Supplemental Data

Vaccine Adjuvants Alter

TCR-Based Selection Thresholds

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Supplemental References

1. Samelson, L.E., Germain, R.N. and Schwartz, R.H. Monoclonal antibodies against the antigen receptor on a cloned T-cell hybrid. Proc Natl Acad Sci U S A. 80(22), 6972-6976 (1983).
2. Nakano, N., Rooke, R., Benoist, C. and Mathis, D. Positive selection of T cells induced by viral delivery of neopeptides to the thymus. Science. 275(5300), 678-683 (1997).
3. Liu, C.P., Parker, D., Kappler, J. and Marrack, P.J. Selection of antigen-specific T cells by a single IEk peptide combination. J. Exp Med. 186(9):1441-1450 (1997).

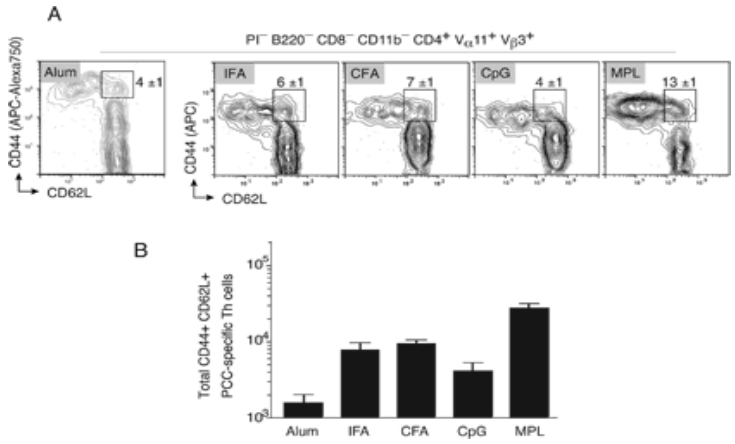


Figure S1. Accumulation of CD44^{hi}CD62L^{hi} Antigen-Specific Th Cells across Different Adjuvants

(**a**) As displayed in Figure 1, PCC-specific Th cells (Vα11$^+$Vβ3$^+$CD44^{hi}CD62L^{hi}) at day 7 in lymph nodes from B10.BR mice immunized with the indicated adjuvant. Profiles gated on propidium iodide (PI) negative cells that are CD4$^+$B220$^-$CD8$^-$CD11b$^-$ and Vα11$^+$Vβ3$^+$ as indicated with mean ±SEM (n≥3) percent of cells within insert box (**b**) Total number of CD44^{hi}CD62L^{hi} PCC-specific Th cells 7 days after immunization with Alum, IFA, CFA, CpG and MPL; means ±SEM n≥3 number of animals used is the same as in Figure 1.

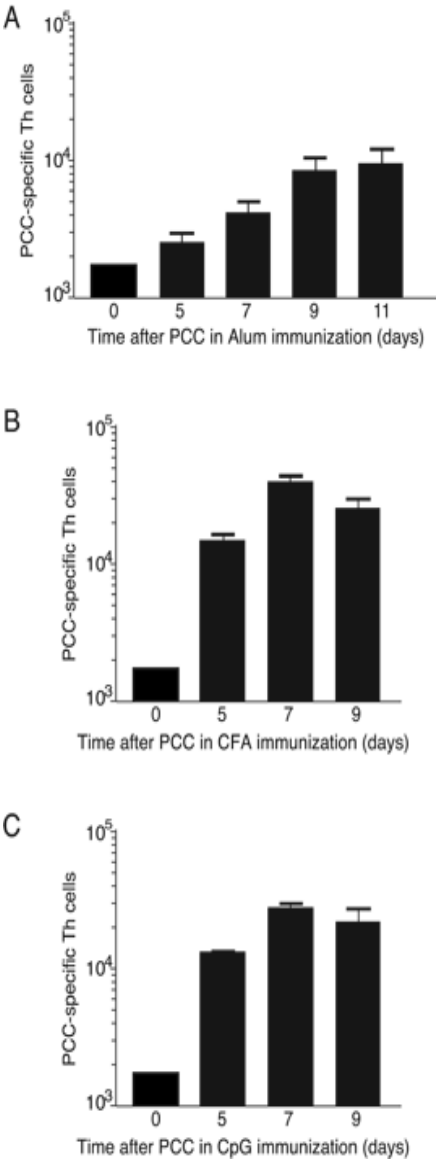


Figure S2. Dynamics of Antigen-Specific Th Cell Response across Different Adjuvants

Total number of PCC-specific Th cells 0, 5, 7, 9 and 11 days after immunization with (a) Alum and PCC and 0, 5, 7 and 9 days after immunization with (b) CFA or (c) CpG. Mean ±SEM for at least three animals at each timepoint.

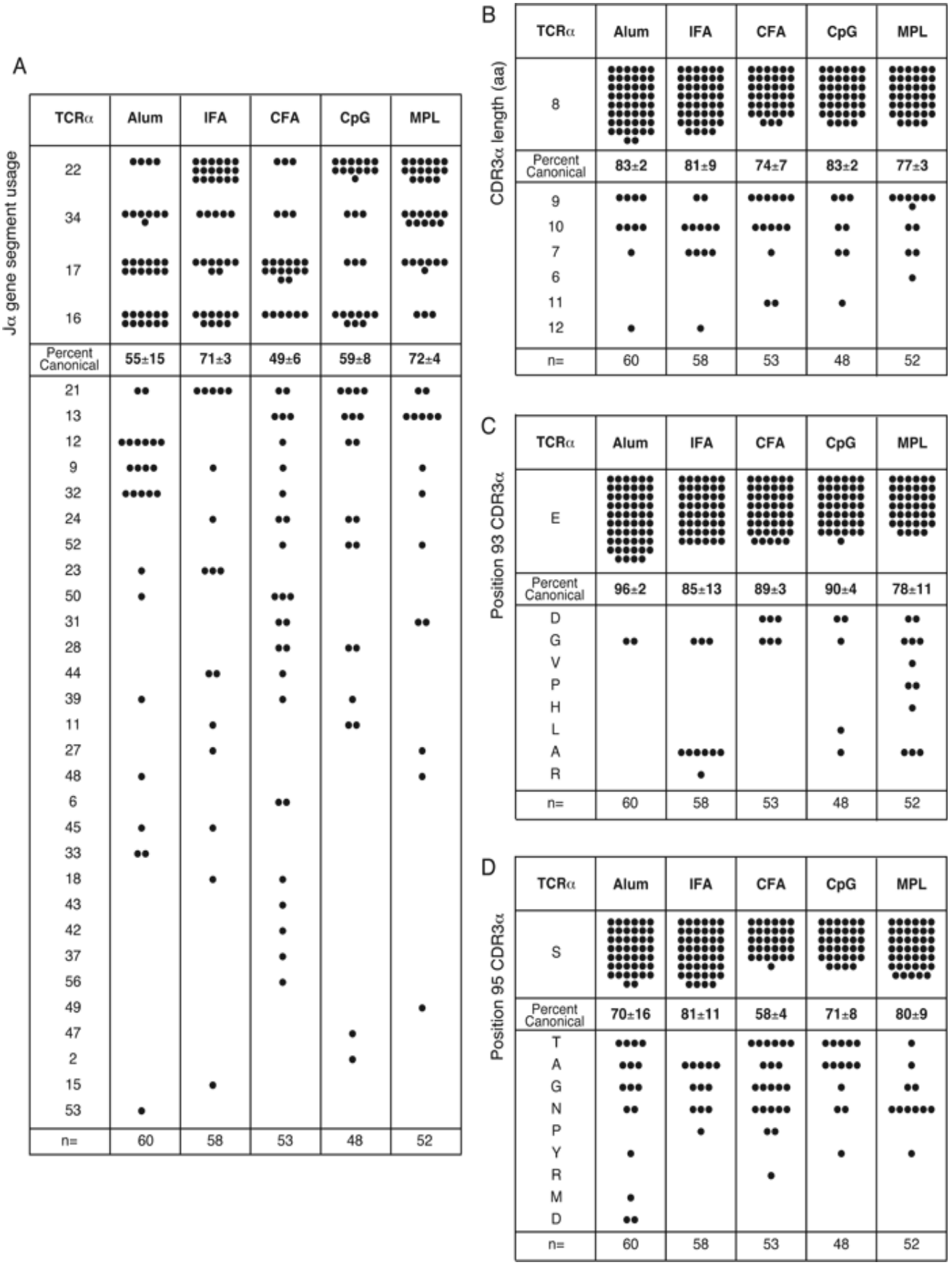

A
Jα gene segment usage
TCRα
Alum
IFA
CFA
CpG
MPL
22
34
17
16
Percent Canonical
55±15
71±3
49±6
59±8
72±4
21
13
12
9
32
24
52
23
50
31
28
44
39
11
27
48
6
45
33
18
43
42
37
56
49
47
2
15
53
n=
60
58
53
48
52
B
CDR3α length (aa)
TCRα
Alum
IFA
CFA
CpG
MPL
8
Percent Canonical
83±2
81±9
74±7
83±2
77±3
9
10
7
6
11
12
n=
60
58
53
48
52
C
Position 93 CDR3α
TCRα
Alum
IFA
CFA
CpG
MPL
E
Percent Canonical
96±2
85±13
89±3
90±4
78±11
D
G
V
P
H
L
A
R
n=
60
58
53
48
52
D
Position 95 CDR3α
TCRα
Alum
IFA
CFA
CpG
MPL
S
Percent Canonical
70±16
81±11
58±4
71±8
80±9
T
A
G
N
P
Y
R
M
D
n=
60
58
53
48
52

Figure S3. Impact of Adjuvant on TCRα Chain Selection

Ag-driven selection for preferred CDR3 features in α-chain of the TCR. Single PCC-specific Th cells ($V\alpha11^{+}V\beta3^{+}CD4^{+}CD44^{hi}CD62L^{lo}$) were sorted from draining lymph nodes of B10.BR mice immunized with PCC and the indicated adjuvant. Single-cell repertoire analysis was undertaken as described in Materials and Methods. Each dot represents the sequence information from a single-cell. The y-axis represents (a) Jα usage (b) CDR3α length (c) aa present at position α93 and (d) α95. The numbers of sequences used in the analysis are displayed (across 3 separate mice for each condition).

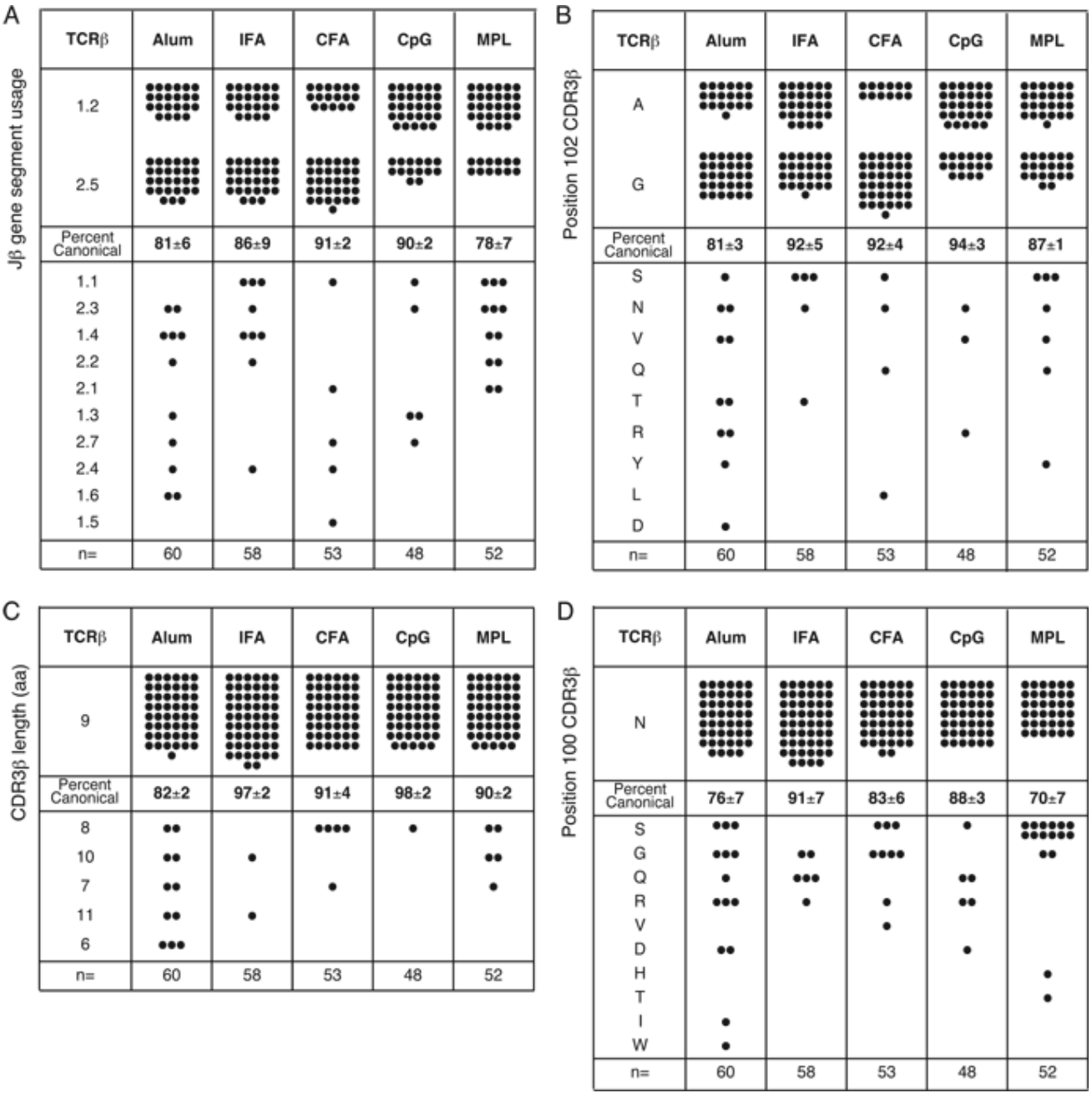
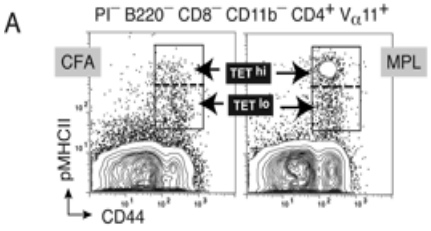
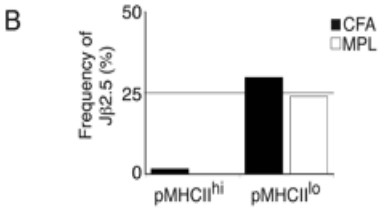


Figure S4. Impact of Adjuvant on TCRβ Chain Selection

Ag-driven selection for preferred CDR3 features in β-chain of the TCR. Single PCC-specific Th cells ($V\alpha11^{+}V\beta3^{+}CD4^{+}CD44^{hi}CD62L^{lo}$) were sorted from draining lymph nodes of B10.BR mice immunized with PCC and the indicated adjuvant. Single-cell repertoire analysis was undertaken as described in Materials and Methods. Each dot

represents the sequence information from a single-cell. The y-axis represents (a) Jβ usage (b) aa present at position β102 (c) the CDR3β length and (d) aa present at position β100. The numbers of sequences used in the analysis are displayed (across 3 separate mice for each condition).





C

CFA

CDR3β (100 102)	Jβ	$V\alpha11^+ V\beta3^+$ $CD44^{hi}$ $CD62L^{lo}$	$V\alpha11^+$ pMHCII$^+$ $CD44^{hi}$
S L N N A N S D Y	1.2	25	54
S L N R G Q D T Q	2.5	8	13
S L N S A N S D Y	1.2	6	7
S L N R A Q D T Q	2.5	10	6
S L N W G E D T Q	2.5	19	6
S L V G G Y A E Q	2.1	15	1
S S N W G E D T Q	2.5	6	0
S L N W G K D T Q	2.5	2	0
S R N W G E D T Q	2.5	2	0
S P N W G E D T Q	2.5	2	0
S P N W G Q D T Q	2.5	4	0

Figure S5. pMHCII Labeling Discriminates Jβ Usage

(**a**) Single $CD4^+V\alpha11^+pMHCII^{hi}CD44^{hi}$ (mfi>500) and $CD4^+V\alpha11^+pMHCII^{lo}CD44^{hi}$ (40<mfi<500) cells were sorted from mice immunized with CFA (left) or MPL-based adjuvant (right) and (**b**) the relative abundance of Jβ1.2 and Jβ2.5 gene segments was evaluated by single-cell PCR using Jβ specific primers ($pMHCII^{hi}$ CFA, n=3 with a total of 65 cells; $pMHCII^{hi}$ MPL, n=2 with a total of 61 cells; $pMHCII^{lo}$ CFA, n=2 with a total of 68 cells; $pMHCII^{lo}$ MPL, n=2 with a total of 42 cells). (**c**) Predicted amino acid sequences of CDR3β regions for $CD4^+V\alpha11^+V\beta3^+CD44^{hi}CD62L^{lo}$ and $CD4^+V\alpha11^+pMHCII^+CD44^{hi}$ Th cells sorted from the same CFA immunized B10.BR mice. The sequences are sorted according to their relative enrichment using pMHCII tetramer staining.

Hybridoma	V_α11 CDR3α										Jα	V_β3 CDR3β									Jβ	Adjuvant	Reference
	93	94	95	96	97	98	99	100	101	102		98	99	100	101	102	103	104	105	106			
2B4	L	R	V	T	G	G	N	N	K	L	56	S	L	**N**	W	S	Q	D	T	Q	**2.5**	CFA	1
2C2	L	R	V	T	G	G	N	N	K	L	56	S	L	**N**	W	**G**	Q	D	T	Q	**2.5**	CFA	1
MCC2	**E**	A	T	G	G	Y	K	V			12	S	L	**N**	R	**G**	Q	D	T	Q	**2.5**	CFA	2
15	**E**	G	A	G	G	Y	K	V			12	S	L	**N**	R	**G**	Q	D	T	Q	**2.5**	CFA	2
8	**E**	A	**S**	G	S	W	Q	L			22	S	L	**N**	R	**G**	Q	D	T	Q	**2.5**	CFA	2
1	**E**	P	G	G	N	N	K	L			56	S	L	**N**	W	**G**	Q	D	T	Q	**2.5**	CFA	2
KMAC-92	G	A	**S**	S	F	N	K	L			4	S	L	**N**	W	**G**	G	D	T	Q	**2.5**	CFA	3

Figure S6. PCC-Specific Hybridomas Expressing TCRβ Using Jβ2.5

CDR3α and CDR3β regions of TCR sequences from known PCC-specific hybridomas using Jβ2.5. Columns (left to right): hybridoma designation; CDR3 α, with positions α93E and α95S 'highlighted' in black as canonical, and motif length; J α gene segment usage; CDR3β, with positions β100N and β102G 'highlighted' in black as canonical, and motif length; Jβ gene segment usage; adjuvant used to promote PCC-specific Th cell response and isolate T cell hybridomas.