**Supplementary Legends**

**Supplementary Figure 1: Fractionation-based proteomics analysis for D8 early-differentiated cardiomyocytes**

(A) A volcano plot is given depicting the significantly upregulated genes in cardiomyoblasts and D8 cardiomyocytes using the fractionation based approach. A t-test (FDR=0.01 and S0=1) was performed for pairwise comparison. Results revealed an increase in structural proteins at D8, which was to the results from the shotgun analysis at D6. (**B**) Results from the GO analysis using Gorilla and a ranked list of higher expressed proteins at D8 (compared to undifferentiated H9C2 cardiomyoblasts) revealed pathway enrichment that is similar to the results from D6 (see Figure 1).

**Supplementary Figure 2: Fractionation-based proteomics analysis for D8 early-differentiated cardiomyocytes**

(A) A volcano plot is given depicting the significantly upregulated genes in D8 versus D13 cardiomyocytes using the fractionation-based approach. A t-test at relaxed criteria (FDR=0.05 and S0=1) was performed for pairwise comparison.

**Supplementary Figure 3: Graphical depiction of the results of Fig 5**

Analysis with Reactome was performed as shown in Figure 5 and the most decisively regulated pathways for each cluster were depicted graphically as given by Reactome. Results in the left and right columns related to clusters with up and down-regulated proteins, while rows describe clusters with early, late, continuously and transiently downregulated proteins. Yellow coloured branches of pathways indicate those where proteins that relate to the respective cluster are involved.

**Supplementary Table 1: Data from differential shotgun proteomics between undifferentiated and D6 differentiated H9C2 cells**

Proteins that were found to be significantly up or downregulated in cardiomyocyte-like cells at day 6 differentiation compared to undifferentiated H9C2.1 cardiomyoblasts are depicted. Gene names and detailed protein description are given along their statistical significance (Lop P value) and the logarithmic values of their relative expressions (D6 vs. undifferentiated). Further details of the proteome analysis including the number of identified peptides, the sequence coverage and the molecular weights are given.

**Supplementary Table 2: Data from fractionation-based proteomics between undifferentiated and day 8 differentiated H9C2**

Differential proteomics results as in Supplementary Table 1 depicting relative expressions between D8 differentiated and undifferentiated H9C2 cells during the fractionation proteomics run.

**Supplementary Table 3: Data from fractionation-based proteomics between undifferentiated and day 13 differentiated H9C2**

Differential proteomics results as in Supplementary Table 1 depicting relative expressions between D13 differentiated and undifferentiated H9C2 cells during the fractionation proteomics run. Only proteins that were not differentially regulated at D8 were depicted.

**Supplementary Data 1: Results from two-way ANOVA and Reactome analysis**

The first sheet gives the raw data from the two-way ANOVA analysis for each protein. Thereby, the association of the individual protein to its cluster and the p-values for belonging to the differentiation, the timing and the interaction groups are given (Columns AE, AF, AG). Proteins with either p<0.05 in at least one of these groups were selected. The other sheets present the REACTOME analysis for analysing the entire proteins for each of the clusters, together with the graphs that were given in Figure 5. Clusters were organised according the sequence as obtained from hierarchical clustering, depicting early, late, continuously, and transiently upregulated (Clusters 4, 1, 3, 2; in the given sequence) and downregulated proteins (Clusters 8, 6, 5, 7). Only pathways containing 2% of the cluster proteins and were considered and ranked according the enrichment ratio in Column D.