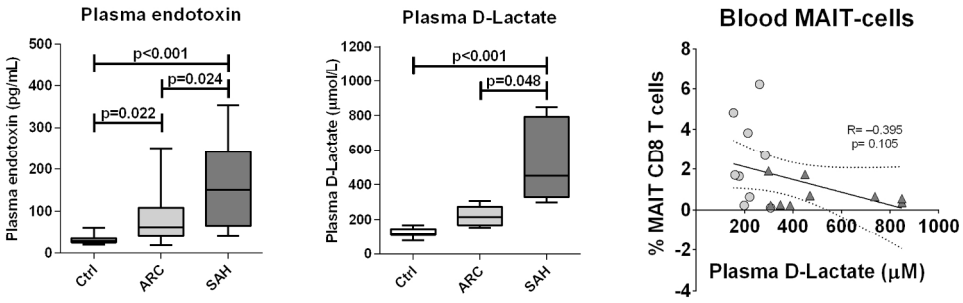

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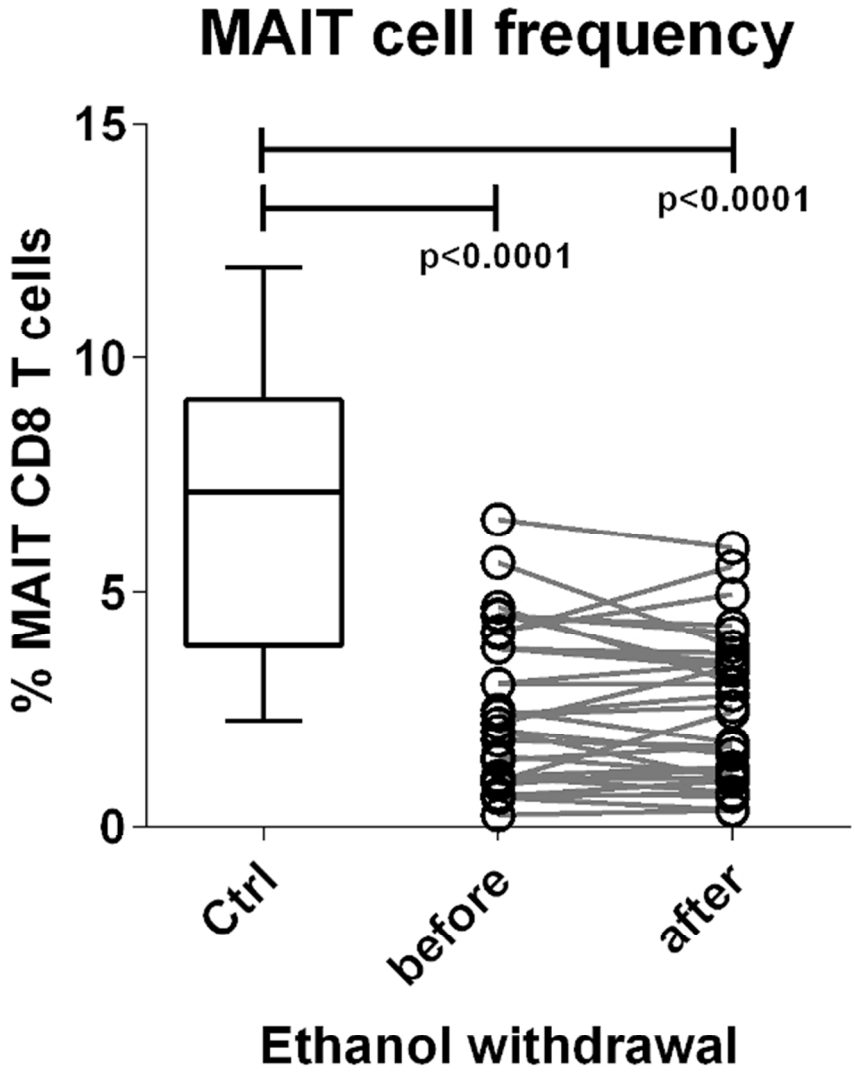

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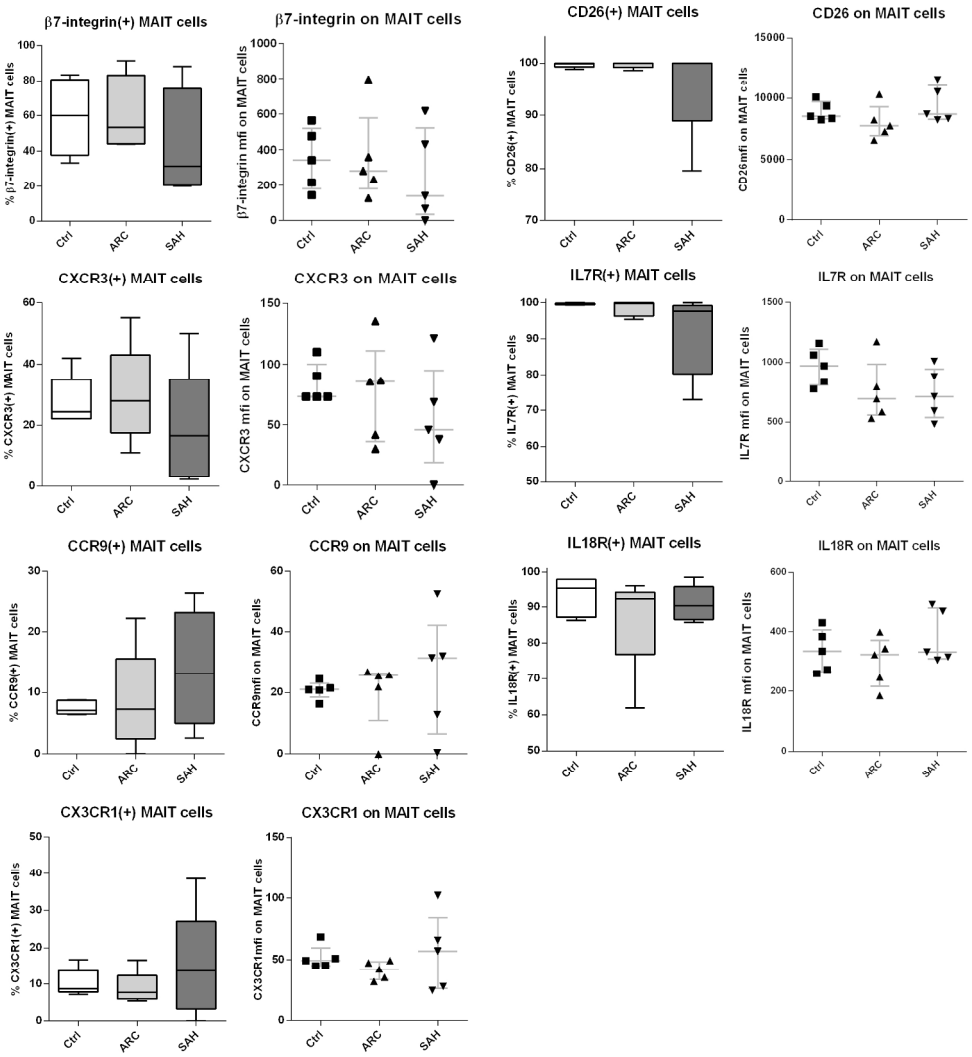

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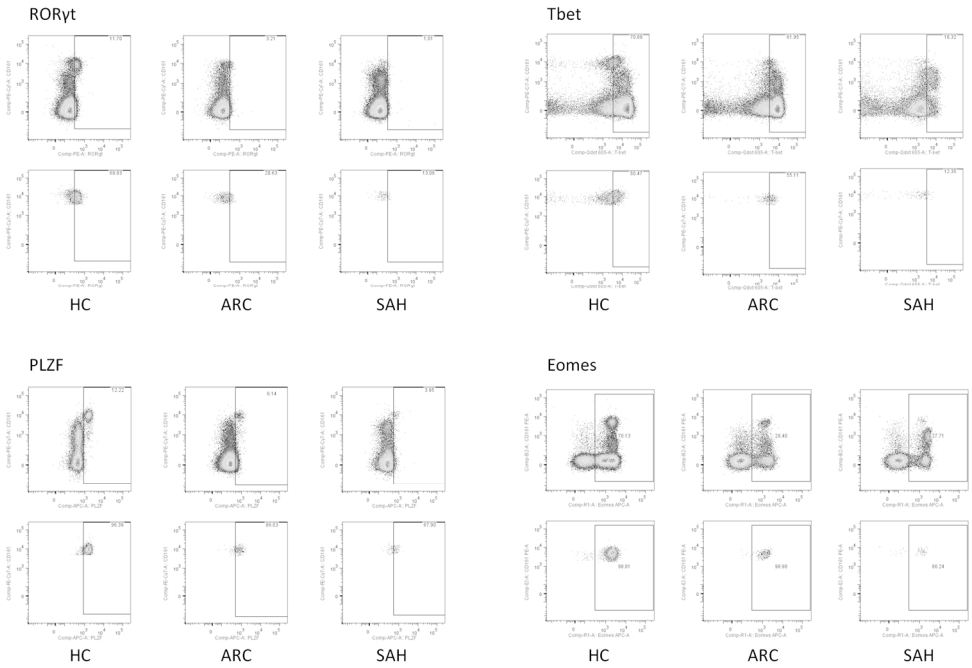

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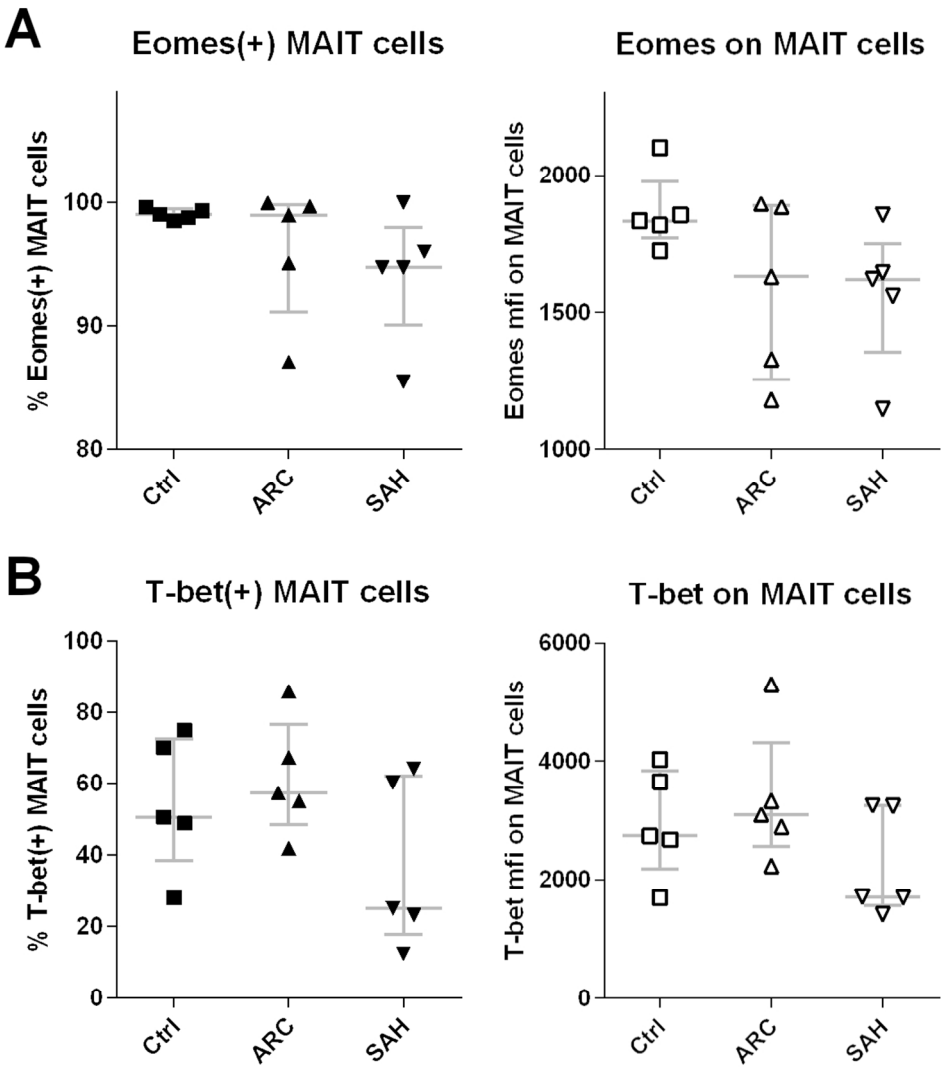
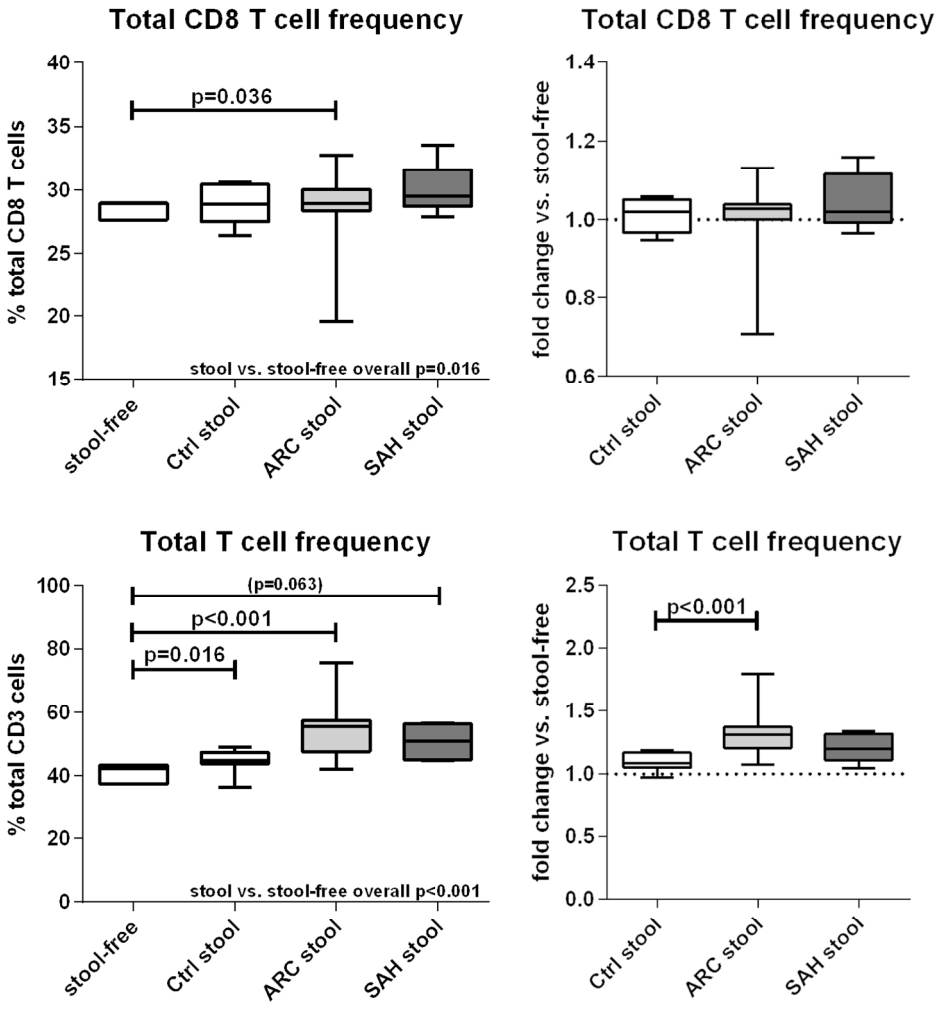


104x116mm (300 x 300 DPI)



127x136mm (300 x 300 DPI)

Marker	Colour	Clone	Company
CD3	Brilliant Violet 510	UCHT1	BioLegend
	PE-Cy7	UCHT1	eBioscience
	Pacific Orange	UCHT1	Invitrogen
CD4	Alexa Fluor 700	RPA-T4	eBioscience
	APC-eFluor 780	OKT4	eBioscience
	VioGreen	VIT4	Miltenyi Biotec
CD8a	PerCP-eFluor 710	SK1	eBioscience
CD161	APC	191B8	Miltenyi Biotec
	FITC	191B8	Miltenyi Biotec
	PE-Cy7	HP-3G10	eBioscience
TCR Vα7.2	Brilliant Violet 421	3C10	BioLegend
	FITC	3C10	BioLegend
	PerCP-Cy5.5	3C10	BioLegend
CD69	PE	FN50	eBioscience
HLA-DR	FITC	LN3	eBioscience
PD-1	PE	eBioJ105	eBioscience
TIM-3	FITC	F38-2E2	eBioscience
LAG3	APC	3DS223H	eBioscience
IFNγ	PE	4S.B3	eBioscience
TNFα	Alexa Fluor 488	MAb11	BD Biosciences
IL-17	Alexa Fluor 647	SCPL1362	BD Biosciences
CD107a	APC	H4A3	BD Biosciences
GrB	PE	GB11	BD Biosciences
Perf	Alexa Fluor 488	dG9	BD Biosciences
RORγt	PE	Q21-559	BD Biosciences
PLZF	APC	6318100	R&D Systems
Eomes	eFluor 660	WD1928	eBioscience
T-bet	Brilliant Violet 605	4B10	BioLegend
Integrin β7	APC	FIB504	BioLegend
CXCR3	PE	1C6/CXCR3	BD Biosciences
CCR9	PE	L053E8	BioLegend
CX3CR1	FITC	2A9-1	BioLegend
CD26	PE	2A6	eBioscience
CD57	FITC	HCD57	BioLegend
CD127	FITC	MB15-18C9	Miltenyi Biotec
IL-18R	PE	H44	eBioscience
Ki67	FITC	B56	BD Biosciences
live-dead dye	near IR	NA	Invitrogen

Supplementary Table 1

Common name	Gene Symbol	Gene Title	Representative Public ID	Affymetrix Probe Set ID
Integrin αE, CD103	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	NM_002208	205055_at
Integrin α4, CD49D	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	NM_000885	205884_at
Integrin α4, CD49D	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	BG532690	213416_at
CXCL10, IP10	CXCL10	chemokine (C-X-C motif) ligand 10	NM_001565	204533_at
MR1	MR1	major histocompatibility complex, class I-related	NM_001531	207565_s_at
MR1	MR1	major histocompatibility complex, class I-related	AF010446	210223_s_at
MR1	MR1	major histocompatibility complex, class I-related	AF031469	210224_at
MR1	MR1	major histocompatibility complex, class I-related	AI270356	235352_at
RORγt	RORC	RAR-related orphan receptor C	NM_005060	206419_at
RORγt	RORC	RAR-related orphan receptor C	AI218580	228806_at
PLZF	ZBTB16	zinc finger and BTB domain containing 16	NM_006006	205883_at
Eomesodermin	EOMES	eomesodermin	NM_005442	231776_at
Galectin 1	LGALS1	lectin, galactoside-binding, soluble, 1	NM_002305	201105_at

Supplementary Table 2

Supplementary materials and methods

Study ethical approval.

All local recruiting centres had individual ethical approval: the North East London Research Ethics Committee (08/H0702/52); the National Research Ethics Service (NRES) Committees South West (13/SW/0219), Portsmouth South Central (12/SC/0359), London Riverside (135979), London Westminster (12/LO/1417); the Local Research Ethics Committee (LREC) South Birmingham (98/CA5192 and 06/Q2708/11); the Steroids or Pentoxifylline for Alcoholic Hepatitis trial (STOPAH) (09/MRE09/59); the Richmond VA Medical Center IRB (bajaj004).

Immunophenotyping, intracellular staining and functional apoptosis.

MAIT-cells were identified by flow cytometry using the following panel: CD3/CD4/CD8/TCR_Vα7.2/CD161. We measured (i) activation markers and immune-checkpoint receptors (CD69/HLA-DR/PD1/TIM3/LAG3); (iii) intracellular cytokines/cytotoxicity markers (IFNγ/TNFα/IL17/GranzymeB/Perforin/CD107a); (iv) homing-related markers (beta7-integrin/CCR9/CXCR3/CX3CR1/CD26); (v) cytokine receptors (IL7R/IL18R); (vi) proliferation/senescence markers (Ki67/CD57); (vii) transcription factors (RORγt/PLZF/Eomes/T-bet). The impact of stool on MAIT-cell apoptosis was assessed by exposing healthy PBMC cultures with FEB (as described above) and measuring apoptosis rates using the Vybrant-FAM Poly-Caspase kit (ThermoFisher-Scientific, UK) following the manufacturer's instructions. Supplementary Table 1 provides the comprehensive list of antibody clones, fluorochromes and manufacturers.

Cells were stained with surface antibodies (20 minutes, 4°C), then either fixed with BD Cytofix buffer and acquired, or fixed/permeabilised with BD Cytofix/Cytoperm (BD Biosciences, UK) (20 minutes, 4°C), stained with intracellular antibodies (30 minutes, 4°C), and finally acquired in FACS-staining buffer (foetal bovine serum 1% in PBS). The cytotoxicity-related marker CD107a was pre-stained for the whole duration of the cultures (allophycocyanin/APC, clone H4A3, BD Biosciences, UK). Samples were acquired on a FACSCanto-II (BD Biosciences, UK).

Detection of TCR_Vα7.2 (TRAV1-2) by TaqMan PCR.

RNA from PBMC and colon pinch-biopsies was extracted by firstly solubilising in TriReagent (Ambion/ThermoFisher Scientific, UK). Samples were then extracted with chloroform (15 minutes, RT) followed by isopropanol precipitation (10 minutes, RT), washed with ethanol 75% in RNAse-free water, resuspended in RNAse-free water and stored at -80°C. RNA concentrations were determined with a NanoDrop spectrophotometer (Thermo Scientific, UK). cDNA was transcribed with RNA QuantiTect Reverse Transcription kit (Qiagen, UK). Real-time TaqMan PCR was performed on an ABI 7500 system (Applied Biosystems/ThermoFisher Scientific, UK) using TaqMan Universal Master Mix II with UNG (Applied Biosystems/ThermoFisher Scientific, UK), 100 ng/reaction of cDNA and the following previously published primers and probe(1,2): TRAV1-2 forward primer: 5'-TCCTTAGTCGGTCTAAAGGGTACAG-3' (18 µmol/L); TRAV1-2 probe: 5'-FAM-CTCCAGATGAAAGACTCTGCCTCTTACCTCTGTGC-NFQ-3' (5 µmol/L); Constant-alpha reverse primer: 5'-CATCAGAATCCTTACTTTGTGACACATTTG-3' (18 µmol/L). The TaqMan PCR protocol was as follows: 1 cycle at 50°C, 2 minutes (UNG incubation); 1 cycle at 95°C, 10 minutes (polymerase activation); 99 two-step cycles with denaturation at 95°C, 15 seconds, followed by annealing/extension at 60°C, 1 minute. Human large ribosomal protein (RPLP0) was used as reference gene (Applied Biosystems/ThermoFisher Scientific, UK).

Identification of TCR_Vα7.2-expressing cells via immunohistochemistry and imaging.

Immunohistochemistry was performed as previously reported(3). In brief, cubes of liver tissue (1-1.5 cm^3) were cut, snap frozen in liquid nitrogen and stored at -80°C. Tissue was then embedded in Cryoembed (Leica Biosystems, UK) and 7 µm-thick sections cut using a cryostat (specimen temperature: -13°C; chamber: -20°C). Sections were air-dried, fixed in acetone onto poly-L-lysine-coated slides (5 minutes), air-dried again and stored at -20°C in the dark until staining. Thawed sections were re-fixed in acetone (5 minutes), washed twice with 0.1% Tween-PBS buffer and endogenous peroxidase activity was quenched in 0.3% hydrogen peroxide-methanol (Sigma Aldrich, UK) (20 minutes). Slides were blocked with casein (1:10 in

PBS, Vector Laboratories, Burlingame, CA) (20 minutes), stained with purified primary antibody anti-TCR_Vα7.2 (clone 3C10, BioLegend, UK) or IgG1-isotype-control (1 hour, both at 50 µg/mL in PBS) followed by Impress secondary reagent (Vector Laboratories) (30 minutes, RT), then developed using ImmPACT™ DAB reagent (Vector Laboratories) (10 minutes) following manufacturer's instructions. Sections were finally counterstained with filtered haematoxylin (Leica Biosystems) (2 minutes) and developed in water (cold: 2.5 minutes; hot: 2.5 minutes). Once dry, slides were mounted using DPX (Cellpath, UK) and imaged on a Zeiss Axioskop 40 Microscope with 20x-40x magnification. Parenchymal and portal tract regions were identified and their areas measured using AxioVision SE64 v4.9. Numbers of TCR_Vα7.2(+) cells/mm^2 were counted.

References for supplementary methods

1. Van Rhijn I, Kasmar A, de Jong A, Gras S, Bhati M, Doorenspleet ME, et al. A conserved human T cell population targets mycobacterial antigens presented by CD1b. Nat Immunol. 2013 Jul;14(7):706–13.

2. Tilloy F, Treiner E, Park S-H, Garcia C, Lemonnier F, de la Salle H, et al. An Invariant T Cell Receptor α Chain Defines a Novel TAP-independent Major Histocompatibility Complex Class Ib–restricted α/β T Cell Subpopulation in Mammals. J Exp Med. 1999 Jun 21;189(12):1907–21.

3. Jeffery HC, van Wilgenburg B, Kurioka A, Parekh K, Stirling K, Roberts S, et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. J Hepatol. 2016 May;64(5):1118–27.

Supplementary Figure and Supplementary Table legends

Supplementary Figure 1. *Markers of bacterial translocation in ALD patients and correlation with blood MAIT-cells*. Patients with ARC and SAH had increased levels of plasma endotoxin and plasma D-Lactate, two surrogate markers of bacterial translocation and dissemination, compared to controls. We observed a trend for an association between increasing concentrations of plasma D-Lactate and reduced frequencies of blood MAIT-cells in patients overall (ARC, light grey circles; SAH, dark grey triangles).
Box-whiskers plots represent $25^{th}/50^{th}/75^{th}$ %ile (box) and min/max (whiskers). Controls= white, ARC= light grey, SAH= dark grey.

Supplementary Figure 2. *Short-term alcohol abstinence does not restore blood MAIT-cell frequencies*. In a cohort of excess-alcohol-users undergoing alcohol abstinence, short-term (2-5 days) ethanol withdrawal did not cause quantitative recovery of blood MAIT-cells, which remained as low compared to healthy controls as they were at baseline. All the MAIT-cell frequencies were evaluated as percentages relative to total CD8 T-cells, normalising for any absolute or relative quantitative alteration in total T-cells or total CD8 T-cells which may have occurred as a consequence of excess alcohol consumption.
The box-whiskers plot represents 25th/50th/75th %ile (box) and min/max (whiskers) of healthy controls (white). The line plot represents individual values of excess-alcohol-users on an alcohol abstinence programme (clear circles and lines).

Supplementary Figure 3. *Homing markers on blood MAIT-cells in ALD*. Expression of homing markers CCR9, CX3CR1, beta7-integrins and CXCR3 on blood MAIT-cells were comparable between groups. Blood MAIT-cells from all patients and controls also expressed similar levels of the immunoenzyme CD26 and of the cytokine receptors for IL7 and IL18.
Box-whiskers plots represent $25^{th}/50^{th}/75^{th}$ %ile (box) and min/max (whiskers). Dot-whiskers plots represent $25^{th}/50^{th}/75^{th}$ %ile. Controls= white or squares, ARC= light grey or up-triangles, SAH= dark grey or down-triangles.

Supplementary Figure 4. *Representative FACS plots representing expression of transcription factors RORγt, PLZF, Tbet and Eomes in blood MAIT-cells*. For each transcription factor, representative FACS plots were chosen from one HC, one ARC and one SAH patient. FACS plots on each top row indicate the gating threshold defined on CD161/transcription_factor biparametric plots. FACS plots on each bottom row indicate the same gating threshold referred to total MAIT-cells only, which was used for the derivation of cell percentages and MFI analysed.

Supplementary Figure 5. *Expression of transcription factors Eomes and T-bet in blood MAIT-cells in ALD*. (A) Expression of transcription factor Eomes on blood MAIT-cells was comparable

between groups. (B) Expression of transcription factor T-bet on blood MAIT-cells was also comparable between groups.
Dot-whiskers plots represent $25^{th}/50^{th}/75^{th}$ %ile. Controls= squares, ARC= up-triangles, SAH= down-triangles.

Supplementary Figure 6. *Exposure of healthy PBMC to crude stool extracts does not affect total T-cells or total CD8(+) T-cells*. Total T-cells and total CD8(+) T-cells were not depleted by crude stool extracts, indicating lack of non-specific toxicity in this experimental setup.
Box-whiskers plots represent $25^{th}/50^{th}/75^{th}$ %ile (box) and min/max (whiskers). Dotted lines in box-whiskers plots indicate the no-response threshold for the respective parameter. Controls= white, ARC= light grey, SAH= dark grey.

Supplementary Table 1. *Comprehensive list of all antibody markers, clones, fluorochromes and manufacturers used for flow cytometry*.

Supplementary Table 2. *Comprehensive list of all genes of interest queried from publicly available microarray datasets*.