

841 **Supplemental Figure 1. Metabolite abundance in 2D co-cultures.** Ion counts after  
842 normalization to total ng DNA of: (A) pyruvate, (B) Acetyl-CoA, (C) Propionyl-CoA, (D) Lactate,  
843 (E) NAD<sup>+</sup>, (F) NADH, and (H) AMP, ADP, ATP nucleotides. (G) Ratio of NAD<sup>+</sup> to NADH ion  
844 counts. (I) Calculated energy charge of each co-culture group based on ion counts of AMP,  
845 ADP, and ATP. Significance tested using unpaired t-test, comparison HJ vs SJH or SJ vs SJH,  
846 and corrected for multiple comparisons using Benjamini-Hochberg method. \*: p adj. < 0.05,  
847 \*\*: p adj. < 0.01, \*\*\*: p adj. < 0.001, \*\*\*\*: p adj. < 0.0001.

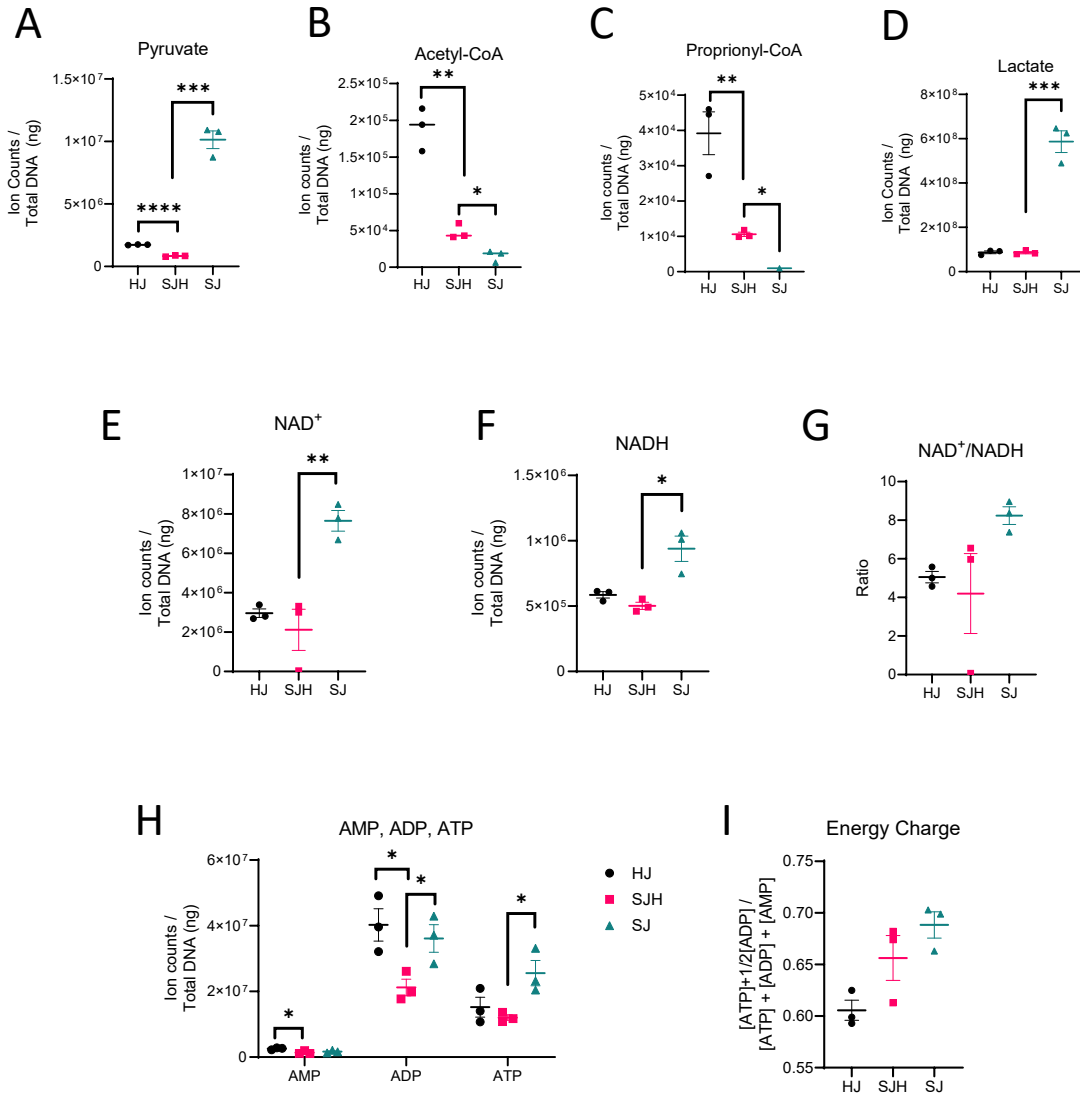
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849 **Supplemental Figure 2. Lactate abundance in co-culture and 1T1 dilution.** Bar graph of  
850 total ion counts after normalization to DNA compared to analytical dilution (1T1).  
851 Significance tested using unpaired t-test, comparison HJ vs SJH or SJ vs SJH, and 1T1 vs SJH;  
852 corrected for multiple comparisons using Benjamini-Hochberg method. \*: p adj. < 0.05, \*\*:  
853 p adj. < 0.01

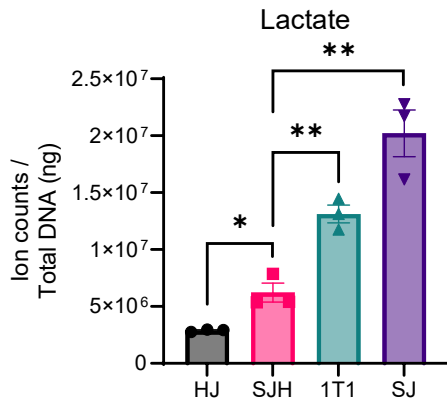
854  
855 **Supplemental Figure 3. Percent change in media purines.** Percent difference in media  
856 after 24h incubation with co-cultured cells where time point 0 represents starting media  
857 abundance prior to cell exposure. Dotted lines represent accumulation of hypoxanthine  
858 (Hpx) and uric acid (UA) after 24h at 37°C in media in absence of cells. Statistical comparison  
859 by unpaired t-test; letters indicate significance in comparison to HJ controls (“a”) or SJ  
860 controls (“b”).

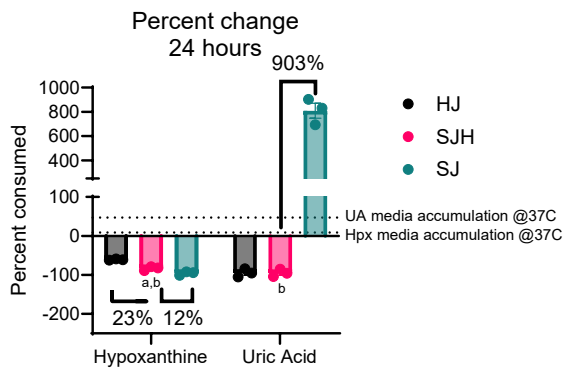
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862 **Supplemental Figure 4. Correlation matrix of SJH vs HJ ITUM in 2D co-cultures.**  
863 Correlation matrix assessing co-enriched isotopologues in response to presence of  
864 hepatocytes (SJ, SJH cultures). Red gradient represents positive associations while blue  
865 represents negative associations. Correlations measuring by Pearson correlation method.

866  
867 **Supplemental Figure 5. Distribution of <sup>13</sup>C enrichment.** Percent enrichment of total pools  
868 of (A) Uridine diphosphate N-acetylglucosamine and (B) glutathione after 24h incubation  
869 with [U-<sup>13</sup>C<sub>6</sub>]glucose at 37°C. \*: p adj. < 0.05

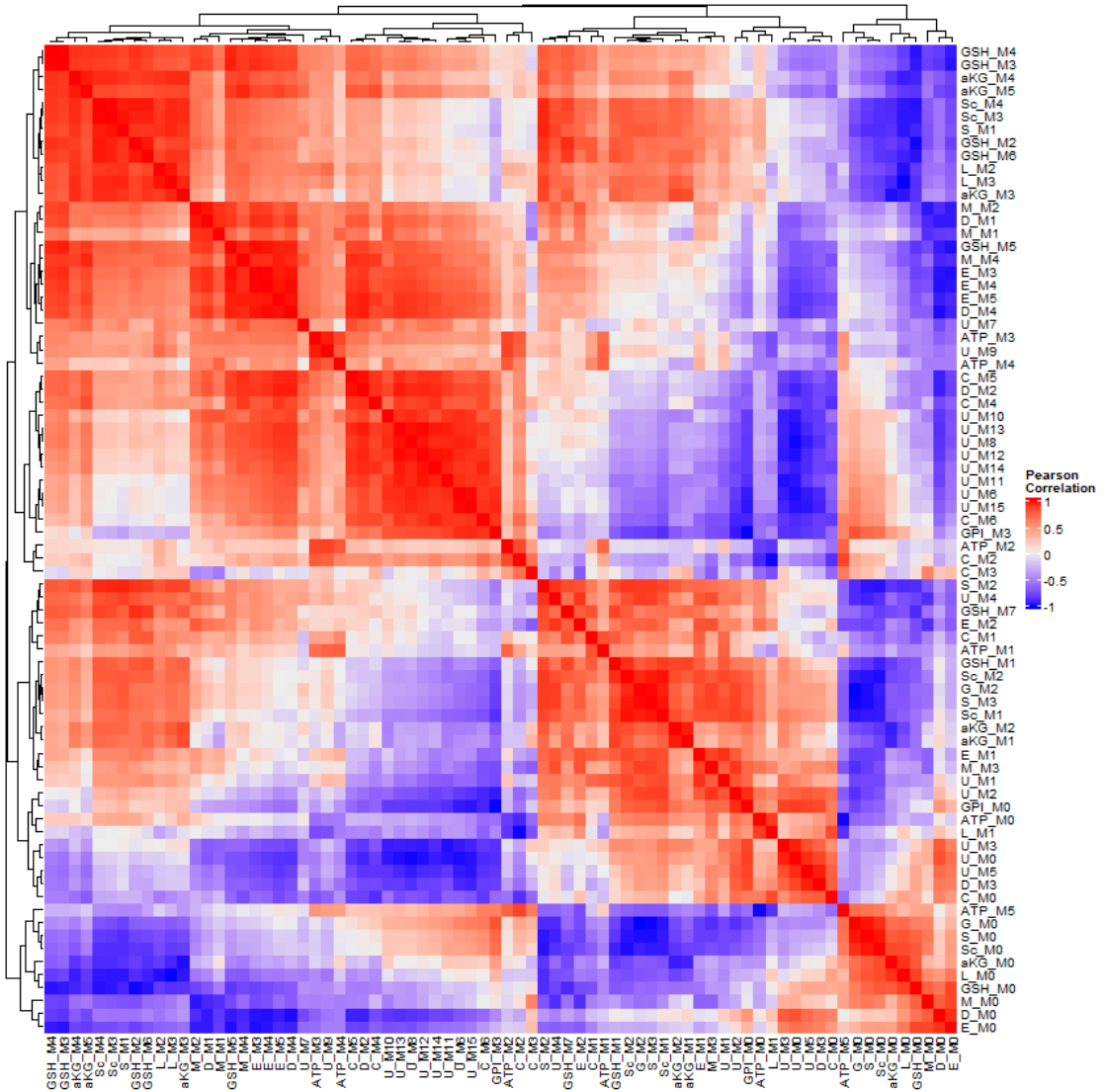
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871 **Supplemental Figure 6. Multiomic pathway analysis.** (A) Gene counts with functional  
872 group membership of 151 genes found to correlate strongly with glutamyl-glycine (Glu-Gly),  
873 orotic acid, lactate, uridine monophosphate, and malate. (B) Functional network of shared  
874 genes in represented transcriptional profile with strong metabolite-gene associations;  
875 analysis performed using ExpressAnalyst. (C) Scatter plot of joint pathway analysis from  
876 MetaboAnalyst v4.0 using DEGs and full static metabolomics dataset based on associated  
877 fold changes. X and y axes show enrichment score in genes and metabolite peaks,  
878 respectively.

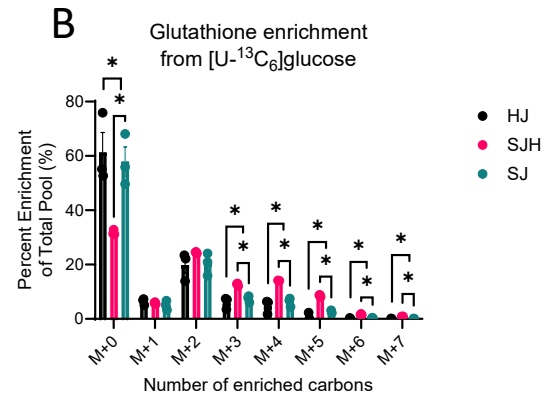
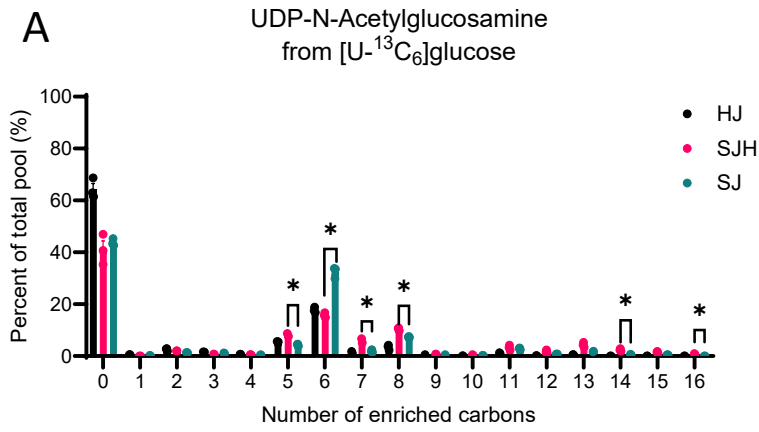




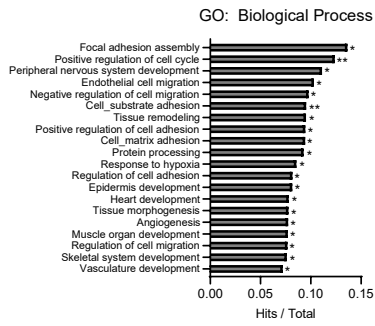


ITUM Pearson Correlation Matrix: SJH vs HJ





A



B

