

Supplemental Material

Investigating *LMNA*-related dilated cardiomyopathy using human induced pluripotent stem cell-derived cardiomyocytes

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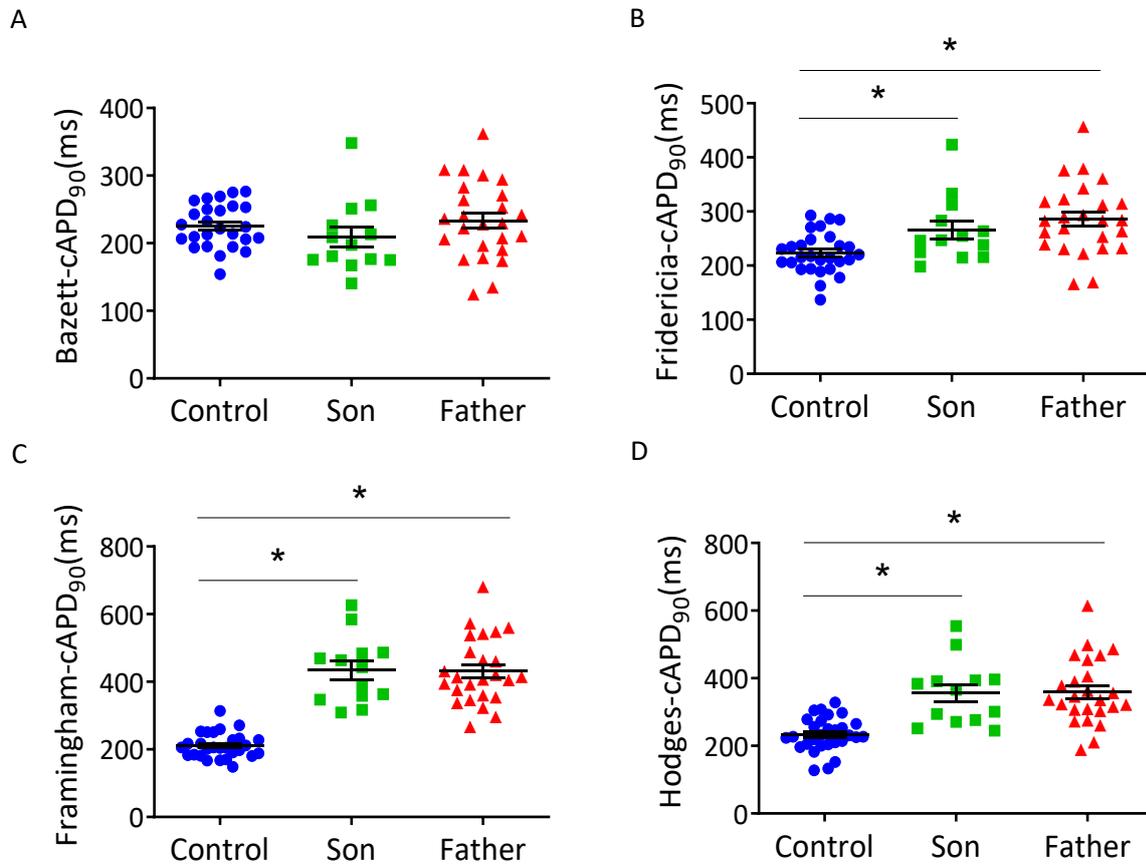
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Supplementary Figures and Figure Legend

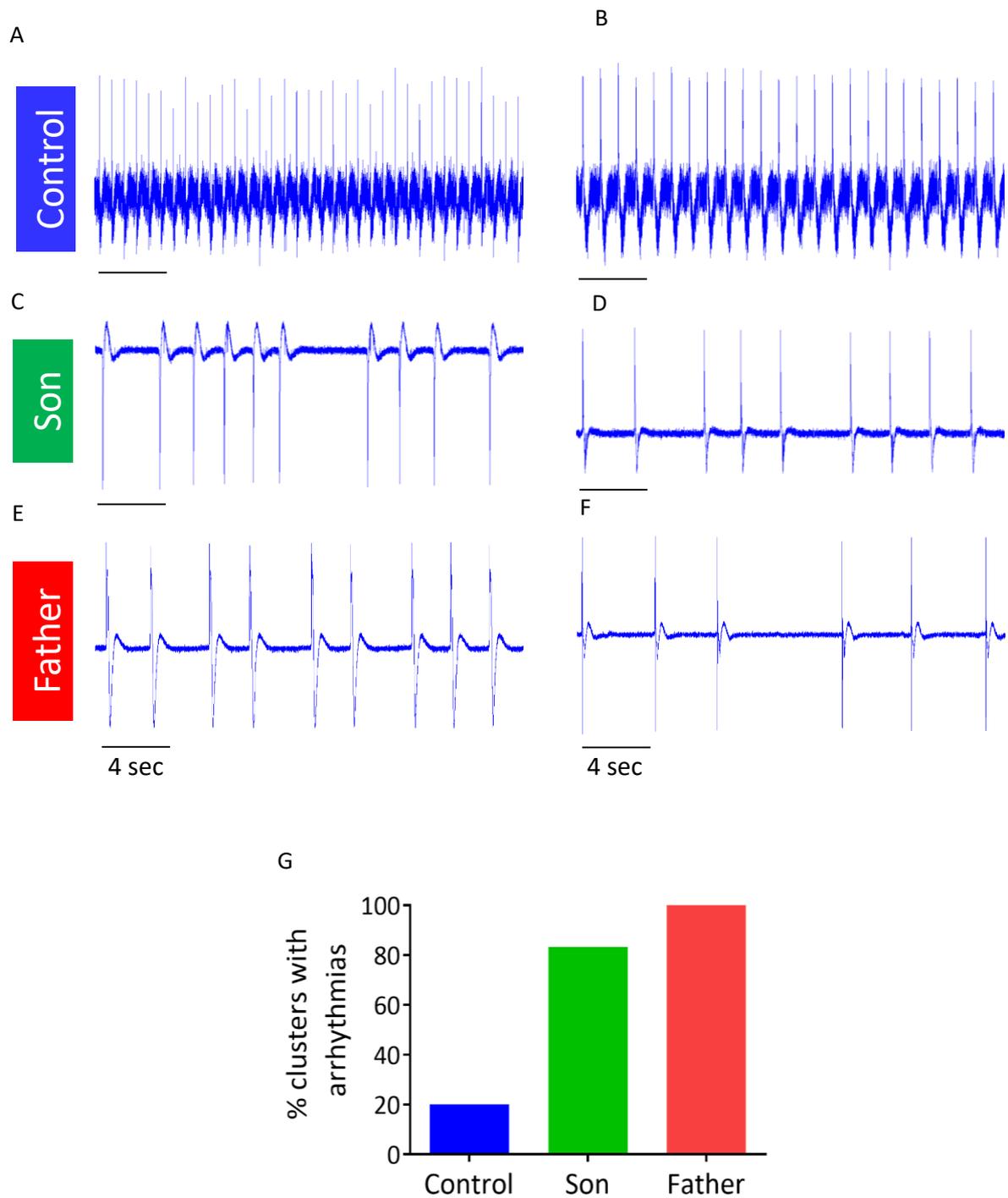
Supplementary Figure S1



Correction of APD₉₀ (cAPD₉₀) for beat rate changes in control, and *LMNA*-mutated father and son iPSC-CMs. (A) cAPD₉₀ with Bazett formula. (B) cAPD₉₀ with Fridericia formula. (C) cAPD₉₀ with Framingham formula. (D) cAPD₉₀ with Hodges formula. Control, n=30; son, n=14; father, n=27. One-way ANOVA was performed followed by Holm-Sidak *post hoc* analysis.

*p<0.05.

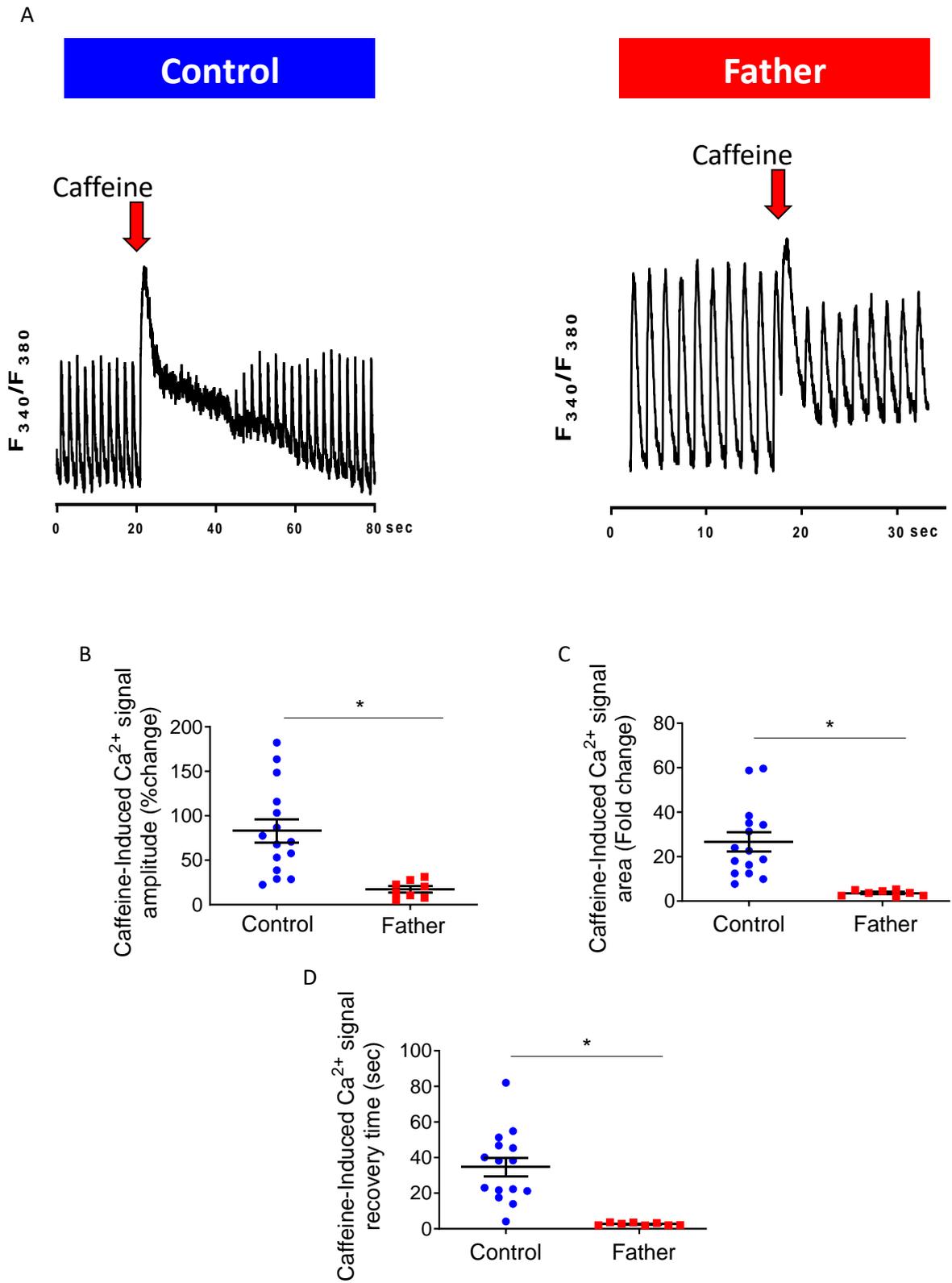
Supplementary Figure S2



Arrhythmias in *LMNA*-mutated iPSC-CMs at the network level. (A-F) Representative recordings of extracellular electrograms from control (A, B), son (C, D) and father (E, F)

spontaneous beating clusters, using the MEA data acquisition system. Note arrhythmias in father and son iPSC-CMs. (G) Percentage of spontaneously beating clusters showing arrhythmias in control, father and son iPSC-CMs (Control, n=5; son, n=6; father, n=4).

Supplementary Figure S3



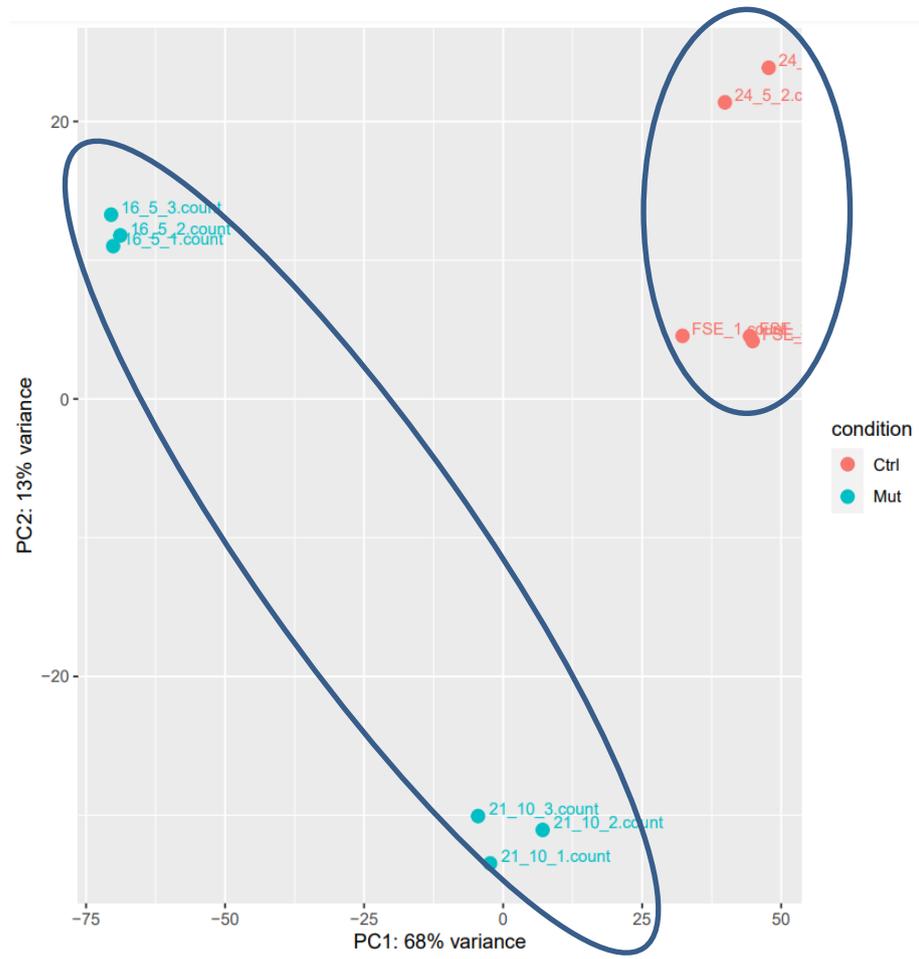
The response of $[Ca^{2+}]_i$ to caffeine (10 mM) in control and father iPSC-CMs. (A)

Representative $[Ca^{2+}]_i$ transients from control and father *LMNA*-mutated iPSC-CMs

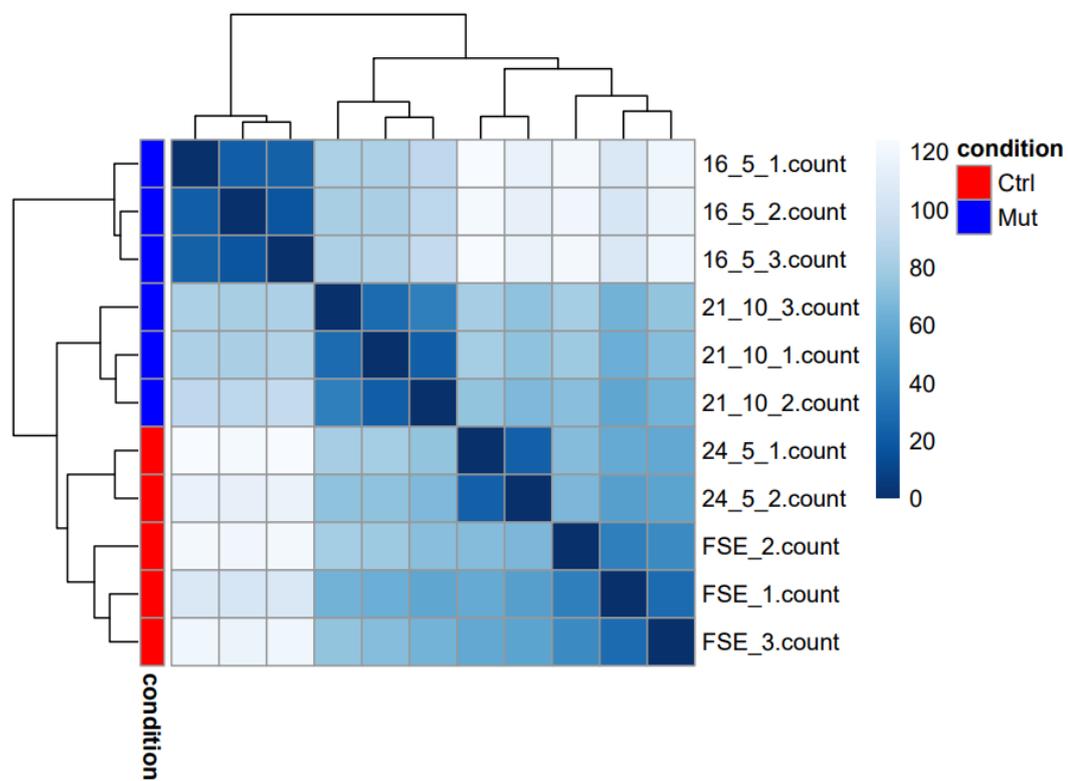
demonstrate the effect of caffeine. (B) Percent change in caffeine-induced Ca^{2+} signal amplitude compared to the pre-caffeine amplitude; (C) Percent change in area of the caffeine-induced $[\text{Ca}^{2+}]_i$ signal compared to the pre-caffeine area; (D) The mean recovery time, calculated as the time from the peak of caffeine-induced $[\text{Ca}^{2+}]_i$ rise to the first measurable $[\text{Ca}^{2+}]_i$ transient. Control, n=16; father, n=8. One-way ANOVA was performed followed by Holm-Sidak *post hoc* analysis. *p<0.05.

Supplementary Figure S4

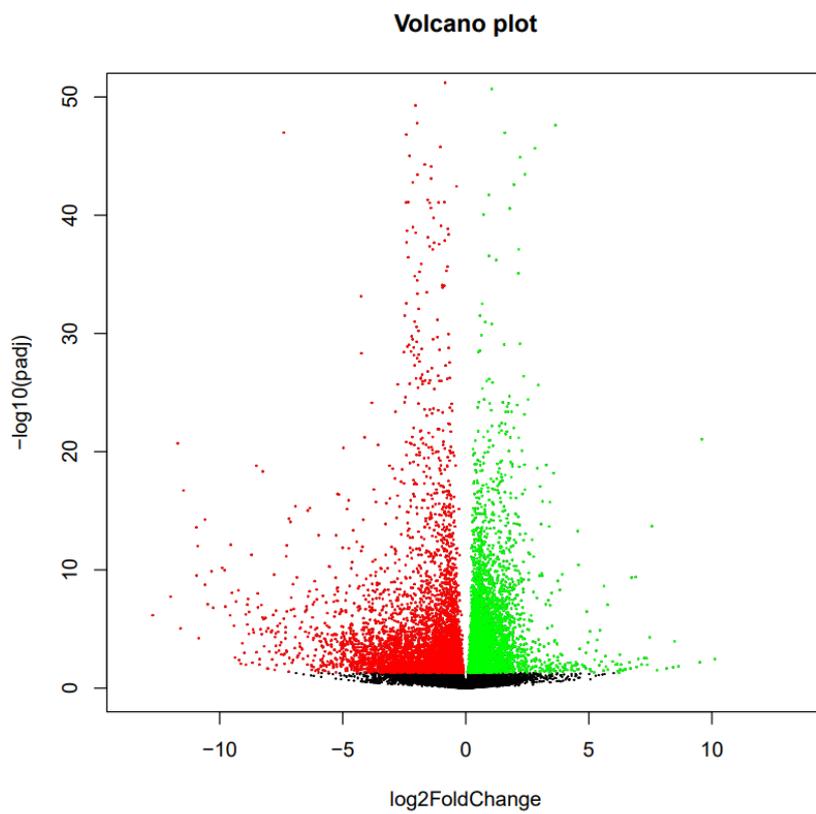
A



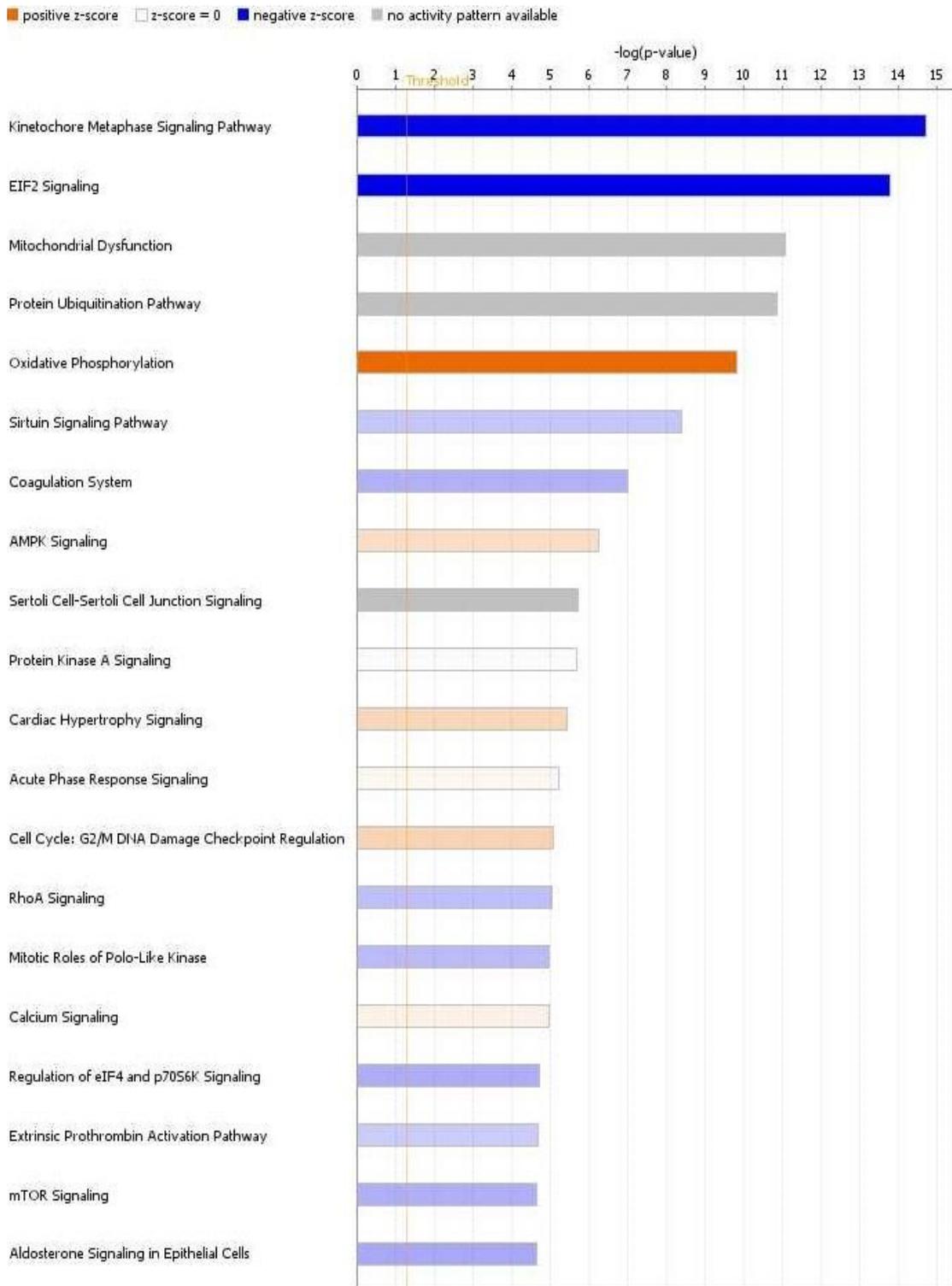
B



C



D



Altered gene expression in *LMNA*-mutated iPSC-CMs. (A) PCA and (B) heatmap of gene expression profile in control (clones 24.5 and FSE-5m), and father (clone 16.5) and son (clone

21.10) *LMNA*-mutated iPSC-CMs. (C) Volcano plot showing gene expression changes in *LMNA*-mutated iPSC-CMs compared to control iPSC-CMs. The x-axis shows the fold-change in gene expression between the two groups. The y-axis shows the statistical significance of the differences. The black dots represent genes without significant different expression. The green dots represent significantly upregulated genes. The red dots represent significantly downregulated genes. A total of 9,794 differentially expressed genes (DEGs) were identified ($p_{adj} < 0.05$). (D) Ingenuity Pathway Analysis (IPA) showing the top 20 canonical pathways enriched with DEGs in *LMNA*-mutated iPSC-CMs compared to control iPSC-CMs.

Supplementary Tables

Table S1: Shared rare variants between both father and son iPSCs

Chr.	HGVS	Protein	Gene	Type	Classification ¹	ID	MAF*
1	c.1024G>A	p.(Glu342Lys)	LMNA	Missense	Likely pathogenic	-	-
3	c.233C>T	p.(Thr78Met)	CAV3	Missense	Uncertain significance	rs72546668	0.0027

Table S2: Additional rare variant only found in the father iPSCs

Chr.	HGVS	Protein	Gene	Type	Classification ¹	ID	MAF*
2	c.52384C>T	p.(Leu17462Phe)	TTN	Missense	Uncertain significance	-	-

*Genome Aggregation Database (gnomAD; <https://gnomad.broadinstitute.org/>) (accessed on September 2019).

Gene Transcripts: LMNA (NM_170707.3), TTN(NM_001267550.2), CAV3 (NM_033337.2).

Abbreviations: Chr., chromosome; HGVS, *Human Genome Variant Society*-nomenclature; ID, identification number of reference SNP; MAF, minor allele frequency; LMNA, lamin A/C; CAV3, caveolin-3; TTN, titin [1].

References

1. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; Voelkerding, K.; Rehm, H.L. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **2015**, *17*, 405–424.