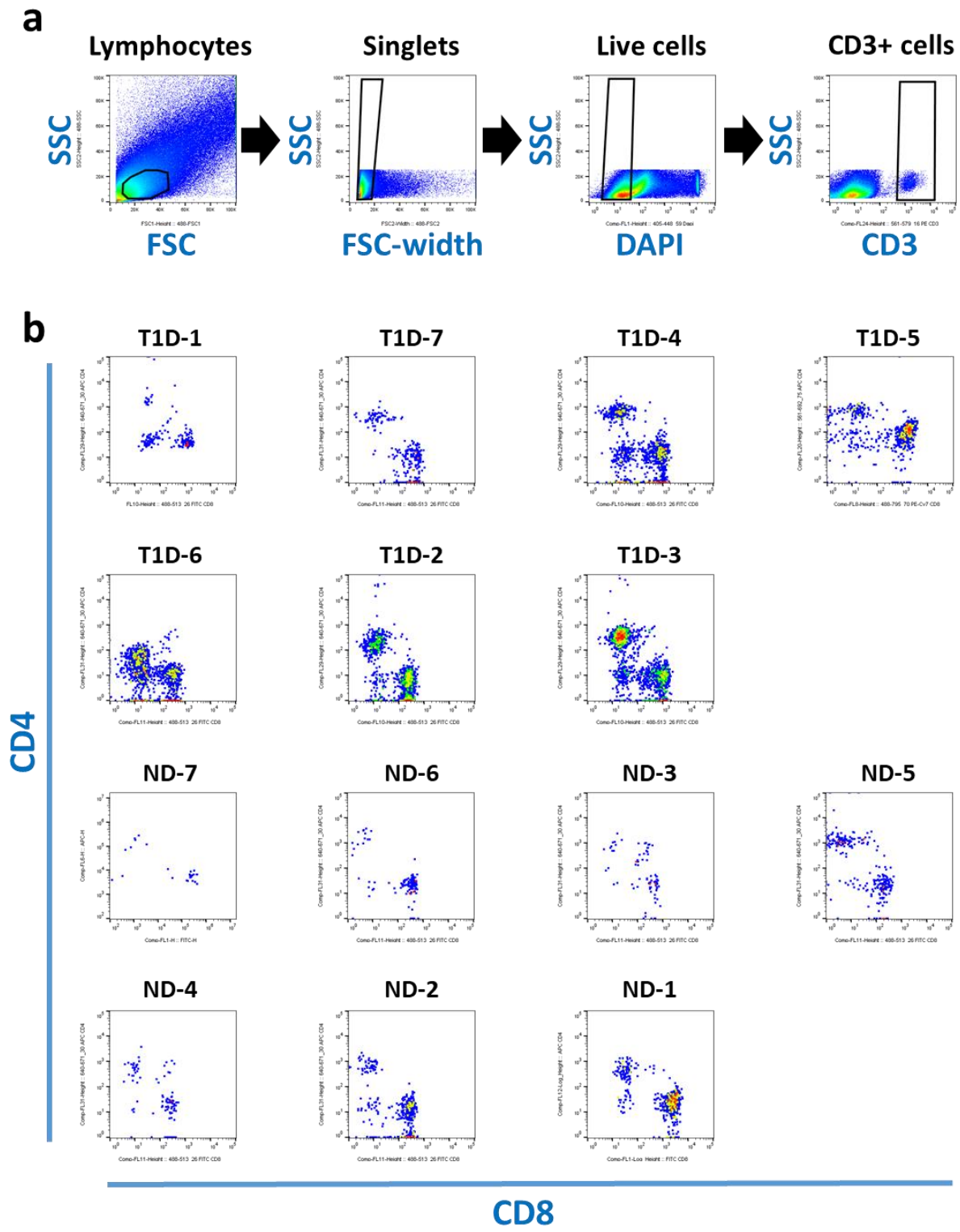
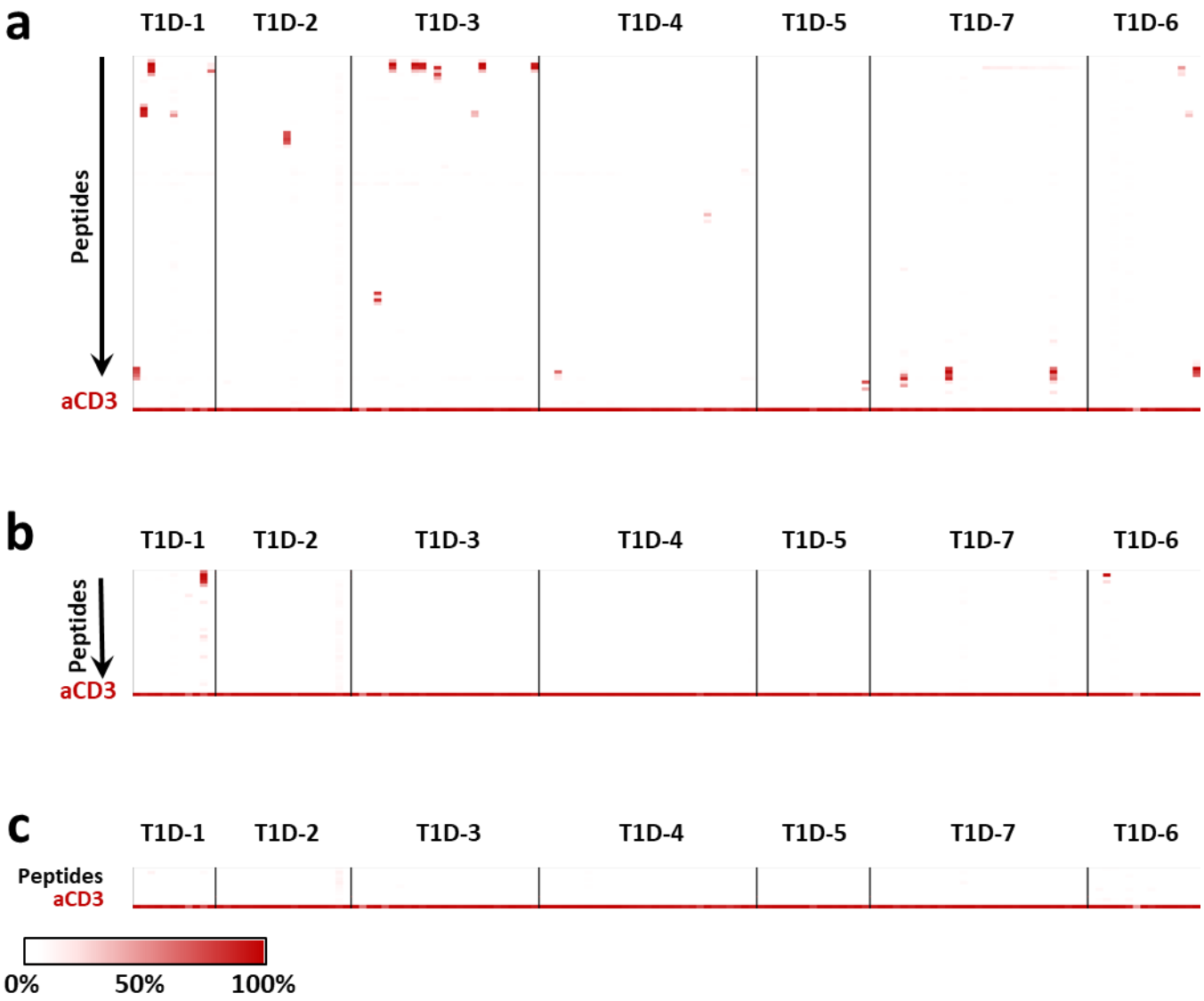
Supplementary Figure 1



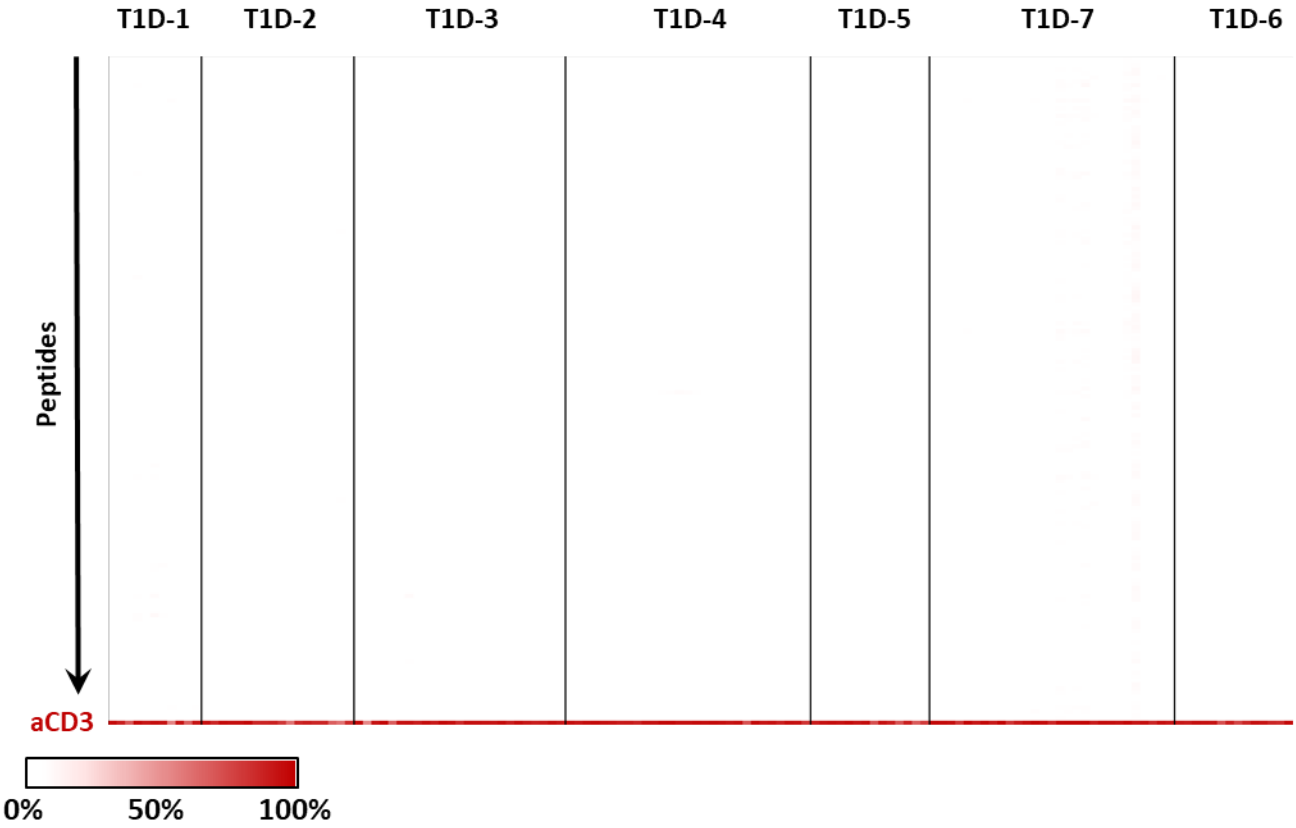
Supplementary Figure 1: Flow cytometry sorting of CD4 and CD8 T-cells. (a) Islet tissues were dispersed into single cells, followed by staining with anti-CD3, anti-CD4, and anti-CD8 antibodies and DAPI. Single lymphocytes were determined in the forward and side scatter view, living cells were selected by DAPI-negative cells, and $CD3^+$ cells were identified to find $CD3^+CD4^+$ and $CD3^+CD8^+$ cells. **(b)** Individual panels show $CD3^+$ cells determined by the gating strategy indicated in (**a**). Cells were stained with anti-CD4 and anti-CD8 antibodies.

Supplementary Figure 2



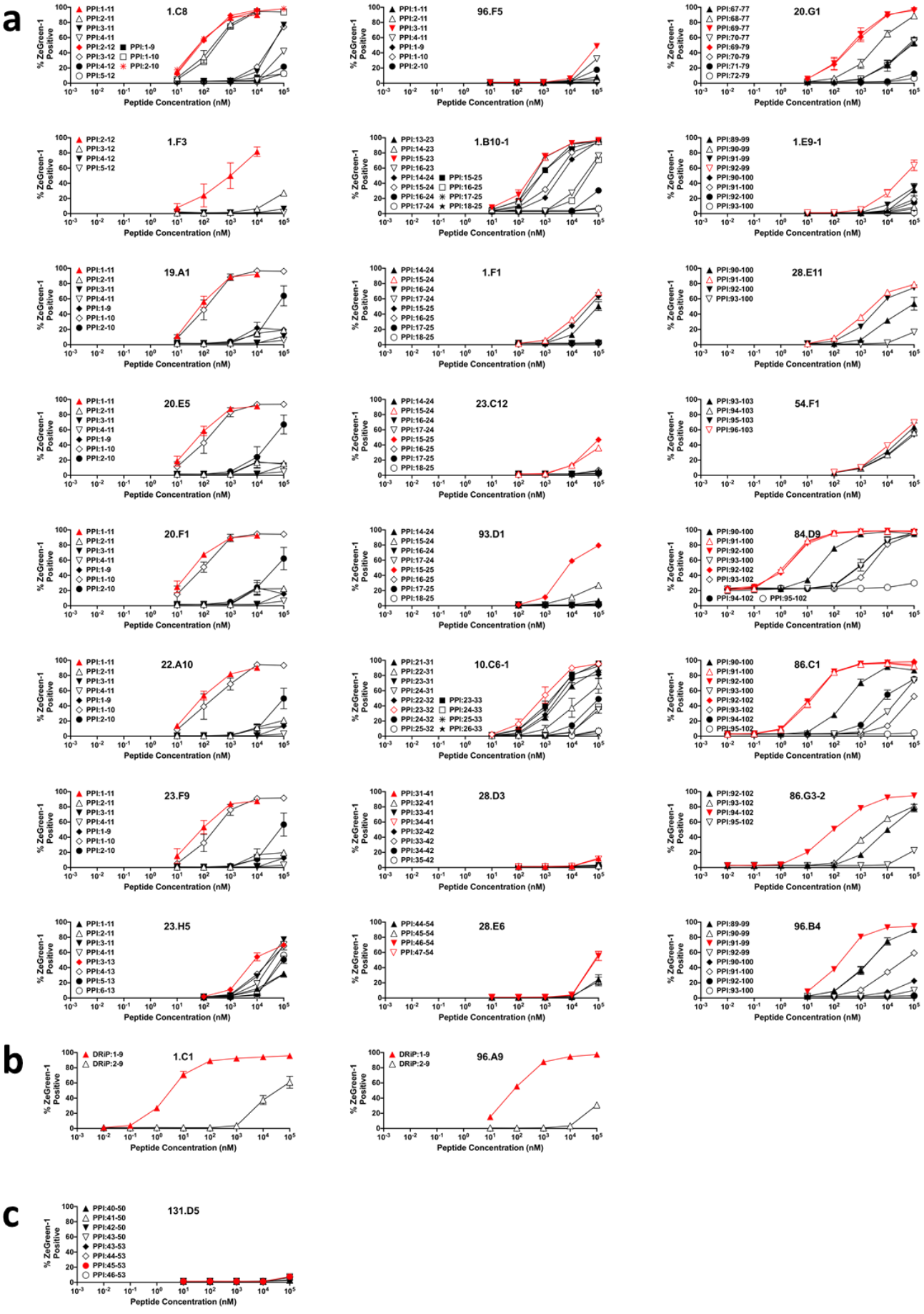
Supplementary Figure 2: Screening of TCR clonotypes for the response to preproinsulin and other candidate peptides. TCR transductants were cultured with (**a**) truncated preproinsulin peptide pools, (**b**) truncated insulin DRiP peptide pools, or (**c**) peptides known to be antigens for CD8 T-cells in peripheral blood of T1D patients, in the presence of autologous EBV-transformed B cells, followed by evaluation of ZsGreen-1 expression. Individual rows indicate each peptide pool (**a** & **b**) and peptide (**c**) designated in Supplementary Tables 2, 3, and 4, respectively, and individual columns indicate each TCR transductant from 7 T1D organ donors. Responses to each peptide or peptide pool by each TCR transductant were evaluated by percentages of ZsGreen-1 positive cells and are shown in heat maps. The bottom rows in individual heat maps show activation levels elicited by anti-CD3 antibodies.

Supplementary Figure 3



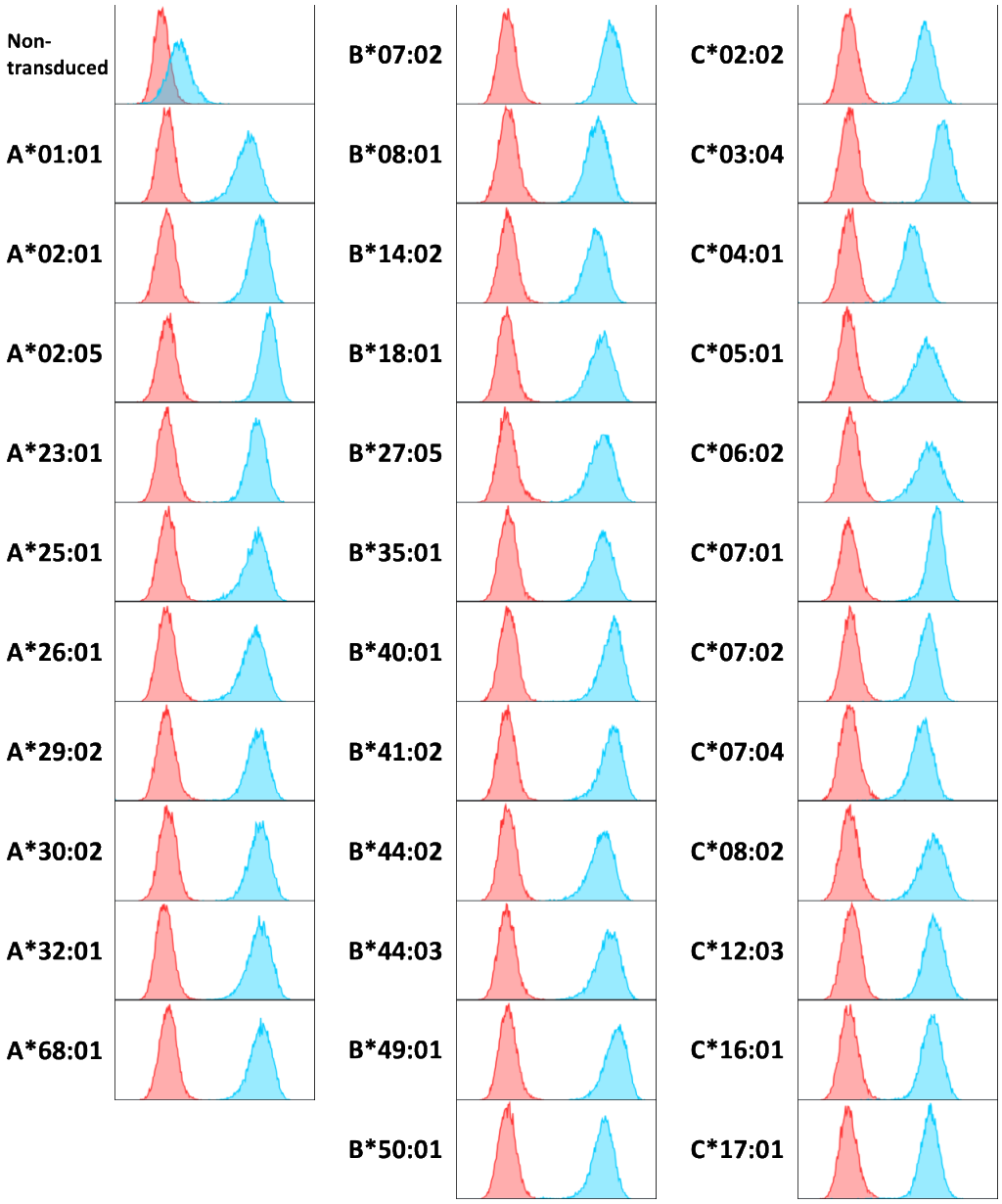
Supplementary Figure 3: Screening of TCR clonotypes for the response to glucagon peptides. TCR transductants were cultured with 173 truncated glucagon peptide pools in the presence of autologous EBV-transformed B cells, followed by evaluation of ZsGreen-1 expression. Individual rows indicate each peptide pool designated in Supplementary Table 5, and individual columns indicate each TCR transductant from 7 T1D organ donors. Responses to each peptide or peptide pool by each TCR transductant were evaluated by percentages of ZsGreen-1 positive cells and are shown in heat maps. The bottom rows in individual heat maps show activation levels elicited by anti-CD3 antibodies.

Supplementary Figure 4



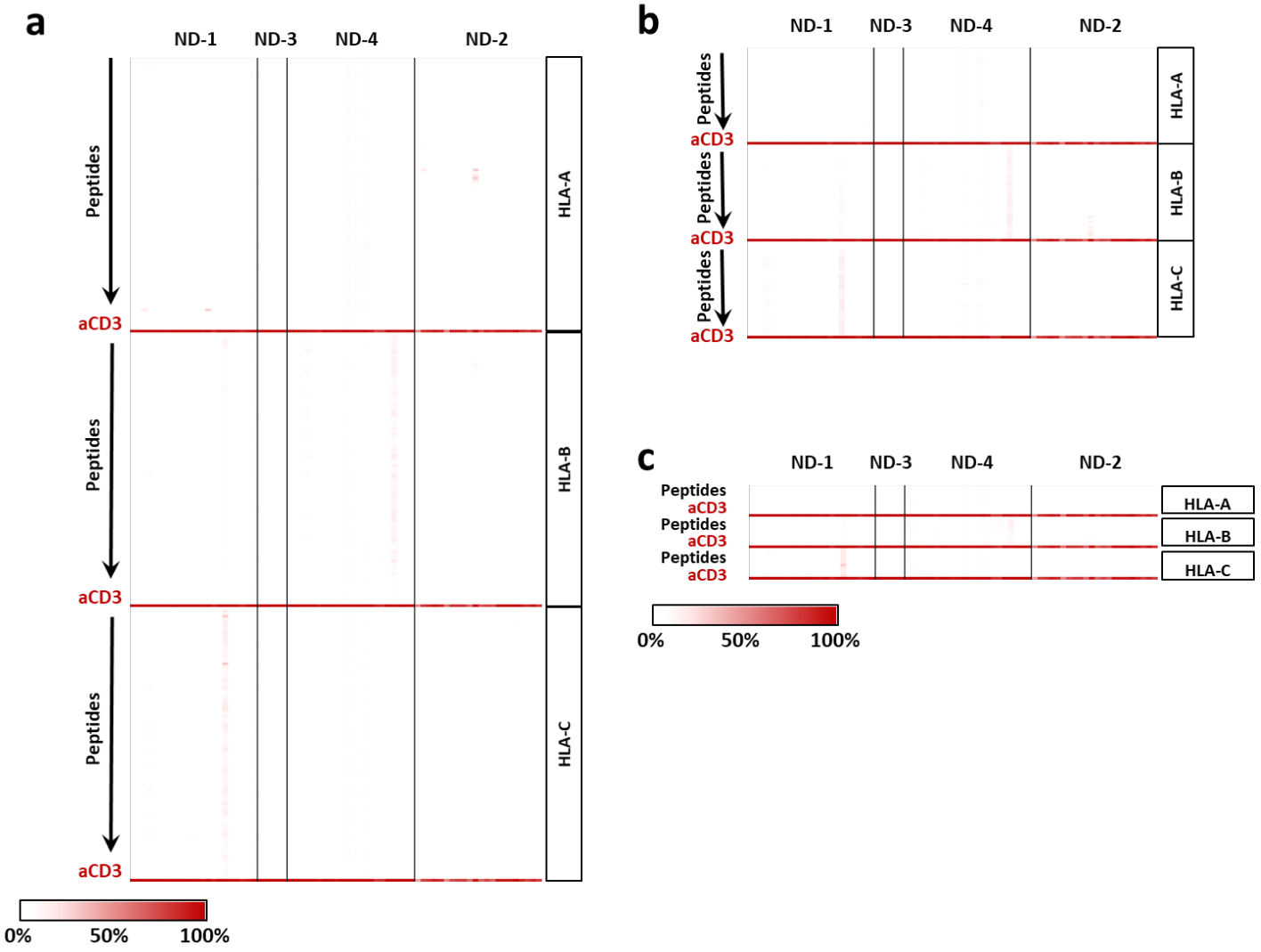
Supplementary Figure 4: Titration analysis determining responses to preproinsulin and insulin DRiP peptides. Preproinsulin and insulin DRiP-reactive TCR transductants were cultured with different concentrations of individual peptides contained in peptide pools that stimulated TCR transductants in the screening test. K562 cells expressing a cognate HLA class I molecule were used as antigen-presenting cells. Peptide sequences are indicated in Supplementary Table 6. Panels in **a**, **b**, and **c** show ZsGreen-1 expression levels in response to peptide stimulation for individual transductants expressing preproinsulin-reactive TCRs derived from T1D donors, insulin DRiP-reactive TCRs, and a preproinsulin-reactive TCR derived from a non-diabetic control donor, respectively.

Supplementary Figure 5



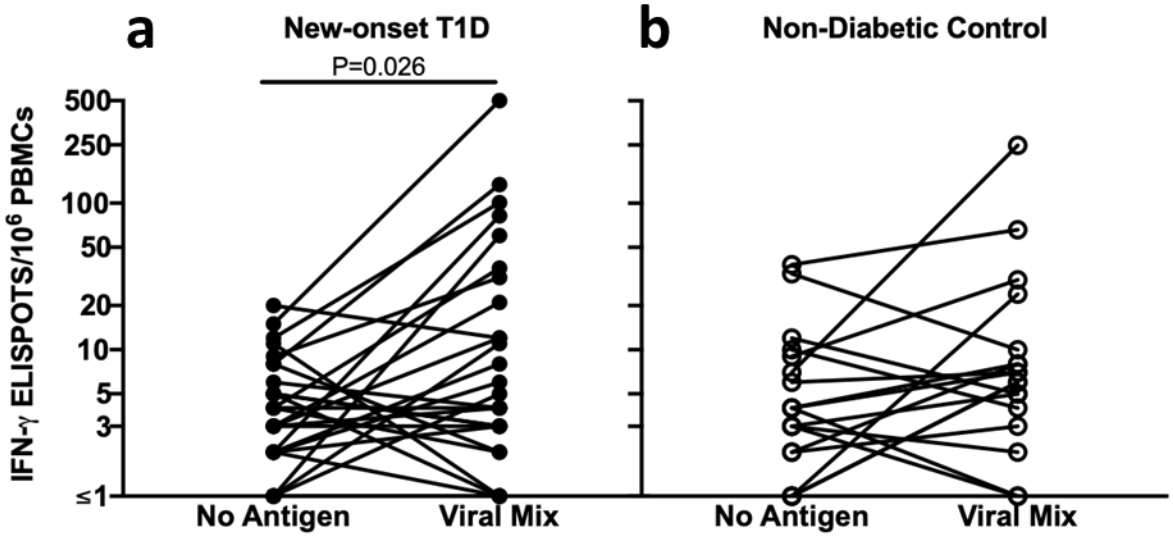
Supplementary Figure 5: Expression of HLA class I molecules in antigen-presenting K562 cells. K562 cells transduced with each HLA class I variant were stained with anti-beta2 microglobulin antibodies. Blue and red forms represent cells stained with and without the antibodies. Flow cytometry image data from one representative experiment of two independent experiments are shown.

Supplementary Figure 6



Supplementary Figure 6: Screening of donors TCR clonotypes derived from non-diabetic control donors. TCR transductants were cultured with (**a**) truncated preproinsulin peptide pools, (**b**) truncated insulin DRiP peptide pools, or (**c**) peptides known to be antigens for CD8 T-cells in peripheral blood of T1D patients, followed by evaluation of ZsGreen-1 expression. Mixture of two K562 cells expressing HLA-A, B, and C molecules of each cognate donor was used as antigen-presenting cells. HLA-A, B, and C typing results are indicated in Table 1. Individual rows indicate each peptide pool (**a** & **b**) and peptide (**c**) designated in Supplementary Tables 2, 3, and 4, respectively, and individual columns indicate each TCR transductant. Responses to each peptide or peptide pool by each TCR transductant were evaluated by percentages of ZsGreen-1 positive cells and are shown in heat maps. The bottom rows in individual heat maps show activation levels elicited by anti-CD3 antibodies.

Supplementary Figure 7



Supplementary Figure 7: IFN-γ ELISPOT responses to viral peptides using peripheral blood mononuclear cells. Cryopreserved PBMCs were cultured in the presence or absence of a viral mix of peptides (CD8 T-cell epitopes from CMV, EBV, and Measles) for 48 hours, washed, and then cells transferred to an IFN-γ monoclonal antibody-coated plate for overnight culture followed by development and enumeration of ELISPOTs. Comparison of no antigen to viral peptides for **(a)** new-onset T1D subjects (n=30) and **(b)** non-diabetic subjects (n=18). P=0.026 for new-onset T1D and p=0.164 for non-diabetic subjects using a Wilcoxon matched-pairs signed rank test.

Supplementary Table 1: TCR clonotypes identified and studied

Donor Type	Donor ID	Donor Reference	No of islets sampled	No of T cells for which TCR α and/or β identified	No of Unique clones	No of Clones to make transductants	No of TCR-αβ pairs to make transductants	No of TCR-αβ pairs not expressed	No of TCR-αβ pairs having non-specific responses	No of TCR-αβ pairs screened	No of unique clones screened
T1D	T1D-1	nPOD 69	500	46	18	10	12	1	0	11	10
T1D	T1D-2	nPOD 6323	1750	161	108	17	18	0	0	18	17
T1D	T1D-3	nPOD 6342	500	209	154	25	27	2	0	25	24
T1D	T1D-4	nPOD 6367	5390	199	142	27	32	3	0	29	26
T1D	T1D-5	nPOD 6414	1000	145	117	15	15	0	0	15	14
T1D	T1D-6	nPOD 6472	~120	304	191	14	17	1	1	15	14
T1D	T1D-7	VUMC/ Pittsburgh	6000	238	125	28	30	0	1	29	27
T1D		Total		1302	855	136	151	7	2	142	132
Non diabetic Control	ND-1	iidp 9657	4000	200	84	21	24	2	0	22	21
Non diabetic Control	ND-2	iidp 1726	4000	221	101	20	22	0	0	22	19
Non diabetic Control	ND-3	R300	4000	63	49	6	7	1	1	5	5
Non diabetic Control	ND-4	R301	4000	179	138	23	24	0	2	22	21
Non diabetic Control	ND-5	IIDP 5642	4000	91	54	ND	ND	ND	ND	ND	ND
Non diabetic Control	ND-6	IIDP 7400	8000	243	136	ND	ND	ND	ND	ND	ND
Non diabetic Control	ND-7	R283	3000	TCR sequencing not performed							
Non diabetic Control		Total		663	372	70	77	3	3	71	66

Supplementary Table 2: Preproinsulin Truncated peptide pools

Pool No	11 Mer	10 Mer	9 Mer	8 Mer
1	-	-	-	MALWMRLL
2	-	-	MALWMRLLP	ALWMRLLP
3	-	MALWMRLLPL	ALWMRLLPL	LWMRLLPL
4	MALWMRLLPLL	ALWMRLLPLL	LWMRLLPLL	WMRLLPLL
5	ALWMRLLPLLA	LWMRLLPLLA	WMRLLPLLA	MRLLPLLA
6	LWMRLLPLLAL	WMRLLPLLAL	MRLLPLLAL	RLLPLLAL
7	WMRLLPLLALL	MRLLPLLALL	RLLPLLALL	LLPLLALL
8	MRLLPLLALLA	RLLPLLALLA	LLPLLALLA	LPLLALLA
9	RLLPLLALLAL	LLPLLALLAL	LPLLALLAL	PLLALLAL
10	LLPLLALLALW	LPLLALLALW	PLLALLALW	LLALLALW
11	LPLLALLALWG	PLLALLALWG	LLALLALWG	LALLALWG
12	PLLALLALWGP	LLALLALWGP	LALLALWGP	ALLALWGP
13	LLALLALWGPD	LALLALWGPD	ALLALWGPD	LLALWGPD
14	LALLALWGPDP	ALLALWGPDP	LLALWGPDP	LALWGPDP
15	ALLALWGPDPA	LLALWGPDPA	LALWGPDPA	ALWGPDPA
16	LLALWGPDPAA	LALWGPDPAA	ALWGPDPAA	LWGPDPAA
17	LALWGPDPAAA	ALWGPDPAAA	LWGPDPAAA	WGPDPAAA
18	ALWGPDPAAAF	LWGPDPAAAF	WGPDPAAAF	GPDPAAAF
19	LWGPDPAAAFV	WGPDPAAAFV	GPDPAAAFV	PDPAAAFV
20	WGPDPAAAFVN	GPDPAAAFVN	PDPAAAFVN	DPAAAFVN
21	GPDPAAAFVNQ	PDPAAAFVNQ	DPAAAFVNQ	PAAAFVNQ
22	PDPAAAFVNQH	DPAAAFVNQH	PAAAFVNQH	AAAFVNQH
23	DPAAAFVNQHL	PAAAFVNQHL	AAAFVNQHL	AAFVNQHL
24	PAAAFVNQHLC	AAAFVNQHLC	AAFVNQHLC	AFVNQHLC
25	AAAFVNQHLCG	AAFVNQHLCG	AFVNQHLCG	FVNQHLCG
26	AAFVNQHLCGS	AFVNQHLCGS	FVNQHLCGS	VNQHLCGS
27	AFVNQHLCGSH	FVNQHLCGSH	VNQHLCGSH	NQHLCGSH
28	FVNQHLCGSHL	VNQHLCGSHL	NQHLCGSHL	QHLCGSHL
29	VNQHLCGSHLV	NQHLCGSHLV	QHLCGSHLV	HLCGSHLV
30	NQHLCGSHLVE	QHLCGSHLVE	HLCGSHLVE	LCGSHLVE
31	QHLCGSHLVEA	HLCGSHLVEA	LCGSHLVEA	CGSHLVEA
32	HLCGSHLVEAL	LCGSHLVEAL	CGSHLVEAL	GSHLVEAL
33	LCGSHLVEALY	CGSHLVEALY	GSHLVEALY	SHLVEALY
34	CGSHLVEALYL	GSHLVEALYL	SHLVEALYL	HLVEALYL
35	GSHLVEALYLV	SHLVEALYLV	HLVEALYLV	LVEALYLV
36	SHLVEALYLVC	HLVEALYLVC	LVEALYLVC	VEALYLVC
37	HLVEALYLVCG	LVEALYLVCG	VEALYLVCG	EALYLVCG
38	LVEALYLVCGE	VEALYLVCGE	EALYLVCGE	ALYLVCGE
39	VEALYLVCGER	EALYLVCGER	ALYLVCGER	LYLVCGER
40	EALYLVCGERG	ALYLVCGERG	LYLVCGERG	YLVCGERG
41	ALYLVCGERGF	LYLVCGERGF	YLVCGERGF	LVCGERGF
42	LYLVCGERGFF	YLVCGERGFF	LVCGERGFF	VCGERGFF
43	YLVCGERGFFY	LVCGERGFFY	VCGERGFFY	CGERGFFY

44	LVCGERGFFYT	VCGERGFFYT	CGERGFFYT	GERGFFYT
45	VCGERGFFYTP	CGERGFFYTP	GERGFFYTP	ERGFFYTP
46	CGERGFFYTPK	GERGFFYTPK	ERGFFYTPK	RGFFYTPK
47	GERGFFYTPKT	ERGFFYTPKT	RGFFYTPKT	GFFYTPKT
48	ERGFFYTPKTR	RGFFYTPKTR	GFFYTPKTR	FFYTPKTR
49	RGFFYTPKTRR	GFFYTPKTRR	FFYTPKTRR	FYTPKTRR
50	GFFYTPKTRRE	FFYTPKTRRE	FYTPKTRRE	YTPKTRRE
51	FFYTPKTRREA	FYTPKTRREA	YTPKTRREA	TPKTRREA
52	FYTPKTRREAE	YTPKTRREAE	TPKTRREAE	PKTRREAE
53	YTPKTRREAED	TPKTRREAED	PKTRREAED	KTRREAED
54	TPKTRREAEDL	PKTRREAEDL	KTRREAEDL	TRREAEDL
55	PKTRREAEDLQ	KTRREAEDLQ	TRREAEDLQ	RREAEDLQ
56	KTRREAEDLQV	TRREAEDLQV	RREAEDLQV	REAEDLQV
57	TRREAEDLQVG	RREAEDLQVG	REAEDLQVG	EAEDLQVG
58	RREAEDLQVGQ	REAEDLQVGQ	EAEDLQVGQ	AEDLQVGQ
59	REAEDLQVGQV	EAEDLQVGQV	AEDLQVGQV	EDLQVGQV
60	EAEDLQVGQVE	AEDLQVGQVE	EDLQVGQVE	DLQVGQVE
61	AEDLQVGQVEL	EDLQVGQVEL	DLQVGQVEL	LQVGQVEL
62	EDLQVGQVELG	DLQVGQVELG	LQVGQVELG	QVGQVELG
63	DLQVGQVELGG	LQVGQVELGG	QVGQVELGG	VGQVELGG
64	LQVGQVELGGG	QVGQVELGGG	VGQVELGGG	GQVELGGG
65	QVGQVELGGGP	VGQVELGGGP	GQVELGGGP	QVELGGGP
66	VGQVELGGGPG	GQVELGGGPG	QVELGGGPG	VELGGGPG
67	GQVELGGGPGA	QVELGGGPGA	VELGGGPGA	ELGGGPGA
68	QVELGGGPGAG	VELGGGPGAG	ELGGGPGAG	LGGGPGAG
69	VELGGGPGAGS	ELGGGPGAGS	LGGGPGAGS	GGGPGAGS
70	ELGGGPGAGSL	LGGGPGAGSL	GGGPGAGSL	GGPGAGSL
71	LGGGPGAGSLQ	GGGPGAGSLQ	GGPGAGSLQ	GPGAGSLQ
72	GGGPGAGSLQP	GGPGAGSLQP	GPGAGSLQP	PGAGSLQP
73	GGPGAGSLQPL	GPGAGSLQPL	PGAGSLQPL	GAGSLQPL
74	GPGAGSLQPLA	PGAGSLQPLA	GAGSLQPLA	AGSLQPLA
75	PGAGSLQPLAL	GAGSLQPLAL	AGSLQPLAL	GSLQPLAL
76	GAGSLQPLALE	AGSLQPLALE	GSLQPLALE	SLQPLALE
77	AGSLQPLALEG	GSLQPLALEG	SLQPLALEG	LQPLALEG
78	GSLQPLALEGS	SLQPLALEGS	LQPLALEGS	QPLALEGS
79	SLQPLALEGSL	LQPLALEGSL	QPLALEGSL	PLALEGSL
80	LQPLALEGSLQ	QPLALEGSLQ	PLALEGSLQ	LALEGSLQ
81	QPLALEGSLQK	PLALEGSLQK	LALEGSLQK	ALEGSLQK
82	PLALEGSLQKR	LALEGSLQKR	ALEGSLQKR	LEGSLQKR
83	LALEGSLQKRG	ALEGSLQKRG	LEGSLQKRG	EGSLQKRG
84	ALEGSLQKRGI	LEGSLQKRGI	EGSLQKRGI	GSLQKRGI
85	LEGSLQKRGIV	EGSLQKRGIV	GSLQKRGIV	SLQKRGIV
86	EGSLQKRGIVE	GSLQKRGIVE	SLQKRGIVE	LQKRGIVE
87	GSLQKRGIVEQ	SLQKRGIVEQ	LQKRGIVEQ	QKRGIVEQ
88	SLQKRGIVEQC	LQKRGIVEQC	QKRGIVEQC	KRGIVEQC
89	LQKRGIVEQCC	QKRGIVEQCC	KRGIVEQCC	RGIVEQCC

90	QKRGIVEQCCT	KRGIVEQCCT	RGIVEQCCT	GIVEQCCT
91	KRGIVEQCCTS	RGIVEQCCTS	GIVEQCCTS	IVEQCCTS
92	RGIVEQCCTSI	GIVEQCCTSI	IVEQCCTSI	VEQCCTSI
93	GIVEQCCTSIC	IVEQCCTSIC	VEQCCTSIC	EQCCTSIC
94	IVEQCCTSICS	VEQCCTSICS	EQCCTSICS	QCCTSICS
95	VEQCCTSICSL	EQCCTSICSL	QCCTSICSL	CCTSICSL
96	EQCCTSICSLY	QCCTSICSLY	CCTSICSLY	CTSICSLY
97	QCCTSICSLYQ	CCTSICSLYQ	CTSICSLYQ	TSICSLYQ
98	CCTSICSLYQL	CTSICSLYQL	TSICSLYQL	SICSLYQL
99	CTSICSLYQLE	TSICSLYQLE	SICSLYQLE	ICSLYQLE
100	TSICSLYQLEN	SICSLYQLEN	ICSLYQLEN	CSLYQLEN
101	SICSLYQLENY	ICSLYQLENY	CSLYQLENY	SLYQLENY
102	ICSLYQLENYC	CSLYQLENYC	SLYQLENYC	LYQLENYC
103	CSLYQLENYCN	SLYQLENYCN	LYQLENYCN	YQLENYCN

Supplementary Table 3: Insulin DRiP Truncated peptide pools

Pool No	11 Mer	10 Mer	9 Mer	8 Mer
1				MLYQHLLP
2			MLYQHLLPL	LYQHLLPL
3		MLYQHLLPLP	LYQHLLPLP	YQHLLPLP
4	MLYQHLLPLPA	LYQHLLPLPA	YQHLLPLPA	QHLLPLPA
5	LYQHLLPLPAG	YQHLLPLPAG	QHLLPLPAG	HLLPLPAG
6	YQHLLPLPAGE	QHLLPLPAGE	HLLPLPAGE	LLPLPAGE
7	QHLLPLPAGEL	HLLPLPAGEL	LLPLPAGEL	LPLPAGEL
8	HLLPLPAGELL	LLPLPAGELL	LPLPAGELL	PLPAGELL
9	LLPLPAGELLQ	LPLPAGELLQ	PLPAGELLQ	LPAGELLQ
10	LPLPAGELLQL	PLPAGELLQL	LPAGELLQL	PAGELLQL
11	PLPAGELLQLD	LPAGELLQLD	PAGELLQLD	AGELLQLD
12	LPAGELLQLDA	PAGELLQLDA	AGELLQLDA	GELLQLDA
13	PAGELLQLDAA	AGELLQLDAA	GELLQLDAA	ELLQLDAA
14	AGELLQLDAAC	GELLQLDAAC	ELLQLDAAC	LLQLDAAC
15	GELLQLDAACR	ELLQLDAACR	LLQLDAACR	LQLDAACR
16	ELLQLDAACRQ	LLQLDAACRQ	LQLDAACRQ	QLDAACRQ
17	LLQLDAACRQP	LQLDAACRQP	QLDAACRQP	LDAACRQP
18	LQLDAACRQPH	QLDAACRQPH	LDAACRQPH	DAACRQPH
19	QLDAACRQPHT	LDAACRQPHT	DAACRQPHT	AACRQPHT
20	LDAACRQPHTR	DAACRQPHTR	AACRQPHTR	ACRQPHTR
21	DAACRQPHTRR	AACRQPHTRR	ACRQPHTRR	CRQPHTRR
22	AACRQPHTRRL	ACRQPHTRRL	CRQPHTRRL	RQPHTRRL
23	ACRQPHTRRLL	CRQPHTRRLL	RQPHTRRLL	QPHTRRLL
24	CRQPHTRRLLH	RQPHTRRLLH	QPHTRRLLH	PHTRRLLH
25	RQPHTRRLLHR	QPHTRRLLHR	PHTRRLLHR	HTRRLLHR
26	QPHTRRLLHRE	PHTRRLLHRE	HTRRLLHRE	TRRLLHRE
27	PHTRRLLHRER	HTRRLLHRER	TRRLLHRER	RRLLHRER
28	HTRRLLHRERW	TRRLLHRERW	RRLLHRERW	RLLHRERW
29	TRRLLHRERWN	RRLLHRERWN	RLLHRERWN	LLHRERWN
30	RRLLHRERWNK	RLLHRERWNK	LLHRERWNK	LHRERWNK
31	RLLHRERWNKA	LLHRERWNKA	LHRERWNKA	HRERWNKA
32	LLHRERWNKAL	LHRERWNKAL	HRERWNKAL	RERWNKAL
33	LHRERWNKALE	HRERWNKALE	RERWNKALE	ERWNKALE
34	HRERWNKALEP	RERWNKALEP	ERWNKALEP	RWNKALEP
35	RERWNKALEPA	ERWNKALEPA	RWNKALEPA	WNKALEPA
36	ERWNKALEPAK	RWNKALEPAK	WNKALEPAK	NKALEPAK

Supplementary Table 4: A2-restricted epitopes known to be associated with T1D

Peptide Name	Sequence
GAD:536-545	RMMEYGTTMV
GAD:114-123	VMNILLQYVV
IA2:797–805	MVWESGCTV
IA2:172-180	SLSPLQAEL
IA2:482-490	SLAAGVKLL
IAPP:9-17	FLIVLSVAL
IGRP:215-223	FLFAVGFYL
IGRP:228-236	LNIDLLWSV
IGRP:265-273	VLFGLGFAI
ZnT8:153–161	VVTGVLVYL
ZnT8:186–194	VAANIVLTV

Supplementary Table 5: Glucagon Truncated peptide pools

Pool No	11 Mer	10 Mer	9 Mer	8 Mer
1	MKSIYFVAGLF	KSIYFVAGLF	SIYFVAGLF	IYFVAGLF
2	KSIYFVAGLFV	SIYFVAGLFV	IYFVAGLFV	YFVAGLFV
3	SIYFVAGLFVM	IYFVAGLFVM	YFVAGLFVM	FVAGLFVM
4	MKSIYFVAGLF	KSIYFVAGLF	SIYFVAGLF	IYFVAGLF
5	KSIYFVAGLFV	SIYFVAGLFV	IYFVAGLFV	YFVAGLFV
6	SIYFVAGLFVM	IYFVAGLFVM	YFVAGLFVM	FVAGLFVM
7	IYFVAGLFVML	YFVAGLFVML	FVAGLFVML	VAGLFVML
8	YFVAGLFVMLV	FVAGLFVMLV	VAGLFVMLV	AGLFVMLV
9	FVAGLFVMLVQ	VAGLFVMLVQ	AGLFVMLVQ	GLFVMLVQ
10	VAGLFVMLVQG	AGLFVMLVQG	GLFVMLVQG	LFVMLVQG
11	AGLFVMLVQGS	GLFVMLVQGS	LFVMLVQGS	FVMLVQGS
12	GLFVMLVQGSW	LFVMLVQGSW	FVMLVQGSW	VMLVQGSW
13	LFVMLVQGSWQ	FVMLVQGSWQ	VMLVQGSWQ	MLVQGSWQ
14	FVMLVQGSWQR	VMLVQGSWQR	MLVQGSWQR	LVQGSWQR
15	VMLVQGSWQRS	MLVQGSWQRS	LVQGSWQRS	VQGSWQRS
16	MLVQGSWQRSL	LVQGSWQRSL	VQGSWQRSL	QGSWQRSL
17	LVQGSWQRSLQ	VQGSWQRSLQ	QGSWQRSLQ	GSWQRSLQ
18	VQGSWQRSLQD	QGSWQRSLQD	GSWQRSLQD	SWQRSLQD
19	QGSWQRSLQDT	GSWQRSLQDT	SWQRSLQDT	WQRSLQDT
20	GSWQRSLQDTE	SWQRSLQDTE	WQRSLQDTE	QRSLQDTE
21	SWQRSLQDTEE	WQRSLQDTEE	QRSLQDTEE	RSLQDTEE
22	WQRSLQDTEEK	QRSLQDTEEK	RSLQDTEEK	SLQDTEEK
23	QRSLQDTEEKS	RSLQDTEEKS	SLQDTEEKS	LQDTEEKS
24	RSLQDTEEKSR	SLQDTEEKSR	LQDTEEKSR	QDTEEKSR
25	SLQDTEEKSRS	LQDTEEKSRS	QDTEEKSRS	DTEEKSRS
26	LQDTEEKSRSF	QDTEEKSRSF	DTEEKSRSF	TEEKSRSF
27	QDTEEKSRSFS	DTEEKSRSFS	TEEKSRSFS	EEKSRSFS
28	DTEEKSRSFSA	TEEKSRSFSA	EEKSRSFSA	EKSRSFSA
29	TEEKSRSFSAS	EEKSRSFSAS	EKSRSFSAS	KSRSFSAS
30	EEKSRSFSASQ	EKSRSFSASQ	KSRSFSASQ	SRSFSASQ
31	EKSRSFSASQA	KSRSFSASQA	SRSFSASQA	RSFSASQA
32	KSRSFSASQAD	SRSFSASQAD	RSFSASQAD	SFSASQAD
33	SRSFSASQADP	RSFSASQADP	SFSASQADP	FSASQADP
34	RSFSASQADPL	SFSASQADPL	FSASQADPL	SASQADPL
35	SFSASQADPLS	FSASQADPLS	SASQADPLS	ASQADPLS
36	FSASQADPLSD	SASQADPLSD	ASQADPLSD	SQADPLSD
37	SASQADPLSDP	ASQADPLSDP	SQADPLSDP	QADPLSDP
38	ASQADPLSDPD	SQADPLSDPD	QADPLSDPD	ADPLSDPD
39	SQADPLSDPDQ	QADPLSDPDQ	ADPLSDPDQ	DPLSDPDQ
40	QADPLSDPDQM	ADPLSDPDQM	DPLSDPDQM	PLSDPDQM
41	ADPLSDPDQMN	DPLSDPDQMN	PLSDPDQMN	LSDPDQMN
42	DPLSDPDQMNE	PLSDPDQMNE	LSDPDQMNE	SDPDQMNE
43	PLSDPDQMNED	LSDPDQMNED	SDPDQMNED	DPDQMNED

44	LSDPDQMNEDK	SDPDQMNEDK	DPDQMNEDK	PDQMNEDK
45	SDPDQMNEDKR	DPDQMNEDKR	PDQMNEDKR	DQMNEDKR
46	DPDQMNEDKRH	PDQMNEDKRH	DQMNEDKRH	QMNEDKRH
47	PDQMNEDKRHS	DQMNEDKRHS	QMNEDKRHS	MNEDKRHS
48	DQMNEDKRHSQ	QMNEDKRHSQ	MNEDKRHSQ	NEDKRHSQ
49	QMNEDKRHSQG	MNEDKRHSQG	NEDKRHSQG	EDKRHSQG
50	MNEDKRHSQGT	NEDKRHSQGT	EDKRHSQGT	DKRHSQGT
51	NEDKRHSQGTF	EDKRHSQGTF	DKRHSQGTF	KRHSQGTF
52	EDKRHSQGTFT	DKRHSQGTFT	KRHSQGTFT	RHSQGTFT
53	DKRHSQGTFTS	KRHSQGTFTS	RHSQGTFTS	HSQGTFTS
54	KRHSQGTFTSD	RHSQGTFTSD	HSQGTFTSD	SQGTFTSD
55	RHSQGTFTSDY	HSQGTFTSDY	SQGTFTSDY	QGTFTSDY
56	HSQGTFTSDYS	SQGTFTSDYS	QGTFTSDYS	GTFTSDYS
57	SQGTFTSDYSK	QGTFTSDYSK	GTFTSDYSK	TFTSDYSK
58	QGTFTSDYSKY	GTFTSDYSKY	TFTSDYSKY	FTSDYSKY
59	GTFTSDYSKYL	TFTSDYSKYL	FTSDYSKYL	TSDYSKYL
60	TFTSDYSKYLD	FTSDYSKYLD	TSDYSKYLD	SDYSKYLD
61	FTSDYSKYLDS	TSDYSKYLDS	SDYSKYLDS	DYSKYLDS
62	TSDYSKYLDSR	SDYSKYLDSR	DYSKYLDSR	YSKYLDSR
63	SDYSKYLDSRR	DYSKYLDSRR	YSKYLDSRR	SKYLDSRR
64	DYSKYLDSRRA	YSKYLDSRRA	SKYLDSRRA	KYLDSRRA
65	YSKYLDSRRAQ	SKYLDSRRAQ	KYLDSRRAQ	YLDSRRAQ
66	SKYLDSRRAQD	KYLDSRRAQD	YLDSRRAQD	LDSRRAQD
67	KYLDSRRAQDF	YLDSRRAQDF	LDSRRAQDF	DSRRAQDF
68	YLDSRRAQDFV	LDSRRAQDFV	DSRRAQDFV	SRRAQDFV
69	LDSRRAQDFVQ	DSRRAQDFVQ	SRRAQDFVQ	RRAQDFVQ
70	DSRRAQDFVQW	SRRAQDFVQW	RRAQDFVQW	RAQDFVQW
71	SRRAQDFVQWL	RRAQDFVQWL	RAQDFVQWL	AQDFVQWL
72	RRAQDFVQWLM	RAQDFVQWLM	AQDFVQWLM	QDFVQWLM
73	RAQDFVQWLMN	AQDFVQWLMN	QDFVQWLMN	DFVQWLMN
74	AQDFVQWLMNT	QDFVQWLMNT	DFVQWLMNT	FVQWLMNT
75	QDFVQWLMNTK	DFVQWLMNTK	FVQWLMNTK	VQWLMNTK
76	DFVQWLMNTKR	FVQWLMNTKR	VQWLMNTKR	QWLMNTKR
77	FVQWLMNTKRN	VQWLMNTKRN	QWLMNTKRN	WLMNTKRN
78	VQWLMNTKRNR	QWLMNTKRNR	WLMNTKRNR	LMNTKRNR
79	QWLMNTKRNRN	WLMNTKRNRN	LMNTKRNRN	MNTKRNRN
80	WLMNTKRNRNN	LMNTKRNRNN	MNTKRNRNN	NTKRNRNN
81	LMNTKRNRNNI	MNTKRNRNNI	NTKRNRNNI	TKRNRNNI
82	MNTKRNRNNIA	NTKRNRNNIA	TKRNRNNIA	KRNRNNIA
83	NTKRNRNNIAK	TKRNRNNIAK	KRNRNNIAK	RNRNNIAK
84	TKRNRNNIAKR	KRNRNNIAKR	RNRNNIAKR	NRNNIAKR
85	KRNRNNIAKRH	RNRNNIAKRH	NRNNIAKRH	RNNIAKRH
86	RNRNNIAKRHD	NRNNIAKRHD	RNNIAKRHD	NNIAKRHD
87	NRNNIAKRHDE	RNNIAKRHDE	NNIAKRHDE	NIAKRHDE
88	RNNIAKRHDEF	NNIAKRHDEF	NIAKRHDEF	IAKRHDEF
89	NNIAKRHDEFE	NIAKRHDEFE	IAKRHDEFE	AKRHDEFE

90	NIAKRHDEFER	IAKRHDEFER	AKRHDEFER	KRHDEFER
91	IAKRHDEFERH	AKRHDEFERH	KRHDEFERH	RHDEFERH
92	AKRHDEFERHA	KRHDEFERHA	RHDEFERHA	HDEFERHA
93	KRHDEFERHAE	RHDEFERHAE	HDEFERHAE	DEFERHAE
94	RHDEFERHAEG	HDEFERHAEG	DEFERHAEG	EFERHAEG
95	HDEFERHAEGT	DEFERHAEGT	EFERHAEGT	FERHAEGT
96	DEFERHAEGTF	EFERHAEGTF	FERHAEGTF	ERHAEGTF
97	EFERHAEGTFT	FERHAEGTFT	ERHAEGTFT	RHAEGTFT
98	FERHAEGTFTS	ERHAEGTFTS	RHAEGTFTS	HAEGTFTS
99	ERHAEGTFTSD	RHAEGTFTSD	HAEGTFTSD	AEGTFTSD
100	RHAEGTFTSDV	HAEGTFTSDV	AEGTFTSDV	EGTFTSDV
101	HAEGTFTSDVS	AEGTFTSDVS	EGTFTSDVS	GTFTSDVS
102	AEGTFTSDVSS	EGTFTSDVSS	GTFTSDVSS	TFTSDVSS
103	EGTFTSDVSSY	GTFTSDVSSY	TFTSDVSSY	FTSDVSSY
104	GTFTSDVSSYL	TFTSDVSSYL	FTSDVSSYL	TSDVSSYL
105	TFTSDVSSYLE	FTSDVSSYLE	TSDVSSYLE	SDVSSYLE
106	FTSDVSSYLEG	TSDVSSYLEG	SDVSSYLEG	DVSSYLEG
107	TSDVSSYLEGQ	SDVSSYLEGQ	DVSSYLEGQ	VSSYLEGQ
108	SDVSSYLEGQA	DVSSYLEGQA	VSSYLEGQA	SSYLEGQA
109	DVSSYLEGQAA	VSSYLEGQAA	SSYLEGQAA	SYLEGQAA
110	VSSYLEGQAAK	SSYLEGQAAK	SYLEGQAAK	YLEGQAAK
111	SSYLEGQAAKE	SYLEGQAAKE	YLEGQAAKE	LEGQAAKE
112	SYLEGQAAKEF	YLEGQAAKEF	LEGQAAKEF	EGQAAKEF
113	YLEGQAAKEFI	LEGQAAKEFI	EGQAAKEFI	GQAAKEFI
114	LEGQAAKEFIA	EGQAAKEFIA	GQAAKEFIA	QAAKEFIA
115	EGQAAKEFIAW	GQAAKEFIAW	QAAKEFIAW	AAKEFIAW
116	GQAAKEFIAWL	QAAKEFIAWL	AAKEFIAWL	AKEFIAWL
117	QAAKEFIAWLV	AAKEFIAWLV	AKEFIAWLV	KEFIAWLV
118	AAKEFIAWLVK	AKEFIAWLVK	KEFIAWLVK	EFIAWLVK
119	AKEFIAWLVKG	KEFIAWLVKG	EFIAWLVKG	FIAWLVKG
120	KEFIAWLVKGR	EFIAWLVKGR	FIAWLVKGR	IAWLVKGR
121	EFIAWLVKGRG	FIAWLVKGRG	IAWLVKGRG	AWLVKGRG
122	FIAWLVKGRGR	IAWLVKGRGR	AWLVKGRGR	WLVKGRGR
123	IAWLVKGRGRR	AWLVKGRGRR	WLVKGRGRR	LVKGRGRR
124	AWLVKGRGRRD	WLVKGRGRRD	LVKGRGRRD	VKGRGRRD
125	WLVKGRGRRDF	LVKGRGRRDF	VKGRGRRDF	KGRGRRDF
126	LVKGRGRRDFP	VKGRGRRDFP	KGRGRRDFP	GRGRRDFP
127	VKGRGRRDFPE	KGRGRRDFPE	GRGRRDFPE	RGRRDFPE
128	KGRGRRDFPEE	GRGRRDFPEE	RGRRDFPEE	GRRDFPEE
129	GRGRRDFPEEV	RGRRDFPEEV	GRRDFPEEV	RRDFPEEV
130	RGRRDFPEEVA	GRRDFPEEVA	RRDFPEEVA	RDFPEEVA
131	GRRDFPEEVAI	RRDFPEEVAI	RDFPEEVAI	DFPEEVAI
132	RRDFPEEVAIV	RDFPEEVAIV	DFPEEVAIV	FPEEVAIV
133	RDFPEEVAIVE	DFPEEVAIVE	FPEEVAIVE	PEEVAIVE
134	DFPEEVAIVEE	FPEEVAIVEE	PEEVAIVEE	EEVAIVEE
135	FPEEVAIVEEL	PEEVAIVEEL	EEVAIVEEL	EVAIVEEL

136	PEEVAIVEELG	EEVAIVEELG	EVAIVEELG	VAIVEELG
137	EEVAIVEELGR	EVAIVEELGR	VAIVEELGR	AIVEELGR
138	EVAIVEELGRR	VAIVEELGRR	AIVEELGRR	IVEELGRR
139	VAIVEELGRRH	AIVEELGRRH	IVEELGRRH	VEELGRRH
140	AIVEELGRRHA	IVEELGRRHA	VEELGRRHA	EELGRRHA
141	IVEELGRRHAD	VEELGRRHAD	EELGRRHAD	ELGRRHAD
142	VEELGRRHADG	EELGRRHADG	ELGRRHADG	LGRRHADG
143	EELGRRHADGS	ELGRRHADGS	LGRRHADGS	GRRHADGS
144	ELGRRHADGSF	LGRRHADGSF	GRRHADGSF	RRHADGSF
145	LGRRHADGSFS	GRRHADGSFS	RRHADGSFS	RHADGSFS
146	GRRHADGSFSD	RRHADGSFSD	RHADGSFSD	HADGSFSD
147	RRHADGSFSDE	RHADGSFSDE	HADGSFSDE	ADGSFSDE
148	RHADGSFSDEM	HADGSFSDEM	ADGSFSDEM	DGSFSDEM
149	HADGSFSDEMN	ADGSFSDEMN	DGSFSDEMN	GSFSDEMN
150	ADGSFSDEMNT	DGSFSDEMNT	GSFSDEMNT	SFSDEMNT
151	DGSFSDEMNTI	GSFSDEMNTI	SFSDEMNTI	FSDEMNTI
152	GSFSDEMNTIL	SFSDEMNTIL	FSDEMNTIL	SDEMNTIL
153	SFSDEMNTILD	FSDEMNTILD	SDEMNTILD	DEMNTILD
154	FSDEMNTILDN	SDEMNTILDN	DEMNTILDN	EMNTILDN
155	SDEMNTILDNL	DEMNTILDNL	EMNTILDNL	MNTILDNL
156	DEMNTILDNLA	EMNTILDNLA	MNTILDNLA	NTILDNLA
157	EMNTILDNLAA	MNTILDNLAA	NTILDNLAA	TILDNLAA
158	MNTILDNLAAR	NTILDNLAAR	TILDNLAAR	ILDNLAAR
159	NTILDNLAARD	TILDNLAARD	ILDNLAARD	LDNLAARD
160	TILDNLAARDF	ILDNLAARDF	LDNLAARDF	DNLAARDF
161	ILDNLAARDFI	LDNLAARDFI	DNLAARDFI	NLAARDFI
162	LDNLAARDFIN	DNLAARDFIN	NLAARDFIN	LAARDFIN
163	DNLAARDFINW	NLAARDFINW	LAARDFINW	AARDFINW
164	NLAARDFINWL	LAARDFINWL	AARDFINWL	ARDFINWL
165	LAARDFINWLI	AARDFINWLI	ARDFINWLI	RDFINWLI
166	AARDFINWLIQ	ARDFINWLIQ	RDFINWLIQ	DFINWLIQ
167	ARDFINWLIQT	RDFINWLIQT	DFINWLIQT	FINWLIQT
168	RDFINWLIQTK	DFINWLIQTK	FINWLIQTK	INWLIQTK
169	DFINWLIQTKI	FINWLIQTKI	INWLIQTKI	NWLIQTKI
170	FINWLIQTKIT	INWLIQTKIT	NWLIQTKIT	WLIQTKIT
171	INWLIQTKITD	NWLIQTKITD	WLIQTKITD	LIQTKITD
172	NWLIQTKITDR	WLIQTKITDR	LIQTKITDR	IQTKITDR
173	WLIQTKITDRK	LIQTKITDRK	IQTKITDRK	QTKITDRK

Supplementary Table 6a: HLA alleles and peptides analyzed with TCRs having positive screening response

TCR ID	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	Antigen								
1.C8	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	PPI:2-12	PPI:3-12	PPI:4-12	PPI:5-12	PPI:1-10	PPI:2-10	PPI:3-10	
1.F3	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	PPI:2-12	PPI:3-12	PPI:4-12	PPI:5-12				
19.A1	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:1-10	PPI:2-10	PPI:3-10					
20.E5	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:1-10	PPI:2-10	PPI:3-10					
20.F1	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:1-10	PPI:2-10	PPI:3-10					
22.A10	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:1-10	PPI:2-10	PPI:3-10					
23.F9	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:1-10	PPI:2-10	PPI:3-10					
23.H5	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:1-11	PPI:2-11	PPI:3-11	PPI:4-11	PPI:3-13	PPI:4-13	PPI:5-13	PPI:6-13
96.F5	A*02:01	A*32:01	B*08:01	B*18:01	C*05:01	C*07:01	No Ag	PPI:1-11	PPI:2-11	PPI:3-11	PPI:4-11	PPI:1-9	PPI:1-10	PPI:2-10	PPI:3-10
1.B10-1	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	PPI:13-23	PPI:14-23	PPI:15-23	PPI:16-23				
1.F1	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	PPI:14-24	PPI:15-24	PPI:16-24	PPI:17-24	PPI:15-25	PPI:16-25	PPI:17-25	PPI:18-25
23.C12	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:14-24	PPI:15-24	PPI:16-24	PPI:17-24	PPI:15-25	PPI:16-25	PPI:17-25	PPI:18-25
93.D1	A*02:01	A*32:01	B*08:01	B*18:01	C*05:01	C*07:01	No Ag	PPI:14-24	PPI:15-24	PPI:16-24	PPI:17-24	PPI:15-25	PPI:16-25	PPI:17-25	PPI:18-25
10.C6-1	A*0101	A*2501	B*0801	B*1801	C*0701	C*1203	No Ag	PPI:21-31	PPI:22-31	PPI:23-31	PPI:24-31	PPI:22-32	PPI:23-32	PPI:24-32	PPI:25-32
28.D3	A*02:01	A*29:02	B*18:01	B*44:03	C*07:01	C*16:01	No Ag	PPI:31-41	PPI:32-41	PPI:33-41	PPI:34-41				
28.E6	A*02:01	A*29:02	B*18:01	B*44:03	C*07:01	C*16:01	No Ag	PPI:44-54	PPI:45-54	PPI:46-54	PPI:47-54				
20.G1	A*02:01	A*68:01	B*40:01	-	C*03:04	-	No Ag	PPI:67-77	PPI:68-77	PPI:69-77	PPI:70-77	PPI:69-79	PPI:70-79	PPI:71-79	PPI:72-79
1.E9-1	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	PPI:89-99	PPI:90-99	PPI:91-99	PPI:92-99	PPI:90-100	PPI:91-100	PPI:92-100	PPI:93-100
28.E11	A*02:01	A*29:02	B*18:01	B*44:03	C*07:01	C*16:01	No Ag	PPI:90-100	PPI:91-100	PPI:92-100	PPI:93-100				
54.F1	A*01:01	A*23:01	B*07:02	B*08:01	C*07:01	C*07:02	No Ag	PPI:93-103	PPI:94-103	PPI:95-103	PPI:96-103				
84.D9	A*02:05	A*23:01	B*35:01	B*41:02	C*04:01	C*17:01	No Ag	PPI:90-100	PPI:91-100	PPI:92-100	PPI:93-100	PPI:92-102	PPI:93-102	PPI:94-102	PPI:95-102
86.C1	A*02:05	A*23:01	B*35:01	B*41:02	C*04:01	C*17:01	No Ag	PPI:90-100	PPI:91-100	PPI:92-100	PPI:93-100	PPI:92-102	PPI:93-102	PPI:94-102	PPI:95-102
86.G3-2	A*02:05	A*23:01	B*35:01	B*41:02	C*04:01	C*17:01	No Ag	PPI:92-102	PPI:93-102	PPI:94-102	PPI:95-102				
96.B4	A*02:01	A*32:01	B*08:01	B*18:01	C*05:01	C*07:01	No Ag	PPI:89-99	PPI:90-99	PPI:91-99	PPI:92-99	PPI:90-100	PPI:91-100	PPI:92-100	PPI:93-100
1.B2	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	DRiP:5-15	DRiP:6-15	DRiP:7-15	DRiP:8-15				
1.C1	A*02:01	A*26:01	B*35:01	B*50:01	C*04:01	C*06:02	No Ag	DRiP:1-9	DRiP:2-9						
96.A9	A*02:01	A*32:01	B*08:01	B*18:01	C*05:01	C*07:01	No Ag	DRiP:1-9	DRiP:2-9						
130.D11	A*02:01	A*29:02	-	-	-	-	No Ag	PPI:30-40	PPI:31-40	PPI:32-40	PPI:33-40	PPI:40-50	PPI:41-50	PPI:42-50	PPI:43-50
131.D5	A*02:01	A*29:02	-	-	-	-	No Ag	PPI:40-50	PPI:41-50	PPI:42-50	PPI:43-50	PPI:43-53	PPI:44-53	PPI:45-53	PPI:46-53
110.D4	A*01:01	A*02:01	-	-	-	-	No Ag	PPI:93-103	PPI:94-103	PPI:95-103	PPI:96-103				
111.D11-2	A*01:01	A*02:01	-	-	-	-	No Ag	PPI:93-103	PPI:94-103	PPI:95-103	PPI:96-103				

Supplementary Table 6b: Preproinsulin and insulin DRiP peptide sequences

Peptide Name	Sequence		Peptide Name	Sequence
PPI:1-9	MALWMRLLP		PPI:34-41	HLVEALYL
PPI:1-10	MALWMRLLPL		PPI:40-50	YLVCGERGFFY
PPI:2-10	ALWMRLLPL		PPI:41-50	LVCGERGFFY
PPI:3-10	LWMRLLPL		PPI:42-50	VCGERGFFY
PPI:1-11	MALWMRLLPLL		PPI:43-50	CGERGFFY
PPI:2-11	ALWMRLLPLL		PPI:43-53	CGERGFFYTPK
PPI:3-11	LWMRLLPLL		PPI:44-53	GERGFFYTPK
PPI:4-11	WMRLLPLL		PPI:45-53	ERGFFYTPK
PPI:2-12	ALWMRLLPLLA		PPI:46-53	RGFFYTPK
PPI:3-12	LWMRLLPLLA		PPI:44-54	GERGFFYTPKT
PPI:4-12	WMRLLPLLA		PPI:45-54	ERGFFYTPKT
PPI:5-12	MRLLPLLA		PPI:46-54	RGFFYTPKT
PPI:3-13	LWMRLLPLLAL		PPI:47-54	GFFYTPKT
PPI:4-13	WMRLLPLLAL		PPI:67-77	ELGGGPGAGSL
PPI:5-13	MRLLPLLAL		PPI:68-77	LGGGPGAGSL
PPI:6-13	RLLPLLAL		PPI:69-77	GGGPGAGSL
PPI:13-23	LLALWGPDPAA		PPI:70-77	GGPGAGSL
PPI:14-23	LALWGPDPAA		PPI:69-79	GGGPGAGSLQP
PPI:15-23	ALWGPDPAA		PPI:70-79	GGPGAGSLQP
PPI:16-23	LWGPDPAA		PPI:71-79	GPGAGSLQP
PPI:14-24	LALWGPDPAAA		PPI:72-79	PGAGSLQP
PPI:15-24	ALWGPDPAAA		PPI:89-99	RGIVEQCCTSI
PPI:16-24	LWGPDPAAA		PPI:90-99	GIVEQCCTSI
PPI:17-24	WGPDPAAA		PPI:91-99	IVEQCCTSI
PPI:15-25	ALWGPDPAAAF		PPI:92-99	VEQCCTSI
PPI:16-25	LWGPDPAAAF		PPI:90-100	GIVEQCCTSIC
PPI:17-25	WGPDPAAAF		PPI:91-100	IVEQCCTSIC
PPI:18-25	GPDPAAAF		PPI:92-100	VEQCCTSIC
PPI:21-31	PAAAFVNQHLC		PPI:93-100	EQCCTSIC
PPI:22-31	AAAFVNQHLC		PPI:92-102	VEQCCTSICSL
PPI:23-31	AAFVNQHLC		PPI:93-102	EQCCTSICSL
PPI:24-31	AFVNQHLC		PPI:94-102	QCCTSICSL
PPI:22-32	AAAFVNQHLCG		PPI:95-102	CCTSICSL
PPI:23-32	AAFVNQHLCG		PPI:93-103	EQCCTSICSLY
PPI:24-32	AFVNQHLCG		PPI:94-103	QCCTSICSLY
PPI:25-32	FVNQHLCG		PPI:95-103	CCTSICSLY
PPI:30-40	LCGSHLVEALY		PPI:96-103	CTSICSLY
PPI:31-40	CGSHLVEALY		DRiP:1-9	MLYQHLLPL
PPI:32-40	GSHLVEALY		DRiP:2-9	LYQHLLPL
PPI:33-40	SHLVEALY		DRiP:5-15	HLLPLPAGELL
PPI:31-41	CGSHLVEALYL		DRiP:6-15	LLPLPAGELL
PPI:32-41	GSHLVEALYL		DRiP:7-15	LPLPAGELL
PPI:33-41	SHLVEALYL		DRiP:8-15	PLPAGELL

Supplementary Methods and Materials

T-cell isolation and TCR sequencing

Isolation of T-cells from islet tissues were performed using a protocol as described previously (1). Briefly, islet tissues were cultured in RPMI supplemented with Penicillin-Streptomycin, human AB+ serum, interleukin-2, interleukin-15, interleukin-7 for one-four days except those from T1D-1, which were cultured for approximately 10 days. The islet tissues were dispersed to single cells by treating with Liberase DL, followed by staining with anti-CD3, anti-CD4, and anti-CD8 antibodies along with 4'6-diamidino-2-phenylindole. CD3ε+CD4+ and CD3ε+CD8+ cells were identified and sorted into an each well of 96-well plates using the MoFlo Astrios EQ flow cytometric sorter. Single cells were then lysed for reverse-transcriptase reaction using mixture of random hexamers and primers specific for the constant regions of T-cell-receptor alpha and beta chains. TCR alpha and beta chain genes were amplified by the multiplex polymerase chain reaction (PCR) using primers targeting individual variable region genes and the constant region-specific primer. Individual PCR products were further amplified using primers conjugated with the Illumina adaptor oligonucleotides and index sequences for high-throughput sequencing on Illumina MiSeq. Sequences were demultiplexed and analyzed by the IMGT-HighV-QUEST algorithm to identify variable gene, joining gene, and junction sequences for individual cells. Primer sequence information was published previously (1).

Generation of TCR and HLA transductants

Transductants expressing TCR or HLA molecules were generated using a recently published protocol (2, 3). Briefly, TCR alpha and beta gene fragments, in which human variable regions are followed by the murine constant regions, were connected by the porcine teschovirus-1 2A (P2A) peptide and inserted into a murine stem cell virus-based retroviral vector, and introduced into 5KC T-hybridoma reporter cells using a standard retroviral expression protocol. The 5KC reporter cells were engineered with a nuclear factor activated T-cells (NFAT)-driven fluorescent reporter, ZsGreen-1, along with the human CD8 gene and fluorescent protein genes as identifiers, and thus express ZsGreen-1 upon activation, and cells expressing individual TCRs can be distinguished by fluorescent protein identifiers. To generate HLA transductants, individual HLA class I genes connected to the human beta-2 microglobulin with the P2A peptide in between were engineered into a human immunodeficiency virus 1 (HIV-1)-based lentiviral vector with a spleen focus-forming virus (SFFV) promoter, and transduced into human myeloma-derived K562 cells using a standard spinfection protocol.

Screening for reactivity to preproinsulin, insulin DRiP, glucagon, and other islet antigen peptides

We used a multiplex T-cell stimulation assay system to test reactions to peptides and truncated peptide pools using a recently published protocol (2, 3). In this system, up to eight TCR transductants expressing different combinations of fluorescent protein identifiers were mixed and cultured in the presence or absence of antigens in each well of 96-well plates, and demultiplexed to assess ZsGreen-1 reporter protein expressions by each TCR transductant. For screening of reactivity to preproinsulin, insulin DRiP, and glucagon, peptide pools, each of which contains 8- to 11-mers of crude peptides ending at a same position of preproinsulin, insulin DRiP, and glucagon, were custom-ordered from Mimotopes (Mulgrave, Australia) and added at approximately 200 μg/ml. Amino acid sequences contained in each peptide pool are shown in **Supplementary Tables 2, 3, and 5**. To assess responses to known islet antigen-derived epitopes (**Supplementary Table 4**), each peptide was added at

100 µM. We used autologous EBV-transformed B cells, generated using spleen cells of individual donors that were co-cultured with EBV-containing supernatants produced from B95-9 cells, as antigen-presenting cells to stimulate TCR transductants derived from organ donors having T1D (100,000 cells per well). For screening of TCRs derived from non-diabetic organ donors, we used mixtures of transductants expressing individual HLA-A, B, and C alleles (50,000 cells per well). Mixtures of TCR transductants (20,000 cells per well for each TCR transductant) were cultured with or without peptides in the presence of antigen-presenting cells for overnight, followed by evaluating ZsGreen-1 expression on a Cytoflex flow cytometer (Beckman Coulter). Cultures with 5 µg/ml of anti-mouse CD3ε antibody (Clone 125-2C11, BD) were included in the assay as positive control.

Confirming positive responses and determining HLA restriction

Peptides contained in truncated peptide pools that stimulated TCR transductants were synthesized by Genemed Synthesis (San Antonio, Texas). Individual TCR transductant cells (20,000 cells per well) were cultured with or without each peptide (100 µM) in the presence of HLA transductant cells transduced with each HLA class I allele of the donor (50,000 cells per well) for overnight, followed by evaluating ZsGreen-1 expression on a Cytoflex flow cytometer (Beckman Coulter). Peptides and HLA class I alleles tested for each TCR clonotype are shown in **Supplementary Tables 6a and 6b**. Cultures with 5 µg/ml of anti-mouse CD3ε antibody (Clone 125-2C11, BD) were included in the assay as positive control.

Determining potency of responses to optimal epitopes

TCR transductant cells (20,000 cells per well) were cultured with peptides at concentrations ranging from 10 pM to 100 µM in the presence of HLA transductant cells expressing a cognate HLA class I molecule (50,000 cells per well) for overnight, followed by evaluating ZsGreen-1 expression on a Cytoflex flow cytometer (Beckman Coulter). Peptide sequences are shown in **Supplementary Table 6b**. EC_{50} values were calculated using GraphPad Prism 8, the nonlinear regression log (agonist) vs. response (three parameters) equation model. For TCR clonotypes, 28.D3, 96.F5, 28.E6, and 131.D5, EC_{50} values were not determined due to weak responses, and therefore the nonlinear regression log (agonist) vs. normalized response equation model was used to determine EC_{50} values.

Cytokine ELISPOT assays

Assays were conducted as previously described using the human IFN-γ ELISPOT kits (UCyTech Biosciences) (4). Briefly, cryopreserved PBMCs were thawed and 10^6 cells cultured in 250 µl of serum free AIM-V® Medium (Invitrogen) in a 48-well polystyrene culture plate with 20 µg/ml of preproinsulin truncated peptide pools dissolved in dimethyl sulfoxide (DMSO) at 37°C with 5% CO_2. Cells were harvested after 48 hours, washed, then resuspended in 300 µl medium and transferred as three 100 µl aliquots to 96-well clear polystyrene culture plates coated with an IFN-γ capture monoclonal antibody. Seventeen hours later, the cells were removed by decanting and the wells washed. Spots were then developed by sequential incubations with the biotinylated 2nd site anti-IFN-γ, gold-labeled goat anti-biotin, and a precipitating silver substrate. Spots were enumerated with a Bioreader 4000 Pro X (BIOSYS GmbH). For each assay, negative control wells had no antigen but received the same final concentration of DMSO as the PPI wells, and the Pediarix vaccine (GlaxoSmithKline), a childhood vaccine containing five different immunogens, was used as a positive control.

The viral mix contained known CD8 T-cell epitopes from Epstein-Barr virus (CLGGLLTMV), cytomegalovirus (NLVPMVATV), and measles (SMYRVFEVGV) at a final concentration of 20 μg/ml.

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2. S. E. Mann *et al.*, Multiplex T Cell Stimulation Assay Utilizing a T Cell Activation Reporter-Based Detection System. *Frontiers in immunology* **11**, 633 (2020).
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