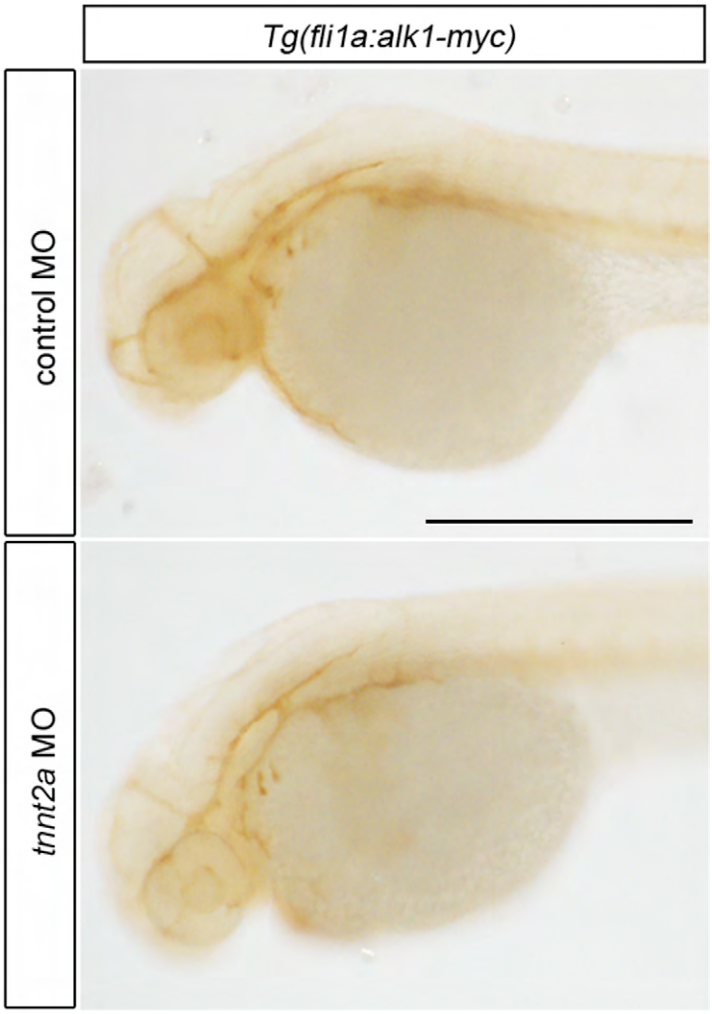

Fig. S1. *Tg(fli1a:alk1-myc)* embryos express Alk1-myc in all endothelial cells in the presence or absence of flow. Immunohistochemistry for myc in 36 hpf *Tg(fli1a:alk1-myc)* control and *tnnt2a* morphant embryos. Scale bar: 500 μm.

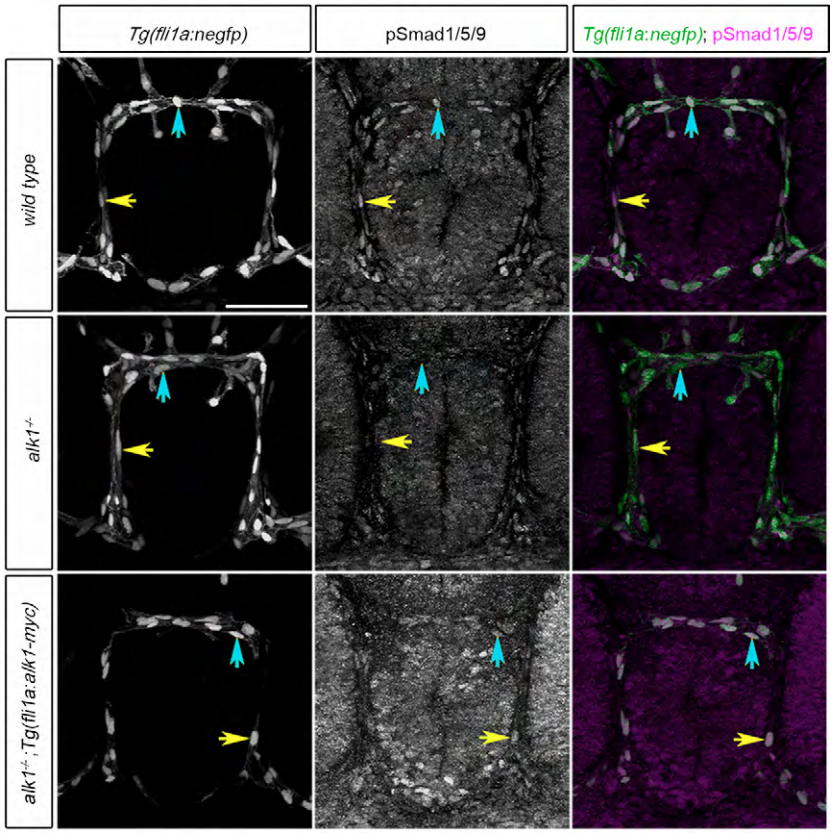

Fig. S2. pSmad1/5/9 expression in *alk1*-positive endothelial cells depends on *alk1*. pSmad1/5/9 expression (middle column) in endothelial cells (nuclei marked by *fli1a:negfp* transgene, left column) in wild-type, *alk1*$^{-/-}$ and *alk1*$^{-/-}$*;Tg(fli1a:alk1-myc)* embryos at 36 hpf. In merge, EGFP-expressing endothelial cell nuclei are green, pSmad1/5/9 immunofluorescence is magenta. Yellow and blue arrows indicate endothelial cells in the CaDI and BCA, respectively. 2D confocal projections of 50 µm frontal sections, dorsal upwards. Scale bar: 50 µm. See Table S1 for fluorescence quantitation.

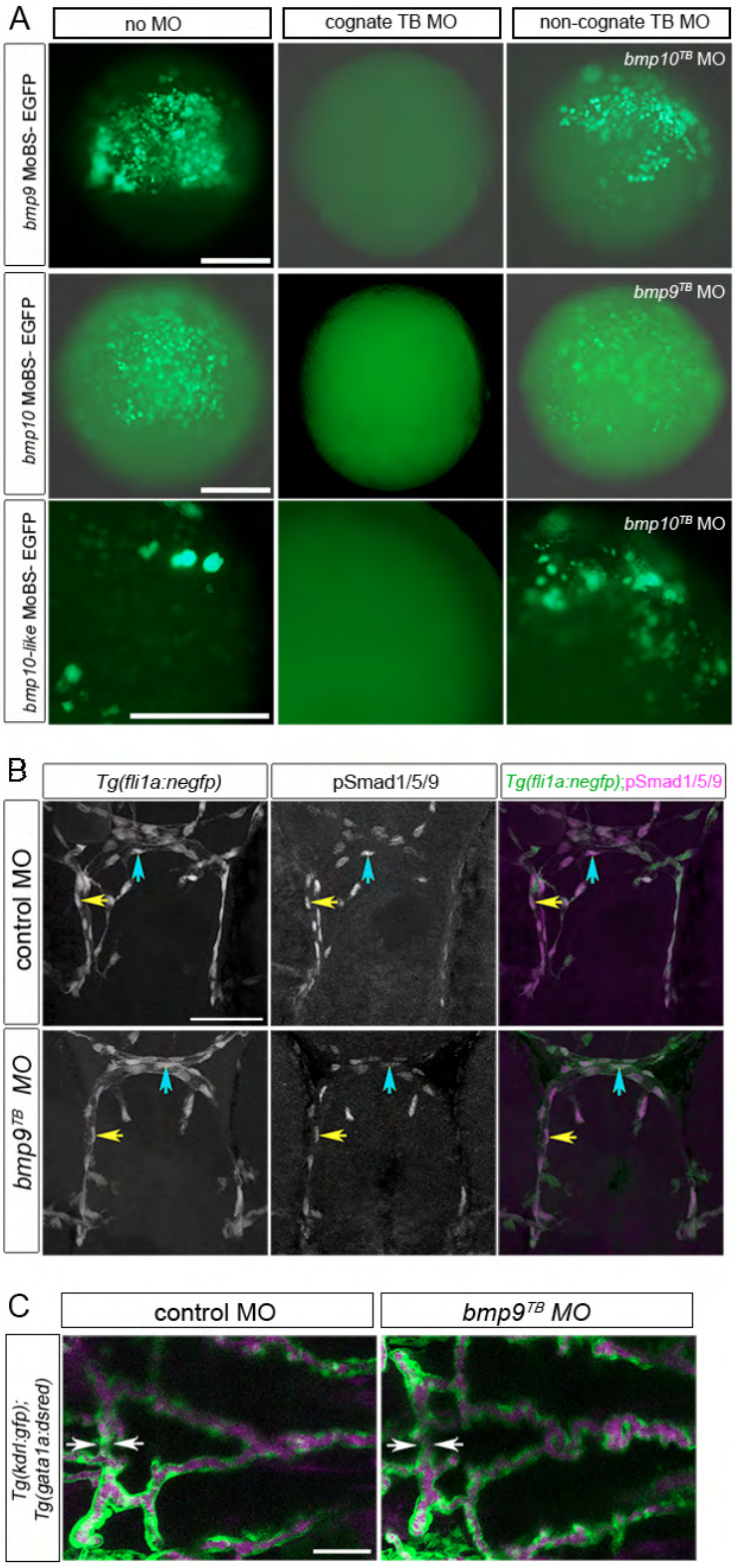

Fig. S3. *bmp9* knockdown does not phenocopy zebrafish *alk1*$^{-/-}$ mutants. (**A**) Translation blocking (TB) morpholino validations. Wild-type embryos injected at the one-cell stage with 50 pg CMV-driven EGFP constructs modified with morpholino-binding sites (MoBS) inserted upstream of the ATG, with or without cognate or non-cognate morpholinos. *bmp9*^{TB} MO, 7 ng; *bmp10*^{TB} MO, 20 ng; *bmp10-like* MO, 3 ng. Embryos were observed at ~6 hpf for EGFP fluorescence. Scale bar: 500 μm (top two rows) or 250 μm (bottom row). (**B**) pSmad1/5/9 expression (middle column) in endothelial cells (nuclei marked by *fli1a:negfp* transgene, left column) in 36 hpf embryos injected with 7 ng control or *bmp9*^{TB} morpholino. Higher amounts of this morpholino were overtly toxic. In merge (right column), EGFP-expressing endothelial cell nuclei are green, pSmad1/5/9 immunofluorescence is magenta. Yellow and blue arrows indicate endothelial cells in the CaDI and BCA, respectively. 2D confocal projections of 50 μm frontal sections, dorsal upwards. Scale bar: 50 μm. See Table S1 for fluorescence quantitation. (**C**) Cranial vasculature in 48 hpf *Tg(kdrl:gfp);Tg(gata1a:dsRed)* embryos injected with 7 ng control or *bmp9*^{TB} morpholino. Arrows highlight width of BCA. Endothelial cells are green, red blood cells are magenta. 2D confocal projections, dorsal views, anterior leftwards. Scale bar: 50 μm. *n*=207 over five experiments.

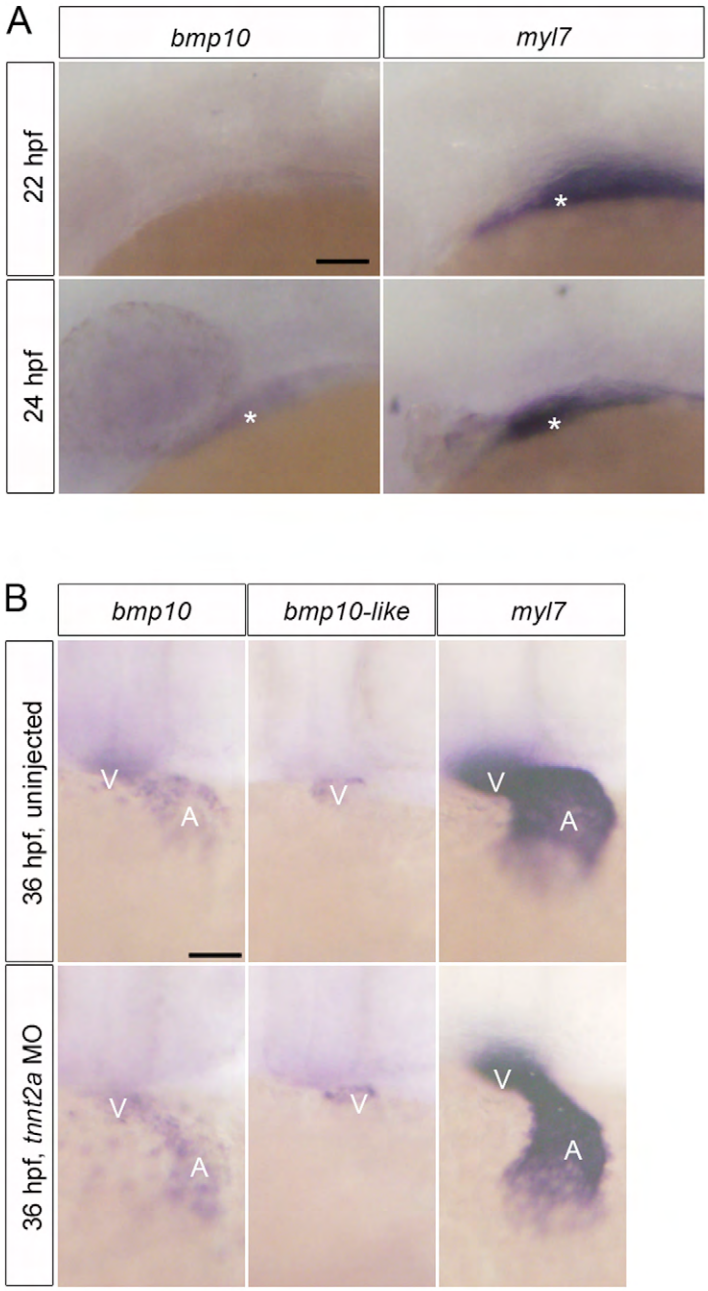

Fig. S4. Expression of *bmp10* and *bmp10-like* mRNA does not depend on heartbeat. (**A**) *bmp10* is not expressed at 22 hpf but is faintly expressed in the heart at 24 hpf. Lateral views, anterior leftwards. Scale bar: 100 μm. Asterisk indicates developing heart. (**B**) *bmp10* and *bmp10-like* expression in control and *tnnt2a* morphants, which lack heartbeat. Images are representative of n=60 embryos per group. A, atrium; V, ventricle. Ventral views, anterior upwards. Scale bar: 50 μm. *myl7* is shown as a pan-myocardial control.

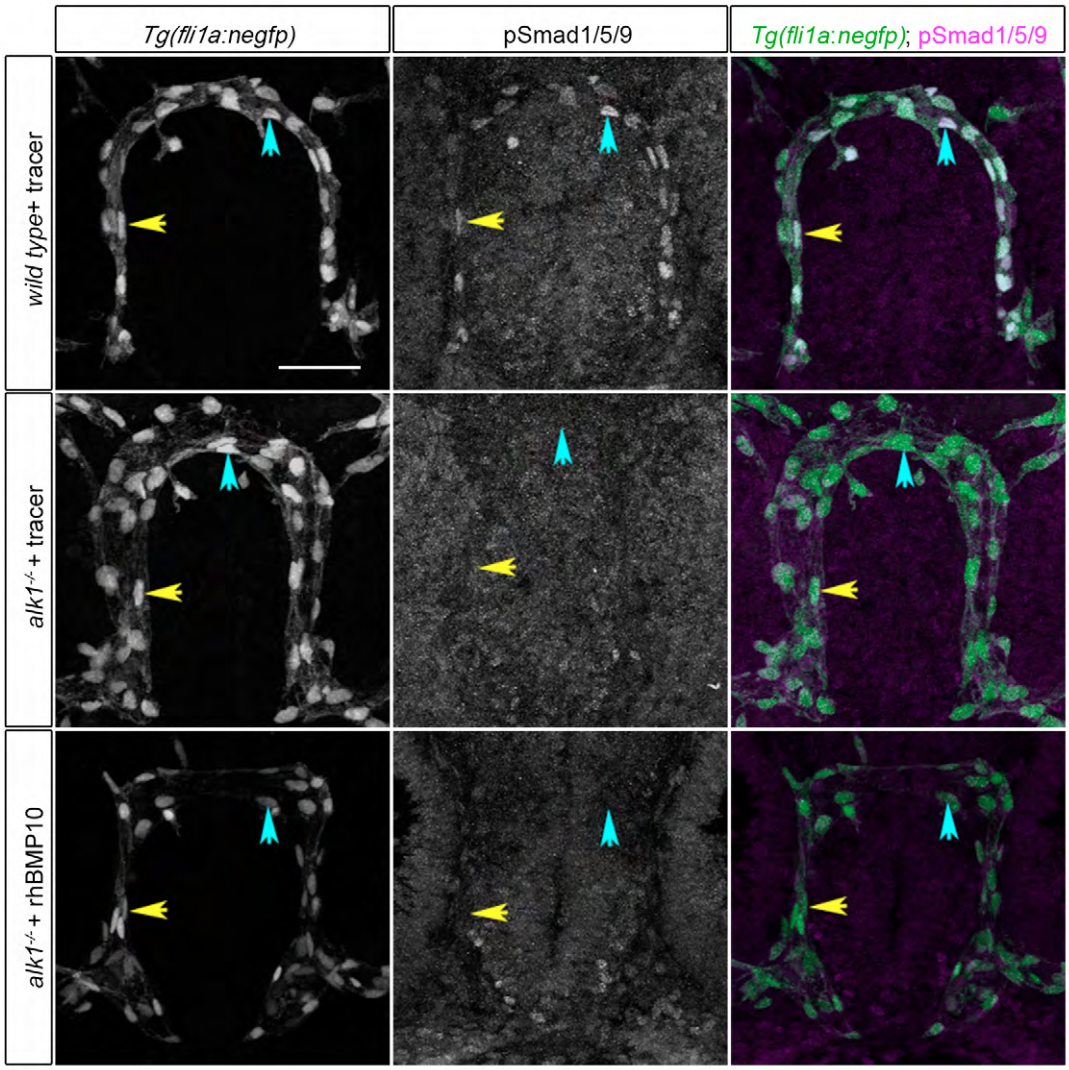

Fig. S5. rhBMP10 cannot induce pSmad1/5/9 in the absence of *alk1*. Intravascular injections of tracer alone or tracer + 2 nl 10 μM rhBMP10 were performed at 28 hpf into wild-type or *alk1* mutant embryos. Images show pSmad1/5/9 expression (middle column) in endothelial cells (nuclei marked by *fli1a:nEGFP* transgene, left column) at 36 hpf. In merge (right column), EGFP-expressing endothelial cell nuclei are green, pSmad1/5/9 immunofluorescence is magenta. Yellow and blue arrows indicate endothelial cells in the CaDI and BCA, respectively. 2D confocal projections of 50 μm frontal sections, dorsal upwards. Scale bar: 50 μm. See Table S1 for fluorescence quantitation.

Table S1. Quantitation of average nuclear phospho-Smad1/5/9 intensity

Corresponding figure	Sample	Average nuclear pSmad1/5/9 intensity (% of control)	*n*
Fig. 2	Wild type, 24 hpf	23.4±3.9***	6
	tnnt2a MO, 36 hpf	49.9±8.0***	7
	Uninjected control, 36 hpf	**100±8.0**	7
	tnnt2a MO*; Tg(fli1a:alk1-myc)*, 36 hpf	43.6±7.3***	7
	Tg(fli1a:alk1^{CA}-mCherry), 36 hpf	164.9±25*	6
Fig. 3C, Fig. S3B	**Control MO, 36 hpf**	**100±8.1**	11
	Bmp10 MO, 36 hpf	52±5.5***	10
	Bmp9 MO, 36 hpf	118.6±25	6
Fig. 5B	***tnnt2a* MO;*Tg(fli1a:alk1-myc)* + tracer, 36 hpf**	**100±3.5**	7
	tnnt2a MO + rhBMP10, 36 hpf	75.7±4.4	6
	tnnt2a MO; Tg(*fli1a:alk1-myc*) + rhBMP10, 36 hpf	317±27.7**	8
Fig. S2	**Wild type, 36 hpf**	**100±10.2**	14
	alk1$^{-/-}$, 36 hpf	48.5±6.4***	13
	alk1$^{-/-}$*;Tg(fli1a:alk1-myc)*, 36 hpf	120.9±10.2	7
Fig. S5	**Wild type + tracer, 36 hpf**	**100±5.5**	4
	alk1$^{-/-}$ + tracer, 36 hpf	40.9±4.5**	4
	alk1$^{-/-}$ + rhBMP10, 36 hpf	32.8±5.7**	4

Controls for each experiment were normalized to 100% and are in bold. Values are mean±s.e.m. *P<0.05; **P<0.01; ***P<0.001.