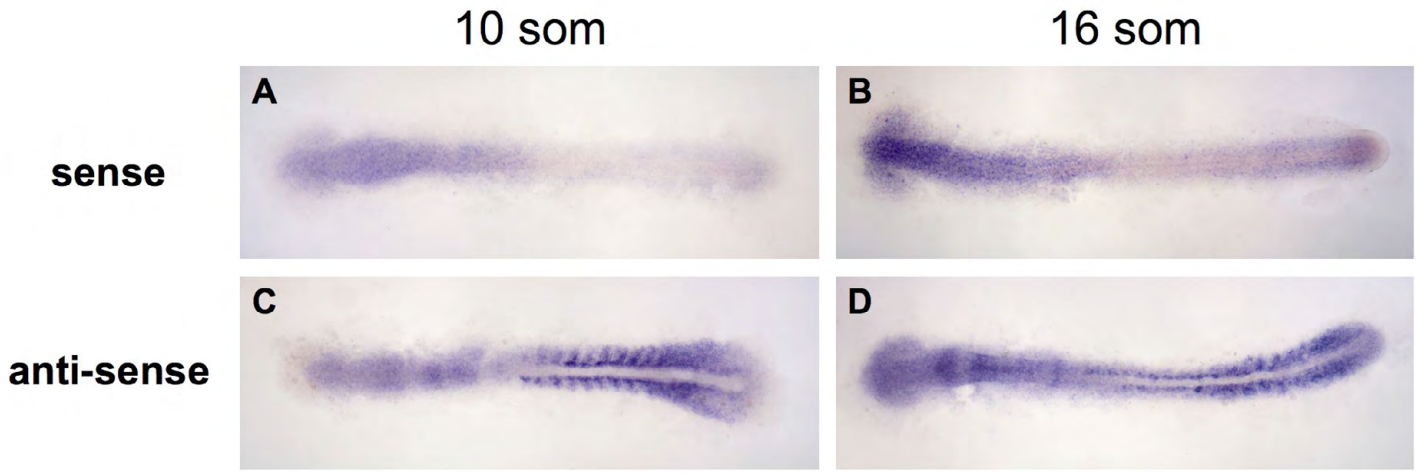


**Fig. S1. The *cdh15* transgenic muscle promoter is expressed in developing somites during early and mid-somitogenesis.** (**A-D**) Whole-mount RNA *in situ* hybridization was performed on 10 somite (A,C) and 16 somite (B,D) *cdh15:GFP* transgenic embryos using *GFP* anti-sense (C,D) RNA probes or sense (A,B) control probes. Flat-mounted embryos are depicted, with anterior on the left. Significant background staining is seen in the anterior embryo in sense controls. High levels of somite-specific staining are seen only in antisense probe-treated embryos.

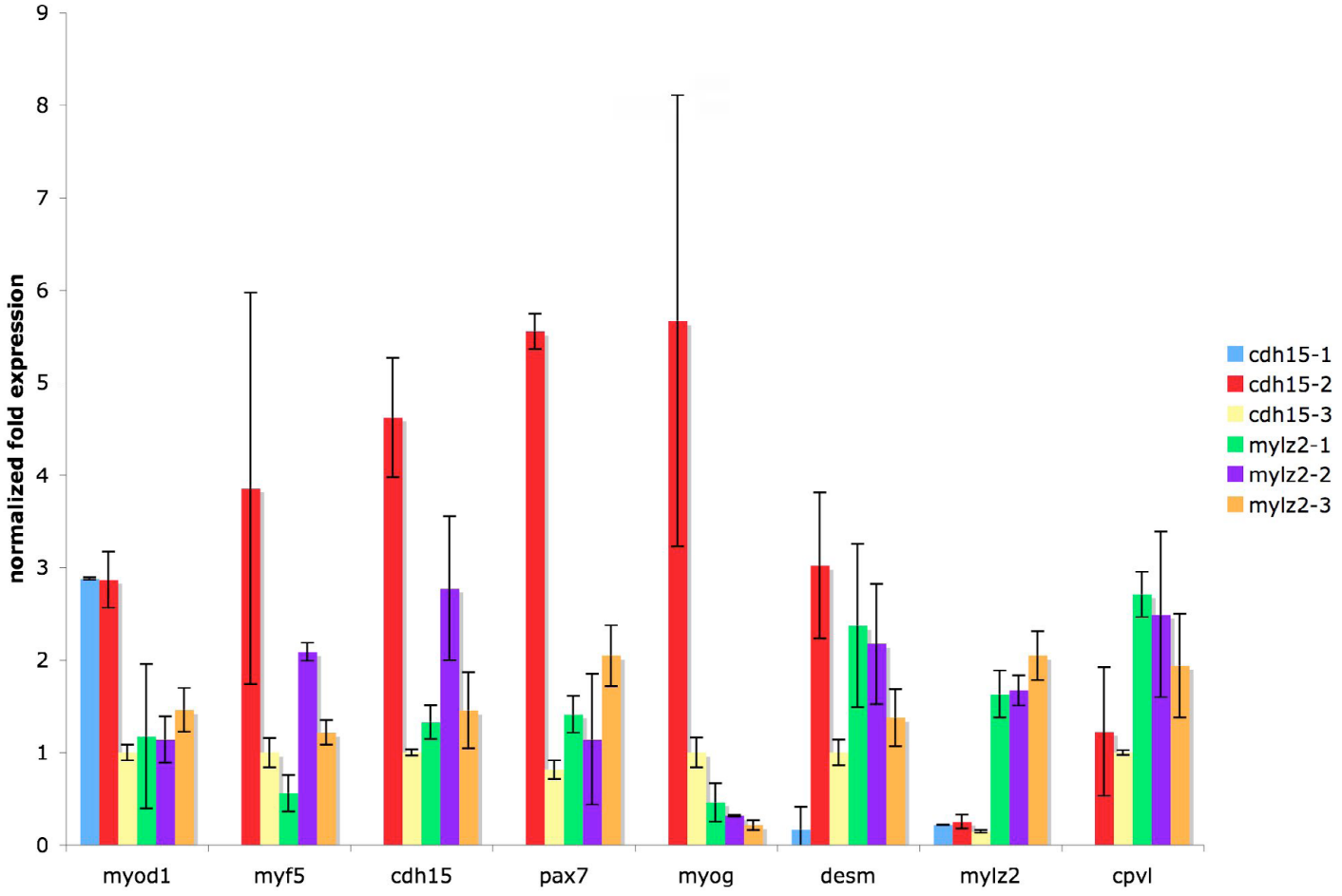


**Fig. S2. Gene expression of muscle markers in sorted cell populations from *cdh15:gfp* and *mylz2:mCherry* fish, quantified by QPCR.** Cells from three adult *cdh15:gfp* and three adult *mylz2:mCherry* fish were double-sorted for GFP or mCherry positivity, respectively, to relative purity (>90%). RNA was isolated and QPCR was performed to quantify expression of muscle genes in the fluorophore-positive populations. There was a significant increase ($P$=0.007, Student's $t$-test) in *mylz2* expression in mCherry-positive cells from *mylz2:mCherry* fish, and a non-significant increase in *cpvl*, a marker of muscle differentiation. There was a non-significant increase in *myod1* and *myog* expression in GFP-positive cells from *cdh15:gfp* fish. Expression of *myf5, cdh15, pax7, myog* and *cpvl* could not be evaluated in one *cdh15:gfp* fish ('cdh15-1') owing to insufficient RNA. The average of three experimental replicates is depicted per sample. Error bars represent s.e.m.

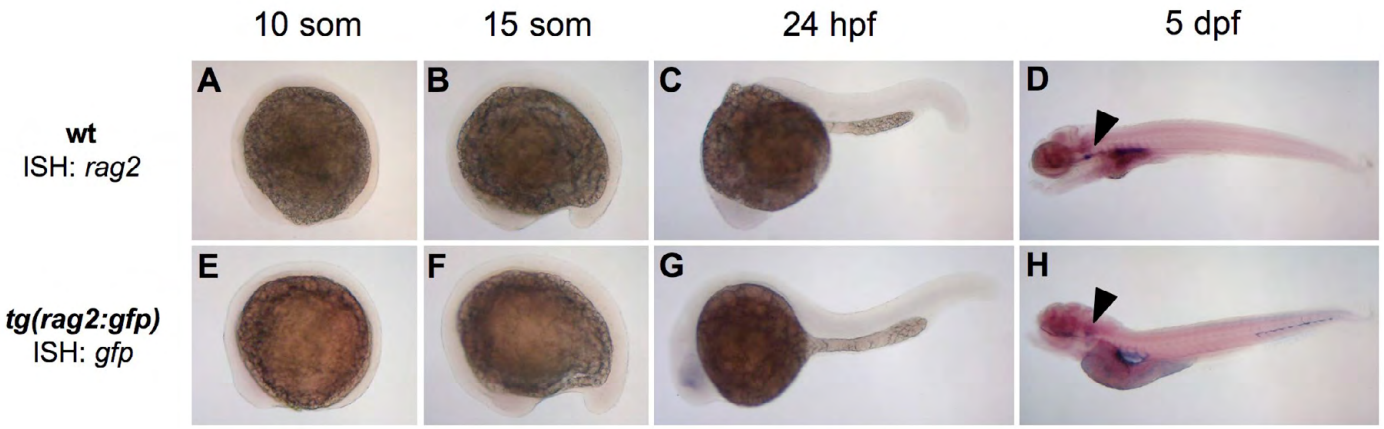


**Fig. S3. *rag2* transgenic muscle promoter recapitulates endogenous expression patterns during development.** (**A-H**) Whole-mount RNA *in situ* hybridization was performed on endogenous *rag2* transcript in AB strain wild-type embryos (A-D), or on *GFP* transcript in transgenic embryos (E-H). (A,E) 10 somite stage. (B,F) 15 somite stage. (C,G) 24 hours post fertilization (hpf). (D,H) 5 days post fertilization (dpf). Anterior is on the left. Arrowheads denote the location of the thymus in 5 dpf larvae.

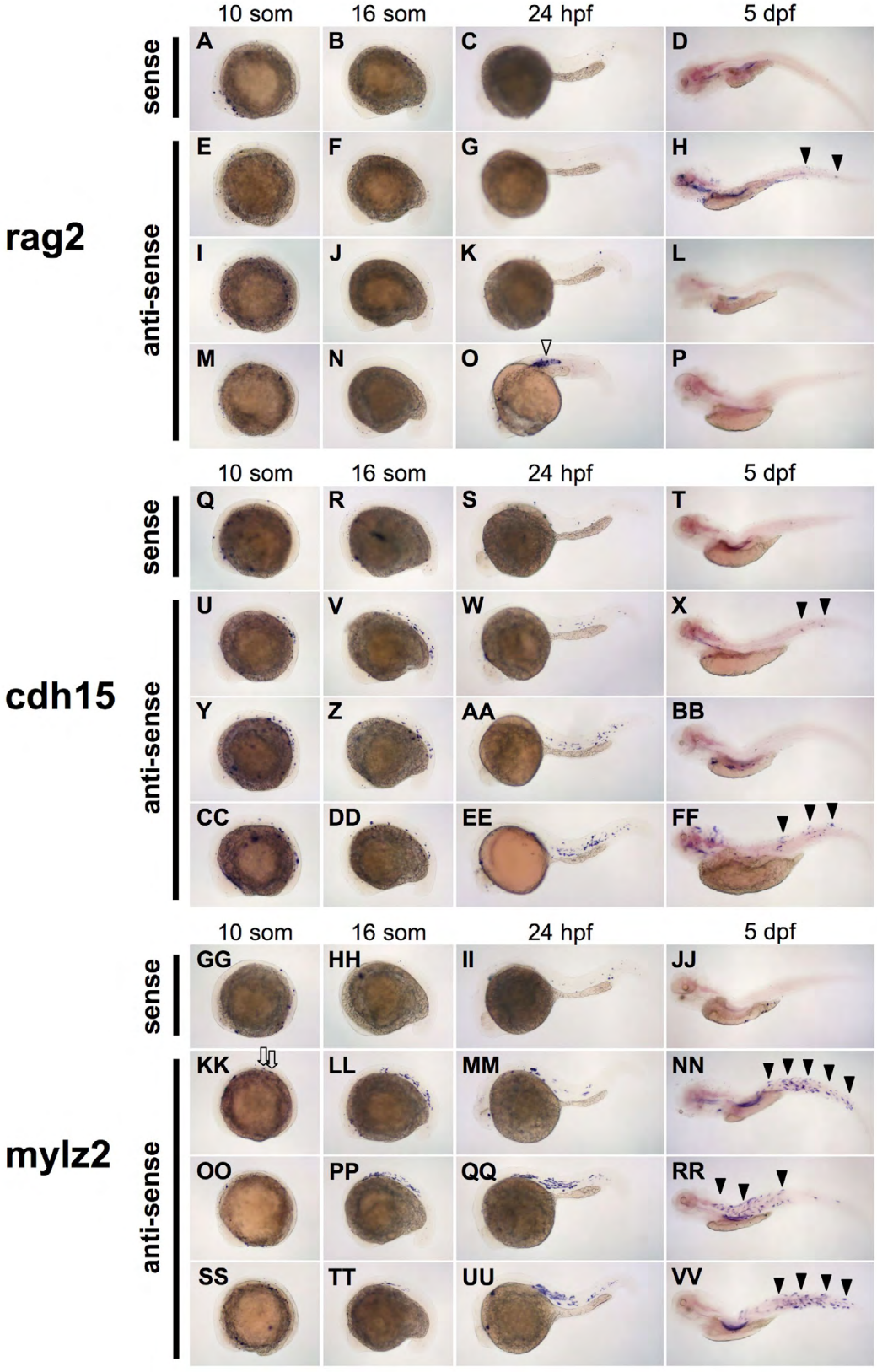


**Fig. S4. Muscle promoter-driven *KRAS*$^{G12D}$ expression in mosaic transgenic embryos and larvae are largely consistent with endogenous expression patterns.** (**A-VV**) Whole-mount RNA *in situ* hybridization was performed using *KRAS* anti-sense RNA probes (E-P,U-FF,KK-VV) or *KRAS* sense controls (A-D,Q-T,GG-JJ) on embryos or larvae that had been injected at the one-cell stage with *rag2:KRAS*$^{G12D}$ (A-P), *cdh15:KRAS*$^{G12D}$ (Q-FF), or *mylz2:KRAS*$^{G12D}$ (GG-VV) vectors. For each time point, one representative sense control staining and three representative anti-sense probe stainings are depicted. (A,E,I,M,Q,U,Y,CC,GG,KK,OO,SS) 10 somite stage. (B,F,J,N,R,V,Z,DD,HH,LL,PP,TT) 16 somite stage. (C,G,K,O,S,W,AA,EE,II,MM,QQ,UU) 24 hpf. (D,H,L,P,T,X,BB,FF,JJ,NN,RR,VV) 5 dpf. Black arrowheads indicate areas of muscle staining at 5 dpf. Unfilled arrowhead indicates area in one *rag2* embryo (1 out of 71 embryos) that displayed a population of cells with anti-sense staining. Arrows represent positive muscle fibers in a 10 somite *mylz2* embryo.

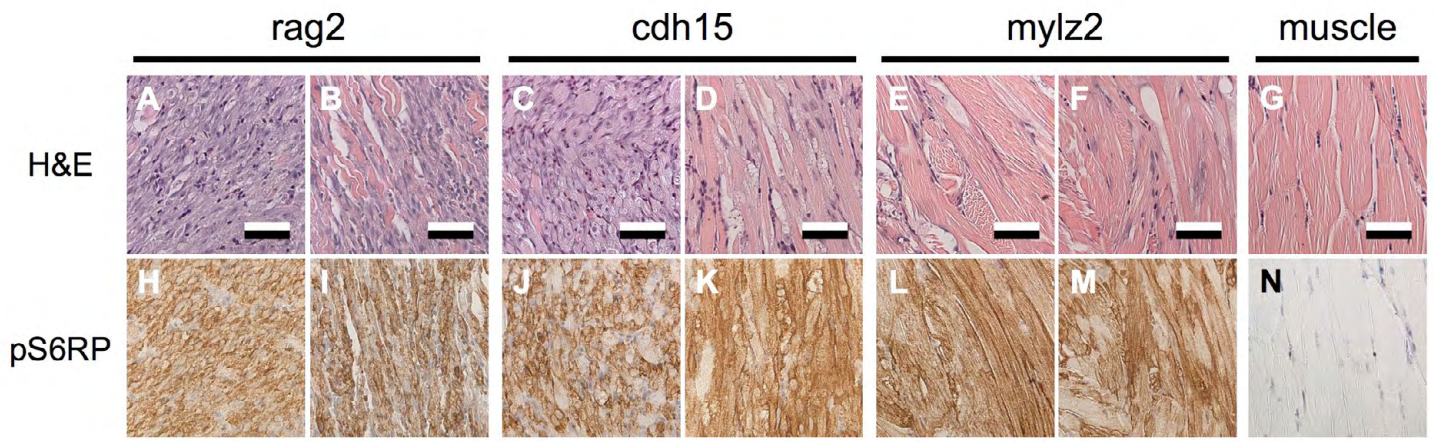


**Fig. S5. *rag2:KRAS$^{G12D}$*, *cdh15:KRAS$^{G12D}$* and *mylz2:KRAS$^{G12D}$* tumors express phospho-S6 ribosomal protein (pS6RP) at similar levels.** (**A-N**) H&E staining (A-G) and immunohistochemistry staining of S6RP (H-N) of two representative *rag2* (A,B,H,I), *cdh15* (C,D,J,K) and *mylz2* (E,F,L,M) tumors, or one normal muscle (G,N) section. Scale bars: 50 μm. All images are at the same magnification.

**Table S1. Up- and downregulated gene lists for the following comparisons.** Wild-type versus *cdh15* tumors (sheet A), wild-type versus *mylz2* tumors (sheet B), wild-type versus *rag2* tumors (sheet C), *cdh15* versus *mylz2* tumors (sheet D), *cdh15* versus *rag2* tumors (sheet E) and *mylz2* versus *rag2* tumors (sheet F). Venn diagrams depicting the overlap of dysregulated genes in tumors compared to wild type are shown in sheet G. GSEA plots showing enrichment of *mylz2* signature compared with *rag2* (sheet H) or *cdh15* (sheet I) in human well-differentiated (WD) RMS. Heatmaps of the GSEA results are also included.

**Download Table S1**

**Table S2. GFP-positive cells are enriched for tumor-propagating capability in secondary transplantations of *rag2* and *cdh15* tumors.** Results are depicted as number of fish showing tumor engraftment out of number of total fish transplanted for each dilution.

| tumor | cell population | # cells transplanted | | |
|---|---|---|---|---|
| | | 1,000 | 100 | 10 |
| rag2 | G+R- | - | 6 of 10 | 1 of 9 |
| | G+R+ | - | 2 of 10 | 0 of 9 |
| | G-R+ | 0 of 8 | - | - |
| | G-R- | 0 of 10 | - | - |
| cdh15 | G+R- | - | - | 2 of 16 |
| | G+R+ | - | 4 of 7 | 1 of 14 |
| | G-R+ | - | 0 of 14 | - |
| | G-R- | 0 of 13 | - | - |

## Table S3. Oligonucleotide primer sequences

| **Primers for cloning promoter elements** | |
|---|---|
| cdh15-XhoI-F | 5'-AATTCCCTCGAGTCATCTTGTGTGCAACTACTGTGTCG-3' |
| cdh15-BamHI-R | 5′-GGAATTGGATCCCCTCAATGACCACAGTAAACCACTGC-3′ |
| mylz2-XhoI-F | 5′-AATTCCGTCGACCTCGAGATTCGCCACAGAGGAATGAGCCACCA-3′ |
| mylz2-BamHI-R | 5′-GGAATTGGATCCGTCGAGACGGTATGTGTGAAGTCT-3′ |

| **Primers for generating in situ hybridization probes** | |
|---|---|
| GFP-T7-F | 5′-TTCCGTAATACGACTCACTATAGGGGAAGGTGATGCAACATACGG-3′ |
| GFP-SP6-R | 5′-TTCCGATTTAGGTGACACTATAGAACCATGTGGTCTCTCTTTTCG-3′ |
| mCherry-T7-F | 5′-TTCCGTAATACGACTCACTATAGGGACATGGCCATCATCAAGGAG-3′ |
| mCherry-SP6-R | 5′-TTCCGATTTAGGTGACACTATAGAAGTTCCACGATGGTGTAGTCC-3′ |
| mylz2-T7-F | 5′-TTCCGTAATACGACTCACTATAGGGTCATCAGCAAAGACGACCTTAGG-3′ |
| mylz2-SP6-R | 5′-TTCCGATTTAGGTGACACTATAGAATTACTCCTCCTTCTCCTCTCCGTG-3′ |
| cdh15-T7-F | 5′-TTCCGTAATACGACTCACTATAGGGAACAAACTGATTGCGGAGGTTG-3′ |
| cdh15-SP6-R | 5′-TTCCGATTTAGGTGACACTATAGAATGTCTTCATCCAAGGCACTAAGC-3′ |
| rag2-T7-F | 5'-TTCCGTAATACGACTCACTATAGGGAGCTGTCCTCCAGTCTCTACAT-3' |
| rag2-SP6-R | 5'-TTCCGATTTAGGTGACACTATAGAAAGACTCCATATGGACTCCCACA-3' |
| hKRAS-SP6-F | 5'-TTCCATTTAGGTGACACTATAGCTTGTGGTAGTTGGAGCTGA-3' |
| hKRAS-T7-R | 5'-TTCCGTAATACGACTCACTATAGGGCACTAATGTATAGAAGGCATC-3' |

| **Primers for quantitative RT-PCR** | |
|---|---|
| EF1a-QRT-F | 5′-CATCGAGAAGTTCGAGAAGGAAGC-3′ |
| EF1a-QRT-R | 5′-GTCAATGGTGATACCACGCTCAC-3′ |
| hKRAS-QRT-F | 5′-TTGATGGAGAAACCTGTCTCTTGG-3′ |
| hKRAS-QRT-R | 5′-CAAATACACAAAGAAAGCCCTCCC-3′ |
| pax7b-QRT-F | 5′-CAGTATTGACGGCATTCTGGGAG-3′ |
| pax7b-QRT-R | 5′-TCTCTGCTTTCTCTTGAGCGGC-3′ |
| myf5-QRT-F | 5′-CCAGACAGTCCAAACAACAGACC-3′ |
| myf5-QRT-R | 5′-TGAGCAAGCAGTGTGAGTAAGCG-3′ |
| cdh15-QRT-F | 5'-CTAAGGAAAGATGCACCCCATTAC-3′ |
| cdh15-QRT-R | 5′-TCAGAGCTGTGTCGTATGGTGG-3′ |
| myog-QRT-F | 5′-GTGGACAGCATAACGGGAACAG-3′ |
| myog-QRT-R | 5′-TCTGAAGGTAACGGTGAGTCGG-3′ |
| desmin-QRT-F | 5′-CGAGATTGACTCTCTCAAGGGCAC-3′ |
| desmin-QRT-R | 5′-GGGCGATAGTGTCCTGATAACCAC-3′ |
| mylz2-QRT-F | 5′-TTGACCACTCAGTGCGACAGGTTC-3′ |
| mylz2-QRT-R | 5′-AACATTGCCAGCCACATCTGGG-3′ |
| cpvl-QRT-F | 5′-TTGGAGCTGACCCGGGCAAA-3′ |
| cpvl-QRT-R | 5′-CACCAGGAAGGGGACCCACC-3′ |