Apoptotic beta-cells induce macrophage reprogramming under diabetic conditions

Meliza G. Ward, Ge Li, Mingming Hao

Supporting figures

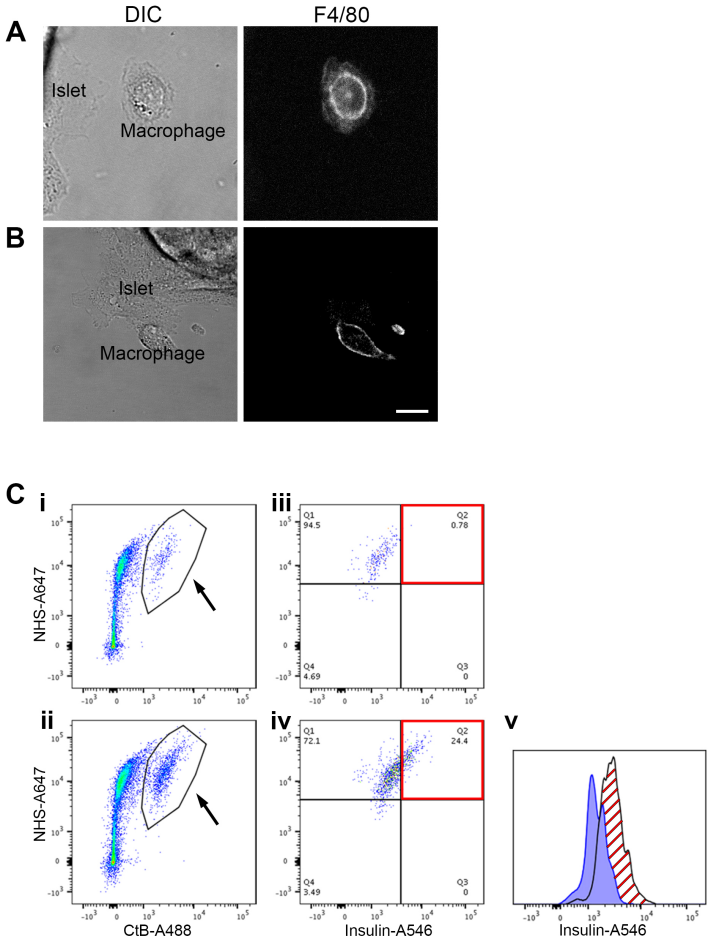

Figure S1. (A, B) Identification of islet macrophages by F4/80. Isolated islets containing islet macrophages were individually seeded in imaging dishes. Here are two examples of a macrophage that crawled out of the islet. One was separate from the islet (A) and one was still attached (B). Islet purification went through three rounds of manual picking to ensure no contamination of other cells was present when an islet was seeded in the imaging dish. F4/80, an extensively used surface marker for mouse macrophages, was used to identify macrophages. DIC, differential interference contrast. Scale bar, 10 μm. **(C)** Flow cytometry analysis of efferocytosis-induced insulin accumulation. (i, ii) Gate setting for the analysis of J7 cells. J7 cells were identified by CtB (x-axis), and MIN6 cell fragments were labeled with NHS (y-axis). Arrows point to J7 cells containing MIN6 fragments. (iii, iv) Analysis of insulin contents in J7 cells. Q2 (red) shows NHS-containing J7 cells that were also positive for insulin staining. Panels i and iii show samples without insulin antibody; panels ii and iv show samples with insulin antibody. (v) Histogram plot of insulin staining showing negative (lavender) and positive (red) samples. This figure is supplementary to Figure 1.

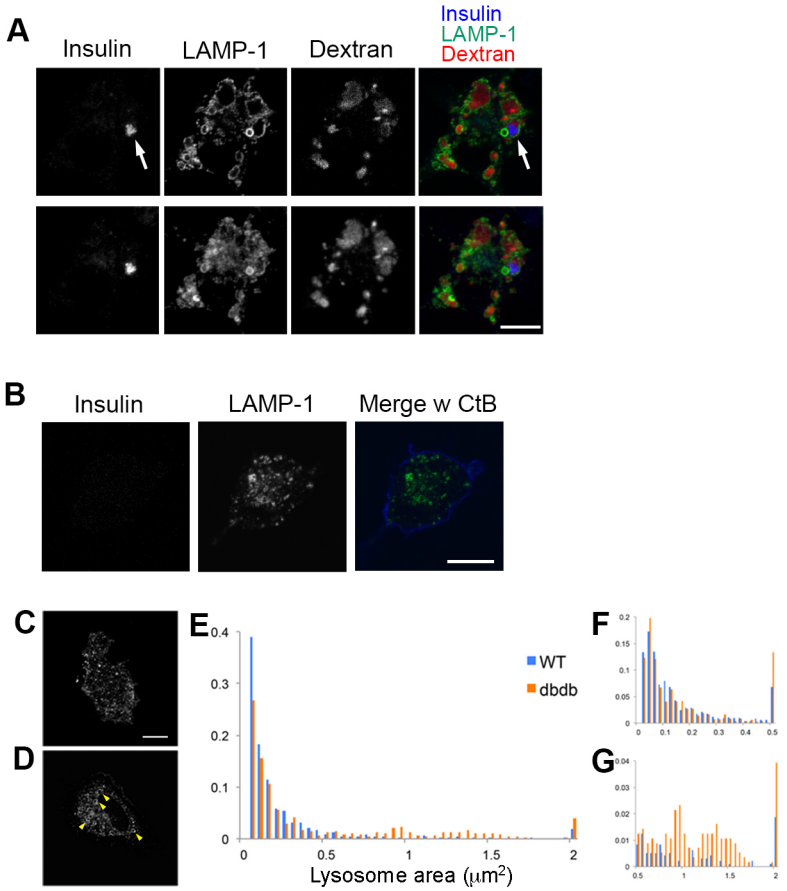

Figure S2. Phagocytosis of apMIN6 cells leads to insulin accumulation and enlarged lysosomes. (A) BMMs incubated overnight with apMIN6 cells displayed enlarged lysosomes, similar to J7 cells. Dextran was loaded to fill the lysosomes. The arrow points to an enlarged lysosome containing dextran and insulin aggregate. Not all enlarged lysosomes contain insulin labeling, most likely due to limited accessibility of the insulin antibody. **(B)** J7 cells incubated with monomeric insulin in solution for 3 d did not induce insulin accumulation or lysosome enlargement. **(C-G)** Isolated islets from wile-type (WT, C) and db/db (D) mice were dissociated and stained with F4/80 to identify islet macrophages (not shown) and LAMP1 to label the lysosomes (C, D). The LAMP1-postive structures selected by intensity thresholding were plotted as a distribution of area size (E), with areas <0.5 μm^2 and >0.5 μm^2 separated further in F and G, respectively. n=2 mice each for WT and db/db. Scale bars, 10 μm. This figure is supplementary to Figure 2.

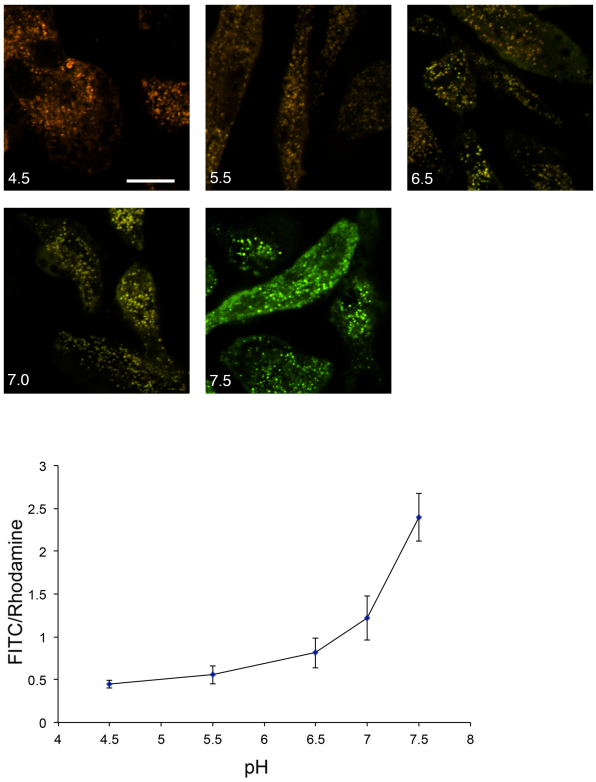

Figure S3. Calibration curves for the pH measurements. Calibration curves were generated by equilibrating the FITC-rhodamine dextran in fixed cells to a range of pH buffers. The ratio of FITC to rhodamine in individual lysosomes was measured. The curve shown here is representative of three experiments. Scale bar, 10 μm. This figure is supplementary to Figure 4.

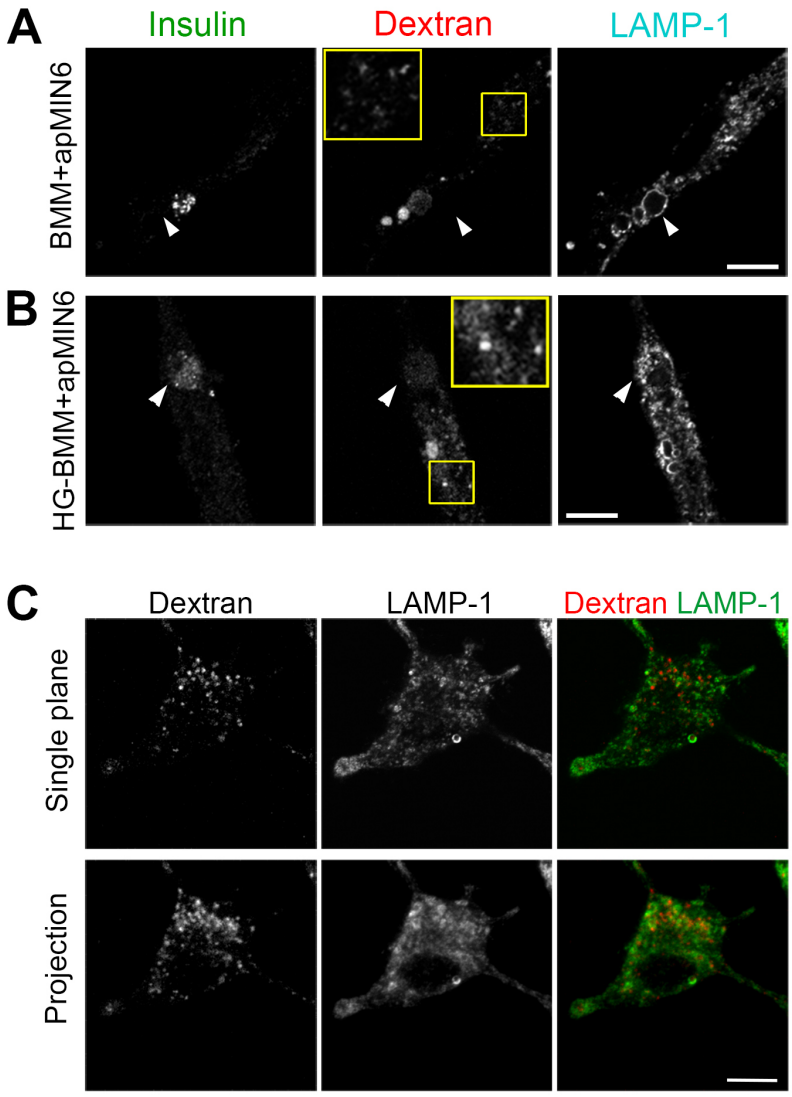

Figure S4. High glucose induces lysosomal permeabilization in phagocytic macrophages. (**A**, **B**) BMMs were incubated with apMIN6 cells and cultured in either normal medium (A, BMMs+apMIN6) or medium with 30 mM glucose for 3 d (B, HG-BMMs+apMIN6). They were then loaded with lysine-fixable rhodamine-dextran and labeled with insulin and LAMP-1 antibodies. Shown here are single plane images by confocal microscopy. The yellow boxes show a region of the cell in which the dextran shows punctate staining in the lysosomes (A) or additional diffuse staining in the cytoplasm (B). **(C)** High glucose alone did not induce lysosomal permeabilization. BMMs were cultured in medium with 30 mM glucose for 3 d. They were then loaded with lysine-fixable rhodamine-dextran and labeled with LAMP-1 antibodies. Shown here are single plane and summation projection images by confocal microscopy. Scale bar, 10 μm. This figure is supplementary to Figure 5.

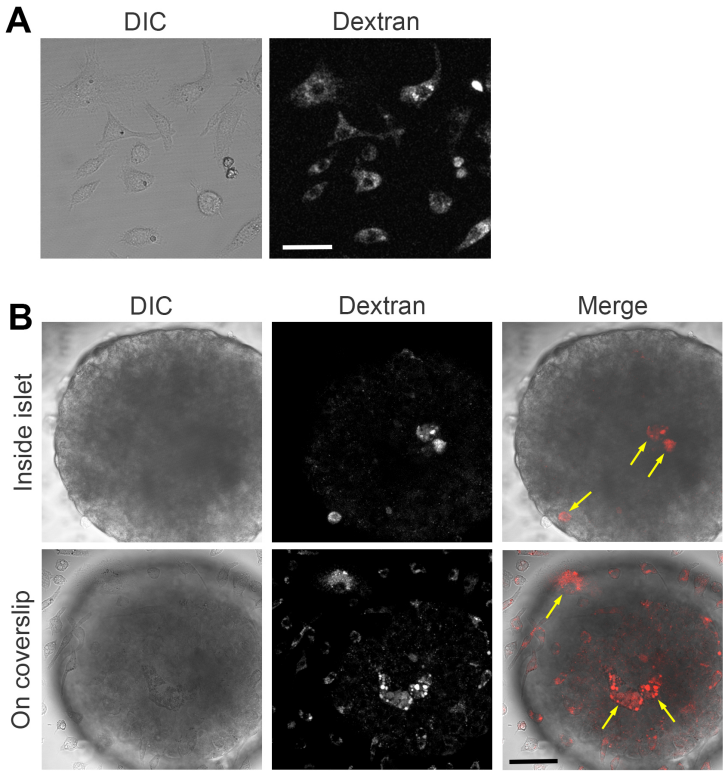

Figure S5. Dextran staining in HG-BMMs. (A) BMMs were cultured in HG and labeled with rhodamine-dextran similarly to the conditions used for Figure 6C, D. They were imaged live by confocal microscopy. (**B**) HG-BMMs loaded with rhodamine-dextran were added to apoptotic islets and imaged live by confocal microscopy. Images in the top and bottom panels focused on the BMMs that were inside the islet (above the coverslip) and on the coverslip, respectively. Yellow arrows show diffuse dextran in the cytoplasm. Scale bars, 50 μm. This figure is supplementary to Figure 5.

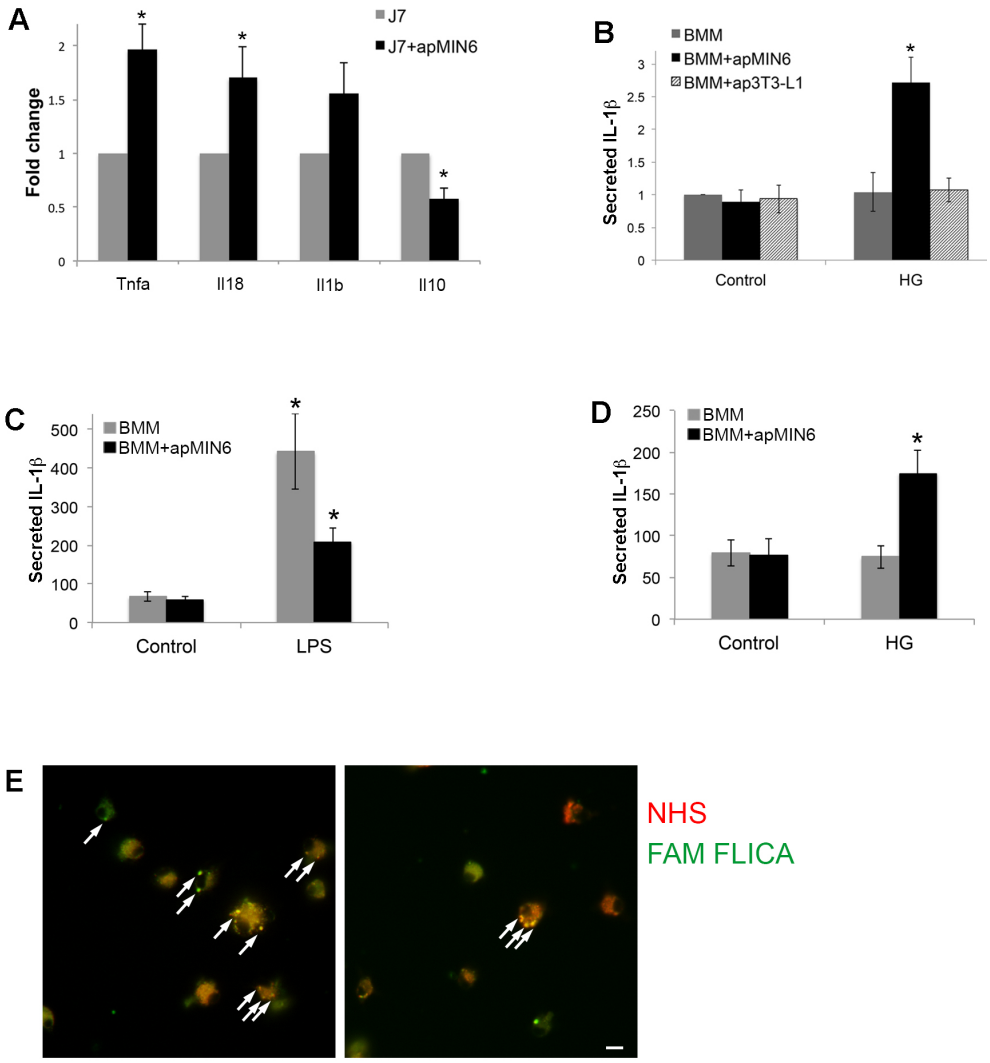

Figure S6. Inflammation induced by apMIN6 cells. (A). RT-PCR of inflammatory cytokine genes upon exposure to apMIN6 in J7 cells. **(B)** BMMs in control or HG medium were cultured overnight alone, with apMIN6 or with ap3T3-L1. Medium was collected after 24 h for ELISA assay of secreted IL-1β. *$p<0.05$. **(C, D)** BMMs kept in control (C) or HG (D) medium were cultured alone or overnight with apMIN6, and incubated for another 24 h with LPS (C) or in HG (D). Medium was collected for ELISA assay of secreted IL-1β (pg/ml). *$p<0.05$ by Student t test. **(E)** Caspase-1 activation visualized using FAM FLICA. HG-BMMs co-cultured with NHS-labeled apMIN6 (red) were probed with FAM FLICA (green) and imaged by wide-field microscopy. Arrows point to the specks where active caspase-1 was concentrated. Two examples are presented here to show that the colocalization between FAM FLICA and NHS fluorescence was not due to signal crossover. Scale bar, 10 μm. This figure is supplementary to Figure 6.

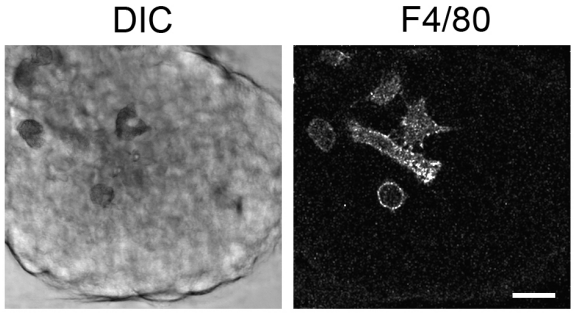

Figure S7. Islet macrophages stand out in DIC images. Isolated islets were cultured for two weeks to induce apoptosis. Islet purification went through three rounds of manual picking to ensure no contamination of other cells was present when an islet was seeded in the imaging dish. F4/80 was used to identify macrophages. DIC, differential interference contrast. Scale bar, 20 μm. This figure is supplementary to Figure 7.