Islet vascularization is regulated by primary endothelial cilia via VEGF-A dependent signaling

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Summary

Islet vascularization is essential for intact islet function and glucose homeostasis. We have previously shown that primary cilia directly regulate insulin secretion. However, it remains unclear whether they are also implicated in islet vascularization. At eight weeks, murine Bbs4−/− islets show significantly lower intra-islet capillary density with enlarged diameters. Transplanted Bbs4−/− islets exhibit delayed re-vascularization and reduced vascular fenestration after engraftment, partially impairing vascular permeability and glucose delivery to β-cells. We identified primary cilia on endothelial cells as the underlying cause of this regulation, via the vascular endothelial growth factor A (VEGF-A)/VEGF receptor 2 (VEGFR2) pathway. In vitro silencing of ciliary genes in endothelial cells disrupts VEGF-A/VEGFR2 internalization and downstream signaling. Consequently, key features of angiogenesis including proliferation and migration are attenuated in human BBS4 silenced endothelial cells. We conclude that endothelial cell primary cilia regulate islet vascularization and vascular barrier function via the VEGF-A/VEGFR2 signaling pathway.

Key words

Islet vascularization, endothelial cell, primary cilium, VEGF-A
**Introduction**

Pancreatic islets rely on their morphologically unique vascular network for functional support and efficient delivery of oxygen and nutrients, as well as rapid dissemination of islet derived hormones. In addition, blood vessels are important to establish microenvironments or “niches” required for stem or progenitor cell maintenance. Proper islet re-vascularization has also been suggested to be a critical determinant in graft survival following islet transplantation in the treatment of Type 1 Diabetes. As the functional barrier between tissues and the blood stream, vascular endothelial cells in both central and peripheral organs of insulin action have been implicated in insulin resistance and diabetes etiology. However, functional assessment of nutrient delivery and insulin disposal across the endothelial barrier has been difficult, since blood vessels are not preserved in vitro after islet isolation, while pancreas is difficult to access in situ with long-term, non-invasive live imaging techniques.

Anatomically, the exocrine pancreas is organized by branching ducts while the endocrine islets of Langerhans are scattered throughout the exocrine tissue and interconnected by blood vessels. During development, pancreatic bud and blood vessel formation are initiated at the same time and, although both endocrine and exocrine pancreatic tissue are derived from the same progenitors, vessel density in the exocrine portion of the organ is five times lower compared to intra-islet vessel density after maturity. Moreover, islet capillaries have ten times more diaphragm-covered fenestrae than the exocrine pancreas- a distinctive feature that facilitates rapid exchange of fluids and small solutes between tissue and blood stream. This suggests that specific local factors play a role in endocrine pancreatic vascularization.

One of the main players in islet vascularization is vascular endothelial growth factor A (VEGF-A)/ VEGF receptor 2 (VEGFR2) dependent signaling. VEGF-A is produced by islet endocrine cells (both α- and β-cells) and binds to VEGFR2 on endothelial cells, which is
crucial for the maintenance of a dense and leaky islet microvasculature\textsuperscript{3,10,11}. While pancreas-specific VEGF-A ablation does not completely block islet vascularization, islet fenestration is crucially dependent on VEGF-A/VEGFR2-dependent signaling. Delivery of nutrients to β-cells and disposal of insulin into the blood vessels is modulated by fenestrated blood vessels that are fine tuning the system to optimize glucose tolerance\textsuperscript{10}. Constitutive, targeted deletion of Vegfa from β-cells at embryonic development revealed a critical role for VEGF-A dependent signals in islet vessel formation, maintenance and function\textsuperscript{3}. β-cell specific Vegfa deficient mice are severely glucose intolerant but show normal glucose response and insulin secretion in isolated islets, suggesting that either nutrients (such as glucose) are not delivered efficiently to the β-cells or insulin disposal into the blood stream is significantly hampered \textit{in vivo}\textsuperscript{3,10}. In contrast, removal of Vegfa from mature β-cells at a later stage has a less severe impact on glucose tolerance although intra-islet capillary density is reduced by 50\%\textsuperscript{12}. This may suggest that, after islet maturation, local VEGF-A/VEGFR2 dependent signaling is less important for the maintenance of intra-islet capillaries and insulin disposal compared to during development. Changing the biomechanical properties such as actomyosin cortex tension of epithelial cells in developing islets by deletion of Integrin-linked kinase (Ilk) specifically blocks intra-islet capillary formation completely and leads to glucose intolerance. Instead, blood vessels accumulate in the islet periphery and form an envelope-like structure suggesting that biomechanics of epithelial tissues are important determinants of blood vessel formation\textsuperscript{13}.

Primary cilia are present on roughly eighty percent of the cells in the adult mammalian organism\textsuperscript{14}. In metabolically active organs, they play a role in sensing metabolic signals and energy homeostasis\textsuperscript{15,16}. We have shown that β-cell cilia play a role in insulin signaling, insulin secretion and glucose homeostasis\textsuperscript{17,18}. Endothelial cells are also ciliated, and endothelial cilia have been implicated in flow-sensing and vascular hypertension,
intracranial blood vessel formation, and atherosclerosis prevention\(^\text{19-22}\). Recent studies further unveiled a novel role of primary cilium in preventing vascular regression\(^\text{23}\). Here we address the role of endothelial cilia in re-vascularization of transplanted islets and regulation of vascular barrier function.

**Results**

*Intra-islet capillary density is reduced in Bbs4\(^{-/-}\) pancreata*

To test if primary cilia play a role in pancreatic islet vascularization, we characterized intra-islet capillaries in pancreatic cryosections of two-month old Bbs4\(^{-/-}\) mice (N=8). Bbs4\(^{-/-}\) mice are a model of Bardet-Biedl Syndrome (OMIM \#209900), a ciliopathy characterized mainly by polydactyly, renal and gonadal malformations and truncal obesity. Bbs4 encodes a protein that is a component of the BBSome complex, a protein super complex thought to be involved in the sorting of membrane proteins to and from the ciliary compartment\(^\text{24,25}\). While Bbs4\(^{-/-}\) mice form primary cilia, they are not fully functional\(^\text{26}\). The symptoms show good overlap with what has been observed in BBS patients and include obesity, male sterility, and impaired glucose handling. Platelet endothelial cell adhesion molecule (PECAM-1) immunofluorescence, as a marker of endothelial cells, revealed 35% reduced intra-islet capillary density compared to wildtype (wt) littermate controls (Fig. 1A,B). To calculate intra-islet capillary density, the relative PECAM-1 positive volume was normalized to insulin positive islet volume (Fig. 1B, \(P=0.0019\)). In addition, the average vessel diameter was increased by 18% compared to that in wt controls (Fig. 1C, \(P<0.0001\)). Because tissue integrity is often compromised in cryopreserved samples, we corroborated our results by whole mount staining and imaging of freshly isolated islets of two-month old mice (Fig. 1D-1F). In good agreement with our previous observations, capillary density was reduced by 34% and vessel diameter increased by 20% in islets of Bbs4\(^{-/-}\) mice (\(P<0.0001\) for both). Of
We observed no change in pericyte coverage of intra-islet capillaries based on Neuron-glial 2 (NG2) immunofluorescence ((Figure 1-figure supplement 1A, 1B, P=0.7045). In four-month old Bbs4<sup>−/−</sup> mice, however, the difference in intra-islet capillary density and diameter in Bbs4<sup>−/−</sup> mice approximated that of wt littermates (Figure 1-figure supplement 1C-1E, P=0.0676 and 0.0736 respectively). There is no evidence of significant changes in β-cell mass or insulin content in Bbs4<sup>−/−</sup> mice, and the observation of only slightly but not statistically significantly larger islets in Bbs4<sup>−/−</sup> mice at 4 weeks of age rules out effects of islet size on vessel density<sup>18</sup>. These dynamics implicate that primary cilia and centrosome/basal body integrity play a role during the vascularization of pancreatic islets. Importantly, there was no detectable difference in vascularization of the exocrine portion of the pancreas at two months of age (Figure 1-figure supplement 1F). Therefore, Bbs4 function seems to be mostly restricted to the endocrine pancreas and not relevant for the exocrine compartment.

Bbs4<sup>−/−</sup> endothelial cells exhibit blunted angiogenic response during islet re-vascularization

To test if cilia play a role in intra-islet capillary formation during engraftment of transplanted islets, we transplanted murine islets into the anterior chamber of the eye (ACE)<sup>27</sup>. This approach allows for longitudinal and non-invasive in vivo monitoring of islet engraftment and re-vascularization. We and others have previously shown that endothelial cells disappear over prolonged periods of cultivation of isolated islets. Immediately after isolation, endothelial cells maintain the intra-islet capillary network. After two days in culture, endothelial cell clusters remain, whereas seven days post isolation, intra-islet endothelial cells are generally lost (Figure 1-figure supplement 2A). Thus, when transplanted shortly after isolation, a significant number of endothelial cells from the donor survives and contributes to revascularization of the islet graft<sup>4,28,29</sup>. Importantly, both islet cells and endothelial cells are ciliated<sup>19,22,30,31</sup>. Therefore, to determine whether the role of primary ciliary/centrosomal
function in islet endothelial or endocrine cells is underlying the reduction in intra-islet capillary density in $\text{Bbs}4^{-/-}$ mice, we capitalized on the temporal differences in the ratio of donor or recipient endothelial cells in regenerated islet capillary network (Figure 1-figure supplement 2B).

In one experimental setting, islets isolated from $\text{Bbs}4^{-/-}$ and wt littermate controls were cultivated for two days before transplantation into the ACE of wt B6 albino recipients (Fig. 1G, Figure 1-figure supplement 2B, upper panel). We determined the rate of re-vascularization by intravenous (i.v.) injection of fluorescently labeled 70 KDa dextran once a week for a total of twelve weeks (Fig. 1G). The percentage of dextran-labeled vascular structure in total islet volume (based on backscatter signal$^{32}$) was used as a measure of islet vascularity, and re-vascularization rate of each islet was calculated by normalizing its dextran-labeled volume at each individual week to the volume at twelve weeks. Two weeks after transplantation, $\text{Bbs}4^{-/-}$ islets showed significantly less re-vascularization compared to wt controls (Fig. 1H). Until four weeks after transplantation, re-vascularization remains lower than that of wt islets (Fig. 1H). Twelve weeks post transplantation, relative intra-islet vascular density is similar in both $\text{Bbs}4^{-/-}$ and wt islets (Fig. 1I). Morphologically, the newly formed blood vessels also differ among the two different genotypes during the first few weeks of engraftment. The vessel diameter is greater in $\text{Bbs}4^{-/-}$ islets, suggesting that there are fewer, wider islet capillaries in these islets compared to the wt controls during the first four weeks of engraftment (Fig. 1J), similar to the results obtained from cryosections of young $\text{Bbs}4^{-/-}$ animals.

In the other experimental setting, islets were kept in culture for seven days after isolation before being transplanted to the ACE of wt recipients. This treatment ablates endothelial cells in the donor tissue and favors almost exclusively revascularization by the recipients’ vascular system (Figure 1-figure supplement 2B, lower panel). We did not observe
significant differences in re-vascularization rate over time or islet vascular density at the
12-week endpoint (Fig. 2A-2C). In addition, vessel morphology and diameter were
similar in Bbs4\(^{-}\) and wt control islets (Fig. 2D). Importantly, under these experimental
conditions, the majority of donor endothelial cells were lost during the seven-day cultivation.
In conclusion, our findings suggest that Bbs4\(^{-}\) endothelial cells underlie the blunted
angiogenic response, thus impeding islet re-vascularization.

To verify our conclusion, we reversed the transplantation strategy by transplanting wt
islets to Bbs4\(^{-}\) recipients either directly after isolation or after keeping them in culture for
two and seven days respectively (Fig. 3). To minimize confounding effects of diabetes on
islet engraftment, we transplanted islets into the eyes of four-month-old Bbs4\(^{-}\) mice that
were obese and glucose intolerant, but not overtly diabetic yet (Fig. 3A-3C). Based on the
apparent role of endothelial primary cilia in the angiogenic response, we expected more
severe impairment in re-vascularization compared to wt recipients. Indeed, we observed
significantly slower re-vascularization in this transplantation scheme and the rates correlated
with the time islets were kept in culture (Fig. 3D, 3E). The re-vascularization rate of wt islets
transplanted to the ACE of Bbs4\(^{-}\) mice seven days post-isolation was significantly lower than
that of wt islets two days post-isolation (P=0.0053), and both were significantly lower than
that of wt islets transplanted to wt ACE (P=0.0002 for two-day group and P<0.0001 for
seven-day group respectively). Interestingly, intra-islet vessel density after completion of re-
vascularization was not significantly lower than that of wt islets transplanted into wt
recipients at the twelve-week endpoint (Fig. 3F). The diameter of intra-islet capillaries,
however, was markedly wider in wt islets transplanted to Bbs4\(^{-}\) recipients after seven days in
culture than the other two groups (Fig. 3G).

To further distinguish donor and recipient endothelial cells in ACE islet grafts and
trace their dynamics during engraftment, we used a tdTomato reporter mouse line (Ai14) that
carries a loxP flanked STOP codon preventing expression of a CAG-driven red fluorescent protein (tdTomato) in the Rosa26 locus. Crossing the floxed allele to an endothelial specific transgenic Cdh5-Cre mouse line rendered all VE-Cadherin expressing-endothelial cells labeled with tdTomato (Cdh5-tdT). Directly after isolation, red fluorescence efficiently labels the intact intra-islet capillary network, which is demonstrated by PECAM-1 staining (Figure 3-figure supplement 1A). As expected, this network is partially preserved when transplanted shortly after isolation, and forms a mosaic neo-vasculature in islet grafts together with recipient endothelial cells (Figure 3-figure supplement 1B, left panel). Twelve weeks post-transplantation, we quantified the surface area ratio of tdTomato-positive structures to the lumen of the blood vessels as marked by fluorescein-labeled dextran and found it to be 41.7% in wt and 45.9% in Bbs4 islets transplanted two days after culture. This is in good agreement with another study, in which freshly transplanted islets were under the kidney capsule; here, the authors observed that donor endothelial cells constituted 40±3% of the newly formed vasculature between three to five weeks post-transplantation. On the contrary, only little tdTomato fluorescence was detected in the Cdh-tdT islets cultivated over seven days, suggesting that re-vascularization is mostly derived from non-fluorescent recipient endothelial cells (Figure 3-figure supplement 1B, right panel). To confirm previous findings and better understand the relative contribution of donor versus recipient endothelial cells during islet graft vascularization, we transplanted Ai14;Cdh5-Cre animals with islets isolated from Bbs4 and wt littermate controls which were cultivated for two days and monitored their re-vascularization (Figure 3-figure supplement 1C). While delayed re-vascularization and altered vascular morphology of Bbs4 islets was observed as before in wt recipients (Figure 3-figure supplement 1D, 1E, Fig. 1H, 1J), the ratio of recipient-derived tdTomato positive endothelial cells stayed significantly higher in Bbs4 islets during the first four weeks of engraftment (Figure 3-figure supplement 1F). Taken together, these results support
the conclusion that $Bbs4^{+/}$ endothelial cells are indeed less responsive to the angiogenic signals than wt endothelial cells.

**Primary cilia of endothelial cells regulate islet re-vascularization**

Because Bbs4 protein might have additional roles in cellular processes unrelated to the basal body, we used two additional mutant mouse models. *Pitchfork* (*Pifo*~*−/−*) mice have dysfunctional cilia that cannot be properly disassembled$^{34}$. *PIFO* does not localize to the ciliary axoneme but accumulates at the base of the cilium during cilia disassembly and interacts with ciliary targeting complexes. Laterality defects in *Pifo*~*−/−* mice suggest that ciliary signaling is impaired in these animals. We determined intra-islet vascular density in three-month-old *Pifo*~*−/−* mice. PECAM-1 staining revealed 19% reduction in intra-islet vascular density and 38% increase in vessel diameter in *Pifo*~*−/−* mice compared to littermate controls, similar to our observation in $Bbs4^{−/−}$ islets (Figure 3-figure supplement 2A-2C, $P=0.0421$ and $P=0.0032$ respectively). Transplantation of *Pifo*~*−/−* islets into wt ACE showed a delay in relative re-vascularization compared to wt islets, also similar to what we observed for $Bbs4^{−/−}$ mice (Figure 3-figure supplement 2D-2G). Although the effects on re-vascularization were less severe than those in $Bbs4^{−/−}$ islets, the findings support a role for primary cilia in islet vascularization and the angiogenic response in islet engraftment.

To further clarify the contributions of β-cell vs. endothelial cilia to the vascularization phenotype and confirm the findings from $Bbs4^{−/−}$ mice, we specifically ablated *Intraflagellar transport 88* (*Ift88*), a core ciliary protein, in β-cells by crossing a floxed *Ift88* mouse line to an inducible cyclic recombinase line under the control of *Pdx1*- promoter. This generates a β-cell specific, inducible ciliary knockout mouse (*Ift88*~*loxP/loxP*; *Pdx1-CreER*) with an efficiency of 50%, that we will refer to as βICKO (pronounced BICKO) from here on$^{17}$(Fig. 4A). *Ift88* is essential to ciliary maintenance and formation, and cells depleted of *Ift88* do not have
ciliary axonemes\textsuperscript{35}, although the early stages of ciliogenesis such as vesicle docking to centrosomes are still completed\textsuperscript{36}. Recombination was induced between postnatal day 25 and 35, and we quantified intra-islet vascular density in Tamoxifen-treated βICKO mice six weeks after induction. We found no observable difference between induced βICKO mice and oil-treated controls (Fig. 4B, 4C). In addition, we isolated βICKO islets two months after Tamoxifen treatment and transplanted them into wt mice after overnight culture. We did not observe differences in the re-vascularization rate between vehicle and Tamoxifen-treated βICKO islets (Fig. 4D-4G). Overall, our observations strongly suggest that cilia in endothelial cells regulate re-vascularization during islet engraftment.

\textit{Intra-islet vasculature plays a role in glucose metabolism}

Besides a significantly higher vascular density than exocrine pancreas, another hallmark of intra-islet capillary are fenestrae as gateways for substance exchange between islets and the blood stream\textsuperscript{9}. Thus, we further examined the barrier function of \textit{Bbs4\textsuperscript{-/-}} islet vessels four months after engraftment, when the intra-islet vascular density is comparable with wt islets. As previously reported, in isolated islets or \textit{Bbs4} depleted MIN6m9 cells, we did not observe a difference in β-cell glucose uptake or glucose metabolism. However, the role of \textit{Bbs4} in islet endothelial cell barrier function remains uncharacterized. Blinded analysis of electron micrographs showed significantly reduced fenestration in the capillaries of \textit{Bbs4\textsuperscript{-/-}} compared to wt islet grafts in the ACE four months after transplantation (Fig. 5A, 5B, 27% lower, P=0.0006). Although the basement membrane seems slightly thicker in \textit{Bbs4\textsuperscript{-/-}} islets, we did not observe significant thickening of this structure (Fig. 5C, P=0.1414). To test if reduced fenestration could affect the permeability and thus barrier function of \textit{Bbs4\textsuperscript{-/-}} islet capillaries, we intravenously injected 40 kDa fluorescently labeled dextran and measured the diffusion of dextran-related fluorescence outside the islet graft over time (inside the dashed area not
including the part of vessel, Fig. 5D). Fluorescently labeled dextran of different sizes were
tested prior to the experiments, and we chose 40 KDa dextran due to its specific leakage
through fenestrated islet vessels instead of surrounding iris vessels (Figure 5-figure
supplement 1). During the time of the experiments, fluorescence intensity immediately
increases outside the islet graft followed by a plateau that is established at comparable times
after dextran injection due to the active drainage of aqueous humor. Fluorescence intensity is
normalized to islet size for better comparison. Importantly, both the initial rate and the
plateau are decreased in Bbs4′ islet grafts (Fig. 5E, P=0.0478). Simulation of this process
also supports different kinetics of dye leakage (Fig. 5F), suggesting that the delivery of
medium sized molecules out of the blood stream is less efficient in Bbs4′ intra-islet
capillaries. Nutrients such as glucose or fatty acids are small molecules that often rely on
active transport across cell membranes. But in the case of highly fenestrated capillaries,
solutes including small molecules, ions and hormones pass freely through the pores between
the blood and luminal surface. To test if glucose delivery to islet cells was affected by the
ultrastructural changes in islet capillaries, we injected fluorescently labeled glucose analog
(2-NBDG) into wt animals transplanted with Bbs4′ and wt islets. 2-NBDG is rapidly
transported into islet cells from intra-islet capillaries and simultaneously leaked into the
surrounding aqueous humor from iris vessels. We followed 2-NBDG uptake by measuring
fluorescence intensity originating from islet cells over time, and normalized it to the
fluorescence intensity originating from the aqueous humor (Fig. 5G), since the 2-NBDG
leaking from iris vessels is determined by the recipient animal and not by the individual eye.
Importantly, wt islet cells internalized significantly more 2-NBDG than Bbs4′ islet cells
under the same period of time (Fig. 5H, P=0.0118). This result points to a critical role of
ciliary/ basal body proteins in maintaining islet capillary fenestration with implications for
the delivery of glucose and potentially other nutrients to islet cells. To investigate the
physiological relevance of our findings, we utilized our transplantation model in combination
with \textit{Bbs4}–or wt islets to better understand how reduced intra-islet capillary density and
permeability may affect islet output and glucose tolerance. It was previously shown that 100
islets (diameter between 150 and 200 μm) transplanted into the ACE of chemically induced
diabetic mice were sufficient to revert blood glucose concentration to normoglycemic levels.
This amount of pancreatic islets is defined as marginal islet mass\textsuperscript{37}. C57Bl/6 J mice were
treated with streptozotocin and subsequently transplanted with marginal islet mass of wt or
\textit{Bbs4}–islets. Four weeks after transplantation, mice transplanted with \textit{Bbs4}–islets were
glucose intolerant compared to those mice transplanted with wt islets (Fig. 5I). Eight weeks
after transplantation, mice showed impaired glucose tolerance and, 12 weeks post-
transplantation, only mildly impaired glucose handling compared to those transplanted with
wt islets (Fig. 5J, 5K). Overall, the phenotype is ameliorated over time, which is consistent
with our previous observations in re-vascularization (Fig. 1, 2), suggesting that a lack of
capillaries in \textit{Bbs4}–islets indeed negatively affects whole body glucose homeostasis.

Glucose handling is mildly impaired by the end of 12 weeks, which is probably caused by
impaired vascular barrier function in combination with defective 1\textsuperscript{st} phase insulin secretion\textsuperscript{18}.

\textit{Impaired VEGFA-VEGFR2 dependent signaling in Bbs4-depleted endothelial cells}

We have observed a role for endothelial cilia in the islet vascularization process; the signaling
pathways involved in this process, however, remained elusive. To complement the \textit{in vivo}
data and to better understand the cell-autonomous role of ciliary/basal body proteins in
endothelial cells, we used a murine pancreatic endothelial cell line, MS-1. Suppressing \textit{Bbs4}
by RNA interference reduces \textit{Bbs4} expression up to two-fold (Figure 6-figure supplement
1A). Expression analysis of key angiogenic factors revealed no observable changes in Tie1,
Tie2, endothelial Nitric oxide synthase (eNOS) or Notch ligand Delta-4 (Dll4) (Fig. 6A).
However, VEGFR2, Ephrin A1 and B2 are downregulated by 20% when MS-1 cells are depleted of Bbs4. Interestingly, Bbs4 depletion has no effect on PECAM-1 expression and therefore does not change the characteristic properties of this endothelial cell line. Of note, intra-islet capillary density and fenestration is primarily maintained by VEGF-VEGFR2-signaling\textsuperscript{10,38,39}. 

Angiogenesis is not exclusively regulated on the transcriptional level but also relies on a complex network of signaling cascades. Therefore, we tested if VEGFR2-dependent phospho-activation of downstream signaling molecules, including Phospholipase C\textgamma{}1 (PLC\textgamma{}1), Protein Kinase B (Akt) and Mitogen-activated protein kinase (MAPK 42/44) were affected. We used primary human endothelial cells HDMEC stably transduced with lentivirus encoding shRNA targeting \textit{BBS4}, and tested before (t=0), seven and fifteen minutes after VEGF-A addition (Fig. 6B-6D, Suppl Fig. 6B, 6C). In control cells (scrambled), VEGFR2 Tyr 1175 phosphorylation is markedly increased at seven minutes (4.3-fold) and sustained until 15 minutes (3.4-fold) after stimulation (Fig. 6B, 6D). By comparison, pTyr1175 levels increased minimally in \textit{BBS4}-depleted HDMECs (2-fold and 1.8-fold respectively, Fig. 6B, 6D). At the same time, total levels of VEGFR2 are also lowered in absence of Bbs4 by 20% (Fig. 6B, Figure 6-figure supplement 1B, P=0.0101). In addition, we found that EphrinB2 protein level is slightly reduced in \textit{BBS4}-depleted HDMECs (Fig. 6B, Figure 6-figure supplement 1B, 15%, P=0.0051), corroborating our previous finding of decreased gene expression (Fig. 6A). While PLC\textgamma{}1 phosphorylation peaks at seven minutes and decreases fifteen minutes after stimulation in control cells, loss of Bbs4 leads to a 40% attenuation in PLC\textgamma{}1 phosphorylation at seven minutes (Fig. 6C, Figure 6-figure supplement 1C, P=0.0336). Similarly, pAkt and pMAPK 42/44 were significantly reduced in \textit{BBS4}-depleted cells seven and fifteen minutes after VEGF-A addition (Fig. 6C, Figure 6-figure supplement 1C, P=0.0222 and P=0.0010 respectively). These \textit{in vitro} experiments are suggestive but do
not necessarily reflect *in vivo* processes of islet vascularization. Therefore, we further validated these results in freshly isolated islets from wt and $Bbs4^{-/}$ animals, which still contain endothelial cells (Figure 6-figure supplement 1D). In unstimulated islet cells, pAkt and pMAPK baseline levels did not differ between wt and $Bbs4^{-/}$ cells. Ten minutes after addition of 100 ng/ml VEGF-A, pAkt levels increased 2.2-fold in wt but only 1.4-fold in $Bbs4^{-/}$ cells. Likewise, pMAPK levels increased 3-fold in wt but only 1.5-fold in $Bbs4^{-/}$ cells, demonstrating that VEGF-A triggered downstream signaling is not sufficiently activated in these cells (Figure 6-figure supplement 1D, 1E). Importantly, similar experiments on wt islets cultured for 7d to diminish intra-islet endothelial cells showed markedly lower response to VEGF-A stimulation; therefore, VEGF-A/ VEGFR2 dependent signaling cascades in endothelial cells contribute significantly to overall VEGF-A dependent signaling in whole murine islets (Figure 6-figure supplement 1F, 1G).

Binding of VEGF-A to receptors triggers their endocytosis instantly, which is the initial step for subsequent intracellular signaling, and mainly regulated by EphrinB2\textsuperscript{40-42}. Several studies have shown that VEGFR2 does not signal from the plasma membrane but from other intracellular compartments, mainly early endosomes and the endosomal sorting complex required for transport (ESCRT)\textsuperscript{42,43}. The observed attenuation of VEGFR2 downstream signaling may stem from dysregulation of these initial steps. Therefore, we examined the uptake of biotinylated VEGF-A in $BBS4$-depleted HDMECs. After 30 min incubation, $BBS4$-depleted cells had only internalized 45% VEGF-A compared to controls (Fig. 6E, 6F, $P=0.0101$). We also tested VEGF-A uptake dynamics in a pancreatic endothelial cell line, MS-1 cells. Similar to our observations in HDMECs, control MS-1 cells efficiently and rapidly internalized VEGF-A, while by comparison, $Ift88$-depleted MS-1 cells internalized less VEGF-A after 30 min (61% compared to controls, Figure 6-figure supplement 1H-1J, $P=0.0027$). We further tested if VEGFR2 internalization was impaired in
BBS4-depleted HDMECs (Fig. 6G). Plasma membrane bound VEGFR2 was evident prior to VEGF-A stimulation in both control and BBS4-depleted HDMECs. After ten min incubation with VEGF-A, a significant number of VEGFR2 was internalized in control cells, while 25% more VEGFR2 remained on the cell surface in BBS4-silenced cells, which indicates decreased internalization of VEGFR2 (Fig. 6H, P=0.0019) and may be explained by our previous finding of lowered EphrinB2 levels (Fig. 6A, 6B). These results suggest that the VEGFR2 internalization upon ligand binding is regulated by ciliary and basal body proteins.

VEGF-A signaling triggers a complex array of biological processes including endothelial cell proliferation and migration, two characteristic processes of angiogenesis. We tested the effect of Bbs4-depletion on proliferation by ethynyldeoxyuridine (EdU) incorporation in MS-1 cells. Under the chosen conditions, VEGF-A induced cell proliferation in Bbs4-depleted cells was reduced by 27% compared to controls cells (Fig. 6I, 6J, P=0.0024). A scratch assay revealed that Bbs4-depleted cells covered only 73% of the distance of control cells in the same time frame (Fig. 6K, P<0.0001), similar to previous reports of ciliary involvement in cell migration. These independent lines of evidence, i.e. reduced endothelial cell proliferation, migration, and impaired vessel fenestration, all point to a role for cilia and basal body proteins in VEGF-A/ VEGFR2-dependent regulation of endothelial cell function.

An important negative regulator of VEGF-A dependent signaling is Notch1 – dependent signaling. Therefore, we tested the ability of Ift88 depleted pancreatic endothelial cells to respond to Notch1- dependent signaling (Figure 6-figure supplement 1K). Placing firefly luciferase under the control of multimerized CSL response elements upstream of a minimal promoter showed that the Notch1 intracellular domain (NICD) elicited a weaker response in Ift88 depleted cells compared to controls. We did not observe different responses in the absence of NICD suggesting that the phenotype was specific to Notch1- dependent
signaling. Because Notch1 signaling is a negative regulator of VEGF-A dependent signaling, loss of Notch1 signaling cannot explain the observed phenotype.

We also examined the relative expression of the same set of angiogenic factors in freshly isolated islets and found, while most expression levels remain unchanged, VEGF-A expression was reduced by 20% in \textit{Bbs4}\textsuperscript{-/-} islets compared to wt controls. \textit{Von-Hippel-Lindau} gene expression, a known ciliary protein mutated in Von Hippel-Lindau disease and a known regulator of Hypoxia induced factor 1a, remains unchanged (Figure 6-figure supplement 1L). VEGF-A gene expression was also slightly decreased in \(\beta\text{ICKO}\) islets (Figure 6-figure supplement 1M). To test if this reduction was physiologically relevant, we tested VEGF-A secretion in \(\textit{Bbs4}\textsuperscript{-/-}\) and \(\beta\text{ICKO}\) islets. Loss of Bbs4 or Ift88 function respectively decreased VEGF-A secretion by 33% and 28% (Figure 6-figure supplement 1N, \(P=0.0063\) and Figure 6-figure supplement 1O, \(P=0.0070\)), which could be explained by the reduction in VEGF-A gene expression. Altogether, these results consistently suggest that the intra-islet VEGF-A signaling pathway is impaired in \(\textit{Bbs4}\textsuperscript{-/-}\) islets.

**Discussion**

Several recent studies have addressed the role of primary cilia in endothelial cell function\textsuperscript{20-23}. However, the precise role of primary cilia in islet vascularization remains unclear. Here, we reveal the role of the ciliary/ basal body machinery in islet vascularization during islet islet engraftment upon transplantation. \(\textit{Bbs4}\textsuperscript{-/-}\) islets exhibited lower intra-islet capillary density and enlarged capillaries at two months of age. Similarly, \(\textit{Pifo}\textsuperscript{-/-}\) islets also have a reduced intra-islet capillary density and increased vessel diameter compared to littermate controls at the same age. This suggests that the observed defects are probably due to dysfunctional cilia and not related to other, non-ciliary functions of \(\textit{Bbs4}\). Of note, although Bbs4, Pifo and Ift88 are linked to ciliary function, their individual roles do not
overlap precisely. Bbs4 localizes to the BBSome complex at the basal body and is involved in sorting of proteins to and from the ciliary compartment, but cilia still form. By comparison, Pifo mediates Aurora Kinase A (AurA)-dependent cilium disassembly resulting in persistent ciliation and bifurcation of cilia. In addition, cilia function is impaired as evidenced by laterality defects in Pifo<sup>−/−</sup> mice<sup>34</sup>. By contrast, Ift88 depletion ablates all cilia. Importantly, though, vesicle docking still occurs<sup>36</sup>, resulting in an abnormal cell that should not be interpreted as a wildtype, unciliated cell. Phenotypic variations could be explained by these different roles, but the fact that they are overlapping strongly implicates the cilia/ basal machinery in endothelial function.

Previous work has shown hyperglycaemia and elevated circulating insulin levels in Bbs4<sup>−/−</sup> mice at 4-6 months of age. This suggests increased insulin demand and thus insulin resistance, which could potentially affect islet vascularity. Indeed, studies with three independent insulin resistance mouse models showed that intra-islet vessel area significantly increased, primarily due to morphological changes, namely vessel dilation but not angiogenesis<sup>45</sup>. Similarly, studies in Zucker diabetic fatty rats (ZDF) also demonstrated that early stages of insulin resistance and islet hyperplasia are accompanied by hypervascularization<sup>46</sup>. Of note, Bbs4<sup>−/−</sup> mice only become significantly overweight at 3-4 months of age, while we observe changes in islet vascularization at two months of age. We believe that we can exclude confounding effects of increasing body weight and insulin resistance on islet vascularization at the time of our investigation because the intra-islet capillary density is decreased, and not increased as is the case for models of insulin resistance.

Islet vascularization is typically regulated by paracrine signaling between endocrine cells (including α-, β- and δ-cells) and endothelial cells in the tissue surrounding the islet. Islet transplantation into the ACE and subsequent non-invasive and longitudinal monitoring
of re-vascularization recapitulated this phenotype. In addition, this approach allows us to observe islets depleted of Bbs4 in a wildtype environment similar to targeted knockout islets. Given the problems with β-cell or islet specific Cre lines such as leakiness\(^{47}\) or activity in the nervous system\(^{48}\) our approach avoids these confounding factors. On the other hand, removing cilia from all endothelial cells also makes it difficult to exclude secondary, indirect effects that stem from endothelial cells in another tissue. In our experimental setup, both control and Bbs4\(^{-/-}\) islets are transplanted into the eyes of the wt animal, and vice versa, which minimizes the variability between different recipient animals and therefore makes it ideal for comparison.

Several lines of evidence suggest that the observed phenotype is largely driven by impaired ciliary and basal body function in endothelial cells. First, Bbs4\(^{-/-}\) islets that were transplanted two days after isolation were re-vascularized at a lower rate than those transplanted after seven days. Prolonged islet cultivation depletes donor endothelial cells (in this case Bbs4\(^{-/-}\) endothelial cells). Bbs4\(^{-/-}\) islet endothelial cells are less efficient at islet revascularization compared to wt endothelial cells of the recipient. Second, wt islets transplanted into the ACE of Bbs4\(^{-/-}\) mice were more slowly re-vascularized when transplanted after seven days compared to two days of tissue culture. The former condition shifts the composition of the islet vasculature toward a higher proportion of ciliary/basal body deficient Bbs4\(^{-/-}\) endothelial cells, which are less responsive to the angiogenic demands than wt endothelial cells. Third, native and transplanted βICKO islets (β-cell specific Ift88 depletion) show no obvious defect in capillary density, diameter or revascularization rate. Although we cannot exclude a minor contribution by reduced VEGF-A secretion from both Bbs4\(^{-/-}\) and βICKO islets, the effect on islet vascularization is likely minimal and mostly compensated for by circulating VEGF-A. Islet vascularization is in part modulated by changing the actomyosin cortex of islet cells; while we have shown EMT-like polarization...
defects in β-cells depleted of Ift88, we did not observe a complete loss of intra-islet capillary formation nor an enveloping structure of blood vessels surrounding the islets in βICKO mice\textsuperscript{13,17}. Similarly, primary cilia are also present on some α- and δ-cells of the murine pancreatic islet\textsuperscript{30}; we cannot fully exclude that they might play a role in islet re-vascularization, and that this might explain the difference between the findings in global knockout models \textit{Bbs4}\textsuperscript{-/-} and \textit{Pifo}\textsuperscript{-/-} or βICKO respectively. Because previous work has shown that β-cell VEGF-A secretion is responsible for the bulk of islet vascularization we believe that α-cell cilia are unlikely key denominators of islet vascularization. Here we suggest that there is an important, albeit not exclusive role for the endothelial ciliary/ basal body apparatus in islet vascularization.

VEGF-A/ VEGFR2 is a key signaling pathway for maintaining the especially dense and leaky islet microvasculature. Our results suggest that dysfunctional primary cilia alter the VEGF-A/ VEGFR2 paracrine signaling activity in \textit{Bbs4}\textsuperscript{-/-} islets by impairing internalization of ligand-bound VEGFR2 receptor. VEGF-A/ VEGFR2 internalization and subsequent phosphorylation is disrupted in endothelial cells depleted of \textit{BBS4} and \textit{Ift88}, respectively. Several downstream signaling components are insufficiently activated in good agreement with previous observations. We conclude that defective VEGF-A/ VEGFR2 dependent signaling in endothelial cells is the underlying cause of the observed phenotypes, namely reduced intra-islet capillary density and impaired fenestration of intra-islet micro-vessels. However, we cannot rule out a possible role of the Notch1 signaling pathway in the observed vascular phenotypes, although we did not detect significant changes in Dll4 expression in \textit{Bbs4}\textsuperscript{-/-} islets. Extensive studies are required to further pinpoint the involvement of other angiogenic signals which could also be affected by cilia dysfunction.

Intra-islet capillary networks provide endocrine cells with nutrients, oxygen and growth factors which support the development and maturation of islets. In addition, the
microvasculature constantly adapts its barrier properties to the dynamic metabolic rates. Finely tuned interplay between nutrient delivery to islet endocrine cells, endocrine cell function and insulin disposal into the blood stream is essential for the maintenance of glucose homeostasis. Whereas intra-islet capillary density normalizes over time in $Bbs4^{-/-}$ islets, vessel function remains impaired when cilia/basal function is compromised more than four months after transplantation. Blood vessel fenestration is significantly reduced in $Bbs4^{-/-}$ islets. Fenestrae are key features of islet capillaries that enable efficient nutrient delivery from and insulin disposal into blood vessels. In vivo 2-NBDG diffusion is less efficient and leakage of 40 KDa dextran diminished when $Bbs4$ is deleted from intra-islet capillaries. With respect to glucose handling, decreased vessel permeability in $Bbs4^{-/-}$ islets could impose an additional barrier on insulin disposal, adding to the observed defects in 1st phase insulin secretion. We thus suggest that defective delivery of nutrients such as glucose to β-cells and the disposal of insulin from β-cells into the blood stream may contribute to impaired glucose handling of $Bbs4^{-/-}$ mice.

Functional imaging of micro-vessels remains challenging and is largely limited to easily accessible tissues such as cornea and skin. Transplantation of islets into the ACE not only renders established intra-islet capillaries more accessible for standard imaging techniques but also provides the opportunity to follow dynamic processes such as islet re-vascularization under physiological as well as pathological conditions. This technique may also prove to be a useful resource for further investigations into blood vessel function in other tissues when transplanted into the ACE.

Finally, re-vascularization and establishing a functional interface between islet cells and blood supply are not only important in the context of islet transplantation but will likely prove vital for all approaches to β-cell replacement therapy. Moreover, considering the scarcity of organ donations worldwide, it is of paramount importance to optimize the
outcomes of organ transplants. Revascularization rates in liver transplants correlate with long
term graft survival, renal function and early allograft dysfunction. Understanding the
signaling role of primary cilia in this process provides us with novel targets to improve tissue
re-vascularization and hence graft survival.

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Materials and Methods

| Key Resources Table |
|---------------------|
| **Reagent type (species) or resource** | **Designation** | **Source or reference** | **Identifiers** | **Additional information** |
|---------------------|-----------------|------------------------|----------------|--------------------------|
| Cell line (Homo sapiens) | HEK 293FT | Thermo Fisher Scientific | # R70007 RRID:CVCL_6911 | Human embryonic kidney |
| Cell line (Homo sapiens) | HDMEC | Promocell | C-12212 | Primary human dermal microvascular |
| Cell line (Mus musculus) | Antibody Details | Antibody Details | Antibody Details |
|--------------------------|------------------|------------------|------------------|
| MS-1                     | Goat polyclonal anti-PECAM-1 | R&D systems | AF3628 (IF 1:400) |
| RRID:CVCL_D134           |                  |                  |                  |
| Pancreas/islet of Langerhans; endothelium |                  |                  |                  |
| RRID:CVCL_D134           |                  |                  |                  |
| Rabbit polyclonal anti-NG2 | Merck | AB5320 | (IF 1:200) |
| Rabbit polyclonal anti-BBS4 | Proteintech | 12766-1-AP | (WB 1:1000) |
| Rabbit polyclonal anti-EphrinB2 | Thermo Fisher Scientific | BS-10659R | (WB 1:500) |
| Rabbit monoclonal anti-phospho-VEGFR2 Y1175 | Cell Signaling Technology | 2478 | (WB 1:1000) |
| Rabbit monoclonal anti-PLCγ1 Y783 | Cell Signaling Technology | 2821 | (WB 1:1000) |
| Rabbit monoclonal anti-PLCγ1 | Cell Signaling Technology | 2822 | (WB 1:1000) |
| antibody                  | Cell Signaling Technology | 4051 | (WB 1:1000) |
|--------------------------|---------------------------|------|-------------|
| Rabbit monoclonal anti-phospho-Akt Thr308/473 | Cell Signaling Technology | 4691 | (WB 1:1000) |
| Rabbit monoclonal anti-pan Akt | Cell Signaling Technology | 4370 | (WB 1:2000) |
| Rabbit monoclonal anti-phospho-p44/42 MAPK Thr202/Tyr204 | Cell Signaling Technology | 4695 | (WB 1:2000) |
| Mouse monoclonal anti-α-tubulin | Sigma-Aldrich | T8203 | (WB 1:2000) |
| Donkey monoclonal anti-Rabbit IgG, peroxidase-linked species-specific whole antibody Secondary Antibody | GE Healthcare | 10794347 | (WB 1:6000) |
| Sheep monoclonal anti-mouse IgG, peroxidase-linked species-specific whole antibody Secondary Antibody | GE Healthcare | 10196124 | (WB 1:6000) |
| Category                  | Description                                                                 | Supplier            | Catalog Number | Concentration          |
|---------------------------|------------------------------------------------------------------------------|---------------------|----------------|------------------------|
| peptide, recombinant      | Recombinant Human VEGF 165                                                   | Peprotech           | 100-20         | 100 µg/ml in 1XPBS     |
| protein                   | Reombinant Human VEGF 165, Biotinylated Protein                              | R&D systems         | BT293          | 100 µg/ml in 1XPBS     |
| commercial assay or kit   | Mouse VEGF Quantikine ELISA Kit                                              | R&D systems         | MMV00          |                        |
| commercial assay or kit   | PowerUp™ SYBR™ Green Master Mix                                              | Thermo Fisher       | A25742         |                        |
| commercial assay or kit   | Dextran, Texas Red™, 70,000 MW, Neutral                                       | Thermo Fisher       | D1830          | 5 mg/ml in 1X PBS      |
| commercial assay or kit   | Dextran, Fluorescein, 40,000 MW, Anionic                                      | Thermo Fisher       | D1844          | 2.5 mg/ml in 1X PBS    |
| commercial assay or kit   | Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 488 dye       | Thermo Fisher       | C10337         |                        |
| chemical compound, drug   | 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose)      | Thermo Fisher       | N13195         | 5 mg/ml in 1X PBS      |
| chemical compound, drug   | Streptozotocin                                                               | Sigma-Aldrich       | S0130-50MG     |                        |
Animal models

Experimental procedures involving live animals were carried out in accordance with animal welfare regulations and with approval of the Regierung Oberbayern (Az 55.2-1-54-2532-187-15 and ROB-55.2-2532.Vet_02-14-157) or in accordance with the Karolinska Institutet’s guidelines for the care and use of animals in research, and were approved by the institute’s Animal Ethics Committee respectively (Ethical permit number 19462-2017). C57Bl/6J B6 albino and C57B1/6J mice were obtained from The Jackson Laboratory (Maine, USA). Ai14 mice and Cdh5-Cre mice were obtained from the Jackson laboratory (stock number 007914 and 017968). Bbs4\(^{-/+}\) mice were generated in the Lupski lab and backcrossed to C57Bl/6J\(^{34}\). Heterozygous offspring were crossed to produce homozygous Bbs4\(^{-/-}\) and littermate control. Pifo\(^{wt/-}\) mice were crossed to produce homozygous Pifo\(^{-/-}\) mice and littermate controls\(^{34}\). βlCKO animals were generated by crossing Pdx1-CreER mice\(^{55}\) with Ifit8\(^{loxP/loxP}\) mice\(^{17,35}\).

Islet isolation and transplantation

Islets were isolated by cannulation of the common bile duct of donor animals and infusion of 2.5ml collagenase P, 1.0 mg/ml in HBSS containing 25mM HEPES and 0.2% BSA (Sigma-Aldrich, USA). Inflated pancreata were dissected out and digested at 37℃ for 10 min, and cultured in RPMI 1640 Medium (11mM D-glucose) supplemented with 10% fetal bovine
serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM L-Glutamine (all from Thermo Fisher Scientific, USA). Transplanted islets in all experimental setups have an average diameter of 200 μm.

4-month-old female Bbs4/- mice and their wildtype littermates were used as islet donors. 3-4-month-old female mice were used as recipients in all the in vivo transplantations except for Bbs4/- animals (Fig 3D-3G, 4 males and 1 female knockout animals were used as recipients). In all experiment setups, wt and Bbs4/- islets were transplanted into each eye of the same recipient mouse to minimize the variations caused by different recipients. Similarly, wt islets cultivated for different periods were transplanted into each eye of the same Bbs4/- recipient for comparison. For transplantation, recipient mice were anesthetized with 2% isoflurane (Baxter, USA) and fixed with a custom-made head holder (Narishige, Japan) as described previously. A small incision was made in the cornea with a 25 G needle and a glass cannula containing islets was inserted through the opening into the anterior chamber of the eye. Around 4-8 islets were carefully positioned on the iris around the pupil. Post-operative analgesia was done by subcutaneous injection of 2 μg Temgesic (RB Pharmaceuticals Limited, UK).

Streptozotocin treatment and glucose tolerance test

For Streptozotocin (STZ, Sigma-Aldrich, USA) treatment, 4-month-old female C57BL/6J B6 albino mice were fasted for 6 hours and a single dose of 200 mg STZ per kg body weight in phosphate buffered saline (PBS, Thermo Fisher Scientific, USA) was injected intraperitoneally. For glucose tolerance tests, animals were fasted for 6 hours and 2 g D-glucose (20% solution in PBS+/+) per kg body weight was injected intraperitoneally. Blood glucose values were measured afterwards at specified time points using an Accu-Chek Aviva system (Roche, Switzerland).
In vivo imaging of islet grafts

Imaging was performed between one to twelve weeks post transplantation by confocal microscopy. Images of islet grafts were obtained by a Leica SP5 system with 25× objective (N.A.0.95, Leica Microsystems, Germany). Viscotears (Laboratoires Théa, France) was used as an immersion medium between the lens and the mouse eyes. For visualization of islet vascularization, animals were anesthetized with isoflurane (Baxter, USA). 100 μl of PBS solution containing 5 mg/ml of 70-kDa Texas Red-conjugated dextran (Thermo Fisher Scientific, USA) was injected intravenously prior to imaging. Z-stacks of 2 μm thickness were acquired for every islet graft (Ex.: 561 & 594nm, Em.: 558-564 nm & 600-700 nm). For quantification of vascular leakage, animals were anesthetized with a mixture of 1:1 Hypnorm and midazolam (Roche, Switzerland). 100 μl of PBS solution containing 2.5 mg/ml of 40-kDa FITC-conjugated dextran (Thermo Fisher Scientific, USA) was injected intravenously prior to imaging. Time series of z-stacks (2 μm) were acquired every second (Ex.: 488 & 633nm, Em.: 500-550 nm & 630-636 nm). For monitoring the uptake of glucose analogue, animals were anesthetized with a mixture of 1:1 Hypnorm and midazolam (Roche, Switzerland). 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose (2-NBDG, Thermo Fisher Scientific, USA) was dissolved in PBS at 5 mg/ml. Recipient mice were anesthetized and a tail vein catheter containing 50 μl 2-NBDG solution was installed. Time series of z-stacks (2 μm) were acquired every second (Ex.: 488 & 633nm, Em.: 500-600nm & 630-636nm) and 2-NBDG solution was injected during imaging.

Image analysis

Unprocessed original images were used in all quantifications of in vivo re-vascularization. Graft volume was estimated from backscatter signals using Volocity image analysis software.
Dextran-labeled islet capillaries were identified at different time points, using the same thresholds for all groups at all times, and structures smaller than 10 \( \mu m^3 \) were automatically excluded. Their diameters and volumes were calculated using Volocity. Re-vascularization rate at 1 to 8 weeks post transplantation was calculated as the percentage of islet vascular volume at each individual time point to that of the same islet at 12-week end point. Relative vascularity of each islet graft at 12 weeks post transplantation was calculated from dividing the volume of islet vessels by the estimated graft volume. For FITC-dextran and 2-NBDG leakage analysis, images were first movement corrected in MATLAB (MathWorks, USA). Afterwards, time lapse confocal image stacks were analyzed and fluorescence intensity was quantified using Fiji with the plugin Time Series Analyzer V3. Islet cells were identified by backscatter signals. The simulation of in vivo leakage kinetics was done in GraphPad Prism using the model of plateau followed by one phase association.

Image analysis for histological sections were performed with Volocity. The region of interest per stack (ROI) was drawed according to insulin staining. The surface area and volume of PECAM-1, NG2, and insulin-positive structures were calculated using Volocity. Islet capillary density was obtained by counting the PECAM-1-positive volume and dividing it by the insulin-positive volume enclosed in this region. Pericyte coverage was calculated by counting NG2-positive surface area and dividing it by PECAM-1-positive surface area in the same insulin-positive region. Vessel diameters were taken from 6-10 randomly chosen segments per islet.

**Cell culture**

HEK 293FT cells were purchased from Thermo Fisher Scientific (USA). MS-1 cells were purchased from ATCC (USA). Human Dermal Microvascular Endothelial Cells (HDMECs) were purchased from PromoCell (Germany). HEK293FT cells were cultured in Dulbecco’s...
Modified Eagle’s Medium (DMEM) supplemented with 2 mM L-Glutamine and 10% fetal bovine serum (all from Thermo Fisher Scientific, USA). MS-1 cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 2 mM L-Glutamine and 5% fetal bovine serum. HDMECs were cultured in Endothelial Cell Growth Medium MV2 with Endothelial Cell Growth Supplement (PromoCell, Germany). All cells have been tested negative for mycoplasma contamination. The identities of HEK 293FT and MS-1 haven’t been re-authenticated, but they have been used within limited passages upon purchasing.

Construction of lentiviral vectors

We used a commercial lentiviral system from Sigma-Aldrich for designing and constructing shRNA containing lentiviral vectors. Two verified sequences (*Mus musculus*) and one verified sequence (*homo sapiens*) per gene of interest (*Mus musculus*) were selected from MISSION shRNA database (https://www.sigmaaldrich.com/life-sciences/herbal-genomics-and-rnai/shRNA/individual-genes.html). For *Bbs4*: 5’-CCTTGTATTAAGAACCTAG-3’ (shBbs4-1) and 5’-GCATGACCTGACTTACATAAT-3’ (shBbs4-2). For *Ift88*: 5’-TCAGATGCCATCAACTCATTT-3’ (shIft88-1) and 5’-CTATGAGTACCATACAGGTATTT-3’ (shIft88-2). For human *Bbs4*: 5’-TAGTCCTCAGAGTGCTGATAA-3’. Control scrambled sequence: 5’-CCTAAGGTTAAGTGCCTCG-3’. The corresponding oligonucleotides were cloned into pLKO.1-puro vector (Sigma-Aldrich, USA) according to manufacturer’s instructions. The efficacy was assessed by relative level of message RNA by qPCR. For reasons of clarity, we refer to more effective shRNA construct shBbs4-1 as shBbs4 and shIft88-1 as shIft88 in the text.

Lentiviral production and endothelial cell transduction
Lentivirus was produced in 293FT cells and harvested following the manufacturer’s guidelines (https://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/trc-shrna-products/lentiviral-packaging-mix.html). 1×10^6 MS-1 or 2×10^5 HDMEC cells were plated in 25 cm^2 flasks and 1 ml of crude virus containing medium was used for transduction. Transduced cells were further screened in 1.0 μg/ml puromycin (Sigma-Aldrich, USA) for a week before use.

**VEGF-A uptake**

1×10^5 transduced HDMECs or 5×10^5 MS-1 cells were plated on coverslips in 6 well plates. Confluent cells were starved in DMEM supplemented with 0.5% BSA for 6 hours, and incubated with 50 ng/ml biotinylated VEGF-A (R&D Systems, USA) for 30 min at 37ºC. Treated cells were subsequently fixed in 4% paraformaldehyde solution at room temperature for 15 min and permeabilized in 0.1% Triton-X (Sigma-Aldrich, USA) in PBS for 10 min. Fixed cells were then washed 3 times in PBS and blocked with 2% BSA in PBS for 1 hour. To detect the internalized biotinylated VEGF-A, cells were further incubated with Streptavidin- FITC conjugate (1:1000, Thermo Fisher Scientific, USA) at room temperature for 1 hour.

**VEGFR-2 internalization**

1×10^5 transduced HDMECs were plated on coverslips in 6 well plates. Confluent cells were starved overnight in DMEM supplemented with 1% BSA. Starved cells were gently washed twice with PBS, and stimulated with 50 ng/ml human VEGF-A (Peprotech, Sweden) for 10 min at 37ºC. VEGF-A containing medium was quickly removed and cells were washed twice in ice-cold PBS. Subsequently, cells were fixed in 4% paraformaldehyde solution at room temperature for 15 min, and blocked with 2% BSA in PBS for 1 hour without
permeabilization. Further incubation steps with primary and secondary antibodies were described as below.

VEGF-A ELISA

Isolated islets from control and Bbs4−/− animals were cultured overnight. 30 islets from each group were picked into 4 well plates containing 450 µl culture medium per well. 72 hours later, supernatants were collected and stored at -80°C. Islets were collected and lysed in 50 µl M-PER buffer (Thermo Fisher Scientific, USA), for quantification of DNA contents by Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, USA) later according to manufacturer’s instructions. Secreted VEGF-A in the supernatants was measured with mouse VEGF Quantikine ELISA Kit (R&D Systems, USA), according to manufacturer’s instructions.

EdU labeling

Cell proliferation was detected with Click-iT™ EdU Alexa Fluor™ 488 Imaging Kit (Thermo Fisher Scientific, USA). Briefly, 2×10^5 transduced MS-1 cells were plated on coverslips in 6 well plates. After attachment, cells were starved overnight in DMEM supplemented with 1% BSA, gently washed twice with PBS, and further incubated with 50 ng/ml mouse VEGF-A (Peprotech, Sweden) and 10 µM EdU for 4 hours. Treated cells were fixed in 4% paraformaldehyde solution at room temperature for 15 min and EdU detection was carried out according to manufacturer’s instructions.

Endothelial cell migration assay

2.5×10^5 transduced MS-1 cells were plated in 12 well plates. Confluent cells were washed gently with PBS, and incubated with 50 ng/ml VEGF-A in DMEM supplemented with 1%
BSA. A scratch was made with 1 ml tip in the middle of each well, cells were washed again with PBS, and images were acquired for documentation of the initial distances between the separated cells. 18 hours later, cells were fixed in 4% paraformaldehyde solution at room temperature for 15 min, and the same areas were photographed again for calculating the distances of migration in individual wells. Images were acquired with BD Pathway 855 Bioimaging Systems using a Olympus 10x objective (BD, USA).

**Notch1 activity assay**

3×10⁴ transduced MS-1 cells were plated in 96 well plates for transfection of Notch1 reporter. Notch1 signaling pathway activity was detected according to manufacturer’s instructions (BPS Bioscience, #60509).

**Transmission electron microscopy (TEM)**

Islet grafts were dissected out from euthanized animals which have been transplanted previously, and peri-islet iris tissues were removed carefully. Grafts were fixed in 2.5% glutaraldehyde+1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 at 4 °C overnight. Samples were further washed in 0.1 M phosphate buffer, pH 7.4 and post-fixed in 2% osmium tetroxide 0.1 M phosphate buffer, pH 7.4 at 4 °C for two hours, dehydrated in ethanol and acetone, and embedded in LX-112 (Ladd Research Industries, Burlington, USA).

Ultra-thin sections of 50–60 nm were cut by Leica EM UC 6 (Leica Microsystems, Germany) and contrasted with uranyl acetate, followed by lead citrate treatment and checked in Tecnai 12 Spirit Bio TWIN transmission electron microscope (FEI company, The Netherlands) at 100 kV. Digital images were acquired by Veleta camera (Olympus Soft Imaging Solutions, Germany).
Pancreatic sections and immunofluorescent staining

Pancreata were swiftly removed from euthanized animals, rinsed in PBS and fixed in 4% paraformaldehyde in PBS at room temperature for 2 hours. Tissues were then washed with PBS twice and transferred stepwise to 10%, 20% and eventually 30% sucrose in PBS. Tissues were then embedded in O.C.T. freezing medium (Thermo Fisher Scientific, USA) at -80 ºC and subsequently sectioned into 20 µm thick sections.

Cells and islets were fixed in 4% paraformaldehyde solution at room temperature for 15 min or 1 hour, followed by permeabilization in 0.1% Triton-X in PBS, and blocking with 2% BSA or 10% goat serum in PBS respectively. Samples were incubated with primary antibodies, including anti-PEACAM-1 antibody (goat, 1:400, R&D Systems, USA), purified rat anti-mouse CD31 antibody (rat, 1:100, BD Bioscience, USA), anti-NG2 antibody (rabbit, 1:100, Millipore, USA), anti-insulin antibody (guinea pig, 1:1000, Dako, Sweden) and anti-VEGFR2 N-terminal antibody (rabbit, 1:100, R&D Systems, USA), at room temperature for 1 hour or at 4 ºC overnight. After washing with PBS, cells and tissues were further incubated with secondary antibodies (goat anti-rabbit IgG H+L Alexa Fluor 633, goat anti mouse IgG H+L Alexa Fluor 488 or 633, goat anti guinea pig IgG H+L Alexa Fluor 561 and donkey-anti goat IgG H+L 488; all at 1:1000, Thermo Fisher Scientific, USA) at room temperature for 1 hour, and mounted with ProLong Antifade mountant with DAPI (Thermo Fisher Scientific, USA).

Western Blots

Confluent HDMECs were stimulated by 50 ng/ml VEGF-A for indicated lengths of time and lysed in modified RIPA buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.4, 1% NP-40, 0.1% sodium deoxycholate, 1 mM EDTA, all from Sigma-Aldrich, USA) supplemented with protease and phosphatase inhibitors (Mini Complete/PhosphoStop, Roche Diagnostics,
Germany). Protein contents were determined using the BCA Protein Assay Kit (Pierce, USA). Protein extracts were separated on 7.5% stain-free precast gels (Bio-Rad, USA) and transferred to 0.22 \( \mu \)m PVDF membranes (GE Healthcare, USA). Blots were probed with primary antibodies, including phospho-VEGFR2 Y1175 antibody (rabbit, 1:1000), VEGFR2 antibody (rabbit, 1:1000), EphrinB2 antibody (rabbit, 1:500), phospho-PLC\( \gamma \)1 Y783 antibody (rabbit, 1:1000), PLC\( \gamma \)1 antibody (rabbit, 1:1000), phospho-Akt Thr308/473 antibody (rabbit, 1:1000), Akt1/2 antibody (rabbit, 1:1000), phospho-p44/42 MAPK Thr202/Tyr204 antibody (rabbit, 1:2000) and Erk1/2 antibody (rabbit, 1:2000; all from Cell Signaling Technology, USA), and \( \alpha \)-tubulin antibody (mouse, 1:2000, Sigma-Aldrich, USA), and subsequently with secondary anti-mouse/-rabbit IgG (H+L)-HRP conjugates (1:6000, GE Healthcare, USA). Quantification was carried out on original scanned images using Image Lab software (Bio-Rad, USA) and normalization to total protein.

Quantitative RT-PCR

Total RNA samples were collected from isolated islets of control and \( Bbs4^{+/c} \) animals, or transduced MS-1 cells. Tissue and cell samples were lysed and total RNA was isolated using the GeneJET RNA purification kit (Thermo Fisher Scientific, USA) according to manufacturer’s instructions. Extracted mRNA was transcribed into cDNA using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) following manufacturer’s instructions. \( Bbs4, \) \( Ift88, \) \( VEGF-A, \) \( VEGFR2, \) \( Ang-1, \) \( Ang-2, \) \( Tie-1, \) \( Tie-2, \) \( VHL, \) \( EphrinA1, \) \( EphrinB2, \) \( eNOS \) and \( Dll4 \) messages were measured using the SYBR Green Master Mix (Thermo Fisher Scientific, USA) with an QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA). Relative gene expression level was quantified against the average of \( 18S, HMBS \) and \( TBP \) messages, and normalized to those of control cells. All primer sequences are listed in Table 1.
Statistical tests

All results are presented as either original data points or mean ± SEM. Measurements and calculated results obtained from the WT and control groups were subjected to normality test, and most of them pass Kolmogorov-Smirnov test. Two-way ANOVA test was used for all time course analysis, One-way ANOVA test was used for comparison of multiple groups, and Mann-Whitney test was used to assess statistical significance for other experiments, with a p value < 0.05 considered to indicate significance (GraphPad Prism).

Table 1. Sequences of qPCR primers

| Gene | Sequence |
|------|----------|
| 18S  | L:5’-AACCCGTTGAACCCCATT-3’ R:5’-CATCCAATCGGTAGTAGCG-3’ |
| HMBS | L:5’-CGGAGTCATGTCCCGTAAC-3’ R:5’-GGTGCCCACCTCGAATCAC-3’ |
| TBP  | L:5’-TGCTGTTGGTGTGGTTG-3’ R:5’-CTGGCTTTGTGGGAAAGAT-3’ |
| Bbs4 | L:5’-AATGCACTGACCTACGACCC-3’ R:5’-ATGCTGATGCATACTGCTGC-3’ |
| Ift88| L:5’-TGAAGTGGCAGCTGATGGTA-3’ R:5’-CTGTGCAGAGACGAACCAAG-3’ |
| VEGF-A| L:5’-CAGGCTGCTGTAACGATGAA-3’ R:5’-GCATTCACATCTGCTGTGCT-3’ |
| VEGFR2| L:5’-GGCGGTGACGACTATCTT-3’ R:5’-GTCACTGACAGGCGATGA-3’ |
|   | L:5'–GATCTTACGCGGTGCCGATT-3' R:5'–TTAGATTGGAGGGCCACAG-3' |
|---|---------------------------------------------------------|
| Ang-1 | L:5’-TCCAAGAGCTCGTTGCTAT-3’ R:5’-AGTTGGGGAAGGTCAAGTGTG-3’ |
| Ang-2 | L:5’-TCAACTGCAGCTCCAAAATG-3’ R:5’-TGACAGCTCTGTCCAAAACG-3’ |
| Tie-1 | L:5’-TCCAAGAGCTCGTTGCTAT-3’ R:5’-AGTTGGGGAAGGTCAAGTGTG-3’ |
| Tie-2 | L:5’-GTGAGAAGAAAGGAGCAGGC-3’ R:5’-GTAGGTAGTGGCCACCCAG-3’ |
| VHL | L:5’-CAGGAGACTGGACATCGTCA-3’ R:5’-TCCTCTCCAGTGCTGACT-3’ |
| EphrinA1 | L:5’-CAGGAGACTGGACATCGTCA-3’ R:5’-TCCTCTCCAGTGCTGACT-3’ |
| EphrinB2 | L:5’-AGGAATCACGGTCCAACAAG-3’ R:5’-GTCTCCTGCGGTACTTGAGC-3’ |
| eNOS | L:5’-GCAAGACCTCCTGAGGACAG-3’ R:5’-TGCAAAAGAAAGCTCTGATG-3’ |
| Dll4 | L:5’-CAGGAGACTTCGCCAGGAAAC-3’ R:5’-TCATTTTGTCTGCTGTTCG-3’ |

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**Fig 1.** *Bbs4*<sup>−/−</sup> islets show delayed vascularization and enlarged capillary diameter in the pancreas and the anterior chamber of the eye upon transplantation.

(A) Immuno-fluorescence staining of pancreatic sections from 2-month-old wt and *Bbs4*<sup>−/−</sup> mice showing islets (insulin, red) and intra-islet capillaries (PECAM-1, green). (B-C) Quantification of relative intra-islet PECAM-1 positive volume, normalized to insulin positive volume (B) and average intra-islet capillary diameters (C) in wt and *Bbs4*<sup>−/−</sup> pancreatic sections. Individual data points are shown, **p<0.01, n=8 for animals and n=2-3 islets per animal.** (D) Immuno-fluorescence staining of freshly isolated and fixed pancreatic islets from 2-month-old wt and *Bbs4*<sup>−/−</sup> mice, showing PECAM-1 (green) labelled islet capillaries. (E-F) Quantification of relative CD31 positive volume within each islet normalized to islet volume estimated by backscatter signal (E) and average capillary diameters (F) in wt and *Bbs4*<sup>−/−</sup> islets. Individual data points are shown, **p<0.01 by Mann-Whitney test, n=3 for animals and n=8-12 islets per animal.** (G) Re-vascularization of 2 day-cultivated wt (upper) and *Bbs4*<sup>−/−</sup> (lower) islets in wt recipient eyes at 2, 4, 8- and 12-weeks post transplantation, visualized by intravenous injection of Texas Red-conjugated dextran. (H) Quantification of re-vascularization rates of wt and *Bbs4*<sup>−/−</sup> islet grafts in wt recipients. Results are shown as mean ± SEM. (I) Relative vascular density of wt and *Bbs4*<sup>−/−</sup> islet grafts at the end of 12 weeks post transplantation. Individual data points are shown, n.s. means not significant by Mann-Whitney test. (J) Average diameters of newly formed capillaries in wt and *Bbs4*<sup>−/−</sup> islet grafts in wt recipients. Results are shown as mean ± SEM, *p<0.05, **p<0.01 by two-way-ANOVA, n=6 for animals and n=4-8 islets per animal. Islets were encircled by dashed lines. The same is for all the other figures. Scale bars: 50 μm.
Fig 2. Bbs4−/− islets after prolonged culture exhibits normal re-vascularization patterns in wt recipient eyes.

(A) Re-vascularization of 7 day-cultivated wt (upper) and Bbs4−/− (lower) islets in wt recipient eyes at 2, 4, 8- and 12-weeks post transplantation, visualized by intravenous injection of Texas Red-conjugated dextran. Donor islets have been cultured for 7 days prior to transplantation. (B) Quantification of re-vascularization rates of wt and Bbs4−/− islet grafts in wt recipients. Results are mean±S.E.M. (C) Relative vascular density of wt and Bbs4−/− islet grafts at the end of 12 weeks post transplantation. Individual data points are shown, n.s. means not significant by Mann-Whitney test. (D) Average diameters of newly formed
capillaries in wt and $Bbs4^+$ islet grafts in wt recipients. Results are mean±S.E.M., n=4 for animals and n=2-6 islets per animal. Scale bar: 50 μm.
**Fig 3.** Wildtype islet grafts transplanted into *Bbs4/-* recipient eyes show stronger impairment in re-vascularization.
(A) Non-fasting glycemic levels in Bbs4-/ mice during islet engraftment. (B) Body weight measurements of Bbs4-/ mice during engraftment. (C) Intraperitoneal glucose tolerance test 12 weeks post transplantation. Results are mean±S.E.M. *p<0.05, **p<0.01 by two-way-ANOVA, n=5. (D) Re-vascularization of wt islets (upper) after 2 (middle) or 7 days (lower) of culture in wt or Bbs4-/ recipients at 2, 4, 8- and 12-weeks post transplantation, visualized by intravenous injection of Texas Red-conjugated dextran. (E) Quantification of re-vascularization rates of WT islet grafts. Results are mean±S.E.M. (F) Relative vascular density of wt islet grafts in different recipients at the end of 12 weeks post transplantation. Individual data points are shown, n.s. means not significant by one-way-ANOVA. (G) Average diameters of newly formed capillaries in wt islet grafts. Results are mean±S.E.M. Comparisons were made between 2-day and 7-day groups by two-way-ANOVA, *p<0.05, **p<0.01. n=4 for animals and n=2-4 islets per animal for wt recipients. n=5 for animals and n=1-4 islets per animal for Bbs4-/ recipients. Scale bar: 50 µm.
Fig 4. βICKO mice display no visible islet vascular phenotypes

(A) Efficiency of *Ift88* silencing by tamoxifen induction. Individual data points are shown, **p<0.01 by Mann-Whitney test, n=4 for animals. (B) Immuno-fluorescence staining of
pancreatic sections from 2-month-old control (vehicle) and βICKO mice, showing islets (Nkx 6.1, cyan) and intra-islet capillaries (PECAM-1, magenta). (C) Quantification of relative intra-islet PECAM-1 positive area, normalized to islet area in control and βICKO pancreatic sections. Individual data points are shown, n.s. means not significant by Mann-Whitney test, n=6 for animals and n=5-6 islets per animal. (D) Re-vascularization of overnight-cultured control (upper) and βICKO (lower) islets in wt recipient eyes at 2, 4, 8- and 12-weeks post transplantation, visualized by intravenous injection of Texas Red-conjugated dextran. (E) Quantification of re-vascularization rates of control and βICKO islet grafts in wt recipients. Results are mean±S.E.M. n=6 for animals and n=6-8 islets per animal. (F) Relative vascular density of control and βICKO islet grafts at the end of 12 weeks post transplantation. Individual data points are shown. n=3 for animals and n=2-3 islets per animal. (G) Average diameters of newly formed capillaries in control and βICKO islet grafts in wt recipients. Results are mean±S.E.M. and n.s. means not significant by Mann-Whitney test. n=6 for animals and n=6-8 islets per animal. Scale bars: 50 μm.
**Fig 5.** Dysfunctional intra-islet vasculature in $Bbs4^{−/−}$ islets undermines glucose metabolism

(A) Electron microscopic images of wt and $Bbs4^{−/−}$ islet graft dissected from wt recipient eyes, showing fenestrated islet capillaries (arrows) and basement membrane (asterisks). Scale bar:
(B-C) Quantification of average fenestrae density (B) and basement membrane thickness (C) of the capillaries in wt and Bbs4−/− islet grafts. Individual data points are shown, **p<0.01, n.s. means not significant by Mann-Whitney test, n=7 for animals, and n=1-2 for islets. (D) Representative image showing leakage of 40 KDa FITC conjugated dextran from wt islet grafts in mouse eyes at 1 min after injection. Scale bar: 50 μm. (E-F) Quantification of FITC fluorescence intensity in the region circles by the dashed lines outside wt and Bbs4−/− islet grafts (E) and simulated curves showing different kinetics (F). Arrow indicates injection time point. Results are mean±S.E.M. *p<0.05 by two-way-ANOVA, n=12 for animals. (G) Representative image showing 2-NBDG leakage from wt islet vasculature and uptake by islet cells in vivo. Times points are 3 s (left) and 60 s (right) after injection. Scale bar: 50 μm. (H) Real time ratio of 2-NBDG fluorescence intensity in islet cells of wt and Bbs4−/− islet grafts to aqueous humor. Arrow indicates injection time point. Results are mean± S.E.M. *p<0.05, n=8 for animals and n=1-2 for islets. (I-K) Intraperitoneal glucose tolerance test of wt recipient mice which were transplanted with WT or Bbs4−/− islets, at 4 weeks (I), 8 weeks (J) and 12 weeks (K) post transplantation. Results are mean±S.E.M. *p<0.05, **p<0.01 by two-way-ANOVA, n=6 for animals.
Figure 6

**Fig 6.** VEGFA-VEGFR2 induced signalling pathway and angiogenic response is disrupted in *Bbs4* silenced endothelial cells

(A) qPCR analysis of VEGF-A signaling related gene expression in *Bbs4* silenced MS-1. Results are presented as relative mRNA levels normalized to scrambled shRNA treated cells, *p*<0.05, **p**<0.01 by one-way-ANOVA, n=3. (B) and (C) Western blots showing VEGF-A
signaling pathway in scrambled or shBbs4 treated HDMECs. (D) Quantification of VEGFR2 activation. (E) Representative images showing biotinylated VEGF-A uptake in scrambled or ShBbs4 treated HDMECs and quantification (F). (G) Representative cell surface staining of VEGFR2 in scrambled (Scr) or ShBbs4 treated HDMECs, prior to (0 min) and after VEGF-A stimulation (10 min). Scale bar: 50 µm. (H) Quantification of the percentage of remaining membrane-bound VEGFR2. (I) VEGF-A induced proliferation in Scr or ShBbs4 treated MS-1 by EdU assay (green) and quantification (J). Scale bars: 50 µm. (K) VEGF-A induced endothelial cell migration in Scr or ShBbs4 treated MS-1. Individual data points are shown, *p<0.05, **p<0.01 by Mann-Whitney test, n=3.

Supplementary figure legends

Figure 1-figure supplement 1. Pericyte coverage of intra-islet capillaries and capillary density in exocrine pancreas are not affected in Bbs4−/− mice (A) Immuno-fluorescence staining of pancreatic sections from 2-month-old wt and Bbs4−/− animals showing pericyte coverage (NG2, red) on intra-islet capillaries (PECAM-1, green). (B) Quantification of surface ratio of NG2 positive area to PECAM-1 positive area. n=4 for animals and n=2-4 islets per animal. (C) Immuno-fluorescence staining of pancreatic sections from 4-month-old wt and Bbs4−/− animals showing islets (insulin, red) and intra-islet capillaries (PECAM-1, green). (D-E) Quantification of relative intra-islet PECAM-1 positive volume, normalized to insulin positive volume (D) and average intra-islet capillary diameters (E) in wt and Bbs4−/− islets from 4-month-old animals. n=4 for animals and n=2-4 islets per animal. (F) Immuno-fluorescence staining of pancreatic sections from 2 months old wt and Bbs4−/− animals, showing PECAM-1 (green) labeled capillaries in exocrine pancreas. Individual data points are shown, n.s. means not significant by Mann-Whitney test. Scale bars: 50 µm.
**Figure 1-figure supplement 2.** Number of donor endothelial cells participating in islet re-vascularization decreases under prolonged cultivation

(A) Remaining endothelial cells in islets which were freshly isolated (left), or cultured for 2 days (middle) or 7 days (right), indicated by whole islet PECAM-1 staining. Scale bar: 50 μm. (B) Schematic illustration of two scenarios of islet revascularization in the anterior chamber of mouse eyes. Upper panel indicates that when transplanted after minimum *in vitro* culture, the newly formed islet microvasculature is composed of both donor islet vascular cells (red) and recipient cells (green). Lower panel indicates that when transplanted after prolonged culture, the number of donor islet vascular cell decreases, and islet re-vascularization is mainly derived from recipient cells.

**Figure 3-figure supplement 1.** *Bbs4-/-* endothelial cells are less responsive to the angiogenic signals during islet re-vascularization

(A) Immuno-fluorescence staining of a freshly isolated islet from *Cdhltd*T mice showing that tdTomato fluorescence (red) and intra-islet capillaries (PECAM-1, green) overlaps. (B) Representative images showing that 2-day and 7-day culture of *Cdhltd*T islets leads to different amounts of donor endothelial cells constituting islet vasculature after engraftment. (C) Re-vascularization of 2 day-cultured wt (upper) and *Bbs4-/-* (lower) islets in *Cdhltd*T recipient eyes at 2, 4, 8- and 12-weeks post transplantation, visualized by intravenous injection of FITC-conjugated dextran. (D) Quantification of re-vascularization rates of wt and *Bbs4-/-* islet grafts in *Cdhltd*T recipients. (E) Average diameters of newly formed capillaries in wt and *Bbs4-/-* islet grafts in wt recipients. (F) Proportion of recipient to donor endothelial cells in wt and *Bbs4-/-* islet grafts indicated by the surface area ratio of tdTomato labelled cells to total dextran labelled islet vessels during engraftment. Results are mean±S.E.M. *
p<0.05, **p<0.01 by two-way ANOVA, n=5 for animals and n=2-4 islets per animal. Scale bars: 50 μm.

**Figure 3-figure supplement 2.** *Pifo/-* mice exhibit similar islet vascular phenotypes as *Bbs4/-* mice

(A) Immuno-fluorescence staining of pancreatic sections from 3-month-old wt and *Pifo/-* mice showing islets (insulin, red) and intra-islet capillaries (PECAM-1, green). (B-C) Quantification of relative intra-islet PECAM-1 positive volume, normalized to insulin positive volume (B) and average intra-islet capillary diameters (C) in wt and *Pifo/-* pancreatic sections. Individual data points are shown. *p<0.05, **p<0.01 by Mann-Whitney test, n=3 for animals and n=4-6 islets per animal. (D) Re-vascularization of 2 day-cultured wt (upper) and *Pifo/-* (lower) islets in wt recipient eyes at 2, 4, 8- and 12-weeks post transplantation, visualized by intravenous injection of Texas Red-conjugated dextran. (E) Quantification of re-vascularization rates of wt and *Pifo/-* islet grafts in wt recipients. Results are mean±S.E.M. (F) Relative vascular density of wt and *Pifo/-* islet grafts at the end of 12 weeks post transplantation. Individual data points are shown. (G) Average diameters of newly formed capillaries in wt and *Pifo/-* islet grafts in wt recipients. Results are mean±S.E.M., **p<0.01 by two-way ANOVA, n.s. means not significant by Mann-Whitney test, n=5 for animals and n=2-5 islets per animal. Scale bars: 50 μm.

**Figure 5-figure supplement 1.** Dextran leakage kinetics from the capillaries of islet grafts or the iris vessels in mouse eyes

Kinetics of fluorescence intensity outside islet grafts in transplanted eyes (Tx) or in the aqueous humour of non-transplanted control eyes (Non-Tx) after intravenously injection of
fluorescently labelled dextran at sizes of 10 KDa (A), 40 KDa (B) and 70 KDa (C), respectively. Results are mean±S.E.M, n=4.

Figure 6-figure supplement 1. VEGF-A gene expression, secretion and signalling in Bbs4 or Ift88 depleted cells

(A) Efficiency of shRNA silencing of Bbs4 in MS1 endothelial cells. Individual data points are shown, **p<0.01 by one-way-ANOVA, n=4. (B-C) Quantification of western blots showing BBS4, VEGFR2 and EphrinB2 protein levels (B), and activation of PLCγ1, Akt and MAPK 42/44 (C) in scrambled or shBbs4 treated HDMECs. *p<0.05, **p<0.01 by two-way-ANOVA, n=3. (D) Western blots showing VEGF-A signaling pathway in freshly isolated wt and Bbs4/- islets, and (E) quantification of activation of Akt and MAPK 42/44. *p<0.05 by two-way-ANOVA, n=3 for animals. (F) Western blots showing VEGF-A signaling pathway in isolated wt islets cultivated for 7 days, and (G) quantification of activation of Akt and MAPK 42/44, *p<0.05 by Mann-Whitney test, n=2 for animals. (H) Efficiency of shRNA silencing of Ift88 in MS-1 cells. **p<0.01 by one-way-ANOVA, n=3. (I) Representative images showing biotinylated VEGF-A uptake in scrambled or ShIft88 treated MS-1 cells and quantification (J), **p<0.01 by Mann-Whitney test, n=3. Scale bar: 10 μm. (K) Notch signaling activity in scrambled or ShIft88 treated MS-1 cells and indicated by luciferase assay. *p<0.05 by Mann-Whitney test, n=3. (L) qPCR analysis of angiogenic gene expression in freshly isolated wt and Bbs4/- islets. **p<0.01 by one-way-ANOVA, n=4 for animals. (M) qPCR analysis of VEGF-A and VEGFR2 gene expression in freshly isolated control and βICKO islets. Results are presented as relative mRNA levels normalized to control. *p<0.05 by one-way-ANOVA, n=4 for animals. (N) VEGF-A secretion from cultured wt and Bbs4/- islets, normalized to total DNA quantity of islets. **p<0.01 by Mann-Whitney test, n=5 for animals. (O) VEGF-A secretion from cultured control and βICKO
islets, normalized to total DNA quantity of islets. **p<0.01 by Mann-Whitney test, n=4 for animals.
