Apelin signaling drives vascular endothelial cells toward a pro-angiogenic state

Christian SM Helker, Jean Eberlein, Kerstin Wilhelm, Toshiya Sugino, Julian Malchow, Annika Schuermann, Stefan Baumeister, Hyouk-Bum Kwon, Hans-Martin Maischein, Michael Potente, Wiebke Herzog, Didier YR Stainier

Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; Philippus-University Marburg, Faculty of Biology, Cell Signaling and Dynamics, Marburg, Germany; Angiogenesis and Metabolism Laboratory, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; University of Muenster, Muenster, Germany; DZHK (German Center for Cardiovascular Research), partner site Frankfurt Rhine-Main, Berlin, Germany; Max Planck Institute for Molecular Biomedicine, Muenster, Germany

Abstract To form new blood vessels (angiogenesis), endothelial cells (ECs) must be activated and acquire highly migratory and proliferative phenotypes. However, the molecular mechanisms that govern these processes are incompletely understood. Here, we show that Apelin signaling functions to drive ECs into such an angiogenic state. Zebrafish lacking Apelin signaling exhibit defects in endothelial tip cell morphology and sprouting. Using transplantation experiments, we find that in mosaic vessels, wild-type ECs leave the dorsal aorta (DA) and form new vessels while neighboring ECs defective in Apelin signaling remain in the DA. Mechanistically, Apelin signaling enhances glycolytic activity in ECs at least in part by increasing levels of the growth-promoting transcription factor c-Myc. Moreover, APELIN expression is regulated by Notch signaling in human ECs, and its function is required for the hypersprouting phenotype in Delta-like 4 (Dll4) knockdown zebrafish embryos. These data provide new insights into fundamental principles of blood vessel formation and Apelin signaling, enabling a better understanding of vascular growth in health and disease.

Introduction Endothelial cell sprouting is a fundamental process of physiological and pathological blood vessel growth. Attracted by growth factors such as vascular endothelial growth factor-A (VEGF-A) secreted from hypoxic tissues, endothelial cells (ECs) break out of the quiescent vessel wall to form new vessel branches (Ferrara et al., 2003; Koch and Claesson-Welsh, 2012). ECs with higher levels of VEGF-A signaling become invasive tip cells that lead new vascular sprouts, while neighboring ECs with lower VEGF-A signaling become trailing stalk cells (Gerhardt et al., 2003). This process is coordinated by Delta-like 4 (Dll4)/Notch signaling. Activation of Notch receptors by their ligand DLL4, expressed by tip cells, represses tip cell behavior in stalk cells (Hellström et al., 2007; Leslie et al., 2007; Siekmann and Lawson, 2007; Suchting et al., 2007). Loss of Notch signaling, on the other hand, causes excessive tip cell formation and vascular overgrowth (Hellström et al., 2007; Leslie et al., 2007; Siekmann and Lawson, 2007; Suchting et al., 2007).

Apelin (Apln) is a small secreted peptide, which was initially identified because of its inotropic activity (Szokodi et al., 2002). Apelin was subsequently described as a tip cell-enriched gene
Apelin signaling is required for angiogenic sprouting

To examine the expression pattern of the apelin ligand and receptor genes during angiogenic sprouting in zebrafish embryos, we first performed whole-mount in situ hybridization during inter-segmental vessel (ISV) formation. We detected clear apln, but no apela, expression within the sprouting ISVs (Figure 1—figure supplement 1 arrowheads). For the receptor genes, we could only detect aplnrb expression in the ISVs (Figure 1—figure supplement 1 arrowheads).

To visualize apln and aplnrb expression at single cell resolution, we developed reporters using Bacterial artificial chromosome (BAC) recombinaseering (Figure 1—figure supplement 2). To this end, we replaced the ATG of an apln containing BAC with an EGFP cassette. Similarly, we replaced the stop codon of an aplnrb containing BAC with a tandem fluorescent timer (TagRFP-sfGFP) cassette leading to a fusion protein. We injected both modified BACs into one-cell stage zebrafish embryos to generate stable transgenic lines, Tg(apln:EGFP) and Tg(aplnrb:aplnrb-sfGFP) (hereafter referred to Tg(apln:aplnrb-sfGFP)) (Figure 1—figure supplement 2). We first detected weak apln: EGFP expression in sprouting ISVs at 30 hpf (Figure 1A). At 54 hpf, all ECs within the dorsal longitudinal anastomotic vessel (DLAV) – a vessel formed by tip cells – were labeled (Figure 1A, arrowheads) while some stalk cells also exhibited weak apln:GFP expression (Figure 1A, arrows). Of note, aplnrb:Aplnrb-sfGFP expression at 26 hpf was visible in the entire ISV sprout (Figure 1B arrowheads), but it was absent from non-angiogenic ECs within the dorsal aorta (DA). At 54 hpf, aplnrb:Aplnrb-sfGFP expression was detected in all ECs that had sprouted out of the DA but also weakly in ECs within the DA (Figure 1B). These results suggest that apln is expressed in tip cells while aplnrb is expressed in all sprouting ECs.

To examine the function of Apelin signaling during sprouting angiogenesis in zebrafish, we used mutants for aplnra (Helker et al., 2015), aplnrb (Helker et al., 2015), apln (Helker et al., 2015) and apela (Chng et al., 2013). Homozygous aplnra mutant embryos exhibited no obvious defects during ISV formation (Figure 1—figure supplement 3). However, homozygous aplnrb mutant embryos exhibited reduced ISV length and failed to form the DLAV (Figure 1—figure supplement 3). This phenotype was more severe in embryos lacking both aplnra and aplnrb (Figure 1C, Figure 1—figure supplement 3), indicating partial compensation. We also analyzed apln and apela mutants. Homozygous apela mutant embryos displayed only a mild delay in ISV outgrowth (Figure 1—figure supplement 3), while homozygous apln mutant embryos exhibited defects in ISV outgrowth and failure to form the DLAV (Figure 1—figure supplement 3). Loss of both ligands increased the severity of the phenotype leading to ISV stalling at the horizontal myoseptum (Figure 1C, Figure 1—figure supplement 3). Consistent with studies in the mouse retina (del Toro et al., 2010), our studies identify apln expression as a marker of endothelial tip cells in zebrafish and show that Apelin signaling is required for Notch-controlled angiogenesis.
Apelin signaling regulates tip cell morphology

To investigate when the sprouting defects in Apelin signaling-deficient embryos first appear, we analyzed developmental time points when tip cells start to sprout out of the DA. However, no differences in sprout initiation or tip cell specification were observed in double homozygous receptor or ligand mutants (Figure 2—figure supplement 1, Figure 2A). Instead, we found that sprout...
Figure 2. Apelin signaling regulates endothelial filopodia formation and endothelial cell numbers. (A) Still images from confocal time-lapse movies of vascular development in wild-type and aplnra+/-; aplnr-/- embryos. During sprouting, wild-type tip cells send out filopodia (arrowheads). aplnra+/-; aplnr-/- embryos exhibit smaller sprouts and fail to form filopodia. (B) Confocal images of the blood vasculature in 24 hpf Tg(kdrl:HsHRAS-EGFP) embryos injected with Ctr MO and aplnr MO. aplnr morphant embryos exhibit smaller sprouts and fail to form filopodia (arrowheads). (C) aplnr

Figure 2 continued on next page
morphant embryos exhibit a reduction in the number of endothelial filopodia (Ctr MO, n = 10; aplnrb MO, n = 15). (D) Confocal images of the blood vasculature of 30 and 52 hpf Tg(fli1a:nEGFP) wild-type and aplnra -/-; aplnrb -/- embryos showing EC cell nuclei. (E) aplnra +/+; aplnrb -/- embryos exhibit reduced EC numbers in the ISVs (30 hpf: apln +/+; n = 3; apln +/−, n = 10; apln −/−, n = 8; 52 hpf: apln +/+; n = 5; apln +/−, n = 10; apln −/−, n = 9). n.s. not significant (two-tailed t-test). Scale bars: A, D, 20 μm; B, 40 μm; B, inset 10 μm.

The online version of this article includes the following video and figure supplement(s) for figure 2:

**Figure supplement 1.** No obvious defects during initiation of EC sprouting in Apelin signaling-deficient embryos.

**Figure supplement 2.** No obvious defects in EC polarity in Apelin deficient embryos.

**Figure supplement 3.** Overexpression of apln does not cause ectopic EC sprouting.

**Figure 2—video 1.** Confocal time-lapse-imaging of a Tg(fli1a:EGFP) wild-type embryo from 23 to 32 hpf. 
https://elifesciences.org/articles/55589#fig2video1

**Figure 2—video 2.** Confocal time-lapse-imaging of a Tg(fli1a:EGFP) aplnra +/+; aplnrb -/- embryos from 23 to 35 hpf. 
https://elifesciences.org/articles/55589#fig2video2

Elongation was slower in these mutant embryos, resulting in an overall reduction of sprout length (Figure 2A, Figure 2—video 1, Figure 2—video 2). Furthermore, while endothelial tip cells in wild-type embryos formed long filopodia which extended toward the dorsal side of the animal (Figure 2A I, II, Figure 2—video 1), aplnra mutant embryos (aplnra +/−; aplnrb -/− and aplnra -/−; aplnrb -/) as well as aplnrb morpholino (MO) injected embryos (morphants) displayed a blunted tip cell morphology (Figure 2A III, 2A IV, 2B, C, Figure 2—video 2), a phenotype which did not recover over time.

Previously, we reported a role for Apelin signaling in establishing blood flow-induced EC polarity (Kwon et al., 2016). To determine whether the observed defects during sprouting were caused by defects in EC polarity, we analyzed the location of the Golgi apparatus during ISV formation in wild-type and mutant embryos. However, we could not detect obvious differences in EC polarity during angiogenic sprouting (Figure 2—figure supplement 2, arrowheads point to polarized ECs). Next, we asked whether Apelin signaling regulates the number of ECs, and so combined aplnr mutants with the Tg(fli1a:nEGFP) reporter line (Roman et al., 2002) to visualize EC nuclei. Compared to controls, aplnra mutants exhibited a reduction in ISV EC numbers of 1 cell at 30 hpf (4 instead of 5) and 2 cells at 52 hpf (5 instead of 7) (Figure 2D, E). We next assessed whether apln overexpression leads to ectopic sprouting. To this end, we generated an inducible transgenic line to overexpress apln under the control of the hsp70l1 promoter. However, global overexpression of apln did not lead to ectopic sprouting of blood vessels but led to mispatterned lymphatic vessels (Figure 2—figure supplement 3, arrows). Altogether, these data indicate that the angiogenic defects in Apelin signaling-deficient embryos are caused by filopodia defects and impaired cell migration. Apelin signaling also regulates the number of ECs within the ISV sprouts.

**Apelin signaling drives the sprouting behavior of ECs**

We hypothesized that aplnrb expression (Figure 1B) provides an advantage for ECs to sprout. To test this hypothesis, we generated chimeric embryos using wild-type and aplnrb deficient embryos (Figure 3A). Upon transplantation of wild-type donor cells into wild-type hosts, 34.5% of the donor-derived ECs were present in the ISVs at 24 hpf (Figure 3B, C). In contrast, upon transplantation of wild-type donor cells into aplnrb-deficient hosts, 80% of the donor-derived ECs were present in the ISVs at 24 hpf (Figure 3B, C). Together these data show that the apelin receptors function cell-autonomously in endothelial sprouting. The Apelin receptor has been shown to signal mainly through the G-protein Gαi (Habata et al., 1999). Therefore, we blocked Gαi function through the mosaic and vascular-specific overexpression of pertussis toxin (PTX). Our results show that ECs deficient for signaling though Gαi behave similarly to aplnra mutant ECs indicating that the Apelin receptor mediates its angiogenic effect through Gαi (Figure 3—figure supplement 1). Notably, wild-type donor-derived ECs in aplnrb deficient embryos populated the entire dorsal part of the vasculature which is usually missing in these mutants, further confirming the cell-autonomous function of the Apelin receptors during angiogenesis (Figure 3—figure supplement 2). Together, these results indicate that apelin signaling primes ECs toward a sprouting state.
Apelin signaling functions downstream of Notch signaling

It has been previously reported that Notch-deficient ECs outcompete wild-type ECs during ISV sprouting (Siekmann and Lawson, 2007), an observation consistent with data in mouse (Jakobsson et al., 2010; Pitulescu et al., 2017). Since wild-type ECs similarly outcompete aplnr mutant ECs, we wanted to investigate potential links between Apelin and Notch signaling. Hence, we first blocked Notch signaling in Tg(BAC(apln:EGFP)) embryos by injecting a dll4 MO. As previously reported (Leslie et al., 2007; Siekmann and Lawson, 2007), dll4 morphants exhibited a hyper-sprouting ISV phenotype (Figure 4A). Notably, we also observed a clear increase in apln:EGFP expression in the ectopic sprouts (Figure 4A). To test whether Apelin signaling is required as a downstream effector of Notch signaling during angiogenesis, we injected the dll4 MO into the offspring of apln and aplnrb heterozygous parents and compared the phenotype in homozygous mutant embryos versus their wild-type siblings. Strikingly, the hypersprouting phenotype of dll4 morphants was not present when Apln or Aplnrb function was lost (Figure 4B, Figure 4—figure supplement 1). To examine whether other hypersprouting phenotypes require Apelin signaling, we analyzed aplnrb, plexinD1 (plxnd1) double mutant embryos (Figure 4C,E). Notably, aplnrb mutant embryos exhibit reduced sprouting and plxnd1 mutant embryos exhibit ectopic sprouting in line with published data (Torres-Vázquez et al., 2004; Figure 4C,E). Loss of aplnrb function in the background of the plxnd1 mutant did not alter its hypersprouting phenotype (Figure 4C,E), suggesting that it is
Figure 4. Apelin signaling functions downstream of Notch signaling in endothelial cells. (A - D) Confocal projection images of the blood vasculature in the trunk region of Tg(fli1a:tdTomato) (A) and Tg(fli1a:EGFP) (B–D) animals at 54 (B–D) and 72 (A) hpf. (A) Injection of a dll4 morpholino leads to an increase in TgBAC(apln:EGFP) expression. (B) Loss of Apelin function can block excessive endothelial sprouting in dll4 morphants. (C, E) Angiogenic response in aplnr−/−, plxnd1−/−, and aplnr−/−; plxnd1−/− embryos (arrowheads) (n = 95). (D, F) Angiogenic response to bmp2b overexpression in aplnr−/− embryos.
**Apelin signaling positively regulates EC metabolism**

Because EC sprouting requires an increase in metabolic activity (Dobrina and Rossi, 1983; Krützfeldt et al., 1990; Mertens et al., 1990; De Bock et al., 2013a; Vandekere et al., 2015) and Apelin signaling has been shown to control cell metabolism in other contexts (Dray et al., 2008; Sawane et al., 2013), we asked whether Apelin signaling promotes EC metabolism. Previous studies have demonstrated that ECs rely on glycolysis for sprouting (De Bock et al., 2013a; Vandekere et al., 2015). Therefore, we measured the extracellular acidification rate (ECAR) as a surrogate parameter of glycolysis in Apelin signaling-deficient HUVECs (Figure 5A,B). Notably, we observed a marked reduction in glycolysis after knockdown of Apelin signaling (Figure 5A), whereas mitochondrial oxygen consumption appeared unchanged (Figure 5B). To gain insight into the underlying mechanisms, we analyzed key regulators of metabolism and found a reduction in c-MYC protein levels after depletion of Apelin signaling (Figure 5C). Furthermore, expression of PFKFB3, which encodes an enzyme that sustains high glycolytic rates, was also reduced in Apelin signaling-deficient HUVECs (Figure 5D). In order to analyze whether a reduction in EC metabolic activity causes the vascular phenotype observed in apla−/− mutants, we performed mosaic rescue experiments and overexpressed pfkfb3 in ECs. In agreement with our in vitro data, we found that overexpression of pfkfb3 in endothelial tip cells leads to a partial rescue of the vascular phenotype in apla−/− mutants (Figure 5E, Figure 4—source data 1). Thus, Apelin signaling controls the expression of regulators of glucose metabolism as well as glycolytic activity in developing endothelial cells.

**Discussion**

During the formation of the first embryonic blood vessels, angioblasts migrate to the midline where they coalesce to form the future DA and cardinal vein. We have previously reported that vasculogenesis relies on the function of the ligand Apela (Helker et al., 2015). Here, we show that angiogenesis depends mostly on the function of the ligand Apln. However, Apln can partially compensate for the loss of Apln. This stage-specific ligand usage is in agreement with previous studies showing that apela expression is reduced by the end of vasculogenesis when apln starts to be expressed (Chng et al., 2013; Pauli et al., 2014).

During angiogenesis in embryos lacking Apelin signaling, we observed a severe sprouting defect with a reduction in EC numbers and filopodia. As ECs proliferate, extend filopodia, and migrate during ISV formation, it is challenging to assign the cause of the sprouting defect to the EC proliferation or filopodia formation defects. However, Phng et al., 2013 reported that the inhibition of filopodia
Figure 5. Apelin signaling positively regulates EC metabolism. (A - B) Extracellular acidification rate (ECAR) (A) and oxygen consumption rates (OCR) (B) in siScr and APLN+APLNR siRNA-treated HUVECs under basal conditions and in response to oligomycin, fluoro-carbonyl cyanide phenylhydrazone (FCCP) and antimycin A (AA)/rotenone. (A) Reduced basal and maximal glycolytic activity in APLN+APLNR siRNA-treated compared to siScr-treated HUVECs. (B) No significant difference in oxygen consumption in APLN+APLNR siRNA-treated compared to siScr-treated HUVECs. (C) Reduced c-MYC expression in APLN+APLNR siRNA-treated compared to siScr-treated HUVECs. (D) Reduced mRNA relative fold change in PFKFB3 in APLN+APLNR siRNA-treated compared to siScr-treated HUVECs. (E) Reduced formation of DLAV segments in aplnrb-/- embryos compared to control embryos. (F) Reduced formation of DLAV segments in pfkfb3*-/- embryos compared to control embryos.

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levels in APLN+APLNR siRNA-treated compared to siScr-treated HUVECs. (D) RT-qPCR analysis of PFKFB3 mRNA levels in APLN+APLNR siRNA-treated compared to siScr-treated HUVECs. (E) Confocal projection images of the blood vasculature in the trunk region of a 54 hpf Tg(kdr:HSRAS-mCherry) animal injected with an EGFP:fltl:pfkb3 plasmid. Arrowheads point to formed DLAV fragments while asterisks indicate missing DLAV fragments. (F) Quantification of the rescue of the DLAV fragment by mosaic pfkb3 overexpression in aplnrb−/− embryos. n.s. not significant (two-tailed t-test). Scale bar: E, 50 μm.

The online version of this article includes the following figure supplement(s) for figure 5:

Figure supplement 1. Schematic model.

formation by Latrunculin B treatment reduces ISV sprout length, suggesting that the ISV sprouting defects in apln mutants is caused by the filopodia defects. However, one cannot exclude the possibility that defects in EC numbers are also contributing to the ISV sprouting defects.

While we observed a severe angiogenesis phenotype when Apelin signaling was impaired, global overexpression of apln did not lead to ectopic sprouting. However, these experiments were done in the presence of endogenous Apelin, and thus, it is possible that the endogenous Apelin gradient prevents ECs from ectopic sprouting. In addition, Apelin might need to be expressed from a discrete source, rather than globally, to elicit a sprouting response.

During sprouting angiogenesis, ECs within a sprout are highly heterogenous in their shape, gene expression and function, which led to the model of tip and stalk cells (Gerhardt et al., 2003). While differences in expression between tip and stalk cells have been reported for several genes (Tammela et al., 2008), (Hellström et al., 2007; Siekmann and Lawson, 2007; Leslie et al., 2007; Suchting et al., 2007; del Toro et al., 2010; Bussmann et al., 2011; Herbert et al., 2012), little is known about the molecular differences between sprouting and resting ECs (Scherer et al., 2018). By analyzing novel reporter lines for apln and aplnrb expression, we observed high apln expression in tip cells while we could not observe any difference in aplnrb expression between tip and stalk cells (Figure 5—figure supplement 1). Interestingly, aplnrb is highly expressed in spraying ECs in ISVs while being absent from non-angiogenic ECs in the DA (Figure 5—figure supplement 1). These observations are in line with a recent study showing that ECs during tumor angiogenesis can be labeled by a CreERT2 transgene in the Aplnr locus while quiescent blood vessels in the surrounding tissue are not labeled (Zhao et al., 2018).

At the molecular level, vascular spraying and cell positioning within the sprout is tightly regulated by VEGF and Notch signaling (Hellström et al., 2007; Lobov et al., 2007; Siekmann and Lawson, 2007; Suchting et al., 2007; Jakobsson et al., 2010). In addition to these signaling pathways, we propose Apelin signaling as a molecular switch to drive ECs into a pro-angiogenic state. In line with the expression of aplnrb in spraying but not quiescent ECs, we show that aplnrb function regulates the ability of ECs to sprout or stay quiescent. Similarly, Notch signaling regulates the behavior of ECs (Siekmann and Lawson, 2007): rbpj deficient ECs contribute to the ISVs while wild-type ECs stay within the DA (Siekmann and Lawson, 2007). Of note, we found that Notch signaling regulates the expression of apln in vitro as well as in vivo and that Apelin signaling is a key downstream effector of Notch signaling during angiogenesis (Figure 5—figure supplement 1). However, it is very unlikely that apln is a direct Notch target gene since activation of Notch signaling leads to a downregulation of APLN expression. Thus far, two downstream effectors of Notch signaling have been reported to control angiogenesis namely PTEN (Serra et al., 2015) and CXCR4, another GPCR (Hasan et al., 2017; Pitulescu et al., 2017). While PTEN has been shown to be required for Notch induced arrest in EC proliferation (Serra et al., 2015), CXCR4 mediates Notch-controlled EC migration (Hasan et al., 2017; Pitulescu et al., 2017). PTEN and Apelin both regulate AKT phosphorylation (Davies et al., 1998; Masri et al., 2004). Thus, one might speculate that AKT function is a common effector of PTEN and Apelin signaling in EC proliferation. Furthermore, we found that Apelin was required for EC migration in the absence of Notch signaling. Similarly, CXCR4 is required for EC migration in the absence of Notch signaling (Hasan et al., 2017; Pitulescu et al., 2017). CXCR4 and APLNR both signal through the G-protein Gαi (Moepps et al., 1997; Habata et al., 1999), and they might therefore have similar effects. Gpr124, another GPCR, has been reported to be required in tip cells during zebrafish angiogenesis (Vanhollebeke et al., 2015), similar to Aplnr. However,
Gpr124 is required in tip cells only in the brain (Vanhollebeke et al., 2015), while Aplnr is required in tip cells in the ISVs, where it is most highly expressed.

Sprouting angiogenesis is controlled by genetically encoded signal transducers as well as by the metabolic state. However, how environmental signals modulate the metabolic activity of ECs is incompletely understood. Here, we show that Apelin signaling regulates the expression of PFKFB3 and c-MYC, two powerful drivers of EC metabolism (Wilhelm et al., 2016; De Bock et al., 2013b). Recently it has been shown that Apelin signaling promotes FOXO1 phosphorylation (Hwangbo et al., 2017), which negatively regulates its activity. Consistent with these findings, FOXO1 has been shown to suppress c-MYC expression (Wilhelm et al., 2016). Together these data raise the possibility that Apelin signals through FOXO1 to regulate c-MYC levels. Of note, genetic deletion of Pfkfb3 in mouse ECs leads to a reduction in their number as well as defects in filopodia formation and extension (De Bock et al., 2013b), phenocopying aplnr mutant embryos.

Taken together, our findings provide novel insights into a druggable pathway regulating angiogenesis and suggest that manipulating the angiogenic state of ECs by controlling Apln signaling might have therapeutic potential to control vascular growth in pathological settings.

Materials and methods

Key resources table

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| genetic reagent (D. rerio)        | Tg(fli1a:EGFP)\(^7\) | Lawson and Weinstein, 2002 | ZFIN: y1   |
| genetic reagent (D. rerio)        | Tg(fli1a:nEGFP)\(^7\) | Roman et al., 2002, 2006 | ZFIN: y7   |
| genetic reagent (D. rerio)        | Tg(kdrl:HsHRAS-mCherry)\(^{16,16}\) | Chi et al., 2008 | ZFIN: s896 |
| genetic reagent (D. rerio)        | aplnra\(^{mu296}\) | Helker et al., 2015 | ZFIN: mu296 |
| genetic reagent (D. rerio)        | aplnrb\(^{mu281}\) | codes for another allele of aplnrb from Helker et al., 2015 | ZFIN: mu281 |
| genetic reagent (D. rerio)        | apln\(^{mu267}\) | Helker et al., 2015 | ZFIN: mu267 |
| genetic reagent (D. rerio)        | Tg(hsp70:apln)\(^{mu269}\) | This manuscript | ZFIN: mu269 |
| genetic reagent (D. rerio)        | Tg(fli1a:Hsa.B4GALT1-mCherry)\(^{bns9}\) | Kwon et al., 2016 | ZFIN: bns9 |
| genetic reagent (D. rerio)        | Tg(fli1a:hsa.B4GALT1-mCherry)\(^{bns9}\) | Kwon et al., 2016 | ZFIN: bns9 |
| genetic reagent (D. rerio)        | Tg(hsp70:apln)\(^{mu269}\) | This manuscript | ZFIN: mu269 |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| genetic reagent (D. rerio)        | Tg(kdrl:HsHRAS-EGFP)    | This manuscript    | ZFIN: mu280  |
|                                   |             |                     |             |                        |
| genetic reagent (D. rerio)        | Tg(apln:EGFP)    | This manuscript    | ZFIN: bns157 |
|                                   |             |                     |             |                        |
| genetic reagent (D. rerio)        | Tg(aplnrb:aplnrb-TagRFP-sGFPP) | This manuscript | ZFIN: bns309  |
| antibody                          | anti-FOXO1 (rabbit monoclonal) | Cell Signaling Technology | Cat#2880 (1:1000) |
| antibody                          | anti-pThr24FOXO1/pThr32FOXO3a (rabbit monoclonal) | Cell Signaling Technology | Cat#9464 (1:1000) |
| antibody                          | anti-c-MYC (rabbit polyclonal) | Cell Signaling Technology | Cat#9402 (1:1000) |
| antibody                          | anti-Tubulin (rabbit polyclonal) | Cell Signaling Technology | Cat#2148 (1:1000) |
| other                            | Taqman probe APLN | Thermo Fisher Scientific | Hs00175572_m1 |
| other                            | Taqman probe APLNR | Thermo Fisher Scientific | Hs00270873_s1 |
| other                            | Taqman probe PFKFB3 | Thermo Fisher Scientific | Hs00270873_s1 |
| other                            | Taqman probe ACTB | Thermo Fisher Scientific | Hs01060665_g1 |
| commercial assay or kit          | In-Fusion HD Cloning Plus | Takara Bio | Cat# 638910 |
| transfected construct (human)    | APLN         | Dharmacon          | Cat# L-017023-01-0005 50 nM |
| transfected construct (human)    | APLNR        | Dharmacon          | Cat# L-005430-00-0005 50 nM |
| transfected construct (human)    | ON-TARGETplus Non-targeting Pool | Dharmacon | Cat# D-001810-10-05 50 nM |
| commercial assay or kit          | mMessage mMachine SP6 Transcription Kit | Thermo Fisher Scientific | Cat# AM1340 |
| commercial assay or kit          | DIG RNA labelling kit | Roche | Cat# 11277073910 |
| commercial assay or kit          | SuperScript III First-Strand Synthesis System | Thermo Fisher Scientific | Cat#18080051 |
| commercial assay or kit          | RNA Clean and Concentrator Kit | Zymo Research | Cat# R1013 |
| software, algorithm              | ZEN Blue 2012 | Zeiss, Germany |             |
Zebrafish husbandry and strains

All zebrafish housing and husbandry were performed under standard conditions in accordance with institutional (Max Planck Society) and national ethical and animal welfare guidelines approved by the ethics committee for animal experiments at the Regierungspräsidium Darmstadt, Germany, as well as the FELASA guidelines (Alestro¨m et al., 2020). Embryos were staged by hours post fertilization (hpf) at 28.5˚C (Kimmel et al., 1995). The following lines were used: Tg(fli1a:EGFP)y1 (Lawson and Weinstein, 2002), Tg(fli1a:nEGFP)y7 (Roman et al., 2002), Tg(kdrl:HsHRAS-mCherry)s896 (Chi et al., 2008), aplnraμ296 (Helker et al., 2015), the aplnrbμ281 allele was generated using the same CRISPR as in Helker et al., 2015 and contains a 4 bp insertion 137 bp downstream of the ATG leading to a premature stop codon 196 bp downstream of the ATG, aplnμ267 (Helker et al., 2015), Tg(hsp70:bmp2b)y13 (Chocron et al., 2007), apelaβ13 (Chng et al., 2013), Tg(fli1a:LIFEACT-GFP)y10 (Hamm et al., 2016), Tg(fli1a:Hsa.B4GALT1-mCherry)y109 (Kwon et al., 2016), Tg(hsp70:apln)μ269 (this study), Tg(kdrl:HsHRAS-EGFP)μ280 (this study), Tg(apln:EGFP)μ157 (this study) and Tg(aplnrb: aplnrb-TagRFP-sfGFP)μ309 (this study).

Generation of the TgBAC(apln:EGFP)Bns157, TgBAC(aplnrb:aplnrb-TagRFP-sfGFP)Bns309, Tg(kdrl:HsHRAS-EGFP)mu280, and Tg(hsp70:apln)mu269 lines

To generate the apln and aplnrb bacterial artificial chromosome (BAC) constructs, we used the BAC clones RP71-2G21 containing the apln locus and CH211-102K containing the aplnrb locus. All recombineering steps were performed as described in Bussmann and Schulte-Merker, 2011 with the modifications as described in Helker et al., 2019. The following homology arms were used to generate the targeting PCR products of the EGFP_Kan, and TagRFP-sfGFP_Kan cassettes: apln-HA1: 5’-ccactacagtatatcagctagcgactggcagggaaacggagggagagcaaccatggtgagcaagggcgaggag-3’ and apln-HA2: 5’-ccacgcagaaacccacagcaaccagcagctgtaatctccacatattttcacagatgtagtgagag-3’. aplnrb-HA1: 5’-gccctttcttcagacagagggaccagcagcactgtctggagctagggctctcagctggagc-3’ and aplnrb-HA2: 5’-taatttgctgactgtctcccaaatctccatcttgctctctcagctgtaatctccagacagagggaccagcagcactgtctggagctagggctctcagctggagc-3’.

To generate the Tg(kdrl:HsHRAS-EGFP) and Tg(hsp70:apln) lines, the gateway recombination system (Invitrogen) using entry vectors and the pTolDest destination vector (Villefranc et al., 2007) was used. The apln coding sequence was amplified from cDNA. 100 pg DNA of the plasmids and 50 pg of tol2 mRNA were injected into one-cell stage zebrafish embryos for stable germline transmission.

Morpholino injections

Morpholinos were obtained from Gene Tools, resuspended in distilled H2O and around 2 nl was injected into 1 cell stage embryos. The following morpholinos were used: aplnrb MO (Helker et al., 2015) at 0.5 ng/embryo, dll4 MO (Hogan et al., 2009) at 3 ng/embryo. An equal amount of the standard control MO: 5’-CCTCTTACCTCAGTTACAATTTATA-3’ was used for each experiment.

Transplantation experiments

At the sphere stage, cells were removed from Tg(kdrl:HsHRAS-mCherry) donor embryos and transferred to Tg(fli1a:EGFP) aplnr mutant hosts using a glass capillary. Transplanted ECs were identified by transgenic mCherry expression.
Whole-mount in situ hybridization
Single in situ hybridizations were performed as described (Thisse and Thisse, 2008; Helker et al., 2013). The following probes were synthesized: apln (Helker et al., 2015), apela (Chng et al., 2013), aplnra (Helker et al., 2015), and aplnrb (Helker et al., 2015).

Confocal microscopy
Zebrafish larvae were mounted in 1% low melt agarose. Egg water and agarose were supplemented with 19.2 mg/l Tricaine. All fluorescent images were acquired using an upright Zeiss LSM 780, 800 or 880 or a Leica SP5 or SP8 confocal microscope. Maximum projection images were analyzed and generated using Imaris (Bitplane).

Quantification of mutant phenotypes
For every embryo, somites 5 to 15 were analyzed (normal: 10 fully developed ISVs and connected DLAV; mild: 10 ISVs fully developed but no DLAV; strong: 1 to 6 ISVs shortened; severe: 1 to 10 ISVs shortened).

Quantification of filopodia
Only filopodia with more than 10 μm in length were used for quantification.

Cell culture
Pooled human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (#CC-2519) and cultured in endothelial basal medium (EBM, Lonza) supplemented with hydrocortisone (1 μg/ml), bovine brain extract (12 μg/ml), gentamicin (50 μg/ml), amphotericin B (50 ng/ml), epidermal growth factor (10 ng/ml), and 10% fetal bovine serum (FBS, Life Technologies). HUVECs were tested for mycoplasma and cultured until the fourth passage. Cells were maintained at 37˚C in a humidified atmosphere with 5% CO₂.

RNA interference
To silence APLN and APLNR gene expression, HUVECs were transfected with 50 nM APLN and APLNR ON-TARGET SMARTpool siRNA (Dharmacon). As a control, a non-targeting siRNA pool was used (Dharmacon). HUVECs were grown to 70% confluency and transfected with Lipofectamine RNAiMAX (Life Technologies) according to manufacturer’s instructions.

Western blot analysis and antibodies
Western blot analyses were performed with precast gradient gels (Bio-Rad) using standard methods. Briefly, cells were lysed in RIPA buffer (Sigma; 150 mM NaCl, 1.0% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0) supplemented with Complete Protease Inhibitor Cocktail (Roche) and 1 mM PMSF. Proteins were separated by SDS-PAGE (Tris-glycine/SDS buffer, Bio-Rad) and transferred onto nitrocellulose membranes using the Trans Turbo Blot system (Bio-Rad). Membranes were probed with specific primary antibodies and then with peroxidase-conjugated secondary antibodies. The following primary antibodies were used: FOXO1 (Cell Signaling Technology, #2880, 1:1000), pThr24FOXO1/pThr32FOXO3a (Cell Signaling Technology, #9464, 1:1000), c-MYC (Cell Signaling Technology, #9402, 1:1000), Tubulin (Cell Signaling Technology, #2148, 1:1000), Secondary antibodies are peroxidase-conjugated Goat IgGs (1:5000) purchased from Jackson Immuno Research Labs. The target proteins were visualized by chemiluminescence...
using an ECL detection kit (Clarity Western ECL Substrate, Bio-Rad) and a ChemiDoc MP Imaging System (Bio-Rad).

**RT-qPCR**

Total RNA from HUVECs was extracted using a RNeasy Mini Kit (Qiagen). Reverse transcription polymerase chain reaction (RT-PCR) was performed using a SuperScript III First-Strand Synthesis System (Invitrogen) according to manufacturer’s instructions. RT-qPCR was carried out to quantify gene expression levels on a CFX connect Realtime System (Bio-Rad) with the following Taqman probes: APLN Hs00175572_m1, APLNR Hs00270873_s1, PFKFB3 Hs00270873_s1. Each sample was normalized to the housekeeping probe ACTB Hs01060665_g1.

**Metabolic assay**

The metabolism of cells was assessed by the measurement of extracellular acidification (ECAR) and oxygen consumption rates (OCR) using a Seahorse XFe96 analyser (Agilent). Four hours before the measurement, 40,000 HUVECs per well were seeded in a fibronectin-coated XFe96 microplate. The measurement was done following manufacturer’s protocol. To monitor glycolysis, the glycolysis stress test kit was used. The following substances were sequentially injected after a baseline measurement: Glucose (10 mM), Oligomycin (3 μM) and 2-Deoxyglucose (2-DG; 100 mM). The oxygen consumption rate was assessed using the Mito stress test kit. After a baseline measurement, the following substances were sequentially injected: Oligomycin (3 μM), the mitochondrial uncoupler carbonyl cyanide-4-(trifluoromethoxy)phenyl-hydrazone (FCCP; 1 μM) as well as a mixture of antimycin A (1.5 μM) and rotenone (3 μM).

**Statistics**

Standard error of the mean and P-values from a two-tailed t-test were calculated using Prism.

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**Author ORCIDs**
Christian SM Helker [https://orcid.org/0000-0003-0427-5338](https://orcid.org/0000-0003-0427-5338)
Toshiya Sugino [http://orcid.org/0000-0002-6330-7275](http://orcid.org/0000-0002-6330-7275)
Didier YR Stainier [https://orcid.org/0000-0002-0382-0026](https://orcid.org/0000-0002-0382-0026)

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Animal experimentation: Ethics Statement All zebrafish husbandry was performed under standard conditions, and all experiments were conducted in accordance with institutional (MPG) and national ethical and animal welfare guidelines (Proposal numbers: B2/1017, B2/1041, B2/1218, B2/1138). All procedures conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

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**Additional files**
Supplementary files
- Transparent reporting form

**Data availability**
All data generated or analysed during this study are included in the manuscript and supporting files.

**References**
Alastalo TP, Li M, Perez VJ, Pham D, Sawada H, Wang JK, Koskenvuo M, Wang L, Freeman BA, Chang HY, Rabinovitch M. 2011. Disruption of PPARγ/β-catenin-mediated regulation of apelin impairs BMP-induced
mouse and human pulmonary arterial EC survival. Journal of Clinical Investigation 121:3735–3746. DOI: https://doi.org/10.1172/JCI43382, PMID: 21821917

Aleström P, D’Angelo L, Midtlyng PJ, Schorderet DF, Schulte-Merker S, Sohm F, Warner S. 2020. Zebrafish: housing and husbandry recommendations. Laboratory Animals 54:213–224. DOI: https://doi.org/10.1177/0023677219869037

Bussmann J, Wolfe SA, Siemann AF. 2011. Arterial-venous network formation during brain vascularization involves hemodynamic regulation of chemokine signaling. Development 138:1717–1726. DOI: https://doi.org/10.1242/dev.098981, PMID: 21429983

Bussmann J, Schulte-Merker S. 2011. Rapid BAC selection for tol2-mediated transgenesis in zebrafish. Development 138:4327–4332. DOI: https://doi.org/10.1242/dev.068080, PMID: 21865323

Chandra SM, Razavi H, Kim J, Agrawal R, Kundu RK, de Jesus Perez V, Zamanian RT, Quertermous T, Chun HJ. 2011. Disruption of the apelin-APJ system worsens hypoxia-induced pulmonary hypertension. Arteriosclerosis, Thrombosis, and Vascular Biology 31:814–820. DOI: https://doi.org/10.1161/ATVBAHA.110.219980, PMID: 21233449

Chen T, Wu B, Lin R. 2017. Association of apelin and apelin receptor with the risk of coronary artery disease: a meta-analysis of observational studies. Oncotarget 8:57345–57355. DOI: https://doi.org/10.18632/oncotarget.17360, PMID: 28915675

Chi NC, Shaw RM, De Val S, Kang G, Jan LY, Black BL, Stainier DY. 2008. Foxn4 directly regulates tbx2b expression and atrioventricular canal formation. Genes & Development 22:734–739. DOI: https://doi.org/10.1101/gad.1629908, PMID: 18347092

Chng SC, Ho L, Tian J, Reversade B. 2013. ELABELA: a hormone essential for heart development signals via the apelin receptor. Developmental Cell 27:672–680. DOI: https://doi.org/10.1016/j.devcel.2013.11.002, PMID: 24316148

Chocron S, Verhoeven MC, Rentzsch F, Hammerschmidt M, Bakkers J. 2007. Zebrafish Bmp4 regulates left-right asymmetry at two distinct developmental time points. Developmental Biology 305:577–588. DOI: https://doi.org/10.1016/j.ydbio.2007.03.001, PMID: 17395172

Chun HJ, Ali ZA, Kojima Y, Kundu RK, Sheikh AY, Agrawal R, Zheng L, Leeper NJ, Pearl NE, Patterson AJ, Anderson JP, Tsao PS, Lenardo MJ, Ashley EA, Quertermous T. 2008. Apelin signaling antagonizes ang II effects in mouse models of atherosclerosis. Journal of Clinical Investigation 118:3343–3354. DOI: https://doi.org/10.1172/JCI34871, PMID: 18769630

Cox CM, D’Agostino SL, Miller MK, Heimark RL, Krieg PA. 2006. Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. Developmental Biology 296:177–189. DOI: https://doi.org/10.1016/j.ydbio.2006.04.452, PMID: 16750822

Davies MA, Lu Y, Sano T, Fang X, Tang P, LaPушin R, Koul D, Bookstein R, Yung WK, Mills GB, Steck PA. 1998. ‘Adenoviral transgene expression of MMAC/PTEN in human glioma cells inhibits Akt activation and induces anoikis’. Cancer Research 58:5285–5290. PMID: 9850049

De Bock K, Georgiadou M, Carmeliet P. 2013a. Role of endothelial cell metabolism in vessel sprouting. Cell Metabolism 18:634–647. DOI: https://doi.org/10.1016/j.cmet.2013.08.001, PMID: 23973331

De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquière B, Cauwenberghs S, Phng LK, Tembuyser B, Brepoels K, Welti J, Geudens I, Segura I, Cruys B, Bifari F, Decimo I, et al. 2013b. Role of PFKFB3-driven glycolysis in vessel sprouting. Developmental Cell 29:295–301. DOI: https://doi.org/10.1016/j.devcel.2013.01.002, PMID: 23911327

del Toro R, Prahst C, Mathivet T, Siegfried G, Kaminker JS, Lariviere B, Breant C, Duarte A, Takakura N, Fukamizu A, Penninger J, Eichmann A. 2010. Identification and functional analysis of endothelial tip cell-enriched genes. Blood 116:4025–4033. DOI: https://doi.org/10.1182/blood-2010-02-270819, PMID: 20705756

Dobrina A, Rossi F. 1983. Metabolic properties of freshly isolated bovine endothelial cells. Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research 76:95–101. DOI: https://doi.org/10.1016/0167-4889(83)90084-8

Dray C, Knauf C, Daviaud D, Waget A, Boucher J, Buléon M, Cani PD, Attané C, Guigné C, Carpeé C, Burcelin R, Castan-Laurell I, Valet P. 2008. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metabolism 8:437–445. DOI: https://doi.org/10.1016/j.cmet.2008.10.003, PMID: 19046574

Ferrara N, Gerber HP, LeCouter J. 2003. The biology of VEGF and its receptors. Nature Medicine 9:669–676. DOI: https://doi.org/10.1038/nm0603-669, PMID: 12778165

Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Altitol K, Shima D, Betsholtz C. 2003. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. Journal of Cell Biology 161:1163–1177. DOI: https://doi.org/10.1083/jcb.200302047, PMID: 12810700

Goetze JP, Rehfeld JF, Carlens J, Videbaek R, Andersen CB, Boesgaard S, Friis-Hansen L. 2006. Apelin: a new plasma marker of cardiopulmonary disease. Regulatory Peptides 133:134–138. DOI: https://doi.org/10.1016/j.regpep.2005.09.032, PMID: 16263185

Habata Y, Fujii R, Hosoya M, Fukusumi S, Kawamata Y, Hinuma S, Kitada C, Nishizawa N, Muroasaki S, Kurokawa T, Onda H, Tatemento K, Fujino M. 1999. Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research 1452:25–35. DOI: https://doi.org/10.1016/S0167-4889(99)00114-7

Hamm MJ, Kirchmaier BC, Herzog W. 2016. Semaphorin3d controls collective endothelial cell migration by distinct mechanisms via NrP1 and Pnxn1. Journal of Cell Biology 215:415–430. DOI: https://doi.org/10.1083/jcb.201603100, PMID: 27799363
et al. eLife 2020;9:e55589. DOI: https://doi.org/10.7554/eLife.55589

Helker et al. eLife 2020;9:e55589. DOI: https://doi.org/10.7554/eLife.55589

Hasan SS, Tsaryk R, Lange M, Wisniewski L, Moore JC, Lawson ND, Wojciechowska K, Schnittler H, Siekmann AF. 2017. Endothelial notch signalling limits angiogenesis via control of artery formation. Nature Cell Biology 19: 928–940. DOI: https://doi.org/10.1038/nccb3574, PMID: 28714969

Hashimoto T, Kihara M, Imai N, Yoshida S, Shimosayama H, Yasuzaki H, Ishida J, Toya Y, Kiuchi Y, Hirawa N, Tamura K, Yazawa T, Kitamura H, Fukamizu A, Umemura S. 2007. Requirement of apelin-apelin receptor system for oxidative stress-linked atherosclerosis. The American Journal of Pathology 171:1705–1712. DOI: https://doi.org/10.1016/j.ajpath.2007.07.0471, PMID: 17884970

Helker CS, Schuermann A, Karpen T, Zeuschner D, Belting HG, Affolter M, Schulte-Merker S, Herzog W. 2013. The zebrafish common cardinal veins develop by a novel mechanism: lumen ensheathment. Development 140: 2776–2786. DOI: https://doi.org/10.1242/dev.091876, PMID: 23698350

Helker CSM, Schuermann A, Pollmann C, Chng SC, Kiefer F, Reversade B, Herzog W. 2015. The hormonal peptide elabela guides angioblasts to the midline during vasculogenesis. eLife 4:e06726. DOI: https://doi.org/10.7554/eLife.06726

Helker CSM, Mullapudi ST, Mueller LM, Preussner J, Tunaru S, Skog O, Kwon HB, Kreuder F, Lacman JJ, Bonnavion R, Dong PDS, Looso M, Offermanns S, Korsgren O, Spagnoli FM, Stainier DYR. 2019. A whole organism small molecule screen identifies novel regulators of pancreatic endocrine development. Development 146:dev172569. DOI: https://doi.org/10.1242/dev.172569, PMID: 31245359

Hesslström M, Phng LK, Hofmann JJ, Wallgard E, Coutlas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalén M, Gerhardt H, Betsholtz C. 2007. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature 445:776–780. DOI: https://doi.org/10.1038/nature05571, PMID: 17259973

Herbert SP, Cheung JY, Stainier DYR. 2012. Determination of endothelial stalk versus tip cell potential during angiogenesis by H2.0-like homeobox-1. Current Biology 22:1789–1794. DOI: https://doi.org/10.1016/j.cub.2012.07.037, PMID: 22921365

Hogan BM, Herpers R, Witte M, Helotère H, Altalalio K, Duckers HJ, Schulte-Merker S. 2009. Vegfc/Ft4 signalling is suppressed by Dil4 in developing zebrafish intersegmental arteries. Development 136:4001–4009. DOI: https://doi.org/10.1242/dev.039990, PMID: 19906867

Hwangbo C, Wu J, Papangeli I, Adachi T, Sharma B, Park S, Zhao L, Ju H, Go GW, Cui G, Inayathullah M, Job JK, Rajadas J, Kweli SL, Li MO, Morrison AR, Quertermous T, Mani A, Red-Horse K, Chun HJ. 2017. Endothelial APLNR regulates tissue fatty acid uptake and is essential for apelin’s glucose-lowering effects. Translational Medicine 9:eaad4000. DOI: https://doi.org/10.1100/scttranslmed.aad4000, PMID: 28904225

Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, Rosewell I, Busse M, Thurstorn G, Medvinsky A, Schulte-Merker S, Gerhardt H. 2010. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. Nature Cell Biology 12:943–953. DOI: https://doi.org/10.1038/nccb2103, PMID: 20871601

Kärin RE, Kretz MP, Meyer AM, Kispert A, Heppner FL, Brändli AW. 2007. Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. Developmental Biology 305:599–614. DOI: https://doi.org/10.1016/j.ydbio.2007.03.004, PMID: 17412318

Kidoya H, Ueno M, Yamada Y, Mochizuki N, Nakata M, Yano T, Fujii R, Takakura N. 2008. Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. The EMBO Journal 27:522–534. DOI: https://doi.org/10.1038/sj.emboj.7601982, PMID: 18200044

Kidoya H, Naito H, Takakura N. 2010. Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. Blood 115:3166–3174. DOI: https://doi.org/10.1182/blood-2009-07-232306, PMID: 20185589

Kidoya H, Kunii N, Naito H, Muramatsu F, Okamoto Y, Nakayama T, Takakura N. 2012. The apelin/APJ system induces maturation of the vascular vasculature and improves the efficiency of immune therapy. Oncogene 31: 3254–3264. DOI: https://doi.org/10.1038/onc.2011.489, PMID: 22037214

Kidoya H, Naito H, Muramatsu F, Yamakawa D, Jia W, Ikawa M, Sonobe T, Tsuchimochi H, Shirai M, Adams RH, Fukamizu A, Takakura N. 2015. APJ regulates parallel alignment of arteries and veins in the skin. Developmental Cell 33:247–259. DOI: https://doi.org/10.1016/j.devcel.2015.02.024, PMID: 25920569

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. 1995. Stages of embryonic development of the zebrafish. Developmental Dynamics 203:253–310. DOI: https://doi.org/10.1002/aja.1002030302, PMID: 8589427

Koch S, Claesson-Welsh L. 2012. Signal transduction by vascular endothelial growth factor receptors. Cold Spring Harbor Perspectives in Medicine 2:a006502. DOI: https://doi.org/10.1101/cshperspect.a006502

Kojima Y, Kundu RK, Cox CM, Leeper NJ, Anderson JA, Chun HJ, Ali ZA, Ashley EA, Krieg PA, Quertermous T. 2010. Upregulation of the apelin–APJ pathway promotes neointima formation in the carotid ligation model in mouse. Cardiovascular Research 87:156–165. DOI: https://doi.org/10.1093/cvr/cvq052, PMID: 20176814

Krützfeldt A, Spahr R, Mertens S, Siegmund B, Piper HM. 1990. Metabolism of exogenous substrates by coronary endothelial cells in culture. Journal of Molecular and Cellar Cardiology 22:1393–1404. DOI: https://doi.org/10.1016/0022-2828(90)90984-A, PMID: 2089157

Kwon HB, Wang S, Helker CS, Rasouli SJ, Maischein HM, Offermanns S, Herzog W, Stainier DY. 2016. In vivo modulation of endothelial polarization by apelin receptor signalling. Nature Communications 7:11805. DOI: https://doi.org/10.1038/ncomms11805, PMID: 27248505

Lawson ND, Weinstein BM. 2002. In vivo imaging of embryonic vascular development using transgenic zebrafish. Developmental Biology 248:307–318. DOI: https://doi.org/10.1006/dbio.2002.0711
Leslie JD, Ariza-McNaughton L, Bermange AL, McDow R, Johnson SL, Lewis J. 2007. Endothelial signalling by the notch ligand Delta-like 4 restricts angiogenesis. Development 134:839–844. DOI: https://doi.org/10.1242/dev.003244, PMID: 17251261

Lobev IB, Renard RA, Papadopoulos N, Gale NW, Thurston G, Yiacoupolous GD, Wiegand SJ. 2007. Delta-like ligand 4 (DLL4) is induced by VEGF as a negative regulator of angiogenic sprouting. PNAS 104:3219–3224. DOI: https://doi.org/10.1073/pnas.0611206104, PMID: 17296940

Masri B, Morin N, Cornu M, Knibiehler B, Audigier Y. 2004. Apelin (65-77) activates p70 S6 kinase and is mitogenic for umbilical endothelial cells. The FASEB Journal 18:1909–1911. DOI: https://doi.org/10.1096/fj.04-19305e, PMID: 15385434

Mertens S, Noll T, Spahr R, Krutzfeldt A, Piper HM. 1990. Energetic response of coronary endothelial cells to hypoxia. American Journal of Physiology-Heart and Circulatory Physiology 258:H689–H694. DOI: https://doi.org/10.1152/ajpheart.1990.258.3.H689

Moepps B, Frodl R, Rodewald HR, Baggioni M, Gierschik P. 1997. Two murine homologues of the human chemokine receptor CXCR4 mediating stromal cell-derived factor 1alpha activation of Gi2 are differentially expressed in vivo. European Journal of Immunology 27:2102–2112. DOI: https://doi.org/10.1002/eji.1830270839, PMID: 9295051

Papangeli I, Kim J, Maier I, Park S, Lee A, Kang Y, Tanaka K, Khan OF, Ju H, Koijima Y, Red-Horse K, Anderson DG, Siekmann AF, Chun HJ. 2016. MicroRNA 139-5p coordinates APLNR-CXCR4 crosstalk during vascular maturation. Nature Communications 7:11268. DOI: https://doi.org/10.1038/ncomms11268, PMID: 27068335

Paula A, Norris ML, Valen E, Chew GL, Gagnon JA, Zimmerman S, Mitchell A, Ma J, Dubulle J, Reyon D, Tsai SQ, Joung JK, Saghatelian A, Schier AF. 2014. Toddler: an embryonic signal that promotes cell movement via apelin receptors. Science 343:1248636. DOI: https://doi.org/10.1126/science.1248636, PMID: 24407481

Phng L-K, Stanchi F, Gerhardt H. 2013. Filopodia are dispensable for endothelial tip cell guidance. Development 140:4031–4040. DOI: https://doi.org/10.1242/dev.097352

Pitkin SL, Maguire JJ, Kuc RE, Davenport AP. 2010. Modulation of the apelin/APJ system in heart failure and atherosclerosis in man. British Journal of Pharmacology 160:1785–1795. DOI: https://doi.org/10.1111/j.1476-5381.2010.01082.x, PMID: 20649580

Pitulescu ME, Schmidt I, Giaimo BD, Antoine T, Berkenfeld F, Ferrante F, Park H, Ehling M, Blajes D, Rocha SF, Langen UN, Stehling M, Nagasawa T, Ferrara N, Borggreve T, Adams RH. 2017. DLL4 and notch signalling couples sprouting angiogenesis and artery formation. Nature Cell Biology 19:915–927. DOI: https://doi.org/10.1038/nccb3555, PMID: 28714968

Roman BL, Pham VN, Lawson ND, Kulik M, Childs S, Lekven AC, Garrity DM, Moon RT, Fishman MC, Lechleider RJ, Weinstein BM. 2002. ‘Disruption of acvrl1 increases endothelial cell number in zebrafish cranial vessels’. Development 129:3009–3019. PMID: 12050147

Sawane M, Kajiya K, Kidoya H, Takagi M, Muramatsu F, Takakura N. 2013. Apelin inhibits diet-induced obesity by enhancing lymphatic and blood vessel integrity. Diabetes 62:1970–1980. DOI: https://doi.org/10.2337/db12-0604, PMID: 23378608

Schlereth K, Weichenhan D, Bauer T, Heumann T, Giannakouri E, Chivite I, Angulo-Urarte A, Soler A, Sutherland JD, Arruabarrena-Aristoena A, Ragab A, Lim R, Malumbres M, Fruttiger M, Potente M, Serrano M, Fabra A, Viñals F, Casanovaos O, Pandolfo PP, Bigas A, Cancedo D, Onda H, Fujino M. 1998. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Circulation Research 83:585–598. DOI: https://doi.org/10.1161/01.res.0000033522.37861.69, PMID: 9295051

Tammela T, Zarkada G, Wallgard E, Murtomäki A, Suchting S, Wirzenius M, Waltari M, Hellström M, Schomber T, Peltonen R, Freitas C, Duarte A, Isomoi H, Laakonen P, Christofori G, Ylä-Herttuala S, Shibuya M, Pytowski B, Eichmann A, Betsholtz C, et al. 2008. Blocking VEGFR-3 suppresses angiogenic sprouting and vessel branching. PNAS 104:3225–3230. DOI: https://doi.org/10.1073/pnas.0611177104, PMID: 17296941

Tatemoto K, Hosoya M, Habata Y, Fuji R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, Fujino M. 1998. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochemical and Biophysical Research Communications 251:471–476. DOI: https://doi.org/10.1016/S0006-291X(98)91970-9, PMID: 9792798

Tempel D, de Boer M, van Deel ED, Haasdijk RA, Duncker DJ, Cheng C, Schulte-Merkel S, Duckers HJ. 2012. Apelin enhances cardiac neovascularization after myocardial infarction by recruiting aplnr+ circulating cells. Circulation Research 111:585–598. DOI: https://doi.org/10.1161/CIRCRESAHA.111.262097, PMID: 22753078

Thiss C, Thisse B. 2008. High-resolution in situ hybridization to whole-mount zebrafish embryos. Nature Protocols 3:59–69. DOI: https://doi.org/10.1038/nprot.2007.514
Torres-Vázquez J, Gitler AD, Fraser SD, Berk JD, Pham VN, Fishman MC, Childs S, Epstein JA, Weinstein BM. 2004. Semaphorin-plexin signaling guides patterning of the developing vasculature. Developmental Cell 7:117–123. DOI: https://doi.org/10.1016/j.devcel.2004.06.008, PMID: 15239959

Uribesalgo I, Hoffmann D, Zhang Y, Kavirayani A, Lazovic J, Berta J, Novatchkova M, Pai TP, Wimmer RA, László V, Schramek D, Karim R, Tortola L, Deswal S, Haas L, Zuber J, Szücs M, Kuba K, Dome B, Cao Y, et al. 2019. Apelin inhibition prevents resistance and metastasis associated with anti-angiogenic therapy. EMBO Molecular Medicine 11:e9266. DOI: https://doi.org/10.15252/emmm.201809266, PMID: 31267692

Vandekeere S, Dewerchin M, Carmeliet P. 2015. Angiogenesis revisited: an overlooked role of endothelial cell metabolism in vessel sprouting. Microcirculation 22:509–517. DOI: https://doi.org/10.1111/micc.12229, PMID: 26250801

Vanhollebeke B, Stone OA, Bostaille N, Cho C, Zhou Y, Maquet E, Cabochette P, Fukuhara S, Mochizuki N, Nathans J, Stainier DYR. 2015. Tip cell-specific requirement for an atypical Gpr124- and Reck-dependent wnt/β-catenin pathway during brain angiogenesis. eLife 4:e06489. DOI: https://doi.org/10.7554/eLife.06489

Villefranc JA, Amigo J, Lawson ND. 2007. Gateway compatible vectors for analysis of gene function in the zebrafish. Developmental Dynamics 236:3077–3087. DOI: https://doi.org/10.1002/dvdy.21354, PMID: 17948311

Wang W, McKinnie SM, Patel VB, Haddad G, Wang Z, Zhabyeyev P, Das SK, Basu R, McLean B, Kandalam V, Penninger JM, Kassiri Z, Vederas JC, Murray AG, Oudit GY. 2013. Loss of apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic apelin analogues. Journal of the American Heart Association 2:e000249. DOI: https://doi.org/10.1161/JAHA.113.000249, PMID: 23817469

Wiley DM, Kim JD, Hao J, Hong CC, Bautch VL, Jin SW. 2011. Distinct signalling pathways regulate sprouting angiogenesis from the dorsal aorta and the axial vein. Nature Cell Biology 13:686–692. DOI: https://doi.org/10.1038/ncb2232, PMID: 21572418

Wilhelm K, Happel K, Eelen G, Schoors S, Oellerich MF, Lim R, Zimmermann B, Aspalter IM, Franco CA, Boettger T, Braun T, Fruttiger M, Rajewsky K, Keller C, Brüning JC, Gerhardt H, Carmeliet P, Potente M. 2016. FOXP1 couples metabolic activity and growth state in the vascular endothelium. Nature 529:216–220. DOI: https://doi.org/10.1038/nature16498, PMID: 26735015

Zhang X, Hu W, Feng F, Xu J, Wu F. 2016. Apelin-13 protects against myocardial infarction-induced myocardial fibrosis. Molecular Medicine Reports 13:5262–5268. DOI: https://doi.org/10.3892/mmr.2016.5163, PMID: 27109054

Zhao H, Tian X, He L, Li Y, Pu W, Liu Q, Tang J, Wu J, Cheng X, Liu Y, Zhou Q, Tan Z, Bai F, Xu F, Smart N, Zhou B. 2018. Apj+ vessels drive tumor growth and represent a tractable therapeutic target. Cell Reports 25:1241–1254. DOI: https://doi.org/10.1016/j.celrep.2018.10.015, PMID: 30204115