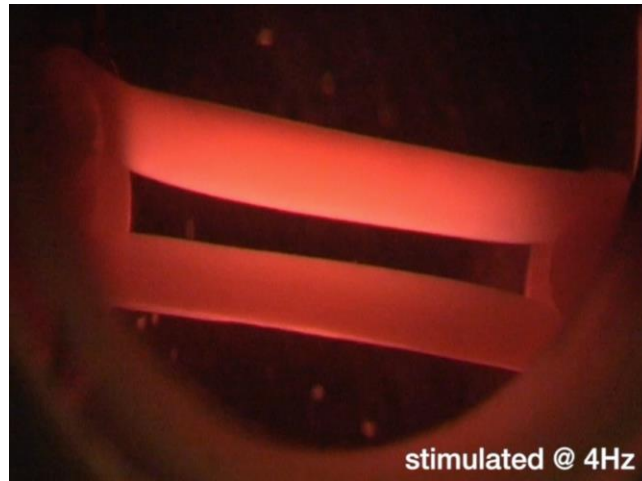
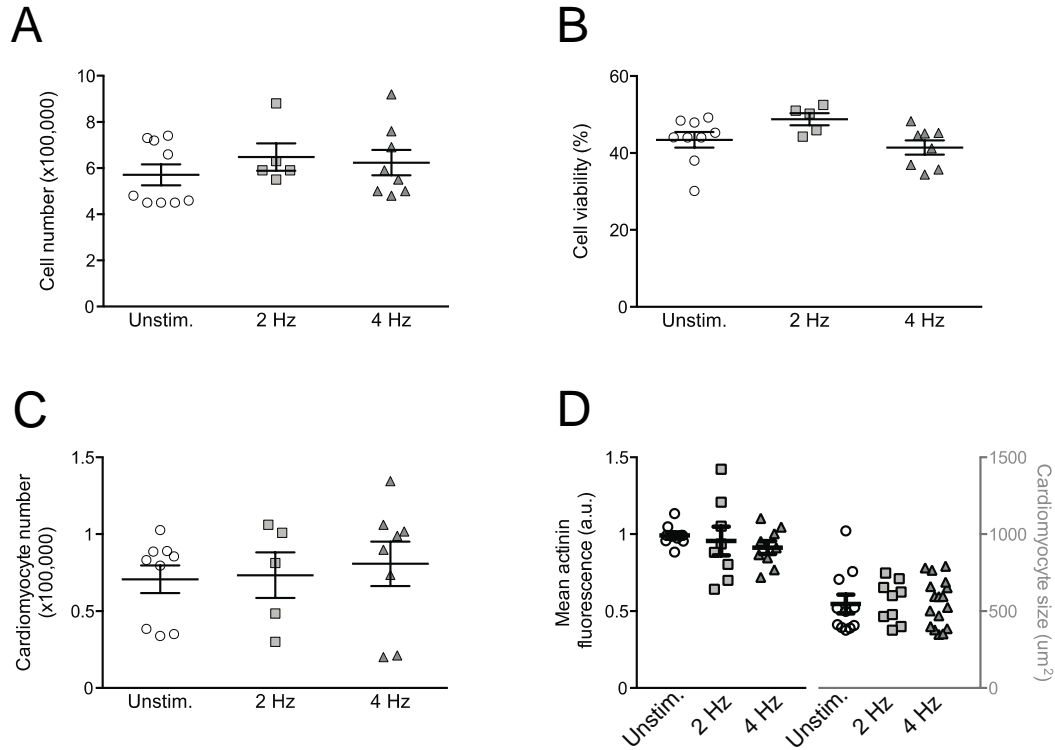


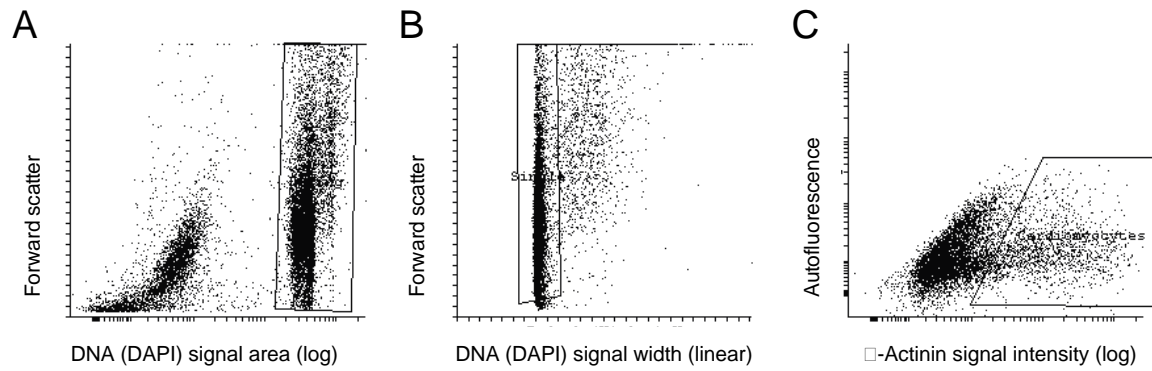
## Supporting Information



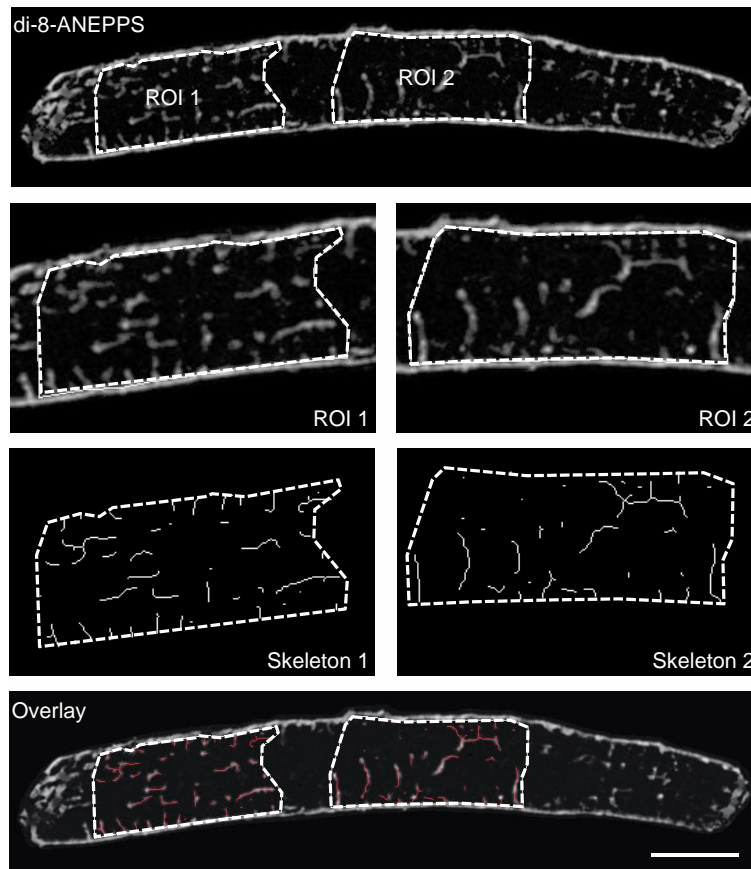
**Supplemental Movie.** Positioning of the C-dish connected to the C-Pace EP Culture Pacer (Ionoptix) for electro-mechanical support of EHM followed by video sequences showing unstimulated, 2 Hz and 4 Hz stimulated auxotonically contracting EHM on culture day 8.



**Figure S1. Cell characteristics in EHM on culture day 13.** Analyses were performed in cardiomyocytes enzymatically isolated from EHM. **A.** Total cell number per EHM measured by the current occlusion method; n=9/5/8 EHM per group. **B.** Cell viability measured using the highly sensitive current exclusion method; n=9/5/8 EHM per group. **C.** Cardiomyocyte number per EHM enumerated by flow cytometry after staining for  $\alpha$ -actinin; n=9/5/8 EHM per group (**Figure S1**). **D.** Mean  $\alpha$ -actinin fluorescence signal per cell served as a surrogate parameter for cardiomyocyte size (cardiomyocyte pools from 8-10 EHM/group); the data was confirmed by assessing the cardiomyocyte area in n=11/9/17 EHM-derived cardiomyocytes per group. Scatter plots with mean  $\pm$  SEM in **A-C**; symbols represent measures from individual EHMs. Box plots in **D** with 25% confidence interval.



**Figure S2. Flow cytometry gating strategy for cardiomyocyte analysis.** After enzymatic dispersion of EHM, cells were labeled with WGA, fixed, permeabilized, and stained for  $\alpha$ -actinin to specifically label cardiomyocytes. The cardiomyocyte gating strategy was: **A.** Exclusion of cell debris based on DNA (DAPI) signal area; **B.** Single cells were selected based on DNA (DAPI) signal width; **C.** Cardiomyocytes were identified based on  $\alpha$ -actinin signal.



**Figure S3. Analysis of T-tubulation.** Cardiomyocytes were labeled with di-8-ANEPPS and image analysis was performed using Fiji (<http://fiji.sc/>). Representative regions of interest (ROIs) which excluded the sarcolemma and nuclei were selected followed by automated tracing of the di-8-ANEPPS labeled intracellular membranes; representative cardiomyocyte from a 13 day old rat (also displayed in Fig. 7C); scale bar: 10  $\mu\text{m}$ .