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Tetramer reagents and tetramer-guided epitope mapping

For epitope mapping, Phl p 1, Phl p 5a and Phl p 5b derived-peptides were divided into pools of 5 peptides each, as previously described (E1). Cells were cultured with peptide pools for 14 days and then stained with pooled peptide tetramers. Cells from wells that resulted in positive staining were stained again with individual pMHCII tetramers from the positive pool. pMHCII tetramers loaded with irrelevant peptides were used as negative controls.

Intracellular cytokine staining. CD4+ T cells cultured for 14 days with specific immunodominant peptide were stained with corresponding PE-conjugated pMHCII tetramers for 60 minutes at 37°C. Cells were then restimulated with 50 ng/mL phorbol 12-myristate 13-acetate and 1 mg/mL ionomycin in the presence of 10 mg/mL monensin in 1 mL of complete medium for 6 hours at 37°C and 5% CO_2. After restimulation, surface staining was performed first, followed by fixation/permeabilization, as per the manufacturer's protocol (eBioscience). Cells were then stained with various combinations of antibodies for IFN-g, IL-17, IL-10, (all from BioLegend, San Diego, CA) and IL-4 (eBioscience) or corresponding isotype-matched mAbs. After 30 minutes at 4°C, cells were washed and immediately analyzed by flow cytometry.

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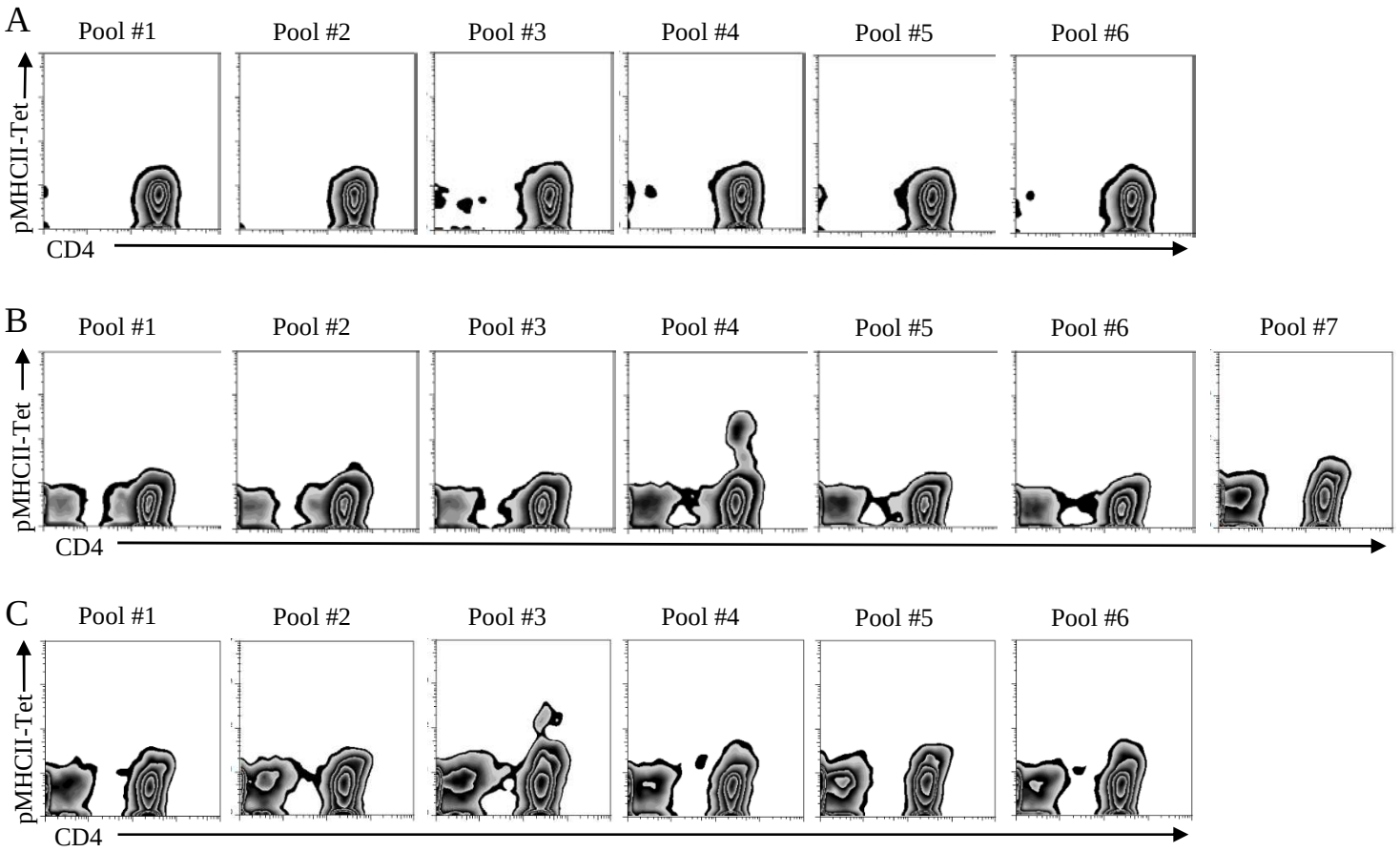
(E1) Novak EJ, Liu AW, Gebe JA, Falk BA, Nepom GT, Koelle DM, et al. Tetramer-guided epitope mapping: rapid identification and characterization of immunodominant CD4+ T cell epitopes from complex antigens. J Immunol 2001 Jun 1;166(11):6665-70.

Supplementary Table EI: Clinical characteristics of the DRB1*04:01 individuals enrolled in the study.

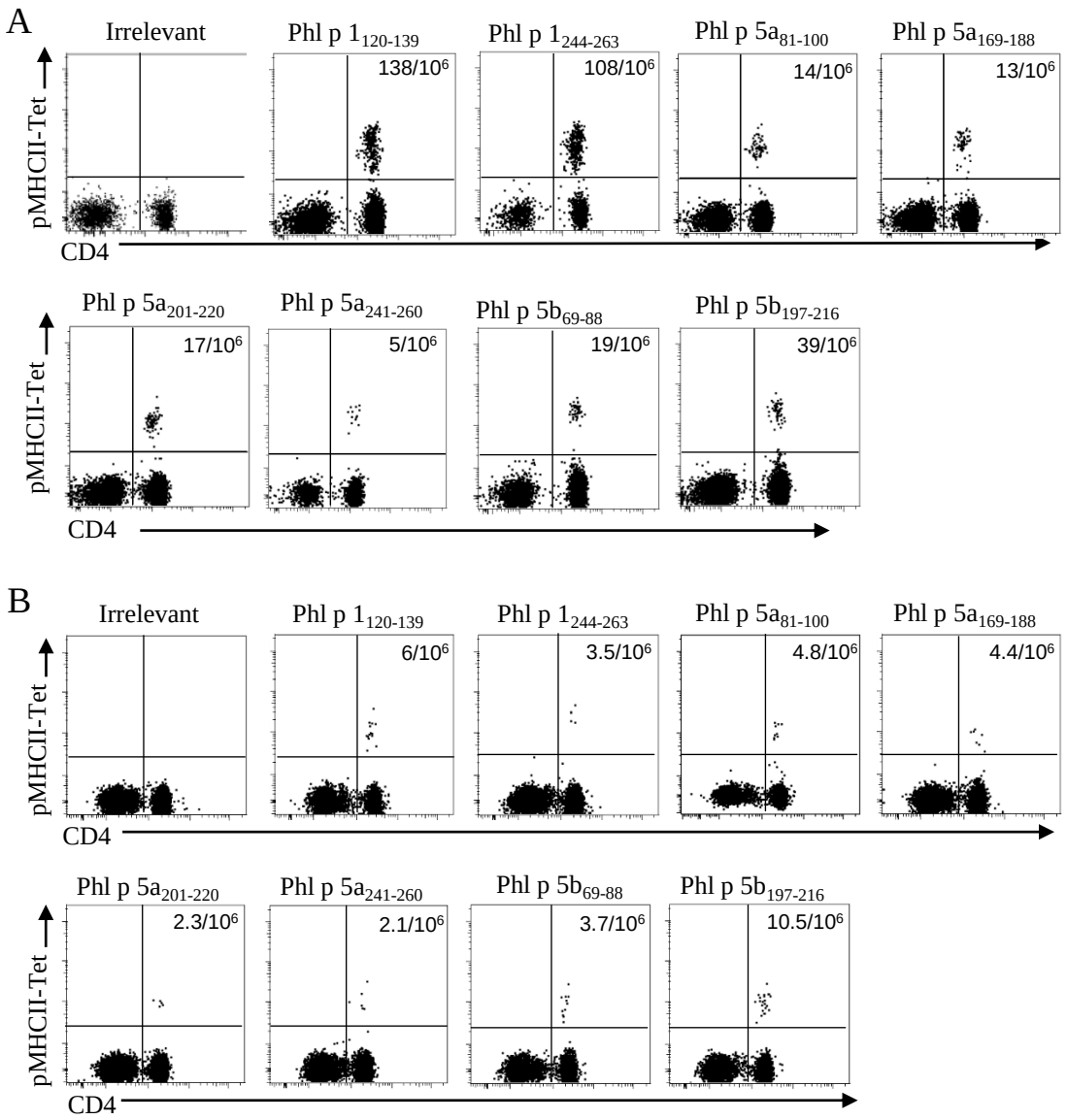
Group	ID	HLA-DR typing	Age	M/F	Phl p 1 (IgE kUA/L)	Phl p 5 (IgE kUA/L)	Skin Prick Test	Asthma
Grass pollen allergic	GP-1	DR4/DR15	33	F	16.9	26.2	4++	Y
	GP-2	DR4/DR13	58	M	1.97	2.06	4+	N
	GP-3	DR4/DR3	36	F	>100	41.3	4+	N
	GP-4	DR4/DR3	60	F	5.72	6.06	4+	N
	GP-5	DR4/DR13	26	M	45.4	31.3	4+	N
	GP-6	DR4/DR7	36	M	18.8	21.5	4+	Y
	GP-7	DR4/DR1	38	F	15.7	8.14	4+	N
	GP-8	DR4/DR3	38	M	-	-	4+	N
	GP-9	DR4/DR1	33	M	19.3	21.9	4+	N
	GP-10	DR4/DR4	34	F	6.57	8.38	3+	Y
	GP-11	DR7/DR15	27	M	-	-	4+	Y
	GP-12	DR7/DR1	29	M	17.2	26.7	4++	Y
Non allergic	H-1	DR4/DR15	28	M	-	-	0	N
	H-2	DR4/DR11	36	M	-	-	-	N
	H-3	DR4/DR3	29	F	-	-	-	N
	H-4	DR4/DR14	33	F	-	-	0	N
	H-5	DR4/DR13	36	M	-	-	-	N
Post ASIT	ASIT-1	DR4/DR15	56	M	-	-	-	N
	ASIT-2	DR4/DR7	61	M	-	-	1+	Y
	ASIT-3	DR4/DR11	30	M	-	-	2+	Y
	ASIT-4	DR4/DR15		F	-	-	-	N
	ASIT-5	DR4/DR11		M	-	-	2+	Y
	ASIT-6	DR4/DR7		F	-	-	2+	N

Supplementary Table EII: Binding capacities of Phl p 1, Phl p5a and Phl p 5b peptides to immuno-purified HLA-DR04:01 and HLA-DR07:01 molecules.

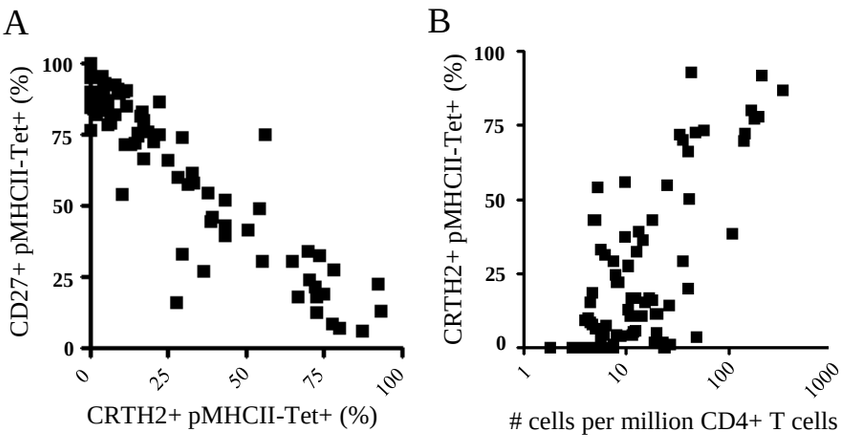
HLA-DRB1	Allergen epitope	Sequence	IC50 (nM)	Immunogenicity	Induced response
*04:01	Phl p $1_{120-139}$	EEPIAPYHFDLSGHAFGAMA	76	Dominant	T_H2 epitope
	Phl p $1_{244-263}$	TEAEDVIPEGWKADTSYESK	232	Dominant	$T_H2$epitope
	Phl p $5a_{81-100}$	FAEGLSGEPKGAAESSSKAA	297	Non dominant	T_H2 epitope
	Phl p $5a_{169-188}$	DAAFKVAATAANAAPANDK	177	Non dominant	T_H2 epitope
	Phl p $5a_{201-220}$	KASTGGAYESYKFIPALEAA	565	Non dominant	T_H2 epitope
	Phl p $5a_{241-260}$	ETALKKAITAMSEAQKAAKP	595	Non dominant	T_H1/T_R1 epitope
	Phl p $5b_{69-88}$	KFKTFEAAFTSSSKAAAAKA	104	Non dominant	T_H1/T_R1 epitope
	Phl p $5b_{197-216}$	GGAYDTYKCIPSLEAAVKQA	123	Non dominant	T_H1/T_R1 epitope
*07:01	Phl p $5a_{121-140}$	PEAKYDAYVATLSEALRIIA	81	Dominant	T_H2 epitope
	Phl p $5b_{89-108}$	ATPEAKFDSFVASLTEALRV	194	Dominant	T_H2 epitope



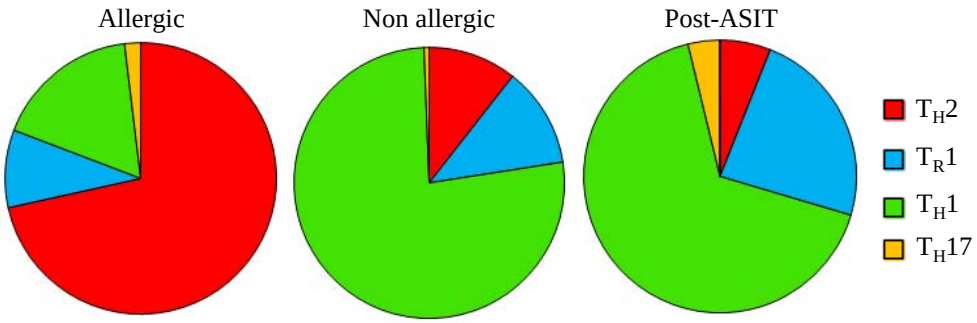
Supplemental Figure E1: Tetramer-guided epitope mapping of TGP major allergen. T cells from DRB1*07:01 TGP-allergic individuals were stimulated with overlapping peptide mixtures spanning the entire Phl p 1 (**a**), Phl p 5a (**b**) or Phl p 5b (**c**) protein and stained using pooled peptide-loaded tetramers after 2 weeks. Data are representative of 3 DRB1*07:01-restricted TGP-allergic individuals.



Supplemental Figure E2: Representative flow cytometric analysis of *ex vivo* PE-conjugated DRB1*04:01/Phl p 1, /Phl p 5a and /Phl p 5b pMHCII tetramer staining in allergic (**A**) and non-allergic (**B**) subject after magnetic bead enrichment for PE-positive cells. Plots are gated on live $CD3^+$ $CD14^-$ $CD19^-$ T lymphocytes. Frequencies of specific T cells per million $CD4^+$ T cells are as indicated.



Supplemental Figure E3: A, Correlation between the proportion of CRT_H2^+ cells and the proportion of $CD27^-$ cells in TGP allergen-specific T cells. B, Scatter plot showing the correlation between the *ex vivo* frequency and the proportion of CRT_H2^+ cells in TGP allergen-specific T cells. (A,B) Each data point represents the value for each single TGP epitope from ten DR04:01-restricted TGP-allergic donors.



Supplemental Figure E4: Global DR04:01-restricted TGP-specific $CD4^+$ T cell response in allergic, non-allergic and post-ASIT individuals. Data are representative of at least 5 individuals and are presented as the mean values from each group in pie charts.