

## Supplementary Materials

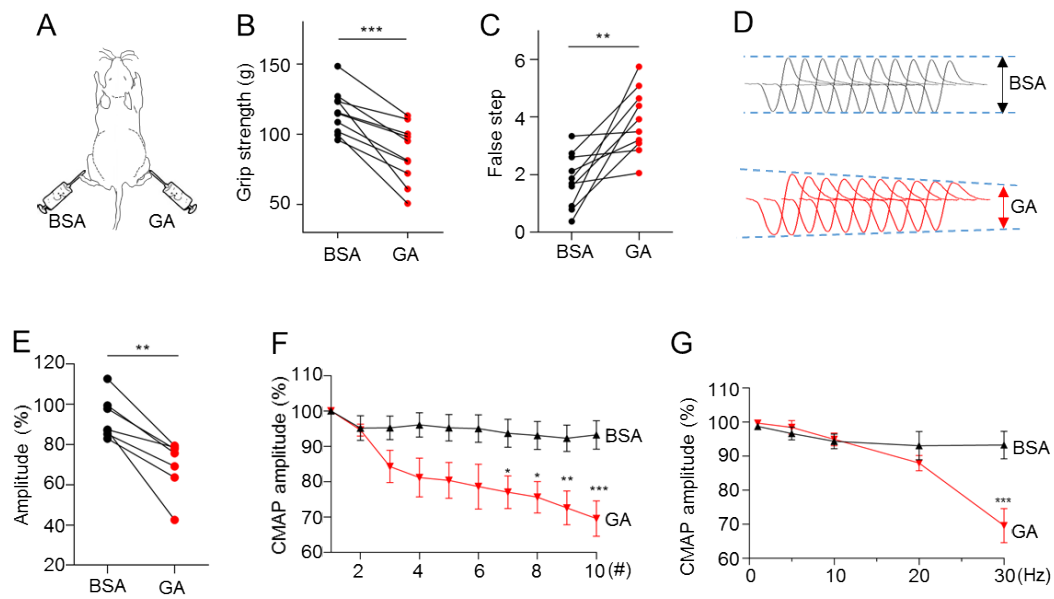
### **C9orf72 poly-GA proteins impair neuromuscular transmission**

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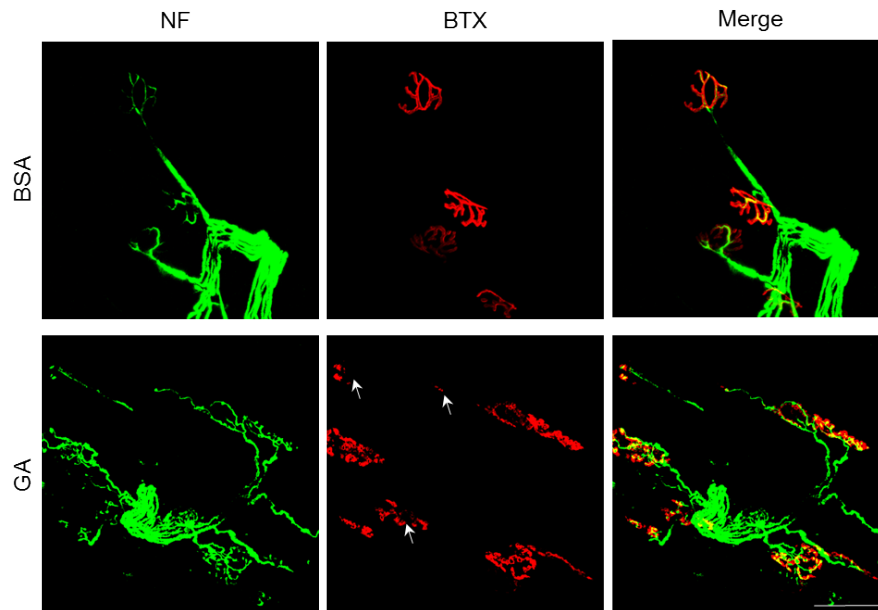
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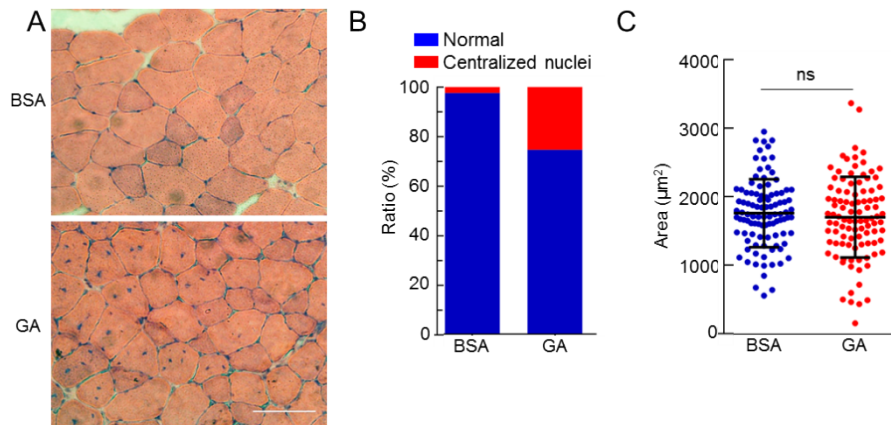
### Supplementary Figure S1 Poly-GA proteins impair motor function and neurotransmission using alternative control injectant (related to Figure 1)

A: Diagram of local administration of poly-GA. Same volume of BSA-containing injectant was administered in the same mouse. B: Grip strength of two hindlimbs in mice injected with BSA-containing injectant and poly-GA.  $n=10$  mice,  $***P<0.001$ . C: Foot faults of two hindlimbs in mice injected with BSA-containing injectant and poly-GA.  $n=10$  mice,  $**P<0.01$ . D: Ten CMAP traces were stacked in succession for better comparison. With successive stimulations, CMAP amplitude was reduced in GA-injected muscles. E: Reduced CMAP amplitudes in GA-injected muscles at 10th stimulation at 30 Hz.  $n=7$  mice,  $**P<0.01$ . F: Reduced CMAP amplitudes in GA-injected muscles at 10th stimulation at 30 Hz.  $n=7$  mice,  $*P<0.05$ ,  $**P<0.01$ ,  $***P<0.001$ . G: Reduction in CMAP amplitudes was stimulation frequency-dependent.  $n=7$  mice,  $***P<0.001$ . Unless otherwise specified, data are presented as mean $\pm$ SEM. At least three independent experiments were performed.  $*P<0.05$ ,  $**P<0.01$ ,  $***P<0.001$ ; Two-tailed paired Student's  $t$ -test in B, C, and E. Two-way ANOVA in F and G.



**Supplementary Figure S2 Innervation of broken NMJs**

TA muscle was stained with R-BTX (red) to label AChR and antibodies against NF and SV2 (NF; green) to label nerve branches and terminals. White arrows indicate broken NMJs, which are innervated. NF, green; BTX, red. Scale bar: 50  $\mu$ m.



### Supplementary Figure S3 Muscle fibers in poly-GA mice

A: Number of nuclei was centralized in GA-injected muscles compared to control. Scale bar: 100  $\mu\text{m}$ . B: Quantification of centralized nuclei. About 25% of muscle fibers analyzed showed centralized nuclei in GA-injected muscles. In total, 100 myotubes from three animals were analyzed. C: Quantification of muscle area. In total, 100 myotubes from three animals were analyzed. ns, not significant. Unless otherwise specified, data are presented as mean $\pm$ SEM. At least three independent experiments were performed. Two-tailed unpaired Student's *t*-test in C.