The role of transcription factor FOXO in maintaining homeostasis at the neuromuscular junction in *Drosophila melanogaster*

Allison Birnbaum, Hua Bai[1]

[1] Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011

Abstract

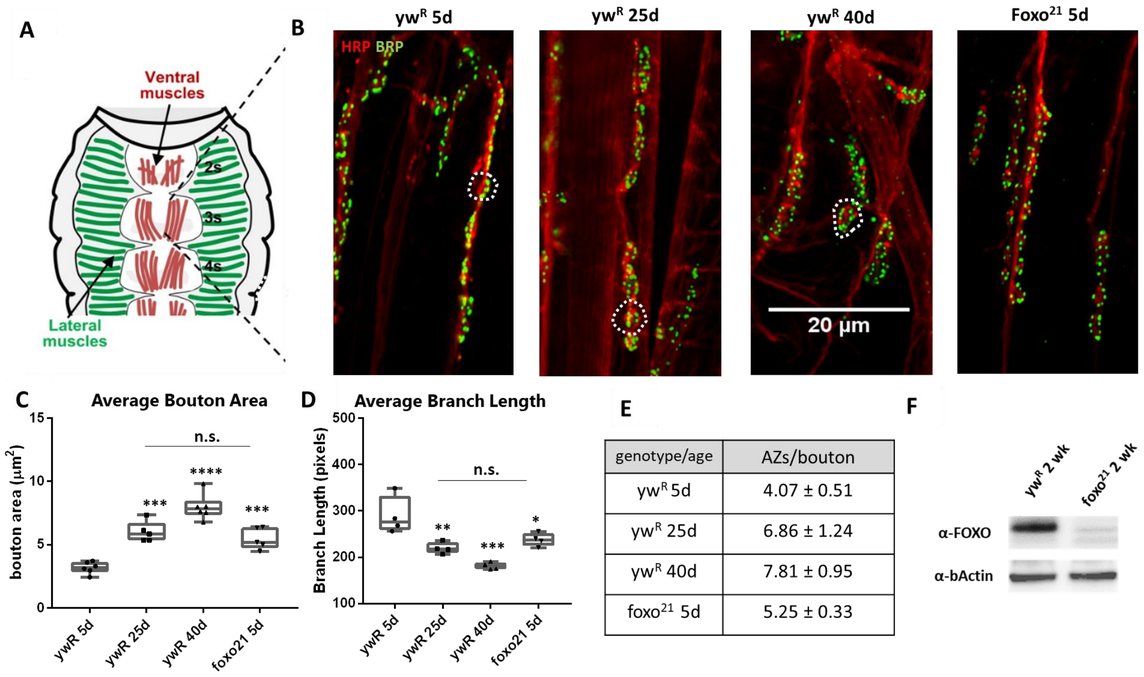
The transcription factor FOXO is a known regulator of tissue homeostasis and animal lifespan[1,2]. It has been linked to the maintenance of neuronal processes across many species, and has been shown to influence axonal integrity and synaptic plasticity at the neuromuscular junction (NMJ)[3,4]. However, the role of FOXO on aging at the NMJ has yet to be evaluated. We profiled adult Drosophila NMJs in FOXO-null mutant abdominal ventral longitudinal muscles and found that these mutants exhibited morphological characteristics similar to those of aging wild-type flies. Surprisingly, we observed an accumulation of Rab7-marked late endosomes associated with the axon with age as well as upon specific knock-down of motor neuron FOXO. Overexpressed FOXO in the motor neuron and were able to rescue the accumulation of Rab7 with aging, suggesting FOXO is a regulator of this phenotype and has a positive effect on neuronal homeostasis. In order to determine the downstream targets of FOXO, we used FOXO ChIP-seq and RNA-seq data to evaluate FOXO activity[5]. A genetic screen revealed members of MAPK signaling act downstream of FOXO to control NMJ homeostasis during aging. We also observed increased activation of p38 upon foxo knockdown. Our work reveals that FOXO is a key regulator for NMJ homeostasis, and it maintains NMJ integrity by repressing MAPK signaling during aging.

Highlights

1) Loss of FOXO results in altered NMJ morphology in adult Drosophila with enlarged boutons and shorter branches, comparable to those observed with aging
2) Knockdown of FOXO in the motor neuron results in an increase in late endosomes at axon branch points in young adult flies
3) Overexpression of FOXO in the motor neuron reduces middle aged NMJ late endosome accumulation and bouton enlargement
4) Members of MAPK and activin signaling rescue foxo-knockdown induced aging phenotypes
5) FOXO acts as a repressor of p38 activation in the motor neuron, illustrating FOXO's role as a regulator of NMJ integrity

Results

Knockout of FOXO cause age-related morphological changes at the abdominal ventral muscle



genotype/age	AZs/bouton
yw^R 5d	4.07 ± 0.51
yw^R 25d	6.86 ± 1.24
yw^R 40d	7.81 ± 0.95
$foxo^{21}$ 5d	5.25 ± 0.33

Figure 1. A. A cartoon representation of the adult ventral longitudinal abdominal muscle[6] **B.** Immunofluorescence staining of the Abdominal VLM A3 segment for $foxo^{21}$ mutant flies and ywR control flies at 5 days old, 25 days old, and 40 days old. Flies were stained with anti-HRP (red) to detect neuronal tissue and active zone protein BRP (green). **C.** Bouton area for 5 day *foxo* flies is significantly larger than 5 day controls, but is comparable to 25 day control flies. ** P<0.01. **D.** Branch length for 5 day old *foxo* mutants is significantly shorter than 5 day controls, but is comparable to 25 day control flies. *P<0.05, ** P<0.01, ns: not significant. **E.** Quantification of changes in active zone number per bouton. **F.** *Foxo[21]* mutants show reduced FOXO protein expression throughout whole body tissue.

Aging and loss of FOXO cause changes in endocytic activity at the adult NMJ

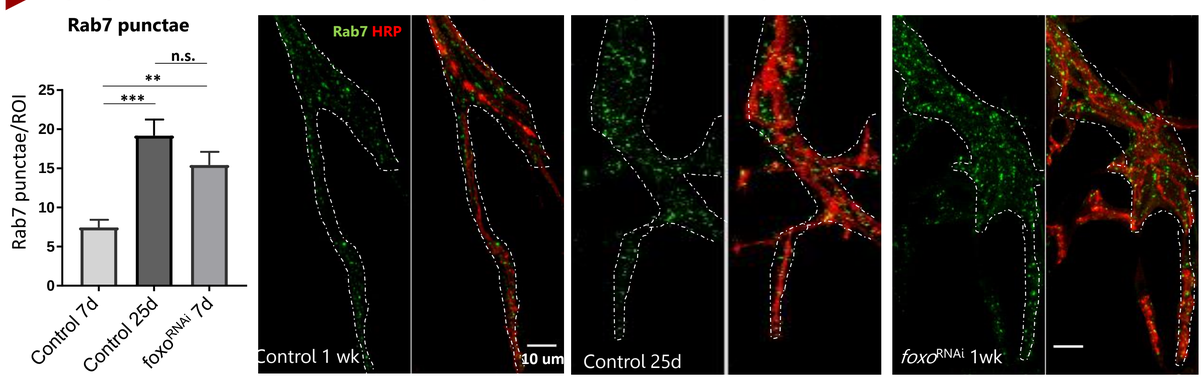


Figure 2. A. A significant increase in the number of Rab7 punctae was detected with control (Ok6> BL35783) aging and at a young age in adult flies containing a knockdown of motor neuron FOXO at axon branchpoints in the A3 VLM segment. **P< 0.01, ***P< 0.001, n.s.- not significant. **B.** Representative immunofluorescence images of main axon branchpoints with indicated genotypes and ages. Co-labeled with Anti-Rab7 and anti-HRP. Bar 10 µM.

Acknowledgments

We thank Bloomington Stock Center, Harvard Medical School TRiP project, Nan Liu and Xiaofen Wu (Chinese Academy of Sciences, China) Aurelio Teleman (German Cancer Research Center (DKFZ), Germany), and Robert Dubreuil (University of Illinois at Chicago, USA) for fly lines for this project. Thank you to Elizabeth McNeill (Iowa State University, USA) for fly lines, antibodies, and experimental design. This work is supported by NIH/NIA Research grant R00AG to Hua Bai.

Working Model

Overexpression of FOXO in motor neurons results in a rescue of mid-aged Rab7 accumulation

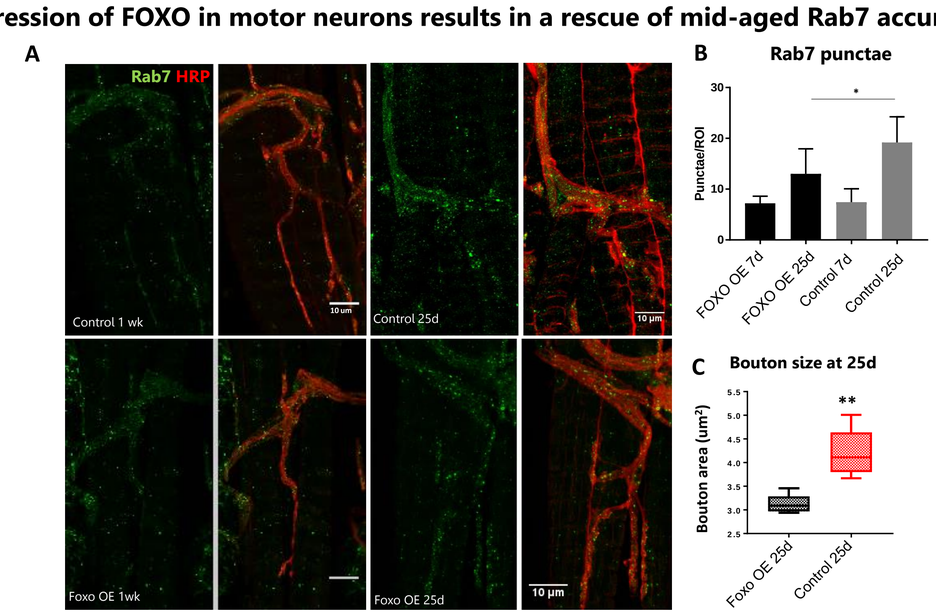


Figure 3. A. Overexpression of FOXO in motor neurons (Ok6-Gal4>BL42221) reduces age-dependent rab7 accumulation compared to control flies. (ok6-Gal4>BL35783) Quantification shown in B. **B**. 25 day old FOXO-OE flies have less rab7 accumulation than control flies. *p<0.05 **C.** FOXO overexpression in the motor neuron results in significantly smaller boutons than controls at 25 days post eclosion. **p<0.01.

A genetic screen reveals MAPK and Activin signaling can rescue foxo-induced processes

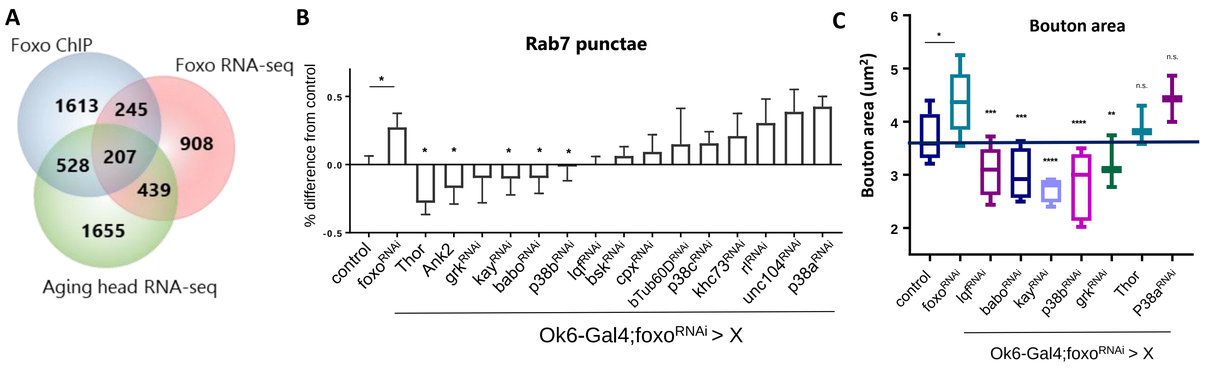


Figure 4. A. Venn diagram overlap of ChIP-seq, head foxoC431 RNA-seq, and aged head RNA-seq: For RNA-seq data {FC >±1.25, P < 0.05}. {FOXO ChIP FC >1.5, FDR< 0.05}. Reveals 207 genes shared between all three datasets. **B**. Double mutant flies rescue late endosome through MAPK and Activin pathway. Values set as a percentage with baseline control (Ok6-Gal4>ywR). Significance compared to foxoRNAi (Ok6-GAL4;BL32993) *P<0.05. **C.** Double mutant lines rescue foxo induced bouton expansion. Significance compared to foxoRNAi. *P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, ns: not significant.

Knockdown of motor neuron foxo results in increased phosphorylated p38

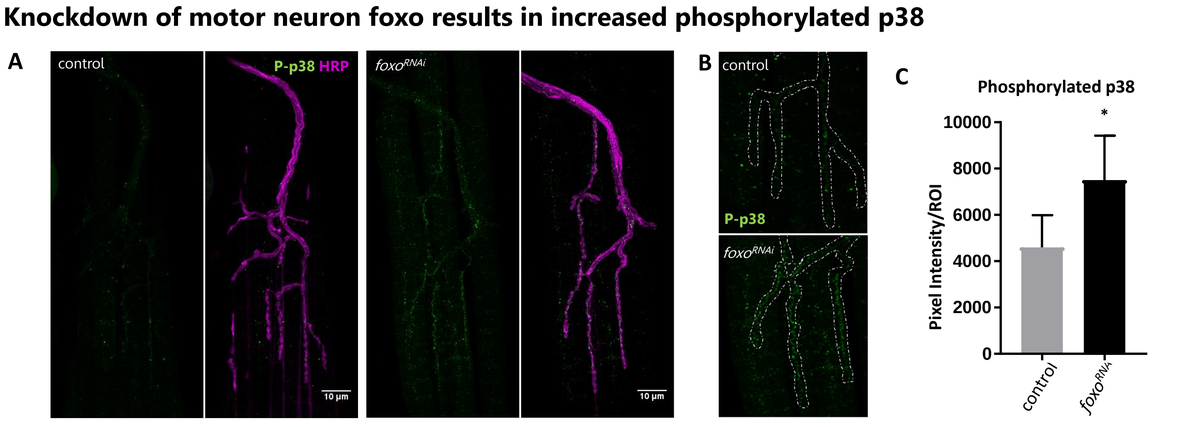


Figure 5. A. Representative images show increased phosphorylation of p38 upon the knockdown of foxo in the motor neuron. **B.** A close up of the axon terminal regions (white dotted outline). **C P**hosphorylated p38 pixel intensity associated with HRP is significantly increased upon foxo knockdown. . *P<0.05.

References:

[1] Kenyon et al, 1993. Nature.
[2] Salih and Brunet, 2008. Curr Opin Cell Biol.
[3] McLaughlin and Broihier, 2018. Trends Genet
[4] Paik et al, 2018. Cell Stem Cell.
[5] Birnbaum et al, 2019. Front Genet.
[6] López-Arias et al, 2017. PloS One.
[7] Deleidi et al, 2015. Europe PMC.