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ELECTRICAL STIMULATION OF THE MYOSCREEN™ PLATFORM WITH THE FDSS µCELL/EFS: A NEW HIGH-THROUGHPUT SCREENING PLATFORM FOR EARLY MUSCLE DRUG DISCOVERY

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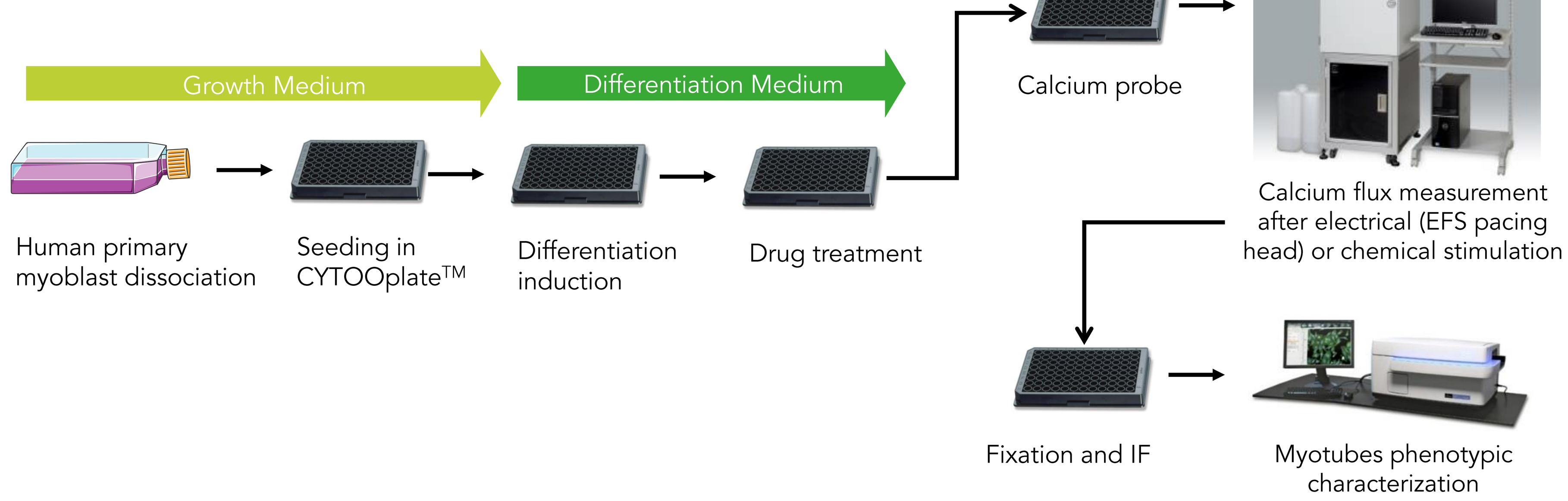
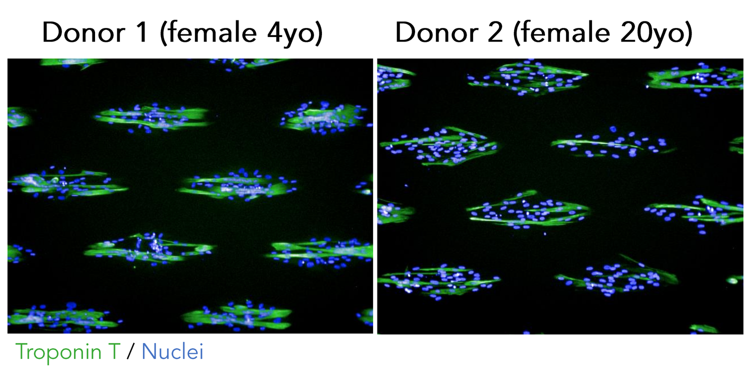
MYOSCREEN™, A VERSATILE R&D PLATFORM TO RUN MUSCLE DRUG DISCOVERY

CYTOO has developed **MyoScreen™**, a versatile high-throughput drug discovery engine that provides a physiological human *in vitro* model of skeletal muscle. The model relies on micropatterns that control the cellular microenvironment and thus guide and orientate the differentiation of human primary myoblasts. **MyoScreen™** myotubes have been shown to demonstrate characteristics of mature muscle, including typical striated myofibrils, acetylcholine receptor clustering and response to chemical contraction-inducing agents like acetylcholine and KCl.
Using the **FDSS/µCELL/EFS** from Hamamatsu **Photonics**, a response to electrical **field** stimulation of MyoScreen™ human healthy donors was observed by measuring intracellular calcium transients in real-time. Then, the functionality of the excitation-contraction coupling (ECC) machinery in MyoScreen™ myotubes was investigated. Finally, a novel stimulator of myofiber regeneration/repair, recently identified by CYTOO in an "in-house" drug screen, was further characterized on the FDSS-MyoScreen™ system.

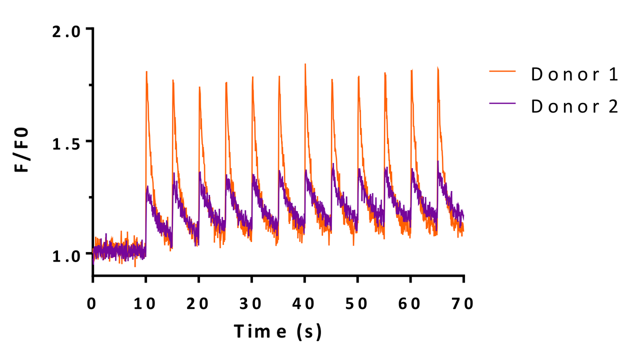
FULLY AUTOMATED SCREENING PROTOCOL



MYOTUBE RESPONSIVENESS TO ELECTRICAL STIMULATION

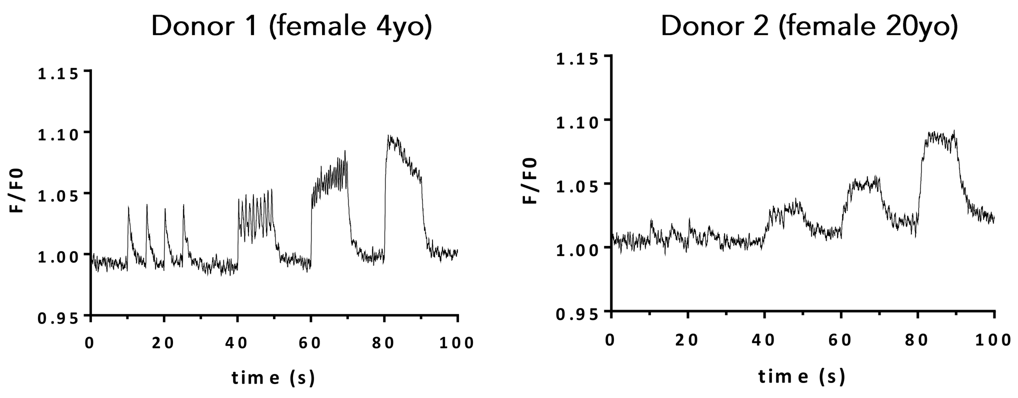


	Fusion index	Myotube Mean Area
Donor 1	0.38	14 190 µm²
Donor 2	0.28	11 141 µm²



Donors 1 and 2 myotubes differ in differentiation and morphological characteristics. Both donors respond to multi-pulses low frequency stimulation. Calcium flux is higher in Donor 1 compared to Donor 2.

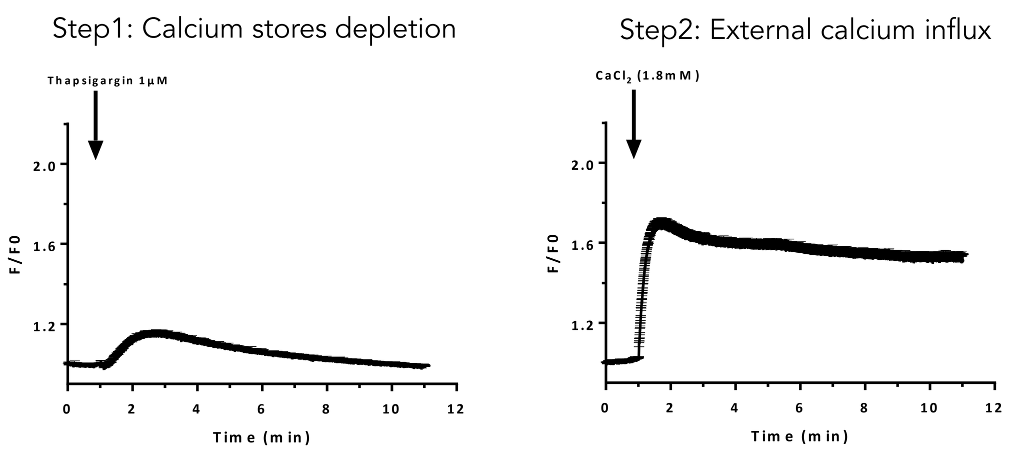
FROM TWITCH TO TETANUS STATES



Increasing stimuli frequency results in wave summation and finally complete tetanus reflecting *in vivo* phenomenon.

STORE OPERATED CALCIUM ENTRY DETECTION

SOCE corresponds to the influx of extracellular Ca^{2+} into cells subsequent to the depletion of internal to Ca^{2+} stores. This phenomenon can be disturbed in specific muscle disease which makes it an interesting readout to detect SOCE modulators.

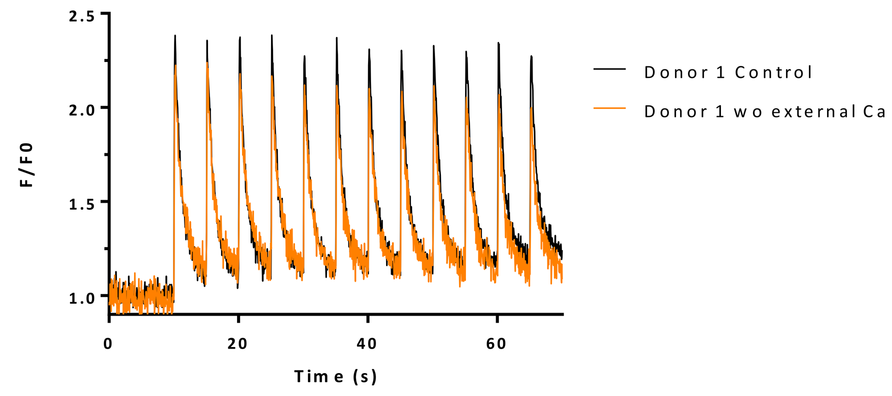


SOCE is observed in the MyoScreen™ model.
The FDSS equipment allows the detection of SOCE in a high throughput manner.

EXCITATION-CONTRACTION COUPLING CHARACTERIZATION

Influence of external calcium

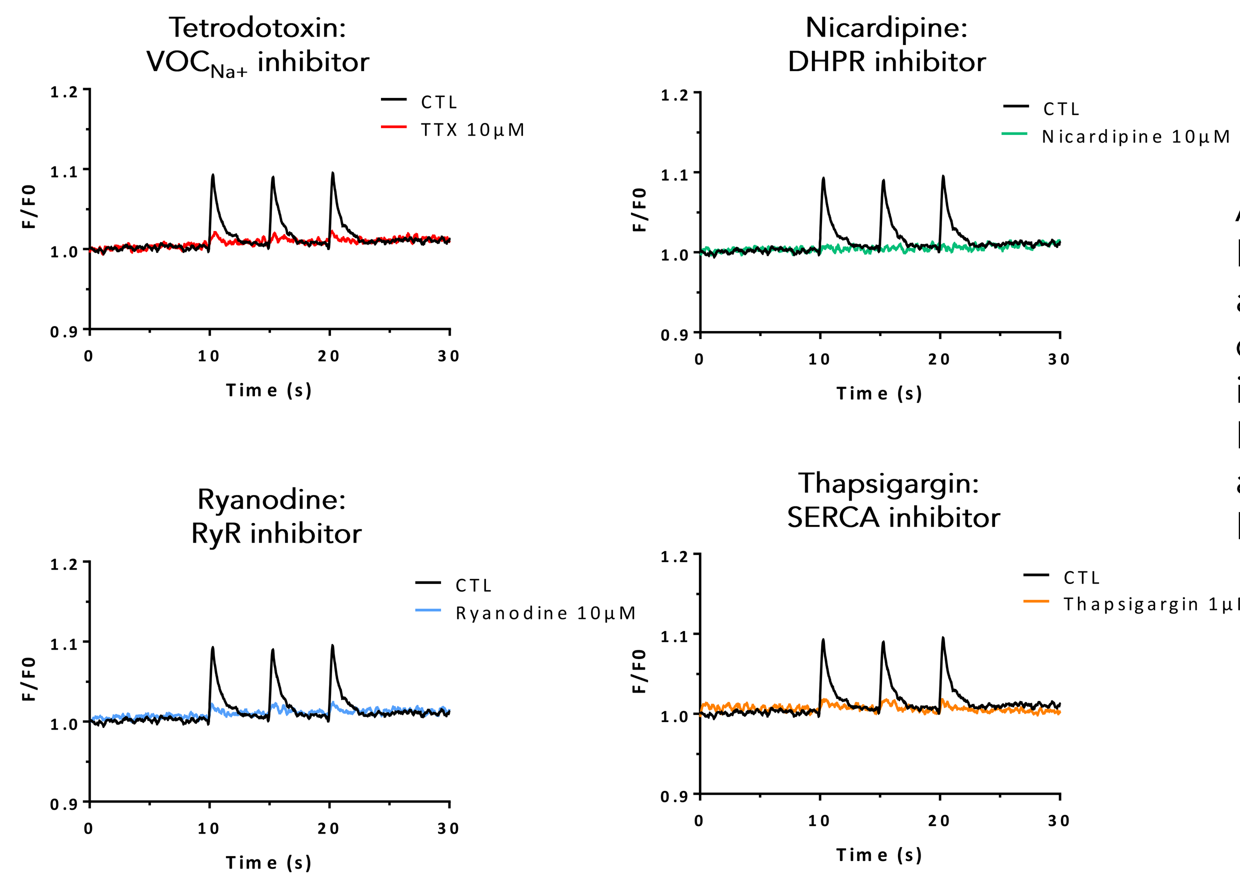
In order to check that the calcium release induced by electrical stimulation does not come from an external source, calcium flux were measured in presence or in absence of external calcium.



Electrical induced sarcoplasmic calcium release is observed in absence of external calcium, a mode of operation of the ECC apparatus specific to striated muscle.

EC-coupling characterization

Calcium release upon electrical stimulation was observed on pre-incubated myotubes with major EC-coupling components inhibitors in order to prove their functionality.



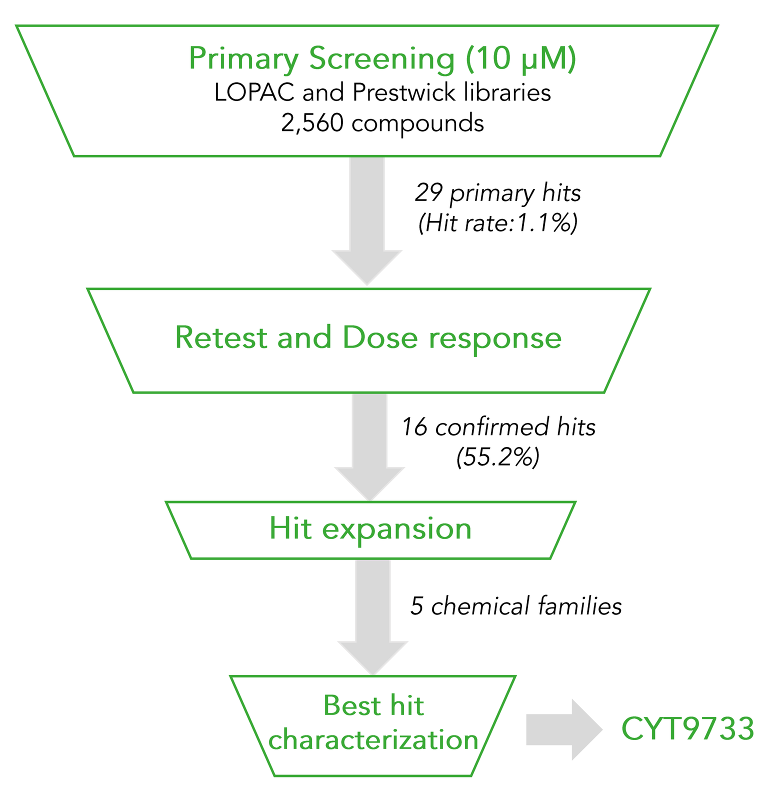
As expected Tetrodotoxin, Ryanodine, Thapsigargin and Nicardipine drastically decrease Ca^{2+} flux indicating that these major EC-coupling constituents are functional in MyoScreen™ myotubes.

SMALL MOLECULES PROMOTING MUSCLE REGENERATION IMPACT EFS INDUCED CALCIUM FLUX

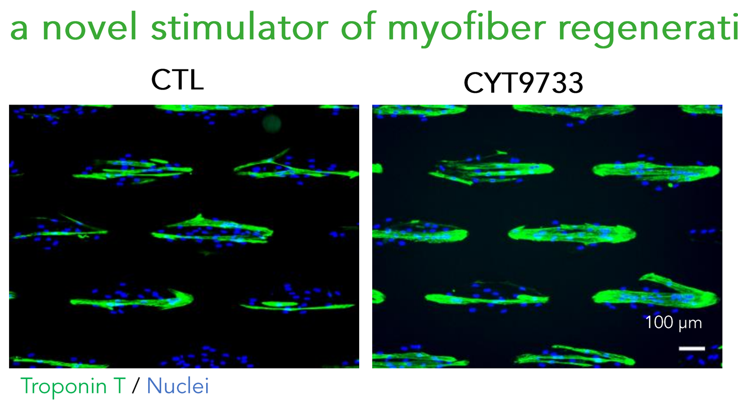
To find novel alternative compounds that promote healthy muscle regeneration/repair, a methodical screening strategy was undertaken: MyoScreen myotubes were exposed to 2560 FDA-approved drugs or well-defined pharmacologically active compounds. 29 primary hits molecules were identified. Follow-up retesting at expanded doses confirmed 16/29 of these compounds. These diverse chemicals grouped into 5 families based on molecular target and included novel and known pathways with links to muscle regeneration.

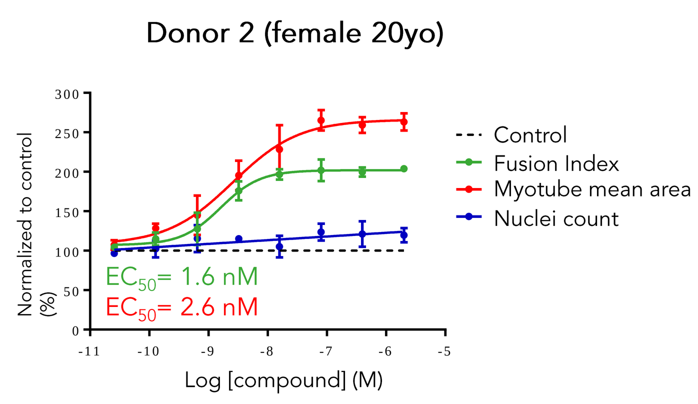
Among them CYT9733 was identified as a novel stimulator of myofiber regeneration/repair that increases myotube differentiation and size, acetylcholine receptors clustering, as well as calcium flux induced by acetylcholine. This compound was further characterized on the FDSS-MyoScreen™ system.

Screening overview



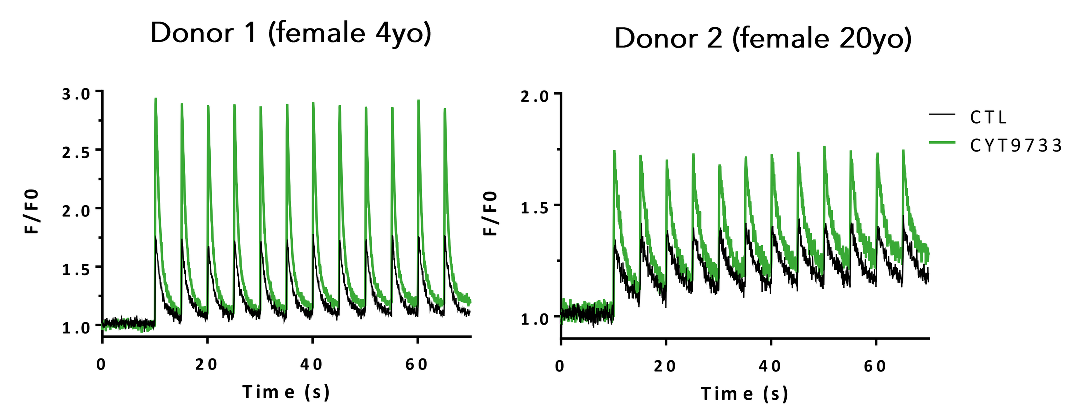
CYT9733 a novel stimulator of myofiber regeneration and repair





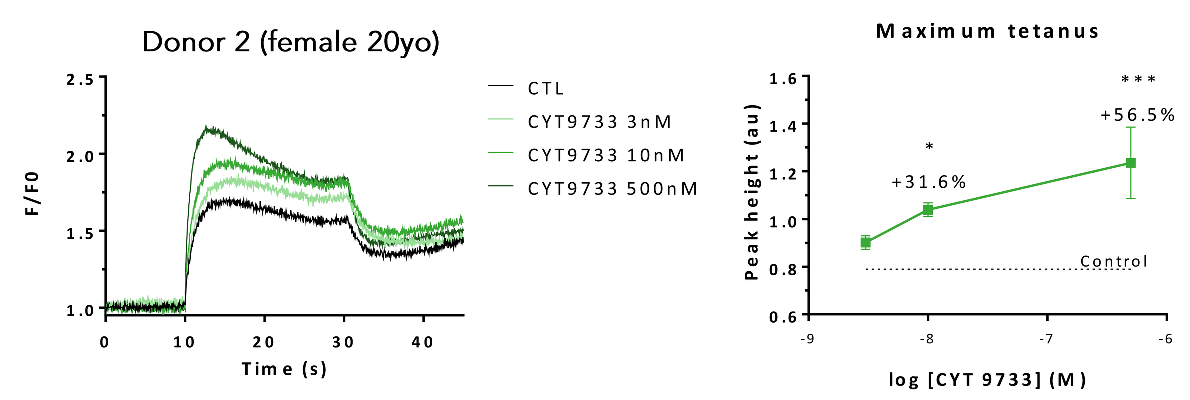
CYT9733 increases myotube differentiation and area in a dose-dependent manner without impacting the cell viability, indicating a positive impact on pathways associated with myogenesis.

CYT9733 impact on twitch stimulation response



CYT9733 highly increases calcium flux upon twitch stimulation in both donors, as well as upon tetanus stimulation in a dose-dependent way in Donor 2.

CYT9733 impact on tetanus stimulation response



CONCLUSION

- MyoScreen™ myotubes respond to single and multiple electrical stimulations induced by the Hamamatsu FDSS/µCELL/EFS in 96-well plates. Twitch and tetanus states can be modeled.
- Major components of the EC-coupling apparatus are functional in MyoScreen™ myotubes and can be easily assessed in a high throughput way thanks to the Hamamatsu FDSS/µCELL system.
- CYT9733, a novel stimulator of myofiber regeneration/repair, significantly increases calcium flux upon electrical stimulation.
- The Hamamatsu FDSS/µCELL electrical **field** stimulation and calcium imaging platform combined with the MyoScreen™ *in vitro* myotube model provides a relevant screening system for modulators of calcium levels in the context of skeletal muscle exercise and performance.

PHYSIOLOGICAL CELL-BASED ASSAYS FOR HCS

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