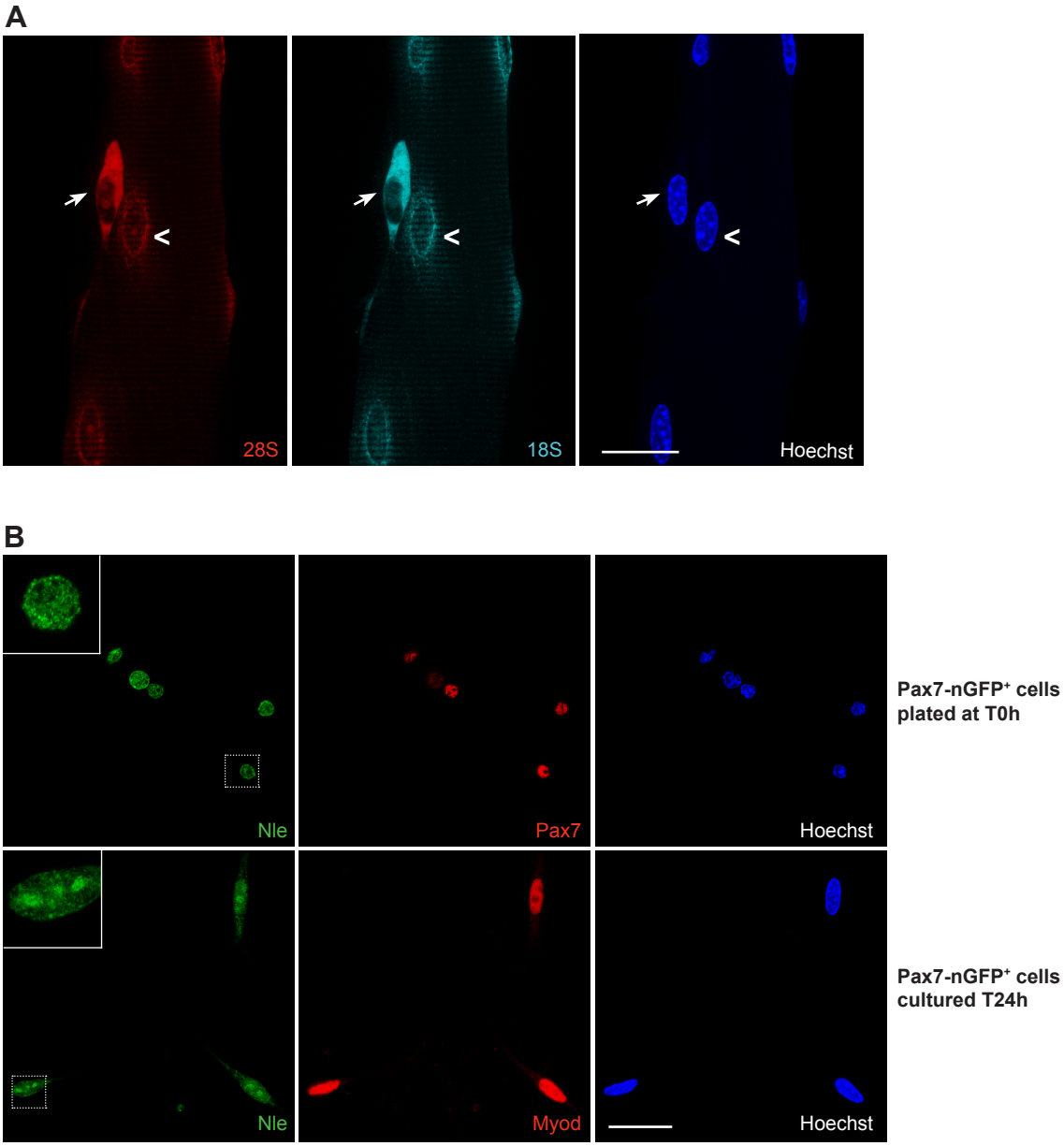


Figure S1. Nle protein and ribosomal RNA expression during myogenesis.

A) Representative images of rRNA FISH on cultured (T24h) *Tg:Pax7-nGFP* myofibres. Note high levels of total 18S and 28S in activated satellite cells (arrows); rRNAs indicate ribosomes located throughout myofibre, and enriched around myonuclei (arrowhead).

B) Representative images of Nle immunostaining on T0h and 24h cultured satellite cells, co-stained with Pax7 and Myod respectively. Note Nle nucleolar staining in activated satellite cells, and faint staining in nuclei of quiescent cells. Scale bar A and B, 20μm.

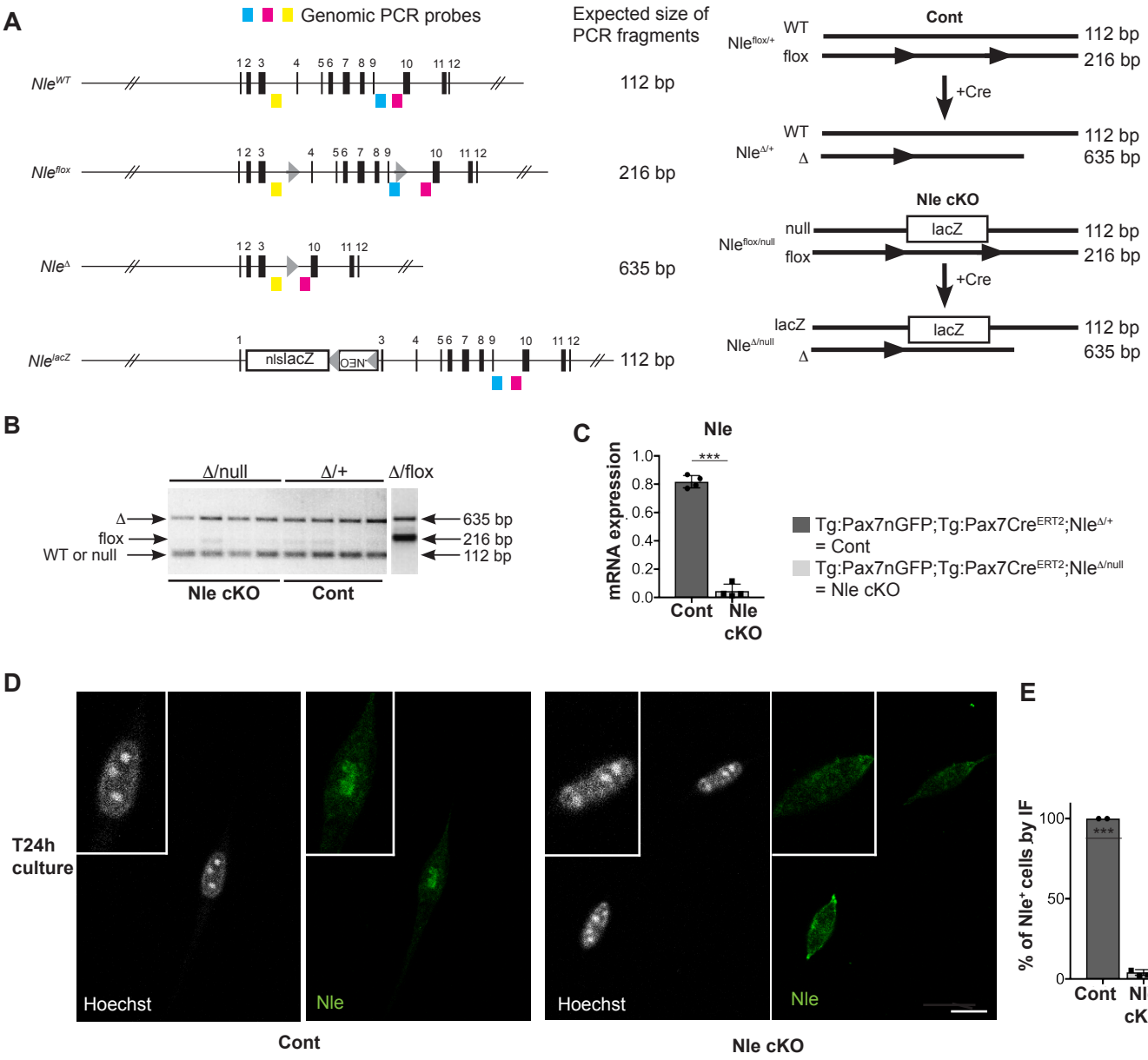


Figure S2. *Nle* deficiency in satellite cells.

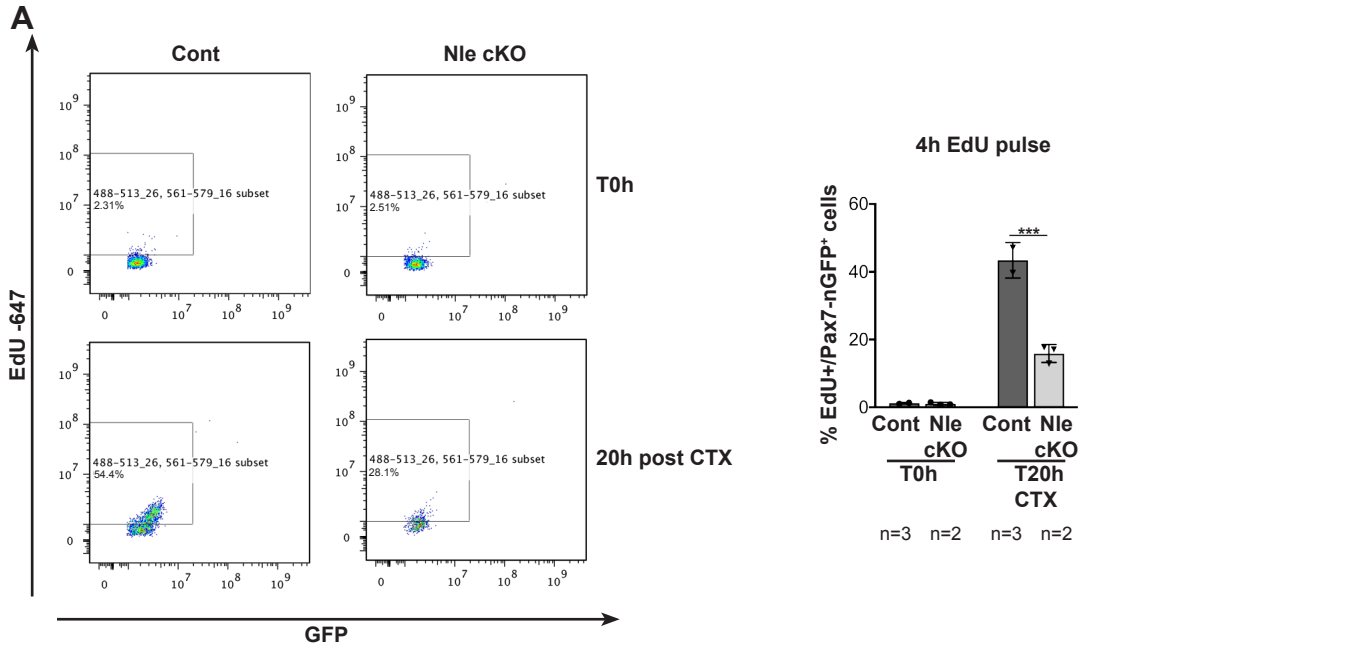
A) Scheme of WT and recombined *Nle* alleles. Upon tamoxifen treatment and nuclear expression of *Cre* recombinase, control and mutant mice recombined the floxed allele deleting exons 4-9(Δ), generating a non-functional allele. WT and null (*lacZ*) alleles are indicated.

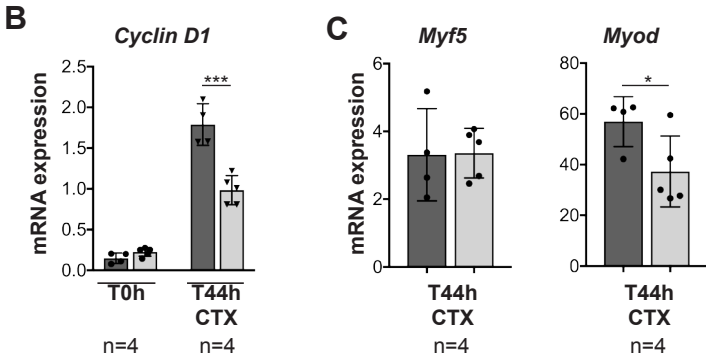
B) Genomic PCR for *Nle* deletion on isolated satellite cells. After tamoxifen treatment, the floxed allele was efficiently recombined in quiescent satellite cells. Far right lane (Δ/flox), internal PCR control detecting the floxed and deleted alleles (Le Bouteiller et al., 2013).

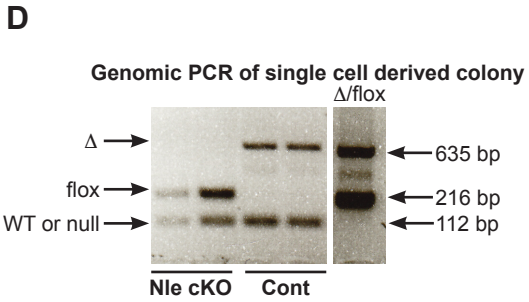
C) *Nle* expression in control and mutant quiescent satellite cells; reduction of 95% of *Nle* transcripts after *Cre*-recombination.

D) Immunofluorescence of Nle protein in 24h cultured satellite cells. Note nuclear staining in control-activated cells, but not in mutant cells.

E) Quantification of Nle^{+} cells by immunofluorescence at 24h on cells cultured *in vitro*.







Tg:Pax7nGFP;Tg:Pax7Cre^{ERT2};Nle$^{\Delta/+}$
= Cont

Tg:Pax7nGFP;Tg:Pax7Cre^{ERT2};Nle$^{\Delta/null}$
= Nle cKO

Figure S3. Failure of proliferation of *Nle* cKO satellite cells.

A) FACS profiles of *in vitro* EdU labeling of control and mutant injury-activated satellite cells with 4h EdU pulse of TA muscles collected 20h after CTX injury. Upper leg muscles were used to isolate control quiescent cells; 20h after CTX injury, mutant cells showed a lower number of EdU-labeled cells.

B, C) RT-qPCR of markers for cell cycle (*Cyclin D1*) and myogenic regulatory factors *Myod*, *Myf5*, in control and *Nle*-deleted satellite cells.

D) Genomic PCR single cell cloning colonies after 3 weeks in culture; note rare single cell derived colonies in *Nle* cKO cultures contained only the undeleted Nle^{flox} allele (flox) and the null allele, indicating that these cells are escapers (did not recombine the floxed allele); single cell-derived colonies from control muscles showed WT and recombined floxed allele (Δ).

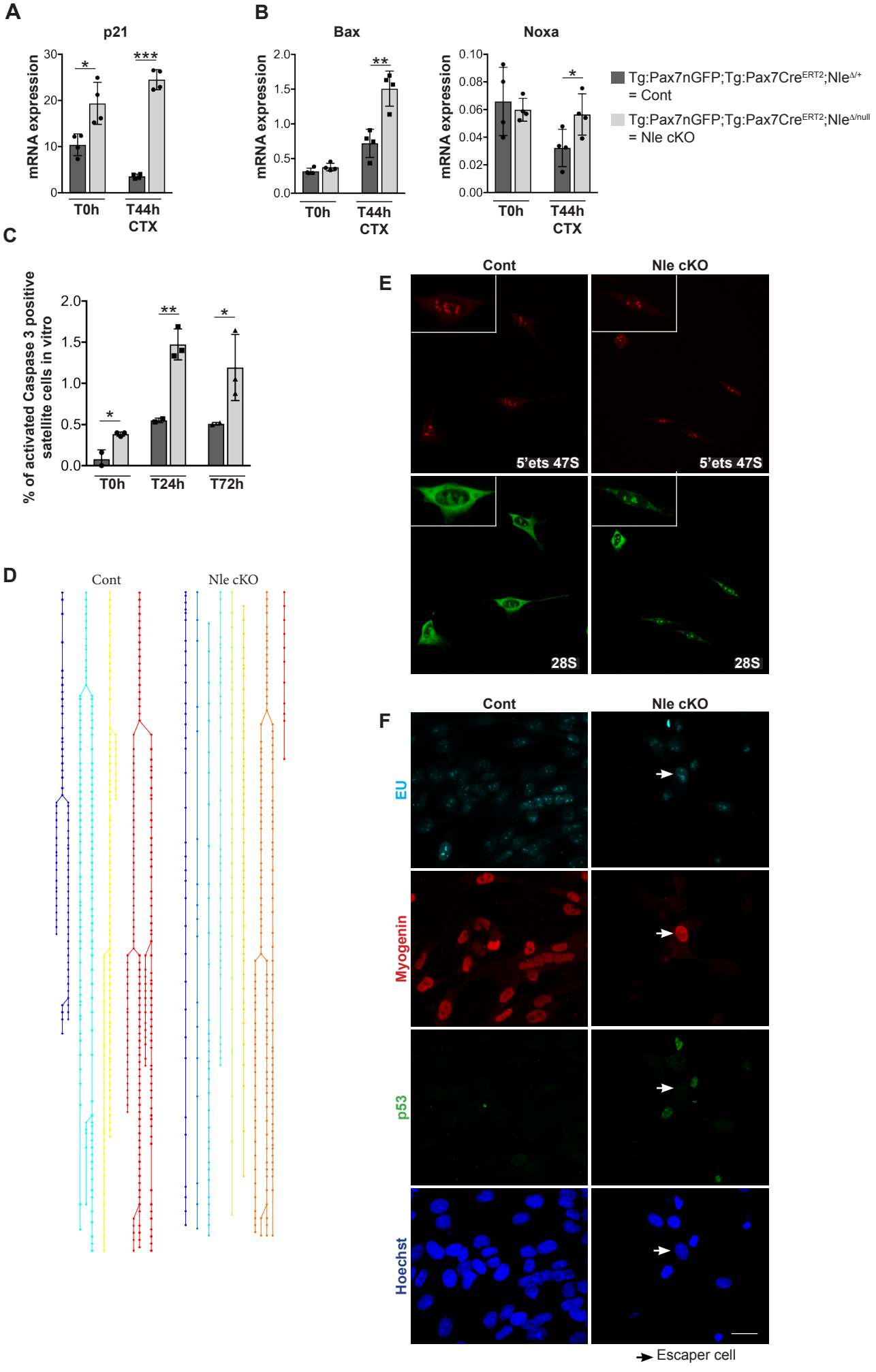


Figure S4. Apoptosis and nucleolar integrity of Nle cKO cells.

A) Quantification by RT-qPCR of *p21* mRNA expression in quiescent and cardiotoxin-activated Cont and Nle cKO cells.

B) Quantification by RT-qPCR of apoptotic genes *Bax* and *Noxa* mRNA expression in quiescent and cardiotoxin-activated Cont and *Nle* cKO cells.

C) Percentage of activated-Caspase 3 positive satellite cells at T0h, T24h and T72h after in vitro culture.

D) Cell tracking overview of Cont (Movie 1) and *Nle* cKO (Movie 2) cells. Note that Cont cells divide several times and most *Nle* cKO cells do not divide. Among *Nle* cKO cells, one cell is undergoing cell division and corresponds likely to a non-recombined escaper cell.

E) Representative images of rRNA FISH in cultured Cont and *Nle* cKO cells at T24h. The 5'ets probe hybridized only unprocessed 47S rRNA located in nucleoli. The 28S probe localized rRNAs in all cellular compartments. Both show a similar well defined nucleoli in Cont and *Nle* cKO cells.

F) Representative staining of 5-Ethynyl–Uridine (EU) staining of cells cultured for 4 days. Both Cont and *Nle* cKO cells show a nucleolar staining for newly generated rRNAs after 4h pulse with EU. After 4d in culture, only Cont cells expressed the differentiation marker Myogenin, while *Nle* cKO cells are positives for p53. Arrow indicates an escaper cell, positive for Myogenin and negative for p53.

For RT-qPCR 3-4 mice were used for each time point; TBP was used as reference gene. Scale bar, 20μm

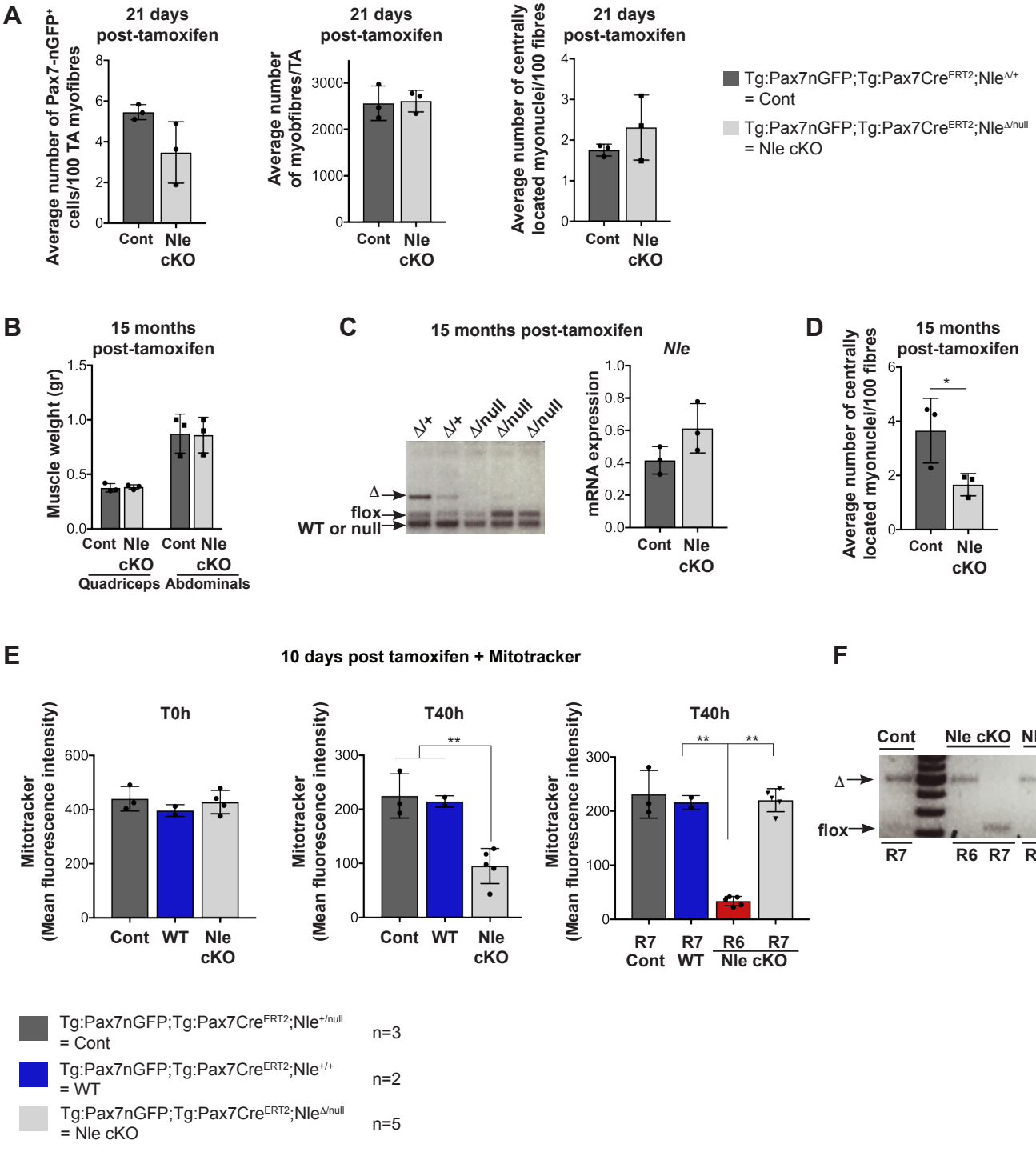


Figure S5. Long-term loss of *Nle* cKO satellite cells during homeostasis.

A) Analysis of satellite cells and myofibres 21 days post-tamoxifen treatment.

B) Quadriceps and abdominal muscle weight 15-months post-tamoxifen treatment.

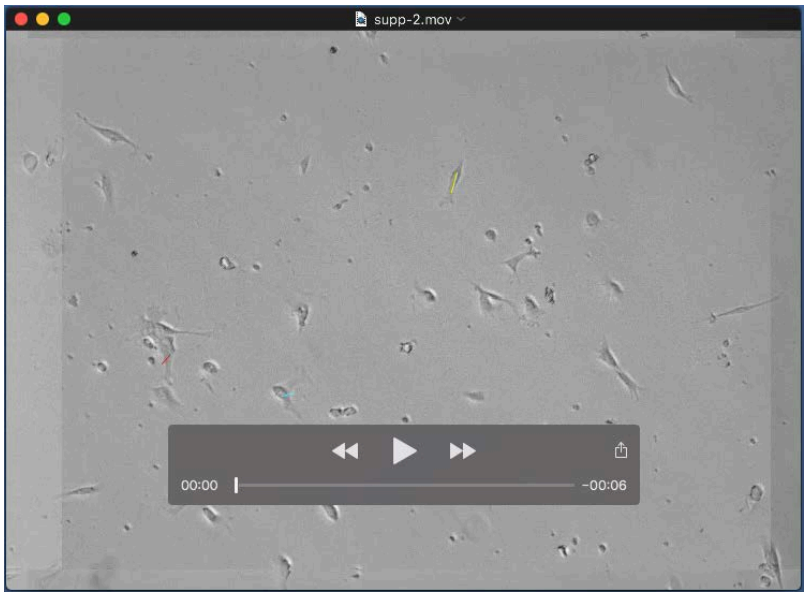
C) Representative genomic PCR of isolated Pax7-nGFP+ satellite cells from 15-month post-tamoxifen treated muscles. Only the non-recombined allele was found 15-months post-tamoxifen treatment, indicating the prevalence of escaper cells after a long period in homeostatic muscles. Expression of *Nle* mRNA 15-months post-tamoxifen treatment (right graph).

D) Average number of centrally located myonuclei/100 TA myofibres from Cont and *Nle* cKO 15 months old mice. n=3 mice, around 2000 myofibres on 2 different sections were counted.

E) Mitochondria quantification on WT, heterozygous and mutant cells. The number of mitochondria was unchanged in controls (WT and flox/+) and mutant (Null/Δ) cells in quiescence but the number of mitochondria decreased dramatically in the *Nle* mutant (flox/Δ) cells (Red R6).

F) Genomic PCR of floxed and deleted (Δ) alleles of cells isolated by FACS after mitotrackerT staining. Only the R7 population was observed in Cont cells, showing the deleted allele (Δ/+). In *Nle* cKO cells, both R6 and R7 were observed. The R6 population had a low mitochondrial content, showed the deleted (Δ) allele, and corresponded to the (*Nle* null cells), whereas R7 represents a minor population with a mitochondrial content equivalent to control and showed the floxed allele corresponding to escaper cells.

Movie 1. Time lapse imaging of Nle cKO cells in culture after isolation by FACS.



Movie 2. Time lapse imaging of control satellite cells in culture after isolation by FACS.

Table S1. Sequences of RT-qPCR primers

Target gene	Forward primer	Reverse primer
Nle	TCCTTAGCATATCCTGGTCCC	GCACTCGGGGTTCATGTGA
its1	TCTGACCTCGCCACCCTA	CCTCGTAGACACGGAAGAGC
Its2	TGTGTGTGTTTGGGTCTTGC	GGATACCACCTCTCTCCGTTC
18S	CGGCTACCACACATCCAAGGAA	GCTGGAATTACCGCGGCT
28S	TCATCAGACCCCAGAAAAGG	GATTCGGCAGGTGAGTTGTT
CyclinE	CAGTCCGCTCCAGAAAAAGG	GGTCCACGCATGCTGAATTA
CyclinD1	CACACGGACTACAGGGGAG	CACAGGAGCTGGTGTTCCAT
TroponinT	CCTCATTGACAGCCACTTTG	TCTCAGCGCAATTCTTTG
p21	CCCTCTATTTTGGAGGGTTAATCT	GTACCCTGCATATACATTCCCTTC
Bax	GCCGGAGCTACCTGTTTTTAG	GACAAAGCCACACATGACCAG
Noxa	GCAGAGCTACCACCTGAGTTC	CTTTTGCGACTTCCCAGGCA
TBP	AGAACAATCCAGACTAGCAGC	GGGAACTTCACATCACAGCTC

Target gene	Taqman primer
Myogenin	Mm00446195_g1
Myod	Mm01203489_g1
Myf5	Mm00435125_m1
HeyL	Mm00516555_m1
TBP	Mm00446971_m1

Key resources

Antibody	Reference	Dilution
Anti GFP, Chicken polyclonal	Abcam, ab13970	1:1000
Anti-Laminin, Rabbit polyclonal	Sigma, L9393	1:400
Anti-Myogenin, Mouse monoclonal	DSHB, F5D	1:50
Anti-Pax7, Mouse monoclonal	DHSB, Pax7	1:50
Anti-Myod, Mouse monoclonal	Dako, Clone 5.8A, M3512	1:100
Anti-phospho-S6 RP, Rabbit polyclonal	Cell Signaling, 2F9, 4856	1:2000
Anti-S6 RP, Rabbit polyclonal	Cell Signaling, 5G10, 2217	1:2000
Anti-Notchless, Rabbit Polyclonal	(Le Bouteiller et al., 2013)	1:100

Mice	References
Tg:Pax7-nGFP	(Sambasivan et al., 2009)
Tg:Pax7-Cre^{ERT2}	(Mourikis et al., 2012)
Nle$^{flox/null}$	(Cormier et al., 2006)
Tg:HSA-Cre	(Miniou et al., 1999)