Supplementary data:

Supplementary Table 1

Supplementary Figures, 1-4

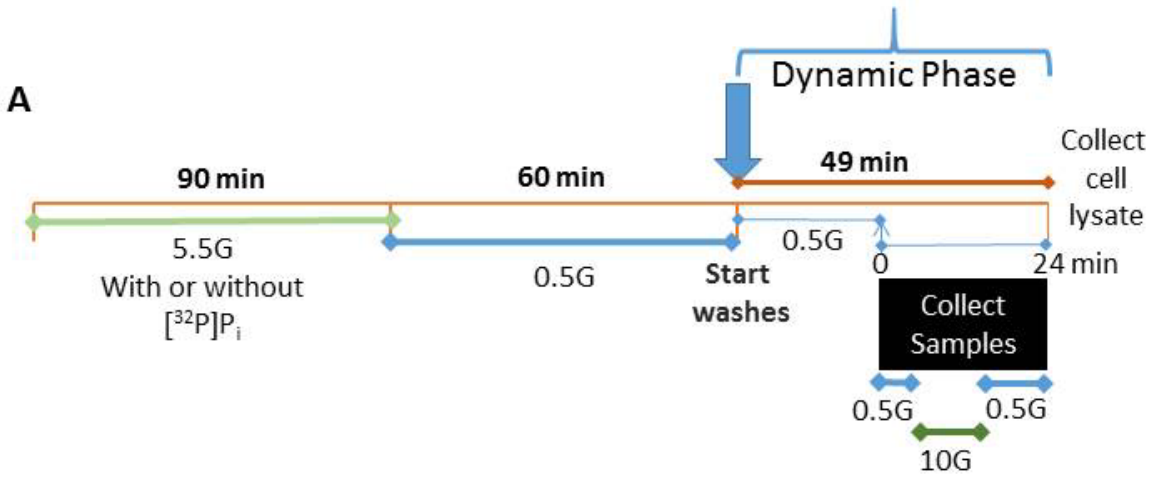
Supplementary Reference

Supplemental Table 1

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

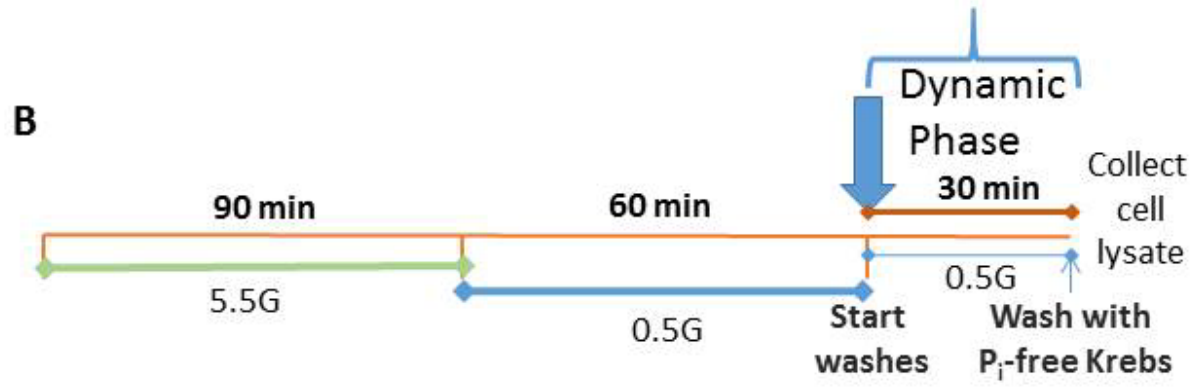
Data set	Source of variation	Bonferroni post-test
Fig. 2*D*, (n = 4)	Interaction, F(23,144) = 2.01, **p = 0.007 Time, F(23,144) = 3.71, ***p < 0.0001 siRNA, F(1,144) = 89.90, ***p < 0.0001	3 min after 10G stimulation, Control siRNA vs. XPR1 siRNA, ***p < 0.001 4 min after 10G stimulation, Control siRNA vs. XPR1 siRNA, **p < 0.01 5 min after 10G stimulation, Control siRNA vs. XPR1 siRNA, **p < 0.01 7 min after 10G stimulation, Control siRNA vs. XPR1 siRNA, **p < 0.01 10 min after 10G stimulation, Control siRNA vs. XPR1 siRNA, *p < 0.05
Fig. 2*G*, (n = 4)	Interaction, F(23,144) = 1.08, n.s. Time, F(23,144) = 10.91, ***p < 0.0001 siRNA, F(1,144) = 2.25, n.s.	4 min after 10G stimulation Control siRNA vs. XPR1 siRNA, *p < 0.05
Fig. 3*B*, (n = 4)	Interaction, F(23,144) = 0.87, n.s. Time, F(23,144) = 8.77, ***p < 0.0001 siRNA, F(1,144) = 23.69, ***p < 0.0001	
Fig. 3*D*, (n = 4)	Interaction, F(23,144) = 2.93, ***p < 0.0001 Time, F(23,144) = 6.40, ***p < 0.0001 TNP, F(1,144) = 36.24, ***p < 0.0001	3 min after 10G stimulation, DMSO vs. TNP, *p < 0.05 6 min after 10G stimulation, DMSO vs. TNP, **p < 0.01

(0.5G, 0.5 mmol/L glucose; 10G, 10 mmol/L glucose; n.s., not significant).

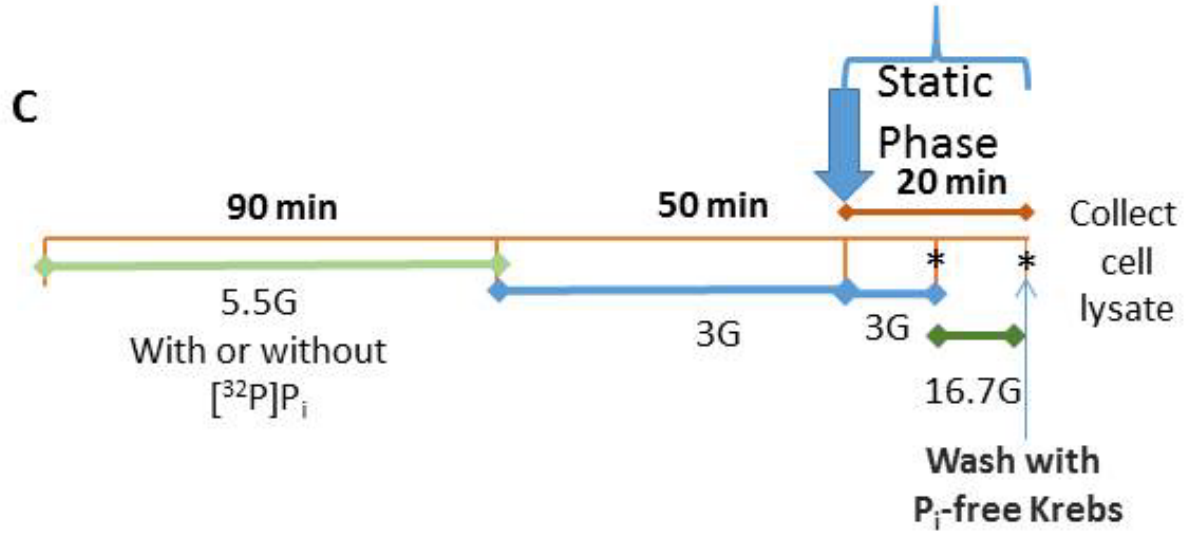


Supplementary Figure 1—Schemes of protocols used in experiments with MIN6m9 cells (*A* and *B*) and pseudoislets (*C*). All of the experiments were performed at 37°C, using a modified KREBS buffer containing 119 mmol/L NaCl, 4.6 mmol/L KCl, 2.8 mmol/L $CaCl_2$, 1 mmol/L $MgSO_4$, 0.15 mmol/L Na_2HPO_4, 0.4 mmol/L KH_2PO_4, 5 mmol/L $NaHCO_3$, 0.5 mg/mL BSA and 20 mmol/L HEPES, pH 7.4. *A* and *B*: MIN6m9 cells were seeded in Falcon® 6-well Clear Flat Bottom plate dishes (21,000-26,000 cells/cm^2) in complete DMEM medium with 10% dialyzed fetal bovine serum. In the RNA silencing experiments, the cells were treated with siRNA 24 h after seeding. After 3 days the cells were subjected to the protocols described in *A* or *B*.

Supplementary Figure 1*A*: MIN6m9 cells were incubated for 90 min with 25-50 µCi carrier-free [^{32}P]P_i (for phosphate efflux measurements) or without this label (for measurements of insulin secretion) in KREBS buffer containing 5.5 mmol/L glucose. The cells were washed extensively with unlabelled KREBS buffer containing 0.5 mmol/L glucose and subsequently incubated in this buffer for 60 min. This was followed by the Dynamic Phase, when the KREBS buffer with 0.5 mmol/L glucose covering the cells (0.9 mL) was exchanged, every min, for 30 min, followed by 10 minutes stimulation with 10 mmol/L glucose, again exchanging solution (0.9 mL) every minute. This was followed by a return to 0.5 mmol/L glucose KREBS buffer for remaining time, continuing to exchange (0.9 mL) every minute. Samples, consisting of the exchanged buffer, were collected from the final 5 min of incubation in basal glucose (0.5 mmol/L) to the end of the Dynamic Phase and used for [^{32}P]P_i or insulin quantification. In the [^{32}P]P_i experiments the total [^{32}P] phosphate labelled material was extracted from the cells by adding 1% Triton X-100 for 5 min at room temperature (1) and scraping off the dishes cellular material. The radioactivity of the exchanged buffer samples and the scraped cells was determined using a liquid scintillation counter. In the unlabelled experiments insulin was measured utilizing the PerkinElmer AlphaLISA assay and normalized to protein, extracted using M-PER and determined by the BCA Protein assay. G = Glucose (mmol/L).

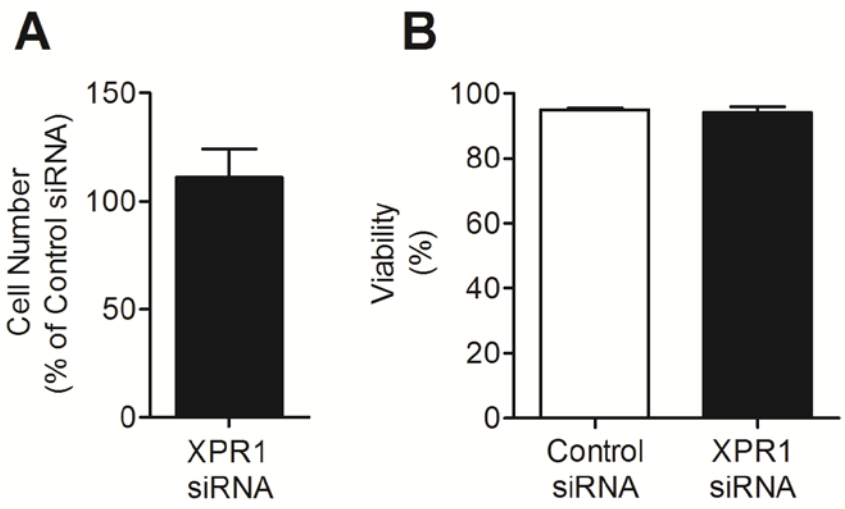


Supplementary Figure 1*B*—In experiments performed to measure inorganic intracellular phosphate (P_i) in MIN6m9 cells the same protocol was followed as in panel (*A*). However, the incubations were terminated at the end of the 30 min in 0.5 mmol/L glucose of the Dynamic Phase by briefly washing the cells with P_i-free KREBS buffer and then adding 0.5 mL of ice-cold 5% perchloric acid (PCA) to extract both the free phosphate and proteins. The cells were left on ice for 15 min and then scrapped off the plate. The extract was spun down at 4,080*g* in a microfuge, at 4°C for 5 min, to collect the cellular debris (containing the total protein). The supernatant was removed and neutralized with 50 µL of 5 M KCO_3 solution, then the resulting precipitate was spun down at 14,240*g* for 5 min at 4°C. The final supernatant was stored at -20°C until P_i measurement with the PiColorLock assay. The pellet from the first centrifugation, containing the cellular debris, was solubilized with 100 µL of solution containing 0.5 M NaOH and 0.1% SDS and stored at -20°C for subsequent protein determination with the BCA Protein assay. P_i values were normalized to total protein. G = Glucose (mmol/L).

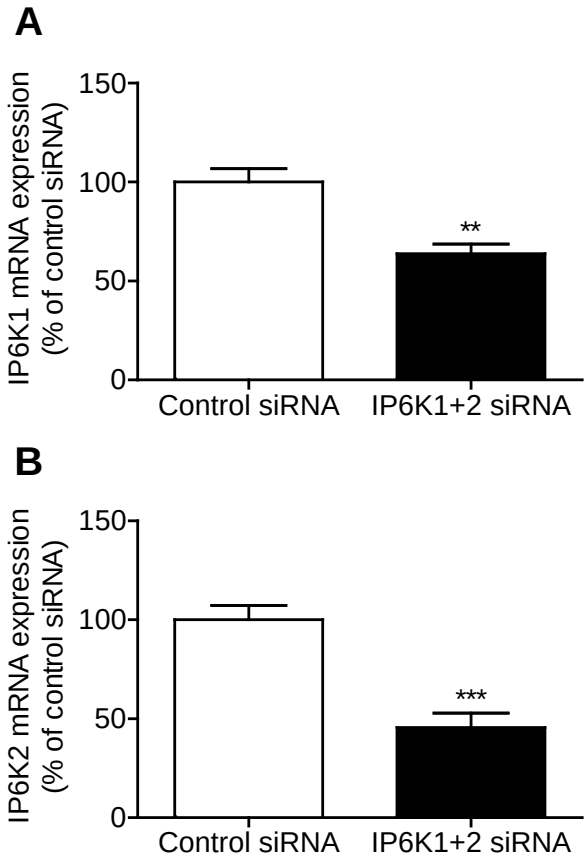


Supplementary Figure 1*C*—Pseudoislets were incubated in modified KREBS buffer for the determination of phosphate efflux (80 pseudoislets/tube) or insulin secretion and intracellular P_i (40 pseudoislets/tube) in microfuge tubes placed in a temperature-controlled heating block at 37ºC. Washing or buffer exchange of the pseudoislets was achieved using brief centrifugation (30 s, 2,000*g*). After incubation for 90 min in 5.5 mmol/L glucose KREBS buffer with 25-50 µCi carrier-free [^{32}P]P_i (for phosphate efflux measurements) or without (for insulin and intracellular phosphate measurements), pseudoislets were washed extensively with 3 mmol/L glucose KREBS buffer and kept in the same buffer for additional 50 min. Phosphate efflux or insulin release were measured during the Static Phase by replacing the buffer with KREBS containing 3 mmol/L basal glucose for 10 min, followed by KREBS containing 16.7 mmol/L stimulatory glucose for 10 min. The supernatants were collected by centrifugation for either [^{32}P]P_i efflux or insulin determination. The radioactivity of the supernatants obtained at basal and stimulatory glucose was determined by liquid scintillation counting. In the unlabeled experiments, insulin release was measured in the supernatants utilizing the PerkinElmer AlphaLISA assay. The pseudoislets were washed once with P_i-free KREBS buffer and extracted using 5% ice-cold PCA to obtain the intracellular P_i and the total cellular protein. Measurement of P_i was carried out using the PiColorLock assay. Total protein was determined by the Pierce BCA Protein assay and used to normalize the insulin and P_i data. *Time points when samples were collected. G = Glucose (mmol/L).

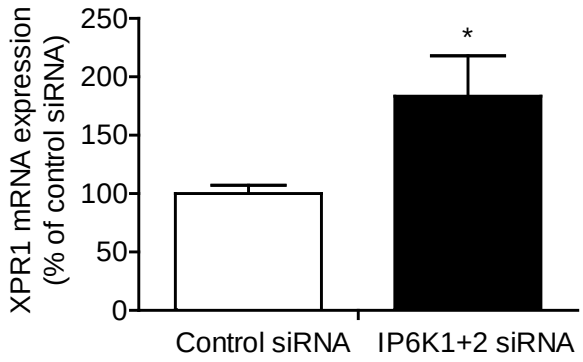
Supplementary Figures



Supplementary Figure 2—Cell characteristics following XPR1 knockdown. MIN6m9 cells treated with control or XPR1 siRNA were trypsinized and *A:* Cell number or *B*: Cell viability (% of total cells), were assessed using the trypan blue exclusion assay. Cells were stained with 0.2% trypan blue. Means ± SEM, n = 3 independent experiments. There is no statistically significant difference either in cell number (95% Confidence Interval) or cell viability (Student's t-test).



Supplementary Figure 3—Assessment of the efficacy of IP6K1 and IP6K2 knockdown in the experiment shown in Fig. 3. Effect of combined silencing IP6K1 and IP6K2 on mRNA expression of *A*: IP6K1 or *B*: IP6K2 in MIN6m9 cells, relative to control siRNA. Cells were treated with either control siRNA or a combination of IP6K1 and IP6K2 siRNAs. The RNA was extracted and the relative expression of the individual IP6Ks determined by RT-PCR, using 18S as endogenous control. Values expressed as means ± SEM, n = 6 (*A*) or 7 (*B*), $^{**}p < 0.005$ and $^{***}p < 0.001$, respectively, Student's t-test. The n values represent independent experiments.



Supplementary Figure 4—Effect of combined silencing of IP6K1 and IP6K2 on XPR1 mRNA expression in MIN6m9 cells. Cells were treated with either control siRNA or a combination of 2 different IP6K1 siRNAs and 2 different IP6K2 siRNAs, following the same protocol as in the experiments shown in Fig. 3*B*. The RNA was extracted and the relative XPR1 mRNA expression was determined by RT-PCR, using 18S rRNA as endogenous control. The values are expressed as percentage of control siRNA, means ± SEM, n = 8, **p* < 0.05, Student's t-test. The n values represent independent experiments.

Supplementary Reference

1. Giovannini D, Touhami J, Charnet P, Sitbon M, Battini JL. Inorganic phosphate export by the retrovirus receptor XPR1 in metazoans. Cell Rep 2013;3:1866-1873