

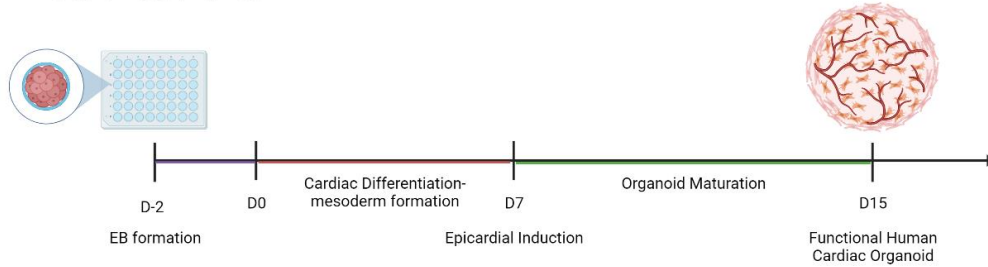
Supplementary Figures

“Improving Human Cardiac Organoid Design Using Transcriptomics”

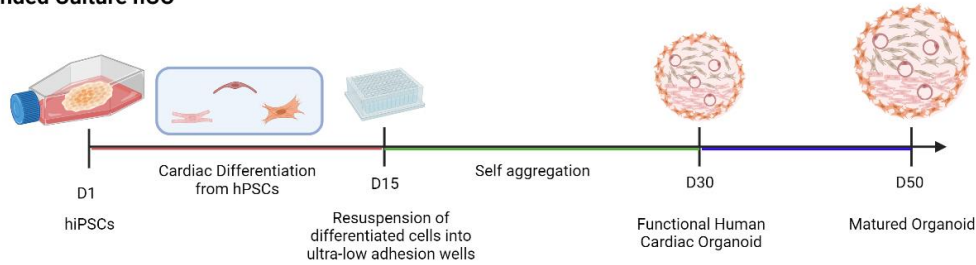
Hyams, Nathaniel A., Kerr, Charles M., Arhontoulis, Dimitrios C., Ruddy, Jean Marie, Mei, Ying

Figure S1

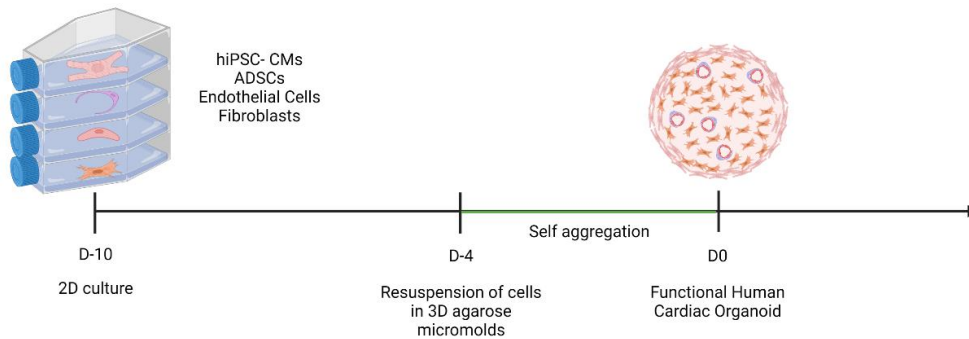
A. Embryoid Body (EB) hCO



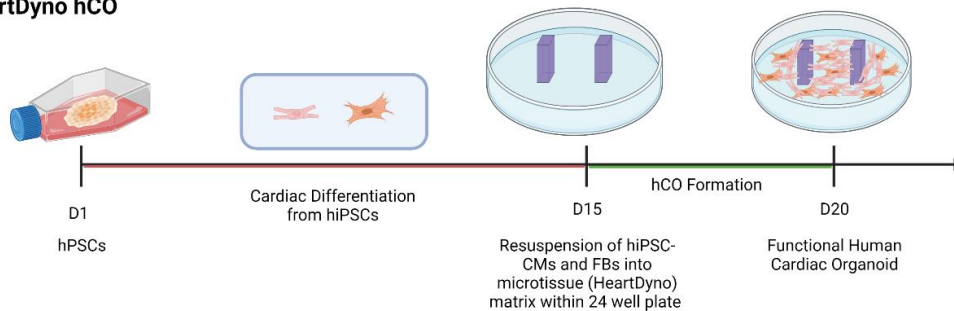
B. Extended Culture hCO



C. Primary Stroma hCO



D. HeartDyno hCO



Cell Key



Figure S1. Characterization of hCO models chosen. (A) Embryoid Body hCO formation. hiPSCs are seeded into ultra-low attachment 96-well plates, allowing EB formation to take place. After 48 hours, EB's are exposed to CHIR99021 and Wnt-C59 sequentially, inducing cardiac mesoderm. EB hCOs are established in 7 days, then allowed to mature, taking on a mature profile at D15 of the protocol. (B) Extended Culture hCO formation. hiPSCs are differentiated in 2D within 12-well plates, where cells are treated with CHIR99021 and IWP-2, sequentially to induce cardiac mesoderm. Differentiation was ended at D15, and cells were dissociated and seeded into ultra-low adhesion 96-well plates. hCOs were kept in culture past D50 of culture for maturation. (C) Primary Stroma hCO formation. hiPSC-CMs, human cardiac ventricular fibroblasts (FBs), human umbilical vein endo-thelial cells (ECs), and human adipose-derived stem cells (ADSCs) which act as PCs were kept in culture. All cell types were dissociated and seeded into agarose micro-molds at a 50% hiPSC-CM to 50% non-myocyte ratio. hCOs are allowed 4 days to self-aggregate and form a hCO. (D) HeartDyno hCO formation. hiPSCs are differentiated in 2D, utilizing CHIR99021 followed by IWP4, then dissociated, and seeded alongside Collagen I into custom-built 96-well plates, with inserts to in-fluence hCO formation. hCOs self-aggregate and form functional hCOs in 5 days, by D20.

Figure S2

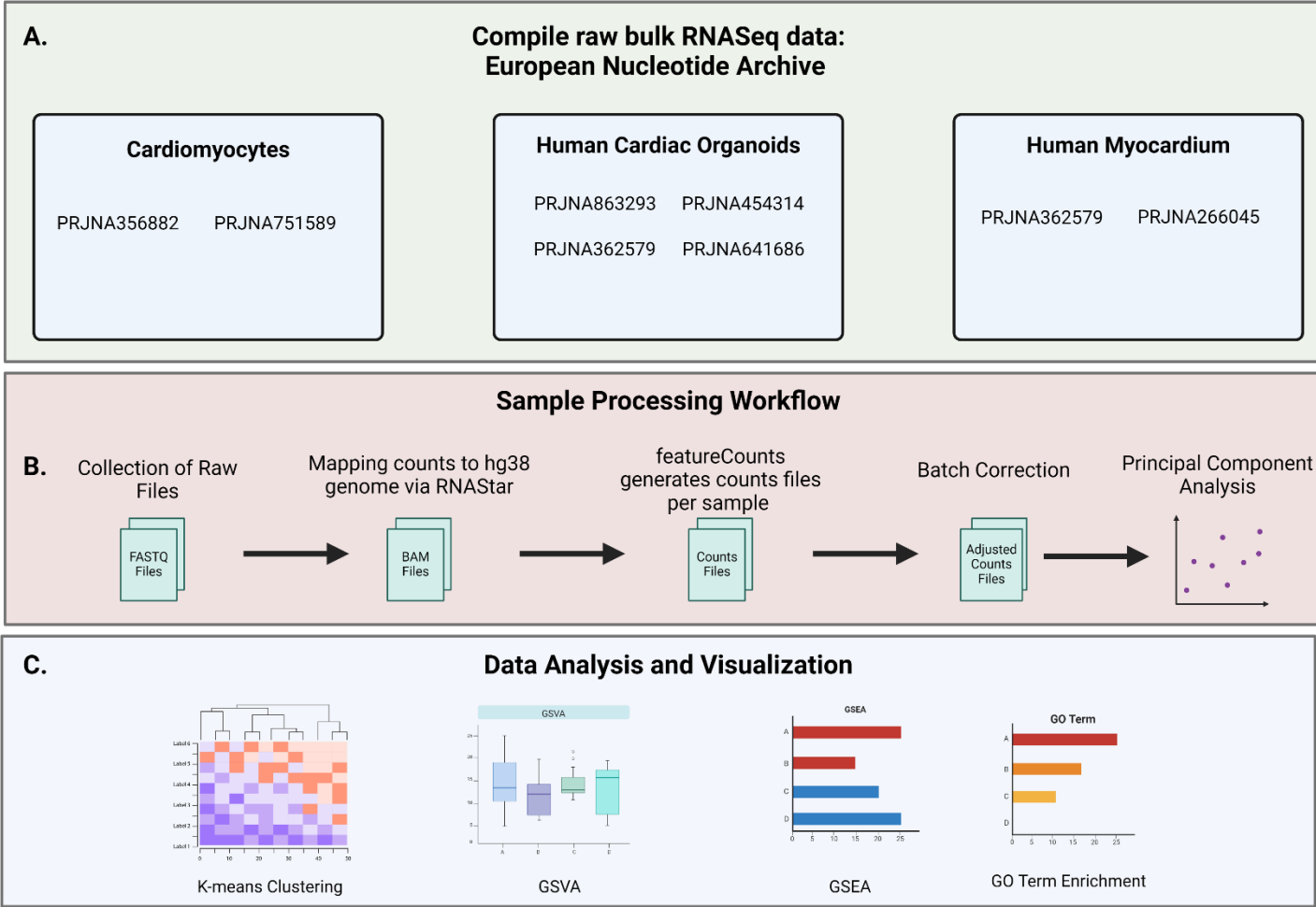


Figure S2. Schematic outline of experimental workflow. (A) European Nucleotide Archive (ENA) project identification numbers for each set of samples used in the bulk RNA sequencing study. (B) Experimental workflow: obtaining raw FASTQ files from public databases, mapping raw files to hg38, generating counts files, batch correction and normalization, and producing a principal component analysis (PCA) output. (C) Overview of the various analyses within this study: K-means clustering analysis, gene set variation analysis (GSVA), and gene set enrichment analysis (GSEA).

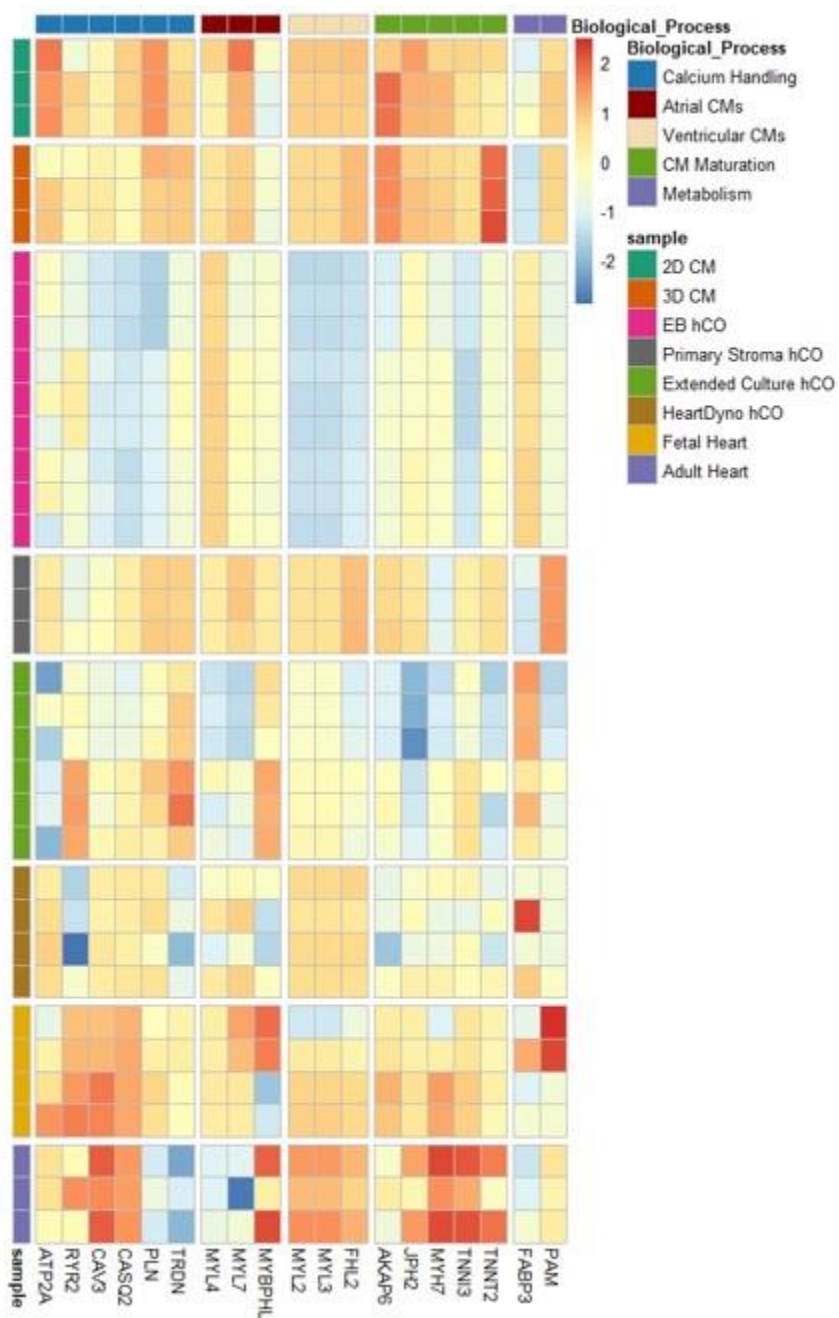


Figure S3. Heatmap displaying expression across entire study for selected cardiac biological processes of interest. Scale bar is Z-score. Genes indicated within each biological process shown at the bottom. Primary Stroma hCOs and Fetal Heart samples profiled similarly across all biological processes, while Extended Culture hCOs scored well with Calcium Handling.

Gene Sets for GSVA				
Cardiac Fibroblasts	Endothelial Cells	Immune Cells	Atrial CMs	Ventricular CMs
SCN7A	DLC1	GNLY	KCNMB2-AS1	LINC02552
ABCA8	PDGFRB	PRF1	MYL4	NLGN1
APOD	VWF	NKG7	PLCB1	ABHD18
ABCA6	HEY1	GZMB	LINC02147	PCDH7
ACSM3	DNAJB1	KLRD1	MALAT1	CTNND2
MALAT1	HSPA1A	LILRA1	HTR4	DLC1
SPARCL1	HSPA1B	APOBEC3A	MYL7	FHL2
ABCA9	NDUFA4L2	FCN1	KCNMB2	CPVL
ABCA10	AGT	IFI30	PANCR	INPP4B
DCN	RG55	CDC42EP2	ADGRL2	TRDN-AS1
CFH	SLC9A3R2	IL32	HAMP	PRELID2
NPPA	RGCC	IL7R	ZNF385B	GRXCR2
KCNT2	ZFH3	CD3D	FHL2	AC107068.2
NAV3	MYH11	TRAC	CACNB2	SH3RF2
PLA2G2A	ACTA2	PTPRC	SLIT3	LINC02147
DAAM1	TPM2	F13A1	ASTN2	FGF12
KA2N	CASQ2	NEURL2	NTM	IL1RAPL1
FBLN1	RAMP3	PLTP	LINC01170	LINC02137
TIMP3	ACKR1	LYVE1	LINC01880	SGCD
TBX20	PLVAP	C1QB	PRELID2	CDH13
JUNB	TSHZ2	CD3D	CALD1	ANKRD1
NAMPT	CCL2	CCL5	ANKRD1	ACTA1
MT2A	ICAM1	CD3E	PALLD	MYO18B
ZFP36	CXCL2	TRAC	MYO18B	FHL1
NR4A1	LDB2	F13A1	MYOM1	PALLD
GADD45B	GMD5	FGF13	ACTN2	CREB5
FOS	LINC02147	RBPI	MYPN	XIRP2
EGR1	CEMIP2	APOD	SAMD4A	ROR1
THBS1	ST6GALNAC3	STAB1	SVIL	MYPN
SAT1	PCDH7	S100A9	PDLIM3	ACTN2
FN1	MYH11	LYZ	GAPDH	MYL2
PLXDC2	ACTA2	S100A8	CRYAB	ACTC1
COL8A1	TPM2	VCAN	ACTC1	CRYAB
CTGF	LMOD1	S100A12	CSRP3	ACTB
TNC	TAGLN	SAT1	FTH1	MB
CCDC80	MYL9	MT-CO2	NDUFA4	MYL12A
SERPINE2	TTN	KLRD1	UQCRB	TNNI3
NOX4	RYR2	NKG7	MB	PLN
COL1A2	MYBPC3	GNLY	PLN	MYL3
PCOLCE2	SLC8A1	PRF1	CKM	TCAP
MFAP5	FGF12	CD7	EPS8	EBF1
FBN1	FHL2	CD74	PDGFRB	PDGFRB
RYR2	CCL21	MS4A1	DLC1	DLC1
DCN	MMRN1	IGKC	EBF1	PLA2G5
IGFBP6	PKHD1L1	BANK1	SPARCL1	EPS8
S100A6	SEMA3A	IGHM	IGFBP7	NR2F2-AS1
UAP1	PTPRE	CCR7	PLA2G5	SOX5
GSN	HP	LTB	MAML2	SPARCL1
GFPT2	PRG4	LEF1	UNC5C	FRMD3

Table S1. List of top 50 expressed gene across each cell type according to the Human Heart Atlas. These top 50 genes are used within the GSVA analysis.