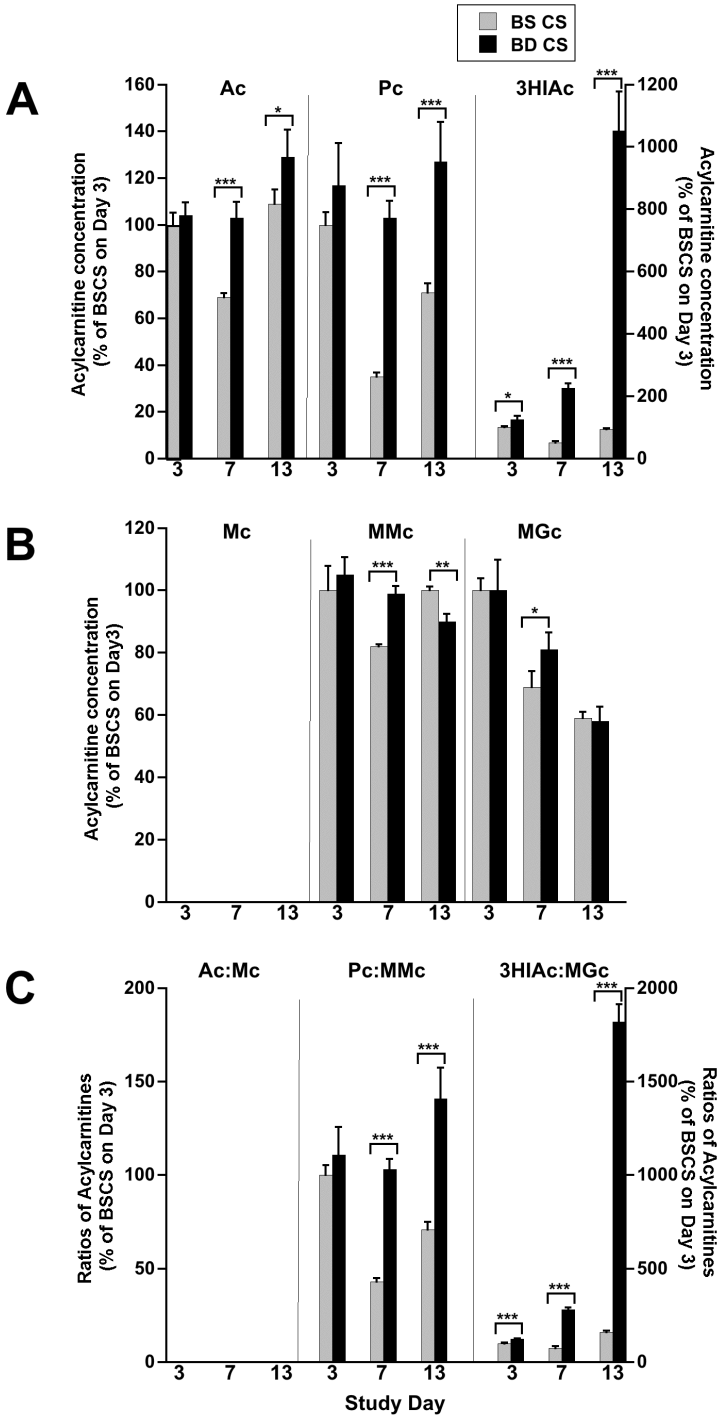

Supplemental Figure 2. Metabolic blocks caused by progressive biotin deficiency in HepG2 cells are reflected in changing **extracellular** acylcarnitine concentrations and acylcarnitines ratios. A. Concentration of acylcarnitines arising from the substrate CoAs: Ac, Pc, and 3HIAc. Data for the 3HIAc were plotted against the secondary, right y-axis. B. Concentration of acylcarnitines arising from the products CoAs: Mc, MMc, and MGc. C. Substrate:product ratios of the acylcarnitines - Ac:Mc, Pc:MMc, and 3HIAc:MGc. Data for the 3HIAc:MGc were plotted against the secondary, right y-axis. Results are presented as % increase relative to BS CS cells on day 3. For all three substrate-derived acylcarnitines and the two ratios, two-way ANOVA revealed highly significant interactions ($P < 0.0001$) between biotin status and time. Pair-wise contrasts between BS CS and BD CS cells at each time point revealed a significant effect of biotin status at both day 7 and 13. For product-derived acylcarnitines, interactions were also significant ($P < 0.01$) for MMc and MGc. Pair-wise comparisons between two groups by time point showed significant effect of biotin status at day 7 (MMc and MGc) and day 13 (MMc). Values are means ± SDs, n = 4. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. Ac, acetylcarnitne; 3HIAc, 3-hydroxyisovalerylcarnitine; Mc, malonylcarnitine; MGc, 3-methylglutarylcarnitine; MMc, methylmalonylcarnitine; Pc, propionylcarnitine; BD CS, biotin deficient carnitine sufficient; BS CS, biotin sufficient carnitine sufficient.