SUPPLEMENTAL DATA

Supplemental Materials and methods

Static incubation assays

The hamster HIT-T15 cells (ATCC, UK) were maintained in RPMI 1640 medium as previously described [1, 2]. HIT-T15 cells (40,000 cells/cm^2) were labelled for 120 h with 10 µCi/ml [^{3}H] *myo*-inositol in a custom-made RPMI 1640 [1, 2]. Cells were pre-incubated for 30 min in KREBS buffer containing 0.1 mM glucose, then stimulated with 10 mM glucose in KREBS buffer for various time up to 5 min and lysed by 5 % TCA to extract inositol polyphosphates.

The human derived HCT116 colon cancer cells were cultured in DMEM/F12 medium as described previously [3]. Cells (1.0 x 10^6) were seeded in a 10 cm dish in 8 ml of DMEM/F12 medium supplemented with 10 % FBS plus 10 µCi/ml [^{3}H] *myo*-inositol and cultured for three days. On the day of the experiment, the cells were pre-incubated in DMEM medium without glucose supplemented with 10 % FBS for 3.5 h. HCT116 cells were then stimulated with 10 mM glucose for different time intervals as described in Supplemental Fig. S7. Inositol phosphates were extracted and purified before analysis using a carboPac HPLC column as previously described [4].

FACS analysis of dispersed islet cells

Pancreatic islets were isolated from 3 month-old lean and ob/ob mice after 12 h fasting. The islets were maintained in complete RPMI (5.5 mM glucose) for 1-2 h prior dispersion into single cells by Accutase digestion, as previously described [5]. Next, cells were incubated in fixation/permeabilization buffer (#FC007, R&D system, Abingdon, United Kingdom) for 30 min. Then, they were washed with PBS and stained with either an Allophycocyanin

(APC)-conjugated antibody against insulin (#IC1417A, R&D system, dilution 1:10) or an APC-control antibody (Rat IgG2A, #IC00A, R&D system, dilution 1:10) in flow cytometry permeabilization/wash buffer I (#FC005, R&D system) at 4 ºC overnight. After two washes with the permeabilization/wash buffer I, the cells were stained with 5 μg/ml Hoechst (Invitrogen, Lidingö , Sweden) in flow cytometry staining buffer (#FC001, R&D system) and analyzed by FACS, using the Influx cell sorter (BD Biosciences, San Jose, CA, USA) equipped with a solid state laser for 635 nm excitation and a 670/30 nm emission filter for APC fluorescence detection. Positive antibody staining was discriminated from negative staining based on APC intensity threshold.

Immunocytochemistry

MIN6m9 cells were seeded at the density of 12,500 cells/cm^2 on glass coverslips in complete DMEM medium. Cells were grown for ~72 h before fixation for the detection of the endogenous IP6K2. In order to visualize IP6K1 we overexpressed a Myc-tagged version of the protein. MIN6m9 cells grown for ~24 h were transfected with a plasmid carrying IP6K1-Myc [1] using Lipofectamine 3000 Transfection Reagent (ThermoFisher, Stockholm, Sweden) and cultured for additional ~72 h. Cells were fixed with 4 % paraformaldehyde, then they were washed with PBS twice and kept in blocking solution (PBS containing 0.1 % BSA and 0.3 % Triton-X100) for 1 h. This was followed by the incubation with the primary antibody (rabbit anti-IP6K2 antibody, # 179921, Abcam, Cambridge, UK, diluted 1:100; mouse anti-c-Myc, 9E10, # sc-40, Santa Cruz Biotechnology Inc., AH diagnostics AB, Sweden, diluted 1: 100) in blocking solution, at 4 °C overnight. The coverslips were washed 3 times, 10 min each, with rinse buffer (PBS containing 0.1 % Triton-X100). Then the cells were incubated with the appropriate polyclonal secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG, # A-11034; Alexa Fluor 488 goat anti-mouse IgG, # A-11029, Invitrogen,

ThermoFisher) diluted 1:300 in blocking solution, for 2 h at room temperature. The cells were washed with rinse buffer 3 times, 10 min each, prior to nuclear staining with 3.3 μg/ml 7-aminoactinomycin D (7-AAD, Molecular Probes, Life Technologies, ThermoFisher) in rinse buffer for 30 min. The coverslips were mounted on slides using Vectashield mounting medium (Vector Laboratories, BioNordika, Stockholm, Sweden). Cells were examined with a Leica SP2/SP5 confocal microscope (LEICA Microsystems). Immunofluorescence was imaged using sequential scanning with the following settings: Alexa Fluor 488, 488 nm excitation, 506-536 nm detection; 7-AAD, 543 nm or 561 nm excitation, 585-677 nm detection.

Quantification of IP6K1 protein levels using immunoblotting

IP6K1 protein levels from MIN6m9 cells were quantified essentially as described in [6]. In brief, MIN6m9 cells were preincubated and stimulated with 10 mM glucose as described under Dynamic incubation assays. Then, the cells were lysed with RIPA buffer (150 mM NaCl, 1 % NP-40, 0.1 % sodium deoxycholate, 1 mM EDTA, 0.1 % SDS, 50 mM Tris, pH 7.6) supplemented with complete mini protease inhibitor cocktail and PhosSTOP phosphatase inhibitors (Roche Diagnostics, Stockholm, Sweden). Equal amounts of protein extracts (20 μg/lane) were separated using pre-cast NuPAGE™ 4- 12 % Bis-Tris gel (Thermofisher Scientific) and transferred to nitrocellulose membranes. The membranes were then blocked with 5 % skim milk and probed with rabbit-anti IP6K1 antibody (Sigma-Aldrich HPA040825) followed by HRP-conjugated donkey anti-rabbit antibody (NA934, GE Healthcare, Uppsala, Sweden) or HRP-conjugated mouse anti-β-actin (Sigma-Aldrich®A3854). The blots were developed with Amershan ECL Prime Western Blotting Detection Reagent (GE Healthcare) [6]. The chemiluminescence signal was detected by ChemiDoc™ Imaging System and analyzed by densitometry using Image J (version 1.48) software.

Statistical analysis

Data are expressed as means ± SEM. The data were statistically analyzed with GraphPad Prism software version 5.0, using the statistical tests described in figure legends and tables.

Supplemental references

[1] Illies, C., Gromada, J., Fiume, R., Leibiger, B., Yu, J., Juhl, K., et al.,. Requirement of inositol pyrophosphates for full exocytotic capacity in pancreatic beta cells. Science 318(5854) (2007) 1299-1302.

[2] Larsson, O., Barker, C.J., Sjoholm, A., Carlqvist, H., Michell, R.H., Bertorello, A., et al.,. Inhibition of phosphatases and increased Ca2+ channel activity by inositol hexakisphosphate. Science 278(5337) (1997) 471-474.

[3] C. Gu, H.N. Nguyen, D. Ganini, Z. Chen, H.J. Jessen, Z. Gu, H. Wang, S.B. Shears, KO of 5-InsP7 kinase activity transforms the HCT116 colon cancer cell line into a hypermetabolic, growth-inhibited phenotype, Proc Natl Acad Sci U S A 114 (45) (2017) 11968-11973.

[4] Gu, C., Wilson, M.S., Jessen, H.J., Saiardi, A., Shears, S.B. Inositol Pyrophosphate Profiling of Two HCT116 Cell Lines Uncovers Variation in InsP8 Levels. PLoS One. 2016 Oct 27;11(10):e0165286.

[5] Köhler, M., Darè, E., Ali, M.Y., Rajasekaran, S.S., Moede, T., Leibiger, B., et al.,. One-step purification of functional human and rat pancreatic alpha cells. Integrative Biology 4(2) (2012) 209-219.

[6] Rajasekaran, S.S., Illies, C., Shears, S.B., Wang, H., Ayala, T.S., Martins, J.O., Darè, E., Berggren, P.O., Barker, C.J. Protein kinase- and lipase inhibitors of inositide metabolism deplete IP7 indirectly in pancreatic beta-cells: Off-target effects on cellular bioenergetics and direct effects on IP6K activity, Cell Signal 42 (2018) 127-133.

Supplemental Table

Table S1. Two-way ANOVA of various data sets followed by Bonferroni post hoc test.

Data set	Source of variation	Bonferroni post-test
Fig. 1H, (n = 3)	Interaction, F(1,8) = 5.98, *p < 0.05 Glucose, F(1,8) = 6.19, *p < 0.05 siRNA, F(1,8) = 51.13, ***p < 0.001	siControl, 0.5G vs. 10G, *p < 0.05 siIP6K1, 0.5G vs. 10G, n.s. 0.5G, siControl vs. siIP6K1, *p < 0.05 10G, siControl vs. siIP6K1, ***p < 0.001
Fig. 2A, (n = 3)	Interaction, F(48, 150) = 1.99, ***p < 0.001 Time, F(24, 150) = 64.96, ***p < 0.001 siRNA, F(2, 150) = 1.56, n.s.	2 min after 10G stimulation, siControl vs. siIP6K1, ***p < 0.001

(0.5G, 0.5 mM glucose; 10G, 10 mM glucose; n.s., not significant).

Supplemental figures

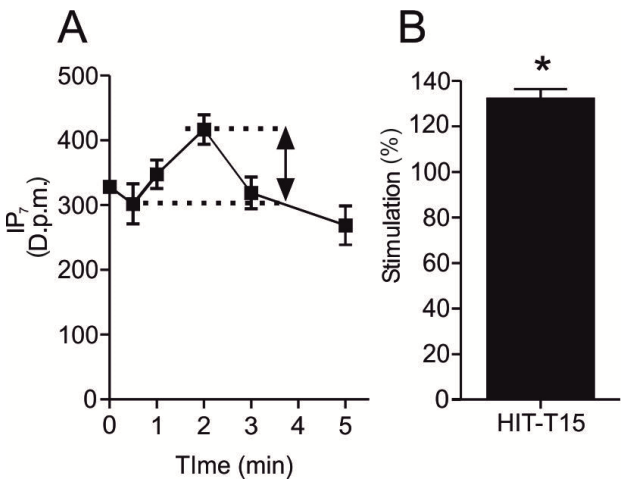

Fig. S1. Glucose responsiveness of IP_7 from hamster derived HIT-T15 insulin secreting cells. (A) IP_7 levels obtained at various time points following 10 mM glucose stimulation, data from a representative experiment with triplicates performed on HIT-T15 cells. (B) Response from dip to peak (*see panel* A) of IP_7 expressed as percentage of stimulation, n = 3, *$p < 0.05$, 95 % confidence interval. Plot and bar, means ± SEM.

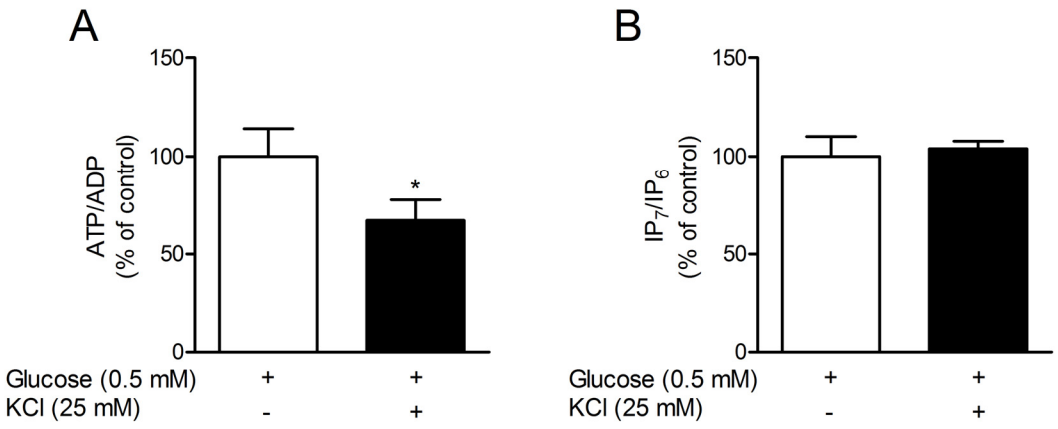

Fig. S2. Effect of KCl stimulation on ATP/ADP and IP_7/IP_6. MIN6m9 cells were stimulated in KREBS buffer containing 0.5 mM glucose or 0.5 mM glucose plus additional 25 mM KCl for 3 min. KCl stimulation decreased ATP/ADP levels significantly (A), whereas IP_7/IP_6 levels remained unchanged (B). Under these conditions there was as expected a 2.8-fold increase in insulin secretion (insulin release, pg/ml, normalized to inositol lipids d.p.m.: 0.5 mM glucose, 0.0124 ± 0.0023; 0.5 mM glucose plus 25 mM KCl, 0.0334 ± 0.0043). Means ± SEM, n = 3, *$p < 0.05$, Student's *t*-test.

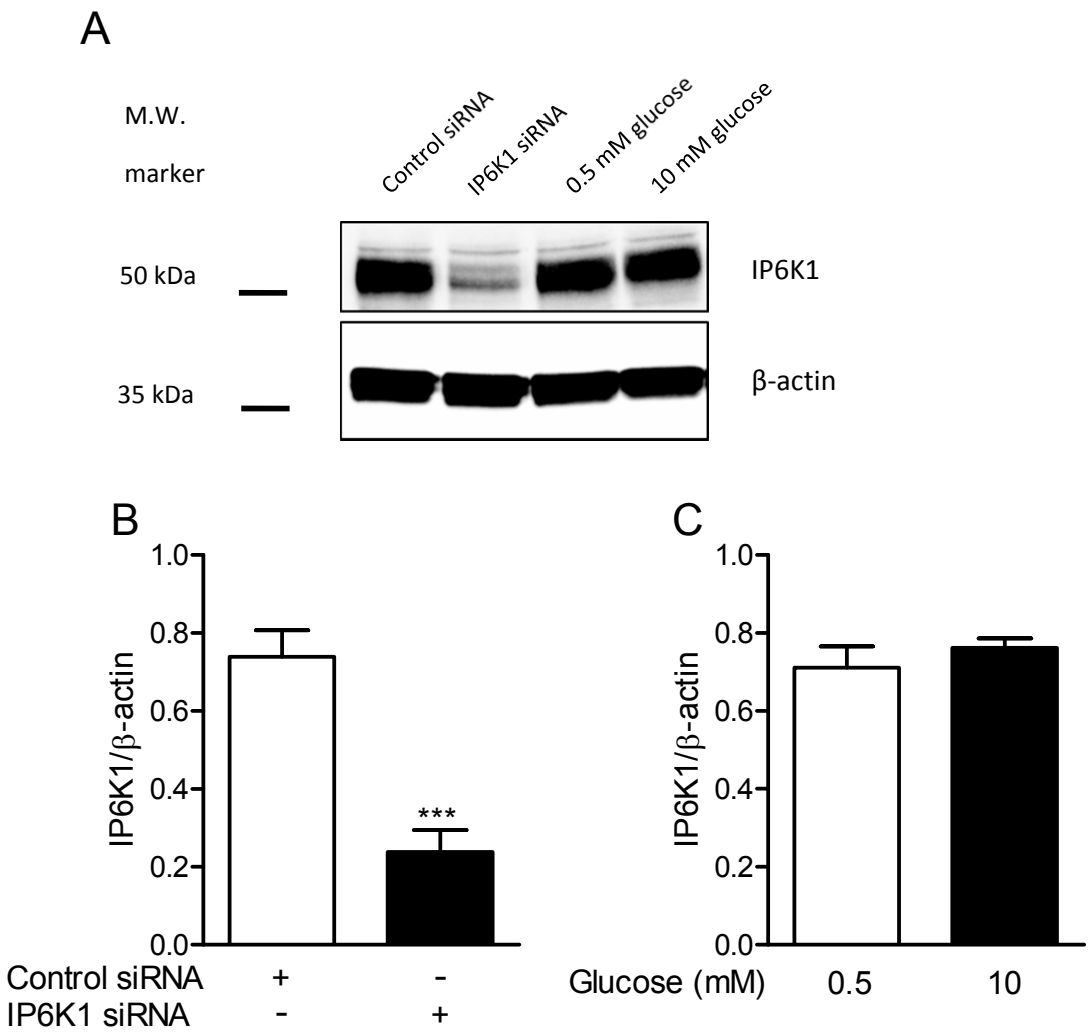

Fig. S3. The level of the IP6K1 protein was evaluated by immunoblotting in MIN6m9 cells. (A) Representative immunoblot out of three showing IP6K1 expression in MIN6m9 cells upon different conditions as indicated in the panel. The β-actin signal was visualized in the same membranes. Densitometry analysis of IP6K1 immunoblots showed that there was a 69 % decrease in IP6K1 protein levels upon treatment with IP6K1 siRNA (B) and there was no difference in IP6K1 protein levels upon treatment with 10 mM glucose for 3 min (C). IP6K1 protein level was quantified and expressed as a ratio of IP6K1/β-actin. Data are presented as means ± SEM, n = 3 experiments, ***$p < 0.001$, Student's paired *t*-test.

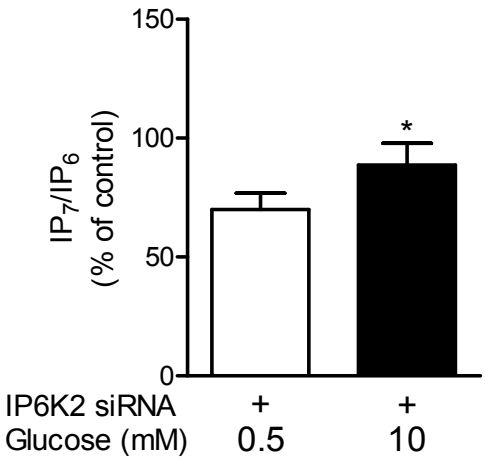

Fig. S4. IP_7 measurements in IP6K2-silenced cells. The rise in IP_7 induced by glucose stimulation was maintained in IP6K2-silenced MIN6m9 cells, means ± SEM, n = 3, *$p < 0.05$, Student's paired *t*-test. Data are presented as a percentage of unstimulated control siRNA.

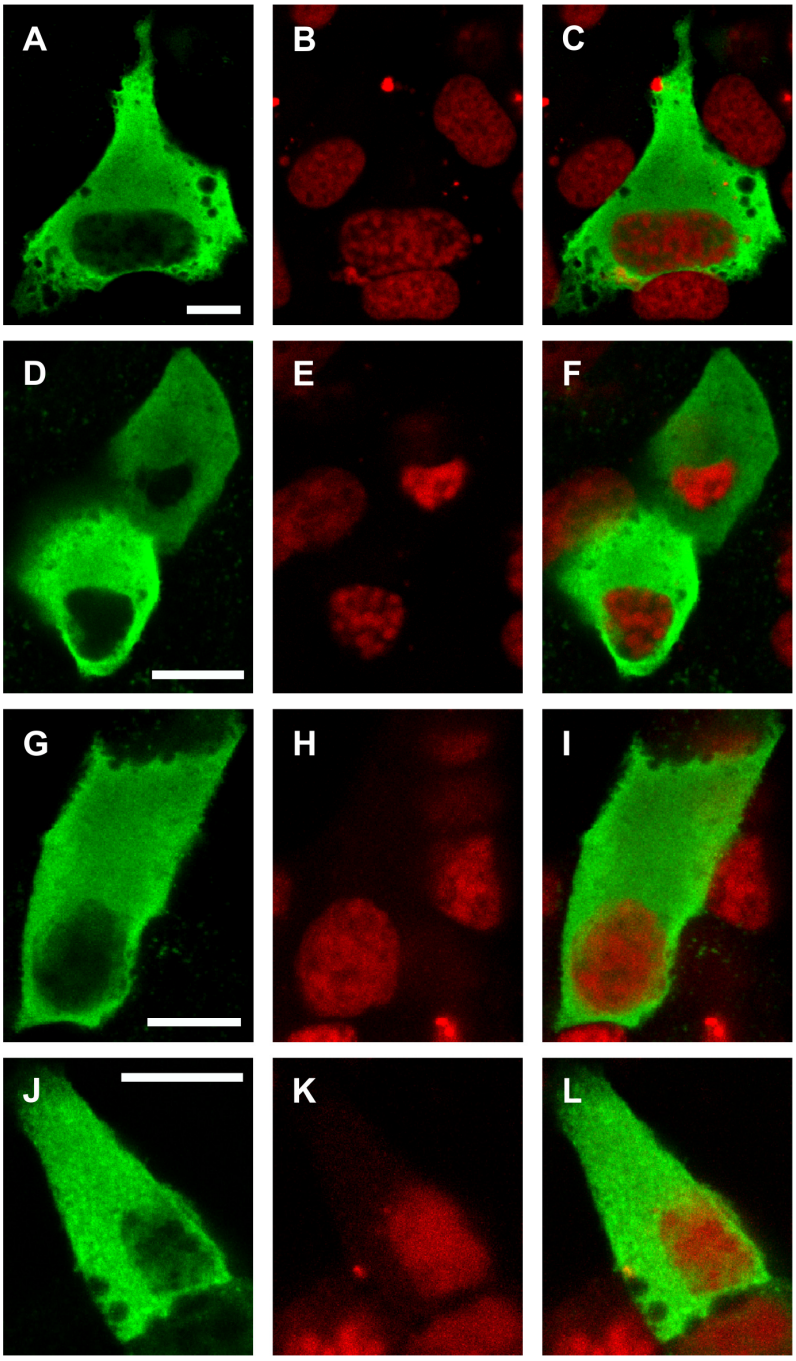

Fig. S5. Detection of IP6K1 by immunocytochemistry in MIN6m9 cells. (A, D, G and J) Myc-immunoreactivity was used to detect IP6K1 in MIN6m9 cells overexpressing Myc-IP6K1. (B, E, H and K) Visualization of the cell nuclei by 7-AAD staining. (C) Overlay of A and B. (F) Overlay of D and E. (I) Overlay of G and H. (L) Overlay of J and K. Scale bar = 10 μm.

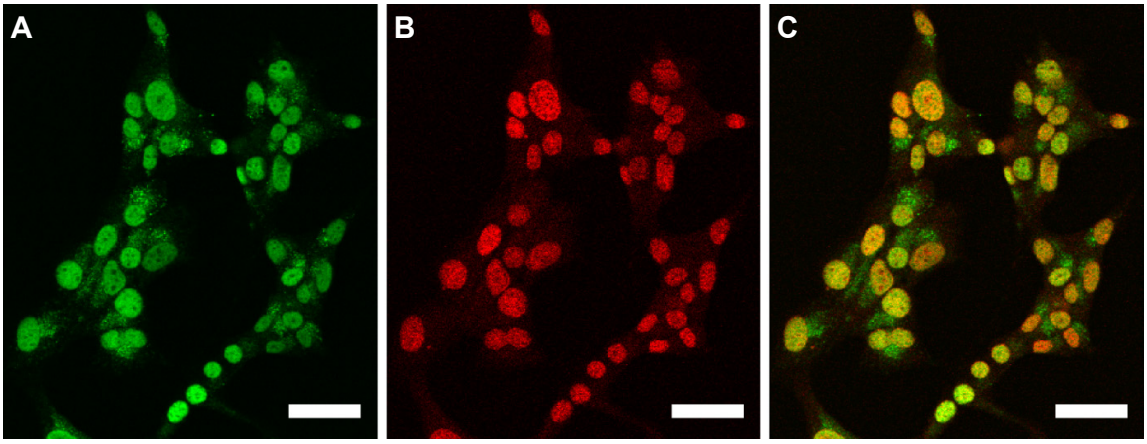

Fig. S6: Detection of IP6K2 by immunocytochemistry in MIN6m9 cells. (A) IP6K2 immunoreactivity in MIN6m9 cells. (B) Visualization of the cell nuclei by 7-AAD staining. (C) Overlay of A and B. Scale bar = 30 µm.

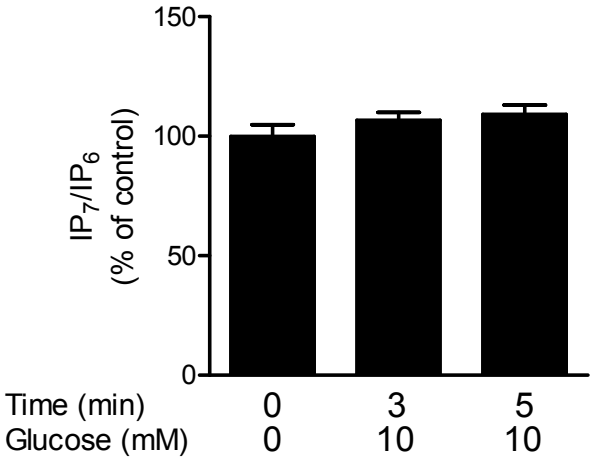

Fig. S7. Effect of glucose on IP_7 levels in human derived HCT116 colon cancer cell line. IP_7 levels were obtained at various time points following 10 mM glucose stimulation in HCT116 cells. Using one-way ANOVA, there is no significant increase in IP_7 levels upon glucose stimulation at 3 or 5 min. Means ± SEM, n = 5 experiments.