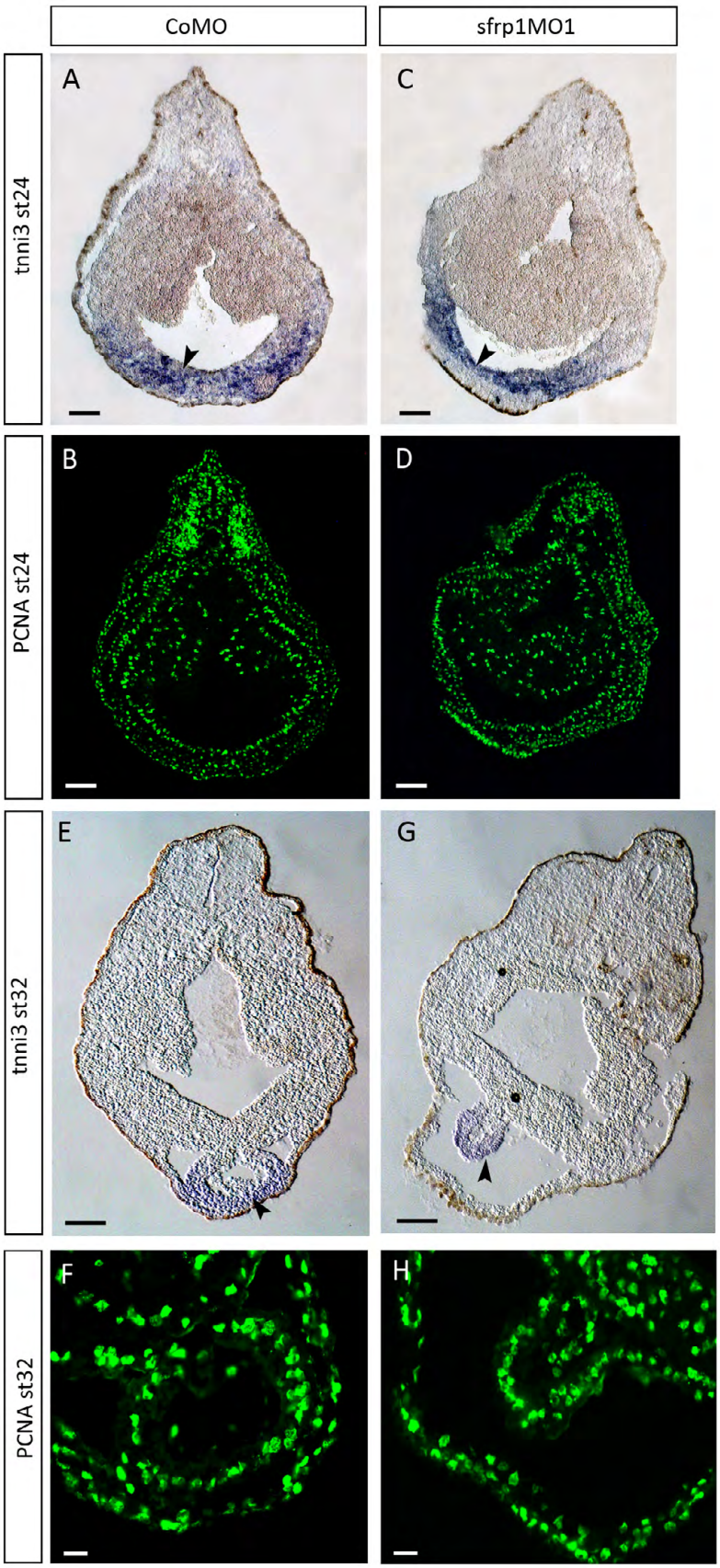
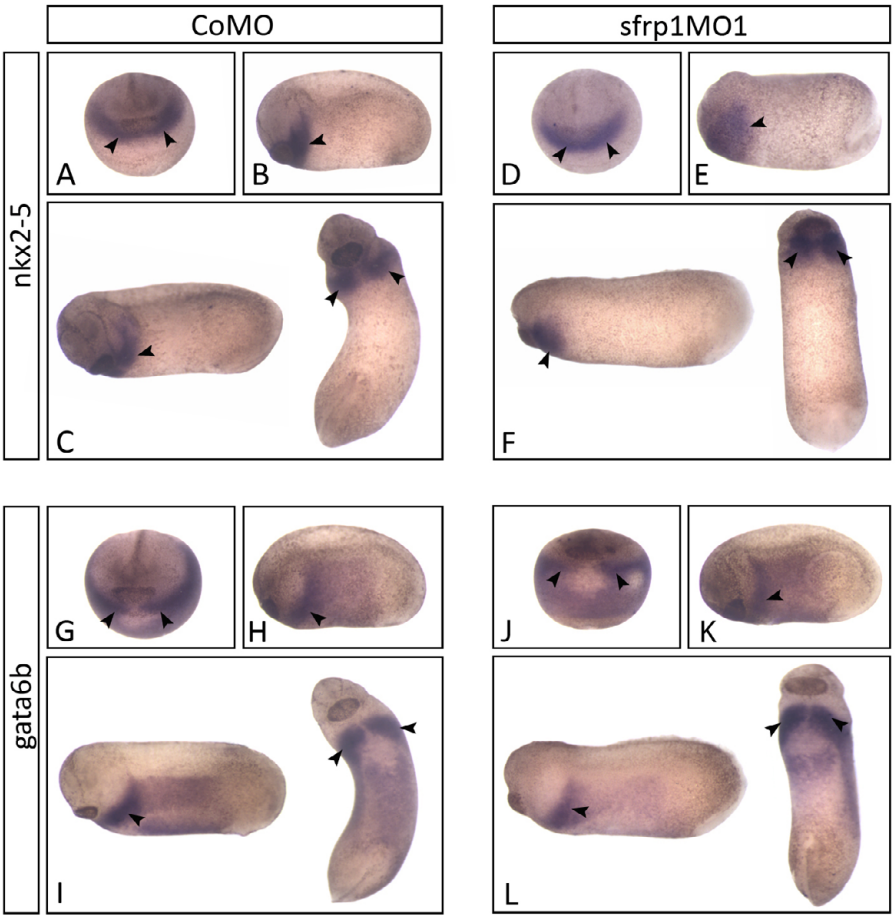


Fig. S1. *sfrp1* is not required for regulating cell proliferation. (**A**,**C**) RNA *in situ* sections at stage 24 show *nkx2-5* expression highlighting cardiac progenitor cells. (**E**,**G**) RNA *in situ* sections at stage 32 show *tnni3* expression within the myocardium of the differentiated heart. Note some expression remaining after loss of *sfrp1* (C), whereas *tnni3* expression after loss of *sfrp1* is clearly confined due to the reduction in the amount of differentiated heart muscle (G). (**B**,**D**,**F**,**H**) Immunofluorescence staining of PCNA protein highlighting proliferating cells throughout the same sections as A,C,E,G. Note there is no obvious reduction in the amount of PCNA at stage 24 or at stage 32, indicating that proliferation in the heart was not the cause for loss of heart muscle in G. Scale bars: 200 μm.



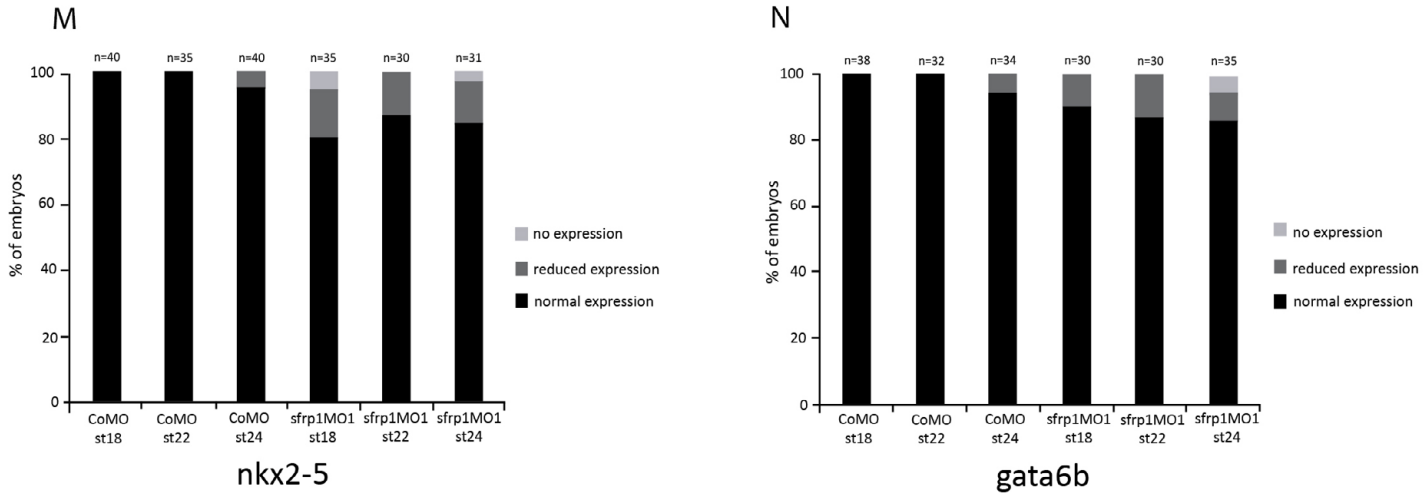


Fig. S2. Loss-of-function experiments show no change to cardiogenic gene expression during embryonic stages 18-24. (**A-F**) Whole-mount RNA *in situ* hybridisation analysis showing *nkx2-5* expression at stages 18 (A,D), 22 (B,E) and 24 (C,F) in CoMO-injected (A-C) or sfrp1MO1-injected (D-F) embryos. (**G-L**) Whole-mount RNA *in situ* hybridisation analysis showing *gata6b* expression at stages 18 (G,J), 22 (H,K) and 24 (I,L) in CoMO-injected (G-I) or sfrp1MO1-injected (J-L) embryos. (**M**,**N**) Quantitative analysis of *nkx2-5* (M) and *gata6b* (N) *in situ* hybridisation. Note there is minimal to no loss of gene expression in embryos with loss of sfrp1 compared with controls at the indicated stages.

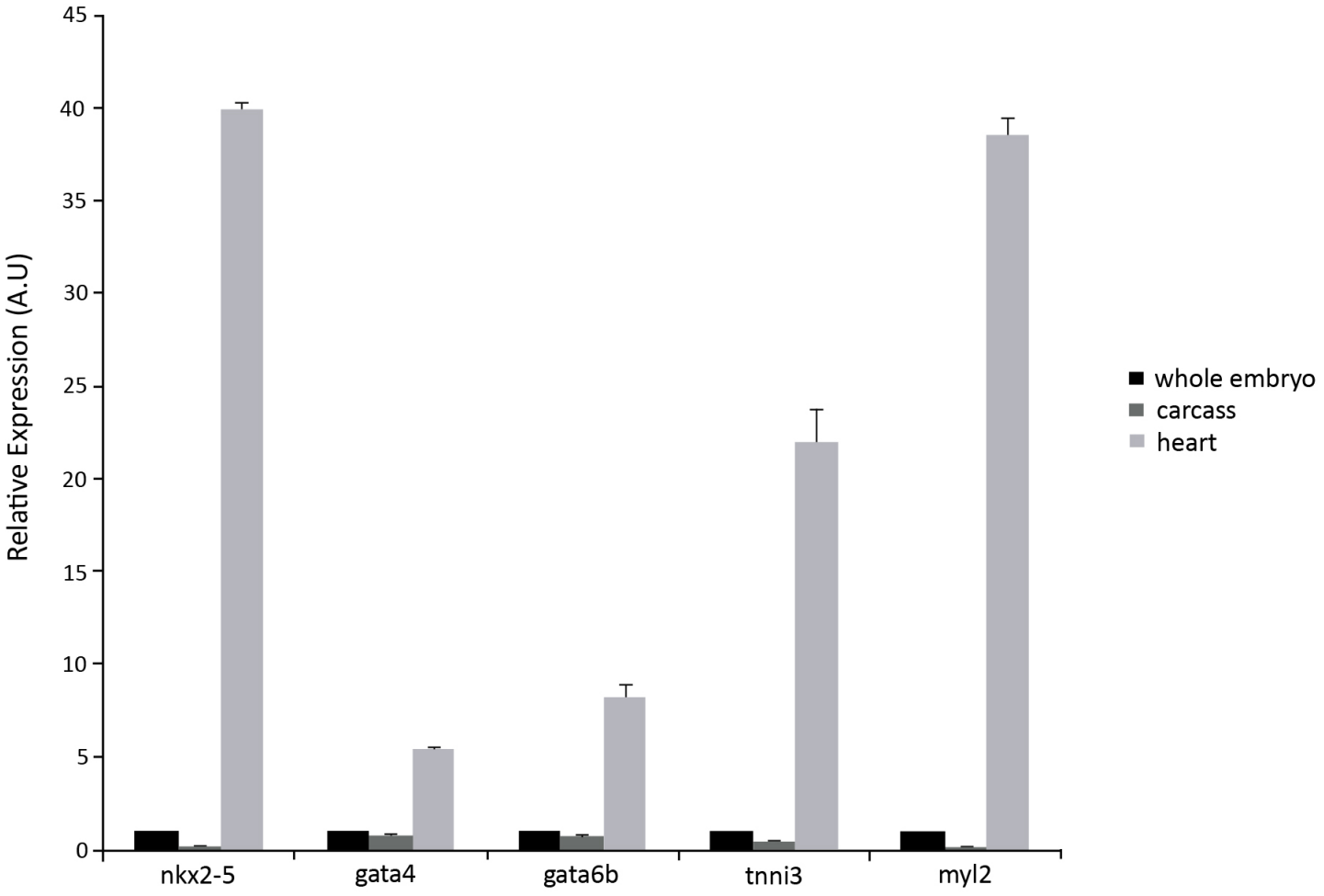


Fig. S3. Tissue specificity of cardiac marker gene expression. qPCR analysis of the relative expression of cardiogenic marker genes from dissected embryonic hearts at stage 33. The heart region was dissected out from eight uninjected embryos. The heart tissue was collected in one tube, the remaining carcasses in another tube and eight whole embryos were collected separately as a control. The tissues were snap frozen using liquid nitrogen then RNA was extracted for qPCR analysis. Gene expression levels were normalised to *odc1*. Whole-embryo gene expression was normalised to 1, with gene expression in the heart and the carcass sample expressed relative to the controls. Note the considerable enrichment of *gata4* and *gata6b* expression in the heart sample, and high levels of heart specificity for *nkx2-5, tnni3* (TnIc) and *myl2* (MLC2) expression.

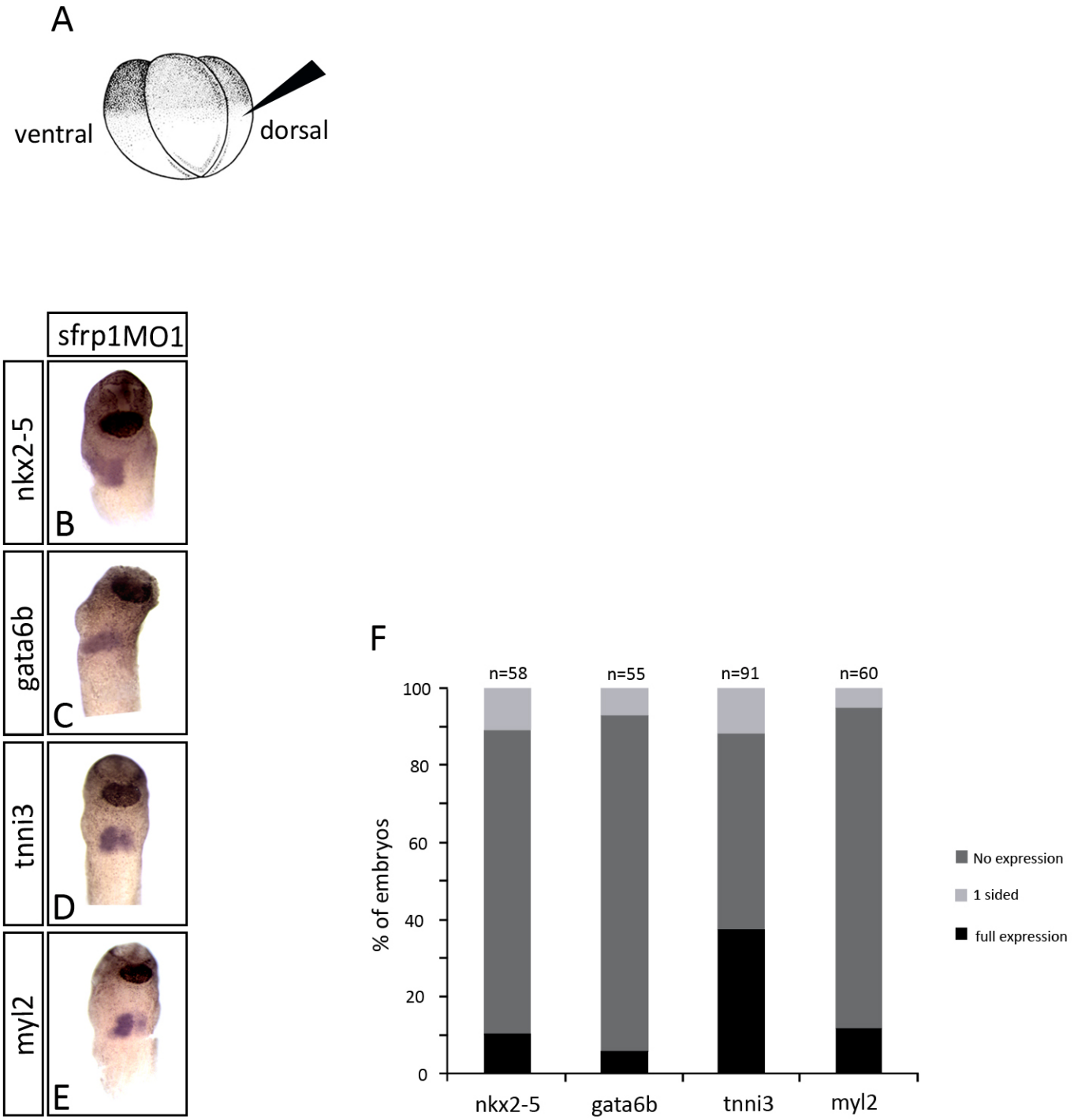


Fig. S4. Unilateral sfrp1MO-injection experiment. (**A**) sfrp1MO1 was injected into the marginal zone of the dorsal blastomere on the prospective left side of the embryo. (**B-E**) Whole-mount RNA *in situ* hybridisation analysis on stage 32 embryos showing cardiac marker gene expression: *nkx2-5* (B), *gata6b* (C), *tnni3* (D) and *myl2* (E). Note reduced gene expression on the left side of the embryos where sfrp1MO1 had been injected, but incomplete loss of detectable expression possibly due to diffusion of secreted sfrp1 protein from the uninjected right side of the embryo and cell mixing across the ventral midline. (**F**) Quantification of *in situ* hybridisation analysis.

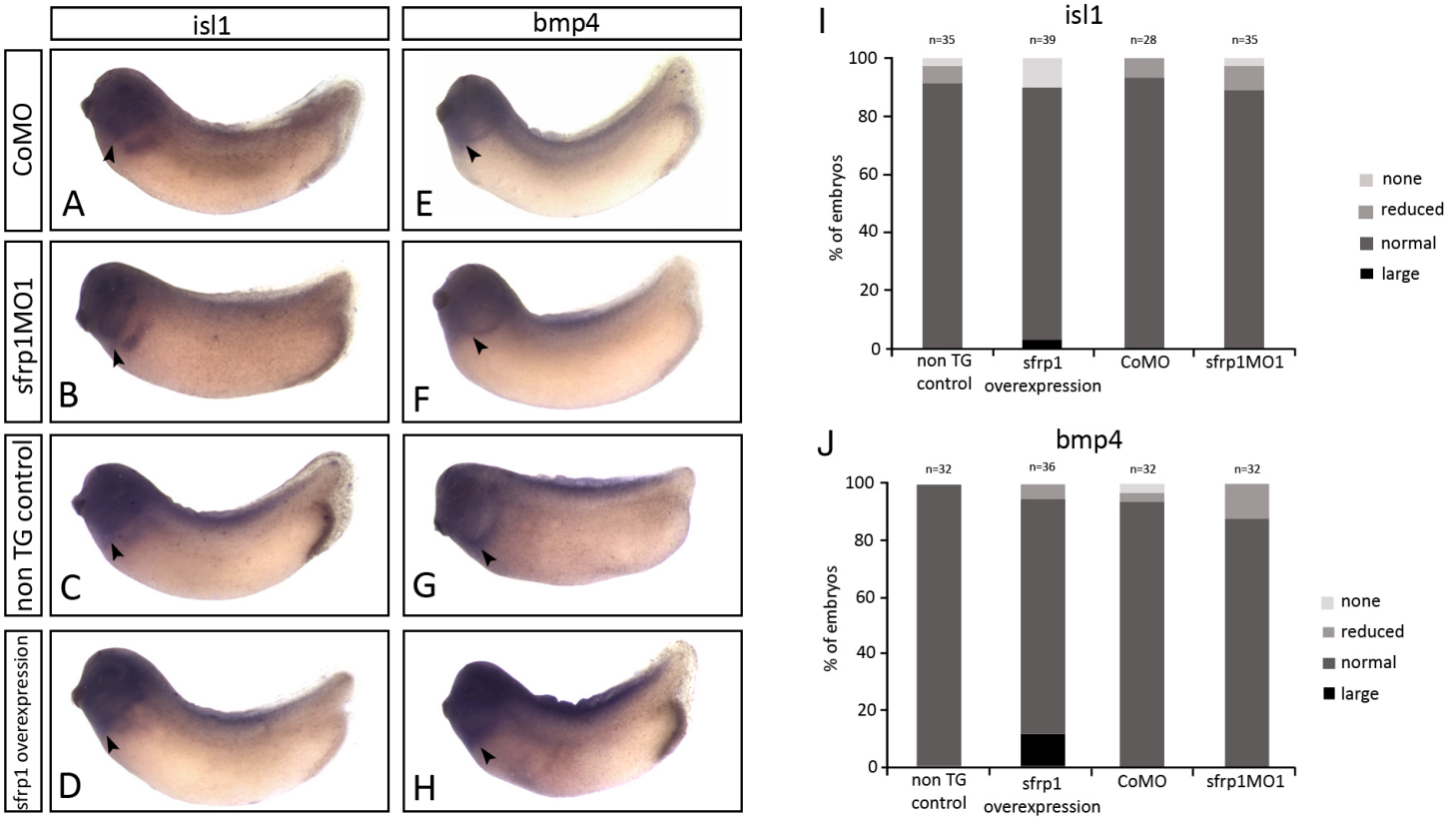


Fig. S5. Second heart field molecular marker gene expression appears unaffected in sfrp1 experiments. (**A-H**) Whole-mount RNA *in situ* hybridisation analysis on stage 32 embryos showing second heart field marker gene expression: *isl1* (A-D) and *bmp4* (E-H). (**I**,**J**) Quantification of *isl1* (I) and *bmp4* (J) *in situ* hybridisation results. Note mostly unaffected *isl1* and *bmp4* expression in embryos from experiments in which sibling embryos show altered expression of *gata6b, nkx2-5, tnni3* and *myl2* expression (as in Fig. 3), suggesting regulation of first heart field but not second heart field development at this stage.

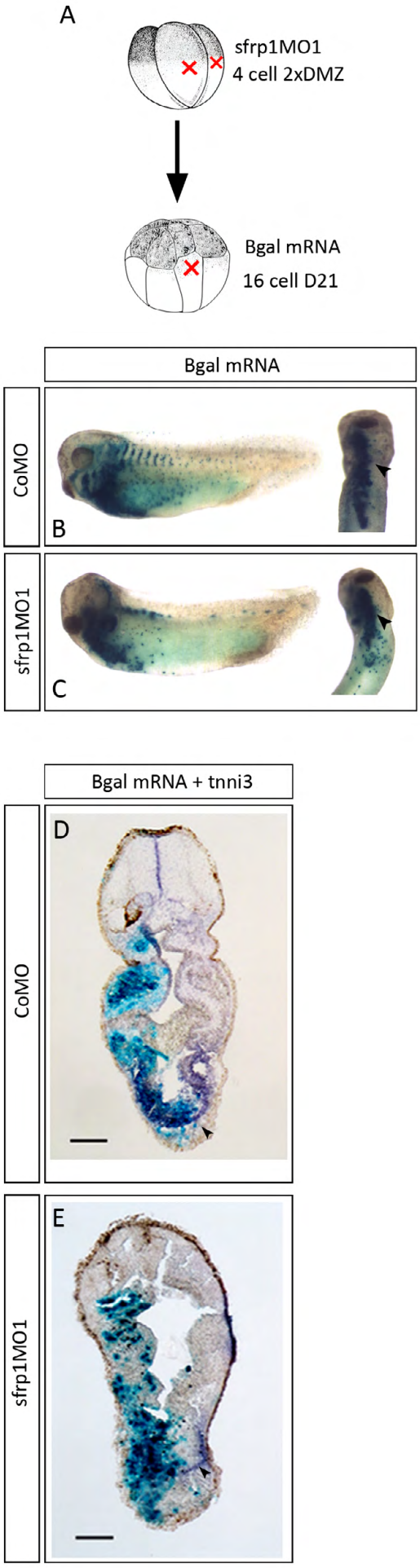


Fig. S6. β-gal lineage tracing reveals cell fate changes within the embryo. (**A**) sfrp1MO1 was injected into both dorsal blastomeres at the 4-cell stage followed by 400 pg β-gal-encoding mRNA into one D21 cell at the 16-cell stage. (**B**,**C**) X-gal staining in whole embryos at stage 32 showing the lineage patterning of injected β-gal. (**D**,**E**) Whole-mount RNA *in situ* hybridisation for *tnni3* expression on β-gal-injected embryos. CoMO (D) and sfrp1MO1 (E) injected embryos were sectioned at 14 µm. *tnni3* expression was located at the myocardium (arrowhead) with co-expression of β-gal on the injected side. Note reduced *tnni3* expression domain in E compared with D but equal β-gal expression. Scale bars: 100 µm.

Movie 1. Morpholino experiments. Compare CoMO-injected embryo at stage 42 of development, showing heart with rhythmic contractions and visible blood flow through the chambers, with sfrp1MO1-injected embryo at the same stage (in the inset bottom right), with reduced beating heart tissue and absence of visible blood flow through the chambers due to loss of heart structures.

Movie 2. Overexpression experiments. Compare non-transgenic control embryo at stage 42 of development on the left with transgenic embryo at the same stage on the right following sfrp1 overexpression at stage 22, first at low and then at higher magnification. Note the normal size heart, beating efficiently and surrounded by an adequately sized pericardial cavity in the control. Following sfrp1 overexpression the heart has increased dramatically in size, which is evident by the absence of pericardial cavity and by the ventral wall of the embryo being pushed out with every heart beat. However, the increase in the amount of myocardial tissue has not apparently compromised cardiac function at the stages examined.

Table S1. Summary of capped RNA constructs for microinjection

Plasmid construct	Restriction enzyme	Transcription enzyme	References
wnt6 (pCS2^{+}xWnt6)	*Asp*718	SP6	(Lavery et al., 2008a; Lavery et al., 2008b)
sfrp1 (xFrzA-pXT7~1)	*Sal*I	T7	(Xu et al., 1998)
nβgal (pSP6nβgal)	*Xho*I	SP6	(Smith and Harland, 1991)

Plasmid vector templates used to generate *in vitro* RNA constructs for microinjection into *Xenopus* embryos (see Fig. 6).

Table S2. Summary of antisense RNA constructs for probe synthesis

Plasmid construct	Gene	Restriction enzyme	Transcription enzyme	References
GATA5 (pXGATA-5)	*gata5*	*Xba*I	T7	(Jiang and Evans, 1996)
GATA6B (pXGATA-6B)	*gata6*	*Not*I	T7	(Gove et al., 1997)
Fz7 (pBSXfz7(λ2bi))	*fzd7*	*Not*I	T3	(Wheeler and Hoppler, 1999)
MLC2 (XMLC2)	*myl2*	*Bam*HI	T7	(Evans et al., 1995)
Nkx2.5 (pNkx2.5)	*nkx2-5*	*Hin*dIII	T7	(Tonissen et al., 1994)
sfrp1 (xFrzA-pBSKS)	*sfrp1*	*Acc*I	T7	(Xu et al., 1998)
TnIc (pXTnIc)	*tnni3*	*Not*I	T7	(Drysdale et al., 1994)

Plasmid vectors for generating *in vitro* RNA probes for whole-mount in situ hybridisation analysis of gene expression.

Table S3. Quantitative RT-PCR primer sequences

Gene	Forward primer	Reverse primer	NCBI reference
gata4	GTGCCACCTATGCAAGCCC*	GGGTTGACATTGCTCCACAG	NM_001090629.1
gata6b	CAGTCTCGCTGTCAGTGG*	GACCGGTGCCATCTCGTCTCC	NM_001090256.1
myl2 (MLC2)	CAGGGAAGGCGGCTGCCAAA	GCTTCTCGCCAAACAACGACAGG	NM_001086846.1
nkx2-5	CCCCTACCCTGCAGGATCCCAA	AGTTCCCGGCTGCACACGAGA	NM_001086721.1
odc1	ACCAACGTGTGATGGGCTGGA	AGCAGGTGCAAGTTCCATTCCGCT	NM_001086698.1
sfrp1	GAGCGAGTATGACTATGTGAGC	CAGCTACTTGCCTGGTACTTCAC	NM_001087488.1
tnni3 (TnIc)	CCTCTCTGCCGACGCCATGA	CGGCCTTCGAACACAAACAAAGA	NM_001094653.1

Sequences of primers used for quantitative RT-PCR analysis of gene expression.

*Forward primers for *gata4* and *gata6b* were obtained from Lavery et al. (Lavery et al., 2008b).