SUPPLEMENTAL MATERIAL

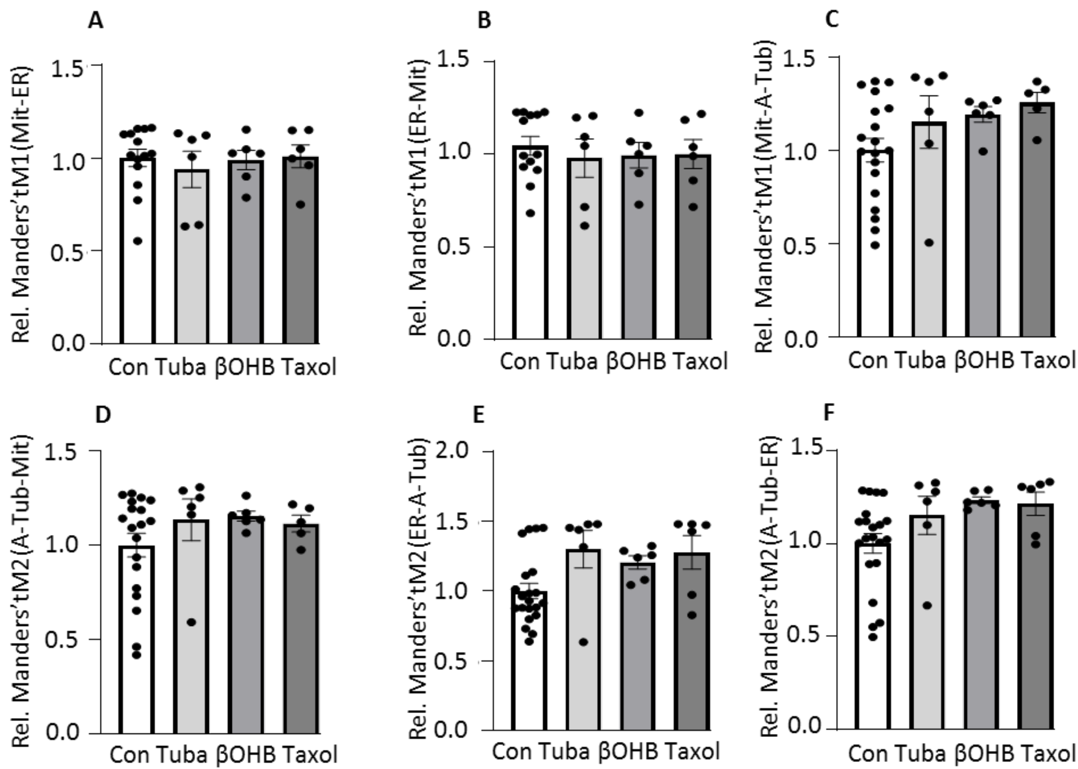
Data S1. Supplemental Methods

Mitochondrial associated membrane isolation

To isolate crude mitochondria, HL-1 cardiomyocyces were rinsed with cold PBS (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM anhydrous sodium phosphate dibasic, and 1.8 mM anhydrous potassium phosphate monobasic), and ice-cold IB_{cells}–1 buffer (containing 225 mM mannitol, 75 mM sucrose, 0.1 mM EGTA, 30 mM Tris-HCl pH=7.4, 0.1 mM EGTA) was added, and cells were homogenized using a Teflon pestle at 2,000 rpm. Homogenization was checked under a light microscope and the homogenate was transferred to a 15 ml tube and centrifuged at 600 *g* for 5 min at 4 °C. Afterwards, the supernatant was collected in a new 15 ml tube and centrifuged at 600 *g* for 5 min at 4 °C. Next, the supernatant was collected into a new 15 ml tube and centrifuged at 7,000 *g* for 10 min at 4 °C, and the pellet was gently resuspended in 2 ml ice-cold IBcells-2 buffer (containing 225 mM mannitol, 75 mM sucrose, 0.1 mM EGTA, 30 mM Tris-HCl pH=7.4), followed by centrifugation at 7,000 *g* for 10 min at 4 °C. The supernatant was discarded and the mitochondrial pellet resuspended in 20 ml of ice-cold IBcells-2 buffer. The mitochondrial suspension was centrifuged at 10,000 *g* for 10 min at 4 °C, the supernatant discarded and the mitochondrial pellet was gently re-suspended in 2 ml of ice-cold mitochondria resuspending buffer (MRB) (250-mM mannitol, 5-mM HEPES pH 7.4 and 0.5-mM EGTA). For fractionation of crude mitochondria, the mitochondrial suspension was layered above 8 ml of percoll medium (Sigma-Aldrich) in the 14 ml thin-wall polyallomer ultracentrifuge tubes (Beckman-Coulter). Afterwards, MRB solution (about 3.5 ml) was gently layered on top of the mitochondrial suspension to fill up the centrifuge tube (the suspension should remain 4-5 mm below the top of the tubes), and the tube was centrifuged at 95,000 *g* for 30 min at 4 °C. A dense band containing purified

mitochondria is localized approximately at the bottom of the ultracentrifuge tube, and MAM is visible as the diffused white band located above the purified mitochondria. The MAM fraction was carefully collected from the percoll gradient with a Pasteur pipette, diluted ten times with MRB and centrifuged at 6,300 *g* for 10 min at 4 °C. The supernatant containing MAM was transferred to a 10 ml polycarbonate tube with cap (Beckman-Coulter) and centrifuged at 100,000 *g* for 1 h at 4 °C, the supernatant was discarded and the MAM containing pellet was re-suspended in a small volume of MRB (200 µl) and stored at -20 freezer for further use.

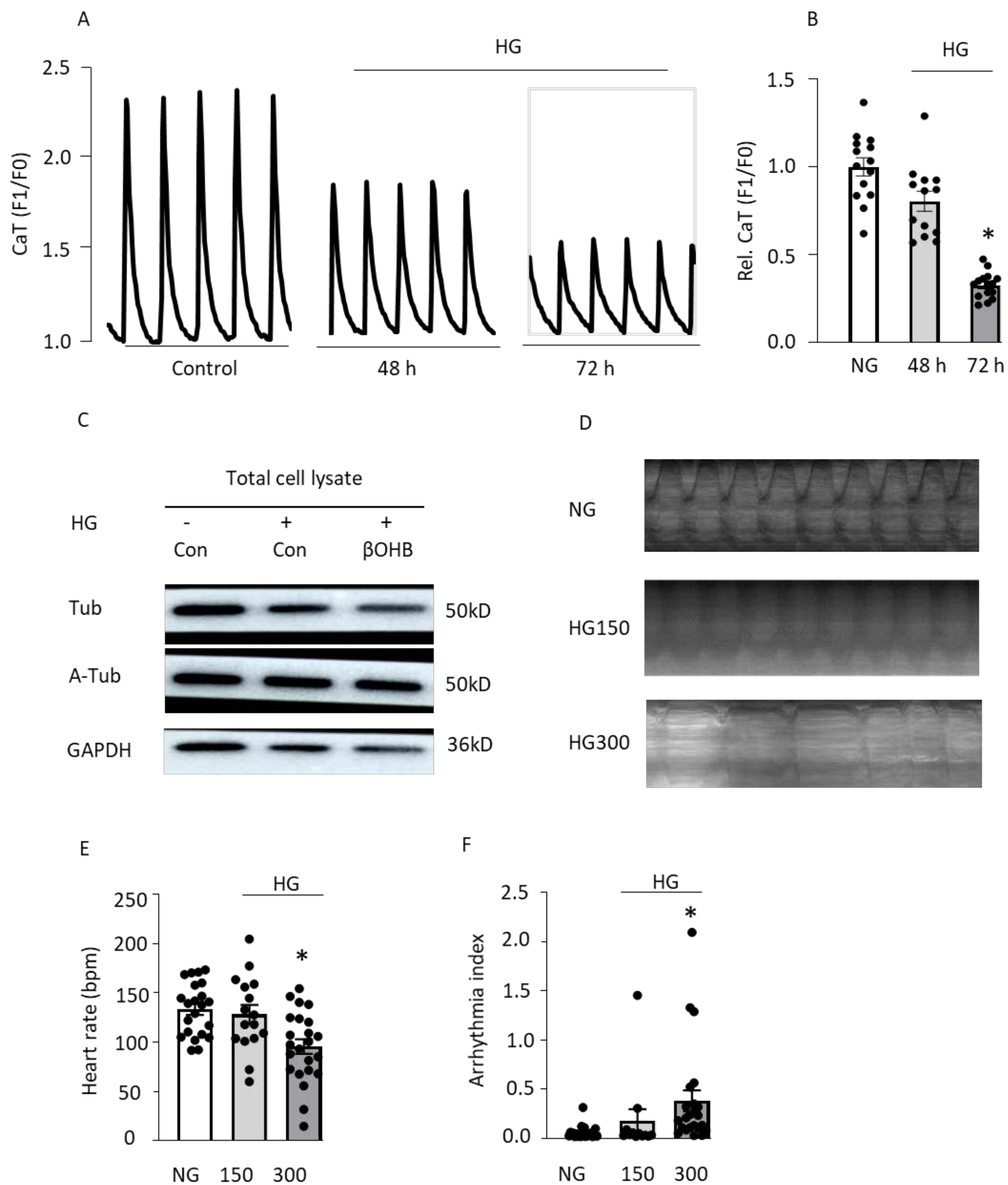
Figure S1. Microtubule stabilizers tubacin, taxol and β-hydroxybutyrate **(βOHB) do not affect the co-localization between ER/SR, mitochondria, and microtubules in control HL-1 cardiomyocytes without tachypacing.**



A-B) No differences were observed in the percentage of co-localization of mitochondria (Mit) and ER/SR among the various groups. Manders' tM1: the fraction of mitochondria colocalized with ER. Manders' tM2: the fraction of ER colocalized with mitochondria. C-D) No difference was found in the percentage of colocalization between mitochondria and microtubule among the various groups. Manders' tM1: the fraction of mitochondrial colocalized with microtubule. Manders' tM2: the fraction of microtubule colocalized with mitochondria. E-F) No change was observed in the percentage of colocalization between ER and microtubule among the various groups. Manders' tM1: the fraction of ER colocalized with microtubule. Manders' tM2: the

fraction of microtubule colocalized with ER. Data are shown as mean ± SEM. Statistical analysis was performed with one-way ANOVA followed by Tukey's multiple comparisons test.

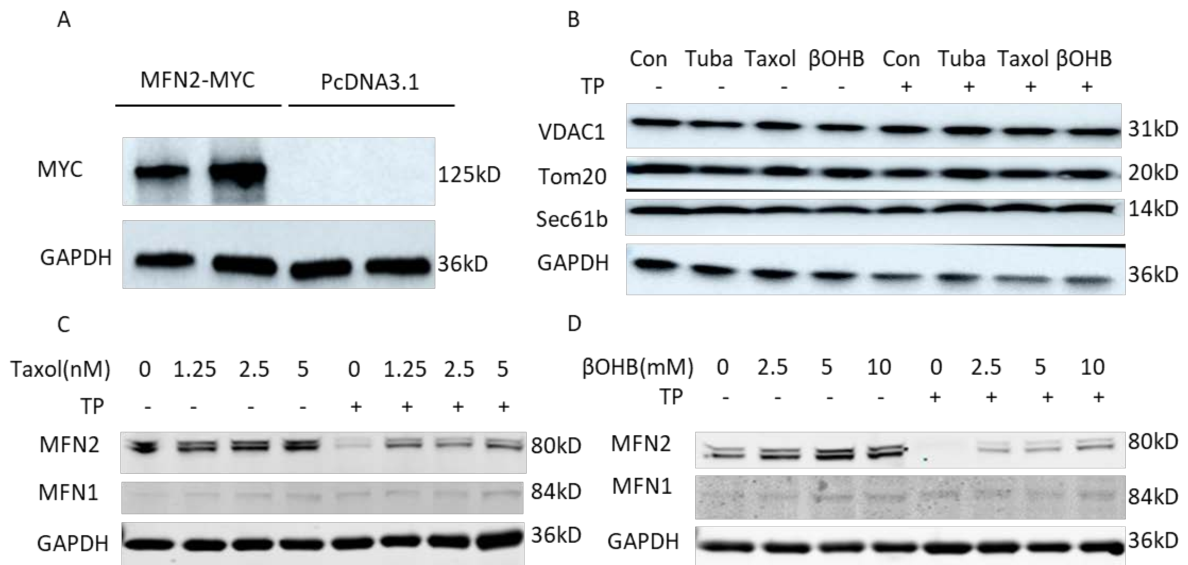
Figure S2. High glucose induces contractile dysfunction in HL-1 cardiomyocytes and *Drosophila.*



A-B) HL-1 cardiomyocytes were treated with 25 mM high glucose (HG) for 48 h and 72 h, following by calcium transient (CaT) measurement. After 72 h HG stimulation, CaT in HL-1 cardiomyocytes was significantly reduced. *P<0.05 vs Control with normal glucose (NG). C) HG had no effect on the levels of α-tubulin and acetylated α-tubulin in HL-1 cardiomyocyte, and pretreatment with βOHB did not alter microtubule proteins as well. D-F) Heart wall contraction of prepupa exposed to normal food and the food with high HG 150g/L and 300

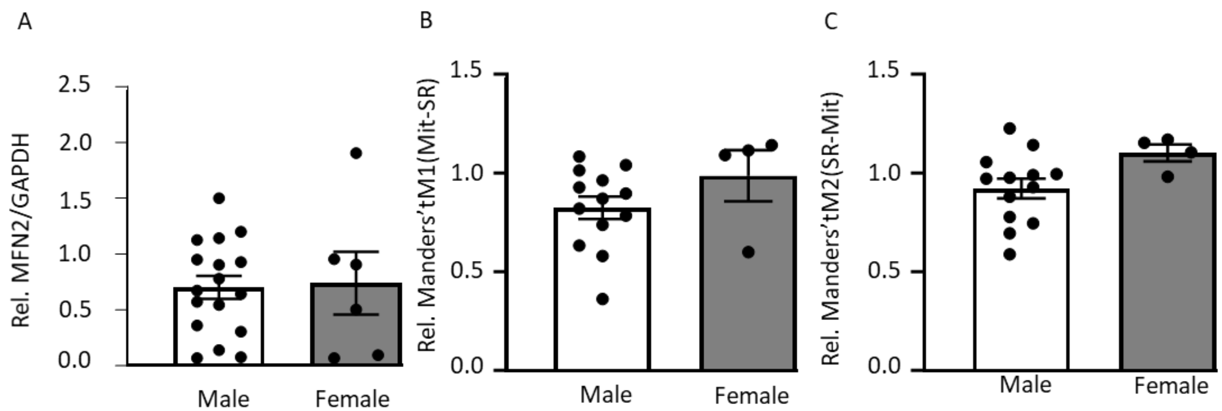
g/L) were monitored. HG reduced heart rate and increased arrhythmia index in *Drosophila* prepupa. Statistical analysis was performed with one-way ANOVA followed by Tukey's multiple comparisons test. *$P<0.05$ vs Control (NG).

Figure S3. Representative Western blot images of MFN2-MYC overexpression, effect of microtubule stabilizers on total ER/SR and mitochondrial markers, and dose-dependent) effect of microtubule stabilizers on MFN2/MFN1 levels in HL-1 cardiomyocytes.



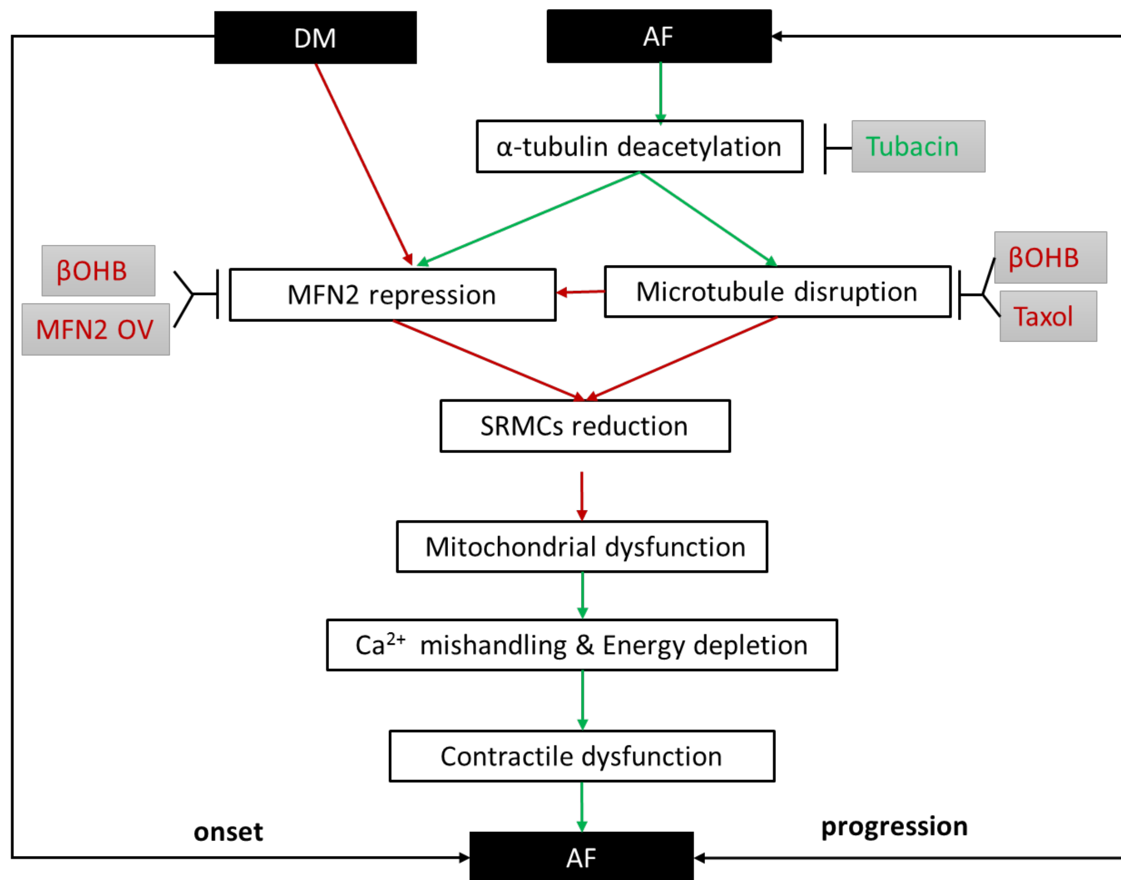
A) Representative Western blot showing successful overexpression of MFN2 in HL-1 cardiomyocytes. HL-1 cardiomyocytes were transfected with either MFN2-MYC or control PcDNA3.1 plasmid for 48 h, and then Western blot experiments were performed after transfection. B) Representative Western blot showing that in whole cell lysate the protein levels of mitochondrial markers VDAC1 and TOM20, and ER marker SEC61b were not affected by the microtubule stabilizers including 1 μM Tubacin (Tuba), 5 nM taxol and 10 mM β-hydroxybutyrate (βOHB). C and D) Representative Western blot showing the dose-dependent effect of microtubule stabilizers Taxol and βOHB on MFN2 but not on MFN1. HL-1 cardiomyocytes were pretreated with the different doses of microtubule stabilizers: Taxol (0, 1.25, 2.5, 5 nM) (C) or βOHB (0, 2.5, 5, 10 mM) (D) with or without tachypacing (TP) and then Western Blot experiment were performed to check MFN2 and MFN1 expression. At least two independent experiments have been performed, similar results were observed.

Figure S4. Sex comparison of SR mitochondria contacts (SRMCs) in patients.



No significant difference was found of the levels of SRMC tether protein MFN2 (A) and SR-mitochondrial colocalization between male and female patients (B, C). Manders' tM1: the fraction of mitochondria colocalized with SR. Manders' tM2: the fraction of SR colocalized with mitochondria. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons test.

Figure S5. Proposed schematic model for the role of SR mitochondria contacts (SRMCs) in the onset and progression of atrial fibrillation (AF).



Tachypacing induces the deacetylation of α-tubulin, followed by disruption of microtubules and MFN2 repression, both of which contributed to SRMC reduction, and subsequently lead to contractile dysfunction and AF progression. Pretreatment with microtubule stabilizers (taxol or βOHB) or MFN2 overexpression (MFN2 OV) inhibited tachypacing-induced SRMC reduction and contractile dysfunction. High glucose (HG, mimicking DM) induced MFN2 reduction and consequent loss of SRMCs, and thereby contributing to contractile dysfunction and AF onset. Furthermore, βOHB or MFN2 OV also attenuated HG-induced loss of SRMCs and contractile dysfunction. Green arrows: discovered previously. Red arrows: discovered in this study.