

Supplementary information

Running title: CRISPR/Cas9-mediated MSTN^{-/-} medaka

Using CRISPR/Cas9-mediated gene editing to further explore growth and trade-off effects in myostatin-mutated F4 medaka (*Oryzias latipes*)

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Figure legends

Figure S1. No off-target effect was found in MSTN^{-/-} F5 medaka. (A) A search using the online tool CHOPCHOP (<https://chopchop.rc.fas.harvard.edu>) identified 2 possible binding target of the MSTN sgRNA used in this study. The gene sequence chr21:18493824 was from our gene of interest, ie MSTN, while the gene sequence chr4:31195001 was a possible off-target site with 3 mismatching base pairs. (B) A primer set, chr4:31195001F and chr4:31195001R (arrows), was designed to amplify the possible off-target site on chr4:31195001 (boxed). (C) Off-target chr4:31195001 PCR amplicons were subcloned and sequenced. Sequencing data showed that no insertion or deletion occurred on chr4:31195001 in any of the tested MSTN^{-/-} F5 medaka.

Figure S2. 22-bp insertions and 61-bp deletions were found in MSTN mRNA from MSTN^{-/-} F5 medaka. (A) A primer set, MSTN-Q-F and MSTN-R3 (arrows), was designed to amplify a MSTN cDNA fragment that included the MSTN-sgRNA target site (boxed). (B) The resulting PCR amplicons were subcloned and sequenced. In all of the tested fish, sequencing data showed the expected 22-bp insertion in the C-terminus of the MSTN mRNA of the knockout fish. However, in two of the knockout medaka (MSTN^{-/-} #5 and #8), an unexpected 61-bp deletion was also sometimes detected even

though the genotypes of these CRISPR/Cas9 treated medaka all showed the 22-bp insertion in the C-terminus of MSTN. (C) Corresponding amino acid sequences translated from the PCR amplicons of the WT and two mutant types (22-bp insertion and 61-bp deletion) showed that both mutant types caused a frame-shifted mutation of MSTN.

Figure S3. Spinal deformity in MSTN^{-/-} F5 medaka. (A) Severe spinal curvature was observed in several adult MSTN^{-/-} F5 medaka. (B) PCR followed by sequencing of the 450 bp amplicon that included the MSTN-sgRNA target site (underlined) was used to check the genotype of the three MSTN^{-/-} F5 medaka in Suppl. Fig. S3A and two other MSTN^{-/-} F5 medaka that also presented with the same spinal deformity. All of the tested deformed fish showed a homozygous 22 bp insertion at the target site

A

MSTN specific part of sgRNA: TGAAGATCTCAGAGGGCCCCAGG

Off-targets

Genomic location	Number of mismatches	Sequence (including mismatches)
chr21: 18493824	0	TGAAGATCTCAGAGGGCCCCAGG
chr4: 31195001	3	TGAAGgTCTCtGAGGGCCcCAGG

B

chr4 :	GGGGGATTCAACTCGTGATGTTTACCAAAAGAGCCGTGCTGGGGTTCCTCCTATGTACATATATGGTCAACAGGAAATGAGTCACAGCTTCTCATAAGATTTCAGGATTTC	: 112
chr4 :	GGGGGATTCAACTCGTGATGTTTACCAAAAGAGCCGTGCTGGGGTTCCTCCTATGTACATATATGGTCAACAGGAAATGAGTCACAGCTTCTCATAAGATTTCAGGATTTC	
chr4 :	GTAATAATTAAGCCAGCAGAAACCCACTGCTCTGACTAGTCCAGACCCCTCCCAACAGTACTAATCACTAGAGAATAATTTTCAGTAAATACATAAACCTTCAGACCGCC	: 224
chr4 :	GTAATAATTAAGCCAGCAGAAACCCACTGCTCTGACTAGTCCAGACCCCTCCCAACAGTACTAATCACTAGAGAATAATTTTCAGTAAATACATAAACCTTCAGACCGCC	
chr4 :	ACTTCCCGAGAGCTGATCTCCCTTTTCTCATTGAGGTACAGAGAAACAAAGGAGATATCCAGAGTCCGGATGTTCCCGACGCCCTCCACTGGCTCTCCCGTGGCGTCT	: 336
chr4 :	ACTTCCCGAGAGCTGATCTCCCTTTTCTCATTGAGGTACAGAGAAACAAAGGAGATATCCAGAGTCCGGATGTTCCCGACGCCCTCCACTGGCTCTCCCGTGGCGTCT	
chr4 :	CATCAACGACTAACCGGTGCATGGAGTGAGAACCGGTGACTAAGAACTAGCTTACATATTAACAGCATGAGTCACCTGGGTCTGCTTCTGCAGATTTTCACACACAAG	: 448
chr4 :	CATCAACGACTAACCGGTGCATGGAGTGAGAACCGGTGACTAAGAACTAGCTTACATATTAACAGCATGAGTCACCTGGGTCTGCTTCTGCAGATTTTCACACACAAG	
Chr4: 31195001	CCCCTCTGGACCTTCTATCTGCCCCAGGATCCGGTCTCAAGCGTAGAGACGTTTCAGAGGGACGAGCAGCGCTGAAGGTGCTCAACGTTCTGCGGGTGGGATTGGGAC	: 560
Chr4: 31195001	CCCCTCTGGACCTTCTATCTGCCCCAGGATCCGGTCTCAAGCGTAGAGACGTTTCAGAGGGACGAGCAGCGCTGAAGGTGCTCAACGTTCTGCGGGTGGGATTGGGAC	
chr4 :	CGTTCACACGTTGGAGGAGTGCAGGCGGATCCGCTGACGGTGAAGGTCTCTGAGGGCCGAGGACCAGATGTTCCGGTGCACACCTCTCCAGCTCTGCTCGGTGCGCAG	: 672
chr4 :	CGTTCACACGTTGGAGGAGTGCAGGCGGATCCGCTGACGGTGAAGGTCTCTGAGGGCCGAGGACCAGATGTTCCGGTGCACACCTCTCCAGCTCTGCTCGGTGCGCAG	
chr4 :	CTTCCTTCACTCTCCATCTCCACCTCCACCCCTGGCTGATGGTCTGTGGGTCTGCCCCGTGACCCGCTGGGCGGCTCAGAGAAGAAAGCCCTCGGGCTGTTTTCTCT	: 784
chr4 :	CTTCCTTCACTCTCCATCTCCACCTCCACCCCTGGCTGATGGTCTGTGGGTCTGCCCCGTGACCCGCTGGGCGGCTCAGAGAAGAAAGCCCTCGGGCTGTTTTCTCT	
chr4 :	TAGAAATGGAATAATGGGATTTTCTTCTCTGCTCTTAATGCACCCCTCCATGCATGTCCTAGAGACTTGGTCTGCTGTTGGTGCAATTTTCCATTTCTACTTCGCC	: 896
chr4 :	TAGAAATGGAATAATGGGATTTTCTTCTCTGCTCTTAATGCACCCCTCCATGCATGTCCTAGAGACTTGGTCTGCTGTTGGTGCAATTTTCCATTTCTACTTCGCC	
chr4 :	CTTT	: 900
chr4 :	CTTT	

C

Genomic tracks showing DNA sequences for WT #2, MSTN⁺ #2, and MSTN⁺ #3 across two regions (10-150 and 160-300). The sequences are aligned to a reference chr4 sequence. A box highlights a "Possible off-target site" in the 280-300 region.

Region 10-150:

WT #2: chr4, No. 1, No. 2, No. 3

MSTN⁺ #2: No. 1, No. 2, No. 3

MSTN⁺ #3: No. 1, No. 2, No. 3, No. 4, No. 5

Region 160-300:

WT #2: chr4, No. 1, No. 2, No. 3

MSTN⁺ #2: No. 1, No. 2, No. 3

MSTN⁺ #3: No. 1, No. 2, No. 3, No. 4, No. 5

Possible off-target site: A box highlights a region around position 280-300, indicating a potential off-target site for the sequence.

Figure S1

A

MSTN^{-/-} with
normal morphology

Deformed MSTN^{-/-}



B

Deformity #1

WT TGAGGTGAAGATCTCAGAGGGCCCCAGGCGTGCCAGGAAGG

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

Deformity #2

WT TGAGGTGAAGATCTCAGAGGGCCCCAGGCGTGCCAGGAAGG

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

Deformity #3

WT TGAGGTGAAGATCTCAGAGGGCCCCAGGCGTGCCAGGAAGG

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

Deformity #4

WT TGAGGTGAAGATCTCAGAGGGCCCCAGGCGTGCCAGGAAGG

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

Deformity #5

WT TGAGGTGAAGATCTCAGAGGGCCCCAGGCGTGCCAGGAAGG

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

+22 TGAGGTGAAGATCTCAATGAAGATCTCCTGGCACGCCTGAGTCTCCCAGGCGTGCCA

Figure S3