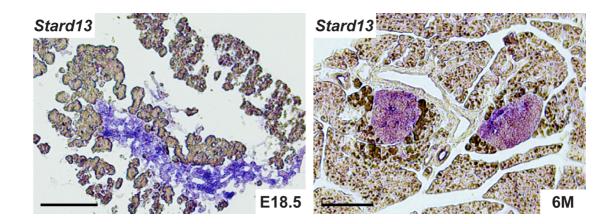
## SUPPLEMENTARY MATERIAL

## The RhoGAP Stard13 controls insulin secretion through F-actin remodeling

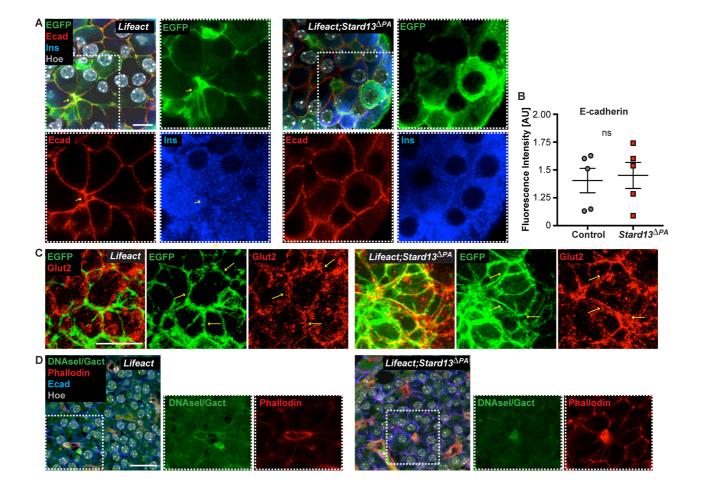
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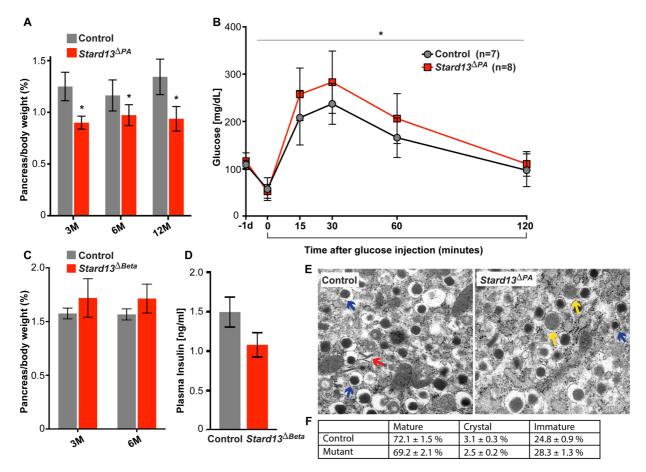


Supplementary Figure 1. Stard13 expression in mouse islets. In situ hybridization analysis for Stard13 on cryosections of mouse E18.5 and adult (6 M) pancreatic tissue. Stard13 transcript accumulates exclusively in the islets after E17.5 in the mouse. Purple color indicates the expression of the digoxigenin-labelled Stard13 in situ RNA probe; in brown, the unstained exocrine pancreatic tissue. Bar, 200um.



Supplementary Figure 2. Characterization of islet cells organization in the absence of Stard13. A) Representative single plane confocal images of whole-mount immunofluorescence staining of Lifeact control *Lifeact; Stard13*<sup>△PA</sup> mouse islets for E-cadherin (Ecad, red), insulin (Ins., blue) and in green Lifeact-EGFP. Hoechst 33342 was used as nuclear counterstain. Insets show split channels of boxed area; arrows indicate cellular vertex. Bar, 10 um. B) Dot plot quantification of E-cadherin fluorescence intensity in control and Stard13 $^{\Delta PA}$  islets. **C**) Whole-mount immunofluorescence staining of Lifeact control and Lifeact; Stard13<sup>APA</sup> mouse islets for GLUT2 (red) and in green Lifeact-EGFP. Arrows indicate staining along the cell membranes. Bar, 20 um. D) Immunofluorescence analysis on cryosections of control and Stard13<sup>∆PA</sup> adult pancreatic tissues. Phalloidin-Alexa Fluor™ 555 (red) was

used for the detection of filamentous actin (F-actin), Deoxyribonuclease I-Alexa Fluor™ 488 (green) for unpolymerized actin (G-actin) along with E-cadherin (blue) and Hoechst nuclear counterstain (grey). Insets show split channels of boxed area at higher magnification. Bar, 20 um.



Supplementary Figure 3. Stard13 regulates glucose metabolism

**A)** Pancreas weight normalized by body weight in control and  $Stard13^{\Delta PA}$  mice at indicated age. M, months. (n = 6-10 per age group, \*\*p < 0.001). **B)** Glucose tolerance test (GTT) was carried out on twelve-month-old male animals that had been fasted overnight, before the day of experimentation (time point -1d on the chart). Animals were injected i.p. with glucose (2g /kg body weight). Glucose levels were measured from blood collected from the tail immediately before the glucose challenge (time point 0) and 15, 30, 60 and 120 minutes after the glucose injection. Data are expressed as means  $\pm$  s.e.m. ANOVA test, \* p <0.05. **C)** Pancreas weight normalized by body weight in control and  $Stard13^{\Delta Beta}$  mice at six-month age. (n = 5). **D)** Plasma insulin in the random-fed condition is similar between control and  $Stard13^{\Delta Beta}$  mice.

(n = 7). **E)** Representative TEM micrographs of islets from control and  $Stard13^{\Delta PA}$  adult mice. Yellow arrows point to immature vesicles; blue arrows point to vesicles containing mature insulin-dense core granules; red arrows point to vesicles containing insulin crystal structures. Magnification x8900. **F)** The percentage of different vesicle morphologies in control and  $Stard13^{\Delta PA}$  β-cells. Quantification was performed by counting the number of each type of vesicle with respect to the total number of vesicles per field (magnification x8900). In total 50 β-cells were quantified from three different pancreas per genotype. Data are expressed as means  $\pm$  s.e.m.

## **Supplementary Table 1. List of Primary Antibodies**

Antibody	Raised in	Dilution	Source and catalog number
Deoxyribonuclease		1:100	Invitrogen, #D12371
I-Alexa Fluor™ 488			
E-cadherin	Rat	1:500	Sigma, #U3254
Glucagon	Rabbit	1:500	Immunostar, #20076
Glut2	Goat	1:200	Santa Cruz, #sc7580
Insulin	Guinea pig	1:250	Invitrogen, #PAI-26938
Phalloidin-Alexa		1:200	Invitrogen, #A34055
Fluor™ 555			