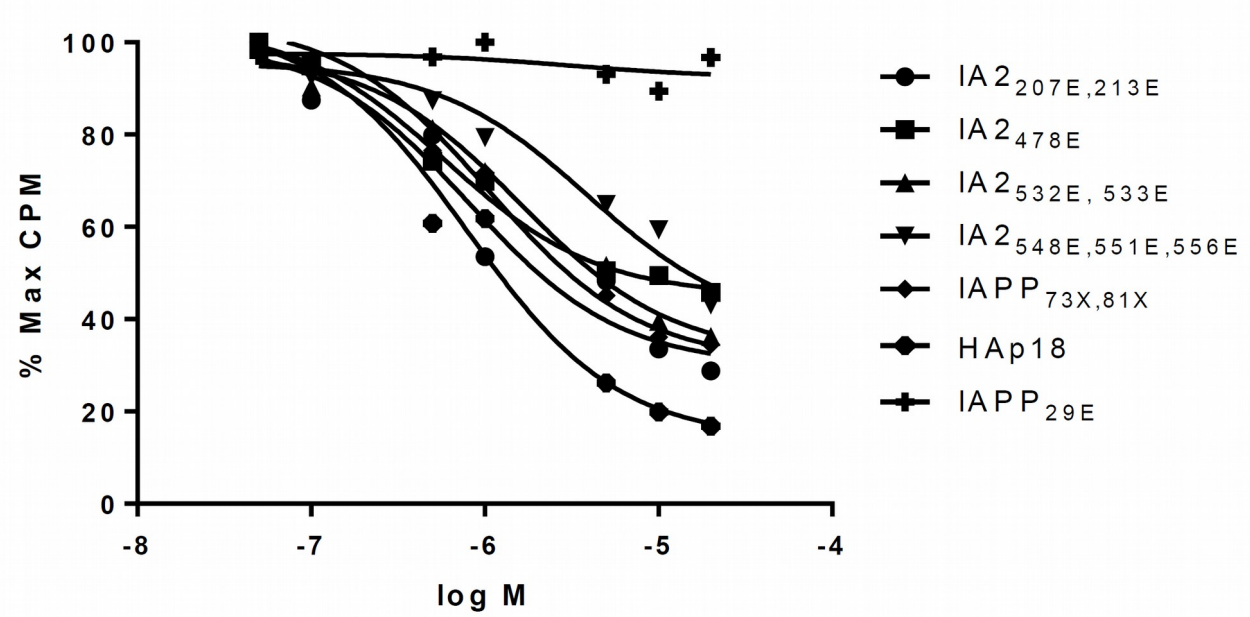
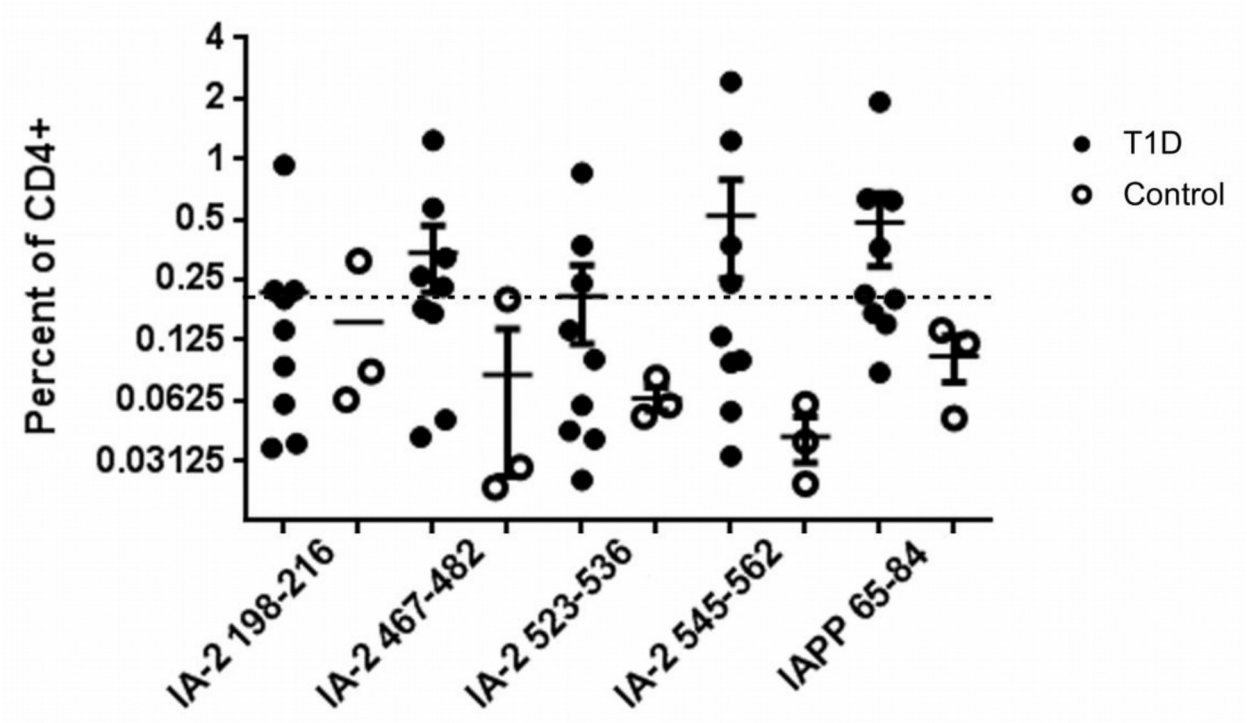
SUPPLEMENTARY DATA

Supplementary Figure 1. Binding affinity of modified peptides to HLA-DQ8. Increasing concentrations of modified peptides that contained predicted motifs were bound to DQ8 protein in competition with a biotinylated reference peptide. From these curves, IC_{50} values were determined as the concentration of peptide needed to displace 50% of the reference peptide (a lower IC_{50} value indicates stronger binding). The five putative epitopes shown (IA-$2_{207E, 213E}$, IA-2_{478E}, IA-$2_{532E, 533E}$, IA-$2_{548E, 551E, 556E}$, and IAP-$P_{73X, 81X}$ bound with affinities that were clearly higher than a non-binding control peptide ($IAPP_{29E}$) and that approached or exceeded the binding of a positive control influenza peptide (HA p18).



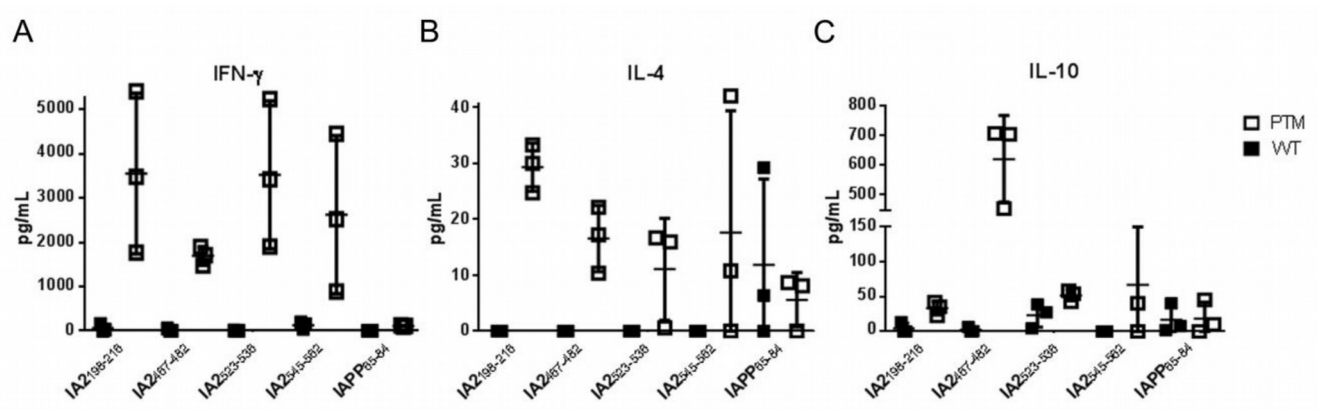
SUPPLEMENTARY DATA

Supplementary Figure 2. In vitro responses to PTM peptides. To identify immunogenic PTM peptides derived from IA-2 and IAPP, PBMC from 9 subjects with type 1 diabetes (T1D) and 3 controls (all with DQ8 haplotypes) were expanded by stimulating for two weeks with modified peptide (deamidated or citrullinated as indicated in Table 1) and then stained with the corresponding DQ8 tetramer. In general, the modified peptides elicited comparatively higher percentages of tetramer positive CD4+ T cells for subjects with diabetes than controls. Using twice the mean value of the controls as a cutoff (0.22%, indicated by dotted line), at least 3 positive responses were observed for each epitope from subjects with diabetes but only a single positive response was observed from controls (p=0.005, Fisher's exact test).



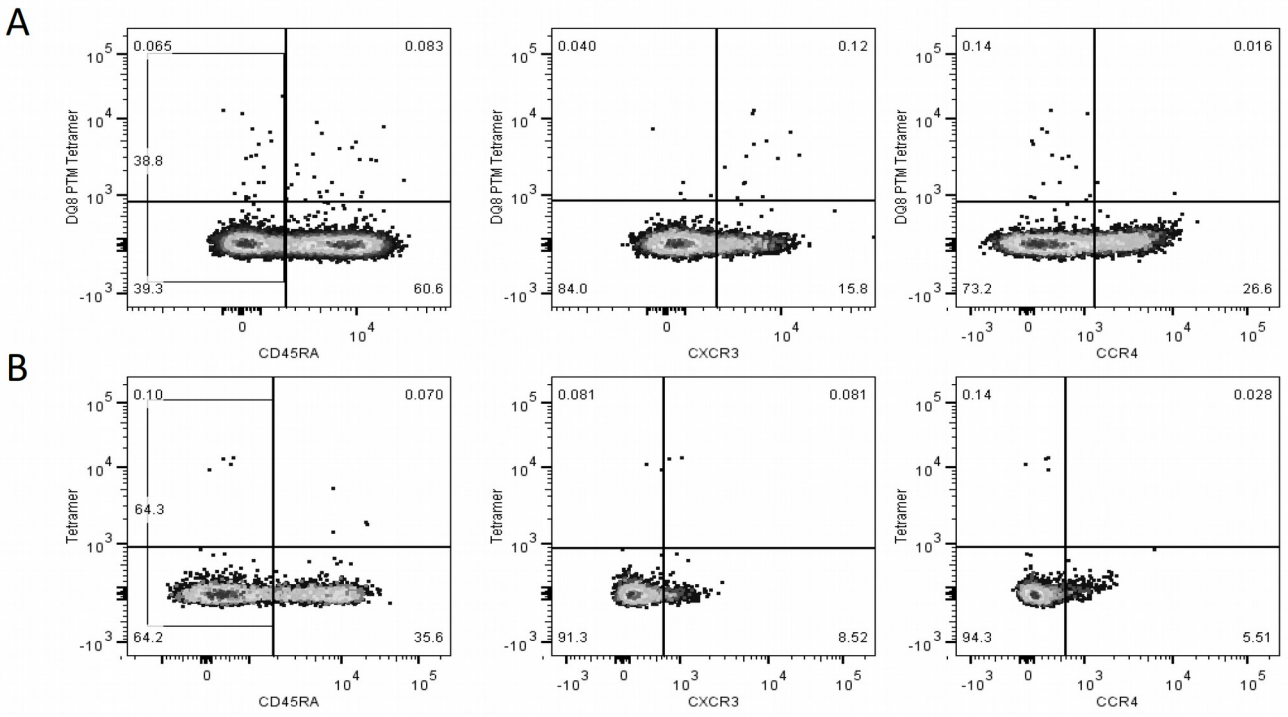
SUPPLEMENTARY DATA

Supplementary Figure 3. Functional profiling of PTM specific T cell clones. To characterize their cytokine profiles, T cell clones were stimulated in triplicate with modified peptide (open squares) or unmodified peptide (solid squares) and secreted concentrations of IFN-γ, IL-4, and IL-10 were determined by ELISA. Levels of secreted of IFN-ɣ, IL4, and IL-10 were modest for unmodified peptides. The modified peptides elicited comparatively higher levels of IFN-ɣ than IL4 or IL-10.



SUPPLEMENTARY DATA

Supplementary Figure 4. Ex vivo phenotype of PTM specific T cells. A magnetic enrichment procedure was used to visualize T cells specific for modified epitopes directly ex vivo. PBMCs were isolated and co-stained ex vivo with tetramer and antibodies to characterize their surface phenotype. Each of the panels shown has been gated based on size, viability, lack of CD14/CD19 expression and CD4 expression. CXCR3 and CCR4 versus tetramer plots are further gated to include only CD45RA- (memory) cells. The percentages shown in each quadrant indicate the percentage of tetramer positive cells that are positive for the corresponding surface marker (A) Ex vivo phenotype of tetramer positive T cells for an unmanipulated sample from a DQ8+ subject with T1D. (B) Ex vivo phenotype of tetramer positive T cells for an unmanipulated sample from a DQ8+ healthy subject.



SUPPLEMENTARY DATA

Supplementary Table 1. Information on DQ8+ subjects with diabetes used for study

Subject	Age[a]	Gender	Time since	Auto-antibody status[b]
T1D #1	48	M	4.6 years	IAA, ZnT8
T1D #2	12	F	0.9 years	IAA, ZnT8
T1D #3	29	M	13.0 years	IA2, IAA, GAD
T1D #4	13	M	1.6 years	IA2, IAA, GAD, ZnT8
T1D #5	18	M	14.6 years	IAA, ZnT8
T1D #6	17	M	11.0 years	IAA, GAD, ZnT8
T1D #7	28	M	4.4 years	IAA, ZnT8
T1D #8	16	M	4.5 years	IA2, IAA
T1D #9	27	M	2.0 years	GAD, IAA

a The mean age of patients was 23.1 years. The average time since diagnosis was 6.3 years.
b GAD denotes glutamic acid decarboxylase 65, IAA denotes insulin, IA2 denotes tyrosine phosphatase-related islet antigen 2, ZnT8 denotes zinc transporter 8

SUPPLEMENTARY DATA

Supplementary Table 2. Information on DQB1*03:02 healthy subjects used for study

Subject ID	Age	Gender
Control #1	64	F
Control #2	57	F
Control #3	33	M
Control #4	56	F
Control #5	36	M
Control #6	44	M
Control #7	40	M
Control #8	33	F

The mean age of controls was 45.3 years
Healthy subjects under the age of 18 could not be recruited

SUPPLEMENTARY DATA

Supplementary Table 3. Summary of characteristics of human donors.

Islet Donors			
ID	**Age**	**Gender**	**Islet Purity**
H116	46 years	Male	80%
HP391	46 years	Female	80%
T14001	1 day	Female	30%
PLN Donors			
ID	**Age**	**Gender**	**Disease Duration**
T1D.7	27 years	Male	17 years
T1D.12	23 years	Male	8 years

The characteristics of islet donors (non-diabetic) and PLN donors (diabetic) are shown.

SUPPLEMENTARY DATA

Supplementary Table 4. Prediction Matrix for binding to DQ8

P1[a] residu	C_{p1}[b]	P4[a] residue	C_{p4}[b]	P6[a] residue	C_{p6}[b]	P7[a] residue	C_{p7}[b]	P9[a] residue	C_{p9}[b]
G	0.12	G	0.43	G	0.10	G	0.36	G	0.21
A	0.31	A	1.00	A	1.14	A	0.96	A	0.05
V	0.58	V	0.64	V	0.70	V	0.87	V	0.10
L	0.70	L	0.84	L	0.50	L	0.67	L	0.13
I	1.03	I	0.55	I	0.64	I	0.68	I	0.15
M	0.65	M	0.58	M	0.91	M	0.77	M	0.42
P	0.08	P	0.21	P	0.85	P	0.33	P	0.24
F	0.51	F	1.03	F	0.10	F	0.90	F	0.51
W	0.36	W	0.51	W	0.28	W	0.76	W	0.29
S	0.38	S	0.60	S	0.62	S	0.90	S	0.02
T	0.43	T	0.60	T	1.12	T	0.86	T	0.08
N	0.22	N	0.39	N	0.18	N	0.81	N	0.38
Q	0.49	Q	0.15	Q	0.61	Q	0.45	Q	0.20
Y	0.71	Y	0.56	Y	0.34	Y	0.89	Y	0.17
C	0.30	C	0.45	C	0.28	C	0.42	C	0.34
K	0.03	K	0.02	K	0.47	K	0.53	K	0.38
R	0.03	R	0.01	R	0.35	R	0.35	R	0.15
H	0.24	H	0.46	H	0.25	H	0.52	H	0.42
D	0.63	D	0.28	D	0.56	D	0.89	D	1.28
E	0.92	E	0.10	E	0.83	E	0.93	E	2.00
X	0.09	X	0.09	X	0.1	X	0.1	X	0.15

[a] P1, P4, P6, P7, and P9 denote binding pocket 1, binding pocket 4, binding pocket 6, binding pocket 7, and binding pocket 9 of HLA-DQ8. [b] C_{p1}, C_{p4}, etc. denotes the predicted influence of each residue on binding to the corresponding pocket. The relative binding affinity of any peptide is predicted as $C_{p1} \times C_{p4} \times C_{p6} \times C_{p7} \times C_{p9}$

SUPPLEMENTARY DATA

Supplementary Table 5. Sequences and binding affinity for peptides that bind DQ8

Peptide	Modification[a]	Sequence[b]	Modified IC_{50} (μM)[c]
GAD 17-36	31E, 34E	SGDSENPGTARAWC**E**VA**E**KF	3.9
GAD 97-116	112E	LLPACDGERPTLAFL**E**DVMN [d]	2.3
GAD 145-164	152E, 154E, 164E	YNWELAD**E**P**E**NLEEILMHC**E**	3.5
GAD 177-196	181E	RYFN**E**LSTGLDMVGLAADWL	2.3
GAD 313-332	324E	SDLERRILEAK**E**KGFVPFLV	5.5
IGRP 89-103	100E	IYPNHSSPCLE**E**FPT	1.4
IGRP 188-203	200E	LVAEAFEHTPGI**E**TAS	14.8
IA2 49-66	55E, 61E, 63E	HLEVCI**E**DGLFG**E**C**E**VGV	1.6
IA2 64-83	68E, 74E, 81E	VGVG**E**ARPLL**E**VTSPVL**E**RL [d]	1.5
IA2 98-114	103E, 108E	HDDLT**E**YVIS**E**EMERIP	2.8
IA2 198-216	207E, 213E	SLSYEPALL**E**PYLFH**E**FGS	3.0
IA2 319-333	330E	DRGEKPASPAV**E**PDA	0.7
IA2 467-482	478E	AAEEYGYIVTD**E**KPLS [d]	6.8
IA2 523-536	532E, 533E	QNLSLADVT**E**EAGL [d]	2.8
IA2 545-562	548E, 551E, 556E	TGL**E**IL**E**TGVG**E**REEAAA	16.8
IA2 709-723	714E, 720E	LAKEW**E**ALCAY**E**AEP	1.2
IA2 778-792	784E	PAYIAT**E**GPLSHTIA	5.8
IAPP 1-20	7E	MGILKL**E**VFLIVLSVALNHL	17.2
ChgA 4-18	15E	AAVLALLLCAG**E**VTA	16.8
ChgA 100-113	111E	FEDELSEVLEN**E**SS	3.4
ChgA 141-155	152E	KSGEATDGARP**E**ALP	11.6
ChgA 197-211	198E, 208E	P**E**AEGDSEGLS**E**GLV	1.7
ChgA 303-322	214E	KEEEEEMAVVP**E**GLFRGGKS	2.2
ChgA 325-339	327E	LE**E**EEERLSKEWEDS	9.0
Proinsulin 49-68	62E, 65E	FYTPKTRREAEDL**E**VG**E**VEL [d]	12.2
Proinsulin 65-84	65E, 78E	**E**VELGGGPGAGSL**E**PLALEG	10.0
Proinsulin 73-92	78E, 87E	GAGSL**E**PLALEGSL**E**KRGIV	17.8
Proinsulin 89-108	94E, 104E	RGIVE**E**CCTSICSLY**E**LENY	2.13
GAD 89-108	105X	YAFLHATDLLPACDGE**X**PTL	3.7
GAD 97- 116	105X	LLPACDGE**X**PTLAFLQDVMN	1.2
GAD 249-268	255X	YAMMIA**X**FKMFPEVKEKGMA	2.6
GAD 529-548	536X	VAPVIKA**X**MMEYGTTMVSYQ	1.6
IA2 551-565	557X	QTGVGQ**X**EEAAAVLP	4.2
IAPP 65-84	73X, 81X	VGSNTYGK**X**NAVEVLK**X**EPL	23.1
ChgA 81-95	90X	DLALQGAKE**X**AHQQK	18.1

[a] X denotes citrulline. [b] The residue that modulates recognition when modified is bolded in each sequence. [c] IC_{50} represents the peptide concentration that displaces half of the reference peptide. [d] Reported as a candidate HLA-DQ8 epitope by van Lummel et al. (*23*).

SUPPLEMENTARY DATA

Supplementary Table 6. Binding affinities for substituted IA2 and IAPP peptides

Peptide	Modification	Amino acid sequence[a,b]	IC_{50} (μM)[c,d]
$IA2_{198-216}$	207E, 213E	SLSYEPALL**E**PYLFH**E**FGS	3.0
	207E, 213Q	SLSYEPALL**E**PYLFHQFGS	n.b.
	207Q, 213E	SLSYEPALLQPYLFH**E**FGS	6.0
$IA2_{523-536}$	532E, 533E	QNLSLADVT**EE**AGL	2.8
	532E, 533Q	QNLSLADVT**E**QAGL	4.2
	532Q, 532E	QNLSLADVTQ**E**AGL	n.b.
$IA2_{545-562}$	548E, 551E, 556E	TGL**E**IL**E**TGVG**E**REEAAA	16.8
	548E, 551Q, 556Q	TGL**E**ILQTGVGQREEAAA	18.9
	548Q, 551E, 556Q	TGLQIL**E**TGVGQREEAAA	31.3
	548E, 551Q, 556E	TGLQILQTGVG**E**REEAAA	18.6
$IAPP_{65-84}$	73Cit, 81Cit	VGSNTYGK**X**NAVEVLK**X**EPL	23.1
	73Cit, 81R	VGSNTYGK**X**NAVEVLKREPL	31.3
	73R, 81Cit	VGSNTYGKRNAVEVLK**X**EPL	n.b.

[a] The modified residue is bolded in each sequence. [b] X indicates citrulline. [c] IC50 represents the peptide concentration that displaces half of the reference peptide. [d] n.b. indicates non-binding, or >50 μmol/L in our assay.

SUPPLEMENTARY DATA

Supplementary Table 7. Responsiveness of PTM-reactive T cells to primary human islets

Patient Sample	T Cell Clones									
	C74 ($IA2_{198-216}$)		C84 ($IA2_{467-482}$)		C86 ($IA2_{523-536}$)		C90 ($IA2_{545-562}$)		C107 ($IAPP_{65-84}$)	
	Ctrl.[a]	Thaps[a]	Ctrl.[a]	Thaps[a]	Ctrl.[a]	Thaps[a]	Ctrl.[a]	Thaps[a]	Ctrl.[a]	Thaps[a]
H116	0.0 ± 0.0	16.2 ± 3.4	7.8 ± 3.5	46.0 ± 3.0	0.0 ± 0.0	26.2 ± 1.3	0.1 ± 0.2	42.5 ± 1.9	35.6 ± 4.0	971.3 ± 69.6
HP391	4.7 ± 1.2	483.5 ± 12.8	0.5 ± 0.9	1278.0 ± 36.5	0.0 ± 0.0	408.7 ± 2.7	0.0 ± 0.0	466.9 ± 2.6	4.7 ± 1.2	1068.3 ± 26.8
T14001	0.0 ± 0.0	404.6 ± 22.7	4.4 ± 2.4	1752.8 ± 57.2	2.3 ± 0.4	246.1 ± 6.2	0.0 ± 0.0	442.4 ± 30.3	0.0 ± 0.0	1017.7 ± 18.2

[a]Data shown are mean IFNγ secretion ± S.D. of triplicates. Similar to what is shown in Figure 3B-F, experimental controls lacking T cells, APC, or islets did not lead to IFNγ secretion for any of the assay conditions shown here.