

Supplemental Information

Relayed signaling between mesenchymal progenitors and muscle stem cells ensures adaptive stem cell response to increased mechanical load

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Cell Stem Cell

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Supplemental Figures

S. Figure 1

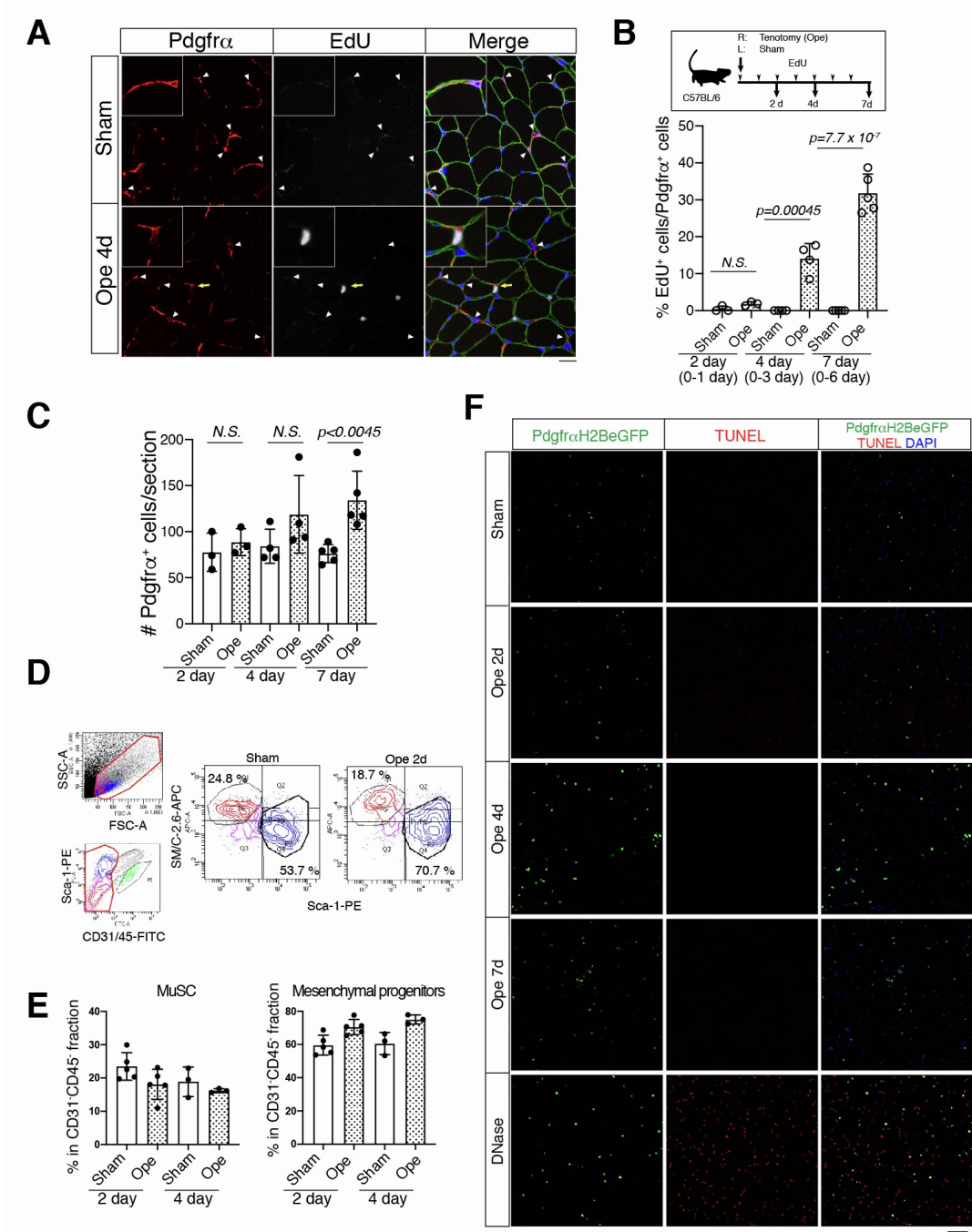


Figure S1. Behaviors of Pα⁺ cells in overloaded muscles; Related to Figure 1

(A) Immunostaining of Pdgfr α (red), laminin α 2 (green), and EdU in Sham or Ope muscles on day 4 after tenotomy. Arrows and arrowheads indicate Pdgfr α ⁺EdU⁺ and Pdgfr α ⁺EdU⁻ cells, respectively. Nuclei were counterstained with DAPI. Scale bar: 25 μ m

(B) Experimental scheme for analyzing Pdgfr α ⁺ cells in plantaris muscle in C57BL/6 mice on day 2, 4, and 7 after tenotomy (Ope; R: right). Contralateral left plantaris muscle was used as sham control (L: Sham). Graph indicates the percentage of EdU⁺ Pdgfr α ⁺ cells in Sham and Ope muscles on day 2 (n=3), 4 (n=4), and 7 (n=5) after tenotomy. The number in parentheses refer to the duration of EdU injections.

(C) Number of Pdgfr α ⁺ cells per muscle section on day 2, 4, and 7 after tenotomy.

(D) FACS profiles for detecting MuSCs and mesenchymal fractions. The upper left panel shows the gate for the following analyses. The lower left panel shows the gate for CD31⁻CD45⁻ fraction. The right two panels show MuSCs (SM/C-2.6⁺Sca-1⁻) and mesenchymal progenitor fractions (Sca-1⁺) in CD31⁻CD45⁻ fraction.

(E) Frequency of MuSC (left) or mesenchymal progenitors (right) in CD31⁻CD45⁻ fraction in sham or overloaded muscles.

(F) TUNEL staining in sections of Sham and Ope muscles of *Pdgfra*^{H2BeGFP} mice on day 2-7 after tenotomy. DNase was used as a positive control of TUNEL staining. Scale bar: 75 μ m

S. Figure 2

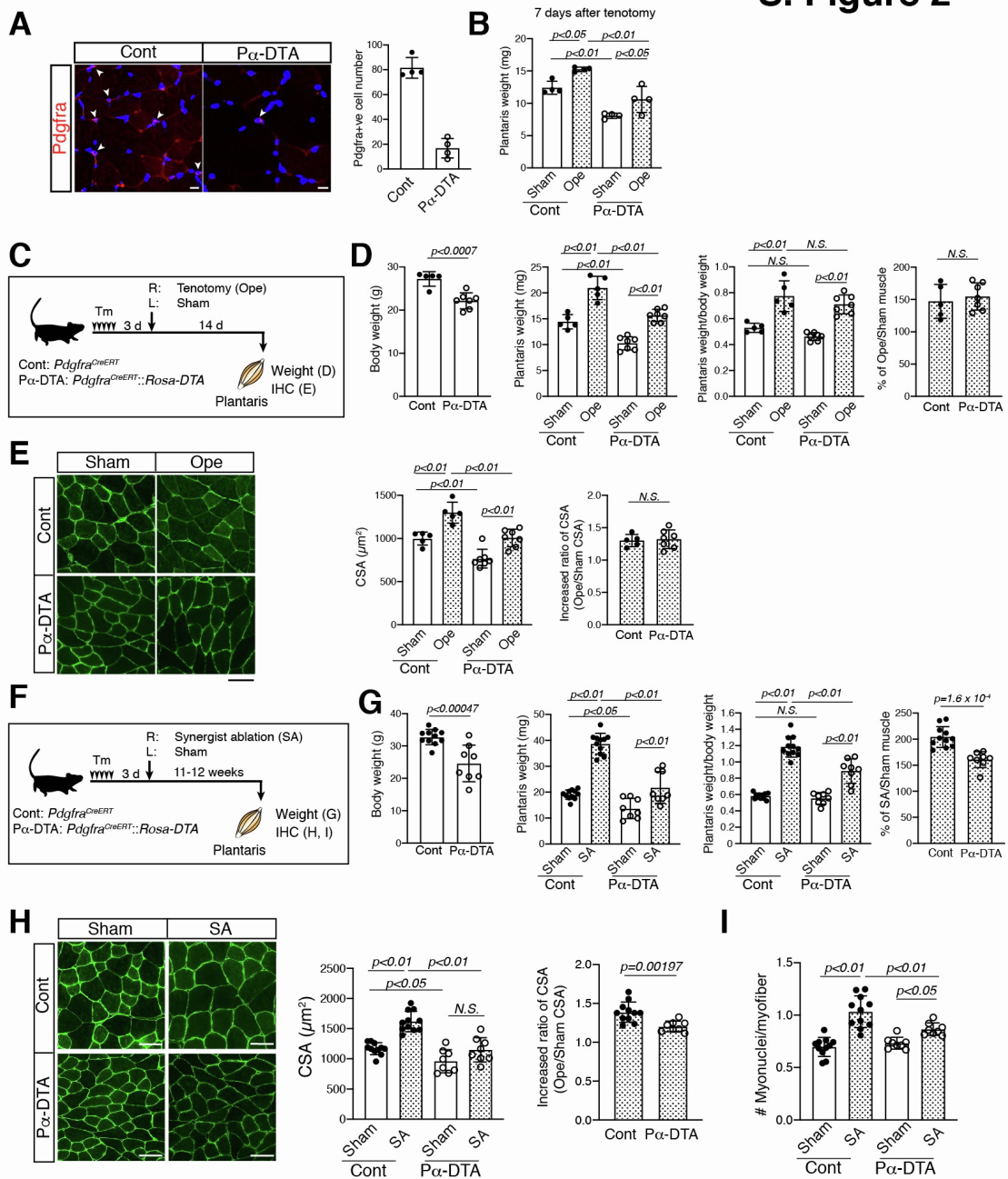


Figure S2. Ablation of Pa cells blunts muscle hypertrophy at 11–12 weeks after synergist ablation (SA); Related to Figure 2

(A) Immunostaining of Pdgfra (red) in plantaris muscles of Cont and Pα-DTA mice. Arrowheads indicate Pdgfra⁺ cells. Scale bar: 10 μm. The graph shows the number of

Pdgfra⁺ cells per section of plantaris muscles of female Cont (n=4) and Pα-DTA (n=4) mice.

(B) Plantaris muscle weight of sham or overloaded muscle on day 7 after tenotomy of female Cont (n=4) and Pα-DTA (n=4) mice.

(C) Experimental scheme for analyzing the effects of Pα⁺ cell-depletion on muscle hypertrophy in plantaris muscle two weeks after tenotomy (Ope; R: right). Contralateral left plantaris muscle was used as sham control (L: Sham).

(D) Body weight, plantaris muscle weight, plantaris muscle weight per body weight, or increased ratio of plantaris muscle weight (Ope/Sham) of male control (n=5) or Pα-DTA mice (n=7)

(E) Representative muscle sections stained with anti-laminin α2 (green) antibody for calculating CSA. The graphs show CSA (left) or increased ratio of CSA (Ope/Sham, right) of male control (n=5) or Pα-DTA (n=7) mice. Scale bar: 50 μm

(F) Experimental scheme for analyzing the effects of Pα⁺ cell-depletion on muscle hypertrophy in plantaris muscle 11–12 weeks after SA (R: SA). Contralateral left plantaris muscle was used as sham control (L: Sham).

(G) Body weight, plantaris muscle weight, plantaris muscle weight per body weight, or increased ratio of plantaris muscle weight (SA/Sham) of male control (n=11) or Pα-DTA (n=8) mice.

(H) Representative muscle sections stained with anti-laminin α2 (green) antibody for calculating CSA. The graphs indicate CSA (left) or increased ratio of CSA (SA/Sham, right) of male control (n=11) mice or Pα-DTA (n=8) mice. Scale bar: 50 μm

(I) Myonuclear number/myofiber in Sham or SA plantaris muscles of male control (n=11) or Pα-DTA (n=8) mice.

S. Figure 3

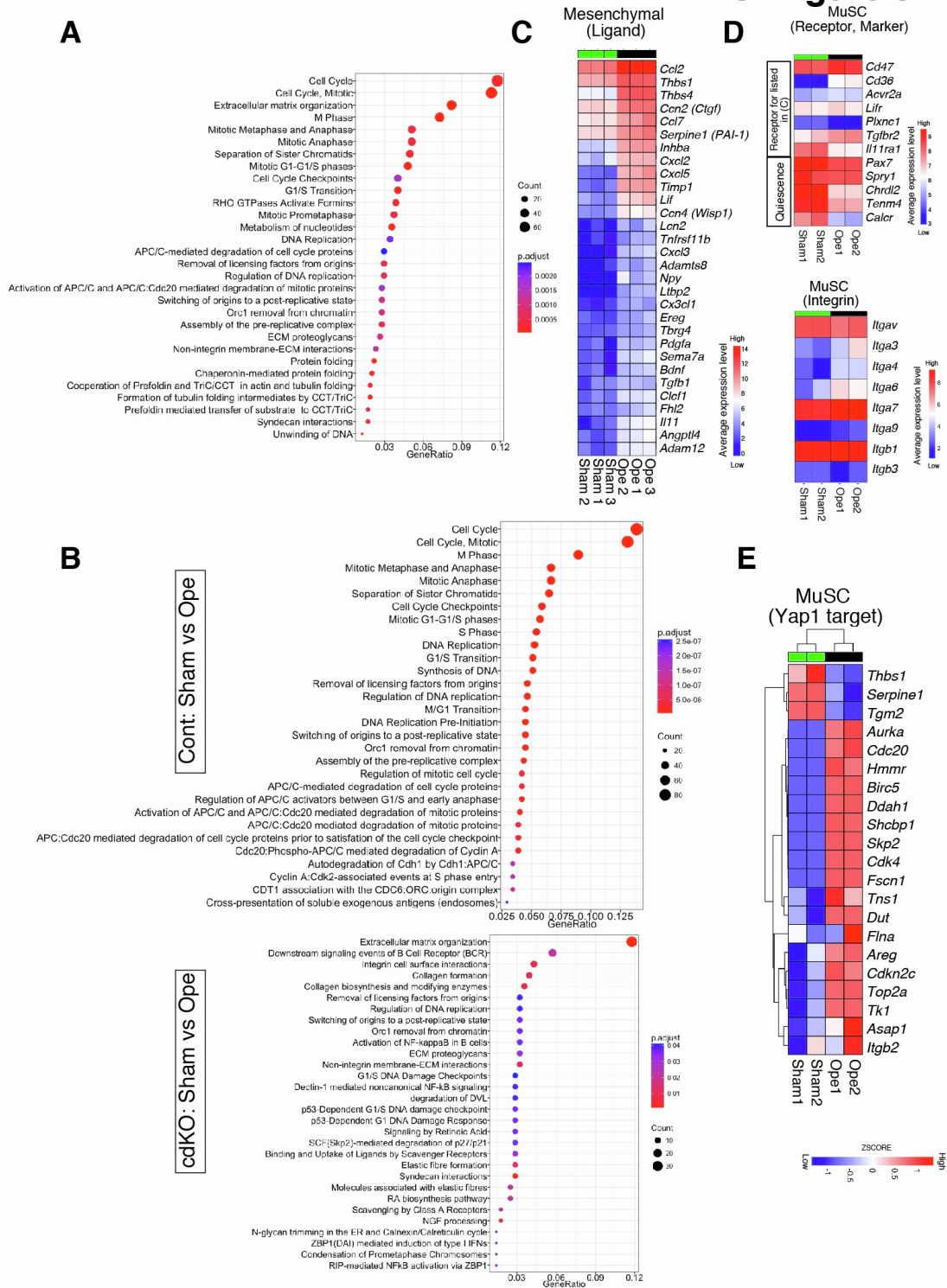


Figure S3. RNA-seq analyses of overloaded mesenchymal progenitors and muscle satellite cells (MuSCs); Related to Figure 3 and 4

(A) Upregulated Biological Process categories ($|\log_2FC| \geq 1$ and $p < 0.05$) in mesenchymal progenitors from overloaded muscle on day 2 after tenotomy compared to those from sham muscle.

(B) Upregulated Biological Process categories ($|\log_2FC| \geq 1$ and $p < 0.05$) in mesenchymal progenitors from overloaded muscle of Cont (upper) or cdKO (lower) mice on day 2 after tenotomy compared to those from sham muscle.

(C) Heatmap of genes encoding secreted proteins that were highly expressed ($|\log_2FC| \geq 2$ and $p < 0.05$) in mesenchymal progenitors from overloaded plantaris muscle. Ope1–3: mesenchymal progenitors derived from overloaded plantaris muscles, Sham1–3: mesenchymal progenitors derived from sham plantaris muscles.

(D) Heatmap of genes encoding receptors related to (C), quiescence genes (*Spry1*, *Chrdl2*, *Tenm4*, *Calcr*), or integrins (*Itga7* and *Itgb1*: positive controls) in MuSCs from overloaded muscle on day 4 after tenotomy (Ope1-2) and sham muscle (Sham1–2). Data deposited with accession number GSE135903 were used in these analyses.

(E) Heatmap of genes involved in Yap1 signature (annotated gene sets in MSigDB) in MuSCs from overloaded muscle on day 4 after tenotomy (Ope1-2) and sham muscle (Sham1–2).

S. Figure 4

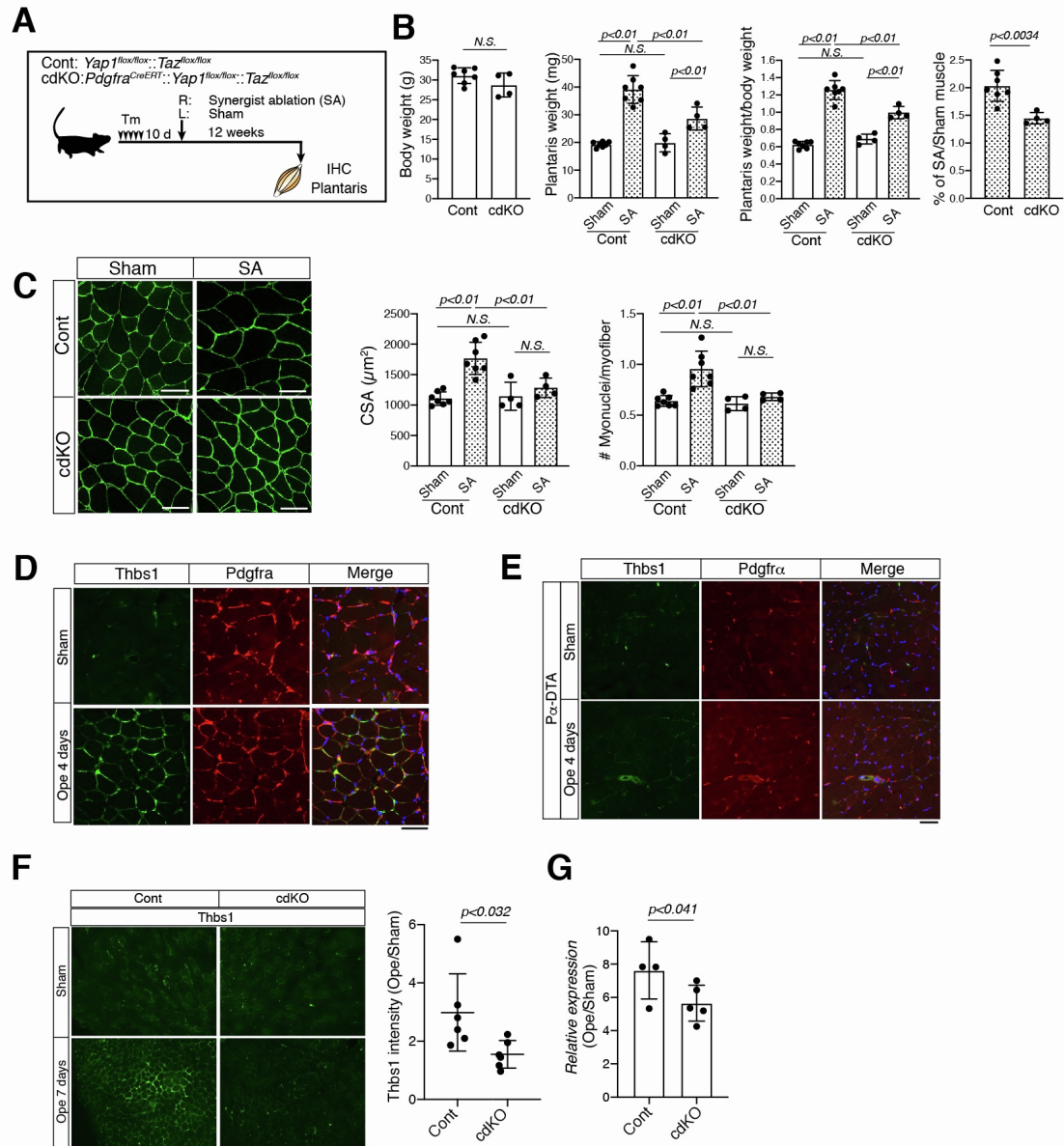


Figure S4. Blunted muscle hypertrophy and reduced expression of Thbs1 in mesenchymal progenitor-specific inactivation of Yap1/Taz; Related to Figure 4 and

(A) Experimental scheme for analyzing effects of *Pdgfra*-specific *Yap1*/*Taz*-depletion on plantaris muscle hypertrophy 12 weeks after SA (R: SA). Contralateral left plantaris muscle was used as sham control (L: Sham).

(B) Body weight, plantaris muscle weight, plantaris muscle weight per body weight, or increased ratio of plantaris muscle weight (SA/Sham) of male control (n=7) or cdKO (n=4) mice.

(C) Representative muscle sections stained with anti-laminin $\alpha 2$ (green) antibody for calculating CSA. The graphs indicate CSA (left) or myonuclear number/myofiber (right) of male control (n=7) or cdKO (n=4) mice. Scale bar: 50 μ m

(D) Immunostaining of *Pdgfra* (red) and *Thbs1* (green) in sections of Sham and overloaded plantaris muscle 4 days after tenotomy (Ope 4d) of control mice (Cont: *Pdgfra*^{CreERT/+}). Scale bar: 50 μ m

(E) Immunostaining of *Pdgfra* (red) and *Thbs1* (green) in sections of sham or overloaded plantaris muscles in $\text{P}\alpha$ -DTA mice on day 4 after tenotomy (Ope 4d). Nuclei were counterstained with DAPI. Scale bar: 50 μ m

(F) Immunostaining of *Thbs1* (green) in sections of sham and overloaded plantaris muscle 7 days after tenotomy (Ope 7d) from control (Cont, *Pdgfra*^{CreERT}) and cdKO mice. Scale bar: 100 μ m. The graph indicates *Thbs1*⁺ area in sham per Ope 7d muscle of Cont (n=6; five male and one female) and cdKO (n=6; four male and two female) mice.

(G) The graph indicates *Thbs1* mRNA expression in Ope 4d muscle per that in Sham muscle from Cont (n=4; two male and two female) or cdKO (n=5; two male and three female).

S. Figure 5

A

Female

Male

Body weight (g)

Heart weight (mg)

Muscle weight (mg)/Body weight (g)

TA/BW

Soleus/BW

PLA/BW

GC/BW

Qu/BW

WT KO

N.S.

p<0.05

p<0.01

p<0.05

p<0.05

p<0.05

p<0.05

p<0.01

N.S.

p<0.01

p<0.01

p<0.05

B

Yap1

DAPI

WT-P α

KO-P α

Cultured P α ⁺

Thbs1

C

Relative proliferation

WT

KO

2 hr

24 hr

48 hr

72 hr

D

Sta-APC only

Anti-CD47 + Sta-APC

SSC

FSC

CD47

Gated YFP+

CD47

0.2%

60.3%

E

Gated YFP+

Sham

Ope 4d

Ope 7d

CD47

56.2%

81.6%

90.5%

CD47-ckO

SSC

FSC

CD47

Gated YFP+

CD47

0.4%

4.5%

in MuSCs; Related to Figure 5 and 6

- (A) Body weight, heart weight or normalized muscle weight of Thbs1-KO mice.
- (B) Immunostaining of Yap1 (left) or Thbs1 (right) in cultured Pdfgr α^+ cells from WT or Thbs1-KO. Nuclei were counterstained with DAPI. Scale bar: 50 μ m
- (C) Proliferation of mesenchymal progenitors *in vitro*. The Y-axis indicates the relative intensity of luminescence with respect to the beginning (2 hours after seeding) of the cultivation.
- (D) CD47 expression in MuSCs of control (*Pax7^{CreERT2}::Cd47^{+/+}::Rosa-YFP*) (upper) or CD47-cKO (*Pax7^{CreERT2}::Cd47^{flx/flx}::Rosa-YFP*) mice (lower). CD47 expression was detected in control MuSC fraction gated R1 (FCS/SSC plot) & R2 (YFP $^+$ fraction), but not in CD47-cKO MuSCs. Other CD47 $^+$ cells (green dots) were detected in both control and CD47-cKO MuSCs, indicating MuSC-specific deletion of CD47 in CD47-cKO mice. Blue dots show MuSC fraction in all panels.
- (E) The histograms show CD47 expression in MuSCs from Sham or loaded plantaris muscle 4 or 7 days after tenotomy of control (*Pax7^{CreERT2}::Cd47^{+/+}::Rosa-YFP*) mice.

S. Figure 6

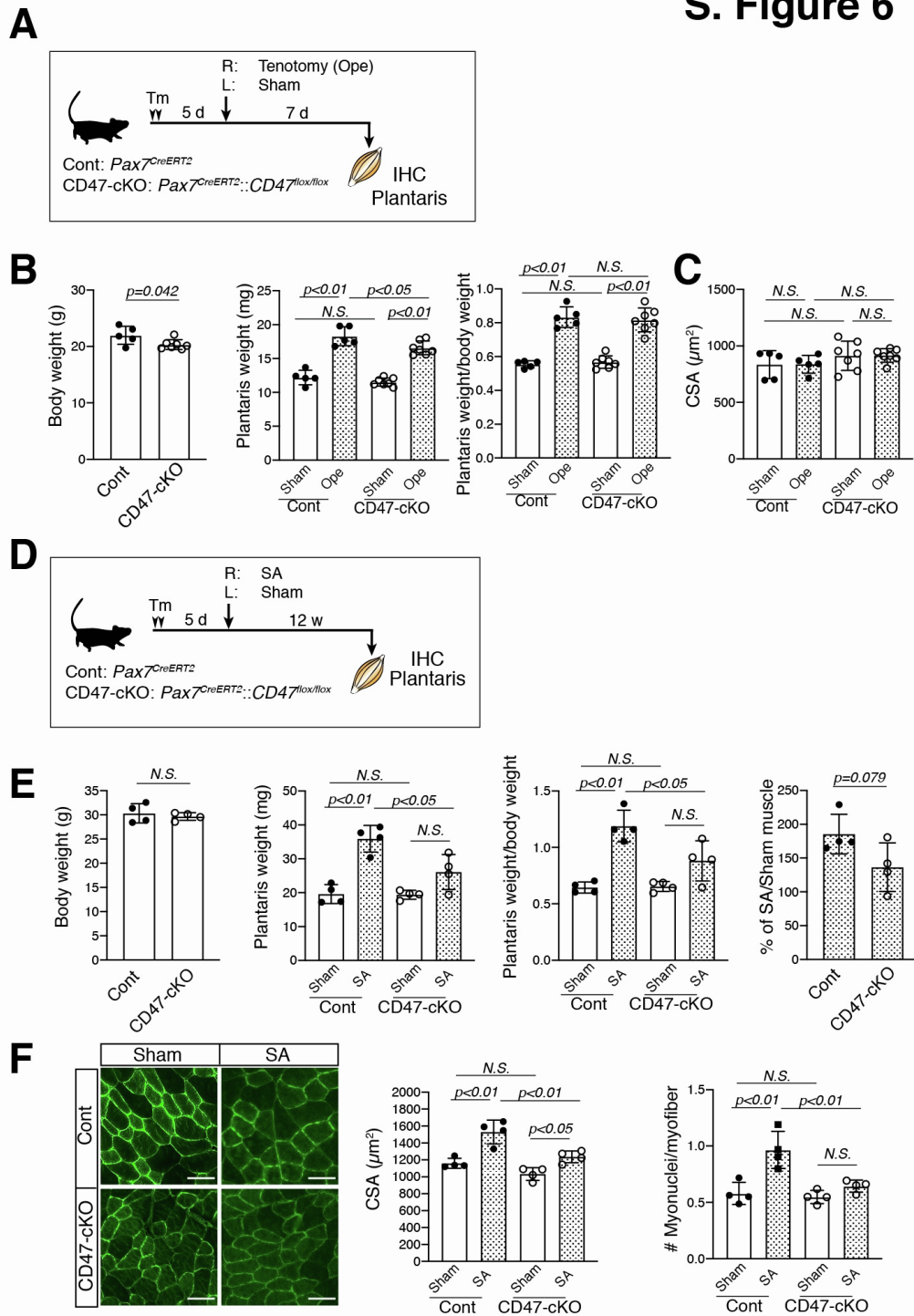


Figure S6. Loss of CD47 in MuSC blunts muscle hypertrophy at 12 weeks after synergist ablation (SA); Related to Figure 6

(A) Experimental scheme for analyzing effects of MuSC specific CD47-depletion on

plantaris muscle hypertrophy 7 days after tenotomy (R: Ope). Contralateral left plantaris muscle was used as sham control (L: Sham).

(B) Body weight (left), plantaris muscle weight (middle), or plantaris muscle weight per body weight (right) of female control (n=5) or CD47-cKO (n=7) mice.

(C) Myofiber size (CSA: cross sectional area) of female control (n=5) or CD47-cKO (n=7) mice.

(D) Experimental scheme for analyzing effects of MuSC specific CD47-depletion on plantaris muscle hypertrophy 12 weeks after SA (R: SA). Contralateral left plantaris muscle was used as sham control (L: Sham).

(E) Body weight, plantaris muscle weight, plantaris muscle weight per body weight, or increased ratio of plantaris muscle weight (SA/Sham) of male control (n=4) or CD47-cKO (n=4) mice.

(F) Representative muscle sections stained with anti-laminin $\alpha 2$ (green) antibody for calculating CSA. Scale bar: 50 μm . The graphs indicate CSA (left) or myonuclear number/myofiber (right) of male control (n=4) or CD47-cKO (n=4) mice.

S. Figure 7

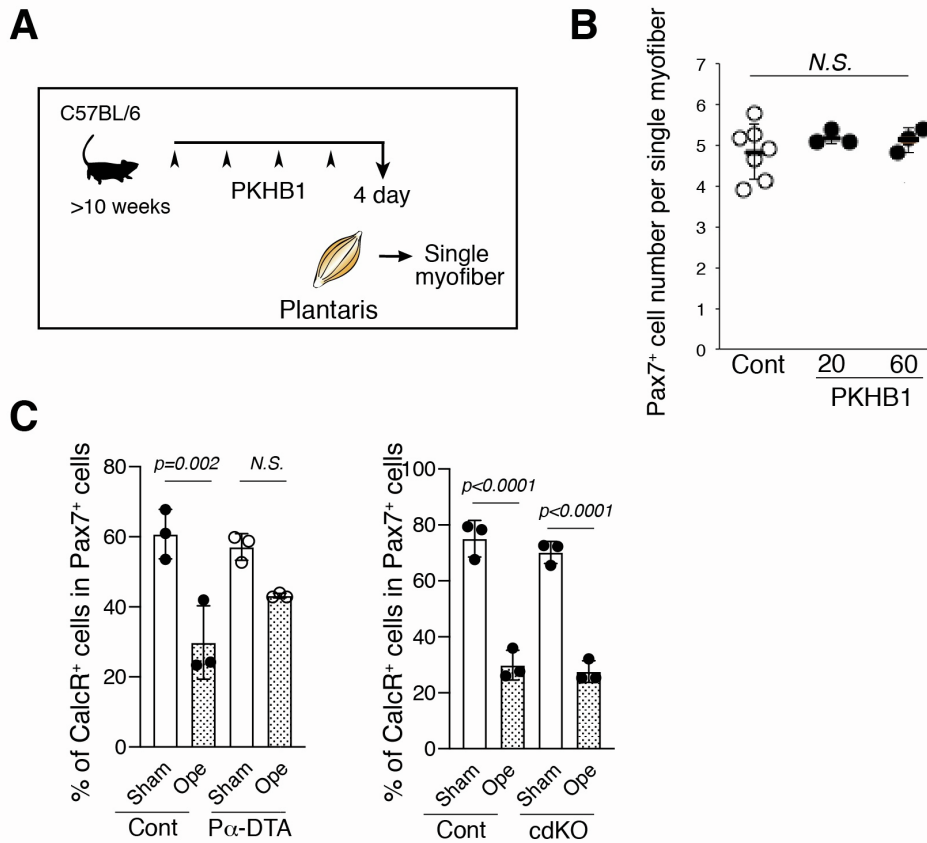


Figure S7. Function of PKHB1 in sedentary C57BL/6 or expression of CalcR in Pα-DTA or Pα-Yap1/Taz -cdKO mice; Related to Figure 7

(A) Experimental scheme for analyzing the effect of CD47 agonist (PKHB1) on myonuclear accretion in sedentary C57BL/6 mice.

(B) The graph indicates the average number of MuSCs on single myofiber of control (PBS-treated, open circle, n=7) and PKHB1-treated mice (20 mg/kg, n=3; 60 mg/kg, n=3). Data are presented as mean ± S.D.; ANOVA. N.S., not significant. 26-30 myofibers were observed per mouse.

(C) Frequency of Pax7⁺CalcR⁺ MuSC in Sham and Ope muscle of Pα-DTA (left, n=3; two male, one female) or cdKO (right, n=3; three male) mice. Rosa-DTA (n=3; two male,

one female) or *Yap1^{flox/flox}Taz^{flox/flox}* mice (n=3; three male) were used for each control.

18-30 myofibers were observed for calculating the frequency of CalcR⁺ cell in Pax7 cells per mouse.

Supplementary Table S1: List of genotyping primers used in this study, related to STAR Methods.

Strain	Primer name	Primer sequence
<i>Pax7-CreERT2</i>	Pax7-CE Fwd	ACT AGG CTC CAC TCT GTC CTT C
	Pax7-CE Rev	GCA GAT GTA GGG ACA TTC CAG TG
<i>Calcr-floxed</i>	Calcr loxp Fwd	CAA CTA TAC TCT GTG CAA CGC
	Calcr loxp Rev	TAA TAC GCT TCA GAA ACC
<i>Rosa-DTA</i>	oIMR8052	GCG AAG AGT TTG TCC TCA ACC
	oIMR8545	AAA GTC GCT CTG AGT TGT TAT
	oIMR8546	GGA GCG GGA GAA ATG GAT ATG
<i>CD47-floxed</i>	CD47-F	AGA TAA GGA GGT CCA CTT CT
	CD47-F4	TGA AGC TCC TCA CTC TCC AGT G
	CD47-R2	TGT TCT CTC TGC TCC AGT GCT TAC
<i>Pdgfra-CreERT</i>	pdgfra ex2 (forward)	TCA GCC TTA AGC TGG GAC AT
	cre (reverse)	ATG TTT AGC TGG CCC AAA TG
<i>Yap1-floxed</i>	Yap1Fwd-29878	AGG ACA GCC AGG ACT ACA CAG
	Yap1Rev-29879	CAC CAG CCT TTA AAT TGA GAA C
<i>Taz-floxed</i>	SWg0005 Taz	GGG CAA AGT TGT GAT GCC CTG GAC
	SWg0006 Taz	CCA ATG GCC TGG ATC TCT TAG GGC
<i>Pdgfra-H2B-eGFP</i>	oIMR7801	CCC TTG TGG TCA TGC CAA AC
	oIMR7802	GCT TTT GCC TCC ATT ACA CTG G
	oIMR7919	ACG AAG TTA TTA GGT CCC TCG AC
<i>Rosa-YFP</i>	oIMR8545	AAA GTC GCT CTG AGT TGT TAT
	oIMR4982	AAG ACC GCG AAG AGT TTG TC
	oIMR8546	GGA GCG GGA GAA ATG GAT ATG
<i>Thbs1-KO</i>	oIMR5186	GAG TTT GCT TGT GGT GAA CGC TCA G
	oIMR5187	AGG GCT ATG TGG AAT TAA TAT CGG
	oIMR5188	TGC TGT CCA TCT GCA CGA GAC TAG