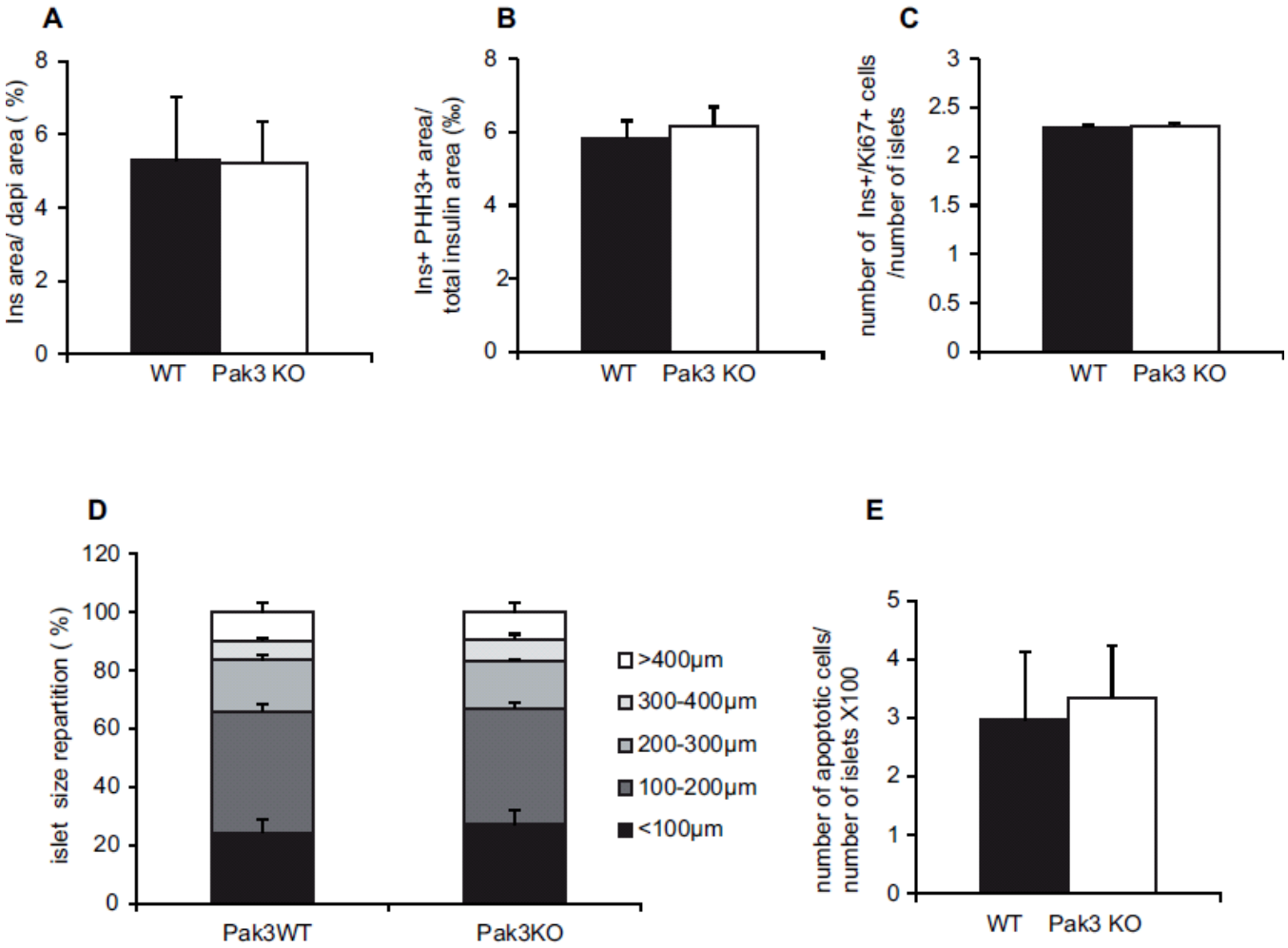
SUPPLEMENTARY DATA

Supplementary Figure 1. Pak3 deficiency does not affect the number of beta-cells, islet formation and size or apoptosis at birth (P0). (**A**) Quantification of the insulin^{+} area. (**B-C**) Quantification of beta-cell proliferation with the G2/M marker Phospho-histoneH3 (**B**) or Ki67 labelling all cell cycle phases (**C**). (**D**) Analysis of islets size based on the perimeter of at least 215 islets per animal. **(E)** Quantification of apoptotic (TUNEL) islet cells. (A-E) Data are represented as mean ± SD of n=3.



Method: Pancreas were dissected shortly after birth, fixed overnight in PFA4% and cryo-embedded using standard procedures. Immunofluorescence experiments were performed on 10µm thick sections covering the whole pancreas (each 50µm). Image acquisition was performed with the slide scanner NanoZoomer 2HT and fluorescence module L11600-21 (Hamamatsu Photonics, Japan). The light source LX2000 (Hamamatsu Photonics, Japan) consisted in an ultra high-pressure mercury lamp coupled to an optical fiber. Single RGB acquisition was made in epifluorescence mode with the 3-chip time delay integration (TDI) camera equipped with a filter-set optimized for DAPI, fluorescein and tetramethylrodamine detection. The scanner performed line scanning that offered fast acquisition at high resolution of the fluorescent signal. The acquisition was performed using a dry 20x objective (NA:0.75) The 40X resolution was achieved with a lens converter. The latter mode used the full capacity of the camera (resolution 0.23µm/pixel). Counting was done directly on the viewer software NDP.view (Hamamatsu). For area quantification, regions of interests were exported in tiff format and analysed with ImageJ. Apoptosis was measured with the In Situ Cell Death Detection Kit (Roche) according to the manufacturer instructions. The number apoptotic cells was counted in at least 120 islets per animal and normalised by the number of islets counted.

SUPPLEMENTARY DATA

Supplementary Figure 2. Normal mass and proliferation rate of alpha- and beta- cells in adult *Pak3*-deficient mice under high fat diet (HFD). 9-10 weeks old mice under HFD were sacrificed and the pancreas was dissected and fixed in PFA4%. After a few hours the pancreas was rolled in a "sausage"-like shape in a Tissue-Tek biopsy bag (Sakura) and further fixed overnight at room temperature. After washing in PBS the pancreas was processed for paraffin embedding using standard protocols. After infiltration the pancreas "sausage" was cute in 8 pieces 2mm thick and embedded in paraffin. One 3µm thick section of each piece was then stained and analysed. Mass (**A**) and proliferation (**B-C**) of alpha- and beta-cells were explored by Immunofluorescence, image analysis was as described in supplementary figure 2. **(A)** Quantification of the beta- (left) and alpha-cell mass (right). Cell mass was calculated as ((% insulin (or glucagon) in total tissue/100) x pancreas weight(g) x 1000)/(mouse weight(g)/1000) **(B)** Proliferative index of insulin$^+$ using either the S-phase marker BrdU (pulse 24h) (left) or the global proliferation marker Ki67 (right). **(C)** Proliferative index of glucagon$^+$ cells. **(D)** RT-qPCR for *Pak1-3* in Pak3 KO and control pancreas from 9-10 weeks old mice under HFD (n=3). (A-D) Data are represented as mean ± SD of n=3; ** $p<0.01$; AU stands for Arbitrary Unit. Proliferative index = number of BrdU+ or Ki67+ beta cells/total insulin area X100 in **(B)** and number of BrdU+ or Ki67+ alpha--cells/total glucagon area X100 in **(C).**

SUPPLEMENTARY DATA