

Figure S1: The gene expression of mitochondrial cytochrome oxidase 1 (mtCOI) is studied to quantify the mitochondrial copy number. The representative results are shown using A) gel RT-PCR. B) Representative data from qRT-PCR averaged from three technical replicates was used to quantify the change in mRNA levels of mtCOI (relative to GAPDH). ΔCt method was used to compare expression of mtCOI (relative to GAPDH) between 1m and 3m hPSC-CMs. Data is shown from three biological replicates and error bars represent SEM. The primers used for mtCOI: Forward: 5'-ACGTTGTAGCCCACCTTCCAC-3', Reverse: 5'-CATCGGGGTAGTCCGAGTAA-3', GAPDH: Forward: 5'-CTGATTTGGTCGTATTGGGC-3', Reverse: 5'-TGGAAGATGGTGATGGGATT-3'. The annealing temperature used for PCR was 60°C.

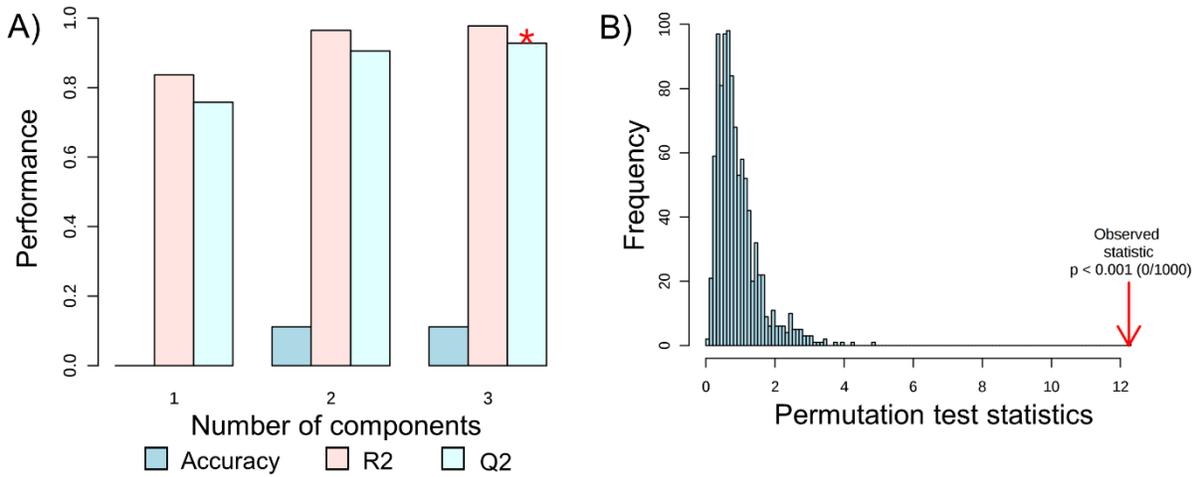


Figure S2: Model statistics and validation for PLS-DA model used in Figure 3. (A) Performance of PLS-DA model using cross validation method: leave one out cross validation (LOOCV) and * represents maximum quality assessment statistic (Q2) indicating high consistency between the predicted and original data. (B) The significance of the model was tested using separation distance (B/W) test statistic and 1000 permutations.

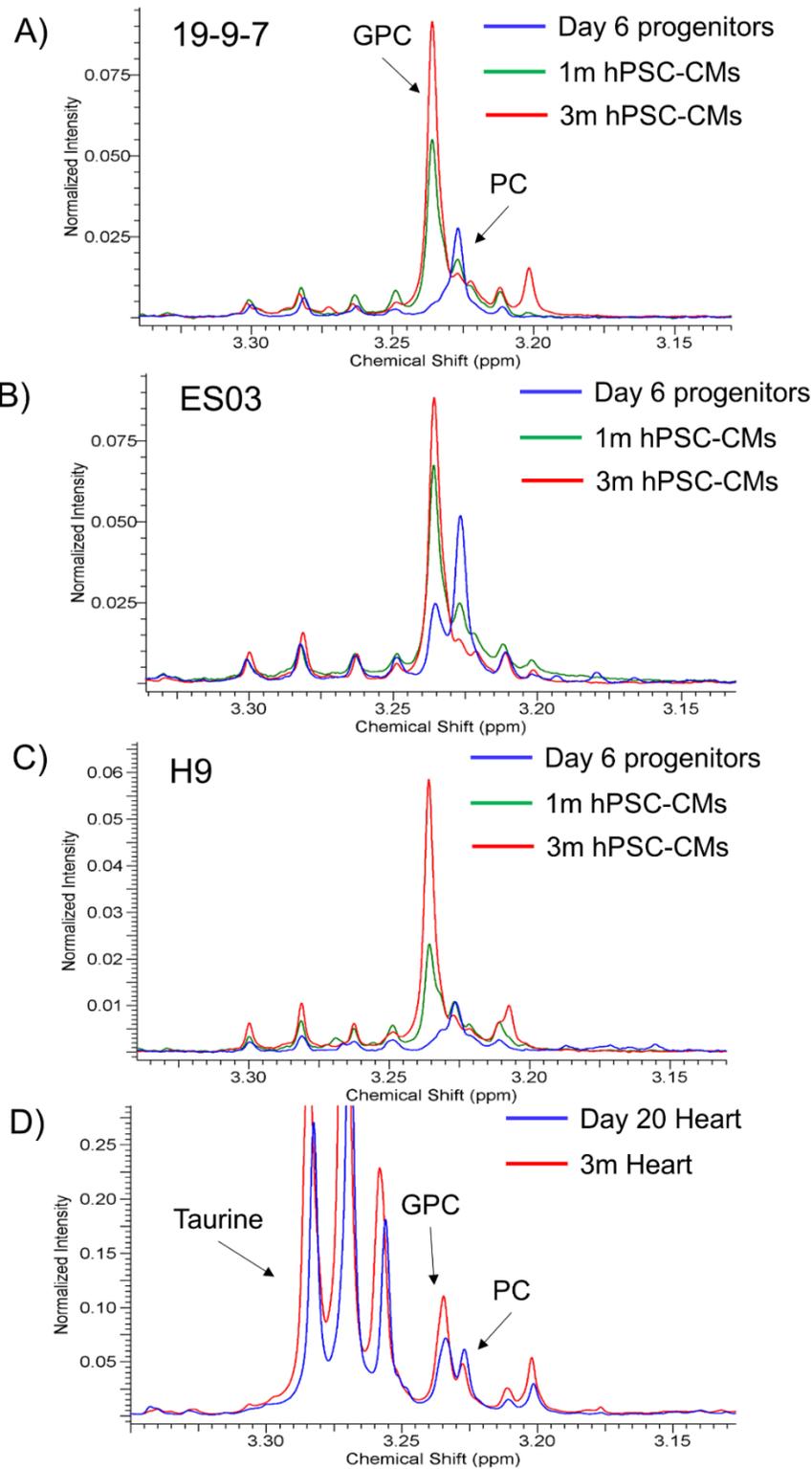


Figure S3: Representative NMR spectra for *in vitro* hPSC-CMs and murine cardiac tissue metabolite quantification. The region from 3.14 to 3.32 is shown here for A) 19-9-7 derived, B) ES03 derived, and C) H9 derived CMs at three different time points (day 6, 1m and 3m). D) Heart tissue from day 20 and 3m old mouse.

Supplementary Table 1: List of antibodies used for immunostaining (IS) and flow cytometry (FC)

Antibody	Isotype/source/cat.no./clone	Dilution
cTnT	Mouse IgG1/Lab Vision/ms-295-p1/13-11	1:200 (IS)
MF20	Mouse IgG2b/DSHB/MF20	1:20 (FC)
α-actinin	Mouse IgG1/Sigma-Aldrich/A7811/EA-53	1:200 (IS)
MLC2a	Mouse IgG2b/Synaptic systems/311011/56F5	1:400 (IS)
MLC2v	Rabbit polyclonal/ProteinTech Group/PTG10906-1-AP	1:200 (IS)
Ki-67	Mouse IgG1/BD Biosciences/550609	1:100 (FC)