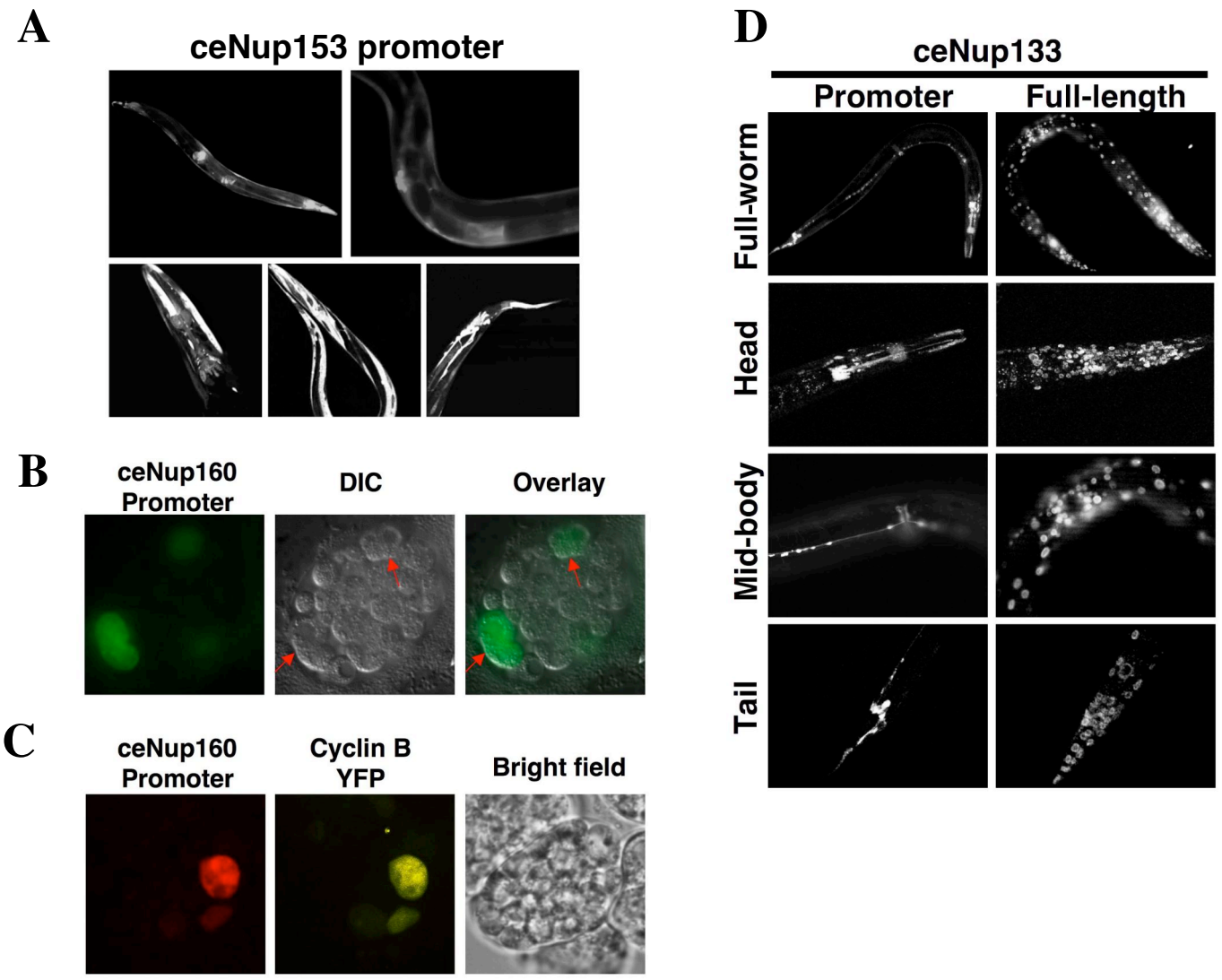
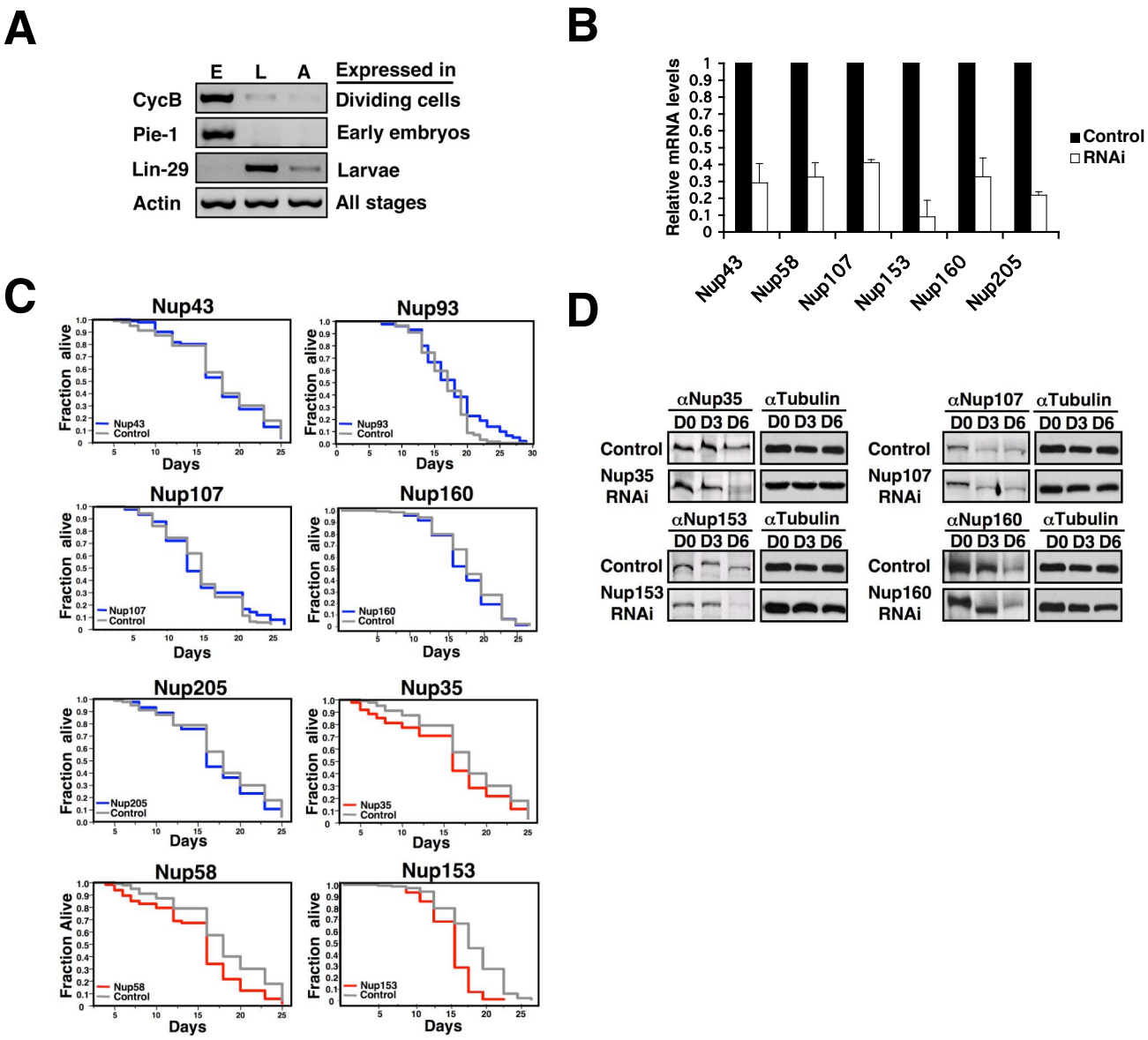


Supplemental figure 1. Analysis of C. elegans transgenic strains

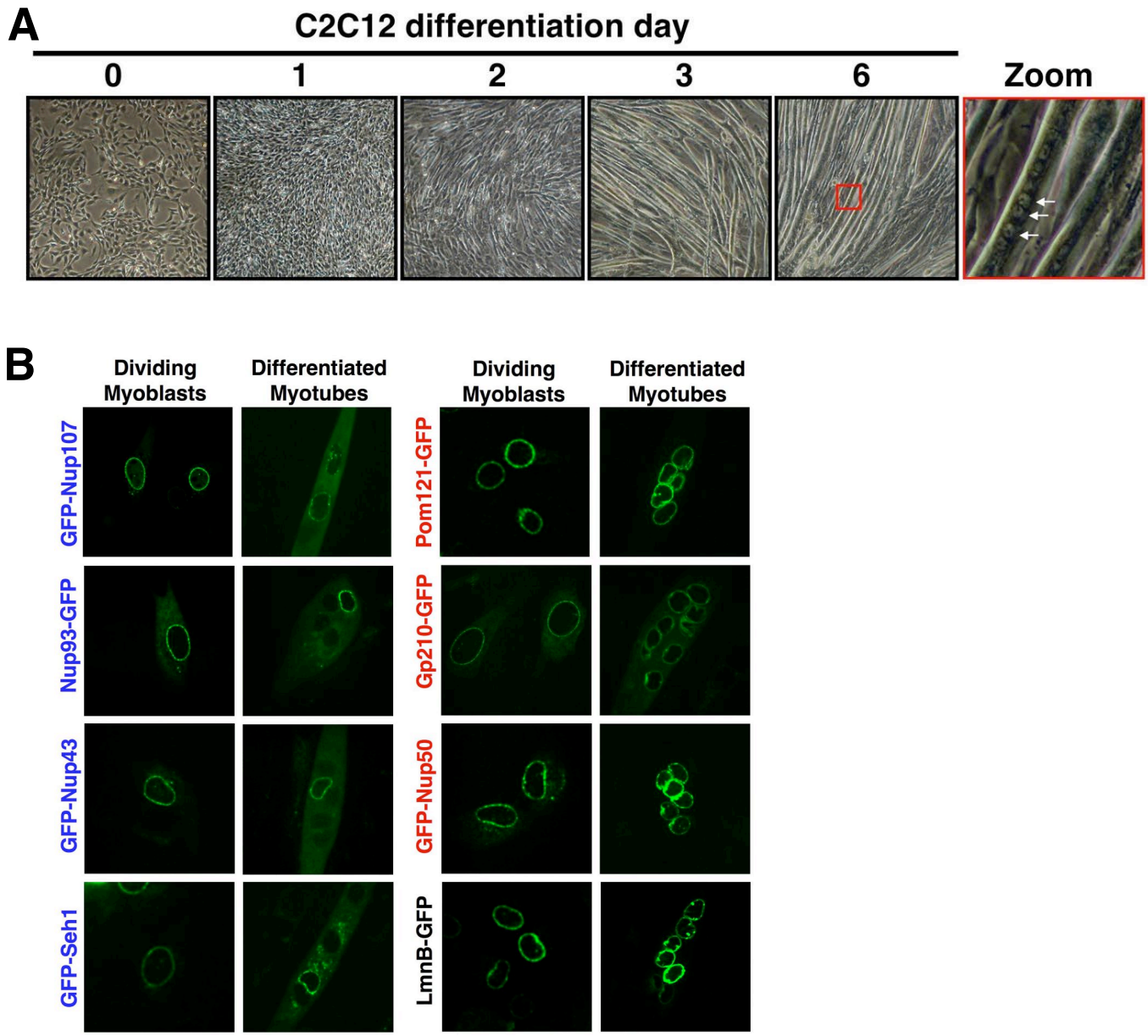
(A) The activity of the ceNup153 promoter was analyzed by microscopy in transgenic worms expressing *GFP* under the control of nucleoporin promoter. Images show expression of ceNup153 in adult worms. Nup153 is expressed in all tissues with a higher expression in pharinx, nervous system and body wall muscle. The activity the of ceNup153 promoter was also detected in intestinal cells at lower levels. The lack of expression in the germline is likely due to transgene silencing. **(B)** The activity of the ceNup160 promoter was analyzed by microscopy in transgenic worms expressing *GFP* under the control of nucleoporin promoter. Arrows show the expression of *GFP* in dividing embryonic cells. **(C)** To confirm that ceNup160 is expressed in dividing cells a new transgenic worm was generated by injecting a worm line expressing Cyclin B-YFP under its own promoter with a vector expressing the Tomato fluorescent protein under ceNup160 promoter. The confocal images show that the activity of ceNup160 promoter correlates with the expression of Cyclin B. **(D)** *C. elegans* N2 wild type strain was injected with a vector expressing Tomato under the control of either the *ceNup133* promoter (Promoter) or with a vector expressing ceNup133-Tomato under its endogenous promoter (Full-length). Expression of the reporter protein was analyzed by microscopy. Note that while ceNup133 promoter is only active in a few neurons, the pharinx and a few cells from the vulva, the protein is present in every nuclei of the adult worm

D'Angelo et al, Supplemental Fig. 1



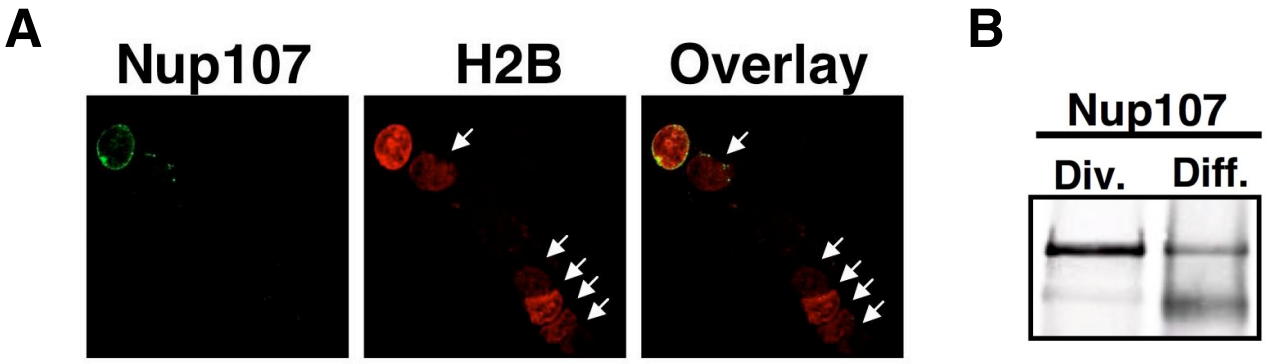
Supplemental figure 2. Expression of core nucleoporins is not required during adulthood
(A) *C. elegans* developmental stage controls. RNA was extracted and cDNA was prepared from SS104 embryos (E), synchronized larvae (L) or adult worms (A). Cyclin B (CycB, *CYB-1*) was used as a marker for proliferating stage, *PIE-1 (Pie-1)* is an early embryonic marker and *LIN-29 (Lin-29)* has increasing expression from L1 to L4 that decreases in adulthood. Actin (*ACT-1*) was used as loading control. **(B)** SS104 adult day 1 worms were grown in the corresponding RNAis for 6 days. Total RNA was extracted and the efficiency of RNAi knock-down was verified by Q-PCR analysis. mRNA levels were normalized to the empty vector. **(C)** Lifespans of wild type N2 worms grown on bacteria expressing empty vector (gray lines), scaffold nucleoporins ceNup43, ceNup93, ceNup107, ceNup160 or ceNup205 (blue lines) or dynamic nucleoporins ceNup35, ceNup58 or ceNup153 (red lines). All statistical data can be found in supplementary Table 2. **(D)** Total protein was extracted from sterile SS104 adults worms (25°C) subject to the indicated RNAis for 3 or 6 days and protein levels were assayed by western blot. Tubulin was used as loading control.

D'Angelo et al, Supplemental Fig. 2



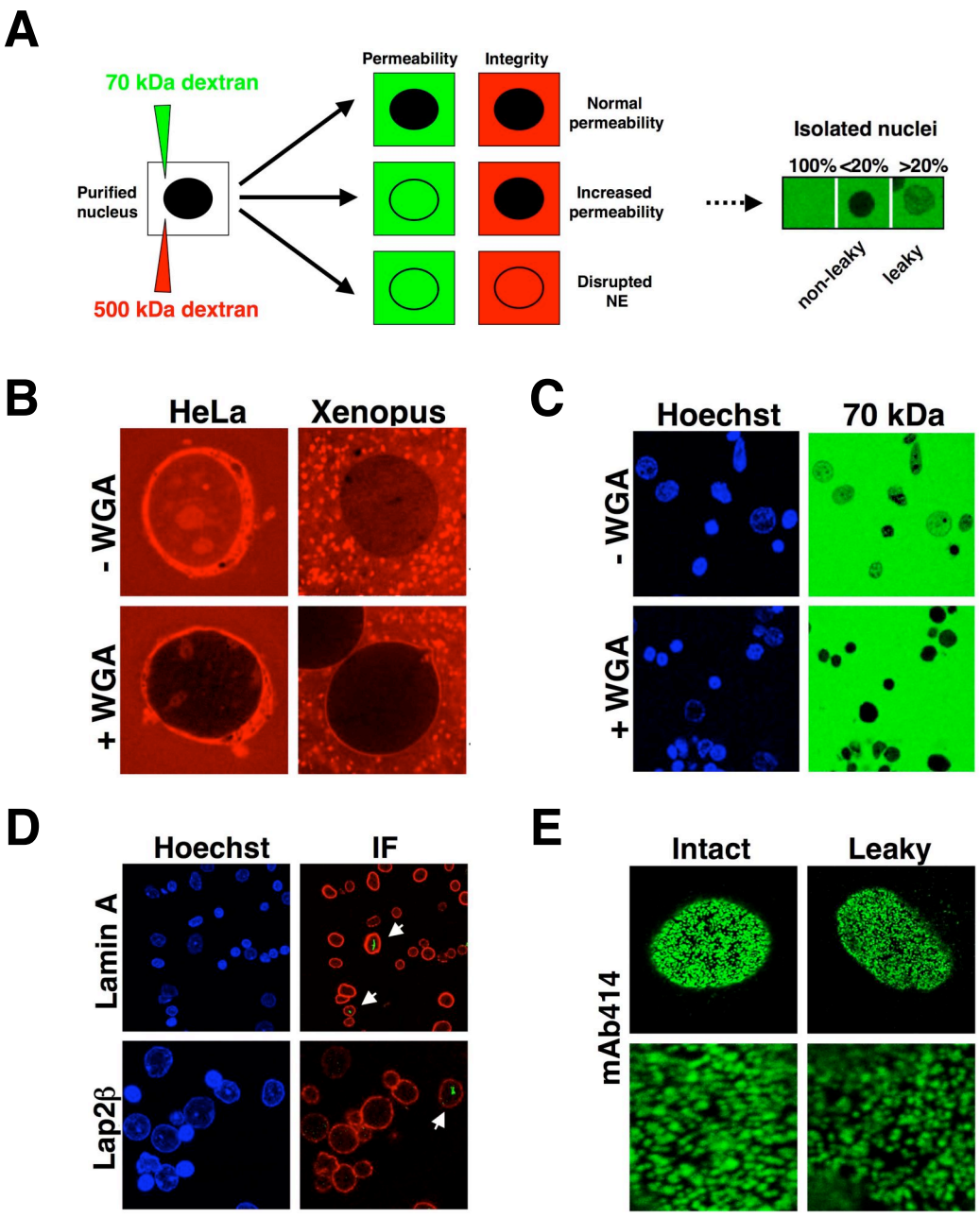
Supplemental figure 3. Nucleoporin expression levels during C2C12 differentiation
(A) Differentiation of C2C12 myoblasts into myotubes. C2C12 myoblast were grown to 90% confluency and induced to differentiate into myotubes in low serum containing media. Bright field images were taken at the indicated days. Zoom image shows a magnification of a day 6 myotube. Arrowheads indicate nuclei inside the same cell. (**B)** C2C12 myoblast were transfected with GFP-Nup107, Pom121-GFP and Lamin B1-GFP expression vectors and the localization of the tagged proteins in dividing myoblasts and differentiated myotubes was analyzed by live confocal microscopy. The published residence times at the NPC for the analysed proteins are Nup107 40hs, Nup93 70 hs, Nup43 61 hs, Seh1 40 hs, Pom121 21 hs, Nup50 20 sec and 3.3 min, Gp210 4.4 min, Lamin B >45 hs (Daigle et al., 2001; Rabut et al., 2004b).

D'Angelo et al, Supplemental Fig. 3



Supplemental figure 4. Analysis of H2B and Nup107 in post-mitotic myotubes
(A) Proliferating myoblasts were co-transfected with GFP-Nup107 and H2B-Tomato expressing vectors. Transfected myoblasts were induced to differentiate and fuse with untransfected cells. Protein exchange between nuclei was analyzed using confocal microscopy. Arrows show myotube nuclei that came from untransfected myoblasts and have exchanged H2B but not Nup107. **(B))** Nup107 protein was inmmunoprecipitated from the cytoplasm of dividing and differentiated C2C12 cells and protein levels were analyzed by western blot. Quantification showed that 54.1 ± 9.7 % of Nup107 is degraded in postmitotic cells compared to 10.6 ± 1.8%in dividing cells.

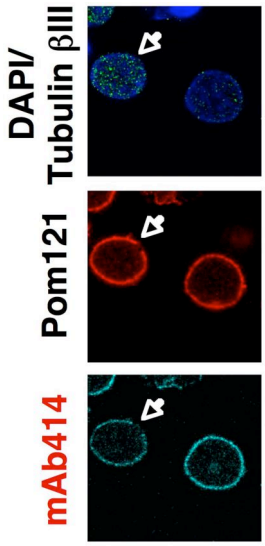
D'Angelo et al, Supplemental Fig. 4



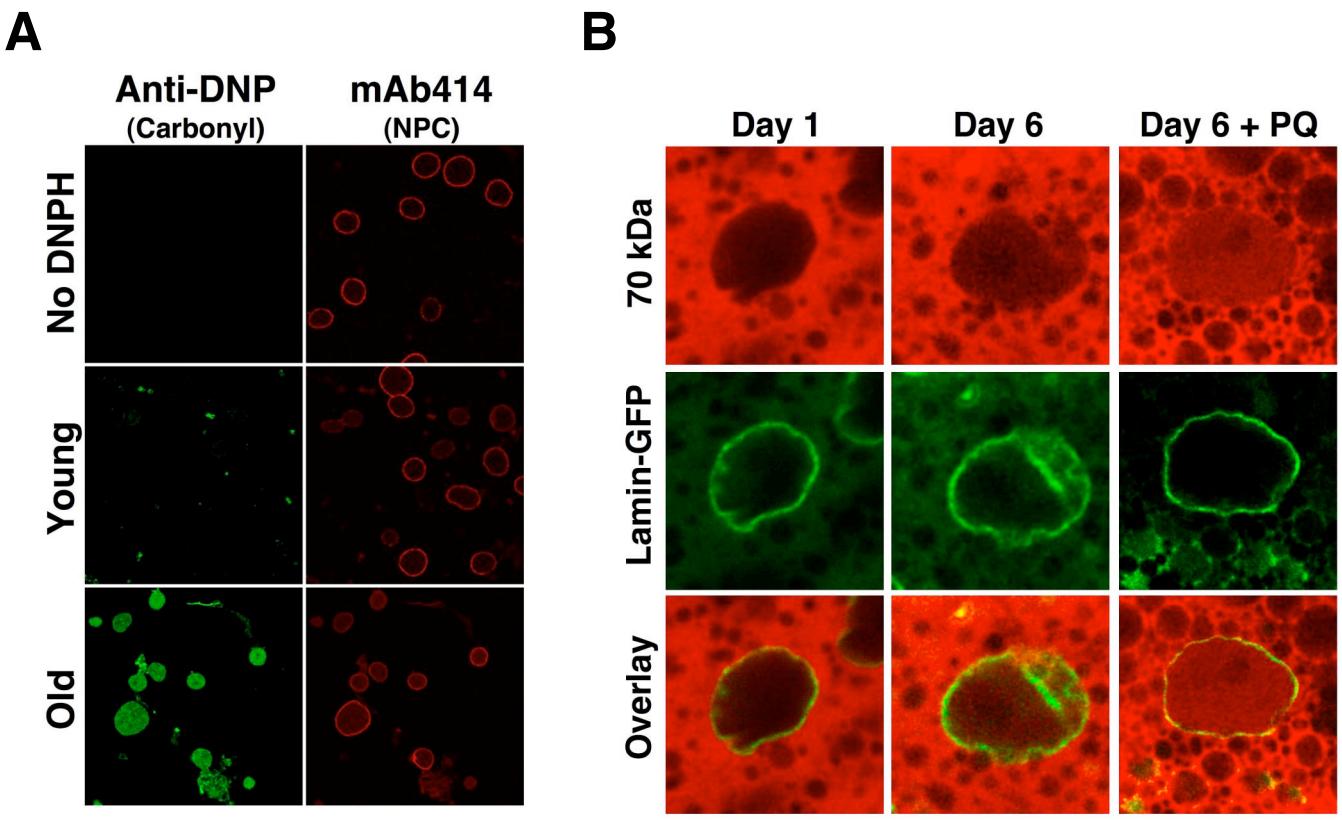
Supplemental figure 5. Nuclear permeability assays and structural analysis of isolated post-mitotic nuclei.

(A) Scheme of the assay used to analyze the permeability barrier of isolated nuclei. Nuclei with a nuclear fluorescent signal >20% of the extranuclear one were considered leaky. **(B)** Isolated HeLa or in vitro assembled nuclei were pre-bloked with a combination of byotinilated-WGA and streptavidin to plug pores and then incubated with a 10kDa fluorescent dextran. Diffusion of the dextran inside the nucleus was analyzed by microscopy. **(C)** The permeability of nuclei isolated from brains of old rats to a 70kDA fluorescent dextran was analyzed in the presence or absence of a WGA to block pores. **(D)** The structure of the nuclear lamina and distribution of the inner nuclear membrane protein Lap2β was analyzed by immunofluorescence in nuclei purified from brains of old rats. Arrows show leaky nuclei with intranuclear tubulin aggregates (green). **(E)** NPc distribution in intact and leaky nuclei, identified by the presence of intranuclear tubulin, was analyzed by immunofluorescence using the mAb414 antibody.

D'Angelo et al, Supplemental Fig. 5



Supplemental figure 6. Analysis of Pom121 and FG-nucleoporins levels in old nuclei.
Nuclei were isolated from brains of old (28 months) rats, fixed and stained with antibodies against tubulin βIII, Pom121 and FG-nucleoporins (mAb414). The staining of intact and leaky nuclei (the latter identified by the intranuclear accumulation of intranuclear tubulin βIII) was compared.



Supplemental figure 7. Characterization of oxidative protein damage levels in young and old post-mitotic cells and its effect on nuclear permeability i*n vivo*.
(A) Nuclei isolated from brains of young and old rats were fixed and treated with 2,4-dinitrophenylhydrazine to derivatize carbonyl groups to 2.4-dinitrophenylhydrazone (DNP-hydrazone). The DNP-derivatized proteins were detected by immunofluorescence using an anti-DNP antibody. **(B)** Adult day 1 worms expressing a Lamin-GFP fusion or adult day 6 worms grown in the presence or absence of 1mM paraquat were injected in the intestine with a 70 kDa fluorescent dextran. Twenty minutes after injection, the diffusion of the dextran inside the nucleus was analyzed by fluorescence microscopy using Lamin as a NE marker. Images show a crossection of intestinal nuclei. Note that in nuclei that have an increased permbeanility show an intranuclear accumulation of the 70 kD dextran with a clear exclusion from the nucleolus.

D'Angelo et al, Supplemental Fig. 6