Islet Macrophages Shift to a Reparative State following Pancreatic Beta-Cell Death and Are a Major Source of Islet Insulin-like Growth Factor-1

HIGHLIGHTS

- Macrophages are a major source of IGF-1 protein within mouse pancreatic islets.
- Post-beta-cell death islet macrophages shift to a reparative state.
- Beta-cell death causes macrophage transcriptome changes consistent with efferocytosis.
- This change can occur even in the presence of HFD feeding or severe hyperglycemia.

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Islet Macrophages Shift to a Reparative State following Pancreatic Beta-Cell Death and Are a Major Source of Islet Insulin-like Growth Factor-1

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SUMMARY

Macrophages play a dynamic role in tissue repair following injury. Here we found that following streptozotocin (STZ)-induced beta-cell death, mouse islet macrophages had increased Igf1 expression, decreased proinflammatory cytokine expression, and transcriptome changes consistent with macrophages undergoing efferocytosis and having an enhanced state of metabolism. Macrophages were the major, if not sole, contributors to islet insulin-like growth factor-1 (IGF-1) production. Adoptive transfer experiments showed that macrophages can maintain insulin secretion in vivo following beta-cell death with no effects on islet cell turnover. IGF-1 neutralization during STZ treatment decreased insulin secretion without affecting islet cell apoptosis or proliferation. Interestingly, high-fat diet (HFD) combined with STZ further skewed islet macrophages to a reparative state. Finally, islet macrophages from db/db mice also expressed decreased proinflammatory cytokines and increased Igf1 mRNA. These data have important implications for islet biology and pathology and show that islet macrophages preserve their reparative state following beta-cell death even during HFD feeding and severe hyperglycemia.

INTRODUCTION

Macrophages are versatile, plastic, innate immune cells essential to numerous biological processes. They participate in host defense, recognition of pathogens, initiation and resolution of inflammation, and maintenance of tissue homeostasis (Okabe and Medzhitov, 2016). Following tissue injury, macrophages are involved in the three main stages of tissue regeneration: inflammation, repair, and resolution. During the inflammatory phase, macrophages disrupt the basement membrane, secrete chemotactic factors to recruit inflammatory cells, and act as scavengers to phagocytose cellular debris. This is followed by a period of wound repair, wherein macrophages produce numerous growth factors, including insulin-like growth factor-1 (IGF-1), platelet-derived growth factors, and vascular endothelial growth factors to stimulate blood vessel development and proliferation of neighboring parenchymal and stromal cells. Transforming growth factor-β is also produced during this stage and activates tissue fibroblasts to facilitate wound closure and extracellular matrix deposition. In the last stage, macrophages assume an anti-inflammatory or pro-resolving phenotype characterized by anti-inflammatory cytokines (interleukin [IL]-10) and immune checkpoint inhibitor expression (PD-L2) (Vannella and Wynn, 2017).

In recent years, pancreatic islet macrophages have become increasingly well characterized in their resting state. Islet macrophages have an M1-like phenotype; they express Il1b and Tnf transcripts, express major histocompatibility complex (MHC) class II, present antigens to T cells, are negative for CD206/CD301, and are derived from definitive hematopoiesis (Calderon et al., 2015; Ferris et al., 2017). In the presence of aggregates of islet amyloid polypeptide (IAPP) (Masters et al., 2010; Westwell-Roper et al., 2016), or when exposed to toll-like receptor (TLR) ligands (Nackiewicz et al., 2014), the proinflammatory state of islet macrophages is enhanced, leading to IL-1 secretion that causes beta-cell dysfunction (Nackiewicz et al., 2014; Westwell-Roper et al., 2014). In contrast, in transgenic models of pancreatic beta-cell regeneration, islet macrophages can produce factors that support beta-cell replication (Brissova et al., 2014; Riley et al., 2015).

Pancreatic beta-cell death is a feature of both type 1 and 2 diabetes, contributing to inadequate insulin secretion and clinical hyperglycemia in both diseases. In type 1 diabetes, apoptotic and necrotic beta-cell death occurs. The immunological consequences of apoptotic beta-cell death are unexplored, whereas
necrotic beta-cell death is thought to initiate or further enhance the activation of antigen-presenting cells in response to released beta-cell factors, causing T cell priming and activation and promoting autoimmunity (Wilcox et al., 2016). In contrast, in type 2 diabetes apoptotic beta-cell death is mainly associated with disease pathology (Halban et al., 2014).

Very little is known about the dynamic role of islet macrophages following beta-cell death. We tested the hypothesis that islet macrophages could be skewed to a tissue repair phenotype in response to beta-cell death, because apoptotic cells promote a tissue repair program in macrophages (Bosurgi et al., 2017) and other tissue macrophages have been shown to be locally programmed for silent clearance of apoptotic cells (Roberts et al., 2017). Here, we thoroughly characterized resident islet macrophage and recruited monocyte cell populations and gene signatures in response to streptozotocin (STZ)-induced cell death, in high-fat diet (HFD)-STZ-treated mice and db/db mice. Macrophages were the major source of IGF-1 protein within pancreatic islets, and transcriptome changes post STZ indicated an enhanced state of cellular metabolism and lysosome activity important in efferocytosis. Adoptive transfer of macrophages maintained circulating insulin levels following beta-cell death in vivo, and IGF-1 neutralization resulted in reduced second-phase glucose-stimulated insulin secretion post STZ in vivo.

RESULTS
Islet Macrophages in STZ-Treated Mice Exhibit a Gene Set Shift Indicative of Enhanced Metabolism and Lysosome Activity and Secrete IGF-1

STZ is a toxin that specifically kills pancreatic beta-cells (Lenzen, 2008) and is commonly used to study islet inflammation. We recently used STZ to investigate the role of gp130 cytokine signaling in pancreatic alpha-cells in a model mimicking type 2 diabetes (STZ + HFD; Chow et al. [2014]). When given at a repeated low dose (<30 mg/kg) it induces mild effects on insulin secretion, glucose tolerance, and beta-cell mass (Chow et al., 2014). Here we used STZ to study the dynamic role of islet macrophages and monocytes following beta-cell death.

We initially analyzed islet macrophages and recruited monocytes at various time points post STZ treatment. Body weight and non-fasting blood glucose were unchanged up to 3 weeks post STZ (Figures S1A and S1B). Islet CD45+ cells, islet macrophages, and recruited monocytes were increased following STZ, with significant increases starting at 1 week and peaking at 2 weeks (Figures 1A–1D). By 3 weeks, no further increases were detected (Figures 1A–1D). Changes in macrophage numbers correlated with gene expression changes, which showed the most difference at 2 weeks post STZ (Figure 1E). At 2 weeks post STZ, Tnf mRNA expression was decreased and Il1rn, Igf1, and Tgfbi mRNA expression were increased in islet macrophages (Figure 1E). No differences in mRNA expression of these genes were detected in recruited monocytes (Figure S1C), and Igf1 was consistently detected only in islet macrophages (see also Figures 1E and S1C).

To obtain a broader unbiased view of the changes in islet macrophages following STZ, we performed transcriptome analysis of isolated islet macrophages at 2 weeks post STZ. The obtained gene expression profile was consistent with phagocytic immune cells, which are known to express high levels of Cd74 (encoding part of MHC class II), Lyz2 (also known as LysM, associated with lysozyme), and Ctsd (cathepsin D gene, associated with lysozyme; highlighted in Figure 1F). According to DEseq2 analysis 128 genes were found to be upregulated and 45 were downregulated (thresholds of Log2 fold change >1 and false discovery rate [FDR] <0.05). Gene set enrichment analysis (GSEA) highlighted gene sets from various pathways enriched in macrophages from STZ-treated mice (Table S1). There were no gene sets downregulated with an FDR q value <0.05. A Cytoscape enrichment map (Cline et al., 2007) of the main altered pathways highlighted multiple overlapping gene sets involved in increased oxidative phosphorylation, increased p450 metabolism, and increased lysosome and protein degradation activity (Figure 1G). Increased lipid metabolism and PPAR signaling genes were also enriched in macrophages from STZ-treated mice. There were no inflammatory gene sets enriched, and prototypical anti-inflammatory genes were not differentially expressed (Il10, Arg1, Mr1, Jag1, Il4ra). A number of growth factors showed consistent upregulation in all three samples based on fragments per kilobase of transcript per million mapped reads (FPKM) values (Igf1, Ngf, Pdgfc, Tgfb, Vegfb, Vgf). Interestingly, a heatmap of the top 25 enriched genes resulted in Igf1 having the highest score (3.47) among all enriched genes (Figure 1H). Cathepsin D (Ctsd) and matrix metalloproteinase-2 (Mmp2), both involved in the proteolysis of IGF-binding proteins (Mutgan et al., 2018), were also among the top 25 enriched genes. Taken together, the transcriptome of islet macrophages at 2 weeks...
Figure 1. Islet Macrophages in Mice Challenged with Multiple Low-Dose STZ Exhibit a Gene Shift toward Enhanced Metabolism and Lysosome Activity and Secrete IGF-1

C57BL/6J male mice were given multiple low-dose STZ (30 mg/kg, 5 times daily intraperitoneal [i.p.] injections) or acetate buffer as an injection control (referred to as “control”) at 16–20 weeks of age.
Ins2 expression were unchanged, whereas STZ-treated islets had increased numbers of TUNEL+Insulin+ cells (Figures 2B and 2C), with no effect on Roper et al., 2014), islet macrophages are also the main contributors to islet following DT was confirmed by flow cytometry (Figure S2B). Depletion of islet macrophages completely (DT). CD11C+ cells are exclusively macrophages in the islet (Ferris et al., 2017). Macrophage depletion was confirmed by flow cytometry (Figure S2B). Depletion of islet macrophages completely abolished the STZ-induced increase in Igf1 mRNA expression (Figure 2A), whereas it had no effect on beta-cell mRNA levels (Ins1, Ins2, Pdx1). Our data also indicate that macrophages are the main source of Tgfbi transcript levels in islets (Figure 2A), which is interesting in the context of recent studies on TGFBI studies, expression of macrophages in islets (CD45-Ly6C+ and CD45-Ly6C- cells) did not (Figures 1I and 1J). Finally, to confirm that STZ was causing beta-cell apoptosis, numbers of TUNEL”Insulin” cells were assessed at 1 and 2 weeks post STZ. STZ induced a 3-fold increase in beta-cell apoptosis at 2 weeks (Figures S1D and S1E).

Islet Macrophage Depletion Decreases Igf1 Expression following STZ-Induced Beta-Cell Death Ex Vivo

To isolate direct beta-cell STZ effects from indirect effects (e.g., elevated postprandial glucose) that may modulate islet macrophage gene expression in vivo, experiments were performed on isolated islets. Treatment of islets with increasing concentrations of STZ (0.25–4 mM) gave similar results to our in vivo studies, causing a decrease in Il1b mRNA expression, while significantly increasing Tgfbi and Pdgfa mRNA expression (Figure S2A). Whole-islet Il1m and Igf1 mRNA expression also tended to increase. Beta-cell Ins1 and Inss2 expression were unchanged, whereas Pdx1 expression was increased by 4 mM STZ treatment (Figure S2A). To determine the contribution of islet macrophages to these gene expression changes, islet macrophages were depleted using islets isolated from CD11c-DTR mice treated with diphtheria toxin (DT). CD11C+ cells are exclusively macrophages in the islet (Ferris et al., 2017). Macrophage depletion following DT was confirmed by flow cytometry (Figure S2B). Depletion of islet macrophages completely abolished the STZ-induced increase in Igf1 mRNA expression (Figure 2A), whereas it had no effect on beta-cell mRNA levels (Ins1, Ins2, Pdx1). Our data also indicate that macrophages are the main source of Tgfbi transcript levels in islets (Figure 2A), which is interesting in the context of recent studies on TGFBI in islets (Han et al., 2014, 2011). As previously shown (Ferris et al., 2017; Nackiewicz et al., 2014; Westwell-Roper et al., 2014), islet macrophages are also the main contributors to islet Il1b and Tnf expression. STZ-treated islets had increased numbers of TUNEL”Insulin” cells (Figures 2B and 2C), with no effect on EdU”Insulin” cells (Figures 2D and 2E). Depletion of islet macrophages reduced EdU”Insulin” cells (Figure 2E).

Finally, STZ did not increase Igf1 mRNA expression or protein secretion, or affect proliferation or apoptosis in bone marrow-derived macrophages (BMDMs; Figures S3A–S3D). In summary, these data support the
Tent was severely reduced due to STZ (Figure 3N), whereas no differences in TUNEL+ islet cells or EdU+ phages had significantly increased non-fasting insulin levels versus STZ controls (Figure 3M). Insulin concentrations both with and without injected macrophages (Figures 3K and 3L), but only mice receiving macrophages showed improved glucose tolerance (Figure S4G). These data support the conclusion that macrophages are the major source of IGF-1 within islets and that beta-cell death directly stimulates islet macrophage Igf1 mRNA expression.

Macrophones and IGF-1 Positively Regulate Insulin Levels in Mice following STZ

We next set out to further investigate the role of islet macrophages in vivo. Because islet macrophages already expressed elevated Igf1 at 1 week post STZ, we depleted phagocytic cells with clodronate-loaded liposomes during, and immediately following, STZ (see study design in Figure 3A). We recently used this protocol to deplete islet macrophages in mice (Nackiewicz et al., 2014; Westwell-Roper et al., 2014). There were no differences in body weight (Figure 3B) between treatment groups, whereas non-fasting blood glucose was significantly elevated at the end of treatment in STZ-treated groups compared with their controls (Figure 3C). Liposome-treated mice all tended to have decreased non-fasting insulin levels (Figure 3D). Insulin secretion was significantly decreased in islets obtained from control mice treated with clodronate-loaded liposomes and in islets from STZ-treated mice (Figure 3E), whereas islet insulin content was significantly reduced only in mice receiving STZ (Figure 3F). Clodronate-liposome-treated mice also tended to show worse glucose tolerance (Figures S4A and S4B). No differences in TUNEL+ islet cells or pHH3+ islet cells were observed between groups (Figures S4C and S4D). Effects of PBS-liposomes were consistent with a previously described effect of liposomes themselves on macrophage function (Ma et al., 2011; Pervin et al., 2016), or might have been due to macrophage depletion with PBS-liposomes (Weisser et al., 2011). These data show that islet macrophages help maintain beta-cell insulin secretion.

Next, we investigated if adoptively transferred macrophages could protect mice from STZ-induced hyperglycemia. Similar to islet macrophages, BMDMs are a rich source of IGF-1 protein (Figure S3B) and secrete increased IGF-1 in response to phagocytosis of apoptotic cells (Han et al., 2016). We injected BMDMs intraperitoneally during, and immediately following, STZ (see study design in Figure 3G). Injected macrophages home to the pancreas (Figure 3H), but could not be found in the spleen (data not shown). Macrophones had no effect on body weight (Figure 3I) but significantly decreased non-fasting blood glucose (Figure 3J). STZ-treated mice had elevated proinsulin levels and proinsulin:insulin ratios both with and without injected macrophages (Figures 3K and 3L), but only mice receiving macrophages had significantly increased non-fasting insulin levels versus STZ controls (Figure 3M). Insulin content was severely reduced due to STZ (Figure 3N), whereas no differences in TUNEL+ islet cells or EdU+ islet cells were observed between groups (Figures S4E and S4F). Mice receiving macrophages also showed improved glucose tolerance (Figure S4G). These data support the conclusion that macrophages that home to the pancreas can increase insulin secretion in vivo following STZ-induced beta-cell death and implicate a role for IGF-1.

Finally, because islet macrophages are the major, if not the sole, source of IGF-1 in islets and its expression is upregulated following beta-cell death, we investigated if IGF-1 neutralization affects glucose homeostasis during STZ-induced beta-cell death. We injected an IGF-1 antibody intraperitoneally during, and immediately following, STZ (see study design in Figure 4A). IGF-1 neutralization had no effect on body weight, whereas non-fasting blood glucose was increased in the STZ + IgG group only (Figures 4B and 4C). During a glucose challenge, only STZ + IGF-1 Ab mice had significantly impaired glucose tolerance versus IgG control mice (Figures 4D and 4E). Significantly lower insulin levels at 30 min in STZ + IGF-1 Ab mice versus STZ controls coincided with the time point where the glucose tolerance test curves separated (Figures 4F and 4D). Similar to the adoptive transfer experiments, both groups of STZ-treated mice had elevated proinsulin levels and proinsulin:insulin ratios versus their controls (Figures 4G and 4H), whereas non-fasting
Figure 3. Macrophages Positively Regulate Insulin Levels in Mice during Multiple Low-Dose STZ
(A) Experimental design of macrophage depletion study. Multiple low-dose STZ (30 mg/kg, 5 times daily i.p. injections) treatments were administered to C57BL/6J males two weeks before sacrifice; 200 μL clodronate-loaded liposomes (CLOD-lip) or PBS-loaded liposomes (PBS-lip) were injected i.p. on days 3, 6, and 10 from the first dose of STZ/buffer.
(B) Body weights; n = 5 mice/control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/STZ group, and n = 3 mice/control + CLOD-lip group.
(C) Non-fasting blood glucose measurements; n = 5 mice/control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/STZ group, and n = 3 mice/control + CLOD-lip group.
(D) Cardiac puncture non-fasting insulin levels at the day of sacrifice; n = 5 mice/control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/STZ group, and n = 3 mice/control + CLOD-lip group.
(E) E islet insulin secretion in 2.8 mM glucose; n = 5 mice/control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/STZ group, and n = 3 mice/control + CLOD-lip group.
(F) Islet insulin content; n = 5 mice/control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/STZ group, and n = 3 mice/control + CLOD-lip group.

*p < 0.05, **p < 0.01, versus control, one-way ANOVA with Tukey's multiple comparisons test.

*p < 0.01 for control versus STZ, #p < 0.01 for PBS-lip versus STZ + PBS-lip,
$ p < 0.01 for CLOD-lip versus STZ + CLOD-lip, two-way ANOVA with Tukey's multiple comparisons test.
insulin levels tended to be lower in IGF-1 Ab-treated mice (Figure 4I). Growth hormone [GH] levels were unchanged between groups (Figure 4J). Finally, no differences in TUNEL+ islet cells or EdU+ cells were observed between groups (Figures S5A and S5B). Thus, post STZ, IGF-1 signaling helps maintain second-phase insulin secretion in vivo.

High-Fat Diet Further Increases Islet Macrophage Numbers and Growth Factor Gene Expression following Beta-Cell Death

Beta-cell death and increased islet macrophages are usually associated with obesity in individuals with type 2 diabetes (Butler et al., 2003; Ehses et al., 2007). Therefore, we made mice obese by feeding them HFD. All mice were sacrificed at the same time point, 2 weeks post STZ, and at the same age (see study design in Figure 5A). At 12 weeks, HFD and HFD + STZ mice had increased body weight compared with chow-fed mice (Figure 5B). Non-fasting blood glucose was increased in 12-week HFD + STZ mice (Figure 5C), and glucose tolerance was impaired (Figures S6A and S6B). Interestingly, numbers of CD45+ cells in islets were significantly increased in 12-week HFD + STZ mice versus STZ mice, due to increased numbers of islet macrophages and other CD45+Ly6C+CD11B−/low cells (Figures 5D–5G and S6C–S6E). Numbers of CD45-Ly6C−/low cells were also higher in 12-week HFD + STZ compared with STZ islets (Figure S6D), whereas no differences in islet CD45-Ly6C+ cell numbers were found (Figure S6E). HFD did not increase islet monocytes versus STZ mice, similar to findings from Ying and colleagues (Ying et al., 2018).

Similar to islet macrophage gene expression post STZ alone, 12-week HFD + STZ macrophages also had increased Igf1 mRNA expression versus HFD control (Figure 5H). However, HFD did not further increase Igf1 expression. Interestingly, HFD did further increase Tgfb1 mRNA versus STZ alone (Figure 5H). HFD + STZ macrophages also had significantly increased Pdgfa mRNA versus chow controls (Figure 5H). No differences in mRNA expression of these genes were detected in recruited monocytes, CD45-Ly6C− cells, or CD45-Ly6C+ cells (Figures S6F–S6H). Igf1 mRNA was consistently detected only in islet macrophages (Figures 5H and S6F–S6H). In summary, HFD combined with STZ further increased numbers of islet macrophages and skewed islet macrophages to a state of increased growth factor expression.

Islet Macrophages in Diabetic db/db Mice Express Increased Igf1 and Decreased Proinflammatory Cytokines

Because STZ is a chemical toxin that might not be relevant for human disease, we also studied islet macrophages in a genetic rodent model of type 2 diabetes, the db/db mouse. At age 6 weeks, db/db mice had elevated body weight, were hyperglycemic, were hyperglucagonemic, and were hyperinsulinemic compared with BKS controls (Figures 6A–6D). However, between 8 and 11 weeks of age insulin levels declined (Figure 6D), indicative of beta-cell dysfunction and death (Medarova et al., 2005; Puff et al., 2011). Therefore, we investigated islet macrophages and monocytes at 8 and 11 weeks of age. A trend toward increased numbers of CD45+ cells in db/db islets at 8 weeks of age was mainly due to significantly increased numbers of islet macrophages (Figures 6E–6G). Similar to islets post STZ, monocytes also tended...
to be increased (Figure 6H). CD45^Ly6C^- cell numbers were increased and CD45^Ly6C^+ cell numbers were significantly reduced (Figures S7A and S7B).

Assessment of cytokine (Il1a, Il1b, Il6, Tnf, Il1rn) and growth factor (Igf1, Pdgfa, Tgfbi) mRNA expression in islet macrophages showed significantly reduced Il6 and Tnf expression with 6-fold increased Igf1 mRNA expression (Figure 6I). No differences in mRNA expression of these genes were detected in monocytes (Figure S7C). Igf1 mRNA was consistently detected only in islet macrophages (see also Figures 6I and S7C).
Figure 5. High-Fat Diet Further Increases STZ-Induced Islet Macrophages and Growth Factor Gene Expression
(A) Experimental design. C57BL/6J male mice were fed regular chow or HFD for 12 weeks. Multiple low doses of STZ (30 mg/kg, 5 times daily i.p. injections) or acetate buffer (referred to as “Control”) were administered 2 weeks before sacrifice.

(B) Body weights; n = 12–22 mice/group, ###/**p < 0.001 HFD/HFD + STZ group versus Control, two-way ANOVA with Dunnett’s multiple comparisons test.

(C) Non-fasting blood glucose measurements; n = 12–22 mice/group, ***p < 0.001 HFD/HFD + STZ group versus Control, two-way ANOVA with Dunnett’s multiple comparisons test.

(D) Representative flow cytometry plots and gating strategy for cell sorting of dispersed islets from mice that received HFD for 12 weeks with acetate buffer injections (left panel) or with multiple low-dose STZ injections (right panel).
At 11 weeks of age, absolute numbers of CD45+ cells in islets also tended to be increased in db/db mice (Figures S7D and S7E). Interestingly, this difference was no longer due to differences in islet macrophage or monocyte numbers (Figures S7F and S7G) and was mainly due to an increase in other immune cell populations (CD45+LY6C−/CD11B−/CD11C−, CD45+LY6C+CD11B−/CD11C−, Figure S7D). Trends in other non-immune cells were similar to those seen at 8 weeks (Figures S7H and S7I). Islet macrophage cytokine and growth-factor mRNA expression showed a similar trend to data from 8-week-old db/db mice (Figure S7J) with elevated Igf1 mRNA.

In summary, similar to STZ-treated and HFD + STZ mice, islet macrophage numbers are increased in 8-week-old db/db mice, and gene expression indicates a state of increased Igf1 expression, and decreased proinflammatory cytokine expression.

**DISCUSSION**

A number of studies, including our own, have shown that islets in humans with type 2 diabetes have increased numbers of macrophages (Ehses et al., 2007; Lundberg et al., 2017; Marchetti, 2016; Richardson et al., 2009). These cells express markers of both classical pro-inflammatory macrophages (CD68) and non-inflammatory macrophages (CD163) (Ehses et al., 2007). However, their functional role is unclear at present and can only be inferred from preclinical studies and clinical studies targeting the pro-inflammatory cytokine, IL-1 (Larsen et al., 2007; Everett et al., 2018).

Our findings here show that beta-cell death results in a dynamic increase in islet macrophage and recruited monocyte cells within 1 week, returning back to normal levels by 3 weeks. This is kinetically similar to effects seen in other tissues, such as cardiac tissue following injury (Walter et al., 2018). Selected pro-inflammatory, anti-inflammatory, and growth factor genes showed changes at 2 weeks, mainly indicative of a state of wound repair (Vannella and Wynn, 2017), and this was confirmed in our whole-transcriptome analysis. Changes in macrophages paralleled significant increases in TUNEL+ beta-cells, potentially providing insight into how they shift to this state.

Phagocytosis of dead cells, also called efferocytosis, is known to induce an anti-inflammatory, reparative state in macrophages and has been increasingly studied in cardiovascular diseases (Brophy et al., 2017). Macrophages rapidly recognize and engulf apoptotic cells via the so-called eat-me signals, the most fundamental of which is phosphatidylserine (PtdSer) (Lemke, 2019). Efferocytotic macrophages are characterized by a state of increased lysosome activity coupled with a state of increased energy needs (Henson, 2017; Voll et al., 1997). Recent studies have shown that fatty acid oxidation fuels the energy requirements of macrophages undergoing efferocytosis (Zhang et al., 2019). Macrophages undergoing phagocytosis of apoptotic cells are also known to secrete increased levels of IGF-1, which increases phagocytosis of local non-professional phagocytes and minimizes inflammation (Han et al., 2016). Our islet macrophage transcriptome data at 2 weeks post beta-cell death (increased expression of genes involved in oxidative phosphorylation, lysosome and protease activity, lipid transport and oxidation, and increased Igf1) fits well with these known effects of efferocytosis on macrophages in other tissues. Future mechanistic studies should determine the phagocytic receptor responsible for shifting islet macrophages to this reparative state.

The energy requirements of macrophages undergoing efferocytosis may also help explain the changes seen in islet macrophages when beta-cell death was combined with HFD feeding. Enhanced skewing toward a reparative state under HFD feeding could be the result of an increased lipid energy source. Indeed, changes in cellular metabolism that lead to functional programming of phagocytic macrophages are a subject of considerable current interest; our data highlight potential pathways that could be targeted to promote islet macrophages with tissue regenerative properties.
**Figure 6. Islet Macrophages in Diabetic db/db Mice Express Igf1 and Decreased Proinflammatory Cytokines**

(A) Body weights of 6- to 11-week-old male BKS and db/db mice.
(B) Non-fasting blood glucose levels of 6- to 11-week-old BKS and db/db mice. (A and B) n = 17–18 mice for 6- to 8-week-old groups, n = 4 mice for 11-week-old group; ***p < 0.001 db/db versus BKS, Student’s t test.
(C) Non-fasting glucagon levels; n = 8 mice for 6- to 8-week-old groups, n = 4 mice for 11-week-old group; **p < 0.01 6-week-old db/db versus BKS control, 8- and 11-week-old db/db versus 6-week-old db/db, one-way ANOVA with Tukey’s multiple comparisons test.
(D) Non-fasting insulin levels; n = 8 mice for 6- to 8-week-old group, n = 3–4 mice for 11-week-old group; **p < 0.01, ****p < 0.0001 6-week-old db/db versus BKS control, 8- and 11-week-old db/db versus 6-week-old db/db, one-way ANOVA with Tukey’s multiple comparisons test.
(E) Representative flow cytometry profiles and gating strategy for cell sorting of dispersed islets from 8-week-old BKS and db/db mice.
(F–H) Fractions of (F) CD45+ cells, (G) islet macrophages, and (H) recruited monocytes in islets of 8-week-old BKS and db/db mice; (F–H) n = 4, 2–4 mice pooled to obtain 556 ± 52 islets per sample (n); ***p < 0.001 db/db versus BKS, Student’s t test.
(I) qPCR of islet macrophages in (G). Relative expression levels of Il1a, Il1b, Il6, Tnf, Il1m, Igf1, Pdgfa, and Tgfbi expressed as fold control (BKS); n = 4, 2–4 mice pooled per sample (n); *p < 0.05 db/db versus BKS, Student’s t test. All data represent mean ± SEM.
The liver is the main source of circulating IGF-1; however, it was recently proposed that macrophages could be the main source of extrahepatic IGF-1 (Gow et al., 2010). Indeed, numerous studies have identified macrophages as major producers of local tissue IGF-1 in the brain, in skeletal muscle, in the lung, and in various other tissues in studies investigating tumor-associated macrophages in cancer biology (Forbes, 2016; Han et al., 2016; Ireland et al., 2016; Tonkin et al., 2015). Here we found that islet macrophages are the major source, if not the sole source, of IGF-1 within pancreatic islets. This has important implications for islet biology.

IGF-1 has long been known to have a beneficial role in diabetes and cardiovascular disease (Higashi et al., 2019). Beta-cell IGF-1 overexpression protects from STZ-induced diabetes (George et al., 2002; Robertson et al., 2008) and exogenous IGF-1 protects NOD mice from developing type 1 diabetes, likely via effects on T cells (Bergerot et al., 1995; Kaino et al., 1996). However, overexpression of IGF-1 locally within islets does not lead to an overt phenotype or effects on beta-cell mass, suggesting that its actions on beta-cells in diabetes are mainly indirect (George et al., 2002; Robertson et al., 2008). The only beta-cell effect observed in transgenic RIP-IGF-1 mice was increased second-phase (30-min) insulin secretion in response to a glucose challenge (Guo et al., 2005). This insulin secretory effect is in agreement with studies knocking out the IGF-1 receptor (IGF-1R) from beta-cells. The absence of a beta-cell IGF-1R did not impair beta-cell development or have effects on beta-cell mass, but it did result in increased fasting insulin levels and impaired first- and second-phase insulin secretion in mice (Kulkarni et al., 2002; Xuan et al., 2002). These data agree well with our current findings, where the primary effect of neutralizing IGF-1 was to decrease second-phase insulin secretion post beta-cell death. We propose that macrophages are the major local source of IGF-1 within pancreatic islets and that paracrine and autocrine effects of macrophage IGF-1 are critical in dampening islet inflammation and maintaining insulin secretion under pathological conditions (Figure 7).
The present findings may seem counterintuitive given the established deleterious role of islet inflammation and IL-1 in humans with type 2 diabetes (Donath and Shoelson, 2011). Indeed, our own work has shown that IAPP induces IL-1β in islet macrophages and TLR ligands elevated in type 2 diabetes activate islet macrophages and have negative effects on beta-cell Ins gene expression (Nackiewicz et al., 2014; Westwell-Roper et al., 2014). However, in the absence of IAPP aggregates or certain TLR ligands islet macrophages clearly skew to a reparative phenotype following beta-cell death. We also observed a pro-resolution/anti-inflammatory phenotype in islet macrophages in ex vivo studies on GK rat islets isolated when beta-cell function was declining in vivo (not shown).

When considering our data in the context of a chronic disease such as type 2 diabetes, it is important to keep in mind how dynamic inflammation and the function of a macrophage is. This is supported by our studies on the db/db mouse, where increased numbers of islet macrophages at 8 weeks where no longer evident at 11 weeks of age, when increases in other immune cell subsets were observed. It is also important to bear in mind that in our own work and that of others, global unbiased transcriptome analysis of islet macrophages was never conducted. It is entirely possible that islet macrophages exhibit a “mixed” pro-inflammatory and reparative phenotype in type 2 diabetes in response to phagocytosis of dead beta-cells following cell death induced by IAPP and other factors (glucose, free fatty acids, endoplasmic reticulum stress, local and/or circulating cytokines). Regardless, our findings are in agreement with a recent study in db/db mice, wherein an NLRP3 inhibitor had no effects on glycemia (Kammoun et al., 2018). Ying and colleagues also found a mixed phenotype of islet macrophages during HFD feeding, not simply defined by M1 or M2 skewing (Ying et al., 2018). Similar to our findings, they also saw increased Pdgfa expression during HFD feeding (Ying et al., 2018).

Our data may have important therapeutic implications relevant to type 2 diabetes. Despite the observance of a significant decrease in HbA1c, IL-1β inhibition has not shown prolonged effects on glycemic control in the studies conducted until now (Everett et al., 2018; Larsen et al., 2007). This could be due to the study population investigated until now or due to the dynamics of islet inflammation. Because inflammation is dynamic and islet macrophages can still change their phenotype to a reparative state even in the presence of HFD feeding or extreme hyperglycemia, increasing their beneficial effects might be an alternative approach to preserving functional beta-cell mass in type 2 diabetes.

Limitations of the Study
A few limitations should be considered when interpreting our data. Currently available techniques do not allow for manipulation of islet-specific macrophages in vivo due to lack of tissue-specific gene expression. Thus, macrophage depletion and adoptive transfer experiments should be interpreted with this in mind. To minimize this limitation, we made use of a toxin that specifically kills beta-cells (Lenzen, 2008), to enable study of the effects of macrophages in islet tissue. We also cannot completely exclude a role for liver IGF-1 in our neutralization experiments, despite effects on insulin secretion that were only evident following beta-cell death. GH levels were also not changed so there did not appear to be any impact of short-term IGF-1 neutralization on insulin sensitivity (Kim and Park, 2017). Although the data suggest that islet macrophage-derived IGF-1 helps maintain beta-cell insulin secretion post beta-cell death, this cannot be conclusively proven in the absence of macrophage IGF-1 knockout studies.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

DATA AND CODE AVAILABILITY
All relevant data are available from the authors upon request. RNA sequencing data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-7234.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.100775.
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AUTHOR CONTRIBUTIONS

Conceptualization, D.N. and J.A.E.; Methodology, D.N., M.D., and J.A.E; Investigation, D.N., M.D., M.S., S.Z.C., Y.-C.C.; Formal analysis, D.N., J.A.P., and J.A.E.; Resources J.A.P., J.A.E., and C.B.V.; Writing – Original Draft, D.N. and J.A.E.; Writing – Review & Editing, D.N., J.A.E., J.A.P., and C.B.V.; Visualization, D.N. and J.A.E.; Funding Acquisition, J.A.E. and C.B.V.; Supervision, J.A.E. and C.B.V.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Islet Macrophages Shift to a Reparative State following Pancreatic Beta-Cell Death and Are a Major Source of Islet Insulin-like Growth Factor-1

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Supplemental Information

Figure S1. Body weight, glycemia, gene expression of recruited monocytes and beta cell apoptosis following STZ treatment in vivo, related to Figure 1
C57BL/6J male mice were given multiple low-dose STZ (30 mg/kg, 5 x daily i.p. injections) or acetate buffer as an injection control (referred to as “control”) at 16-20 weeks of age.

(A) Body weights; n = 9-11 mice/group.

(B) Non-fasting blood glucose levels; n = 9-11 mice/group.

(C) qPCR of recruited monocytes 2 weeks post-STZ. Relative mRNA expression levels of \( \text{Il1b, Tnf, Il1rn, Igf1, Pdgfa, and Tgfbi} \) expressed as fold over islet macrophage control; n = 3 for 0.5, 2, and 3-week treatments, and n = 5 for 1-week treatment. For each sorting sample (n), islets were pooled from 2-4 mice (average of 911 +/- 198 islets).

(D) Representative sections of TUNEL\(^*\)Insulin\(^*\) cells harvested from control or multiple low dose STZ treated mice 2 weeks post STZ. DAPI stain is shown in blue, insulin is green and TUNEL is visualized as a red color; scale bar = 20µm. Colocalization of DAPI and TUNEL is shown in purple. On the right, outlined region is enlarged 4 times.

(E) Quantification of TUNEL\(^*\)Insulin\(^+\) cells harvested from control or multiple low-dose STZ treated mice after 1 and 2 weeks from the start of treatment. Between 567-9870 nuclei per section were counted; n = 3-5, \( **p < 0.01 \) STZ versus control, Student’s t test.
Figure S2. Dose response of STZ induced gene changes and flow cytometry of macrophage depletion in isolated islets, related to Figure 2

(A) qPCR of islets from male C57Bl/6 mice incubated in vitro with increasing doses of STZ for 40 min followed by 48 h recovery in islet media; n = 3, *p < 0.05, **p < 0.01 for STZ versus control, one-way ANOVA with Dunnett’s multiple comparisons test.
Figure S3. Bone marrow derived macrophages do not secrete IGF-1 following STZ treatment, related to Figure 2

(A) qPCR of BMDMs incubated in vitro with increasing doses of STZ for 40 min followed by 48 h recovery in islet media. Relative mRNA expression levels of \( \text{Il1b}, \text{Tnf}, \text{Il1m}, \text{Igf1}, \) and \( \text{Tgfbi} \) shown as fold over control; \( n = 3, \, ^*p < 0.05 \) STZ versus 0 mM STZ, one-way ANOVA with Dunnett’s multiple comparisons test.

(B) IGF-1 secretion from BMDMs incubated in vitro with increasing doses of STZ for 40 min followed by 48 h recovery in islet media; \( n = 3 \).
(C) Quantification of TUNEL+ and (D) EdU+ BMDMs incubated in vitro with increasing doses of STZ for 40 min followed by 48 h recovery in islet media; n = 2.

Figure S4. Glucose tolerance, islet-cell proliferation, and islet-cell apoptosis during macrophage depletion and adoptive transfer in the absence or presence of STZ, related to Figure 3.
(A) Intraperitoneal glucose tolerance test (IPGTT, 1 g glucose/kg body weight) 13 days following the first dose of STZ/acetate buffer; n = 5 mice/ control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/ STZ group, and n = 3 mice/control + CLOD-lip group.

(B) Incremental area under the curve (AUC) for mice in (A); n = 5 mice/control, control + PBS-lip, STZ + PBS-lip, STZ + CLOD-lip groups; n = 4 mice/STZ group, and n = 3 mice/control + CLOD-lip group, *p < 0.05 STZ versus control, one-way ANOVA with Tukey’s multiple comparisons test.

(C) Quantification of TUNEL+ islet cells and (D) pHH3+ islet cells harvested from control or multiple low-dose STZ treated mice 2 weeks from the start of treatment. Between 394-16144 nuclei per section were counted; n = 3-5, One-way ANOVA with Tukey’s multiple comparisons test.

(E) Quantification of TUNEL+ islet cells and (F) EdU+ islet cells in pancreatic sections from control or multiple low-dose STZ (50 mg/kg) treated mice 28 days from the start of the STZ/control treatment. EdU (1 mg) was injected daily i.p. for the last five days before the sacrifice. Between 370-2615 islet cells per section were counted; n = 3-5, One-way ANOVA with Tukey’s multiple comparisons test.

(G) Oral glucose tolerance test (OGTT, 2 g glucose/kg body weight) 25-26 days following administration of the first dose of STZ or acetate buffer; n = 5-6 mice, *p < 0.05, STZ + BMDM versus STZ + DPBS, two-way ANOVA with Bonferroni’s multiple comparisons test.
Figure S5. Islet-cell apoptosis and proliferation during IGF-1 neutralization in the absence or presence of STZ, related to Figure 4

(A) Quantification of TUNEL+ islet cells and (B) pH3+ islet cells harvested from control or multiple low-dose STZ treated mice 2 weeks from the start of treatment. Between 931-13095 nuclei per section were counted; n = 3-5, **p < 0.01 STZ versus control. One-way ANOVA with Tukey’s multiple comparisons test.
Figure S6. Glucose tolerance, islet immune cell populations and mRNA expression in mice challenged with multiple low-dose STZ + HFD, related to Figure 5

A) Glucose tolerance test (IPGTT, 1.5 g/kg) one week after administration of the first dose of STZ or vehicle control; n = 7-9 mice/group, *p < 0.05, **p < 0.01, ***p < 0.001 test group versus control, two-way ANOVA with Dunnett’s multiple comparisons test.

(B) Incremental area under the curve (AUC) for mice in (A); **p < 0.01 test group versus control, one-way ANOVA with Dunnett’s multiple comparisons test.

Fractions of (C) CD45+Ly-6C- cells, (D) CD45-Ly-6C- cells, (E) CD45-Ly-6C+cells from mice described in Figure 5; n = 5 for control, STZ groups; n = 5-6 for 12 weeks HFD, 12 weeks HFD+STZ groups. * p<0.05, Student’s t-test.

(F-H) qPCR of recruited monocytes and non-immune cells. Relative mRNA expression levels of \textit{Il1a}, \textit{Il1b}, \textit{Tnf}, \textit{Il6}, \textit{Il1m}, \textit{Igf1}, \textit{Pdgfa}, and \textit{Tgfbi} expressed as fold over islet macrophage control; n = 4-6. For each sorting sample (n) islets from 3 mice were pooled together (average of 828 +/- 164 islets).
**Figure S7:** Gene expression and islet immune cell populations in 8- and 11-week old diabetic db/db mice, related to Figure 6

Fractions of (A) CD45-Ly-6C- cells and (B) CD45-Ly-6C+ cells in islets of 8-week-old BKS and db/db mice; (A-B) n = 4, 2-4 mice pooled to obtain 556 +/- 52 islets per sample (n); **p < 0.01, ***p < 0.001 db/db versus BKS, Student’s t test.

(C) qPCR of recruited monocytes from 8-week-old BKS and db/db mice. Relative expression levels of *Il1a, Il1b, Tnf, Il6, Il1m, Igf1, Pdgfa,* and *Tgfbi* presented as fold control BKS islet macrophages; n = 4, 2-4 mice pooled per sample (n).

(D) Representative flow cytometry profiles and gating strategy for cell sorting of dispersed islets from 11-week-old BKS and db/db mice.

Fractions of (E) CD45+ cells, (F) islet macrophages, (G) recruited monocytes, (H) CD45-Ly-6C-, and (I) CD45-Ly-6C+ cells cells in 11-week-old BKS and db/db mice.

(J) qPCR of islet macrophages. Relative expression levels of *Il1a, Il1b, Tnf, Il6, Il1m, Igf1, Pdgfa,* and *Tgfbi* shown as fold control (BKS). For BKS mice, 2 mice per sample were pooled to obtain 520 +/- 26 islets and 4 sorting samples in total, n = 4; for *db/db* mice, 4-5 mice were pooled to obtain 464 +/- 127 islets and 2 sorting samples in total, n = 2.
Table S1. Gene sets enriched in STZ versus control treated islet macrophages with FDR q-value < 0.05, related to Figure 1

| NAME                                      | SIZE | ES   | NES   | NOM p-val | FDR q-val | FWER p-val | RANK AT MAX | LEADING EDGE |
|-------------------------------------------|------|------|-------|-----------|-----------|------------|-------------|--------------|
| KEGG_PPAR_SIGNALING_PATHWAY               | 37   | 0.605| 2.203 | 0         | 0.040     | 0.000      | 355         | tags = 24%, list = 3%, signal = 25% |
| KEGG_ALZHEIMERS_DISEASE                   | 130  | 0.628| 2.194 | 0         | 0.040     | 0.040      | 1740        | tags = 54%, list = 16%, signal = 63% |
| KEGG_HUNTINGTONS_DISEASE                  | 140  | 0.627| 2.102 | 0         | 0.040     | 0.040      | 1740        | tags = 54%, list = 16%, signal = 64% |
| KEGG_LYOSOME                               | 108  | 0.574| 2.043 | 0         | 0.040     | 0.040      | 1624        | tags = 40%, list = 15%, signal = 46% |
| KEGG_DRUG_METABOLISM_CYTOCHROME_P450      | 20   | 0.748| 1.992 | 0         | 0.040     | 0.040      | 1189        | tags = 55%, list = 11%, signal = 61% |
| KEGG_GLUCOSAMINOGLYCAN_DEGRADATION        | 16   | 0.709| 1.987 | 0         | 0.040     | 0.040      | 746         | tags = 44%, list = 7%, signal = 47% |
| REACTOME_GLUCONEOGENESIS                  | 21   | 0.713| 1.975 | 0         | 0.040     | 0.040      | 1658        | tags = 57%, list = 15%, signal = 67% |
| BIOCARTA_BAD_PATHWAY                      | 22   | 0.569| 1.952 | 0         | 0.040     | 0.040      | 438         | tags = 18%, list = 4%, signal = 19% |
| KEGG_PARKINSONS_DISEASE                   | 97   | 0.707| 1.912 | 0         | 0.045     | 0.091      | 1815        | tags = 69%, list = 16%, signal = 82% |
| REACTOME_REGULATION_OF_APOPTOSIS          | 54   | 0.635| 1.930 | 0         | 0.045     | 0.091      | 2494        | tags = 63%, list = 22%, signal = 81% |
| KEGG_OXIDATIVE_PHOSPHORYLATION           | 100  | 0.744| 1.923 | 0         | 0.044     | 0.091      | 1744        | tags = 72%, list = 16%, signal = 85% |
| KEGG_CARDIAC_MUSCLE_CONTRACTION           | 41   | 0.685| 1.920 | 0         | 0.044     | 0.091      | 1876        | tags = 59%, list = 17%, signal = 70% |
| REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT | 107 | 0.713| 1.908 | 0         | 0.044     | 0.091      | 1740        | tags = 68%, list = 16%, signal = 80% |
| REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES | 44  | 0.665| 1.897 | 0         | 0.043     | 0.091      | 2494        | tags = 70%, list = 22%, signal = 90% |
| KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450 | 18  | 0.794| 1.893 | 0         | 0.043     | 0.091      | 1189        | tags = 67%, list = 11%, signal = 75% |
| REACTOME_ACTIVATION_OF_NF_KAPPA_B_CELL    | 60   | 0.528| 1.891 | 0         | 0.043     | 0.091      | 1917        | tags = 50%, list = 17%, signal = 60% |
| REACTOME_ANTIGEN_PRESENTATION_OF_NF_KAPPA_B_CELL | 15  | 0.565| 1.890 | 0         | 0.043     | 0.091      | 2532        | tags = 53%, list = 23%, signal = 69% |
| REACTOME_ANTIGEN_PROCESSING_OF_NF_KAPPA_B_CELL | 64  | 0.641| 1.876 | 0         | 0.043     | 0.091      | 2532        | tags = 64%, list = 23%, signal = 82% |
| REACTOMEDESTABILIZATION_OF_MRNA_BYAUFI_HNRNP_D0 | 50  | 0.613| 1.867 | 0         | 0.043     | 0.091      | 1917        | tags = 56%, list = 17%, signal = 67% |
| REACTOME_AUTODEGRADATION_OF_THE_E3_UBIQUITIN_LIGASE_COP1 | 46  | 0.679| 1.867 | 0         | 0.042     | 0.091      | 2494        | tags = 72%, list = 22%, signal = 92% |
| REACTOME_P53_DEPENDENT_G1_DNA_DAMAGE_RESPONSE | 51  | 0.656| 1.852 | 0         | 0.045     | 0.091      | 2494        | tags = 69%, list = 22%, signal = 88% |
| REACTOME_CDK_MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CDC6 | 45  | 0.670| 1.846 | 0         | 0.047     | 0.091      | 2494        | tags = 69%, list = 22%, signal = 88% |
| KEGG Ether_Lipid_Metabolism                | 20   | 0.633| 1.843 | 0         | 0.046     | 0.091      | 971         | tags = 25%, list = 9%, signal = 27% |
| REACTOME_LIPID_DIGESTION_METABOLISM       | 25   | 0.575| 1.842 | 0         | 0.046     | 0.091      | 1005        | tags = 28%, list = 9%, signal = 31% |
Transparent Methods

Mice

BKS.Cg-Dock7m +/- Leprdb/J (db/db), C57BLKS/J (BKS), B6.FVB-1700016L21RikTg(Itgax-DTR/EGFP)57Lan/J (CD11c- DTR) and C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed and bred (C57BL/6J and Cd11c-DTR) in the BC Children’s Hospital Research Institute Animal Care Facility in compliance with Canadian Council on Animal Care guidelines with a 12h light/12h dark cycle and fed ad libitum chow diet or where indicated high fat diet (58 kcal% fat w/sucrose Surwit Diet). Mature male mice were used in all experiments and were sacrificed between 14-20 weeks of age to ensure consistency between animal cohorts.

BKS and db/db mice were 8-11 weeks old at sacrifice to coincide with declining beta cell function and death (Medarova et al., 2005; Puff et al., 2011). Mice were given either 30 mg/kg STZ or acetate buffer (control) i.p. for 5 consecutive days, or for 2 consecutive days if sacrificed on day 3. Following the first STZ or buffer injection mice were sacrificed as indicated in figures on day 3, 7 or 14 and islets were isolated by collagenase digestion. In vivo IGF-1 neutralization was achieved by injecting IGF-1 neutralizing antibody at 0.1 µg/g body weight i.p. Macrophages were depleted in vivo using clodronate loaded liposomes (or PBS loaded liposomes as controls) from Dr. Nico van Rooijen. Liposomes were allowed to reach room temperature, loaded into syringes, and the syringes inverted at least 10 times prior to injecting 200 µl of the solution i.p. per mouse. To adoptively transfer bone-marrow derived macrophages (BMDMs), mice were treated with either 50 mg/kg STZ or acetate buffer (control) i.p. for 5 consecutive days.
and ~0.5 x 10^6 BMDMs were injected i.p. on day 3 followed by injection of ~1 x 10^6 BMDMs i.p. on day 7 from the start of STZ/ buffer treatment. Mice were sacrificed on day 28 post STZ/buffer treatment. The numbers of animals studied are specified in each experiment. The University of British Columbia Animal Care Committee approved all animal studies.

**Mouse islet culture**

After isolation, mouse islets were cultured in islet medium (RPMI 1640 medium (11.1 mM glucose, 2 mM L-Glutamine, Phenol Red) containing 10% FBS, 2 mM L-alanyl-L-glutamine dipeptide (GlutaMAX), 1% penicillin/streptomycin, 10 µg/ml of gentamicin) at 37°C in 5% CO₂ and allowed to recover overnight prior to any *in vitro* experiments. For all *in vitro* experiments, 120-140 healthy-looking islets (round shape, absence of necrotic core, uniform brownish color) from multiple age matched males were pooled together for each n. To determine the optimal dose of STZ *in vitro*, islets were subjected to increasing concentrations of STZ in Krebs Ringer Bicarbonate Buffer (KRB) for 40 min and returned to mouse islet media for 48 h. Corresponding concentrations of acetate buffer were used as controls. To deplete CD11c cells from Cd11c- DTR islets, 10 ng/ml of diphtheria toxin was added to cultured islets for 24 h. Thereafter, islets were treated with 4 mM of STZ or acetate buffer in KRB for 40 min and returned to mouse islet media with 10 ng/ml of diphtheria toxin or vehicle (0.9% NaCl) for 48 h.
Bone-marrow derived macrophages (BMDMs)

Bone marrow was spun down out of mouse femurs and tibias and BMDMs prepared as previously reported (Nackiewicz et al 2014). Red Blood Cell Lysis solution (0.155 M NH₄Cl, 10 mM KHCO₃, 0.127 mM EDTA) was used to deplete erythrocytes and the remaining cells were passed through 40 µm pore size strainers. Around 6 x 10⁶ cells were plated in 12 ml of DMEM supplemented with 1% penicillin/streptomycin, 10 mM HEPES, 10% FBS, and 15% L929-conditioned media in each 100 mm non-tissue culture treated dish and maintained at 37°C in 5% CO₂. Fresh medium was added on days 3 and 5. On day 7, vigorous pipetting with Cell Dissociation Buffer was used to detach adherent BMDMs. Cells were plated in DMEM supplemented with 1% penicillin/streptomycin, 10% FBS at a density described in figures. After 24 h, BMDMs were used in experiments. BMDMs that were starved of L929 conditioned media (a source of macrophage colony-stimulating factor (M-CSF), nerve growth factor (NGF) and other undefined factors (Moore et al., 1980; Pantazis et al., 1977; Warren and Ralph, 1986)) for at least 24 h, were used for adoptive transfer experiments. Qtracker 655 Cell Labeling Kit (Invitrogen) was used to label BMDMs with fluorescent Qdot nanocrystals that allowed subsequent tracking of adoptively transferred cells in dispersed spleen, and exocrine pancreas by flow cytometry.

Islet isolation

Mice anesthetized with isoflurane were sacrificed by cervical dislocation and islets isolated as previously reported (Nackiewicz et al 2014). After clamping the common bile duct, the pancreas was injected intraductally with approximately 2 mL of collagenase XI
(1000 U/ml) in Hanks balanced salt-solution (HBSS) and placed in 50 mL tubes with an additional 3 mL of collagenase solution. The tube was incubated at 37°C for 14 minutes followed by gentle shaking to obtain a homogenously dispersed pancreas. Digestion was stopped with cold HBSS supplemented with 1 mM calcium chloride (CaCl₂). Islets were washed two times in cold HBSS with CaCl₂ and filtered through a 70 µM prewetted cell strainer. After flushing with 20 mL of HBSS with CaCl₂, the strainer was turned upside-down over a Petri dish and rinsed with 10 mL of islet media to wash the islets into the dish. Islets were handpicked under the Nikon SMZ800 microscope into a fresh Petri dish with islet media.

**Physiological measurements**

Non-fasting blood glucose levels were measured from tail bleeds at room temperature using a hand-held blood glucose meter and test strips (OneTouch® UltraMini®, OneTouch® Ultra®2, OneTouch® Ultra® Blue Test Strips, LifeScan Canada). Body weights were recorded at the same time. Mice were fasted 5 hours and injected intraperitoneally (i.p.) with 1.5 g glucose/kg of body weight or 1 g glucose/kg of body weight for i.p. glucose tolerance tests (IPGTT), or mice were given 2 g of glucose/kg of body weight for oral glucose tolerance tests (OGTT). Area under the curve (AUC) was calculated from baseline (time 0 min) for each animal and then used to determine the mean. Blood glucose levels during GTTs were measured from saphenous bleeds just before glucose injection and after 15, 30, 60 and 120 mins. Blood for serum insulin measurement was collected during 0, 15, and 30 min and measured using ELISA (Alpco). For plasma glucagon levels, aprotinin (250 kallikrein inhibitor units/mL plasma;
Sigma-Aldrich) and dipeptidyl peptidase-4 inhibitor (50 μmol/L; Millipore) were added to the collection tubes and measured by ELISA (Merckodia). Insulin (Alpco), proinsulin (Merckodia), and growth hormone (Sigma-Aldrich) were measured by ELISA in sera from cardiac punctures.

**Immunocytochemistry**

Isolated islets were fixed with 4% paraformaldehyde for 15 minutes at room temperature, washed with DPBS, set in agarose, embedded in paraffin and sectioned. Apoptosis was assessed by TUNEL staining with the In Situ Cell Death Detection Kit (Roche) according to the manufacturer’s directions. Proliferation was determined by EdU incorporation (islets were incubated with 10 μM EdU in islet media for 48 h or 72 h prior the fixation) and using the Click-iT™ EdU Alexa Fluor™ 594 Imaging Kit (Invitrogen) following the manufacturer’s directions or by anti-pHH3 antibody. Islet sections were blocked for 30 minutes at room temperature in 2% normal goat serum, incubated overnight at 4 °C with polyclonal guinea pig anti-insulin antibody (1:100 in 1% BSA in DPBS, DAKO) followed by 1 h room temperature incubation either with Alexa Fluor® 488 AffiniPure donkey anti-guinea pig or with DyLight™ 594 AffiniPure donkey anti-guinea pig secondary antibody (1:100 in 1% BSA in DPBS, Jackson ImmunoResearch Laboratories) and mounted using Vectashield with DAPI (Vector Laboratories). Imaging was acquired with a BX61 microscope and quantified using virtual slide microscope OlyVIA, ImageJ software and Image-Pro Analyzer.
**Pancreatic insulin content**

Mice were sacrificed, and the pancreas was isolated. A small piece from the pancreatic tail was excised, weighed, homogenized in acid ethanol, and extracted overnight at 4°C. Samples were spun to remove debris. Supernatants were diluted, and insulin content measured by insulin ELISA (Alpco).

**Islet insulin secretion and content**

Mice were sacrificed, and the islets were isolated by collagenase digestion as described above. After 24 h rest in islet media, 30 similarly-sized islets in duplicate per sample were pre-incubated for 30 min in a Krebs-Ringer Bicarbonate buffer containing 0.5% BSA, pH 7.2 (KRBH) and 2.8 mM glucose at 37°C. Subsequently, islets were transferred to fresh solution of 2.8 mM glucose in KRBH for 30 min at 37°C and supernatant was collected at the end of the incubation. Islet insulin content was extracted during overnight incubation in acid ethanol at 4°C. Insulin concentrations in supernatants were measured by insulin ELISA (Alpco).

**Flow cytometry and cell sorting**

Islet macrophages were sorted as previously published (Nackiewicz et al., 2014) with additional antibodies used to differentiate recruited monocytes. Freshly isolated islets were dispersed in 0.02% Trypsin-EDTA for 3 minutes followed by up to 1 minute of pipetting under a stereomicroscope to obtain a single cell solution. Islet media was added to stop the reaction. Dispersed islets were washed with FACS buffer (1% heat inactivated FBS, 1 mM EDTA, 11 mM glucose in PBS). Cells were kept on ice and pre-
incubated with Fc Block (1:100) for 5 minutes, followed by 30 min incubation with CD45-eFluor 450 (1:250; clone 30-F11), Ly-6C-APC (1:1,200; clone HK1.4), CD11b-PE (1:1,200; clone M1/700, F4/80-FITC (1:150; clone BM8), CD11c-PECy7 (1:150; clone N418), and the viability dye 7AAD (1:2,000). Unstained, single stains, and fluorescence minus one controls were used for setting gates and compensation, and cells were gated on single, live cells. The detailed gating strategy is shown in figures. A BD LSR II was used for flow cytometry and a BD Aria Ii instrument (BD Biosciences) was used for cell sorting with the help of the BC Children's Hospital Research Institute FACS core facility.

**ELISPOT**

Islets from 2 C57BL/6J males aged 16-20 weeks were pooled to obtain enough macrophages for one sample (n). Mice were treated 14 days earlier with 5 daily i.p. injections of 30 mg/kg STZ or acetate buffer and cell sorting was performed as described above. 2500 cells from each group were sorted, plated for 40 h on a 96-well PVDF plate pre-coated with IGF-1 capture antibody (Peprotech Inc.) and maintained in islet media at 37°C in 5% CO2. To detect secreted IGF-1, biotinylated anti-murine IGF-1 (Peprotech Inc.) and streptavidin-ALP were used according to the manufacturer’s instructions. BMDMs served as a positive control. The plate was developed with BCIP/NBT substrate and read on an ELISPOT reader AID Autoimmun Diagnostika GMBH (Germany). Spots were quantified with Image-Pro Analyzer. Pictures were converted to black and white and the number of pixels per well were measured. For visualization the black and white colors were inverted.
**Real-time PCR**

Total RNA was isolated from whole islets and BMDMs using the NucleoSpin® RNA II kit (Macherey-Nagel), and from FACS-sorted cells using the RNeasy Micro Kit (Qiagen) following the manufacturer’s instructions. RNA was quantified using a NanoDrop 2000c (Thermo Scientific). cDNA from whole islets and BMDMs was generated using Superscript II (Invitrogen). cDNA from FACS-sorted cells was prepared using Superscript III (Invitrogen). Quantitative PCR was performed using PrimeTime primers and probes (Integrated DNA Technologies) and TaqMan MasterMix (ThermoFisher/ Applied Biosystems) in the ViiA7 Real-Time PCR System (ThermoFisher/ Applied Biosystems). Differential gene expression was determined by the $2^{-\Delta\Delta Ct}$ method with Rplp0 used as a reference gene.

**Bulk RNA-seq**

Male C57BL/6J mice aged 16-20 weeks were given either 30 mg/kg STZ or acetate buffer i.p. for 5 consecutive days. On day 14 following the first STZ/buffer injection mice were sacrificed and islets isolated. Islets from 10 mice were pooled per sample (n). Islets were hand-picked under the microscope, dispersed, and FACS-sorted as described above. Viable, single CD45+Ly6c-Cd11b+Cd11c+F4/80+ cells were sorted using a BD FACS Aria IIu directly into lysis buffer, and the RNeasy Plus Micro Kit from Qiagen was used to isolate total RNA. Total RNA quality control quantification was performed using an Agilent 2100 Bioanalyzer. All RNA samples had an RNA integrity number (RIN) ≥9.1. The NeoPrep Library Prep System (TruSeq Stranded mRNA Kit) from Illumina was used for library preparation followed by sequencing using standard
Illumina methods and Illumina NextSeq500. RNA-Seq Alignment (BaseSpace Workflow) 1.0.0, TopHat (Aligner) 2.1.0, were used to map raw reads to the reference genome of Mus musculus (UCSC mm10). Cufflinks 2.2.1, BLAST 2.2.26+, DEseq2 (Love et al., 2014), VisR (Younesy et al., 2015), gene set enrichment analysis (GSEA 3.0, the pathway gene sets:
gseaftp.broadinstitute.org://pub/gsea/gene_sets_final/c2.cp.v6.2.symbols.gmt (Mootha et al., 2003; Subramanian et al., 2005) were used to analyze the transcriptome. Cytoscape v3.7.0 with the enrichment map plugin was used to generate a gene set enrichment map based on GSEA analysis (Cline et al., 2007). A node cut-off Q-value of 0.05 and an edge cut-off of 0.5 were used.

**Statistical analysis**

Data are reported as mean ± SEM. Statistical analysis with normality tests were performed, and graphs were created with GraphPad Prism version 7.00. Two-tailed Student’s t test was used when comparing two means. One-way ANOVA or Kruskal-Wallis test with Dunn’s multiple comparisons test was applied when comparing more than two groups, and two-way ANOVA was used when comparing two independent variables in at least two groups. To compare every mean with a control, Dunnett’s post-test was employed. Bonferroni’s or Tukey’s post-hoc test was used to compare different sets of means. Differences were considered significant at p<0.05. The n value and details on statistical analyses of each experiment are indicated in the figure legends.
Contact for reagent and resource sharing

Further information and requests for reagents may be directed to and will be fulfilled by the corresponding author, Dr. C. Bruce Verchere (bverchere@bcchr.ca).

Commercially available reagents and mouse strains used in this study.

| REAGENT                                                      | SOURCE                | IDENTIFIER            |
|--------------------------------------------------------------|-----------------------|-----------------------|
| **Antibodies**                                               |                       |                       |
| CD45 Monoclonal Antibody (30-F11), eFluor 450                | ThermoFisher/ eBioscience | 48-0451-82, RRID: AB_1518806 |
| Ly-6C Monoclonal Antibody (HK1.4), APC                       | ThermoFisher/ eBioscience | 17-5932-82, RRID: AB_1724153 |
| CD11b Monoclonal Antibody (M1/70), PE                        | ThermoFisher/ eBioscience | 12-0112-82, RRID: AB_2734869 |
| CD11c Monoclonal Antibody (N418), PE-Cyanine7                | ThermoFisher/ eBioscience | 25-0114-82, RRID: AB_469590 |
| F4/80 Monoclonal Antibody (BM8), FITC                       | ThermoFisher/ eBioscience | 11-4801-82, RRID: AB_2637191 |
| CD16/CD32 Monoclonal Antibody (93) Fc Block                  | ThermoFisher/ eBioscience | 14-0161-85, RRID: AB_467134 |
| Polyclonal Guinea Pig Anti-Insulin                           | Dako                  | A0564, RRID: AB_10013624 |
| Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L) | Jackson ImmunoResearch Laboratories Inc. | 706-545-148, RRID: AB_2340472 |
| DyLight™ 594 AffiniPure F(ab')2 Fragment Donkey Anti-Guinea Pig IgG (H+L) | Jackson ImmunoResearch Laboratories Inc. | 706-516-148, RRID: AB_2340471 |
| Goat Anti-Mouse IGF-1 Antigen Affinity-purified Polyclonal Antibody (for neutralization) | R&D Systems | AF791, RRID: AB_2248752 |
| Normal Goat IgG Control                                      | R&D Systems           | AB-108-C, RRID: AB_354267 |
| Anti-phospho-Histone H3 (pHH3; Ser10) Antibody, Mitosis Marker | Sigma- Aldrich/ Millipore Sigma | 06-570, RRID: AB_310177 |
| Anti-Murine IGF-1                                            | Peprotech Inc.        | 500-P157G             |
| Chemicals, Peptides, and Recombinant Proteins | Supplier | RRID |
|---------------------------------------------|---------|------|
| Biotinylated Anti-Murine IGF-1 | Peprotech Inc. | 500-P157GBt, RRID: AB_2737301 |

| Chemical | Supplier | Part Number |
|----------|----------|-------------|
| 7-AAD (7-Aminoactinomycin D) | ThermoFisher/Invitrogen | A1310 |
| HBSS (10X), no calcium, no magnesium, no phenol red | ThermoFisher/Gibco | 14185052 |
| Collagenase from Clostridium histolyticum, Type XI, 2-5 FALGPA units/mg solid, ≥800 CDU/mg solid | Sigma- Aldrich/Millipore Sigma | C7657 |
| RPMI 1640 Medium | ThermoFisher/Gibco | 11875119 |
| DMEM Medium | ThermoFisher/Gibco | 11995065 |
| DPBS, no calcium, no magnesium | ThermoFisher/Gibco | 14190144 |
| Gentamicin (10 mg/mL) | ThermoFisher/Gibco | 15710064 |
| GlutaMAX™ Supplement | ThermoFisher/Gibco | 35050061 |
| Penicillin-Streptomycin (10,000 U/mL) | ThermoFisher/Gibco | 15140122 |
| Fetal Bovine Serum, qualified, heat inactivated, Canada origin | ThermoFisher/Gibco | 12484028 |
| Cell Dissociation Buffer, enzyme-free, Hanks' Balanced Salt Solution | ThermoFisher/Gibco | 13150016 |
| Trypsin-EDTA (0.25%), phenol red | ThermoFisher/Gibco | 25200056 |
| Recombinant Murine IGF-1 | Peprotech Inc. | 250-19 |
| RNaseOUT™ Recombinant Ribonuclease Inhibitor | ThermoFisher/Invitrogen | 10777019 |
| Streptozocin, ≥75% α-anomer basis, ≥98% (HPLC), powder | Sigma- Aldrich/Millipore Sigma | S0130 |
| Diphtheria Toxin, Unnicked, Corynebacterium diphtheriae | EMD Millipore/Calbiochem | 322326 |
| UltraPure™ DNase/RNase-Free Distilled Water | ThermoFisher/Invitrogen | 10977-015 |
| Agarose | Fisher Scientific | BP160-500 |
| VECTASHIELD Antifade Mounting Medium with DAPI | Vector Laboratories | H-1200 |

**Critical Commercial Assays**

| Assay | Supplier | Part Number |
|-------|----------|-------------|
| RNeasy Micro Kit | QIAGEN | 74004 |
| Item                                                                 | Manufacturer                  | Code       |
|----------------------------------------------------------------------|-------------------------------|------------|
| RNeasy Plus Micro Kit                                                | QIAGEN                        | 74034      |
| NucleoSpin® RNA II                                                   | Macherey-Nagel                | 740955.250 |
| rDNase Set                                                           | Macherey-Nagel                | 740963     |
| SuperScript™ II Reverse Transcriptase                                | ThermoFisher/Invitrogen       | 18064-014  |
| SuperScript™ III Reverse Transcriptase                               | ThermoFisher/Invitrogen       | 18080-044  |
| TaqMan™ Universal Master Mix II, no UNG                              | ThermoFisher/Applied Biosystems | 4440040    |
| TaqMan™ Fast Advanced Master Mix                                     | ThermoFisher/Applied Biosystems | 4444557    |
| Qtracker™ 655 Cell Labeling Kit                                      | ThermoFisher/Invitogen        | Q25021MP   |
| Murine IGF-1 Standard ABTS ELISA Development Kit                    | Peprotech Inc                 | 900-K170   |
| Mouse Ultrasonensive Insulin ELISA                                   | Alpco                         | 80-INSMSU-E01 |
| STELLUX Rodent Insulin ELISA                                         | Alpco                         | 80-INSMR-CH01 |
| Click-iT™ EdU Alexa Fluor™ 594 Imaging Kit                          | ThermoFisher/Invitrogen       | C10339     |
| In Situ Cell Death Detection Kit, Fluorescein                        | Sigma-Aldrich/Millipore Sigma/Roche | 11684795910 |
| Rat/Mouse Growth Hormone ELISA                                       | Sigma-Aldrich/Millipore Sigma/Roche | EZRMGH-45K   |
| Mercodia Rat/Mouse Proinsulin ELISA                                  | Mercodia                      | 10-1232-01 |
| Mercodia Glucagon ELISA                                              | Mercodia                      | 10-1281-01 |
| Deposited Data                                                       |                                |            |
| RNAseq                                                               | This paper                    | EMBL-EBI   |
|                                                                    |                               | E-MTAB-7234 |
| Experimental Models: Organisms/Strains                               |                                |            |
| Mouse: C57BLKS/J                                                     | The Jackson Laboratory        | JAX stock  |
|                                                                    |                               | #000662    |
| Mouse: BKS.Cg-Dock7m +/- Leprdb/J                                    | The Jackson Laboratory        | JAX stock  |
|                                                                    |                               | #000642    |
| Mouse: B6.FVB-1700016L21RikTgltgax-DTR/EGFP)57Lan/J                  | The Jackson Laboratory        | JAX stock  |
|                                                                    |                               | #004509    |
| Mouse: C57BL/6J                                                      | The Jackson Laboratory        | JAX stock  |
|                                                                    |                               | #000664    |
| Oligonucleotides                                                     |                                |            |
| 50 nm Random Hexamer, sequence: NNNNN                                 | IDT                           | rrmdnm     |
| dNTP Set (100 mM)                                                    | ThermoFisher/Invitrogen       | 10297-018  |
| Gene   | Probe | Primer 1 | Primer 2 | Source | Notes |
|--------|-------|----------|----------|--------|-------|
| Rplp0  | 5'-/56-FAM/TGTCTTCCC/ZEN/TGGGCATCACGTC/3IABkFQ/-3' | 5'-TGACATCGTCTTTAAACCCCG-3' | 5'-TGTCTGCTCCACAATGAAG-3' | IDT   | N/A   |
| Il1a   | 5'-/56-FAM/TCCAACCCA/ZEN/GATCAGCACCTTACAC/3IABkFQ/-3' | 5'-TGCAGTCCATAACCCATGATC-3' | 5'-ACAAACTTCTGCCTGACGAG-3' | IDT   | N/A   |
| Il1b   | 5'-/56-FAM/AGAGCATCC/ZEN/AGCTTCAAATCTCGA/3IABkFQ/-3' | 5'-ACGGACCCAAAGATGAAG-3' | 5'-TTCTCCACAGCCAATGAG-3' | IDT   | N/A   |
| Il1m   | 5'-/56-FAM/TCATAGTGT/ZEN/GTTCTTGGGCATCCACG/3IABkFQ/-3' | 5'-TCATTGCTGGGTACTTACAAGG-3' | 5'-ATCTCCACAGCCAATGAG-3' | IDT   | N/A   |
| Tnf    | 5'-/56-FAM/ATCTGAGTG/ZEN/TGAGGGTCTGGGC/3IABkFQ/-3'  | 5'-CTTCTGTCTACTGAACCTTCC-3' | 5'-CAGGCTTGTCACTCGAAATTTG-3' | IDT   | N/A   |
| Il6    | 5'-/56-FAM/CCTACCCCA/ZEN/ATTTCATGCTCTC/T3IABkFQ/-3' | 5'-CAAAGCAGACGTCTCCAGAG-3' | 5'-GTCCTTAGCCACTCCTTCTG-3' | IDT   | N/A   |
| Gene  | Probe: 5’-/56- | Primer 1: 5’- | Primer 2: 5’- | IDT | N/A |
|-------|----------------|--------------|--------------|-----|-----|
| Igf1  | FAM/AGAAGTCCC/ZEN/CGTCCCTATCGAC | GAGACTGGAGATGTACTGTGC | CTCCTTTTCAGCAGCTTCGTTTTTC | IDT | N/A |
| Tgfbi | FAM/TGTGCGACT/ZEN/TGCCCCGTCTCTATC | AACCGACCACAAGAAGGAG | CTCCATCTCCTCCAGTAAAACC | IDT | N/A |
| Pdgfa | FAM/CGCAGGAAG/ZEN/AGAAGTATTGAGG AAGCC | TTACCATGTGCCCGAGAAG | ATCAGGAAGTTTGCCGATG | IDT | N/A |
| Itgam | FAM/CCACACTCT/ZEN/GTCCAAAGCCTTT GC | CATCCCATGACCTTCCAGAG | GTGCTGTAGTCACACTGGA | IDT | N/A |
| Itgax | FAM/ACACAGGCC/ZEN/GGGAAGAGCAA | TTCAAGGAGACAAAGACCC | AGAGAAAAGTTGAGGCGAAG | IDT | N/A |
| Pdx1  | FAM/ACAAGAGGA/ZEN/CCCGTACTGCCTAAC | CCCTTTTCCCGTGATGAAATC | GAATTCCTTCCAGCTCCAG | IDT | N/A |
### Ins1

| Probe: 5’-/56-FAM/TGTGGTGC/ZEN/ACTTCCTACCCCTG/3IABkFQ/-3’ | Primer 1: 5’-ATCAGAGACCATCAGCAAGC-3’ | Primer 2: 5’-GTGGACAAAGCCTGGGTG-3’ |
|----------------------------------------------------------|--------------------------------------|--------------------------------------|
| IDT                                                      | N/A                                  | N/A                                  |

### Ins2

| Probe: 5’-/56-FAM/CCTCCACC/ZEN/AGCTCCAGTTGT/3IABkFQ/-3’ | Primer 1: 5’-GGCTTCTTCTACACACCAGT-3’ | Primer 2: 5’-TGATCTACAATGCCACGCTTC-3’ |
|------------------------------------------------------|-------------------------------------|--------------------------------------|
| IDT                                                  | N/A                                 | N/A                                  |

### Software and Algorithms

| Software and Algorithms                  | URL                                      | RRID:                      |
|------------------------------------------|------------------------------------------|----------------------------|
| FlowJo 7.6                                | https://www.flowjo.com/solutions/flowjo  | SCR_008520                 |
| BD FACSDiva 6                             | http://www.bdbiosciences.com             |                            |
| OlyVIA 2.8                                | https://www.olympus-lifescience.com/     | SCR_0161                   |
| GraphPad Prism 7                          | http://www.graphpad.com/                 | SCR_0027                   |
| Image-Pro Analyzer 6.2                   | http://www.mediacy.com/                  |                            |
| ImageJ software                           | https://imagej.nih.gov/ij/               | SCR_003070                 |
| Gene Set Enrichment Analysis (GSEA) 3.0   | https://www.broadinstitute.org/gsea      | SCR_003199                 |
| DESeq2                                    | https://bioconductor.org/packages/release/bioc/html/DESeq2.html | SCR_015687                 |
| VisR 0.9.37                               | https://visrsoftware.github.io/          | SCR_016658                 |
| Cytoscape v3.7.0                          | https://cytoscape.org/                   | SCR_003032                 |

### Other

| Other                                         | URL                                      | RRID:                      |
|------------------------------------------------|------------------------------------------|----------------------------|
| PBS Liposomes and Clodronate Liposomes         | www.ClodronateLiposomes.com               | N/A                        |
| 58 kcal% fat w/sucrose Surwit Diet             | Research Diets                           | D12331                     |
| OneTouch Ultra® test strips                    | LifeScan                                 | Blue, code 25              |
| Product Description                                      | Supplier                  | Code    |
|----------------------------------------------------------|---------------------------|---------|
| OneTouch Ultra® UltraMini®, OneTouch® Ultra2® Blood glucose meters | LifeScan                  | N/A     |
| Fisherbrand™ Microhematocrit Capillary Tubes, Heparinized | Fisher Scientific         | 22-362566 |
| Fisherbrand™ Microhematocrit Capillary Tubes, Not Heparinized | Fisher Scientific         | 22-362574 |
| Falcon™ Cell Strainer, 40 μm                              | Fisher Scientific/ Corning Life Sciences | 08-771-1 |
| Falcon™ Cell Strainer, 70 μm                              | Fisher Scientific/ Corning Life Sciences | 08-771-2 |
| L929 conditioned media                                   | AbLab, Biomedical Research Centre, Core Media Facility- Vancouver, UBC | N/A     |

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