Calorie restriction suppresses aging alpha cell pro-inflammatory signaling.

Michael Schleh[1], Amanda Cambraia[1], Melanie Cutler[1], Gabriel Ferguson[1], Rafael Arrojo e Drigo[1,2,3,4]

[1] Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN
[2] Vanderbilt Diabetes Research and Training Center, Vanderbilt University Medical Center, Nashville, TN
[3] Center for Cell Systems Biology, Vanderbilt University School of Medicine, Nashville, TN
[4] Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, TN

Address of Correspondence:
Rafael Arrojo e Drigo
718 Light Hall
2215 Garland Ave
Nashville, Tennessee 37232
r.drigo@vanderbilt.edu

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Abstract

Aging is a major risk factor for insulin resistance and type 2 diabetes, driven in part by declining pancreatic beta cell function. While calorie restriction (CR) initiated early in life improves metabolic health and preserves beta cell function, its impact when initiated later in life remains unclear. We implemented a two-month, 20% CR intervention in 72-week-old male mice and assessed *in vivo* glucose homeostasis and beta cell function using meal tolerance tests, single cell RNA-sequencing, and confocal microscopy. We found that old mice exposed to CR have significantly improved glucose tolerance due to higher insulin sensitivity, which drives a two-fold reduction in glucose-stimulated beta cell insulin secretion. At the molecular level, CR enhanced beta cell proteostasis by downregulating ER stress pathways. Remarkably, CR reprogrammed the alpha cell transcriptome to suppress antigen presentation pathways and cytokine signaling pathways, including major histocompatibility complex class I (MHC-I) signaling. These transcriptional changes correlated with profound reduction in the density of adaptive immune cells in aging islets, including a near-complete loss of cytotoxic $CD8^+$ T cells due to weakened intercellular communication between antigen-presenting cells with T and B lymphocytes. Importantly, aging human alpha cells from non-diabetic donors display similar proinflammatory phenotypes, including higher expression of MHC-I antigens that correlates with higher density of $Cd8^+$ cells in aging human pancreases. Together, these findings demonstrate that CR remodels aging alpha and beta cell structure-function to modulate immune cell interactions in aging pancreases to mitigate age-related immune activation and islet inflammation.

-243 words

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Introduction

Aging is a major risk factor for type 2 diabetes (T2D), affecting 29.2% of adults 65 and older in the United States[1]. Aging is associated with poor glycemic control due to progressive loss of peripheral insulin sensitivity and pancreatic beta cell function[2-4]. Beta cells are located in the islet of Langerhans next to glucagon-secreting alpha cells and somatostatin-secreting delta cells; together, these major islet cell types regulate glucose homeostasis[5]. These major islet cell types remain largely post-mitotic throughout most of their host organism lifespan[6, 7], and therefore are under constant pressure to maintain function that supports normal glucose homeostasis. Moreover, postmitotic cells are continuously exposed to age-associated stressors that can increase accumulation of molecular and cellular damage, which causes stress that impairs cell structure-function and contribute to age-associated disease. Accordingly, aging beta cells suffer from loss of cellular identity markers, increased DNA damage and senescence, up regulation of ER stress, compromised calcium homeostasis, impaired autophagy, and increased transcriptional noise, amyloid deposition, and islet fibrosis[8-11]. These molecular signatures are linked changes to beta cell function in aging mice and humans[9]. It remains unknown how aging impacts alpha cell and delta cell function.

Calorie restriction (CR) is a well-established dietary intervention that extends life- and health-spans by delaying the onset of age-related cell dysfunction and disease[12-15]. CR regimens of typically 10–40% restriction of energy intake enhance metabolic homeostasis in young and aged animal models, where higher restriction amounts can lead to adverse effects such as muscle wasting and immunosuppression[16]. At the molecular level, CR acts via modulation of major nutrient-sensing pathway nodes, namely the suppression of insulin and IGF1 signaling[17], inhibition of mammalian target of rapamycin complex (mTORC1) activity and activation of AMPK[18], reduction of oxidative stress and inflammatory tone[19], and activation of autophagy[20, 21]. While many of these pathways have been explored for their therapeutic potential via pharmacological targeting using rapamycin (mTORC1 inhibition)[22], metformin

(AMPK activation)[23], or resveratrol[24]. However, many of these interventions exhibit significant off-target effects, including immunosuppression and beta cell failure[25, 26], that complicate translation of these approaches to delay or treat age-related human disease.

CR and CR-mimicking interventions (i.e., intermitted fasting and time restricted feeding) in young adult mice promotes beta cell health, function, and longevity by enhancing peripheral insulin sensitivity that in turn reduces the metabolic demand for beta cell insulin secretion[27-30]. These pro-longevity effects are associated with increased expression of beta cell identity and circadian gene expression regulators, improved autophagy and protein homeostasis, mitochondrial homeostasis and ATP production, and reduced beta cell DNA damage and senescence[27-30]. Similar adaptive changes to beta cell function are also observed in other insulin-sensitizing strategies, such as exercise[31, 32], SGLT2 inhibition[33], and incretin agonist treatments[34]. In contrast, CR has blunted benefits when initiated in mid- to late-life[35], and as beta cells age they lose their relative plasticity to adapt to changes to whole body metabolism and dietary interventions may be limited due to irreversible epigenetic changes[36]. Whether CR and other pro-longevity approaches can rescue beta cell aging signatures in mature and aged organisms remains unclear.

Here, we investigated whether short-term CR would improve systemic glucose homeostasis and modulate beta cell structure-function to reverse the hallmarks of beta cell aging in old mice. Two months of 20% CR significantly improved glucose homeostasis and triggered a 2-fold reduction in beta cell insulin release. Single-nucleus RNA sequencing (snRNA-seq) of isolated islets revealed that CR has a limited effect on aging beta cell gene expression and marked by downregulation of a small set of genes mainly associated with heat-shock protein (HSP) folding machinery. Strikingly, CR triggered a significant re-organization of the aging alpha cell transcriptome with lower expression of several pro-inflammatory genes, including key mediators of immune signaling via enhanced MHC class I-dependent antigen presentation. Importantly, aging human islets and alpha cells from non-diabetic donors have

significantly higher expression of several pro-inflammatory genes, which correlates with increased density of activated $CD8^+$ T cells in aging human pancreases.

Together, our findings suggest that old alpha cells could be involved in the regulation of immune cell infiltration in the aging pancreas and contribute to age-associated pancreas and islet inflammation phenotypes. These aspects are suppressed by CR, which remodels the structure-function of aging islet cells and immune cells to reduce organ inflammation. Therefore, CR may offer a dual mechanism for improving metabolic health and reverse islet inflammation in aging pancreases.

Results and Discussion

Calorie restriction improves glucose tolerance and reduces insulin secretion in old mice. To investigate the potential of CR to reverse beta cell aging signatures in old mice, we exposed 72-week-old C57/BL6 male mice to a 2-month 20% CR feeding regimen, while age-matched controls remained on ad libitum (AL) diet (**Figure 1A**). As expected, CR mice consumed 80% of total kcal intake compared to AL-fed controls (**Figure 1B, S1A**). This intervention led to a steady decline in body weight over 2 months, with an average of ~12% body weight loss (**Figure 1C-D, S1B-C**) due to a predominant loss of body fat mass (**Figure 1E-F**). Tissue-specific weight loss was also observed in the liver and epididymal white adipose tissue (eWAT) of CR mice (**Figure 1G, S1-G**).

To investigate the impact of CR on whole body metabolism, we placed old AL and CR mice in metabolic cages for the last week of the diet intervention. While overall energy expenditure and oxygen consumption were comparable between CR or AL mice (**Figure S2A-D**), carbon dioxide production and carbohydrate oxidation were significantly increased in CR mice during the dark period (**Figure S2E-H**). These results indicate that CR mice have a higher preference for carbohydrate metabolism during their active phase and quickly shift towards fatty acid metabolism in phases of inactivity and when food is unavailable. Next, we performed mixed meal tolerance tests (MTTs; 2 g glucose/kg body weight using, Ensure Original) to investigate

the impact of CR on old mouse glucose homeostasis and basal and glucose-stimulated beta cell insulin release mechanisms. We performed a baseline MTT before starting the CR intervention at 72 weeks. After 2 months of CR, old mice had significantly improved glucose tolerance post-intervention at 80 weeks (**Figure 1H**). This phenotype was accompanied by increased insulin sensitivity, and a ~50% reduction in fasting and in glucose-stimulated insulin secretion (**Figure 1I-J**). Like in younger CR mice[27], CR in old mice preserved beta cell function by significantly reducing the relative amount of beta cell insulin secretion required to support normoglycemia (**Figure 1K-L**).

Together, these data demonstrate that CR in old mice leads to significant weight loss and improvement of systemic glucose homeostasis mechanisms via enhanced peripheral insulin sensitivity. This also reduces the amount of basal and glucose-stimulated beta cell insulin secretion, thus suggesting that the potential of old beta cells to adapt to significant improvements in insulin sensitivity are not impacted during aging.

CR downregulates ER-dependent protein and antigen processing in aging islet cells. In young adult mice, CR re-wires the landscape of beta cell gene regulatory networks to increase the expression of beta cell identity and longevity genes. To investigate how CR impacted old beta cell transcriptional heterogeneity and gene expression, we applied single nucleus RNA sequencing (snRNA-seq, 10X genomics) of frozen islet pellets extracted from AL or CR mice (**Figure 2A**). A total of 32,380 cells were sequenced (AL=17,344, CR=15,306) to identify all major islet cell types, including beta cells (*Ins1*, *Ins2*, and *Ero1b*), alpha cells (*Gcg*), delta cells (*Sst*), endothelial cells (*Pecam1*), and pancreatic polypeptide cells (*Ppy*) (**Figure 2B-C**). Approximately 65% of the sequenced cells were beta cells, and CR did not impact the relative composition of the heterogeneous beta cell population (**Figure 2D**), as well as the ratio of beta cells to alpha and delta cells in islets (**Figure S3A**).

Pseudobulk analysis of beta cells, followed by KEGG and STRING pathways analysis, revealed downregulation of genes related to ER protein processing and folding (*Hspa1a/b/HSP70, Hspa8, Hsp90ab1, Dnaja1/HSP40, and Calr)*, as well as cell stress (*Ypel2*, *Txnip*) and insulin synthesis (*Ins1*) genes (**Figure 2E-H**). CR also up-regulated a small number of genes involved in AKT signaling cascade regulation (*Kctd20, Fkbp5*), and the transcription factor *Stat3* and the transcription co-activator Pgc1a (*Ppargc1a*) (**Figure 2E**). Remarkably, CR induced a stronger effect on alpha cells, where it modulated ~5-times more genes than beta cells (184 vs. 36 differentially expressed genes; **Table S1-2**). Differential gene expression and pathway enrichment analysis of old CR alpha cell gene signatures revealed upregulation of pathways associated with glucagon, glucose and fatty acid metabolism (i.e., *Gcg, Gys2, and Acacb*) and Pgc1a (*Ppargc1a)* (**Figure 3A-B**). In addition, CR suppressed the expression of n=159 genes involved in T cell receptor (TCR) signaling, antigen presentation, and immune cell recruitment pathways (i.e., *Cd44, Cd28, Il7r, Il18r1, and Ccr7;* **Figure 3B-C**), as well as genes involved in cell-cell signaling and MHC class I (*H2-Q7*, *H2-Q6*, *H2-K1*) and class II (*H2-Aa*, *H2-Ab1*, *H2-Ob*) antigens **(Figure 3D–E)**.

These findings indicate that CR has a limited effect on the transcriptome of aging beta cells, which contrasts with our previous findings in young adult mice exposed to CR[27]. These results could be explained to age-dependent changes on the beta cell epigenome that limit the plasticity of aging beta cell gene expression patterns[36]. Moreover, our data suggests that CR-mediated remodeling of the beta cell transcriptome may not be required for the functional beta cell adaptations observed *in vivo* in young[27] and old mice (**Figure 1**). More importantly, CR-mediated suppression of age-associated pro-inflammatory responses in aging alpha cells indicates that this cell type could play a role in mediating islet and pancreas immune responses, as recently suggested in spatial mapping experiments in NOD mice[37].

CR alpha cells have reduced adaptive immunity and TCR signaling. To further investigate the impact of CR-mediated changes to alpha cell pro-inflammatory gene expression, we analyzed the relative composition and transcriptional signatures of immune cell types identified in our snRNA-seq dataset (**Figure 4A**). This revealed a significant decrease in B cells, T cells, proliferating B cells, and antigen presenting cells (APCs) in CR mice (**Figure 4B**). Notably, the number and transcriptional profile of islet-resident macrophages remained relatively constant between AL or CR diets (**Figure 4B**), thus indicating that CR could modulate the recruitment of immune cells to the aging pancreas via adaptive immune signatures and does not impact resident immune cells. Accordingly, CR mice had a marked reduction in islet-associated T cells, including cytotoxic $CD8^+$ T cells that were nearly ablated (**Figure 4C-D**). The remaining CD8+ cells had lower expression of granzyme K (*Gzk*), IL-2 receptor (*Cd25*), and *Txk*; all markers of activated T cells (**Figure 4E**). Similar changes were observed in B cells **(Figure 4F-G)**.

Next, we reconstructed cell-cell communication networks to investigate how CR impacts intercellular communication between islet endocrine and immune cells in AL and CR mice (**Figure 4H**)[38]. This approach revealed an overall downregulation of intercellular communication between immune cells and alpha cells, beta cells, and delta cells. This was underscored by a significant reduction in Cd45- and MHC-based communication between old alpha cells and T- and B-cells in CR mice (**Figure 4I-J**).

These findings suggest that suppression of age-related pro-inflammatory gene expression in alpha cells by CR alters receptor-ligand interactions between alpha cells and immune cells. This phenotype is marked by reduced $CD8^+$ cytotoxic T cells and reduced expression of granzyme K, which suggests that CR could reduce age-associated islet inflammation phenotypes. It is unclear whether alpha and beta cells respond to CR through cell-autonomous mechanisms or whether intercellular crosstalk between neighboring endocrine cells contributes to this response.

Upregulation of TCR activation pathways in aging non-diabetic human islets. To translate our findings to the human setting and to determine if aging non-diabetic human islets have similar pro-inflammatory signatures, we leveraged a comprehensive human islet proteomics dataset recently made public by the Human Islet Data Analysis and Sharing (HI-DAS) consortium (**Figure 5A**)[39, 40]. Here, data from non-diabetic young adults (27±5 years) and old adults (71±4 years) was analyzed to identify proteins significantly enriched in aged samples (**Table S2**). Pathway enrichment analysis (KEGG) uncovered increased signatures of amino acid metabolism, senescence, and ER stress in old islets (**Figure 5B**), as expected. Specifically, these changes mapped to age-associated increase of beta cell immaturity markers (RBP4 and upregulated mTORC1 activity regulators, **Figure 5C**), senescence (CDKN1A/p21, **Figure 5D**), impaired proteostasis and/or protein misfolding by the UPR (ATF6, CHOP, and PERK; **Figure 5E**), and increased amino acid metabolism (BCAT1) and mTORC1 activation proteins (**Figure 5F**). In addition, we also identified an activated immune system response in aging islets, which had significantly higher expression of MHC-I and TCR proteins, including HLA-A, CD28, and CD44 (**Figure 5G**), which were also enriched in aging AL mice (**Figure 3C-E**). These results demonstrate that aging human islets experience an active cellular stress response associated with increased amino acid metabolism signatures and links this phenotype with increased expression of pro-inflammatory signatures of TCR activation and immune cell recruitment.

Aging non-diabetic human pancreases have several signs associated with active pro-inflammatory signaling, including increased fibrosis, higher incidence of acinar-to-ductal metaplasia (ADM), angiopathy, and pancreatic adiposity[41]. To validate our previous isolated mouse and human islet analysis *in situ*, we analyzed high-dimensional imaging mass cytometry (IMC) data from the human pancreas analysis program (HPAP)[42, 43]. Here, non-diabetic human pancreases were stratified by age to divide samples into two age groups: young adult (22-40 years) and old adult (47-58 years). Individual cells were clustered into unique cell types based on marker classification; these included alpha (GCG, 7.8%), beta (C-peptide, 6.8%), delta (SST,

0.8%), pancreatic polypeptide (PP; 12%), endothelial cells (CD31, 6%), Epithelial/Acinar cells (CK19/CA2, 48%), Macrophages (CD68, 9%), and T cells (CD4=0.1%, CD8=1.2%). As expected, we observed an age-associated decrease in beta cell identity markers (PDX-1 and NKX6-1; **Figure 5H**), and up-regulation of islet amyloid polypeptide (IAPP) in both alpha and beta cells (**Figure 5I**). In alpha cells, aging was associated with increased expression of MHC-I proteins HLA-ABC and attenuated abundance of MHC-2 HLA-DR protein, whereas beta cells had only attenuated HLA-ABC abundance during aging (**Figure 5J**). Next, we subset the IMC dataset to identify $CD45^+$ immune cells and found no changes to overall macrophage density ($CD68^+$ cells expressing CD45) or in the expression of the anti-inflammatory macrophage marker CD163 (**Figure 5K**). We also observed an age-induced increase in the proportion of $CD8^+$ T cells specifically, whereas the $CD4^+$ T cell population density was not affected in aging pancreases (**Figure 5L**).

These findings support our isolated islet analysis to demonstrate that human islet and pancreas aging is associated with increased expression of pro-inflammatory markers in alpha cells, which correlates with increased density of $CD8^+$ T cells in the aging pancreas. Since MHCs present antigenic peptides to $CD4^+$ and $CD8^+$ T cells via TCR complexes, age-associated increases in alpha cell pro-inflammatory signatures in mice and humans suggests that alpha cells may be involved in the process of human islet and pancreas inflammation that occurs during aging.

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Figure Legends

Figure 1: Late-life calorie restriction improves glucose tolerance and lowers insulin secretory demand. A) 72-week-old C57Bl/6J mice were fed ad libitum (AL, control) or subjected to 20% calorie restriction (CR) for 2 months. B) Cumulative energy intake over the intervention period. C) Body weight measured before (Pre) and after (Post) 2 months CR. D) Percent change in body weight relative to baseline. E-F) Fat mass and lean body mass (E), and fat-to-lean mass ratio after 2 months on diet. G) Pancreas, liver, and epididymal white adipose tissue (eWAT) weight expressed relative to body mass. H) Mixed meal tolerance tests (MTTs) were performed at baseline (72 weeks) and after the intervention (80 weeks); Area under curve (AUC) is presented for each test. I) HOMA-IR, calculated as [glucose (mM) × insulin (µU/mL)]/405. J) Basal insulin levels. K) Insulin levels measured during the MTT. L) Insulin to glucose ratio. Student's *t-test* was used when comparing two groups (panels B,D,G,H,K-L). Two-way ANOVA with diet and intervention as main effects was used in panels C,E-F,I-J). n=13 per group. Data are mean ± SE. * $p < 0.05$ for dietary intervention (pre vs. post); # $p < 0.05$ for group effect (AL vs. CR). Panel A was created by BioRender.com.

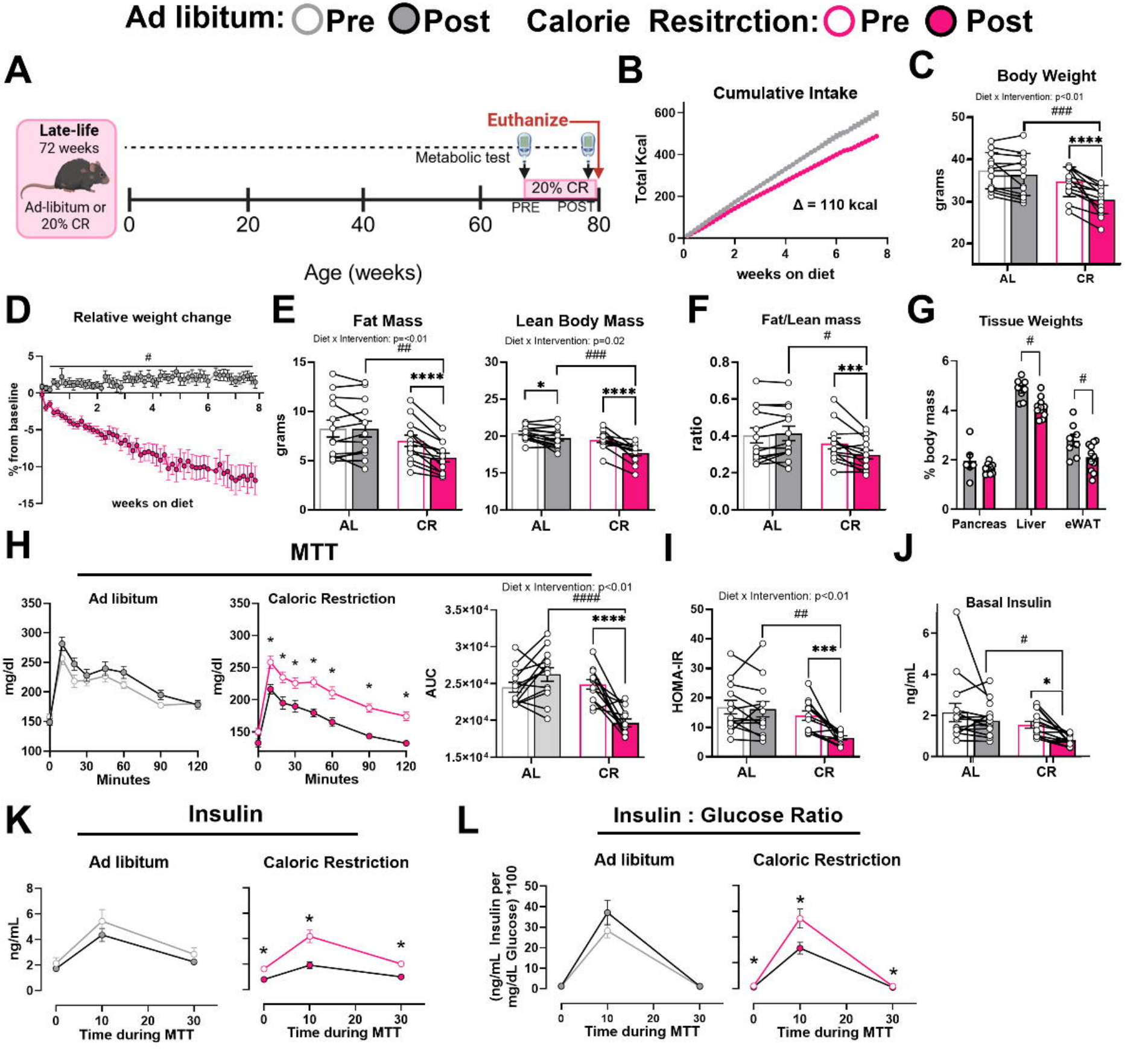
Figure 2: Two months calorie restriction downregulates ER-dependent protein processing in beta cells and antigen presentation in alpha and beta cells. A) Single-nucleus RNA sequencing (SnRNA-seq) was performed on islets from 80-week-old mice fed either AL or CR for 2 months. B) Selected marker genes for non-immune islet subsets based on gene expression. C) UMAP of 32,380 islet nuclei with beta cells segmented from the population. D) Beta cell subclusters and the proportion within each subcluster. E) Differential gene expression in beta cells by pseudobulk analysis. F) KEGG pathways significantly downregulated by CR in beta cells. G) Protein-protein interaction networks shows predicted and known

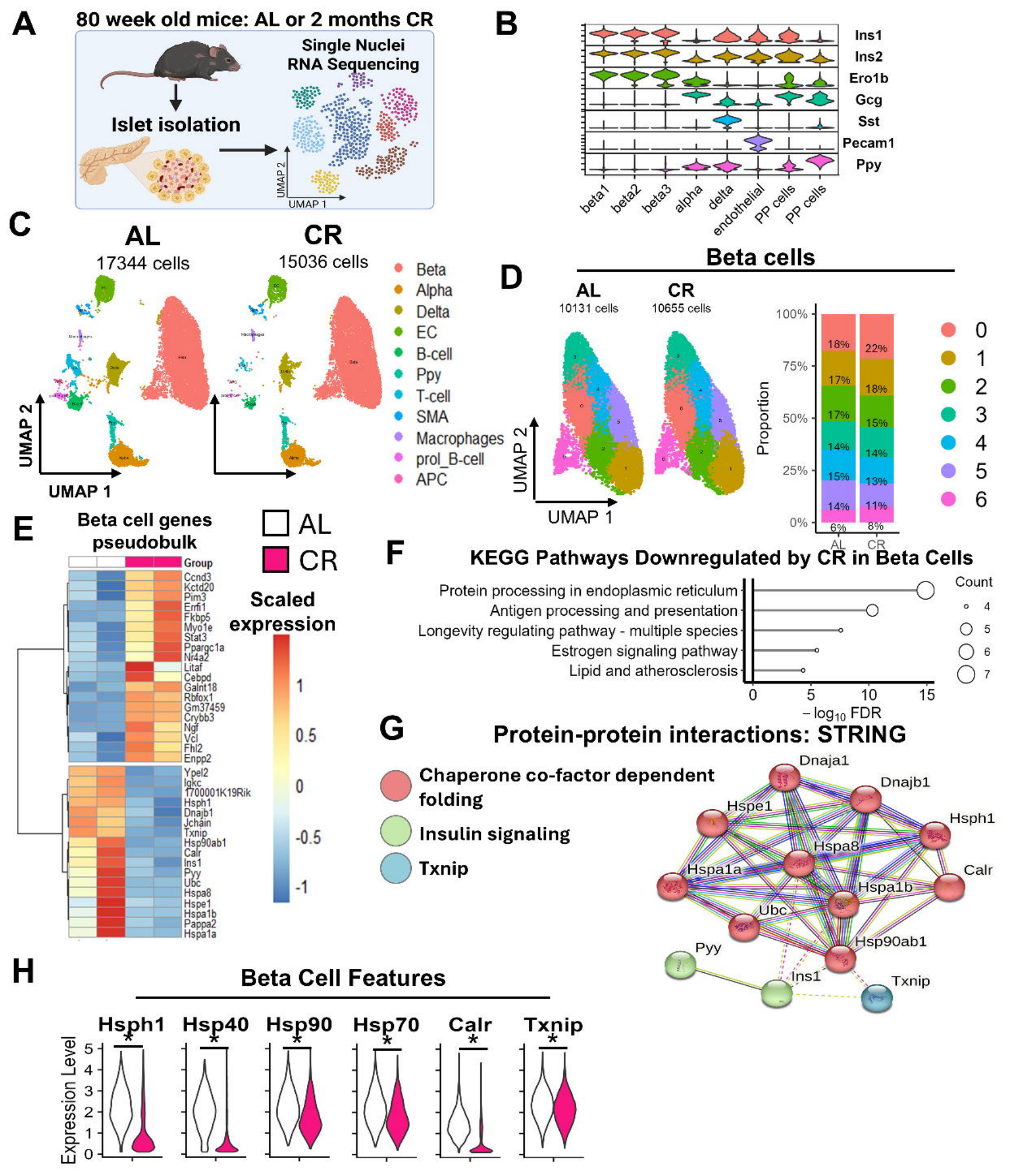
associations among genes downregulated by CR (STRING). H) Expression of beta cell genes related to protein processing and antigen presentation. KEGG, Kyoto encyclopedia of genes and genomes; UMAP, Uniform Manifold Approximation and Projection. * $p < 0.05$ for dietary intervention (AL vs. CR). Panel A was created by BioRender.com.

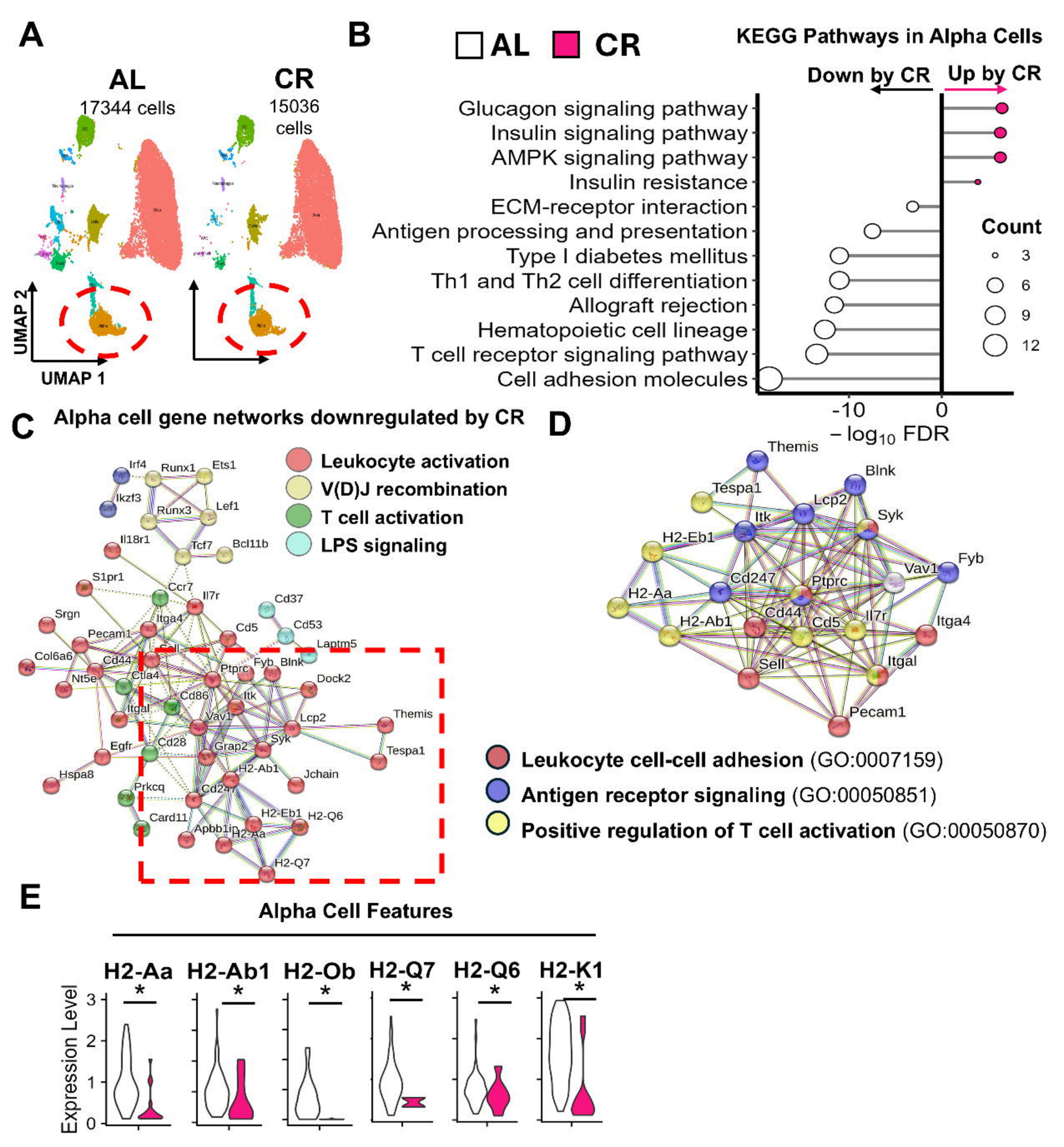
Figure 3: Calorie restriction downregulates TCR activation and antigen presentation in alpha cells. A) Alpha cell subpopulations were analyzed from snRNA-seq dataset. B) KEGG pathways significantly modified by CR in alpha cells. C) Protein-protein interaction networks predicted from genes downregulated by CR in alpha cells. D) Gene ontology networks subset from the 'Leukocyte activation' subcluster in panel C. E) Expression of MHC class I and II genes in alpha cells. * $p < 0.05$ for dietary intervention (AL vs. CR). Panel A was created by BioRender.com.

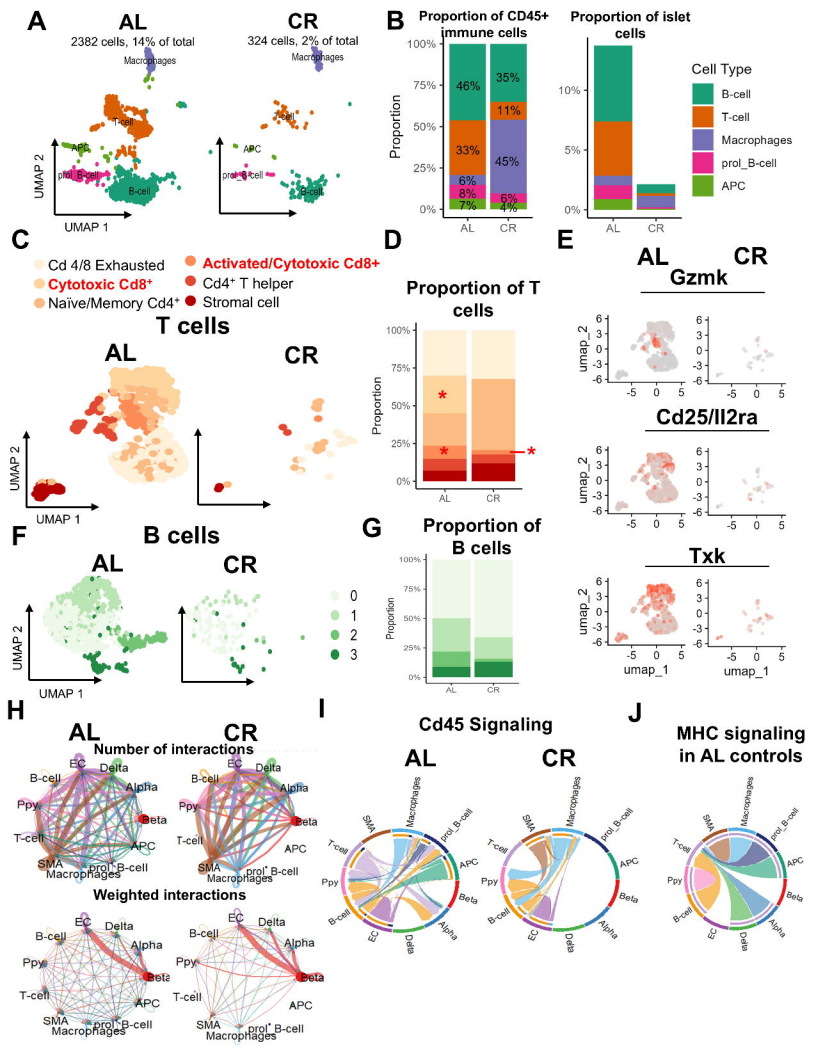
Figure 4: Two months calorie restriction dampens islet adaptive immunity, and alpha cell-specific TCR signaling. A) UMAP of immune cell populations, including B cells, T cells, macrophages, proliferating B cells, and antigen presenting cells (dendritic cells). B) Proportion of immune cell types shown relative to total immune cells (left) and total islet cells (right). C) UMAP of T cell populations. D) Proportion of total T cells. E) Expression of Th1 associated genes; *Gzmk*, *Cd25*, and *Txk*. F-G) UMAP of B cell clusters and the proportion of B cells within each cluster. H) Intercellular communication networks visualized by total number of interactions (absolute), and interaction strength (relative). Edge thickness represents interaction strength. I) Network plot of inferred CD45 signaling interactions. J) Network plot of inferred MHC signaling, which was only present in AL-fed control mice. UMAP, Uniform Manifold Approximation and Projection.

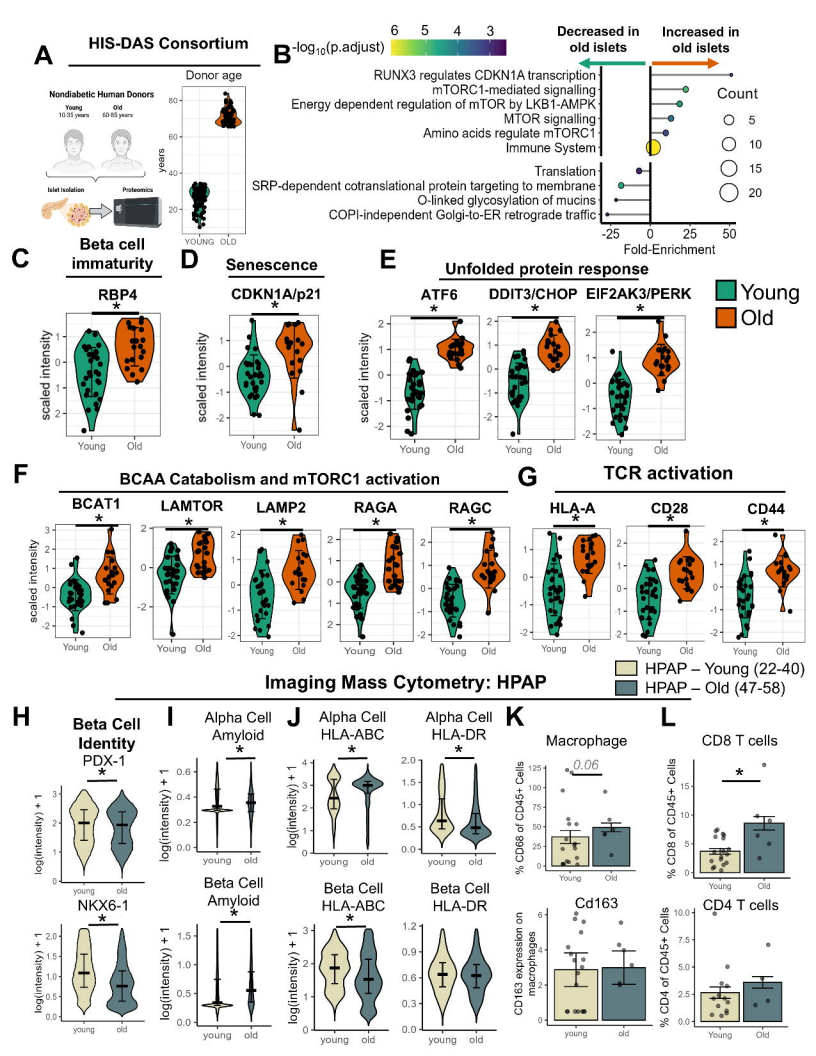
Figure 5: Aging independently increases beta cell immaturity, protein catabolism ER stress, mTORC1, and TCR activation pathways in human islets. A) Islets from young (27±5 years, n=30), and old (71±4 years, n=27) nondiabetic donors in the Human Islet Data Analysis and Sharing (HI-DAS) Consortium and were compared by proteomics. B) Reactome pathways present from significantly abundant proteins between young and old nondiabetic donors. C-D) Protein expression for RBP4 (C) and CDKN1A [p21] (D). E) Significantly abundant proteins for UPR sensors, ATF6, CHOP, and PERK. F) Significantly abundant proteins for BCAA catabolism (BCAT1) and mTORC1 activation (LAMTOR, LAMP2, RAGA, RAGC). F) Significantly abundant proteins for TCR activation proteins (HLA-A, CD28, and CD44). G) Antigen presenting and TCR activation proteins HLA-A, CD28, and CD44. Imaging mass cytometry from the Human Pancreas Analysis Program (HPAP) database were stratified into young 22-40 year old and old 47-58 year old adults, and spatial segmentation was completed. H) Beta cell identity markers, PDX-1 and NKX6-1. I) Islet amyloid polypeptide (IAPP) abundance in alpha cells and beta cells. J) Alpha cell and beta cell MHC-I (HLA-ABC) and MHC-II (HLA-DR) abundance. K) Relative abundance of macrophages within $Cd45^+$ immune cells, and anti-inflammatory macrophage, CD163, abundance on macrophages. L) Relative abundance of $CD4^+$ and $CD8^+$ T cells within $Cd45^+$ immune cells. Error bars are mean±SD. * $p<0.05$, young vs. old donors.


Ad libitum: Pre Post Calorie Resitrction: Pre Post
A
Late-life
72 weeks
Ad-libitum or
20% CR
Euthanize
Metabolic test
20% CR
PRE
POST
0
20
40
60
80
Age (weeks)
B
Cumulative Intake
Total Kcal
Δ = 110 kcal
weeks on diet
C
Body Weight
Diet x Intervention: p<0.01
grams
AL
CR
D
Relative weight change
% from baseline
weeks on diet
E
Fat Mass
Diet x Intervention: p=<0.01
grams
Lean Body Mass
Diet x Intervention: p=0.02
F
Fat/Lean mass
ratio
G
Tissue Weights
% body mass
Pancreas
Liver
eWAT
H
MTT
Ad libitum
Caloric Restriction
mg/dl
Minutes
Diet x Intervention: p<0.01
AUC
I
Diet x Intervention: p<0.01
HOMA-IR
J
Basal Insulin
ng/mL
K
Insulin
Ad libitum
Caloric Restriction
ng/mL
Time during MTT
L
Insulin : Glucose Ratio
Ad libitum
Caloric Restriction
(ng/mL Insulin per mg/dL Glucose) *100
Time during MTT


A
80 week old mice: AL or 2 months CR
Single Nuclei RNA Sequencing
Islet isolation
UMAP 2
UMAP 1
B
Ins1
Ins2
Ero1b
Gcg
Sst
Pecam1
Ppy
beta1
beta2
beta3
alpha
delta
endothelial
PP cells
PP cells
C
AL
17344 cells
CR
15036 cells
Beta
Alpha
Delta
EC
B-cell
Ppy
T-cell
SMA
Macrophages
prol_B-cell
APC
UMAP 2
UMAP 1
D
Beta cells
AL
10131 cells
CR
10655 cells
UMAP 2
UMAP 1
Proportion
100%
75%
50%
25%
0%
18%
17%
17%
14%
15%
14%
6%
22%
18%
15%
14%
13%
11%
8%
AL
CR
0
1
2
3
4
5
6
E
Beta cell genes pseudobulk
AL
CR
Group
Ccnd3
Kctd20
Pim3
Errfi1
Fkbp5
Myo1e
Stat3
Ppargc1a
Nr4a2
Litaf
Cebpd
Galnt18
Rbfox1
Gm37459
Crybb3
Ngf
Vcl
Fhl2
Enpp2
Ypel2
Iqkc
1700001K19Rik
Hsph1
Dnajb1
Jchain
Txnip
Hsp90ab1
Calr
Ins1
Pyy
Ubc
Hspa8
Hspe1
Hspa1b
Pappa2
Hspa1a
Scaled expression
1
0.5
0
-0.5
-1
F
KEGG Pathways Downregulated by CR in Beta Cells
Protein processing in endoplasmic reticulum
Antigen processing and presentation
Longevity regulating pathway - multiple species
Estrogen signaling pathway
Lipid and atherosclerosis
0
5
10
15
− log10 FDR
Count
4
5
6
7
G
Protein-protein interactions: STRING
Chaperone co-factor dependent folding
Insulin signaling
Txnip
Dnaja1
Dnajb1
Hspe1
Hsph1
Hspa8
Hspa1a
Calr
Hspa1b
Ubc
Hsp90ab1
Pyy
Ins1
Txnip
H
Beta Cell Features
Hsph1
Hsp40
Hsp90
Hsp70
Calr
Txnip
Expression Level
0
1
2
3
4
5


A
AL
17344 cells
CR
15036 cells
UMAP 2
UMAP 1
B
AL
CR
KEGG Pathways in Alpha Cells
Down by CR
Up by CR
Glucagon signaling pathway
Insulin signaling pathway
AMPK signaling pathway
Insulin resistance
ECM-receptor interaction
Antigen processing and presentation
Type I diabetes mellitus
Th1 and Th2 cell differentiation
Allograft rejection
Hematopoietic cell lineage
T cell receptor signaling pathway
Cell adhesion molecules
Count
3
6
9
12
-10
0
– log10 FDR
C
Alpha cell gene networks downregulated by CR
Leukocyte activation
V(D)J recombination
T cell activation
LPS signaling
Irf4
Runx1
Ets1
Ikzf3
Runx3
Lef1
Il18r1
Tcf7
Bcl11b
S1pr1
Ccr7
Il7r
Cd37
Srgn
Itga4
Cd5
Cd53
Pecam1
Laptm5
Col6a6
Cd44
Fyb
Blnk
Nt5e
Ctla4
Ptprc
Dock2
Cd86
Itk
Itgal
Vav1
Lcp2
Themis
Egfr
Cd28
Grap2
Syk
Tespa1
Hspa8
H2-Ab1
Jchain
Prkcq
Cd247
Card11
H2-Eb1
H2-Q6
Apbb1ip
H2-Aa
H2-Q7
D
Themis
Blnk
Tespa1
Lcp2
Itk
Syk
H2-Eb1
Fyb
Vav1
Cd247
Ptprc
H2-Aa
Cd44
Il7r
H2-Ab1
Cd5
Itga4
Sell
Itgal
Pecam1
Leukocyte cell-cell adhesion (GO:0007159)
Antigen receptor signaling (GO:00050851)
Positive regulation of T cell activation (GO:00050870)
E
Alpha Cell Features
H2-Aa
H2-Ab1
H2-Ob
H2-Q7
H2-Q6
H2-K1
Expression Level
0
1
2
3


A
AL
2382 cells, 14% of total
CR
324 cells, 2% of total
Macrophages
T-cell
APC
prol_B-cell
B-cell
UMAP 2
UMAP 1
B
Proportion of CD45+ immune cells
Proportion of islet cells
Proportion
100%
75%
50%
25%
0%
46%
33%
6%
8%
7%
35%
11%
45%
6%
4%
AL
CR
10%
5%
0%
Cell Type
B-cell
T-cell
Macrophages
prol_B-cell
APC
C
Cd 4/8 Exhausted
Cytotoxic Cd8+
Naïve/Memory Cd4+
Activated/Cytotoxic Cd8+
Cd4+ T helper
Stromal cell
T cells
AL
CR
UMAP 2
UMAP 1
D
Proportion of T cells
Proportion
100%
75%
50%
25%
0%
AL
CR
E
AL
CR
Gzmk
Cd25/Il2ra
Txk
umap_2
umap_1
6
3
0
-3
-6
-5
0
5
F
B cells
AL
CR
UMAP 2
UMAP 1
0
1
2
3
G
Proportion of B cells
Proportion
100%
75%
50%
25%
0%
AL
CR
H
AL
CR
Number of interactions
Weighted interactions
EC
Delta
B-cell
Alpha
Ppy
Beta
T-cell
APC
SMA
Macrophages
prol_B-cell
I
Cd45 Signaling
AL
CR
Macrophages
prol_B-cell
APC
Beta
Alpha
Delta
EC
B-cell
Ppy
T-cell
SMA
J
MHC signaling in AL controls
Macrophages
prol_B-cell
APC
Beta
Alpha
Delta
EC
B-cell
Ppy
T-cell
SMA


A
HIS-DAS Consortium
Nondiabetic Human Donors
Young 10-35 years
Old 60-85 years
Islet isolation
Proteomics
Donor age
years
YOUNG
OLD
B
-log10(p.adjust)
6 5 4 3
Decreased in old islets
Increased in old islets
RUNX3 regulates CDKN1A transcription
mTORC1-mediated signalling
Energy dependent regulation of mTOR by LKB1-AMPK
MTOR signalling
Amino acids regulate mTORC1
Immune System
Translation
SRP-dependent cotranslational protein targeting to membrane
O-linked glycosylation of mucins
COPI-independent Golgi-to-ER retrograde traffic
Count
5
10
15
20
Fold-Enrichment
C
Beta cell immaturity
RBP4
scaled intensity
Young
Old
D
Senescence
CDKN1A/p21
E
Unfolded protein response
ATF6
DDIT3/CHOP
EIF2AK3/PERK
Young
Old
F
BCAA Catabolism and mTORC1 activation
BCAT1
LAMTOR
LAMP2
RAGA
RAGC
G
TCR activation
HLA-A
CD28
CD44
HPAP – Young (22-40)
HPAP – Old (47-58)
Imaging Mass Cytometry: HPAP
H
Beta Cell Identity
PDX-1
NKX6-1
log(intensity) + 1
young
old
I
Alpha Cell Amyloid
Beta Cell Amyloid
J
Alpha Cell HLA-ABC
Alpha Cell HLA-DR
Beta Cell HLA-ABC
Beta Cell HLA-DR
K
Macrophage
0.06
% CD68 of CD45+ Cells
Cd163
CD163 expression on macrophages
L
CD8 T cells
% CD8 of CD45+ Cells
CD4 T cells
% CD4 of CD45+ Cells