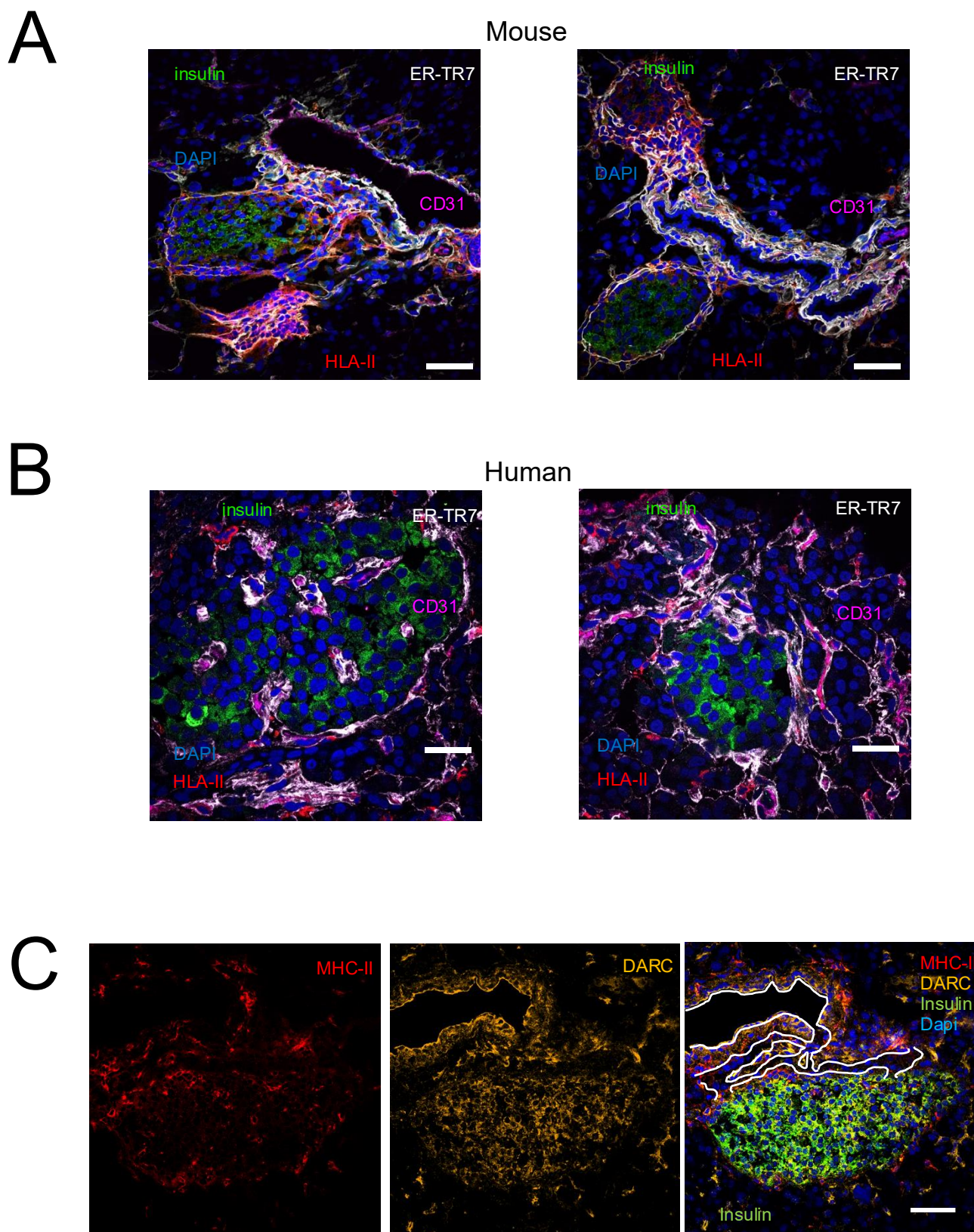


**Cell Reports, Volume 44**

## **Supplemental information**

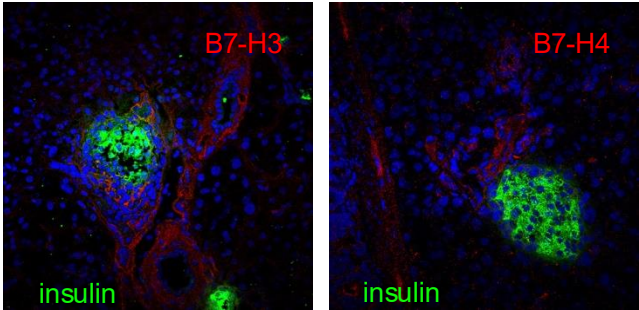
### **A vascular-associated fibroblastic cell controls pancreatic islet immunity**

**Don Clarke, Anne Costanzo, Siddhartha Sharma, Lisa Kain, Kelley W. Moremen, Jeremy Pettus, Alain Domissy, Peng Wu, Kim-Vy Nguyen-Ngoc, Denise Berti, Steven C. George, Christopher C.W. Hughes, Maïke Sander, and Luc Teyton**

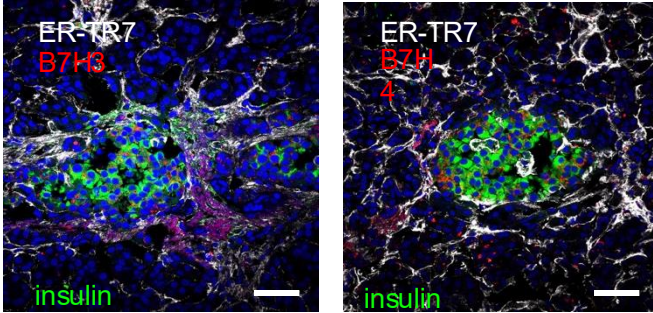
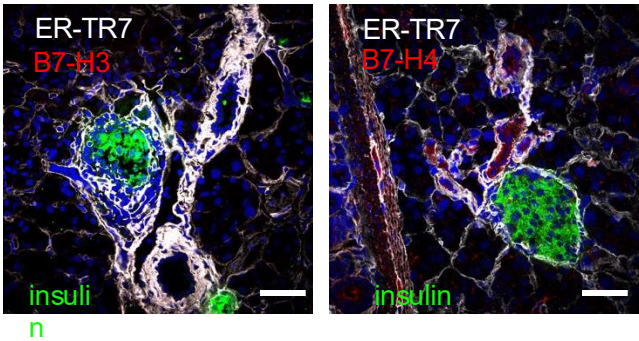
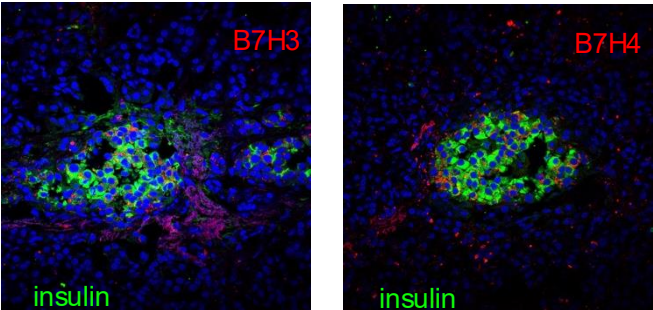


**Supplementary Figure 1: MHC class II expression in healthy islets from human and mice is associated to the vasculature.** Both in mice (**A**) and humans (**B**), MHC class II expressing cells were intimately associated to the vasculature as shown here with the CD31 staining, and the basement membrane stained with ER-TR7 that separated islets from vessels. (**C**) In mice, the same cells were shown to be closely associated to venules as signified by DARC/ACKR1 staining. In the overlay, the contour of the venules is highlighted in white. Scale bars are 50  $\mu\text{m}$ .

A- Mouse



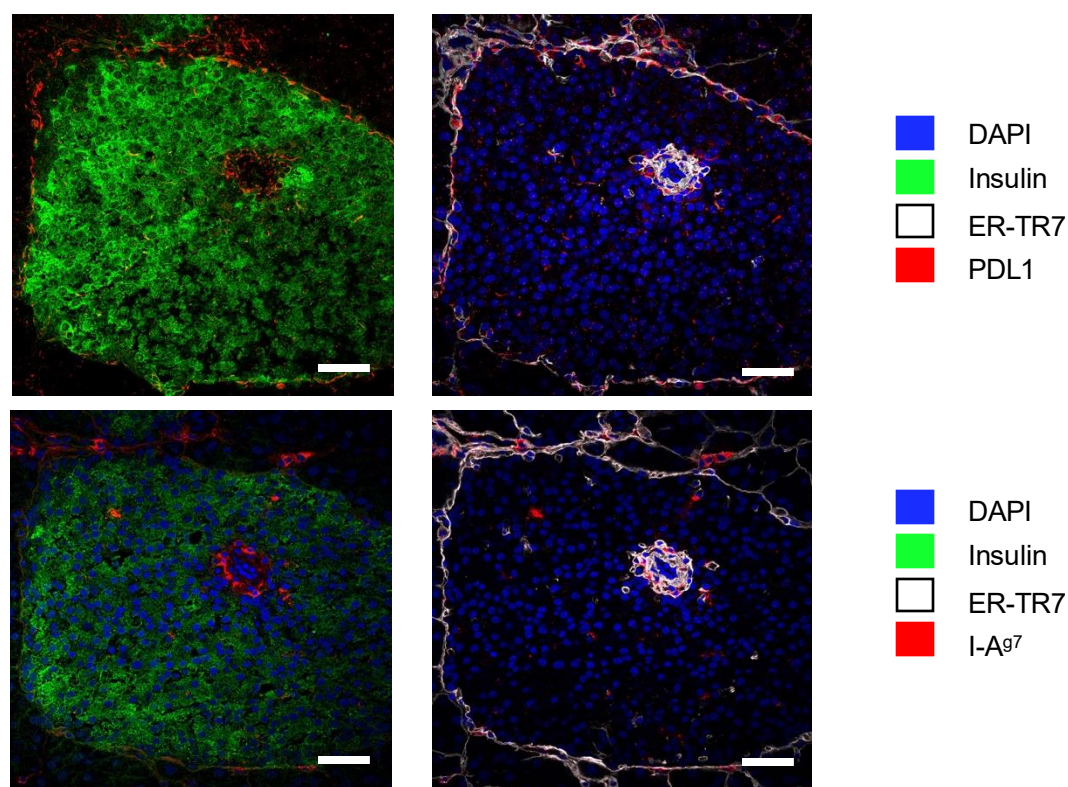
B-Human



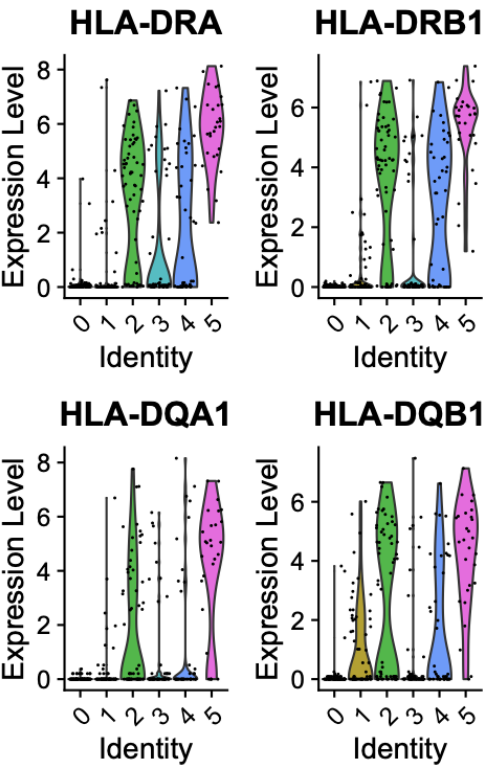
**Supplemental Figure 2: B7 family members expression in mouse and human islets.** Staining of murine (A) and human (B) islets with anti-B7-H3 and B7-H4 antibodies. In both species, the other two B7 family inhibitory receptors were expressed and associated to the vasculature but not directly associated to the islet as evaluated by the lack of costaining between the ER-TR7 boundary of the islet and B7-H3 and B7-H4. Scale bars are 50  $\mu$ m.



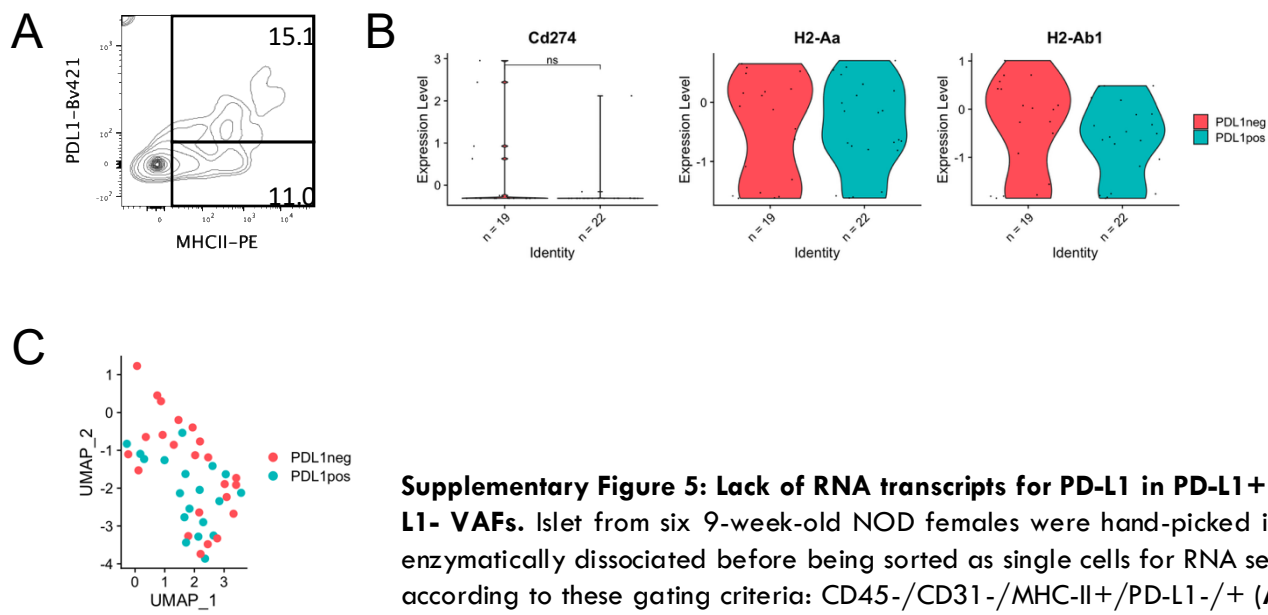
Figure S3



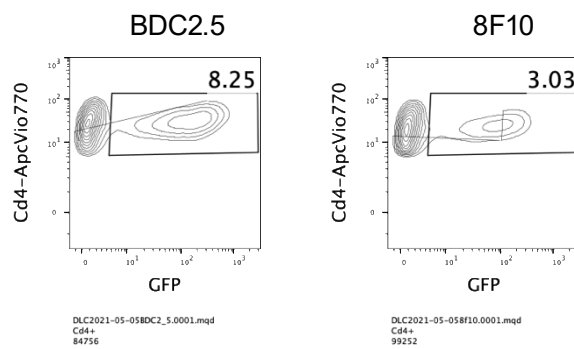
**Supplemental Figure 3: Co-localization of ER-TR7, PD-L1, and MHC-II in murine islets.** ER-TR7-PD-L1, and ER-TR7-MHC-II colocalization are shown in two successive sections of the same block (anti-PD-L1 and anti-MHC-II antibodies could not be used for direct costain as they are from the same species and same isotype. Scale bar is 50 μm.



**Supplementary Figure 4:**  
**HLA-DR and HLA-DQ expression across all 6 human clusters.**  
Clusters 0, 1, and 3 were not expressing HLA class II. The violins plots are centered log-ratio normalized counts.

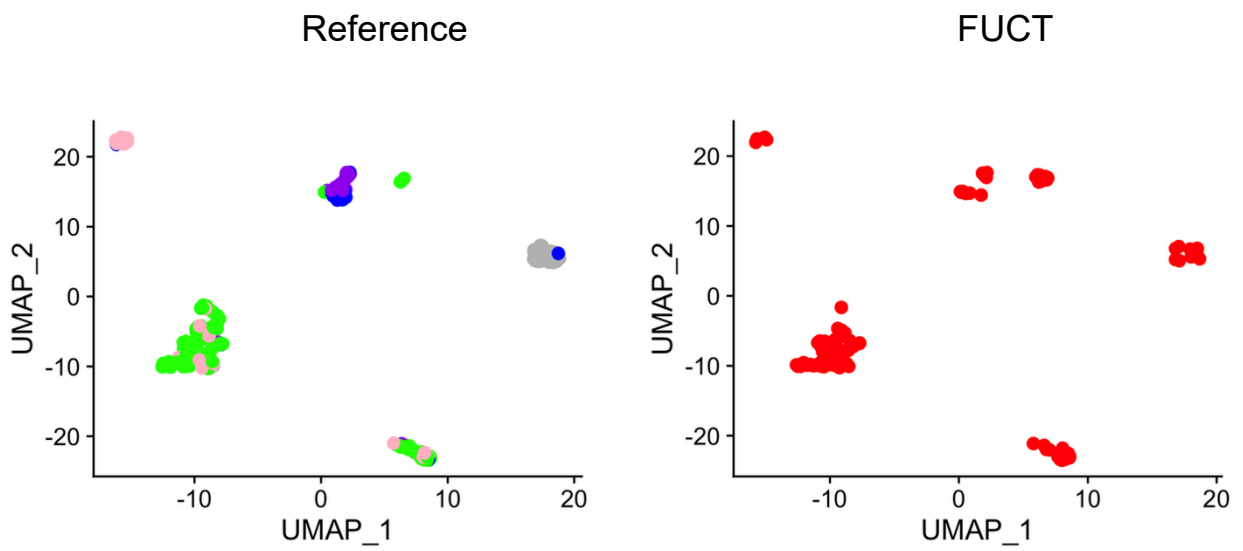


**Supplementary Figure 5: Lack of RNA transcripts for PD-L1 in PD-L1+ and PD-L1- VAFs.** Islet from six 9-week-old NOD females were hand-picked islets and enzymatically dissociated before being sorted as single cells for RNA sequencing according to these gating criteria: CD45-/CD31-/MHC-II+/PD-L1-/ + (**A**). Blood derived cells and cells without MHC-II transcript were removed prior to transcriptional analysis. (**B**) Violin plots show scaled expression of Cd274 (PD-L1), H2-Aa, and H2-Ab1 in PD-L1+ and PD-L1- VAFs. (**C**) In the UMAP built using all genes from PD-L1+ and - VAFs, both cell types are indistinguishable.



**Supplementary Figure 6: Transduction of primary BDC2.5 and 8F10 CD4 T cells with FucT-GFP.** The GFP-positive cells were sorted to >90% purity before being used in labeling experiments of dissociated islets.

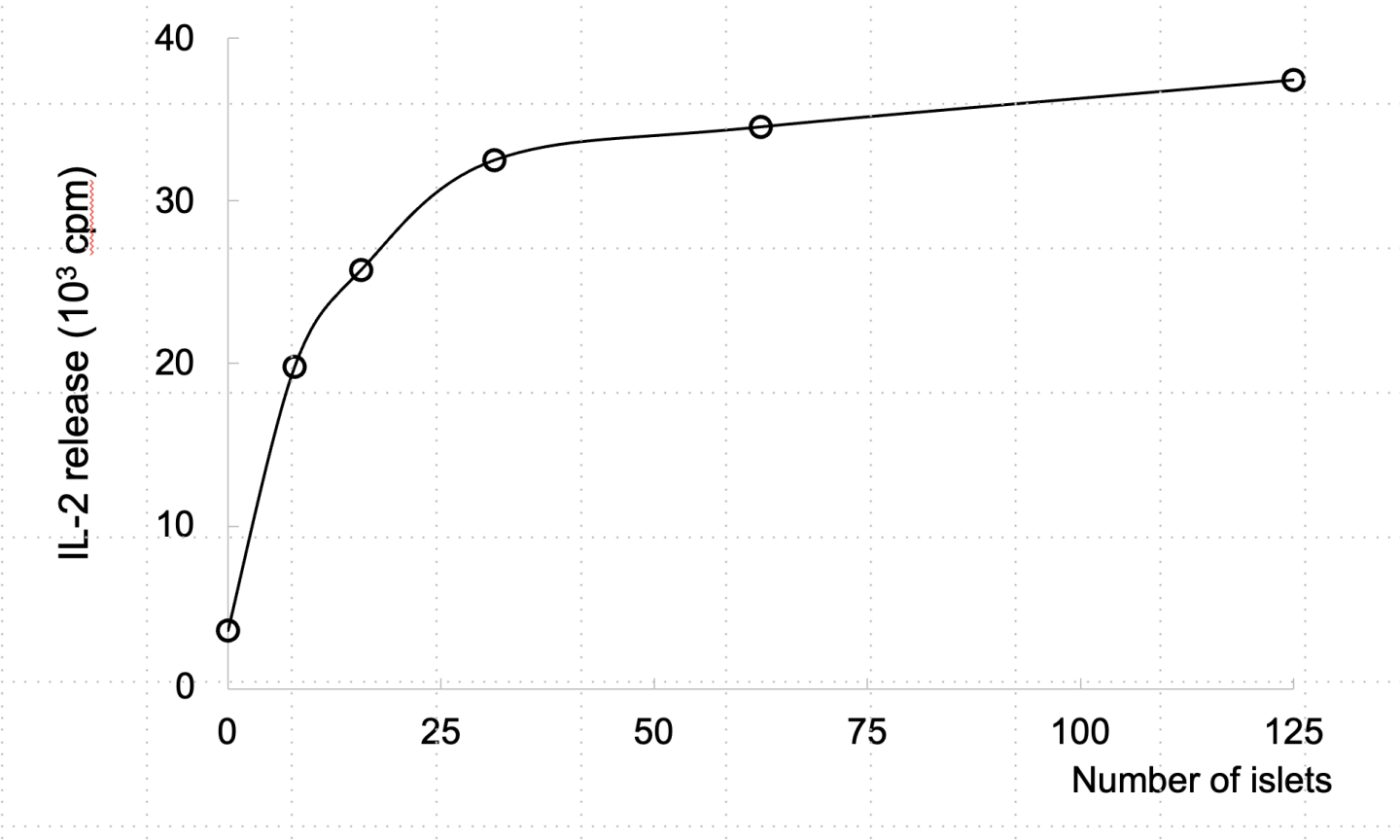
Figure S7



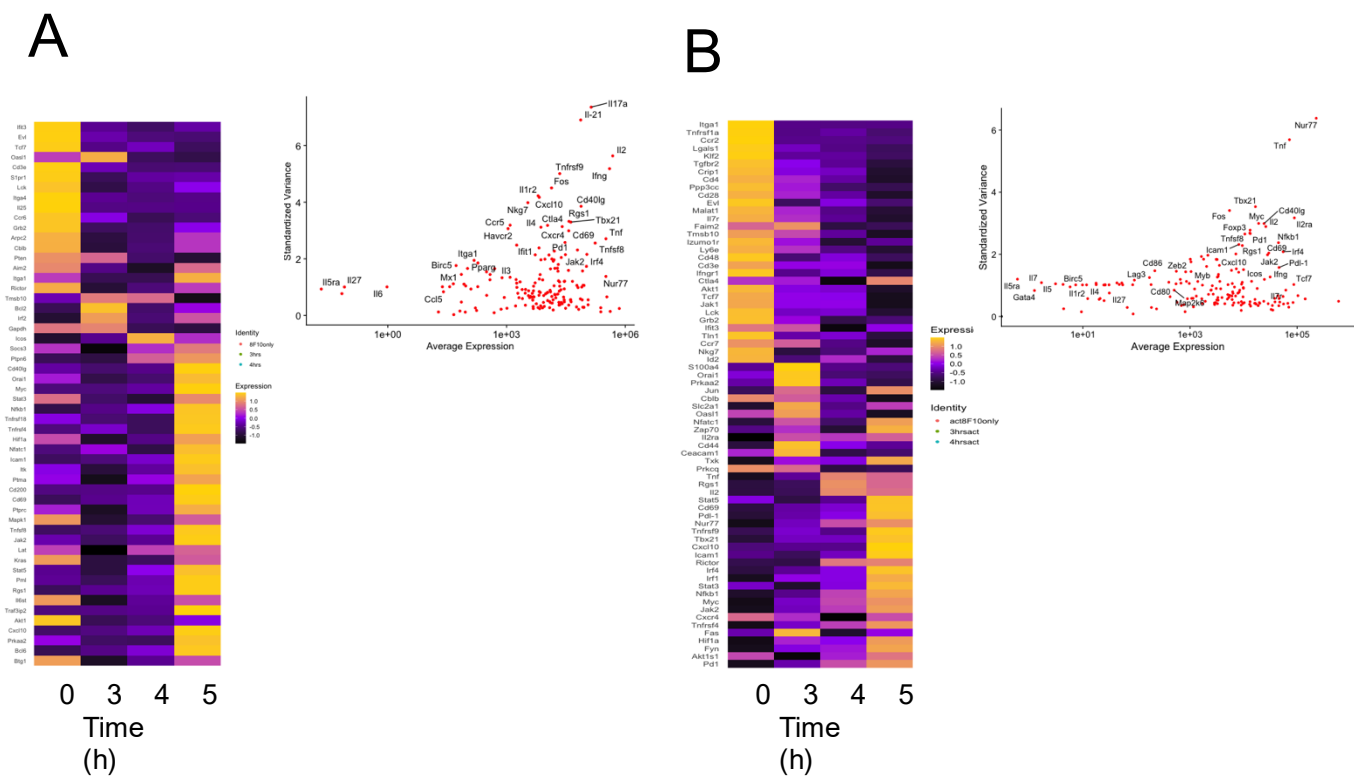
**Supplementary Figure 7: UMAPs of the fucosyl transferase labeling experiments.** On the left is the reference UMAP built with CD45+/CD11b+ (purple), CD45+/CD19+ (blue), CD45-/CD31+ (pink), and CD45-/CD31-/gp38+/- (green) flow sorted single cells. The UMAP on the right shows the location of the FucT labeled cells (red) in each cluster.



Figure S8

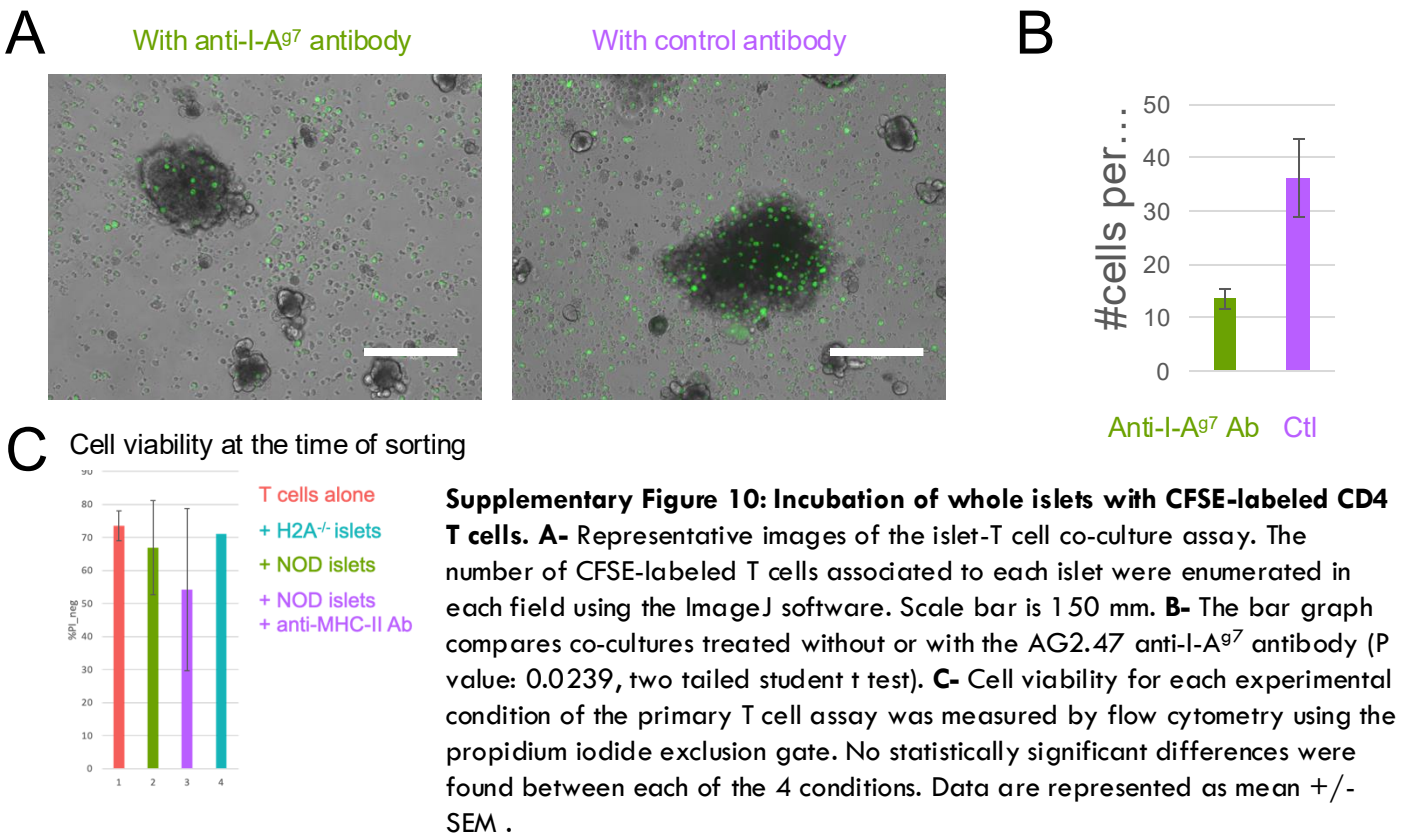


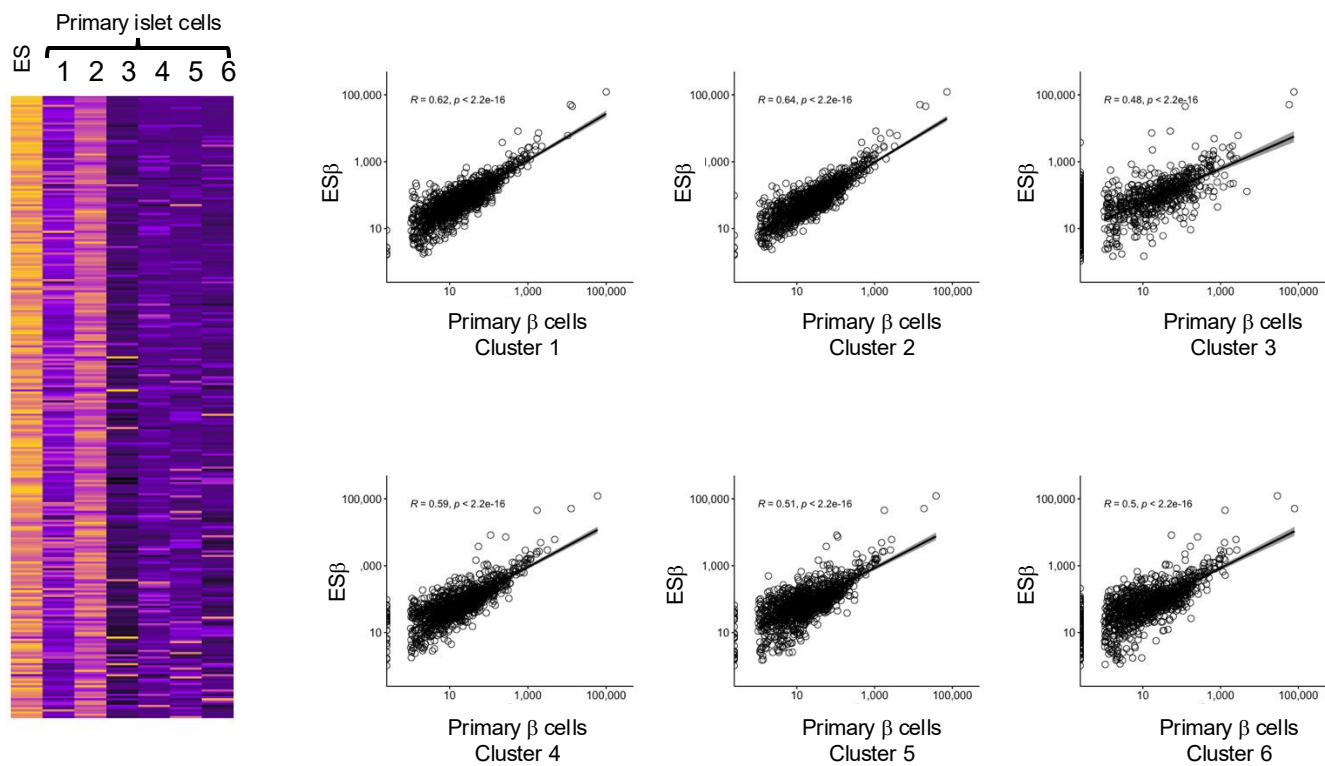
**Supplementary Figure 8: Whole islet stimulation of diabetogenic CD4 T cells.** Whole islet titration against a BDC2.5 clone (clone 33, TRAV16D/DV11\*01 F/TRAJ23\*01/TRBV15\*01/TRBJ27\*01/TRBD1\*01). IL-2 production was measured in supernatants using an IL-2-dependent NK cell line, after a 16 hr incubation of islets and hybridoma T cells.



**Supplementary Figure 9: A single cell-based T cell assay for the detection of primary T cell activation. A-** Primary naive 8F10 T cells from a 6-week-old animal were stimulated for various times on an anti-CD3/anti-CD28 plastic surface and examined with a panel of 183 T cell activation genes after single cell sorting in 96-well plates. **B-** In vitro pre-activated and rested (7 days) 8F10 primary T cells were stimulated on the same anti-CD3/anti-CD28 plastic surface and analyzed using the same 183 genes. For each of the experiment, the results are presented as heatmap and violin plots of scaled expression.

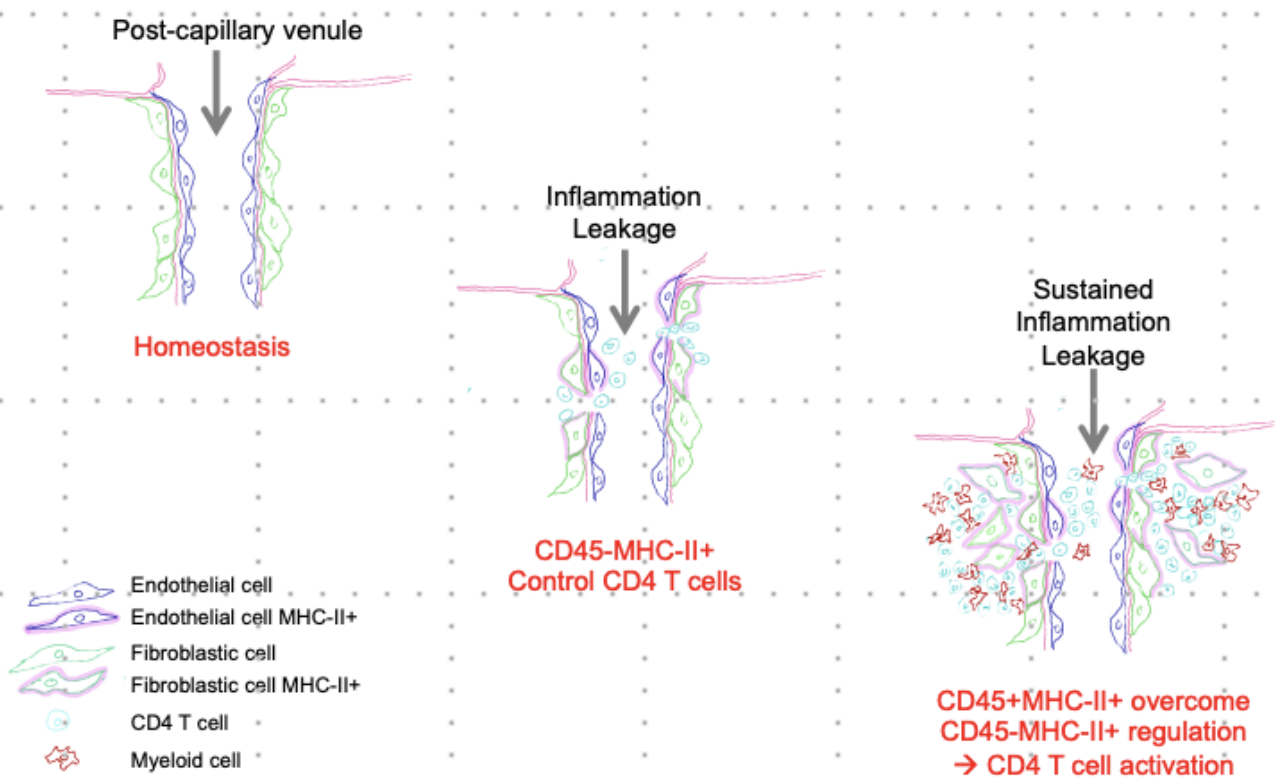
Figure S10





**Supplementary Figure 11: Comparison primary and ES-derived islets cells.** Heatmap of the single cell RNAseq of CD45- HLA-DR+ cells isolated from hESC-pseudo-islets (ES) and primary islets. Unsupervised clustering defined 6 clusters. The comparison of each of the 6 clusters with ES cells is presented as correlation plots on the right side of the figure.

Figure S12



**Supplementary Figure 12: Schematic representation of the elemental lesion leading to type 1 diabetes.** Three phases are represented at the post-capillary venule: Homeostasis, Stage 1, and Stage 2. During stage 1, inflammatory signals increase adherence and permeability of the endothelial barrier, and extravasation of T cells. As MHC-II is induced, the fibroblastic cells control CD4 T cell activation and block disease progression. Stage 2 is marked by the consequences of the sustained inflammation and the influx of professional antigen presenting cells capable of driving CD4 T cells to pathogenic phenotype and function.

## Supplementary Tables

Table S1: Gene panels used for RT-qPCR on the Biomark instrument.

### T cell panel 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bcl6	Cd4	Gata4	Il17A	Il4	Ppara	Aim2	Cxcr3	Ifngr1	Isg15	Oas1B	Stat4
B	Ccr2	Cd40	Gsk3A	Il1R2	Il4Ra	Pparg	Bcl2	Cxcr4	Il12Rb	Jak1	Oas2	Stat5
C	Ccr3	Cd80	Gsk3B	Il2	Il5	Ppargc1A	Ccr1	Fyn	Il18R1	Jak2	Oas1	Tgfb2
D	Ccr4	Cd86	Hprt	Il-21	Il5Ra	Pten	Ccr7	Icos	Il27R	Ly6E	Pd1	Tnfaip3
E	Ccr5	Cd8A	Icam1	Il25	Il6	Tbx21	Pdl-1	Ifi44	Irf1	Map2K6	Rsad2	Traf2
F	Ccr6	Ctla4	Ifng	Il27	Il7	Tnf	Cd44	Ifi44L	Irf2	Mapk8	Socs3	Vav1
G	Cd28	Foxp3	Il10	Il2Ra	Il7R	Tnfrsf1A	Ceacam1	Ifit1	Irf4	Mx1	Stat1	Zap70
H	Cd3E	Gapdh	Il12B	Il3	Nfkb1	Tnfrsf1B	Cxcl10	Ifit3	Irf7	Nur77	Stat3	Zeb2

### T cell panel 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Akt1	Cd200	Dnm1L	Gata3	Ikzf2	Jun	Lypd6	Myc	Ppp3Cc	Rgs1	Slc2A1	Tnfrsf18
B	Akt1S1	Cd200R1	Dpp4	Grb2	Il6St	Klf2	Maf	Nfatc1	Prdm1	Rictor	Slc2A3	Tnfrsf4
C	Arpc2	Cd27	Evl	H2-Aa	Il7R	Kras	Malat1	Nkg7	Prkaa2	Rptor	Srebf1	Tnfrsf9
D	Birc5	Cd40Lg	Faim2	H2-Ab1	Itga1	Lag3	Mapk1	Nras	Prkcq	Runx1	Stmn1	Tnfsf8
E	Btg1	Cd48	Fas	Havcr2	Itga4	Lat	Mapk3	Orai1	Ptma	S100A4	Tcf7	Traf3lp2
F	Calm1	Cd69	Fos	Hif1A	Itgb1	Lck	Mapk8	Pik3Ca	Ptpn6	S100A6	Tigit	Traf5
G	Cblb	Crip1	Foxo1	Hras	Itk	Lef1	Mtor	Plcg1	Ptpnc	S1Pr1	Tln1	Txk
H	Ccl5	Cxcr6	Foxp3	Id2	Izumo1R	Lgals1	Myb	Pml	Rap1A	Sell	Tmsb10	Zbtb16

### Mouse cell ID panel

	1	2	3	4	5	6	7	8	9	10	11	12
A	Acta2	Cd36	Cd8A	Csf2Rb	Fgfr3	H2-Dma	Igf2	Kdr	Mmp9	Ppy	Tek	Tnc
B	Acvr1	Cd3E	Clec7A	Cxcl13	Fgr	Hif1A	Il1A	Klf5	Nfatc1	Ptgs2	Tgfb1	Tnfsf11
C	Angpt1	Cd4	Col11A1	Des	Flt4	Iapp	Il1B	Lck	Nlrp3	Ptk2	Timp1	Vcam1
D	Anpep	Cd44	Col1A1	Egfr	Gcg	Icam1	Il34	Lepr	Pdgfa	Rspo1	Timp2	Vegfa
E	Bmp5	Cd74	Col1A2	Adgre1	Gfap	Icam2	Ins1	Ly75	Pdgfb	Sele	Tlr3	Vegfb
F	Bmp7	Cd80	Csf1	Fap	Ghrl	Icosl	Ins2	Mmp1A	Pdgfrb	Sfrp1	Tlr4	Wnt2B
G	Cd14	Cd83	Csf1R	Fcgr1	Gm13889	Ifng	Itgax	Mmp2	Pdpn	Spp1	Tlr7	Wnt4
H	Cd24A	Cd86	Csf2Ra	Fgfr1	H2-Aa	Igf1	Itgb1	Mmp3	Pecam1	Sst	Tlr9	Zap70



**Table S2: Statistical evaluation  
p values Figure 5a**

corrected p_val	condition	gene	corrected p_val	condition	gene	corrected p_val	condition	gene
7.137E-52	T cells only	Il7r	2.729E-38	NOD islets	Stat3	1.862E-13	H2Ako	Ifit1
9.666E-41	T cells only	Itga4	4.274E-31	NOD islets	Tnfaip3	2.097E-07	H2Ako	Irf7
1.391E-39	T cells only	Tmsb10	3.203E-28	NOD islets	Ifit3	1.302E-06	H2Ako	Rsad2
1.419E-28	T cells only	Jak1	1.934E-25	NOD islets	Socs3	1.487E-06	H2Ako	Isg15
8.401E-26	T cells only	Lef1	1.556E-22	NOD islets	Ifit1	2.854E-05	H2Ako	Stat1
6.000E-25	T cells only	Evl	4.092E-20	NOD islets	Isg15	7.266E-03	H2Ako	Slc2a1
1.442E-24	T cells only	Cd3e	2.254E-18	NOD islets	Gapdh	4.782E-02	H2Ako	Gapdh
5.137E-24	T cells only	Lck	8.916E-12	NOD islets	Tnfsf8	4.969E-02	H2Ako	Ptma
1.245E-20	T cells only	Tcf7	2.711E-11	NOD islets	Oas2	9.964E-21	Anticl2	Slc2a1
1.679E-16	T cells only	Cd28	3.858E-11	NOD islets	Rgs1	4.545E-19	Anticl2	Oasl1
9.833E-16	T cells only	Pik3ca	1.942E-10	NOD islets	Ctla4	7.658E-16	Anticl2	Tmsb10
2.389E-15	T cells only	Klf2	3.963E-09	NOD islets	Nfkb1	1.266E-12	Anticl2	Dpp4
1.786E-14	T cells only	Kras	4.282E-09	NOD islets	Stat5	2.184E-12	Anticl2	Ptma
6.940E-14	T cells only	Cd4	1.421E-08	NOD islets	Tnfrsf1b	2.146E-09	Anticl2	Il4ra
5.944E-13	T cells only	Cd40lg	3.524E-08	NOD islets	Icos	4.793E-06	Anticl2	Calm1
8.844E-12	T cells only	Fyn	5.452E-08	NOD islets	Ccr2	1.928E-04	Anticl2	Irf7
1.144E-11	T cells only	Lat	3.184E-07	NOD islets	Rsad2	5.212E-04	Anticl2	Socs3
3.257E-08	T cells only	Plcg1	1.614E-06	NOD islets	Id2	5.709E-04	Anticl2	Isg15
5.088E-08	T cells only	Tln1	3.715E-06	NOD islets	Pml	1.828E-03	Anticl2	Pml
6.947E-08	T cells only	Arpc2	6.572E-06	NOD islets	Tnfrsf18	2.366E-03	Anticl2	Il12b
7.064E-08	T cells only	Nfatc1	1.109E-05	NOD islets	Oas1b	3.304E-03	Anticl2	Rsad2
7.681E-08	T cells only	Ptpcr	2.314E-05	NOD islets	Bcl6	1.165E-02	Anticl2	Stat1
1.001E-07	T cells only	Sell	2.638E-05	NOD islets	Cxcl10	1.922E-02	Anticl2	Oas2
8.978E-07	T cells only	Bcl2	3.503E-05	NOD islets	Il2ra	2.891E-02	Anticl2	Ccr2
1.380E-06	T cells only	Itgb1	1.351E-04	NOD islets	Hif1a	4.170E-02	Anticl2	Ifit3
1.127E-05	T cells only	Crip1	2.444E-04	NOD islets	Irf7			
1.326E-05	T cells only	Pten	2.902E-04	NOD islets	Cxcr6			
2.659E-05	T cells only	Traf5	3.230E-04	NOD islets	Ifngr1			
5.825E-05	T cells only	Lgals1	8.065E-04	NOD islets	Ly6e			
1.911E-04	T cells only	Runx1	1.725E-03	NOD islets	Irf2			
2.903E-04	T cells only	Ccr6	2.477E-03	NOD islets	Cd69			
3.192E-04	T cells only	Malat1	2.963E-03	NOD islets	Stat1			
5.142E-04	T cells only	Il25	4.778E-03	NOD islets	Ceacam1			
6.314E-04	T cells only	Mtor	6.131E-03	NOD islets	Tnf			
7.469E-04	T cells only	Tgfb2	7.653E-03	NOD islets	Cxcr4			
1.808E-03	T cells only	Akt1	8.125E-03	NOD islets	Txk			
2.731E-03	T cells only	Gsk3a	8.768E-03	NOD islets	Mx1			
7.376E-03	T cells only	Orai1	1.020E-02	NOD islets	Myc			
9.011E-03	T cells only	Mapk3	1.463E-02	NOD islets	Il18r1			
1.306E-02	T cells only	Cd48	1.496E-02	NOD islets	Oasl1			
2.078E-02	T cells only	Faim2						

**Tables S3, and S4: Excel files**

## Supplementary methods:

### ***Method S1: Imaging islets co-cultured with T cells***

Preactivated 8F10 splenocytes were cultured for 7 days in DMEM-10% FBS with 1 u/ml IL-2. Cell labeling was performed with 5 $\mu$ M Vybrant CFDA SE (Invitrogen, CFSE) for 10 minutes at RT in DMEM-10% FBS. Cells were washed once with DMEM 10% FBS. 3x10<sup>4</sup> T cells were cultured with 150 islets in flat bottom 96 well plates for 5 hours at 37°C. Bright field and fluorescent images were captured with a EVOS M5000 inverted microscope (Invitrogen). Number of CFSE labeled cells per islet treated with MHC class II blocking antibodies (AG2.47) or IgG2a control (MOPC-173, Biolegend) were counted using the ImageJ software. Automatic thresholding and binary filters were used to build masks of the islets. Islets extending past the edge of the frame and small debris were erased. The inverse of each islet mask was subtracted from each image. CFSE+ cells were selected with color thresholding and counted, then divided by the number of islets in each mask.

### ***Method S2 Data analysis***

For Figure S11, the average counts per million for x number of ES derived endocrine cell specific genes were used to perform Kendall tau-b correlations between the 6 clusters of cells from Figure 7.