

Fig. S1

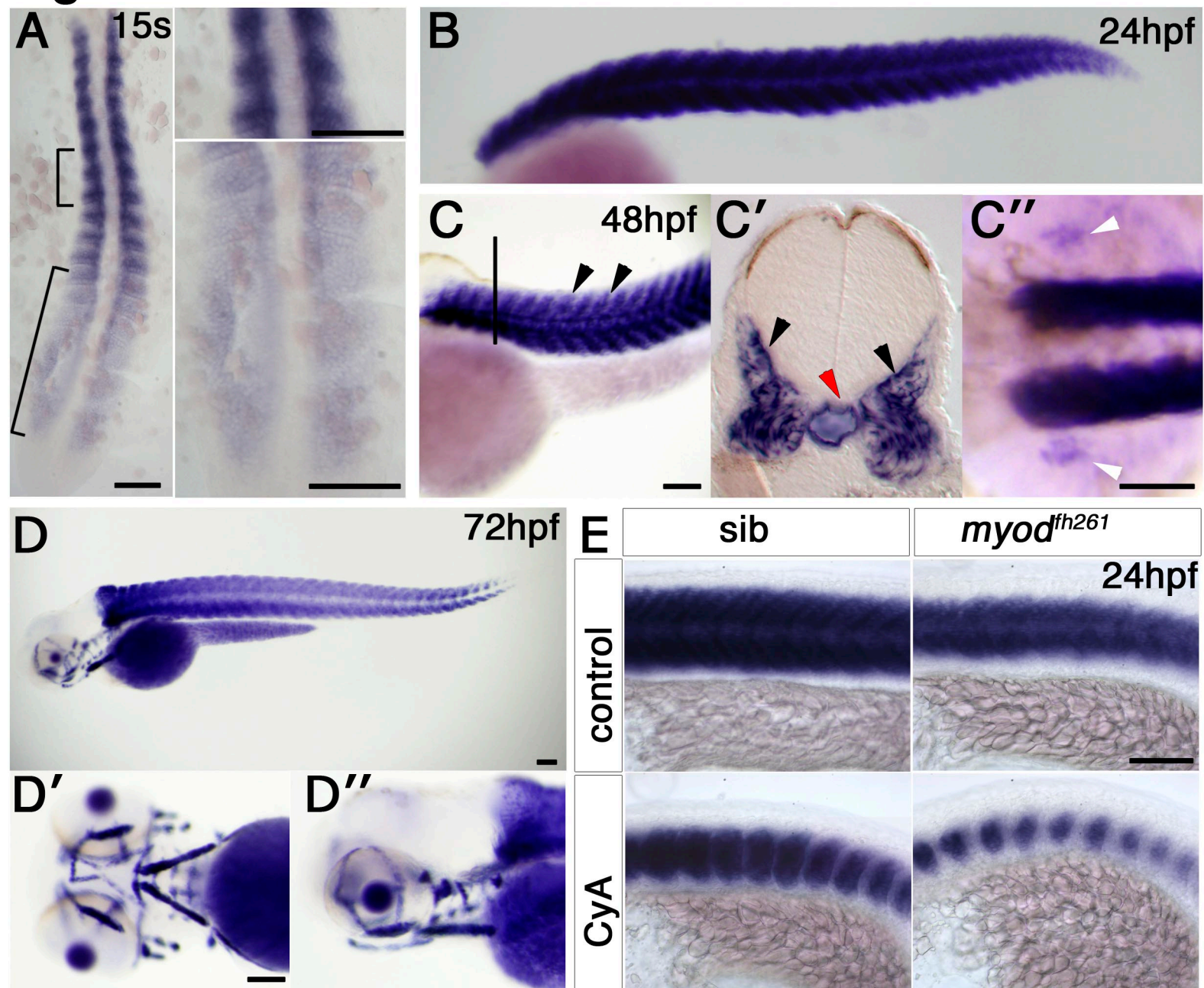


Fig. S1. Expression of miR-206 in zebrafish development.

In situ RNA hybridization for miR-206. Dorsal (A) or lateral (E) flatmounts, lateral (B,C,D,D''), dorsal (C'') or ventral (D') wholemounts, anterior to top (A) or left (B-E). **A.** Expression is detected late in adaxial slow fibre differentiation and early in fast myogenesis. **B.** At 24 hpf, expression is restricted to the differentiated somitic muscle. **C.** By 48 hpf, expression is abundant in somitic fast muscle (arrowheads). A transverse section at the level indicated by the line in C, shows signal in somitic fast muscle (C', black arrowheads). Notochord signal (red arrow) is detected in some embryos. Pectoral fin muscle expression is also detected (C'', white arrowheads). **D.** At 72 hpf, the forming cranial muscles also contain miR-206. **E.** miR-206 expression is reduced in somitic muscle of *myod*^{fh261} mutant and further reduced after cyclopamine treatment, indicating that miR-206 is expressed in slow muscle fibres. Bars = 100 μ m.

Fig. S2: Myf5 or Myod is required for myogenesis.

Dorsal flatmounts (A,D-bottom panel), ventral (D-top panel) and lateral (B,C) wholemounts or transverse cryosections (E) of zebrafish embryos from *myf5*^{hu2022/+};*myod*^{fh261/+} in-cross analysed at the indicated stage by immunohistochemistry for slow MyHC (A) or all MyHC (E) or *in situ* mRNA hybridization for *smyhc1* (B) or *mylz2* (C,D), anterior to top (A -D), dorsal to top (E). **A.** At 15s, most embryos show strong slow muscle differentiation, but approximately 3/16ths (genotyped as *myod*^{fh261/fh261};*myf5*^{hu2022/+} or *myod*^{fh261/fh261};*myf5*^{+/+}) have less muscle and 1/16th (genotyped as *myod*^{fh261/fh261};*myf5*^{hu2022/hu2022}) have no detectable muscle. **B.** mRNAs encoding slow myosin is reduced in putative *myod*^{fh261} mutants and absent in doubles. **C,D.** At 72 hpf, no *mylz2* mRNA is detected in genotyped double mutants, in which the trunk and tail is severely reduced in size. **E.** At 120 hpf, double mutant lacks MyHC in the gut extension region and has a reduced somite area. Note the similar sizes of non-muscle tissues. Bars = 100 μ m.

Fig. S2

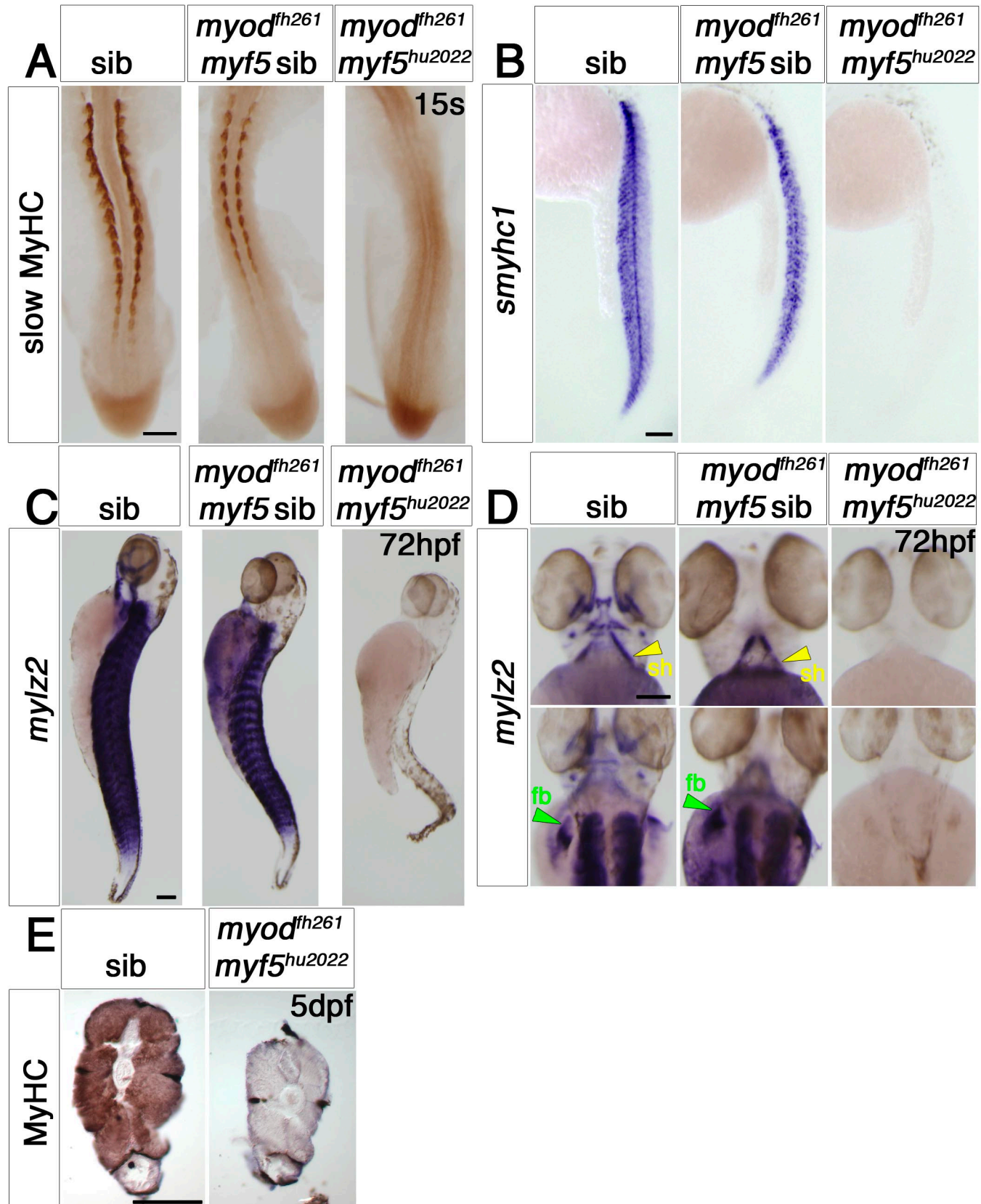


Table S1 Quantification of effects of mutations, morpholinos and drug treatments.

Fig. No.		<i>myod</i> ^{+;flx261} incross	<i>myog</i> ^{+;flx265} incross	<i>myog</i> ^{+;flx265} incross + <i>myod</i> MO	<i>myf5</i> ^{+;flx2022} incross	<i>myf5</i> ^{+;flx2022} ; <i>myod</i> ^{flx261} incross	control	<i>myod</i> MO
1B	slow MyHC(F59); Myod IHC*	5/22 (22%) n, 10/22 (46%) r, 7/22 (32%) sr(MyHC), a(Myod)						
not in Figs	Myod IHC 15s	8/30 (27%) n, 13/30 (43%) r, 9/30 (30%) a						
1C	<i>myod</i> * 10s	14/43 (33%) n, 20/43 (47%) r, 9/43 (21%) sr						
1D	<i>smyhc1</i> 10s	12/40 (30%) r						
1D	<i>actc</i> 10s	7/34 (21%) r						
1D	miR-206 15s	19/56 (34%) r						
1D, 2B	<i>myog</i> * 10s	7/32 (22%) sr	20/46 (44%) r, 13/46 (28%) sr					
1E	<i>eng2a</i> 24s	13/46 (28%) r						
1F	<i>mylz2</i> 24s	10/39 (26%) w						
1F	DP312 IHC 24s	sibs 4/4 (100%) n; mutants 3/3 (100%) ur						
1F, 2C	<i>myhz1</i> , 24hpf	2/9 (22%) rl	15/15 (100%) n	19/28 (68%) rl 9/28 (32%) srl				
3A	miR-206* 48hpf	20/74 (27%) srl			20/20 (100%) n			
3B,C	miR-206 72hpf	13/50 (26%) rl					35/35 (100%) n	70/70 (100%) rl
3C	<i>myog</i> 72hpf	5/25 (20%) rl						
3C	<i>myhz1</i> 72hpf	9/36 (25%) rl						
4A	miR-206 72hpf	13/50 (26%) a					35/35 (100%) n	70/70 (100%) a
4B	<i>myog</i> 72hpf	5/25 (20%) a						
4C	<i>myhz1</i> 72hpf	9/36 (25%) a						
4D	MyHC (MF20) 96hpf	6/46 (13%) a						
5A	Phenotype (bright field), 5-6 dpf*	38/149 (26%) ab					40/40 (100%) n	63/83 (76%) ab
5B,C	alcian blue+ alizarin red, 5-6 dpf	9/54 (17%) r						
6A	<i>myod</i> ; <i>dlx2a</i> , 52hpf	18/50 (36%) a (<i>myod</i>); 50/50 (100%) n (<i>dlx2a</i>)						
6B	<i>myhz1</i> ; <i>kif2b</i> 72hpf	6/38 (16%) a (<i>myhz1</i>); 38/38 (100%) n (<i>kif2b</i>)						
6C	MyHC IHC, DAF-2 DA	12/52 (23%) a (MyHC); 52/52 (100%) n (DAF-2DA)						
7A	phenotype at 24hpf					5/75 (7%) ab	41/41 (100%) n	
7B	<i>mylz2</i> , 24hpf					76/99 (77%) n, 16/99 (16%) r, 7/99 (7%) a	134/134 (100%) n	
7C	MyHC; smooth myh11; Hoescht, 4dpf					39/54 (72%) n, 11/54 (20%) r, 4/54 (7%) a		
7D	MyHC; smooth myh11; Hoescht, 4dpf					39/54 (72%) n, 11/54 (20%) asn, 4/54 (7%) a		
7E	alcian blue+ alizarin red, 6.5 dpf					51/82 (62%) n, 23/82 (28%) r, 8/82 (10%) sr		
S2A	slow MyHC (F59) IHC, 15s*				117/117 (100%) n	63/86 (73%) n, 20/86 (23%) r, 3/86 (4%) a		
S2B	<i>smyhc1</i> , 24hpf					48/59 (81%) n, 7/59 (12%) r, 6/59 (10%) a	41/41 (100%) n	
S2C	<i>mylz2</i> , 72hpf					49/66 (74%) n, 12/66 (18%) r, 5/66 (8%) a		
S2D	<i>mylz2</i> , 72hpf					49/66 (74%) n, 12/66 (18%) asn, 5/66 (8%) a		

1B) Fraction of 15s embryos showing normal, reduced, strongly reduced or absent slow MyHC and Myod immunostaining.

1C-F, 2B,C) Fraction of embryos showing normal, reduced, strongly reduced, reduced laterally or strongly reduced laterally mRNA expression of the indicated genes.

1F) Fraction of embryos showing normal or upregulated immunostaining of DP312 in sections of 24s embryos stained for *mylz2* mRNA.

3A-C) Fraction of embryos showing normal, reduced laterally or strongly reduced laterally mRNA expression of the indicated genes.

4A-D) Fraction of embryos showing normal or absent mRNA or immunostaining of the indicated muscle markers in head muscles (excluding for the sternohyoides muscle).

5A) Fraction of 5-6 dpf embryos showing normal or abnormal craniofacial morphology.

5B,C, 7E) Fraction of 5-6 dpf embryos showing reduced or strongly reduced head cartilage and bone staining.

6A,B) Fraction of embryos showing normal or absent *myod* or *myhz1* mRNA in cranial muscle (excluding for the sternohyoides muscle), and *dlx2a* or *kif2b* mRNA in cartilage and bone cells.

6C) Fraction of embryos with normal or absent MyHC immunostaining in head muscles (excluding for the sternohyoides muscle) and DAF-2DA staining in bone.

7A) Fraction of 24hpf embryos showing abnormal phenotype (curved embryos lacking motility).

7B,C, S2B,C) Fraction of embryos showing normal, reduced laterally or strongly reduced laterally mRNA expression of the indicated genes or MyHC immunostaining in somitic muscle.

7D, S2D) Fraction of embryos showing normal, absent (except for the sternohyoides muscle) or absent (with no exceptions) *myhz1* mRNA or MyHC immunostaining in head muscles.

S2A) Fraction of 15s embryos showing normal, reduced or absent slow MyHC immunostaining.

Abbreviations: n, normal, ab, abnormal, r, reduced, sr, strongly reduced, rl, reduced laterally, srl, strongly reduced laterally, ur, upregulated, a, absent, asn, absent except for the sternohyoides muscle.

* Results verified by genotyping.