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# **Fat1 deletion enhances Fibro-Adipogenic Differentiation and Adipogenic expansion following injury in skeletal muscle**

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1           **Fat1 deletion enhances Fibro-Adipogenic Differentiation and**  
2           **Adipogenic expansion following injury in skeletal muscle**

3  
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17 Running title: Fat1 limits intramuscular adipose tissue expansion induced by glycerol lesions

22 **Abstract :**

23 Skeletal muscles regenerate following injury, owing not only to myogenic stem cells, but  
24 also to non-myogenic cells such as fibro-adipogenic progenitors (FAPs). Quiescent in healthy  
25 muscles, FAPs transiently proliferate in response to tissue-damage, to support myogenic repair.  
26 Aside from their pro-myogenic role in healthy muscles, FAPs are also the origin of intramuscular  
27 fibro-adipose tissue that infiltrate muscles with chronic inflammation and degeneration in  
28 various muscle pathologies. Here, we investigate how the Fat1 Cadherin, previously identified as  
29 a regulator of embryonic muscle morphogenesis, influences FAP biology during damage-induced  
30 muscle regeneration. *Fat1* expression is transiently induced in FAPs and myogenic cells after  
31 muscle damage. We found that mesenchyme-specific *Fat1* ablation leads to increased fibro-  
32 adipogenic infiltrations following glycerol injury, while minimally affecting myogenic repair.  
33 Using an inducible *Pdgfra-cre/ERT* line, we further demonstrated that *Fat1* restricts FAP  
34 adipogenic differentiation through both cell-autonomous and non-cell-autonomous mechanisms.  
35 These findings identify *Fat1* as a novel regulator of FAP biology, essential for limiting FAP  
36 differentiation and the development of fibro-fatty infiltrations after muscle injury.

37

38

## 39 **Introduction**

40 Skeletal muscles have a remarkable capacity to regenerate after acute tissue lesions. This  
41 regenerative capacity is permitted not only by myogenic stem cells (satellite cells), which can  
42 replace damaged fibers, but also by an array of non-myogenic muscle-resident cells constituting  
43 a supportive niche (Biferali *et al*, 2019; Helmbacher & Stricker, 2020; Relaix & Zammit, 2012;  
44 Theret *et al*, 2021). Among those, fibro-adipogenic progenitors (FAPs) are muscle-resident  
45 mesenchymal cells that have gained considerable interest since their initial description (Joe *et al*,  
46 2010; Uezumi *et al*, 2010). While they do not directly contribute to the myogenic lineage, they  
47 are necessary for skeletal muscle mass maintenance, homeostasis and regeneration (Wosczyzna  
48 *et al*, 2019). Mostly quiescent in healthy muscles, FAPs proliferate in response to tissue lesions  
49 to support myogenic repair and ECM remodelling (Joe *et al*, 2010; Uezumi *et al*, 2010), thus  
50 massively invading interstitial space in the lesioned area, and leading to a state of transient  
51 fibrosis (Joe *et al*, 2010). In this phase, they secrete various factors, ECM components and ECM-  
52 associated proteins that promote myogenic repair (Helmbacher & Stricker, 2020; Joe *et al*,  
53 2010; Lukjanenko *et al*, 2019; Theret *et al*, 2021; Uezumi *et al*, 2010; Uezumi *et al*, 2021).  
54 Blocking FAP expansion by pharmacological inhibition (Fiore *et al*, 2016), by genetic ablation  
55 experiments (Wosczyzna *et al*, 2019), or by altering their secretome (Uezumi *et al*, 2021),  
56 compromises the efficiency of muscle regeneration, and leads to muscle atrophy and depletion  
57 of the satellite cells pool. FAPs also orchestrate the dynamic contributions of inflammatory cells  
58 to muscle repair, by promoting the clearance of debris of degenerating myofibers, and  
59 subsequently by secreting cytokines promoting the pro- to anti-inflammatory switch in  
60 macrophage phenotype (Heredia *et al*, 2013; Lemos *et al*, 2015). Once the repair process  
61 induced by acute damage has started, FAPs are subsequently eliminated by apoptosis, triggered  
62 by signals produced by inflammatory macrophages (Lemos *et al*, 2015), thus making space for  
63 growth, fusion, and maturation of de-novo fibers.

64 Besides their pro-myogenic activity, FAPs are multipotent progenitors known for their  
65 inherent capacity to give rise to adipocytes, tissue-fibroblasts/myofibroblasts, osteoblasts, and a  
66 few other stromal derivatives (Eisner *et al*, 2020; Santini *et al*, 2020; Uezumi *et al*, 2011). While  
67 naturally-occurring differentiation is minimal in steady-state healthy muscles, ectopic  
68 differentiation can occur in regenerating muscle, but is strictly limited and transient in healthy  
69 context (Lukjanenko *et al*, 2013). Instead, ectopic differentiation is unleashed in the context of a  
70 number of muscle pathologies, including Duchenne Muscular Dystrophy (DMD) (Contreras *et al*,  
71 2016; Mozzetta *et al*, 2013; Trenszt *et al*, 2010; Uezumi *et al*, 2011), Amyotrophic lateral  
72 sclerosis (ALS) (Gonzalez *et al*, 2017; Madaro *et al*, 2018), Facio-scapulo-humeral dystrophy  
73 (FSHD) (Bosnakovski *et al*, 2017; Dandapat *et al*, 2014), or limb girdle muscular dystrophy  
74 (LGMD) (Hogarth *et al*, 2019). Progression of this pathological differentiation leads to the  
75 replacement of the muscle mass by fibrosis and adipose infiltrations, commonly referred to as  
76 fibro-fatty infiltrations, or as inter- and intra-muscular adipose tissue (IMAT), jointly referred to  
77 as myosteatorsis (Correa-de-Araujo *et al*, 2020; Flores-Opazo *et al*, 2024). The extent of  
78 myosteatorsis in diseased muscles, visualized in patients by magnetic resonance imaging (MRI)  
79 (Correa-de-Araujo *et al*, 2020; Godi *et al*, 2016; Kan *et al*, 2009; Lareau-Trudel *et al*, 2015), is  
80 inversely correlated with regenerative capacities (Norris *et al*, 2024; Norris *et al*, 2025),  
81 implying that these fibro-fatty infiltrates compromise the efficacy of myogenic regeneration and  
82 fiber growth, directly or through their effects on the physical properties of muscle. Thus, aside  
83 from their respective genetic causes, a common feature of chronic muscle pathologies is that  
84 they interfere with a gatekeeping system that allows maintaining FAP quiescence, suppressing  
85 excessive FAP differentiation, ultimately eliminating excess FAPs and their differentiation  
86 byproducts in healthy muscles. Despite being under intense scrutiny by the biomedical  
87 community since the discovery that FAPs are the cell type of origin of fibro-fatty deposition, the  
88 mechanisms involved in suppressing FAP differentiation are still poorly understood. Thus,  
89 identifying novel regulatory mechanisms limiting the formation of fibro-fatty tissue represents a  
90 key unmet medical need to delay disease progression.

91 In the present study, we investigated the role exerted by the Fat1 Cadherin in FAPs during  
92 skeletal muscle regeneration induced by acute lesions. Fat-like Cadherins are adhesion  
93 molecules that regulate coordinated cell behaviors such as planar cell polarity in epithelia  
94 (Bosveld *et al*, 2012; Sharma & McNeill, 2013a, b), polarized cell movements (Caruso *et al*, 2013;  
95 Zakaria *et al*, 2014), and oriented cell divisions (Mao *et al*, 2016; Saburi *et al*, 2008), and that  
96 control tissue growth via the Hippo pathway (Cho *et al*, 2006; Lawrence & Casal, 2013; Matis &  
97 Axelrod, 2013). Among them, we previously identified *Fat1* as a regulator of neuromuscular  
98 morphogenesis during mouse development (Caruso *et al*, 2013; Helmbacher, 2018). *Fat1*  
99 influences the growth and the shape of subsets of muscles in the embryo, through  
100 complementary activities in different cell types including myogenic cells, motor neurons and  
101 muscle-associated mesenchymal cells (Helmbacher, 2018). In humans, alterations of *FAT1*  
102 expression were observed in FSHD patients (Caruso *et al*, 2013; Mariot *et al*, 2015).  
103 Furthermore, pathological *FAT1* variants disrupting its splicing or predicted to alter its functions  
104 were identified in patients with FSHD-like symptoms in absence (Puppo *et al*, 2015), but also in  
105 association with (Park *et al*, 2018) traditional FSHD-causing mutations, implicating *FAT1* as a  
106 putative modifier of FSHD severity (Caruso *et al*, 2013; Caruso *et al*, 2022; Mariot *et al*, 2015;  
107 Puppo *et al*, 2015). Guided by the observation that mesenchymal *Fat1* activity represented the  
108 predominant component of its role in muscle morphogenesis during development, and by the  
109 fact that adult FAPs derive from the developing connective tissue (Stumm *et al*, 2018; Vallecillo-  
110 Garcia *et al*, 2017), we enquired whether *Fat1* signaling might be required in adult FAPs to  
111 ensure proper muscle regeneration and homeostasis.

112 We found that *Fat1* deletion in the mesenchymal lineage enhanced the fibro-adipogenic  
113 expansion induced by glycerol injury in skeletal muscle, with minimal impact on myogenic  
114 repair. Owing to an inducible CRE line leading to low amounts of recombined FAPs, we found  
115 *Fat1* is required to prevent FAP differentiation, and that loss of *Fat1* in FAPs enhances  
116 intramuscular adipogenesis and IMAT deposition both cell-autonomously and non-cell-  
117 autonomously. Distinguishing phenotypes by sex uncovered that enhancement of adipose

118 infiltrations in mutants was more robust in females, whereas enhancement of transient fibrosis  
119 was only observed in males. Furthermore, the cell-autonomous and non-cell autonomous  
120 components of the adipogenesis phenotype were unequally associated with sex. Altogether,  
121 these data identify *Fat1* as a modulator of FAP differentiation, fate choice, and homeostasis,  
122 required to limit the expansion of glycerol-injury-induced adipose infiltrates, with sex-  
123 associated features that might guide future assessment of patient data.

124

## 125 **Results**

### 126 ***Fat1* expression is induced by skeletal muscle injury in subsets of FAPs and myogenic cells**

127 *Fat1* is broadly expressed in the musculo-skeletal system during development,  
128 encompassing myogenic progenitors and their derivatives, muscle-associated connective  
129 tissues, as well as tendons, bones and cartilage (Caruso *et al.*, 2013; Helmbacher, 2018; Mariot *et*  
130 *al.*, 2015; Smith *et al.*, 2007). *Fat1* expression levels vary not only between tissue types, but also  
131 between muscle subtypes, with distinct intensities of *Fat1<sup>LacZ</sup>* signal detected in different  
132 muscles at E13.5 (Caruso *et al.*, 2013; Mariot *et al.*, 2015). While *Fat1* expression is still relatively  
133 high at early postnatal stages, it undergoes a major drop in expression level at adult stages  
134 (Mariot *et al.*, 2015). Thus, *Fat1* expression levels are highest at stages of active developmental  
135 and postnatal myogenesis, whereas our studies in humans and mice illustrated that in  
136 homeostatic adult muscles, *Fat1* dosage, although lowered, keeps distinguishing several muscle  
137 groups (Mariot *et al.*, 2015).

138 Given the strong impact that deletion of *Fat1* activities in myogenic progenitors and  
139 mesenchymal cells exerts on muscle development (Caruso *et al.*, 2013; Helmbacher, 2018), and  
140 given that *Fat1* expression levels in adult muscles are considerably lower than in embryonic,  
141 fetal and juvenile muscle (Mariot *et al.*, 2015), we asked whether *Fat1* expression might be  
142 reactivated during injury-induced muscle regeneration, and whether it might play functions  
143 analogous to those played during development. The *Fat1<sup>LacZ</sup>* mutant allele represents a

144 convenient tool to further monitor *Fat1* expression, and to study its distribution in various  
145 tissue-types (Caruso *et al.*, 2013; Helmbacher, 2018, 2022). We exposed young adult mice (2-6  
146 months) carrying the *Fat1<sup>LacZ</sup>* allele to muscle injury, triggered by Cardiotoxin (Figure 1B, C,  
147 Figure S2) or by glycerol injections (Figure 1C, D-F, Figure S1). We injured either the *tibialis*  
148 *anterior* (TA, Figure 1B), a classical site for the study of muscle regeneration, or the *triceps*  
149 *brachii* (TB, Figure 1D-F), a humeral muscle belonging to the panoply of muscles affected in  
150 FSHD patients at early disease stages, which also expresses high levels of *Fat1* during muscle  
151 development (Helmbacher, 2018; Mariot *et al.*, 2015). *Fat1<sup>LacZ</sup>* expression was monitored at 5  
152 and 7 days post-injury (dpi), by visualizing  $\beta$ -galactosidase activity with the substrate salmon  
153 gal (Figure 1B,D, Figure S2B), or by immunohistochemistry with anti- $\beta$ -galactosidase antibodies  
154 combined with markers of distinct cell types (Figure 1, Figures S1, S2).

155 *Fat1<sup>LacZ</sup>* expression was strongly induced in the lesion area after both cardiotoxin (5dpi) and  
156 glycerol (7dpi) injuries (Figure 1B,D, Figure S1), whereas  $\beta$ -galactosidase activity or protein  
157 levels were below detection levels in unaffected ipsilateral muscle areas or contralateral  
158 uninjured muscles. These observations are consistent with previously published transcriptome  
159 data from regenerating mouse muscle (Figure 1C, data from (Lukjanenko *et al.*, 2013)). *Fat1<sup>LacZ</sup>*  
160 expression was highest at the interface between the central degenerating area (containing  
161 degenerating/necrotic fibers, or ghosts of degenerated fibers) and the more peripheral area  
162 occupied by new fibers (recognizable by centrally located nuclei). This interface corresponds to  
163 the region of active regenerative myogenesis (Figure 1D, E), where  $\beta$ -galactosidase was  
164 detected in the youngest de-novo produced myofibers (yellow arrows in Figure 1E), and in  
165 interstitial mesenchymal cells expressing PDGFRA, co-expression being highest in the areas with  
166 ongoing regeneration and surrounding necrotic fibers (white arrows in Figure 1E). Instead,  
167 *Fat1<sup>LacZ</sup>* expression was relatively lower in FAPs surrounding regenerated myofibers in the  
168 periphery of the lesioned area where regeneration is more advanced and de-novo fiber diameter  
169 is larger, indicating that *Fat1* induction is transient (compare crops 2-3 and 4-5 in Figure 1E).  
170 The resulting gradient of *Fat1* expression levels was most obvious when following staining

171 intensities along a band spanning from the uninjured area to the center of the necrotic area  
172 (Figure S1B, C, D). This contrasts with levels of *Pdgfra*, which are elevated throughout the lesion  
173 area compared to the uninjured area, with a progressive increase in intensity between  
174 peripheral and central FAPs (Figure 1E, Figure S1C, D), such that *Pdgfra* levels are higher than  
175 those of *Fat1* in peripheral FAPs, and lower in central FAPs (Figure S1D). *Fat1<sup>LacZ</sup>* expression was  
176 also low in PAX7-expressing satellite cells (Figure S2C), and present in small subsets of  
177 macrophages (Figure 1F and Figure S2E). This topological gradient also matches the evolution  
178 over time of *Fat1* RNA levels at later timepoints after muscle injury in published datasets (Figure  
179 1C, (Lukjanenko *et al.*, 2013)), with a sharp increase at 5 and 7 dpi, followed by a progressive  
180 return to baseline levels at 14 and 21 dpi. In GEO transcriptome datasets GDS1956 from  
181 references (Bakay *et al.*, 2006; Dadgar *et al.*, 2014), *FAT1* levels are elevated in both DMD and  
182 FSHD muscle biopsies vs. controls (Figure 1G), correlating with RNA levels of *PDGFRA* and  
183 *COL3A1*, hence with the degree of FAP amplification and fibrosis in these samples. Overall, these  
184 data support the possibility that *Fat1* signaling might play a role during skeletal muscle  
185 regeneration, acting predominantly in the myogenic and mesenchymal lineages.

186 Since *Fat1* was not uniformly detected across all FAPs in the lesion area, we wondered if its  
187 expression was particularly enriched in a specific FAPs subtype or associated with a specific  
188 regenerative-like state. Multiple recent single cell RNAseq (scRNA-seq) studies carried out on  
189 mouse muscle, including both homeostatic and post-injury conditions, have illustrated the  
190 remarkable molecular heterogeneity among FAP subtypes (Collins & Kardon, 2021; De Micheli *et*  
191 *al.*, 2020; Oprescu *et al.*, 2020). These datasets have been conveniently integrated in the joint  
192 scMuscle database (McKellar *et al.*, 2021), providing a valuable resource for comparative  
193 analyses. We extracted from this scMuscle dataset the scRNAseq expression levels of *Fat1*  
194 alongside those of established markers of distinct FAP subsets (Figure 2). These included *Pdgfra*,  
195 a common marker for all stromal cells, and selected subset-specific markers, such as *Hic1*, *Pi16*,  
196 *Mme*, or *TnC*. Consistent with our observations, *Fat1* RNA was detected in the FAP cluster, in  
197 Smooth muscle cells, in scattered cells among the myoblast and myogenic stem cell clusters

198 (Figure 2A,B). Focusing on FAPs, we color-converted *Fat1* in green and the other markers in red  
199 to examine their overlap (Figure 2D). This highlighted that although *Fat1* levels are not uniform  
200 among *Pdgfra*-expressing FAPs, *Fat1* expression was not restricted to a specific FAP subtype, but  
201 displayed instead a salt-and-pepper distribution throughout all subtypes. Consistent with the  
202 transient increase of *Fat1* expression after injury, high *Fat1* levels were detected in a subgroup  
203 labelled as “pro-remodelling” FAPs, corresponding to a FAP subtype most abundant at 5 dpi (De  
204 Micheli *et al.*, 2020; McKellar *et al.*, 2021), and characterized by high Tenascin C (TnC)  
205 expression (Figure 2D). TnC is known as a tenocyte marker in developing and adult tendons  
206 (Edom-Vovard *et al.*, 2002; Kardon, 1998), but its injury-induced expression in pro-remodelling  
207 FAP is characteristics of its activity as damaged-induced molecular pattern (Midwood *et al.*,  
208 2009; Piccinini & Midwood, 2010).

209

### 210 ***Impact of mesenchymal Fat1 deletion on glycerol-induced muscle regeneration and fibro-*** 211 ***adipogenic infiltration***

212 Guided by *Fat1* expression in pro-remodelling FAPs, and by our work dissecting how tissue-  
213 specific *Fat1* activities control muscle development, we focused on the mesenchymal lineage  
214 (Helmbacher, 2018). To explore the role played by *Fat1* in muscle-resident mesenchymal cells,  
215 we induced muscle injury in a cohort of adult *Fat1<sup>Prx1</sup>* and control mice. We explored this in the  
216 context of glycerol-induced lesions, a model permissive to regeneration, while also leading to  
217 some persistent fibrosis and adipose infiltrations (Lukjanenko *et al.*, 2013; Norris *et al.*, 2024;  
218 Pisani *et al.*, 2010). We chose to perform these experiments in the triceps brachii (medial), a  
219 muscle with high *FAT1* levels (Caruso *et al.*, 2013; Mariot *et al.*, 2015), that belongs to the clinical  
220 map of early disease stages in FSHD patients (Mariot *et al.*, 2015), rather than in the tibialis  
221 anterior muscle typically used in most regeneration studies. The Triceps Brachii (TB) muscle is  
222 also large enough for the injected myotoxic compound (i.e., glycerol) not to damage the whole  
223 muscle mass, preserving fibers in the periphery of the lesion (Figure 1E,F, and Figure 3D).

224 Inflammatory invasion and myogenic regeneration start from the outer limits of the lesion, and  
225 progress towards the center, such that in a same muscle/stage, regenerated myofibers at the  
226 periphery are the oldest and largest, whereas younger regenerated myofibers present in more  
227 central areas are smaller (Figure 1E, Figure S1). A ring of active regeneration occurs at the  
228 transition between the outer area containing regenerated myofibers, and the central area with  
229 necrotic/degenerating myofibers (Figure 1E-F, Figure S1, Figure 3). As regeneration proceeds,  
230 this active zone progresses by moving towards the center of the lesion, such that the necrotic  
231 area progressively shrinks, while the lesion area expands and is progressively filled with *de novo*  
232 regenerated myofibers, which also expand in diameter until completion of the process. In  
233 healthy mice, while the necrotic area is still present at 7dpi, it has largely disappeared at 14dpi  
234 (Figure 4C,D).

235 Given this positional evolution (large fibers in the periphery and small towards the active  
236 zone) and the variability of the initial lesion size, we established a systematic procedure for  
237 unbiased image quantitative analyses of the phenotypes of our samples. This involves acquiring  
238 a mosaic image of the entire muscle cross section, and systematically including phalloidin  
239 staining in one color channel. This strategy enabled us to unambiguously delineate the limits of  
240 the lesion area, identified by the clear boundary between undamaged fibers with peripheral  
241 nuclei and regenerated fibers featuring central (internal) nuclei (Figure 3). The region  
242 containing undamaged fibers was manually excluded from all channels including phalloidin but  
243 also channels with other antibody signals, so that subsequent ImageJ/Fiji analyses were  
244 restricted solely to the lesion area (Figure 3B). This approach allowed us to perform  
245 morphometric analyses across the entire muscle lesion, thereby avoiding the selection bias  
246 inherent to analyzing smaller, arbitrarily chosen, muscle areas. This method was used to  
247 quantify myofiber cross section area, as well as additional markers used, such as Perilipin-1  
248 (PLIN1) to assess adipogenic infiltrations and Tenascin-C (TnC) as a marker of pro-remodelling  
249 FAPs, characteristic of transient fibrosis. In all cases, measurements were compared between  
250 genotypes, either as raw value or normalized to the muscle or to the lesion area.

251 To investigate the impact of *Fat1* deletion in the mesenchymal lineage, we used first the  
252 *Prx1-cre* line, which drives recombination throughout the lateral plate-derived mesenchymal  
253 lineage from embryonic stages onward (Logan *et al*, 2002). Although *Prx1-cre; Fat1<sup>Flox/Flox</sup>* mice  
254 (referred to as *Fat1<sup>Prx1</sup>* mice hereafter) display developmental phenotypes affecting the shape  
255 and size of several muscles (Helmbacher, 2018), the TB muscle is relatively unaffected. Our  
256 cohort includes mice aged 4 to 9 months (with no difference in age distribution between  
257 controls and mutants). This enabled us to assess damage-induced regeneration, in a context with  
258 minimal pre-injury phenotypes.

259 To evaluate muscle regeneration following *Fat1* ablation in the *Prx1-cre* lineage, we  
260 measured the cross-section area (CSA) of the regenerated fibers (Figure 4E,F), and the total area  
261 occupied by regenerated myofibers within the lesion (Figure 4G). Unlike the robust non-cell  
262 autonomous impact of mesenchymal *Fat1* ablation on developmental muscle growth during  
263 development (Helmbacher, 2018), the impact on adult regeneration was modest. At 7 dpi,  
264 *Fat1<sup>Prx1</sup>* mice exhibited a small increase in the proportion of Fibers with the smallest caliber  
265 (<300 $\mu\text{m}^2$ ) compared to controls (Figure 4E-G). This did not impact the overall median fiber CSA  
266 (Figure 4G top), and was a transient effect not observed at 14dpi. Furthermore, at 14dpi, *Fat1<sup>Prx1</sup>*  
267 mice exhibited a subtle but significant increase in the percentage of lesion area covered by  
268 regenerated fibers relative to controls (Figure 4G bottom). These regeneration-associated  
269 changes are distinct from pre-existing (pre-injury) phenotypes, which manifested as a reduction  
270 of the whole muscle section area in *Fat1<sup>Prx1</sup>* mice compared to controls at 7 but no longer at 14  
271 dpi (Figure S3A-C), whereas the size of the lesion (measured as % of the whole muscle area) was  
272 not significantly affected by the genotype.

273 While myogenic repair was largely preserved, quantifications of PLIN1 and TnC localization  
274 and abundance uncovered more pronounced effects on FAP-derived fibro-adipogenic cell types:  
275 *Fat1<sup>Prx1</sup>* mice exhibited enhanced adipose infiltrations compared to controls (Figure 4H), evident  
276 both at 7 dpi, and 14 dpi, and a substantial increase in the TnC+ area at 7dpi. Consistent with the  
277 fact that TnC labels a subpopulation of FAPs characteristic of early post lesion stages, this

278 increase in mutants was only observed a 7 dpi and was no longer present at 14dpi, a stage at  
279 which the only residual TnC staining was restricted to intramuscular tendon structures (Figure  
280 4D), also present in uninjured muscles (not shown). Further analyses carried out separately on  
281 males and female cohorts uncovered that the effect of *Fat1<sup>Prx1</sup>* ablation on adipose infiltrations  
282 were more pronounced in females than in males (Figure 5A), whereas the increase in TnC+ area  
283 only reached statistical significance in male mutants (Figure 5B,E). The subtle and transient  
284 reduction of fiber CSA was likewise only significant in males (Figure 5D), whereas the  
285 proportion of the lesion occupied by regenerated fibers at 14 dpi was increased only in females  
286 (Figure 5C). These observations indicate that these phenotypes are independent of each-other.  
287 Interestingly, the latter increase in proportion of the lesion area covered by regenerated fibers  
288 in females co-occurred with an increase in adipocyte-covered area, indicating that in the present  
289 study, enhanced adipogenesis was not inversely correlated with regeneration as previously  
290 described (Norris *et al.*, 2025).

291 Overall, our results show that mesenchymal *Fat1* activity is largely dispensable for the  
292 promyogenic activity of FAPs during skeletal muscle repair, as its deletion minimally impacts the  
293 efficacy of muscle regeneration. Instead, *Fat1* activity in the mesenchymal lineage is required to  
294 limit the expansion of fibro-adipose infiltrates triggered by acute glycerol injury. Our analyses  
295 also uncovered sex-specific aspects of the phenotypes resulting from mesenchymal *Fat1*  
296 depletion, with a female-bias for the enhanced adipose infiltrations and the slightly enhanced  
297 regenerated area, and a male bias for the enhanced TnC expansion and for the transient effect on  
298 fiber CSA. These observations also imply that at the stages studied, the *Fat1*-dependent  
299 enhancement of adipose infiltrations is not sufficient to interfere with the efficacy of  
300 regeneration.

### 301 ***Cell-autonomous and cell-non-autonomous effects of inducible Fat1 deletion in FAPs***

302 To further understand the role played by *Fat1* in the formation and expansion of fibro-fatty  
303 infiltrates, and completely suppress the confounding pre-injury phenotypes, we used a second

304 CRE line, referred to as *Pdgfra<sup>icre</sup>*, in which expression of the Tamoxifen-inducible Cre/ERT2  
305 (iCRE) is driven by exogenous *Pdgfra* regulatory genomic locus in a BAC Transgene, allowing  
306 inducible deletion in the *Pdgfra* lineage (Rivers *et al*, 2008). Although *Pdgfra*-positive  
307 mesenchymal cells are also present outside muscles, *Pdgfra* expression in muscle encompasses  
308 all FAP subtypes and is the most widely used marker of FAPs used for FACS studies. A cohort of  
309 adult control (*Fat1<sup>Ctrol</sup>; Pdgfra<sup>icre</sup>; R26<sup>YFP/+</sup>*, referred to as *Ctrol<sup>iPdgfra-YFP</sup>*) and mutant (*Fat1<sup>Flox/Flox</sup>;*  
310 *Pdgfra<sup>icre</sup>; R26<sup>YFP/+</sup>*, referred to as *Fat1<sup>iPdgfra-YFP</sup>*) mice (Figure 6A), was subjected to a regime of 5  
311 consecutive Tamoxifen injections (1 per day), starting 2 days prior to Glycerol muscle lesions in  
312 the triceps brachii muscle at day 0 (Figure 6B). Analyses were carried out at 7 and 14 dpi.

313 In these mice, the *R26<sup>YFP</sup>* reporter line was used as a readout of recombination, allowing to  
314 visualize YFP-positive cells in the interstitial space between muscle fibers, labelling FAPs and  
315 their differentiation progeny (Figures 6 and 7), and to compare the fate of recombined cells in  
316 control and mutant contexts. Most YFP<sup>+</sup> cells were concentrated in the lesion, while largely  
317 absent from unaffected muscle areas, likely because 3 of the 5 Tamoxifen injections were done  
318 after glycerol injection, which triggers FAP expansion, thereby mobilizing the *Pdgfra* locus in the  
319 lesion. This regime resulted in low recombination efficacy, with few YFP<sup>+</sup> cells per section, much  
320 lower than the recombination rate observed with the same *Pdgfra<sup>icre</sup>* line at embryonic and post-  
321 natal studies (Helmbacher, 2018, 2022), and also lower than the recombination rate described  
322 for *Pdgfra<sup>icre</sup>* lines used in other FAPs studies (Kajabadi *et al*, 2023; Norris *et al*, 2023). We had  
323 excluded working with these alternative lines, because they involved knockout alleles of the  
324 endogenous *Pdgfra* locus, raising concerns that *Pdgfra* heterozygosity could influence or  
325 exacerbate phenotypes related to the pathway analyzed. Importantly, the low numbers of  
326 *Pdgfra*-derived YFP<sup>+</sup> cells per section obtained in our study offered us the advantage to allow  
327 distinguishing cell autonomous from non-cell autonomous phenotypes.

328 As previously observed in our work on migrating retinal astrocytes (Helmbacher, 2022),  
329 inducible deletion of *Fat1* in the *Pdgfra* lineage led to an increased the number of YFP<sup>+</sup> cells in  
330 *Fat1<sup>iPdgfra</sup>* mice compared to *Ctrol<sup>iPdgfra</sup>* mice (Figure 6E), consistent with an effect on cell

331 proliferation. Given the enhanced adipogenesis phenotype observed in the *Prx1-cre* model, we  
332 next analyzed the adipogenic fate of recombined cells in the inducible *Pdgfra* model, combining  
333 anti-GFP/YFP with anti-perilipin1/PLIN1 antibodies, and quantified the ratio of PLIN<sup>+</sup>;YFP<sup>+</sup>  
334 versus total YFP<sup>+</sup> area. This uncovered a significant increase in adipogenic fate of recombined  
335 cells in *Fat1<sup>iPdgfra-YFP</sup>* mice compared to *Ctrol<sup>iPdgfra-YFP</sup>* mice (Figure 6C,D,F). The severity of this  
336 phenotype was variable, with the percentage of PLIN<sup>+</sup>;YFP<sup>+</sup> area ranging from 5% to 30%  
337 (median 10%) in *Fat1<sup>iPdgfra</sup>* mice at 7 dpi, while *Ctrol<sup>iPdgfra</sup>* mice ranged between 0 and 6%  
338 (median 3%). At 14 dpi, these values increased from 30% to 48% in *Fat1<sup>iPdgfra</sup>* mice (median  
339 32%), while *Ctrol<sup>iPdgfra</sup>* mice ranged between 0 and 21% (median 7,8%).

340 This increase in the propensity of recombined cells to undergo adipogenic differentiation  
341 in *Fat1<sup>iPdgfra-YFP</sup>* mice was accompanied with a decrease in the proportion of YFP<sup>+</sup> cells  
342 maintaining PDGFRA protein expression (Figure 7). The loss of *Pdgfra* expression likely reflects  
343 the loss of progenitor characteristics and is consistent with the fact that mature adipocytes do  
344 not maintain *Pdgfra* expression (Contreras *et al*, 2019). The proportion of PDGFRA-negative;  
345 YFP<sup>+</sup> cells in *Fat1<sup>iPdgfra-YFP</sup>* mice was higher than the proportion of PLIN<sup>+</sup>; YFP<sup>+</sup> cells, suggesting  
346 that *Fat1* deletion may also enhance alternative FAP differentiation outcomes, such as  
347 myofibroblasts.

348 Unexpectedly, the analysis of PLIN1/YFP double stainings also uncovered a robust non-cell-  
349 autonomous enhancement of adipogenesis: The total PLIN1<sup>+</sup> area in the lesion, consisting of a  
350 large majority of non-recombined cells (YFP-negative), was also significantly increased (Figure  
351 6G). Although it is theoretically possible that recombination at the *R26* and *Fat1* loci may not  
352 systematically occur in the same cells, if this increase in adipocyte number resulted from the cell  
353 autonomous phenotype described above, we would nevertheless expect an increase in the YFP<sup>+</sup>  
354 proportion of the total PLIN<sup>+</sup> area in mutants. Instead, the proportion of YFP<sup>+</sup> among PLIN1<sup>+</sup>  
355 cells remained constant, at around 5% in both control and mutant mice (Figure 6H), supporting  
356 the idea that *Fat1*-deficient FAPs exert a pro-adipogenic influence on neighbouring non-  
357 recombined FAPs, or have lost an inhibitory (anti-adipogenic) property.

358 In line with our observations in the *Fat1<sup>Prx1</sup>* model, analysis by sex revealed that enhanced  
359 adipogenesis was more pronounced in female *Fat1<sup>iPdgfra-YFP</sup>* mice compared to *Ctrol<sup>iPdgfra-YFP</sup>*  
360 females than in males (Figure 8A middle graph). The effect size was approximately 5 folds in  
361 females, versus 2.3 folds in males. In contrast, the non-cell autonomous increase in adipogenesis  
362 was similar (around 2,5 folds) between males and female (Figure 8A right graph), supporting  
363 the idea that the two phenotypes are distinct from each other and not equally sensitive to sex.

364 Finally, given the strong non-cell-autonomous effect on adipogenesis, despite modest  
365 recombination rates, we next assessed whether inducible *Fat1* deletion in FAPs impacts muscle  
366 regeneration by myofiber CSA and total regenerated area. We did not detect any effect on fiber  
367 CSA or total area covered by regenerated fibers at 7dpi, contrasting with the changes observed  
368 in the *Fat1<sup>Prx1</sup>* model. Instead, at 14 dpi, this analysis uncovered a significant reduction of the  
369 median fiber CSA in *Fat1<sup>iPdgfra-YFP</sup>* mice compared to *Ctrol<sup>iPdgfra-YFP</sup>* mice, resulting not only from an  
370 increased proportion of fibers with small caliber (<300 $\mu\text{m}^2$ ), but also from a decreased  
371 proportion of large diameters (>600 $\mu\text{m}^2$ ; <2100 $\mu\text{m}^2$ ) (Figure 8B). Although we cannot  
372 distinguish whether this effect is caused by the enhanced adipogenesis, or by the loss of a  
373 promyogenic *Fat1* activity in FAPs, this observation suggests that inducible *Fat1* deletion in  
374 FAPs negatively interferes with myogenic repair, an effect that was possibly masked in the  
375 *Fat1<sup>Prx1</sup>* model by pre-injury (developmental) phenotypes (which are absent in the inducible  
376 *Fat1<sup>iPdgfra-YFP</sup>* model (Figure S3), as Tamoxifen-induction is coincident with the lesion).

377

## 378 Discussion

379 The appearance of intramuscular fibrosis and adipose tissue is a hallmark of advanced  
380 symptoms in muscles of patients with muscular dystrophy (Flores-Opazo *et al.*, 2024; Theret *et*  
381 *al.*, 2021). Although common to multiple diseases, the appearance of fibro-fatty infiltrates is  
382 frequently a consequence of chronic inflammation, itself being a secondary to the primary cause  
383 of each disease. So far, to our knowledge, conditions in which FAP dysfunction is the primary

384 event have not been identified in human patients. Therefore, animal models engineered to  
385 primarily affect FAP homeostasis represent alternative sources of key information to  
386 understand what contributes to fibro-fatty development and to identify novel therapeutic  
387 approaches, potentially applicable to multiple disease conditions. While FAP-specific deletion of  
388 *Fat1* was initially aimed to explore the impact on the efficiency of muscle regeneration, we made  
389 the unexpected discovery that it enhanced fibro-adipogenic differentiation in regenerating  
390 muscles after glycerol injury, with a strong bias towards adipogenesis, including both a cell  
391 autonomous and a non-cell-autonomous component. This uncovered a new role of *Fat1* as an  
392 inhibitor of intramuscular fibro-adipogenesis, and positions *Fat1<sup>Prx1</sup>* mice as a new model of  
393 enhanced IMAT formation in response to injury.

#### 394 ***Distinguishing developmental from regenerative promyogenic activities of FAPs***

395 FAPs constitute a promyogenic niche that supports regeneration of adult skeletal muscles  
396 by secreting factors promoting satellite cell expansion or myogenic repair (Joe *et al.*, 2010;  
397 Lukjanenko *et al.*, 2019; Madaro *et al.*, 2018), by secreting chemokines orchestrating key steps in  
398 the FAP-immune cross-talk (Heredia *et al.*, 2013; Lemos *et al.*, 2015; Nawaz *et al.*, 2022), and by  
399 structuring the extracellular matrix, influencing myogenesis through its composition and its  
400 stiffness (Kotsaris *et al.*, 2023). Likewise, during embryonic development, a population of  
401 muscle-associated mesenchymal cells plays similar promyogenic functions (Helmbacher &  
402 Stricker, 2020) and was shown to represent the developmental progenitors of adult FAPs  
403 (Vallecillo-Garcia *et al.*, 2017), the two lineages sharing expression of the transcription factor  
404 *Osr1* (Kotsaris *et al.*, 2023; Stumm *et al.*, 2018; Vallecillo-Garcia *et al.*, 2017). Genetic ablation of  
405 adult FAPs resulted in reduced myogenic stem cell expansion after muscle injury (Murphy *et al.*,  
406 2011; Wosczyzna *et al.*, 2019), and consequently in muscle atrophy and weakness, or in impaired  
407 revascularization after hindlimb ischemia (Santini *et al.*, 2020). Similarly, abrogating fate or  
408 functions of developmental FAPs has an influence on muscle growth and patterning, illustrated  
409 in multiple mesenchyme-specific mouse mutants (Helmbacher & Stricker, 2020). Conditioned  
410 media from adult FAPs extracted from injured muscles release factors that promote myogenesis

411 (Joe *et al.*, 2010; Madaro *et al.*, 2018; Mozzetta *et al.*, 2013). While FAP-derived secretomes have  
412 been explored in several studies (Florin *et al.*, 2020; Kotsaris *et al.*, 2023; Vumbaca *et al.*, 2021),  
413 only a small fraction of these promyogenic factors have so far been functionally identified and  
414 confirmed to promote muscle stem cell growth or myofiber growth. This is the case for WISP1,  
415 BMP3a/GDF10, or Follistatin, three factors produced by FAPs in young but not old mice, which  
416 decreased production in aged dystrophic mice is responsible for myofiber atrophy and muscle  
417 weakness (Lukjanenko *et al.*, 2019; Nawaz *et al.*, 2022; Uezumi *et al.*, 2021). Similarly, exploring  
418 the promyogenic transcriptome or secretome of developmental FAPs has yielded a number of  
419 potential mediators of their promyogenic activity (Besse *et al.*, 2020; Orgeur *et al.*, 2018;  
420 Vallecillo-Garcia *et al.*, 2017).

421 During development, abolishing mesenchymal *Fat1* activity had a strong effect of muscle  
422 development, impacting progenitor migration and fiber extension in the cutaneous maximus  
423 (CM) muscle, but also leading to the presence of ectopic muscles with unconventional fiber  
424 orientation and attachment sites (Helmbacher, 2018). These phenotypes recapitulated a large  
425 subset of the muscle phenotypes observed in the constitutive knockout (Caruso *et al.*, 2013). We  
426 thus anticipated that abolishing mesenchymal *Fat1* in adult muscles would likewise interfere  
427 with the promyogenic activity of FAPs. However, mesenchymal *Fat1* ablation (in both  
428 constitutive and inducible models) only had a relatively modest (even though significant) effect  
429 on myogenic repair, limited to a small increase in the proportion of small caliber fibers, and to  
430 the reduced myofiber CSA observed at 14 dpi in the inducible *Fat1<sup>iPdgfra</sup>* model. Although at fetal  
431 stages, *Fat1* levels were higher in the TB muscle than in other developing muscles (Mariot *et al.*,  
432 2015), it remains possible that in adult mice, such a promyogenic activity might vary between  
433 muscle types, and would be relatively mild in the triceps brachii, while more pronounced in  
434 other muscles (that we have not sought to identify). An alternative possibility could be that the  
435 *Fat1*-regulated mesenchymal factors influencing muscle morphogenesis during development  
436 might be distinct from the FAP-derived pro-myogenic factors that promote myogenic repair in  
437 adults. Considering that ablation of mesenchymal *Fat1* resulted instead in a robust increase in

438 the amount of intramuscular adipose differentiation, resulting from the combination of cell-  
439 autonomous and non-cell autonomous component, this suggests that *Fat1* activity in adult FAPs  
440 may have been redirected toward inhibiting adipogenesis, at the expense of a role in promoting  
441 secretion of promyogenic factors. It is also possible that the nature of *Fat1*-regulated FAP  
442 transcriptome (or secretome) might depend on the molecular properties of FAPs, and that the  
443 FAP subtypes present in TB muscle after glycerol injury might be distinct from embryonic FAPs  
444 surrounding *Fat1*-dependent muscles during development. In support of this possibility, it is  
445 interesting to consider *Osr1*, another example of genes acting in both embryonic and adult FAPs  
446 (Kotsaris *et al.*, 2023; Stumm *et al.*, 2018; Vallecillo-Garcia *et al.*, 2017). Unlike *Fat1* however,  
447 *Osr1* deletion had an impact on both developmental and regenerative myogenesis (Kotsaris *et*  
448 *al.*, 2023; Vallecillo-Garcia *et al.*, 2017). Nevertheless, as postulated above, the *Osr1*-dependent  
449 transcriptome signatures appear qualitatively different between developmental and adult stages  
450 (Kotsaris *et al.*, 2023; Vallecillo-Garcia *et al.*, 2017). Consistently, the single cell transcriptome  
451 studies that have characterized molecularly distinct FAPs subpopulations, representing phases  
452 of regeneration and modalities of FAP activities, differently represented in healthy or  
453 pathological environments, have also identified differences between ages, accounting for  
454 differences between young and aged FAPs, but also differences between juvenile and adult FAPs.

#### 455 ***Fat1 is a negative regulator of intramuscular adipogenesis***

456 The present findings identify *Fat1* as a new modulator of fibro-adipogenic differentiation.  
457 This adds to the recent identification of a series of other signaling pathways that significantly  
458 moderate adipogenic FAP differentiation, among which interleukin-4 (IL4) (Dong *et al.*, 2014)  
459 Nitric oxid (NO) and Notch signaling (Marinkovic *et al.*, 2019), Desert Hedgehog (DHH) (Kopinke  
460 *et al.*, 2017; Norris *et al.*, 2023), Wnt5A, and Wnt7A (Fu *et al.*, 2023; Santiago *et al.*, 2025). After  
461 muscle injury, FAPs transiently harbor cilia, which represent a hotspot of active Hedgehog  
462 signaling and are necessary for intramuscular adipogenesis (Kopinke *et al.*, 2017). Genetically  
463 preventing cilia formation in FAPs, deleting Patched1 activity in FAPs, or activating Hedgehog  
464 signaling with the Smoothened agonist SAG, equally resulted in a reduction of intramuscular

465 adipogenesis, and led to increased diameter of regenerated fibers (Kopinke *et al.*, 2017). A  
466 follow-up study identified Desert hedgehog as the ligand, produced by endothelial cells and  
467 schwann cells, which limits adipogenesis in regenerating muscle (Norris *et al.*, 2023). In another  
468 study, an in vitro screen using FAPs from dystrophic mice led to the finding that inhibitors of  
469 GSK3 were overrepresented among anti-adipogenic compounds, suggesting that canonical Wnt  
470 signaling could inhibit intramuscular adipogenesis (Reggio *et al.*, 2020). ScRNAseq analyses  
471 indicated that FAPs were the main source of Wnt ligands. Among them, WNT5a was expressed in  
472 resting and lesion-activated FAPs, downregulated in MDX FAPs, and capable of inhibiting  
473 adipogenesis of FAPs in vitro, (Reggio *et al.*, 2020). Another FAP-derived Wnt, WNT7A,  
474 previously known to promote muscle regeneration induced by injury by inducing expansion of  
475 satellite cells (Le Grand *et al.*, 2009), and to improve regeneration in dystrophic mice (von  
476 Maltzahn *et al.*, 2012), while its ablation exacerbated muscular dystrophy symptoms (Gurriaran-  
477 Rodriguez *et al.*, 2024), was recently shown to suppress intramuscular adipogenesis in vitro and  
478 in vivo (Fu *et al.*, 2023). Furthermore, injection of lipid nanoparticles loaded with WNT7A-mRNA  
479 in mice were shown to be effective in reducing IMAT in vivo (Santiago *et al.*, 2025).

480 In the pathways leading to adipogenesis, adipogenic stem cells are multipotential, and the  
481 first step is a step of commitment, restricting their potential to one fate only (Ferrero *et al.*, 2020;  
482 Ghaben & Scherer, 2019). This is followed by differentiation and maturation, two subsequent  
483 steps that allow maturation of adipocytes into lipid producing cells (Ferrero *et al.*, 2020; Ghaben  
484 & Scherer, 2019). The master regulator of adipogenic differentiation and maturation is PPAR $\gamma$ , a  
485 nuclear receptor acting as transcription factor. Suppression of PPAR $\gamma$  activity blocks adipogenic  
486 differentiation, and its ablation in all cells except the placenta (Dammone *et al.*, 2018) or its  
487 inducible ablation in FAPs (Norris *et al.*, 2025), abrogate intramuscular adipose tissue formation  
488 induced by glycerol lesions. The mechanism by which the anti-adipogenic signals described  
489 above act is likely converging on PPAR $\gamma$ . WNT5A appears to block expression of PPAR $\gamma$  in a  $\beta$ -  
490 catenin-dependent manner (Reggio *et al.*, 2020). Interestingly, the anti-adipogenic activity of  
491 WNT7A correlates with its capacity to promote nuclear accumulation of YAP/TAZ, both of which

492 acting as anti-adipogenic transcription factors in non-muscular adipose tissue: TAZ acts as a  
493 direct suppressor of PPAR $\gamma$  activity (El Ouarrat *et al*, 2020; Jung *et al*, 2009), whereas YAP may  
494 act by inducing WNT5A expression (Lee *et al*, 2022). Paradoxically, loss of *Fat1* activity leads to  
495 cell-autonomous nuclear accumulation of YAP and TAZ in skin cancer (Pastushenko *et al*, 2021),  
496 and to reduced YAP/TAZ degradation in cancer cells and endothelial cells (Li *et al*, 2023). Thus,  
497 based on knowledge mentioned above from non-muscular adipose tissue (El Ouarrat *et al*,  
498 2020; Jung *et al*, 2009; Lee *et al*, 2022), loss-of-*Fat1* would be predicted to cell-autonomously  
499 suppress adipogenesis, which is the opposite of what we observed in the present study.  
500 However, activities of Fat cadherins, but also YAP/TAZ, vary with parameters such as stiffness  
501 changes, position along expression gradients, cell type and activity of neighboring cells, and  
502 several arguments support the idea of cell specificity in *Fat1* mode of action. First, *FAT1* is both  
503 known as a tumor suppressor (Morris *et al*, 2013; Pastushenko *et al*, 2021) or oncogene (Chen  
504 *et al*, 2022; de Bock *et al*, 2012; Meng *et al*, 2021; Zhao *et al*, 2025), depending on the cancer  
505 type. Second, FAP-derived adipocytes are not identical to adipose-tissue-derived adipocytes, as  
506 they differ for example in their insulin sensitivity (Arrighi *et al*, 2015). Third, in the *Fat1<sup>Prx1</sup>*  
507 model, we did not observe any sign of obesity (not even a difference in weight), suggesting that  
508 the increase in intramuscular adipogenesis after glycerol lesion in *Fat1<sup>Prx1</sup>* mice is not associated  
509 with enhanced adipogenesis in non-muscular adipose tissue. Finally, it is interesting to notice  
510 that in our tibialis anterior muscle samples, adipogenic infiltrations are regionalized and always  
511 found centered around a hotspot of perivascular Fat on the posterior side of the muscle (bottom  
512 of full size muscle sections in Figures 3, 4, and 6), while we never found adipocytes in the bone-  
513 associated side of the muscle (top of images). This suggest that *Fat1*-restricted adipogenesis is a  
514 regionalized process potentially reflecting heterogeneity in FAP populations. Thus, future work  
515 will be necessary to characterize the mechanisms by which *Fat1* activity negatively regulates  
516 intramuscular adipogenesis and how it may differ from *Fat1*-regulated fibrosis or *Fat1* activity  
517 in non-muscular stromal cells.

518 ***Fat1 non-cell-autonomously inhibits intramuscular adipogenesis***

519 An unexpected aspect of our work is the discovery that in addition to cell-autonomously  
520 preventing adipogenic differentiation of FAPs, *Fat1* also exerts its anti-adipogenic role through a  
521 non-cell-autonomous or paracrine activity. This was discovered owing to the limited  
522 recombination efficiency of the *Pdgfra-icre* line we used and the Tamoxifen-regime we applied.  
523 The fact that we could distinguish adipocytes derived from either recombined cells (YFP+) or  
524 non-recombined (YFP-negative) cells, in both controls and mutants, meant that we could  
525 differentially quantify cell autonomous and non-cell autonomous phenotypes resulting from  
526 *Fat1* ablation. This led to the clear discovery that *Fat1* exerted both types of activities. The non-  
527 cell-autonomous phenotype suggests that *Fat1*-deficient FAPs promote adipogenesis of  
528 neighboring wild-type FAPs. This effect could result either from an increased secretion of pro-  
529 adipogenic signals or from a decrease in secretion of anti-adipogenic signals. Interestingly, while  
530 the cell autonomous phenotype was more pronounced in females, the non-cell autonomous part  
531 equally affected males and females (Figure 8B). This suggests that the two activities involve  
532 distinct molecular mechanisms, not equally sensitive to sex-dependent parameters.

533 This non-cell autonomous anti-adipogenic paracrine activity is interesting, in the light of  
534 studies on adipose tissue, carried out in non-muscular adipose tissue, that identified a subtype of  
535 adipogenic stem cells endowed with an anti-adipogenic activity, referred to as Areg (adipogenic  
536 regulators), and distinguishable through its expression of F3/CD142, GDF10 (Schwalie *et al*,  
537 2018). The same group later identified several possible mediators of this anti-adipogenic  
538 activity, among which GDF10 or Retinoic acid signaling (Zachara *et al*, 2022). Another study  
539 later identified a similar subtype of GDF10-expressing FAPs, and found reduced proportions of  
540 GDF10+ FAPs in Mdx mice, correlating with enhanced intramuscular fat (Camps *et al*, 2020).  
541 These elements suggest that GDF10 exerts a protective role against pathological intramuscular  
542 adipose tissue expansion, in addition to its role, mentioned earlier, as FAP-derived signal  
543 promoting myofiber growth, and NMJ integrity (Uezumi *et al*, 2021). It will be interesting in  
544 future studies to determine how *Fat1* signaling in FAPs is linked to the various players

545 modulating adipogenesis cell-autonomously, and if it indeed modulates secretion of paracrine  
546 acting anti-adipogenic factors.

### 547 ***Sex bias in Fat1-dependent phenotypes***

548 An interesting aspect of our work is that separating male and female cohorts allowed  
549 uncovering sex-specific aspects of the *Fat1*-dependent phenotypes. As discussed above, the cell-  
550 autonomous phenotype leading to enhanced adipogenicity tends to be associated with females,  
551 whereas the increased secretion of the damage-associated molecular pattern TNC is a male-  
552 specific response to *Fat1* deficiency. Instead, the non-cell autonomous increase in adipogenesis  
553 seen in the inducible model similarly affects both sexes. In contrast, the transient effect on fiber  
554 CSA (higher proportion of small caliber fibers) was specifically seen in males, whereas the  
555 increased area covered by regenerated fibers at 14dpi was only seen in females. Although  
556 modest, these myogenic phenotypes also uncover sensitivity to sex-associated parameters.  
557 While there are no reports to our knowledge of sex-specific differences in signaling by Fat-like  
558 cadherins, sex-specific associations with IMAT have previously been reported, such as in the  
559 remodeling of IMAT in response to muscle injury (Norris *et al.*, 2024) or to high fat diet (HFD)  
560 (Smith *et al.*, 2025).

### 561 ***Conclusion***

562 Altogether, our results link FAT1 dysfunction in FAPs to IMAT formation, the hallmark of  
563 advanced disease progression associated with several pathologies. Although FAT1 itself is not an  
564 easily targetable gene for therapy, the discovery of its participation to the gatekeeping system  
565 negatively modulating IMAT formation and progression opens new avenues for the search of  
566 therapies to block or delay disease progression in patients.

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## 571 **Methods**

### 572 ***Ethics statement***

573 All animal care and procedures involving mice have been approved by the Ethics committee  
574 for animal experimentation of Marseille (committee N° 14, project number 2022011100527909-  
575 V2 #34611) and by the French ministry of research and higher education (project number  
576 APAFIS #34611-2022011100527909 v3), in accordance with the European Community Council  
577 Directives (2010/63/EU) and with the French law and institutional guidelines on animal  
578 research. Animal housing, care and experimental protocols were carried out in the IBDM animal  
579 facility under an agreement for (G1305521) delivered by the “Prefecture de la region Provence-  
580 Alpes-Cotes-d’Azur et des Bouches du Rhône”.

### 581 ***Mice***

582 The mouse lines used in this study are maintained with occasional backcrosses with  
583 commercial C57BL/6J or B6D2-F1J/RJ mice. We used combinations of the following genetic  
584 modifications: *Fat1<sup>Flox</sup>* (*Fat1<sup>tm1.1Fhel</sup>* conditional allele, MGI: 5524120, (Caruso *et al.*, 2013;  
585 Helmbacher, 2018)); *Prx1-cre* (Transgenic line Tg(Prrx1-cre)1Cjt, MGI: 2450929, (Logan *et al.*,  
586 2002)); *Pdgfra-CRE/ERT2* (referred to as *Pdgfra-iCRE*, BAC transgenic line Tg(Pdgfra-  
587 cre/ERT2)1Wdr, MGI: 3832569, (Rivers *et al.*, 2008)); R26-YFP (reporter line for cre activity,  
588 *Gt(ROSA)26sor<sup>tm1(EYFP)Cos</sup>*, MGI: 2449038, (Srinivas *et al.*, 2001)); *Fat1<sup>LacZ</sup>* (Bay Genomics Gene trap  
589 line *Fat1<sup>Gt(KST249)Byg</sup>*, MGI: 4124012, (Caruso *et al.*, 2013; Helmbacher, 2018; Mitchell *et al.*, 2001)).  
590 To produce Mice for the *Prx1-cre* cohort, *Prx1-cre; Fat1<sup>Flox/+</sup>* males were crossed with *Fat1<sup>Flox/Flox</sup>*  
591 females or *Prx1-cre; Fat1<sup>Flox/Flox</sup>* males were crossed with *Fat1<sup>Flox/+</sup>* females. The absence of  
592 germline activity of Prx1-cre in males (in contrast to Females) ensures that males always  
593 transmit unrecombined *Fat1<sup>Flox</sup>* alleles. This cross produces *Prx1-cre; Fat1<sup>Flox/Flox</sup>* mutants, and we  
594 used *Prx1-cre; Fat1<sup>Flox/+</sup>* mice as controls (occasionally *Fat1<sup>Flox/Flox</sup>*). To produce mice for the

595 *Pdgfra-icre* cohort, there was no specific requirement the cre line to be provided in males or  
596 females, as there is no recombination in absence of Tamoxifen activation. *Pdgfra-icre; Fat1<sup>Flox/+</sup>;*  
597 *R26<sup>YFP/+</sup>* mice were crossed with *Fat1<sup>Flox/Flox</sup>; R26<sup>YFP/YFP</sup>* mice. Controls (referred to as *Ctr<sup>l<sup>i</sup>Pdgfra-</sup>*  
598 *YFP*) include *Pdgfra-icre; Fat1<sup>Flox/+</sup>; R26<sup>YFP/+</sup>* and *Pdgfra-icre; Fat1<sup>+/+</sup>; R26<sup>YFP/+</sup>* mice. Genotyping was  
599 done as previously described for each line (Caruso *et al.*, 2013; Helmbacher, 2018, 2022).

### 600 **Muscle lesions.**

601 For muscle injuries, mice were anesthetized by intraperitoneal injection of Ketamine  
602 (100mg/Kg) and Xylazine (10mg/Kg) and receive a subcutaneous injection of buprenorphin  
603 (0,1mg/kg). They were then placed on a heated pad, shaved around the area of interest, and  
604 disinfected with betadine. A small scalpel incision was made to open the skin above the target  
605 muscle, in order to displace the subcutaneous adipose tissue and visualize the muscle. This  
606 allows entering the needle in a well-defined position in the muscle, thereby also avoiding to  
607 damage large vessels. Lesions were made by intramuscular injections of glycerol (50% glycerol  
608 in sterile water), or Cardiotoxin (10  $\mu$ M, dissolved in 0.9% NaCl solution). Injections were  
609 performed in the triceps brachii (TB) muscle in most cases, or occasionally in the tibialis  
610 anterior (TA) muscle, using an insulin syringe 0.3ml (30G). We injected a volume of 30 to 50  $\mu$ l  
611 per muscle. Injections were always carried out in the left Triceps brachii, and the right side was  
612 used as uninjured control. After injection, the skin is sutured using Michel suture clips, applied  
613 with adequate clip-applying forceps (Fine science tools). Mice were kept under observation until  
614 they were fully awake and had recovered from the anesthesia.

### 615 **Tamoxifen induction.**

616 For the inducible model, CRE activation by Tamoxifen is carried out at adult stage.  
617 Tamoxifen (T5648, Sigma) is dissolved first in ethanol (100mg/ml) under agitation for 2h at  
618 42°C, then diluted in Corn Oil (C8267, Sigma), at 10 mg/ml, kept under a chemical hood at room  
619 temperature, for the ethanol to evaporate, then aliquoted and stored at -20°C. Mice are injected  
620 intraperitoneally with a volume corresponding to 10 $\mu$ l/g (per gram of tissue weight) for mice

621 weighing less than 30g (dosage 100µg/g). For mice weighing more than 30g, we limited the  
622 injected volume to 300 µl, to avoid liver toxicity of the Tamoxifen/oil mix. Each mouse (in both  
623 the control and mutant group) received 5 consecutive injections, with two injections before the  
624 lesion (day -2 and -1), and 3 injections after muscle lesion (days 0, +1, +2). The weight at first  
625 injection is considered as reference, and mice are weighed every day to evaluate potential  
626 weight loss. Any mouse having lost more than 15% after the two first injections is excluded from  
627 the experiment. Efficiency of recombination was assessed using the reporter line R26YFP, by  
628 immunohistochemistry with anti-YFP antibodies on muscle cryo-sections. Both *Ctrod<sup>l</sup>iPdgfra-YFP* and  
629 *Fat1<sup>iPdgfra-YFP</sup>* mice carry one copy of the CRE allele and one copy of the R26-YFP allele, and have  
630 been exposed to the same dose and number of Tamoxifen injections, thus making *Fat1* the only  
631 varying factor.

### 632 ***Tissue harvesting and immunohistochemistry.***

633 Muscles were collected after trans-cardiac perfusion of anesthetized mice with 4% PFA (in  
634 PBS). Mice were anesthetized with a lethal dose (2 to 5 fold the amount used for normal  
635 anesthesia) of Ketamine (200mg/Kg) and Xylazine (20mg/Kg) and received a subcutaneous  
636 injection of buprenorphin (0,1mg/kg). After perfusion, muscles were dissected and postfixed in  
637 4% PFA (in PBS) for 4 to 6h at 4°C, rinsed 3 times in PBS, and cryoprotected by immersion in  
638 25% sucrose (in PBS), at 4°C until the tissues sank to the bottom (attesting equilibrium). Each  
639 muscle was transversally cut in two halves, the cut being made at the level of the center of the  
640 lesion. Tissue samples were embedded in gelatin/sucrose mix (7,5% gelatin, 15% sucrose, in  
641 PBS) in plastic molds. For embedding, the mix was preheated at 42°C, 2ml of gelatin/sucrose mix  
642 was poured in the molds. Excess liquid surrounding tissue fragments was dried on absorbing  
643 tissue, the fragments were deposited and oriented in the gelatin/sucrose mix, and the blocks  
644 were allowed to solidify on ice. A color mark indicating the axis and limits of tissue fragments  
645 was added on the solidified blocks prior to freezing in an isopentane bath cooled in dry ice  
646 ethanol mix. Samples were serially sectioned with a cryostat (10 µm sections), deposited on  
647 superfrost plus Gold microscope slides (K5800AMNZ72), which were stored at -20C. For

648 immunocytochemistry, the slides were thawed, rinsed in PBS for 5 minutes, incubated with PBS  
649 0,3% triton (P0.3T) for 5 minutes, then with 6% H2O2 in PBS 0,3% triton for 30 minutes, rinsed  
650 3 times with P0.3T, and incubated overnight at 4°C with primary antibodies in blocking solution  
651 with 20% newborn calf serum, 0,3% triton in PBS. Slides are then washed by multiple  
652 incubations in with P0.3T under gentle agitation (minimum 5 x 20 minutes), and incubated in  
653 blocking solution (as above) with secondary antibodies conjugated with fluorophores, either  
654 overnight at 4° or at room temperature for at least 1h30. After multiple washes with P0.3T, the  
655 slides are mounted under coverslips with mounting medium with ProlongGold antifade reagent  
656 and DAPI (P36935, Thermo Fisher Scientific), and kept at 4°C. The list of primary antibodies  
657 used is provided below. Images are acquired using a Zeiss Z1 microscope, equipped with an  
658 Apotome, using the Zen software. After initial treatment with Zen, final images are exported in  
659 tif, and treated with ImageJ and Fiji for image analyses and with Photoshop for figure mounting.

660 Primary antibodies and reagents: Chicken anti- $\beta$ -galactosidase, Abcam ab9361, RRID:  
661 [AB 307210](#). Purified rat anti-mouse CD104a (Pdgfra) monoclonal antibody, clone APA5, BD  
662 Biosciences #558774, RRID: [AB 397117](#); XP Rabbit anti-Perilipin monoclonal antibody, clone  
663 D1D8; Cell signaling technology, #9349, RRID: [AB 10829911](#); Chicken anti-GFP, Aves, #GFP-  
664 1020, RRID: [AB 10000240](#); Rat monoclonal anti mouse Tenascin-C (TnC) antibody, clone Mtn-  
665 12, Thermofisher Scientific, #MA1-26778, RRID: [AB 2256026](#); Mouse monoclonal anti-Chicken  
666 PAX7, IgG1, Developmental studies hybridoma Bank, #Pax7, RRID: [AB 528428](#); Rabbit anti-Iba1  
667 polyclonal antibody, Wako Chemicals, #019-19741, RRID: [AB 839504](#); Alexa-Fluor-conjugated  
668 Phalloidin, Thermofisher Scientific, Alexa-Fluor-488: A12379; Alexa-Fluor-546: A22283; Alexa-  
669 Fluor-647: A22287.

### 670 ***$\beta$ -galactosidase assays***

671 Staining for  $\beta$ -galactosidase activity on cryosections was done as described previously  
672 (Caruso *et al*, 2014; Helmbacher, 2018), using Salmon Gal (6-chloro-3-indolyl- $\beta$ -D-  
673 galactopyranoside from Appolo scientific, Manchester, UK) as substrate. Briefly, slides with

674 cryostat sections were thawed in PBS, incubated overnight at 37°C in a solution containing 4 mM  
675 Potassium Ferricyanide, 4 mM Potassium Ferrocyanide, 4 mM MgCl<sub>2</sub>, and 0,04% NP40  
676 substitute, without substrate, to inactivate endogenous enzymes, rinsed 3 times in PBS, and  
677 incubated in a solution with Salmon Gal (1mg/ml), nitro blue tetrazolium (NBT, N6876 Sigma,  
678 330 µg/ml), 2 mM MgCl<sub>2</sub>, 0,04% NP40 substitute, in PBS. Staining intensity was monitored  
679 under a dissection microscope. If the solution became purple, it was replaced with fresh staining  
680 solution until completion. After staining (1-2h for sections of *Fat1<sup>LacZ</sup>* muscles) the slides were  
681 fixed overnight with 4% PFA in PBS, and mounted.

### 682 ***Image analyses and quantifications***

683 For every mouse included in the experimental cohorts, muscle samples were serially cryo-  
684 sectioned, so as to generate a collection of representative slides to use for multiple  
685 immunohistochemistry experiments. This allowed performing multiple assays on the full cohort.  
686 After performing a given IHC experiment, we acquired a 10X mosaic image of at least one slice  
687 containing the entire muscle section. Quantifications were systematically done on 10X images of  
688 the entire muscle section rather than portions of it acquired at higher resolution to avoid  
689 selecting areas of interest in a biased manner. Instead, we proceeded in a completely unbiased  
690 manner, following a systematic set of steps described below:

691 All IHC included a channel with phalloidin staining. Single channel TIF images exported  
692 from ZEN were assembled as distinct layers in Photoshop, with fusion modes for each layer set  
693 to “lighten”, on top of a background layer filled with black. Each layer was duplicated so as to  
694 maintain unmodified images, while modifications were done on duplicates. An extra duplication  
695 of the phalloidin layer was done with enhanced midtones, so as to visualize all fibers equally  
696 well irrespective of phalloidin intensity. The lasso tool was used to manually select areas  
697 containing undamaged fibers, and the content of selected areas was deleted in all (duplicate)  
698 layers. After completing the step by step deletion of all undamaged fibers, each remaining  
699 content of each layer was individually merged with the black background layer, thus generating

700 for each color a tif document to be analyzed in Fiji/ImageJ, containing the staining  
701 corresponding, for each color/antibody, to the content of the lesion. These documents were  
702 opened in ImageJ, converted to 8bit, and thresholded (using the same thresholding parameters  
703 and exposure time for all compared images). The area of the objects contained in each image  
704 were analyzed using the “analyze particles” tool (not including holes) and exported to excel. The  
705 area, measured in square pixels, was converted in real dimension by multiplying the object area  
706 by multiplying with the square resolution (96 ppi, pixels per inch), and with the square pixel size  
707 corresponding to the magnification used (0,65 for 10x images, and 0,32 for 20X images). Area  
708 ( $\mu\text{m}^2$ ) = Area (px<sup>2</sup>)x(96)<sup>2</sup>x(pixel size)<sup>2</sup>. For each image/sample/antibody-color, the objects  
709 measured were ranked by size (from larger to smaller), a fixed area cutoff (of 50 $\mu\text{m}^2$ ) was  
710 applied to delete artefacts smaller than the cutoff size. For analyses of PLIN1, TnC, YFP,  
711 stainings, the total area of remaining objects was then calculated, and expressed as raw area or  
712 relative to the lesion area (measured in axiovision software). For analyses of fiber cross section  
713 areas, we defined ranges of 300  $\mu\text{m}^2$ , and counted the percentage of fibers in each size range. For  
714 analyses of PLIN/YFP double staining, the YFP-positive layer was divided in PLIN+ or PLIN1-  
715 negative, using the color selection tool, to selectively delete PLIN+ cells or Plin1-negative cells,  
716 from the YFP+ layer, thus generating two distinct images, in which we applied the method  
717 described above (threshold, particle analyses, cutoff, sum), to quantify for each sample the total  
718 PLIN1+;YFP+ area, and the total PLIN1-negative;YFP+ areas. For analyses of PDGFRA/YFP  
719 double staining, this was done manually, by scanning the image for YFP+ cells, and for each cell,  
720 defining it as PDGFRA-negative, intermediate, or high.

721 Quantification of signal intensities in Figure S1D were performed using Fiji as previously  
722 described (Caruso *et al.*, 2014; Fan *et al.*, 2015). Briefly, the area shown in Figure S1C was  
723 cropped from larger images, to span an area going from the uninjured muscle to the center of the  
724 lesion, where degenerating fibers undergo necrosis. Each color channel was opened in Fiji and  
725 converted to 8bit images. Staining intensity was measured using the function > analyze > Plot  
726 profile, along an area matching the whole length of the cropped image, and a few pixel in height.

727 This measurement was done 4 times for each color (on windows of full width, and a few pixels  
728 heights). The plot values were subjected to background subtraction, threshold subtraction (to  
729 avoid negative values), and expressed as a percentage of the max amplitude (defined as the  
730 difference between max intensity and background intensity; the max intensity value being  
731 defined as the average value in a manually defined window matching the active zone.

### 732 ***Statistical analyses***

733 All measurements above yielded one single numerical value per mouse, allowing to have in  
734 each cohort, and for each genotype, sufficient numbers of mice to perform comparisons and  
735 statistical analyses. Sample size corresponds to the number of independent animal in each  
736 group/genotype/stage. No specific method was used to define sample size. As we did not know  
737 prior to initiating, whether the phenotypes were the same irrespective of sex or if there were  
738 sex-specific phenotypes, we included in each cohort sufficient (and equal) numbers of males and  
739 females, so as to perform statistics on separated sexes and conclude reliably on sex as  
740 parameter. Results are presented either by pooling males and females, or by separating them  
741 (supplementary figures 5 and 8). All statistics tests were done on comparisons between two  
742 genotypes, using unpaired Student's t-Test, when the data showed a normal distribution and  
743 equal variance between the two groups, and Mann-Whitney test otherwise. Differences were  
744 considered significant when  $p < 0,05$ . All p-values are indicated in the figures, unless the  
745 comparison yielded a radically non-significant result (in which case we indicated ns).

### 746 **Data availability**

747 The datasets supporting the conclusions of this article are included within the article and its  
748 additional files, with the exception of figures 1C, and 1H, and Figure 2, for which we used the  
749 following published gene expression datasets. Data on Fat1 expression after CTX and Glycerol  
750 injury (Figure 1C) were extracted from ref (Lukjanenko *et al.*, 2013), and are available in the  
751 Gene Expression Omnibus Repository, under the accession number GSE45577. Data on gene  
752 expression in biopsies from human muscular dystrophy patients (Figure 1G) were extracted

753 from references (Bakay *et al.*, 2006; Dadgar *et al.*, 2014), and are available in the Gene  
754 Expression Omnibus Repository under the accession number GDS1956, or GSE3307. ScRNAseq  
755 data used in Figure 2 were extracted from the ScMuscle dataset, a set of ScRNAseq data  
756 integrated in a common visualization tool published in ref (McKellar *et al.*, 2021), initially  
757 available as a web application ([scmuscle.bme.cornell.edu](http://scmuscle.bme.cornell.edu)) or currently available as fully  
758 processed Seurat and CellChat objects that can be downloaded from the datadryad platform  
759 (doi: [10.5061/dryad.t4b8gtj34](https://doi.org/10.5061/dryad.t4b8gtj34)). The full list of accession numbers, including a novel dataset  
760 GSE162172 (transcriptomic sampling of regenerating aged mouse hindlimb muscle after notexin  
761 injury), as well as several previously published datasets, is available in the supplementary Table  
762 1 of ref (McKellar *et al.*, 2021).

763

764

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## 790 **Contributions**

791 **Pierre-Antoine Ferracci**: investigation, data analysis, manuscript reviewing and editing.

792 **Françoise Helmbacher**: Conception, investigation, data analysis, mouse experimentation,  
793 funding acquisition, supervision, figure assembly, manuscript original draft writing, reviewing  
794 and editing.

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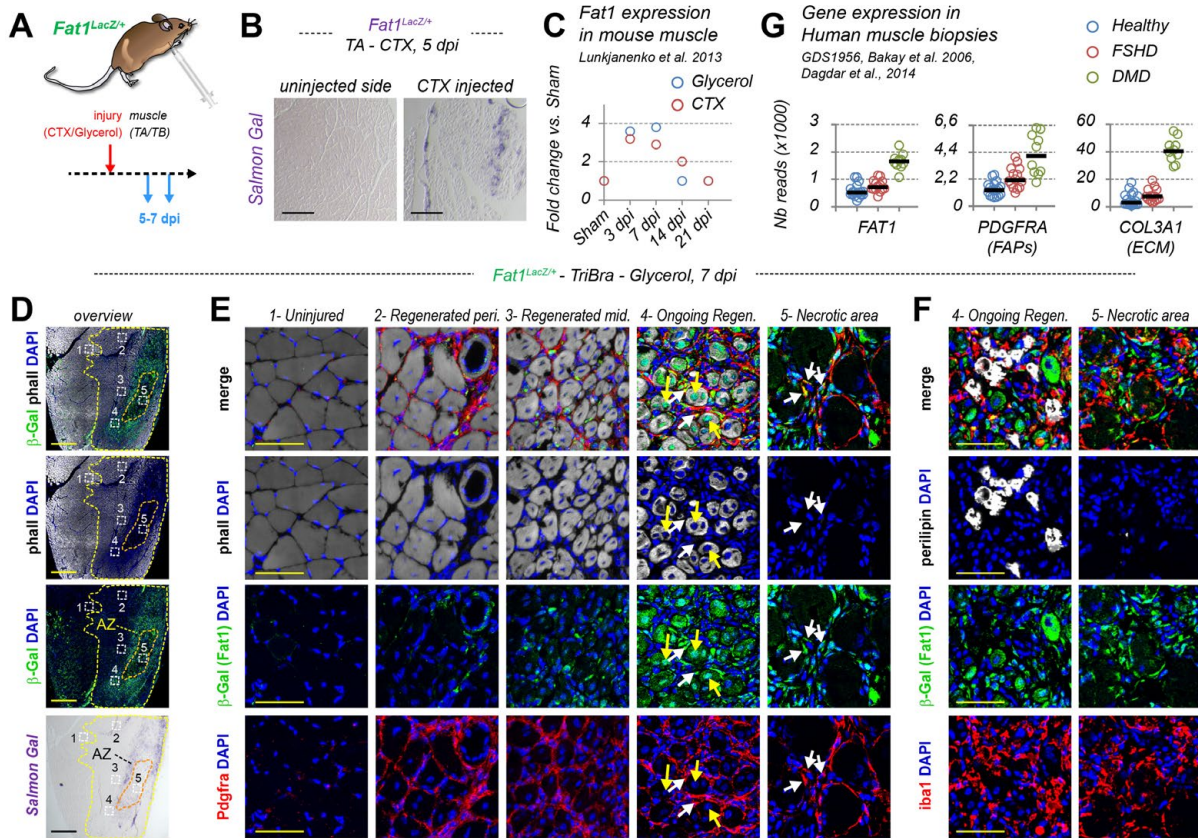
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1099 **Figure Legends**

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1101

1102 **Figure 1: Fat1 expression is transiently induced in FAPs and myogenic cells in regenerating**

1103 **muscle after injury.** (A) Scheme of the experiment, in which adult *Fat1<sup>LacZ/+</sup>* mice were injured either

1104 with glycerol injected in the Triceps Brachii muscle, 14, or with Cardiotoxin (CTX) injected in the

1105 Tibialis anterior muscle, and the injured muscles were collected respectively at 7 or 5 days post-injury

1106 for histological analyses. (B) Salmon Gal staining of muscle cross-sections from the lesion side or the

1107 uninjured side, showing that *Fat1<sup>LacZ/+</sup>* expression is undetectable in the uninjured adult muscle, but

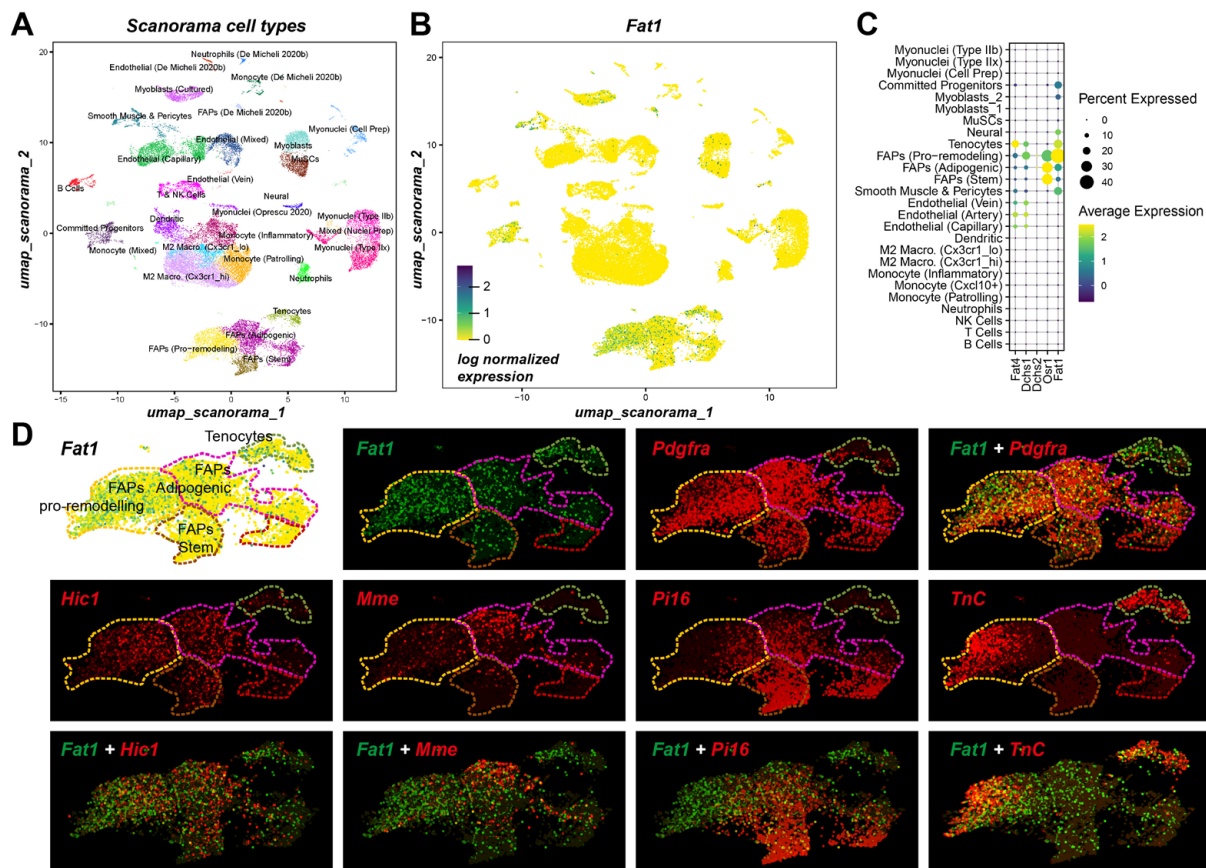
1108 induced by the CTX injury. (C) Plots of the evolution of Fat1 mRNA levels in mouse muscle after

1109 Glycerol or CTX injury at the indicated stages. Transcriptomic data were extracted from Lukjanenko

1110 et al., 2013. (D, E, F) Expression of *Fat1<sup>LacZ</sup>* after glycerol injury at 7 dpi was visualized by salmon

1111 Gal staining (purple, D) or by immunohistochemistry with anti-beta-galactosidase antibodies (green),

1112 combined with phalloiding (white), and antibodies against *Pdgfra* (red, E), or against *iba1* (red, F).  
1113 Images in (D) represent vues of the entire muscle cross-section, while panels in (E, F) are high  
1114 magnification images of areas highlighted by dotted squares in (D). The position of high magnification  
1115 panels is indicated in (D) by numbers (1- uninjured; 2- Regenerated peripheral; 3- regenerated medial;  
1116 4- ongoing regeneration; 5- necrotic area), and are placed relative to the outer limit of the lesion area  
1117 (transition between fibers with peripheral and central nuclei), and to the limits of the necrotic area  
1118 (where degenerating fibers exhibit reduced or absent phalloidin staining). The active zone (AZ) is the  
1119 area at the transition between degenerating and regenerated fibers, where satellite cells and FAPs are  
1120 mobilized to start the myogenic repair processed. While *Pdgfra* labels FAPs throughout the lesion,  
1121 *Fat1<sup>LacZ</sup>* expression levels are highest in the active zone with ongoing regeneration, and in cells  
1122 surrounding degenerating fibers in the necrotic area, including FAPs and a few macrophages. (G)  
1123 Comparison of levels of FAT1, PDGFRA and COL3A1 levels in human muscle biopsies from healthy  
1124 patients or patients with DMD or FSHD, extracted from GDS1956 Gene expression dataset from  
1125 Bakay et al. 2006, Dagdar et al., 2014). Scalebars: (B) 250  $\mu\text{m}$ ; (D) 500  $\mu\text{m}$ ; (E, F) 50  $\mu\text{m}$ .  
1126



1127

1128 **Figure 2: Fat1 expression in published mouse muscle ScRNAseq datasets: Distinguishing**

1129 **FAP subsets. (A, B)** Image showing the umap scanorama plot with cell type annotations (A), or with

1130 Fat1 expression (B) from the ScMuscle web interface from McKellar et al (McKellar *et al.*, 2021),

1131 which integrated ScRNAseq data from several published sources focusing on healthy or injured mouse

1132 muscle. In (B) the cell cluster with highest Fat1 levels include FAPs, also shown magnified in (D). (C)

1133 Dot plot (generated in scMuscle) showing percent of cells from each annotated cell type, and mRNA

1134 levels (colors) for Fat1, Fat4, Dchs1, Dchs2 and Osr1. (D) Umap Scanorama plots zooming on FAPs

1135 for Fat1, and through a color conversion, its overlap with several other fibro-adipogenic progenitors or

1136 Mesenchymal stromal cell markers (converted to red, whereas *Fat1* is converted to green), including

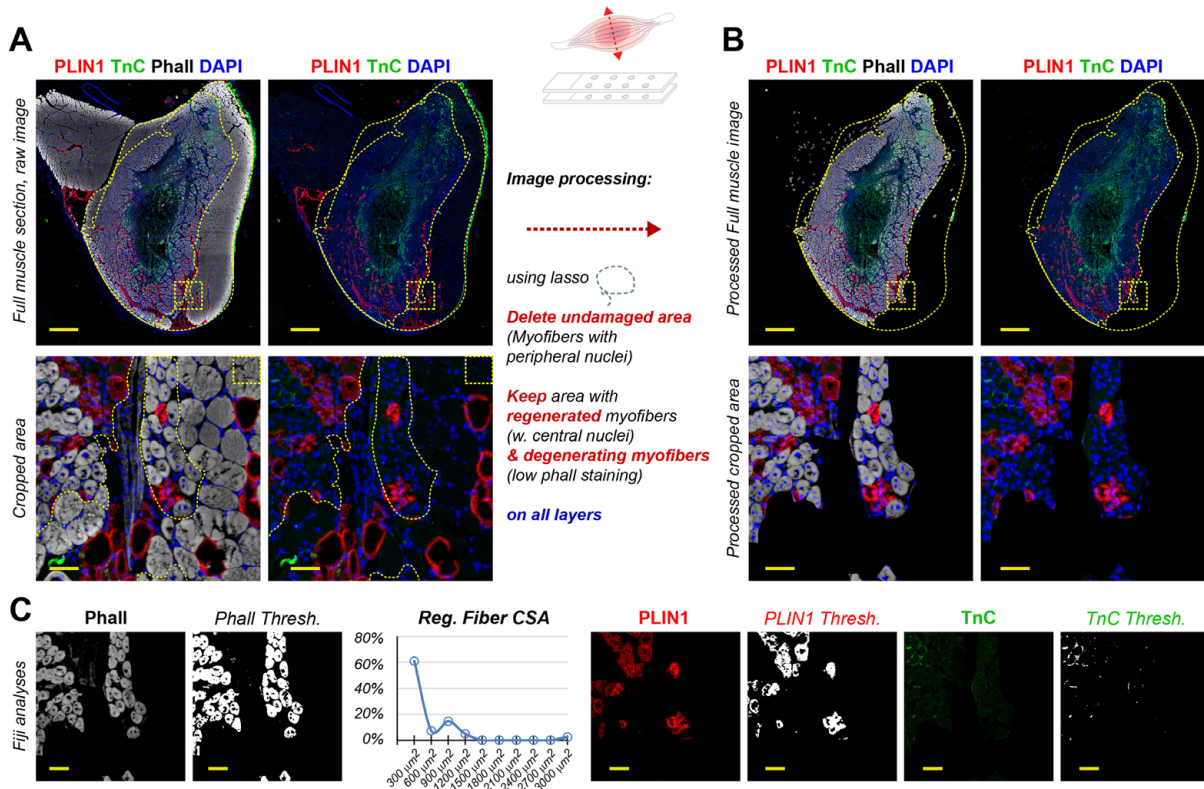
1137 *Pdgfra*, *Hic1*, *Mme*, *Pi16*, and *TnC*. While *Fat1*-expressing cells are detected among all FAP subtypes,

1138 the highest percentage of expressing cells and levels match with the subset expressing *TnC*,

1139 corresponding (according to (McKellar *et al.*, 2021)) the FAP pro-remodelling subtype and to

1140 tenocytes.

1141



1142

1143

**Figure 3: Image analysis pipeline to explore muscle regeneration and IMAT formation in**

1144

**muscle samples after glycerol injury.** Muscles collected from injured mice are embedded and

1145

serially sectioned, and immunostained with antibodies for morphometric analyses (the examples

1146

shown here were stained with phalloidin (white), perilipin1 (PLIN1, red), TnC (green), and with DAPI

1147

(blue). The example shown here shows a muscle cross-section from a control mouse, 7 days after

1148

glycerol injury, to illustrate the image analysis pipeline used for the following quantifications. (A)

1149

Unprocessed images of the whole transverse muscle section (top), or high magnification (bottom) of

1150

an area indicates with the dotted square in top images), with (left) or without (right) the phalloidin

1151

staining, highlighting with dotted lines the full muscle area analyzed, and the lesion limits as deduced

1152

from the transition between unaffected myofibers and regenerated myofibers. (B) Image processing

1153

steps applied to each muscle section in order to restrict morphometric quantifications to the lesion

1154

only. (C) Images of the same muscle section as in (A), after the image processing steps described in

1155

(B), with (left) or without (right) the phalloidin staining, showing the whole muscle section (top) and

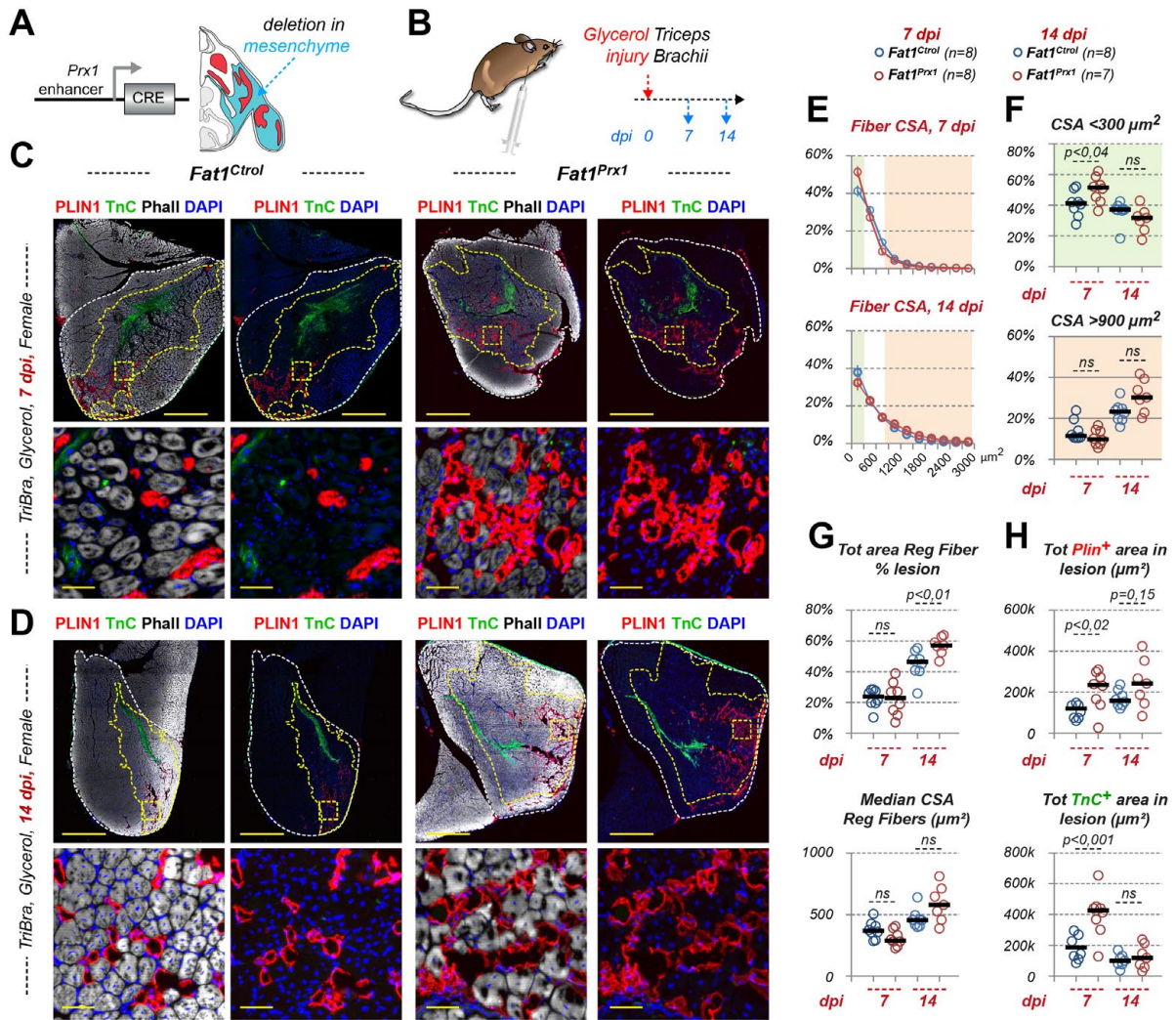
1156

high magnification images (bottom). (D) view of the same area indicated by dotted square, separating

1157

the color channels and showing raw colours or thresholded images (Fiji or ImageJ) from which

1158 quantifications are made, as well as an example of plot of the distribution of fiber cross-section areas  
 1159 corresponding to the phalloidin staining in this small image. Scalebars: (A,B, top images) 500  $\mu\text{m}$ ;  
 1160 B, bottom images) 50  $\mu\text{m}$ ; (C) 50  $\mu\text{m}$ .  
 1161



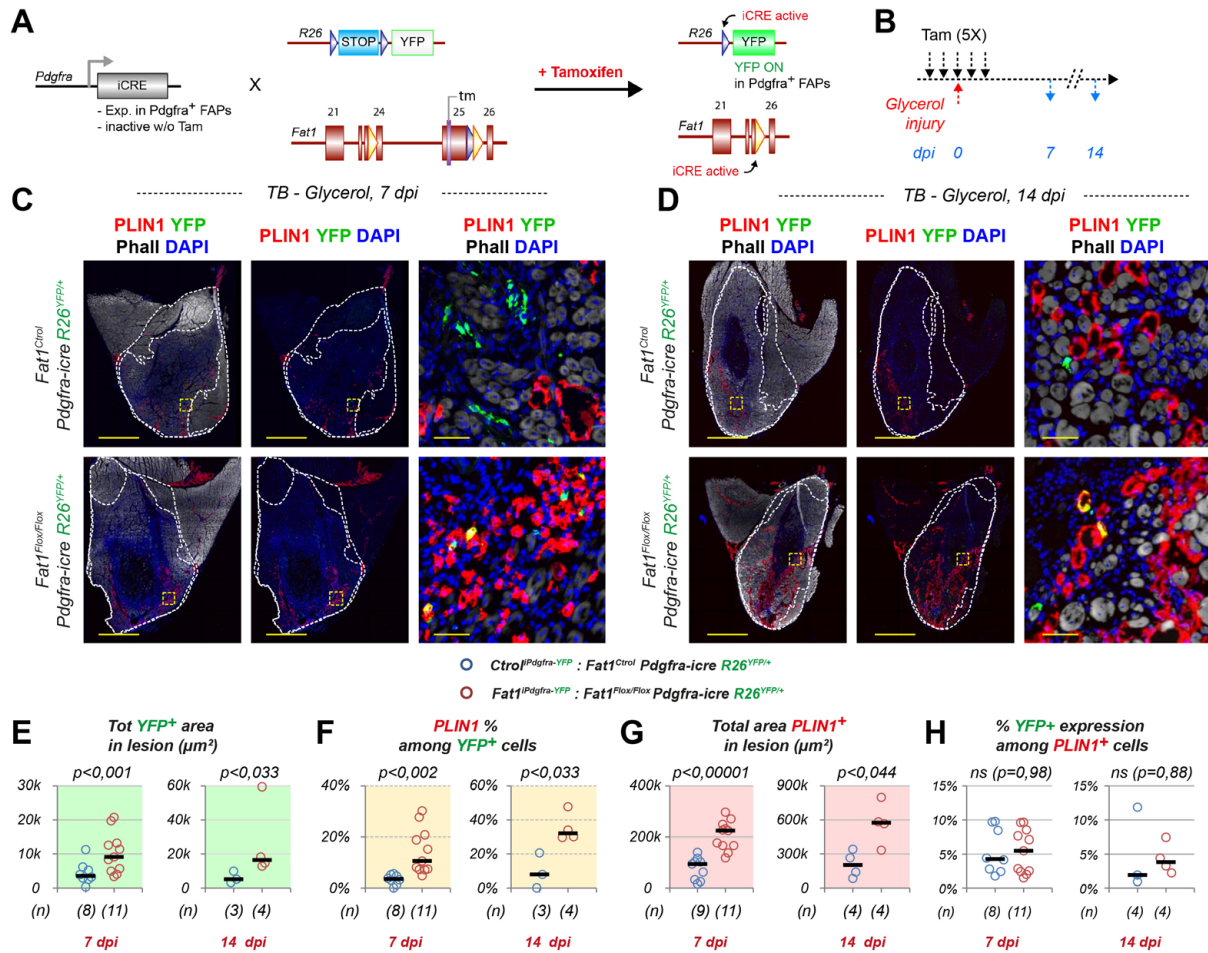
1162  
 1163 **Figure 4: Mesenchyme-specific *Fat1*-deletion enhances glycerol-induced fibro-adipose**  
 1164 **infiltrations while preserving muscle regeneration.** (A) Scheme of the *Prx1*-*cre* driver used for  
 1165 recombination. The domain of activity of *Prx1*-*cre* at embryonic stages includes mesenchyme derived  
 1166 from the lateral plate mesoderm, represented in blue, whereas muscles, derived from paraxial  
 1167 mesoderm (somites) are shown in red (the scheme is based on previously published data in  
 1168 (Helmbacher, 2018)). (B) Experimental procedure and timeline: glycerol injury was performed in 4-8  
 1169 months old mice, and muscles were harvested at 7 or 14 dpi, cryoembedded and serial sectioned. (C,

1170 D) Muscle sections were immunostained with phalloidin (white) and antibodies against Perilipin  
1171 (PLIN1, red) and against Tenascin C (TnC, green) to visualize muscle fibers, adipocytes and transient  
1172 fibrosis, respectively, in *Fat1<sup>Ctrol</sup>* and *Fat1<sup>Prxl</sup>* female mice, 7 days (C), and 14 days (D) after Glycerol-  
1173 induced damage in the triceps brachii. Yellow dotted lines highlight the outer limit of the triceps  
1174 brachii muscle and the limit of the lesion area, while the squared-dotted areas highlight the areas  
1175 shown at higher magnification in panels below the full muscle views. For each genotype, images are  
1176 shown with (left) or without (right) the phalloidin staining. The images shown are representative of the  
1177 female phenotype detailed in Figure 5A-D. (E) Distribution of myofiber cross section areas (CSA),  
1178 showing ranges up to 3000  $\mu\text{m}^2$  (higher CSA ranges, above 3000  $\mu\text{m}^2$ , were skipped because of low  
1179 percentages at these stages). (F) Proportions of myofibers in the 0 to 300  $\mu\text{m}^2$  range (upper plot,  
1180 highlighted in green in C), and in the 900 to 2500  $\mu\text{m}^2$  range (lower plot, highlighted in light orange in  
1181 C), showing individual data points for each mouse. (G) Quantifications of the percentage of the lesion  
1182 area covered by regenerated fibers (top) or median fiber Cross-section-area, showing individual data  
1183 points for both genotypes and stages. (H) Quantifications of the total area in the lesion covered by  
1184 PLIN1 staining (top), or by TnC staining (bottom), showing individual data points for both genotypes  
1185 and stages. Scalebars: (C,D, top images) 1000  $\mu\text{m}$ ; (C,D, bottom images) 50  $\mu\text{m}$ .

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1202

**Figure 6: inducible *Fat1*-deletion in the *Pdgfra* lineage leads to cell-autonomous and non-cell-**

1203

**autonomous enhancement of adipogenic differentiation in glycerol-injured muscles. (A) Genetic**

1204

**paradigm used for inducible-recombination in the *Pdgfra* lineage, with *Pdgfra*-iCRE line, combined**

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**with the *Fat1*-Flox and Reporter allele R26-YFP. Upon addition of Tamoxifen, activated CRE acts on**

1206

**both alleles, and YFP expression is used to follow cells in which recombination occurred. (B) Scheme**

1207

**of the experimental design, with d0 representing the onset of experimentation, on adult mice of the**

1208

**indicated genotypes. Tamoxifen is applied 5 consecutive days, starting 2 days before muscle damage.**

1209

**Analysis is performed 7 or 14 days post-damage. (C, D) Sections of damaged triceps brachii muscles**

1210

**collected from control (*Fat1*<sup>control</sup>; *Pdgfra* -icre; *R26*<sup>YFP/+</sup>; abbreviated as *Ctrl*<sup>*iPdgfra-YFP*</sup>, top images)**

1211

**and FAP-specific *Fat1* mutant mice (*Fat1*<sup>Flox/Flox</sup>; *Pdgfra* -icre; *R26*<sup>YFP/+</sup>, abbreviated as *Fat1*<sup>*iPdgfra-YFP*</sup>,**

1212

**bottom images), at 7dpi (C) or 14 dpi (D). Sections were immunostained with antibodies to GFP/YFP**

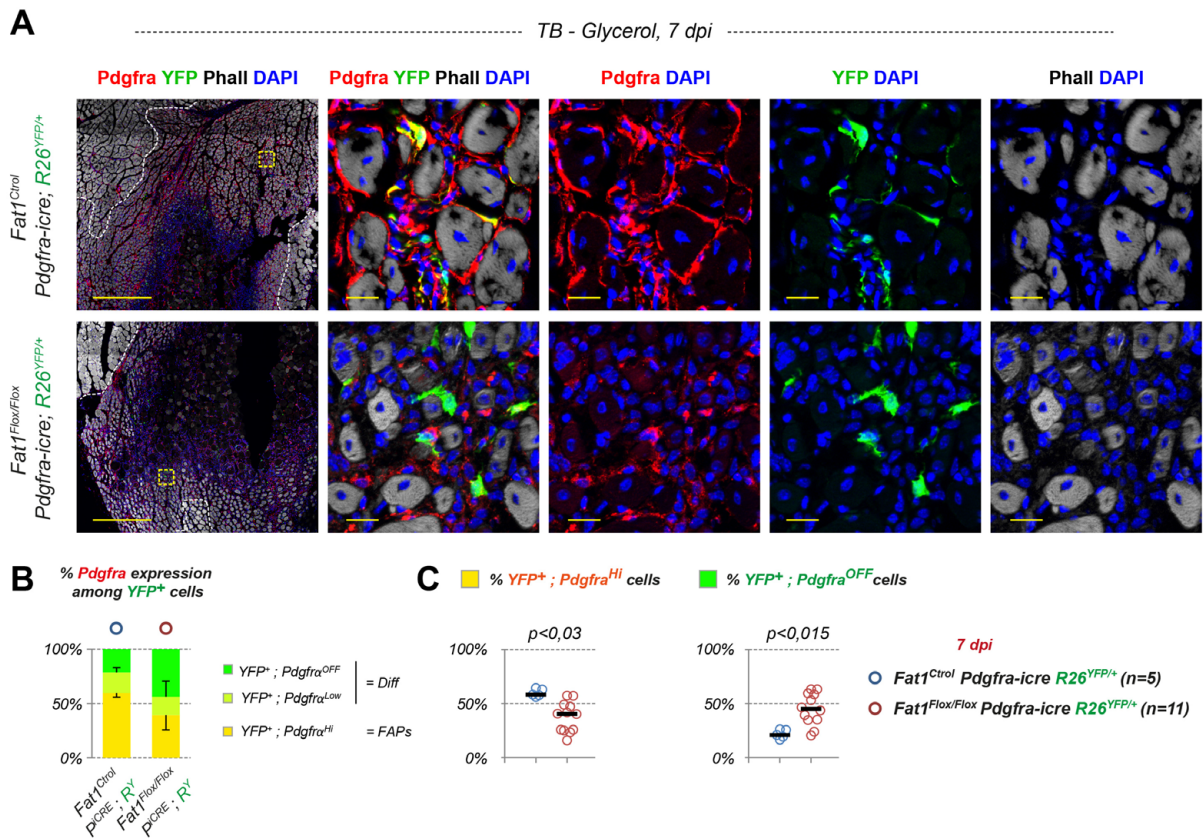
1213

**(green), Perilipin1 (PLIN1, red), with Fluorescent-Phalloidin (white) and DAPI (blue). For each**

1214

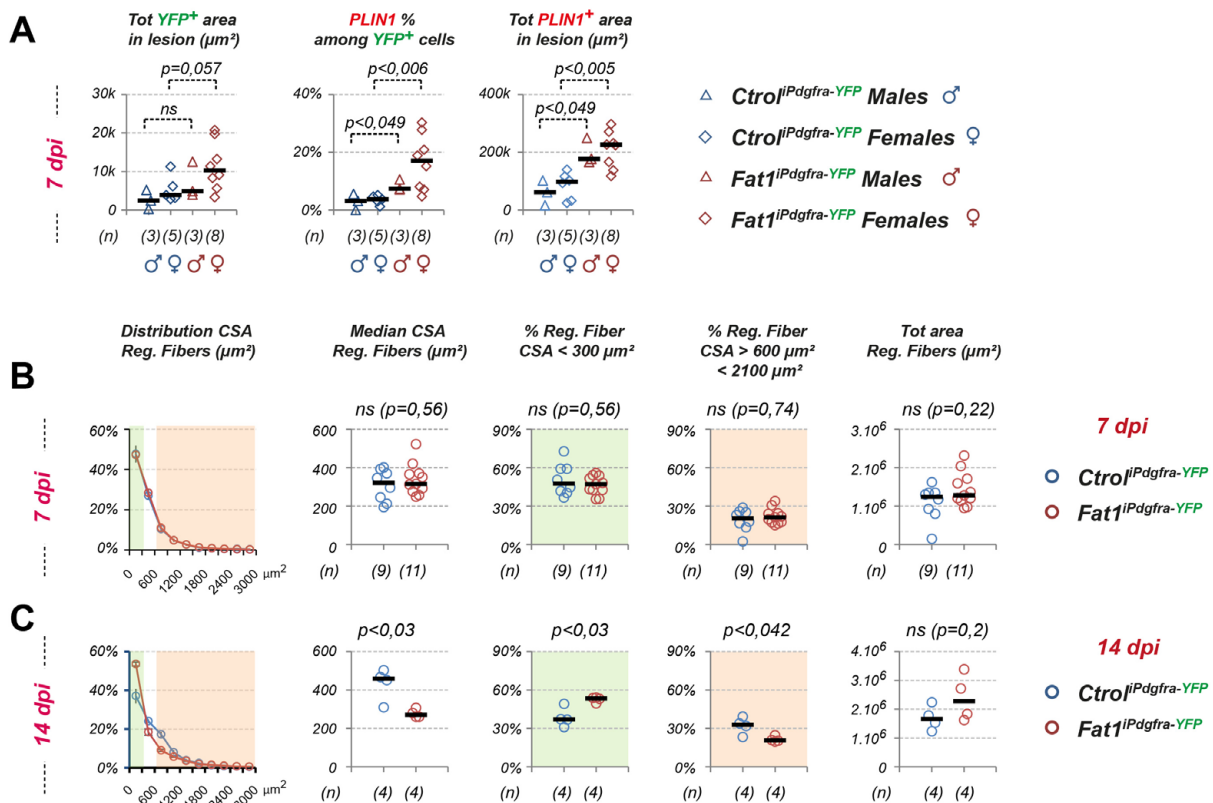
**genotype/stage, the two left panels show an overview of the entire muscle cross-section, while the**

1215 right panel show a higher magnification of the area highlighted with squared dotted lines. For each  
 1216 muscle, we highlighted with dotted lines the outer limit of the studied muscle (triceps brachii, median),  
 1217 and the lesion area (defined by the presence of myofibers with central nuclei (indicating regeneration).  
 1218 (E, F, G, H) Quantifications of the total YFP+ area (E) as a proxi for the number of *Pdgfra-icre*-  
 1219 derived YFP+ cells, of the PLIN1+ percentage among the YFP+ area (F), the total PLIN1+ area (G),  
 1220 and the YFP+ percentage among PLIN1+ area (H), in the lesion, comparing *Ctrl*<sup>*iPdgfra-YFP*</sup>, and  
 1221 *Fat1*<sup>*iPdgfra-YFP*</sup> mice. Each dot represents an individual mouse, and the number of mice is indicated by  
 1222 the numbers between brackets below the plots. Scalebars: (C,D, for each genotype, the first two  
 1223 images (left and center)) 1000  $\mu$ m; (C, D, for each genotype, right side image) 50  $\mu$ m.  
 1224



1225  
 1226 **Figure 7: inducible *Fat1*-deletion in the *Pdgfra* lineage leads to expansion of *Pdgfra*-derived**  
 1227 **lineage and loss of *Pdgfra* expression.** (A) Sections of damaged triceps brachii muscles from control  
 1228 (*Fat1*<sup>control</sup>; *Pdgfra-icre*; *R26*<sup>YFP/+</sup>, top images) and FAP-specific *Fat1* mutant mice (*Fat1*<sup>Flox/Flox</sup>;  
 1229 *Pdgfra-icre*; *R26*<sup>YFP/+</sup>, bottom images), immunostained with Phalloidin (white), DAPI (blue), and

1230 antibodies to YFP (green), and Pdgfra (red). The yellow dotted lines demarcate the lesion area from  
 1231 unaffected muscle tissue. White squares indicate the position of higher magnification images shown in  
 1232 Figure 4. (D) Quantification of percentages among YFP<sup>+</sup> cells, that express high levels (yellow), low  
 1233 levels (light green) or no Pdgfra (dark green, Pdgfra<sup>OFF</sup>) in *Ctrol*<sup>iPdgfra-YFP</sup> and *Fat1*<sup>iPdgfra-YFP</sup> mice. (D)  
 1234 Plots of the Pdgfra<sup>High</sup> percentage (left), and Pdgfra<sup>OFF</sup> percentage (right) among the YFP<sup>+</sup> area,  
 1235 showing individual data points for each mice. Scalebars: (A, overview) 500 μm; (A, crops) 20 μm.  
 1236  
 1237

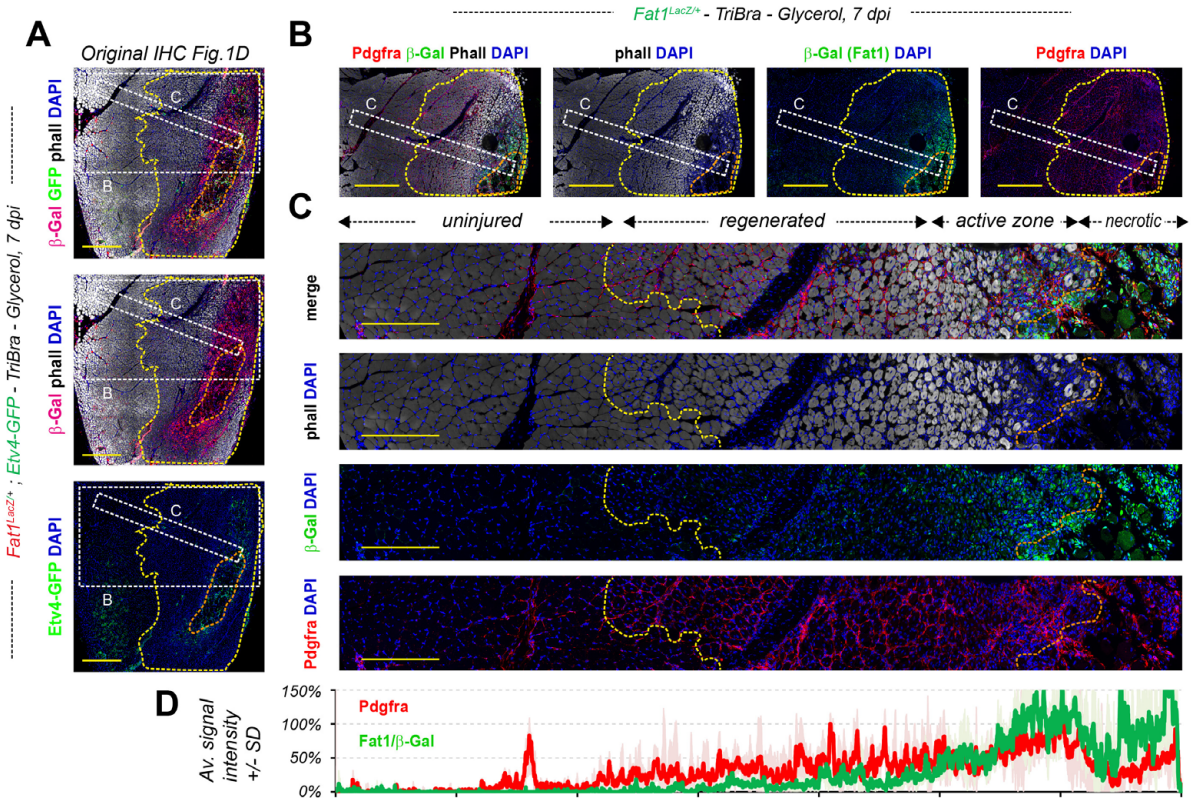


1238  
 1239 **Figure 8: Female-specific enhancement of intramuscular adipogenesis and effects on**  
 1240 **myogenic repair in the inducible *Pdgfra-icre* model.** (A) The same quantifications as in Figure 5E-  
 1241 G, of the total YFP<sup>+</sup> area in the lesion (Left plot), the PLIN1<sup>+</sup> proportion of the YFP<sup>+</sup> area (middle  
 1242 plot), and the total PLIN1<sup>+</sup> area in the lesion (right plot), showing individual data points for each  
 1243 mouse, and separating males and females (genotypes and number of mice area shown below the  
 1244 graph), comparing *Ctrol*<sup>iPdgfra-YFP</sup> and *Fat1*<sup>iPdgfra-YFP</sup> male and female mice, at 7 dpi. (B, C)  
 1245 Quantifications of morphometric parameters related to regeneration in *Ctrol*<sup>iPdgfra-YFP</sup> and *Fat1*<sup>iPdgfra-YFP</sup>

1246 mice, at 7 dpi (B) and 14 dpi (C), including from left to right: 1) the distribution of myofiber cross  
1247 section areas (CSA), showing ranges up to  $3000 \mu\text{m}^2$ , 2) the median CSA, 3) the proportions of  
1248 myofibers in the  $0$  to  $300 \mu\text{m}^2$  range (highlighted in green in the left plots), 4) the proportion of  
1249 myofibers in the  $600$  to  $2100 \mu\text{m}^2$  range (highlighted in light orange in the left plots), 5) the total area  
1250 covered by regenerated fibers in the lesion, showing individual data points for both genotypes and  
1251 stages.  
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1254 **Supplementary data**

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1257 **Figure S1: Fat1 expression is transiently induced in FAPs and myogenic cells in regenerating**

1258 **muscle after injury.** (A) The IHC experiment shown in Figure 1D is presented here in its original

1259 setting. The sample was a Triceps Brachii muscle from a *Fat1<sup>LacZ/+</sup>; Etv4-GFP<sup>+</sup>* mouse, injured with

1260 glycerol, and collected at 7dpi. The section was immunostained with antibodies against beta-

1261 galactosidase (here in Red), and against GFP (Green, highlighting Etv4-GFP expression), combined

1262 with Alexa-conjugated phalloidin (white). The images presented in Figure 1D were color converted

1263 for simplicity of the message. (B, C) From the same animal, additional IHC were performed on

1264 alternate sections of the same muscle, with antibodies against beta-galactosidase (here in Green), and

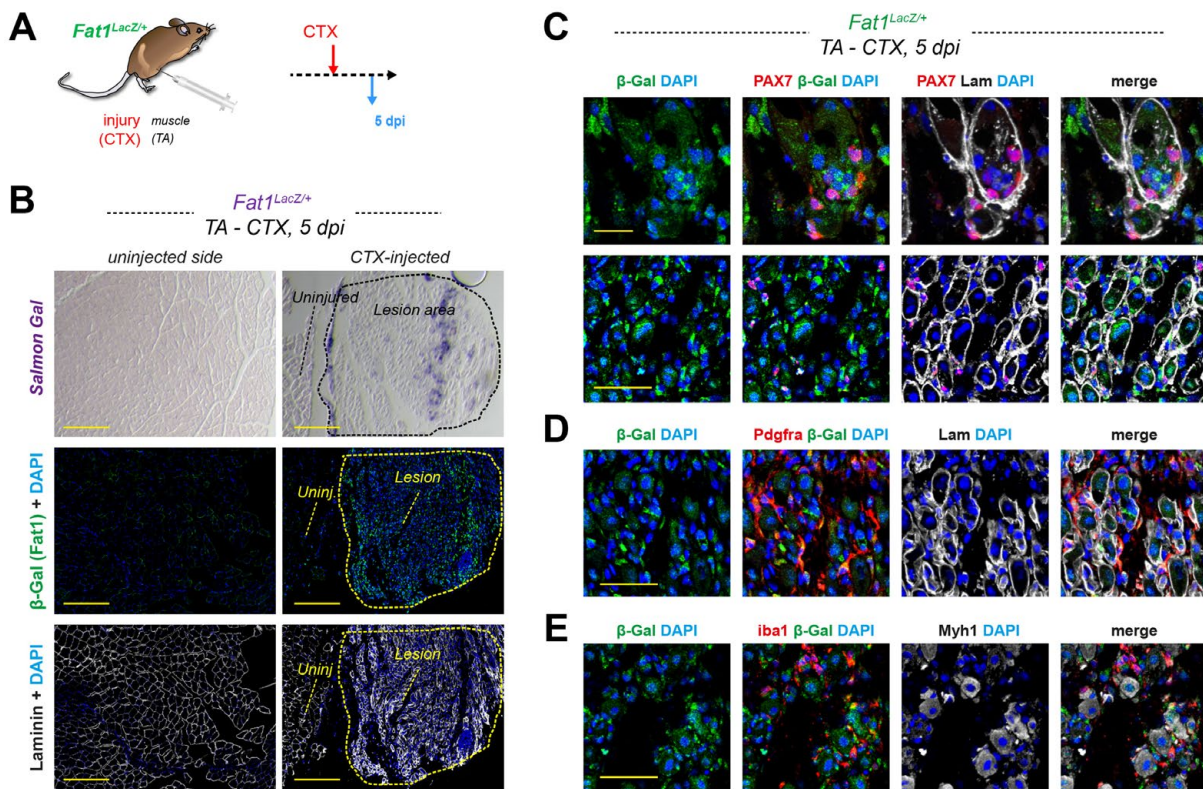
1265 against Pdgfra (Red), combined with Alexa-conjugated phalloidin (white). Acquisitions at the scale

1266 equivalent to those in (A) were not done, but we acquired images with 20X magnification, shown full

1267 size in (B), and at higher magnification in Figure 1E (crops of defined size). An alternative way of

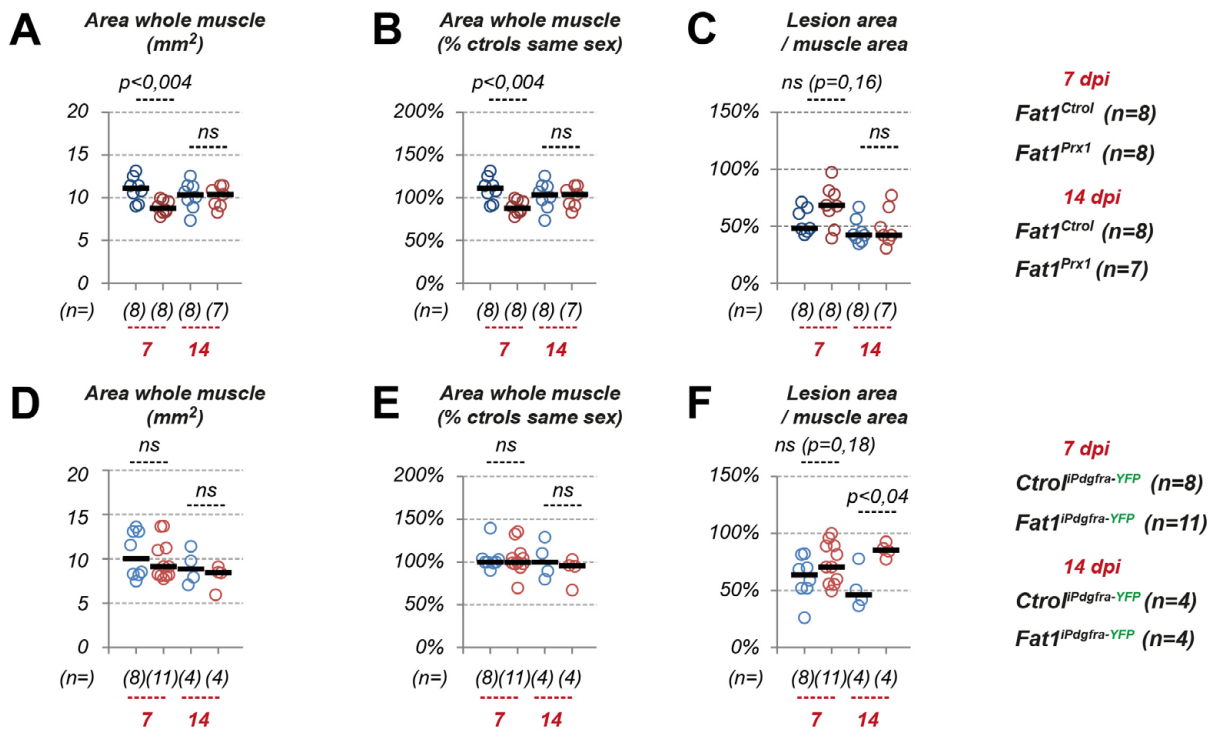
1268 showing how expression levels vary across different areas of the section is shown here in (C),

1269 representing crops of a band the size of which is indicated as white dotted lines in (A). (D)  
 1270 quantification of signal intensities for Fat1/ $\beta$ -Gal (green) and Pdgfra (red) along images in (C): each  
 1271 plot represents the average intensity (+/- standard deviation in light green and light red, respectively)  
 1272 as percentage of Max, for 4 narrow bands along the images. Scalebars: (A) 500  $\mu$ m; (B) 500  $\mu$ m; (C)  
 1273 200  $\mu$ m.  
 1274



1275  
 1276 **Figure S2: *Fat1<sup>LacZ</sup>* induction by Cardiotoxin (CTX), 5 days after injury .** (A) Scheme of the  
 1277 experiment, in which adult *Fat1<sup>LacZ/+</sup>* mice were injured by injection of CTX in the Tibialis anterior  
 1278 muscle (TA), and showing the timeline, with injury occurring at day 0, and muscle collection for  
 1279 histological analyses at 5 days post-injury. (B) *Fat1<sup>LacZ</sup>* expression is visualized on the same muscle  
 1280 cross-sections as in Figure 1B of a *Fat1<sup>LacZ/+</sup>* mouse 5 days after CTX injury in the TA, with uninjured  
 1281 (left) and injected (right) sides, by Salmon gal staining (top), or immunohistochemistry with anti- $\beta$ -  
 1282 galactosidase (green) and laminin (white) antibodies (with DAPI, blue), showing that expression is  
 1283 undetectable in the uninjured adult muscle, but induced by the CTX injury. (C, D, E) high  
 1284 magnification images at the level of the actively regenerating area of the lesion, to show at different

1285 magnifications the combination of anti- $\beta$ -galactosidase (green) and laminin (white) with Pax7 (red, C),  
 1286 *Pdgfra* (red, D, and *iba1* (red, E). Scalebars: (B) 500  $\mu$ m; (C) top images: 20  $\mu$ m, bottom images 50  
 1287  $\mu$ m, (D,E) 50  $\mu$ m.  
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 1289



1290  
 1291 **Figure S3: Presence and absence of pre-injury phenotypes in the Prx1-cre and inducible**  
 1292 ***Pdgfra-icre* models.** Quantifications of (A, D), the whole muscle area (in mm<sup>2</sup>), (B, E) the whole  
 1293 muscle area (normalized to the median area of controls of the same sex), and of (C, F) the ratio  
 1294 between lesion area and whole muscle area, in *Fat1*<sup>Ctrl</sup> and *Fat1*<sup>Prx1</sup> mice (A, B, C), and in *Ctrl*<sup>*iPdgfra-*</sup>  
 1295 <sup>*YFP*</sup>, and *Fat1*<sup>*iPdgfra-YFP*</sup> mice treated with Tamoxifen as described in Figure 5B (D, E, F) showing  
 1296 individual data points for each mouse, at 7 and 14 dpi. The reduction in muscle area observed in  
 1297 *Fat1*<sup>Prx1</sup> mice at 7dpi, associated with an increased L/M ratio (not significant), indicates that a  
 1298 phenotype specific to the uninjured part of the muscle pre-existed the lesion. This phenotype is  
 1299 transient, and no longer detected at 14 dpi, indicating that myogenic repair in the lesion rescues the  
 1300 muscle mass equally well in *Fat1*<sup>Ctrl</sup> and *Fat1*<sup>Prx1</sup> mice. In contrast, in the inducible *Pdgfra-icre*  
 1301 model, the lack of reduction in whole muscle area shows that there are no pre-injury phenotype before

1302 Tamoxifen treatment. This allows uncovering an effect on lesion size at 14dpi, potentially accounted  
1303 for by enhanced adipogenesis in *Fat1*<sup>Pdgfra-YFP</sup> mice.