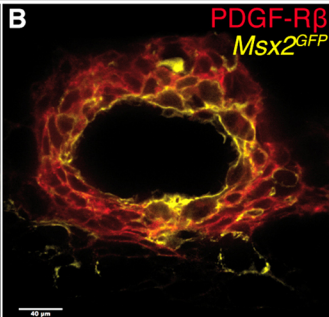
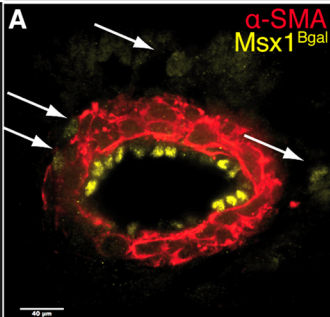
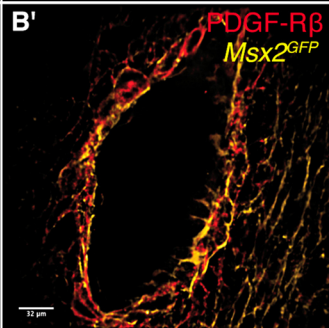
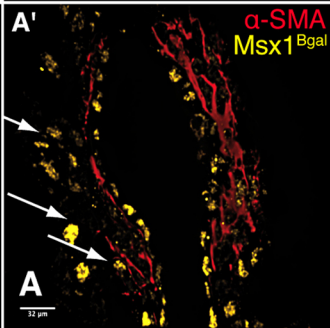


Msx1^{+/-}, *Msx2*^{+/-lox}, α -Sm22Cre

Carotid artery - E12.5

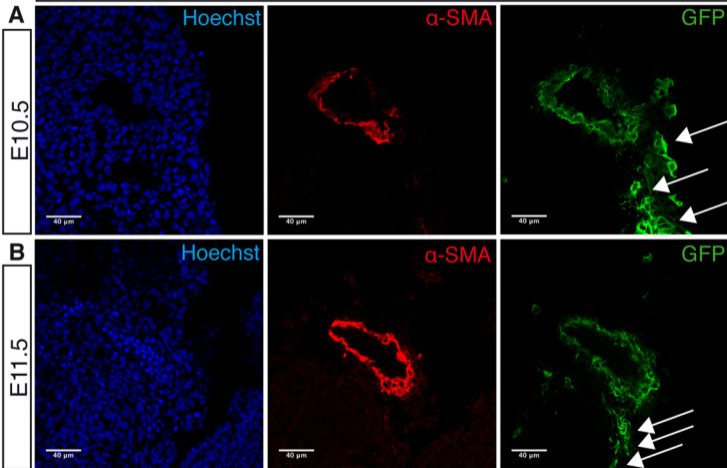


Vertebral artery - E12.5

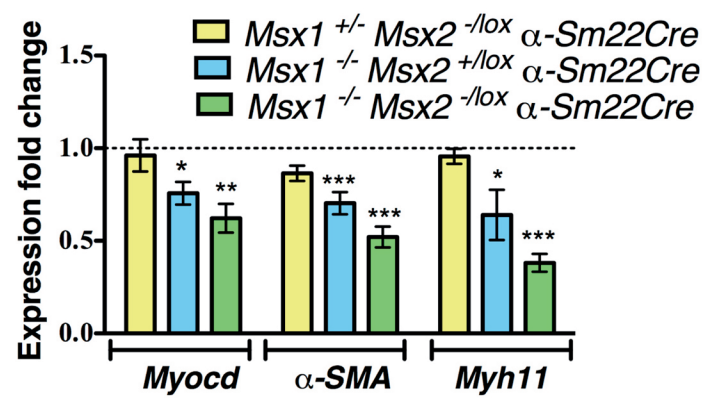


Sup Fig S1

Carotid artery - *Sm22Cre Rosa^{mT/mG}*

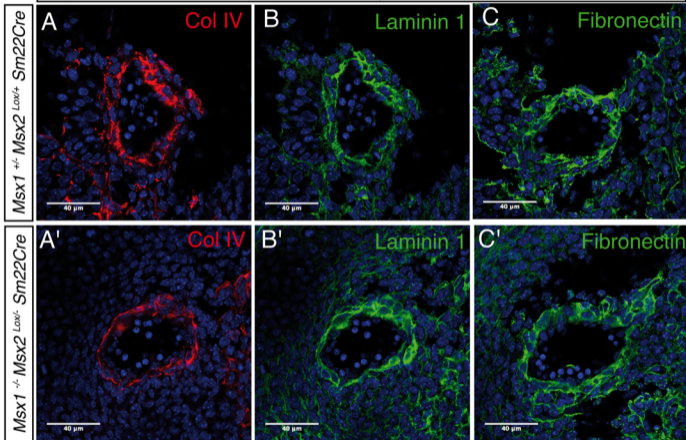


Sup Fig. S2



Sup Fig. S3

Carotid Artery - E12.5



Sup. Fig S4

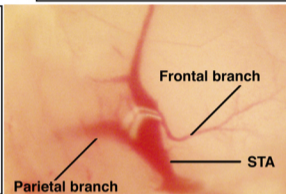
Superior temporal artery (STA) - P0

A



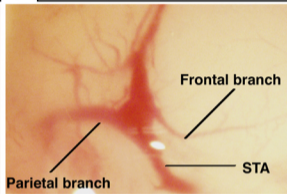
B

Msx1^{+/-}, *Msx2*^{+/-lox}, α -Sm22Cre



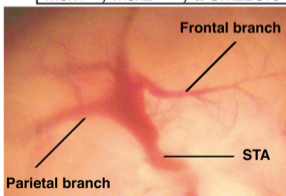
C

Msx1^{+/-}, *Msx2*^{-/-lox}, α -Sm22Cre



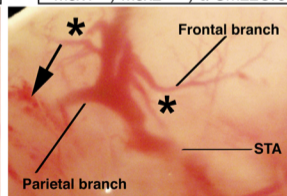
D

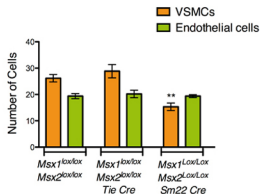
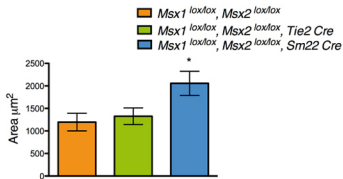
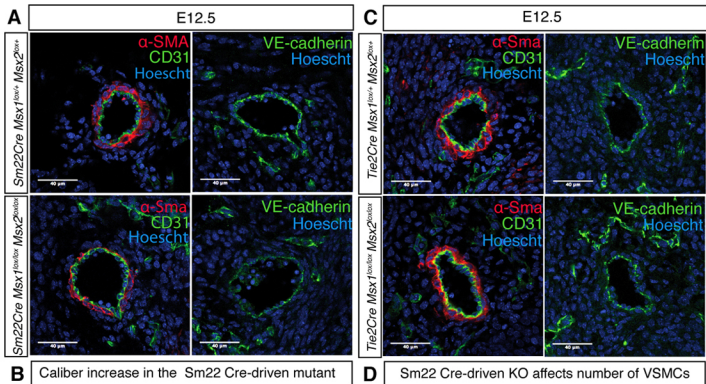
Msx1^{-/-}, *Msx2*^{+/-lox}, α -Sm22Cre



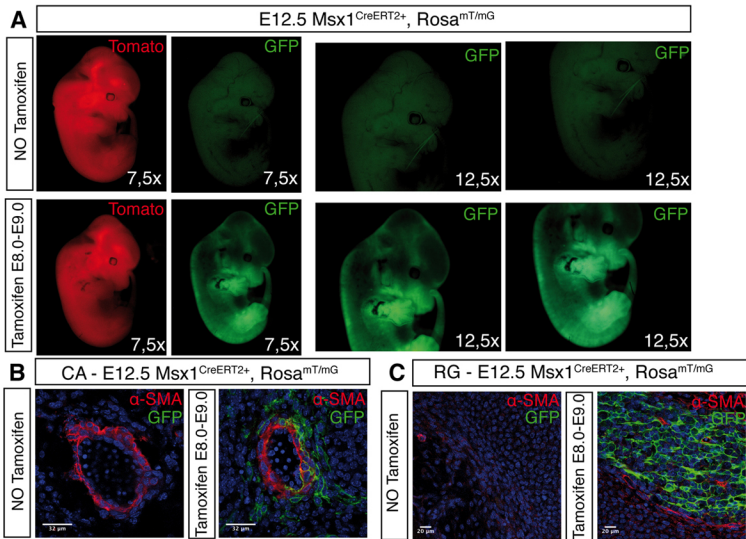
E

Msx1^{-/-}, *Msx2*^{-/-lox}, α -Sm22Cre



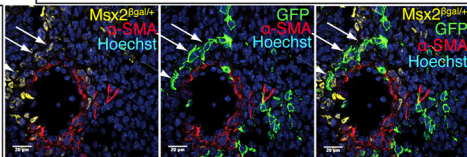


Sup Fig. S6

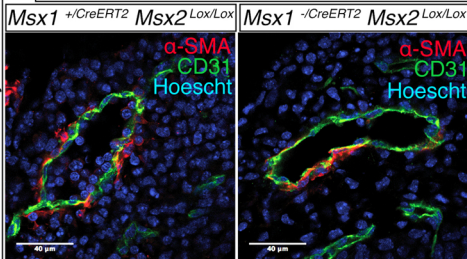


Sup Fig. S7

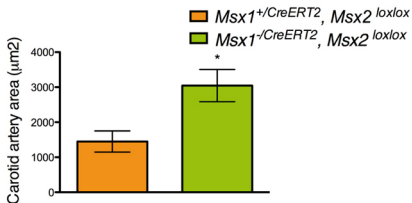
A E11.5 - *Msx1*^{CreERT2/+} *Msx2*^{LacZ/+} *Rosa*^{mT/mG}

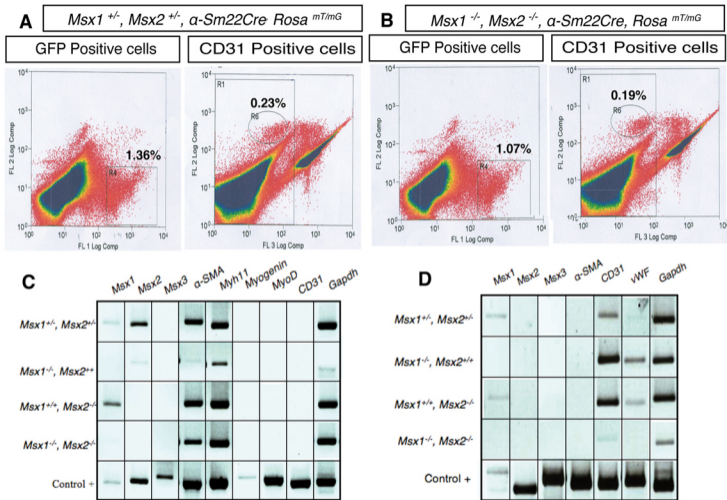


B E11.5



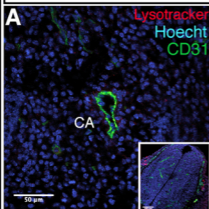
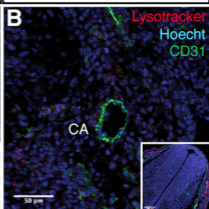
C



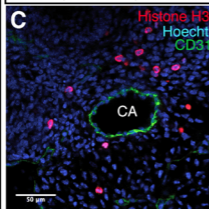
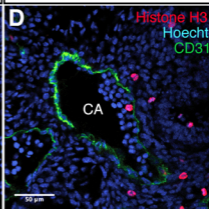


Sup Fig. S9

Apoptosis - E11.5

Msx1^{+/-}, *Msx2*^{+/-lox}, *Sm22Cre**Msx1*^{-/-}, *Msx2*^{-/-lox}, *Sm22Cre*

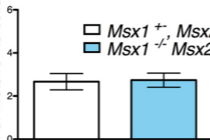
Proliferation - E12.5

Msx1^{+/-}, *Msx2*^{+/-lox}, *Sm22Cre**Msx1*^{-/-}, *Msx2*^{-/-lox}, *Sm22Cre***E**

Cell proliferation - Quantification

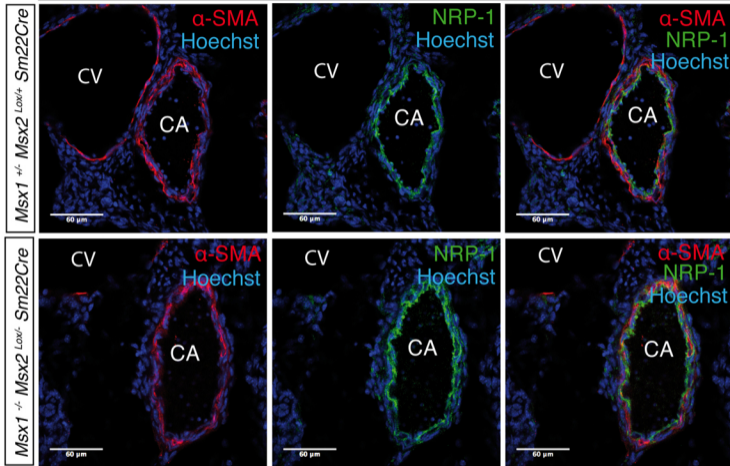
% of Histone H3 cells

□ *Msx1*^{+/-}, *Msx2*^{+/-lox}, α -*Sm22cre*
■ *Msx1*^{-/-}, *Msx2*^{-/-lox}, α -*Sm22Cre*



Sup. Fig S10

E14.5



Sup. Fig. S11

Legends to Supplementary figures and videos

Supplementary video S1A – An OPT 3D representation of the E11.5 control *Msx1*^{+/-}, *Msx2*^{+/lox}, *α-Sm22Cre* embryo head (embryo is from the same litter as the one in supplementary video S1B). The CD31 fluorescent signal is figured in brown, the anatomical data obtained by the embryo auto fluorescence signal in grey. The major artery observed in the head is the carotid artery.

Supplementary video S1B – An OPT 3D representation of an E11.5 *Msx1*^{-/-}, *Msx2*^{-/lox}, *α-Sm22Cre* embryo head vasculature (embryo is from the same litter as the one in supplementary video S1A). The CD31 fluorescent signal is figured in brown, the anatomical data obtained by the embryo auto fluorescence signal in grey. Major differences are visible at the carotid artery branching level (cf. supplementary video S1A).

Supplementary Figure S1 – *Msx1*^{βgal} and *Msx2*^{GFP} patterns of expression in the carotid (**A, B**) and vertebral (**A', B'**) arteries. All panels show different artery sections at E12.5 with an optical thickness of 3μm. The nuclear *Msx1*^{βgal} is represented in yellow (**A-A'**) as is *Msx2*^{GFP} (**B-B'**). Mural markers *α-Sma* (**A, A'**) and PDGF-Rβ (**B, B'**) are represented in red. At this stage the *Msx1*^{βgal} protein is detected in the endothelial layer of both arteries and also in some mural cells (arrows), and in mesenchymal cells around the artery (arrows). *Msx2*^{GFP} activity is only detected in the innermost layer of vascular smooth muscle.

Supplementary Figure S2 – Transverse sections of E10.5 (**A**) and E11.5 (**B**) embryo CAs. Nuclei are labeled with Hoechst (Blue), VSMCs with *α-SMA* (red) and *α-Sm22Cre*-activated GFP-positive cells are labeled in green. At E10.5 (**A**), we observe a weak mural coverage of the CA that is typical of this stage. All VSMCs express GFP. In addition, cells that express GFP but not *α-Sma* are detected outside the vascular smooth muscle layer (arrows). As expected, at E11.5 (**B**) the mural coverage of the CA is more intense. GFP is expressed by all VSMCs and also by cells that are not yet part of the mural layer. These results indicate an early activation of the *α-Sm22* promoter at least in the head mural lineage.

Supplementary Figure S3 – Quantitative RT-PCR using RNA from cephalic tissues dissected as described in Fig. 2H (red-outlined area). Expression of *α-Sma*, *Myh11* and *Myocd* decreases when *Msx1* alleles, and even more when both *Msx1* and *Msx2* alleles, are inactivated. This reduction is proportional to the decrease in the number of head VSMCs (*) P<0.05, (**) P<0.01 and (***) P<0.001.

Supplementary Figure S4 – Transverse sections of E12.5 embryo CAs. (**A, B, C**) show sections from control embryos and (**A', B', C'**) from *Sm22Cre Msx1/2* mutants. Nuclei are labeled with Hoechst

(blue), type IV Collagen in red (**A, A'**), Laminin1 in green (**B, B'**) and Fibronectin in green (**C, C'**). The number of ColIV and Laminin1-surrounded nuclei appears reduced in *Sm22Cre Msx1/2* mutant (**A', B'**) as compared to controls (**A, B**). In contrast, the number of Fibronectin-surrounded nucleus does not look affected by *Msx* mutations (**C, C'**). The intensity of Col IV, Laminin1 and Fibronectin labeling is not affected by *Msx* mutations.

Supplementary Figure S5 – Schematic representation of the superior temporal artery (STA) localization (**A**). The *Sm22Cre Msx1/2* mutant presents aneurysms and hemorrhages of the STA at birth (**B-E**). *Msx* double heterozygous (**B**), *Sm22Cre Msx2* (**C**) and *Msx1* (**D**) simple mutants do not show abnormal phenotypes. In the double *Sm22Cre Msx1/2* mutant (**E**) we observe extra-branches from the STA (asterisks) as well as hemorrhagic regions (arrow). For all genotypes, n=5.

Supplementary Figure S6 – *Msx1^{lox}* and *Msx2^{lox}* alleles were both inactivated using *α-Sm22Cre* (**A**) or *Tie2Cre* (**C**) transgenes. *α-SMA* is revealed in red, CD31 and VE-cadherin in green, Hoechst-labeled nuclei in blue. *α-Sm22Cre*-driven *Msx1* and *Msx2* knock-out (**A**) significantly reduces the number of *α-SMA* positive cells covering the CA and results in an increase in the CA caliber (**B**). The number of CD31-positive cells does not decrease, but the intensity of VE-Cadherin staining is reduced. *Tie2Cre*-driven *Msx1* and *Msx2* knock out does not result in a significant change in smooth muscle coverage or CA caliber, and VE-Cadherin staining is not affected (**C**). Quantification of *α-SMA* and CD31-surrounded nuclei in either mutant is presented in (**D**). In B and D n=6, (*) P<0.05 and (**) P<0.01.

Supplementary Figure S7 – *Msx1^{CreERT2/+}; Rosa^{mT/mG}* analysis. In order to rule out possible leakiness of the *Msx1^{CreERT2/+}* and *Rosa^{mT/mG}* alleles, we performed either injections of 3.5mg of Tamoxifen or mock-injections of oil and ethanol. Mice were injected three times at embryonic stages 8.0, 8.5 and 9.0. At E12.5, mice injected with oil and ethanol carried embryos that do not show any GFP-positive cells either in whole mount (**A**) or in vertebral artery (**B**) or dorsal root ganglion (**C**) sections. After Tamoxifen injection, we observe strong GFP expression in whole mount (**A**), mainly in the limbs and branchial arches, as expected. On sections, a high number of GFP-positive cells are observed both in the vertebral artery (**B**) and the dorsal root ganglion (**C**).

Supplementary Figure S8 – *Msx1^{CreERT2} Rosa^{mT/mG}* mesenchymal cells co-express *Msx2^{βgal}* before CA integration (**A**). Nuclear *Msx2^{βgal}* staining is represented in yellow, *Msx1^{CreERT2}*-driven GFP in green, *α-SMA* in red and Hoechst in Blue (**A**). Either *Msx2^{βgal}* or GFP are expressed in *α-SMA*-positive as well as *α-SMA*-negative mesenchymal cells (arrows). Some of the GFP-positive cells co-express *Msx2^{βgal}* (arrows). *Msx1^{CreERT2}*-driven inactivation of *Msx2*, in an *Msx1* mutant context, leads to a

vascular phenotype similar to the α -Sm22 *Msx1/2* mutation (**B**). *Msx1^{lacZ}* was used as the *Msx1* null allele. α -SMA is shown in red, CD31 in green and Hoechst in blue. The number of α -SMA-positive cells is reduced in the CA of the *Msx1^{CreERT2/-} Msx2^{lox/lox}* mutant as compared to the control. The caliber of the CA is increased in mutant (**B**). Quantification is presented in (**C**). n=5 (*) P<0.05. We performed 2 injections of 3.5mg of Tamoxifen at 12 hours of interval.

Supplementary Figure S9 – Cell sorting data and semi-quantitative RT-PCR. Cells were sorted from different mutants: *Msx1^{+/-}*, *Msx2^{+/-}* (**A**) or *Msx1^{-/-}*; *Msx2^{-/-}* (**B**). The mutants carry the α -Sm22*Cre* and *Rosa^{mT/mG}* alleles that allow VSMCs sorting. Endothelial cells from all mutants were sorted using an anti-CD31 PE-coupled antibody. The identity of sorted cells was confirmed by semi quantitative RT-PCR. The α -Sm22-sorted cells express Myosin Heavy chain (*Myh11*), Smooth Muscle Actin (α -*Sma*) (**C**). The endothelial sorted cells express *CD31* and von Willebrand's Factor (*vWF*) (**D**).

Supplementary Figure S10 – Head transverse sections of *Msx1*; *Msx2* double heterozygous (**A, C**) and *Sm22Cre* *Msx1/2* mutants (**B, D**) E11.5 embryos. Apoptosis was analyzed using lysotracker staining (**A, B, red**), endothelial cells were labeled in green for CD31. At E11.5 no lysotracker-positive cells were observed around the CA in the *Sm22Cre* *Msx1/2* mutant (**B**) or in the control (**A**). As a positive control we have analyzed lysotracker staining of dorsal root ganglia (lower right corner of A, B). No difference was observed between the two genotypes. Proliferation studies were performed using Phospho-Histone H3 immunofluorescence (**C, D, red**). Endothelial CD31-positive cells were labeled in green and nuclei Hoechst-labeled in blue. The number of Phospho-Histone H3-positive cells is similar in the control and mutant context. These results were confirmed by quantification of the percentage of Histone H3/Hoechst positive nuclei in the CA field (**E**). For both genotypes n=6.

Supplementary Figure S11 – Transversal sections of E14.5 α -Sm22 *Msx1/2* mutant and control embryos. α -SMA is shown in red, neuropilin1 (NRP1) in green and Hoechst-labeled nuclei in blue. In the control embryo, NRP1 labeling corresponds to the endothelial layer, localized at the luminal side of the smooth muscle layer. There is no obvious decrease in NRP1 labeling in the CA of the α -Sm22 *Msx1/2* mutants. As expected, the carotid vein (CV) is not labeled by the NRP1 antibody in either case.

Supplementary Tables

Supplementary table S1

Genotyping Primers sequence

Allele name	Forward primer sequence	Forward primer 2 sequence	Reverse primer sequence
<i>Msx1^{LacZ}</i>	TTCTCCAGCTCGCTCAGCCTCACCGAGACC	GACCGCCAAGAGGAAAAGAGAGGCCGAAGGC	GACCGCCAAGAGGAAAAGAGAGGCCGAAGGC
<i>Msx2^{lox} Msx2^{GFP}</i>	GTCTTCGCTTGAGAGTTGCCAGCCGAGTCGC	GTCGGACATGAGCGCCTCCACGCTGAAGG	TTCCGCTGCCTGCAAAGGGTCGCTACAGACG
<i>Sm22^{Cre}</i>	GGACATGTTTCAGGGATCGCCAGGCG		GCATAACCAGTGAAACAGCATTGCTG
<i>Msx2^{LacZ}</i>	GGCAAAGCGCCATTCGCCATTCAGGC	GTCTTCGCTTGAGAGTTGCCA	GTCGGACATGAGCGCCTC
<i>Rosa^{mT/mG}</i>	AAAGTCGCTCTCTGAGTTGTTAT	GCGAAGAGTTTGTCTCAACC	GGAGCGGGAGAAATGGATATG
<i>Msx1^{CreERT2}</i>	GGCTGTCTCGAGCTGCGGCTGGAGGG	CCATGGCGGTTGCGGTGGCCGCAGC	GCTGGATAGTTTTTACTGCCAGACCGCGCGCC

Supplementary table S2

Primary antibodies:

Antibody	Application	Brand	Dilution
Monoclonal rat anti-mouse α -SMA cy3 conjugate	IF	Sigma	1/1000
Polyclonal rabbit anti-mouse PDGF-R β	IF	Santa Cruz	1/200
Monoclonal rat anti-mouse CD31 Biotin coupled	IF	BD Pharmigen	1/100
Monoclonal rat anti-mouse CD31	IF - OPT	BD Pharmigen	1/100
Polyclonal rat anti-mouse CD144	IF	BD Pharmigen	1/100
Polyclonal rabbit anti-mouse Tie-2	IF	Santa Cruz	1/200
Polyclonal rabbit anti- β -Galactosidase	IF	Cappel	1/1500
Monoclonal rabbit anti-mouse GFP	IF	Invitrogen	1/500
PE rat anti-mouse CD31	Cell Sorting	BD Pharmingen	1/400
Monoclonal mouse anti-mouse GFP	IF	Chemicon	1/500

Supplementary table S3

Secondary antibodies:

Antibody	Brand	Dilution
Alexa fluor 488 goat anti mouse	Invitrogen	1/300
Alexa fluor 488 goat anti rabbit	Invitrogen	1/300
Alexa fluor 568 goat anti rabbit	Invitrogen	1/300
Alexa fluor 647 goat anti mouse	Invitrogen	1/300
Alexa fluor 635 goat anti rabbit	Invitrogen	1/300
Alexa fluor 488 streptavidin	Invitrogen	1/1000

Supplementary tables – All antibodies and primers used in this work. **Table S1** lists all the primer sequences used to genotype the different transgenic lines. **Table S2** lists the primary antibodies used in different approaches. With the exception of the PE rat anti-mouse CD31 (used only in flow cytometry), all others were used for immunohistochemistry of embryo sections. The monoclonal rat anti-mouse CD31 was also used to perform OPT. The secondary antibodies are listed in the **Table S3**. Quantitative PCR was performed with primers listed in **Table S4**.

Supplementary table S4

qPCR primers sequence

Gene name	Forward primer sequence	Reverse primer sequence
<i>Jag1</i>	TGCTCACACCTGAAAGACCACT	ATGGGGACCACAGACGTTAGAA
<i>VE-cadherin</i>	CTGCTTTTGC GAACGGAA	GTGGTTTGGTTTGGGCTGGAAT
<i>Cd31</i>	CTGGTGCTCTATGCAAGCCTC	CGGTGCTGAGACCTGCTTT
<i>vWF</i>	CTTCTGTACGCCTCAGCTATG	GCGGTTGTAATTCCCACACAAG
<i>Sma</i>	GTCCCAGACATCAGGGAGTAA	TCCGATACTTCAGCGTCA GGA
<i>Myocd</i>	GATGGGCTCTCTCCAGATCAG	GGCTGCATCATTCTTGTCACCT
<i>Myh11</i>	ACGACAACCTCCTCTCGCTTT	GCCTGTCTGAATAGCCCTAGA
<i>PDGF-Rβ</i>	GGAGTCGAGTTGGAAAGCTCAT	AGGTTCCGAGATCTGGAACA
<i>VEGF-A</i>	TGTAACGATGAAGCCCTGGAGT	ATGTGCTGGCTTTGGTGAGGTT
<i>TGF-β1</i>	TACGCCTGAGTGGCTGTCTTTT	ACAAGAGCAGTGAGCGCTGAAT
<i>Angpt 1</i>	CGGGAAGATGGAAGCCTGGATT	TCCATCAGCTCAATCCTCAGCA
<i>Klf2</i>	ACACCAAGAGCTCGCACCTAAA	GCATCCTTCCCAGTTGCAATGA
<i>Mmp2</i>	GTGGCAGTGTATCCTCAAGA	AGGGTCATAGTCGAAGTGGCAT
<i>Mmp9</i>	ACCTTCAAAGGCCTCAAGTG	GAAGGCGTCATCGATCATGT
<i>BMP1a</i>	GGTTGTGCTCATTTCATGGCT	TTGTAACGACCCCTGCTTGAGA
<i>BMP1b</i>	AGCTGGTTCCGAGAGACTGAGATA	GGGAGCCGTTTTTCATGATAGTCTG
<i>BMP2</i>	TTTGCTGGCCCAGGGATGACTT	TGCACACAGCCGCTTGATTCT
<i>BMP2</i>	TGCGCAGCTTCCATCACGAAGA	ACTCGTCACTGGGGACAGAACTTA
<i>BMP4</i>	AGGAAGAGCAGAGCCAGGGAA	TGCTGAGGTTGAAGAGGAAACG
<i>BMP6</i>	GGACATGGTCATGAGCTTTGTG	ACCCTCAGGAATCTGGGATAGGTT
<i>BMP8</i>	CTCCAAGACGCCAAGAACCAAGA	GGAAGCTGACGTACAGCTCATGTT
<i>Msx1</i>	AAGATGCTCTGGTGAAGGCCGAAA	CTTGCGGTTGGTCTTGTGCTT
<i>Msx2</i>	ATACAGGAGCCCGGCAGATACT	AACCTGCGCTCCAAGGCTAGAA
<i>Gapdh</i>	GGCAAAGTGGAGATTGTTGC	AATTTGCCGTGAGTGGAGTC