Supplementary Material

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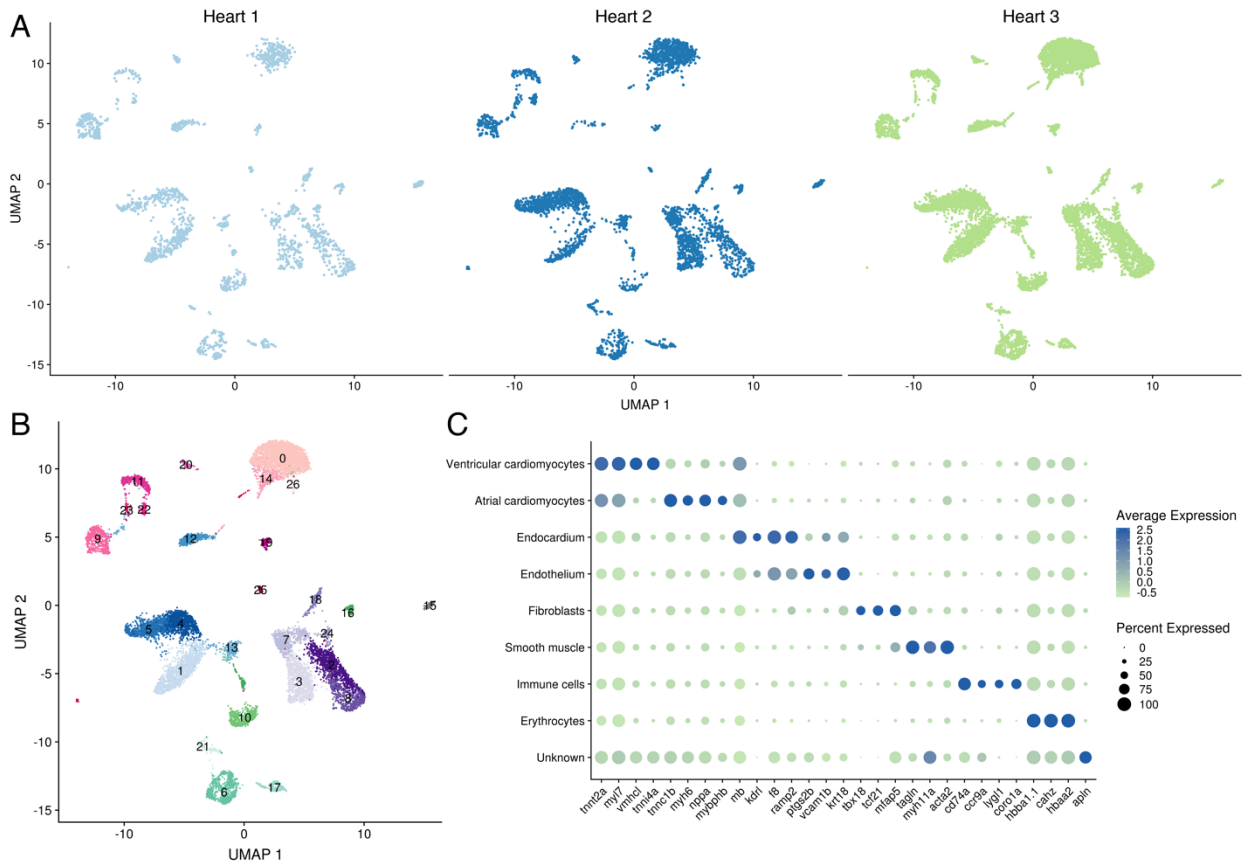
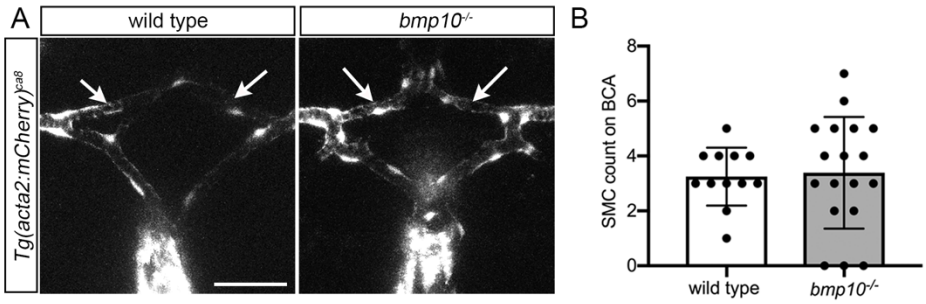
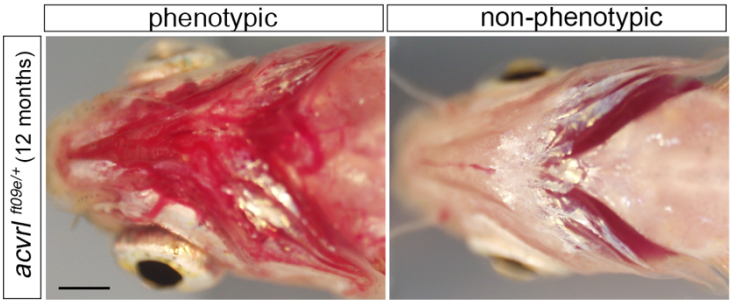


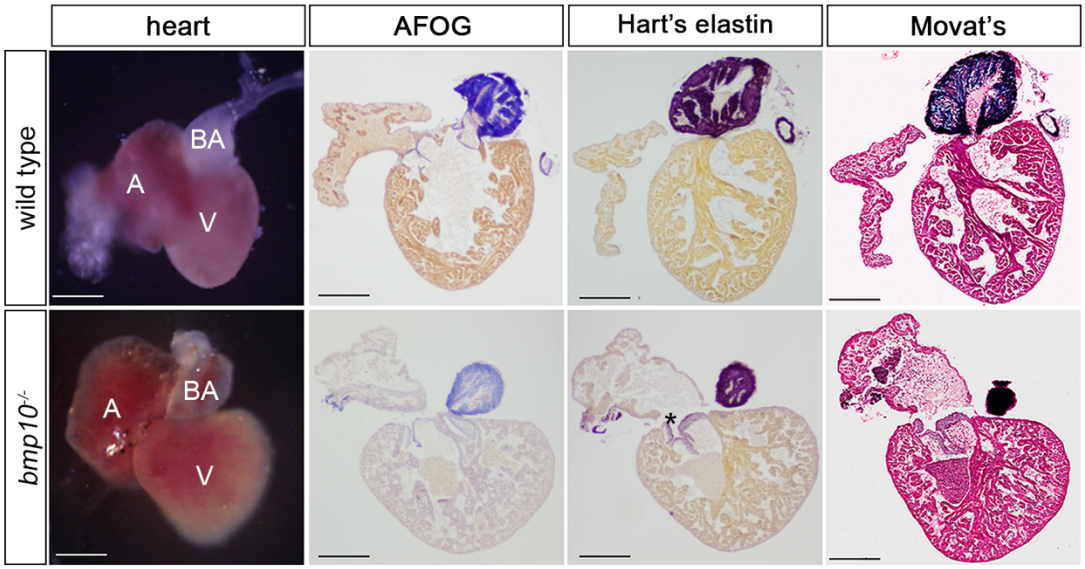
Supplementary Fig. 1 **a** UMAP plots of individual hearts (Heart 1 = 2086 cells, Heart 2 = 3924 cells, Heart 3 = 8462 cells). **b** UMAP plot of the three integrated heart datasets. Cluster numbers correspond to the original cluster IDs from the FindClusters function (res 0.8) and colors correspond to cell type as annotated in Supplementary Dataset 1. Cluster identities are as follows: 0, erythrocytes; 1, atrial cardiomyoctyes; 2, endothelium; 3, endocardium; 4, ventricular cardiomyocytes; 5, ventricular cardiomyocytes; 6, fibroblasts; 7, endocardium; 8, endothelium; 9, immune cells; 10, smooth muscle; 11, immune cells; 12, atrial cardiomyoctyes; 13, atrial cardiomyoctyes; 14, erythrocytes; 15, unknown; 16, smooth muscle; 17, fibroblasts; 18, endothelium; 19, immune cells; 20, immune cells; 21, fibroblasts; 22, immune cells; 23, immune cells; 24, endothelium; 25, immune cells; 26, erythrocytes. **c** Dotplot demonstrating the scaled expression values of select genes used to characterize the clusters. The size of each dot represents the percentage of cells within the cluster that express the gene.



Supplementary Fig. 2 *bmp10* mutant embryos have normal smooth muscle cell coverage on the basal communicating artery (BCA) at 5 dpf. **a** Cranial vasculature in *bmp10*^{pt527};*Tg(acta2:mcherry)*^{ca8} mutant and wild type siblings at 5 dpf. Arrows, BCA. 2D confocal projections, dorsal views, anterior top. Scale bar: 50 μm. **b** Quantification of smooth cell number on BCA. N= 12 wild types, 18 mutants over 3 experiments. Bars shown mean ± SD. Not significant by unpaired Student's *t* test.



Supplementary Fig. 3 *acvrl1*$^{ft09e/+}$ fish phenocopy *bmp10* mutants with low penetrance. 12-month fish. Scale bar: 1 mm.



Supplementary Fig. 4 *bmp10* mutants have abnormally shaped hearts at 3 months. Hearts from 3-month wild type and *bmp10*^{pt527} mutant siblings, whole mount or sections stained with acid fuchsin orange G (AFOG) for collagen (blue) and fibrin (red); Hart's elastin (purple); or Movat's pentachrome for muscle (red), collagen (yellow), elastin (blue-black), and ground substance (light blue). A, atrium; V, ventricle; BA, bulbus arteriosus; asterisks, valves. Scale bars, 200 μm. Images representative of N = 6 hearts per genotype from 3 independent lines.

Supplementary Table 1: TALENs and gRNAs

Gene	TALEN (Addgene #)	Target sequence (5'-3')
bmp9/gdf2	TAL3010 (36002) TAL3011 (36003)	TTGAACAAGGTGGAGAGTttcttaggctttatgaAGGAAGATTTTTTGAGGA
bmp10	TAL3032 (41206) TAL 3033 (41207)	TCAGCTCCCCGGAGAGGCaccgcactgctccagggTTGGATGATGGACATGGA
Gene	**gRNA name**	**Target sequence**
bmp10-like	gRNA bmp10l.3	AGTGGAGGACTGCAGAATAG

Supplementary table 2: Genotyping assays

Gene	Allele	Forward primer	Reverse primer	Enzyme	Result
bmp9	pt533	CACTTAAGGAACCC CGATTTC	ACTCACCCTGAAC GACAAAGC	DdeI	WT 80+95+255 bp M 95+327 bp
bmp9	pt536	CACTTAAGGAACCC CGATTTC	ACTCACCCTGAAC GACAAAGC	DdeI	WT 80+95+255 bp M 95+328 bp
bmp10	pt527	CAAAGTAGCCCCAT CAGCTC	CTTCAGGGTCTCC ATCAAGC	NA	WT 138 bp M 130 bp*
bmp10	pt543	CAAAGTAGCCCCAT CAGCTC	CTTCAGGGTCTCC ATCAAGC	BstNI	WT 44+94 bp M 131 bp
bmp10-like	pt544	GAGTTCGGCGCAGC GCTAAAGTGAAG	TGGGGACTCTTCA GATTGAGCAGCG	MboII	WT 208 bp M 155+35 bp
bmp10-like	pt545	CGCAGCGCTAAAGT GGAGGACTGCTGA	TGGGGACTCTTCA GATTGAGCAGCG	DdeI	WT 200 bp M 168+24 bp
bmp10-like	sa11654	CAGAACTGCGCATT CACATGTTTC	GATGCCGACTTTT CTCCAGTCTC	DdeI	WT 197 bp M 176+21 bp

*Separation requires high-resolution gel e.g. 4% Metaphor agarose (Lonza, Walkersville MD USA).

Supplementary Table 3: PCR primers

Gene	Forward primer	Reverse primer
bmp9	CGCAGGAACACAGAAAGGTT	GTTGGGTTTTGTTGCCTTGT
bmp10	ATGCCTTCGGCAAACATCATACGC	TTGAAGAGAAGTGGGTGTCGTCTCAC
bmp10-like	GGCAGCTAACATCATCAGGAGCTTC	AGATGTTGAACTGGAGACGCTGC
acvrl1	GGGTCTCGTCTTGTGGGAGA	GTCAGAGGGCACCATGTCAA
actb2	CGTGCTGTCTTCCCATCCA	TCACCAACGTAGCTGTCTTTCTG

Supplementary Table 4: Primers used to generate templates for riboprobes

Gene	Forward primer	Reverse primer (with T7 site)
ltbp3	CCTGGGGCCAAAATAAATGCTACA	TAATACGACTCACTATAGGGAGAAATGGG GACTTCCGGAGGCTTGAC
nkx2.5	CATTAACCCTCACTAAAGGGAAGTG CGGGACATACTGAACCT	TAATACGACTCACTATAGGGTGCCTCTTG CACTTGTATCG
tbx20	AGCCGCTCATCCCGACGACTC	TAATACGACTCACTATAGGGAGAGACGCG GTGTGATCTTTCTTCTTG