Electronic supplementary material

Methods

Serotonylation After 24 h of incubation with or without 500 ng/ml PL, MIN6 cells were trypsinised and washed with PBS. The cells were lysed by sonication in serotonin buffer and ascorbic acid (0.1% wt/vol.) was added to the supernatant fraction after centrifugation (20,000 *g*) for 15 min at 4°C. Next 7.2% (wt/vol.) trichloroacetic acid and 0.015% (wt/vol.) deoxycholate were added to the supernatant fraction and incubated on ice. After centrifugation the supernatant fraction was stored at −20°C until measurement and the pellet was washed with ice-cold acetone and resolved in serotonin buffer with 0.1% (wt/vol.) ascorbic acid for serotonin determination via ELISA (LDN, Nordhorn, Germany).

Immunohistochemistry Pancreases were dissected from pregnant wild-type and KO female C57BL6/J mice and fixed in 4% (wt/vol.) paraformaldehyde. Two-colour immunohistochemistry was used to determine the percentage of proliferating beta cells, using guinea pig anti-insulin (a gift from C. F. H. Van Schravendijk, Diabetes Research Center, Vrije Universiteit Brussel, Brussels, Belgium) and rabbit anti MKi67 (Acris Antibodies, Hiddenhausen, Germany). Binding of primary antibodies was detected with biotinylated anti-guinea pig and anti-rabbit Ig in combination with streptavidin horseradish peroxidase and alkaline phosphatase complex. Diaminobenzidine and fuchsin-plus were used as substrates (all reagents from Dako, Glostrup, Denmark). Quantification of MKi67-insulin double positive cells was performed at 400× magnification. A minimum of 1000 cells per animal were evaluated (wild-type, n=3, *Tph1*-KO, n=2 animals).