

Supplementary Materials

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Supplementary Table 1. Comparison of clinical parameters between participants with positive and negative results of IFN- γ ELISpot assays

	Participants with AT1D (n=42)			Participants with KPD (n=33)		
	Positive result of ELISpot assay (n = 16)	Negative result of ELISpot assay (n = 26)	<i>P</i> -value	Positive result of ELISpot assay (n = 12)	Negative result of ELISpot assay (n = 21)	<i>P</i> -value
Demographic characteristics						
Age (years)	51 (16–61)	36.5 (16–72)	N.S. ^a	39 (23–55)	48 (18–70)	N.S. ^a
Sex (Male/Female)	9/7	13/13	N.S. ^b	11/1	21/0	N.S. ^c
Disease duration (years)	0 (0–3)	0 (0–3)	N.S. ^a	0 (0–2.8)	0 (0–3)	N.S. ^a
BMI (kg/m ²)	19.7 (14.9–27.3)	21.2 (16.0–29.1)	N.S. ^a	29.5 (18.3–38.1)	26.6 (22.8–36.5)	N.S. ^a
BMI < 25 / \geq 25	15/1	23/3	N.S. ^c	2/10	6/15	N.S. ^c
Laboratory findings at the time of sampling						
<i>Ad lib</i> plasma glucose level (mmol/L)	9.2 (5.3–70.3)	13.8 (2.7–59.2)	N.S. ^a	14.2 (6.1–31.7)	17.3 (6.1–52.0)	N.S. ^a
(mg/dL)	166.5 (96–1,266)	248 (48–1,067)		255 (110–571)	311 (110–936)	
HbA1c (mmol/mol)	104.4 (39.9–139.4)	106.6 (41.0–141.5)	N.S. ^a	112.0 (88.0–131.7)	114.2 (85.8–138.3)	N.S. ^a
(%)	11.7 (5.8–14.9)	11.9 (5.9–15.1)		12.4 (10.2–14.2)	12.6 (10.0–14.8)	
<i>Ad lib</i> serum C-peptide level (nmol/L)	0.18 (0.00–0.89)	0.30 (0.00–0.66)	N.S. ^a	0.47 (0.26–1.20)	0.78 (0.36–1.43)	< 0.05 ^a
(ng/mL)	0.54 (0.00–2.70)	0.89 (0.01–1.99)		1.42 (0.78–3.63)	2.35 (1.09–4.33)	
GADA (Positive/Negative)	12/4	24/2	N.S. ^c	0/12	0/21	N.S. ^c
HLA						
Presence of HLA DRB1*04:05 (Yes/No)	6/10	13/13	N.S. ^b	3/9	5/16	N.S. ^c

Presence of HLA DRB1*08:02 (Yes/No)	1/15	4/22	N.S. ^c	0/12	0/21	N.S. ^c
Presence of HLA DRB1*09:01 (Yes/No)	9/7	14/12	N.S. ^b	2/10	4/17	N.S. ^c
Presence of HLA DRB1*15:01 and/or DRB1*15:02 (Yes/No)	0/16	1/25	N.S. ^c	4/8	6/15	N.S. ^c
Presence of HLA A*02:01 (Yes/No)	6/10	3/23	N.S. ^c	2/10	1/20	N.S. ^c
Presence of HLA A*24:02 (Yes/No)	11/5	19/7	N.S. ^c	8/4	10/11	N.S. ^b
Presence of HLA A*02:01 and/or A*24:02 (Yes/No)	14/2	20/6	N.S. ^c	9/3	11/10	N.S. ^c

Data are shown as the median (minimum-maximum). AT1D, acute-onset type 1 diabetes; BMI, body mass index; ELISpot, enzyme-linked immunospot; GADA, anti-glutamic acid decarboxylase antibody; HbA1c, glycated hemoglobin; HLA, human leukocyte antigen; IFN- γ , interferon- γ ; KPD, ketosis-prone type 2 diabetes. N.S., not significant.

^aMann-Whitney *U*-test, ^bChi-square test, ^cFischer's exact test.

Supplementary Table 2. Spearman's rank correlation coefficient (r_s) and P -values between IFN- γ SFC numbers and clinical parameters.

	Participants with AT1D (n = 42)		Participants with KPD (n = 33)	
	r_s	P -value	r_s	P -value
Age	0.274	N.S.	-0.385	< 0.05
BMI	-0.162	N.S.	0.230	N.S.
Diabetes duration	0.054	N.S.	0.081	N.S.
<i>Ad lib</i> plasma glucose	-0.045	N.S.	-0.194	N.S.
HbA1c	0.065	N.S.	0.064	N.S.
<i>Ad lib</i> serum C-peptide	-0.168	N.S.	-0.399	< 0.05

AT1D, acute-onset type 1 diabetes; BMI, body mass index; HbA1c, glycated hemoglobin; IFN, interferon; KPD, ketosis-prone type 2 diabetes; N.S., not significant; r_s , Spearman's rank correlation coefficient; SFC, spot-forming cell.

Supplementary Table 3a. Frequencies of HLA class I and class II alleles associated with T1D in participants with AT1D

AT1D (n = 84)	Positive result of ELISpot assay (n = 32)		Negative result of ELISpot assay (n = 52)		Positive vs. Negative results of ELISpot assay	
	n	%	n	%	P-value	OR
HLA class I						
*02:01	6	18.8	3	5.8	N.S. ^a	3.77
*24:02	13	40.6	24	46.2	N.S. ^b	0.80
*02:01 and/or *24:02	19	59.4	27	51.9	N.S. ^b	1.35
HLA class II						
Susceptible						
*04:05	7	21.9	14	26.9	N.S. ^b	0.76
*08:02	1	3.1	4	7.7	N.S. ^a	0.39
*09:01	13	40.6	17	32.7	N.S. ^b	1.41
*04:05, *08:02 and/or *09:01	21	65.6	35	67.3	N.S. ^b	0.93
Protective						
*15:01	0	0	1	1.9	N.S. ^a	0.00
*15:02	0	0	0	0	N.S. ^a	–
*15:01 and/or *15:02	0	0	1	1.9	N.S. ^a	0.00

AT1D, acute-onset type 1 diabetes; ELISpot, enzyme-linked immunospot; HLA, human leukocyte antigen; OR, odds ratio; T1D, type 1 diabetes.

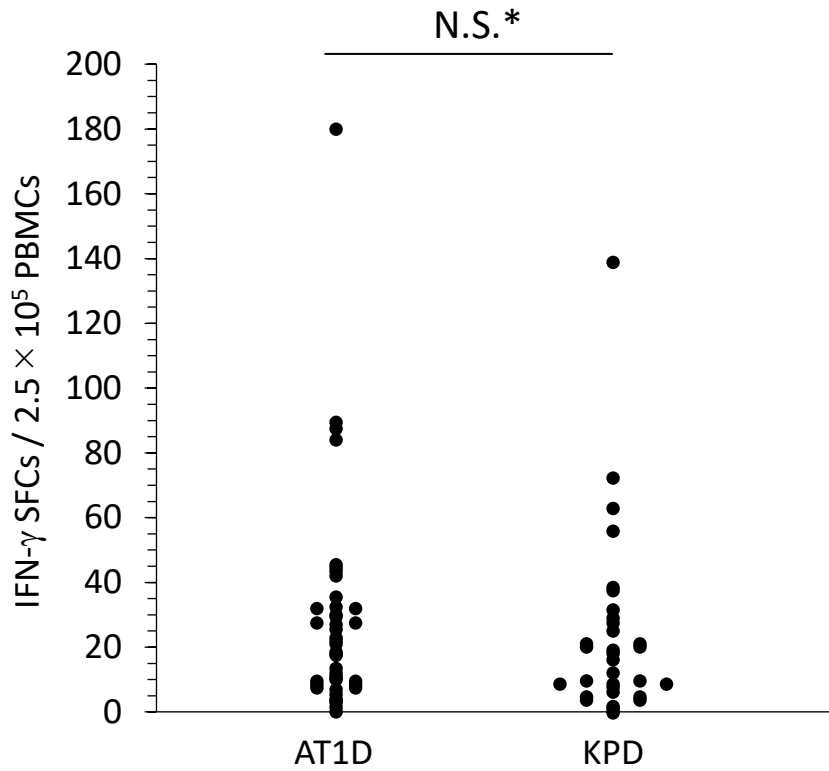
^aFischer's exact test, ^bChi-square test.

Supplementary Table 3b. Frequencies of HLA class I and class II alleles associated with T1D in participants with KPD

KPD (n = 66)	Positive result of ELISpot assay (n = 24)		Negative result of ELISpot assay (n = 42)		Positive vs. Negative results of ELISpot assay	
	n	%	n	%	<i>P</i> -value	OR
HLA class I						
*02:01	2	8.3	2	4.8	N.S. ^a	1.82
*24:02	12	50.0	13	31.0	N.S. ^b	2.23
*02:01 and/or *24:02	14	58.3	15	35.7	N.S. (<i>P</i> = 0.075) ^b	2.52
HLA class II						
Susceptible						
*04:05	3	12.5	6	14.3	N.S. ^a	0.95
*08:02	0	0	0	0.0	N.S. ^a	–
*09:01	2	8.3	4	9.5	N.S. ^a	0.86
*04:05, *08:02 and/or *09:01	6	25.0	10	23.8	N.S. ^b	1.07
Protective						
*15:01	0	0	2	4.8	N.S. ^a	0.00
*15:02	4	16.7	4	9.5	N.S. ^a	1.90
*15:01 and/or *15:02	4	16.7	6	14.3	N.S. ^a	1.20

ELISpot, enzyme-linked immunospot; HLA, human leukocyte antigen; KPD, ketosis-prone type 2 diabetes; OR, odds ratio; T1D, type 1 diabetes.

^aFischer's exact test, ^bChi-square test.

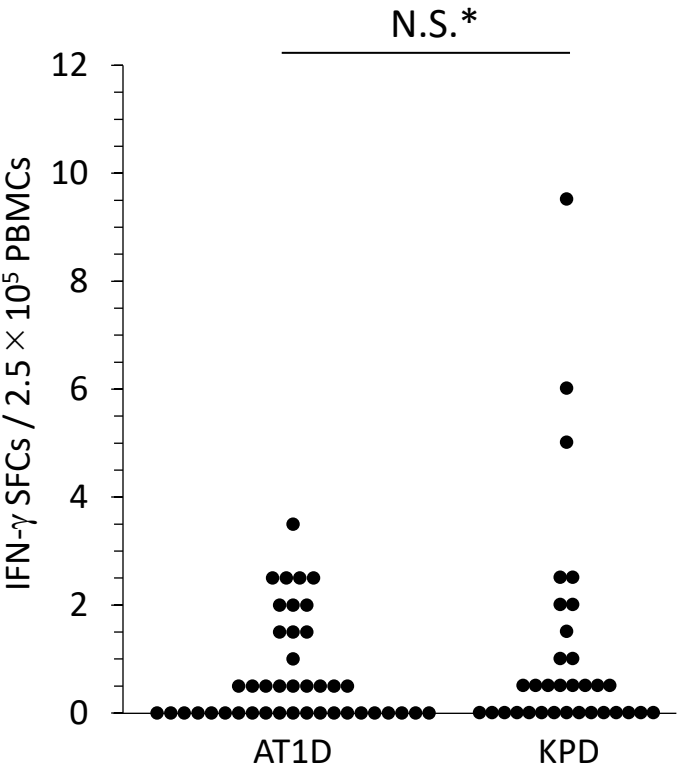


Supplementary Figure 1. ELISpot assay showing SFC numbers of PPD-specific IFN- γ -producing PBMCs in the two categories of diabetes.

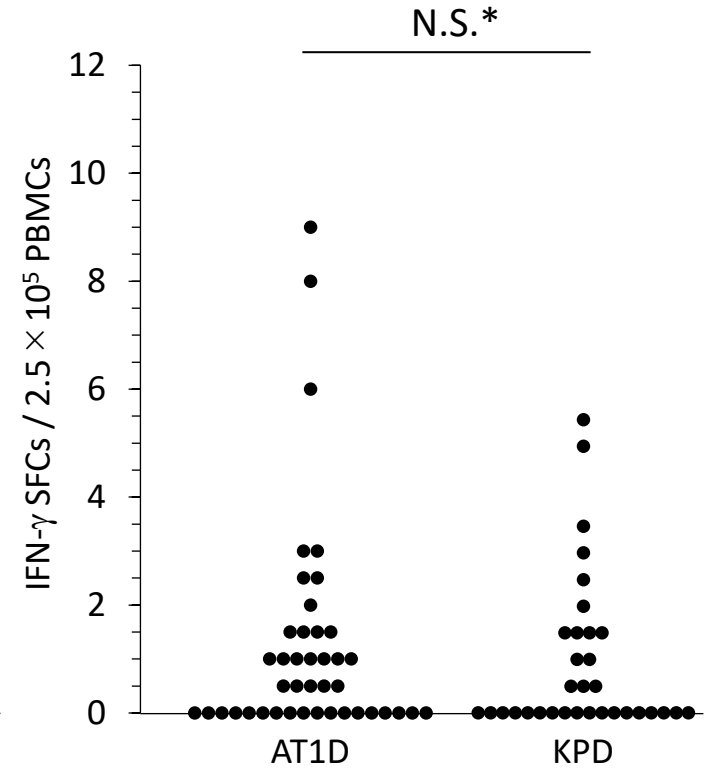
Each dot indicates the number of PPD-specific IFN- γ SFCs determined by ELISpot analysis. Participants with AT1D (n = 42); participants with KPD (n = 33). *Mann-Whitney *U*-test.

AT1D, acute-onset type 1 diabetes; ELISpot, enzyme-linked immunospot; IFN, interferon; KPD, ketosis-prone type 2 diabetes; N.S., not significant; PBMC, peripheral blood mononuclear cell; PPD, purified protein derivative; SFC, spot forming cell.

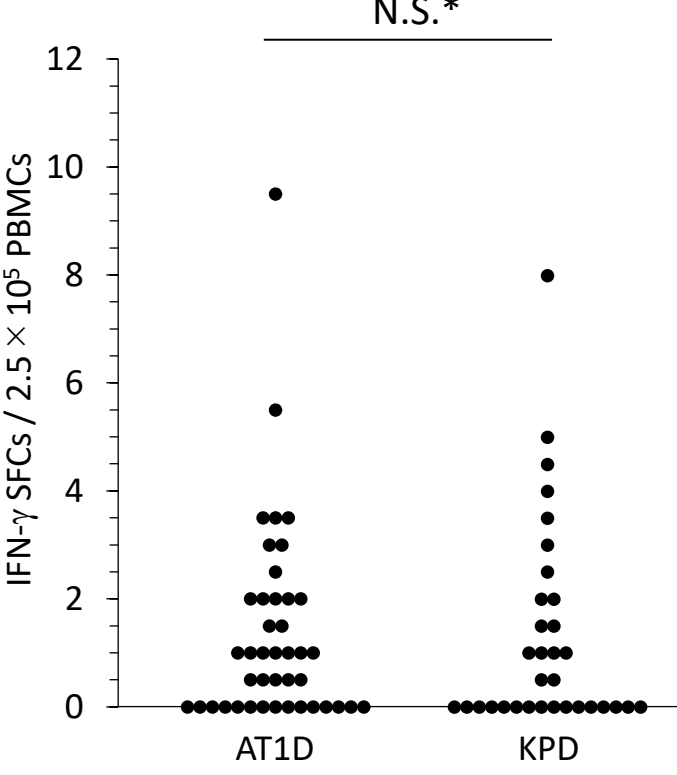
a (B:9-23)



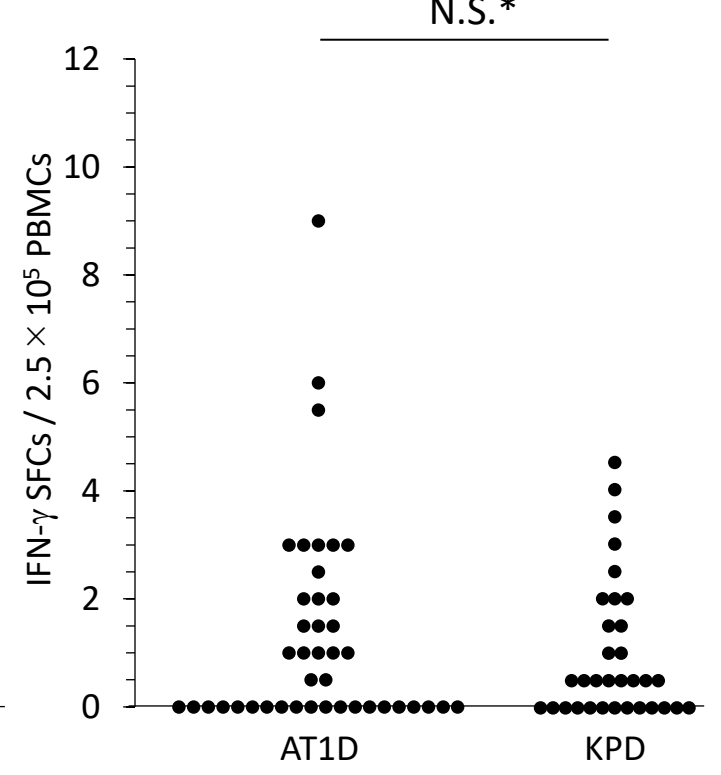
B (B:10-24)



C (B:11-25)

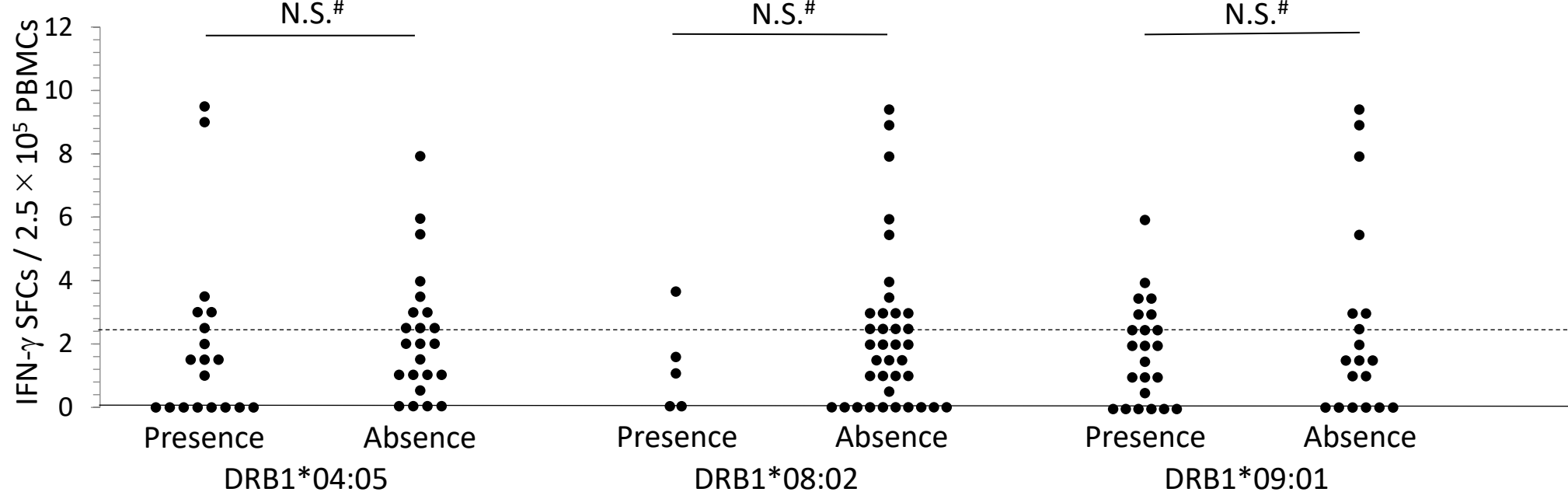


D (B:12-26)

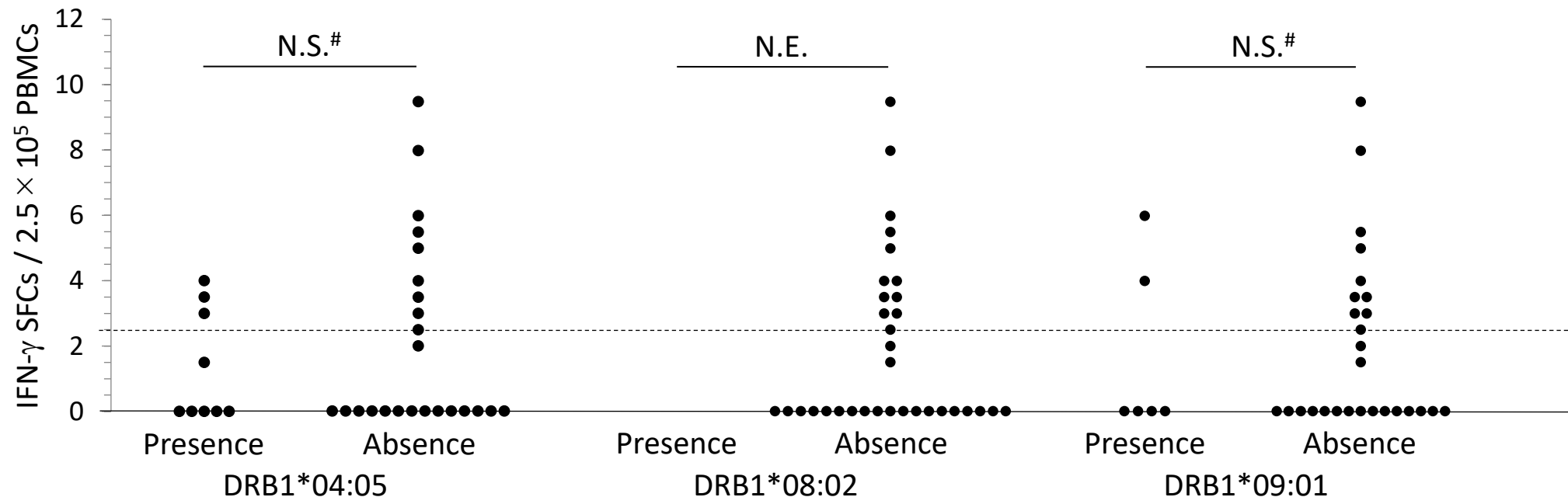


Supplementary Figure 2. ELISpot assay showing SFC numbers of B:9-23-, B:10-24-, B:11-25-, and B:12-26-specific IFN- γ -producing PBMCs in the two categories of diabetes
 Each dot indicates the number of IFN- γ SFCs. (a) B:9-23, (b) B:10-24, (c) B:11-25, (d) B:12-26. Participants with AT1D (n = 42); participants with KPD (n = 33). *Mann-Whitney *U*-test.
 AT1D, acute-onset type 1 diabetes; ELISpot, enzyme-linked immunospot; IFN, interferon; KPD, ketosis-prone type 2 diabetes; N.E., not examined due to zero value in the KPD participants with HLA-DRB1*08:02; N.S., not significant; PBMC, peripheral blood mononuclear cell; SFC, spot forming cell.

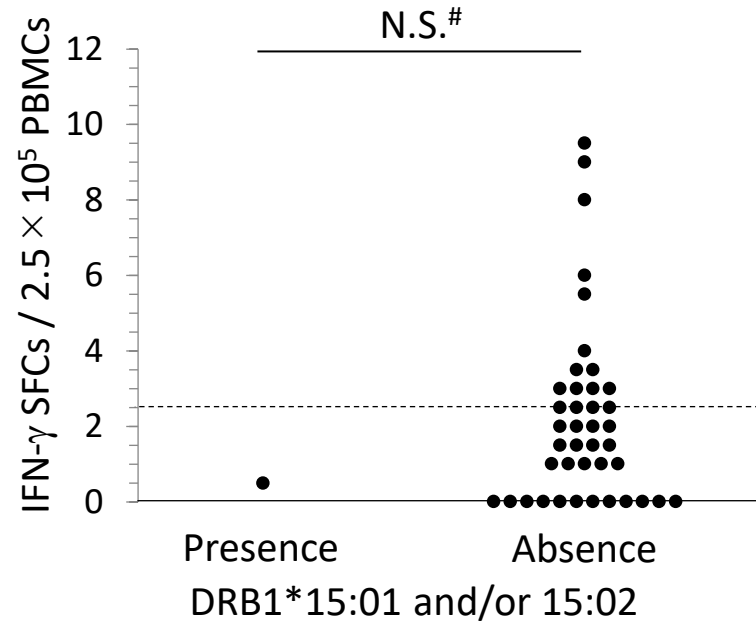
AT1D



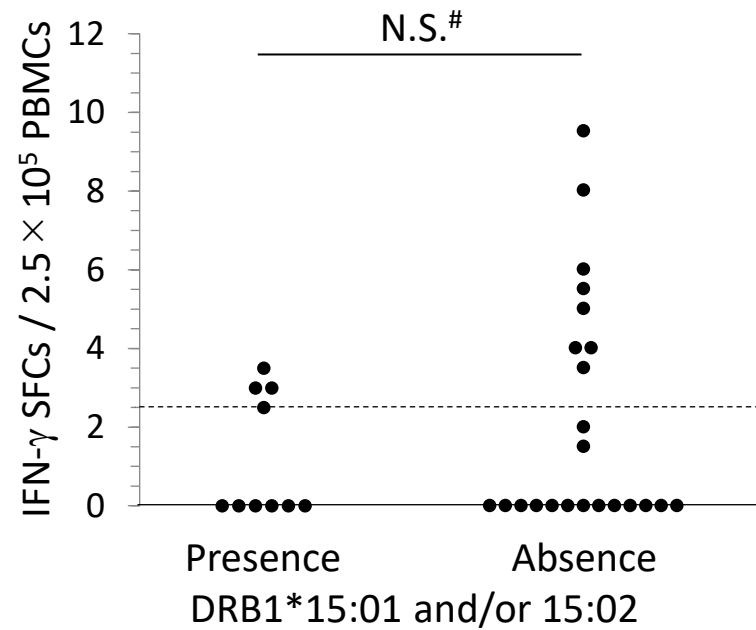
KPD



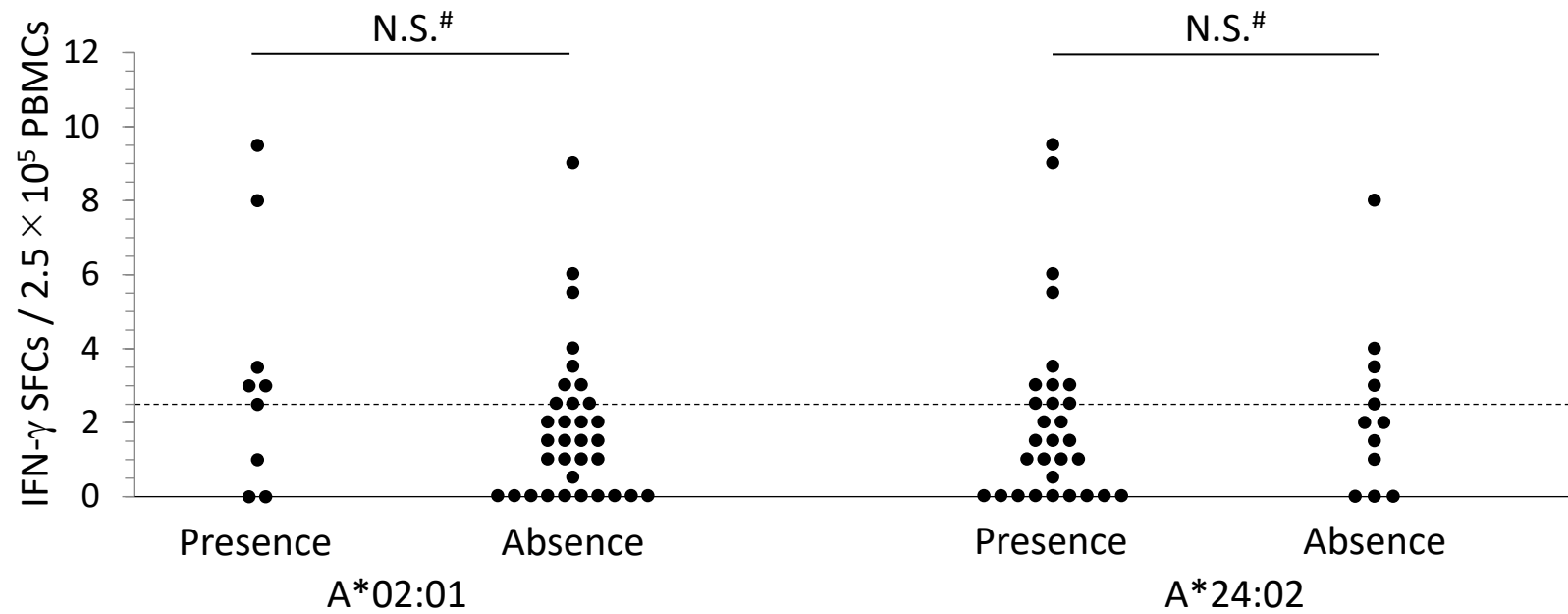
AT1D



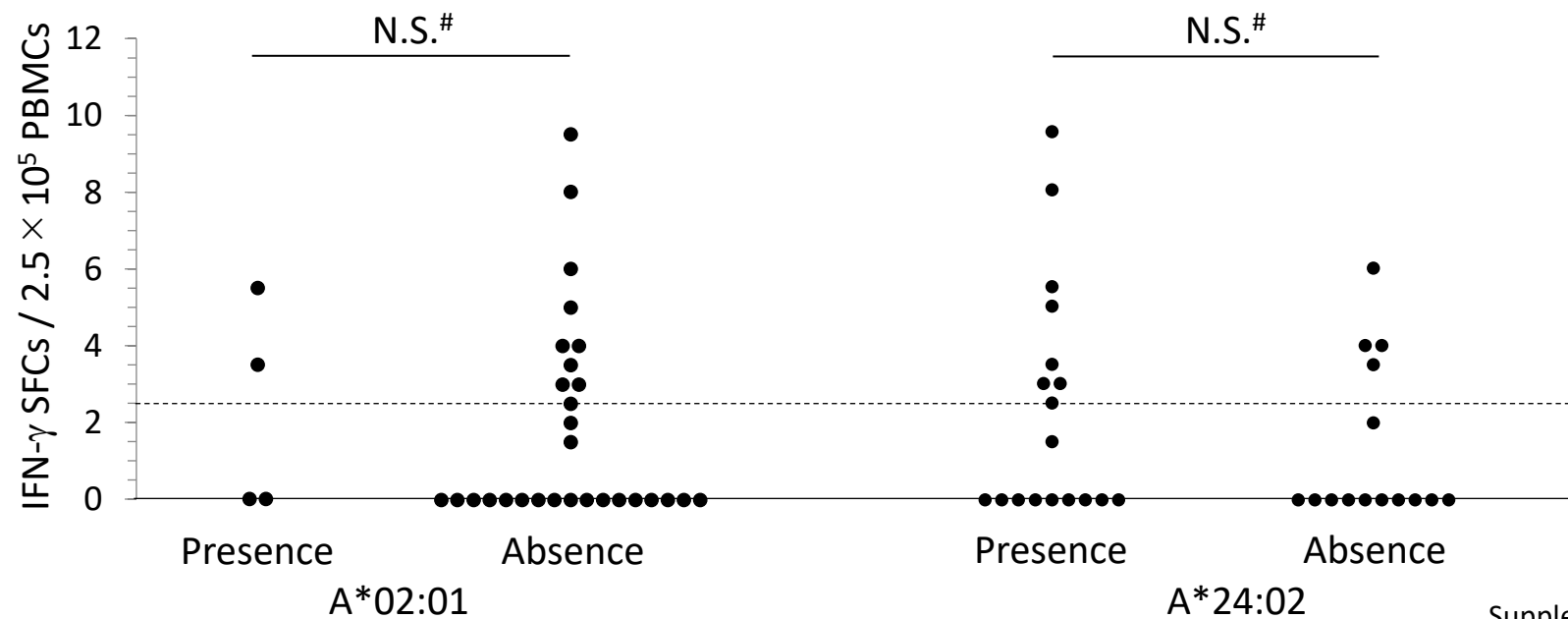
KPD



AT1D



KPD



Supplementary Figure 3. ELISpot assay in participants showing SFC numbers of B:9-23rPep-specific IFN- γ -producing PBMCs according to the presence/absence of HLA alleles linked to type 1 diabetes.

The participants were divided into the following three categories according to presence/absence DRB1*04:05, presence/absence DRB1*08:02 and presence/absence DRB1*09:01 (**a**), the following the category according to presence/absence DRB1*15:01 and/or DRB1*15:02 (**b**), and the following two categories according to presence/absence A*02:01 and presence/absence A*24:02 (**c**), and IFN- γ SFC numbers were evaluated. Each dot indicates the maximum number of IFN- γ SFCs from participants upon stimulation with each of the four B:9-23-related peptides using ELISpot assay. Horizontal dotted line represents a cut-off value of IFN- γ SFC numbers. **Supplementary Table 1** shows the number of positive and negative results of IFN- γ ELISpot assay according to each presence/absence HLA allele. #Mann-Whitney *U*-test.

AT1D, acute-onset type 1 diabetes; ELISpot, enzyme-linked immunospot; IFN, interferon; KPD, ketosis-prone type 2 diabetes; N.E., not examined; N.S., not significant; PBMC, peripheral blood mononuclear cell; SFC, spot forming cell.