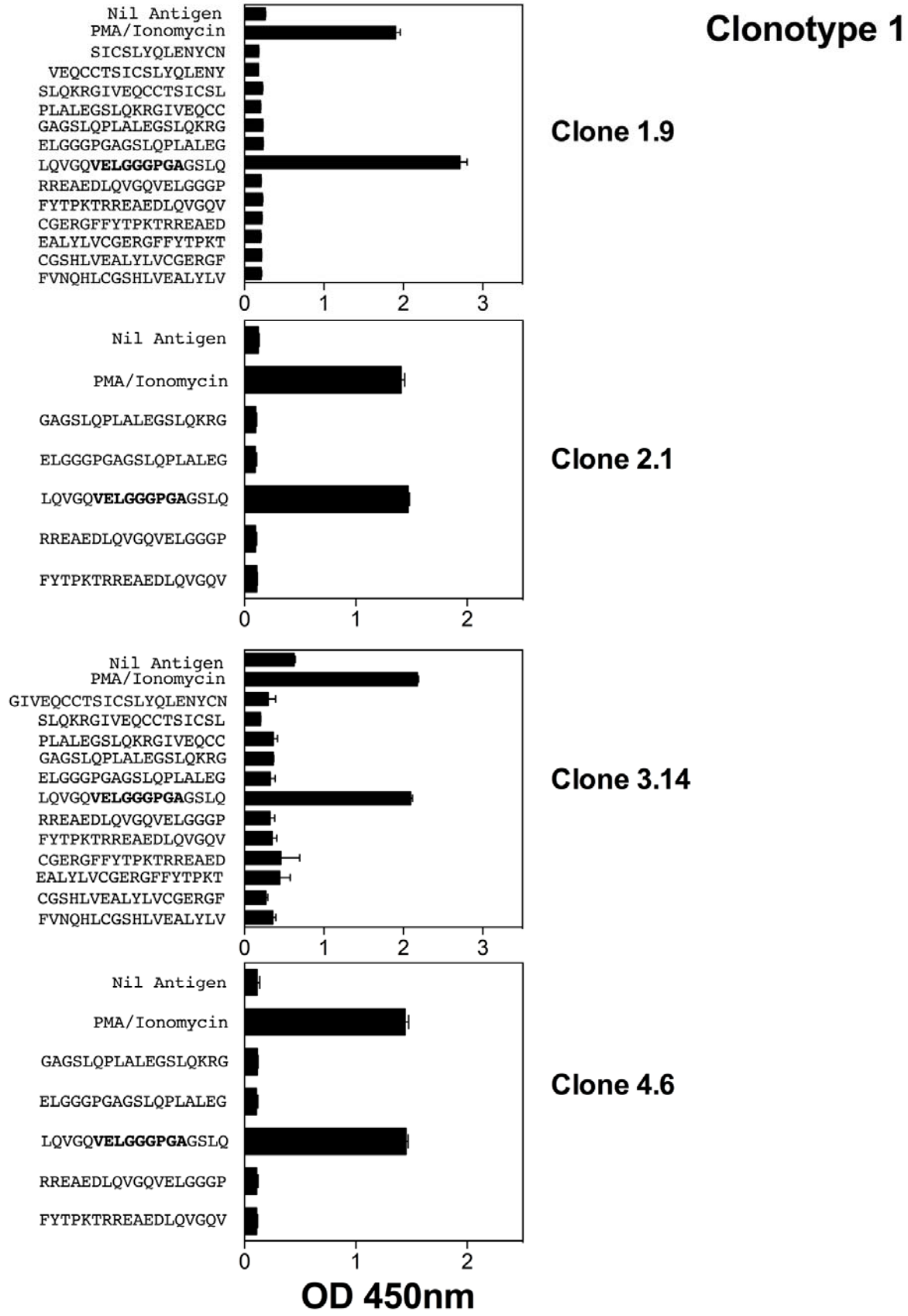
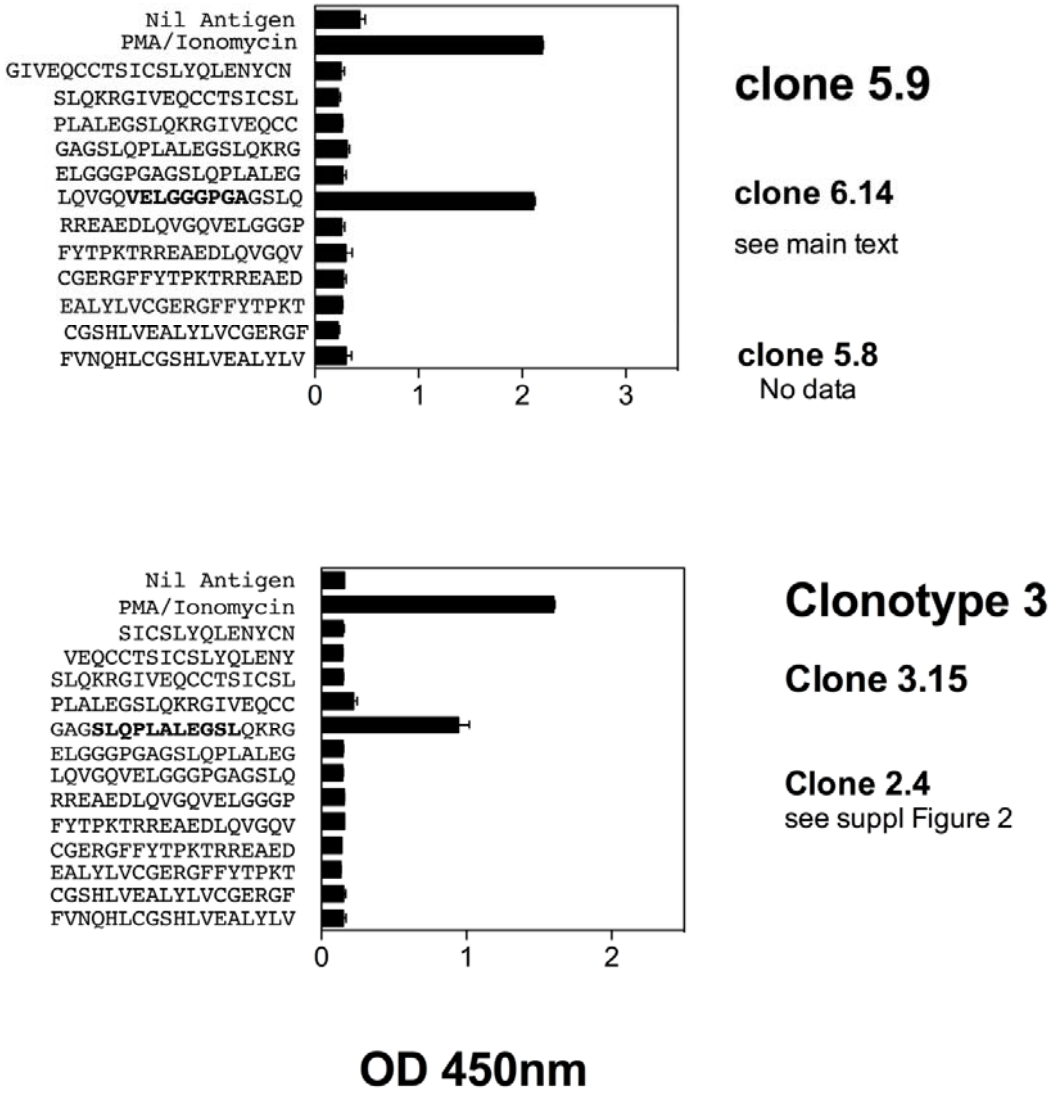
SUPPLEMENTARY DATA

Supplementary Figure 1. Coarse epitope mapping. This was performed using a panel of 18mer pronsulin peptides (amino acids listed in single letter code) overlapping by 12 amino acids. Each peptide was tested at a final concentration of 10μM in triplicate with the B-cell line (KJ EBV) as antigen-presenting cells (20-50,000/ well) and cloned T cells (50,000/well). T cells and antigen-presenting cells, cultured in the absence of antigen (labeled 'Nil antigen') and T cells cultured with phorbol 12-myristate 13-acetate/ionomcyin (labeled 'PMA/Ionomycin') were included as negative and positive controls respectively. Each experimental treatment was conducted in triplicate and the mean +/-SEM of the absorbance at 450nm for an IFNγ ELISA is shown for each clone. OD ~1.0 is equivalent to ~500pg/ml of IFNγ. Where several clones with identical TCR sequences were isolated results from each one are shown. The epitope is showin in bold font. Each experiment was repeated at least twice and a representative experiment is shown.

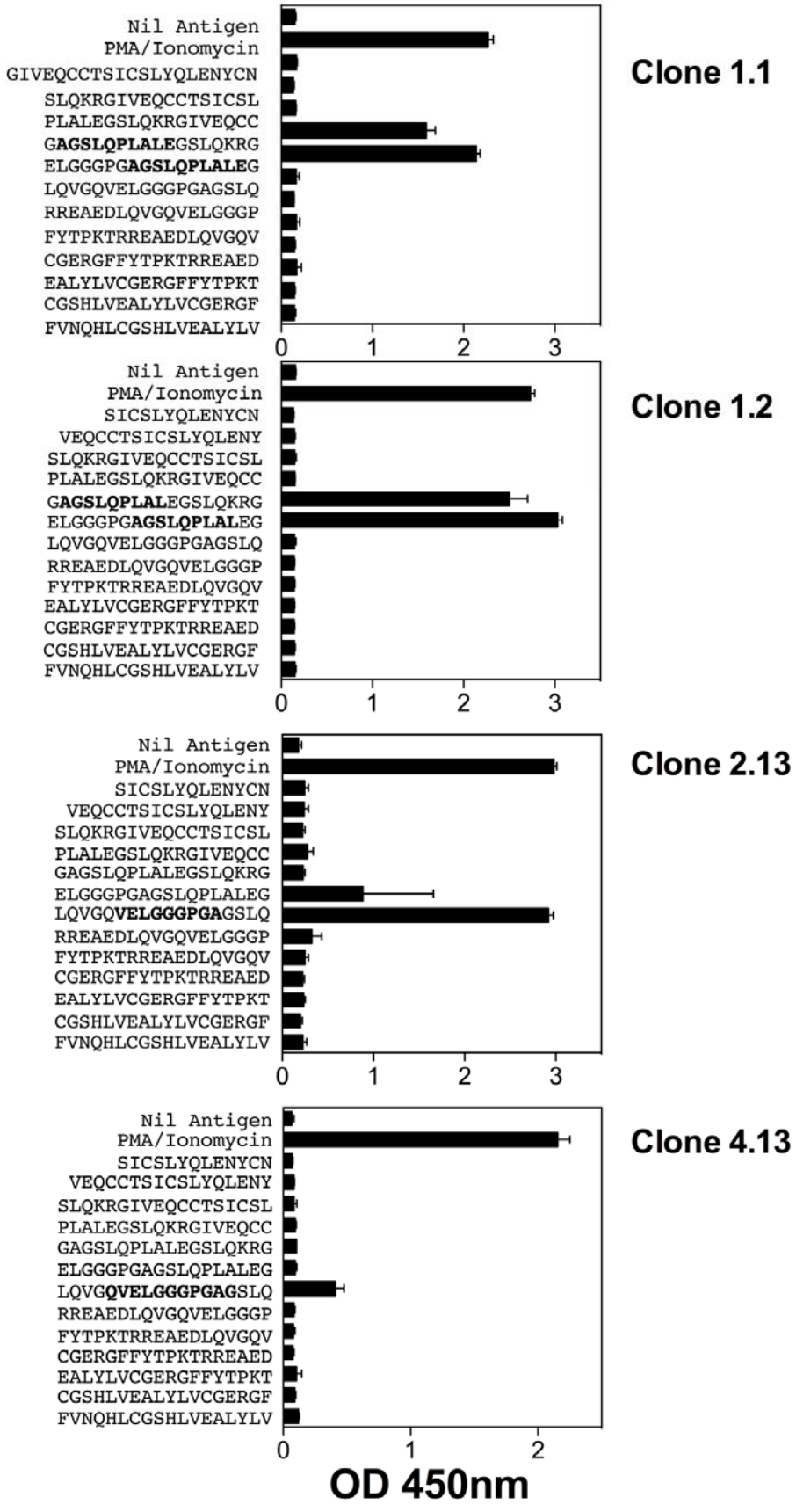
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA



SUPPLEMENTARY DATA



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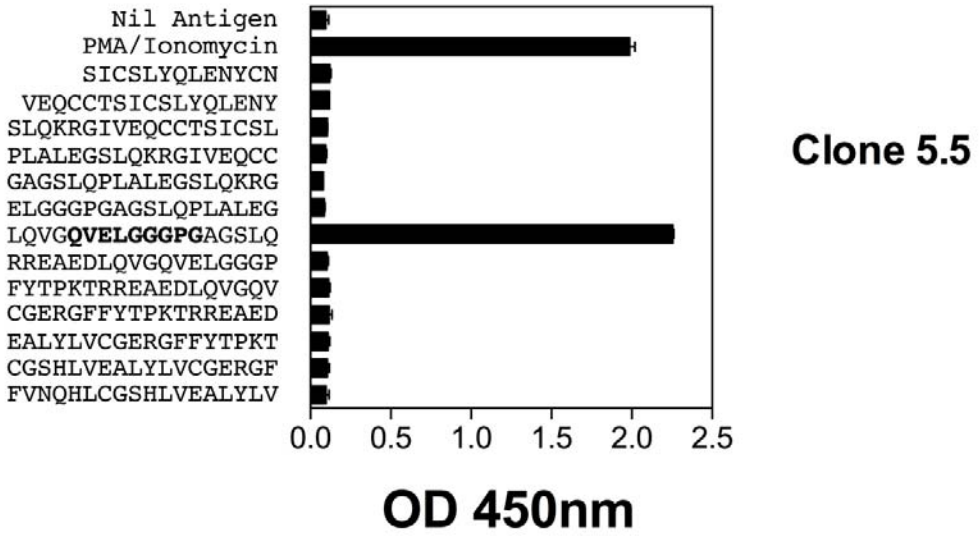
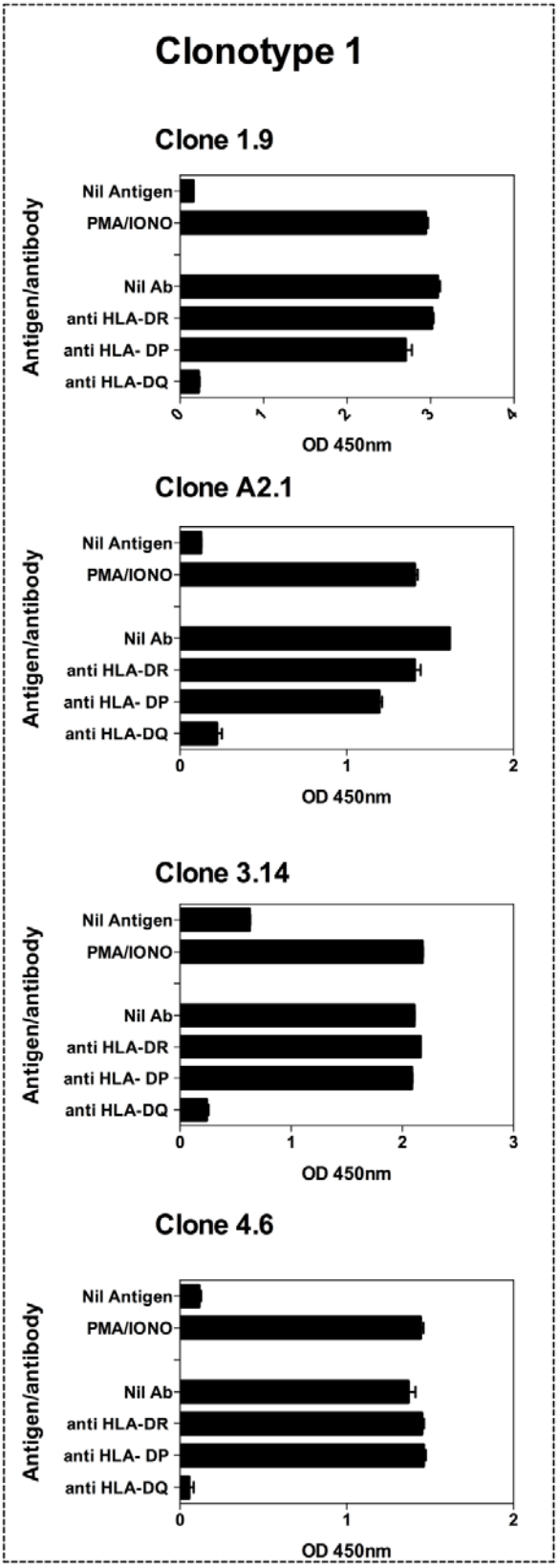
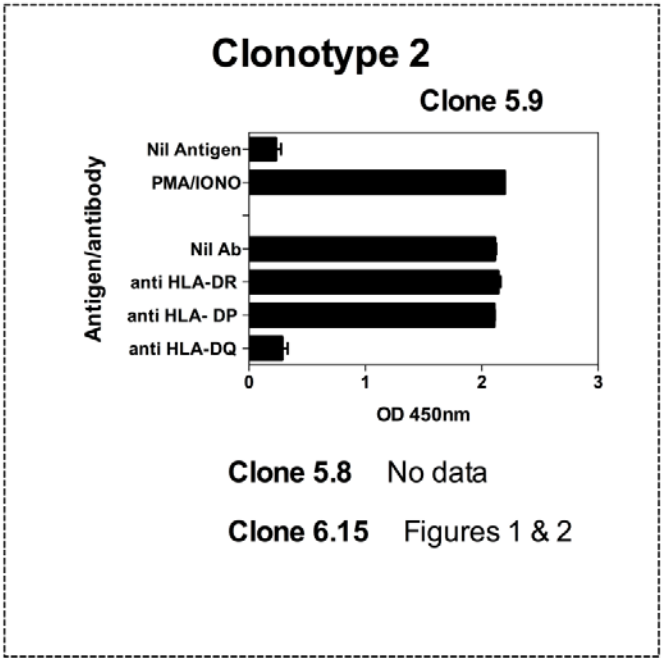
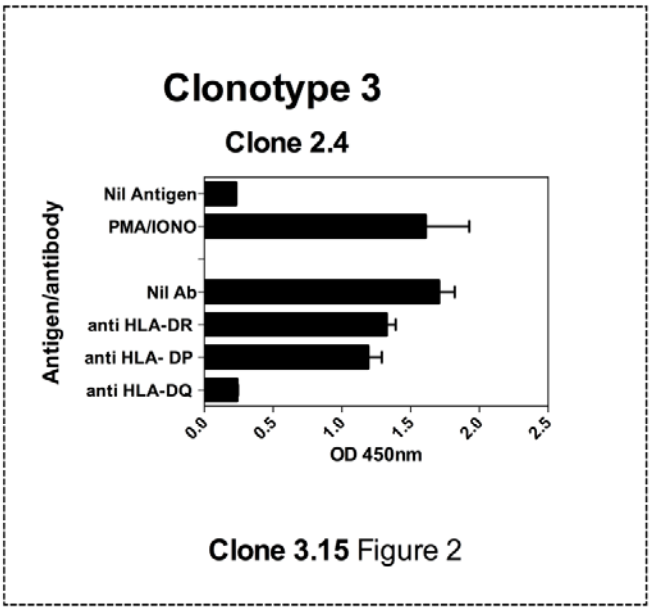


Supplementary Figure 2. Fine epitope mapping. A panel of 12-13 peptides sequentially truncated from the N-, or C-terminus were tested. The '-' represents a position from which an amino acid has been deleted relative to the full-length peptide. Each peptide was tested at a final concentration of 10μM in triplicate with the B-cell line (KJ EBV) as antigen-presenting cells (20,000 -50,000/ well) and cloned T cells (50,000/well). T cells and antigen presenting cells, cultured in the absence of antigen (labeled 'Nil antigen'), and T cells cultured with phorbol 12-myristate 13-acetate/ionomcyin (labeled 'PMA/Iono') were included as negative and positive controls respectively. Each experiment was conducted in triplicate and the mean +/- SEM of the absorbance at 450nm for an IFNγ ELISA is shown for each clone. OD ~1.0 is equivalent to ~500pg/ml of IFNγ. Where several clones with identical TCR sequences were isolated and single representative is shown. A representative of duplicate experiments is shown. The vertical lines represent the minimal epitope deduced from the results.

SUPPLEMENTARY DATA



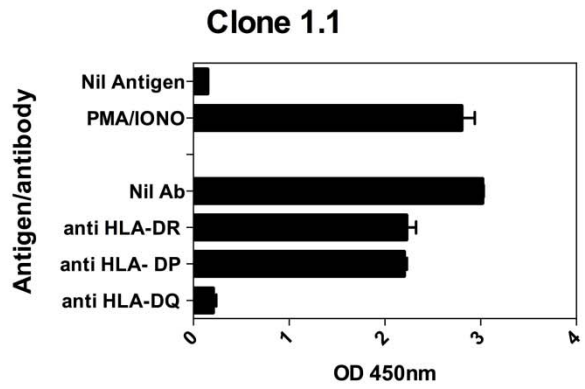


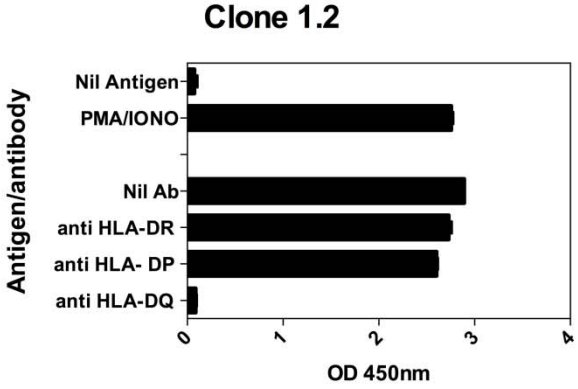


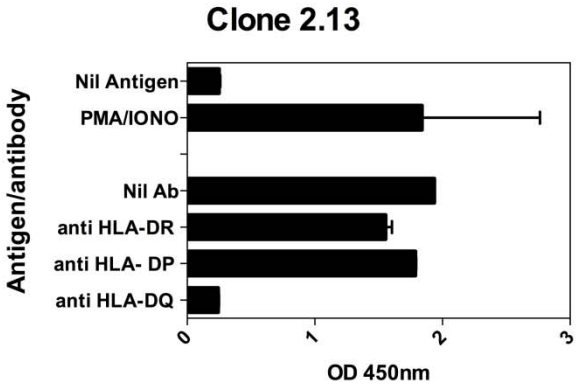
SUPPLEMENTARY DATA

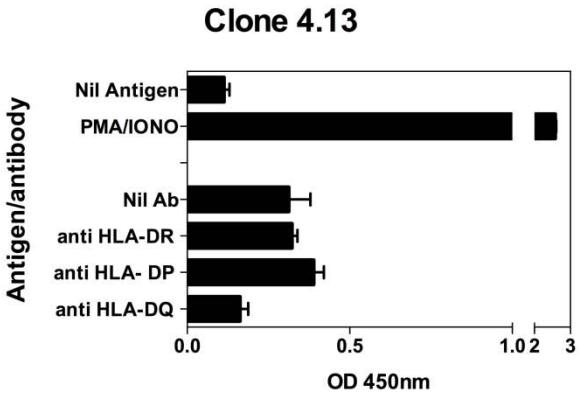
Supplementary Figure 3. HLA restriction antibody blocking. The effect of antibodies specific for HLA-DR, -DQ and-DP (final concentration of 0.3-10μg/ml) on peptide stimulated IFNγ secretion was determined for each clone. Some clone required higher concentrations of mAb than others. The appropriate stimulatory peptide (proinsulin 37-55, or 47-62) for each clone was included at a final concentration of 10μM in triplicate with the B-cell line (KJ EBV) as antigen-presenting cells (20,000 - 50,000/ well) and cloned T cells (50,000/well). T cells cultured with peptide and B cells, but no antibody (Nil Ab); T cells and antigen-presenting cells, cultured in the absence of antigen (labeled 'Nil antigen') and T cells cultured with phorbol 12-myristate 13-acetate/ionomcyin (labeled PMA/Iono) were included as controls. Each experiment was conducted in triplicate and the mean +/- SEM of the absorbance at 450nm for an IFNγ ELISA is shown for each clone. An optical density (OD) of 1.0 is equivalent to ~500pg/ml of IFNγ. Where several clones with identical TCR sequences were isolated the results from each clonotype are grouped together. and single representative is shown.

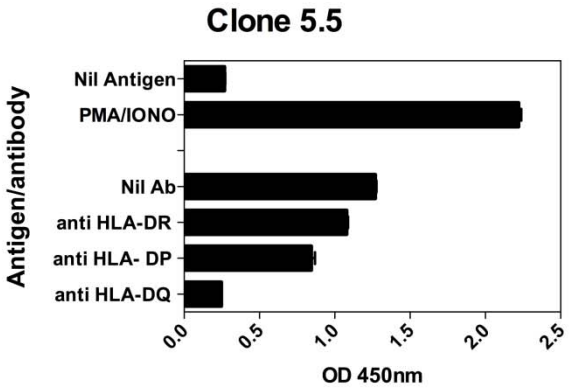
SUPPLEMENTARY DATA







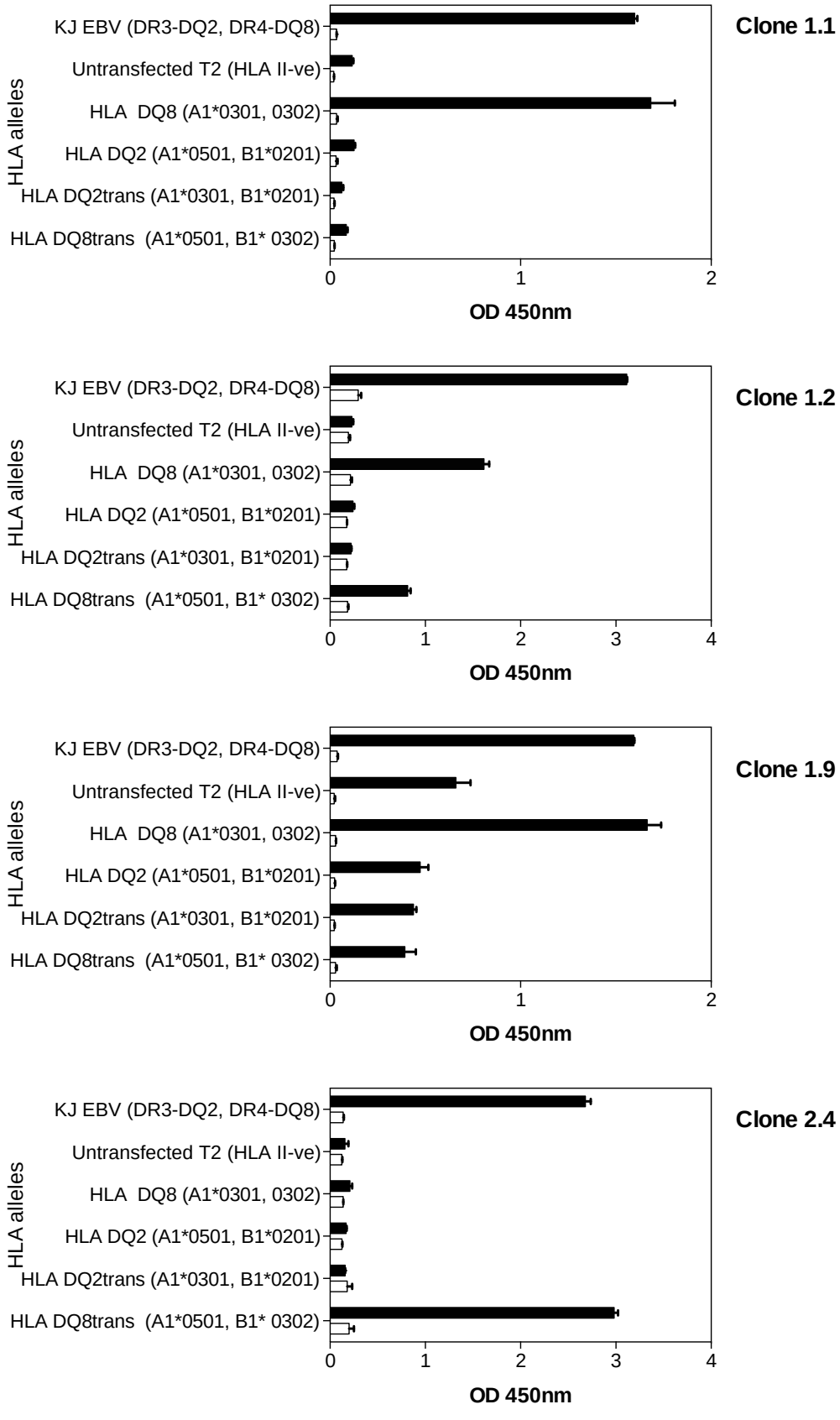




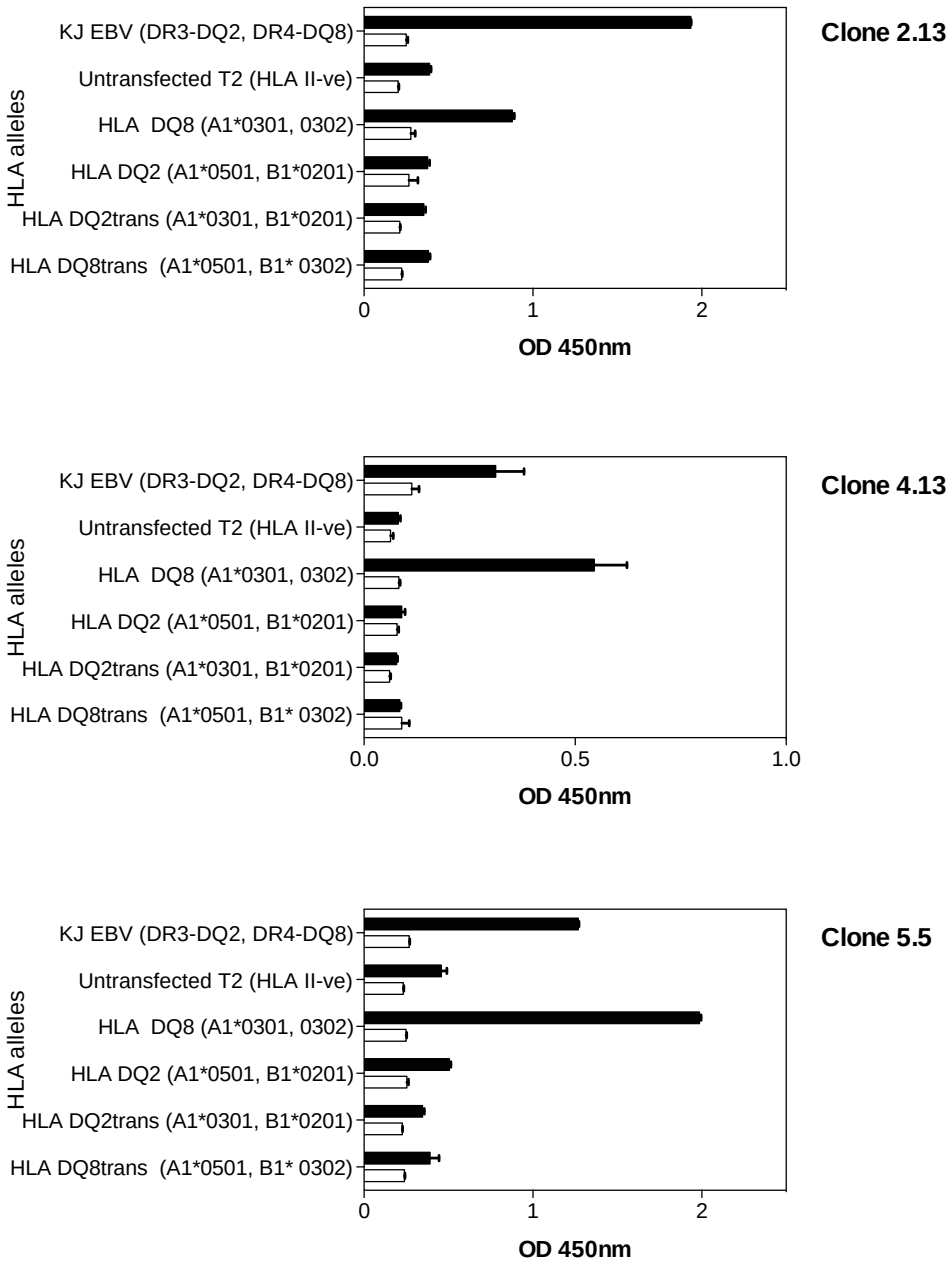
SUPPLEMENTARY DATA

Supplementary Figure 4. HLA restriction transfected cell lines. Presentation of proinsulin peptides to T-cell clones by antigen presenting cells that express a single HLA class II molecule. The requirement for different HLA DQ molecules was determined using a panel of HLA class II deficient T2 cells (20,000 - 50,000/ well) transfected with the different combinations of HLA DQA1 and DQB1 chains. The HLA DQ2 (A1*05:01, B1*02:01) DQ8 (A1*03:01, 03:02) heterozygous B-cell line (KJ) was used a positive control. Each antigen-presenting cell was cultured with cloned T cells (50,000/well) in the presence (filled bars), or absence (open bars) of the appropriate stimulatory peptide (proinsulin 37-55, or 47-62) for each clone, at a final concentration of 10μM in triplicate. Each experiment was conducted in triplicate and the mean +/- SEM of the absorbance at 450nm for an IFNγ ELISA is shown for each clone. An OD of 1.0 is equivalent to ~500pg/ml of IFNγ. Where several clones with identical TCR sequences were isolated and single representative is shown. A representative of duplicate experiments is shown.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Table 1. Summary of recent onset T1D donor's responses to proinsulin epitopes

Subject	**Duration (Months)**	**HLA**		**Responses to peptides CDI**		
		DRB1*	DQB1*	PI **40-52**	PPI **48-62**	Tetanus toxoid
1	1	03:01, 04:05	02:01; 03:02	**6.8**	**4.1**	155
2	3	03:01; 04:01	02:01; 03:02	0.9	**2.0**	97
3	2	04:04; 15:01	03:02; 06:02	0.8	0.7	737
4	2	04:01; -	03:01; 03:02	**119.1**	**11.4**	128
5	6	03:01; 04:01	02:01; 03:02	1.0	1.1	1,4106
6	11	03:01; 04:05	02:01; 03:02	1.1	1.5	59
7	2	03:01; 04:01	02:01; 03:02	0.8	1.3	93
8	1	04:01; 04:02	03:02;-	0.5	0.6	260

Supplementary Table 2. Summary of healthy donors' responses to proinsulin epitopes

Subject	**HLA**		**Responses to peptides CDI**		
	DRB1*	DQB1*	PPI **40-52**	PPI **48-62**	Tetanus toxoid
1	04; -	03:01, **0302**	0.8	0.7	737
2	04:04, 12:01	03:01; **03:02**	1.9	1.1	8
3	04,15	**03:02,** 06:02	1.5	1.5	46
4	02; 04	**03:02** ;06:02	1.1	1.6	48
5	04; 09	**03:02**; 03:03	1.2	1.5	68
6	04, 15	01:01; **0302**	0.9	1.1	16

SUPPLEMENTARY DATA

Supplementary Table 3. Summary of islet-infiltrating T-cell clone TCR sequencing from Donor A

Clone	TRAV	TRAJ	CDR3 α	TRA CDR3 length	TRBV	TRBJ	TRBD	TRB CDR3	TRB CDR3 length
A1.1	25*01	16*01	CAGGFSDGQKLLF	11	20-1*03to07	2-7*01	1*01	CSARTEAYEQYF	10
A1.2	20*01-04	58*01	CAVIETSGSRLTF	11	20-1*03to07	2-3*01	2*01	CSARDQQRVDTQYF	12
A1.3	19*01	21*01	CALSEQ#TFNKFYF	-	12-3*01	1-5*01	1*01	CASSLAWGADQPQHF	13
A1.4	13-1*01	39*01	CAAPHNAGNMLTF	11	2*01/02	1-5*01	1*01	CASRNRDGELPQHF	12
A1.5	8-2*01/*02	5*01	CVVSDLDTGRRALTF	13	7-2*01/04	1-2*01	2*01	CASSSTYGYTF	9
A1.7	35*02	28*01	CAGQEPGAGSYQLTF	13	5-1*01	1-3*01	1*01	CASSSRIGNTIYF	11
A1.8	13-2*01	11*01	CAENIGYSTLTF	10	10-3*01 /02/04	2-7*01	1*01	CAISGPNRGSSYEQYF	14
A1.9	20*02/*04	7*01	CAVQAGGNNRLAF	11	5-1*01	1-2*01	1*01	CASSLERDGYTF	10
A1.10	8-3*02	10*01	CAVGVTGGGNKLTF	12	5-4*01/03	1-2*01	1*01	CASSLRDRGNYGYTF	13
A2.1	20*02/*04	7*01	CAVQAGGNNRLAF	11	5-1*01	1-2*1	1*01	CASSLERDGYTF	10
A2.3	19*01	57*01	CALSDSQGGSEKLVF	13	6-5*01	2-1*01	2*01	CASRRTSGFNEQFF	12
A2.4	19*01	49*01	CALSRAGTGNQFYF	12	5-1*01	2-4*01	2*01	CASSLGLRGENIQYF	13
A2.5	6*03	44*01	CALSMFLTSGTASKLTF	15	30*01/*05	2-7*01	2*02	CAWSLRGGTFTYEQYF	14
A2.7	13-1*01	43*01	CAANPHNDMRF	9	6-5*01	2-3*01	1*01	CASSYFSGAGTDTQYF	14
A2.8	2*01/*02	6*01	CAVQAGGSYIPTF	11	7-2*01/04	2-5*01	1*01	CASSLQGARDGETQYF	14
A2.9	13-1*01	10*01	CAAKVGVTGGGNKLTF	14	20-1*03to07	2-3*01	1*01	CSARDAGASTDTQYF	13
A2.10	27*01	53*01	CAG#GSNYKLTF	-	5-1*01	2-1*01	ND	CASSLEDPQYNEQFF	13
A2.11*	38-1*03	54*01	CAFMGAGAQKLVF	11	4-3*01	2-3*01	2*01	CASSQILRGGPPDTQYF	15
A2.13	26-1*01	39*01	CIVSHNAGNMLTF	11	5-1*01	2-5*01	2*01	CASSLERETQYF	10
A2.14	26-1*01/02	24*02	CIVRVIGSWGKLQF	12	6-1*01	1-1*01	1*01	CASIVTGGNTEAFF	12
A3.4	8-2*01	37*01/2	CVVSDLVRNTGKLIF	13	7-9*01	2-4*01	1*01	CASSRVGAAKNIQYF	13
A3.5	23/D6*01	47*01	CAASGVGGNKLVF	11	20-1*01	2-1*01	ND	CSASGNEQFF	8
A3.6	9-2*02	56*01	CALIGANSKLTF	10	20-1*01	2-7*01	1*01	CSATQGQGNSYEQYF	13
A3.10	38-1*03	54*01	CAFFGQGAGKLVF	11	5-1*01	2-3*01	2*01	CASSLSASGGATDTQYF	15
A3.11	22*01	18*01	CAVERDRGSTLGRLYF	14	20-1*01	1-4*01	2*02	CSARDGRGLGELFF	12
A3.12**	12-1*01	15*01	CVVDGNQAGTALIF	12	9*01	2-1*01	1*01	CASSAAQDTNEQFF	12
	20*02	11*01	CAVRGYSTLTF	9	-	-	-	-	
A3.14	20*02	7*01	CAVQAGGNNRLAF	11	5-1*01	2-1*01	1*01	CASSLERDGYTF	10
A3.15	19*01	49*01	CALSRAGTGNQFYF	12	5-1*01	2-4*01	2*01	CASSLGLRGENIQYF	13
A4.5	13-1*01	42*01	CAALGGSQGNLIF	11	15*02	2-1*01	1*01	CATSRGQSLVYNEQFF	14
A4.6	20*02	7*01	CAVQAGGNNRLAF	11	5-1*01	1-2*01	1*01	CASSLERDGYTF	10

SUPPLEMENTARY DATA

A4.7	36/DV7*04	53*01	CATRRGSNYKLTF	11	20-1*01	2-3*01	2*01	CSAPLSGGSTDTQYF	13
A4.10	8-6*01	34*01	CAVSDPYTDKLIF	11	12-3*01	2-7*01	1*01	CAVSDPYTDKLIF	11
A4.11	38-1*01/04	52*01	CASGGTSYGKLTF	11	27*01	1-2*01	1*01	CASSAISQPPTGMSGYTF	16
A4.12	12-2*01	11*01	CAVRNSGYSTLTF	11	29-1*01/*03	1-5*01	2*02	CSVVDGSNQPQHF	11
A4.13	6*03	36*01	CALKYGANNLFF	10	18*01	1-1*01	1*01	CASSPTTGGDEAFF	12
A4.14	41*01	34*01	CAVLESGDKLIF	10	6-1*01	1-2*01	2*01	CASSEAFRDYGYTF	12
A4.16	9-2*02	3*01	CALSDQDSSASKIIF	13	2*01	1-6*02	1*01	CASRTGGSGNSPLHF	13
A5.1**	5*01	15*01	CAETPNQAGTALIF	12	30*01	1-6*01	1*01	CAWSVEGAQNSPLHF	
	13-1*01	11*01	CATHSGYSTLTF	10	-	-	-	-	-
A5.3	8-6*02	4*01	CAVNPGGYNKLIF	11	4-1*01	2-5*01	2*02	CASSQDSRRETQYF	12
A5.4	24*01	22*01	CASLSGCARQLTF	11	25-1*01	1-2*01	1*01	CASRGDSLLGGYTF	12
A5.5	26-1*01/02	54*01	CIVRVEIQGAQKLVF	13	5-1*01	2-5*01	1*01	CASSLGPGQRETQYF	13
A5.6	34*01	3*01	CGADW#SSASKIF	-	27*01	1-6*02	2*01	CASGTGDSPLHF	10
A5.7	26-1*01/02	42*01	CIVRVVHYGGSQGNLIF	15	29-1*01/*03	1-1*01	1*01	CSVEGVGWSAFF	10
A5.8	26-1*02	21*01	CIAIYNFNKFYF	10	5-1*01	1-6*02	2*01	CASSLEASSYNSPLHF	14
A5.9	26-1*02	21*01	CIAIYNFNKFYF	10	5-1*01	1-6*02	2*01	CASSLEASSYNSPLHF	14
A6.1	41*01	47*02	CAVRVTGNKLVF	10	5-4*01	2-3*01	2*01	CASSLGLAGSTDTQYF	14
A6.2	1-2*01	22*01	CAVRVR#F	-	12-3*01	1-5*01	1*01	CASSLSQQGGQPQHF	13
A6.4	27*01	40*01	CAGPHSGTYKYIF	11	16*01 to 03	2-7*01	1*01	CASSQLNSYHEQYF	12
A6.5	27*01	36*01	CAGPSQTGANNLFF	12	5-1*01	1-4*01	2*02	CASSLVHGRNEKLFF	13
A6.6	8-4*01/04	23*01	CAVSEAYNQGGKLIF	13	5-1*01	2-2*01	1*01	CASSYTGPAGELFF	12
A6.7	8-4*01/04	23*01	CAVSEAYNQGGKLIF	13	7-9*01	1-1*01	1*01	CASRSGQVNTEAFF	12
A6.11	17*01	18*01	CATDDKDRGSTLGRLYF	15	5-1*01	2-3*01	1*01	CASSADRVTDTQYF	12
A6.15	26-1*01/02	21*01	CIAIYNFNKFYF	10	5-1*01	1-6*02	2*01	CASSLEASSYNSPLHF	14

**Two TCRA genes were detected in these clones.

Full TRA and TRB DNA sequences for each proinsulin specific clone, islet infiltrating clone are available on request (smannering@svi.edu.au).

Supplementary Table 4. Summary of proinsulin peptides

```
B chain                              C-peptide                A-chain
                1    5    10   15   20   25   30   35   40   45   50   55   60   65   70   75   80   85
                FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN
1-18            FVNQHLCGSHLVEALYLV
7-25                  CGSHLVEALYLVCGERGF
13-3                        EALYLVCGERGFFYTPKT
19-36                             CGERGFFYTPKTRREAED
25-42                                   FYTPKTRREAEDLQVGQV
31-48                                         RREAEDLQVGQVELGGGP
37-54                                               LQVGQVELGGGPGAGSLQ
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SUPPLEMENTARY DATA

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43-60                                                ELGGGPGAGSLQPLALEG
49-66                                                      GAGSLQPLALEGSLQKRG
55-72                                                            PLALEGSLQKRGIVEQCC
61-78                                                                  SLQKRGIVEQCCTSICSL
67-84                                                                        IVEQCCTSICSLYQLENY
73-86                                                                              TSICSLYQLENYCN
```

SUPPLEMENTARY DATA

Supplementary Table 5. GAD-65 derived $CD4^+$ T-cell epitopes

GAD-65	Sequence
$GAD_{115-130}$	MNILLQYVVKSFDRST
$GAD_{247-279}$	NMYAMMIARFKMFPEVKEKG
$GAD_{260-279}$	PEVKEKGMAALPRLIAFTSE
$GAD_{274-286}$	IAFTSEHSHFSLK
$GAD_{506-518}$	FWYIPPSLRTLED
$GAD_{521-535}$	ERMSRLSKVAPVIKA
$GAD_{555-570}$	NFFRMVISNPAATHQ

Supplementary Table 6. HSP-60 derived $CD4^+$ T-cell epitopes

HSP 60	Sequence
$HSP60_{437–}$	VLGGGCALLRCIPALDSLTPANED
$HSP60_{466–}$	EIIKRTLKIPAMTIAKNAGV

Supplementary Table 7. IA-2 derived $CD4^+$ T-cell epitopes

IA-2	Sequence
$IA\text{-}2_{654–674}$	VSSVSSQFSDAAQASPSSHSS
$IA\text{-}2_{709–732}$	LAKEWQALCAYQAEPNTCATAQGE
$IA\text{-}2_{752–775}$	KLKVESSPSRSDYINASPIIEHDP
$IA\text{-}2_{797–817}$	MVWESGCTVIVMLTPLVEDGV
$IA\text{-}2_{854–872}$	FYLKNVQTQETRTLTQFHF
$IA\text{-}2_{955–975}$	SKDQFEFALTAVAEEVNAILK

Supplementary Table 8. IGRP-derived $CD4^+$ T-cell epitopes

IGRP	Sequence
$IGRP_{8–27}$	GVLIIQHLQKDYRAYYTFLN
$IGRP_{17–36}$	KDYRAYYTFLNFMSNVGDPR
$IGRP_{225–244}$	LRVLNIDLLWSVPIAKKWCA
$IGRP_{241–260}$	KWCANPDWIHIDTTPFAGLV

SUPPLEMENTARY DATA

Supplementary Table 9. ZnT8-derived $CD4^+$ T-cell epitopes

ZnT8	**Sequence**
$ZnT8_{1-27}$	MEFLERTYLVNDKAAKMHAFTLESVEL
$ZnT8_{64-91}$	ANEYAYAKWKLCSASAICFIFMIAEVV
$ZnT8_{120-147}$	SLWLSSKPPSKRLTFGWHRAEILGALL
$ZnT8_{253-281}$	FIFSILVLASTITILKDFSILLMEGVP
$ZnT8_{267-293}$	LKDFSILLMEGVPKSLNYSGVKELILA
$ZnT8_{280-307}$	SLNYSGVKELILAVDGVLSVHSLHIWS
$ZnT8_{309-335}$	TMNQVILSAHVATAASRDSQVVRREIA