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1 ***Naa15* knockdown enhances c2c12 myoblast fusion and induces**
2 **defects in zebrafish myotome morphogenesis**

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10 **Abstract**

11 The comprehension of muscle tissue formation and regeneration is essential to develop
12 therapeutic approaches against muscle diseases or loss in muscle mass and strength during
13 ageing or cancer. One of the critical steps in muscle formation is the fusion of muscle cells to
14 form or regenerate muscle fibres. To identify new genes controlling myoblast fusion, we
15 undertook an siRNA screen in c2c12 myoblasts and found that N-alpha-acetyltransferase 15
16 (*Naa15*) knockdown enhanced c2c12 myoblast fusion suggesting that *Naa15* negatively
17 regulated myogenic cell fusion. We identified two *Naa15* orthologous genes in zebrafish
18 genome: *naa15a* and *naa15b*. These two orthologs are both expressed in myogenic domain of
19 the somite. Knockdown of zebrafish *naa15a* and *naa15b* genes induced a “U” shaped
20 segmentation of the myotome and alteration of myotome boundaries resulting in the
21 formation of abnormally long myofibres spanning adjacent somites. Taken together these
22 results show that Naa15 regulates myotome formation and myogenesis in fish.

23 **Keywords**

24 siRNA screen, morpholino, trout, muscle regeneration, evolution

25 **1. Introduction**

26 The fusion process is a critical step for the formation and reparation of muscle. Indeed,
27 skeletal muscle is mainly composed of multinucleated cells, called myofibres. To form those
28 myofibres, progenitor cells (myoblast) proliferate, differentiate into myocytes, fuse to form
29 multinucleated myotubes and finally mature into functional myofibres. The process leading to
30 the formation of skeletal muscle is usually divided into a primary, a secondary, and an adult

31 phases (1). The primary phase is powered by cells originating from dermomyotome lips and
32 results in the formation of the primary myotome. Then, during the secondary phase, cells
33 emanating from the central region of the dermomyotome, differentiate and fuse either with
34 each other to form secondary fibres (hyperplastic growth) or with primary fibres (hypertrophic
35 growth). Through adult phase, satellite cells allow muscle growth, and regeneration after
36 damage and injury.

37 Myocyte fusion is a very coordinated event requiring that two cells get close, recognize
38 each other, adhere their membrane, open fusion pore and finally merge together into one
39 multinucleated cell (2). This process implies many known molecular components studied in
40 multiple model organisms like *Drosophila*, zebrafish and mouse.

41 In the fruit fly *Drosophila melanogaster*, each muscle is composed of a single myofibre
42 formed by the fusion of a unique founder cell (FC) with fusion competent myocyte (FCM).
43 Recognition and adhesion between FC and FCM are mediated by immunoglobulin domain
44 containing cell adhesion molecules. Among them, are Kin of IrreC (Kirre) and Roughest (Rst)
45 which are expressed in FC (3,4) as well as ticks and stones (Sns) and Hibris (Hbs) both
46 expressed in FCM (5–8). In vertebrate species the presence of two types of muscle cells has
47 not yet been demonstrated, nevertheless, some genes initially identified in *Drosophila* have
48 homologs in vertebrates. For example Kirrel (Kirre homologue) and nephrin (Sns homologue)
49 are both involved in cell recognition/adhesion process (9,10). The fusion of myocytes in
50 vertebrates also implies specific factors such as myomaker, myomerger and myomixer (11–
51 15) and some, species-specific factors such as Jamb and Jamc in zebrafish (16) or Itgb1 in
52 mouse (17).

53 After the initial recognition/adhesion step, greater membrane proximity is required and
54 reached by a reorganization of actin cytoskeleton. This is achieved, in *Drosophila*, by

55 regulators like WASP and Scar that affect actin polymerization mediated by the Arp2/3
56 complex (18–22). In vertebrate, this step is dependent of Dock1 and Dock5 (23,24), Rac1
57 (23,25), and N-WASP (26).

58 At last the lipid bilayer needs to be destabilized to allow the cells to merge. Some studies
59 performed in c2c12 cells and in chicken show that the family of brain angiogenesis inhibitor
60 molecules (BAI) play a major role during this step of the myocyte fusion (27–29).

61 The regulation of myocytes fusion is highly complex and is far to be completely
62 understood. To identify new genes implicated in the myocyte fusion process in vertebrates, we
63 performed an *in vitro* functional screen in c2c12 cell line based on siRNA knockdown. We
64 found that *Naa15* knockdown led to the formation of myotube larger than those found in
65 c2c12 control cells. In line with this observation, knockdown of the two zebrafish orthologous
66 genes *naa15a* and *naa15b* induced the production of giant myofibres spanning two somites in
67 zebrafish embryos suggesting that *Naa15* negatively regulated myofibre formation.

68 **2. Materials and Methods**

69 ***Zebrafish husbandry***

70 Wild-type zebrafish (*Danio rerio*) were raised in our facilities (INRA LPGP, Rennes) and
71 maintained under oxygen saturation in a recirculating water system at 27 ± 1 °C, pH 7.5.
72 Zebrafish were exposed to a photoperiod of 16 h light/8 h dark. Fish used in this study were
73 reared and handled in strict accordance with French and European policies and guidelines of
74 the Institutional Animal Care and Use Committee (DDSV approval #35-47).

75 ***siRNA screen***

76 c2c12-MCK:GFP mouse myoblast cells (ATCC-CRL-1772 modified) were maintained at
77 37°C and seed in 96 wells plates at a density of 10.000 cells/well in Growth Medium
78 (Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated
79 fetal bovine serum (FBS) and Antibiotic-Antimycotic solution (BIOWEST)). Two hundred
80 fifty five genes expressed in proliferating or differentiating C2C12 cells (Moran et al 2002;
81 Tomczack et al., 2003) but with unknown function, were tested in this screen. Each gene was
82 knocking down with two different siRNA (Flexiplate siRNA, Quiagen). Each plate included
83 two negative controls (no siRNA) and two positive controls treated with anti IL4 siRNA. The
84 cells were transfected at day 1 and day 4 with DMEM including 5nM of siRNA and 1µl per
85 well of INTERFERin (Polyplus) and placed in differentiation medium (DMEM containing
86 2% FBS and Antibiotic-Antimycotic solution). The medium was changed every day. After six
87 days of differentiation, cells were washed two times with phosphate-buffered saline (PBS)
88 before fixing in PBS with 4% paraformaldehyde for 15 min. Then, the cells were
89 permeabilized 3 minutes in PBS with 0.1% TritonX100, stained with 0.1µg/ml of DAPI for 5
90 minutes and stored at 4°C in the dark.

91 Image acquisition was made using an HCS Arrayscan VTI (Cellomics/Thermofisher
92 Scientific) with a ZEISS EC plan NEOFluar 10x ON objective and an ORCA-ER 1.00
93 camera. A macrocommand was edited for visilog 6.7 to monitor automatically the fusion
94 index, mean nuclei number per GFP+ cells, and the GFP+ cells area.

95 ***Cell culture for QPCR analysis***

96 The c2c12-MCK:GFP mouse myoblast cells were maintained at 37°C and seeded in 12
97 wells plates at a density of 100.000 cells/well in growth medium. *Naa15* was knocking down
98 with two different siRNA (Flexiplate siRNA, Quiagen). The cells were transfected at day 1
99 with DMEM including 5nM of siRNA and 3.5µl per well of INTERFERin (Polyplus) and

100 place in differentiation medium. Each plate includes 4 wells transfected with anti-GFP siRNA
101 as negative control, and 4 wells transfected with each of the 2 anti-*Naa15* siRNA. The
102 medium was changed every day. One plate was stopped after 0, 1, 2 or 3 days of
103 differentiation. Total RNA was isolated using TRIzol (Invitrogen) according to the
104 manufacturer's instructions and relative RNA concentration was determined by
105 spectrophotometric analysis. 0.3 µg of total RNA was reverse-transcribed into cDNA using
106 the high-capacity cDNA reverse transcription kit (Applied Biosystems). Realtime PCR was
107 performed in duplicate using 1/10, 1/40 or 1/400 dilution of RT-cDNA from c2c12 cells.
108 Samples were amplified in a 96 well plate using SYBR Green on a StepOnePlus Real Time
109 PCR system (Applied Biosystems) with specific primers for mouse *Naa15*, and *Myog* genes at
110 a concentration of 300nM. The expression of *Hprt* and B-Actin genes were used as
111 endogenous control to normalize each sample. Relative mRNA expression was assessed by
112 relative standard curve method.

113

114 ***In situ hybridization***

115 Embryos were removed from their chorion by a 3 min incubation in a 1/100 (wt/vol)
116 Pronase solution (Sigma, P6911) pre-warmed to 28 °C. Then, they were fixed in 4%
117 paraformaldehyde overnight and stored in methanol at -20 °C. Anti-sense RNA probes
118 labelled with digoxigenin (DIG) were prepared from PCR-amplified templates using
119 appropriate RNA polymerases. Whole mount in situ hybridization were performed with an
120 INSITU PRO VS automate (INTAVIS AG) using standard protocol **(30)**. Whole mount in
121 situ images were obtained using a macroscope NIKON AZ 100 coupled with NIKON Digital
122 Sight DSRi1 camera and using NIS-Elements D 3.2 software.

123

124 **Morpholino injection**

125 Freshly fertilized eggs were injected with morpholinos at one to two-cell stage.
126 Morpholinos (Gene Tools) were dissolved in sterile water at a concentration of 2.5 mM. Anti-
127 *naal15a*, anti-*naal15b*, anti-*p53* and anti-*naal15a* mismatch control morpholinos were used.
128 Anti-Naa15a and anti Naa15b were designed to bind the area of the predicted start codon.
129 Morpholinos sequences were as follows: anti-*naal15a*:
130 TCTTGAGGGTTGTCCACCGCGACTT; anti-*naal15b*:
131 CGGCATCCTGTTCACCTCTCTATTTC; anti-*naal15a* mismatch control:
132 TATTGACGGTTGTACACCCCGAATT. 300 to 450 eggs were injected for each experiment.
133 Embryos were injected with approximately 4 nl of morpholinos (8.8 ng) diluted in sterile
134 water with 0.1% phenol red. About the same number of eggs was injected with the anti-
135 *naal15a* mismatch control. To ensure that phenotype specificity is due to knock down of
136 *Naa15* orthologues and not to nonspecific induction of apoptosis, embryos were co-injected
137 with anti-*p53* morpholino (6.5ng of anti-*naal15a*+6.5ng of anti-*p53*). The injected eggs were
138 cultured at 28 °C, and embryos were fixed in 4% paraformaldehyde overnight at 30h of
139 development. Zebrafish embryos were permeabilized in 0.3% Triton in PBS solution for 3 h.
140 Embryos were stained with a solution including 5µg/ml of WGA-Alexa 488 and TOPRO 3
141 (1:1000 dilution) overnight. Confocal microscopy images were collected using an OLYMPUS
142 BX61WI FV 1000 microscope and FluoView 3.0 software. Entire images were adjusted for
143 contrast, brightness, and dynamic range using ImageJ software.

144

145 **Statistical analyses**

146 For the siRNA screen, statistical analysis was made using a One-way ANOVA (ANalysis
147 Of Variance) with post-hoc Tukey test in SigmaStat 3.5. For QPCR experiment statistical
148 analysis was made using a One-way ANOVA in Past 3.15.

149

150 **3. Results**

151 ***Naa15* knockdown enhanced *c2c12* myoblast fusion**

152 An *in vitro* functional screen was performed based on siRNA knockdown (KD) in
153 MCK:GFP C2C12 myoblasts. Those myoblasts express GFP under the muscle creatine kinase
154 (MCK) promoter, GFP is therefore expressed only in differentiated myoblasts allowing an
155 easy monitoring of cells differentiation. Our functional screening allowed the identification of
156 genes which *in vitro* knock-down significantly impacted myoblast fusion without affecting the
157 differentiation capacity of the cells, various functional parameters were assessed among which
158 the fusion index, mean nuclei number per GFP+ cells, and the GFP+ cells area.

159 One of the more strong phenotype was observed after the KD of the N (Alpha)-
160 Acetyltransferase 15 (*Naa15* also referred to as *Tbdn*, *Narg1*, *mNat1*, *NATH*) gene (Fig 1A).
161 The c2c12 cells transfected with two different anti-*Naa15* siRNA exhibited a significant
162 increase in the fusion index. This index (number of nuclei in myotubes / total number of
163 nuclei) was around 75% greater in GFP+ (differentiated) cells after treatment with both
164 *Naa15* siRNA than in control cells (Fig 1B). Specifically, the mean number of cells including
165 more than 10 nuclei after 7 days of differentiation was five times higher for cell cultures
166 treated with anti-*Naa15* siRNA when compared to control cell cultures. No significant
167 difference was observed in the number of small myotubes (<4 nuclei) (Fig 1C).

168 QPCR experiment showed that both siRNA treatment induced a 50% reduction of *Naa15*
169 expression (Fig 1D). As shown by differentiation index, no significant changes in the
170 expression of *Myogenin* (*MyoG*), a differentiation marker, was observed in the cells
171 transfected with anti-*Naa15* siRNA as compare to controls (Fig 1E). This confirm that the
172 differentiation process was not impacted by *Naa15* knockdown.

173

174 ***The two Naa15 orthologous genes were expressed in*** 175 ***zebrafish somites***

176 To study the function of *naa15* during myogenesis in zebrafish, we looked for orthologous
177 genes. We performed BLAST in public databases and identified two orthologous genes
178 namely *naa15a* and *naa15b*. Those genes encode proteins with respectively 85% and 90% of
179 homology with the murine NAA15 protein. Protein sequences were used to deduce a
180 phylogenetic tree from maximum likelihood method (Fig 2) and show an *naa15* duplication
181 occurring in teleost and in cyprinid as expected.

182 *In situ* hybridization showed that *naa15a* (Fig 3A) and *naa15b* (Fig 3B) were both
183 expressed in somites during late somitogenesis (24 hpf), when myoblast fusion process
184 occurs. There are also expressed in eyes and midbrain. It could be noticed that only a weak
185 signal was observed for *naa15b* *in situ* hybridization.

186

187 ***naa15a and naa15b knockdown in zebrafish led to*** 188 ***curvature of the body***

189 To determine whether *naa15a* and *naa15b* are required for fish myogenesis *in vivo*, we
190 performed morpholino knockdown of *naa15a* and *naa15b*. Zebrafish embryos at 1 cell stage
191 were injected with either an anti-*naa15a*, an anti-*naa15b*, or an anti-*naa15a* mismatch
192 morpholino (4 mismatches) used as a control. The control embryos injected with anti-*naa15a*
193 mismatch morpholino exhibited a normal morphology at 30hpf and no phenotype was
194 observed as compare to wild type uninjected embryos. At 30hpf, *naa15a* or *naa15b* morphant
195 exhibited morphological defect that could be classified in two classes. The class 1 embryos
196 (C1) had reduction of the body size and small curvature of the trunk. The phenotype of the
197 class 2 embryos (C2) is more severe, and a greater curvature of the trunk is observed (Fig 4).
198 According to the experiment, the C1 embryos represent 30-50% of the *naa15a* and 15-25% of
199 the *naa15b* morphants, and the C2 embryos represent 30-50% of the *naa15a* and 40-56% of
200 the *naa15b* morphants. Wild type phenotype is observed in 5-20% of the *naa15a* morphants
201 and in 20-30% of the *naa15b* morphants.

202 Co-injection with anti-*naa15a* and anti-*naa15b* morpholino was also performed. Most of
203 the embryos co-injected with the two morpholino died in the first 24 hours after fertilization.
204 The survivors *naa15a* + *naa15b* morphants presented similar phenotypes to embryos injected
205 with anti-*naa15a* or anti-*naa15b* alone (C1 and C2 phenotypes).

206 To confirm that the observed phenotypes did not result from unspecific induction of
207 apoptosis (31) , the experiments were replicated with embryos co-injected with anti-*naa15a*
208 and anti-p53 morpholinos. In all experiments, the *naa15a/p53* morphants present the same
209 phenotype than the embryos injected with anti-*naa15a* or anti-*naa15b* morpholinos alone.

210

211 ***naa15a and naa15b knockdown in zebrafish led to***
212 ***intersegmental boundary defect***

213 Confocal microscopy of the morphants after staining of the nuclei and the plasma
214 membrane and connective tissue revealed defect in the segmentation of the C1 and C2
215 embryos as compare to control mismatch *naa15* morphants. Wheat Germ Agglutinin (WGA)
216 conjugate with alexa488 was used for the plasma membrane and connective tissue staining.
217 This protein is a lectin that selectively binds to N-acetylglucosamine and N-acetylneuraminic
218 acid (sialic acid) residues and allows fast, and convenient methodology for connective tissue,
219 and plasma membrane visualization (32). This methodology allowed us to shown that C1 and
220 C2 embryos did not exhibit the usual chevron shape segmentation of the myotomes (Fig 5)
221 (33). Further, the myotome of the *naa15a* and *naa15b* morphants presented a lack or
222 reduction of the horizontal myoseptum and interruption of myotome boundaries along the
223 dorso/ventral and medio/lateral axis (Fig 5). Those interruptions were observed in all the
224 tested C1 and C2 embryos (n>15) in about 30% of the myotome boundaries. They formed
225 holes through which some of the myofibres, displaying twice the normal fibre length,
226 stretched out (figure 5, figure 6). Some of those longer myofibres could contain up to 11
227 nuclei but altogether, their mean number of nuclei per fibres was not significantly increased as
228 compared to normal size fibres (not shown).

229 ***naa15a and naa15b knockdown in zebrafish led to a***
230 ***reorganization of the myosin pattern***

231 In the C1 an C2 *naa15a* and *naa15b* morphants, the myosin proteins had not the same
232 expression pattern than in the wild type embryos, as shown by myosins immunostaining
233 performed using MF20 antibody. Myosin is mainly located at the peripheries of the wildtype
234 control embryos myofibres, close to the myotome boundary (Fig 6A, E) whereas in the C1

235 and C2 morphants, it was uniformly present within the whole fibre (Fig 6B, F). This abnormal
236 myosin localization was also observed in long fibre spanning the intersegmental boundaries
237 (Fig 6B, F).

238 **4. Discussion**

239 The fusion of muscle progenitor cells is one of the critical steps in muscle formation and
240 regeneration. This process requires several steps including cell recognition, adhesion, and
241 membrane fusion. To identify new genes implicated in myocyte fusion in vertebrates, we
242 undertook an siRNA screen in c2c12 myoblasts followed by *in vivo* functional studies of
243 relevant candidate genes in zebrafish.

244 Among the tested genes, we identified *Naa15* as an inhibitor of c2c12 fusion since its
245 knockdown enhanced fusion of myoblasts *in vitro*. *Naa15* is part of the family of N-terminal
246 acetyltransferase subunits. The NAA15 protein is highly expressed during embryogenesis
247 (34–36) and it binds to the catalytic subunit Naa10 to form the NatA complex (for review of
248 NAT complex see (36)). In mammals, the NatA complex interact with various substrates and
249 is implicated in a broad range of cellular processes from cell growth to cellular differentiation
250 (35–44). The major function of the NatA complex is the proteins N-terminal acetylation (36).
251 The N-terminal acetylation has various consequence for a protein: it could determine the
252 subcellular localization (45–48), module the protein-protein interactions (49,50) and is also
253 crucial for protein folding. Neither Naa15 or the NatA complex is currently described to have
254 a function during myogenesis. Nevertheless, other N-terminal acetyltransferase was reported
255 to play a key role in tropomyosin-actin complex formation, increasing actin binding, and
256 promoting the regulation of specific myosin activity (51,52).

257 Our *in silico* analysis revealed that the *Naa15* gene is found in two copies (*naa15a* and
258 *naa15b*) in the zebrafish genome as a result of the Teleost Genome Duplication (TGD) (53).
259 In situ hybridization analysis indicated that *naa15a* and *naa15b* were expressed in somites at
260 24h post fecundation, when myoblast fusion occurred (16). To further assess the role of
261 *Naa15* in myogenesis, we undertook knockdown experiments in zebrafish using morpholinos.
262 We observed that zebrafish embryos injected with anti *naa15* Morpholinos didn't exhibit
263 classical chevron-shaped myotomes. Further, interruptions in the intersegmental boundaries
264 allowing long myofibres to span over two (rarely 3) segments. This phenotype is reminiscent
265 with those observed after the knockdown of genes encoding components of the Notch
266 signalling pathway, especially *her1* and *her7* (54,55).

267 NAA15 is known to be a binding partner of cortactin [31], a protein regulating the F-actin
268 polymerization and as such could be involved in process such as migration, permeability or
269 elongation of cells. The knockdown of *Naa15* in retinal endothelial cells induces activation of
270 the c-SRC kinase resulting in the phosphorylation and activation of the cortactin by a still
271 unknown mechanism (39). This activation of cortactin resulting of the *naa15a* or *naa15b*
272 knockdown could be partially responsible of the phenotype we observed. Indeed, increasing
273 cell permeability, adhesion and migration could lead to the *in vitro* enhancement of myoblast
274 fusion, and the presence of longer myofibres *in vivo*. Nevertheless, we did not detect any
275 significant modifications in cells migration or adhesion after *Naa15* knockdown (not shown).

276 In conclusion our results showed that *Naa15* not only inhibits c2c12 myoblast fusion *in*
277 *vitro*, but also is expressed in zebrafish developing myotome where it appears to be essential
278 for proper myotome formation. Further research is needed to decipher the possible functional
279 link between *Naa15* activity and the notch pathway or the cortactin activity that could explain
280 the phenotype of zebrafish embryos injected with anti *naa15* morpholinos. Altogether the

281 better understanding of the acetylation process leading to the formation and reparation of
282 muscle fibres will be useful to enhance muscle repair therapy.

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290 [puces-a-cellules.univ-rennes1.fr](http://imagerie-puces-a-cellules.univ-rennes1.fr)).

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457 **Figure Captions**

458

459 **Fig1. SiRNA screen.**

460 *Naa15* act as a fusion inhibitor in c2c12 myoblast. c2c12-MCK:GFP Cell cultures
461 transfected with anti-*Naa15* siRNA and stained with DAPI (blue) present more nuclei in
462 differentiated (GFP+) cells (A). 1B. The fusion index of the GFP+ cells is significantly higher
463 in cultures treated with the anti-*Naa15* siRNA compared to control cell cultures. 1C. The
464 mean number of cells including more than 10 nuclei after 7 days of differentiation is five

465 times higher in cell cultures treated with anti-*Naa15* siRNA compared to control cell cultures.
466 No significant difference is observed in the number of small myotubes (<4 nuclei). QPCR
467 experiment show that, siRNA treatment reduces to half the *Naa15* expression (D). The
468 expression of *Myogenin* (*MyoG*) is not affected by the anti-*naa15* siRNA transfection (E).
469 Different letters indicate significant differences between groups (t-test $p < 0.05$). Values
470 represent means \pm SD (N=4). Blue and red curves and histograms represent results of cell
471 cultures treated by 2 different siRNA, control group is represented by green curves and
472 histograms.

473 **Fig2. *Naa15* orthologous genes in zebrafish.**

474 The evolutionary history of NAA15 related proteins was inferred by using the maximum
475 likelihood method based on the JTT matrix-based model. The bootstrap value calculated out
476 of 500 replicates is indicated for each node. A discrete gamma distribution was used to model
477 evolutionary rate differences among sites. *Naa15* duplicate into *naa15a* and *naa15b* during
478 the Teleost Genome Duplication (TGD).

479 **Fig3. *naa15a* and *naa15b* are expressed in somite at 24hpf.**

480 *naa15a* and *naa15b* expression in 24 hpf zebrafish embryos. *naa15a* is strongly expressed
481 in somites as well as in eyes and midbrain (left panel). *naa15b* is also expressed in somite,
482 eyes and midbrain but the signal appears weaker (right panel).

483 **Fig4. *Naa15* knockdown induce curvature of the body.**

484 *Naa15* KD induces curvature of the body. Most of the eggs injected with anti-*naa15*
485 morpholino gave rise to embryos with curvature of the trunk and reduction of body size.
486 Injection of mismatch morpholino do not induce apparent phenotype. The majority of the

487 *naa15a* morphants presents reduction of body size and moderate curvature of the trunk
488 (bottom left) whereas most of the *naa15b* morphant presents greater curvature (bottom right).

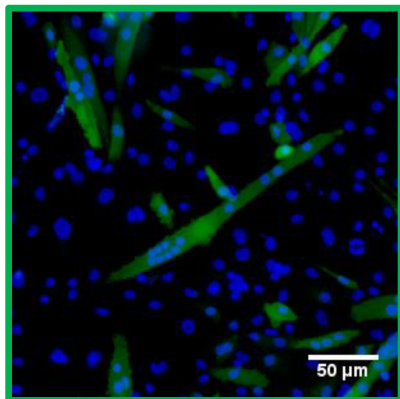
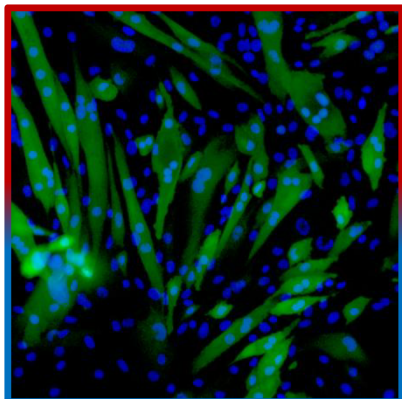
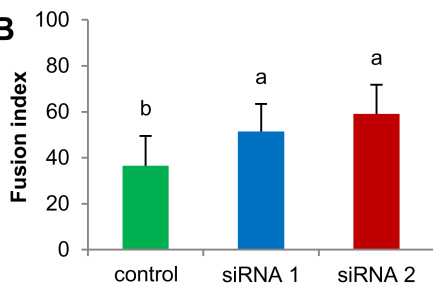
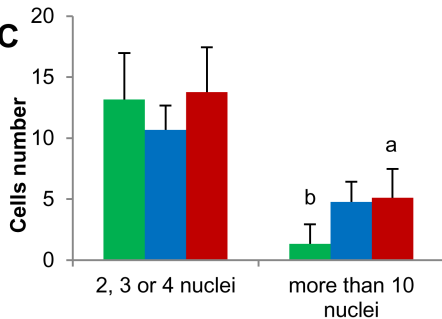
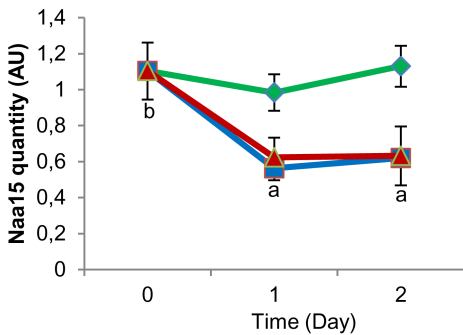
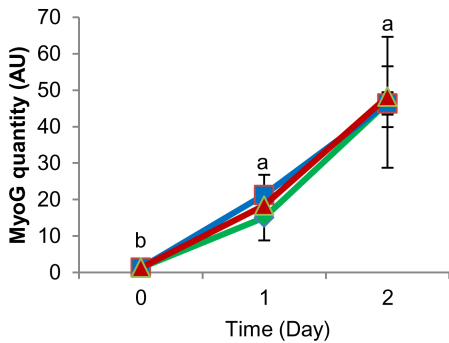
489 **Fig5. *Naa15* knockdown induce myotome defect.**

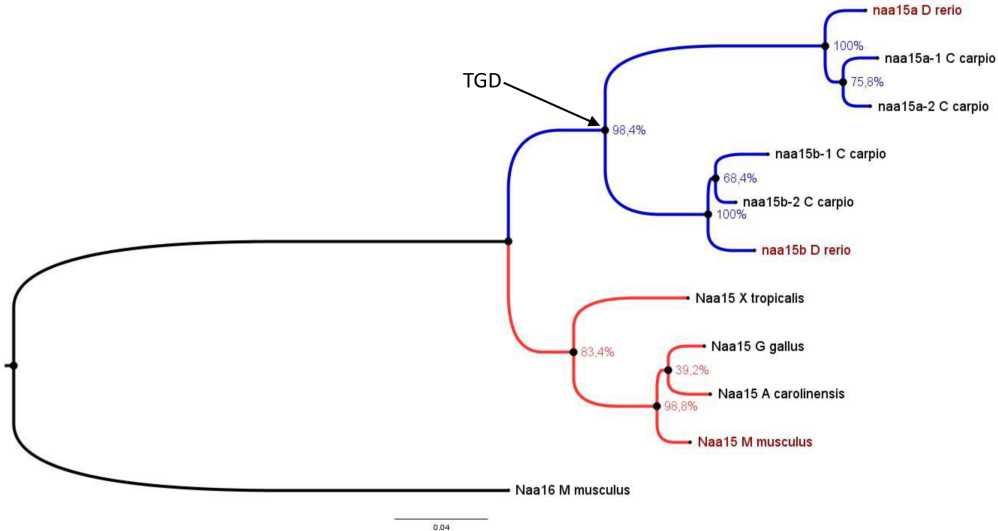
490 *naa15* KD induces myotomal boundary defects. The embryos were stained with TOPRO 3
491 (nuclei in red) and WGA-alexa488 (plasma membranes and myotome boundaries in green).
492 Confocal microscopy of the group 1 and 2 embryos showed a loss of the classical chevron
493 shaped segments with a lack or reduction of the horizontal myoseptum (asterisk) and
494 interruption of myotome boundaries along the dorso/ventral and medio/lateral axis (arrow).
495 Few myofibres stretch out within two somites/myotomes dues to interruption in somite
496 boundaries (arrow head).

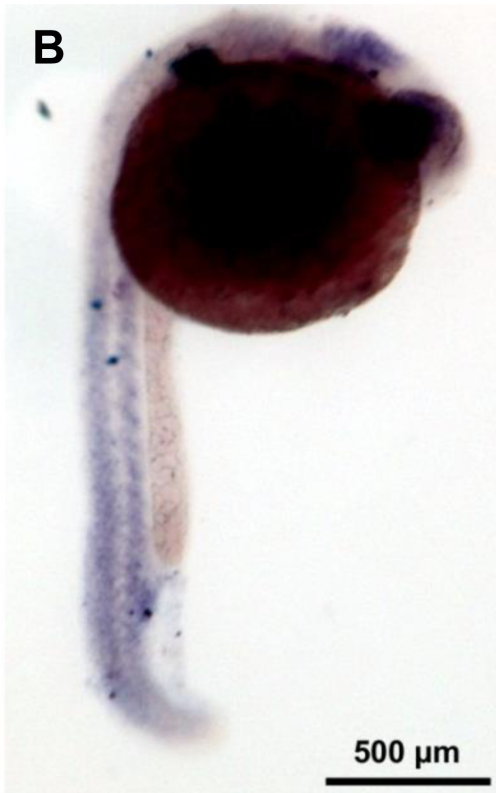
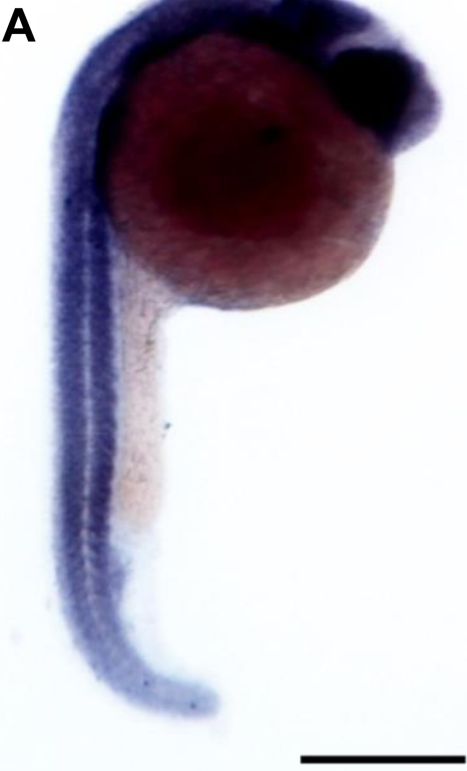
497 **Fig6. *Naa15* knockdown induce defect in the myosine organization.**

498 *naa15* KD induce defect in myosin organisation. Confocal microscopy of morphants after
499 staining with TOPRO 3 (nuclei in blue), WGA-alexa488 (plasma membranes and myotome
500 boundaries in green) (panel A and B), and immunocytofluoresence staining with an antibody
501 against myosin heavy chain (MF20, myosin in red) (panel C and D), revealed a
502 disorganization of the myosin localization in the *naa15* morphants (D) as compared to
503 embryos injected with *naa15* mismatch *naa15a* morpholino (C). Some long myofibres project
504 beyond the intersegmental boundaries (white circle).

505

A**control****anti-*Naa15* SiRNA****B****C****D****E**







Wt

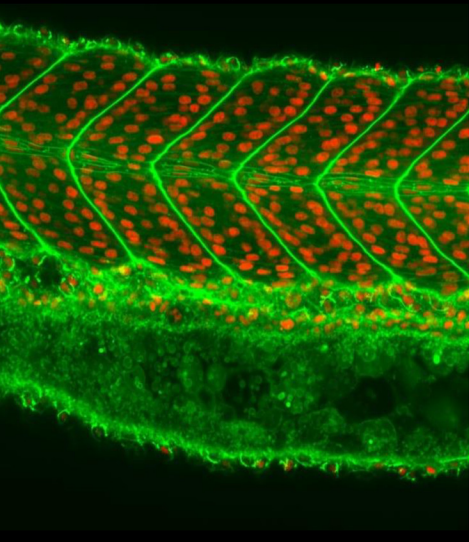


MO Naa15a

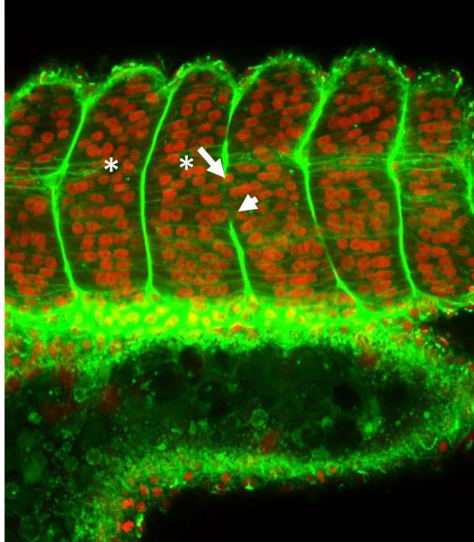


MO Naa15b

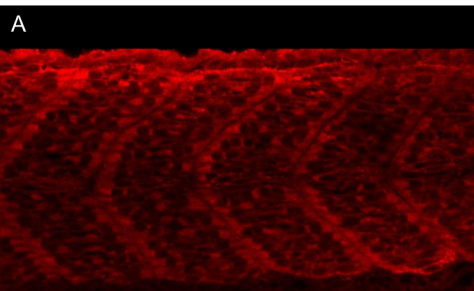
uninjected



MO Naa15b



MO Naa15a scramble control



MO Naa15a

