CD4+CCR6+ T cells dominate the BCG-induced transcriptional signature

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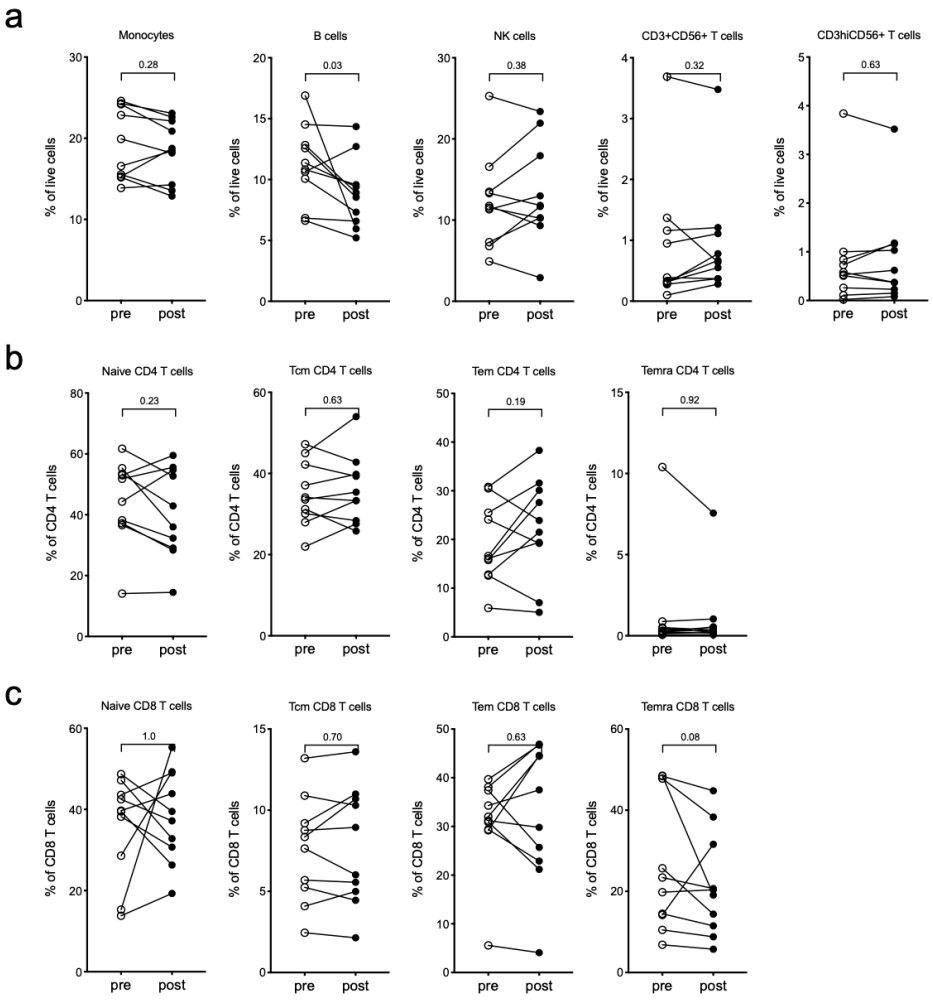
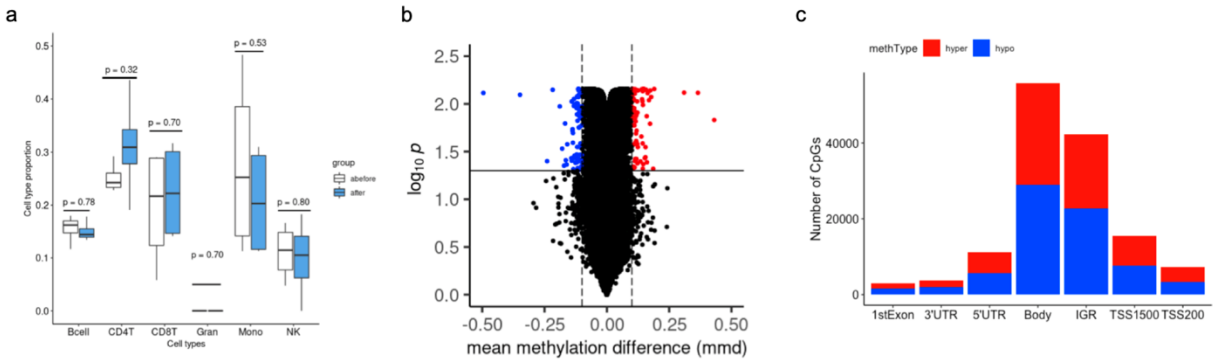
Supplementary material

Supplementary Figures



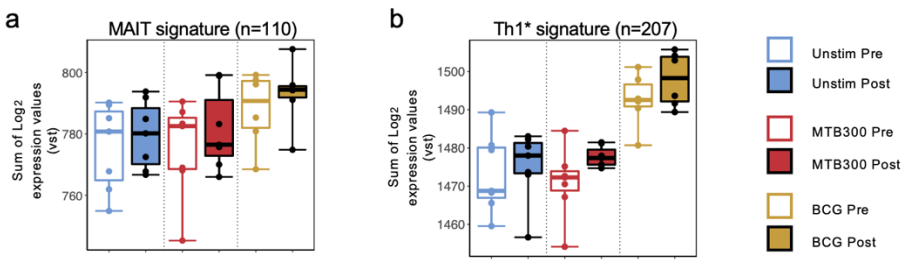
Supplementary Figure 1

SUPPLEMENTARY FIGURE 1. Cell subset frequencies pre- and post- BCG vaccination. **a-c** Frequencies of cell subsets pre- (open circles) and post- (closed circles) BCG vaccination. Each point represents one participant (n=10), Wilcoxon matched pair signed rank test. **a** Major lymphocyte subsets not shown in figure 3. **b, c** Memory T cell subsets defined by CCR7 and CD45RA; Naïve cells (CD45RA+CCR7+), Tcm (CD45RA-CCR7+), Tem (CD45RA-CCR7-), and Temra (CD45RA+CCR7-). **b** CD4 T cell subsets. **c** CD8 T cell subsets.



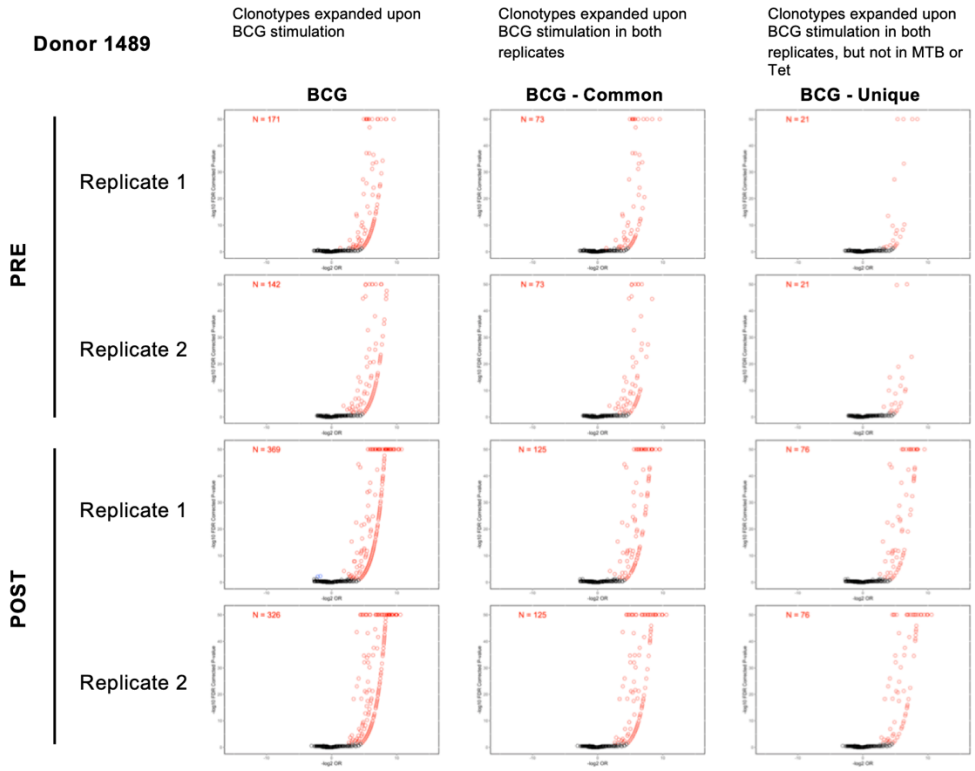
Supplementary Figure 2

SUPPLEMENTARY FIGURE 2. Characterization of the DNAm alterations. **a**. Cell type frequency analysis applying the Houseman algorithm to the DNA methylation dataset of samples obtained pre- and post- BCG vaccination. The y-axis shows the frequency of cell types in the group of samples and the x-axis denotes the calculated cell type in the PBMC sample. Student's t-test. **b**. Volcano plot illustrating the hyper- and hypomethylated CpGs. The x-axis represents the mean methylation difference (mmd). The y-axis denotes the significance level (p-value) at the logarithm scale. The solid horizontal line sets the threshold of p-value < 0.05 and the dashed vertical lines used for the cut-off value, mmd ≥ |0.1|. **c**. Number of CpGs present in different chromosomal locations for hypermethylated (red) and hypomethylated (blue) CpGs.



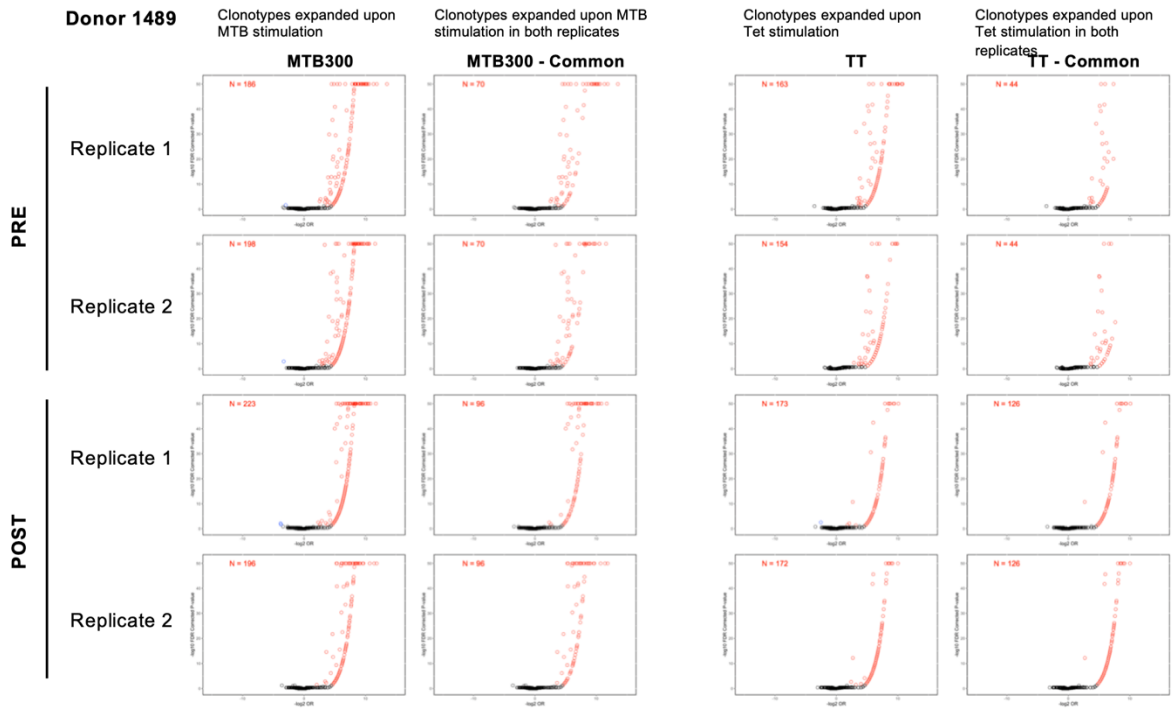
Supplementary Figure 3

SUPPLEMENTARY FIGURE 3. **Cell subset-specific gene signatures pre- and post- BCG vaccination a, b** Boxplots of RNA-sequencing data depicting the sum of log_2 expression values (variance stabilizing transformation, VST) for all genes in the **(a)** MAIT cell signature, and **(b)** Th1* cell signature. Empty and filled boxplot represent pre- and post-vaccination, respectively, and color represents stimulation condition. Each dot represents an individual donor.



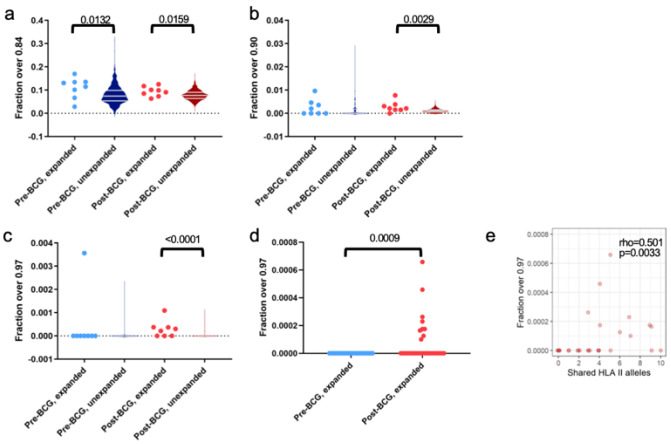
Supplementary Figure 4

SUPPLEMENTARY FIGURE 4. Representative volcano plots showing clonotype expansion in response to BCG stimulation. Analysis from Donor 1489 is shown. Column 1 indicates clonotypes expanded upon BCG stimulation in each replicate, pre- and post- vaccination. Column 2 indicates only those clonotypes that expanded upon BCG stimulation in both replicates, pre- and post-vaccination. Column 3 further indicates a subset to show only those clonotypes that expanded upon BCG stimulation in both replicates, but not in MTB300 or Tetanus stimulation, pre- and post-vaccination.



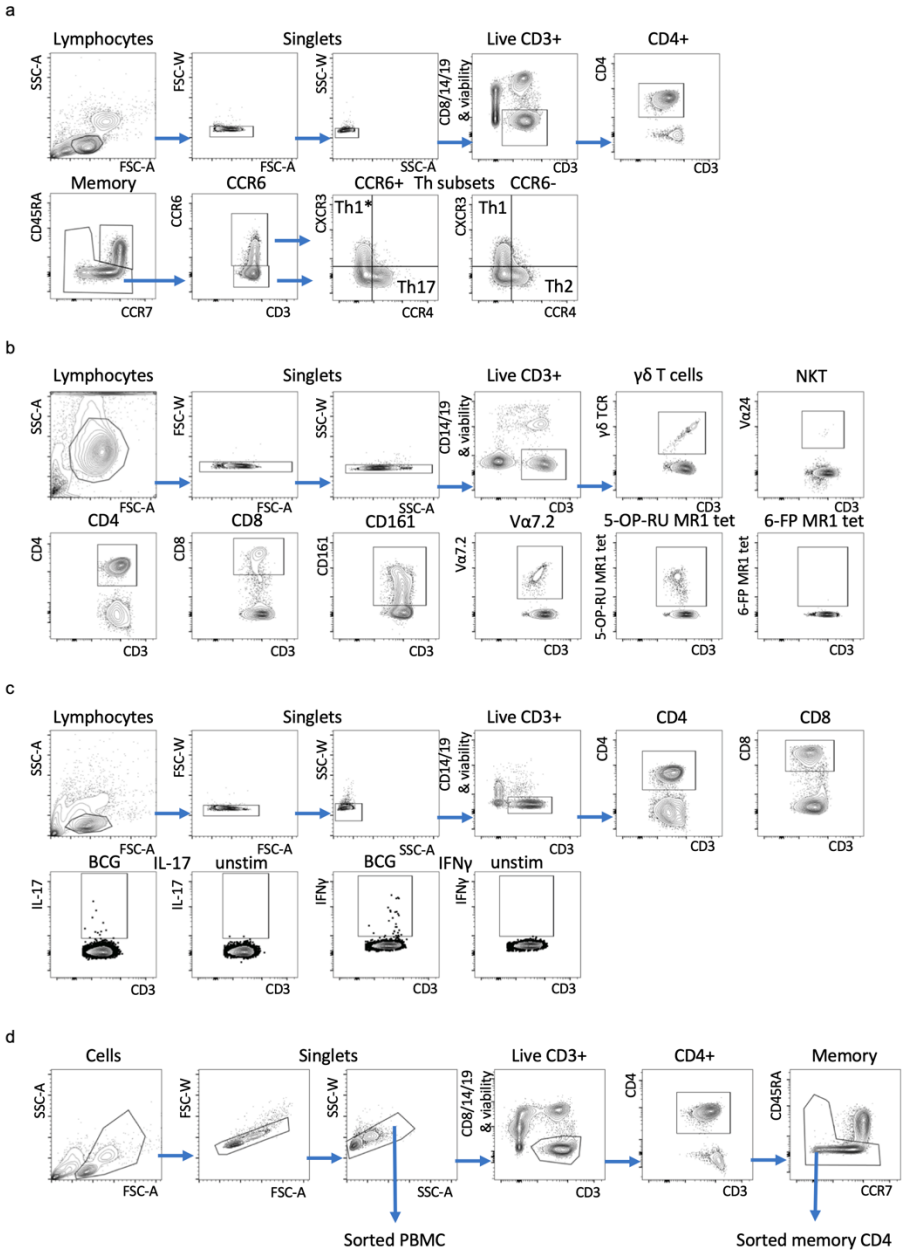
Supplementary Figure 5

SUPPLEMENTARY FIGURE 5. Representative volcano plots showing clonotype expansion in response to MTB300 and Tetanus stimulation. Analysis from Donor 1489 is shown. Column 1 indicates clonotypes expanded upon MTB300 stimulation in each replicate, pre- and post-vaccination. Column 2 indicates only those clonotypes that expanded upon MTB300 stimulation in both replicates, pre- and post- vaccination. Column 3 indicates clonotypes expanded upon Tetanus stimulation in each replicate, pre- and post- vaccination. Column 4 indicates only those clonotypes that expanded upon Tetanus stimulation in both replicates, pre- and post- vaccination.



Supplementary Figure 6

SUPPLEMENTARY FIGURE 6. Comparison of TCRMatch scores within and across T cell repertoires from individuals pre- and post-BCG vaccination. a-c Comparison of CDR3β similarity within individuals before and after BCG vaccination (n=8) and expanded or unexpanded in response to BCG stimulus in vitro, as assessed by the fraction of scores (**a**) >0.84, (**b**) >0.90, (**c**) >0.97. 100 random samplings of each individual's unexpanded TCRs are represented by violin plots. Gray lines indicate first, second and third quartiles. One-tailed Mann-Whitney test. **d** Comparison of CDR3β similarity in expanded T cells across individuals pre- and post-BCG vaccination. One-tailed Mann-Whitney test. **e** Comparison of the fraction of TCRMatch scores >0.97 between two individuals and the number of HLA class II alleles shared by the individuals. One-tailed Spearman test.



SUPPLEMENTARY FIGURE 7. Gating strategies for flow cytometry analyses and cell sorting. a Th-subset gating, **b** non-conventional T cells, MAITs were defined as MR1 5-OP-RU tetramer+CD4-Vα24-$\gamma\delta$TCR-Vα7.2+CD161+. A representative control 6-FP MR1 tetramer stain is shown for comparison. **c** Intracellular cytokine staining. BCG stimulated and unstimulated samples for both IL-17 and IFNγ is shown. **d** Cell sorting of PBMC (excluding doublets) and live memory CD4 T cells.

Supplementary Tables

Supplementary table 1-4 provided as excel files

SUPPLEMENTARY TABLE 1 Differentially expressed genes identified in the different comparisons for PBMC and CD4 memory T cells.

SUPPLEMENTARY TABLE 2 Pathway enrichment for upregulated genes in PBMCs and CD4 memory T cells.

SUPPLEMENTARY TABLE 3 MAIT and Th1* signatures

SUPPLEMENTARY TABLE 4 TCR repertoire dataset

SUPPLEMENTARY TABLE 5 Number of clonotypes per subject

	Subject	Total Clonotypes		Clonotypes expanded upon BCG stimulation		Clonotypes expanded in both replicates				
		Replicate 1	Replicate 2	Replicate 1	Replicate 2	BCG stim	BCG stim - tet	BCG stim only	MTB300 stim only	BCG and MTB300 stim
PRE	1489	30544	24621	171	142	73	36	30	33	6
	1491	18875	17343	183	157	54	23	18	19	5
	1492	4616	5515	105	209	22	20	19	13	1
	1497	5597	6524	174	184	68	44	32	35	12
	1499	9248	8944	205	147	84	62	46	27	16
	1500	10476	12232	369	382	90	55	40	30	15
	1502	13174	17269	235	289	55	40	34	34	6
	2466	7930	11635	89	85	41	25	15	27	10
AVERAGE	-	*12558*	*13010*	*191*	*199*	*61*	*38*	*29*	*27*	*9*
POST	1489	22744	19449	369	326	125	98	82	49	16
	1491	17607	14725	363	298	131	79	59	48	20
	1492	9533	12276	357	563	109	92	74	62	18
	1497	6756	6840	299	277	117	85	68	85	17
	1499	5408	5296	288	301	147	116	97	47	19
	1500	12347	10739	602	658	170	145	127	66	18
	1502	9916	11308	590	520	155	126	103	79	23
	2466	7042	4341	225	232	98	76	61	41	15
AVERAGE	-	*11419*	*10622*	*387*	*397*	*132*	*102*	*84*	*60*	*18*

SUPPLEMENTARY TABLE 6 GLIPH analysis within and across donors to identify similarities in clonotypes that expanded pre- and post- vaccination, upon BCG stimulation in both replicates, but not in MTB300 or Tetanus stimulation.

	Subject	Clonotypes expanded in			GLIPH analysis				
		Pre	Post	Common in Pre & Post	3-mers	4-mers	Convergence groups	Global convergences	Local convergences
Similarity between Pre and Post within each donor in BCG stim only	2466	9	55	6	-	-	3 groups with 2 peptides each	3	-
	1502	20	89	14	-	-	7 groups with 2 peptides each	7	-
	1500	27	114	13	-	-	2 groups with 2 peptides each	2	-
	1499	12	63	34	2	-	1 group with 6 peptides 2 groups with 5 peptides each 3 groups with 2 peptides each	23	20
	1497	19	55	13	1	-	1 group with 5 peptides 4 groups with 2 peptides each	7	6
	1492	14	69	5	-	-	1 group with 5 peptides 3 groups with 2 peptides each	4	6
	1491	11	52	7	-	-	-	-	-
	1489	13	65	17	-	-	1 group with 2 peptides	1	-

SUPPLEMENTARY TABLE 7 HLA alleles expressed by each subject

Subject	A	B	C	DPB1	DQA1	DQB1	DRB1	DRB3/4/5
1489	A*01:01 / A*02:01	B*08:01 / B*15:01	C*01:02 / C*07:01	DPB1*02:01 / DPB1*04:01	DQA1*04:01 / DQA1*05:01	DQB1*02:01 / DQB1*04:02	DRB1*03:01 / DRB1*08:01	DRB3*01:01 /
1490	A*02:01 / A*03:01	B*07:02 / B*44:05	C*02:02 / C*07:02	DPB1*03:01 / DPB1*11:01	DQA1*02:01 / DQA1*03:01	DQB1*02:02 / DQB1*03:02	DRB1*04:04 / DRB1*07:01	DRB4*01:01 / DRB4*01:01
1491	A*02:01 / A*68:01	B*15:01 / B*27:05	C*02:02 / C*03:03	DPB1*02:01 / DPB1*04:01	DQA1*03:01 / DQA1*03:01	DQB1*03:01 / DQB1*03:02	DRB1*04:01 / DRB1*04:01	DRB4*01:01 / DRB4*01:01
1492	A*02:01 / A*32:01	B*15:01 / B*44:02	C*04:01 / C*05:01	DPB1*03:01 / DPB1*20:01	DQA1*03:01 / DQA1*04:01	DQB1*03:02 / DQB1*04:02	DRB1*04:01 / DRB1*08:01	DRB4*01:01 /
1493	A*03:01 / A*03:01	B*35:01 / B*56:01	C*01:02 / C*04:01	DPB1*04:01 / DPB1*04:02	DQA1*01:01 / DQA1*01:02	DQB1*05:01 / DQB1*06:02	DRB1*01:01 / DRB1*15:01	DRB5*01:01 /
1494	A*01:01 / A*02:01	B*15:01 / B*55:01	C*03:03 / C*03:04	DPB1*04:01 / DPB1*04:02	DQA1*03:01 / DQA1*05:01	DQB1*03:01 / DQB1*03:02	DRB1*04:01 / DRB1*11:04	DRB3*02:02 / DRB4*01:01
1495	A*25:01 / A*26:01	B*18:01 / B*51:07	C*12:03 / C*14:02	DPB1*04:01 / DPB1*19:01	DQA1*01:02 / DQA1*01:03	DQB1*06:03 / DQB1*06:09	DRB1*13:01 / DRB1*13:02	DRB3*02:02 / DRB3*03:01
1496	A*02:01 / A*33:03	B*40:23 / B*50:01	C*03:04 / C*06:02	DPB1*04:02 / DPB1*14:01	DQA1*03:01 / DQA1*03:01	DQB1*03:01 / DQB1*03:02	DRB1*04:01 / DRB1*09:01	DRB4*01:01 / DRB4*01:01
1497	A*01:01 / A*02:01	B*44:02 / B*44:02	C*05:01 / C*05:01	DPB1*04:01 / DPB1*04:01	DQA1*01:02 / DQA1*05:01	DQB1*03:01 / DQB1*06:02	DRB1*12:01 / DRB1*15:01	DRB3*02:02 / DRB5*01:01
1498	A*03:01 / A*68:01	B*44:02 / B*57:01	C*06:02 / C*07:12	DPB1*01:01 / DPB1*04:01	DQA1*01:01 / DQA1*02:01	DQB1*03:03 / DQB1*05:01	DRB1*01:01 / DRB1*07:01	DRB4*01:01 /
1499	A*01:01 / A*02:01	B*08:01 / B*15:01	C*03:04 / C*07:01	DPB1*04:01 / DPB1*04:01	DQA1*03:01 / DQA1*05:01	DQB1*02:01 / DQB1*03:02	DRB1*03:01 / DRB1*04:01	DRB3*01:01 / DRB4*01:01
1500	A*03:01 / A*03:01	B*07:02 / B*07:02	C*07:02 / C*07:02	DPB1*03:01 / DPB1*03:01	DQA1*01:01 / DQA1*01:02	DQB1*05:01 / DQB1*06:02	DRB1*10:01 / DRB1*15:01	DRB5*01:01 /
1501	A*02:01 / A*02:01	B*15:01 / B*40:01	C*03:03 / C*03:04	DPB1*02:01 / DPB1*03:01	DQA1*03:01 / DQA1*03:01	DQB1*03:01 / DQB1*03:02	DRB1*04:01 / DRB1*04:07	DRB4*01:01 / DRB4*01:01
1502	A*02:01 / A*03:01	B*08:01 / B*44:02	C*05:01 / C*07:01	DPB1*04:01 / DPB1*04:01	DQA1*01:02 / DQA1*03:01	DQB1*03:01 / DQB1*06:02	DRB1*04:01 / DRB1*15:01	DRB4*01:01 / DRB5*01:01
1503	A*02:01 / A*03:01	B*07:02 / B*15:01	C*03:03 / C*07:02	DPB1*02:01 / DPB1*04:01	DQA1*01:02 / DQA1*03:01	DQB1*03:02 / DQB1*06:04	DRB1*04:01 / DRB1*13:02	DRB3*03:01 / DRB4*01:01
1504	A*01:01 / A*02:01	B*08:01 / B*15:01	C*03:04 / C*07:01	DPB1*03:01 / DPB1*04:01	DQA1*03:01 / DQA1*05:01	DQB1*02:01 / DQB1*03:02	DRB1*03:01 / DRB1*04:01	DRB3*01:01 / DRB4*01:01
2466	A*02:01 / A*02:01	B*40:01 / B*44:02	C*03:04 / C*05:01	DPB1*02:01 / DPB1*04:02	DQA1*02:01 / DQA1*03:01	DQB1*02:02 / DQB1*03:01	DRB1*04:01 / DRB1*07:01	DRB4*01:01 / DRB4*01:01