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Supplementary Materials for

Plakophilin-2 truncating variants impair cardiac contractility by disrupting sarcomere stability and organization

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The PDF file includes:

Figs. S1 to S4
Legends for movies S1 to S12

Other Supplementary Material for this manuscript includes the following:

Movies S1 to S12

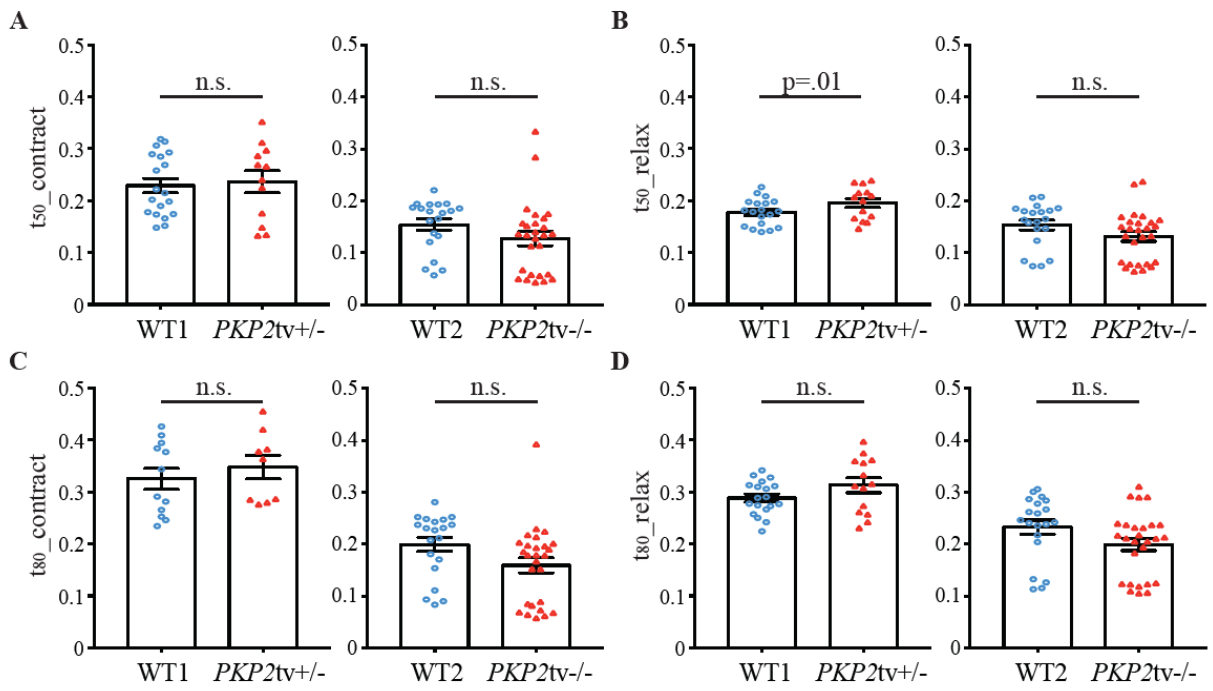
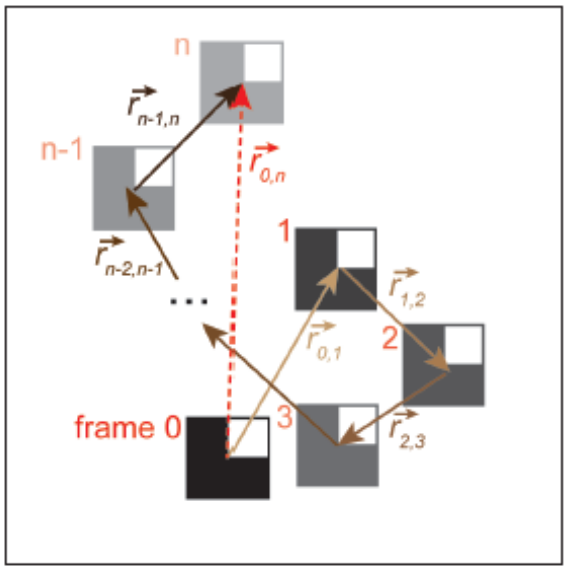


Fig. S1. Contraction and Relaxation Kinetics of Cardiac Micro-Tissues Electrically Paced at 1Hz. Related to Figure 4. Quantification of **(A)** t50_contract, **(B)** t50_relax, **(C)** t80_contract and **(D)** t80_relax, shows comparable contraction and relaxation kinetics of cardiac micro-tissues made with *PKP2*tv+/- (n = 22) vs. WT1 (n = 25) and *PKP2*tv-/- (n = 27) vs. WT2 (n = 20), when the tissues are electrically paced at 1Hz. **Statistics:** individual data points across three independent experiments are shown with mean ± s.e.m. on plots; Studen's t-tests, $\alpha = 0.05$.



Net Displacement (µm)

$$\overrightarrow{r_{0,n}} = \sum \overrightarrow{r_{i,i+1}}$$

Mean Displacement (µm/frame)

$$\frac{1}{n}\sum \left|\overrightarrow{r_{i,i+1}}\right|$$

Ramble (µm/frame)

$$\frac{1}{n}\left[\sum \left|\overrightarrow{r_{i,i+1}}\right| - \left|\overrightarrow{r_{0,n}}\right|\right]$$

Fig. S2. Diagram of Optical Flow Tracking Algorithm Method and Outputs. Related to Figure 8 and Figure 9.

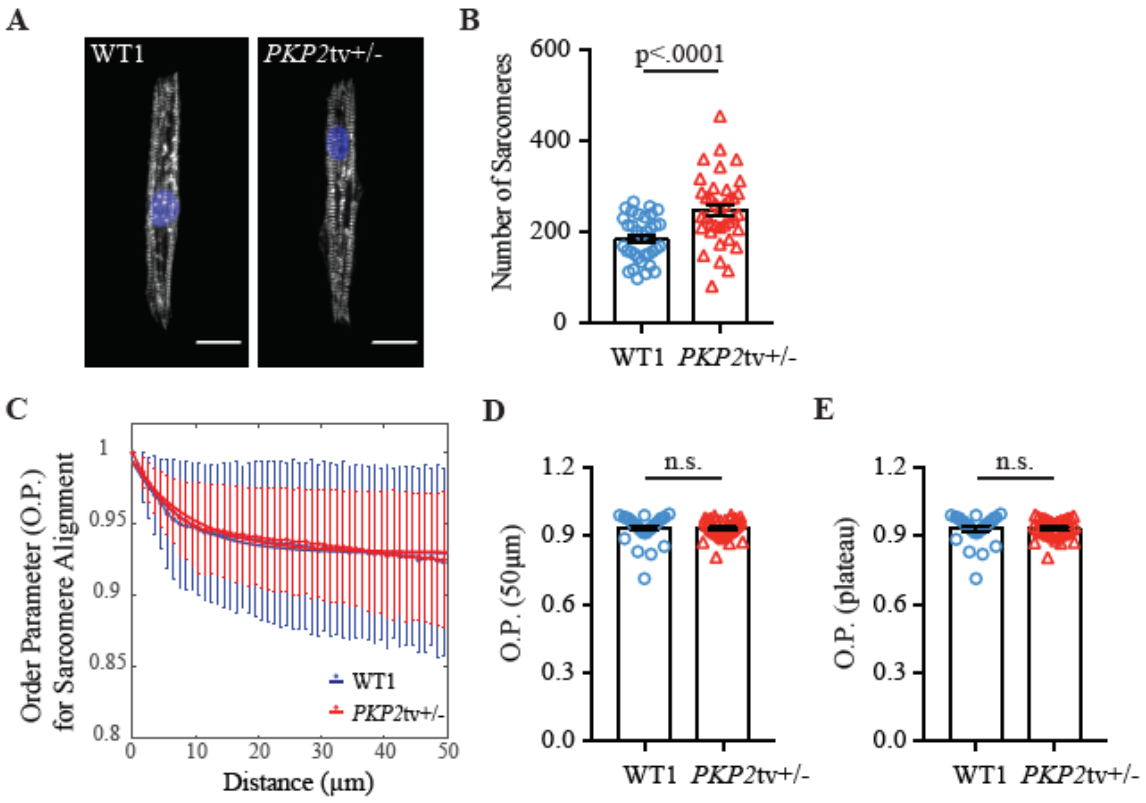


Fig. S3. *PKP2*tv Lead to Increase in Sarcomere Content in Single Cardiomyocytes. Related to Figure 10. (A) Representative images of single iPSC-CMs micropatterned in rectangular shape, immune-stained for sarcomeric α-actinin-2 (shown in grey) and nuclei (shown in blue). Scale bars, 20 μm. **(B)** Quantification of the number of α-actinin-2-positive sarcomeric Z-discs in single cardiomyocytes shows significantly higher sarcomere content in *PKP2*tv+/- (n = 20) vs. WT1 (n = 21). **(C)** Order parameter (O.P.) for sarcomere alignment plotted over distance from any sarcomere Z-disc comparing single cardiomyocytes harboring *PKP2*tv+/- (n = 20) vs. WT1 (n = 21). Data shown as mean ± standard deviation, with the solid lines showing data fitted in an exponential decay model. **(D)** Quantification of O.P. at 50 μm away from any sarcomere Z-disc shows comparable sarcomere alignment in single cardiomyocytes harboring *PKP2*tv+/- (n = 20) vs. WT1 (n = 21). **(E)** Quantification of plateau of O.P. decay model shows significantly comparable sarcomere alignment in long range in single cardiomyocytes harboring *PKP2*tv+/- (n = 20) vs. WT1 (n = 21). **Statistics:** individual data points across three independent experiments are shown with mean ± s.e.m. on plots, unless otherwise specified; Student's t-tests, α = 0.05. Immunostains are representative of at least three independent experiments.

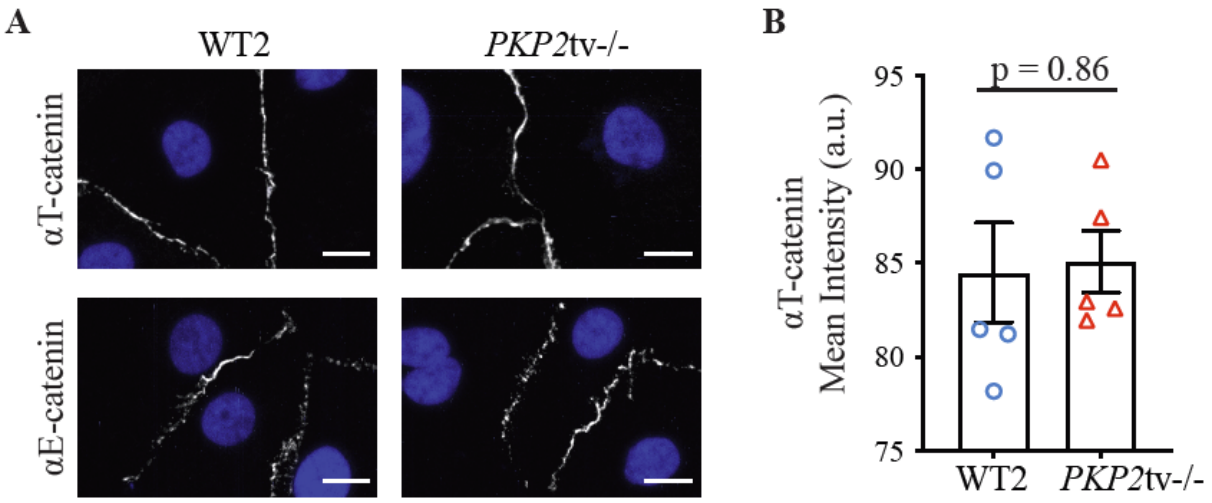


Fig. S4. Junctional expression of αT-catenin and αE-catenin in *PKP2*tv and WT cardiomyocytes. (A) Representative images of iPSC-CM monolayers fixed and stained for nuclei (blue), αT-catenin (grey, top) and αE-catenin (grey, bottom). Scale bars, 10μm. **(I)** Mean fluorescence intensity of junctional αT-catenin is comparable in *PKP2*tv-/- (n = 5) vs. WT2 (n = 5). **Statistics:** individual data points are shown with mean ± s.e.m. on plots; Student's t-tests, α = 0.05.

Movie S1. Fluorescence Recovery After Photobleaching of Junctional N-cadherin signal in WT1 iPSC-CMs, Related to Figure 7. Representative FRAP imaging of WT1 cells expressing N-cadherin-mApple cultured as a multicellular cardiac patch. Images were taken before and immediately after 1 minute of photobleaching of a user defined ROI, at a 30-second frame rate for 30 minutes. Scale bar, 5 μm.

Movie S2. Fluorescence Recovery After Photobleaching of Junctional N-cadherin signal in *PKP2*tv+/- iPSC-CMs, Related to Figure 7. Representative FRAP imaging of *PKP2*tv+/- cells expressing N-cadherin-mApple cultured as a multicellular cardiac patch. Images were taken before and immediately after 1 minute of photobleaching of a user defined ROI, at a 30-second frame rate for 30 minutes. Scale bar, 5 μm.

Movie S3. Fluorescence Recovery After Photobleaching of Junctional N-cadherin signal in WT2 iPSC-CMs, Related to Figure 7. Representative FRAP imaging of WT2 cells expressing N-cadherin-EGFP cultured as a multicellular cardiac patch. Images were taken before and immediately after 1 minute of photobleaching of a user defined ROI, at a 30-second frame rate for 30 minutes. Scale bar, 5 μm.

Movie S4. Fluorescence Recovery After Photobleaching of Junctional N-cadherin signal in *PKP2*tv-/- iPSC-CMs, Related to Figure 7. Representative FRAP imaging of *PKP2*tv-/- cells expressing N-cadherin-EGFP cultured as a multicellular cardiac patch. Images were taken before and immediately after 1 minute of photobleaching of a user defined ROI, at a 30-second frame rate for 30 minutes. Scale bar, 5 μm.

Movie S5. Dynamics of N-cadherin Junctions in WT1 iPSC-CMs, Related to Figure 8. Representative time-lapse imaging of WT1 cells expressing N-cadherin-mApple cultured in a monolayer. Images were taken at a 10-minute frame rate for 8 hours. Scale bar, 10 μm.

Movie S6. Dynamics of N-cadherin Junctions in *PKP2*tv+/- iPSC-CMs, Related to Figure 8. Representative time-lapse imaging of *PKP2*tv+/- cells expressing N-cadherin-mApple cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.

Movie S7. Dynamics of N-cadherin Junctions in WT2 iPSC-CMs, Related to Figure 8. Representative time-lapse imaging of WT2 cells expressing N-cadherin-EGFP cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.

Movie S8. Dynamics of N-cadherin Junctions in *PKP2*tv-/- iPSC-CMs, Related to Figure 8. Representative time-lapse imaging of *PKP2*tv-/- expressing N-cadherin-EGFP cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.

Movie S9. Dynamics of N-cadherin Junctions and Sarcomeric α-Actinin-2 in WT1 iPSC-CMs, Related to Figure 9. Representative time-lapse imaging of WT1 cells expressing N-cadherin-mApple (shown in green) and EGFP-α-Actinin-2 (shown in magenta) cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.

Movie S10. Dynamics of N-cadherin Junctions and Sarcomeric α-Actinin-2 in *PKP2*tv+/- iPSC-CMs, Related to Figure 9. Representative time-lapse imaging of *PKP2*tv+/- cells expressing N-cadherin-mApple (shown in green) and EGFP-α-Actinin-2 (shown in magenta) cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.

Movie S11. Dynamics of N-cadherin Junctions and Sarcomeric α-Actinin-2 in WT2 iPSC-CMs, Related to Figure 9. Representative time-lapse imaging of WT2 cells expressing N-cadherin-EGFP (shown in green) and mApple-α-Actinin-2 (shown in magenta) cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.

Movie S12. Dynamics of N-cadherin Junctions and Sarcomeric α-Actinin-2 in *PKP2*tv-/- iPSC-CMs, Related to Figure 9. Representative time-lapse imaging of *PKP2*tv-/- cells expressing N-cadherin-EGFP (shown in green) and mApple-α-Actinin-2 (shown in magenta) cultured in a monolayer. Images were taken at a 10-minute frame frame rate for 8 hours. Scale bar, 10 μm.