AWARD NUMBER: W81XWH-18-1-0684

TITLE: Restoring Neuromuscular Junction Integrity to Alleviate ALS Progression

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REPORT DATE: October 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2020	Annual	30Sep2019-29Sep2020

4. TITLE AND SUBTITLE

Restoring Neuromuscular Junction Integrity to Alleviate ALS Progression

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0684

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Arlington, TX 76019-0145

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

ALS is a fatal neuromuscular disease. Failure of the respiratory muscle is a main cause of mortality in ALS patients. Defects in neuromuscular junctions (NMJs) and progressive NMJ loss occur at early stages, thus stabilizing and preserving NMJs represents a potential therapeutic strategy to slow ALS disease progression. This project is aim to explore a potential role of a muscle-derived protein MG53 in maintaining the NMJ and diaphragm muscle membrane integrity, and to establish the efficacy and safety for using recombinant human MG53 protein (rhMG53) to treat ALS in the mouse model. Since MG53 is already present in human blood circulation under normal physiologic conditions, therapeutic approach with modulation of MG53 function or systemic administration of rhMG53 can potentially be a safe biologic reagent for treatment of ALS.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

ALS is a fatal neuromuscular disease. Failure of the respiratory muscle is a main cause of mortality in ALS patients. Defects in neuromuscular junctions (NMJs) and progressive NMJ loss occur at early stages, thus stabilizing and preserving NMJs represents a potential therapeutic strategy to slow ALS disease progression. While mitochondria-mediated reactive oxidative species (ROS) links to the etiology of ALS, the mechanisms that underlie NMJ degeneration in ALS are largely unknown. During respiration, the diaphragm constantly undergoes contraction-relaxation, a process that leads to injury to the muscle membrane. Inadequate repair of injury to the sarcolemma can disrupt NMJ integrity and contribute to diaphragm wasting in ALS. MG53 is an endogenous protein in human body that serves essential roles in nucleating assembly of repair patches at membrane injury sites. Genetic ablation of MG53 results in defective membrane repair and tissue regenerative capacity. A series of studies have shown that recombinant human MG53 (rhMG53) protein protects various cell types against membrane disruption when applied to the extracellular environment in animal models. We ***hypothesize that MG53-mediated membrane repair contributes to maintenance of NMJ integrity in ALS***. Since NMJ is an active site of neuron/muscle crosstalk, we postulate that membrane repair defects originate from NMJ. A vicious cycle of mitochondrial dysfunction/membrane repair defects leads to increased vulnerability of NMJ to stress-induced injury as part of ALS pathology. Thus, this project has two specific aims: (1) To elucidate the physiological role of MG53-mediated membrane repair in ALS. Specifically, the SOD1G93A mice will be cross-bred with MG53 knockout and ctPA-MG53 mice to evaluate MG53's physiological role in regulating the degeneration of NMJ associated with ALS progression. These studies will test whether elevated level of MG53 in circulation has protective role for NMJ integrity in ALS. (2) To conduct proof-of-concept study testing rhMG53 as a novel therapeutic means for improving NMJ integrity to treat ALS. We propose to establish the efficacy and safety for using rhMG53 protein to treat ALS in the mouse model. Since MG53 is already present in blood circulation under normal physiologic conditions, therapeutic approach with modulation of MG53 function or systemic administration of rhMG53 can potentially be a safe biologic reagent for treatment of ALS.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

ALS (Amyotrophic Lateral Sclerosis), NMJ (neuromuscular junction), MG53, rhMG53 (recombinant human MG53), Mitochondria, Skeletal muscle

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project? *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*
What was accomplished under these goals? *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project*

progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

What do you plan to do during the next reporting period to accomplish the goals? *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

Major activities, specific objectives, significant results, and the future plan

Aim 1: Elucidate the physiological role of MG53-mediated membrane repair in ALS.

Subtask 1.1: Cross-breeding of *SOD1^{G93A}* mice with *mg53-/-* and ctPA-MG53 mice (1-18 months)
Subtask 1.2: Dissect the role of MG53 in preservation of muscle and NMJ integrity in ALS (6-18 months)
Subtask 1.3: Dissect the function of MG53 in maintenance of mitochondria function in ALS (12-24 months)

Milestones:
1) Physiologic role of MG53 in ALS established. (On going)
2) ALS mice with knockout of MG53 show exacerbated diaphragm muscle injuries. (on going)
3) Elevated MG53 in circulation preserves NMJ integrity in ALS mice. (On going)

1. Back-cross the *mg53-/-* and ctPA-MG53 mice (with genetic background of C57) to the same gene background (B6SJL) of the ALS SOD1^{G93A} mice used in our lab: As stated in the previous Annual Technical Report, Dr. Zhou's lab was relocated from Kansas City University (KCU) to UTA in July 2018. The DOD grant was relinquished from KCU to UTA. The fund started on March 2019. Following the MTA (Material Transfer Agreement between the Ohio State University (OSU) and UTA) signed on Feb 11, 2019, the *mg53-/-* and ctPA-MG53 mice were transferred to UTA in March 2019. After the quarantine and foster breading, we initiated the process of back-crossing those mouse models to the genetic background (B6SJL), which is the genetic background of our G93A mouse model. In our previous Annual Technical Report submitted in Dec 2019, we reported that we had completed 1st round of back-crossing. The Covid-situation had some impact on our progress this year, however, we managed to perform another three procedures of cross-breeding. The table below is the summary of the cross-breeding *mg53-/-* and ctPA-MG53 mice with B6SJL mice.

tPA-MG53 back-crossing with B6SJL mice

1. 2nd backcrossing

Litters/Date of Birth	tPA-MG53^{+}	tPA-MG53^{-}
1, DOB 1/27/2020	Male: 1; Female: 4	Male: 1 ; Female: 1
2, DOB 2/18/2020	Male: 1 ; Female: 1	Male: 1; Female: 3
3, DOB 3/20/2020	Male: 3; Female: 2	Male: 5; Female: 0

2. 3rd backcrossing

Litters/Date of Birth	tPA-MG53^{+}	tPA-MG53^{-}
DOB 8/27/2020	Male: 3; Female: 0	Male: 1 ; Female: 2

3. 4th backcrossing: DOB 11/17/2020, 8 pups (genotype pending)

MG53KO back-crossing with B6SJL mice

1. 2nd backcrossing

Litters/Date of Birth	MG53$^{+/-}$(hemizygous)	MG53$^{+/+}$(wild type)
DOB 1/27/2020	Male: 4; Female: 9	Male: 3 ; Female: 3

2. 3rd backcrossing

Litters/Date of Birth	MG53$^{+/-}$(hemizygous)	MG53$^{+/+}$(wild type)
DOB 8/27/2020	Male: 2; Female: 0	Male: 1; Female: 3

3. 4th backcrossing: DOB 11/18/2020, 7 pups (genotype pending)

2. Evaluating the physiological role of MG53 in preserving NMJ in ALS: After 6 processes of back-crossing to B6SJL genetic background, we will be ready to cross G93A with *mg53-/-* and ctPA-MG53 to generate G93A/*mg53-/-* and G93A-ctPA-MG53 mice, which should limit any potential influence of different genetic background on ALS disease progression in the mouse models. After we obtained the double transgenic mice, we will conduct physiological study to evaluate whether missing or elevated MG53 in circulation affect the muscle function, NMJ integrity and the life span of ALS G93A mice.

Aim 2: Conduct proof-of-concept study testing efficacy and safety of rhMG53 to treat ALS in mice.

Subtask 2.1: Production and quality controls of rhMG53 and PEG-rhMG53. (1-12 months)
Subtask 2.2: Pharmacokinetic (PK) assessment of PEG-rhMG53 in ALS mice. (6-12 months)
Subtask 2.3: In vivo efficacy and safety assays with rhMG53 and PEG-rhMG53 in ALS mice. (6-24 months)

Milestones:

1) Produce 2 grams of rhMG53, sufficient for pre-clinical studies (Completed-last report)
2) In vitro QC assays to ensure purity and function of rhMG53. Endotoxin level of rhMG53 < 10 EU/mg (Completed-last report)
3) PEGylation improves PK of rhMG53 in ALS mice (Completed-last report)
4) Intravenous or subcutaneous administration of rhMG53 or PEG-rhMG53 improves integrity of diaphragm and NMJ in ALS mice. (completed-this report)
5) Repetitive intravenous or subcutaneous administration of rhMG53 does not produce adverse effects in ALS mice. (completed-this report).

1. We have produced 2 grams of the rhMG53 protein, which is sufficient for the preclinical studies (Last report).

2. We conducted quality control assays with SDS-PAGE and RP-HPLC to ensure that the rhMG53 protein is >98% purity. In vitro membrane damage assay was used to determine the function of the rhMG53 in repair of cell membrane injuries; the efficacy is comparable to our published studies in Science Translational Medicine (Weisleder N, et. al., 2012; Duann P, et. Al., 2015). The endotoxin level of the rhMG53 protein is ~2-5 EU/mg. (Last report) (**Fig. 1A, and 1B**)

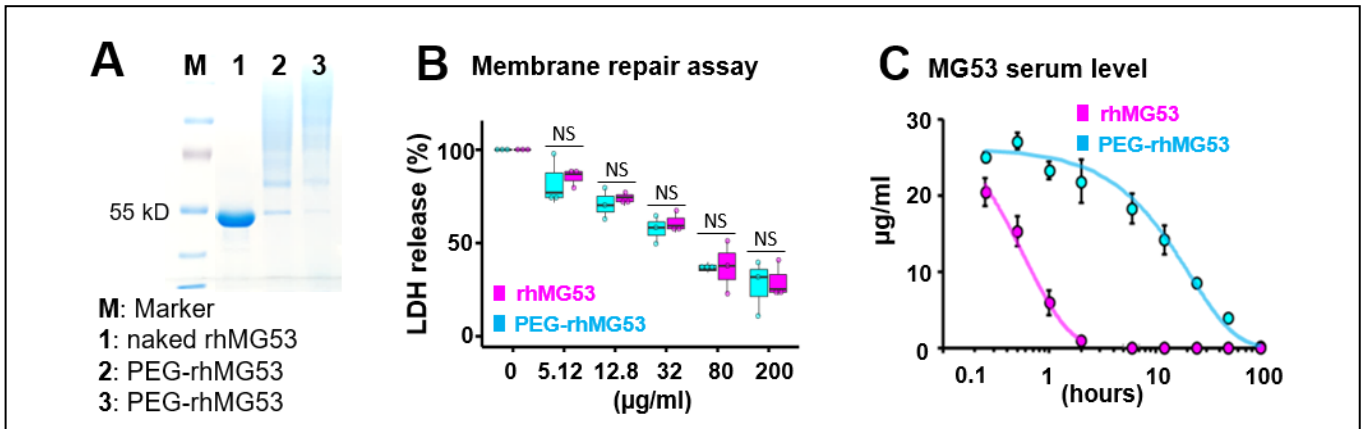


Fig. 1: (**A**) PEGylation of rhMG53 is successful based on the oligomerization pattern of the protein running on SDS-PAGE. Lanes 2 and 3 represent two independent rhMG53 PEGylation experiments. (**B**)The chemical modification did not affect the membrane repair function of rhM53 as there were no difference in EC50 of LDH release from the cultured C2C12 cells following a mechanical membrane damage with glass beads between the rhMG53 and PEG-rhMG53 (n=3 independent experiments). (**C**) Pharmacokinetic (PK) assessment revealed that PEGylation increased serum half-life of rhMG53 in rats from 0.5 hour (rhMG53, n = 4) to 12 hours (PEG-rhMG53, n = 3).

3. We performed PK studies with the PEGylated rhMG53 in rats and found that PEGylation improved the serum half-life of rhMG53 from 0.5 hour to 12 hours. (Last report) (**Fig. 1C**)

4. Intravenous administration (IV) of rhMG53 in ALS G93A mice improved the integrity of diaphragm and NMJ, and extended the life span of ALS muscle. (Started before the last report and further validated in the study of this year)
   a) We confirmed that increased susceptibility of diaphragm injury is an early pathological event in the ALS G93A mouse model. As illustrated in **Fig. 2A**, we performed intraperitoneal (IP) injections of Evans blue (EB) dye to wild type (WT) and G93A littermates at 2 months of age (pre-symptomatic stage, prior to ALS onset). We harvested the diaphragm muscles 16 hours later, immediately after the mice performed a 30 min running protocol (18 m/min, 15^{o} downhill), and evaluated EB retention within muscle fibers. The amount of EB fluorescence measures the extent of muscle membrane leakage. The G93A diaphragm muscles displayed significantly higher EB levels under resting condition compared to WT (**Fig. 2B**). The 30 min downhill protocol dramatically enhanced EB accumulation in the G93A diaphragm (**Fig. 2C**), but there was no change in the WT mice. Measurement of serum creatine kinase (CK) showed significant elevation in G93A mice after down-hill running (**Fig. 2D**), further indicating the enhanced muscle membrane damage in the 2-month-old G93A mice following this modest exercise.

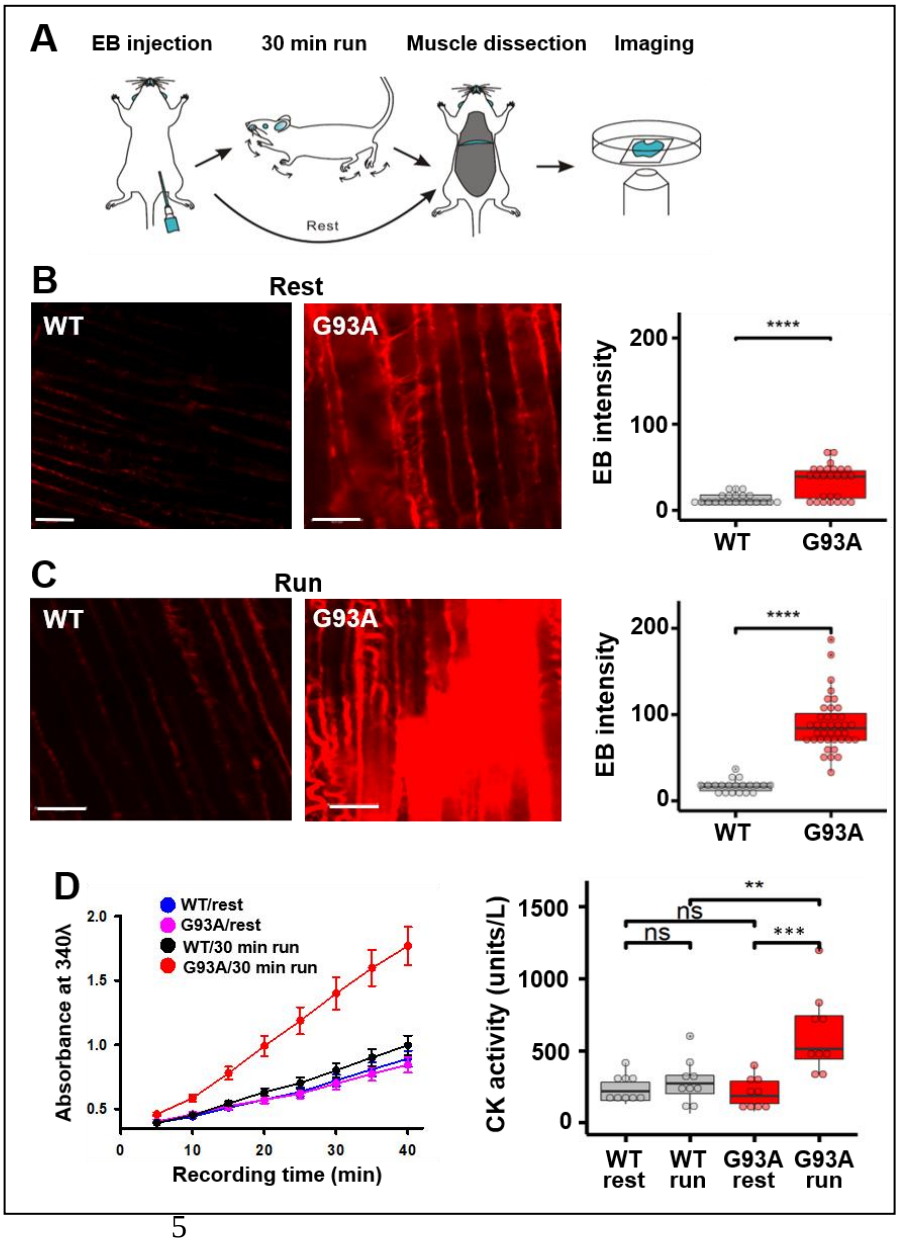


Fig 2. (**A**) Schematic diagram for evaluation of diaphragm integrity in ALS mice. (**B**) Compared with WT mice, diaphragm muscle derived from G93A littermates (2-month-old) showed increased EB intensity without running (WT: 12.8 ± 1.5, n = 21 from 4 mice; G93A: 33.4 ± 3.7, n = 26 from 2 mice, **** P < 0.0001). Scale bars: 20 μm. (**C**) 30 min downhill running caused drastic elevation of EB in diaphragm derived from the G93A mice (WT17.30 ± 1.69, n = 20 from 3 mice; G93A: 88.49 ± 4.92, n = 39 from 4 mice, **** P < 0.0001). Scale bars: 20 μm. (**D**) Quantification of CK activity in serum samples derived from G93A and WT littermate mice (2-month-old) before and after 30 min running. n = 9 for each group, **P < 0.01, ***P < 0.001, ns: not significant. Left panel shows the time-dependent reading of the absorbance (at 340 nm) of serum samples in a 96-well plate; Right panel shows the calculated CK activity derived from the absorbance reading.

b) We had a new finding, in which the neuromuscular junction (NMJ) is an active site of injury-repair by MG53 under physiological conditions that is lost in ALS. Our published work has shown that mitochondrial defects first appeared locally at NMJs in G93A mice. Thus, we examine whether the NMJ itself is an active site of contraction-induced membrane injury, and if MG53 plays a role in repair of NMJ injury. Immediately after the 30 min running, freshly isolated flexor digitorum brevis (FDB) muscle fibers from the 2-month-old mice were fixed for staining with α-Bungarotoxin (BTX) to mark NMJ and anti-MG53 antibodies. MG53 accumulated at the NMJ area and formed patches in WT myofibers (n=130, 4 mice) (**Fig. 3A, left**), suggesting that MG53 contributes to the maintenance of NMJ integrity under physiologic conditions. While MG53 also accumulated at the NMJ area in G93A myofibers (n=109, 3 mice), there were also separate intracellular MG53 aggregates in proximity to NMJs in about 10% of G93A myofibers (**Fig. 3A, right**). Moreover, FDB myofibers derived from the G93A mice at an advanced stage of ALS (4-month-old) showed increased abnormal MG53 aggregation near NMJs even without running (**Fig. 3B**). Such MG53 aggregation suggests potential impaired tissue-repair capacity that may manifest into NMJ degeneration during ALS disease progression.

Using the entry of a cell-impermeable fluorescent dye FM 1-43 as a measure of muscle fiber integrity, we tested the possibility that ALS-associated muscle injury originates at NMJs. **Fig. 3C** shows an overlay of FM 1-43 and BTX, indicating FM 1-43 enriches at NMJ when applied to the medium. In WT myofibers, FM 1-43 was restricted to the NMJ and there were barely any FM 1-43 signals inside myofibers after a 20 min dye incubation (**Fig. 3D**). In contrast, 59% ± 7% of FDB myofibers from three 4-month-old G93A mice showed intracellular uptake of FM 1-43 dye, which formed a gradient centered around the NMJ (**Fig. 3E**), indicating that FM 1-43 dye entered the cells preferentially through injured NMJs. We next examined whether exercise could exacerbate membrane injury at NMJs of G93A muscle. For this purpose, three pairs of 2-month-old WT and G93A littermate mice performed the running protocol for 30 minutes. 72%±2% of G93A FDB myofibers showed NMJ-centered intracellular FM 1-43 gradient, whereas only 6%±2% WT myofibers exhibited this phenomenon ($P < 0.001$). These data substantiate the finding that NMJs are focally more susceptible to injury than other regions of the sarcolemma, and exercise-induced NMJ injury is dramatically exacerbated in the G93A ALS mice.

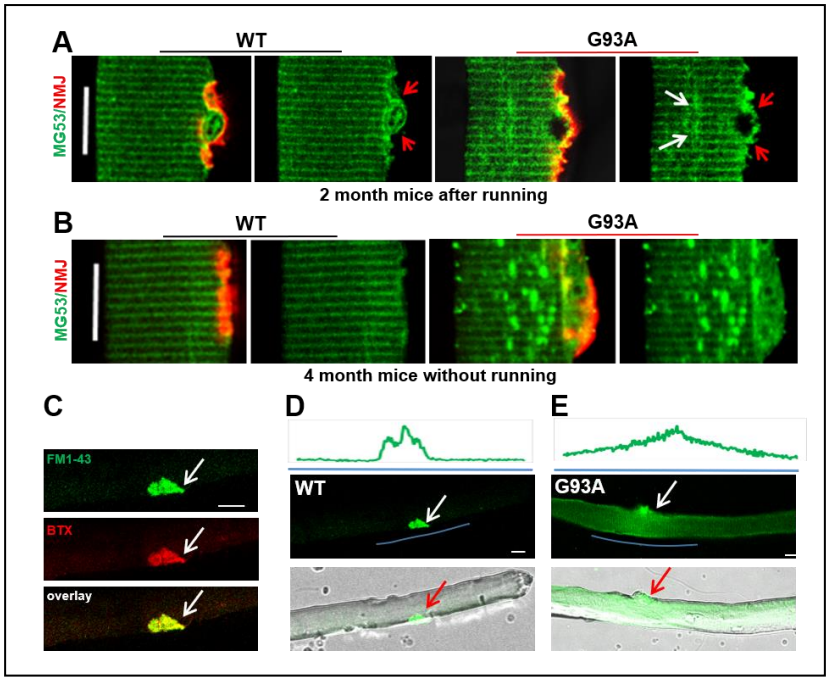


Fig. 3. (**A**) FDB myofibers, obtained from WT and G93A mice (2-month-old) subjected to 30 min running, were co-stained with MG53 antibody (green) and BTX (red). MG53 formed patches covering the NMJ site in both WT and G93A myofibers. However, intracellular MG53 aggregates start to appear near the site of NMJ in the G93A myofibers. Scale bars: 20 μm. (**B**) FDB myofiber derived from G93A mice at the advanced stage of ALS (4-month-old) displayed extensive MG53 aggregates near NMJ without running. FDB myofiber derived from WT littermates showed a uniform pattern of MG53. Scale bars: 20 μm. (**C**) FM 1-43 and BTX co-localized at the NMJ of FDB muscle fibers (white arrows). (**D**) No intracellular FM 1-43 fluorescence was observed in the WT fiber at 20 min after incubation with FM 1-43. (**E**) FM 1-43 entered the G93A fiber via the NMJ region. Green line highlights the region for fluorescence intensity profiling. Scale bars: 20 μm.

c) The excessive mitochondrial ROS production is likely the underlying mechanism for muscle membrane and NMJ injury in ALS mice. We and others have shown that mitochondrial dysfunction is associated

with enhanced reactive oxygen species (ROS) production in the mouse ALS muscle, which could impact the intrinsic membrane repair function of MG53. Using our established live cell imaging method, we examined whether oxidative stress impact the traffic of MG53 vesicles inside C2C12 cells overexpressing GFP-MG53. The 2D x-y time-lapse images were continuously recorded for 10 seconds in the presence or absence (basal) of 1 mM H_2O_2. Representative images at 0 sec (0s, pseudo color green), 5 sec (5s, pseudo color red) and 10 sec (10s, pseudo color red) were selected for generating the overlay images (**Fig. 4**). The overlay images of 0s with 5s or 0s with 10s provide a visualization of GFP-MG53 vesicle dynamics. In the overlay images, non-moved vesicles are marked by yellow color (completely overlap), while moved vesicles are indicated by the red and green colors (no overlap). Note that there is almost no detectable movement of GFP-MG53 vesicles in the presence of H2O2, while moved GFP-MG53 vesicles were detected under basal condition.

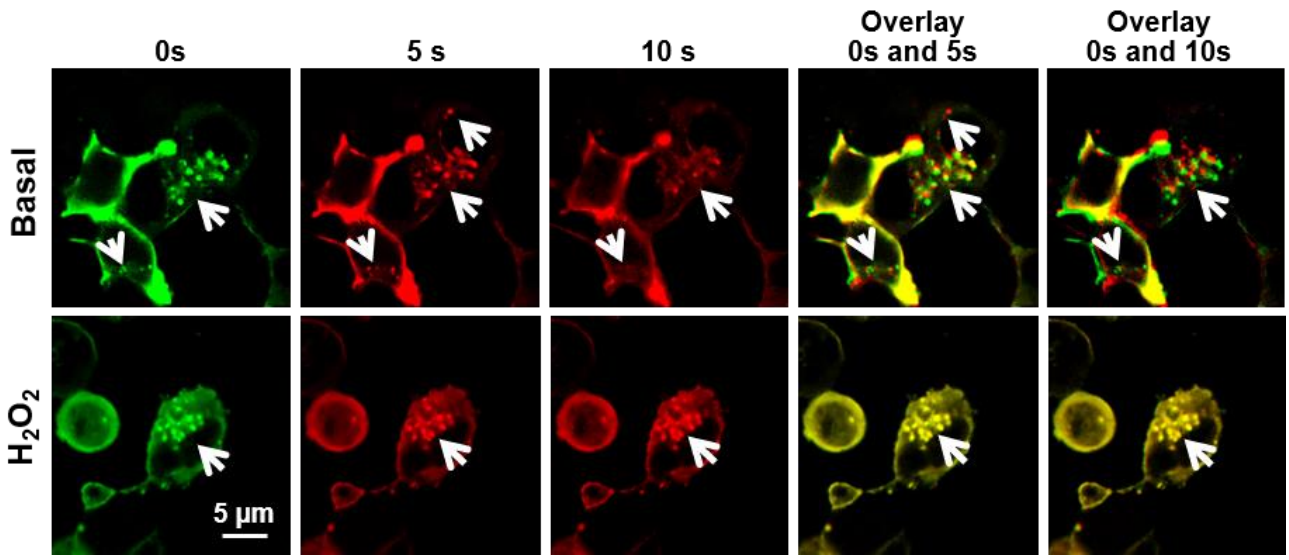


Fig. 4. The representative time-lapse images of C2C12 cells with overexpression of GFP-MG53 in the presence and absence (basal) of 1 mM H_2O_2. The overlay images were generated using images at 0s and 5s, or 0s and 10s. White arrows indicate GFP-MG53 vesicles.

d) Impaired MG53 membrane repair function is a common pathological feature of ALS muscle. With the support of the Target ALS Human Postmortem Tissue Core, we obtained paraffin-embedded diaphragm and psoas muscle sections from both sporadic and familial ALS decedents, and non-ALS controls. Both longitudinal and transverse sections of human ALS diaphragm and psoas muscle showed dramatic abnormal sarcolemmal and intracellular MG53 aggregates. In contrast, the muscle samples of non-neurological control decedents showed only a few scattered MG53 aggregates (**Fig. 5A and Supplementary fig. S2**). It is not unexpected to see a few MG53 aggregates in non-ALS muscle, as MG53-mediated membrane repair also occurs in normal conditions, but to a much lesser extent. We are aware of the limitations of using the very end-stage human disease samples. Importantly, the postmortem ALS muscle samples are not "end-stage" per pathological criteria, but rather show variable degrees of acute and chronic neurogenic atrophy, as is typical with ALS – with different muscles, and even different areas within muscles showing variable severities of denervation. This same staining pattern was observed in longitudinal (**Fig. 5B, left**) and cross-sectional (**Fig. 5B, right**) staining of diaphragm muscle from 4-month-old G93A mice. The abnormal intracellular MG53 aggregation was observed in all 4-month-old G93A muscles examined, including EDL, soleus, and (TA) (**Supplementary fig. S1**). The data from both familial and sporadic ALS decedents, and the G93A mouse model suggest that compromised MG53-mediated muscle membrane repair function could be a common pathology in ALS.

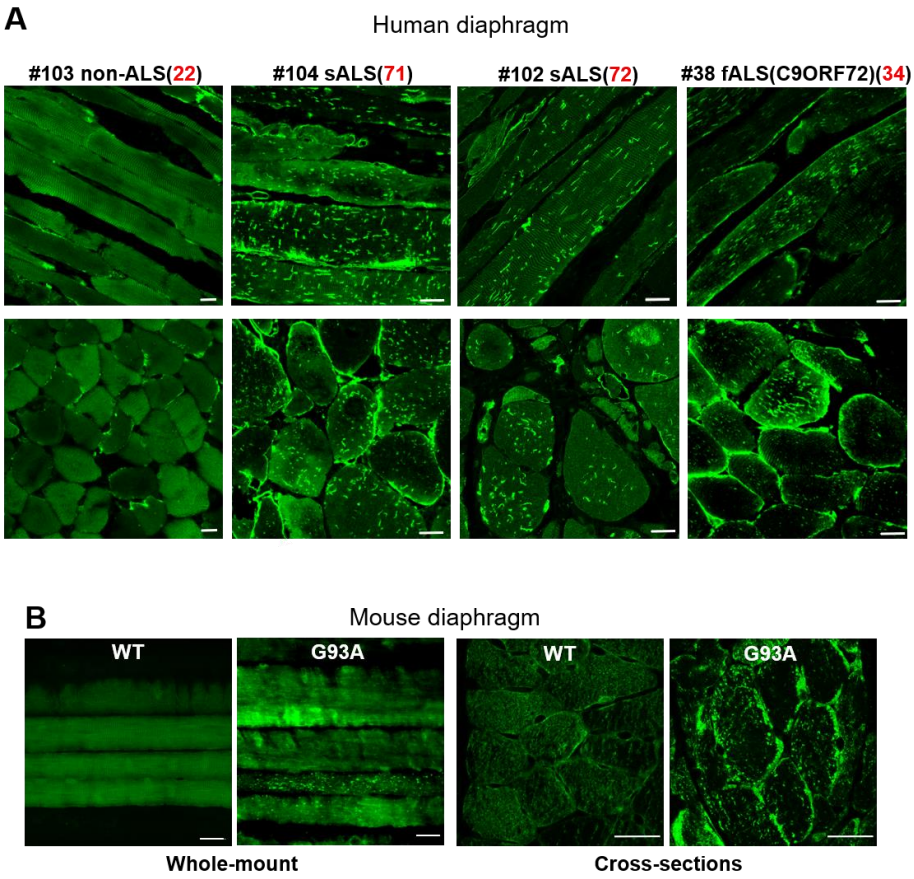
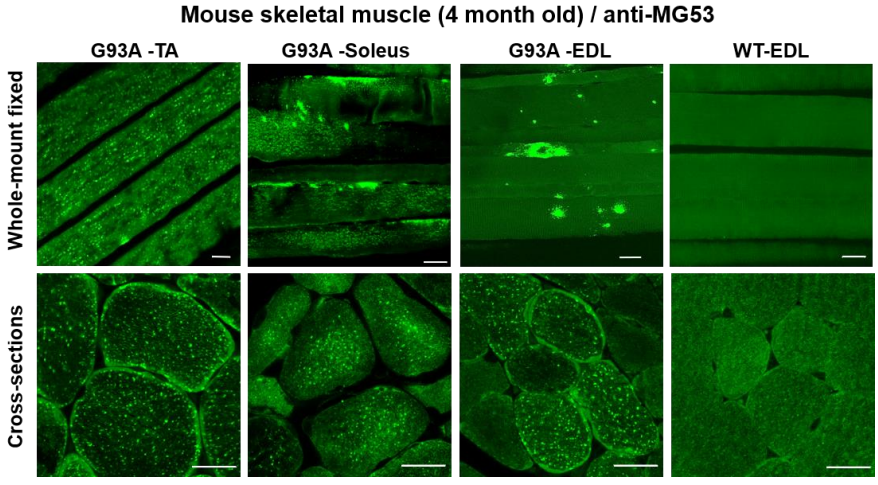
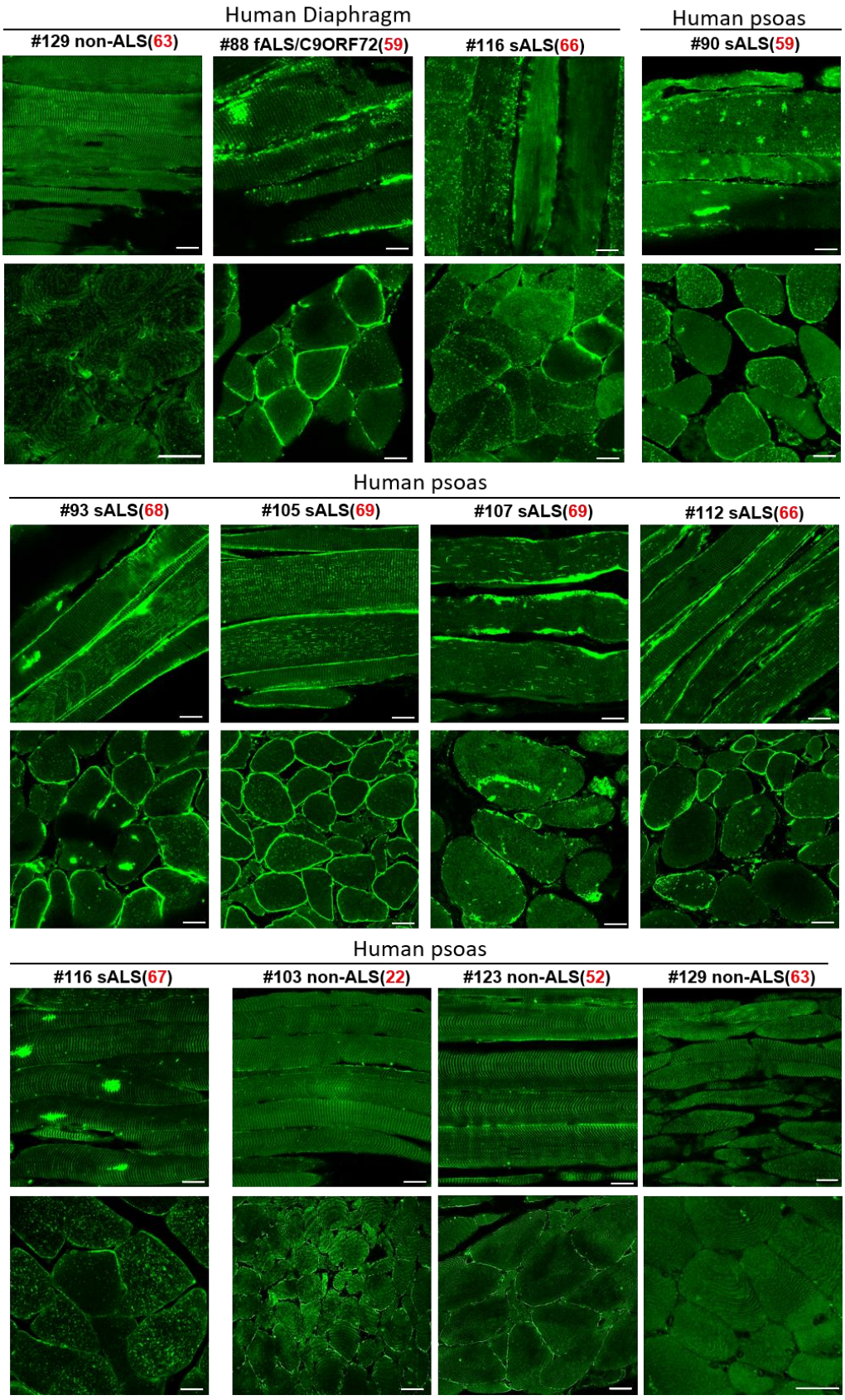


Fig. 5. **Impaired MG53 membrane repair function is a common pathological feature of ALS**. (**A**) Longitudinal (top panels) and cross-sectional (bottom panels) staining of MG53 demonstrated uniform patterns in non-ALS human diaphragm (left); whereas human ALS diaphragms (sALS: sporadic ALS: fALS: familial ALS) displayed extensive intracellular aggregates of MG53. The age at the death was indicated in red fonts. Scale bars: 20 μm. (**B**) Diaphragm muscle derived from a 4-month-old G93A mouse (post-ALS onset) also showed intracellular aggregates of MG53.



Supplementary fig. S1. MG53 immunostaining of skeletal muscles isolated from multiple anatomic locations in 4-month old WT and G93A mice in cross sections and whole-mount preparations. There were prominent intracellular MG53 protein aggregates in all G93A muscles examined, while there was no apparent MG53 aggregation inside the WT muscles.



Supplementary fig. S2. Additional images of anti-MG53 immunostaining of ALS and non-ALS human muscle samples. Bar: 20μm. The same as demonstrated in **Fig. 5A**. both human ALS diaphragm and psoas muscles displayed extensive intracellular aggregates of MG53 or enhanced sarcolemma membrane localization. The age of patients was indicated with red fonts. Scale bars: 20 μm.

e) We validated that the recombinant human (rhMG53) preserves the membrane integrity of diaphragm in G93A mice. We first conducted *ex vivo* studies with isolated myofibers from G93A mice and found that G93A myofibers showed higher laser-induced membrane fragility compared to the WT fibers, and application of rhMG53 in the culture medium could prevent the laser-induced membrane damage to the G93A myofibers (**Fig. 6**). We then conducted the *in vivo* studies by intravenous (IV) administration rhMG53 in G93A mice and demonstrated (1) rhMG53 attenuated the exercise-induced membrane injury in diaphragm muscle of G93A mice (**Fig. 7**); (2) 2 weeks of rhMG53 IV treatment in 3-month-old G93A mice significantly preserved NMJ integrity and the survived numbers of motor neurons in the spinal cord (**Fig. 8**).

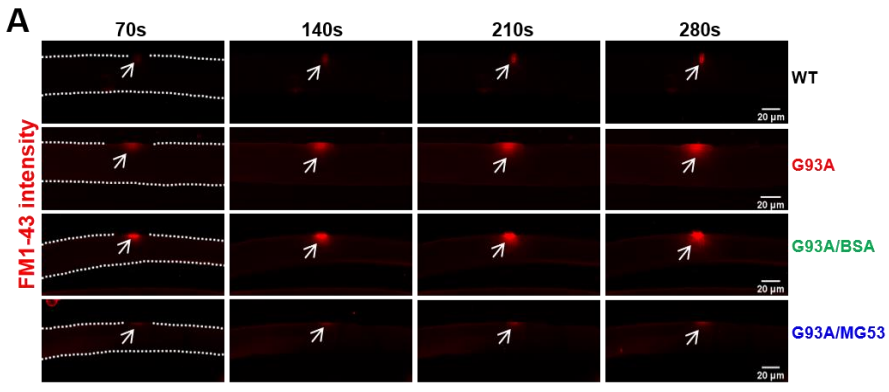


Fig. 6. (**A**) Time-lapse recordings of FM 1-43 accumulation in FDB fibers after laser-induced membrane damage. The dashed lines highlight the fiber outline. (**B**) Plotting of FM 1-43 intensity inside FDB fibers over time after laser-induced membrane injury. Between G93A and WT (n = 6-9 fibers/group; P < 0.001), and between G93A/BSA (9 fibers) and G93A/MG53 (10 fibers) (P < 0.001).

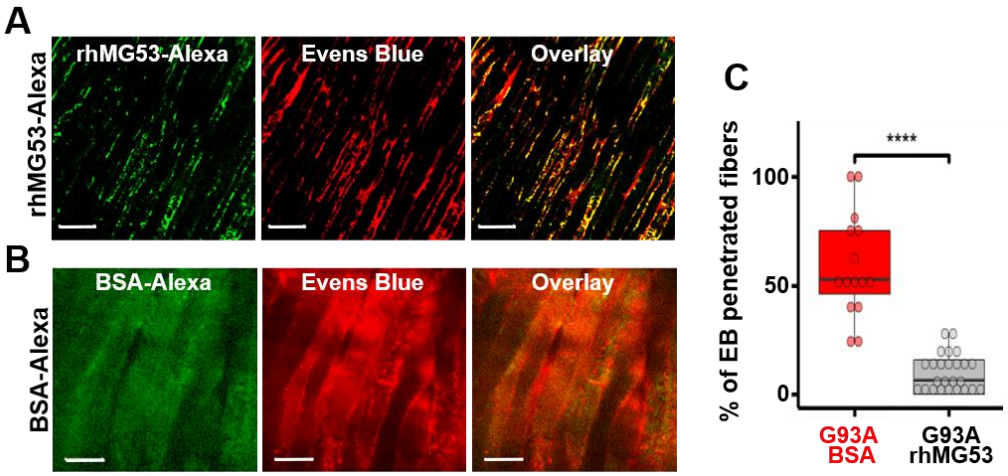


Fig. 7. (**A**) Exercise-induced accumulation of EB in diaphragm derived from G93A mice (2-month-old) was reduced with IV administration of rhMG53-Alexa (2 mg/kg). rhMG53-Alexa targeted to the sarcolemma of the diaphragm. Scale bars: 20 μm. (**B**) Diaphragm derived from G93A mice receiving BSA-Alexa showed extensive accumulation of EB. BSA-Alexa did not target to the sarcolemma. Scale bars: 20 μm. (**C**) Percentage of myofibers with EB penetration was significantly reduced with administration of rhMG53-Alexa (n = 14 for BSA-Alexa488, n = 25 for rhMG53-Alexa488 from 3 pair of G93A mice, ****P < 0.0001).

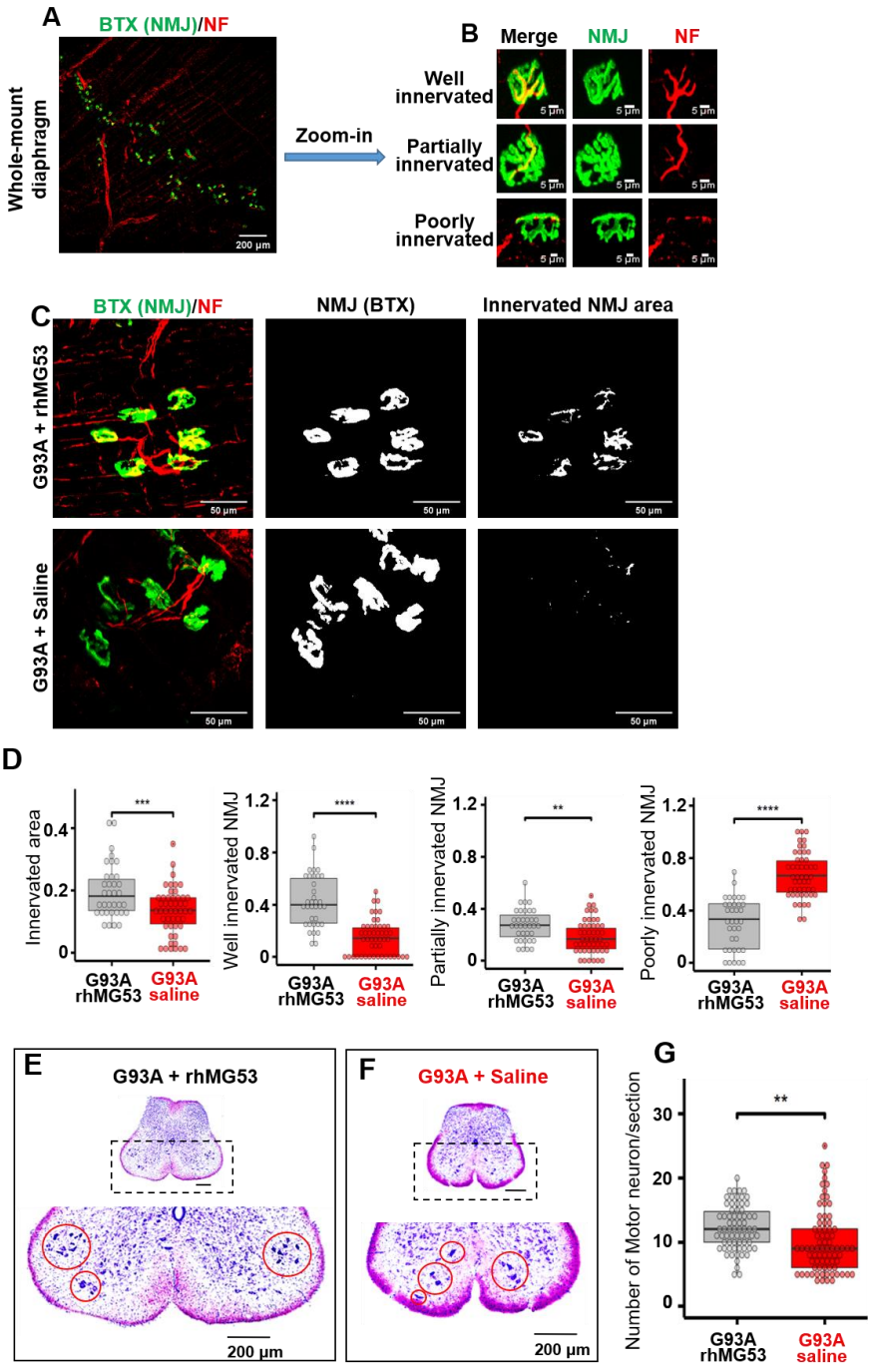


Fig. 8. (**A)** Whole-mount fixed diaphragm muscle stained with the NF antibody (red) for marking axonal terminals and BTX (green) for detecting NMJ. (**B**) Z-stack images of well (the ratio of innervated area >20%), partially (the ratio of innervated area between 10%~20%), and poorly innervated NMJs (the ratio of innervated area <10%) in diaphragm muscle. (**C**) Z-stack images of diaphragm muscle from G93A mice receiving 2-week rhMG53 treatment or saline-control (*left panels*). The area of individual NMJ defined by BTX was presented (*central panels*). The innervated area of NMJ was defined by the area overlapping with NF (*right panels*). (**D**) Comparing the ratio of innervated NMJ area in rhMG53 (n = 39, 4 mice) and saline (n = 45, 5 mice) treated diaphragm muscles of G93A littermate mice, as well as the ratio of well, partially, poorly innervated NMJs. rhMG53 treatment significantly preserved the innervation of NMJ in diaphragm muscle of G93A littermate mice. **P < 0.01, ***P < 0.001, ****P < 0.0001. (**E, F**) Images of the lumbar spinal cord section of G93A mice (with 2-weeks of rhMG53 or saline treatment from the age of 3 months). (**G**) The number of surviving motor neurons per section in G93A littermate mice after two-weeks of treatment with rhMG53 (12.2 ± 0.4) or saline (10.1 ± 0.5) (rhMG53, n = 70 spinal cord sections*;* saline, n = 77 spinal cord sections; 4 pairs of G93A mice per cohort, ** P < 0.01).

5. Repetitive intravenous or subcutaneous administration of PEG-rhMG53 does not show adverse effects in ALS mice. (started before the last report and further validated in the study of this year)

a). We confirmed the effect of PEG-rhMG53 in prolonging the life span of G93A mice in both genders. 26 G93A littermate mice (with gender balanced) at the age of disease onset (3-month-old) were divided into two groups, one receiving PEG-rhMG53 (2 mg/kg, every other day for 30 days), and the other receiving saline as a control. One-month treatment of PEG-rhMG53 significantly extended the life span of G93A mice from 124±2 days (saline) to 137±2 days (PEG-rhMG53). (**Fig. 9A, 9B**)

b). G93A mice received the one-month PEG-rhMG53 treatment revealed a maintained bodyweight (**Fig. 9C**), and significantly improved their motor activity demonstrated with the tail suspension test (**Fig. 9 E**) and the mouse movement tracking (**Fig. 9D**).

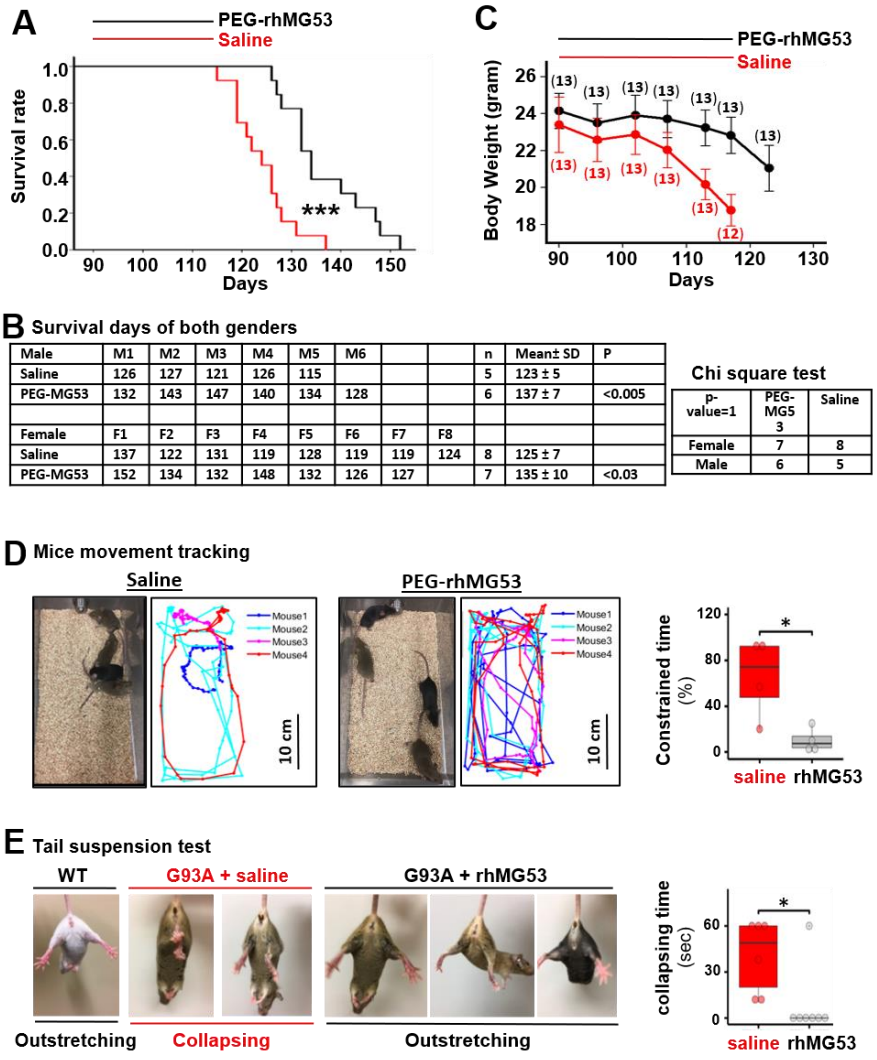


Fig. 9. (**A**) Survival curve of G93A mice with one-month PEG-rhMG53 or saline treatment. (n=13 pairs of G93A littermates, ***P < 0.001). (**B**) List of the survival days of individual tested G93A mice. The Chi square test further confirmed that the significant difference between PEG-rhMG53 (PEG-MG53) and saline treated groups is independent of the gender. (**C**) Bodyweight changes of G93A mice receiving PEG-rhMG53 or saline treatment. The numbers of mice included in each data point were indicated on the plot. (**D**) Movement tracking was performed using 1-minute video recording files of the saline and PEG-rhMG53 treated mice. The time showing constrained movement is defined as the percentage of the recording time in which the mouse moved at less than 1 cm/sec speed for 3 sec or longer. (**E**) Representative photos and Collapsing time measurement of tail suspension tests of G93A mice at the age of 104 day after receiving rhMG53 (n = 7 mice) or saline (n = 6 mice) for 2 weeks. An age-matched WT mouse was included for comparison (* P < 0.05).

c). In a separated study (not directly supported by this grant), we also published a work in Nat Communications (Bian et al, 2019, https://doi.org/10.1038/s41467-019-12483-0https). In this study, we demonstrated that sustained elevation of MG53 in the bloodstream of our mouse model (ctPA-MG53) increases tissue regenerative capacity without compromising metabolic function. The vital organs (including muscle, heart, liver, brain, lung and kidney) of ctPA-MG53 mice show normal morphology at the age of 32 months.

NOTE: We have submitted a manuscript to Science Translational Medicine, which is currently under review.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No significant changes

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The quarantine and back-crossing of MG53$^{-/-}$ and ctPA-MG53 mouse models to B6SJL genetic background caused some delay. We filled a no-cost extension to continue in this study. Please also see the report for Aim 1 for the future plan.
The Covid-19 pandemic has tremendous impact on our research. The Texas Governor put the state on lockdown for several weeks. UTA was also closed for several weeks prior to implementing tele/remote working. Research laboratories were closed for a longer period of times before the university executive staff executed a plan for continued operations. Even after the lab reopening, the research activity has been impacted due to taking turns to working in the lab to keep social distance, working from home, limiting the animal numbers in the facility center, delayed purchase deliveries, etc. We hope the vaccine will be available to the UTA community, so we can restore our research activity back to normal. Based on Tarrant county public health website, at the current time, the vaccine is only available to hospital workers, outpatient care staffs, people older than 65 and people with chronic medical situations. Majority of our lab members are not covered in those categories. We are looking forward to receiving the vaccine when it becomes available to general populations.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No significant changes

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes

Significant changes in use or care of vertebrate animals

No changes

Significant changes in use of biohazards and/or select agents

No changes

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. One manuscript submitted to ***Nature Communication***

Title: "MG53 preserves neuromuscular junction integrity and prolongs the survival of ALS mice"
Authors: Jianxun Yi, Ang Li, Xuejun Li, Ki-Ho Park, Xinyu Zhou, Frank Yi, Yajuan Xiao, Dosuk Yoon, Tao Tan, Lyle W. Ostrow, Jianjie Ma and Jingsong Zhou. (DOD support acknowledged)

2. Ang Li, Jianxun Yi, Xuejun Li and Jingsong Zhou. (2020). Physiological Ca^{2+} transients versus pathological steady-state Ca^{2+} elevation, who flips the ROS coin in skeletal muscle mitochondria. ***Frontiers in Physiology***. 11: 595800. PMCID: PMC7642813. (DOD support acknowledged).

3. A Li, J Zhou, RB Widelitz, RH Chow, CM Chuong. (2020). Integrating Bioelectrical Currents and Ca^{2+} Signaling with Biochemical Signaling in Development and Pathogenesis. ***Bioelectricity***. https://doi.org/10.1089/bioe.2020.0001 (DOD support acknowledged).

4. Xinyu Zhou, Ki Ho Park, Daiju Yamazaki, Pei-hui Lin, Miyuki Nishi, Zhiwei Ma, Liming Qiu, Takashi Murayama, Xiaoqin Zou, Hiroshi Takeshima, Jingsong Zhou, Jianjie Ma. (2020). TRIC-A Channel Maintains Store Calcium Handling by Interacting With Type 2 Ryanodine Receptor in Cardiac Muscle. ***Circulation Research***. https://doi.org/10.1161/CIRCRESAHA.119.316241 (DOD support not acknowledged)

5. Zehua Bian, Qiang Wang, Xinyu Zhou, Tao Tan, Ki Ho Park, H. Fritz Kramer, Alan McDougal, Nicholas J. Laping, Sanjay Kumar, T.M. Ayodele Adesanya, Matthew Sermersheim, Frank Yi, Xinxin Wang, Junwei Wu, Kristyn Gumpper, Qiwei Jiang, Duofen He5, Pei-Hui Lin, Haichang Li, Fangxia Guan, Jingsong Zhou, Mark J. Kohr, Chunyu Zeng, Hua Zhu & Jianjie Ma. (2019). Sustained elevation of MG53 in the bloodstream increases tissue regenerative capacity without compromising metabolic function. ***Nature Communications***. https://doi.org/10.1038/s41467-019-12483-0 (DOD support not acknowledged)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as*

noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to report

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

None

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None

- **Other Products**
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Jianjie Ma, Co-I, from the Ohio State University.
No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No changes

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*