Supplemental Figure Legends

Supplemental Figure 1. **Presence of insulin receptors in mouse and human β-cells.** (A) Mouse pancreas section stained with antibodies to the phosphorylated insulin receptor (IR). (B) Immunofluorescence imaging of phosphorylated IR, total IR in mouse β-cells showing localization in the cytoplasm and nucleus. (C) Overlay of confocal immunofluorescence of dispersed human islet cells using antibodies to IR (red) and early endosomal antigen-1-positive endosomes (EAA-1, green) demonstrates that a large proportion of IR co-localize with EAA1-positive endosomes, whereas IR in the plasma do not. Similar results were found with labelling of lysosomal and endosomal compartments. Bars in A = 50 μm, B = 5 μm C = 3μm.

Supplemental Figure 2. **Insulin promotes Raf-1 and Erk activation in mouse and human islets.** (A) Acute insulin signaling stimulation for 10 minutes in primary mouse islets with 0.02 nM, 0.2 nM, and 200 nM insulin caused an increase of the stimulatory phosphorylation of Raf-1 at serine 338 and a pro-survival phosphorylation of Erk (B). (C) Acute 0.2 nM insulin treatment (30 minutes) in human islets showed a significant decrease in the inhibitory phosphorylation site of Raf-1 at serine 259 and an increasing trend of phosphorylated Erk (D).

Supplemental Figure 3. **Endogenous B-Raf and Bad sub-cellular localization in mouse and human islets.** Immunofluorescence imaging of B-Raf showing localization in the nucleus and cytoplasm, and a moderate co-localization with Bad in primary human and mouse β-cells. Pearson correlation r values between B-Raf and Bad in human and mouse β-cells were 0.5 and 0.3 respectively. Scale bars in A are 10 μm.
Supplemental Figure 4. **Effect of insulin on ER Ca\(^{2+}\).** MIN6 cells transfected with D1ER were exposed to an insulin ramp and ER Ca\(^{2+}\) was measured. 100 \(\mu\)M carbachol was added to assess the releasable ER Ca\(^{2+}\) pool. 71 cells were imaged over 3 separate experiments.

Supplemental Figure 5. **The effects of insulin on \(\beta\)-cell proliferation require ER Ca\(^{2+}\) homeostasis.** BrdU positive MIN6 cells treated with insulin in the presence or absence of thapsigargin (Thap, 1 \(\mu\)M). *Denotes significant difference (p<0.05) between the serum-free control and treatment and ^between insulin and insulin with thapsigargin.

Supplemental Figure 6. **Simplified model of anti-apoptotic signaling of insulin via Raf-1/Erk and Bad pathways.** Activation of the insulin receptor may lead to the following pathways to promote \(\beta\)-cell survival: 1) signaling through the Raf/Mek and Erk cascade, 2) the Bcl-2-mediated translocation of Raf-1 to the mitochondria, where Bad is phosphorylated and inactivated, 3) signaling via Ca\(^{2+}\) mobilization via NAADP-sensitive intracellular stores. Green and red circles denote stimulatory and inhibitory phosphorylation respectively. Many signaling molecules have been omitted for clarity.
Supplemental Figure 1

A) Mouse Pancreas

B) Mouse β-cell

C) Human β-cell
Supplemental Figure 3

A

<table>
<thead>
<tr>
<th>Human β-cell</th>
<th>Mouse β-cell</th>
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<tbody>
<tr>
<td>Bad</td>
<td>B-Raf</td>
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<tr>
<td></td>
<td>Insulin</td>
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<td>Merge</td>
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Supplemental Figure 4

A

ER Ca^{2+} (FRET/CFP)

[Insulin] nM

0.2 200

100μM Carbachol
Supplemental Figure 5

A

Proliferation (Fold Increase)

10% Serum  +  +  -  -  -  -  -  -  -  -  -  -
Insulin (nM)  -  -  -  .2  .2  2  2  20  20  200  200
Thap (1µM)  -  +  -  +  -  +  -  +  -  +  +

*  ^