

Supplementary Information

Diverse roads taken by ¹³C-glucose-derived metabolites in tumor cells exposed to limiting glucose and glutamine conditions

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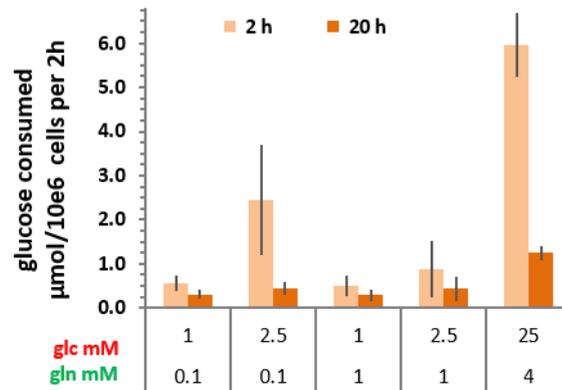


Figure S1: Cellular glucose consumption rate at 2h and 20 h of incubation after medium replenishment with the indicated glucose / glutamine conditions. Cells were cultured in 6-well plates as described in Material and Methods. Consumption was calculated from the amount of glucose removed from the supernatant (Fig. 1B), i.e. the difference to the initial concentration in the medium, and related to the number of cells in the culture. The value of consumed glucose at 20 h was normalized to a 2 h increment. The graph shows that glucose consumption rate decreased with incubation time.

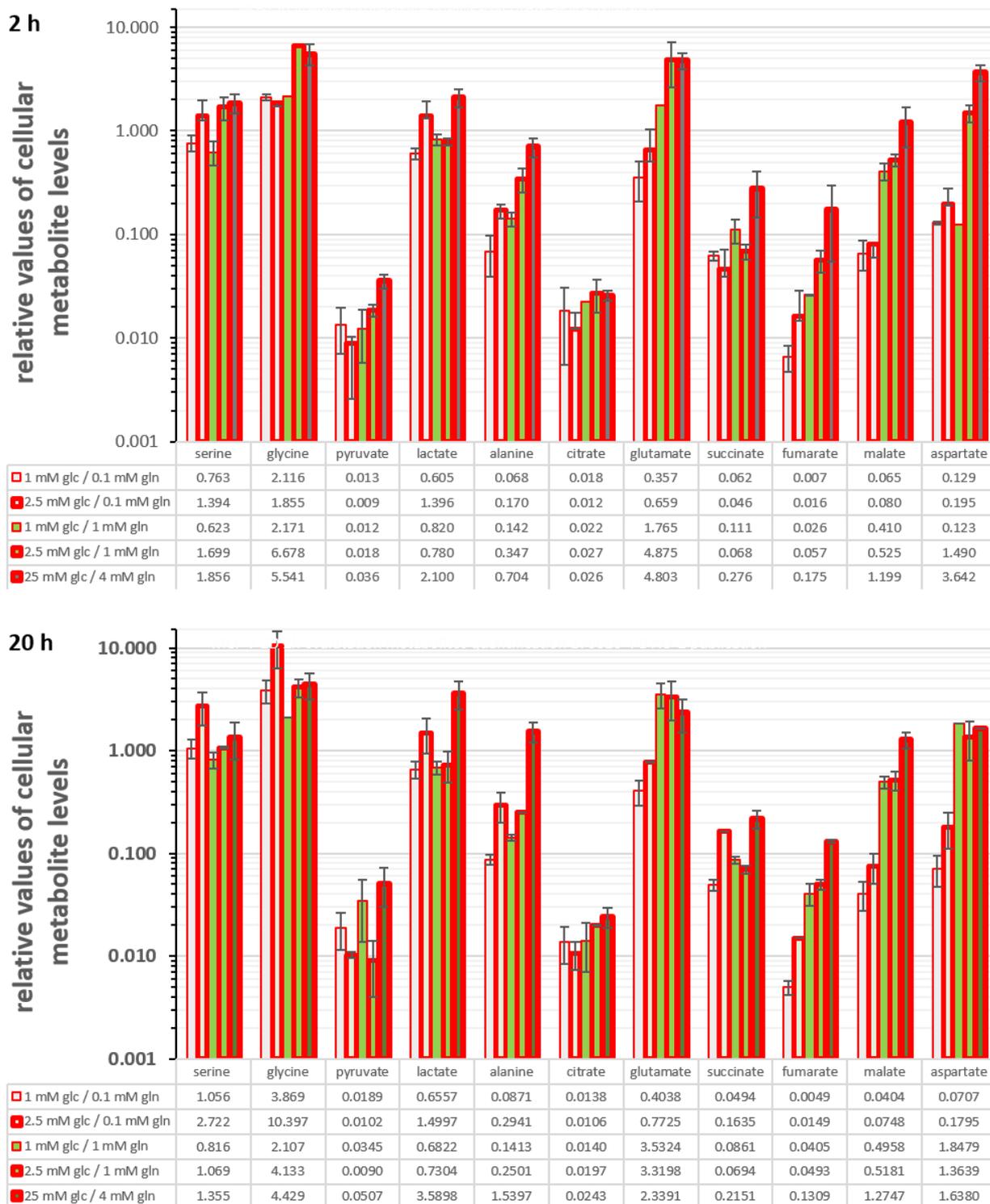


Figure S2: Relative cellular levels of metabolites detectable at 2 hr and 20 hr after medium change with the indicated ¹³C-glucose / glutamine concentrations. The metabolite peaks from GC-MS analysis were quantified relative to 0.4. mM norvaline (=1) added as a reference compound. The relative quantities are depicted in a logarithmic scale. The corresponding graphs at 2 and 20h are depicted in Fig 2 and Fig. S3.

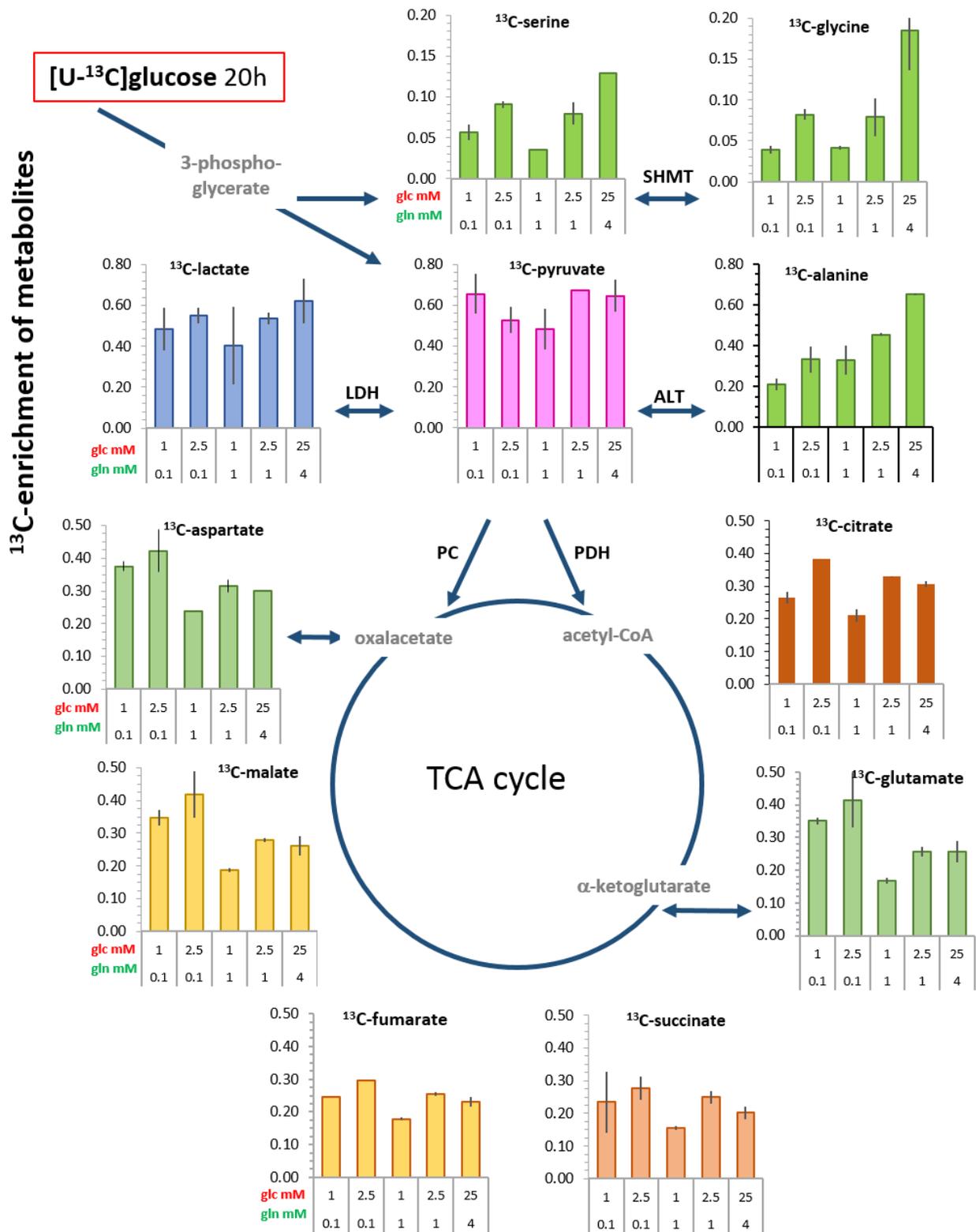


Figure S4: ¹³C-enrichment of metabolite pools in cells incubated for 20 h with [U-¹³C₆]glucose in growth conditions with different combinations of glucose and glutamine concentrations at the indicated concentrations. Abbreviations as in Fig. S3.

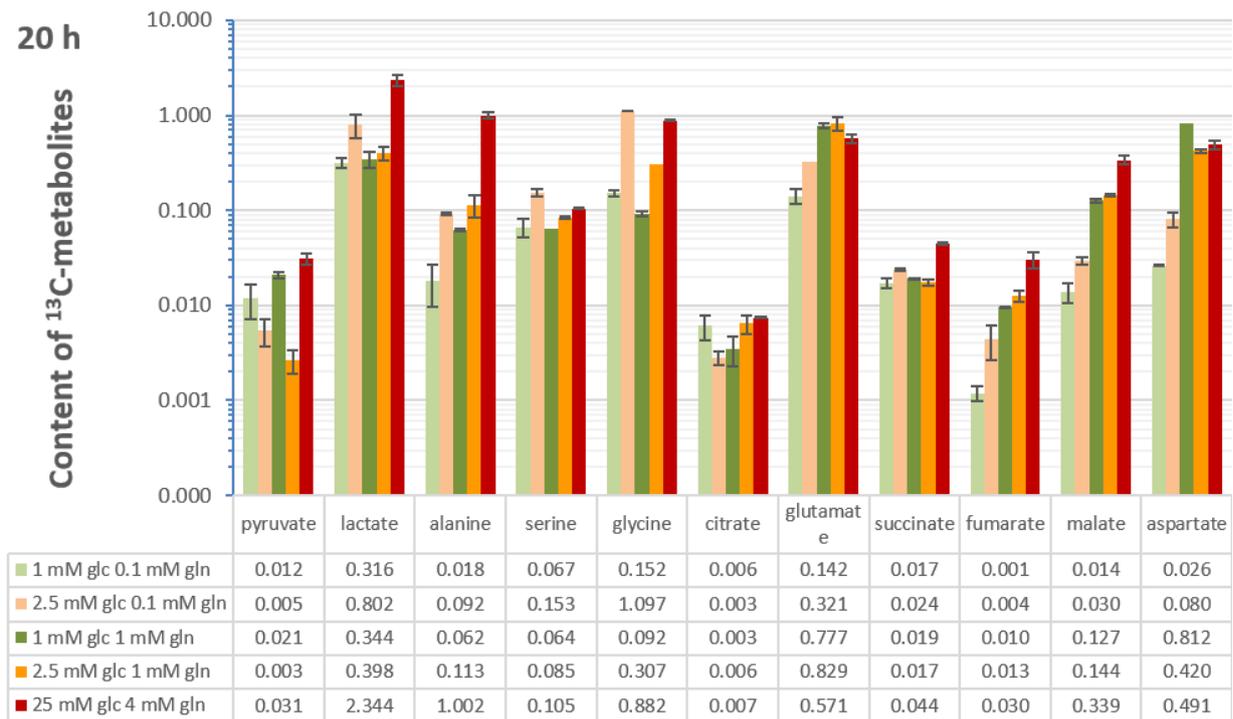
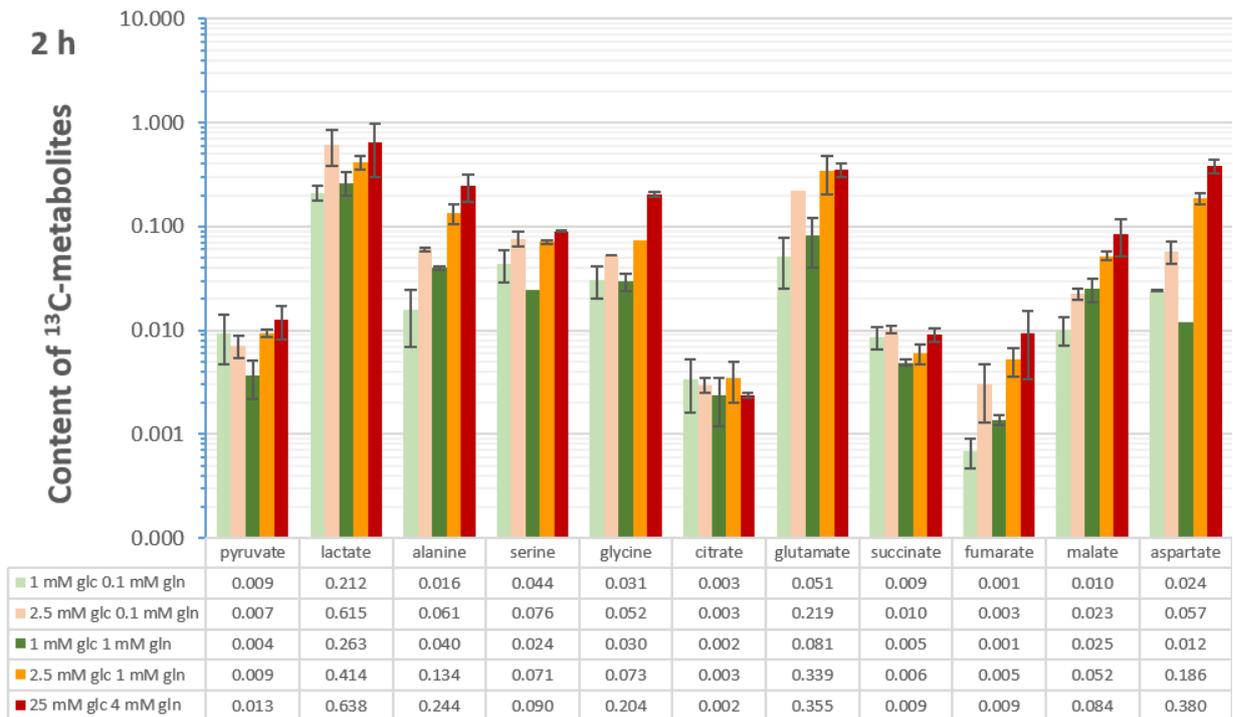


Figure S5: Cellular quantification of the ^{13}C -labelled metabolites after 2 h and 20 -incubations of MCF-7 cells with $[\text{U-}^{13}\text{C}_6]\text{glucose}$. The ^{13}C -enrichment value of each C_6 metabolite (Fig. S3) was multiplied with the total level of the metabolite determined (Fig. S2). The values give a semi-quantitative indication of the level of ^{13}C -metabolites in the cells (see scheme in Fig. S7C). The corresponding graphs at 2 and 20h are depicted in Fig 4 and Fig. S6, respectively.

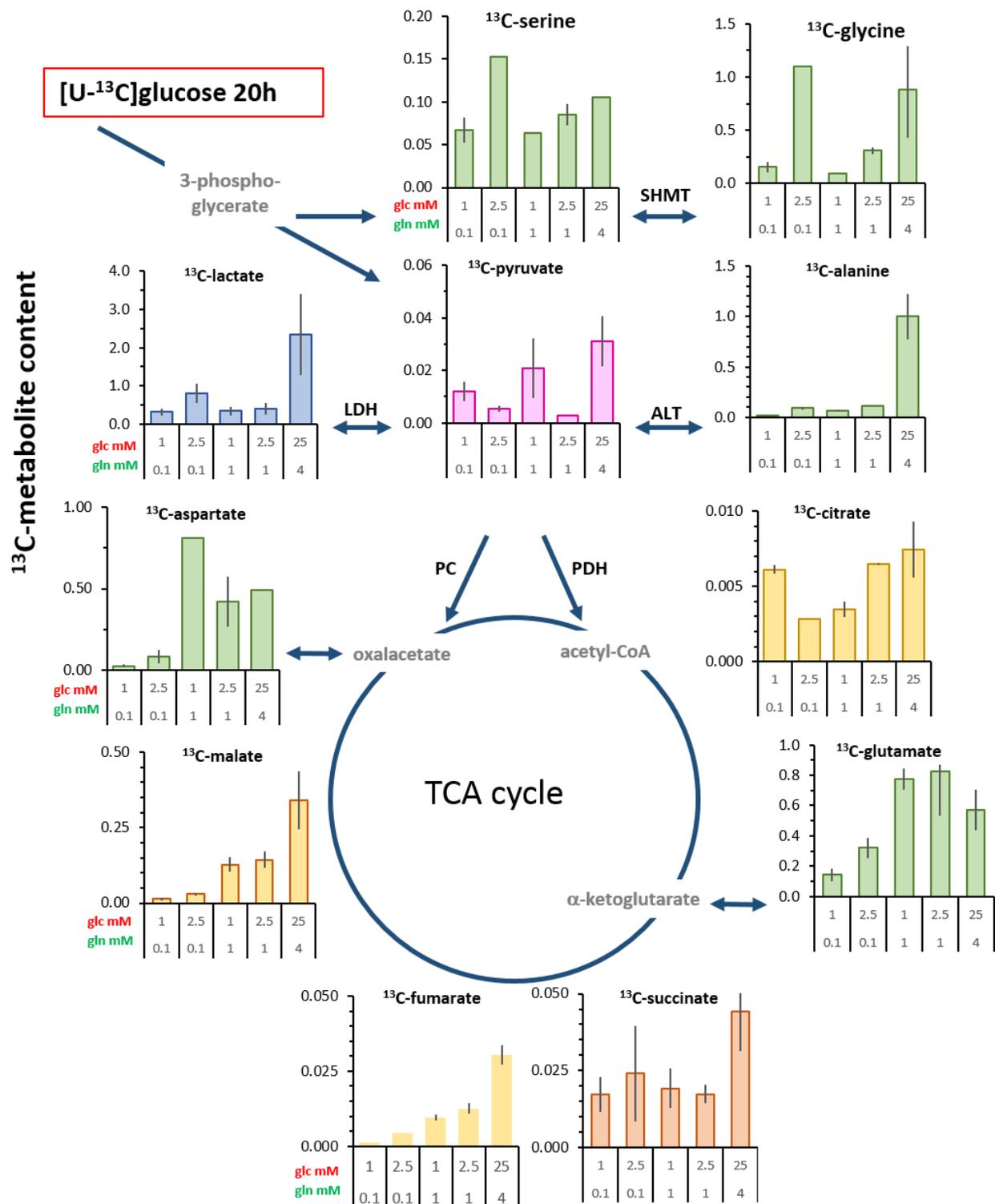


Figure S6: Estimated cellular content of *de novo* glucose-derived metabolites after a 20 h - incubation of MCF-7 cells with [U-¹³C₆]glucose at the indicated glucose and glutamine concentrations. The values for each metabolite are provided in Fig. S5.

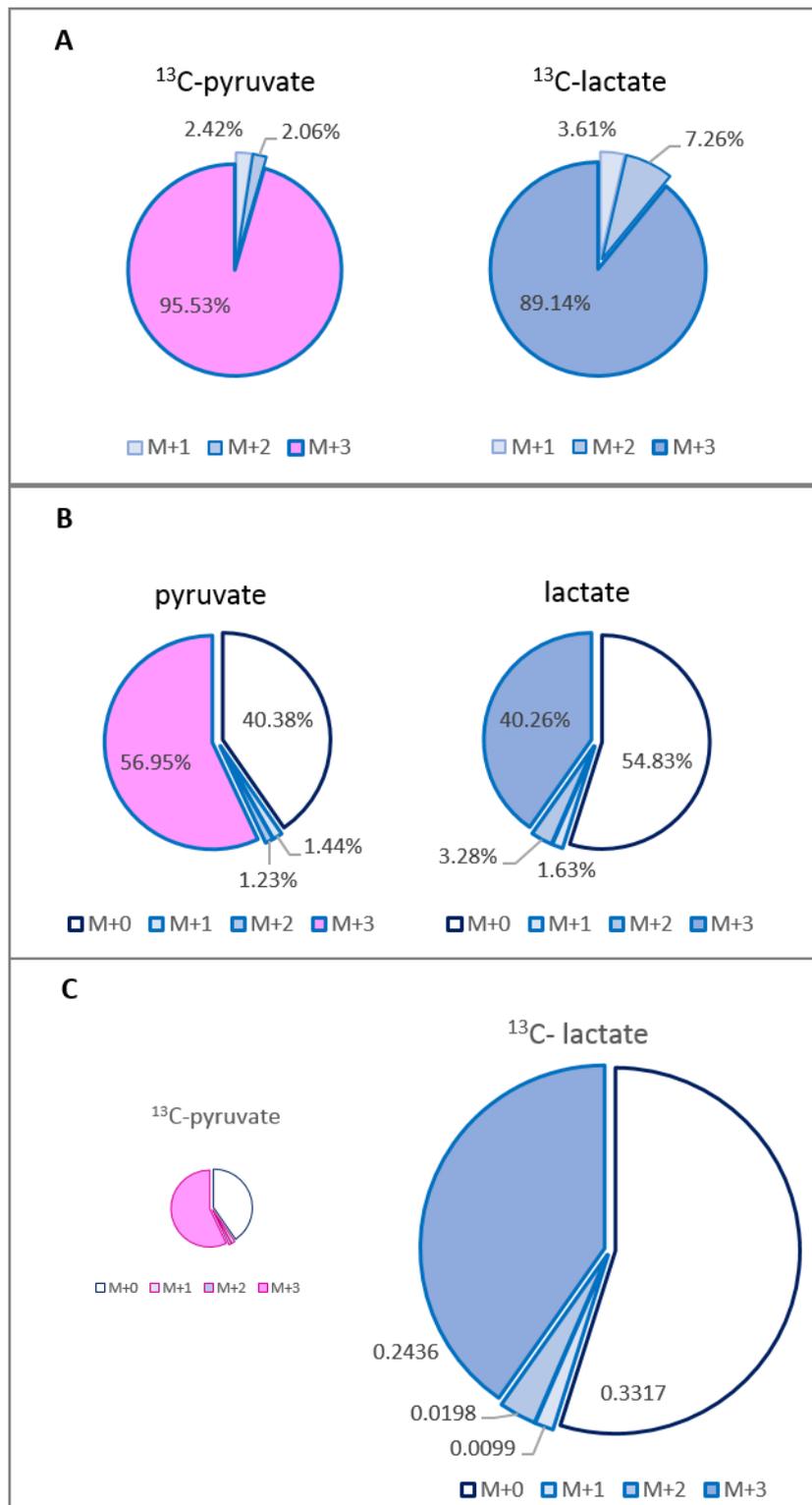


Figure S7: Comparison of different ¹³C-isotopologue evaluations and their quantitative implications. A) Fraction of different ¹³C-isotopologues within the ¹³C-labelled metabolite pool. B) Fraction of ¹³C- isotopologues within the total metabolite pool (incl. M+0 metabolites). C) ¹³C- isotopologues content taking in to account the cellular metabolite pool (diameter of the spheres are drawn approximately to scale).

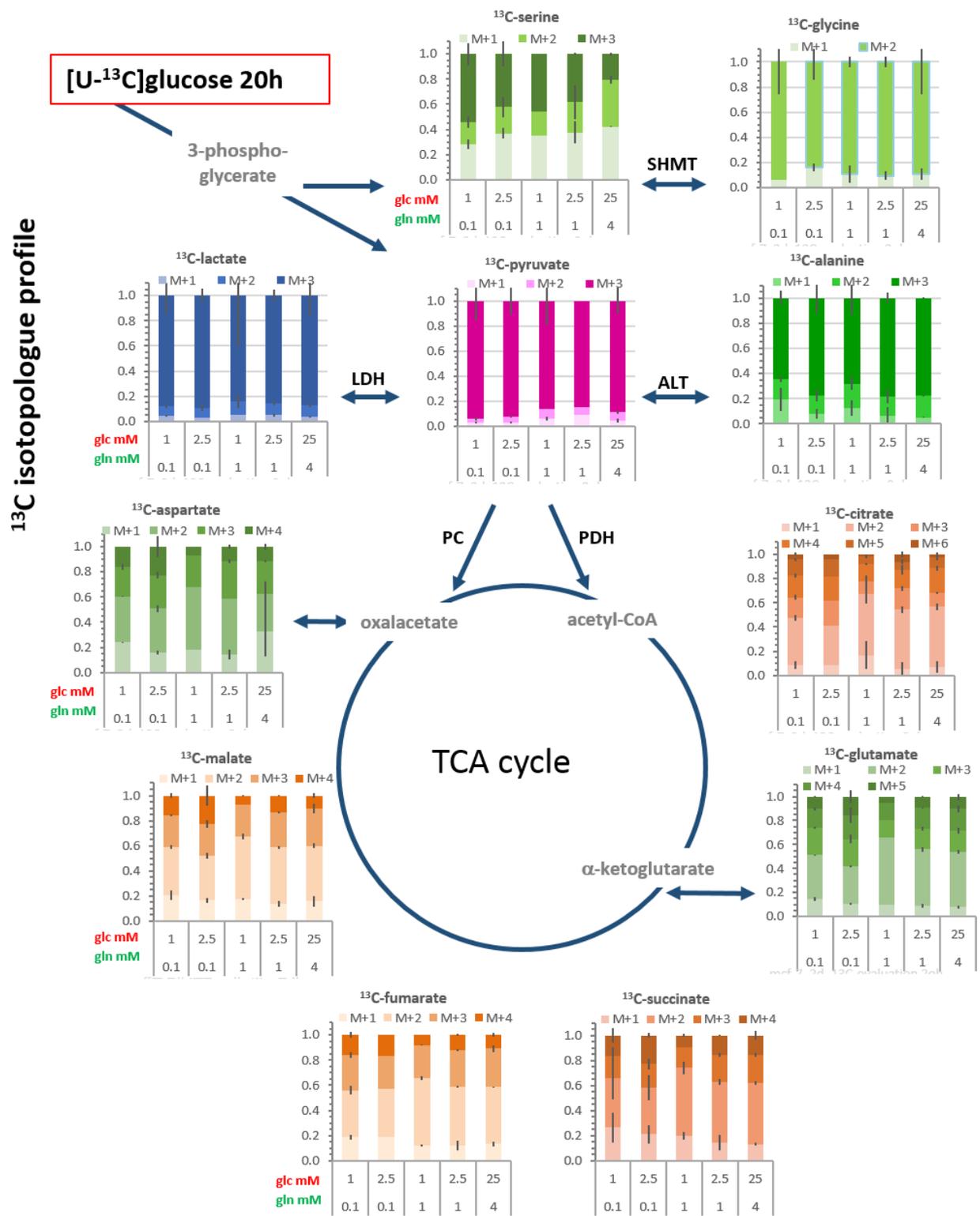


Figure S9: ¹³C-isotopologue profiles of metabolites after 20 h of [U-¹³C₆]glucose incubation in different glucose / glutamine conditions. Shown are the mass distribution of the ¹³C-labelled metabolites evaluated as in Fig. S7A.

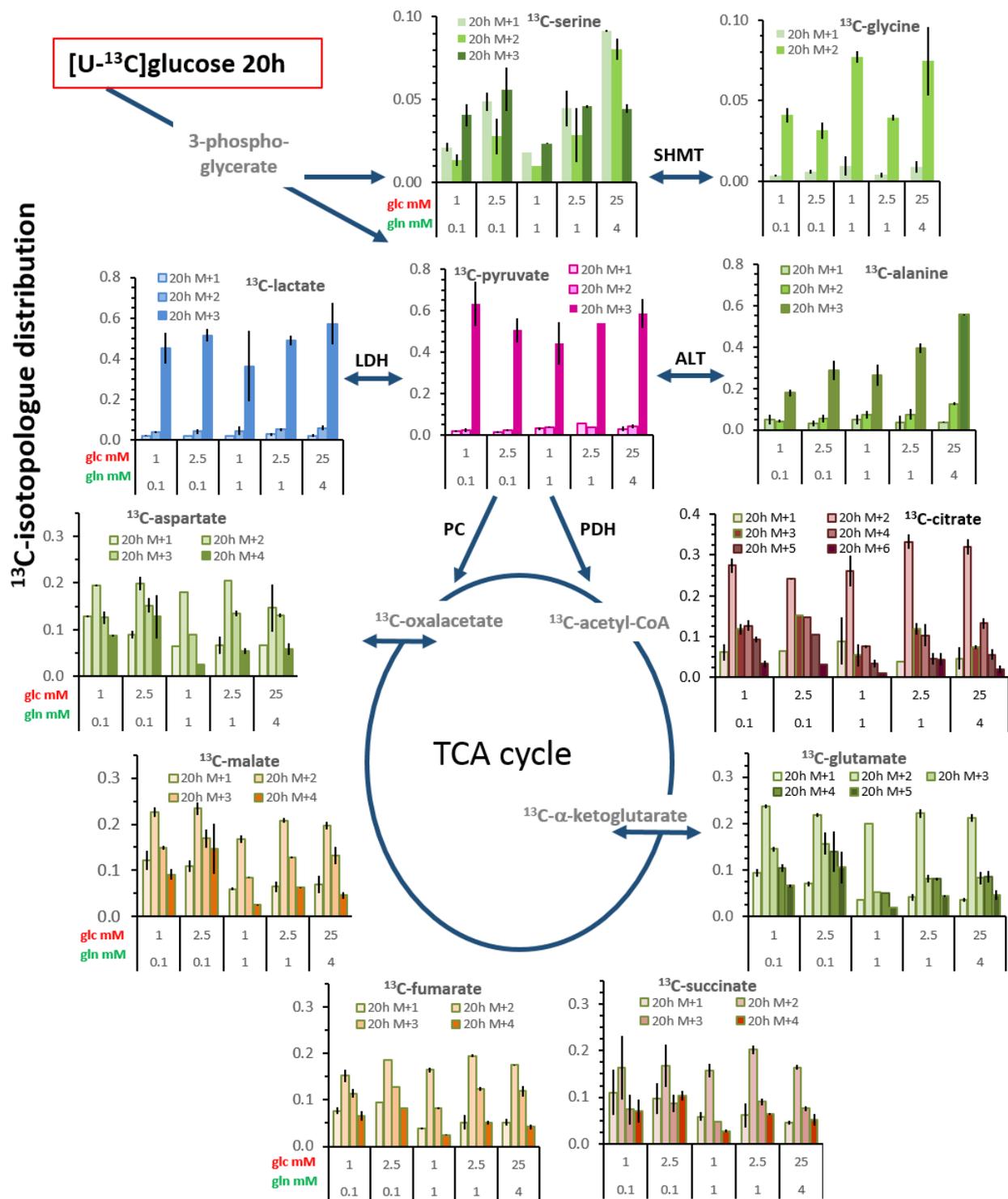


Figure S10: Isotopologue profiles of ¹³C-labeled metabolites associated with glycolysis and the TCA-cycle after a 20 h-incubation with [U-¹³C₆]-glucose in different glucose /glutamine conditions. The isotopologue fraction was calculated from the respective total metabolite pools as depicted in Fig. S7B.