Supporting Information

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SI Materials and Methods

Gata6 DNA Injections. Full-length Gata6 cDNA coding sequence was amplified with primers incorporating the attB1 or attB2 sites listed in Table 2, and recombined into pDON221 by a Gateway cloning BP reaction. This construct was then recombined into pDestTol2pA2 with either the cardiac myosin light chain 2 [CMLC2; (1)] or smooth muscle actin (SMA) promoter cloned in pDONRp4p1r, and the p3E-polyA 3′UTR in pDONRp2p3 by using a Gateway cloning LR reaction as described (2). One-cell zebrafish embryos were injected with 1 nL of 50 ng/μL transposon DNA combined with a 100 ng/μL transposase RNA.

The αSMA promoter was cloned by fusing a putative enhancer from the first intron of the αSMA gene upstream of the promoter. Three hundred base pairs of promoter was cloned using primers SMAp-f3-attb1r and SMAp-r5, and a 2,165-bp intron fragment cloned using SMAp-f4-attb4 and SMAp-r4. The two fragments were fused by PCR and Gateway cloned into pDONRp4p1r.

SMAp-f4attb4 TATAGAAAAGTTGgccacctagcttctctca
SMAp-r4 tagcccatgacaacaatgtg
SMAp-f3 cacattgttgtcatgggctatgagcgtctgtgtagcagatcag
SMAp-r5-attb1r GTACAAACTTGgtgtacacctcactggtttgtg

miR-145 Target Identification. Potential miR-145 targets were identified from miRBASE: http://microrna.sanger.ac.uk

1. Huang CJ, Tu CT, Hsiao CD, Hsieh FJ, Tsai HJ (2003) Germ-line transmission of a myocardium-specific GFP transgene reveals critical regulatory elements in the cardiac myosin light chain 2 promoter of zebrafish. *Dev Dyn* 228:30–40.
2. Kwan KM, et al. (2007) The Tol2kit: A multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. *Dev Dyn* 236:3088–3099.

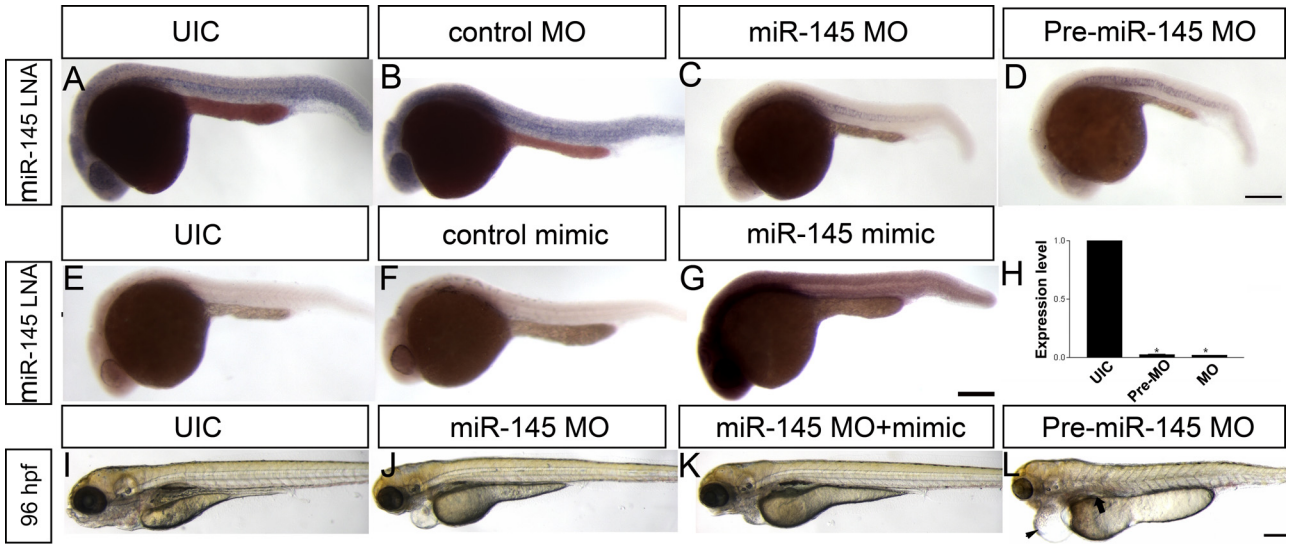

Fig. S1. Phenotype after injection of miR-145 morpholino and mimic. (*A*) LNA in situ hybridization shows ubiquitous expression of miR-145 in uninjected wild-type embryos (UIC) and (*B*) control MO-injected embryos. (*C*) miR-145 expression is significantly reduced by miR-145 MO and (*D*) pre-miR-145 morpholino. (*E*) Expression of miR-145 in uninjected or (*F*) control mimic expressing embryos is low in comparison to (*G*) miR-145 mimic-injected embryos. (*H*) qPCR indicates significant knocking down of miR-145 in 24 hpf embryos treated with mature miR-145 and pre-miR-145 morpholino. (*I–K*) Co-injection of miR-145 mimic with miR-145 morpholino can partially rescue the phenotype of miR-145 morphants. (*L*) pre-miR-145 MO induces a similar phenotype to miR-145 MO with gut morphological defects (arrow) and pericardial edema (arrowhead) in 96 hpf embryos. Bars, mean ± SEM. *, $P < 0.05$. (Scale bars, 200 μm.)

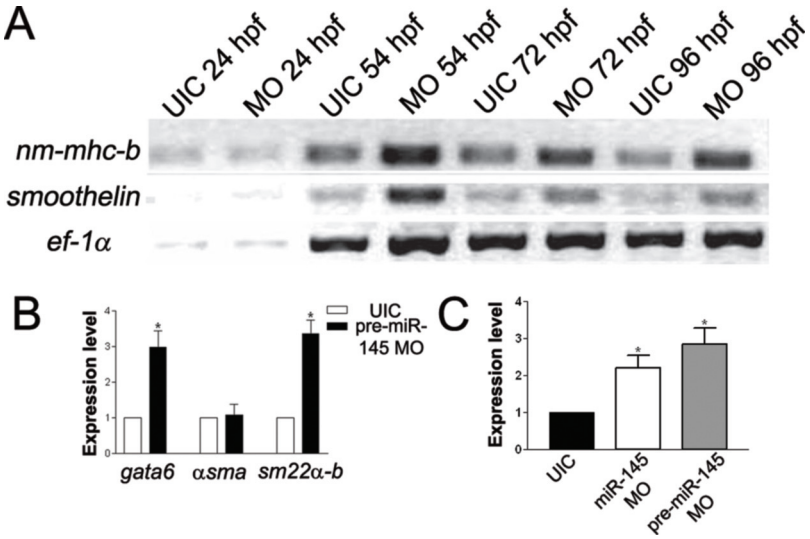

Fig. S2. Loss of miR-145 leads to smooth muscle marker expression changes. (*A*) RT-PCR shows increased expression of *nm-mhc-b* and *smoothelin* in miR-145 morphants at 54, 72, and 96 hpf in comparison with the housekeeping gene EF1α. (*B*) qPCR indicates significantly increased expression of *gata6* and *sm22α-b* but similar level of α*sma* in 48 hpf embryos treated with pre-miR-145 morpholino. (*C*) qPCR shows the inceased expression of *nm-mhc-b* in miR-145 MO or pre-miR-145 morpholino treated embryos at 48 hpf. Bars, mean $\pm$ SEM. *, $P < 0.05$.

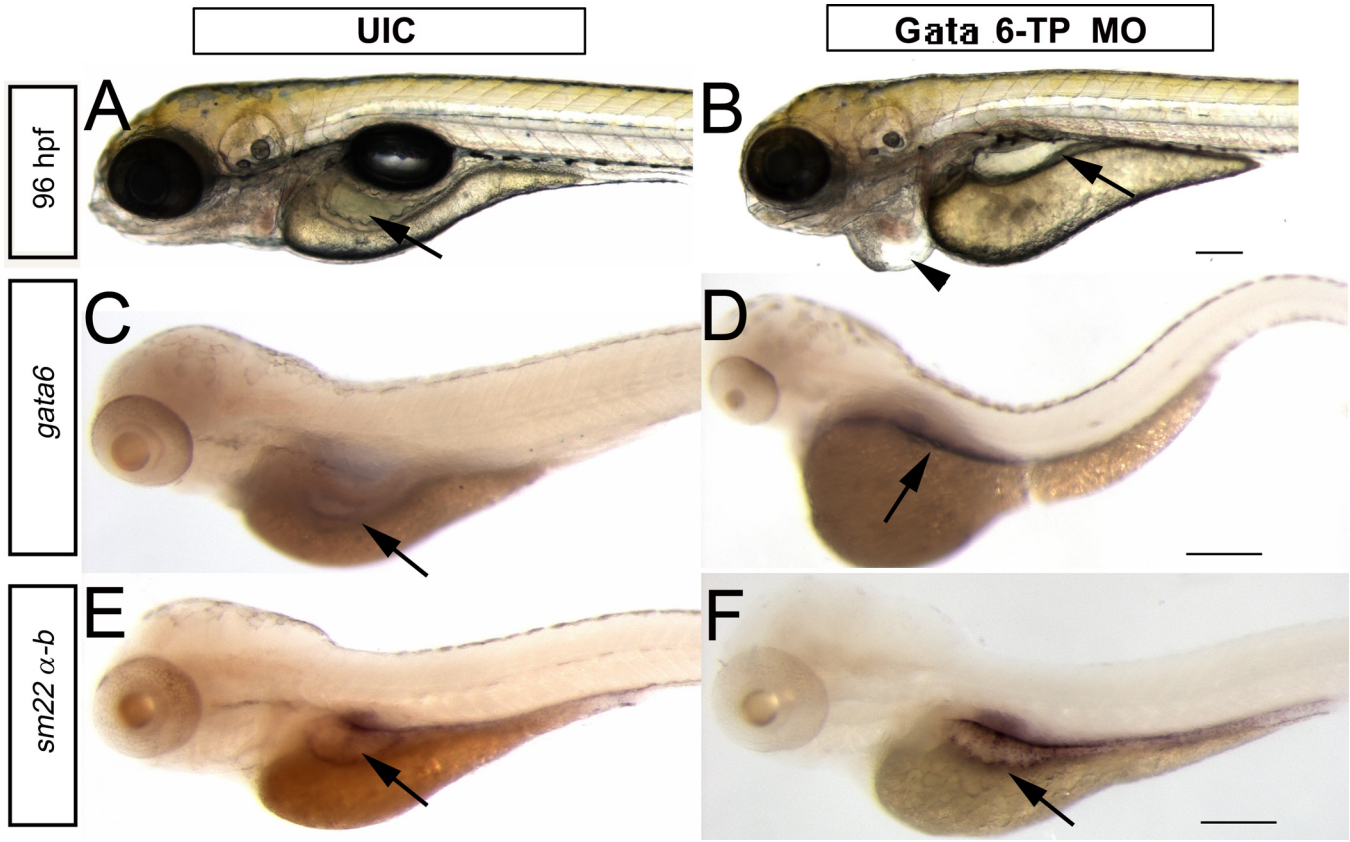

Fig. S3. Injection of the Gata6-TP^{miR145} target protector morpholino mimics the miR-145 morphant phenotype. (*A* and *B*) Gata6-TP^{miR145} target protector-injected embryos display gut morphological defects and pericardial edema (arrowhead) at 96 hpf as compared to uninjected wild-type embryos. Expression of *gata6* (*C* and *D*) and *sm22α-b* (*E* and *F*) is increased in Gata-6-TP^{miR145} morphants. Arrows, gut. (Scale bars, 200 μm.)

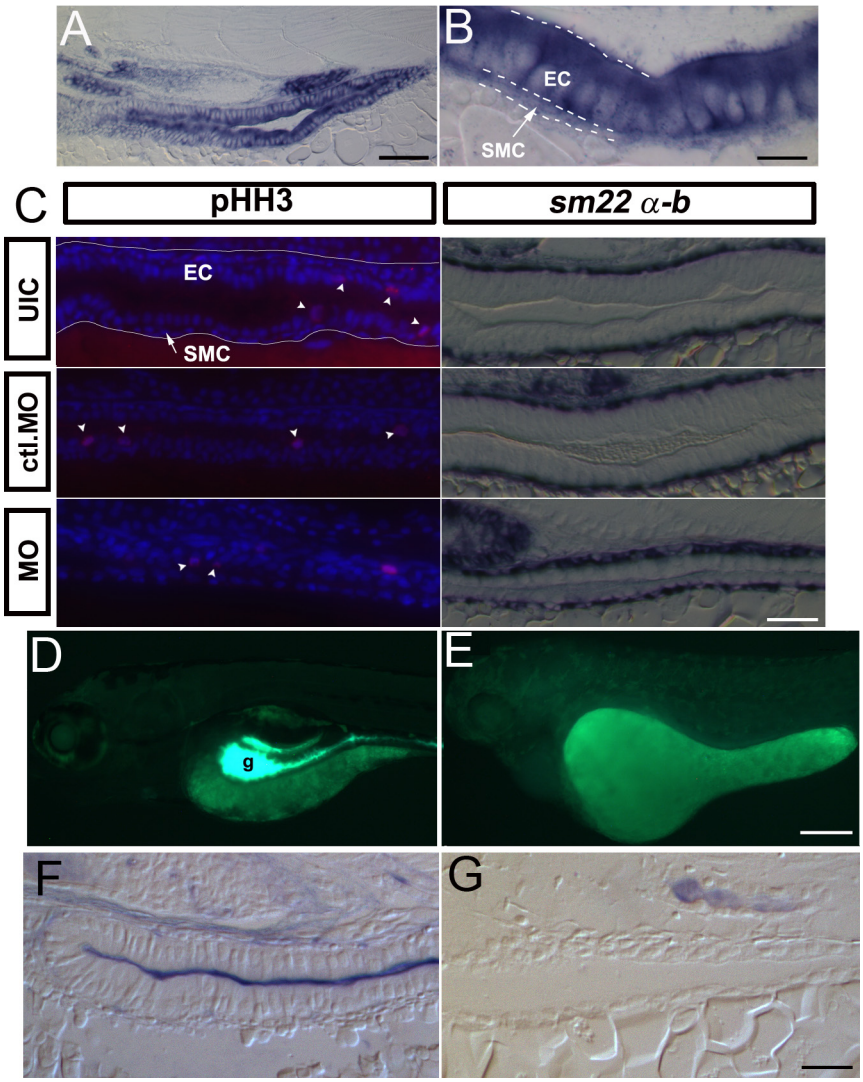

Fig. S4. Expression of *gata6* in the gut and characterization of differentiation defects in the miR-145 morphants. (*A*) A longitudinal section of a 96 hpf wild-type embryo shows strong expression of *gata6* in gut epithelial and smooth muscle cells. (*B*) Enlargement of (*A*) highlights staining of *gata6* in both layers. White dashed lines distinguish the smooth muscle cell layer and epithelial cell layer. (*C*) Longitudinal sections of phosphohistone H3 (pHH3) staining (red) in the 96 hpf gut. Nuclei were counterstained with DAPI (blue). No significant difference of pHH3 positive cells (white arrowheads) was found in the different experimental groups in either epithelial or smooth muscle layers. The white line in the uninjected embryo highlights the gut epithelium. In the second column, longitudinal sections of *sm22α-b* in situ hybridization in 96 hpf embryos shows specific staining in the smooth muscle cell layer, and highlights abnormal morphology of both epithelial and smooth muscle cells in miR-145 morphants as compared to controls. (*D* and *E*) The wild-type gut (g) is strongly fluorescent at 120 hpf after DAF-2DA staining (Green) in comparison with miR-145 morphants. (*F* and *G*) Strong alkaline phosphatase activity is observed in the gut lumen of wild-type embryos but is absent in miR-145 morphants. SMC, smooth muscle cell (white arrow); EC, epithelial cell; ctl. MO, control morpholino. (Scale bars, *A*: 50 μm; *B*: 10 μm; *C* and *G*; 25 μm; *D*: 200 μm.)

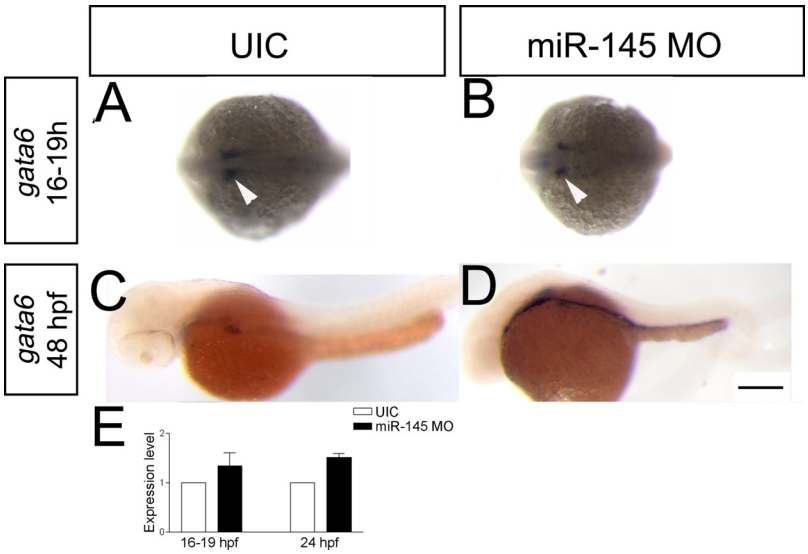

Fig. S5. *gata6* expression level are not modulated by miR-145 at early embryonic stages. (*A* and *B*) *gata6* expression levels (white arrowhead) are unchanged in myocardial precursors of uninjected wild-type embryos and miR-145 morphants at 16–19 hpf. (*C* and *D*) *gata6* expression is significantly increased in miR-145 morphants at 48 hpf. (Scale bars, 200 μm.) (*E*) There is no significant change in gata6 expression in miR-145 morphants at 16 hpf and 24 hpf by qPCR.

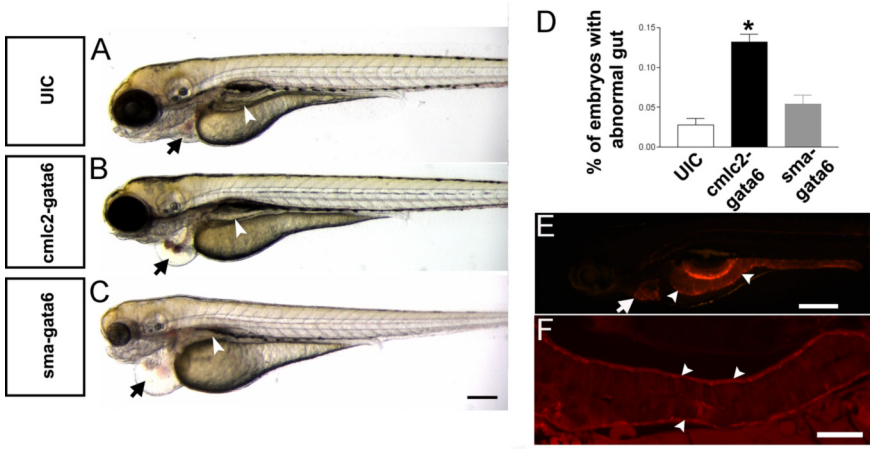

Fig. S6. Characterization of tissue-specific *gata6* overexpression on development. (*A–C*) Pericardial edema (black arrow) results from injection of either *SMA:Gata6* or *CMLC2:Gata6*, but only *SMA:Gata6*-injected embryos show an abnormal gut tube (white arrowhead) at 96 hpf. (*D*) The percentage of embryos exhibiting the abnormal gut morphology was analyzed by using Student's *t* test ($P < 0.05$). A significantly higher percentage of embryos with abnormal gut development is found in *SMA:Gata6* injected embryos. Bars, mean ± SEM. *, $P < 0.05$ ($n > 50$ per group). (*E*) Lateral view of a control *Tg (SMA: mCherry)* transgenic embryo at 96 hpf. The heart (white arrow) and gut smooth muscle (white arrowheads) show expression of mCherry. (*F*) Longitudinal section of a 96 hpf *Tg (SMA: mCherry)* embryo shows that mCherry is specifically expressed in visceral smooth muscle cells (white arrowheads). (Scale bars, *C*: 200 μm; *E*: 400 μm; *F*: 50 μm.)

Table S1. Morpholino and mimic sequences

MO name	MO Sequence	Dose	Ref.
miR-145 MO	TTGCCCAAGGGATTCCTGGGAAAACTGGACC	3 ng	This paper
miR-145 precursor MO	AGTATTTCCAGGAATCCCCCTTTCG	15 ng	This paper
Mismatch miR-145 MO	CCAAGAACAGTATTTCCAGGAATCC	3 ng	This paper
Control TP	TTGTCCAAACTCATCAATGTATCTT	0.4 ng for sensor test	This paper
GATA-6-TP $^{miR-145}$	TCTTCCAGTTTCAGAACGATCAATC	15 ng for phenotype assay/0.4 ng for sensor test	This paper
p53 MO	GCGCCATTGCTTTGCAAGAATTG	1.5 (wt/wt) folds along with other MOs	1
GATA6 MO	AGCTGTTATCACCCAGGTCCATCCA	0.4 ng for rescue	2
hsa-miR-145 miRIDIAN mimic	GUCCAGUUUUCCCAGGAAUCCCU	3 ng for overexpression assay/0.3 ng for sensor test	This paper
Negative control mimic	UCACAACCUCCUAGAAAGAGUAGA	3 ng for overexpression assay	This paper

1. Robu ME, et al. (2007) p53 activation by knockdown technologies. *PLoS Genet* 3:e78.
2. Peterkin T, Gibson A, Patient R (2003) GATA-6 maintains BMP-4 and Nkx2 expression during cardiomyocyte precursor maturation. *EMBO J* 22:4260–4273.

Table S2. Primer sequences

Primer name	Primer Sequence (forward/reverse)	Fragment length, nt
SM22α -b	AGACGGATGCGTGTTGTG/TCTGCTTAAAGGCCATGCTG	92
α SMA	TCAAGATAATCGCTCCACCTG/TTGCTGATCCACATCTGCTG	96
EF-1α	AGCTGATCGTTGGAGTCAAC/TGCGCTGACTTCCTTGGTG	89
GATA6–3′UTR-BamHI forward/GATA6–3′UTR-BamHI reverse	CGCGGATCCGGCATCACGGGGACCATTC/ CGCGGATCCTTCAGTCCTATTACTTTATTG	465
GATA6–3′UTR-HindIII forward/GATA6–3′UTR-SacI reverse	CCCAAGCTTGGCATCACGGGGACCATTC/ GGTGGTGAGCTCTTCAGTCCTATTACTTTATTG	465
GATA6-CDS-attB1-forward/ GATA6-CDS-attB2 reverse	AAAAAGCAGGCTTCATGTATCAGACCCTGGCCAT/ AGAAAGCTGGGTGGTGATGCCAGGCTTTAGGC	1,163
GATA6 QPCR	AAACCTCAGAAGCGCATGTC/AGACCACAGGCGTTGCAC	125