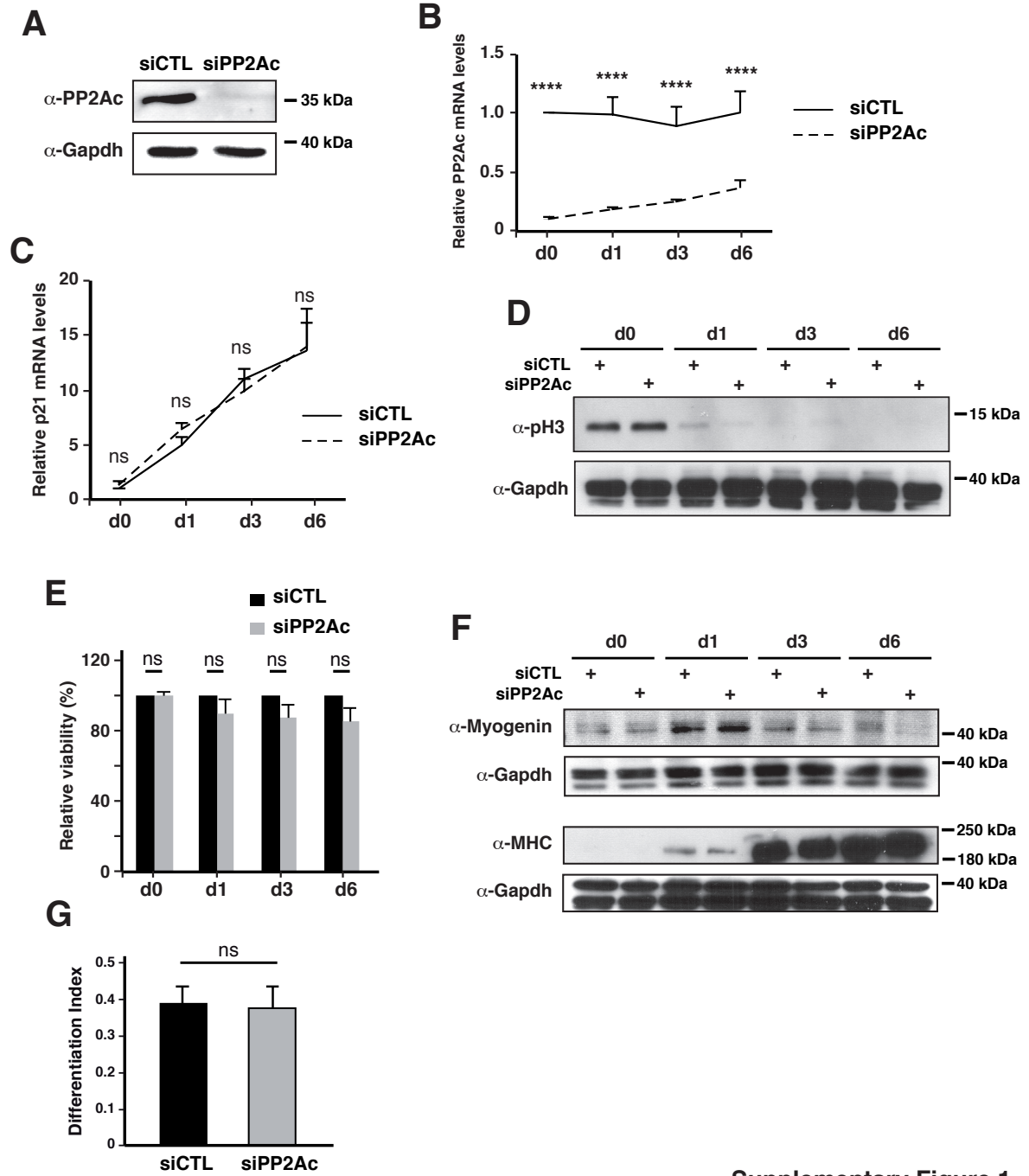


Supplementary Figures and Legends



Supplementary Figure 1

Supplementary Figure 1: Knockdown of PP2A catalytic activity does not impact C2C12 proliferation, viability nor differentiation.

(A) Western blot analysis (representative of 3 independent experiments) of PP2A catalytic subunit (α -PP2Ac) in C2C12 myoblasts transfected with control siRNA (siCTL) or siRNA against the catalytic subunit of PP2A (siPP2Ac) protein levels at day 1 during differentiation. Levels of GAPDH (α -GAPDH) were used as loading control.

(B-C) RT-qPCR analysis of (B) PP2A catalytic subunit and (C) p21 in differentiating C2C12 myoblasts transfected with a control (siCTL) or a PP2Ac (siPP2Ac) siRNA. Values are mean $\pm$ SD from 3 independent experiments. Two-way anova, with Bonferroni correction, **** $P < 0.0001$, ns: not significant.

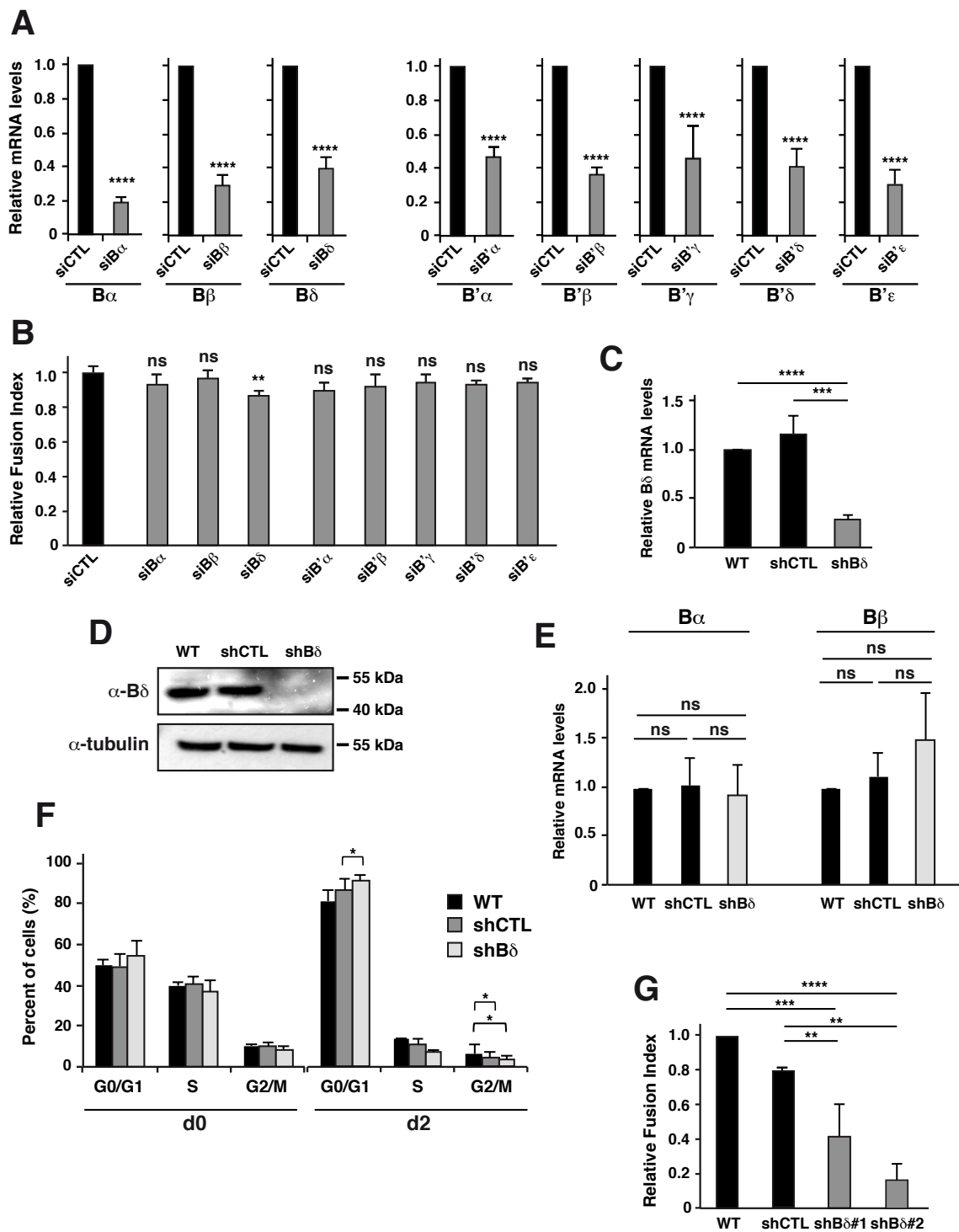
(D) Western blot analysis of phospho-Histone H3 (α -pH3) in differentiating C2C12 myoblasts transfected with a control (siCTL) or a PP2Ac (siPP2Ac) siRNA. Levels of GAPDH (α -GAPDH) were used as loading control. Images are representative of 2 independent experiments.

(E) Relative cell viability levels assessed by MTS assay in differentiating C2C12 myoblasts transfected with a control (siCTL) or a PP2Ac (siPP2Ac) siRNA. Values are mean $\pm$ SD from 4 independent experiments. Two-way anova, with Bonferroni correction, ns: not significant.

(F) Western blot analysis of the early differentiation marker myogenin (α -Myogenin) and the late differentiation marker myosin high chain (MHC), in differentiating C2C12 myoblasts transfected with a control (siCTL) or a PP2Ac (siPP2Ac) siRNA. Levels of

GAPDH (α -GAPDH) were used as loading control. Images are representative of 2 independent experiments.

(G) Differentiation index at day 6 during differentiation (see methods for details) of C2C12 cells transfected with a control (siCTL) or a PP2Ac (siPP2Ac) siRNA. Values are mean $\pm$ SD from 3 independent experiments. Unpaired two-tailed t-test, ns: not significant.



Supplementary Figure 2

Supplementary Figure 2: Knockdown of PP2A-B δ in C2C12 using siRNA and shRNA approaches.

(A) Knockdown efficiencies of siRNA against B- (left panel, siB α , siB β , siB δ) and B'-(right panel, siB' α , siB' β , siB' δ , siB' γ , siB' ϵ) subunits in C2C12 cells, as measured by RT-qPCR and expressed relatively to a control siRNA (siCTL). Values are mean $\pm$ SD from 3 independent experiments. One-way anova, with Tukey's post hoc test. **** $P < 0.0001$.

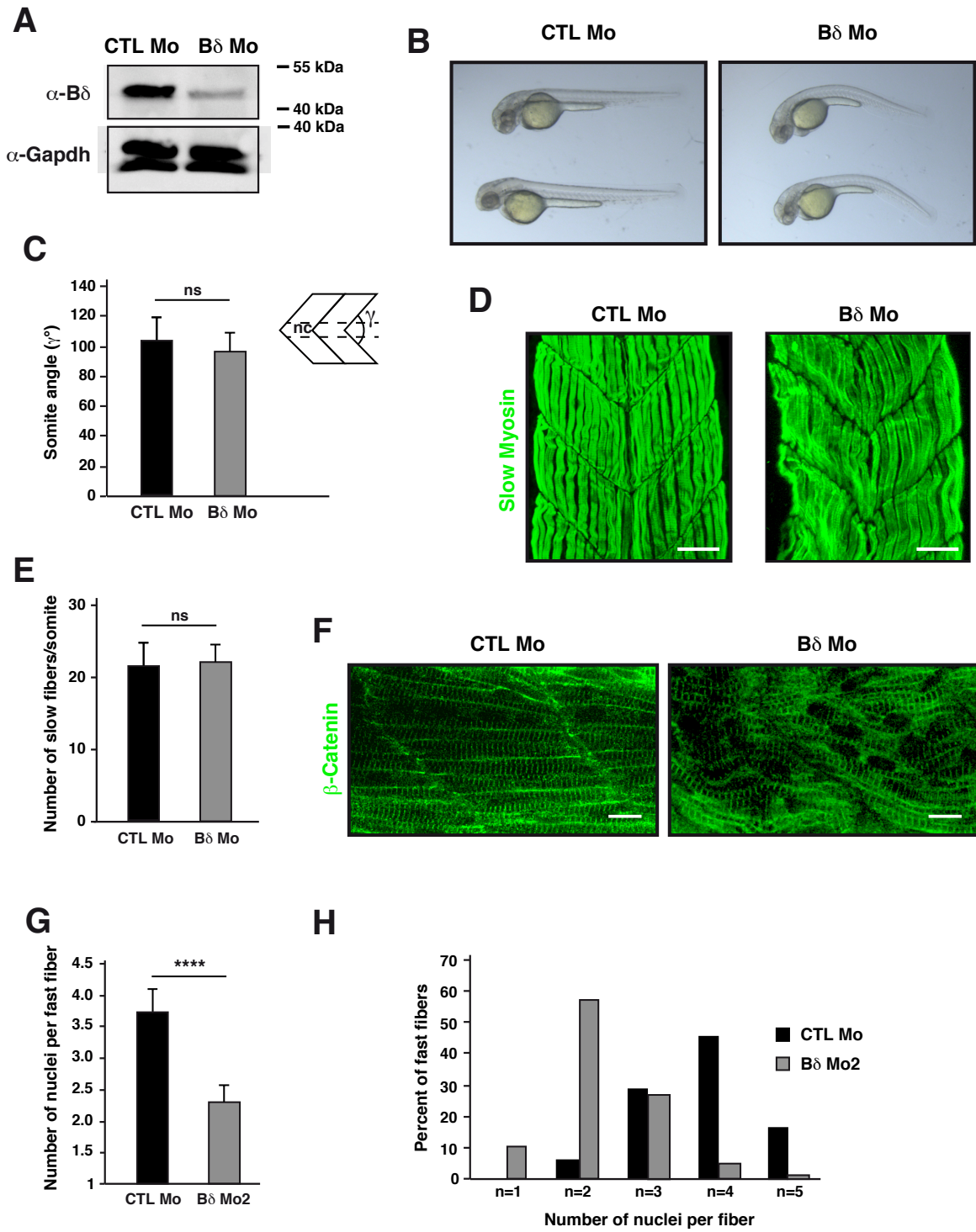
(B) Fusion indexes of C2C12 cells transfected with the indicated siRNA against B- (left panel, siB α , siB β , siB δ) and B'-(right panel, siB' α , siB' β , siB' δ , siB' γ , siB' ϵ) subunits. Results are expressed relatively to the fusion index of C2C12 cells transfected with a control siRNA (siCTL). Values are mean $\pm$ SD from at least 3 independent experiments, one-way anova, with Tukey's post hoc test, ** $P < 0.01$, ns: not significant.

(C-D) (C) mRNA and (D) protein levels of PP2A-B δ in wild-type (WT), control (shCTL) and stable B δ -knocked down (shB δ) C2C12 myoblasts as assessed by qRT-PCR and western blotting, respectively. Tubulin (α -tubulin) was used as loading control for western blot. Values are mean $\pm$ SD from at least 3 independent experiments, one-way anova, with Tukey's post hoc test, **** $P < 0.0001$, *** $P < 0.001$.

(E) QRT-PCR analysis of B α and B β levels in wild-type (WT), control (shCTL) and stable B δ -knocked down (shB δ) C2C12 myoblasts. Values are mean $\pm$ SD from at least 4 independent experiments, one-way anova, with Tukey's post hoc test, ns: not significant.

(F) Cell cycle analysis of wild-type (WT, black bars), control (shCTL, dark grey bars) and stable B δ -knocked down (shB δ , light grey bars) C2C12 myoblasts at day 0 and day 2. Percent of cells in G1, S and G2/M are indicated as mean $\pm$ SD from 3 independent experiments, one-way anova, with Tukey's post hoc test, * $P < 0.05$.

(G) Fusion indexes (see methods for details) of wild-type (WT) C2C12 cells or cells transduced by a control (shCTL), or two independent B δ -specific (shB δ #1 and shB δ #2) shRNA lentiviral vectors. Values are mean $\pm$ SD from 3 independent experiments, one-way anova, with Tukey's post hoc test, *** $P < 0.001$, ** $P < 0.01$.



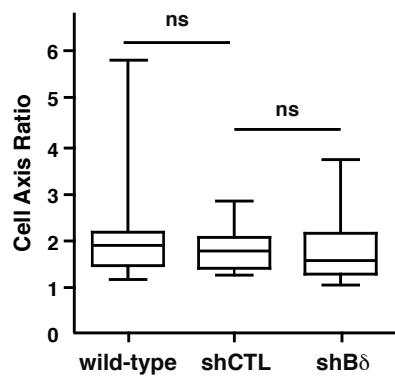
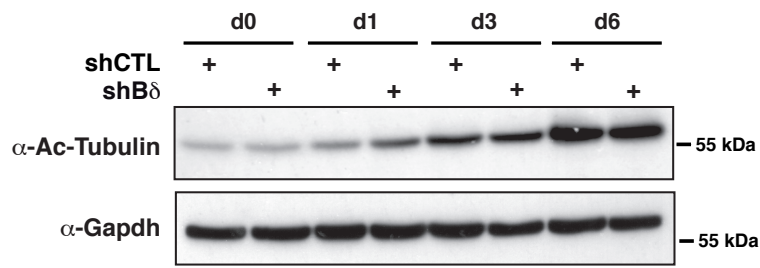
Supplementary Figure 3

Supplementary Figure 3: Knockdown of PP2A-B δ in zebrafish embryos.

(A) Western blot analysis of B δ (α -B δ) in 48 hpf zebrafish embryos injected with control morpholino (CTL Mo) and a morpholino targeting zebrafish B δ orthologue (B δ Mo). GAPDH (α -GAPDH) was used as loading control. Images are representative experiments from 2 independent experiments.

(B-E) **(B)** Bright field pictures representative of at least 10 independent experiments, **(C)** quantification of somite angles (γ ; nc: notochord), **(D)** visualization of slow twitch myotome by confocal microscopy and staining for slow skeletal myosin (green) and **(E)** quantification of slow fibers in each somite of 48 hpf zebrafish embryos injected with control morpholino (CTL Mo, n=8 for (C,D) and n=10 for (E)) or a morpholino against B δ (B δ Mo, n=17 for (C,D) and n=9 for (E)). Unpaired two-tailed t-test, *** $P < 0.001$, ns: not significant.

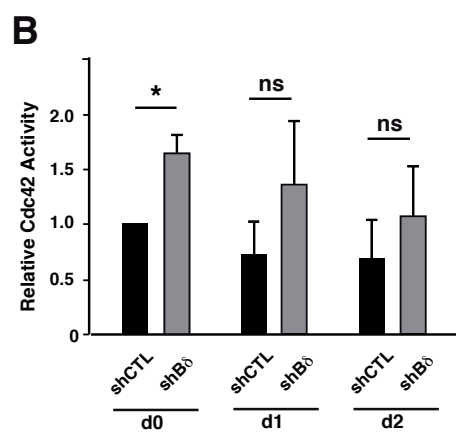
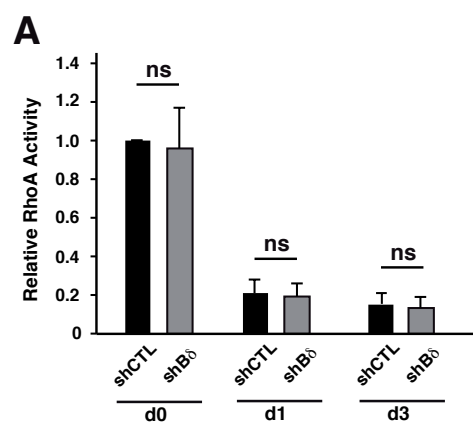
(F-H) **(F)** Confocal pictures of β -catenin (green) highlighting striations, **(G)** quantification of the number of nuclei per fiber and **(H)** proportion of fibers with the indicated number of nuclei in fast myofibers of control (CTL Mo, n=8 for (F) and n=5 for (G,H)) or PP2A-B δ (B δ Mo, n=17 for (F) and n=7 for (G,H)) morphant embryos. Unpaired two-tailed t-test, **** $P < 0.0001$, ns: not significant.

A**B**

Supplementary Figure 4: Knockdown of PP2A-B δ induces morphogenic defects in C2C12 myoblasts.

(A) Major/minor cell axis ratio in wild-type (n=31), control (shCTL, n=16) and B δ -knocked down (shB δ , n=34) C2C12 myoblasts grown in GM. Values are mean $\pm$ SD. Kruskal-Wallis with Dunn's correction. ns: not significant.

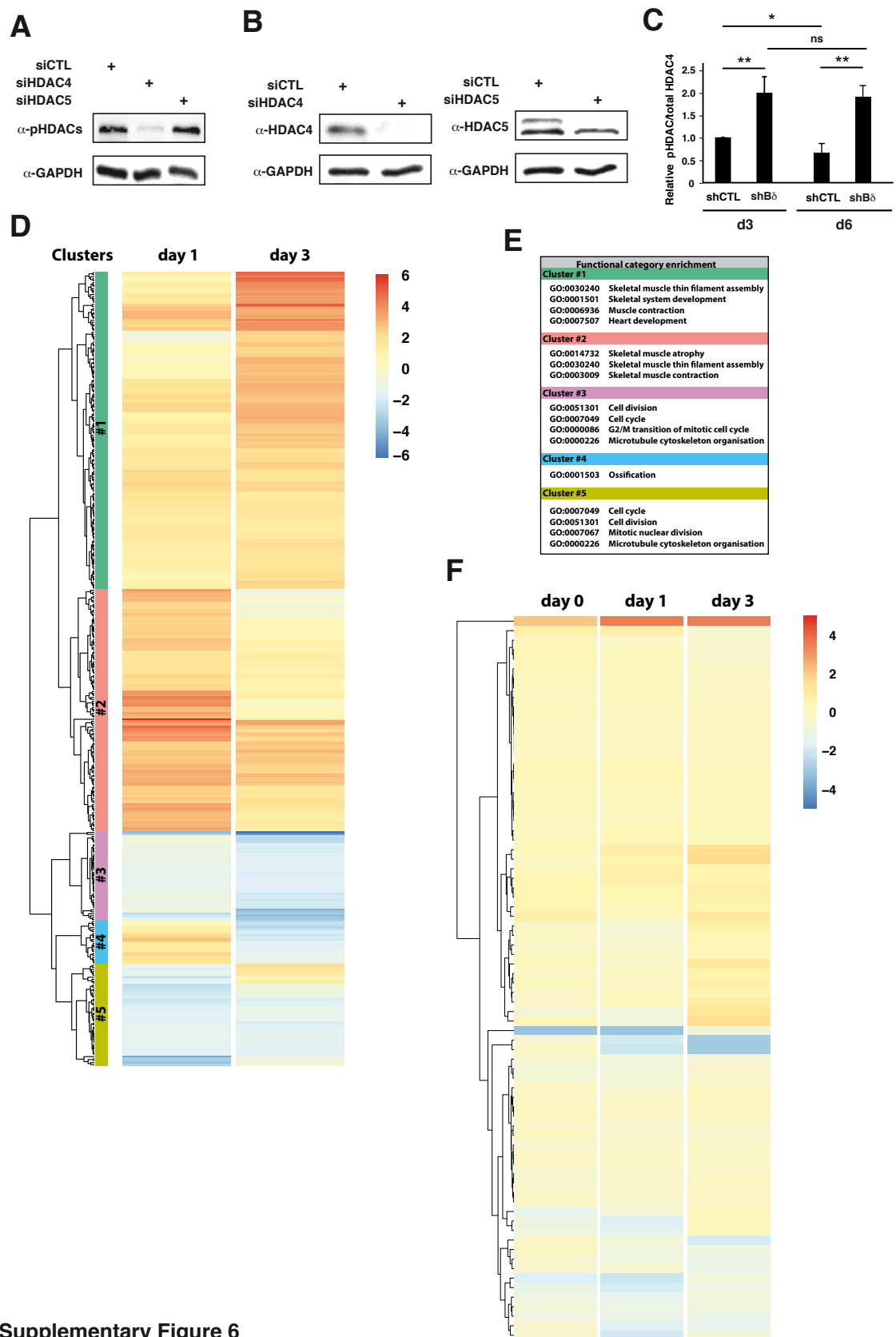
(B) Western blot analysis of acetylated tubulin (α -Ac-Tubulin) in control (shCTL) and B δ -knocked down (shB δ) C2C12 myoblasts at the indicated time points during the differentiation process. GAPDH (α -GAPDH) was used as loading control. Images are representative of 2 independent experiments.



Supplementary Figure 5

Supplementary Figure 5: RhoA and Cdc42 activity in PP2A-Bd KD C2C12.

(A-B) **(A)** RhoA and **(B)** Cdc42 activities were measured by GST pull-down assay in control (shCTL) and B δ -knocked down (shB δ) C2C12 myoblasts at the indicated time points during the differentiation process. Values are mean $\pm$ SD from 3 independent experiments, two-way anova, with Bonferroni correction. , ns: not significant



Supplementary Figure 6

Supplementary Figure 6: Knockdown of PP2A-B δ induces hypophosphorylation of HDAC4 in C2C12 myoblasts.

(A) Analysis of class IIa HDAC phosphorylation by western blotting using a phospho-specific antibody (α -pHDACs) in C2C12 myoblasts transfected with a control (siCTL), anti-HDAC4 (siHDAC4) or anti-HDAC5 (siHDAC5) siRNA. GAPDH (α -GAPDH) was used as loading control.

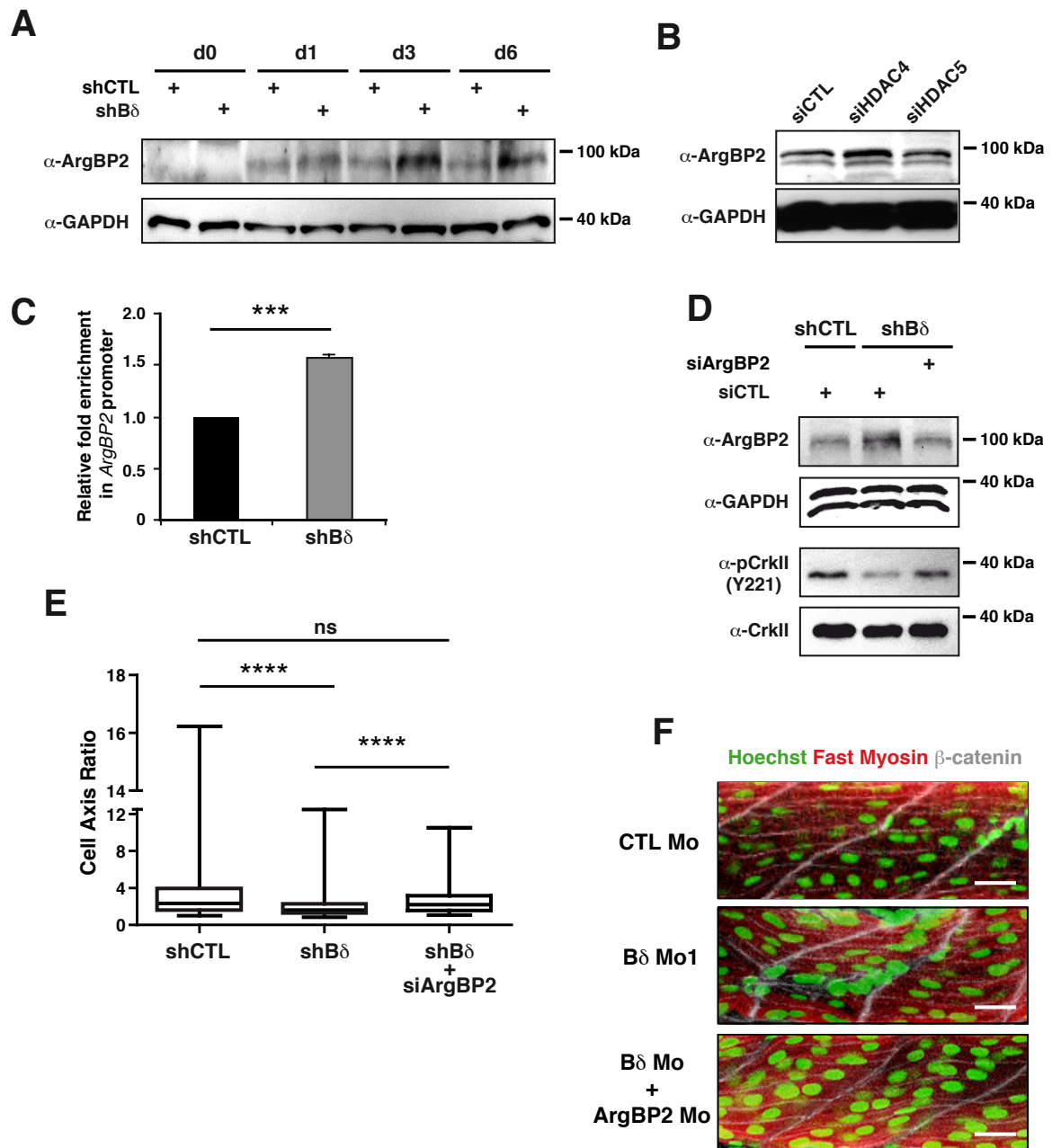
(B) Western blot analysis of (**left panel**) HDAC4 or (**right panel**) HDAC5 using the corresponding antibody (α -HDAC4 and α -HDAC5, respectively) in C2C12 cells transfected with the indicated siRNA. GAPDH (α -GAPDH) was used as loading control.

(C) Quantification of pHDAC4 levels relative to total HDAC4 levels (pHDAC4/HDAC4) in control (shCTL) and B δ -KD (shB δ) C2C12 myoblasts at day 3 and day 6 during differentiation using a phospho-specific antibody against its S246 (α -pHDAC4 (S246)). Total HDAC4 (α -HDAC4) was used as loading control. Results are means from 3 independent experiments.

(D) Hierarchical clustering and heatmap of the expression levels (log₂ normalized changes relative to day 0) of genes in control C2C12 myoblasts after induction of differentiation for the indicated times.

(E) Top significant GO terms (biological processes) in clusters from (D).

(E) Hierarchical clustering and heatmap of the expression levels (log₂ normalized changes in B δ -KD vs control C2C12 myobalsts) of genes annotated as "skeletal muscle differentiation", after induction of differentiation for the indicated times. The list of genes associated with skeletal muscle cell differentiation (GO:0035914), was obtained from Mouse Genome Informatics (<http://www.informatics.jax.org/>)".



Supplementary Figure 7

Supplementary Figure 7: PP2A-B δ controls expression of *ArgBP2* via HDAC4.

(A) Western blot analysis of *ArgBP2* levels in C2C12 myoblasts transfected with a control (siCTL), anti-HDAC4 (siHDAC4) or anti-HDAC5 (siHDAC5) siRNA. GAPDH (α -GAPDH) was used as loading control.

(B) ChIP analysis of MEF2 binding to the *ArgBP2* promoter in control (shCTL) and B δ -KD (shB δ) C2C12 myoblasts at day 3. Immunoprecipitations were realized with a control IgG or an anti-MEF2 antibody. Results are expressed as mean percent of input $\pm$ SD, relative to the control IgG and standardized to shCTL cells, from 3 independent experiments, unpaired two-tailed t-test, *** $P < 0.001$.

(C) Western blot analysis of *ArgBP2* (α -*ArgBP2*) in control (shCTL) and B δ -knocked down (shB δ) C2C12 myoblasts at the indicated time points during the differentiation process. GAPDH (α -GAPDH) was used as loading control. Images are representative of 2 independent experiments.

(D) Analysis of *ArgBP2* (α -*ArgBP2*) and activity of CrkII as assessed by phosphorylation of its inhibitory Y221 (α -pCrkII (Y221)) in control (shCTL) and B δ -knocked down (shB δ) C2C12 myoblasts transfected with a control siRNA or a siRNA against *ArgBP2* (si*ArgBP2*). GAPDH (α -GAPDH) and total CrkII (α -CrkII) were used as loading control.

(E) Major/minor cell axis ratio in control (shCTL) and B δ -knocked down (shB δ) C2C12 myoblasts grown in GM and transfected with a control siRNA or an siRNA against *ArgBP2* (si*ArgBP2*). Results were calculated on >200 cells/experiment from 2 independent experiments, unpaired two-tailed t-test, **** $P < 0.0001$.

(F) Representative confocal pictures of fast skeletal myosin (red) and β -catenin (white) in control (CTL Mo, n=6 independent experiments) or PP2A-B δ (B δ Mo, n=6

independent experiments) morphant embryos, injected with morpholino against ArgBP2 (ArgBP2 Mo, n=10 independent experiments). Nuclei of fast skeletal fibers were stained with Hoechst (green). Scale bars are 100 μ m.