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Notch induced mir-708 antagonizes satellite cell migration and maintains quiescence

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37 **SUMMARY**

38 Critical features of stem cells include anchoring within a niche and activation upon
39 injury. Notch signalling maintains skeletal muscle satellite (stem) cell quiescence by
40 inhibiting differentiation and inducing expression of extracellular components of the
41 niche. However, the complete spectrum of how Notch safeguards quiescence is not
42 well understood. Here we perform Notch ChIP-sequencing and small RNA sequencing
43 in satellite cells and identify the Notch-induced microRNA-708, which is a mirtron that
44 is highly expressed in quiescent cells and sharply downregulated in activated cells. We
45 employ *in vivo* and *ex vivo* functional studies, in addition to live imaging, to show that
46 miR-708 regulates quiescence and self-renewal by antagonizing cell migration through
47 targeting the transcripts of the focal adhesion-associated protein, Tensin3. Therefore,
48 this study identifies a Notch-miR708-Tensin3 axis and suggests that Notch signaling
49 can regulate satellite cell quiescence and transition to the activation state through
50 dynamic regulation of the migratory machinery.

51

52

53 **INTRODUCTION**

54 The regenerative ability and plasticity of adult skeletal muscle is largely due to its
55 resident muscle satellite cells (Sambasivan and Tajbakhsh, 2015). In resting muscle,
56 satellite cells are quiescent and express the paired-box transcriptional factor *Pax7*
57 (Seale et al., 2000). Following injury, they re-enter the cell cycle, proliferate to generate
58 myoblasts that further differentiate and fuse to restore the damaged fibre, while a
59 subpopulation of myogenic cells returns to quiescence for self-renewal of the satellite
60 cell pool (Baghdadi and Tajbakhsh, 2018).

61

62 Notch is a crucial regulator of satellite cells as the specific depletion of RBPJ, the DNA
63 binding factor essential for mediating canonical Notch signalling, leads to spontaneous
64 differentiation that leads to progressive loss of satellite cells (Mourikis et al., 2012b;
65 Bjornson et al., 2012). Notch activity antagonizes myogenesis by induction of
66 transcriptional repressors (members of the HES/HEY family) (Mourikis et al., 2012b;
67 Bjornson et al., 2012) and we recently showed that Notch-induced production of
68 Collagen V by satellite cells is a critical component of the quiescent niche, as its
69 depletion leads to gradual diminution of the stem cell pool (Baghdadi et al., 2018).
70 However, it remains unclear if this pleiotropic signalling pathway maintains stem cells
71 by controlling additional cellular processes other than differentiation and niche
72 composition.

73

74 MicroRNAs (miRNAs) are essential for muscle homeostasis and regeneration upon
75 injury as the conditional deletion of *Dicer* (an RNase III endonuclease required for
76 maturation of miRNAs) in the PAX7+ population, results in a depletion of satellite cells
77 and a quasi-absence of repair upon injury (Cheung et al., 2012). Although numerous
78 miRNAs were reported to regulate myoblast proliferation and differentiation (Kirby et
79 al., 2015), only miR-489 (Cheung et al., 2012) was shown to regulate satellite cell
80 quiescence and/or self-renewal.

81

82 We performed RNA-sequencing on primary skeletal muscle cells (Castel et al., 2018)
83 and identified the quiescence specific miR-708 that is regulated by Notch signaling.
84 We show that this micro-RNA plays a critical role in satellite cell maintenance and
85 migration in the niche by targeting *Tensin3* (*Tns3*) mRNA, which encodes a fibrillar
86 adhesion protein that physically links integrins and the actin cytoskeleton (Qian et al.,
87 2009). Therefore, we identify a miR-708 that acts as a gatekeeper of quiescence by
88 modulating muscle stem cell migration.

89 **RESULTS**90 **A quiescence-specific microRNA is regulated by Notch signalling in satellite**
91 **cells**

92 To identify miRNAs that regulate cellular quiescence, we performed RNA-deep
93 sequencing on freshly isolated satellite cells (QSC), *in vitro* activated satellite cells for
94 60h (ASC) and differentiated cells cultured for 7 days (DIFF) (Fig. 1A; (Castel et al.,
95 2018)). We found that miR-708 was enriched in quiescent satellite cells (Fig. 1A). Real-
96 time quantitative PCR (RT-qPCR) analysis showed miR-708 expression to be
97 significantly decreased *in vivo* in ASCs 5 days post injury, and in freshly isolated
98 myofibres compared to freshly isolated satellite cells (Fig. S1A). Interestingly, miR-708
99 is a highly conserved mirtron (Ruby et al., 2007) encoded in the quiescence-specific
100 gene *Odz4/Tenm4* (Fig. S1B). As is the case with other mirtrons, miR-708 does not
101 have its own promoter, therefore its expression depends on that of *Odz4*.

102

103 Interestingly, *Odz4* expression decreases in Notch-depleted myogenic progenitors in
104 embryos (*Pax3^{Cre/+}; Rbpj^{-/-}; Myod^{+/-}*) (Brohl et al., 2012) suggesting a potential link with
105 Notch signalling. We confirmed this hypothesis by interrogating genome-wide ChIP-
106 seq data in adult murine myoblasts (Castel et al., 2013). Notably, two NICD and RBPJ
107 binding sites were identified as most proximal to the *Odz4* locus (Fig. 1B). The
108 combination of histone modifications H3K4me1, H3K27ac, and the acetyltransferase
109 p300 (Fig. 1B), in addition to their ability to induce transcription upon Notch activation
110 in cell-based luciferase assays (Fig. 1C), indicates that these sequences are in *bona*
111 *fide* enhancers.

112

113 Using genetically modified mice for Notch ablation (*Tg:Pax7-CT2; Rbpj^{Fllox}* (Mourikis et
114 al., 2012b), Fig. 1D and Fig. S1C), or gain-of-function (*Myf5^{Cre}; R26^{NICD-nGFP}* (Mourikis et
115 al., 2012a), Fig. 1E and Fig. S1D) we showed that transcription of miR-708 (but not
116 miR-489, another quiescent miRNA (Cheung et al., 2012)) and *Odz4* tightly followed
117 Notch activity. We confirmed their transcriptional responses in satellite cells isolated
118 from *Tg:Pax7-nGFP* postnatal day 8 (P8) pups in which endogenous Notch activity
119 gradually declines as cells transit from an upstream *Pax7^{High}* to a committed *Pax7^{Low}*
120 state (Mourikis et al., 2012b; Rocheteau et al., 2012) (Fig. 1F and Fig. S1E). Taken
121 together, these data demonstrate that RBPJ/NICD signalling regulates the expression
122 of *Odz4*, and by consequence miR-708, in satellite cells *in vivo* by direct binding on
123 distal transcriptional enhancers.

124

125

126 miR-708 retains stemness and self-renewal capacities of satellite cells *ex vivo*

127 To assess whether the sustained expression of miR-708 affects satellite cell
128 behaviour, we transfected freshly isolated satellite cells with a synthetic miR-708
129 molecule (Mimic-708; Fig. S1F-G for validation, specificity) and scored for proliferation
130 (EdU+) and differentiation (Myogenin+). Interestingly, miR-708 overexpression
131 exhibited a delay in cell cycle entry (24h) and proliferative capacity (48h) (Fig. 1G), and
132 a decrease in the number of MYOG+ cells compared to control at 72h and 4 days (Fig.
133 1H). Taken together, these results show that miR-708 significantly impedes satellite
134 cell proliferation and differentiation, properties reminiscent of activation of Notch
135 signalling.

136

137 In a complementary loss-of-function assay, we depleted miR-708 using short-
138 interfering RNA (AntimiR-708, Fig. S1H) in an *ex vivo* system where resident satellite
139 cells on isolated myofibres exit quiescence, enter the myogenic program and form
140 clusters composed of proliferating (PAX7+/MYOD+/MYOG-), differentiated (PAX7-
141 /MYOG+) and self-renewed (PAX7+/MYOG-) cells (Zammit et al., 2004). Targeting
142 specifically miR-708-5p increased significantly the number of differentiated cells per
143 fibre, and reduced the number of self-renewed cells (Fig. 1I).

144

145 A concern with experiments involving miR-708 manipulations is the putative effect on
146 *Odz4* expression, as germline deletion of this gene results in reduced numbers of
147 myofibres and satellite cells (Ishii et al., 2015). However, no miR-708 binding sites
148 were noted in the 3'UTR of *Odz4*, indicating that it is not a miR-708 target. Furthermore,
149 *Odz4* levels were not altered following miR-708 overexpression (Fig. S1I). Finally, the
150 induction of miR-708 in *Odz4*-deficient primary satellite cells (Fig. S1J) rescued their
151 precocious differentiation phenotype (Fig. 1J). Thus, we conclude that in this context,
152 the anti-myogenic effect of miR-708 was not due to *Odz4* function.

153

**154 Antagonism of miR-708 *in vivo* induces spontaneous exit from quiescence and
155 premature differentiation of satellite cells**

156 To investigate the functional role of miR-708 *in vivo*, we synthesized a miR-708
157 antagonist (AntagomiR-708) with an antisense sequence to mature miR-708-5p, as
158 well as control Scramble. Importantly, RT-qPCR analysis on different cell populations
159 extracted from skeletal muscles, namely endothelial cells, fibro-adipogenic
160 progenitors, resident and infiltrating macrophages revealed that only satellite cells
161 expressed miR-708 (data not shown). We then labelled the majority of satellite cells
162 with a tamoxifen inducible membrane-GFP using *Tg:Pax7-CreERT2; R26^{mTmG}* lineage-

163 traced mice (~95% recombination efficiency, Fig. S2A). Surprisingly, 10 days after
164 injection (Fig. 2A), isolated GFP+ cells from resting muscle showed a significant
165 reduction in miR-708 and miR-489 levels, whereas miR-92 expression (activation
166 enriched miRNA, Fig. 1A) was strongly upregulated (Fig. 2B). In addition, a significant
167 decrease in *Pax7* expression was noted, whereas expression of the activation (*Myod*)
168 and differentiation (*Myogenin*) genes were strongly upregulated following AntagomiR-
169 708 treatment (Fig. 2C). Accordingly, *in vivo* immunostaining showed that 18% of
170 mGFP+ cells lost PAX7 expression in mice that received AntagomiR-708 during this
171 period (Fig. 2D).

172

173 We previously showed that compromised Notch signalling *in vivo* induces satellite cells
174 to differentiate spontaneously without entering S-phase (Mourikis et al., 2012b).
175 However, satellite cells lacking miR-708 incorporated BrdU following activation (Fig.
176 2E), indicating that miR-708 knock-down induces their entry into S-phase prior to
177 differentiation. In addition, satellite cells isolated from AntagomiR-708 treated mice
178 exhibited premature expression of Myogenin (Fig. 2F) and consequently an increase
179 in myotube formation as indicated by a higher fusion index (Fig. 2G). Taken together,
180 these results indicate that reduced miR-708 levels in satellite cells leads to
181 spontaneous exit from quiescence, activation, and premature differentiation.

182

183 Interestingly, long-term inhibition of miR-708 (28 days; Fig. 2A) showed a 50%
184 reduction in satellite cell numbers *in vivo* (Fig. 2H and Fig. S2B). Furthermore,
185 immunostaining with cleaved-caspase 3 showed no significant change in the number
186 of apoptotic cells following AntagomiR-708 treatment (Fig. S2C). In contrast, numerous
187 GFP+ fibres were observed in resting muscle indicating that mGFP+ satellite cells
188 spontaneously fused with pre-existing myofibres (Fig. 2I). Taken together, these
189 results demonstrate that miR-708 is necessary for maintaining satellite cell quiescence
190 *in vivo*. Of note, transcript analysis of this residual population showed normal levels of
191 miR-708 as well as quiescence and differentiation markers (Fig. S2D) suggesting that
192 they escaped AntagomiR-708 treatment.

193

194 In the context of muscle damage, AntagomiR-708 treated mice displayed a slight delay
195 in regeneration at 14 days post-injury (dpi) as indicated by smaller myofibres (Fig. S2E)
196 however, this delay was not prominent by 31 dpi (Fig. S2F). In keeping with this finding,
197 PAX7+ cells were observed at this stage, albeit at much reduced numbers, suggesting
198 that they were escapers. Notably, the severe defect in self-renewal of AntagomiR-

199 treated mice following injury and regeneration (Fig. 2J, K, S2G) indicates that miR-708
200 also regulates re-entry into quiescence.

201

202 **miR-708 promotes satellite cell niche occupancy *in vivo* and regulates their**
203 **motility and migration capacities**

204 Suppression of miR-708 has been shown to promote invasion and metastasis of
205 numerous cancer cells types by targeting different genes or pathways involved in the
206 regulation of cell migration (Lin et al., 2015; Ryu et al., 2013). During homeostasis,
207 satellite cells are localized between the myofibre membrane and the basal lamina
208 (Sambasivan and Tajbakhsh, 2015). Surprisingly, we observed abnormal localization
209 of PAX7+ cells in the interstitial space in the TA muscle of AntagomiR-708 treated mice
210 (Fig. 3A) suggesting that loss of miR-708 perturbed the anchorage of satellite cells and
211 resulted in their release from their niche.

212

213 We confirmed this phenotype by overexpressing miR-708 in freshly isolated satellite
214 cells combined with videomicroscopy (Fig. 3B). In addition to the decrease in the
215 number of dividing cells (Fig. 1G), the migration distance and velocity of myogenic cells
216 treated with miR-708 were strongly diminished (Fig. 3C-D; see Supplementary Movie
217 1). Notably, miR-708 also altered the motility of *Odz4*-depleted satellite cells (Fig. S3A)
218 underscoring its specific involvement in this process. Taken together, these results
219 show that miR-708 actively inhibits the migration and motility of satellite cells.

220

221 Recently, intravital imaging showed that satellite cell properties can be monitored *in vivo*
222 (Webster et al., 2016). To confirm the observed *in vitro* phenotype directly in
223 muscle, we optimized live microscopy on freshly isolated mouse hind limb explants
224 containing the *Extensor digitorum longus* (EDL) muscle from Scramble- or
225 AntagomiR-treated mice using 2-photon microscopy. Intriguingly, over the 10-12h
226 imaging period, no displacement was detected in control Scramble (n=6 mice, ≥ 250
227 cells, Fig. 3E). In contrast, miR-708 silencing *in vivo* resulted in 9% of satellite cells
228 spontaneously exiting their niche and migrating an average distance of $8.7 \pm 6.5 \mu\text{m}$
229 with an average velocity of $7.8 \pm 4.4 \text{ nm/min}$ (Fig. 3E, F and Supplementary Movies 2-
230 5). These experiments provide direct evidence for a role of miR-708 as an active
231 repressor of satellite cell migration.

232

233 To identify miR-708 putative targets, we combined three target prediction algorithms
234 (see Fig. 3G) and identified 24 overlapping genes, among which 3 were highly
235 expressed in activated compared to quiescent satellite cells (Liu et al., 2013): Tensin-

236 3 (*Tns3*), Dickkopf3 (*Dkk3*) and Syndecan-1 (*Sdc1*) (Fig. S3B). Their relevance as
237 potential targets was tested by transfection of HEK293 cells with a luciferase reporter
238 vector containing the 3'-untranslated region (UTR) of *Tns3*, *Dkk3* or *Sdc1* (Table S2).
239 Notably, miR-708 repressed luciferase activity of both *Dkk3* and *Tns3*, but not *Sdc1*
240 (Fig. 3H). Interestingly, only *Tns3* inhibition (Fig. S3C) phenocopied the
241 overexpression of miR-708 and significantly decreased cell migration (Fig. 3I),
242 whereas *Dkk3* knock-down showed no overt cell motility phenotype (data not shown).

243

244 Mutations in *Tns3* miRNA-binding sites significantly decreased miR-708-mediated
245 repression (Fig. 3H), as confirmed by a Target Protector assay combined with
246 videomicroscopy (Fig. S3D). In keeping with these observations, overexpression of
247 TNS3 in satellite cells was sufficient to rescue the inhibition of migration mitigated by
248 mir-708 (Fig. 3J and Supplementary Movie 6). In summary, these findings demonstrate
249 that miR-708 exerts its repressive activity on cell migration primarily through *Tns3*.

250

251 **Inhibition of Focal Adhesion Kinase represses migration and G1-S transition**

252 *Tns3* is a member of focal adhesion (FA)-associated proteins that are important
253 regulators of cell adhesion and migration (Blangy, 2017). Moreover, the Tensin SH2
254 domain forms a complex with the tyrosine phosphorylated protein FAK allowing its
255 phosphorylation and activation (Qian et al., 2009) (Fig. 4A). Notably, miR-708
256 overexpression in myogenic cells showed a reduction in TNS3 protein levels (Fig. 4B
257 and S4A for antibody validation) whereas FAK phosphorylation (p-FAK) levels
258 decreased (Fig. 4B). The presence of miR-708 did not affect the ratio of ERK/p-ERK
259 suggesting that p-FAK was inhibited at the local level, rather than by interference of
260 upstream effectors of the migration pathway (Fig. 4B and S4B). As observed with miR-
261 708 gain-of-function, specific inhibition of *Tns3* resulted in downregulation of p-FAK
262 (Fig. 4C). In contrast, the absence of miR-708 in primary satellite cells (AntagomiR),
263 resulted in increased levels of TNS3 and p-FAK (Fig. 4D and S4C). This decrease in
264 p-FAK was verified by immunostaining satellite cells transfected with either Mimic-708
265 or siTns3 (Fig. 4E-E'). Notably, temporal *in vivo* activation showed a rapid increase in
266 *Tns3* expression within 3h following injury (Fig. S4D) and appearance of TNS3
267 preceded that of MYOD and cell cycle entry (Fig. S4E) indicating that release of cell
268 migration is an early event during activation.

269

270 We note that miR-708 overexpression resulted in a delay in cell proliferation of cultured
271 satellite cells (Fig. 1G), however, analysis of miR-708 putative targets did not identify
272 cell cycle regulators. Thus, we propose that the inhibition of migration indirectly inhibits

273 cell cycle progression. To uncouple proliferation and migration properties, we induced
274 miR-708 in cycling satellite cells isolated from Fucci-Green mice (Fluorescence
275 ubiquitination-based cell cycle indicator), which allows temporal patterns of cell-cycle
276 dynamics using Azami green to label S/G2/M phases (Sakaue-Sawano et al., 2008),
277 combined with live videomicroscopy. Intriguingly, total duration of cell cycle was
278 extended by 25% following miR-708 overexpression (Fig. 4F and Supplementary
279 Movie 7) and this was due to a significant extension of G1 (Fig. 4G and H). These
280 findings indicate that miR-708 exerts cell cycle regulation by modulating the G1 phase
281 of the cell cycle.

282

283 **DISCUSSION**

284 A major question in stem cell biology is how cells enter and exit their niche, and how
285 this interaction is stabilised to maintain the stem cell pool for extended periods. Active
286 cell migration is a key property of satellite cells (Siegel et al., 2009), and studies have
287 shown that stimulation of migration improves myogenic cell dispersal following
288 transplantation, thereby resulting in enhanced engraftment efficiency (Bentzinger et
289 al., 2014). Interestingly, it was reported that reduced levels of Fibronectin and β 1-
290 integrin during ageing results in loss of satellite cells from their niche (Lukjanenko et
291 al., 2016) thereby underscoring the notion that multiple pathways converge to maintain
292 the stem cell pool. However, it remains unclear how intrinsic regulators modulate the
293 transition into and out of cellular quiescence and how cell division and migration are
294 orchestrated with the anchoring of stem cells in their niche.

295

296 In this study, we identified miR-708 as a quiescence-specific mirtron in the *Odz4* gene,
297 where this miRNA acts as a downstream target of Notch signalling to maintain satellite
298 cells within their quiescent niche. Although the mechanism of action of *Odz4* has not
299 been determined in satellite cells, it is intriguing that miR-708 is harboured within this
300 gene, and under the regulation of Notch. Although examples of mirtrons performing
301 regulatory functions have been reported for skeletal and cardiac muscle-specific genes
302 (Cheung et al., 2012; van Rooij et al., 2009), here we provide the first example of such
303 a mirtron that is under the regulation of Notch signalling, and a rare example of a
304 miRNA that controls the maintenance of an adult stem cell population.

305

306 In silico analysis of miR-708 potential target genes identified *Tns3* that responded in
307 functional assays to this miRNA and phenocopied the functional readouts of miR-708.
308 The role of this Tensin isoform on cell migration is context dependent, and it can act to
309 promote or inhibit cell mobility depending on the presence of co-factors (Qian et al.,

310 2009). However, our findings lead us to propose that TNS3 is maintained at low levels
311 by the action of miR-708 in the anchored quiescent satellite cells via the inhibition of
312 FAK. Upon activation, Notch signaling and hence miR-708 are downregulated,
313 allowing *Tns3* to accumulate and participate in the migration of the activated cells.

314

315 Interestingly, FAK has been shown to regulate cell cycle progression by modulating
316 the G1/S transition (Zhao et al., 1998). Therefore, we propose that indirect inhibition of
317 FAK by miR-708 could be responsible of this extension of the G1/S transition. Our
318 observations are concordant with a previous study showing that FAK signalling
319 regulates cell cycle progression via the activation of *cyclin D1* (*Ccnd1*) transcription
320 through EtsB binding site of the *Ccnd1* promoter (Zhao et al., 2001).

321

322 In summary, we provide the first evidence that inhibition of the migration capacity of a
323 stem cell is essential for maintaining the quiescent cell within its niche. We uncover a
324 regulatory axis where Notch induces transcription of the quiescence specific mirtron
325 miR-708 that represses Tensin3, a major component of the focal adhesion complex,
326 to inhibit FAK activation, which in turn stabilises satellite cells within their niche. For
327 quiescent stem cells to enter or exit their niche, they need to coordinate their final
328 mitosis as well as cell migration. Given that satellite cells are rarely found to be
329 subjacent, our results lead us to speculate that satellite cells continue to migrate after
330 their last mitosis, then migration is suppressed through miR-708 as cells are then
331 anchored in their niche. These findings suggest that mitosis and migration might be
332 negotiated sequentially in other quiescence stem cell populations.

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344

345 **Authors contributions**

346 S.T. and M.B.B. proposed the concept and designed the experiments. M.B.B.
347 performed and analysed most of the experiments. D.C. performed the RNA-
348 sequencing. D.D.G. initiated the Western Blots experiments. J.F. processed and
349 analysed the *in vitro* videomicroscopy. P.M. generated the enhancer plasmids. B.E
350 optimized section immunostainings and designed the cloning strategies. K.S.
351 performed and analysed the *ex vivo* explant imaging. M.B.B. and S.T. wrote the paper.
352 All authors read and agreed on the manuscript.

353

354 **Competing Financial Interests statement**

355 The authors declare no competing interests.

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Figure legends

Fig. 1. miR-708, a Notch pathway target mirtron specifically expressed in quiescent satellite cells, suppresses proliferation and differentiation while its inhibition impairs self-renewal.

(A) Gene expression from RNA deep sequencing of freshly isolated satellite cells from *Tg:Pax7-nGFP* adult mice (QSC, n=2), *in vitro* activated satellite cells for 60h (ASC, n=3) and differentiated cells cultured for 7 days (DIFF, n=2).

(B) ChIP-seq tracks showing NICD/RBPJ occupancy on enhancers associated with mouse *Odz4* locus. Orange rectangle indicates RBPJ binding positions.

(C) Transcriptional response measured by Firefly luciferase signal of two enhancers of *Odz4* to activation of Notch signalling in C2C12 cells cultured on Dll1-Fc. Control Fc is set at 1; n=3 independent assays.

(D) RT-qPCR analysis of miR-708 in Control (*Tg:Pax7-CT2; Rbpj^{+/+}*) and *Rbpj* conditional KO (*Rbpj* null; *Tg:Pax7-CT2; Rbpj^{flox/flox}*) satellite cells isolated from resting muscles.

(E) Induction of miR-708 in E14.5 control (*Myf5^{Cre/+}; R26^{mTmG/+}*) and *Myf5^{Cre}-NICD* (*Myf5^{Cre/+}; R26^{stop-NICD-nGFP/+}*) myogenic cells isolated by FACS, assessed by RT-qPCR. *let-7e* is not a Notch target; n=4 mice or fetuses/genotype.

(F) Transcript expression levels of miR-708 targeted by Notch in cells fractionated by FACS based on GFP-intensity from *Tg:Pax7-nGFP* mice at 8 postnatal day (P8): *Pax7^{High}* (20%), *Pax7^{Mid}* (40%) and *Pax7^{Low}* (20%); n=3 pups.

(G) EdU reaction and GFP staining of satellite cells isolated from *Tg:Pax7-nGFP* mice 24h after Mimic-708 or Control (Ctr) transfection. Time course of proliferation by quantification of EdU 24h to 4days following miR-708 overexpression (Mimic-708) or Control.

(H) Myogenin staining of satellite cells isolated from *Tg:Pax7-nGFP* mouse 72h after Mimic-708 or control Scramble transfection. Quantification of Myogenin+ cells at 72h and 4 days following treatment; n=4 mice, ≥400 cells, 2 wells/condition.

(I) miR-708 knock-down using AntimiR-708 transfection of single EDL myofibres from *Tg:Pax7-nGFP* mice cultured for 72h and immunostained for GFP and Myogenin. Quantification of *PAX7⁺/MYOG⁻* (21% vs. 5%), *PAX7⁻/MYOG⁻* (36% vs. 15%) and *PAX7⁻/MYOG⁺* (43% vs. 79% for Scramble and AntimiR-708 respectively) populations; 4 mice/condition, n=25 fibres.

(J) Satellite cells isolated by FACS from control and *Odz4* null mice, treated with Mimic or Control for 72h and stained for Myogenin. Quantification of Myogenin+ cells; n=3 mice/condition, ≥250 cells.

Error bars, mean \pm SD; two-sided unpaired t-test; #p-value: two-sided paired t-test.

Scale bar: 50 μ m. See also Supplementary Figure S1.

Fig. 2. miR-708 maintains the satellite cell quiescent state.

(A) Experimental scheme of tamoxifen, AntagomiR and BrdU administration to *Tg:Pax7-CT2; R26^{mTmG}* mice. AntagomiR-708 and Scramble control were injected every day in the tail vein for 4 days after the end of tamoxifen treatment (D0) and mice were analysed 10 days (B-G) and 28 days (H-I) post-treatment.

(B) miRNA expression in control (Scramble) and miR-708 knock-down (AntagomiR-708) cells isolated by FACS 10 days post-treatment.

(C) mRNA expression in control and AntagomiR-708 cells isolated by FACS 10 days post-treatment.

(D) Representative images of satellite cells on *Tibialis anterior* muscle section from Scramble and AntagomiR-708 treated mice stained for membrane-GFP and PAX7. Quantification of mGFP+/PAX7- cells (arrow) relative to total mGFP+ cells; n=4 mice/treatment.

(E) membrane-GFP+ satellite cells on TA muscle section from Scramble and AntagomiR-708 treated mice stained for BrdU. Quantification of mGFP+/BrdU+ cells relative to total mGFP+ cells. Asterisk shows mGFP-/BrdU+ cells; arrows indicate mGFP+/BrdU+ cell; n=4 mice/treatment.

(F) Myogenin and GFP immunostainings of satellite cells cultured for 72h and quantification; n=3 mice/treatment; ≥150 cells.

(G) Myosin Heavy Chain (MyHC) staining of satellite cells from Scramble and AntagomiR-708 treated mice isolated by FACS, cultured for 5 days and quantification of fusion index (nuclei in MyHC+ myotubes with ≥2 nuclei/total number of nuclei); n=3 mice/treatment, ≥500 nuclei, 2 wells/condition.

(H) Laminin and PAX7 immunostaining on quiescent TA muscle section from Scramble and AntagomiR-treated mice (10dpa) and quantification of PAX7+ cells/mm²; n=6 Scramble and n= 8 AntagomiR treated mice.

(I) Immunostaining for Laminin and GFP on sections from TA muscles of mice 28 days post-Scramble and AntagomiR-708 treatment, inset shows whole TA section.

(J) Schemes of *Tg:Pax7-CT2; R26^{mTmG}* mice fed with tamoxifen for 2 weeks, injured with Cardiotoxin (CTX) at D0 followed by daily AntagomiR-708, or Scramble injections for 4 days. Muscles were collected 14 days post-injury.

(K) Laminin and PAX7 immunostaining on injured TA muscle section from Scramble and AntagomiR-treated mice (14 days post-injury) and quantification of PAX7+ cells/mm²; n=3 Scramble and n= 4 AntagomiR treated mice.

Error bars, mean ± SD; two-sided unpaired t-test. Scale bar: 25µm (D, F), 50µm (E, G, H, K), 100µm and 300µm in inset (I). See also Supplementary Figure S2.

Fig. 3. miR-708 regulates myogenic cell migration and motility.

(A) Immunostaining for Laminin and PAX7 on sections from resting TA muscles 10 days post Scramble and AntagomiR-708 treatment and quantification of PAX7+ cells in interstitial space (2% for Scramble vs. 38% for AntagomiR-708); n=4 mice; 2 sections/TA.

(B) Scheme of miR-708 overexpression on membrane-GFP+ satellite cells isolated from *Tg:Pax7-CT2; R26^{mTmG}* mice treated 2 weeks with tamoxifen.

(C) Maximum projection of 48h-time-lapse experiment of mGFP+ cells overexpressing miR-708 (Mimic-708) and Control. Coloured line depicts trajectory of a cell for every condition. See Supplementary Movie 1.

(D) Distance and velocity of miR-708 overexpressing cells (Mimic-708) and control were scored for 48h; n=30 cells.

(E) Maximum projection of EDL muscle from Scramble or AntagomiR-708 treated mice imaged on an explant at 0h and 12h (n=6 mice/treatment). Arrows point tracked cells. See Supplementary Movies 2-5.

(F) Migration distance of *in vivo* AntagomiR or Scramble-treated satellite cells on an explant imaged for 12h (0 cells displace in Scramble vs. 13 cells in AntagomiR); n=6 mice/treatment; n=250 cells tracked.

(G) Venn Diagram displaying putative targets of miR-708 as predicted by TargetScan, TargetRank and MiRDB.

(H) Schemes of *Tns3* (*Tensin-3*), *Dkk3* (*Dickkopf-3*), *Sdc1* (*Syndecan-1*) constructs and mutated *Tns3* 3'UTR with relative luciferase activity associated with each in presence (Mimic-708) or absence (Mimic-Ctr) of miR-708. Note, analysis using full 3'UTR length of *Sdc1* and *Dkk3* and truncated *Tns3* 3'UTR; n=6 independent experiments, 2 wells/condition.

(I) Migration properties assessed by transwell assay, of miR-708-overexpressing (Mimic-708) or upon *Tns3* knock-down (si*Tns3*) compared to control: Mimic-Ctr, and siCtr respectively. Quantification of number of migrating satellite cells through pores of the membrane upon treatment; n=4 mice, 2 fields counted/membrane.

(J) Velocity delayed in presence of miR-708 is rescued by overexpression of TNS3. See Supplementary Movie 6.

Error bars, mean \pm SD; two-sided unpaired t-test (A), Fisher (F), Mann-Whitney test (H, J), two-sided paired t-test (I). Scale bar: 50 μ m (A-C), 20 μ m (E) and 10 μ m (A inset and I). See also Supplementary Figure S3.

Fig. 4. Tensin-3 inhibition reduces FAK activation and phenocopies miR-708 overexpression.

(A) Signaling pathway for cell migration mediated by ERK. Growth factors (PDGF, EGF and FGF) trigger downstream signaling Ras-Raf-MEK that further activate ERK. Activated ERK regulates focal adhesion dynamics by phosphorylation of FAK and Paxillin. Tensin-3 can interact with FAK (Chen and Lo, 2003).

(B) Western blot showing protein levels and phosphorylation state of FAK, TNS3, Paxillin and ERK after miR-708 overexpression (Mimic-708) and Control (Ctr) in C2C12 myoblasts; n=4 independent experiments.

(C) Protein levels and phosphorylation of FAK upon *Tns3* knock-down (siTns3) in C2C12 cells; n=4 independent experiments.

(D) TNS3 and p-FAK protein levels in freshly isolated satellite cells from Scramble and AntagomiR-treated mice.

All Western-blots were normalized to GAPDH levels; n=3-4 mice/condition.

(E-E') Actin staining with Phalloidin and immunostaining of p-FAK on satellite cells isolated from *Tg:Pax7-nGFP* mouse and transfected with Mimic-708 (E) or siTns3 (E'). Arrowheads show p-FAK positive signal. Two fields from the same culture dish are shown, separated by line; n=4 mice/condition.

(F) Cell cycle duration (hours) of satellite cells isolated from Fucci-green mouse treated with Mimic-708 or control and tracked for one division.

(G) Cell cycle repartition of Fucci-green satellite cell following control and miR-708 overexpression (Mimic-708). Cells were tracked for one cell cycle.

(H) Time series of a Fucci-green satellite cell transfected with Mimic-708 or control with corresponding kymograph. Distance between two arrows represents one cell cycle; n=3 mice, 15 cells tracked/condition. See Supplementary Movie 7.

Error bars, mean \pm SD; two-sided unpaired t-test (B-D); Mann-Whitney test (F-G). Scale bar: 25 μ m. See also Supplementary Figure S4.

STAR Methods

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Shahragim Tajbakhsh (shahragim.tajbakhsh@pasteur.fr).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

Animals housing, husbandry and handling were performed in the animal facility of Institut Pasteur in accordance with the national and European community guidelines. The health and immune status of all mice used were normal, and they were not involved in any previous procedures. Food and water were administered *ad libitum*. For Notch *in vivo* gain-of-function, *Myf5^{Cre}* mice (Haldar et al., 2008) were crossed with *R26^{stop-NICD-nGFP}* (Murtaugh et al., 2003), and fetuses were recovered 14.5 days post-fertilization. For Notch *in vivo* knock-down, *Tg:Pax7-CreERT2* (Mourikis et al., 2012b) was crossed with *Rbpj^{fllox/fllox}* (Han et al., 2002) and the lineage tracing mouse line *R26^{mTmG}* (Muzumdar et al., 2007). The *Tg:Pax7-nGFP* mouse line was generated in the Tajbakhsh lab (Sambasivan et al., 2009) with a BAC containing approximately 200 kbp of mouse genomic DNA including the locus encoding *Pax7* and sequences both upstream (~55 kbp with respect to *Pax7* initiator ATG) and downstream (~60 kbp from terminator codon). The targeting vector was designed to introduce nGFP (nuclear GFP) into the first exon of *Pax7* gene (mutated initiator ATG and deleted bases 58-94 of exon 1). *Odz4* KO mice were kindly provided by Drs. Chihiro Akazawa and Nobuharu Suzuki (Ishii et al., 2015) and Fucci-Green mice (Sakaue-Sawano et al., 2008) by Dr. Atsushi Miyawaki. All experiments were performed indistinguishably with mice of both sexes from 6 to 8 weeks of age. All protocols were approved by ethics committee at Institut Pasteur and the French Ministry.

METHOD DETAILS

Muscle injury, tamoxifen and BrdU administration

For muscle injury, mice were anesthetized with 0.5% Imalgene/2% Rompun and the TA muscle was injected with 50 μ l of Cardiotoxin (10mM; Latoxan, L8102). *Tg:Pax7-CreERT2*; *Rbpj^{fllox}*; *R26^{mTmG}* and *Tg:Pax7-CreERT2*; *R26^{mTmG}* were fed with tamoxifen containing diet for two weeks (Envigo, TD55125). Five days prior sacrifice mice were given the thymidine analogue 5-Bromo-2'-deoxyuridine (BrdU, 0.5mg/ml, Sigma, B5002) in the drinking water supplemented with sucrose (25mg/ml). Comparisons were done between age-matched littermates using 8-12-week old mice.

Satellite cell dissociation and fluorescence activated cell sorting (FACS)

Adult and foetal limb muscles were dissected, minced and incubated with a mix of Dispase II (Roche, 04942078001) 3U/ml, Collagenase A (Roche, 11088793001) 100ug/ml and DNase I (Roche, 11284932001) 10mg/ml in Hank's Balanced Salt Solution (HBSS, Gibco) supplemented with 1% Penicillin-Streptomycin (PS; Gibco) at 37°C at 60rpm in a shaking water bath for 2h. The muscle suspension was successively filtered through 100µm and 70µm cell strainers (Milteny, 130-098-463 and 130-098-462) and then spun at 50g for 10min at 4°C to remove large tissue fragments. The supernatant was collected and washed twice by centrifugation at 600g for 15min. Prior to FACS, the final pellet was resuspended in cold Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 1%PS and 2% Fetal Bovine Serum (FBS, Gibco) and the cell suspension was filtered through a 40µm strainer (BD, 352340).

For satellite cell isolation from Fucci-green and *Odz4* KO mice, cells were stained with α7-integrin-APC (Ablab, clone R2F2, 1/1000), CD34-e450 (eBioscience, clone RAM34, 1/50), Sca1-Pe-Cy7 (eBioscience, clone D7, 1/400), CD45-Pe-Cy7 (eBioscience, clone 30-F11, 1/100) and CD31-PE (BD, clone MEC13.3, 1/50). Satellite cells were sorted with an Aria III (BD Biosciences).

RNA extraction and quantitative PCR (RT-qPCR)

Micro-RNAs from cells or tissue were purified using (Qiagen miRNAeasy® Micro Kit) and reverse transcribed in cDNA using miRCURY LNA® universal RT kit (Exiqon, 203301). Expression of mature miRNAs was determined using ExiLent SYBR® green master mix (Exiqon) and miRNA LNA™ PCR primers (Exiqon; hsa-miR-708-5p, 204490; mmu-miR-489-3p, 205036; hsa-let-7e-5p, 205711; mmu-miR-92-5p, 205947). Two snoRNA; RNU5G (Exiqon, 308014) and SNORD65 (Exiqon, 308016) were used for normalization.

Total mRNAs were isolated using (Qiagen RNAeasy® Micro Kit) and reverse transcribed using SuperScriptIII® enzyme (Invitrogen, 18080093). The eventual remaining RNAs were degraded by incubation 20 min at 37°C with RNase H endonuclease (Roche, 10786357001). Expression of mature mRNAs was assessed with SYBR green master mix (Roche, 04913914001) and analysis were performed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All RT-qPCR samples were normalized with *Tbp* and *Rpl13*. Specific forward and reverse primers used for RT-qPCR are listed in Table S1.

Satellite cell culture and transfection

Satellite cells were isolated by FACS, and total muscle preparations were seeded at 3×10^3 cells/cm² on Matrigel® (Corning, 354248) coated dishes for 30 min at 37°C. Cells were cultured

in a growth medium (GM) containing DMEM/F12 (50:50; Gibco), 1% PS, 20% FBS, 2% Ultrosor (Pall, 15950-017) and incubated at 37°C, 3% O₂, 5% CO₂ for the indicated time. Half of the medium was changed every 3 days. To assess proliferation, cells were pulsed with the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU), 1x10⁻⁶ M, 2h prior to fixation (ThermoFisher Click-iT Plus EdU kit, C10640).

Freshly isolated satellite cells from *Tg:Pax7-nGFP* were transfected in suspension with miRIDIAN microRNA mmu-miR-708-5p mimic (AAGGAGCUUACAAUCUAGCUGGG, Dharmacon, C310987) and Scramble Control#1 (UCACAACCUCCUAGAAAGAGUAGA; Dharmacon, CN-001000) at 200nM final concentration using Lipofectamine 2000 (ThermoFisher, 11668) in Opti-MEM (Gibco). Four hours after transfection, 3 volumes of fresh growth medium were added and cells were cultured for the indicated time. Cells were fixed with 4% paraformaldehyde (PFA) in PBS 10 min at room temperature (RT).

Single myofibre isolation and antimir transfection

Single myofibres were isolated from *Extensor digitorum longus* (EDL) muscles following the previously described protocol (Shinin et al., 2006). Briefly, EDL muscles were dissected and incubated in 0.1% w/v collagenase (Sigma, C0130)/DMEM for 1h in a 37°C shaking water bath at 40rpm. Following enzymatic digestion, mechanical dissociation was performed to release individual myofibres that were then transferred to serum-coated petri dishes. Single myofibres were transfected with miRCURY LNATM mmu-miR-708-5p inhibitor (AAGGAGCUUACAAUCUAGCUGGG, Exiqon, 4101225) or Scramble Control A (Exiqon, 199096) at a final concentration of 250nM, using Lipofectamine 2000 (ThermoFisher, 11668) in Opti-MEM (Gibco). Four hours after transfection, 6 volumes of fresh satellite cell growth medium was added and fibres were cultured for 72h at 37°C, 3%O₂. Fibres were fixed with 4%PFA/PBS 15 min at room temperature.

Immunostaining of cells, myofibres and sections

Following fixation, cells and myofibres were washed three times with PBS, then permeabilised and blocked at the same time in buffer containing 0.25% Triton X-100 (Sigma), 10% goat serum (GS; Gibco) for 30min at RT. For BrdU immunostaining, epitopes were unmasked with DNase I (1000 U/ml, Roche, 04536282001) for 30 min at 37°C. Cells and fibres were then incubated with primary antibodies overnight at 4°C. Samples were washed with 1X PBS three times and incubated with Alexa-conjugated secondary antibodies (Life Technologies, 1/1000) and Hoechst (Life Technologies, 1/10000) for 45 min at RT. EdU staining was chemically revealed using the Click-iT Plus kit according to manufacturer's recommendations (Life Technologies, C10640). For Phalloidin staining, cells were fixed in 3%PFA/2% Sucrose for 15

min at RT, permeabilised with 0.1% Triton for 10 min and incubated with Rhodamine Phalloidin for 30 min at RT.

Isolated *Tibialis anterior* (TA) muscles were frozen in liquid-nitrogen cooled isopentane and sectioned transversely at 8µm. Sections were post-fixed with 4%PFA overnight at 4°C and washed 3 times with 1X PBS. For antibody staining of transcription factors, antigen retrieval was performed by incubating sections in boiling 10mM Tris-EDTA buffer pH9 in a 2100 Retrifer device.

Confocal images were acquired with Zeiss LSM700 microscope and Zen Blue 2.0 software. Quantifications were done on the whole TA muscle section taken from two distally located regions in the muscle for each mouse acquired with Zeiss Axioscan Z1.

AntagomiR synthesis and administration

AntagomiR and Scramble were designed as described before (Krutzfeldt et al., 2005). PAGE-purified AntagomiR were synthesized with the following modifications (Dharmacon):

AntagomiR-708:

5'mC*mC*mCmAmGmCmUmAmGmAmUmUmGmUmAmAmGmCmU*mC*mU*mU*3'-Chl;

Scramble:

5'mU*mU*mUmCmUmAmAmUmCmAmAmGmGmGmUmCmUmGmUmG*mG*mC*mU*3'-

Chl. Where * represents phosphothiotate linkage at given position; m, 2'OMethyl-modified nucleotides; Chl, cholesterol linked through a hydroxyprolinol linkage. AntagomiR molecules were resuspended in saline and injected every day for 4 days into tail veins at a dose of 8µg/g of mouse.

Transwell Assay

The bottom part of a transwell membrane with 8µm pores size (Corning, 3428) was coated with Matrigel 15min at 37°C. FACS isolated satellite cells from *Tg:Pax7-nGFP* mouse were culture as described before for 24h prior to Mimic or siRNA transfection. Twenty-four hours (for Mimic) or 48h (for siRNA) post-transfection, myogenic cells were trypsinized (Gibco, 25200) 10 min at 37°C. Trypsin was washed away by the addition of DMEM/10% FCS and cells were centrifuged 15min at 600g. Cell pellets were resuspended in a low serum medium DMEM/2% FCS and seeded on the upper part of the transwell. Cells were allowed to migrate in a vertical direction through the pores of the membrane into the lower compartment, in which higher serum content was present (growth medium). Six hours after seeding, the membrane was fixed 15min with methanol and non-migrated cells remaining on the topside of the filter were removed with a cotton swab. The migrated cells were stained with Crystal Violet 0.5%/25% Methanol for 5min (Sigma, C0775) and washed 5 times in 1X PBS.

Construction of luciferase reporters, mutagenesis and luciferase assays

For the generation of luciferase reporters, candidate enhancers of *Odz4* were amplified by PCR from genomic DNA of C2C12 cells. The enhancers were then cloned into the firefly-luciferase pGL3-Basic vector (Promega, E1751) upstream of a minimal thymidine kinase promoter (minTK) as described previously (Castel et al., 2013). Twenty-four hours after transfection, C2C12 cells were trypsinized and plated on Dll-Fc or control Fc for 24h before luciferase activity measurement. For Dll1-Fc and Fc protein production, conditioned medium was prepared from stable 293T cell lines, transfected with the corresponding plasmid as previously described (Hicks et al., 2002).

The full 3'UTR length of mouse *Dkk3* (2.5kb) and *Sdc1* (1.8kb) (<http://genome.ucsc.edu>) were amplified by PCR from reverse transcribed cDNA from total RNA extract of E17.5 mouse foetuses (Table S2). Partial *Tns3*-3'UTR (500bp) containing miR-708 potential binding site of interest was obtained from SourceBioscience (EST clone: IMAGp998D088514Q) (Table S2). 3'UTR were cloned in the pGL3-Control vector (Promega, E1741) at the XbaI site downstream of the luciferase gene. Targeted mutagenesis was performed using the Q5[®] site-directed mutagenesis kit (NEB, E0554S) according to manufacturer's guidelines. HEK293T were co-transfected with the plasmid of interest and Mimic-708 or Scramble negative control and cultured in DMEM/10%FCS, at 37°C, 5% CO₂, and 20% O₂ for 48h before luciferase assay. In both assays, a Renilla luciferase plasmid (pCMV-Renilla) was also co-transfected as transfection control and empty pGL3 vector was used as a background negative control. Firefly and renilla luciferase activities were detected with Dual Glo[®] luciferase assay system (Promega, 2920) and the results were expressed as firefly luciferase activity relative to Renilla luciferase activity.

Target Protector assay

Freshly isolated satellite cells from *Tg:Pax7-nGFP* mice were transfected using miScript Target Protector according to the manufacturer's recommendation (Qiagen, 219000). Briefly, Script Target Protector (10nM final) and miRNA Mimic (500nM final) were transfected using Lipofectamine 2000 as described above.

Western Blot

C2C12 cells were transfected with Mimic (final concentration 200nM) or siRNA (siTns3 #L063982, siDkk3 #L060631, Scramble #D0018100205, Dharmacon, final concentration 250nM) as described above and cultured in DMEM/10%FCS, at 37°C, 5% CO₂, and 20% O₂ for 48h. Primary satellite cells were sorted directly in lysis buffer (150mM NaCl, 50mM Tris pH8, 5mM EDTA, 1% NP-40 (Sigma, I8896), 0.5% sodium deoxycholate, 0.1% SDS supplemented with 1X proteases (Sigma, S8820) and phosphatases (Roche, 4906845001) inhibitors) and

5×10^4 cells were loaded/lane. Total protein extracts were run on a 4-12% Bis-Tris Gel NuPAGE (Invitrogen, NP0322) and transferred on a PVDF Amersham Hybond-P transfer membrane (Ge Healthcare, RPN303F). The membrane was then blocked with 5% BSA (Sigma, A9418) in Tris-Buffer Saline (TBS) for 1h at RT and probed with specific primary antibodies overnight at 4°C. After three washes in TBS1X-Tween 0.2%, membrane was incubated with HRP-conjugated goat anti-rabbit (1:3000, Pierce, 31460) or anti-mouse (1:10000, Pierce, 31430) IgG secondary antibodies, and revealed by chemiluminescence (Pierce ECL2 western blotting substrate, Thermo Scientific, 80196). All Western blots bands were quantified using ImageJ software.

Ex vivo videomicroscopy

Cells were transfected and seeded as indicated above. The plate was then incubated at 37°C, 5% CO₂, and 3% O₂ (Zeiss, Pecon). A Zeiss Observer.Z1 connected to an LCI PlnN 10x/0.8 W objective and Hamamatsu Orca Flash 4 camera piloted with Zen (Zeiss) was used. Cells were imaged every 13 min for the time indicated. Distance and velocity plots were obtained using the Manual tracking plugin available in Fiji (Fig. 3D) or automatic tracking (remaining experiments) provided by TrackMate (Tinevez et al., 2017) after contrast enhancement using a median filter followed by “Detect Edges” functions available in FIJI. Track parameters were kept within the same datasets; track segment splitting was allowed with a maximal distance between time frames of 50µm. For the kymograph, cells were manually tracked and a fixed ROI of 150px by 150px containing the cell in its center was extracted for each timepoint. A line encompassing the cell nucleus was drawn and the Dynamic Reslice plugin in Fiji was used to obtain the corresponding kymograph.

Live videomicroscopy of muscle explants

Live imaging was performed on the *Extensor digitorum longus* (EDL) in an explant culture system. The EDL was kept attached to the bone from tendons on both sides while the remaining leg muscles were carefully removed. The explant was immobilized on a 50mm glass bottom dish (Mattek Co, P50G-0-30-F) coated with 1% low melting agarose (Sigma, A9414) and cultured in satellite cell GM supplemented with 20mM NaHCO₃ at 37°C, 5% CO₂. Imaging was conducted on a Zeiss LSM7MP microscope with a 2-photon laser (Chameleon Laser from Coherent) at 860nm and a Zeiss Plan-Apochromat 20x/0.8 M27 objective. Z stacks of 160-200 um were acquired with a step of 2.5 µm. GFP and tdTomato were simultaneously detected using 2 NDD detectors with the following bandpass filters: BP 575-610 and BP 640-710. Movies had a duration of 10-12h with a 30min interval. A maximum intensity projection (MIP) of all optical sections was done using Zen Blue software from Carl Zeiss. The “Correct 3D drift”

plugin available in Fiji was then applied to the MIP and afterwards the “Manual Tracking” plugin in Fiji was employed to obtain the relevant data (velocities and distance).

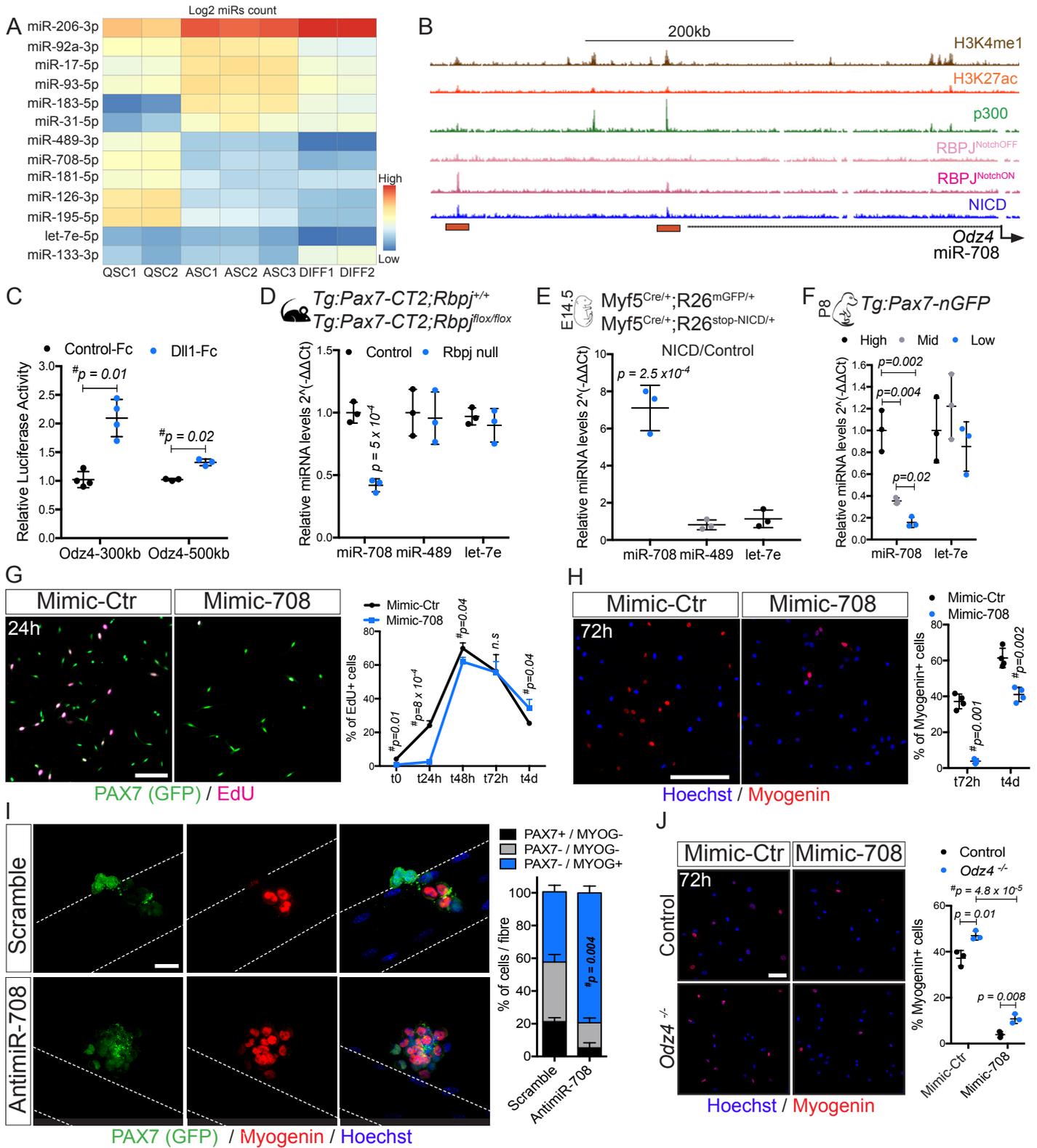
QUANTIFICATION AND STATISTICAL ANALYSIS

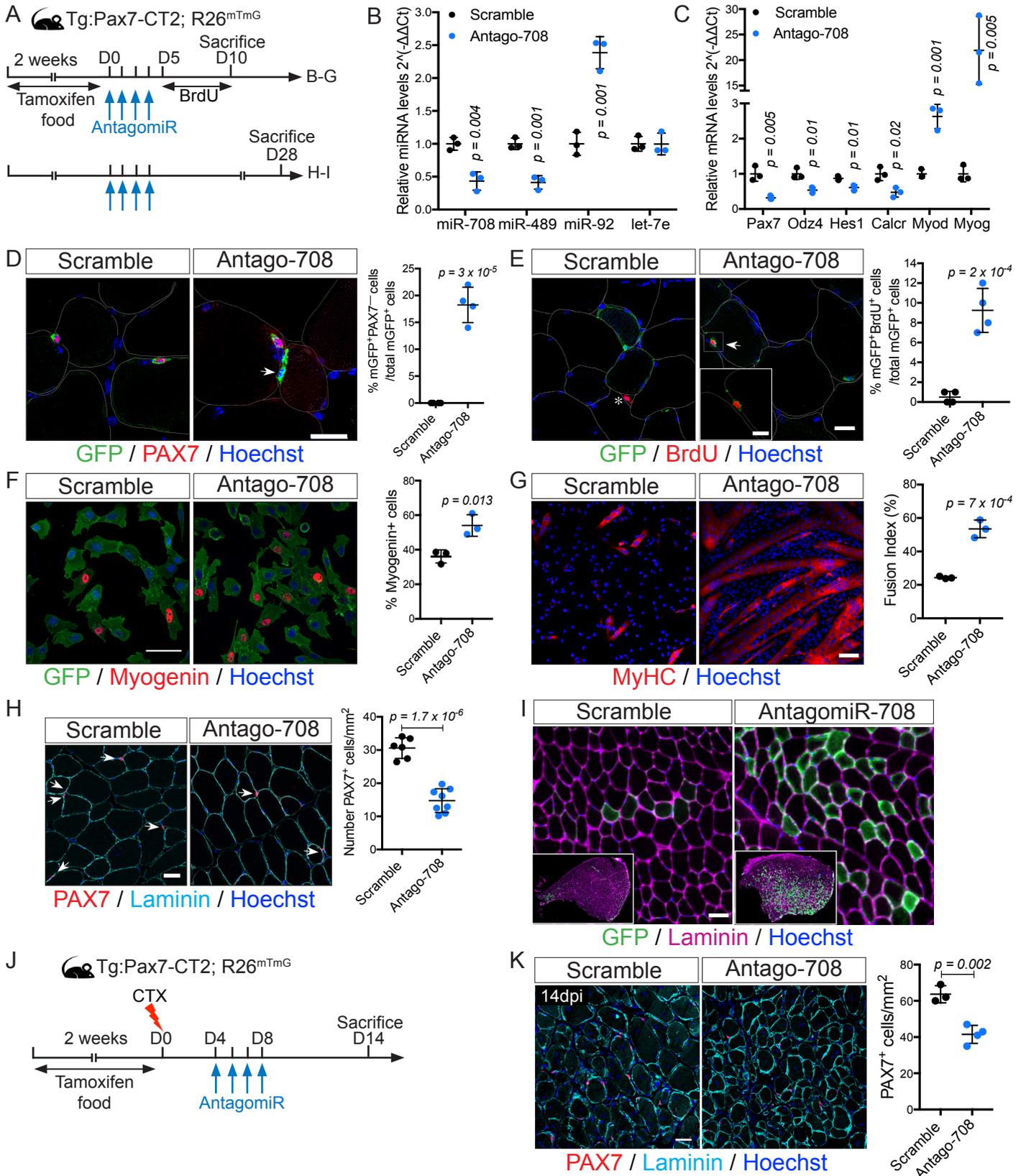
Statistical analysis

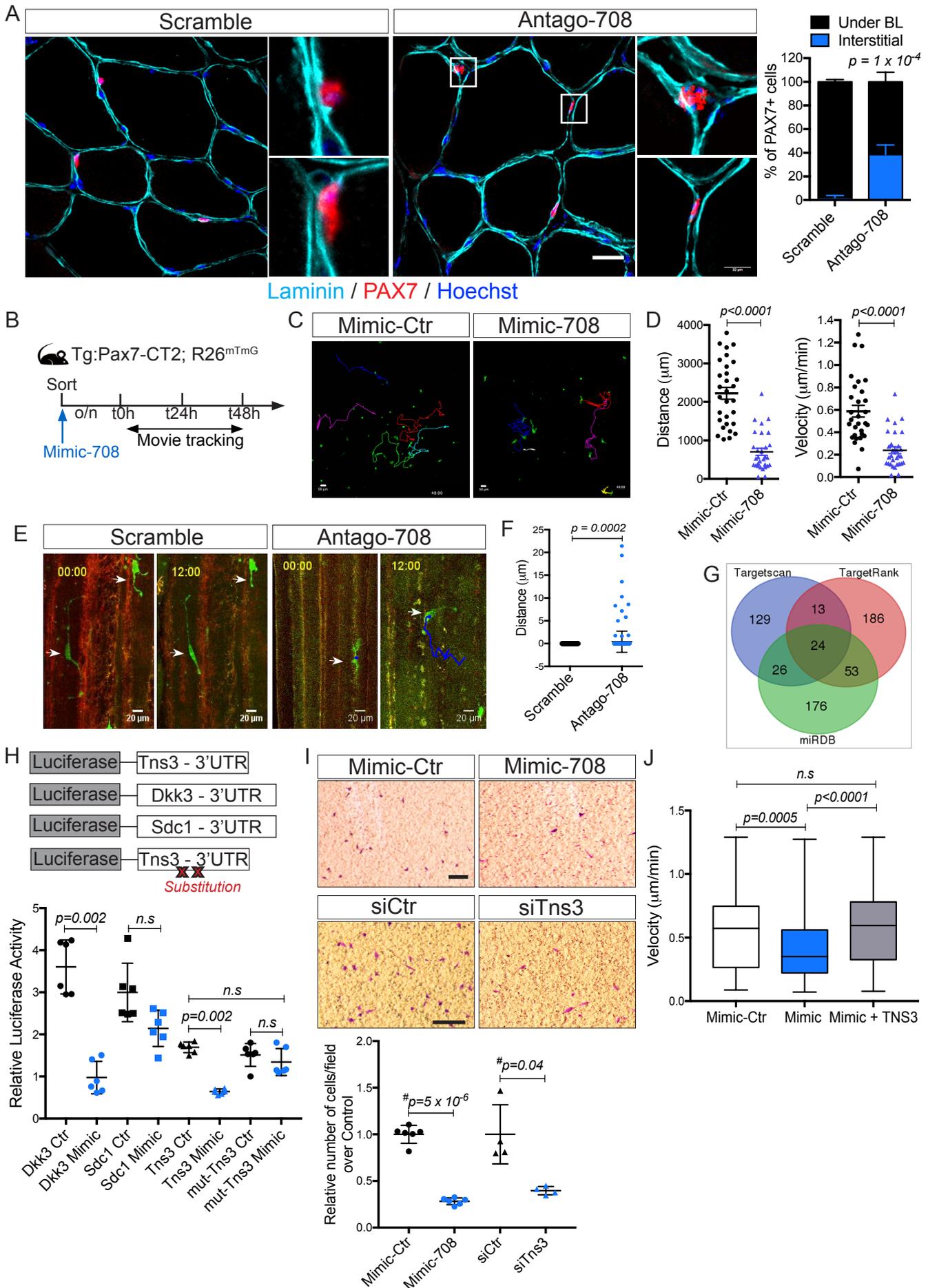
No statistical methods were used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment. No animal has been excluded from analysis and no randomization method has been applied in this study. For comparison between two groups, two-tailed paired, unpaired Student's t-tests or Mann-Whitney test were performed to calculate P values and to determine statistically significant differences. The number of independent experimental replications (n value ≤ 3 : mice, fetuses, experiments, wells or counted cells/muscles), the definition of center, variation (mean \pm SD) and statistical test (P value) are reported in each corresponding figure legend. Fisher test was used to compare phenotypic variations in Fig. 3F. All statistical analyses were performed with GraphPad Prism software.

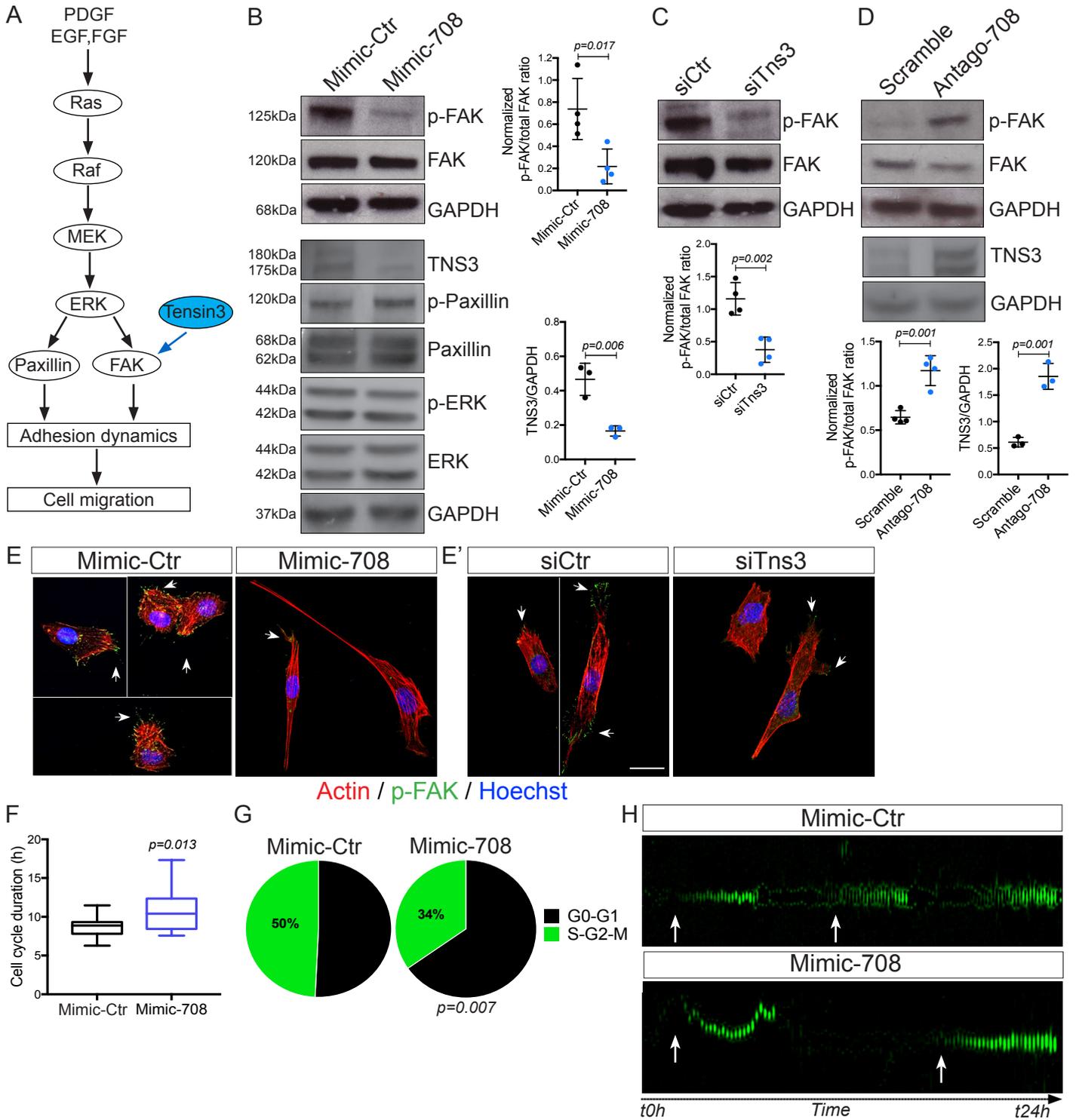
Data and software availability

The small RNA-seq are available in the ArrayExpress database at EMBL-EBI under accession no. E-MTAB-5955 (Castel et al., 2018). Notch ChIP-seq data are available under the accession no. GEO Accession no. GSE37184 (Castel et al., 2013). Microarray analysis on freshly isolated and activated satellite cells data are available under the GEO accession no. GSE52192 (Liu et al., 2013). Supplementary movies are available at <http://dx.doi.org/10.17632/xhrjsbwg92.1#folder-16a2cd73-967e-4fda-9fb8-a2009a1cf4fa>.









Supplementary Tables related to STAR Methods

Table S1: RT-qPCR primers used in this study. Related to STAR Methods.

Mouse RT-PCR primer	Sequence (5' > 3')
Odz4_F	GTGGGATGGAGGTTAGCTCG
Odz4_R	ATGGGTTCTACTGCCCAAGTG
Hey1_F	CACCTGAAAATGCTGCACAC
Hey1_R	ATGCTCAGATAACGGGCAAC
HeyL_F	GTCTTGCAGATGACCGTGGA
HeyL_R	CTCGGGCATCAAAGAACCCT
Myod_F	CACTACAGTGGCGACTCAGATGCA
Myod_R	CCTGGACTCGCGCGCCGCCTCACT
Myogenin_F	GTGAATGCAACTCCCACAGC
Myogenin_R	CGCGAGCAAATGATCTCCTG
Pax7_F	GACAAAGGGAACCGTCTGGAT
Pax7_R	TATCTTGTGGCGGATGTGGTTA
Col4a2_F	GATACCCGCGTAATCTCAA
Col4a2_R	ATGAGCACCTTGGAATCCTG
Rpl13_R	GTGGTCCCTGCTGCTCTCAAG
Rpl13_F	CGATAGTGCATCTTGGCCTTTT
Tbp_F	ATCCAAGCGATTTGCTG
Tbp_R	CCTGTGCACACCATTTTCC
Odz4 KO_F	TGAAGGAGAGGAAGCCCTATCG
Odz4 KO_R	TCACAAAGAAGCCGTCGTAGCC

Table S2: 3'-UTR of miR-708 predicted target genes. In bold: miR-708 seed sequence. Related to STAR Methods

Gene	3'UTR sequence
Dickkopf-3 (Dkk3)	GCCCAGACCCAGCTGAGTCACTGGTAGATGTGCAATAGAAATGGCTAATTTAT TTTCCCAGGAGTGTCCCAAGTGTGGAATGGCCGCAGCTCCTTCCCAGTAGCT TTTCTCTGGCTTGACAAGGTACAGTGCAGTACATTTCTTCCAGCCGCCCTGCT TCTCTGACTTGGGAAAGACAGGCATGGCGGGTAAGGGCAGCGGTGAGTCGTC CCTCGCTGTTGCTAGAAACGCTGTCTTGTTCATGGATGGAAAGATTTGTTTG AAGGGAGAGGATGGGAAGGGGTGAAGTCTGCTCATGATGGATTTGGGGGATA CAGGGAGGAGGATGCCTGCCTTGCAGACGTGGACTTGGCAAAATGTAACCTTT GCTTTTGTCTTGCGCCGCTCCCATGGGCTGAGGCAGTGGCTACACAAGAGCTA TGCTGCTCTGTGGCTCCACATATTCATCCCTGTGTTT CAGCTCCTACCTCAC TGTCAGCACAGCCCTTCATAGCCACGCCCCCTTGTCTCACCACAGCCTAGGA GGGGACCAGAGGGGACTTCTCTCAGAGCCCCATGCTCTCTCTCAACCCCAT ACCAGCCTCTGTGCCAGCGACAGTCTTCCAAATGGAGGGAGTGAAATCCTTT GGTTTTATTATTTCTCCTTCAAGGCACGCCTGCCACTAAGGTCAGGCTGACTT GCATGTCCCTCTAACGTTTCGTAGCAGTGTGGTGGACACTGTCTTCCACCGACTG CTTCAATACCTCTGAAAGCCAGTGTCTGGAGTGCAGTTCGTGTAATTAATTT GCAGGAAGTATACTTGGCTAATTGTAGGGCTAGGATTGTGAATGAAATTTGCA AAGTCGCTTAGCAACAATGGAAAGCCTTTCTCAGTCACACCGAGAAGTCACAA CCAAGCCAGGTTGTGTAGAGTACAGCTGTGACATACAGACAGAAGAAGGCTG GGCTGGATGTCAGGCCTCAGATGACGGTTTCAGGTGCCAGGAATAATTACCA TCTGTATCTATCCAGAGTTATTAATAATTGAAAGTTGCACACATTTGTATAAGCA TGCCTTTCTCCTGAGTTTTAAATTAATATGTATACACAACATGTGGCCCTCAA GATCATGCACAAACCACTACTCTTTGTCTAATTCTTGGACTTTTCTCTTTGATTTT CAATAAATACAAATCCCTTCATGCAAAAAAATTAATAAATCTGTAGTATAA AGAGACAAAAAATCCATAGAGCAGATTTTCCAGGCATCTGCAGTTTCCCT CTTTTAGAATCGGAATTCGTTGGAACCTCTCATCCTTGTCTGGATGGGAATTAGC TTTAACAGAGAACTACTTACCCTCTCCTGAAAGAACAATGGAATATATGA GTCTTCTTGGAGGCTCTTCCACTCAAATGCAGTTCGGGGCTGTGCTAGCA TTGATACTGTAACAAAACGGCTGAAGCAATGAACTTATATTTAAAAAGTTA GGTTAATTGGGTTACCATTTTCAGGTTTCAGTCTGATCCCATGGGGTTGAAAC TAAGGAGAGGCAGCACAGCGTGGCAAGGGAATGTGGTAGAGTCAAGCTGCTC CCTTCTGGCTAACAGGAGAGTGGGCAATGTGCAGTCTTGTGAGAATGCCAG GTCCTGGGGGAAGGGAGTGCCTGGACATCACCTTAAAGGTGGAGACTTCTG CAGCTTTGGTTTTAGTTACTTCTGGGTGCTACAATCAAACGCCCAACAAGA AGCCACCTGAGGGATGAGGGTTATTTGGCTCCTGGTTCAAGCAGGGAGTCC TTCGTGGCAGGAGTGCAAGGTTGCTTCTGCAAGTGTGGAGGATCAGGAAGCAA AGAAAGAGCAATGCAAGACTCAGCTTCTCTTCCCTGATTATTTATTCTGG AACCCCAACCTTGGGGTGGTGCCGACCGCAGTAAGAGTGTGCTCTTCTCCT TAGAACCTCTGAAAACCTTGGCCCTCATAGAAAATGTGCAGAGGTGTGTCACC TAAATGTTCAAATCCATTCTGTTCCAAGACATGGGAGCGCTATGTGCTAAGT CTCCACATAAGAGCACCGAGTACCTCTTAAACGCCTGTAATTCGCATCTGAA GATACCACAGTAAAGAGATGTAAACATTTAGGAAAACAATAAATGTAACCTGA TGAAGTCACC
Syndecan-1 (Sdc1)	TGGGGAAATAGTTCTTCTCCCCCACAGCCCCTGCCACTCACTAGGCTCCCAC TTGCCTCTTCTGTGAAAAAATTCAAGCCCTGGCCTCCCCACCACTGGGTCAATGT CCTCTGCACCCAGGCCCTTCCAGCTGTTCCTGCCCGAGCGGTCCCAGGGTGTG CTGGGAAGTACTTCCCCTCCTTTGACTTCTGCCTAGAAGCTTGGGTGCAAAGG GTTCTTGCATCTGATCTTCTACCACAACCACACCTGTCTGCTCACTCTTCTGA CTTGGTTTCTCCAAATGGGAGGAGACCCAGCTCTGGACAGAAAGGGGACCCG ACTGCTTTGGACCTAGATGGCCTATTGCGGCTGGAGGATCTGAGGACAGGAG AGGGGCTTCGGCTGACCAGCCATAGCACTTACCCATAGAGACCGCTAGGGTTG GCCGTGCTGTGGTGGGGGATGGAGGCCTGAGCTCCTTGGAAATCCACTTTTCAT TGTGGGGAGGTCTACTTTAGACAACCTGGTTTTTGCACATAATTTCTCTAATTT TCTGTTTCAAGAGCCCAGCAGACCTTATTACTGGGGTAAGGCAAGTCTGTTGAC TGGTGTCCCTCACCTCGCTTCCCTAATCTACATTCAGGAGACCGAATCGGGGG TTAATAAGACTTTTTTTGTTTTTTGTTTTTTAACCTAGAAGAACCAAATC TGGACGCCAAAACGTAGGCTTAGTTGTGTGTGCTCTGAGTTTGTCTGCTCAT

	<p>AAGTAGGCTGGCTAGTCCAGGATACTGTGGAATAGCCACCTCTTGACCAGTCA TGCCTGTGTGCATGGACTCAGGGCCACGGCCTTGGCCTGGGCCACCGTGACAT TGGAAGAGCCTGTGTGAGAACTTACTCGAAGTTCACAGTCTAGGAGTGGAGG GGAGGAGACTGTAGAGTTTTGGGGGAGGGGTGGCAAGGGTGCCCAAGCGTCT CCCACCTTTGGTACCATCTCTAGTCATCCTTCCCTCCCGGAAGTTGACAAGACAC ATCTTGAGTATGGCTGGCACTGGTTCCTCCATCAAGAACCAAGTTCACCTTCA GCTCCTGTGGCCCCGCCCCAGGCTGGAGTCAGAAATGTTTCCCAAAGAGTG AGTCTTTTGCTTTTGGCAAACGCTACTTAATCCAATGGGTTCTGTACAGTAGA TTTTGCAGATGTAATAAACTTTAATAATAAAGGAGTCCTATGAACTCTACTGCTT CTGCTTCTTCTCTCTGGACTGGTGGTATAGATATAGCCACCCTTTGCCCAAAC CCTGGTAGCTCGGGGAAGCTTGGCTTAAGGCTGCACGCCTCCAATCCCCAAA GGGTAGGATCCTGGCTGGGTCCAGGGTCCCTCTGATTTATTTGGTTTTGTGTG TTGTGTTGTGTTTTCTTTTTGGCTAAACTTCTTTTTGGAAAGTTGGTAAGTTCAGCC AAGTTTTACAGGCCCTGATGTCTGTTCTTAAATGGTTAAAGTAATGGGGAC TCTAGCACATCTTGACCTAGGGTCACTAGAGCTAAGCTTGCTTTGCAGGGCAG ACACCTGGGACAGCCTTCCCTCATGTTGCTGGGACACTGCTGAGCACCC CTTGCTTACTTAGCTCAGTGATGTTCCAGCTCCTGGCTAGGCTGCTCAGCCACT CAGCTAGACAAAAGATCTGTGCCCTGTGTTTCATCCAGAGCTTGTTGCCAGA TCACATGGCTGGATGTGATGTGGGGTGGGGGTGGGGTCATATCTGAGACAGCC CTCAGCTGAGGGCTTGTGGGACAGTGTCCAAGCCTCAGGCTGGGCTCATTAT ATAATTGCAATAA</p>
<p>Tensin-3 (Tns3)</p>	<p>GTCTGTGTGTATACAGGTGGACCATTCCACTTTATGCTCATGTATGTCTGTGTG TATACAGGTGGACCATTCCACTTTTGCTCATGTATGTCTGTGTGTATACAGGTG GACTATCCACTTTTTAGCTCCTATTGATGCACCAAAAAGCAAGTGCCTCATT CTGTGCCAAATGTTTGCCTTGGTCTTTAAGGACCTCCTTCGTGGACACTCTGAT GTGCCTGTTAGAGGGAATGTGCCACCATTCCCTAGAGGCCCCATGTCTTCCAC AGAGGCTTCTAGTGTTCAGTTACTCATATGCAGCTAAACTCCAGATGGGGGC AGGGGTGGGGCTGAAGTTGTGCTCTAAGAAGTATCACATCCTATGATTATAAG TTTATATGCAGATGTGGCCCAGAGATCACAGCCCCGCACTCTTTTCCCTCCCGCT GGAGGGGGGTGGGGGTGGGGGGAGAGGGCCTAATTAGAAACTCAGCTGGGCT CTGCTGAAGCCCAGCTTTCCGGTGAATTGAATGCCACAAAAGGTTGGCATGGA ATGGCATCCAAGAAGCCACAACGAATGTGCGTTTCAAAAACTGACCGGGAGGG TATGATTTACTCCAGGATACAAGTCAGTCCAGGGTATCCAGGATCGACTGA GGGAACCCAGGGAGACCGTCCACATGGTACAAACACTGGGGGCGGCCGGAAC GAGGGAAGCGGGTTGACAACACAACGGACTACACACCGGGGCCACACGGAC GAATACACAGT</p>
<p>Mutated Tensin-3</p>	<p>GTCTGTGTGTATACAGGTGGACCATTCCACTTTATGCTCATGTATGTCTGTGTG TATACAGGTGGACCATTCCACTTTTGCTCATGTATGTCTGTGTGTATACAGGTG GACTATCCACTTTTTAGCGAATATTGATGCACCAAAAAGCAAGTGCCTCATT CTGTGCCAAATGTTTGCCTTGGTCTTTAAGGACCTCCTTCGTGGACACTCTGAT GTGCCTGTTAGAGGGAATGTGCCACCATTCCCTAGAGGCCCCATGTCTTCCAC AGAGGCTTCTAGTGTTCAGTTACTCATATGCAGCTAAACTCCAGATGGGGGC AGGGGTGGGGCTGAAGTTGTGCTCTAAGAAGTATCACATCCTATGATTATAAG TTTATATGCAGATGTGGCCCAGAGATCACAGCCCCGCACTCTTTTCCCTCCCGCT GGAGGGGGGTGGGGGTGGGGGGAGAGGGCCTAATTAGAAACTCAGCTGGGCT CTGCTGAAGCCCAGCTTTCCGGTGAATTGAATGCCACAAAAGGTTGGCATGGA ATGGCATCCAAGAAGCCACAACGAATGTGCGTTTCAAAAACTGACCGGGAGGG TATGATTTACTCCAGGATACAAGTCAGTCCAGGGTATCCAGGATCGACTGA GGGAACCCAGGGAGACCGTCCACATGGTACAAACACTGGGGGCGGCCGGAAC GAGGGAAGCGGGTTGACAACACAACGGACTACACACCGGGGCCACACGGAC GAATACACAGT</p>

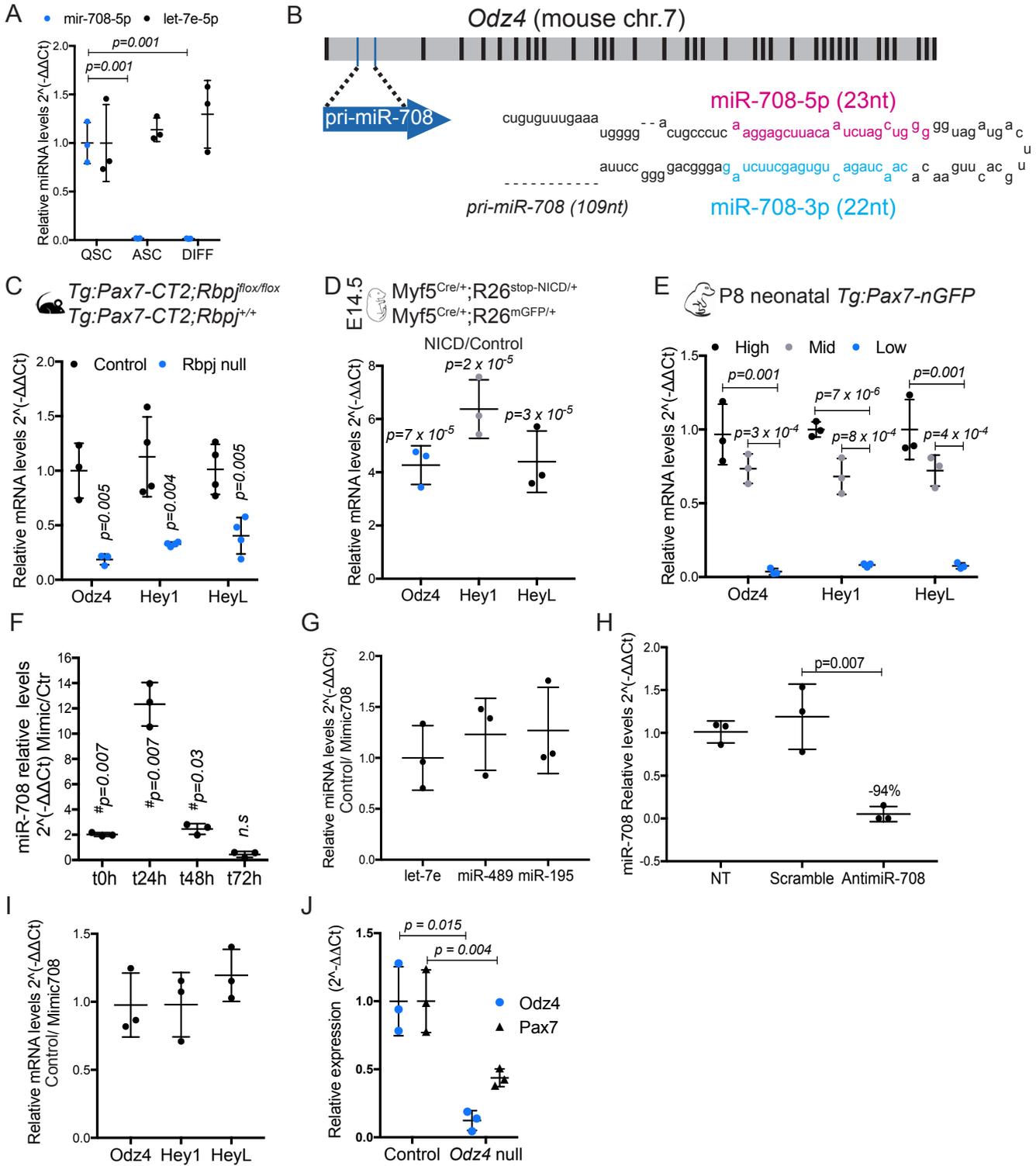


Fig. S1 related to Fig.1. Assessment of miR-708 expression in subpopulations of satellite cells and *ex vivo* gain and loss of function validations.

(A) RT-qPCR validation of miR-708 expression on freshly isolated satellite cells (QSC), *in vivo* activated (ASC) satellite cells at 5 days post-injury of *Tibialis anterior* (TA) muscle, and freshly isolated myofibres from *Extensor digitorum longus* (EDL) muscle (DIFF). Let-7e expression was stable in every condition (see Fig. 1A) and was used as a negative control; (n=3 mice/condition).

(B) Scheme of mouse *Odz4* gene; black boxes represent exons. miR-708 is encoded by the first intron of *Odz4*. Stem loop sequence of pri-miR-708 (109 nt) including miR-708-5p (pink; aaggagcuuacaauacuagcuggg), the mature strand in satellite cells and the passenger strand, miR-708-3p (blue; caacuagacugugagcuucuag). The miR-708-5p strand constitutes the major mature form of miR-708 in satellite cells, while the passenger strand miR-708-3p was in minority. Therefore, we focused on miR-708-5p (Accession MIMAT0004828) for the remaining experiments in this study.

(C) RT-qPCR analysis of *Odz4* gene in *Rbpj* conditional KO (*Rbpj* null) and Control satellite cells. Cells were isolated from resting muscles by FACS 2-weeks post-tamoxifen treatment. Control: *Tg:Pax7-CT2*; *Rbpj*^{+/+}; *R26^{mTmG}* and *Rbpj* null: *Tg:Pax7-CT2*; *Rbpj*^{fllox/fllox}; *R26^{mTmG}*; n=4 mice/genotype.

(D) Induction of *Odz4* gene in E14.5 control (*Myf5^{Cre/+}*; *R26^{mTmG/+}*) and *Myf5^{Cre}-NICD* (*Myf5^{Cre/+}*; *R26^{stop-NICD-nGFP/+}*) myogenic cells isolated by FACS assessed by RT-qPCR. *Hey1* and *HeyL* are reporters of Notch activity; n=4 fetuses/genotype.

(E) Transcript expression levels of *Odz4* targeted by Notch in cells fractionated by FACS based on GFP-intensity from *Tg:Pax7-nGFP* at postnatal day 8 (P8) where Notch activity gradually decreases from the more committed (high) to more differentiated (low) cells: Pax7^{High} 20% of population (black), Pax7^{Mid} 40% (grey) and Pax7^{Low} 20% (blue); n=3 pups.

(F) RT-qPCR of miR-708 expression at 4h, 24h, 48h and 72h after Mimic-708 transfection of satellite cells isolated from adult *Tg:Pax7-nGFP* mice; n=3 mice.

(G) RT-qPCR of quiescent-specific miRNAs, miR-489 and miR-195, showing that miR-708 overexpression is specific to a single miRNA; n=3 mice.

(H) Transcript levels of miR-708 in satellite cells isolated from *Tg:Pax7-nGFP* at 12h after miR-708 inhibition using AntimiR-708 transfection; n=3 mice.

(I) Transcripts levels of *Odz4* following miR-708 overexpression (24h post-transfection).

(J) RT-qPCR of *Odz4* and *Pax7* in Control and knock-out (*Odz4* KO) mice. Note that *Pax7* is downregulated in *Odz4* KO satellite cells; n=3 mice/genotype.

Error bars, mean \pm SD; two-sided unpaired t-test; #p-value: two-sided paired t-test.

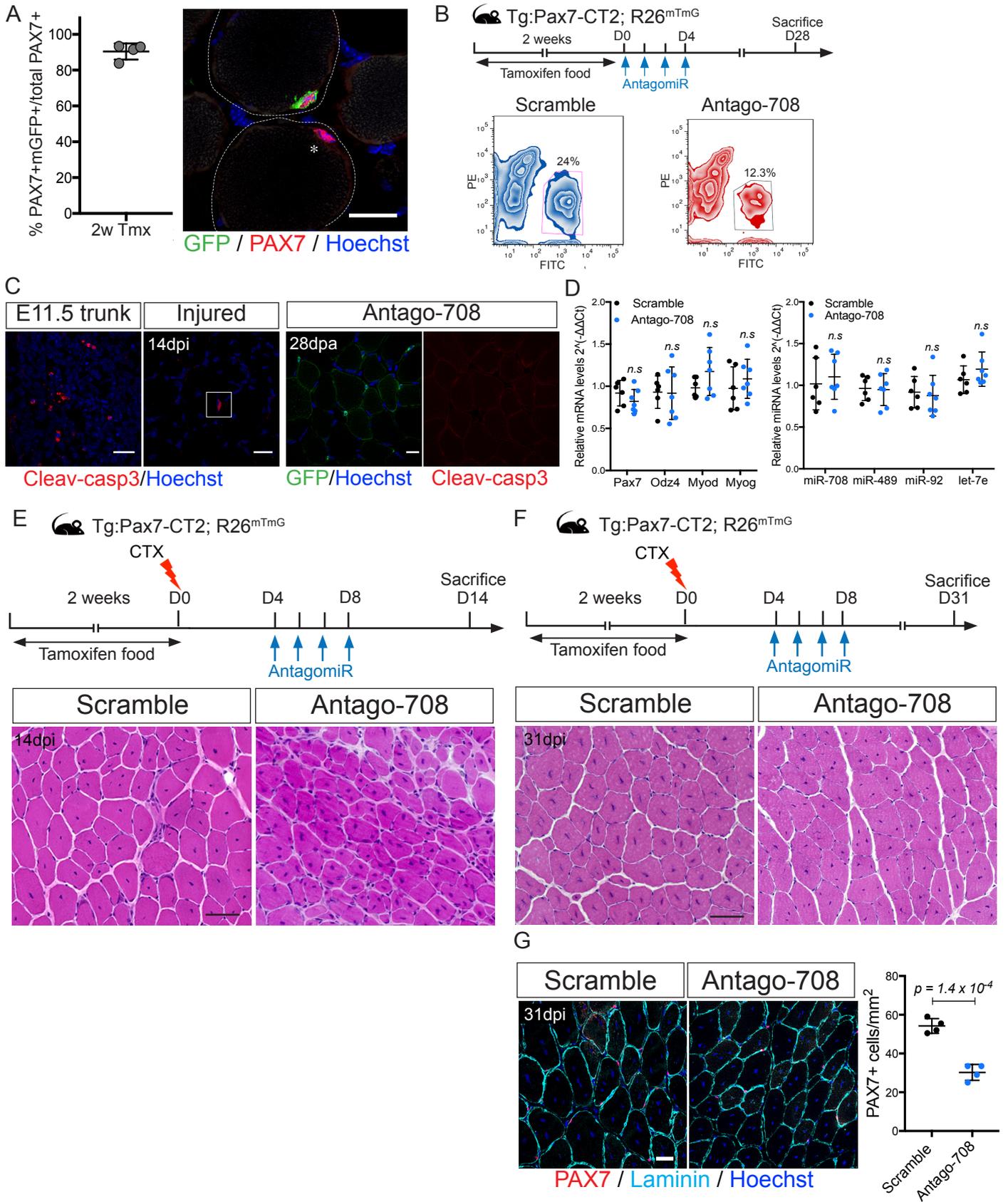


Fig. S2 related to Fig.2. *In vivo* inhibition of miR-708 induces spontaneous differentiation.

(A) Recombination efficiency of *Tg:Pax7-CT2; R26^{mTmG}* mice fed two weeks with tamoxifen food and assessed by quantification of mGFP+/ PAX7+ cells over total PAX7+ cells in TA sections. After two weeks of treatment, 95-97% of satellite cells (PAX7+) were recombined (mGFP+). Asterisk shows a non-recombined cell (PAX7+mGFP-); n=4 mice.

(B) Scheme of tamoxifen and AntagomiR administration to *Tg:Pax7-CT2; R26^{mTmG}* mice. AntagomiR-708 and Scramble control were injected daily for 4 days after the end of tamoxifen treatment (D0) and mice were sacrificed 28 days post-AntagomiR treatment. FACS profiles of mGFP-positive cells from resting muscles of control (Scramble) or AntagomiR-708 treated mice show decrease in satellite cell numbers 28 days after miR-708 *in vivo* inhibition.

(C) Cleaved-caspase3 immunostaining showed no apoptosis in quiescent satellite cells treated with AntagomiR-708. Sections from E11.5 trunk embryo and injured TA are shown as positive controls; n=3 mice.

(D) RT-qPCR of mGFP-positive cells isolated from resting muscle of Scramble or AntagomiR-708 treated mice after 28 days (see also Fig. S2B). Expression of quiescence (*Pax7, Odz4*), activation (*Myod*) and differentiation (*Myogenin*) genes was not affected (left); quiescent miR-708, miR-489 and activated miR-92 expression levels are similar in Scramble and AntagomiR-708 treated satellite cells (right); n=6 Scramble and n=7 AntagomiR treated mice.

(E)(F) Schemes of miR-708 *in vivo* knock-down. *Tg:Pax7-CT2; R26^{mTmG}* mice were fed with tamoxifen for 2 weeks. Four days following Cardiotoxin injury (CTX), AntagomiR-708, or Scramble were injected every day for 4 days and injured muscles were collected 14 days (E) or 31 days (F) post-injury. Hematoxylin and Eosin staining of transverse sections of regenerating TA muscles 14 days (E) or 31 days (F) post-injury show miR-708 *in vivo* knock down induced a delay in muscle regeneration at 14 days post injury, but histology looks similar in both conditions at 31 days post-injury; n= 4 mice/treatment.

(G) Quantification of number of satellite cells/mm² on TA sections from Scramble and AntagomiR-treated mice 31dpi stained with Laminin and PAX7; n=4 mice/treatment.

Error bars, mean \pm SD; two-sided unpaired t-test. Scale bar 10 μ m (A), 50 μ m (C, G) and 100 μ m (E, F).

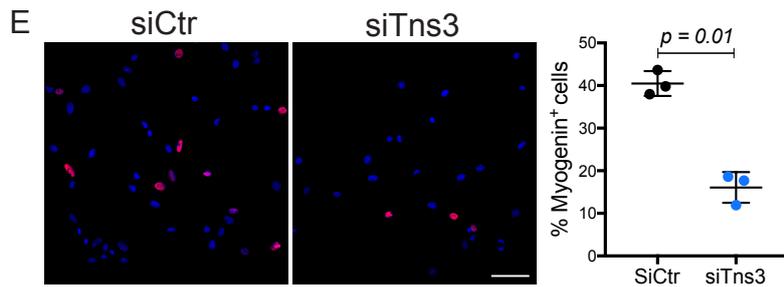
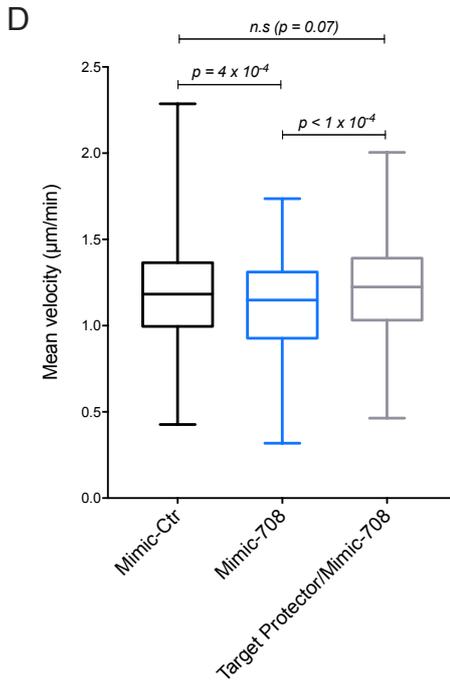
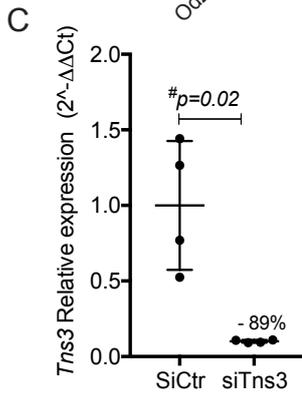
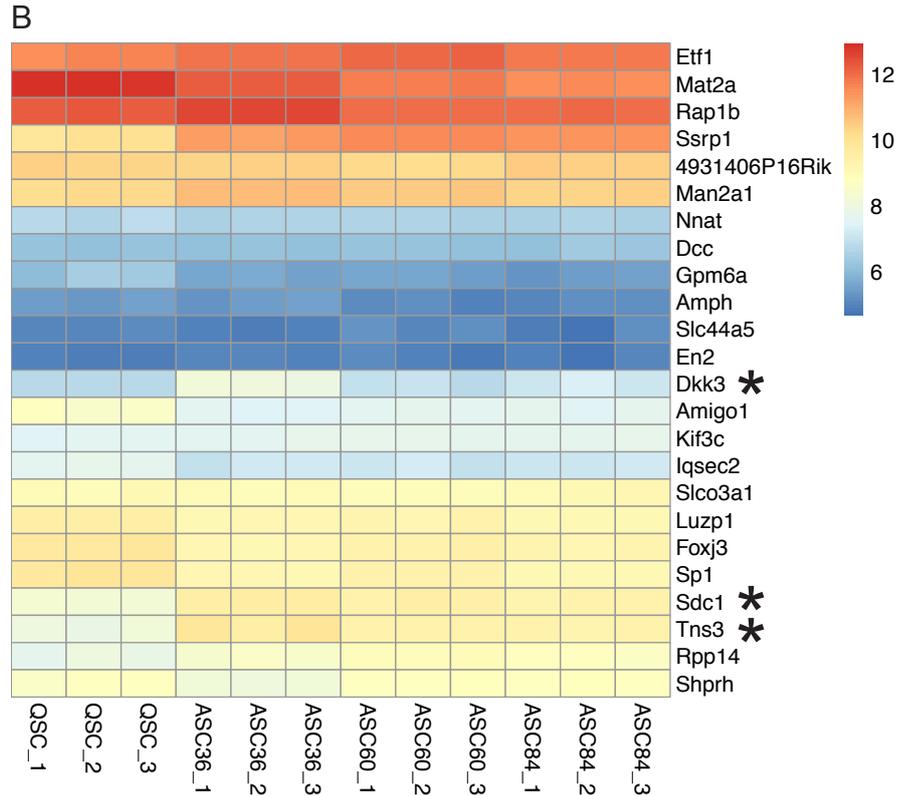
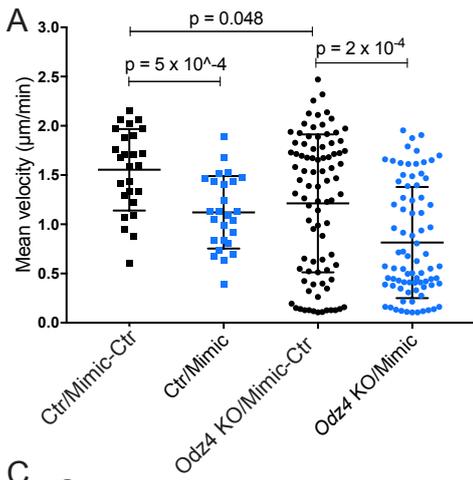


Fig. S3 related to Fig.3. miR-708 predicted target genes.

(A) Velocity of control and *Odz4*-null satellite cells overexpressing miR-708 (Mimic) or control RNA oligonucleotide (Ctr) assessed by live videomicroscopy for 32h. Note that motility in presence (control) and absence (*Odz4* KO) of *Odz4* is similar; n=3 mice/ genotype/treatment.

(B) Gene expression microarray data show 24 genes predicted by the 3 logarithms (TargetScan, TargetRank and Miranda) in satellite cells. The data are shown as a heatmap of normalized transcripts expressed in quiescence (QSC) and different time points post injury (36h (ASC36), 60h (ASC60) and 84h (ASC84); GEO accession number GSE52192 (Liu et al., 2013)). Note that only *Dkk3*, *Tns3* and *Sdc1* were upregulated during activation. Asterisks show genes of interest.

(C) Efficiency of knock-down shown by RT-qPCR on C2C12 cells transfected with siTns3 and siRNA control; n= 4 independent experiments.

(D) Target Protector Assay (where *Tns3* 3'UTR is occupied by a morpholino masking miR-708 binding site; see Methods) on satellite cells isolated from *Tg:Pax7-nGFP*, imaged and tracked for 8h upon transfection; n≤200 cells tracked from 2 independent experiments.

(E) Knock-down of *Tns3* (siTns3) on freshly isolated satellite cells from *Tg:Pax7-nGFP* mice decreased differentiation at 72h of culture; n=3 mice/condition.

Error bars, mean \pm SD; Mann-Whitney test (A, D), two-sided paired (C), unpaired t-test (E). Scale bar: 50 μ m.

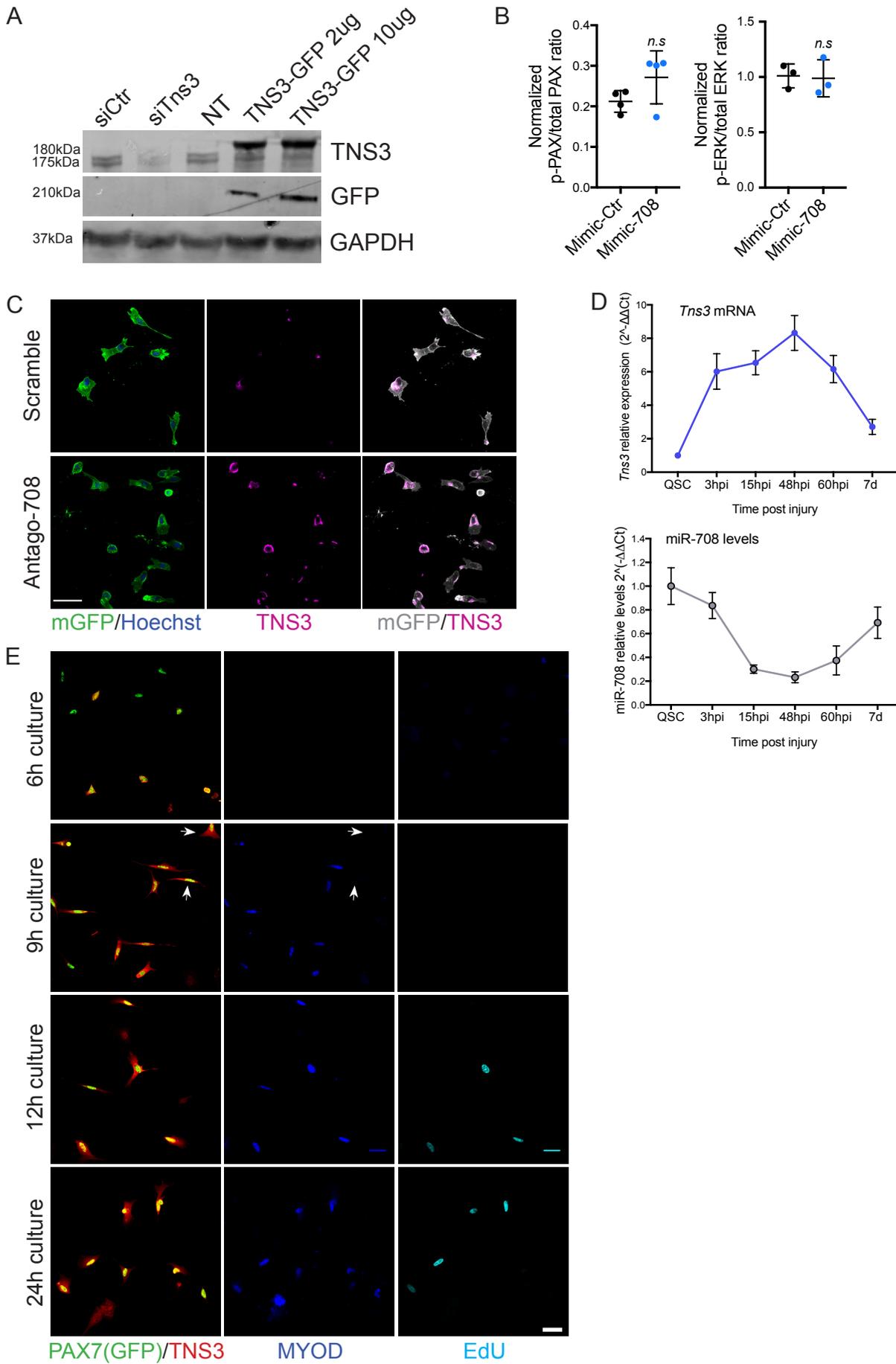


Fig. S4 related to Fig.4. miR-708 targets Tns3 to release cell migration and promote activation.

(A) Validation of Tns3 (see Table 2) antibody by knock-down of *Tns3* (siTns3) in C2C12 myoblasts 48h after siRNA transfection. Scramble siRNA and non-transfected (NT) cells were used as negative controls; overexpression of TNS3-GFP expressing plasmid served as positive control. Two specific bands about 175 and 180kDa in size correspond to TNS3, the higher one likely resulting from post-translational modification. Note that both bands are decreased upon *Tns3* knock-down.

(B) Quantification of ERK and Paxillin phosphorylation state upon miR-708 induction compared to control in C2C12 myoblasts. Western-blot were normalized to GAPDH levels; n=3-4 independent experiments. Error bars, mean \pm SD; two-sided unpaired t-test.

(C) Immunostaining of TNS3 and mGFP on satellite cells isolated by FACS from Scramble and AntagomiR-708 treated mice (10d post treatment) plated for 12h before fixation. TNS3 is strongly upregulated when miR-708 is repressed (see Fig. 4D).

(D) Time course of *Tns3* and miR-708 expression performed by RT-qPCR on freshly isolated satellite cells (QSC), 3h, 15h, 48h, 60h or 7 days after Cardiotoxin injury of TA muscle of *Tg:Pax7-nGFP* mice; n=4 mice/condition.

(E) Representative images of PAX7-nGFP⁺ satellite cells from total muscle preparations plated for indicated times and stained for EdU (2h pulse), GFP, TNS3, and MYOD. At 6h, no MYOD⁺ or EdU⁺ cells were detected. At 9h, arrows point TNS3⁺/MYOD⁻ cells. Scale bar 25 μ m.