

## Abstract

Skeletal muscle is one of the main tissues in the human body in normal-weight subjects. The integrity of this tissue depends on the balance between protein synthesis and degradation. Foxo proteins correspond to a family of transcription factors that participate as a negative regulator promoting muscle atrophy.

Flexor digitorum brevis (FDB) skeletal muscle fibers were isolated from adult (4–6 weeks old) female CD1 mice. Foxo-GFP trafficking was determined as a function of time and nucleus-to-cytoplasm (N/C) ratio.

Our laboratory has shown that trophic factors such as IGF-1 or insulin promote Foxo efflux from the nucleus, preventing atrogen expression. Foxo activation in this pathway is Akt-dependent. On the other hand, the use of trains of 5 s duration of repeated electrical stimulation at low frequency (10 Hz) decreases Foxo entry into the nucleus in isolated fibers compared to a basal condition without electrical stimulation.

Other studies have shown that mechanical stress in muscle without electrical stimulation can phosphorylate Foxo through an Akt-dependent pathway, allowing it to efflux from the nucleus. To study the effect of this type of stress on muscle, we will use a passive mechanical deformation system on individual fibers to determine Foxo trafficking.

Our findings suggest that Foxo may be a key regulator in skeletal muscle adaptation to mechanical stress and stimulation frequency. Studying these mechanisms could be relevant to better understand how skeletal muscle fibers respond to different environmental stimuli, which could have therapeutic implications in diseases such as muscular dystrophy and sarcopenia.

## Introduction

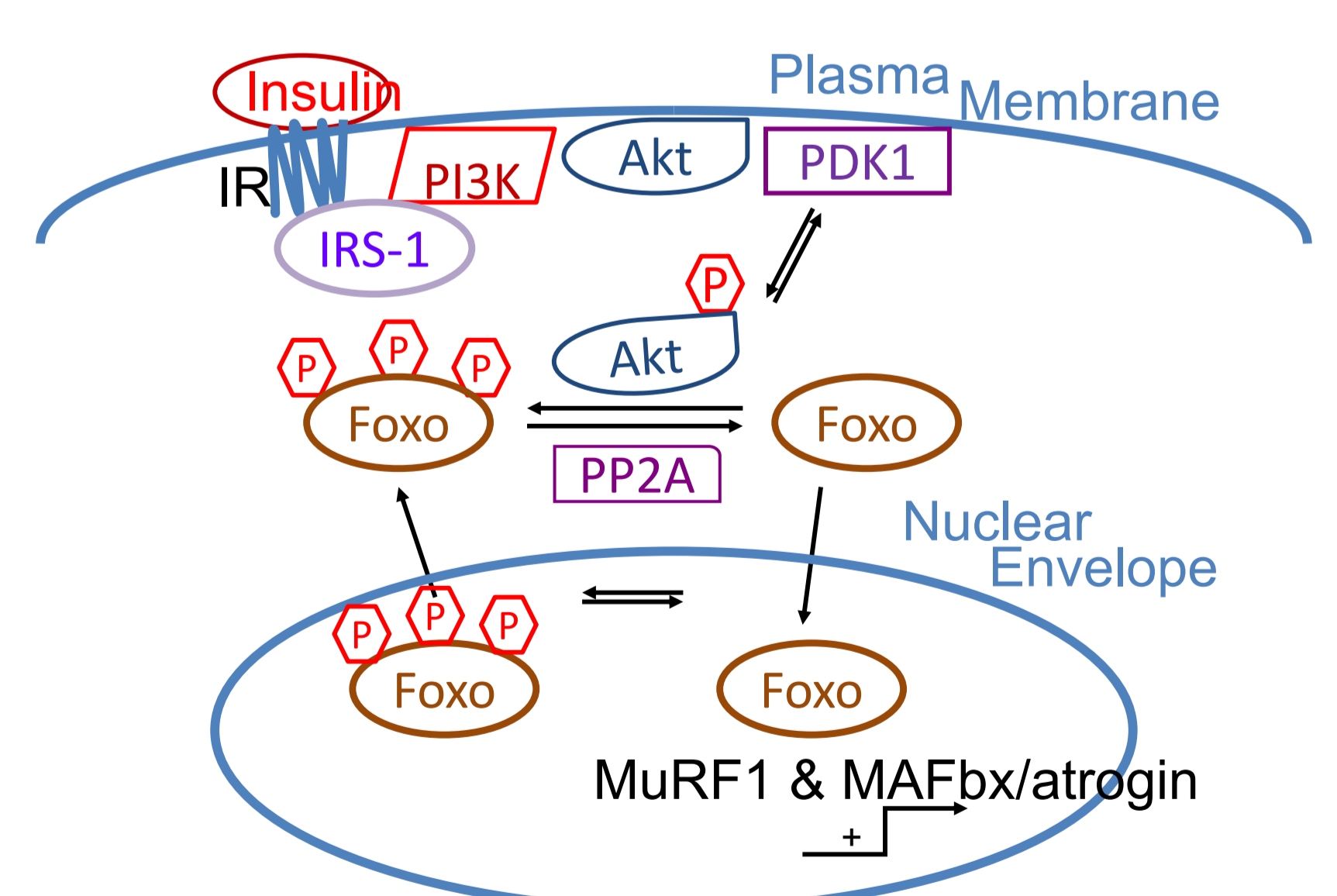
Skeletal muscle atrophy and wasting occurs during disuse, inactivity and aging or as an accompaniment of cancer, diabetes, heart disease, septicemia or other severe system disease states and causes debilitating limitations on mobility and breathing. The forkhead box, class O (Foxo) transcription factors, including Foxo1, serve as a key activators of muscle protein breakdown by promoting the transcription of atrophy related ubiquitin ligases.

Foxo proteins shuttle into and out of muscle fiber nuclei primarily depending on phosphorylation status (see figure 1). In response to insulin-like growth factor 1 or insulin by a “canonical pathway” involving phosphoinositide 3-kinase (PI3K) – Akt, leads to phosphorylation of Foxo on three conserved residues, thus preventing nuclear entry.

Phosphorylation of nuclear Foxo by Akt, generates a conformational changes causing an unbinding of Foxo from DNA, that allows binding of Foxo to the chaperone protein 14-3-3, resulting in transport of the Foxo out of the nucleus via the nuclear export system.

Dephosphorylation of Foxo allows it to enter the nucleus and can bind to DNA sites causing transcriptional activation. Nuclear Foxo promotes the expression of ubiquitin ligases MuRF1 and MAFbx/atrogen1, which increase muscle protein breakdown and this contribute to atrophy and muscle wasting.

Figure 1: Molecular basis for Foxo transport and signaling



## Aim

To investigate if Foxo1 signaling can be regulated by electromechanical activity and identify the mechanisms responsible for this regulation in mammalian muscle.

Figure 2: Insulin promotes Foxo1-GFP nuclear efflux

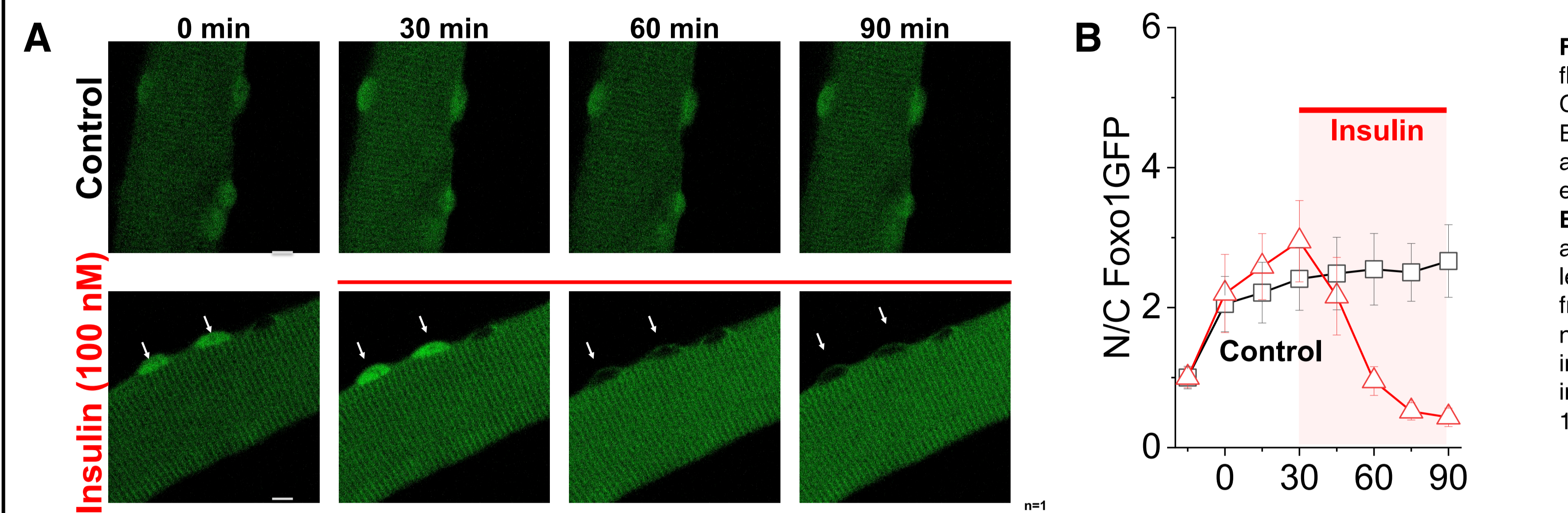


Fig. 2. A, representative fluorescent images of FOXO1-GFP in skeletal muscle fibers. Each row shows the same fiber at different times during the experiment. B, time course experiment analysis of FOXO1-GFP N/C levels (average of N/C values from images shown in A, normalized to the average N/C) in the presence or absence of insulin (100 nM). 10-mm scale bar

Figure 3: Brief repetitive field stimulation at 10 but not 50 Hz promotes Foxo1-GFP nuclear efflux.

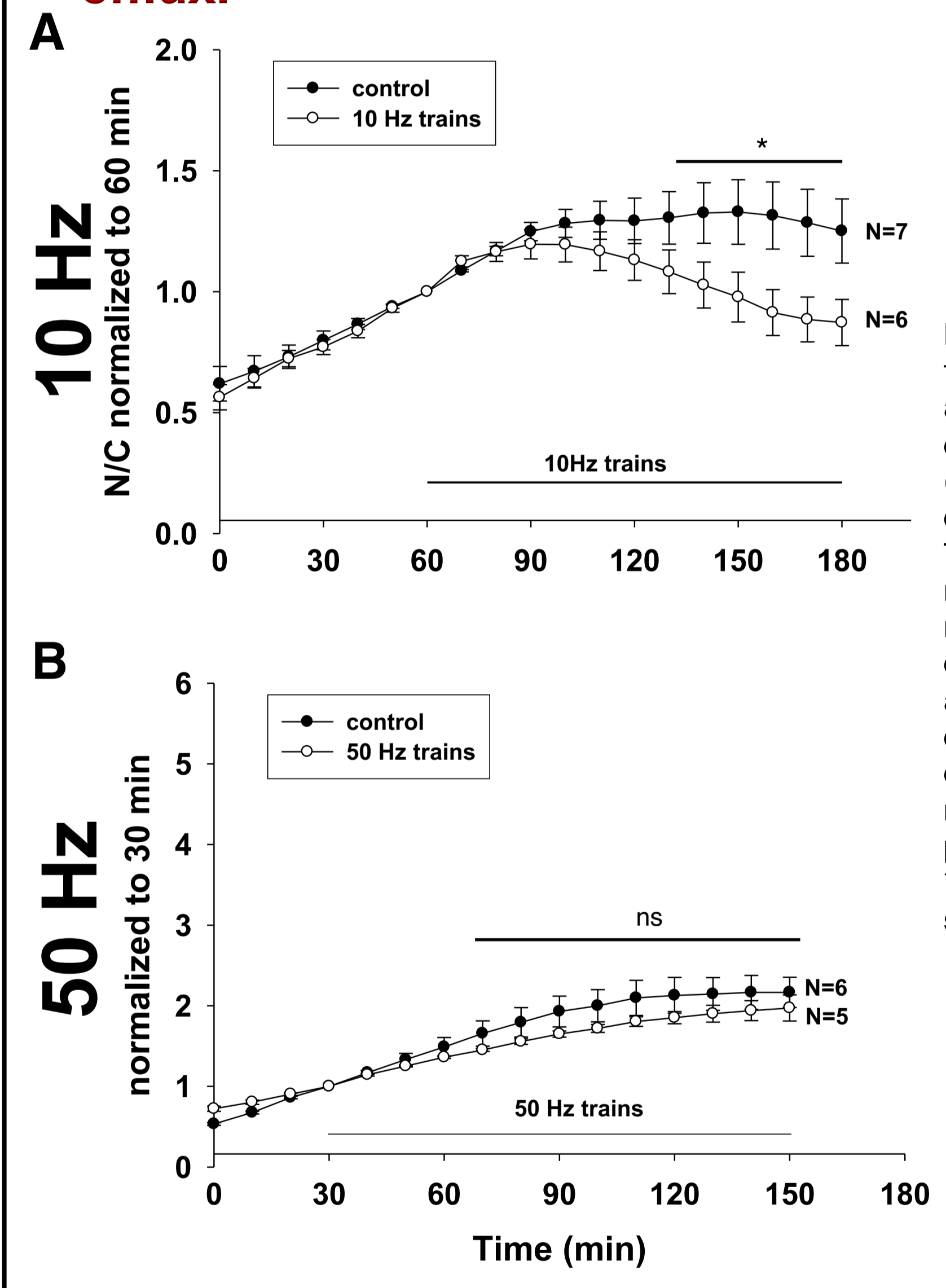


Fig. 3. Repetitive fiber stimulation using a 5 sec duration train of 10 Hz (A) or 50 Hz (B) stimuli delivered once every 50 sec. These data may represent a first recording of the effect of fiber electrical activity on the time course of nuclear cytoplasmic movements of Foxo proteins. \* p < 0.05; ns: not significant

Figure 4: Ca<sup>2+</sup> signal in response to repetitive field stimulation at 10 or 50Hz

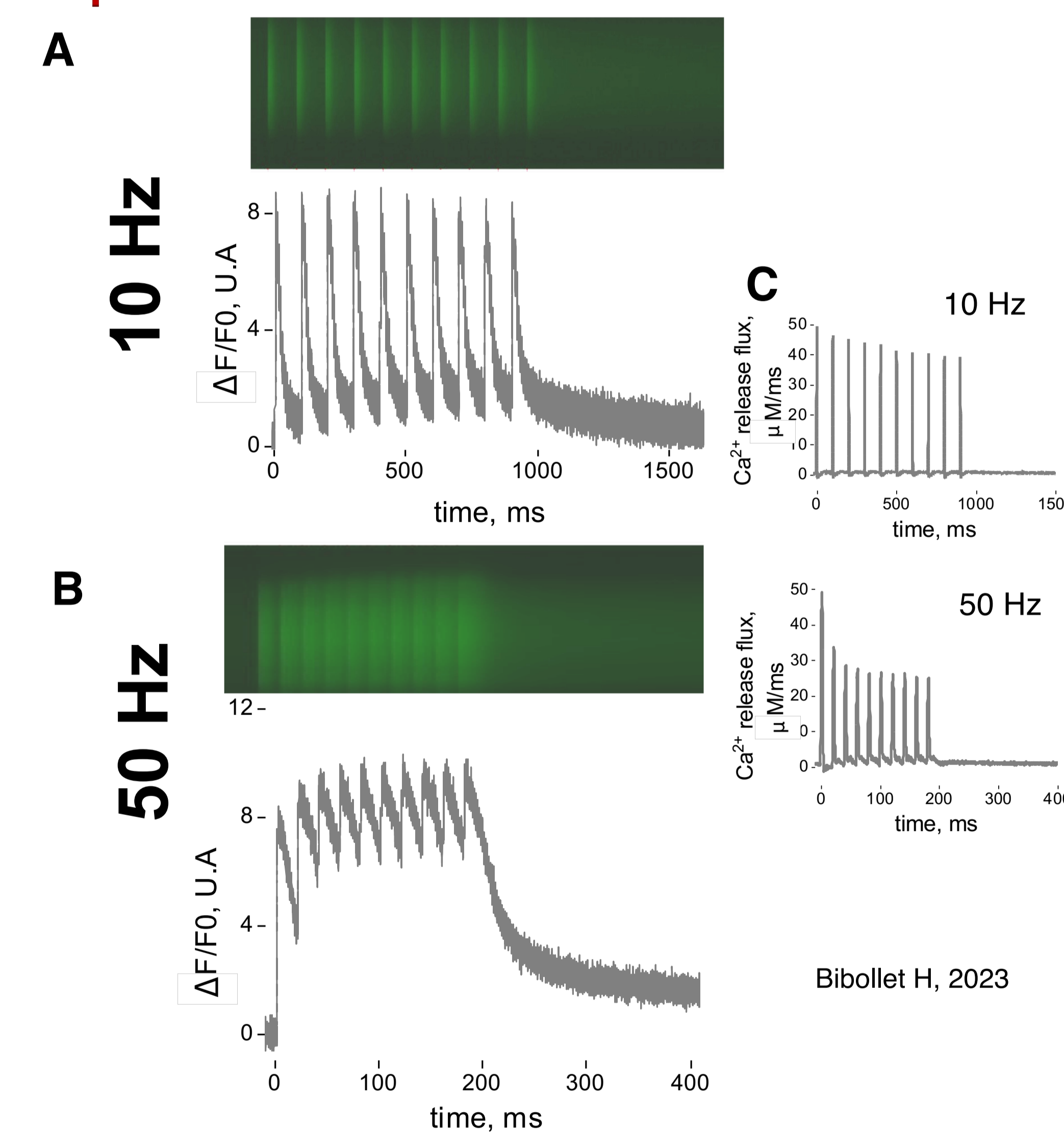
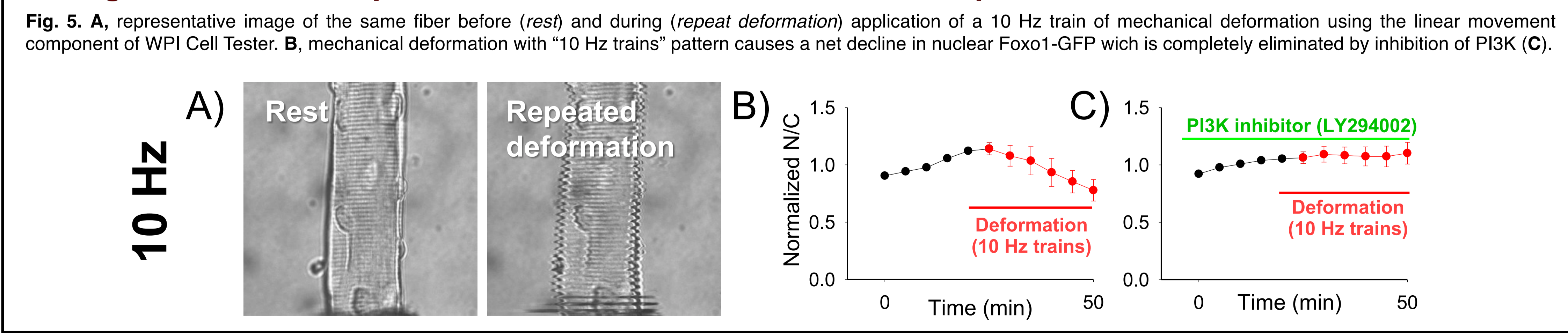


Fig. 4. Representative line scan confocal microscopy Ca<sup>2+</sup> transients (x-t; top) elicited by field stimulation at 10 (A) and 50 Hz (B) in FDB fibers loaded with Fluo-4 AM and corresponding representative ΔF/F0 time courses (bottom). C, representative estimated Ca<sup>2+</sup> release flux trajectories from signals presented in A, B. Adapted from Bibollet, H. (2023). DOI: 10.14814/phy2.15675

Figure 5: Passive repetitive mechanical stimulation at 10 Hz promotes Foxo1-GFP nuclear efflux

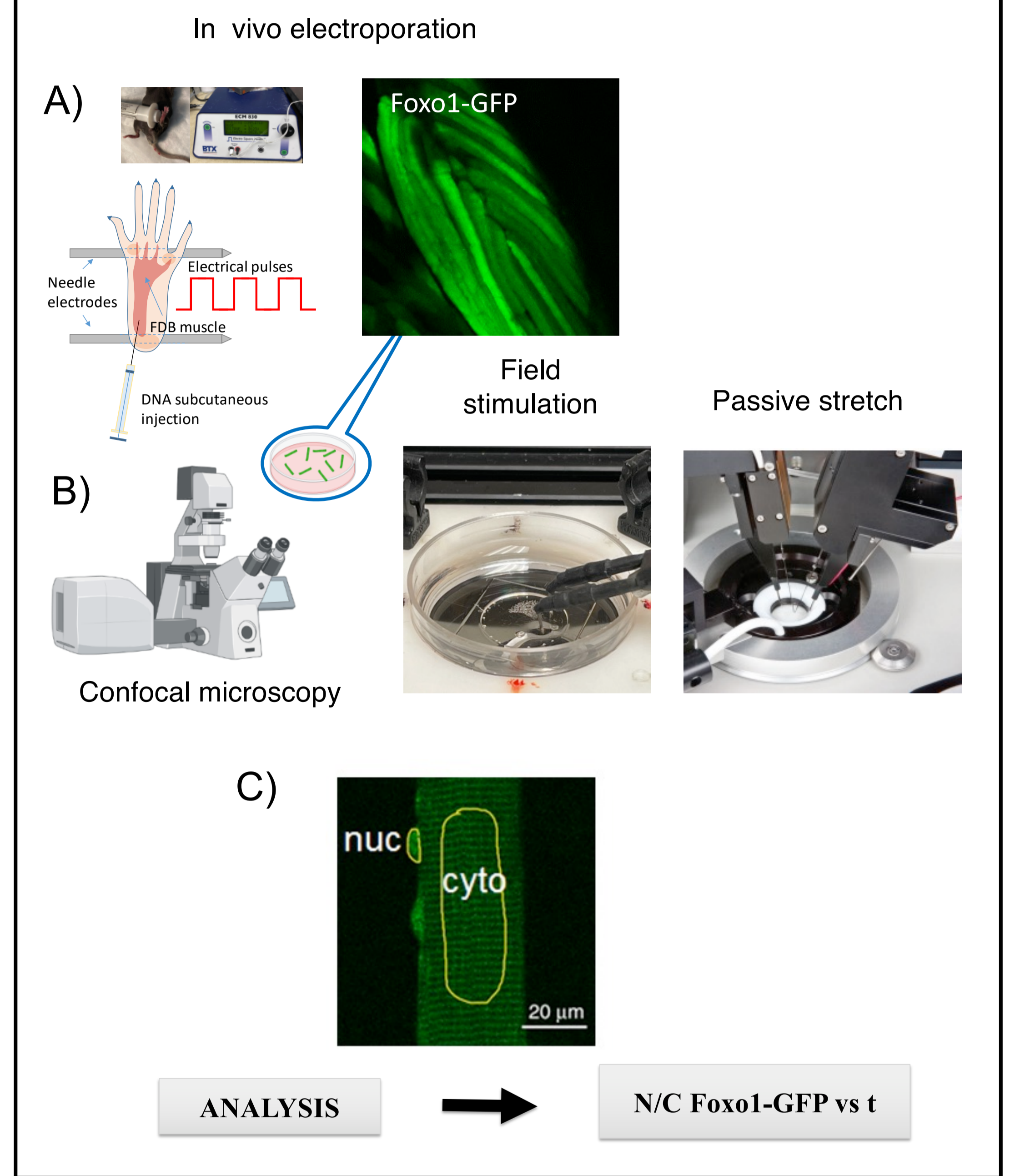


## Acknowledgments

This work was supported by grants R01-AR-075726 and R01-NS-103777 (M.F.S) from National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## Methods

Field stimulation was carried out using 5 sec duration train of 10 Hz or 50 Hz stimuli delivered once every 50 sec. Passive stretch using 10 Hz train of mechanical deformation through the linear movement component of WPI Cell Tester. Model for Foxo1-GFP nuclear-cytoplasmic movements previously described (Schachter, TN. et al. 2012).



## Conclusions

- In cultured adult skeletal muscle fibers:
  - Electrical stimulation with 10 Hz trains, but not 50 Hz trains, decreased the nuclear translocation of Foxo1-GFP.
  - Passive mechanical deformation also promoted nuclear efflux of Foxo1-GFP.
  - This Foxo1-GFP efflux evoked by passive membrane deformation was prevented by PI3K inhibition.

## Future directions

1. Future studies will explore and identify the signaling events involved in this activity and mechanosensitive dependent Foxo1-GFP efflux.

## Contact information

Giovanni Rosales-Soto: grosales-soto@som.umaryland.edu  
Erick O. Hernández-Ochoa: ehernandez-ochoa@som.maryland.edu  
Martin F. Schneider: mschneider@som.umaryland.edu