

# The L27 Domain of MPP7 enhances TAZ-YY1 Cooperation to Renew Muscle Stem Cells

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## Article

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1 **The L27 Domain of MPP7 enhances TAZ-YY1 Cooperation to Renew Muscle Stem Cells**

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17 The authors declare no conflict

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19 **SUMMARY**

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21

22 Stem cells regenerate differentiated cells to maintain and repair tissues and organs. They also

23 replenish themselves, i.e. self-renewal, for the regenerative process to last a lifetime. How stem

24 cells renew is of critical biological and medical significance. Here we use the skeletal muscle stem

25 cell (MuSC) to study this process. Using a combination of genetic, molecular, and biochemical

26 approaches, we show that MPP7, AMOT, and TAZ/YAP form a complex that activates a common

27 set of target genes. Among these targets, *Carm1* can direct MuSC renewal. In the absence of

28 MPP7, TAZ can support regenerative progenitors and activate *Carm1* expression, but not to a

29 level needed for self-renewal. Facilitated by the actin polymerization-responsive AMOT, TAZ

30 recruits the L27 domain of MPP7 to up-regulate *Carm1* to the level necessary to drive MuSC

31 renewal. The promoter of *Carm1*, and those of other common downstream genes, also contain

32 binding site(s) for YY1. We further demonstrate that the L27 domain of MPP7 enhances the

33 interaction between TAZ and YY1 to activate *Carm1*. Our results define a renewal transcriptional

34 program embedded within the progenitor program, by selectively up-regulating key gene(s)

35 within the latter, through the combination of protein interactions and in a manner dependent on

36 the promoter context.

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## 41 INTRODUCTION

42  
43 Stem cells are critical for tissue homeostasis. Depending on the tissue, stem cells either cycle  
44 constantly, periodically, or rarely under normal physiological conditions (reviewed in Fuchs and  
45 Blau, 2020). Their activities are regulated by their microenvironment or niche. Upon injury, stem  
46 cells can enter a faster or longer proliferative state to produce differentiated cells for repair. Like  
47 any given cell, stem cells interpret mechanical and biochemical signals to enter or exit the cell  
48 cycle, but, in addition, they are tasked with giving rise to differentiated cells and to replenishing  
49 themselves, i.e. self-renewal. Here we focus on skeletal muscle stem cells (MuSCs), which are  
50 profoundly important to muscle homeostasis and regeneration, as well as to muscle diseases,  
51 cancers, and aging (reviewed in Relaix et al., 2021; Sousa-Victor et al., 2022).

52         The main source of MuSCs is the muscle resident PAX7-expressing (PAX7<sup>+</sup>) cells, also  
53 known as satellite cells (Mauro, 1961), as elucidated by lineage tracing (Lepper et al., 2009) and  
54 cell ablation studies (Lepper et al., 2011; Murphy et al., 2011; Sambasivan et al., 2011). They are  
55 attached to the muscle fiber via the apical adherens junction (AJ) and situated on the basal  
56 extracellular matrix (ECM) surrounding the muscle fiber. Loss of the AJ proteins M- and N-  
57 cadherins leads to MuSC activation and incorporation into myofiber, as well as increased MuSC  
58 numbers (Goel et al., 2017). Loss of the ECM-receptor  $\beta$ 1-integrin leads to minimal incorporation  
59 of MuSC into the myofiber and loss of MuSCs (Rozo et al., 2016). As cadherins and integrins  
60 organize actin, the role of actin in MuSC activity has been investigated. Live imaging of quiescent  
61 MuSCs revealed elaborate cellular projections that retract during early activation by injury or  
62 myofiber isolation (Kann et al., 2022; Ma et al., 2022). Rac and Rho, two small GTPases regulating  
63 actin polymerization at cell projections and cortex, are implicated in regulating MuSC quiescence

64 and activation, respectively (Kann et al., 2022). Moreover, Rho stimulates the nuclear entry of  
65 the G-actin sensing co-activator, myocardin-related transcription factor (MRTF), in activated  
66 MuSCs. The actin-tethered mechanosensitive  $\text{Ca}^{2+}$  channel Piezo1, which regulates Rho and  
67 MuSC cell projections, also facilitates MuSC activation (Hirano et al., 2023; Ma et al., 2022).

68 Non-canonical Wnt4 signaling has also been implicated in MuSC quiescence by  
69 suppressing the mechano-responsive yes-associated transcriptional co-factor (YAP; Eliazar et al.,  
70 2019). YAP and related TAZ (also known as WWTR1, and collectively, YAP/TAZ) are co-activators  
71 for the TEAD family (TEAD1-4) of DNA-binding transcription factors that drive cell proliferation  
72 (reviewed in Ma et al., 2019; Pan, 2022). YAP overexpression promotes the proliferation of  
73 activated MuSCs and myoblasts (Judson et al., 2012; Tremblay et al., 2014). Conditional  
74 inactivation of *Yap* in mouse MuSCs (*Yap* cKO) compromises muscle regeneration, whereas *Taz*  
75 germline mutant mice do not appear to display regeneration defects (Sun et al., 2017). On the  
76 other hand, inactivating *Yap* and *Taz* in MuSCs after muscle injury promotes MuSC quiescence  
77 (Silver et al., 2021). By contrast, inactivating *Yap* and *Taz* in MuSCs prior to muscle injury has not  
78 been reported. YAP/TAZ are well-recognized mechano-responsive co-activators (reviewed in  
79 Panciera et al., 2017), but the players that promote their mechano-sensitivity are incompletely  
80 understood in the MuSC.

81 An evolutionarily conserved regulatory pathway that restrains YAP/TAZ is the Hippo  
82 kinase cascade (reviewed in Ma et al., 2019; Pan, 2022). When the Hippo pathway is activated  
83 (typically at cell-cell junctions), the most downstream kinases LATS1/2 phosphorylate YAP/TAZ  
84 to promote retention in the cytoplasm and proteasomal degradation. In mammals, Angiomotin  
85 (AMOT) family members (AMOT, AMOTL1, and AMOTL2, collectively AMOTs) interact with

86 multiple Hippo pathway components (reviewed in Moleirinho et al., 2014). At tight junctions  
87 (TJs), they interact with Merlin, an upstream kinase of the Hippo pathway. AMOT can be  
88 phosphorylated at serine 175 (S175) by LATS1/2, and lose its actin-binding property. Reciprocally,  
89 AMOT can stimulate the kinase activity of LATS1/2. Lastly, AMOTs contain LPTY and PPxY motifs  
90 that bind to YAP/TAZ. At all these intersection points, AMOTs act to retain YAP/TAZ at cell  
91 junctions, on actin, or in the cytoplasm, and promote their degradation. In certain cell types  
92 however AMOT increases the level of nuclear YAP (Yi et al., 2013), a role which appears to be  
93 carried out by the non-phosphorylated AMOT (Moleirinho et al., 2017). Whether the non-  
94 phosphorylated AMOT also modulates YAP's transcriptional activity and helps target gene  
95 selection is unknown.

96         Before being identified as associated with the Hippo pathway, AMOT was identified as an  
97 angiostatin-binding protein involved in endothelial cell migration and proliferation (reviewed in  
98 Moleirinho et al., 2014). In the case of migration, AMOT helps localize Rho activity to the leading  
99 edge of endothelial cells. At TJs, AMOT binds and promotes Rich1 (a GTPase activating protein)-  
100 mediated hydrolysis of Rac1 and Cdc42 to compromise TJs. Several key TJ proteins, including  
101 membrane palmitoylated protein 5 (MPP5, also known as Pals1), interact with AMOT. MPP7,  
102 related to MPP5, also exists in an AMOT-containing protein complex (Wells et al., 2006). MPP7  
103 has been implicated in the maintenance of TJs and AJs through binding to MPP5/Crumb (Stucke  
104 et al., 2007) and DLG/LIN7 (Bohl et al., 2007), respectively, but AMOT was not included in those  
105 studies. On the other hand, we have suggested an unconventional mode of action for MPP7 in  
106 MuSC renewal: transcriptional regulation through interaction with AMOT and YAP (Li and Fan,

107 2017). The mechanisms underlying their tri-partite interaction and target gene activation in  
108 MuSCs are not clear.

109         Although YAP is a well-recognized transcription co-activator, it can also function as a  
110 transcriptional repressor of cell cycle inhibitor genes in human Schwann cells (Hoxha et al., 2020).  
111 In this context, YAP was localized to genomic regions occupied by the transcription repressor  
112 Ying-Yang 1 (YY1) and the enhancer of zeste homolog 2 (EZH2) in the polycomb repressive  
113 complex 2 (PRC2). In C2C12 myoblasts, YY1 was shown to inhibit muscle differentiation by  
114 repressing myogenic loci (Lu et al., 2013; Wang et al., 2007). During embryogenesis, the  
115 inactivation of *Ezh2* in the myogenic lineage led to the de-repression of non-muscle genes (Caretti  
116 et al., 2004). A genetic study of *Yy1* function in MuSC revealed that it represses mitochondrial  
117 genes during MuSC activation (Chen et al., 2019). Whether and how YAP1 and YY1 coordinate to  
118 repress select genes in the MuSC is yet to be explored.

119         Here we provide evidence that a complex containing MPP7, AMOT, TAZ(YAP), and YY1,  
120 activates high levels of *Carm1* expression, which is necessary for MuSC renewal (Kawabe et al.,  
121 2012). We determined the roles of *Mpp7*, *Amot*, and *Yap/Taz* in MuSCs for muscle regeneration  
122 genetically, and defined their downstream target genes. Promoters of these downstream genes,  
123 including that of *Carm1*, contain TEAD binding sites and surprisingly, YY1 binding sites. We  
124 dissected the biochemical interactions of the aforementioned proteins and deciphered the  
125 mechanism underlying their convergence to *Carm1* activation. Central to the enhanced  
126 transcriptional activity of this complex are the AJ-targeting L27 domain of MPP7 and the F-actin-  
127 regulated nuclear shuttling of AMOT. Together, we have uncovered an unexpected layer of

128 YAP/TAZ-regulated MuSC renewal, through incorporating MPP7 and AMOT and cooperating with  
129 YY1, to activate *Carm1* to a sufficiently high levels that are necessary for self-renewal.

130

## 131 RESULTS

### 132 ***Mpp7* plays a role in SC self-renewal**

133 To determine the role of *Mpp7* genetically, we generated a mouse model with a conditional  
134 *Mpp7<sup>fllox</sup>* knockout allele (Figure 1A). The loxP sites flank exon 3, which encodes a part of MPP7's  
135 PDZ domain. Cre-mediated recombination predicts a frameshift with an early stop codon. We  
136 combined this allele with a *Pax7*-CreER<sup>T2</sup> allele (*Pax7<sup>CE</sup>*; Lepper et al., 2009) for tamoxifen (TMX)  
137 inducible conditional knockout (cKO), referred to as *Mpp7* cKO (Figure 1B); *Rosa26<sup>YFP</sup>* (Srinivas et  
138 al., 2001) was included for marking the recombined cells. The cKO efficiency was ~ 92% based on  
139 immunofluorescence (IF) for MPP7 in control and *Mpp7* cKO MuSCs (Figure S1A, B). Thirty days  
140 (d) after TMX (without injury), PAX7<sup>+</sup> MuSC numbers in the tibialis anterior (TA) muscles were  
141 similar between control and *Mpp7* cKO animals (Figure S1C). To assess regeneration, we used the  
142 procedure outlined in Figure 1B; 5 additional TMX injections after injury were included to further  
143 increase cKO efficiency. At 5 days post-injury (dpi), the *Mpp7* cKO had smaller regenerated  
144 myofibers and lower PAX7<sup>+</sup> MuSC density (Figure 1C-F) compared to the control. At 21 dpi (Figure  
145 1G, H; Figure S1D, E), regenerated myofibers remained smaller and PAX7<sup>+</sup> SC was density lower  
146 in the *Mpp7* cKO than those in the control. Using EdU incorporation *in vivo*, we found fewer  
147 proliferated YFP<sup>+</sup> cells in the *Mpp7* cKO than those in the control (Figure 1I; Figure S1F). YFP<sup>+</sup>  
148 MuSCs (isolated by fluorescence-activated cell sorter, FACS, and cultured *in vitro*) also showed a  
149 smaller EdU<sup>+</sup> fraction of the *Mpp7* cKO compared to that of the control, but no difference in



150 programmed cell death (PCD) was found (Figure S1G-I). Lastly, we used a single myofiber culture  
151 assay to assess self-renewal, which is based on the relative fractions of three MuSC-derived cell  
152 fates: self-renewal (PAX7<sup>+</sup>), progenitor (PAX7<sup>+</sup>MYOD<sup>+</sup>), and differentiation-committed (MYOD<sup>+</sup>)  
153 (Figure 1J; Figure S1J). *Mpp7* cKO had fewer progenitor and renewed cells and more  
154 differentiation-committed cells, compared to the control. Together, *Mpp7* functions to support  
155 MuSC proliferation and self-renewal after injury/activation.

156

### 157 **MPP7's PDZ and L27 domains are critical for its function in SCs**

158 MPP7 is known to be required for maintaining AJs and TJs in epithelial cells (Bohl et al., 2007;  
159 Stucke et al., 2007). We therefore examined whether *Mpp7* inactivation affected apical proteins.  
160 Immediately after single myofiber isolation, *Mpp7* cKO MuSCs showed normal levels of apically  
161 localized M-cadherin, N-cadherin,  $\beta$ -catenin, and PAR3 (Figure S2A). This is consistent with  
162 normal MuSC numbers at 30d after *Mpp7* inactivation.

163 We next investigated the domains of MPP7 required in the MuSC. MPP7 is composed of  
164 an L27, a PDZ, an SH3, and a GUK (last two combined as SH3GUK) domain (Figure 2A; reviewed  
165 Chytla et al., 2020). The L27 domain interacts with DLG and LIN7 for AJ targeting (Bohl et al.,  
166 2007), the SH3 domain interacts with MPP5 and Crumb for TJ targeting (Stucke et al., 2007), but  
167 the PDZ domain has no assigned partner nor known function to date. We transfected expression  
168 constructs for full-length (WT), L27-deleted ( $\Delta$ L27), PDZ-deleted ( $\Delta$ PDZ), and SH3GUK-deleted  
169 ( $\Delta\Delta$ SH3GUK) *Mpp7* (Figure 2A) into the *Mpp7* cKO MuSCs and assessed their ability to rescue  
170 defects in single myofiber culture (Figure 2B). All deletion forms of *Mpp7* exhibit similar cellular  
171 distribution as WT *Mpp7*: i.e. nuclear localization in the majority of MuSCs (Figure S2B). WT and

172  $\Delta\Delta$ SH3GUK Mpp7 rescued both progenitor and renewed cells (compared to the control),  $\Delta$ L27  
173 MPP7 rescued the progenitor but not renewed cells and  $\Delta$ PDZ MPP7 rescued neither (Figure 2C).  
174 Thus, MPP7's PDZ domain is critical for all protein functions assessed, while the L27 domain is  
175 uniquely required for renewal. The SH3 and GUK domains appear to be dispensable.

176

### 177 **The PDZ-binding motif (PDM) of AMOT binds to the PDZ of MPP7 and is critical for function**

178 We previously showed an interaction between MPP7 and AMOT by co-immunoprecipitation (co-  
179 IP) in 293T cells (Li and Fan, 2017). How they interact and whether *Amot* plays a role *in vivo* were  
180 unknown. Using the co-IP assay, we found that MPP7 and AMOT bind to each other via their  
181 respective PDZ and PDM domains (Figure 2D; Figure S2E; domain organization of AMOT reviewed  
182 in Moleirinho et al., 2014). Importantly, *Amot* cKO mice (same strategy as for *Mpp7* cKO mice  
183 described above) showed muscle regeneration defects similar to those of *Mpp7* cKO mice:  
184 Smaller myofibers, fewer PAX7<sup>+</sup> SCs, and reduced EdU incorporation of lineage-marked YFP<sup>+</sup> cells  
185 (Figure 2E-H). In the single myofiber culture, *Amot* cKO MuSCs also had reduced progenitor and  
186 renewed cell fractions, which could be rescued by expressing WT *Amot* but not by  $\Delta$ PDM *Amot*  
187 (Figure 2I). Because of their interaction, we examined whether their protein levels depended on  
188 each other. MPP7 level was not affected in cultured *Amot* cKO MuSCs (Figure 2J). By contrast,  
189 the AMOT level was reduced in *Mpp7* cKO MuSCs in culture (Figure 2K) as well as on single  
190 myofiber (Figure S2C). Thus, not only the interaction domains of MPP7 and AMOT share a  
191 common role in progenitor and renewal fates, their interaction also appears critical to maintain  
192 AMOT level ( summarized in Figure S2D).

193

194 ***Mpp7* cKO and *Amot* cKO MuSCs share differentially expressed genes**

195 We next performed RNA-seq to identify differentially expressed genes (DEGs) in *Mpp7* and *Amot*  
196 cKO MuSCs, compared to the control. The experimental design is outlined in Figure 3A and the  
197 principle component analysis (PCA) of RNA-seq data is in Figure 3B. *Mpp7* cKO had 58 DEGs and  
198 *Amot* cKO had 66 DEGs compared to WT cells (Figure 3C; Table S1). Thirty-five of their DEGs  
199 intersect, with 15 of these being downregulated (Figure 3D; Table S1). *Amot* is not a DEG in the  
200 *Mpp7* cKO, indicating that its reduced protein levels in this background are post-transcriptionally  
201 regulated. As such, some DEGs of *Mpp7* cKO might be a consequence of reduced AMOT levels.  
202 GO-term analysis revealed enrichment for estrogen receptor (ESR) signaling, mitochondria  
203 biogenesis, and small GTPases (Figure 3E). Genes in our GO-term list have not been studied in  
204 the MuSC, except for *Carm1* (or *Prmt4*). CARM1 is an arginine methyl transferase that methylates  
205 PAX7, which then recruits epigenetic regulators to activate de novo committed satellite myogenic  
206 cells (Kawabe et al., 2012). *Carm1* cKO also has reduced regenerative myofiber size and PAX7<sup>+</sup>  
207 MuSC number. We confirmed that CARM1 level was reduced in *Mpp7* cKO and *Amot* cKO MuSCs  
208 (Figure 3F, G), per its downregulated mRNA; the same results were seen in the single myofiber  
209 culture (Figure S3A).

210 To determine if *Carm1* plays a role in the *Mpp7*-operated pathway, we force-expressed  
211 *Carm1* in *Mpp7* cKO MuSCs in single myofiber culture and found that it was sufficient to rescue  
212 progenitor and renewed fates (Figure 3H). We next tested whether *Mpp7* could regulate *Carm1*  
213 transcription. For this, we made a luciferase reporter fused to a putative promoter region (-630  
214 to +15 bp) of *Carm1* (i.e., a *Carm1*-reporter) and found that overexpression of *Mpp7* could  
215 activate the *Carm1*-reporter in 293T cells (Figure 3I). By contrast, *Mpp5* could not activate the

216 *Carm1*-reporter, indicating a selectivity for *Mpp7* (Figure S3B). In addition,  $\Delta\Delta$ SH3GUK *Mpp7*  
217 activated the reporter similarly to the WT *Mpp7*,  $\Delta$ L27 *Mpp7* weakly activated, and  $\Delta$ PDZ *Mpp7*  
218 did not activate the reporter (Figure S3C). Thus, among the DEGs of *Mpp7* cKO, *Carm1* is the chief  
219 effector gene for progenitor and renewal fates.

220

### 221 **The regulatory network of *Yap* and *Taz* overlaps with those of *Mpp7* and *Amot***

222 *Mpp7* knock-down reduced nuclear YAP in myoblasts, suggesting MPP7 increases YAP activity (Li  
223 and Fan, 2017). AMOT is known to bind to YAP/TAZ directly, and can either increase or decrease  
224 nuclear YAP/TAZ in different contexts (reviewed in Moleirinho et al., 2014). Following these  
225 threads, we examined an existing set of DEGs obtained by overexpressing YAP or TAZ in  
226 myoblasts (Sun et al., 2017)(Figure S3D). We found very few of those overlap with DEGs of *Mpp7*  
227 cKO and/or *Amot* cKO, likely due to different experimental approaches. We next asked whether  
228 DEGs of *Mpp7* or *Amot* cKOs harbor TEAD-binding sites in their promoters. Indeed, most of their  
229 promoters have TEAD-binding sites (Fishilevich et al., 2017; Keenan et al., 2019), including that  
230 of *Carm1* (Figure S3E). This encouraged us to employ *Taz<sup>fllox</sup>* and *Yap<sup>fllox</sup>* (Reginensi et al., 2013) as  
231 double cKO in the MuSC (*YapTaz* cKO) and compare with *Mpp7* cKO and *Amot* cKO.

232 We confirmed that *Yap* cKO had compromised muscle regeneration (Sun et al., 2017), and  
233 found that *YapTaz* cKO had a more severe defect (Figure 4A). Previously, *Taz* mutant was  
234 reported to have no muscle regeneration defects (Sun et al., 2017), and our *YapTaz* cKO data  
235 indicate that *Taz* partially compensates for *Yap* in muscle regeneration. To not miss DEGs due to  
236 compensation, we proceeded with RNA-seq using *YapTaz* cKO MuSCs. The *YapTaz* cKO showed  
237 564 DEGs, compared to the control (Figure 4B). As expected, many cell growth- and proliferation-

238 associated pathways were impacted (Figure S4A). Thirty-three *Mpp7* cKO DEGs and 31 *Amot* cKO  
239 DEGs overlapped with *YapTaz* cKO DEGs, and 19 were common in all 3 sets of DEGs (Figure 4B;  
240 Figure S4B). During our search for transcription factor binding sites, we noticed a congruence of  
241 TEAD- and YY1-binding sites in these DEGs' promoters (Figure S4C). These analyses suggest that  
242 MPP7 and AMOT help YAP and/or TAZ to selectively regulate a small set of downstream genes,  
243 and YY1 is likely involved in their regulation (see below).

244         Neither *Yap* nor *Taz* were a DEG in *Mpp7* or *Amot* cKOs, but their protein levels were  
245 reduced in both cKOs; with TAZ levels being the most reduced in *Mpp7* cKO MuSCs (Figure 4C,  
246 D). The addition of proteasome inhibitor MG132 to *Mpp7* cKO MuSCs restored the levels of TAZ,  
247 YAP, and AMOT near to those in the control, but the level of CARM1 was only partially restored  
248 (Figure S4D). This is puzzling if *Carm1* is a target of YAP/TAZ (via TEAD). We reasoned that MPP7  
249 must have a role other than stabilizing TAZ, YAP, and AMOT. Using TAZ as a representative for  
250 TAZ and YAP (because TAZ level is most affected in *Mpp7* cKO MuSCs), we showed that MPP7  
251 could interact with TAZ, and their interaction was enhanced by AMOT (Figure 4E; Figure S4F).  
252 Using a TEAD-reporter (Dupont et al., 2011), we found that MPP7 co-expression substantially  
253 increased reporter activation by TAZ, and adding AMOT further boosted the reporter activity  
254 (Figure 4F). AMOT alone however reduced TAZ's activity, consistent with it being a negative  
255 regulator in most studies. We conclude that MPP7 reverses AMOT's negative role for TAZ, and  
256 MPP7 and AMOT together facilitate TAZ function as a co-activator.

257         We next asked if the TEAD binding site in the *Carm1* reporter mediated the  
258 responsiveness to *Mpp7*. The *Carm1*-reporter with the TEAD binding site mutated was no longer  
259 responsive to *Mpp7* (Figure 4G). Given that *Carm1* is sufficient to rescue the defects of *Mpp7* cKO

260 MuSCs, we asked whether *Taz* could also do so. Both TAZ and TAZ S89A (a stabilized form of TAZ;  
261 Kanai et al., 2000) could only increase CARM1 levels by ~2-fold in *Mpp7* cKO MuSCs, and TAZ  
262 S89A performed slightly better than TAZ (Figure 4I; Figure S4E). However, TAZ S89A could not  
263 rescue the renewal fate of *Mpp7* cKO MuSCs, even though it rescued the progenitor fate (Figure  
264 4J). Thus, while TAZ can up-regulate *Carm1* to rescue the progenitor fate, MPP7 is additionally  
265 needed to boost *Carm1* expression to a higher level required for the renewal fate.

266

### 267 **L27 domain of MPP7 enhances TAZ-mediated transcription and SC renewal**

268 To dissect the mechanism underlying MPP7-enhanced TAZ activity, we examined the domains of  
269 MPP7 required for their synergy. By co-IP, we found that  $\Delta$ PDZ MPP7 failed to interact with TAZ,  
270  $\Delta$ L27 MPP7 had a diminished interaction with TAZ, and  $\Delta$  $\Delta$ SH3GUK MPP7 had the same level of  
271 interaction with TAZ as WT MPP7 (Figure 5A). When tested on the TEAD-reporter, both  $\Delta$ PDZ  
272 *Mpp7* and  $\Delta$ L27 *Mpp7* had little activity, even though a low level of interaction was observed for  
273  $\Delta$ L27 MPP7 and TAZ (Figure 5B). The interaction between MPP7 and TAZ is likely indirect via  
274 endogenous AMOT in 293T cells: 1)  $\Delta$ PDZ MPP7 cannot bind AMOT; 2)  $\Delta$ PDM AMOT, not able to  
275 bind MPP7, interfered with MPP7-TAZ interaction, likely by competing TAZ away from  
276 endogenous AMOT (Figure S5A); 3) TAZ with a mutated WW domain (TAZ WWm) unable to  
277 interact with AMOT, failed to interact with MPP7 (Figure S5B).

278 The above results suggest that the L27 domain of MPP7 (MPP7-L27) enhances the  
279 interaction between MPP7 and TAZ only when they are brought into proximity via two distinct  
280 parts of AMOT (Figure 5C), and the interaction between MPP7-L27 and TAZ is key to enhancing  
281 TAZ's transcriptional activity. If so, a fusion of MPP7-L27 to TAZ (L27-TAZ; Figure 5D) should

282 bypass their interaction via the obligatory AMOT and have enhanced transcriptional activity.  
283 Indeed, L27-TAZ activated the TEAD-reporter to the same level as TAZ and MPP7 together (Figure  
284 5E). L27-TAZ also activated the Carm1-reporter effectively (Figure S5C). We further dissected  
285 MPP7's L27 domain, which consists of two L27 repeats, L27N and L27C. L27N-TAZ and L27C-TAZ  
286 fusions showed slightly reduced transcriptional activity than the full L27-TAZ. The L27N binds DLG  
287 and L27C, to LIN7 (Bohl et al., 2007). Mutations of Lysine 38 (L38) in L27N and L95 in L27C should  
288 interrupt interactions with DLG and LIN7, respectively. Yet neither mutation in L27-TAZ affected  
289 transcriptional activity (Figure S5C), indicating that DLG and LIN7 are not involved in this context.

290       Importantly, L27-TAZ increased CARM1 levels in MuSCs more than TAZ did (Figure S5D),  
291 and rescued the progenitor and renewal fates of *Mpp7* cKO SCs (Figure 5F). Uncovering the role  
292 of MPP7-L27 for MuSC renewal by enhancing TAZ's transcriptional activity brings us two  
293 outstanding questions: 1) Is AMOT's role only to bring TAZ (YAP) to the proximity of MPP7-L27?  
294 2) How does MPP7-L27 enhance TAZ function?

295

### 296 **AMOT acts as a F-actin-regulated shuttling factor in MuSCs**

297 AMOT is proposed to function as a scaffold protein to promote the localization of YAP to the  
298 cytoplasm, AJs, TJs, and F-actin, thereby precluding nuclear YAP. However, in some cell types  
299 AMOT promotes nuclear YAP (reviewed in Moleirinho et al., 2014). When MuSCs were isolated  
300 on single myofibers in the presence of a ROCK inhibitor Y-27632 (Kann et al., 2022), AMOT, MPP7,  
301 and YAP (TAZ not detectable) were localized to the apical side and cell projections (Figure 6A);  
302 ROCK acts downstream of Rho to promote actin polymerization. We next subjected MuSCs to  
303 pharmacological reagents that affect actin polymerization (Figure 6B) and examined the

304 localization of AMOT and MPP7. Immediately after FACS isolation, MuSCs treated with  
305 Jasplakinolide (Jasp) and Narciclasine (Nar) to stabilize F-actins had increased nuclear AMOT and  
306 MPP7, compared to mock-treated cells (Figure 6C). Conversely, MPP7 and AMOT were mostly  
307 nuclear in activated MuSCs at 48 h of culture but shuttled out to the cytoplasm when treated  
308 with Blebbistatin (Bleb), Cytochalasin B (Cyto B), or Y-27632 to weaken or disrupt F-actin (Figure  
309 6D; Figure S6A).

310 Curiously, MPP7 and AMOT did not show strict co-localization in the MuSC, but their  
311 cytoplasmic versus nuclear localization are similarly regulated by actin states and MPP7 helps  
312 stabilize AMOT. The AMOT-MPP7 complex is likely dynamic, while their cell compartment  
313 localization depends on AMOT and F-actin interaction. AMOT and F-actin interaction (Ernkvist et  
314 al., 2006) is modulated by phosphorylation of serine 175 (S175): S175A AMOT can bind actin,  
315 whereas phosphor-mimetic S175E AMOT cannot (Chan et al., 2013; Dai et al., 2013). We  
316 expressed both forms of AMOT in *Amot* cKO MuSCs. Similar to those reported in cell lines  
317 (Moleirinho et al., 2017), higher percentages of WT and S175A AMOT were present in the nucleus  
318 compared to S175E AMOT in transfected MuSCs (Figure 6E). Unlike WT *Amot*, S175A *Amot* could  
319 only rescue the progenitor but not the renewal fate; S175E *Amot* bore no rescue activity (Figure  
320 6F). Furthermore, AMOT binding to TAZ/YAP is critical for MuSC renewal, as AMOT with all 3  
321 TAZ/YAP binding motifs mutated (denoted as 3PY) was largely cytoplasmic (Figure 6E) and did  
322 not rescue *Amot* cKO MuSCs (Figure 6F). These results together suggest that the dynamic  
323 association of AMOT with F-actin (and with MPP7), instead of a strictly phosphorylated or non-  
324 phosphorylated form of AMOT per se, is critical for MuSC renewal, and its binding to YAP/TAZ is  
325 indispensable.



326

327 **YY1 adds another dimension to MPP7-L27 and TAZ for transcriptional activity**

328 We next addressed how MPP7-L27 and TAZ acquire higher transcriptional activity than TAZ alone.

329 As mentioned before, YY1 binding sites are prevalent in the promoter regions of genes commonly

330 regulated by MPP7, AMOT, and YAP/TAZ (Figure S4B). The *Yy1* cKO has been shown to cause

331 dysregulated mitochondrial and glycolic genes in MuSCs (Chen et al., 2019). We found significant,

332 though not extensive, overlaps between our DEGs and *Yy1* cKO DEGs (Figure S7A). Four examples

333 of the promoters of these overlapping DEGs are depicted in Figure 7A.

334 To test the relevance of YY1, we examined its ability to activate the *Carm1*-reporter. Both

335 TAZ and YY1 can activate the reporter, and L27-TAZ displayed higher activity than TAZ with or

336 without YY1, but no synergy was observed (Figure 7B). When we mutated either the TEAD or the

337 YY1 binding site, no combinations of YY1, TAZ, and L27-TAZ showed any appreciable activity

338 (Figure 7C and D); WT *Mpp7* also could not activate the mutated *Carm1*-reporters (Figure S7B).

339 One explanation is that exogenous YY1 or TAZ activates the promoter by cooperating with

340 endogenous TAZ (or YAP) and YY1, respectively. That is, their ubiquitous expression masks their

341 cooperativity, and their co-existing binding sites enhance the efficiency of co-occupancy to

342 activate transcription.

343 To have a higher efficiency of promoter co-occupancy, they likely interact with each other.

344 Indeed, TAZ and YY1 could interact with each other by co-IP, and L27-TAZ performed better than

345 TAZ (Figure 7E). Furthermore, YY1 interacted with MPP7 indirectly through endogenous AMOT,

346 as their interaction also required the AMOT-binding PDZ domain of MPP7 (Figure 7F). YY1-MPP7

347 interaction (through endogenous AMOT) was also diminished when the L27 domain was deleted,

348 consistent with its diminished interaction with TAZ. Although L27-TAZ and YY1 together activated  
349 the Carm1-reporter the most, we could not exclude a possible contribution of AMOT in  
350 transcriptional activity as TAZ could recruit AMOT. To test this, we made an L27-TAZ WWm (which  
351 precludes AMOT binding) and found it to activate the reporter equally well as L27-TAZ, either  
352 with or without YY1 (Figure S7C). We summarized these findings in Figure 7G and propose that  
353 the MPP7-AMOT-TAZ(YAP)-YY1 protein complex is a stronger activation complex targeting a  
354 select set of genes intersecting the YY1 and YAP/TAZ pathways. Although AMOT does not  
355 contribute to the transcriptional activation, it is key to bridging the complex together, and its  
356 sensitivity to the actin polymerization state endows this complex to respond to mechanical  
357 changes of MuSCs from quiescence to activation.

358

359

360 **Discussion**

361  
362 Here we show that an array of protein interactions converges to upregulate *Carm1* to a level  
363 necessary for MuSC self-renewal. MPP7, AMOT, and YAP are localized to quiescent MuSCs' lateral  
364 projections, poised for nuclear entry. As the actin cytoskeleton undergoes re-arrangement during  
365 activation, AMOT and MPP7 enter the nucleus and help stabilize nuclear YAP/TAZ. The L27  
366 domain of MPP7 plays a central role in enhancing the interaction between TAZ and YY1 on the  
367 *Carm1* promoter for high levels of expression. We propose that MuSC renewal through  
368 upregulating *Carm1* is tied to a mechano-responsive mechanism in the damaged and  
369 regenerative muscle environment where MuSCs experience a dynamic flux of mechanical forces.

370

371 **Tiered regulation of *Carm1* levels for MuSC renewal**

372 Here we uncovered a mechanism underlying transcriptional regulation of *Carm1* in MuSC  
373 renewal. CARM1 was first described as having the ability to methylate PAX7 thus enabling  
374 activation of de novo MuSCs during asymmetric division (Kawabe et al., 2012), and its activity  
375 was regulated by p38 MAP kinase (Chang et al., 2018). Our RNA-seq data reveal that *Carm1* is a  
376 shared DEG among the cKOs of *Mpp7*, *Amot*, *Yap/Taz*, and *Yy1*. We focused on *Carm1* in this  
377 study, but other common DEGs may also contribute to MuSC activity. All the above cKOs,  
378 including *Carm1* cKO (Kawabe et al., 2012) display muscle regeneration defects. In particular,  
379 *Mpp7* and *Amot* support MuSC proliferation and renewal in vivo and in vitro, as does *Carm1*  
380 (Kawabe et al., 2012). *Carm1* promoter harbors indispensable TEAD and YY1 binding sites for  
381 transcriptional activation, and MPP7 contributes to *Carm1* regulation by providing its L27  
382 domain, via AMOT as an intermediate bridge protein, to facilitate TAZ-YY1 interaction.

383 Curiously, most *YapTaz* cKO's DEGs are unaffected in either *Mpp7* cKO or *Amot* cKO. We  
384 reason that the remaining levels of YAP/ TAZ in *Mpp7* cKO or *Amot* cKO are sufficient to  
385 activate those non-overlapping DEGs to allow a reduced level of muscle regeneration. Such  
386 levels of YAP/ TAZ are however insufficient to activate high levels of *Carm1*, leading to reduced  
387 progenitors and renewed MuSCs. Without YAP and TAZ, i.e. *YapTaz* cKO, cell growth and  
388 proliferation are severely compromised, leading to a severe regenerative deficit. Our results  
389 extend previous studies of YAP and/or TAZ (Silver et al., 2021; Sun et al., 2017; Tremblay et al.,  
390 2014) concerning the proliferative state of MuSCs, by including MPP7, AMOT, and YY1, and  
391 linking them to regulate *Carm1* transcription. While TAZ(YAP) can activate *Carm1*, it needs to  
392 cooperate with MPP7 (via AMOT) and YY1 to activate *Carm1* to a higher tier of expression level  
393 to drive de novo generation of satellite myogenic cells.

394

#### 395 **AMOT is a conduit for YAP and TAZ's mechano-responsive transcriptional regulation**

396 AMOT and MPP7 bind each other, their cKO MuSCs share many DEGs, and their nuclear versus  
397 cytoplasmic localization is co-regulated by actin polymerization states. Given AMOT's  
398 association with F-actin and Rich1, suggests it plays the actin-sensor role within the AMOT-  
399 MPP7 partnership. Reciprocally, MPP7 plays a chaperone-like role in the stability of AMOT.  
400 Their IF signals are not strictly co-localized in the MuSC, and yet their interaction was visualized  
401 by PLA (Li and Fan, 2017). Furthermore, WT *Amot* can rescue *Amot* cKO MuSC renewal, but  
402  $\Delta$ PDM-*Amot*, F-actin-binding-able S175A *Amot*, F-actin-binding-disabled S175E *Amot*, and  
403 TAZ/YAP-binding-defective 3PY *Amot* cannot. Together, our results suggest that dynamic

404 association with MPP7 and F-actin, as well as binding to YAP/TAZ are all critical for AMOT  
405 function.

406           Recent work revealed that actin regulation by Rac at the MuSC's lateral projections and  
407 by Rho at the cortex controls MuSC's quiescence and activation, respectively (Kann et al., 2022).  
408 There, the nuclear entry of MRTF, a G-actin sensing co-activator, was found to respond to Rho  
409 activation. Nuclear YAP is also regulated by Rho activation (Dupont et al., 2011). Our results  
410 showing that AMOT can respond to actin polymerization states and bring MPP7 into the  
411 nucleus to do YAP/TAZ's bidding, support that AMOT is a direct mechano-sensor for YAP/TAZ's  
412 mechano-responsive transcription. The distinct and overlapping transcriptomes governed by  
413 YAP/TAZ and MRTF have been explored in cancer cells (Foster et al., 2017). How they operate  
414 inter-dependently in the MuSC is of great future interest.

415

#### 416 **The L27 domain of MPP7 plays a key role in MuSC renewal**

417 We reported that MPP7 was detected in activated MuSC nuclei (Li and Fan, 2017), contrasting  
418 prior reports of its localization at AJ and TJ (Bohl et al., 2007; Stucke et al., 2007). Our data here  
419 indicate that multiple regions of MPP7 can mediate its nuclear entry, possibly via other partner  
420 proteins. While the essential interaction between MPP7-PDZ and AMOT-PDM motif is  
421 predictable, the role of its L27 domain for MuSC renewal and enhancing TAZ's transcriptional  
422 activity is unexpected. By co-IP and *Carm1*-reporter assays, we determine that MPP7's L27  
423 domain is brought to the proximity of TAZ and YY1 through AMOT, and it strengthens the  
424 interactions between TAZ and YY1 to enhance *Carm1* transcription via TEAD and YY1 binding  
425 sites. The finding that TAZ, L27-TAZ, and YY1 can only activate the *Carm1*-reporter with both

426 TEAD and YY1 binding sites intact and that no synergy was observed by their exogenous co-  
427 expression (though a higher level of reporter activity was found than each single expression)  
428 suggest that the primary mode of L27 is to enhance TAZ-YY1 interaction for higher occupancy  
429 efficiency on *Carm1* promoter through DNA binding, instead of enhancing cooperativity of the  
430 transcription activating domains of TAZ and YY1.

431

### 432 **YY1 coordinates with YAP/TAZ to activate high levels of *Carm1* activation**

433 YY1 contains distinct activation and repression domains and can function either way in a  
434 context-dependent manner (reviewed in Verheul et al., 2020). YAP/TAZ have also been shown  
435 to act as co-repressors via their DNA-binding partner TEAD (Kim et al., 2015). YY1 and YAP  
436 together repress cell cycle inhibitor genes in cancer cells by recruiting the repressive chromatin  
437 remodeler, EZH2 (Hoxha et al., 2020). In the context of MuSCs, YY1 has been studied as a  
438 repressor to regulate mitochondria function and glycolysis during MuSC activation (Chen et al.,  
439 2019). We were led to connect YAP/TAZ and YY1 by the co-existence of TEAD and YY1 binding  
440 sites in their common DEGs. Although we focused on the cooperative activation by YAP/TAZ  
441 and YY1 herein, they may cooperatively repress certain common DEGs. For example, 2  
442 mitochondrial genes (*Slc25a29* and *Cmc1*; Table S1) are upregulated in the *Yy1* cKO and the  
443 *YazTaz* cKO and may be co-repressed by YY1 and YAP/TAZ. We chose the downregulated  
444 *Carm1* for an in-depth study because of YAZ/TAZ's activator function in MuSCs (Judson et al.,  
445 2012; Sun et al., 2017; Tremblay et al., 2014) and *Carm1*'s role in MuSC renewal (Chang et al.,  
446 2018; Kawabe et al., 2012). Given the co-IP and *Carm1*-reporter results, the rescue of *Mpp7*

447 cKO MuSCs by L27-TAZ but not TAZ suggests that the L27 domain of MPP7 is needed for TAZ  
448 (YAP) to cooperate with endogenous YY1 for MuSC renewal.

449           The logic of a multi-tiered control of *Carm1* expression, in addition to its post-  
450 transcriptional regulation (Chang et al., 2018), may lie in a checkpoint by the convergence of  
451 YY1's and YAP/TAZ's transcriptional programs. YY1 largely controls mitochondrial and glycolytic  
452 genes (Chen et al., 2019), whereas YAP/TAZ largely controls cell growth and proliferation genes  
453 (Sun et al., 2017 and herein). These cellular functions have to be coordinated for robust stem  
454 cell activation and progenitor expansion. Once these cellular conditions are coordinated, the  
455 convergence to renewal gene(s), e.g. *Carm1*, is set in motion. The inclusion of AMOT and MPP7  
456 renders a mechano-checkpoint for MuSCs to sense their local regenerative physical  
457 environment and adjust the renewal rate accordingly. Lastly, both YY1 and YAP/TAZ pathways  
458 have also been extensively studied in cancer cells. Whether *Mpp7* and *Amot* contribute to  
459 cancers originating from MuSCs, e.g., rhabdomyosarcoma, deserves attention.

460

461

462

463

464

## 465 MATERIALS AND METHODS

466

### 467 Mice

468 Animal treatment and care followed NIH guidelines and the requirements of Carnegie  
469 Institution, and approved by Carnegie Institutional Animal Care and Use Committee. *Mpp7<sup>flox</sup>*  
470 mice was generated via contractual service with ALSTEM Inc., and available upon request.  
471 *Pax7<sup>CreERT2</sup>* mice (Lepper et al., 2009) were donated by Dr. C Lepper. *Amot<sup>flox</sup>* mice (Shimono and  
472 Behringer, 2003) was obtained from Dr. J Kissil. *Yap<sup>flox</sup>*, *Taz<sup>flox</sup>* (Reginensi et al., 2013), and  
473 *Rosa26<sup>YFP</sup>* (Srinivas et al., 2001) mice were obtained from The Jackson Laboratory. Mice were  
474 genotyped by PCR using tail DNA by allele-specific oligonucleotides (information available upon  
475 request). Appropriate mating schemes were performed to obtain control and experimental  
476 mice stated in text, figures and legends. Both male and female mice were used and included in  
477 data analysis unless specified otherwise.

478

### 479 Animal procedures

480 Mice (3-6 month of age) were administered intraperitoneally for 5 consecutive days with 250  
481  $\mu$ L tamoxifen (10 mg/mL corn oil; Millipore Sigma), followed by 3 days of chase. For muscle  
482 injury, mice were anesthetized and 50  $\mu$ L of 10  $\mu$ M cardiotoxin (CTX; Millipore Sigma) in PBS  
483 was injected into tibialis anterior (TA) muscles using the BD insulin syringe (Becton Dickenson).  
484 For EdU (5-ethynyl-2'-deoxyuridine; Millipore Sigma) incorporation in vivo, 10  $\mu$ L of EdU (0.5  
485 mg/ml in PBS) per gram of weight was used per intraperitoneal injection. Time lines of  
486 experimental procedure and muscle sample harvest are detailed in figures and legends.

487

### 488 Histology and Immunofluorescence (IF)

489 TA muscles were fixed in 4% PFA (Electron Microscopy Sciences) immediately after harvesting.  
490 They were processed through 10% sucrose/PBS, 20% sucrose/PBS, and FSC 22 frozen section  
491 media (Leica) before mounted onto a cork and flash-frozen in liquid nitrogen cooled isopentane  
492 (VWR). Frozen samples were stored in -80°C until sectioning by a cryostat (Leica CM3050 S).  
493 Sections of 10  $\mu$ m thickness were collected on Superfrost plus slides (VWR), dried, and stored at  
494 -20°C for future use. For histology, Hematoxylin Gill's II and Eosin (H&E) were used following  
495 instructions of the manufacturer (Surgipath), and mounted in Permount (VWR). For IF,  
496 sections were permeabilized with 0.6% TritonX-100/PBS for 20 min, blocked in Mouse on  
497 Mouse (M.O.M; Vector) blocking reagent, and then in blocking buffer (10% normal goat serum  
498 (Gibco) or normal donkey serum (Sigma-Aldrich), 10% carbo-free blocking solution (Vector) in  
499 PBS). Harvested single myofibers and cultured cells (see below) were fixed and permeabilized  
500 the same way and blocked in blocking buffer without M.O.M. reagent. Tissues and cells were  
501 incubated with primary antibodies overnight at 4°C and secondary antibodies for 1 h at room  
502 temperature. DAPI was used to detect nuclei. EdU incorporation was detected by Click-iT Alexa  
503 Fluor 647 Imaging kit (Thermo Fisher Scientific). Brightfield microscope (Nikon Eclipse E 800),  
504 fluorescence microscope (Nikon E800), and confocal microscope (Leica TCS SP5) were used for  
505 imaging.

506



507 **MuSC isolation by fluorescence activated cell sorter (FACS)**

508 YFP-labeled MuSCs were isolated by FACS from control and cKO mice specified in text, figures,  
509 and legends. Briefly, hindlimb muscles were minced and digested with 0.2% collagenase  
510 (Worthington Biochemical) for 90 min followed by 0.2% dispase (Thermo Fisher Scientific  
511 ) for 30 min in 37°C shaking water bath. Triturated muscle suspension was filtered through a 40  
512 µm cell strainer (Corning) and subjected to isolation by BD FACSAriaIII. For culture,  
513 mononuclear cells were seeded on Matrigel (Corning) coated plates in growth media (DMEM  
514 with 20% FBS, 5% horse serum, 1% pen-strep, 1% glutamax (above from Gibco), 0.1% chick  
515 embryo extract (MPbio) and 2 ng/mL FGF2; R&D systems) for specified time in text and legends,  
516 before fixation and analysis.

517

518 **Single myofiber isolation**

519 Single myofibers were isolated from extensor digitorum longus (EDL) muscles as described (Li  
520 and Fan, 2017). Isolated MuSCs were fixed in 4% PFA immediately or cultured in DMEM with  
521 10% horse serum and 0.5% chick embryo extract for specified time in text, figures, and legends.  
522 To preserve MuSC projections, modifications were made to the procedure in (Kann et al., 2022).  
523 Knee tendon was cut prior to ankle tendon to remove the EDL muscle. Tugging and pulling were  
524 avoided to prevent muscle stretching and loss of projections. EDL muscles were digested in 2.6  
525 mg/mL collagenase in DMEM with Y-27632 (50 µM; Tocris Bioscience) for 55 min in 37°C  
526 shaking water bath, and transferred to DMEM with Y-27632 (50 µM) for trituration to liberate  
527 individual myofibers. These single myofibers were immediately fixed in 4% PFA. After fixation,  
528 myofibers and their associated MuSCs were subjected IF and imaging analysis.

529

530 **RNA-seq and analyses**

531 For RNA-seq, 3-month old female mice were used. YFP-labeled MuSCs were purified by FACS  
532 and processed for RNA extraction using Direct-zol RNA Miniprep Kit (Zymo Research). Total RNA  
533 was processed by ribosomal RNA depletion using the Ribo-Zero rRNA Removal Kit (Illumina) and  
534 sequencing library generated using the TruSeq RNA Library Prep Kit (Illumina) with omission of  
535 PolyA selection. Raw data from FastQ were processed using standard method (Pertea et al.,  
536 2016) and the reads were mapped to the mouse mm9 genome. Differentially expressed gene  
537 (DEG) analysis was performed with DESeq2 (Love et al., 2014) with default parameters.  
538 Transcription factors binding sites in gene promoters were identified by using ChEA3 (Keenan et  
539 al., 2019) and GeneHancer prediction (Fishilevich et al., 2017). To cross-compare our DEGs were  
540 with *Yap/Taz* over-expression and *Yy1 cKO*, we extracted DEGs from (Sun et al., 2017) and  
541 (Chen et al., 2019), respectively for analyses. Significance of the rate of enrichment was  
542 assessed using hypergeometric test, and *P*-values stipulated in figures.

543

544 **Plasmid transfection of single myofibers and 293T cells**

545 For single myofiber transfection, myofibers isolated from Con, *Mpp7* cKO or *Amot* cKO were  
546 cultured for 12 hr and transfected with indicated expression plasmids (Table S2) using TransfeX  
547 reagent (ATCC). Myofibers were then cultured and harvested at indicated time; 1 µM 4-OH-  
548 TMX (Tocris Bioscience) was added to sustain knockout efficiency. For 293T cells, indicated  
549 plasmids were transfected using Lipofectamine 3000 (Thermo Fisher Scientific). After 24 hr,  
550 cells were lysed by RIPA buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1% NP-40, 0.5%

551 deoxycholic acid, and 0.1% SDS) supplemented with complete protease inhibitor cocktail  
552 (Roche) and 1 mM PMSF (Millipore Sigma) processed for Western blot detection.

553

#### 554 **Co-immunoprecipitation (co-IP) and Western blot**

555 Cell lysates from transfected 293T were incubated with anti-FLAG M2 magnetic beads  
556 (Millipore Sigma) or anti-V5 agarose beads (Millipore Sigma) at 4°C for 4 h or overnight. Beads  
557 were then washed 3 times with NP-40 cell lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl,  
558 1% NP-40 supplemented with 0.5 mM dithiothreitol) and 1 time with PBS. 5% input and  
559 immunoprecipitated fractions were boiled in Laemmli SDS sample buffer (Thermo). Protein  
560 samples were processed for SDS-PAGE (4-15% gel; Bio-Rad), transferred to PDMF membrane  
561 (Bio-Rad) for detection using rabbit anti-HA, rabbit anti-V5, or rabbit anti-FLAG antibodies (Cell  
562 Signaling), followed by HRP-conjugated goat anti-rabbit antibodies (Bio-Rad). ECL substrate  
563 (Thermo Fisher Scientific) was used for detection. Exposure and images were performed using  
564 LI-COR Fc imager (LI-COR biosciences).

565

#### 566 **Luciferase assay**

567 Carm1 promoter region (-630 to +15) was cloned to pGL4 luciferase reporter vector (Promega)  
568 to be the Carm1-reporter. Carm1 promoter region was analyzed by PROMO (Farre et al., 2003;  
569 Messeguer et al., 2002) and JASPAR (Castro-Mondragon et al., 2022) to identify TEAD and YY1  
570 binding sites. Carm1-reporter with TEAD and YY1 binding site mutated were generated by PCR  
571 and the nucleotide sequences are indicated in figures. The Carm1-reporter or its mutated  
572 reporters was co-transfected into 293T with the pRL-TK plasmid (Promega) expressing renilla  
573 for normalization. Combinations of cDNA expression plasmids (Table S2) were indicated in  
574 figures and legends. Twenty-four h after transfection, 293T cells were harvested and luciferase  
575 and renilla activities were detected using the Dual-luciferase reporter assay kit (Promega) in a  
576 Glowmax 20/20 luminometer (Promega). The 8XGTIIc-luciferase vector (Dupont et al., 2011)  
577 was used as the TEAD-reporter using the same procedure. For reporter assays, 3 independent  
578 biological replicates were performed for each combination of reporters and cDNA expression  
579 plasmids.

580

#### 581 **Pharmacological treatments**

582 For EdU incorporation in cultured MuSCs, EdU was mixed in media to a final concentration of  
583 10  $\mu$ M for 24 hr, followed by the Click-reaction (Thermo Fisher Scientific) for detection. FACS-  
584 isolated MuSCs were immediately treated with DMSO (mock-treatment), Jasplakinolide (100  
585 nM; Tocris Bioscience) or Narciclasine (100 nM; Tocris Bioscience) at for 2 hr, cytospun to  
586 coverslip, and processed for IF. For activated MuSCs, they were cultured for 48 h after FACS-  
587 isolation, and treated by DMSO, Blebbinstatin (10  $\mu$ M; Tocris Bioscience), Cytochalasin B (10  
588  $\mu$ M; Tocris Bioscience), and Y-27632 (10  $\mu$ M) for 2 hr and processed for IF.

589

#### 590 **Quantifications and statistical analysis**

591 For cryosections,  $\geq$  50 cells from 10 sections per animal (5 animals per genotype) were imaged  
592 using a Nikon E800 fluorescence microscope at 40X magnification. For MuSC fractions on single  
593 myofibers, total 150-200 cells were assessed from 2-3 animals using the same microscopy  
594 above. For quantification of IF signal intensity, 50 cells on myofibers (from 2-3 mice) or 200

595 dish-cultured MuSCs (from 2-3 mice) were imaged at 63x/1.4 oil fluorescent objectives on a  
596 Leica TCS SP5 confocal microscope. Gain and exposure settings were consistent between  
597 experiments. Z-stacks were collected to capture full objection lengths of MuSCs. Images were  
598 exported to Fiji and CellProfiler for analysis.

599 For quantification of IF signal intensity and nuclear vs. cytoplasmic distribution, single myofibers  
600 or cultured cells were IF-stained for protein of interest (i.e., MPP7, AMOT, YAP, TAZ, CARM1, or  
601 tagged epitopes), YFP and DAPI, and multi-channel images were acquired. A custom CellProfiler  
602 pipeline was used to set threshold on YFP and DAPI channels to identify primary and secondary  
603 objects, respectively. The primary objects from YFP channel were then used as masks on  
604 protein-of-interest channel images, and the integrated intensity of the masked image was used  
605 for total signal intensity. The secondary objects from DAPI channel were used as masks on  
606 protein-of-interest channel images and the intensity of the masked part was quantified for  
607 nuclear signal intensity.

608 For co-IP quantification, the samples were analyzed in three independent biological replicates.  
609 Intensity of blotting bands were measured using Fiji. Co-IPed target proteins were then  
610 normalized to their primary IP proteins.

611 For statistics, error bars represent means  $\pm$  SD. Data analyses were performed by Prism 9  
612 software. Data comparison of two independent groups was performed by two-tailed unpaired  
613 Student's t test. Multiple group analysis was performed using one-way ANOVA followed by the  
614 Tukey or Dunnet post hoc test for multiple comparison per figure legends. To test significance  
615 of cell population fraction, the total SC population over all the experimental repeats were  
616 included and comparisons were performed by Chi-squared test. \*\*\*,  $P \leq 0.001$ ; \*\*,  $P \leq 0.01$ ; \*,  $P$   
617  $\leq 0.05$ ; n.s, not significant,  $P > 0.05$ . For RNA-seq,  $P < 0.05$  was considered significant. To  
618 calculate the statistical significance of overlapped genes in Venn diagrams,  $P$ -values were  
619 calculated based on hypergeometric test.

620

#### 621 **Data Availability**

622 RNA-seq data in this study have been deposited to Gene Expression Omnibus (GEO) database  
623 under the accession GSE241340.

624

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#### 633 **Author Contributions**

634 A.S. and C.-M.F. conceived and designed the study and wrote the manuscript. A.S. carried out  
635 all the experiments and data analyses. J.L.K. provided comments and suggestions, crucial  
636 expression plasmids, the *Amot*<sup>flox</sup> mouse, and extensive manuscript editing.

637 **REFERENCES**

638

639 Bohl, J., Brimer, N., Lyons, C., and Vande Pol, S.B. (2007). The stardust family protein MPP7  
640 forms a tripartite complex with LIN7 and DLG1 that regulates the stability and localization of  
641 DLG1 to cell junctions. *J Biol Chem* 282, 9392-9400.

642 Caretti, G., Di Padova, M., Micales, B., Lyons, G.E., and Sartorelli, V. (2004). The Polycomb Ezh2  
643 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes*  
644 *Dev* 18, 2627-2638.

645 Castro-Mondragon, J.A., Riudavets-Puig, R., Rauluseviciute, I., Lemma, R.B., Turchi, L., Blanc-  
646 Mathieu, R., Lucas, J., Boddie, P., Khan, A., Manosalva Perez, N., *et al.* (2022). JASPAR 2022: the  
647 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids*  
648 *Res* 50, D165-D173.

649 Chan, S.W., Lim, C.J., Guo, F., Tan, I., Leung, T., and Hong, W. (2013). Actin-binding and cell  
650 proliferation activities of angiomin family members are regulated by Hippo pathway-  
651 mediated phosphorylation. *J Biol Chem* 288, 37296-37307.

652 Chang, N.C., Sincennes, M.C., Chevalier, F.P., Brun, C.E., Lacaria, M., Segales, J., Munoz-Canoves,  
653 P., Ming, H., and Rudnicki, M.A. (2018). The Dystrophin Glycoprotein Complex Regulates the  
654 Epigenetic Activation of Muscle Stem Cell Commitment. *Cell Stem Cell* 22, 755-768 e756.

655 Chen, F., Zhou, J., Li, Y., Zhao, Y., Yuan, J., Cao, Y., Wang, L., Zhang, Z., Zhang, B., Wang, C.C., *et*  
656 *al.* (2019). YY1 regulates skeletal muscle regeneration through controlling metabolic  
657 reprogramming of satellite cells. *EMBO J* 38.

658 Chytla, A., Gajdzik-Nowak, W., Olszewska, P., Biernatowska, A., Sikorski, A.F., and Czogalla, A.  
659 (2020). Not Just Another Scaffolding Protein Family: The Multifaceted MPPs. *Molecules* 25.

660 Dai, X., She, P., Chi, F., Feng, Y., Liu, H., Jin, D., Zhao, Y., Guo, X., Jiang, D., Guan, K.L., *et al.*  
661 (2013). Phosphorylation of angiomin by Lats1/2 kinases inhibits F-actin binding, cell  
662 migration, and angiogenesis. *J Biol Chem* 288, 34041-34051.

663 Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le  
664 Digabel, J., Forcato, M., Bicciato, S., *et al.* (2011). Role of YAP/TAZ in mechanotransduction.  
665 *Nature* 474, 179-183.

666 Eliazer, S., Muncie, J.M., Christensen, J., Sun, X., D'Urso, R.S., Weaver, V.M., and Brack, A.S.  
667 (2019). Wnt4 from the Niche Controls the Mechano-Properties and Quiescent State of Muscle  
668 Stem Cells. *Cell Stem Cell* 25, 654-665 e654.

669 Ernkvist, M., Aase, K., Ukomadu, C., Wohlschlegel, J., Blackman, R., Veitonmaki, N., Bratt, A.,  
670 Dutta, A., and Holmgren, L. (2006). p130-angiomin associates to actin and controls  
671 endothelial cell shape. *FEBS J* 273, 2000-2011.

672 Farre, D., Roset, R., Huerta, M., Adsuara, J.E., Rosello, L., Alba, M.M., and Messeguer, X. (2003).  
673 Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN.  
674 *Nucleic Acids Res* 31, 3651-3653.

675 Fishilevich, S., Nudel, R., Rappaport, N., Hadar, R., Plaschkes, I., Iny Stein, T., Rosen, N., Kohn, A.,  
676 Twik, M., Safran, M., *et al.* (2017). GeneHancer: genome-wide integration of enhancers and  
677 target genes in GeneCards. Database (Oxford) 2017.

678 Foster, C.T., Gualdrini, F., and Treisman, R. (2017). Mutual dependence of the MRTF-SRF and  
679 YAP-TEAD pathways in cancer-associated fibroblasts is indirect and mediated by cytoskeletal  
680 dynamics. *Genes Dev* 31, 2361-2375.

681 Fuchs, E., and Blau, H.M. (2020). Tissue Stem Cells: Architects of Their Niches. *Cell Stem Cell* 27,  
682 532-556.

683 Goel, A.J., Rieder, M.K., Arnold, H.H., Radice, G.L., and Krauss, R.S. (2017). Niche Cadherins  
684 Control the Quiescence-to-Activation Transition in Muscle Stem Cells. *Cell Rep* 21, 2236-2250.

685 Hirano, K., Tsuchiya, M., Shiomi, A., Takabayashi, S., Suzuki, M., Ishikawa, Y., Kawano, Y.,  
686 Takabayashi, Y., Nishikawa, K., Nagao, K., *et al.* (2023). The mechanosensitive ion channel  
687 PIEZO1 promotes satellite cell function in muscle regeneration. *Life Sci Alliance* 6.

688 Hoxha, S., Shepard, A., Troutman, S., Diao, H., Doherty, J.R., Janiszewska, M., Witwicki, R.M.,  
689 Pipkin, M.E., Ja, W.W., Karetka, M.S., *et al.* (2020). YAP-Mediated Recruitment of YY1 and EZH2  
690 Represses Transcription of Key Cell-Cycle Regulators. *Cancer Res* 80, 2512-2522.

691 Judson, R.N., Tremblay, A.M., Knopp, P., White, R.B., Urcia, R., De Bari, C., Zammit, P.S.,  
692 Camargo, F.D., and Wackerhage, H. (2012). The Hippo pathway member Yap plays a key role in  
693 influencing fate decisions in muscle satellite cells. *J Cell Sci* 125, 6009-6019.

694 Kanai, F., Marignani, P.A., Sarbassova, D., Yagi, R., Hall, R.A., Donowitz, M., Hisaminato, A.,  
695 Fujiwara, T., Ito, Y., Cantley, L.C., *et al.* (2000). TAZ: a novel transcriptional co-activator  
696 regulated by interactions with 14-3-3 and PDZ domain proteins. *EMBO J* 19, 6778-6791.

697 Kann, A.P., Hung, M., Wang, W., Nguyen, J., Gilbert, P.M., Wu, Z., and Krauss, R.S. (2022). An  
698 injury-responsive Rac-to-Rho GTPase switch drives activation of muscle stem cells through rapid  
699 cytoskeletal remodeling. *Cell Stem Cell* 29, 933-947 e936.

700 Kawabe, Y., Wang, Y.X., McKinnell, I.W., Bedford, M.T., and Rudnicki, M.A. (2012). *Carm1*  
701 regulates *Pax7* transcriptional activity through *MLL1/2* recruitment during asymmetric satellite  
702 stem cell divisions. *Cell Stem Cell* 11, 333-345.

703 Keenan, A.B., Torre, D., Lachmann, A., Leong, A.K., Wojciechowicz, M.L., Utti, V., Jagodnik, K.M.,  
704 Kropiwnicki, E., Wang, Z., and Ma'ayan, A. (2019). ChEA3: transcription factor enrichment  
705 analysis by orthogonal omics integration. *Nucleic Acids Res* 47, W212-W224.

706 Kim, M., Kim, T., Johnson, R.L., and Lim, D.S. (2015). Transcriptional co-repressor function of the  
707 hippo pathway transducers YAP and TAZ. *Cell Rep* 11, 270-282.

708 Lepper, C., Conway, S.J., and Fan, C.M. (2009). Adult satellite cells and embryonic muscle  
709 progenitors have distinct genetic requirements. *Nature* 460, 627-631.

710 Lepper, C., Partridge, T.A., and Fan, C.M. (2011). An absolute requirement for *Pax7*-positive  
711 satellite cells in acute injury-induced skeletal muscle regeneration. *Development* 138, 3639-  
712 3646.

713 Li, L., and Fan, C.M. (2017). A CREB-MPP7-AMOT Regulatory Axis Controls Muscle Stem Cell  
714 Expansion and Self-Renewal Competence. *Cell Rep* 21, 1253-1266.

715 Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and  
716 dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550.

717 Lu, L., Sun, K., Chen, X., Zhao, Y., Wang, L., Zhou, L., Sun, H., and Wang, H. (2013). Genome-wide  
718 survey by CHIP-seq reveals YY1 regulation of lincRNAs in skeletal myogenesis. *EMBO J* 32, 2575-  
719 2588.

720 Ma, N., Chen, D., Lee, J.H., Kuri, P., Hernandez, E.B., Kocan, J., Mahmood, H., Tichy, E.D.,  
721 Rompolas, P., and Mourkioti, F. (2022). Piezo1 regulates the regenerative capacity of skeletal  
722 muscles via orchestration of stem cell morphological states. *Sci Adv* 8, eabn0485.  
723 Ma, S., Meng, Z., Chen, R., and Guan, K.L. (2019). The Hippo Pathway: Biology and  
724 Pathophysiology. *Annu Rev Biochem* 88, 577-604.  
725 Mauro, A. (1961). Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 9, 493-495.  
726 Messeguer, X., Escudero, R., Farre, D., Nunez, O., Martinez, J., and Alba, M.M. (2002). PROMO:  
727 detection of known transcription regulatory elements using species-tailored searches.  
728 *Bioinformatics* 18, 333-334.  
729 Moleirinho, S., Guerrant, W., and Kissil, J.L. (2014). The Angiomotins--from discovery to  
730 function. *FEBS Lett* 588, 2693-2703.  
731 Moleirinho, S., Hoxha, S., Mandati, V., Curtale, G., Troutman, S., Ehmer, U., and Kissil, J.L.  
732 (2017). Regulation of localization and function of the transcriptional co-activator YAP by  
733 angiomotin. *Elife* 6.  
734 Murphy, M.M., Lawson, J.A., Mathew, S.J., Hutcheson, D.A., and Kardon, G. (2011). Satellite  
735 cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration.  
736 *Development* 138, 3625-3637.  
737 Pan, D. (2022). The unfolding of the Hippo signaling pathway. *Dev Biol* 487, 1-9.  
738 Panciera, T., Azzolin, L., Cordenonsi, M., and Piccolo, S. (2017). Mechanobiology of YAP and TAZ  
739 in physiology and disease. *Nat Rev Mol Cell Biol* 18, 758-770.  
740 Pertea, M., Kim, D., Pertea, G.M., Leek, J.T., and Salzberg, S.L. (2016). Transcript-level  
741 expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* 11,  
742 1650-1667.  
743 Reginensi, A., Scott, R.P., Gregorieff, A., Bagherie-Lachidan, M., Chung, C., Lim, D.S., Pawson, T.,  
744 Wrana, J., and McNeill, H. (2013). Yap- and Cdc42-dependent nephrogenesis and  
745 morphogenesis during mouse kidney development. *PLoS Genet* 9, e1003380.  
746 Relaix, F., Bencze, M., Borok, M.J., Der Vartanian, A., Gattazzo, F., Mademtoglou, D., Perez-  
747 Diaz, S., Prola, A., Reyes-Fernandez, P.C., Rotini, A., *et al.* (2021). Perspectives on skeletal  
748 muscle stem cells. *Nat Commun* 12, 692.  
749 Rozo, M., Li, L., and Fan, C.M. (2016). Targeting beta1-integrin signaling enhances regeneration  
750 in aged and dystrophic muscle in mice. *Nat Med* 22, 889-896.  
751 Sambasivan, R., Yao, R., Kissenpfennig, A., Van Wittenberghe, L., Paldi, A., Gayraud-Morel, B.,  
752 Guenou, H., Malissen, B., Tajbakhsh, S., and Galy, A. (2011). Pax7-expressing satellite cells are  
753 indispensable for adult skeletal muscle regeneration. *Development* 138, 3647-3656.  
754 Shimono, A., and Behringer, R.R. (2003). Angiomotin regulates visceral endoderm movements  
755 during mouse embryogenesis. *Curr Biol* 13, 613-617.  
756 Silver, J.S., Gunay, K.A., Cutler, A.A., Vogler, T.O., Brown, T.E., Pawlikowski, B.T., Bednarski, O.J.,  
757 Bannister, K.L., Rogowski, C.J., McKay, A.G., *et al.* (2021). Injury-mediated stiffening persistently  
758 activates muscle stem cells through YAP and TAZ mechanotransduction. *Sci Adv* 7.  
759 Sousa-Victor, P., Garcia-Prat, L., and Munoz-Canoves, P. (2022). Control of satellite cell function  
760 in muscle regeneration and its disruption in ageing. *Nat Rev Mol Cell Biol* 23, 204-226.  
761 Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., Tanabe, Y., Jessell, T.M., and Costantini, F.  
762 (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26  
763 locus. *BMC Dev Biol* 1, 4.

764 Stucke, V.M., Timmerman, E., Vandekerckhove, J., Gevaert, K., and Hall, A. (2007). The MAGUK  
765 protein MPP7 binds to the polarity protein hDlg1 and facilitates epithelial tight junction  
766 formation. *Mol Biol Cell* 18, 1744-1755.

767 Sun, C., De Mello, V., Mohamed, A., Ortuste Quiroga, H.P., Garcia-Munoz, A., Al Bloshi, A.,  
768 Tremblay, A.M., von Kriegsheim, A., Collie-Duguid, E., Vargesson, N., *et al.* (2017). Common and  
769 Distinctive Functions of the Hippo Effectors Taz and Yap in Skeletal Muscle Stem Cell Function.  
770 *Stem Cells* 35, 1958-1972.

771 Tremblay, A.M., Missiaglia, E., Galli, G.G., Hettmer, S., Urcia, R., Carrara, M., Judson, R.N.,  
772 Thway, K., Nadal, G., Selfe, J.L., *et al.* (2014). The Hippo transducer YAP1 transforms activated  
773 satellite cells and is a potent effector of embryonal rhabdomyosarcoma formation. *Cancer Cell*  
774 26, 273-287.

775 Verheul, T.C.J., van Hijfte, L., Perenthaler, E., and Barakat, T.S. (2020). The Why of YY1:  
776 Mechanisms of Transcriptional Regulation by Yin Yang 1. *Front Cell Dev Biol* 8, 592164.

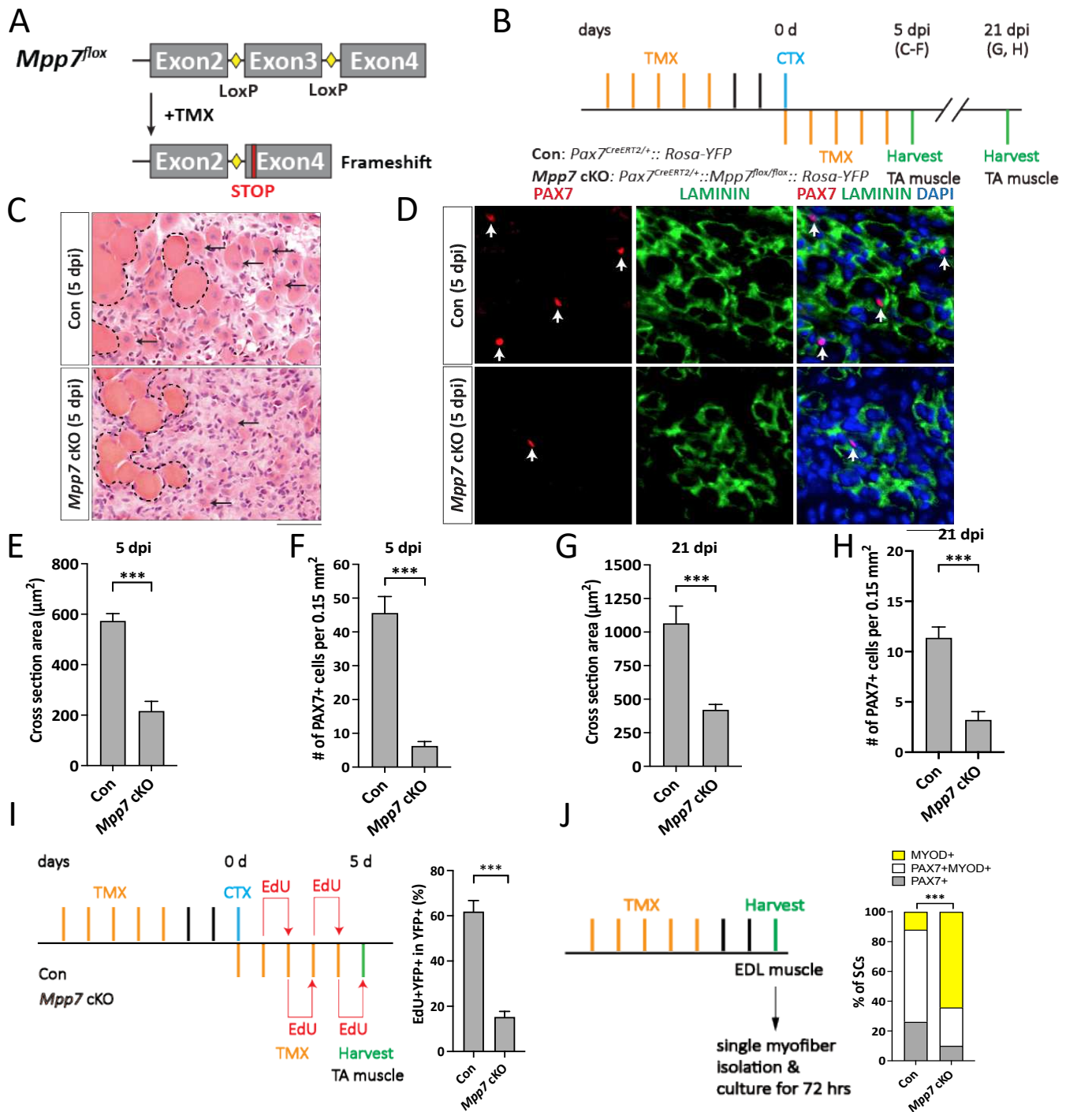
777 Wang, H., Hertlein, E., Bakkar, N., Sun, H., Acharyya, S., Wang, J., Carathers, M., Davuluri, R.,  
778 and Guttridge, D.C. (2007). NF-kappaB regulation of YY1 inhibits skeletal myogenesis through  
779 transcriptional silencing of myofibrillar genes. *Mol Cell Biol* 27, 4374-4387.

780 Wells, C.D., Fawcett, J.P., Traweger, A., Yamanaka, Y., Goudreault, M., Elder, K., Kulkarni, S.,  
781 Gish, G., Virag, C., Lim, C., *et al.* (2006). A Rich1/Amot complex regulates the Cdc42 GTPase and  
782 apical-polarity proteins in epithelial cells. *Cell* 125, 535-548.

783 Yi, C., Shen, Z., Stemmer-Rachamimov, A., Dawany, N., Troutman, S., Showe, L.C., Liu, Q.,  
784 Shimono, A., Sudol, M., Holmgren, L., *et al.* (2013). The p130 isoform of angiominin is required  
785 for Yap-mediated hepatic epithelial cell proliferation and tumorigenesis. *Sci Signal* 6, ra77.

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Fig.1



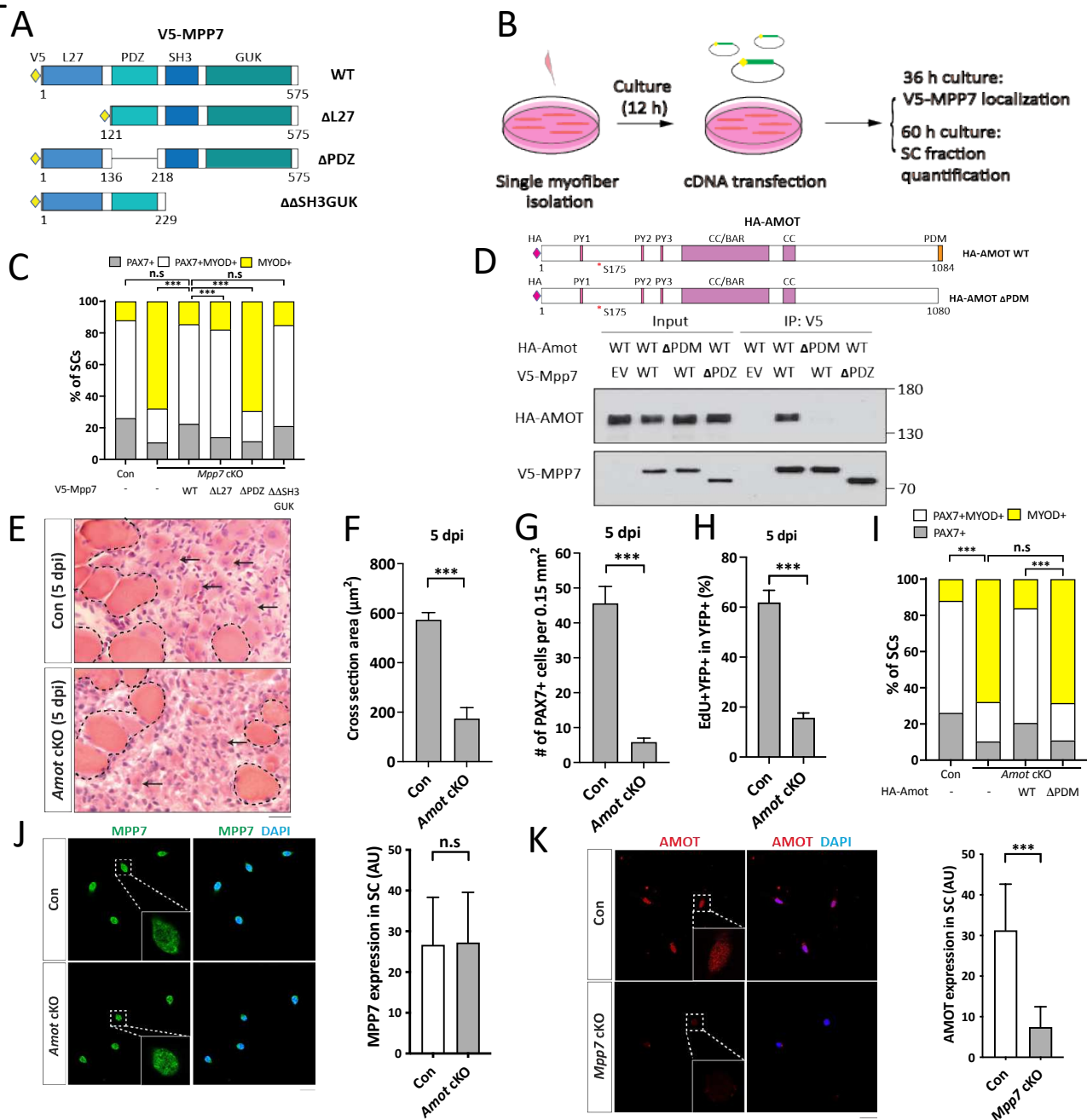
**Figure 1. *Mpp7* cKO in Pax7<sup>+</sup> MuSCs shows defects in regeneration and MuSC self-renewal.**

- (A) Diagram of *Mpp7* floxed allele (*Mpp7<sup>fllox</sup>*; loxP, yellow diamond) for tamoxifen (TMX) inducible Cre-mediated cKO. After recombination, out of-frame (Frameshift) joining of Exons 2 and 4 introduces an early stop codon (STOP).
- (B) Regimen of TMX administration, cardiotoxin (CTX) injury, and tibialis anterior (TA) muscle harvest; d, day; dpi, days post injury. Genotypes of control (Con) and *Mpp7* cKO are indicated.
- (C-F) *Mpp7* cKO regeneration defects at 5 dpi. Representative images of H&E histology are in (C), immunofluorescence (IF) for PAX7 and LAMININ in (D, with DAPI), and quantification of regenerated myofiber cross sectional area in (E) and of PAX7<sup>+</sup> MuSC density in (F). Black arrows indicate regenerated myofibers; dashed lines, boundary of injury; white arrows, PAX7<sup>+</sup> MuSCs. N = 5 mice, each.
- (G, H) Quantifications of regenerated myofiber cross sectional area (G) and PAX7<sup>+</sup> MuSC density (H) at 21 dpi. N = 5 mice, each.
- (I) Regimen of in vivo EdU incorporation to assess the percentage of proliferated YFP-marked cells at 5 dpi; quantification to the right. N = 5 mice, each.
- (J) Regimen of cell fate determination using single myofiber culture. Cell fates were assessed by IF of PAX7 and MYOD; quantification to the right; keys to cell fates at the top. N = 3 Con mice, of total 601 cells; N = 4 *Mpp7* cKO mice, of total 517 cells.

Data information: Scale bars = 25  $\mu\text{m}$  in (C-D). (E-I) Error bars represent means  $\pm$  SD; Student's *t*-test (two-sided). (J) Chi-square test. \*\*\*,  $P < 0.001$ .



Fig. 2

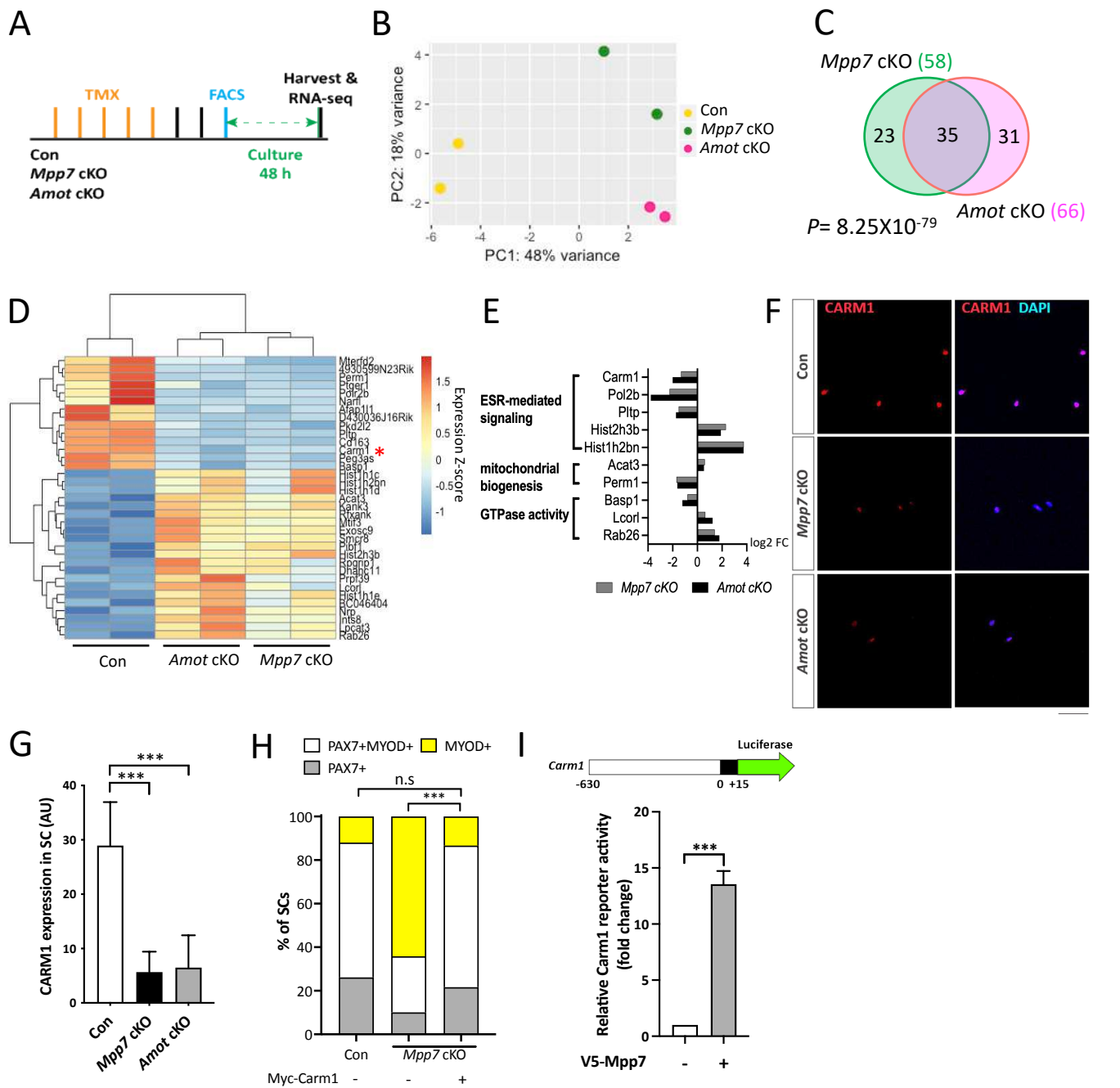


**Figure 2. Interacting domains of MPP7 and AMOT are critical for SC renewal.**

- (A) Depiction of MPP7 domain architecture and V5-tagged wild type (WT) and domain deletion mutants (listed on the right) of *Mpp7* expression constructs used in (B, C).
- (B) Flowchart to force-express various *Mpp7* constructs (A) in *Mpp7* cKO MuSCs.
- (C) Quantifications of cells fate from experiments depicted in (B); IF of V5, PAX7 and MYOD was performed to determine the fate of transfected cells. Expression constructs used are in x-axis; (-), empty vector; keys to cell fate at the top;  $\geq 168$  transfected cells were assessed per group.
- (D) Co-IP assays to determine interaction domains between MPP7 and AMOT in 293T cells. HA-tagged WT Amot (HA-Amot) and  $\Delta$ PDM Amot are depicted. V5-tagged WT MPP7 and  $\Delta$ PDZ MPP7 were used for co-IP with HA-AMOT and  $\Delta$ PDM AMOT using an anti-V5 antibody, followed by Western blotting with anti-HA or anti-V5 antibodies.
- (E-H) *Amot* cKO regenerative defects at 5 dpi. Representative H&E staining images of TA muscles from Con and *Amot* cKO are in (E), quantifications of regenerated muscle fiber cross sectional areas in (F), PAX7+ MuSC densities in (G), and percentages of EdU+ YFP-marked cells in (H). Experimental design is the same as in Figure 1. N = 5 mice, each.
- (I) Single myofiber transfection assays with WT or  $\Delta$ PDM HA-Amot constructs as depicted in (B); (-), empty vector. IF of PAX7, MYOD and HA was performed to determine the fate of transfected cells; keys at the top;  $\geq 180$  cells per group.
- (J, K) IF of MPP7 in Con and *Amot* cKO MuSCs in (J) and of AMOT in Con and *Mpp7* cKO MuSCs in (K), at 48 h of culture after FACS isolation. Qualified fluorescent signals (arbitrary units, AU) are to the right of corresponding representative images; 200 cells per group.

Data information: Scale bars = 25  $\mu\text{m}$  in (E, J-K). (F-H, J, K): Error bars represent means  $\pm$  SD. Student's *t*-test (two-sided). (C, I): Chi-square tests were performed. n.s.,  $P > 0.05$ ; \*\*\*,  $P < 0.001$ .

Fig. 3



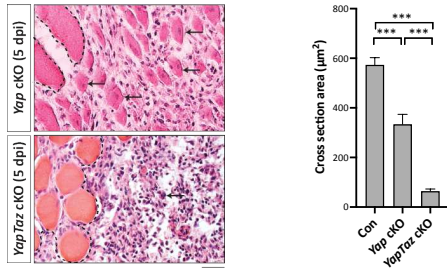
**Figure 3. CARM1 is one of the genes commonly regulated by *Mpp7* and *Amot* in the SC.**

- (A) Flowchart for bulk RNA-sequencing (RNA-seq).
- (B) PCA analysis of transcriptome data of Con, *Mpp7* cKO, *Amot* cKO MuSCs.
- (C) Venn diagram summarizes (35) overlapping DEGs between the *Mpp7* cKO (58 DEGs) and the *Amot* cKO (66 DEGs).
- (D) Hierarchical clustering and heatmap of RNA-seq expression z-scores computed for the 35 DEGs in the *Mpp7* cKO and the *Amot* cKO; red asterisk, *Carm1*.
- (E) Expression fold-changes ( $\log_2$  FC,  $\log_2$  fold change) of genes in GO-term enriched pathways (y-axis) are displayed for the *Mpp7* cKO (grey bars) and the *Amot* cKO (black bars).
- (F, G) Representative IF images of CARM1 in Con, *Mpp7* cKO and *Amot* cKO MuSCs at 48 h in culture are in (F). Quantified CARM1 signals (in AU) are in (G); 200 MuSCs from 2-3 mice in each group.
- (H) Expressing a Myc-tagged Carm1 (Myc-Carm1) rescues *Mpp7* cKO MuSCs in single myofiber culture; (-), empty vector. IF of Myc, PAX7, and MYOD was performed to determine the fate of transfected cells. Quantification of cell fate fractions are shown; keys at top;  $\geq 530$  cells in each group.
- (I) V5-Mpp7 expression construct activates the Carm1-reporter (a luciferase reporter driven by a promoter region (-630 to +15) of *Carm1*, depicted at the top) in 293T cells; (-), empty vector. N=3.

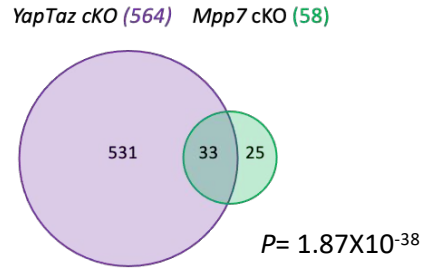
Data information: RNA seq data have been deposited in NCBI. Programs used to generate (B, D, E) are described in Methods. Scale bar = 50  $\mu$ m in (F). Hypergeometric test was used in (C). Error bars represent means  $\pm$  SD. One-way ANOVA with Tukey's post hoc test was performed in (G), Chi-square test in (H), and Student's *t*-test (two-sided) in (I). n.s.,  $P > 0.05$ ; \*\*\*,  $P < 0.001$ .

**Fig. 4**

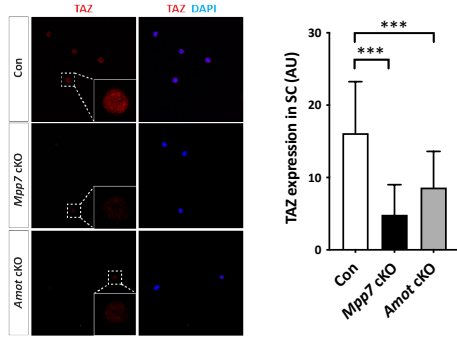
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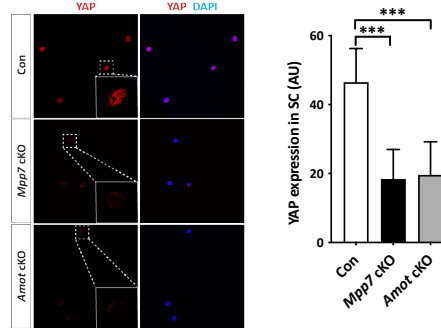
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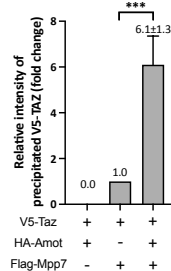
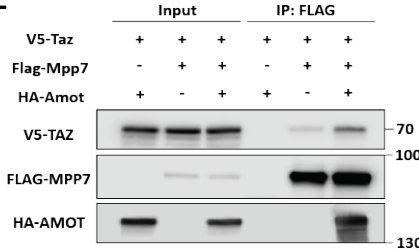
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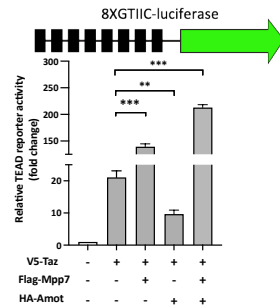
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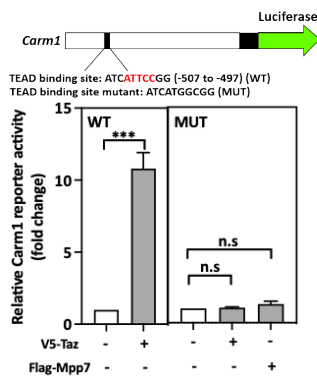
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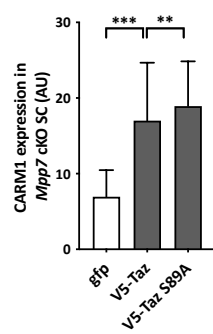
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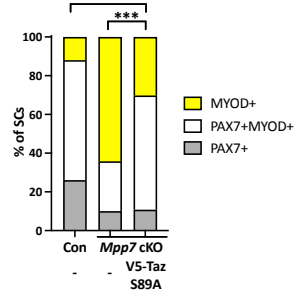
**G**



**H**



**I**

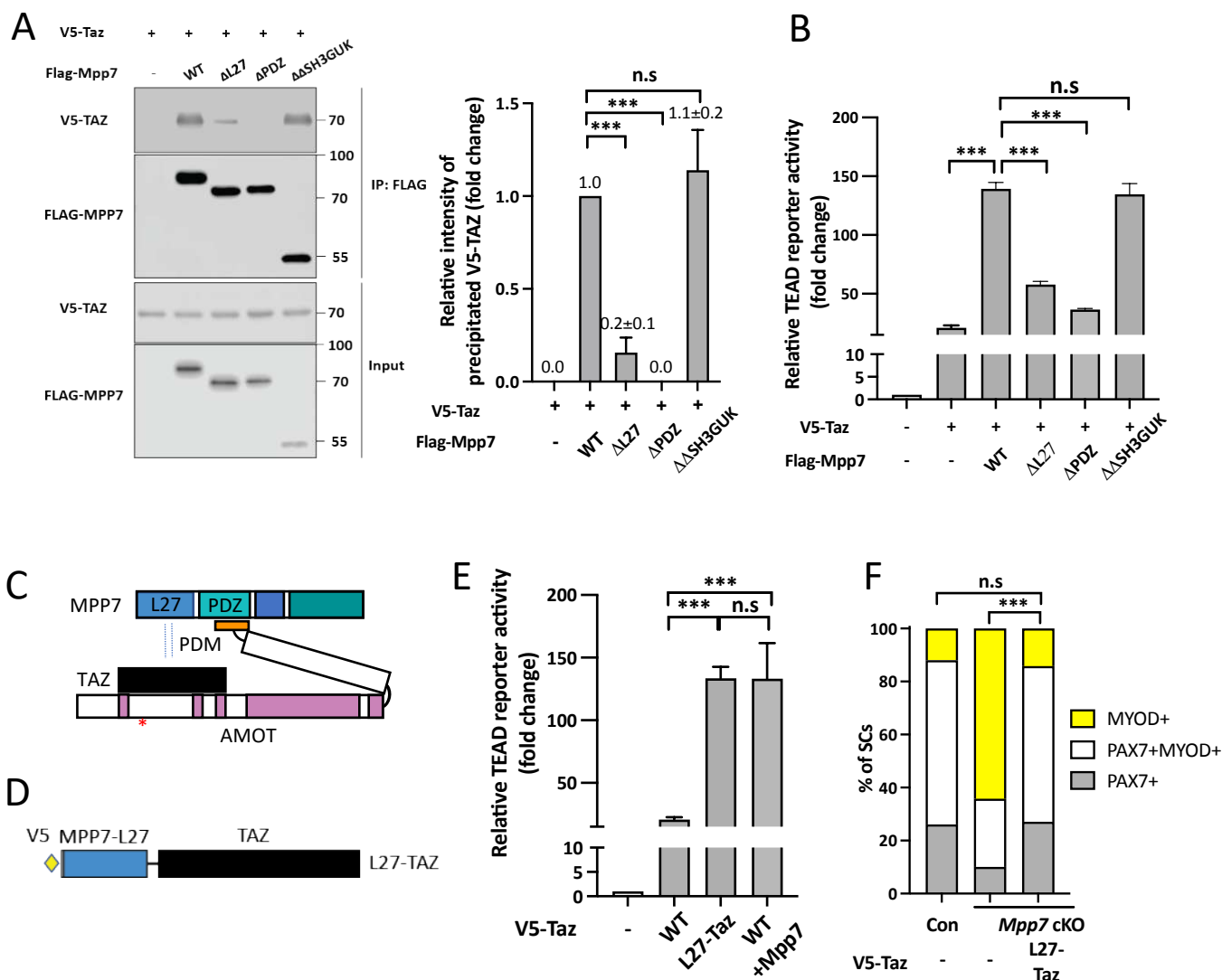


**Figure 4. *Mpp7/Amot* regulatory network intersects with that of *Yap/Taz*.**

- (A) Representative H&E histology of *Yap* cKO and *YapTaz* cKO muscles at 5 dpi (Con histology not included); quantifications of regenerated myofiber cross sectional area to the right. N = 5, each genotype.
- (B) Venn diagram shows overlapping DEGs between the *YapTaz* cKO and the *Mpp7* cKO.
- (C, D) Representative IF images of TAZ (C) and YAP (D) in FACS-isolated and cultured Con, *Mpp7* cKO, and *Amot* cKO MuSCs at 48 h; quantified fluorescent signals (AU) to the right of corresponding images; 200 MuSCs from 2-3 mice in each group.
- (E) Co-IP of V5-TAZ and HA-AMOT by FLAG-MPP7 expressed in 293T cells. Expression constructs and tagged epitopes for detection are indicated; (-), empty vector. Quantification of relative levels of co-IPed V5-TAZ is to the right. N = 3.
- (F) Relative TEAD-reporter (8XGTIIIC-luciferase, depicted at top) activities when co-transfected with V5-Taz, Flag-Mpp7, and/or Ha-Amot expression constructs in 293T cells; (-), empty vector. N = 3.
- (G) Relative activities of WT and TEAD-binding site mutated (MUT) *Carm1*-reporters co-transfected with V5-Taz or Flag-Mpp7 expression constructs; (-), empty vector. N = 3.
- (H) Quantified IF signals (AU) of CARM1 in *Mpp7* cKO MuSCs transfected with *gfp* (as control), V5-Taz WT and V5-Taz S89A expression constructs; 200 MuSCs from 2-3 mice in each group.
- (I) Comparison of cell fate fractions among Con, *Mpp7* cKO, and *Mpp7* cKO MuSCs transfected with V5-Taz S89A expression construct in single myofiber culture; (-), empty vector; keys at the top;  $\geq 217$  cells in each group.

Data information: Scale bar = 25  $\mu$ m in (A) and 50  $\mu$ m in (C, D). Error bars represent means  $\pm$  SD. Hypergeometric test was used in (B); One-way ANOVA with Tukey's post hoc test was performed in (A, C, D, F-H); Chi-square test in (I). n.s.,  $P > 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Fig. 5

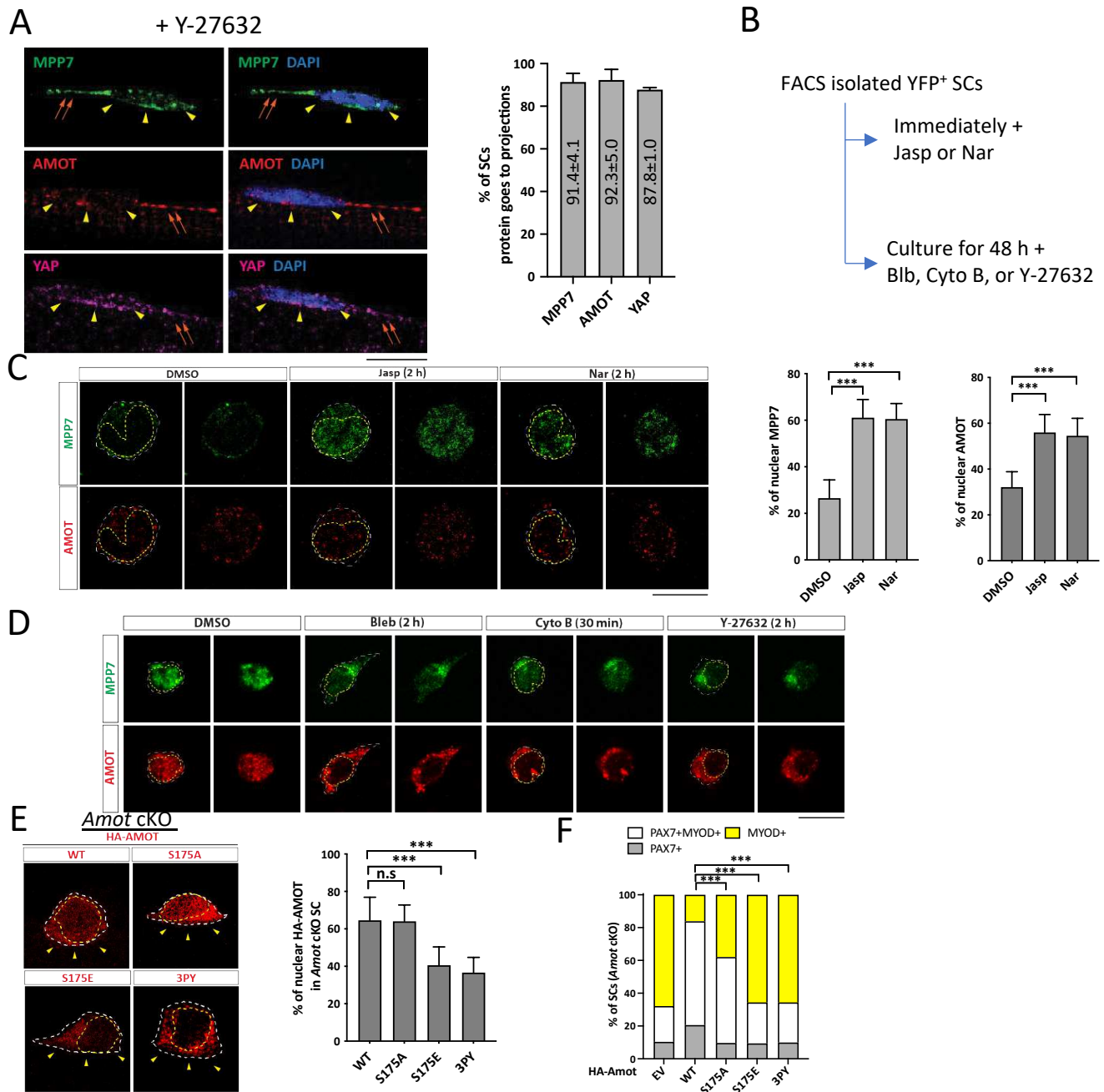


**Figure 5. AMOT links TAZ to the MPP7-L27 domain for enhanced transcriptional activity.**

- (A) MPP7-L27 contributes to MPP7-TAZ interaction by co-IP assay in 293T cells. Expression constructs and tagged epitopes for detection are indicated; (-), empty vector. Quantification of co-IPed V5-TAZ are to the right. N = 3.
- (B) Relative TEAD-reporter activities when co-transfected with various *Mpp7* expression constructs (x-axis); (-), empty vector. N = 3.
- (C) A model summarizes the co-IP results in Figures 5A, S5A and S5B.
- (D) Diagram of the fusion construct between V5-tagged *Mpp7*-L27 and Taz (i.e., L27-Taz) used in (E, F).
- (E) Relative TEAD-reporter activities when co-transfected with expression constructs indicated in the x-axis; (-), empty vector. N = 3.
- (F) Quantification of cell fates of Con and *Mpp7* cKO MuSCs in single myofiber assay; expression constructs in the x-axis; (-), empty vector;  $\geq 155$  cells in each group.

Data information: Error bars represent means  $\pm$  SD. One-way ANOVA with Tukey's post hoc test was performed in (A, B, E), and Chi-Square test, in (F). n.s,  $P > 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Fig. 6

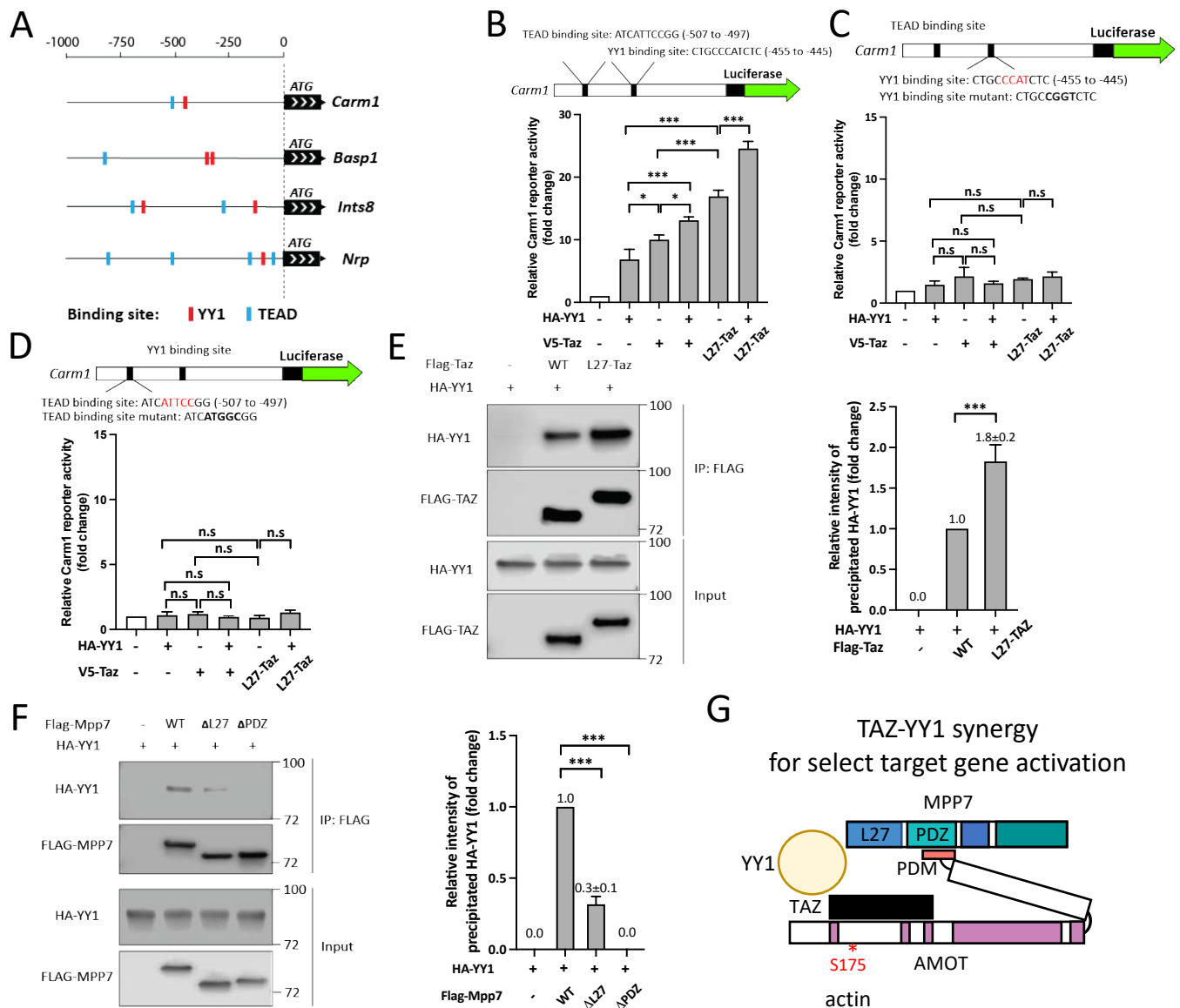


**Figure 6. Actin polymerization state impacts AMOT localization and function through binding to TAZ or YAP.**

- (A) Representative IF images of MPP7, AMOT, and YAP in wild type MuSCs on single myofibers isolated in the presence of 50  $\mu$ M Y-27632. Percentages of MuSCs with MPP, AMOT and YAP signals in cellular projections were quantified: 77 cells for MPP7, 73 cells for AMOT, and 66 cells for YAP, from 3 mice.
- (B) Experimental flowchart to investigate the impact of actin polymerization state on the cellular localization of MPP7 and AMOT in FACS-isolated MuSCs in culture.
- (C) Representative IF images of MPP7 and AMOT of freshly isolated MuSCs treated with DMSO (control), 100 nM Jasplankinoline (Jasp), or 100 nM Narciclasine (Nar) for 2 h. Percentages of MPP7 or AMOT IF signals in the nucleus (versus total signals) in each SC were quantified; 200 MuSCs in each group.
- (D) Representative IF images of MPP7 and AMOT in MuSCs cultured for 48 h and treated with DMSO (control), 10  $\mu$ M Blebbistatin (Bleb), 10  $\mu$ M Cytochalasin B (Cyto B), or 10  $\mu$ M Y-27632 for indicated time prior to assay. See Figure S6A for quantified data.
- (E) Localization of HA-tagged AMOT WT, AMOT S175A (S175A), AMOT S175E (S175E), and AMOT 3PY (3PY) expressed (via transfection) in *Amot* cKO MuSCs on single myofibers by IF of HA; yellow arrowheads, apical side. Percentages of nuclear signals (of total signal) of each variant were quantified; 50 MuSCs in each group.
- (F) HA-tagged AMOT variants in (E) were transfected into *Amot* cKO SCs on single myofibers; (-), empty vector. Cell fates of transfected cells were determined by IF of HA, PAX7 and MYOD and quantified;  $\geq$  145 cells in each group.

Data information: Scale bars = 10  $\mu$ m in (A, C-E). Error bars represent means  $\pm$  SD. One-way ANOVA with Tukey's post hoc test was performed in (A, C, E), and Chi-square test in (F). n.s.,  $P > 0.05$ ; \*\*\*,  $P < 0.001$ .

Fig. 7



**Figure 7. MPP7's L27 domain cooperates with TAZ and YY1 to enhance transcription.**

- (A) Schematics for promoter regions of 4 DEGs among *Mpp7*, *Amot*, *YapTaz*, and *Yy1* cKO (Chen *et al.*, 2019) data sets. Putative YY1 binding sites are shown as red blocks and TEAD binding sites, blue blocks.
- (B) The location and sequence of the TEAD and YY1 binding sites are indicated in the *Carm1* promoter. *Carm1*-reporter was co-transfected with expression constructs in x-axis to assess transcriptional activity; (-), empty vector. N = 3.
- (C) Same as in (B), except that the YY1 binding site was mutated (top). N=3.
- (D) Same as in (B), except that the TEAD binding site was mutated (top). N=3.
- (E) Co-IP assay shows the interaction between TAZ and YY1 can be enhanced by fusing MPP7's L27 domain to TAZ in 293T cells. Expression constructs and tagged epitopes for detection are indicated. Quantification of co-IPed HA-YY1 is to the right. N = 3.
- (F) Co-IP assay to determine the relative contributions of L27 and PDZ domains of MPP7 to its interaction with HA-YY1 in 293T cells. Expression constructs and tagged protein detection are indicated. Quantification of co-IPed HA-YY1 is shown to the right. N = 3.
- (G) A model for TAZ-YY1 cooperation mediated by AMOT and MPP7. S175 of AMOT is subjected to phosphorylation and regulation by actin dynamics.

Data information: Error bars represent means  $\pm$  SD. One-way ANOVA with Tukey's post hoc test was performed in (B-F). n.s,  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [110123Mpp7supplementalfigures.pdf](#)
- [TableS1DEGsandcrosscomparison.xlsx](#)
- [TableS2Reagentsresource.xlsx](#)