Supplementary Information

So et al (2018) **"Proinsulin C-peptide is an Autoantigen in People with Type 1 Diabetes"**

SUPPLEMENTARY METHODS

FIGURES

TABLES

Supplementary Methods

HLA typing

All HLA typing was performed by the Australian Red Cross Blood Services (ARCBS). Clinical details of all subjects are shown in Supplementary Tables 1–4. Healthy controls were defined as individuals who had either an HLA-DQ2 or HLA-DQ8 allele and had no personal or family history of T1D. The absence of autoantibodies against insulin, IA-2 or GAD-65 was confirmed in the majority of healthy controls.

Isolation of PBMC

Peripheral blood mononuclear cells (PBMC) were isolated over Ficoll-paque (GE Healthcare, Sweden) and washed twice in phosphate buffered saline (PBS) as described (20).

CFSE-based proliferation and functional assays

PBMC were labelled with 0.1μM CFSE (5,6- carboxylfluorescein diacetate succinimidyl ester). Tetanus toxoid was used at 10LfU/ml and supplied by Statens Serum Institut, Copenhagen, Denmark). Clones that retained antigen specificity after expansion were stored in 10% dimethyl sulfoxide (DMSO) and fetal calf serum (FCS) (Bovogen, Victoria, Australia) at $5x10^6$ cells/mL in liquid nitrogen.

Synthetic peptides

Peptides were synthesized by Purar Chemicals (Wuxi New District, China) and reconstituted in 40% acetonitrile, 0.5% acetic acid and water, or DMSO, to 2.5–5.0mM and stored at $^{-}80^{o}$C. Full length C-peptide (PI_{33-63}): EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ (31 amino acids).

Insulin VNTR genotyping

Genomic DNA was purified using Genomic DNA purification NucleoBond kit (Macherey-Nagel, Germany) from thawed 0.5mL of whole blood that had been snap frozen and stored at -80^{o}C at the time of venepuncture. Genotyping of the single nucleotide polymorphism, rs689 (Cat# 4351379), was performed using an inventoried Taqman SNP Genotyping Assay obtained from Applied Biosystems (Life Technologies, Carlsbad, CA, USA). The assay was run on a Roche LightCycler 480 II using Roche Probe Master Mix (Roche Applied Science, Penzberg, Germany) according to manufacturer's instructions

CD4^{+} T-cell cloning

The CFSE-based T cell assay responses from which C-peptide specific CD4^{+} T cell clones were isolated are highlighted in Figure 1. Several factors determined from which CFSE-based T cell proliferation assays T-cell cloning was attempted. These included: the volume of blood and number of PBMC available from each donor and the strength of the response in the CFSE assay. We did not attempt to clone if the CDI was less than 3.0.

Functional Assays

Cloned CD4^{+} T cells were thawed and used directly in functional assays. APC (20,000 cells/well) were either the The Epstein Barr Virus (EBV) transformed B-cell line KJ (HLA-DRB1*03:01, 04:04; DQB1*02:01, 03:02) or PBMC. APCs were irradiated: 2,000 rad PBMC and 10,000 rad KJ-EBV After 2 days, ^{3}H-thymidine (0.5μCi/ well) was added for 18 hours after which the cells were harvested, and incorporated radioactivity was measured by β-scintillation counting.

Analysis of HLA restriction

HLA-blocking monoclonal antibodies specific for HLA-DR (clone L243), HLA-DP (clone B7/21) and HLA-DQ (clone SPV-L3) were used to define the restricting HLA isotype. Second, the HLA allele(s) were determined using a panel of T2 cells transfected with HLA-DQA and HLA-DQB alleles (indicated in the figure legends). For HLA-DR restricted clones, the alleles were determined using a panel of HLA-DR-transfected Bare Lymphocyte Syndrome (BLS) cells.

TCR sequencing

Expanded C-peptide specific clones were sorted in 0.5% FCS/PBS into 96-well PCR plates to give five cells/well. Plates were stored at $^{-}80^{o}$C overnight. PCR product was sequenced and the TCR genes identified by alignment with the IMGT database (http://www.imgt.org/IMGT_vquest). The TRA or TRB gene was amplified and its presence confirmed using gene-specific primers. Sanger sequencing was performed by Australian Genome Research Facility (AGRF), Melbourne, Australia.

Preparation of islet and acinar extracts

Snap-frozen human islets from five donors, supplied by the Tom Mandel Islet Transplantation Program, were pooled and homogenized in a pre-prepared buffer (60% Tris 20mM/NaCl 50mM, sucrose 0.3M, methionine 1μM, protease inhibitor 1:200 (Sigma-Aldrich); 30% acetonitrile, 10% butanol). Acinar tissue from the same donors was prepared in the same way to use as a negative control. Protein quantity was estimated using the bicinchoninic acid (BCA) protein assay (Thermo

Scientific, Rockford, IL, USA). Aliquots were stored at $^{-}80^{o}$C. Islet and acinar lysates were lyophilized and diluted in culture media to the required concentration.

Statistical analysis

The statistical significance of the comparison of the proportions of individuals who had responses to C-peptide was determined using Fischer's exact test. CDIs from the CFSE-based proliferation assay were log transformed then differences between groups were analyzed using a Student's t test. For analysis of T-cell clone experiments, comparisons between group data (mean ± SEM) were made using unpaired Welch's two-tailed *t* test. Statistical significance was defined as $p<0.05$.

Supplementary Figure 1

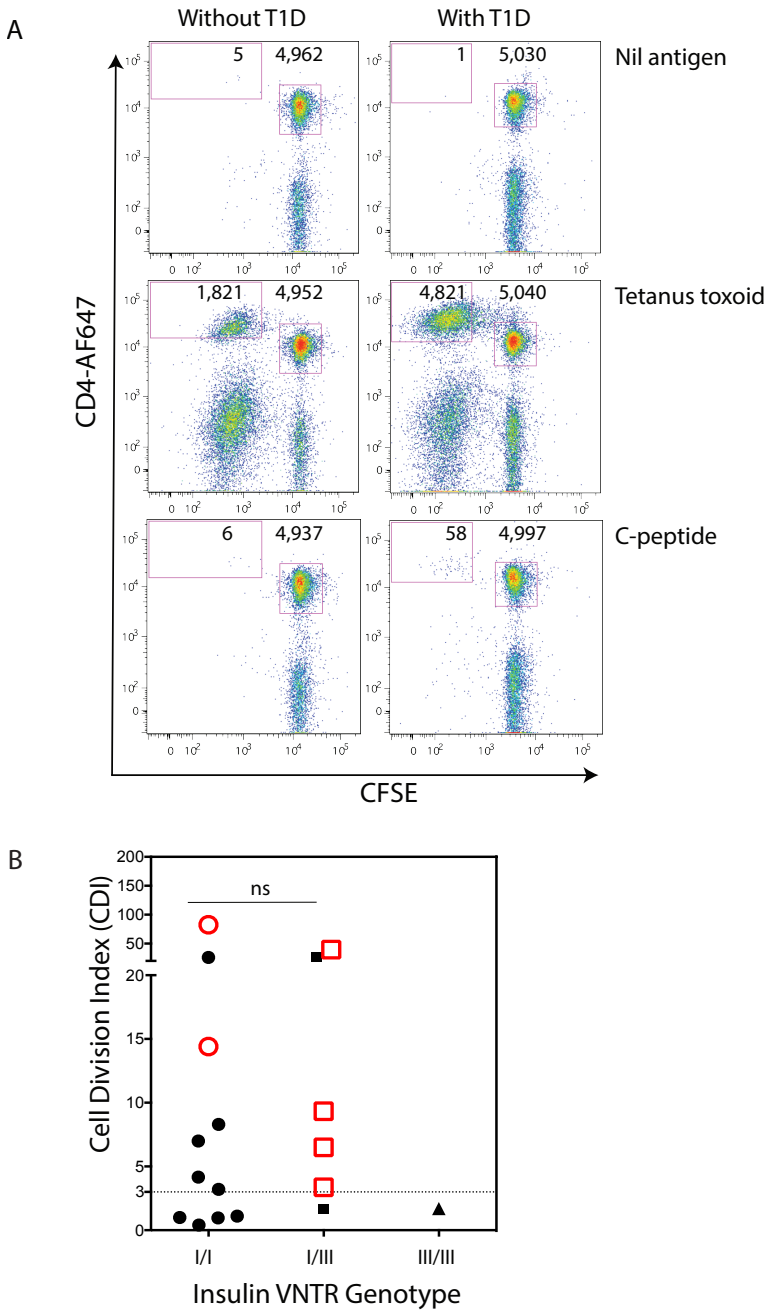


Figure S1. *CD4⁺ T-cell responses to C-peptide in PBMC*. (A) Representative FACS plots from a CFSE-based proliferation assay with PBMC from donors with and without T1D. Nil antigen - without antigen, tetanus toxoid (10LfU/mL), C-peptide; PI_{33-63} (10µM). (B) PI_{33-63} specific $CD4^+$ T-cell responses plotted against the subject's VNTR genotype. Open red symbols indicate samples

from which clones were isolated. Statistical significance was determined using unpaired Student's *t* test, *= $p<0.05$ after log transformation of the CDIs.

Supplementary Figure 2

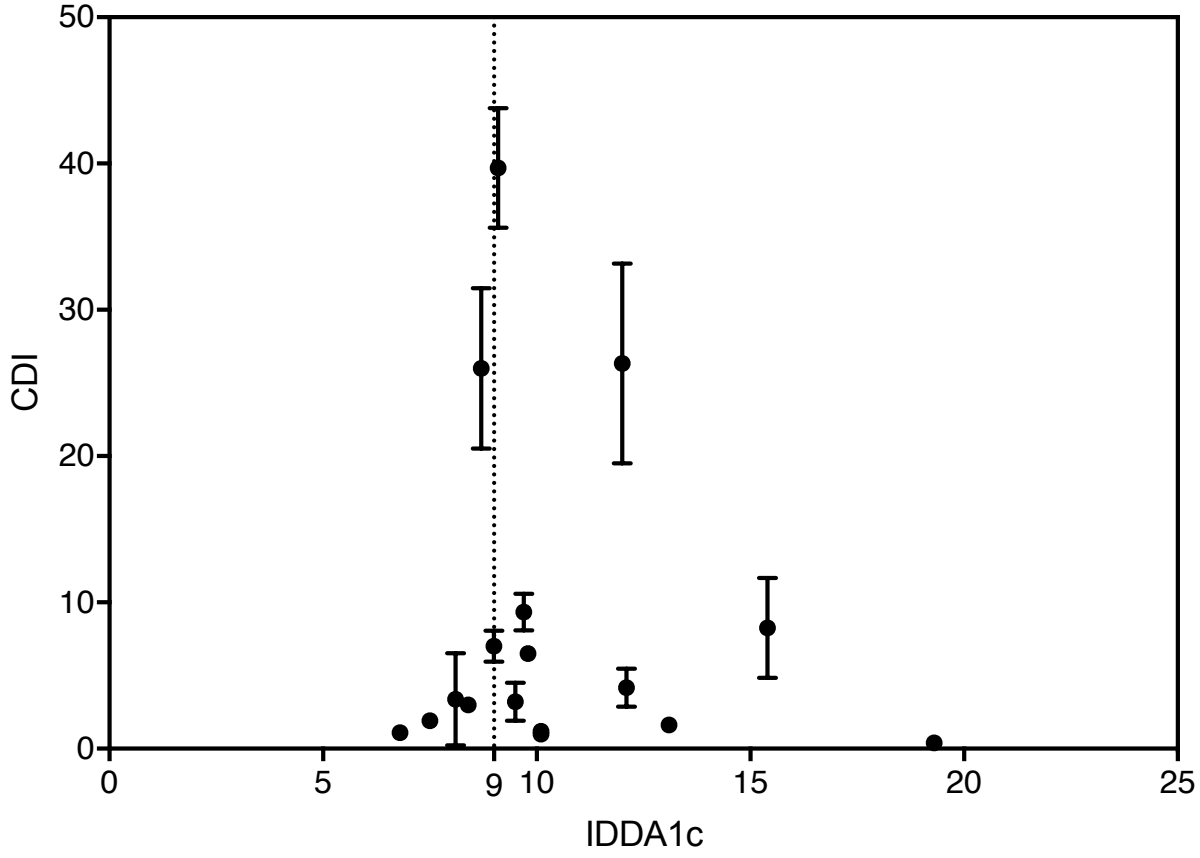
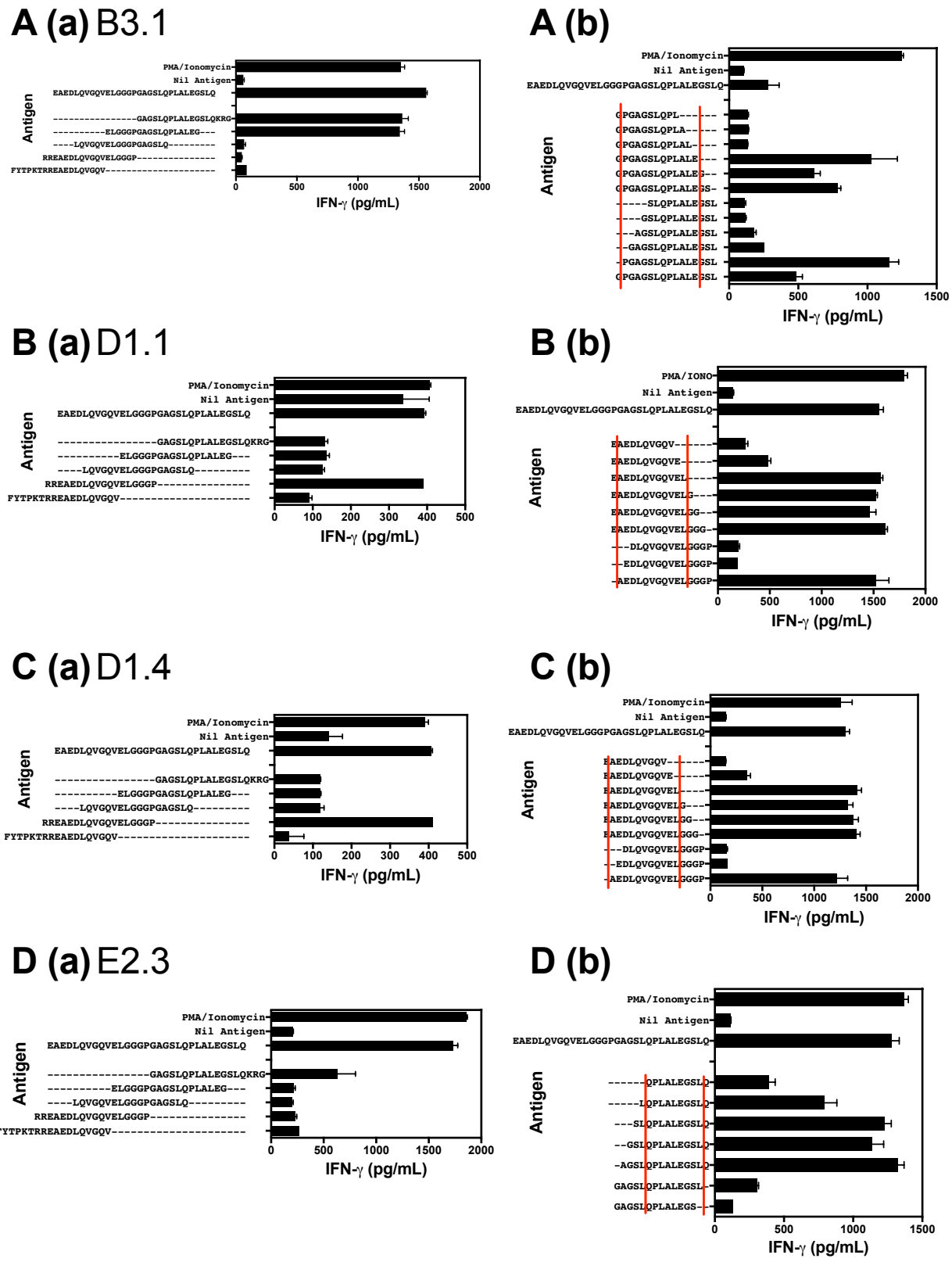


Figure S2. *CD4$^+$ T-cell responses to C-peptide compared to insulin dose-adjusted HbA1c*

CD4$^+$ T-cell responses to C-peptide of subjects with recent-onset T1D were plotted against insulin dose-adjusted HbA1c (IDAA1c). Insulin dose-adjusted HbA1c is used as a surrogate measure for residual beta-cell function and calculated as HbA1c (%) + 4 x insulin dose (U/kg/24 hours) (Max Andersen, M. L., et al *Pediatr Diabetes, 15*(7), 469-476. doi:10.1111/pedi.12208). Partial remission of beta-cell function is defined as IDAA1c ≤9 and is indicated by the dotted line. Partial remission was defined in 6/17 (70.6%) subjects analyzed. No correlation was seen between CD4$^+$ T-cell responses to C-peptide (CDI) and residual beta-cell function (IDAA1c), $r^2 = 0.01$, $p = 0.68$).

Supplementary Figure 3



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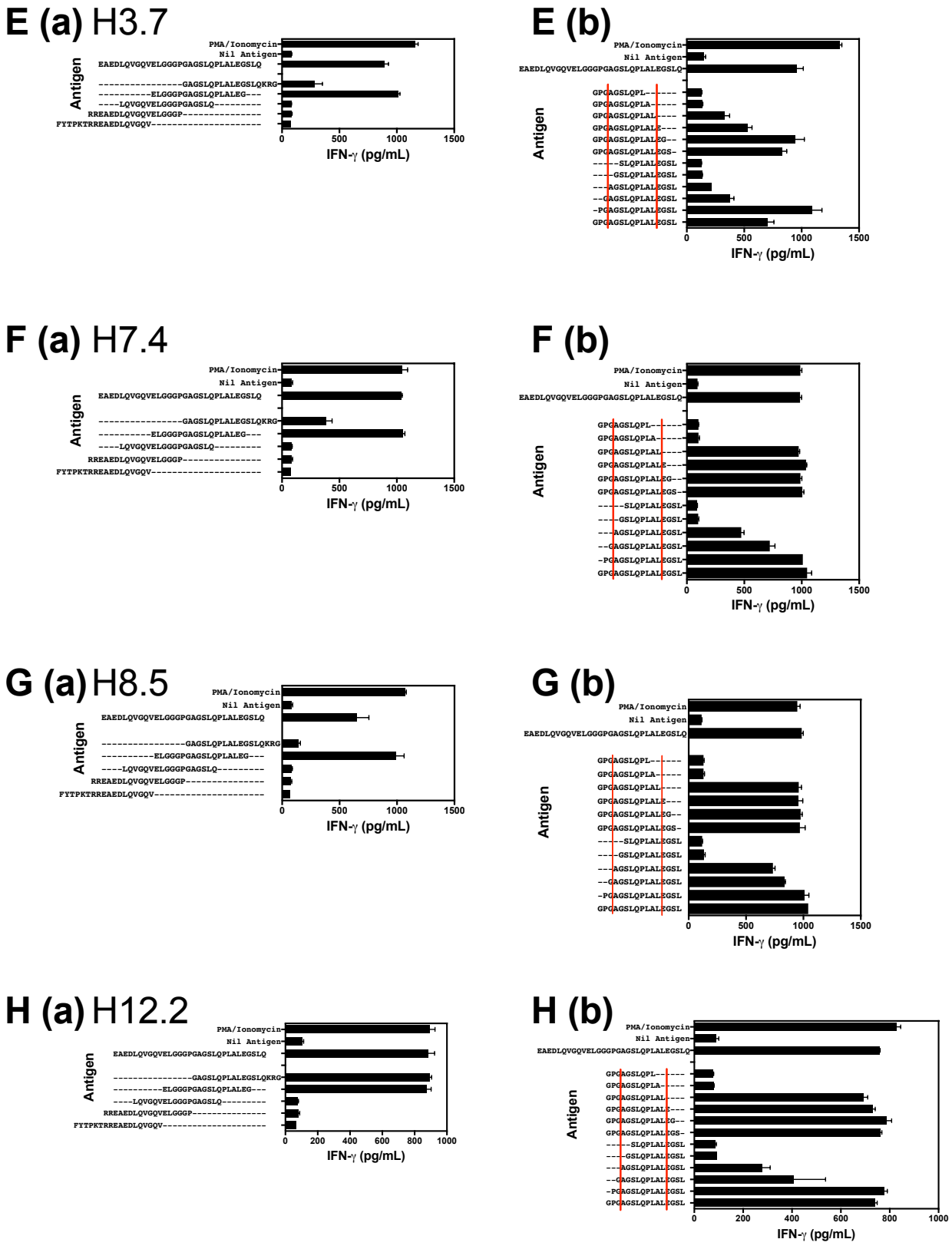

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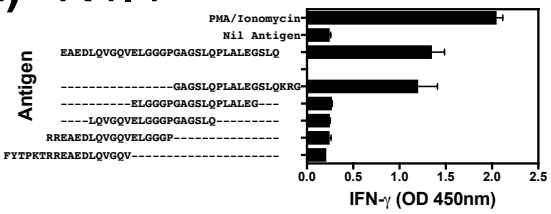

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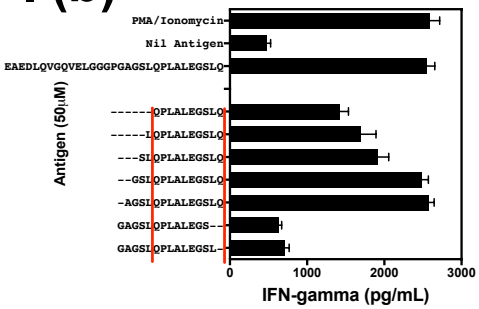

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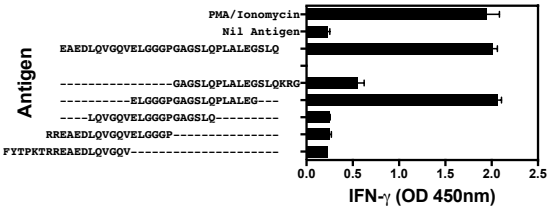

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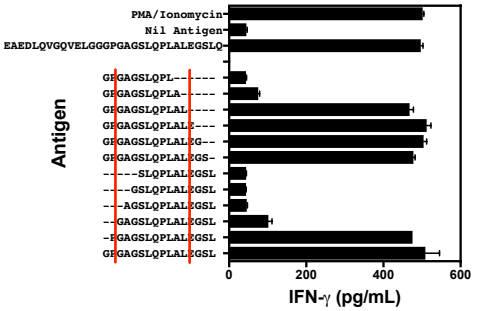

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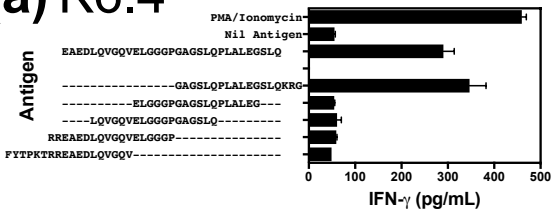

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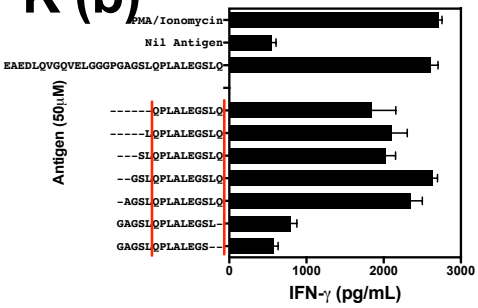

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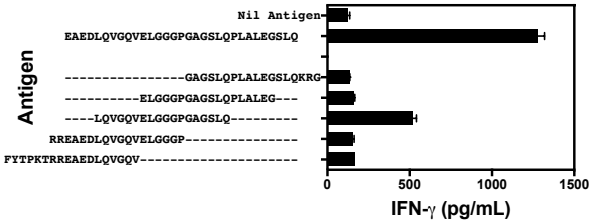

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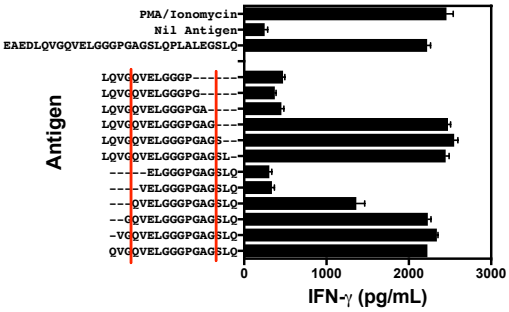

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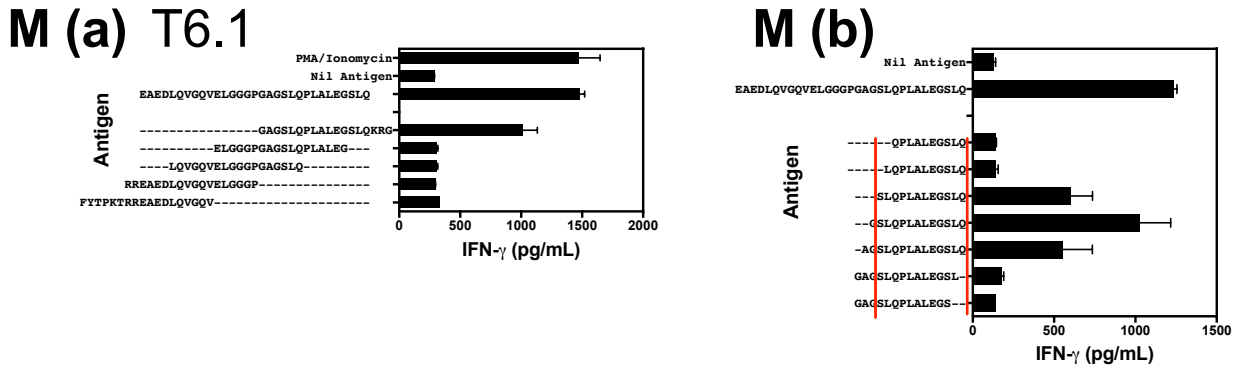
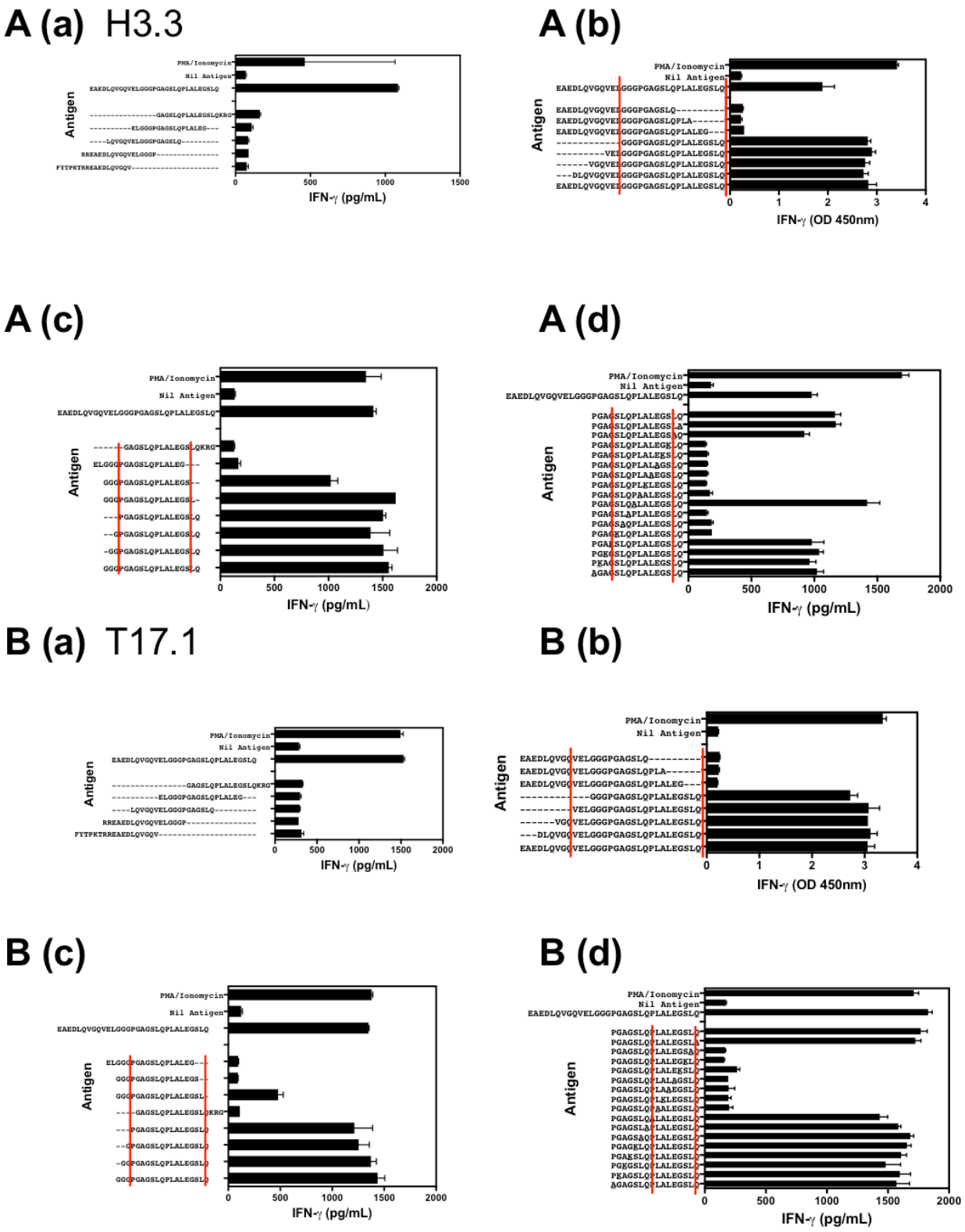


Figure S3. *Epitope mapping of C-peptide specific clones.* Responses of $CD4^+$ T-cell clones to antigen were measured by the secretion of IFN-γ measured by ELISA. The means (+/- SEM) of triplicate IFN-γ measurements are shown. (a) "Coarse" epitope mapping. Clones were tested in triplicate against 18mer peptides, at a final concentration of 10μM, spanning the length of C-peptide, overlapping by 12 amino acids. Where a response was not elicited, peptide concentration was increased to 50μM (as indicated on the figure). (b) "Fine" epitope mapping of clone. Epitope specificity was further refined using peptides truncated by a single amino acid from either the N- or the C-terminus. The parallel lines in red delineate the sequence of the minimum epitope determined by the results of the experiment. (A) B3.1, (B) D1.1, (C) D1.4, (D) E2.3, (E) H3.7, (F) H7.4, (G) H8.5, (H) H12.2, (I) K4.4, (J) K6.2, (K) K6.4, (L) K9.6, (M) T6.1

Supplementary Figure 4



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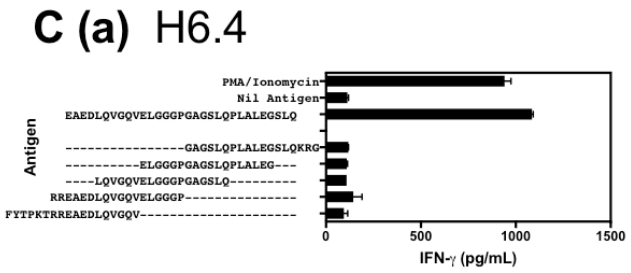

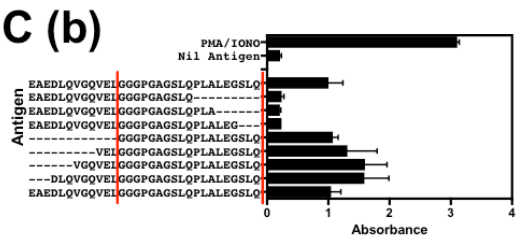

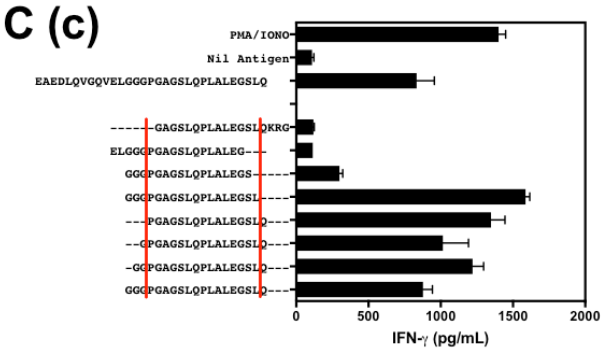

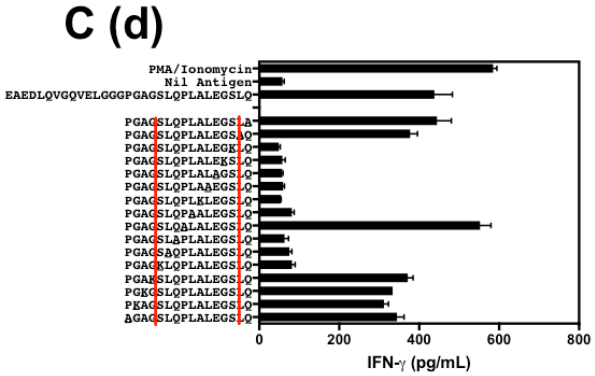

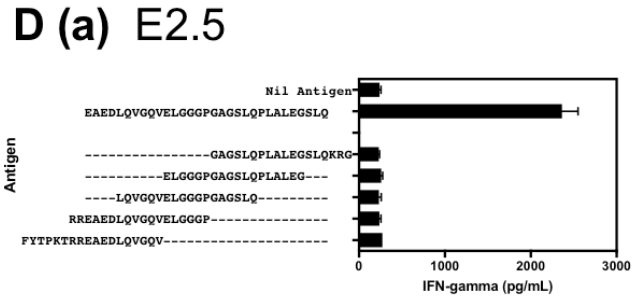

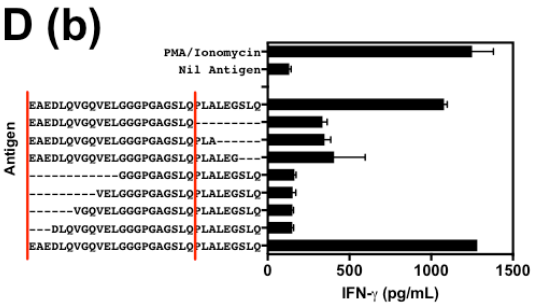

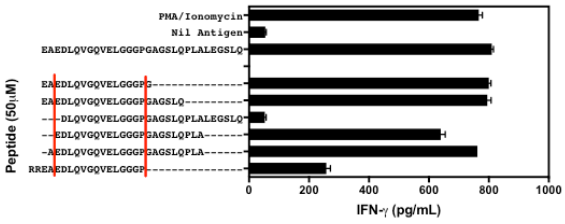

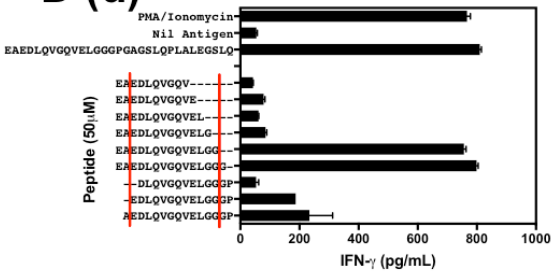

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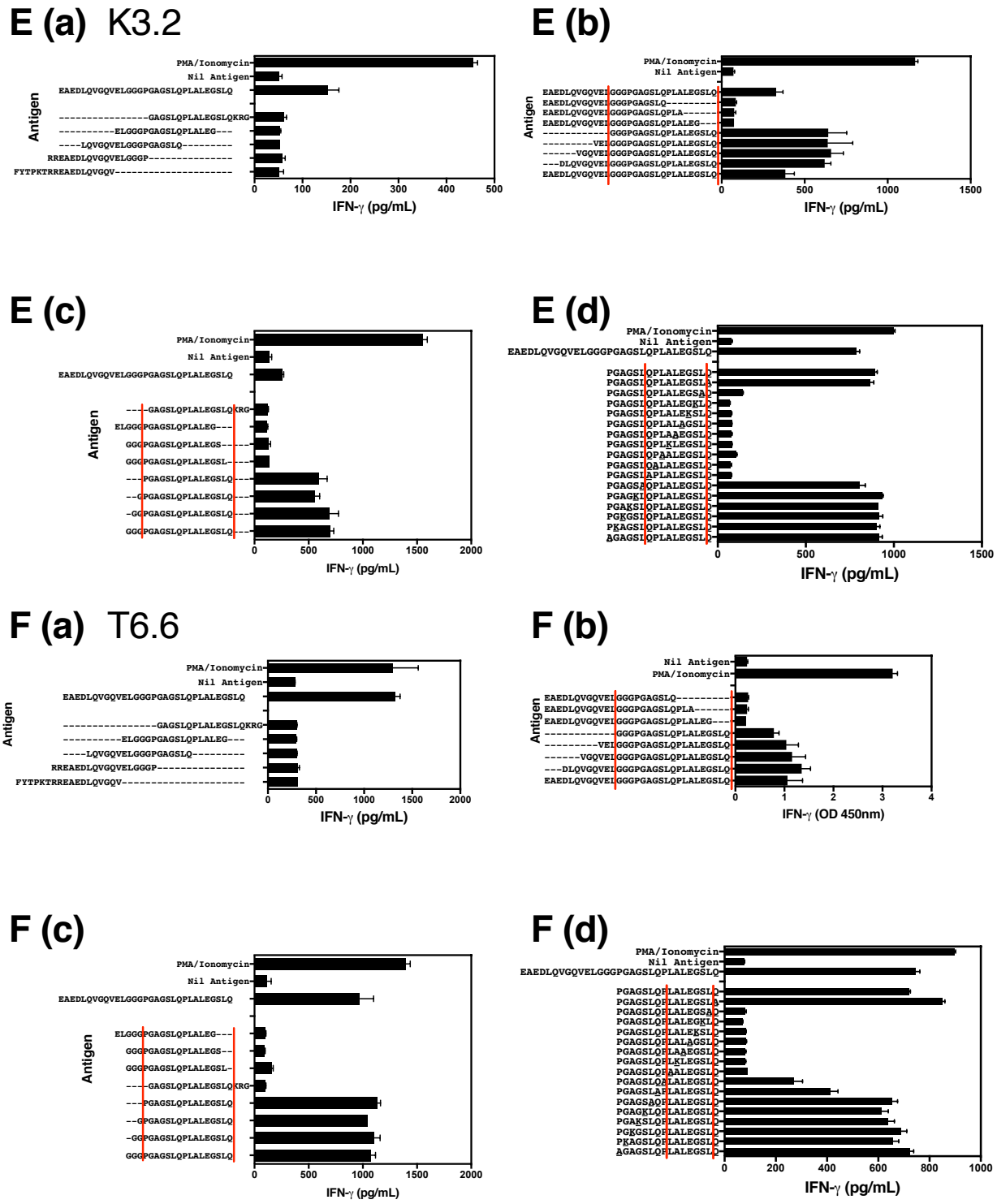

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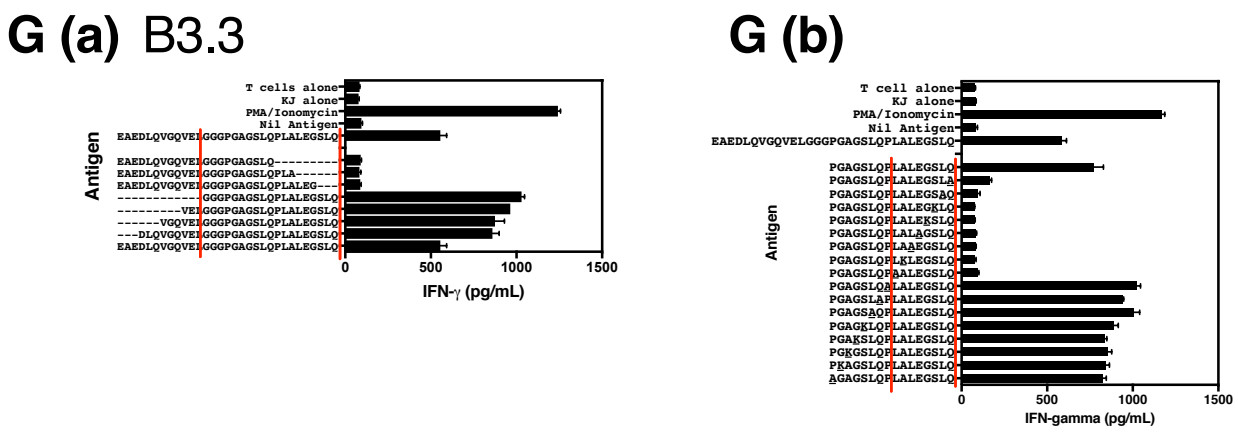
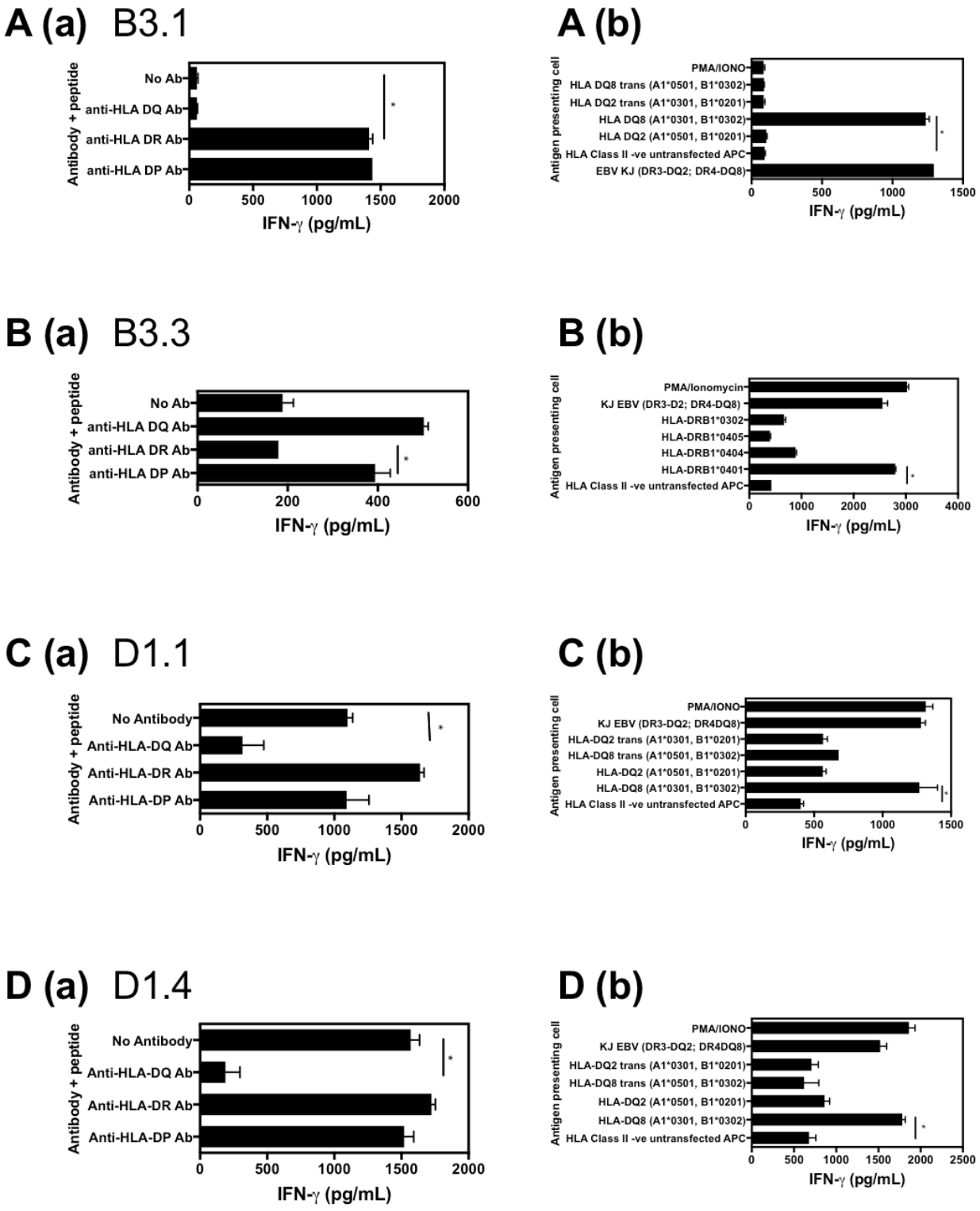


Figure S4 *Epitope mapping of C-peptide specific clones that could not be determined with 18mer peptides*. The responses of $CD4^+$ T-cell clones to antigen were measured by the secretion of IFN-γ measured by ELISA in triplicate. Means +/- SEM are shown. (a) "Coarse" epitope mapping: Clones were tested in triplicate against 18mer peptides, at a final concentration of 10μM, spanning the length of C-peptide, overlapping by 12 amino acids. (b) Alternative "coarse" epitope mapping of clone. These clones did not respond to any of the five 18mer peptides and were mapped using variants of the full-length C-peptide which were sequentially truncated from either the N- or C-terminus by three amino acids. (**c**) "Fine" epitope mapping: the minimum epitope was refined by using a panel of peptides sequentially truncated by one amino acid from either the N- or C-terminus (d) Further "fine" epitope mapping: The minimum epitope was then determined by testing the clones against a panel of peptides with a single amino acid substitution. The substitutions were chosen to disrupt the putative HLA-binding or TCR recognition. For clone E2.5 (D) substitution peptides were not required as a minimum epitope was determined using higher concentrations of a panel of truncated peptides. (A) H 3.3, (B) T17.1, (C) H6.4, (D) E2.5, (E) K3.2, (F) T6.6, (G) B3.3.

Supplementary Figure 5



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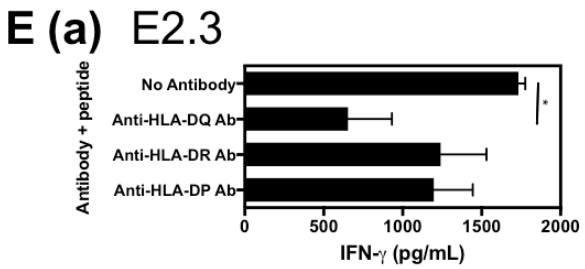

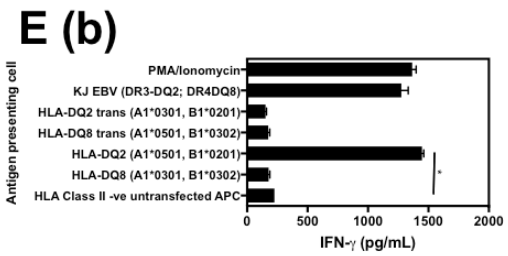

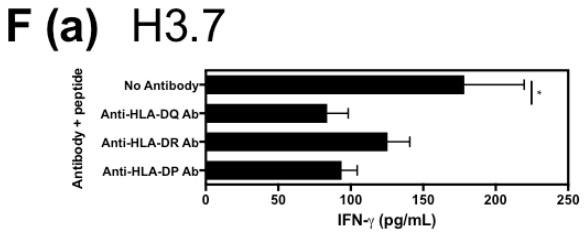

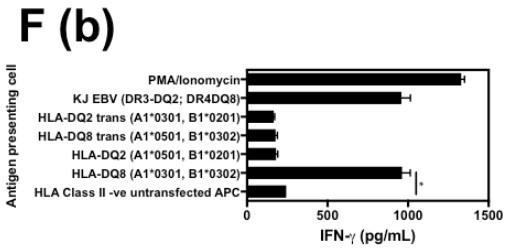

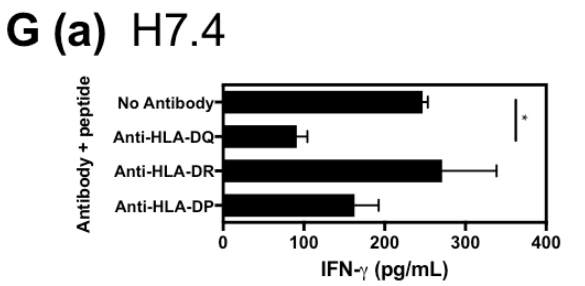

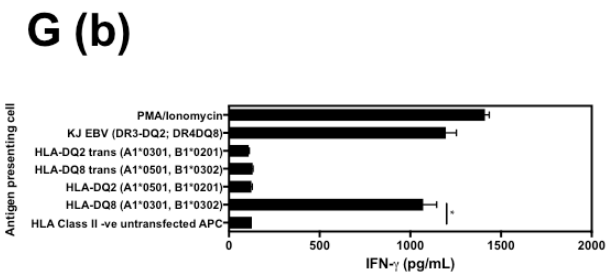

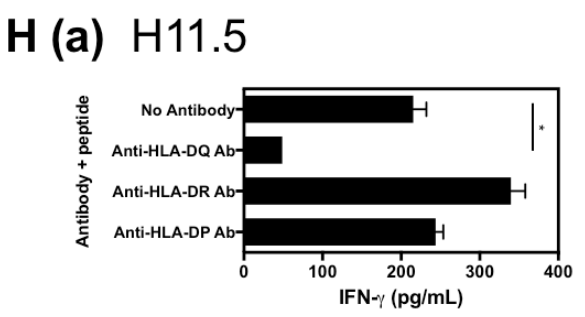

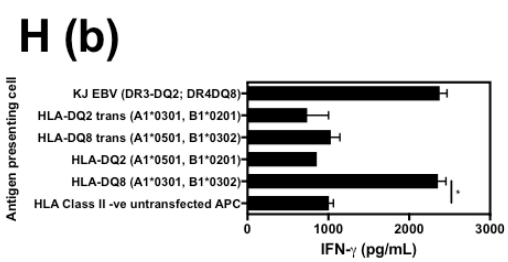

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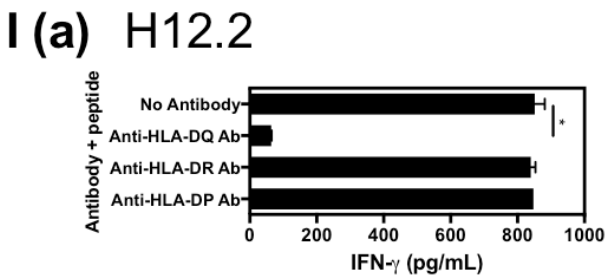

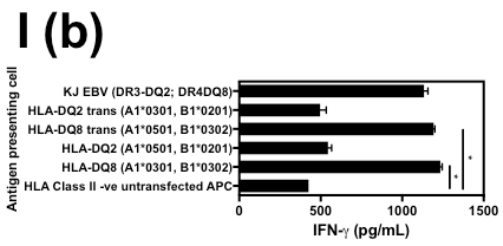

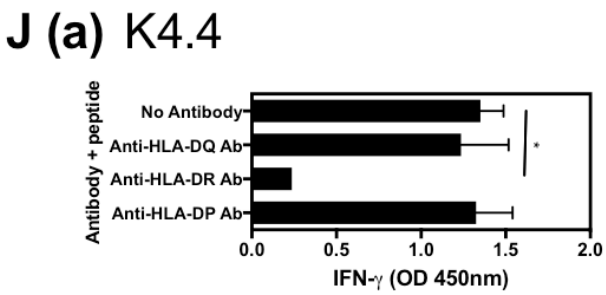

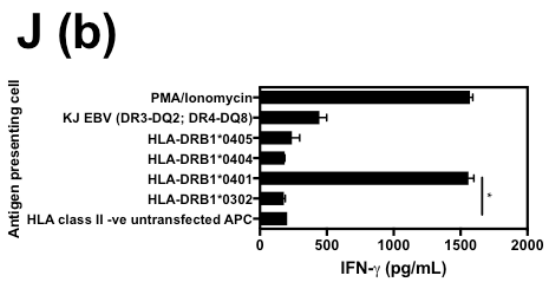

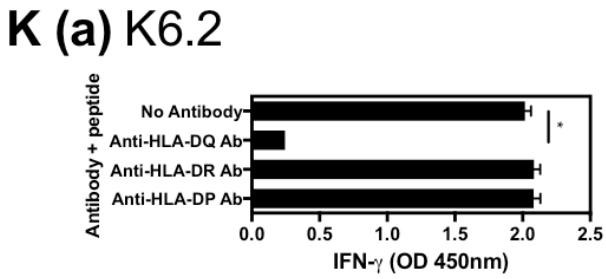

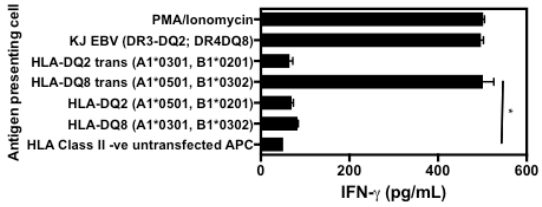

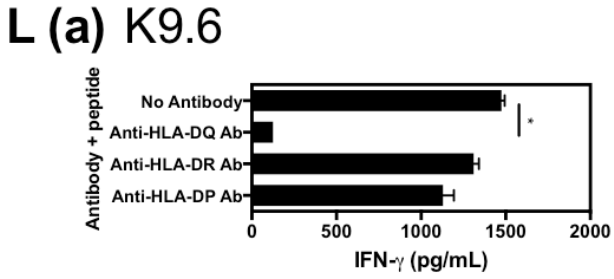

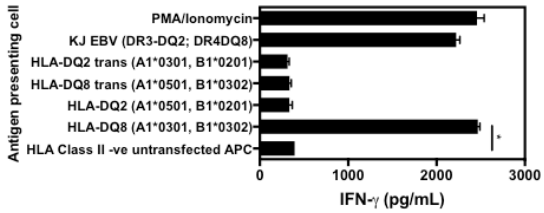

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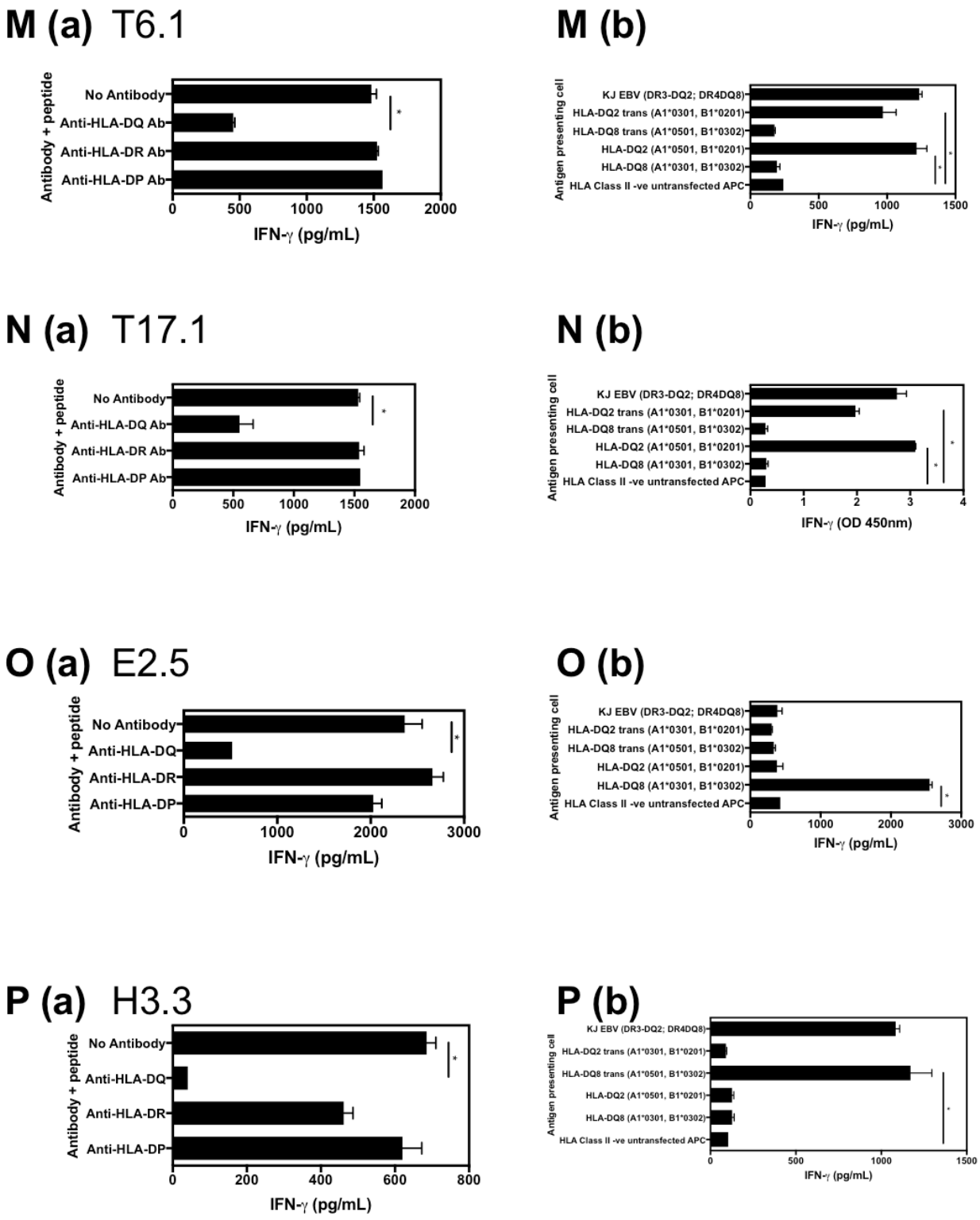
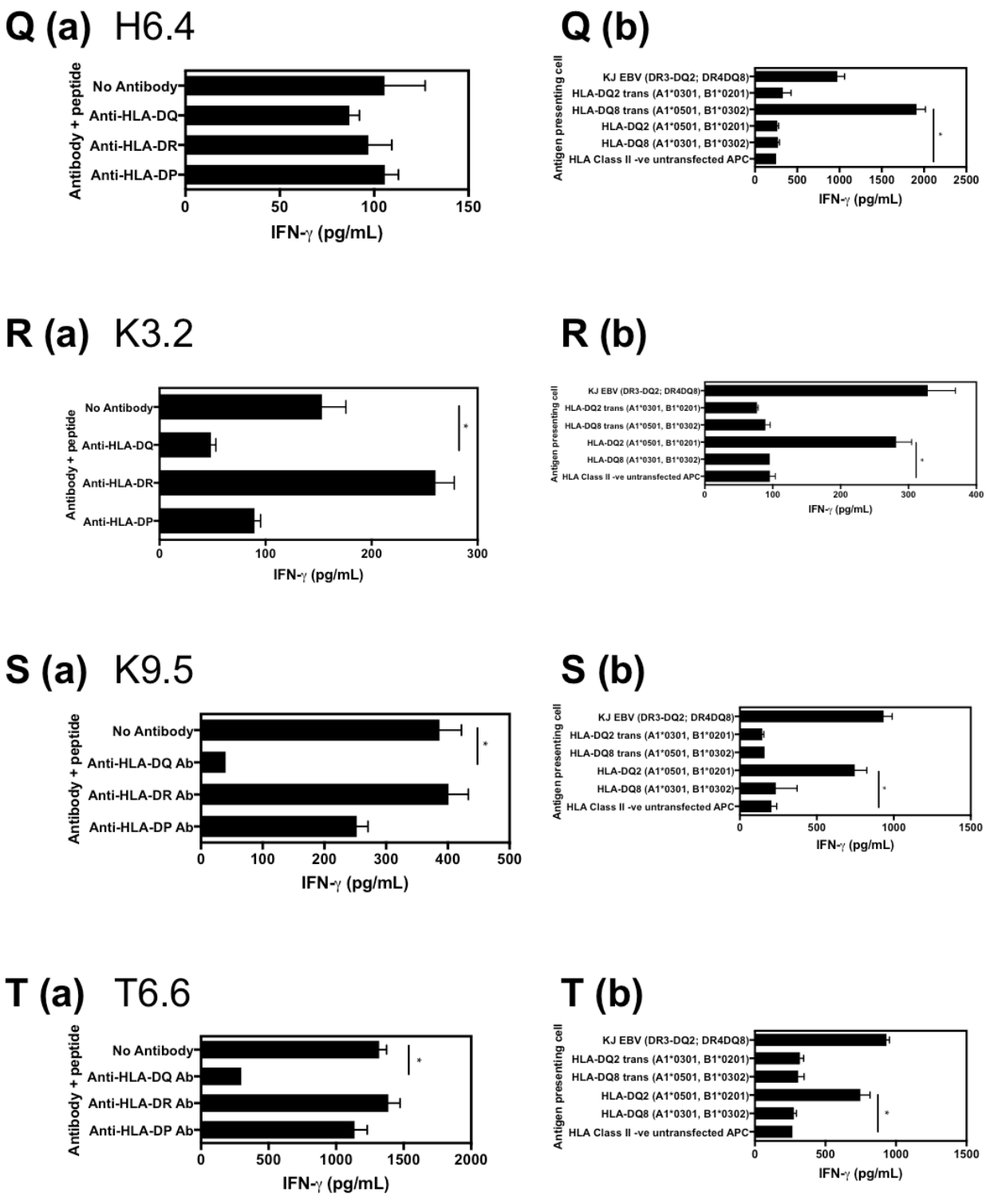


Supplementary Figure 5. Cont.



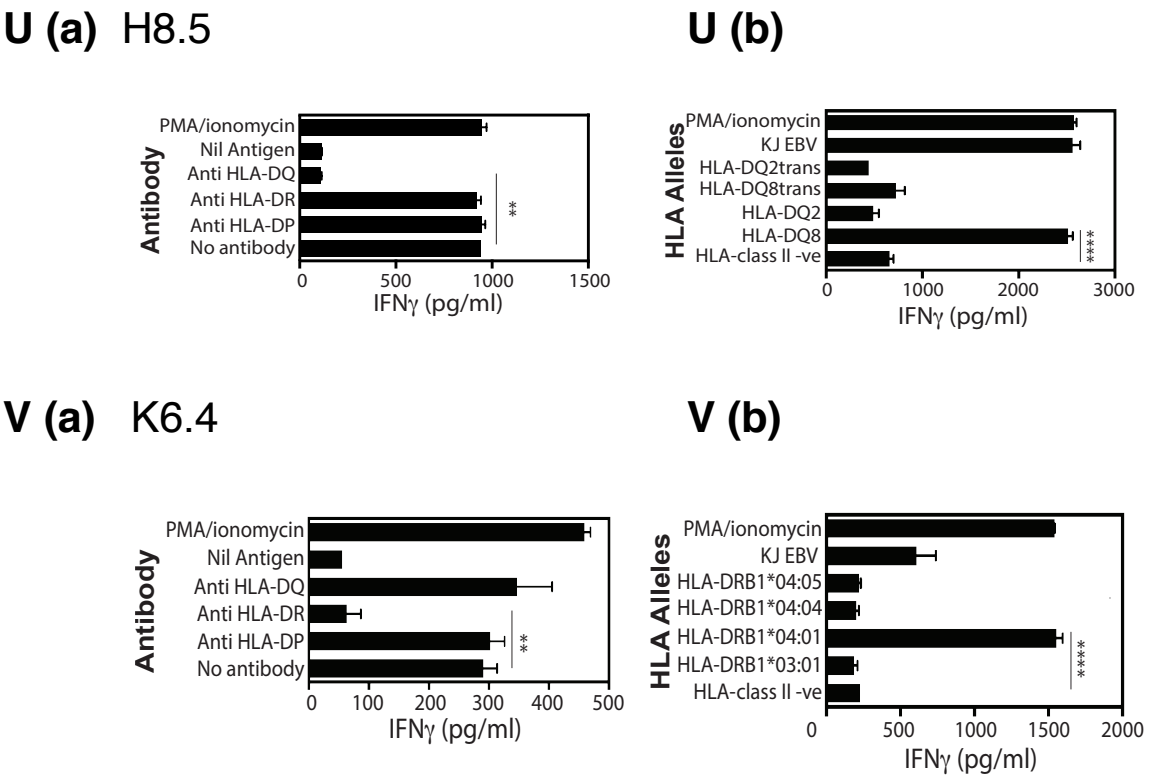


Figure S5. *HLA restriction of C-peptide specific clones.* The HLA restriction of the C-peptide specific $CD4^+$ T-cell clones was determined in two steps. First, (a) antibodies against HLA-DR, -DP, and -DQ were included in the peptide stimulation assays at a final concentration of 5.0μg/mL. To define the HLA allele, HLA-class II negative cells transduced with different HLA alleles were tested (b) The EBV line, KJ EBV (HLA-DRB1*03:01, 04:04; DQB1*02:01, 03:02) was used as a positive control APC. A representative of duplicate experiments is shown. Statistical significance was determined using unpaired Welch's two-tailed *t* test and defined as $p<0.05$ as represented by *. (A) B3.1, (B) B3.3, (C) D1.1, (D) D1.4, (E) E2.3, (F) H3.7, (G) H7.4, (H) H11.5, (I) H12.2,(J) K4.4, (K) K6.2, (L) K9.6, (M) T6.1, (N) T17.1, (O) E2.5, (P) H3.3, (Q) H6.4, (R) K3.2, (S) K9.5, (T) T6.6 (U) H8.5, (V) K6.4.

Supplementary Figure 6

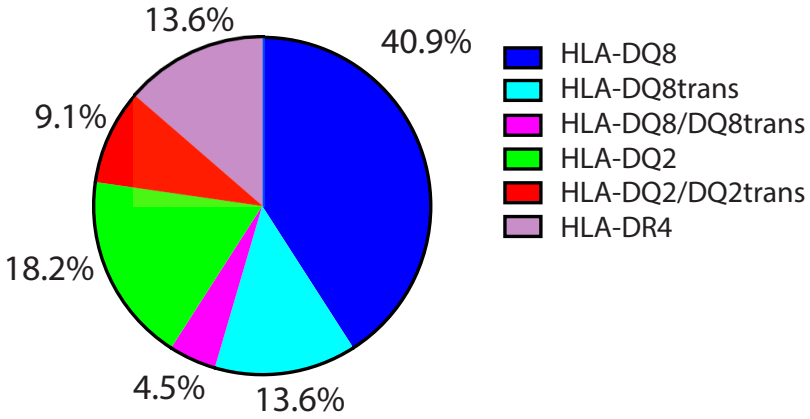


Figure S6. *Most clones are HLA-DQ2 or DQ8 restricted.* Summary of percentage of clones with the indicated HLA restriction out of 22 C-peptide-specific $CD4^+$ T-cell clones.

Supplementary Figure 7

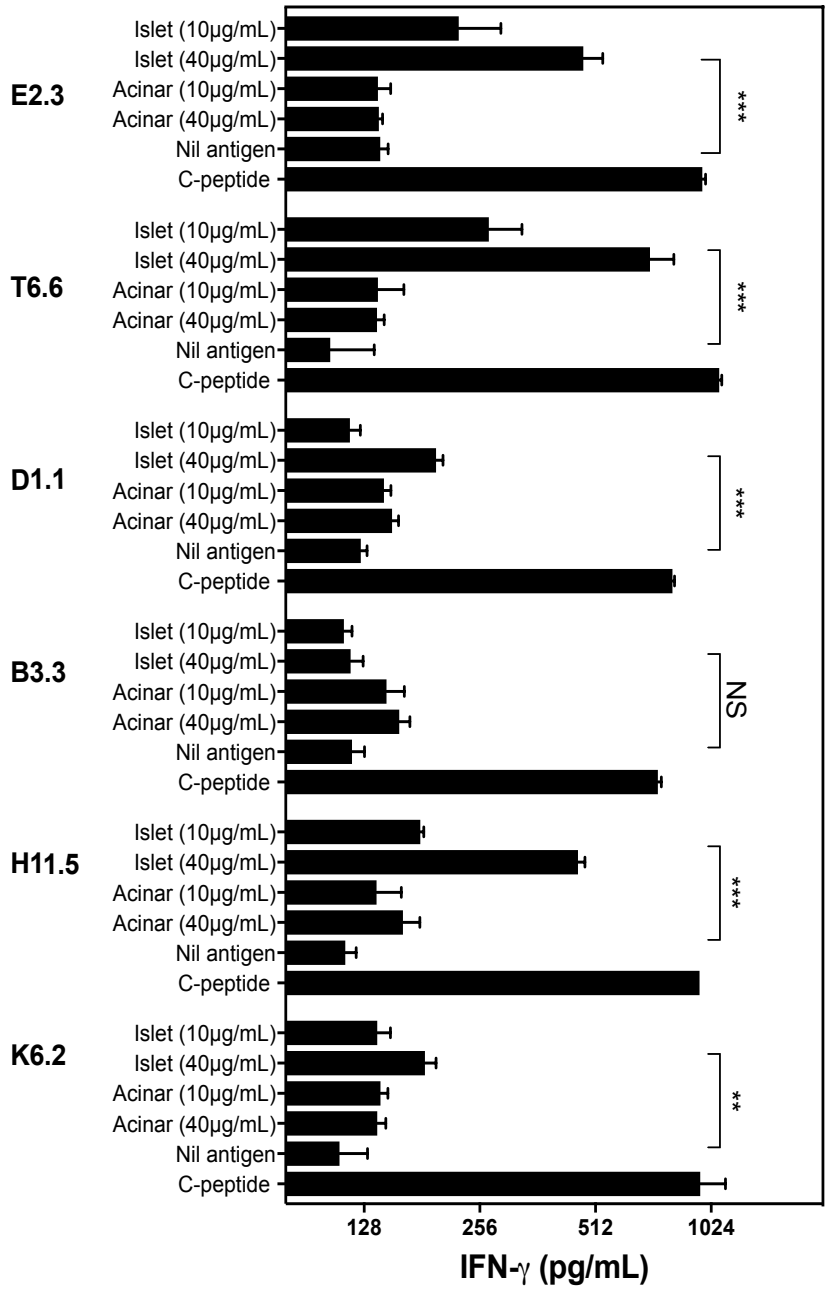


Figure S7. *Response of C-peptide specific CD4⁺ clones to human pancreatic islet extract*

Human pancreatic islet or acinar lysate were diluted in culture media (concentrations as indicated) and incubated with EBV line KJ and $CD4^+$ T-cell clones. Responses were detected by IFN-γ ELISA.

Table S1. Characteristics of recent-onset (<100 days) type 1 diabetes subjects.

Subject	Clone ID	Age*	HLA alleles		Autoantibodies					CDI
			HLA-DRB1	HLA-DQB1	ZnT8†	Insulin	GAD	IA2	ICA	
1	B	23	03:01; 04:01	02:01; 03:02	-	-	**27.3**	**142.9**	**X**	3.4
2	D	20	04:01	03:01; 03:02	**X**	**X**	**1780**	**>4000**	**X**	6.5
3	H	21	03:01; 04:01	02:01; 03:02	-	**X**	**729**	**14**	-	39.7
4		26	04:01; -	03:02; -	**X**	**X**	**>2000**	**>4000**	**X**	7.0
5		24	03:01; 04:01	02:01; 03:02	**X**	**X**	**>2000**	**306**	**X**	1.6
6		47	04:01; 04:08	03:01; 03:02	-	-	**641.3**	-	-	1.9
7	K	12	03:01; 04:01	02:01; 03:02	-	**1.0**	**>2000**	**168**	-	82.5
8		31	03:01; 04:01	03:02; 02:01	-	**X**	**543**	**2519**	**X**	3.2
9		25	03:01; 04:05	02:01; 03:02	-	**X**	-	-	-	1.0
10		33	03:01; 04:01	02:01; 03:01	**X**	-	**805**	-	-	1.1
11		45	01:01; 04:02	05:01; 03:02	**X**	**X**	**71**	-	**X**	1.0
12	E	36	03:01; 04:04	02:01; 03:02		-	**167**	**374**		9.5
13		14	04:05; 13:02	03:02; 06:04		-	-			1.7
14		14	03:01; 07:01	02:01; -		**2.5**	**>2000**	-		26.3
15		15	03:01; 13:02	02:01; 06:04		**0.7**	-	-		0.4
16	T	16	01:01; 03:01	02:01; 05:01		**1.0**	**581**	-		9.3
17		14	01:01; 04:01	03:02; 05:01		-	**17.5**			3.5
18		15	03:01; 04:02	02:01; 03:02		-	**375**			4.2
19		13	01:02; 09:01	02:01; 05:01		-	**>2000**			1.7
20		40	04:05; 15:02	04:01; 05:01		-	-			8.3
21		29	03:01; -	02:01; -			**1262**	14.1		1.2
22		22	04:01; 13:02	03:02; 06:09			-	**>4000**		26.0
23		24	03:01; 04:03	02:01; 03:02		-	**>200**	-		3.0

*Age in years

†X indicates the detection of the antibody; where the quantitative antibody level was available, this has been included in bold when the value was above the laboratory reference range; - refers to a negative result; empty cells indicate the antibody result was not available.

Table S2. Baseline characteristics of long-standing type 1 diabetes subjects

Subject	Age*	Age* at diagnosis	Time since diagnosis†	HLA-DRB1	HLA-DQB1
1	22	16	5	01:01; 04:05	03:02; 05:01
2	14	14	<1 (256 days)	04:01; 13:02	03:02; 06:04
3	15	14	1	04:01; 15:01	03:01; 06:02
4	25	14	12	04:05; 15:02	04:01; 05:02
5	63	13	11	03:01; 04:04	02:01; 03:02
6	39	29	29	03:01; 04:01	02:01; 03:02
7	40	33	32	03:01; 04:01	02:01; 03:01
8	31	11	20	03:01, 04:04	02:01, 03:02
9	24	21	21	03:01; 04:01	02:01; 03:02
10	25	15	18	03:01; 04:01	02:01; 03:02
11	25	23	2	03:01; 04:08	02:01; 03:04
12	35	31	4	03:01; 04:01	02:01; 03:01
13	23	8	15	03:01; 04:05	02:01; 03:02
14	47	28	19	03:01; 07:01	02:01; -
15	29	15	14	01:01; 03:01	02:01; 05:01

*Age in years,

†Time in years

Table S3. Characteristics of subjects without type 1 diabetes

Subject	Age*	DRB1*	DQB1*	Autoantibodies		
				Insulin (<0.7)†	GAD (<5)†	IA-2 (<15)†
1	43	04; 15	03:02; 06:02	0.3	<0.6	<0.3
2	24	03:01; 07:01	02:01; 03:03	0.3	<0.6	<0.3
3	31	03:01; -	02:01; -	ND	ND	ND
4	29	03:01; -	02:01;-	ND	ND	ND
5	29	04:01; 07:01	03:03; 03:02	0.4	<0.6	<0.3
6	50	02:01; 04:01	03:02; 06:02	0.4	<0.6	<0.3
7	61	04:04; 15:01	03:02; 06:02	0.4	<0.6	<0.3
8	34	03:01; 14:04	02:01; 05:03	0.4	<0.6	<0.3
9	24	03:01; 07:01	02:01; 03:03	0.3	<0.6	<0.3
10	ND	04:09; -	03:02; 03:03	ND	ND	ND
11	27	04:04; 11:04	03:01; 03:02	0.3	<0.6	<0.3
12	39	04:04; -	03:01; 03:02	0.4	<0.6	<0.3
13	12	04:01; 01:01	03:02; 05:01	0.3	<3.5	<0.4

*Age in years

†Reference range

ND, not determined, or disclosed.

Table S4. Date of diagnosis and date of collection for insulin antibody positive individuals

Subject	Clone ID	Age*	Date of Diagnosis (dd/mm/yy)	Date of collection of Insulin Ab	Days from diagnosis to collection	Insulin Antibodies†	CDI
1	B	23	17/08/15	17/08/15	0	-	3.4
2	D	20	26/11/15	26/11/15	0	**X**	6.5
3	H	21	16/12/15	16/12/15	0	**X**	39.7
4		26	11/05/15	12/05/15	1	**X**	7.0
5		24	04/01/15	04/01/15	0	**X**	1.6
6		47	12/08/15	12/08/15	0	-	1.9
7	K	12	16/10/15	16/10/15	0	**1.0**	82.5
8		31	02/07/15	08/07/15	6	**X**	3.2
9		25	29/05/15	13/03/15	-77	**X**	1.0
10		33	03/06/15	03/06/15	0	-	1.1
11		45	16/04/15	14/05/15	28	**X**	1.0
12	E	36	6/04/16	07/04/16	1	-	9.5
13		14	22/07/15	14/07/15	-8	-	1.7
14		14	14/08/15	07/09/15	24	**2.5**	26.3
15		15	17/08/15	23/10/15	67	**0.7**	0.4
16	T	16	19/11/15	16/11/15	-3	**1.0**	9.3
17		14	02/04/16	04/04/16	2	-	3.5
18		15	8/07/16	19/07/15	11	-	4.2
19		13	20/9/16	20/9/16	0	-	1.7
20		40	13/08/16	13/08/16	0	-	8.3

*Age in years

†X indicates the detection of the antibody; where the quantitative antibody level was available, this has been included in bold when the value was above the laboratory reference range; - refers to a negative result.

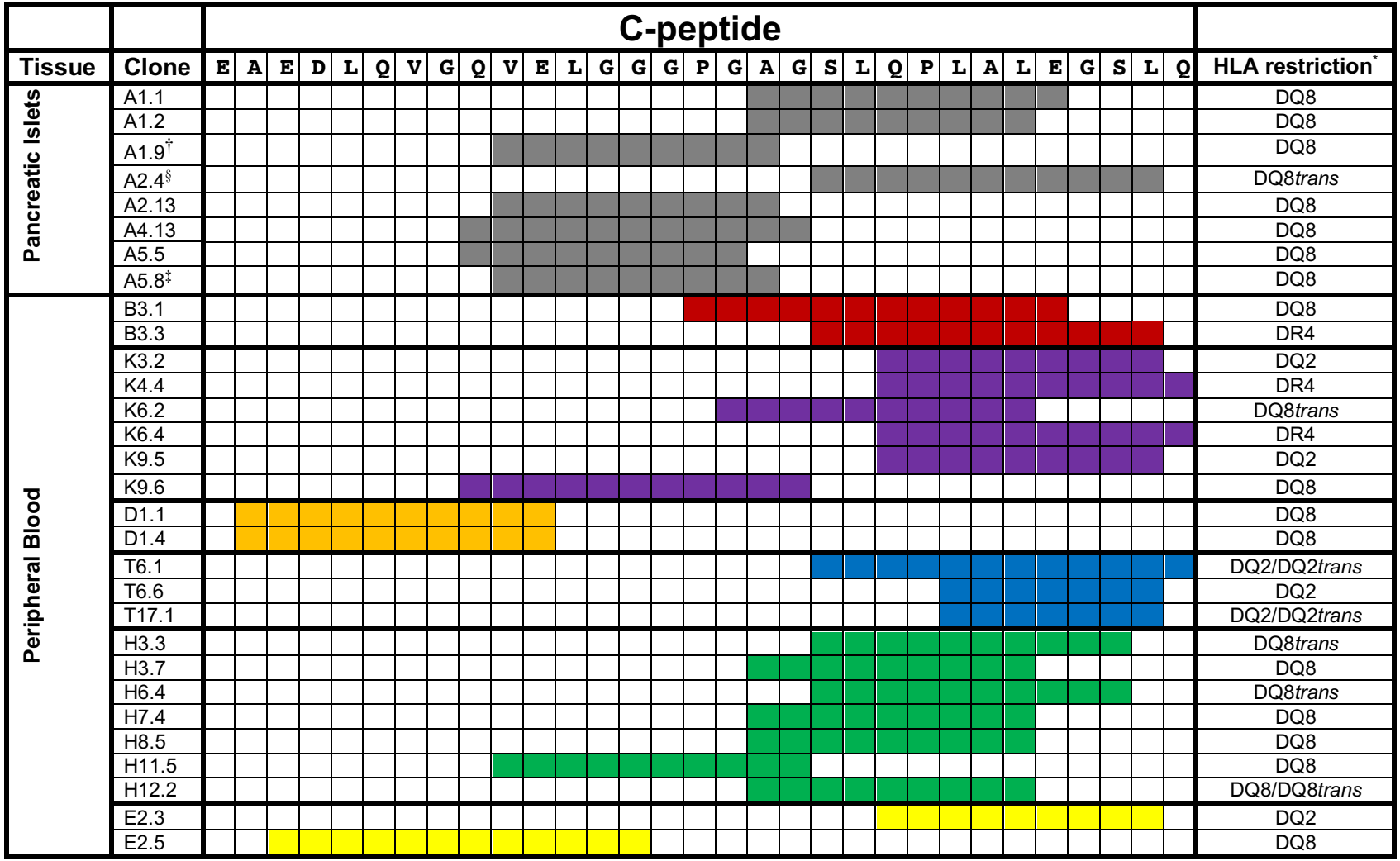
Table S5. ROC curve analysis of C-peptide specific $CD4^+$ T-cell responses

CDI*	**Sensitivity (%)†**	**Specificity (%)†**
>2.0	60.9	54.0
>2.5	60.9	69.0
>2.9	60.9	92.3
>3.5	47.8	100

*Cell Division Index (CDI) calculated from CFSE-based proliferation assay.

†All subjects were included in the analysis (recent-onset type 1 diabetes subjects n=23, healthy control subjects n=13)

Table S6. Comparison of epitope specificity of islet-infiltrating and peripheral blood-derived C-peptide specific CD4$^+$ T cell clones



*Nomenclature referenced by abbreviated HLA type is as follows (indicated in brackets): HLA-DQ8 (HLA-DQA1*03:01, HLA-DQB1*03:02), HLA-DQ2 (HLA-DQA1*05:01, HLA-DQB1*02:01), HLA-DQ8*trans* (HLA-DQA1*05:01, HLA-DQB1*03:02), HLA-DQ2*trans* (HLA-A1*03:01, HLA-B1*02:01), HLA-DR4 (HLA-DRB1*04:01).

†Three additional clones from donor A (2.1, 3.14, 4.6) had identical TRAV and TRBV genes – see reference (16).

§One additional clone from donor A (A3.15) had identical TRAV and TRBV genes – see reference (16)

‡Two additional clones from donor A (5.9, 6.15) had identical TRAV and TRBV genes – see reference (16)

Table S7. Summary of TCR gene sequencing of C-peptide specific $CD4^+$ T-cell clones

Clone	TRAV	TRAJ	TRAV CDR3		TRBV	TRBJ	TRBD	TRBV CDR3	
			AA sequence	AAs				TRBV CDR3	AAs
B3.1	12-1*01	9*01	CVVKSTGGFKTIF	11	20-1*01-05	2-5*01	2*01	CSAGGLAGASQETQYF	14
B3.3	17*01	54*01	CATGPIQGAQKLVF	12	6-5*01	1-1*01	1*01	CASSYAWGRATEAFF	13
K3.2	3*01	31*01	CAVRGDNNARLMF	11	7-2*01/04	2-2*01	1*01	CASSPIIWGTGELFF	13
K4.4	10*01	17*01	CVVSAKAAGNKLTF	12	7-8*01/03	2-7*01	1*01	CASSLAGTDHYEQYF	13
K6.2	8-2*01/8-4*01	11*01	CAVTPKSGYSTLTF	12	20-1*01-05	2-3*01	2*01	CSARDLAIPDTQYF	12
K6.4	10*01	17*01	CVVSAKAAGNKLTF	12	7-8*01/03	2-7*01	1*01	CASSLAGTDHYEQYF	13
K9.5	3*01	31*01	CAVRGDNNARLMF	11	7-2*01-04	2-2*01	1*01	CASSPIIWGTGELFF	13
K9.6	26-1*01/02	54*01	CIVRVEIQGAQKLVF	13	3-2*02	2-1*01	1*01	CASSSPGTEYNEQFF	13
D1.1	13-1*01	38*01	CAARNAGNNRKLIW	12	4-2*01/4-3*01	2-3*01	2*01	CASSFRGLGGGTDTQYF	15
D1.4	13-1*01	38*01	CAARNAGNNRKLIW	12	4-2*01/4-3*01	2-3*01	2*01	CASSFRGLGGGTDTQYF	15
T6.1[†]	10*01	45*01	CVVSAAG#GGGADGLTF	NF	9*02	2-1*01	2*02	CASSVDPGVYNEQFF	13
T6.6	35*02	28*01	CAAALSGAGSYQLTF	13	19*01	2-3*01	2*01	CASRLDPSTDTQYF	12
T17.1	35*02	28*01	CAAALSGAGSYQLTF	13	19*01-03	2-3*01	2*01	CASRLDPSTDTQYF	12
H3.3	19*01	57*01	CALSGRGSEKLVF	11	5-1*01/02	2-7*01	1*01	CASSTRTGQGGNEQYF	14
H3.7	12-1*01	20*01	CVVNPTDDYKLSF	11	20-1*01-05	2-3*01	2*01	CSARSLASGGPDTQYF	14
H6.4	19*01	57*01	CALSGRGSEKLVF	11	5-1*01	2-7*01	1*01	CASSTRTGQGGNEQYF	14
H7.4	12-1*01	20*01	CVVNPTDDYKLSF	11	20-1*01-05	2-3*01	2*01	CSARSLASGGPDTQYF	14
H8.5	12-1*01	20*01	CVVNPTDDYKLSF	11	20-1*01-05	2-3*01	2*01	CSARSLASGGPDTQYF	14
H11.5	26-1*01/02	36*01	CIVRVVTGANNLFF	12	5-1*01/02	2-5*01	1*01	CASSLERETQYF	10
H12.2	ND	ND	ND	ND	ND	ND	ND	ND	ND
E2.3	30*01	37*01	CGTEKPGSGNTGKLIF	14	20-1*01-05	1-4*01	2*01	CSARDGARGEKLFF	12
E2.5	12-3*01/02	5*01	CVISPPGRRALTF	11	5-4*02	2-2*01	2*01	CASSSGTSAGTGELFF	14

ND - not determined. A TCR sequence couldn't be obtained for this clone.

[†]The only TRAV gene detectable had a mutation ('#') in the CDR3 that rendered it non-functional (NF).

Table S8. Summary of TCR usage of sister clones

Donor	No. Clones isolated	No. Identical TCRs	% of all clones with identical TCRs	TRAV	TRBV
B	2	0	0	N/A	N/A
K	6	2	33	10*01	7-8*01
		2	33	3*01	7-2*01
D	2	2	100	13-1*01	4-2*01/4-3*01
T†	3	2	66	35*02	19*0119*01
H*	7	3	43	12-1*01	20-1*01-05
		2	29	19*01	5-1*01
E	2	0	0	N/A	N/A

*Two clones with identical TRBVs, but different TRAVs

†Despite having identical TCRs, one clone responded to both HLA-DQ2 and DQ2trans, while the other only responded to HLA-DQ2

Table S9. Relative potency of full-length C-peptide compared to 18mer peptides

Fig. 3 Panel	Clone	C-peptide EC_{50} (μM)	18mer EC_{50}(μM)	Fold difference*	18mer sequence†	HLA restriction‡
3A	K9.6	2.8	2.8	1.0	LQVG**QVELGGGPGAG**SLQ	HLA-DQ8
3B	H11.5	1.2	1.2	1.0	LQVGQ**VELGGGPGAG**SLQ	HLA-DQ8
3C	D1.1	4.1	13.7	3.3	RRE**AEDLQVGQVE**LGGGP	HLA-DQ8
3D	E2.3	0.3	24.9	76.2	GAGSL**QPLALEGSL**QKRG	HLA-DQ2
3E	T17.1	0.3	101.2	292.0	GAGSLQP**LALEGSL**QKRG	HLA-DQ2/DQ2tr
3F	K9.5	4.9	>>100.0§	>>100.0	GAGSL**QPLALEGSL**QKRG	HLA-DQ2
3G	B3.3	0.7	>>100.0§	>>100.0	GAG**SLQPLALEGSL**QKRG	HLA-DR4
3H	H3.3	8.4	>>100.0§	>>100.0	GAG**SLQPLALEGS**LQKRG	HLA-DQ8tr

*Fold increase in the EC_{50} from full-length C-peptide to 18mer peptide (μM).

†Bold text indicates the core epitope

‡HLA-DQ8 (DQA*03:01; DQB*03:02), HLA-DQ2 (DQA*05:01, DQB*02:01), HLA-DQ8tr (DQA*05:01; DQB*03:02), HLA-DR4 (DRB1*04:01), HLA-DQ2tr (DQA*03:01, DQB*02:01).

§EC_{50} could not be calculated, but it is greater than 100μM.

Table S10. Responses of CD4+ T cells to full-length and 18mer fragments of C-peptide

	C-peptide																													
Clone	**E**	**A**	**E**	**D**	**L**	**Q**	**V**	**G**	**Q**	**V**	**E**	**L**	**G**	**G**	**G**	**P**	**G**	**A**	**G**	**S**	**L**	**Q**	**P**	**L**	**A**	**L**	**E**	**G**	**S**	**L**	**Q**	**Fold Δ†**	**HLA‡**
K9.6 (A)*																																1.0	DQ8
H11.5 (B)																																1.0	DQ8
D1.1(C)																																3.3	DQ8
E2.3 (D)																																76.2	DQ2
T17.1 (E)																																292.0	DQ2/DQ2tr
K9.5 (F)																																>100.0	DQ2
B3.3 (G)																																>100.0	DR4
H3.3 (H)																																>100.0	DQ8tr

*The panel showing this titration in Fig. 3 is in parentheses.

†Fold increase in the EC_{50} from full-length C-peptide to 18mer peptide.

‡HLA restriction of clones as shown in Table 1.