



Agrin Nanoparticles in a Nanofiber Hydrogel-based Drug Delivery System Improve Neuromuscular Junction Reinnervation and Functional Recovery After Peripheral Nerve Injury

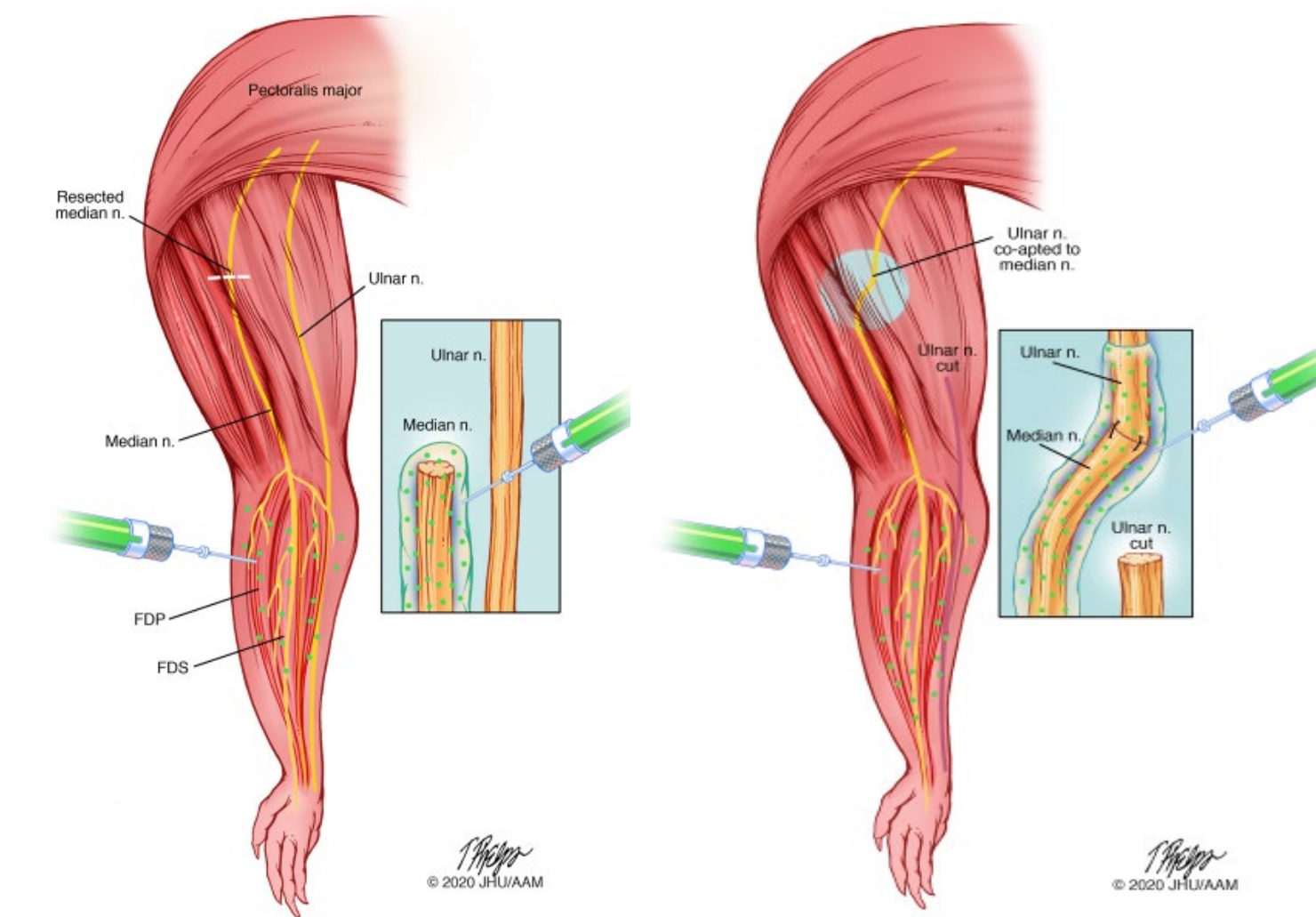


Purpose

Following peripheral nerve injury, neuromuscular junctions (NMJs) within denervated muscle rapidly destabilize. Agrin, a proteoglycan essential to NMJ formation, may have an essential role in preserving NMJ receptivity to reinnervation. Our lab has previously formulated and optimized nanoparticle encapsulated agrin (Agrin-NP) embedded within a nanofiber hydrogel composite (NHC) for sustained local delivery. We demonstrated that Agrin-NP/NHC maintains healthy NMJ morphology for at least 6 weeks in the setting of denervation. This study aimed to evaluate the efficacy of Agrin-NP/NHC therapy on functional motor recovery in a rodent forelimb chronic denervation model.

Methods - Study Design

Model: The median nerve was denervated for 12 weeks until ulnar-to-median nerve transfer. Following nerve transfer, all animals underwent 16 weeks of regeneration and grip strength assessment.



Chronic Denervation Model with Injection

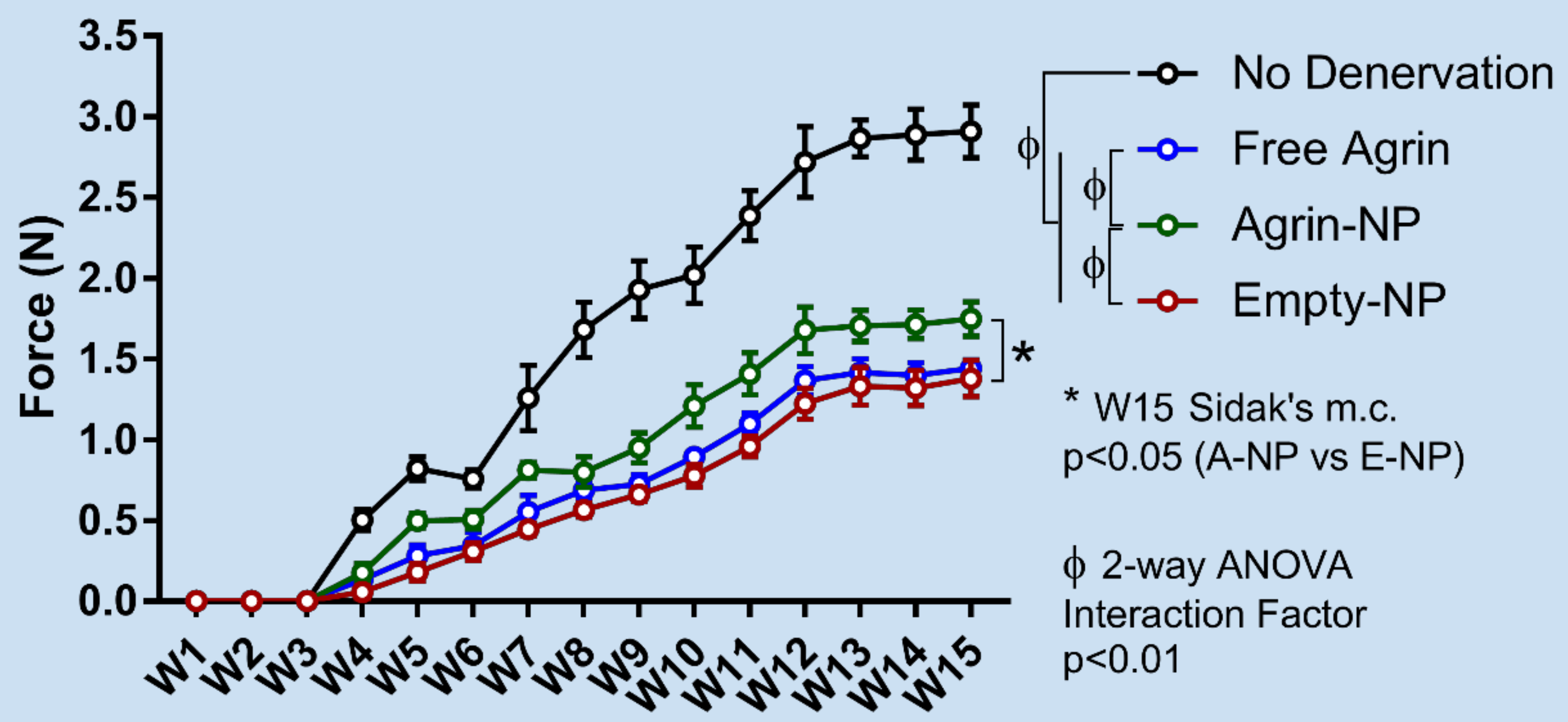
Denervated animals were injected with agrin-NP/NHC, free agrin, or empty-NP/NHC every six weeks from time of median nerve injury. A total of five injections were given:

- 1) at the time of median nerve injury,
- 2) after 6 weeks of denervation
- 3) at the time of nerve transfer after 12 weeks of denervation
- 4) week 6 post nerve transfer
- 5) week 12 post nerve transfer

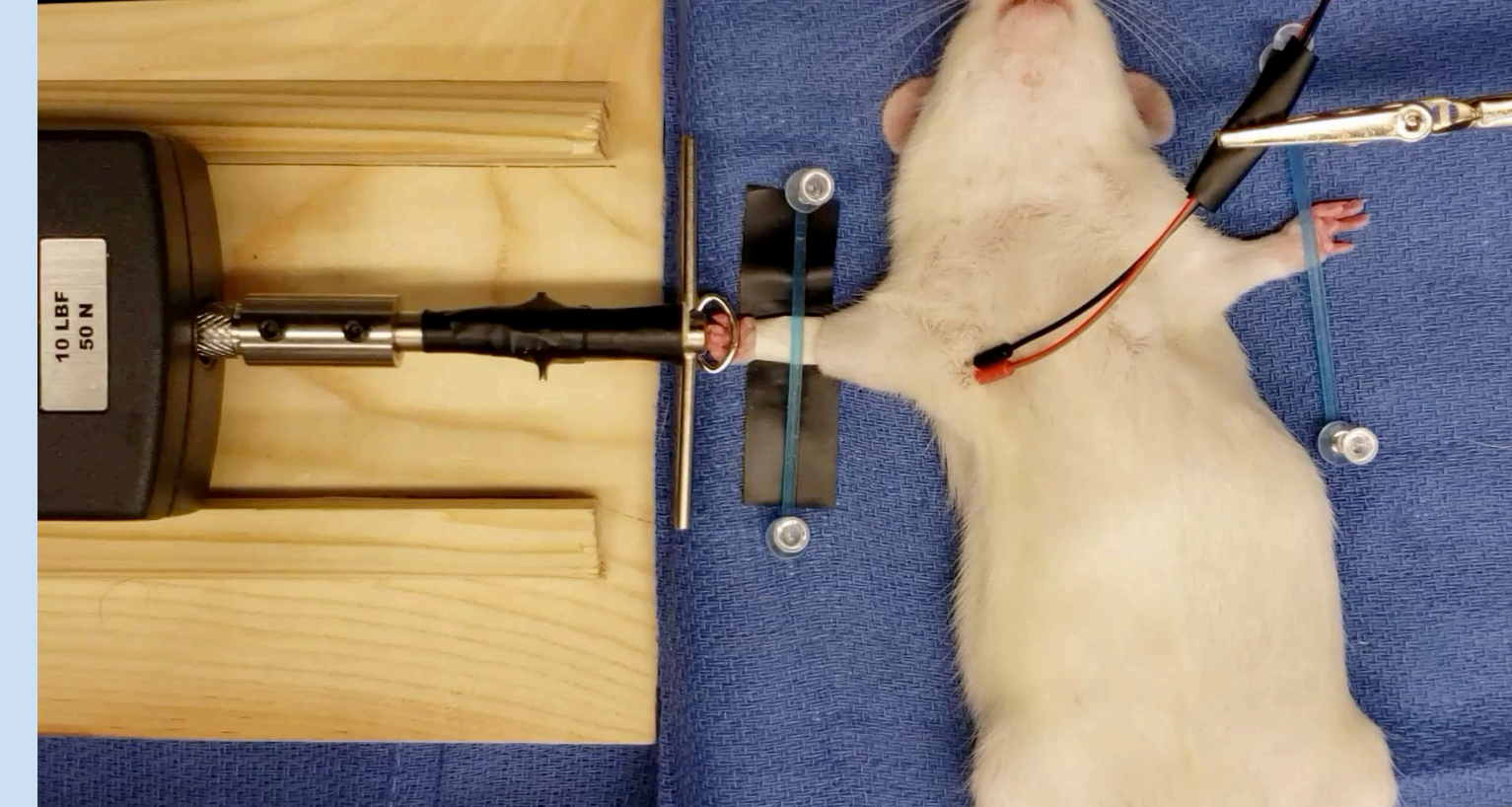
The “no denervation” control group only received the ulnar-to-median nerve transfer and saline injections.

Results

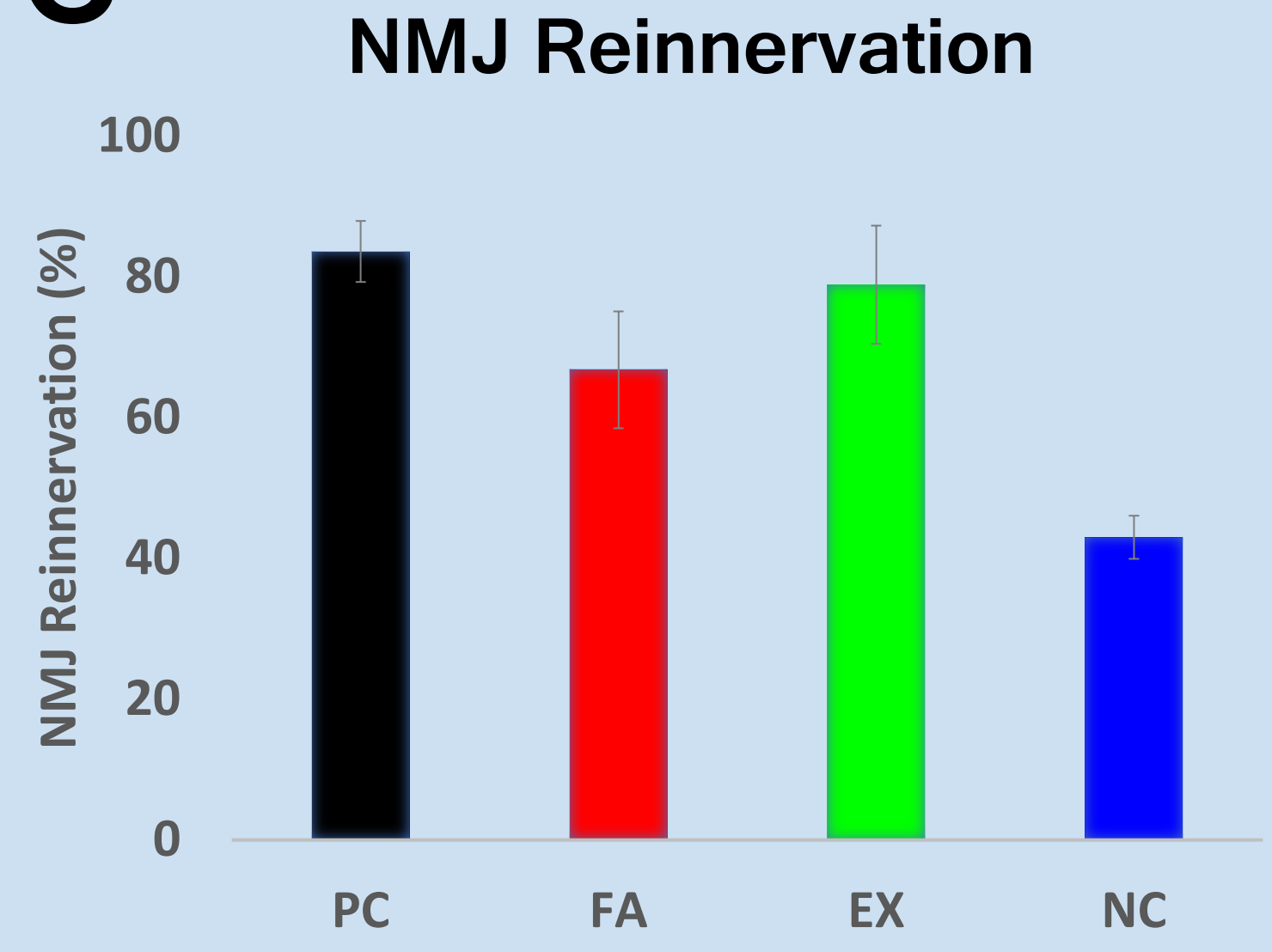
A Stimulated Grip Strength Test (sGST)



B



C



A) **Agrin-NP/NHC** treated animals exhibited significantly increased functional recovery throughout the regeneration period compared to **free agrin** ($p < 0.01$ 2-way ANOVA) and **empty-NP/NHC** treated animals ($p < 0.01$ 2-way ANOVA) At Week 15, **Agrin-NP/NHC** treated animals demonstrated an increase of 21.2% compared to **free agrin** ($p = 0.056$) and of 26.8% compared to **empty-NP/NHC** animals ($p < 0.05$).

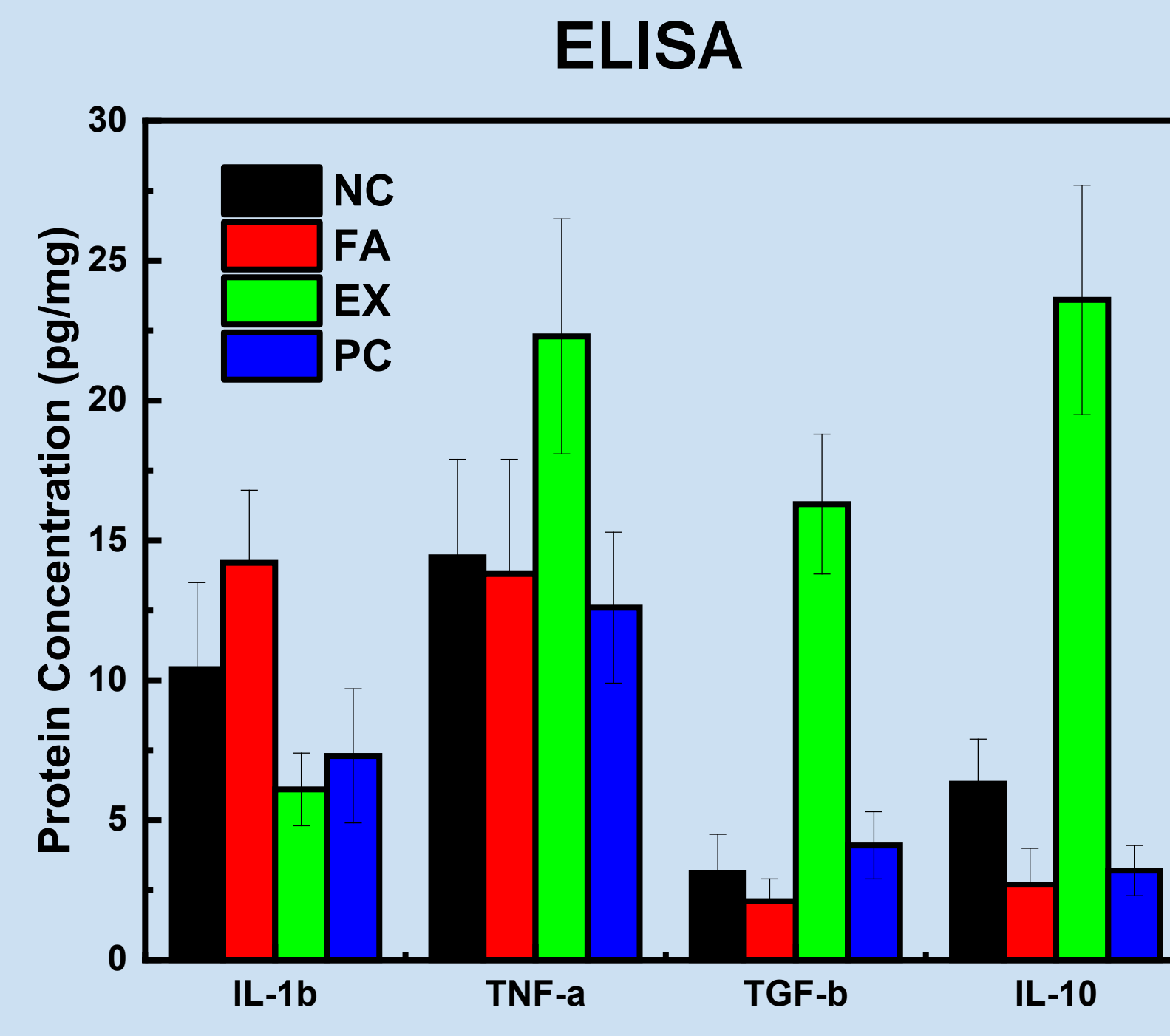
B) Stimulated grip strength testing involves percutaneously stimulating the median nerve to elicit maximal flexion of the digital flexors for serial assessment of functional recovery.

C) **Agrin-NP/NHC (EX)** treated animals demonstrated significantly greater NMJ reinnervation than **free agrin (FA)** (78.9% vs 66.8%, $p < 0.05$) and **empty-NP/NHC (NC)** groups (78.9% vs 39.2%, $p < 0.001$).

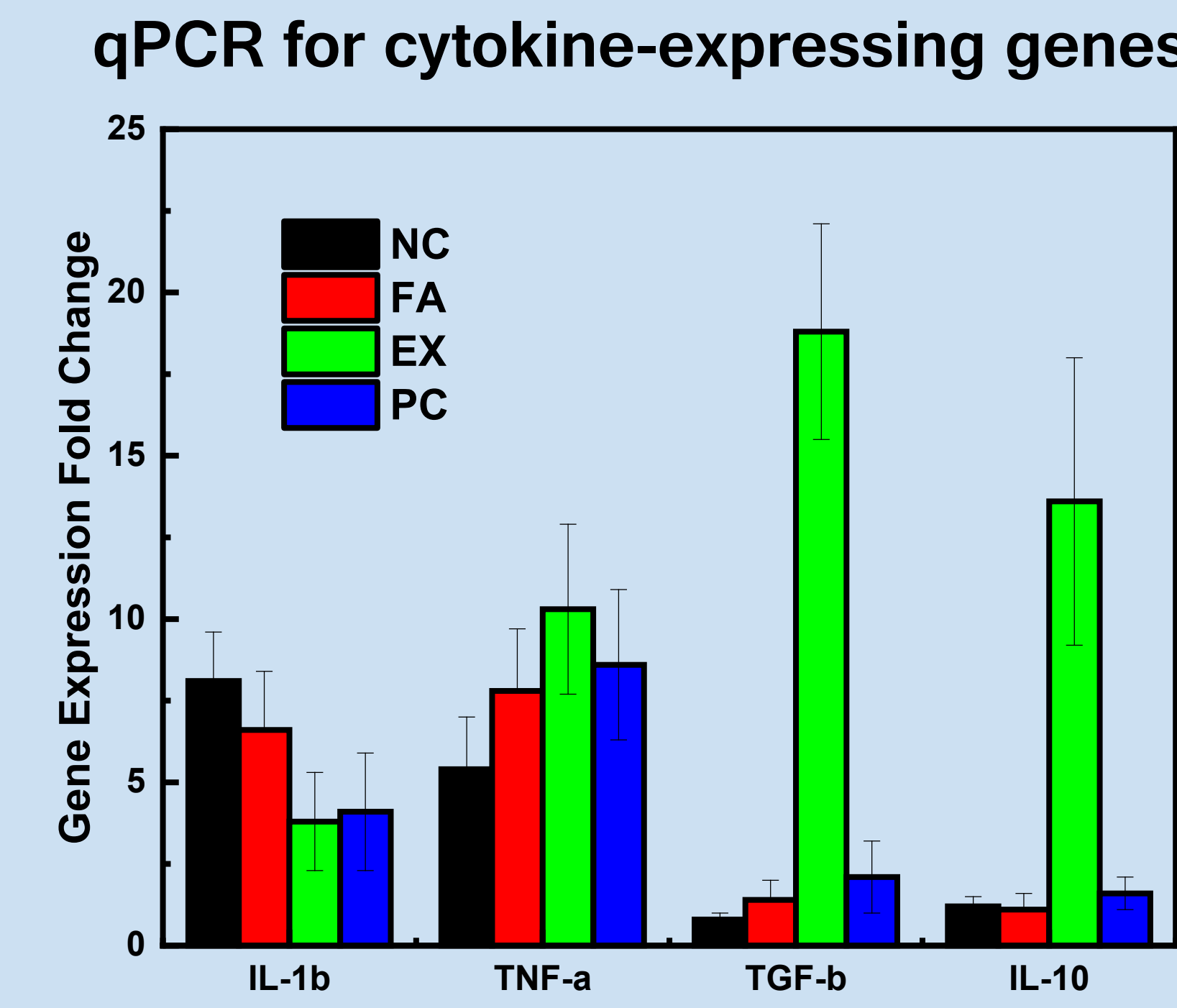
D1 & D2) **Agrin-NP/NHC (EX)** treatment promotes anti-inflammatory cytokines. This appears to be independent of the anti-inflammatory properties inherent to the NHC.

E) **Agrin-NP/NHC (EX)** treatment reduced intramuscular collagen deposition as assessed through Masson's Trichrome.

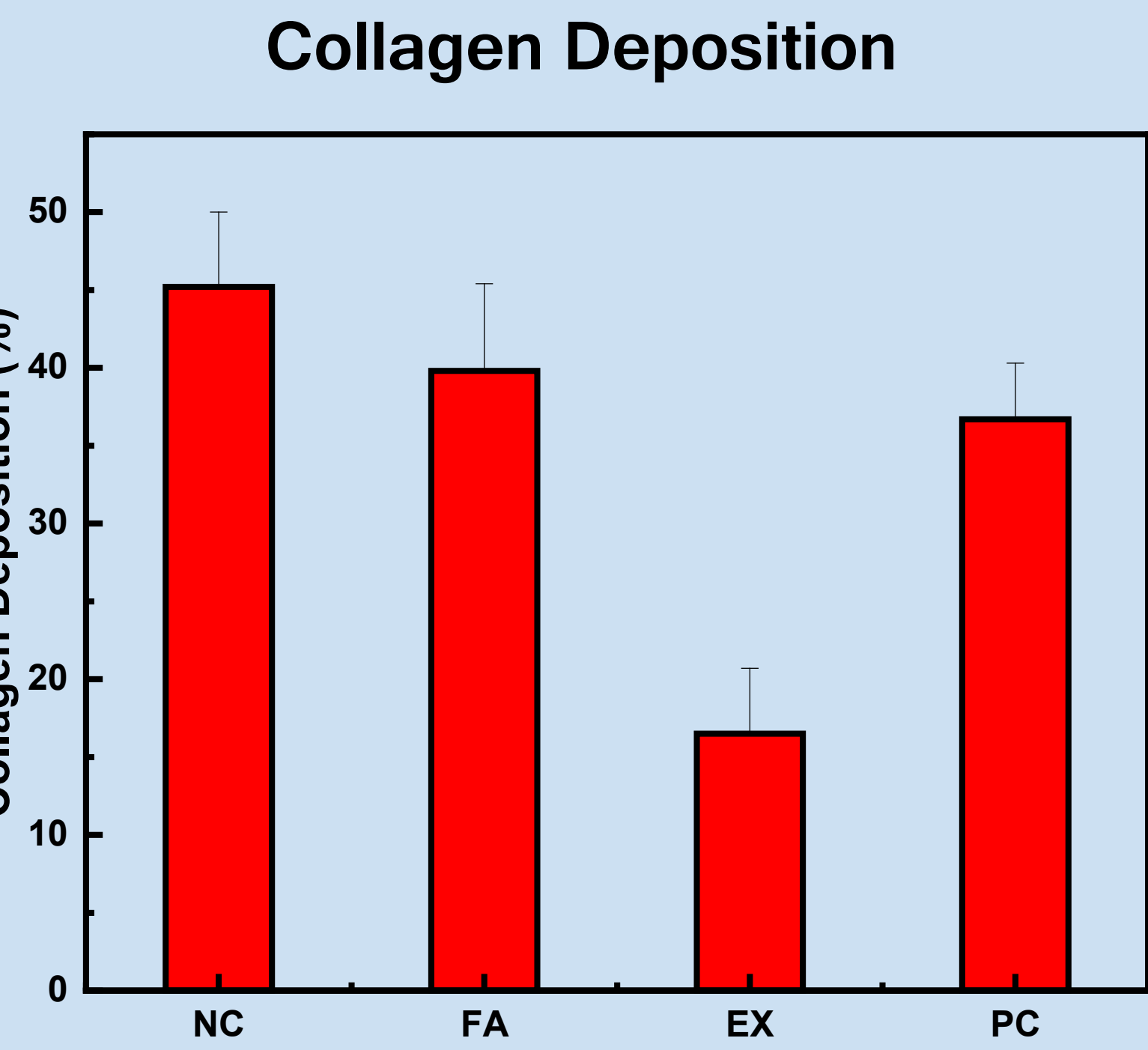
D-1



D-2



E



Conclusions

Our findings demonstrate Agrin-NP/NHC treatment in vivo improves functional recovery of forelimb grip strength and promotes neuromuscular junction reinnervation. Histologic analyses are currently ongoing.

