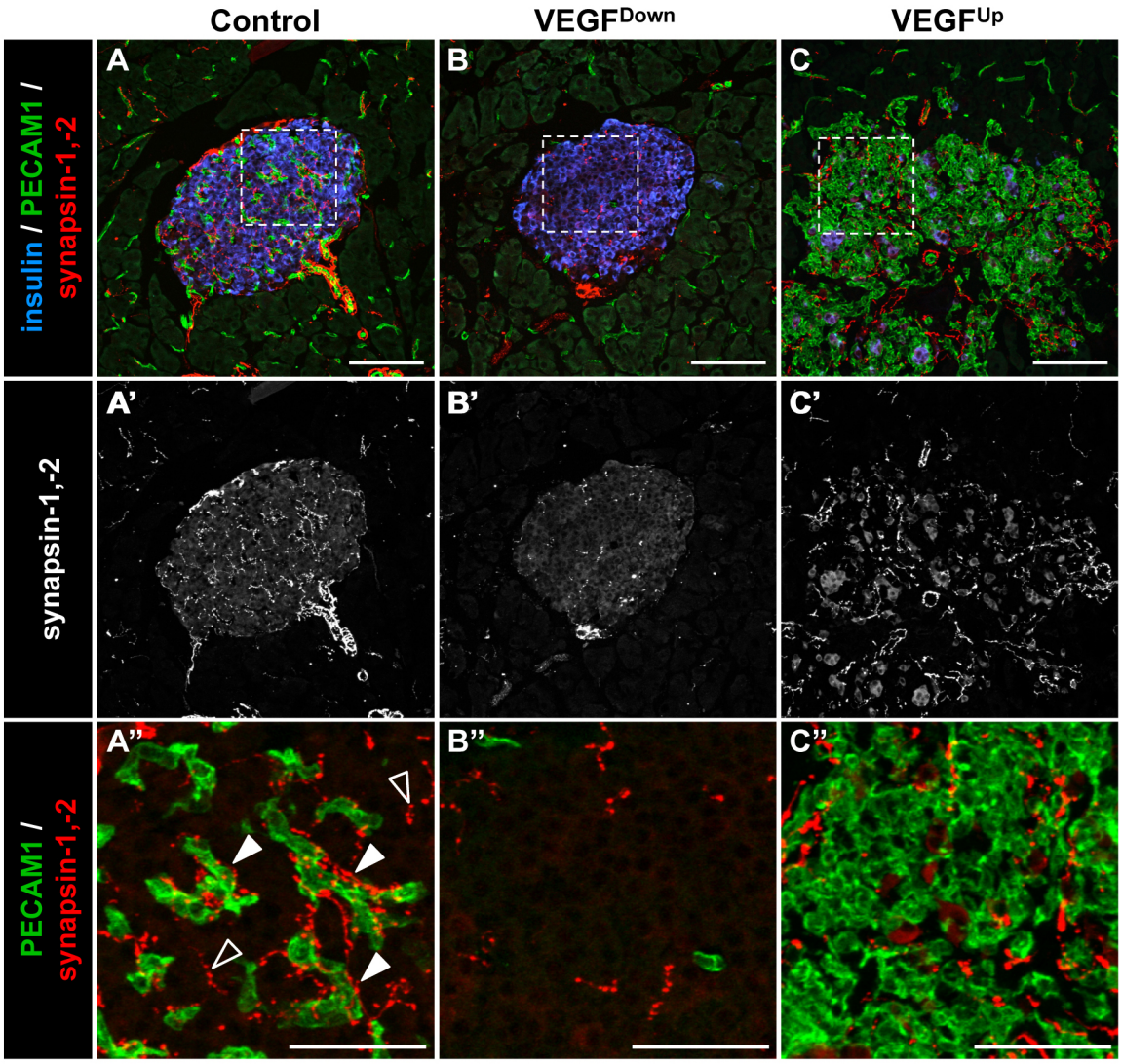
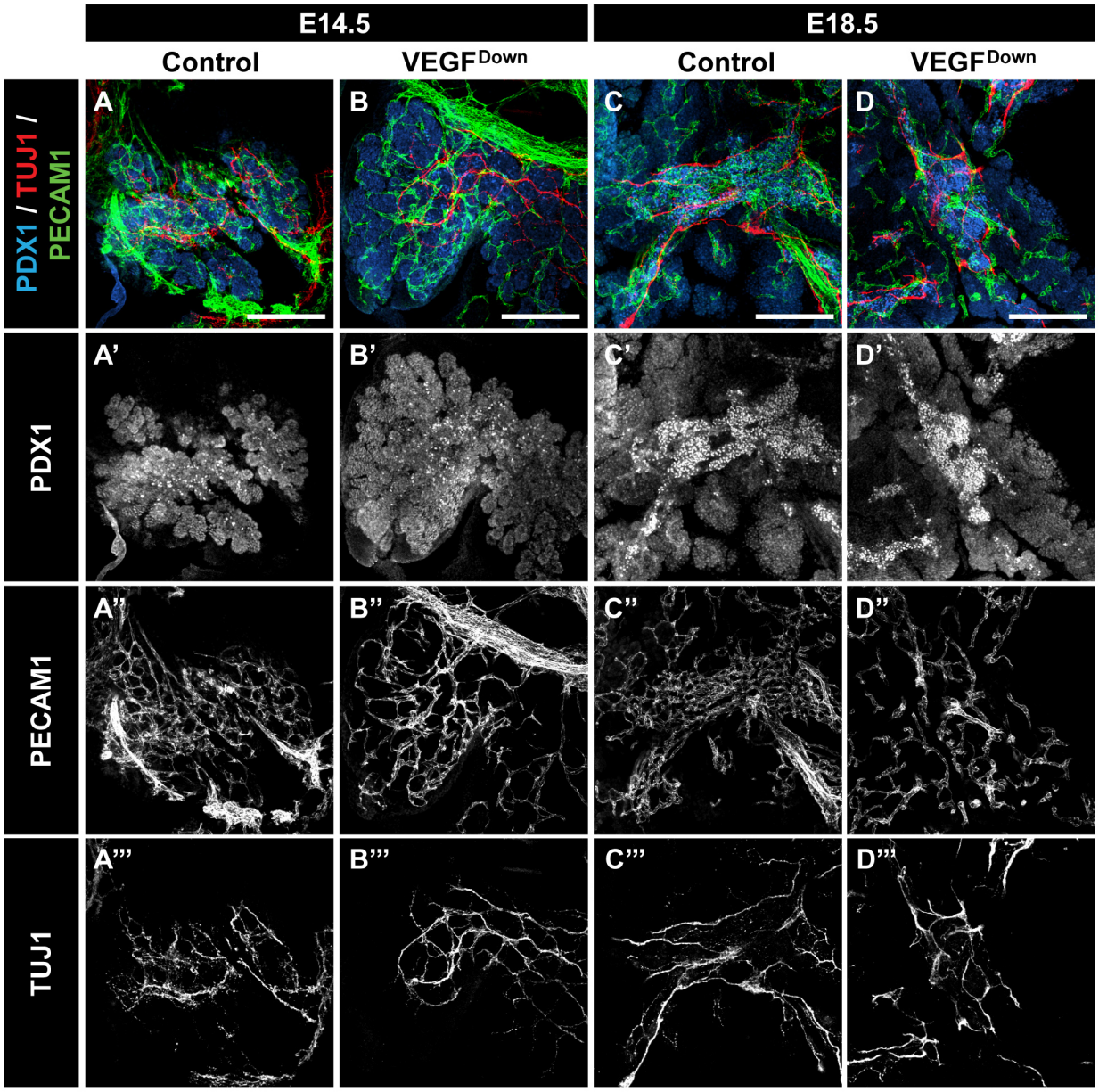
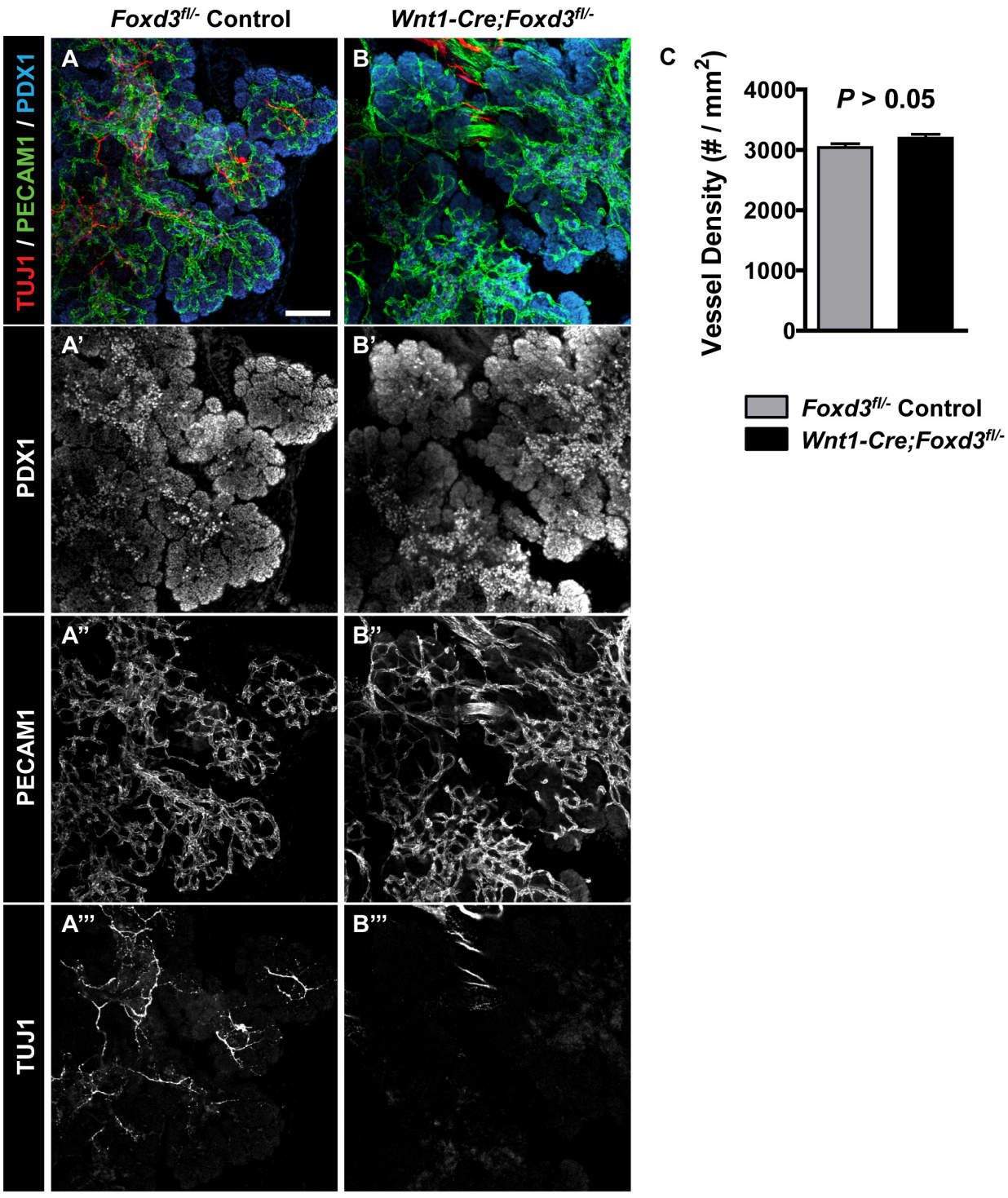
Reinert Fig. S1

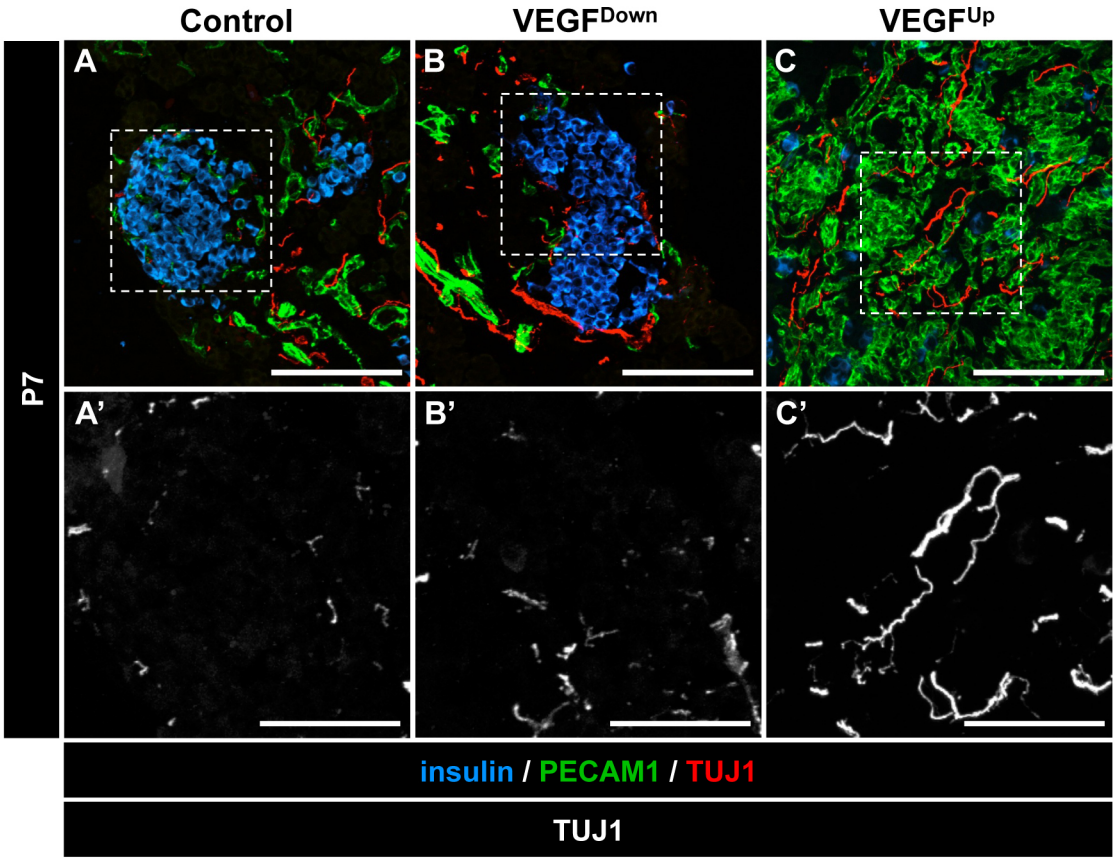


Reinert Fig. S2

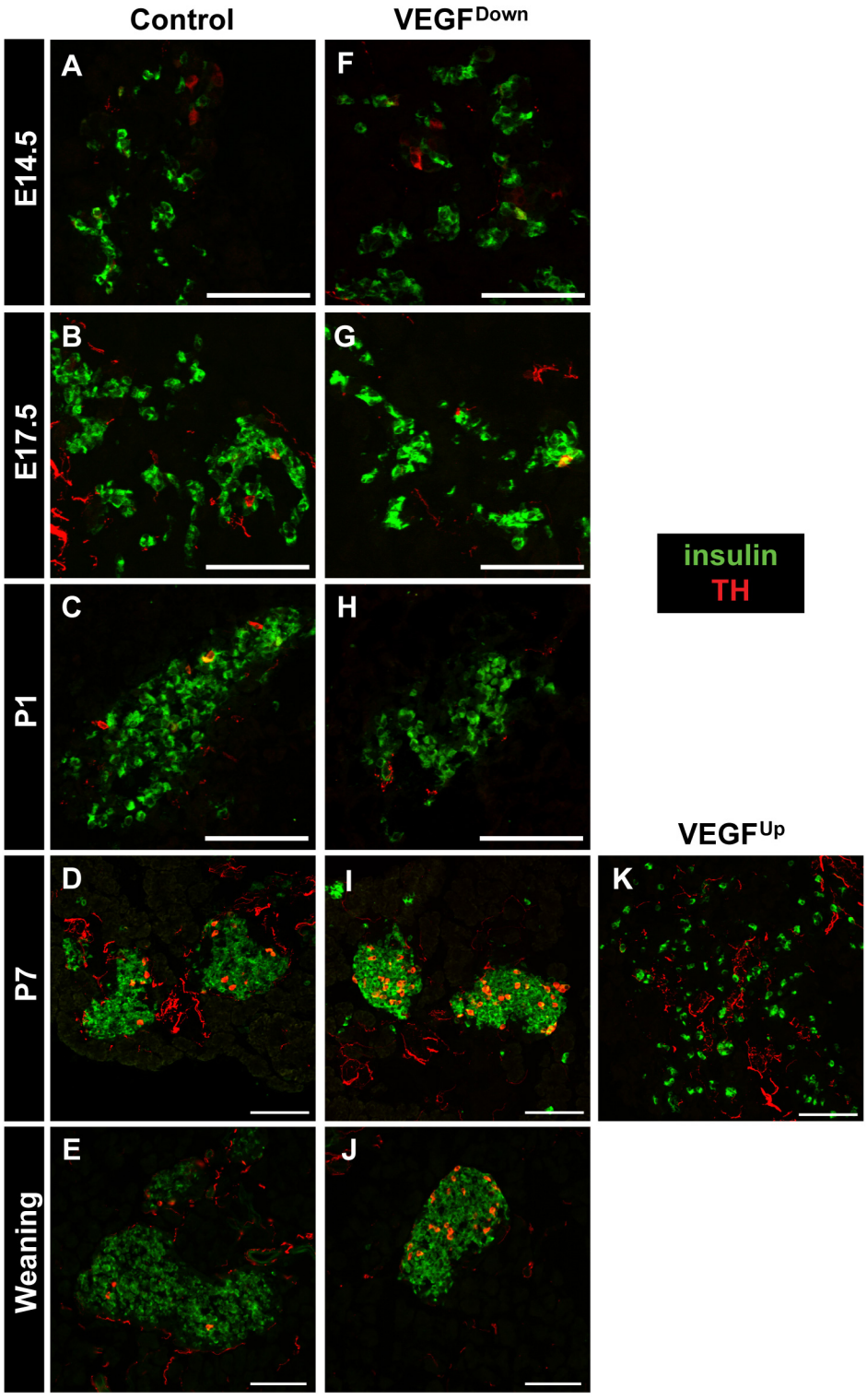



Foxd3fl/- Control
Wnt1-Cre;Foxd3fl/-
TUJ1 / PECAM1 / PDX1
PDX1
PECAM1
TUJ1
A
B
A'
B'
A''
B''
A'''
B'''
C
Vessel Density (# / mm2)
4000
3000
2000
1000
0
P > 0.05
Foxd3fl/- Control
Wnt1-Cre;Foxd3fl/-

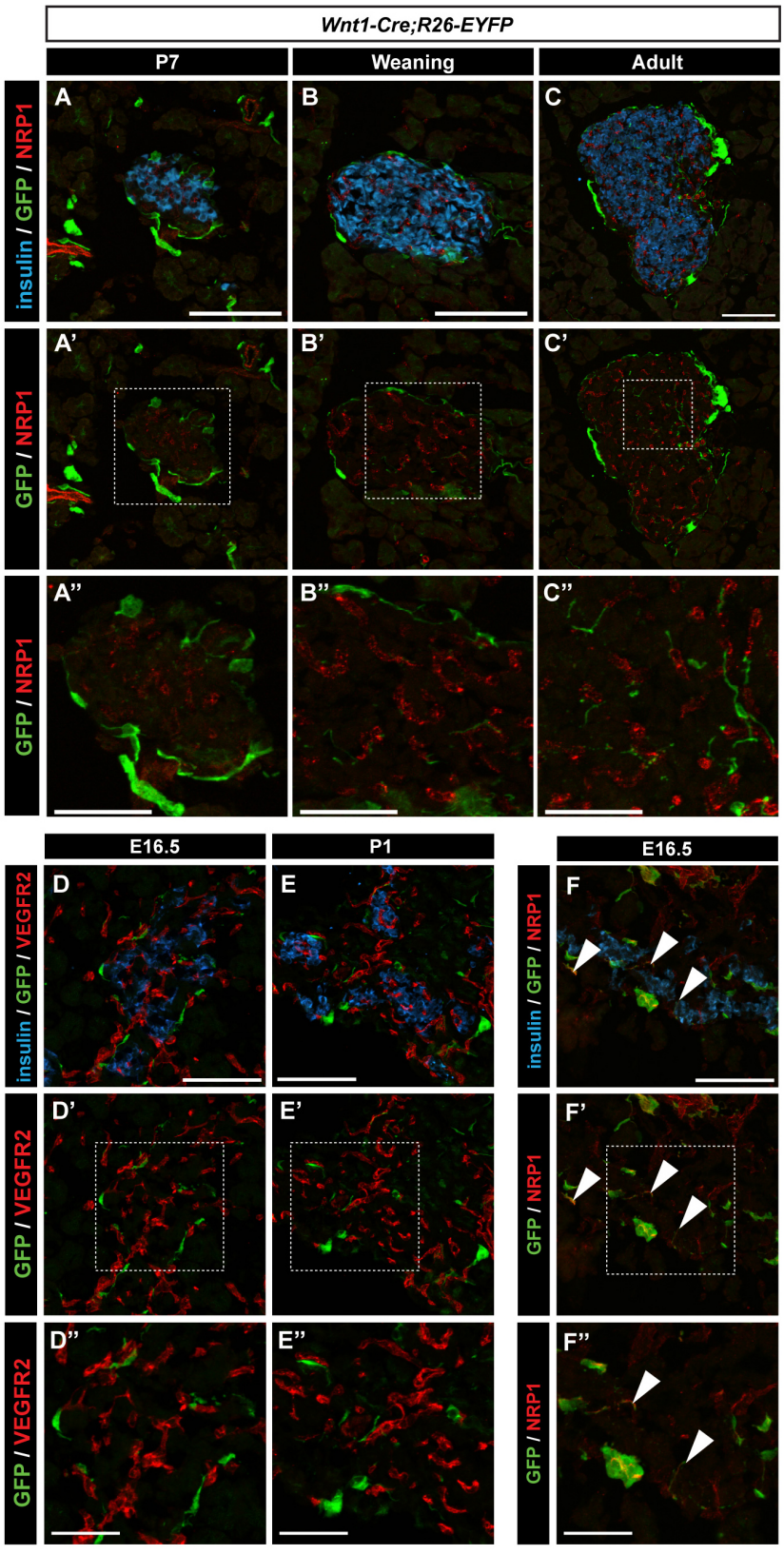
Reinert Fig. S4



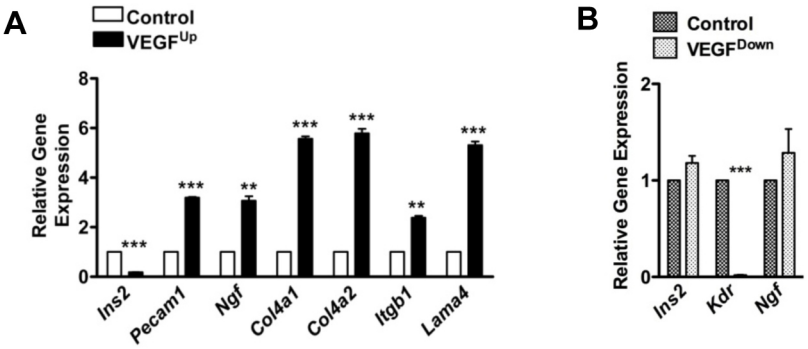
Reinert Fig. S5



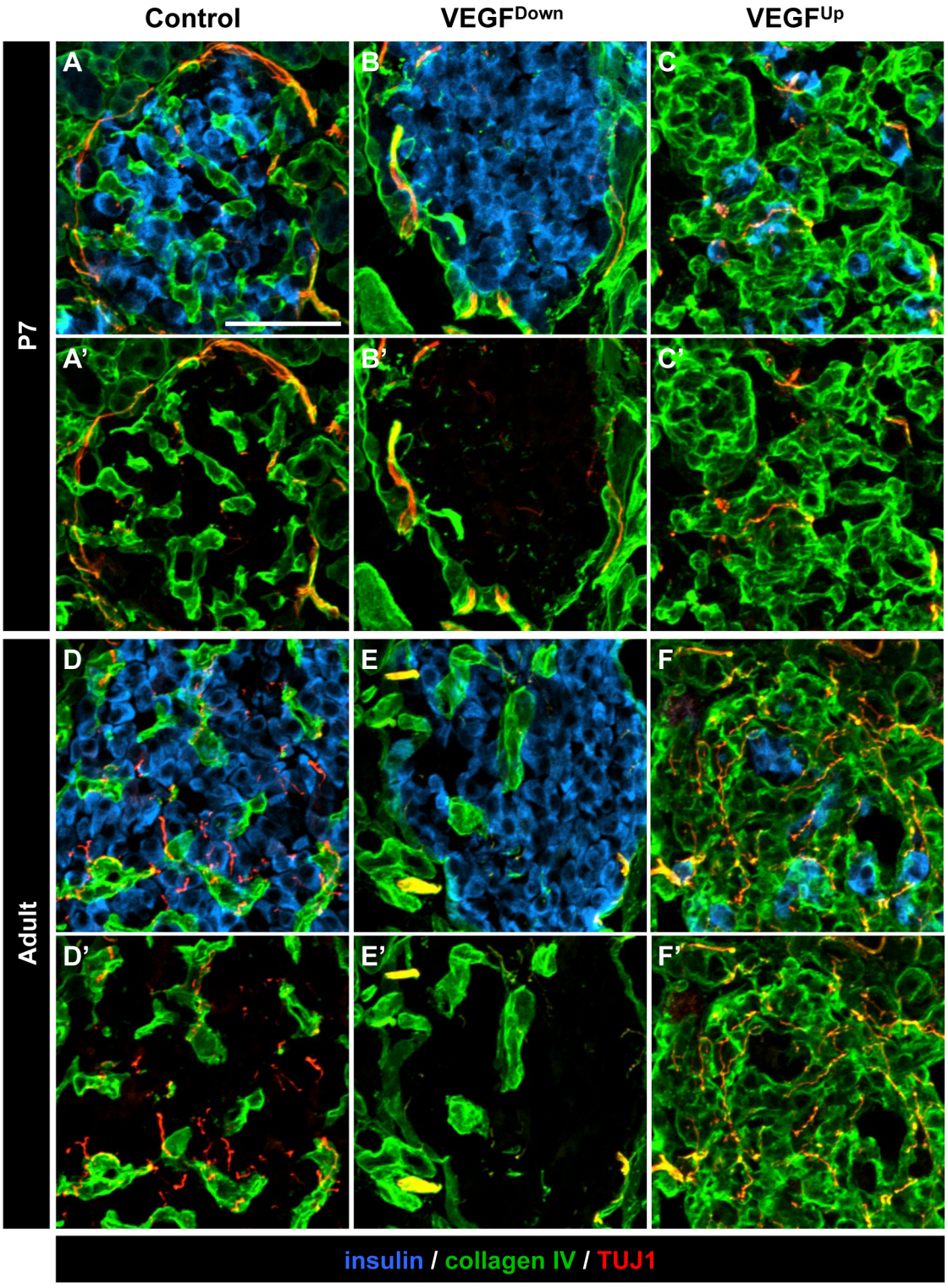
Reinert Fig. S6



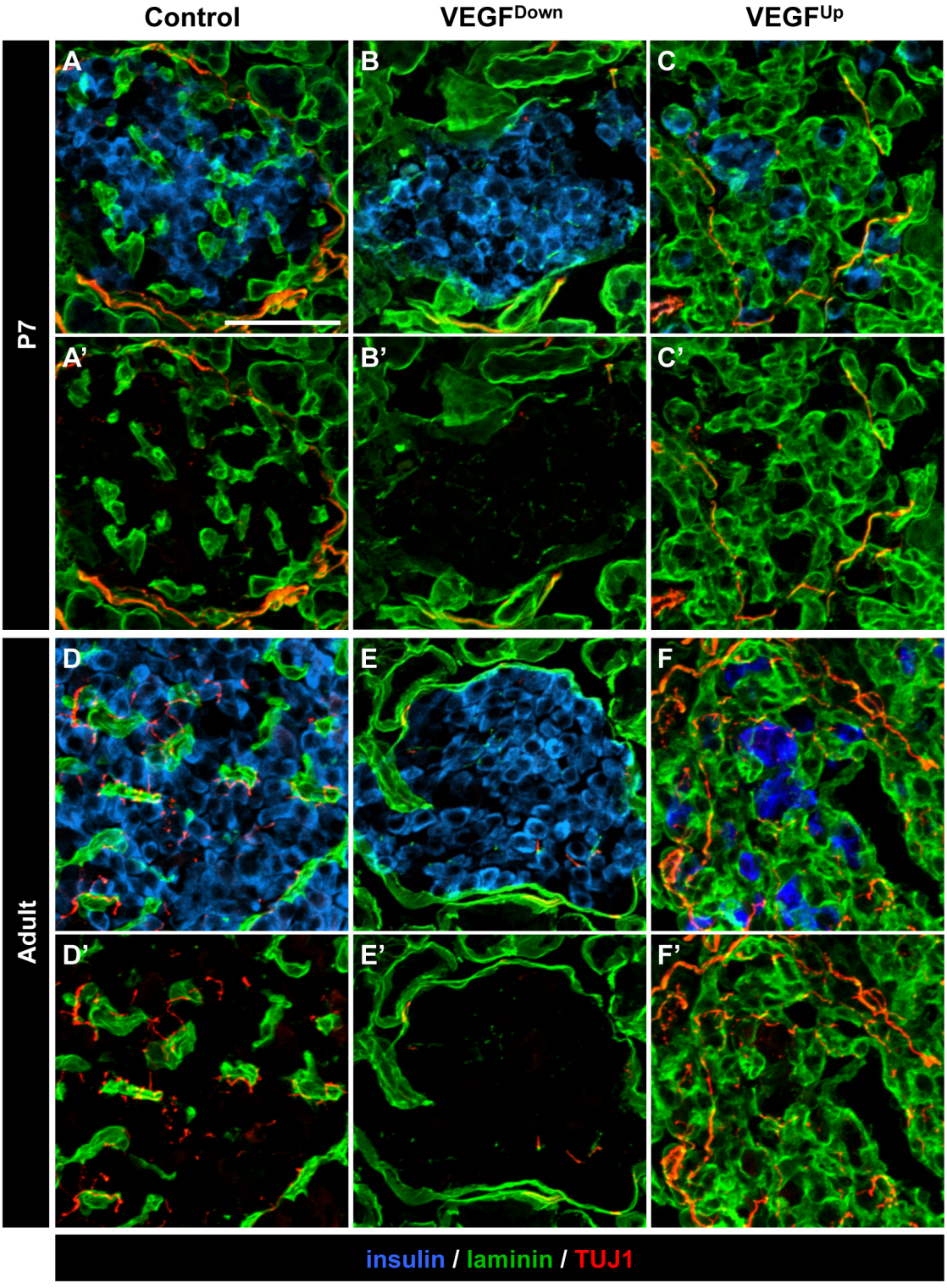
Reinert Fig. S7



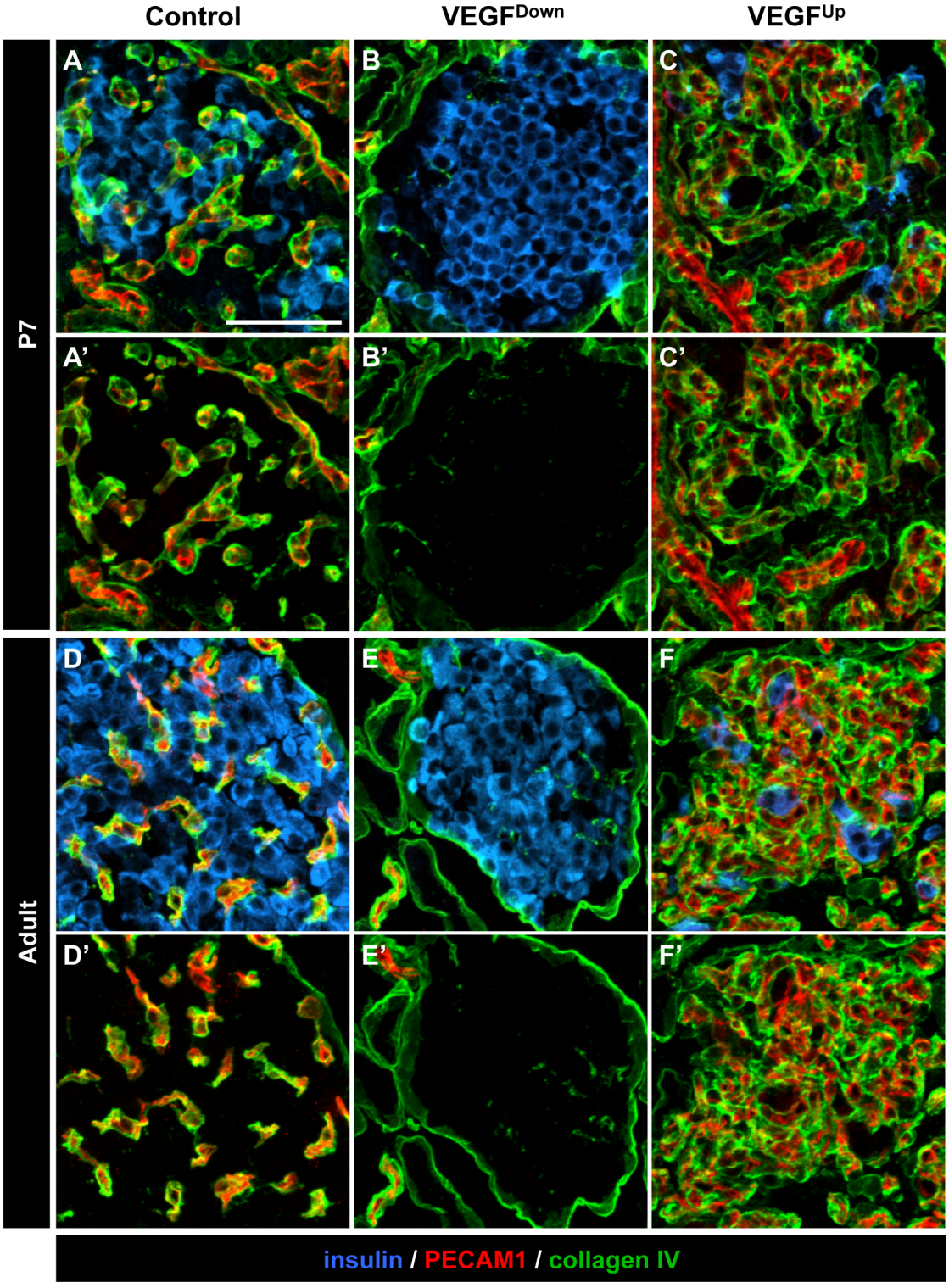
Reinert Fig. S8



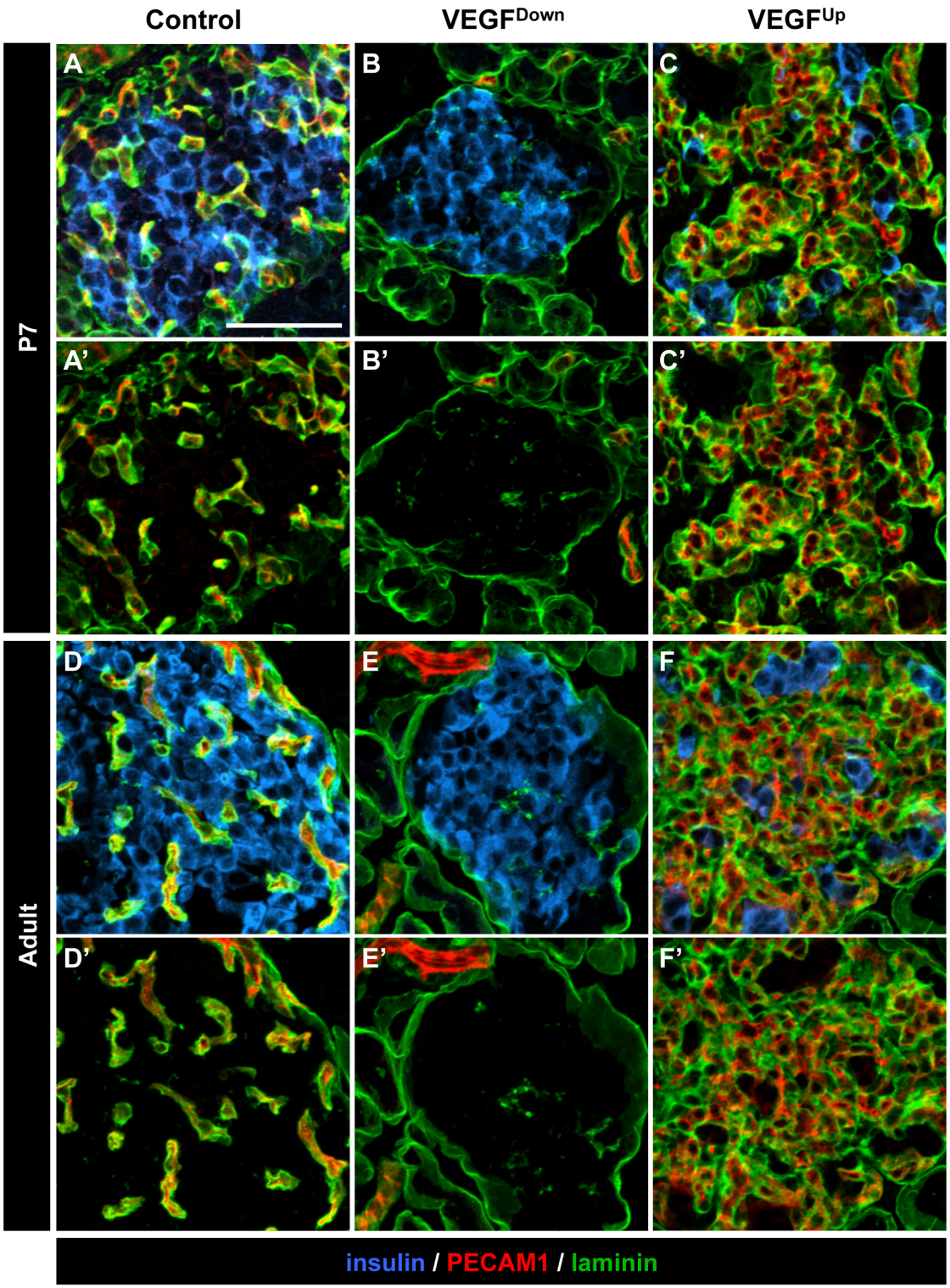
Reinert Fig. S9



Reinert Fig. S10



Reinert Fig. S11



SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Islet innervation follows islet VEGF production and vascularization. A-**C**. Representative islets from adult control (**A**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ (VEGF^{Down}; **B**), and doxycycline-treated (for one week) *RIP-rtTA;TetO-hVegfa* (VEGF^{Up}; **C**) mice, immunolabeled for insulin (blue), PECAM1 (green) and synapsin-1,-2 (red). Panels **A'**-**C'** show grayscale images of synapsin-1,-2 labeling in **A**-**C**. Regions denoted by the dashed line in **A**, **B**, and **C** are shown in **A''**, **B''** and **C''**, respectively. Closed arrowheads point to synapsin-1,-2+ neuronal structures in a complete or partial alignment with PECAM1+ capillaries. Open arrowheads designate synapsin-1,-2+ structures that were not adjacent to endothelial cells. Scale bars in **A**-**C** are 100 μm. Scale bars in **A''**-**C''** are 50 μm.

Fig. S2. Pancreatic innervation during embryogenesis. A-**D**. Images are 3D reconstructions of confocal z-stacks (30 μm-thick) of embryonic pancreata from control (**A, C**) and *Pdx1-Cre;Vegfa*$^{fl/fl}$ (VEGF^{Down}; **B, D**) mice at embryonic day 14.5 (E14.5; **A, B**) and E18.5 (**C, D**). Pancreata were immunolabeled in whole mount with antibodies to PDX1 (blue), PECAM1 (green), and TUJ1 (red). Scale bars in **A**-**D** are 100 μm, and correspond to all panels below.

Fig. S3. **Pancreatic vascularization is not altered by reduced innervation. A**, **B**. Images are 3D reconstructions of confocal z-stacks (15 μm-thick) of embryonic pancreata from *Foxd3*$^{fl/-}$ (**A**) and *Wnt1-Cre;Foxd3*$^{fl/-}$ (**B**) mice at embryonic day 16.5 (E16.5), immunolabeled in whole mount with antibodies to PDX1 (blue), PECAM1 (green), and TUJ1 (red). **A'**, **B'**. Grayscale images of PDX1 labeling from respective panels **A**, **B**. **A''**, **B''**. Grayscale images of PECAM1 labeling from respective panels **A**, **B**. **A'''**, **B'''**. Grayscale images of TUJ1 labeling from respective panels **A**, **B**. Scale bar in **A** is 50 μm, and corresponds to all other panels. **C**. Quantification of vessel density. Data are summarized as mean ± standard error of the mean (SEM).

Fig. S4. **Pancreatic islet innervation matures postnatally, and depends on VEGF expression.** Representative islets from control (**A**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ (VEGF^{Down}; **B**), and doxycycline-treated (from E5.5) *RIP-rtTA;TetO-hVegfa* (VEGF^{Up}; **C**) mice at postnatal day 7 (P7). Pancreatic sections were immunolabeled for insulin (blue), PECAM1 (green) and TUJ1 (red/grayscale). Scale bars in **A**-**C** are 100 μm. Regions denoted by the dashed line in **A**-**C** are shown in **A'**-**C'**, respectively. Panels **A'**-**C'** show grayscale images of TUJ1 labeling in **A**-**C**. Scale bars in **A**'-**C**' are 50 μm.

Fig. S5. The number of β-cells expressing tyrosine hydroxylase is elevated in VEGF-deficient islets during postnatal development. A-**J**. Representative islets from *Vegfa*$^{fl/fl}$ (Control, **A**-**E**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ (VEGF^{Down}; **F**-**J**), and doxycycline-treated (from embryonic day E5.5) *RIP-rtTA;TetO-hVegfa* (VEGF^{Up}; **K**) mice at E14.5 (**A, F**), E17.5 (**B**, **G**), postnatal day 1 (P1; **C, H**), P7 (**D, I, K**) and weaning (**E, J**), immunolabeled for insulin (green) and TH (red). Scale bars are 100 μm.

Fig. S6. Islet neural crest-derived cells do not express VEGF receptors in postnatal life. A-**C**. Representative islets from *Wnt1-Cre;R26-EYFP* mice at postnatal day 7 (P7; **A**), weaning (P21-P28; **B**), and adult (**C**) stages labeled for insulin (blue), GFP (green), and neuropilin 1 (NRP1, red). Regions denoted by the dotted line in **A'**, **B'**, and **C'** are shown in **A''**, **B''**, and **C''**, respectively. Scale bars in **A**-**C** are 100 μm. Scale bars in **A''**-**C''** are 50 μm. **D**-**F**. Representative islets from *Wnt1-Cre;R26-EYFP* mice at embryonic day 16.5 (E16.5; **D, F**) and postnatal day 1 (P1; **E**). Images are labeled for insulin (blue), GFP (green), and VEGFR2 (red in **D**-**E**) or neuropilin 1 (NRP1, red in **F**). Regions denoted by the dotted line in **D'**, **E'**, and **F'** are shown in **D''**, **E''** and **F'**, respectively. Arrowheads in **F** denote fibers with colocalization of GFP and NRP1. Scale bars in **D**-**F** are 100 μm. Scale bars in **D''**-**F''** are 50 μm.

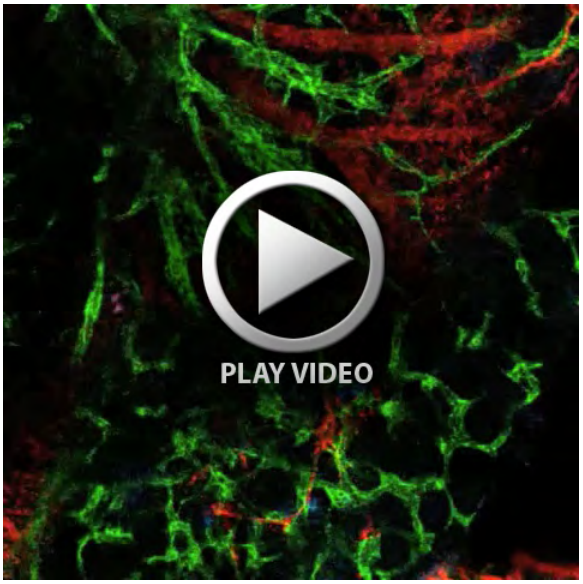
Fig. S7. Intraislet endothelial cells modulate islet innervation via synthesis of the vascular basement membrane. A. Relative gene expression of *Ins2* (encoding insulin), *Pecam1* (encoding PECAM1), *Ngf*, (encoding nerve growth factor) *Col4a1* (encoding collagen IV α1), *Col4a2* (encoding collagen IV α2), *Itgb1* (encoding integrin β1), and *Lama4* (encoding laminin α4) in islets from doxycycline-treated (for one week) *RIP-rtTA;TetO-hVegfa* ($VEGF^{Up}$) mice compared to untreated *RIP-rtTA;TetO-hVegfa* controls, evaluated by quantitative RT-PCR. **B**. Relative gene expression of *Ins2*, *Kdr* (encoding VEGFR2), and *Ngf* in islets from *Pdx1-Cre;Vegfa*$^{fl/fl}$ ($VEGF^{Down}$) mice compared to *Vegfa*$^{fl/fl}$ controls, evaluated by quantitative RT-PCR. Data are summarized as mean ± standard error of the mean (SEM); $n = 4$; $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. S8. Intraislet nerve fibers align with endothelial cells expressing collagen IV. Representative islets from *Vegfa*$^{fl/fl}$ (Control, **A**, **D**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ ($VEGF^{Down}$; **B, E**), and doxycycline-treated (from embryonic day E5.5) *RIP-rtTA;TetO-hVegfa* ($VEGF^{Up}$; **C, F**) mice at postnatal day 7 (P7; **A-C**) and adult (**D-F**) stages, immunolabeled for insulin (blue), collagen IV α1 (green) and TUJ1 (red). Images **A** and **D** are replicates of Fig. 7C-D. Panels **A'-F'** highlight collagen IV and TUJ1 labeling in islets from panels **A-F**. Scale bar in **A** is 50 μm and applies to all other panels.

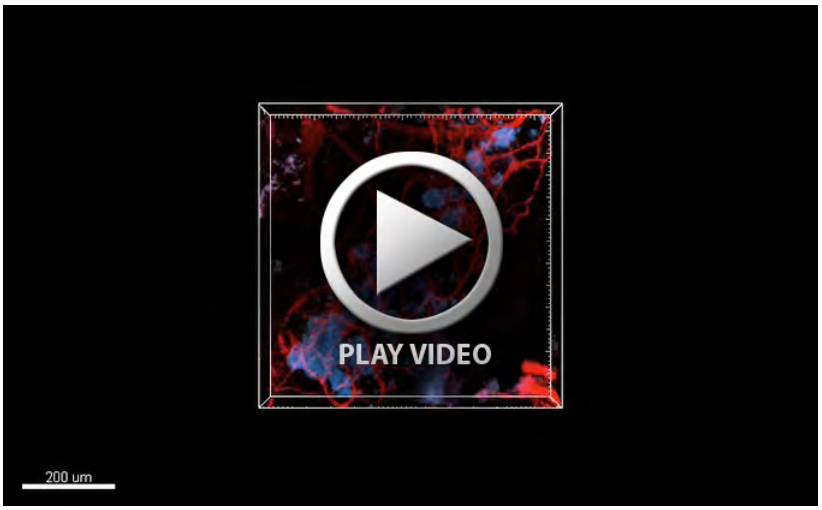
Fig. S9. Intraislet nerve fibers align with endothelial cells expressing laminin. Representative islets from *Vegfa*$^{fl/fl}$ (Control, **A**, **D**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ ($VEGF^{Down}$; **B, E**), and doxycycline-treated (from embryonic day E5.5) *RIP-rtTA;TetO-hVegfa* ($VEGF^{Up}$; **C, F**) mice at postnatal day 7 (P7; **A-C**) and adult (**D-F**) stages, immunolabeled for insulin (blue), laminin (green) and TUJ1 (red). Panels **A'-F'** highlight laminin and TUJ1 labeling in islets from panels **A-F**. Scale bar in **A** is 50 μm and applies to all other panels.

Fig. S10. Endothelial cells produce collagen IV of the intraislet vascular basement membrane. A-F. Representative islets from *Vegfa*$^{fl/fl}$ (Control, **A**, **D**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ (VEGF^{Down}; **B, E**), and doxycycline-treated (from embryonic day E5.5) *RIP-rtTA;TetO-hVegfa* (VEGF^{Up}; **C, F**) mice at postnatal day 7 (P7; **A-C**) and adult (**D-F**) stages, immunolabeled for PECAM1 (red), and collagen IV α1 (green). Panels **A'-F'** highlight collagen IV and TUJ1 labeling in islets from panels **A-F**. In addition to the endothelial cell-associated collagen IV labeling, we noted some fine collagen IV+ fibers within VEGF^{Down} islets; however, these fibers were rarely aligned with TUJ1+ nerve fibers. Scale bar in **A** is 50 μm and applies to all other panels.

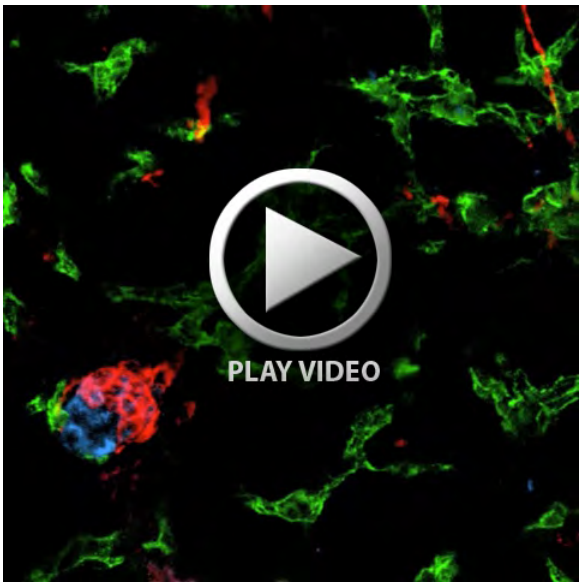
Fig. S11. Endothelial cells produce laminin of the intraislet vascular basement membrane. A-F. Representative islets from *Vegfa*$^{fl/fl}$ (Control, **A**, **D**), *Pdx1-Cre;Vegfa*$^{fl/fl}$ (VEGF^{Down}; **B, E**), and doxycycline-treated (from embryonic day E5.5) *RIP-rtTA;TetO-hVegfa* (VEGF^{Up}; **C, F**) mice at postnatal day 7 (P7; **A-C**) and adult (**D-F**) stages, immunolabeled for PECAM1 (red) and laminin (green). Panels **A'-F'** highlight laminin and TUJ1 labeling in islets from panels **A-F**. In addition to the endothelial cell-associated laminin labeling, we noted some fine laminin+ fibers within VEGF^{Down} islets; however, these fibers were rarely aligned with TUJ1+ nerve fibers. Scale bar in **A** is 50 μm and applies to all other panels.



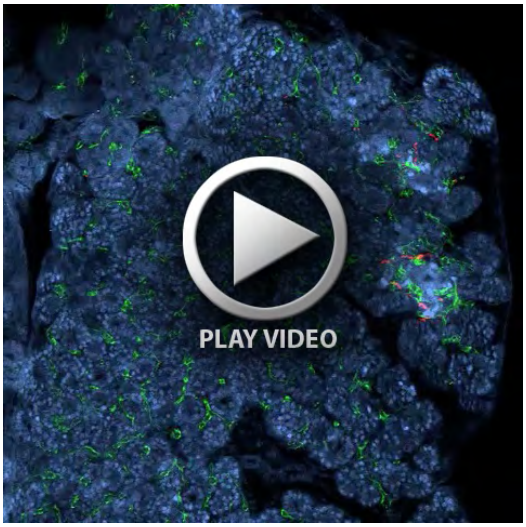
Movie 1A.



Movie 1B.

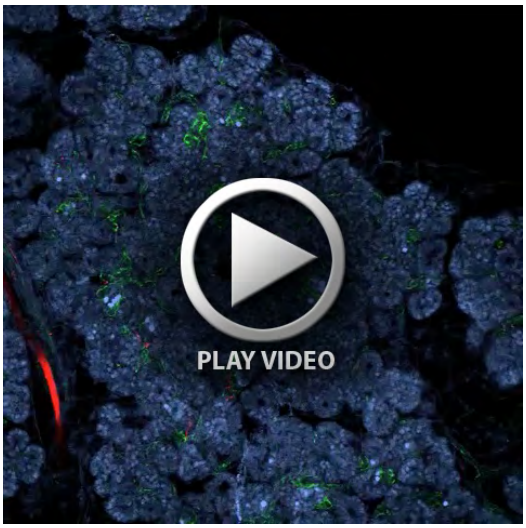


Movie 2.



Movie 3.

Movie 4.



Movie 5.

SUPPLEMENTARY MOVIE LEGENDS

Movie S1A. Developing pancreatic islets are interconnected by a network of nerves during embryogenesis. Movie displays sequential confocal z-stack of a 100 μm-thick portion of pancreas from a control embryo at E14.5, immunolabeled in whole mount with antibodies to insulin and glucagon (blue), PECAM1 (green), and TUJ1 (red).

Movie S1B. Three-D visualization of neuronal network interconnecting developing pancreatic islets. Movie displays 3D reconstructed sequential confocal z-stack of a 100 μm-thick portion of pancreas from a control embryo at E14.5 (shown in Movie S1A), immunolabeled in whole mount with antibodies to insulin and glucagon (blue), and TUJ1 (red).

Movie S2. Nerve processes are adjacent to, but do not expand, into developing pancreatic islets during embryogenesis. Movie displays sequential confocal z-stack of a 67 μm-thick portion of pancreas from a control embryo at E14.5, immunolabeled in whole mount with antibodies to insulin and glucagon (blue), PECAM1 (green), and TUJ1 (red).

Movie S3. The pancreas is well innervated in late embryogenesis. Movie displays sequential confocal z-stack of a 30 μm-thick portion of pancreas from the control embryo shown in Fig. 4A. The pancreas was harvested at E16.5 and immunolabeled in whole mount with antibodies to PDX1 (blue), PECAM1 (green), and TUJ1 (red).

Movie S4. VEGF is not required for pancreatic innervation during embryogenesis. Movie displays sequential confocal z-stack of a 30 μm-thick portion of pancreas from the *Pdx1-Cre; Vegfa*$^{fl/fl}$ (VEGF^{Down}) embryo shown in Fig. 4B. The pancreas was harvested at E16.5 and immunolabeled in whole mount with antibodies to PDX1 (blue), PECAM1 (green), and TUJ1 (red).

Movie S5. VEGF enhances pancreatic innervation during embryogenesis. Movie displays sequential confocal z-stack of a 30 µm-thick portion of pancreas from the doxycycline-treated (from E5.5) *RIP-rtTA; TetO-hVegfa* ($VEGF^{Up}$) embryo shown in Fig. 4C. The pancreas was harvested at E16.5 and immunolabeled in whole mount with antibodies to PDX1 (blue), PECAM1 (green), and TUJ1 (red).