Inhibiting C1q improves compound muscle action potential and reduces neurodegenerative damage in the SOD1^{G93A} mouse model of ALS

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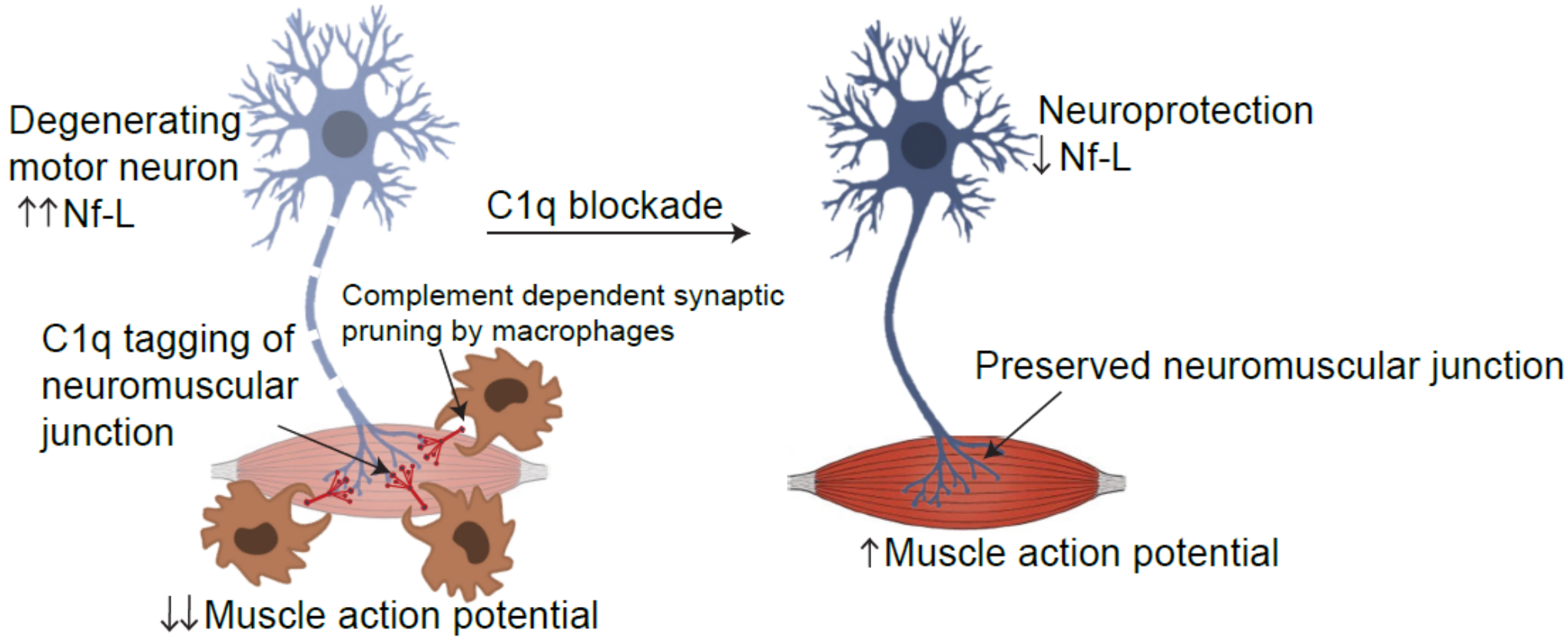
Hypothesis

We evaluated the hypothesis that C1q and the classical complement cascade contribute to neuronal damage and loss of motor function in the SOD1^{G93A} mouse model of ALS by systemic blockade of C1q using an anti-C1q antibody.



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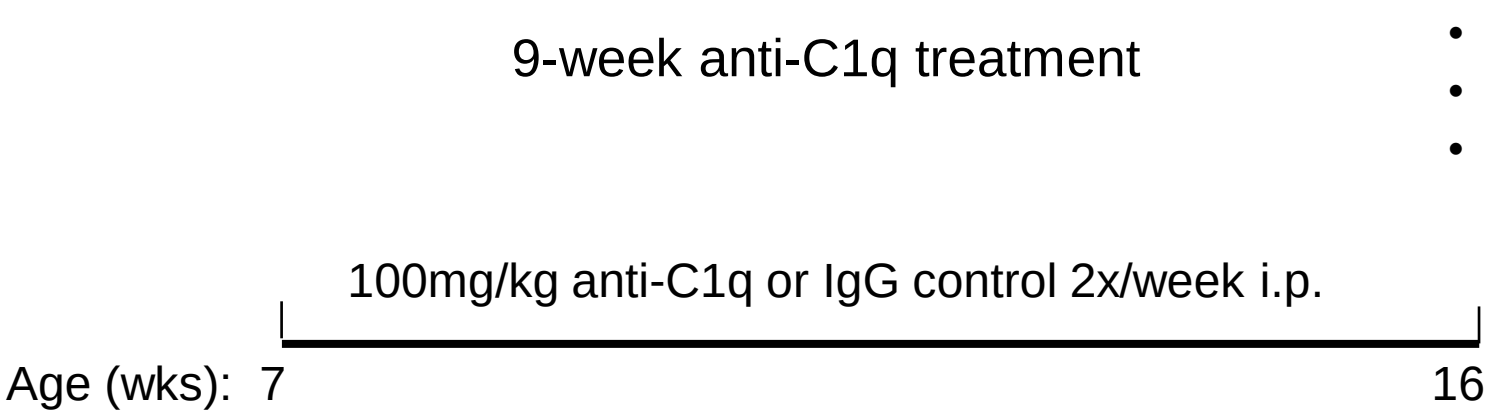
Study Design



- Measured muscle function
- Measured NfL
- Collected CSF, plasma, brain, spinal cord, gastrocnemius

SOD1^{G93A} mice were dosed twice per week intraperitoneally with 100mg/kg anti-C1q antibody or isotype control antibody for nine weeks (from ages 7 to 16 weeks; n=16-17/group).

Wildtype (WT) mice at 16 weeks of age (n=17) were used for baseline control measures.

Compound action muscle potential was measured 3 days after the last dose (drug nadir), then plasma was collected, and the mice were perfused before collection of CSF, spinal cord and gastrocnemius muscles.

In vivo animal work was done at Psychogenics Inc. CSF and plasma Nf-L were measured at Quanterix Corp using their Single Molecule Array (SIMOA) platform.

Free drug (anti-C1q) detected in plasma and tissue lysates

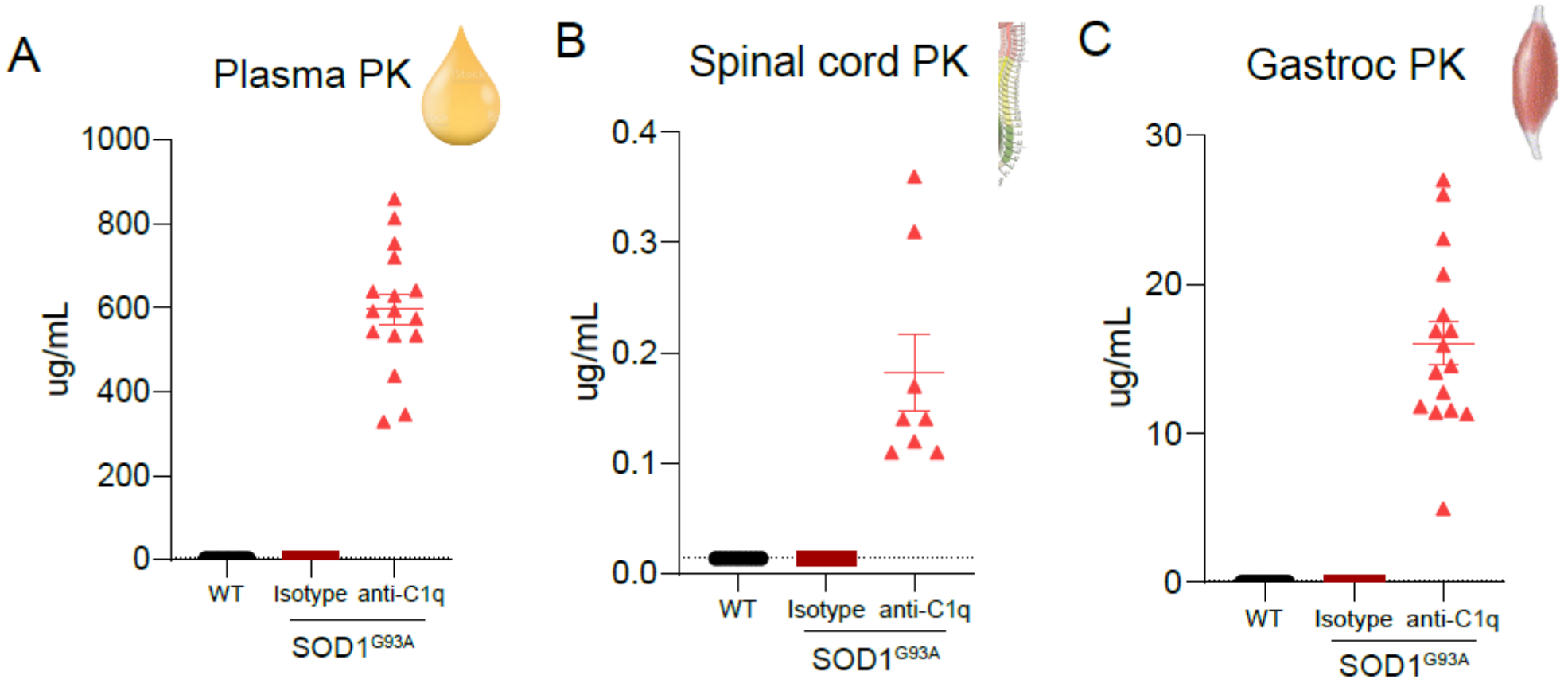


Fig.1: Spinal cord and gastrocnemius tissues were lysed in TBS with protease inhibitors. Plasma and lysates were appropriately diluted, and anti-C1q antibody levels were measured using standard ELISA. Free antibody levels averaging 600ug/mL in plasma (A), 0.2 ug/mL in spinal cord (B), and 15 ug/mL in gastrocnemius muscle (C) were measured in anti-C1q antibody treated $SOD1^{G93A}$ mice, but not in WT or isotype treated $SOD1^{G93A}$ mice. Dotted line shows lower limit of quantification (0.014-8.2 ug/mL).

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C1q target engagement in plasma, spinal cord and muscle tissues

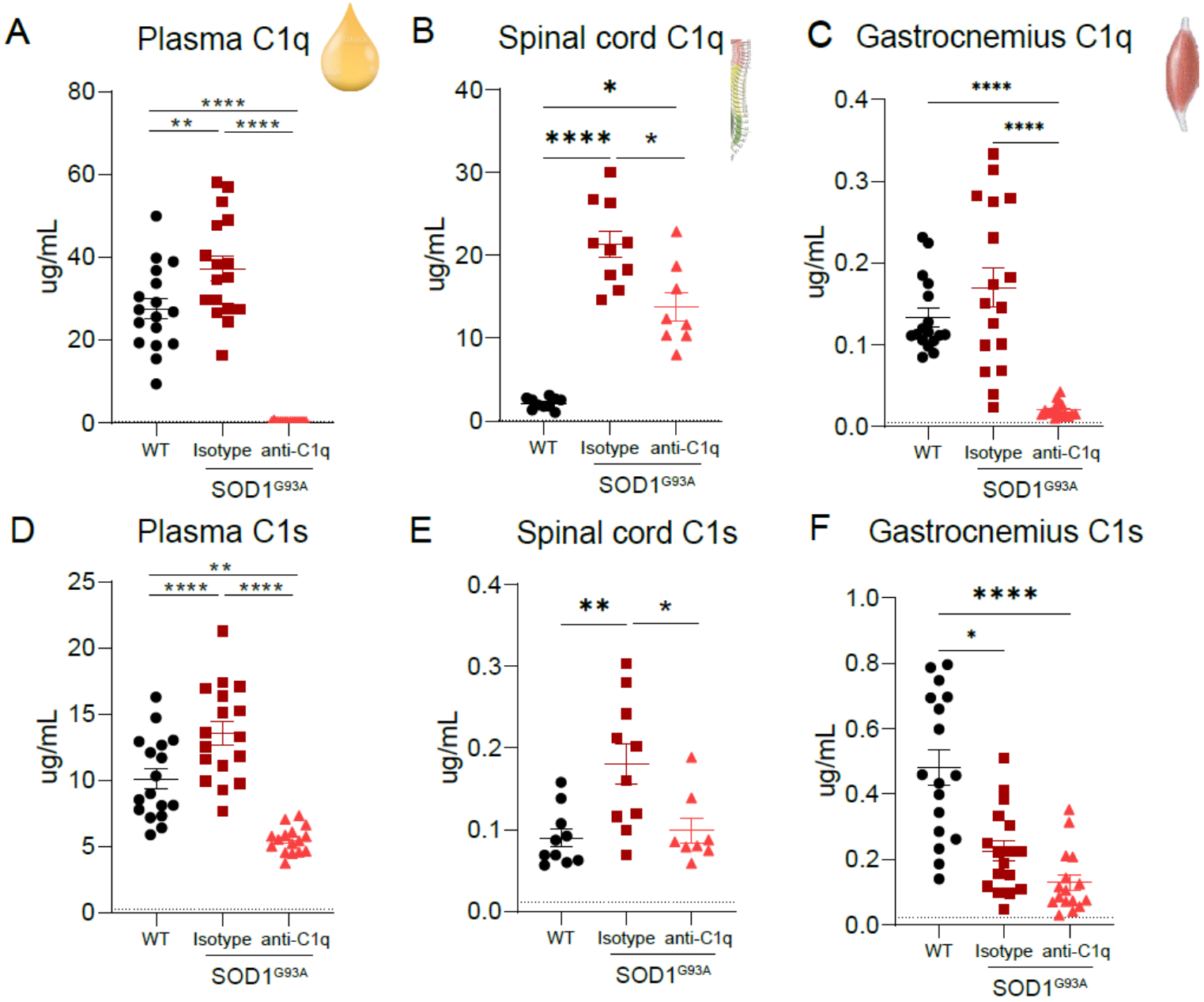

Fig.2: Both C1q (A-C) and C1s (D-F) levels were increased in SOD1^{G93A} tissues compared to WT.

Anti-C1q antibody treatment significantly reduced C1q as well as the downstream C1s in SOD1^{G93A} mice, indicating C1q engagement/inhibition within the spinal cord. The level of C1q engagement within the interstitial fluid of the spinal cord is likely to be higher than depicted since both intracellular and extracellular C1q will be exposed in tissue lysate.

Statistical analysis: One-way ANOVA with repeated measures *P<0.05, **P<0.01, ****P<0.0001. Dotted line shows lower limit of quantification (C1q= 0.004-0.2 ug/mL, C1s= 0.02-0.2 ug/mL).

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C1q inhibition reduces neuronal damage in SOD1^{G93A} mice

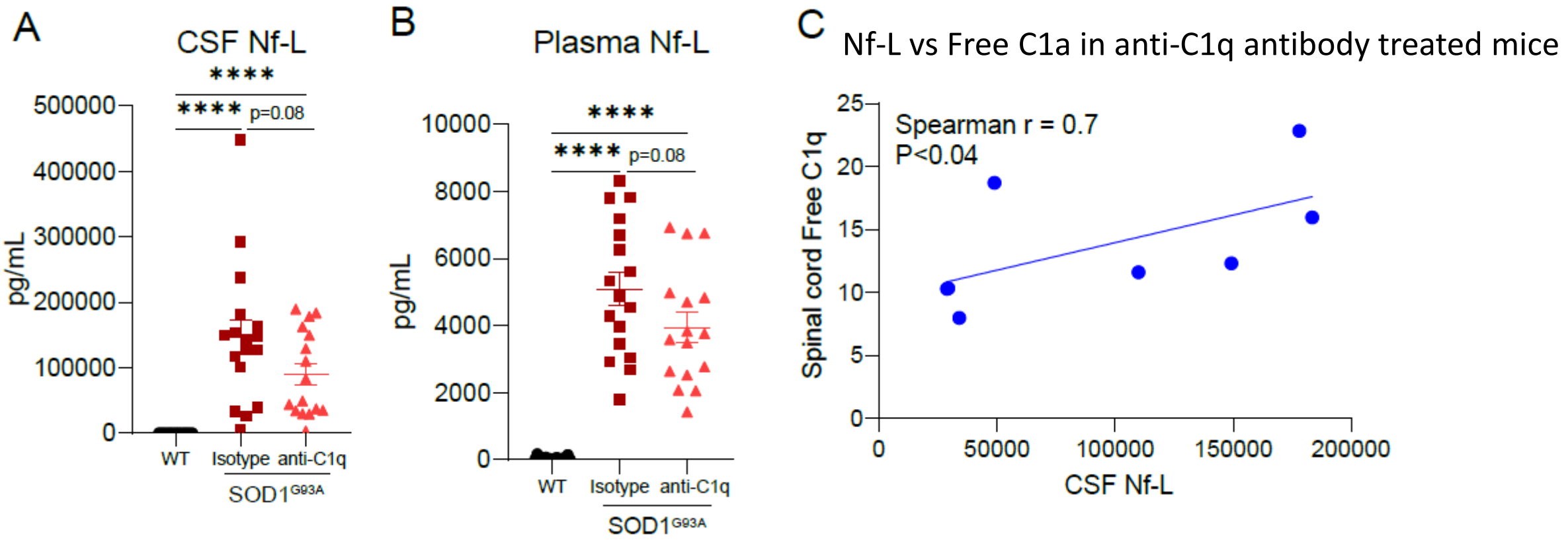

Fig.3: Neurofilament-light chain (Nf-L) was measured as a marker of neuronal damage in mouse CSF and plasma after 9 weeks of anti-C1q antibody treatment.

SOD1^{G93A} mice showed ~600-fold and ~100-fold upregulation of Nf-L in CSF (A) and plasma (B) respectively.

Anti-C1q antibody treatment reduced Nf-L in both CSF and plasma by 40%, suggesting that blocking the classical complement pathway reduces neuronal damage in SOD1^{G93A} mice. CSF Nf-L correlated significantly with reduced C1q levels in spinal cord of SOD1^{G93A} mice (C).

Statistical analysis: One-way ANOVA with repeated measures ****P<0.0001. Dotted line shows lower limit of quantification (1.52-7.6 pg/mL).

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C1q inhibition improves CMAP in SOD1^{G93A} mice

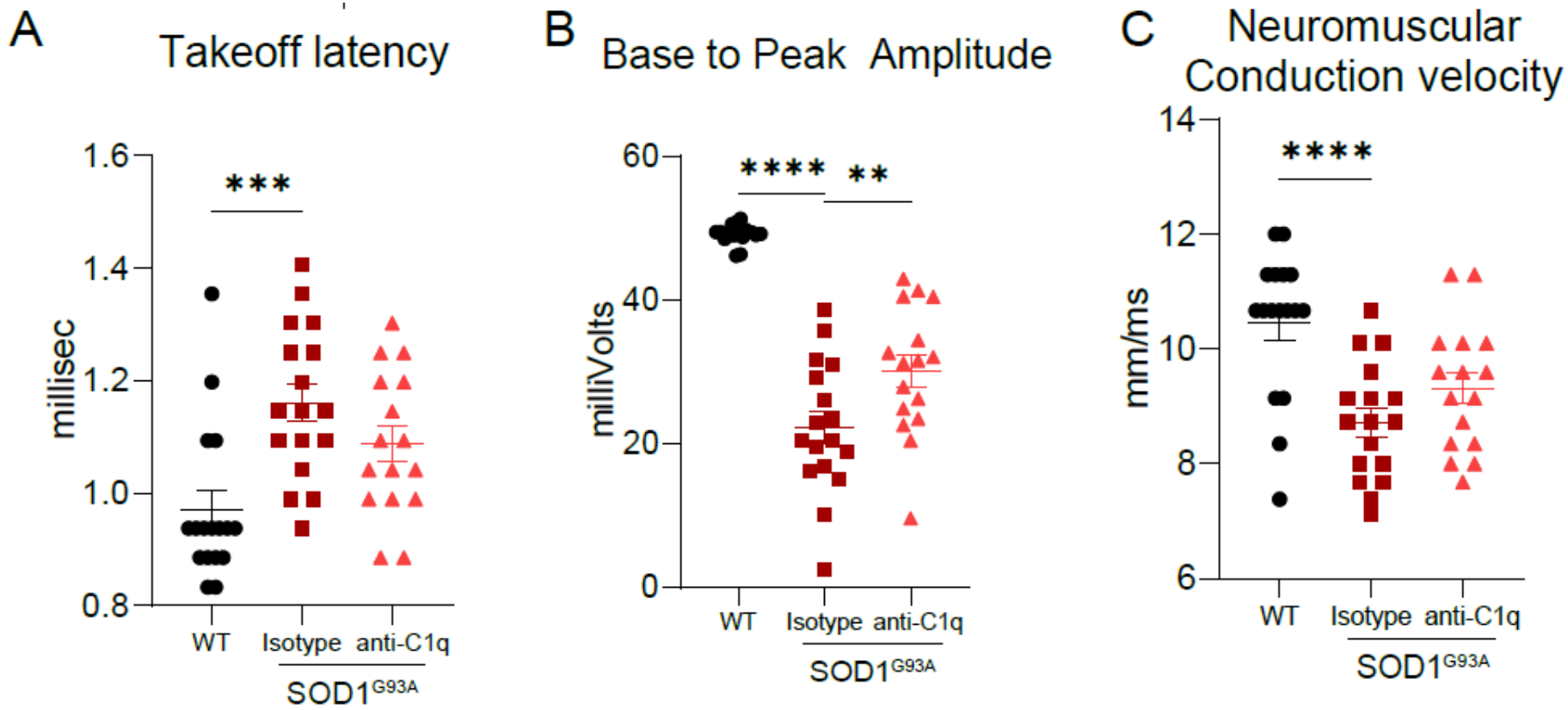

Fig.4: Compound action muscle potential (CMAP) was measured to assess nerve conduction in the gastrocnemius muscle after 9 weeks of anti-C1q antibody treatment in SOD1^{G93A} mice. The stimulating electrode was placed along the axis of the sciatic nerve proximal to the sciatic notch and the recording electrode was placed where the gastrocnemius muscle has maximum diameter. A reference electrode was placed just beneath the recording electrode. For all analyses, five responses evoked by stimulation were averaged to generate a smooth CMAP. SOD1^{G93A} mice showed significantly increased latency (A), reduced amplitude (B) and reduced conduction velocity (C). Anti-C1q antibody treatment significantly improved CMAP amplitude (B) in SOD1^{G93A} mice which marks improvement in axonal preservation and/or synaptic connections at the NMJ. Statistical analysis: One-way ANOVA with repeated measures **P<0.01, ***P<0.001, ****P<0.0001.

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Conclusions

We show that inhibition of classical complement pathway resulted in reduced neuronal damage, as measured by Nf-L, and improved muscle nerve conduction in the SOD1^{G93A} mouse model of ALS.

These results support a mechanism of classical complement-mediated neurodegeneration and support further study of anti-C1q as a potential therapeutic for ALS.

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