

# Mucosal-associated invariant T cells are functionally impaired in pediatric and young adult patients following allogeneic hematopoietic stem cell transplantation and their recovery correlates with clinical outcomes

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## **SUPPLEMENTARY MATERIAL FIGURE LEGENDS**

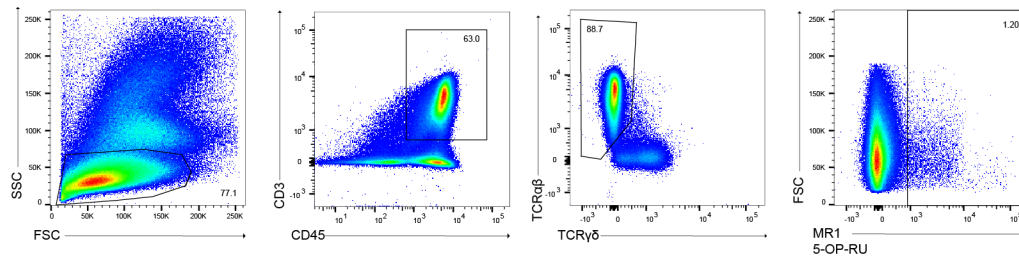
Fig.S1 | Representative staining and gating strategy of MAIT cells among CD45<sup>+</sup>CD3<sup>+</sup> T-cells, using MR1-5OP-RU-tetramer (upper panel) and 6-FP-tetramer (lower panel) as a negative control, in the peripheral blood of HD.

Fig.S2 | Clinical outcomes of the whole study population. (A) Cumulative incidence of aGvHD and aGvHD 2-4. (B) Cumulative incidence of cGvHD and moderate-severe cGvHD. (C) Cumulative incidence of late BSI and CMV reactivation. (D) Probability of DFS and CIR. (E) Cumulative incidence of NRM and probability of OS. (F) Probability of GRFS.

Fig.S3 | Representative manual gating strategy used for the evaluation of functional markers among MAIT cells subsets.

# SUPPLEMENTARY FIGURES

## MR1-5-OP-RU-tetramer



## MR1-6-FP-tetramer

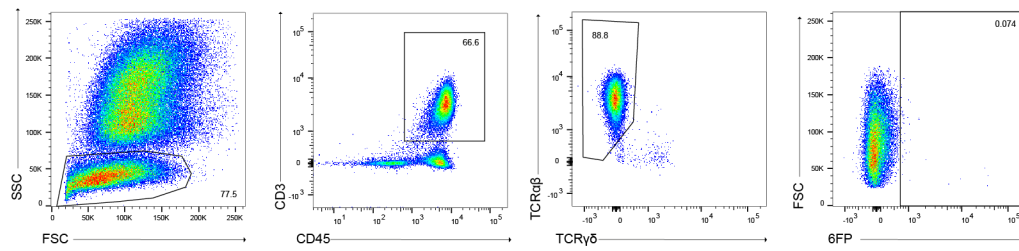


Fig. S1

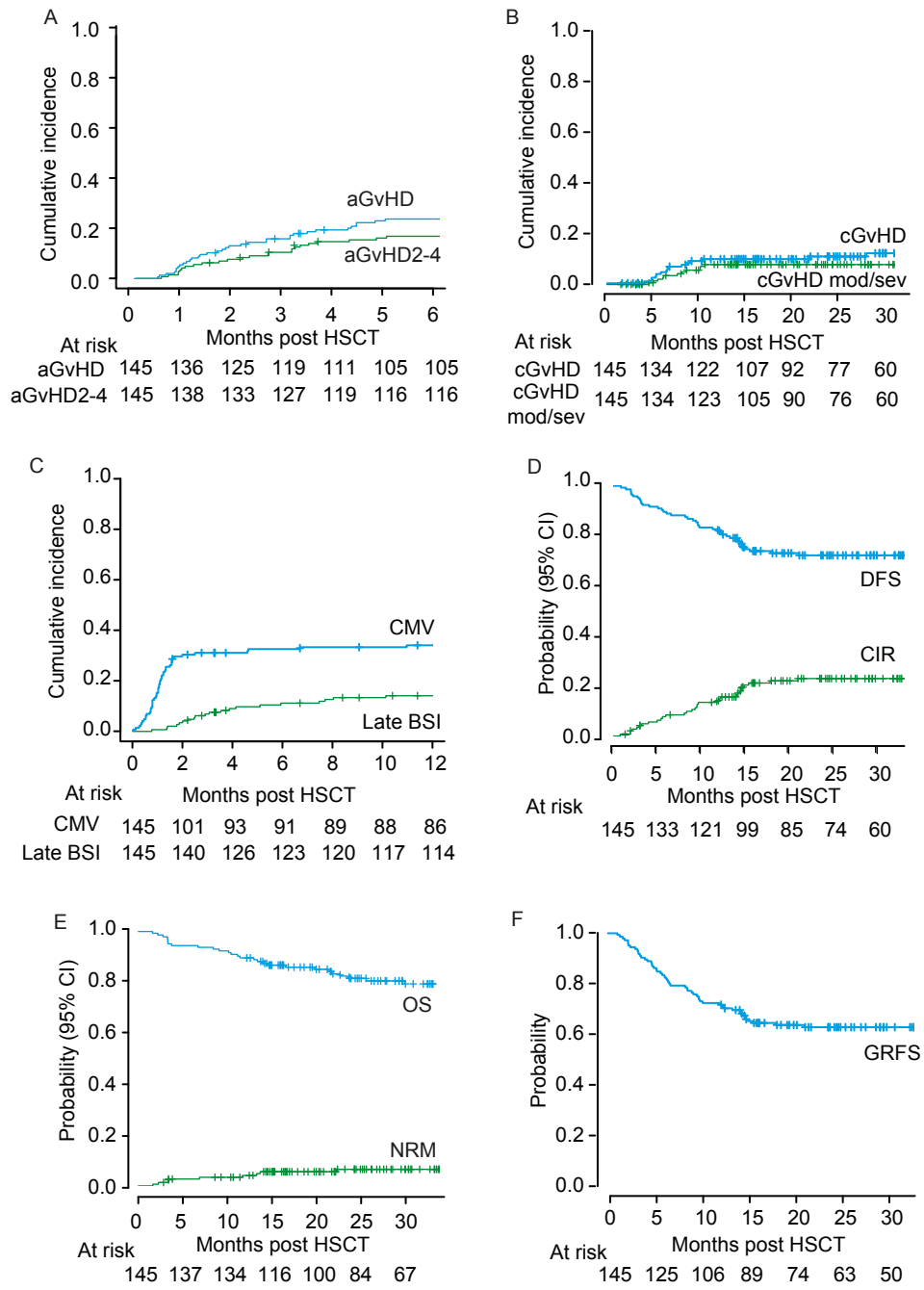


Fig. S2

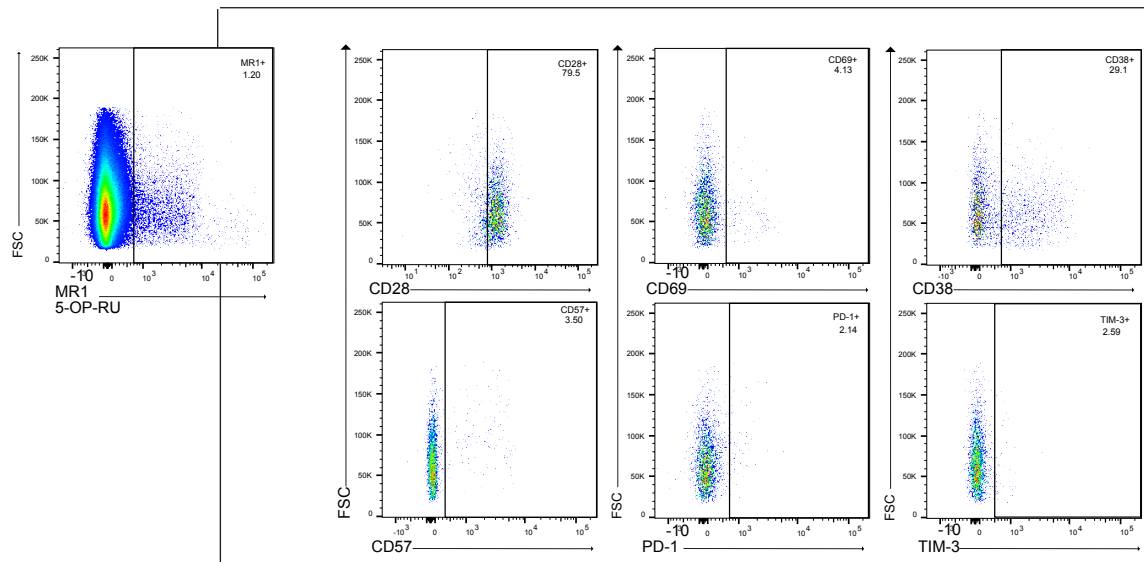


Fig. S3

## SUPPLEMENTARY TABLES

**Table S1. Antibody list**

Antigene	Fluorochrome	Clone	Cat number	Manufacturer
Fixable Dye	BUV440	FVS440UV	566332	BD
Tetramer hMR1 5-OP-RU	BV421		45163	NIH
Tetramer hMR1 6-FP-RU	BV421		45164	NIH
CD183 (CXCR-3) *	BV650	1C6/CXCR3	740603	BD
CD197 (CCR-7)	BUV395	2-L1-A	749655	BD
CD161 *	APC	DX12	550968	BD
CD56	PE-Cy7	NCAM 16	335826	BD
CD3	BUV496	UCHT1	612941	BD
CD127 (IL-7R $\alpha$ ) *	PE-CF594	HIL-7R-M21	562397	BD
CD45RO	BV605	UCHL1	562791	BD
CD4 *	BUV737	SK3	612749	BD
CD45	BUV805	HI30	612892	BD
CD27 *	PE	O323	59455	BD
CD152 (CTLA-4) *	PE-Cy5	BNI3	561717	BD
CD223 (LAG-3) *	APC-eFluor780	3DS223H	47-2239-42	Invitrogen
CD25 *	BB700	M-A251	566448	BD
CD95	BV480	DX2	746675	BD
CD69 *	BV750	FN50	747522	BD
HLA-DR *	APC-R700	G46-6	565127	BD
CD28 *	BUV563	L293	748476	BD
CD8 *	BV570	SK1	624298	BD
CD31	BB790-P	WM59	624296	BD
TCR $\alpha$	BB755	IP26	624391	BD
TCR $\gamma$	BUV661	11F2	750019	BD
CD366 (TIM3) *	BB660-P	7D3	624295	BD
CD137 (4-1BB) *	BB630-P2	4B4-1	624294	BD
CD279 (PD-1) *	BV786	EH12.1	563789	BD
CD38 *	BV711	HIT2	563965	BD
CD57 *	FITC	HNK-1	333169	BD
CD3	Purified	OKT3	16-0037-85	Invitrogen
CD28 *	Purified	CD28.2	555275	BD

Markers used for UMAP generation in Fig. 2A and B are marked with asterisks

**TABLE S2: UNIVARIATE AND MULTIVARIATE ANALYSIS OF VARIABLES AFFECTING aGVHD**

	Univariate	Multivariate Analysis		
	p-value	HR	95% CI	p-value
Age ≤ 2	0.87			
Donor type	0.66			
Disease risk score	0.93			
Source of HSCs	0.47			
Conditioning regimen	0.19			
Pre-engraftment BSI	0.13			
Day+30 abTCR T cell counts* (> vs < median value)	0.08	1.97	0.6-3.3	0.25
Day+30 MAIT cell counts (> vs < median value)	0.06	1.67	0.5-5.3	0.39
Day+30 MAIT cell % of abTCR+ cells (> vs < median value)	0.65			
Day+30 CD161+ MAIT cell counts (> vs < median value)	0.23			
Day+30 CD161+ cells % of MAIT cells (> vs < median value)	0.81			

**TABLE S3 UNIVARIATE AND MULTIVARIATE ANALYSIS OF VARIABLES AFFECTING cGVHD**

	Univariate	Multivariate Analysis		
	p-value	HR	95% CI	p-value
Age ≤2	0.14			
Donor type	0.65			
Disease risk score	0.7			
Source of HSCs	0.65			
Conditioning regimen	0.08	0.5	0.2-1.4	0.19
aGvHD II-IV	0.08	1.9	0.6-5.8	0.26
Pre-engraftment BSI	0.59			
Day+30 abTCR T cell counts* (> vs < median value)	0.55			
Day+30 MAIT cell counts (> vs < median value)	0.07	2.2	0.7-7.3	0.16
Day+30 MAIT cell % of abTCR+ cells (> vs < median value)	0.21			
Day+30 CD161+ MAIT cell counts (> vs < median value)	0.81			
Day+30 CD161+ cells % of MAIT cells (> vs < median value)	0.78			

**TABLE S4 UNIVARIATE AND MULTIVARIATE ANALYSIS OF VARIABLES AFFECTING GRFS**

	Univariate	Multivariate		
	p-value	HR	95% CI	p-value
Age ≤2	0.70			
Donor type	0.89			
Disease risk score	0.16	(INT) 0.91 (LOW) 0.24	0.35-2.3 0.04-1.2	0.84 0.09
Source of HSCs	0.74			
Conditioning regimen	0.27			
Pre-engraftment BSI	0.67			
Day+30 abTCR T cell counts* (> vs < median value)	0.34			
Day+30 MAIT cell counts (> vs < median value)	0.05	1.38	0.9-3.4	0.06
Day+30 MAIT cell % of abTCR+ cells (> vs < median value)	0.47			
Day+30 CD161+ MAIT cell counts (> vs < median value)	0.14			
Day+30 CD161+ cells % of MAIT cells (> vs < median value)	0.49			

**TABLE S5 UNIVARIATE AND MULTIVARIATE ANALYSIS OF VARIABLES AFFECTING LATE BSI INCIDENCE**

	Univariate	Multivariate Analysis		
	p-value	HR	95% CI	p-value
Age ≤2	0.86			
Donor type	0.03	(MUD) 0.73	0.1 - 5.8	0.77
Disease risk score	0.83			
Source of HSCs	0.04	1.57	0.4 - 17.6	0.71
Conditioning regimen	0.75			
Pre-engraftment BSI	0.13			
Day+30 abTCR T cell counts* (> vs < median value)	0.05	0.73	0.2-2.7	0.64
Day+30 MAIT cell counts (> vs < median value)	0.08	0.42	0.1-1.3	0.14
Day+30 MAIT cell % of abTCR+ cells (> vs < median value)	0.77			
Day+30 CD161+ MAIT cell counts (> vs < median value)	0.13			
Day+30 CD161+ cells % of MAIT cells (> vs < median value)	0.47			



**TABLE S6 UNIVARIATE AND MULTIVARIATE ANALYSIS OF VARIABLES AFFECTING CMV REACTIVATION**

	Univariate	Multivariate Analysis		
	p-value	HR	95% CI	p-value
Age ≤2	0.26			
Donor type	0.01	(MUD) 0.53	0.2-1.7	0.31
Disease risk score	0.87			
Source of HSCs	0.01	(PBSC) 1.4	0.4-5.4	0.59
Conditioning regimen	0.12			
Pre-engraftment BSI	0.02	2.3	1.1-5	0.03
Day+30 abTCR T cell counts* (> vs < median value)	0.66			
Day+30 MAIT cell counts (> vs < median value)	0.02	2.1	1.2-3.8	0.01
Day+30 MAIT cell % of abTCR+ cells (> vs < median value)	0.86			
Day+30 CD161+ MAIT cell counts (> vs < median value)	0.92			
Day+30 CD161+ cells % of MAIT cells (> vs < median value)	0.78			

**Table S7 : Statistical analysis of plots in Fig. 1B-E and 2C-D**

abT cells			
Counts (Fig 1B)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	<0.001	<0.001	<0.001
60	<0.001	<0.001	<0.001
90	<0.001	<0.001	<0.001
180	<0.001	<0.001	<0.001
360	0.025	<0.001	0.0505
720	0.786	0.016	0.088
frequency of T cells (Fig 1D)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	<0.001	<0.001	0.008
60	<0.001	<0.001	0.004
90	<0.001	<0.001	0.0018
180	<0.001	<0.001	<0.001
360	<0.001	0.275	0.001
720	0.046	0.681	0.001

MAIT			
Counts (Fig 1C)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	0.162	<0.001	<0.001
60	<0.001	<0.001	<0.001
90	0.009	<0.001	<0.001
180	0.017	<0.001	<0.001
360	0.54	<0.001	<0.001
720	0.687	<0.001	<0.001
Frequency of abT cells (Fig. 1E)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	0.558	<0.001	<0.001
60	0.056	<0.001	<0.001
90	0.535	<0.001	<0.001
180	0.310	<0.001	<0.001
360	0.715	<0.001	<0.001
720	0.097	<0.001	<0.001

CD161+ MAIT cells			
Frequency of MAIT cells (Fig. 2C)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	<0.001	<0.001	0.093
60	<0.001	<0.001	0.077
90	<0.001	<0.001	0.023
180	<0.001	<0.001	<0.001
360	<0.001	<0.001	<0.001
720	0.418	<0.001	0.022

CD8+ MAIT cells			
Frequency of MAIT cells (Fig. 2D)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	<0.001	<0.001	0.651
60	0.013	0.02	0.307
90	0.004	<0.001	0.380
180	<0.001	0.005	0.517
360	0.72	0.028	0.014
720	0.549	0.716	0.922

CD4+ MAIT cells			
Frequency of MAIT cells (Fig. 2D)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	0.013	0.015	0.717
60	0.619	0.667	0.946
90	0.301	0.524	0.962
180	0.047	0.005	0.124

360	0.608	<0.001	0.001
720	0.603	0.005	0.026

CD4-CD8- DN MAIT cells			
Frequency of MAIT cells (Fig. 2D)			
days post-HSCT	MUD vs Haplo	HD vs Haplo	HD vs MUD
30	0.001	0.11	0.334
60	0.337	0.678	0.272
90	0.449	0.557	0.061
180	0.056	0.075	<0.001
360	0.687	<0.001	0.001
720	0.712	<0.001	0.002