Supporting Information

Comprehensive Isotopic Targeted Mass Spectrometry (CIT-MS): Reliable Metabolic Flux Analysis with Broad Coverage

Xiaojian Shi[1], Bowei Xi[2], Paniz Jasbi[1], Cassidy Turner[1], Yan Jin[1], Haiwei Gu[1]*

[1]Arizona Metabolomics Laboratory, College of Health Solutions, Arizona State University, Scottsdale, AZ 85259, USA

[2]Department of Statistics, Purdue University, West Lafayette, IN 47907, USA

*Corresponding Author:
Haiwei Gu, PhD
Arizona Metabolomics Laboratory
College of Health Solutions
Arizona State University
13208 E. Shea Blvd
Scottsdale, AZ 85259
Tel: 480-301-6016
Fax: 480-301-7017
Email: haiweigu@asu.edu

Separate Excel Table S1. The in-house metabolite library containing 310 unlabeled chemical standards and their LC-MS/MS parameters.

Separate Excel Table S2. All isotopic MRM transitions in CIT-MS.

Page S-3: Table S3. Linear regression analysis of the data in **Figure 2**.

Page S-4: Figure S1. Pathway view showing only 179 (red dots) of the 310 metabolites in the detection panel.

Page S-5: Figure S2. Enriched carbohydrate metabolites from U-$^{13}C_6$-glucose that were detected by CIT-MS in a steady state study using Myc-On and Myc-Off Tet21N cells.

Page S-6: Figure S3. Enriched TCA cycle metabolites from U-$^{13}C_6$-glucose that were detected by CIT-MS in a steady state study using Myc-On and Myc-Off Tet21N cells.

Page S-7: Figure S4. Enriched amino acid metabolites from U-$^{13}C_6$-glucose that were detected by CIT-MS in a steady state study using Myc-On and Myc-Off Tet21N cells.

Page S-8: Figure S5. Exponential fitting of the time-course data of nucleotide metabolites for the comparison of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.

Page S-9: Figure S6. Exponential fitting of the time-course data of carbohydrate metabolites for the comparison of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.

Page S-10: Figure S7. Exponential fitting of the time-course data of TCA cycle metabolites for the comparison of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.

Page S-11: Figure S8. Exponential fitting of the time-course data of amino acid metabolites for the comparison of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.

Table S3. Linear regression analysis of the data in **Figure 2**.

Compound	Linear Equation	R^2
G	Y=1.0025X-0.1388	0.9987
1-^{13}C-G	Y=0.9915X-0.5353	0.9996
1,6-$^{13}C_2$-G	Y=1.0014X-0.6499	0.9995
U-$^{13}C_6$-G	Y=0.9378X+1.7373	0.9981

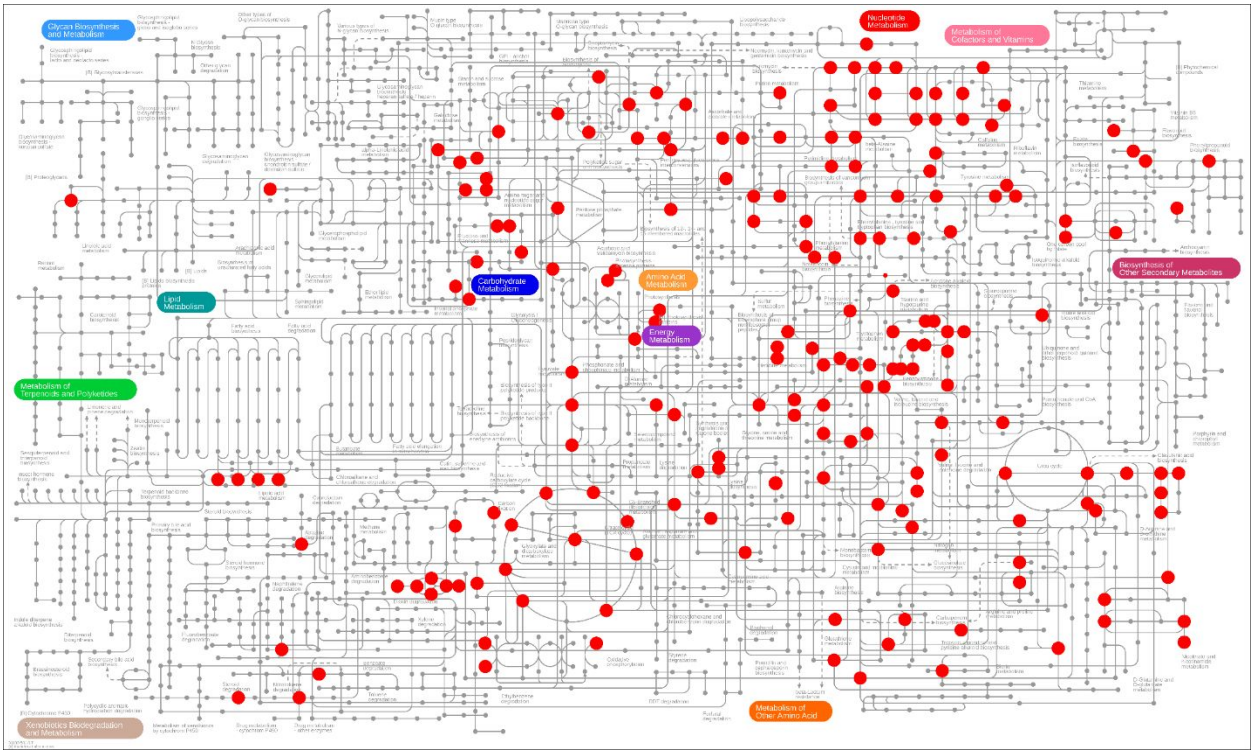


Figure S1. Pathway view showing only 179 (red dots) of the 310 metabolites in the detection panel.

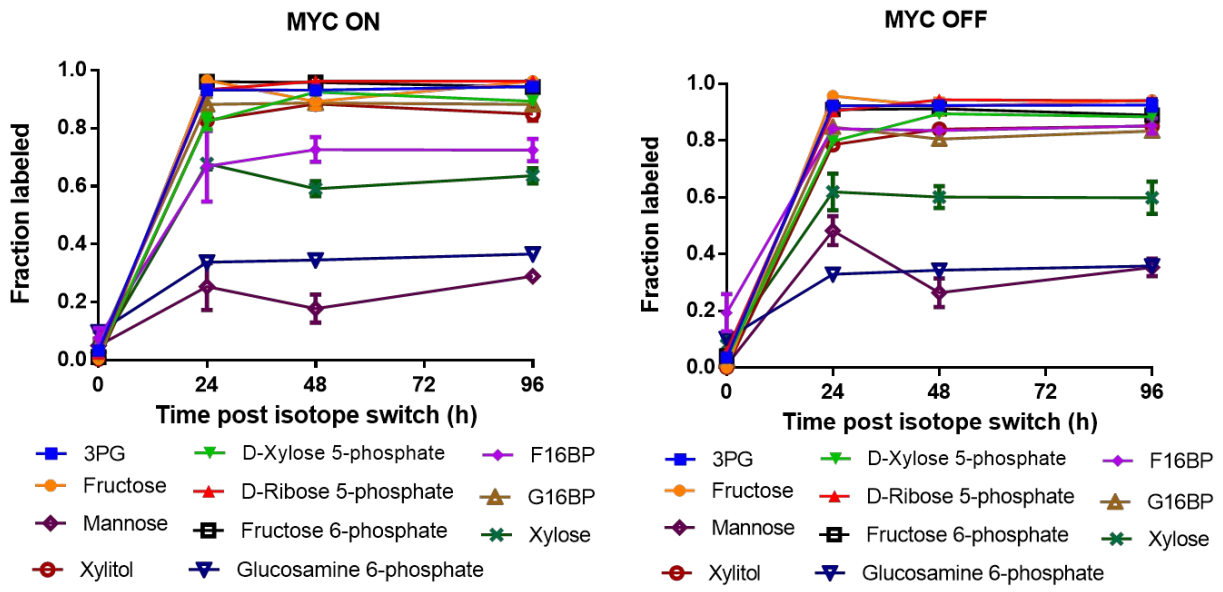
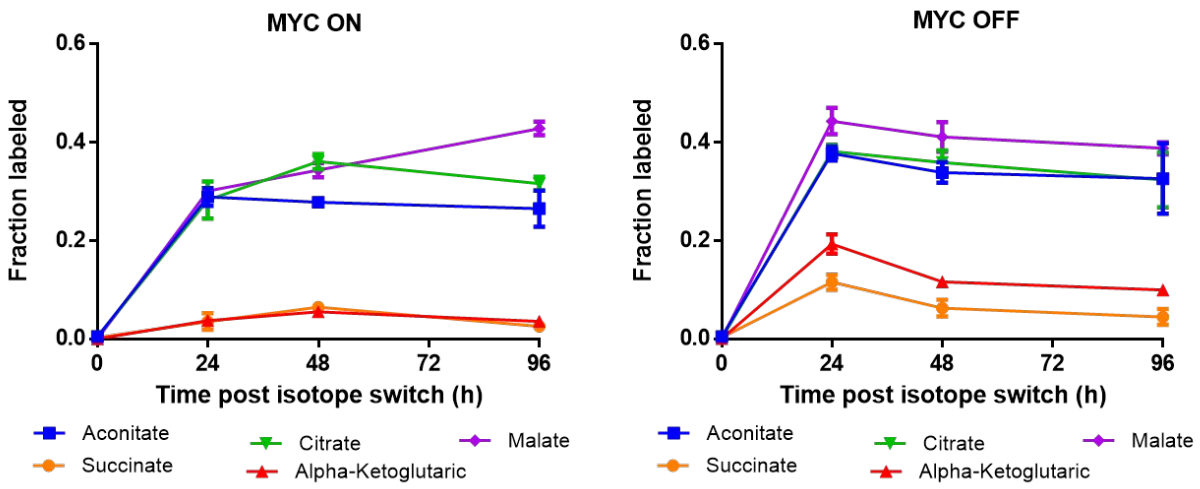


Figure S2. Enriched carbohydrate metabolites from U-$^{13}C_6$-glucose that were detected by CIT-MS in a steady state study using Myc-On and Myc-Off Tet21N cells.



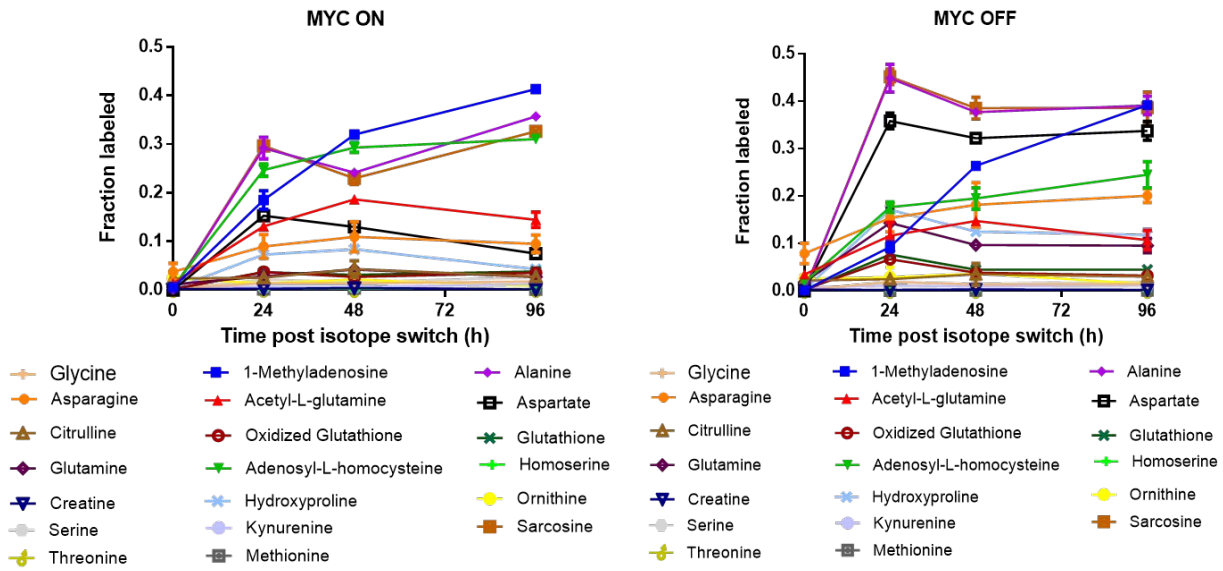
68

69 **Figure S3.** Enriched TCA cycle metabolites from U-$^{13}C_6$-glucose that were detected by CIT-MS

70 in a steady state study using Myc-On and Myc-Off Tet21N cells.

71

72

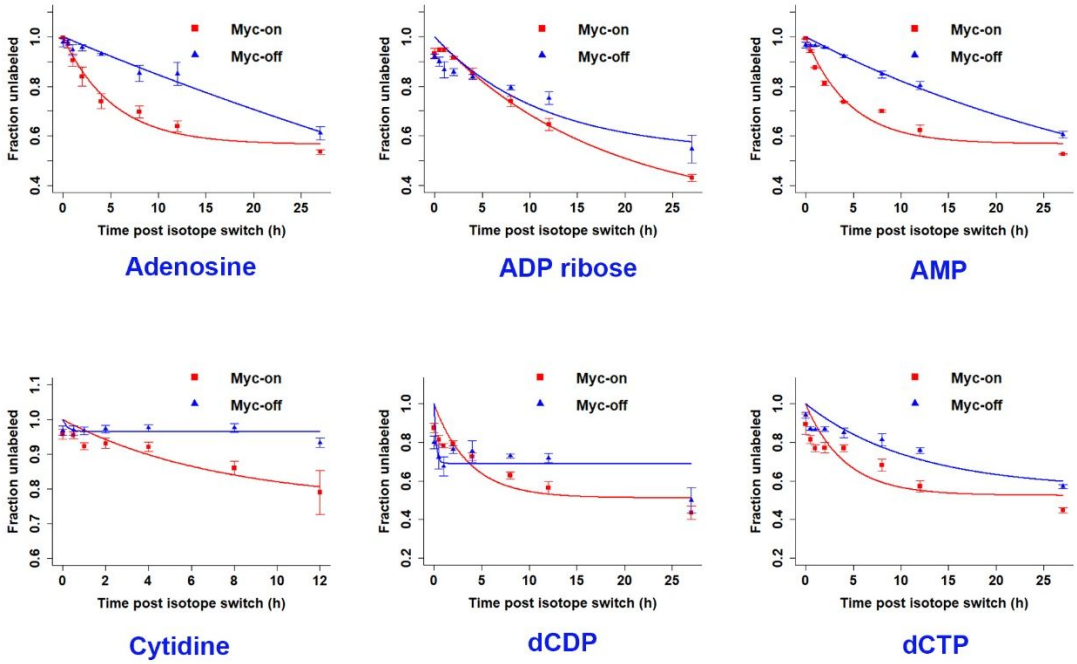


73

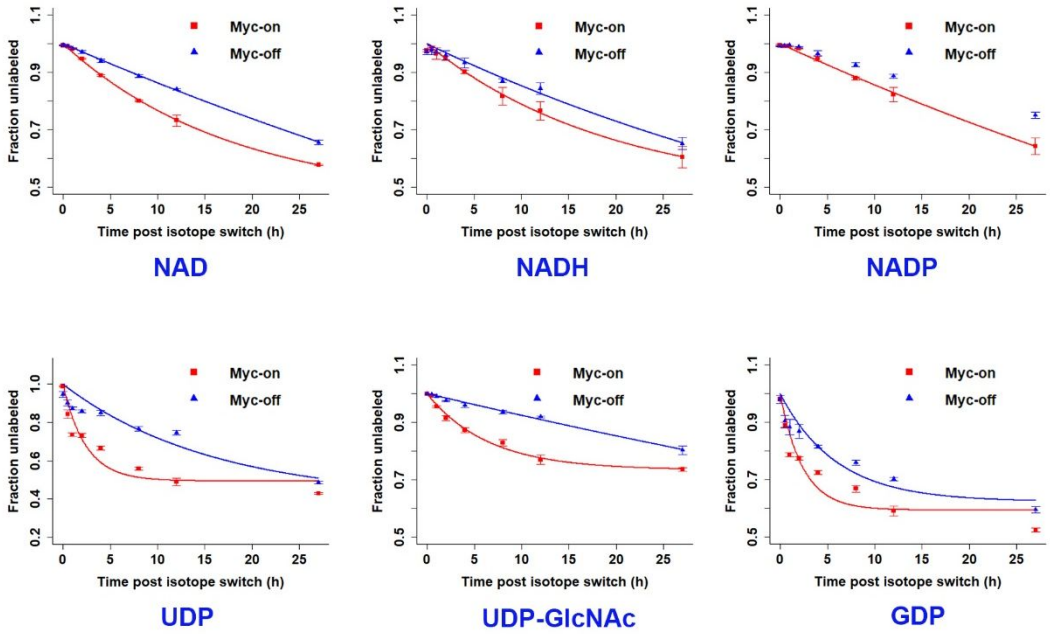
74 **Figure S4.** Enriched amino acid metabolites from U-$^{13}C_6$-glucose that were detected by CIT-MS

75 in a steady state study using Myc-On and Myc-Off Tet21N cells.

76



77



78

79 **Figure S5.** Exponential fitting of the time-course data of nucleotide metabolites for the comparison

80 of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.

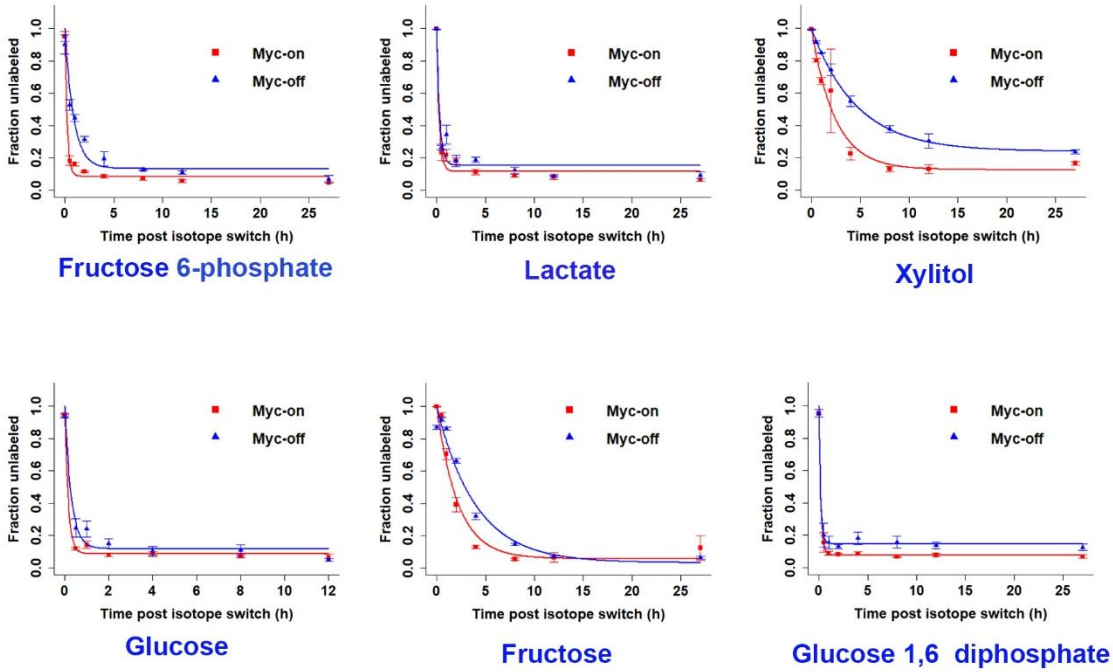
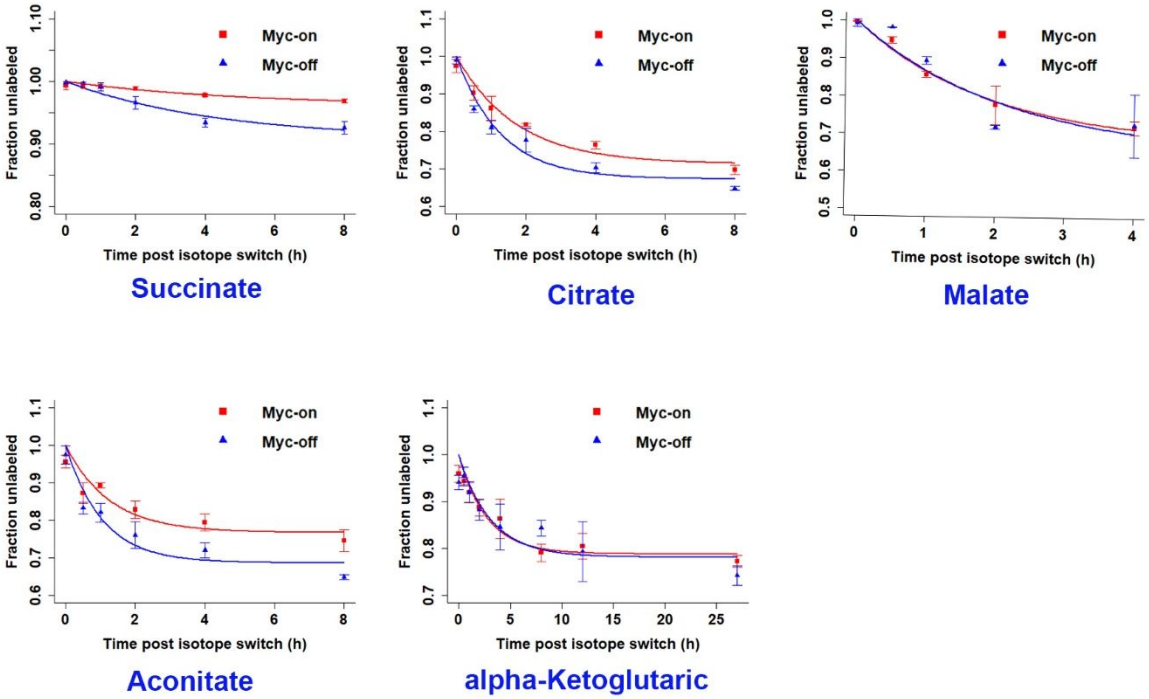


Figure S6. Exponential fitting of the time-course data of carbohydrate metabolites for the comparison of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.



85

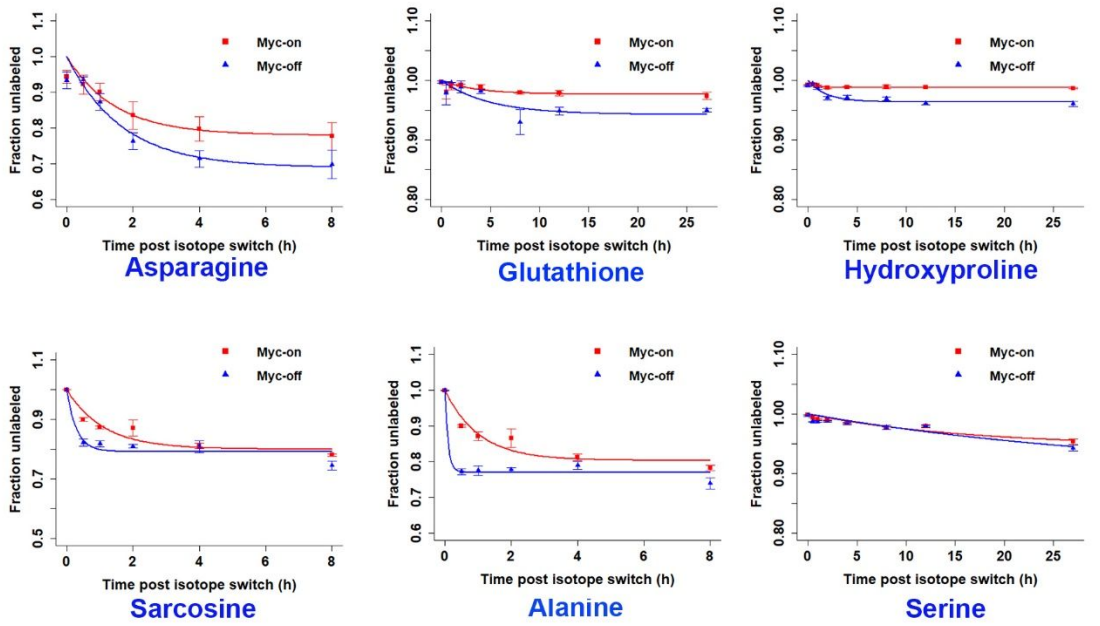
86 **Figure S7.** Exponential fitting of the time-course data of TCA cycle metabolites for the comparison

87 of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-glucose labeling.

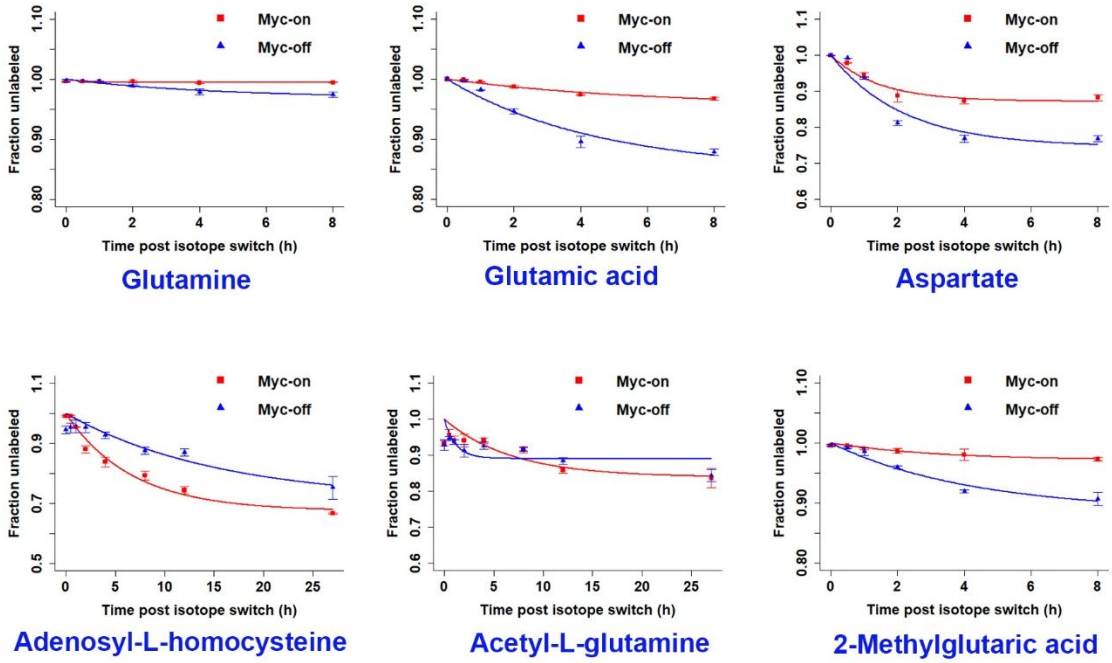
88

89

90



91



92

93 **Figure S8.** Exponential fitting of the time-course data of amino acid metabolites for the
94 comparison of Myc-On and Myc-Off Tet21N cells under the pseudosteady state post U-$^{13}C_6$-
95 glucose labeling.