti-Helios eFluor 450	WD1928 22F6	**eBioscience**
anti-IL-18Rα PE anti-PLZF APC	70625 6318100	**R&D Systems**
anti-CD3 PE-Texas Red anti-CD56 PE-Texas Red anti-NKG2A PE Vβ repertoire kit (IOTest® Beta Mark TCR Vbeta Repertoire Kit)	UCHT1 N901 (NKH-1) Z199	**Beckman Coulter**
anti-CD3 PE-Texas Red anti-CD8 Qdot 605 LIVE/DEAD® Fixable Aqua, Green, and Near-IR Dead Cell Stain Kits	S4.1 3B5	**Invitrogen / Life Technologies / Thermo Fisher**
anti-BDCA-2 FITC anti-SLAN (M-DC8) FITC	AC144 DD1	**Miltenyi Biotec**
anti-CD14 FITC	TÜK4	**Dako**
anti-α4β7 integrin*	Act-1	**The AIDS Reagent Program, Division of AIDS, NIAID, NIH**
Human MR1 5-OP-RU PE, BV421 Human MR1 6-FP PE, AF680	MR1 tetramers	**The NIH Tetramer Core Facility at Emory University**

* The anti-α4β7 integrin antibody was biotinylated as per the manufacturer's instructions (FluoReporter® Mini-Biotin-XX Protein Labeling Kit, Life Technologies).