

SUPPLEMENTAL MATERIALS

Non-negative blind deconvolution for signal processing in a CRISPR-edited iPSC-cardiomyocyte model of dilated cardiomyopathy

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SUPPLEMENTAL METHODS

Maintenance of human iPSCs. Protocols for studies involving human iPSCs were approved by the Goettingen University Ethical Board. Human iPSCs as described [1-3] were a kind gift by Joseph C. Wu (Stanford University, CA). WT and MUT iPSCs were grown on Matrigel-coated plates with chemically defined E8 medium as published [4]; the medium was changed daily. Human iPSCs were passaged every 3-4 days as described previously [2, 3]; in brief, iPSCs were detached using a PBS wash and incubation with EDTA (Thermo Fisher Scientific) for 5-10 minutes at 37°C. Next, cells were pelleted at 200 g for 3 min and plated on a matrigel-coated culture dish in E8 medium and Y-27632 (SelleckChem). Cells were allowed to attach overnight at 37°C.

Immunohistochemistry. Human iPSCs or iPSC-CMs were plated onto 10 mm coverslips and stained for pluripotency and cardiac markers as described before [1, 3, 5, 6]. In short, after fixing cells in 4% PFA, permeabilization in 0.2% Triton X-100 –PBS and a blocking step using 5% BSA, cells were subjected to immunostaining with antibodies against anti-troponin T (rabbit, Thermo Fisher Scientific), anti-myosin heavy chain (mouse, Developmental Studies Hybridoma Bank), anti-myosin light chain 2A (mouse, Synaptic Systems) and anti-myosin light chain 2V (mouse, Synaptic Systems). Next, coverslips were incubated with AlexaFluor 488 anti-mouse (Fisher Scientific) and AlexaFluor 568 anti-rabbit (Fisher Scientific) secondary antibodies. Image acquisition was performed using a Zeiss LSM510 Meta inverted confocal microscope with a 63X oil-immersed objective.

High-speed motion-traction in human iPSC-CMs. iPSC-CMs were seeded onto 10-mm coverslips and allowed to recover for three days prior to experiment. Two hours prior to recording, coverslips were transferred to the imaging chamber and supplied with RPMI

medium plus B27 supplement at 37°C throughout the entire experiment. Contractility of single beating iPSC-CMs was recorded using a high-speed microscopy camera (ORCA II Digital CCD camera C11090-22B) and Olympus software.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Characterization of CRISPR/Cas9-edited iPSCs carrying the TnT-R141W mutation

CRISPR/Cas9-edited iPSCs homozygous for the TnT-R141W mutation were tested for expression of pluripotency markers octamer-binding protein 3/4 (Oct3/4, POU5F1) and podocalyxin (Tra-1-81, PODXL) (A-D), compared to WT controls. (A-C) Relative mRNA expression analysis for the pluripotency markers NANOG (A), POU5F1 (B), and SOX2 (C) was performed using quantitative real-time PCR (qRT-PCR). Data are shown as mean $\pm$ SEM for n=3 independent experiments with n=3 technical replicates per sample in each experiment; differences between the groups (WT and MUT) are not significant as calculated via two-tailed Student's t-test. (D) Immunohistochemistry-based assessment and confocal imaging of pluripotency marker expression for octamer-binding protein 3/4 (Oct3/4, POU5F1) and podocalyxin (Tra-1-81, PODXL) in MUT iPSC-CMs compared to WT; scale bar, 20 μ m

Figure S2: Characterization of CRISPR/Cas9-edited iPSC-CMs carrying the TnT-R141W mutation compared to WT controls

(A-C) Protein expression levels of troponin T (TnT), troponin C (TnC) and tropomyosin (TM) showed no significant difference for TnT-R141W iPSC-CMs (MUT) compared with WT control. Normalization to loading controls was performed (GAPDH or tubulin as indicated). Bargraph presents averages of n=3 experiments for both groups (WT and MUT); representative immunoblots are shown. Data are expressed as mean $\pm$ SEM; ns = not significant as calculated by an unpaired Student's t-test (D-F) Immunohistochemistry followed by confocal imaging detected expression of the cardiac markers troponin T (TNNT2), myosin heavy chain (MYH7)

(D), myosin light chain 2a (MYL2) (E), myosin light chain 2v (MYL7) (F) in MUT iPSC-CMs versus WT controls; scale bar, 20 μ m.

Figure S3: Simplifying integration of dysfunctional Ca²⁺ handling, reduced beating force and abnormal contractility in the TnT-R141W iPSC-CM model

(A-C) Shown are peak shapes as obtained via averaging of all data presented in Figures 3-5, respectively. Markers indicate the average value and whiskers present the SEM. Transients of 0-0 to 100-100 are shown for WT control iPSC-CMs (blue) and MUT iPSC-CMs (orange). (A) The averaged peak shape of Ca²⁺ transients for the Ca²⁺ traces presented in Figure 3 is shown for WT control and MUT iPSC-CMs. WT control (WT), n=82 out of 82 Ca²⁺ traces were analyzed corresponding to 82 individual cells; MUT, n=73 out of 84 Ca²⁺ traces were analyzed corresponding to 84 individual cells. (B) Averaged peak shape of force recordings shown in Figure 4 for WT control and MUT iPSC-CMs. WT control (WT), n=60 out of 60 force recordings were analyzed, corresponding to 60 individual cells; MUT, n=49 out of 49 force recordings were analyzed, corresponding to 49 individual cells. (C) Averaged peak shape of all contractility measurements shown in Figure 5 for WT control and MUT iPSC-CMs. WT control (WT), n=73 out of 85 contraction recordings were analyzed corresponding to 85 individual cells; MUT, n=68 out of 107 contraction recordings were analyzed corresponding to 107 individual cells. Duration times for all amplitude transients were averaged and shown as mean $\pm$ SEM.

Figure S4: Application of least-squares fitting for assessing dysfunctional Ca²⁺ handling in TnT-R141W iPSC-CMs

The original data as used for the analysis presented in Figure 3 were analyzed with an established method, least-square fitting. (A, B) Ca²⁺ transient signals were fitted using least-square fitting (red) superimposed on raw data (grey), shown for WT control (A) and MUT

iPSC-CMs (B). (C-N) Results of least-square fitting-based analysis of Ca^{2+} transients in MUT iPSC-CMs compared to WT for assessment of Ca^{2+} handling properties in WT control versus MUT iPSC-CMs. (C, E) Time-to-peak; (D, F) amplitude; (G, I) decay time; (H, J) transient duration; (K, M) baseline; as well as (L, N) beating rate. Data in boxplots are shown for individual cells (dots), plus 0th, 25th, 50th, 75th, and 100th percentile quantiles (box) as well as range of all points (whiskers). A total n=58 out of 84 individual cells were fitted and analyzed in WT control and n=35 out of 82 in MUT iPSC-CMs; n=1 Ca^{2+} transient was recorded per cell. *** $P < 0.001$ as calculated by unpaired Student's test in both boxplots and bar graphs. Data are expressed as mean $\pm$ SEM.

Figure S5: Alternative analysis of beating force measurements in CRISPR/Cas-edited iPSC-CMs carrying the DCM mutation TnT-R141W

Original data as used for the analysis presented in Figure 4 were analyzed with an established approach, force curve analysis. (A-L) Analysis of beating force properties in MUT vs. WT iPSC-CMs for time-to-maximum (A, C), force (B, D), maximal decay (E, G), event duration (F, H), baseline (I, K), as well as beating rate (J, L). Data in boxplots are shown for individual cells (dots), plus 0th, 25th, 50th, 75th, and 100th percentile quantiles (box) as well as range of all points (whiskers). A total n=60 out of 60 individual cells were recorded and analyzed in WT control and n=49 out of 49 in MUT iPSC-CMs; n=1 beating force recording per cell was recorded. *** $P < 0.001$ and ns = not significant as calculated by unpaired Student's test in both boxplots and bar graphs. Data are expressed as mean $\pm$ SEM.

Figure S6: Single-cell motion-traction analysis in MUT vs WT iPSC-CMs via the MuscleMotion software

The original data used for the analysis presented in Figure 5 were analyzed with a previously published software, MuscleMotion. (A, B) Contractility recordings were analyzed with

MuscleMotion for WT control (A) and MUT iPSC-CMs (B). (C-N) Results of contractility data analysis for WT and MUT iPSC-CMs. (C-N) Statistics of contractility properties. Differences in contractility are shown for MUT iPSC-CMs vs. WT controls regarding time-to-maximum (C, E), amplitude (D, F), maximal decay (G, J), contraction duration (I, H), baseline (K, M), as well as beating rate (L, N). Data in boxplots are shown for individual cells (dots), plus 0th, 25th, 50th, 75th, and 100th percentile quantiles (box) and for range of all points (whiskers). A total n=36 out of 85 individual cells were analyzed in WT control and n=29 out of 107 in MUT iPSC-CMs; n=1 contraction recording was performed per cell. *** $P < 0.001$ and ns = not significant as calculated by unpaired Student's test in both boxplots and bar graphs. Data are expressed as mean $\pm$ SEM.

Table S1: Analysis of all individual events for Ca²⁺ transients, beating force and contractility in MUT and WT control iPSC-CMs as presented in Fig. 3-5.

Data presented in Fig. 3 - 5 were analyzed considering all individual events separately in individual Ca²⁺ transients, beating force measurements, and contractility recordings, respectively. 1) Averaged parameters for Ca²⁺ transients: time-to-peak, amplitude, decay time, and transient duration. WT control (WT), 766 single events were averaged for n=82 out of 82 Ca²⁺ traces corresponding to 82 individual cells; MUT, 317 single events were averaged for n=73 out of 84 Ca²⁺ traces corresponding to 84 individual cells. 2) Averaged parameters for beating force measurements: time-to-maximum, beating force, maximal decay, and event duration. WT control (WT), 2858 single events from n=60 out of 60 force recordings corresponding to 60 individual cells were averaged; MUT, 1568 single events from n=49 out of 49 force recordings corresponding to 49 individual cells were averaged. 3) Averaged parameters for contractility recordings: time-to-maximum, amplitude, maximal decay, and

contraction duration. WT control (WT), averages of 583 single events from n=73 out of 85 contraction recordings corresponding to 85 individual cells; MUT, averages of 254 single events from n=68 out of 107 contraction recordings corresponding to 107 individual cells. Data are shown as mean $\pm$ SEM.

Table S2: Comparison between NNBD-based and previous methods for Ca²⁺ transients, beating force and contractility analysis in MUT and WT control iPSC-CMs.

Previous analysis methods and the NNBD-based strategy were compared regarding successful fitting and analysis. 1) Successful fitting of Ca²⁺ traces with least-square fitting as well as subsequent analysis was completed for n = 35 out of 82 Ca²⁺ traces in WT control iPSC-CMs and 58 out of 84 Ca²⁺ traces in MUT iPSC-CMs. All Ca²⁺ traces in WT control iPSC-CMs and 73 out of 84 Ca²⁺ traces in MUT iPSC-CMs were successfully fitted and analyzed with the NNBD-based method. 2) Force recordings were analyzed via force curve analysis by defining the start point as well as end point and peak position for each individual peak and then calculating the respective parameters; n = 60 out of 60 measurements were analyzed in WT control iPSC-CMs and n = 49 out of 49 measurements in MUT iPSC-CMs. Using the NNBD-based method, n = 60 out of 60 force measurements in WT control iPSC-CMs and n = 49 out of 49 force measurements in MUT iPSC-CMs were fitted and analyzed. 3) Applying MuscleMotion for contractility data analysis and fitting, n = 36 out of 85 single-cell recordings in WT control iPSC-CMs and n = 29 out of 107 single-cell recordings in MUT iPSC-CMs were analyzed and fitted. With the NNBD-based method, n = 73 out of 85 single-cell recordings in WT control iPSC-CMs and n = 68 out of 107 single-cell recordings in MUT iPSC-CMs were fitted and analyzed.

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