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small methods

Supporting Information

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Combined Effects of Electric Stimulation and Microgrooves in Cardiac Tissue-on-a-Chip for Drug Screening

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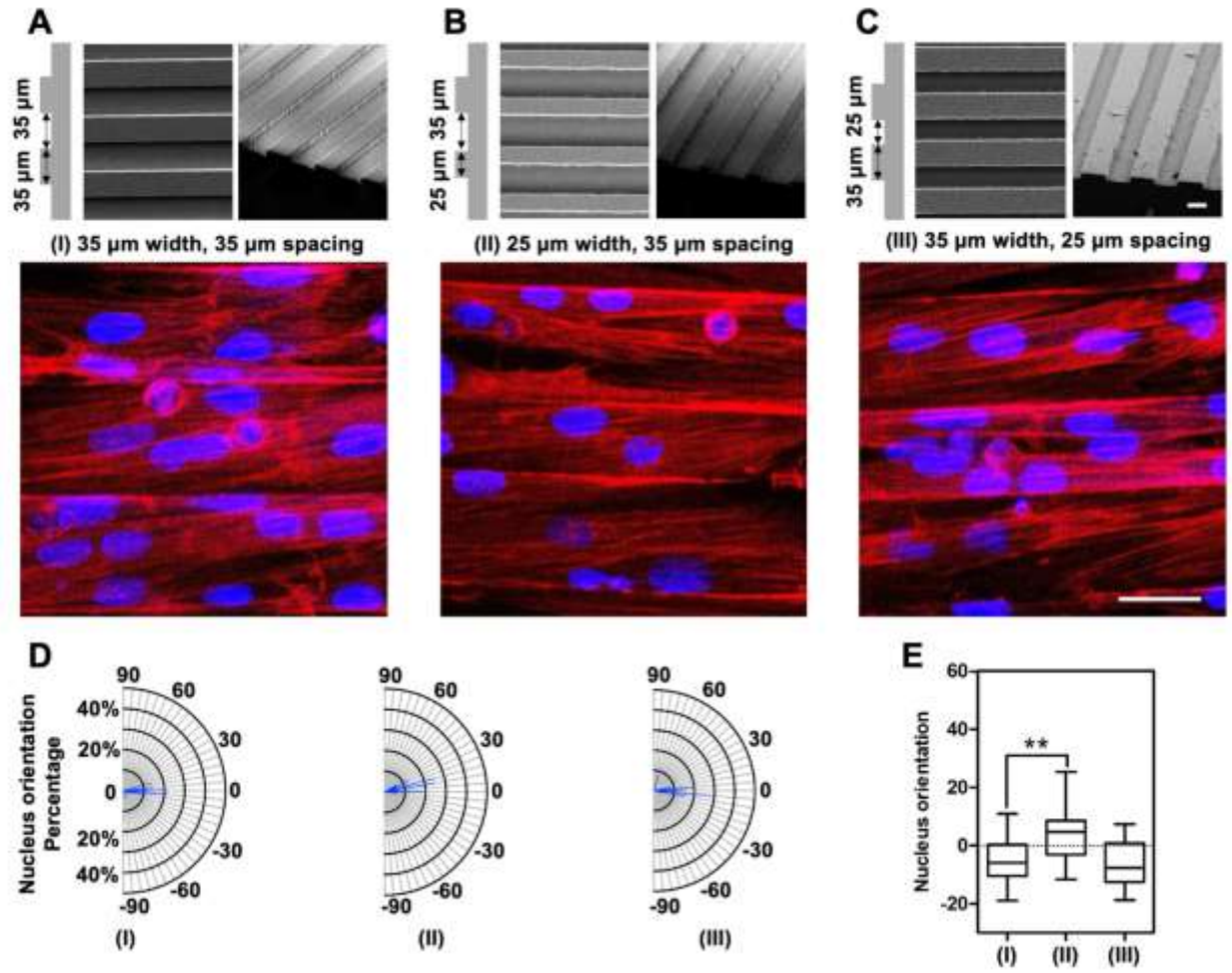

Figure S1. Optimization of the size of PDMS MGs to culture CMs. A, B, C. SEM images of PDMS MGs with different groove sizes (scale bar = 25 µm), and corresponding F-actin staining (red) of cultured CMs (nucleus in blue) (scale bar = 25 µm). D. Frequency of CMs with different nucleus orientations and E. Nucleus orientation analysis of cultured CMs under (I) 35 µm width and 35 µm spacing, (II) 25 µm width and 35 µm spacing, and (III) 35 µm width and 25 µm spacing. The nucleus orientation is the sum of CMs angles shown in D. (~ 30 nuclei were analyzed for each group, $^{**}p < 0.01$).

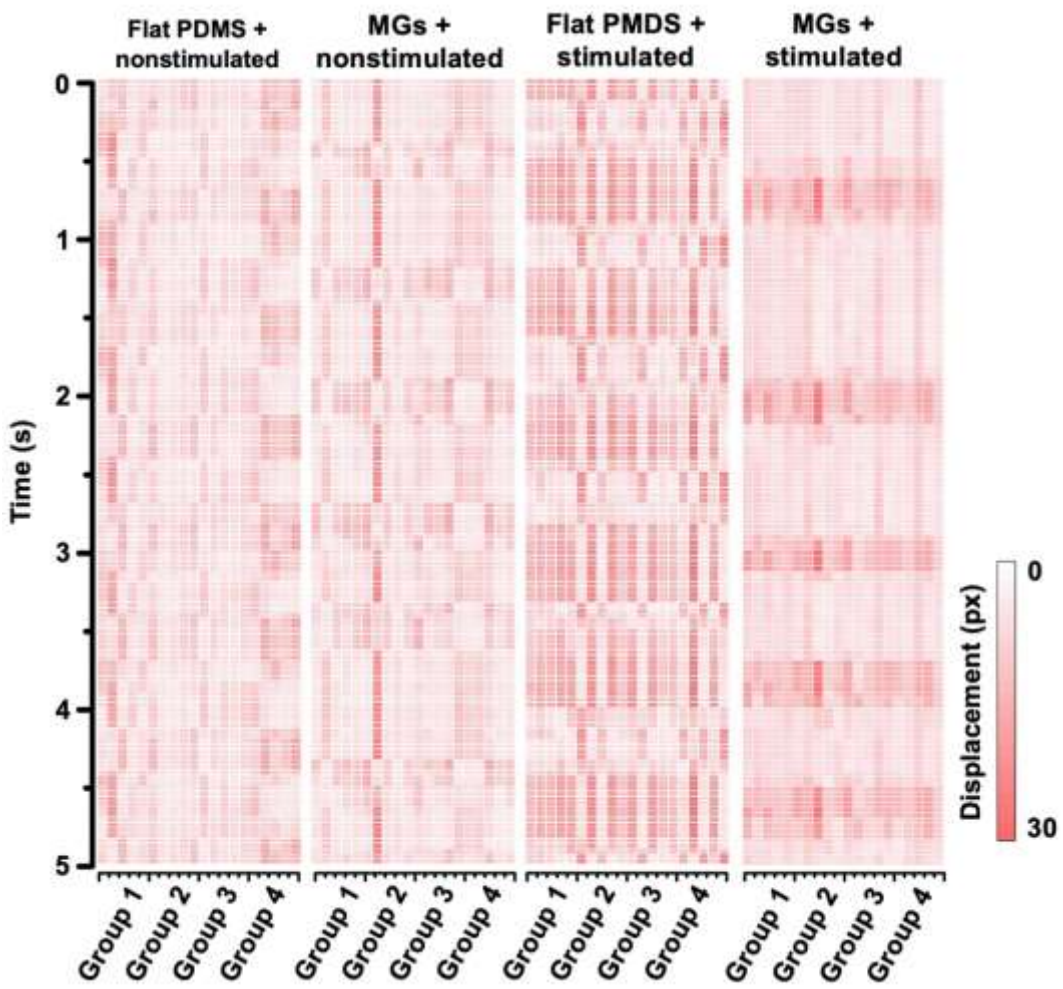

Figure S2. Heatmap analysis of CM beating under: Flat PDMS + nonstimulated, MGs + nonstimulated, flat PDMS + nonstimulated, and MGs + stimulated conditions. The beating behavior is represented as a displacement change of the cells, which is measured as the change in pixels (px). 5 cells from each independent testing group were analyzed (n = 4).

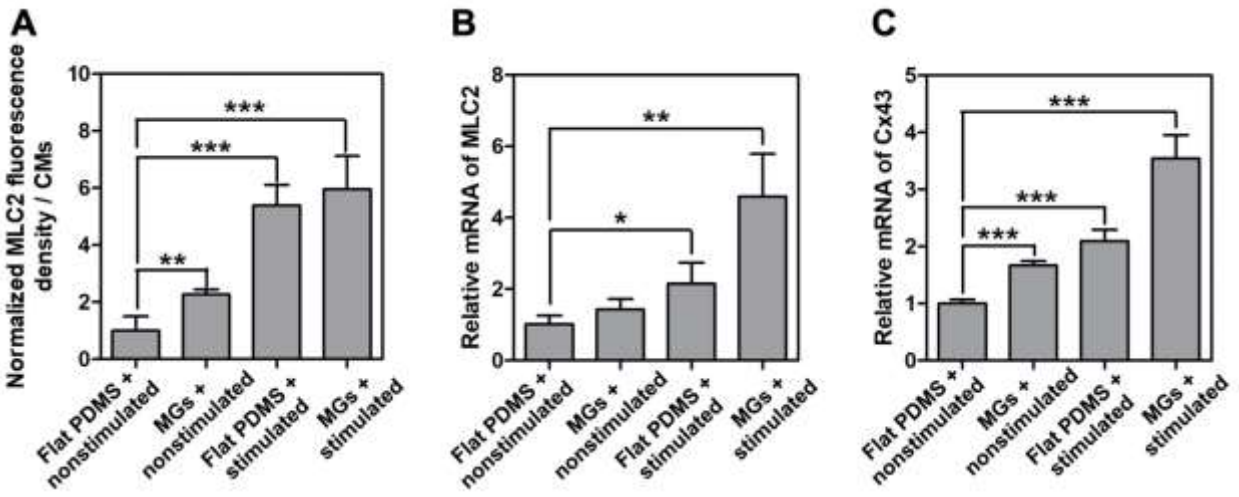

Figure S3. A. Quantitative analysis of MLC2 fluorescence intensities per cell. B. RT-qPCR analysis of MLC2 mRNA levels relative to the housekeeping gene GADPH. C. RT-qPCR analysis of CX43 expressions relative to the housekeeping gene GADPH. CMs were cultured cultured under: Flat PDMS + nonstimulated, MGs + nonstimulated, PDMS + nonstimulated, and MGs + stimulated condition. (Data represents mean ± SD, n ≥ 3; *p<0.05, **p<0.01, ***p<0.001)

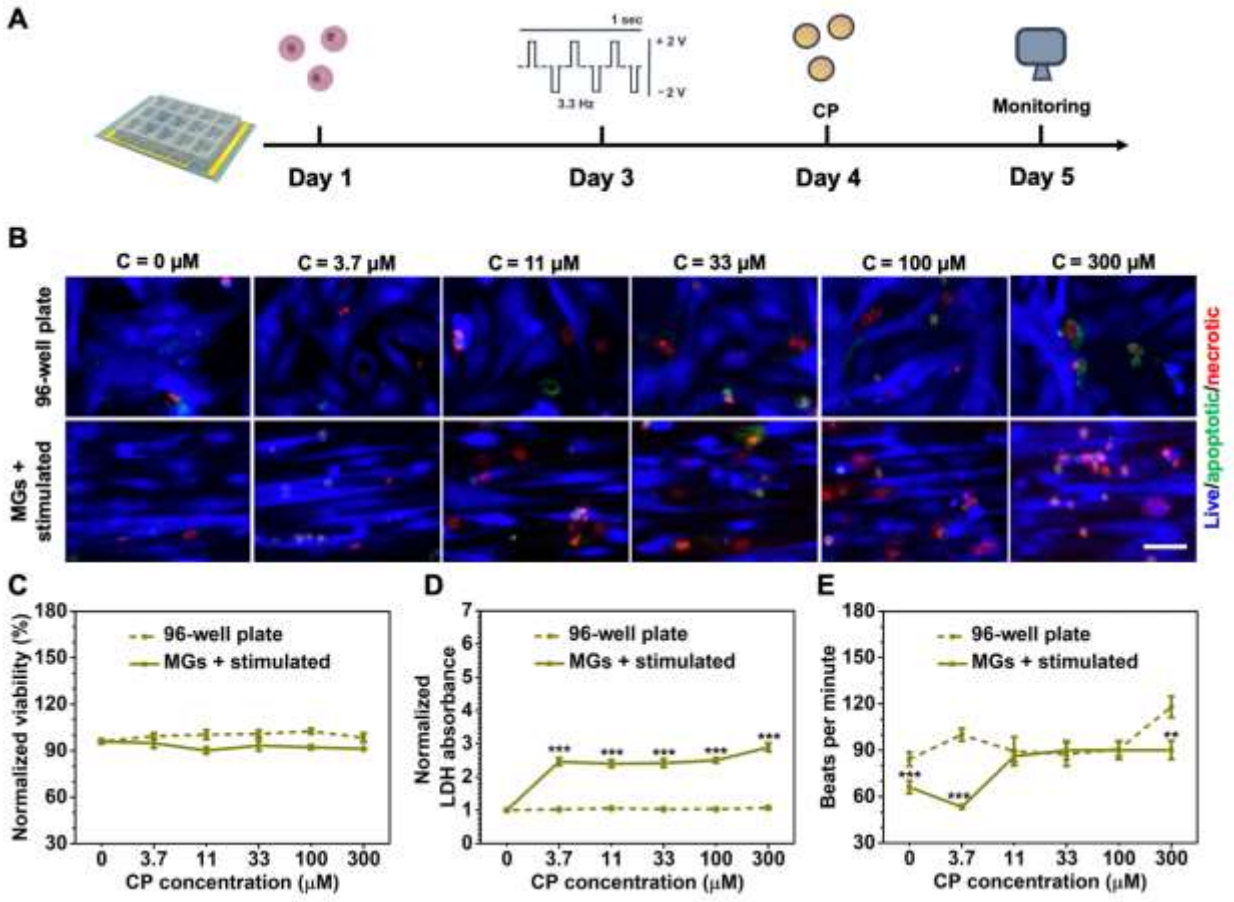

Figure S4. Cardiotoxicity assessment induced by CP. A. Schematic timeline of cardiotoxicity evaluations enabled by Heart-Chip. B. Representative fluorescent images of CMs viability treated by different dosages of CP (Scale bar = 50 μm, CMs were seeded on Day 1, stimulated on Day 3, followed by the addition of the CP on Day 4, and imaged on Day 5). C. CCK-8 assay for quantitative analysis of CM viability treated by increasing doses of CP. D. LDH assay for quantitative analysis of CMs viability treated by increasing dosage of CP. E. Quantification of beating behavior of CMs treated by increasing dosage of CP represented as beats per minute. (S4C and S4D data are mean ± SEM, n ≥ 3; S4E data are mean ± SD, n = 3; $^{**}p < 0.01$ and $^{***}P < 0.001$).

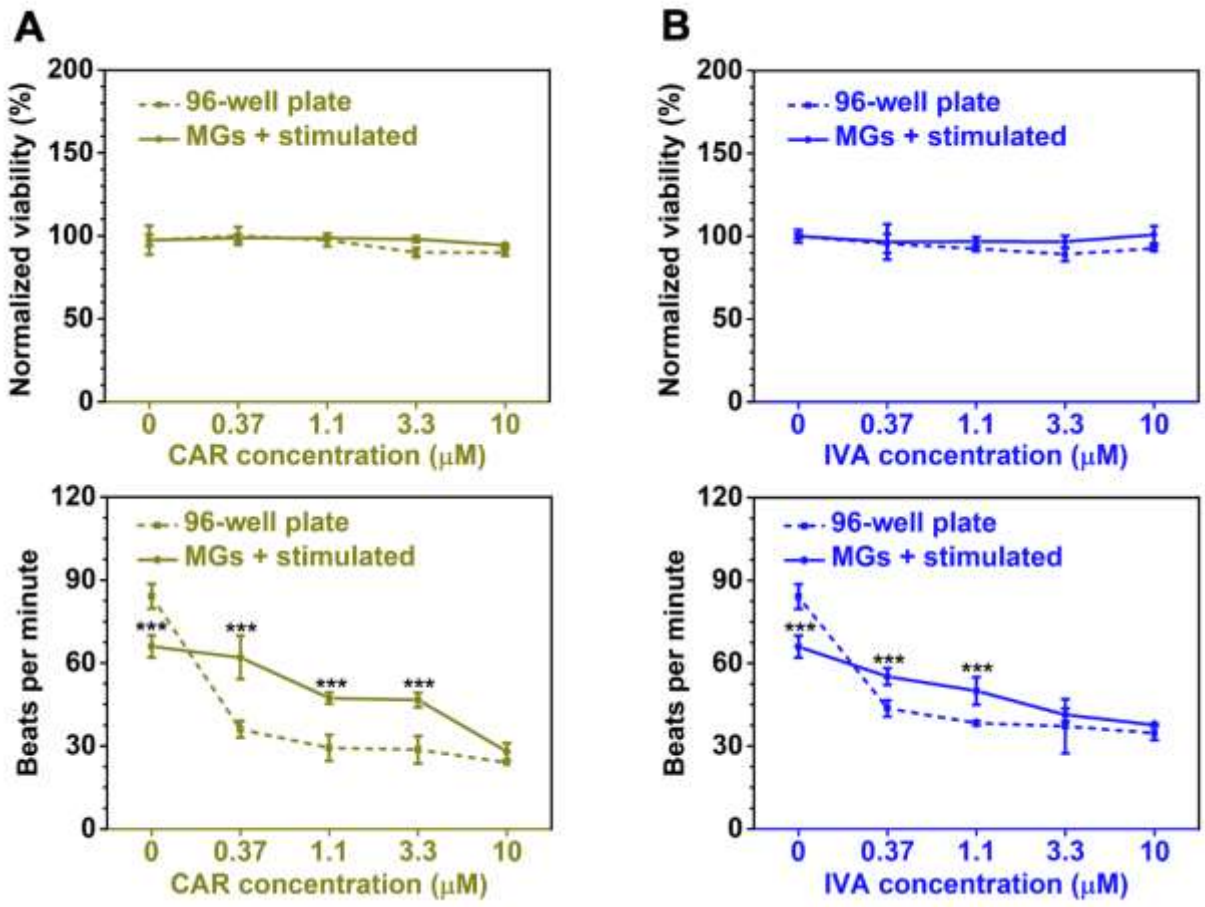

Figure S5. Cardioprotective efficacy assessment of CAR and IVA only treatment. A. CCK-8 viability assay and beating behavior quantification of CMs treated under increasing dosages of CAR. B. CCK-8 viability assay and beating behavior quantification of CMs treated under increasing dosages of IVA. (Normalized viability data are mean ± SEM, n ≥ 3; beats per minute data are mean ± SD, n ≥ 3; ***$p < 0.001$).