A High-Throughput Contractility Assay for Human Cardiac Spheroids: a Translational Platform for Cardiomyopathy and Drug Discovery

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SUMMARY

- Contractile dysfunction is a hallmark of **cardiomyopathies** that can be studied with hPSC-derived cardioids
- A **video-based method** was developed to produce pixel displacement, **contraction, velocity, and beat frequency measurements** that can be used as proxies for **force readouts**
- We validated this model using **pacing frequency**, **acute isoproterenol,** and **chronic doxorubicin**
- We applied this method to a **compound library** and found novel drugs which increase contractility
- We applied this method to validate a **panel of genes** predicted to increase contractility by the **ML model GeneFormer** and found key genes that increase contractility
- We were able to recapitulate the abnormal contractile function in a **DCM model of titinopathy**.
- This method meets the increasing demand for **high-throughput, large-scale, functional validation** in the study of cardiomyopathies at a **fraction of the cost** of current technologies

MODEL DEVELOPMENT

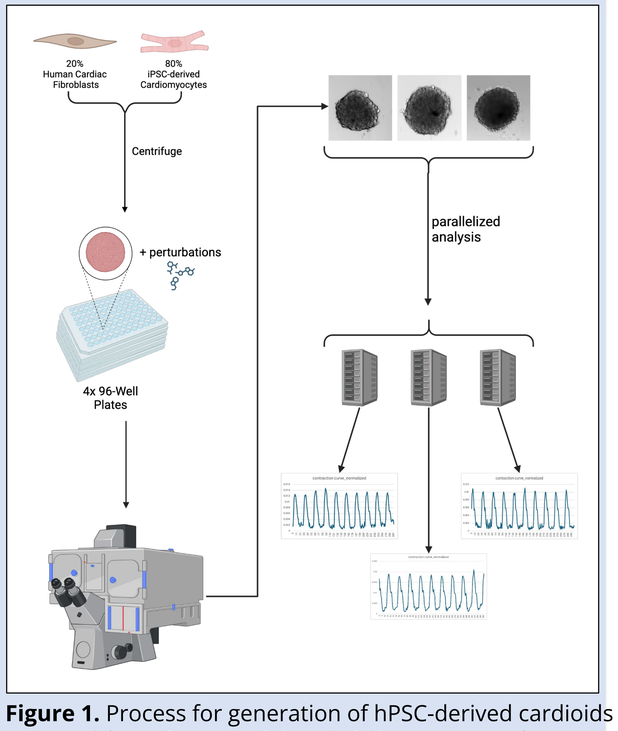

Figure 1. Process for generation of hPSC-derived cardioids (adapted from Campostrini et al. 2021), capture of cardioid videos, steps of parallelized video-based analysis, and expected contractility curve outputs.

MODEL VALIDATION

Contractility measurements recapitulate responses to known treatments

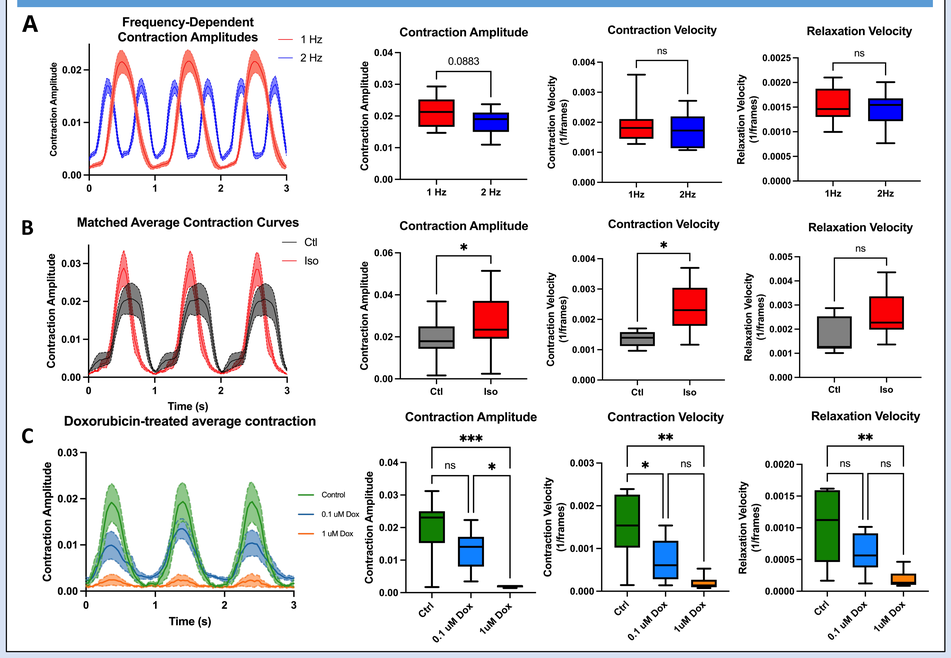

Figure 2. (A) Comparison of amplitude, contraction and relaxation velocity between 1Hz and 2Hz-paced cardioids. **(B)** Comparison of amplitude, contraction and relaxation velocity between control and 1 μM Isoproterenol treated cardioids. **(C)** Comparison of amplitude, contraction and relaxation velocities in doxorubicin treated cardioids.

Functional compound screen identifies novel drug that increases contractility

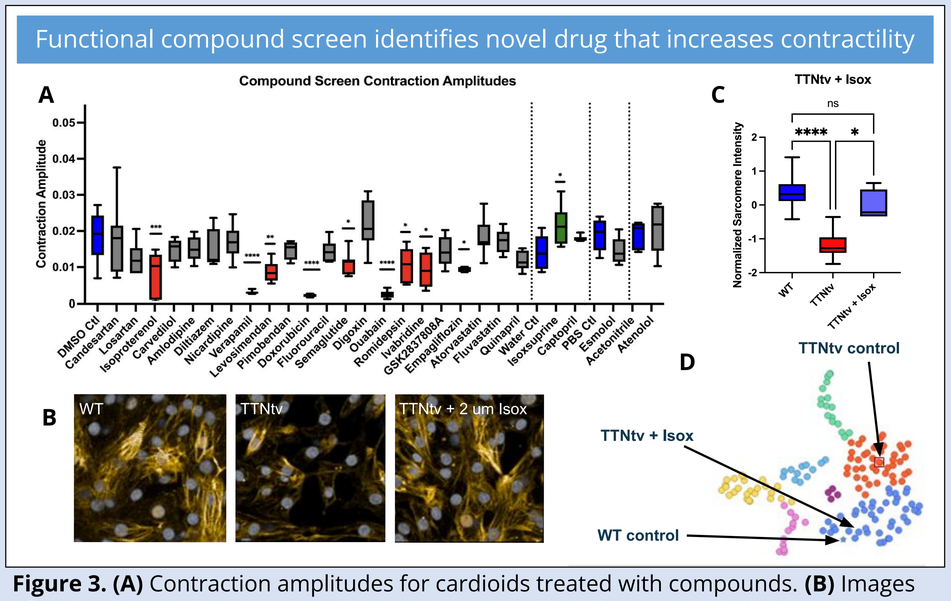

Figure 3. (A) Contraction amplitudes for cardioids treated with compounds. **(B)** Images of WT, TTNtv, and TTNtv + 2 μM isoxsuprine treated cardiomyocytes. **(C)** Normalized sarcomere intensity of WT, TTNtv, and TTNtv + 5 μM isoxsuprine-treated cardiomyocytes. **(D)** UMAP of TTNtv cardiomyocytes treated with compounds.

TTNtv cardioids have reduced contractility

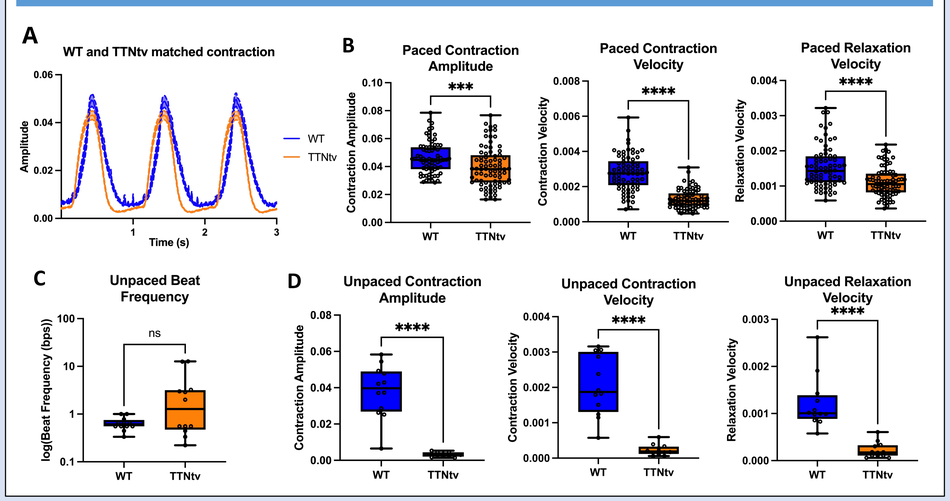

Figure 4. (A) Pacematched, average contraction curves of WT and TTNtv cardioids. **(B)** Relative amplitude, contraction velocity, and relaxation velocity of paced WT and TTNtv cardioids, $n \geq 74$. **(C)** Beat frequency of unpaced WT and TTNtv cardioids, $n=12$. **(D)** Relative amplitude, contraction velocity, and relaxation velocity of unpaced WT and TTNtv cardioids, $n=12$.

Cardioids capture gene knockdown effects

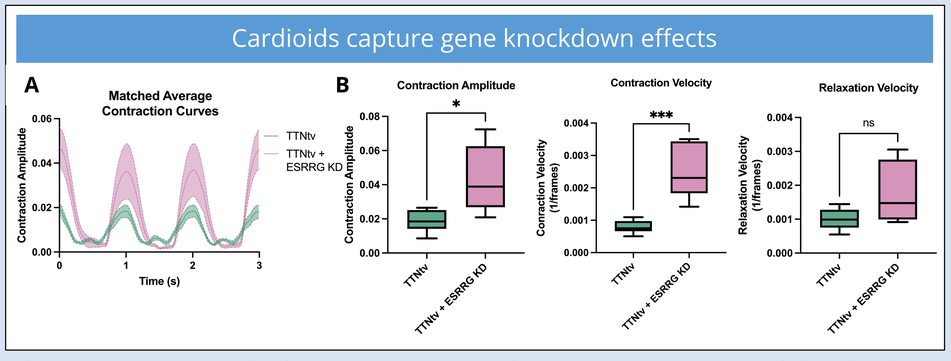

Figure 5. (A) Pacematched contraction curves of TTNtv and TTNtv+ESRRG kd cardioids. **(B)** Relative amplitude, contraction velocity, and relaxation velocity of ESRRG knockdown in TTNtv cardioids. $n \geq 5$.

INNOVATIONS

- High-throughput generation of hPSC-derived cardioids lead to reproducible contractility readouts
- Development of a fast and automated analysis pipeline provides four unbiased readouts of contractility
- Put together, this model provides a first-in-class high-throughput, cost effective, functional validation method for analysis of contractility

DISCLOSURES

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