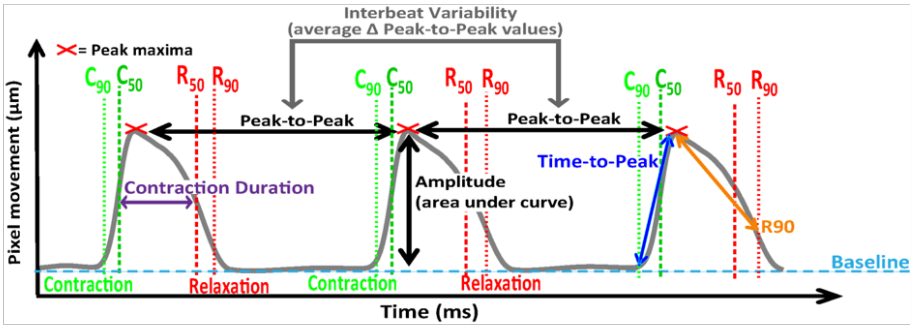
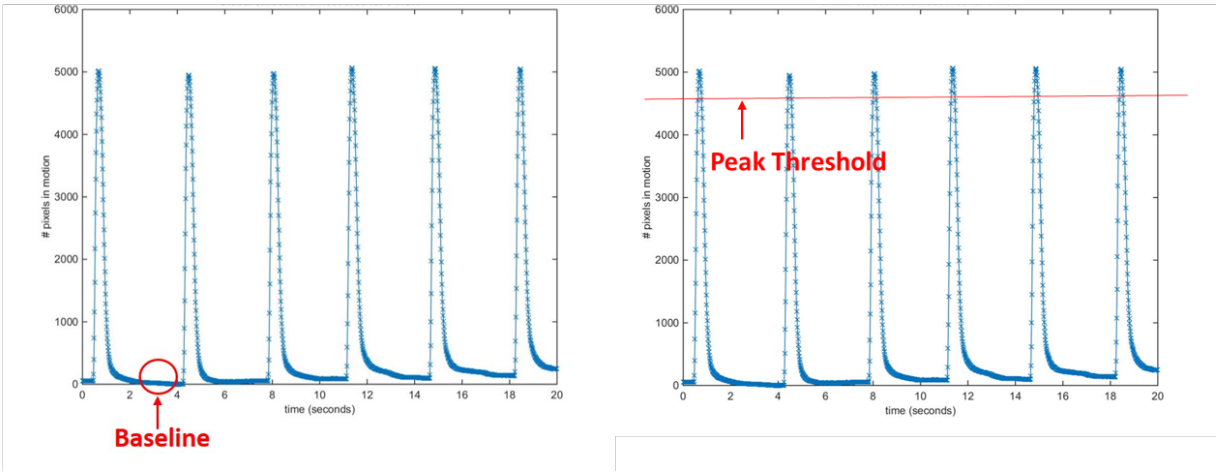
SUPPLEMENTARY INFORMATION

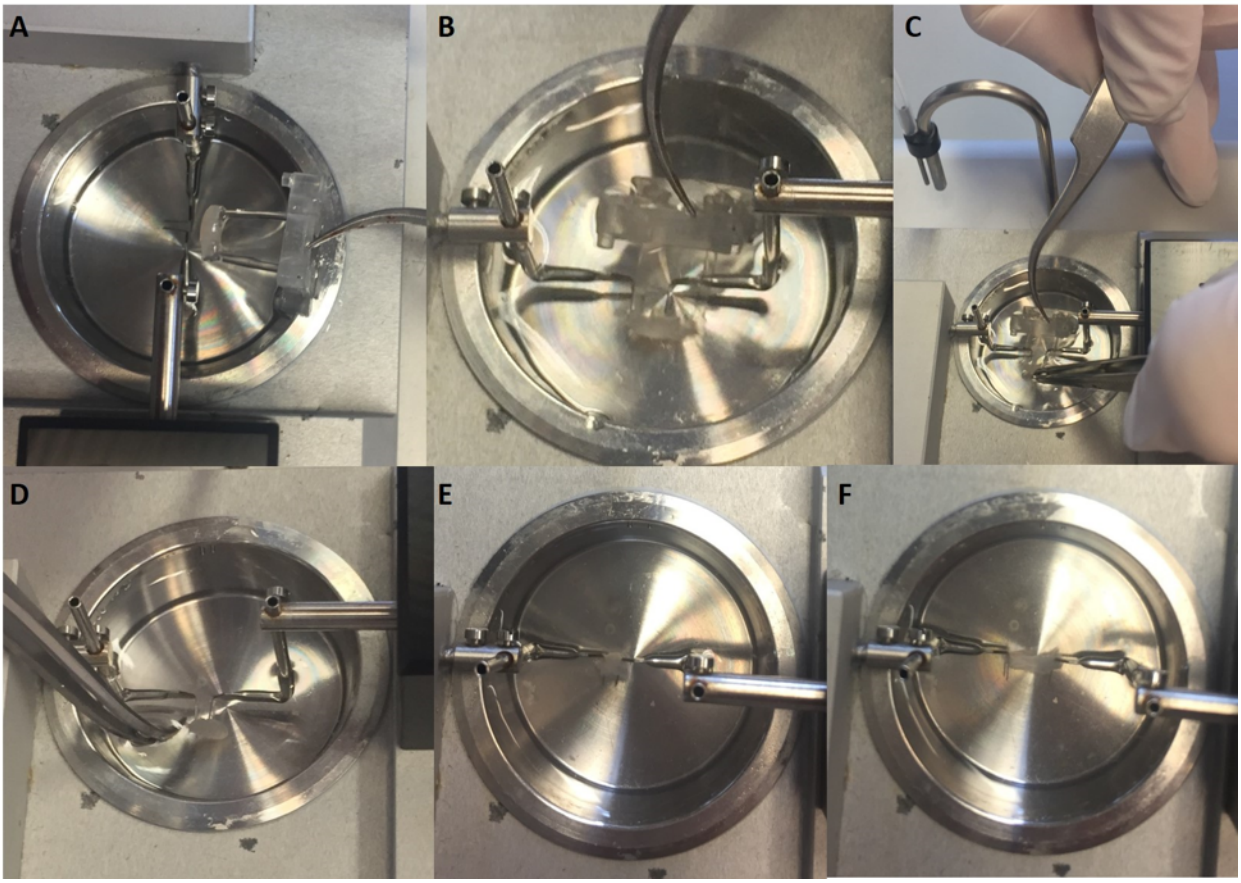
A



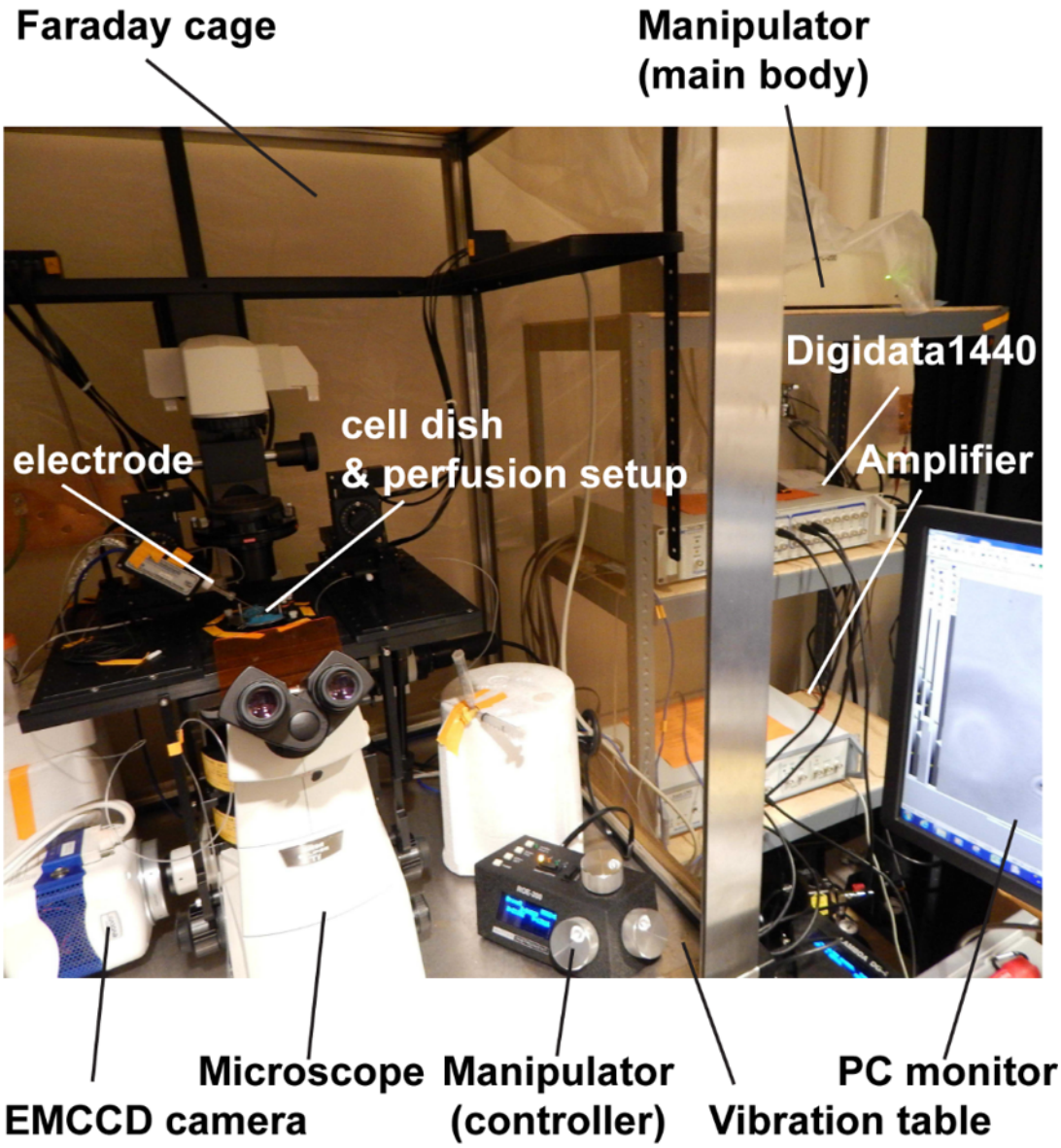
B



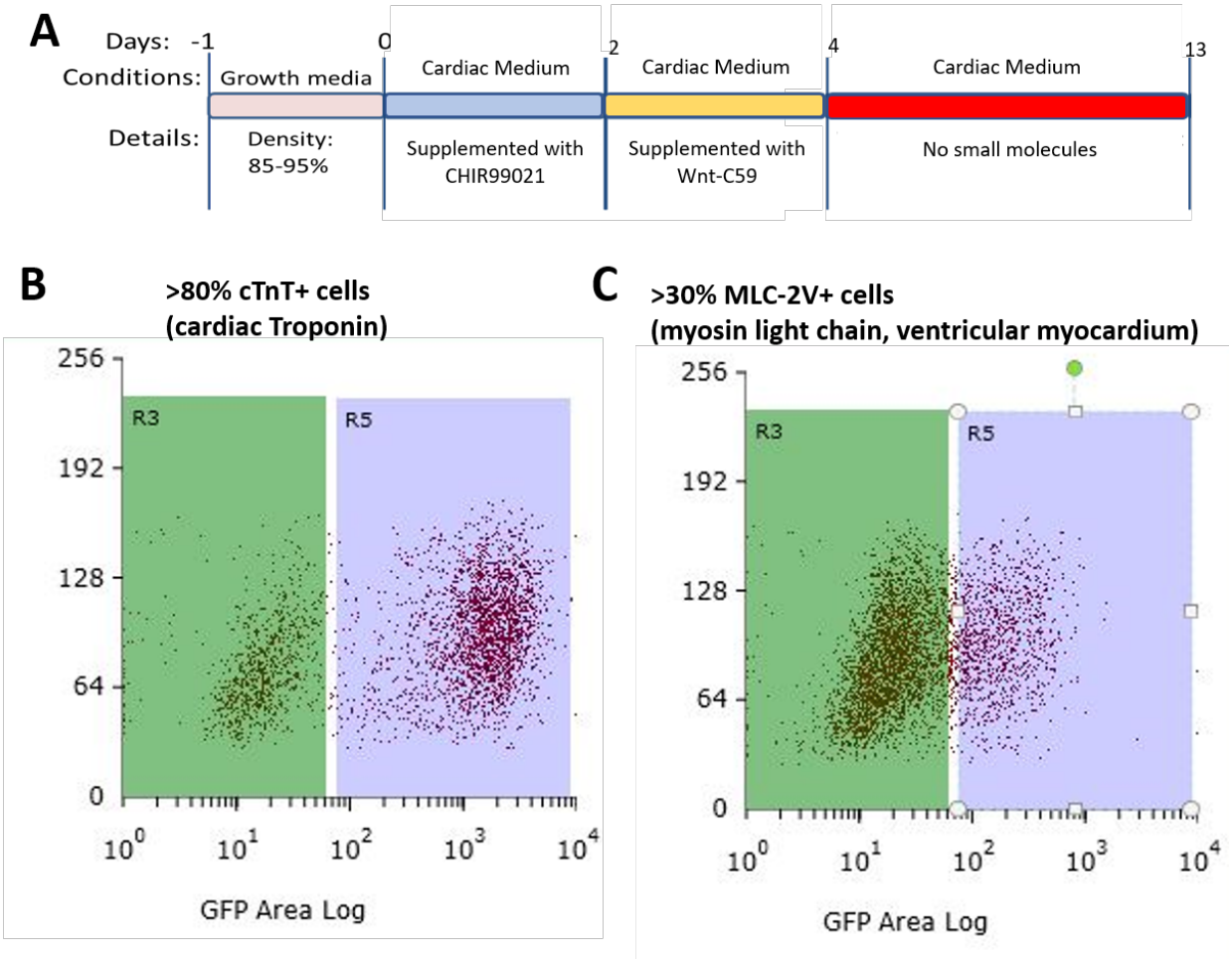
Supplementary Figure 1 - Contractility assessment of cardiac tissues. **A)** Parameters evaluated by the custom contractility code. **B)** Baseline and peak threshold of an example of a contractility trace.



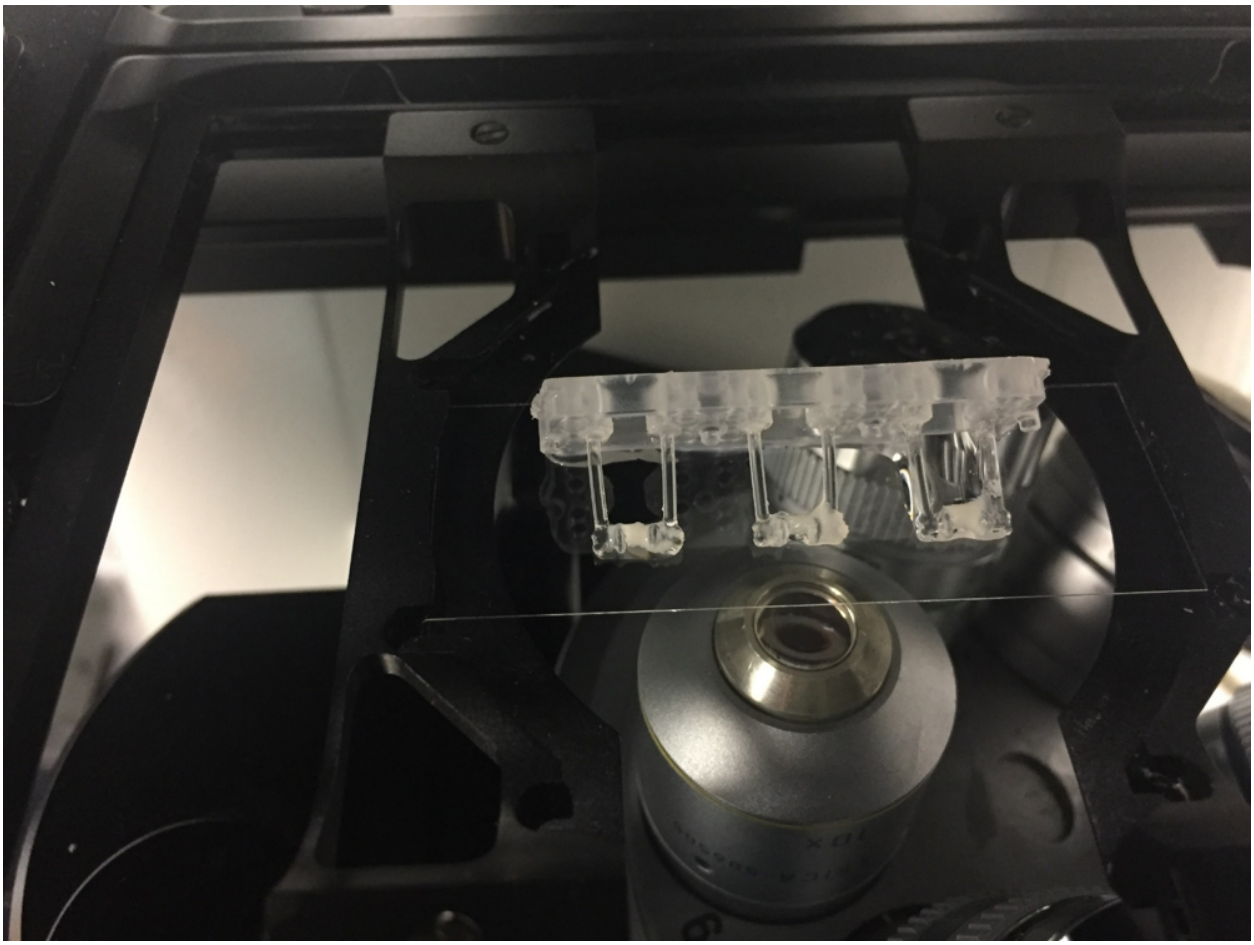
Supplementary Figure 2. Organ bath setup of the cardiac tissues. A) Bring the hooks of the force transducer and tissue holder closer together until they are separated by a length similar to the length of the tissue from the inside on once PDMS pillar to the inside of the opposite PDMS pillar. **B)** Load the cardiac tissue by piercing the cardiac tissue on the inside of each PDMS pillar with the attachment hooks. **C)** Using an additional set of forceps to hold the tissue in place, remove the cardiac tissue from the PDMS pillars. **D)** Use forceps to ensure cardiac tissues are adequately attached to attachment hooks. **E-F)** Record the initial length of the cardiac tissue (**E**) and stretch the cardiac tissue by turning the positioning dial on the organ bath to find the optimal force-length relationship (**F**).



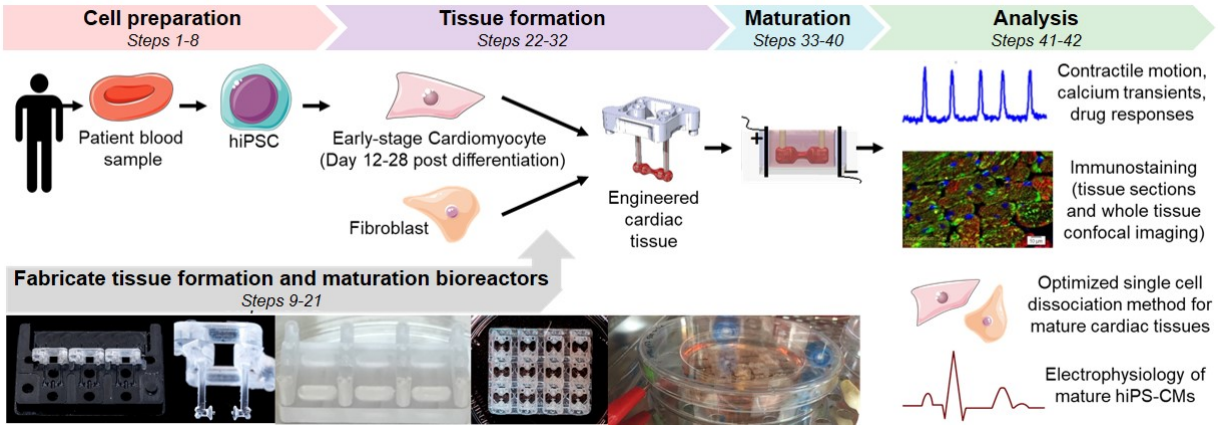
Supplementary Figure 3. Electrophysiological recording setup. A glass-bottom dish containing cardiomyocytes was replaced in the dish holder with perfusion and temperature controllers on the microscopic stage. Eye pieces were used to find cells and computer monitor (~40-60x objective lens) was used to seal the cells with an electrode.



Supplementary Figure 4. Flow cytometry of early-stage hiPS-CM. A) Differentiation regimen used in the differentiation of C2A hiPS-CM shown below. **B-C)** Flow cytometry of an early-stage differentiation containing C2A cells labelled with cardiac troponin T(cTnT) reveals an efficiency greater than 80% **(B)** and myosin light chain ventricular isoform (MLC-2V) reveals an efficiency greater than 30% **(C)**.



Supplementary Figure 5. Confocal imaging setup. Confocal images of the whole tissues are difficult to obtain without having enough working distance to image the tissue portion, as opposed to the pillar regions. Subsequently, we found that laying the tissues on their side and keeping them hydrated in PBS during imaging enabled confocal images of the cardiac tissue to be obtained.



Supplementary Figure 6. Schematic detailing the overall workflow to engineer adult-like cardiac tissues and the anticipated analysis of their functionality, calcium, force and drug response, metabolism, electrophysiology and ultrastructure that can be performed.

Supplementary Table 1. Genes used for gene expression analysis. The following genes were used for the cardiac specific gene expression analysis in Figure 3 B-E.

Conduction genes	Maturation genes	Energetics genes	Calcium handling
SCN5A	NPP	PRKAA1	CACNA1C
KCNJ2	NPPB	TFAM	RYR2
KCND3	MAPK1	PPARGC1A	CASQ2
ITPR3	MPRKACA	PPA1	PLN
HCN2		PTPA	CAMK2B
SCN1B		SLC2A4	TRDN
HCN4			CAV3
HCN1			BIN1
KCNJ8			AMPH
KCNH2			ATP2A2

SUPPLEMENTARY DATA:

Supp Data 1: A zip file labelled “Electrical_Stimulation_Cardiac_Maturation.ino” containing the code and libraries need for the Arduino based electrical stimulator detailed in **Box 2**.

Supp Data 2: A .zip file labelled “CardiacContractileMotion” containing the code and libraries need for the Matlab based analysis detailed in **Box 3**.