Cell Reports, Volume *32*

Supplemental Information

Modeling Type 1 Diabetes *In Vitro* Using Human Pluripotent Stem Cells

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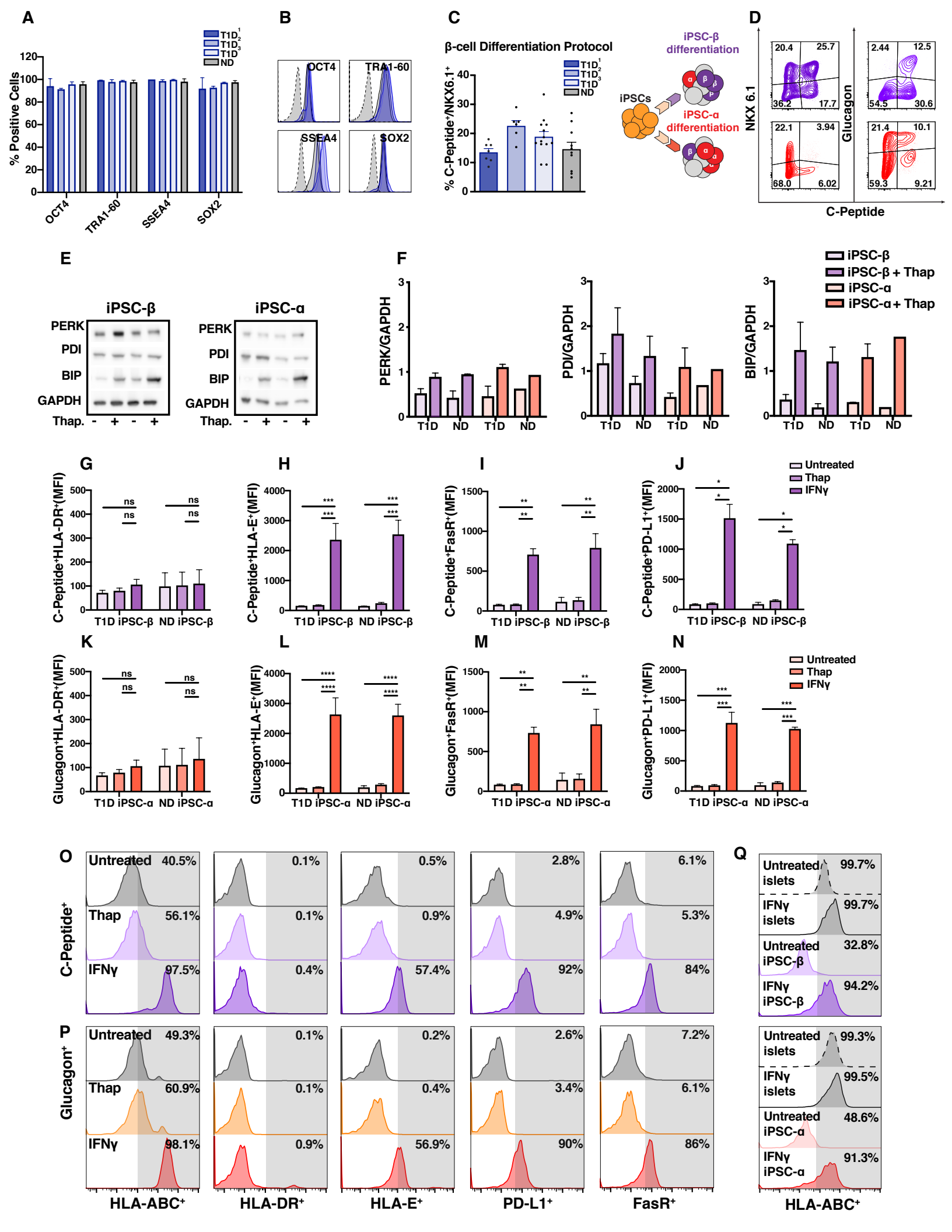
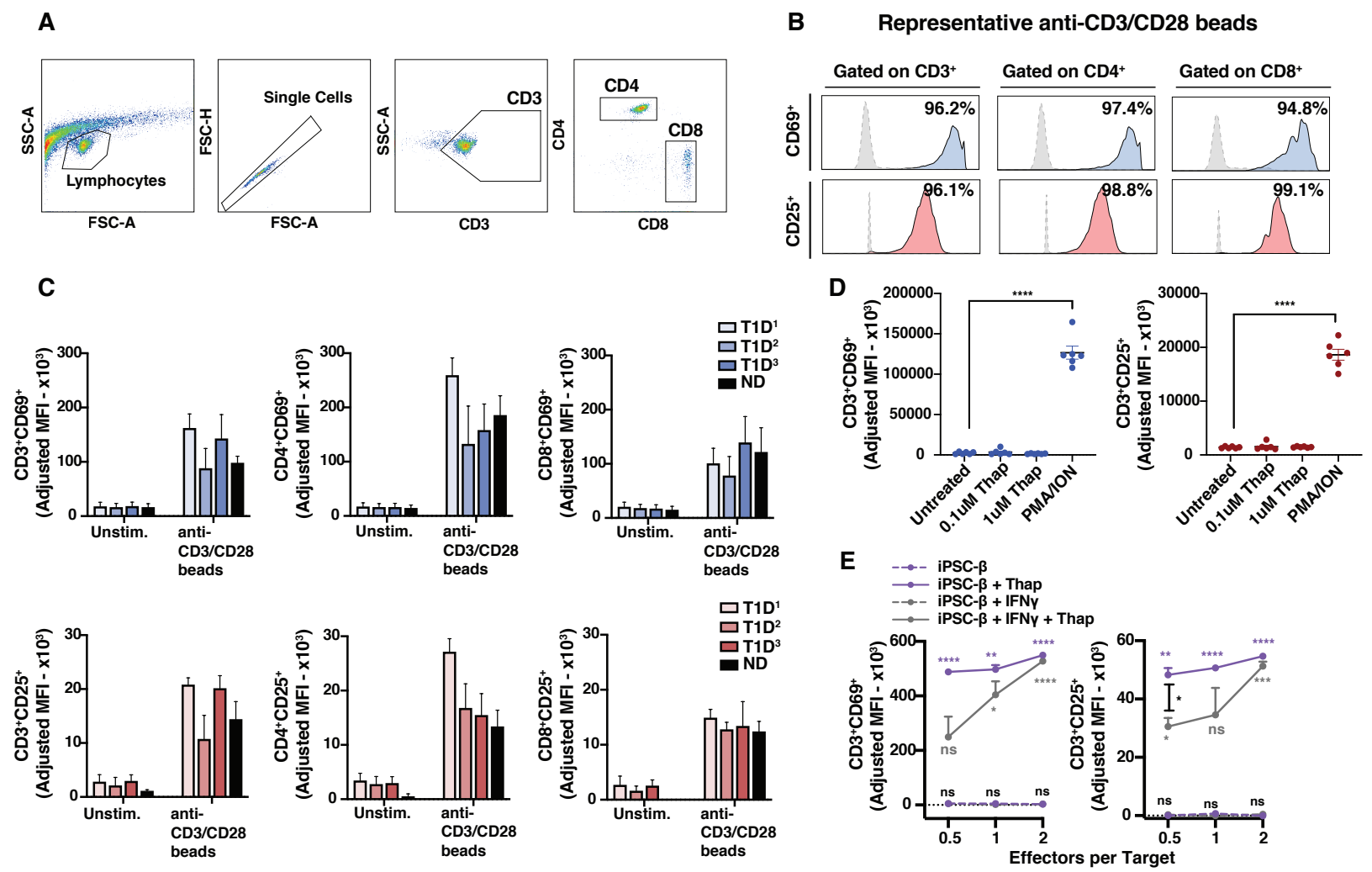

A
% Positive Cells
T1D1
T1D2
T1D3
T1D
ND
OCT4
TRA1-60
SSEA4
SOX2
B
OCT4
TRA1-60
SSEA4
SOX2
C
β-cell Differentiation Protocol
% C-Peptide+/NKX6.1+
iPSCs
iPSC-β differentiation
iPSC-α differentiation
D
NKX 6.1
Glucagon
C-Peptide
20.4
25.7
36.2
17.7
2.44
12.5
54.5
30.6
22.1
3.94
68.0
6.02
21.4
10.1
59.3
9.21
E
iPSC-β
iPSC-α
PERK
PDI
BIP
GAPDH
Thap. - + - +
F
iPSC-β
iPSC-β + Thap
iPSC-α
iPSC-α + Thap
PERK/GAPDH
PDI/GAPDH
BIP/GAPDH
T1D ND T1D ND
G
C-Peptide+HLA-DR+(MFI)
H
C-Peptide+HLA-E+(MFI)
I
C-Peptide+FasR+(MFI)
J
C-Peptide+PD-L1+(MFI)
Untreated
Thap
IFNγ
T1D iPSC-β ND iPSC-β
K
Glucagon+HLA-DR+(MFI)
L
Glucagon+HLA-E+(MFI)
M
Glucagon+FasR+(MFI)
N
Glucagon+PD-L1+(MFI)
T1D iPSC-α ND iPSC-α
O
C-Peptide+
Untreated 40.5% 0.1% 0.5% 2.8% 6.1%
Thap 56.1% 0.1% 0.9% 4.9% 5.3%
IFNγ 97.5% 0.4% 57.4% 92% 84%
P
Glucagon+
Untreated 49.3% 0.1% 0.2% 2.6% 7.2%
Thap 60.9% 0.1% 0.4% 3.4% 6.1%
IFNγ 98.1% 0.9% 56.9% 90% 86%
HLA-ABC+ HLA-DR+ HLA-E+ PD-L1+ FasR+
Q
Untreated islets 99.7%
IFNγ islets 99.7%
Untreated iPSC-β 32.8%
IFNγ iPSC-β 94.2%
Untreated islets 99.3%
IFNγ islets 99.5%
Untreated iPSC-α 48.6%
IFNγ iPSC-α 91.3%
HLA-ABC+

Figure S1. iPSC-β and iPSC-α are sensitive to ER-stress induction *in vitro*. Related to Figure 1.
(A-B) iPSC lines characterization.
(A) Flow cytometric analysis of pluripotent markers in iPSCs (n=3 T1D and n=1 iPSC ND donor, n=3 batches per donor line), quantification of positive cells for OCT4, TRA1-60, SSEA4, SOX2 in comparison to IgG isotype control.
(B) Representative flow cytometry histograms. Each color represents a different donor's iPSCs. Dashed histogram represents the IgG isotype control.
(C) Flow cytometry quantification of C-Peptide and NKX6.1 double positive cells in the iPSC-β protocol. Data are mean ± SEM. n=3 T1D iPSC donors and n=1 ND iPSCs line, n=3 to 7 differentiation batches per donor line.
(D) Representative flow cytometry plots for β and α cell markers, C-Peptide, NKX6.1 and glucagon in cells produced from the β cell differentiation protocol (top panels, as presented in the scheme S1D) or α cell differentiation protocol (bottom panels, as presented in the scheme S1D).
(E and F) Western blot analysis of PERK, PDI and BIP, in differentiated iPSC-derived endocrine cells (β or α as indicated), untreated or treated with thapsigargin (thap, 5μm for 5 h).
(F) Quantification of western blot analysis. n=3 T1D iPSC donors, n=1 iPSC ND donor, n=3 differentiation batches per donor line. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together. Data are mean ± SEM.
(G-N) Flow cytometry analysis gates of differentiated iPSC-derived endocrine cells treated with thap (5μm for 5 h) or IFNγ (100U/ml, 48h). Median fluorescence intensity (MFI) in (C-F) C-peptide positive or (G-J) glucagon positive cells, co-stained with (C and G) HLA-DR, (D and H) HLA-E, (E and I) FasR or (F and J) PD-L1. n=3 T1D iPSC donors, n=1 iPSC ND donor, n=3 batches per donor line. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together.
(O and P) Representative flow cytometry histograms gates of (K) C-peptide positive cells or (L) glucagon positive cells, co-stained with HLA-A, B, C, HLA-DR, HLA-E, PD-L1 or FasR.
(Q) Representative flow cytometry histograms gates of C-peptide positive cells (purple) or glucagon positive cells (red) of iPSC-β compared to human islets. (n=3 T1D iPSC donors, n=1 iPSC ND donor, n=1 differentiation batch per donor line).
**p<0.005, ***p<0.0005, ****p<0.0001 Ordinary one-way ANOVA. Data are mean ± SEM.



Gating strategy for Activation Assays

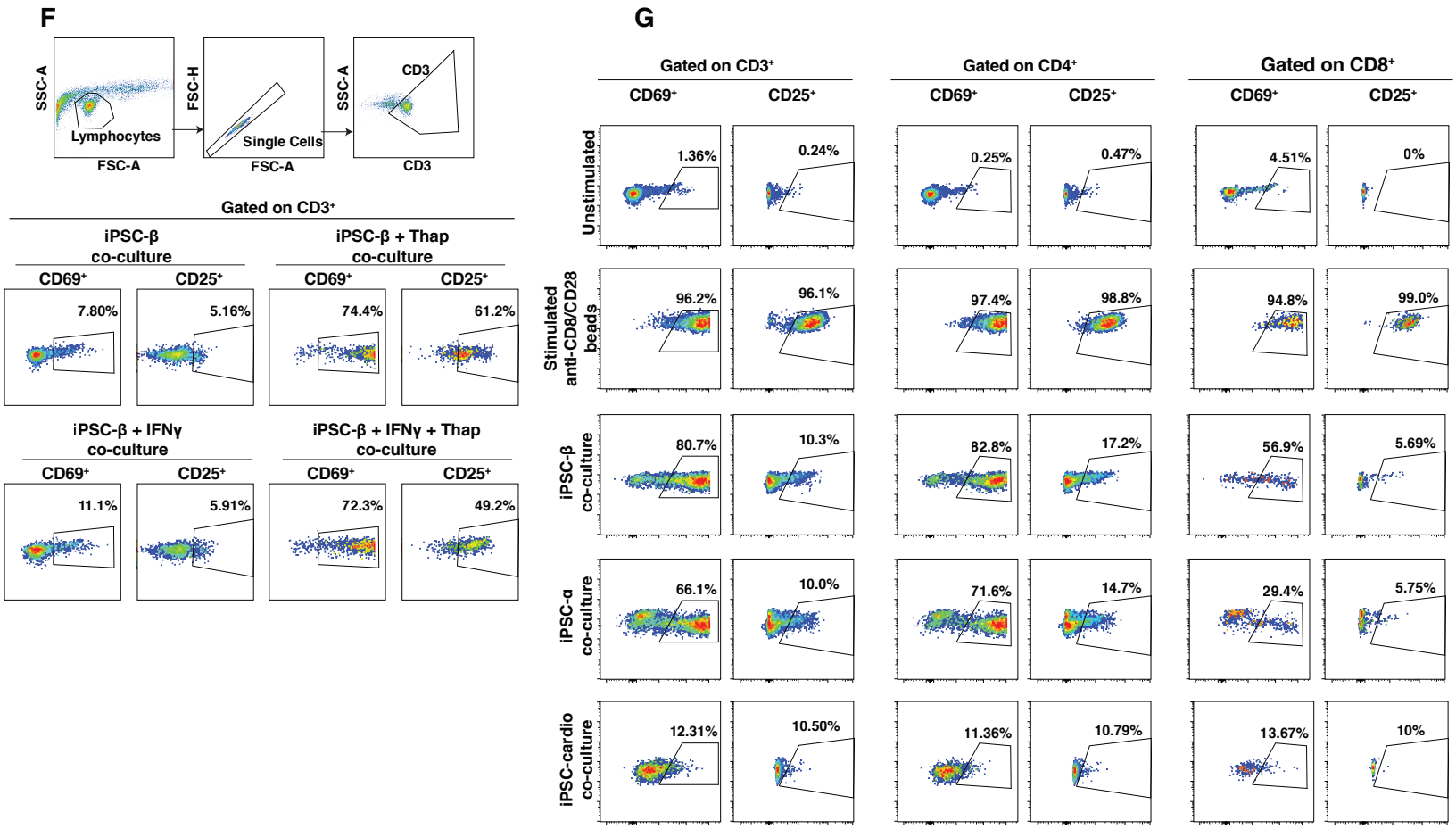
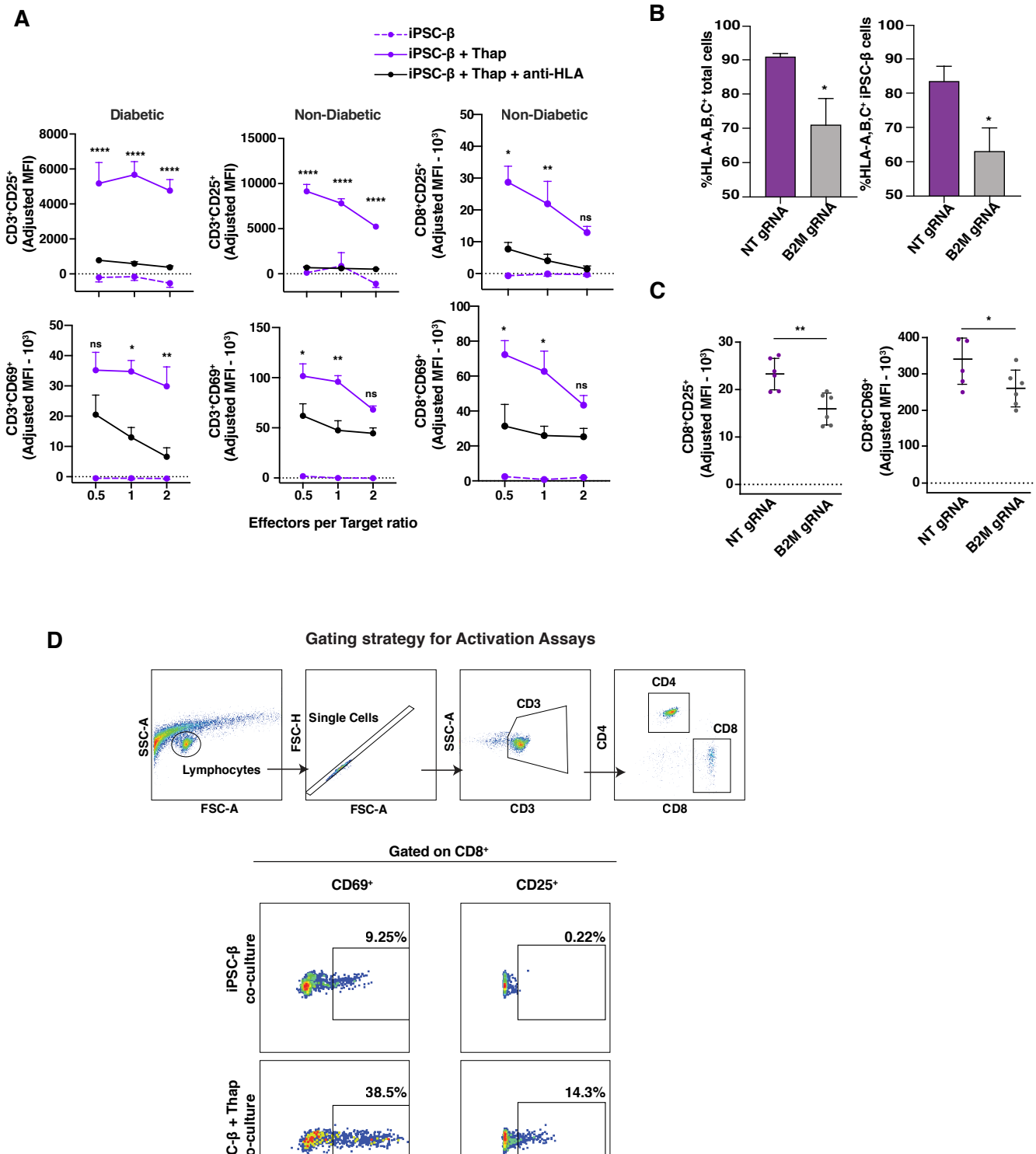


Figure S2. Positive and negative controls for the PBMCs activation assays. Related to Figure 2.
(A) Representative gating strategy for lymphocytes, single cells, $CD3^+$, and $CD4^+$ or $CD8^+$ cell populations; used in all T cell activation gating presented in Figures 2 to 4.
(B-C) Flow cytometry analysis of T cell activation markers, $CD25^+$ and $CD69^+$ on $CD3^+$, $CD4^+$ and $CD8^+$ populations activated with anti-CD3/anti-CD28 beads.
(B) Representative flow cytometry plots of $CD3^+$, $CD4^+$ and $CD8^+$ cells showed in B, expressing CD25 or CD69, unstimulated (gray histogram) or stimulated with anti-CD3/CD28 activation beads (in blue: CD69 and in red: CD25 positive control).
(C) PBMC negative and positive controls. Positive control stimulations were performed using anti-CD3/CD28 beads. Data are mean ± SEM.
(D) Flow cytometry representation of T cell activation markers, $CD25^+$ and $CD69^+$ on CD3 population from PBMCs treated with thap (0.1 or 1uM for 5h) or PMA/Ionomycin, as a positive control (n=3 T1D PBMCs preparation).
(E) Flow cytometry analysis of T cell activation markers after co-culture, gated on $CD3^+$ cells. Autologous PBMCs co-cultured with iPSC-β untreated or pre-treated with thap (5μm for 5 h) ± IFNγ (100U/ml for 48h) (n=1 T1D donor, n=3 differentiation batches per donor line.
$T1D^1$, $T1D^2$ and $T1D^3$ were pooled together).
(F) Representative gate strategy of data presented on E.
(G) Gating strategy for activation assays. Representative gating strategy for lymphocytes, single cells, $CD3^+$, and $CD4^+$ or $CD8^+$ cell populations, and activation markers CD25 and CD69; used in all T cell activation gating presented in Figures 2 and 4.
Data are mean ± SEM. *<0.05, **p<0.005, ***p<0.0005, ****p<0.0001 Two-way ANOVA: (E) Purple: thaps vs. untreated. Grey: Thap+IFNγ vs. IFNγ. Black: IFNγ vs. no IFNγ. ns, non-significant. Effector to target ratio are indicated.


A
iPSC-β
iPSC-β + Thap
iPSC-β + Thap + anti-HLA
Diabetic
Non-Diabetic
Non-Diabetic
CD3+CD25+ (Adjusted MFI)
CD3+CD25+ (Adjusted MFI)
CD8+CD25+ (Adjusted MFI - 10³)
CD3+CD69+ (Adjusted MFI - 10³)
CD3+CD69+ (Adjusted MFI - 10³)
CD8+CD69+ (Adjusted MFI - 10³)
Effectors per Target ratio
B
%HLA-A,B,C+ total cells
%HLA-A,B,C- iPSC-β cells
NT gRNA
B2M gRNA
C
CD8+CD25+ (Adjusted MFI - 10³)
CD8+CD69+ (Adjusted MFI - 10³)
NT gRNA
B2M gRNA

D
Gating strategy for Activation Assays
SSC-A
Lymphocytes
FSC-A
FSC-H
Single Cells
FSC-A
SSC-A
CD3
CD3
CD4
CD4
CD8
CD8
Gated on CD8+
CD69+
CD25+
iPSC-β co-culture
9.25%
0.22%
iPSC-β + Thap co-culture
38.5%
14.3%
iPSC-β + Thap + anti-HLA co-culture
11.0%
0.54%

Figure S3. iPSC-β induced T cell activation is mediated by direct T cell-HLA interaction in T1D and ND donors. Related to Figure 3.

(A) PBMCs co-cultured for 48h with autologous iPSC-β (n=3 T1D donors, 3 differentiation batched per donor. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together) in increasing PBMCs to iPSC-β ratio. iPSC-β were untreated or pre-treated with thapsigargin (thap, 5μM for 5h) and/or anti-HLA antibody for 30min prior to co-culture. Flow cytometry analysis after co-culture of T cell activation markers, $CD25^+$ and $CD69^+$ on $CD3^+$ gated cells and $CD8^+$ gated cells as indicated.

Data are mean ± SEM. *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001. Two-way ANOVA. ns, non-significant. n=3 T1D iPSC donors, n=1 iPSC ND donor, n=3 batches per donor line. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together.

(B) Percentage of HLA-A, B, C^+ expression in total cells in iPSC-β differentiation and C-$peptide^+$/$glucagon^-$ cells (iPSC-β cells). Samples were collected 10 days after transduction with a lentivirus vector targeting beta 2 microglobulin (B2M) gRNA or non-targeting control gRNA and expressing Cas9.

(C) Expression of the activation marker CD25 (left) and CD69 (right) in $CD8^+$ gated cells after co-culture with autologous iPSC-β transduced with a lentivirus vector expressing a non-targeting or B2M gRNA and Cas9. n=1 ND iPSC donor, n=3 differentiation batches per donor line. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together. *p<0.05, **p<0.01, student t-test. Data are mean ± SEM.

(D) Representative gating strategy for lymphocytes, single cells, $CD3^+$, and $CD8^+$ cell populations, and activation markers CD25 and CD69; used in T cell activation gating presented in Figure 3.

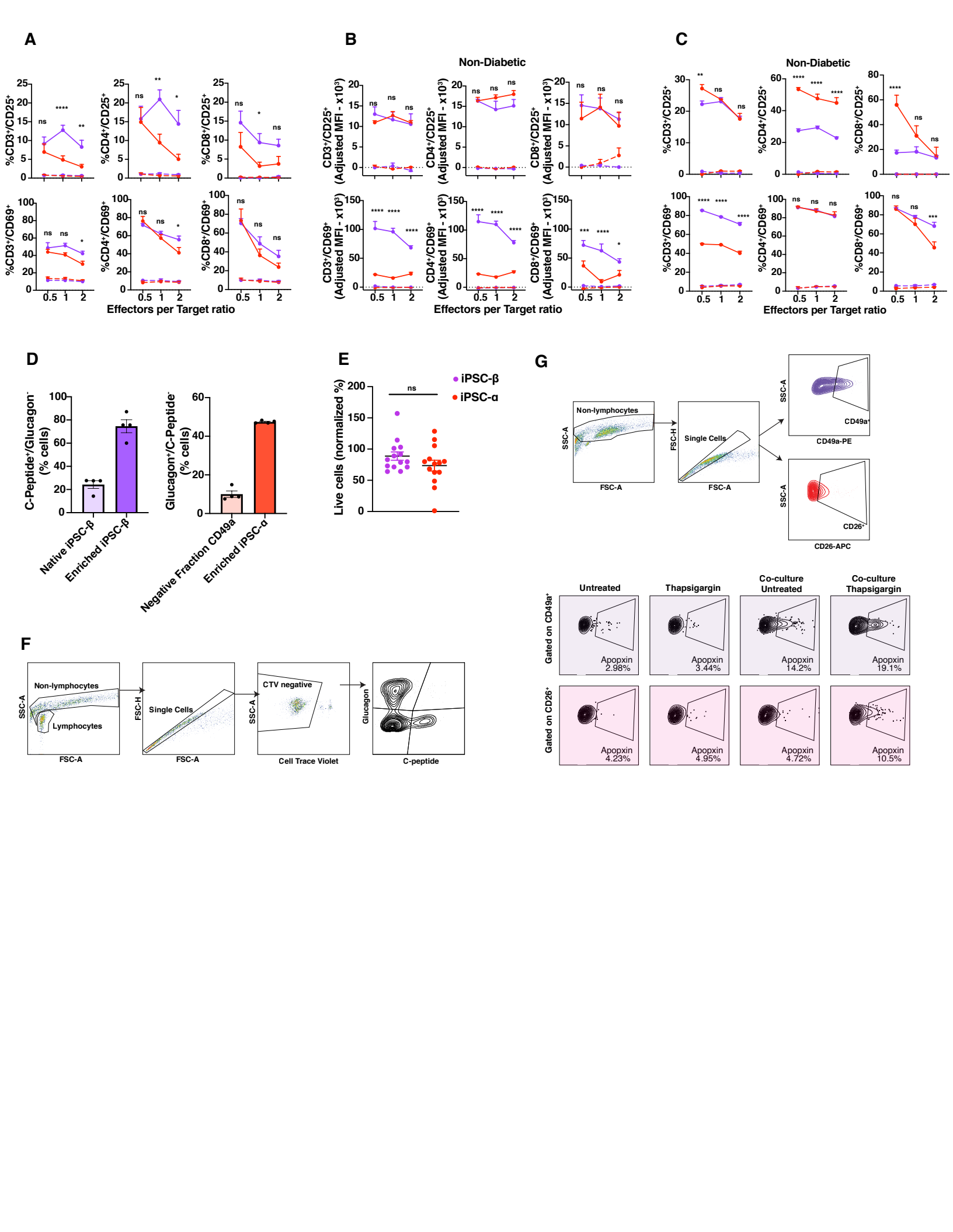

A
B
C
Non-Diabetic
Non-Diabetic
%CD3+/CD25+
%CD4+/CD25+
%CD8+/CD25+
%CD3+/CD69+
%CD4+/CD69+
%CD8+/CD69+
CD3+/CD25+ (Adjusted MFI - x10³)
CD4+/CD25+ (Adjusted MFI - x10³)
CD8+/CD25+ (Adjusted MFI - x10³)
CD3+/CD69+ (Adjusted MFI - x10³)
CD4+/CD69+ (Adjusted MFI - x10³)
CD8+/CD69+ (Adjusted MFI - x10³)
Effectors per Target ratio
Effectors per Target ratio
Effectors per Target ratio
D
C-Peptide+/Glucagon- (% cells)
Glucagon+/C-Peptide- (% cells)
Native iPSC-β
Enriched iPSC-β
Negative Fraction CD49a
Enriched iPSC-α
E
Live cells (normalized %)
iPSC-β
iPSC-α
ns
G
Non-lymphocytes
SSC-A
FSC-A
FSC-H
Single Cells
CD49a+
CD49a-PE
CD26-
CD26-APC
Untreated
Thapsigargin
Co-culture Untreated
Co-culture Thapsigargin
Gated on CD49a+
Gated on CD26+
Apopxin 2.98%
Apopxin 3.44%
Apopxin 14.2%
Apopxin 19.1%
Apopxin 4.23%
Apopxin 4.95%
Apopxin 4.72%
Apopxin 10.5%
F
Non-lymphocytes
Lymphocytes
SSC-A
FSC-A
FSC-H
Single Cells
CTV negative
Cell Trace Violet
Glucagon
C-peptide

Figure S4. Activation and killing by T cells is selective for iPSC-β in T1D and ND donors. Related to Figure 4.
(A-C) Cell surface T cell activation marker expression showed as (A and C) frequency or (B) MFI of $CD25^+$ or $CD69^+$ cells. Expression of the activation marker CD25 (top) and CD69 (bottom) on $CD3^+$, $CD4^+$ or $CD8^+$ gated T cells as indicated. PBMCs (gated on $CD3^+$, $CD4^+$ and $CD8^+$ populations) co-cultured with autologous iPSC-β or iPSC-α in increasing PBMCs:iPSC-β or iPSC-α ratios. (A) n=3 T1D iPSC donors, n=3 differentiation batches per donor line. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together. (B and C) n=1 ND iPSC donor, n=3 differentiation batches per donor line. Data are mean ± SEM. Two-way ANOVA. *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001, ns, not significant.
(D) Flow cytometry quantification of C-peptide and glucagon positive cells generated using the β differentiation protocol and enriched using CD49a and CD26 magnetic sorting. Percentage of enriched iPSC-β or iPSC-α cell populations are shown. n=1 T1D iPSC donor, n=3 differentiation batches per donor line.
(E) Percentage of live iPSC-β ($C\text{-peptide}^+/Glucagon^-$) or iPSC-α ($C\text{-peptide}^-/Glucagon^+$) from β or α differentiation protocols, treated with thap, and co-cultured with autologous PBMCs (2:1 effector to target ratio). Values are normalized from control wells without PBMCs. n=3 T1D. $T1D^1$, $T1D^2$ and $T1D^3$ were pooled together. Data are mean ± SEM. ns, not significant, student t-test.
(F) Representative gating strategy for acquiring absolute counts of live iPSC-β or iPSC-α after co-culture, gated for $C\text{-peptide}^+/Glucagon^-$ or $C\text{-peptide}^-/Glucagon^+$, respectively. Co-cultured PBMCs were stained with Cell Trace Violet (CTV) dye to distinguish from iPSC-β or iPSC-α.
(G) Gating strategy for apoptosis assay. Representative gating strategy for non-lymphocytes, single cells, $CD49a^+$, $CD26^+$, and $apopxin^+$.

Table S1. Patient samples. Related to figure 1.

Donor number	Age	Gender	Disease diagnosis	Age of diagnosis	BMI (CDC calculator)	HLA-A2	HLA risk alleles
T1D[1]	42	F	T1D	5	25.2	Positive	HLA-DR3, -DR4, -DQ8
T1D[2]	27	F	T1D	25	23.6	Positive	None
T1D[3]	44	M	T1D	25	26.4	Positive	HLA-DR3
ND	27	F	ND	N/A	21.4	Positive	None

Table S2. List of transcripts and target sequences used for Nanostring expression profiling. Related to STAR methods.

Gene Name	Accession	Position	Target Sequence
ARX	NM_139058.2	2622-2721	TGCACTCAGCGTGGTATGGTAAAAGTTTGTCCTCCCGTAGATTCTTACTGTGTTGTAGATACGGTAGGGTTCCTAGACAAATATTTATGTACTCAAGCCC
CHGA	NM_001275.3	293-392	CTGCGCCGGGCAAGTCACTGCGCTCCCTGTGAACAGCCCTATGAATAAAGGGGATACCGAGGTGATGAAATGCATCGTTGAGGTCATCTCCGACACACTT
GCG	NM_002054.2	296-395	TGGACTCCAGGCGTGCCCAAGATTTTGTGCAGTGGTTGATGAATACCAAGAGGAACAGGAATAACATTGCCAAACGTCACGATGAATTTGAGAGACATGC
INS	NM_000207.2	309-408	GGGTCCCTGCAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGACGCAGCCCGCAGGCA
NKX6-1	NM_006168.2	661-760	CTGGCCTGTACCCCTCATCAAGGATCCATTTTGTTGGACAAAGACGGGAAGAGAAAACACACGAGACCCACTTTTTCCGGACAGCAGATCTTCGCCCTGG
PDX1	NM_000209.3	414-513	GGGAGCCGAGCCGGGCGTCCTGGAGGAGCCCAACCGCGTCCAGCTGCCTTTCCCATGGATGAAGTCTACCAAAGCTCACGCGTGGAAAGGCCAGTGGGCA

SST	NM_001048.3	286-385	AGCTGCTGTCTGAACCCAACCAGACGGAGAATGATGCCCTGGA ACCTGAAGATCTGTCCCAGGCTGCTGAGCAGGATGAAATGAGG CTTGAGCTGCAGAG
ActB	NM_001101.2	1011-1110	TGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAATGAAGAT CAAGATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGATC GGCGGCTCCATCCT
G6PC2 (IGRP)	NM_021176.2	693-792	ACGGCCAGTCTGGGCACATACCTGAAGACCAACCTCTTTCTCTT CCTGTTTGCAGTTGGCTTTTACCTGCTTCTTAGGGTGCTCAACA TTGACCTGCTGT
GAD1	NM_000817.2	576-675	CAAAGGACCAACAGCCTGGAAGAGAAGAGTCGCCTTGTGAGT GCCTTCAAGGAGAGGCAATCCTCCAAGAACCTGCTTTCCTGTG AAAACAGCGACCGGG
GAD2	NM_000818.2	1246-1345	TGTATGCCATGATGATCGCACGCTTTAAGATGTTCCCAGAAGT CAAGGAGAAAGGAATGGCTGCTCTTCCCAGGCTCATTGCCTTC ACGTCTGAACATAG
IAPP	NM_000415.1	311-410	ATTCTCTCATCTACCAACGTGGGATCCAATACATATGGCAAGA GGAATGCAGTAGAGGTTTTAAAGAGAGAGCCACTGAATTACTT GCCCCTTTAGAGGA
PTPRN (IA2)	NM_001199763.1	477-576	TTCTCCAACGCTTACAAGGTGTGCTCCGACAACTCATGTCCCAA GGATTGTCCTGGCACGATGACCTCACCCAGTATGTGATCTCTCA GGAGATGGAGCG
SLC30A8 (ZNT8)	NM_173851.2	2166-2265	CAGATGCAACCAATTCATTCAGTCCACGAGCATGATGTGAGCA CTGCTTTGTGCTAGACATTGGGCTTAGCATTGAAACTATAAAG AGGAATCAGACGCA