Supplemental Information

METHODS

Human subjects

Subjects were recruited from the Virginia Mason Medical Center Allergy Clinic and Benaroya Research Institute. Subjects with allergy and a documented record of shrimp allergy symptoms and positive ImmunoCAP scores for shrimp-specific IgE (Phadia AB, Sweden) were recruited. Non-allergic subjects with no clinical symptoms of allergy and negative ImmunoCAP scores for shrimp were also recruited. DNA samples were HLA-typed by using Dynal Unitray SSP Kits (Invitrogen, CA) according to the manufacturer's instructions. All subjects were recruited with informed consent and institutional review board approval. The attributes of these human subjects are summarized in Table E1.

TGEM

Biotinylated HLA-DR proteins were purified as described (E1). A total of 34 Pen m 1 peptides (p1 to p34), which were 20 amino acids in length with a 12 amino acid overlap spanning the entire Pen m 1 sequence (including the signal peptide), were synthesized (Mimotopes, Australia). A total of 41 Pen m 2 peptides (p1 to p41), which were 20 amino acids in length with a 12 amino acid overlap spanning the entire Pen m 2 sequence were also synthesized. Pen m 1 peptides were divided into 6 pools of 5 peptides each plus a 7^{th} pool of 4 peptides. Pen m 2 peptides were divided into 8 pools of 5 each plus a 9^{th} of 3 peptides. These peptides mixtures were loaded into the biotinylated HLA-DR proteins to generate pooled tetramers as described (1). Cells were cultured for 14 days and then stained with pooled peptide tetramers. Cells from wells

that gave positive staining were stained again by using tetramers loaded with each individual peptide from the positive pool.

Ex vivo analysis of Pen m 1 and Pen m 2-reactive $CD4^+$ T cells

Briefly, 30 million PBMCs in 200 µL T-cell culture medium were stained with 20µg/mL phycoerythrin-labeled tetramers at room temperature for 100 minutes. Cells were washed and incubated with anti-phycoerythrin magnetic beads (Miltenyi Biotec) at 4°C for 20 minutes, and a 1/10 fraction was saved for analysis. The other fraction was passed through a magnetic column (Miltenyi Biotec). After enrichment, cells were stained with percp-cy5.5 anti-CD14 (HCD14, Biolegend), percp-cy5.5 anti-CD19 (HIB19, Biolegend), V500 anti-CD4 (RPA-T4, BD biosciences), Alexa fluor 700 anti-CD45RA (HI100, BD Biosciences), Alexa fluor 488 anti-CD183 (CXCR3) (G025H7, Biolegend), Pe-cy7 anti-CD194 (CCR4) (TG6/CCR4, Biolegend), Alexa fluor 647 anti-CD294 (CRTH2) (BM16, BD Biosciences), APC-cy7 anti-CD27 (O323, Biolegend), Brilliant Violet 421 anti-CD196 (CCR6) (G034E3, Biolegend) antibodies and with Cell viability solution (BD Via-Probe, BD Biosciences). Data were acquired on a FACS LSR II flow cytometer (BD Biosciences) using FACSDiva software (BD Biosciences) and were analyzed with FlowJo 8.8.2 software (Treestar). The frequency of Pen m 1 and Pen m 2-specific T cells was determined by using the formula F = n/N. Where n designates the number of tetramer positive cells in the bound fraction after enrichment and N is the total number of $CD4^+$ T cells, calculated as 10 × the number of $CD4^+$ T cells in $1/10^{th}$ pre-enriched fraction that was saved for analysis.

Intracellular cytokine staining

For *in vitro* intracellular cytokine staining of IFN-γ, IL-4, IL-17, IL-5 and IL-10, PBMCs were stimulated for 2 weeks with specific peptide and then stained with the corresponding phycoerythrin-labeled tetramers for 60 minutes at 37°C. Cells were then restimulated with 25ng/mL phorbol 12-myristate 13-acetate and 1µg/mL ionomycin in the presence of 10µg/mL Brefeldin-A for 4h at 37°C. After stimulation, cells were stained with APC-cy7 anti-CD4 (OKT3, Biolegend), Alexa Fluor 488 anti-IL-4 (8D4-8, eBioscience), Alexa Fluor 700 anti-IFN-γ (4S.B3, Biolegend), Alexa Fluor 647 anti-IL21 (3A3-N2.1, BD Biosciences), Percp-cy5.5 anti-IL-17 (BL168, Biolegend), Pe-cy7 anti-IL-10 (JES3-9D7, Biolegend) antibodies and with fixable viability stain 450 (BD biosciences). Intracellular staining were performed using fixation buffer and permeabilization buffer (eBiosciences). Data were acquired on a FACS LSR II flow cytometer (BD Biosciences) using FACSDiva software (BD Biosciences) and were analyzed with FlowJo 8.8.2 software (Treestar).

Statistical analysis

Statistical analysis was performed using the appropriate tests, as indicated in the figure legends, using Prism 5.0 software (GraphPad).

REFERENCES

E1. Novak EJ, Liu AW, Gebe JA, Falk BA, Nepom GT, Koelle DM, et al. Tetramer-guided epitope mapping: rapid identification and characterization of immunodominant CD4+ T cell epitopes from complex antigens. Journal of immunology. 2001;166(11):6665-70.
E2. Chattopadhyay PK, Yu J, Roederer M. A live-cell assay to detect antigen-specific CD4+ T cells with diverse cytokine profiles. Nature medicine. 2005;11(10):1113-7.

LEGENDS

FIG E1. TGEM studies of DR0301-restricted Pen m 1 and Pen m 2-reactive $CD4^+$ T cells. PBMCs from a DR0301 subject with shrimp allergy were stimulated with 7 pools of Pen m 1 peptides and 9 pools of Pen m 2 peptides for two weeks and subsequently stained with corresponding DR0301/Pen m 1 or /Pen m 2 pooled peptide tetramers (top 2 rows of top and bottom panels respectively) . Cells that were stimulated with Pen m 1 pool 1 or Pen m 2 pool 3 were re-stained with individual peptides from the corresponding pool (third row of top and bottom panels respectively). The staining identified Pen m 1 p2 (Pen m $1_{9\text{-}28}$) and Pen m 2 p11 (Pen m $2_{81\text{-}100}$) as DR0301-restricted Pen m 1 and Pen m 2 T-cell epitopes.

FIG E2. Frequency of Pen m 1 and Pen m 2 epitope-specific $CD4^+$ T cells in each subjects. (A, B) *Ex vivo* frequency of Pen m 1 (A) and Pen m 2 (B) epitope-specific $CD4^+$ T cells in DRB5*01:01-DQA*01:02/DQB*06:02 adults with or without shrimp allergy. The frequencies of specific T cells per million $CD4^+$ T cells are as indicated. (C) Frequencies of naïve Pen m 1 and Pen m 2 epitope-specific $CD4^+$ T cells for 9 allergic (right) and 12 non-allergic (left) subjects. Each symbol denotes a DRB1*03:01, DRB5*01:01 or DQA*01:02/DQB*06:02-restricted T-cell epitope as indicated on the figure.

FIG E3. Pen m 1 and Pen m 2 specific $CD4^+$ T cell responses in allergic subjects with CD154 assays. For the CD154 expression assay (E2), $30x10^6$ PBMC (at $6x10^6$ /mL) were stimulated for 3h at 37°C with 5µg/mL of synthesized peptide pools (Mimotopes, Australia) spanning the entire Pen m 1 or Pen m 2 sequences, in the presence of 1µg/ml anti-CD40 (HB14, Miltenyi Biotec). After stimulation, PBMC were

labeled with PE-conjugated CD154 and $CD154^+$ cells were enriched using anti-PE magnetic beads (Miltenyi Biotec). (A) CD154 expression on gated $CD4^+$ T cells after 3h PBMC stimulation with Pen m 1 or Pen m 2. Results shown are after anti-PE bead enrichment. Numbers indicate frequency of $CD154^+$ $CD45RA^-$ $CD4^+$ T cells per 10^6 $CD4^+$ T cells. (B) Frequency of total $CD154^+$ $CD45RA^-$ $CD4^+$ T cells after Pen m 1 (white bar) or Pen m 2 (black bar) stimulation of PBMC in 7 allergic and 2 non-allergic subjects.

FIG E4. DRB1*03:01 and DRB5*01:01/Pen m 1 and Pen m 2-specific T cells lines. (A, B) DRB1*03:01 and DRB5*01:01/Pen m 2-specific T cells lines were analyzed by tetramer staining after stimulation of PBMCs from allergic (A) or non-allergic (B) adults with antigenic Pen m 2 peptides for 2 weeks. (C, D) DRB1*03:01 and DRB5*01:01/Pen m 1-specific T cells lines were analyzed after stimulation of PBMCs from allergic (C) or non-allergic (D) adults with antigenic Pen m 1 peptides for 2 weeks. (E) Examples of cytokine profile of DRB1*03:01/Pen m 2, DRB1*03:01/Pen m 1 and DRB5*01:01/Pen m 1-specific T cells lines from allergic adults (top) and non-allergic adults (bottom).

	Sample	HLA-DR	Immunocap score Shrimp IgE	Dust Mite P IgE	Symptomes
shrimp-allergic	#1	1501/1115	3	3	hives
	#2	1501/0101	3	3	hives
	#3	1501/1501	1	1	anaphylaxis
	#4	1501/0301	3	4	wheezing
	#5	0101/0301	2	1	anaphylaxis
	#6	0301/0301	2	2	anaphylaxis
	#7	0301/0301	2	2	anaphylaxis
	#8	0301/0404	3	4	anaphylaxis
	#9	0301/1104	3	2	anaphylaxis
Shrimp non- allergic	#1	0301/0401	0	0	
	#2	0301/0401	0	0	
	#3	0301/0401	0	0	
	#4	1501/07**	0	0	
	#5	1501/1501	0	0	
	#6	0401/1501	0	0	
	#7	0101/1501	0	0	
	#8	0301/0701	0	0	peanut anaphylaxis
	#9	0301/0701	0	0	peanut anaphylaxis
	#10	0401/1501	0	0	peanut anaphylaxis
	#11	0701/1501	0	3	peanut allergy
	#12	0101/1501	0	0	peanut anaphylaxis

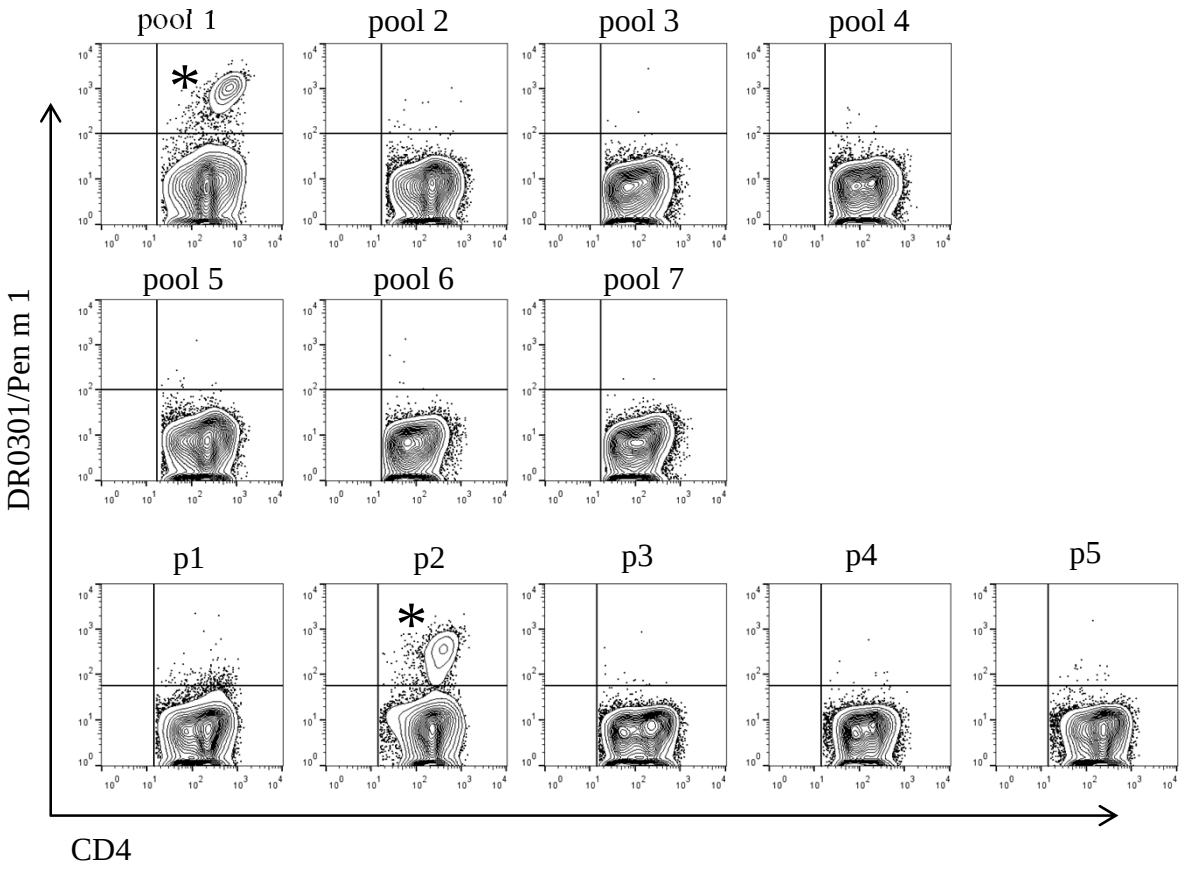
Immunocap score: 0 < 0.35 IU/ml; 1 = 0.35 - 0.69 IU/ml; 2 = 0.70 - 3.49 IU/ml; 3 = 3.50 - 17.49 IU/ml; 4 = 17.50 - 49.99 IU/ml

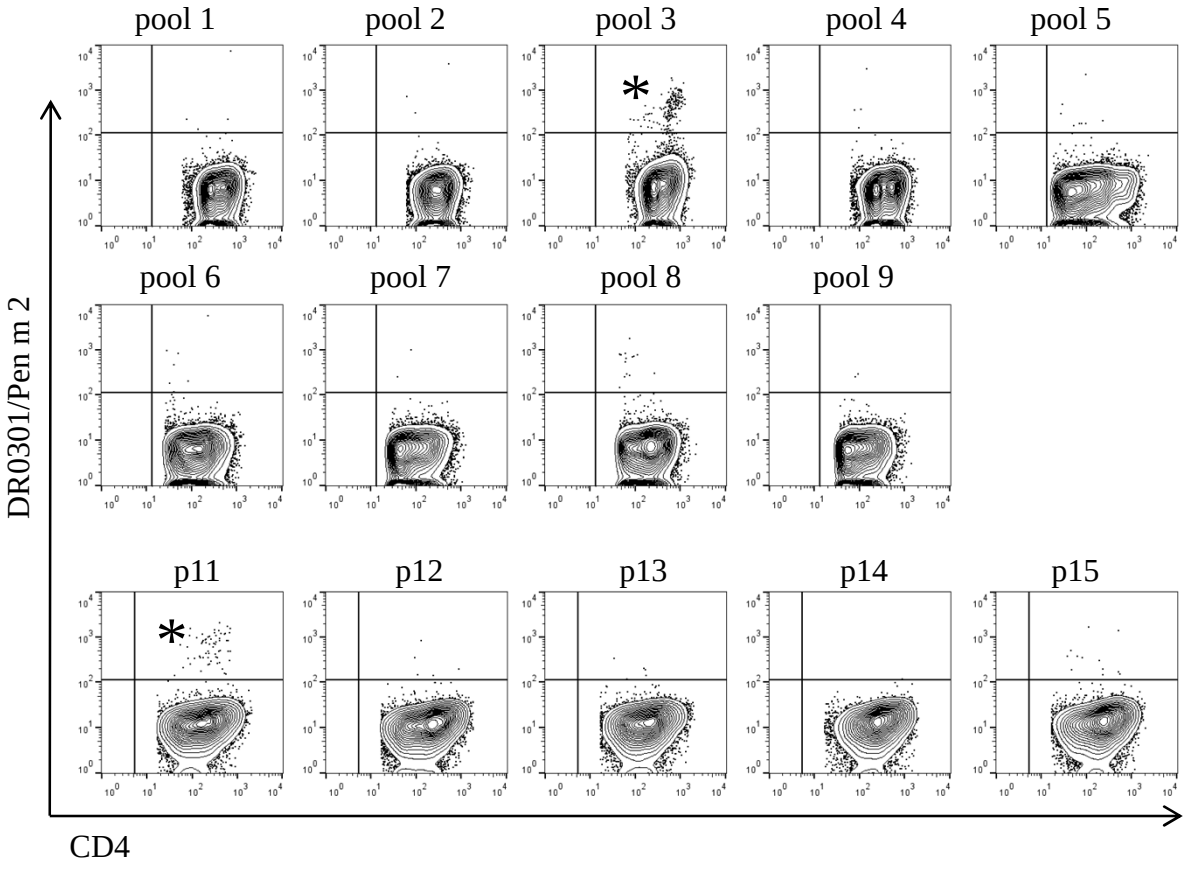
**all subjects with the DR1501 haplotype also have the DRB5*01:01-DQA*01:02/DQB*06:02 haplotype*

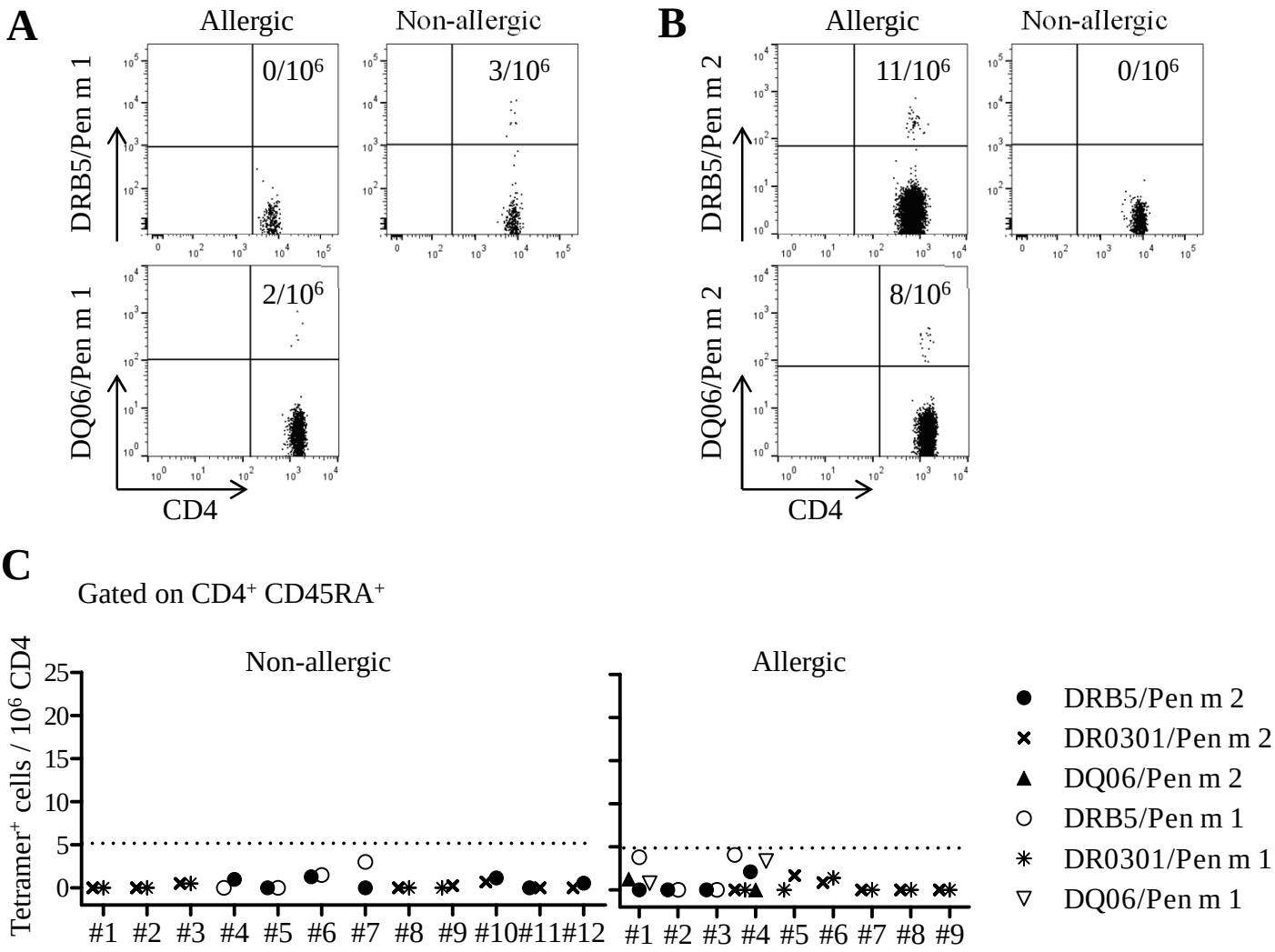
Table E1. Study subjects' characteristics

HLA restriction	Epitope	AA sequence
DRB1*03:01	Pen m1 $_{9 - 28}$	QAMKLEKDNAMDRADTLEQQ
DRB1*03:01	Pen m2 $_{81 - 100}$	LFDPIIEDYHVGFKQTDKHP
DRB5*01:01	Pen m1 $_{161 - 180}$	KYDEVARKLAMVEADLERAE
DRB5*01:01	Pen m2 $_{193 - 212}$	RFLQAANACRYWPAGRGIYH
DQA*01:02/DQB*06:02	Pen m1 $_{97 - 116}$	EDLERSEERLNTATTKLAEA
DQA*01:02/DQB*06:02	Pen m2 $_{265 - 284}$	LGFLTFCPTNLGTTVRASVH

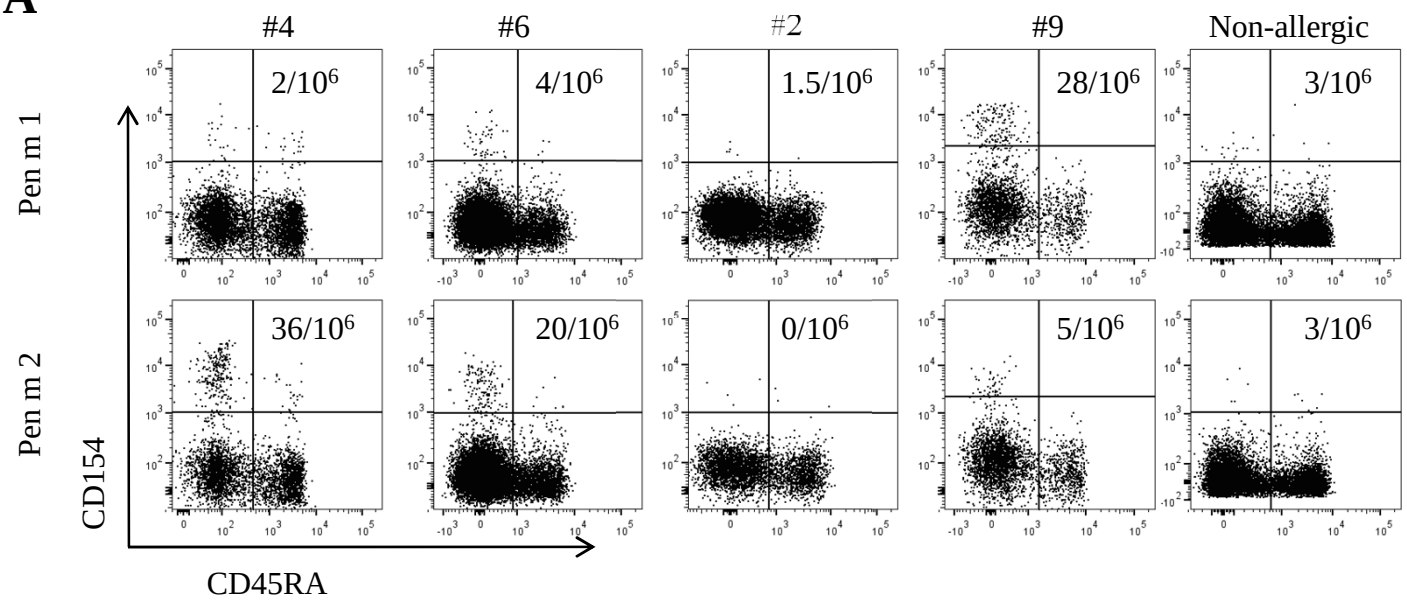
Table E2. Pen m 1 and Pen m 2 T-cell epitopes


pool 1
pool 2
pool 3
pool 4
pool 5
pool 6
pool 7
p1
p2
p3
p4
p5
DR0301/Pen m 1
CD4

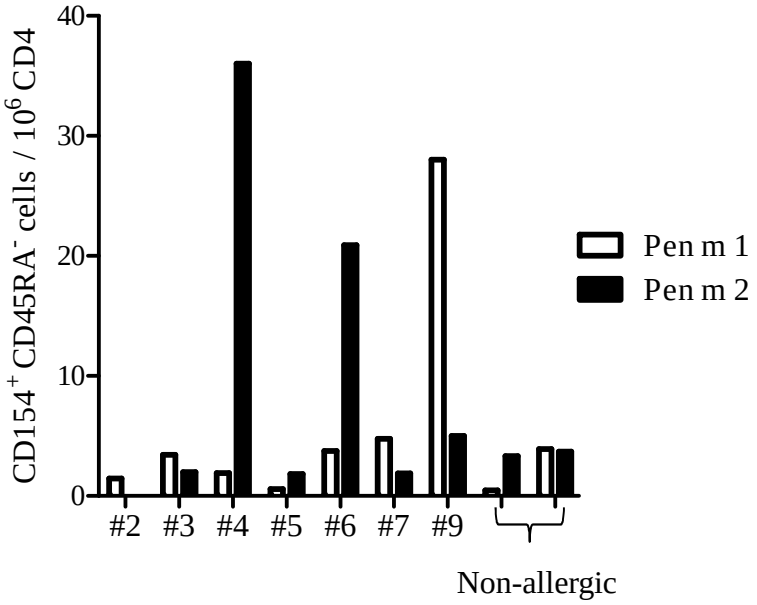

pool 1
pool 2
pool 3
pool 4
pool 5
pool 6
pool 7
pool 8
pool 9
p11
p12
p13
p14
p15
DR0301/Pen m 2
CD4

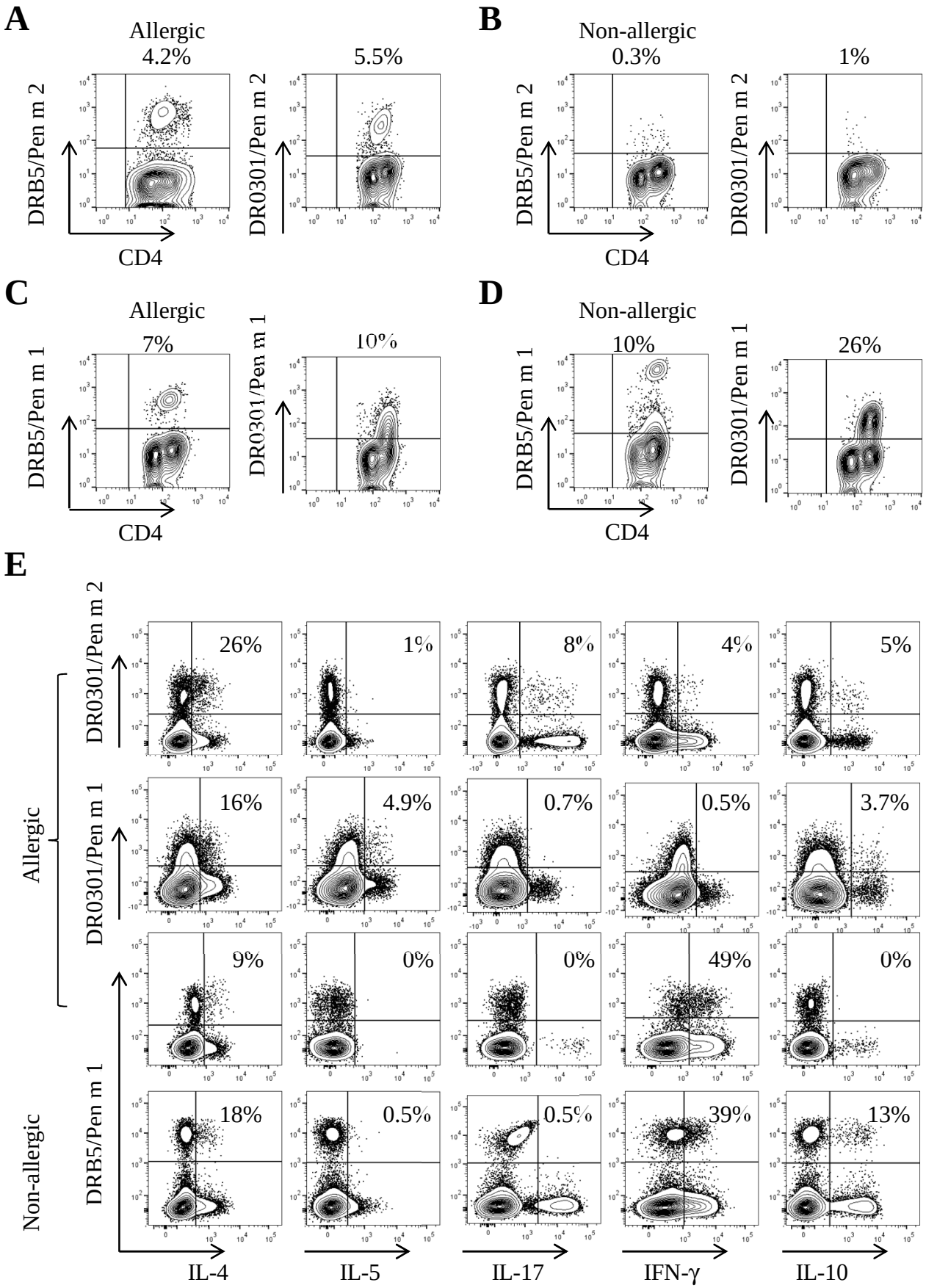

A
Allergic
Non-allergic
DRB5/Pen m 1
0/10^6
3/10^6
DQ06/Pen m 1
2/10^6
CD4
B
Allergic
Non-allergic
DRB5/Pen m 2
11/10^6
0/10^6
DQ06/Pen m 2
8/10^6
CD4
C
Gated on CD4+ CD45RA+
Non-allergic
Allergic
Tetramer+ cells / 10^6 CD4
25
20
15
10
5
0
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12
#1 #2 #3 #4 #5 #6 #7 #8 #9
DRB5/Pen m 2
DR0301/Pen m 2
DQ06/Pen m 2
DRB5/Pen m 1
DR0301/Pen m 1
DQ06/Pen m 1

A


#4
#6
#2
#9
Non-allergic
Pen m 1
Pen m 2
2/10^6
4/10^6
1.5/10^6
28/10^6
3/10^6
36/10^6
20/10^6
0/10^6
5/10^6
3/10^6
CD154
CD45RA

B


CD154+ CD45RA- cells / 10^6 CD4
40
30
20
10
0
#2
#3
#4
#5
#6
#7
#9
Non-allergic
Pen m 1
Pen m 2


A
Allergic
4.2%
5.5%
DRB5/Pen m 2
DR0301/Pen m 2
CD4
B
Non-allergic
0.3%
1%
DRB5/Pen m 2
DR0301/Pen m 2
CD4
C
Allergic
7%
10%
DRB5/Pen m 1
DR0301/Pen m 1
CD4
D
Non-allergic
10%
26%
DRB5/Pen m 1
DR0301/Pen m 1
CD4
E
DR0301/Pen m 2
DR0301/Pen m 1
DRB5/Pen m 1
Allergic
Non-allergic
26%
1%
8%
4%
5%
16%
4.9%
0.7%
0.5%
3.7%
9%
0%
0%
49%
0%
18%
0.5%
0.5%
39%
13%
IL-4
IL-5
IL-17
IFN-γ
IL-10