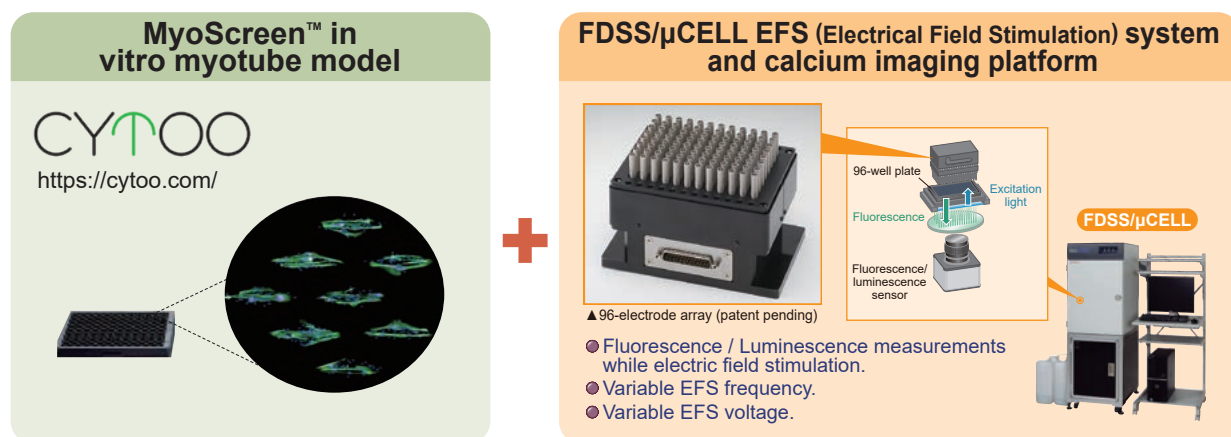


## A new HTS platform (96-well plate) for early muscle drug discovery

The FDSS/ $\mu$ CELL from Hamamatsu was used to measure intracellular calcium transients in real-time, the response to electrical stimulation of MyoScreen™, a patient-derived skeletal muscle in vitro high-throughput platform, as well as the functionality of the excitation-contraction coupling (ECC) machinery were evaluated (Fig. 1, 2, 3). 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. We found that the hit compound significantly increased levels of myoplasmic calcium compared to non-treated controls upon electrical stimulation (Fig. 4). Overall, the Hamamatsu FDSS/ $\mu$ CELL electrical 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.



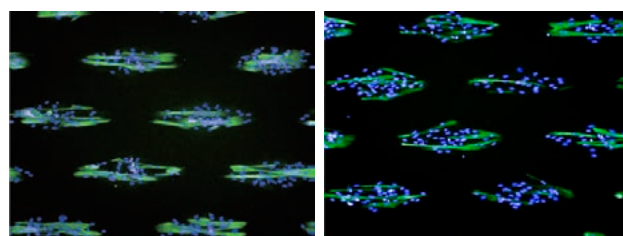
Hamamatsu FDSS/ $\mu$ CELL EFS system and calcium imaging platform combined with the MyoScreen™ in vitro myotube model

### Calcium flux of MyoScreen™ using the FDSS/ $\mu$ CELL 96 ch EFS

MyoScreen™ responsiveness to electrical stimulation (Fig. 1)

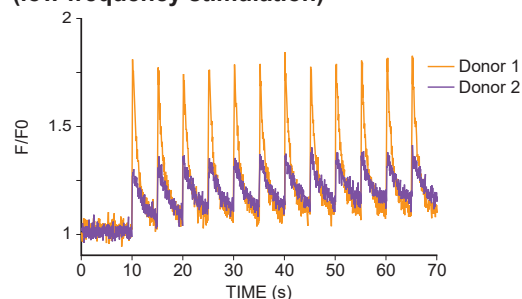
#### Captured images of MyoScreen™

\* Microscopy images offered by CYTOO.



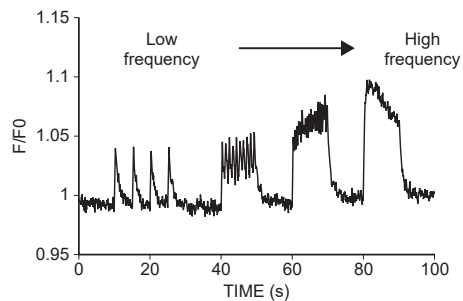
\* Green: Troponin T/ Blue: Nuclei

#### Calcium flux recording with EFS (low frequency stimulation)



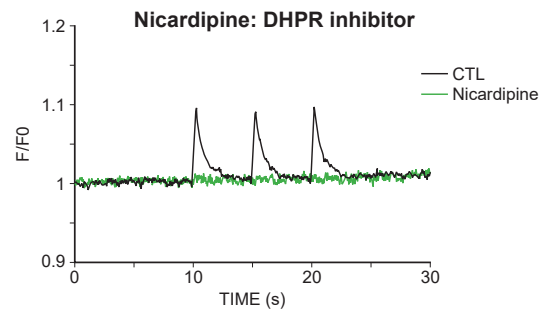
Responsiveness of two donors presenting different maturation stages were observed using the FDSS/ $\mu$ CELL system.

## From twitch to tetanus states (Fig. 2)



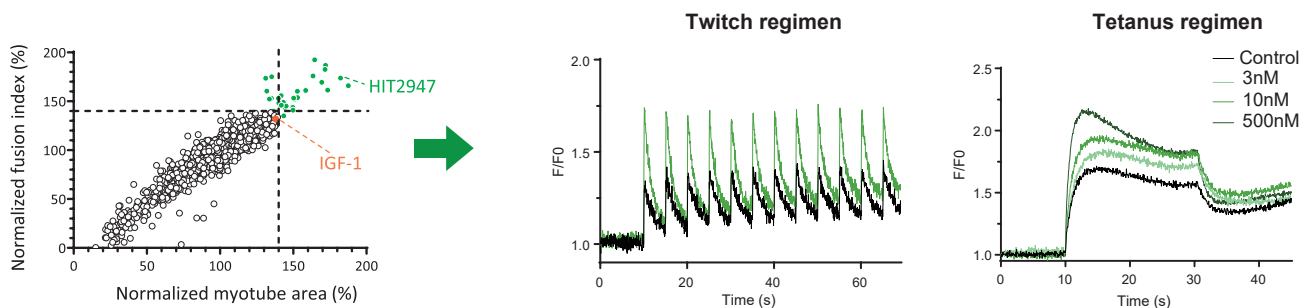
Increasing stimuli frequency results in wave summation and finally complete tetanus reflecting in vivo phenomenon.

## Screening of calcium flux modulators (Fig. 3)



Calcium flux modulators can be easily assessed in a high throughput way.

## MyoScreen™ drug screen hits identification and response to electrical stimulation (Fig. 4)



Out of 2560 pharmacologically active or FDA-approved drugs, 29 increased fusion index and/or myotube size by >40 %. Among them, a novel stimulator of myofiber regeneration/repair, HIT2947 was further characterized on the FDSS-MyoScreen™ system. We found that the hit compound significantly increased levels of myoplasmic calcium compared to non-treated controls upon electrical stimulation.

\* The FDSS/ $\mu$ CELL EFS system should not be used for optically detecting/monitoring change in transmembrane potential of the cells.

The FDSS/ $\mu$ CELL EFS system should not be used on any cell or cells in which the user or anyone else has expressed target ion channels.

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Systems Division

812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan, Telephone: (81)53-431-0124, Fax: (81)53-433-8031, E-mail: [export@sys.hpk.co.jp](mailto:export@sys.hpk.co.jp)

U.S.A.: Hamamatsu Corporation: 360 Foothill Road, Bridgewater, NJ 08807, U.S.A., Telephone: (1)908-231-0960, Fax: (1)908-231-1218 E-mail: [usa@hamamatsu.com](mailto:usa@hamamatsu.com)

Germany: Hamamatsu Photonics Deutschland GmbH: Arzbergerstr. 10, D-82211 Herrsching am Ammersee, Germany, Telephone: (49)8152-375-0, Fax: (49)8152-265-8 E-mail: [info@hamamatsu.de](mailto:info@hamamatsu.de)

France: Hamamatsu Photonics France S.A.R.L.: 19, Rue du Saule Trapu, Parc du Moulin de Massy, 91882 Massy Cedex, France, Telephone: (33)1 69 53 71 00, Fax: (33)1 69 53 71 10 E-mail: [infos@hamamatsu.fr](mailto:infos@hamamatsu.fr)

United Kingdom: Hamamatsu Photonics UK Limited: 2 Howard Court, 10 Tewin Road, Welwyn Garden City, Hertfordshire AL7 1BW, UK, Telephone: (44)1707-294888, Fax: (44)1707-325777 E-mail: [info@hamamatsu.co.uk](mailto:info@hamamatsu.co.uk)

North Europe: Hamamatsu Photonics Norden AB: Torshamnsgatan 35 16440 Kista, Sweden, Telephone: (46)8-509 031 00, Fax: (46)8-509 031 01 E-mail: [info@hamamatsu.se](mailto:info@hamamatsu.se)

Italy: Hamamatsu Photonics Italia S.r.l.: Strada della Moia, 1 int. 6, 20020 Arese (Milano), Italy, Telephone: (39)02-93 58 17 33, Fax: (39)02-93 58 17 41 E-mail: [info@hamamatsu.it](mailto:info@hamamatsu.it)

China: Hamamatsu Photonics (China) Co., Ltd.: 1201 Tower B, Jiaming Center, 27 Dongsanhuan Beilu, Chaoyang District, 100020 Beijing, P.R. China, Telephone: (86)10-6586-6006, Fax: (86)10-6586-2866 E-mail: [hpc@hamamatsu.com.cn](mailto:hpc@hamamatsu.com.cn)

Taiwan: Hamamatsu Photonics Taiwan Co., Ltd.: 8F-3, No.158, Section 2, Gongdao 5th Road, East District, Hsinchu, 300, Taiwan R.O.C. Telephone: (886)3-659-0080, Fax: (886)3-659-0081 E-mail: [info@hamamatsu.com.tw](mailto:info@hamamatsu.com.tw)