

SUPPLEMENTAL MATERIALS

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Supplemental Method

Detection of the injected human *MYH6* cRNA in zebrafish embryos

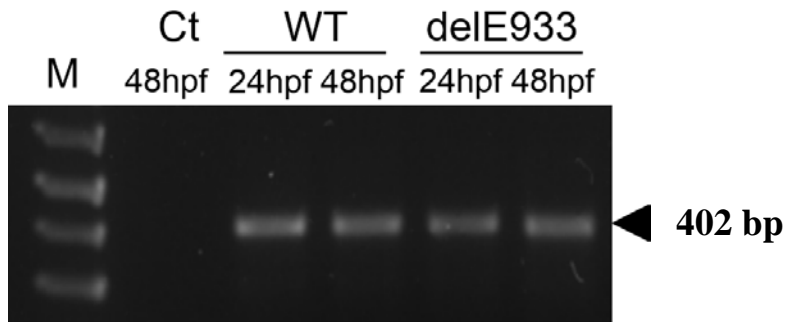
Myh6 ATG-MO (0.5-1 ng/embryo) was co-injected with WT or delE933 *MYH6* cRNA (0.4 ng/embryo) at the 1- to 2-cell stage. After the injection, embryos were collected at 24 and 48 hpf. The uninjected embryos were collected at 48 hpf. To validate the expression of the exogenously injected human *MYH6* cRNAs in the embryonic bodies, total RNA was extracted from whole embryo using protocol based on QIAGEN RNeasy Mini Kit (Qiagen, Venlo, Netherlands). cDNA libraries were constructed by reverse transcriptase-PCR (RT-PCR) using the Ambion RETROscript Kit (Life Technologies). PCR was performed using *MYH6* primers (MYH6-2555F and MYH6-2935R; Supplemental Table S1) designed to specifically amplify human *MYH6* (402 bp) but not intrinsic zebrafish *myh6*. An aliquot of PCR product was loaded on an agarose gel, and then sequenced using ABI 3130 genetic analyzer (Life Technologies).

Supplemental Result

Expression of the injected human *MYH6* cRNA in zebrafish embryos

As shown in the supplemental figure, primer set of MYH6-2555F and MYH6-2935R successfully amplified a single human *MYH6* fragment (402 bp, arrowhead) from whole zebrafish embryos (lane 2, 3: co-injected with myh6 ATG-MO and WT-*MYH6* cRNA; lane 4, 5: co-injected with myh6 ATG-MO and delE933-*MYH6* cRNA) both at 24 and 48 hpf, but not from uninjected embryo (Ct). WT and delE933 human *MYH6* sequences of the PCR products were confirmed (not shown). These data support that the inability of delE933 to rescue heart rate suppression by myh6 ATG-MO is attributable to the properties of overexpressed delE933-*MYH6*, rather than technical inadequacy such as RNA degradation.

Supplemental Figure



RT-PCR shows the presence of human *MYH6* RNAs in zebrafish embryos at 24h and 48h after co-injection of *myh6* ATG-morpholino and *MYH6* cRNA of either WT or delE933. Primers were designed to specifically amplify a 402 bp fragment (arrowhead) of human *MYH6*, but not zebrafish *myh6*. Ct: Control (uninjected embryos), M: 100 bp marker

Table S1. Nucleotide sequences of the primers1. Genetic screening of *MYH6*

Analyzed region	Forward primer (5' to 3')	Reverse primer (5' to 3')
Exon 3	AGAGGACAAAGCCACTCGCTG	TGCAAGTGGCTCCACCTCTG
Exons 4-6	AATGGGAAGGGAAATTACCTG	CTAGGCATCAGCGTGTCTGC
Exons 7-8	CCCTGTATGGAGAACAGTAG	TGGGTGTGGCAAACAGCAC
Exons 9-10	CATTTCCAGAACCATCCAGG	CCTGCATGCAGGAGTCGTTG
Exons 11-12	TTGCCTGGTGCAGACATGCTG	AGAGAGCCTGGTCAGCACCTC
Exon 13	GTGCTCACTTATCCTTTCCC	CTCTCAGCAAATGGCTGTTG
Exon 14	CAACAGCCATTTGCTGAGAG	CTCTAGTTTCTTGGGTGTAG
Exon 15	TGTCAGGGTATGGGACTGTG	GTGCTTTGAAGCAGCAGGAC
Exons 16-20	AAAGTCTCAGAGCTACCAAGCG	CTTCTGACCCACACTAGTTGAC
Exons 21-23	AGTCTACGTGCCTACGAACTTG	CAGGACTTTCTGGGCCATTGG
Exons 24-25	GAAGGAGGCAAAGAGCATAAC	CTGCAGCCTCAGTTACCTCAG
Exons 26-28	TTCTGGTAGCTTTTCAGAGC	TCCATTTCTGGCACTGAGATG
Exons 29-30	AAGGCTGGGCTTGGTTGAAG	AGCCGCATGTCCAAGATCTG
Exons 31-32	CAGATCTTGGACATGCGGCT	AGATTTTGTCTGGGGTCAG
Exons 33-34	ACCGTGTATCTTCTCATCCTC	ACTCAGTAGGTTTCCACAAGG
Exons 35-36	ACCACCTTTAATTCTTTCTGG	TAAATCTACCAACAGCATCTC
Exon 37	GGGAAAGGTGATTGCATTTGC	AGCAAACCTTTGTCCAGGCC
Exon 38	GTTGCAGGAATATGCATGAGG	ACATATAGGGCAAGCAGTGCC
Exon 39	ACCACAAGTGCCTCTAACGTG	CTACTGCCCTGATCCAGGATG

2. Plasmid constructions of α -MHC and MyBP-C

Name	Sequence (5' to 3')
MYH6-F-EcoRV	TCCGGACTCAGATCGATATCAATGACCGATGCCAGATGGCTG
MYH6-R-Sal1-N	GCGGTACCGTCGACGTGTCACTCCTCATCGTGCATTTTTTGC
MYH6-933del-A-RN	CCGCGTTCATCTCCTCATCCTCCAGCCTCTCATTTCATC
MYH6-933del-P-FN	GGATGAGGAGATGAACGCGGAGCTCACTGCCAAGAAG
MYH6-R721W-A-R	CGGAAGTCCCCATAGAGGATGCGGTTG
MYH6-R721W-P-F	ATCCTCTATGGGGACTTCCGGCAGAGGTATC
MYH6-F-Cla1	TCCGGACTCAGATATCGATAAATGCACCATGCCAG
MYH6-R-Xho1	TGGATCCCGGGCTCGAGGTACCGTCGACGTGTCACTC
MYH6-pIRES-Nhe1-F	CCGGACTCAGCTAGCCCACCATGACCGATGCCAGATGGCTG
MYH6-pIRES-Sal1-R	TACCGTCGACGTGTCACTCCTCATCGTGCATTTTTTGCTTG
MYH6-S2-F-Bam	ACTTCAAGGATCCGCCGCTGCTGAAGAGCGCAGAG
MYH6-S2-R-Eco	TCTCCTTGAATTCCTTGGCCAGTGTGCTCAGCTCCAG
MYBPC3-C1C2-F-Eco	CCCGGGCGAATTCTGTCCACGAGGCCATG
MYBPC3-C1C2-R-Sal	TTGATATCGTCGACTGTGCTCTTCTTCTC

3. RT-PCR of human *MYH6* in zebrafish embryos

Name	Sequence (5' to 3')
MYH6-2555F	CCACCATGAAGGAAGAGTTTCG
MYH6-2935R	CAGCCATCTCCTCTGTTAGGTT

4. ATG-blocking morpholino antisense oligonucleotide targeting zebrafish *myh6*

Name	Sequence (5' to 3')
<i>myh6</i> ATG-MO	ACTCTGCCATTAAAGCATCACCCAT

Table S2. GenBank accession numbers of human MYH paralogs

Genes	GenBank accession number
<i>MYH1</i>	NP_005954
<i>MYH2</i>	NP_060004
<i>MYH3</i>	NP_002461
<i>MYH4</i>	NP_060003
<i>MYH6</i>	NP_002462
<i>MYH7</i>	NP_000248
<i>MYH8</i>	NP_002463
<i>MYH13</i>	NP_003793
<i>MYH14</i>	NP_079005
<i>MYH15</i>	NP_055796

Table S3. GenBank accession numbers of α -MHC orthologs

Species	GenBank accession number
human	NP_002462
macaque	XP_001102827
mouse	NP_001157643
rat	NP_058935
bovine	XP_002690549
dog	XP_003435121
chicken	NP_990097
xenopus	NP_001085070
zebrafish	NP_942118

Table S4. MYH6 exon variations in nine SSS probands

Exon	Nucleotide position	Changes of amino acid and nucleotide	Number of patients	dbSNP
17	2151	Y717Y (TAC to TAT)	1	rs76202964
22	2797-2799	delE933 (del_GAG)	1	-
25	3302	A1101V (GTG to GCG)	3	rs365990
32	4527	E1509E (GAG to GAA)	1	rs34855944
33	4777	Q1593L (CAG to CTG)	1	rs45574136
33	4838	V1613A (GTC to GCC)	4	rs61742476
33	4914	A1638A (GCT to GCC)	1	rs178640

Table S5. Cell counting on multi-electrode arrays

Line	Control pIRES2-EGFP	WT- <i>MYH6</i>	delE933- <i>MYH6</i>
1	2.45	2.12	2.34
2	2.00	2.55	2.11
3	2.91	2.13	2.23
4	2.56	2.38	2.41

x10⁵ cells