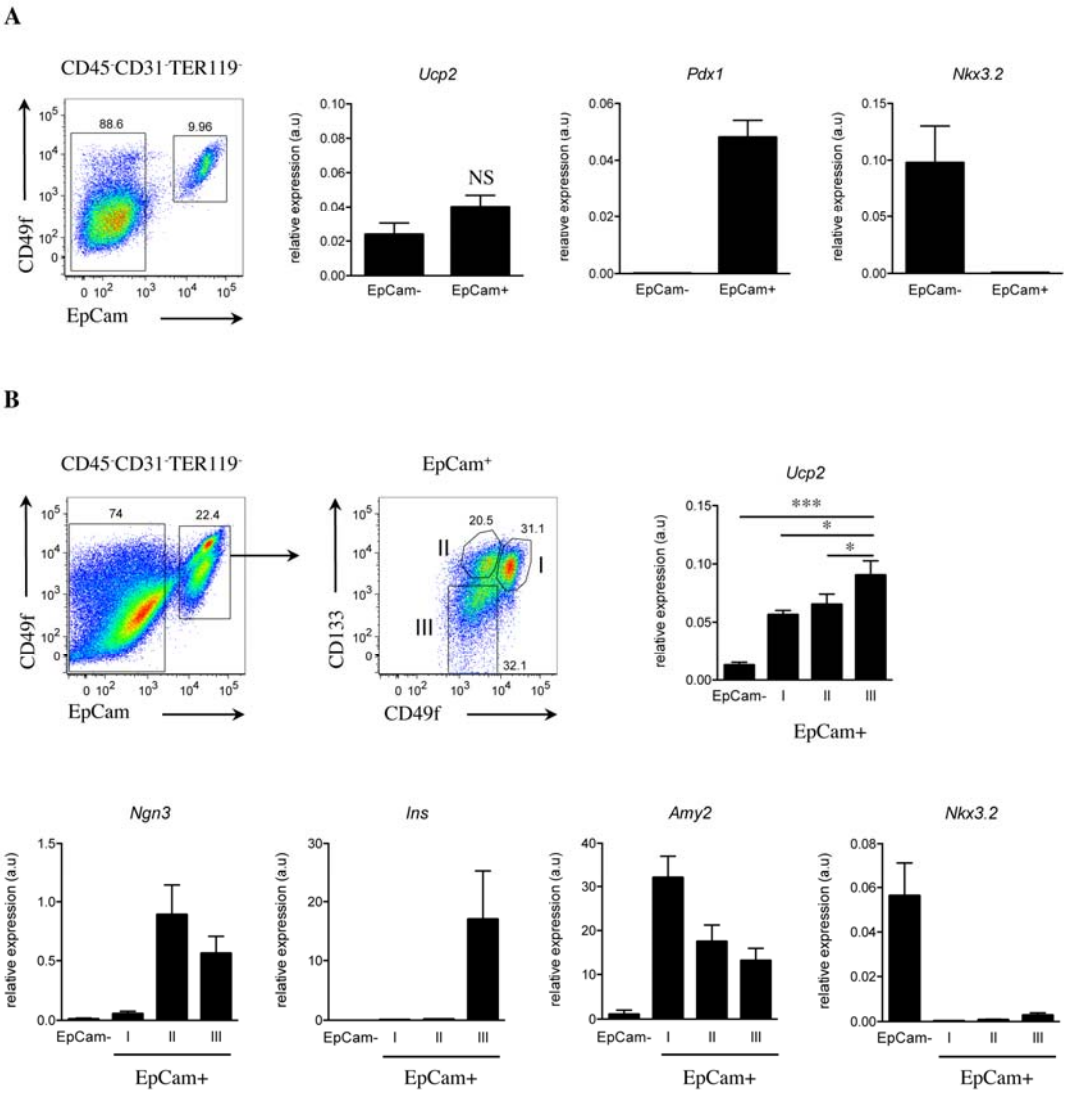
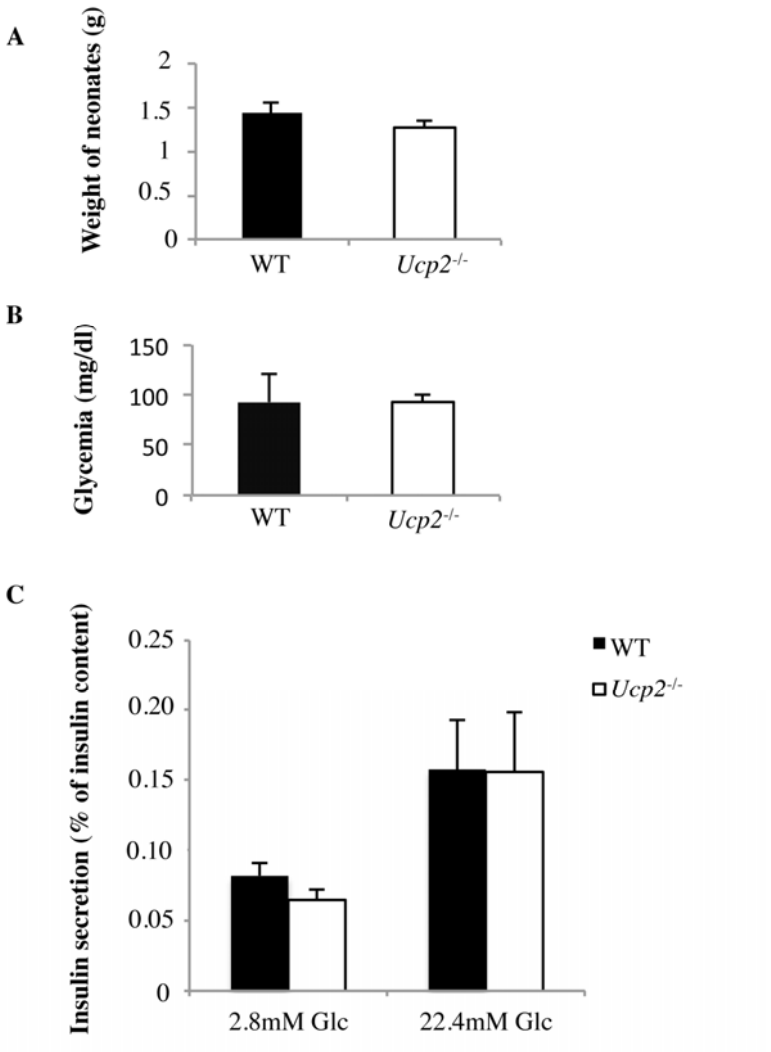
SUPPLEMENTARY DATA

Supplementary Figure. 1. FACS analysis of the expression of *Ucp2*.
(A) Analysis of *Ucp2* expression at E12.5. (B) Analysis of *Ucp2* expression at E16.5. E12.5 and E16.5 fetal pancreata were dissociated and stained as described in Supplementary Materials with anti-CD45, anti-CD31, anti-TER119, anti-EpCam, anti-CD133 and anti-CD49f antibodies. CD45, CD31 and TER119 were used to exclude hematopoietic cells, endothelial cells and erythrocytes, respectively. The remaining CD45- CD31- TER119- fraction was subdivided into mesenchymal (EpCam-) and epithelial (EpCam+) enriched fractions. At E16.5, the EpCam+ epithelial enriched fraction was further subdivided into 3 fractions using anti-CD133 and anti-CD49f antibodies: fraction I (CD133+ CD49f high) is enriched in acinar cells, fraction II (CD133+ CD49f intermediate) is enriched in NGN3 positive cells, fraction III (CD133- CD49f intermediate) is enriched in hormone positive cells. Dot plots are representative of 3 independent stainings of 7 to 15 pooled fetal pancreata. Quantitative RT-PCR gene expression analysis of *Ucp2*, *Pdx1* and *Nkx3.2* was done in E12.5 pancreas CD45-CD31-TER119- EpCam- and EpCam+ fractions. Quantitative RT-PCR gene expression analysis of *Ucp2*, *Nkx3.2*, *Ins*, *Amy2*, *Ngn3* was done in E16.5 pancreas (CD45-CD31-TER119-) EpCam- and EpCam+ (I, II and III) fractions. Histograms display relative quantity of expression normalized to *Ppia*. Data is a pool of 3 independent experiments. * $P<0.05$.



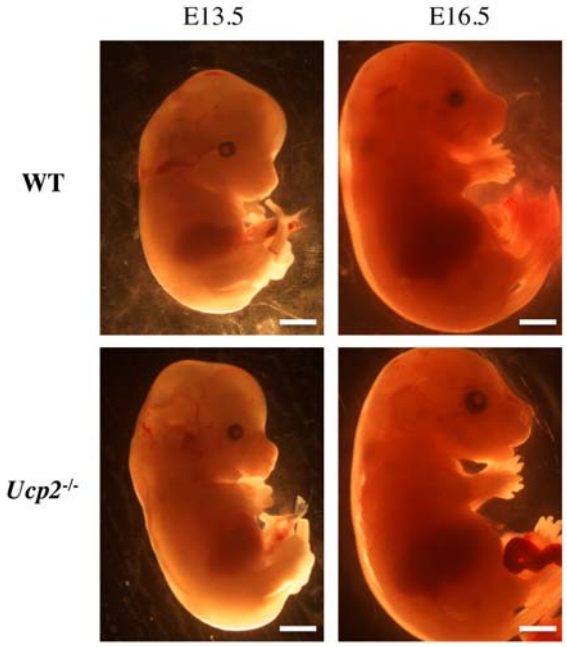
SUPPLEMENTARY DATA

Supplementary Figure. 2. Physiological parameters of the WT and *Ucp2*$^{-/-}$ neonates.
(A) WT and *Ucp2*$^{-/-}$ neonates were weighed at birth. Graph represents the mean± S.E.M of n≥5 animals. (B) Glycemia was measured using a glucometer (in mg/dl). Each point represents the mean of n≥5 animals ± S.E.M. (C) Pancreatic islets were isolated one day after birth from *Ucp2*$^{-/-}$ and WT pancreata. Glucose stimulated insulin secretion is represented. Data are expressed as average percentage of secreted insulin. Results are representative of two independent experiments performed.

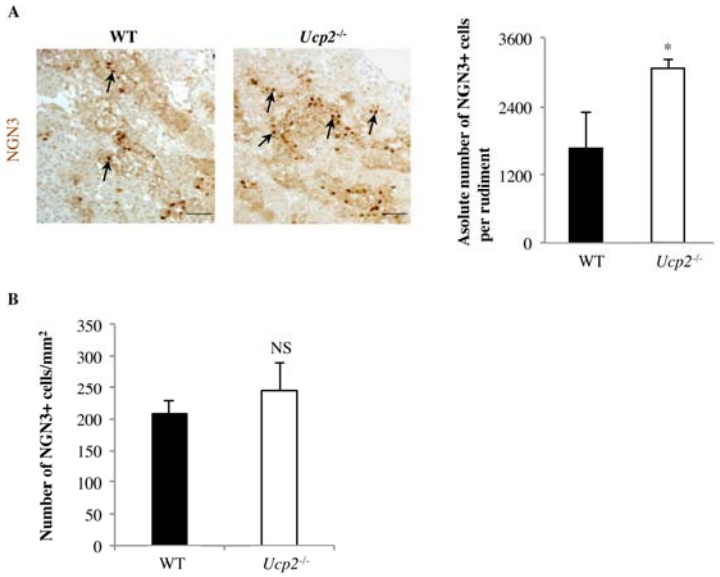


SUPPLEMENTARY DATA

Supplementary Figure. 3. The external morphology of the WT and *Ucp2*$^{-/-}$ animals is normal during embryonic and fetal development.
E13.5 and E16.5 WT and *Ucp2*$^{-/-}$ fetuses from the same litter were photographed using a binocular microscope (Leica, France). Bar: 2000 µm.



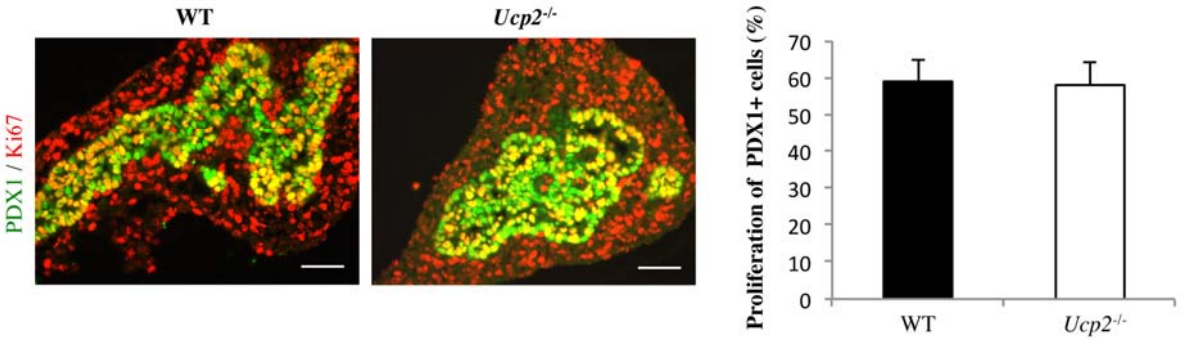
Supplementary Figure. 4. The number of endocrine progenitor cells is increased in the *Ucp2*$^{-/-}$ E16.5 pancreata.
Fetal pancreata were analyzed at E16.5. (A) NGN3 expression (in brown) was detected by immunohistochemistry and the absolute number of NGN3-positive cells was quantified. Arrows indicate positive nuclei. (B) The number of NGN3-positive cells per surface of pancreas (mm^2) is represented. Each point represents the mean± S.E.M of three individual pancreata. * P<0.05. NS: not significant. Scale bar: 50 µm.



SUPPLEMENTARY DATA

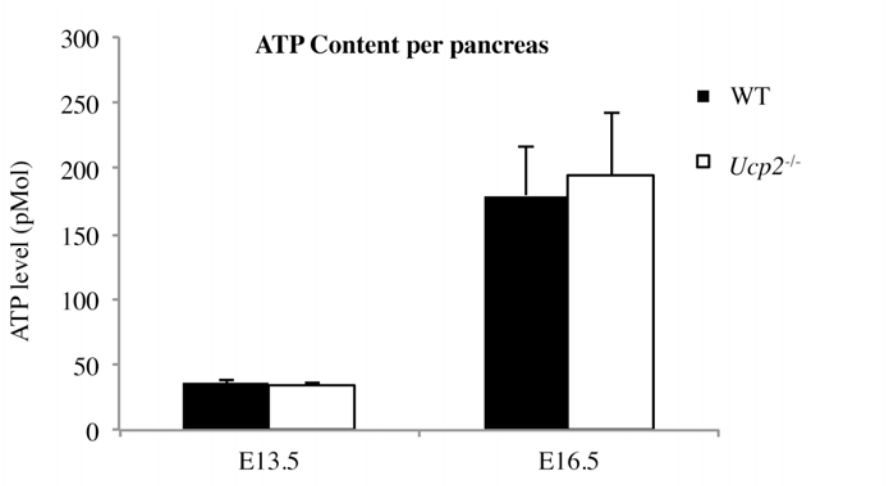
Supplementary Figure. 5. The proliferation of the progenitor cells is unaltered in the *Ucp2*$^{-/-}$ E12.5 pancreata.

The proliferation of the PDX1+ progenitor cells was analyzed using anti-PDX1 (green) and anti-Ki67 (red) antibodies. Proliferation percentage was also quantified. Each point represents the mean ± S.E.M of three individual pancreata. Scale bar: 50 µm.



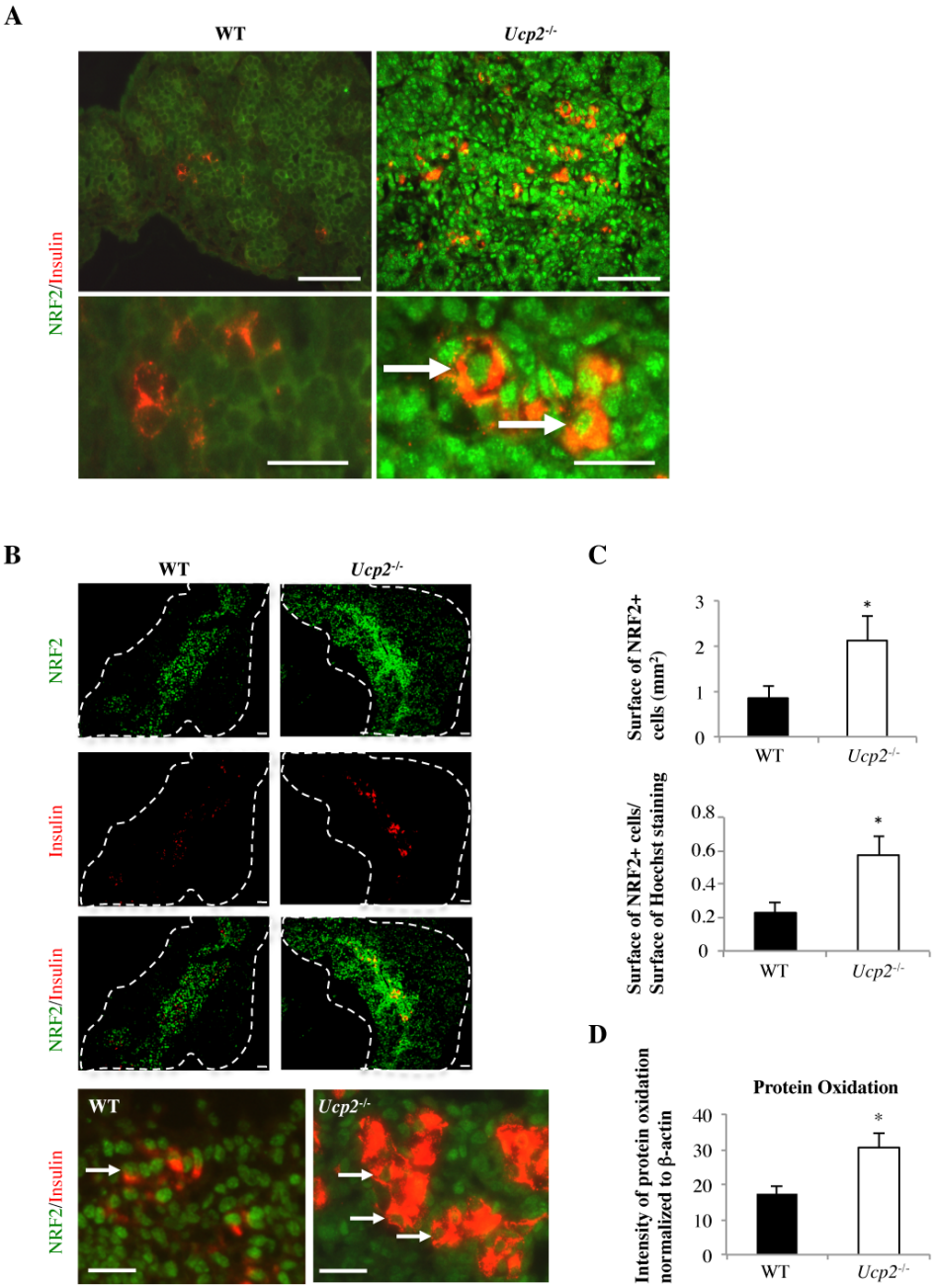
Supplementary Figure. 6. The pancreatic ATP content is unaltered by *Ucp2* deficiency.

ATP was extracted from n=5 pancreatic rudiments from E13.5 and E16.5 *Ucp2*$^{-/-}$ and WT fetuses. Each point represents the mean ± S.E.M of five individual pancreata.



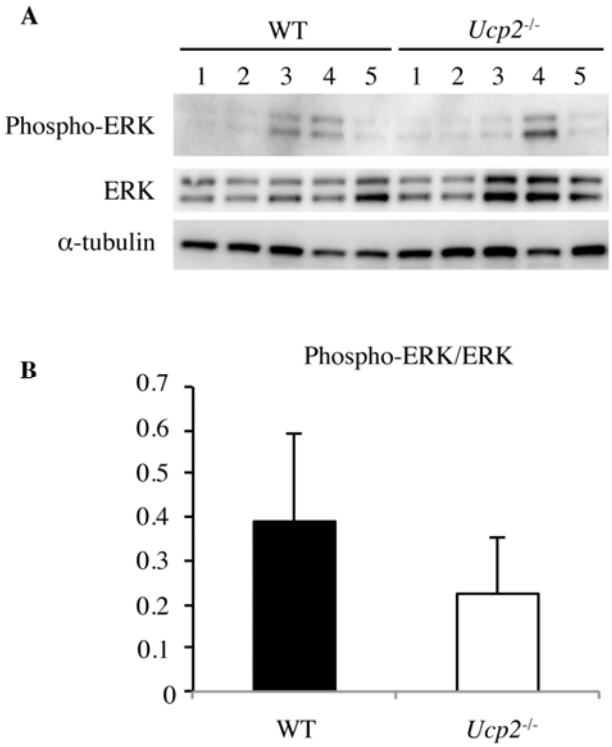
SUPPLEMENTARY DATA

Supplementary Figure. 7. NRF2 translocation analysis.
(A) Photomicrographs showing immunohistochemical staining for NRF2 (green) and insulin (red) on E13.5 pancreatic sections. NRF2 was found at the periphery of the nuclei in WT pancreata while it was detected in the nuclei of the *Ucp2*$^{-/-}$ pancreata. Arrows indicate the presence of NRF2 in the insulin-positive cells. **(B)** Immunohistochemical staining for NRF2 (green) and insulin (red) on E16.5 pancreatic sections. Higher magnification shows the presence of NRF2 in the nuclei. Arrows indicate the NRF2+ Insulin+ cells. The white dotted lines demarcate the limits of the pancreas. (C) Quantification of the total surface of immunoreactive cells for NRF2, and the surface of NRF2-positive cells per mm^2 of pancreas surface. (D) Quantification of protein oxidation in extracts from WT and *Ucp2*$^{-/-}$ pancreata. Each point represents the mean ± S.E.M of five individual pancreata. * P<0.05. Scale bar: 50 µm. For higher magnification, bar: 10 µm.



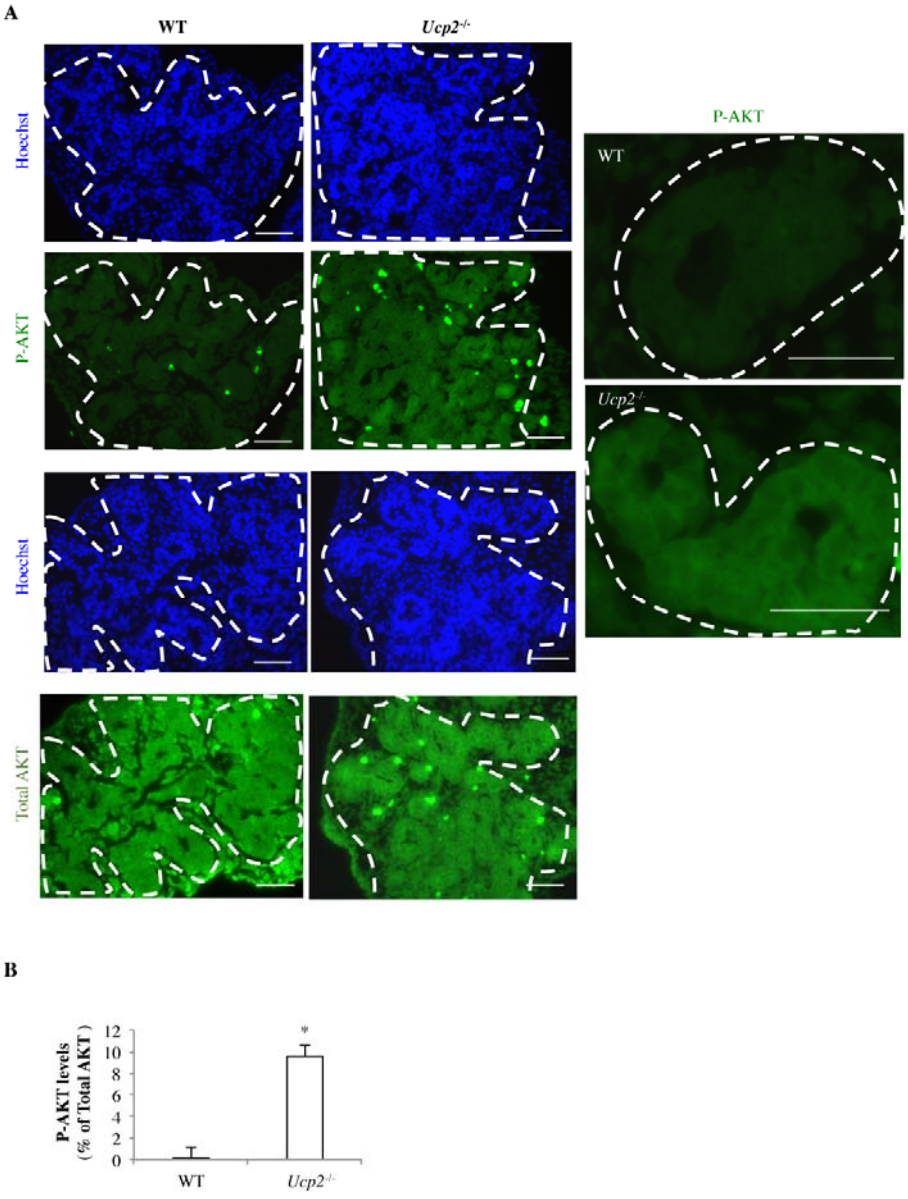
SUPPLEMENTARY DATA

Supplementary Figure. 8. The ERK1/2 pathway is not modified in the *Ucp2*$^{-/-}$ pancreata.
(A) Protein extracts from WT and *Ucp2*$^{-/-}$ pancreata were analyzed by western blot to quantify Phospho-ERK and Total ERK. (B) Ratio of Phospho-ERK/ERK was quantified for each group of pancreata. Each point represents the mean ± S.E.M n=5.



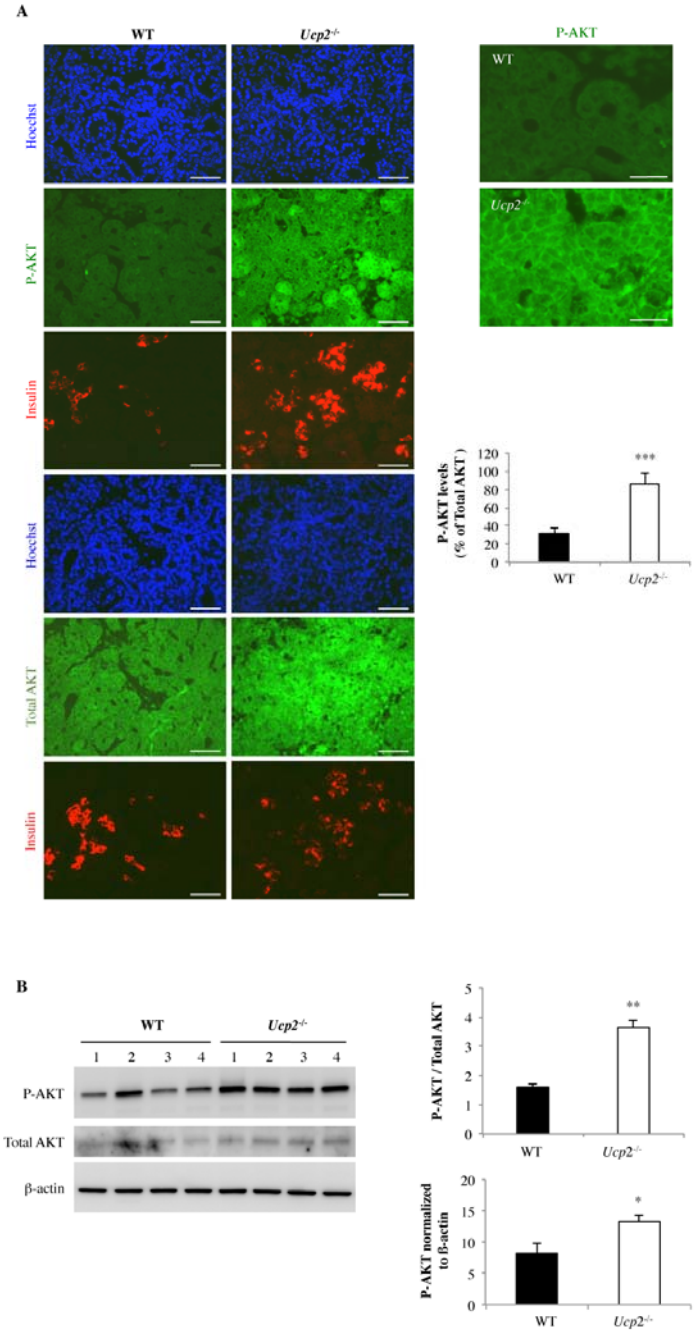
SUPPLEMENTARY DATA

Supplementary Figure. 9. Phosphorylation of AKT is activated in the *Ucp2*$^{-/-}$ E13.5 pancreata. (A) Phospho-AKT and Total AKT were analyzed by immunofluorescence on WT and *Ucp2*$^{-/-}$ consecutive sections. Higher magnification shows increased staining of P-AKT in the *Ucp2*$^{-/-}$ pancreata versus WT. The white dotted lines demarcate the limits of the pancreas. (B) The signals were quantified using ImageJ software and the P-AKT percentage with regards to Total AKT was calculated. Each point represents the mean ± S.E.M n=3 pancreata. Scale bar: 50 µm. For higher magnification, scale bar: 10 µm. * P<0.05.



SUPPLEMENTARY DATA

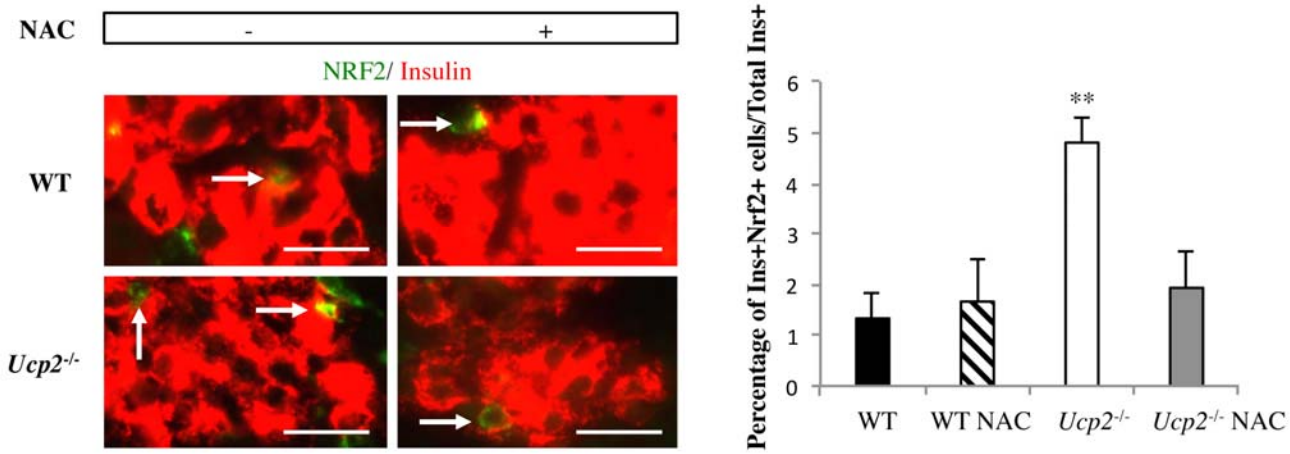
Supplementary Figure. 10. Phosphorylation of AKT is activated in the *Ucp2*$^{-/-}$ E16.5 pancreata. (A) Phospho-AKT and Total AKT were analyzed by immunofluorescence on WT and *Ucp2*$^{-/-}$ consecutive sections. The signals were quantified using ImageJ software and the P-AKT percentage with regards to Total AKT was calculated. Higher magnification shows increased staining of P-AKT in the *Ucp2*$^{-/-}$ pancreata versus WT. Each point represents the mean ± S.E.M n=3 pancreata. * P<0.05. Scale bar: 50 µm. For higher magnification, bar: 10 µm. (B) Protein extracts from WT and *Ucp2*$^{-/-}$ E16.5 pancreata were analyzed by western blot to quantify P-AKT and Total AKT. Densitometry was performed and P-AKT was normalized to ß-actin and quantified for each group of tissue. Each point represents the mean ± S.E.M of n=4. * P<0.05. Ratio P-AKT/ Total AKT was also determined. Each point represents the mean ± S.E.M of n=4. ** P<0.01.



SUPPLEMENTARY DATA

Supplementary Figure. 11. NAC treatment decreases expression of nuclear NRF2 protein in the *Ucp2*$^{-/-}$ beta-cells.

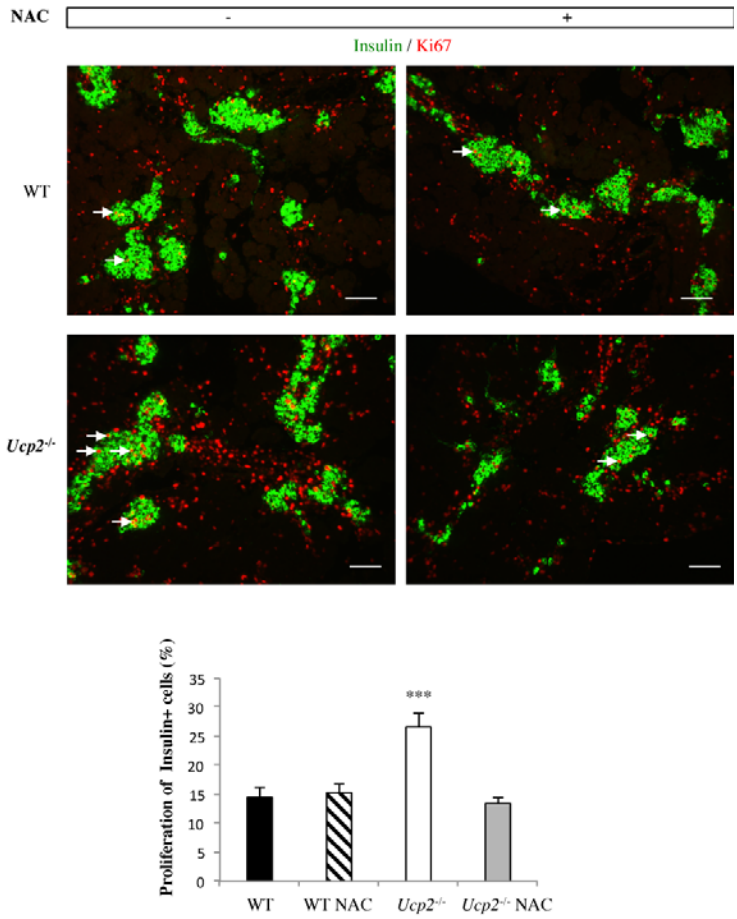
Pregnant WT and *Ucp2*$^{-/-}$ mice were treated with 10 mM NAC (drinking water) from E12.5 to E19.5 days post-coïtum. Fetal pancreata were analyzed at E19.5. NRF2 was detected by immunofluorescence (green) and beta-cells were detected with anti-insulin (red). The NRF2+ Ins+ cell percentage of the total number of insulin-positive cells is shown. Each point represents the mean ± S.E.M of three individual pancreata. * P<0.05. Scale bar: 10 µm.



SUPPLEMENTARY DATA

Supplementary Figure. 12. The proliferation of beta-cells is increased in the *Ucp2*$^{-/-}$ E19.5 pancreata and depends on oxidative stress.

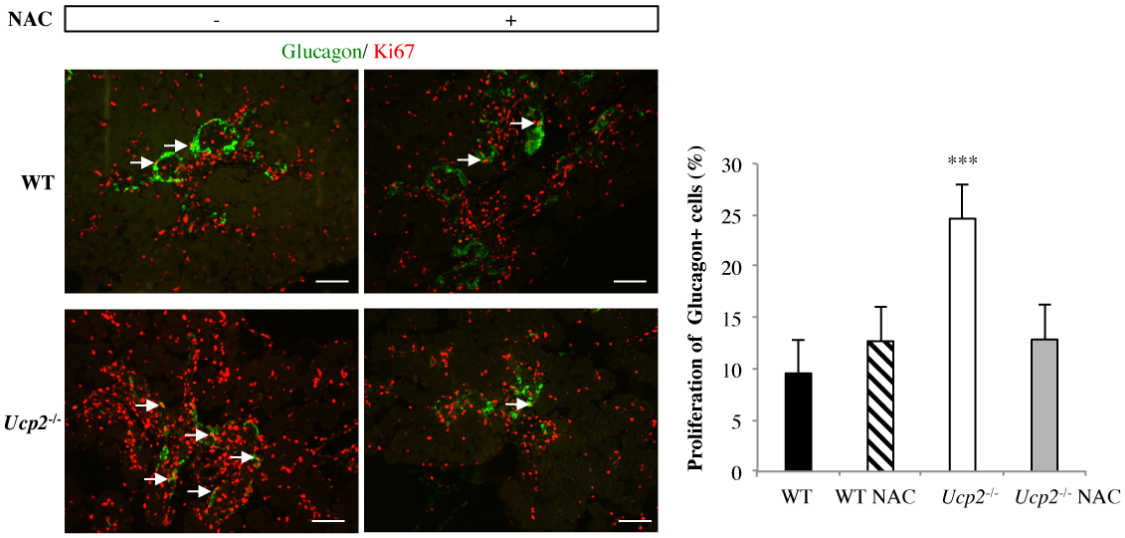
The proliferation of the beta-cells was analyzed using anti-insulin (green) and anti-Ki67 (red) antibodies. When treated with NAC from E12.5 to E19.5, the proliferation of beta-cells decreased in *Ucp2*$^{-/-}$ but not in WT pancreata. Each point represents the mean ± S.E.M of three individual data pools. *** P<0.001. Scale bar: 50 µm.



SUPPLEMENTARY DATA

Supplementary Figure. 13. The proliferation of alpha-cells is increased in the *Ucp2*$^{-/-}$ E19.5 pancreata and decreased after NAC treatment.

The proliferation of the alpha-cells was analyzed using anti-glucagon (green) and anti-Ki67 (red) antibodies. When treated with NAC from E12.5 to E19.5, the proliferation of alpha-cells decreased in *Ucp2*$^{-/-}$ but not in WT pancreata. Each point represents the mean ± S.E.M of five individual pancreata. *** P<0.001. Scale bar: 50 µm.



SUPPLEMENTARY DATA

Supplementary Figure. 14. Fractions of alpha and beta cells in *Ucp*2-/- and wild type pancreata at different developmental stages.

(A) The insulin staining percentage of the total pancreatic surface was quantified at E16.5. Each point represents the mean ± S.E.M of five individual pancreata. (B) The glucagon staining percentage of the total pancreatic surface was quantified. Each point represents the mean ± S.E.M of five individual pancreata. (C-D) Percentage of beta- and alpha-cell mass at E19.5 in *Ucp2*$^{-/-}$ and WT pancreata treated or not with NAC. (C) The beta-cell mass percentage of the total pancreatic mass was quantified. Each point represents the mean ± S.E.M of five individual pancreata. (D) The alpha-cell mass percentage of the total pancreatic mass was quantified. Each point represents the mean ± S.E.M of five individual pancreata. (E-F) beta- (E) and alpha-cell (F) masses normalized to pancreas weight at PN2. Each point represents the mean ± S.E.M of four individual pancreata. (F-G) beta- (F) and alpha-cell (G) masses normalized to body weight at PN2. Each point represents the mean ± S.E.M of four individual pancreata.