

SUPPLEMENTARY DATA

Supplementary Table 1. Study participant demographic, tetramer, and autoantibody data (ex vivo samples)

Sample	Diabetes Status	Age (years)	T1D duration (years) [§]	Gender	# InsB tetramer ⁺ cells	# CD4 ⁺ T cells (X 10 ⁶)	# CD4 ⁺ T cells/ Tetramer ⁺ cells	% T _{EM}	% T _{CM}	% T _{naive}	mIAA+	GAD AA+	IA-2 AA+	ZnT8 AA+	blood volume (mL)	Total # of PBMCs (X10 ⁶)
ExVivo1	New onset	3	0.10	M	16	12.75	0.79	0	0	100	0.049	193	314	0.121	22.5	60.2
ExVivo2	New onset	11	0 (0 days) [§]	M	14	26.89	1.96	0	75	25	N/A	N/A	N/A	N/A	37.5	81.3
ExVivo3	New onset	22	0 (0 days) [§]	F	12	14.77	1.22	0	50	50	N/A	N/A	N/A	N/A	45	86.4
ExVivo4	New onset	18	0.10	M	7	16.82	2.38	0	50	50	0.017	443	361	0.428	24.5	64
ExVivo5	New onset	16	0 (2 days) [§]	M	6	67.66	11.50	0	100	0	-0.001	0	366	0.150	51	110
ExVivo6	New onset	14	0 (3 days) [§]	M	0	6.82	0				0.000	14	168	0.109	36.5	25
ExVivo7	T1D	3	1.70	M	21	16.81	0.80	66.7	16.7	16.7	2.466	37	83	0.014	25	99.6
ExVivo8	T1D	5	1.70	F	15	23.40	1.55	33.3	66.7	0	0.682	31	1	-0.001	26	71
ExVivo9	T1D	12	0.50	F	13	24.36	1.89	0	100	0	0.325	37	0	0.003	49	99.6
ExVivo10	T1D	9	4.70	M	12	9.88	0.82	0	33.3	66.7	2.759	0	2	-0.001	17	57.8
ExVivo11	T1D	44	25.00	F	10	49.14	5.17	33.3	66.6	0	3.220	0	0	0.002	49	130
ExVivo12	T1D	23	14.00	F	9	12.62	1.40	100	0	0	1.824	320	68	0.005	32	69.5
ExVivo13	T1D	10	1.00	F	8	14.20	1.78	100	0	0	4.009	693	365	0.026	32	50.7
ExVivo14	T1D	51	48.00	M	7	27.98	4.16	66.6	33.3	0	1.082	0	0	0.000	48	94
ExVivo15	T1D	6	3.00	M	6	22.91	3.80	0	0	100	0.119	37	40	0.010	23.5	63
ExVivo16	T1D	16	0.50	F	6	10.79	1.79	100	0	0	0.450	607	277	0.318	35	61
ExVivo17	T1D	50	37.00	F	4	27.77	6.52	0	0	100	N/A	N/A	N/A	N/A	52.5	85.5
ExVivo18	T1D	38	35.00	M	4	29.36	7.89	0	0	100	1.829	13	10	0.003	43	94
ExVivo19	T1D	49	21.00	F	3	22.30	6.68	0	0	100	N/A	N/A	N/A	N/A	55	70
ExVivo20	T1D	35	29.00	F	3	25.72	8.69	0	0	100	0.003	0	0	0.004	55	145
ExVivo21	T1D	15	1.80	M	3	23.61	8.99	0	0	100	1.271	113	145	0.102	38	70
ExVivo22	T1D	4	3.00	M	0	9.93	0				0.285	751	10	0.156	12.5	32.1
ExVivo23	T1D	10	3.00	F	0	4.46	0				0.072	4	0	0.006	16	36.3
ExVivo24	T1D	10	3.00	F	0	32.68	0				0.064	11	0	-0.002	42	66
ExVivo25	T1D	11	3.00	F	0	8.64	0				0.257	0	58	0.009	52	57
ExVivo26	T1D	38	25.00	F	0	19.69	0				0.018	134	0	0.008	43	80.6
ExVivo27	T1D	47	33.00	F	0	24.74	0				0.180	0	22	-0.002	55.5	83.6
ExVivo28	T1D	35	31.00	F	0	27.83	0				-0.001	12	0	0.000	51	77
ExVivo29	T1D	39	15.00	F	0	31.46	0				0.145	112	100	0.037	54	100
ExVivo30	T1D	58	43.00	M	0	35.43	0				0.011	41	2	0.002	54	146
ExVivo31	T1D	61	0.50	M	0	16.94	0				0.011	114	445	0.077	55	69
ExVivo32	T1D	50	41.00	F	0	24.35	0				3.017	33	8	0.004	32	98
ExVivo33	T1D	30	13.50	M	0	35.33	0				0.007	0	2	0.005	54	99
ExVivo34	T1D	67	41.00	F	0	9.04	0				2.906	14	58	0.000	14	33
ExVivo35	T1D	60	36.00	M	0	8.16	0				0.002	1	0	-0.001	21	31
ExVivo36	T1D	57	55.00	M	0	31.17	0				0.882	0	0	-0.002	51	93
ExVivo37	T1D	19	17.00	F	0	44.25	0				0.089	0	93	-0.001	54	96

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Sample	Diabetes Status	Age (years)	T1D duration (years ^{&})	Gender	# InsB tetramer ⁺ cells	# CD4 ⁺ T cells (X 10 ⁶)	# CD4 ⁺ T cells/ Tetramer ⁺ cells (X 10 ⁶)	% T _{EM}	% T _{CM}	mIAA+	GAD AA+	IA-2 AA+	ZnT8 AA+	blood volume (mL)	Total # of PBMCs (X10 ⁶)
ExVivo38	Non-diabetic (At-risk)	3		F	2	19.91	9.96	100	0	0.002	0	0	-0.001	26	58
ExVivo39	Non-diabetic (At-risk)	11		F	0	35.67	0			-0.006	617	41	0.015	46.5	126
ExVivo40	Non-diabetic	12		F	5	37.83	7.57	0	0	-0.001	0	0	0.000	28	55.7
ExVivo41	Non-diabetic	46		F	4	44.66	11.16	0	0	-0.003	0	0	-0.002	52	112
ExVivo42	Non-diabetic	25		M	3	22.53	7.51	0	0	0.002	0	0	-0.001	44	135
ExVivo43	Non-diabetic	45		F	0	28.83	0			-0.002	0	0	0.001	54	117
ExVivo44	Non-diabetic	39		F	0	49.87	0			-0.001	0	0	0.002	46	160
ExVivo45	Non-diabetic	36		F	0	23.23	0			-0.002	0	0	-0.001	57	116.5
ExVivo46	Non-diabetic	18		M	0	30.77	0			-0.008	0	0	-0.001	51.5	110
ExVivo47	Non-diabetic	43		M	0	12.49	0			0.002	11	0	-0.001	55.5	153
ExVivo48	Non-diabetic	10		F	0	18.76	0			-0.005	0	0	0.000	35.6	77.5
ExVivo49	Non-diabetic	17		F	0	64.04	0			-0.001	0	0	0.002	40	111.6
ExVivo50	Non-diabetic	48		F	0	23.39	0			-0.010	0	1	0.000	41	91.5
ExVivo51	Non-diabetic	19		F	0	2.97	0			-0.001	0	0	-0.001	26.5	27
ExVivo52	Non-diabetic	49		M	0	11.86	0			0.001	0	1	0.000	42.5	69.6
ExVivo53	Non-diabetic	33		F	0	17.67	0			-0.001	1	0	-0.001	54.9	91.5
ExVivo54	Non-diabetic	45		F	0	19.37	0			-0.003	0	0	-0.002	53.5	93
ExVivo55	Non-diabetic	13		F	0	15.84	0			0.000	0	0	0.000	38.5	78.5
ExVivo56	Non-diabetic	39		F	0	38.42	0			0.041	0	0	-0.002	52	97.5
ExVivo57	Non-diabetic	63		M	0	42.16	0			0.005	14	0	-0.001	54	94
ExVivo58	Non-diabetic	62		F	0	19.75	0			-0.005	0	0	-0.002	52.5	94
ExVivo59	Non-diabetic	36		M	0	42.16	0			0.000	0	0	-0.002	54	121
ExVivo60	Non-diabetic	58		F	0	23.67	0			0.002	0	0	-0.002	49	108
ExVivo61	Non-diabetic	27		F	0	25.93	0			N/A	N/A	N/A	N/A	47	123
ExVivo62	Non-diabetic	21		F	0	30.06	0			0.003	0	0	-0.003	31	83
ExVivo63	Non-diabetic	58		F	0	46.23	0			-0.006	0	0	0.000	54	125

* cut-off for mIAA=0.01, GAD AA=20, IA-2 AA=5, ZnT8 AA=0.02

& T1D duration in years unless listed in days as indicated for newly diagnosed

N/D=not determined

N/A=not available

New onset (within 100 days of T1D diagnosis)

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Supplementary Table 2. Study participant demographic, tetramer, and autoantibody data (*in vitro* samples)

Sample	Diabetes Status	T1D		Gender	# PBMCs (X 10 ⁶)	%InsB ₁₀₋₂₃	%HA ₃₀₆₋₃₁₈										
		Age (years)	duration (Days)			tetramer ⁺ of CD4 ⁺ T cells	tetramer ⁺ of CD4 ⁺ T cells	mIAA*	GAD AA*	IA-2 AA*	ZnT8 AA*	DR1	DQA1	DQB1	DR2	DQA1	DQB2
new onset T1D #1	New onset	8	8	F	9.1	0.009	0.32	0.07	212	239	0.001	0301	0501	0201	0401	0301	0302
new onset T1D #2	New onset	13	10	M	12.8	0.081	0.01	0.14	113	334	0.448	0301	0501	0201	0405	0303	0302
new onset T1D #3	New onset	5	13	M	11.6	0	0.00	0.41	0	312	0.398	0405	0301	0302	1301	0103	0603
new onset T1D #4	New onset	10	8	F	10.2	0.001	9.21	0.02	185	12	-0.003	0301	0501	0201	0401	0301	0302
new onset T1D #5	New onset	11	15	M	10.3	0.001	7.07	0.23	642	267	0.84	0101	0101	0501	0401	0301	0302

* cut-off for mIAA=0.01, GAD AA=20, IA-2 AA=5, ZnT8 AA=0.02

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Supplementary Table 3. Primer and probes used for HLA typing.

Identifier	Primer/Probe	Final Concentration	Sequence (5'-3')
GDR1	DRA Forward Primer	225 nM	AGGCCGAGTTCTATCTGAATCCT
GDR2	DRA Reverse Primer	225 nM	CGCCAGACCGTCTCCTTCT
GDR3	DRA Probe	50 nM	VIC-CATAAACTCGCCTGATTG-MGB
GDR4	Pan DR4 Forward Primer	225 nM	CGTTTCTTGGAGCAGGTTAAACA
GDR5	Pan DR4 Reverse Primer	225 nM	GCACGTACTCCTCTTGGTGATAGA
GDR6	DR4 Probe	50 nM	FAM-TCCGTCCCGTTGAAGA-MGB
GDQ1	DQB Forward 132	225 nM	GGGCATGTGCTACTTCACCAA
GDQ2	DQB Reverse 209	225 nM	GCGTACTCCTCTCGGTTATAGATGT
GDQ3	DQB 167 Probe 1	50 nM	FAM-TGCGTCTTGTGACCAG-MGB
GDQ4	DQB 167 Probe 2	50 nM	VIC-TGCGTCTTGTACCAG-MGB
GDQ5	DQB Forward 155	225 nM	GGACGGAGCGCGTGCGTCTT
GDQ6	DQB Reverse 294	225 nM	TTCCTTCTGGCTGTTCCAGTACT
GDQ7	DQB 245 Probe 1	50 nM	FAM-ACCGCGCGGTACA-MGB
GDQ8	DQB 246 Probe 2	50 nM	VIC-CACCGCCCGATAC-MGB
GDR7	DR4 Subtyping Forward	225 nM	CGTTTCTTGGAGCAGGTTAAACA
GDR8	DR4 Subtyping Reverse	225 nM	CTCGCCGCTGCACTGTG
GDR9	DRB codon 57-gat Probe	50 nM	FAM-CAGTACTCGGCATCAG-MGB
GDR10	DRB codon 57-agc Probe	50 nM	VIC-ACTCGGCGCTAGG-MGB
GDR11	DRB codon 71-aag Probe	50 nM	FAM-AGCAGAAGCGGGC-MGB
GDR12	DRB codon 71-agg Probe	50 nM	VIC-TGGAGCAGAGGCG-MGB
GDR13	DRB codon 71-gag Probe	50 nM	FAM-GAAGACGAGCGGGC-MGB
GDR14	DRB codon 74-gcg Probe	25 nM	FAM-TGTCCACCGCGGC-MGB
GDR15	DRB codon 74-gag Probe	100 nM	VIC-TGTCCACCTCGGC-MGB
GDR16	DRB codon 86-ggt Probe	50 nM	FAM-AAGCTCTACCAACC-MGB
GDR17	DRB codon 86-gtg Probe	50 nM	VIC-AAGCTCTCACAACC-MGB
GDQ9	*03 Forward	225 nM	TAACCGAGAGGAGTACGCACGCT
GDQ10	*03 Reverse	225 nM	GTGTCTGCACACCGTGTCCAACCT
GDQ11	*0302	25 nM	FAM-ACTCGGCGGCAGGC-MGB
GDQ12	*0303	50 nM	VIC-ACTCGGCGTCAGGC-MGB
GDQ13	*0301	50 nM	FAM-CCGGTACACCTCCACGT-MGB

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Supplementary Table 4. Antibodies used for cell staining and flow cytometry

Antigen	Target species	Clone	Fluorochrome	Manufacturer
CD4	Human	SK3	BUV395	BD Biosciences
ICOS	Human	DX29	BV711	
CD8 α	Human	RPA-T8	BV605	BioLegend
CD45RO	Human	UCHL1	BV650	
CCR7	Human	G043H7	FITC	
CXCR3	Human	G025H7	PerCp/Cy5.5	
PD-1	Human	EH12.2H7	BV786	
Human TruStain FcX (CD16/32)	Human			
CD3	Human	SK7	APC-ef780	eBioscience
CD20	Human	2H7	PE-Cy7	
CD33	Human	WM-53	PE-Cy7	
CD123	Human	6H6	PE-Cy7	
Ghost violet 510				Tonbo bioscience
B220	Mouse	RA3-6B2	FITC	BD Biosciences
CD3 \square	Mouse	145-2C11	BUV395	
CD8 α	Mouse	53-6.7	PerCp/Cy5.5	
CD11b	Mouse	M1/70	FITC	
CD11c	Mouse	N418	FITC	
CD44	Mouse	1M7	BV786	
CD4	Mouse	RM4-5	APC-ef780	eBioscience
PD-1	Mouse	J43	PE-Cy7	