

BIO101 accelerates differentiation and enhances mitochondrial function in skeletal muscle cells

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Introduction

- Muscle wasting diseases such as sarcopenia, myopathies and cachexia are associated with the decline in differentiation of muscle cells into functional myofibers. This leads to a decrease in mobility and poor quality of life.
- Ecdysteroids are natural compounds found in insects and plants which increase protein synthesis in mammals, improving skeletal muscle strength and endurance.
- The drug candidate BIO101 is a pharmaceutical-grade preparation of the ecdysteroid 20-hydroxyecdysone purified from *Stemmacantha carthamoides*. BIO101 previously demonstrated its potential on muscle quality and function in different in vitro and in vivo models.
- BIO101 is the API of Sarconeos currently being tested in a clinical IIb trial in sarcopenic patients.
- The aim of this study was to characterize the impact of BIO101 on muscle cells differentiation.

Methods

Cell line: C2C12 murine myoblasts were induced to differentiate for 6 days. Appropriate treatment was administered from the first day of differentiation.

Immunofluorescence: C2C12 were plated on microscopy coverslips in 12-well culture plates. Differentiation was induced in sub-confluent cultures by changing the medium for DMEM/F-12 with 2% of horse serum. Cells were allowed to differentiate with or without drugs for 6 days, then they were fixed with 4% paraformaldehyde. The cells were immunostained with MHC (MF20) or myogenin. Staining was revealed with Alexa Fluor 488-conjugated secondary antibody and counterstained with DAPI. The cells were then observed under an epifluorescence microscope. Image analysis was performed using ImageJ software. Number of DAPI-stained nuclei per myotube was counted and diameters of MHC-positive myotubes were measured. The fusion index was calculated as the ratio of nuclei in fused MHC positive cells (≥ 2 nuclei per cells) to the total nuclei number.

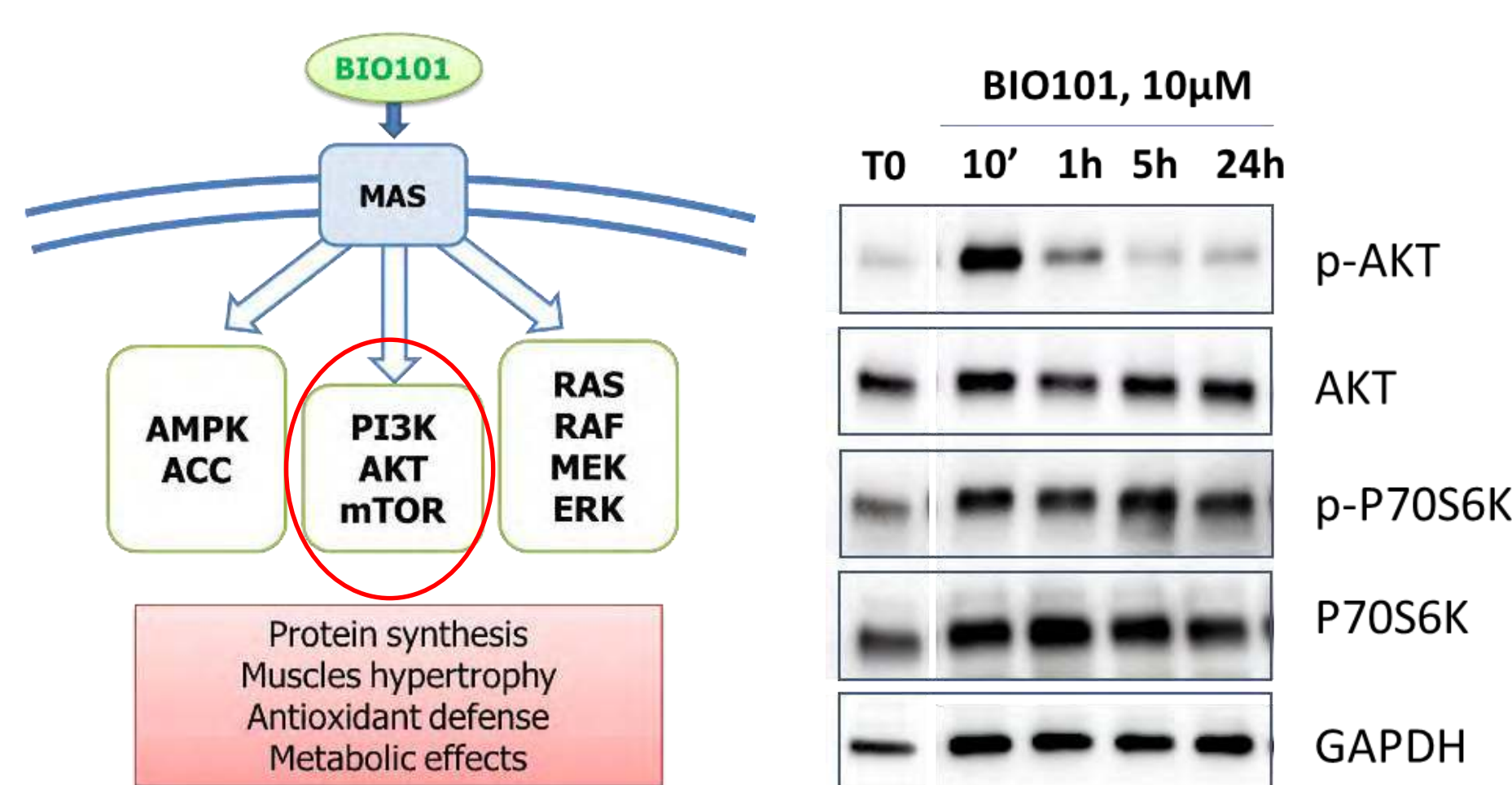
Mitotracker Red staining: After 24h, 72h or 8 days of differentiation, with or without BIO101, C2C12 cells were incubated with 300 nM mitotracker deep red FM (Invitrogen M22426) for 30 min at 37°C in cell medium. The cells were then briefly washed with PBS and imaged with fluorescence microscope. Fluorescence intensity was then measured using ImageJ software.

Western blot: Cells were lysed, equal amounts of proteins were electrophoresed on 4-12% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% BSA and incubated with specific antibodies overnight. Immunostaining was visualized using ECL. Band intensity was quantified using ImageJ software.

OCR measurements: Oxygen consumption was recorded using a Seahorse XF24 Analyzer.

Results

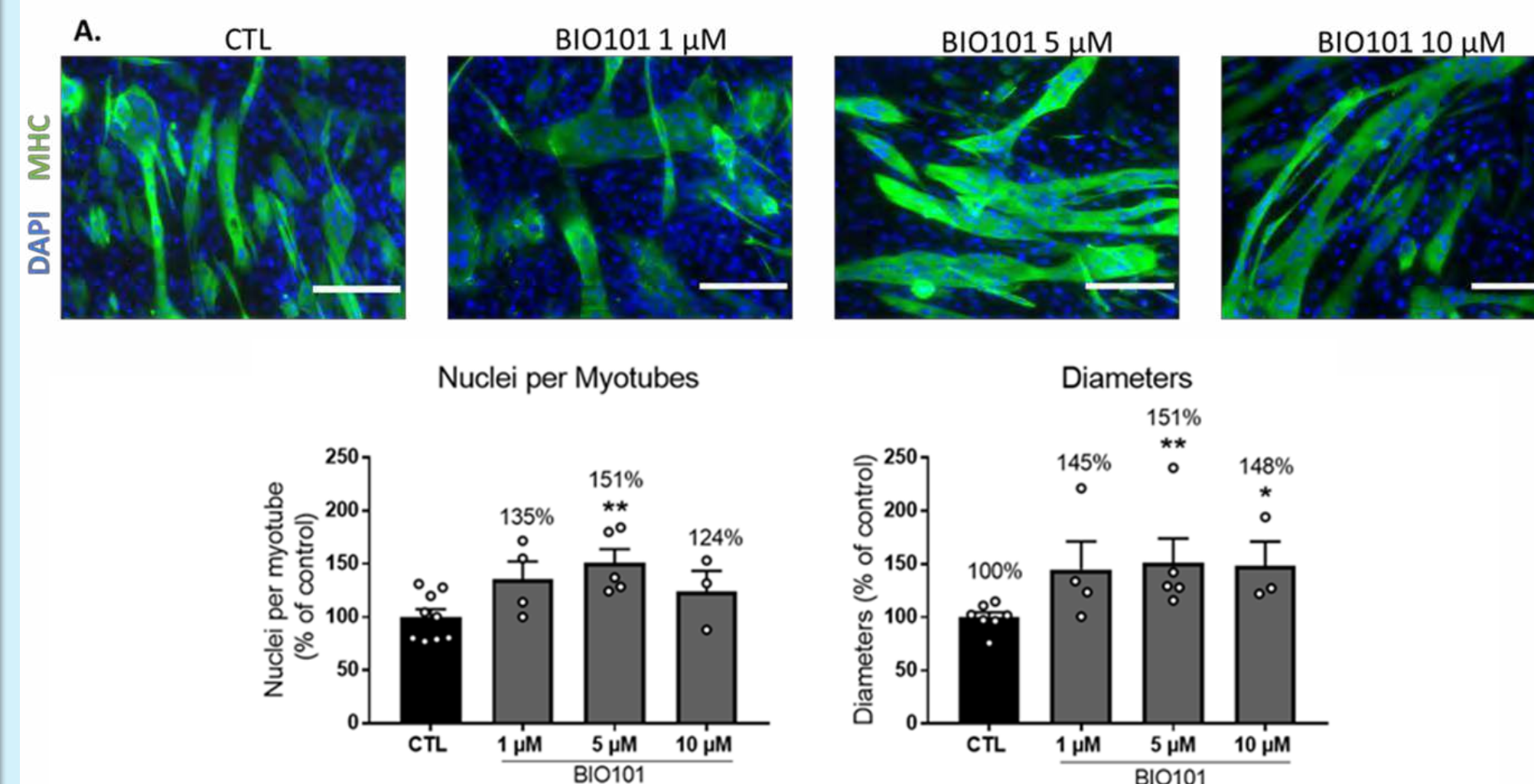
Effects of BIO101 on AKT/mTOR signaling



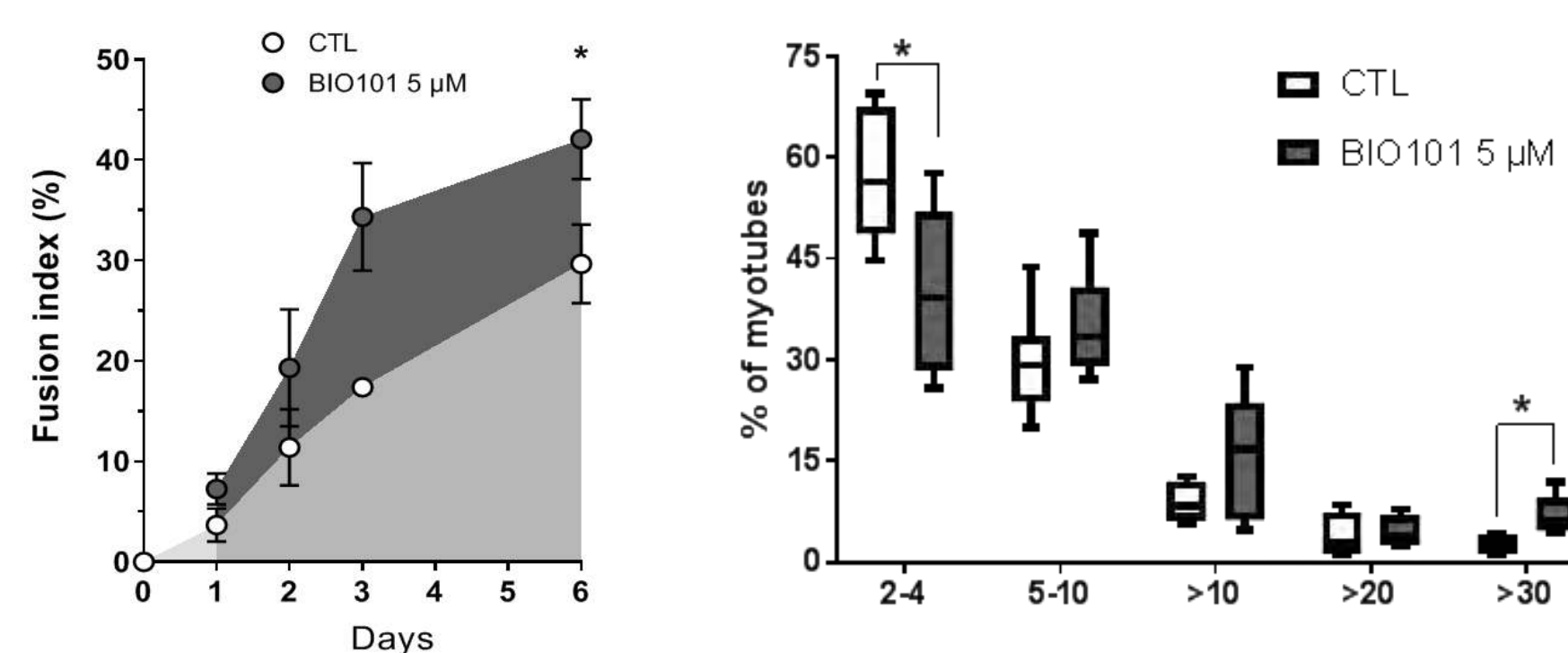
→ **BIO101 increases phosphorylation of major kinases of AKT/mTOR pathway in C2C12 cells**

Results

Anabolic effects of BIO101

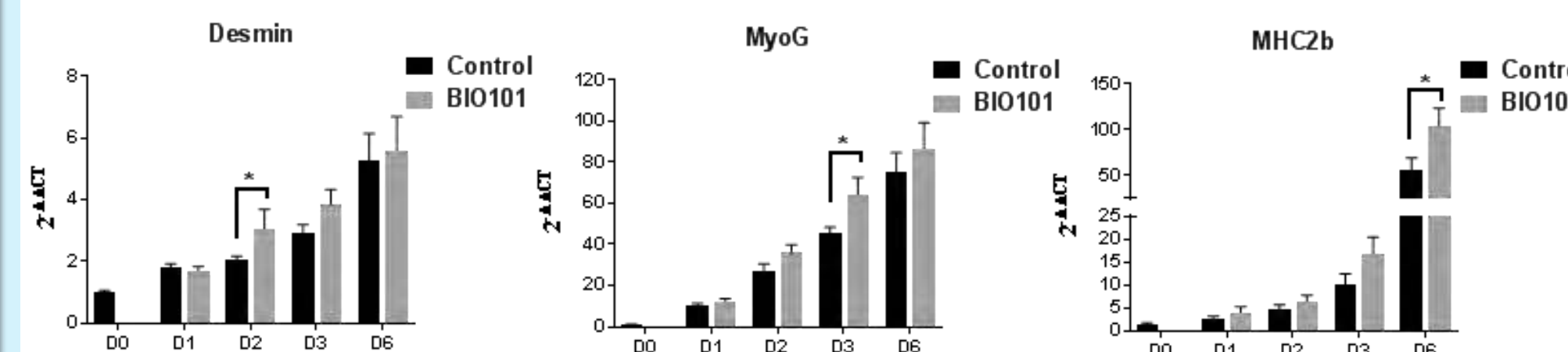
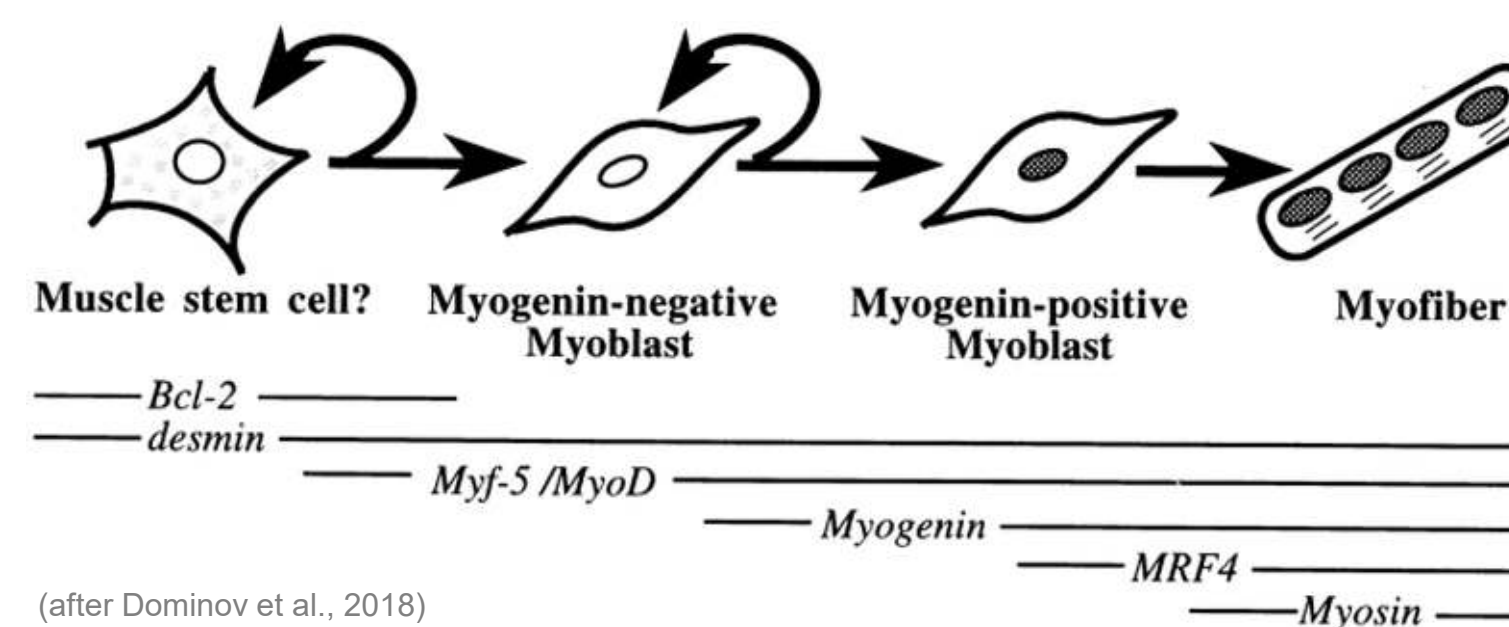


→ **BIO101 displays anabolic and pro-differentiating effects on C2C12**



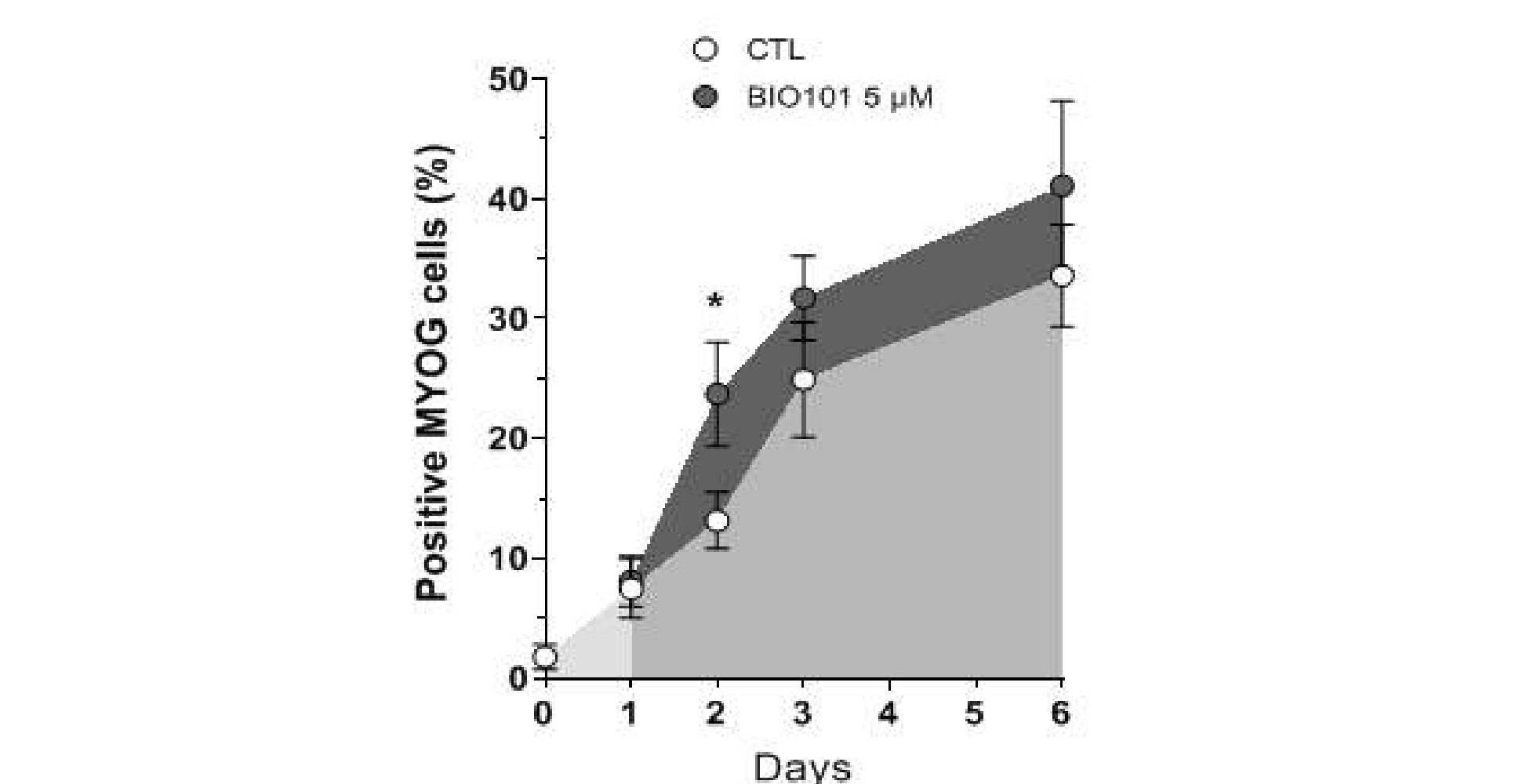
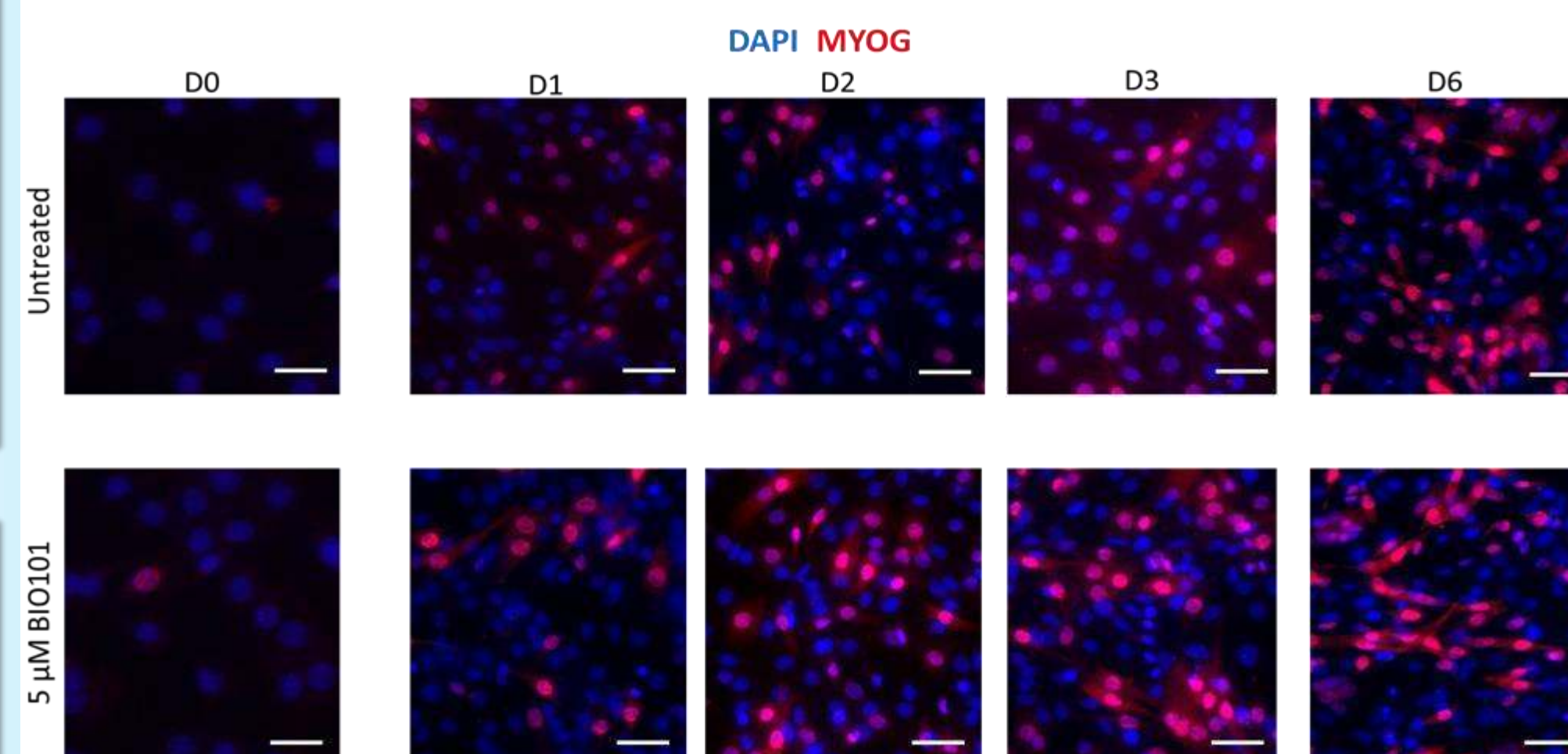
→ **BIO101 accelerates the kinetic of myoblasts' differentiation into myotubes and promotes the formation of multinucleated myotubes**

Effects of BIO101 on mRNA expression of differentiation markers



→ **BIO101 induces mRNA expression of early and late differentiation markers**

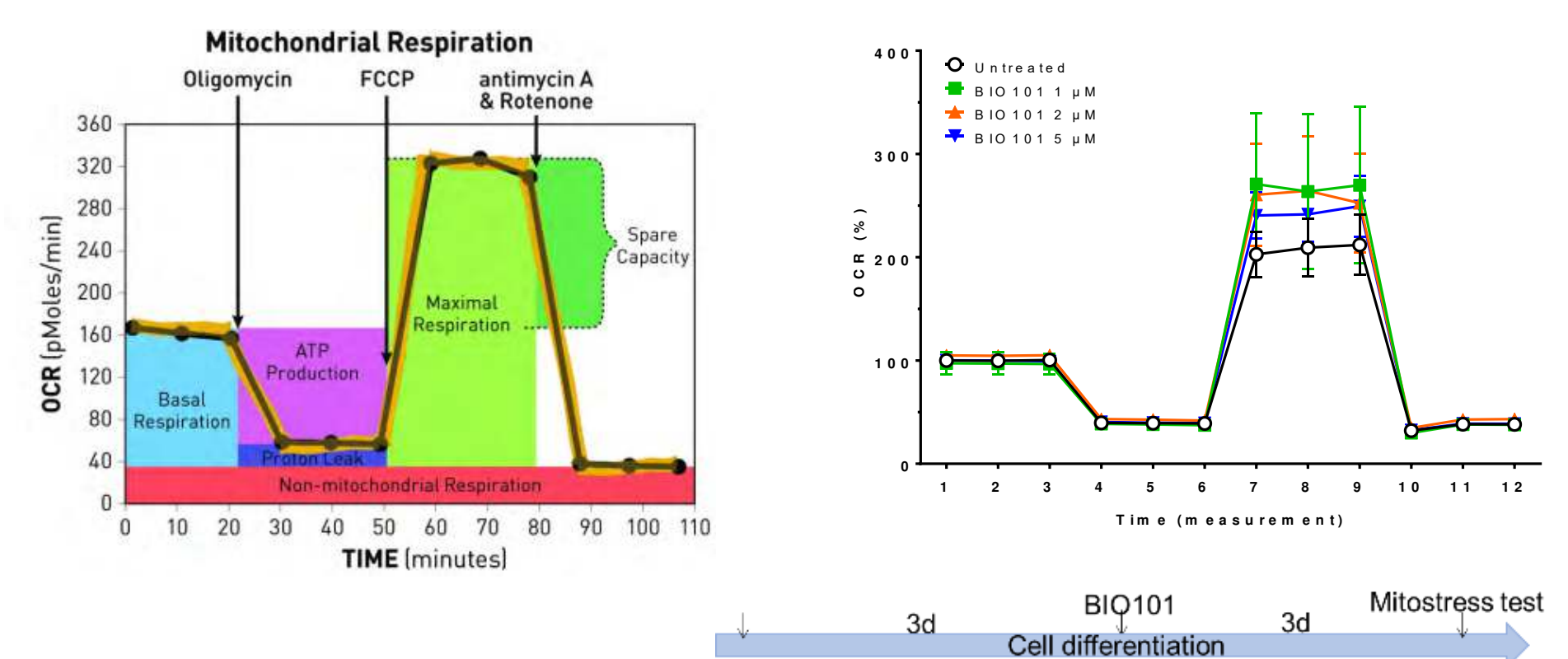
Effects of BIO101 on myogenin expression



→ **BIO101 induces early increase in myogenin expression**

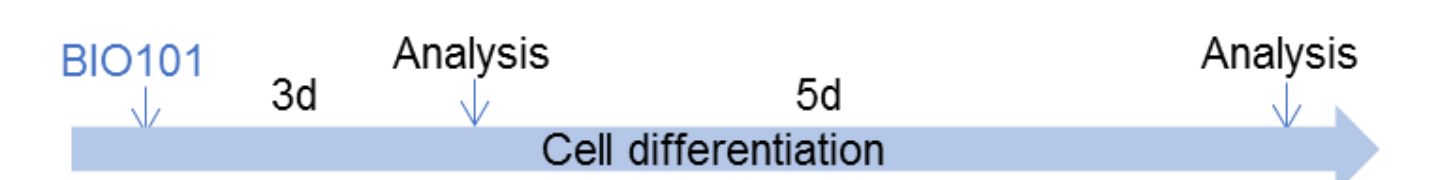
Results

Effects of BIO101 on oxygen consumption (Seahorse)

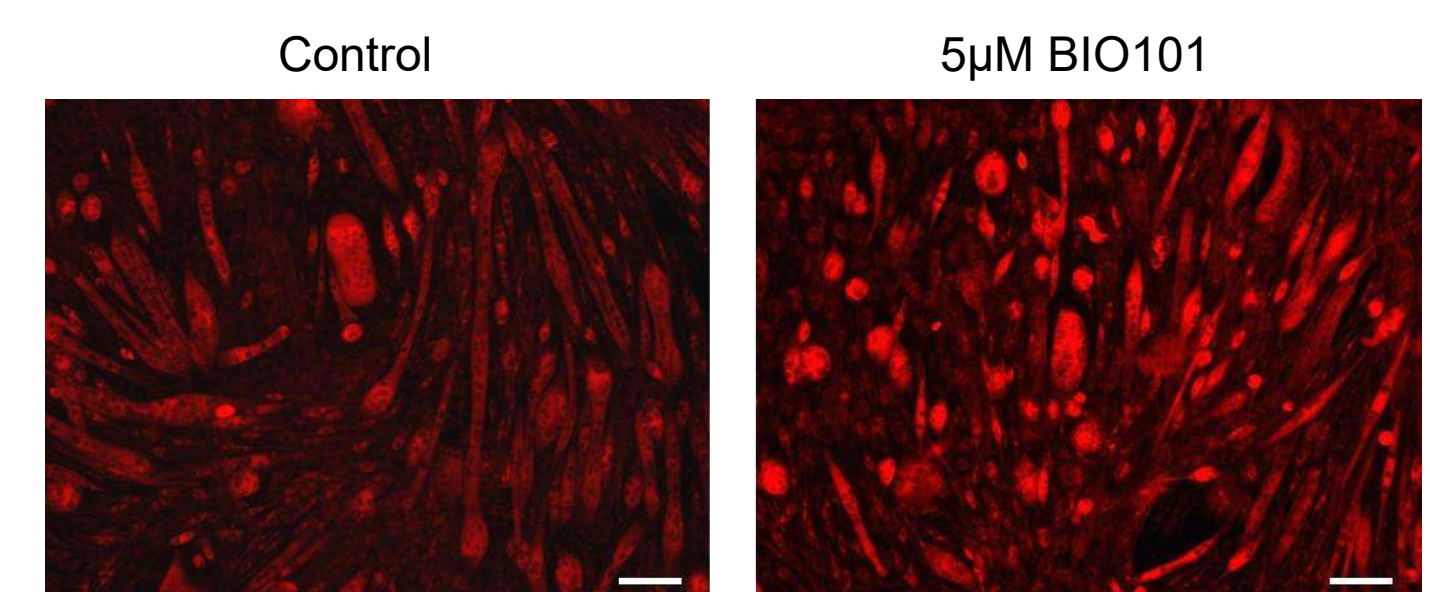


→ **BIO101 treatment for 72 hours during differentiation increases mitochondrial spare respiratory capacity of C2C12 cells**

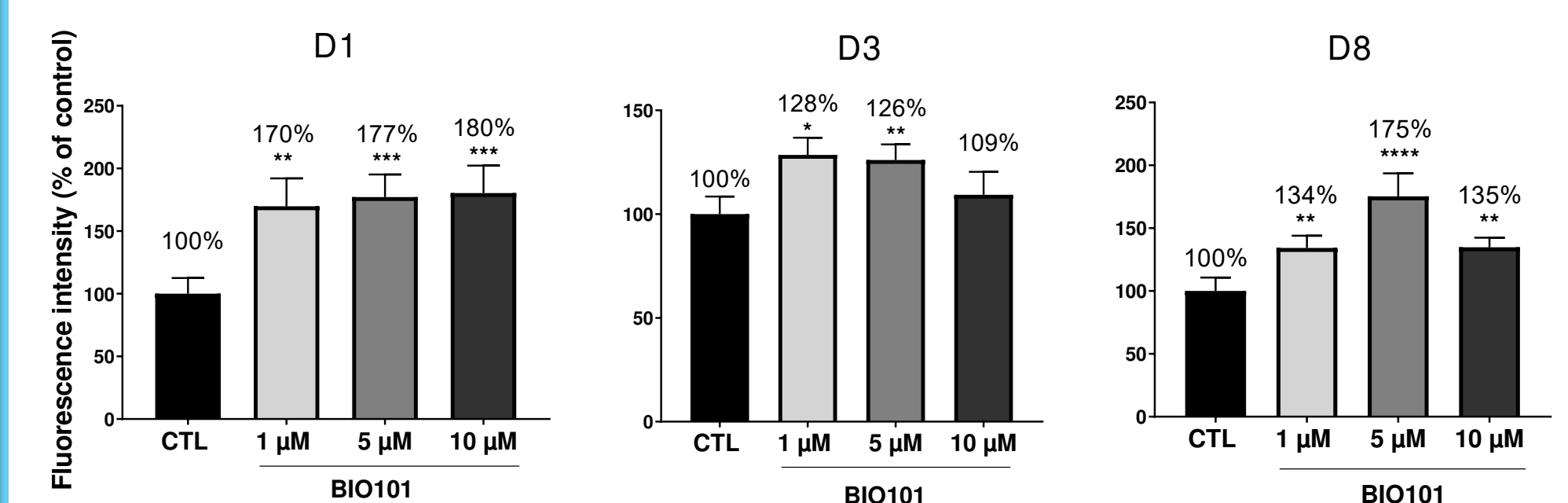
BIO101 effects on mitochondrial biogenesis



Mitotracker red staining



Mitotracker fluorescence intensity



→ **BIO101 treatment during differentiation increases mitochondrial content of C2C12 cells**

Conclusions

Our study demonstrates the overall beneficial effects of BIO101 on muscle cell differentiation.

- BIO101 induces an hypertrophy of myofibers in muscle cells associated with AKT/mTOR pathway activation
- Desmin, myogenin, and myosin expression are progressively increased with BIO101 treatment.
- Increases in mitochondrial spare respiratory capacity and mitochondrial mass in response to BIO101 exposure are believed to be responsible, at least in part, for improved muscle function.
- These results may support the clinical development of Sarconeos in sarcopenic patients.