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Supplementary Materials for

Plasmid-Encoded Proinsulin Preserves C-Peptide While Specifically Reducing Proinsulin-Specific CD8$^+$ T Cells in Type 1 Diabetes

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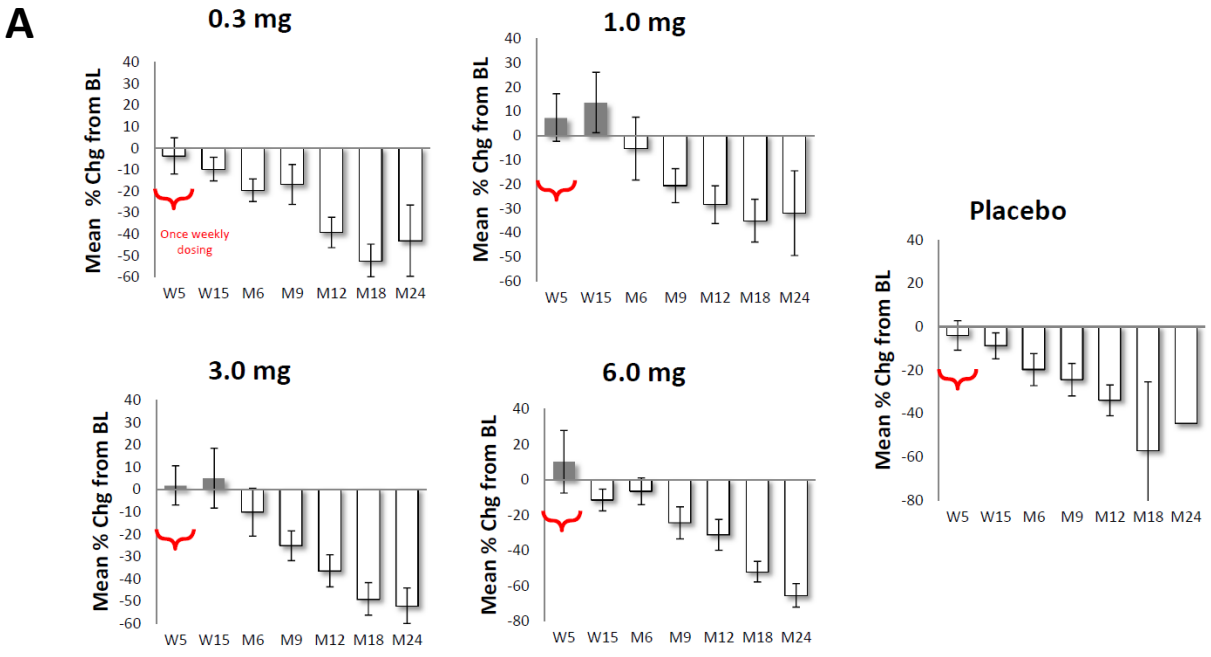
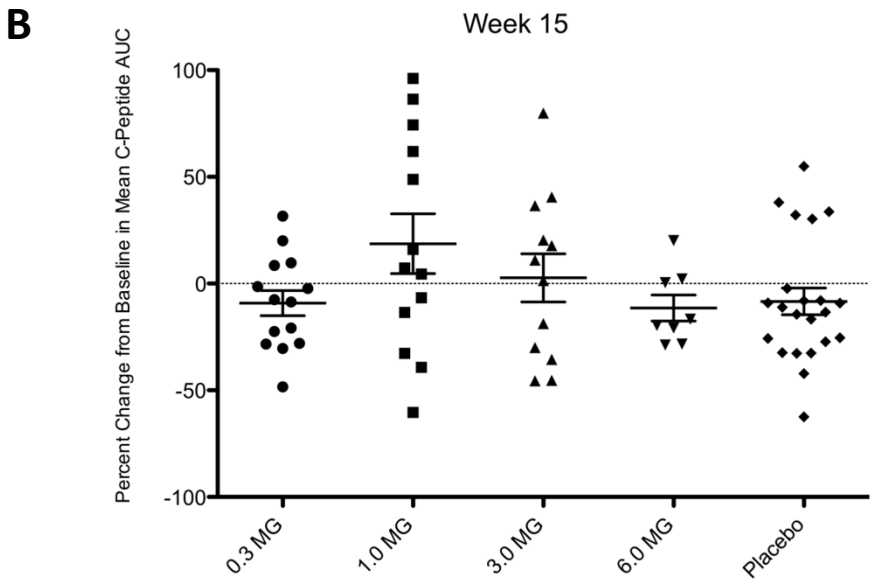
The PDF file includes:

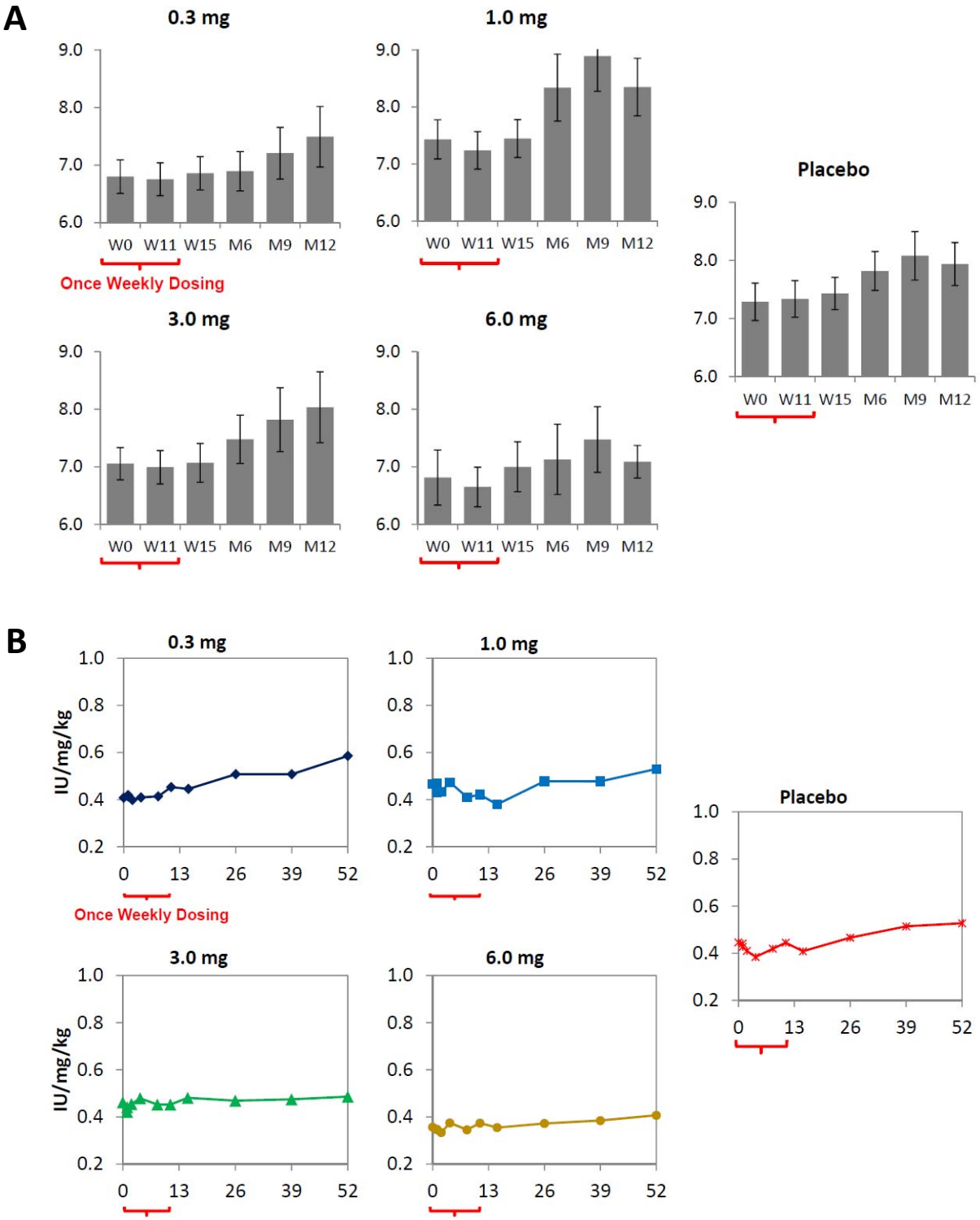
Fig. S1. (A) Mean percent change in C-peptide from baseline over 24 months. (B) Scatter plot to visualize the individual patient's mean change in C-peptide levels at all doses at 15 weeks.
Fig. S2. $HgbA_{1c}$ and mean total insulin usage at various doses of BHT-3021 versus BHT-placebo.
Fig. S3. Changes in antigen-specific T cells over time for vaccine- and viral-related epitopes.
Fig. S4. Frequencies of antigen-specific CD8$^+$ T cells in treated (squares) and placebo (circles) patients at week 0.
Fig. S5. Number of IL-10– and IFN-γ–positive ELISpots compared to baseline for four different antigen-specific T cell subgroups.
Table S1. Incidence of precipitous decline in C-peptide.
Table S2. Change in antibody status at week 15.
Table S3. TEAEs by treatment group (ITT population).
Table S4. Combinations of Qdot-labeled HLA-A2, HLA-A3, and HLA-B7 multimers.





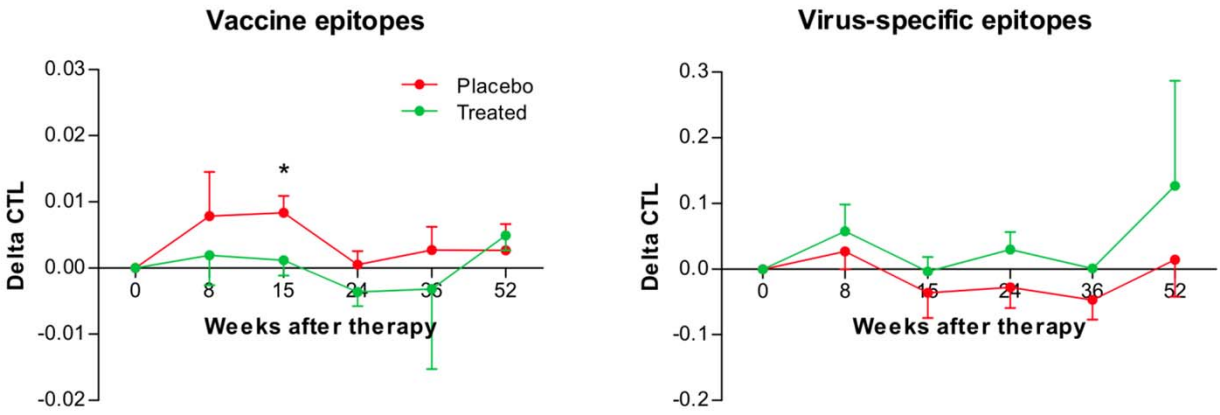
Supplementary Figure S1. (A) Mean Percent Change in C-Peptide from Baseline over 24 Months. C-peptide was measured as described in methods (*18-20*). Through 12 months N=14 for 0.3 mg dose; n=15 for 1.0 mg dose; n=13 for 3.0mg dose; n=8 for 6.0 mg dose; n=23 for placebo. For 0.3 mg N=14 at M18 and N=7 at M24; 1.0 mg N=13 at M18 and N=7 at Month 24; 3mg N=12 at M18, N=9 at M24; 6.0 mg N=7 at M18 and n=6 at M24; Placebo n=3 at M18 and N=1 at M24. The mean percentage change from baseline +/- CI is displayed.

(B) Scatter plot to visualize the individual patient's mean change in C-peptide levels at all doses at 15 weeks.

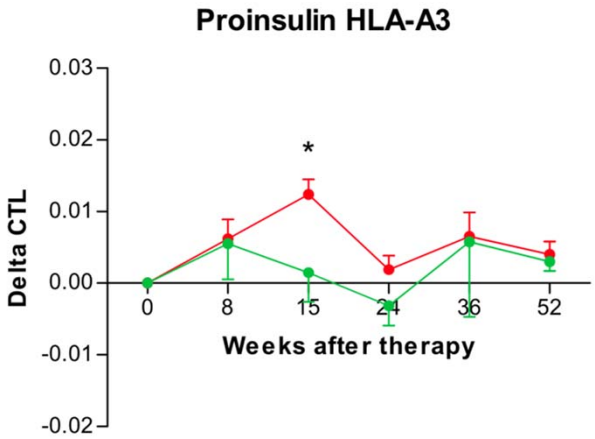


Supplementary Figure S2. $HgbA_{1c}$ and mean total insulin usage at various doses of BHT-3021 versus BHT-Placebo. (A) Hemoglobin A1C is shown on the y axis, and time in months (M) on the x axis. (B) Mean total insulin usage in IU/mg/kg is depicted on the y axis, and time in weeks on the x axis. The 104 week data are not statistically significant, and this is now mentioned in the text. Further N=3 at week 104, as mentioned in the figure legend for Supplementary fig. 2A.

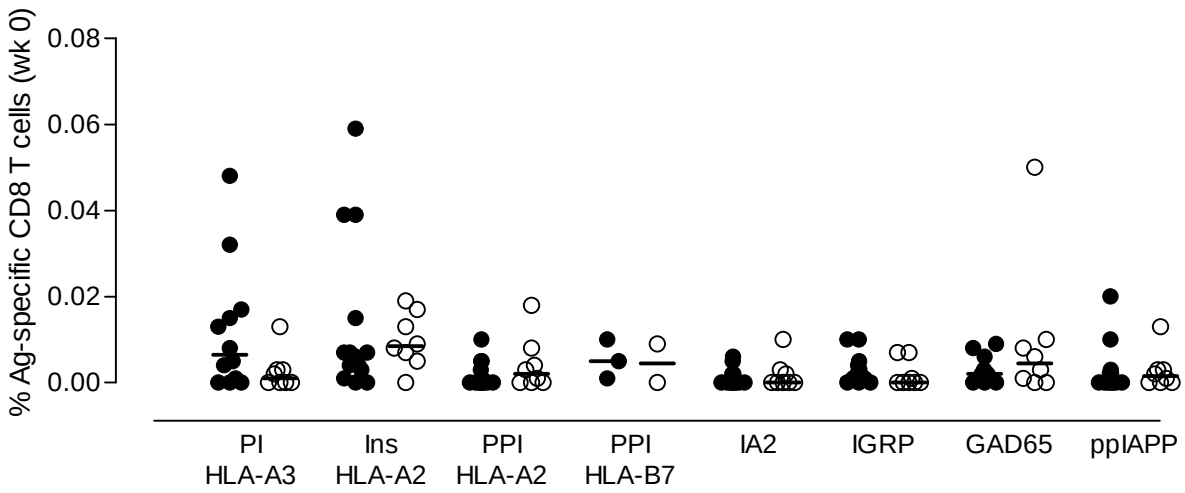
A



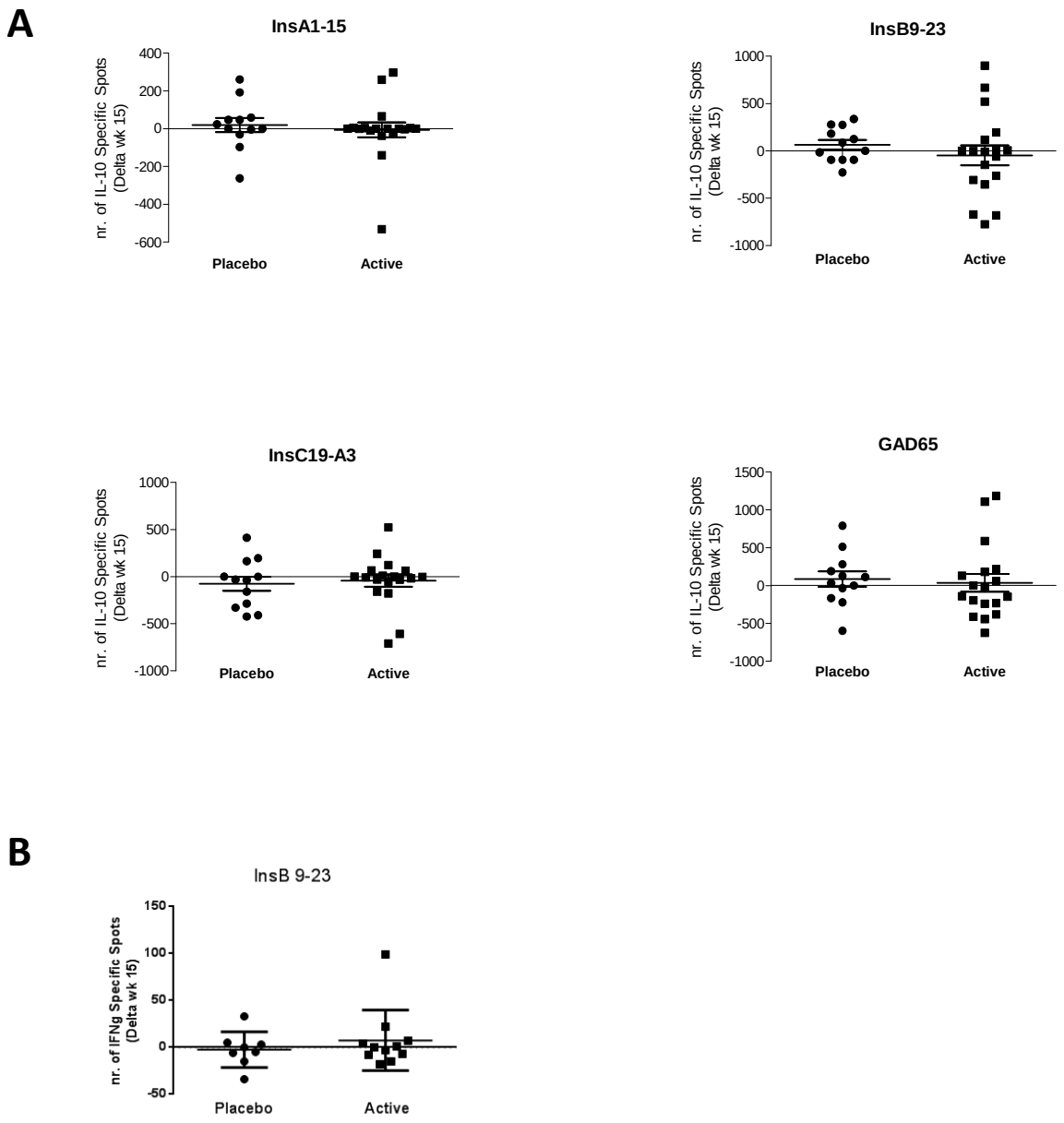
B



Supplementary Figure S3. Changes in antigen-specific T cells over time for vaccine- and viral-related epitopes.
Antigen specific CD8 T cells against vaccine related epitopes (Proinsulin and Insulin) and virus-specific epitopes (EBV, CMV and measles); or (B) Proinsulin alone were enumerated with peptide-HLA multimers at baseline and weeks 8, 15, 24, 36 and 52. Deltas were calculated by subtracting the baseline values from values at each time point.



Supplementary Figure S4. Frequencies of antigen-specific CD8$^+$ T cells in treated (squares) and placebo (circles) patients at week 0. Antigen-specific CD8 T cells were enumerated with Qdot-multimer technology using class I HLA multimers loaded with various antigens21-24. CTL frequencies are defined as percentage of antigen-specific CD8 T cells. PI: Proinsulin; Ins: Insulin; PPI: Preproinsulin.



Supplementary Figure S5. Number of IL-10- and interferon-gamma positive ELISpots compared to baseline for four different antigen-specific T cell subgroups. Change in the number of IL-10 (A) and interferon gamma (B) specific spots is measured on the y axis, comparing changes between week 0 and week 15, both for BHT-placebo and for BHT-3021 treated subjects.

Supplementary Table S1: Incidence of precipitous decline* in C-peptide.						
Timepoint	**0.3 mg (n=14)**	**1.0 mg (n=15)**	**3.0 mg (n=12)**	**6.0 mg (n=8)**	**Pooled Active (n=49)**	**Placebo (n=23)**
Month 6						
Decrease ≥75%, n (%)	0	1 (6.7%)	0	0	1 (2.0%)	0
Month 12						
Decrease ≥75%, n (%)	1 (7.1%)	1 (6.7%)	1 (8.3%)	0	3 (6.1%)	3 (13%)

* Decrease ≥75% in C peptide

Supplementary Table S2. Change in antibody status at week 15.		
Dose Level5	**GAD65[1]**	**IAA[2]**
(mg)	**NEG to POS**	**NEG to POS**
0.3	0/14	1/14 (Subject 42-005)
1	0/15	0/15
3	0/13	1/13 (Subject 42-003)
6	0/8	0/8
0 (placebo)	1/23 (42-004)	2/23 (Subjects 31-007 and 42-004)

[1]GAD65- measured by RIA using index value of >0.032 as positive cut-off

[2]IAA - measured by RIA using index value of >0.01 as positive cut-off

Supplementary Table S3. TEAEs by treatment group (ITT population).					
MedDRA Body System	**0.3 mg n=14 n (%)**	**1.0 mg n=18 n (%)**	**3.0 mg n=14 n (%)**	**6.0 mg n=8 n (%)**	**Placebo n=26 n (%)**
Subjects with Any AE	12 (85.7%)	18 (100%)	11 (78.6%)	7 (87.5%)	25 (96.2%)
Blood and Lymphatic System Disorders	1 (7.1%)		3 (21.4%)		1 (3.8%)
Cardiac Disorders				1 (12.5%)	1 (3.8%)
Ear and Labyrinth Disorders			1 (7.1%)		
Eye Disorders	1 (7.1%)	2 (11.1%)	1 (7.1%)		2 (7.7%)
Gastrointestinal Disorders	2 (14.3%)	6 (33.3%)	5 (35.7%)	1 (12.5%)	6 (23.1%)
General Disorders and Administration Site Conditions	2 (14.3%)	1 (5.6%)	1 (7.1%)	3 (37.5%)	1 (3.8%)
Immune System Disorders	1 (7.1%)				4 (15.4%)
Infections And Infestations	10 (71.4%)	13 (72.2%)	9 (64.3%)	6 (75.0%)	13 (50.0%)
Injury, Poisoning and Procedural Complications	3 (21.4%)	3 (16.7%)	3 (21.4%)	2 (25.0%)	4 (15.4%)
Metabolism and Nutrition Disorders	3 (21.4%)	2 (11.1%)	3 (21.4%)	3 (37.5%)	12 (46.2 %)
Musculoskeletal and Connective Tissue Disorders	2 (14.3%)	1 (5.6%)	4 (28.6%)	1 (12.5%)	3 (11.5%)
Nervous System Disorders	4 (28.6%)	6 (33.3%)	5 (35.7%)	2 (25.0%)	8 (30.8%)
Psychiatric Disorders			2 (14.3%)		1 (3.8%)
Renal and Urinary Disorders	1 (7.1%)	1 (5.6%)			
Respiratory, Thoracic and Mediastinal Disorders	1 (7.1%)	3 (16.7%)	4 (28.6%)	1 (12.5%)	8 (30.8%)
Skin and Subcutaneous Tissue Disorders	3 (21.4%)	4 (22.2%)	3 (21.4%)	2 (25.0%)	8 (30.8%)
Surgical and Medical Procedures		1 (5.6%)			1 (3.8%)
Metabolism and Nutrition Disorders	3 (21.4%)	2 (11.1%)	3 (21.4%)	3 (37.5%)	12 (46.2 %)
Musculoskeletal and Connective Tissue Disorders	2 (14.3%)	1 (5.6%)	4 (28.6%)	1 (12.5%)	3 (11.5%)
Nervous System Disorders	4 (28.6%)	6 (33.3%)	5 (35.7%)	2 (25.0%)	8 (30.8%)
Psychiatric Disorders			2 (14.3%)		1 (3.8%)
Renal and Urinary Disorders	1 (7.1%)	1 (5.6%)			
Respiratory, Thoracic and Mediastinal Disorders	1 (7.1%)	3 (16.7%)	4 (28.6%)	1 (12.5%)	8 (30.8%)
Skin and Subcutaneous Tissue Disorders	3 (21.4%)	4 (22.2%)	3 (21.4%)	2 (25.0%)	8 (30.8%)
Surgical and Medical Procedures		1 (5.6%)			1 (3.8%)

Supplementary Table S4. Combinations of Qdot-labeled HLA-A2, HLA-A3, and HLA-B7 multimers.

Origin	Islet specific	Insulin Specific	Position/ protein	Sequence	HLA Restriction	Signal
CMV	no	NA	pp65	NLVPMVATV	HLA-A2	Qdot 585 + 655
EBV	no	NA	LMP2	CLGGLLTMV	HLA-A2	Qdot 585 + 655
Measles	no	NA	H250	SMYRVFEVGV	HLA-A2	Qdot 585 + 655
HLA-A2	no	NA	140–149	YAYDGKDYIA	HLA-A2	Qdot 585 + 605
Insulin	yes	yes	B 10–18	HLVEALYLV	HLA-A2	Qdot 605 + 655
PPI	yes	yes	15–24	ALWGPDPAAA	HLA-A2	Qdot 705 + 655
GAD65	yes	no	114–123	VMNiLLQYVV	HLA-A2	Qdot 800 + 655
IA-2	yes	no	797–805	MVWESGCTV	HLA-A2	Qdot 705 + 605
IGRP	yes	no	265–273	VLFGLGFAI	HLA-A2	Qdot 800 + 605
ppIAPP	yes	no	5–13	KLQVFLIVL	HLA-A2	Qdot 705 + 800
PPI_{76-84}	yes	yes	76-84	SLQPLALEG	HLA-A3	Qdot 585 + 800
PPI_{79-88}	yes	yes	79-88	PLALEGSLQK	HLA-A3	Qdot 585 + 705
PPI_{4-13}	yes	yes	4-13	WMRLLPLLAL	HLA-B7	Qdot 585 + Qdot 800 or Qdot 655 + 705*

CMV, cytomegalovirus; EBV, Epstein-Barr virus.

*: depending on the mix with either HLA-A3 or HLA-A2 epitopes combined.