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Data Article

A shotgun proteomic dataset of human mucosal-associated invariant T cells



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ABSTRACT

Mucosal-associated invariant T (MAIT) cells represent a unique unconventional T cell population important in eliciting immunomodulatory responses in a range of diseases, including infectious diseases, autoimmunity and cancer. This innate-like T cell subset predominantly express CD8 in humans. Unlike conventional CD8+ T cells, which recognize peptide antigen presented by polymorphic major histocompatibility complex (MHC) molecules, MAIT cells are restricted by MR1, a non-polymorphic antigen-presenting molecule widely expressed in multiple tissues. Thus, identification of proteomic signature of MAIT cells in relation to conventional T cells is pivotal in understanding it's specific functional characteristics. The high-resolution dataset presents here comprehensively describes and compare the whole cell proteomes of MAIT (TCRV α 7.2⁺CD161⁺) and conventional/non-MAIT T cells (TCR Va7.2-CD161-) in humans. The dataset was generated using the proteomic samples prepared from matched T cell subsets sorted from peripheral blood mononuclear cells (PBMC) of three healthy volunteers. Peptides obtained from trypsin-digested cell

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lysates were analysed using Data-Dependent Mass Spectrometry (DDA-MS). Label-free quantitation of DDA-MS data using MaxQuant and MaxLFQ software identified 4,442 proteins at a 1 % false discovery rate. Of them, 3680 proteins that were detected with single UniProt accession and a minimum of 2 unique or razor peptides were assessed to identify differentially abundant proteins between MAIT cells and conventional T cells, including total T cells and CD8⁺ T cells. The dataset comprises high-quality label-free quantitative proteomic data that can be used to compare the expression pattern of whole cell proteomes between the above-mentioned T cell populations. Further, this can be used as a reference proteome of human MAIT cells for the in-depth understanding of the MAIT cell behaviour among T cells and to discover potential therapeutic targets to modulate MAIT cell function.

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Specifications Table

Subject	Immunology
Specific subject area	Mucosal-associated invariant T (MAIT) cells are unconventional T cells important in human immunity. However, few studies have examined the primary human MAIT cell
	proteome. As majority of MAIT cells express CD3 ⁺ and CD8 ⁺ , this dataset compares
	the proteome of MAIT cells with matched conventional T cells (total CD3 ⁺ and
	CD3 ⁺ CD8 ⁺ T cells) circulating in the blood of healthy volunteers to establish
Town of Jaka	differentially abundant proteins.
Type of data Data collection	Tables, Figures, Raw and Processed data
Data conection	Label-free shotgun data were generated from MAIT, and conventional/non-MAIT T cells purified from the blood of three healthy volunteers. For sorting, CD3 ⁺ , CD161 ^{high} , and
	TCR V α 7.2 ⁺ cells were gated as MAIT cells. Peptide samples obtained from
	trypsin-digested cell lysates were analysed using an Orbitrap Fusion $^{\rm TM}$ Tribrid $^{\rm TM}$ mass
	spectrometer (Thermo Fisher Scientific, USA) inline coupled to nanoACQUITY
	ultra-performance liquid chromatography system (Waters, USA). Peptides were separated using a 160-minute chromatographic gradient at 0.3 µl/min flow rate. Raw
	proteomic data were analysed and normalized using MaxQuant (Release 1.6.0.16) and
	MaxLFQ software respectively.
Data source location	Raw proteomic data are available via ProteomeXchange [1]. Data were generated from
	volunteers recruited at QIMR Berghofer Medical Research Institute -Brisbane,
Data accessibility	Queensland - Australia. Repository name: ProteomeXchange via PRIDE database Data identification number:
Data accessibility	PXD052574 https://www.ebi.ac.uk/pride/archive/projects/PXD052574
	- reviewer_pxd052574@ebi.ac.uk
	- https://www.ebi.ac.uk/pride/review-dataset/3d4ae97c1c1d4edd9dda94c7a9824e23
	https://doi.org/10.1016/J.DIB.2024.110786

1. Value of the Data

- The dataset generated by label-free shotgun proteomic approach enables the comparison of approximately 3600 proteins between human MAIT and conventional T cells (including total CD3⁺ T cells, and CD3⁺CD8⁺ T cells).
- Researchers can use this dataset to explore the phenotypic and functional characteristics of human MAIT cells and differentiate them from conventional T cells.
- As the dataset was generated from peripheral blood mononuclear cells (PBMC) collected from normal healthy adults, it can be used as an exploratory proteome when characterizing changes in MAIT cell proteome associated with multiple conditions.

2. Background

Mucosal-associated invariant T (MAIT) cells are evolutionary conserved, unconventional T cells, characterized by the expression of semi-invariant T cell receptor (TCR) with a canonical TRAV1-2/TRAJ33 (V α 7.2/J α 33) that can recognize vitamin B metabolites derived from some bacteria and fungi [2]. Their immunomodulatory functions are mainly associated with secretion of cytotoxic molecules [3] and cytokines [4–6]. In humans, MAIT cells are found in mucosal tissues [7,8], peripheral blood [9] and liver [10,11]. MAIT cells represent approximately 10 % of circulating T cells and present a memory phenotype that allow them to rapidly respond to stimulus in a range of pathological conditions [10,12]. Since their discovery 15 years ago, omics analysis of human MAIT cells have evidenced their phenotypic and functional characteristics [13–15]. MAIT cells are classified under the common T cell antigen, CD3, and primarily express CD8 in humans, which is a canonical marker for conventional cytotoxic T cells [7]. Therefore, describing the proteomic demarcation of MAIT cells in relation to conventional T cell populations is crucial for identifying their unique functional and phenotypic properties.

3. Data Description

The dataset presented in this article includes label-free quantitative proteomic data for human MAIT (CD3⁺TCR V α 7.2⁺CD161⁺) and conventional T cells (including total CD3⁺ and CD8⁺ T cells bearing a TCR V α 7.2⁻CD161⁻ phenotype). The data were generated using a Data-Dependent Acquisition approach (DDA-MS) with a Orbitrap FusionTM TribridTM mass spectrometer (Thermo Fisher Scientific, USA) inline coupled to nanoACQUITY ultra performance liquid chromatographic (Waters, USA) system. The method used to isolate the cell populations, as well as the key steps for proteomic sample preparation and data acquisition, are summarized in Fig. 1. Raw data were analyzed using MaxQuant software (Release 1.6.0.16) [16], with the analysis conducted against the UniProt human-reviewed proteome. MaxLFQ was employed to normalize the protein expression data for label-free quantification [17]. All raw and processed data are deposited and publicly available through the ProteomeXchange data repository (PXD052574), as summarized in Table 1.

The parameter file deposited with the dataset guides the researchers on the criteria used in the identification and quantification of peptides and proteins. The current analysis has led to the detection and quantification of 4440 protein groups at a peptide and protein false discovery rate (FDR) of 1 %. In future applications, the raw data can be reanalyzed with different parameters depending on the study objectives. Of the identified protein groups in the current analysis, 4110 proteins (93 % of total) had a single UniProt accession name and 3680 proteins (83 % of total) were detected with a minimum of 2 unique or razor peptides (Fig. 2A). To assess the quality of the selected proteins, the data were further analyzed to determine the normalized protein intensity distribution (Fig. 2B), the number of peptide ions

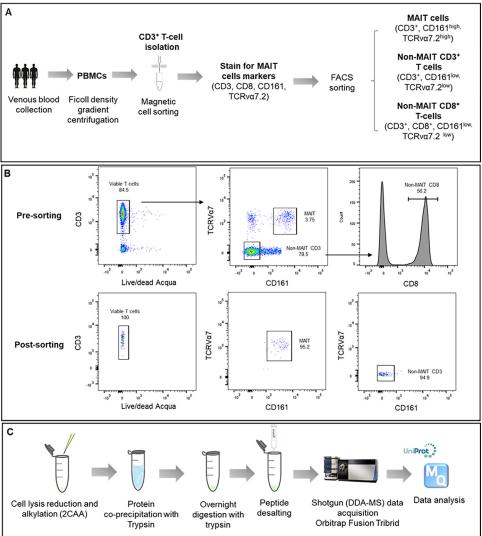


Fig. 1. Experimental design used to generate the proteomic data. **A.** Key steps for isolating T cell populations **B.** Gating strategy used for fluorescence-activated cell sorting (FACS) to obtain MAIT cells and non-MAIT T cell populations at a high purity. Gating strategy is shown for cells pre and post-FACS. **C.** Key steps followed for the obtention of trypsin digested peptide samples and proteomic data acquisition.

Table 1

Data files available through the ProteomeXchange data repository (PXD052574).

	File/folder	Description
1	Rep1_MAIT.raw	.raw file of MAIT cells – Biological Replicate 1
2	Rep2_MAIT.raw	.raw file of MAIT cells – Biological Replicate 2
3	Rep3_MAIT.raw	.raw file of MAIT cells – Biological Replicate 3
4	Rep1_nonMAIT_CD3.raw	.raw file of nonMAIT CD3 ⁺ T cells – Biological Replicate 1
5	Rep2_nonMAIT_CD3.raw	.raw file of nonMAIT CD3 ⁺ T cells - Biological Replicate 2
6	Rep3_nonMAIT_CD3.raw	.raw file of nonMAIT CD3+ T cells – Biological Replicate 3

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(continued on next page)

Table 1 (continued)

	File/folder	Description
7	Rep1_nonMAIT_CD8.raw	.raw file of nonMAIT CD8+ T cells – Biological Replicate 1
8	Rep2_nonMAIT_CD8.raw	.raw file of nonMAIT CD8 ⁺ T cells - Biological Replicate 2
9	Rep3_nonMAIT_CD8.raw	.raw file of nonMAIT CD8 ⁺ T cells – Biological Replicate 3
10	search.zip	MaxQuant ouput files resulted from the analysis of the above raw files against UniProt/SwissProt human reviewed proteome
11	parameters.txt	Parameters used in the data analysis through MaxQuant, MaxLFQ search engine
12	human_proteome_reviewed_25102017.fasta	UniProt/SwissProt proteome database used in the analysis
13	MaxQuant_MaxLFQ_Output_protein group	MaxQuant ouput files giving the protein quantification data
	file.txt	and LFQ normalised protein intensities

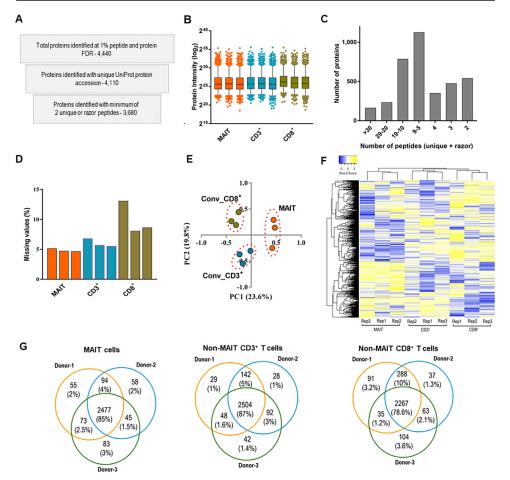


Fig. 2. An overview of the proteomic dataset obtained from MAIT and conventional/non-MAIT T cells. **A.** Number of proteins obtained at various stages of data curation **B.** Distribution of normalized protein intensities across different samples (central lines and boxes represent means and 95 % confidence intervals respectively while whiskers are 2.5 to 97.5 percentiles) **C.** Number of proteins identified and quantified with varying number of peptides **D.** Percentage of proteins with missing values in each cell population (percentage was obtained from the total number of identified proteins) **E.** Principal component analysis of protein intensity data **F.** Heat map showing the hierarchical clustering of quantified protein intensity data. **G.** Venn diagrams ilustrating the variations in protein expression across three donors for all cell populations. Within each T cell group, these diagrams visualize both common and differential proteins across the donors.

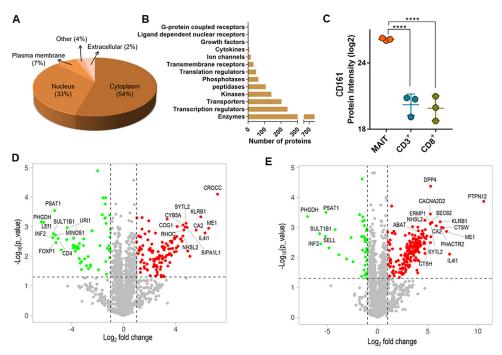


Fig. 3. Gene ontology and differential expression analysis of normalized protein intensity data. **A.** Distribution of proteins in different subcellular compartments is given as a percentage of all proteins selected for differential expression analysis. **B.** Distribution of proteins selected for differential expression analysis across different functional groups (Qiagen, IPA). **C.** Expression of CD161 in three cell subsets as based on DDA-MS data (****q < 0.0001, multiple t-test with false discovery determination by two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli) **D.** Volcano plots labelling the top 20 differentially expressed proteins in MAIT cells compared to non-MAIT CD3⁺ T cells **E.** Volcano plots labelling the top 20 differentially expressed proteins in MAIT cells compared to non-MAIT CD8⁺ T cells.

detected per protein (Fig. 2C), and the percentage of missing protein intensity values in each sample (Fig. 2D). The results of the principal component analysis and unsupervised hierarchical clustering of proteomic data from the different T cell subsets are shown in Fig. 2E and 2F, respectively. The protein expression variation (fold change) across different donors is presented in Fig. 2G.

The current analysis excluded the proteins with missing expression data in more than 50 % of samples and those with m-score of below 5 when calculating differential expression across the three T cell populations. As per the subcellular (Fig. 3A) and functional group (Fig. 3B) analysis, about half (54 %) of the selected proteins (n = 1566) were mainly present in the cytoplasm and 28 % were classified as enzymes (n = 798). As expected, differential expression analysis revealed significant overexpression of CD161 in MAIT cells (Fig. 3C). In total, 243 (\sim 8 %) and 285 (\sim 10 %) proteins were differentially expressed (DE) in MAIT cells compared to conventional CD3⁺ and CD8⁺ T cells, respectively. The top 20 DE proteins in MAIT cells compared to CD3⁺ and CD8⁺ are shown in Fig. 3D and 3E. These figures demonstrate that the majority of DE proteins are overexpressed in MAIT cells (Fig. 3D and 3E). Further, the DE proteins and canonical pathways are summarized in Fig. 4 and Table 2, respectively.

00000	7.21	В	MEN2	A 334	B	00,051	258	B 265	MAPKAPK2	A 204	B 3.43	FKBP1	L A	B 1.47
MEI	653	6.87	HSF1	3.31		UBE3C	256	1.43	SAMD9L	200	1.66	BCAS		1.81
IL411	625	7.18	PTPRJ	329	409	8507	255	3.00	ABAT	1.95	531	EDC		3.52
KL 581	592	624	SEPT8	327	361	UQCC2	252	243	GBP5	1.95	234	PDESC		320
CA2	5.57	585	HELZ	322	3.34	CHP1	252	1.90	LYST	1.95	276	TMEM258	5	3.09
ABCB1	5.09	393	8509	321	333	TSSC1	251		SEC62	1.94	5.43	NAGLU	J	393
CTSH	493	527	INPP1	3.16		PPP1511	251	374	SH2D2A	1.93	1.64	CASP		1.45
SPA1L1	4.88	482	SMAR CB1	3.11		RBM33	251	295	LMF2	1.91	285	C TSV	V	6.55
PHAC TR2	4.81	638	SCRN1	3.10	254	AAR2	249	255	FUK	1.89	307	YAP	2	1.58
SYTL2	479	526	MANEA	3.04	3.49	STARD9	248		TPD52	1.85	203	PPP1 598	3	304
COGI	472	241	GZMK	3.04	279	TBX21	248		STOM	1.85		ZN F830		219
RHCC	4.63	1.68	RPS6KA4	3.03		PTPN18	247	3.28	R.EKHA2	1.84		MORF4L1		3.45
CYB5A	4.55	1.48	MYO5A	3.02	217	R88P6	247	3.42	SERPINA1	1.84	3.09	GOST	2	320
NHSL2	4.55	4.72	ATG16L1	3.00	3.01	APOBR	245		TDRKH	1.84	291	SUGP		213
MAP2K8	4.49	214	DCP18	297	3.27	TRIP4	245	226	FAM91A1	1.83	237	THEM		255
MOV10	4.41		BTAF1	297	3.48	NPER_1	241	3.48	NBEAL2	1.82	3.52	MON		253
LZTFL1	4.37	4.60	ZC3H7B	297	213	GZMA	240		ABCD3	1.81		BLCC 15	·	4.47
NPC1	4.18	298	TRIM56	295	296	TNFAIP3	238	247	FBX06	1.80	293	S100A10		1.59
DPR4	411	529	JUN	293	324	RF28P1	235	1.55	ICAM2 PPP653	1.73	238	AATH		3.57
UGOH	4.07	296	ERMPI	290	5.28	NDC1	235	277		1.72	215	DDB		269
GALC	4.05	4.15	LPCAT4 P5NP	290	3.73 3.53	SNAPN JADE2	233 233	-	SH2D3C MTR	1.66	285	PAP		4.11 3.54
R 5 P9	3.95	-	PTMS	286	335	WAC	233	245	5100A4	1.64	1.00	C2CDS	-	227
TRPV2	3.92		CLPTMI	284	1.96	LGAL53	230	3.14	RPR30	1.62	263	MRR.45		3.89
RAB68	390	432	EOMES	282	4.01	TMEM205	229	1.93	NUR85	1.62	1.48	TRAE		262
KDSR	3.88	4.13	BNP1	278	1.48	ASPSCR1	229	1.30	MAP3K4	1.61	249	DNAJC12		231
JUNE	377	364	TANK	278	300	M5PS21	2.26		TOMMS	1.61	249	TUBGCR		1.43
MMTAG2	372	420	SYI	277		INPR_1	220	2.29	MESOC2	1.59	491	PSMG		207
MPRIP	371	3.59	PUM	276		P2 5 Y8	219	285	COGS	1.59	1.85	APOC		237
FAH	367	3.98	RUN02	276	302	GFER	217	1.62	PRPF388	1.58	215	MA		236
AGPATS	365	379	BRPF1	273		PAR P12	217		TBC1D9B	1.55		GALA		1.76
IT GB7	360		RAB11 FIP1	269	471	EHEP1L1	213		ANXA4	1.55		WPG	2	215
PR KCD	358	245	WDFY1	268	260	MPST	212	403	STRN3	1.54	1.51	PNPLA		225
TNFSF14	355	382	CCDC93	268	422	USP19	211	3.01	CIAOI	1.53		SH2D1/	4	1.29
TMCO1	3.50	262	NUD T16	265	269	ZC3HC1	209		RRAS2		1.25	TMEM214	4	291
5518	3.45		DCTPP1	265	425	SVL	208	248	SETD3		225	MOB	2	3.58
SEC11C	3.45	381	SKAP2	262	1.84	GYGI	205	1.84	SQSTM1	1	3.18	MKEN	2	1.74
PYCRL	3.40	388	UBR1	260	248	GPRN3	205	207	CD84		295	CCDC8	5	1.75
S.AMF1	3.40	402	SGR_1	259	1.62	MYO1F	205	1.61	CWC27		1.65	ENDOC	<u>، ا</u>	226
	3.40 3.37	4.02	SGPL1 CCDC88C	259 258	1.62	MYO1F NEDD9	205 205	1.61 2.18	CWC27 LPP		1.65	ENDOC S100A11		226
SLAMF1		10.58						218			321			1.32
SLAMF1 PTPN12		10.58 B	C CDC88C		В	NED09		218 B	UPP	A		\$100A11	A	
SLAMF1 PTPN12 ANXA1		10.58 B 1.29			B 527	NEDD9		218 B 425	UPP SKAPI	1.81	321	510 0A1	A 323	1.32
SLAMF1 PTPN12 ANXA1 GCHFR		10.58 B 1.29 1.60	C CDC88C C ACNA2D2 FN1		B 5.27 1.59	NEDO9 LAMTOR3 STAU1		2 18 B 4 26 3 16	UPP SKAPI HSPA14	1.81 1.81	321	5100A1 TAB 2 NAA 16	A 323 323	1.32
SLAMF1 PTPN12 ANXA1 GCHFR MRPS27		10.58 B 1.29 1.60 2.32	C CDC88C		B 527 1.59 3.69	NEDD9 LAMTOR3 STAU1 DGUOK		218 B 425 316 434	UPP SKAPI	1.81 1.81 1.89	321 B	S100A1 TAB 2 NAA 16 PRKAR 18	A 323 323 332	1.32
SLAMF1 PTPN12 ANXA1 GCHFR		10.58 B 1.29 1.60 2.32 1.46	C CDC88C C ACNA2D2 FN1 OXR1		B 527 1.59 3.69 4.38	NEDO9 LAMTOR3 STAU1		2.18 B 4.26 3.16 4.34 1.70	UPP SKAPI HSPA14 DSTN	1.81 1.81	321	5100A1 TAB2 NAA16 PRKAR1B SF385	A 323 323 332 332 335	1.32
SLAMF1 PTPN12 ANXA1 GCHFR MRPS27 DENND4C		10.58 B 1.29 1.60 2.32 1.45 1.49	C CDC88C C ACNA2D2 FN1 OXR1 METTL14		B 5.27 1.59 3.69 4.38 3.88	NEDD9 LANITOR3 STAU1 DGUOK GNLY		218 B 425 316 434 1.70 327	UPP SKAPI HSPA14 DSTN EPH02	1.81 1.81 1.89 1.92	321 B 1.26	5100A1 TAB 2 NAA 16 PRKAR 18 SF 38 5 POLR2G	A 323 323 332 332 335 338	1.32 B
SLAMFI PTPN12 ANXA1 GCHFR MRP527 DENND4C LPCATI		10.58 B 1.29 1.60 2.32 1.46	C CDC88C C ACNA2D2 FN1 OXR1 METTL14 TMEM63A		B 527 1.59 3.69 4.38	NEDO9 LAUITOR3 STAU1 DGUOK GNLY RBCK1		2.18 B 4.26 3.16 4.34 1.70	LPP SKAPI HSPA14 DSTN EPH02 NDUFAF3	1.81 1.81 1.89 1.92 1.97	321 B	S100A1 TAB 2 NAA16 PRKAR 1B SF385 POLR2G GANT	A 323 323 332 335 338 340	1.32
SLAMFI PTPN12 ANXA1 GCHFR MRPS27 DENND4C LPCAT1 MBP		10.58 B 1.29 1.60 2.32 1.46 1.49 2.02	C CDC88C C ACNA2D2 FN1 OXR1 METTL14 TMEM63A MT-ND4		B 527 1.59 3.69 4.38 3.88 2.90	NEDD9 LAUITOR3 STAU1 DGUOK GNLY RBCKI MCM3		2 18 B 4 26 3 16 4 34 1.70 3 27 1.50	LPP SKAP1 HSPA14 DSTN EPHX2 NDUFAF3 RGS10	1.81 1.81 1.89 1.92 1.97 1.99	321 B 1.26	S100A1 TAB 2 NAA16 PRKAR 1B SF385 POLR2G GANT CRYL1	A 323 323 332 335 338 340 345	1.32 B
SLAMPI PTPN12 ANXA1 GCHFR MRPS27 DENND4C LPCAT1 MBP DAPS		10.58 B 129 160 2.32 1.45 1.49 2.02 1.74	C CDC88C C ACNA202 FN1 OXR1 METTL14 TMEM63A MT-ND4 APOA1		B 5.27 1.59 3.69 4.38 3.88 2.90 3.10	NEDD9 LAUITOR3 STAU1 DGUOK GNLY RBCKI MCN3 HMGN1		2 18 B 4 26 3 16 4 34 1.70 3 27 1.50 1.58	LPP 9KAP1 HSPA14 DSTN EPH02 NDUFAF3 RGS10 MRP59	1.81 1.89 1.92 1.97 1.99 2.05	321 B 1.26	S100A1 TAB 2 NAA16 PRKAR 1B SF385 POLR2G GANT	A 323 323 332 335 338 340 345 346	1.32 B
SLAMPI PTPN12 ANXA1 GCHFR MRPS27 DENND4C LPCAT1 MBP DAPS NFKBB		10.58 B 129 160 2.32 1.45 1.49 2.02 1.74 3.47	C CDC88C C ACNA2D2 PN1 OXR1 METTL14 TMEM63A MT-ND4 APOA1 BF283		B 527 159 369 438 388 290 310 407	NEDD9 LAUTOR3 STAU1 DGUOK GNLY RSCK1 MCM3 HMGN1 GL/IN		218 B 426 316 434 1.70 327 1.50 1.58 4.73	LPP SKAPI HSPA14 DSTN EPH02 NDUFAF3 RG510 MRP59 RDH11	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09	321 B 1.26	S100A1 TAB 2 NAA16 PRKAR 1B SF385 POLR2G GANT CRYL1	A 323 323 332 335 338 340 345	1.32 B
SLAMPI PTPN12 ANXAI GCHFR MRPS27 DENND4C LPCATI MBP DARS NFKBB CKARI		10.58 B 1.29 1.60 2.32 1.46 1.49 2.02 1.74 3.47 2.34	C CDC88C PN1 OX51 METTL14 TMEM63A MT-ND4 APOA1 BF283 G\$K38		B 527 1.59 3.69 4.38 3.88 2.90 3.10 4.07 3.75	NEDD9 LAUTOR3 STAU1 DGUOK GNLY RBCK1 MCM3 HMGN1 GL/IN C170r59		2 18 B 4 26 3 16 4 34 1.70 3 27 1.50 1.58 4 73 3 22	LPP SKAPI HSPA14 DSTN EPH02 NDUFAF3 RG510 MRP39 RDH11 MBLAC2	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12	321 B 1.26	S100A1 TAB 2 NAA 16 PRKAR 18 SF38 5 POLR2G GANT CRYL1 TRAF1	A 323 323 332 335 338 340 345 346	1.32 B
SLAMFI PTPN12 ANXAI GCHR MRPS27 DENND4C LPCATI NEP DARS NFKBB CKAR TMUBI		1058 B 129 160 232 146 1.49 202 1.44 3.47 2.34 260 1.41 4.50	CCDC88C CACNA202 PN1 OX51 METTL14 TMEM53A MT-ND4 APOA1 BF283 G5K38 DBNND48		B 527 159 369 438 290 310 407 375 294 144 314	NEDO9 LAUITOR3 STAUI DGUOK GNLY RECKI MCM3 HMGN1 GL/IN C170759 PHC3		218 B 425 316 434 1.70 327 1.50 1.58 4.73 322 325 401 2.77	UPP SKAPI HSPAI4 DSTN EPHO2 NDUFAF3 RGS10 MRP59 RDH11 MBLAC2 PRXABI FAM134C WDR89	1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19	321 B 1.26	S100A1 TAB 2 NAA16 PRKAR 1B SF38 5 POLR2G GANT CRYL1 TRAF1 ETS 1	A 323 323 332 335 338 340 345 346 352	1.32 B
S.AMFI PTPN12 ANXAI GCHFR MRPS27 DENND4C LPCATI MBP DAR8 NFKBB CKAR4 TMUBI QL1		1058 B 129 160 232 145 149 202 174 347 234 260 141 450 475	C CDC88C C ACNA202 FN1 OXF1 METTL14 TMEM63A MT-ND4 BF283 G\$K38 DBNND48 TUB88		B 5.27 1.59 3.69 4.38 2.90 3.10 4.07 3.75 2.94 1.44 3.14 4.45	NEDD9 LAUITOR3 STAU1 DGUCK GRUCK MCN3 HMGN1 GLUN C170759 PHC3 HSP90A82P		218 B 425 316 434 170 327 150 158 473 322 325 401 277 392	UPP SKAPI HSPAI4 DSTN EPHO2 NDUFAF3 RGS10 MRP59 RDH11 MBLAC2 PRKABI FAI1134C VOR89 FDXR	1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31	321 B 1.26	S100A1 TAB2 NAA16 PRKAR1B SF385 POLR2G GANIT CRYL1 TRAF1 ETS1 FAM98A	A 323 323 332 335 338 340 345 346 352 3.61	1.32 B
SLAMFI PTPN12 ANXAI GCHFR MRPS27 DENND4C LPCATI MEP DAP3 NFKBB CKAR8 TMUBI QL1 NO65		1058 B 129 160 232 145 149 200 174 347 234 260 141 450 475 464	C COC68C PN1 OX51 METTL14 TMEM63A MT-ND4 APOA1 BF283 G5K38 D BNND48 TUE88 TSPAN14		B 527 159 369 438 290 310 407 375 294 144 314 445 343	NEDD9 LAUTOR3 STAU1 DGUCK GNLY RBCK1 MC0/3 HMC0/3 HMC0/3 C170759 PHC3 HSP00A82P NF51 GSN/P HECA		218 8 425 316 434 170 327 150 158 473 322 325 401 277 392 257	UPP SKAPI HSPAI4 DSTN EPHO2 NDUFAF3 RGS10 MRP59 RDH11 MBLAC2 PRXABI FAM134C WDR89	1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36	321 B 1.26	TAB2 NAA16 PRKAR18 SF385 POLR2G GAMT CRYL1 TRAF1 ETS1 FAM98A WHSC1L1 HST1H1A	A 323 323 332 335 338 340 345 346 352 361 361 370	1.32 B
SLAMPI PTPN12 ANXAI GCHFF MRPS27 DENND4C LPCATI MBP DARS NFKBB CKAR CKAR CKAR CKAR QL1 NO66 UTN NOPPIO SNX17		1058 B 129 160 232 145 149 202 174 347 234 260 141 450 475 464 184	C CCCCEC C ACNA202 RV1 O XR1 METTL14 TIMEM63A METTL14 APOAL BF283 G SK38 D BIND48 TUB88 TSFAN14 GALEB1 C EBP2 NUR97		B 527 159 369 438 290 310 407 375 294 144 314 445 343 321	NEDO9 LAUTOR3 STAU1 DGUOK GRLY RECKI MCM3 HMGN1 GL/IN C170769 PHC3 HSP0A80P NFS1 GSKP HECA SUMP2		218 8 425 316 434 1.70 327 1.50 1.58 4.73 322 325 401 2.77 392 2.57 1.31	UPP SKAPI HSPAIL DSTN EPHO2 NOUFARS RGS10 MILAC2 PRKABI FAU1134C WDR89 FDXR ARAF RCL1	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39	321 B 1.26	S100A1 TAB 2 NAA16 PRKAR18 SF385 POLR20 GAMT CRYL1 TRAF1 ETS1 FAM98A WHSC1L1	A 323 323 332 335 338 340 345 346 352 361 361 370 371	1.32 B
SLAMP PTPN12 ANXAI GCHFR MRP227 DENND4C LPCATI DERND4C LPCATI NC65 CKAR TIMUBI QLI NC66 UTRN NO66 UTRN NO66 UTRN NO66 UTRN		1058 B 129 160 232 146 149 202 1.74 347 247 247 247 247 141 450 143 141 450 141 184 271	C CCC68C PN1 O/RT1 METTL14 TMEM63A MTTND4 FF283 C\$K188 D BNND48 TF98N14 GME81 TG98N14 GME81 TG98N14 GME81 TG98N14 GME81 C\$FP2 DBND45 TG98N14 GME81 C\$FP2 DBND45 C\$FP2 DBND45 C\$FP2 DBND45 C\$FP2		B 527 159 369 438 290 310 407 375 294 144 314 445 343 321 319	NEDO9 LAUTOR3 STAU1 OGUOK GNLY RECKI MCN3 HMGN1 GL/IN C170769 PHC3 C170769 PHC3 C170769 PHC3 SSNP NF51 GSNP HECA SUME2 NOP16		218 B 426 316 434 170 158 473 327 150 158 473 325 401 277 392 257 131 379	UPP HSPAIL DSTN EPHO2 NOUFAR3 RGS10 MRP3 ROH11 MBLAC2 PRKAB1 FAM134C WDR89 FDXR ARAF RCL1 MP3	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39 2.39	321 B 125 153	TAB2 NAA16 PRKAR18 SF385 POLR2G GAMT CRYL1 TRAF1 ETS1 FAM98A WHSCIL1 HST1H1A PDRG1	A 323 323 332 335 338 340 345 346 352 361 361 370 371 382	1.32 B
SLAMP PTPN12 ANXAI GCHR MRPS27 DENNU4C LPCATI MRP DARS NFKBB CKAR TMU81 QL1 NO66 UTRN NOPI0 SV17 CCDC132 CN275K		1058 B 129 160 232 145 149 202 174 347 234 260 141 450 475 464 184 271	C CCC68C RN1 OX51 METTL14 TMEM63A MTTD14 APOA1 EF283 C5K38 DEWD48 TSPAN14 GALEB CEBPZ VUP37 RPAP1 RPAP1 ZNF148		B 527 159 369 438 388 290 310 407 375 294 144 343 344 343 341 445 343 321 319 377	NEDO9 LAUTOR3 STAU1 DGUOK GRUY RECK1 MCN3 HMGN1 GLIM C170769 PHC3 C170769 PHC3 C170769 PHC3 C170769 PHC3 C170769 PHC3 C170769 PHC3 C170769 PHC3 C170769 PHC3 C170769 C170769 PHC3 C170769 NF515 C170769 NF515 C170767 C170769 C170767 C170769 C170767 C170767 C170769 C170767 C170777 C170777 C170777 C170777 C170777 C1707777 C1707777777777		218 8 425 316 434 170 327 150 158 473 322 325 401 277 392 257 131 379 149	UPP SKAPI HSPAIL DSTN EPHO2 NDUFAF3 RCS10 MRP59 RCH11 MBLAC2 PRKABI FAI134C WDR89 FDXR ARAF RCL1 MR8 COTL1	1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39 2.39 2.43	321 B 1.26	TAB 2 NAA16 PRKAR18 SF885 POLR2G GAMT CRYL1 TRAF1 ETS11 FAM98A WHSC1L1 HST1H/A PDR51 PNISR TMEM 245	A 323 322 332 335 338 340 345 346 345 361 361 370 371 382 383	1.32 B
SLAUFI PTPN12 ANXAI GCHFR MRPS27 DENND4C LPCATI MBP DAP3 NFKBB CKAR4 TIMUBI QL1 NO56 UTNN NOP10 SNX17 CCDC122 NP55K TRATI		1058 B 129 160 232 145 149 202 174 347 250 141 450 141 455 464 184 271 132 132	C CCC68C C ACNA202 PN1 NETTL14 TIMEN63A MT-ND4 APOA1 E P283 C SK38 D ENND48 TUB68 TUB68 TSFAN14 GALE81 C EEPZ N N/R7 RPAP1 2 N/143 C N/073		B 527 159 369 438 388 290 310 407 375 294 144 314 445 343 321 319 377 272	NEDOS LAUTOR3 STAU1 DGUOK MC013 HMC011 GUNN C170759 PHC3 C170759 PHC3 C170759 PHC3 C170759 PHC3 C170759 HC2CA SUMP2 SUMP2 NOP16 LONB CD5		218 B 425 316 434 170 327 150 158 473 322 325 401 277 139 257 131 379 145	UPP HSPAI4 DSTNI EPH02 NDUFAF3 RGS10 MIRP99 RDH11 MBLAC2 FRXAB1 FAU134C WDR99 FDXR ARAF RCL1 MIR COTL1 GYPC	1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39 2.39 2.43 2.53	321 B 125 153	TAB 2 NAA16 PRKAR18 SF385 POLR20 GANIT CRYL1 TRAF1 ETS1 FAM98A WHSC1L1 HST1H1A POIR31 PNISR TNEM245 EIF4E3	A 323 322 332 335 338 340 345 346 352 361 370 371 382 383 384	1.32 B
SLAMPT PTPN12 ANXAI GCHFR MRPS27 DENND4C LPCATI DENND4C LPCATI MRPS27 DENND4C LPCATI MRPS47 CCC132 NOPHO SNX17 CCDC132 NPS47 TAP54 TAP54		1058 B 129 160 232 146 149 202 174 347 234 260 141 450 457 464 184 271 294 264 184 132 465 152	C CCC68C NI OXA METTL14 TMEM63A MT NOL APOAI BF283 DBIND48 TSPAII4 GALE81 C CEP2 NJ R7 RPAPI 2NF145 C NOT3 TMEM588		B 527 159 369 438 388 290 310 407 375 294 407 375 294 407 375 294 407 375 294 319 321 319 377 272 325	NEDO9 LAUITOR3 STAU1 DGUGK GNLY RECKI MCM3 HMGN1 GUNN C170F9 PHC3 HSP(0AE39 NF51 GSNP HECA SUMF2 NOP16 LOHB C055 SMAD4		218 8 426 316 434 170 327 150 158 473 322 326 401 277 392 257 131 379 149 149 149	UPP SKAPI HSPAIL DSTNI EPHO2 NUUFAF3 RGS10 MIRPAF3 RGS10 MIRPAF3 ARAF RKABI MR8 ARAF COTL1 GYPC CNST	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39 2.39 2.43 2.53 2.54	321 B 125 153	TAB2 NAA16 PRKAR18 SF385 POLR26 GAMT CRVL1 TRAF1 ETS1 FAM38A WHSC1L1 HST1HHA POR51 PNISR TMEM 245 EIF423 GPA33	A 323 323 332 335 338 340 345 346 352 361 361 370 370 371 382 383 384 387	1.32 B
SLAUFI PTPN12 ANXAI GCHFR MRPS27 DENNDUC LPCATI MEP DARS NFKBB CKARE TMUBI QLI NORS UTRN NOPIO STATI PAFI SRAI		1058 B 129 160 232 146 1.46 1.49 2001 1.47 234 2.06 1.41 4.50 4.64 1.64 2.71 2.94 1.64 2.71 2.94 2.65	CCCC68C PNI METTL14 IMENTL14 IMENGIA APOAI EP28B G\$K38 OBNID4 EP28B G\$K38 OBNID4 EP28B G\$K38 OBNID4 EP28B C\$BNID4 CBE2 NUR7 RPAPI 2NF145 CNOT3 TIMENISSE		B 5.27 1.59 3.69 4.38 3.88 2.90 3.10 4.07 3.75 2.94 1.44 3.14 4.45 3.43 3.21 3.19 3.77 2.72 2.72 3.75 1.34	NEDOS STAUI OGUOK GNLY RBCKI MCNIS HMGNI GLNN C170765 PHC3 H5P0A82P PHC3 SUMP2 SUMP2 L0HB C05 SUMP2 L0HB C05 SUMP2 L0HB		218 B 425 316 434 170 327 150 158 473 322 326 401 277 392 257 401 277 392 257 131 379 149 146 401 385	UPP HSPAIL DSTNI EPHO2 NDUFAF3 RGS10 MIR/PS9 ROH11 MIR/RCS PRKAB1 FAN134C WDR89 FDXR RCL1 MIR RCC1 MIR RCC1 GYPC COTL1 GYPC CN51 THUMIR03	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39 2.39 2.39 2.39 2.39 2.43 2.54 2.54	321 B 125 153	TAB 2 NAA16 PRKAR18 SF385 POLR2G GAMT CRYL1 TRAF1 ETS1 FAM98A WHSC1L1 HST1H1A PORG1 PNSR TMEM 245 EIF4E3 GPA33 COMMD2	A 323 323 332 332 335 338 340 345 346 345 346 352 361 361 370 371 382 384 384 384	3.84
SLAMPT PTPN12 ANXAI GCHR MRP27 DENNO4C LPCATL LPCATL LPCATL MBP CRAR UTAUBI QLI NOPIO SNX17 CCDC132 NOPPSK TRATI PAFI SATI PAFI SATI		1058 B 129 160 232 145 149 200 174 347 260 141 450 464 184 275 464 132 466 286 410	CCCC68C PAIN METTLIA METTLIA METTLIA METTLIA METTLIA METTLIA PAPOA METTLIA PAPOA DENICLAS GMEBI DENICLAS GMEBI CEBPZ NURT NURT SPAPI ZNFLAS CNOTS TIMENISS RPL38A		B 527 159 369 438 388 290 310 407 375 294 144 343 343 321 319 377 272 325 1377 272 325 4283	NEDOS STAUTO STAUTO DGUCK GNLY RECKI MCN3 HMCN1 GLUM C170759 PHC3 HSP00A207 NFS1 GSNP HECA SUIT2 NOP16 LOHB C055 SMADL DNAJC11 BRC62	205 A	218 8 426 316 434 170 327 150 158 473 322 326 401 277 392 257 131 379 149 149 149	UPP HSPAIL DSTN EPHO2 NOUFARS RGS10 MRP99 FOMM MBLAC2 PRVABI FAII134C FAII134C COTL1 GTPC COTL1 GTPC CNST THUMP03 CH03	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.31 2.36 2.39 2.43 2.54 2.54 2.54	321 B 125 153	TAB 2 NAA16 PRKAR 18 SF385 POLR26 GRV11 TRAF1 ETS1 FAM98A VHSCL1 HST1H1A PORG1 PNSR1 PNSR2 EIF453 GPA33 COMIMD2 URI1	A 323 323 323 332 335 338 340 345 346 352 361 370 371 382 383 384 387 430 432	132 B 3.84 4.22
SLAUFI PTPN12 ANXAI GCHFR MR927 DENND4C LPCATI LPCATI LPCATI MEP DAR8 CKAR4 TMUBI CLI NOR6 UTN NOFIO 9X17 CCDC132 NOPRK TRATI PAFI SRAI NUDT2 KAA1429		1058 B 129 160 232 145 149 202 174 347 260 141 260 141 260 475 464 184 271 294 132 465 286 445 3390	CCCCCSC Prill OXRI METTL14 METTL14 METTL14 PP283 CRMSB DBND45 TUB88 TUB88 TUB88 TUB88 TUB88 TUB88 CEBPZ NUR37 RPAPI 2NF143 CNOT3 TNEM1558 RPJ35A TOMING43		B 527 1.57 3.69 4.38 2.90 3.10 4.07 3.75 2.94 1.44 3.43 3.43 3.14 4.45 3.43 3.19 3.77 2.77 2.325 1.34 2.83 3.72	NEDO9 STAUITOR3 STAUITOR3 STAUITOR3 RECKI MC013 HMC011 C170r69 PHC3 C170r69 PHC3 C170r69 PHC3 C170r69 PHC3 C170r69 PHC3 C170r69 PHC3 C170r69 C170 C170r69 C170r69 C170 C170 C170 C170 C170 C170 C170 C	205 A	218 B 426 316 434 1.70 1.50 1.58 4.73 322 326 401 277 392 2.57 1.31 3.79 1.49 1.49 1.49 1.49 1.49 1.49 1.49 1.4	UPP HSPAIL DSTN EPHO2 NDUFAF3 RGS10 MILAC2 PRKABI FAII134C PRKABI FAII134C TAIL134C FAII134C RGS10 NDR3 FOXR ARAF RCL1 MIR3 COTL1 GYPC CNST THUMIPO3 CHO3 CHO3 CHO3 CHO3 CHO3 CHO3 CHO3 CH	1.81 1.81 1.89 1.92 1.97 2.05 2.09 2.12 2.14 2.15 2.19 2.31 2.36 2.39 2.43 2.53 2.54 2.55 2.57	321 B 125 153	TAB 2 NAA16 PRKAA18 SF385 POLR2G GAMT CRYL1 TRAF1 ETS11 FAM98A WHSCL1 HST1HA POR01 PNSR TMEM245 GPA33 COMMD2 UR11 SELL	A 323 323 332 335 338 340 345 352 361 361 370 361 370 361 382 383 384 387 432 436	3.84
SLAMPT PTPN12 ANXAI GCHFR MR927 DENND4C LPCAT1 MEP DARS NFKBS CKAR4 TMUBS QL1 NO66 UTNN NOF10 SWA17 CCDC10 SWA17 CCDC10 SWA17 CCDC10 SWA17 SRA1 ND72 KAA129 SRA1 ND72 KAA129 USE48		1058 B 159 160 232 145 149 202 174 347 234 260 141 450 244 250 141 450 201 141 455 266 410 204 256 410 316	CCCCCSC RN1 OXR1 METL14 TMEM63A MTAD4 PC85 DBND45 TU888 DBND45 TU888 DBND45 TU888 DBND45 CSF2 ORN52 CSF2 ON073 TMEM588 SPL35A CN075 AAA5		B 5.27 1.59 3.69 4.38 2.90 3.10 4.07 3.75 2.94 1.44 3.14 3.43 3.43 3.43 3.43 3.21 3.77 2.72 3.72 1.34 2.83 3.77 4.374	NEDD9 STAU1 DGUOK GALY RECKI MCN3 HIGN1 GLIM C170769 PHC3 HSPQ0AE29 NF51 GSKP HECA SUMF2 NOP16 LDH8 CD5 SMA01 DNA.C11 BRC6 M6RR ABLM1	205 A	218 B 425 316 434 170 327 150 158 473 322 326 401 277 392 257 401 277 392 257 131 379 149 146 401 385	UPP SKAPI HSPAIL DSTN EPHO2 NDUFATS RGS10 MRP39 FOX RCH11 MBLAC2 PRKABI FOXR RCL1 MP3 COTL1 GYPC COTT THUMP03 COTT IFTS PRKABI IFTS PRKABI IFTS PRKABI IFTS PRKABI IFTS PRKABI IFTS PRKABI IFTS PRKABI IFTS PRKABI IFTS I I I I I I I I I I I I I I I I I I I	1.81 1.81 1.89 1.92 1.97 1.99 2.05 2.09 2.12 2.14 2.15 2.19 2.36 2.39 2.39 2.32 2.53 2.54 2.57 2.58	321 B 125 153	TAB 2 NAA16 PRKAR 8 SF385 POLR2G GAWT TRAF1 CRYL1 TRAF1 FAM98A WHSC1L1 HST1H4A PDR51 FM98A WHSC1L1 PNSR TMEM 245 EIF4E3 GPA33 COMMD2 URI1 SELL RAB43	A 323 323 332 335 340 345 346 352 361 361 370 371 382 383 384 387 430 432 436	132 B 3.84 4.22
SLAMPT PTEN12 ANXAI GCHFR MRPS27 DENNDUC LPCATI MRP DARS NFKBB CCATI NOBS UTRN NOBS SVAIT CCDC132 NPPSK TAUTI PAFI SRAI NUDT2 KAA129 UKA		1058 B 129 160 232 146 202 174 347 234 260 141 453 454 1.84 271 1.84 271 1.32 465 286 300 316 325	CCCC68C PRI METTLIA METTLIA METTLIA METTLIA METTLIA METTLIA METRLIA METRLIA METRLIA METRLIA METRLIA CEBP2 NURS CEBP2 NURS CEBP2 NURS CRANI CEBP2 NURS CRANI CONTO TIMEMISSE CONTO TIMEMISSE CONTO TOMINUL CIIOTIS AAAS		B 527 159 369 438 290 310 407 375 294 314 445 343 319 377 272 325 134 283 372 374 272	NEDD9 STAU1 DGUCK GRUCY RBCK1 MCM3 HMGN1 MCM3 C17069 PHC3 GLNN C17069 PHC3 GSUP H5P0A82P PHC3 GSUP H5P0A82P H5P	205 A 151 1.51 1.55 1.59	218 B 426 316 434 1.70 1.50 1.58 4.73 322 326 401 277 392 2.57 1.31 3.79 1.49 1.49 1.49 1.49 1.49 1.49 1.49 1.4	UPP SKAPI HSPAIL DSTN EPHO2 NUFARS RGS10 MRP29 RCH11 MRP3 FONT RKABI FAU132C WOR89 FDKK RCL1 MRP3 COTL1 GYPC CNST THUMP3 IFITS PCNT	181 181 181 182 197 205 209 233 239 233 254 254 255 258	321 B 125 153	TAB 2 NAA16 PRKAR18 SF385 POLR2G GANT CRYL1 TRAF1 ETS11 FAM98A WHSCIL1 HST1HHA PDRG1 PDRG1 PNSR TIMEN245 EIF4E3 GPA33 COMMD2 URI1 SELL RAB43 CD4	A 323 323 332 335 338 340 345 346 352 361 370 371 382 383 384 387 430 432 436	1.32 B 3.84 4.22 5.19
SAMPI PTIPNI2 ANXAI GCHPR MRPSCT LPCATI MRPSCT DARS DARS MRKBE OARS CKAR		1058 B 1260 232 146 149 202 174 234 260 141 450 141 450 264 154 264 1294 2285 266 286 410 300 316 3265	CCCC68C PN1 OXR1 METL141 TMEM63A MTAC4 APOAI EP288 DBNID48 TU888 DBNID48 CRE81 CRE91 CRE81		B 5.27 1.59 3.69 4.38 2.90 3.10 4.07 3.75 2.94 1.44 3.14 4.45 3.43 3.21 3.77 2.72 3.74 2.72 3.74 2.72 3.66	NEDD9 STAU1 DGUCK GNLY RECKI MCN3 HMGN1 GLIM C170F9 PHC3 GSNP HECA SUMF2 GSNP HECA SUMF2 C05 SMA0L DNA.C11 BRC6 MGRR ABUINT NPF	205 A 	218 B 426 316 434 1.70 1.50 1.58 4.73 322 326 401 277 392 2.57 1.31 3.79 1.49 1.49 1.49 1.49 1.49 1.49 1.49 1.4	LPP SKAPI HSPAIL DSTN EPHOL RKASI RKASI FAITS/C RKASI FAITS/C COTLI MIRA ARAF RCL1 MIRA COTLI GYPC CNST THUMROS CHOS C	181 181 181 182 197 199 205 209 212 214 215 239 243 253 254 255 257 258 270	321 B 125 153	TAB 2 NAA16 PRKAR18 SF365 POLP20 GAMT CRYL1 TRAF1 ETS1 FAM98A WHSCL1 HST1HIA PPNSR TMEM 245 EF463 COM MD2 URI1 SELL RA843 CD4 FOXP1	A 323 323 332 335 338 340 345 346 352 361 370 371 382 383 384 383 384 432 432 432 502 519	1.32 B 3.84 4.22
SAMP1 PTPN12 ANXAI CCHR MR921 DENNDUC LPCATP DENNDUC LPCATP DENNDUC LPCATP DAR MR921 DAR MR91 DAR MR921 DA		1058 B 129 160 232 146 149 202 174 234 260 347 234 263 475 464 184 224 254 224 267 234 271 24 286 410 266 316 3365 3365 338 365	CCCC68C PAIL OXRI METLIA METLIA PORT PESS SK38 DENICLE DENICLE CEBP2 DENICLE CREP2 DENICLE CREP2 DENICLE CREP2 DENICLE CREP3 DENIC DENICLE CREP3 DENICLE CREP3 DENICLE CREP3 DENICLE CREP3 DENICLE CREP3 DENIC		8 527 159 369 300 300 300 300 300 300 300 300 300 30	NEDO9 LAUTOR3 STAU1 DGUCK GNLY RBCK1 MCM3 C170F9 PHC3 C170F9 PHC3 C170F9 PHC3 C170F9 PHC3 C170F9 PHC3 C170F9 PHC3 C170F9 HSP0AB2P	205 A 	2 18 8 4 25 3 16 4 34 170 150 150 327 150 150 164 164 164 164 164 164 164 164	UPP SKAPI HSPAIL DSTN EPHO2 NDUFAF3 KGS10 MIRP RKABI FAI1134C WORBS FOXE FAI1134C WORBS FOXE COTL1 GYPC CNST THUMP03 CH03 CH03 CH03 FINEC CH03 FINEC FUNCCS	181 181 189 192 205 209 212 214 215 231 236 239 243 253 254 255 257 258 250 271	321 B 125 153	TAB 2 NAA16 PRKAR18 SF305 POLP20 GANIT CRVL1 TRAF1 ETS1 FAM38A WHSCL1 PNDR31 PNDR31 PNDR31 PNDR31 COM MD2 UR11 SELL RAB43 CD4 FOXP1 MINOS1	A 323 323 332 335 338 340 345 352 361 370 371 382 383 384 387 384 387 430 432 436 437 502 519 522	132 B 3.84 4.22 5.19 4.82
S.AMP1 PTIPN2 CCHR DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL DENDAL SCAR NOPIO SVAT SVAT NOPIO SVAT NOPIO SVAT SVAT SVAT NOPIO SVAT SVAT NOPIO SVAT SVAT SVAT SVAT SVAT SVAT SVAT SVAT		1058 B 129 160 222 145 143 147 247 240 2260 2260 247 244 141 247 245 2260 247 244 154 154 154 251 245 246 240 246 251 245 243 332 3300 345	CCCC68C PRI METTL14 METTL14 METTL14 METTL14 METTL14 METCL14 METCL14 METCL14 METCL14 METCL14 METCL14 CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE CEBP2 NURSE NURSE CEBP2 NURSE NURSE CEBP2 NURSE NURSE CEBP2 NURSE NURSE CEBP2 NURSE NU		B 527 159 369 433 290 310 407 371 341 445 343 321 344 343 321 339 371 372 372 360 371 178	NEDD9 LAUTOR3 STAU1 DGUOK GNLY RBCK1 MCN3 HMIGHT GLNN C170E9 PHC3 GLNN C170E9 PHC3 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUIF2 SUF5	205 A 	218 B 426 316 434 1.70 1.50 1.58 4.73 322 326 401 277 392 2.57 1.31 3.79 1.49 1.49 1.49 1.49 1.49 1.49 1.49 1.4	LPP SKAPI HSPAI4 DSTN EPH02 NOUFAR3 RGS10 MRP29 RCH11 MLA22 WOR89 FONG RCA8 FAU1134C WOR89 FONG CH03 IFITS PCNT MIMA TK FUNO22 USP16	181 181 181 182 197 190 209 212 214 231 236 239 243 254 254 254 257 258 270 271 272	321 B 125 153	TAB 2 NAA16 PRKAR18 SF385 POLR26 GANT GRYL1 TRA18 GRA11 FAM38A WHSCIL1 HST1HHA PDR61 PDR61 PNSR TMEM245 GPA33 COMMD2 URI1 SELL RAB43 COM SELL RAB43 COM	A 223 323 332 335 336 340 345 361 361 361 361 371 382 383 384 430 432 430 432 502 522 528	132 B 3.84 4.22 5.19 4.82 5.13
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Fig. 4. Differentially expressed proteins in MAIT cells compared to CD3⁺ and CD8⁺ conventional/non-MAIT T cell populations. Proteins with $\pm log_2 fc \ge 1$ and a q value of 0.05 were considered differentially expressed.

Table 2

Canonical pathways enriched by differentially expressed proteins in MAIT cells compared to Non-MAIT T cells.

	Ingenuity Canonical Pathways	Log ₂ fold-change	Over-expressed	Under- expressed	Quantified proteins
MAIT cells vs non-MAIT CD3 ⁺ T cells	B cell receptor signalling	1.4	9/192 (5 %)	1/192 (1 %)	MAP2K6, RRAS2, JUN, INPPL1, GSK3A, MAP3K4, INPP5K, GSK3B, MALT1, NFKBIB
	14-3-3-mediated signalling	1.4	8/131 (6 %)	0/131 (0 %)	RRAS2, JUN, TUBB6, TUBB8, PRKCD, EDC3, GSK3A, GSK3B
	CD40 signalling	1.4	5/79 (6 %)	1/79 (1 %)	MAP2K6, TANK, JUN, TNFAIP3, MAPKAPK2, NFKBIB
	TNFR2 signalling	1.4	4/30 (13 %)	0/30 (0 %)	TANK, JUN, TNFAIP3, NFKBIB
	Regulation of IL-2 in activated and anergic T-cells	1.4	6/80 (8 %)	0/80 (0 %)	RRAS2, JUN, CHP1, SMAD4, MALT1, NFKBIB
	Protein kinase A signalling	1.39	11/401 (3 %)	3/401 (1 %)	CHP1, PTPN18, PPP1R11, GSK3A, PTPN12, AKAP11, PTPRJ, PRKCD, SMAD4, LEF1, H1F0, GSK3B, NFKBIB, PDE6D
	TNRF2-mediated oxidative stress response	1.38	8/193 (4 %)	1/193 (1 %)	MAP2K6, DNAJC17, RRAS2, JUN, PRKCD, JUNB, GSK3B, SQSTM1, DNAJC11
	3-phosphoinositide degradation	1.38	7/157 (4 %)	1/157 (1 %)	PTPRJ, PIP4P1, NUDT16, EPHX2, INPPL1, INPP5K, NUDT2, PTPN12
MAIT cells vs non-MAIT CD8 ⁺ T cells	CD40 signalling	1.44	5/79 (6 %)	1/79 (1 %)	MAP2K6, TANK, JUN, TNFAIP3, MAPKAPK2, TRAF1
	TNFR2 signalling	1.44	3/30 (10 %)	1/30 (3 %)	TANK, JUN, TNFAIP3, TRAF1
	Thiosulfate disproportionation III (Rhodanese)	1.44	2/3 (66 %)	0/3 (0 %)	MPST, MOCS3
	Protein kinase A signalling	1.44	6/401 (1 %)	7/401 (2 %)	NFATC3, CHP1, PTPN18, PPP1R11, ITPR1, PTPN12, AKAP11, HIST1H1A, PTPRJ, PRKCD, PRKAR1B, LEF1, H1F0
	CD28 signalling in Th cells	1.42	2/132 (2 %)	5/132 (4 %)	JUN, NFATC3, CD4, CHP1, ITPR1, MALT1, ITK

4. Experimental Design, Materials and Methods

4.1. Purification of primary human MAIT, CD3⁺, and CD8⁺ T cell populations

Human circulating MAIT cells were isolated from peripheral blood mononuclear cells (PBMCs) obtained from three healthy young volunteers aged between 30-35 years (2 males and 1 fe-

male). To isolate MAIT cells, first CD3⁺ T cells were negatively enriched from PBMCs using a pan-human T cell isolation kit (Miltenyi Biotec, USA) and magnetic activated cell sorting. CD3⁺ T cells were surface stained with live/dead Fixable Aqua (Life Technologies, USA), CD3-APCe780 (clone SK7; eBioscience, Thermo Fisher Scientific, USA), CD161-APC (clone HP-3G10; eBioscience, Thermo Fisher Scientific, USA), TCR V α 7.2-FITC (clone 3C10; Biolegend, USA) and CD8-Percp/cy5.5 (clone SK1, Biolegend, USA) by incubating the cells for 20 minutes at 4°C in dark. After washing three times with cold FACS buffer the stained cells were sorted using a FACS Aria III flow cytometer (BD bioscience, USA) to obtain ~ 1×10⁶ CD3⁺, CD8⁺, and MAIT cells from each donor. In the FACS sorting, CD3⁺, CD161⁺, and TCR V α 7.2⁻ cells were sorted as MAIT cells while CD3⁺, CD161⁻ and TCR V α 7.2⁻ and CD3⁺, CD8⁺, CD161⁻ and TCR V α 7.2⁻ were collected as CD3⁺ and CD8⁺ conventional T cells, respectively (Fig. 1A and B). Collected cells were washed three times with cold PBS, pelleted, and stored at – 80°C for proteomic sample preparation.

4.2. Proteomic sample preparation

The steps used in the proteomic sample preparation and data acquisition are shown in Fig. 1C. The cell pellets were thawed and lysed in a lysis buffer composed of 2 % sodium dodecyl sulphate (Biorad, USA) in 100 mM triethylammonium bicarbonate (TEAB, Sigma-Aldrich, USA) and 1 x Roche complete protease inhibitor cocktail (Sigma-Aldrich, USA). Then 200 ng of ovalbumin (Sigma-Aldrich, USA) was added as an internal standard. The amount of protein in each cell lysate was quantified at a wavelength of 562 nm using Pierce bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific, USA), following the manufacturer's instructions. About 20 µg of protein from each cell lysate was reduced in 10 mM of tris (2-carboxyethyl) phosphine hydrochloride (Thermo Fisher Scientific, USA) at 60°C for 30 minutes and alkylated in 40 mM chloroacetamide (Sigma, USA) at 37°C in dark for 45 minutes. Cell lysates were then co-precipitated overnight with sequencing grade modified porcine trypsin (Promega, USA) at a trypsin: protein ratio of 1:100 in cold (-20°C) cold chromAR grade methanol (Honeywell Research Chemicals, USA) as described previously [18]. On the next day, samples with precipitated proteins were further cleaned by washing the pellet three times consecutively with 100 %, 90 %, and 100 %, cold chromAR grade methanol (Honeywell Research Chemicals, USA). Each centrifugation was performed for 15 min at 16,100xg at 4°C and the supernatants were aspirated carefully without disturbing the protein pellets. Resulted protein pellets were then resolubilized in 50 mM TEAB containing 5 % acetonitrile (ACN, Honeywell research chemicals, USA) and were incubated in a thermo-mixture at 37°C for 2 h at 600 rpm after adding 1 μ l of 1 μ g/ μ l sequencing grade trypsin. At the end of the incubation period, another 1 μ l of 1 μ g/ μ l (1:50) trypsin was added, vortexed to mix, and incubated overnight to obtain complete protein digestion. After 12 hours of digestion, the enzymatic reaction was inhibited by adding 25 µl of 5 % formic acid (Sigma-Aldrich, USA), and the resulting acidified tryptic digested peptides were desalted using strata-x polymeric reversed phase 10 mg/ml C18 cartridges (Phenomenex, USA). Desalted peptides were dried using a speedVac vacuum concentrator (Thermo Fisher Scientific, USA) at 35°C and stored at -80°C until tandem mass spectrometry (LC-MS/MS) based proteomic analysis.

4.3. DDA-MS data acquisition

LC-MS/MS analysis of desalted peptide samples was performed on an Orbitrap FusionTM TribridTM mass spectrometer (Thermo Fisher Scientific, USA) inline coupled to nanoACQUITY ultra performance liquid chromatographic (Waters, USA) system. From each sample, $\sim 1 \ \mu g$ of peptides as quantified by micro-BCA (Thermo Fisher Scientific, USA) was loaded onto a Symmetry C18, 2G, VM (100Å, 5 μm particle size, 180 $\mu m \ge 20 \ mm$) trap column (Waters, USA) at

a flow rate of 0.3 μ L/min to separate the peptides on a BEH C18 (130Å, 1.7 μ m particle size, 75 μm x 200 mm) column (Waters, USA). The mobile phase consisted of buffer A (0.1 % formic acid), and buffer B (100 % acetonitrile and 0.1 % formic acid) was used to create three consecutive linear gradients (buffer B, 5 %- 9 % between 3 and 10 min, 9 %-26 % between 10 and 120 min and 26 %-40 % between 120 and 145 min) to elute the peptides. After elution, the column was washed with buffer B at a concentration of 40 %- 80 % between 145 and 152 min, then holding it at 80 % until 157 min and at 1 % until 160 min. The eluted peptides were ionized using Nanospray Flex ion source (Thermo Fisher Scientific, USA) in which the ion spray voltage and heating temperature were held at 1.9 kV and 285°C respectively. In DDA-MS acquisition, Chromeleon software (version 6.8, Dionex) included in Xcalibur software (version 3.0.63, Thermo Fisher Scientific, USA) was used to control the liquid chromatographic system. Peptide ions in the mass range of 380 - 1500 m/z were selected at 120,000 FWHM resolution to generate MS1 spectra. The mass spectrometer was controlled by Xcalibur software to operate "top speed" mode allowing automatic selection of positively charged (+2 to +7) top 15 peptides to trigger MS2. Higher Energy C-trap Dissociation (HCD) was used to fragment the selected peptide ions. In the acquisition of MS2 spectra, the resolution and dynamic exclusion time were set as 30,000 FWHM and 90 seconds respectively. The cycle time was 2 s.

4.3.1. Data processing and statistical analysis

MaxQuant (Release 1.6.0.16) software [16] was used to process the .raw files in which spectral data were searched against UniProt human-reviewed proteome database containing 20,242 entries (downloaded on 25th October 2017). MaxLFQ included in MaxQuant software was used to obtain the normalized label-free peptide and protein intensity data [17]. Trypsin-digested peptides with a maximum of 2 miscleavages were included in the analysis. Only carbamidomethylation of cystine (fixed modification), and oxidation of methionine and N terminal acetylation (variable modifications) peptide modifications were allowed. Precursor and product mass tolerance were set as \pm 20 ppm and \pm 40 ppm respectively to identify the peptides up to the maximum charge of +7. Only the peptide spectral matches and proteins detected at a 1 % of FDR were selected in which the proteins that were detected with at least one unique or razor peptide were quantified between runs.

In the downstream analysis, less reproducible proteins (expression data is missing for > 50 % of samples) and that were quantified at m-score of < 5 were removed from the final quantification and the missing values of the remaining proteins were imputed using maximum likelihood estimate (R package) [19]. In the statistical analysis, mean intensity values of each cell population were compared using multiple t-test with FDR determination by two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli [20] to identify the differentially abundant proteins in MAIT cells compared to conventional CD3⁺ and CD8⁺ Tcells. Expression fold change was obtained in the log₂ fold change (log₂fc) scale to depict proteins expressed at $\pm log_2 fc \ge 1$ at q value < 0.05 as differentially abundant proteins. Qiagen ingenuity pathway analysis (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA) was used to profile the subcellular localization, biological functions of the selected proteins, and the canonical pathways enriched by the differentially abundant proteins [21]. In IPA analysis, the *p*-value corrected for false discoveries in multiple comparisons using Benjamini-Hochberg (B-H p value) equation was used to set the cut-off, in which canonical pathways identified at or above 1.30 –log₁₀ B-H p value (B-H *p* value = 0.05) were considered significant.

Limitations

The present analysis aimed to characterize the proteome of human circulating MAIT cells in three young healthy volunteers. The proteome of MAIT cells can vary, particularly in elderly people, children, and those with different disease conditions and this diversity is not represented in the current dataset. As non-MAIT T cells were sorted from CD3⁺ T cells, a contamination with non-MAIT unconventional T cell populations (e.g. V $\delta 2 \gamma \delta$ T cells), which have some similarities

to MAIT cells can be expected. Further, CD3⁺ and CD8⁺ conventional T cell populations contain T cells of different phenotypes (eg; naïve and memory) while MAIT cells predominantly have memory phenotype. Thus, some differentially expressed proteins will not relate to differences between MAIT and conventional T cells, but rather to differences in the ratio of naïve vs. memory cells. As indexed retention time (iRT) peptides (Biognosys AG, Switzerland) were not added to the samples during DDA-MS data acquisition, the use of this data to develop spectral libraries for data independent analysis (DIA) will be limited.

Ethics Statement

Ethical clearance for this study was obtained from the QIMRB human research ethics committee (HREC, #P2058). Informed consent was obtained from all volunteers and the study adhered to the Declaration of Helsinki of 1975.

CRediT Author Statement

Harshi Weerakoon: Data curation; Formal analysis; Validation; Investigation; Methodology; Writing - original draft. **John J Miles:** Conceptualization; Methodology; Resources; Supervision; Project administration; Funding acquisition, **Michelle M Hill:** Conceptualization; Methodology; Resources; Supervision; Writing-original draft; Project administration. **Ailin Lepletier:** Conceptualization; Methodology; Supervision; Writing-Review & Editing; Project administration.

Data Availability

Human mucosal-associated invariant T (MAIT) cell proteome (Original data) (ProteomeX-change via the PRIDE database)

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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