Charting Unknown Metabolic Reactions by Mass Spectrometry-Resolved Stable-isotope Tracing Metabolomics

Yang Gao[1,2], Mingdu Luo[1,2], Hongmiao Wang[1,2], Zhiwei Zhou[1], Yandong Yin[1], Ruohong Wang[1], Beizi Xing[1,2], Xiaohua Yang[1], Yuping Cai[1,], and Zheng-Jiang Zhu[1,3,*]*

[1] Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, 200032 China

[2] University of Chinese Academy of Sciences, Beijing, 100049 China

[3] Shanghai Key Laboratory of Aging Studies, Shanghai, 201210 China

* Corresponding authors

Corresponding Author

Correspondence should be addressed to Z.J.Z (jiangzhu@sioc.ac.cn); Y. C. (yupingcai@sioc.ac.cn)

List of Supplementary Figures

Supplementary Figure 1. The algorithm for the calculation of isotopologue pattern similarity scores.

Supplementary Figure 2. The workflow of IsoNet. (A) Annotation of labeled metabolites.

Supplementary Figure 3. The isotopologue similarity subnetworks uncovered known metabolic reactions.

Supplementary Figure 4. Discovery of new metabolic reactions through isotopologue similarity networking of iBMDM cells and the mouse liver.

Supplementary Figure 5. Validation of a previously uncharacterized metabolic pathway of γ-Glu-Ser-Gly biosynthesis.

Supplementary Figure 6. Elucidation of S-acetylglutathione.

Supplementary Figure 7. Elucidation of glutathione sulfinamide.

Supplementary Figure 8. Elucidation of deaminated glutathione.

Supplementary Figure 9. Elucidation of γ-Glu-3-sulfamoyl-Ala-Gly.

Supplementary Figure 10. Elucidation of glutathione sulfinic acid.

Supplementary Figure 11. Elucidation of glutathione sulfonic acid.

Supplementary Figure 12. Elucidation of S-(2-succinyl)glutathione.

Supplementary Figure 13. Reaction validation in vitro.

Supplementary Figure 14. Significant changed metabolites in different metabolite treatment.

Supplementary Figure 15. Itaconate-associated isotopologue similarity subnetwork disclose newly identified metabolites

Supplementary Figure 16. ^{1}H (top) and ^{13}C (bottom) NMR spectra for γ-Glu-Ser-Gly.

Supplementary Figure 17. ^{1}H (top) and ^{13}C (bottom) NMR spectra for glutathione sulfonic acid.

Supplementary Figure 18. ^{1}H (top) and ^{13}C (bottom) NMR spectra for glutathione sulfinic acid.

Supplementary Figure 19. ^{1}H (top) and ^{13}C (bottom) NMR spectra for S-acetylglutathione.

Supplementary Figure 20. ^{1}H (top) and ^{13}C (bottom) NMR spectra for γ-Glu-3-sulfamoyl-Ala-Gly.

Supplementary Figure 21. ^{1}H (top) and ^{13}C (bottom) NMR spectra for S-(-2-Succinyl)-glutathione.

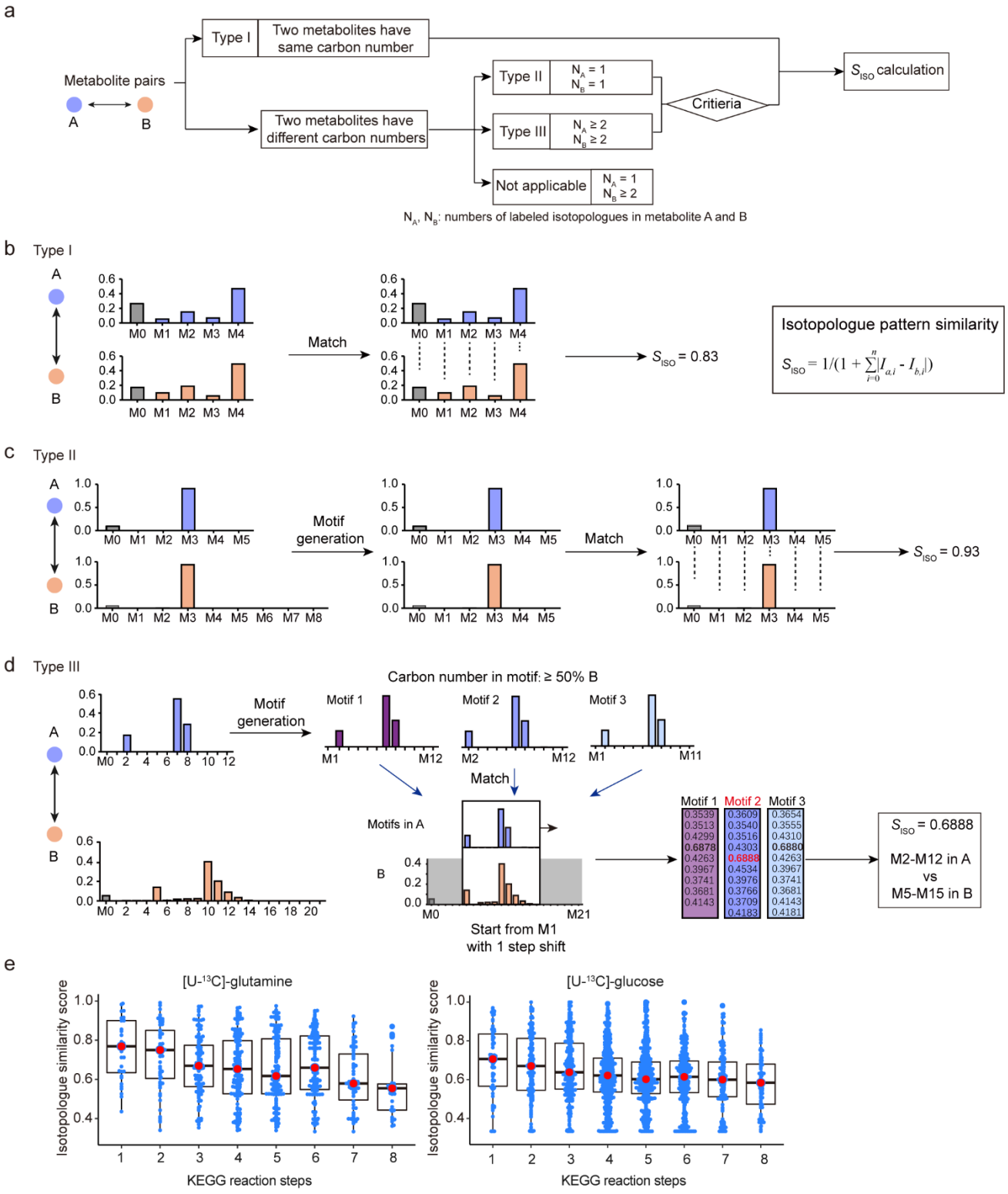
Supplementary Figure 22. ^{1}H (top) and ^{13}C (bottom) NMR spectra for sulfoitaconate.

List of Supplementary Tables

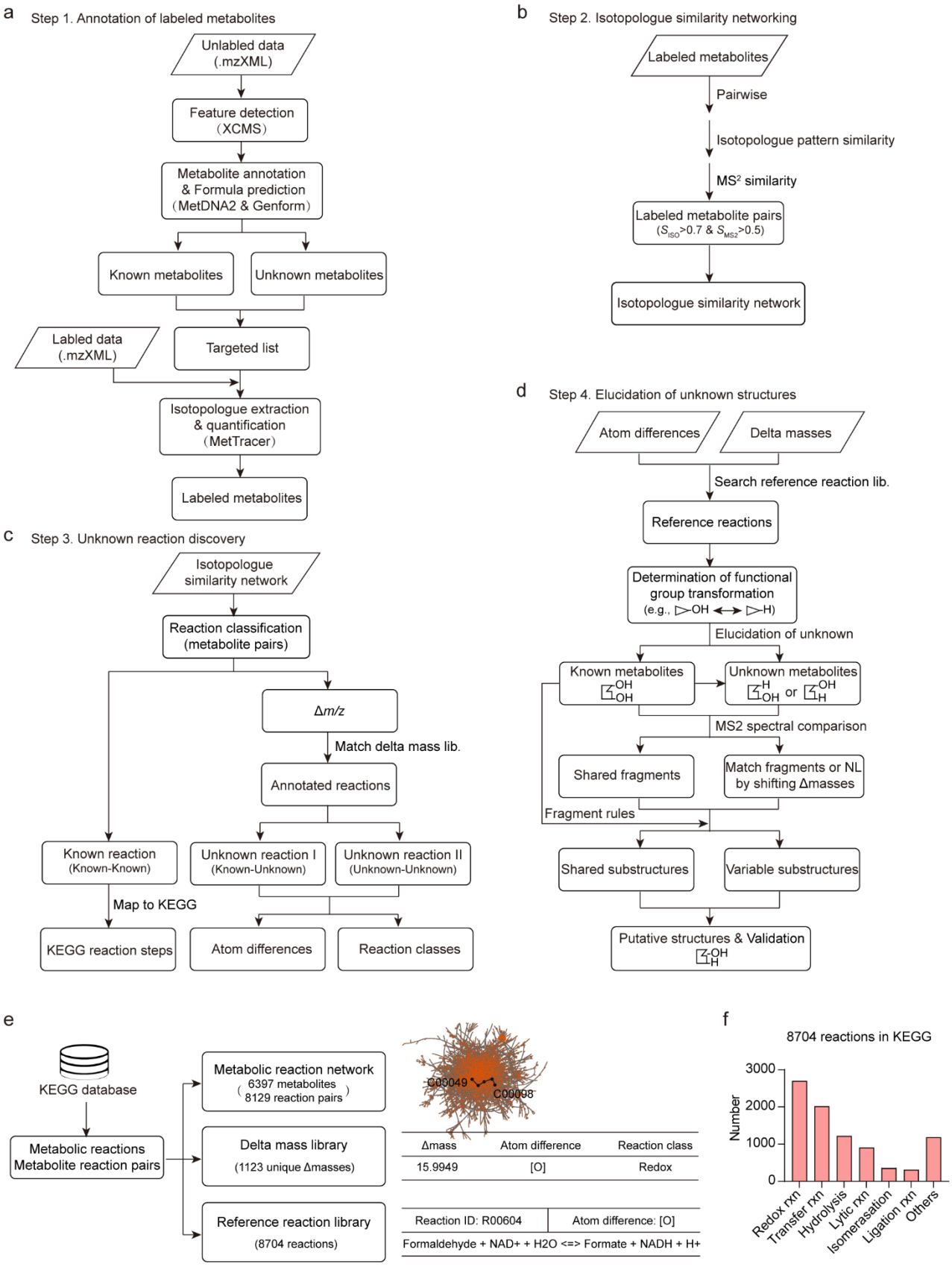
Supplementary Table 1: The database information of unknown metabolites in glutathione-associated metabolic reaction network.

Supplementary Table 2: The database information of reactions in glutathione-associated metabolic reaction network.

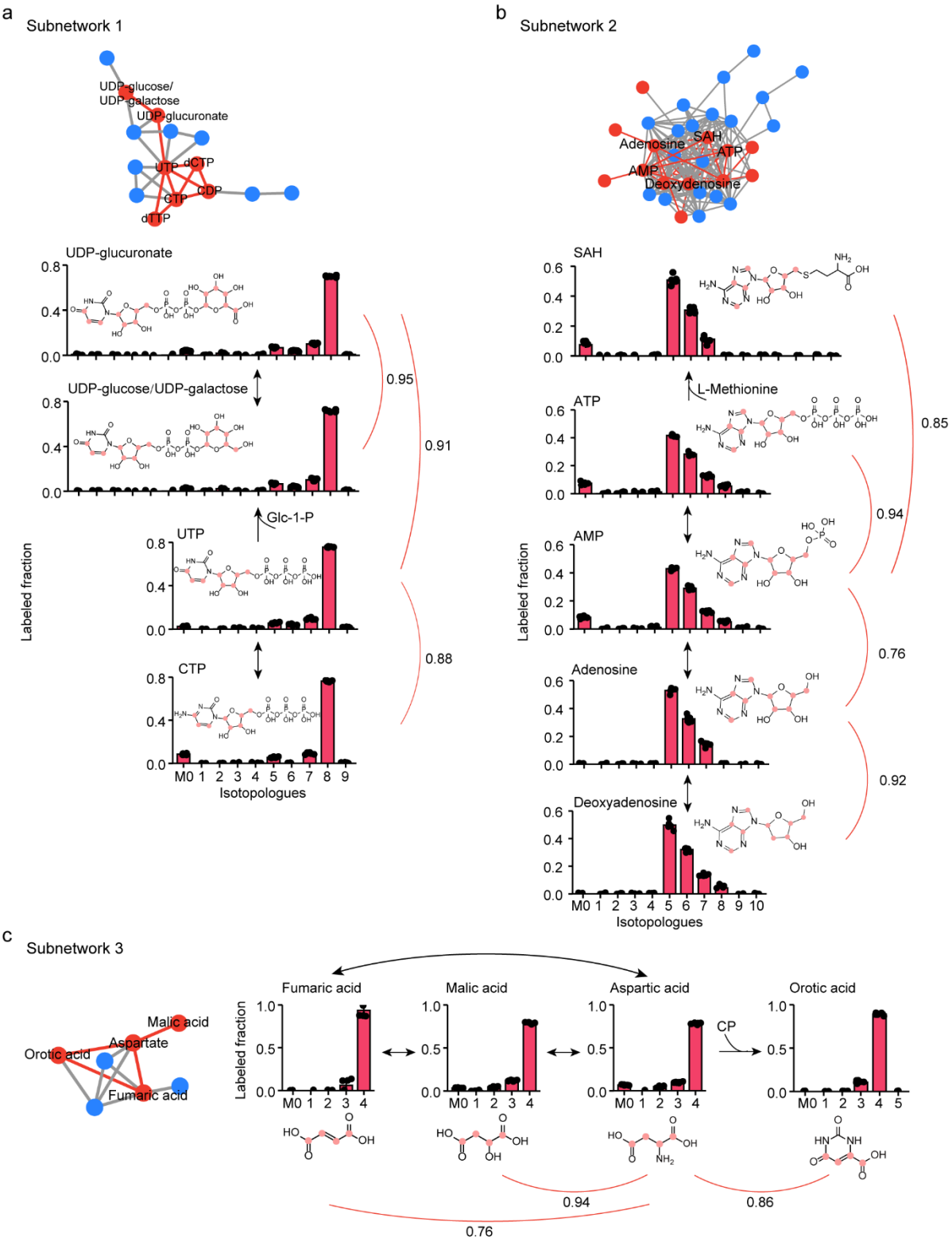
Supplementary Table 3: Significantly enriched pathways of individual metabolite treatment experiments.



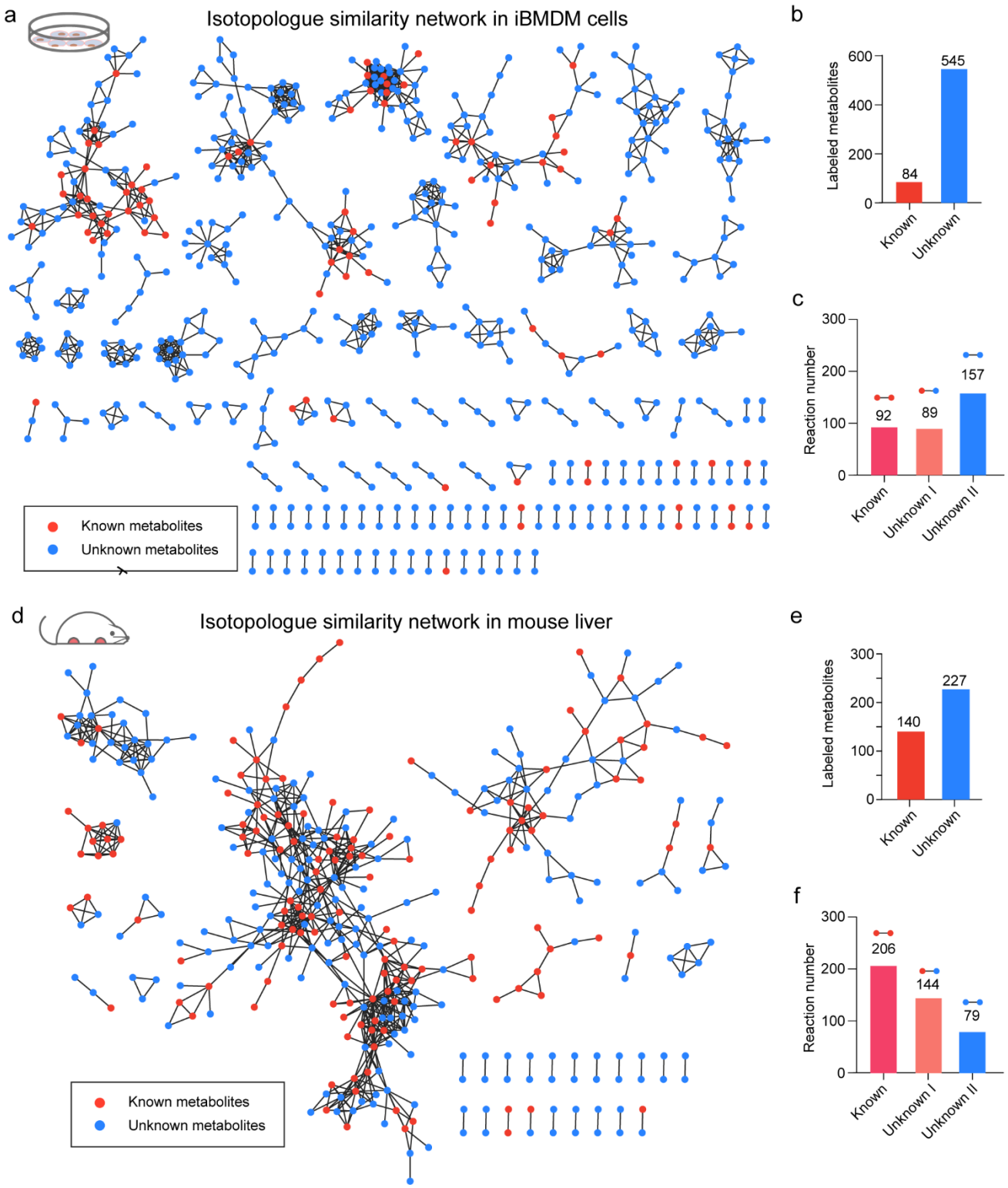
Supplementary Figure 1. The algorithm for the calculation of isotopologue pattern similarity scores. (a) Three types of metabolic scenarios based on the numbers of carbon and labeled isotopologues between labeled metabolite pairs. (b-d) Isotopologue similarity calculation of type I (b), II (c), and III (d) metabolic scenarios. (e) Distribution of isotopologue similarity scores based on metabolic reaction steps in 293T cells labeled with [U-^{13}C]-glutamine or [U-^{13}C]-glucose.



Supplementary Figure 2. The workflow of IsoNet. (a) Annotation of labeled metabolites. (b) Isotopologue similarity networking. (c) Unknown reaction discovery. (d) Elucidation of unknown reactions and structures. (e) The metabolic reaction network, the delta mass library, and the reference reaction library curated from the KEGG database. (f) The reaction class distribution of 8704 reactions in the reference reaction library.

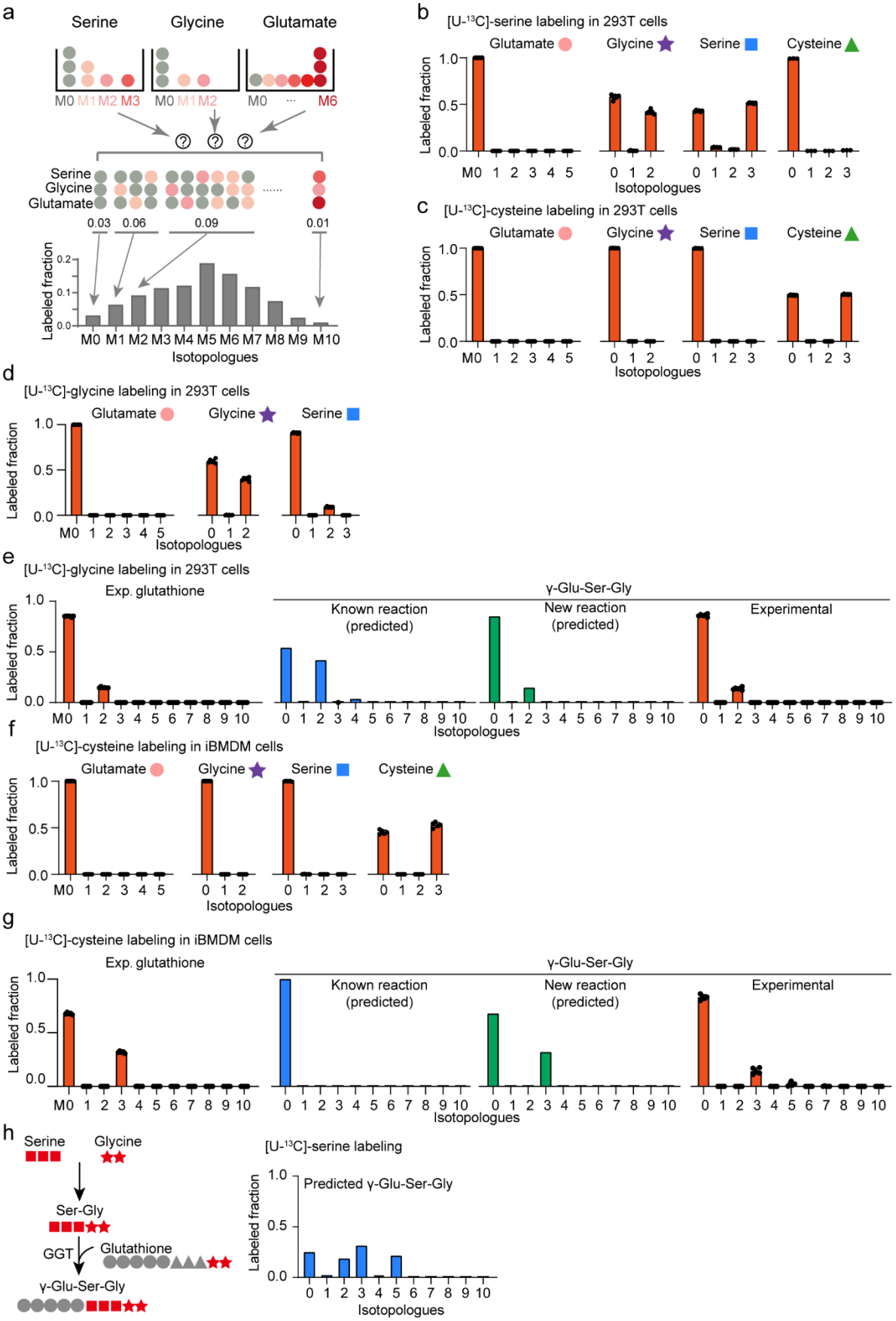


Supplementary Figure 3. The isotopologue similarity subnetworks uncovered known metabolic reactions. (a) The isotopologue similarity subnetwork containing reactions among uridine diphosphate (UDP)-glucuronate, UDP-glucose/UDP-galactose, uridine triphosphate (UTP), and cytidine triphosphate (CTP). (b) The isotopologue similarity subnetwork containing reactions among S-adenosyl-L-homocysteine (SAH), adenosine 5'-triphosphate (ATP), adenosine monophosphate (AMP), adenosine, and deoxyadenosine. (c) The isotopologue similarity subnetwork containing reactions among fumaric acid, malic acid, aspartic acid, and orotic acid. Values represent means ± SEM.



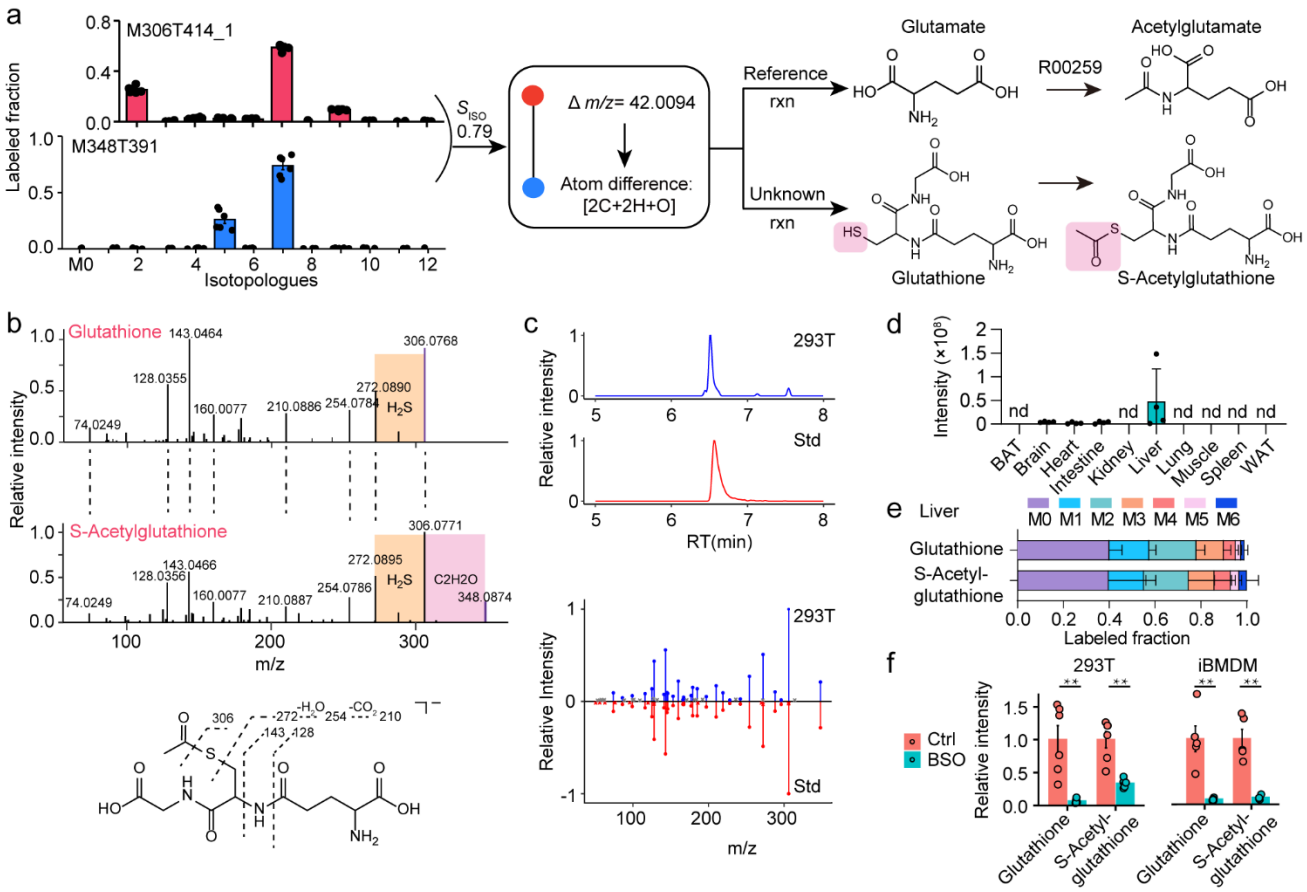
Supplementary Figure 4. Discovery of new metabolic reactions through isotopologue similarity networking of iBMDM cells and the mouse liver. (a) The isotopologue similarity network in iBMDM cells labeled with mixed tracers ([U-^{13}C]-glutamine, [U-^{13}C]-glucose, and [U-^{13}C]-acetate) for 17 h. Nodes represent labeled known (red) and unknown (black) metabolites; edges represent that two metabolites shared similar isotopologue patterns and MS/MS spectra. (b) Numbers of known and unknown labeled metabolites in the network. (c) Numbers of known and unknown reactions in the network. The reactions between known and unknown metabolites were defined as unknown I. The reactions between unknown metabolites were defined as unknown II. (d) The

isotopologue similarity network in mouse liver administered with [U-^{13}C]-glucose for 24 h. Nodes represent labeled known (red) and unknown (black) metabolites; edges represent that two metabolites shared similar isotopologue patterns and MS/MS spectra. (e) Numbers of known and unknown labeled metabolites in the network. (f) Numbers of known and unknown reactions in the network.

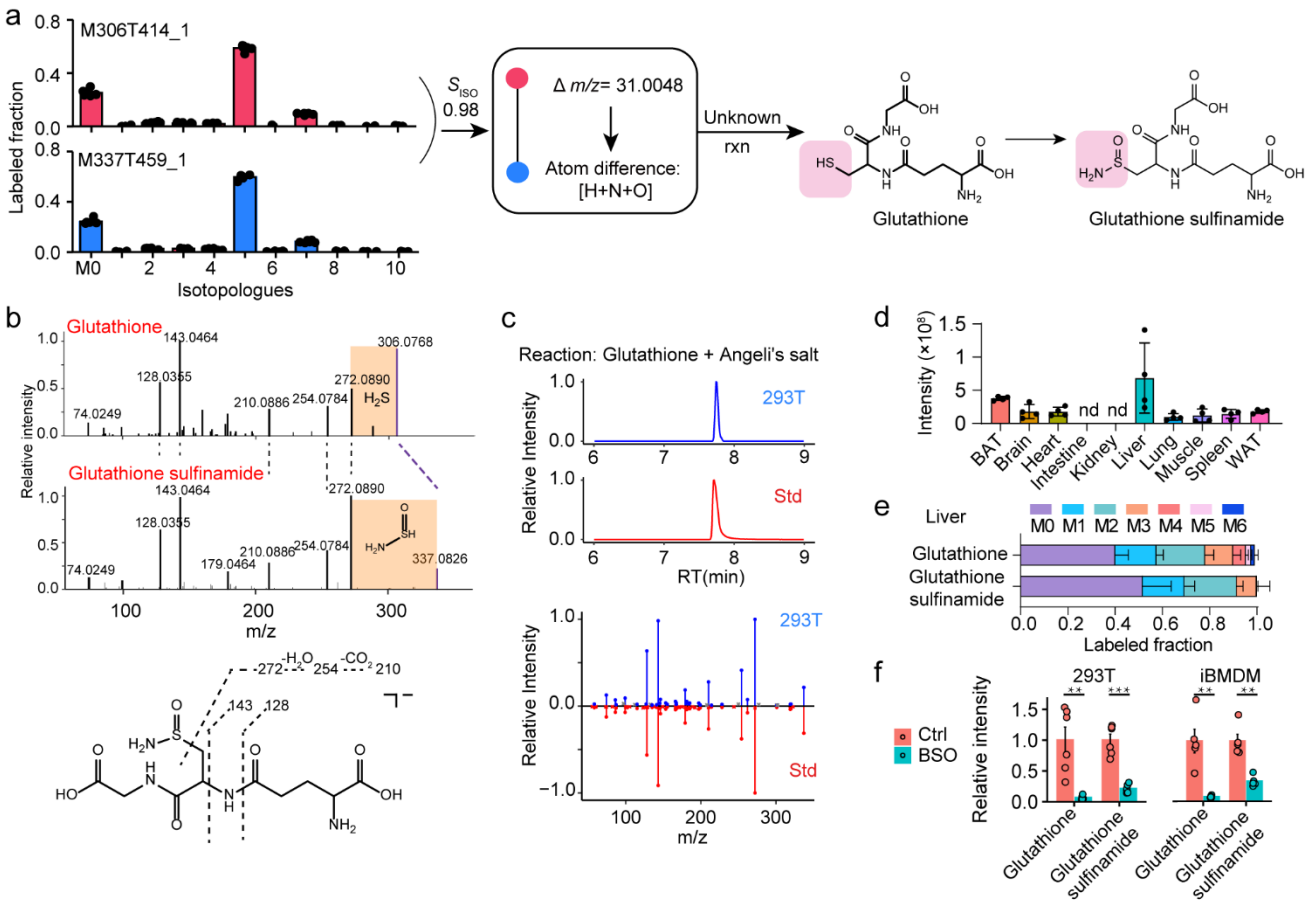


Supplementary Figure 5. Validation of a previously uncharacterized metabolic pathway of γ-Glu-Ser-Gly biosynthesis. (a) Schematic illustration of joint probability to predict isotopologue patterns. (b-c) The

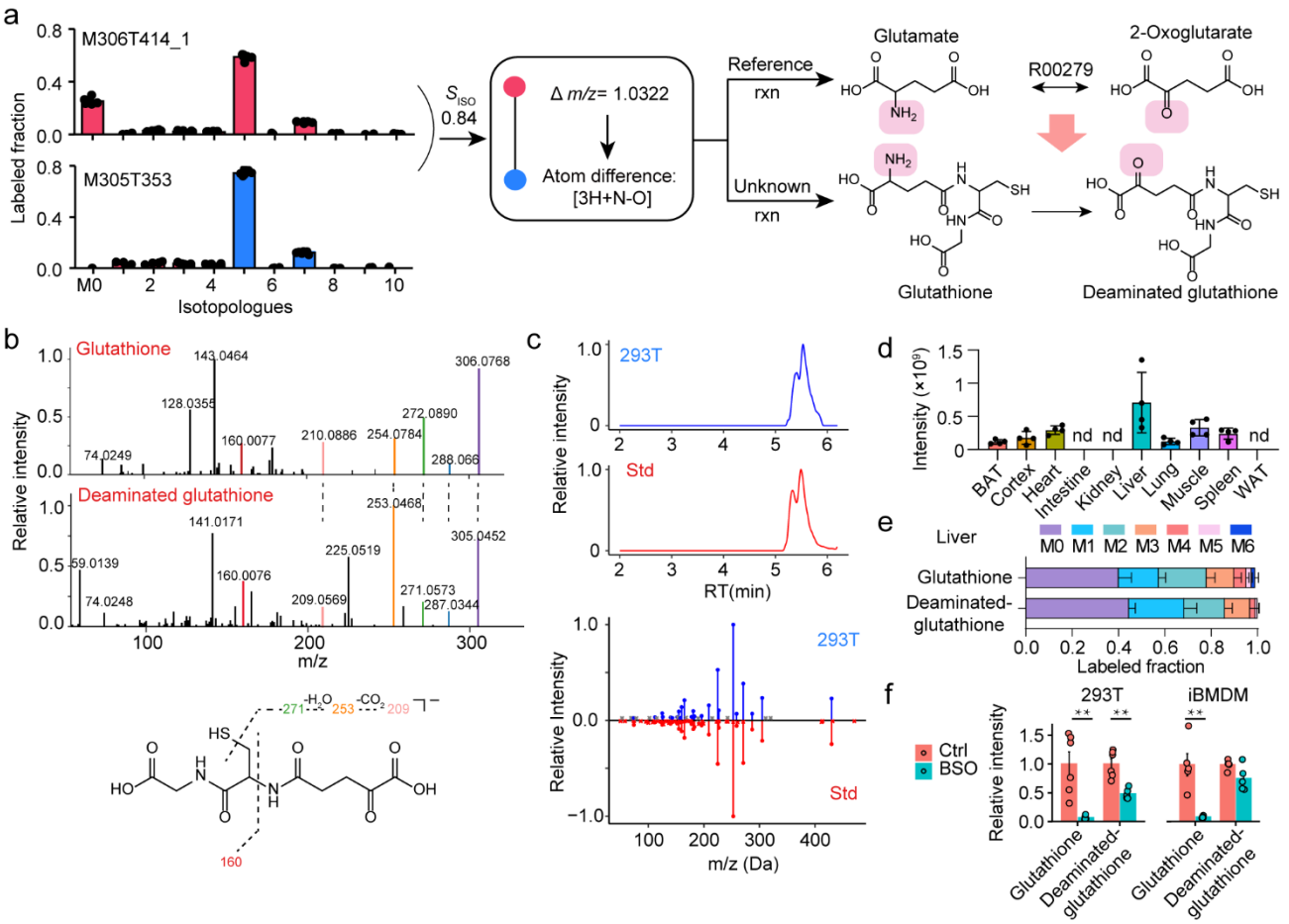
isotopologue patterns of glutamate, glycine, serine, and cysteine in 293T cells labeled with [U-^{13}C]-serine (b) or [U-^{13}C]-cysteine (c) for 6 h. (d) The isotopologue pattern of glutamate, glycine, and serine in 293T cells labeled with [U-^{13}C]-glycine for 6 h. (e) The isotopologue pattern of glutathione and γ-Glu-Ser-Gly (red) and predicted γ-Glu-Ser-Gly (blue and green) in 293T cells labeled with [U-^{13}C]-glycine for 6 h. (f) The isotopologue pattern of glutamate, glycine, serine, and cysteine in iBMDM cells labeled with [U-^{13}C]-cysteine for 6 h. (g) The isotopologue pattern of glutathione and γ-Glu-Ser-Gly (red) and predicted γ-Glu-Ser-Gly (blue and green) in iBMDM cells labeled with [U-^{13}C]-cysteine for 6 h. (h) The reported biosynthesis pathway of γ-Glu-Ser-Gly by GGT (left) and the isotopologue pattern of predicted γ-Glu-Ser-Gly in 293T cells labeled with [U-^{13}C]-serine for 6 h (right). Values represent means ± SEM.



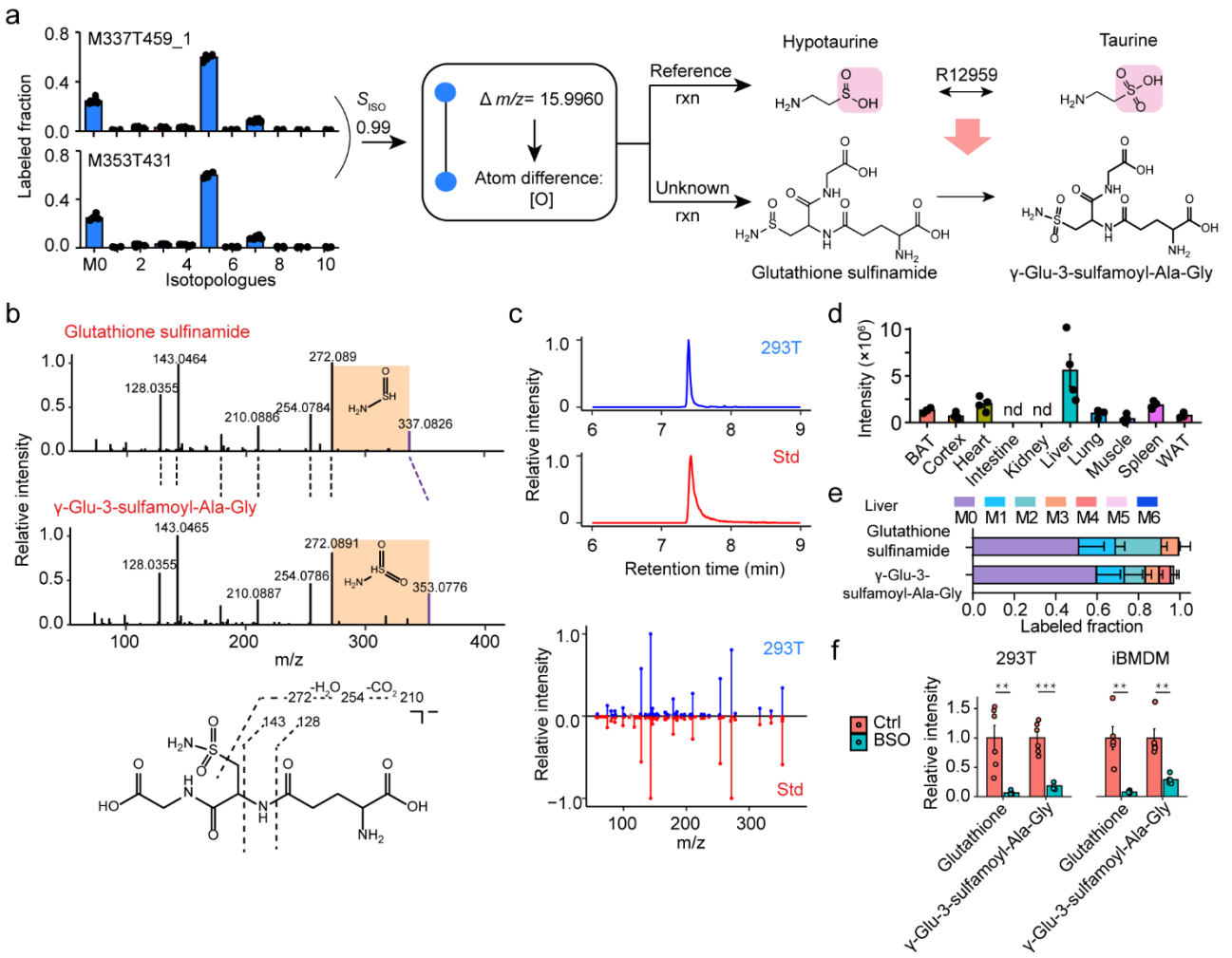
Supplementary Figure 6. Elucidation of S-acetylglutathione. (a) Elucidation of the metabolic reaction between glutathione and M348T391 (S-acetylglutathione). (b) Interpretation of MS/MS spectra between glutathione and S-acetylglutathione. (c) Validation of S-acetylglutathione using chemical standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of S-acetylglutathione in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione and S-acetylglutathione in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular S-acetylglutathione levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p=0.0028 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.0035 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



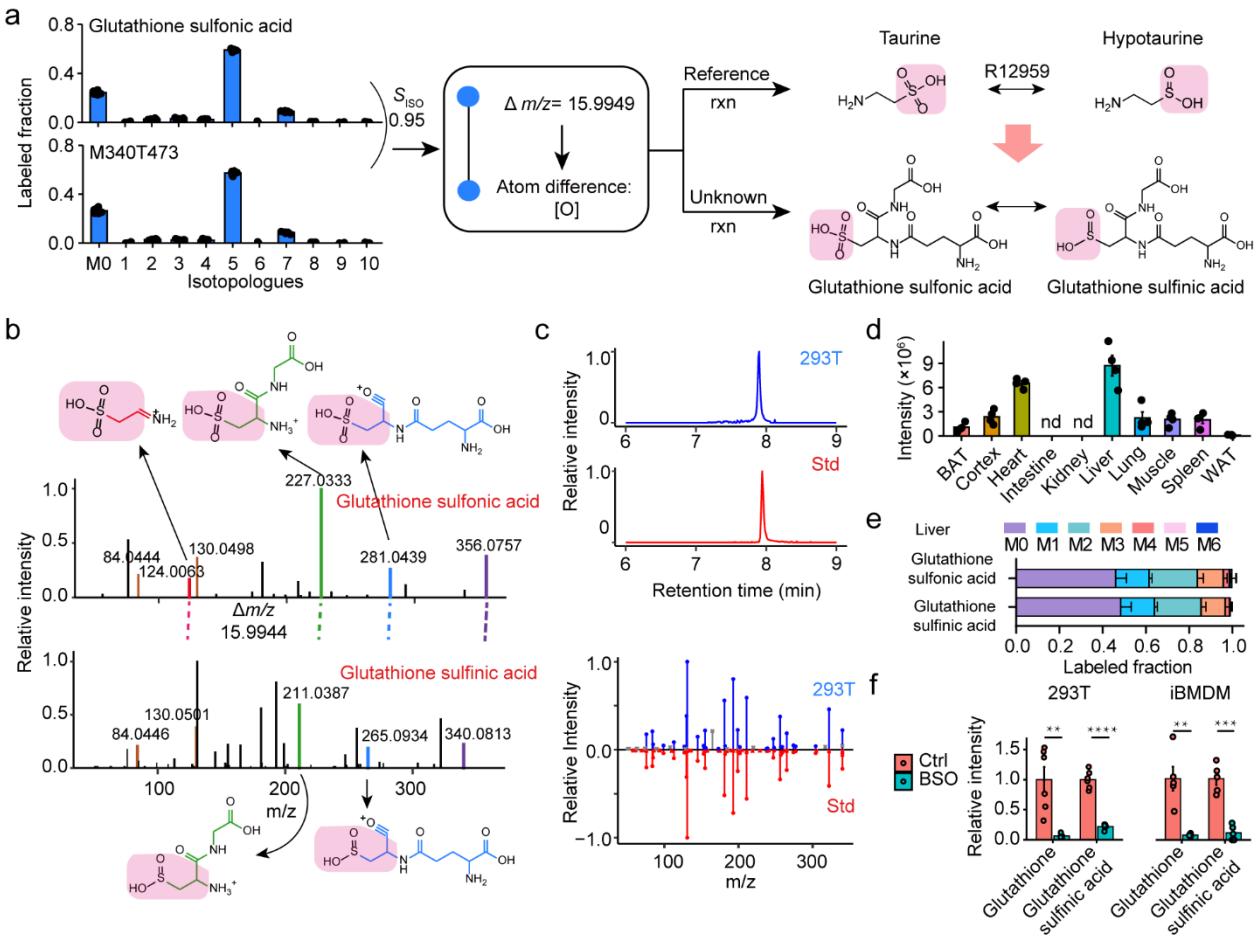
Supplementary Figure 7. Elucidation of glutathione sulfinamide. (a) Elucidation of the metabolic reaction between glutathione and M337T459_1 (glutathione sulfinamide). (b) Interpretation of MS/MS spectra between glutathione and glutathione sulfinamide. (c) Validation of glutathione sulfinamide using in-house synthesized standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of glutathione sulfinamide in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione and glutathione sulfinamide in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular glutathione sulfinamide levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p=0.00029 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.0026 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



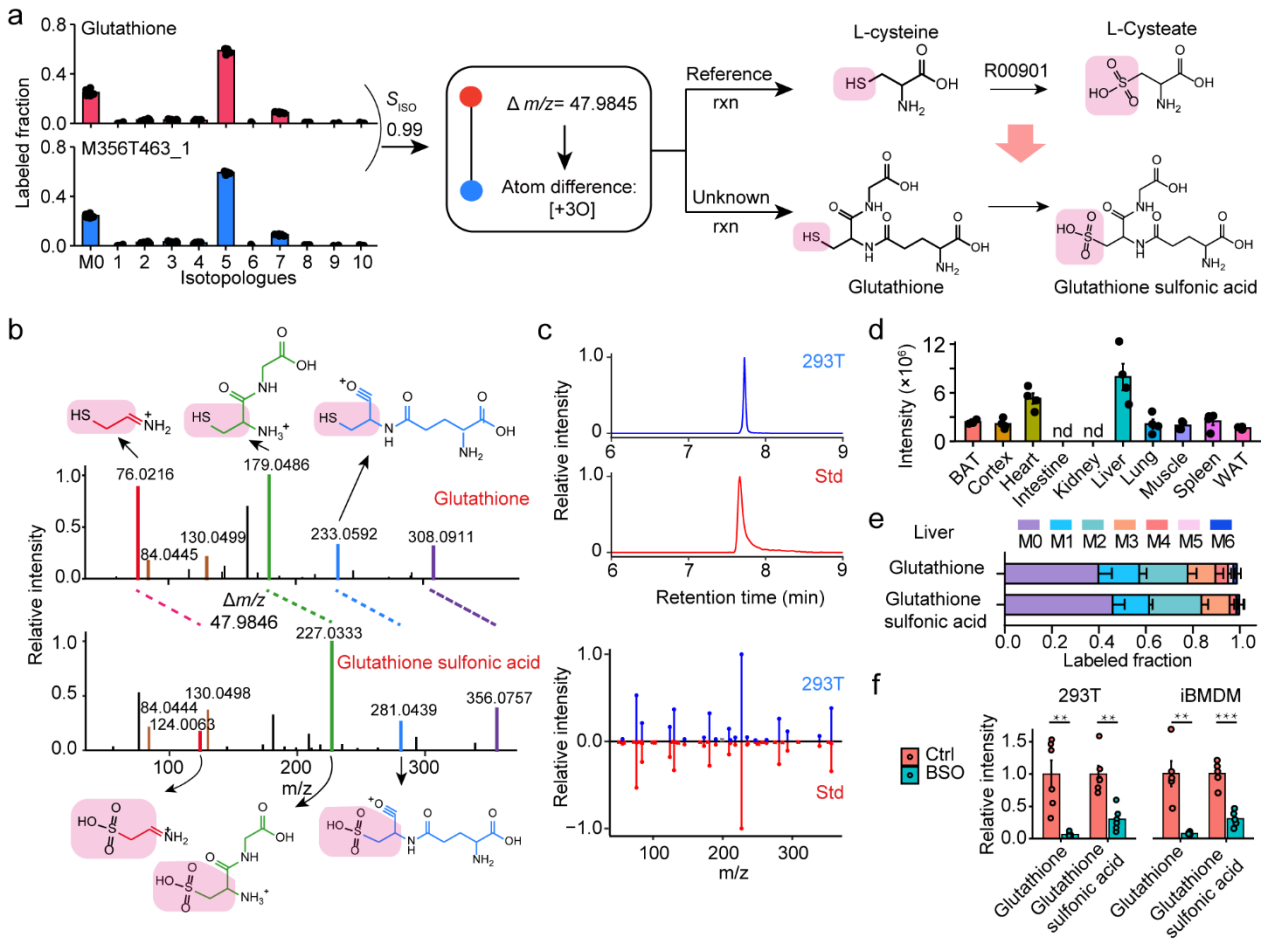
Supplementary Figure 8. Elucidation of deaminated glutathione. (a) Elucidation of the metabolic reaction between glutathione and M305T353 (deaminated glutathione). (b) Interpretation of MS/MS spectra between glutathione and deaminated glutathione. (c) Validation of NEM-derivatized deaminated glutathione using enzymatic synthesized standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of deaminated glutathione in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione and deaminated glutathione in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular Deaminated glutathione levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p=0.0018 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.06 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



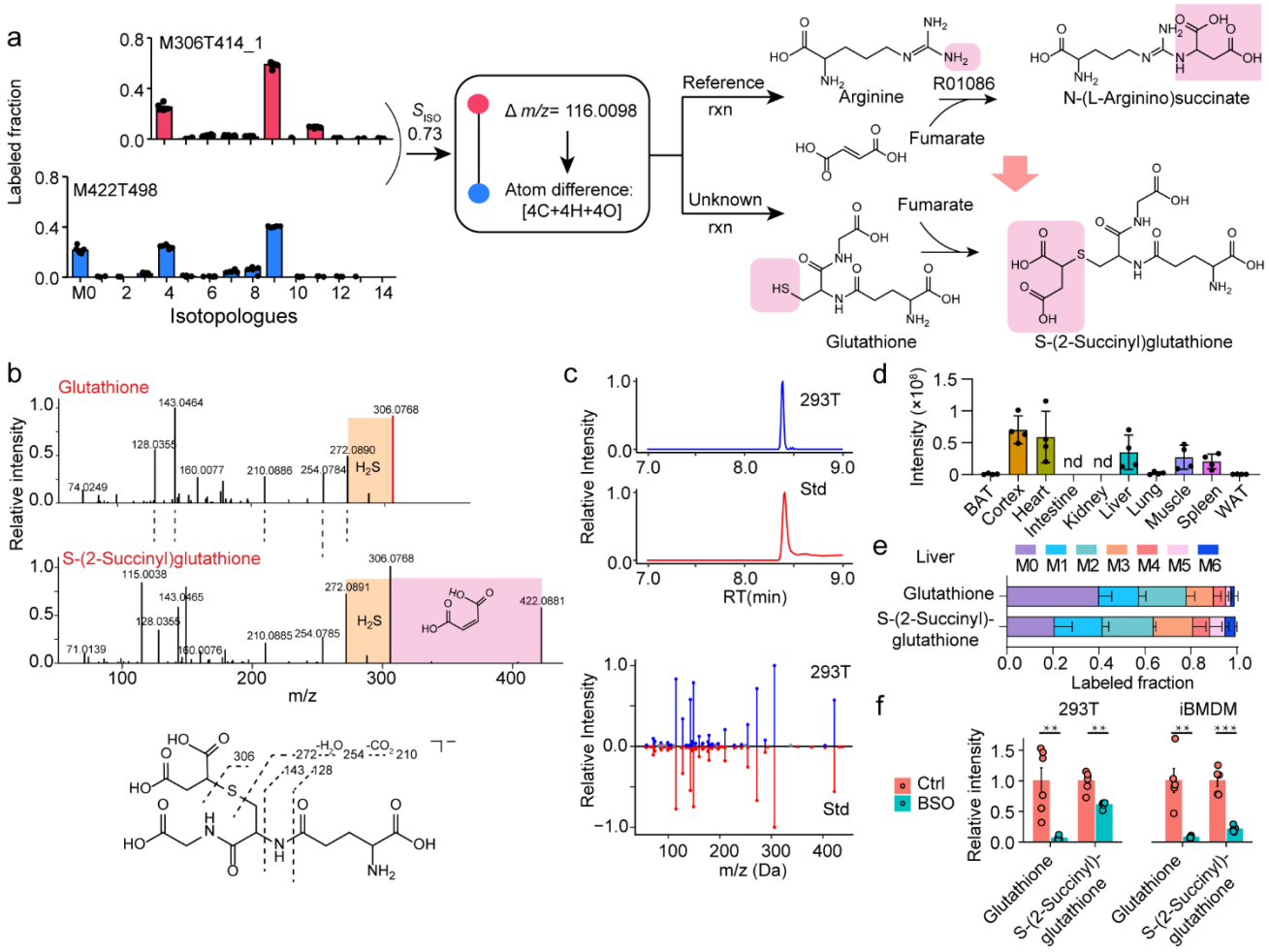
Supplementary Figure 9. Elucidation of γ-Glu-3-sulfamoyl-Ala-Gly. (a) Elucidation of the metabolic reaction between glutathione sulfinamide and M353T431 (γ-Glu-3-sulfamoyl-Ala-Gly). (b) Interpretation of MS/MS spectra between glutathione sulfinamide and γ-Glu-3-sulfamoyl-Ala-Gly. (c) Validation of γ-Glu-3-sulfamoyl-Ala-Gly using chemical standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of γ-Glu-3-sulfamoyl-Ala-Gly in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione sulfinamide and γ-Glu-3-sulfamoyl-Ala-Gly in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular γ-Glu-3-sulfamoyl-Ala-Gly levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p=0.00042 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.0094 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



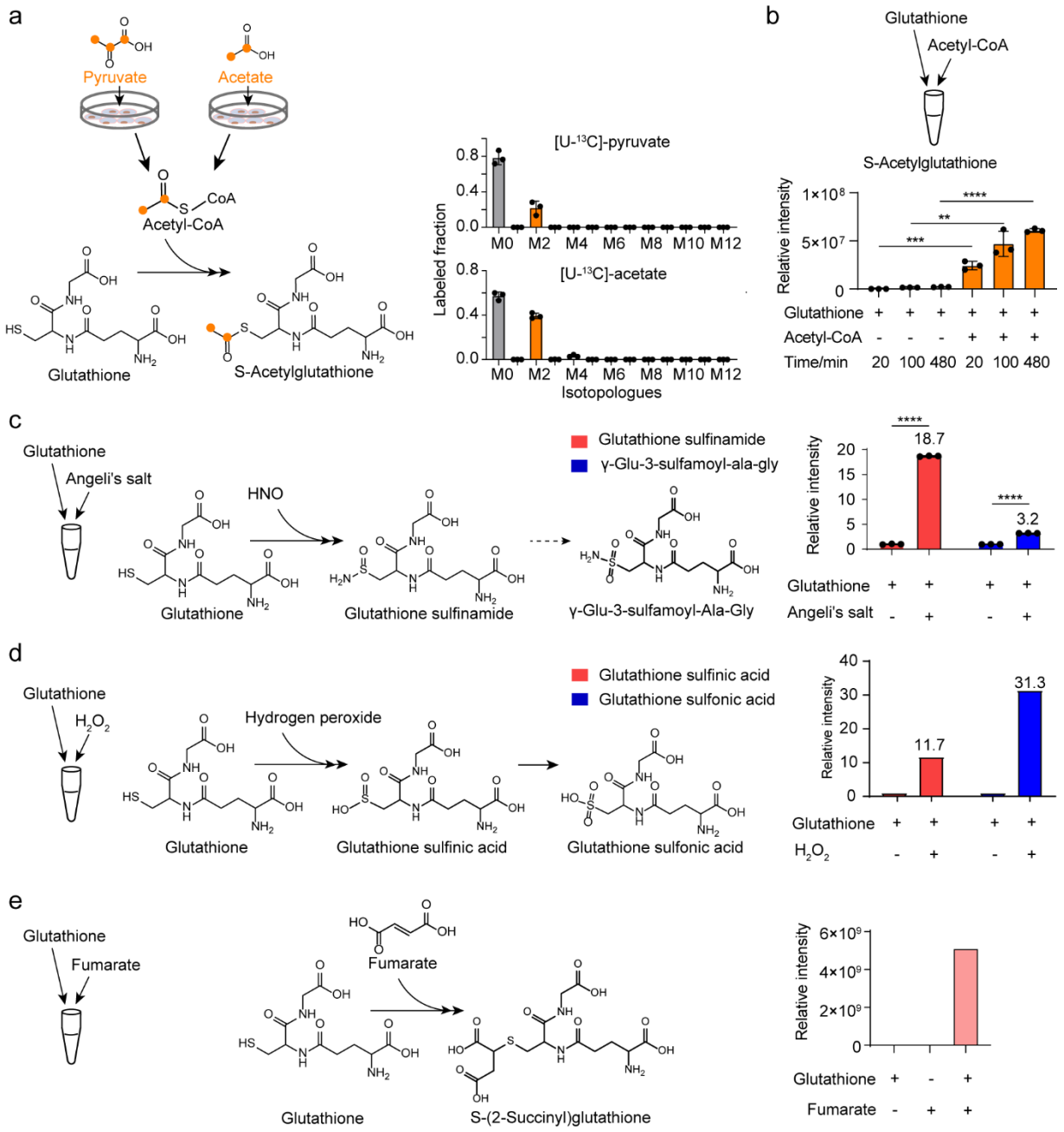
Supplementary Figure 10. Elucidation of glutathione sulfinic acid. (a) Elucidation of the metabolic reaction between glutathione sulfonic acid and M340T473 (glutathione sulfinic acid). (b) Interpretation of MS/MS spectra between glutathione sulfonic acid and glutathione sulfinic acid. (c) Validation of glutathione sulfinic acid using chemical standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of glutathione sulfinic acid in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione sulfonic acid and glutathione sulfinic acid in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular glutathione sulfinic acid levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p<0.0001 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.00027 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



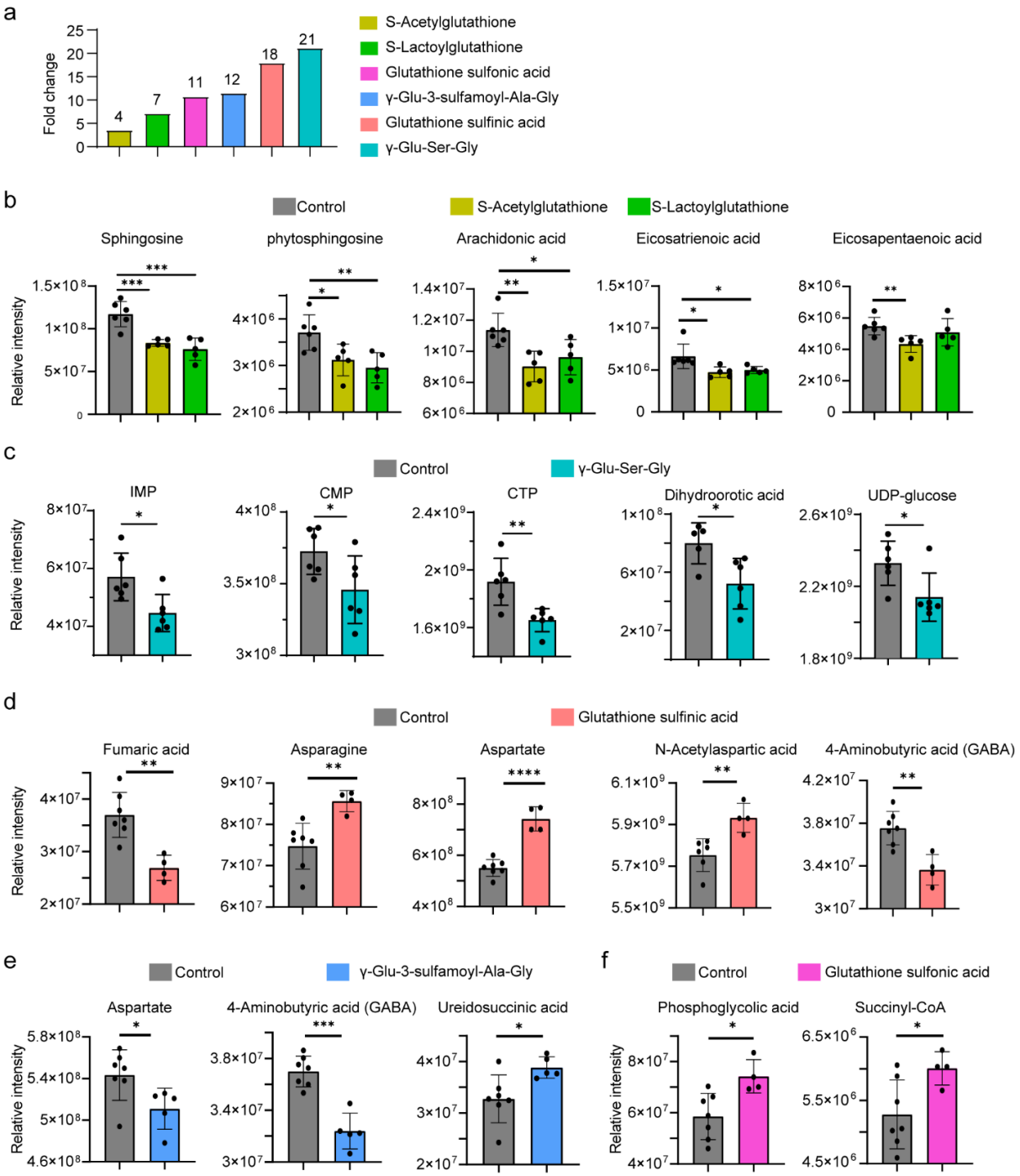
Supplementary Figure 11. Elucidation of glutathione sulfonic acid. (a) Elucidation of the metabolic reaction between glutathione and M356T463_1 (glutathione sulfonic acid). (b) Interpretation of MS/MS spectra between glutathione and glutathione sulfonic acid. (c) Validation of glutathione sulfonic acid using chemical standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of glutathione sulfonic acid in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione and glutathione sulfonic acid in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular glutathione sulfonic acid levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p=0.0015 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.00029 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



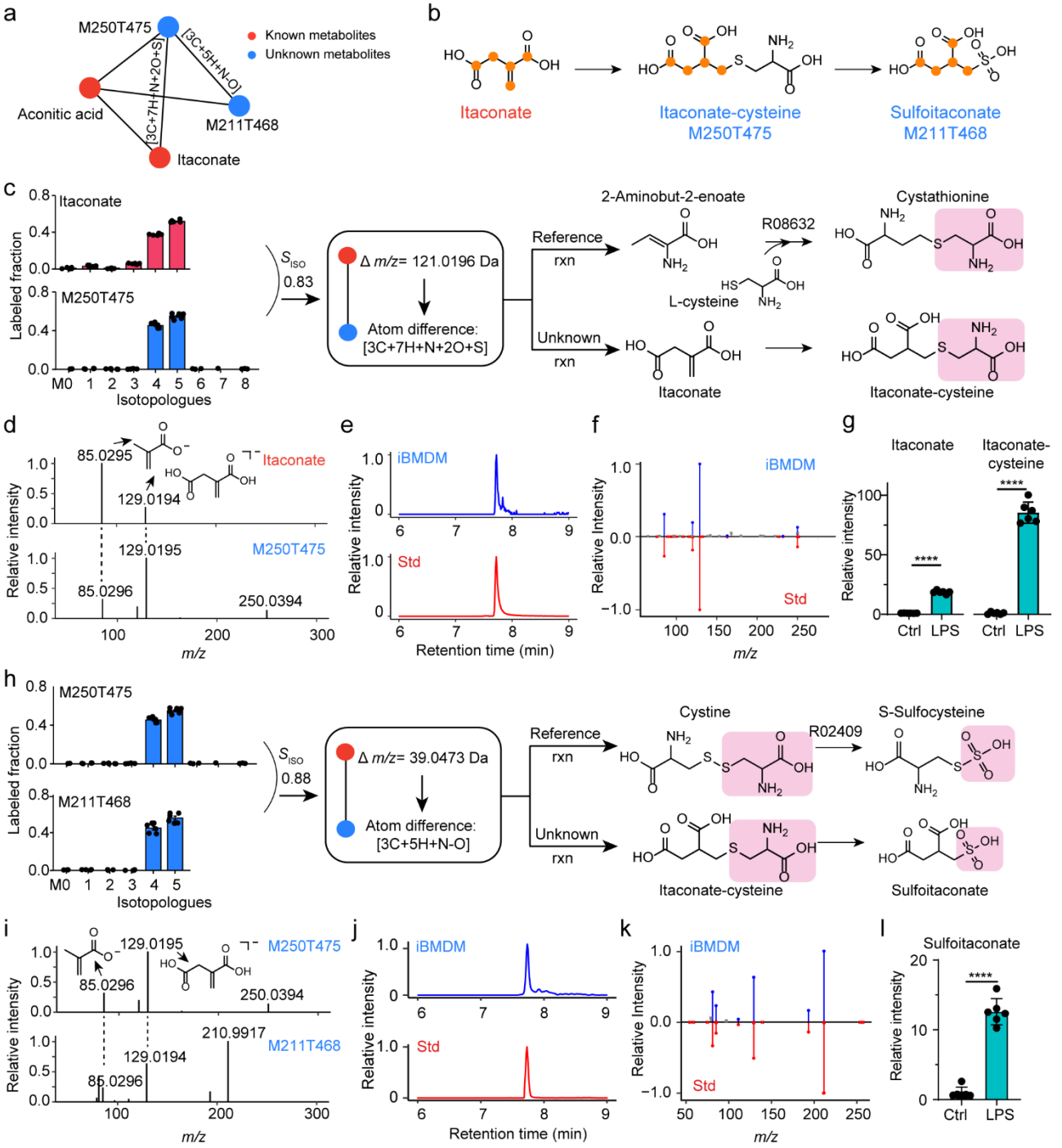
Supplementary Figure 12. Elucidation of S-(2-succinyl)glutathione. (a) Elucidation of the metabolic reaction between glutathione and M422T498 (S-(2-succinyl)glutathione). (b) Interpretation of MS/MS spectra between glutathione and S-(2-succinyl)glutathione. (c) Validation of S-(2-succinyl)glutathione using chemical standard: retention time match (top) and MS2 spectral match (bottom). (d) The relative levels of S-(2-succinyl)glutathione in various mouse tissues (n=4 biological replicates per group). (e) Isotopologue pattern similarity between glutathione and S-(2-succinyl)glutathione in mouse liver tissue. Mice were administered with [U-^{13}C]-glucose for 24 h (n=4 biological replicates per group). (f) Intracellular S-(2-succinyl)glutathione levels in 293T and iBMDM cells after the treatment of buthionine sulfoximine (BSO; 100 μM) for 12 h or 10 h, respectively (n=6 biological replicates for 293T cells per group, p=0.0070 for glutathione, p=0.0012 for γ-Glu-Ser-Gly; n=5 biological replicates for iBMDM cells per group p=0.0093 for glutathione, p=0.00084 for γ-Glu-Ser-Gly). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.



Supplementary Figure 13. Reaction validation in vitro. (a) Reaction scheme (left) and the isotopologue pattern (right) of S-acetylglutathione in 293T cells labeled with [^{13}C]-pyruvate for 6 hours or [^{13}C]-acetate for 33 hours (n=3). (b) The relative intensity of S-acetylglutathione with or without acetyl-CoA in the present of glutathione at 20 min, 100min, 480min respectively (n=3). (c) Reaction scheme and the relative intensity of glutathione sulfinamide and γ-Glu-3-sulfamoyl-Ala-Gly with or without Angeli's salt in the present of glutathione (n=3). (d) Reaction scheme and the relative intensity of glutathione sulfinic acid and glutathione sulfonic acid with or without H_2O_2 in the present of glutathione. (e) Reaction scheme and the relative intensity of S-(2-succinyl)glutathione with or without fumarate in the present of glutathione. Values represent means ± SD.

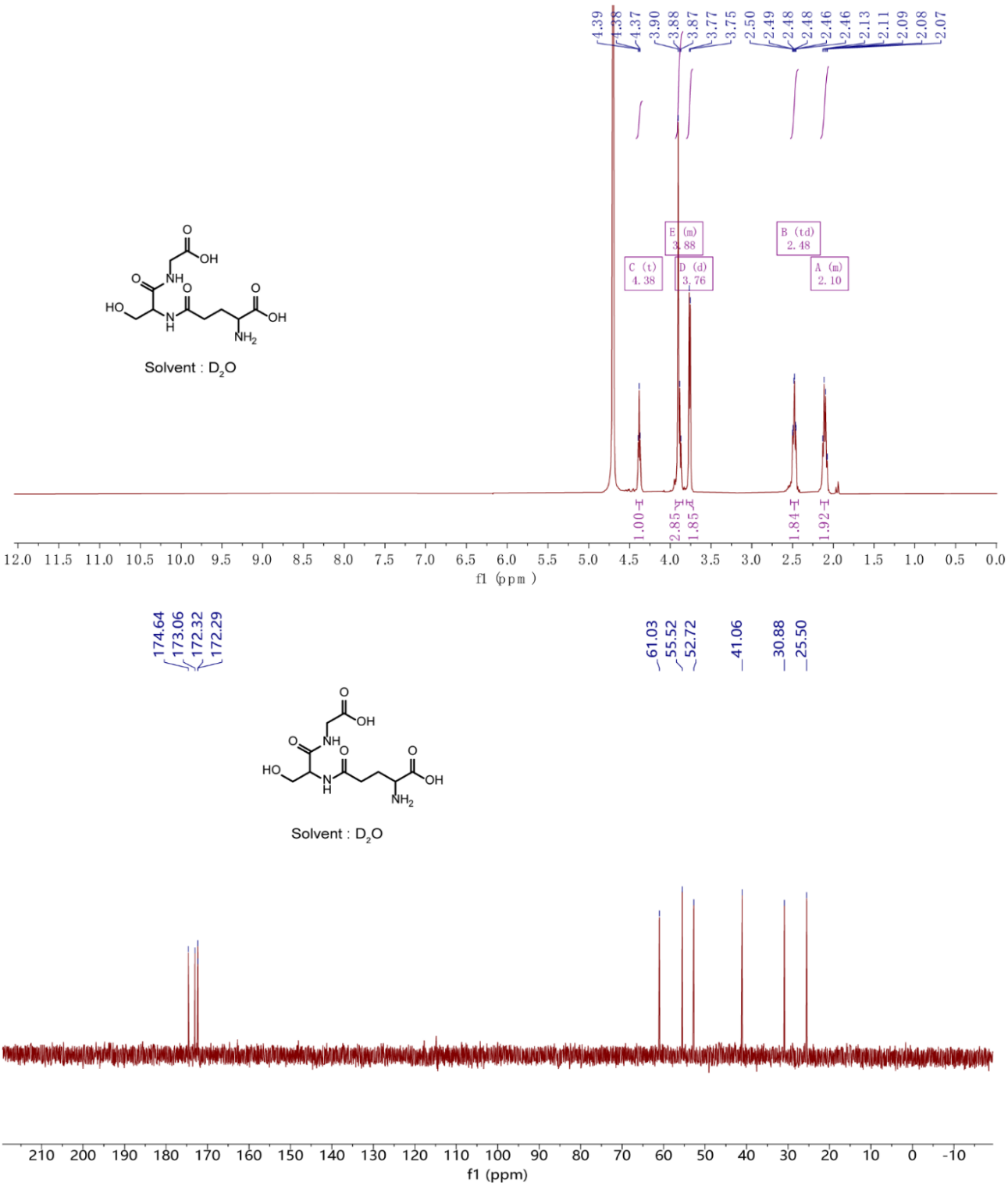


Supplementary Figure 14. Significant changed metabolites in different metabolite treatment. (a) The changed radio of intercellular unknown metabolites after treatment. (b) The significant changed metabolites of S-acetylglutathione and S-lactoylglutathione treatment (n=5 biological replicates for treatment group and n=6 biological replicates for control group). (c) The significant changed metabolites of γ-Glu-Ser-Gly (n=6 biological replicates per group). (d) The significant changed metabolites of glutathione sulfonic acid (n=4 biological replicates for treatment group and n=7 biological replicates for control group). (e) The significant changed metabolites of γ-Glu-3-sulfamoyl-Ala-Gly (n=5 biological replicates for treatment group and n=7 biological replicates for control group). (f) The significant changed metabolites of glutathione sulfinic acid (n=4 biological replicates for treatment group and n=7 biological replicates for control group). P-values were determined by a two-tailed Student's t-test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001. Values represent means ± SD.

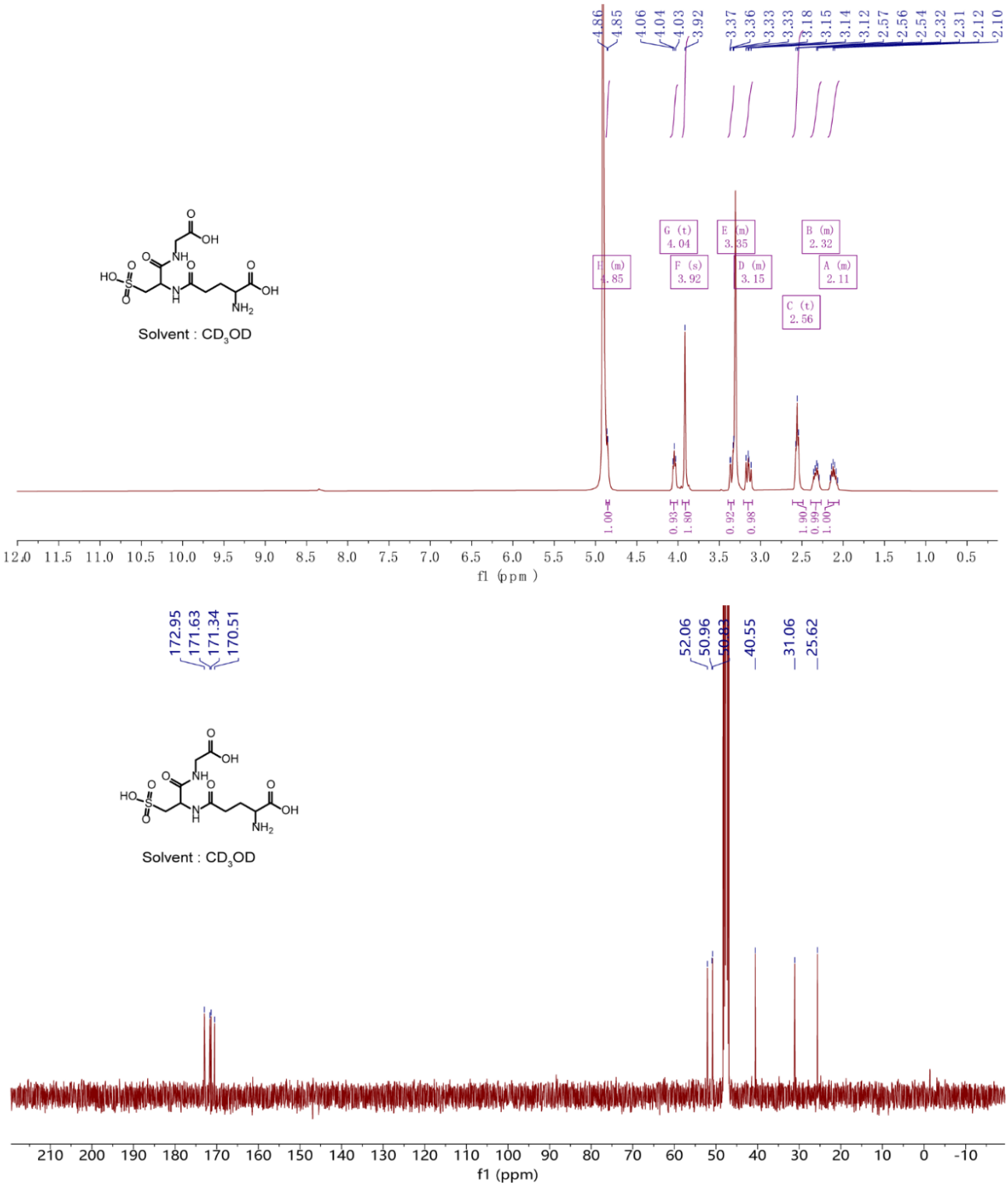


Supplementary Figure 15. Itaconate-associated isotopologue similarity subnetwork disclose newly identified metabolites. (**a**) The itaconate-associated isotopologue similarity subnetwork in negative ionization mode of iBMDM cells with LPS stimulation. (**b**) The unknown metabolic reactions between itaconate, M250T475, and M211T468. Orange points represent the 13C-labeled atoms. (**c**) Elucidation of the metabolic reaction between itaconate and M250T475 (itaconate-cysteine, putative structure). (**d**) Interpretation of MS/MS spectra of itaconate and M250T475. (**e-f**) Validation of itaconate-cysteine using an in-house synthesized chemical standard (Std) by mixing itaconate and cysteine standards: retention time match (**e**) and MS/MS spectral match (**f**). (**g**) Intracellular levels of itaconate and M250T475 (itaconate-cysteine, putative structure) in iBMDM cells after the treatment of LPS (100 ng/ml) for 10 h, respectively (n=6 biological replicates per group, p<0.0001). (**h**) Elucidation of the metabolic reaction between itaconate-cysteine (putative structure) and M211T468

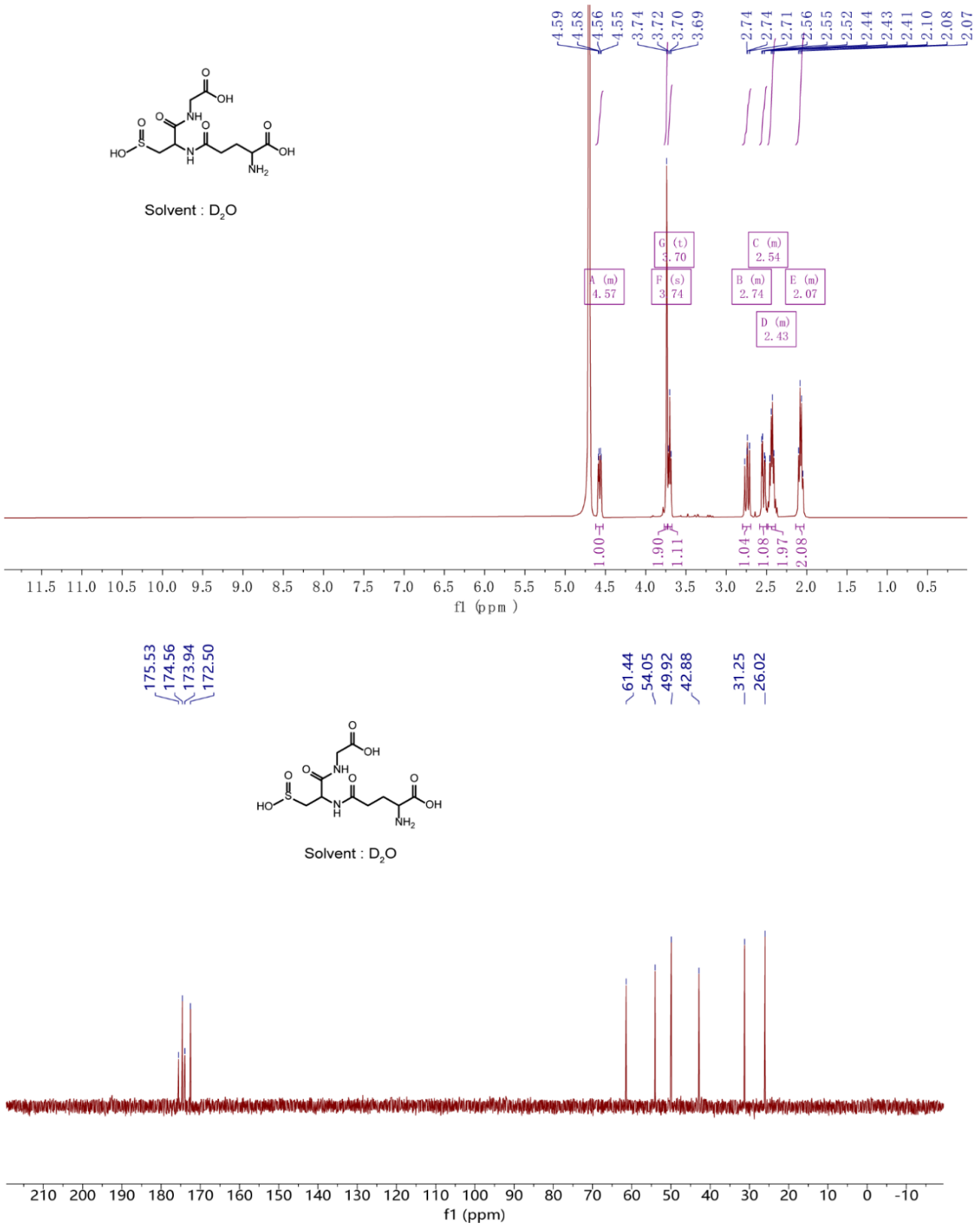
(sulfoitaconate). (**i**) Interpretation of MS/MS spectra of itaconate-cysteine (putative structure) and sulfoitaconate. (**j**-**k**) Validation of sulfoitaconate using chemical standard (Std): retention time match (**j**) and MS/MS spectral match (**k**). (**l**) Intracellular levels of sulfoitaconate in iBMDM cells after the treatment of LPS (100 ng/ml) for 10 h (n=6 biological replicates per group, p<0.0001). P-values were determined by a two-tailed Student's t-test. ****, p-value < 0.0001. Values represent means ± SD.



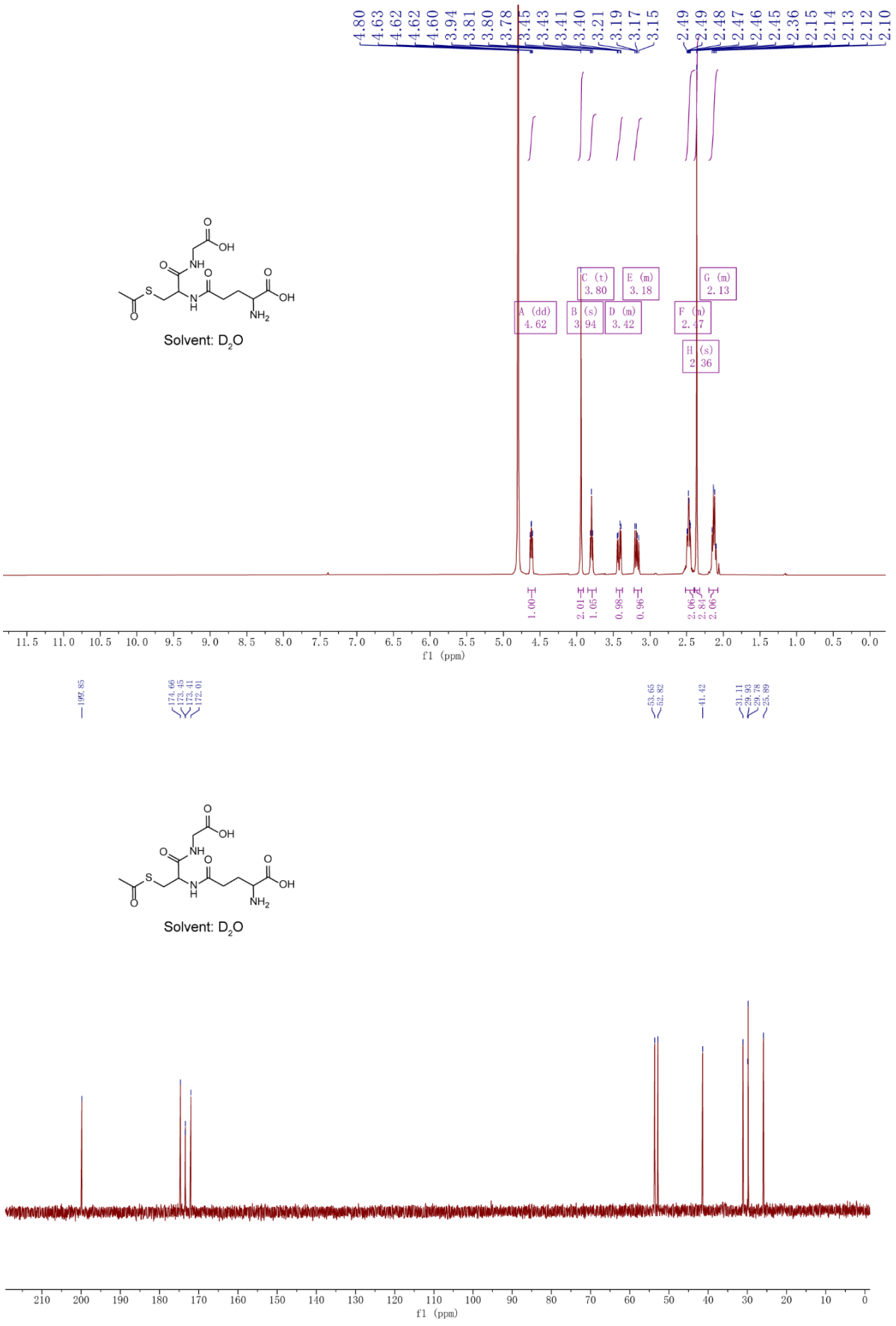
Supplementary Figure 16. ^{1}H (top) and ^{13}C (bottom) NMR spectra for γ-Glu-Ser-Gly.



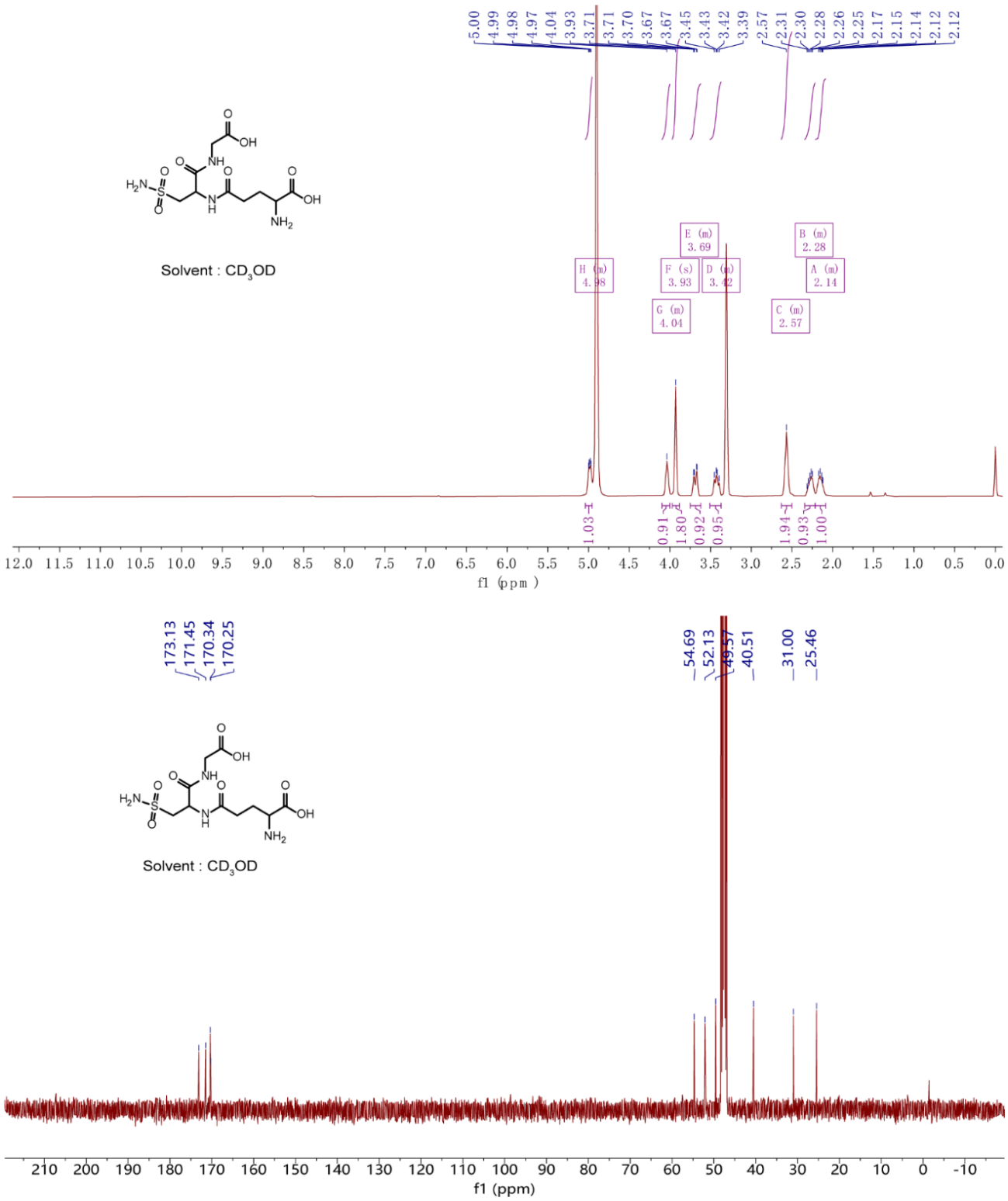
Supplementary Figure 17. ^{1}H (top) and ^{13}C (bottom) NMR spectra for glutathione sulfonic acid.



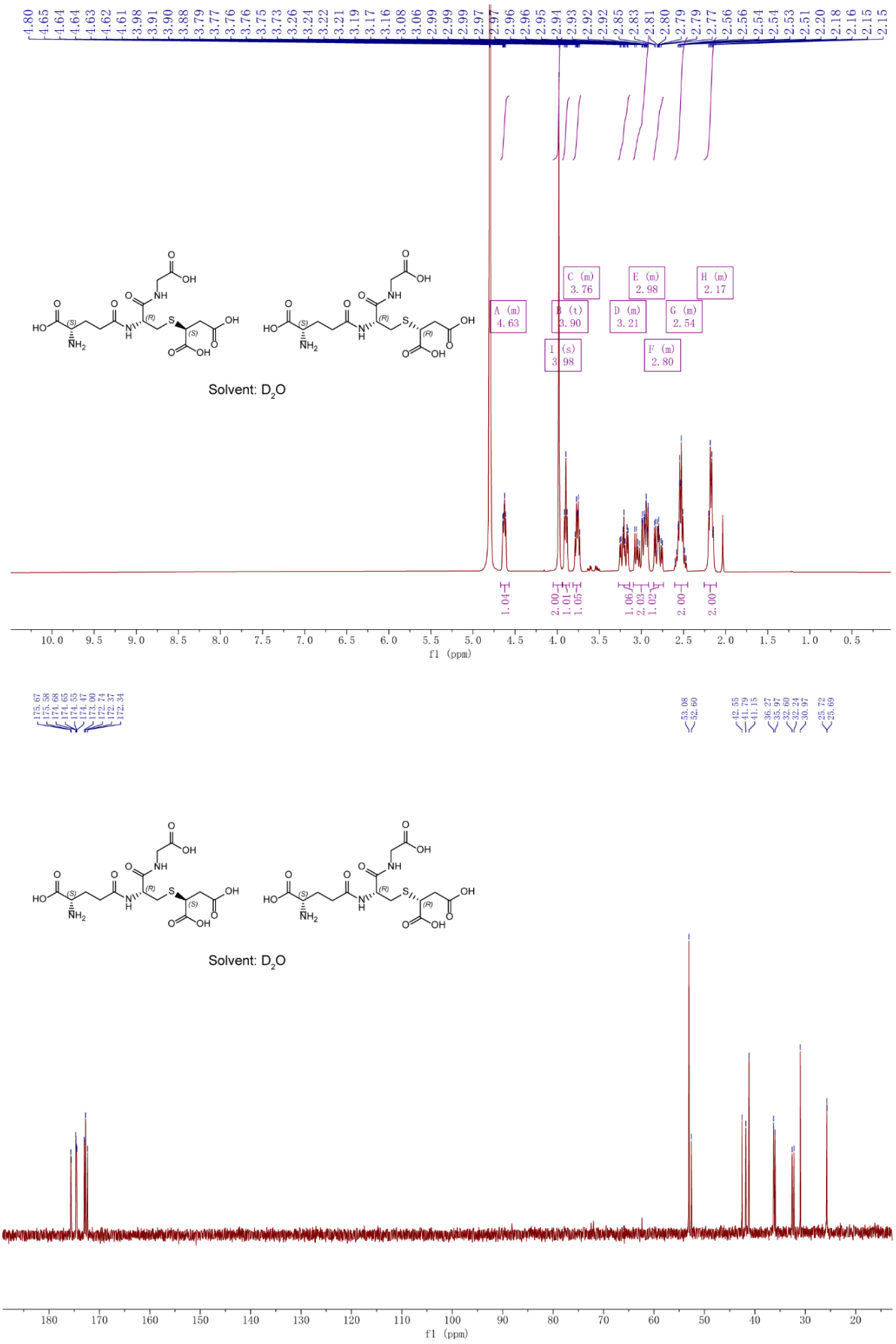
Supplementary Figure 18. ^{1}H (top) and ^{13}C (bottom) NMR spectra for glutathione sulfinic acid.



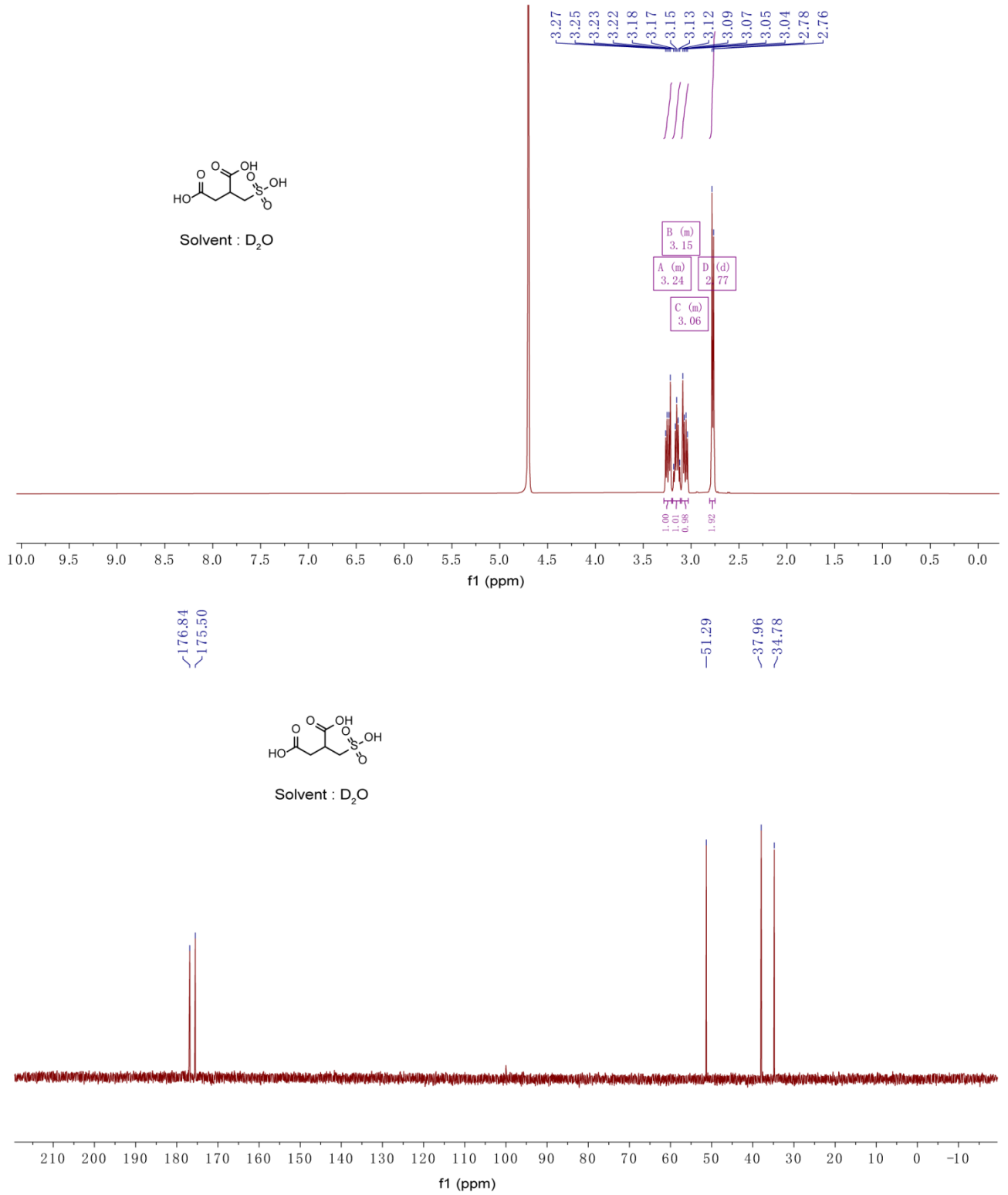
Supplementary Figure 19. ^{1}H (top) and ^{13}C (bottom) NMR spectra for S-acetylglutathione.



Supplementary Figure 20. ^{1}H (top) and ^{13}C (bottom) NMR spectra for γ-Glu-3-sulfamoyl-Ala-Gly.

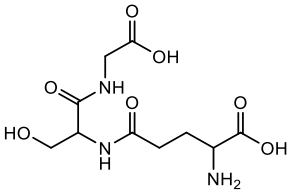
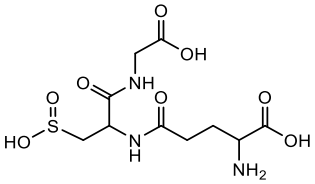
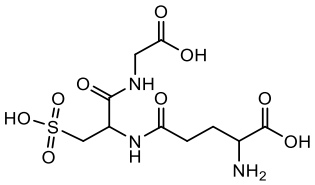
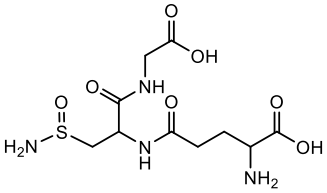
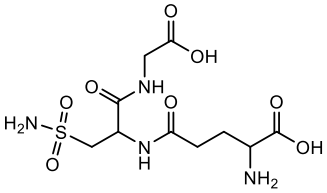
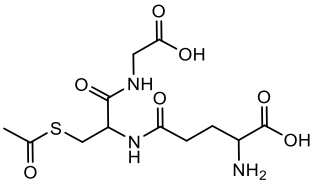
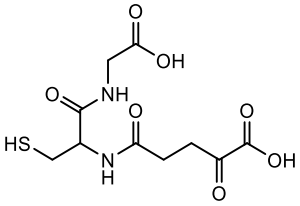


Supplementary Figure 21. ^{1}H (top) and ^{13}C (bottom) NMR spectra for S-(-2-Succinyl)-glutathione.

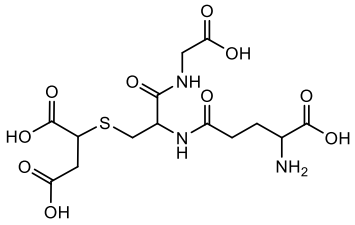
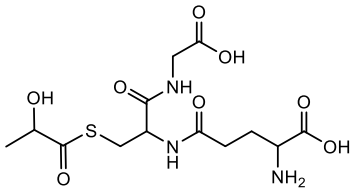
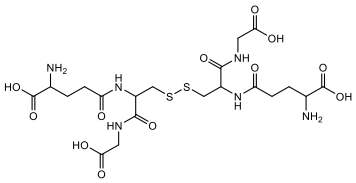


Supplementary Figure 22. ^{1}H (top) and ^{13}C (bottom) NMR spectra for synthesized chemical standard sulfoitaconate.

Supplementary Table 1. The database information of unknown metabolites in glutathione-associated metabolic reaction network.

Name	Structure	Pubchem	KEGG	HMDB
γ-Glu-Ser-Gly		21120626	×	×
Glutathione sulfinic acid		5326960	×	×
Glutathione sulfonic acid		444104	×	×
Glutathione sulfinamide		129320410	×	×
γ-Glu-3-sulfamoyl-Ala-Gly		×	×	×
S-Acetylglutathione		9894372	×	×
Deaminated glutathione		71620685	×	×

Continued Supplementary Table 1

Name	Structure	Pubchem	KEGG	HMDB
S-(2-Succinyl)glutathione		4411483	×	HMDB 0257378
S-Lactoylglutathione		440018	C03451	HMDB 0001066
Glutathione disulfide		65359	C00127	HMDB 0003337

(Access on February 2024) Pubchem [https://pubchem.ncbi.nlm.nih.gov/]; KEGG [https://www.genome.jp/kegg/]; HMDB [https://hmdb.ca/].

Supplementary Table 2. The database information of reactions in glutathione-associated metabolic reaction network.

Reactions	KEGG	Reported
Glutathione ⇋ γ-Glu-Ser-Gly	×	×
Glutathione ⇋ S-Acetylglutathione	×	×
Glutathione ⇋ γ-Glu-3-sulfamoyl-Ala-Gly	×	×
Glutathione ⇋ Glutathione sulfinamide	×	√
Glutathione ⇋ Glutathione sulfinic acid	×	√
Glutathione sulfinic acid ⇋ Glutathione sulfonic acid	×	√
Glutathione ⇋ Deaminated glutathione	×	√
Glutathione ⇋ S-(2-Succinyl)glutathione	×	√
Glutathione ⇋ S-Lactoylglutathione	√	√
Glutathione ⇋ Glutathione disulfide	√	√

Supplementary Table 3. Significantly enriched pathways of individual metabolite treatment experiments.

Pathways	Hits	p values	Metabolite treatment group
Alanine, aspartate and glutamate metabolism	7	0.00001	Glutathione sulfinic acid
Pyruvate metabolism	4	0.00293	Glutathione sulfinic acid
Arginine biosynthesis	3	0.00560	Glutathione sulfinic acid
Nicotinate and nicotinamide metabolism	3	0.00686	Glutathione sulfinic acid
Pentose and glucuronate interconversions	3	0.01354	Glutathione sulfinic acid
Biosynthesis of unsaturated fatty acids	4	0.01504	Glutathione sulfinic acid
Pantothenate and CoA biosynthesis	3	0.01564	Glutathione sulfinic acid
Citrate cycle (TCA cycle)	3	0.01564	Glutathione sulfinic acid
Valine, leucine and isoleucine biosynthesis	2	0.01837	Glutathione sulfinic acid
Valine, leucine and isoleucine degradation	4	0.02155	Glutathione sulfinic acid
Amino sugar and nucleotide sugar metabolism	4	0.02537	Glutathione sulfinic acid
Alanine, aspartate and glutamate metabolism	4	0.00078	γ-Glu-3-sulfamoyl-Ala-Gly
Pentose and glucuronate interconversions	3	0.00290	γ-Glu-3-sulfamoyl-Ala-Gly
Valine, leucine and isoleucine biosynthesis	2	0.00639	γ-Glu-3-sulfamoyl-Ala-Gly
Pyrimidine metabolism	3	0.02216	γ-Glu-3-sulfamoyl-Ala-Gly
Butanoate metabolism	2	0.02239	γ-Glu-3-sulfamoyl-Ala-Gly
Valine, leucine and isoleucine degradation	3	0.02370	γ-Glu-3-sulfamoyl-Ala-Gly
Histidine metabolism	2	0.02534	γ-Glu-3-sulfamoyl-Ala-Gly
Citrate cycle (TCA cycle)	2	0.03860	γ-Glu-3-sulfamoyl-Ala-Gly
Pyruvate metabolism	2	0.04993	γ-Glu-3-sulfamoyl-Ala-Gly
Glyoxylate and dicarboxylate metabolism	4	0.00132	Glutathione sulfonic acid
Citrate cycle (TCA cycle)	3	0.00337	Glutathione sulfonic acid
Alanine, aspartate and glutamate metabolism	3	0.00891	Glutathione sulfonic acid
Arginine biosynthesis	2	0.01959	Glutathione sulfonic acid
Pyrimidine metabolism	3	0.02216	Glutathione sulfonic acid
Valine, leucine and isoleucine degradation	3	0.02370	Glutathione sulfonic acid
Ether lipid metabolism	2	0.03860	Glutathione sulfonic acid
Propanoate metabolism	2	0.04603	Glutathione sulfonic acid
Pyruvate metabolism	2	0.04993	Glutathione sulfonic acid
Glyoxylate and dicarboxylate metabolism	3	0.02132	S-Acetylglutathione
Sphingolipid metabolism	3	0.02132	S-Acetylglutathione
Glycine, serine and threonine metabolism	3	0.02315	S-Acetylglutathione
Biosynthesis of unsaturated fatty acids	3	0.02916	S-Acetylglutathione
Arginine and proline metabolism	3	0.02916	S-Acetylglutathione
Sphingolipid metabolism	3	0.01945	S-Lactoylglutathione
Glyoxylate and dicarboxylate metabolism	3	0.01945	S-Lactoylglutathione
Biosynthesis of unsaturated fatty acids	3	0.02666	S-lactoylglutathione
Pyrimidine metabolism	3	0.03288	S-Lactoylglutathione
Neomycin, kanamycin and gentamicin biosynthesis	1	0.03650	S-Lactoylglutathione
Amino sugar and nucleotide sugar metabolism	3	0.03981	S-Lactoylglutathione
Starch and sucrose metabolism	2	0.04174	S-Lactoylglutathione

Continued Supplementary Table 3

Pathways	Hits	p values	Metabolite treatment group
Glyoxylate and dicarboxylate metabolism	5	0.00040	γ-Glu-Ser-Gly
Pyrimidine metabolism	5	0.00102	γ-Glu-Ser-Gly
Purine metabolism	6	0.00265	γ-Glu-Ser-Gly
Citrate cycle (TCA cycle)	3	0.00750	γ-Glu-Ser-Gly
Amino sugar and nucleotide sugar metabolism	4	0.01020	γ-Glu-Ser-Gly
Valine, leucine and isoleucine biosynthesis	2	0.01102	γ-Glu-Ser-Gly
Arginine biosynthesis	2	0.03311	γ-Glu-Ser-Gly
Arginine and proline metabolism	3	0.03740	γ-Glu-Ser-Gly
Histidine metabolism	2	0.04254	γ-Glu-Ser-Gly

Pathway enrichment p-values were calculated using the hypergeometric test (unadjusted).