

Figure S1

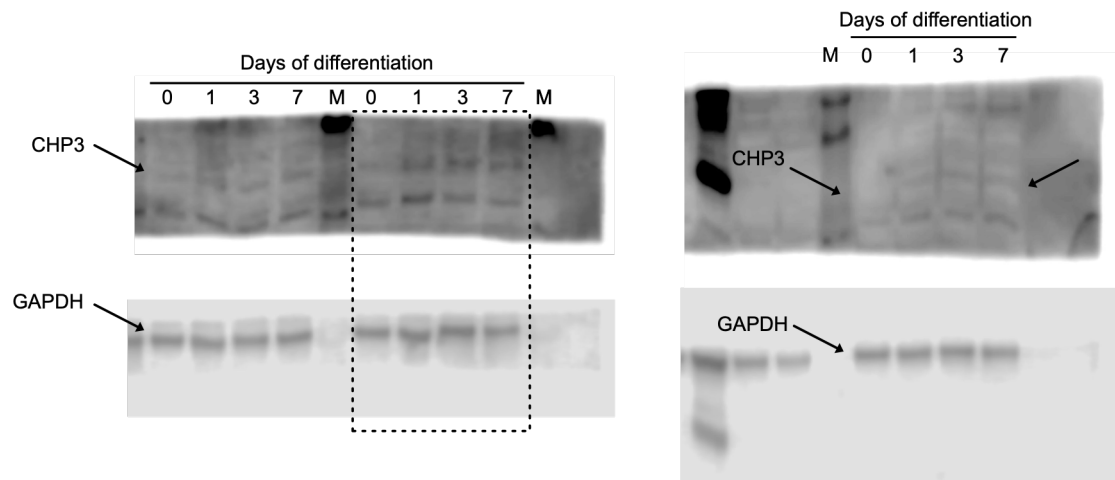


Figure S1 The uncropped Western blot data used in Figure 1A

Protein bands are indicated by arrows. M denotes the molecular weight marker. Bands outlined by dotted lines are shown as representative images.

Figure S2

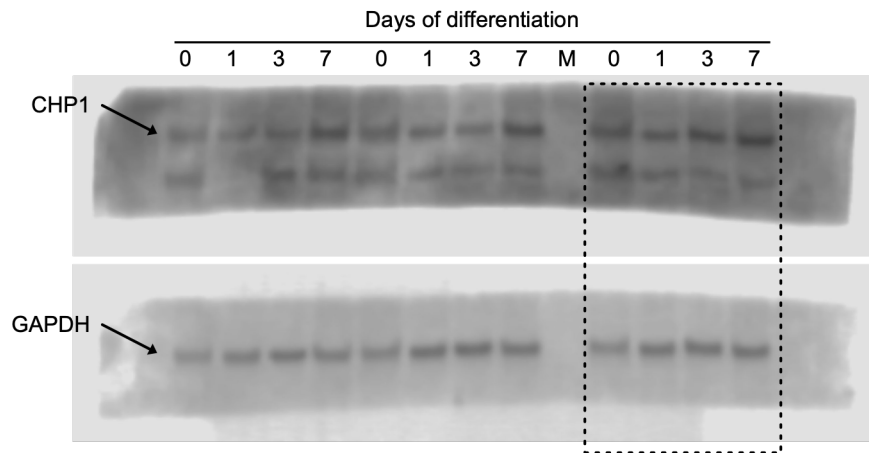


Figure S2 The uncropped Western blot data used in Figure 1C

Protein bands are indicated by arrows. M denotes the molecular weight marker. Bands outlined by dotted lines are shown as representative images.

Figure S3

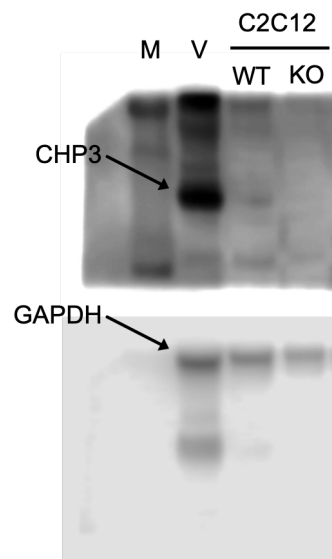


Figure S3 The uncropped Western blot data used in Figure 2A

The CHP3 and GAPDH protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S4

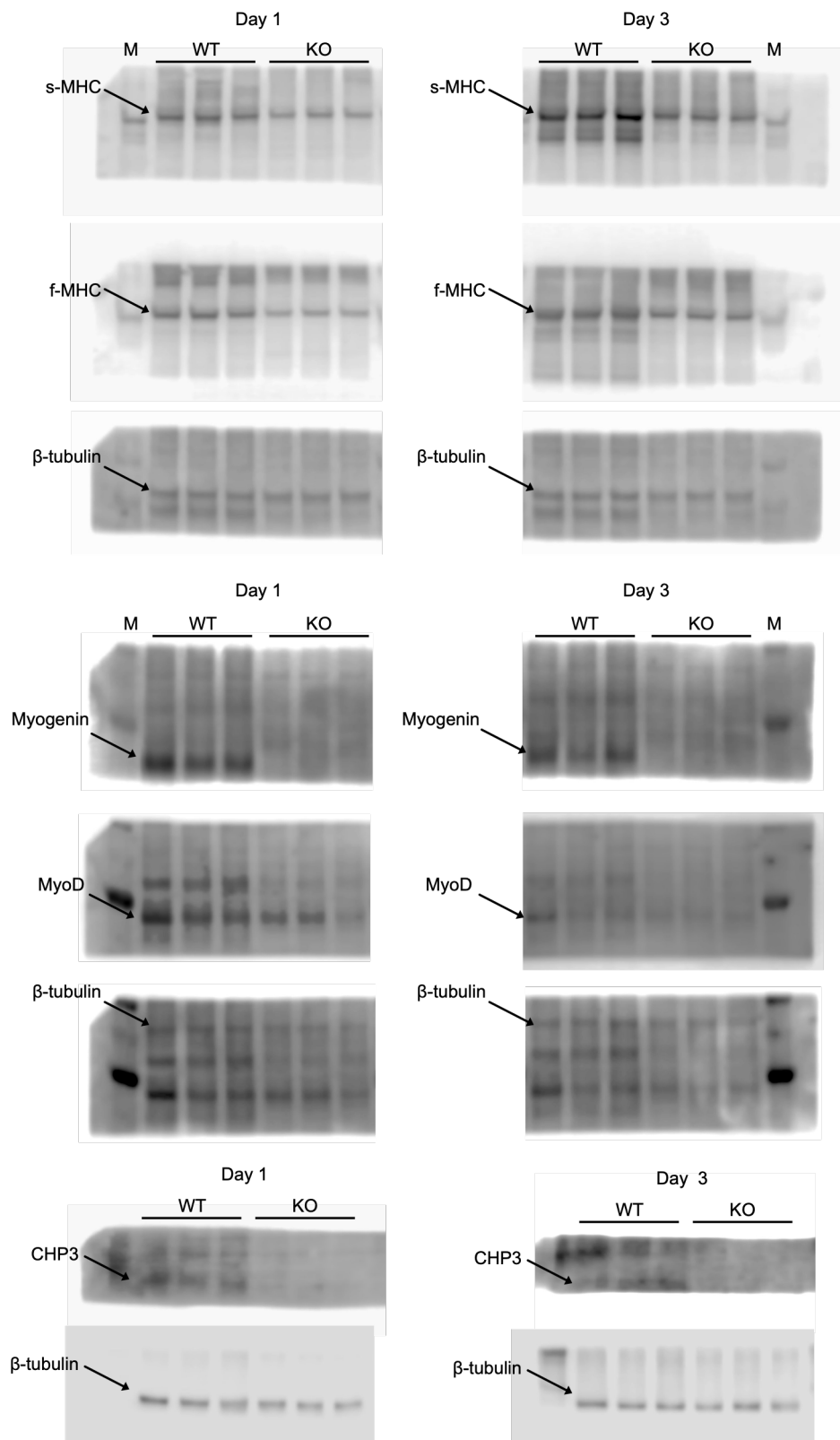


Figure S4 The uncropped Western blot data used in Figure 2B and C

Protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S5

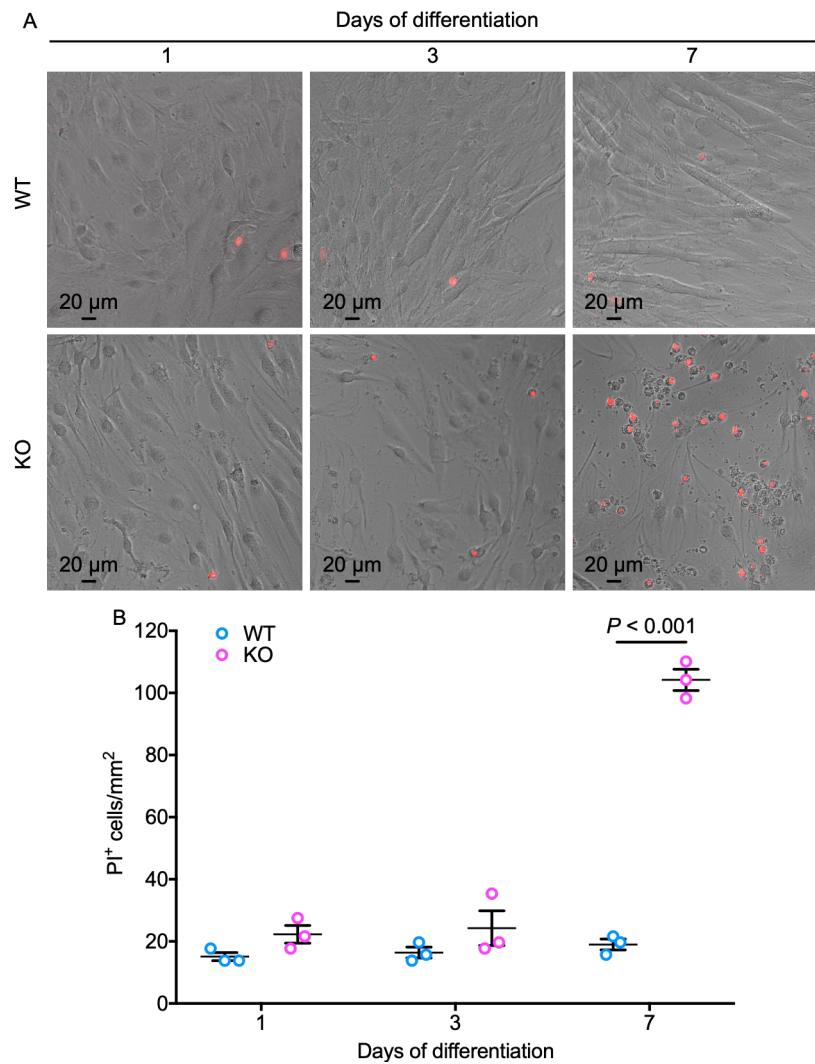


Figure S5 CHP3-KO C2C12 cells exhibit increased propidium iodide (PI)-positive cells on day 7 of differentiation

(A) C2C12 cells were induced to differentiate for 1, 3, and 7 days. PI-positive cells in WT and CHP3-KO C2C12 cells was assessed by fluorescence microscopy. (B) Quantification of PI-positive cells. Statistical significance was determined using Welch's *t*-test (*n* = 3).

Figure S6

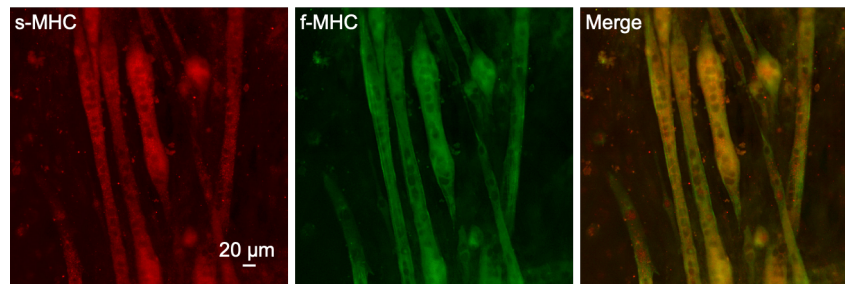


Figure S6 s-MHC-positive differentiated C2C12 cells are also positive for f-MHC

C2C12 cells were induced to differentiate for 3 days. s-MHC and f-MHC were detected using Alexa Fluor 555- and Alexa Fluor 488-conjugated secondary antibodies, respectively.

Figure S7

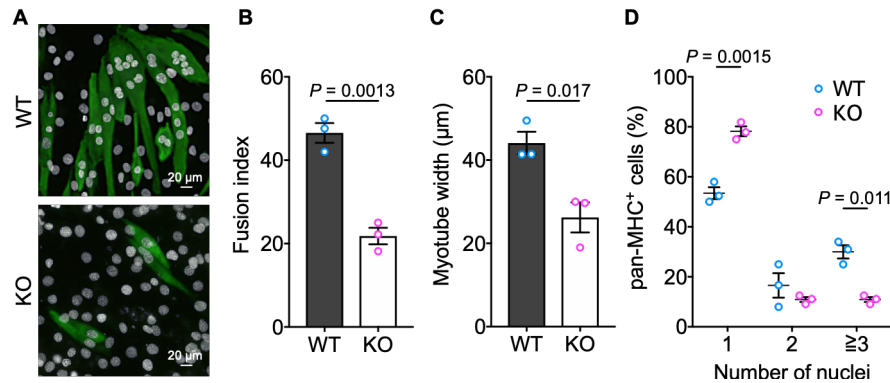


Figure S7 Pan-MHC staining confirms the impaired fusion phenotype in CHP3-KO C2C12 cells

(A) Immunofluorescence analysis of pan-MHC in CHP3-KO C2C12 cells (KO) on day 3 of differentiation. pan-MHC was detected using the pan-MHC antibody MF20 and an Alexa Fluor 555-conjugated secondary antibody. The Alexa Fluor 555 signal is displayed as a green pseudocolor for consistency across the manuscript. (B) Fusion index, (C) myotube width, and (D) number of nuclei per pan-MHC-positive (pan-MHC⁺) cell were quantified. Statistical significance was determined using Welch's *t*-test ($n = 3$).

Figure S8

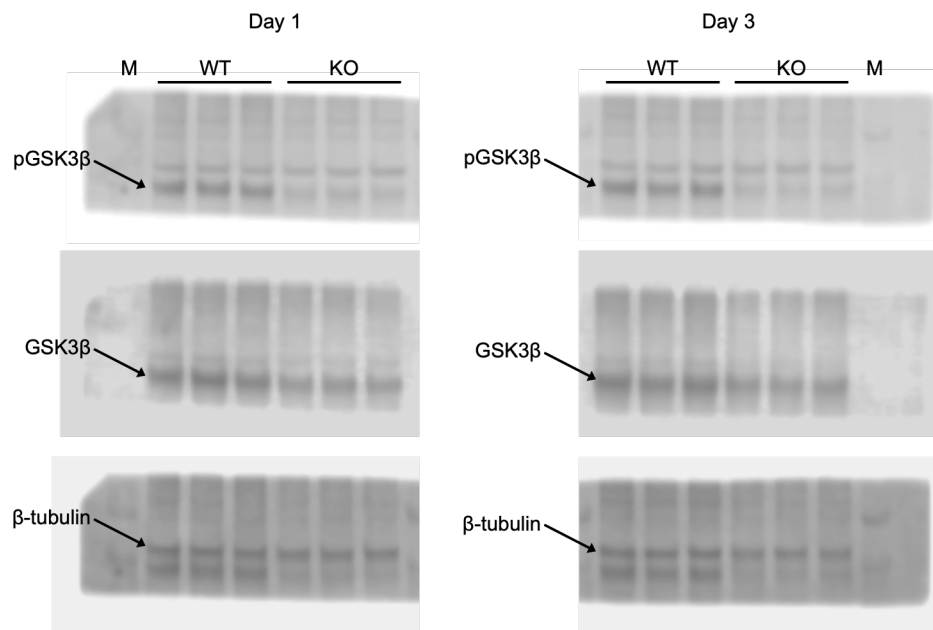


Figure S8 The uncropped Western blot data used in Figure 3A and B

Protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S9

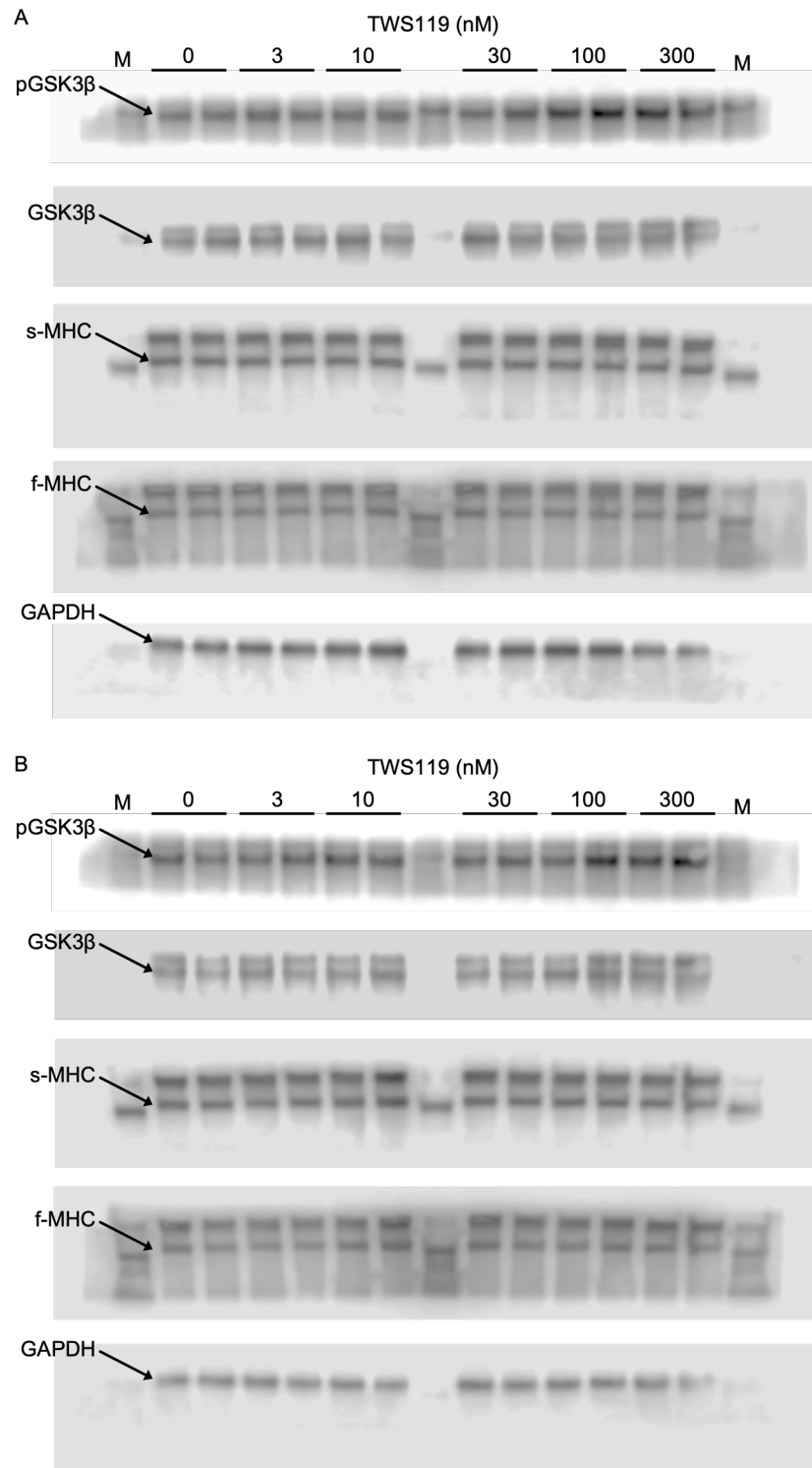


Figure S9 The uncropped Western blot data used in Figure 3C

(A) Representative Western blot images. (B) Additional images used for quantitative analysis. Quantification was performed using data from both (A) and (B). Protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S10

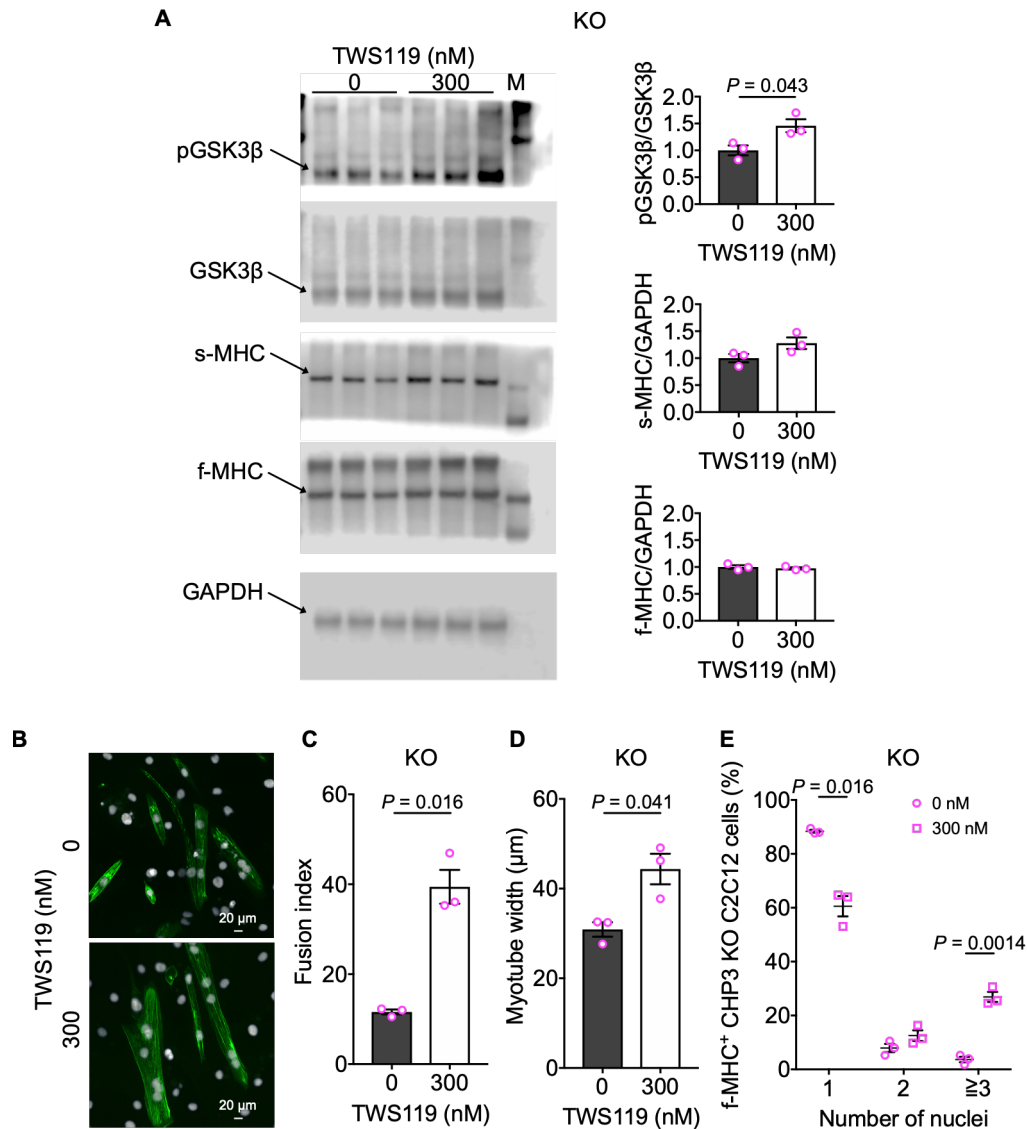


Figure S10 TWS119 improves myogenic fusion and myotube width in CHP3-KO C2C12 cells

(A) CHP3-KO C2C12 cells (KO) were treated with 300 nM TWS119 for 3 days during differentiation. Phosphorylation of GSK3 β at Ser9 and expression of f-MHC and s-MHC were analyzed by Western blotting. pGSK3 β was normalized to total GSK3 β , and MHC levels were normalized to GAPDH. (B) Immunofluorescence analysis of f-MHC in CHP3-KO C2C12 cells treated with 300 nM TWS119 at day 3 of differentiation. f-MHC was detected using an Alexa Fluor 555-conjugated secondary antibody and is displayed as a green pseudocolor for consistency across the manuscript. (C) Fusion index, (D) myotube width, and (E) number of nuclei per f-MHC-positive (f-MHC⁺) cell were quantified. Statistical significance was determined using Welch's *t*-test, with comparisons made against 0 nM TWS119 (n = 3).

Figure S11

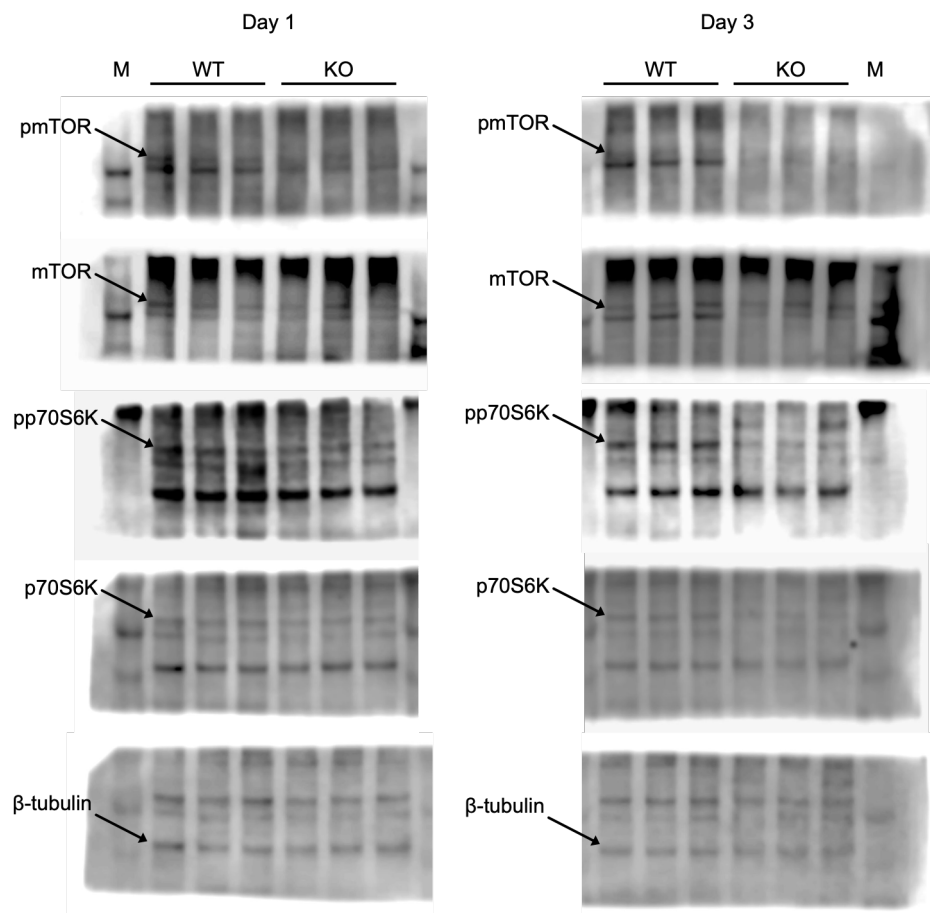


Figure S11 The uncropped Western blot data used in Figure 4A and B

Protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S12

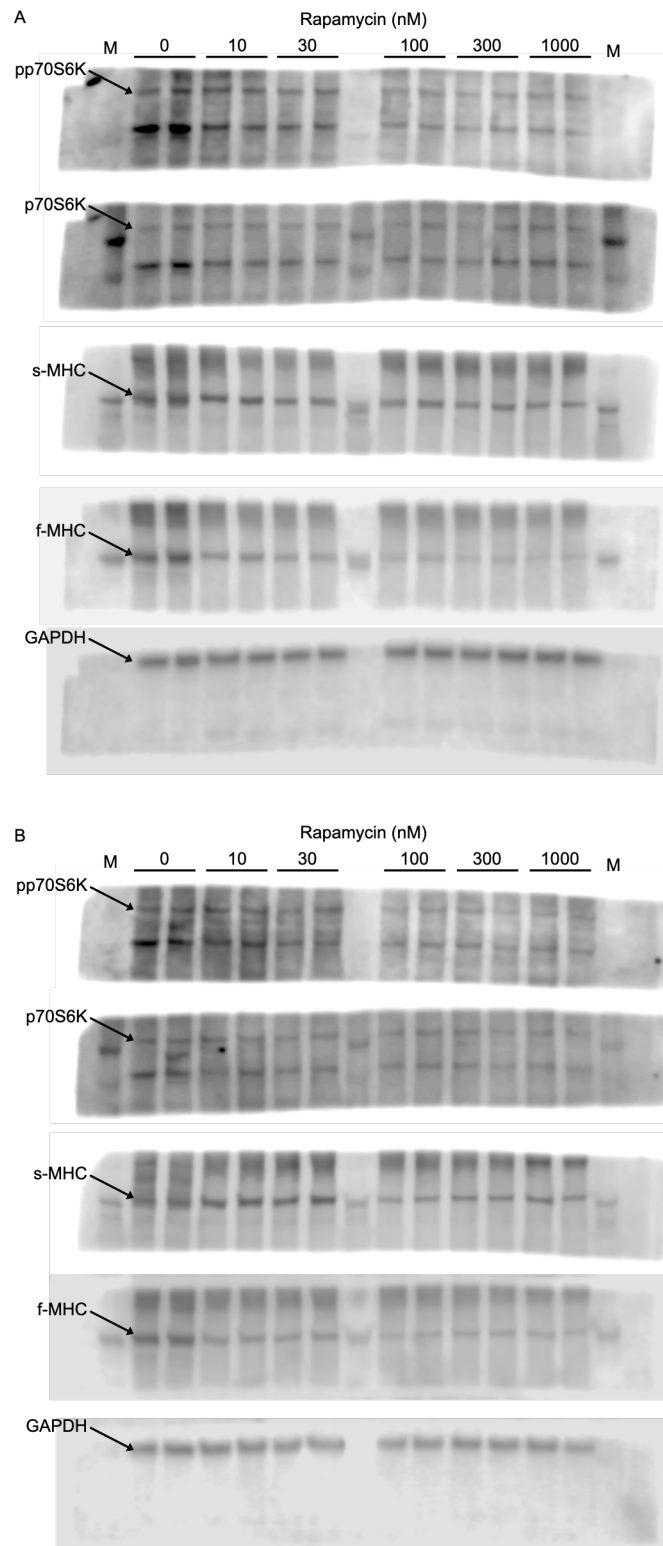


Figure S12 The uncropped Western blot data used in Figure 4C

(A) Representative Western blot images. (B) Additional images used for quantitative analysis. Quantification was performed using data from both (A) and (B). Protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S13

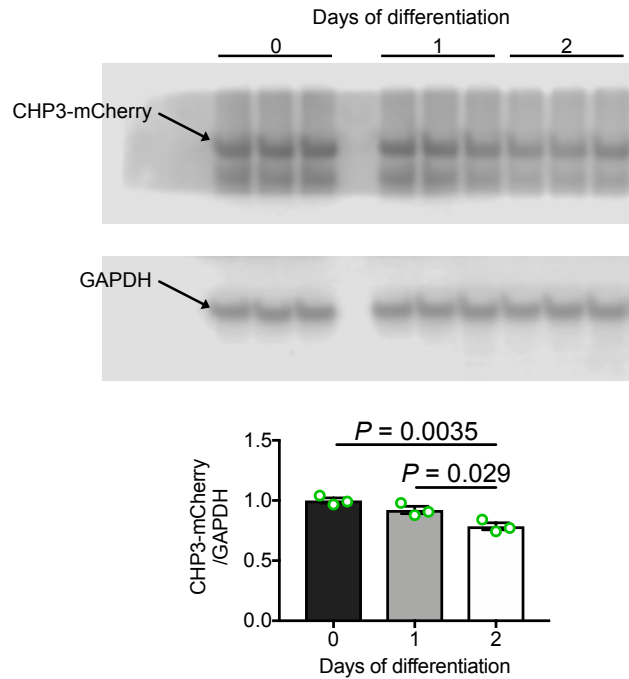


Figure S13 CHP3-mCherry expression declines by day 2 of differentiation

C2C12 cells were transfected with a CHP3-mCherry expression vector and, one day later, switched from growth medium to differentiation medium to induce differentiation. CHP3-mCherry protein levels were analyzed by Western blotting on days 0–2 of differentiation. GAPDH served as a loading control. The CHP3-mCherry and GAPDH protein bands are indicated by arrows. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparisons test against the day 0 ($n = 3$).

Figure S14

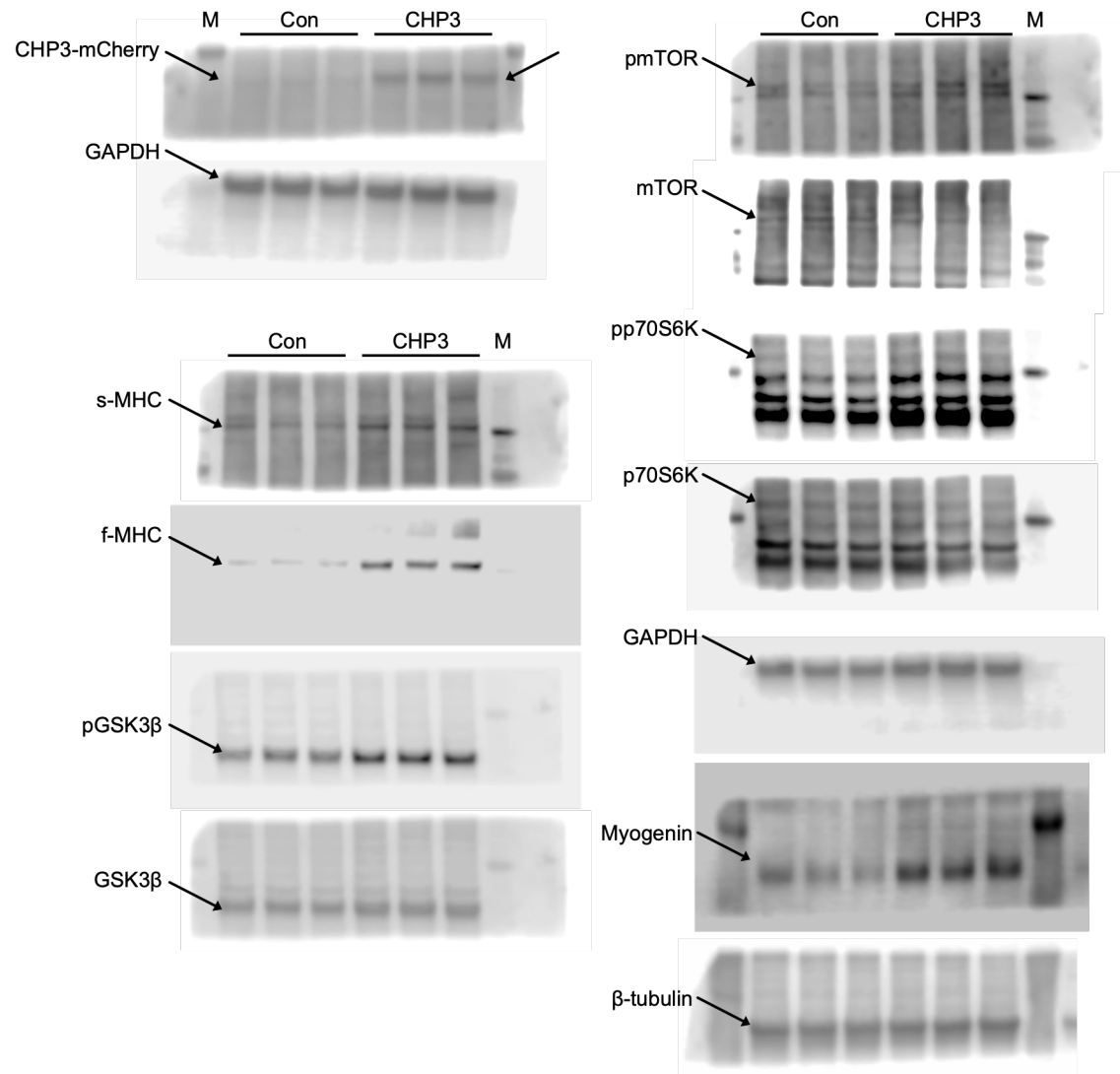


Figure S14 The uncropped Western blot data used in Figure 5A

Protein bands are indicated by arrows. M denotes the molecular weight marker.

Figure S15

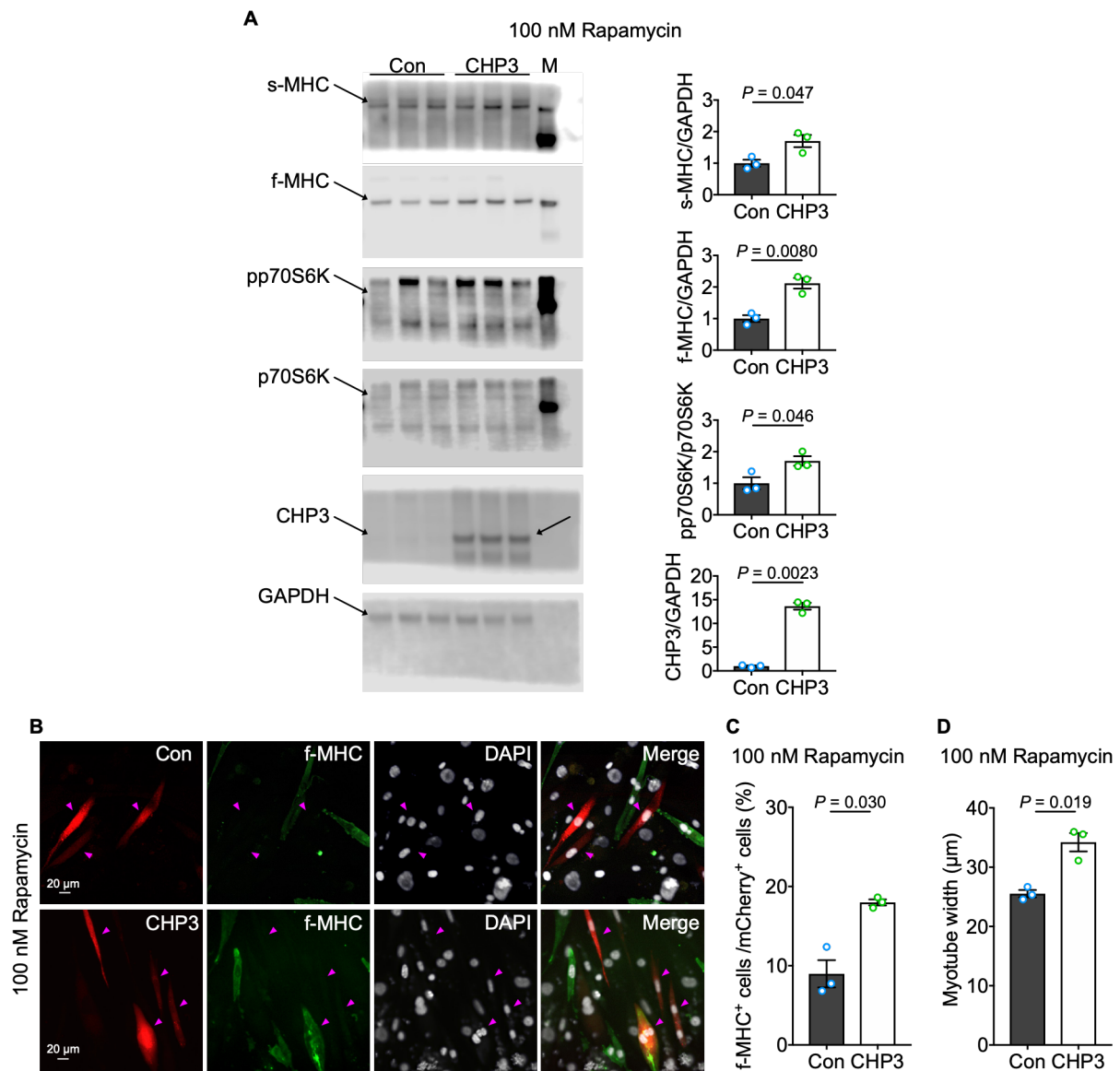


Figure S15 CHP3-mCherry increases C2C12 differentiation and phosphorylation of p70S6K in the presence of rapamycin

(A) CHP3-mCherry cells (CHP3) were differentiated for 2 days. Cells expressing mCherry alone (Con) served as a control. Phosphorylation of p70S6K at Thr389 and expression of f-MHC, s-MHC, and CHP3-mCherry were analyzed by Western blotting. pp70S6K was normalized to total p70S6K, and MHCs and CHP3-mCherry levels were normalized to GAPDH. (B) Immunofluorescence analysis of f-MHC in CHP3-mCherry cells treated with 100 nM rapamycin at day 2 of differentiation. f-MHC was detected using an Alexa Fluor 488-conjugated secondary antibody. (C) The proportion of f-MHC-positive (f-MHC⁺) cells among mCherry-positive (mCherry⁺) cells and (D) myotube width were quantified. Statistical significance was determined using Welch's *t*-test (*n* = 3).

Figure S16

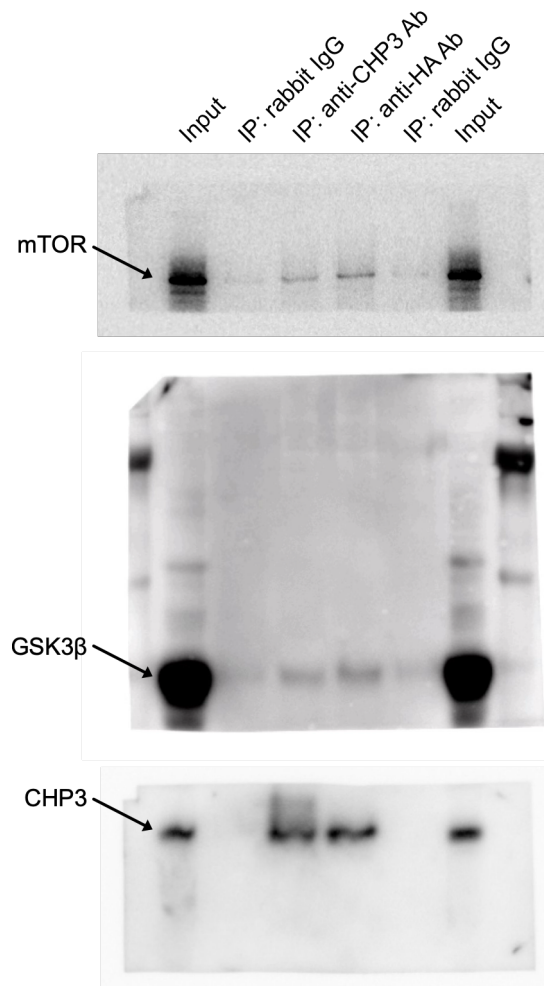


Figure S16 The uncropped Western blot data used in Figure 6A

Protein bands are indicated by arrows.

Figure S17

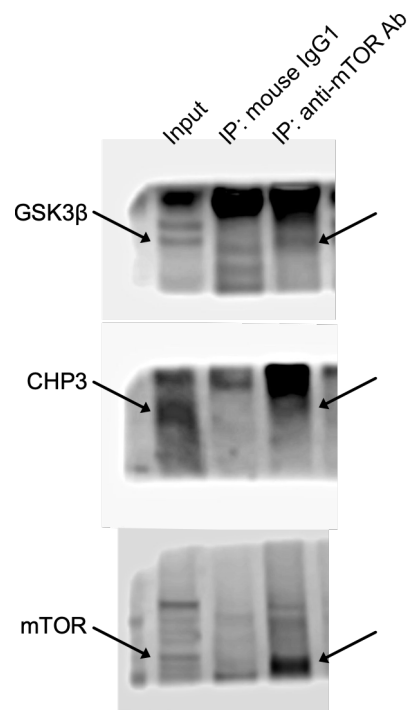


Figure S17 The uncropped Western blot data used in Figure 6B
Protein bands are indicated by arrows.