Supporting Information

von Maltzahn et al. 10.1073/pnas.1307680110

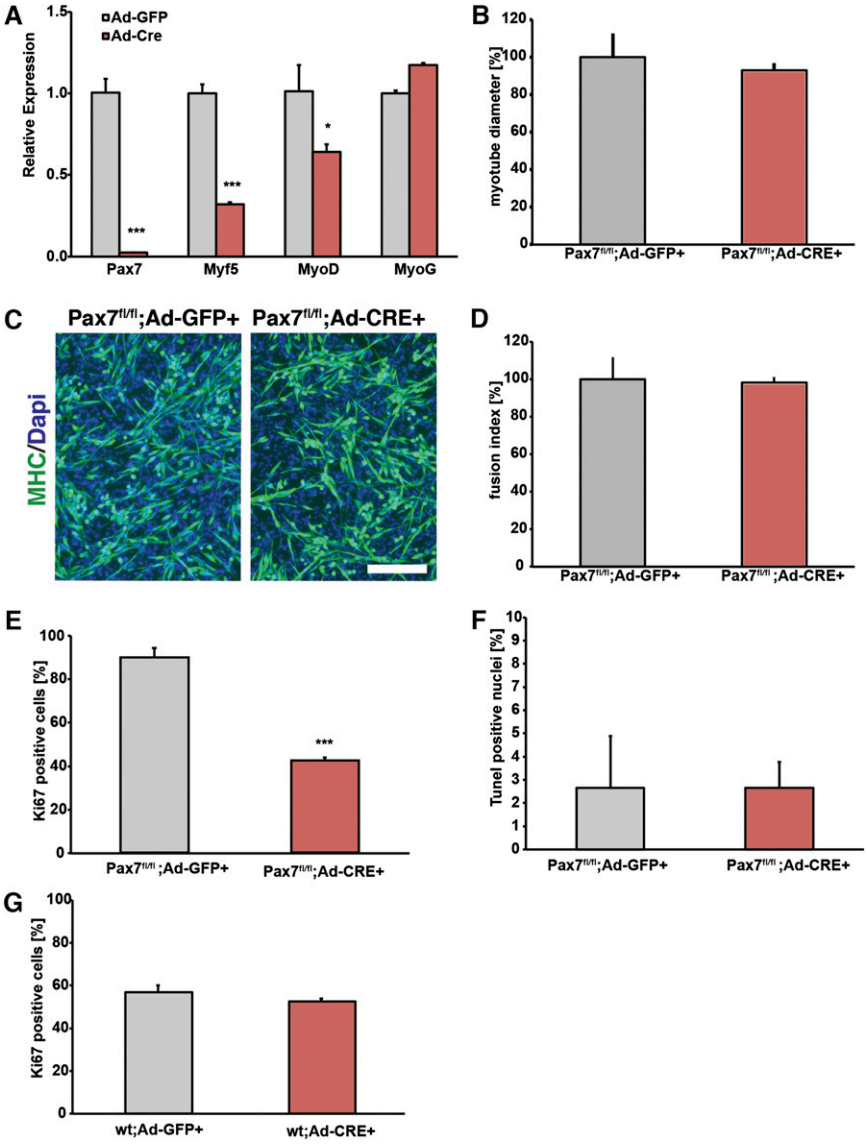

Fig. S1. Paired box 7 (Pax7) is not required for the differentiation of primary myoblasts. (*A*) Quantitative PCR analysis confirms loss of Pax7 expression and dysregulation of Myf5 and MyoD following infection of *Pax7*$^{fl/fl}$ myoblasts with an adenovirus encoding the Cre gene. Data are presented as the mean + SEM, normalized to GAPDH and shown relative to Ad-GFP infected control myoblasts; $n = 4$, $^{*}P < 0.05$, $^{***}P < 0.001$. (*B*) No differences in myotube diameter were observed following infection of *Pax7*$^{fl/fl}$ myoblasts with an adenovirus encoding the Cre gene compared with *Pax7*$^{fl/fl}$ myoblasts infected with an adenovirus encoding the GFP gene; $n = 3$. (*C*) Primary myoblasts depleted for Pax7 expression differentiate normally, myosin heavy chain is shown in green, and nuclei are counterstained with DAPI (in blue). (Scale bar: 100 μm.) (*D*) No differences in the fusion index (nuclei per myotube) were observed after depletion of Pax7 expression and consecutive differentiation, $n = 3$, and cells were differentiated at similar confluencies. (*E*) Analysis of staining for Ki67 demonstrates inhibited proliferation when Pax7 expression is ablated; $n = 3$, $^{***}P < 0.001$. (*F*) Ablation of Pax7 expression does not lead to increased apoptosis in myoblasts; $n = 3$. (*G*) Expression of Ad-Cre in WT myoblasts does not impair proliferation; $n = 3$.

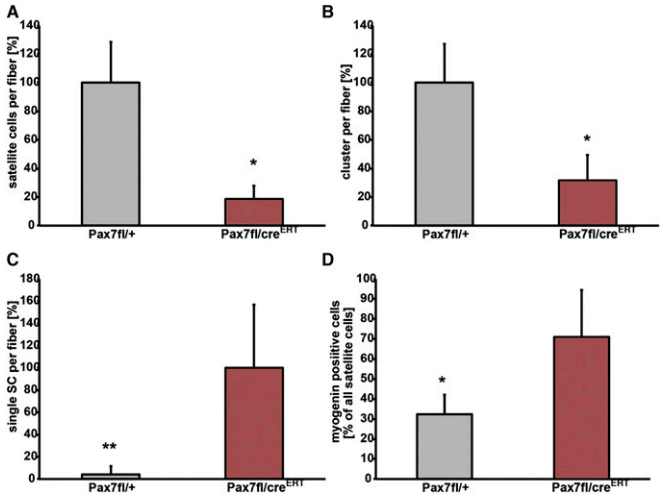

Fig. S2. Pax7 is required for primary myoblasts proliferation. (*A*) Numbers of satellite cells identified by staining for M-Cadherin on single myofibers are significantly decreased when satellite cells do not express Pax7 following tamoxifen-induced induction of the CreERT in $Pax7^{fl/CreERT2}$ mice; $n = 2$, $P < 0.05$, 72 h culture. (*B*) Reduced numbers of satellite cell clusters identified by staining for M-Cadherin on extensor digitorum longus fibers of $Pax7^{fl/CreERT2}$ mice; $n = 2$, $P < 0.05$, 72 h culture. (*C*) Increase in the numbers of single satellite cells marked by expression of M-Cadherin on single fibers from $Pax7^{fl/CreERT2}$ mice; $n = 2$, $P < 0.01$, 72 h culture. (*D*) Increase in the percentage of myogenin-positive M-Cadherin–positive satellite cells on single fibers from $Pax7^{fl/CreERT2}$ mice; $n = 2$, $P < 0.05$, 72 h culture.

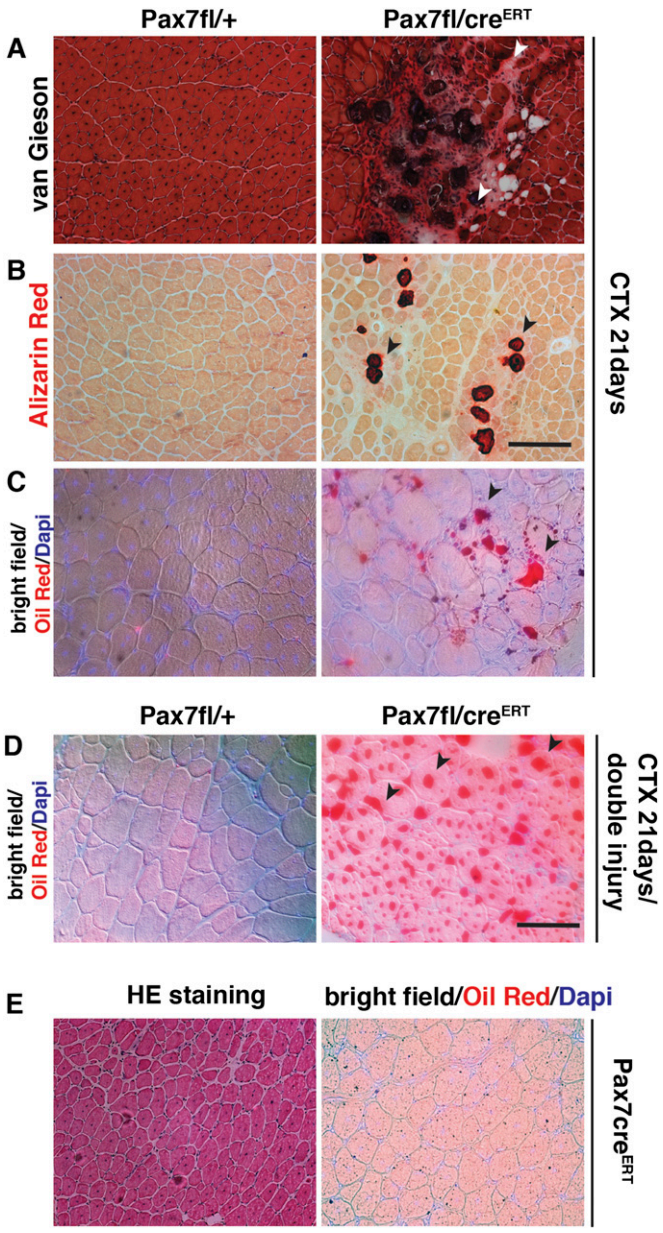

Fig. S3. Maintenance of CreERT2 activity with continuous tamoxifen treatment leads to increased adipogenesis in *Pax7*$^{fl/CreERT2}$ mice. (*A*) van Gieson staining demonstrating increased connective tissue deposition (bright red, marked by arrowheads) in *Pax7*$^{fl/CreERT2}$ mice maintained on tamoxifen diet. (*B*) Alizarin Red staining marking calcium deposition (in red, marked arrowheads) in *Pax7*$^{fl/CreERT2}$ mice maintained on tamoxifen diet. (*C*) Oil Red staining showing lipid deposits (in red, marked by arrowheads) in *Pax7*$^{fl/CreERT2}$ mice maintained on tamoxifen diet. (*D*) Oil Red staining showing lipid deposits (in red, marked by arrowheads) in *Pax7*$^{fl/CreERT2}$ mice maintained on tamoxifen diet. (*E*) H&E staining and Oil Red staining of *Pax7*$^{CreERT2/+}$ mice reveals no impairment of regeneration through expression of Cre-recombinase in satellite cells. (Scale bar: 100 μm.)

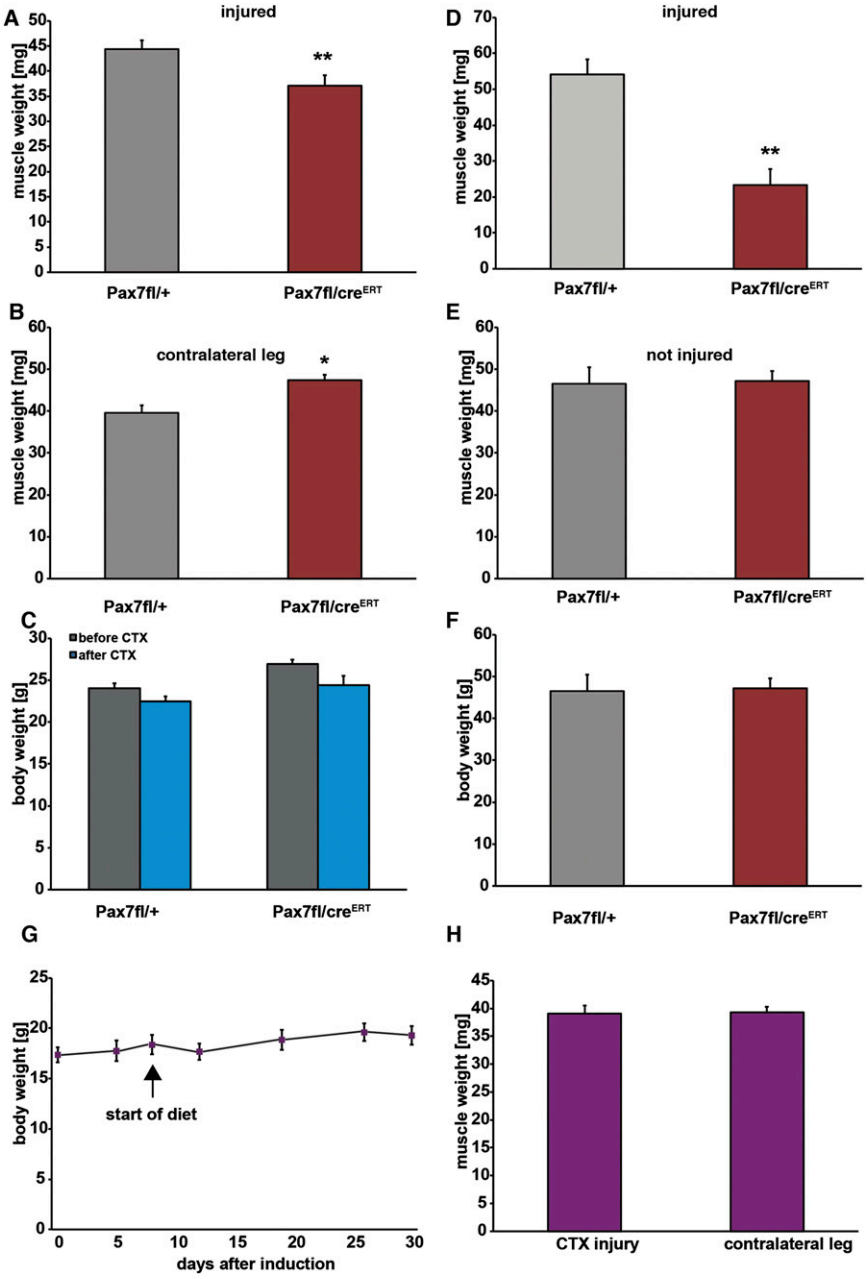

Fig. S4. Decreased weights of CTX-injured muscles following deletion of Pax7 in satellite cells. (*A*) Wet weight of Tibialis anterior muscles from mice 10 d after CTX injury following continuous tamoxifen application; n= 4, $^{**}P < 0.01$. (*B*) Tibialis anterior muscle wet weights of the contralateral leg of mice 10 d after CTX injury following continuous tamoxifen application; $n = 4$, $^{*}P < 0.05$. (*C*) Body weight of mice 10 d after CTX injury following continuous tamoxifen application; $n = 4$. (*D*) Wet weight of Tibialis anterior muscles from mice 21 d after the second CTX injury following continuous tamoxifen application; n= 3, $^{**}P < 0.01$. (*E*) Tibialis anterior muscle wet weights of the contralateral leg of mice 21 d after the second CTX injury following continuous tamoxifen application; $n = 3$. (*F*) Body weight of mice 21 d after the second CTX injury following continuous tamoxifen application; $n = 3$. (*G*) Body weight of $Pax7^{CreERT2/+}$ animals fed with Tamoxifen containing chow; $n = 3$. (*H*) Wet weights of Tibialis anterior muscles from $Pax7^{CreERT2/+}$ animals; $n = 3$.

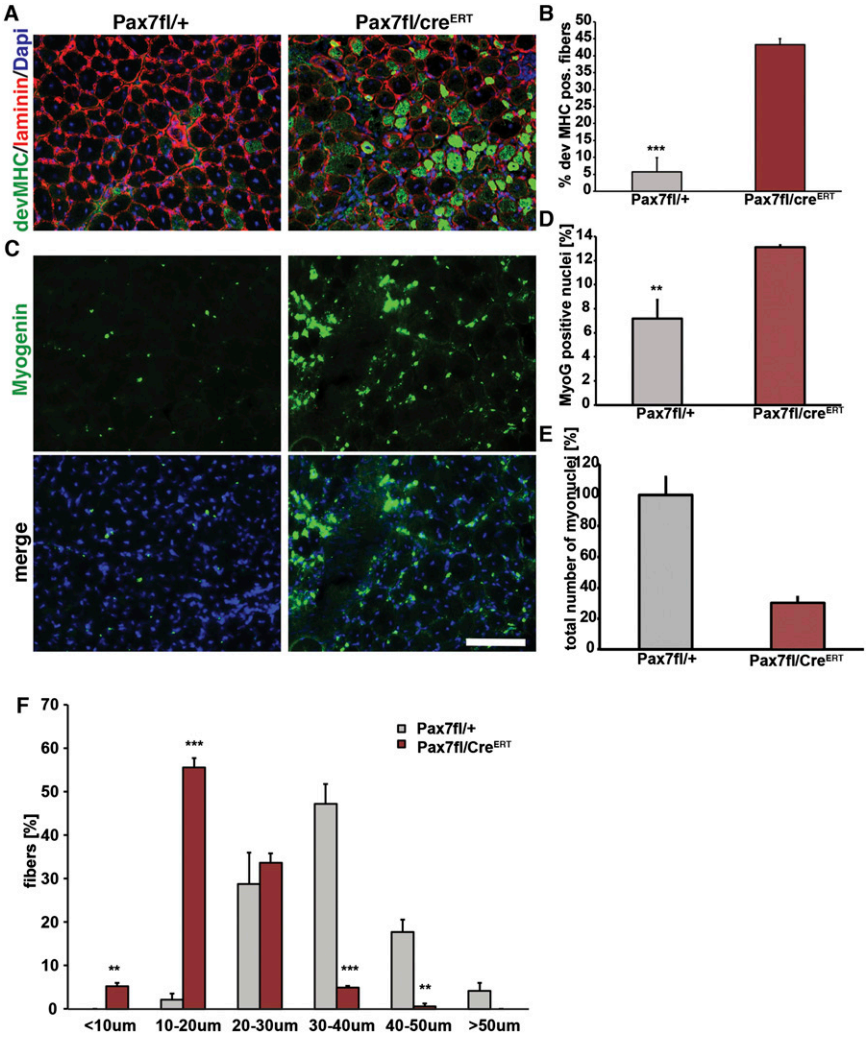

Fig. S5. Impaired muscle regeneration following deletion of Pax7 in satellite cells. (*A*) Deletion of Pax7 in adult satellite cells results in delayed regeneration marked by expression of developmental myosin (in green) at day 10 after acute injury. Laminin staining is shown in red, and nuclei are counterstained with DAPI (in blue). (Scale bar: 100 μm.) (*B*) Quantification of developmental myosin heavy chain fibers at day 10 after CTX injury; $n = 6$, $P < 0.001$. (*C*) Immunostaining for myogenin (in green) demonstrating increased numbers of myogenin-positive nuclei in *Pax7*$^{fl/CreERT2}$ animals compared with *Pax7*$^{fl/+}$ animals at 10 d after acute injury. Nuclei are counterstained with DAPI (in blue). (Scale bar: 100 μm.) (*D*) Quantification of myogenin-positive nuclei relative to the total number of nuclei; $n = 6$, $^{**}P < 0.01$. (*E*) Quantification of the number of myonuclei; $n = 4$, $^{*}P < 0.05$. (*F*) Quantification of the minimal fiber feret; $n = 3$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table S1. Continuous tamoxifen treatment leads to strongly impaired regeneration in *Pax7*$^{fl/CreERT2}$ mice 21 d after acute injury

Animal ID	Genotype	CTX damage*
364	Pax7fl/+	+/++
366	Pax7fl/+	+
370	Pax7fl/+	+/++
372	Pax7fl/+	+
981	Pax7fl/+	+
988	Pax7fl/+	+
989	Pax7fl/+	+
982	Pax7fl/cre	+++
983	Pax7fl/cre	+++
984	Pax7fl/cre	+++
985	Pax7fl/cre	+
987	Pax7fl/cre	++
989	Pax7fl/cre	++
990	Pax7fl/cre	+
365	Pax7fl/cre	++
367	Pax7fl/cre	++
369	Pax7fl/cre	+++
371	Pax7fl/cre	+++

*+, regenerating fibers and no fat; ++, regenerating fibers and few fat depositions; +++, regenerating fibers, extensive fat deposition, and fibrosis.

Table S2. Deletion of Pax7 expression leads to impairment of muscle regeneration 21 d after acute injury

Animal ID	Genotype	CTX damage*
486	Pax7fl/+	+
490	Pax7fl/+	+/++
491	Pax7fl/+	+
487	Pax7fl/cre	++
488	Pax7fl/cre	++
489	Pax7fl/cre	++

*+, regenerating fibers and no fat; ++, regenerating fibers and few fat depositions; +++, regenerating fibers, extensive fat deposition, and fibrosis.