

**CLASS II PHOSPHOINOSITIDE 3-KINASE REGULATES EXOCYTOSIS
OF INSULIN GRANULES IN PANCREATIC BETA CELLS**

**Veronica Dominguez¹, Claudio Raimondi¹, Sangeeta Somanath¹, Marco Bugliani²,
Merewyn K. Loder³, Charlotte E. Edling¹, Nullin Divecha⁴, Gabriela da Silva-Xavier³,
Lorella Marselli², Shanta J. Persaud⁵, Mark D. Turner¹, Guy A. Rutter³, Piero Marchetti²,
Marco Falasca¹ and Tania Maffucci¹**

SUPPLEMENTAL DATA

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. PI3K-C2 α downregulation affects insulin secretion. *A*, Insulin secretion was determined upon stimulation of the indicated stable cell lines with 60 mM KCl for 30 min. Data are means \pm SEM from 21 independent experiments performed in duplicate. Total secreted insulin for each sample (pg insulin) was normalized by the total protein content (μ g protein) and then expressed as fold increase over the unstimulated “sh scrambled” cells. Student’s t-Test $^* = p < 0.001$. *B*, Insulin secretion was determined upon stimulation of the indicated stable cell lines with 20 mM glucose for 30 min. Data are means \pm SEM from 3 independent experiments performed in duplicate. Total secreted insulin for each sample (pg insulin) was normalized by the total protein content (μ g protein) and then expressed as fold increase over each unstimulated condition. Student’s t-Test $^{**} = p < 0.05$.

Fig. S2. Analysis of IRS2 tyrosine phosphorylation. The indicated cells were stimulated with 100 nM insulin, 60 mM KCl for 30 min (*A*) or the secretagogue cocktail for 3h (*B*). Tyrosine phosphorylation of IRS2 was determined in Western blot analysis upon immunoprecipitation. Membranes were then stripped to determine the total amount of immunoprecipitated IRS2.

Fig. S3. Confocal microscopy analysis of syntaxin 1 and VAMP. Representative images of syntaxin 1 and VAMP1/2/3 in the indicated cells analyzed as described in Experimental Procedures.

Fig. S4. Confocal microscopy analysis of syntaxin 1 and SNAP25. Confocal microscopy images of syntaxin 1 and SNAP25 in the indicated cells. DAPI stained the nuclei.

Figure S1

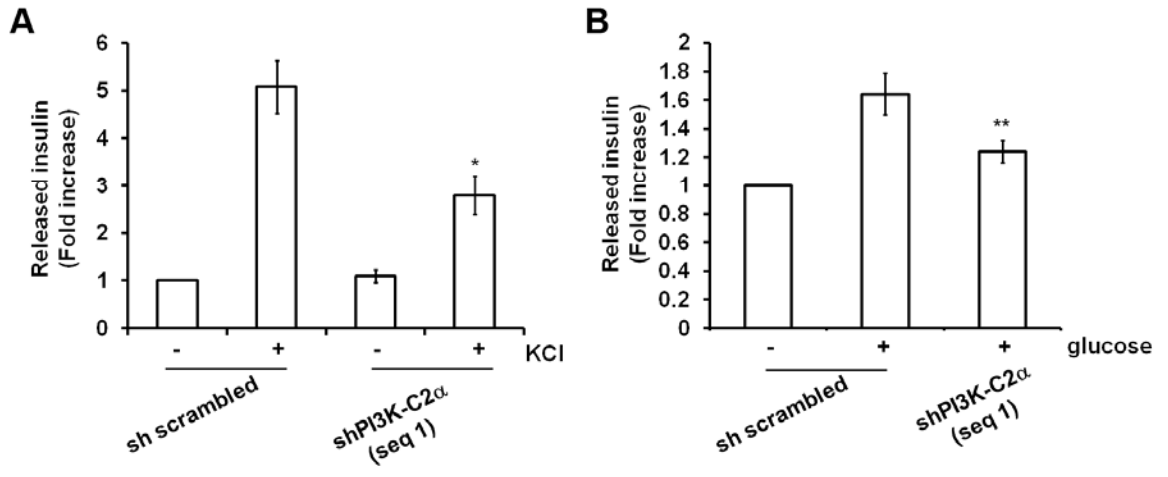
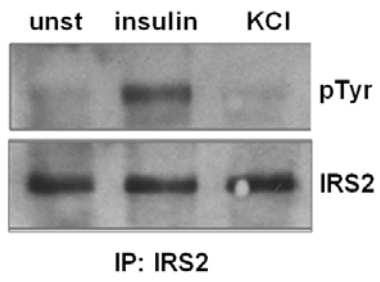


Figure S2

A



B

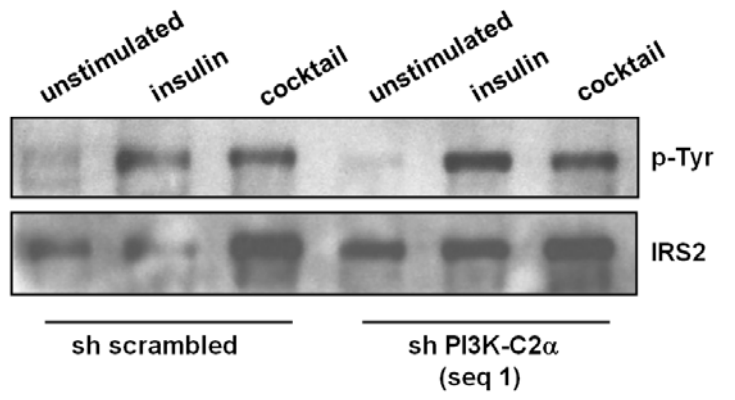
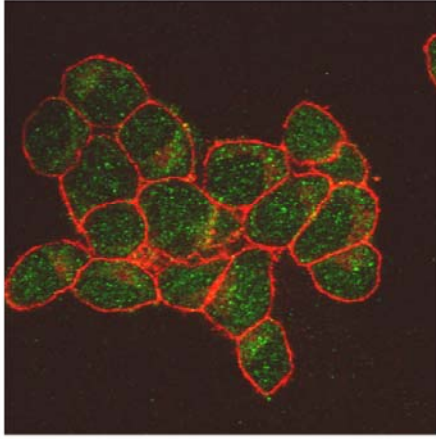
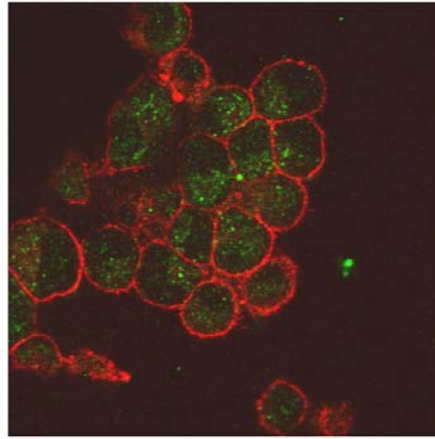


Figure S3



sh scrambled



sh PI3K-C2 α (seq 1)

Syntaxin 1
VAMP

Figure S4

