

Supplementary Figures and Legends

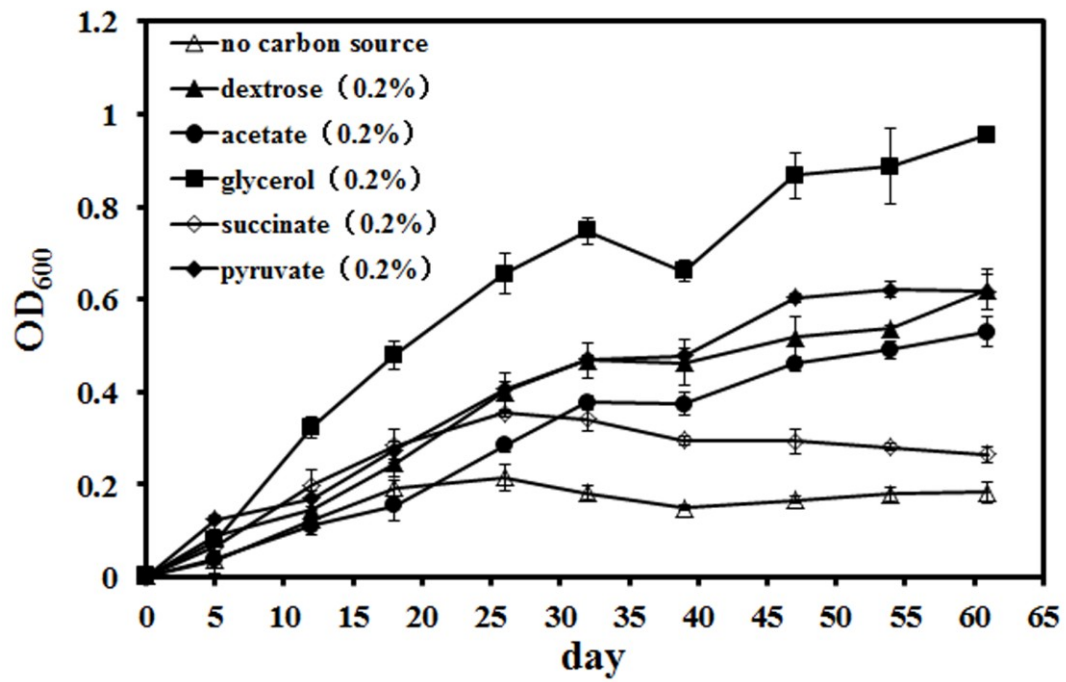


Figure S1. Growth of *M. tuberculosis* H37Rv in culture media containing different carbon sources. Growth curves are representative of at least three independent experiments.

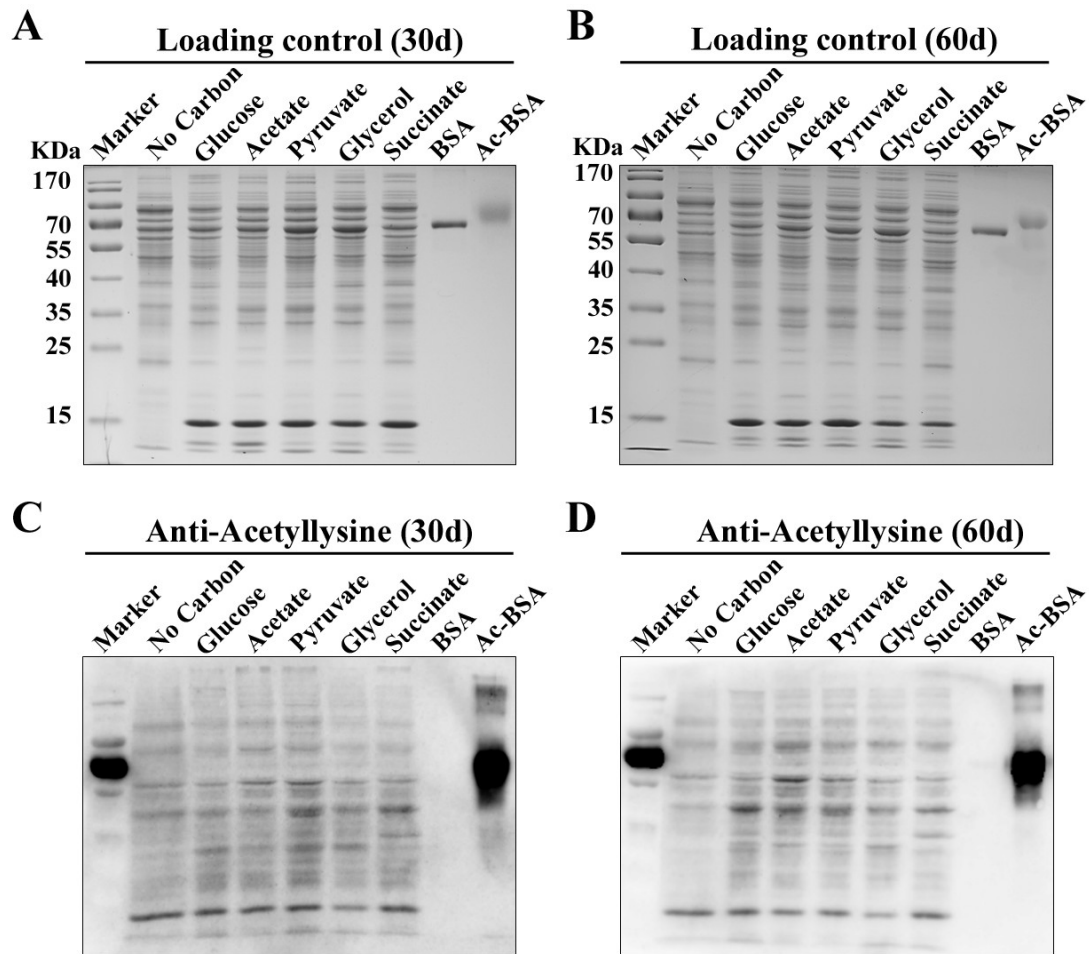


Figure S2. Anti-lysine acetylation immunoblots showing that different carbon sources altered acetylation profiles of *Mtb* H37Rv. Coomassie blue staining of protein lysates from (A) 30 days and (B) 60 days of cultures. Coomassie blue staining was used for the loading control. Western blotting analysis of lysine acetylation level in protein lysates from (C) 30 days and (D) 60 days of cultures with a pan anti-acetyllysine antibody. Specificity of acetylation signals was validated by acetylated or non-acetylated BSA assay.

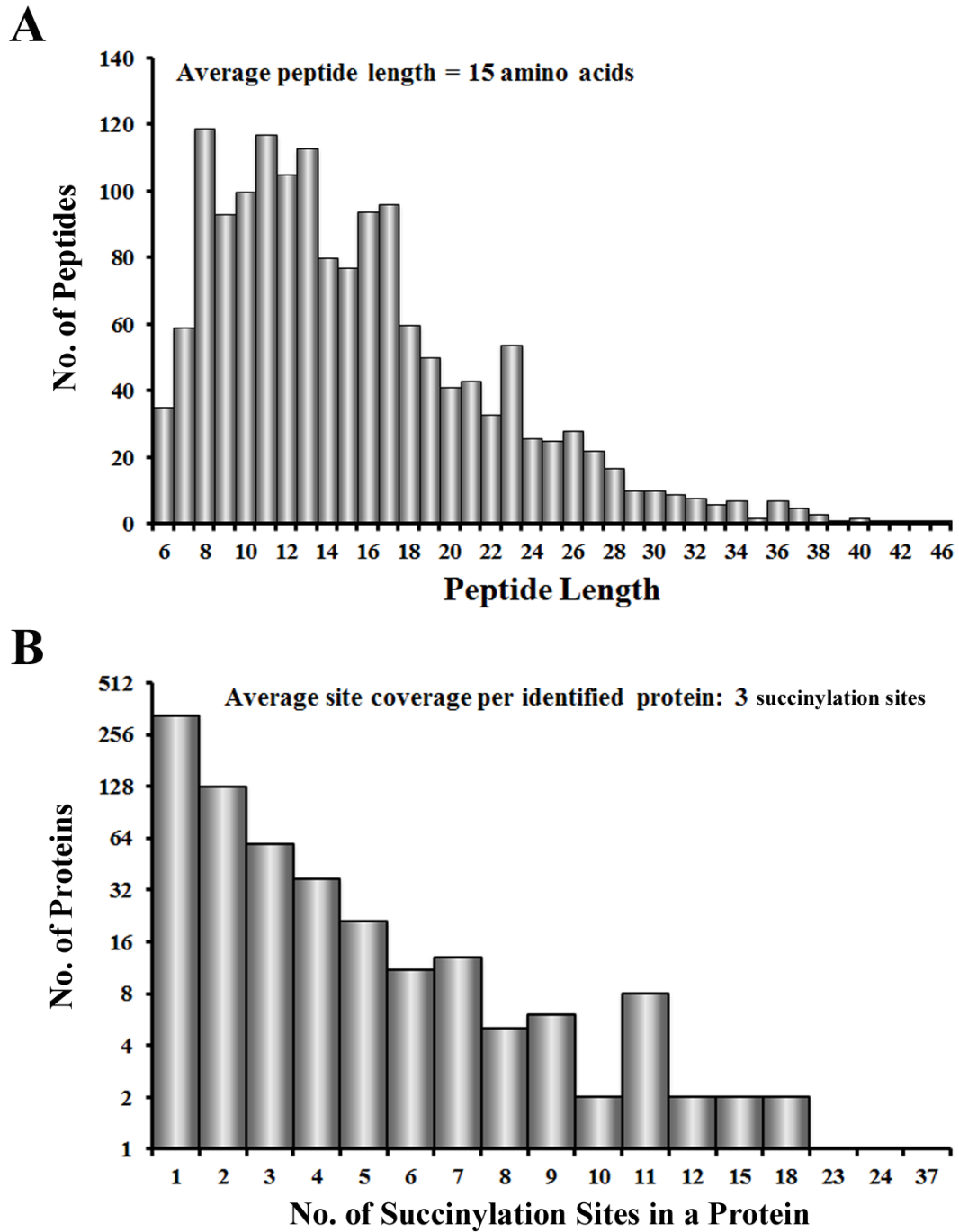


Figure S3. Overview of the *Mtb* succinylome. (A) Histogram depicting the length of identified succinylated peptide. (B) Histogram depicting the number of succinylation sites identified per protein.

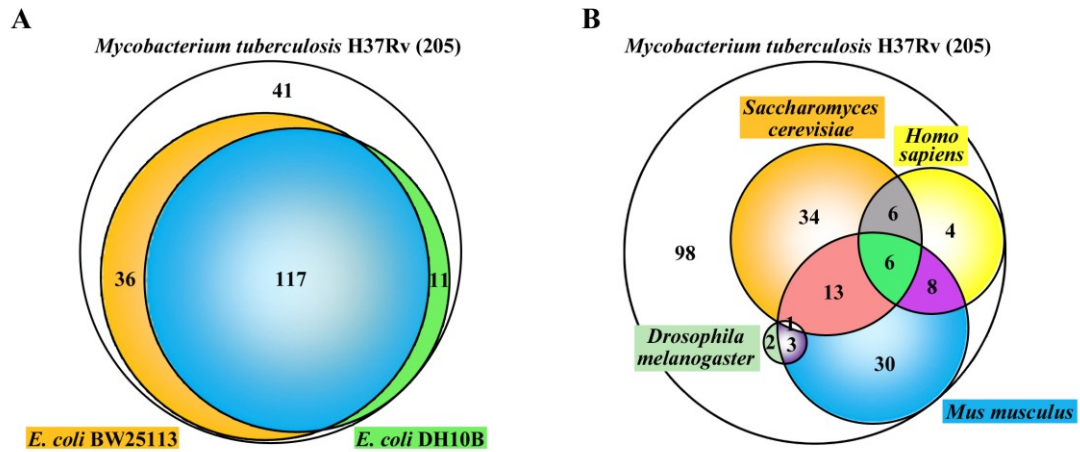


Figure S4. Venn diagrams showing the overlap of *Mtb* succinylated proteins with other published succinylomes. (A) Comparison of *Mtb* succinylome with other published bacterial succinylomes. (B) Comparison of *Mtb* succinylome with other published eukaryotic succinylomes.

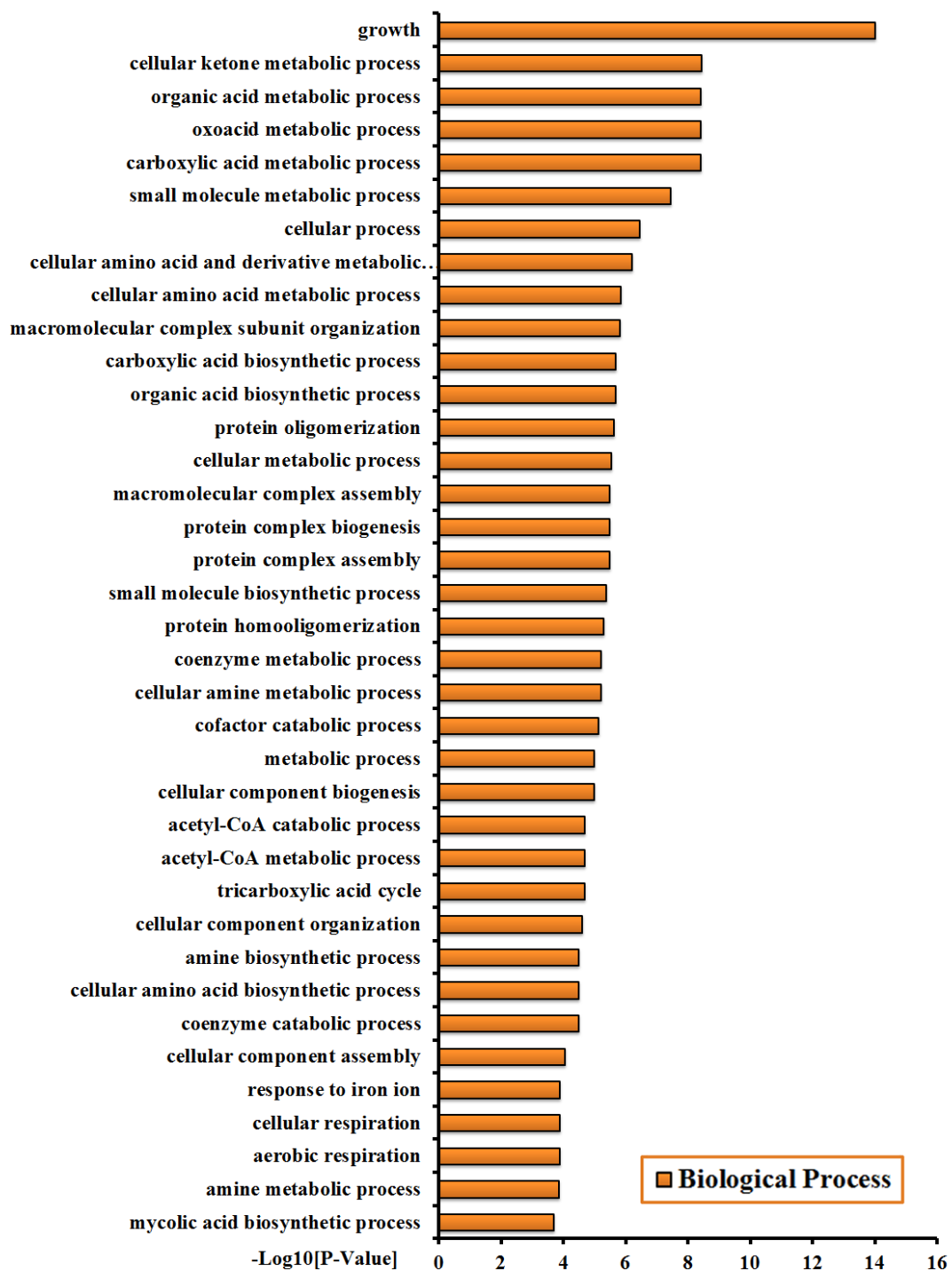


Figure S5. Functional annotation of lysine succinylome. GO enrichment analysis of succinylated proteins for biological process. The enrichment of GO categories was analyzed using Cytoscape plugin BiNGO and the $p < 0.01$ were considered significantly.

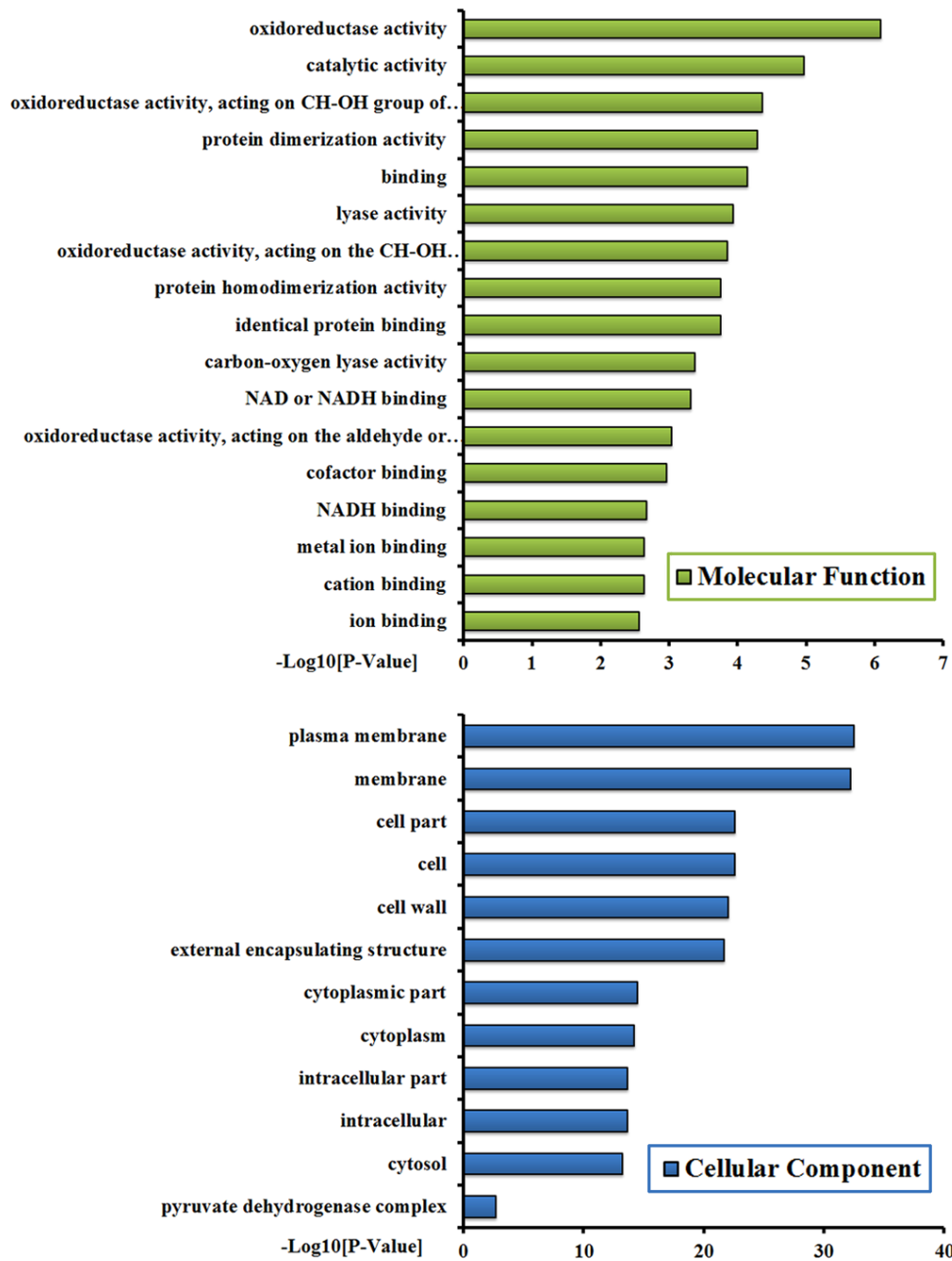


Figure S6. Functional annotation of lysine succinylome. GO enrichment of succinylated proteins for molecular function and cellular component. The enrichment of GO categories was analyzed using Cytoscape plugin BiNGO and the $p < 0.01$ were considered significantly.

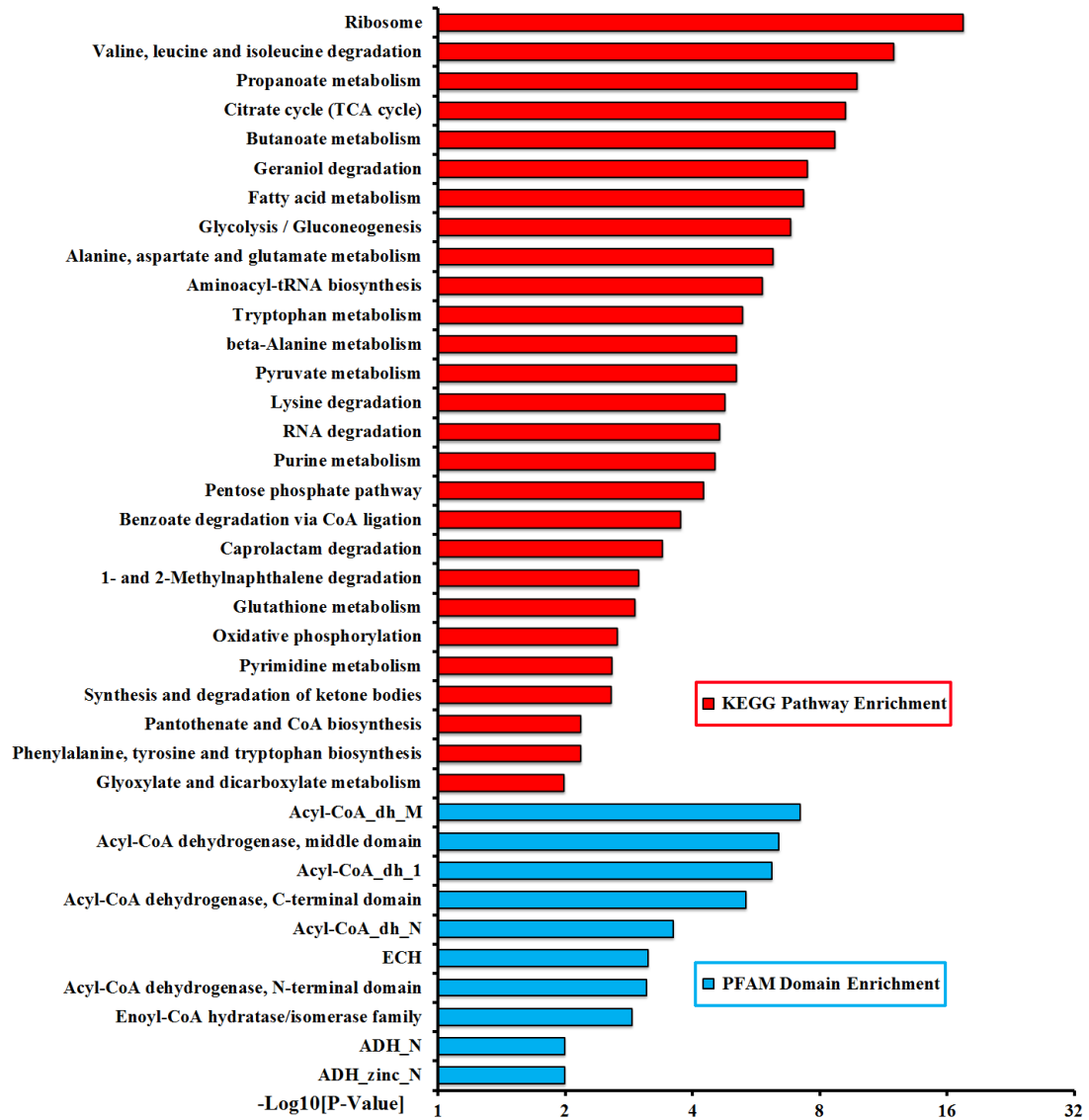


Figure S7. Functional annotation of lysine succinylome revealed the enriched metabolic pathways and PFAM domain. Pathway and domain analysis were performed using DAVID bioinformatics tools ($p < 0.01$).

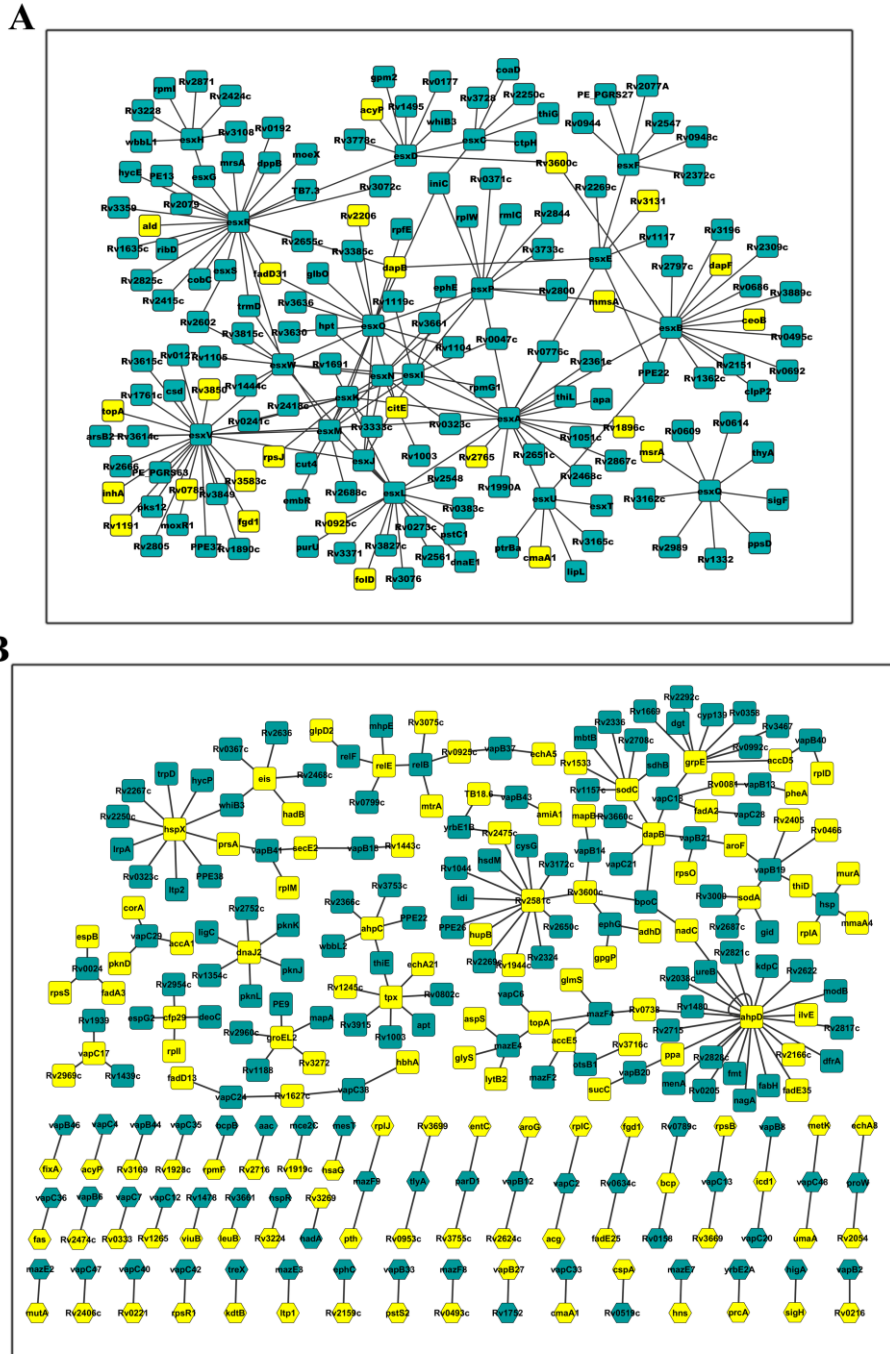


Figure S8. Interaction network of succinylated proteins involved in ESX systems, virulence, detoxification and adaptation in *Mtb* H37Rv. Identified succinylated proteins were highlighted in yellow. (A) The interaction network of some reported secretion proteins in direct linkage with ESX systems of ESAT6 (Rv3875) and CFP10 (Rv3874)-like proteins. (B) The interaction network involved in virulence, detoxification and adaptation function.

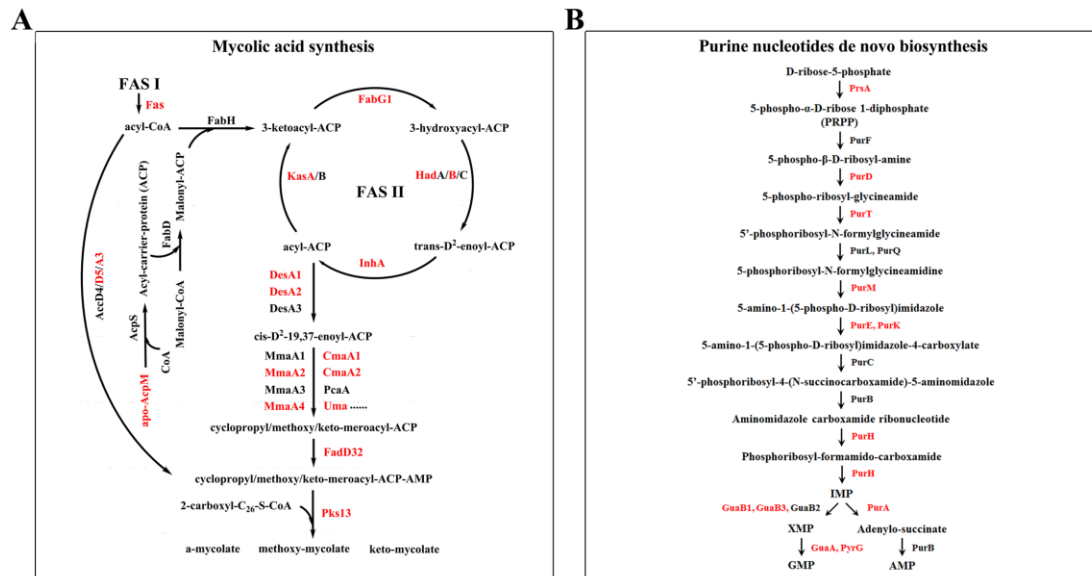


Figure S9. Illustrations of succinylated proteins involved in metabolic pathways in *Mtb* H37Rv. (A) Schematic representation of the mycolic acid biosynthesis pathway. (B) Schematic representation of the purine nucleotides *de novo* biosynthesis pathway.

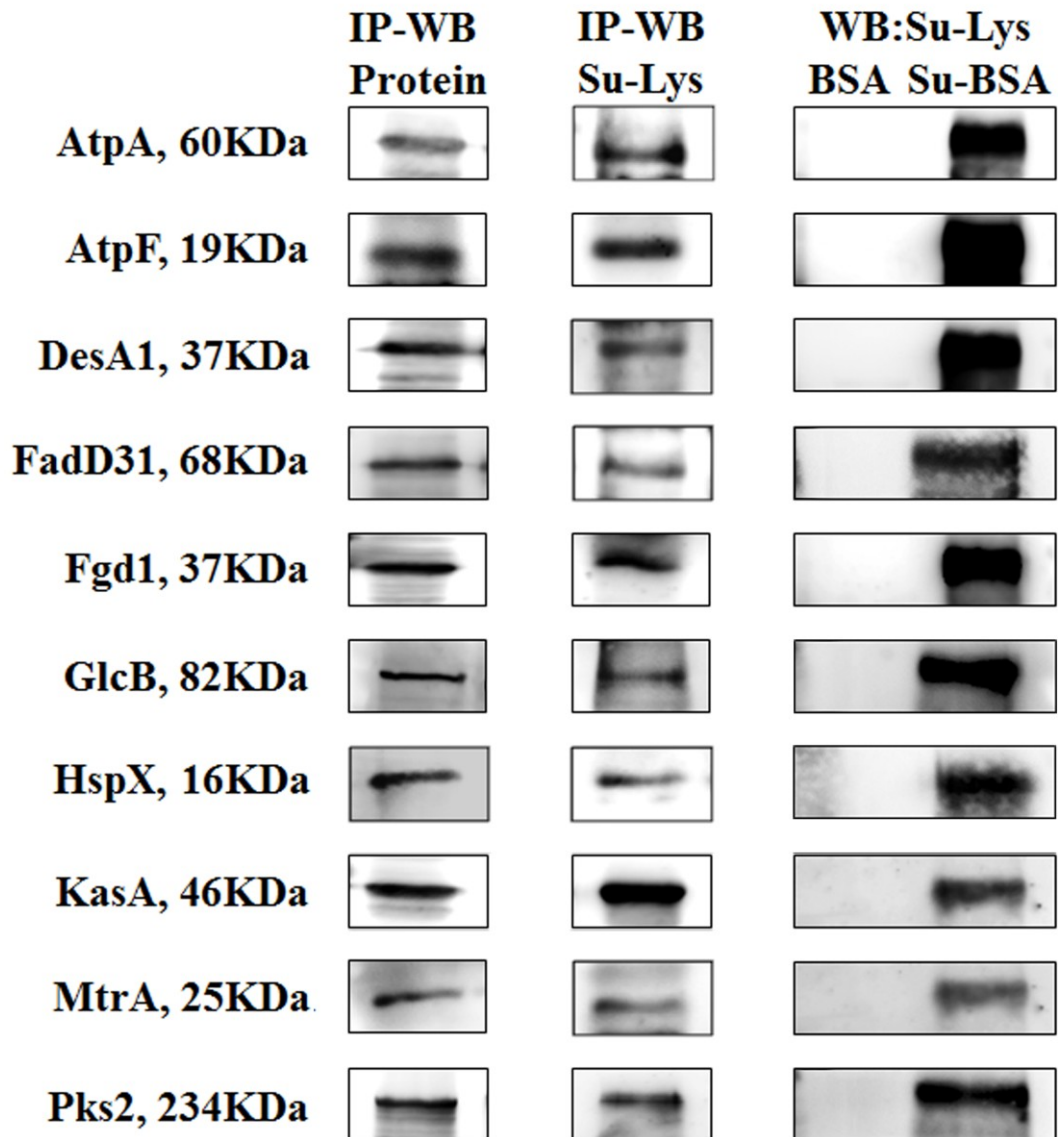


Figure S10. Validation of identified succinylated proteins using immunoprecipitation and Western blotting. AtpA: ATP synthase alpha chain; AtpF: ATP synthase beta chain; KasA: 3-oxoacyl-[acyl-carrier protein] synthase 1; DesA1: acyl-[acyl-carrier protein] desaturase; FadD31: acyl-CoA ligase; Fgd1: F420-dependent glucose-6-phosphate dehydrogenase; GlcB: malate synthase G; HspX: heat shock protein; MtrA: two component system transcriptional regulator; Pks2: polyketide synthase.

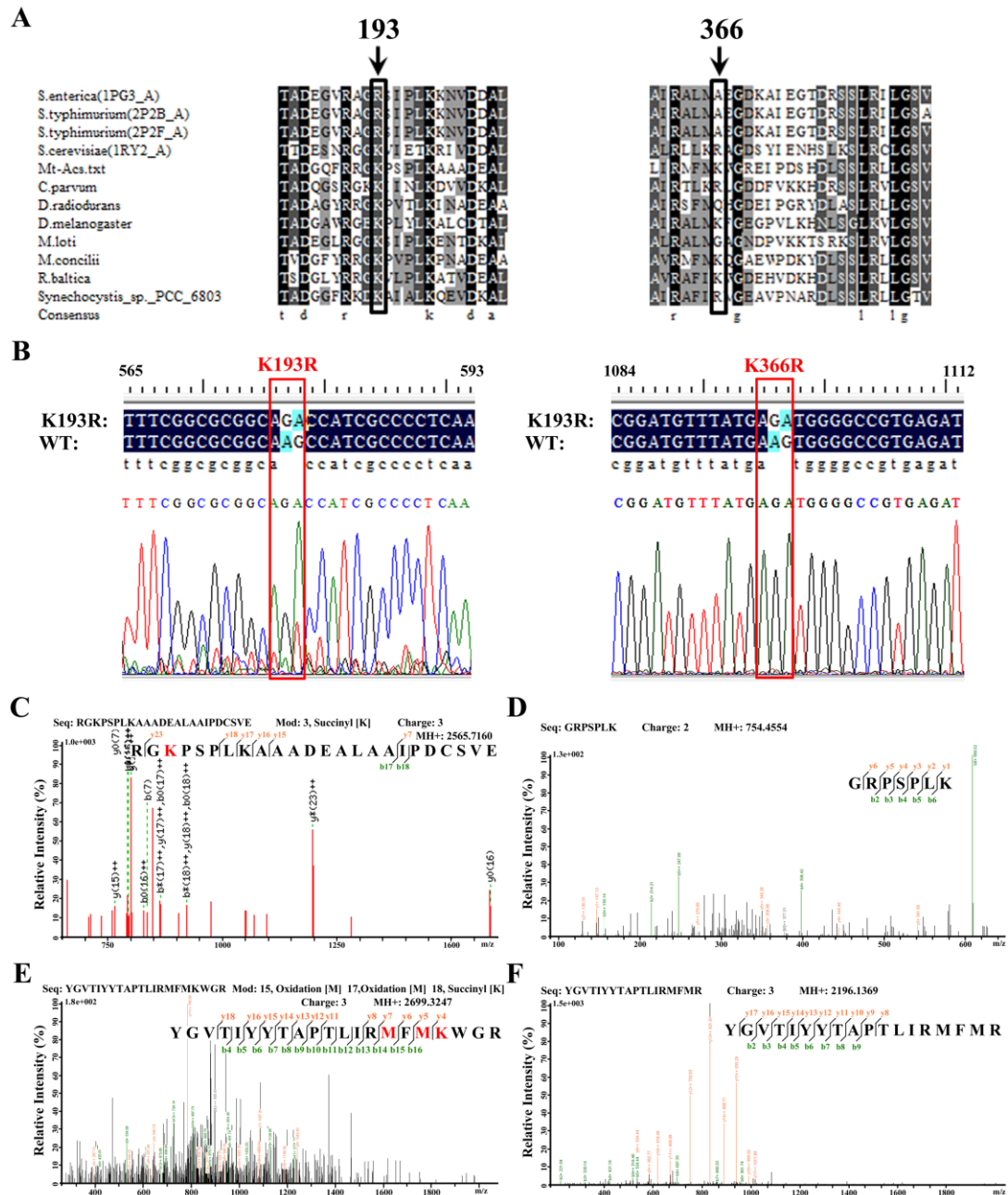


Figure S11. Mutations of AcS were verified by DNA sequencing and MS searches. (A) Multiple sequence alignment of AcS from different species. The conserved active-sites were marked by the black arrow. (B) Site-directed mutations were verified by DNA sequencing. Succinylated peptides of AcS in the wild type and non-succinylated peptides in mutants were further confirmed by MS analysis. (C) K193 succinylation site. (D) K193R mutation site. (E) K366 succinylation site. (F) K366R mutation site.