A human iPSC-based platform to screen therapeutics for ALS using specific and robust phenotypic assays covering disease-relevant readouts.

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Background

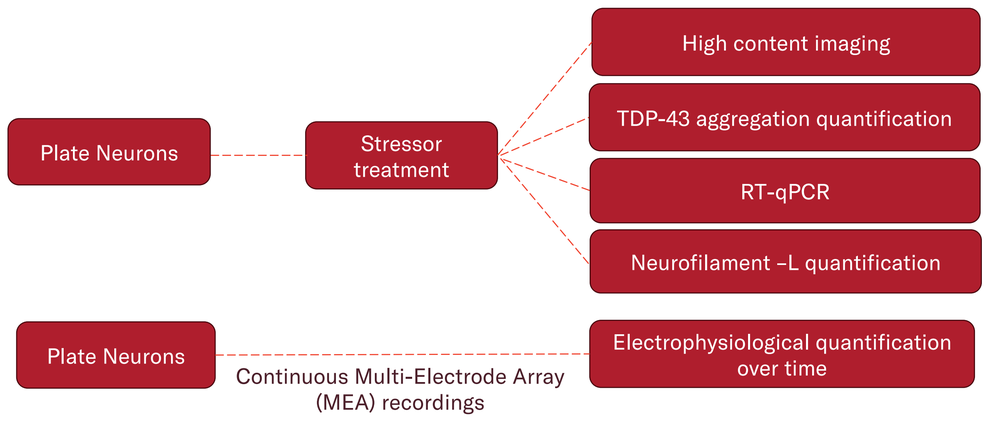
Amyotrophic lateral sclerosis (ALS) is a rare neurological disease that primarily affects the nerve cells (motor neurons) responsible for controlling voluntary muscle movement. ALS is characterized by stiff muscles, muscle twitching, and gradually worsening weakness due to muscles decreasing in size. This results in difficulty speaking, swallowing, and eventually breathing. Most of the cases present mis-localization and pathological aggregates of TDP-43 in the cytoplasm. Furthermore, mis-splicing of *STMN2* and subsequent reduction of STMN2 protein are also core phenotypes associated with ALS.

Ncardia developed relevant assays using human iPSC derived motor neurons (MNs) with a CRISPR engineered TDP-43 point mutation to quantify ALS phenotypes:

- Mis-localization of TDP-43 to the cytoplasm
- Aggregation of TDP-43
- Reduction of STMN2 protein levels
- Mis-splicing of *STMN2*
- Altered electrophysiological properties
- Neurofilament-L secretion

Methods

Ncardia employed hiPSC-derived MNs with a CRISPR engineered TDP-43 mutation* and assessed their ALS relevant phenotypes. All readouts were quantified after 2-3 weeks in culture.



* iCell® Motor Neurons, ALS TDP43, 01279 and iCell® Motor Neurons, 01279 from FUJIFILM Cellular Dynamics, Inc

Mislocalization of TDP-43 to the cytoplasm and pathological TDP-43 aggregate formation

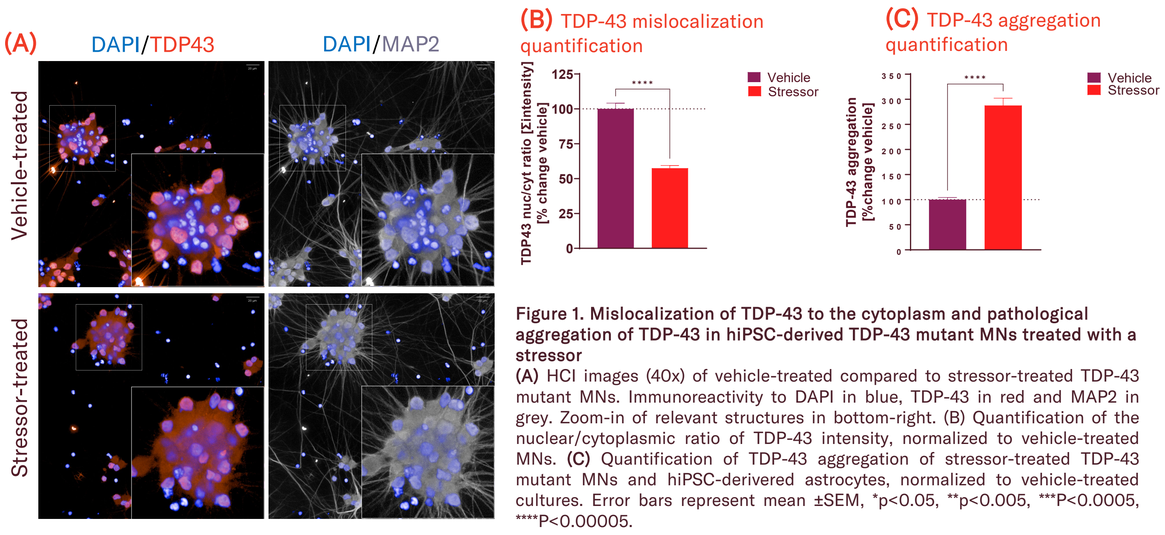


Figure 1. Mislocalization of TDP-43 to the cytoplasm and pathological aggregation of TDP-43 in hiPSC-derived TDP-43 mutant MNs treated with a stressor
(A) HCI images (40x) of vehicle-treated compared to stressor-treated TDP-43 mutant MNs. Immunoreactivity to DAPI in blue, TDP-43 in red and MAP2 in grey. Zoom-in of relevant structures in bottom-right. (B) Quantification of the nuclear/cytoplasmic ratio of TDP-43 intensity, normalized to vehicle-treated MNs. **(C)** Quantification of TDP-43 aggregation of stressor-treated TDP-43 mutant MNs and hiPSC-derivered astrocytes, normalized to vehicle-treated cultures. Error bars represent mean ±SEM, *p<0.05, **p<0.005, ***P<0.0005, ****P<0.00005.

Quantifiable reduction of STMN2 and mis-splicing of *STMN2*

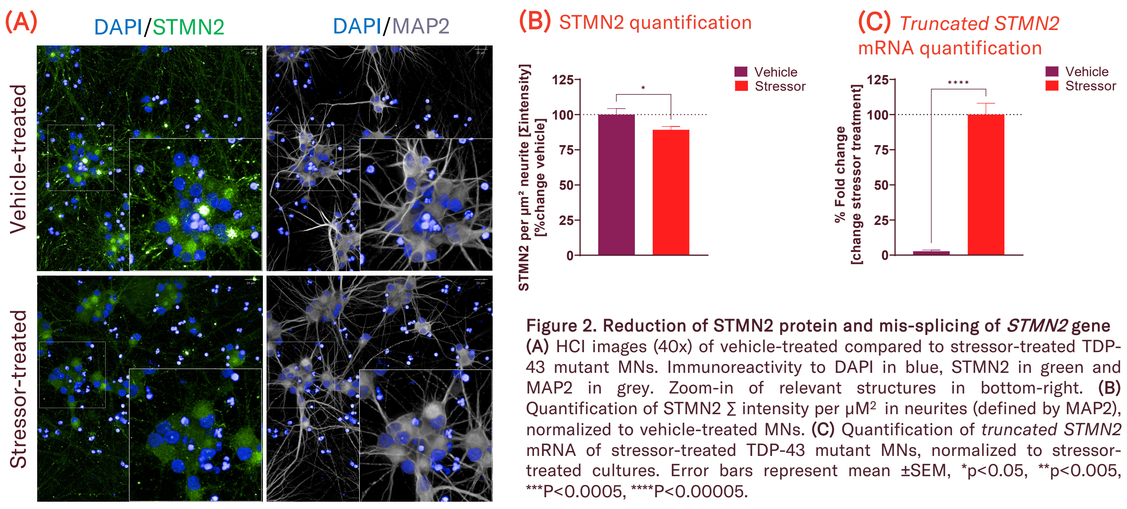


Figure 2. Reduction of STMN2 protein and mis-splicing of *STMN2* gene
(A) HCI images (40x) of vehicle-treated compared to stressor-treated TDP-43 mutant MNs. Immunoreactivity to DAPI in blue, STMN2 in green and MAP2 in grey. Zoom-in of relevant structures in bottom-right. **(B)** Quantification of STMN2 ∑ intensity per µM² in neurites (defined by MAP2), normalized to vehicle-treated MNs. **(C)** Quantification of *truncated STMN2* mRNA of stressor-treated TDP-43 mutant MNs, normalized to stressor-treated cultures. Error bars represent mean ±SEM, *p<0.05, **p<0.005, ***P<0.0005, ****P<0.00005.

Altered electrophysiological properties of TDP-43 MNs in complex coculture with astrocytes

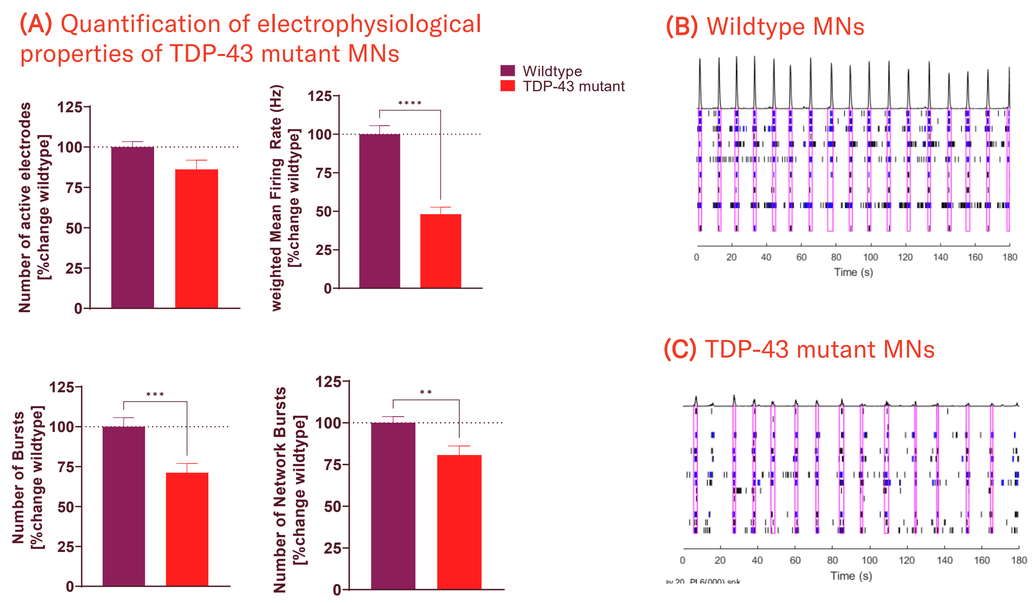


Figure 3. Electrophysiological properties of wildtype and TDP-43 mutant MNs in coculture with hiPSC-derived astrocytes
(A) Quantification of electrophysiological activity of wildtype and TDP-43 mutant MNs. Including number of active electrodes, mean firing rate (Hz), number of bursts and network bursts. **(B)** Representative raster plots of multi-electrode array (MEA) recordings of wildtype MNs in coculture with hiPSC-derived astrocytes. Black lines indicate spikes, blue boxes indicate bursts and network bursts are indicated by pink boxes. Each row represents 1 electrode (total 16 electrodes). **(C)** Representative raster plots of MEA recordings of TDP-43 mutant MNs in coculture with hiPSC-derived astrocytes. Black lines indicate spikes, blue boxes indicate bursts and network bursts are indicated by pink boxes. Each row represents 1 electrode (total 16 electrodes). Error bars represent mean ±SEM, *p<0.05, **p<0.005, ***P<0.0005, ****P<0.00005.

Neurofilament-L release in TDP-43 mutant MNs

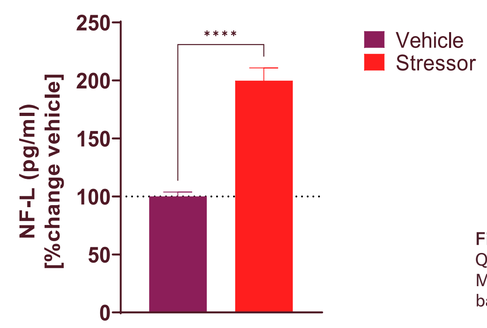


Figure 4. Quantification of neurofilament-L release of TDP-43 mutant MNs
Quantification of Neurofilament-L (NF-L) in supernatants of TDP-43 mutant MNs treated with a stressor normalized to vehicle-treated TDP-43 MNs. Error bars represent mean ±SEM, *p<0.05, **p<0.005, ***P<0.0005, ****P<0.00005.

Succesful transduction of TDP-43 mutant MNs & astrocyte coculture with gene therapy and subsequent reduction of TDP-43 aggregation

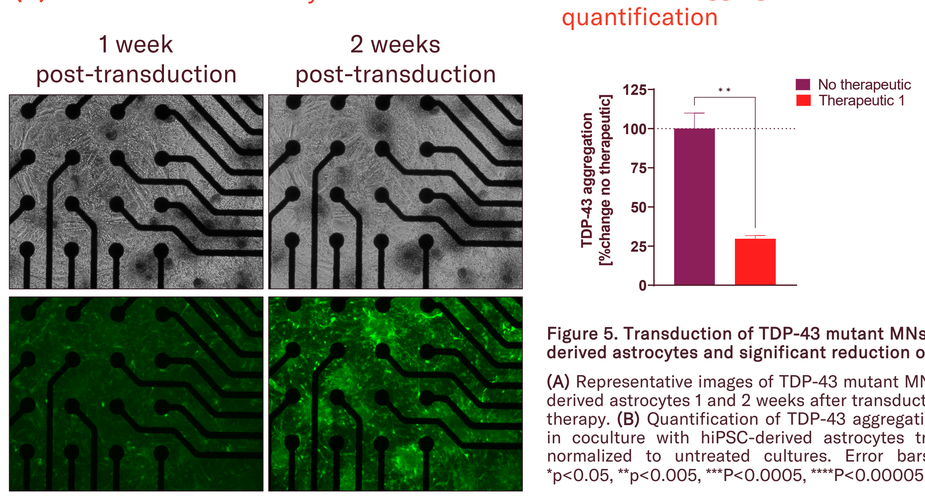


Figure 5. Transduction of TDP-43 mutant MNs in coculture with hiPSC-derived astrocytes and significant reduction of TDP-43 aggregation
(A) Representative images of TDP-43 mutant MNs in coculture with hiPSC-derived astrocytes 1 and 2 weeks after transduction with GFP carrying gene therapy. **(B)** Quantification of TDP-43 aggregation in TDP-43 mutant MNS in coculture with hiPSC-derived astrocytes treated with gene therapy, normalized to untreated cultures. Error bars represent mean ±SEM, *p<0.05, **p<0.005, ***P<0.0005, ****P<0.00005.

Conclusions

- We demonstrate quantifiable TDP-43 mislocalization and STMN2 protein reduction by high-throughput high-content imaging and miniaturized assays. Furthermore, this model exhibits other relevant hallmarks of ALS, such as quantifiable TDP-43 aggregation, *STMN2* mis-splicing, neurofilament-L secretion and electrophysiological deficits as measured by MEA.

- We present a human in vitro model amenable for evaluation of new therapeutic candidates. The cultures were successfully transduced and when treated with a gene therapy, Ncardia was able to significantly reduce TDP-43 aggregation as compared to non-treated cultures.

Comprehensive physiologically-relevant platform, with hiPSC-based miniaturized assays, for screening of new ALS therapeutics!

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