

## **Figure Captions**

**Fig.1.** Detail of the four cell culture steps and comparison of medium renewal frequency between both protocols (P1 and P2), Green: days with culture medium renewal and red: days without culture medium renewal.

**Fig.2.** Characterization of iPS derived hepatocytes at the end of differentiation (end of step 4) of each protocol: (A) cell morphology of hepatocyte like cells, (B) DAPI and CYP3A4 immunostaining, (C) hepatic genes expression compared to initial iPSC and (D) albumin production. **Fig.3.** (A) PLS-DA score plot derived from GC-MS analysis of all samples (four steps of protocols 1 and 2); (B) PLS-DA score plot of step 1 and step 4 in both protocols and (C) heatmap of top 25 metabolites significantly modulated.

**Fig.4.** PLS-DA score plot of step 1 in both protocols (A); volcano plot of metabolite log<sub>2</sub> fold change (P2S1/P1S1) plotted against  $-\log_{10} P$  value, indicating the metabolites significantly changed between both protocols (B, red circles,  $P < 0.01$  and  $FC > 1.4$ ) and heatmap of top 25 metabolites significantly modulated (C).

**Fig.5.** PLS-DA score plot of step 2 (A), step 3 (D) and step 4 (G) in both protocols; volcano plot of metabolite log<sub>2</sub> fold change (P2/P1) plotted against  $-\log_{10} P$  value, indicating the metabolites significantly changed between both protocols in step 2 (B), step 3 (E) and step 4 (H): red circles  $P < 0.01$  and  $FC > 1.4$ , blue circles  $P < 0.05$  and  $FC > 1.4$ ; and heatmap of top modulated metabolites ( $P < 0.01$ ) in step 2 (C), step 3 (F) and step 4 (I).

**Fig.6.** Selective mRNA levels (analyzed by RTqPCR) in step 1 for protocol 1 and protocol 2 (A); Heatmap of transcriptomic profile comparison between both protocols in step 4 (B) and heatmap of GO KEGG pathways modulated at the gene levels.

