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PHYSIOLOGICAL ASSAYS FOR HCS

MYOSCREEN™, A DRUG DISCOVERY PLATFORM FOR DISEASE MODELING AND SCREENING OF COMPOUNDS RESTORING MUSCLE HEALTH

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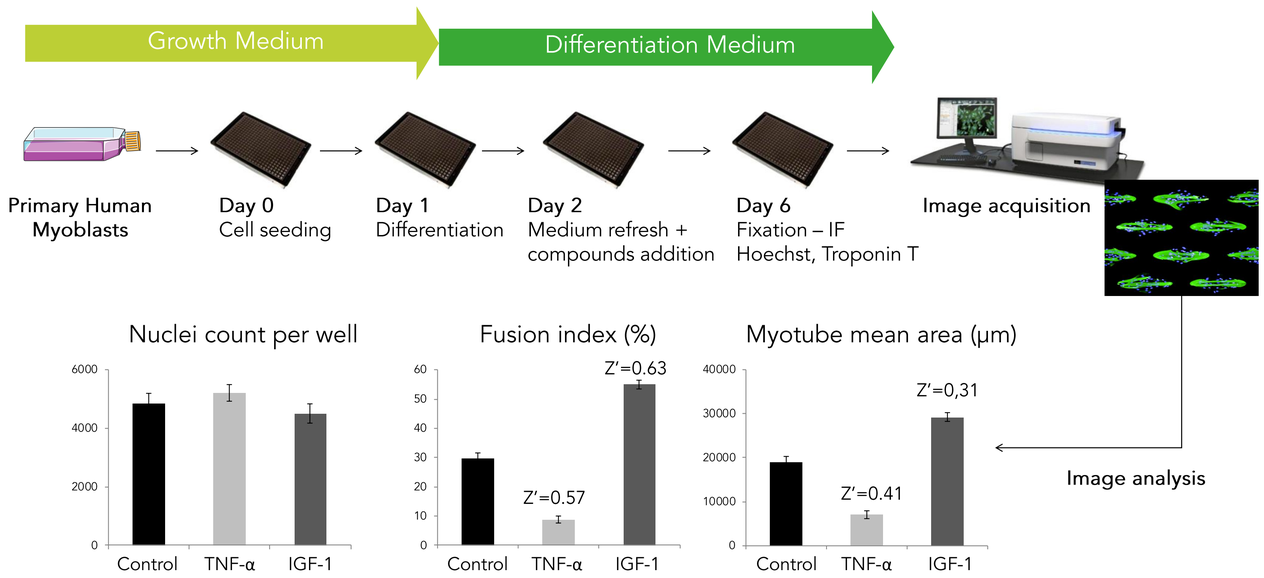
MYOSCREEN™, A VERSATILE R&D PLATFORM TO RUN MUSCLE DRUG DISCOVERY

BACKGROUND: Effective treatments are lacking for degenerative muscle disorders such as DMD. One barrier to finding better medicines is the reliance on *in vitro* muscle models and assays that lack physiology and pathological relevance.

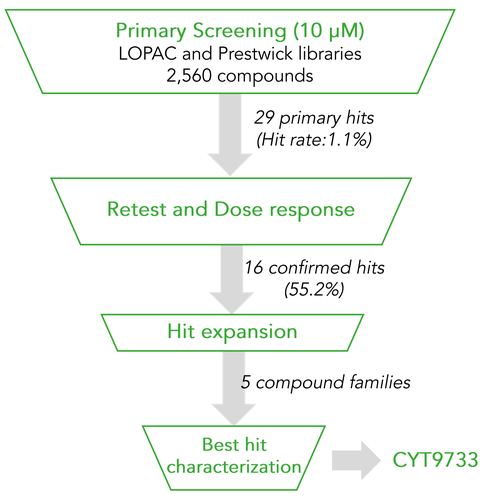
We recently reported **MyoScreen™** human skeletal myotubes that demonstrate sarcomeric organization, membrane-localized dystrophin and acetylcholine receptor (AChR) expression, response to chemical and electrical stimulation and pharmacologically relevant drug responses (SLAS Discov. 2018 23(8):790-806).

OBJECTIVES: Employ our extensive panel of patient derived myoblasts for disease modeling and assays pertinent to muscle biology such as myotube differentiation efficiency, AChR clustering, calcium homeostasis and contraction.

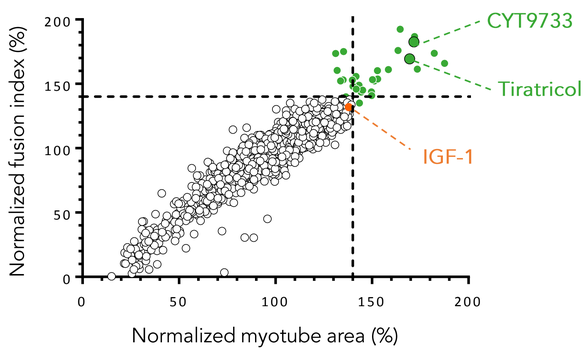
METHOD: A high-content screen for compounds that stimulate healthy myotube differentiation was performed. Hits were confirmed in several donors and potency determined in dose-response studies. Functional assays exploited calcium imaging techniques and the FDSS/µCELL from Hamamatsu for electrical stimulation.



PRIMARY SCREEN STRATEGY AND RESULTS

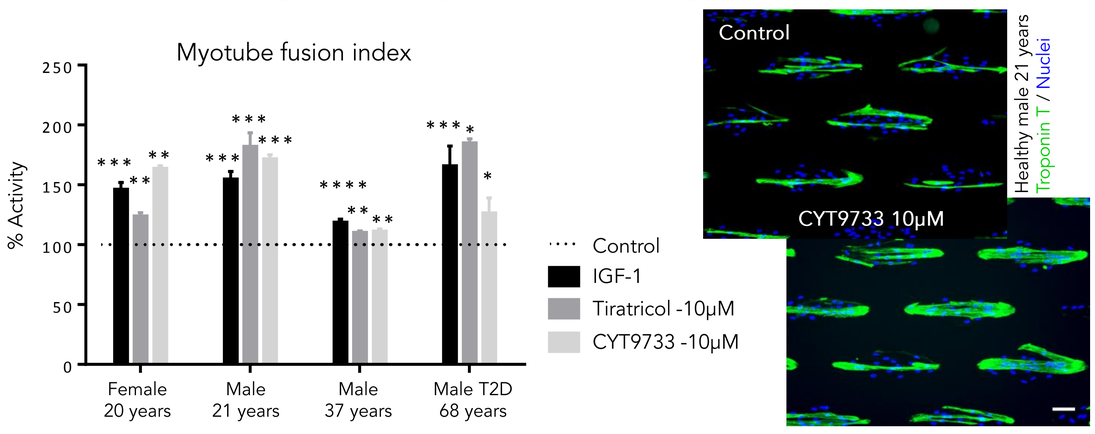


MyoScreen myotubes were exposed to 2560 FDA-approved or pharmacologically active compounds. Primary screening resulted in the identification of 29 hits that increased FI and/or myotube area to >+40% above the control mean.



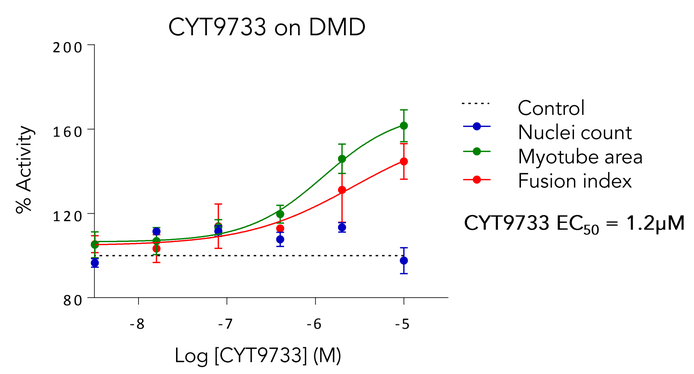
HITS CONFIRMATION ON HEALTHY DONORS

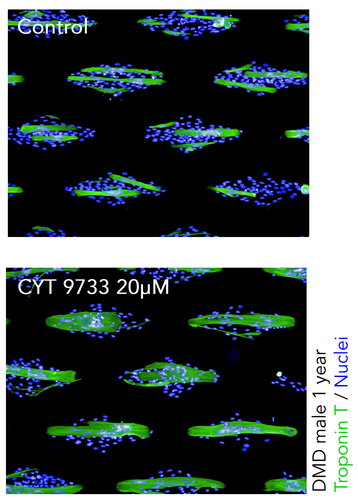
Follow-up retesting in additional healthy donors and at expanded doses confirmed 55% of compounds were dose-responsive. Hits families included novel and known pathways with links to muscle regeneration (thyroid hormone analogs).



CYT9733 REGENERATES DMD MYOTUBES

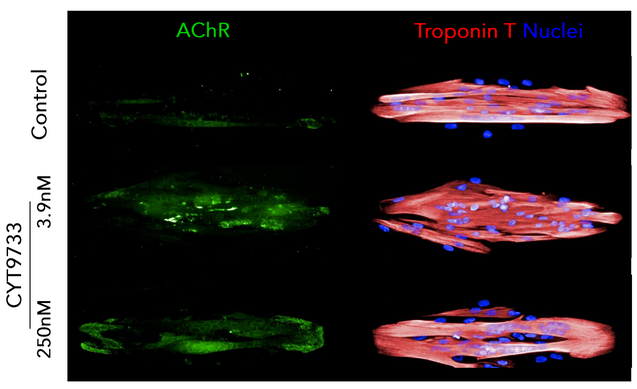
CYT9733 increases DMD myotube differentiation and size in a dose-dependent manner without negatively impacting cell viability, indicating a positive effect on pathways associated with muscle regeneration.

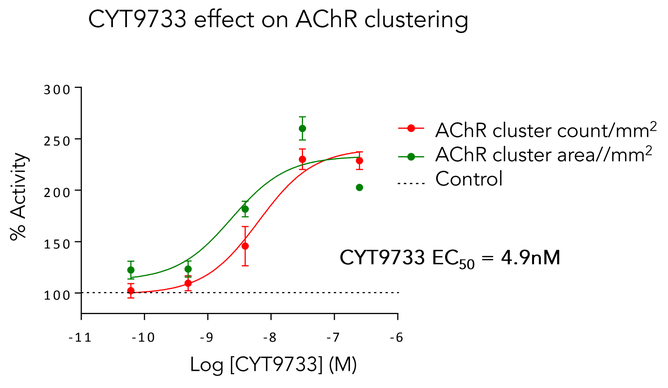




CYT9733 DRIVES ACHR EXPRESSION AND CLUSTERING

After 10 days of differentiation that included 9 days of drug treatment, healthy myotubes treated with a dose response of CYT9733 had more AChR clusters and total AChR area (increase by 2.5-fold at 250nM) compared to non-treated myotubes.

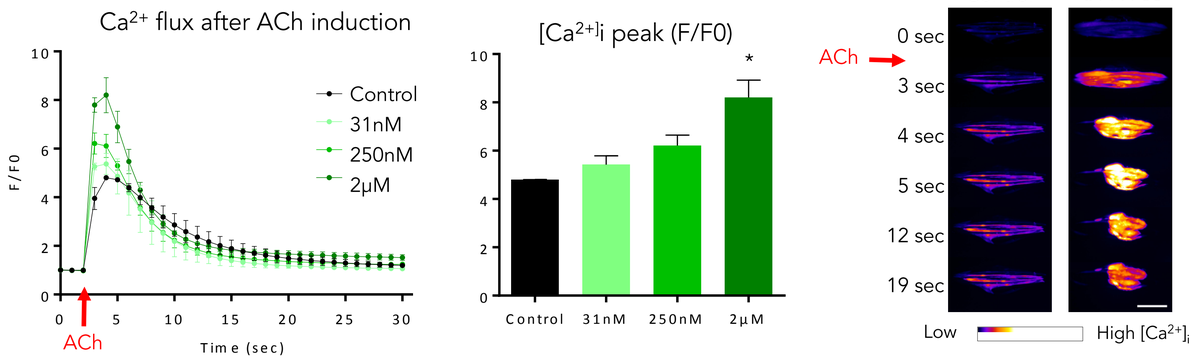




CYT9733 POTENTIATES MYOTUBE EXCITATION CONTRACTION COUPLING

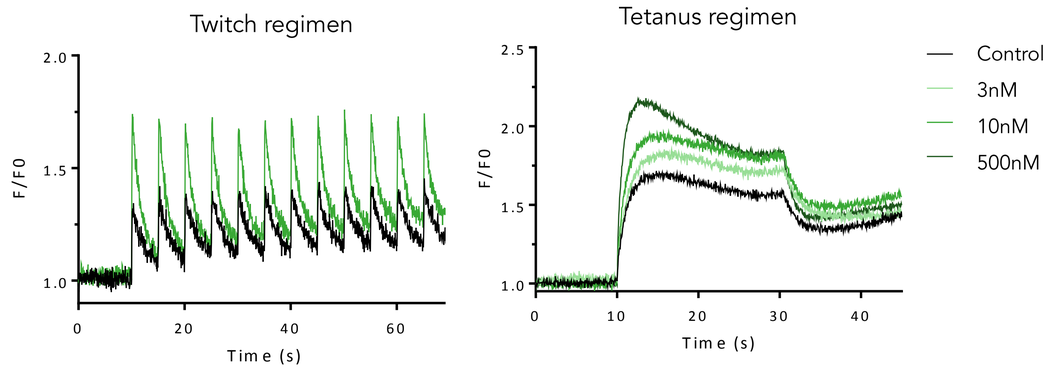
HIGHER INCREASE IN CALCIUM FLUX AND CONTRACTION AFTER ACH STIMULATION

- Myotubes differentiated in the presence of CYT9733 showed a statistically significant increase of 1.6-fold in intracellular [Ca^{2+}] compared to control myotubes after ACh stimulation
- Calcium flux time-lapse videos also implied that myotubes differentiated in the presence of CYT9733 displayed a marked increase in the number of retracting myotubes



CYT9733 INCREASES TWITCH & TETANUS RESPONSES

- MyoScreen™ myotubes respond to single and multiple electrical stimulations induced by the Hamamatsu FDSS/µCELL in 96-well plates
- CYT9733 increases calcium flux upon twitch and tetanus stimulations in a dose-dependent manner



CONCLUSIONS

MyoScreen™ is a robust and versatile high-throughput high-content screening (HT/HCS) platform that integrates a physiologically and pharmacologically relevant human primary skeletal muscle model with a panel of pertinent phenotypic and functional assays.

CYT9733 is a good candidate molecule for regulating changes in muscle regeneration phenotype and of further interest for preclinical testing. These results illustrate predictivity of this disease modeling and drug discovery platform, its capacity to select active compounds and provide early investigation of mechanisms of action involved.

PHYSIOLOGICAL CELL-BASED ASSAYS FOR HCS

This work was supported by Bpifrance through the ISI grant, ETICS

Electrical stimulation results were obtained in collaboration with HAMAMATSU PHOTON IS OUR BUSINESS

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