ESM Materials and Methods

IIDP: The Integrated Islet Distribution Program **(**IIDP), is an initiative funded by the National Institutes of Health, USA that provides human pancreatic islet research specimens to investigators. Human islets provided through this program use a national network of academic laboratories experienced with human islet isolation. Pancreata are obtained from deceased donors after documented consent to use the organ for research purposes. Local organ procurement agencies (OPO) throughout the US coordinate the donation procedure by obtaining consent and providing documentation of the consent along with the medical history of donors in a standardized form to islet processing laboratories. Human islets are then isolated, and shipped to IIDP approved investigators for use in various studies. Researchers obtaining islets provided by the IIDP receive organ donor and islet characterization data preselected by IIDP. All data provided through the IIDP is de-identified to ensure a) the anonymity of the deceased donor, and b) compliance with all applicable laws, regulations, and oversight bodies. The culture, shipment, and assessment of human islets provided through IIDP are standardized to avoid variability among isolation centers. IIDP closely monitors the quality of human islets through post-shipment evaluation by investigators. Further information regarding the standard procedures and policies are available on line at http://iidp.coh.org/. The demographic information and medical history made available are provided in table 1 and ESM table 2. Our criteria for accepting islets were purity and viability above 80% and within 5 days of isolation for both non-diabetic and type 2 diabetic islets. Although not confirmed for each case, systemic infection and active malignancy were generally excluded by OPOs and isolation centers. Two pre diabetic donors (see ESM table 2) were included in type 2 diabetic group to reflect the heterogeneity of type 2 diabetes in the

general population. However, these donors were excluded from the analysis when comparisons between non-diabetic and type 2 diabetic groups were made.

Ex vivo perifusion assay. The profile of insulin secretion for each donor was first plotted as the percentage of insulin secretion/islet insulin contents (ESM Fig. 1a). SI was determined as (the average insulin secretion during the 23 mmol/l glucose ramp/the average secretion during 3 mmol/l glucose). As reflected in the y-axis of ESM Fig. 1a, the percentage of insulin secretion/islet insulin contents varied widely between donors, and showed no correlation with the presence of a first-phase response or SI (ESM Fig. 1c-d). Therefore, the insulin secretion rate was expressed taking the second-phase response (average between time 55 to 60 min) as 1. For samples that did not display a sustained increase in insulin secretion when perifused at high glucose, the highest insulin secretion observed was taken as 1 for Fig. 1a. This conversion did not change the plot pattern of insulin secretion for each donor from ESM Fig. 1a to Fig. 1a, as all data points within each graph were divided by the same denominator unique to each graph determined by the second-phase response. The ratio of the first-phase response and second-phase response (first/second) was defined as (average insulin secretion between times 46 to 48 min)/(average insulin secretion between times 55 to 60 min). $AUC_{44\text{-}67}$ is the AUC of insulin secretion in response to the 23 mmol/l glucose ramp in Fig. 1a. The SI during the first-phase response was determined as (the average insulin secretion during times 46 to 48 min/the average secretion during 3 mmol/l glucose). The SI during the second-phase was determined as (the average insulin secretion during times 55 to 60 min/the average secretion during 3 mmol/l glucose).

RNA extraction and cDNA preparation. RNA was extracted from 500 IEQ of human islets using RNeasy kit (Qiagen, Valencia, CA, USA). cDNA was generated by SprintScriptVILO cDNA synthesis Kit (Invitrogen, Carlsbad, CA, USA).

Flow cytometry

Human islets were acquired from IIDP and Beta-Pro and all islets were incubated overnight in CMRL-1066 supplemented with 10% FBS and 1% pen-strept (Invitrogen) at 37^{o}C and 5% CO_2 to recover from the shipment. 5,000-7,000 IEQ islets were passed through a 40 μm cell strainer (BD Biosciences, San Jose, CA, USA) to remove cell debris and disintegrated islets. The islets retained on the strainer were washed and incubated in 0.025% trypsin-0.2 g/L EDTA/PBS (Invitrogen) at 37°C for 2 min. Thereafter, islets were passed through a 40 μm cell strainer to create a single cell suspension and washed with PBS. Cells from the islet cell suspension were incubated with TruStain fcX (Biolegend, San Diego, CA, USA) in PBS (4°C, 10 min) to block Fc receptors and subsequently stained with primary antibodies (ESM table 3 and listed below) or appropriate isotype or fluorescence minus one (FMO) controls (4°C, 25 min), washed twice with PBS, re-suspended in 2% paraformaldehyde (Sigma Aldrich, St. Louis, MO, USA) in PBS (v/v), and subsequently analyzed. Immunofluorescence was detected with a DXP 8 color upgraded 488/637/407 FACSCalibur (Cytek Development Inc, Fremont, CA, USA) and analyzed using FlowJO software (Tree Star Inc, Ashland, OR, USA). For the analysis of total islet leukocyte content and islet leukocyte subset composition, leukocytes were positively identified by CD45 expression. CD45, a common leukocyte marker, is typically used in order to clearly distinguish total leukocytes from tissue cells. This approach has been successfully used to analyze leukocyte

content within various non-lymphoid tissues [1-4], including murine islets [5]. Thus, we used CD45 as a marker to initially identify leukocytes within the islet cell suspensions. Aggregates of cells and events smaller than 50,000 FSC or larger than 250,000 SSC were excluded from the analysis based on FSC/FSC-A and FSC/SSC variables (Fig. 3). For the analysis of CD11b expression by $CD11c^+$ myeloid cells, $CD11c^+CD45^+$ singlets were sub-gated and examined. To examine the viability of type 2 diabetic and non-diabetic islet leukocytes, Live/Dead Fixable Aqua (Invitrogen) staining was utilized in some preliminary experiments. In these experiments, islet cell suspensions were stained with Live/Dead Fixable Aqua (1 μl of dye/1ml of PBS/ $1x10^6$ cells, 15 min, 4°C) and washed twice with PBS before the TruStain FcX blocking and primary antibody staining steps (ESM Fig. 4). To initially determine whether trypsinization would affect islet antigen stability, cell suspensions were created from the same islet donor either mechanically by breaking apart islets with repeated pipetting, or via enzymatic digestion, as described above. The resulting cell suspensions were stained and analyzed simultaneously (ESM Fig. 5). To examine the endocrine cell content of islet cell suspensions (ESM Fig. 3), islet cell suspensions were stained with CD45, HPa2 and HPi2 primary antibodies (Oregon Health & Science University, Portland, OR, USA). To detect unlabeled HPa2 and HPi2 antibodies, islet cell suspensions were incubated with secondary antibodies (20 min, 4°C), washed twice with PBS, subsequently fixed in 2% paraformaldehyde in PBS (v/v), and analyzed. To assess the islet endocrine cell content, the specimens were gated on singlets and events between 50-250,000 FSC and 5-250,000 SSC, based on FSC/FSC-A and FSC/SSC variables. $Singlet^+FSC^+$ cells were assessed for leukocytes ($CD45^+$ cells), Alpha cells ($HPa2^+HPi2^+$ cells), and beta, delta, and PP ($HPi2^+HPa2^-$) cells (ESM Fig. 3). To determine the placement of the gates, human islet cell suspension aliquots and/or blood from each donor were stained in parallel with appropriate

isotype controls or fluorescence minus one controls. The following antibodies/conjugates were used (summarized in ESM table 3): CD3-PE, CD11b-Pacific Blue, CD11b-FITC, CD11c-APC, CD44-FITC, CD45RA-PE Cy7, CD69-APC, CD86, CD235a-FITC (all from BD Biosciences); CD11b-eFluor 450, CD45-eFluor-450 (Affymetrix eBioscience, San Diego, CA, USA); CD20-Pacific Blue, CD45-Per-CP (Biolegend), HPa2 and HPi2 (Oregon Health & Science University), and Goat Anti-Mouse IgG FCγ fragment specific-Alexa Fluor 647 (HPa2 secondary antibody) and Goat anti-Mouse IgM μ chain specific-PE (HPi2 secondary antibody, Jackson Immunoresearch, West Grove, PA, USA).

EMS references

[1] Butcher MJ, Gjurich BN, Phillips T, Galkina EV (2012) The IL-17A/IL-17RA axis plays a proatherogenic role via the regulation of aortic myeloid cell recruitment. Circulation research 110: 675-687

[2] Festin R, Bjorkland A, Totterman TH (1994) Multicolor flow cytometric analysis of the CD45 antigen provides improved lymphoid cell discrimination in bone marrow and tissue biopsies. Journal of immunological methods 177: 215-224

[3] Galkina E, Kadl A, Sanders J, Varughese D, Sarembock IJ, Ley K (2006) Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. The Journal of experimental medicine 203: 1273-1282

[4] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation 112: 1796-1808

[5] Yin N, Xu J, Ginhoux F, et al. (2012) Functional specialization of islet dendritic cell subsets. J Immunol 188: 4921-4930