Supplementary Information

for

Simultaneous Multi-Omics Analysis by Direct Infusion Mass Spectrometry (SMAD-MS)

Yuming Jiang[1,2,3]*, Ivan Salladay-Perez[4], Amanda Momenzadeh[1,2,3], Anthony J. Covarrubias[4], Jesse G. Meyer[1,2,3]*

[1]Department of Computational Biomedicine, Cedars Sinai Medical Center, Los Angeles, CA 90048, USA.

[2]Advanced Clinical Biosystems Research Institute, Cedars Sinai Medical Center, Los Angeles, CA 90048, USA.

[3]Smidt Heart Institute, Cedars Sinai Medical Center, Los Angeles, CA 90048, USA.

[4]Department of Molecular Biology, Immunology, and Molecular Genetics, University of California, Los Angeles, 90095, USA.

*Correspondence to Yuming Jiang (yuming.jiang@cshs.org) or Jesse G. Meyer (jesse.meyer@cshs.org).

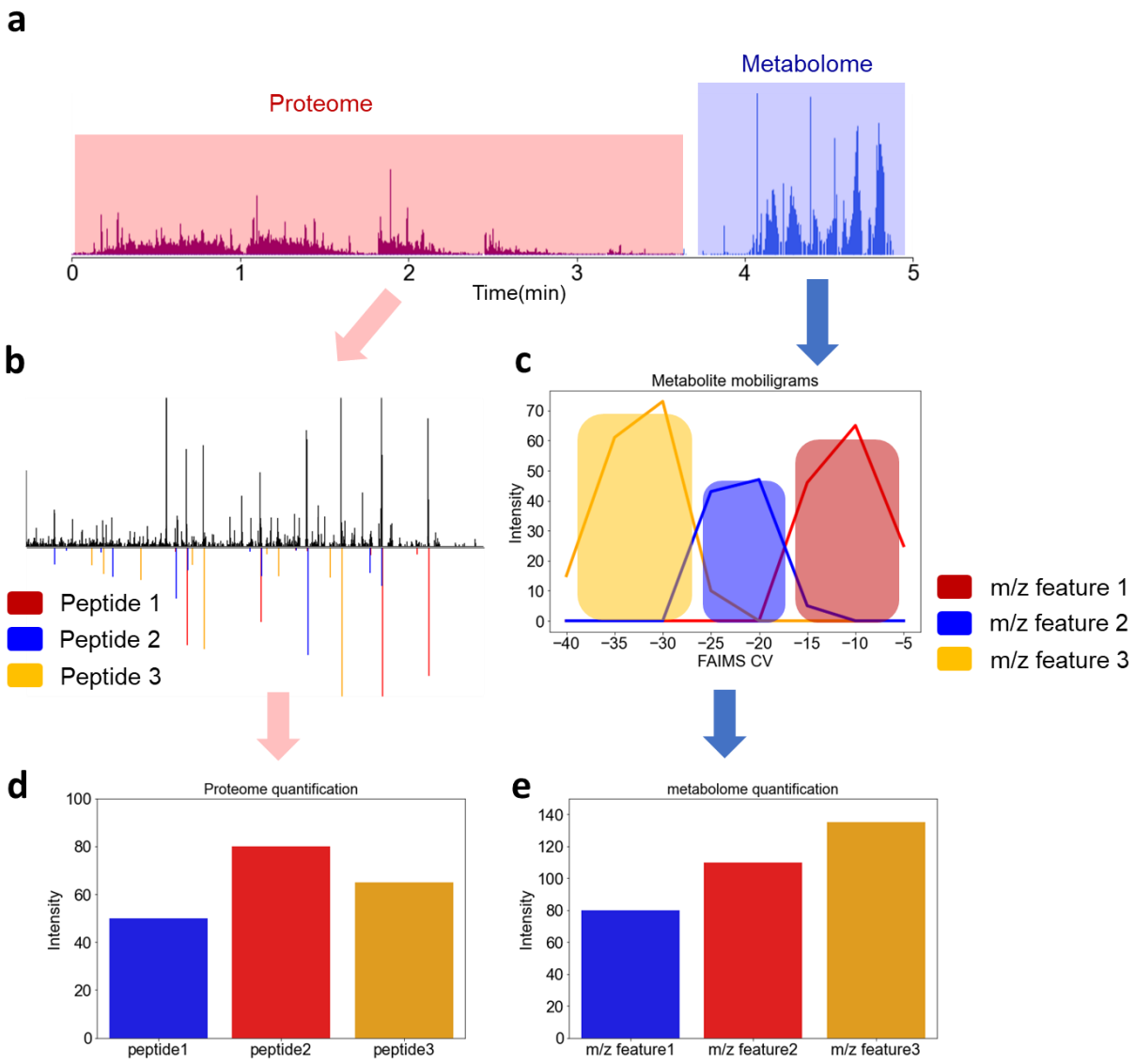

Figure S1. Quantitative and identification schematic diagram of SMAD. a, Typical TIC figure of SMAD. **b,** identification of peptides from tandem mass spectra by spectra-to-spectra match. **c,** extracting intensities with same m/z across all FAIMS compensation voltages and calculate ion mobiligrams (XIMs) for quantification. **d,** Quantification of proteome by summing up all fragment intensities of a specific peptide. **e,** Quantification of metabolome by extracted ion mobiligrams (XIMs) of a specific *m/z* feature.

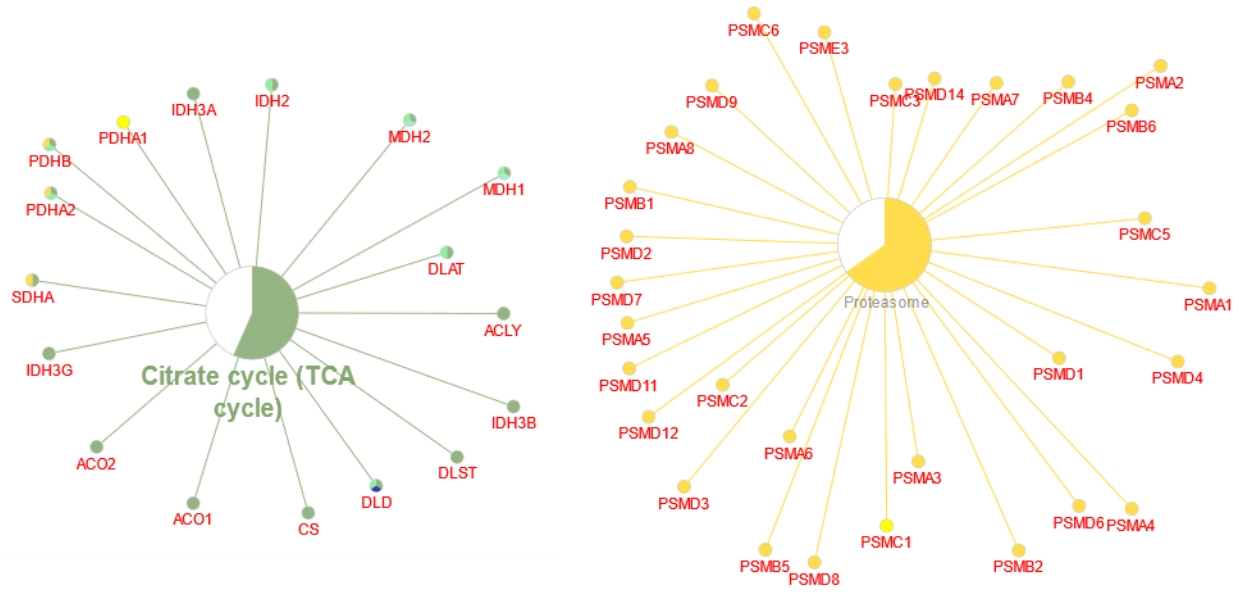

Figure S2. Enriched KEGG pathways including all protein members identified by SMAD of two specific pathways with more than 50% coverage (matching Fig. 2c). Pathway enrichment analysis was done in Cytoscape with the plugin clueGO. Colored portion of the circle gives the proportion of proteins in that pathway that were identified.

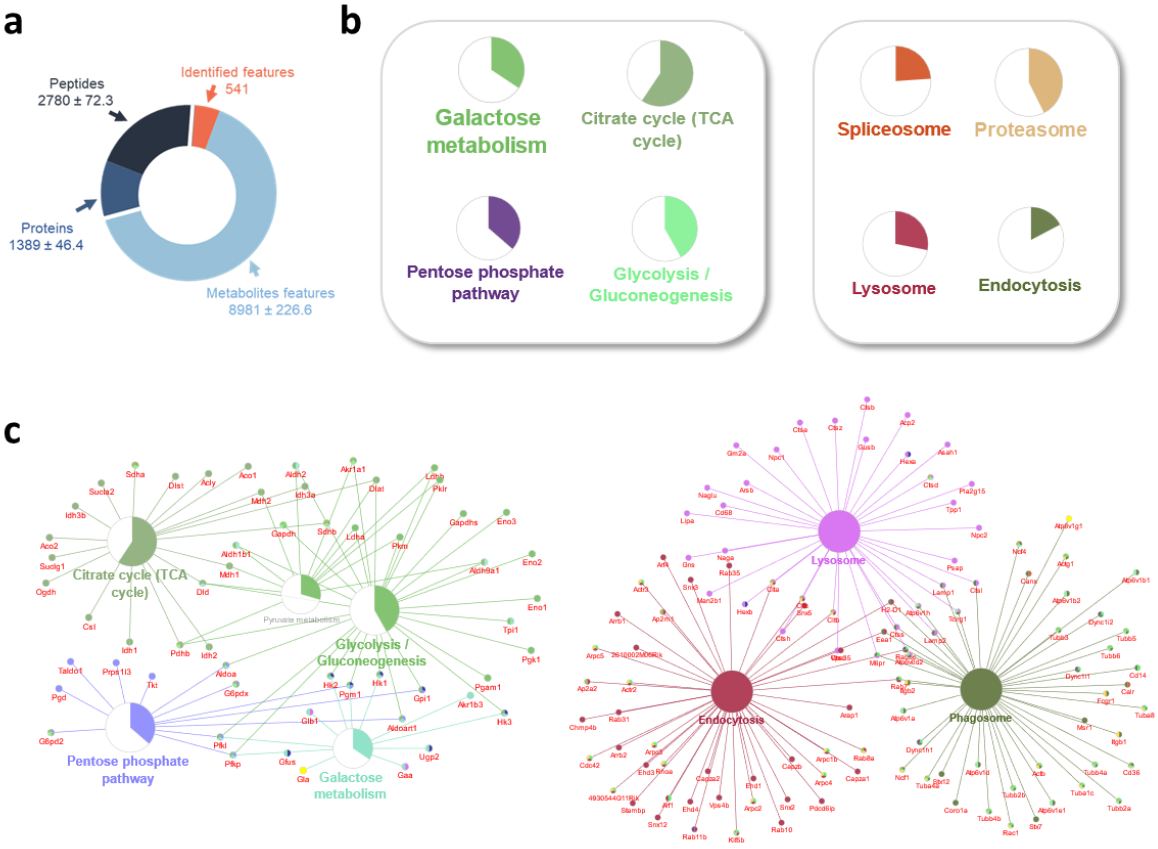

Figure S3. Performance of SMAD in macrophages. a, Number of detected metabolite features, peptides, and proteins of macrophages by SMAD. **b,** KEGG pathway enrichment analysis of the proteins identified from macrophages by SMAD. The bars indicate how many proteins in the pathway were identified, and the colored proportion of the circle reflects the coverage of the proteins in each pathway. **c,** Proteins related to typical KEGG pathways identified by SMAD from macrophages.

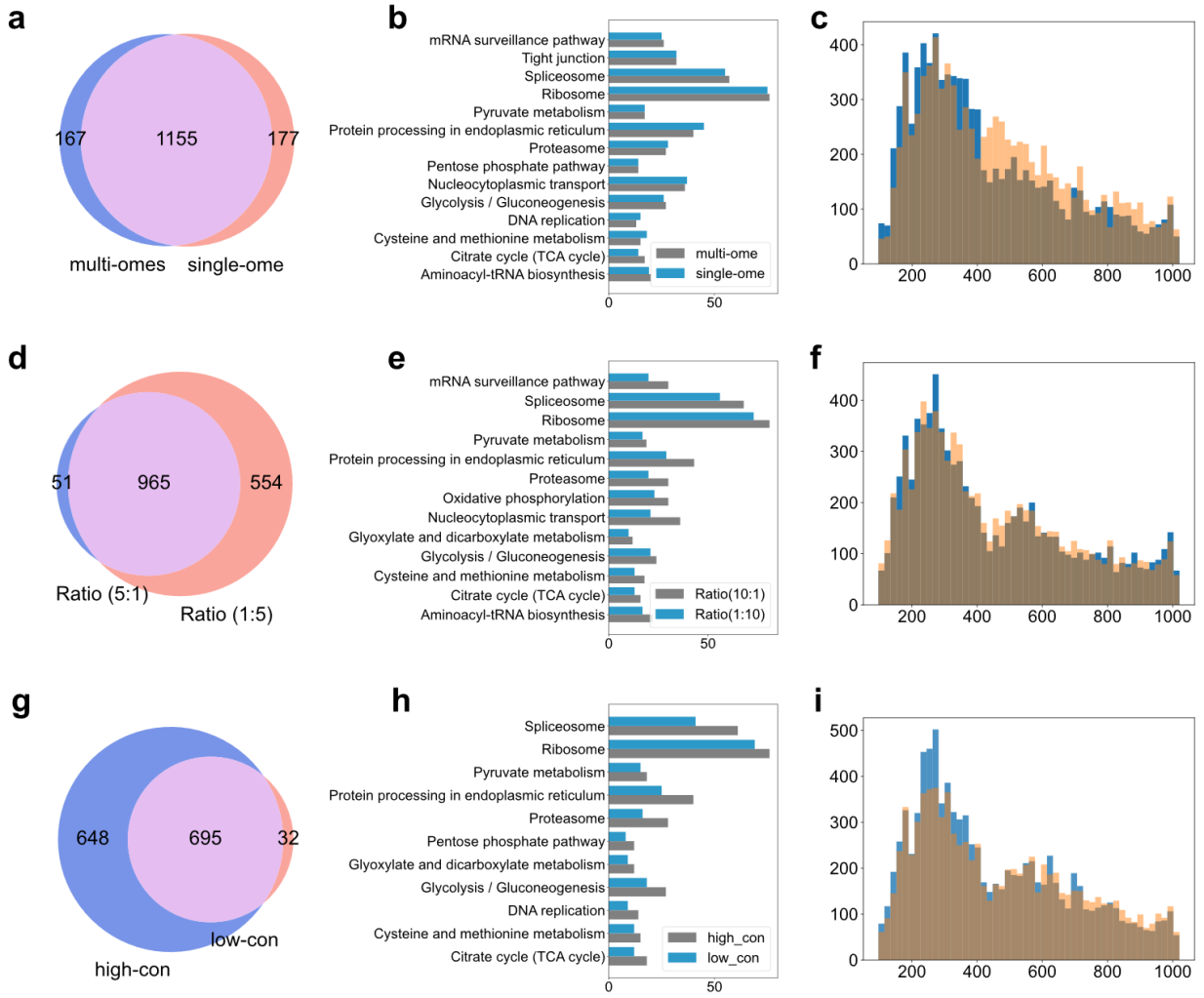

Figure S4. Comparison of protein species, enriched pathways, and m/z distribution between different conditions. **a,** Venn diagram showing the overlap of protein species between the multi-omics method and single-omic methods. **b,** KEGG enriched pathways showing that basically no difference between multi-omics method and single-omic method. **c,** comparison of m/z distribution of detected metabolite features between multi-omics method and single-omic method. **d,** Venn diagram showing the overlap of protein species between high metabolome/proteome ratio (5:1) and low metabolome/proteome ratio (1:5). **e,** KEGG enriched pathways showing that the differences between high metabolome/proteome ratio (:1) and low metabolome/proteome ratio (1:5). **f,** comparison of m/z distribution of detected metabolite features between high metabolome/proteome ratio (5:1) and low metabolome/proteome ratio (1:5). **g,** Venn diagram showing the overlap of protein species between high sample concentration and low sample concentration. **h,** KEGG enriched pathways showing the differences between high sample concentration and low sample concentration. **i,** comparison of m/z distribution of detected metabolite features between high sample concentration and low sample concentration.

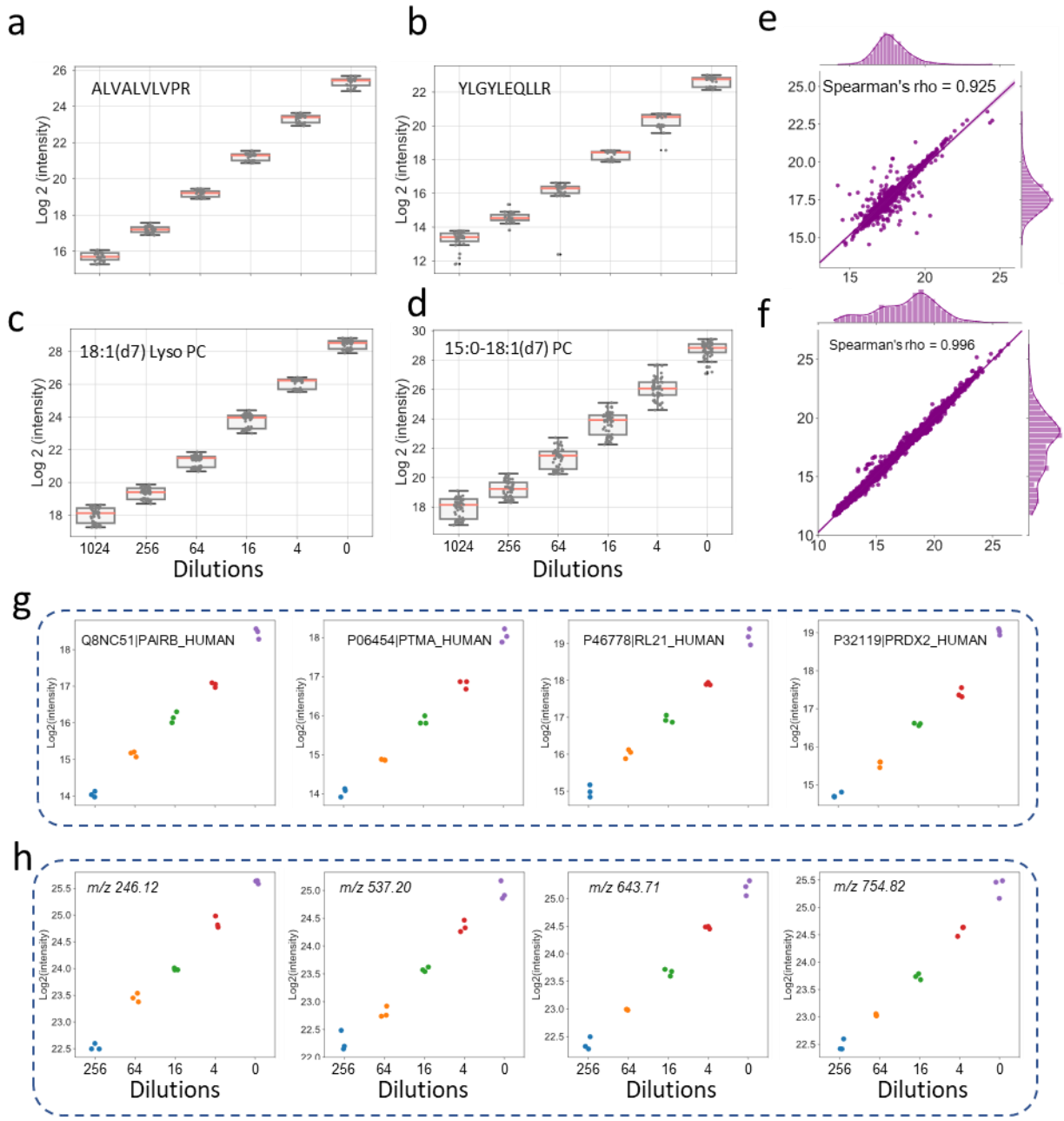

Figure S5. Quantification assessment of SMAD with standards and real samples. a,b, label free quantification curve of two standard peptides from MS-QCAL protein spiked in real samples. (each ratio was measured in triplicate). **c,d,** label free quantification curve of two standard lipids from Avanti spiked in real samples. (each ratio was measured in triplicate). **e,f,** Scatterplot of peptide (e) and metabolite feature intensities (f) quantified by SMAD from two injections of multi-omic samples of 293T cells. **g,** examples of typical quantified proteins from real samples. h, examples of typical quantified metabolite features from real samples.

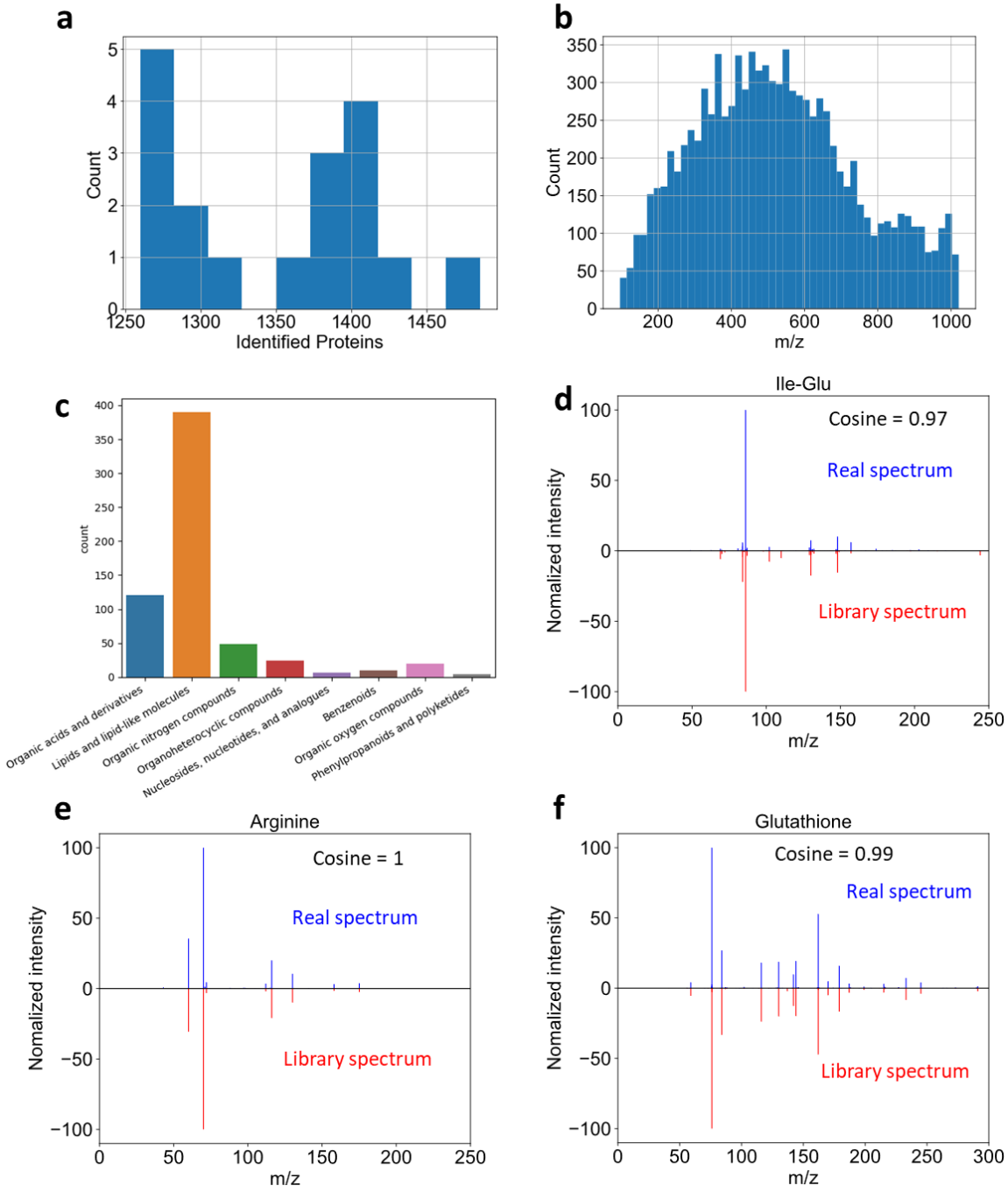

Figure S6. Macrophage dataset evaluation and metabolites identification. a, Histogram of identified proteins in each injection including all treatments and replicates. **b,** Histogram of m/z distribution of all detected m/z features in macrophages. **c,** class of identified metabolite features in macrophages. **d,e,f,** Tandem mass spectra matching plot for typical metabolites including Ile-Glu(d), Arginine(e) and Glutathione(f).

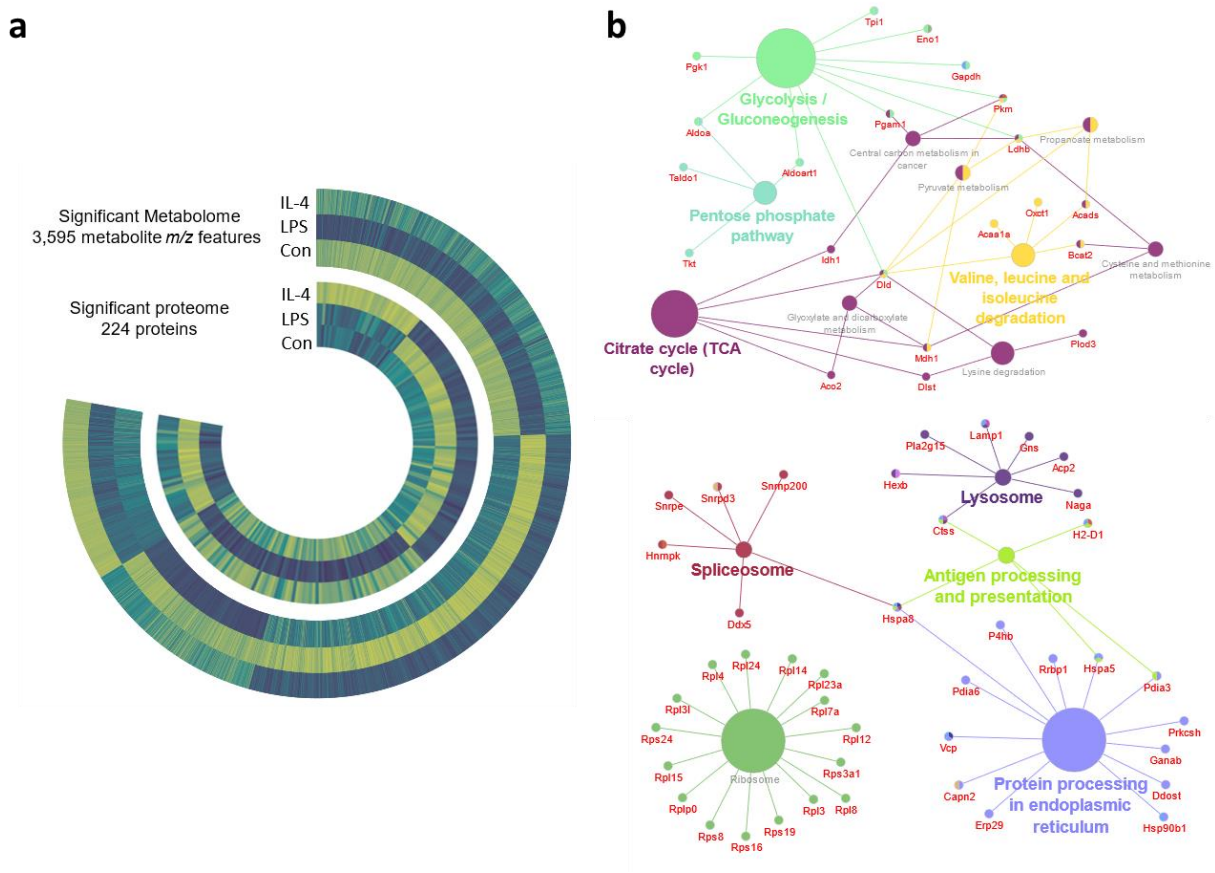

Figure S7. Significantly dysregulated molecules and related pathway analysis of macrophage polarization study. **a,** Heatmap of all significant dysregulated proteins and metabolite m/z features. **b,** Proteins and typical KEGG pathways related to significant dysregulated proteins identified by SMAD of macrophage polarization study.

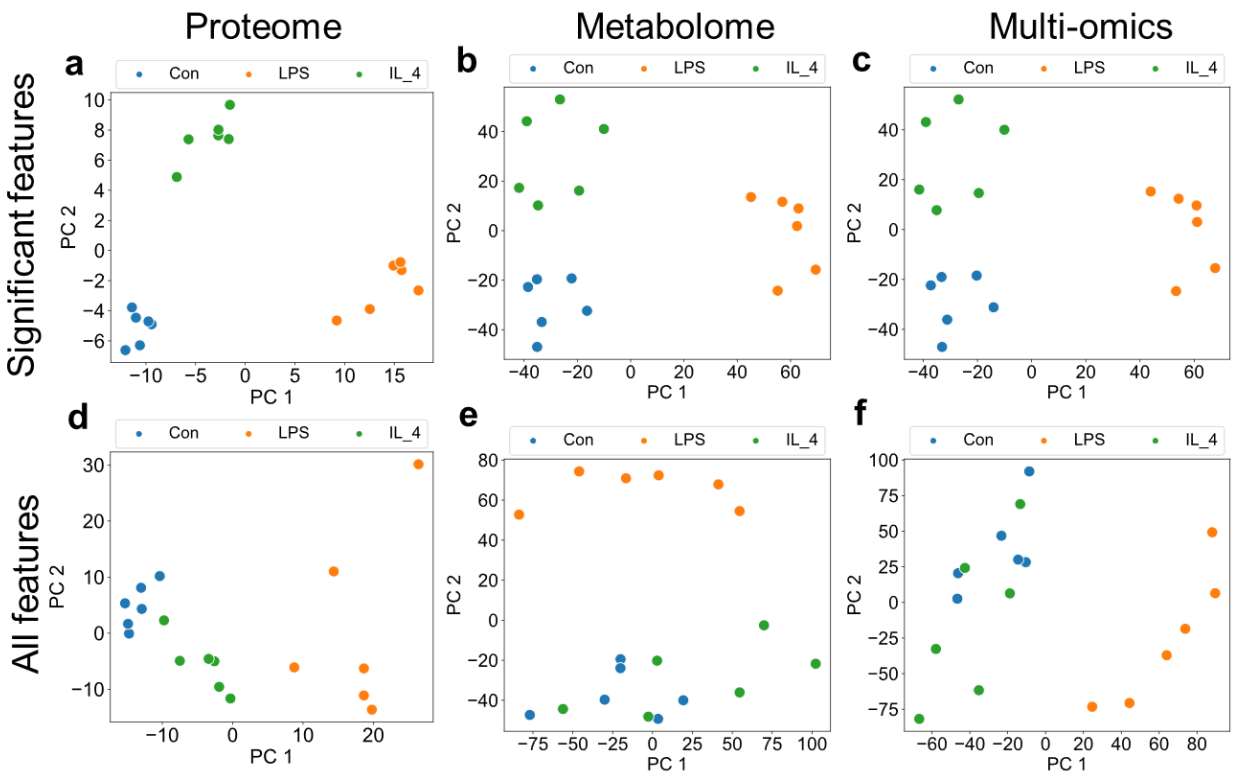

Figure S8. Principal component analysis (PCA) of multi-omics data from macrophage polarization study. a, b, c, scatter plot showing PCA results between treatments and control from significant changed proteins(a), metabolites(b) and together(c). **d,e,f,** scatter plot showing PCA results between treatments and control from all detected proteins(d), metabolites(e) and together(f).

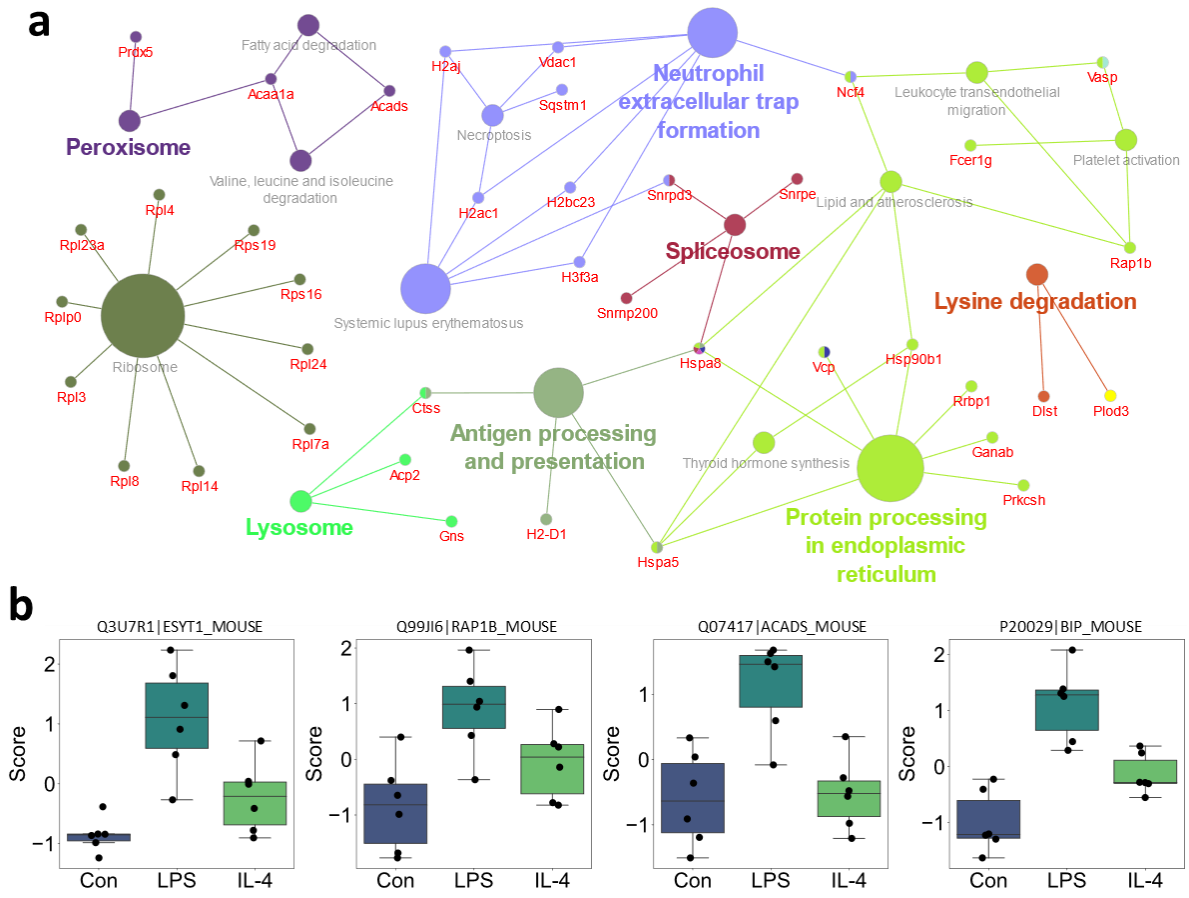

Figure S9. KEGG pathway analysis of cluster 2 (matching Fig. 3d) and typical proteins. **a,** KEGG pathway analysis results of Cluster 2. **b,** Four proteins related to cluster 2 which significantly upregulated in LPS.

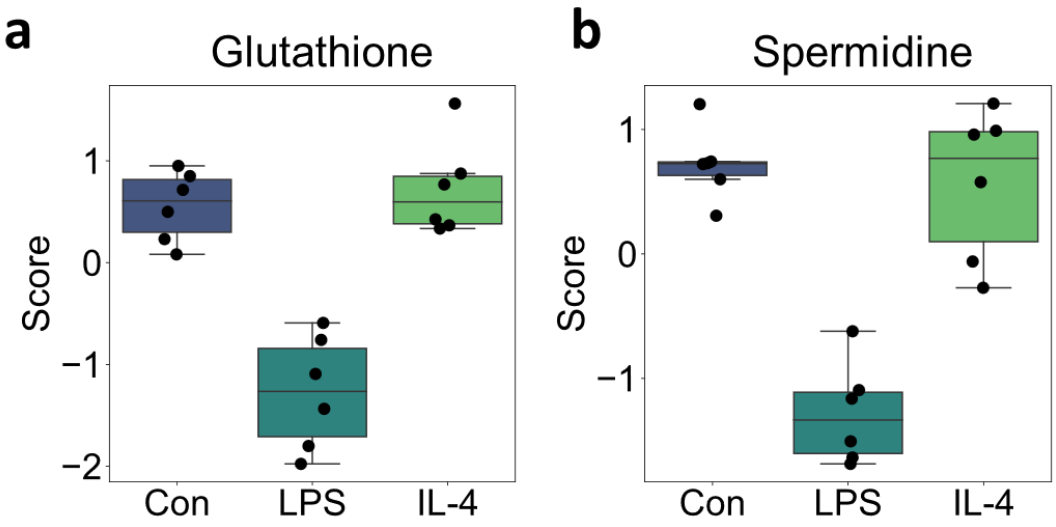

Figure S10. Specific metabolites variation after macrophage polarization. a, Boxplot of glutathione in all treatments and control. **b,** Boxplot of Spermidine in all treatments and control.

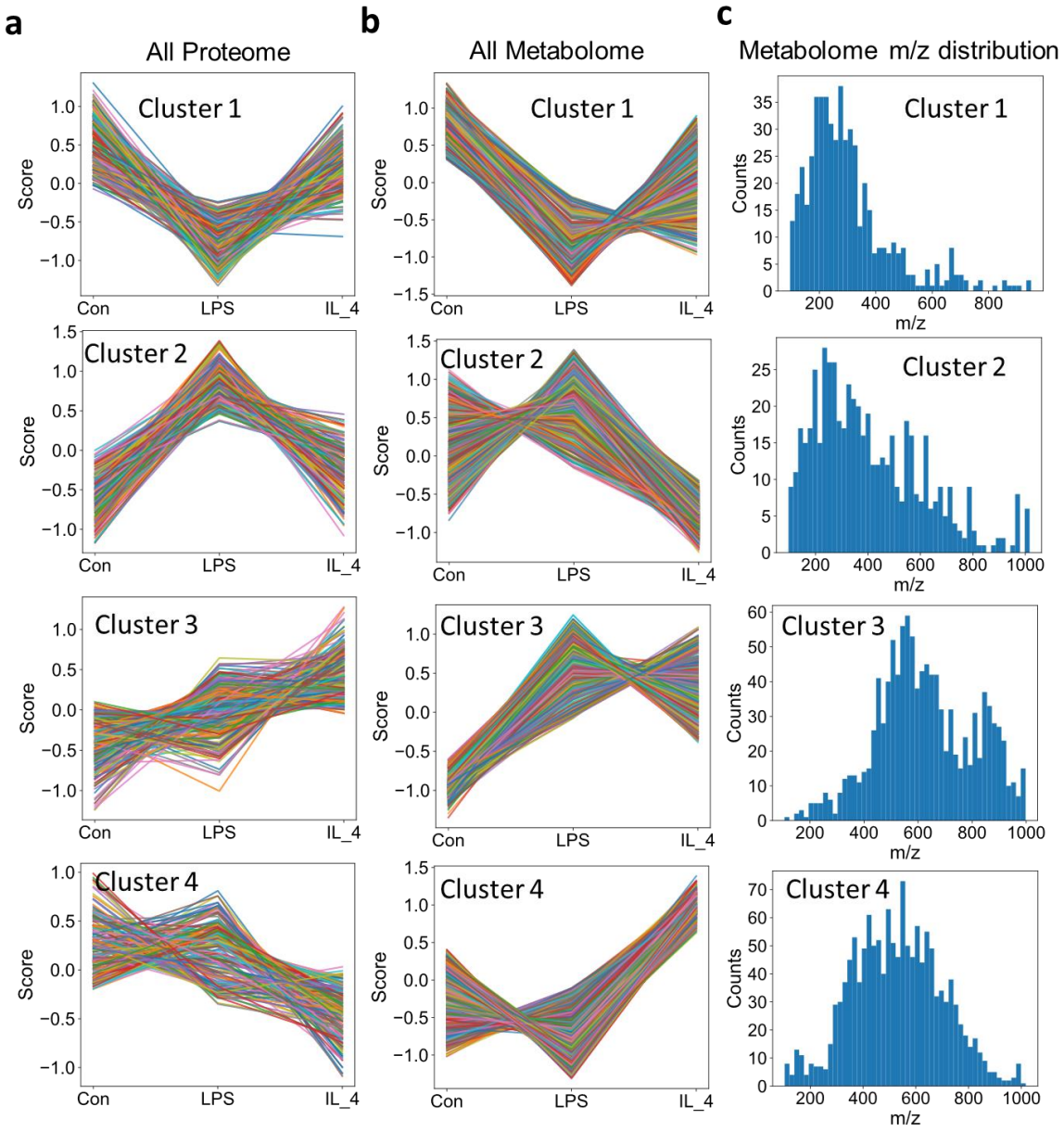

Figure S11. K-means clustering of all detected features in Macrophage study. a, four clusters of all identified proteins. **b,** four clusters of all detected metabolite m/z features. **c,** m/z distribution of metabolite features in each cluster reflect the potential metabolite class difference.

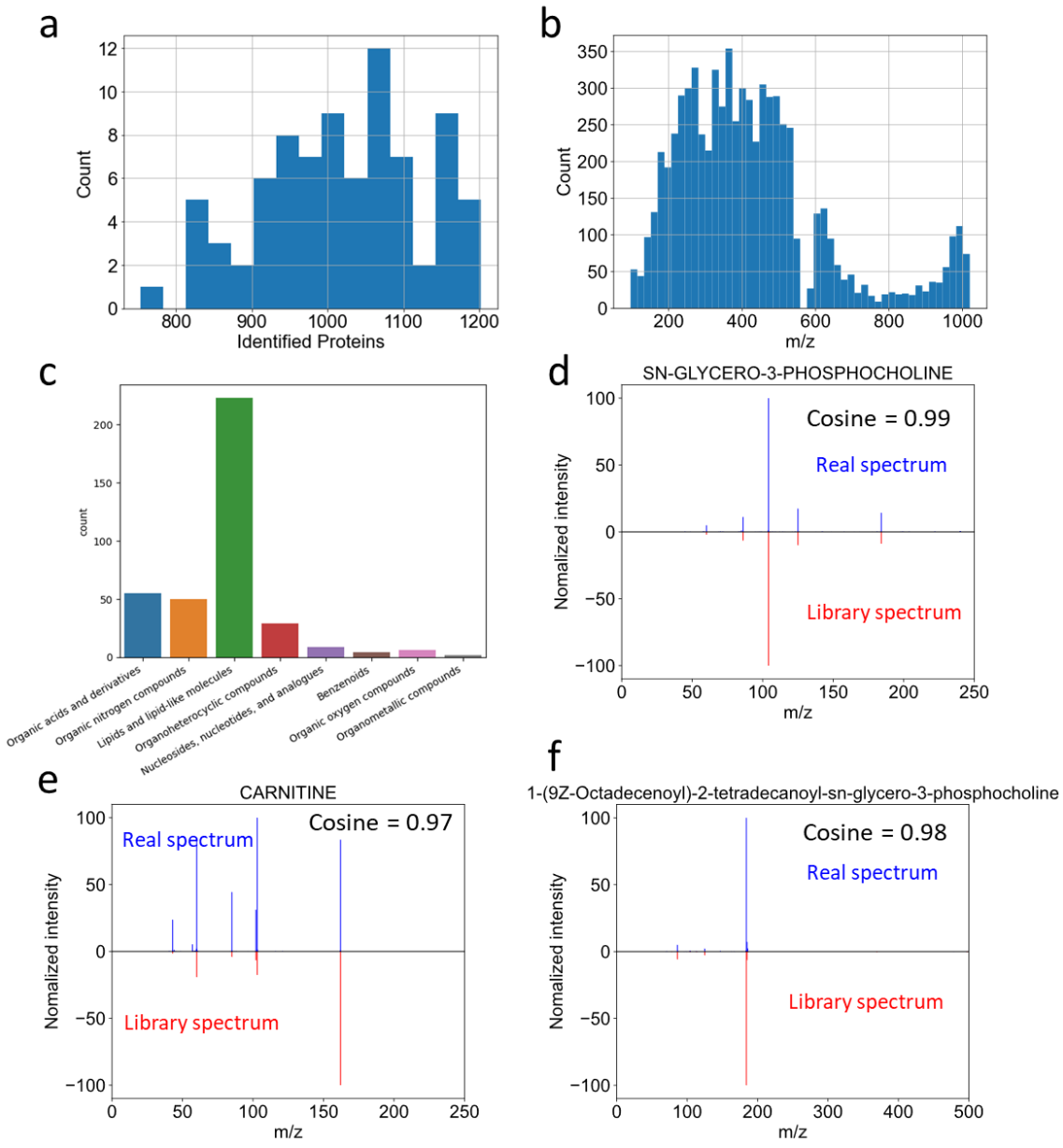

Figure S12. Drug screening study dataset evaluation and metabolites identification. a, Histogram of identified proteins in each injection including all drug treatments and replicates. **b,** Histogram of m/z distribution of all detected m/z features in 293T cells of drug screening study. **c,** Class of identified metabolite features in 293T cells. **d,e,f,** Tandem mass spectra matching plot for typical metabolites including SN-GLYCERO-3-PHOSPHOCHOLINE(d), CARNITINE(e) and 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine(f).

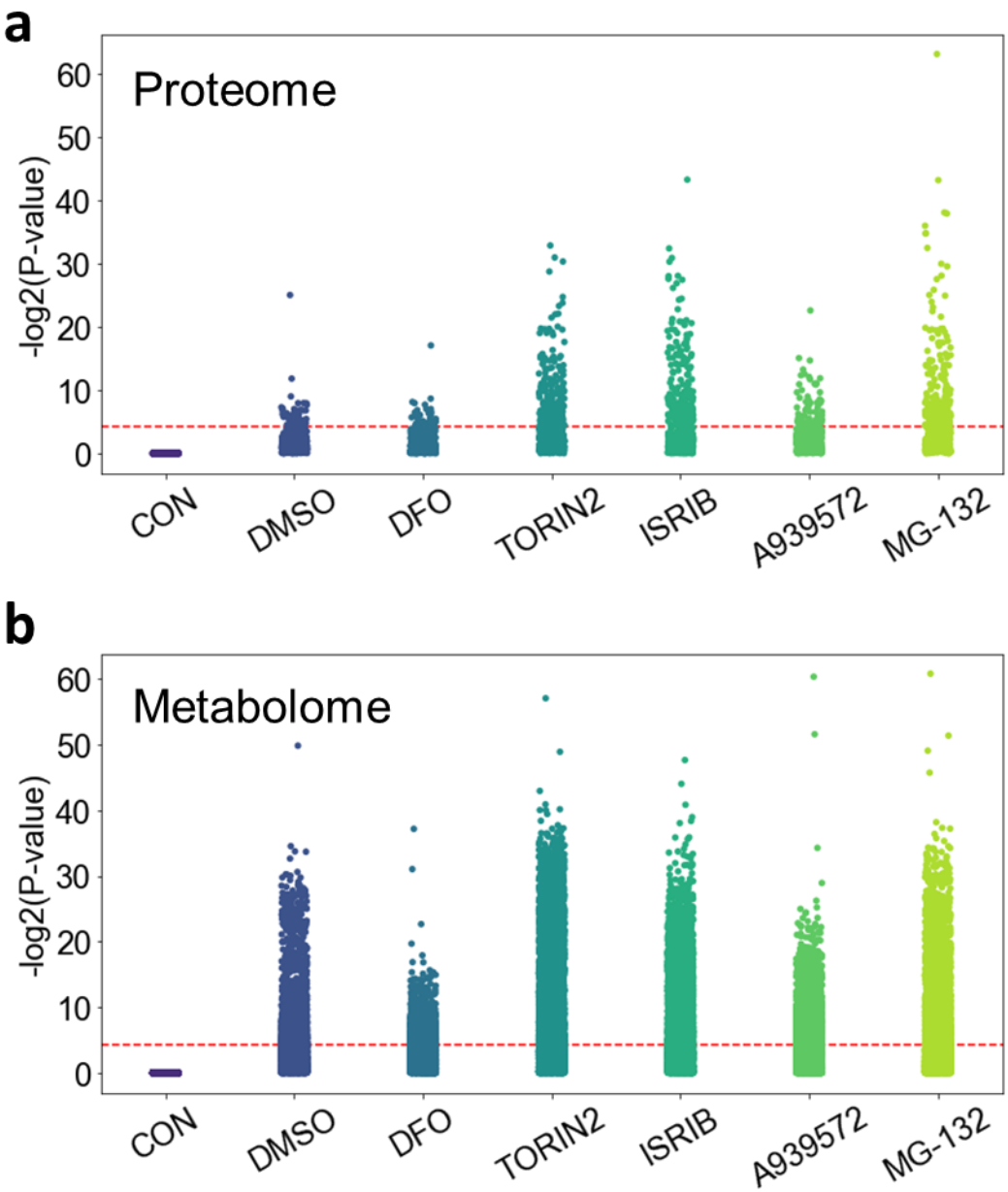

Figure S13. Significant dysregulated features between drug treatments and control. **a,** scatter plot showing significant changed proteins**.** Red line marks P-value = 0.05. **b,** scatter plot showing significant changed metabolites. Red line marks P-value = 0.05. Results come from T-TEST and after Benjamini–Hochberg (BH)-adjusted.

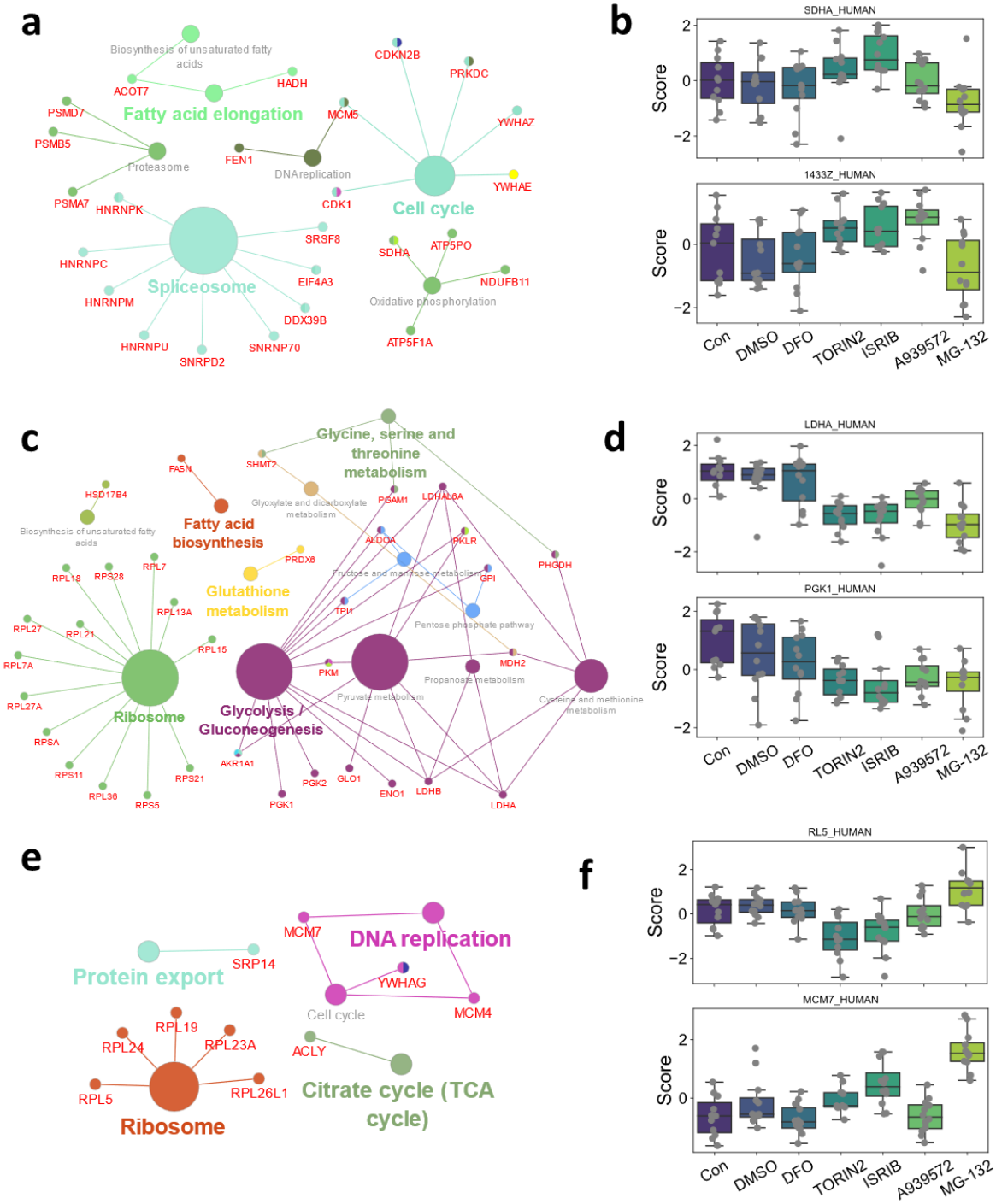

Figure S14. KEGG pathway analysis of different clusters and typical proteins. **a, b,** KEGG pathway analysis results of Cluster 2 and two typical proteins which significantly upregulated in TORIN2 and ISRIB. **c, d,** KEGG pathway analysis results of Cluster 1 and two typical proteins which significantly downregulated in TORIN2 and ISRIB. **e, f,** KEGG pathway analysis results of Cluster 3 and two typical proteins which significantly increased in MG-132.

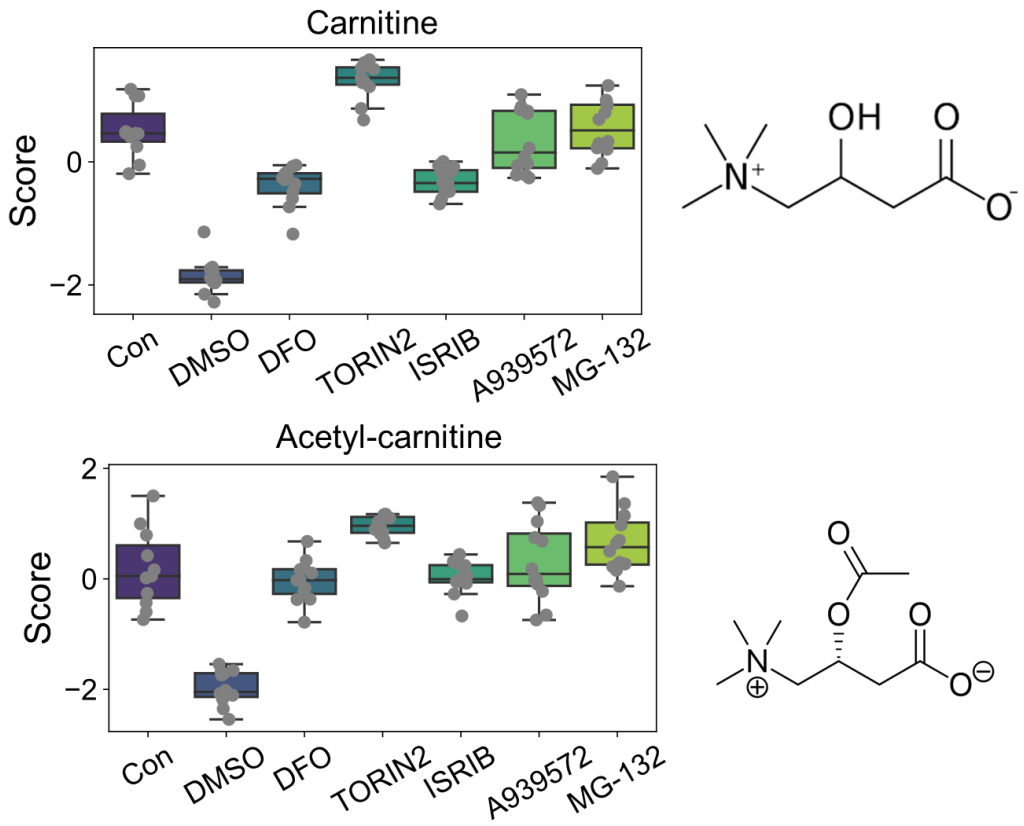

Figure S15. Typical metabolites related to fatty acid and lipid metabolism. a, Boxplot of Carnitine in all drug treatments and controls. **b,** Boxplot of Acetyl-Carnitine in all drug treatments and controls.

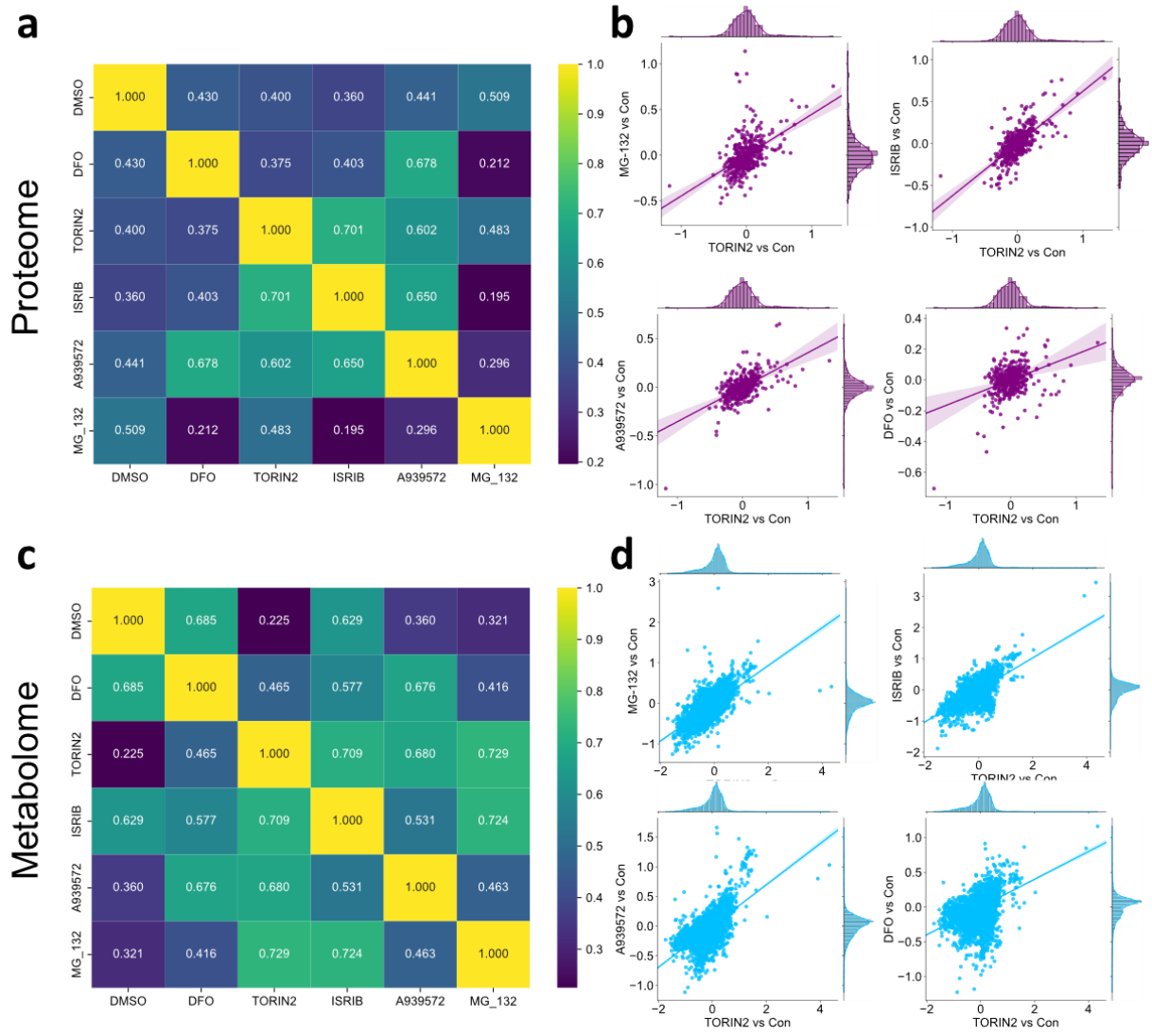

Figure S16. Correlation analysis of different drugs. a, Heatmap of Spearman correlation to show the correlations between different drugs from proteome data. **b,** Scatterplot of comparison between different drugs from proteome data. **c,** Heatmap of Spearman correlation to show the correlations between different drugs from metabolome data. **d,** Scatterplot of comparison between different drugs from metabolome data.