

| Table S1. Quantification of effect of MOs, mutants and drug treatments | | | | | | | |
|--|--|--|---------------------------------------|-------------------------------|--|-------------------------------|--|
| Figure number and panel | Assay | Control | <i>myf5</i> MO | <i>myod</i> MO | <i>myog</i> MO | <i>myf5</i> + <i>myod</i> MOs | |
| Fig. 1-left | <i>smyhc1</i> mRNA, 15s | an0/47 (0%) | an0/15 (0%) | d18/18 (100%) | an0/20 (0%) | ab22/25 (88%) | |
| Fig. 1-left | <i>actin</i> mRNA, 15s | an0/57 (0%) | an0/53 (0%) | an0/66 (0%) | an0/19 (0%) | sr36/41 (88%) | |
| Fig. 1-left | <i>tpma</i> mRNA, 15s | an0/50 (0%) | an0/21 (0%) | an0/21 (0%) | an0/15 (0%) | sr13/16 (81%) | |
| Fig. 1-left | slow MyHC IHC, 15s | an0/40 (0%) | w6/52 (12%) | d46/49 (94%) | an0/40 (0%) | ab31/35 (89%) | |
| Fig. 1-right | <i>myf5</i> mRNA, 15s | an0/54 (0%) | s27/27 (100%) | an0/40 (0%) | an0/40 (0%) | | |
| Fig. 1-right | <i>myod</i> mRNA, 15s | an0/50 (0%) | an0/28 (0%) | s39/41 (95%) | an0/40 (0%) | | |
| Fig. 1-right | <i>mrf4</i> mRNA, 15s | an0/41 (0%) | w12/18 (33%) | an0/34 (0%) | an0/36 (0%) | | |
| Fig. 1-right | <i>myog</i> mRNA, 15s | an0/48 (0%) | an0/29 (0%) | r28/33 (85%) | s28/36 (78.8%) | | |
| | | <i>myf5</i> ^{hu2022} incross | | | | | |
| Fig. 2D | <i>myf5</i> mRNA, 15s | n6/28 (21%), w13/28 (46%), vw9/28 (32%) | | | | | |
| Fig. 2E-left | <i>myog</i> mRNA, 5s | sr15/49 (30.6%) | | | | | |
| Fig. 2E-right | <i>myf5</i> mRNA, 10s + <i>myog</i> | vw20/74 (27%) r20/74 (27%) | | | | | |
| | | Control | <i>myf5</i> ^{hu2022} incross | <i>myod</i> MO | <i>myf5</i> ^{hu2022} incross + <i>myod</i> MO | <i>myog</i> MO | <i>myf5</i> + <i>myod</i> MO |
| Fig. 3B | <i>myf5</i> mRNA, 13s + <i>mef2ca</i> | | | | vw7/34 (21%) <i>myf5</i> sr <i>mef2ca</i> | | |
| Fig. 3B | <i>prdm1</i> mRNA + MyHC IHC, 15s | an0/32 (0%) <i>prdm1</i> an0/32 (0%) MyHC | | | | | an0/61 (0%) <i>prdm1</i> sr58/61 (95%) MyHC |
| Fig. 3B | <i>myf5</i> mRNA, 13s + <i>prdm1</i> | | | | vw8/31 (26%) <i>myf5</i> an0/31 (0%) <i>prdm1</i> | | |
| Fig. 3B | <i>myf5</i> mRNA, 13s + <i>ptc1</i> | | | | vw5/23 (22%) <i>myf5</i> an0/23 (0%) <i>ptc1</i> | | |
| Fig. 3B | <i>smyhc1</i> mRNA, 15s | | an0/87 (0%) | | ab29/96 (30%) | | |
| Fig. 3B | <i>actin</i> mRNA, 15s | | an0/99 (0%) | | ab8/46 (17%) | | |
| Fig. 3B | <i>tpma</i> mRNA, 15s | | an0/97 (0%) | | ab15/67 (22%) | | |
| Fig. 3C | MyHC IHC, 80hpf (cranial) | an0/24 (0%) | an0/40 (0%) | sr20/22 (91%) | sr31/34 (91%) | | |
| Fig. 3C | <i>myhz1</i> mRNA, 72hpf (cranial) | an0/35 (0%) | an0/30 (0%) | sr10/24 (42%) r14/24 (58%) | | r15/18 (83%) | |
| Fig. 3D | <i>myog</i> mRNA, 48hpf (cranial) | an0/17 (0%) | an0/49 (0%) | sr13/21 (62%) r6/21 (29%) | | | |
| Fig. 3E | MyHC IHC, 80hpf (fin) | an0/24 (0%) | an0/40 (0%) | sr12/22 (55%) r8/22 (36%) | 25/34 (sibs): sr12/25 (48%) | | |

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| | | | | | r10/25 (40%) 9/34 (muts') sr9/9 (100%) | | | |
| Fig. 3E | <i>myog</i> mRNA, 48hpf (fin) | an0/17 (0%) | an0/15 (%) | 11/21 (52.3%) (5/21-mild) | | | | |
| Fig. 3E | <i>lhx2</i> mRNA, 48hpf (fin) | an0/52 (0%) | an0/20 (0%) | an1/49 (2%) | an0/45 (0%) | | | |
| Fig. 3F | <i>myf5</i> mRNA, 15s + <i>hsp90a</i> | | vw11/37 (30%) <i>myf5</i> , d <i>hsp90a</i> | | vw3/15 (20%) <i>myf5</i> sr <i>hsp90a</i> | | | |
| Fig. 3F | <i>myf5</i> mRNA, 15s + <i>meox1</i> | | vw5/19(26%) <i>myf5</i> sr0/19 (0%) <i>meox1</i> | | vw9/29 (31%) <i>myf5</i> sr0/29 (0%) <i>meox1</i> | | | |
| | | Control | <i>myf5</i> MO | <i>myod</i> MO | <i>myog</i> MO | <i>myf5</i> + <i>myod</i> MOs | <i>myf5</i> ^{hu2022} incross | <i>myf5</i> ^{hu2022} incross + <i>myod</i> MO |
| Fig. 4A | <i>myog</i> mRNA, 10s | an0/20 (0%) | | | | ab20/21 (95%) | | |
| Fig. 4A | <i>myog</i> mRNA, 20s | an0/30 (0%) | | | | sr24/27 (89%) | | |
| Fig. 4B | MyHC IHC, 24hpf | an0/21 (0%) | | r15/15 (100%) | | sr34/38 (89%) | an0/41 (0%) | r24/36 (67%) sr12/36 (33%) |
| Fig. 4C | Slow + fast MyHC IHC, 24hpf | an0/25 (0%) | | | | sr17/19 (89%), fast ab14/19 (73%) sr4/19 (21%), slow | | |
| | | Control | <i>myod</i> MO | <i>myf5</i> ^{hu2022} incross | <i>myf5</i> ^{hu2022} incross + <i>myod</i> MO | <i>myf5</i> ^{hu2022} incross+ <i>myf5</i> MO | <i>myf5</i> ^{hu2022} incross + <i>myf5</i> + <i>myod</i> MOs | |
| Fig. 4D | <i>myhz1</i> mRNA, 24hpf | an0/49 (0%) | | an0/104 (0%) | r87/117 (74%) sr30/117 (26%) | an0/37 (0%) | sr41/41 (100%) | |
| Fig. 4D | <i>tpma</i> mRNA, 24hpf | | | an0/28 (0%) | r31/43 (72%) sr12/43 (28%) | | | |
| Fig. 4D | <i>mylz2</i> + <i>myog</i> mRNA, 24hpf | | | an0/26 (0%) | r26/30 (87%) sr4/30 (13%) | | | |
| Fig. 4D | <i>mylz2</i> + <i>myog</i> mRNA, 20s | an0/57 (0%) | r33/33 (100%) | an0/64 (0%) | r35/44 (79%) sr9/34 (21%) | | | |
| | | Control | <i>myf5</i> MO | <i>myod</i> MO | <i>myog</i> MO | <i>myf5</i> ^{hu2022} incross | <i>myf5</i> ^{hu2022} incross + <i>myod</i> MO | |
| Fig. 4E | <i>mef2d</i> mRNA, 15-16s | an0/20 (0%) | | r18/20 (90%) | | an0/49 (0%) | r35/43 (81%) sr8/43 (19%) | |
| Fig. 4E | <i>mef2d</i> mRNA, 22s | an0/56 (0%) | an0/13 (0%) | r19/19 (0%) | w12/12 (100%) | | | |
| | | Control | <i>myf5</i> + <i>myod</i> MOs | <i>mrf4</i> ^{hu2041} incross | <i>mrf4</i> ^{hu2041} incross + <i>myf5</i> + <i>myod</i> MOs | <i>myf5</i> ^{hu2022} incross | <i>myf5</i> ^{hu2022} incross + <i>myod</i> MO | |
| Fig. 5C | <i>myog</i> mRNA, 22s + <i>mrf4</i> | an0/14 (0%) | sr16/19 (84%) <i>myog</i> ab <i>mrf4</i> | | | an0/45 (0%) | sr13/37(35%) <i>myog</i> ab <i>mrf4</i> ; r24/37(65%) <i>myog</i> , <i>mrf4</i> | |
| Fig. 5C | <i>myog</i> mRNA, + <i>mrf4</i> , 24hpf | | | | | an0/12 (0%) | sr8/27(29%) <i>myog</i> sr <i>mrf4</i> ; r19/27 (71%) <i>myog</i> , | |

| | | | | | | | <i>mrf4</i> | |
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| Fig. 5D | <i>myhz1</i> mRNA, 24hpf | an0/26 (0%) | sr24/26 (92%) | w1/20 (5%) | sr23/25 (92%) | | | |
| | | Control | <i>myod</i> MOs | <i>myod</i> + <i>myog</i> MOs | <i>myf5^{hu2022}</i> incross | <i>myf5^{hu2022}</i> incross + <i>myod</i> + <i>myog</i> MOs | <i>myf5^{hu2022}</i> incross + <i>myog</i> MOs | <i>myog</i> MOs |
| Fig. 6A | <i>mylz2</i> + <i>smyhc1</i> mRNA, 24hpf | an0/21 (0%) | | sr14/14 (100%) <i>mylz2</i> an0/14 (0%) <i>smyhc1</i> | an0/20 (0%) | sr,n16/24 (67%) <i>mylz2</i> , <i>smyhc1</i> ab8/24 (33%) <i>mylz2</i> , <i>smhyc1</i> | | |
| Fig. 6B | <i>myhz1</i> mRNA, 24hpf | | | | an0/23 (0%) | | an0/23 (0%) | |
| Fig. 6B | <i>smyhc1</i> mRNA, 24hpf | | | | an0/53 (0%) | | an0/67(0%) | |
| Fig. 6C | Tg(acta1: GFP)/slow MyHC/Mef2 IHC, 24hpf | an0/29 (0%) | r15/15 (100%) GFP | 3sr0/30 (100%) GFP, Mef2 (in fast) | | | | |
| Fig. 6D | fast MyHC IHC, 24hpf | an0/20 (0%) | r15/18 (83%) | sr13/16 (81%) | | | | an0/17 (0%) |
| Fig. 6E | <i>myhz1</i> mRNA, 24hpf | | | 1 ng each MO -r43/52 (82%) 2 ng each MO -r8/23 (35%) sr13/23 (56%) | | | | |
| | | Control | <i>myod</i> MO | <i>myf5^{hu2022}</i> incross | <i>myf5^{hu2022}</i> incross + <i>myod</i> MO | <i>myf5^{hu2022}</i> incross + CyA* | <i>myf5^{hu2022}</i> incross + <i>myod</i> MO + CyA* | <i>myod</i> + <i>myog</i> MOs |
| Fig. 7A | <i>myhz1</i> mRNA, 24hpf | | | an0/20 (0%) | r22/30 (73%) sr8/30 (27%) | sl30/30 (100%) | r,sl28/37 (76%) ab9/37 (24%) | |
| Fig. 7B | <i>eng1a</i> mRNA, 26hpf | an0/23 (0%) | an0/24 (0%) | an0/32 (0%) | ab7/42 (17%) | | | |
| Fig. 7B | <i>eng2a</i> mRNA, 23s + <i>myf5</i> | | | vw7/28 (25%) <i>myf5</i> an0/28 (0%) <i>eng2a</i> | r6/12 (50%) ab6/12 (50%) | | | |
| Fig. 7B | <i>eng1a</i> mRNA, 23s | an0/16 (0%) | | | | | | vw1/19 (5%) |
| Fig. 7B | <i>eng2a</i> mRNA, 23s | an0/17 (0%) | | | | | | ab23/27 (85%) |
| Fig. 7C | Tg (<i>mylz2:GFP</i>) + Engrailed IHC, 24hpf | an0/31 (0%) | r35/37 (95%) | | | | | |
| | | <i>myf5^{hu2022}</i> incross | | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>myod</i> | vw14/57 (25%) <i>myf5</i> an0/57 (0%) <i>myod</i> | | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>myog</i> | vw12/57 (21%) <i>myf5</i> an0/57 (0%) <i>myog</i> | | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>mrf4</i> | vw6/28 (21%) <i>myf5</i> | | | | | | |

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| | | an0/28 (0%) <i>mrf4</i> | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>pax3</i> | vw9/33 (27%) <i>myf5</i> an0/28 (0%) <i>pax3</i> | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>mef2ca</i> | vw10/38 (26%) <i>myf5</i> an0/38 (0%) <i>mef2ca</i> | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>prdm1</i> | vw8/31 (26%) <i>myf5</i> an0/31 (0%) <i>prdm1</i> | | | | | |
| Fig. S2C | <i>myf5</i> mRNA, 13s + <i>ptc1</i> | vw4/15 (27%) <i>myf5</i> an0/15 (0%) <i>ptc1</i> | | | | | |
| | | <i>mrf4</i> mRNA (100 pg) | <i>mrf4</i> ^{hu2041} mRNA (100 pg) | | | | |
| Fig. S3B | MyHC, 24hpf | em13/17 (76%) | em0/28 (0%) | | | | |

IHC, immunohistochemistry; CyA, cyclopamine; an, abnormal; r, reduced; sr, strongly reduced; w, weaker; vw, very weak; d, delayed; s, stronger; ab, abolished; sl, smu-like.
Fig. 1, fraction of embryos with abnormal (an), delayed (d), strongly reduced (sr), abolished (ab), stronger (s) or weaker (w) levels of expression in the somites.
Fig. 2D, fraction of embryos with normal (n), weak (w) or very weak (vw) *myf5* mRNA levels.
Fig. 2E-left, fraction of embryos with strongly reduced or absent (sr) *myog* mRNA levels in adaxial cells.
Fig. 2E-right, fraction of embryos with delayed (d) *myog* mRNA levels in fast precursors.
Fig. 3B, fraction of embryos from a *myf5*^{hu2022} incross injected with *myod* MO with very weak (vw) *myf5* mRNA levels and/or abolished (ab), strongly reduced (sr) or abnormal (an) levels of *mef2ca*, *prdm1*, *ptc1*, *smyhc1*, α -*actin* or *tpma* mRNA or fraction of control and *myf5*+*myod* MO embryos with altered *prdm1* mRNA or MyHC IHC.
Fig. 3C-E, fraction of embryos with abnormal (an), reduced (r) or strongly reduced (sr) *myhz1* or *myog* mRNA levels or MyHC IHC in cranial muscle. *Siblings (sibs) and *myf5*^{hu2022} mutants (mut) were distinguished by somitic MyHC expression.
Fig. 3F, fraction of non-injected control or *myod* MO-injected embryos from a *myf5*^{hu2022} incross with very weak (vw) *myf5* mRNA levels and abnormal (an), delayed (d) or strongly reduced (sr) levels of *hsp90a* or *meox1* mRNA.
Fig. 4, Fraction of embryos with abnormal (an), weak (w), reduced (r), strongly reduced (sr) or abolished (ab) levels of *myhz1*, *tpma*, *myl2*, *myog* or *mef2d* mRNA or MyHC, slow MyHC or fast MyHC IHC.
Fig. 5, fraction of embryos with strongly reduced (sr), reduced (r) or abolished (ab) levels of *mrf4*, *myog* or *myhz1* mRNA.
Fig. 6A, fraction of embryos with strongly reduced (sr), abolished (ab) or normal (n) mRNA levels of *myl2* and *smyhc1*.
Fig. 6B, fraction of embryos with reduced mRNA levels of *myhz1* or *smyhc1*.
Fig. 6C, fraction of embryos with reduced (r) or strongly reduced (sr) *acta1*:GFP, Mef2 or slow MyHC (F59) IHC.
Fig. 6D, fraction of embryos with reduced (r) or strongly reduced (sr) fast MyHC (EB165) IHC.
Fig. 6E, fraction of embryos injected with *myod* and *myog* MO (1 ng or 2 ng of each MO) with reduced (r) or strongly reduced (sr) mRNA levels of *myhz1*.
Fig. 7A, Fraction of *myod* MO-injected or uninjected wild type or *myf5*^{hu2022} incross embryos treated with CyA or ethanol vehicle with reduced (r) or strongly reduced (sr) or abolished (ab) mRNA levels of *myhz1*. Smu-like (sl) refers to U-shaped somites, as seen in Hh signalling mutants like *smo*.
Fig. 7B, Fraction of *myod* MO-injected or uninjected wild type or *myf5*^{hu2022} incross embryos with very weak (vw), reduced (r) or abolished (ab) mRNA levels of *myf5*, *eng1a* or *eng2a*.
Fig. 7C, Fraction of *myod* MO-injected or uninjected *Tg(myf5:GFP)* embryos with reduced (r) Engrailed (4D9) IHC.
Fig. S2C, Fraction of embryos from a *myf5*^{hu2022} incross with very weak (vw) *myf5* mRNA levels and abnormal (an) levels of *myod*, *myog*, *mrf4*, *mef2ca*, *pax3*, *prdm1* or *ptc1*.
Fig. S3B, Fraction of embryos injected with *mrf4* mRNA or *mrf4*^{hu2041} mutant mRNA showing ectopic muscle outside the somites (em).