Supporting Information

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SI Materials and Methods

Body and Muscle Weight. Total body weight was measured at 1 mo of age in male mice, and tibialis anterior (TA) muscle weight was measured in adult female mice and reported in grams (g).

Satellite Cell Isolation. Hind-limb quadriceps femoris (QF) muscles were excised and digested with protease XIV (Sigma-Aldrich). Cells were triturated and passed through a 100-μm nylon mesh. The cell suspension was centrifuged at 1,500 × *g* and preplated in DMEM, 2% (vol/vol) HS, 100 μg/mL Primocin (Invivogen) to remove fibroblasts and debris. Satellite cells were grown on Matrigel (BD Biosciences) in Hams F-10, 20% (vol/vol) FBS, 10 ng/mL bFGF (BD Biosciences), 100 μg/mL Primocin. Before use, cells were stained for Pax7 and genotyped.

Genotyping and Genomic qPCR of Satellite Cells. QF muscles from adult mice were used to isolate satellite cells as described. Cells were treated with 4-hydroxytamoxifen (4OH-T) or ethanol. Cells were genotyped to confirm excision of the *Numb* and *Numblike* loci. Primers flanking downstream loxP sites on $Nb^{fl/fl}$ and $Nbl^{fl/fl}$ alleles were used to detect excision. PCR products were run on 3% (wt/vol) agarose gel. Genomic QPCR was used to quantify the percentage of *Numb* loci that are excised. Genomic qPCR was done using Sybergreen (Eurogentec). The control primers provide the total genomic DNA. Samples are compared using mean $\Delta\Delta C_t$ values between the excised and unexcised primer sets to determine the percent of recombined loci. These data indicate that we can efficiently excise both *Numb* and *Numblike* in culture.

Immunofluorescence of Muscle Sections. Muscles were flash frozen in isopentane, and midbelly cryostat sections (8 μM) were stained with hematoxylin/eosin (H&E) or with antibodies against embryonic myosin heavy chain (eMyHC) (cl. *F1.652*, DSHB), or laminin (Sigma-Aldrich) as described (1). For immunofluorescence (IF) of frozen injured muscles, primary antibodies used included rabbit anti-GFP (1:500; Invitrogen), mouse anti-MyoD (1:200; Becton/Dickson), mouse anti-Pax7 (1:200; DSHB), and Alexa 647-conjugated mouse anti-Ki67 (1:20; Becton/DicksonBD Biosciences). Staining with antibodies against Pax7 and MyoD was performed using the Zenon Alexa Fluor 594 Labeling Kit (Life Technologies) per the manufacturer. Species-specific secondary antibodies were conjugated to Alexa 488 or 594 and used at a concentration of 1:1,000 (Invitrogen). DAPI was added during the secondary antibody incubation to stain the nuclei.

Gomorri's Trichrome and H&E Stains. Muscles were fixed at 4 °C in paraformaldehyde and were embedded in paraffin. For staining, 5-μm sections were dewaxed in xylenes and rehydrated through graded alcohols and then postfixed in Bouin's fixative. Sections were stained in modified Wiegert's iron hematoxylin, and then color differentiation was done in acid alcohol followed by trichrome stain. For H&E, the sections were stained with hematoxylin, washed in dH_2O, and stained with eosin. Sections were dehydrated through graded alcohols to xylenes and mounted.

FACS Analysis. Satellite cells were isolated from the hindlimb muscles, and FACS was done as described (2), using a FACSAria III (BD Biosciences). Primary antibodies used to identify the satellite cells were rat anti-CD31 and rat anti-CD45 antibodies conjugated to allophycocyanin (APC; 1:100; BD Biosciences), rat anti-Sca1 conjugated to Pacific Blue (1:100; BioLegend), biotinylated rat anti-CD106 antibody (Vcam; 1:100; BD Biosciences), and phycoerythrin-Cy7 Streptavidin (1:100; BD Biosciences). DAPI dilactate (250 μg/μl; Invitrogen) was used to exclude dead cells.

Western Blots. Muscle satellite cells were isolated then treated with either 4OH-T or ethanol for 48 h. Cells were collected in PBS with 10 mM EDTA, 1% Triton X-100, and Complete Protease Inhibitor (Roche), and total protein lysates were made. Each lane had 5 μg of protein loaded on 10% (wt/vol) SDS/PAGE, as determined by Bradford assay. Proteins were transferred to Immobilon-P membrane. Proteins were detected with mouse anti-Numb (1:600; Cell Signaling) or mouse anti-Mstn (1:1,000; Abcam) and visualized with anti-mouse-AP antibody (1:10,000; Invitrogen) using ECL substrate (GE Healthcare).

1. Quach NL, Biressi S, Reichardt LF, Keller C, Rando TA (2009) Focal adhesion kinase signaling regulates the expression of caveolin 3 and beta1 integrin, genes essential for normal myoblast fusion. *Mol Biol Cell* 20(14):3422–3435.

2. Mourikis P, et al. (2012) A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. *Stem Cells* 30(2):243–252.

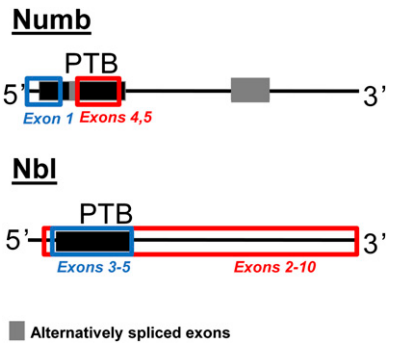

Fig. S1. Schematic representation of the different strategies used to ablate Numb and Nbl. The two different deletion strategies adopted in this study to simultaneously ablate *Numb* and *Nbl* are depicted in red (1, 2) and blue (3, 4). Alternatively spliced exons are colored in gray. The functional PTB domain is also included in the schematic.

1. Zhong W, et al. (2000) Mouse numb is an essential gene involved in cortical neurogenesis. *Proc Natl Acad Sci USA* 97(12):6844–6849.
2. Petersen PH, Zou K, Hwang JK, Jan YN, Zhong W (2002) Progenitor cell maintenance requires numb and numblike during mouse neurogenesis. *Nature* 419(6910):929–934.
3. Zilian O, et al. (2001) Multiple roles of mouse Numb in tuning developmental cell fates. *Curr Biol* 11(7):494–501.
4. Wilson A, et al. (2007) Normal hemopoiesis and lymphopoiesis in the combined absence of numb and numblike. *J Immunol* 178(11):6746–6751.

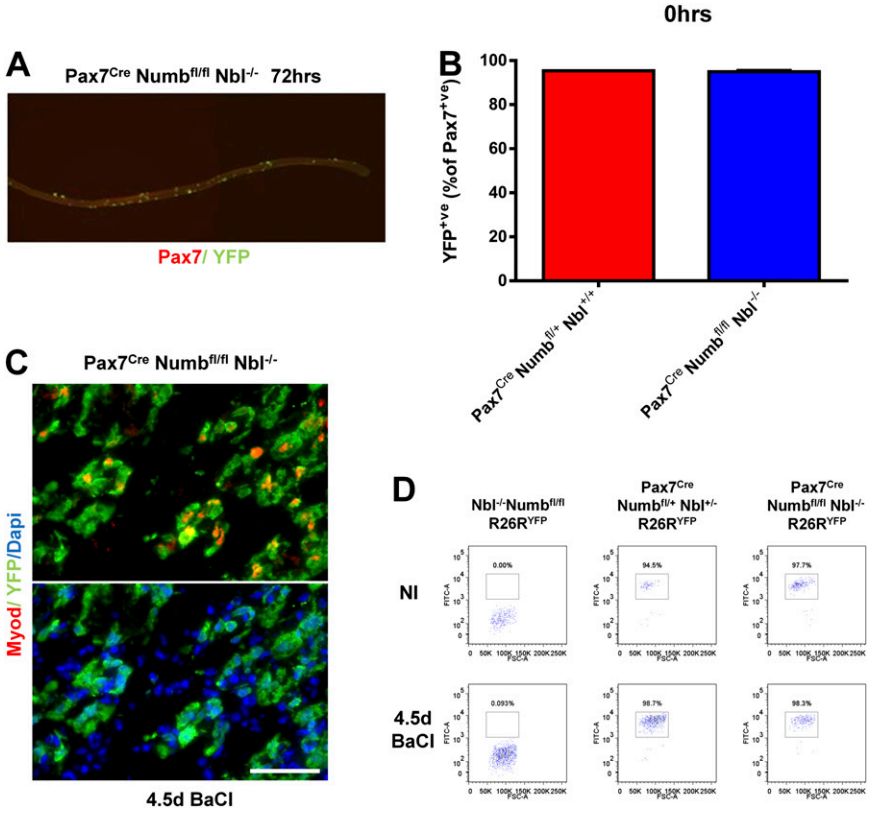

Fig. S2. Efficient recombination in the Pax7^{Cre} strain. (*A*) Representative image of a fiber isolated from EDL muscles of *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$R26R^{YFP}* mice and cultured for 72 h in vitro, showing strong YFP expression in the satellite cell progeny. (*B*) The percentage of Pax7^{+ve} cells expressing YFP was quantified on fibers from *Pax7-Numb$^{fl/+}$Nbl$^{+/+}$R26R^{YFP} and Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$R26R^{YFP}* immediately after isolation. Note that the great majority of Pax7^{+ve} cells coexpress YFP. (*C*) Immunofluorescence analysis with antibodies against MyoD and YFP on TA muscles sections from *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$R26R^{YFP}* mice 4.5 d after $BaCl_2$ injury. Nuclei were counterstained with DAPI. Note that the great majority of MyoD^{+ve} cells are also YFP^{+ve}. (Scale bar: 50 μm.) (*D*) The fraction of the Vcam^{+ve} / Cd45/Cd31/Sca^{-ve} satellite cells expressing the lineage marker YFP (FITC) is quantified by FACS on cells obtained from hindlimb muscles without injury (NI) or 4.5 d after $BaCl_2$ injury. Note the high recombination efficiency in satellite cells *Pax7-Numb$^{fl/+}$Nbl$^{+/-}$R26R^{YFP} and Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$R26R^{YFP}* mice whereas, in the *Numb$^{fl/fl}$Nbl$^{-/-}$R26R^{YFP}* mice, recombination was undetectable in absence of the *Pax7^{ICNm}* allele.

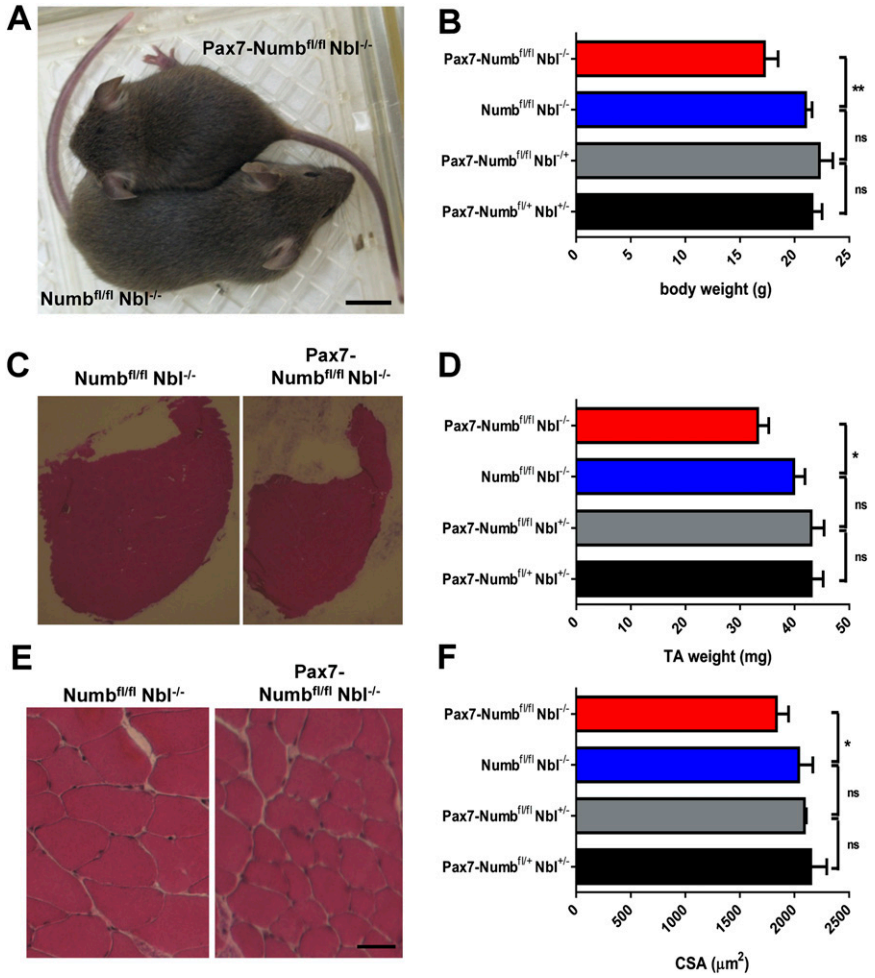

Fig. S3. Hypotrophic muscle in mice lacking Numb in the Pax7-lineage. (*A*) Picture of 1 mo-old *Numb$^{fl/fl}$Nbl$^{-/-}$* and *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$* mice. Bar corresponds to 1 cm. (*B*) Decreased body weight in 1-mo-old *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$* mice ($n = 13$). Note the similar weight of the *Numb$^{fl/fl}$Nbl$^{-/-}$* ($n = 31$), *Pax7-Numb$^{fl/fl}$Nbl$^{+/-}$* ($n = 3$), and *Pax7-Numb$^{fl/+}$ Nbl$^{-/+}$* ($n = 5$) control mice. Error bars represent the SEM. $^{**}P < 0.01$; ns, not significant. (*C* and *D*) Reduced TA mass in *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$* ($n = 10$) compared with *Numb$^{fl/fl}$Nbl$^{-/-}$* ($n = 9$), *Pax7-Numb$^{fl/fl}$Nbl$^{+/-}$* ($n = 5$), and *Pax7-Numb$^{fl/+}$Nbl$^{+/-}$* ($n = 5$) control mice ($^{*}P < 0.05$; $^{**}P < 0.01$). Error bars represent the SEM. (*E*) TA muscles from *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$* and *Numb$^{fl/fl}$Nbl$^{-/-}$* adult mice isolated 4 or 14 d after $BaCl_2$ injury and H&E stained. (Scale bar: 50 μm.) (*F*) Decreased CSA of TA muscle of *Pax7-Numb$^{fl/fl}$Nbl$^{-/-}$* mice.

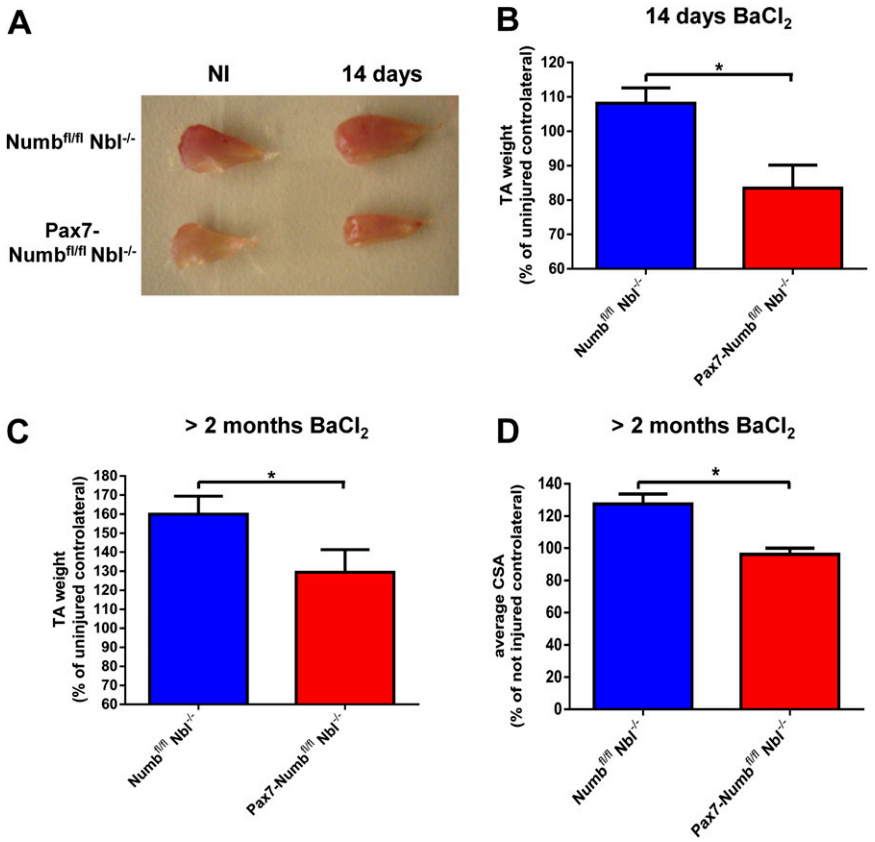

Fig. S4. Reduced muscle mass in mice lacking Numb in the Pax7-lineage 2 mo after injury. (*A*) Picture of TA muscles of *Numb*$^{fl/fl}$*Nbl*$^{-/-}$ and *Pax7-Numb*$^{fl/fl}$*Nbl*$^{-/-}$ mice, without injury (NI) or 14 d after $BaCl_2$ injury. (*B* and *C*) TA mass is expressed as percentage of uninjured controlateral muscle. Note the reduced TA mass in *Pax7-Numb*$^{fl/fl}$*Nbl*$^{-/-}$ compared with *Numb*$^{fl/fl}$*Nbl*$^{-/-}$ control mice both at 14 d ($n \geq 4$) and ≥2 mo ($n = 3$) after $BaCl_2$ injury. Error bars represent the SEM. *$P < 0.05$. (*D*) Average fiber CSA have been quantified and expressed as percentage of the CSA of the uninjured controlateral muscles. Note the reduction in *Pax7-Numb*$^{fl/fl}$*Nbl*$^{-/-}$ compared with *Numb*$^{fl/fl}$*Nbl*$^{-/-}$ controls (*$P < 0.05$; $n = 3$). Error bars represent the SEM.

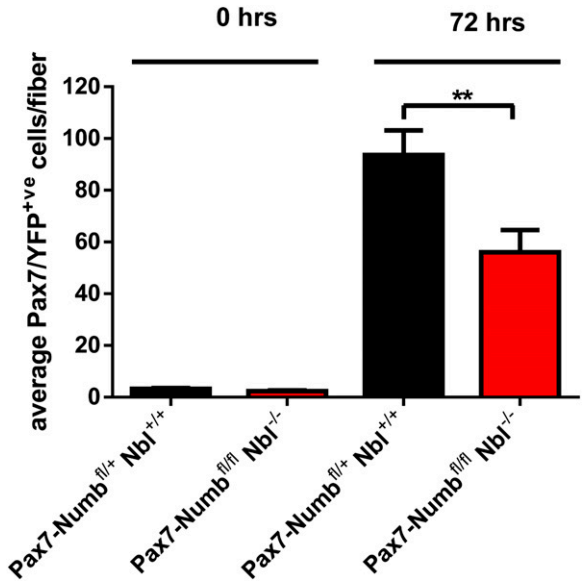

Fig. S5. Numb-deficient satellite cells have a proliferation defect. Single fibers and associated satellite cells from EDL muscles were obtained from *Pax7-Numb*$^{fl/fl}$*Nbl*$^{-/-}$ and *Pax7-Numb*$^{fl/+}$*Nbl*$^{+/+}$ mice bred into the R26R^{YFP} heterozygous background. The number of Pax7/YFP^{+ve} cells was quantified immediately after isolation (0 h) or after 72 h of culture (30 fibers per time point). Significantly fewer satellite cell progeny, $P < 0.01$, were present on the fibers isolated from the *Pax7-Numb*$^{fl/fl}$*Nbl*$^{-/-}$ after 72 h. Genotypes are as indicated.

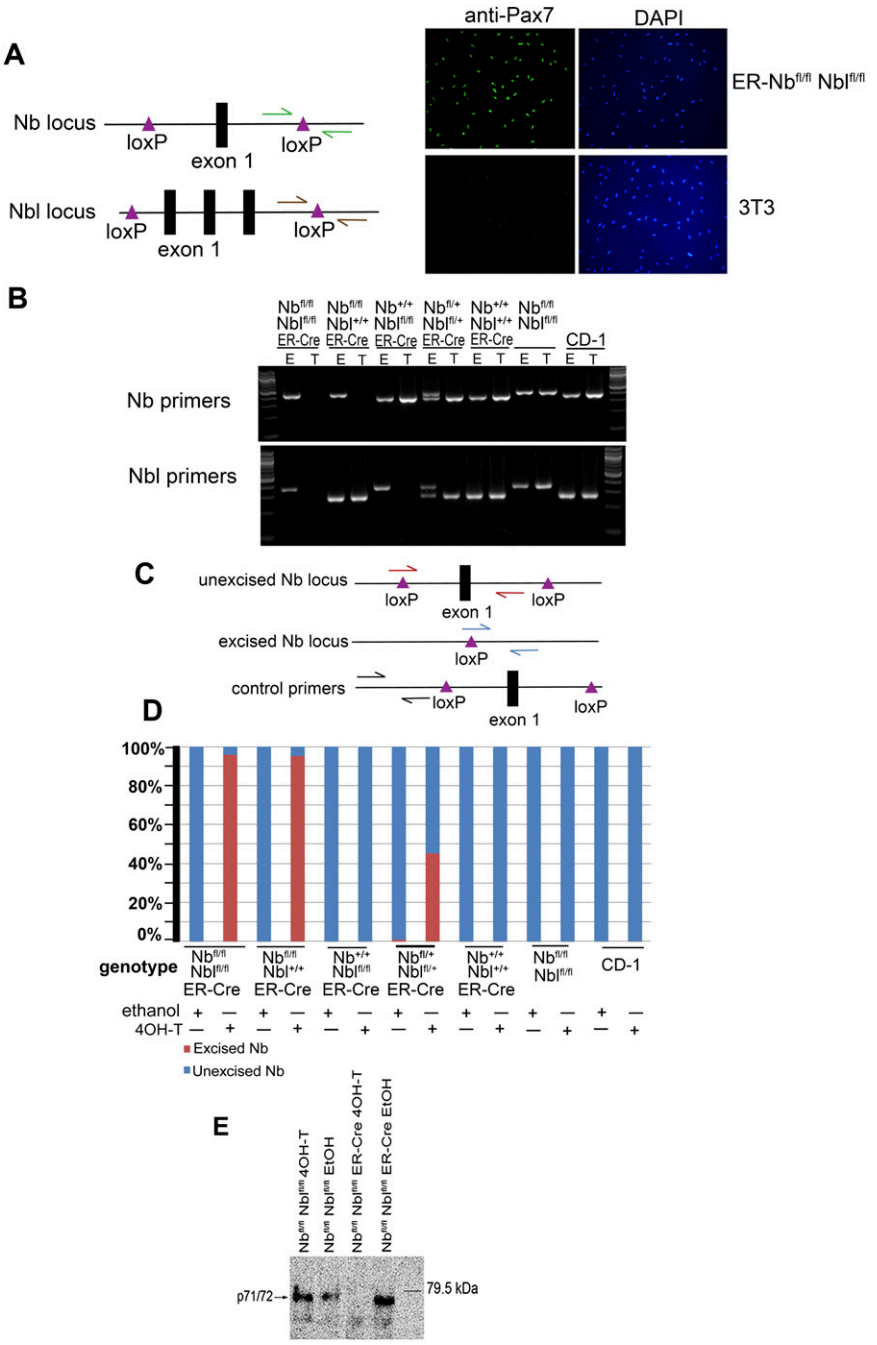

Fig. S6. Isolation and genotyping of satellite cells for excision of Numb. (*A*) (*Right*) Primers flanking downstream loxP sites on *Nb*$^{fl/fl}$ and *Nbl*$^{fl/fl}$ alleles were used to detect excision of *Numb* and *Nbl*. (*Left*) Immediately after 4OH-T or ethanol treatment, cells were plated on Matrigel-coated coverslips and fixed, and Pax7 was detected by immunofluorescence. Cells were counterstained with DAPI. Pax7^{+ve} nuclei were counted. Only those cultures that had >90% Pax7^{+ve} cells were used in analysis. Control 3T3 cells do not express Pax7. (*B*) PCR products were run on 3% agarose gel. E, ethanol-treated; T, 4OH-T-treated. Upon Cre-mediated excision, one loxP site is removed and there is loss of the PCR product. In *ER-Nb*$^{fl/fl}$*Nbl*$^{fl/fl}$ satellite cells treated with 4OH-T, both Numb and Nbl products are lost. Similarly, in *ER-Nb*$^{fl/fl}$*Nbl*$^{+/+}$ satellite cells, only the Numb product is lost whereas *Nbl* is still present. (*C*) Genomic qPCR done with Sybergreen was used to quantify the percentage of *Numb* loci that are excised. The control primers provide are used to determine the total genomic loci. (*D*) The differences of the $\Delta\Delta C_t$ values between the excised and unexcised primer sets were used to determine the percent of recombined loci. These data show that we can efficiently excise both *Numb* and *Nbl* in culture. For all analyses in these studies, we used only cells with >90% rearrangement by genomic qPCR. (*E*) Western blot of satellite cells post-4OH-T or ethanol treatment. Cells were isolated 2 d post-treatment and total protein lysates were analyzed for Numb proteins.

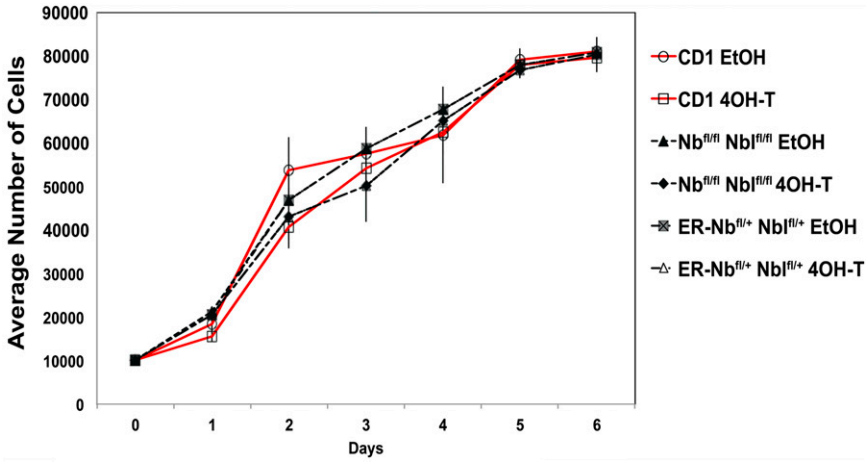

Fig. S7. Control satellite cells proliferate normally. Satellite cells were isolated from mice with the genotypes indicated, treated with 4OH-T or ethanol in vitro, and cultured in growth medium. Triplicate wells were counted daily ($n = 3$).

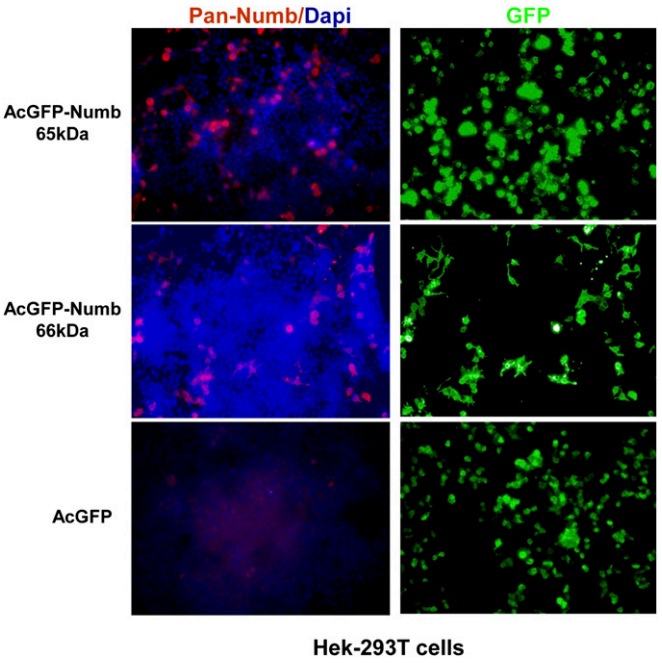

Fig. S8. Expression of AcGFP-Numb fusion proteins in 293T cells. Hek-293T cells were transfected with AcGFP-Numb (65- and 66-kDa isoforms) expressing plasmids and were stained with antibodies recognizing Numb and GFP. A control plasmid expressing AcGFP was transfected in parallel. Note that Numb is expressed exclusively in the GFP^{+ve} cells upon transfection with AcGFP-Numb–expressing plasmids, but not in control cultures.

Table S1. Candidate gene qRT-PCR

Gene	ER-Nb$^{fl/fl}$ Nbl$^{fl/fl}$ cells treated with 4OH-T
Notch1	0.80 ± 0.15, $P = 0.318174$
Deltalike1	0.82 ± 0.12, $P = 0.198402$
Hes1	1.16 ± 0.07, $P < 0.05$
Hey2	1.28 ± 0.35, $P = 0.248351$
Hes6	4.88 ± 0.08, $P < 0.0001$
Hes3	5.74 ± 3.46, $P = 0.079378$
Pax7	0.96 ± 0.78, $P = 0.656296$
CD34	1.59 ± 0.25, $P < 0.05$
Myod1	0.85 ± 0.01, $P = 0.573410$
Mstn	71.56 ± 23.44, $P < 0.05$
P21	199.04 ± 58.93, $P < 0.05$

Nb$^{fl/fl}$Nbl$^{fl/fl}$ and *ER-Nb$^{fl/fl}$Nbl$^{fl/fl}$* satellite cells were treated with 4OH-T or ethanol for 48 h and then maintained in growth medium for 24 h. Total RNA was harvested and cDNA was synthesized. Gene expression was determined using gene-specific primers that span an intron for qRT-PCR using Sybergreen. All C_t values were normalized to Gapdh. Relative gene expression was calculated using ΔΔCq, with *Nb$^{fl/fl}$Nbl$^{fl/fl}$* cells treated with ethanol as the comparison control. Data are relative expression ± SD ($n = 3$).