Characterization of metabolic defects across multiple models of ALS

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that disrupts muscle function and has no cure. TAR DNA Binding Protein (TDP-43) is an RNA binding protein associated with pathological aggregates in 97% of ALS patients. Expressing wild-type or mutant human TDP-43 in the fruit fly *Drosophila* recapitulates several symptoms of ALS, including locomotor dysfunction and reduced survival.[1] We recently found that glycolysis is upregulated in this model as a compensatory mechanism that improves locomotor function and increases lifespan.[2] Increasing the availability of glucose was found to improve several phenotypes in our model of ALS based on TDP-43 overexpression. To determine whether high glucose availability is protective in other ALS types we tested whether a high glucose diet improved locomotor function in C9 and SOD1 models of ALS in flies and found a similar protective effect as was the case with TDP-43 overexpression.[3,4] In addition, we used a recently generated CRISPR model of TDP-43 proteinopathy and found that a high glucose diet mitigates locomotor defects in adults.[5] Finally, to begin understanding the role of PFK at ALS synapses we generated a PFK-GFP CRISPR line and examined PFK localization at the neuromuscular junction (NMJ). Preliminary experiments show that PFK is localized at the NMJs however it is unclear whether or not TDP-43 proteinopathy alters its localization. Higher resolution imaging through expansion microscopy is needed to determine whether TDP-43 proteinopathy has an effect on PFK levels and/or localization in synaptic boutons.

A high glucose diet is protective in *Drosophila* C9 and SOD1 models of ALS
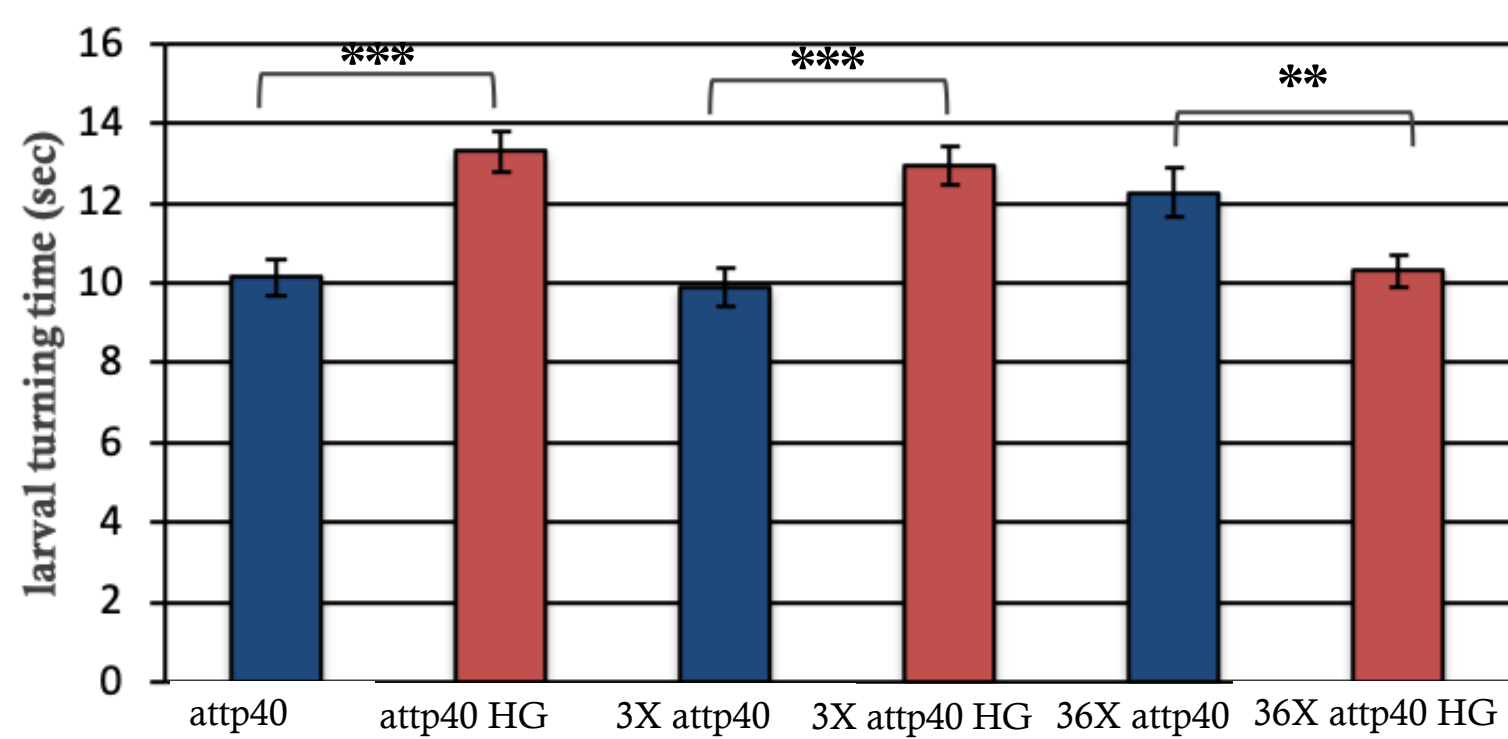

Figure 1: Larval turning measured in *Drosophila* larvae expressing either 3 G4C2 (3X) or 36 G4C2 (36X) repeats in motor neurons. The motor neuron driver, D42-GAL4 was used to drive G4C2 expression. attp40 is the genetic background control. Locomotor function was reduced in 36X compared to 3X or attp40. This phenotype was rescued by a high glucose diet. Significance was determined using student's t-test. n>50 for all genotypes.
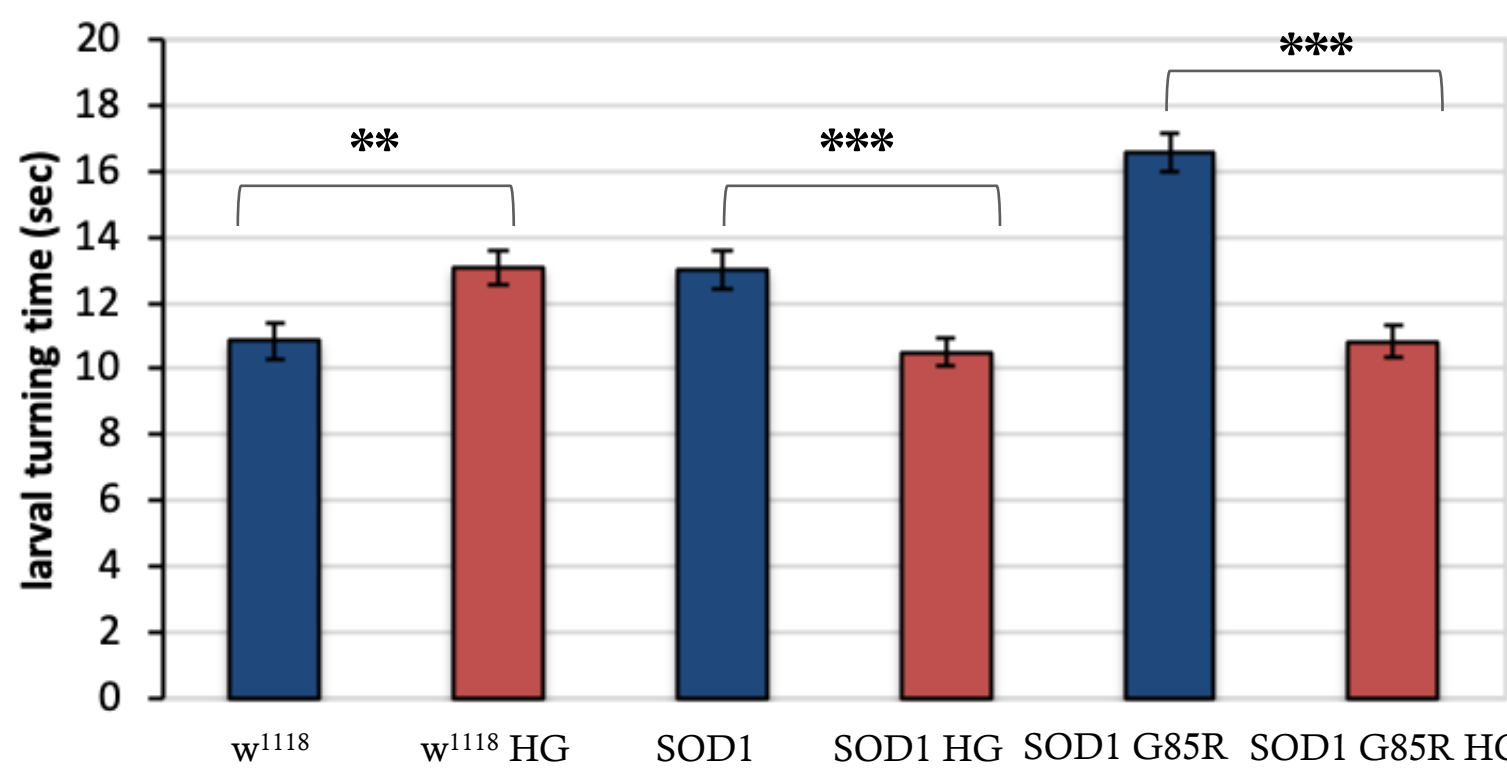
Figure 2: Larval turning measured in *Drosophila* larvae expressing either SOD1 WT or SOD1 G85R. A motor neuron driver, D42-GAL4 was used to drive SOD1 expression. w1118 is the genetic background control. Locomotor function was reduced in all genotypes compared to w1118. This phenotype was then rescued on a high glucose diet. Significance was determined using student's t-test. n>50 for all genotypes.

Locomotor defects in the TDP-43 CRISPR model

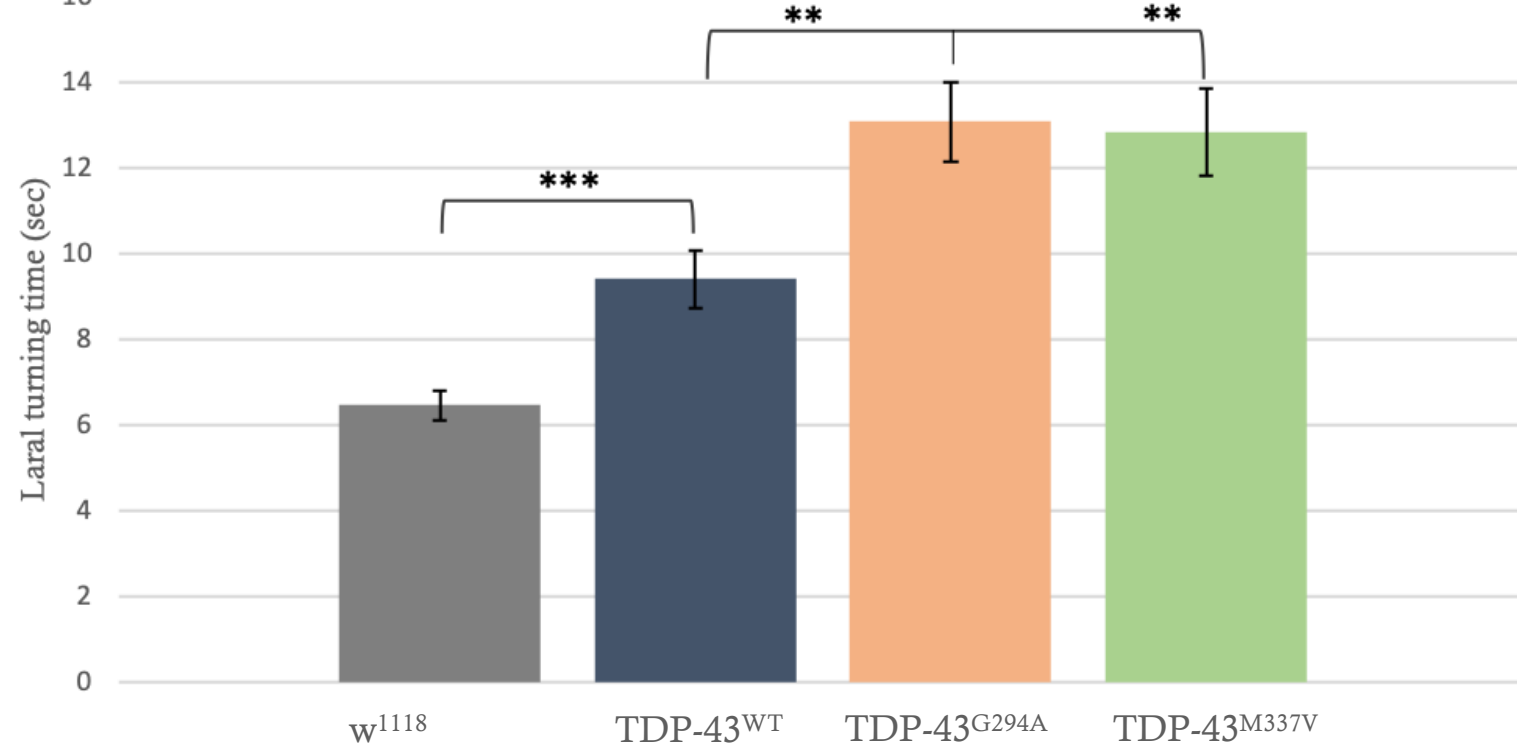**Figure 3:** Larval turning measured in *Drosophila* harboring hTDP-43WT, hTDP-43G294A, and hTDP-43M337V as a replacement for the endogenous fly gene using CRISPR. Increased larval turning times for all genotypes compared to w1118. Significance was determined using a student's t-test. n>30 for all genotypes.
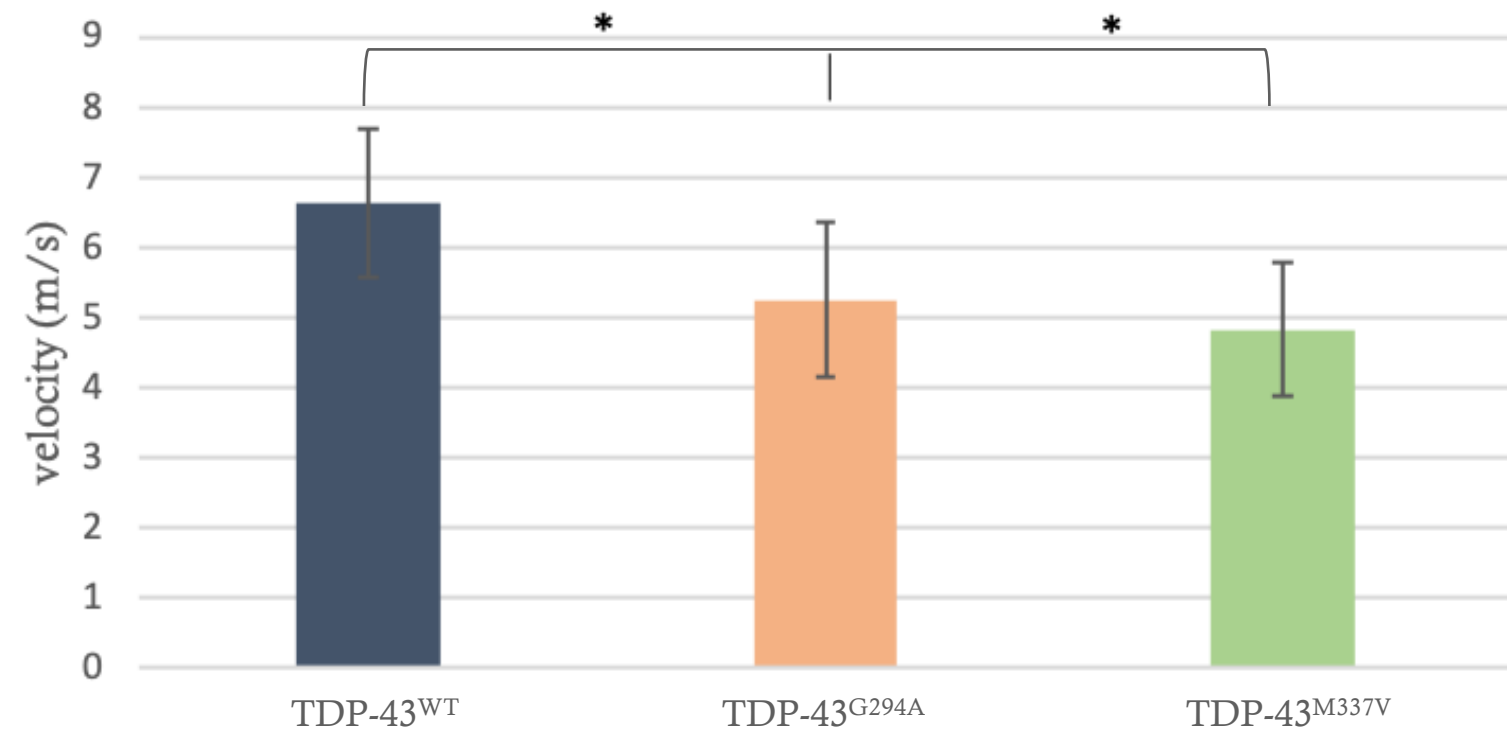
Figure 4: FITR based imaging method (FIM)[6] used to measure motor function in *Drosophila* hTDP-43WT CRISPR, hTDP-43G294A CRISPR, and hTDP-43M337V CRISPR. Significance was determined using a student's t-test. n>30 for all genotypes.
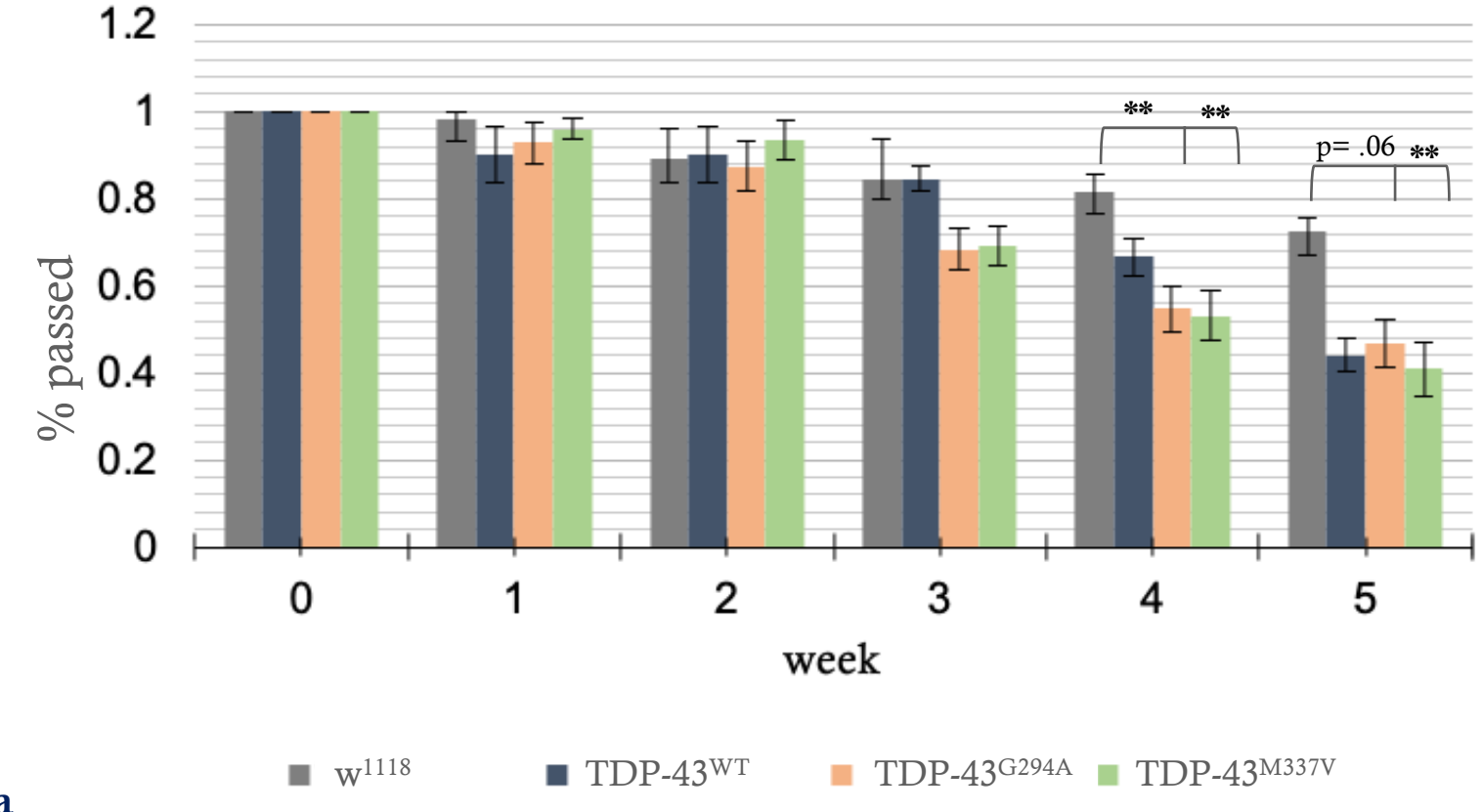
Figure 5a & b: Climbing ability measured in adult *Drosophila* expressing hTDP-43WT CRISPR, hTDP-43G294A CRISPR, and hTDP-43M337V CRISPR. Climbing ability was reduced in all genotypes compared to w1118. This phenotype was then rescued on a high glucose diet. Significance was determined using Fisher's exact test. n=50 for all genotypes

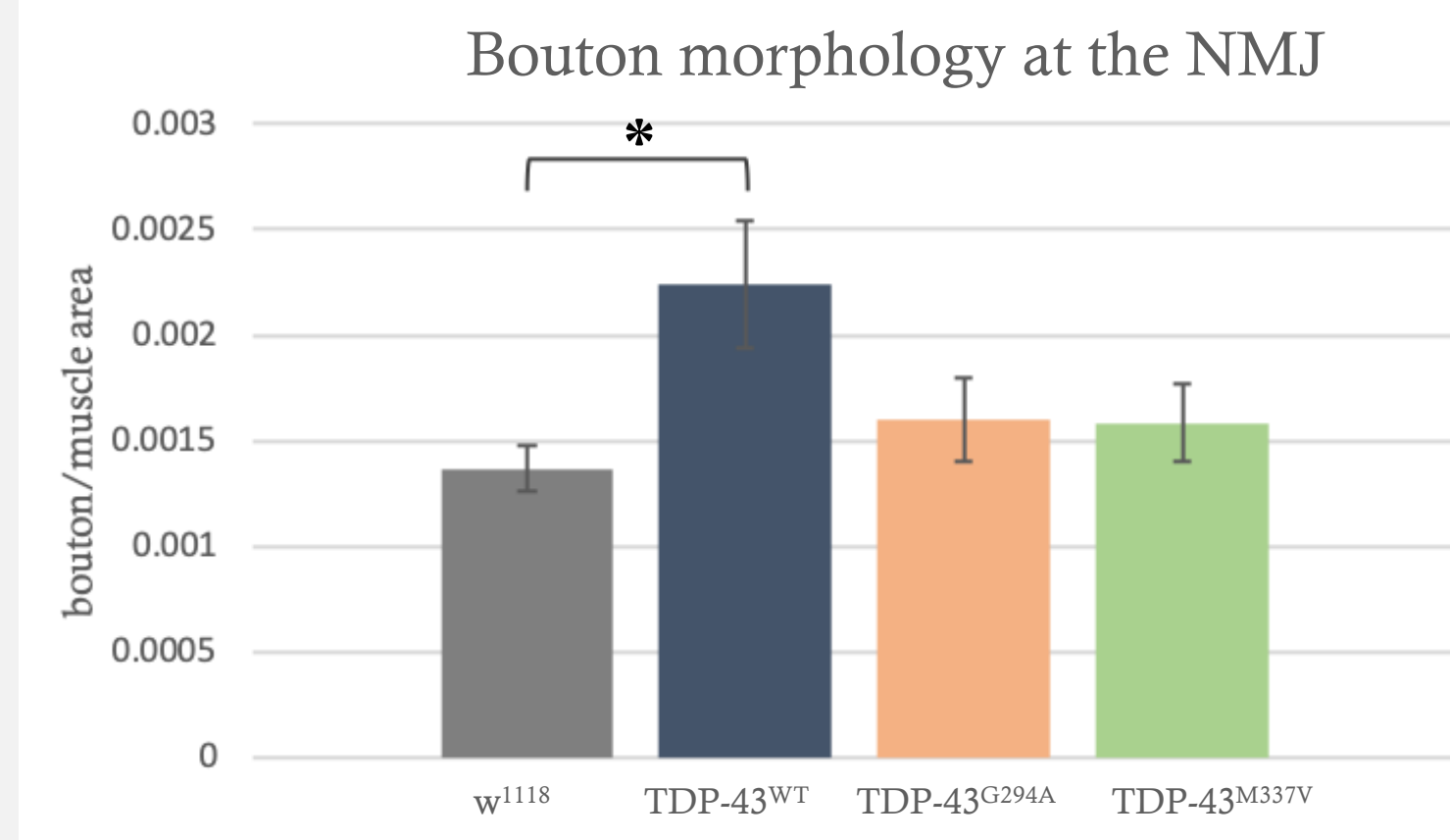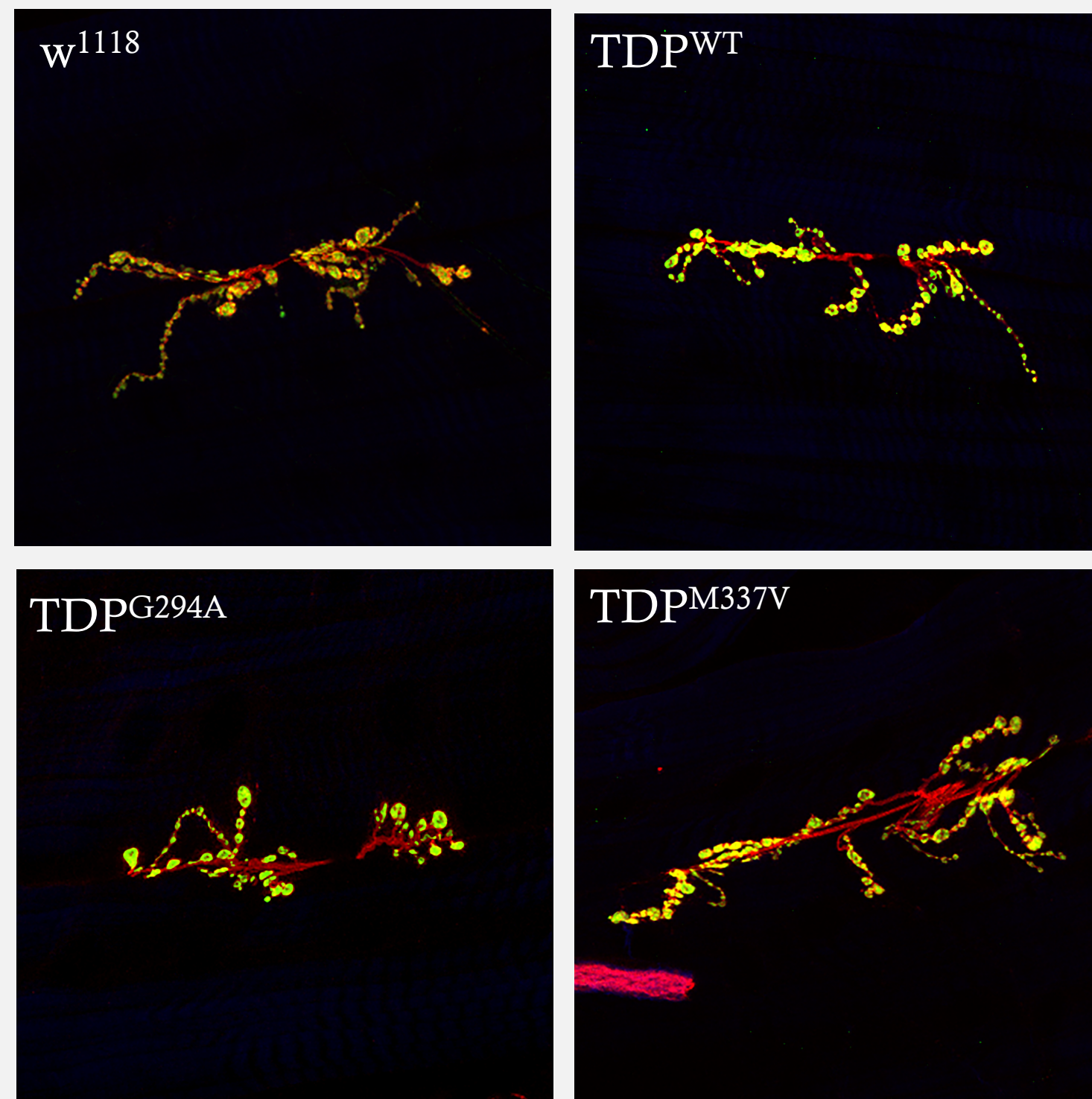## Neuromuscular junction abnormalities in TDP-43 CRISPR flies

Figure 8: NMJ's dissected from third instar larvae. Bouton number was normalized to muscle area. TDP-43WT CRISPR increased bouton number, there was not significance for TDP-43G294A CRISPR or TDP-43M337V CRISPR. A student's t-test was used to determine significance. n= 6 for all genotypes.

Figure 7: The neuromuscular junction (NMJ) dissected from third instar larvae. Stained for CSP, HRP, and GFP.

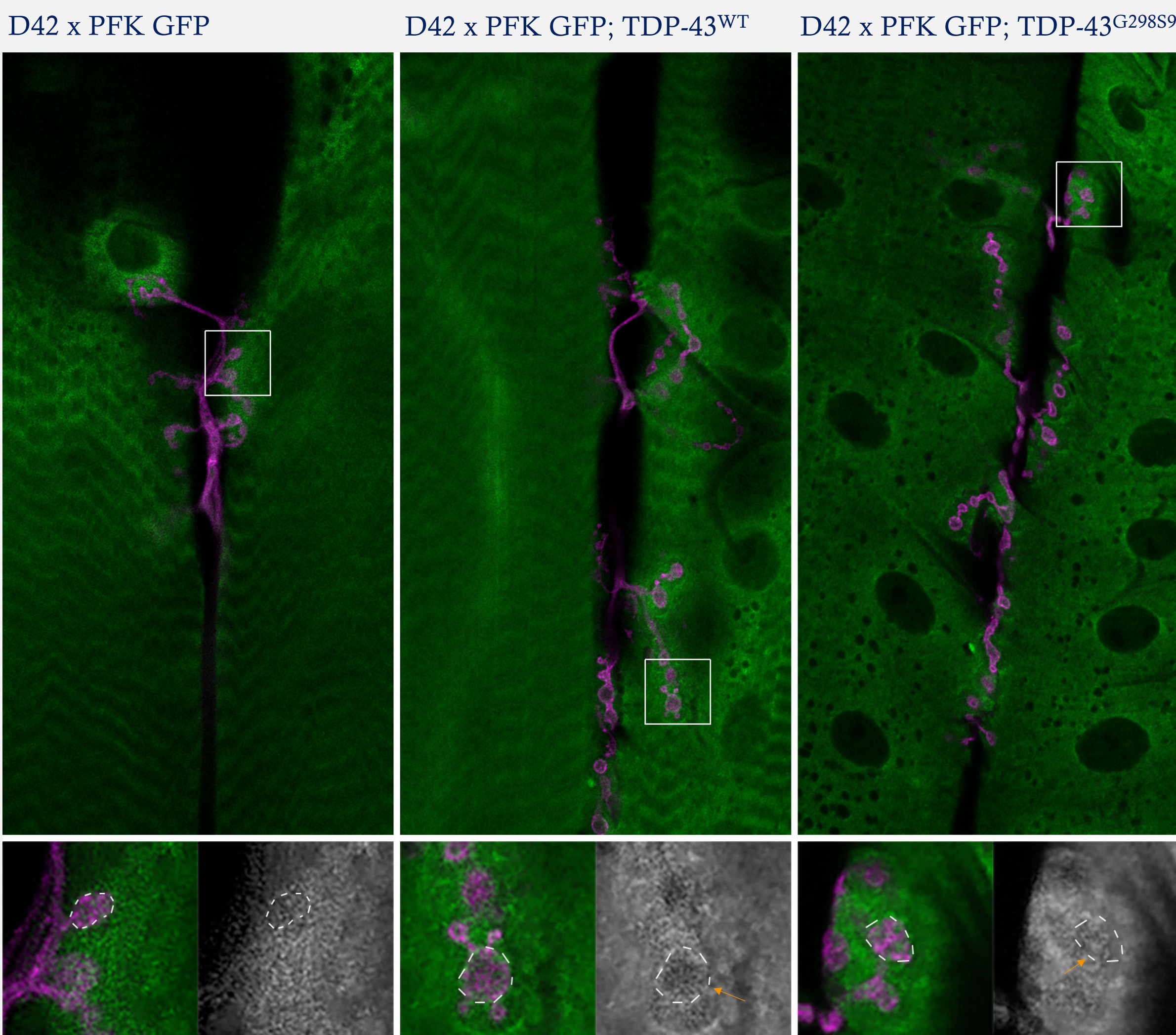## PFK localization in TDP-43 OE models

Figure 9: The neuromuscular junction dissected from third instar larvae stained for GFP and HRP. GFP tagged endogenous PFK is expressed in motor neurons. Enlarged boutons are shown with the in bottom panels with the GFP signal isolated on the right for each genotype.

Conclusions

- A high glucose diet mitigates locomotor deficits in multiple ALS models (SOD1, C9, and TDP-43 CRISPR)
- NMJ abnormalities are seen in TDP-43 CRISPR ALS model when compared to w1118 controls.
- PFK is localized at the synapse, but higher resolution microscopy (ExM) is need to determine whether TDP-43 proteinopathy alters its localization.

References

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