

SUPPLEMENTAL MATERIALS

Increased cytosolic calcium buffering contributes to a cellular arrhythmogenic substrate in iPSC-cardiomyocytes from patients with dilated cardiomyopathy

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Running title: Increased Ca²⁺ buffering in DCM-TnT-R173W iPSC-CM

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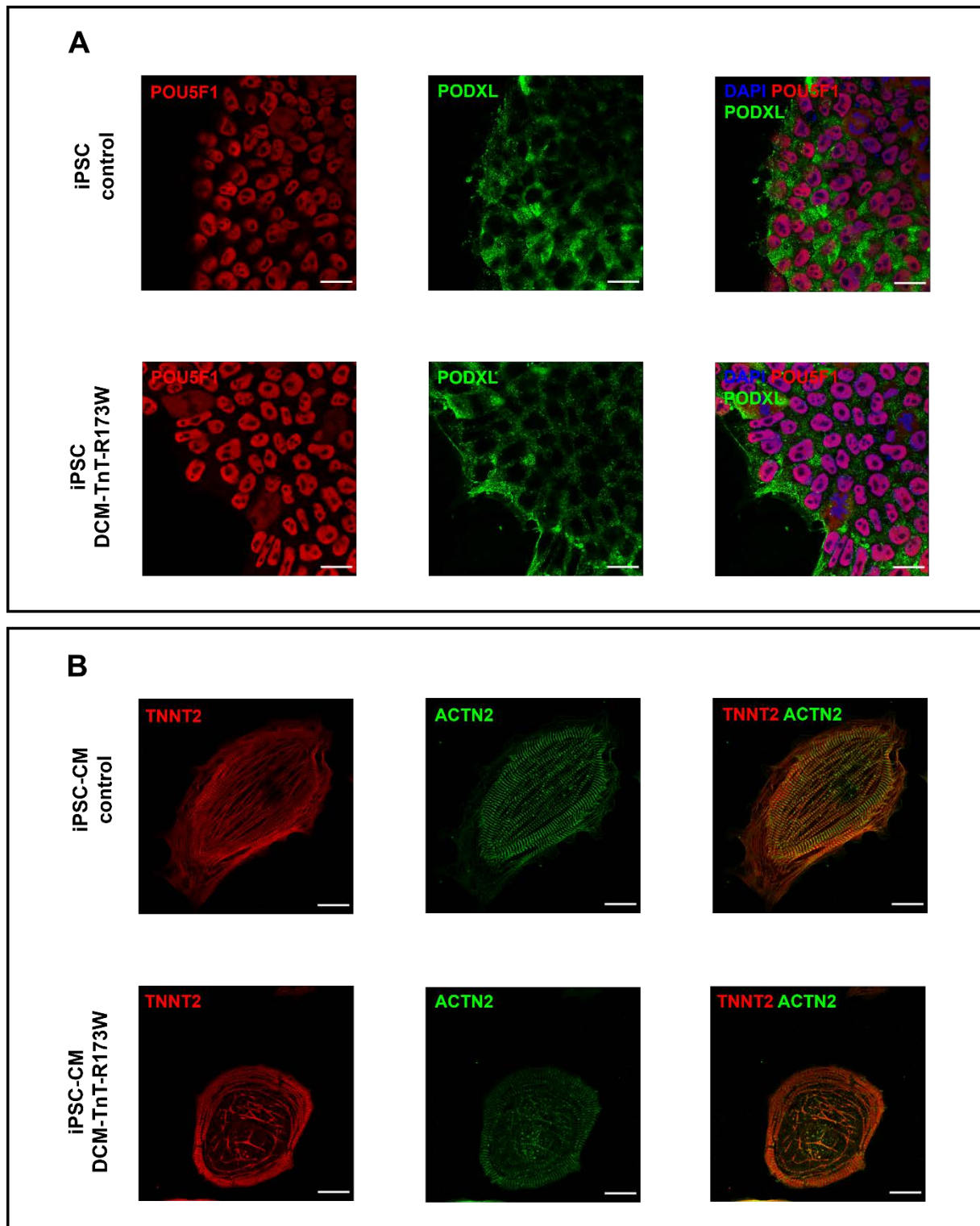
Supplemental Methods

Culture and maintenance of human iPSC. The human iPSC lines used in this study have previously been published [5, 7, 8]. Human iPSC were grown on matrigel-coated plates (VWR), as previously described [1, 3], in chemically defined E8 medium [1] which was changed daily. Cells were passaged every 4 days using EDTA (Life Technologies). Healthy control and DCM iPSC as published in [5, 7] were a kind gift from Joseph C. Wu (Stanford University, CA).

Pluripotency and cardiac marker analysis. Human iPSC or iPSC-CM were grown on matrigel-coated coverslips. For human iPSC, immunofluorescence staining was performed for Oct3/4 (POU5F1, R&D Systems) and Tra-1-81 (PODXL, Stemgent) as previously described [2, 3, 7]. DAPI was used for detection of nuclei. Human iPSC-CM were immunostained with antibodies against cardiac troponin T (Abcam) and sarcomeric alpha-actinin (Sigma). Coverslips were mounted on glass slides using Fluoromount G (SouthernBiotech). Images were acquired with a plan apochromat (63x/1.40 oil DIC M27) objective using an inverted confocal microscope (LSM 710 Meta, Zeiss) and ZEN software (Zeiss).

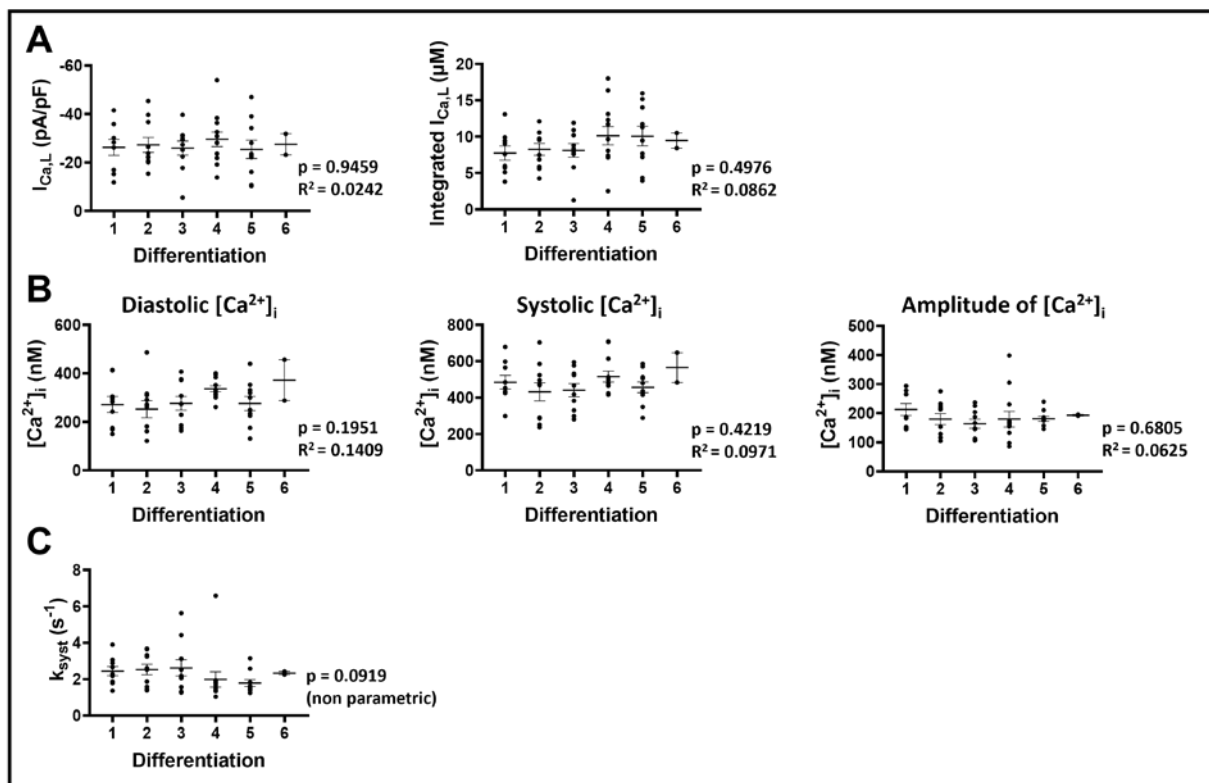
Analysis of mRNA expression levels. Total RNA was isolated from human iPSC-CM using a RNeasy Kit (Qiagen) as per the manufacturer's instructions. RNA was reverse-transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies). Quantitative real-time PCR (qRT-PCR) was performed using Reverse Transcription Supermix (BioRad) and the Hs00167681_m1 as well as Hs03003631_g1 TaqMan probes (Thermo Fisher Scientific), utilising Real-Time PCR Detection System (Life Technologies).

Supplemental Figures



Supplemental Figure 1: Control and DCM-TnT-R173W induced pluripotent stem cells (iPSC) express comparable levels of pluripotency markers. Following cardiac differentiation, iPSC-CM express regular levels of cardiac markers.

A, Immunohistochemistry and confocal imaging for pluripotency markers (Oct3/4 [POU5F1], and Tra-1-81 [PODXL]) in control (Ctrl) and DCM patient-derived (DCM-TnT-R173W) iPSC. Scale bar, 20 μm . **B**, Immunohistochemistry and confocal imaging of cardiac markers (cTnT [TNNT2] and SAA [ACTN2]) in Ctrl and DCM-TnT-R173W iPSC-CM. Scale bar, 20 μm .

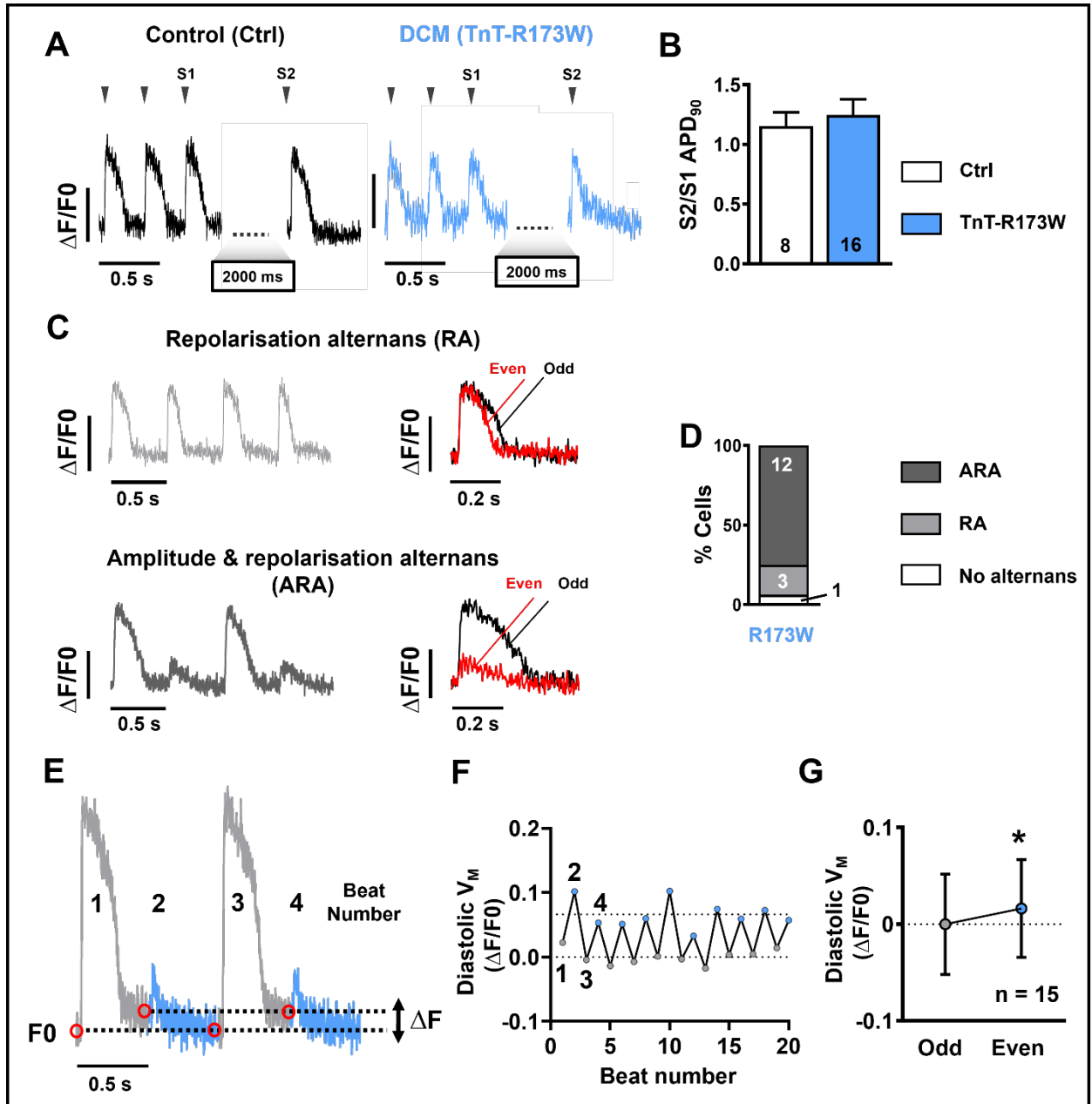


Supplemental Figure 2: Negligible clustering of experimental data in 6 different differentiations of wild type induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM).

A, Peak $I_{\text{Ca,L}}$ amplitude (left) and integrated $I_{\text{Ca,L}}$ (right). **B**, Diastolic $[\text{Ca}^{2+}]_i$ (left), systolic $[\text{Ca}^{2+}]_i$ (middle) and Ca^{2+} transient amplitude (right). **C**, Constant of Ca^{2+} transient decay.

Individual values and scatter plots are shown, which indicate mean $\pm$ SEM. P value and R^2 are

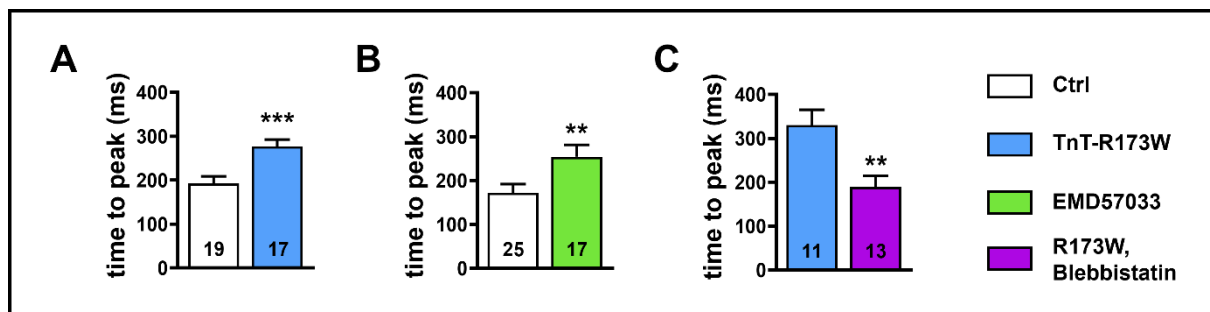
shown beside each plot as determined by one-way ANOVA (A, B), or Kruskal-Wallis test for non-parametric data sets (C).



Supplementary Figure 3: Optical action potential (AP) characteristics in control (Ctrl) and DCM-TnT-R173W induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM).

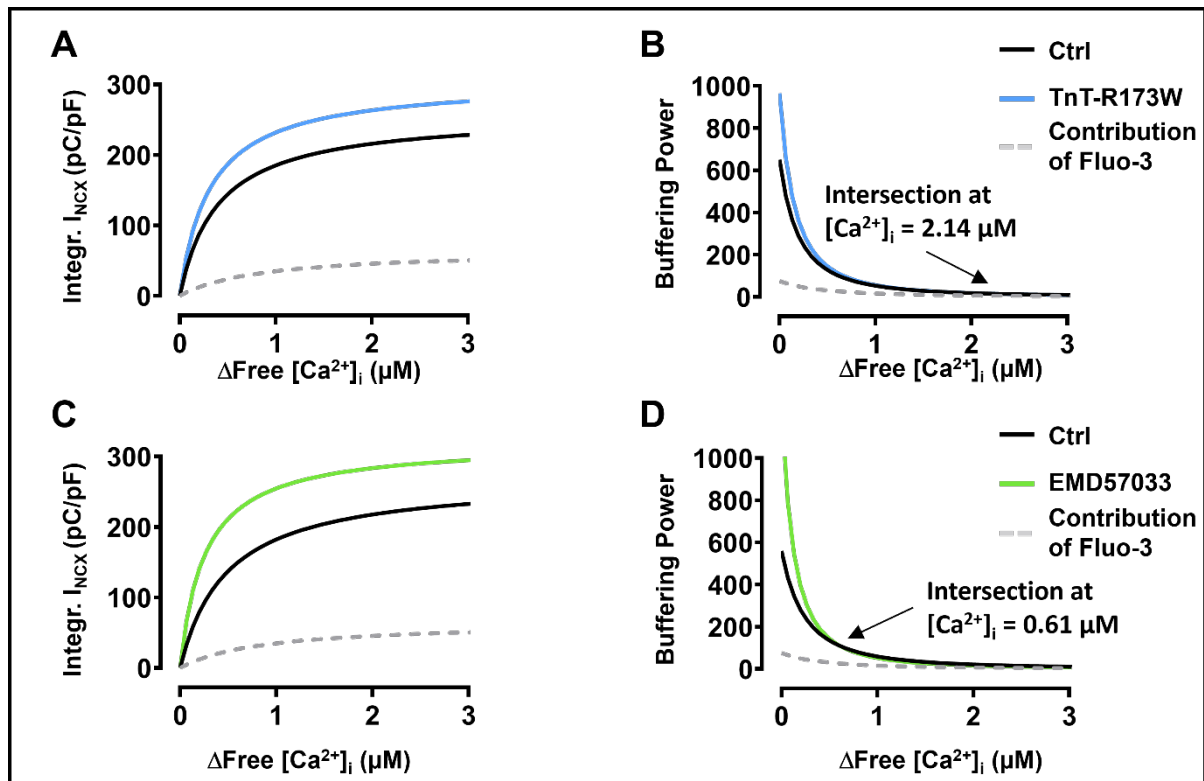
A, Normalised representative traces of optical AP post-rest potentiation in Ctrl (left) and TnT-R173W (right) iPSC-CM. 3 Hz stimulation (S1) was paused for 2 s prior to a post-pause

extrastimulus (S2). **B**, Relative post-pause AP duration at 90% repolarisation (APD_{90} , S2) compared with the pre-pause APD_{90} (S1). **C**, Types of AP alternans observed in TnT-R173W iPSC-CM as quantified following analysis with a discrete Fourier transform spectral method [4, 6]. **D**, Percentage of variation in alternans characteristics in all TnT-R173W iPSC-CM optical AP traces. **E**, Representative trace of TnT-R173W iPSC-CM optical AP and resting membrane potential (V_M) alternans during 2 Hz stimulation. The blue traces indicate the even 'pathological' beats. F0 indicates the optical take off potential of every odd 'physiological' beat which is increased for every even beat (ΔF). **F**, Representative example of beat-to-beat changes in optical diastolic V_M in TnT-R173W iPSC-CM over 20 consecutive beats. **G**, Quantification of diastolic V_M changes for all averaged odd and even beats in TnT-R173W iPSC-CM. n = number of iPSC-CM from 2-3 batches. Data are mean $\pm$ SEM. *P < 0.05 vs. odd using Student's t-test.



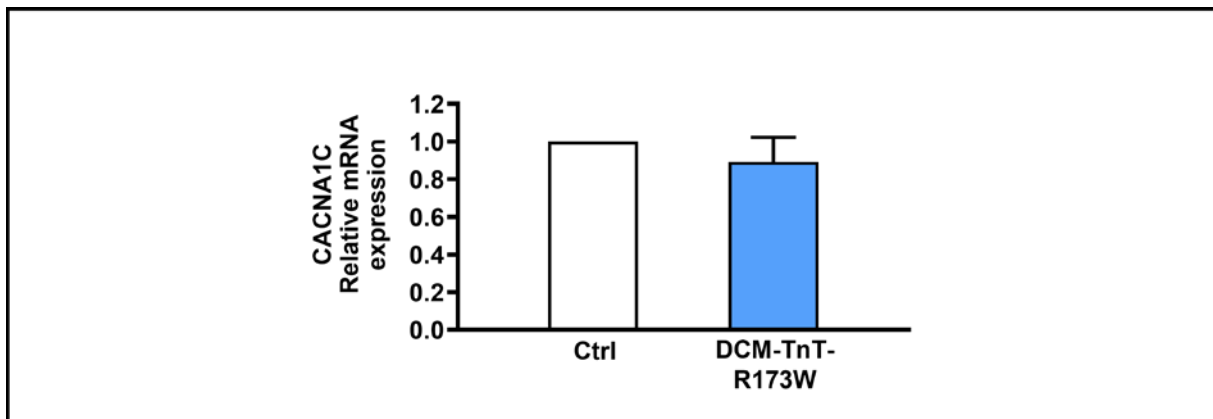
Supplemental Figure 4: Increased time to peak in field stimulation triggered Ca^{2+} -transients in induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM) with increased cytosolic calcium buffering.

A, B, C, Ca^{2+} transient time to peak assigned to experiments shown in **Figure 2, 7, 8**. Time-to-peak values were determined by measuring the time between electrical stimulation and maximum Ca^{2+} level in an averaged trace generated from 10 consecutive traces. n = number of iPSC-CM from 2-5 batches. Data are mean $\pm$ SEM. **P < 0.01 vs. Ctrl using Student's t-test (**A, C**) and the Mann-Whitney U test (**B**).



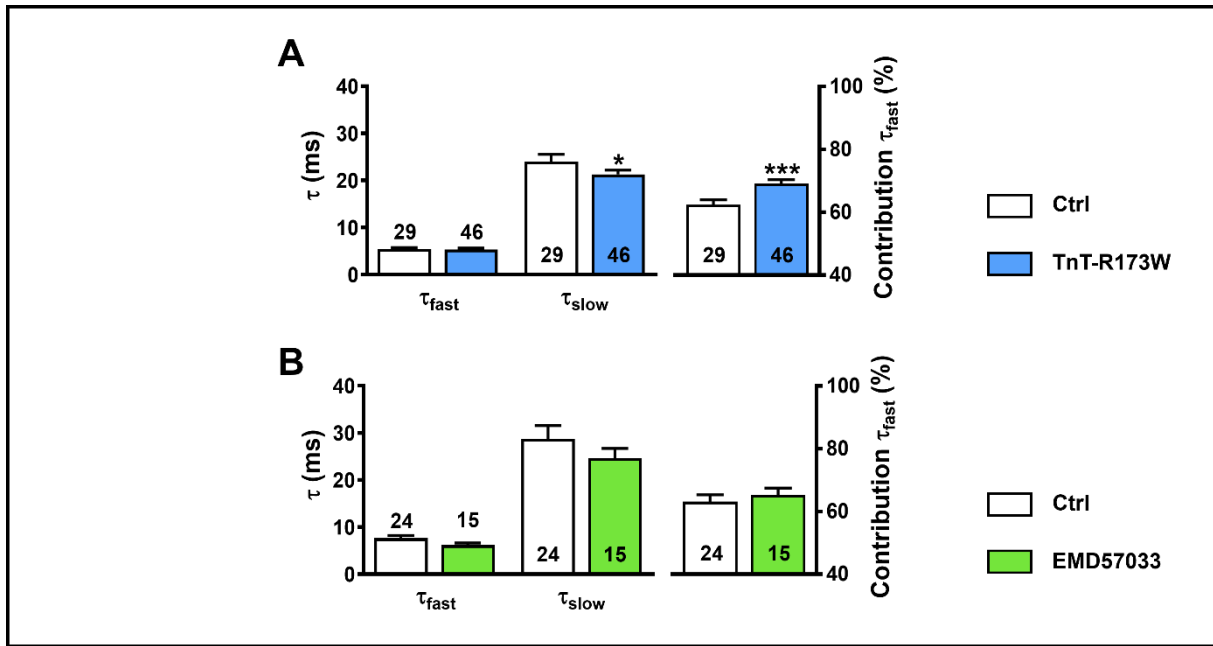
Supplemental Figure 5: Buffer curves and buffer power in control (Ctrl), DCM-TnT-R173W and Ctrl pre-treated with EMD57033 induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM).

A, Cytosolic buffer curves shown as a plot of integrated I_{NCX} against a wide range of $[\text{Ca}^{2+}]_i$ created using the experimental estimated means of K_d and B_{max} values from Ctrl and TnT-R173W iPSC-CM. **B**, Buffering power, i.e. the change of $[\text{Ca}^{2+}]_{\text{total}}$ divided by the change of $[\text{Ca}^{2+}]_i$ ($d[\text{Ca}^{2+}]_{\text{total}}/d[\text{Ca}^{2+}]_i$) as a function of $[\text{Ca}^{2+}]_i$ estimated from the mean buffer curves in Ctrl and TnT-R173W iPSC-CM (**A**). The arrows indicate the calculated point of intersection of two buffer power curves. **C**, Cytosolic buffer curves in Ctrl iPSC-CM and Ctrl iPSC-CM pre-treated with EMD57033. **D**, Buffering power of Ctrl iPSC-CM and Ctrl iPSC-CM pre-treated with EMD57033. Contribution of Fluo-3 to all buffer- and buffer power curves are illustrated as grey dashed lines.



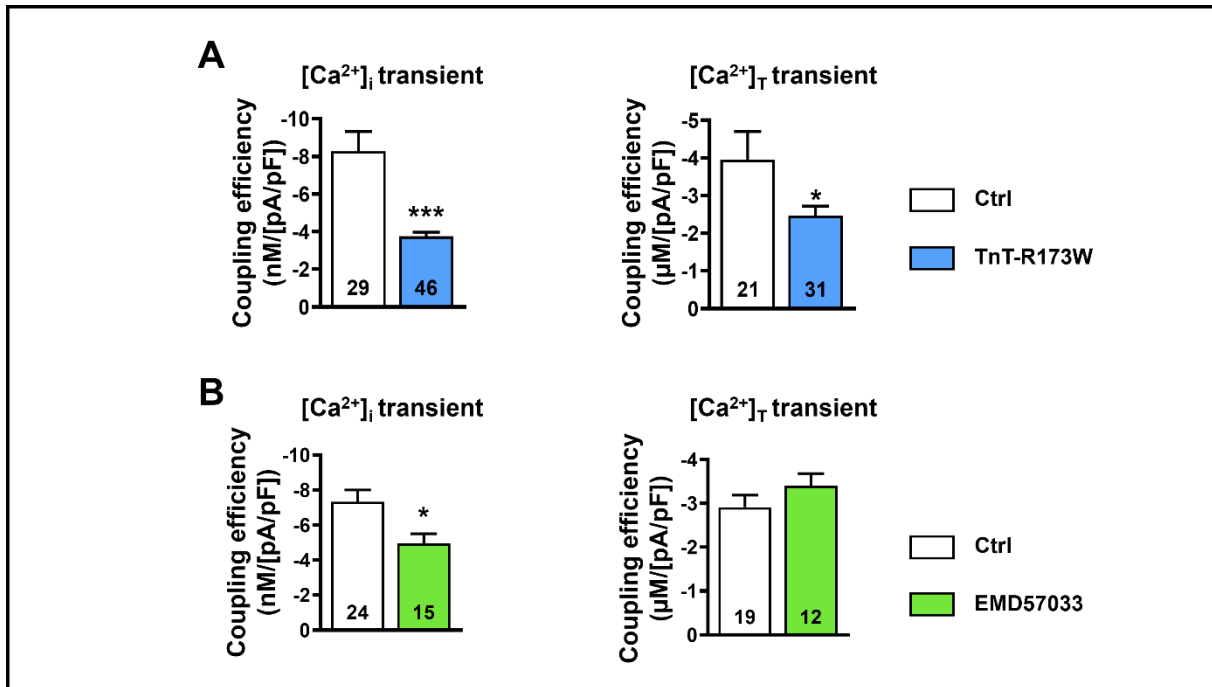
Supplemental Figure 6: mRNA expression of Cav1.2 is not significantly altered in DCM-TnT-R173W iPSC-CM vs. control

Relative mRNA expression of Cav1.2 (CACNA1C) was assessed with quantitative real-time PCR (qRT-PCR) in DCM patient-derived (DCM-TnT-R173W) iPSC-CM and Control (Ctrl) iPSC-CM. n = 3 (independent experiments). Data are mean $\pm$ SEM. P = 0.674 using Student's t-test.



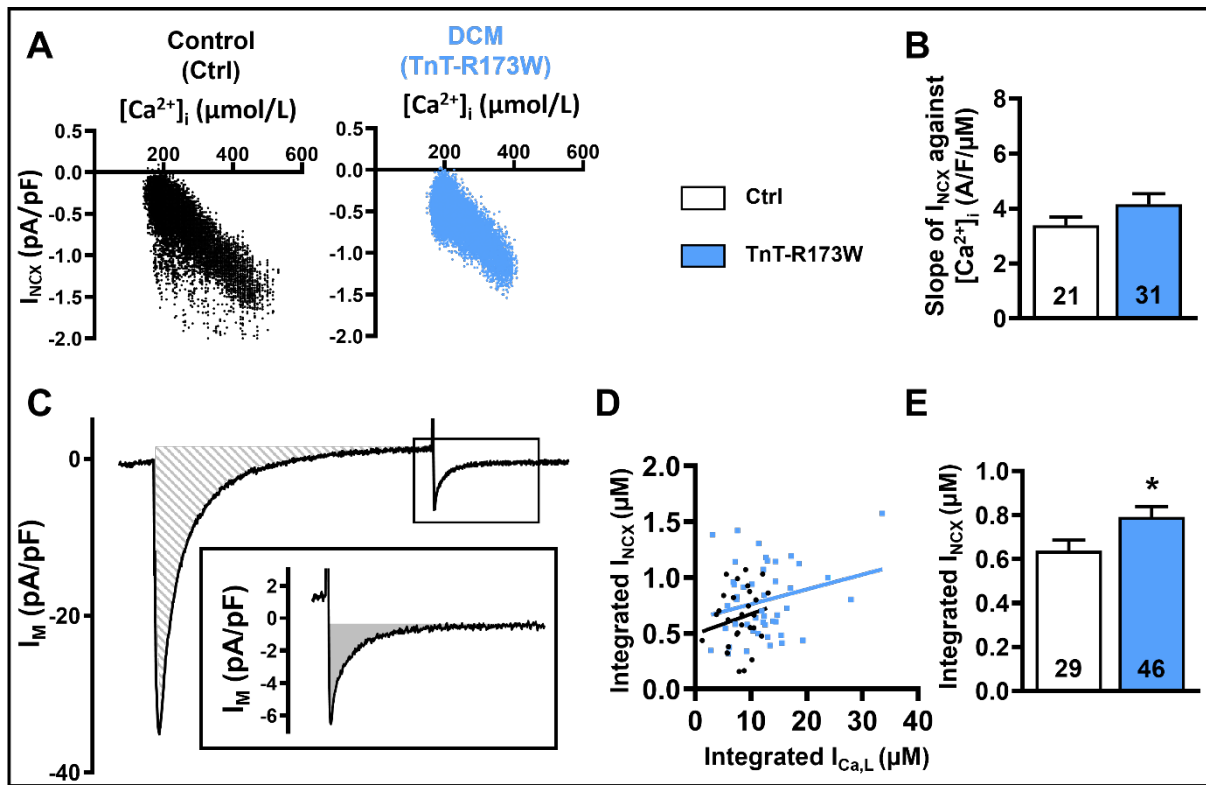
Supplemental Figure 7: Further analysis of the biphasic $I_{\text{Ca,L}}$ inactivation in control (Ctrl), DCM-TnT-R173W and Ctrl pre-treated with EMD57033 induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM).

A, B, Time constant of the fast part of the inactivation (τ_{fast} , left), time constant of the slow part of inactivation (τ_{slow} , middle), and the contribution of τ_{fast} to complete inactivation (% , right). n = number of iPSC-CM from 3-5 batches. Data are mean $\pm$ SEM. *P < 0.05 and ***P < 0.001 vs. Ctrl using Mann-Whitney U test (**A, B left and middle**) and Student's t-test (**B right**).



Supplemental Figure 8: Coupling efficiency in control (Ctrl), DCM-TnT-R173W and Ctrl pre-treated with EMD57033 induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM).

A, B, “coupling efficiency” of Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} release estimated with experimental measured free Ca^{2+} ($[\text{Ca}^{2+}]_i$, left) and calculated total Ca^{2+} ($[\text{Ca}^{2+}]_T$, right). n = number of iPSC-CM from 3-5 batches. Data are mean $\pm$ SEM. *P < 0.05 and ***P < 0.001 vs. Ctrl using Mann-Whitney U test.



Supplemental Figure 9: Comparable NCX function between control (Ctrl) and DCM-TnT-R173W induced pluripotent stem-cell derived cardiomyocytes (iPSC-CM).

A, I_{NCX} in relation to $[\text{Ca}^{2+}]_i$ during decay of caffeine-induced Ca^{2+} transient from representative experiments using Ctrl (left) and TnT-R173W (right) iPSC-CM. **B**, The slope of a fitted linear function using I_{NCX} against $[\text{Ca}^{2+}]_i$ (**A**) is indicating the $[\text{Ca}^{2+}]_i$ -dependence of NCX function. **C**, Representative membrane current according to voltage-clamp protocol showing $I_{\text{Ca,L}}$ (left) and I_{NCX} (right, framed). The striped area visualises the integrated $I_{\text{Ca,L}}$ representing the charge of Ca^{2+} entering the cell during stimulation (**Figure 3B**). The grey area visualises the integrated I_{NCX} representing the charge leaving the cell through the NCX after stimulation. **D**, Integrated I_{NCX} plotted against integrated $I_{\text{Ca,L}}$ and correlated by a linear regression. Slope of Ctrl = 0.018, slope of DCM-TnT-R173W = 0.013. **E**, Integrated I_{NCX} . n = number of iPSC-CM from 3-5 batches. Data are mean $\pm$ SEM. *P < 0.05 vs. Ctrl using Mann-Whitney U test (**B**) and Student's t-test (**E**).

Supplemental References

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