

1 **SUPPLEMENTARY INFORMATION**

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3 **SELF-ASSEMBLING HUMAN HEART ORGANOIDs FOR THE MODELING OF CARDIAC**
4 **DEVELOPMENT AND CONGENITAL HEART DISEASE**

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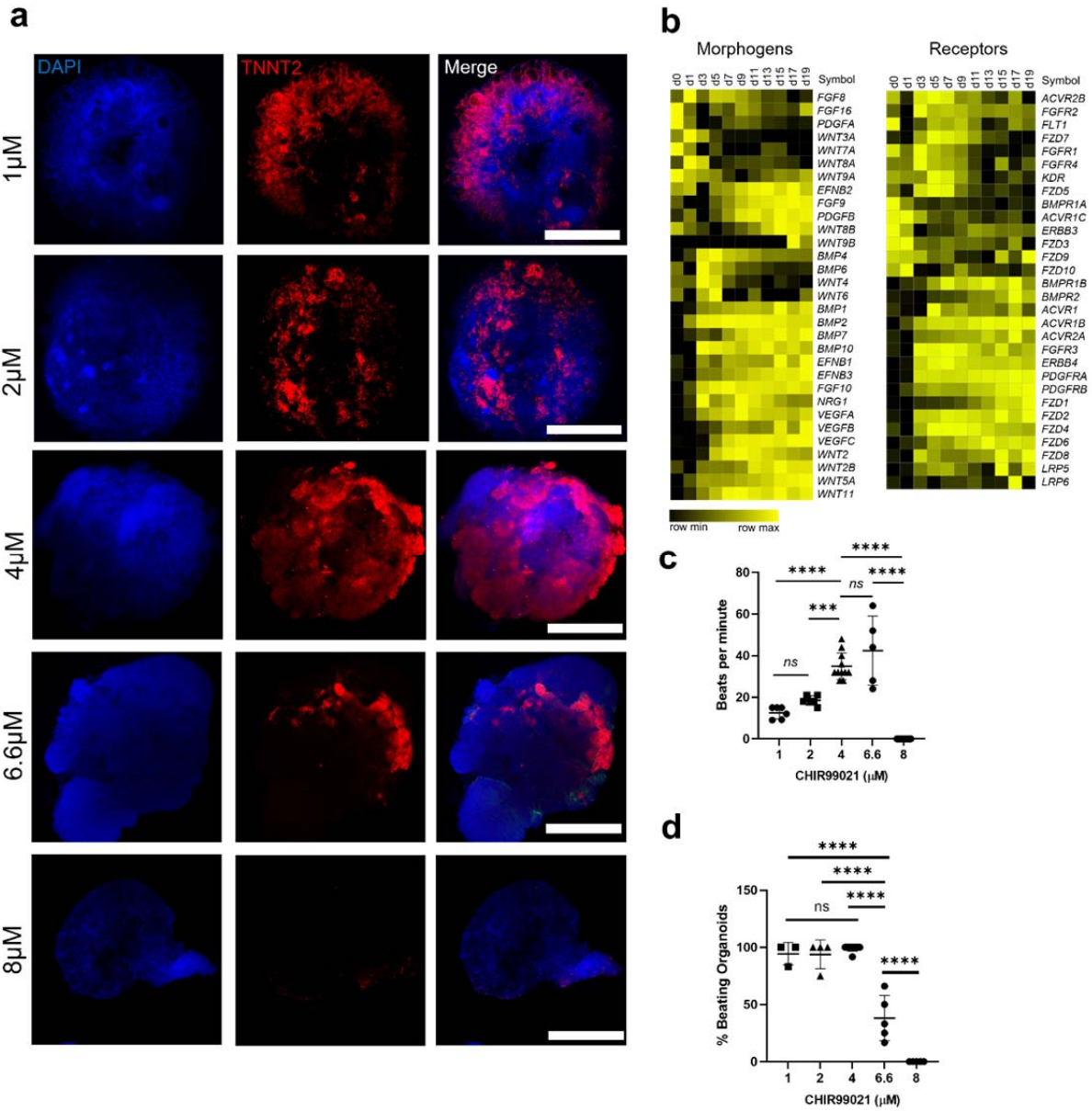
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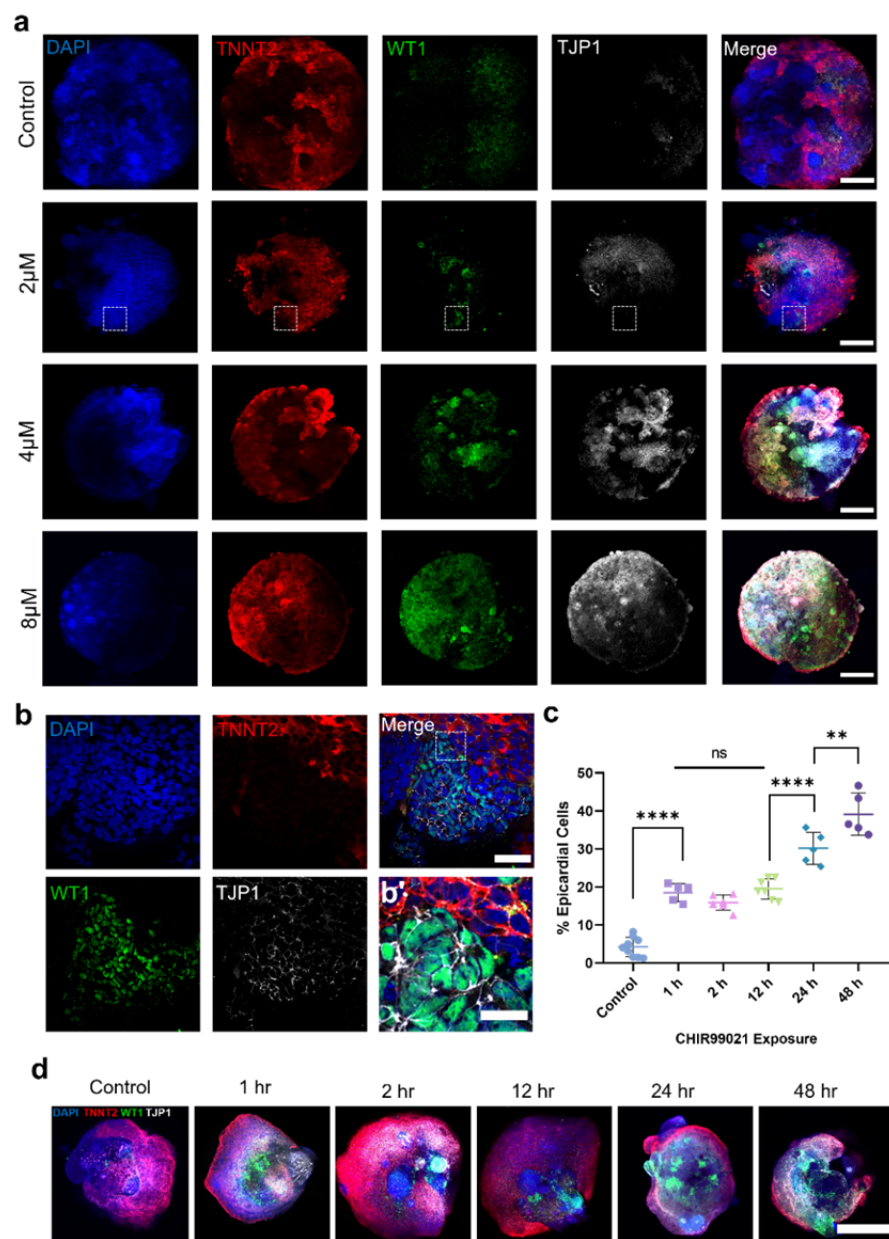
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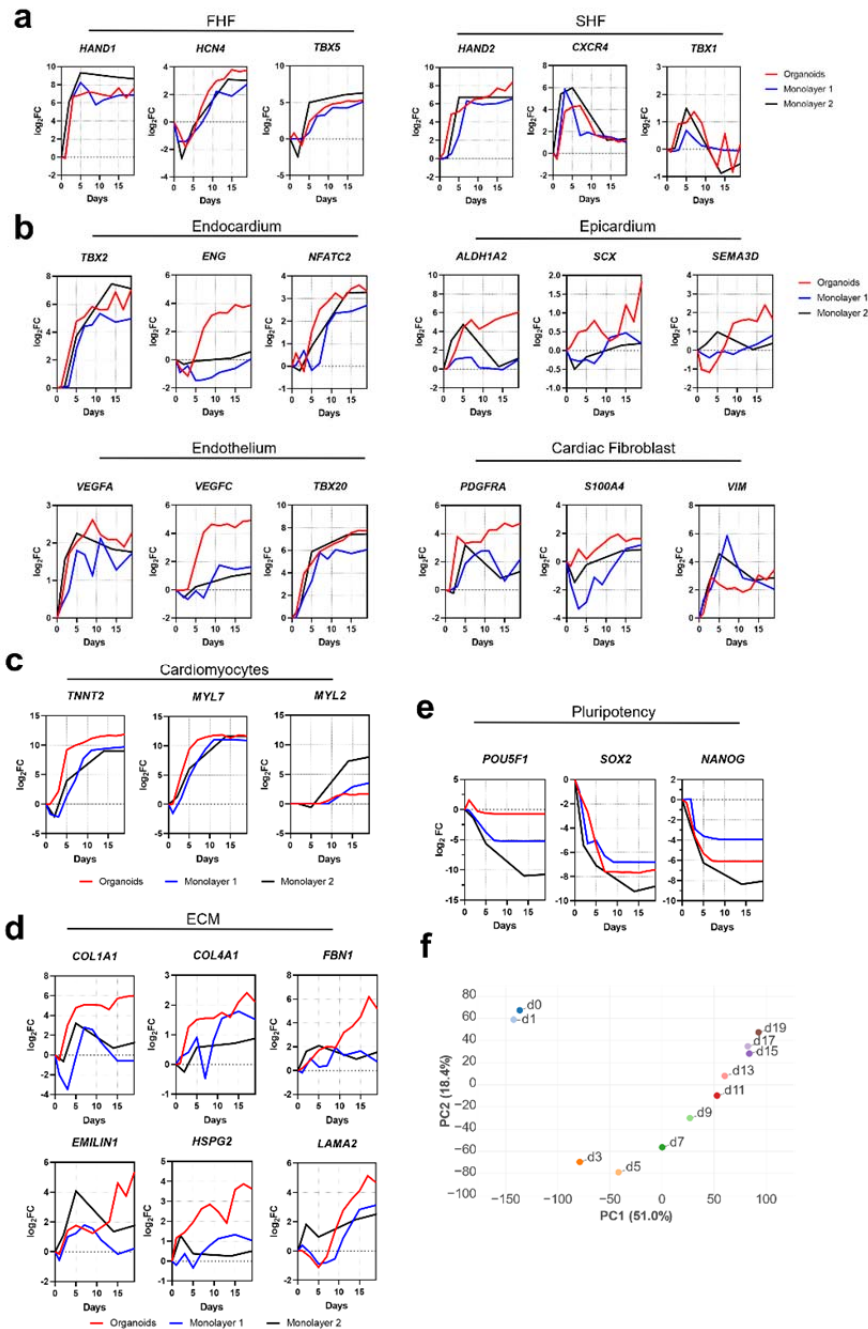
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Supplementary Figure 1. a, Confocal immunofluorescent images for DAPI (blue) and TNNT2 (red), in organoids with CHIR99021 exposure concentrations of 1, 2, 4, 6.6, and 8 μM , at day 15; scale bars, 500 μm ($n=12$). **b**, Morphogen and receptor expression levels as determined by RNA-seq heatmaps in differentiating hHOs from day 0 to day 19. **c**, Quantification of beat frequency in hHOs ($n=6$) and **d**, percentage of beating hHOs per treatment (1 μM $n=288$ organoids, 2 μM $n=384$ organoids, 4 μM $n=960$, 6.6 and 8 μM $n=480$ organoids). Value = mean $\pm$ s.d., 1-way ANOVA multiple comparison test; *** $p=0.0003$, **** $p<0.0001$, otherwise ns: no significance. Heatmap colors are relative intensity representing gene expression. Source data are provided as a Source Data file.

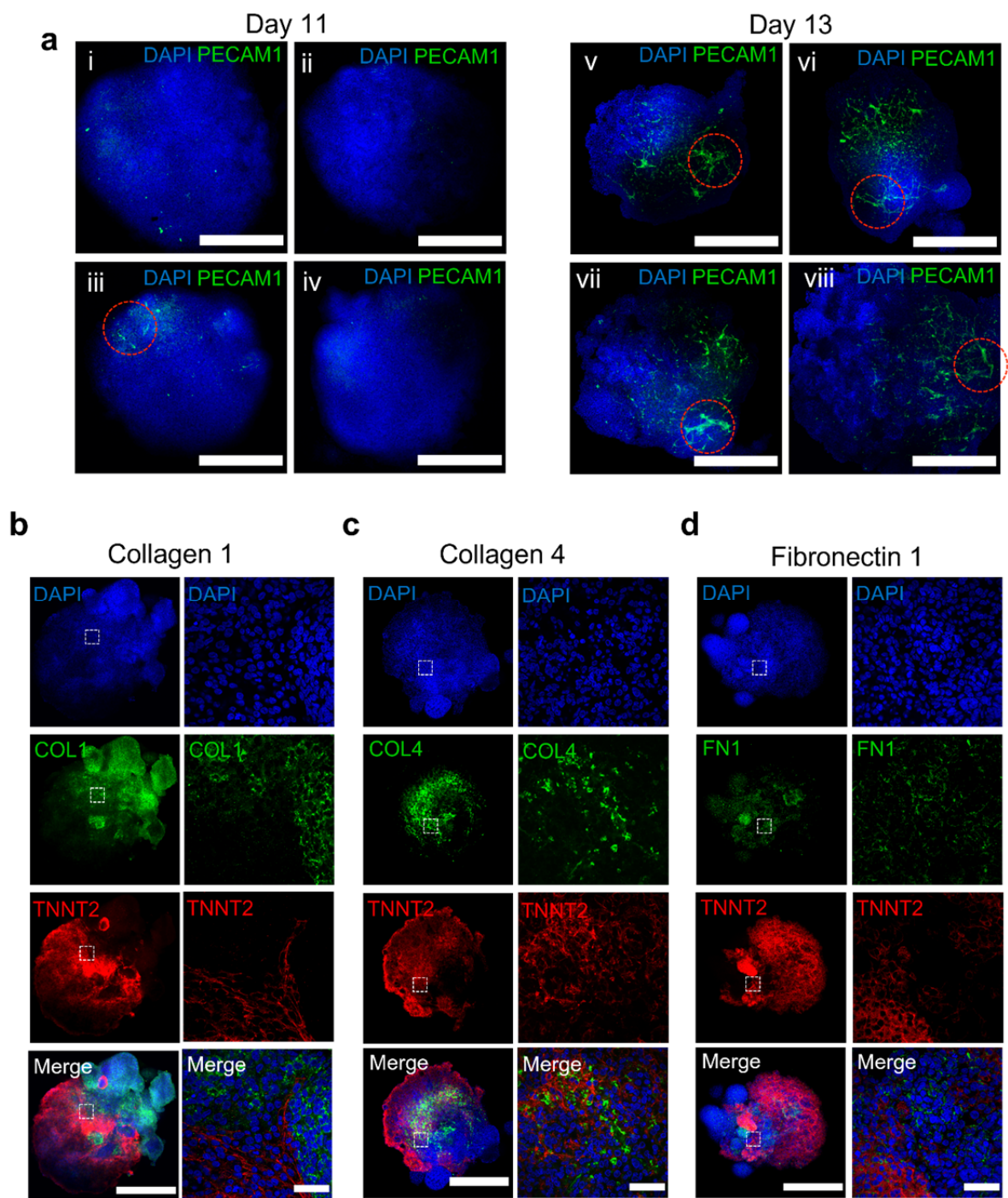


Supplementary Figure 2. **a**, Confocal immunofluorescent images of hHOs at differentiation day 15 for DAPI (blue), WT1 (green), TNNT2 (red), and TJP1 (white), with variable concentrations of the second CHIR exposure at day 7 vs. control with no second CHIR exposure; scale bars: 500 μ m, and **b**, high magnification of hHOs after 2 μ M second CHIR exposure showing an adjacent region of TNNT2⁺ myocardial tissue and WT1⁺/TJP1⁺ epicardial tissue; scale bar: 50 μ m. **c**, Cell quantification analysis of cardiomyocyte (TNNT2⁺) and epicardial cells (WT1⁺ and TJP1⁺) within organoids taken at multiple z-planes as a percentage of DAPI⁺ cells of each organoid treated with CHIR99021 at day 7 for different time durations (control n=9 organoids, 1, 24 and 48 hours n=5 organoids, 2 hours n=6 organoids, 12 hours n = 7 organoids), and **d**, representative confocal immunofluorescent images of organoids from these time durations; scale bar: 500 μ m. Value = mean $\pm$ s.d., 1-way ANOVA multiple comparison test; **p=0.0018, ****p<0.0001, otherwise ns: no significance. Source data are provided as a Source Data file.



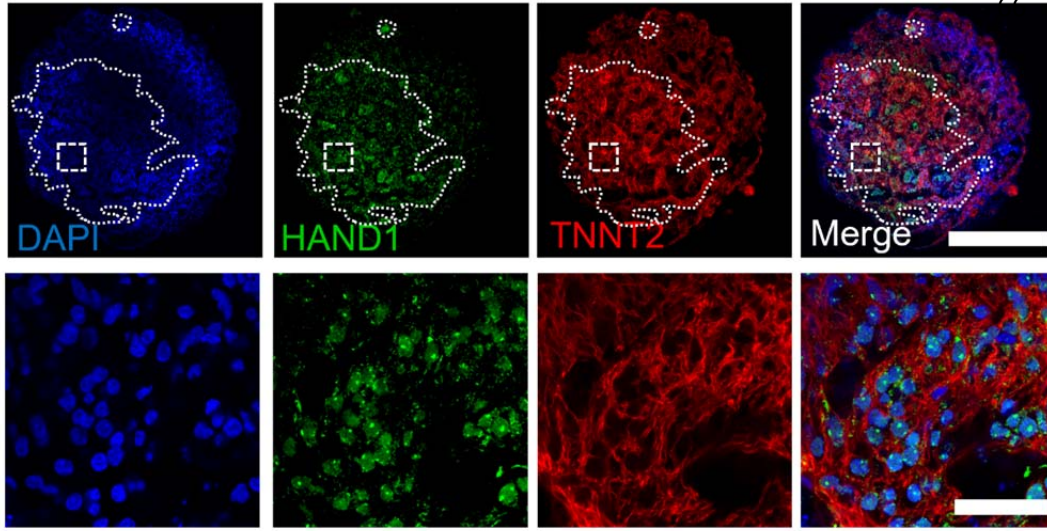
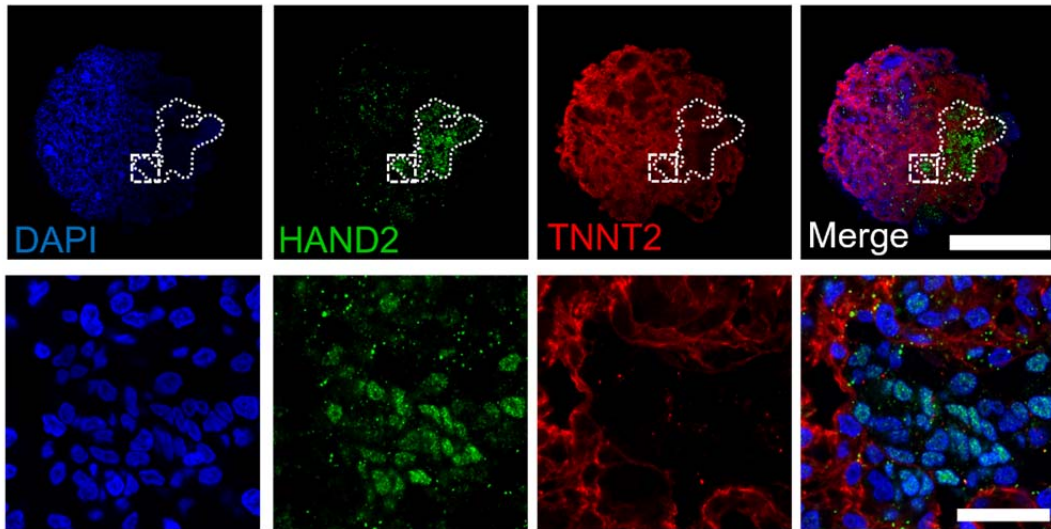
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49 **Supplementary Figure 3. a**, Gene expression analysis of additional first and second heart field
 50 markers over heart organoid differentiation. **b–f**, Gene expression analysis ($\log_2$ fold-change vs.
 51 D0) for **b**, cardiac-specific cell type populations in heart organoids, including (from top left to
 52 bottom right) endocardial cells, epicardial cells, endothelium, and cardiac fibroblasts. **c**,
 53 cardiomyocyte markers, **d**, ECM protein-coding genes that are present in cardiac tissue, and **e**,
 54 for pluripotency markers. **f**, Principal component analysis of heart organoid differentiation over
 55 time. ECM: extracellular matrix, FHF: first heart field, PC: principal component, SHF: second
 56 heart field.

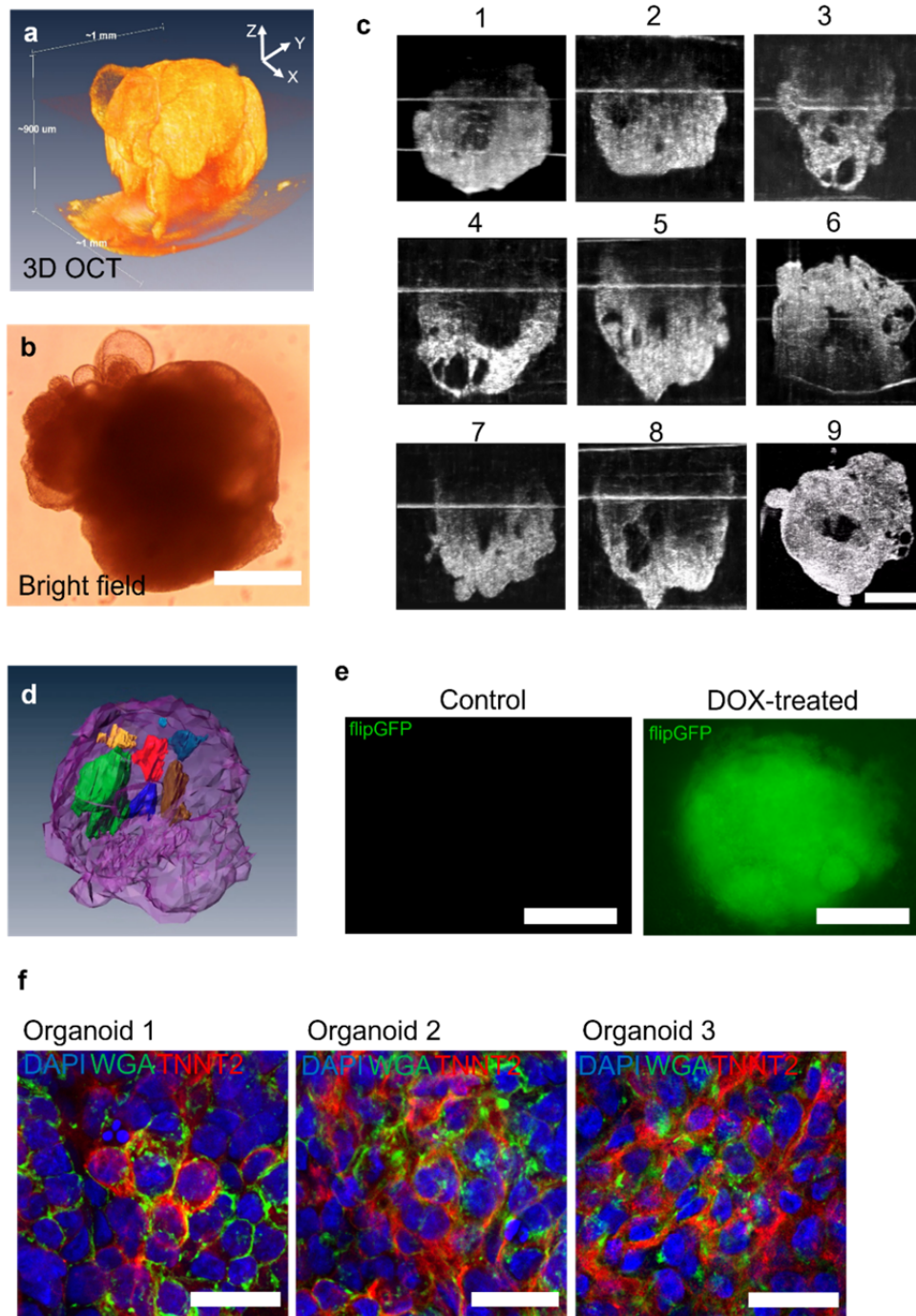


59 **Supplementary Figure 4.** Confocal immunofluorescent images of hHOs, DAPI in blue and
60 TNNT2 in red. **a**, Day 11 and day 13 organoids showing maximum intensity projections of
61 PECAM1 (green) endothelial cells forming robust vascular-like network throughout the hHOs;
62 red dotted circles indicate region of high vascular branching. **b–d**, Presence of ECM proteins in
63 green in day 15 organoids, showing collagen 1 (**b**), collagen 4 (**c**), and fibronectin 1 (**d**); in
64 green; scale bars: 500 μ m, inset: 50 μ m.

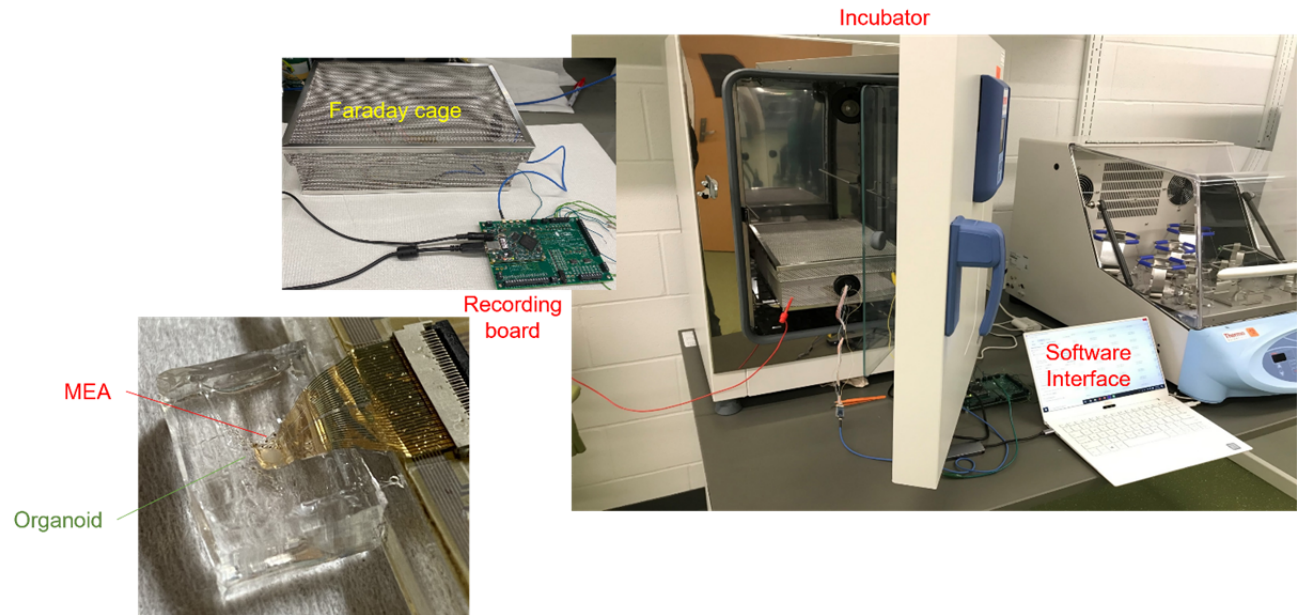
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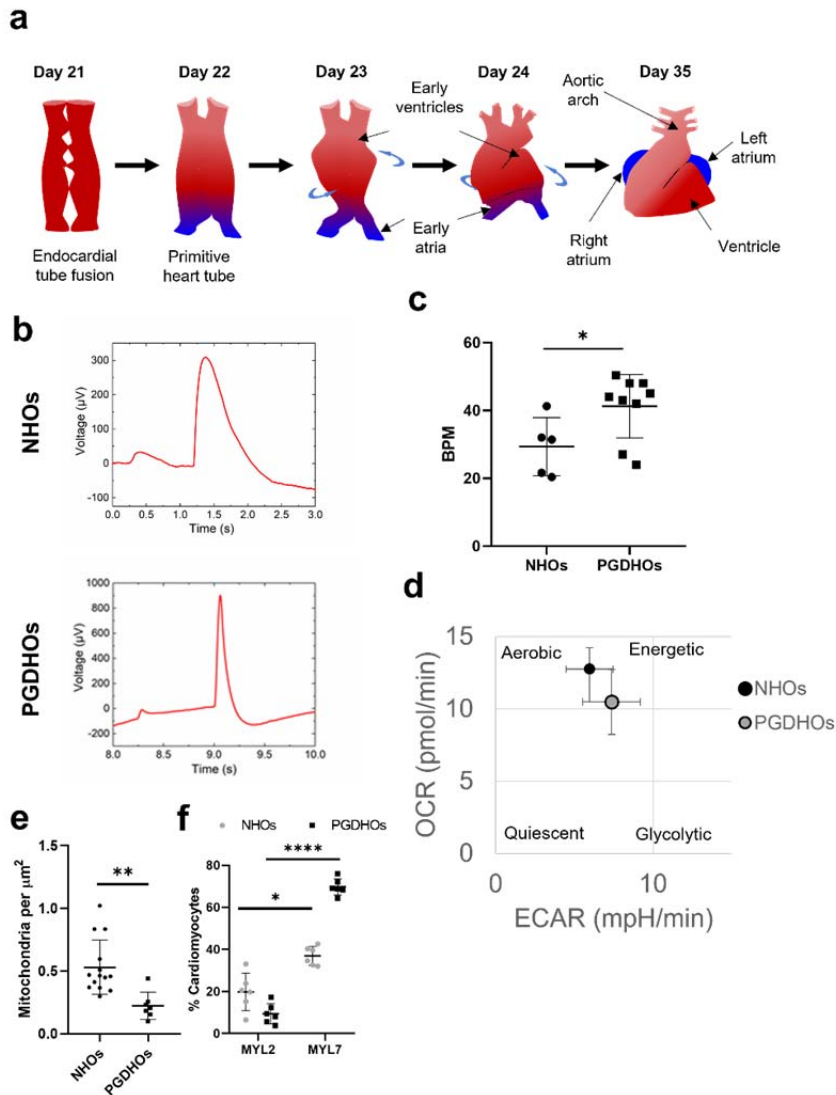
Supplementary Figure 5. a-b, Confocal immunofluorescent images of hHOs at differentiation day 8. **a**, FHF marker HAND1 (green) in defined area of the hHO, and **b**, SHF marker HAND2 (green); DAPI (blue), TNNT2 (red); scale bars: 500 μ m, inset: 50 μ m.



Supplementary Figure 6. **a**, 3D reconstruction of OCT images and **b**, bright field image of hHO. **c**, OCT images showing cross-sections of the center of 9 different organoids, revealing central chambers; scale bar: 500 μm. **d**, 3D reconstruction of chambers observed via OCT imaging. **e**, Immunofluorescence images of organoids derived from a FlipGFP transgenic iPSC line L1 showing no apoptosis in control hHOs (left) and high apoptosis in hHOs treated with 5 μM Doxorubicin (DOX) (right); scale bar: 500 μm. **f**, Representative immunofluorescence imaging of t-tubules-like structures in day 15 hHOs, using WGA (green) as a t-tubule indicator; scale bar: 50 μm.

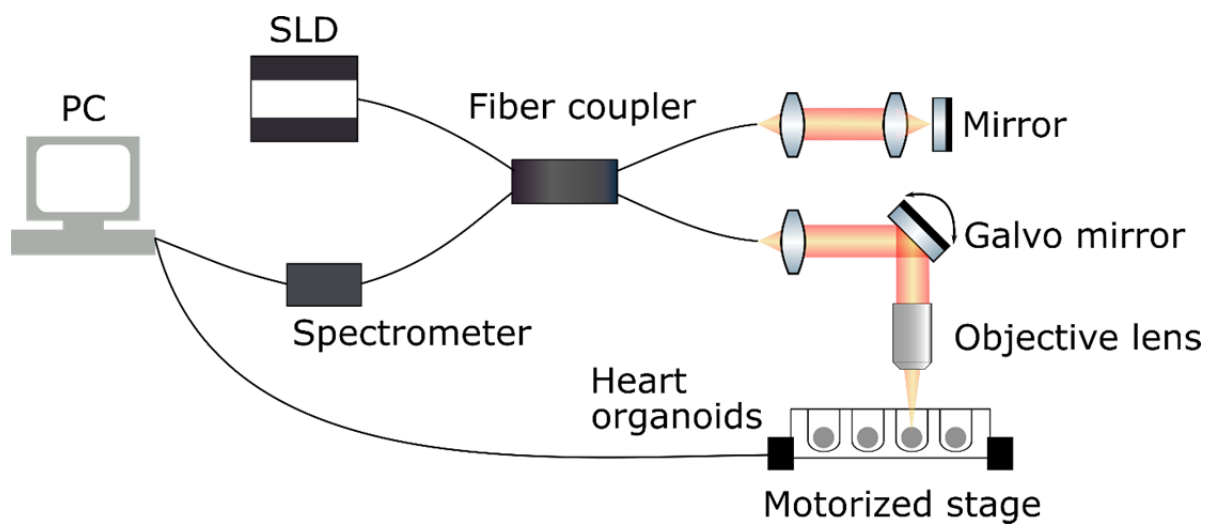


Supplementary Figure 7. Microelectrode array (MEA) recording system showing the gold electrode array in a PDMS chamber where the organoid is placed within a Faraday cage inside an incubator.



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89 **Supplementary Figure 8. Human heart organoids modeling functional features healthy**
 90 **vs. diabetic conditions.** **a**, Schematic of heart tube formation and looping into the four
 91 chambers of the heart. **b**, Representative MEA electrophysiology detail of normal vs. diabetic
 92 organoids. **c**, beating frequency in beats per minute (BPM) in normal and diabetic organoids as
 93 recorded by MEA (value = mean $\pm$ s.d, NHOs n=5 organoids, PGDHOs n=9 organoids; unpaired
 94 t-test, *p=0.03). **d**, Seahorse energy map of normal and diabetic-like organoids (value = mean $\pm$
 95 s.d, n=6). **e**, Number of Mitochondria per μm^2 as seen in TEM images of NHOs and PGDHOs
 96 (value = mean $\pm$ s.d., n=6, two-tailed, unpaired t-test, **p=0.0024). **f**, percentage area of MYL2⁺
 97 and MYL7⁺ regions in NHOs and PGDHOs showing ventricular and atrial cardiomyocytes
 98 markers, respectively (value = mean $\pm$ s.d., n=4, 2-way ANOVA Sidak's multiple comparisons
 99 test). *p=0.012, ****p<0.0001, otherwise ns: no significance). BPM: beats per minute, ECAR:
 100 extracellular acidification rate, NHOs: normal heart organoids, OCR: oxygen consumption rate,
 101 PGDHOs: pregestational diabetes heart organoids. Source data are provided as a Source Data
 102 file.



Supplementary Figure 9. Illustration of a custom spectral-domain Optical Coherence Tomography imaging system, PC: personal computer, SLD: superluminescent diode.

112 **Supplementary Table 1** Antibodies used for immunofluorescence in this report.

	Antibody name	Host Species	Dilution	Catalogue Number	Vendor
Primary	cTnT (TNNT2)	Mouse	1:200	ab8295	Abcam
	WT1	Rabbit	1:200	ab89901	Abcam
	ZO1 (TJP1)	Goat	1:250	PA5-19090	Thermo Fisher Scientific
	HAND1	Rabbit	1:200	ab196622	Abcam
	HAND2	Rabbit	1:200	ab200040	Abcam
	MLC2V (MYL2)	Rabbit	1:200	ab79935	Abcam
	MLC2A (MYL7)	Mouse	1:200	311-011	Synaptic Systems
	Vimentin (VIM)	Goat	1:200	ab11256	Abcam
	CD90/Thy1	Rabbit	1:200	ab133350	Abcam
	NFAT2 (NFATC1)	Rabbit	1:200	ab25916	Abcam
	CD31 (PECAM1)	Rabbit	1:50	ab28364	Abcam
	COL1A1	Mouse	1:200	M-38	DSHB
	COL4A1	Mouse	1:200	M3F7	DSHB
	FBN1	Mouse	1:200	CPTC-FBN1-1	DSHB
Secondary	Alexa Fluor 488	Donkey anti-mouse	1:200	A-21202	Thermo Fisher Scientific
	Alexa Fluor 488	Donkey anti-rabbit	1:200	A-21206	Thermo Fisher Scientific
	Alexa Fluor 594	Donkey anti-mouse	1:200	A-21203	Thermo Fisher Scientific
	Alexa Fluor 594	Donkey anti-rabbit	1:200	A-21207	Thermo Fisher Scientific
	Alexa Fluor 647	Donkey anti-goat	1:200	A32849	Thermo Fisher Scientific

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