MyoScreen™: A high-content-based discovery platform to model and screen key metabolic processes in human skeletal muscle

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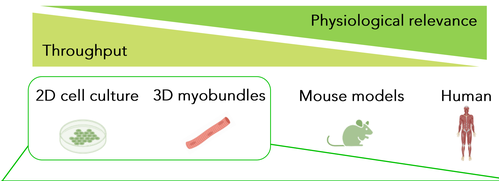
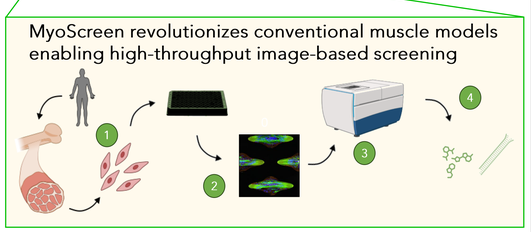
CYTOO

The MyoScreen Discovery Platform

A solution to the need for relevant *in-vitro* human muscle disease models

CYTOO's MyoScreen platform incorporating micropatterned plates and Artificial Intelligence (AI) driven image analysis provides *in-vitro* human muscle screening models that recapitulate inherited muscle diseases for drug development. Capitalizing on this expertise, CYTOO has now developed a panel of high-throughput and predictive functional assays that monitor key metabolic processes and disease hallmarks associated with acquired chronic diseases impacting muscle health. Examples include sarcopenic obesity, diabetes and metabolic syndrome.



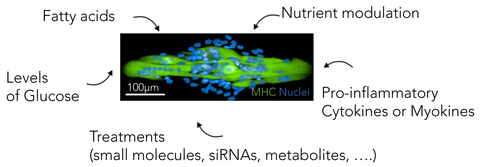


How to build a MyoScreen metabolic disease model:

1 Source the cell line

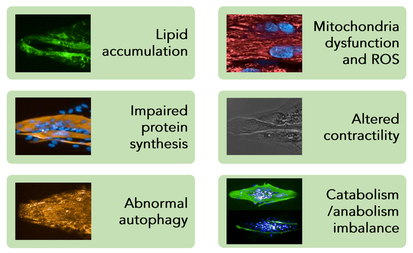
Pre-validated models for:
- Healthy myotubes (male vs. female, young vs. elderly)
- Metabolic diseased myotubes from patients with obesity and/or diabetes

2 Identify the main metabolic disease driver
- Induce specific features by changing cell culture conditions in healthy or diseased cultured cells

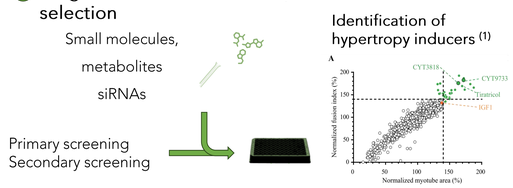


3 Characterize the main hallmarks and additional secondary outcomes

Validated and optimized assays are available to monitor different pathways. Several assays can be multiplexed in the same well.

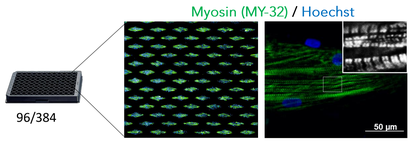


4 Target and Hit identification, Validation and Lead selection

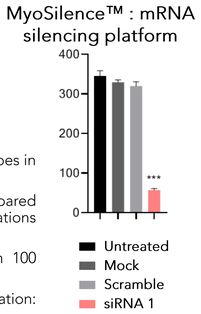


Exploring CYTOO's Integrated High-throughput Robust Discovery Platform

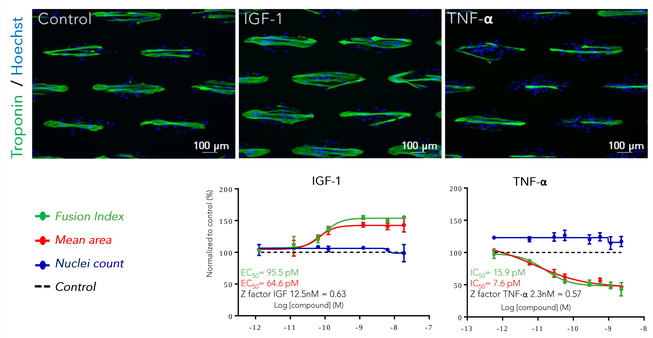
Myoscreen: a predictive, high throughput and robust discovery platform for RNAi and small compound screens



- High throughput and standardized: aligned and linear myotubes in 96 and 384 well format plates
- Predictive: Differentiation of myotubes is more efficient compared to conventional 2D plates and myotubes form sarcomeric striations and acetylcholine receptor clusters
- Robust: low level of variability, high statistical power with 100 myotubes per well (96-well plate), high Z-factors
- MyoSilence siRNA screening platform for target identification: inter-experiment variability < 10%



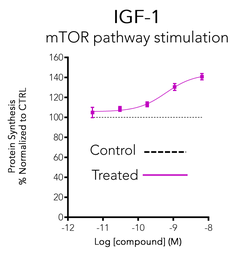
Identify compounds that preserve/increase muscle mass



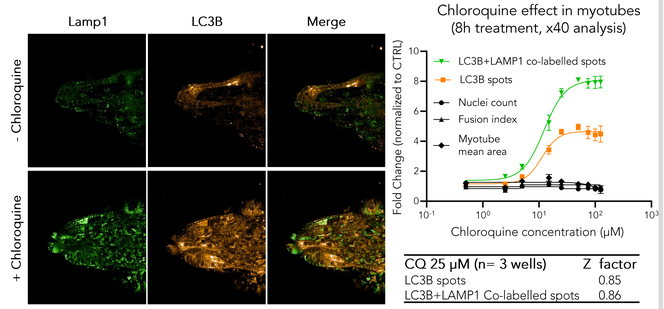
Concentration–response curves showing the stimulatory and inhibitory effects of hypertrophic (IGF-1) or atrophic (TNF-α) signaling compounds on morphological readouts such as fusion index (FI), myotube mean area, and nuclei count after 96h of treatment.

Protein synthesis assay provides additional information about the effect of compounds: here increasing doses of IGF-1 increases protein synthesis levels in myotubes.

Hypertrophy, atrophy and atrophy rescue assays can all be performed in combination with protein synthesis assays to deliver a more comprehensive overview of treatment effects.



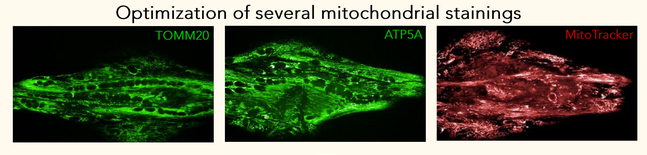
Measure autophagic flux precisely with high content quantification

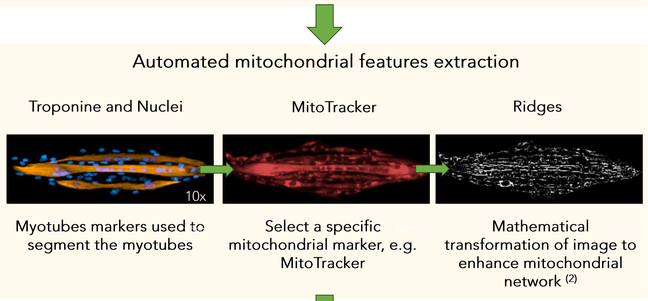


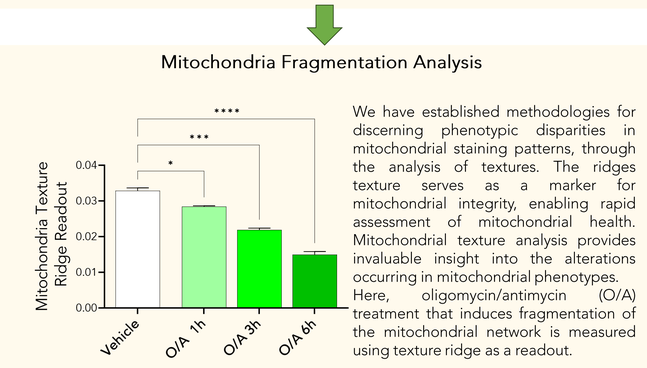
CQ 25 µM (n= 3 wells)	Z factor
LC3B spots	0.85
LC3B+LAMP1 Co-labelled spots	0.86

LC3B and LAMP1 staining can be used to measure autophagic vacuoles and monitor autophagic flux. Here, chloroquine has been used as an inhibitor control to visualize accumulation of autophagic vesicles. Abnormal autophagy activity and lysosomal dysfunction is one of the main hallmarks of metabolic disorders such as diabetes and obesity, as well as in skeletal muscle genetic disorders of metabolism such as Pompe disease.

Explore mitochondrial health through phenotypic screening

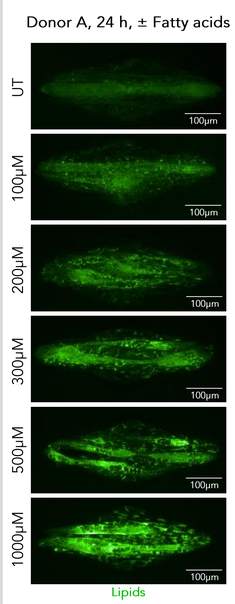


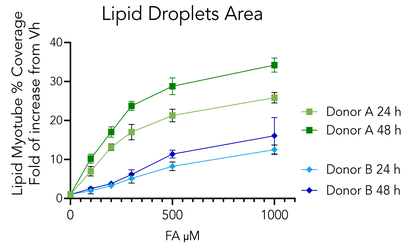




We have established methodologies for discerning phenotypic disparities in mitochondrial staining patterns, through the analysis of textures. The ridges texture serves as a marker for mitochondrial integrity, enabling rapid assessment of mitochondrial health. Mitochondrial texture analysis provides invaluable insight into the alterations occurring in mitochondrial phenotypes. Here, oligomycin/antimycin (O/A) treatment that induces fragmentation of the mitochondrial network is measured using texture ridge as a readout.

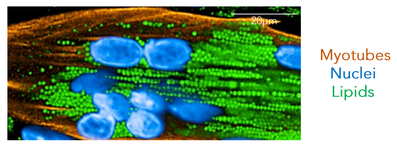
Recapitulate lipid uptake and measure lipid accumulation





Human skeletal muscle myotubes derived from 2 different patient donors were subjected to increasing concentrations of Fatty Acids (FA) added at different time points. Lipid accumulation can be measured through several readouts such as "% of myotube area covered by lipid" or "Mean fluorescent intensity" (data not shown).

Z factor for FA 300 µM at 24 h, Donor A = 0.56.



Structurally, high resolution images reveal intramyocellular lipid droplets organized in a specific linear pattern running parallel with the myotube. Such organization is suggestive of an association of lipid droplets with myofibrils.

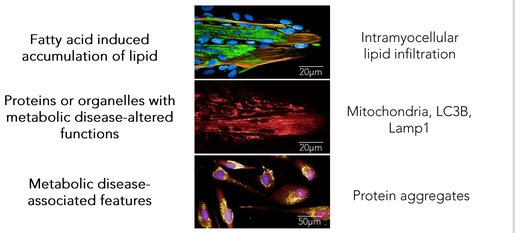
AI-Powered Image Analysis

AI-Powered Cell Profiling: monitor reversal of disease phenotypes upon treatment (3)

AI-powered cell profiling assays are robust, versatile and applicable to primary and immortalized cell lines. One key advantage of cell profiling is that phenotypic features do not require exact knowledge of the disease or of the therapy's mechanism of action.

A. Identify phenotypes that can distinguish
- Healthy versus inherent or induced diseased myotubes
- Myotubes grown in condition culture A versus B
- Myotubes with and without compound treatment

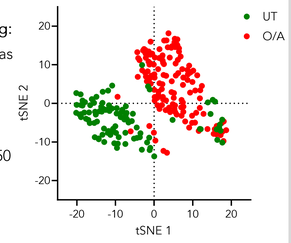
AI-powered cell profiling can be performed with diverse image markers



B. Profile Generation

Example for mitochondrial staining:
- Extract phenotypic features such as intensity, intensity distribution, granularity to characterize mitochondrial morphology
- Machine learning: myotubes (red and green dots in tSNE plot) are separated based on more than 250 phenotypical features.

Model Accuracy
Goal: F-score>0.95



C. Quantify the phenotypical response of healthy donors to a treatment or diseased cells to a specific therapy
- Analysis of treated healthy or disease donors
- Utilize profiles (features) established above

Conclusions

- CYTOO's MyoScreen platform enables the modeling of muscle disease phenotypes and the development of quantitative functional readouts of processes altered in skeletal muscle metabolic dysfunction
- All the muscle metabolism related assays presented are high-throughput compatible
- The robust screening capabilities of MyoScreen reflected in high Z-factor performance are expected to enhance success in target identification and primary screens
- The MyoScreen platform brings new prospects to metabolic disorders by facilitating a predictive research strategy for identifying and validating novel targets and supporting preclinical development to select innovative drug candidates targeting key features of muscle metabolic function

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References
[1]Young *et al.* 2018, [2]Cretin *et al.* 2021, [3] Hariharan, Lorintiu *et al.* 2023

Acknowledgments
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