

Figure S1

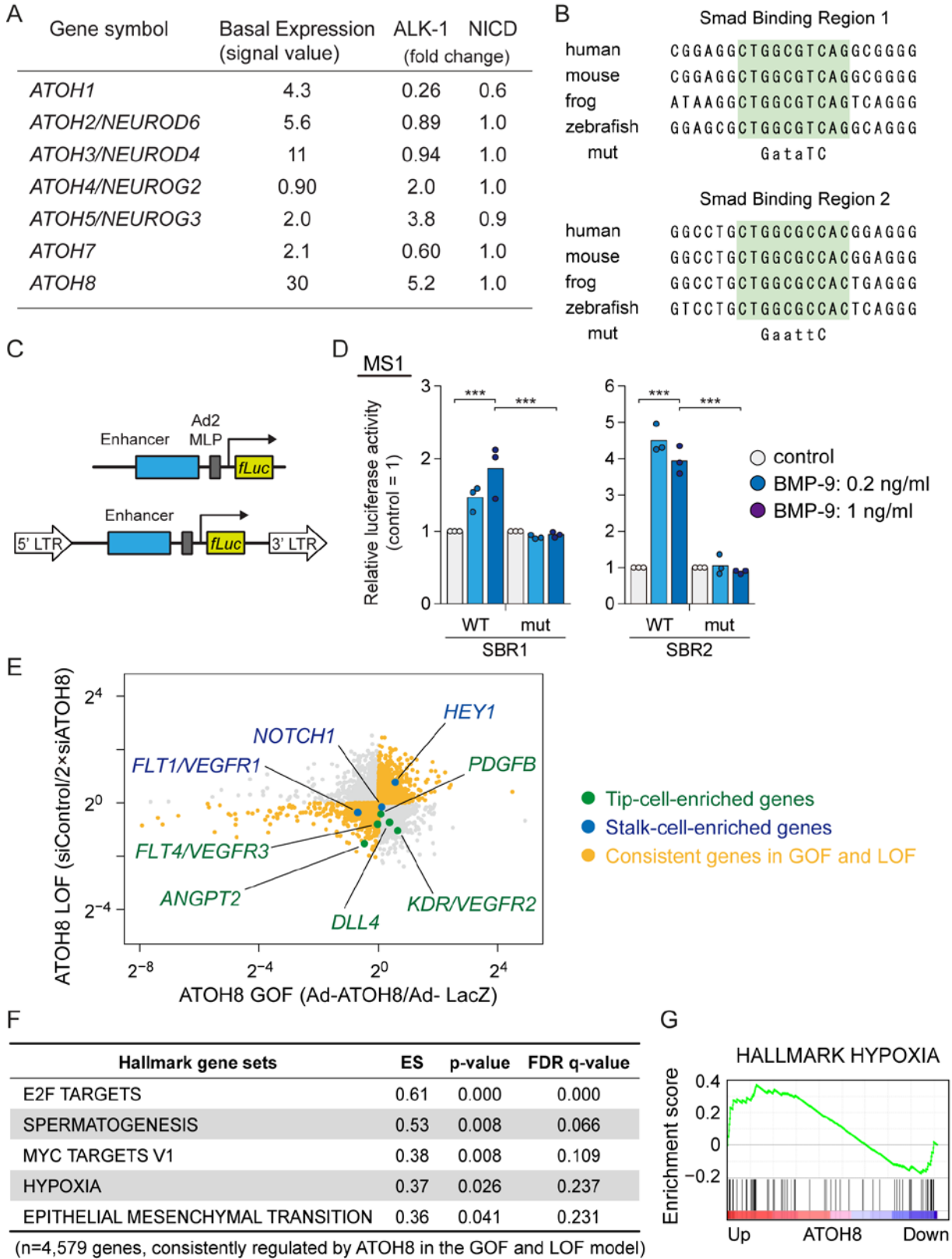


Figure S1. ATOH8 is induced by BMP-9/ALK-1 but not by Notch in ECs

(A) Microarray data of HUVECs treated with BMP-9 (1 ng ml⁻¹) (GSE27661) (5). mRNA expression data of *atonal*-family members are shown .

(B) Multiple sequence alignment of predicted SMAD1/5 binding sites in Smad binding region (SBR) with their vertebrate counterparts. mut: mutations in a SMAD1/5 binding motif, or GC-SBE.

(C) Schematic presentation of BMP reporter constructs. Ad2 MLP; minimal adenoviral major late promoter.

(D) Luciferase reporter assay of MS1 cells transfected with the indicated reporter constructs, and treated with the indicated concentrations of BMP-9 for 16 h. Results of n=3 independent experiments are shown by scatter plots with bars representing the means. Differences between the conditions were analyzed by Tukey's honestly significant difference (HSD) test corrected for multiple comparisons; ***p < 0.001.

(E) Scatter plot presentation of microarray data of ATOH8 gain-of-function (GOF) and loss-of-function (LOF). Genes consistently regulated by ATOH8 in GOF and LOF were colored orange. Genes enriched in tip and stalk cell phenotypes are colored green and blue, respectively.

(F and G) Gene set enrichment analysis (GSEA) of expression changes by ATOH8. The relation between ATOH8-induced and -repressed genes and MsigDB hallmark gene set signatures were assessed. Gene sets with a p-value < 5% and an FDR q-value < 25% were considered significant. ES; enrichment score.

Figure S2

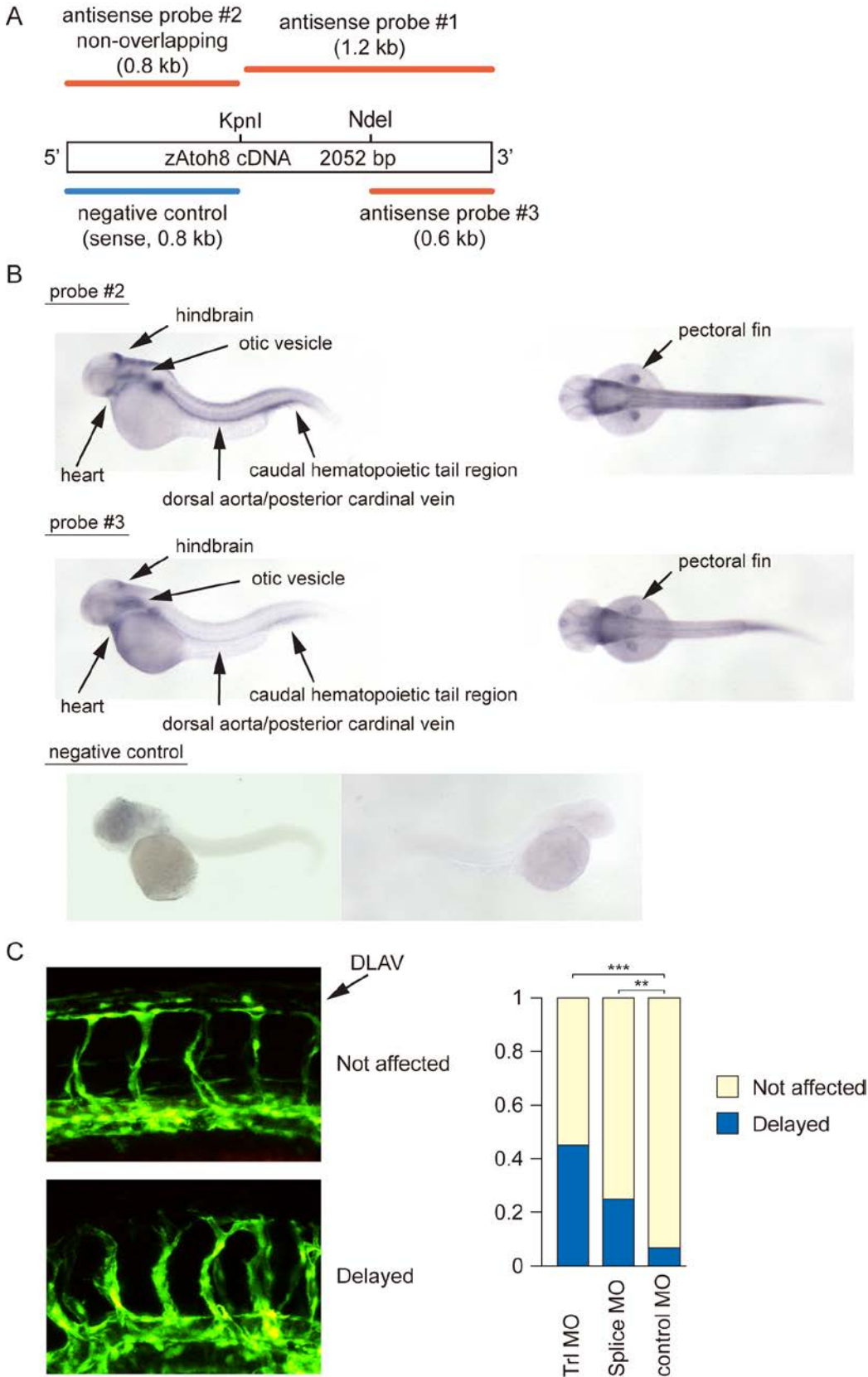


Figure S2. Zebrafish *atoh8* is expressed in large vessels and *atoh8* morphants have a slight delay in ISV formation

(A) Probes for whole-mount *in situ* hybridization of zebrafish *atoh8* used in the present study.

(B) Whole-mount *in situ* hybridization of zebrafish *atoh8* at 48 hpf (hours after fertilization). Related to Fig. 3A.

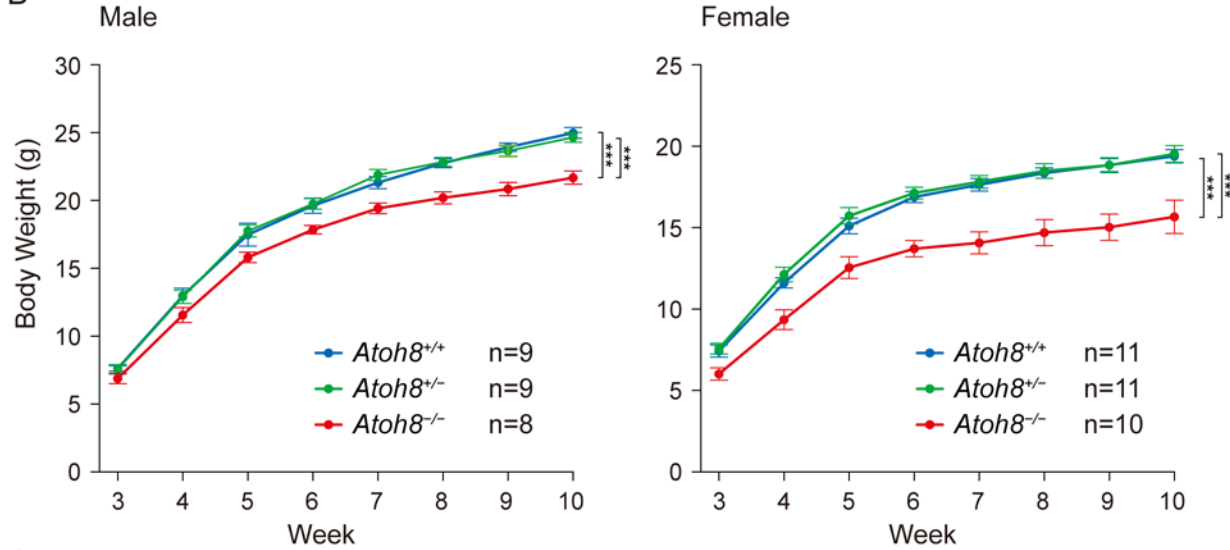
(C) Tg(*fli1:EGFP*) zebrafish were injected with control, translational blocking (Trl), or splice blocking (Splice) morpholinos (MOs) and analyzed for intersegmental vessel (ISV) outgrowth at 30 hpf. Representative images (left) and percentage of fish with delayed sprouting of ISV (right) at 30 hpf are presented. The data is the sum of multiple independent experiments, n=20, 12, and 15 for Trl MO, Splice MO, and control MO, respectively. Differences between the conditions were analyzed by Fisher's exact test with Bonferroni correction; **p < 0.01, ***p < 0.001. DLAV; dorsal longitudinal anastomotic vessels.

Figure S3

A

Cross: <i>Atoh8</i> ^{+/-} × <i>Atoh8</i> ^{+/-}			
Genotype	Number	Observed %	Expected Number
<i>Atoh8</i> ^{+/+}	95	24.4%	97.5
<i>Atoh8</i> ^{+/-}	208	53.3%	195
<i>Atoh8</i> ^{-/-}	87	22.3%	97.5
Total	390		(p = 0.35)

B



C

Female (P14)

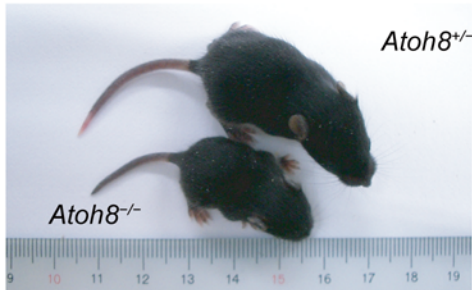


Figure S3. *Atoh8*^{-/-} mice are small in body size but are viable and fertile

(A) Genotypes of progeny from *Atoh8*^{+/-} heterozygous intercrosses at postnatal day 5. p-value was determined by chi-square test.

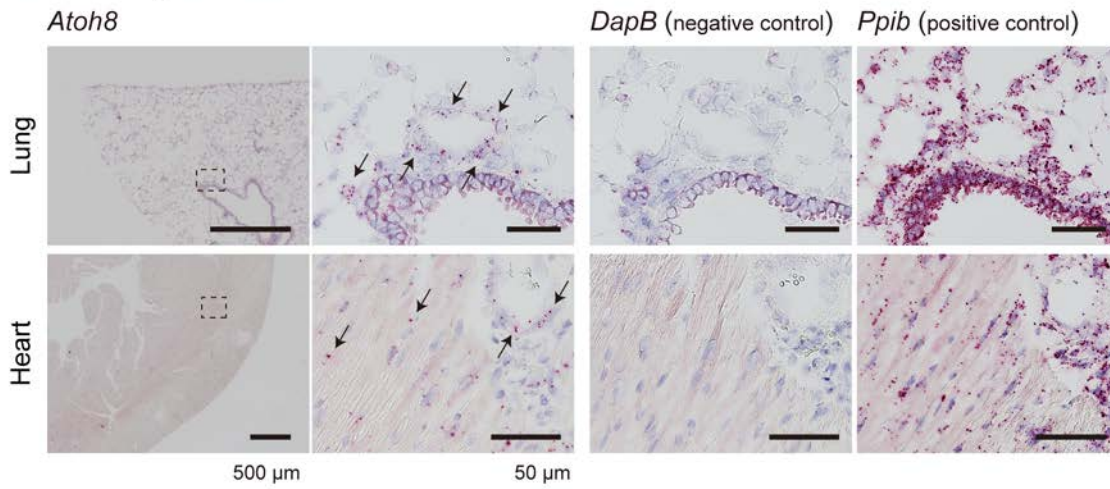
(B) Body weight, mean ± SEM. Differences between the conditions were analyzed by Tukey's HSD test corrected for multiple comparisons; ***p < 0.001.

(C) Representative image of female mice at P14 (or at 2 weeks of age)

Figure S4

A

RNA in situ hybridization



B

n=12 for each group	Normoxia			Hypoxia		
	<i>Atoh8</i> ^{+/+} (a)	<i>Atoh8</i> ^{-/-} (b)	(a) vs (b)	<i>Atoh8</i> ^{+/+} (c)	<i>Atoh8</i> ^{-/-} (d)	(c) vs (d)
Body weight (g)	31.9±0.5	26.2±0.4	***	30.6±0.4	24.2±0.5	***
Systolic BP (mmHg)	98.9±1.5	97.8±2.7	n.s.	95.6±1.4	91.2±1.1	*
Diastolic BP (mmHg)	66.2±2.4	71.1±2.3	n.s.	71.9±2.3	61.8±1.9	**
Mean BP (mmHg)	77.1±1.8	79.8±2.2	n.s.	79.8±1.9	71.4±1.6	**
Heart rate (beats per min)	535.5±21.4	639.5±21.3	**	531.2±15.9	534.4±12.6	n.s.

C

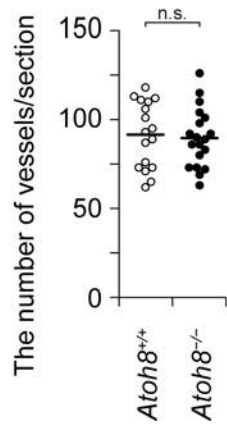


Figure S4. *Atoh8* is expressed in ECs in the lung and *Atoh8*^{-/-} mice exhibits a PAH-like phenotype

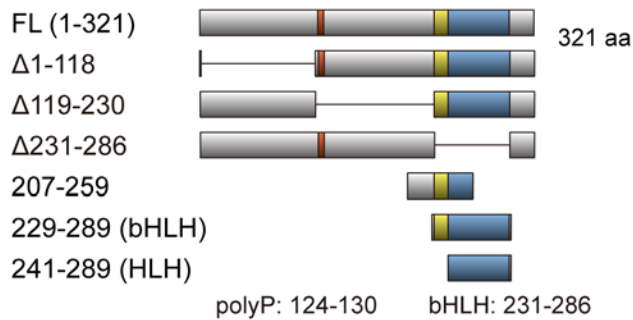
(A) In situ hybridization for expression of mouse *Atoh8* mRNA (red dots, indicated by arrows) and control genes in the lung and heart of aged mice housed in the hypoxic condition. High-magnification images of the dashed-square areas are presented. Images are representative of different experiments (more than n=3 independent samples), Scale bar: 500 μm (left) and 50 μm (right three panels).

(B) Table of results of hemodynamics, mean ± SEM. Differences between the groups were analyzed by Welch's *t*-test, or unequal variances *t*-test, with *p <0.05, **p <0.01, and ***p <0.001 being considered significant.

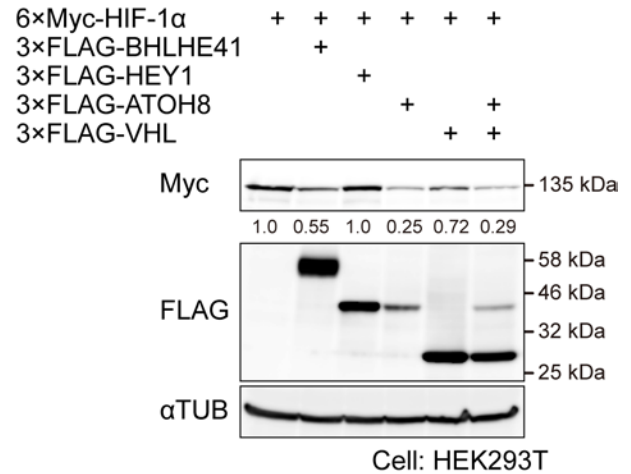
(C) Assessment of vessel density in the lung of *Atoh8*^{-/-} mice. The number of the peripheral pulmonary arteries ranging from 25 to 75 μm in size was counted in *Atoh8*^{+/+} (n=17) and *Atoh8*^{-/-} (n=19) male mice. The data are presented as scatter plot with mean, and difference between the groups was analyzed by Welch's *t*-test, or unequal variances *t*-test.

Figure S5

A



B



C

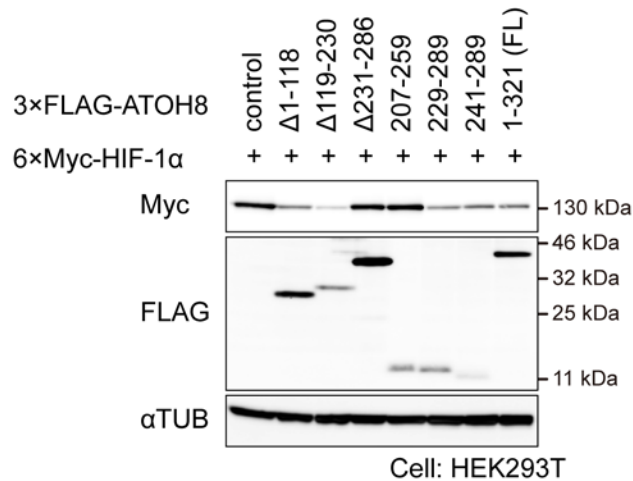


Figure S5. ATOH8 interacts with HIF-1 α and decreases its abundance

(A) Schematic presentation of ATOH8 full-length protein and mutants used in this study.

(B) HEK293T cells were transfected with the indicated plasmids and subjected to blotting. α TUB was used as a loading control. Blots are representative of n=3 independent experiments. The band densities of Myc-HIF-1 α were normalized to those of α TUB, and means from n=3 independent experiments are presented.

(C) HEK293T cells were transfected with the indicated plasmids and subjected to Western blotting. α TUB was used as a loading control. Blots are representative of n=3 independent experiments.

Data File S1. Gene regulation of 70 putative direct target genes of BMP-9/ALK-1

The table contains the regulation of 70 putative direct target genes of BMP-9/ALK-1 (GSE27661) (5), together with fold change calculations after overexpression of Notch1 intracellular domain (GSE29850) (20).

Data File S2. SMAD1/5 binding sites in HUVECs, which are related to blood vessels or the heart

The table contains SMAD1/5 binding sites in HUVECs that are related to blood vessels or the heart (VISTA Enhancer Browser) (22).

Data File S3. Genomic regions commonly bound by FLAG-ATOH8 in HPAEC-FLAG-ATOH8 cells.

The table lists genomic regions commonly bound by FLAG-ATOH8 in HPAEC-FLAG-ATOH8 cells, identified by ChIP-seq experiment. Results of n=2 independent experiments are analyzed (the bed format, hg19).

Data File S4. Primer sets used for qRT-PCR and ChIP-qPCR.

The table lists primer sets used for qRT-PCR and ChIP-qPCR.