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1 **CXCR7 promotes foetal myoblast fusion at muscle fiber tips independently of**
2 **Myomaker via a β 1 integrin-EGFR-dependent mechanism**
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13

14 **Abstract**

15

16 Muscle growth must be tightly regulated during development in order to obtain the final
17 muscle shape. Myoblast fusion is a critical step of muscle growth, driving the formation of
18 syncytial myofibers attaching at both ends to tendons. We investigated the role of the CXCR7
19 chemokine receptor in foetal muscle growth during chicken limb development. We show that
20 CXCR7 displays a regionalized expression at the tips of myofibers close to tendons in foetal
21 limb muscles, which is exclusive to the central location of the fusion gene MYOMAKER
22 (*TMEM8C* in chicken) in foetal muscles. CXCR7 promotes myoblast fusion independently of
23 *TMEM8C* in chicken limb muscles and in foetal myoblast cultures and requires EGF receptor
24 signalling. The CXCR7 ligand, CXCL12, expressed in connective tissue, increases β 1 integrin
25 activation at the myotendinous junction and CXCR7 expression at muscle tips, resulting in a
26 fusion promoting effect independent from a direct binding of CXCL12 to CXCR7 receptor.
27 Our results evidence a CXCR7-dependent/*TMEM8C*-independent fusion mechanism at the
28 myofiber tips that regulates muscle growth at the tendon/muscle interface during foetal
29 myogenesis.

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35 **Introduction**

36

37 During development, skeletal muscle growth must be tightly regulated in order to obtain the
38 final shape of each individual muscle. This process relies on muscle progenitors expressing
39 the PAX3 and PAX7 transcription factors (Relaix et al., 2005; Hutcheson et al., 2009), which
40 enter the myogenic program under the control of the Myogenic Regulatory Factors (MRFs),
41 including the bHLH transcription factors MYF5, MYOD, MYOG and MRF4 (Buckingham
42 and Rigby, 2014). The MRFs trigger the successive steps of muscle specification,
43 differentiation and finally fusion, the ultimate phase of myogenesis, leading to the conversion
44 of mononucleated muscle cells to multinucleated myofibers, the functional unit of skeletal
45 muscle (Comai and Tajbakhsh, 2014; Esteves de Lima and Relaix, 2021). Distinct successive
46 phases of myogenesis are observed during muscle development. Embryonic myogenesis
47 involves fusion events between myoblasts, forming the nascent primary myofibers that define
48 the scaffold of the future muscles (Besse et al., 2020; Kardon, 1998) and is followed by foetal
49 myogenesis which involves myoblast fusion to embryonic myofibers and correspond to
50 muscle growth (Biressi et al., 2007). Finally, muscle satellite cells establish between
51 myofibers and basal lamina and are responsible for postnatal growth and repair of adult
52 muscle (Stockdale, 1992). MYOG-positive cells are recognized to be the fusion competent
53 cells and MYOG function is required for myoblast fusion (Ganassi et al., 2018; Hasty et al.,
54 1993).

55 Myoblast fusion has been shown to depend on the transmembrane protein MYOMAKER
56 during developmental, postnatal and regenerative myogenesis (Petrany and Millay, 2019).
57 During development, MYOMAKER is required and sufficient for myoblast fusion in mice,
58 chicken and zebrafish (Landemaine et al., 2014; Luo et al., 2015; Millay et al., 2013) and in
59 chicken limbs, *TMEM8C* (MYOMAKER in chicken) is enriched in central versus tip regions
60 of foetal muscles, similarly to the preferential central location of MYOG-positive fusion-
61 competent cells (Esteves de Lima et al., 2022), suggesting a regionalization of fusion events
62 in foetal muscles. Apart from the pivotal role of MYOMAKER, extrinsic factors have also
63 been shown to regulate muscle fusion but these have been mostly identified during
64 regenerative myogenesis (Horsley et al., 2003; Sotiropoulos et al., 2006) and are less
65 characterized during developmental myogenesis. TGF β signaling has been identified as a
66 negative regulator of myoblast fusion during muscle regeneration in mice (Girardi et al.,
67 2021) and also during development in chicken embryos (Melendez et al., 2021). Inhibition of
68 ERK pathway has been shown to drive fusion of myoblast to myotubes in myoblast cultures

69 and during muscle regeneration (Eigler et al., 2021).
70 Chemotactic factors, secreted by the muscle environment or the muscle itself are recognized
71 as important regulators of myoblast migration, the prerequisite step required for a correct
72 fusion process during myogenesis as well as adult muscle repair (Abmayr and Pavlath, 2012).
73 During embryonic development, the CXCL12 chemokine has been shown to be expressed in
74 the limb mesenchyme and inactivation of CXCR4 receptor, expressed in muscle progenitors,
75 inhibits their migration from the somite to the limb (Vasyutina et al., 2005). Inactivation of
76 CXCL12 has also been shown to decrease myoblast fusion without affecting myogenic
77 differentiation both in C2C12 myoblasts (Ge et al., 2013) and in mouse primary myoblasts
78 (Griffin et al., 2010) via CXCL12 binding to CXCR4 (Bae et al., 2008; Griffin et al., 2010).
79 CXCL12 chemokine can also signal through CXCR7 receptor. Depending on the cell type or
80 process, CXCR7 has been alternatively described as a scavenger receptor for CXCL12 ligand
81 or a signalling receptor acting with or without CXCR4 (Koch and Engele, 2020). The
82 involvement of CXCR7 during myogenesis is not well documented, as homozygous CXCR7
83 null mutation in mice led to birth lethality, due to ventricular septal defects and semilunar
84 heart valve malformation (Gerrits et al., 2008; Sierro et al., 2007). Nevertheless, sparse *in*
85 *vitro* studies highlight a CXCR7 function in the differentiation steps of C2C12 muscle cells
86 (Hunger et al., 2012; Melchionna et al., 2010). Interestingly, a recent siRNA screen conducted
87 on the C2C12 cell line to determine genes implicated in myoblast fusion identified CXCR7
88 among the genes necessary for this process (Melendez et al., 2021).
89 In this study, we investigated the function of CXCR7 receptor during chicken limb foetal
90 myogenesis. We show that CXCR7 exhibits a regionalized expression at the tips of muscles at
91 the transcript and protein levels, which is exclusive from the central location of *TMEM8C*
92 transcripts in chicken limb foetal muscles. CXCR7 promotes myoblast fusion independently
93 of *TMEM8C* in foetal muscles and involves EGF receptor signaling in myoblast cultures. The
94 CXCR7 ligand, CXCL12, expressed in connective tissue (CT), increases β 1 integrin activation
95 and CXCR7 expression at muscle tips and mimics the fusion promoting effect of CXCR7.
96 Our results evidence a CXCR7-dependent fusion mechanism at the myofiber tips in chicken
97 limb foetal muscles that would regulate muscle growth and elongation at the tendon/muscle
98 interface independently of *TMEM8C*.

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101

102

103 **Results**

104

105 **CXCR7 receptor is expressed in foetal myogenic cells**

106 We and others have previously shown that *CXCL12* is expressed in CT during limb
107 development (García-Andrés and Torres, 2010; Nassari et al., 2017; Vasyutina et al., 2005).
108 Here, we investigated the expression pattern of the two *CXCL12* receptors, *CXCR4* and
109 *CXCR7*, during limb foetal myogenesis by in situ hybridization and immunostaining. *PAX7*
110 and *MF20* antibodies were used to visualize progenitor and differentiated muscle cells. At E5,
111 *CXCR4* was expressed near the mesenchymal region expressing *CXCL12* (Fig. 1a, b), in cells
112 of the nascent limb vasculature and at a lower level in the dorsal and ventral muscle masses
113 (Fig. 1b, (Vasyutina et al., 2005)). At this stage, *CXCR7* transcripts are expressed in
114 developing cartilage and in some muscle progenitors (Fig. 1c). Detection of both receptors
115 revealed that some cells co-expressed *CXCR4* and *CXCR7* (Fig. 1d) and revelation of *PAX7*
116 antibody showed that *CXCR7* was indeed expressed in some *PAX7*-positive progenitors (Fig.
117 1e-g). From E6, revelation of *MEP21* antibody, which stained endothelial cells, underlined
118 the expression of *CXCR4* in limb vessels (Supp. Fig. 1a-c), while it was down-regulated in
119 muscle cells (Supp. Fig. 1b, d). From E8, *CXCR7* transcripts (Fig. 1h, j, k) and protein (Fig.
120 1l) appeared mostly expressed to the tips of differentiated *MF20*-positive myotubes (Fig. 1m,
121 n). At this stage, *CXCL12* expression in CT was faintly expressed near *CXCR7* expression at
122 muscle tips (Fig. 1h, i) but surprisingly, a strict correlation between ligand and receptor
123 expression was not observed. At E10, *CXCR7* expression at the tips of myotubes (Fig. 1o, q)
124 was observed facing the MTJ revealed by the tendon marker *SCLERAXIS (SCX)*, (Fig. 1r, s),
125 while *CXCL12* expression was detected in CT mostly around limb vessels (Fig. 1p, t),
126 corresponding to the vascular *CXCR4* expression (Supp. Fig. 1e, f; Nassari et al., 2017).
127 These results show that during foetal myogenesis, *CXCR7* is first expressed in a sub-
128 population of *PAX7*-positive progenitors, then at the tips of differentiated myotubes while
129 *CXCR4* is downregulated in muscle cells and expressed in limb vessels around which
130 *CXCL12* expression is mostly located.

131

132 **CXCR7 receptor promotes foetal myoblast fusion during chicken limb development**

133 In order to elucidate the role of *CXCR7* in foetal myogenesis, functional assays were
134 performed by overexpressing the wild-type form of the *CXCR7* receptor or a dominant-
135 negative form of the *CXCR7* receptor (*dnCXCR7*) in forelimbs of chicken embryos using the
136 avian RCAS retrovirus system. The *dnCXCR7* construct lacked the carboxy-terminus part of

137 the receptor, impairing *CXCR7* internalization and signalling, without affecting its binding to
138 CXCL12 ligand (Ray et al., 2013). Pellets of RCAS-*CXCR7* or RCAS-*dnCXCR7* producing
139 chicken fibroblasts were grafted into E4 limbs to allow virus spread into dividing cells and
140 induce gene overexpression (Fig. 2A; (Havis et al., 2016)). Embryos were collected 6 days
141 later and muscles from grafted and control limbs were analyzed by performing 3D-
142 reconstruction of MF20 whole-mount immunostaining. Overexpression of RCAS-*dnCXCR7*
143 and RCAS-*CXCR7* both resulted in changes in muscle morphogenesis when compared to
144 controls (Fig. 2Ba-d, Ca-d). Volume and length analyses in 3D-reconstructed muscles showed
145 that most muscles expressing *dnCXCR7* exhibited a reduced volume and length while muscles
146 overexpressing *CXCR7* have mostly an increased volume and length (Fig. 2Be, f, Ce, f).
147 To understand these phenotypes, infected and control wings were transversally sectioned and
148 immunostained with PAX7, MYOG and MF20 antibodies. Analysis of sections showed that
149 muscles overexpressing the *dn-CXCR7* form appeared smaller (Fig. 3c) when compared to
150 controls (Fig. 3a), a phenotype confirmed by area measurements of the cross-sections of each
151 muscle in the infected region (Fig. 3d). Comparison of transverse sections taken at the same
152 longitudinal level in individual muscles (Fig. 3e) showed that *dn-CXCR7* expressing muscles
153 were not only smaller when compared to controls but also shorter, as illustrated by the strong
154 reduction of muscle surface at the tips compared to the bulk (50% versus 30% in the EMU
155 muscle, Fig. 3f). Quantification of muscle cell proliferation, number of PAX7- and MYOG-
156 positive cells on sections revealed no differences between *dnCXCR7*-expressing and control
157 muscles (Supp. Fig. 2A, Fig. 3g), showing that muscle differentiation was not affected by
158 *CXCR7* loss-of-function. However, *dnCXCR7* overexpression induced a significant decrease
159 in the percentage of MYOG-positive myonuclei into myotubes when compared to control
160 (Fig. 3h, i), showing that myoblast fusion was affected. This effect was confirmed by the
161 quantification of the myofiber nuclei versus the total number of nuclei, which showed that the
162 myoblast fusion index was decreased in *dnCXCR7* expressing muscles when compared to
163 controls (Fig. 3j, k). These data showed that inhibition of *CXCR7* receptor in foetal muscles
164 decreases myoblast fusion during chicken limb development.
165 Conversely, most muscles overexpressing *CXCR7* appeared larger (Fig. 4b, c) when
166 compared to control muscles (Fig. 4a), as assessed by the larger muscle areas observed in
167 *CXCR7* expressing muscles when compared to controls (Fig. 4d). Comparison of transverse
168 sections taken at the same longitudinal level (Fig. 4e) showed that the increase in muscle
169 surface in *CXCR7* expressing EDC muscle was stronger at the tips compared to the bulk
170 (150% versus 120%, Fig. 4f), suggesting that infected muscles were not only bigger but also

171 longer. Quantification of muscle cell proliferation, number of PAX7-positive and MYOG-
172 positive cells on sections revealed no differences between *CXCR7*-expressing and control
173 muscles (Supp. Fig. 2B, Fig. 4g), showing that muscle differentiation was not affected by
174 *CXCR7* gain-of-function. *CXCR7* overexpression induced a significant increase in the
175 percentage of MYOG-positive myonuclei into myotubes when compared to control (Fig. 3h,
176 i), showing that myoblast fusion was increased. This effect was confirmed by the
177 quantification of myoblast fusion index, which was increased in *CXCR7* expressing muscles
178 when compared to controls (Fig. 4j, k). Taken together, these results show that *CXCR7*
179 receptor is required for the correct myoblast fusion process in limbs during chicken foetal
180 development.

181

182 ***CXCR7* receptor promotes myoblast fusion in myoblast cultures**

183 To understand *CXCR7* function on myoblast fusion, we turned to *in vitro* experiments using
184 primary cell cultures of foetal myoblasts derived from forelimbs of E10 chicken embryos.
185 After 5 days of culture, *in situ* hybridization and immunostaining for *CXCR7*, PAX7 and
186 MF20 revealed that *CXCR7* mRNA and protein were observed in some but not all PAX7
187 progenitors (Supp. Fig. 3Aa-c), as observed *in vivo*. In myotubes, *CXCR7* was strongly
188 expressed around most myonuclei but not all and did not display a restricted expression at the
189 tips, as observed in chicken limb muscles (Supp. Fig. 3Ad-f; Fig. 1).

190 Foetal myoblasts were transfected with RCAS-*dnCXCR7*, RCAS-*CXCR7* or empty RCAS as
191 a control and grown *in vitro* for 2 days in proliferation medium and 3 days in differentiation
192 conditions. Analysis of the number of EDU-positive cells after 2 days of culture demonstrated
193 that proliferation was not modified in primary cultures or in the PAX7-positive progenitors in
194 *dnCXCR7*-expressing cells compared to controls (Supp. Fig. 3Ba). Consistently, no change in
195 the number of PAX7- (Supp. Fig. 3Bb) and MYOG-positive cells (Fig. 5Ad-f) was observed
196 in these conditions, showing that muscle cell specification and differentiation were not
197 affected. However, *dnCXCR7* overexpression in foetal myoblasts *in vitro* led to a decrease in
198 the number of myotubes (Fig. 5Aa-c) and in the number of MYOG-positive nuclei into the
199 myotubes when compared to controls (Fig. 5Ad, e, g), resulting in a decrease in the myoblast
200 fusion index (Fig. 5Ah) and in the number of nuclei per myotube (Fig. 5Ai). Interestingly,
201 *dnCXCR7* overexpression did not modify the expression of the specific muscle fusogene
202 *TMEM8C* (Fig. 5Aj). These results show that *CXCR7* inactivation in chicken foetal myoblast
203 cultures reduced myoblast fusion without affecting muscle differentiation and *TMEM8C*
204 expression.

205 Conversely, *CXCR7* overexpression in chick foetal myoblasts *in vitro* resulted in an increase
206 in the number of myotubes (Fig. 5Ba-c). Cell proliferation was not affected in the whole
207 culture and in PAX7-positive progenitors (Supp. Fig. 3Ca). No change in the number of
208 PAX7- (Supp. Fig. 3Cb) and MYOG- positive cells (Fig. 5Bd-f) was observed but *CXCR7*
209 overexpression in foetal myoblasts induced a significant increase in the number of MYOG-
210 positive nuclei into the myotubes (Fig. 5Bd, e, g), leading to an increase in myoblast fusion
211 index (Fig. 5Bh) and in the number of nuclei per myotube (Fig. 5Bi). Conversely, *CXCR7*
212 overexpression did not modify *TMEM8C* expression (Fig. 5Bj). These results show that
213 overexpression of *CXCR7* in chicken limbs increased myoblast fusion *in vitro* without
214 affecting differentiation and *TMEM8C* expression.

215 Because myoblast fusion could be affected by the ability of foetal myoblasts to migrate, we
216 tested the migration capacity of myoblasts under *CXCR7* loss- or gain-of-function conditions.
217 *DnCXCR7*- or *CXCR7*-transfected foetal myoblasts were cultured in proliferation conditions
218 until confluence and cells were scratched from the plate to create a wound. The ability of
219 cultured cells and PAX7-positive cells to refill the gap was monitored after 48h of culture and
220 showed that neither *CXCR7* loss- or gain-of-function modified the ability of PAX7-positive
221 muscle progenitors to migrate (Supp. Fig. 4), demonstrating that the *CXCR7* effect on
222 myoblast fusion did not result from changes in the migration capacity of muscle progenitors.

223

224 ***CXCR7* promotes myoblast fusion independently of the muscle specific *TMEM8C*** 225 **fusogene**

226 We observed that *CXCR7* gain- and loss-of-function in myoblast cultures did not affect the
227 expression of the muscle specific *TMEM8C* fusogene (Fig. 5). In order to define whether the
228 *CXCR7* fusion-promoting effect was linked to *TMEM8C*, we analyzed the expression
229 patterns of *CXCR7* and *TMEM8C* during foetal myogenesis. Indeed, we have recently shown
230 that during chicken foetal myogenesis, *TMEM8C* transcripts are preferentially located in
231 central regions of chicken limb foetal muscles and excluded from muscle tips (Esteves de
232 Lima et al., 2022) where *CXCR7* transcripts are expressed (Fig. 1). Detection of both
233 transcripts on transverse sections of E5, E6 and E10 limbs showed that *TMEM8C* and *CXCR7*
234 expression did not overlapped in muscle cells (Fig. 6Aa-i). We then analyzed the
235 consequences of *CXCR7* gain- and loss-of-function on *TMEM8C* expression in chicken limb
236 muscles and found that *TMEM8C* expression was not affected by deregulation of *CXCR7*
237 signalling (Fig. 6Ba-c), as observed in myoblast cultures (Fig. 5). To confirm that the *CXCR7*
238 fusion effect was acting independently of *TMEM8C*, we blocked the *TMEM8C*-dependent

239 fusion by transfecting a specific chick *siTMEM8C* in myoblast cultures (Luo et al., 2015) and
240 tested the consequences of CXCR7 gain- and loss-of-function in these conditions. Cells
241 transfected with *siTMEM8C* showed a 50% reduction in the fusion index when compared to
242 controls (Fig. 6Ca, b), as previously described (Luo et al., 2015). Conversely, when myoblasts
243 were transfected simultaneously with *siTMEM8C* and *CXCR7*, they displayed a fusion index
244 nearly equivalent to control cultures, showing that the CXCR7 fusion effect can occur in the
245 absence of TMEM8C (Fig. 6Ca, b). In addition, myoblast cultures simultaneously transfected
246 with *siTMEM8C* and *dn-CXCR7* exhibited a 10% lower fusion index than the one observed in
247 myoblasts only transfected with *siTMEM8C* (Fig. 6Ca, b), showing that the loss-of-function
248 of CXCR7 can affect myoblast fusion even in the absence of TMEM8C. The non-overlapping
249 expression pattern of *CXCR7* and *TMEM8C* in foetal muscles, the unchanged *TMEM8C*
250 expression levels in *CXCR7* functional experiments and the observations that *CXCR7* had an
251 effect on myoblast fusion even in the absence of functional *TMEM8C* led us to conclude that
252 the fusion promoting effect of *CXCR7* is independent of *TMEM8C*.

253

254 **EGF receptor is required for the CXCR7 fusion promoting effect in myoblast culture:**

255 CXCR7 receptor can act to regulate proliferation and migration processes or as a ligand
256 scavenger (Koch and Engele, 2020), but to date, it has not been described as a fusogen protein
257 and our results show that its fusion effect is independent of TMEM8C. As it has been shown
258 that CXCR7 can physically interact with EGFR (Singh and Lokeshwar, 2011) and that EGFR
259 phosphorylation is increased during myogenesis (Horikawa et al., 1999) and required for
260 myoblast differentiation (Santos-Zas et al., 2016), we investigated whether EGFR could be
261 involved in the CXCR7 effect on myoblast fusion. We first analyzed the expression pattern of
262 the phosphorylated form of EGFR (pEGFR) during chicken limb foetal myogenesis. At E6,
263 pEGFR was not expressed in muscle cells (Fig. 7Aa), while from E8, nuclear pEGFR was
264 observed in most muscle cells (Fig. 7Ab, c, d). At this stage, most myonuclei expressing
265 CXCR7 at the tips of myotubes exhibited a nuclear expression of pEGFR (Fig. 7Ae).
266 Analysis of pEGFR staining in myoblast cultures showed a cytoplasmic expression in PAX7-
267 positive cells (Fig. 7Ba) while a nuclear expression was observed in myotubes (Fig. 7Bb). To
268 test whether CXCR7 acts through EGFR signalling to promote myoblast fusion, we blocked
269 the tyrosine kinase activity of EGFR in CXCR7 gain-of-function experiments in myoblast
270 cultures. Foetal myoblasts were transfected with RCAS-*CXCR7* and treated with PD153035
271 reagent which inhibits the EGFR tyrosine kinase activity by acting as a selective ATP
272 competitive inhibitor of phosphorylation (Fry et al., 1994). The decrease of pEGFR

273 expression in PD153035-treated cultures was a witness of the efficacy of the inhibitor.
274 PD153035-treated cultures showed a decrease in foetal myoblast fusion (Fig. 7Cd-f, m) when
275 compared to controls (Fig. 7Ca-c, m). As described above, *CXCR7* overexpression in cultured
276 chicken foetal myoblasts led to a significant increase in the number of myotubes and in
277 myoblast fusion index (Fig. 7Cg-i, m). However, this effect was not observed when *CXCR7*
278 transfected cultures were treated with PD153035 (Fig. 7Cj-l). Indeed, such cultures exhibited
279 a myoblast fusion index nearly similar to PD153035-treated cultures (Fig. 7Cm). These data
280 show that EGFR is required for the fusion promoting effect of *CXCR7* in chicken foetal
281 myoblasts.

282

283 **CXCL12 overexpression increases myoblast fusion in chicken limb muscles, while** 284 **having no effect on myoblast cultures.**

285 The *CXCL12* ligand of *CXCR7* receptor is expressed in CT surrounding muscles during limb
286 development (Fig. 1; García-Andrés and Torres, 2010; Nassari et al., 2017; Vasyutina et al.,
287 2005). Consequently, we tested whether *CXCL12* coming from the CT could be involved in
288 the effect of *CXCR7* on foetal myoblast fusion of chicken limbs. A stable vector containing
289 the chicken *CXCL12* sequence and the TOMATO gene as a reporter (Nassari et al., 2017) was
290 electroporated in the forelimb lateral plate to overexpress *CXCL12* specifically in limb CT.
291 Electroporation was performed at E2.5 and electroporated and contralateral limbs were
292 analyzed at E9 (Fig. 8Aa, b) by immunostaining on sections with PAX7, MYOG and MF20
293 antibodies. *CXCL12* overexpression was assessed by TOMATO fluorescence (Fig. 8Ab, Bb,
294 e). Analysis of sections showed that muscles surrounded by *CXCL12* overexpression in the
295 CT appeared larger (Fig. 8Bb, e) when compared to controls (Fig. 8Ba, e), as shown by area
296 measurements of each muscle in the electroporated region, which were increased when
297 compared to controls (Fig. 8Bc). Comparison of transverse sections taken at the same
298 longitudinal level (Fig. 8Bd) showed that the increase in muscle surface in EDC muscle
299 surrounded by *CXCL12* expression was stronger at the tips compared to the bulk (150%
300 versus 130%, Fig. 8f), suggesting that muscles in the electroporated region were not only
301 larger but also longer, as observed for *CXCR7*-overexpressing limb muscles (Fig. 4).
302 Quantification of muscle cell proliferation, number of PAX7- (Sup. Fig. 2C) and MYOG-
303 positive cells (Fig. 8Bf) revealed no differences between muscles in control and
304 electroporated forelimbs. *CXCL12* overexpression in CT induced a significant increase in the
305 number of MYOG-positive nuclei inside the myotubes (Fig. 8Bg, h) and in the myoblast
306 fusion index (Fig. 8Bi, j) when compared to controls. In addition, *CXCL12* overexpression in

307 CT did not modify the expression of the fusion gene *TMEM8C* (Fig. 8C). These results show
308 that *CXCL12* overexpression in limb CT increased foetal myoblast fusion.

309 Because *CXCL12* expressed in limb CT mimicked the fusion effect of *CXCR7* in chicken
310 limb muscles (Fig. 4), we tested whether this effect was observed in myoblast cultures.
311 Primary cultures of chicken embryonic fibroblasts were transfected with *RCAS-CXCL12*,
312 cultured for 2 days and the culture medium was collected to prepare *CXCL12*-concentrated
313 supernatant, which was added to foetal myoblast culture medium. In some experiments,
314 *CXCL12* recombinant protein was added to foetal myoblasts grown in N2 minimum medium
315 without serum to avoid a possible effect of *CXCL12* contained in the culture serum. In both
316 cases, primary muscle cells were cultured for 2 days in proliferation medium and 3 days in
317 differentiation conditions (Sup. Fig. 5A). Surprisingly, MF20 immunostaining did not
318 revealed differences in the number of myotubes in the presence of *CXCL12*, compared to
319 controls (Sup. Fig. 5Ba-d). Cell proliferation (Sup. Fig. 5Be, f), number of PAX7-positive
320 cells (Sup. Fig. 5Bg) and myoblast fusion (Sup. Fig. 5Bh, i) were not modified either by the
321 addition of *CXCL12* supernatant or *CXCL12* recombinant protein. These results show that an
322 exogenous source of *CXCL12* does not regulate foetal myoblast fusion in cultures, suggesting
323 that *CXCL12* does not act directly via binding to *CXCR7* receptor to activate myoblast fusion
324 in chicken limb muscles.

325

326 **Fusion effect of *CXCR7* is promoted by a *CXCL12*-dependent activation of $\beta 1$ integrins**

327 We have previously shown that *CXCL12* induces the expression of CT markers and
328 extracellular matrix genes in chicken embryonic limb, among which collagens (Nassari et al.,
329 2017; Vallecillo-García et al., 2017). Collagens bind to integrin receptors that are required for
330 the formation of MTJs (Martin-Bermudo, 2000; Mayer et al., 1997; Valdivia et al., 2017) and
331 $\beta 1$ integrins are recognized as regulators of myoblast fusion (Schwander et al., 2003).
332 Moreover, $\beta 1$ integrin activation consecutive to stromal extracellular matrix remodelling has
333 been shown to increase *CXCR7* expression in tumor cells (Kiss et al., 2013; Windus et al.,
334 2014).

335 To analyze the effect of *CXCL12* on $\beta 1$ integrin activation, *CXCL12* was overexpressed in
336 limb CT by electroporation of the forelimb lateral plate and sections of control and
337 experimental limbs were immunostained with TASC, an antibody that specifically recognized
338 the activated form of $\beta 1$ integrin (Neugebauer and Rekhart, 1991). As expected, activated
339 $\beta 1$ integrins are expressed at the MTJ in control limbs (Fig. 9Ab, f). *CXCL12* overexpression
340 in limb CT induced a significant enlargement of $\beta 1$ integrin activation at the level of the MTJ

341 (Fig. 9Ad, g) where Tomato, reflecting ectopic CXCL12, was observed (Fig. 9Ac, h).
342 β 1integrin activation was also enhanced around the cartilage elements in Tomato-positive
343 regions in electroporated limbs compared to controls (Fig. 9Ad, g). In addition, an
344 enlargement of CXCR7 expression was observed nearby the activated β 1integrin at the MTJ
345 in muscles surrounded by CXCL12 overexpression in electroporated limbs (Fig. 9Aj, k) when
346 compared to controls (Fig. 9Ai). These results show that CXCL12 overexpression in CT
347 enhances β 1integrin activation at MTJ and CXCR7 expression in muscle.

348 To investigate the effect of β 1integrin activation and CXCR7 expression on myoblast fusion,
349 we first stimulated integrin activation with phorbol-myristate-acetate (PMA) or Mn^{2+}
350 treatments in myoblast cultures. Both treatments are known to activate integrins with Mn^{2+}
351 changing the external conformation of integrins in the absence of a bound ligand (Outside-in
352 mechanism) while PMA, by PKC stimulation, allows Talin to activate the cytoplasmic tail of
353 integrins (Inside-out mechanism), (Ye et al., 2012). Foetal myoblasts were submitted to PMA
354 or Mn^{2+} treatments for 24 hours and 3 hours respectively. As expected, TASC
355 immunostaining revealed a strong integrin activation in enlarged myotubes of PMA treated
356 cultures (Fig. 9Bf) and an increase in CXCR7 expression (Fig. 9Bj) when compared to
357 controls (Fig. 9Be, i). Integrin activation induced by PMA treatment led to a massive
358 myoblast fusion (Fig. 9Bb-j), illustrated by an increase of the fusion index when compared to
359 controls (Fig. 9Bk), with large myotubes containing many grouped myonuclei (Fig. 9Bb)
360 compared to the well-aligned myonuclei in control myotubes (Fig. 9Ba). Integrin activation
361 by Mn^{2+} treatment in cultures also increased myoblast fusion and CXCR7 expression (Supp.
362 Fig. 6a-d). We conclude that β 1integrin activation increases CXCR7 expression and activates
363 myoblast fusion.

364 To determine if integrin activation induced myoblast fusion via CXCR7, we blocked CXCR7
365 function in integrin-activated myoblast cultures. Foetal myoblasts were transfected with
366 RCAS-*dnCXCR7* and treated with PMA or Mn^{2+} to activate integrins. We found that the
367 fusion phenotype induced by integrin activation (Fig. 9Bb, f, j, k) was not observed in PMA-
368 treated cultures transfected with *dnCXCR7* (Fig. 9Bd, h, k), which exhibited a fusion index
369 similar to that of *dnCXCR7*-transfected cultures (Fig. 9Bk), which, as expected, presented a
370 reduced myoblast fusion (Fig. 9Bc, g, k). Similar results were obtained with Mn^{2+} treatment
371 on *dnCXCR7*-transfected cultures (Supp. Fig. 6e, f). These results demonstrate that the
372 blockade of CXCR7 function prevented the fusion effect of integrin activation.

373 Taken together, our data show that CXCL12, expressed in limb CT and known to promote CT
374 markers and extracellular matrix components (Nassari et al., 2017), increases β 1integrin

375 activation at the MTJ and CXCR7 expression at muscle tips, which in turn, promotes
376 myoblast fusion. Finally, we analyzed whether the addition of CXCL12 on myoblast cultures,
377 which does not promote myoblast fusion, has an effect on β 1 integrin activation and CXCR7
378 expression and showed that adding CXCL12 on foetal myoblasts *in vitro* did not change
379 β 1 integrin activation and CXCR7 expression (Supp. Fig. 7), confirming that CXCL12 does
380 not act directly via binding to CXCR7 receptor to activate myoblast fusion in chicken limb
381 muscles.

382

383 **Discussion**

384

385 In this study, we show that *CXCR7*, a CXCL12 chemokine receptor, exhibits a restricted
386 expression at the tips of muscle fibers, exclusive from the central location of *TMEM8C* during
387 chicken limb foetal myogenesis. *CXCR7* promotes myoblast fusion independently of the
388 muscle specific *TMEM8C* fusion gene, by cooperating with EGFR signalling. We also show
389 that CXCL12 ligand mimics indirectly the *CXCR7* fusion effect, by increasing β 1 integrin
390 activation at the MTJ and CXCR7 expression at muscle tips.

391

392 **CXCR7 function on myoblast fusion is not related to those of other myofiber tips** 393 **markers**

394 *CXCR7* displays a regionalized expression at muscle tips close to tendon in limb foetal
395 muscles. The molecular specificity of muscle tips facing the tendon has been first highlighted
396 by the localized expression of signalling pathways during development of this region in
397 chicken. FGF and BMP pathways have been shown to be restricted at the tips of foetal
398 myofibers (Edom-Vovard et al., 2001; Wang et al., 2010). However, the deregulation of FGF
399 and BMP pathways in chicken limbs leads to different phenotypes to that of *CXCR7* (Figs. 2,
400 3). FGF4 has been shown to promote the formation of tendon CT at the expense of myogenic
401 cells (Edom-Vovard et al., 2001); (Edom-Vovard et al., 2002), while BMP increases the
402 number of muscle progenitors at the expense of muscle CT in chicken limbs during foetal
403 development (Esteves de Lima et al., 2021). The fusion phenotype of *CXCR7* in myoblast
404 cultures (Fig. 4) is also not mimicked by BMP and FGF, since both factors are recognized as
405 potent inhibitors of muscle differentiation *in vitro* (Lathrop et al., 1985; Ono et al., 2011;
406 Pizette et al., 1996). These phenotypes in chicken limb muscles and myoblast cultures lead us
407 to conclude that FGF and BMP signalling are not directly involved in the *CXCR7*-promoting
408 fusion effect at muscle tips. LoxL3 (Lysyl-oxidase-Like-3) enzyme, known to remodel the

409 extracellular matrix, has also been shown to be specifically localized at the myofiber tips and
410 to regulate integrin-mediated signalling at muscle attachment sites (Kraft-Sheleg et al., 2016)
411 but *LoxL3* mutant mouse displays abnormal myofiber anchorage at the MTJ (Kraft-Sheleg et
412 al., 2016), a phenotype not related to myoblast fusion. Finally, analysis of single-nucleus
413 RNA-sequencing data in post-natal and adult muscles have identified a specific
414 transcriptional signature in myonuclei at the MTJ compartment (Chemello et al., 2020; Dos
415 Santos et al., 2020; Kim et al., 2020; Petraný et al., 2020). However, *CXCR7* has not been
416 pinpointed in these data, underlying the possibility that it is only required for myoblast fusion
417 during the establishment of the MTJ and down-regulated at post-natal stages.

418

419 **A *CXCR7*/EGFR-dependent fusion mechanism occurs at the tips of foetal muscles**

420 We show that *CXCR7* effect on myoblast fusion requires EGFR signaling in cell cultures.
421 The requirement of EGFR signalling for *CXCR7* function has been already shown in normal
422 and tumor prostate epithelial cells, in which *CXCR7* induces EGFR
423 phosphorylation/activation in a *CXCR7* ligand-independent fashion, through a physical
424 association of *CXCR7* with EGFR that is regulated by β -arrestin (Kallifatidis et al., 2016;
425 Salazar et al., 2014; Singh and Lokeshwar, 2011). Interestingly, both *Cxcr7* and *Egfr* genes
426 were identified in a siRNA screen performed on mouse myogenic C2C12 cells to determine
427 genes involved in myoblast fusion (Melendez et al., 2021). In human muscle cell cultures, it
428 has been shown that EGFR activity is down-regulated during myogenesis and that this event
429 is required for muscle differentiation (Leroy et al., 2013). However, EGFR phosphorylation is
430 increased during muscle differentiation in C2C12 muscle cell line (Horikawa et al., 1999) and
431 EGFR activation rescues regeneration defects in dystrophic muscle (Wang et al., 2019). In
432 addition, β -arrestin has been shown to be essential in human myoblasts for cell cycle exit and
433 myoblast fusion through EGFR activation (Santos-Zas et al., 2016) and β -arrestin is a well-
434 known *CXCR7* intracellular relay (Rajagopal et al., 2010). Interestingly, *CXCR7* has been
435 shown to activate EGFR independently of both *CXCR7* and EGFR ligands (Kallifatidis et al.,
436 2016; Moro et al., 2002; Singh and Lokeshwar, 2011), suggesting that *CXCR7*/EGFR-
437 dependent myoblast fusion would operate independently of direct binding of ligands.

438

439 **CXCL12-dependent ECM-integrin interactions are involved in myoblast fusion via** 440 ***CXCR7***

441 We found that *CXCL12* overexpression in CT mimics the fusion phenotype observed with the
442 gain-of-function of *CXCR7* receptor in limb muscles *in vivo*. In addition, *CXCL12*

443 overexpression in CT enhances β 1 integrin activation at the MTJ and CXCR7 expression at
444 muscle tips (Fig. 9). As addition of CXCL12 to cultures of foetal myoblasts expressing
445 CXCR7 has no effect on myoblast fusion and no effect on β 1 integrin activation and CXCR7
446 expression, we suggest that the CXCL12-dependent fusion effect does not result from a direct
447 binding of CXCL12 to CXCR7 but from the positive action of CXCL12 on extracellular
448 matrix which would enhance β 1 integrin activation at the MTJ and increase CXCR7
449 expression at muscle tips, resulting in myoblast fusion. Consistent with this hypothesis are our
450 data showing that CXCL12 transcripts in chicken limb are strongly expressed around
451 CXCR4-expressing vessels and faintly near MTJs where CXCR7 expression is observed (Fig.
452 1). We also previously showed that CXCL12 overexpression in limb CT promotes the
453 expression of CT markers and extracellular matrix genes by altering vascular network via
454 binding to CXCR4 and not to CXCR7 (Nassari et al., 2017). In addition, CXCR7 expression
455 is closely associated with muscle tips at the MTJ, where integrins are specifically enriched
456 and have been shown to participate in myoblast fusion both *in vitro* and *in vivo* (McClure et
457 al., 2019; Quach et al., 2009; Schwander et al., 2003). We show that myoblast fusion induced
458 by β 1 integrin activation in cultures requires CXCR7 (Fig. 6), putting CXCR7 downstream of
459 β 1 integrin activation at MTJ. Finally, EGFR signalling can be transactivated by integrins
460 independently of EGFR ligand (Moro et al., 2002), leading to the possible scenario that
461 β 1 integrins could induce CXCR7/EGFR transactivation and myoblast fusion independently of
462 a direct binding of CXCR7 to CXCL12 ligand. However, the question remains why CXCL12
463 would be unable to bind CXCR7. One possibility could be that most CXCL12 would be
464 trapped by the neighboring CXCR4-expressing endothelial cells. Alternatively, it has been
465 shown that glycosaminoglycans are crucial partners in CXCL12 presentation to CXCR4
466 receptor (Panitz et al., 2016), underlying the possibility that CXCL12 in limb mesenchyme
467 interacts with proteoglycans, favoring its binding to CXCR4 at the expense of CXCR7.

468

469 **A TMEM8C-independent/CXCR7-dependent fusion mechanism occurs at the tips of** 470 **foetal muscles**

471 Numerous transmembrane proteins have been involved in myoblast fusion (Demonbreun et
472 al., 2015) but to date, only MYOMAKER (TMEM8C in chicken) has been described to be
473 muscle specific during developmental and regenerative myogenesis (Petrany and Millay,
474 2019). We identified CXCR7 as a novel transmembrane protein involved in foetal myoblast
475 fusion in chicken limbs and myoblast cultures (Figs. 3, 4, 6). However, the expression of
476 CXCR7 transcripts at muscle tips is exclusive to that of *TMEM8C* mainly located centrally

477 (Fig. 4, Esteves de Lima et al., 2022). The mutually exclusive expression of *CXCR7* and
478 *TMEM8C* transcripts and the fact that *TMEM8C* expression is not modified in *CXCR7* gain-
479 and loss-of -function experiments in chicken limbs and myoblast cultures (Figs. 3, 4, 6)
480 indicate that myoblast fusion occurs at muscle tips independently of *TMEM8C*. This is
481 confirmed by the results showing that *CXCR7* can modulate myoblast fusion *in vitro* in the
482 absence of functional *TMEM8C*. Although MYOMAKER is a critical factor for myoblast
483 fusion and muscle formation (Millay, 2022), some MYOMAKER-independent fusion
484 pathways have been already reported. Complete defective myoblast fusion is not observed in
485 *Myomaker* mutant in fish (Shi et al., 2018) and inactivation of *Myomaker* in fibroblast-derived
486 myonuclei at the MTJ has no effect on their fusion capacity (Yaseen et al., 2021). Similarly,
487 fusion index is increased in co-cultures of human tenocytes and myoblasts without any
488 change in *Myomaker* expression (Tsuchiya et al., 2022). These data support our observations
489 that other factors can control the fusion mechanisms at the muscle tips. Our observations
490 suggest two different types of fusion within foetal muscles, one located at myofiber tips and
491 the other one in central regions of muscles. The reasons for these two fusion mechanisms
492 remain elusive, but one can speculate that it is related to the spindle shape of the growing
493 muscle, with a large diameter in the central region compared to the tips. *TMEM8C*-dependent
494 fusion in the muscle bulk would support growth in diameter while *CXCR7*-dependent fusion
495 at the tips would support growth in length or elongation. Consistent with this hypothesis are
496 the observations that *CXCR7* misregulation has an effect on myofiber length (Figs. 2, 3, 4, 8)
497 and that reducing the number of myonuclei by MYOMAKER inactivation in satellite cells
498 during post-natal muscle growth in mice leads mostly to the reduction in muscle diameter and
499 volume rather than myofiber length (Cramer et al., 2020).

500

501 In summary, we propose a model in which β 1 integrin activation at the MTJ would lead to the
502 interaction of *CXCR7* receptor with the EGF receptor to promote myoblast fusion specifically
503 at the tips of muscle fibers and independently of the muscle-specific fusion gene *TMEM8C*
504 (Fig. 10). *CXCL12*, expressed in CT, would participate indirectly to this process by regulating
505 extracellular matrix genes and β 1 integrin activation at the MTJ. This molecular network
506 would enable muscle growth and elongation at the tendon/muscle interface.

507

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511

512 **Competing interests**

513 The authors declare no competing or financial interests.

514

515 **Material and methods**

516

517 **Chick embryos**

518 Fertilized chick eggs from commercial sources (JA 57 strain, Institut de Sélection Animale,
519 Lyon, France, and White Leghorn, HAAS, Strasbourg) were incubated at 38°C in a
520 humidified incubator until appropriate stages. Embryos were staged according to the number
521 of days in ovo (E).

522

523 **Constructs**

524 The chicken *CXCL12*, *dnCXCR7* and *CXCR7* coding regions were amplified by PCR from a
525 RT-PCR-derived cDNA library made from E5 chick limb, using primers containing the ClaI
526 enzyme restriction site. The *dnCXCR7* coding region is a truncated form of *CXCR7* lacking
527 the C-terminal part of the sequence ((Ray et al., 2013)). Amplified *dnCXCR7* and *CXCR7*
528 sequences were inserted into pCR-II TOPO vector using TOPO-TA cloning kit (Invitrogen)
529 or pGEM vector using pGEM-T easy vector system kit (Promega). Inserted sequences were
530 excised by digestion with ClaI and inserted into the ClaI site of the replication-competent
531 retroviral vector RCASBP(A), ((Hughes et al., 1987)), previously digested with ClaI enzyme.
532 Clones containing the *dnCXCR7* or *CXCR7* coding regions in the sense orientation were
533 selected. For pT2AL-CMV/Tomato-T2A-CXCL12 construct, the chicken *CXCL12* coding
534 sequence was amplified by PCR from a RT-PCR-derived cDNA library made from E5 chick
535 limb, using a forward primer containing the BstbI enzyme restriction site and a reverse primer
536 comprising the PmlI enzyme restriction site. Purified PCR products were included into the
537 PCR-II TOPO vector using TOPO-TA cloning kit (Invitrogen), and clones containing
538 *CXCL12* sequence with BstbI and PmlI restriction sites respectively in 5' and 3' ends of the
539 coding sequence were selected. The TOPO/BstbI-CXCL12-PmlI and TOPO/BstbI-CXCL14-
540 PmlI were then digested with BstbI and PmlI enzymes. Purified digested products were
541 finally inserted into the PT2AL-CMV/Tomato-T2A-GFP plasmid, from which GFP was
542 previously extracted by BstbI and PmlI enzymatic digestion and clones containing *CXCL12*
543 coding sequence were selected.

544

545 **Primary muscle cell cultures**

546 Forelimbs of E10 chicken embryos were used to establish primary myoblast cultures as
547 previously described ((Havis et al., 2012)). Limb muscles were cut in small pieces in Minimal
548 Essential Medium (MEM) followed by mechanical dissociation. Homogenate was then
549 centrifuged and the supernatant was filtered into a 40µm filter to collect muscle cells.
550 Centrifugation and filtration steps were repeated several times. Chicken myoblasts were
551 plated on a 0,1% gelatin coated plastic dish in MEM complemented with 10% of foetal calf
552 serum for proliferation conditions during 48 hours. For differentiation conditions, MEM was
553 complemented with 2% of foetal calf serum and cells cultured for 3 or 5 more days. Primary
554 myoblasts were transfected at around 30-40% confluence with the Calcium Phosphate
555 transfection kit (Invitrogen). To control the effect of CXCL12 supernatant collected from
556 infected chicken fibroblasts in vitro, its chemotactic effect was tested on chicken fibroblast
557 cultures in a Boyden chamber. To confirm the effect of CXCL12 supernatant, some cultures
558 were conducted in N2 minimum medium without serum (R&D System) and recombinant
559 CXCL12 protein (R&D System) was added at 50ng/ml. For pharmacological experiments,
560 primary myoblasts were transfected at around 30-40% confluence, cultured in proliferation
561 medium until confluence and treated with pharmacological agents after being transferred into
562 differentiation medium. For experiments testing β 1 integrin activation, PMA (Sigma Merck,
563 5µg/ml) or Manganese Chloride (Sigma Merck, 2mM) was added to proliferating myoblasts
564 for 24 hours or 3 hours respectively. For experiments testing the implication of the
565 phosphorylated form of EGF receptor, PD15035 inhibitor (Sigma Merck, 6µM) was added to
566 proliferating myoblasts for 1 hour.

567

568 **Myoblast migration assay**

569 Migration of myoblasts was analysed using scratch wound healing assay. Briefly, control and
570 transfected cells were plated in the culture dish without gelatin and cultured until they reached
571 90% of confluency. Cells were scratched from the plate using a plastic tip to create the
572 wound. The wound healing manifested by the ability of the cells to refill the created gap was
573 monitored after 48h of culture.

574

575 **Production and grafting of recombinant RCAS-expressing cells**

576 Primary chicken fibroblasts were transfected with RCAS-*CXCR7*, RCAS-*dnCXCR7* or empty
577 RCAS as a control, using Calcium Phosphate transfection kit (InVitrogen) and grown for one
578 week. One day before grafting, transfected fibroblasts were plated into bacteria plastic dishes

579 in order to induce cell aggregate formation. Pellets of approximately 50 μm in diameter were
580 grafted into the right wing bud of E4 chick embryos. Embryos were harvested at various times
581 after grafting and processed for whole mount or section staining or RT-q-PCR. The left wing
582 was used as an internal control. Owing to certain variability in virus spread among embryos,
583 the ectopic gene expression was systematically checked by in situ hybridization.

584

585 **Lateral plate mesoderm electroporation**

586 E2.5 chicken embryos were electroporated as previously described ((Bourgeois et al., 2015)).
587 PT2AL-CMV CXCL12 (1.5-2 $\mu\text{g}/\mu\text{l}$) construct was mixed with the transposase vector CMV-
588 T2TP (molar ratio 1/3) to allow stable integration of genes in the chicken genome, in a
589 solution containing 0.33% carboxymethyl cellulose, 1% Fast green, 1mM MgCl_2 in PBS.
590 DNA mix was injected with a glass capillary in the coelomic cavity between somatopleural
591 and splanchnopleural mesoderm, at the level of the forelimb territory. Homemade platinum
592 electrodes were placed above and below the embryos, with the negative electrode inserted
593 into the yolk and the positive electrode localized above the presumptive forelimb region.
594 Electroporation was delivered using a Nepagene NEPA21 electroporator using the following
595 parameters: 2 pulses of 70V, 1ms duration with 100 ms interpulse interval followed by 5
596 pulses of 40V, 2ms duration with 500 ms interpulse interval.

597

598 **In situ hybridization and immunostaining to tissue sections and cultures**

599 Forelimbs were fixed in a 4% paraformaldehyde solution in PBS, successively embedded in a
600 4% and 15% sucrose solution, and then frozen in chilled isopentane. Cryostat-cut sections of
601 12-20 μm were collected on Superfrost/Plus slides (CML, France). Immunostaining and in
602 situ hybridization were proceeded as previously described ((Escot et al., 2013)). For grafted
603 and electroporated embryos, the electroporated and control forelimbs from the same embryo
604 were embedded together in order to allow comparison. For in situ hybridization, the following
605 digoxigenin-labeled mRNA probes were used: chicken *CXCL12* ((Escot et al., 2013)),
606 chicken *CXCR7* ((Escot et al., 2013)). The chicken *TMEM8C* (*MYOMAKER*) probe was
607 produced from a RT-PCR-derived cDNA library made from chicken primary muscle cell
608 cultures. In some cases, fluorescent in situ hybridization was performed according to
609 (Wilmerding et al., 2022)). For immunostaining, the following primary antibodies were used:
610 anti-myosin MF20 (Developmental Studies Hybridoma Bank, non-diluted supernatant), anti-
611 CXCR4 (1:1000, (Escot et al., 2013)), anti-CXCR7 (1:200, Abcam), anti-MEP21 (1:200,
612 generous gift from T. Jaffredo), anti-PAX7 (1:200, Developmental Studies Hybridoma Bank),

613 anti-MYOGENIN (1:200, generous gift from C. Marcelle), anti-activated β 1 integrin TASC
614 (1:100, Millipore), anti-phospho EGFR Tyr-1068 (1:100, In Vitrogen). Proliferation analysis
615 (EdU) was performed using the Click-iT kit (Thermo Fisher Scientific, France) or the anti-
616 phospho-histone-3 antibody (1:1000, Upstate Biotechnology). Immunolabelings were
617 performed using secondary antibodies conjugated to Alexa Fluor 488 and 555 (InVitrogen).
618 Nuclei were stained using DAPI (1:1000, Sigma). Stained tissue sections and cultures were
619 observed with an inverted Leica microscope, images were collected with the Leica software
620 and processed using Adobe Photoshop software.

621

622 **3D reconstructions of muscles**

623 Grafted and control forelimbs were immunostained in toto for MF20. Whole-mount
624 immunostained forelimbs were imaged with a Zeiss biphoton microscope and generated files
625 were analysed with the IMARIS software (Bitplane) to perform 3D muscle reconstructions.
626 Reconstructed images were then processed using Adobe Photoshop software.

627

628 **RNA isolation, reverse transcription and quantitative real-time PCR**

629 Total RNAs were extracted from chick limbs or muscle cell cultures using the RNeasy mini
630 kit from Qiagen. 500ng to 1 μ g RNAs were reverse-transcribed using the High Capacity
631 Retrotranscription kit (Applied Biosystems). RT-qPCR was performed using SYBR Green
632 PCR Master Mix (Applied Biosystems). Primer sequences used for TMEMC8 were the
633 following: Forward 5'-TGGGGTGTCCCTGATGGC-3', Reverse 5'-
634 CCCGATGGGTCCTAGTAG-3'. The relative mRNA levels were calculated using the 2⁻ Δ
635 Δ Ct method ((Livak and Schmittgen, 2001)). The Δ Cts were obtained from Ct normalized
636 with chick *S17* levels in each sample. Each sample was analyzed in duplicate. Results were
637 expressed as Standard Deviation (SD). Statistical analysis was performed with Graphpad
638 Prism V6 software using the non-parametric Mann-Whitney test to determine p-values.
639 Statistical significance was set at p<0.05.

640

641 **Quantification and statistical analyses**

642 For enumeration of myoblasts and myotubes in vitro, pictures of cultures stained with PAX7,
643 MYOG and MF20 antibodies were assembled and counted. MYOG-positive nuclei were
644 counted outside and inside the MF20-positive myotubes. Myoblast fusion was estimated by
645 counting the number of DAPI-positive nuclei inside the MF20-positive myotube compared to
646 the total number of DAPI-positive nuclei. Proliferation was quantified by estimating the

647 number of EDU-positive cells among the total number of DAPI-positive nuclei. Results
648 shown are the mean of at least six biological samples coming from at least three independent
649 cultures. Quantification in vivo was realized on transverse sections of control, grafted and
650 electroporated limbs immunostained with PAX7, MYOG or MF20 antibodies. Muscle area
651 measurement and determination of the number of PAX7-positive cells and MYOG-positive
652 nuclei were performed on five to eight successive sections of five different muscles in four to
653 six different embryos. The number of PAX7-positive cells and MYOG-positive nuclei was
654 estimated on the total number of DAPI-positive nuclei and compared between experimental
655 and control muscles. Proliferation was quantified by estimating the number of PH3-positive
656 cells among the total number of DAPI-positive nuclei or among the total number of PAX7-
657 positive nuclei. Results shown are the mean of quantification on all sections for each muscle.
658 Quantification analysis was realized with the Cell Counter plug-in of the free software Fiji
659 (Rasband,W.S., ImageJ,U.S.National Institute of Health, Bethesda, Maryland,USA,
660 <http://imagej.nih.gov/ij/>, 1997–2012). Statistical analysis was performed with Graphpad
661 Prism V6 software using the non-parametric Mann-Whitney test to determine p-values.
662 Statistical significance was set at $p < 0.05$.

663

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883 33.

884 885 886 **Legends to Figures**

887

888 **Figure 1: CXCR7 receptor is expressed in foetal myogenic cells in chicken embryonic**
889 **limb.** In situ hybridization for *CXCL12* (a, i, p, t), *CXCR7* (c, d, e, h, j, k, o, q, s), *SCX* (r) and
890 immunostaining for CXCR4 (b, d), CXCR7 (f, g, l-n), PAX7 (f, g, m) and MF20 (l, n, o-t) on
891 transverse sections of E5 (a-g), E8 (j-n), E10 (o, p, r, s) and longitudinal section of E8 (h, i)
892 and E10 (q) chicken embryo limbs. At E5, CXCR4+ muscle masses express CXCR7 in a few
893 PAX7+ cells. From E8, CXCR7 was restricted to the tips of MF20+ myotubes, facing the
894 SCX+ MTJ at E10. At all stages, CXCL12 was expressed in CT with a strong expression
895 around limb vessels at E10. Dorsal at the top, posterior at the left, u: ulna, r: radius. Bars: 100
896 μm in a-c, e, f, j, l, o, p, r-t; 50 μm in d, g, h, i, k, m, n, q.

897

898 **Figure 2: Misregulation of CXCR7 receptor induces muscle defaults in chicken**
899 **embryonic limb.** (A) Pellets of CEF transfected with RCAS-*CXCR7* or *dnCXCR7* were
900 grafted in the forelimb of E4 chicken embryos. Infected and control (contralateral) limbs were

901 collected at E10 and analysed. (B, C) Dorsal views of whole mount MF20 immunostaining
902 (Ba, b, Ca, b) revealed differences in muscle patterning between infected limbs (Bb, Cb) and
903 controls (Ba, Ca) confirmed by 3D reconstructions of individual muscles in *dnCXCR7*- (Bd)
904 and *CXCR7*- (Cd) infected limbs when compared to controls (Bc, Cc). Volume and length
905 analysis in reconstructed muscles shown in B and C shows that *dnCXCR7* overexpression
906 induced a decrease in muscle volume and length (Be, f), while *CXCR7* overexpression
907 resulted in an increase in muscle volume and length (Ce, f). Colors of arrows, individual
908 muscles and histograms indicate the same muscle in each condition. ANC: anconeus, EDC:
909 extensor digitorum communis, EMU: extensor metacarpi ulnans, EMR: extensor metacarpi
910 radialis, EIL: extensor incidus longus, FCU: flexor carpi ulnaris. n= 2 embryos for *dnCXCR7*
911 and one embryo for *CXCR7*. P values were analysed by non-parametric Mann-Whitney test
912 using the Graphpad Prism V6 software. Error bars indicate the standard deviation. Bar: 2 mm
913

914 **Figure 3: Overexpression of a dominant negative form of CXCR7 inhibits myoblast**
915 **fusion *in vivo*.** Immunostaining for MF20 (a, c, f) and in situ hybridization for *CXCR7* (b) on
916 transverse sections of E10 *dnCXCR7*-infected (b, c, f) and control (a, f) limbs, showing that
917 infected dorsal limb muscles exhibited decreased surfaces when compared to control muscles
918 (d, f). Dorsal at the top, posterior at the left, u: ulna, r: radius. f illustrates transverse sections
919 taken from the same longitudinal level in control and infected EMU muscle (section levels of
920 the limb are schematized in e). *DnCXCR7*-infected muscles differentiated normally, as shown
921 by the unchanged number of MYOG+ cells (g), but exhibited a decrease in the number of
922 MYOG+ nuclei into the myotubes (h, i, arrows: MYOG+ nuclei in myotubes, arrowheads:
923 MYOG+ nuclei outside myotubes) and in myoblast fusion index (j, k) when compared to
924 controls. Arrow in k highlighted the few myotubes in control exhibiting two neighbour nuclei,
925 which are never observed in *Dn-CXCR7* expressing muscles. n= 7 embryos. ANC: anconeus,
926 EDC: extensor digitorum communis, EML: extensor medius longus, EMU: extensor
927 metacarpi ulnans, EIL: extensor incidis longus. P values were analysed by non-parametric
928 Mann-Whitney test using the Graphpad Prism V6 software. Error bars indicate the standard
929 deviation. Bars: 200 μ m in a-c; 100 μ m in d; 50 μ m in f, 25 μ m in i, k.

930

931 **Figure 4: CXCR7 overexpression promotes myoblast fusion *in vivo*.** Immunostaining for
932 MF20 (a, c, f) and in situ hybridization for *CXCR7* (b) on transverse sections of *CXCR7*-
933 infected (b, c, f) and control (a, f) limbs, showing that infected dorsal limb muscles exhibited
934 increased surfaces when compared to control muscles (d, f). Dorsal at the top, posterior at the

935 left, u: ulna, r: radius. f illustrates transverse sections taken from the same longitudinal level
936 in control and infected EDC muscle (section levels of the limb are schematized in e). *CXCR7*-
937 infected muscles differentiated normally, as shown by the unchanged number of MYOG+
938 cells (g), but exhibited an increase in the number of MYOG+ nuclei into the myotubes (h, i,
939 arrows: MYOG+ nuclei in myotubes, arrowheads: MYOG+ nuclei outside myotubes) and in
940 myoblast fusion index (j, k) when compared to controls. Arrows in k highlighted the increase
941 in myotubes exhibiting two neighbour nuclei in *CXCR7* expressing muscles, when compared
942 to controls. n=6 embryos. ANC: anconeus, EDC: extensor digitorum communis, EIL:
943 extensor incidis longus, EMU: extensor metacarpi ulnans. P values were analysed by non-
944 parametric Mann-Whitney test using the Graphpad Prism V6 software. Error bars indicate the
945 standard deviation. Bars: 200 μ m in a-c; 100 μ m in d; 50 μ m in f, 25 μ m in i, k.

946
947 **Figure 5: CXCR7 receptor controls myoblast fusion *in vitro*.** (A) *dnCXCR7*
948 overexpression in cultured foetal myoblasts decreases the number of myotubes compared to
949 controls, as shown by MF20 immunostaining (Aa, b) and myotube quantification (Ac),
950 without changing the total number of MYOG+ cells (Ad-f). A decrease in the number of
951 MYOG+ myonuclei into myotubes (Ad, e, g), in myoblast fusion index (Ah) and in the
952 number of nuclei per myotube (Ai) shows that *dnCXCR7* overexpression induced a decrease
953 in myoblast fusion *in vitro*. *TMEM8C* expression is not affected by *dnCXCR7* overexpression
954 (Aj). Quantification and mRNA levels of controls were normalized to 1. n=24 cultures, 8
955 independent experiments. (B) *CXCR7* overexpression increases the number of myotubes
956 compared to control cultures, as shown by MF20 immunostaining (Ba,b) and myotube
957 quantification (Bc), without modifying the total number of MYOG+ cells (Bd-f). An increase
958 in the number of MYOG+ myonuclei into myotubes (Bd, e, g), in myoblast fusion index (Bh)
959 and in the number of nuclei per myotube (Bi) illustrates that *CXCR7* overexpression led to an
960 increase in myoblast fusion *in vitro*. *TMEM8C* expression is not affected by *CXCR7*
961 overexpression (Bj). Quantification and mRNA levels of controls were normalized to 1. n=18
962 cultures, 6 independent experiments. P values were analysed by non-parametric Mann-
963 Whitney test using the Graphpad Prism V6 software. Error bars indicate the standard
964 deviation. Bars: 100 μ m

965
966 **Figure 6: TMEM8C and CXCR7 regulate independently myoblast fusion events during**
967 **foetal myogenesis.** (A) In situ hybridization of *TMEM8C* and *CXCR7* on transverse sections
968 of chick forelimbs at E5 (a-c, dorsal at the top), E6 (d-f, dorsal muscle mass) and E10 (g-I,

969 FCU muscle) with simultaneous detection of DAPI in d,e and MF20 in g, h. c, f, i: Artificial
970 superposition of *TMEM8C* and *CXCR7* expression from two adjacent sections. c, f: Higher
971 magnification of the region squared in a,b and d,e. Arrows in g, i delineates *CXCR7*
972 expression at the MTJ in E10 FCU muscle while arrowhead in h, i shows *TMEM8C*
973 expression in the central region of the muscle. (B) *TMEM8C* expression is not affected by
974 deregulation of *CXCR7* signaling. (a) RT-qPCR for *CXCR7* and *TMEM8C* on control and
975 *dnCXCR7*-transfected (a) or *CXCR7*-transfected (b) limbs. Quantification and mRNA levels
976 of controls were normalized to 1. P values were analysed by non-parametric Mann-Whitney
977 test using the Graphpad Prism V6 software. (b) In situ hybridization for *TMEM8C* and
978 *CXCR7* on transverse limb sections of *CXCR7*-infected and contralateral E8 limbs. n=6
979 embryos, 3 independent experiments. (C) Fusion effect of *CXCR7* occurs independently of
980 *TMEM8C*. Expression of *SiRNA* against *TMEM8C* in cultured foetal myoblasts decreases
981 myoblast fusion when compared to controls, as shown by MF20 immunostaining (Ca) and
982 fusion index quantification (Cb), while coexpression of *SiTMEM8C* and *CXCR7* restores
983 myoblast fusion at a higher level than *SiTMEM8C* but at a lower level than *CXCR7* (Ca, b).
984 n=4 cultures. Bars: 100 μ m in Aa, b, d-f; 50 μ m in Ag-i; 200 μ m in Bc.

985
986 **Figure 7: CXCR7 modulates myoblast fusion through EGF receptor signaling.** (A)
987 Immunostaining for MF20 and pEGFR on transverse sections of E6 chicken limbs (Aa, dorsal
988 muscle mass) and immunostaining for MF20, pEGFR, DAPI and *CXCR7* on transverse
989 sections of E8 limbs (Ab-e) showing the expression of pEGFR in muscle nuclei from E8 of
990 development. c, d represent high magnification of the squared region in b. ANC: anconeus,
991 EIL: extensor incidis longus, EMU: extensor metacarpi ulnans. (B) Immunostaining for
992 PAX7 (Ba), MF20 (Bb) and pEGFR (Ba, b) on chicken foetal myoblasts cultured for 3 days
993 showing the diffuse expression of pEGFR in the cytoplasm of PAX7+ myoblasts and the
994 nuclear pEGFR expression in differentiated myotubes. (C) Inhibition of pEGFR blocks the
995 *CXCR7*-dependent myoblast fusion. Control (Ca-c), PD153035-treated (Cd-f), *CXCR7*-
996 transfected cultures (Cg-i) and *CXCR7*-transfected cultures treated with PD153035 (Cj-l) and
997 stained with MF20 and pEGFR. (m) Quantification of myoblast fusion index in control,
998 PD153035-treated, *CXCR7*-transfected cultures and *CXCR7*-transfected cultures treated with
999 PD153035. Myoblast fusion was blocked by inhibition of EGFR phosphorylation and *CXCR7*
1000 gain-of-function was not able to restore the myoblast fusion defect demonstrating that
1001 *CXCR7* effect on myoblast fusion acts through EGFR phosphorylation. n=24 cultures, 4
1002 independent experiments. Bars: 100 μ m. b, c, e, f and h, i, k, l represent high magnifications of

1003 the squared regions in a, d and g, j respectively. P values were analysed by non-parametric
1004 Mann-Whitney test using the Graphpad Prism V6 software. Error bars indicate the standard
1005 deviation.

1006

1007 **Figure 8: CXCL12 overexpression in CT promotes myoblast fusion in chicken limb**

1008 **muscles** (A) Lateral plate electroporation was performed in E2.5 embryos and electroporated

1009 limbs expressing TOMATO were collected at E9 (Ab). (B) MF20 immunostaining in

1010 electroporated (Bb, e) and control (Ba, e), showing that limb muscles exhibited increased

1011 surfaces upon CXCL12 overexpression in limb CT when compared to control muscles (c, e). f

1012 illustrates transverse sections taken from the same longitudinal level in control and EDC

1013 muscle upon CXCL12 overexpression in limb CT (section levels of the limb are schematized

1014 in d). Dorsal at the top, posterior at the left, u: ulna, r: radius. CXCL12 expression in CT did

1015 not change the total number of MYOG+ cells (Bf), but induced an increase in the number of

1016 MYOG+ nuclei into the myotubes (Bg, h, arrows: MYOG+ nuclei in myotubes, arrowheads:

1017 MYOG+ nuclei outside myotubes) and in myoblast fusion index (Bi,j) when compared to

1018 controls. Arrows in j highlighted myotubes exhibiting two neighbour nuclei in nearby muscles

1019 of *CXCL12* overexpression in CT. ANC: anconeus, EDC: extensor digitorum communis,

1020 EML: extensor medius longus, EMU: extensor metacarpi ulnans, FCU: flexor carpi ulnaris.

1021 n= 9 embryos. Bars: 200 μ m in a, b; 100 μ m in c; 50 μ m in e, 25 μ m in h, j. (C) RT-qPCR for

1022 *CXCL12* and *TMEM8C* on control and electroporated limbs with *CXCL12* showing that

1023 *TMEM8C* expression is not affected by CXCL12 overexpression in limb CT. Quantification

1024 and mRNA levels of controls were normalized to 1. n=6 embryos, 3 independent experiments.

1025

1026 **Figure 9: CXCL12 overexpression in CT increases β 1 integrin activation at the MTJ and**

1027 **promotes CXCR7 expression in muscle.** (A) MF20 (a, d) and TASC (b, c) immunostaining

1028 on transverse sections of electroporated (c, d) and control (a, b) limbs, showing the increase in

1029 TASC expression in CXCL12 overexpressing limb regions (arrows in b, c). FCU: flexor carpi

1030 ulnaris. Dorsal at the top left, posterior at the bottom left, u:ulna, r: radius. (e) Quantification

1031 of FCU muscle surface in the electroporated limb overexpressing CXCL12 in CT, showing

1032 the expected surface increase attesting of the CXCL12 phenotype. The surface represents the

1033 average of all FCU muscle transverse sections in control and electroporated limb. (f-h) MF20

1034 and TASC immunostaining (f, g) on transverse sections of control (f) and electroporated (g, h)

1035 limb regions expressing CXCL12 in CT (h). TASC expression is increased and enlarged at

1036 the MTJ in FCU muscle (arrows in f, g). Asterisk in f, g shows the increased TASC

1037 expression around the cartilage of electroporated limb. CXCR7 and TASC immunostaining (i,
1038 j) on transverse sections of control (i) and electroporated (j) limb region expressing CXCL12
1039 in CT (k), showing the increase and enlargement of CXCR7 expression in FCU muscle
1040 (arrows in i, j). Asterisks highlight β 1 integrin activation at MTJ nearby CXCL12
1041 overexpression in CT. n= 5 embryos. Bars: 200 μ m in Aa-d; 100 μ m in Af-k. (B) Integrin
1042 activation enhances myoblast fusion and CXCR7 expression *in vitro*. Control, PMA-treated,
1043 *dnCXCR7*-transfected cultures and *dnCXCR7*-transfected cultures treated with PMA and
1044 stained with MF20 (Ba, b, g, i), TASC (Bc, d, h, j) and CXCR7 (Be, f). Myoblast fusion,
1045 TASC and CXCR7 staining were increased after integrin activation by PMA (Bb, d, f) but
1046 integrin activation had no effect on myoblast fusion after CXCR7 loss-of-function (Bi, j). (k)
1047 Quantification of myoblast fusion index in control, PMA-treated, *dnCXCR7*-transfected
1048 cultures and *dnCXCR7*-transfected cultures treated with PMA. n=24 cultures, 4 independent
1049 experiments. Bars: 100 μ m. P values were analysed by non-parametric Mann-Whitney test
1050 using the Graphpad Prism V6 software. Error bars indicate the standard deviation.

1051
1052 **Figure 10: A proposed model for the regulation of myoblast fusion at foetal muscle fiber**
1053 **tips via CXCR7.**

1054
1055 **Legends to Supplemental Figures**

1056
1057 **Supplemental Figure 1: CXCR4 receptor is expressed in vessels during limb**
1058 **development in chicken embryos.** CXCR4 (a, b), MEP21 (c) and MF20 (d) immunostaining
1059 on transverse sections of E6 chick embryo limbs. At E6, CXCR4 is no more expressed in
1060 muscle masses (b, d) but its expression is reminiscent of MEP21 staining, corresponding to
1061 endothelial cells of the limb vessels (b, c). b, c, d : high magnification of the squared region in
1062 a. Arrows indicate CXCR4 expression in vessels and arrowheads the lack of CXCR4
1063 expression in muscle masses. Dorsal at the top, posterior at the left. *CXCL12* (e) and CXCR4
1064 (f) staining on transverse sections of E10 chick embryo limbs showing the strong *CXCL12*
1065 expression in CT surrounding vessels (arrows). Bars : 100 μ m.

1066
1067 **Supplemental Figure 2: Deregulation of CXCL12/CXCR7 signaling does not modify**
1068 **myoblast proliferation and specification in chicken limb muscles** (A) *dnCXCR7*-infected
1069 limb muscles exhibited no change in cell proliferation (Aa) and number of PAX7+ cells (Ab)
1070 when compared to controls. n= 7 embryos. (B) *CXCR7*-infected limb muscles exhibited no

1071 change in cell proliferation (Ba) and number of PAX7+ cells (Bb) when compared to controls.
1072 n= 6 embryos. (C) CXCL12 overexpression in limb CT did not modify cell proliferation (Ca),
1073 and number of PAX7+ cells (Cb) in nearby muscles when compared to controls. n= 9
1074 embryos. P values were analysed by non-parametric Mann-Whitney test using the Graphpad
1075 Prism V6 software. Error bars indicate the standard deviation.

1076

1077 **Supplemental Figure 3: Deregulation of CXCR7 receptor does not modify myoblast**
1078 **proliferation and specification in chicken foetal myoblast cultures.** (A) Expression of
1079 CXCR7 receptor in foetal muscle cells *in vitro*. In situ hybridization for *CXCR7* (a) and
1080 immunostaining for PAX7 (b, c), MF20 (b, d) and CXCR7 (c, d) in foetal myoblasts cultured
1081 under differentiation conditions for 3 days. Both *CXCR7* mRNA and CXCR7 protein are
1082 expressed in a few PAX7-positive cells (arrows in a, c) and around some nuclei in MF20-
1083 positive myotubes (arrowheads in a, b, d). Bars: 100 μ m in a, b, d, e, f; 50 μ m in c. (B)
1084 *dnCXCR7* expression did not induce changes in total and PAX7+ cell proliferation (Ba) and
1085 in total number of PAX7+ cells (Bb). n=24 cultures, 8 independent experiments. (C) *CXCR7*
1086 overexpression did not induce changes in total and PAX7+ cell proliferation (Ca) and in total
1087 number of PAX7+ (Cb). n=18 cultures, 6 independent experiments. P values were analysed
1088 by non-parametric Mann-Whitney test using the Graphpad Prism V6 software. Error bars
1089 indicate the standard deviation.

1090

1091 **Supplemental Figure 4: CXCR7 deregulation does not impair *in vitro* migration of foetal**
1092 **myoblasts** (Aa) Measurement of gap closure in wound healing assays 48 hours after the
1093 scratch in primary cultures of chicken foetal myoblasts. (Ab) Measurement of gap closure of
1094 PAX7-positive cells in wound healing assays 48 hours after the scratch in primary cultures of
1095 chicken foetal myoblasts. (B) Phase-contrast views of gap closure in control (a), *dnCXCR7*-
1096 transfected (c) and *CXCR7*-transfected (e) cultures. Black lines delineate the gap borders.
1097 Phase-contrast views of and PAX7 immunostaining in control (b), *dnCXCR7*-transfected (d)
1098 and *CXCR7*-transfected (f) cultures. P values were analysed by non-parametric Mann-
1099 Whitney test using the Graphpad Prism V6 software. Error bars indicate the standard
1100 deviation. n=12 cultures, 3 independent experiments. Bars: 1mm in a, c, e, 100 μ m in b, d, f.

1101

1102 **Supplemental Figure 5: CXCL12 does not impair *in vitro* foetal myogenesis.** (A) Chicken
1103 embryonic fibroblasts were transfected with RCAS-*CXCL12* or control empty RCAS and
1104 transfected CEF were grown for 2 days to generate CXCL12 or control concentrated medium.

1105 Mediums were used to culture myoblasts under proliferation or differentiation conditions
1106 before staining. (B) Immunostaining for MF20 (Ba, b), quantification of myotubes (Bc, d),
1107 cell proliferation (Be, f), PAX7⁺ cells (Bg), number of nuclei per myotube (Bh) and fusion
1108 index (Bi) all showed that CXCL12 has no effect on *in vitro* myogenesis. P values were
1109 analysed by non-parametric Mann-Whitney test using the Graphpad Prism V6 software. Error
1110 bars indicate the standard deviation. n=36 cultures, 12 independent experiments. Bar: 100 μ m
1111

1112 **Supplemental Figure 6: Integrin activation enhances CXCR7 expression and myoblast**
1113 **fusion in vitro.** Chicken foetal myoblasts were cultured for 2 days in proliferation and Mn2⁺
1114 was added to proliferating myoblasts for 3 hours. Control, Mn2⁺-treated, *dnCXCR7*-
1115 transfected cultures and *dnCXCR7*-transfected cultures treated with Mn2⁺ and stained with
1116 MF20 (a, c, e, f) and CXCR7 (b, d). Myoblast fusion and CXCR7 staining were increased
1117 after integrin activation by Mn2⁺ but integrin activation had no effect on myoblast fusion
1118 after CXCR7 loss-of-function. (g) Quantification of myoblast fusion index in control, Mn2⁺-
1119 treated, *dnCXCR7*-transfected cultures and *dnCXCR7*-transfected cultures treated with Mn2⁺.
1120 P values were analysed by non-parametric Mann-Whitney test using the Graphpad Prism V6
1121 software. Error bars indicate the standard deviation. n=12 cultures, 3 independent
1122 experiments. Bars: 100 μ m.

1123
1124 **Supplemental Figure 7: CXCL12 does not impair β 1 integrin activation and CXCR7**
1125 **expression in *in vitro* foetal myogenesis.** Chicken foetal myoblasts were grown with control
1126 or CXCL12 concentrated medium for 2 days under proliferation conditions and 5 days under
1127 differentiation conditions. (A) Immunostaining for MF20 (Aa, c) and TASC (Ab, d), showing
1128 that CXCL12 has no effect on β 1 integrin activation in foetal myoblast cultures. (B) RT-
1129 qPCR for *CXCR7* on foetal myoblasts cultured in control conditions or with CXCL12
1130 concentrated medium showing that CXCL12 has no effect on *CXCR7* expression.
1131 Quantification and mRNA levels of controls were normalized to 1. P values were analysed by
1132 non-parametric Mann-Whitney test using the Graphpad Prism V6 software. Error bars
1133 indicate the standard deviation. n=18 cultures, 3 independent experiments. Bar: 100 μ m

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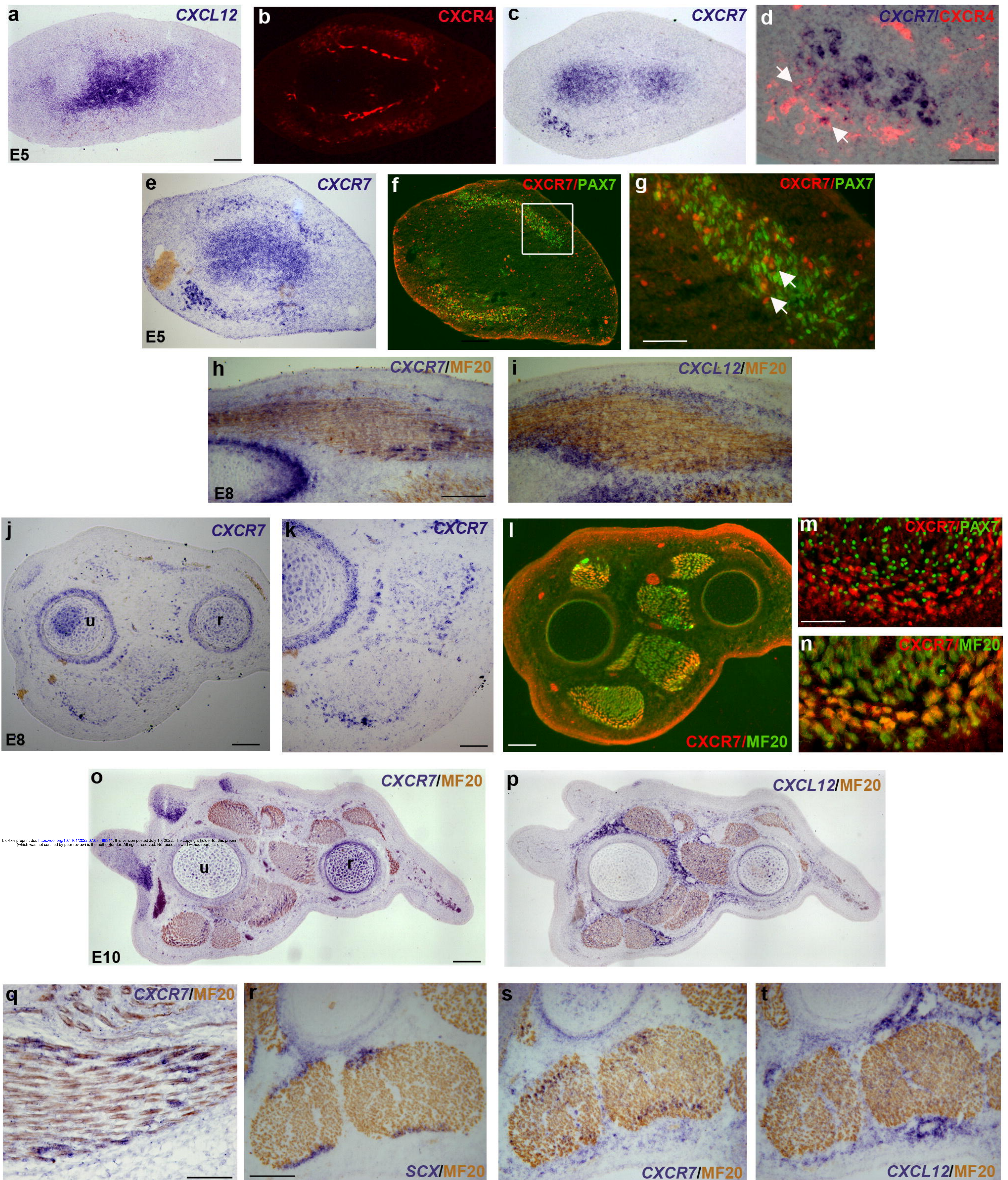
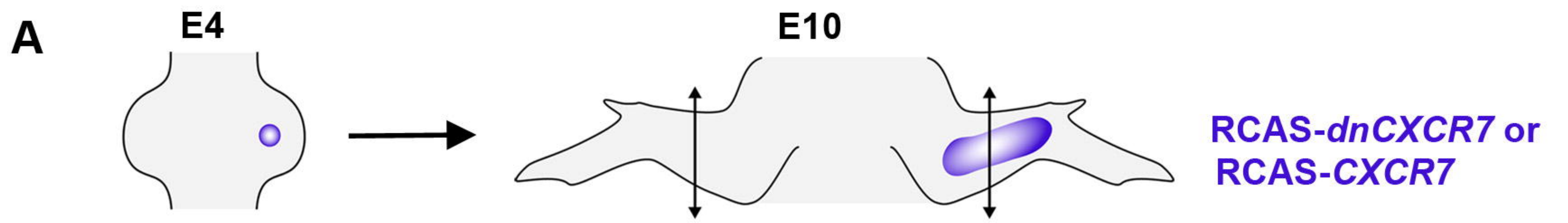
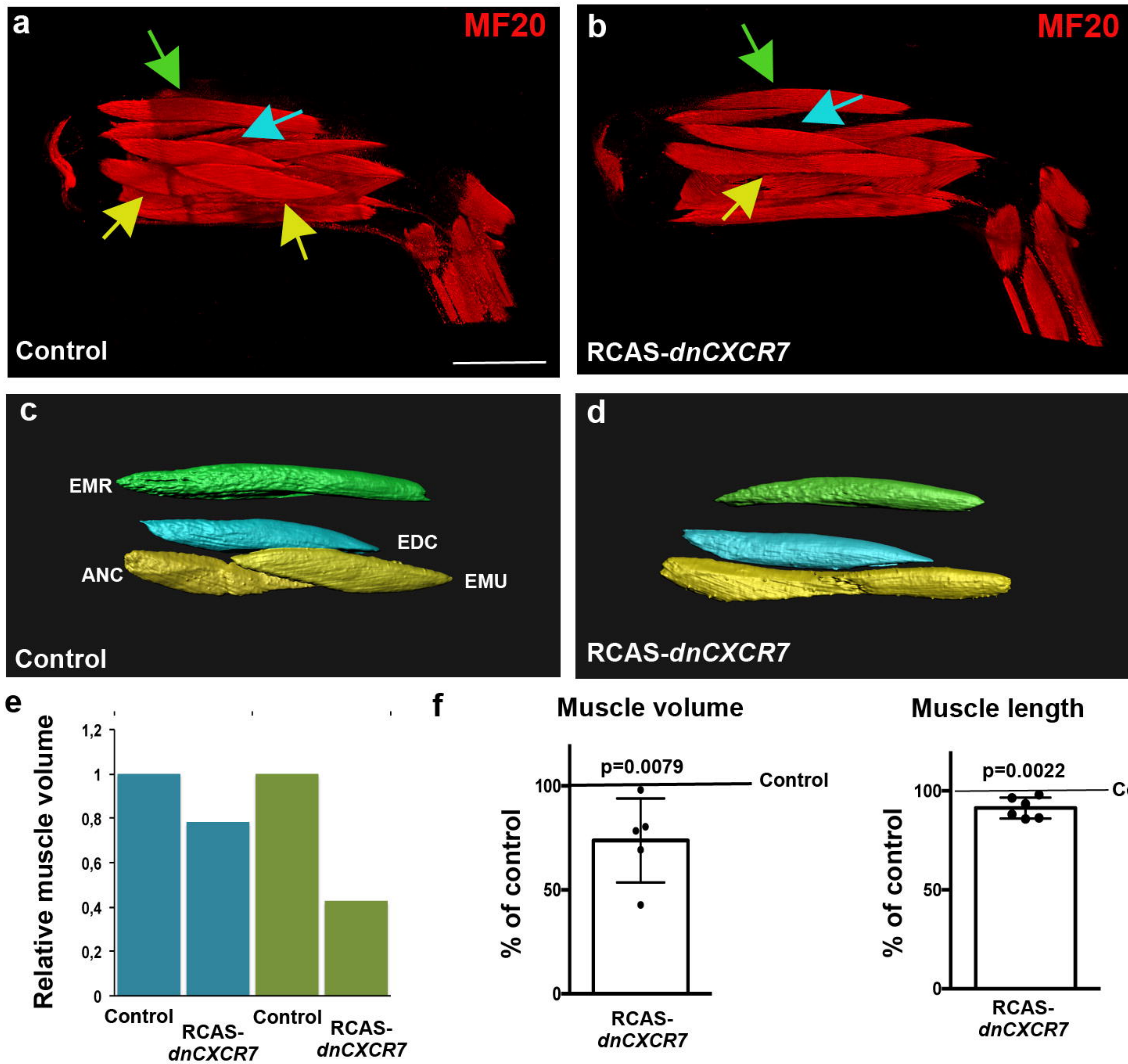


Figure 1



B



C

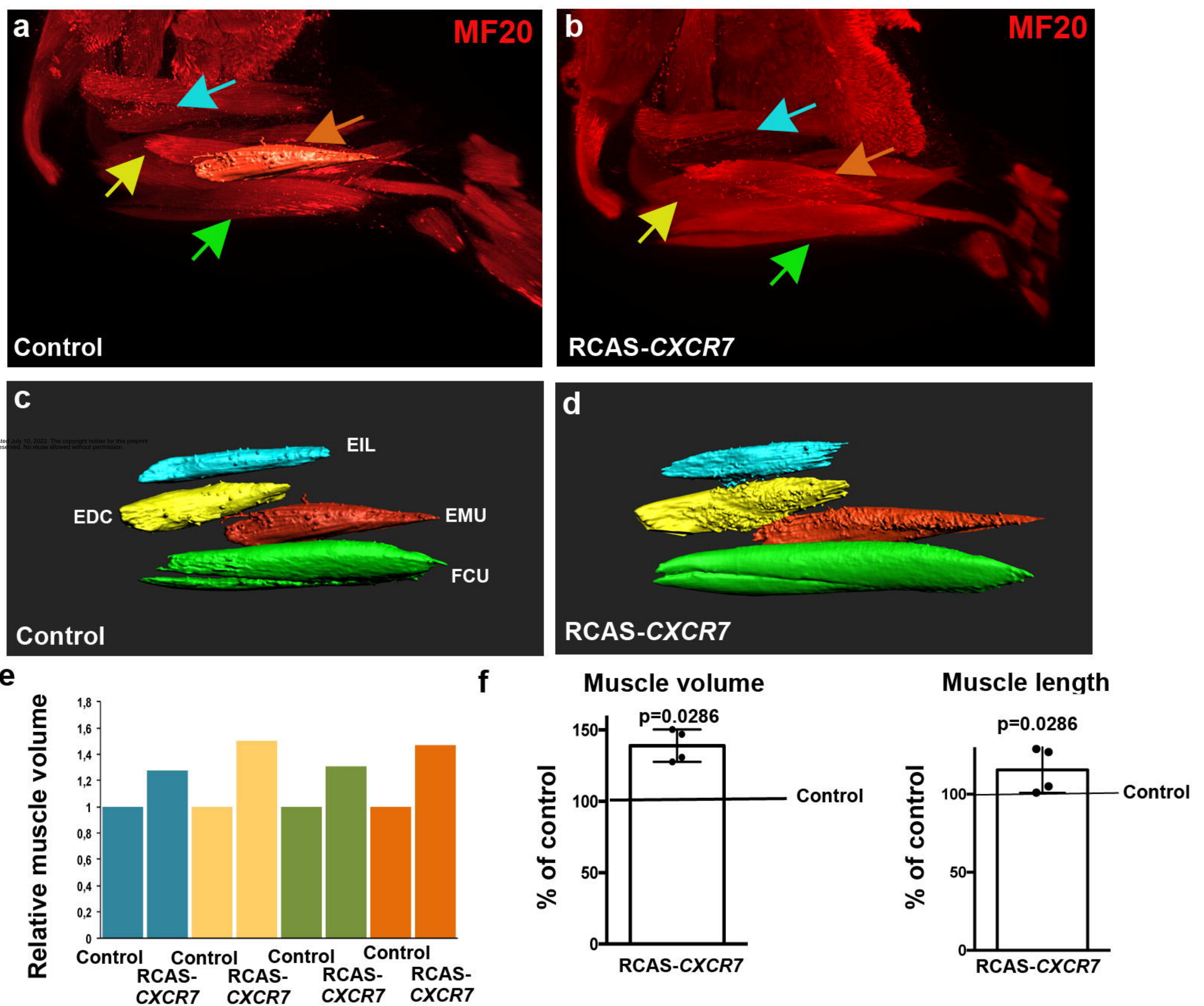


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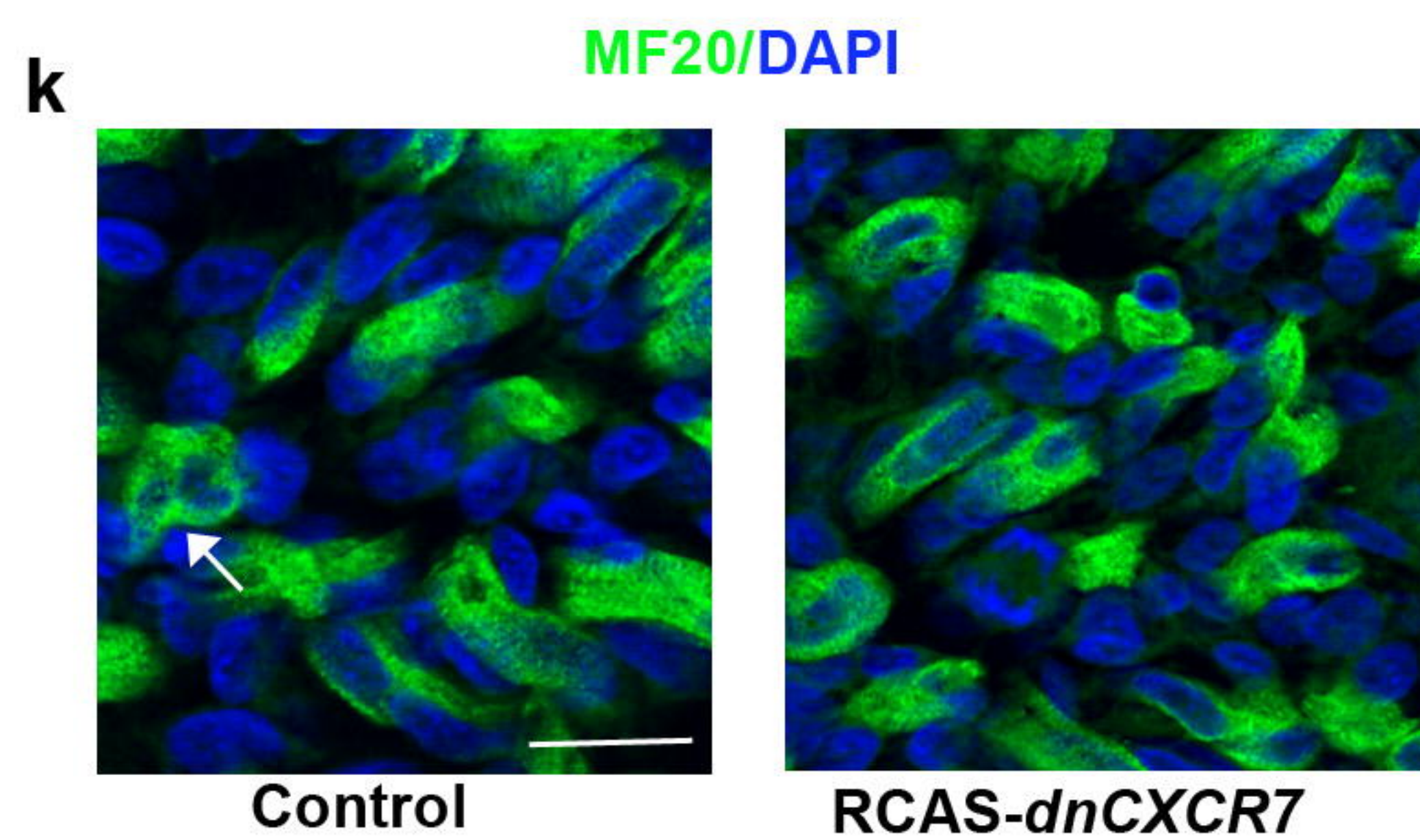
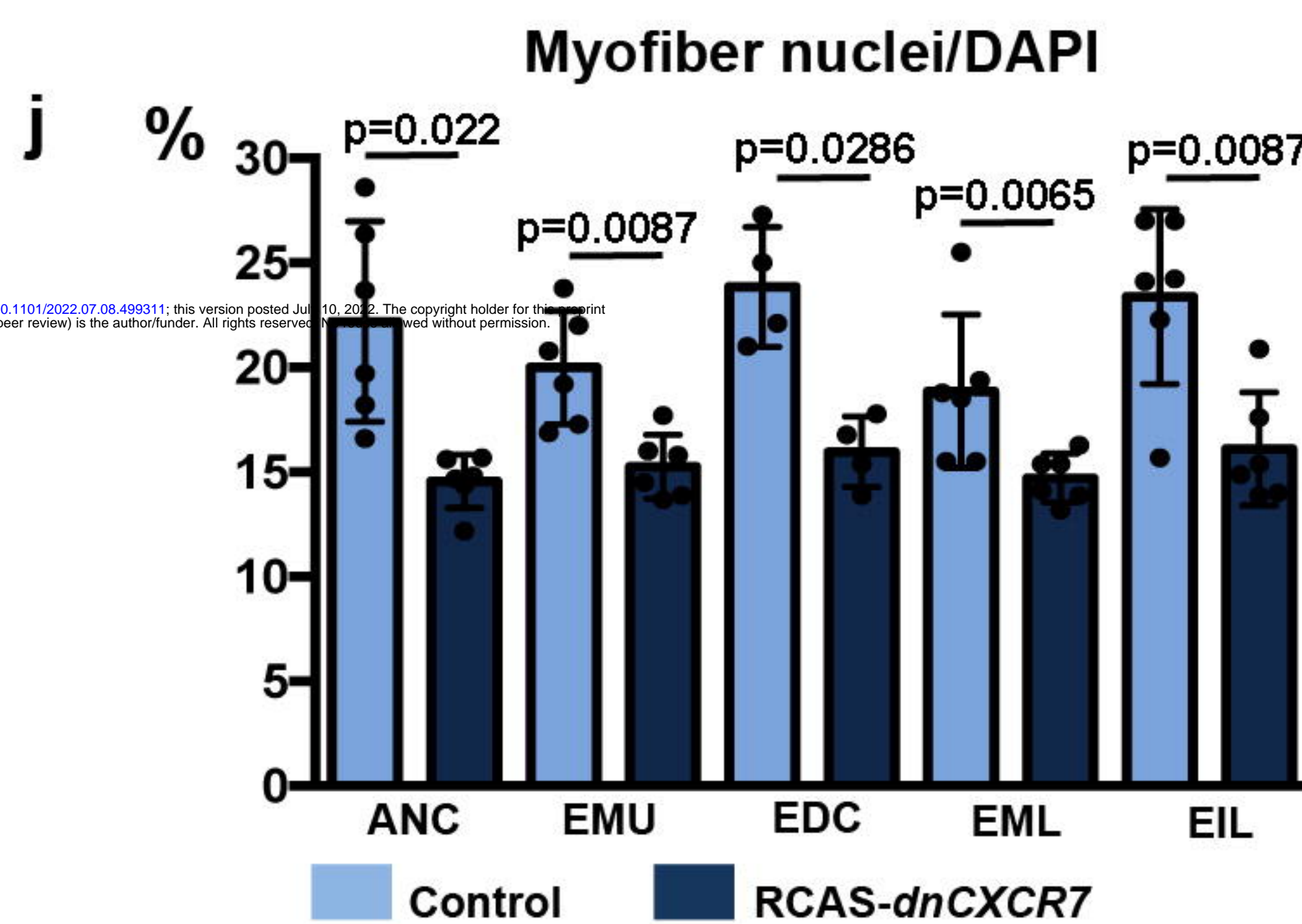
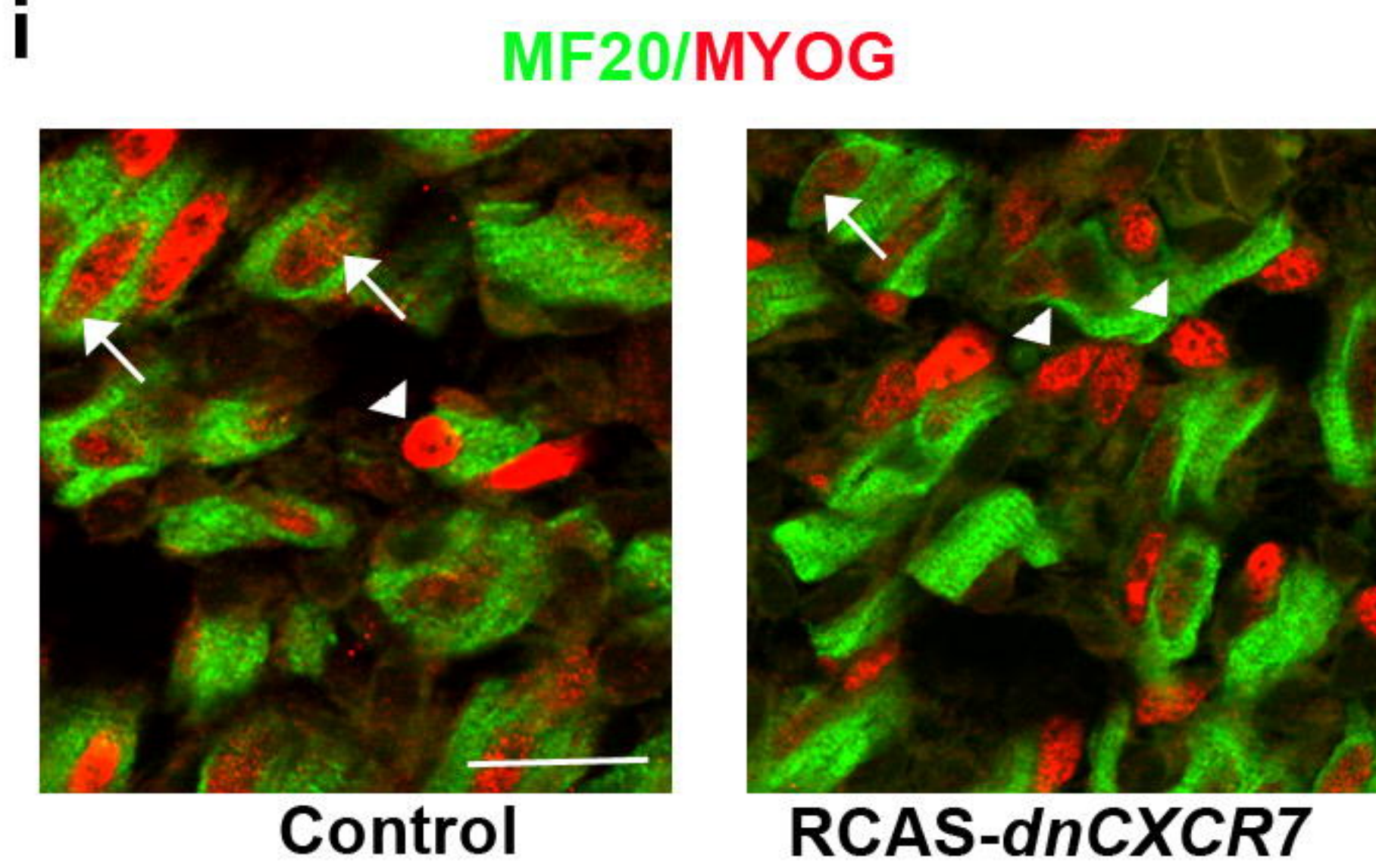
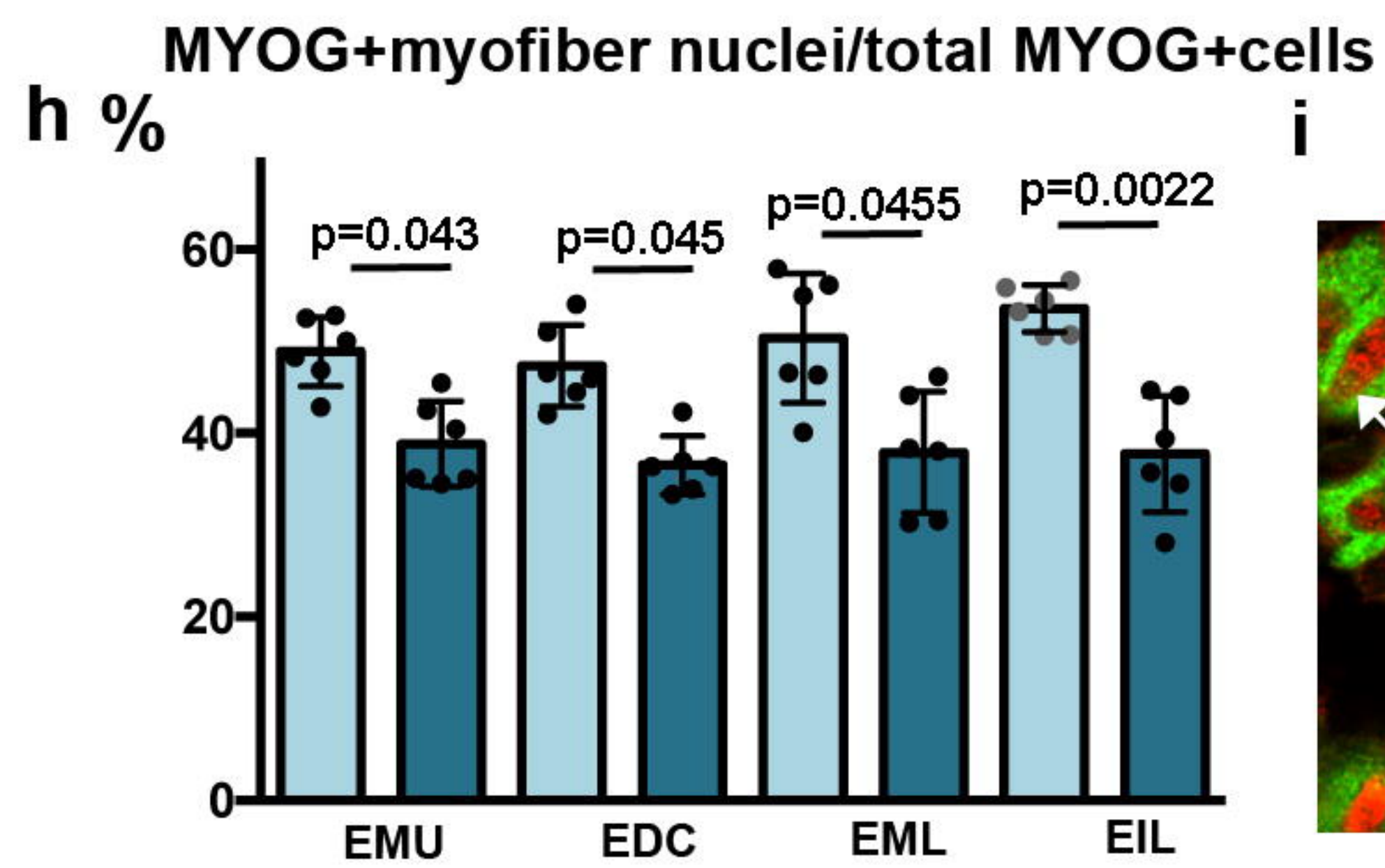
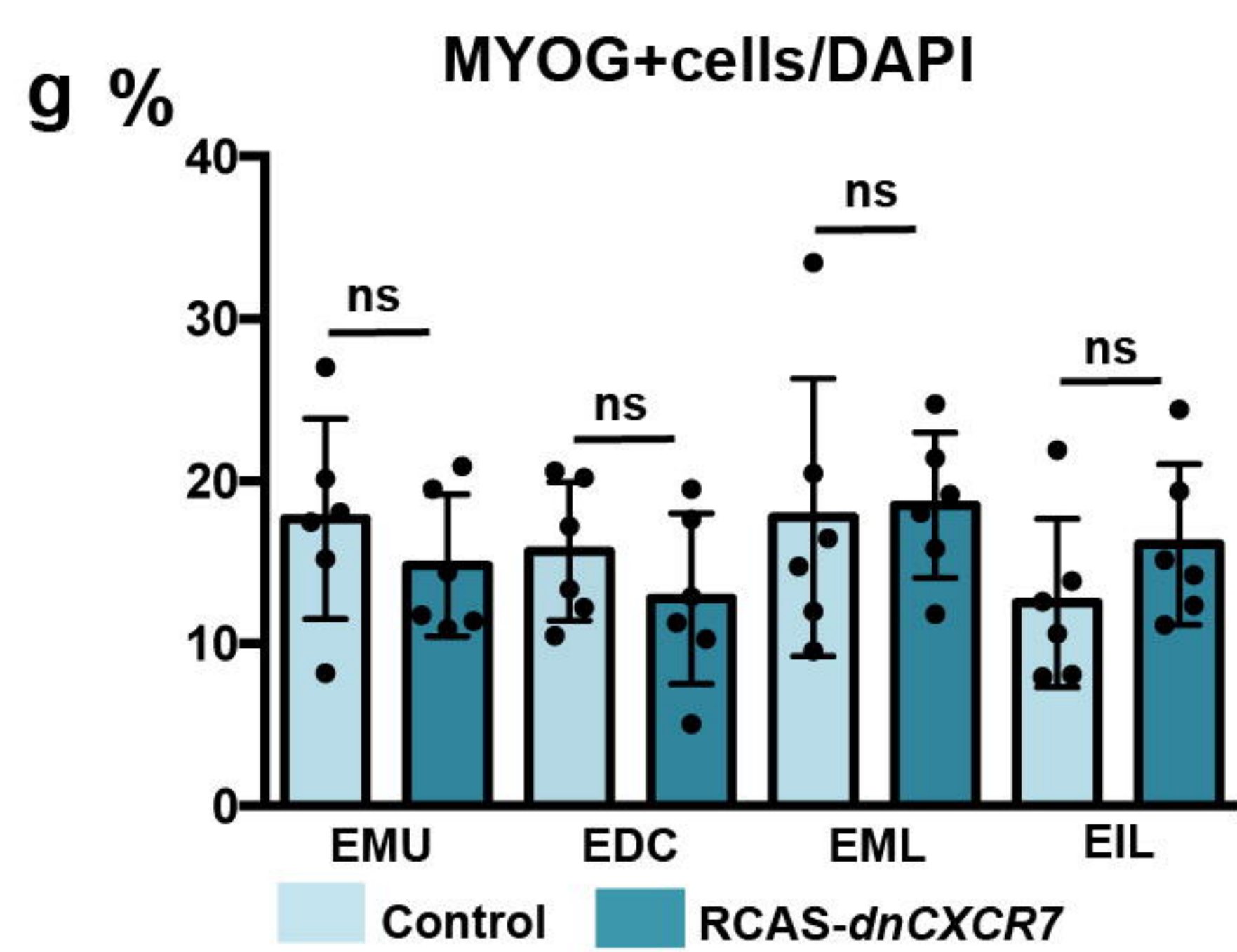
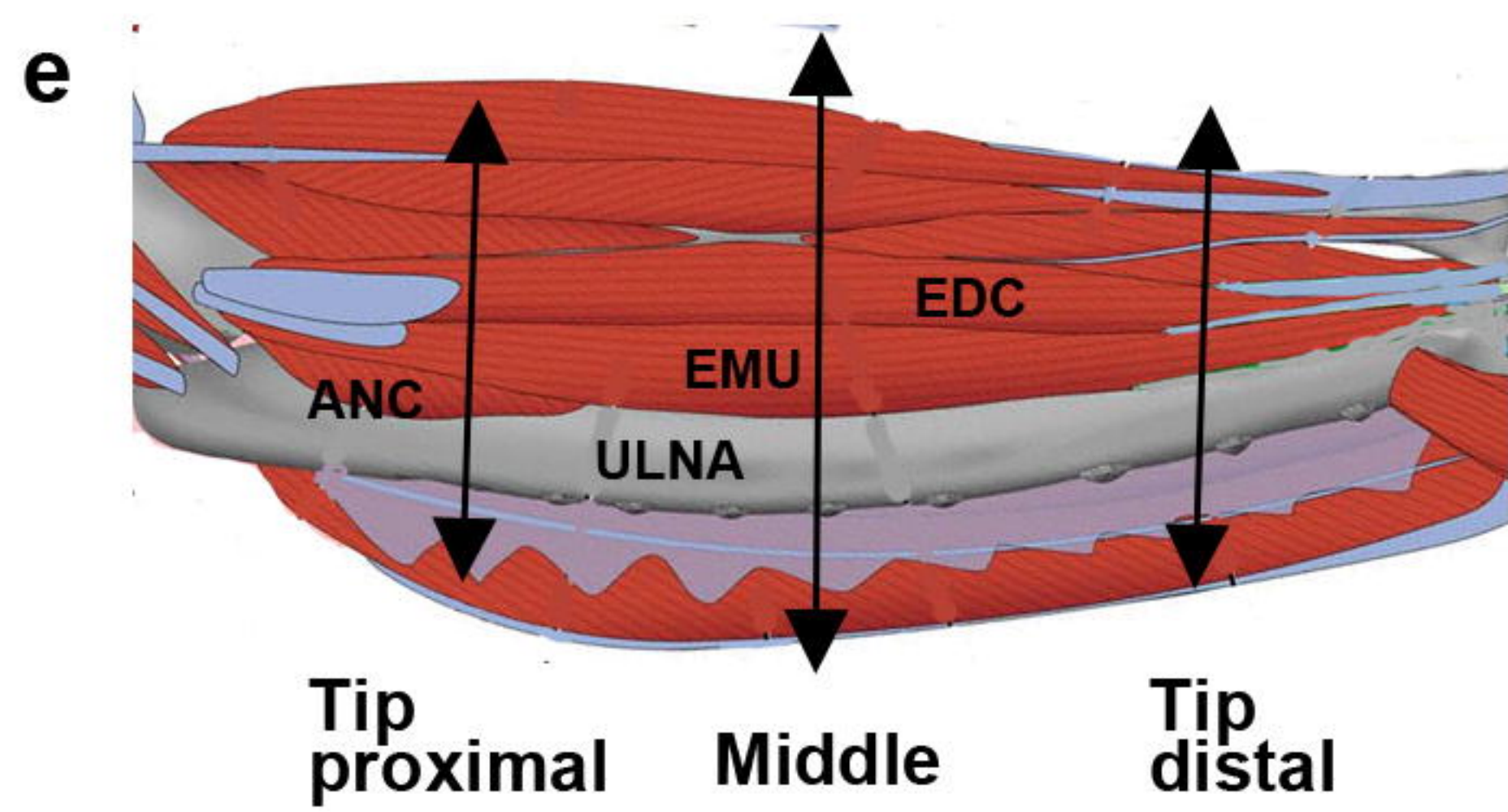
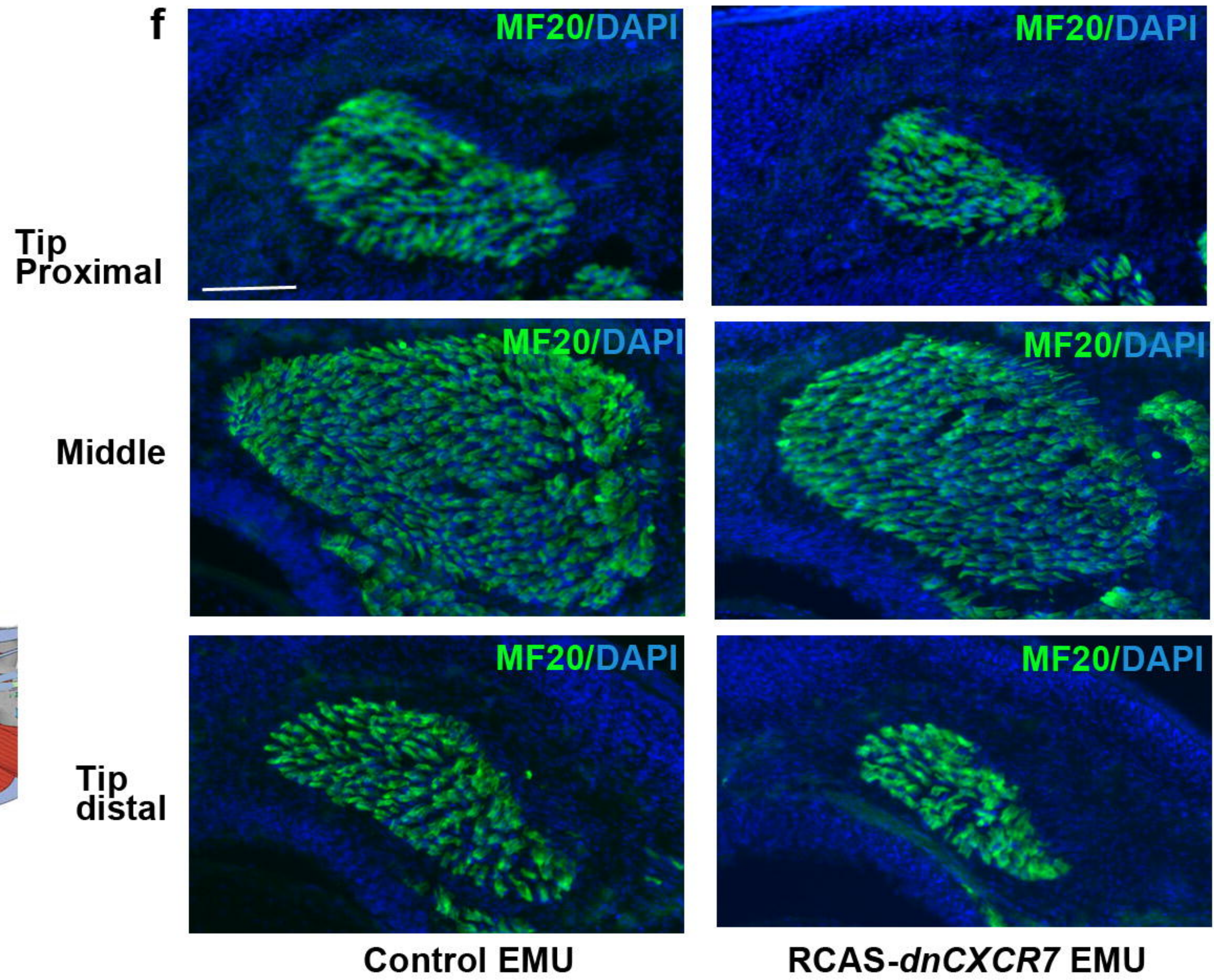
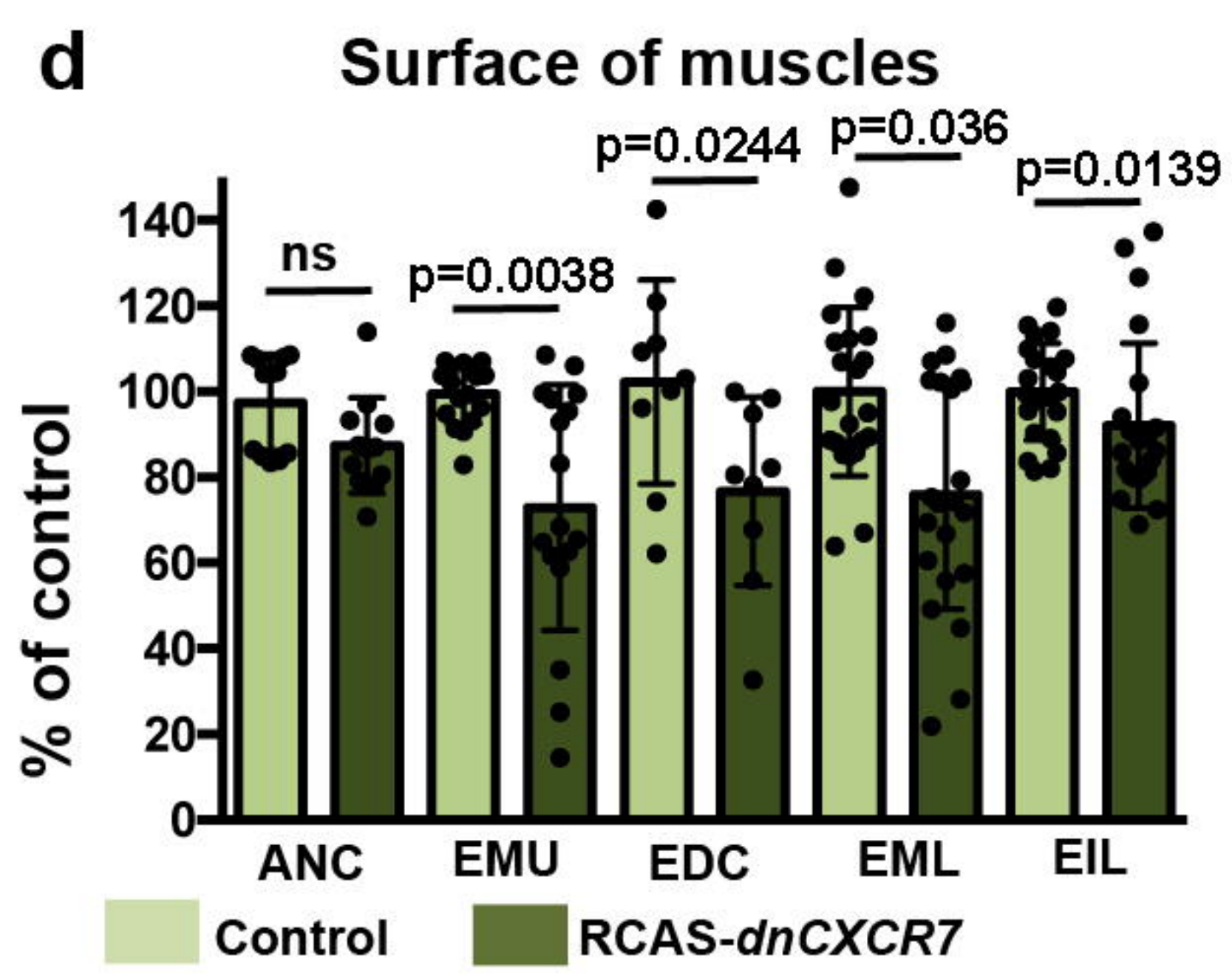
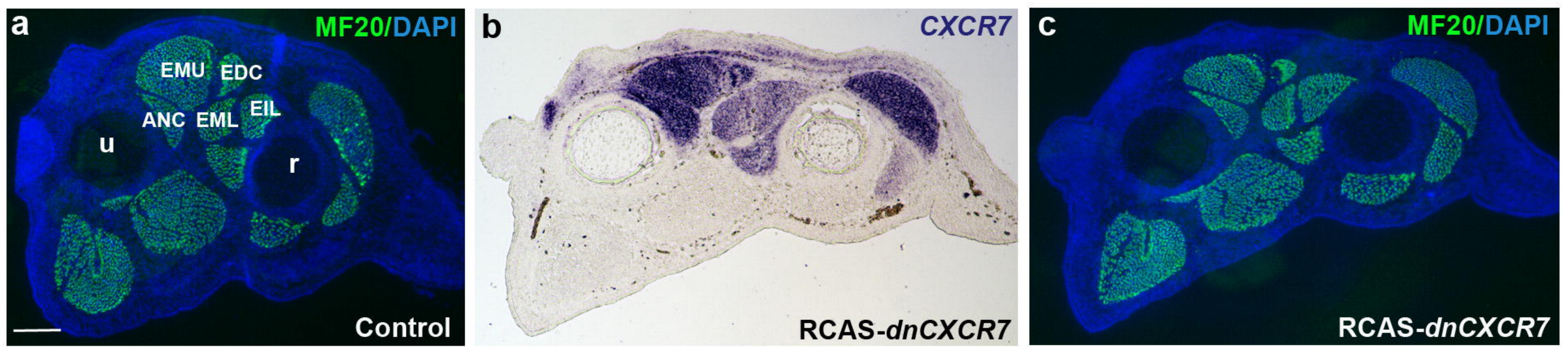


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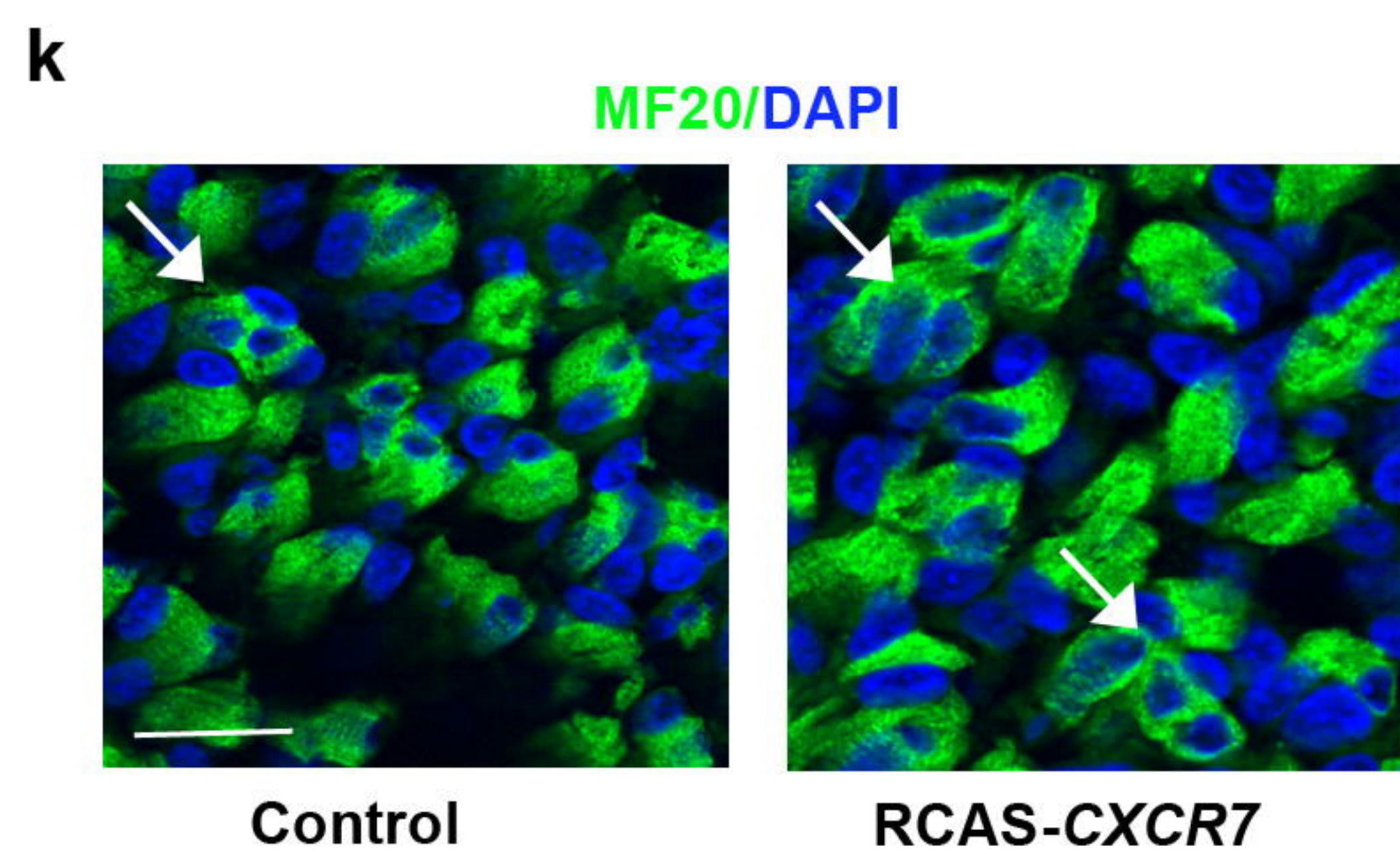
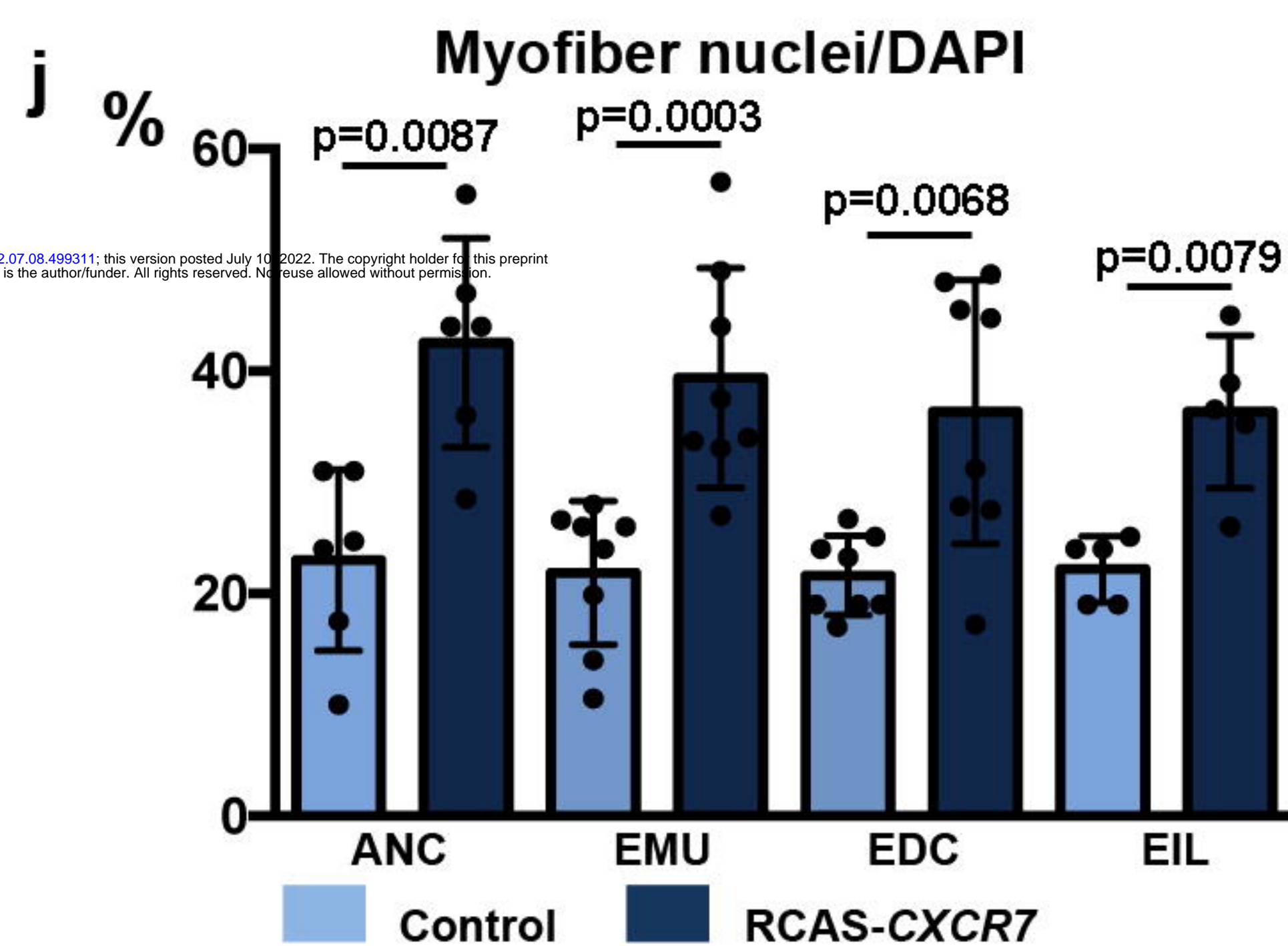
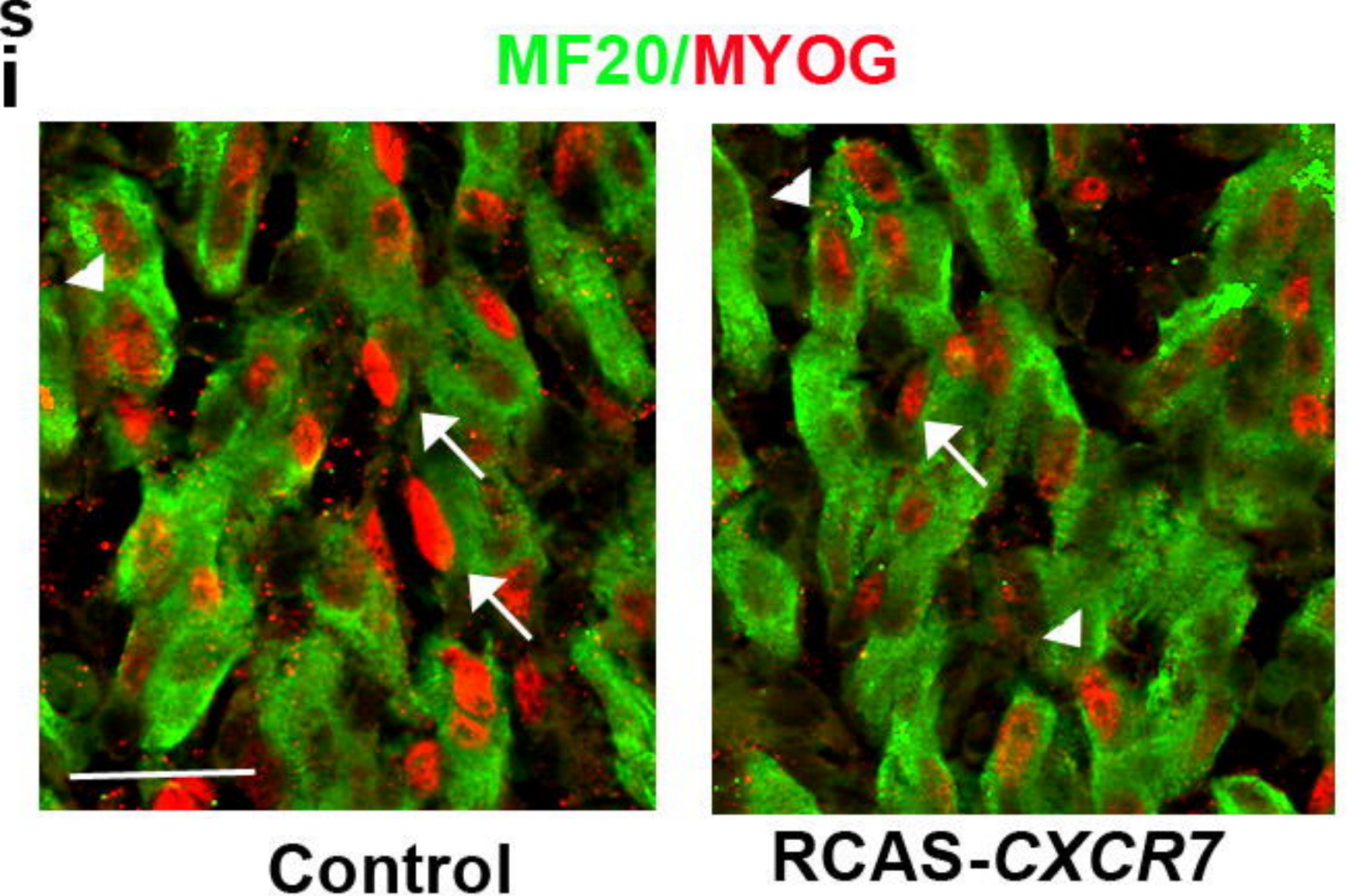
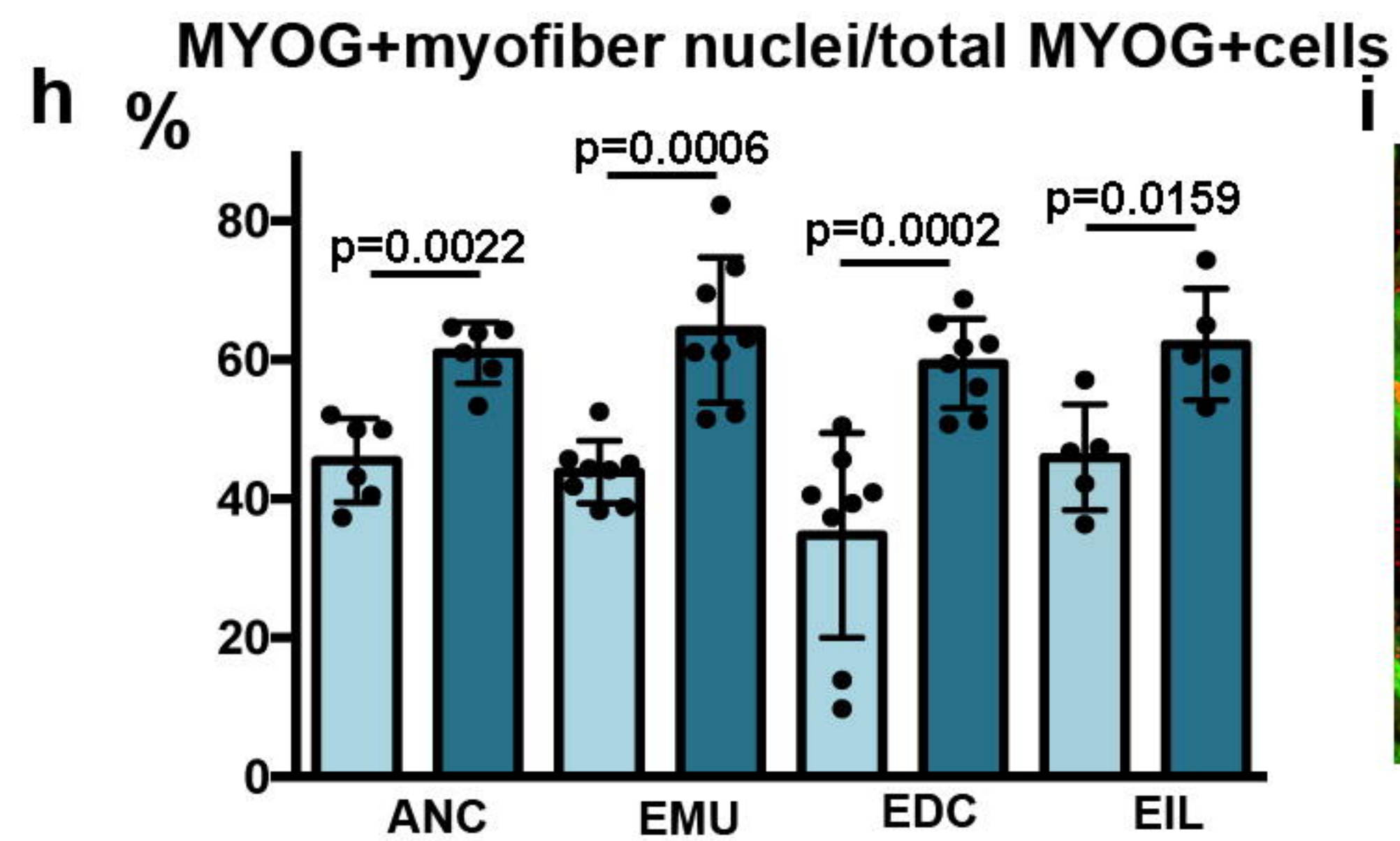
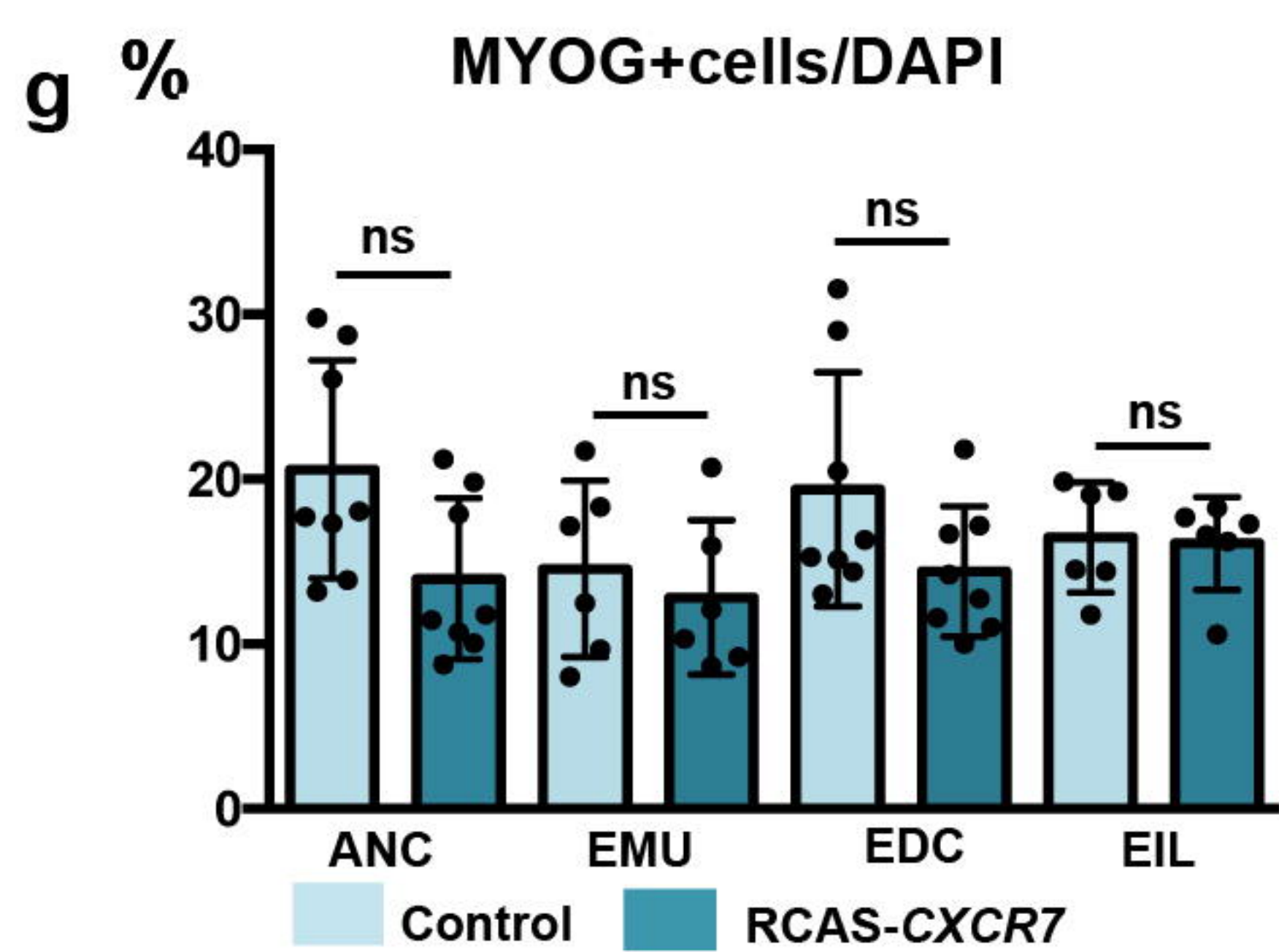
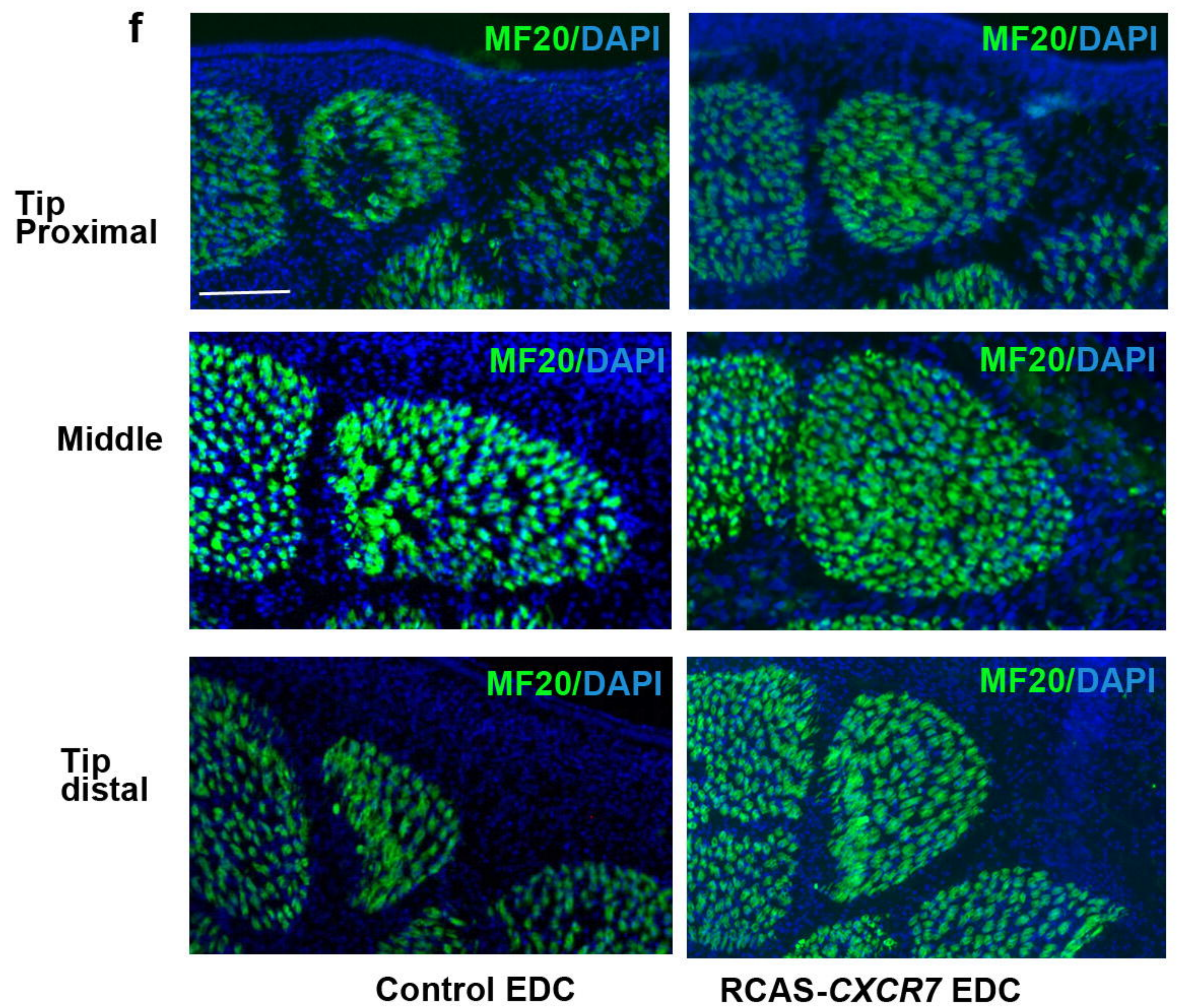
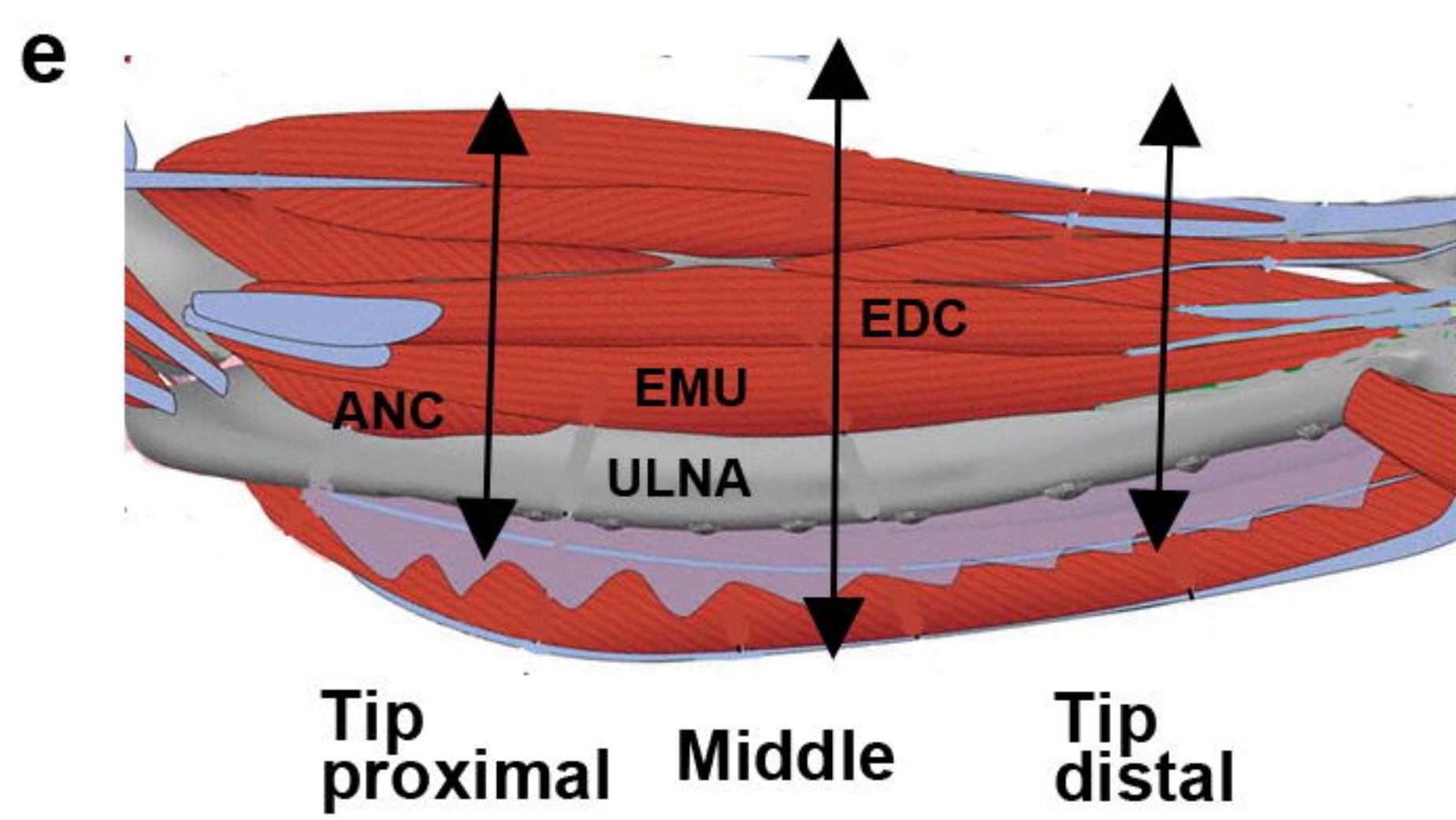
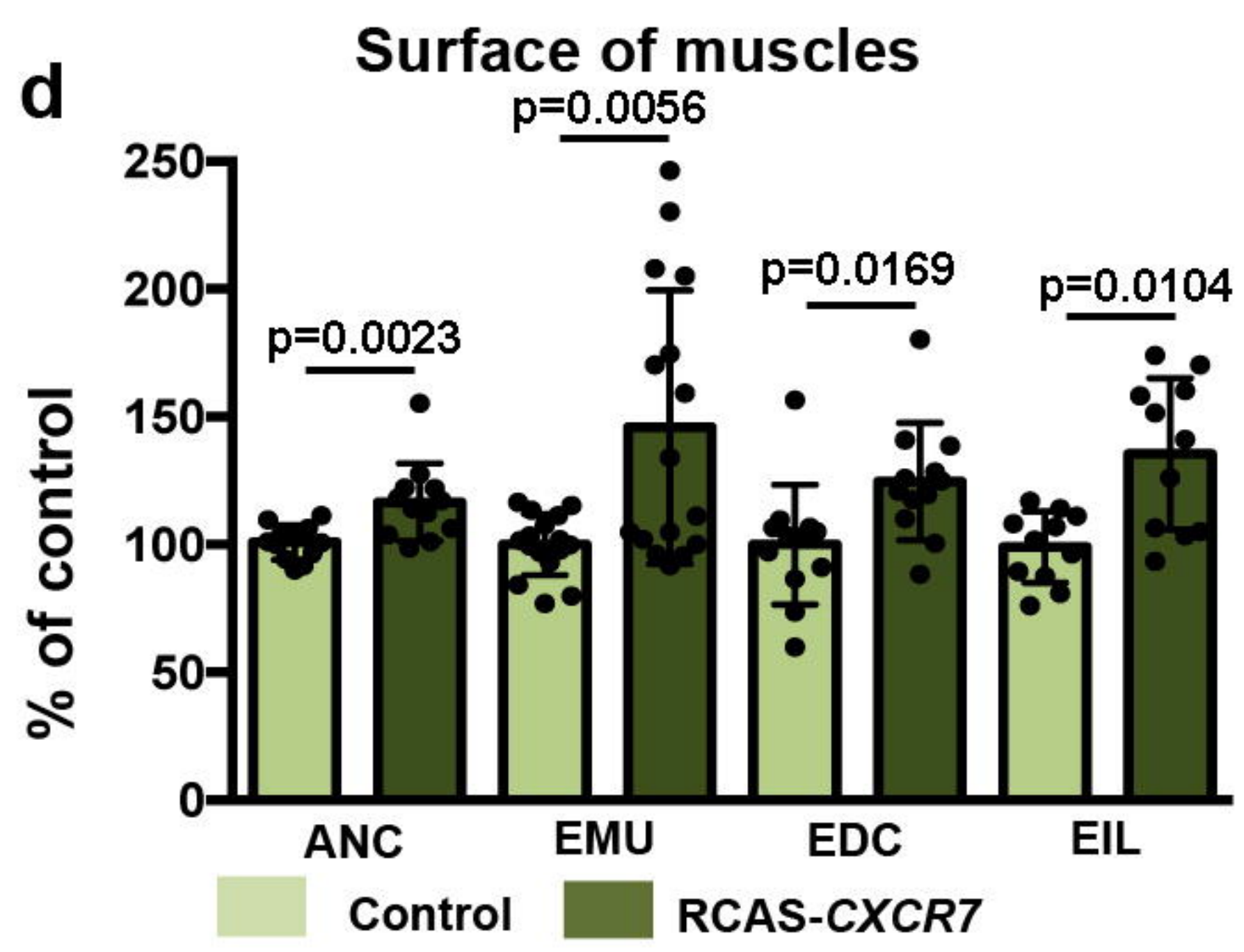
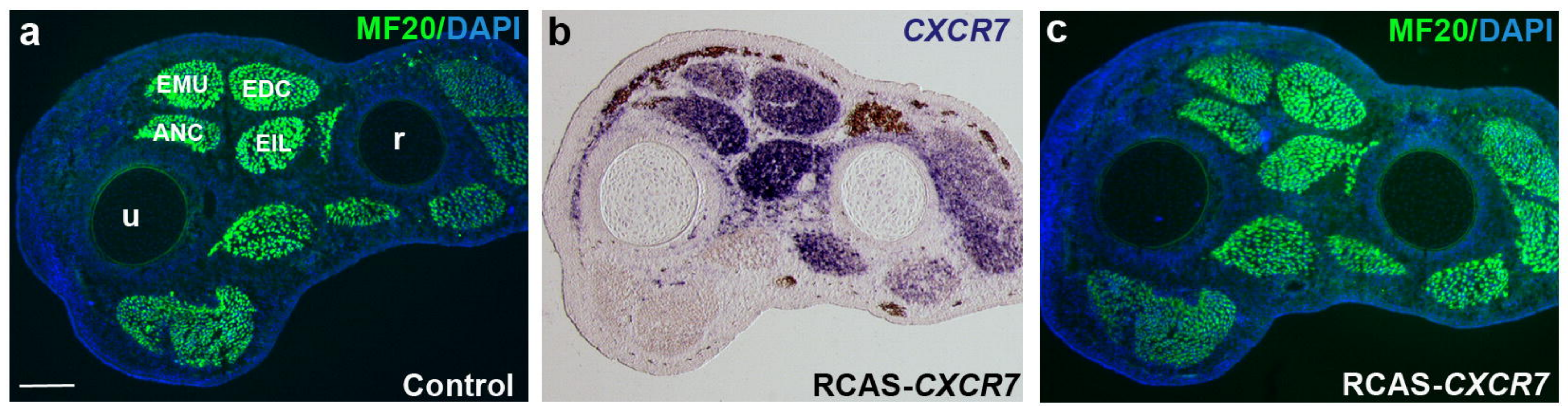
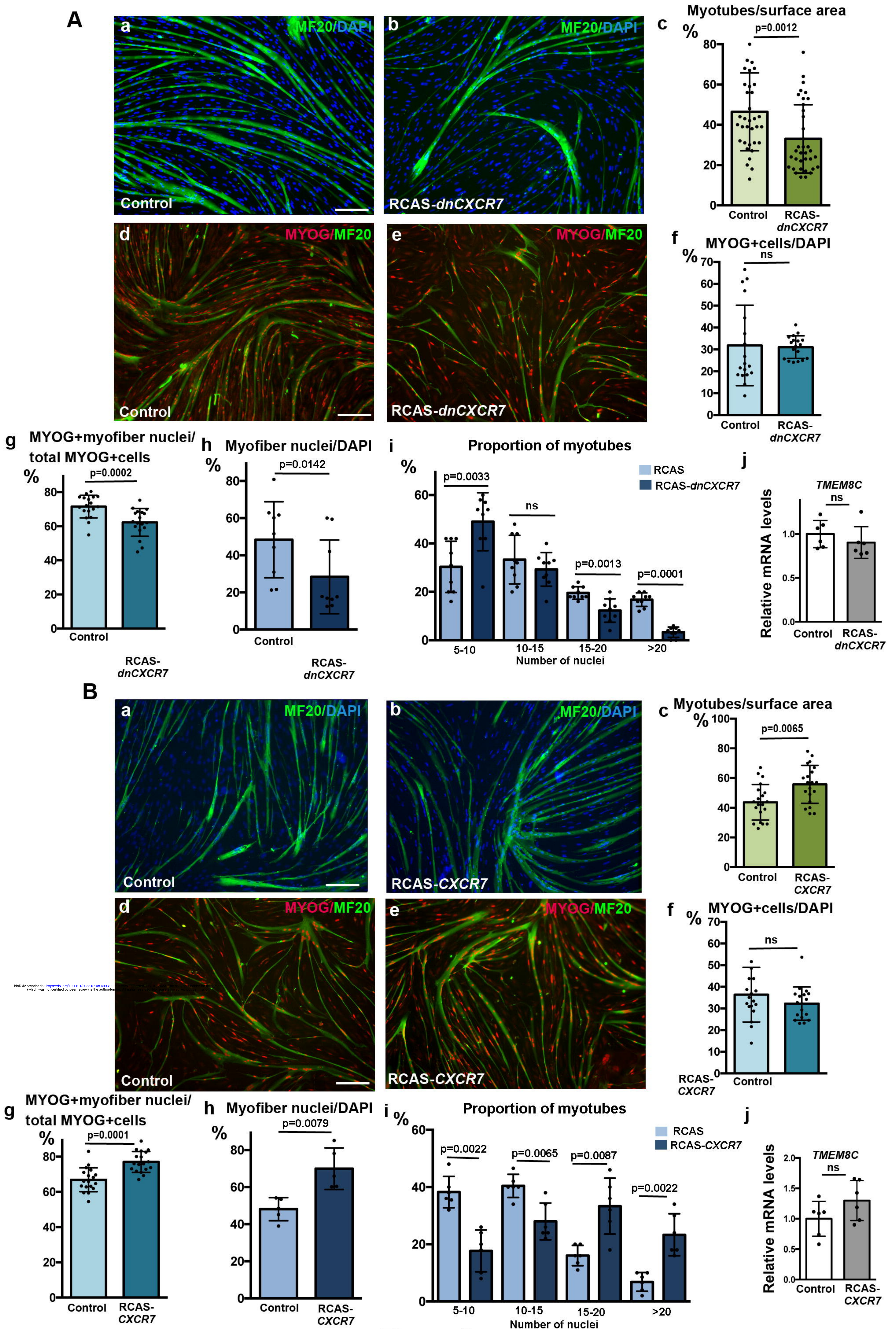


Figure 4



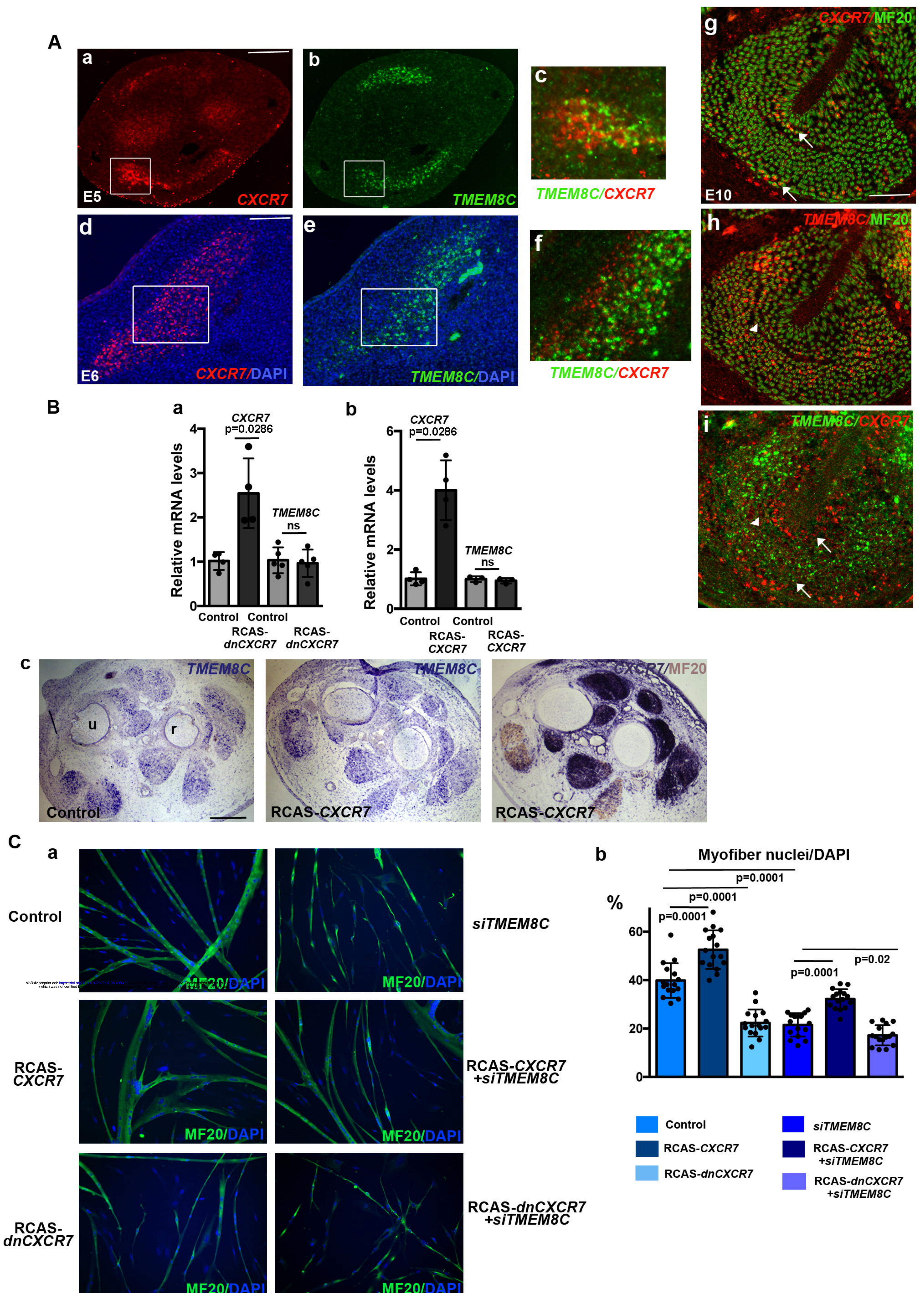


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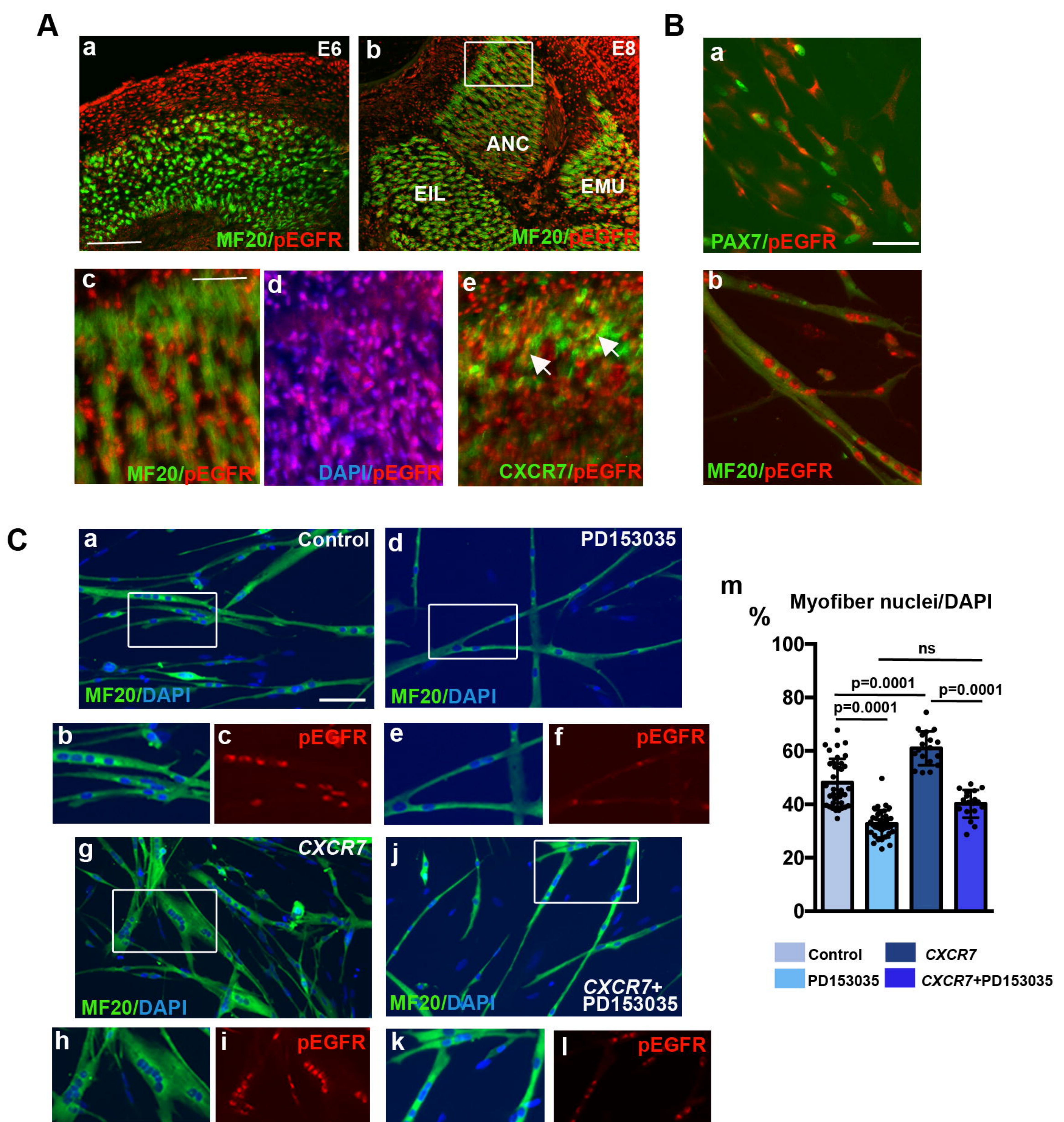


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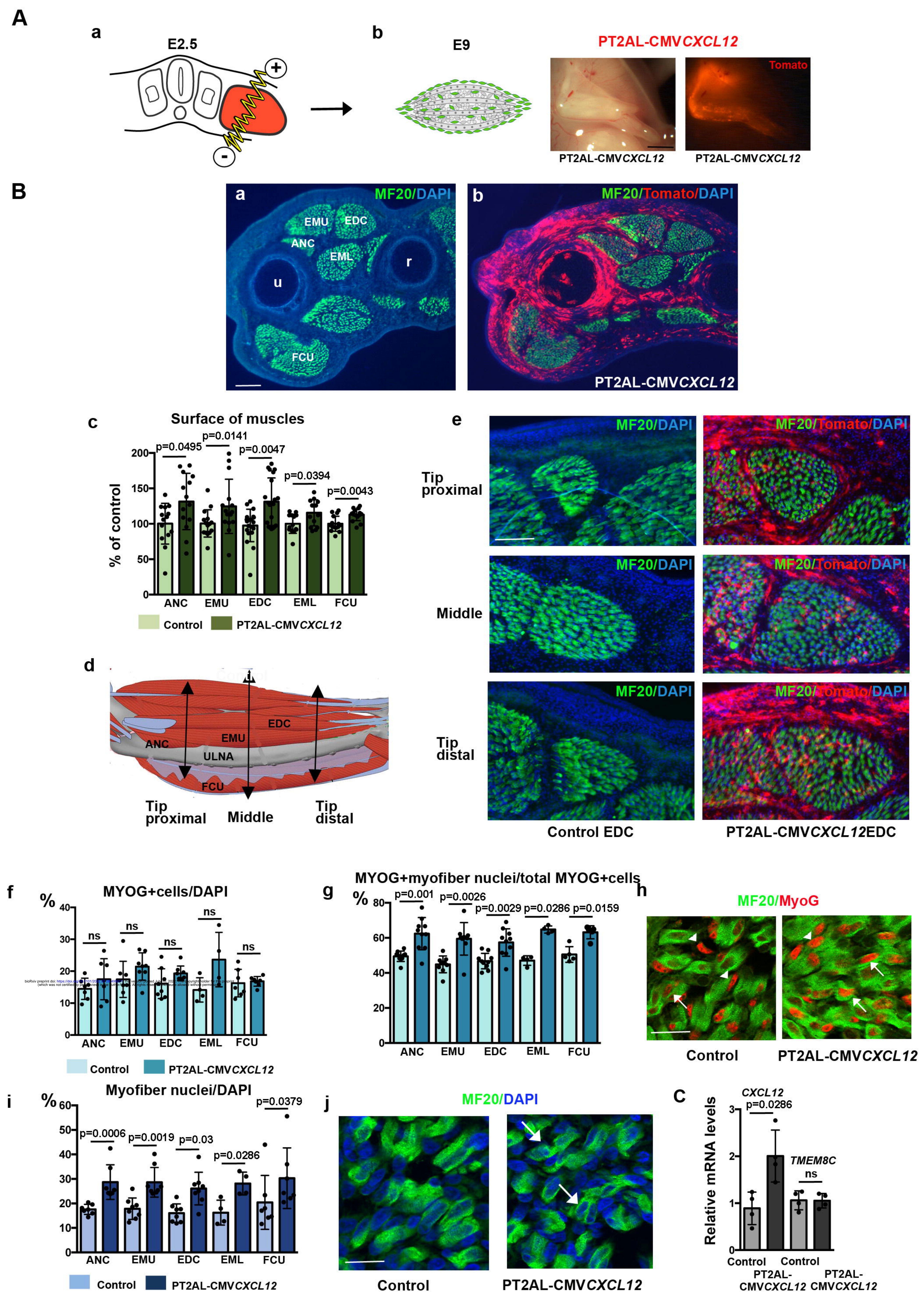
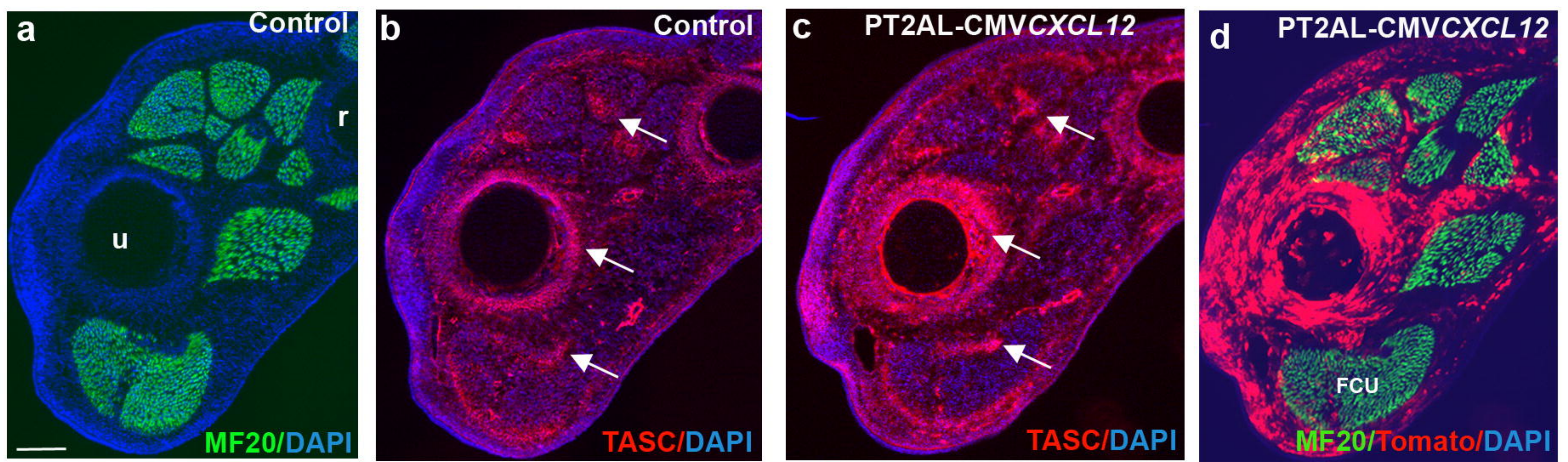
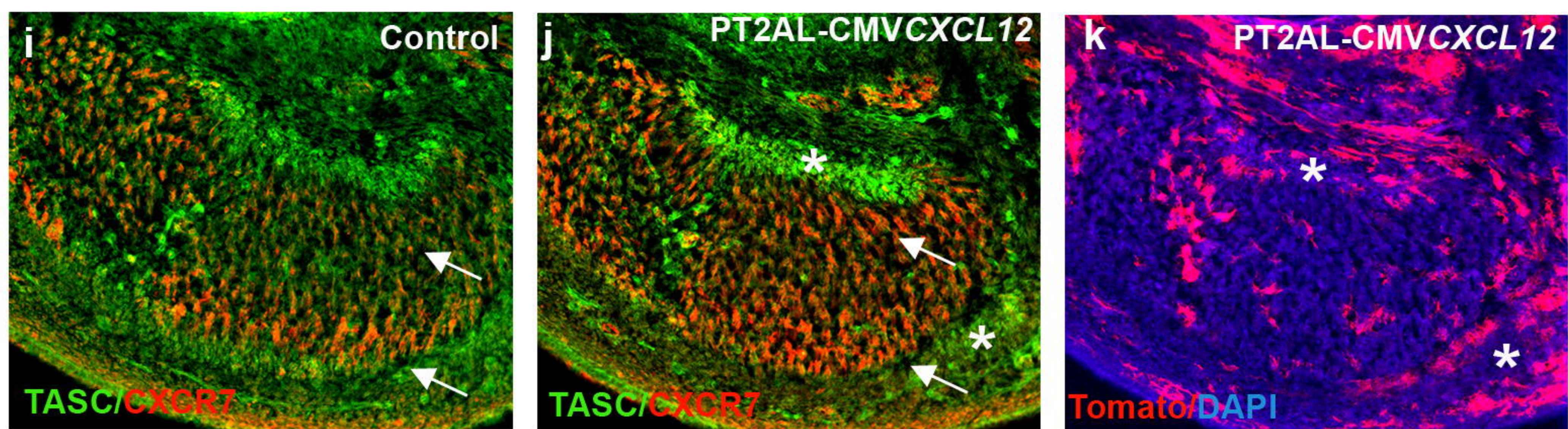
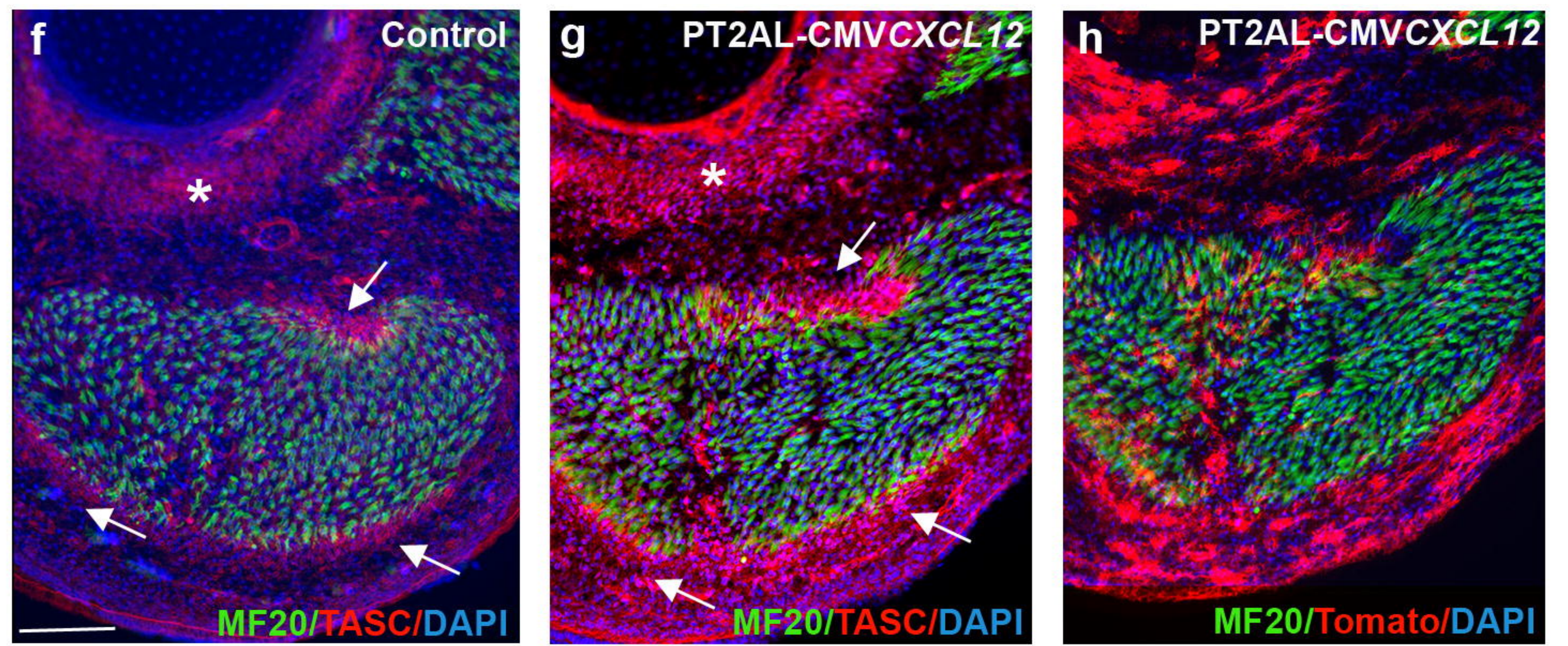
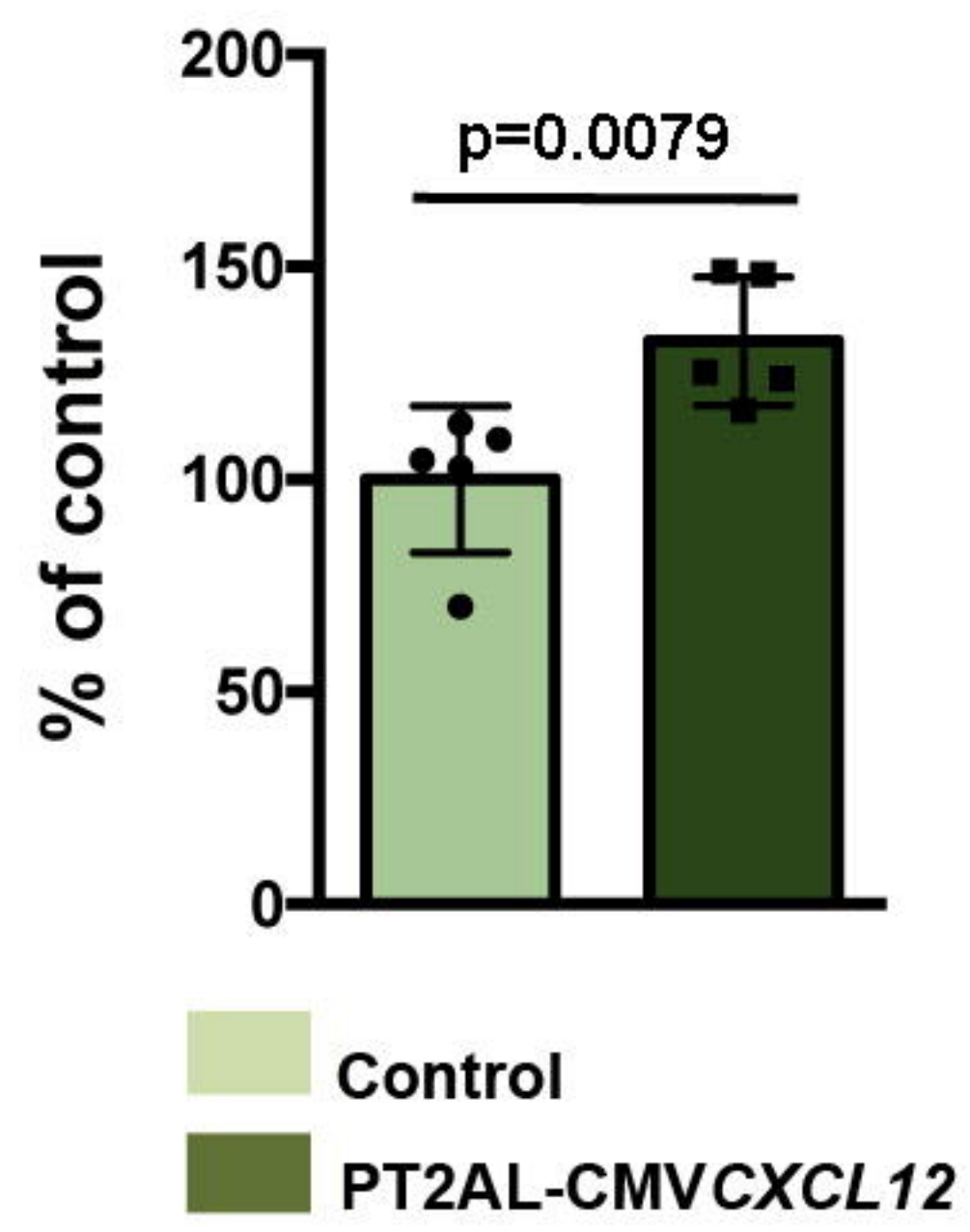


Figure 8

A



e Surface of FCU muscle



B

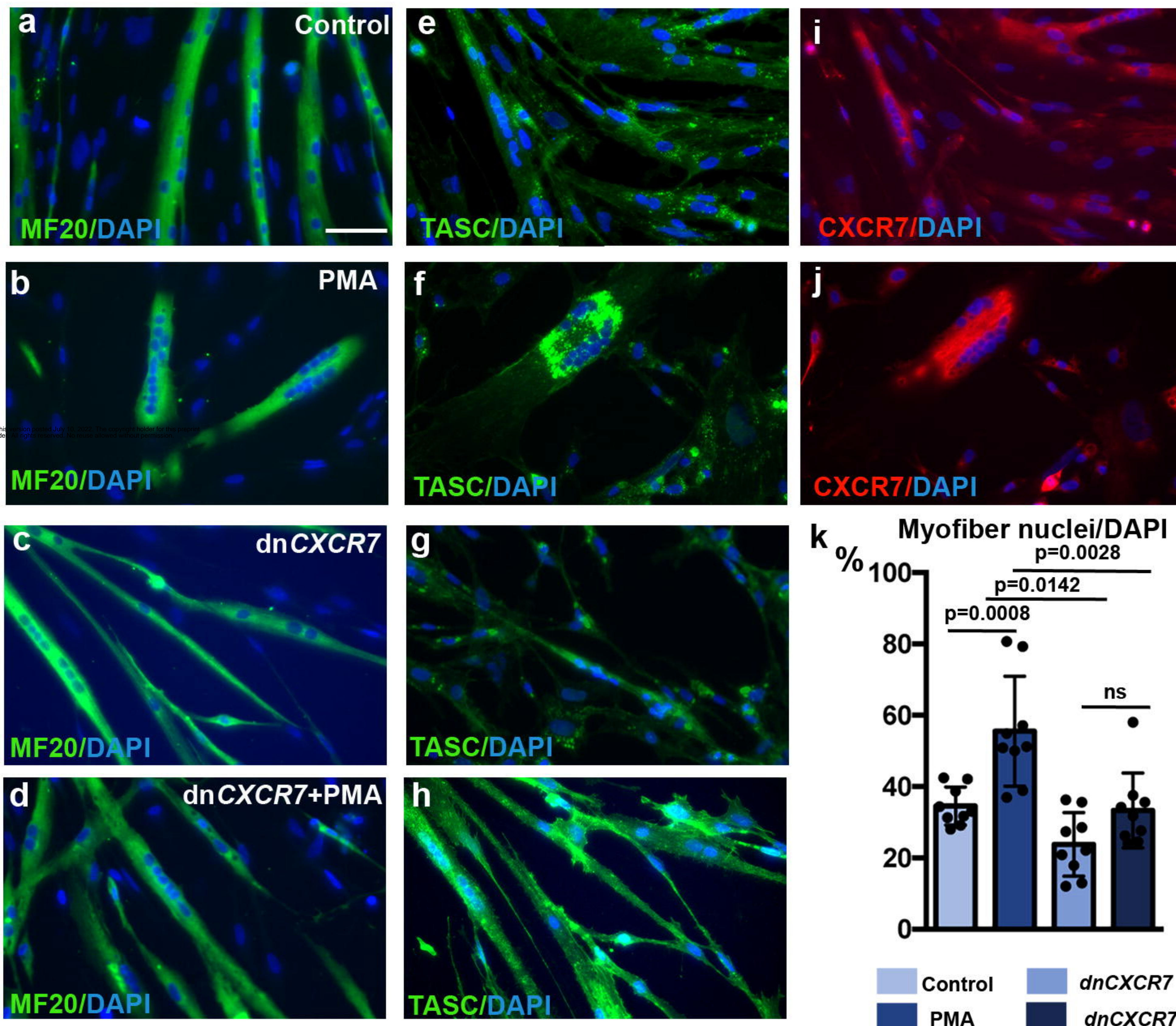


Figure 9

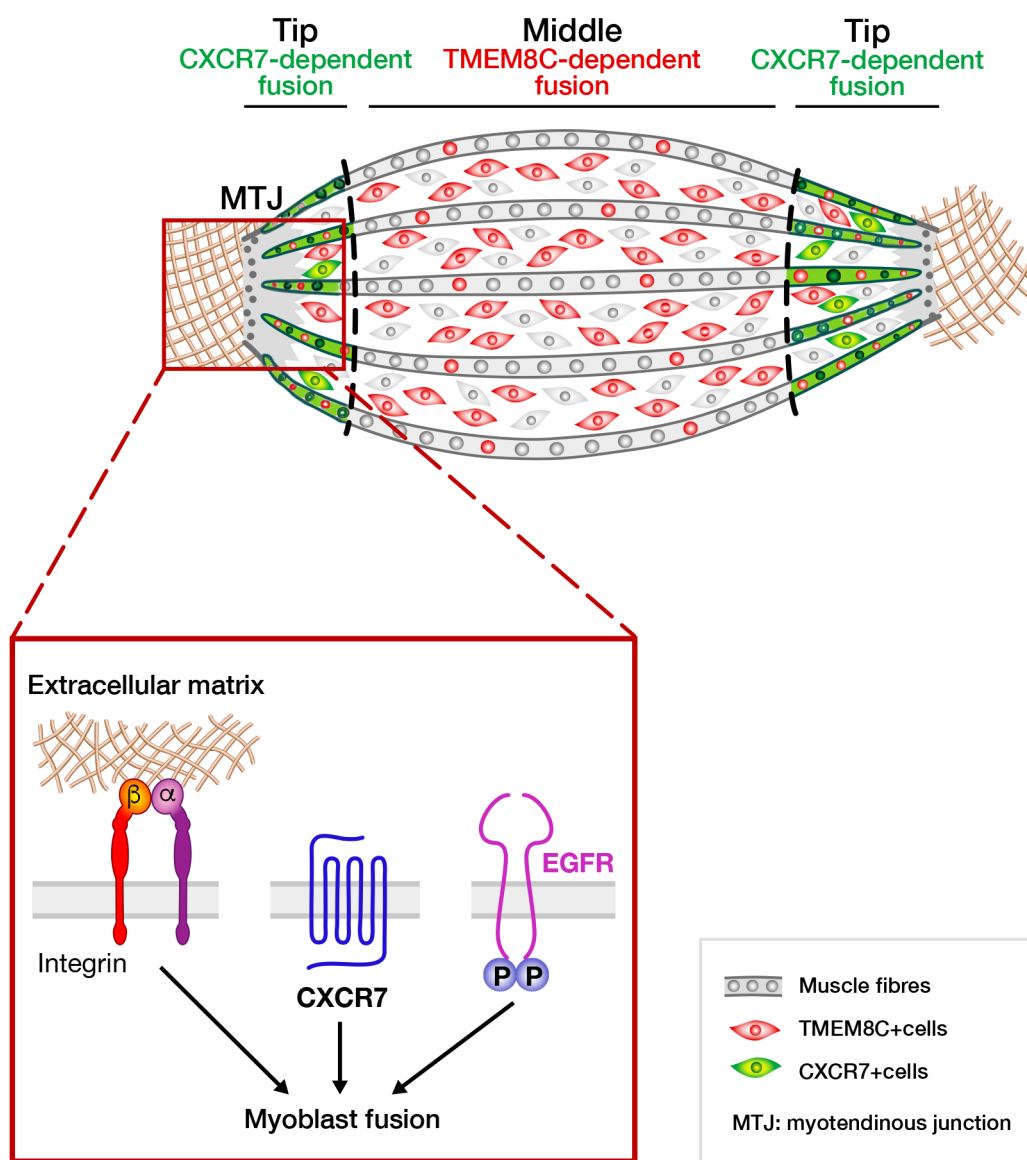


Figure 10