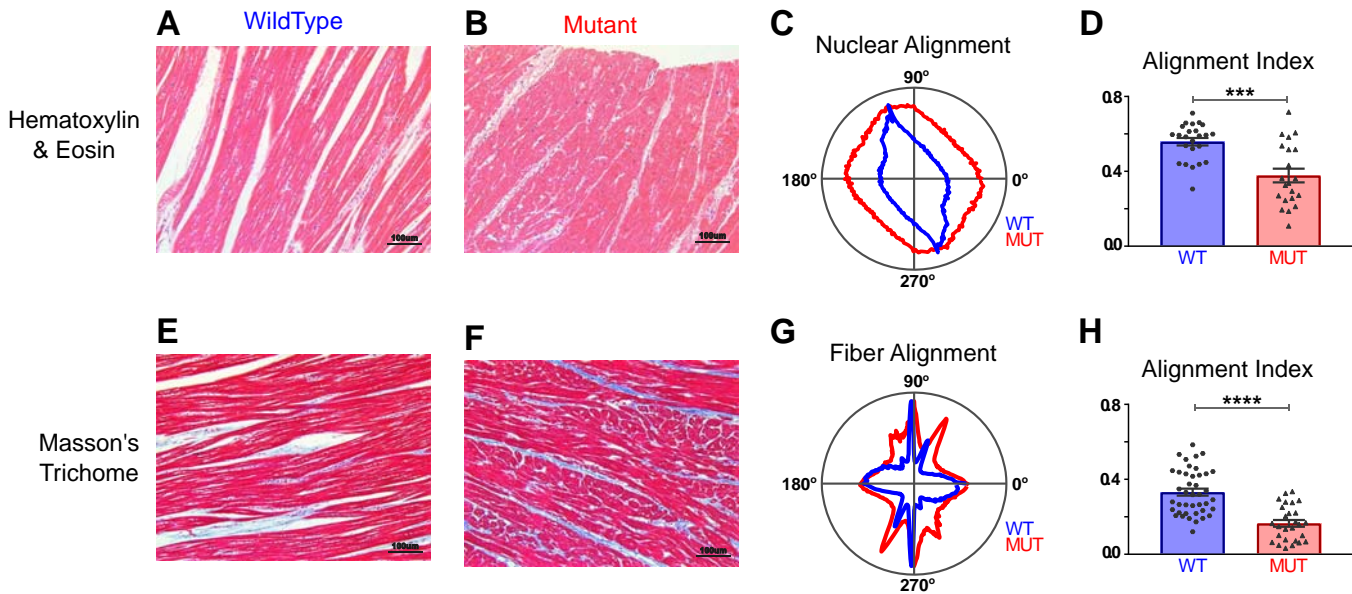


Online Appendix

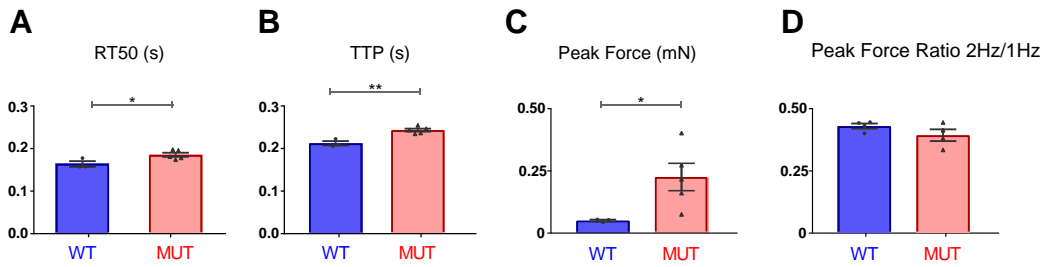
Supplemental Table 1. qPCR Primers

	Description	cDNA size (bp)	Forward	Reverse	gDNA size (bp)
GAPDH	Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	117	GAAGGTGAAGGTCGGAGTCA	TTGAGGTCAATGAAGGGGTC	1749
NPPB	Natriuretic Peptide B	109	TTTGGGAGGAAGATGGACC	TGTGGAATCAGAAGCAGGTG	651
ATP2A2	ATPase Sarcoplasmic/Endoplasmic Reticulum Ca ²⁺ transporter 2 (ATP2A2)	138	GTGAATCTGTCTCTGTCAATC	CTCCAGTTGCTACCACCACT	1215
CACNA1C	Calcium Voltage-Gated Channel Subunit Alpha 1C	141	ACTTCATCATCCTCTTCATCTGTG	CCAGCTTCTTTCTCTCCTTCTC	1701
TGFB2	transforming growth factor beta 2	146	CTACAGACTTGAGTCACAAC	CGTGTATCCATTCCACCCT	1341
PLN	Phospholamban	138	CTGCCAAGGCTACCTAAAAG	AGCTGAGCGAGTGAGGTATT	10570



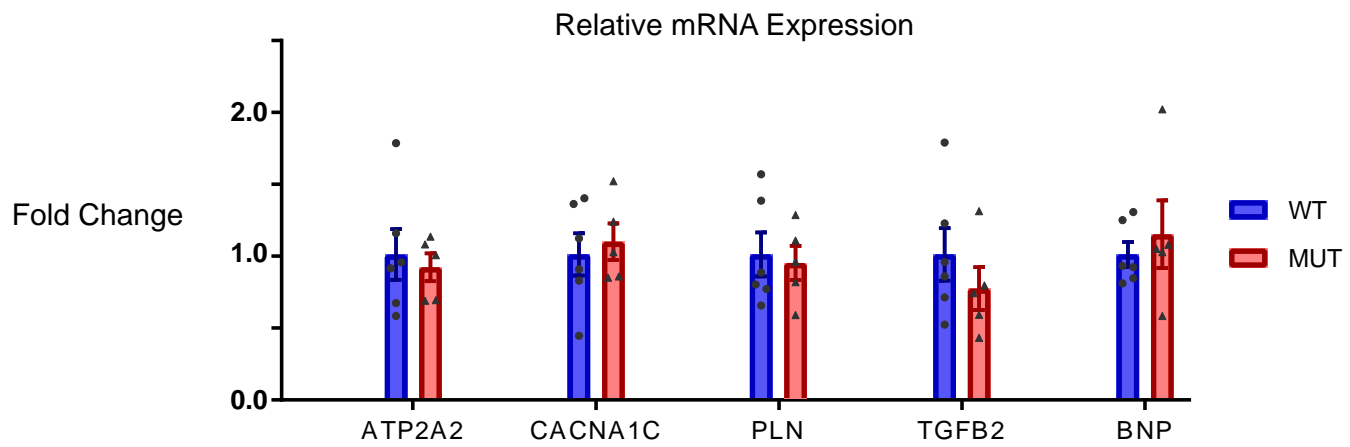
Supplemental Figure 1. Additional Histological findings in wildtype and mutant ECM.

(A/B) Representative H&E imaging of WT and MUT porcine extracellular matrix (pECM). (E/F) Representative Trichrome imaging of WT and MUT pECM. (C/G) Representative fiber alignment quantification using FFT method. (D) Tissue alignment index calculated using nuclear orientation segmentation showed statistically significant decrease in MUT samples (n=21) compared to WT samples (n=24), 0.337 v. 0.5914, $p < 0.0001$ by Mann-Whitney test. (H) Tissue alignment index calculated using fiber orientation segmentation showed statistically significant decrease in MUT samples (n=27) compared to WT samples (n=41), 0.3179 v. 0.1597, $p = 0.001$ by Mann-Whitney test.



Supplemental Figure 2. Active mechanics of EHTs at 16 days.

(A) R403Q mutant ECM EHTs that were cultured for and additional 6 days continued to show an increase in early relaxation (RT50, $p=0.0423$). (B) Time to Peak Tension (TTP) in mutant ECM EHTs was slower ($p=0.0033$). (C) Peak force was significantly increased in mutant ECM EHTs ($p=0.0357$). (D) Change in active force from 1Hz to 2Hz pacing was not significantly different ($p=0.2$).



Supplemental Figure 3. qPCR Analysis of Gene Expression in EHTs.

Transcripts were not found to be significantly different between R403Q mutant ECM EHTs and wild-type ECM EHTs for ATP2A2 ($p=0.68$), CACNA1C ($p=0.67$), PLN ($p=0.77$), TGFB2 ($p=0.35$), and BNP ($p=0.56$).