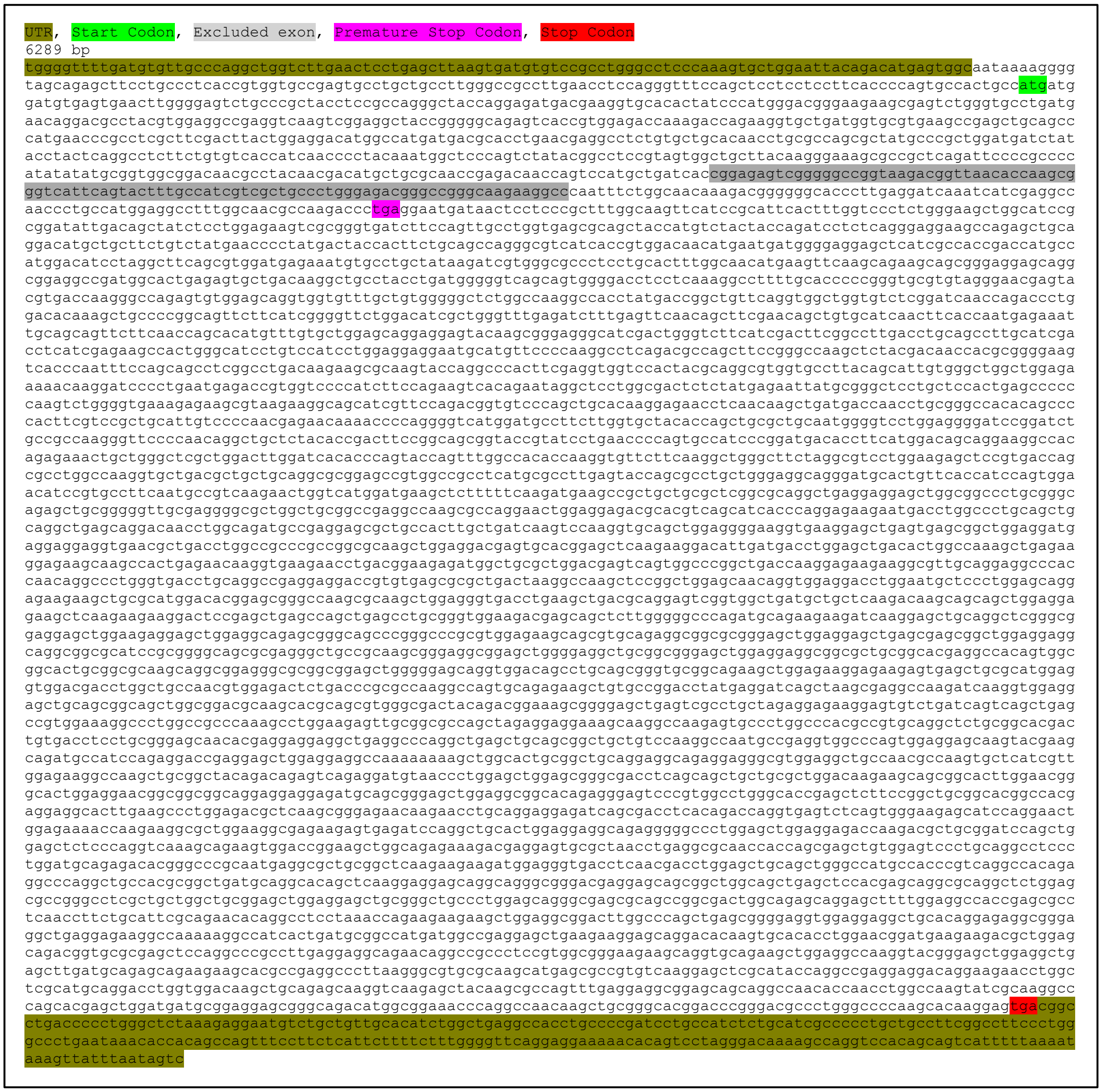
SUPPORTING INFORMATION



```
UTR, Start Codon, Excluded exon, Premature Stop Codon, Stop Codon
6289 bp
tggggttttgatgtgttgcccaggctggtcttgaactcctgagcttaagtgatgtgtccgcctgggcctcccaaagtgctggaattacagacatgagtggcaataaaaggggg
tagcagagcttcctgccctcaccgtggtgccgagtgcctgctgccttgggccgccttgaacctccagggtttccagctcctcctccttcaccccagtgccactgccatgatg
gatgtgagtgaacttggggagtctgcccgctacctccgccagggctaccaggagatgacgaaggtgcacactatcccatgggacgggaagaagcgagtctgggtgcctgatg
aacaggacgcctacgtggaggccgaggtcaagtcggaggctaccggggcagagtcaccgtggagaccaaagaccagaaggtgctgatggtgcgtgaagccgagctgcagcc
catgaacccgcctcgcttcgacttactggaggacatggccatgatgacgcacctgaacgaggcctctgtgctgcacaacctgcgccagcgctatgcccgctggatgatctat
acctactcaggcctcttctgtgtcaccatcaacccctacaaatggctcccagtctatacggcctccgtagtggctgcttacaagggaaagcgccgctcagattccccgcccc
atatatatgcggtggcggacaacgcctacaacgacatgctgcgcaaccgagacaaccagtccatgctgatcaccggagagtcgggggccggtaagacggttaacaccaagcg
ggtcattcagtactttgccatcgtcgctgccctgggagacgggccgggcaagaaggcccaatttctggcaacaaagacgggggcacccttgaggatcaaatcatcgaggcc
aaccctgccatggaggcctttggcaacgccaagaccctgaggaatgataactcctcccgctttggcaagttcatccgcattcactttggtccctctgggaagctggcatccg
cggatattgacagctatctcctggagaagtcgcgggtgatcttccagttgcctggtgagcgcagctaccatgtctactaccagatcctctcagggaggaagccagagctgca
ggacatgctgcttctgtctatgaaccccctatgactaccacttctgcagccagggcgtcatcaccgtggacaacatgaatgatggggaggagctcatcgccaccgaccatgcc
atggacatcctaggcttcagcgtggatgagaaatgtgcctgctataagatcgtgggcgccctcctgcactttggcaacatgaagttcaagcagaagcagcgggaggagcagg
cggaggccgatggcactgagagtgctgacaaggctgcctacctgatgggggtcagcagtggggacctcctcaaaggccttttgcaccccgggtgcgtgtagggaacgagta
cgtgaccaagggccagagtgtggagcaggtggtgtttgctgtggggctctggccaaggccacctatgaccggctgttcaggtggctggtgtctcggatcaaccagaccctg
gacacaaagctgccccggcagttcttcatcggggttctggacatcgctgggtttgagatctttgagttcaacagcttcgaacagctgtgcatcaacttcaccaatgagaaat
tgcagcagttcttcaaccagcacatgtttgtgctggagcaggaggagtacaagcgggagggcatcgactgggtcttcatcgacttcggccttgacctgcagccttgcatcga
cctcatcgagaagccactgggcatcctgtccatcctggaggaggaatgcatgttccccaaggcctcagacgccagcttccgggccaagctctacgacaaccacgcggggaag
tcacccaatttccagcagcctcggcctgacaagaagcgcaagtaccaggcccacttcgaggtggtccactacgcaggcgtggtgccttacagcattgtgggctggctggaga
aaaacaaggatcccctgaatgagaccgtggtccccatcttccagaagtcacagaataggctcctggcgactctctatgagaattatgcgggctcctgctccactgagccccc
caagtctggggtgaaagagaagcgtaagaaggcagcatcgttccagacggtgtcccagctgcacaaggagaacctcaacaagctgatgaccaacctgcgggccacacagccc
cacttcgtccgctgcattgtccccaacgagaacaaaaccccaggggtcatggatgccttcttggtgctacaccagctgcgctgcaatggggtcctggaggggatccggatct
gccgccaagggttccccaacaggctgctctacaccgacttccggcagcggtaccgtatcctgaaccccagtgccatcccggatgacaccttcatggacagcaggaaggccac
agagaaactgctgggctcgctggacttggatcacacccagtaccagtttggccacaccaaggtgttcttcaaggctgggcttctaggcgtcctggaagagctccgtgaccag
cgcctggccaaggtgctgacgctgctgcaggcgcggagccgtggccgcctcatgcgccttgagtaccagcgcctgctgggaggcagggatgcactgttcaccatccagtgga
acatccgtgccttcaatgccgtcaagaactggtcatggatgaagctctttttcaagatgaagccgctgctgcgctcggcgcaggctgaggaggagctggcggccctgcgggc
agagctgcgggggttgcgagggcgctggctgcggccgaggccaagcgccaggaactggaggagacgcacgtcagcatcacccaggagaagaatgacctggccctgcagctg
caggctgagcaggacaacctggcagatgccgaggagcgctgccacttgctgatcaagtccaaggtgcagctggaggggaaggtgaaggagctgagtgagcggctggaggatg
aggaggaggtgaacgctgacctggccgcccgccggcgcaagctggaggacgagtgcacggagctcaagaaggacattgatgacctggagctgacactggccaaagctgagaa
ggagaagcaagccactgagaacaaggtgaagaacctgacggaagagatggctgcgctggacgagtcagtggcccggctgaccaaggagaagaaggcgttgcaggaggcccac
caacaggccctgggtgacctgcaggccgaggaggaccgtgtgagcgcgctgactaaggccaagctccggctggagcaacaggtggaggacctggaatgctccctggagcagg
agaagaagctgcgcatggacacggagcgggccaagcgcaagctggagggtgacctgaagctgacgcaggagtcggtggctgatgctgctcaagacaagcagcagctggagga
gaagctcaagaagaaggactccgagctgagccagctgagcctgcgggtggaagacgagcagctcttgggggcccagatgcagaagaagatcaaggagctgcaggctcggcg
gaggagctggaagaggagctggaggcagagcgggcagcccgggcccgcgtggagaagcagcgtgcagaggcggcgcgggagctggaggagctgagcgagcggctggaggagg
caggcggcgcatccgcggggcagcgcgagggctgccgcaagcgggaggcggagctggggaggctgcggcgggagctggaggaggcggcgctgcggcacgaggccacagtggc
ggcactgcggcgcaagcaggcggagggcgcggcggagctgggggagcaggtggacagcctgcagcgggtgcggcagaagctggagaaggagaagagtgagctgcgcatggag
gtggacgacctggctgccaacgtggagactctgacccgcgccaaggccagtgcagagaagctgtgccggacctatgaggatcagctaagcgaggccaagatcaaggtggagg
agctgcagcggcagctggcggacgcaagcacgcagcgtgggcgactacagacggaaagcggggagctgagtcgcctgctagaggagaaggagtgtctgatcagtcagctgag
ccgtggaaaggccctggccgcccaaagcctggaagagttgcggcgccagctagaggaggaaagcaaggccaagagtgccctggcccacgccgtgcaggctctgcggcacgac
tgtgacctcctgcgggagcaacacgaggaggaggctgaggcccaggctgagctgcagcggctgctgtccaaggccaatgccgaggtggcccagtggaggagcaagtacgaag
cagatgccatccagaggaccgaggagctggaggaggccaaaaaaaagctggcactgcggctgcaggaggcagaggagggcgtggaggctgccaacgccaagtgctcatcgtt
ggagaaggccaagctgcggctacagacagagtcagaggatgtaaccctggagctggagcgggcgacctcagcagctgctgcgctggacaagaagcagcggcacttggaacgg
gcactggaggaacggcggcggcaggaggaggagatgcagcgggagctggaggcggcacagagggagtcccgtggcctgggcaccgagctcttccggctgcggcacggccacg
aggaggcacttgaagccctggagacgctcaagcgggagaacaagaacctgcaggaggagatcagcgacctcacagaccaggtgagtctcagtgggaagagcatccaggaact
ggagaaaaccaagaaggcgctggaaggcgagaagagtgagatccaggctgcactggaggaggcagagggggccctggagctggaggagaccaagacgctgcggatccagctg
gagctctcccaggtcaaagcagaagtggaccggaagctggcagagaaagacgaggagtgcgctaacctgaggcgcaaccaccagcgagctgtggagtccctgcaggcctccc
tggatgcagagacacgggcccgcaatgaggcgctgcggctcaagaagaagatggagggtgacctcaacgacctggagctgcagctgggccatgccacccgtcaggccacaga
ggcccaggctgccacgcggctgatgcaggcacagctcaaggaggagcaggcagggcgggacgaggagcagcggctggcagctgagctccacgagcaggcgcaggctctggag
cgccgggcctcgctgctggctgcggagctggaggagctgcgggctgccctggagcagggcgagcgcagccggcgactggcagagcaggagcttttggaggccaccgagcgcc
tcaaccttctgcattcgcagaacacaggcctcctaaaccagaagaagaagctggaggcggacttggcccagctgagcggggaggtggaggaggctgcacaggagaggcgga
ggctgaggagaaggccaaaaaggccatcactgatgcggccatgatggccgaggagctgaagaaggagcaggacacaagtgcacacctggaacggatgaagaagacgctggag
cagacggtgcgcgagctccaggcccgccttgaggaggcagaacaggccgccctccgtggcgggaagaagcaggtgcagaagctggaggccaaggtacgggagctggaggctg
agcttgatgcagagcagaagaagcacgccgaggcccttaagggcgtgcgcaagcatgagcgccgtgtcaaggagctcgcataccaggccgaggaggacaggaagaacctggc
tcgcatgcaggacctggtggacaagctgcagagcaaggtcaagagctacaagcgccagtttgaggaggcggagcagcaggccaacaccaacctggccaagtatcgcaaggcc
cagcacgagctggatgatgcggaggagcgggcagacatggcggaaacccaggccaacaagctgcgggcacggacccgggacgccctgggccccaagcacaaggagtgacggc
ctgacccccctgggctctaaagaggaatgtctgctgttgcacatctggctgaggccacctgccccgatcctgccatctctgcatcgcccccctgctgccttcggccttccctgg
gccctgaataaacaccacagccagtttccttctcattcttttctttggggttcaggaggaaaaacacagtcctagggacaaaagccaggtccacagcagtcatttttaaaat
aaagttatttaatagtc
```

Figure S1. Sequence originating from the *MYH7b* gene locus. Full sequence of the RNA transcribed from the *MYH7b* locus, with important features like the skipped exon annotated.

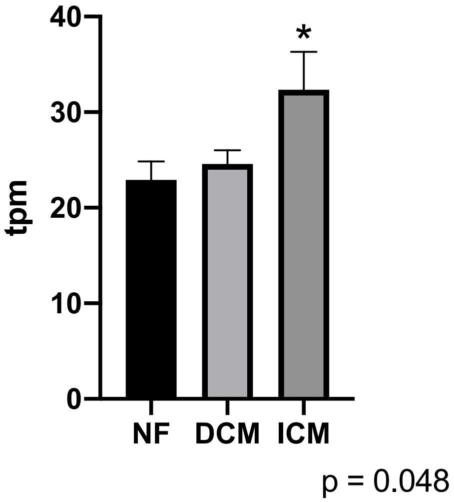


Figure S2. MYH7b increases in heart disease. Analysis of dataset GSE116250 shows a modest increase in MYH7b tpm in DCM hearts and a significant increase in ICM hearts.

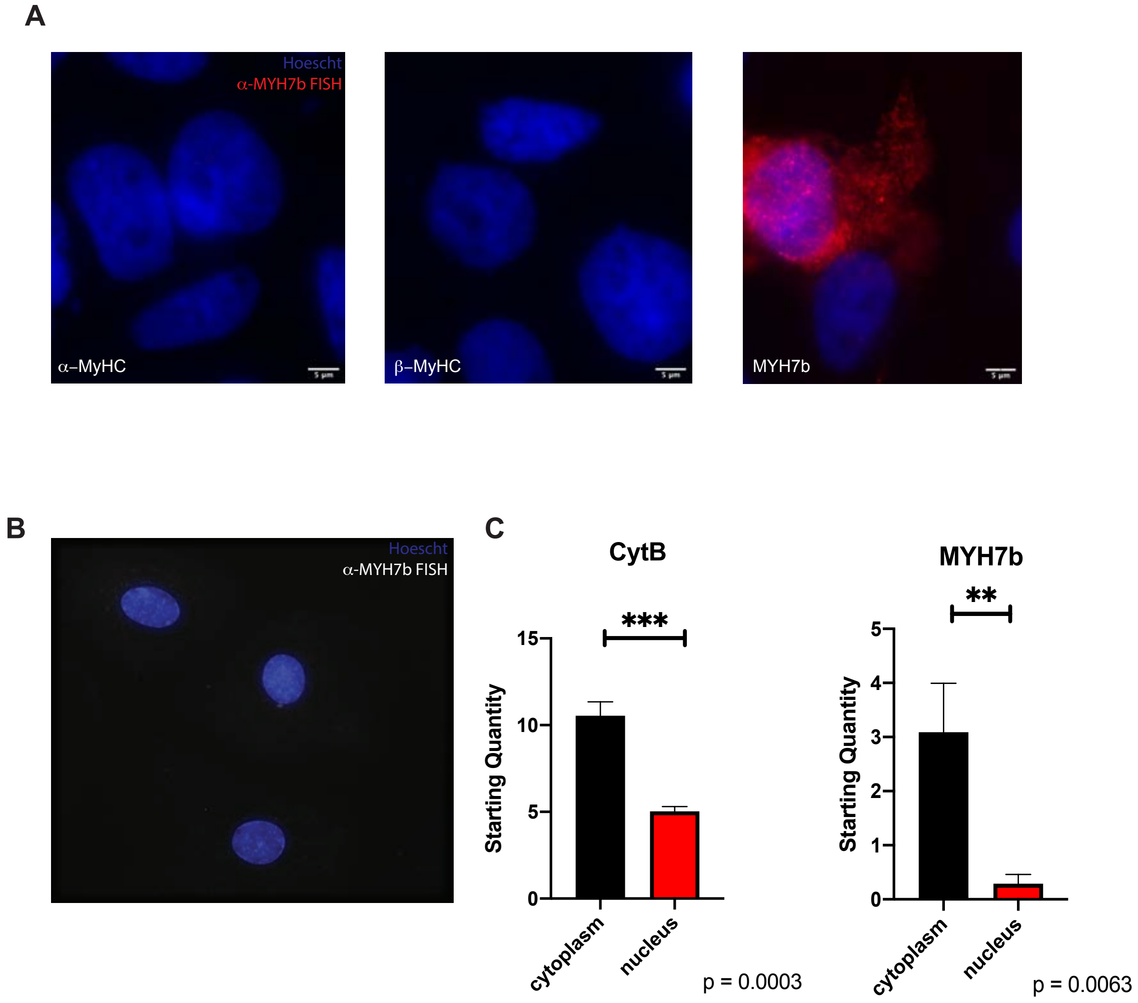


Figure S3. α-MYH7b FISH probes are specific to MYH7b and reflect biochemical fractionation data. A) Cos7 cells were transfected with the cDNA of α-MyHC, β-MyHC, and MYH7b, individually, then subjected to FISH using probes against MYH7b. There was no cross-reaction with either α-MyHC or β-MyHC. **B)** There is no FISH signal in hiPS-CMs treated with the MYH7b ASO. **C)** qPCR done on a biochemical fractionation of hiPS-CMs shows strong cytoplasmic localization of MYH7b RNA, agreeing with the FISH data.

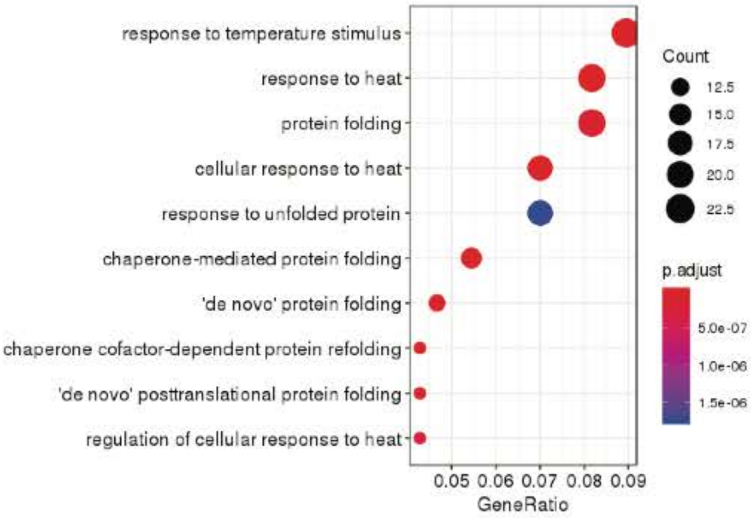


Figure S4. Pathways associated with MYH7b_sp expression. GO analysis of RNA-seq in iPS-CMs infected with adenovirus expressing MYH7b_sp shows pathways associated with adenoviral infection and folding of a foreign peptide. This indicates that MYH7b_sp is not typically expressed in cardiomyocytes.

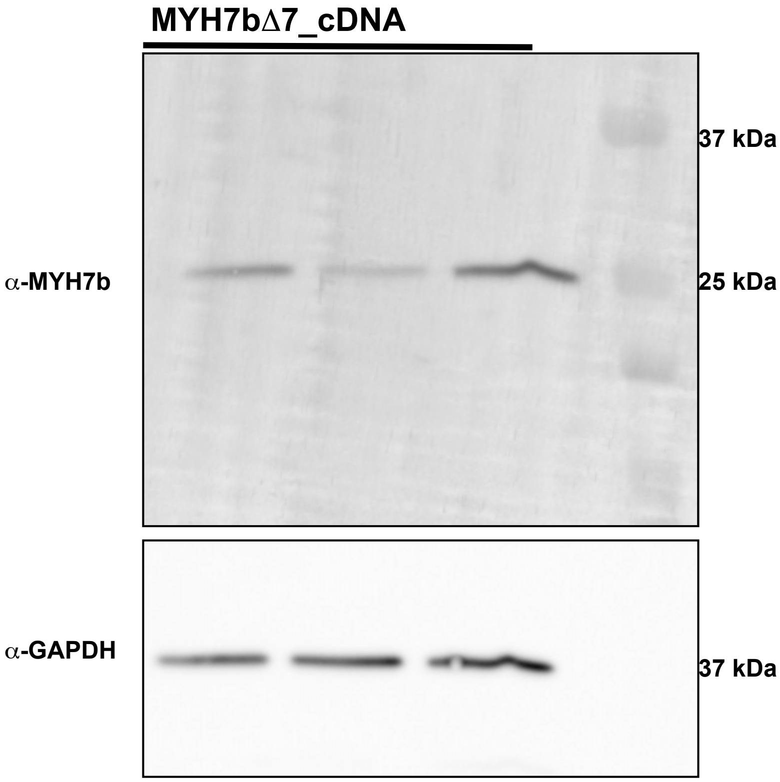


Figure S5. A MYH7b exon-skipped cDNA (MYH7bΔ7_cDNA) produces MYH7b_sp. Western blotting reveals that overexpression of MYH7bΔ7_cDNA via adenovirus infection causes translation of MYH7b_sp, which is not normally detectable in human hearts. We have also shown MYH7b_sp to not be relevant to the β-MyHC/α-MyHC ratio, and therefore moved on to a loss-of-function approach.

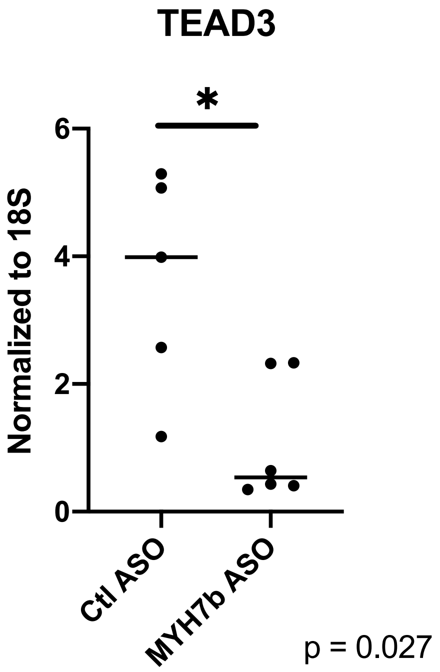


Figure S6. TEAD3 RNA is significantly down by qPCR. Changes in TEAD3 expression were confirmed across three differentiations by SYBR Green qPCR.

Type	Name	Sequence
anti-MYH7b	12150	GCTTCTCTGCACTG

Table S1. Sequence of anti-MYH7b ASO.

Sequence	Position	GC Content	# Matches
cttttgtccctaggactgtg	6128	50.00%	0
cgtctttgttgccagaaatt	747	40.00%	2
gatgcagagatggcaggatc	6016	55.00%	3
cagatgtgcaacagcagaca	5978	50.00%	4
aagttcactcacatccatca	220	40.00%	6
cccgcataattctcatagag	1991	45.00%	7
ttcttacgcttctctttcac	2042	40.00%	8
ttgtcttgagcagcatcagc	3341	50.00%	8
gtacttgcgcttcttgtcag	1833	50.00%	8
atgggatagtgtgcaccttc	284	50.00%	9
actgagactcacctggtctg	4680	55.00%	9

ctccagtaagtcgaagcgag	460	55.00%	11
cttatagcaggcacatttct	1164	40.00%	11
acaatgctgtaaggcaccac	1886	50.00%	11
tgtagcaccaagaaggcatc	2195	50.00%	11
tggtactcaaggcgcatgag	2528	55.00%	11
ttagctgatcctcataggtc	3892	45.00%	11
tgtaagcagccactacggag	623	55.00%	12
tggtagtagacatggtagct	974	45.00%	12
ggttcatagacagaagcagc	1030	50.00%	12
tgacttctggaagatgggga	1950	50.00%	12
ctgtccatgaaggtgtcatc	2336	50.00%	12
ttacatcctctgactctgtc	4408	45.00%	12
tatcattcctcagggtcttg	829	45.00%	13
ccagagggaccaaagtgaat	878	50.00%	13
caggcaactggaagatcacc	946	55.00%	13
catcattcatgttgtccacg	1087	45.00%	13
tgaagcctaggatgtccatg	1135	50.00%	13
gtctggttgatccgagacac	1448	55.00%	13
gctggaaattgggtgacttc	1804	50.00%	13
acttgatcagcaagtggcag	2854	50.00%	13
agctgactgatcagacactc	4025	50.00%	13
ttcttggttttctccagttc	4715	40.00%	13
ctggatctcactcttctcgc	4746	55.00%	13
ccaggagatagctgtcaata	916	45.00%	14
acaatgcagcggacgaagtg	2144	55.00%	14
cgttcaggtgcgtcatcatg	488	55.00%	14
tcttccaggacgcctagaag	2447	55.00%	14
tatagactgggagccatttg	599	45.00%	15
tatatatggggcggggaatc	660	50.00%	15
tagtggaccacctcgaagtg	1859	55.00%	15
ggacaccgtctggaacgatg	2067	60.00%	15

atgttccactggatggtgaa	2576	45.00%	15
gttcttgacggcattgaagg	2601	50.00%	15
atggcatctgcttcgtactt	4262	45.00%	15
ctgcgaatgcagaaggttga	5280	50.00%	15
ttcttctgctctgcatcaag	5618	45.00%	15
caaactggcgcttgtagctc	5770	55.00%	15
taggtatagatcatccagcg	546	45.00%	16
cagaaccccgatgaagaact	1491	50.00%	16
tcaaggccgaagtcgatgaa	1658	50.00%	16
cgtggttgtcgtagagcttg	1780	55.00%	16
cggaagtcggtgtagagcag	2279	60.00%	16
atgtcgttgtaggcgttgtc	690	50.00%	16
acggtctcattcaggggatc	1928	55.00%	16
tatgcgagctccttgacacg	5678	55.00%	16

Table S2. Sequences of the MYH7b FISH probes.

Gene	Forward Primer	Reverse Primer
18S	GAATTCCCAGTAAGTGGGGG	GGGCAGGGACTTAATCAACG
MYH7b	CCATGATGACGCACCTGAA	AGAAGAGGCCTGAGTAGGTATAG
MYH7	GACCTCAAGAAGGATGTCTTCG	GGTCACTGTCTTGCCATACTC
CytB	CCTTCCACCCTTACTACACAATC	CCTAGGAGGTCTGGTGAGAATA
TEAD3	GATGTGCGCCAGATCTATGA	CCCAGAACTTGACAAGGAAGA

Table S3. qPCR primer sequences.