**SUPPLEMENTARY DATA**

**SELF-ASSEMBLING HUMAN HEART ORGANOIDS FOR THE MODELING OF CARDIAC DEVELOPMENT AND CONGENITAL HEART DISEASE**

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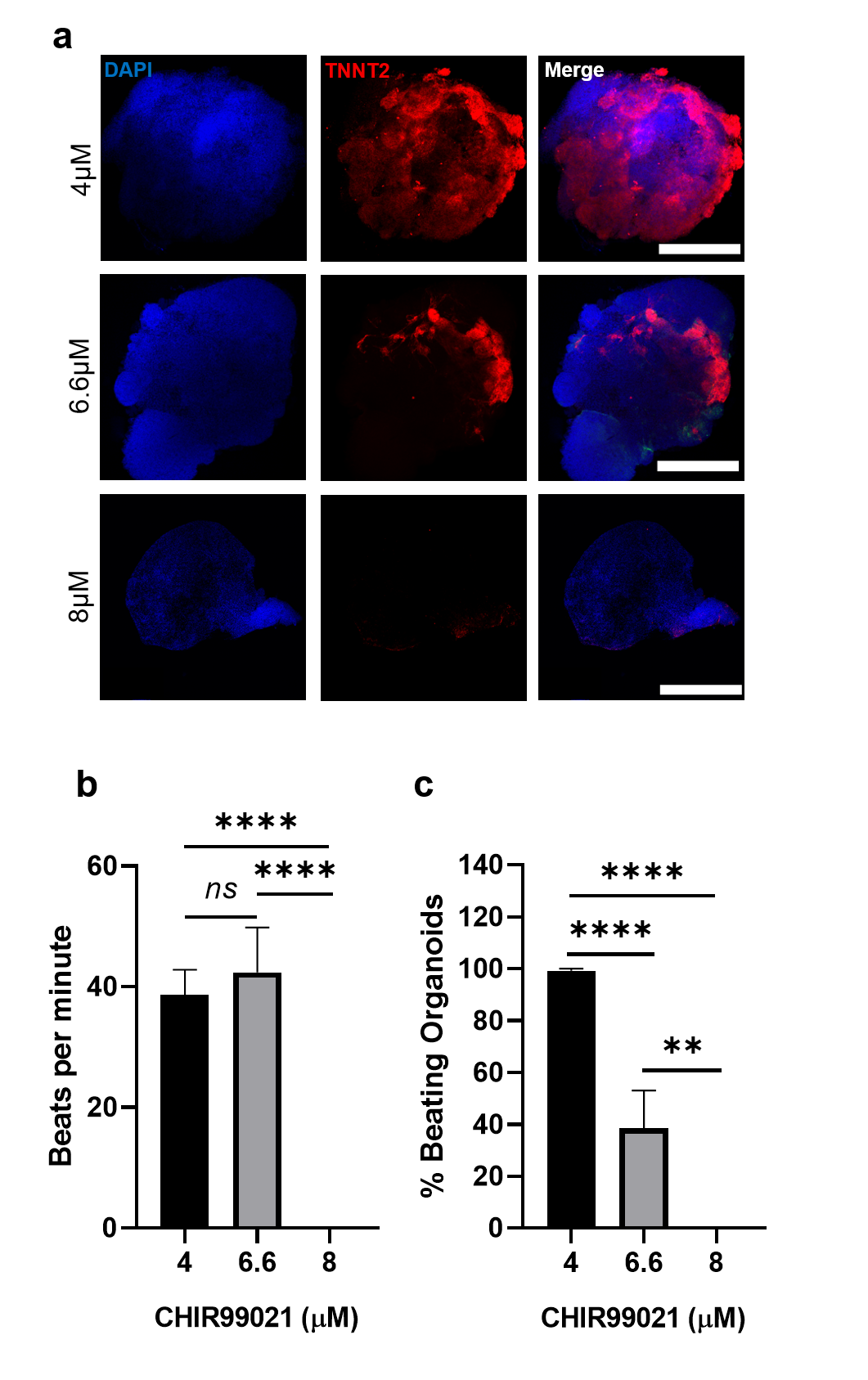
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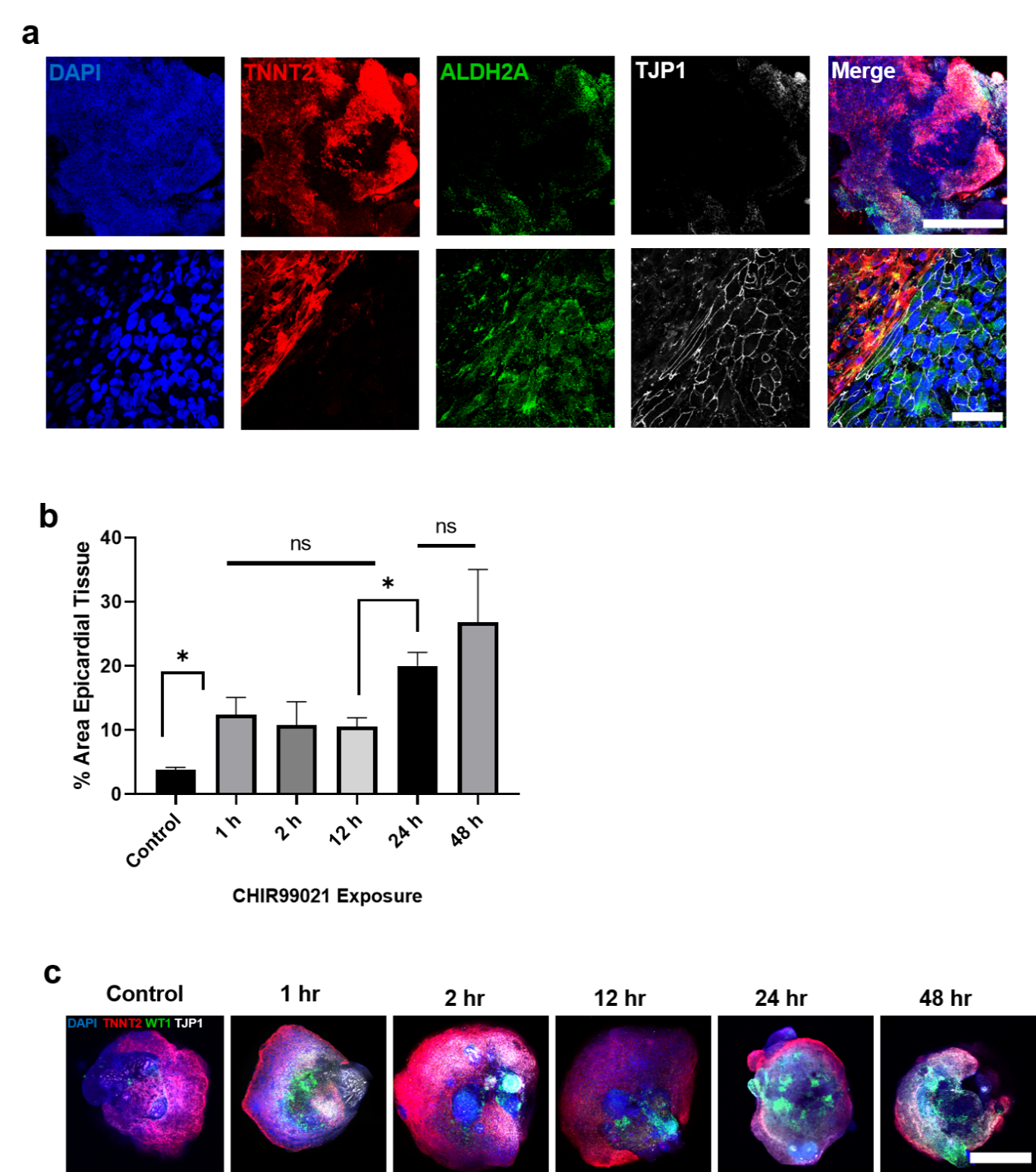
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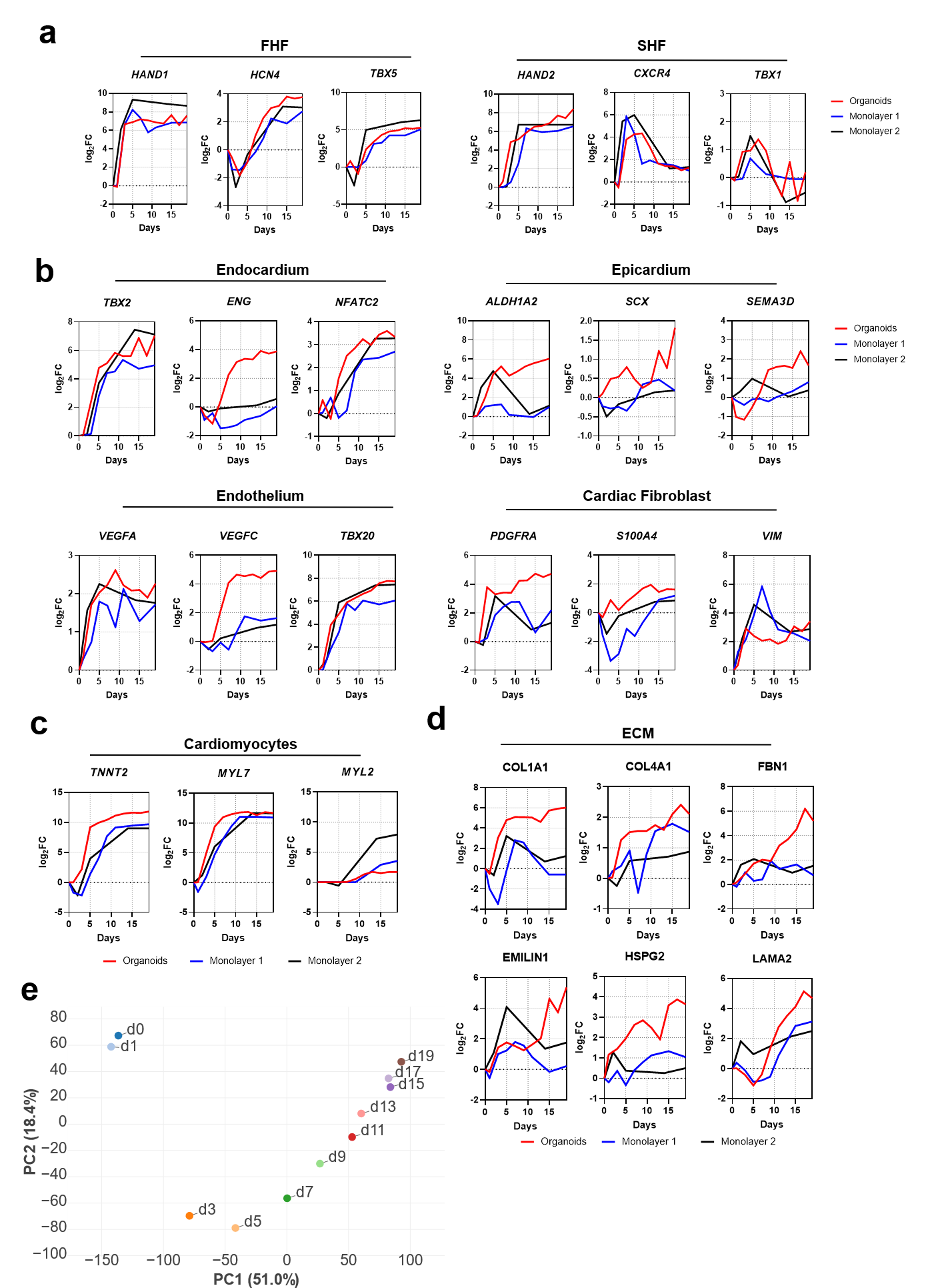
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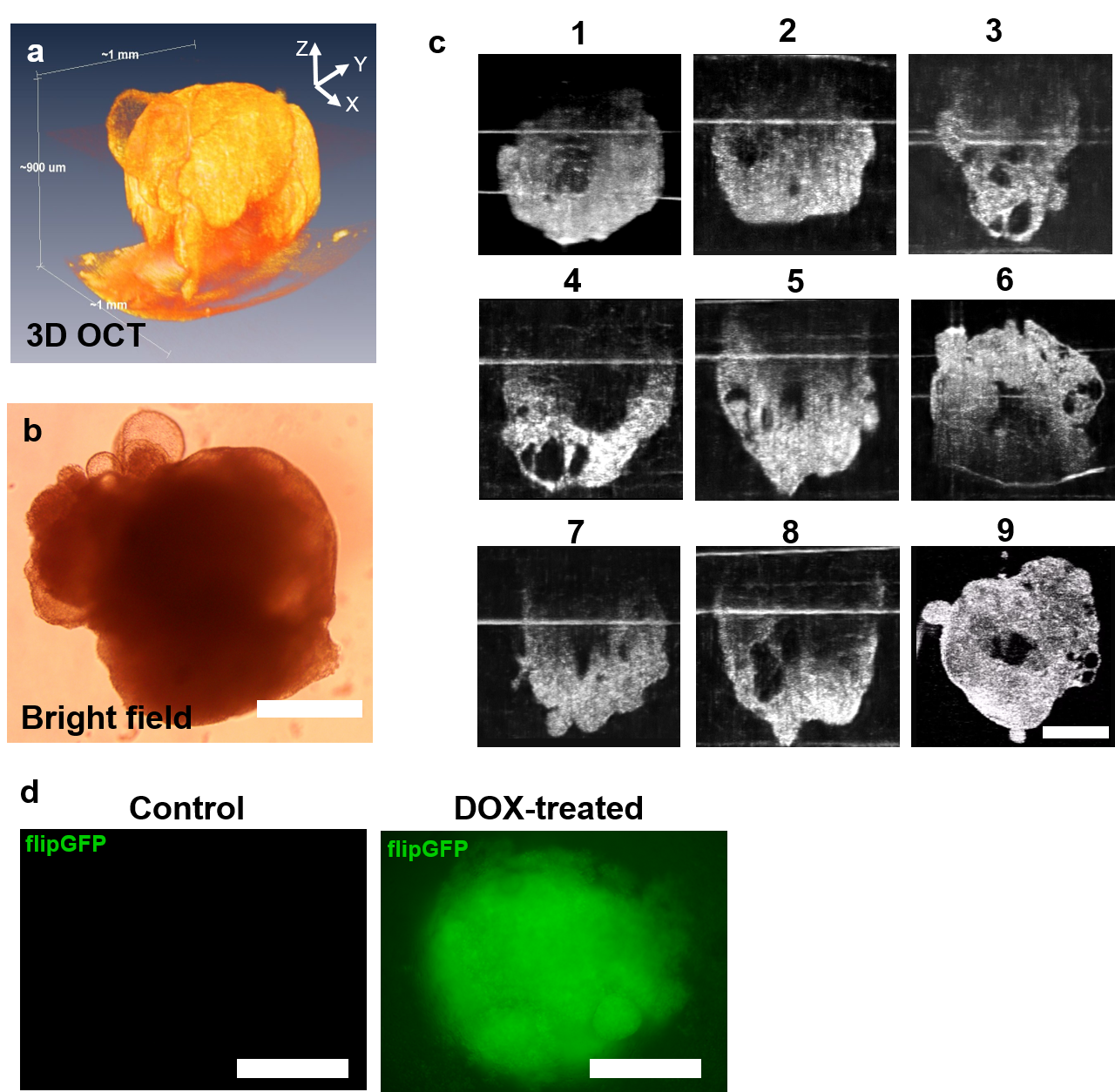
**Supplementary Figure 1. a,** Confocal immunofluorescent images for DAPI (blue) and TNNT2 (red), in organoids with CHIR99021 exposure concentrations of 4µM (top), 6.6µM (middle), and 8µM (bottom) at day 15. Scale bars, 500µm. **b,** Frequency of beats per minute of the hHOs and **c**, percentage of beating hHOs per treatment. (Value = mean ± s.d., 1-way ANOVA multiple comparison test; \*\*p<0.01, \*\*\*\*p<0.0001).



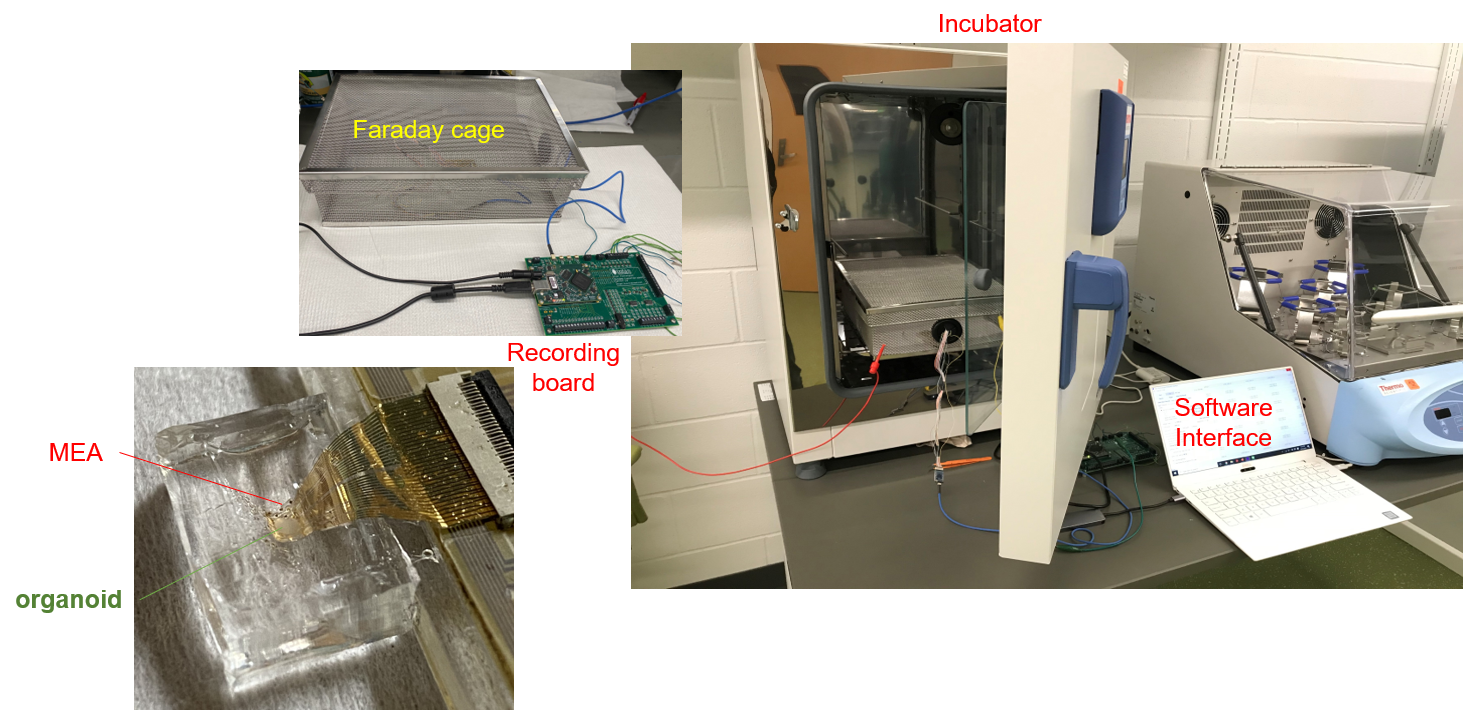
**Supplementary Figure 2. a,** Confocal immunofluorescent images for DAPI (blue) and TNNT2 (red), in hHOs showing epicardial markers ALDH2A (green) and TJP1 (white) near edge of the organoid. **b,** Area analysis of cardiomyocyte regions (TNNT2+) and epicardial regions (WT1+ and TJP1+) within organoids taken at multiple z-planes as a percentage of DAPI+ regions of each organoid treated with CHIR99021 at day 7 for different time durations, and **c,** representative confocal immunofluorescent images of organoids from these time durations: Scale bar: 500 µm. (Value = mean ± s.d., 1-way ANOVA multiple comparison test; \*p<0.05).



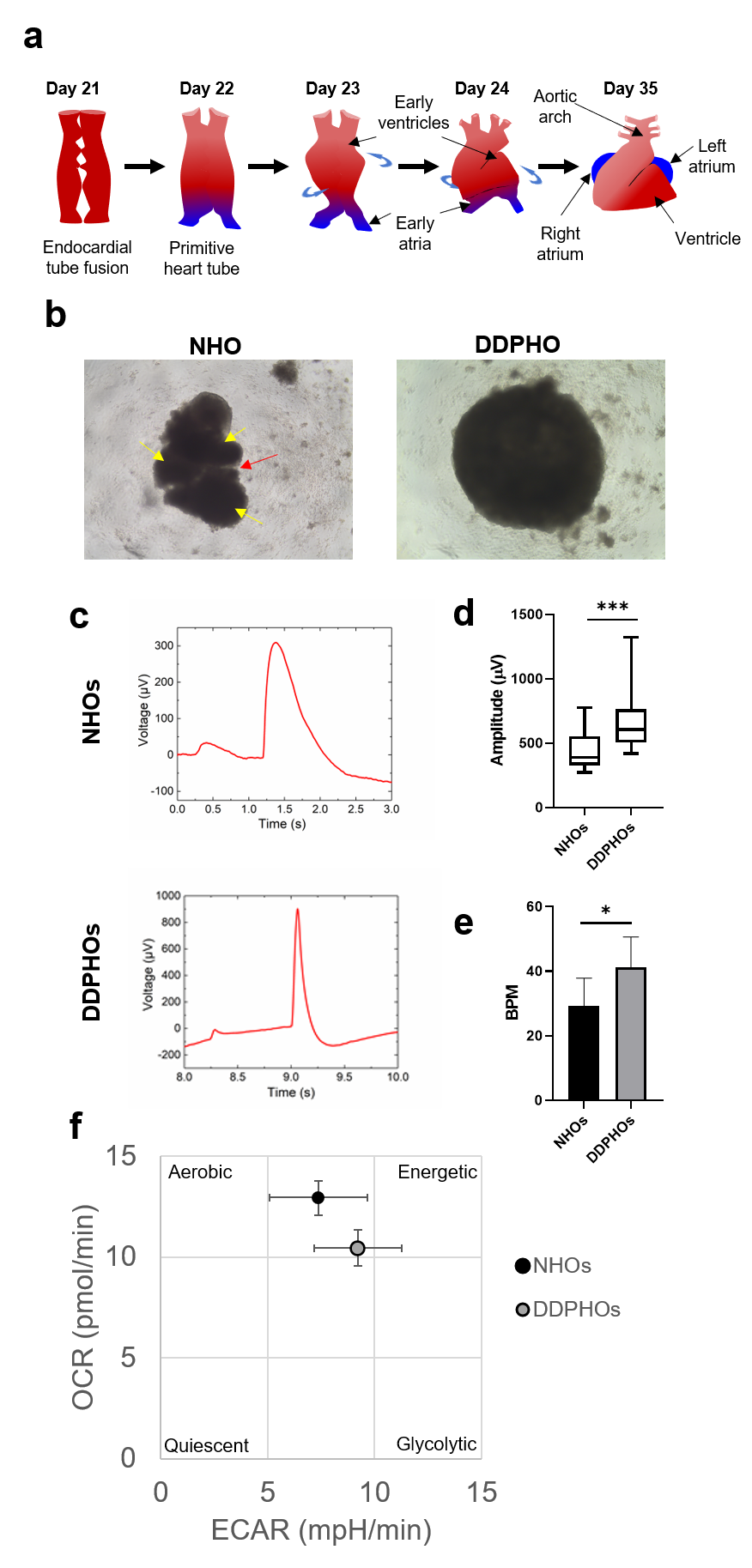
**Supplementary Figure 3.a,** Gene expression analysis indicating of more first and second heart field markers over heart organoid differentiation process. **b**, Gene expression analysis (log2 fold-change vs. D0) for cardiac-specific cell type populations in heart organoids, including (from top left to bottom right) endocardial cells, epicardial cells, endothelium and cardiac fibroblasts. **c**, Gene expression analysis (log2 fold-change vs. D0) for cardiomyocyte markers. **d**, Gene expression analysis (log2 fold-change vs. D0) for ECM protein coding genes that are present in cardiac tissue. **e**, Principal component analysis of heart organoid differentiation over time.



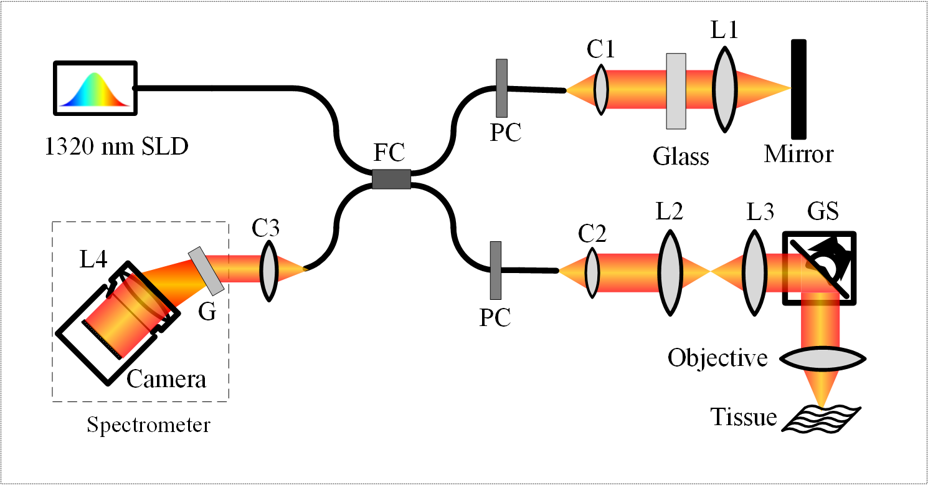
**Supplementary Figure 4. a,** 3D reconstruction of OCT images and **b,** bright field image of hHO. **c**, OCT images showing cross-section of center of 9 different organoids, revealing central chambers; scale bar: 500µm. **d**, Immunofluorescence images of organoids derived from a flipGFP transgenic iPSC line L1 showing no apoptosis in control hHOs (left) and high apoptosis in hHOs treated with 5µM Doxorubicin (DOX) (right); scale bar: 500µm.



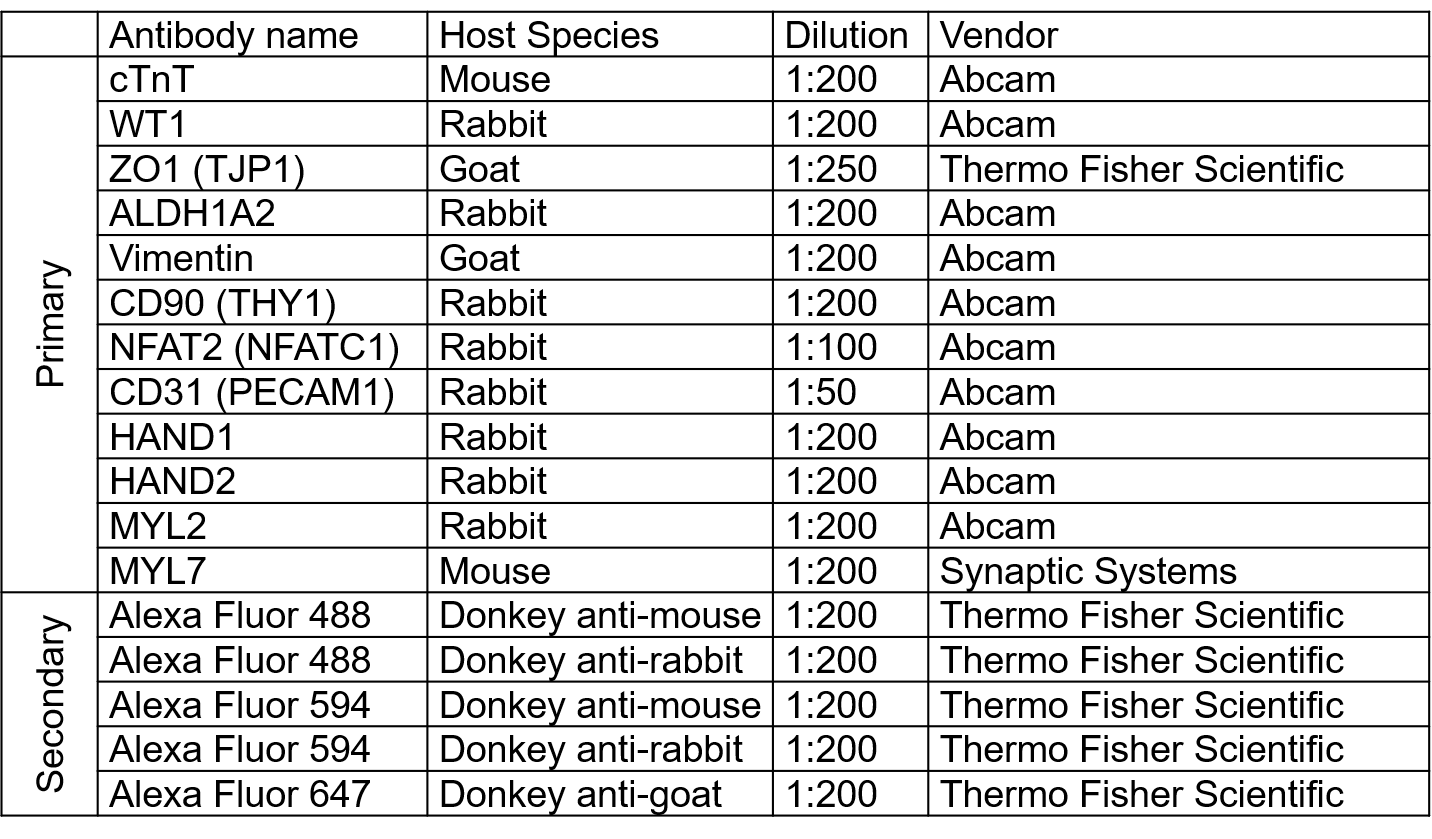
**Supplementary Figure 5.** Microelectrode array (MEA) recording system showing the gold electrode array in a PDMS chamber where the organoid is placed within a Faraday cage inside an incubator.



**Supplementary Figure 6. Human heart organoids modeling functional features healthy vs diabetic conditions. a,** Schematic of heart tube formation and looping into the four chambers of the heart. **b,** Brightfield image of NHO and DDPHO at day 15 showing segmentation (red arrow) and separate heart regions resembling early heart structures. **c,** Representative MEA electrophysiology detail of normal vs. diabetic organoids. **d,** Amplitude magnitude in µV of action potentials in normal and diabetic hHOs (n>12 over 3 replicates per condition; unpaired t-test, \*\*\*p<0.001). **e,** Beating frequency in beats per minute (BPM) in normal and diabetic organoids as recorded by MEA (mean±SD, n>5 organoids; unpaired t-test, \*p<0.05,) **f,** Seahorse energy map of normal and diabetic-like organoids (mean±sd).



**Supplementary Figure 7.** Illustration of a custom SD-OCT imaging system, FC: fiber coupler, PC: polarization controller, C1-C3: collimator, L1 - L4: lens, GS: galvo scanner; G: grating.



**Supplementary Table 2.** Antibodies used for immunofluorescence in this report.