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14. ABSTRACT
Skeletal muscle homeostasis is the result of proper maintenance of differentiated muscle fibers and continuous repair of muscle tissue by satellite cells. Alterations in both, or either, of these processes results in progressive muscle damage leading to excessive loss of muscle mass and/or to the development of muscular dystrophies. The identification and characterization of the basic regulators of skeletal muscle maintenance is critical to comprehend the mechanisms that lead to the deterioration of muscle integrity, and a necessary step in the development of therapies to enhance muscle performance and treat muscle diseases.
In our previous work we discovered that the nuclear pore complex component Nup210 is an important regulator of muscle physiology. Of particular importance for this project, we identified that mice lacking Nup210 show a progressive deterioration of muscle structure and function. Yet, the muscle processes that are affected by the absence of Nup210, and the causes of such muscle alterations are poorly understood.

15. SUBJECT TERMS
Musculoskeletal Disorders - Tissue Regeneration

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TABLE OF CONTENTS

1. INTRODUCTION

Muscle growth, maintenance and repair in adulthood requires the constant regeneration of muscle fibers. As we age or in pathological conditions, the deterioration of muscle maintenance mechanisms contributes to the loss of muscle mass (sarcopenia (1)), to reduced muscle repair in response to injury, and to the development of muscular dystrophies. Understanding the basic regulators of skeletal muscle homeostasis is crucial to prevent muscle wasting and to develop new therapies to promote muscle regeneration and treat muscle disorders. Our previous work uncovered that Nup210, a tissue-specific nuclear pore complex (NPC) component, plays a critical role in myoblast differentiation, and in the maturation and survival of skeletal muscle fibers. We also identified that Nup210 knockout mice show progressive deterioration of skeletal muscle structure and function, with features reminiscent of inflammatory muscle disorders. The main goals of this proposal are to understand in detail the skeletal muscle alterations that result from Nup210 depletion, to identify their causes, and to establish if increasing Nup210 levels can promote muscle repair and function in healthy mice and in a model of Duchenne Muscle Dystrophy (DMD).

2. KEYWORDS

Nuclear pore complex, Nup210, skeletal muscle, regeneration, repair, muscle dystrophy, inflammation, vacuoles, immune system

3. ACCOMPLISHMENTS

What were the major goals of the project?
Our hypothesis, based on our previous findings, is that Nup210 is an important regulator of muscle maintenance and that increasing its activity can enhance muscle repair and function. The overarching goal of this project is to establish how inhibiting Nup210 affects the integrity and function of skeletal muscle and to define if modulating its levels could be exploited to enhance muscle performance and regeneration. Below is the list of the major goals of the project pertaining to this reporting period as stated in the project timeline approved SOW and:

Aims/Tasks	Months
Specific Aim 1: Characterize the age-dependent deterioration of skeletal muscle in Nup210 knockout animals.	
Major Task 1: Employ immune profiling, histological, and functional analyses to characterize in detail the age-dependent alterations in the skeletal muscle tissue of Nup210 knockout animals and determine if they represent features of inflammatory myopathies, particularly sIBM	
Subtask 1: Histological characterization of different muscles from control and $Nup210^{-/-}$ mice of different ages.	4-6
Subtask 4: Establish if Nup210 knockout muscles have abnormal fiber type distribution	2-4
Specific Aim 2: Identify the origin/s of muscle alterations in $Nup210^{-/-}$ animals.	
Major Task 2: Combine tissue-specific knockouts in which Nup210 is specifically ablated either in the immune system or in skeletal muscle, with pharmacological interventions to determine the contribution of Nup210 malfunction in each tissue to skeletal muscle degeneration. Investigate if Nup210 loss results in a progressive deterioration of muscle stem cell function and/or in the maturation and survival of differentiated muscle cells.	

Subtask 5: Characterize the function of young and old muscle stem cells and determine if Nup210-depleted differentiated muscle cells have alterations in growth and maturation	6-8
Specific Aim 3: Define if Nup210 up-regulation can stimulate muscle regeneration.	
Major Task 3: Generate and use different mouse models that allow to increase Nup210 levels in skeletal muscle to evaluate if increasing its activity can enhance the endurance, strength, and regeneration capacity of healthy and dystrophic muscle.	
Subtask 1: Generate a conditional Nup210 knockin mouse line (*CRE/C57BL6J$^{wt/CAG\text{-}loxP\text{-}Stop\text{-}loxP\text{-}Nup210}$*)	6-9
Subtask 2: Breed conditional Nup210 knockin mouse line with muscle-specific constitutive and inducible CRE lines (*HSA1-CRE* and *HSA1-CRE^{ERT2}*)	6-8
Subtask 3: Characterize mouse lines overexpressing Nup210 in muscle	2-3

What was accomplished under these goals?

Major Task 1: Employ immune profiling, histological, and functional analyses to characterize in detail the age-dependent alterations in the skeletal muscle tissue of Nup210 knockout animals and determine if they represent features of inflammatory myopathies, particularly sIBM

Subtask 1: Histological characterization of different muscles from control and Nup210$^{-/-}$ mice of different ages.

The specific objective is to characterize the muscle alterations that result from the ablation of the nuclear pore complex component Nup210. The major activities for this goal have been to examine sections from different muscles of control and Nup210 knockout mice of different ages to evaluate alterations tissue integrity. For the past year we have increased the number of animals analyzed to determine obtain results of statistical significance. These additional studies confirmed that young Nup210$^{-/-}$ mice show no obvious differences in the size, structure, or cross-sectional area (CSA) of quad and TA muscles compared to control mice (Fig 1A-D). The lack of muscle alterations in young Nup210$^{-/-}$ mice is consistent our previous findings in Zebrafish showing that this nucleoporin is not required during embryonic muscle development (2). Our previous studies using the Zebrafish model system, had shown that while fish lacking Nup210 do not have alterations in embryonic muscle development, they show a progressive deterioration of skeletal muscle structure as they age. So far, our studies in mice show that by 6-9 months of age, Nup210$^{-/-}$ mice have a significant increase in the percentage of myofibers with centrally located nuclei compared to controls, but no changes in the total number of myofibers (Fig 2A-C). A similar increase was observed in TA muscles (Fig 2D). We found that the higher number of centrally nucleated fibers is also present in the muscles of Nup210$^{f/f}$/Acta1-Cre mice, in which Nup210 is specifically depleted in differentiated adult skeletal muscle cells, indicating that this is a consequence of muscle-intrinsic alterations (Fig 2E). Like constitutive Nup210 knockout mice, these animals showed no significant differences fiber size distribution with control mice (Fig 2F, G) (These experiments are also related to Specific Aim 2, Subtask 4).

Subtask 4: Establish if Nup210 knockout muscles have abnormal fiber type distribution

Our previous work using the C2C12 myogenic model identified that Nup210 regulates the expression of muscle genes by modulating the activity of the Mef2C transcription factor (2). Mef2C is a critical regulator of muscle fiber type composition that is preferentially active in slow oxidative type I fibers (3-5). Depleting Mef2C was found to reduce the number of type I fibers in mice, while increasing its expression levels has the opposite effect (3, 6, 7). The specific objective of this goal is to determine if Nup210 knockout mice shown alterations in their muscle fiber type distribution. The major activities of this aim have been so set up the conditions for muscle staining with fiber type-specific myosin isoforms and to quantify different muscle fibers in soleus of control and Nup210 knockout animals of different ages. To investigate if Nup210-deficient mice show alterations in fiber type composition, in funding years one and two we have performed immunofluorescence for fiber type-specific

myosin isoforms (MyHC-I, MyHC-IIa, and MyHC-IIb) in sections of soleus, a muscle with a large fraction of type I fibers. We found that while young (6-8 weeks old) animals show no significant differences in fiber type distribution (data not shown), 6-9 months old Nup210 knockout mice have a significant decrease in the number of type I fibers and an increased number of type II fibers, particularly type IIx fibers, compared to control mice (Fig 3A-C). The total number of fibers in the soleus of both mice was not different (Fig 3D). Further characterization of soleus muscles exposed an increase in fiber size that was not restricted to a specific fiber type and was observed in both, type I and II fibers (Fig 3E, F), indicating a modest hypertrophy of the soleus muscle. Our previous experiments exposed that Nup210 knockout mice tend to run shorter distances than control mice. Because running has been shown to induce a change in muscle fiber type distribution associate with increased endurance, we tested whether Nup210 knockouts were unable to undergo this switch. For this, control and Nup210 knockout mice were subjected to voluntary running for 5 weeks and fiber type was analyzed in soleus muscle as described above. How running affects fiber type distribution in the soleus muscle of mice is controversial with some studies showing increase in fiber type I while other show increased fiber type II. Our studies in control animals show decreased number of type I fibers and increased type II after voluntary running support the later model. We also determine that Nup210 knockout mice show the same fiber type distribution than control mice, indicating that not only these animals are able to undergo fiber type switching, but also that running seems to decrease the fiber type alterations that result from Nup210 knockout (Fig 4A, B).

Major Task 2: Combine tissue-specific knockouts in which Nup210 is specifically ablated either in the immune system or in skeletal muscle, with pharmacological interventions to determine the contribution of Nup210 malfunction in each tissue to skeletal muscle degeneration. Investigate if Nup210 loss results in a progressive deterioration of muscle stem cell function and/or in the maturation and survival of differentiated muscle cells.

Subtask 5: Characterize the function of young and old muscle stem cells and determine if Nup210-depleted differentiated muscle cells have alterations in growth and maturation.

The specific objective is to establish if the muscle alterations that result from the ablation of the nuclear pore complex component Nup210 are due to abnormalities in the differentiation of muscle progenitor cells or the maturation of differentiated muscle fibers. Our previous work identified that Nup210 is required for the differentiation of the C2C12 myogenic cell line (8). Downregulation of Nup210 in C2C12 myoblasts strongly inhibits their ability to form myotubes in vitro (8). The major activities for this goal have been to investigate their ability of muscle stem cells from control and Nup210 knockout mice to differentiate in vitro. For this, satellite cells were isolated from muscles of young mice and grew them in differentiation media to induce the formation of myotubes as described by Tierney *et al* (9). Consistent with our previous findings using the C2C12 myogenic model, we found that Nup210 is expressed in differentiated myotubes but not in satellite cells/myoblasts (Fig 5A). But in contrast to Nup210-depleted C2C12 cells, satellite cells from *Nup210*$^{-/-}$ mice were able to form myotubes *in vitro* (Fig 5B). Moreover, *Nup210*$^{-/-}$ satellite cells showed no significant decrease in their ability to differentiate compared to muscle progenitors isolated from control mice (Fig 5C). Because Nup210 is constitutively knocked out in these animals, it is possible that compensation during embryonic development could mask myogenic differentiation defects in these animals. To test this possibility, we acutely inactivated Nup210 only in satellite cells before inducing differentiation. For this, we employed a conditional gene targeting approach in which we crossed a satellite-cell specific Cre transgenic mouse line, Pax7-CreER^{T2} (10), with mice carrying a floxed Nup210 allele (11). In the *Nup210*$^{f/f}$/Pax7-CreER^{T2} line, Nup210 ablation in satellite cells is induced by tamoxifen treatment. Control (*Nup210*$^{+/+}$/Pax7-CreER^{T2}) and *Nup210*$^{f/f}$/Pax7-CreER^{T2} mice were injected with tamoxifen (3 times every other day) to knockout Nup210, and satellite cells were isolated 48 hours after the last injection. Satellite cells were then cultured in differentiation conditions and their ability to form myotubes was quantified. Consistent with our findings using the constitutive knockout mice, satellite cells acutely depleted of Nup210 were able to efficiently form myotubes (Fig 5D, E). Nup210 staining after differentiation confirmed the tamoxifen-induced knockout of this nucleoporin (Fig 5D). These findings indicate that Nup210 is not required for muscle development or adult satellite cell differentiation.

Our findings indicate that while *Nup210*$^{-/-}$ mice show no muscle alterations in homeostatic conditions, they have delayed regeneration and the accumulation of centrally nucleated fibers with age. These findings suggest that even though satellite cells from young animals can differentiate in vitro and reconstitute muscle in vivo (Fig 6A), the activity of these cells might become compromised with age possibly due to their continuous need for muscle maintenance. To investigate if continuous muscle challenge could exacerbate the repair defects of *Nup210*$^{-/-}$ mice, we subjected these animals to serial muscle injury. For this, TA muscles of control and *Nup210*$^{-/-}$ mice were injured with $BaCl_2$ three times sequentially. Muscle injuries were spaced by 25 days to allow full muscle recovery before the next injury round, and muscle regeneration was analyzed 25 days after the last injury by measuring CSA. Under these conditions, *Nup210*$^{-/-}$ mice also showed a delay in muscle recovery (Fig 6B). This difference in myofiber CSA was similar to that observed in animals subjected to single injury, indicating no progressive deterioration of satellite cell function with continuous challenge. Altogether, these findings indicate that the alterations in muscle repair observed in Nup210 knockout animals do not result from a decreased myogenic potential of skeletal muscle satellite cells.

Major Task 3: Generate and use different mouse models that allow to increase Nup210 levels in skeletal muscle to evaluate if increasing its activity can enhance the endurance, strength, and regeneration capacity of healthy and dystrophic muscle.

Subtask 1: Generate a conditional Nup210 knockin mouse line (*CRE/C57BL6J*$^{wt/CAG\text{-}loxP\text{-}Stop\text{-}loxP\text{-}Nup210}$*)* & Subtask 2: Breed conditional Nup210 knockin mouse line with muscle-specific constitutive and inducible CRE lines (*HSA1-CRE* and *HSA1-CRE*^{ERT2})

The <u>specific goals</u> of subtask 1 and 2 of this aim is to generate mouse lines that allow us to increase Nup210 levels in muscle and investigate if it can improve muscle function. The <u>major activities</u> have been to generate a mouse line that allow for the Cre-regulated ectopic expression of Nup210 and to cross these animals constitutive and inducible Cre mouse lines. During the past year we have successfully bred our *CRE/C57BL6J*$^{wt/CAG\text{-}loxP\text{-}Stop\text{-}loxP\text{-}Nup210}$ generated as part of this project with a constitutive (CMV-Cre) and an inducible (Cre^{ERT2}) mouse lines which we are using to establish how increasing the levels of this nucleoporin affect the integrity, regeneration and function of skeletal muscle.

Subtask 3: Characterize mouse lines overexpressing Nup210 in muscle & Subtask 4: Assess muscle regeneration in mouse lines overexpressing Nup210 in muscle (healthy and DMD)

The <u>specific goals</u> of subtask 3 and 4 of this aim are to characterize mouse lines overexpressing the nucleoporin Nup210 and to determine if higher levels of this nucleoporin con enhance muscle regeneration and function in health and disease. The <u>major activities</u> of this task have been to analyze the levels of and localization of Nup210 in muscle and other tissues of animal ectopically expressing an additional copy of this nucleoporin, and to investigate if higher levels of this protein increase muscle regeneration in normal mice. Using western blot analyses we determined that Nup210 is significantly overexpressed in muscle, spleen and liver of *CRE/C57BL6J*$^{wt/CAG\text{-}loxP\text{-}Stop\text{-}loxP\text{-}Nup210}$*CMV-Cre* mice (Fig 7A, B). Two copies of the ectopic expression cassette did not seem to significantly upregulate Nup210 levels over the heterozygous animals (Fig 7B), which is consistent with qPCR analyses of Nup210 mRNA levels in muscle and liver tissues (Fig 7C). The ectopic expression of Nup210 can also be observed in mouse embryonic fibroblast (Fig 7D). Fibroblasts do not normally express Nup210, but this nucleoporin can be detected colocalizing with the nuclear pore complex marker mAB414 in cells isolated from in mice carrying one copy of the overexpression cassette (Fig 7D). Preliminary experiments on quad muscles of Nup210 overexpressing mice show no significant differences in fiber size (Fig 8A). As a first approach to determine if increasing the levels of this nucleoporin could enhance muscle regeneration, we subject control and Nup210 overexpressing animals to muscle injury assays as described previously. Oud preliminary studies show that animals with increased levels of this nucleoporin do not seem to have faster regeneration than control animals (Fig 8B). Yet, analysis of Nup210 localization in quad muscles of overexpressing mice show strong cytoplasmic/endoplasmic reticulum signal suggesting that high level expression of this nucleoporin might result in mislocalization that could affect its function (Fig 8C). This is also observed in other tissues overexpressing Nup210 and it is also commonly seen with several nucleoporins (data not shown).

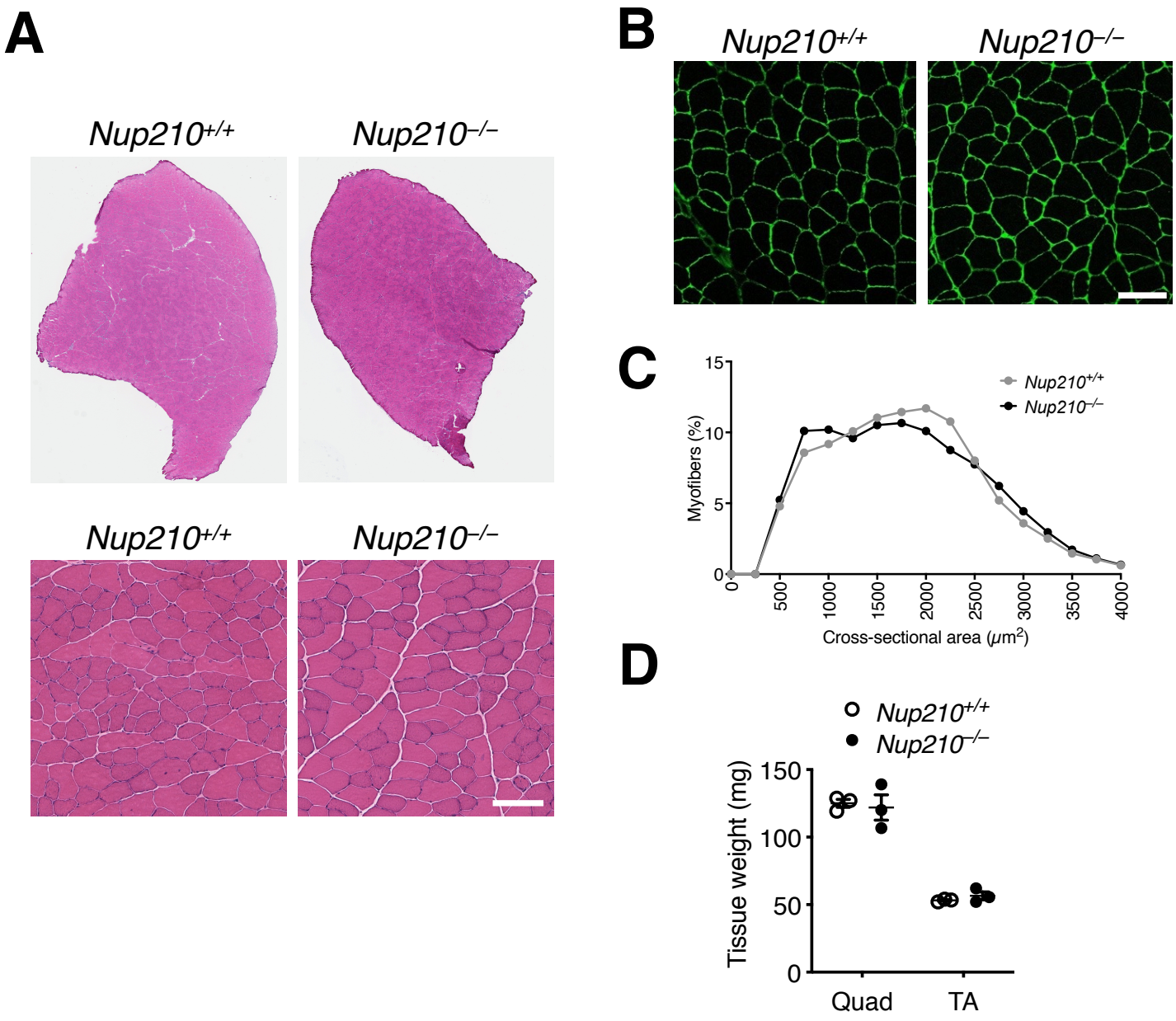

Figure 1: A) Representative images of H&E full projections (top) and detailed views (bottom) of TA muscle transverse sections isolated from 6- to 8-weeks-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice. Scale bar 100 µm. **B)** Representative images of immunofluorescence staining for Laminin in quadricep muscles from 6- to 8-week-old mice. Scale bar, 100 µm. **C)** Quantification of myofiber cross-sectional area distribution in quadricep muscles from 6- to 8-week-old mice (n=3). Data are binned in 250 µm2 bins and plotted as mean. **D)** Fresh tissue weights for Quad and TA muscles isolated from *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n=3).

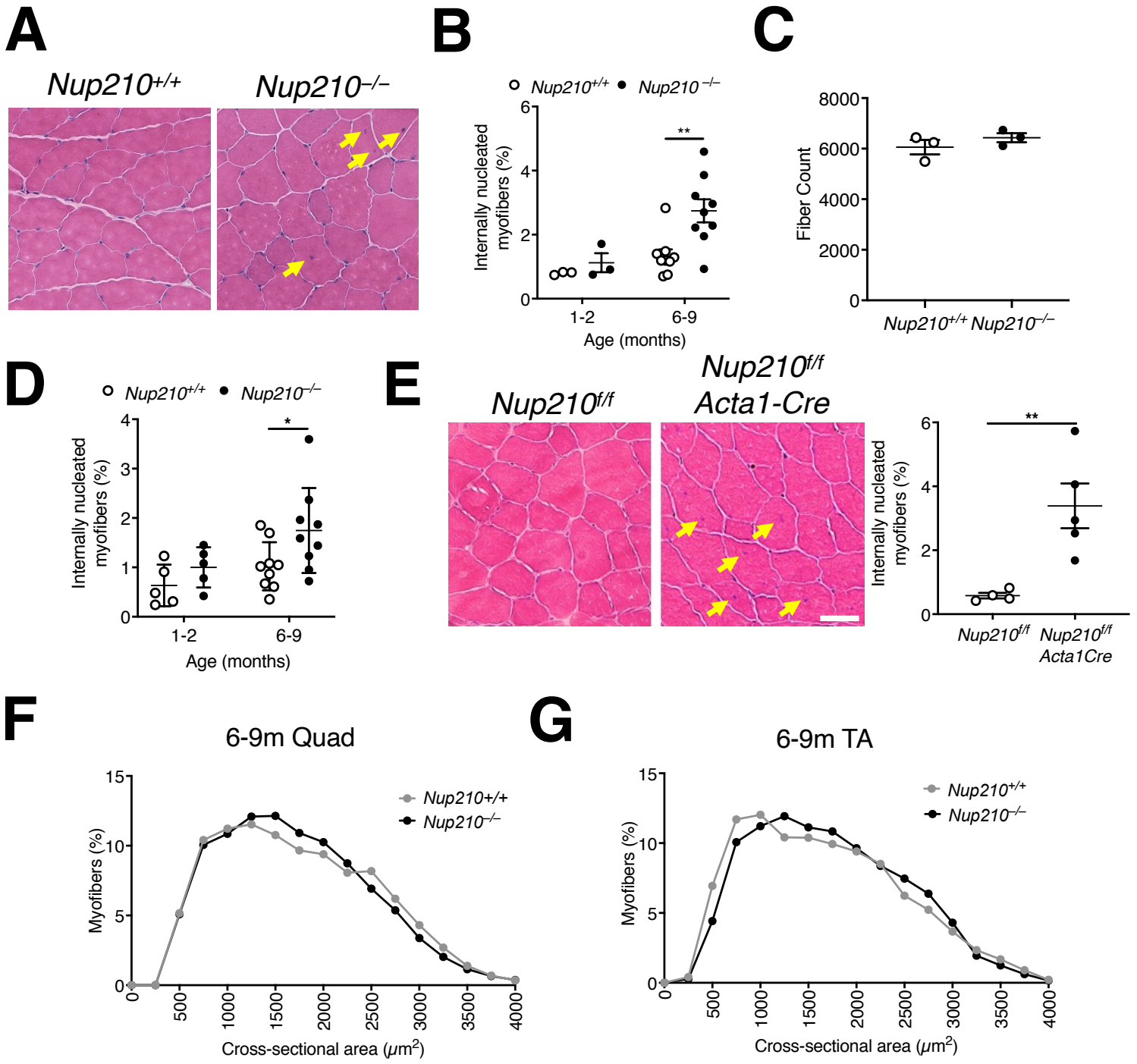

Figure 2: A) Representative H&E images (left) of quadricep muscle from 6- to 9-month-old Nup210$^{+/+}$ and Nup210$^{-/-}$ mice (n=3-9). Arrows denote centrally nucleated fibers. Scale bar 50 μm. **B)** Quantification of centrally nucleated myofibers from A. **C**) Quantification of total fiber count for quadricep muscles from 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 3). **D)** Quantification of centrally nucleated myofibers in TA muscle from *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 5–9). **E)** Representative H&E images (left) and whole section quantification of centrally nucleated myofibers (right) of quadricep muscle from 6- to 9-mo-old *Nup210*$^{f/f}$ and *Nup210*$^{f/f}$/Acta1-Cre mice (n = 4–5). Arrows denote centrally nucleated fibers. Scale bar 50 μm. **F)** Quantification of quadricep myofiber cross-sectional area isolated from 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 3). Data are binned in 250 μm^2 bins. **G)** Quantification of myofiber cross-sectional area distribution in TA isolated from 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 4).

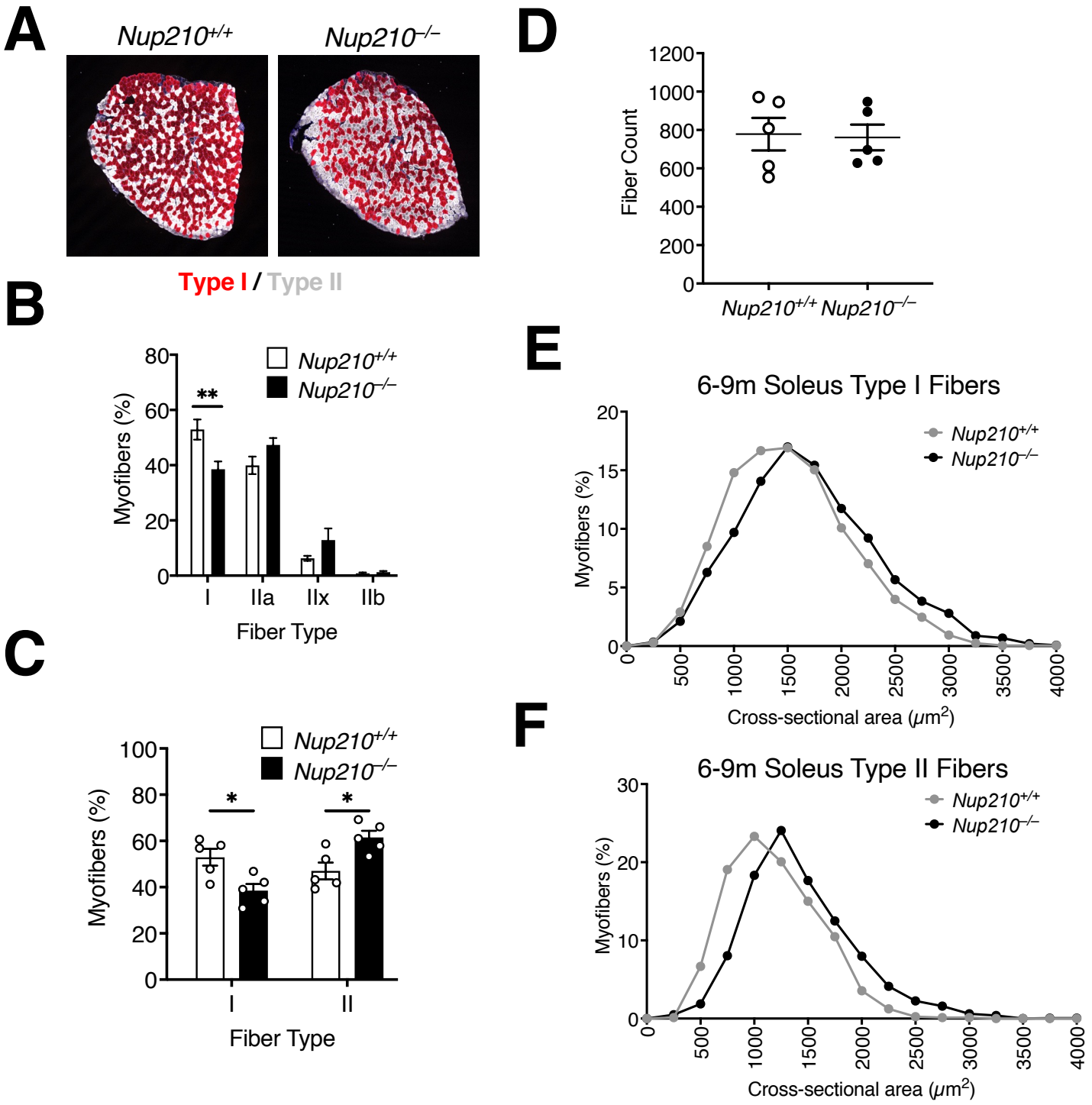

Figure 3: A) Representative immunofluorescence images showing Type I (red) and II (white) fibers in soleus of 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice. **B)** Quantification of fiber type distribution in soleus of 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 5). **C)** Quantification of fiber type distribution in soleus of 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 5). D) Quantification of total fiber count for soleus muscles from 6- to 9-mo-old mice (n = 5). **E, F)** Quantification of myofiber cross-sectional area for Type I fibers (E), and Type II (IIa, IIx, and IIb) fibers (F) in soleus of 6- to 9-mo-old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice (n = 5).

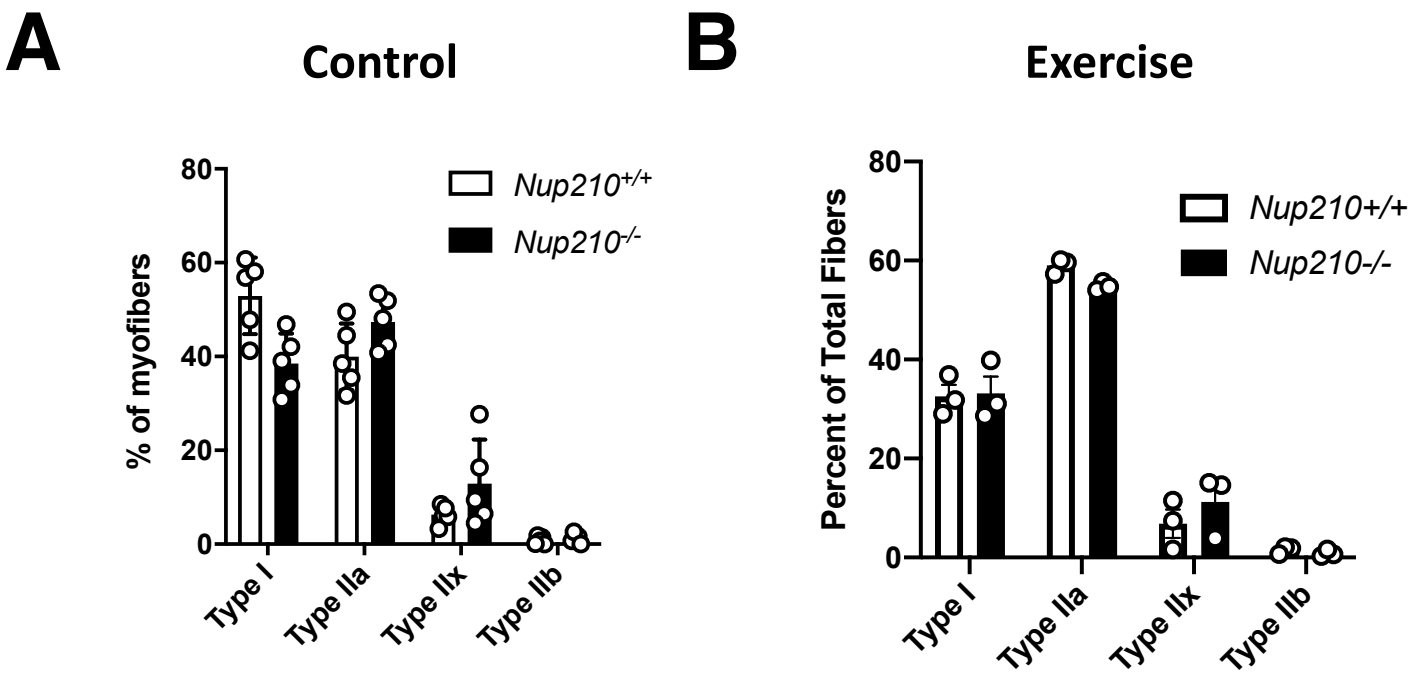

Figure 4: 6-8 weeks old *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice were subjected to voluntary running and fiber type distribution was analyzed as previously described. **A)** Control mice no running. **B)** Muscles from mice after 5 weeks of running.

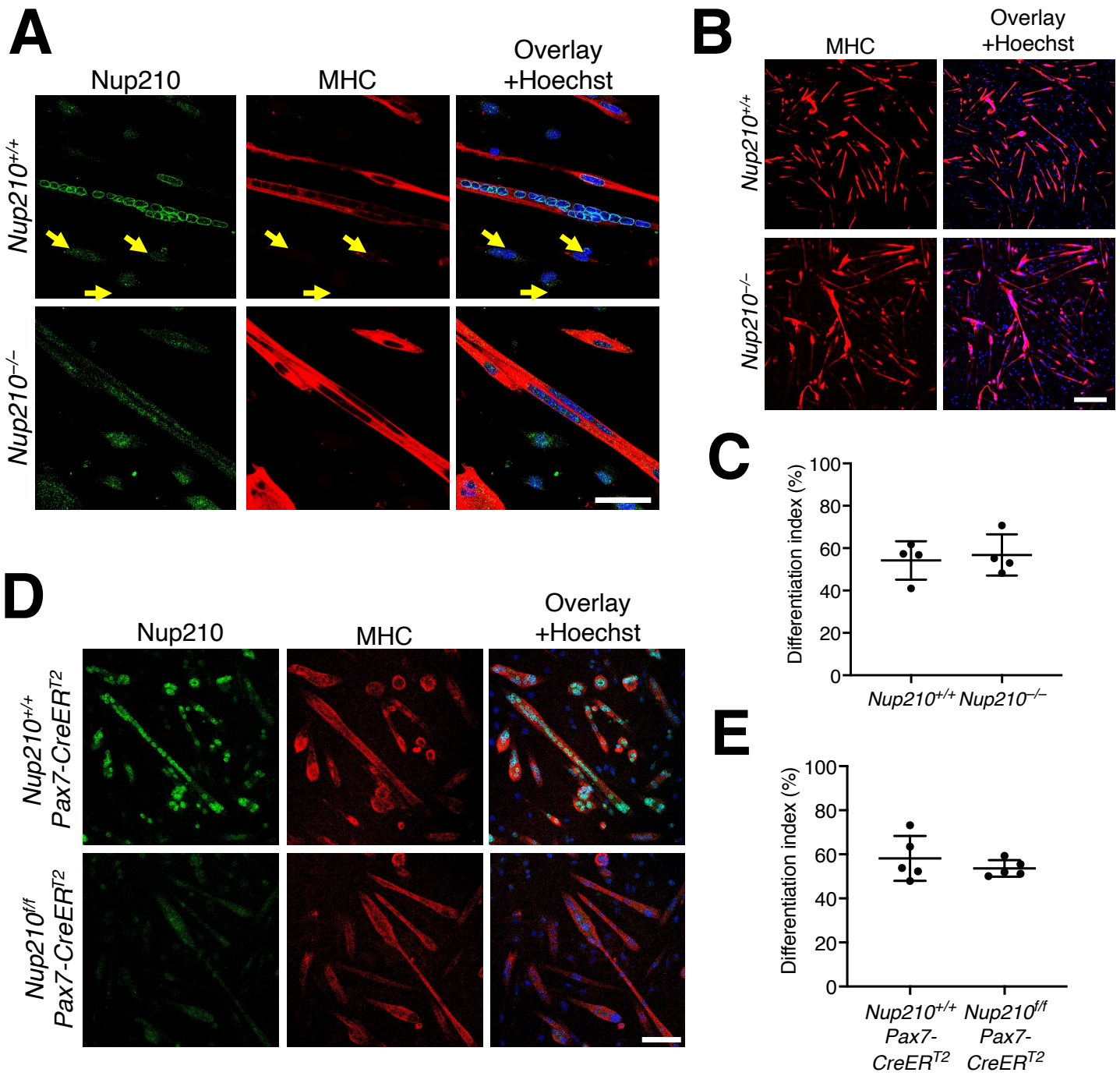

Figure 5: A) Representative images immunofluorescence staining (bottom) of sorted satellite cells from *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice after induction of myogenic differentiation. Arrows indicate undifferentiated muscle progenitor cells which lack Nup210 expression. Scale bar, 50 µm. **B)** Representative immunofluorescence images of differentiated satellite cells from *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice stained for myosin heavy chain (MHC) and Hoechst. Scale bar, 300 µm. **C)** *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ satellite cells were sorted, differentiated, and stained for MHC. The percentage of nuclei in MHC-positive cells (differentiation index) was quantified. **D, E)** *Nup210*$^{+/+}$/Pax7-CreER^{T2} and *Nup210*$^{fl/fl}$/Pax7-CreER^{T2} were treated with tamoxifen and satellite cells were isolated and differentiated in vitro. D) Representative immunofluorescence images of cells stained for Nup210 and MHC. Scale bar, 100 µm. E) The differentiation index (percentage of nuclei in MHC-positive cells) was quantified as in (C).

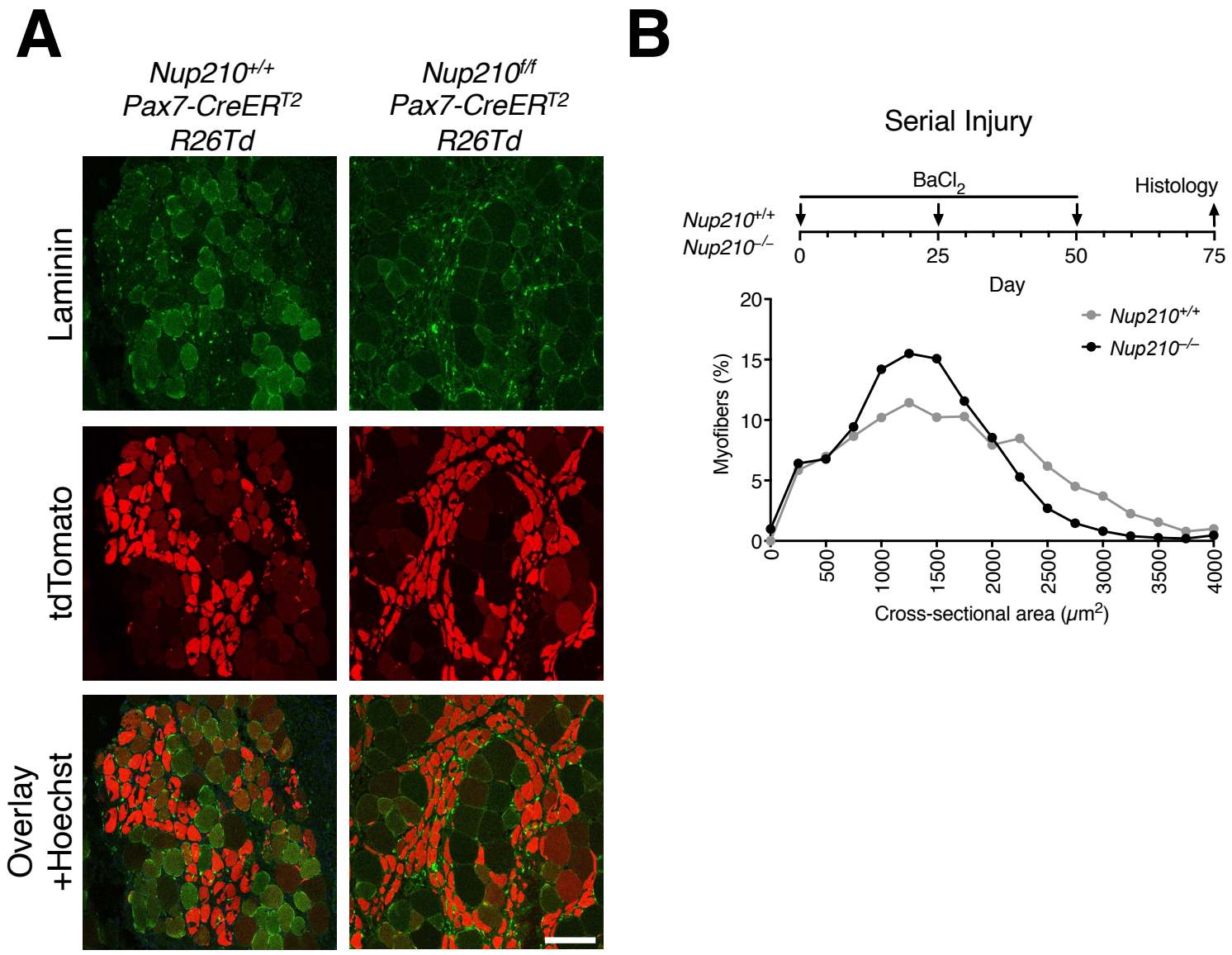

Figure 6: A) *Nup210*$^{+/+}$/Pax7-CreER^{T2} and *Nup210*$^{f/f}$/Pax7-CreER^{T2} mice carrying a Cre-inducible tdTomato reporter (R26Td) were treated with tamoxifen before being subjected to $BaCl_2$ muscle injury. The tdTomato-positive myofibers were analyzed by immunofluorescence in muscle sections. Laminin was used as counterstain to detect muscle fibers. Top: Schematic representation of experimental approach. Bottom: Representative immunofluorescence images from TA sections. Scale bar, 100 µm. **B)** Young *Nup210*$^{+/+}$ and *Nup210*$^{-/-}$ mice were subjected to $BaCl_2$-induced serial muscle injury (three injuries total), and muscle regeneration was analyzed 25 d after last injury. Top: Schematic illustration of experimental approach. Bottom: Quantification of myofiber cross-sectional area distribution. n = 3, data are binned in 250-µm^2 bins and are plotted as mean.

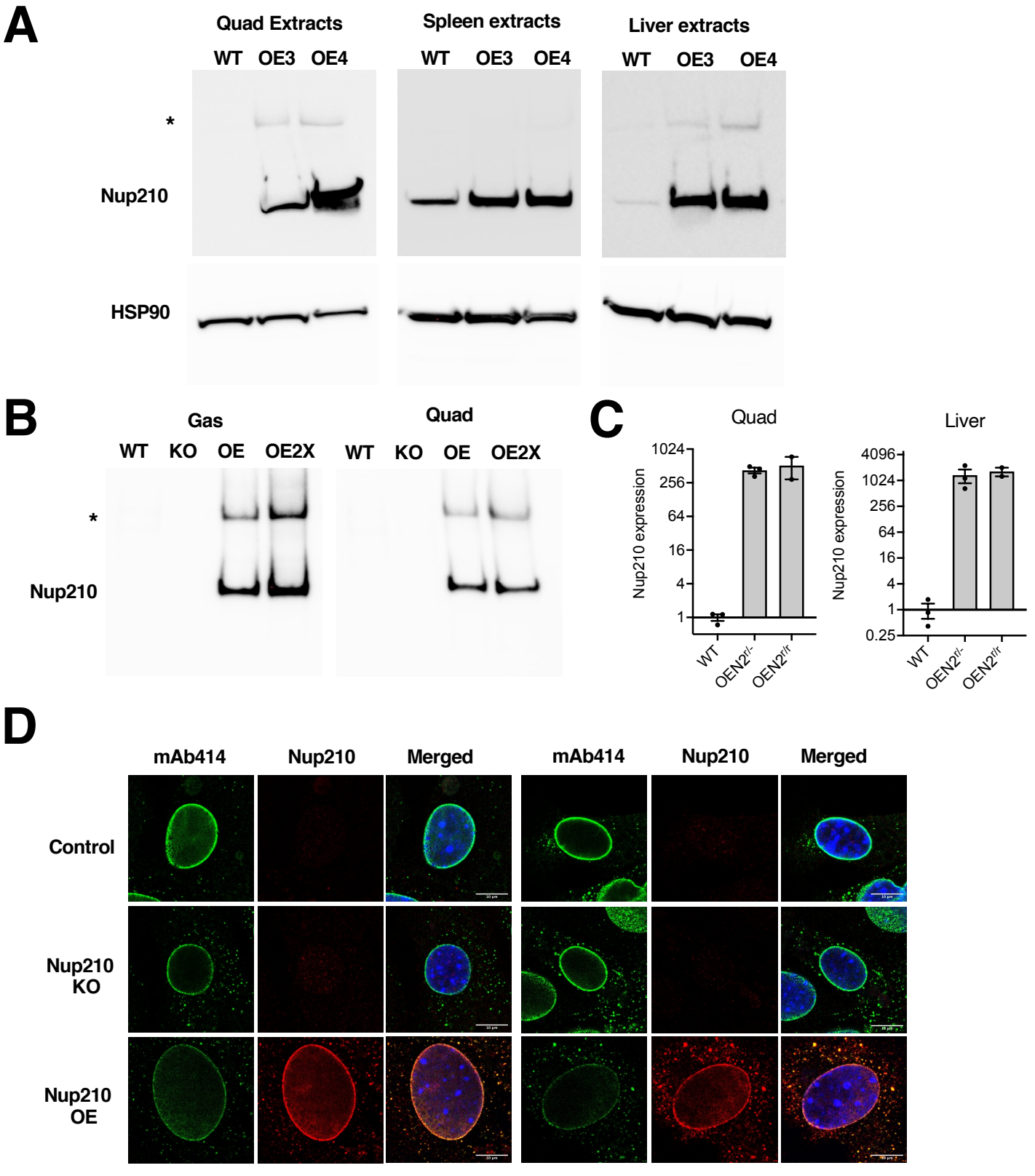

Figure 8: A) Western blot analyses of Nup210 in different tissues. Asterisk shows dimerization bands of Nup210. HSP90 was used as loading control. **B)** Western blot analyses of Nup210 of quadriceps and gastrocnemius muscles of control, Nup210 knockout (KO) and mice carrying one copy (OE) or two copies (OE2X) of the Nup210 overexpression cassette. **C)** The mRNA expression levels of Nup210 were analyzed in quadriceps and liver of control mice and mice carrying one copy (OE) or two copies (OE2X) of the Nup210 overexpression cassette by qPCR. **D)** Mouse embryonic fibroblasts from control, Nup210 knockouts and Nup210 overexpression mice were analyzed by immunofluorescence using antibodies against Nup210 or the nuclear pore marker mAb4141.

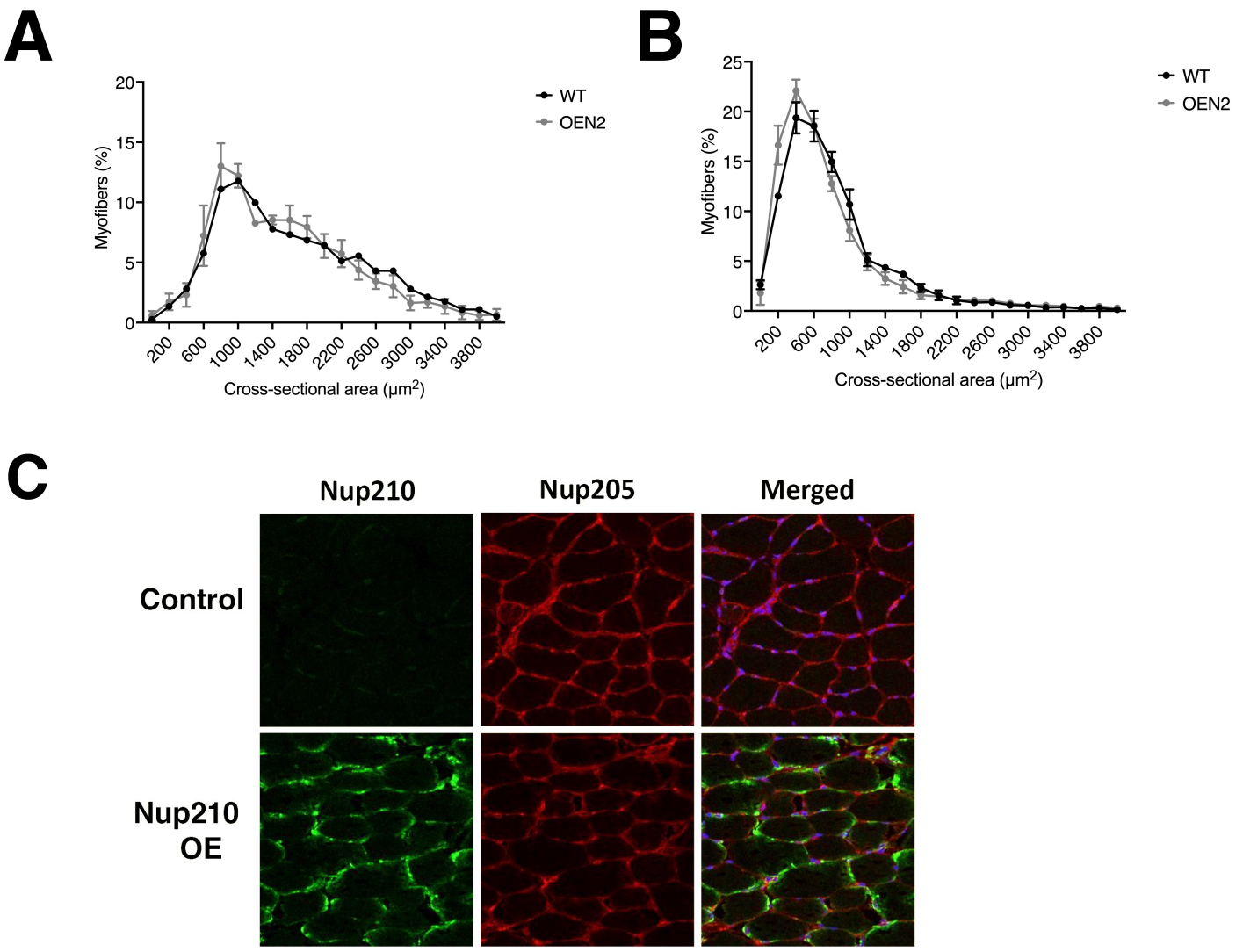

Figure 8: A) Quantification of myofiber cross-sectional area distribution in quadricep muscles from 6- to 8-week-old control or Nup210 overexpression mice (n=3). Data are binned in 200 µm2 bins and plotted as mean. **B)** Control or Nup210 overexpression mice were subjected to $BaCl_2$-induced muscle injury (and muscle regeneration was analyzed 5 d after last injury. Figure shows quantification of myofiber cross-sectional area distribution. n = 3, data are binned in 200-µm^2 bins and are plotted as mean. **C)** Immunofluorescence of Nup210 and Nup205 in quadriceps muscles from control or Nup210 overexpression mice.

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What opportunities for training and professional development has the project provided?

The Sanford Burnham Prebys Medical Discovery Institute (SBP) Office of Education, Training & International Services (OETIS) oversees and coordinates an annual individual development planning (IDP) process for all postdocs at the Institute. The focus of the IDP process at SBP is the career goal of the postdoc; identification of what skills, knowledge, and accomplishments will be necessary for the postdoc to obtain a desired independent position following training; and identification of training and professional development opportunities that are available for the postdoc to obtain the necessary skills and knowledge. The SBP Office of Education, Training & International Services provides guidance and advising to both postdocs and PIs throughout the postdoc's training with respect to developing IDPs and preparing for a successful transition to independence post-training. The SBP Office of Education, Training & International Services also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process.

The SBP IDP process includes two components:

I) First-Year IDP (effective in 2014): Within the first 3 months of beginning postdoctoral training at SBP, all postdocs receive and fill out an initial "planning and expectations" document to discuss with their PI. This document serves as the foundation for their postdoctoral IDP and is designed to facilitate discussion between the PI and new postdoc regarding goals and expectations for the first year of training, as well as stimulate initial discussions about long-term career goals and training plans.

II) Postdoctoral IDP (effective January 2013): At the end of the first year of training SBP postdocs receive notification that it is time to update their IDP, and they receive the information they included in their first-year planning and expectations document in the form of a full IDP that they can update with their accomplishments over the past year and their goals for the coming year, mid-term future, and long-term future. Each subsequent year of their postdoctoral training, postdocs will receive notification and the previous year's IDP form to update and expand. The IDP forms are designed to build upon each previous year as well as provide a solid foundation from which a postdoc can easily build his or her CV/resume.

During the past year, Dr. D'Angelo participated in Part II) of the IDP process with Valeria Guglielmi.

The Sanford Burnham Prebys Medical Discovery Institute's (SBP) Graduate School of Biomedical Sciences (GSBS) oversees and coordinates an annual individual development planning (IDP) process for all graduate students in the SBP GSBS program. The focus of the IDP process within GSBS is the development of the educational pathway of the student through identification of the skills, knowledge, and accomplishments that will be necessary for the student to obtain a PhD. degree; and identification of educational and professional development opportunities that are available for the student to obtain the necessary skills and knowledge. GSBS provides guidance and advising to both students and PIs throughout the student's education with respect to developing IDPs and preparing for a successful transition to the next career level post graduation.

The SBP GSBS IDP process includes two components:

I) Student Mentor Annual Reports. Each year students are required to submit an annual progress report in collaboration with their mentor. This report focuses on the educational goals accomplished through the past school year, highlights the scientific research progress and other accomplishments made by the students, and outlines an academic and research plan for the following year. Students and their mentor complete this form together and each complete sections providing feedback on the topics above. These reports are reviewed by the Graduate Program Executive Committee (GPEC) each year.

II) Annual Thesis Committee Meetings. Beginning in year two of studies, students are required to assemble their Thesis Committee for an annual meeting to be held between June – November of each year. At these meetings, the student outlines their current specific aims for their thesis project, reports progress made in the previous year and outlines a plan for the future of the project. The thesis committee members provide the student feedback and guidance on the progression of the research project and may suggest additional coursework or training if needed. At the completion of the meeting, the student submits a report signed by the faculty mentor

containing a summary of the work they presented, the committee's feedback and plans for continuance to the Graduate Office. This report is then reviewed by GPEC.

Stephen Sakuma joined the Sanford Burnham Graduate School of Biomedical Sciences in Fall 2019. He held his first Annual Thesis Committee Meeting in Fall 2020. He has completed his coursework and held his Qualifying Exam Milestone Meeting in Fall 2021. He is in good academic standing.

How were the results disseminated to communities of interest?
An oral presentation at the Sanford Burnham Prebys Trainee Seminar Series
An oral presentation for Sanford Burnham Prebys Graduate School of Biomedical Sciences

What do you plan to do during the next reporting period to accomplish the goals?
For the next reporting period, we expect to focus in further characterizing how increased levels of Nup210 might affect muscle integrity and function. We will perform additional muscle regeneration experiments as well as subject these animals to voluntary running exercise. To determine if lowering the levels of Nup210 in the overexpressing mice results in better localization of this nucleoporin to nuclear pore complexes we will cross mice carrying one copy of the overexpression cassette with our Nup210 knockout mice. The levels and localization of Nup210 will be analyzed by immunofluorescence and western blots, and muscle function will be analyzed as described. We currently have additional muscle functional analyses underway which include treadmill exercise as well as the characterization of Pax7+ muscle progenitors in injured muscle for Nup210 control and overexpression mice. We expect to finish these experiments in the coming months. We are still working on improving the conditions for immune cell isolation from skeletal muscle tissue as well as characterizing the immune infiltrates using qPCR with specific immune cell markers. We expect to have additional data on the immune landscape of muscles with different levels of Nup210 in the coming year.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS

Publications, conference papers, and presentations

Stephen Sakuma, Ethan Ys Zhu, Marcela Raices, Pan Zhang, Rabi Murad, Maximiliano A D'Angelo. Loss of Nup210 results in muscle repair delays and age-associated alterations in muscle integrity. Life Sci Alliance, 2021 Dec 15;5(3) PMCID: PMC8711851 DOI: 10.26508/lsa.202101216

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Maximiliano D'Angelo, PI – No change

Valeria Guglielmi, Postdoctoral Associate – No change

Stephen Sakuma, Graduate Student – 12 calendar months
Mr. Sakuma has worked with Mr. Zhu to developed most of the reported experiments. Mr. Sakuma also contributed interpretation of data, and experimental planning.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes, current support for Dr. D'Angelo is attached.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES

- PI Current Support
- Award Chart

CURRENT SUPPORT

Name of Individual: D'Angelo, Maximiliano
Current Appointment: Associate Professor, Sanford Burnham Prebys Medical Discovery Institute

Other Support – Project/Proposal

ACTIVE

Title:	Nup210 Roles in Cell Survival and Leukemia
Major Goals:	The goal of this project is to establish the role that nuclear pore complexes play in the development of blood malignancies.
Specific Aims:	1. Determine if increased Nup210 levels enhance the survival, proliferation and invasion capacities of normal and transformed hematopoietic progenitors. 2. Establish the role of Nup210 in leukemia initiation and progression. 3. Uncover the molecular mechanisms of Nup210 function in leukemic transformation.
Project Number:	RSG-17-148-01-CCG
Name of PD/PI:	D'Angelo, Maximiliano
Source of Support:	American Cancer Society
Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period:	01/2018 – 12/2022
Total Award Amount:	(total direct costs)
Time Commitment:	1.80 calendar months
Agency POC:	Charles Saxes, Ph.D.;
Overlap:	None

Title:	Characterizing the Nuclear Pore Complex-T Cell Receptor Connection The
Major Goals:	main goal of this project is to dissect the molecular mechanisms through which nuclear pore complexes regulate TCR signaling.
Specific Aims:	1. Establish the mechanisms that connect TCR activation with Nup210 function at the nuclear envelope. 2. Identify the role of Nup210 in the regulation of NFATc1 and STAT3 activity. 3. Characterize Cav2 function in Nup210 regulation of T cell gene expression
Project Number:	R01 AI148668
Name of PD/PI:	D'Angelo, Maximiliano
Source of Support:	NIH/NIAID
Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period:	12/2019 – 11/2024
Total Award Amount:	(total direct costs)
Time Commitment:	3.60 calendar months
Agency POC:	Jordan A Kindbom;
Overlap:	None

Title:	Nuclear Pore Complexes in the Maintenance of Skeletal Muscle Integrity and Function
Major Goals:	The goal of this project is to characterize in detail the muscle degeneration that results from Nup210 ablation in mice and to establish how this nucleoporin regulates different muscle processes critical for muscle maintenance.

Specific Aims:	1. Characterize the age-dependent deterioration of skeletal muscle in Nup210 knockout animals. 2. Identify the origin/s of muscle alterations in Nup210-/- animals. 3. Define if Nup210 up-regulation can stimulate muscle regeneration.
Project Number:	W81XWH-20-1-0212
Name of PD/PI:	D'Angelo, Maximiliano
Source of Support:	Department of Defense
Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period:	05/2020 – 04/2023
Total Award Amount:	(total direct costs)
Time Commitment:	3.00 calendar months
Agency POC:	Brittany N. Hebb, 301-619-9802
Overlap:	None

Title:	Cancer Center Support Grant (CCSG)
Major Goals:	The mission of Sanford Burnham Prebys Medical Discovery Institute's Cancer Center is to be a national leader in the effort to overcome cancer as a cause of human suffering and death. Our vision is to make paradigm shifting discoveries that will underlie novel therapeutic modalities by creating, translating, and disseminating exceptional basic cancer science.
Project Number:	P30 CA030199
Name of PD/PI:	Ronai, Ze'ev
Source of Support:	NIH/NCI
Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period:	05/2020 – 04/2025
Total Award Amount:	(total direct costs)
Time Commitment:	2.40 calendar months
Agency POC:	Candace M Cofie;
Overlap:	None

<u>INACTIVE</u>

Title:	Nuclear Pore Regulation of Leukemia Cell Metabolism and Survival
Major Goals:	The goal of this project was to uncover how the molecular mechanisms of Nup210 promotes cell survival and leukemic transformation by characterizing its role in the regulation of leukemia cell metabolism, and by establishing the cellular pathways regulated by Nup210.
Specific Aims:	1. Establish the function of Nup210 in leukemia cell metabolism. 2. Define the role of sirtuins in Nup210 regulation of leukemia cell transformation and metabolism.
Project Number:	R21 CA244028
Name of PD/PI:	Raices, Marcela
Source of Support:	NIH/NCI
Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period:	12/2019 – 02/2022
Total Award Amount:	(total direct costs)
Time Commitment:	1.80 calendar months
Agency POC:	Candace M Cofie;
Overlap:	None

W81XWH-20-1-0212: Nuclear pore complexes in the maintenance of skeletal muscle integrity and function

PI: Maximiliano D'Angelo, Sanford Burnham Prebys Medical Research Institution, California

Budget: $1,922,318.00

Topic Area: Medical Research Program **Mechanism:** Investigator-Initiated Research Award

Research Area(s): Musculoskeletal Disorders , Tissue Regeneration **Award Status:** 05/01/2020-04/30/2021

Study Goals:

The rationale of this proposal is based on our previous findings where we identified that Nup210 is important for skeletal muscle physiology. Our hypothesis is that Nup210 is a key regulator of muscle maintenance and that increasing its activity can enhance muscle repair and function. This project seeks to increase our understanding of Nup210 regulation of skeletal muscle homeostasis and is expected to provide novel insights into how specialized NPCs are exploited to regulate tissue physiology. This proposal also aims to define if modulating the levels of Nup210 could be exploited to enhance muscle performance and regeneration.

Specific Aims:

Specific Aim 1. Characterize the age-dependent deterioration of skeletal muscle in Nup210 knockout animals.
Specific Aim 2. Identify the origin/s of muscle alterations in Nup210$^{-/-}$ animals.
Specific Aim *3.* Define if Nup210 up-regulation can stimulate muscle regeneration.

Key Accomplishments and Outcomes:

In the second year of this award, we have worked to further characterize the muscle alterations of Nup210 knockout mice. Using tools we have generated in year 1 and 2 we have also began to analyzed how increasing Nup210 levels affect muscle integrity and function with the aim of establishing whether modulating the levels of this protein could be exploited to enhance muscle performance. We have determine that the progressive muscle alterations and abnormal muscle regeneration that we identified in Nup210 knockout animals are not due to an abnormal function of muscle progenitor cells. We have also discovered that eliminating Nup210 in mice leads to alterations in muscle fiber distribution and decrease muscle performance. We have also perform preliminary characterization of mice with elevated Nup210 levels and establish that increasing the amount of this nucleoporin does not affect the integrity or muscle regeneration ability of animals.

Publications: Stephen Sakuma, Ethan Ys Zhu, Marcela Raices, Pan Zhang, Rabi Murad, Maximiliano A D'Angelo. Loss of Nup210 results in muscle repair delays and age-associated alterations in muscle integrity. Life Sci Alliance, 2021 Dec 15;5(3) PMCID: PMC8711851 DOI: 10.26508/lsa.202101216

Patents: None

Funding Obtained: None