Supplemental material

Parisi et al., http://www.jcb.org/cgi/content/full/jcb.201501053/DC1

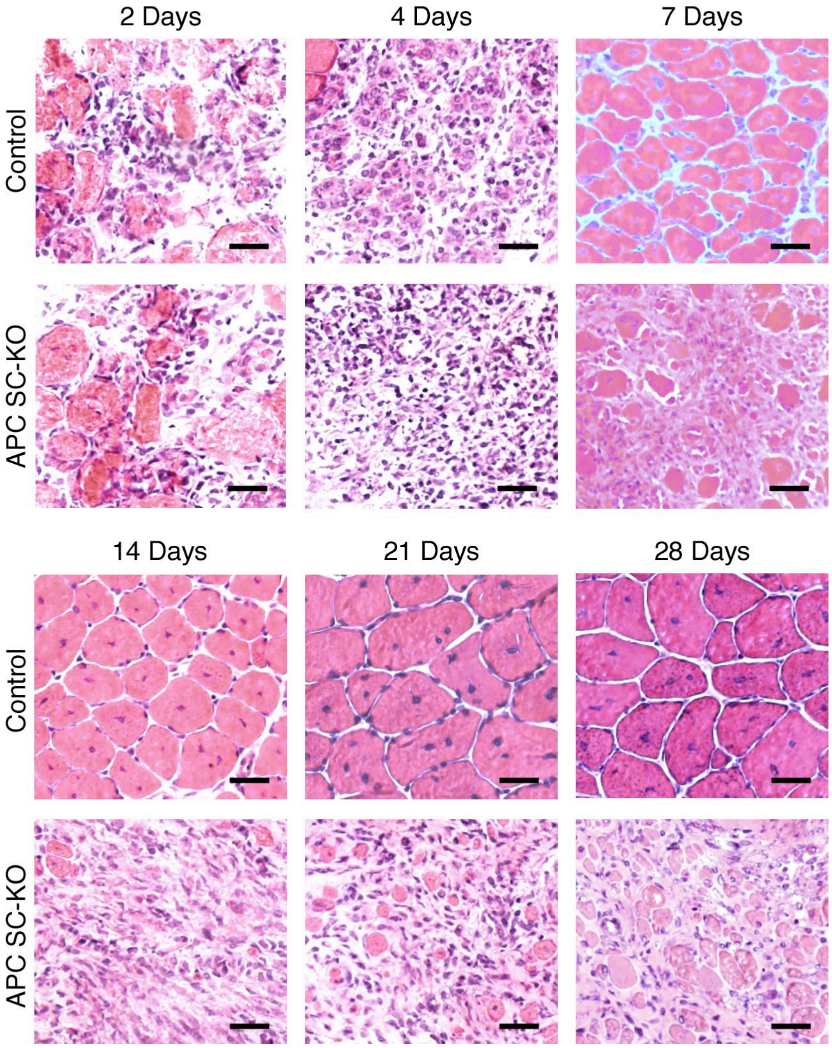


Figure S1. **APC expression by muscle stem cell is required for muscle regeneration, related to Fig. 2.** Hematoxylin and eosin staining of cryosections from control and APC SC-KO muscles sampled at different time point after CTX injection. Control muscles can efficiently regenerate, as shown by the presence of centrally nucleated myofibers, whereas APC SC-KO muscles present complete regeneration failure. Bars, 50 µm.

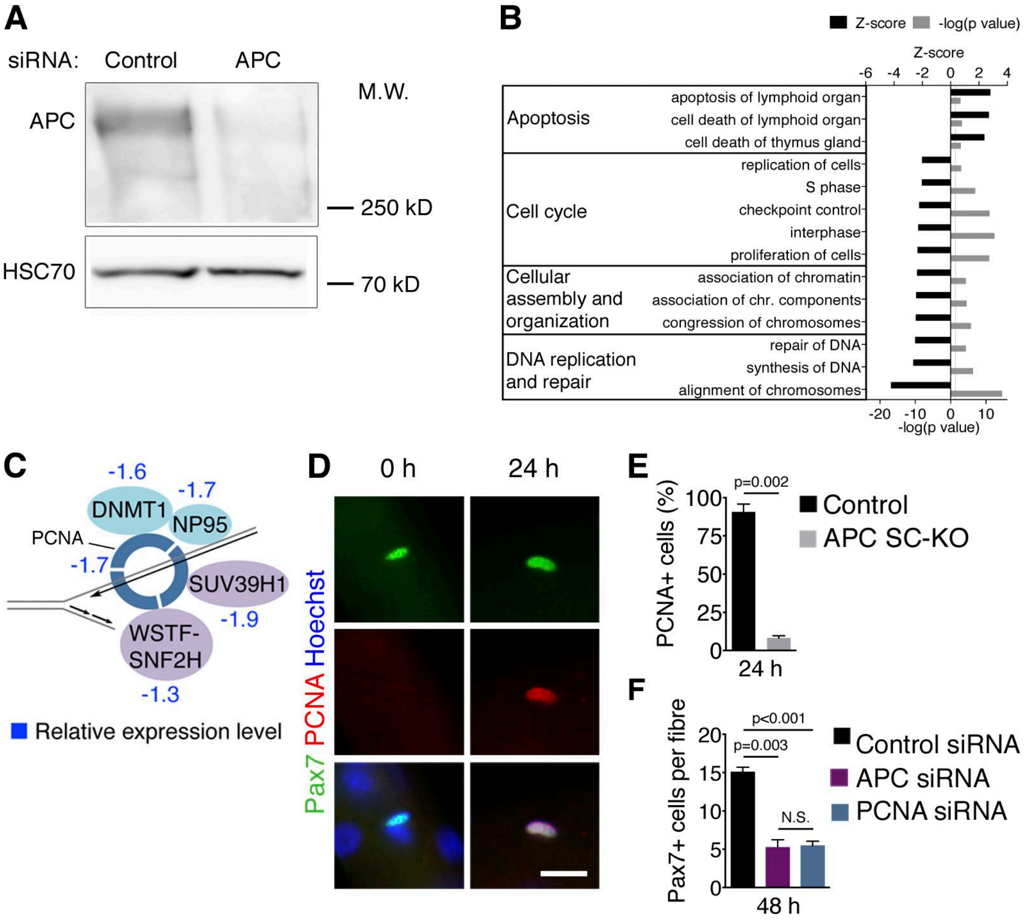
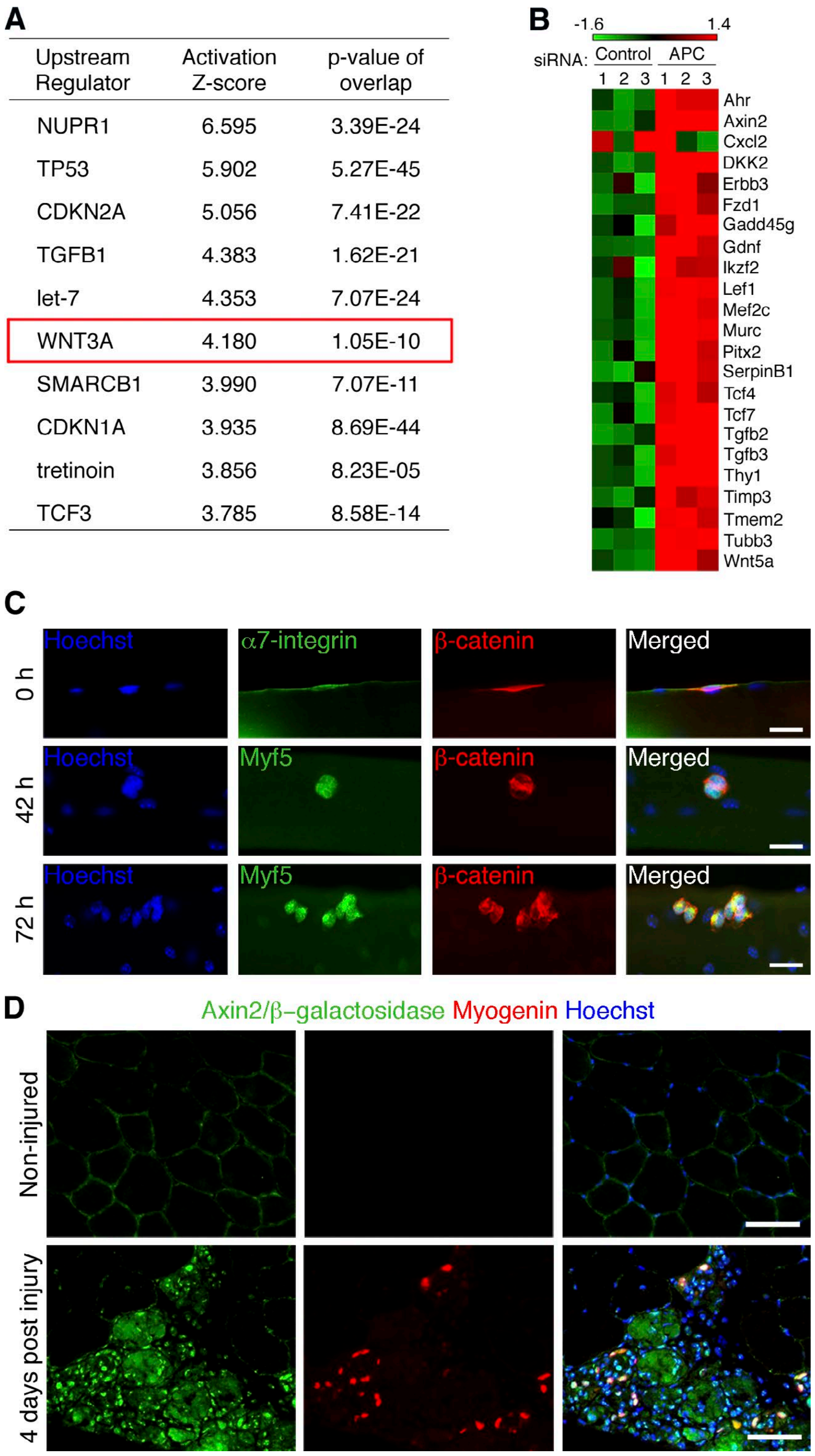


Figure S2. **APC loss affects DNA replication and cell cycle progression, related to Fig. 3.** (A) APC Western blotting shows a decrease in APC protein level on proliferating primary myoblasts transfected with an siRNA targeting APC. (B) Representation of the 14 most relevant biological functions and their categories predicted by microarray analysis to be activated (z-score > 2) or inhibited (z-score < −2) after APC silencing as compared with control proliferating myoblasts. (C) Schematic representation of DNA replication fork and the associated factors. Numbers indicate the fold changes of the expression levels obtained by microarray between APC-silenced and control primary myoblasts. (D) Pax7 and PCNA immunostaining on EDL single myofibers isolated from wild-type animals and directly fixed (0 h) or cultured for 24 h. Nuclei were stained with Hoechst. Bar, 10 µm. (E) Quantification of the percentage of satellite cells expressing PCNA on single fibers isolated from control and APC SC-KO EDL and cultured for 24 h. (F) Quantification of the number of satellite cells per fiber on EDL single fibers transfected with control, APC, or PCNA siRNA and cultured for 48 h. Bars, 50 µm.



Upstream Regulator	Activation Z-score	p-value of overlap
NUPR1	6.595	3.39E-24
TP53	5.902	5.27E-45
CDKN2A	5.056	7.41E-22
TGFB1	4.383	1.62E-21
let-7	4.353	7.07E-24
WNT3A	4.180	1.05E-10
SMARCB1	3.990	7.07E-11
CDKN1A	3.935	8.69E-44
tretinoin	3.856	8.23E-05
TCF3	3.785	8.58E-14

Figure S3. **APC loss activates canonical Wnt signaling, related to Fig. 4.** (A) Table depicting the 10 upstream regulators with highest activation z-score identified by pathway analysis of microarray comparing control and APC-silenced primary myoblasts. (B) Heat map representing the expression level of genes belonging to canonical Wnt pathway (Wnt3a) identified by pathway analysis of microarrays comparing control and APC-silenced primary myoblasts. 1, 2, and 3 indicate three independent experiments. Each row is normalized to have mean zero, variance one. (C) Immunofluorescent staining for α7-integrin, Myf5, and β-catenin on single EDL fibers isolated and fixed (0 h) or cultured for 42 and 72 h. β-Catenin is detected only in the cytoplasm of quiescent satellite cells and accumulates in the nucleus only after 72 h of culture. This indicates that β-catenin subcellular localization is modulated during myogenic lineage progression. Bars, 10 µm. (D) β-galactosidase and Myogenin immunostaining on sections of noninjured TA muscles and injured TA muscles sampled 4 d after CTX injection from Axin2$^{LacZ/+}$ mice, in which the lacZ gene coding for the β-galactosidase protein has been inserted within the Axin2 locus. This enables us to monitor canonical Wnt signaling activity. In noninjured muscles, β-galactosidase is not expressed. Conversely, 4 d after injury, differentiating myocytes that express Myogenin are also positive for β-galactosidase, suggesting that canonical Wnt signaling is only active in differentiating cells and not in quiescent satellite cells. Bars, 50 µm.

Table S1. **Gene ontology (GO) analysis of APC-silenced primary myoblasts**

Category	Term	Count	P-value	Fold enrichment	Bonferroni	Benjamini	FDR
Molecular function	GO:0000166 nucleotide binding	109	2.30×10^{-7}	1.60	1.26×10^{-4}	1.26×10^{-5}	3.36×10^{-4}
	GO:0017076 purine nucleotide binding	101	1.69×10^{-8}	1.73	9.22×10^{-6}	1.02×10^{-6}	2.46×10^{-5}
	GO:0032553 ribonucleotide binding	99	8.73×10^{-9}	1.77	4.77×10^{-6}	6.82×10^{-7}	1.27×10^{-5}
	GO:0032555 purine ribonucleotide binding	99	8.73×10^{-9}	1.77	4.77×10^{-6}	6.82×10^{-7}	1.27×10^{-5}
	GO:0001882 nucleoside binding	91	2.63×10^{-9}	1.87	1.44×10^{-6}	7.20×10^{-7}	3.84×10^{-6}
	GO:0001883 purine nucleoside binding	90	4.25×10^{-9}	1.87	2.33×10^{-6}	7.75×10^{-7}	6.20×10^{-6}
	GO:0030554 adenyl nucleotide binding	88	1.30×10^{-8}	1.84	7.13×10^{-6}	8.92×10^{-7}	1.90×10^{-5}
	GO:0003677 DNA binding	87	1.51×10^{-5}	1.57	8.25×10^{-3}	6.37×10^{-4}	2.21×10^{-2}
	GO:0032559 adenyl ribonucleotide binding	86	5.76×10^{-9}	1.89	3.15×10^{-6}	7.88×10^{-7}	8.40×10^{-6}
	GO:0005524 ATP binding	85	7.31×10^{-9}	1.89	4.00×10^{-6}	6.66×10^{-7}	1.07×10^{-5}
Cellular component	GO:0043228 non-membrane-bounded organelle	160	2.94×10^{-33}	2.59	1.03×10^{-30}	3.44×10^{-31}	4.02×10^{-30}
	GO:0043232 intracellular non-membrane-bounded organelle	160	2.94×10^{-33}	2.59	1.03×10^{-30}	3.44×10^{-31}	4.02×10^{-30}
	GO:0005856 cytoskeleton	95	7.81×10^{-19}	2.63	2.74×10^{-16}	3.05×10^{-17}	1.07×10^{-15}
	GO:0005694 chromosome	78	9.00×10^{-41}	6.40	3.16×10^{-38}	3.16×10^{-38}	1.23×10^{-37}
	GO:0044430 cytoskeletal part	70	5.06×10^{-15}	2.81	1.79×10^{-12}	1.28×10^{-13}	6.98×10^{-12}
	GO:0044427 chromosomal part	69	2.41×10^{-37}	6.73	8.46×10^{-35}	4.23×10^{-35}	3.30×10^{-34}
	GO:0015630 microtubule cytoskeleton	48	7.06×10^{-13}	3.31	2.48×10^{-10}	1.55×10^{-11}	9.66×10^{-10}
	GO:0000775 chromosome. centromeric region	42	4.50×10^{-33}	11.74	1.58×10^{-30}	3.95×10^{-31}	6.15×10^{-30}
	GO:0000793 condensed chromosome	33	9.69×10^{-23}	9.57	3.40×10^{-20}	6.81×10^{-21}	1.33×10^{-19}
	GO:0043292 contractile fiber	28	9.93×10^{-19}	9.14	3.49×10^{-16}	3.49×10^{-17}	1.36×10^{-15}
Biological process	GO:0007049 cell cycle	111	1.46×10^{-50}	5.35	3.20×10^{-47}	3.20×10^{-47}	2.53×10^{-47}
	GO:0022402 cell cycle process	85	3.19×10^{-44}	6.38	6.99×10^{-41}	1.75×10^{-41}	5.54×10^{-41}
	GO:0022403 cell cycle phase	81	1.10×10^{-46}	7.28	2.41×10^{-43}	8.02×10^{-44}	1.91×10^{-43}
	GO:0000279 M phase	77	1.30×10^{-47}	8.02	2.85×10^{-44}	1.42×10^{-44}	2.26×10^{-44}
	GO:0051301 cell division	73	1.53×10^{-43}	7.66	3.35×10^{-40}	5.58×10^{-41}	2.65×10^{-40}
	GO:0000278 mitotic cell cycle	68	1.31×10^{-42}	8.21	2.87×10^{-39}	4.11×10^{-40}	2.28×10^{-39}
	GO:0000087 M phase of mitotic cell cycle	63	7.62×10^{-44}	9.57	1.67×10^{-40}	3.34×10^{-41}	1.32×10^{-40}
	GO:0007067 mitosis	61	4.33×10^{-42}	9.46	9.50×10^{-39}	1.19×10^{-39}	7.53×10^{-39}
	GO:0000280 nuclear division	61	4.33×10^{-42}	9.46	9.50×10^{-39}	1.19×10^{-39}	7.53×10^{-39}
	GO:0048285 organelle fission	61	4.67×10^{-41}	9.13	1.03×10^{-37}	1.14×10^{-38}	8.12×10^{-38}

FDR, false discovery rate.

Table S2 lists the genes that exhibit changes in expression after APC silencing, and is available as a PDF.