Science Advances
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Supplementary Materials for

FUNDC1 interacts with FBXL2 to govern mitochondrial integrity and cardiac function through an IP3R3-dependent manner in obesity

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Published 16 September 2020, *Sci. Adv.* **6**, eabc8561 (2020)
DOI: 10.1126/sciadv.abc8561

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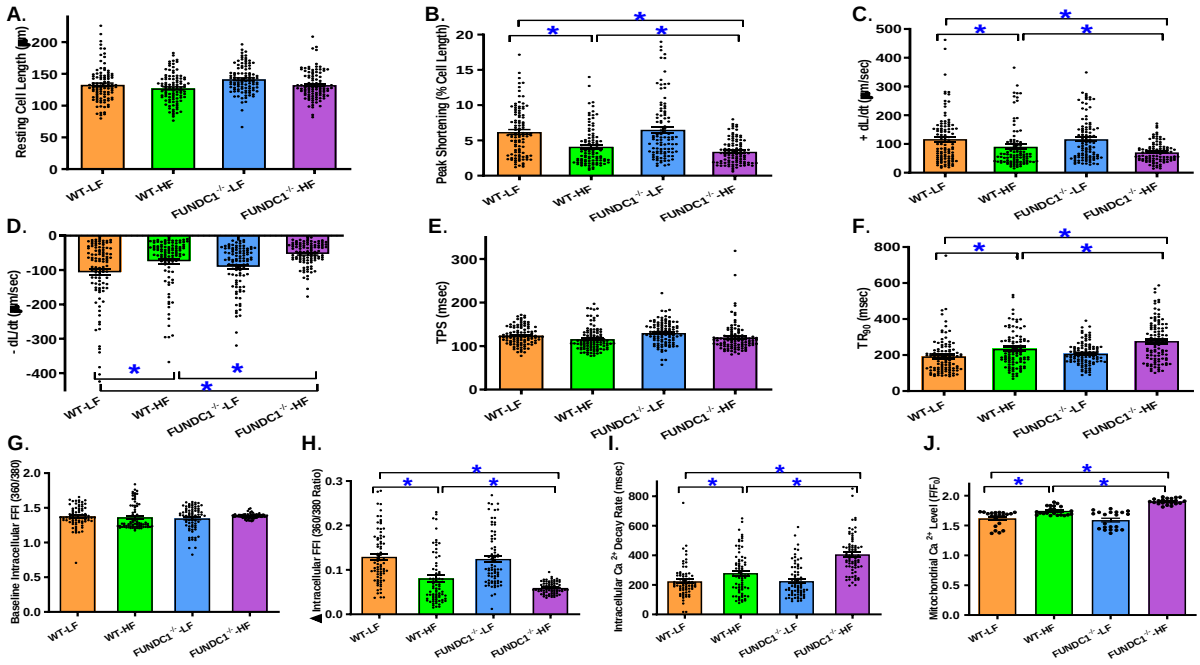
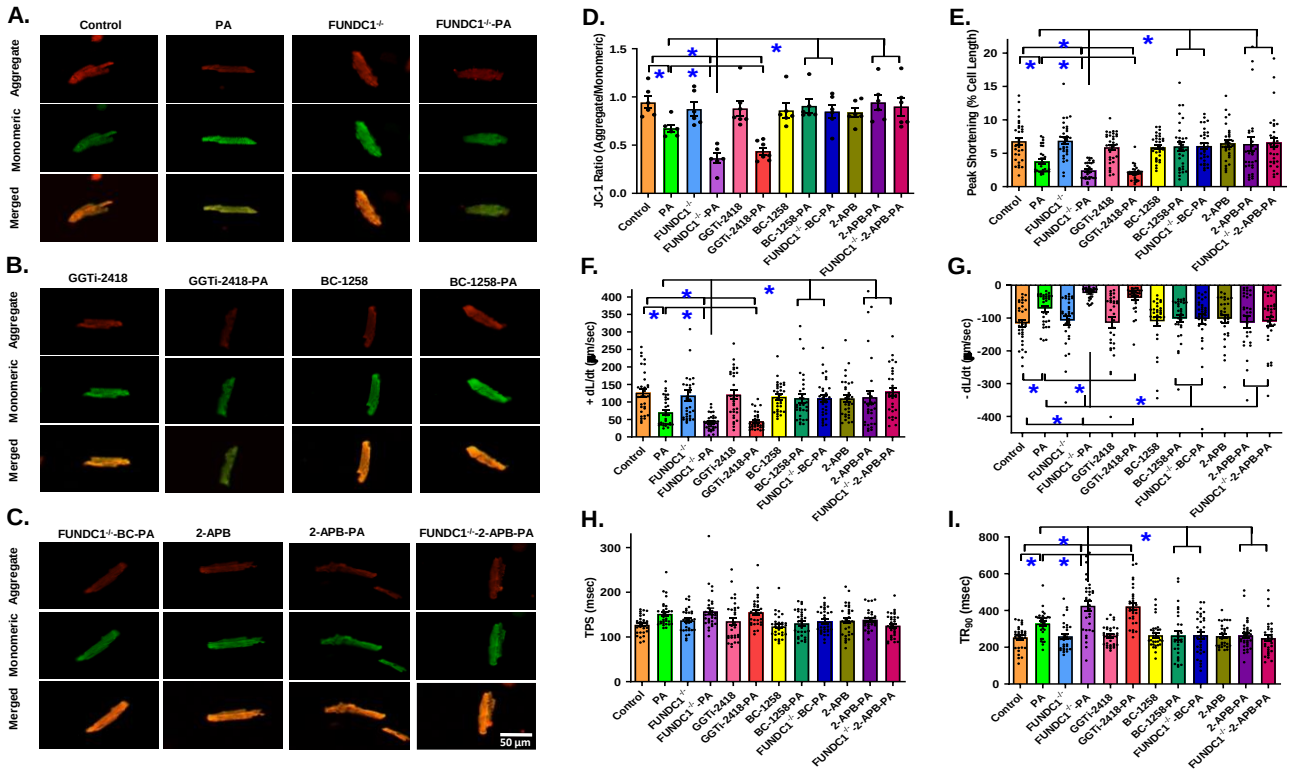
Supplemental Table 1. Primer sequence information for individual genes.

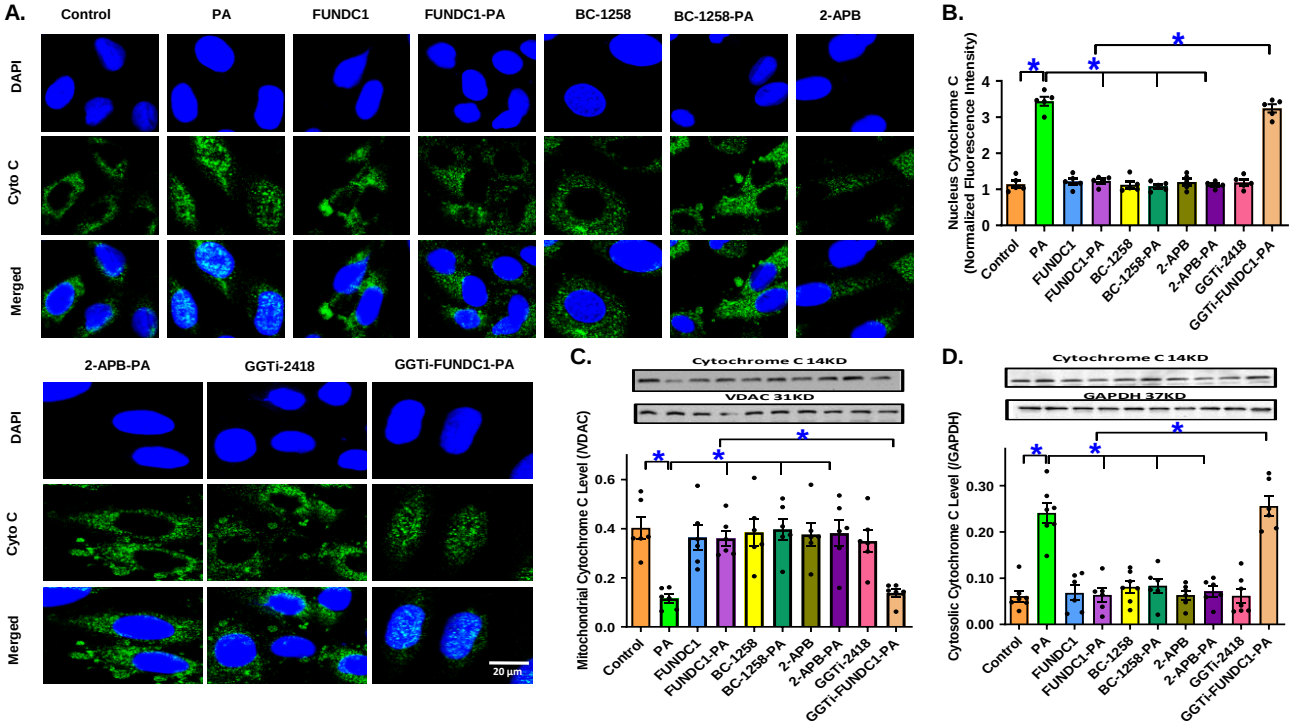
Primers	Forward sequence	Reverse Sequence
ANP	5'-ACAGCCAAGGAGGAAAAGC-3'	5'-CCACAGTGGCAATGTGACCA-3'
BNP	5'-TCCAGAGCAATTCAAGATGCA-3'	5'-CTTTTGTGAGGCCTTGGTCC-3'
GATA4	5'- CCCTACCCAGCCTACATGG-3'	5'- ACATATCGAGATTGGGGTGTCT-3'
GAPDH	5'-ACCACAGTCCATGCCATCAC-3'	5'-TCCACCACCCTGTTGCTGTA-3'



Supplemental Fig. S1: Effect of HF diet intake on cardiomyocyte contractile and intracellular Ca^{2+} properties in WT and FUNDC1$^{-/-}$ mice. A: Resting cell length; B: Peak shortening (% of resting cell length); C: Maximal velocity of shortening (+ dL/dt); D: Maximal velocity of relengthening - dL/dt; E: Time-to-peak shortening TPS; F: Time-to-90% relengthening (TR_{90}); G: Baseline fura-2 fluorescence intensity (FFI); H: Rise in FFI (ΔFFI) in response to electrical stimuli; I: Intracellular Ca^{2+} decay rate; and J: Baseline mitochondrial Ca^{2+} levels using Rhod-2. Mean ± SEM, n = 101 (panel A-F) or 75 (panel G-I) cells from 4 mice per group, * $p < 0.05$ between the indicated groups.



Supplemental Fig. S2: Effect of palmitic acid (PA) on ΔΨm and mechanical properties in cardiomyocytes from WT and FUNDC1$^{-/-}$ mice in the presence or absence of activator of FBXL2 or inhibitors for FBXL2 colocalization and IP3R3. Adult mouse cardiomyocytes were incubated with PA (0.5 mM for 8 hrs) in the presence or absence of the FBXL2 colocalization inhibitor GGTi-2418 (15 µM), the FBXL2 agonist BC-1258 (10 µg/ml) or the IP3R3 inhibitor 2-APB (30 µM) prior to functional assessment. A-C: Representative images of hyperpolarized (J-aggregate) mitochondria (Red fluorescence), depolarized (monomer, Green fluorescence) or merged fluorescence; D: Quantification of ΔΨm expressed as ratio of aggregate/monomer fluorescence; E: Peak shortening; F: Maximal velocity of shortening (+ dL/dt); G: Maximal velocity of relengthening (- dL/dt); H: TPS; and I: TR_{90}. Mean ± SEM, n = 6-7 images (panel D) or 31 cells (panel E-I) per group, * $p < 0.05$ between indicated groups.



Supplemental Fig. S3: Effect of FUNDC1 transfection on palmitic acid (PA)-induced cytochrome C release in neonatal cardiomyocytes in the presence or absence of FBXL2 activator, inhibitors for IP3R3 or FBXL2 colocalization. Neonatal cardiomyocytes were transfected with FUNDC1 overnight prior to incubation with PA (0.5 mM) for another 8 hrs in the presence or absence of the FBXL2 activator BC-1258 (10 µg/ml), the IP3R3 inhibitor 2-APB (30 µM) or the FBXL2 colocalization inhibitor GGTi-2418 (15 µM) prior to assessment of cytochrome C using immunofluorescence or Western blotting. A: Representative images displaying nucleus (DAPI), cytochrome C (Green fluorescence) or merged fluorescence; B: Quantification of cytochrome C; C: Mitochondrial fraction of cytochrome C using Western blot analysis; and D: Cytosolic fraction of cytochrome C using Western blot analysis. Insets: Representative images depicting levels of cytochrome C using VDAC and GAPDH as loading controls for mitochondria and cytosols, respectively. Mean ± SEM, n = 6-8 images or isolations per group, * $p < 0.05$ between indicated groups.