Supplementary Information

Multi-dimensional Bio Mass Cytometry：Simultaneous Analysis of Cytoplasmic Protein and Metabolites on Single Cells

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Table of Content:

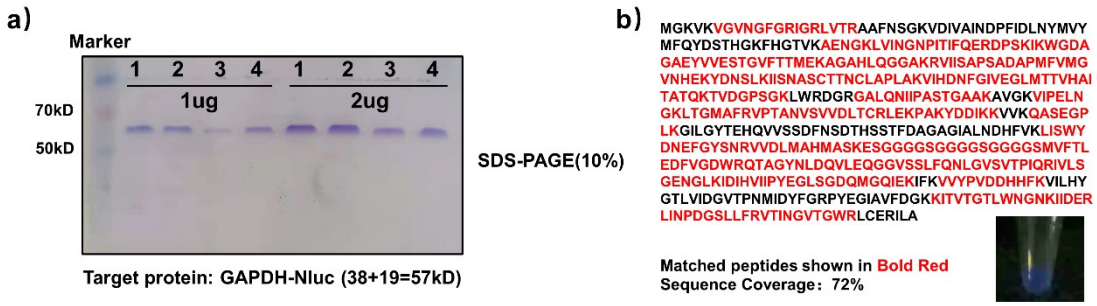

Figure S1. GAPDH-Nluc protein characterization. (a) SDS-PAGE result. Only one lane was detected, which indicated that purified GAPDH-Nluc was obtained (n=4 duplicates, loading amount is 1μg or 2μg) (b) MS identification result. 72% sequence coverage implied success expression of GAPDH-Nluc fusion protein. Illumination of blue light when incubated with substrate also validated the enzymatic activity of fusion protein. (bottom right)

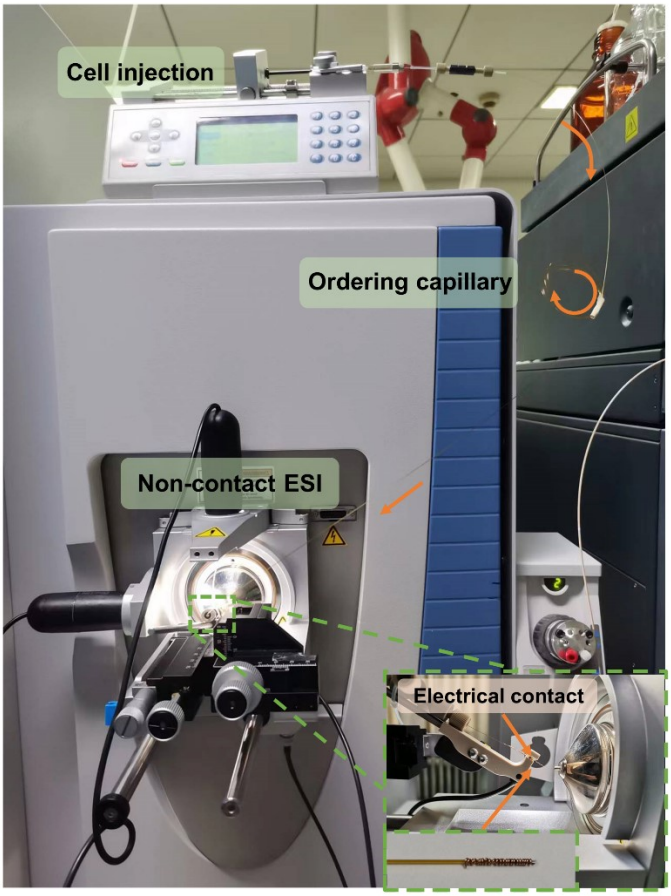

Figure S2. Multi-dimensional single cell mass cytometry setup. Cell was injected into ordering capillary by MS built-in injection syringe. Then non-contact ESI was utilized for subsequent cellular contents ionization. Spray emitter was entwined with Cu wire for electrical contact (bottom right). High-voltage electric field was formed at spray needle to induce electrospray.

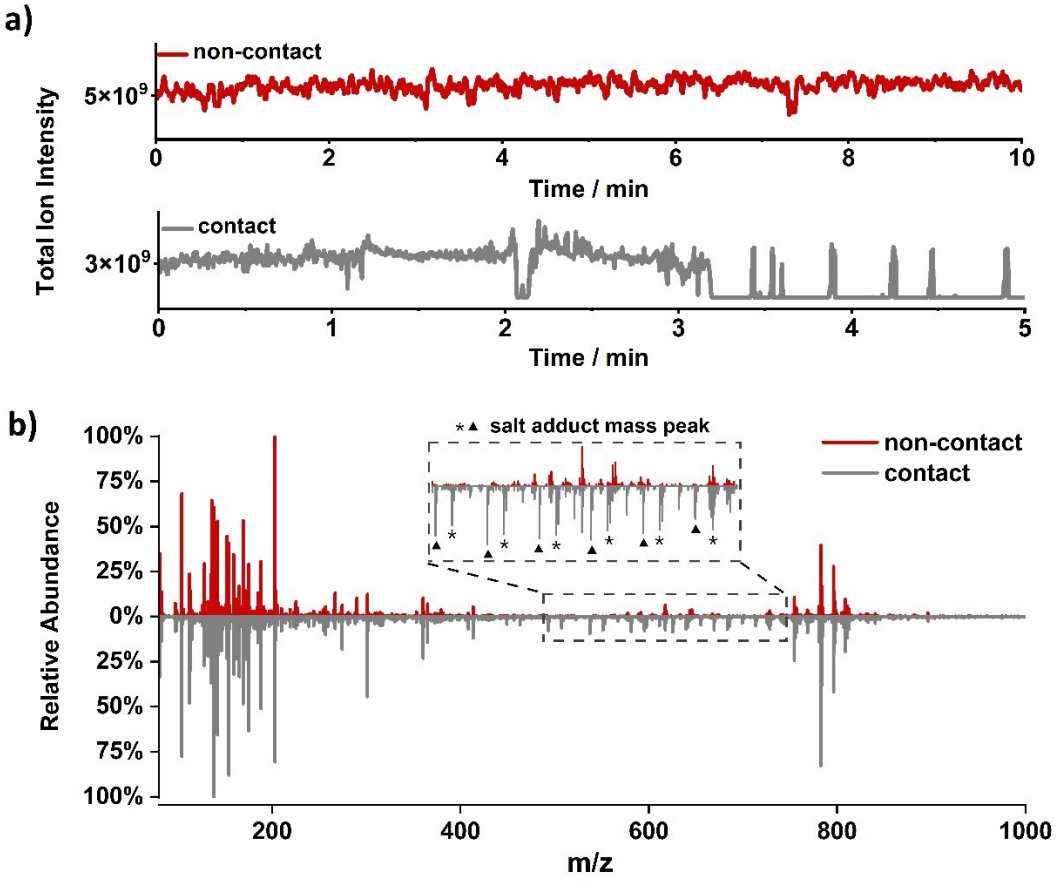

Figure S3. MS detection performance comparison with cell lysate between non-contact ESI mode and contact ESI mode. (a) Total ion chromatogram in single run. Blockage occurred within 5 min at contact mode. (b) MS spectrum comparison. Significant salt interference existed at contact mode.

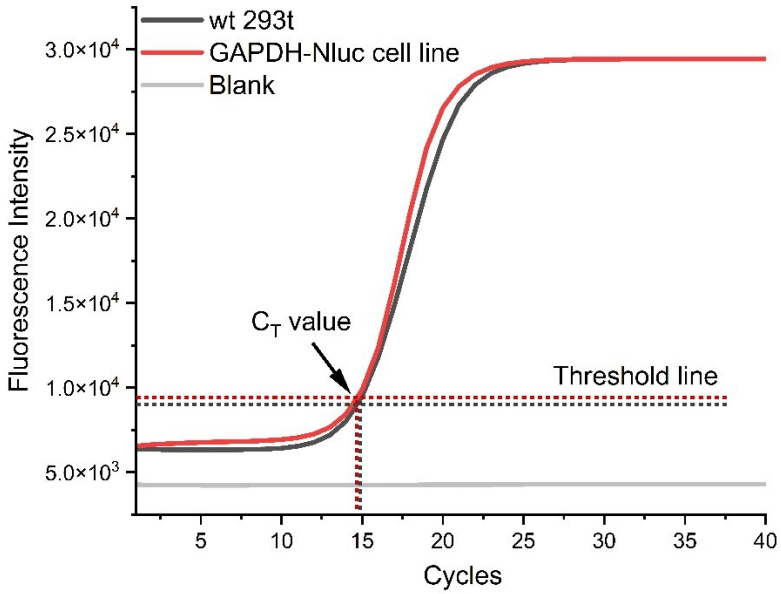

Figure S4. Amplification plots of target gene GAPDH in qPCR quantification assay. (blank indicates no templates added)

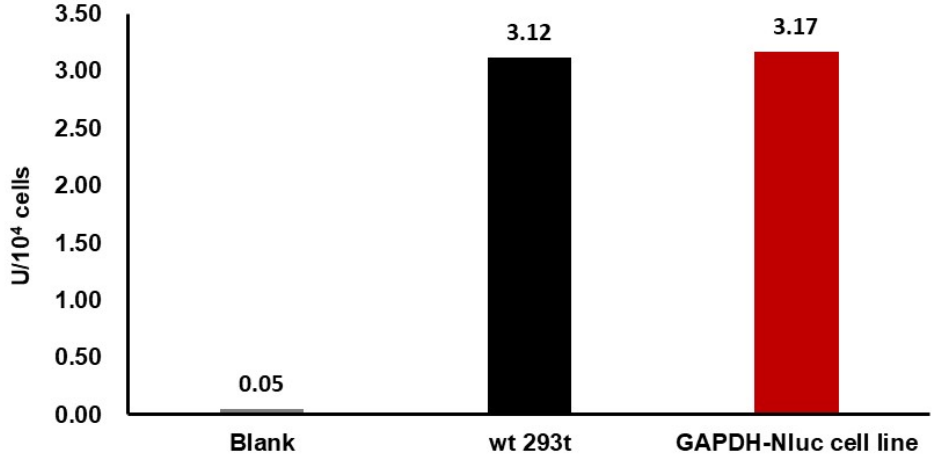

Figure S5. GAPDH enzyme activity evaluation. GAPDH in wt 293t and GAPDH-Nluc cell line exhibits basically the same enzyme activity.

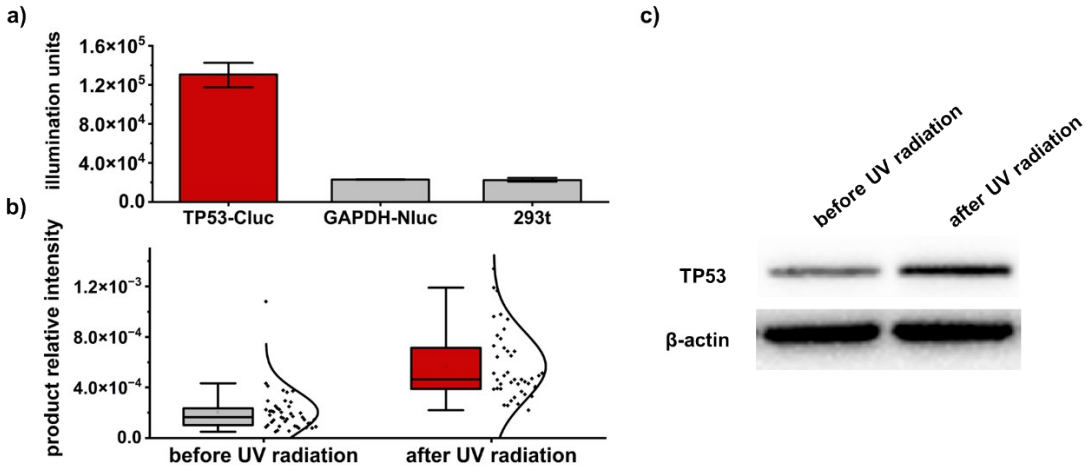

Figure S6. Validation of quantification capability for TP53. (a) Illumination strength comparison among TP53-Cluc, GAPDH-Nluc and 293t cell line when incubated with substrate cypridina luciferin. (n = 3 independent experiments; error bars: mean ± s.d.). Quantification validation using UV-radiation model with (b) single cell mass cytometry and (c) Western Blot assay. It is reported that the expression level of TP53 will increase upon UV irradiation[1].

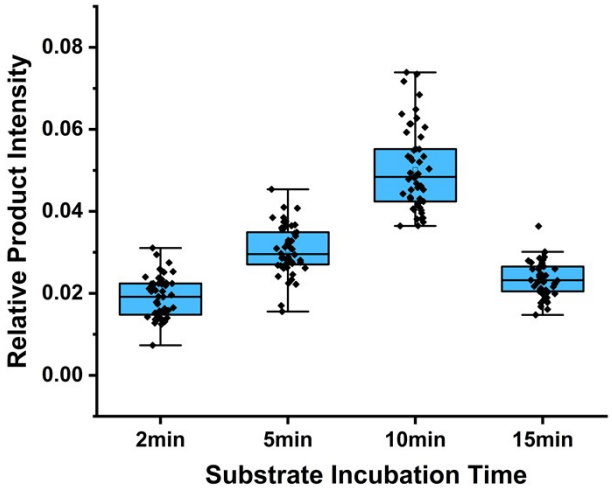

Figure S7. Substrate incubation time optimization result. Product relative intensity reach peak at 10min reaction time. (cell numbers = 200, black lines indicate means)

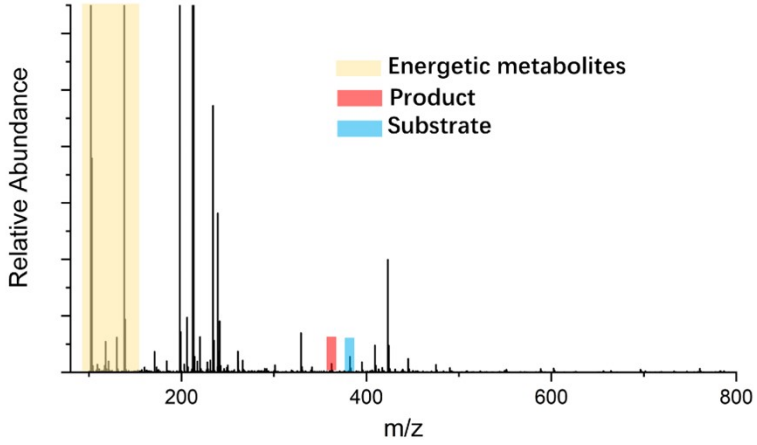

Figure S8. Representative singe-cell cellular MS spectrum in negative mode (mass range 80-800). MS peak of energetic metabolites including pyruvic acid (m/z 87.0088), lactic acid (m/z 89.0239) were highlighted with pale yellow. MS peak of enzymatic substrate and product were highlight with red and blue, respectively.

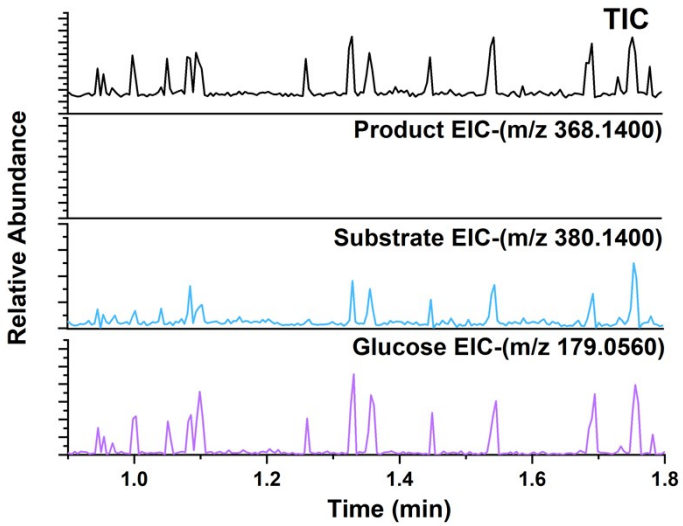

Figure S9. Mass cytometry results of WT293t cells in negative mode with substrate incubation. No obvious EIC signal of product was detected.

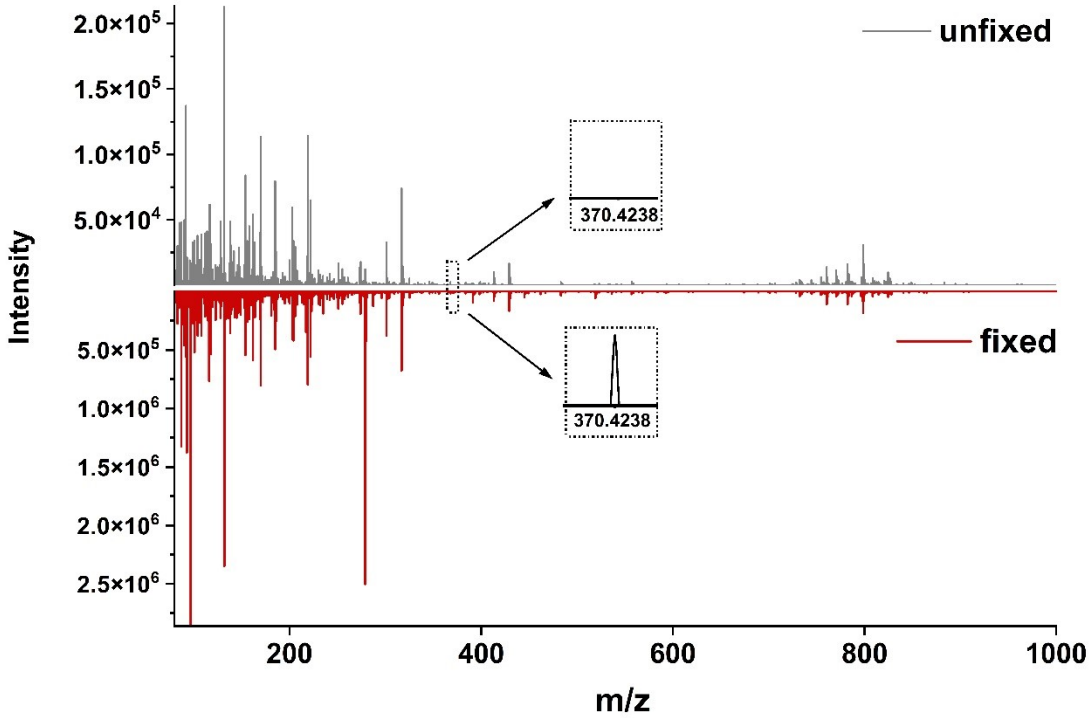

Figure S10. Mass spectrum comparison of single cell events between unfixed cell and fixed cells (mass range: 80-1000). m/z 370.4238 indicates enzymatic product.

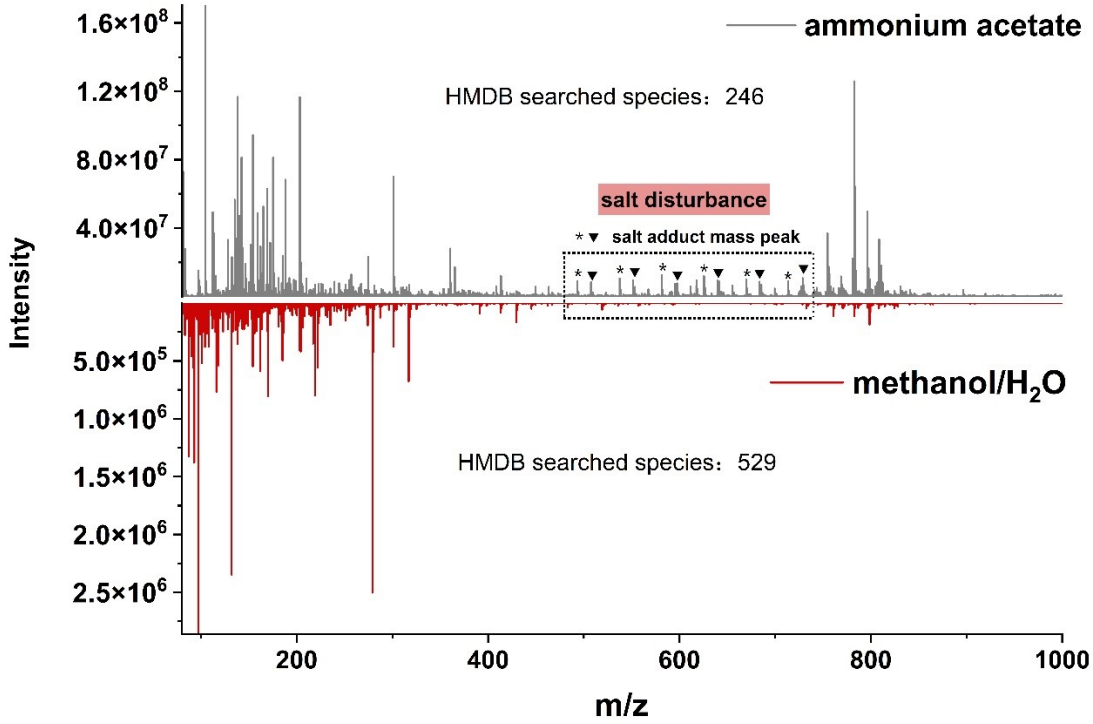

Figure S11. Mass spectrum comparison of single cell events in different solvents. (ammonium acetate and methanol/H_2O) (mass range: 80-1000).

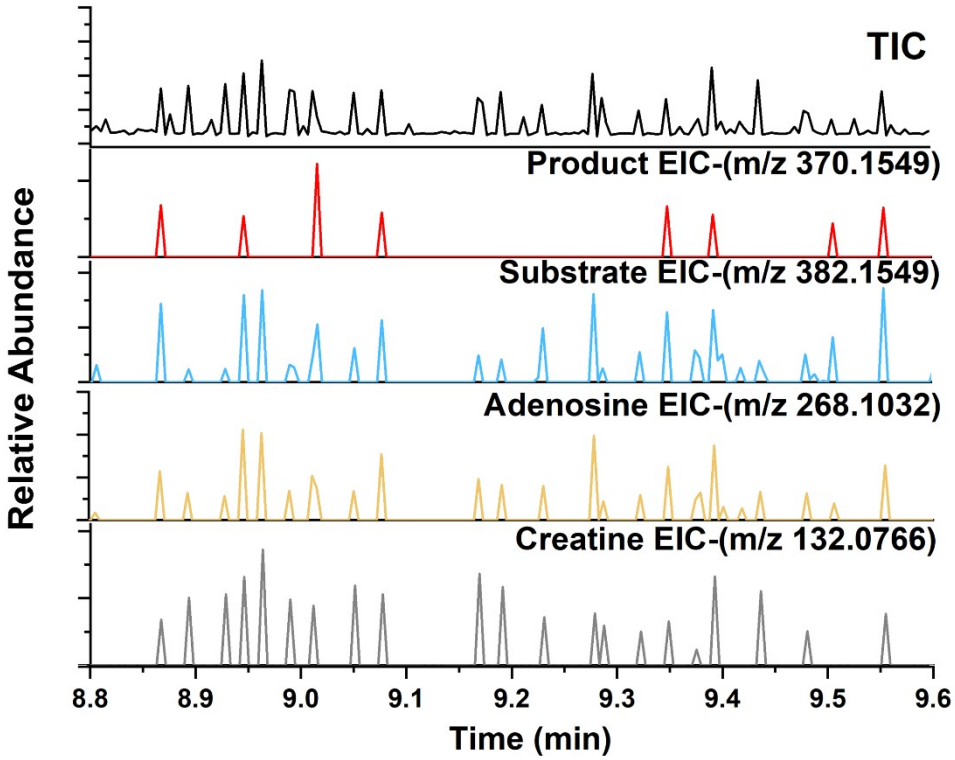

Figure S12. TIC and EICs of metabolites in Nluc knock-in cells in positive mode. Endogenous metabolites adenosine and creatine were detected in single cells.

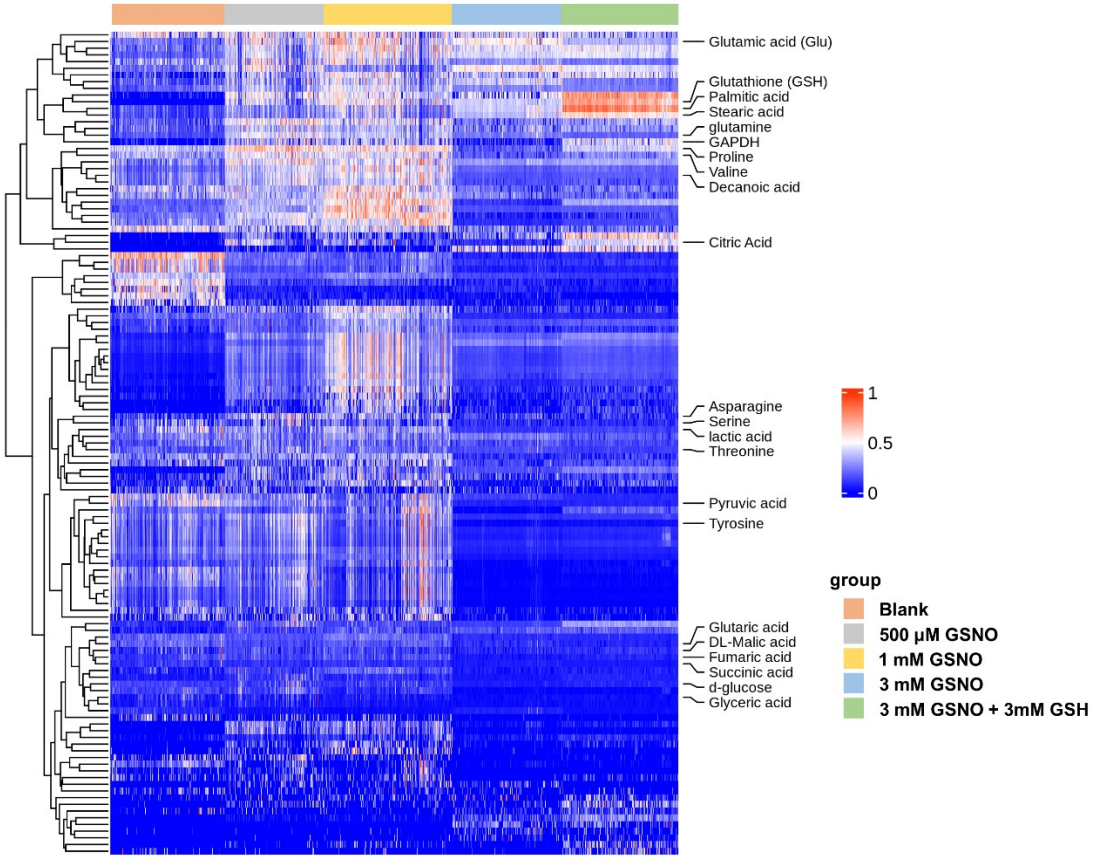

Figure S13. Heat maps of metabolites in single cells under different concentrations of GSNO and GSH (cell numbers = 250).

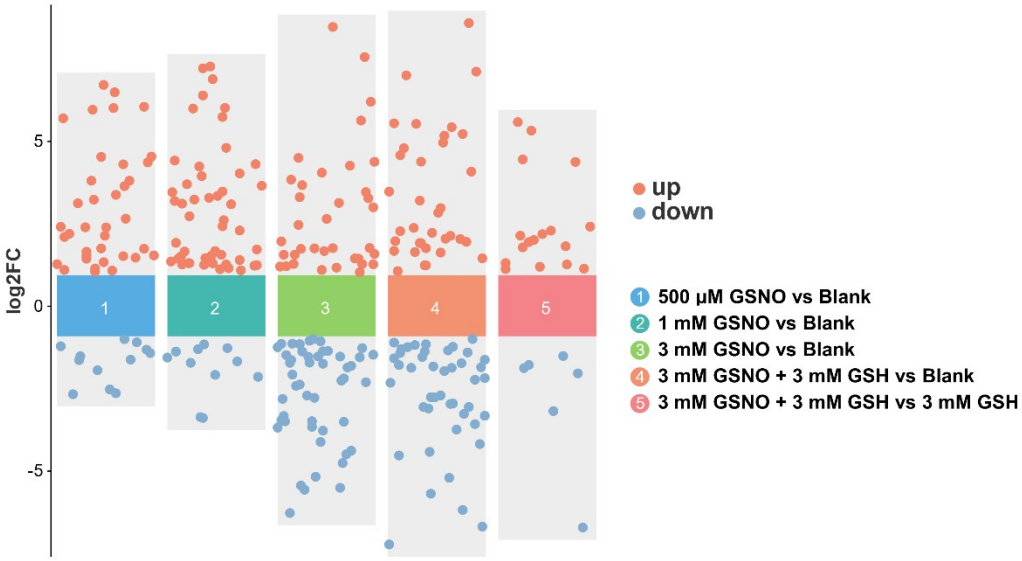

Figure S14. Volcano plot of the P-values and fold changes (FC) of normalized intensities for metabolite signals between different samples; (T test, P value <0.05, -log10 transformation was used for P values; | FC | >2). Dots represent identified metabolites, with up-regulated metabolites marked as orange and down-regulated metabolites marked as blue.

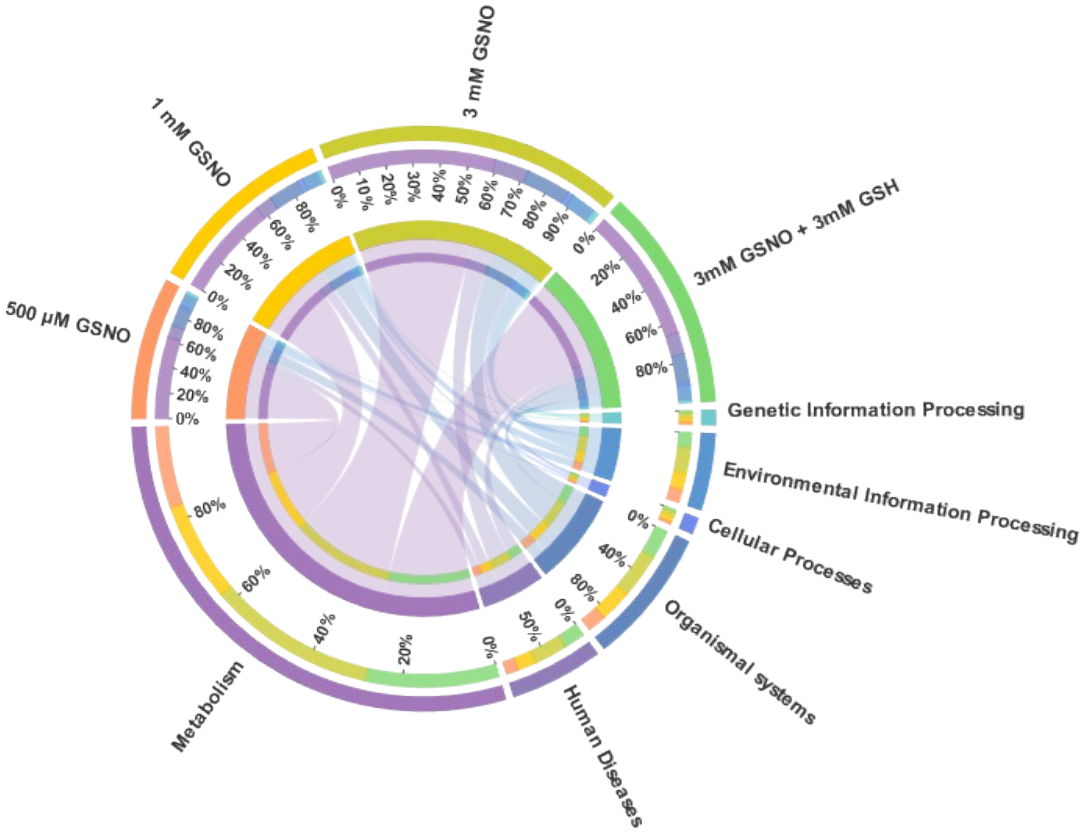

Figure S15. Circus plot of differential metabolites compared with blank group. With the increase of oxidative stress, the number of differential metabolites species gradually increases, which cause the most important effect on the metabolic activity of the cell.

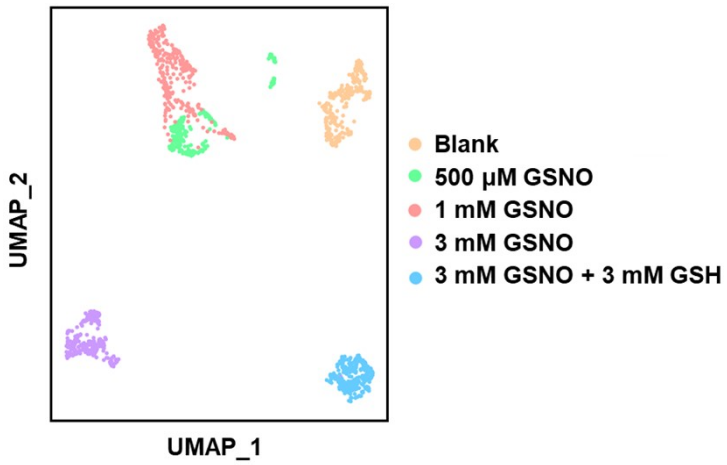

Figure S16. UMAP visualization of single cells under different oxidative stress (cell numbers = 250).

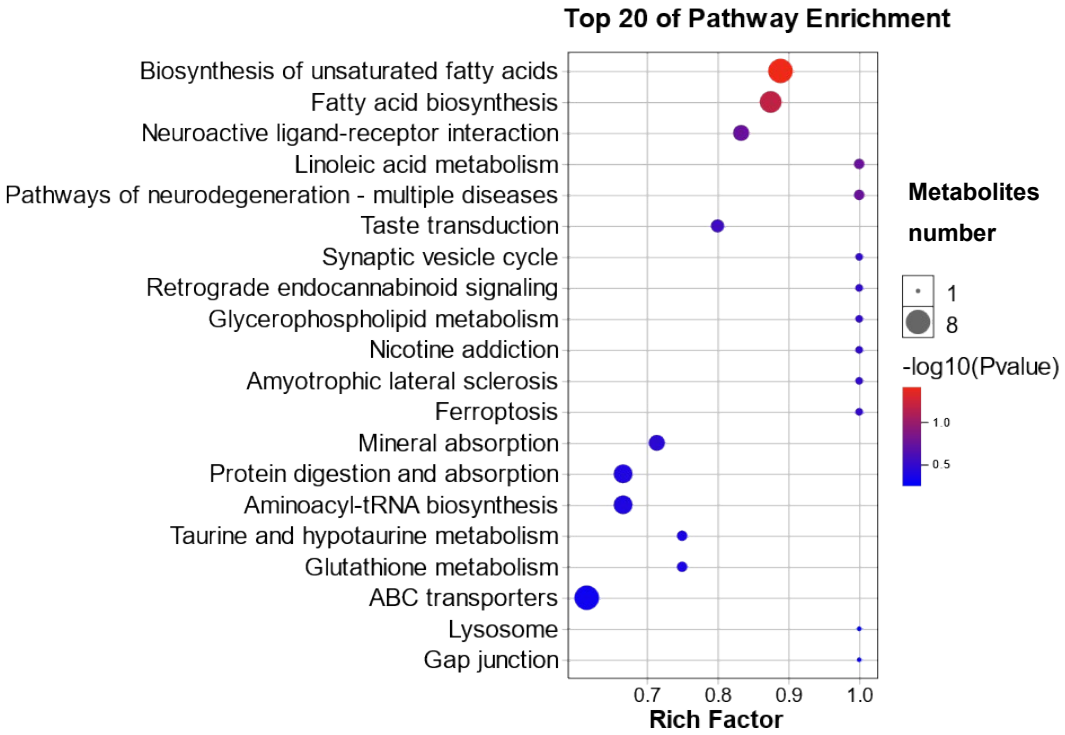

Figure S17. KEGG enrichment pathway analysis. Relevant pathways of fatty acids metabolism were most significantly affected

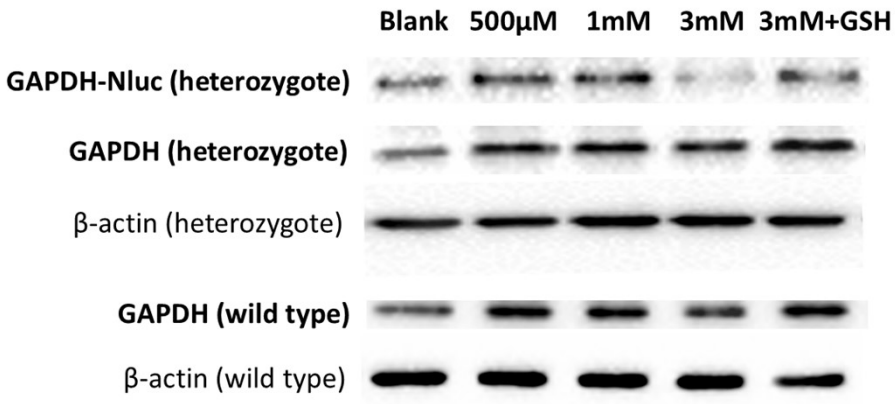

Figure S18. Western Blot results of GAPDH expression level in edited cell line and wt293t cell line.

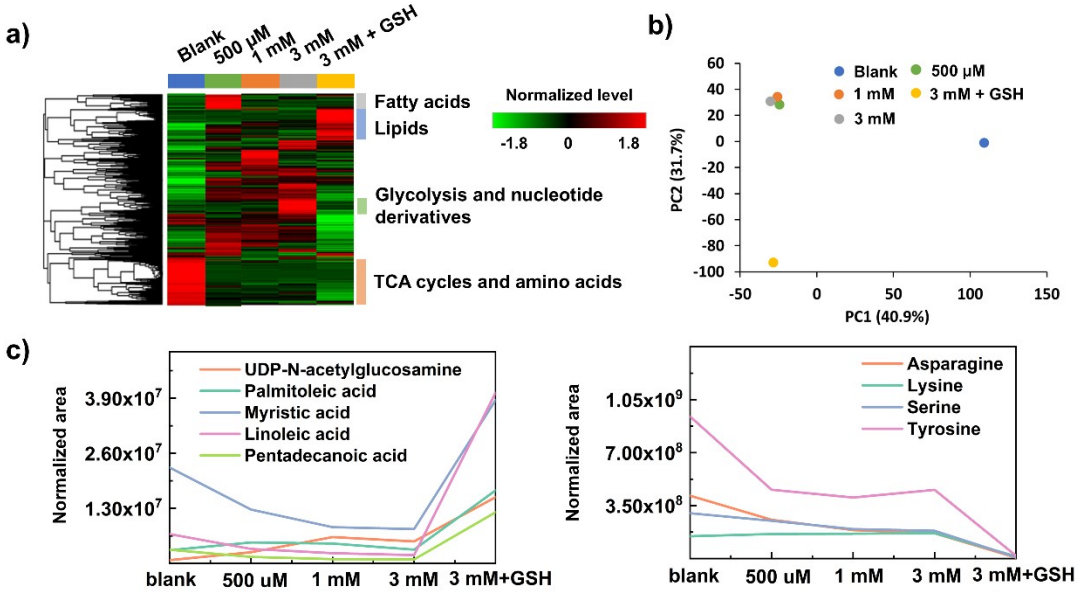

Figure S19. LC-MS/MS validation assay. a) heat map analysis (relevant metabolites are annotated) b) PCA analysis among all samples. c) abundance change of partial metabolites among different samples. Metabolites abundance was normalized by internal standards intensity.

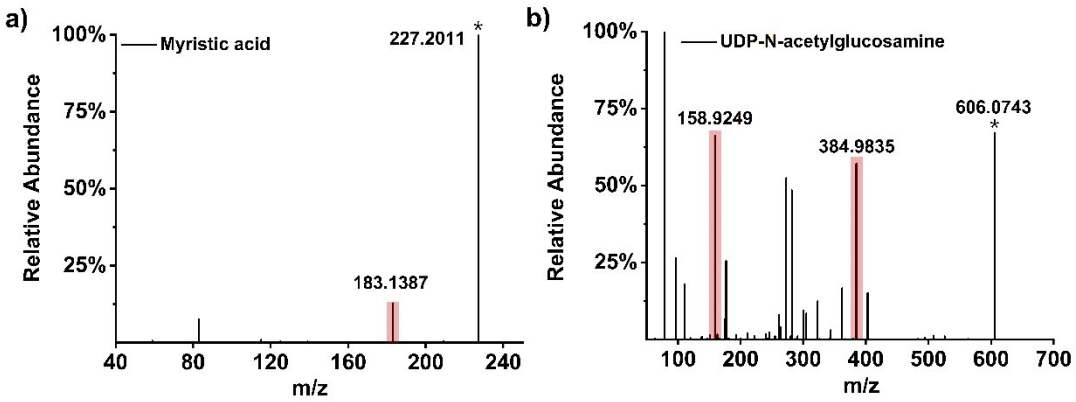

Figure S20. Representative MS/MS spectrum of metabolites a) myristic acid and b) UDP-N-acetylglucosamine obtained from LC-MS/MS validation. (Asterisk indicates molecular ion peak, and MS/MS fragments are highlighted)

Table S1. Sequences of oligonucleotides used in the work

sgRNA design (5’ to 3’)	
sgRNA-1	GGTCCAGGGGTCTTACTCCT
sgRNA-2	CCAGGGGTCTTACTCCTTGG
sgRNA-3	CTTACTCCTTGGAGGCCATG
sgRNA-4	GGTGGACCTCATGGCCCACA
sgRNA-5	CATGGCCCACATGGCCTCCA

Primers for T7E1 assay (5’ to 3’)	
Primer-fw	AGAAGACTGTGGATGGCCCCT
Primer-Rv	GACACAAGCCCAGCTTCCCT

Primers for PCR verification (5’ to 3’)	
Primer-fw	GTCATCCCTGAGCTGAACGG
Primer-Rv	CGCTCAGACCTTCATACGGG

Primers for sequencing verification (5’ to 3’)	
Primer-fw	CCTCAACGACCACTTTGTCAA
Primer-Rv	AGTGAGGGTCTCTCTCTTCC

Insertion sequence (5’ to 3’)	
3xGGGGS linker + Nluc	GGTGGCGGAGGCTCGGGCGGAGGTGGGTCGGGTGGCGGCG GATCAATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGC GACAGACAGCCGGCTACAACCTGGACCAAGTCCTTGAACAGG GAGGTGTGTCCAGTTTGTTTCAGAATCTCGGGGTGTCCGTAAC TCCGATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAA GATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGG CGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTAC CCTGTGGATGATCATCACTTTAAGGTGATCCTGCACTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACATGATCGACTATTT CGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAA GATCACTGTAACAGGGACCCTGTGGAACGGCAACAAAATTATC GACGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGA GTAACCATCAACGGAGTGACCGGCTGGCGGCTGTGCGAACG CATTCTGGCGTAA

Primers for donor (5’ to 3’)	
sgRNA-1 donor Fw	AACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGG AGGGTGGCGGAGGCTCGGGC
sgRNA-1 donor Rv	CTTGTGCTCTTGCTGGGGCTGGTGGTCCAGGGGTCTTACTT TACGCCAGAATGCGTTCGC
sgRNA-2 donor Fw	AGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCA AGGGTGGCGGAGGCTCGGGC
sgRNA-2 donor Rv	GTGCTCTTGCTGGGGCTGGTGGTCCAGGGGTCTTACTCCT TTACGCCAGAATGCGTTCGC

Primers for qPCR quantification assay (5’ to 3’)	
Primer-fw	GATTTGGTCGTATTGGGCGC
Primer-Rv	TTCCCGTTCTCAGCCTTGAC

Table S2. Sequencing result of Nluc knock-in in 293t cell line.

TTCTAGGTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCC
ACATGGCCTCCAAGGAGGGTGGCGGAGGCTCGGGCGGAGGTGGGTCGGGTGGCG
GCGGATCAATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGCGACAGACAGCC
GGCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGTTTCAGAA
TCTCGGGGTGTCCGTAACTCCGATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGC
TGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGGCGACCAAATG
GGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAG
GTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGATCGA
CTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCAACCCC
GACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCTGT
GCGAACGCATTCTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATC
CGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGT
GCCTAATGAGTGAGCTAACTCACAT

(Nluc encoding sequence highlighted with blue color, GGGGS linker highlighted with green color.)

Table S3. Metabolites list of closely related to GAPDH

Metabolites classification	Name	m/z	MS/MS fragments
Amino acids derivates	Taurine	124.0069	79.9558, 106.9298
	Phenylalanine	164.0712	146.9916, 114.9516
	DL-α-Methoxyphenylacetic acid	165.0552	105.0346, 91.0553
Fatty acids	Caffeic acid	179.0344	135.0519, 79.0552
	Citric Acid	191.0192	172.9930, 110.8860
	Myristic acid	227.2011	183.1387
	Pentadecanoic acid	241.2168	197.1541
	Palmitoleic acid	253.2169	235.2069
	Linoleic acid	279.2324	235.1693
	13(S)-HOTrE	293.2117	61.9885
	(+/-)13-HODE	295.2276	59.0133
	11(Z),14(Z)-Eicosadienoic acid	307.2645	199.1715
	(+/-)9-HpODE	311.2228	183.0125
	(+/-)5(6)-DiHET	335.2222	71.0866
Energetic related	ADP	426.0213	158.9240, 134.0468
Glycosylation related	UDP-N-acetylglucosamine	606.0743	78.9587, 158.9249

References:

[1] aS. Parikh, R. Parikh, K. Michael, L. Bikovski, G. Barnabas, M. Mardamshina, R. Hemi, P. Manich, N. Goldstein, H. Malcov-Brog, T. Ben-Dov, O. Glaich, D. Liber, Y. Bornstein, K. Goltseker, R. Ben-Bezalel, M. Pavlovsky, T. Golan, L. Spitzer, H. Matz, P. Gonen, R. Percik, L. Leibou, T. Perluk, G. Ast, J. Frand, R. Brenner, T. Ziv, M. Khaled, S. Ben-Eliyahu, S. Barak, O. Karnieli-Miller, E. Levin, Y. Gepner, R. Weiss, P. Pfluger, A. Weller, C. Levy, *Nature Metabolism* **2022**, *4*, 883-900; bA. Nigro, L. Mauro, F. Giordano, S. Panza, R. Iannacone, G. M. Liuzzi, S. Aquila, F. De Amicis, F. Cellini, C. Indiveri, M. L. Panno, *Molecular Cancer Therapeutics* **2016**, *15*, 1063-1073.