Synaptotagmin-7 is the Principal Ca^{2+}-Sensor for Ca^{2+}-Induced Glucagon Exocytosis in Pancreas by Gustavsson *et al.*,

Supplemental Materials and Methods

Immunostaining and Electron Microscopy

Immunostaining of additional synaptotagmins were performed essentially as described in the main text. Briefly, 10 µm cryo-sections of synaptotagmin-7 KO and control mouse pancreata were mounted on slides and probed with antibodies against glucagon and synaptotagmin-1 (Cl41.1), -2 (I735) or -3 (L181), and then with Alexa488 and Alexa546 conjugated secondary antibodies (Invitrogen). Slides were visualized on a Nikon A1 confocal laser microscope.

RNA Extraction and qPCR

Total RNA was extracted from islets of synaptotagmin-7 KO and control mice using the Trizol method, treated with DNase I (Roche) and reverse-transcribed using Reverse Transcription kit (Applied Bioscience). qPCR for glucagon was performed on an Applied Biosystems Prism using the following primer pair: 5'-ATT CAC CAG CGA CTA CAG CAA-3' and 5'-TCA TCA ACC ACT GCA CAA AAT C-3'. β-Actin was used as an internal standard to determine relative mRNA levels.

Supplemental Legends

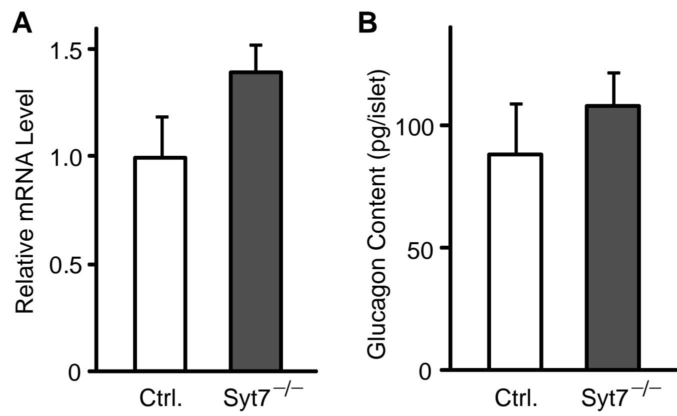
Supplemental Figure 1. Normal Glucagon mRNA Level and Glucagon Content in Synaptotagmin-7 KO Islets.

(A) Glucagon mRNA levels were analyzed by real time PCR from total RNA extracted from isolated islets. Compared with control (Ctrl., white bar), glucagon mRNA level was not altered in synaptotagmin-7 KO ($Syt7^{-/-}$, gray bar) based on three independent qPCR experiments from pooled islets of three to five mice. **(B)** Glucagon content was measured in batches of 10 isolated islets from synaptotagmin-7 KO ($Syt7^{-/-}$, gray bar) or control (Ctrl., white bar) by using RIA. Islets were cultured overnight at 11.1 mM

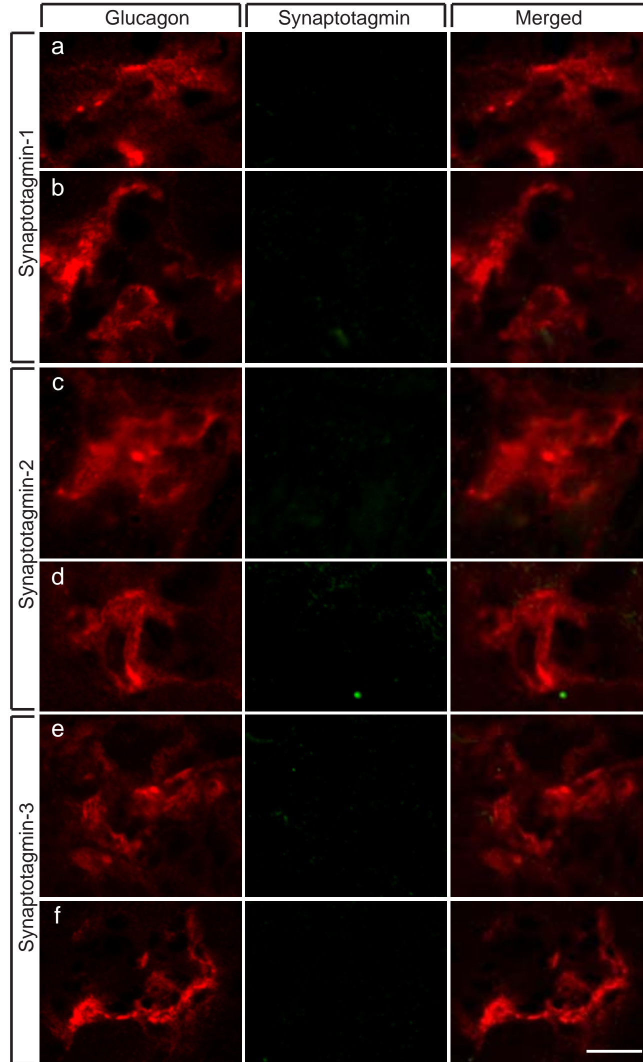
glucose, washed with PBS and lysed in cell lysis buffer by sonication. Data are presented as mean ± SEM of 14 KO and 15 control mice.

Supplemental Figure 2. Synaptotagmin-1, -2 and -3 Are not Present in Mouse Pancreatic α-cells.

Ten micrometer pancreatic sections were stained with glucagon antibody plus an antibody against synaptotagmin-1 (Cl41.1), -2 (I735) or -3 (L181), followed by fluorescence-conjugated secondary antibodies. Representative images of such stained sections, taken on a Nikon A1 confocal laser microscope, are shown. No apparent synaptotagmin-1, -2 or -3 signal was detected in glucagon-positive (red) cells from synaptotagmin-7 KO (b, d, f) or control (a, c, e) mice. Scale bar (10 μm) applies to all panels.



Supplemental Figure 1 (Gustavsson et al.)



Supplemental Figure 2 (Gustavsson et al.)