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Supplemental information

Substrate mechanics unveil early structural and functional pathology in iPSC micro-tissue models of hypertrophic cardiomyopathy

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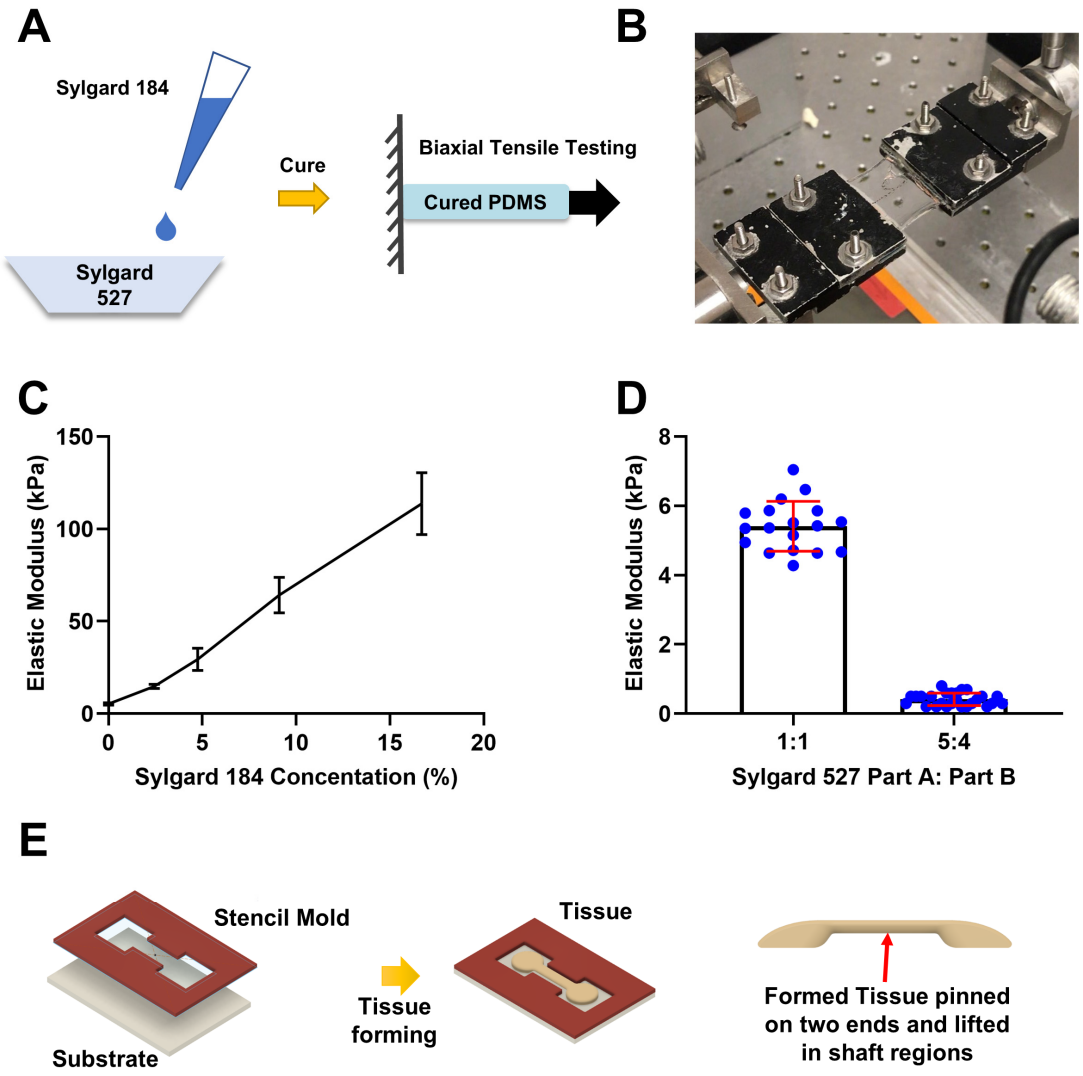


Figure S1. Substrate characterization and tissue formation schematic, relate to Figure 1.

(A) Controlling substrate mechanical properties using the mix of sylgard 184 and sylgard 527 with different ratios to manipulate substrate stiffnesses. (B) PDMS elastic modulus characterization through uniaxial tensile testing. (C) Blended PDMS substrates can achieve tensile elasticity from 5 kPa up to 114 kPa. (D) Ultra-soft 0.4 kPa substrate can be made by blending different ratios of sylgard 527, part A to part B weight ratio of 5 to 4. (E) Schematic of device fabrication and tissue formation[1].

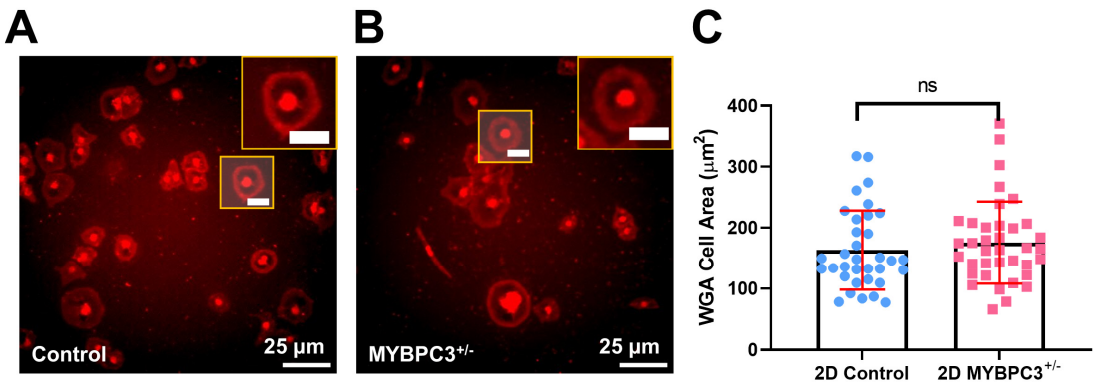


Figure S2. MYBPC3$^{+/-}$ iPSC-cardiomyocytes do not show cellular hypertrophy when cultured in 2D monolayers on tissue culture polystyrene, relate to Figure 2 A, B. (A) Representative WGA staining images of purified 2D control iPSC-cardiomyocytes. (B) Representative WGA images of 2D purified MYBPC3$^{+/-}$ iPSC-cardiomyocytes. (C) Quantitative analysis of cell area measured from both control and MYBPC3$^{+/-}$ cultures. *P* value 0.4336, n>5. Scale bar: 25 µm, insets 10 µm.

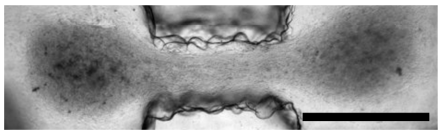
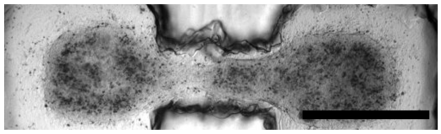
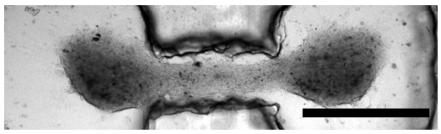
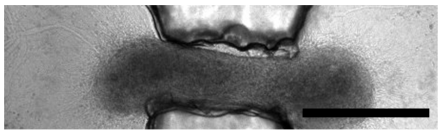
Tissue exclusion criteria based on tissue appearance	
“Good quality” tissue	
Poor quality tissue type 1: Visible cellular death and dark spots within the tissue	
Poor quality tissue type 2: Partially detached tissue at the edges	
Poor quality tissue type 3: completely delaminated from substrate	

Figure S3. Tissue exclusion criteria based upon tissue appearance, relate to Figure 2.

Within 75-95% cardiomyocytes batches, the lower quality tissues were excluded from physiological and electrophysiological studies. Only “good quality” tissues, which are uniformly distributed and compacted tissue with no obvious cellular death and detachment were selected for further analysis. We observe the inclusion/exclusion rate are not dependent on genotypes or stiffnesses. Scale bar: 1000 μm.

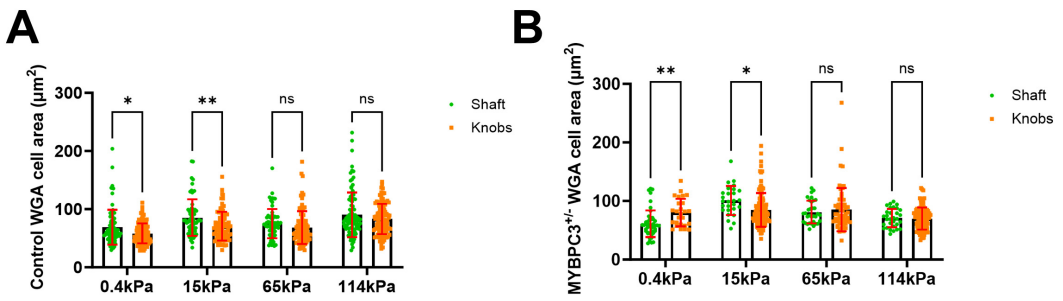


Figure S4. Cellular cross-sectional area at different regions (shaft or knobs) within the tissue, relate to Figure 2 B. (A) Isogenic control tissues cellular size at different regions of the tissue under different stiffness conditions. Cell area at the shaft regions is significantly larger than the knob regions for 0.4 and 15 kPa conditions. However, the differences are not significant with stiffer 65 and 114 kPa conditions. (B) MYBPC3$^{+/-}$ tissues cellular area different regions of the tissue under different stiffness conditions. A similar trend was seen for MYBPC3+/- tissues compared to the isogenic control tissues, except for 0.4 kPa conditions, knob area is significantly larger compared to the shaft, potentially indicating less alignment and maturation. * and ** indicate *p* value less than 0.05 and 0.01. Error bar: *SD*, n represents > 4 individual tissue batches.

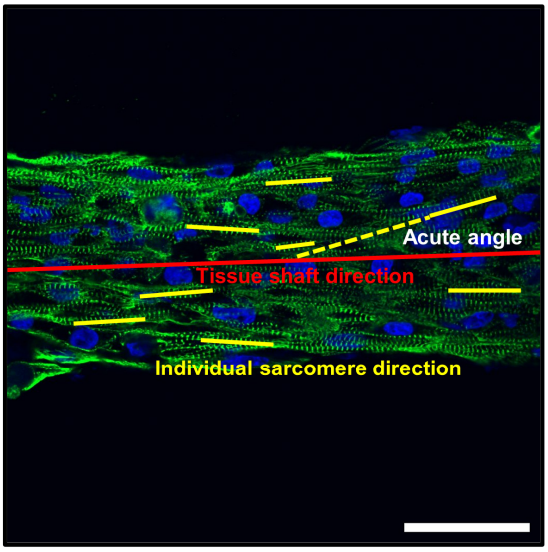


Figure S5. Illustration of sarcomere alignment quantification, relate to Figure 2 D. In custom build MATLAB code, tissue shaft direction (shown in red solid line) and individual sarcomere direction (shown in yellow solid lines) were drawn manually by users and the acute angles between tissue shaft direction and individual sarcomere directions were quantified. 0 degree indicates perfect alignment of the sarcomere within the tissue, while 90 degree indicates sarcomere disarray within the tissue. Scale bar: 50 μm.

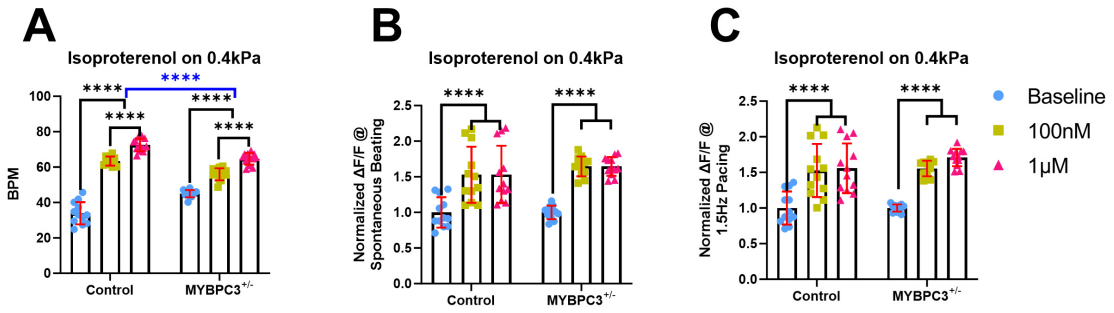


Figure S6. Isoproterenol response for control and MYBPC3$^{+/-}$ tissues under soft 0.4 kPa condition, relate to Figure 2 F-H. (A) Spontaneous beat rate changes for control and MYBPC3$^{+/-}$ 0.4 kPa tissues in response for isoproterenol. (B-C) Spontaneous (B) and 1.5 Hz (C) pacing Ca^{2+} intake for control and MYBPC3$^{+/-}$ 15 kPa tissues in response for isoproterenol. **** indicates *p* value less 0.0001. Error bar: *SD*. n represents >3 individual differentiated tissue batches.

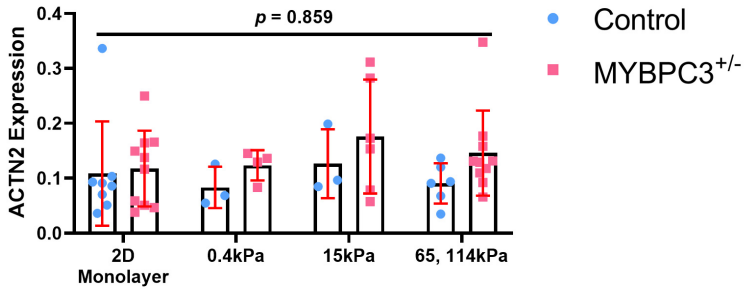


Figure S7. Gene expression of ACTN2 expression between control and MYBPC3$^{+/-}$ at different environmental stiffnesses, relate to Figure 4 A. Sarcomere α-actinin gene expression has no significant differences between conditions.

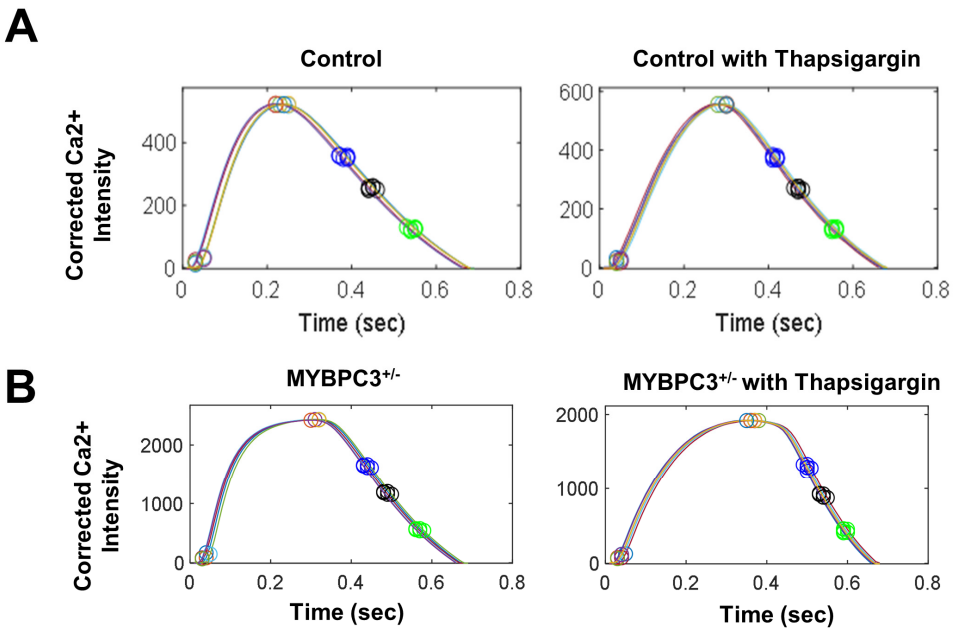


Figure S8. Effects of SERCA inhibition using thapsigargin on control and MYBPC3$^{+/-}$ tissues, relate to Figure 6 C, D. SERCA inhibition causes the prolonged calcium upstroke for both genotypes, indicating both control and MYBPC3$^{+/-}$ tissues have functional SERCA. Meanwhile, SERCA inhibition does not recapitulate prolonged calcium plateau in control tissues.

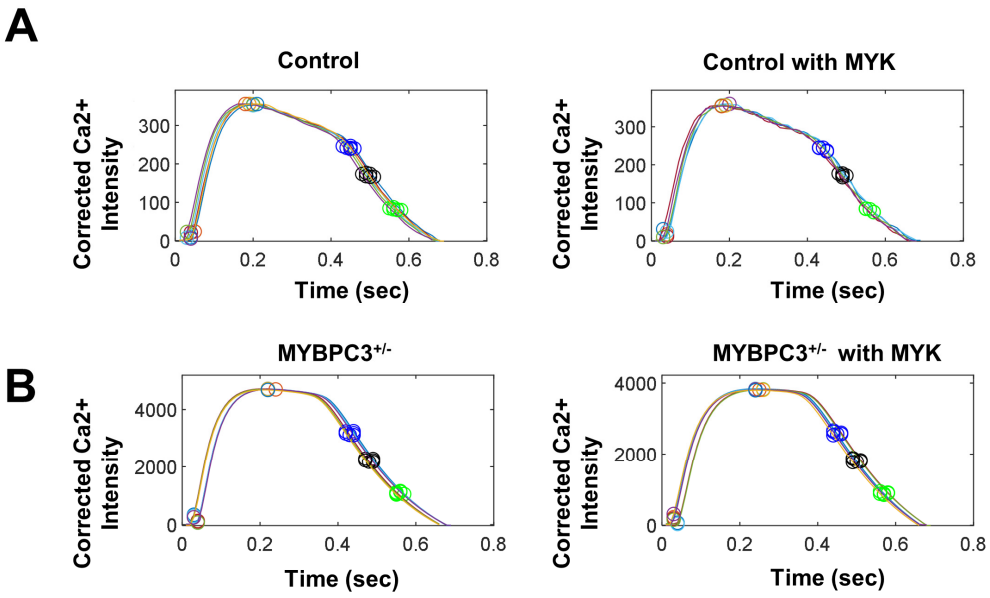


Figure S9. Myosin inhibitor mavacamten (MYK) substantially reduced contractility of the μHM without affecting the calcium transient, relate to Figure 6 E, F. A) Representative calcium transient curves of control 15 kPa tissue before and after treating with 0.5 μM MYK. B) Representative calcium transient curves of MYBPC3$^{+/-}$ 15 kPa tissue before and after treatment with 0.5 μM MYK.

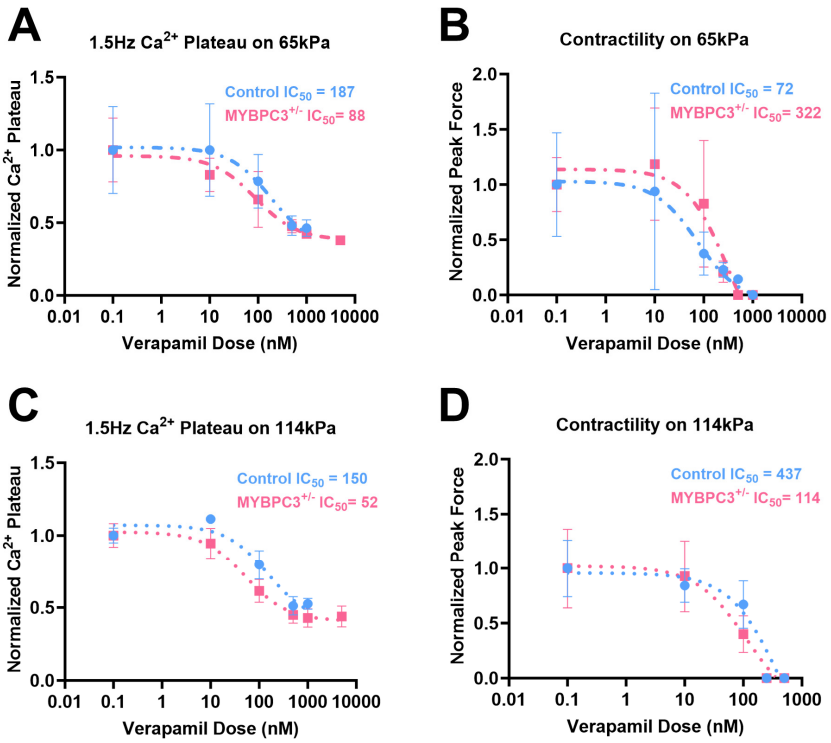


Figure S10. Verapamil responses for isogenic control and MYBPC3$^{+/-}$ tissues at higher stiffnesses, relate to Figure 6 I-L. (A) Ca^{2+} plateau duration in response to verapamil for both control and MYBPC3$^{+/-}$ μHM on 65 kPa conditions. (B) Contractility in response to verapamil for both control and MYBPC3$^{+/-}$ μHM on 65 kPa conditions. (C) Ca^{2+} plateau duration in response to verapamil for both control and MYBPC3$^{+/-}$ μHM on 114 kPa condition. (D) Contractility in response to verapamil for both control and MYBPC3$^{+/-}$ μHM on 114 kPa condition. Error bars: *SD*. n>4.

References:

1. Guo, J., Simmons, D.W., Ramahdita, G., Munsell, M.K., Oguntuyo, K., Kandalaft, B., Rios, B., Pear, M., Schuftan, D., Jiang, H., et al. (2020). Elastomer-Grafted iPSC-Derived Micro Heart Muscles to Investigate Effects of Mechanical Loading on Physiology. ACS Biomater Sci Eng. 10.1021/acsbiomaterials.0c00318.