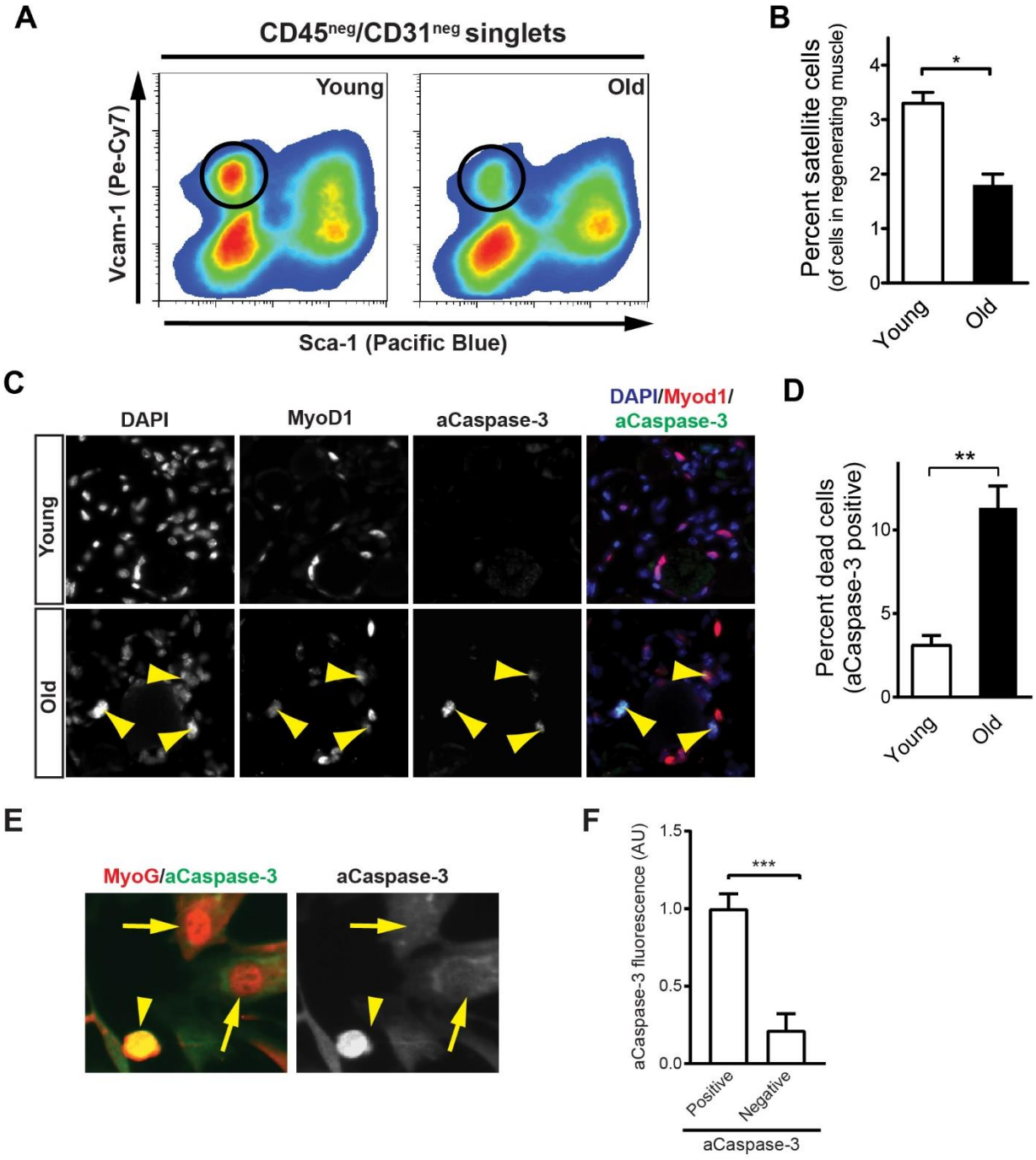

Figure S1. Impaired self-renewal and increased cell death of aged MuSCs, related to Figure 1.
(A) Representative FACS plot depicting surface marker expression in mononuclear cells of injured muscle. The $CD31^{neg}/CD45^{neg}$ population (dark blue) contains $Vcam^{pos}/Sca\text{-}1^{neg}$ MuSCs (orange) and $Sca\text{-}1^{pos}$ fibro-adipogenic progenitors (light blue). (B) Quantification of the number of activated MuSCs in young and old mice 60 hours after injury. The percentage of MuSCs in the total mononucleated cell population was plotted (n=4 for each age). (C) Representative IF images of activated Caspase-3 (aCaspase-3) in activated, Myod1-expressing MuSCs on cross section of the TA muscles from young and old mice 3 days after injury. (D) Quantification of aCaspase-3 staining among Myod1-expressing, activated MuSCs in young and old mice (n = 3 per age). (E) Representative IF images of aCaspase-3 staining in differentiated myocytes expressing Myogenin (MyoG; arrow) and dead cells (arrow head). (F) Relative fluorescent intensity of aCaspase staining in positive and negative cells. Error bars represent SEM. *: $p < 0.05$; **: $p<0.01$; ***: $p < 0.001$.

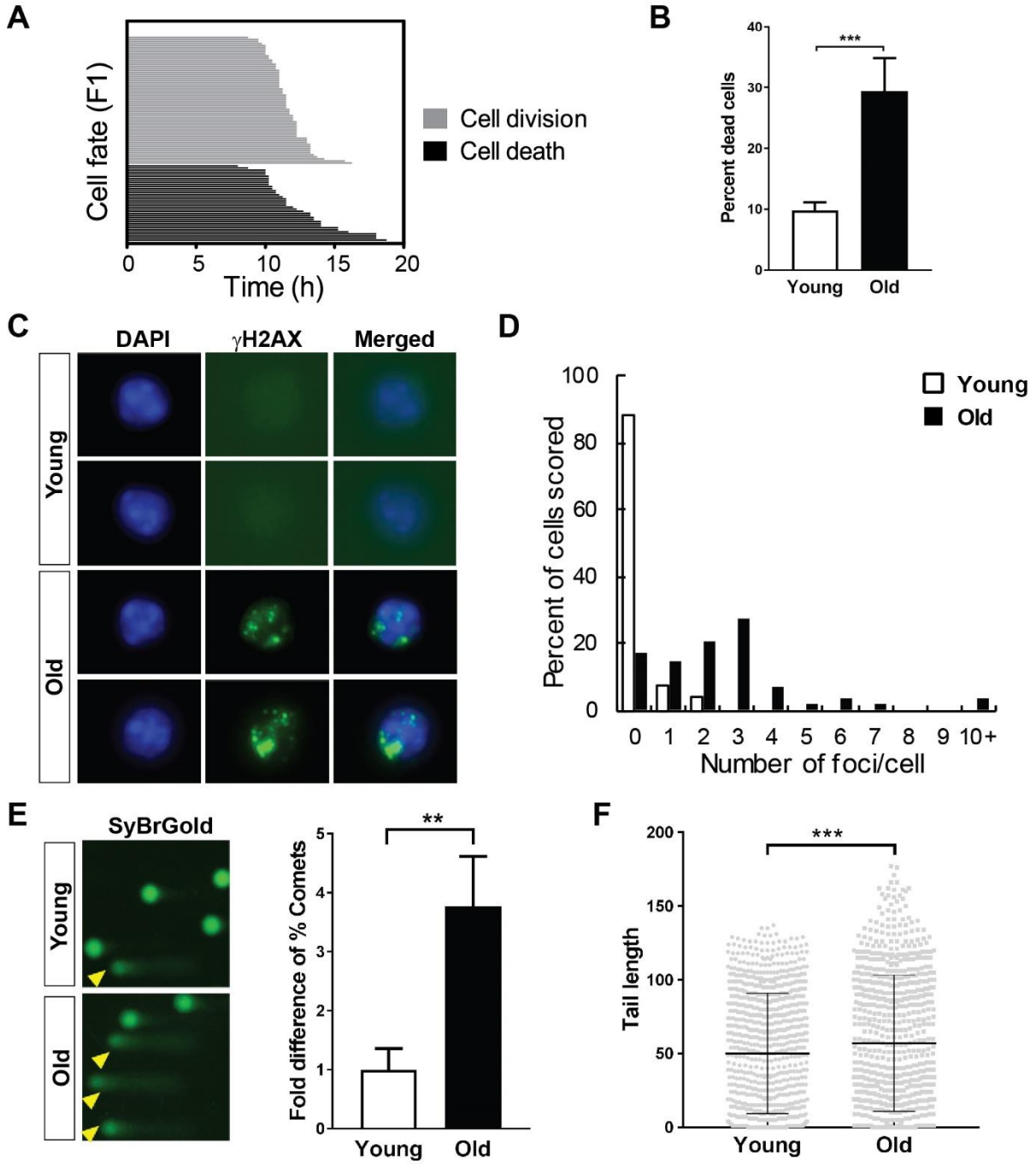

Figure S2. Mitotic catastrophe of activated MuSCs during *in vitro* activation, related to Figure 2.

(A) Representative cell fate analysis of progeny of plated MuSCs after the first cell division by time-lapse microscopy. Each horizontal bar represents a single cell (n=100). The length of a given bar along the x-axis reflects the length of time required to reach the point of cell division (grey lines; top) or cell death (black lines; bottom). (B) Quantification of the frequency of cell death of young and old MuSCs by time-lapse microscopy (n=3). (C) Representative IF images of MuSCs from 2-month- and 24-month-old mice stained with the γH2AX antibody (green). DNA was stained with DAPI. (D) Quantification of γH2AX foci of (C). Histogram of the number of γH2AX foci per cell was shown. (E) Comparison of the level of DNA damage in young and old MuSCs by Comet assay. Left: representative images of nuclei of MuSCs isolated from 2- month- and 24-month-old mice after electrophoresis and SYBR Gold staining. Cells with a Comet tail are indicated by arrow heads. Right: Quantification of frequency of nuclei with Comet tails in MuSCs from young and old mice. (F) Tail length of cells that exhibited a Comet tail from young and old mice. Each dot represents a cell. The center black line represents median tail length.

Error bars represent SEM. **: $p<0.01$; ***: $p < 0.001$.

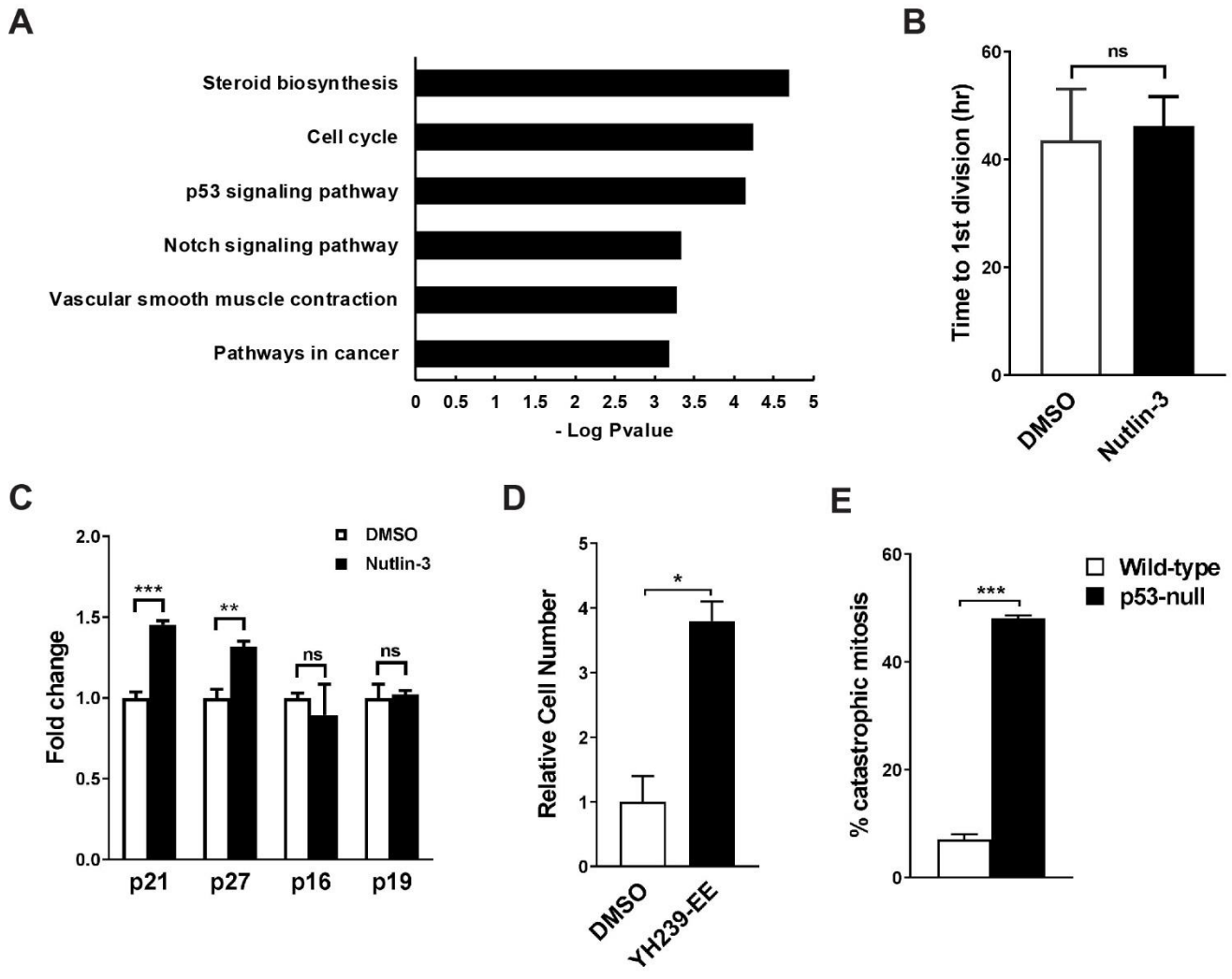

Figure S3. Pharmacologic activation of p53 prevents mitotic catastrophe in MuSCs, related to Figure 3.
(A) Significantly enriched KEGG pathways in genes whose expression change with age in MuSCs. (B) Quantification of the time to the first division of MuSCs untreated or treated with N3 by time-lapse microscopy. (C) Expression of individual cyclin kinase inhibitors of MuSCs without or with N3 treatment. RNA was isolated from MuSCs activated and expanded in vitro for 60 hours in the absence or presence of N3 and used for reverse transcription and Real-time PCR analysis with specific primers for the indicated genes. (D) Quantification of total number of MuSC progeny in the absence of presence of YH239-EE after a 4-day culture. Fold change is shown in the graph (n=3 independent experiments). (E) Quantification using time-lapse microscopy of the frequency of mitotic catastrophe in wild-type and p53-null MuSCs treated 10μM N3 (*n* = 150 cells per condition in 3 independent experiments).
Error bars represent SEM. *: $p < 0.05$; **: $p<0.01$; ***: $p < 0.001$; ns: not significant.

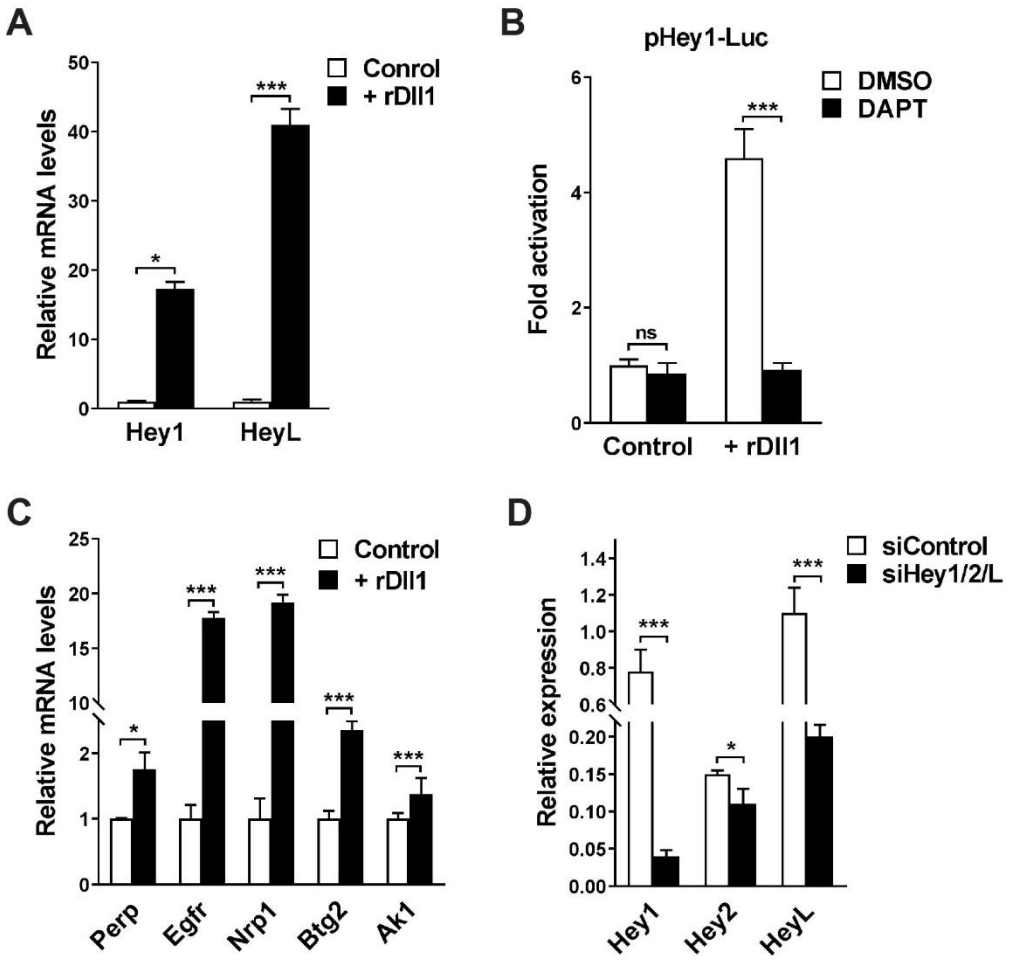

Figure S4. Activation of p53 transcriptional activity by rDll1, related to Figure 4.
(A) Induction of Hey1 and HeyL in MuSCs by rDll1. MuSCs were isolated from wildtype mice and cultured in the absence or presence of rDll1 treatment. RNA was isolated from these cells 60 hours later for reverse transcription and Real-time PCR analysis. (B) γ-secretase-dependent activation of Hey1 transcription by rDll1. MuSCs were transfected with a Hey1 luciferase reporter plasmid and cultured with or without rDll1 in the presence and absence of 25 μM DAPT. Cells were collected 48 hours after transfection and subjected to luminescent assay. Normalized luciferase activity of the control culture was set at 1. Fold change was calculated for other conditions and plotted (n = 3 independent cultures for each condition). (C) Induction of p53 target genes by rDll1 treatment in MuSCs. Freshly isolated MuSCs were cultured in the absence or presence of rDll1 for two days. The expression levels of the indicated genes were determined by RT-qPCR analysis and normalized to GAPDH expression (n = 3 for each condition). (D) Knockdown of Hey1 and HeyL expression using specific siRNA. Freshly isolated MuSCs were transfected with siRNA specific to Hey1, Hey2 and HeyL or a non-specific siRNA control. Transfected cells were cultured for 48 hours in the presence of rDll1 and harvested for RT-PCR analysis. The expression of the individual Hey genes normalized to GAPDH expression is plotted.
Error bars represent SEM. *: $p < 0.05$; **: $p<0.01$; ***: $p < 0.001$; ns: not significant.

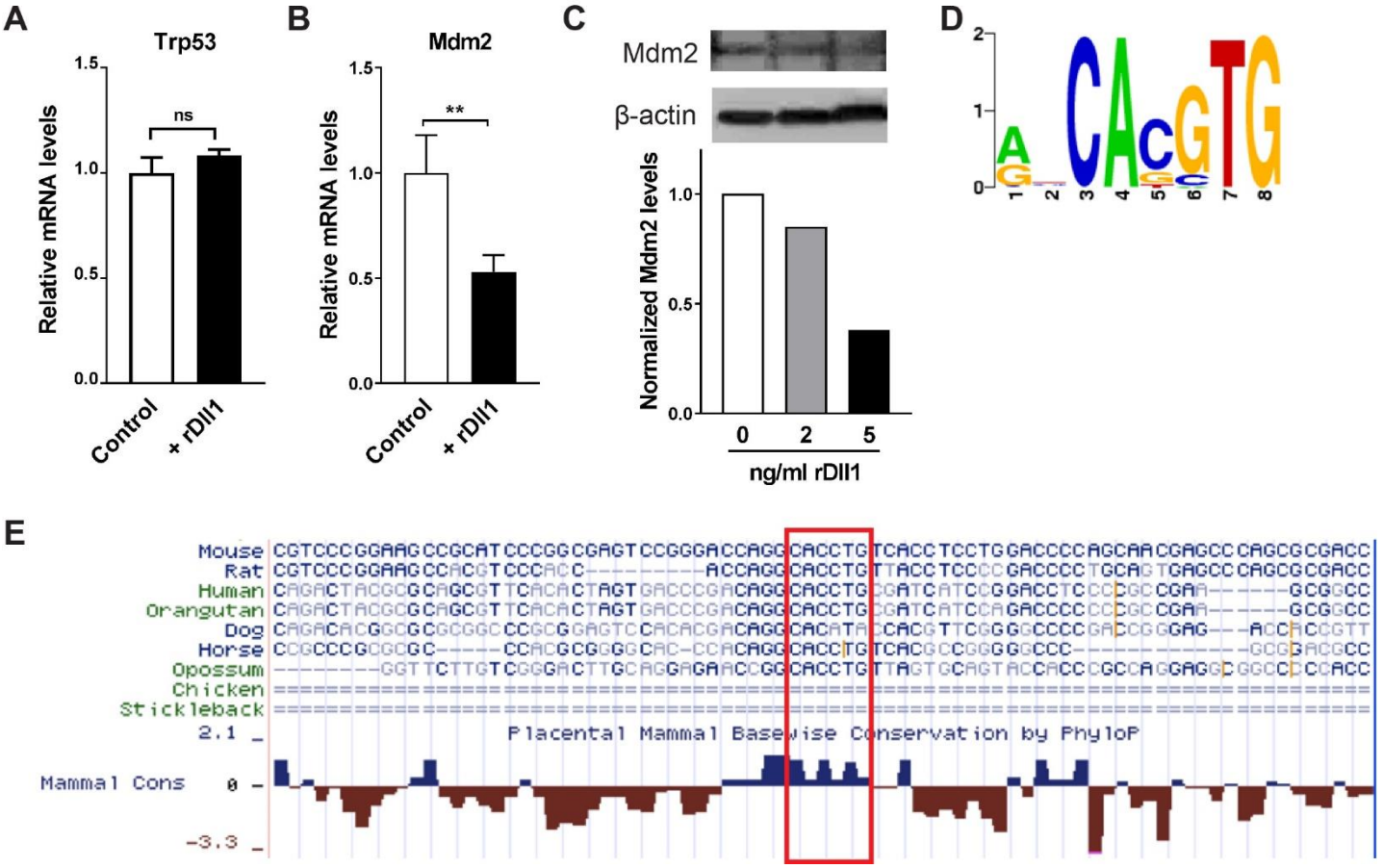

Figure S5: Activation of Mdm2 transcription by Hey1, related to Figure 5.
(A) rDll1 treatment does not induce Trp53 transcription. MuSCs were cultured in the absence or presence of rDll1 for 48 hours and harvested for RT-qPCR analysis. Trp53 expression was normalized to GAPDH and fold change between the conditions is plotted. (B) Suppression of Mdm2 expression by rDll1. MuSCs were cultured in the absence or presence of rDll1 for 48 hours and harvested for RT-qPCR analysis. Mdm2 expression was normalized to GAPDH and fold change between the conditions is plotted. (C) Reduction of Mdm2 proteins by rDll1 treatment. MuSCs were cultured in the absence or presence of various concentration of rDll1 for 48 hours and harvested for Western Blot analysis. Relative level of Mdm2 protein normalized to β-actin expression is shown in the bottom panel. (D) Position weight matrix representing the consensus binding motif of the basic helix-loop-helix transcription factor family which includes Hey1/2/L. (E) Putative Hey1/2/L binding site in the 5' regulatory sequence of *Mdm2* (outlined in red) conserved in different mammalian species.
Error bars represent SEM. **: $p<0.01$; ns: not significant.

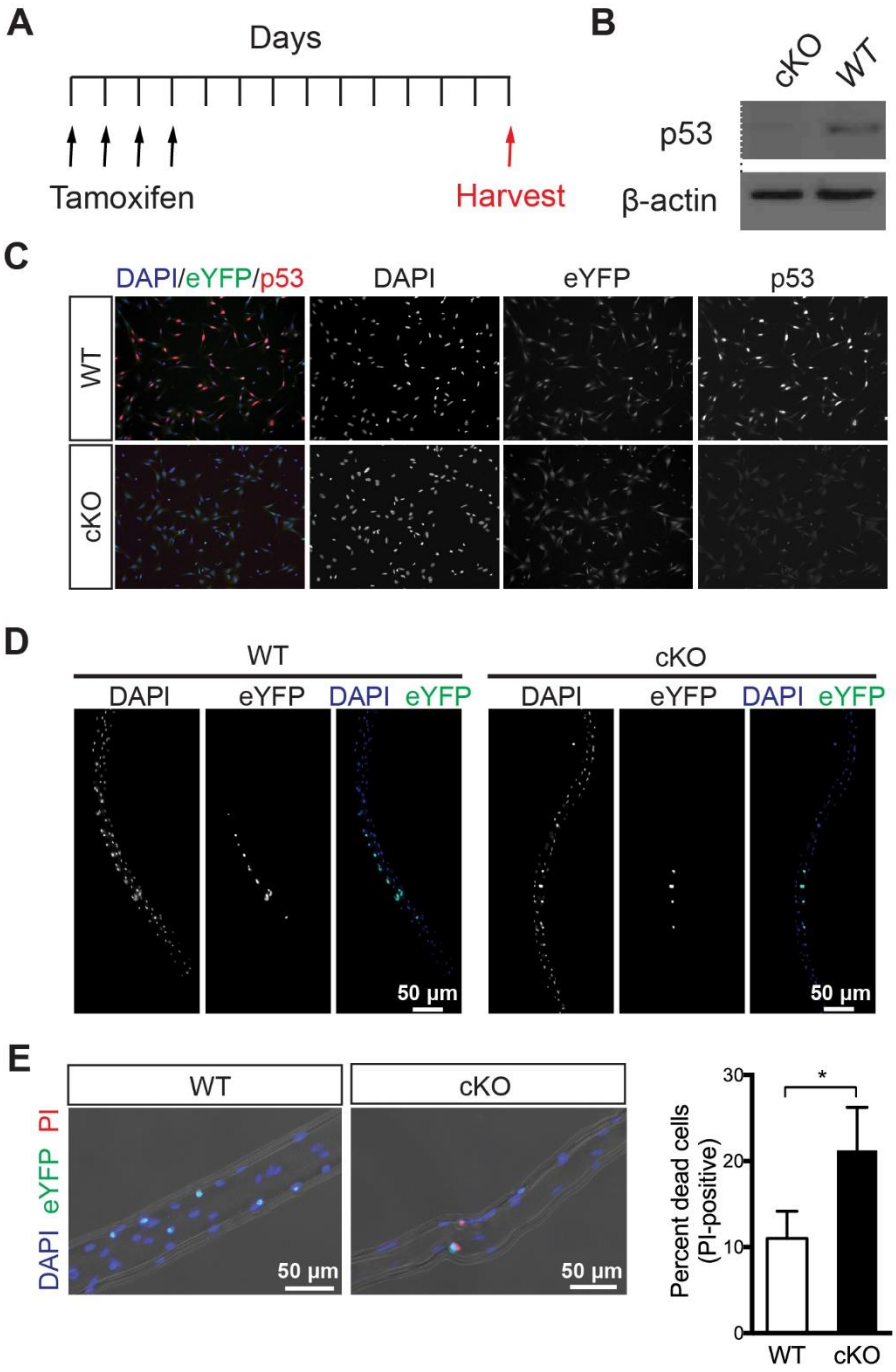

Figure S6: Impairment of MuSCs from conditional p53 cKO mice, related to Figure 6.
(A) Tamoxifen injection scheme. Each vertical line represents one day. (B) Representative western blot analysis of p53 protein levels in purified wild-type and p53 cKO MuSCs. Cells were isolated from uninjured muscle 14 days following tamoxifen treatment. (C) Representative IF images of purified p53 WT and cKO MuSCs that were stained for expression of eYFP (green) and p53 (red). Cells were treated with doxorubicin for 12 hours to induce p53 nuclear localization for better staining. (D) Representative IF images of fiber-associated MuSCs isolated from p53 cKO and control mice that were culture 3 days ex vivo. MuSCs were identified by expression of YFP (green). (E) Representative fluorescence microscopy images of dying MuSCs associated with myofibers isolated from p53 cKO and control mice and cultured for 3 days ex vivo. MuSCs were identified by expression of the YFP (green). Fibers were incubated with PI for 15 minutes prior to fixation to identify dying cells (red). Frequency of dead cells was defined as the ratio between PI-labeled and YFP-expressing cells and plotted in the graph on the right.
Error bars represent SEM. *: $p < 0.05$.

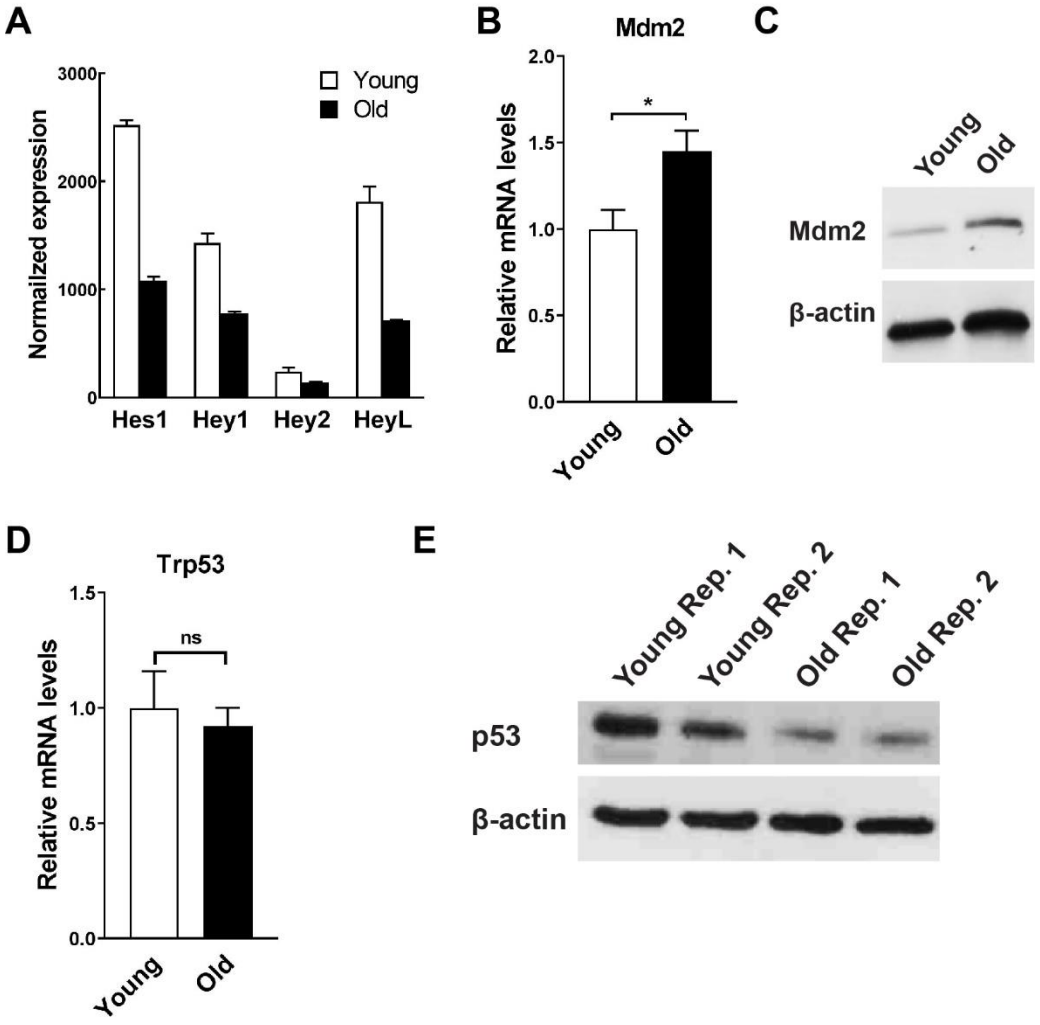

Figure S7. Rejuvenation of the function of aged MuSCs by pharmacological activation of p53, related to Figure 7.

(A) RT-qPCR analysis of *Mdm2* mRNA levels in MuSCs isolated from young and old regenerating muscle 3 days post-injury (n = 4). (B) Western blot analysis of Mdm2 protein levels in MuSCs isolated from young and old regenerating muscle 3 days post-injury. Each lane represents pooled MuSCs from two animals. (C) RT-qPCR analysis of *Trp53* mRNA levels in MuSCs isolated from young and old regenerating muscle 3 days post-injury (n = 4) (D) Western blot analysis of p53 protein levels in MuSCs isolated from young and old regenerating muscle 3 days post-injury. Two replicates, each representing two animals, are shown.

Error bars represent SEM. *: $p < 0.05$; ns: not significant.

Methods S1: List of PCR primers, related to STAR methods

Oligonucleotide sequences	Purpose	Source
CCGACGAGACCGAATCAATAAC	Forward PCR primer for Hey1:	Primer Bank
TCAGGTGATCCACAGTCATCTG	Reverse PCR primer for Hey1:	Primer Bank
AAGCGCCCTTGTGAGGAAAC	Forward PCR primer for Hey2:	Primer Bank
GGTAGTTGTCGGTGAATTGGAC	Reverse PCR primer for Hey2:	Primer Bank
AACCCGGCGGAATTTGTTG	Forward PCR primer for HeyL:	Primer Bank
GGATTGGGACTATGCTCCTGG	Reverse PCR primer for HeyL:	Primer Bank
TCAGCGAGTGCATGAACGAG	Forward PCR primer for Hes1:	Primer Bank
CATGGCGTTGATCTGGGTCA	Reverse PCR primer for Hes1:	Primer Bank
CTAGCATTCAGGCCCTCATC	Forward PCR primer for Trp53:	qPrimerDepot
AATGTCTCCTGGCTCAGAGG	Reverse PCR primer for Trp53:	qPrimerDepot
GGATCTTGACGATGGCGTAAG	Forward PCR primer for Mdm2:	Primer Bank
AGGCTGTAATCTTCCGAGTCC	Reverse PCR primer for Mdm2:	Primer Bank
CAGATCCACAGCGATATCCA	Forward PCR primer for CDKN1A:	qPrimerDepot
ACGGGACCGAAGAGACAAC	Reverse PCR primer for CDKN1A:	qPrimerDepot
GTGGACCAAATGCCTGACTC	Forward PCR primer for CDKN1B:	qPrimerDepot
TTCTTCTGTTCTGTTGGCCC	Reverse PCR primer for CDKN1B:	qPrimerDepot
CGTGAACATGTTGTTGAGGC	Forward PCR primer for CDKN2A:	qPrimerDepot
TCGAATCTGCACCGTAGTTG	Reverse PCR primer for CDKN2A:	qPrimerDepot
TTCTTCATCGGGAGCTGGT	Forward PCR primer for CDKN2D:	qPrimerDepot
TCAGGAGCTCCAAAGCAACT	Reverse PCR primer for CDKN2D:	qPrimerDepot
GACTTCAACAGCAACTCCCAC	Forward PCR primer for GAPDH:	In this paper
TCCACCACCCTGTTGCTGTA	Reverse PCR primer for GAPDH:	In this paper
TGGGGGAGGAAAGGAAGAGCGA	Forward PCR primer for Mdm2 promoter -1414 to -1321:	In this paper
TGGGCATGTCCCAGATCTCCAGT	Reverse PCR primer for Mdm2 promoter -1414 to -1321:	In this paper
ATCATGTCACAACCAGCAGCACCC	Forward PCR primer for Mdm2 promoter -744 to -675:	In this paper
TCCACAGCCCTTCCCCTGACG	Reverse PCR primer for Mdm2 promoter -744 to -675:	In this paper
AAACGACGTCGGCGAGCTGT	Forward PCR primer for Mdm2 promoter -289 to -199:	In this paper
GAGACCGACCGGACACCCCT	Reverse PCR primer for Mdm2 promoter -289 to -199:	In this paper
CGTGTCCCAACTTGACCAGCCC	Forward PCR primer for Mdm2 promoter -100 to -26:	In this paper
CTTCCGGGACGGGT-GGGACT	Reverse PCR primer for Mdm2 promoter -100 to -26:	In this paper
CCCAACTTGACCAGCCCCACAG	Forward PCR primer for Mdm2 promoter -95 to +25:	In this paper
CTGGGGTCCAGGAGGTGACAGG	Reverse PCR primer for Mdm2 promoter -95 to +25:	In this paper
CGGAGGGCGCGGAAAAGAGG	Forward PCR primer for Mdm2 promoter +286 to +473:	In this paper
TAGGAGCGGCCGGTGAGGAG	Reverse PCR primer for Mdm2 promoter +286 to +473:	In this paper
GGTTTTCAGTCACTCACGCCACACA	Forward PCR primer for Mdm2 promoter +1015 to +1105:	In this paper
TGCACACAGGTGCGGTTGCT	Reverse PCR primer for Mdm2 promoter +1015 to +1105:	In this paper
GGAGTTTGGCCTGGACGCTGC	Forward PCR primer for Mdm2 promoter +1639 to +1762:	In this paper
AGGT-GGACCCAGACGGCTGTT	Reverse PCR primer for Mdm2 promoter +1639 to +1762:	In this paper