

## SUPPLEMENTARY FIGURES

**Figure S1. Blood vessel intercalation with developing pancreatic epithelial branches. (A-B)** Flk1-lacZ pancreata stained for  $\beta$ -Galactosidase activity (green) and for E-cadherin with DAB (brown) at E10.5 and E12.0. Black dashed line represents the boundaries of pancreatic epithelium. Scale bars 50  $\mu$ m. **(C-D)** E10.5 and E11.5 wild type pancreata immunostained for epithelial marker E-cadherin (green) and endothelial marker PECAM-1, or PECAM-1 and Endomucin (PE) in the same channel (red). Arrows in **(C)** indicate presence of blood cells detected by autofluorescence (Auto-fluor, blue) without any immunostaining. Scale bars 20  $\mu$ m.

**Figure S2. Peripheral angioblasts in the distal mesenchyme surround the pancreatic epithelium. (A-F)** Whole mount GFP immunostaining performed on Flk1-GFP pancreas to label angioblasts. Panels represent consecutive slices of a Z-stack through the pancreatic bud. Squares designate isolated cells throughout the z stack, while each color marks a single angioblast across the z stack. Scale bar 50  $\mu$ m.

**Figure S3. Pancreatic epithelium expresses VEGF heterogeneously. (A, C)** Whole mount staining on E11.5 and E15.5 VEGF-lacZ pancreata for  $\beta$ -Galactosidase activity (green). Black dashed line represents the boundaries of the pancreatic bud. Scale bars 100  $\mu$ m **(A)** and 200  $\mu$ m **(C)**. **(B, D)** Eosin staining on paraffin sections of E11.5 and E15.5 pancreata stained for  $\beta$ -Galactosidase activity. Black dashed line represents the boundaries of pancreatic bud. Scale bars 50  $\mu$ m **(B)** and 100  $\mu$ m **(D)**. **(E-F')** Eosin staining on paraffin sections of E15.5 pancreata stained for  $\beta$ -Galactosidase activity. Squares in **(E, F)** are shown at high magnification in **(E', F')**. Arrows and arrowheads indicate areas negative and positive for  $\beta$ -Galactosidase activity, respectively. Scale bars 100  $\mu$ m. **(G-H)** Paraffin sections of adult pancreata were immunostained with VEGF **(G)** and VEGFR2 **(H)** antibodies to assess VEGF expression and label endothelial cells, respectively. Scale bars 100  $\mu$ m.

**Figure S4. Vascular remodeling in the pancreas: from plexus to hierarchical vessels. (A-D)** 3D reconstructions of whole mount PECAM-1 stained embryonic guts and budding pancreatic anlagen. Yellow dashed line marks the boundaries of pancreatic bud. **Small vessels coalesce and remodel into the central pancreatic artery (D, yellow arrow)**. All scale bars 100  $\mu$ m.

**Figure S5. Development of the central pancreatic artery and recruitment of mural cells.** (A) High magnification of coalescing patent vessels in Figure 5A' at E11.5. Dashed lines mark luminal areas. (B-C') Cryosections of E11.5, E13.5 and E14.5 wild type pancreata immunostained for Connexin40 (red) and SM22 $\alpha$  (green) to label the main (central) artery and pericytes, respectively. All scale bars 5  $\mu$ m.

**Figure S6. Endothelial heterogeneity in the developing dorsal pancreatic bud.** Expression of the endothelial genes *Sox18*, *ICAM2*, *Pecam-1*, *APJ*, *Endoglin* and *Rasip1* in the pancreas by in situ hybridization at (A-F) E10.5, (A'-F') E12.5-13.5, and (A''-F'') E14.5. Dashed lines represent boundaries of pancreatic bud. White arrows designate the location where the central artery and vein emerge. All scale bars 100  $\mu$ m.

**Figure S7. Summary of endothelial heterogeneity in the developing dorsal pancreatic bud.** (A, D, G) Expression of Flk1-LacZ, APJ and Rasip1 on whole mount E14.5 pancreas by in situ hybridization. Arrows indicate expression in central artery/vein. Scale bars 100  $\mu$ m. (B, E, H) Expression of Flk1-LacZ, APJ and Rasip1 on cross-sectioned E14.5 pancreas by in situ hybridization. Arrows (yellow) and arrowheads (blue) designate expression in central artery and vein, respectively. (B) Note activity of Flk1-LacZ in vessels of all size, both in peripheral and central epithelium. (E) Note that APJ is only expressed in mid-sized vessels, as neither central artery/vein nor capillaries are labeled. (H) Rasip1 marks both central artery and vein, as well as capillaries (in small punctae) across the peripheral pancreatic epithelium. Scale bars 50  $\mu$ m. (C, F, I) Schematics represent expression of each gene in sagittal (left) and cross-section (right) view of the developing pancreas. In cross-section view, capillaries are represented by small punctae/short lines, and mid-sized vessels by larger punctae/longer lines. Spl, spleen; stom, stomach.

**Figure S8. Vascular architecture is disturbed in Pdx1-tet-VEGF pancreas.** (A-B') Whole mount in situ hybridization of E12.5 control or Pdx1-tet-VEGF pancreata for Rasip1 and Endoglin as indicated. Black dashed lines mark pancreatic bud boundaries. (C-C') Whole mount in situ hybridization of E14.5 control or Pdx1-tet-VEGF pancreata for Connexin40 as indicated, shown from the posterior side. Arrows designate bifurcation point of the central artery. (D-E') In situ hybridization on paraffin sections of E14.5 control or Pdx1-tet-VEGF pancreata for Connexin40 or APJ as indicated. Arrowheads indicate ectopic vessels (D') or abnormal coalescence (E'). All scale bars 100  $\mu$ m.

## MOVIES

**Movie 1. 3D reconstruction of the avascular E10.5 pancreatic bud.** Z-stack of whole mount immunofluorescent isolated pancreatic bud and its vasculature within the surrounding mesenchyme. E-cadherin (green), PECAM-1 (red) and auto-fluorescence (blue).

**Movie 2. 3D reconstruction of the branching E11.5 pancreatic bud.** Z-stack of whole mount immunofluorescent isolated pancreatic bud showing blood vessels intercalating between epithelial branches. E-cadherin (green), PECAM-1 (red) and auto-fluorescence (blue).