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**TITLE: Understanding and Enhancing the Regenerative Capacity of Skeletal Muscle to Trauma by Targeting Muscle-Nerve Synergy**

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**CONTRACTING ORGANIZATION:** University of Michigan, Ann Arbor, MI

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT: Background: Poor healing after lower-limb extremity trauma represents an enormous medical problem ($400B / year ascribed to trauma in CONUS and >24M limited duty days in 2005) and recent conflicts in Iraq and Afghanistan have emphasized the prevalence of lower-limb extremity trauma (up to 78 percent of medical disability discharges). These injuries present debilitating consequences, which have been shown to result in pronounced disabilities ranging from declines in limb function, to development of osteoarthritic pathology and delayed or elected limb amputation. Moreover, the effects of lower-limb extremity trauma have significantly reduced Department of Defense (DoD) readiness and performance and as each force begins to downsize, the importance of sustaining Warfighter readiness and recovery from trauma is a priority. Objective/ Hypothesis: While efforts to develop treatments that hasten and improve healing for lower-limb skeletal muscle injuries are ongoing, their development has been inherently limited due largely to our lack of understanding of the basic processes involved in the healing process. Efficient and appropriate repair and regeneration of skeletal muscle is mediated by a pool of muscle stem cells (MuSCs) called satellite cells, which activate, proliferate and differentiate and fuse to form new multinucleated myofibers. After regeneration of myofibers, function must be established by re-formation / attachment of a neuromuscular junction (NMJ). The NMJ connects the axon of a motor neuron to a muscle fiber and is responsible for excitation / contraction coupling and voluntary motor function. The intricate interaction between MuSCs and the NMJ niche is not fully understood and as such, how neural control influences response to trauma remains an open question.Specific Aims and Study Design: The overarching purposes of this project are to understand how the regenerative capacity of MuSCs are altered when the NMJ is disrupted and to utilize this niche to enhance MuSC-based therapies against traumatic injuries. Accordingly, we will perform three specific aims: Aim 1) Identify intrinsic factors in muscle stem cells that modulate muscle repair and regeneration after disruption to the neuromuscular junction. We have developed an animal model that permits study of NMJ disruption and MuSC response and will use this model to administer severe trauma followed by isolation of purified populations of MuSCs using flow cytometry.**

**15. SUBJECT TERMS**

None listed.

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
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## TABLE OF CONTENTS

## 1. INTRODUCTION:

The repair and regeneration of severely damaged soft tissues such as skeletal muscle remains a substantial clinical challenge and relatively few treatments exist. The overarching purposes of this project are to understand how the regenerative capacity of muscle stem cells are altered when the neuro-muscular junction is disrupted and to utilize this niche to enhance muscle stem cell-based therapies against traumatic injuries.

## 2. KEYWORDS:

Neuromuscular Junction, Satellite Cells, Motor Neurons, Regeneration, Single Cells, Signaling

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

**Major Task 1:** Establish mechanistic insights into the intrinsic molecular mechanisms and signaling pathways that couple skeletal muscle regeneration and neural control.

**Major Task 2:** Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury.

**Major Task 3:** Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury.

**What was accomplished under these goals?**

**Major Task 1 – Key Experiments**

Morphologically characterized NMJs before and after muscle injury and nerve injury.

- Assessed NMJ regeneration after muscle injury and nerve injury using confocal microscopy and immunohistochemistry
- Contrasted how cells respond to denervation using RNA sequencing and observed a fibrotic response that impinges on muscle stem cells.
- Performed myonuclear sequencing before denervation and integrated datasets with existing data for comparison.

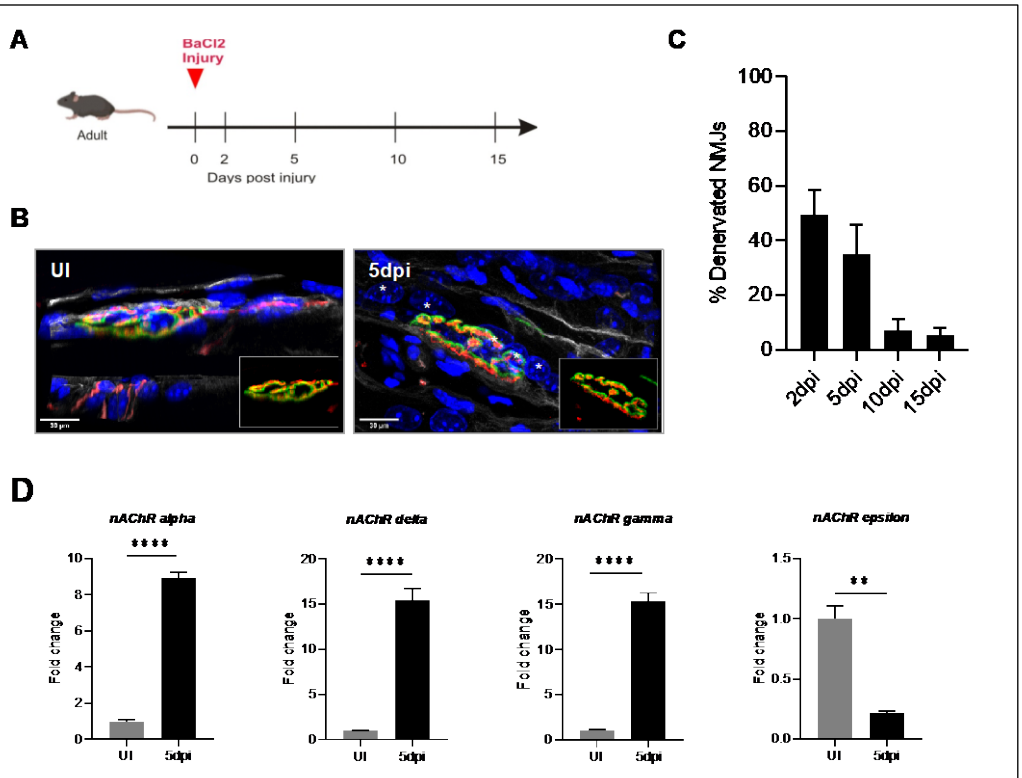
**Summary of major findings**

- Muscle injury results in acute denervation followed by reinnervation

- Denervation induces changes in fibrotic cell signaling that negatively acts on muscle stem cells
- Myonuclear sequencing recovers multiple cell types and will be critical assess after denervation.

**Major Task 1 – Subtasks 2-3: Skeletal muscle degenerative injury is accompanied by denervation and re-innervation.**

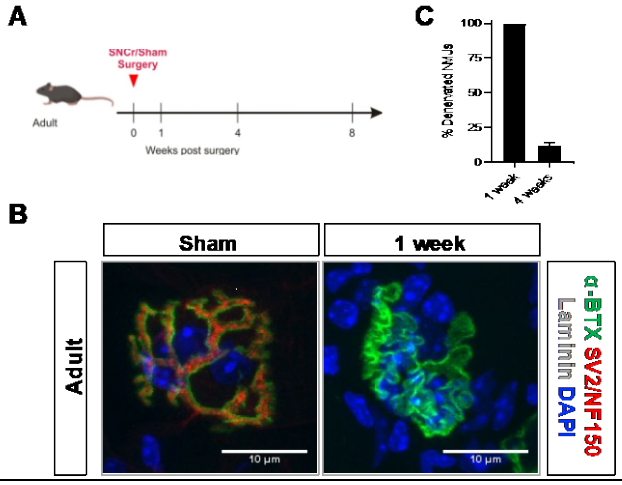
In order to establish cellular and molecular mechanisms that couple skeletal muscle regeneration and neural control, we first utilized an animal model that facilitates insights into muscle regeneration after injury and changes in innervation. Denervation has been observed a few days after muscle injury though the kinetics of this process have not been examined. We administered a muscle injury by intramuscular injection of barium chloride ($BaCl_2$), an experimental and reproducible model of muscle injury that causes widespread myofiber degeneration, but spares MuSCs and preserves the basal lamina. To examine the extent of denervation and the kinetics of reinnervation after $BaCl_2$ mediated skeletal muscle degenerative injury, we employed confocal immunofluorescence (IF) microscopy and 3-D image rendering to assess the presence of nerve terminal markers at the NMJs of tibialis anterior (TA) and extensor digitorum longus (EDL) muscles at various time points during regeneration (Figure. 1a). Regenerating myofibers were recognized based on the characteristic clusters of centrally located nuclei within the remaining basal lamina (Figure. 1b). The NMJs on regenerating myofibers were considered to be; innervated if the vast majority of post-synaptic regions are covered by pre-synaptic terminal markers, and denervated if the postsynaptic apparatus had negligible if any labeling of pre-synaptic terminal markers. At 2 days post degenerative injury (dpi), we observed half of the regenerating adult myofibers to be denervated (49.5 ± 8.9% denervated NMJs) (Figure. 1c). No further increase in the denervation of regenerating myofibers was observed at 5dpi (35 ± 10.9% denervated NMJs); however, by 10dpi and 15dpi, we observed approximately complete reinnervation with less than 10% denervation of regenerating myofibers (Figure. 1c). To verify changes in post-synaptic recruitment of motor neurons and promotion of re-innervation, we assessed expression of nicotinic acetyl-choline receptors (nAChRs) at 5 dpi by qRT-PCR, when the data suggested the process of re-innervation was initiating. Three of the 4 AChR subunits increased in expression at this time indicating muscle was actively trying to recruit motor neurons back after injury.




**Major Task 1 – Subtasks 2-3: Figure 1. Morphological and molecular assessment of re-innervation after muscle injury**. **(A)** Schematic for muscle injury by injection of $BaCl_2$ muscles of adult mice and collected at 2, 5, 10 and 15 days post injury (dpi). **(B)** Representative images of longitudinal sections of un-injured (UI) and 5dpi muscle stained for post-synaptic nAChRs (α-bungarotoxin, BTX; green), nerve terminal markers (SV2,Syt-2, neurofilament; red), basal lamina (Laminin; gray) and nuclei (DAPI; blue). Nuclei aligned in the center of the myofiber are indicated with white asterisks. Magnified inset images show NMJs. Scale bar = 30um. **(C)** Quantification of denervated NMJs in regenerating myofibers (>30 NMJs/mouse). Data are reported as mean ± SEM. **(D)** mRNA expression of nAChR subunits alpha, delta, gamma and epsilon in 5dpi relative to UI muscle reported as fold-change ± SD normalized to an average of GAPDH and relative to UI. **P < 0.01, ****P < 0.0001, unpaired t test with Welch's correction.

**Major Task 1 – Subtasks 2-3: Nerve injury that results in denervation of neuromuscular junctions stimulates changes in inflammatory cell expression**

We next established a neural injury model to determine changes in muscle regeneration that occur after denervation. We employed sciatic nerve crush injury (SNCr), whereby mice were anesthetized, skin was incised and the anterior head of the biceps femoris is opened revealing the sciatic nerve. The nerve is crushed for 15 seconds using hemostatic forceps. The gluteal musculature is re-opposed, and the skin incision is closed using wound clips. Mice were allowed to recover in their cage placed on a heating pad at 37˚C. For a surgical control (sham), the contralateral sciatic nerve is exposed and mobilized, but left intact. We utilized confocal microscopy to determine the kinetics of denervation and reinnervation of adult NMJs after SNCr (Figure 2a). At 1 week post SNCr injury, all adult NMJs were observed to be denervated (Figures 2b-c). At 4 weeks, 89% of adult NMJs were reinnervated (Figure 2c). These results suggest that the SNCr injury model results in overall similar kinetics of denervation and re-innervation as muscle regeneration delivered via $BaCl_2$ injection.

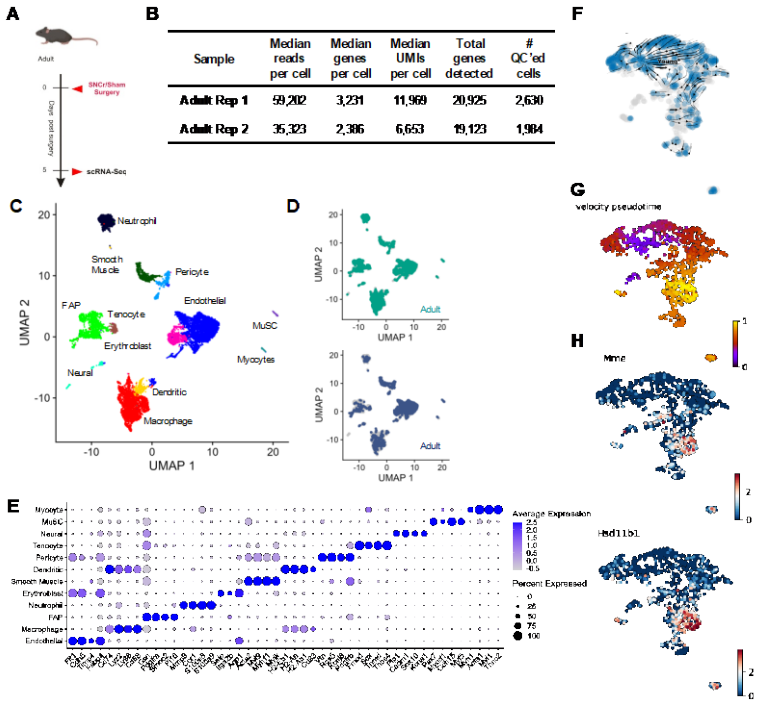



**Major Task 1 – Subtasks 2-3: Figure 2. Peripheral nerve injury results in acute denervation and near complete re-innervation. a** Schematic for nerve injury in adult (2-3 months old, n=3) mice. Sciatic nerve crush (SNCr) surgery was performed along with sham surgery of the contra lateral limb. Muscles from both limbs were collected at 1 and 4 weeks post surgery. **b** Representative confocal IF images of adult NMJs from sham and nerve crush at 1 week stained for post-synaptic nAChRs (α-bungarotoxin, BTX; green), nerve terminal markers (SV2,Syt-2, neurofilament; red) and nuclei (DAPI; blue). Scale bar = 10um. **c** Quantification of denervated NMJs ( >30 NMJs/mouse) in adult TA muscles. Data are reported as mean ± SEM.

**Major Task 1 – Subtasks 5-6: Nerve injury that results in denervation of neuromuscular junctions stimulates changes in fibrotic cell expression**

We previously established molecular changes in muscle stem cells that occur during muscle regeneration after $BaCl_2$ injection (Cell Reports 2020, eLife 2021). We next sought to establish how these regenerative dynamics are altered after denervation. We employed sciatic nerve crush injury (SNCr), and performed single-cell RNA-sequencing (scRNA-Seq) of muscle stem cells and all mononucleated cells at 5 days post SNCr injury from replicate adult tibialis anterior muscles (Figure.

3a). We generated 4,614 single-cell profiles with an average of 2,982 genes and 10,772 unique molecular identifiers per cell (Figure 3b). We integrated the datasets using Seurat and performed dimensionality reduction using Uniform Manifold Approximation and Projection (UMAP) followed by unsupervised Louvain clustering. This analysis revealed 12 cell types (Figure 3c), with consensus among cell types across replicates (Figure 3d). Assessment of marker gene expression from the different cell types revealed overlap with previously published single-cell atlases from muscle (Figure 3e). We found the fibro-adipogenic progenitor (FAP) cells demonstrated considerable changes in expression, which is significant because these cells impinge on muscle stem cell based regeneration. We performed RNA velocity analysis on re-clustered FAPs and found a subset of FAPs expressed MMe and Hsd11b1, which have been found in proximity to the neuromuscular junction after denervation and are strong contributors to fibrosis. These results are important because these fibrotic cells negatively signal to muscle stem cells to prevent their fusion via TGFβ1 and inhibit regeneration.

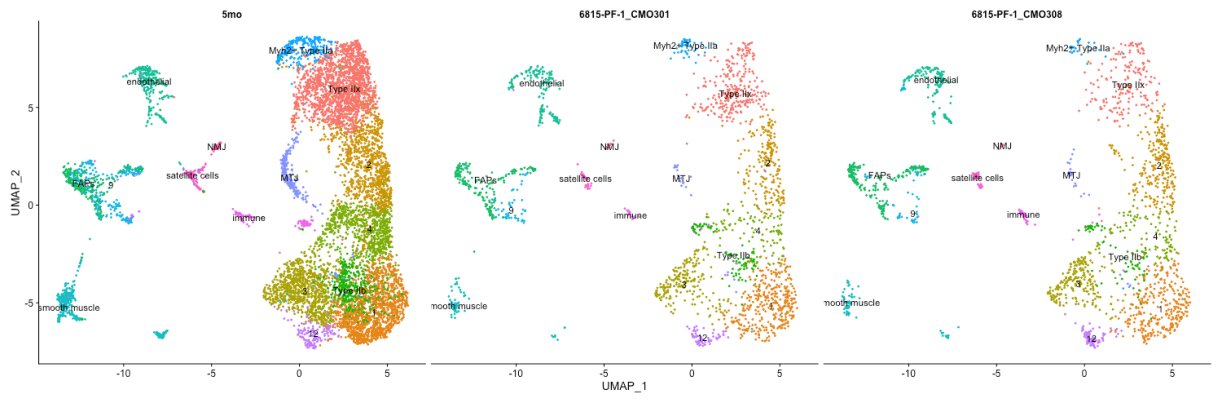



| Sample | Median reads per cell | Median genes per cell | Median UMIs per cell | Total genes detected | # QC'ed cells |
|---|---|---|---|---|---|
| Adult Rep 1 | 59,202 | 3,231 | 11,969 | 20,925 | 2,630 |
| Adult Rep 2 | 35,323 | 2,386 | 6,653 | 19,123 | 1,984 |

**Major Task 1 – Subtask 5: Figure 3. Muscle denervation induces a fibrotic response from fibro-adipogenic progenitors that impinges on muscle stem cells.** a) Experiment schematic for generation of scRNA-seq datasets from mononucleated cells in adult skeletal muscle 5 days after denervation. b) Quality control metrics for scRNA-Seq libraries. c) UMAP embedding colored by cell types recovered from dimensional reduction and unsupervised clustering. d) UMAP embedding split by samples and biological replicates shows shared coverage of all recovered cell types. e) Dot plot of representative marker genes for each cell type. f) Overlay of RNA velocity for re-clustered FAPs shows trajectory towards fibrotic like state. g) Pseudotime of RNA velocity shows trajectory towards fibrotic like state. h) Overlay of expression for Mme (top) and Hsd11b1 (bottom), which are markers of fibrotic like FAPs associated with the NMJ.

**Major Task 1 – Subtasks 5-6: Myonuclear sequencing and data integration**

A limitation of our approach is that a major responder to denervation is myonuclei from muscle fibers, which signal to MuSCs and other cell types. To begin to address this limitation, we developed a protocol to isolate in-tact myonuclei from muscles and subjected the myonuclei to RNA sequencing. As above, we integrated the datasets with previously generated datasets of nuclei from muscle using Seurat and performed dimensionality reduction using UMAP followed by unsupervised Louvain clustering. This analysis revealed 14 cell types with myonuclei from different fiber types (Figure 4a), with consensus among cell types across replicates (Figure 4). We will use this protocol to begin to ask how myonuclei change after denervation and signal to muscle stem cells and other cell types that inhibit their ability to regenerate the tissue.




**Major Task 1 – Subtasks 5-6: Figure 4. Sequencing of myonuclei to understand signaling that promotes regeneration.** UMAP embedding colored by cell types recovered from dimensional reduction and unsupervised clustering. Left – Data from Petrany et al, Nature Comm. 2020. Middle and Right – Generated data from biological replicates showing similar cell types recovered.

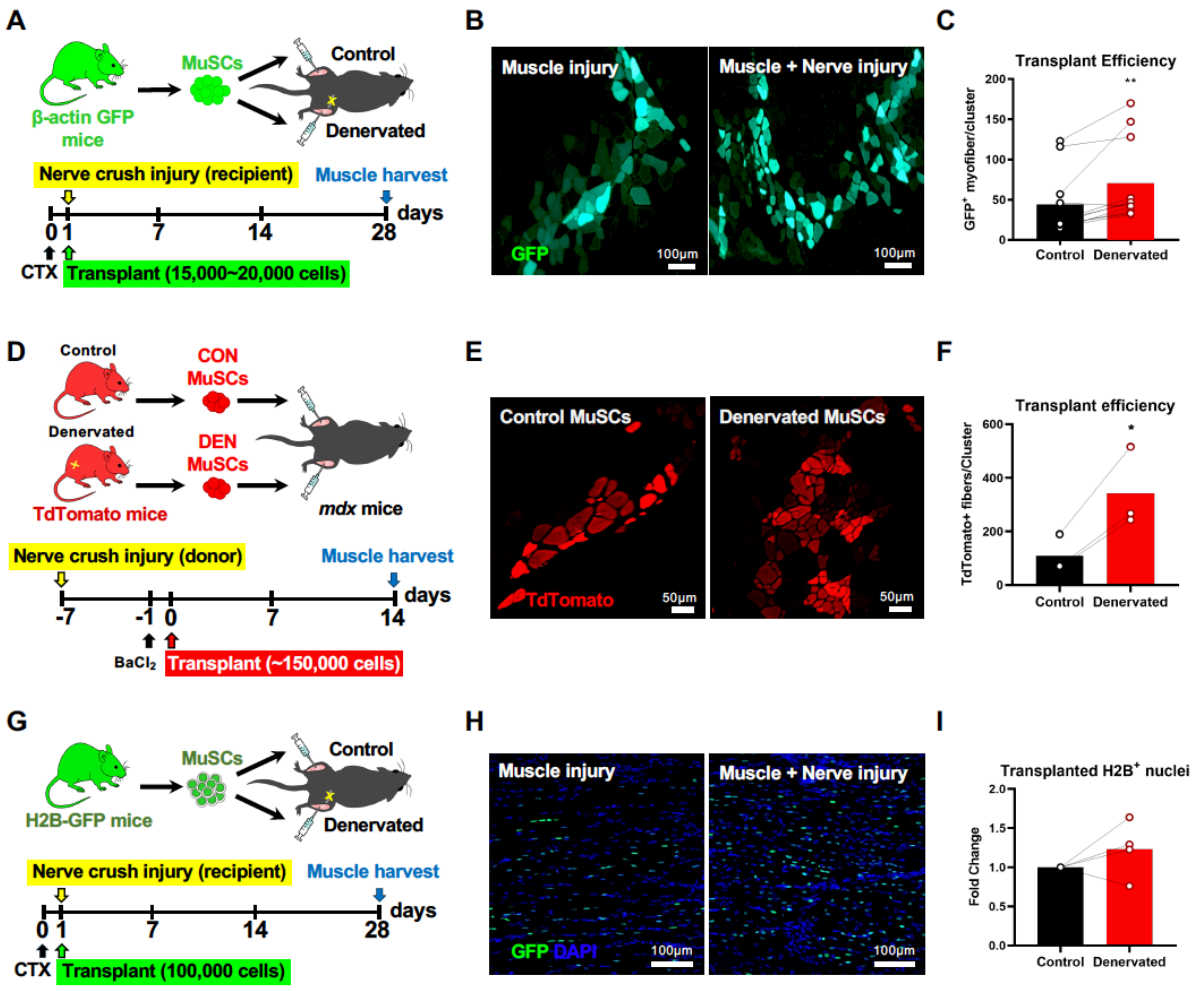
**Major Task 2 – Key Experiments**

Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury.

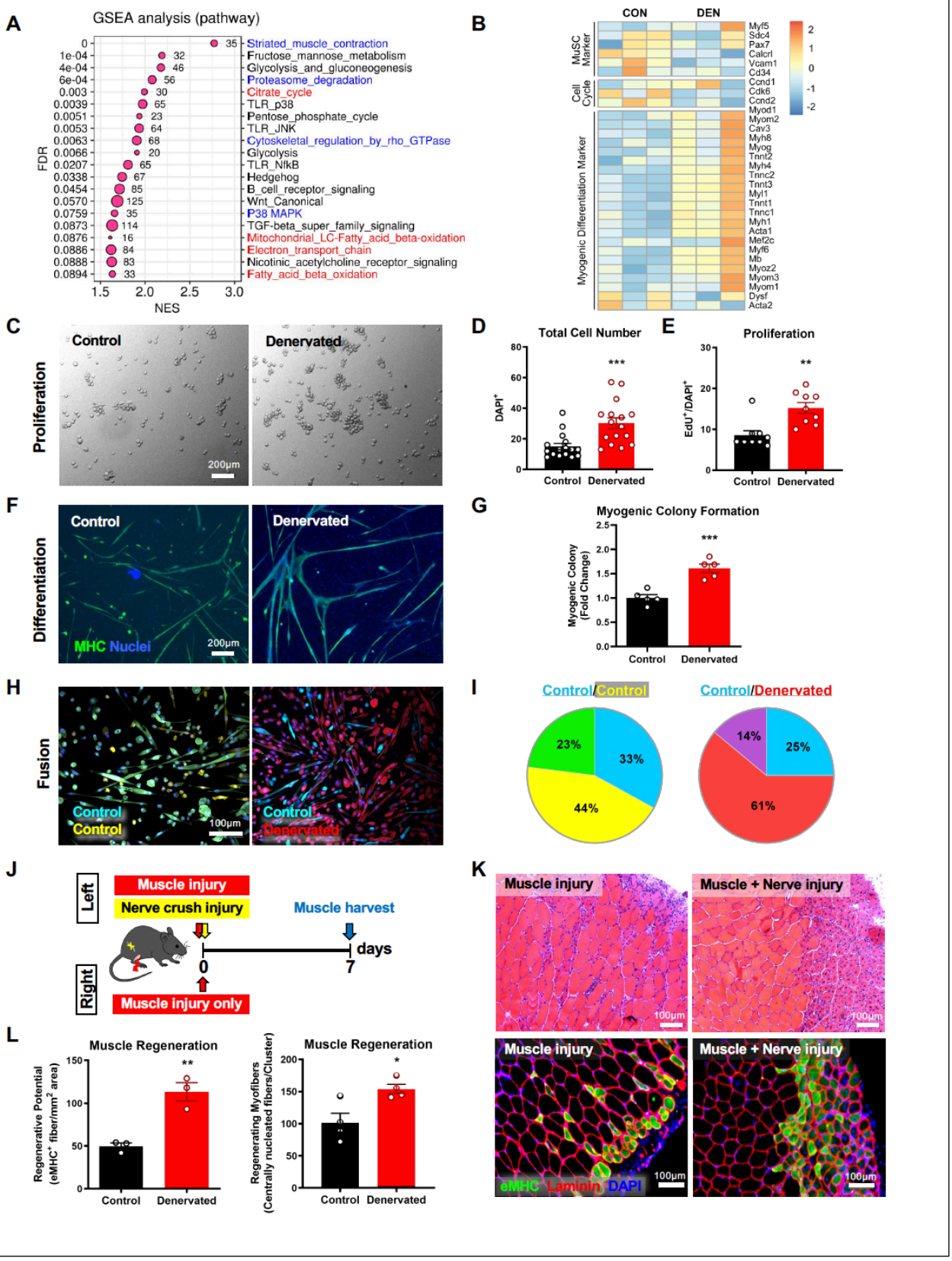
- Assess whether denervation injury (NMJ manipulation) alters muscle stem cell function and regeneration
- Assess engraftment of donor and recipient effects of MuSCs following NMJ perturbation.

**Summary of major findings**

- Nerve perturbation promotes a favorable microenvironment that augments muscle regeneration.
- Mild denervation injury enhances myogenic activity of muscle stem cells *in vitro* and *in vivo*.
- NMJ manipulation through mild nerve injury significantly enhances engraftment of donor muscle stem cells.
- Muscle stem cells from mild denervated muscle exhibit enhanced myogenic activated and engraftment.
- Enhanced myogenic activity of NMJ primed muscle stem cells is in part due to an increase in mitochondrial bioenergetics and elevated protein synthesis.




**Major Task 2 – Subtasks 1: Figure 5. Nerve regeneration synergistically enhances muscle satellite cell transplantation efficiency. (A)** Schematic diagram of experimental design assessing transplantation efficiency of control and denervated recipient muscles. **(B)** Representative images of transplanted $GFP^+$ fibers from only muscle-injured and both muscle- and nerve-injured tissue. Scale bar 100 μm. **(C)** Quantification of transplantation efficiency as measured by the total number of $GFP^+$ fibers per cluster. n = 6. **(D)** Schematic diagram of experimental design assessing denervation effects on donor muscle satellite cells (TdTomato). **(E)** Representative images of transplanted $TdTomato^+$ fibers originated from control and denervated donor muscle satellite cells. Scale bar 50 μm. **(F)** Quantification of transplant efficiency measured by total $TdTomato^+$ fibers. n= 3 biological replicates. **(G)** Schematic diagram of experimental design assessing muscle satellite cell-derived myonuclei. **(H)** Representative images of $H2B^+$ muscle satellite cell-derived nuclei in control and denervated recipient myofibers, and **(I)** quantification of $H2B^+$ nuclei in control and denervated myofibers. n= 4 biological replicates. $*p < 0.05$ and $**p < 0.01$ via paired two-tailed *t* test.


A
GSEA analysis (pathway)
FDR
NES
Striated_muscle_contraction
Fructose_mannose_metabolism
Glycolysis_and_gluconeogenesis
Proteasome_degradation
Citrate_cycle
TLR_p38
Pentose_phosphate_cycle
TLR_JNK
Cytoskeletal_regulation_by_rho_GTPase
Glycolysis
TLR_NfkB
Hedgehog
B_cell_receptor_signaling
Wnt_Canonical
P38 MAPK
TGF-beta_super_family_signaling
Mitochondrial_LC-Fatty_acid_beta-oxidation
Electron_transport_chain
Nicotinic_acetylcholine_receptor_signaling
Fatty_acid_beta_oxidation
B
CON
DEN
MuSC Marker
Cell Cycle
Myogenic Differentiation Marker
Myf5
Sdc4
Pax7
Calcrl
Vcam1
Cd34
Ccnd1
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Muscle injury
Nerve crush injury
Muscle injury only
Muscle harvest
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Muscle injury
Muscle + Nerve injury
100μm
eMHC Laminin DAPI
L
Muscle Regeneration
Regenerative Potential (eMHC+ fiber/mm2 area)
**
Control
Denervated
Muscle Regeneration
Regenerating Myofibers (Centrally nucleated fibers/Cluster)
*
Control
Denervated

**Major Task 2 – Subtasks 1: Figure 6. Nerve injury enhances myogenesis and muscle regeneration. (A)** Gene set enrichment analysis (GSEA) of top twenty pathways enriched in muscle satellite cells 7 days following denervation (Blue: protein synthesis-related pathways, Red: mitochondria-related pathways). $n = 3$ biological replicates derived from RNA-seq data were used. Normalized enrichment score, NES; False discovery rate, FDR. **(B)** Heatmap of key genes associated with muscle satellite cell identity, cell-cycle, and myogenic differentiation. **(C)** Representative images of proliferated muscle satellite cells isolated from control and denervated muscle, respectively. Scale bar, 200 μm. Quantification of **(D)** total muscle satellite cells per cluster and **(E)** proliferating $EdU^+$ muscle satellite cells 48 hours after seeding. $n = 3$ biological replicates. **(F)** Representative images of the differentiated FACS-purified muscle satellite cells. Scale bar, 200 μm. **(G)** Single cell clonal expansion assay of sorted muscle satellite cells from control or denervated muscles. $n = 5$ biological replicates. **(H)** Representative images of myogenic fusion assay. Isolated cyan fluorescent protein$^+$ ($CFP^+$) control MuSCs were either cultured with yellow fluorescence protein$^+$ ($YFP^+$) control MuSCs or TdTomato$^+$ denervated MuSCs. **(I)** The fusion rate was quantified in percentage. CFP/YFP fused cells appear green and CFP/TdTomato fused cells appear magenta. Scale bar, 100 μm. $n = 3$ biological replicates. **(J)** Schematic diagram of experimental design measuring muscle regeneration upon muscle injury between control and denervated muscles *in vivo*. **(K)** H&E (top) and immunofluorescence staining (bottom- embryonic myosin heavy chain, eMHC; laminin; DAPI) cross-sectioned from tibialis anterior (TA) muscle of 7-day cardiotoxin injury (right leg) and cardiotoxin + sciatic nerve pinch injury (left leg). Scale bars, 100 μm. **(L)** Quantifications of muscle regeneration assessed by the $eMHC^+$ and centrally nucleated myofibers. $n = 3$ ($eMHC^+$ fibers) and 4 (centrally nucleated fibers) biological replicates. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ via unpaired two-tailed *t* test. All quantitative data are shown as mean ± SEM.

**Major Task 2 – Subtask 1: Modulation through the NMJ to enhance muscle stem cell engraftment and improve regenerative potential.**

To validate an enhanced myogenic activity of denervated MuSCs, we assessed the proliferation and differential potential *in vitro*. When cells were cultured for 3 days, MuSCs from muscle that underwent denervation injury showed an approximately 2-fold increase in expansion, as measured by total myoblast number. Consistent with a higher frequency of myoblasts, denervated MuSCs exhibited increased proliferation as measured by incorporation of 5-ethynyl-2'-deoxyuridine (EdU). To further validate the improved myogenic activity of denervated MuSCs, we then measured the differentiation of these cells by reducing serum concentration. When an equal number of myoblasts was seeded in a 2% serum condition, myoblasts from denervated muscle formed multinucleated, myosin heavy chain (MHC) expressing myotubes at a much faster rate compared to control myoblasts. To further confirm that denervated MuSCs were primed for myogenesis, enhancing muscle regeneration *in vivo*, we tested muscle regeneration capacity by inducing muscle injury. The left and right tibialis anterior (TA) muscles were both freeze-injured, and a mild nerve injury was added to just the left sciatic nerve. Strikingly, the left nerve/muscle-injured muscle regenerated better than the right muscle injury control leg. Muscle regeneration was quantified by counting the number of centrally nucleated muscle fibers and embryonic myosin heavy chain (eMHC)-expressing fibers from the cross-sectioned muscles collected at 7-, 14-, and 28-day points after the injuries. At all-time points, we confirmed that denervation synergistically enhanced muscle regeneration from the freeze injury. Overall, these results suggest that mild nerve injury alters MuSC transcriptomics and primes MuSCs for myogenesis (**Figure 6**).

We postulated that the positive enrichments in MuSC function induced by nerve injury would result in concomitant enhancements in muscle regeneration. Thus, we tested whether the denervation affected the muscle tissue environment of the recipients to improve MuSC transplant efficiency. To verify enhanced muscle regeneration in the nerve-injured muscle, we transplanted an equal number of $GFP^+$ donor MuSCs into nerve-plus-muscle injury or muscle-injury only recipients. After 28 days of transplantation, TA muscles were harvested and analyzed for engraftment efficiency (**Figure 5A**). As expected, denervated host muscle displayed a significantly higher number of $GFP^+$ myofibers, suggesting that the nerve injury synergistically augmented myogenesis of exogenous MuSCs (**Figures 5B and 5C**). Conversely, when an equal number of control and 7 day-denervated MuSCs ($TdTomato^+$) were transplanted into the barium chloride-injured recipient muscles (**Figure 5D**), the donor MuSCs from denervated muscle showed significantly greater engraftment compared to that of control muscle *ex vivo* (**Figures 5E and 5F**). To further confirm our hypothesis that the increased muscle regeneration is due to newly added myonuclei and subsynaptic nuclei from the MuSCs, we transplanted the same number of MuSCs, isolated from H2B-GFP reporter mice, and traced the number of $H2B^+$ myonuclei in control and denervated muscles, but they were not statistically significant to each other (**Figure 5G**). These results demonstrate that changes in the neuromuscular niche promote regenerative dynamics of MuSCs in muscle fibers.

**Major Task 3 – Key Experiments**

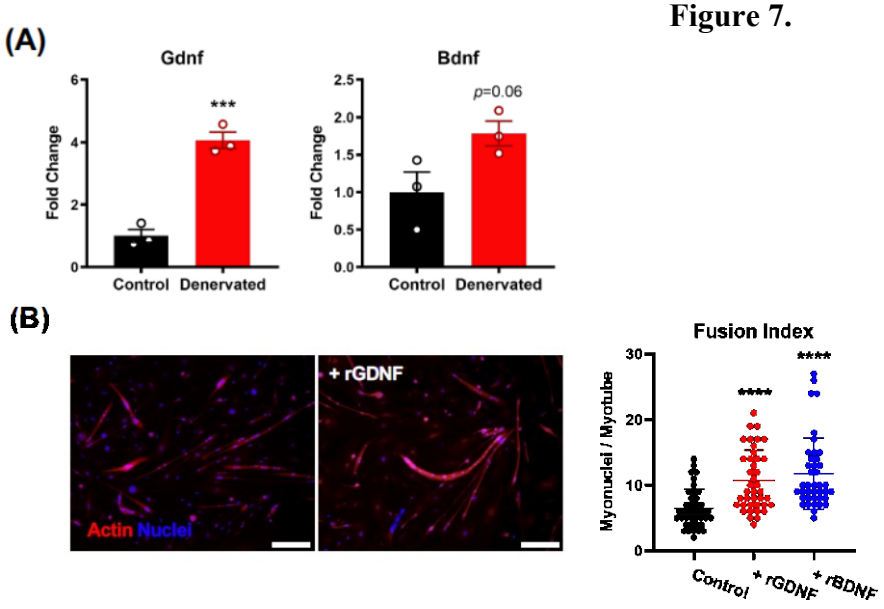
Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury.

- The role of glial-derived neurotrophic factor (GDNF) on myogenesis

**Summary of major findings**

○ Sustained delivery of a potent neurotrophic factor, GDNF significantly enhances myogenic differentiation similar to NMJ perturbation.

**Figure 7.**



**Major Task 3 – Subtasks 1: Figure 7. Glial-derived neurotrophic factor enhances myogenesis similar to NMJ priming (A)** Nerve injured MuSCs express significantly higher levels of *Gdnf* and *Bdnf*. **(B)** Sustained delivery of GDNF recombinant protein significantly myogenic differentiation as measured by fusion index. Fusin index was determined as number of multinucleated and myosin heavy chain positive myotubes. ***$p < 0.001$ via unpaired two-tailed *t* test. All quantitative data are shown as mean ± SEM.

**What opportunities for training and professional development has the project provided?**
Nothing to report

**How were the results disseminated to communities of interest?**
We published results from Major Task 1 in eLife, which is an open access journal. We also have deposited sequencing datasets on the Gene Expression Omnibus (GEO) and are accessible to the community for download. These results were presented at Emory University for a symposium on Musculoskeletal Regeneration in a session focused on the neuromuscular junction.

**What do you plan to do during the next reporting period to accomplish the goals?**

**Major Task 1:** Establish mechanistic insights into the intrinsic molecular mechanisms and signaling pathways that couple skeletal muscle regeneration and neural control.

In the next year, we will perform sequencing of myonuclei after denervation and use bioinformatics to connect how changes in extrinsic signaling converge onto muscle stem cells that help promote re-innervation and muscle regeneration.

- Sequence MuSC-derived myonuclei after 7 and 14 days post denervation and compare to uninjured myonuclei before neural injury.

**Major Task 2:** Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury.

In the next year, we will test whether neuromuscular electrical stimulation (NMES) as a strategy to boost donor muscle stem cell engraftment.

- MuSC engraftment efficiency will be assessed using bioluminescent imaging and NMES will occur multiple times during recovery.

**Major Task 3:** Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury.

In the next year, we will test the effect of MuSCs and neurotrophic factor co-delivery within biofunctional hydrogel on functional recovery following traumatic injury.

- Preparation of PEG-mal hydrogel incorporating different concentrations of GDNF and adhesive ligands for *in vivo* delivery

- FACS isolation of MuSCs and functionalized hydrogels for sustained release of GDNF *in vivo*.
- Assess neuromuscular junction formation, endplate potential, and muscle force generation.

## *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:

Dr. Jang moves universities from Georgia Tech to Emory in 2021-2022 and this has delayed progress on Major Tasks 2 and 3. He has initiated a new animal protocol and ACURO approval and this has taken time to perform.

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

None

**Changes that had a significant impact on expenditures**

None

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

**Significant changes in use or care of human subjects**

None

**Significant changes in use of biohazards and/or select agents**

None

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**

**Journal publications.**

1) Larouche, J. et al. Muscle Stem Cell Response to Perturbations of the Neuromuscular Junction Are Attenuated With Age. *eLife* **10**, e66749 (2021).
2) Choi, JJ et al. Motor neuron injury primes muscle stem cells for myogenesis by enhancing protein synthesis and mitochondrial bioenergetics. *In preparation for submission* (2022)

**Books or other non-periodical, one-time publications.**

None

**Other publications, conference papers and presentations**.

None

- **Website(s) or other Internet site(s)**

https://www.nobel.bme.umich.edu/

https://www.janglabemory.org/

- **Technologies or techniques**

None

- **Inventions, patent applications, and/or licenses**

None

- **Other Products**

None

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

*Name:* *Paula Fraczek*
*Project Role:* *Graduate Student*
*Researcher Identifier (e.g. ORCID ID):*
*Nearest person month worked:* *12*
*Contribution to Project:* *Ms. Fraczek has administered nerve trauma on transgenic animals, imaged single myofibers to glean muscle stem cell contributions and worked on isolating myonuclei from muscle for profiling.*

*Name:* *Jeongmoon Choi*
*Project Role:* *Graduate Student/Postdoc*
*Researcher Identifier (e.g. ORCID ID):*
*Nearest person month worked:* *12*
*Contribution to Project:* *Dr. Choi performed in vitro and in vivo experiments on nerve injured muscle stem cells, muscle stem cell transplantation, and prepared manuscript on these results.*

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

| |
|---|
| No |

**What other organizations were involved as partners?**

| |
|---|
| Young C. Jang – Georgia Institute of Technology/Emory School of Medicine |

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:**

**QUAD CHARTS:**

## 9. APPENDICES: