

SUPPLEMENTARY DATA

Supplementary Figure 1. Proteome analysis of pulsed DC by mass spectrometry. DC pulsed with GAD65 and IA-2 were lysed, digested with trypsin and proteome analysis was conducted using mass spectrometry. Peptides were identified derived from both islet-autoantigens. A: GAD65; B: IA-2.

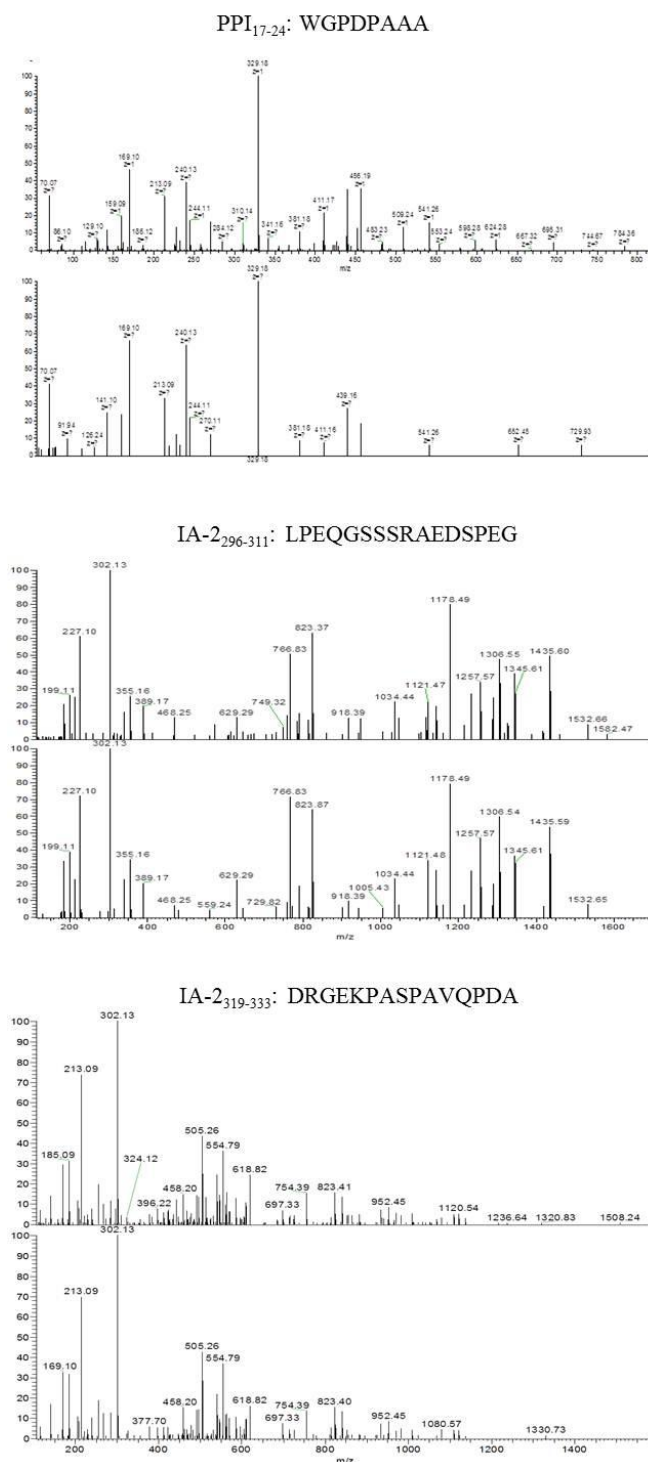
INS_HUMAN (99%), 11,981.1 Da Insulin OS=Homo sapiens GN=INS PE=1 SV=1 1 unique peptides, 2 unique spectra, 10 total spectra, 35/110 amino acids (32% coverage)				
MALWMRL LPL	LALLALWGPD	PAAAFVNQHL	CGSHLVEALY	LVCGERGFFY
TPKTRREAED	LQVGQVELGG	GPGAGSLQPL	ALEGS LQKR	IVEQCCTSI C
SLYQLENYCN				

sp Q16849-2 PTPRN_HUMAN (100%), 102,817.5 Da Isoform 2 of Receptor-type tyrosine-protein phosphatase-like N OS=Homo sapiens GN=PTPRN 17 unique peptides, 20 unique spectra, 24 total spectra, 250/950 amino acids (26% coverage)				
MRRPRRPGGL	GGSGGLRLLL	CLLLLSSRP	GCSAVSAHGC	LFDRRLCSHL
EVCIQDGLFG	QCQVGVGQAR	PLLQVTSPL	QRLQGVLRQL	MSQGLSWHDD
LTQYVISQEM	ERIPRLRPPE	PRPRDRSGLA	PKRPGPAGEL	LLQDIP TGS A
PAAQHRLPQP	PVGKGAGAS	SSLSPLQAE	LPPLLEHLLL	PPQPPHPSLS
YEPALLQPYL	FHQFGSRDGS	RVSEGS PMV	SVGPLPKAEA	PALFSRTASK
GIFGDHPGHS	YGDLPGPSA	QLFQDSGLLY	LAQELPAPSR	ARVPRLPEQG
SSSRAEDSPE	GYEKEGLGDR	GEKPASPAVQ	PDAALQRLAA	VLAGYGVLEL
QLTPEQLSTL	LTLLQLLPKG	AGRNPGGVN	VGADIKKTME	GPVEGRDTAE
LPARTSPMPG	HPTASPTSS	VQQVSPSPSS	EPPKAARPPV	TPV LLEKKSP
LGQSQPTVAG	QPSARPAEEE	YGYIVTDQNV	VGPALTFRIR	HNEQNLSLAD
VTQQAAGLVKS	ELEAQTGLQI	LQTGVGQREE	AAAVLPQTAH	STSPMRSVLL
TLVALAGVAG	LLVALAVALC	VRQHARQQDK	ERLAALGPEG	AHGDTTFEYQ
DLCRQH MATK	SLFNRAEGPP	EPSRVSSVSS	QFSDAAQASP	SSHSS T P SWC
EPAQANMDI	STGHMILAYM	EDHLNRNDR	AKEWQALCAY	QAEPN TCATA
QGEENIKKNR	HPDFLPYDHA	RIKLKVESSP	SRSDYINASP	IEHDP RMPA
YIATQGPLSH	TIADFWQMVW	ESGCTVI VML	TPLVEDGVKQ	CDRYWPD EGA
SLYHVEVNL	VSEHIWCEDF	LVRSFY LKNV	QTQETRTL TQ	FHFLSWPAEG
TPASTRPLLD	FRRKVNKC YR	GRSCPIIVHC	SDGAGRTGT Y	ILIDMVLNRM
AKGVKEIDIA	ATLEHVRDQR	PGLVRSK DQF	EFALTAV AEE	VNAILKALPQ

DCE2_HUMAN (100%), 65,412.9 Da Glutamate decarboxylase 2 OS=Homo sapiens GN=GAD2 PE=1 SV=1 8 unique peptides, 9 unique spectra, 28 total spectra, 139/585 amino acids (24% coverage)				
MASPGSGFWS	FGSE DGSGDS	ENPGTARAWC	QVAQKFTGGI	GNKLCALLYG
DAEKPAESGG	SQPPRAAARK	AACACDQKPC	SCSKVDVNYA	FLHATD L LPA
CDGERPTLAF	LQDVMNILLQ	YVVKSFDRST	KVIDFHYPNE	LLQEYNWELA
DQPQNLEEIL	MHCQTTLKYA	IKTGHPRYFN	QLSTGLDMVG	LAADWLTSTA
NTNMFTYEIA	PVFVLLLEYVT	LKKMREIIGW	PGGSGDGIFS	PGGAI SNMYA
MMIARFKMFP	EVKEKGMAAL	PRLIAFTSEH	SHFSLKKGAA	ALGIGTDSVI
LIKCDERGKM	IPSDLERRIL	EAKQKGFVPF	LVSATAGTTV	YGAFDPL LAV
ADICKKYKI W	MHVDAAWGGG	LLMSRKHKWK	LSGVERANSV	TWNPHKMMGV
PLQCSALLVR	EEGLMQNCNQ	MHASYL FQQD	KHYDLSYDTG	DKALQCGRHV
DVFKLWLMWR	AKGTTGF EAH	VDKCLELA EY	LYNIIKNREG	YEMVFDGK PQ
HTNVCFWYIP	PSLRTL EDNE	ERMSRLSKVA	PVIKARMMEY	GTTMVS YQPL
GDKVNFFRMV	ISNPAATHQD	IDFLIEEIER	LGQDL	

SUPPLEMENTARY DATA

Supplementary Figure 2. Spectrum validation of identified HLA-DQ eluted peptides. Peptides eluted from HLA-DQ2/8 and HLA-DQ8 were synthesized and analyzed by mass spectrometry for validation of the identified eluted peptide-spectra. Shown are the peptide-spectra of PPI₁₇₋₂₄ (A), IA-2₂₉₆₋₃₁₁ (B) and IA-2₃₁₉₋₃₃₃ (C) eluted from HLA-DQ2/8 (upper spectra) and the corresponding spectra of the synthesized peptides (lower spectra in gray). The peptide-spectra of eluted and synthesized peptides fully overlap.



SUPPLEMENTARY DATA

Supplementary Table 1. Cytokine responses of T1D patients against IA-2 naturally epitopes eluted from high-risk HLA-DQ. Cytokine responses (IFN- γ and IL-10) against two immunogenic IA-2 naturally epitopes (IA-2₂₉₃₋₃₁₁ and IA-2₃₁₈₋₃₃₃) were measured using ELISPOT. Data are expressed as the number (#) of spots in triplicate and are compared to the number of spots in triplicate in the presence of diluent alone.

Patient	Cytokine	IA-2 293-311	IA-2 318-333	background	SI IA-2 293-311	SI IA-2 318-333	HLA-DQ
Diab 12	IFN-y	2	6	2	1	3.0	DQ4/8
	IL-10	88	58	43	2.0	1.3	
Diab 13	IFN-y	7	5	5	1.4	1	DQ5/8
	IL-10	331	372	184	1.8	2.0	
Diab 14	IFN-y	13	3	1	13	3.0	DQ2/8
	IL-10	118	93	51	2.2	1.8	
Diab 15	IFN-y	295	238	15	19.5	15.6	DQ2/2
	IL-10	40	51	213	0.2	0.2	
Diab 16	IFN-y	5	1	3	1.7	0.3	DQ2/8
	IL-10	1152	542	167	6.9	3.2	
Diab 17	IFN-y	592	11	1	20	11	DQ6.4/8
	IL-10	245	489	135	1.8	3.6	
Diab 18	IFN-y	1	0	2	0.5	1	DQ6.2/8
	IL-10	30	63	116	0.3	0.5	
Diab 19	IFN-y	6	3	1	6	3	DQ4/8
	IL-10	135	178	154	0.9	1.2	
Diab 20	IFN-y	5	8	3	1.3	2.7	DQ4/8
	IL-10	283	72	70	4.0	1	
Diab 21	IFN-y	0	0	1	0	1	DQ2/5
	IL-10	215	76	107	2.0	0.7	
Diab 22	IFN-y	39	39	2	19	19	DQ6.3/8
	IL-10	194	185	112	1.7	1.7	
Diab 23	IFN-y	3	3	5	0.6	1	DQ5/8
	IL-10	433	390	437	1.0	0.9	
Diab 24	IFN-y	31	45	3	10	15	DQ2/5
	IL-10	107	133	66	1.6	2.1	
Diab 25	IFN-y	0	0	0	0	1	DQ2/2
	IL-10	338	179	105	3.2	1.7	
Diab 28	IFN-y	3	1	1	3	1	DQ4/8
	IL-10	30	24	7	4.3	3.4	
Diab 29	IFN-y	4	4	0	4	4	DQ2/8
	IL-10	21	17	2	11	9	
Diab 30	IFN-y	1	1	0	1	1	DQ2.2/7
	IL-10	13	2	4	3.3	0.5	
Diab 34	IFN-y	0	4	1	0	4.0	DQ2/6.4
	IL-10	59	74	43	1.4	1.7	
Diab 35	IFN-y	1	0	0	1	1	DQ2/8
	IL-10	81	151	74	1.1	2.1	
Diab 37	IFN-y	126	86	6	21	14	DQ5/5
	IL-10	36	42	87	0.4	0.5	
Diab 38	IFN-y	11	3	3	3.7	1	DQ2/8

SUPPLEMENTARY DATA

	IL-10	148	107	182	0.8	0.6	
Diab 39	IFN- γ	29	7	2	15	3.5	DQ2/8
	IL-10	273	182	130	2.1	1.4	
Diab 40	IFN- γ	1	139	7	0.2	19.8	DQ6.4/9
	IL-10	493	478	398	1.2	1.2	
Diab 41	IFN- γ	3	0	1	3	1	DQ5/8
	IL-10	10	2	9	1.1	0.2	
Diab 43	IFN- γ	0	0	1	0	1	DQ8/8
	IL-10	1	0	27	0	0	
Diab 44	IFN- γ	3	3	5	0.6	1	DQ2/8
	IL-10	128	173	67	1.5	2.6	
Diab 45	IFN- γ	1	3	0	1	3	DQ2/2
	IL-10	16	25	22	0.7	1.1	
Diab 46	IFN- γ	0	3	0	0	3	DQ2/8
	IL-10	22	24	24	0.8	1	
Diab 47	IFN- γ	2	5	33	0.1	0.2	DQ8/8
	IL-10	14	13	27	0.5	0.5	
Diab 48	IFN- γ	3	2	6	0.5	0.3	DQ2/6.4
	IL-10	8	5	13	0.6	0.4	

SUPPLEMENTARY DATA

Supplementary Table 2. Cytokine responses of T1D patients against PPI naturally epitope eluted from high-risk HLA-DQ. Cytokine responses (IFN- γ and IL-10) against PPI₁₇₋₂₄ was measured using ELISPOT. Data are expressed as the number (#) of spots in triplicate and are compared to the number of spots in triplicate in the presence of diluent alone.

Patient	Cytokine	PPI 17-24	background	SI PPI 17-24	HLA-DQ
Diab 59	IFN-y	0	1	1	DQ2/8
	IL-10	316	574	0.6	
Diab 60	IFN-y	0	1	1	DQ2/8
	IL-10	1009	1524	1	
Diab 61	IFN-y	5	6	0.8	DQ2.2/8
	IL-10	364	579	0.6	
Diab 63	IFN-y	3	1	3	DQ2/8
	IL-10	724	236	3	
Diab 64	IFN-y	2	1	2	DQ5/8
	IL-10	887	1050	0.9	
Diab 65	IFN-y	3	1	3	DQ2/2
	IL-10	2201	1820	1.2	
Diab 66	IFN-y	0	1	1	DQ5/8
	IL-10	272	326	0.8	
Diab 68	IFN-y	1	1	1	DQ2/8
	IL-10	708	359	1.9	
Diab 69	IFN-y	3	1	3	DQ2/9
	IL-10	648	562	1.2	
Diab 70	IFN-y	25	25	1	DQ2/8
	IL-10	539	1185	0.5	
Diab 71	IFN-y	0	1	1	DQ2/8
	IL-10	932	302	3.1	
Diab 72	IFN-y	0	1	1	DQ4/8
	IL-10	166	631	0.3	
Diab 73	IFN-y	0	1	1	DQ2/2.2
	IL-10	1971	1857	1.1	
Diab 74	IFN-y	1	1	1	DQ2/8
	IL-10	1425	1684	0.9	
Diab 76	IFN-y	4	1	4	DQ2/8
	IL-10	31	80	0.4	
Diab 77	IFN-y	5	1	5	DQ2/8
	IL-10	914	1578	0.6	
Diab 79	IFN-y	0	1	1	DQ6/8
	IL-10	473	483	1	
Diab 80	IFN-y	0	1	1	DQ2/5
	IL-10	18	6	3	
Diab 82	IFN-y	6	1	1	DQ8/8
	IL-10	820	1	820	
Diab 83	IFN-y	1	1	1	DQ5/8
	IL-10	480	161	3	
Diab 84	IFN-y	0	1	1	DQ7/7

SUPPLEMENTARY DATA

	IL-10	241	389	0.6	
Diab 89	IFN- γ	1	1	1	DQ2/8
	IL-10	3	3	1	
Diab 90	IFN- γ	1	1	1	DQ2/4
	IL-10	0	1	0	
Diab 93	IFN- γ	1	1	1	DQ2/8
	IL-10	1512	1261	1.2	
Diab 94	IFN- γ	0	1	0	DQ5/5
	IL-10	1339	1359	1	
Diab 95	IFN- γ	0	1	1	DQ2/5
	IL-10	2	785	1	
Diab 96	IFN- γ	0	1	1	DQ2/8
	IL-10	59	20	3	
Diab 97	IFN- γ	1	1	1	DQ4/7
	IL-10	19	48	0.6	
Diab 98	IFN- γ	0	1	0	DQ2/7
	IL-10	3	1	3	
Diab 99	IFN- γ	0	1	0	DQ2/8
	IL-10	5	1	5	

SUPPLEMENTARY DATA

Supplementary Table 3. Cytokine responses of healthy controls against PPI and IA-2 naturally epitope eluted from high-risk HLA-DQ. Cytokine responses (IFN- γ and IL-10) against PPI₁₇₋₂₄, IA-2₂₉₃₋₃₁₁ and IA-2₃₁₈₋₃₃₃ were measured using ELISPOT. Data are expressed as the number (#) of spots in triplicate and are compared to the number of spots in triplicate in the presence of diluent alone.

Control	Cytokine	PPI 17-24	IA-2 293-311	IA-2 318-333	background	PPI 17-24	SI IA-2 293-311	SI IA-2 318-333	HLA-DQ
HC01	IFN- γ	4	4	0	0	4.0	4.0	0.0	DQ5/6.4
	IL-10	2	3	5	6	0.3	0.5	0.8	
HC03	IFN- γ	5	1	5	1	5.0	1.0	5.0	DQ5/6.2
	IL-10	10	17	3	11	0.9	1.5	0.3	
HC04	IFN- γ	1	3	1	1	1.0	3.0	1.0	DQ5/8
	IL-10	1	1	3	1	1.0	1.0	3.0	
HC05	IFN- γ	0	0	1	4	0.0	0.0	0.3	DQ5/5
	IL-10	2	2	3	4	0.5	0.5	0.8	
HC06	IFN- γ	7	2	7	18	0.4	0.1	0.4	DQ2/6.4
	IL-10	1	1	1	0	1.0	1.0	1.0	
HC07	IFN- γ	1	1	0	0	1.0	1.0	0.0	DQ2/5
	IL-10	4	4	3	3	1.3	1.3	1.0	
HC08	IFN- γ	0	0	0	0	0.0	0.0	0.0	DQ2/8
	IL-10	4	2	2	3	1.3	0.7	0.7	
HC09	IFN- γ	0	0	0	0	0.0	0.0	0.0	DQ3/5
	IL-10	0	1	1	0	0.0	1.0	1.0	
HC10	IFN- γ	6	14	14	16	0.4	0.9	0.9	DQ3/5
	IL-10	4	3	12	11	0.4	0.3	1.1	
HC11	IFN- γ	7	1	2	3	2.3	0.3	0.7	DQ5/8
	IL-10	2	1	0	2	1.0	0.5	0.0	
HC12	IFN- γ	1	0	1	1	1.0	0.0	1.0	DQ3/5
	IL-10	1	4	0	0	1.0	4.0	0.0	
HC13	IFN- γ	1	0	0	0	1.0	0.0	0.0	DQ2/8
	IL-10	1	1	3	0	1.0	1.0	3.0	
HC14	IFN- γ	1	0	1	0	1.0	0.0	1.0	DQ8/9
	IL-10	2	3	3	5	0.4	0.6	0.6	
HC15	IFN- γ	3	4	2	0	3.0	4.0	2.0	DQ2/8
	IL-10	1	2	5	5	0.2	0.4	1.0	
HC16	IFN- γ	0	0	0	1	0.0	0.0	0.0	DQ2/8
	IL-10	6	6	5	2	3.0	3.0	2.5	
HC17	IFN- γ	0	0	0	0	0.0	0.0	0.0	DQ8/8
	IL-10	0	0	0	0	0.0	0.0	0.0	
HC18	IFN- γ	0	1	0	0	0.0	1.0	0.0	DQ7/7
	IL-10	0	4	2	1	0.0	4.0	2.0	
HC19	IFN- γ	0	2	1	2	0.0	1.0	0.5	DQ2/7
	IL-10	0	1	0	0	0.0	1.0	0.0	
HC20	IFN- γ	1	25	19	11	0.1	2.3	1.7	DQ2/8
	IL-10	1	10	3	3	0.3	3.3	1.0	
HC21	IFN- γ	5	7	11	15	0.3	0.5	0.7	DQ3/5
	IL-10	2	3	3	3	0.7	1.0	1.0	
HC22	IFN- γ	161	127	110	92	1.8	1.4	1.2	DQ2/2

SUPPLEMENTARY DATA

	IL-10	3	18	8	18	0.2	1.0	0.4	
HC23	IFN- γ	8	11	12	10	0.8	1.1	1.2	DQ5/8
	IL-10	0	3	2	5	0.0	0.6	0.4	
HC24	IFN- γ	5	6	12	5	1.0	1.2	2.4	DQ3/5
	IL-10	3	7	7	3	1.0	2.3	2.3	
HC25	IFN- γ	4	1	1	0	4.0	1.0	1.0	DQ2/6.2
	IL-10	7	10	6	10	0.7	1.0	0.6	
HC26	IFN- γ	3	8	10	4	0.8	2.0	2.5	DQ6.2/8
	IL-10	4	3	1	3	1.3	1.0	0.3	
HC27	IFN- γ	0	0	1	0	0.0	0.0	1.0	DQ2/7
	IL-10	2	4	4	1	2.0	4.0	4.0	
HC28	IFN- γ	0	1	1	3	0.0	0.3	0.3	DQ8/8
	IL-10	2	4	0	3	0.7	1.3	0.0	
HC29	IFN- γ	1	0	1	2	0.5	0.0	0.5	DQ6.3/8
	IL-10	0	0	0	0	0.0	0.0	0.0	
HC31	IFN- γ	3	0	4	0	3.0	0.0	4.0	DQ2/2
	IL-10	0	0	5	0	0.0	0.0	5.0	
HC32	IFN- γ	3	1	0	0	3.0	1.0	0.0	DQ2/6.3
	IL-10	0	1	0	1	0.0	1.0	0.0	
HC33	IFN- γ	1	3	1	0	1.0	3.0	1.0	DQ3/5
	IL-10	0	5	1	1	0.0	5.0	1.0	
HC34	IFN- γ	6	3	1	4	1.5	0.8	0.3	DQ6.3/8
	IL-10	2	10	4	8	0.3	1.3	0.5	
HC35	IFN- γ	2	5	9	7	0.3	0.7	1.3	DQ2/2
	IL-10	10	16	8	23	0.4	0.7	0.3	
HC36	IFN- γ	6	5	2	3	2.0	1.7	0.7	DQ2/5
	IL-10	15	30	19	10	1.5	3.0	1.9	
HC37	IFN- γ	8	14	11	11	0.7	1.3	1.0	DQ2/6.2
	IL-10	29	39	18	42	0.7	0.9	0.4	
HC38	IFN- γ	3	2	9	5	0.6	0.4	1.8	DQ2/6.4
	IL-10	1	1	1	0	1.0	1.0	1.0	