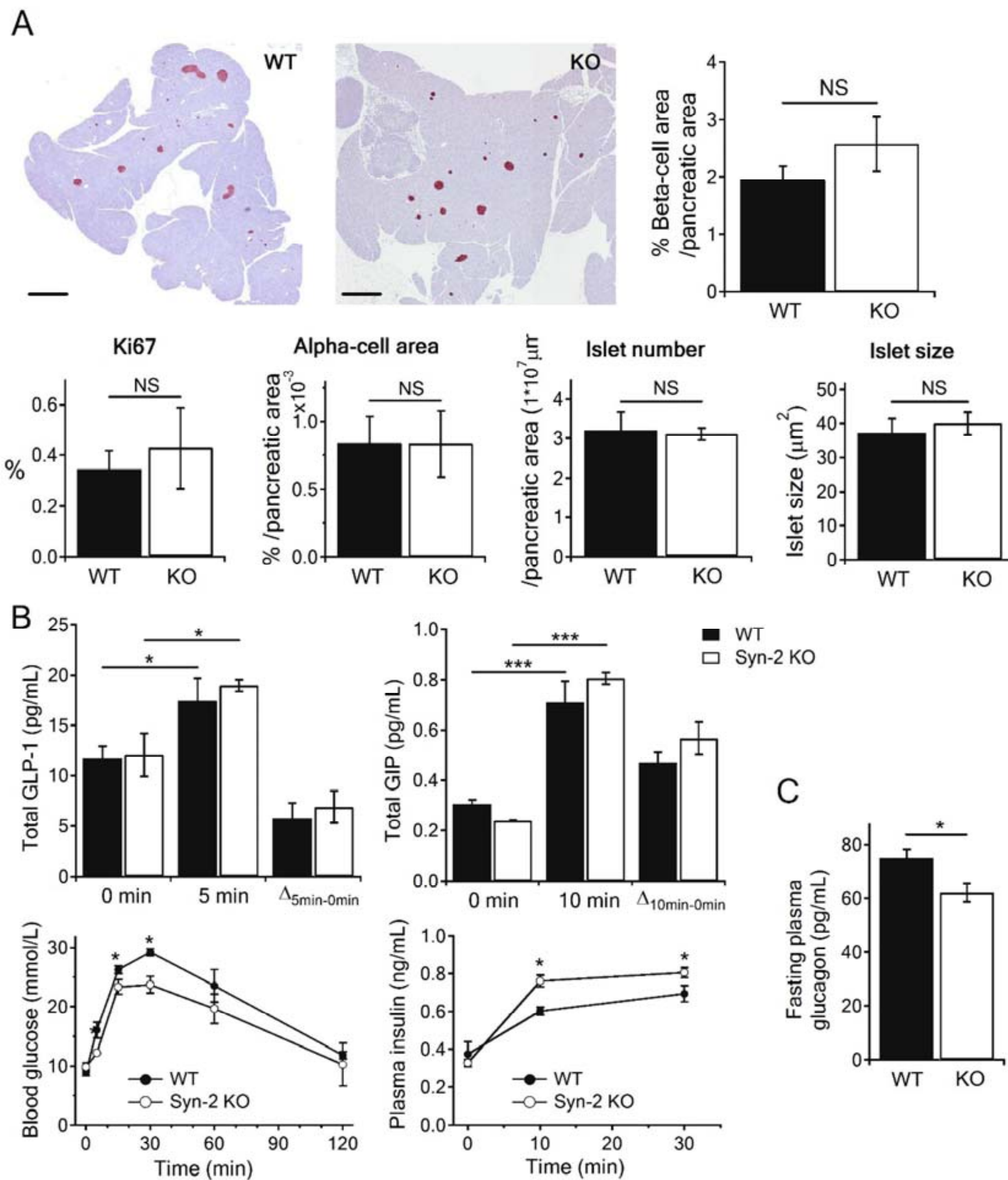
SUPPLEMENTARY DATA

Suppplementary Figure S1. Syn-2 deletion does not affect islet morphology, β-cell mass, or GLP-1/GIP secretion. ***A:*** Left: Insulin-immunostained pancreatic sections. Scale bars, 600 µm; Right: Ratios of β-cell area per pancreatic area on mouse pancreatic sections. Bottom from left to right: Ki67-positive islet β-cells as percentage of total islet β-cells on mouse pancreatic sections; Ratios of α-cell area per pancreatic area; islets numbers per pancreatic area; islet sizes on pancreatic sections. N=8-10 mice for each. ***B*****:** Circulating blood levels of total GLP-1 (*left*) and GIP (*Right*) immunoreactivity (*top*) were measured before and after oral glucose administration, along with determination of blood glucose and insulin levels (*bottom*). N = 6 for each group. ***C*****:** Fasting plasma glucagon levels of WT and Syn-2 mice (N = 3). **p* < 0.05; ****p* < 0.001. Summary graphs shown as means ± SEMs.



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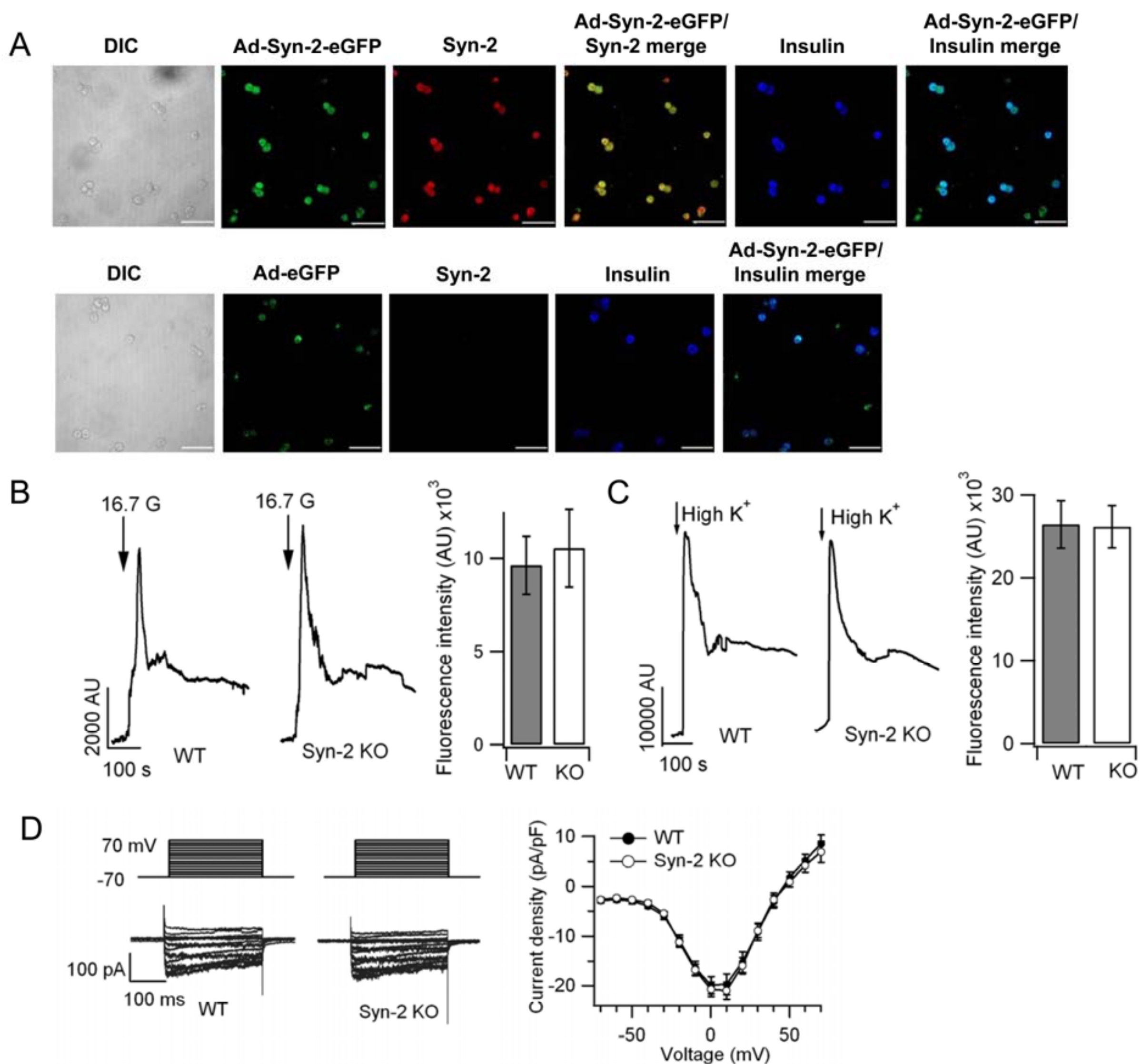
SUPPLEMENTARY DATA

Supplementary Figure S2-related to Figure 2. ***A***: Confocal images of Syn-2-KO mouse islet β-cells whose Syn-2 expression was restored by Ad-Syn-2-eGFP infection. Top: Syn-2-KO islet β-cells infected with Ad-Syn-2-eGFP; Bottom: Syn-2 KO islet β-cells infected with Ad-eGFP as control. Scale bar: 50 μm. ***B-C***: Calcium imaging with fluo-4 showed there was no difference in intracellular Ca^{2+} concentration increase between WT and Syn-2-KO mouse β-cells stimulated with high glucose (B, 16.7 mM) or with high K^{+} (C, 40 mM) stimulation. ***D***: Deletion of endogenous Syn-2 in mouse β-cells did not affect voltage-gated calcium channel currents. Representative traces showing Cav currents recorded in whole-cell mode from -70 to 70 mV with 10-mV increment in WT and Syn-2 KO mice (Left**)**. Current-voltage relationship of Cavs from WT (n=15) and Syn-2 KO (n=11) β-cells (*right*). Current were normalized to cell capacitance to yield current density. Values are means ± SEMs.



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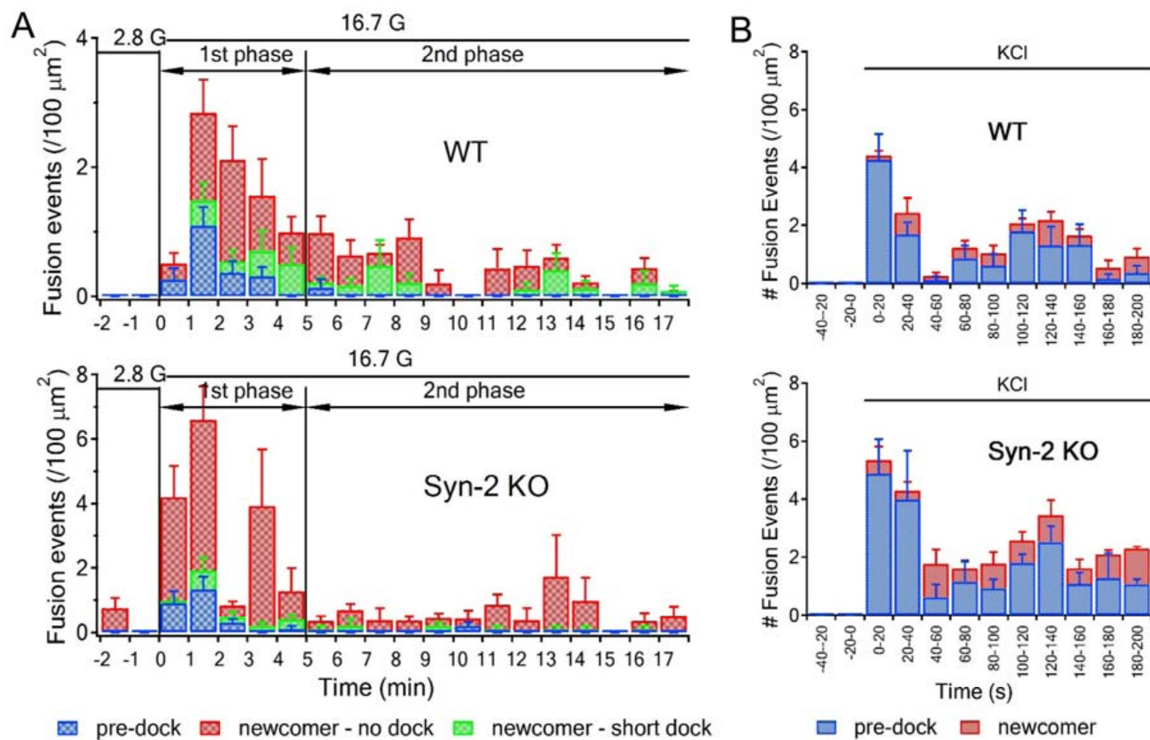
Supplementary Figure S3. Syn-2 deletion increases newcomer SG exocytosis stimulated by 16.7 mM glucose and pre-dock SG exocytosis stimulated by 50 mM KCl. ***A***: Histogram of fusion events in first (first 5 min after 16.7 mM glucose stimulation) and second phases (5–18 min) in WT (top) versus Syn-2-KO β cells (bottom). Three patterns of fusion events (pre-dock, newcomer-no dock, newcomer-short dock) are indicated by blue, red and green bars, respectively. Data obtained from five independent experiments (2-4 cells from each experiment). ***B:*** Histogram of fusion events during 200 seconds of acquisition in WT (top) versus Syn-2-KO β cells (bottom). Pre-dock and newcomer SG fusion events are indicated by blue and red bars, respectively. Data obtained from three independent experiments (3-5 cells from each experiment).



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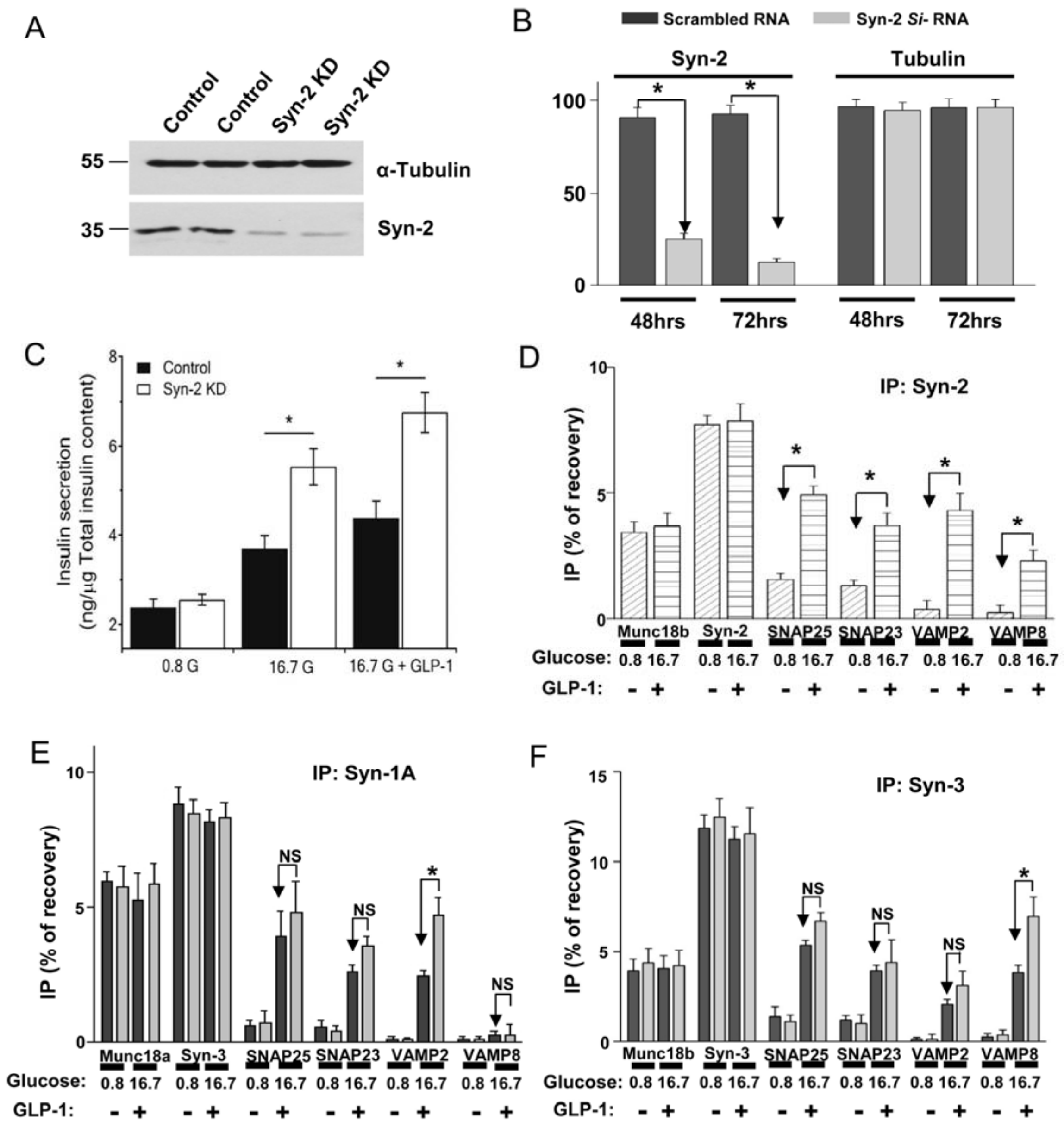
SUPPLEMENTARY DATA

Supplementary Figure S4-Data analysis for Figure 4. ***A*****:** Western blot analysis of Syn-2 knockdown expression in INS-1 cells. INS-1 cells were transfected with Syn-2 siRNA and the scrambled siRNA were used as controls. ***B***: Analysis of Western blots in **Fig. 4*F*** of siRNA knockdown of Syn-2 expression in INS-1 cells at 48 and 72 hrs. ***C:*** Glucose (as indicated) with or without the presence of 10 nm GLP-1 stimulated insulin secretion were performed on Syn-2 siRNA knockdown and scrambled control INS-1 cells. Data from 4 independent experiments. ***D-F:*** Quantitative analysis of indicated co-IPed proteins by Syn-2 (***D*** for **Fig. 4*E***, untreated INS-1), Syn-1A (***E*** for **Fig. 4G**, Syn-2 siRNA-treated INS-1) and Syn-3 (***F*** for **Fig. 4*H***, Syn-2 siRNA-treated INS-1) antibodies. Summary graphs shown are means ± SEMs, N=3. *p< 0.05 per Student's t test; NS, no significant difference.



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