Supplementary materials:

Functional heterogeneity of human tissue-resident memory T cells based on dye efflux capacities

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Donor Number	Age	Sex	Donor Number	Age	Sex
43	60	F	275	31	М
65	69	Μ	277	21	Μ
79	44	F	278	78	F
115	5	F	282	32	F
116	42	Μ	285	53	Μ
138	35	Μ	286	46	Μ
181	46	Μ	287	34	Μ
189	59	Μ	291	26	F
190	69	F	302	56	Μ
191	29	Μ	303	64	Μ
193	50	Μ	304	68	Μ
198	23	F	305	28	F
202	50	Μ	306	71	F
203	70	Μ	309	45	F
206	50	Μ	312	50	F
230	52	F	314	35	F
232	53	Μ	315	63	Μ
233	26	F	320	55	F
235	50	Μ	326	29	Μ
241	19	Μ	327	52	F
257	19	Μ	328	52	М
259	46	Μ	335	23	F
265	24	Μ	341	49	Μ
267	70	F	342	59	Μ
270	23	F	346	32	М
272	8	Μ	352	28	F
273	67	F	354	53	F
			355	61	F

Supplementary Table 1: List of organ donors from which tissues were obtained for this

study

Supplementary Table 2. List of antibodies used in this study.

Poactivity	Targot	Clone	Company
			- Company
Human	CCR7	G043H7	Biolegend
Human	CD101	BB27	Biolegend
Human	CD103	Ber-ACT8	Biolegend
Human	CD45RA	HI100	Biolegend
Human	CD49a	TS2/7	Biolegend
Human	PD-1	EH12.2H7	Biolegend
Human	MDR1	UIC2	Biolegend
Human	Va7.2	3C10	Biolegend
Human	CD69	FN50	Biolegend
Human	CD19	SJ25C1	Biolegend
Human	CD4	RPA-T4	Biolegend
Human	CTLA4	L3D10	Biolegend
Human	CD57	HNK-1	Biolegend
Human	CD127	A019D5	Biolegend
Human	CD161	HP-3G10	eBiosciences
Human	CD8	HIT8a	Biolegend
Human	Ki67	MKI67	Biolegend
Human	CD3	OKT3	eBiosciences
Human	IFN-gamma	4S.B3	eBiosciences
Human	CD107a	eBioH4A3	eBioscience
Human	pSTAT5	pY694	BD Biosciences



Supplementary Figure 1. Viability and MDR1 expression by efflux(+) and efflux(-) TRM cells.

(A) Viability of TRM is not affected by Mitotracker dyes or CSA. TRM were isolated from human spleen by cell sorting and stained with Mitotracker Green Dye in media alone (left) or in the presence of cyclosporine A (CsA, right). Plots show viability as assessed by staining with Live/Dead Fixable Staining Kit (Invitrogen) as a function of mitochondrial dye staining. (B) MDR1 expression by efflux(+) and efflux(-) TRM subsets. Left: histogram shows expression of MDR1 by efflux(-) (black line) and efflux(+) (grey) from the spleen of one representative donor. Right: Compiled data from 6 donors is shown. ***p ≤ 0.001 . Student's T test.



Supplementary Figure 2. MAIT cells are a minor population of TRM phenotype CD8⁺ T cells. Identification of MAIT populations in spleen by CD161 and V α 7.2 TCR clonotype expression. Statistic indicates mean frequency ⁺/- standard error of the mean (SEM) of MAIT (CD161⁺, V α 7.2⁺) cells within CD69⁺ (TRM) CD8⁺ T cells in the spleen; N=7 donors.