SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1. Expression of neurexin and neuroligin mRNA in human pancreatic islets and rat and mouse β–cell lines. To determine whether neurexin and neuroligin transcripts are expressed in islets and β-cell lines, cDNA from human islets, INS-1 cells, NIT cells and human fetal pancreas was analyzed by PCR amplification using gene-specific primers to all neurexin and neuroligin family members. Brain cDNA from the appropriate species was used as a positive control. As expected, products of the expected molecular weight were detected in brain for all neurexin and neuroligin family members analyzed. (A) In human islets, products corresponding to transcripts from the neurexin-1α, −1β, -2α, -2β, -3β and from the neuroligin-1, -2 and -3 genes where detected. (B) In the rat β-cell line INS-1, products corresponding to transcripts from the neurexin-1α, -1β, -2α, -2β and from the neuroligin-1, -2 and -3 genes were detected. (C) In NIT-1 cells, products corresponding to transcripts from the neurexin-1, -3, neuroligin-1 and -2 genes were detected. Faint bands corresponding to neurexin-2 and neuroligin-3 were also observed. (D) In fetal pancreas, products corresponding neurexin-3β and all of the neuroligin genes were detected. Trace amounts of the neurexin-1α and -1β PCR products were detected but the other neurexin genes were not. The lack of expression of neurexin-3α in humans islets, fetal pancreas and INS-1 cells distinguishes their neurexin expression from that in brain, from which neurexin-3α was readily amplified. (Br, brain; Is; human islets; Nt, no template; I, INS-1 cells; N, NIT cells; P, human fetal pancreas; Nrxn, neurexin; NL, neuroligin).

Supplemental Figure 2. Neuroligin and neurexin mRNA expression in human islets. To determine the relative abundance of neuroligin and neurexin transcripts in human islets, RNA was isolated and reverse transcribed to cDNA and analyzed by real time PCR analysis. Neuroligin-1, neuroligin-2, neurexin-1α, neurexin-2β and neurexin-3β were the most abundant transcripts detected. Lower levels of neuroligin-3, neurexin-2α and neurexin-3α transcripts were detected and neurexin-1β was not detected in human islets. Data are expressed as the difference between cycle threshold values for human islet cDNA amplified with either neurexin or neuroligin primers and background signal (RNA not treated with reverse transcriptase).

Supplemental Figure 3. Abundance of Neurexin and Neuroligin transcripts in INS-1 cells relative to rat brain. To determine how transcript levels compare in INS-1 cells and the rat brain, the number of transcripts detected in INS-1 cells was expressed as a percentage of the number of transcripts detected in brain. Statistically, no difference was detected between brain and INS-1 mRNA levels for neuroligin-2 (116%), neurexin-1α (72%) or neurexin-2 (87%). Neuroligin-3 was expressed in INS-1 cells at 14% of brain levels and all other neurexin and neuroligin genes tested were at levels that were at least 10-fold lower than levels detected in brain. Elevated 18s C_T values in rat islets, possibly as a result of differing RNA isolation methods, made us uncomfortable expressing rat islets as a percentage of brain. (*n* of 3 separately-prepared RNA/ cDNA samples per experiment).

Supplemental Figure 4. Neurexin-1 is not expressed in α-cells. To determine whether neurexin was expressed in islet α-cells, immunohistochemical localization was performed using a polyclonal antibody to neurexin-1 and a monoclonal antibody to glucagons. Images were captured with a confocal microscope equipped with a 60x lens. Neurexin was not detected in cells expressing glucagon. (Nrxn, neurexin-1; glu, glucagon).

Supplemental Figure 5. Neuroligin-1, -3 and -4 increase insulin secretion at basal glucose levels. INS-1 cells were transfected with a constructs encoding either full-length neuroligin-1, neuroligin-3 or neuroligin-4, cultured for 48 hours and then conditioned for 1 hour in a Krebs-ringer solution containing 2.75 mM glucose. After conditioning, secreted insulin was measured by radioimmunoassay after treatment with low (2.75 mM) glucose for 1h. Overexpression of neuroligin-1, -3 and -4 led to 61% (n=7, p=.004), 149% (n=12, p=.00009) and 168% (n=12, p=.000006) increases in secreted insulin levels relative to mock-transfected controls.

Supplemental Figure 6. Insulin secretion from dispersed islet cells treated with low and high glucose. Dispersed islets were transfected with a pool of non-targeting SiRNA, cultured for 48 hours and then conditioned for 1 hour in Krebs-ringer solution containing 2.75 mM glucose. After conditioning, basal insulin secretion was measured by radioimmunoassay after treatment with low (2.75 mM) glucose for 1h. Dispersed islets were then treated with high glucose (25 mM) for 1 hour to measure stimulated insulin secretion. Dispersed islet cells treated with high glucose (25 mM) and non-targeting

siRNA secreted 40% more insulin than those treated with low glucose (2.75 mM) and non-targeting siRNA (n=6, p=.01). Secreted insulin was normalized to total insulin in the cell layer.