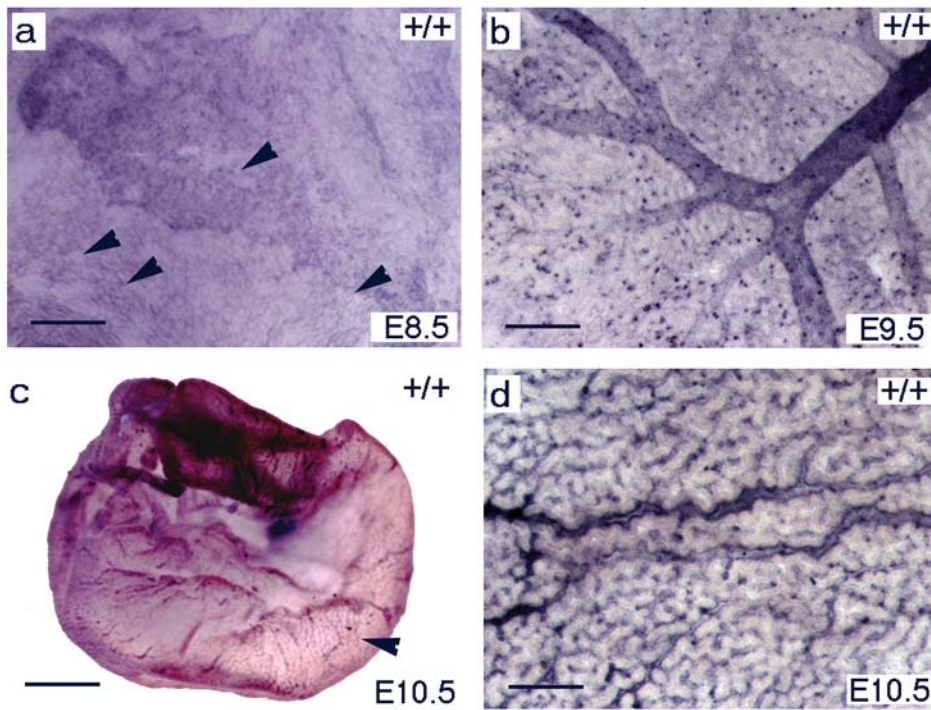


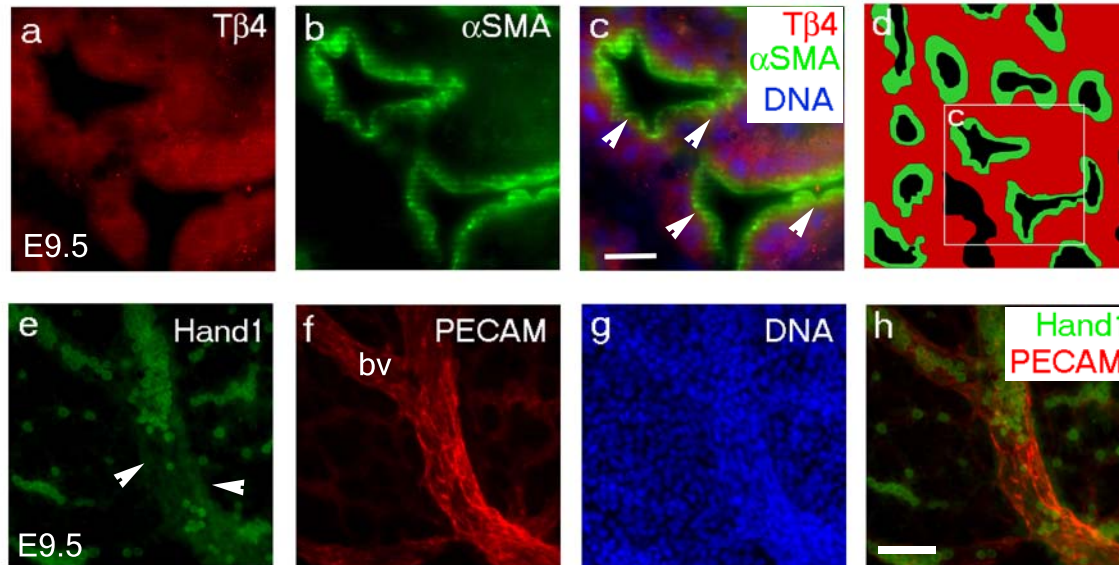
Supplementary Figure S1



***Tbeta4* is expressed in the developing yolk sac vasculature**

In situ hybridisation revealed *Tbeta4* expression in the blood islands of the yolk sac at E8.5 (**a**) and in the developing vessels at E9.5 (**b**) and E10.5 (**c**, **d**). Scale bars, **a**, **b**, 100 μm ; **c**, 250 μm ; **d**, 50 μm .

Supplementary Figure S2



TB4 is not expressed in smooth muscle cells within the developing yolk sac vasculature and Hand1 is down-regulated during vessel maturation

TB4 is not expressed in smooth muscle cells in the yolk sac vasculature at E9.5 (**a-c**) consistent with Hand1 expression at the same stage (see Fig. 4f, g); white arrowheads in **c** highlight α -SMA⁺ cells in green, lining TB4⁺ endothelium in red. Schematic of the representative region encompassing panel **c**, at lower magnification, to highlight plexus and network of vessels in red lined with green smooth muscle cells; black regions represents gaps between neighboring vessels and not individual vessel lumens (**d**). As the yolk sac endothelium matures with ongoing yolk sac angiogenesis Hand1 is down-regulated (**e-h**). Weak Hand1 immunostaining is highlighted in the vessel wall by white arrowheads (**e**). PECAM staining reveals elongated mature endothelial cells within the vessel wall (**f, h**). bv, blood vessel. Scale bars; **c**, 20 μ m; **h**, 10 μ m.

Supplementary Table S1. Primer sequences

Gene		Primer Sequence (5'-3')
qRT-PCR PRIMERS		
Tβ4	Forward	ATGTCTGACAAACCCGATATGGC
	Reverse	CCAGCTTGCTTCTCTTGTTC
Hand1	Forward	TGAGTGCATCCCCAATGTG
	Reverse	GCCAGCACGTCCATCAAGTA
Pecam	Forward	CTGCCAGTCCGAAAATGGAAC
	Reverse	CTTCATCCACCGGGGCTATC
Vegfa	Forward	GCACATAGAGAGAATGAGCTTCC
	Reverse	CTCCGCTCTGAACAAGGCT
Flt1	Forward	CTCAGACAAGTCAAACCTGGAG
	Reverse	GGGAACTTCATCTGGGTCCATAA
Tgfβ1	Forward	CTCCCGTGGCTTCTAGTGC
	Reverse	GCCTTAGTTTGGACAGGATCTG
TgfβR2	Forward	CCGCTGCATATCGTCCTGTG
	Reverse	AGTGGATGGATGGTCCTATTACA
Smad2	Forward	ATGTCGTCCATCTTGCCATTC
	Reverse	AACCGTCCTGTTTTCTTTAGCTT
Smad5	Forward	TTGTTTCAGAGTAGGAACTGCAAC
	Reverse	GAAGCTGAGCAAACCTCCTGAT
Endoglin	Forward	CCCTCTGCCCATTACCCTG
	Reverse	GTAAACGTCACCTCACCCCTT
Notch1	Forward	CCCTTGCTCTGCCTAACGC
	Reverse	GGAGTCCTGGCATCGTTGG
Notch2	Forward	ATGTGGACGAGTGTCTGTTGC
	Reverse	GGAAGCATAGGCACAGTCATC
Notch4	Forward	CTCTTGCCACTCAATTTCCCT
	Reverse	TTGCAGAGTTGGGTATCCCTG
Hand1 GENOTYPING PRIMERS		
WT Forward (proximal exon 2)		CCACTAGGATCGCACGTGCA
Mutant Forward (neo ^r cassette)		GCAAAGCTGCTATTGGCCGC
Common Reverse (distal exon 2)		CCAGCAACGATTGGGAACGC