SUPPLEMENTAL MATERIALS

"Glucose intolerance and impaired insulin secretion in pancreas-specific STAT3 KO mice are associated with microvascular alterations in the pancreas" by Kostromina *et al.*

MATERIALS AND METHODS

Evaluation of vascular density by immunostaining. Five µm cryosections from different parts of the pancreas were immunostained with FITC-conjugated Isolectin-B4 from *Bandeiraea simplicifolia* (BSI-B4, L2895, Sigma) diluted in TBS at 10 µg/ml or antibody against VE-Cadherin (#555289, BD Pharmingen) at 1:200 dilution. After immunostaining, confocal images of 10 serial sections from each animal were acquired using a Zeiss LSM510 META confocal microscope (objectives 40x, 63x; Carl Zeiss, Germany). All images were acquired with identical settings on the laser scanning system. Vascular density was assessed as area of positive Isolectin-B4 or VE-Cadherin signal above a preset threshold value relative to the whole pancreatic area using Image Pro-Plus software.

FIGURE LEGENDS

Figure 1. Reduced islet microvascular density in female STAT3 KO mice

A. Five µm cryosections of p-KO and control mouse pancreas were immuno-labeled with STAT3 (red) and endothelial marker Isolectin-B4 (green). **B.** Vascular density, measured as Isolectin B4-stained area relative to the islet area was reduced in p-KO (grey bar, N = 4 mice) compared with control mice (white bar, N = 4 mice). Images taken from 10 pancreatic sections of each animal were used for analysis. Data are presented as means ± SEM. **, $p < 0.01$.

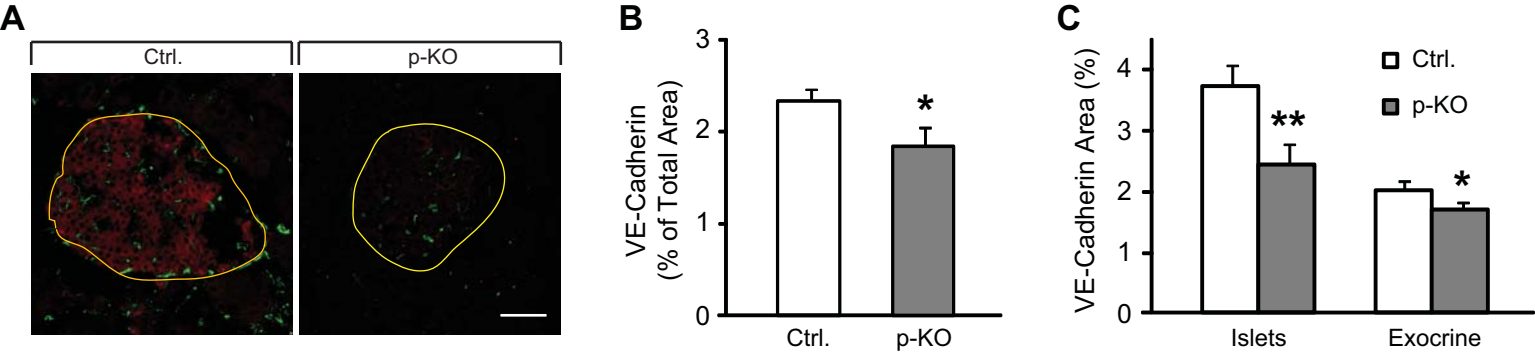
Figure 2. Reduced islet microvascular density in female STAT3 KO mice

A. Five µm cryosections of p-KO and control mouse pancreas were immuno-labeled with STAT3 (red) and endothelial marker VE-Cadherin (green). **B.** Vascular density, measured as VE-Cadherin-stained area relative to the whole pancreas was reduced in p-KO (grey bar, N =

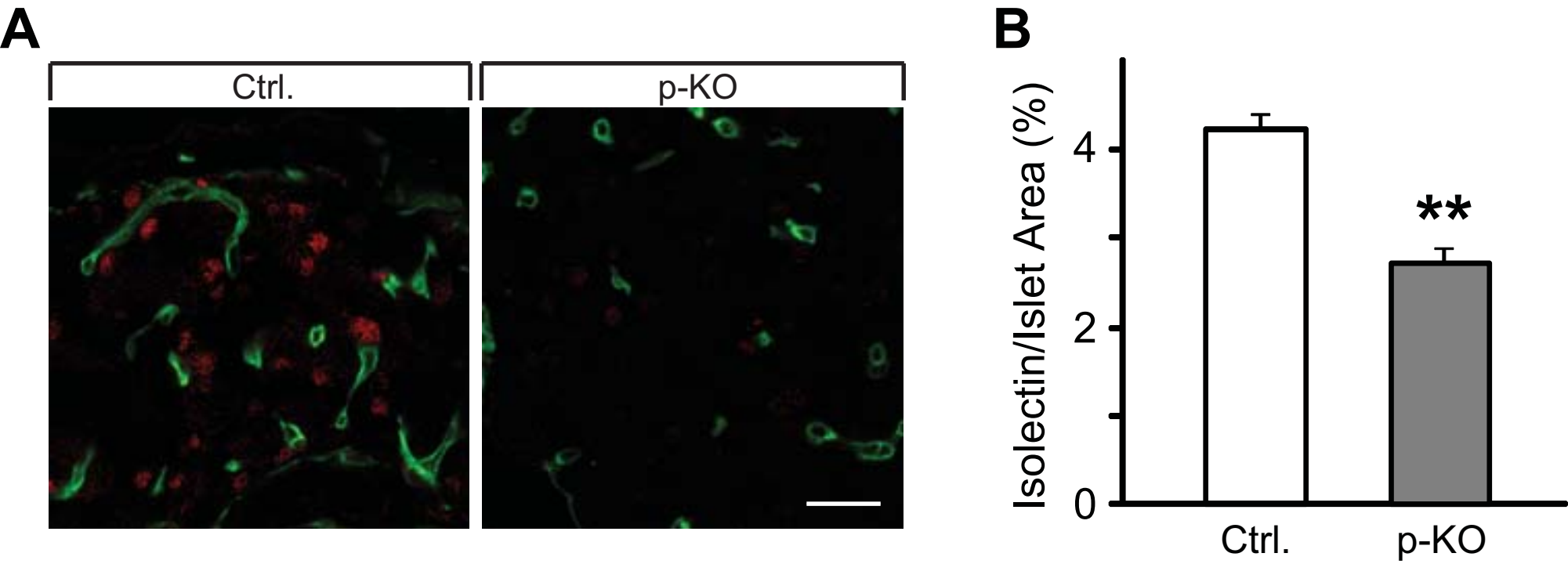
4 mice) compared with control mice (white bar, N = 4 mice). Images taken from 10 pancreatic sections of each animal were used for analysis. **C.** Vascular density in the islets (e.g. inside yellow boundary in **A**) and exocrine pancreas (e.g. outside yellow boundary in **A**) was similarly assessed as in **B.** Vascular density was significantly reduced in the islets and exocrine pancreas. Data are presented as means ± SEM. Images taken from 10 pancreatic sections of each mouse, 4 mice per group were used for analysis. *, $p < 0.05$; **, $p < 0.01$.

Figure 3. Reduced islet microvascular density in male STAT3 KO mice

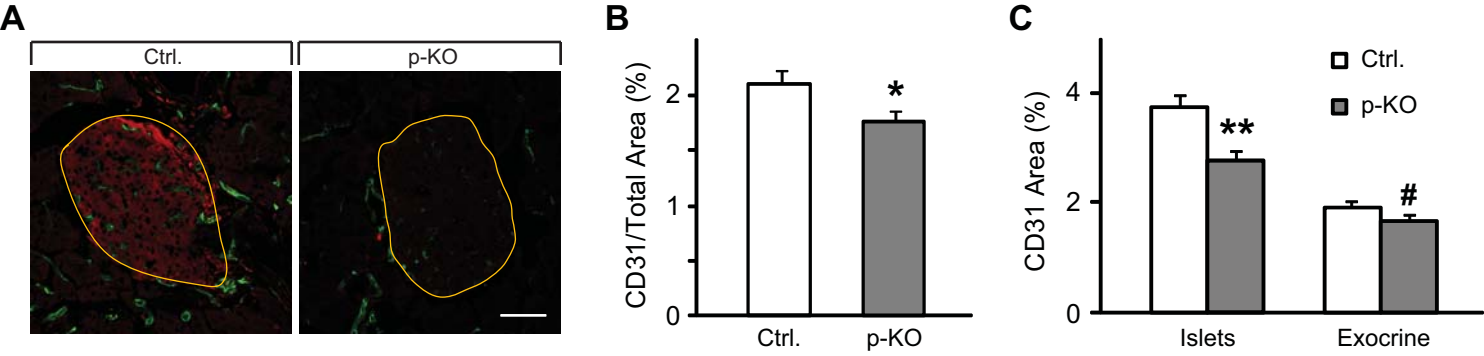
A. Five μm cryosections of p-KO and control mouse pancreas were immuno-labeled with STAT3 (red) and endothelial marker CD31 (green). **B.** Vascular density, measured as CD31-stained area relative to the whole pancreas was reduced in p-KO (grey bar, N = 4 mice) compared with control mice (white bar, N = 4 mice). Images taken from 10 pancreatic sections of each animal were used for analysis. **C.** Vascular density in the islets (e.g. inside yellow boundary in **A**) and exocrine pancreas (e.g. outside yellow boundary in **A**) was similarly assessed as in **B.** Vascular density was significantly reduced in the islets (**, $p < 0.01$), and marginally reduced in the exocrine tissue (#, $p = 0.08$). Data are presented as means ± SEM. Images taken from 10 pancreatic sections of each mouse, 4 mice per group were used for analysis.



Kostromina et al., Figure S2



Kostromina et al., Figure S1



Kostromina et al., Figure S3