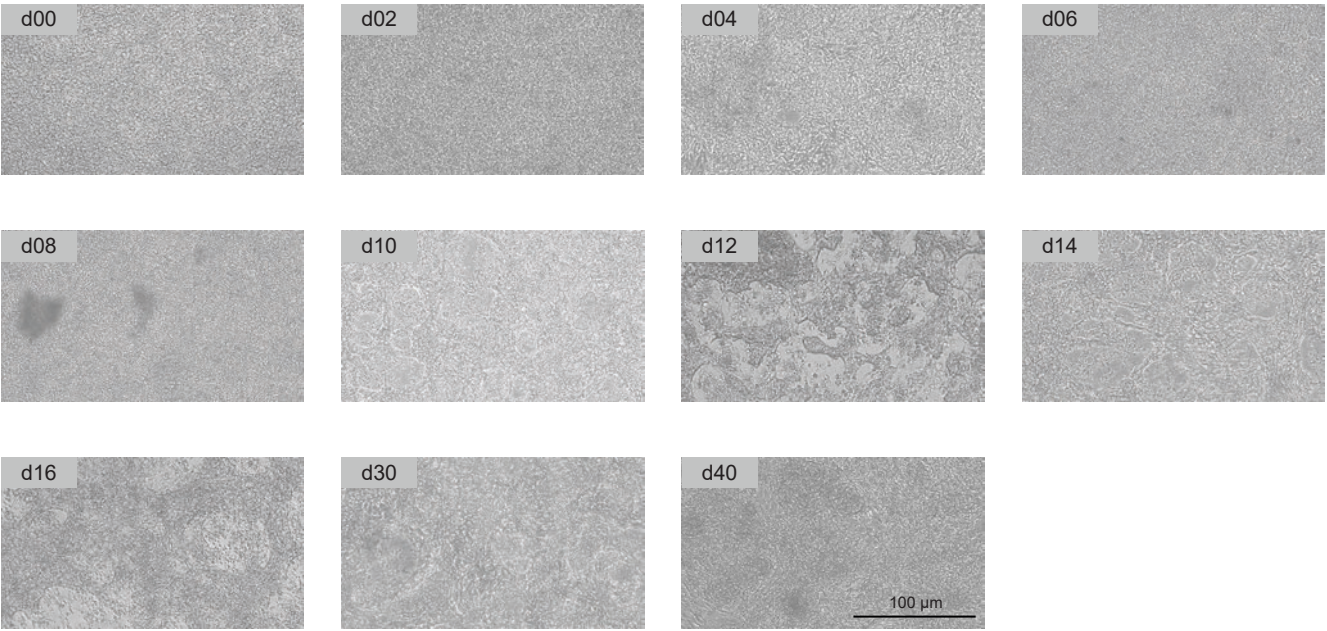


a

1460



b

1460c

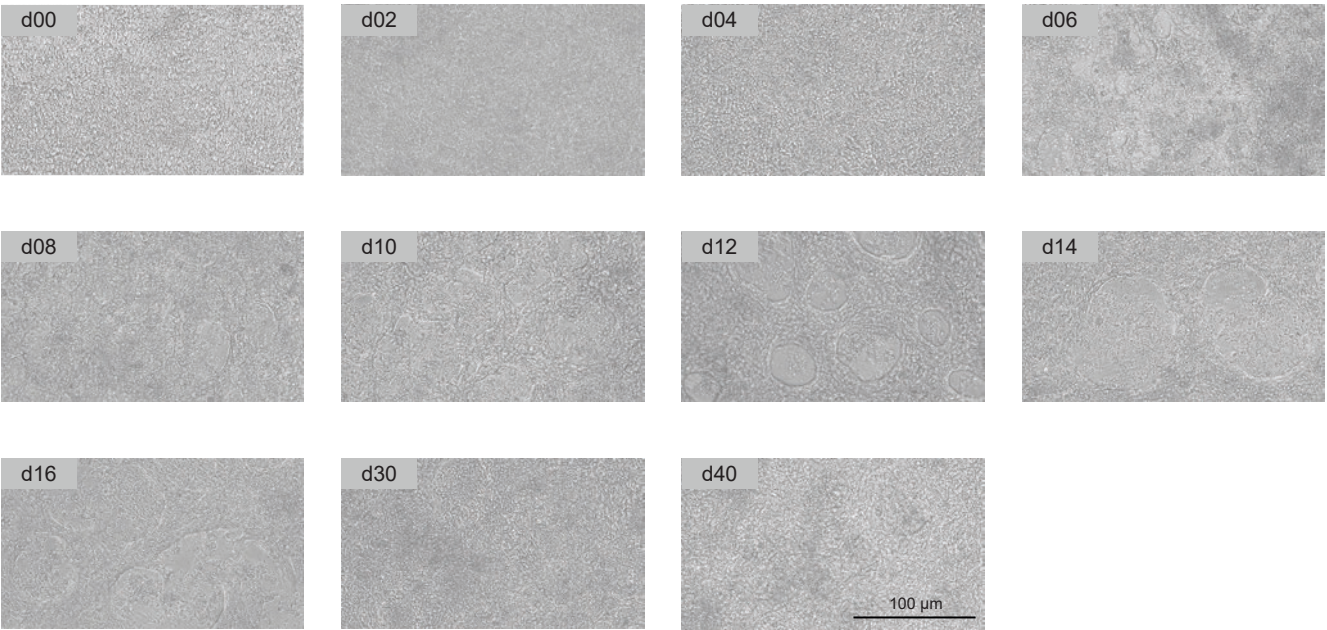


Figure S1

Figure S1. Brightfield images of the *in vitro* phenotype of 2D culture during cardiac differentiation trajectories, related to Figure 2.

a, b Brightfield images of cell lines 1460 (**a**) and 1460c (**b**) monolayers over a 40-day cardiac differentiation trajectory (d00, d02, d04, d06, d08, d10, d12, d14, d16, d30, and d40). Scale bars are shown in the figure.

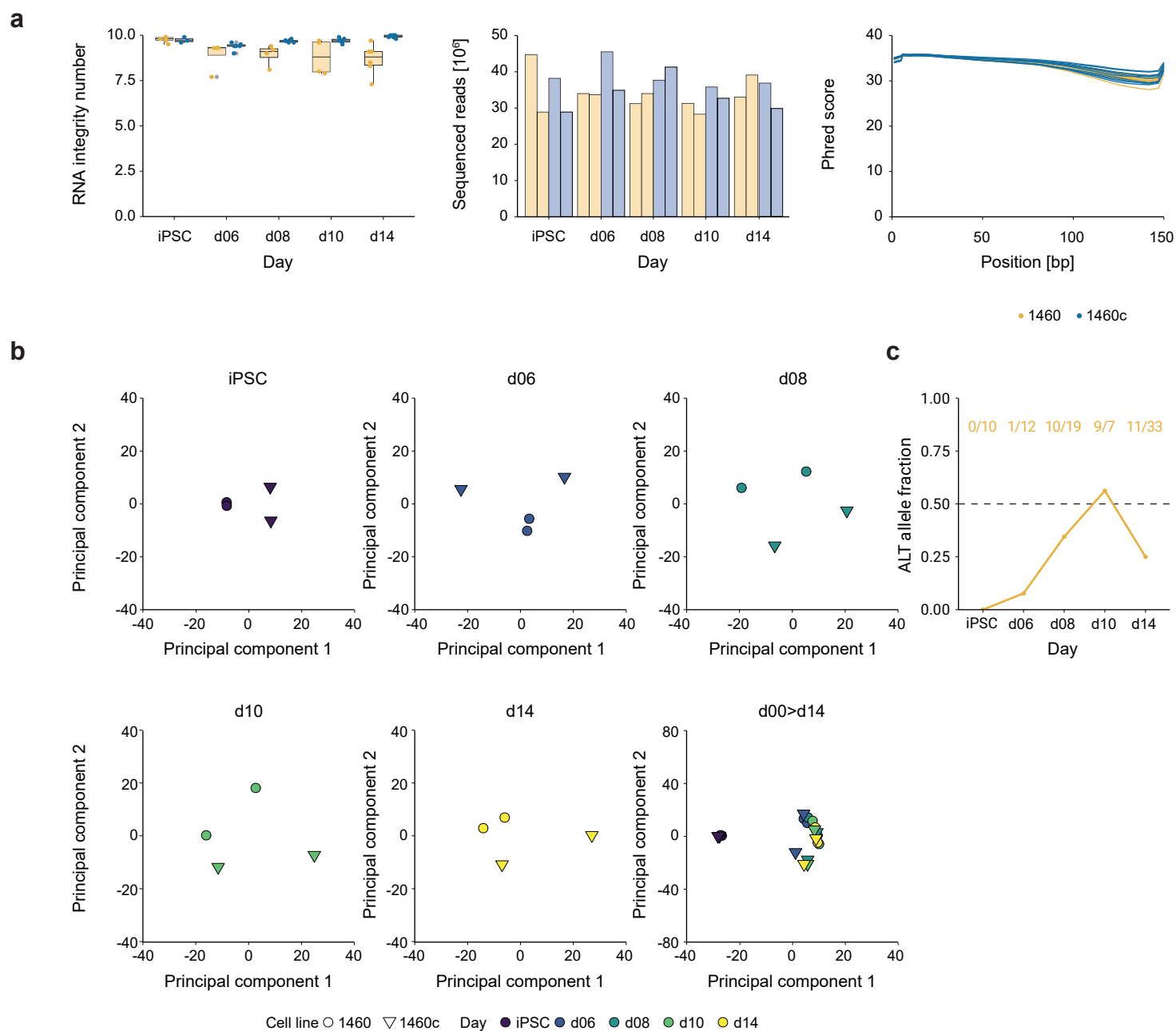
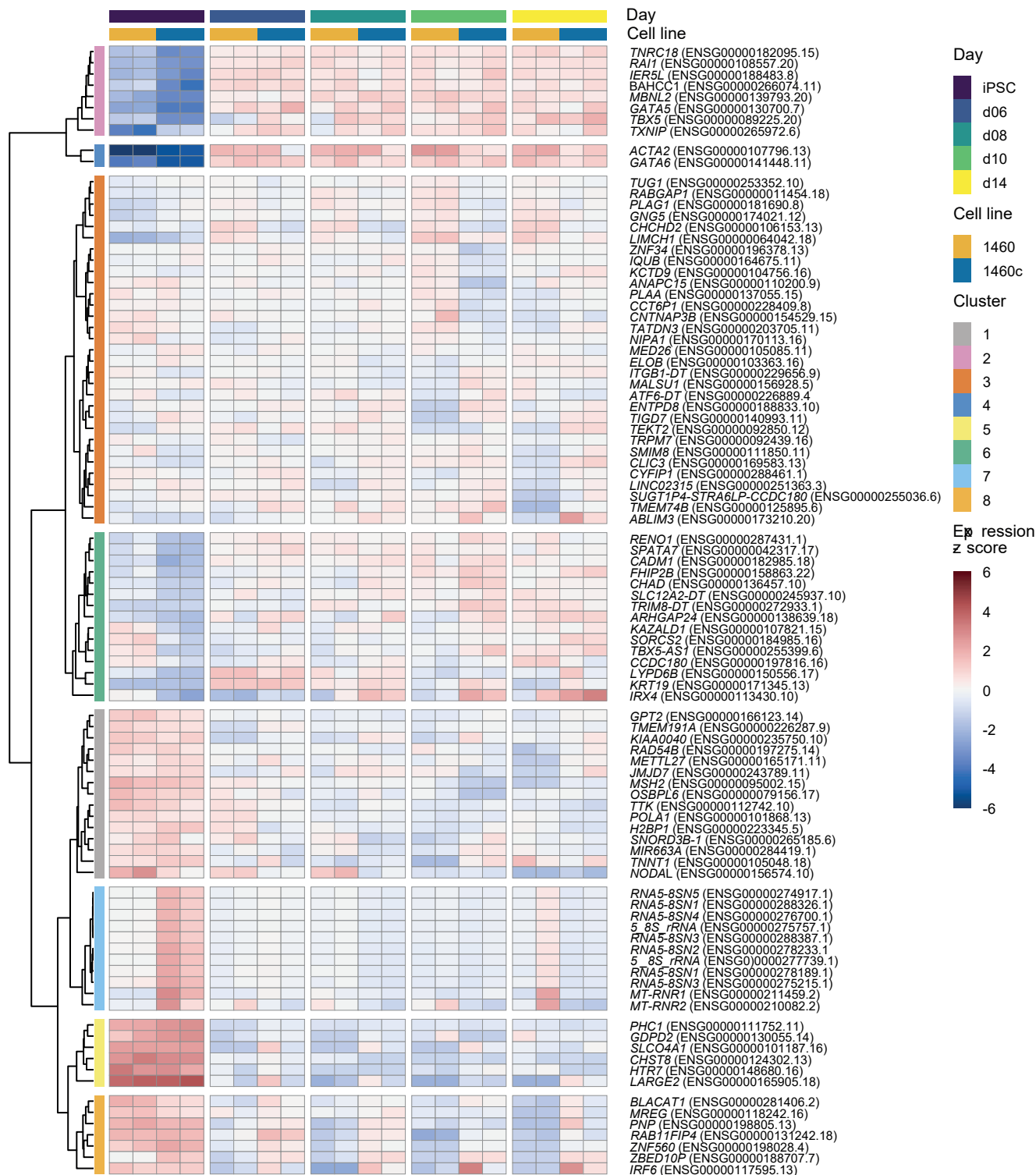


Figure S2

Figure S2. Quality metrics of bulk RNA-seq for 2D differentiation, related to Figure 2.

a RNA integrity numbers for iPSC, d06, d08, d10, and d14 samples, shown as boxplots (left); number of sequenced reads for each sample (1460/1460c) across differentiation stages (middle); and corresponding Phred quality scores across read positions (right). **b** Principal component analysis of RNA-seq confirming similarity among biological replicates within each line across differentiation stages (iPSC, d06, d08, d10, and d14) and progressive transcriptional separation along the differentiation trajectory relative to iPSCs (d00>d14). **c** Allele-specific expression at the heterozygous SNP assessed using reads overlapping the variant. ALT allele fractions were calculated from aggregated informative reads. Numbers above each time point indicate ALT/REF read counts. The dashed line indicates balanced allelic expression (0.5).

a



b

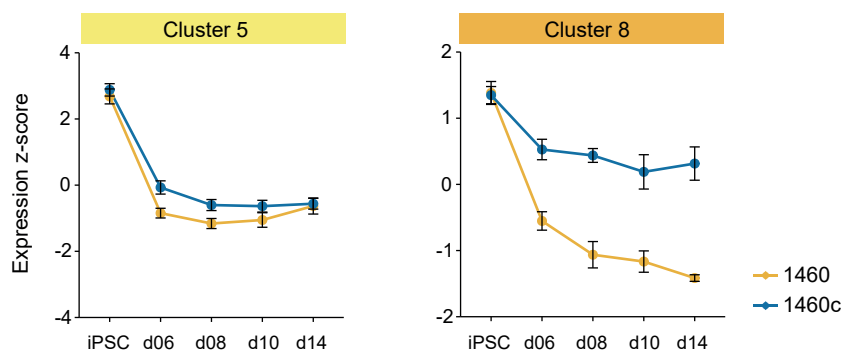


Figure S3

Figure S3. Stage-specific analysis of temporal gene expression dynamics during 2D cardiac differentiation, related to Figure 2.

a Heatmap showing z-score-normalized expression of genes with significant genotype $\times$ time interaction effects identified by likelihood ratio testing (LRT) across cardiac differentiation (iPSC, d06, d08, d10, and d14). Genes were grouped by hierarchical clustering into eight expression clusters. Sample annotations indicate differentiation stage and line (1460/1460c).

b Mean expression profiles of clusters 5 and 8 across differentiation, shown as mean z-scores $\pm$ SEM, reveal early divergence between 1460 and 1460c during differentiation.

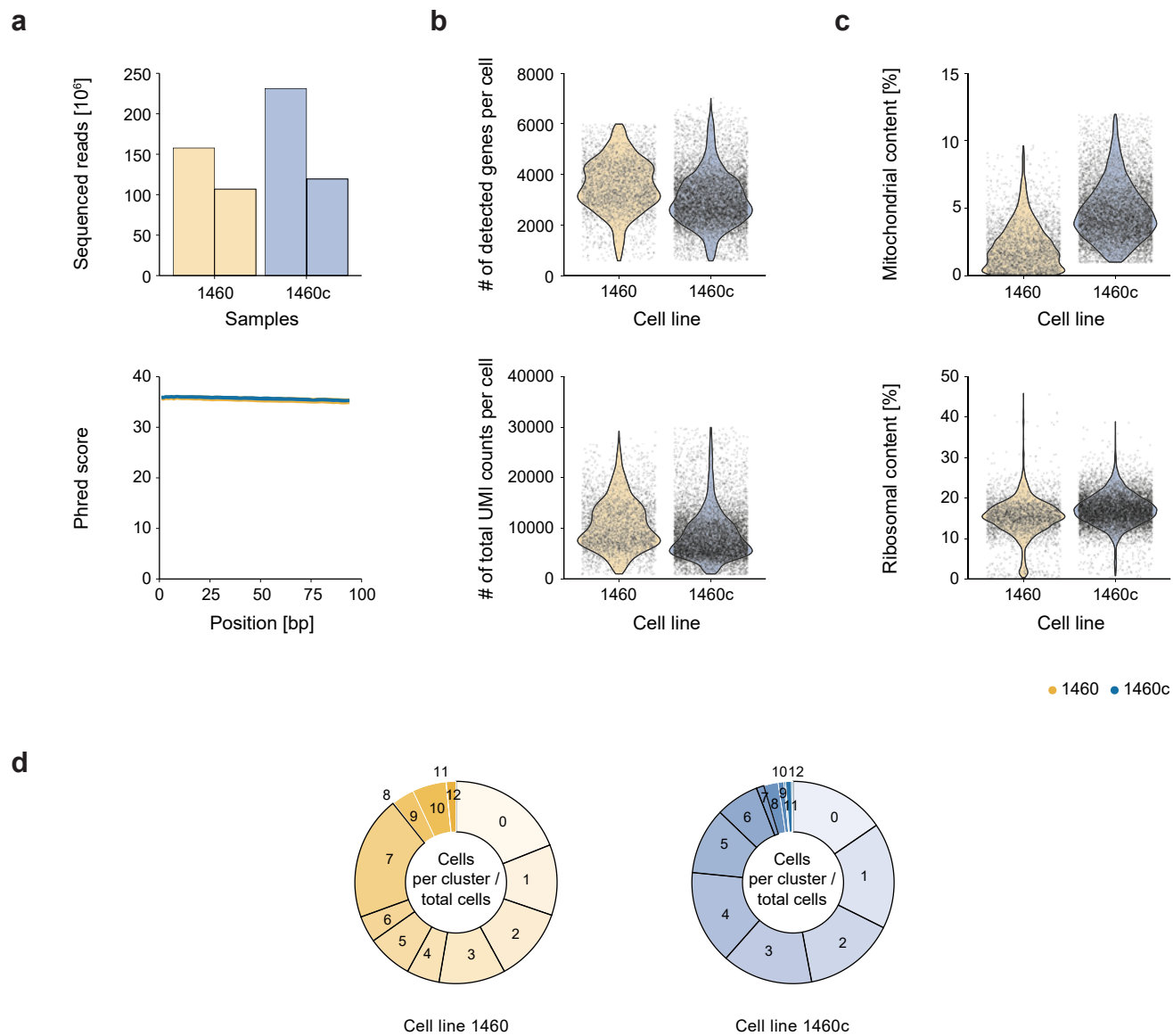


Figure S4

Figure S4. Quality metrics of scRNA-seq for 2D differentiation and cell distribution across the identified cell identities stratified by genotype, related to Figure 3.

a Sequenced reads (upper panel) and Phred quality scores (lower panel) for each cell line and sample. **b** Number of detected genes (upper panel) and total UMI counts (lower panel) per cell, split by line. **c** Percentage of mitochondrial content (upper panel) and ribosomal content (lower panel) across cell lines. **d** Distribution of cells across identified cell identities for 1460 (left) and 1460c (right) showing that clusters 0 to 7 (progenitors/cardiac progenitors/immature cardiomyocytes) comprise 89.6% (1460) and 95.3% (1460c) of total analyzed cells, respectively.

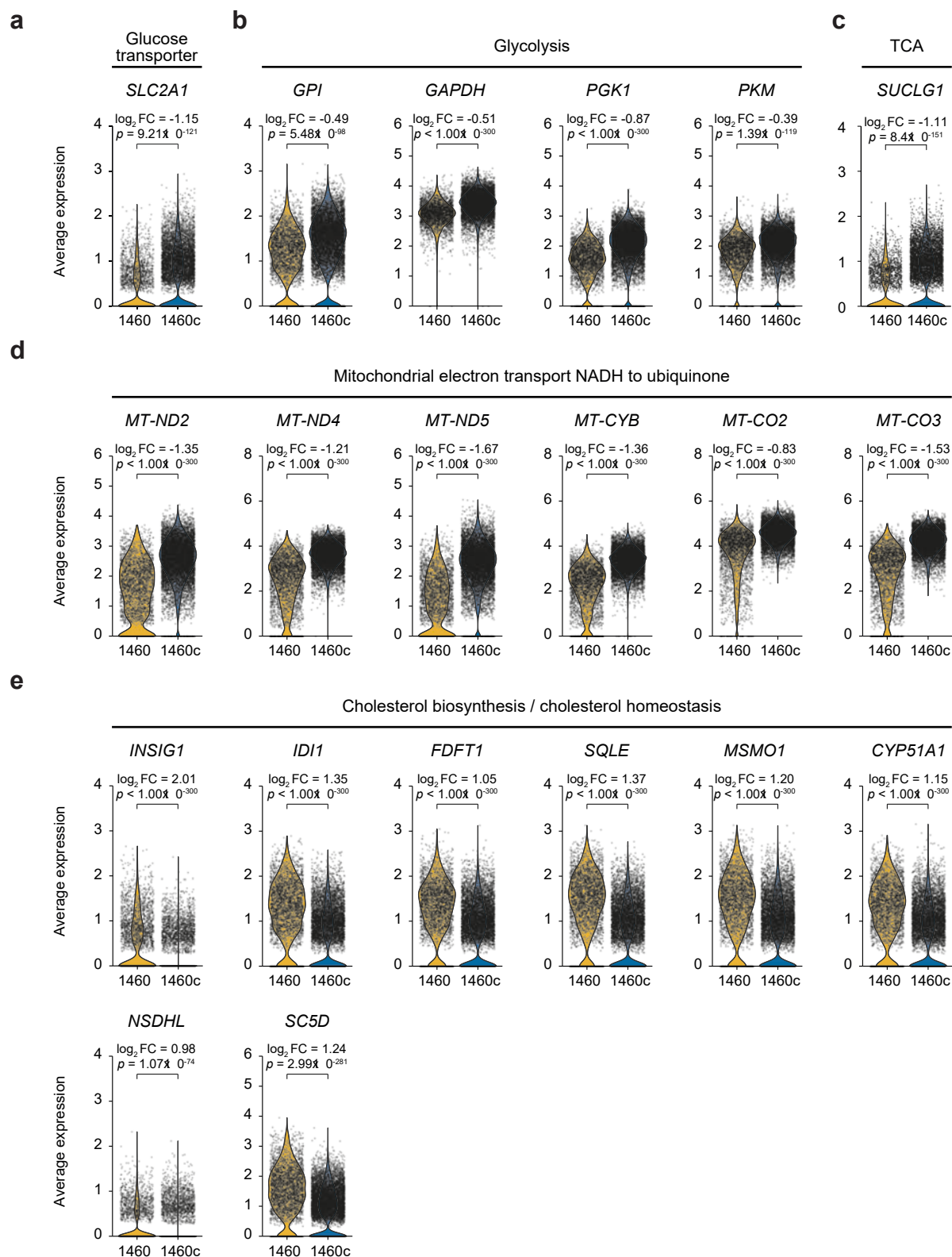


Figure S5

Figure S5. Normalized expression of modulated genes identified by scRNA-seq on d08, related to Figure 3.

a-e Average expression of **(a)** glucose transporter genes (*SLC2A*), **(b)** glycolysis genes (*GPI*, *GAPDH*, *PGK1*, and *PKM*), **(c)** TCA cycle genes (*SUCLG1*), **(d)** genes involved in mitochondrial NADH-to-ubiquinone electron transport (NADH-dehydrogenase/complex I: *MT-ND2*, *MT-ND4*, and *MT-ND5*; cytochrome-c-reductase/complex III: *MT-CYB*; cytochrome-c-oxidase/complex IV: *MT-CO2*, *MT-CO3*), and **(e)** genes of cholesterol biosynthesis and homeostasis (*INSIG1*, *IDI1*, *FDFT1*, *SQLE*, *MSMO1*, *CYP51A1*, *NSDHL*, and *SC5D*) split by genotype for clusters 0-7 are shown. *P* values were calculated using the Wald test. *P* < 0.05 is statistically significant and exact values are specified in the corresponding figures.

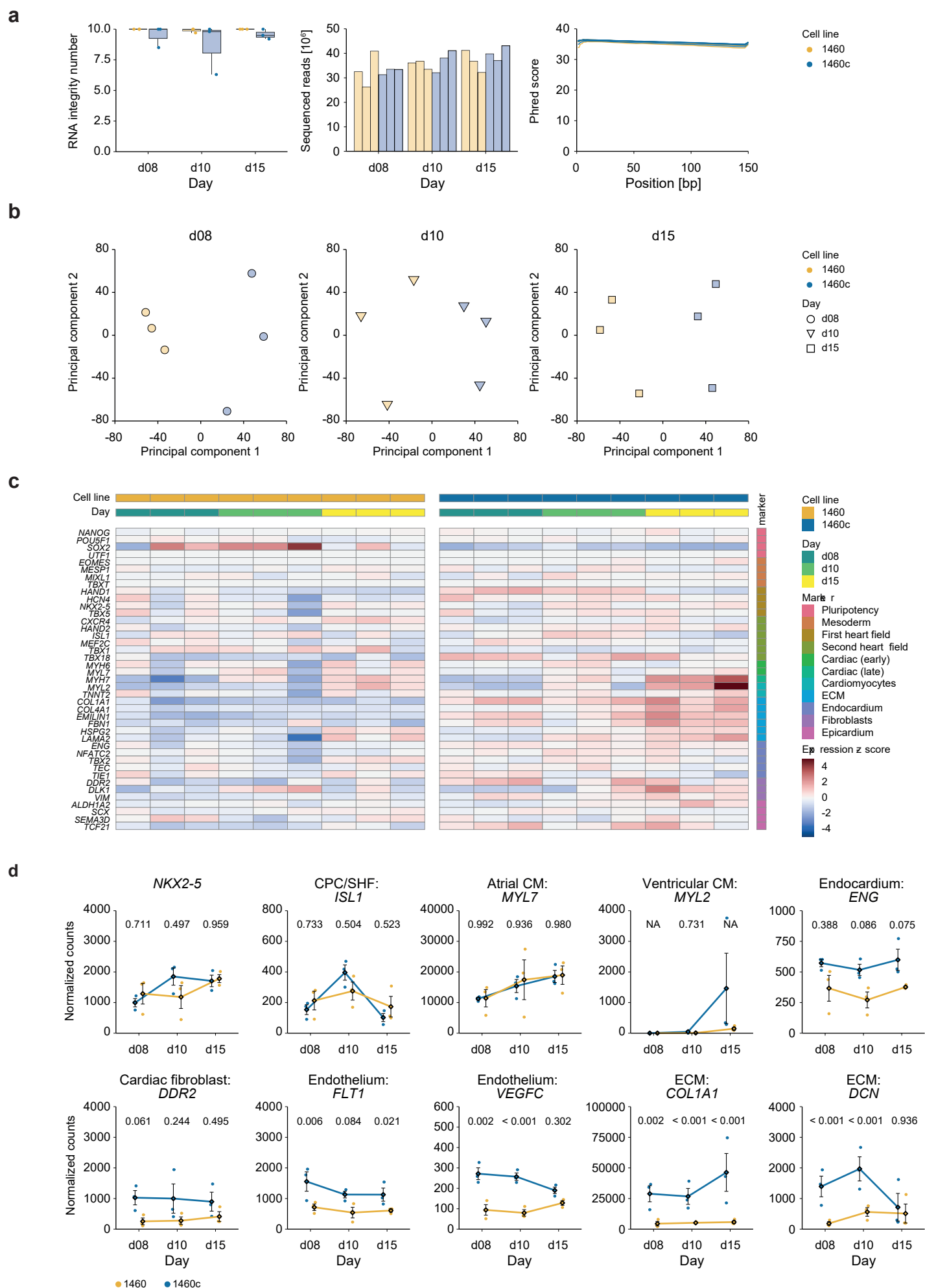
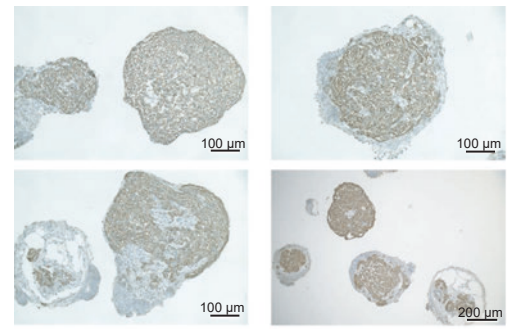
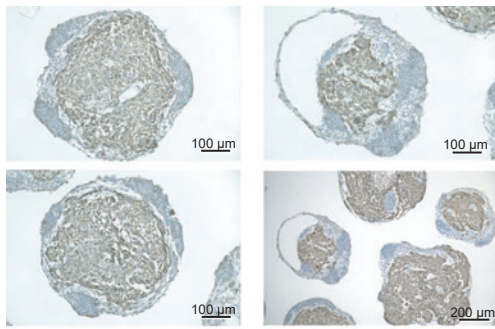


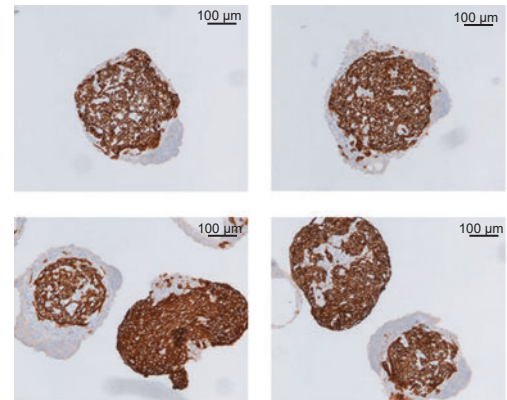
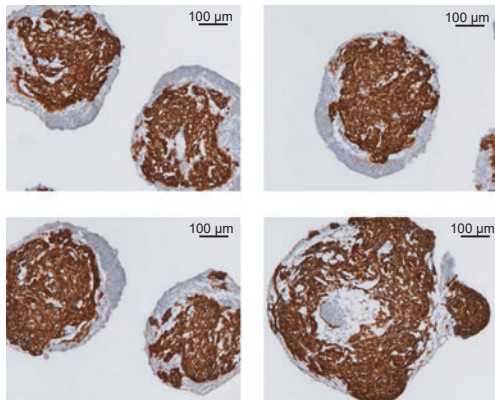
Figure S6

Figure S6. Bulk RNA-seq of 3D human heart organoids from HOS-derived and corrected iPSC lines, related to Figure 4.

a RNA integrity number of d08, d10, and d15 samples for each cell line shown as boxplots (left); number of sequenced reads for each sample (1460/1460c, n=3) per day (d08, d10, and d15) (middle); and Phred quality scores (right). **b** Principal component analysis of RNA-seq showing similarity among biological triplicates within each cell line (d08, d10, and d15). **c** Heatmap showing expression of representative markers for pluripotency, mesoderm, FHF, SHF, early and late cardiac stages, CMs, ECM, endocardium, fibroblasts and epicardium recapitulating hHO formation on d08, d10, and d15 for 1460 and 1460c with three replicates per line, according to Lewis –Israeli et al. **d** Transcriptome analysis (normalized counts) for FHF (*NKX2-5*), CPC/SHF (*ISL1*), atrial/early CMs (*MYL7*), ventricular/late CMs (*MYL2*), endocardium (*ENG*), cardiac fibroblasts (*DDR2*), endothelium (*FLT1*, *VEGFC*), and ECM (*COL1A1*, *DCN*) over the hHO differentiation process (d08, d10, and d15, n=3 per line). All data are presented as mean $\pm$ SEM. *P* values were calculated by Wald test. *P* < 0.05 is statistically significant with precise values specified in the figure. FHF, first heart field. SHF, second heart field. CMs, cardiomyocytes. ECM, extracellular matrix. CPC, cardiac progenitor cells. hHO, human heart organoids. N/A not applicable.

a α -ACTININ**b**

MLC2A

**c**

MLC2V

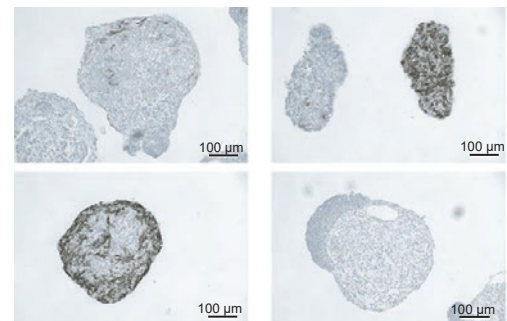
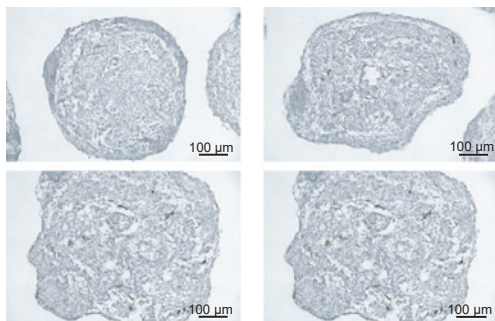
**Figure S7**

Figure S7. Immunohistochemistry of 3D human heart organoids from HOS and corrected iPSC line, related to Figure 4.

a-c Confocal immunofluorescence images of paraffin-embedded organoid samples (d15) stained for α -ACTININ (**a**), MLC2A (**b**), and MLC2V (**c**). Scale bars are shown in the figure.