# A human iPSC-based platform to screen therapeutics for ALS using assays covering disease- relevant readouts.

Benoit Samson-Couterie, Carola van Berkel, Leo Smit, Jessica Koepke, Shushant Jain
Ncardia Services BV, the Netherlands, www.ncardia.com

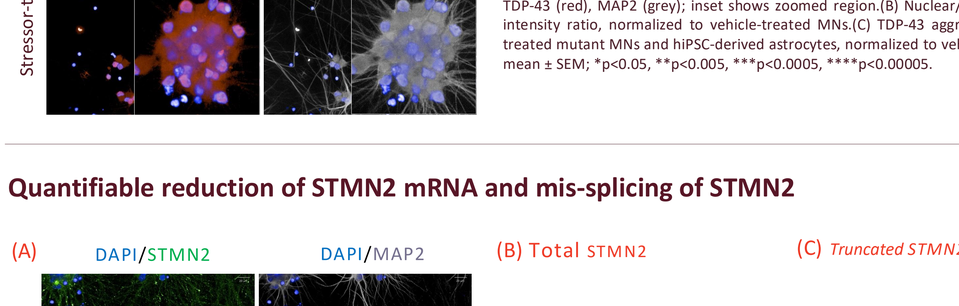
## Background

Amyotrophic lateral sclerosis (ALS) is a rare and progressive neurodegenerative disorder that primarily affects motor neurons—the nerve cells responsible for controlling voluntary muscle movement. Clinically, patients experience increasing difficulty with speaking, swallowing, and mobility, and in advanced stages, respiratory failure due to loss of breathing muscle function.
At the molecular level, most ALS cases are characterized by the mis-localization and pathological aggregation of the RNA-binding protein TDP-43 from the nucleus to the cytoplasm, where it forms insoluble inclusions. This disruption of normal TDP-43 function contributes to widespread defects in RNA processing. Among these, aberrant splicing and reduced expression of STMN2—a protein critical for axonal maintenance and neuronal repair—have emerged as key disease-associated phenotypes and biomarkers of TDP-43 dysfunction.
Here, we demonstrate how human-relevant cellular assays can model these hallmark features of ALS pathology, including TDP-43 mis-localization, aggregation, and STMN2 dysregulation. These platforms enable mechanistic studies and provide quantitative, translational tools to evaluate whether candidate therapies can rescue multiple aspects of disease pathogenesis, thereby supporting more predictive drug discovery efforts.
Ncardia developed relevant assays using human iPSC derived motor neurons (MNs) with a 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.




## Mislocalization of TDP-43 to the cytoplasm and TDP-43 aggregation

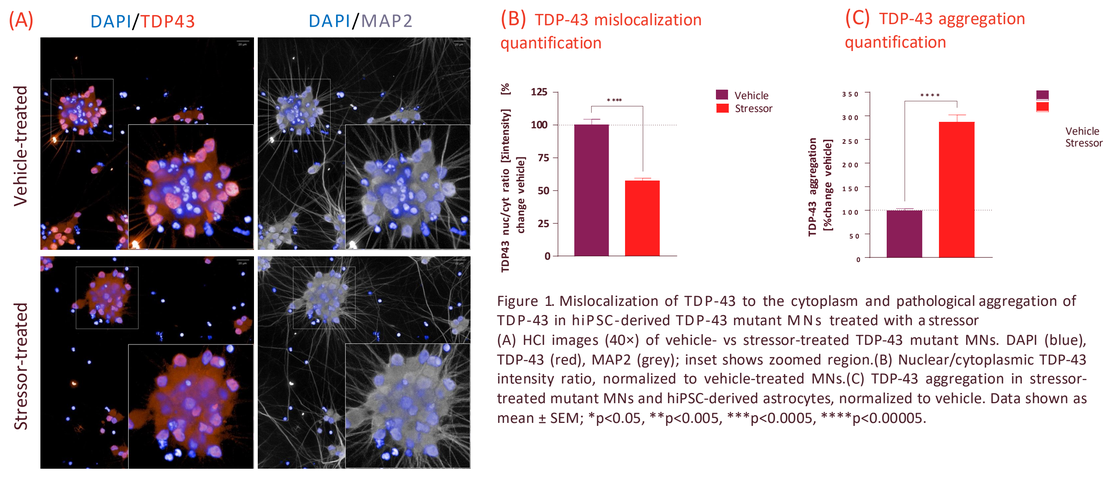



Figure 1. Mislocalization of TDP-43 to the cytoplasm and pathological aggregation of TDP-43 in hiPSC-derived TDP-43 mutant MNs treated with a stressor
(A) HCI images (40×) of vehicle- vs stressor-treated TDP-43 mutant MNs. DAPI (blue), TDP-43 (red), MAP2 (grey); inset shows zoomed region.(B) Nuclear/cytoplasmic TDP-43 intensity ratio, normalized to vehicle-treated MNs.(C) TDP-43 aggregation in stressor-treated mutant MNs and hiPSC-derived astrocytes, normalized to vehicle. Data shown as mean ± SEM; *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005.

## Quantifiable reduction of STMN2 mRNA and mis-splicing of STMN2

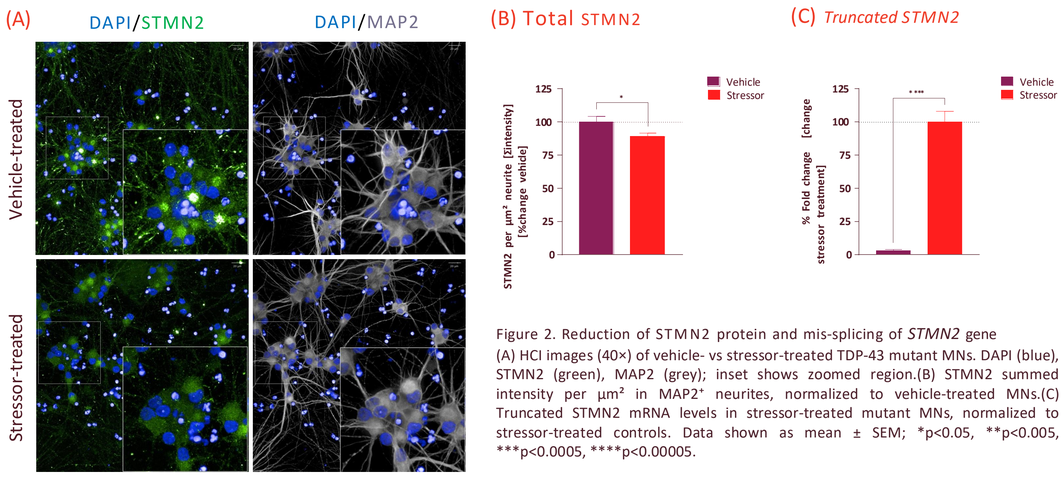



Figure 2. Reduction of STMN2 protein and mis-splicing of *STMN2* gene
(A) HCI images (40×) of vehicle- vs stressor-treated TDP-43 mutant MNs. DAPI (blue), STMN2 (green), MAP2 (grey); inset shows zoomed region.(B) STMN2 summed intensity per µm² in MAP2⁺ neurites, normalized to vehicle-treated MNs.(C) Truncated STMN2 mRNA levels in stressor-treated mutant MNs, normalized to stressor-treated controls. Data shown as mean ± SEM; *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005.

## Altered electrophysiological properties of TDP-43 MNs

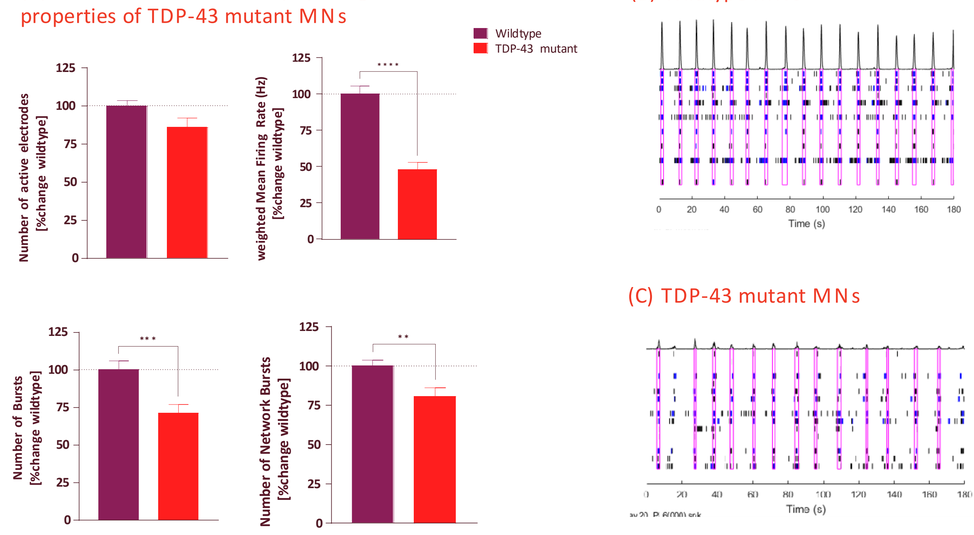



Figure 3. Electrophysiological properties of wildtype and TDP-43 mutant MNs in coculture with hiPSC-derived astrocytes
(A) Quantification of electrophysiological activity in wildtype and TDP-43 mutant MNs, including active electrodes, mean firing rate (Hz), bursts, and network bursts.(B–C) Representative MEA raster plots of wildtype (B) and TDP-43 mutant (C) MNs co-cultured with hiPSC-derived astrocytes. Black lines = spikes, blue boxes = bursts, pink boxes = network bursts; each row represents one electrode (16 total).Data shown as mean ± SEM; *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005.

## Small molecule rescue of TDP-43 aggregation and electrophysiological deficits

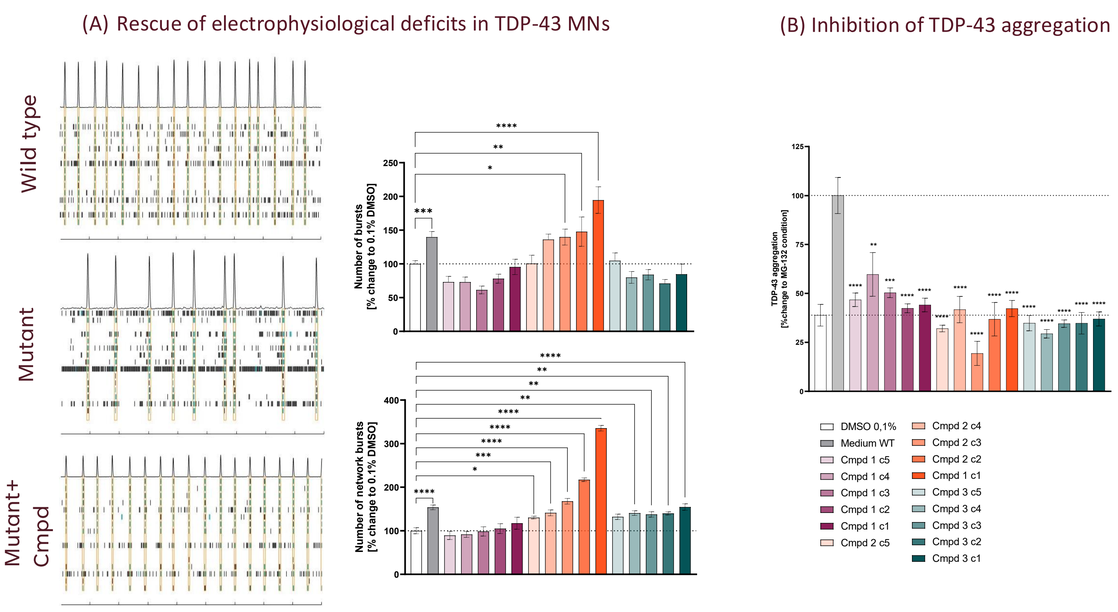



Figure 4. MEA assessment of network activity in wildtype and TDP-43 mutant motor neurons (MNs).
(A) Representative MEA raster plots of MNs co-cultured with hiPSC-derived astrocytes. Each row represents one electrode (16 total). Black ticks = spikes, blue boxes = bursts, yellow boxes = network bursts. Shown are wildtype, TDP-43 mutant, and rescue/treated conditions. Quantification of active electrodes, mean firing rate (Hz), bursts, and network bursts. Data are presented as mean ± SD; *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005.
(B) Quantification of TDP-43 aggregation shown as % change relative to control. Mutant MNs display increased aggregation, which is reduced by treatment. Data are mean ± SEM; **p<0.01, ***p<0.001, ****p<0.0001 vs control.

## Succesful transduction of TDP-43 mutant MNs & astrocyte coculture subsequent decrease of TDP-43 aggregation

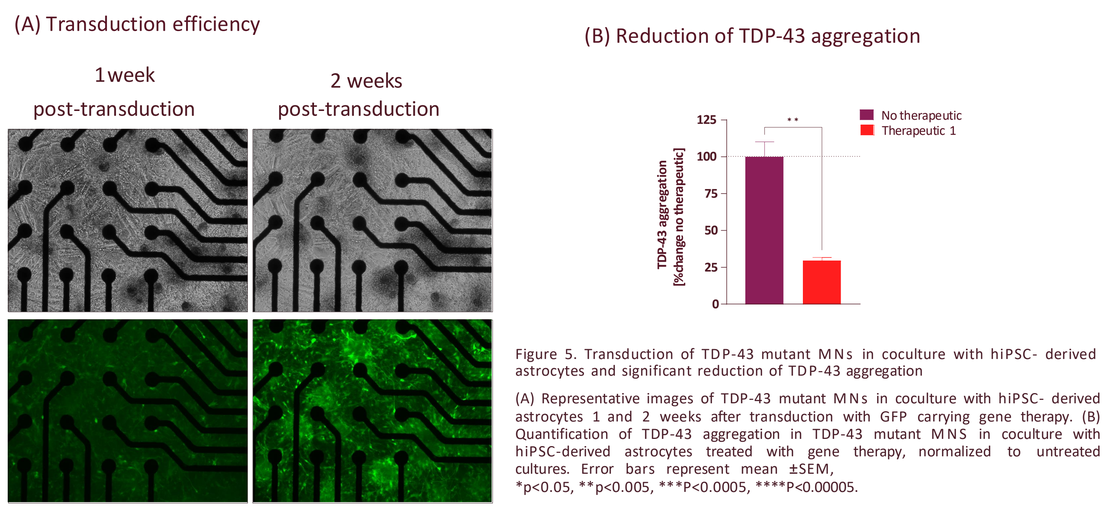



Figure 5. Transduction of TDP-43 mutant MNs in coculture with hiPSC- derived astrocytes and significant reduction of TDP-43 aggregation

(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 ALS therapeutics

Ncardia Services B.V.

Contact us at support@ncardia.com or scan the QR code to download the poster