

# **Human duct cells contribute to $\beta$ -cell compensation in insulin resistance**

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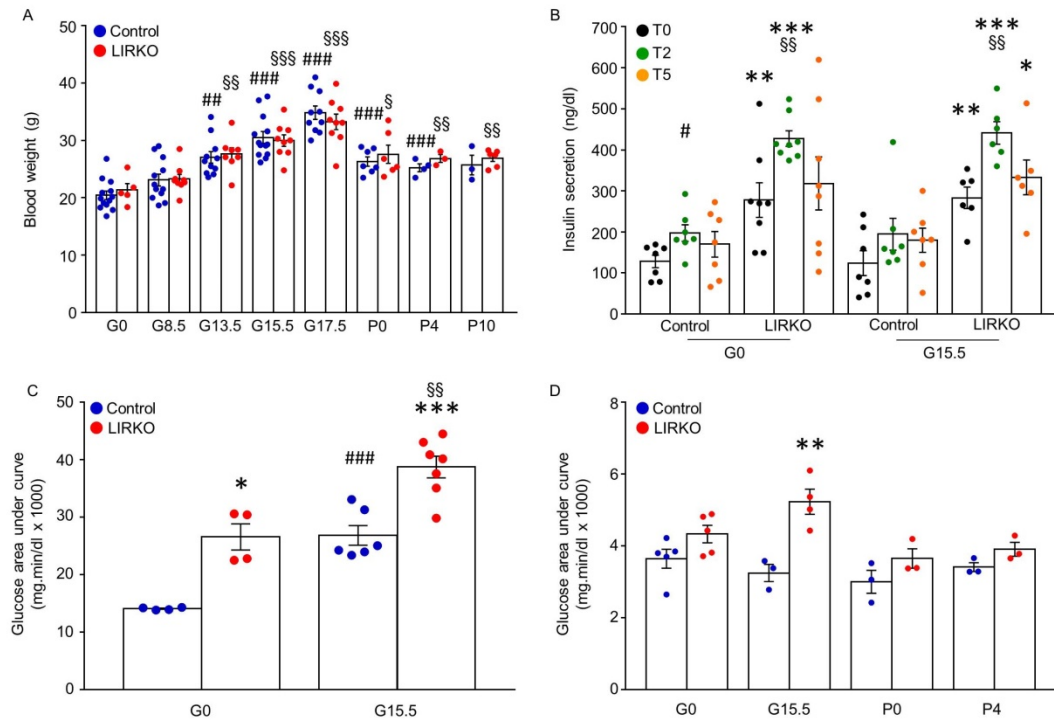
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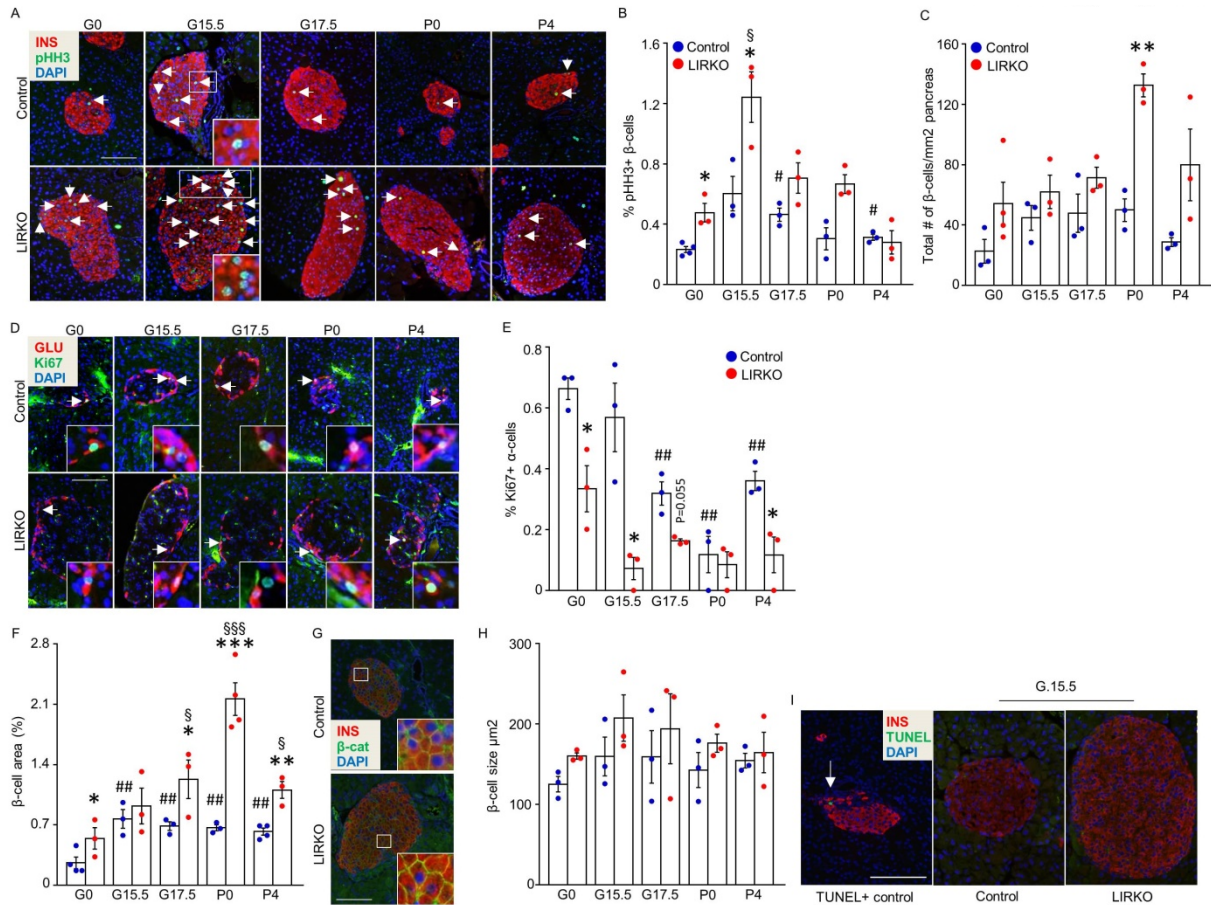
<sup>6</sup>Lead Contact

## Supplemental Figures



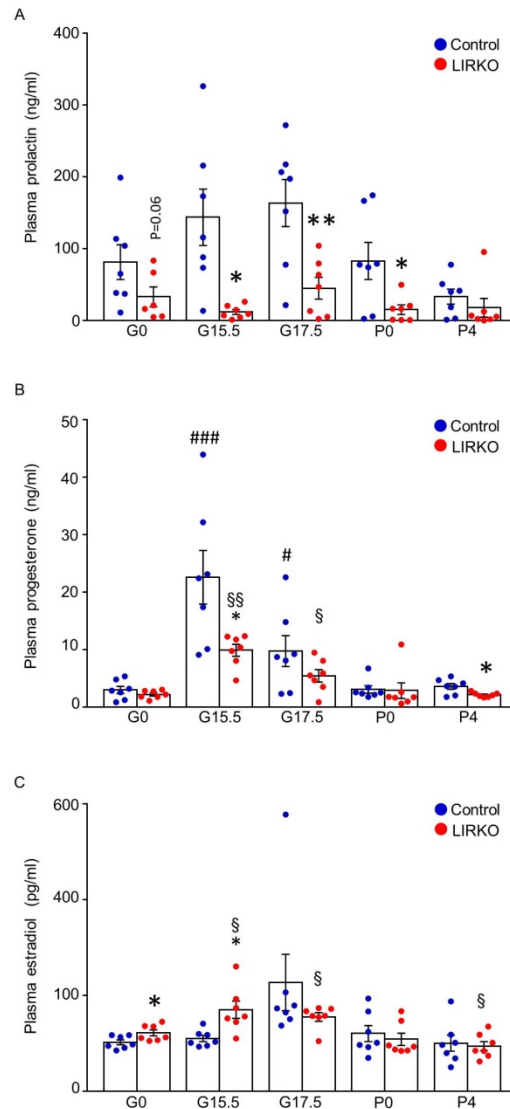
## Supplementary Fig.1

**Supplementary Figure 1.** (A) Body weight gain of control and LIRKO mice before (G0), during (G15.5, G17.5) gestation and post-partum (n=3-13mice per group, Student's *t*-test). (B) Acute (first)-phase insulin secretion following oral glucose load (2.5 g/kg/b.wt; data are expressed as increase in insulin (ng/dl) at 2 and 5 min relative to time 0) (n=6-8 mice per group, Student's *t*-test) (C) Area under the curve (AUC) for glucose following an oral glucose administration (2.5 g/kg. b.wt) in G0 and G15.5 mice (n=4-7 mice per group, Student's *t*-test) (D) AUC following intraperitoneal injection of insulin (1 U/kg. b.wt) in G0, G15.5, P0 and P4 mice (n=3-5 mice per group, Student's *t*-test). #, Control vs Control, \* Control vs LIRKO, and §, LIRKO vs LIRKO. Data were expressed as means ± SEM. #, §, \**P* < 0.05, ##, §§, \*\**P* < 0.01, and §§§, ###, \*\*\**P* < 0.001.



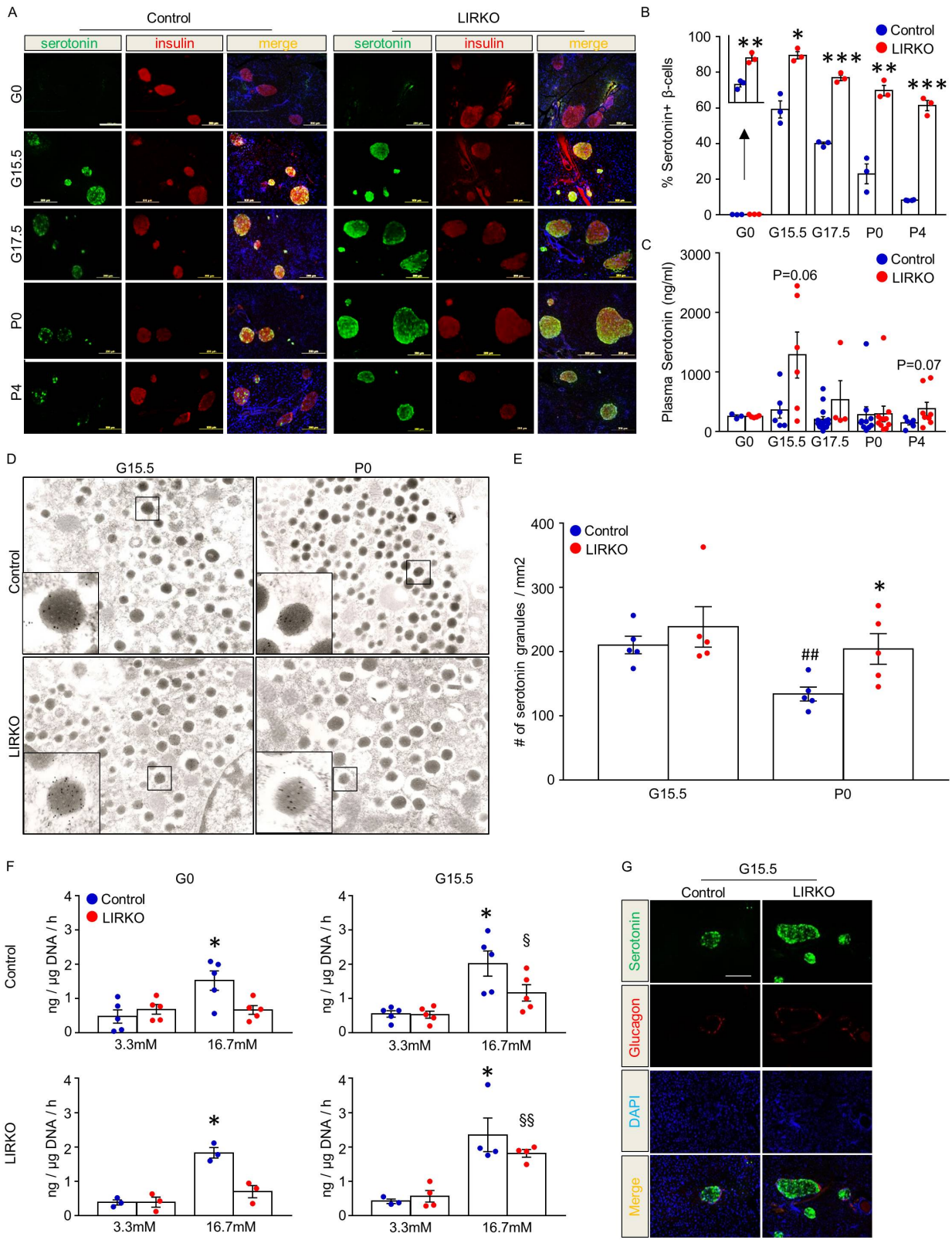
## Supplementary Fig.2

**Supplementary Figure 2.** (A) Representative immunofluorescence images of control and LIRKO mice immunostained for insulin (red), pHH3 (green) and DAPI (blue) before (G0), during (G15.5 and G17.5) and after pregnancy (P0 and P4). White arrows show proliferating  $\beta$ -cells. (B) Quantification of pHH3 positive  $\beta$ -cells (n=3-4 mice per group, Student's *t*-test) (for quantification see Supplementary Table 1). (C) Total number of  $\beta$  cells counted per mm<sup>2</sup> pancreatic area (n=3-4 mice per group, Student's *t*-test). (D) Representative pancreas sections showing  $\alpha$ -cell proliferation and immunostained for glucagon (red), Ki67 (green) and DAPI (blue). White arrows show proliferating  $\alpha$ -cells. (E) Quantification of Ki67 positive  $\alpha$ -cells (n=3 mice, Student's *t*-test). (F) Morphometric analysis of  $\beta$ -cell area as described in Methods (n=3-4 mice per group, Student's *t*-test) (G) Representative images of pancreas sections immunostained for  $\beta$ -catenin (green), insulin (red) and DAPI (blue). Insets show magnified  $\beta$ -catenin staining. (H) Quantification of  $\beta$ -cell size (n=3 mice per group, Student's *t*-test). (I) Representative images of pancreatic islets obtained at G15.5 immunostained for TUNEL (green), insulin (red) and DAPI (blue). White arrows show TUNEL+  $\beta$ -cells. Data are expressed as mean  $\pm$  S.E.M. Scale bars: 100  $\mu$ m. #, Control vs Control, \* Control vs LIRKO, and §, LIRKO vs LIRKO. Data were expressed as means  $\pm$  SEM. #, §, \**P* < 0.05, ##, \*\**P* < 0.01, and §§§, ###, \*\*\**P* < 0.001.



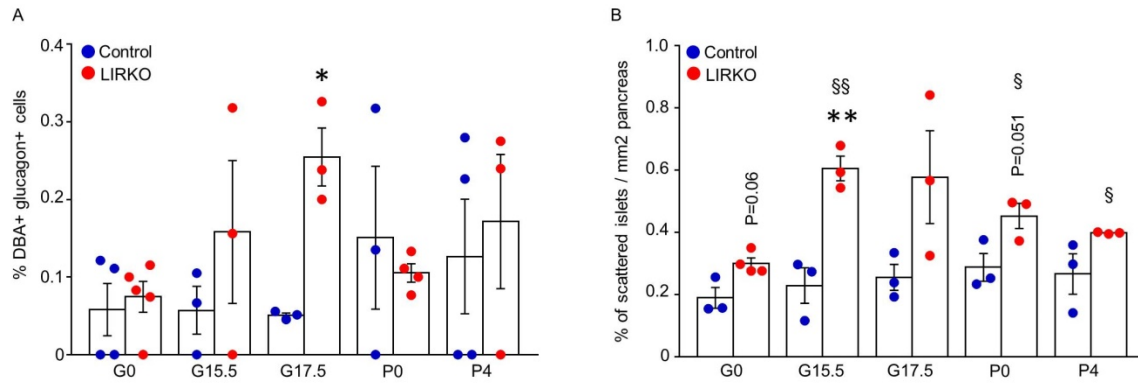
Supplementary Fig.3

**Supplementary Figure 3.** (A) Prolactin (n=6-7 mice per group, Student's *t*-test), (B) Progesterone (n=7 mice per group, Student's *t*-test) and (C) Estradiol (n=7 mice per group, Student's *t*-test) levels measured from plasma obtained from control and LIRKO mice before (G0), during (G15.5 and G17.5) and after (P0 and P4) gestation. #, Control vs Control, \* Control vs LIRKO and §, LIRKO vs LIRKO. Data were expressed as means  $\pm$  SEM. #, §, \* $P < 0.05$ , §§, \*\* $P < 0.01$ , ### $P < 0.001$



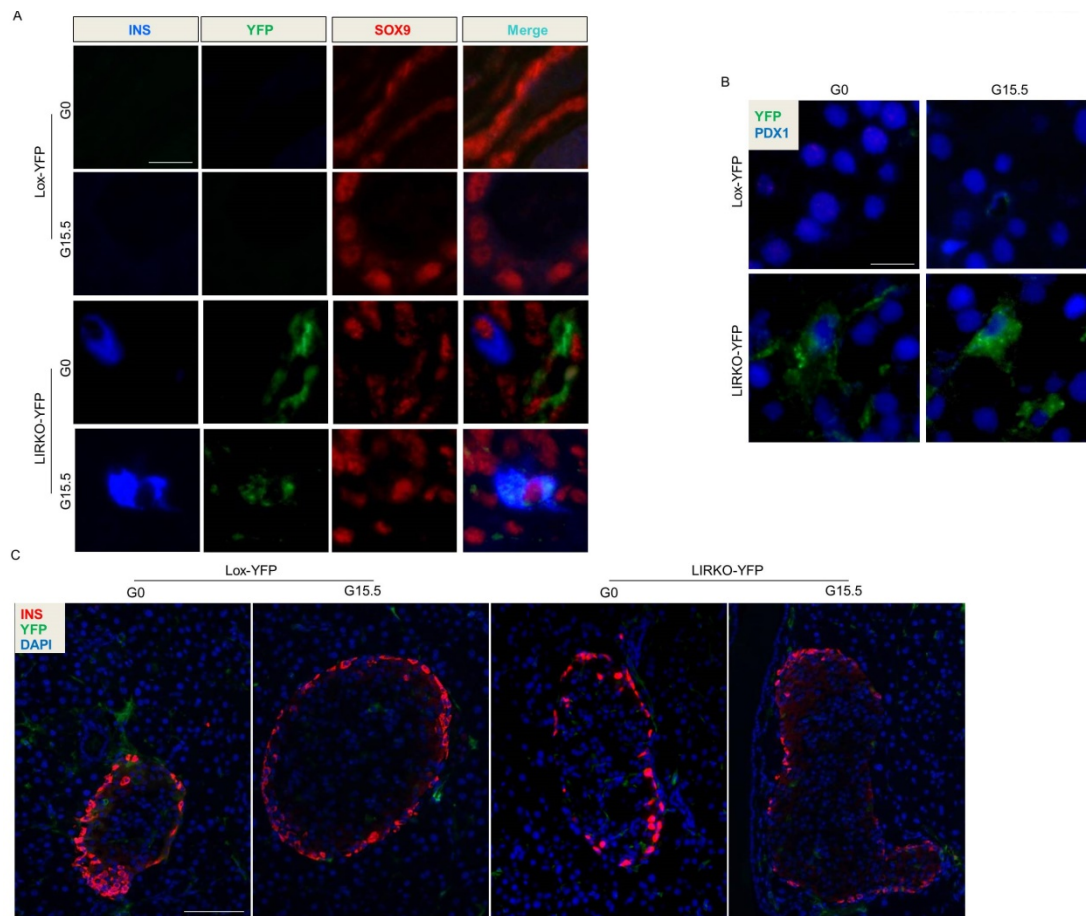
## Supplementary Fig.4

**Supplementary Figure 4.** (A) Immunofluorescent staining of pancreata from control and LIRKOs before (G0), during (G15.5 and G17.5) and after (P0 and P4) gestation for insulin (red) and serotonin (green). (B) Quantification of serotonin+  $\beta$ -cells (n=3-4 mice per group, Student's *t*-test), and (C) Plasma serotonin levels detected by ELISA (n=3-15 mice per group, Student's *t*-test) at the time points as indicated in A. (D) Ultrastructural analysis of pancreatic  $\beta$ -cells using electron microscopy on islets from control and LIRKO pregnant (G15.5) and post-partum (P0) mice (Magnification: 43.860x). (E) Quantification of serotonin granules per mm<sup>2</sup> area at the time points in mice as indicated in A (n=5 mice per group, Student's *t*-test). (F) Secreted insulin and serotonin, as assayed by ELISA, after 1 h incubation of size matched islets isolated from non-pregnant (G0) and pregnant (G15.5) control and LIRKO mice in 3.3 mM or 16.7 mM glucose. Five batches of size-matched 25 islets were used per group. Please note \* and § in F shows comparison of 3.3 mM vs 16.7 mM (n=3-5 mice per group, Student's *t*-test) (G) Representative immunofluorescence image immunostained for serotonin (green), glucagon (red) and DAPI (blue). Scale bars: 200  $\mu$ m. #, Control vs Control, \* Control vs LIRKO, and §, LIRKO vs LIRKO. Data were expressed as means  $\pm$  SEM. #, §, \**P* < 0.05, §§, ##, \*\**P* < 0.01, \*\*\**P* < 0.001.



### Supplementary Fig.5

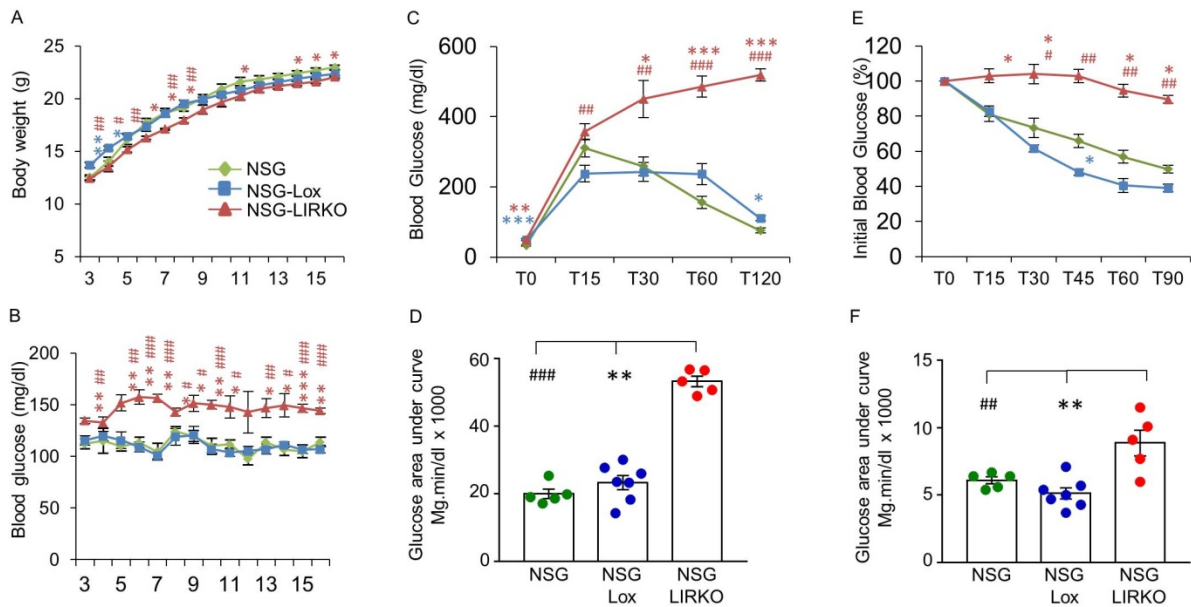
**Supplementary Figure 5.** (A) Percentage of Dolichos Bifloros Agglutinin (DBA) and glucagon double positive cells (n = 3-5 mice per group, Student's *t*-test) and (B) Number of scattered islets per mm<sup>2</sup> (n = 3-4 mice per group, Student's *t*-test) was analyzed in pregnant and non-pregnant control and LIRKO mice. \*, Control vs LIRKO and §, LIRKO vs LIRKO. Data were expressed as means ± SEM. §, \**P* < 0.05, §§, \*\**P* < 0.01.



## Supplementary Fig.6

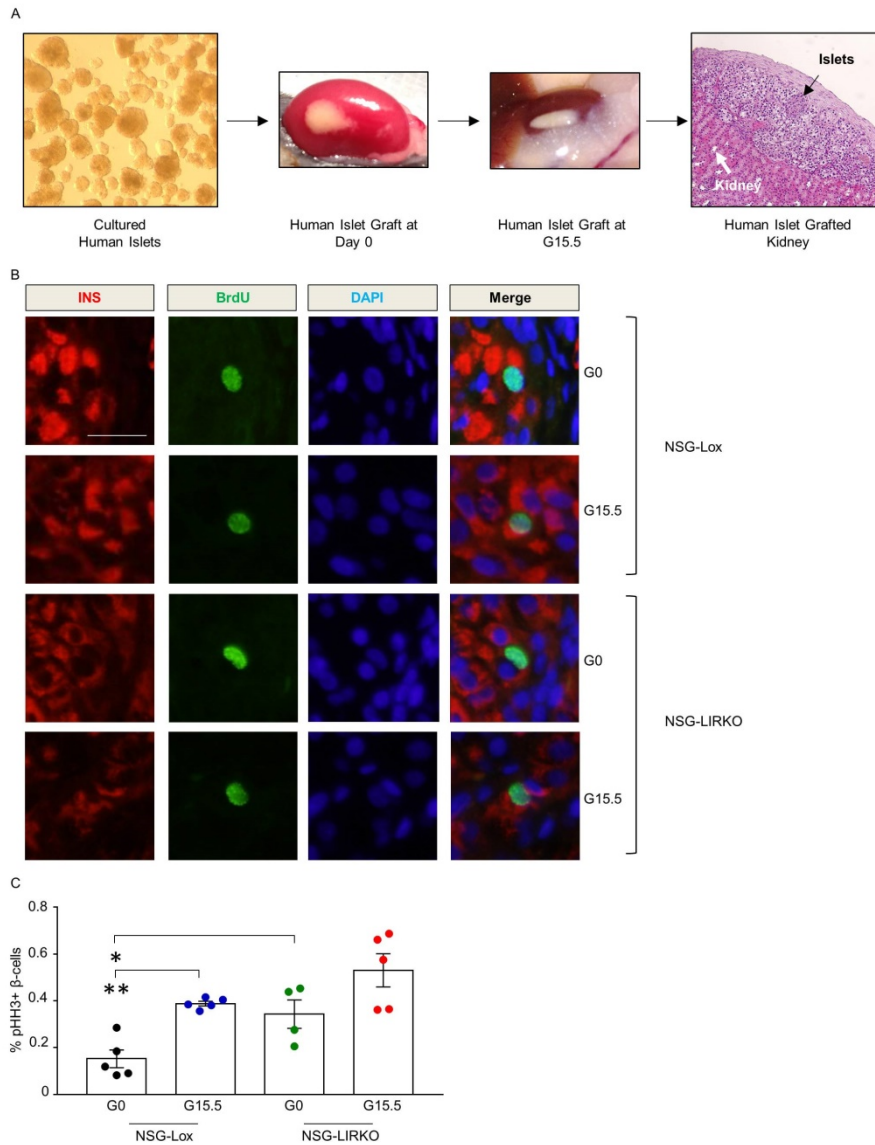
**Supplementary Figure 6.** Representative confocal image of pancreas section obtained from non-pregnant and pregnant Tamoxifen-treated pregnant Lox-YFP and LIRKO-YFP mice stained for **(A)** insulin (blue), YFP (green) and Sox9 (red) **(B)** YFP (green) and PDX1 (blue) and **(C)** Glucagon (red), YFP (green) and DAPI (blue). Scale bars: 10  $\mu$ m (A,B) and 50  $\mu$ m (C)





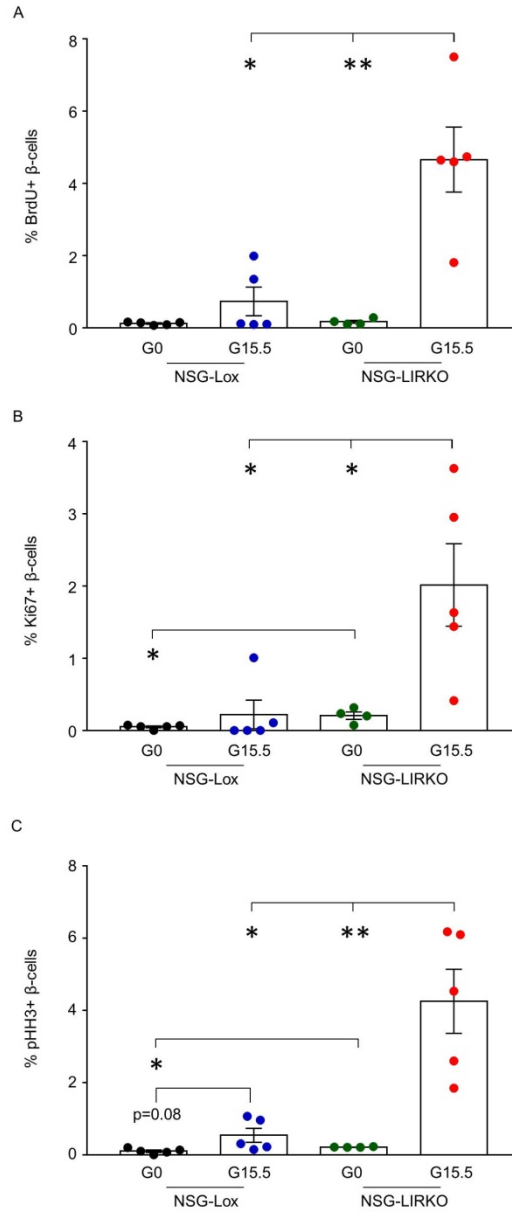
Supplemental Fig.7

**Supplementary Figure 7.** (A) Weekly body weight (n=5-7 mice per group, Student's *t*-test) and (B) blood glucose (n=5-7 mice per group, Student's *t*-test) levels in NSG, NSG-Lox and NSG-LIRKO mice starting at 3 weeks of age and followed up to 16 weeks of age. (C) Blood glucose (n=5-7 mice per group, Student's *t*-test) and (D) area under the curve (AUC) following an oral glucose administration (2.5 g/kg.b.wt) and (E) glucose levels (n=5-7 mice per group, Student's *t*-test), plotted as % of basal values and (F) AUC following intraperitoneal injection of insulin (1 U/kg. b.wt). NSG mice were used as controls. # vs NSG and \* vs NSG-Lox. Data were expressed as means  $\pm$  SEM. #, \**P* < 0.05, ##, \*\**P* < 0.01, and ###, \*\*\**P* < 0.001.



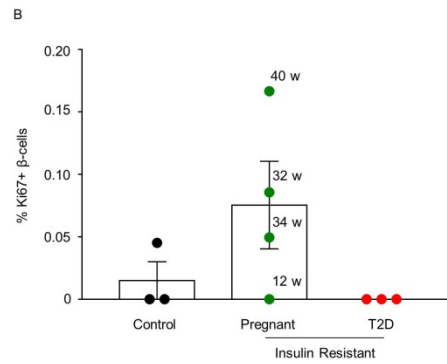
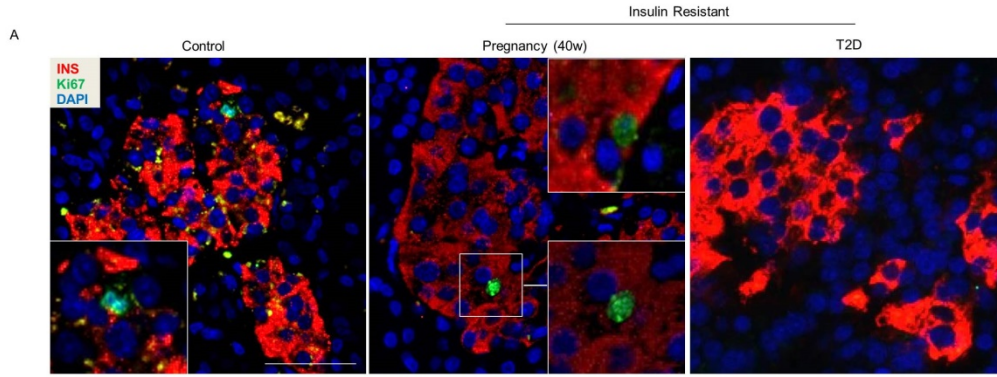
## Supplementary Fig.8

**Supplementary Figure 8.** (A) Overnight cultured human islets were transplanted under the kidney capsule of the mice and grafts were harvested on day G15.5 after mice became pregnant. (B) Individual immunofluorescence images showing proliferating human  $\beta$ -cells (correspond to upper panel in Fig 4B in main text) in non-pregnant and pregnant NSG-Lox and NSG-LIRKO mice stained for insulin (red), proliferation marker BrdU (green) and nuclear stain DAPI (blue). Scale bar: 25  $\mu$ m. (C) Quantification of proliferating  $\beta$ -cells double positive for pHH3 and insulin from human islet grafted kidney sections in samples shown for BrdU and Ki67 (for quantification see Supplementary Table 3) (n = 4-5 mice per group, 1-way ANOVA). Data were expressed as means  $\pm$  SEM. \*  $P < 0.05$ , \*\*  $P < 0.01$ .



## Supplementary Fig.9

**Supplementary Figure 9.** Quantification of proliferating  $\beta$ -cells in endogenous pancreatic islets of mice transplanted with human islets. Samples were analyzed for **(A)** BrdU/insulin (n=4-5 mice per group, Student's *t*-test), **(B)** Ki67/insulin (n=4-5 mice per group, Student's *t*-test), and **(C)** pHH3/insulin (n=4-5 mice per group, Student's *t*-test) double positive cells (for quantification see Supplementary Table 3). Data were expressed as means  $\pm$  SEM. \*  $P < 0.05$ , \*\*  $P < 0.01$ .



## Supplementary Fig.10

**Supplementary Figure 10. (A)** Representative immunofluorescence images of pancreas sections obtained from control, pregnant (40 week of pregnancy) and T2D cases immunostained for insulin (red), Ki67 (green) and DAPI (blue). Scale bar: 50 $\mu$ m. **(B)** Quantification of Ki67 positive  $\beta$ -cells (n=3-4 cases per group) (for quantification see Supplementary Table 3).