SUPPLEMENTAL FIGURES and LEGENDS

Figure S1. The proteome microarray based KPI studies are reproducible and with reasonable signal distribution.
(A). Silver staining of 11 ser/thr protein kinases. **(B).** Detection the activities of ser/thr protein kinases with anti-ser/thr antibody. **(C).** Histogram of protein signals (median of foreground divided by median of background of each spot).The x-axis denotes signal intensity, and the y-axis denotes density of signal intensity. Signals for most of the spots on the array are close to 1, indicating that the majority of the proteins are not interacted with kinases. Only the spots with signals greater than 3 standard deviations above the mean (indicated by black arrows) were considered as potential binding proteins of the kinase. Except PknA has a wider variance, whereas all the others have very consistent distribution.

Figure S2.Validation of PknB-KPIs and PknD-KPIs using BLI.

Figure S3.Validation of PknG-KPIs and PknH-KPIs using BLI.

Figure S4.Validation of STPK-KPIs by Y2H for PknB, PknG, PknD and PknH. For Y2H, all the strains could grow on SD-LW media, while only positive strains could grow on SD-LWHA media. “+” indicates positive interaction, “++” indicates strong interaction.

Figure S5. MPF-C proteineprotein assay. PknB and PknD with its interaction proteins facilitated the reassembly of the [F1, 2] and [F3] domains of mDHFR, enabling growth of Msm strains in the presence of 20 mg/mL TMP. Identical spots on control plates without TMP revealed growth of all strains. Positive control, Saccharomyces cerevisiae GCN4 dimerization domains fused to [F1, 2] and [F3], respectively.

Figure S6. The phosphorylation substrates have been found with mass-spectrometry.

Figure S7. The KPIs network of the 11 STPKs. All the 1,027 KPIs were analyzed by STRING and the landscape was plotted using Cytoscape. The proteins with clear known functions were grouped and colored in the middle.

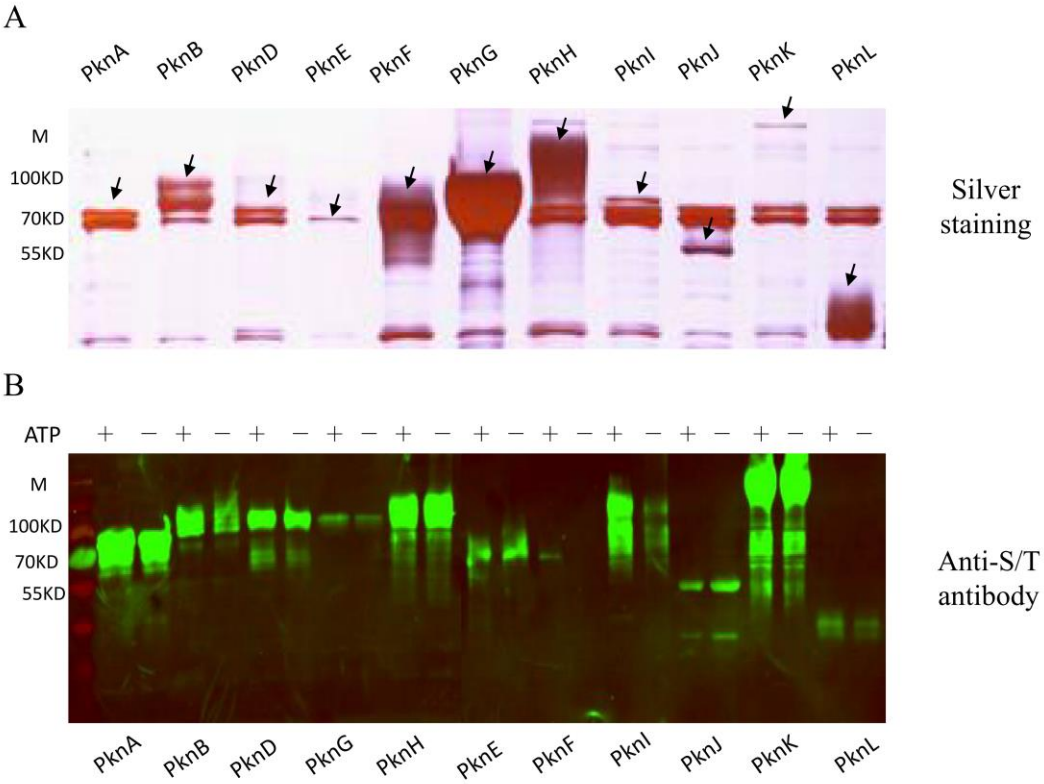
Figure S8. Detection of MurC activity with PknG overexpression in *M. smegmatis.* The consumption of the substrate L-Alanine in the MurC enzyme activity reaction and detected with ESI-MS.

Supplementary Tables and Table Legends

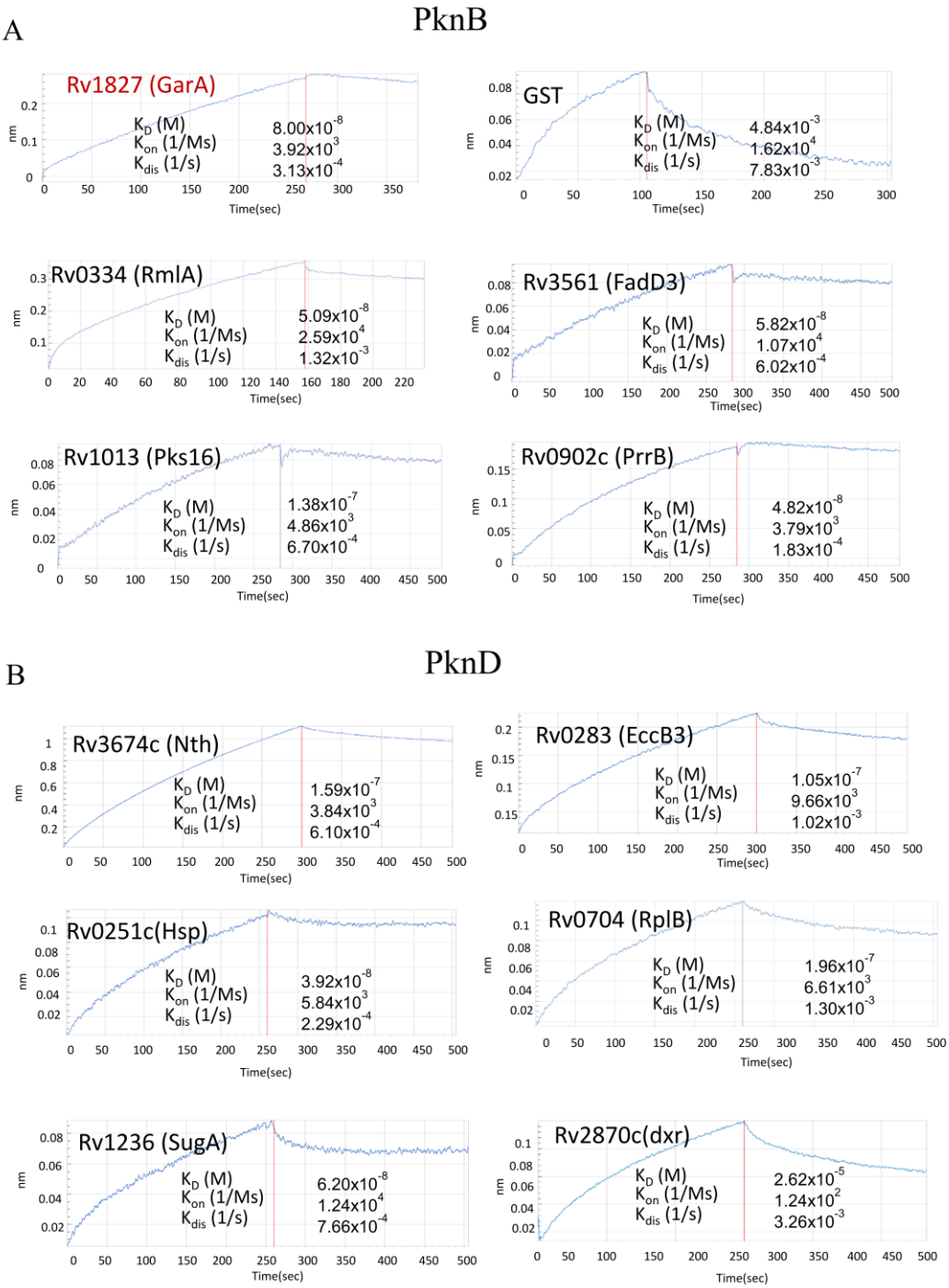
Table S1. The complete list of KPIs identified in this study.

Table S2. Validation of the KPIs with BLI and Y2H.

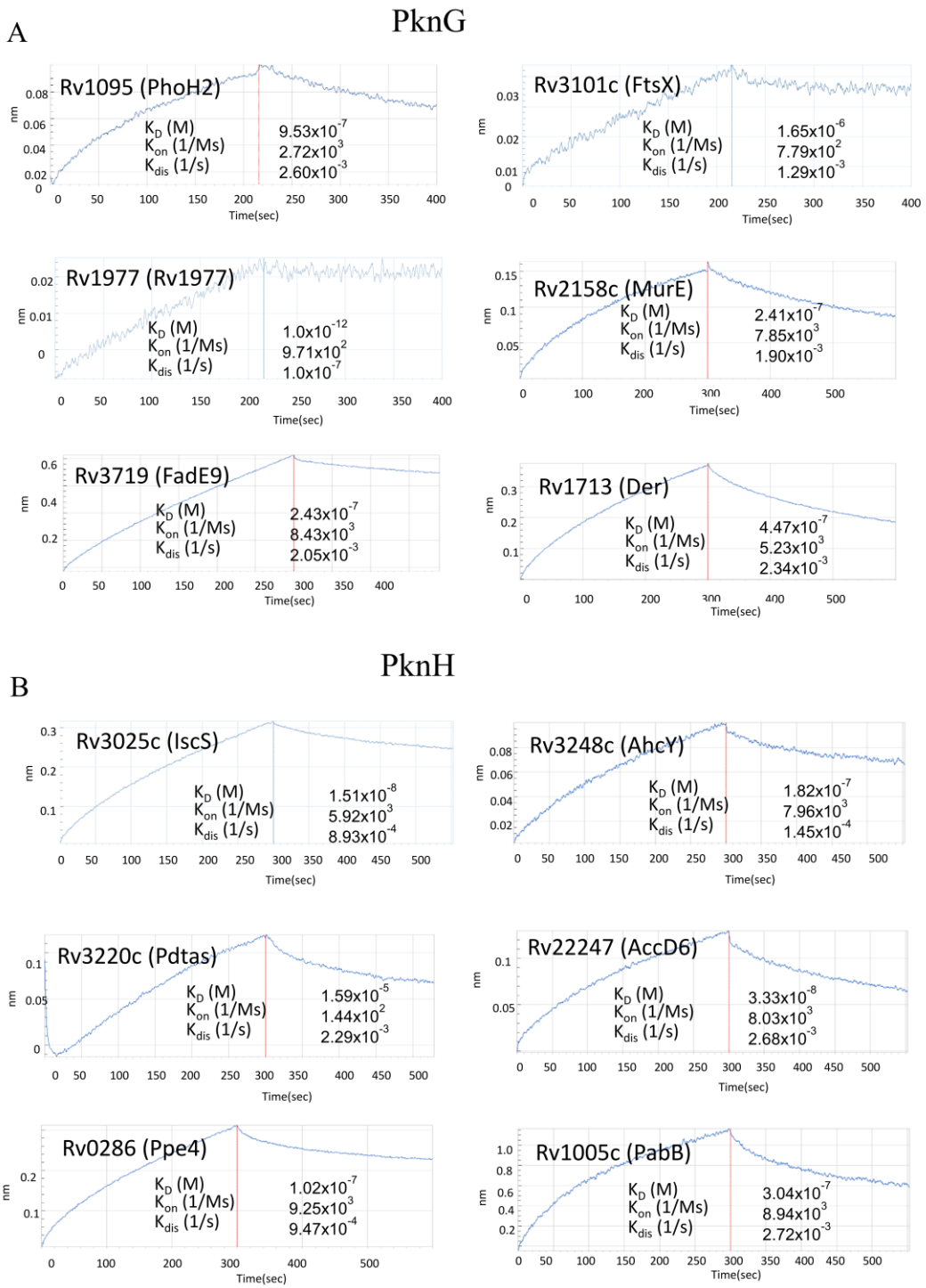
Table S3. New phosphosites identified in this study.



Wu et. al., Figure S1.

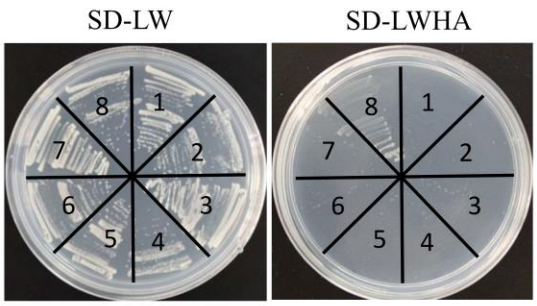


Wu et. al., Figure S2.



Wu et. al., Figure S3.

PknB

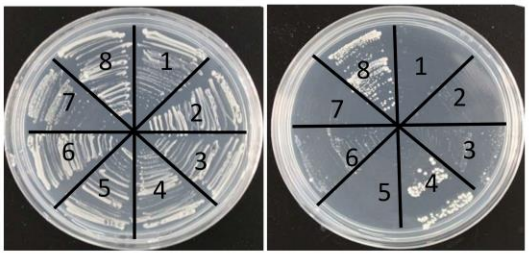


	Y2H	Interaction
1	PknB+pGADT7	−
2	PknB+Rv3155	−
3	PknB+Rv1013	−
4	PknB+Rv0445c	−
5	PknB+Rv0475	−
6	PknB+Rv0852	−
7	PknB+Rv2364c	−
8	PknB+Rv0902c	+

	Y2H	Interaction
1	pknB+Rv1350	−
2	pknB+Rv1584c	−
3	pknB+Rv2837c	−
4	pknB+Rv3308	−
5	pknB+Rv2841c	−
6	pknB+Rv2581c	+
7	pknB+Rv3283	−
8	pknB+Rv1646	+

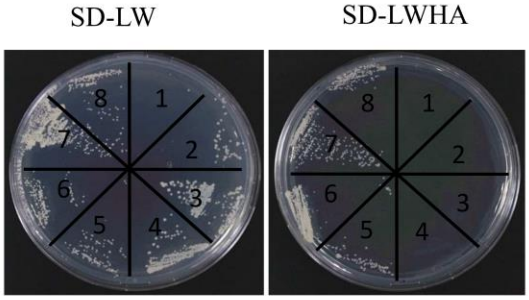
PknG

	Y2H	Interaction
1	PknG+pGADT7	−
2	PknG+Rv1981c	+
3	PknG+Rv3872	+
4	PknG+Rv1931c	+
5	PknG+Rv3101c	+
6	PknG+Rv3719	+
7	PknG+Rv2580c	+
8	PknG+Rv1013	+



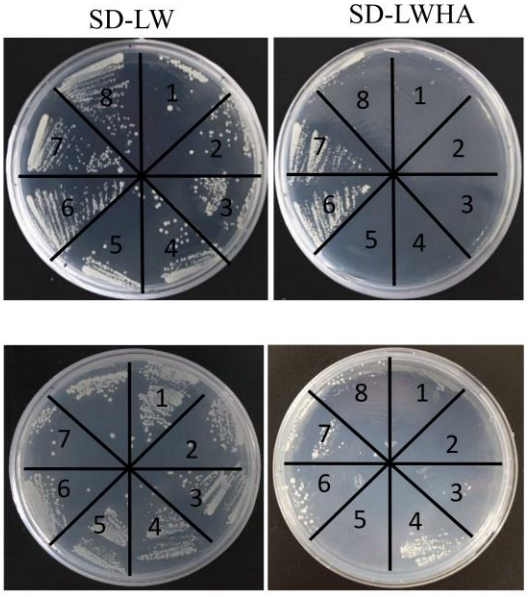
	Y2H	Interaction
1	PknG+pGBADT7	−
2	PknG+Rv2534c	−
3	PknG+Rv0540	−
4	PknG+Rv2703	++
5	PknG+Rv1710	−
6	PknG+Rv1070c	+
7	PknG+Rv1977	−
8	PknG+Rv1095	++

PknD



	Y2H	Interaction
1	pknD+Rv3588c	−
2	pknD+Rv2870c	−
3	pknD+Rv2000	−
4	pknD+Rv3674c	−
5	pknD+Rv2460c	+
6	pknD+Rv0251c	+
7	pknD+Rv1423	+
8	pknD+Rv0384c	+

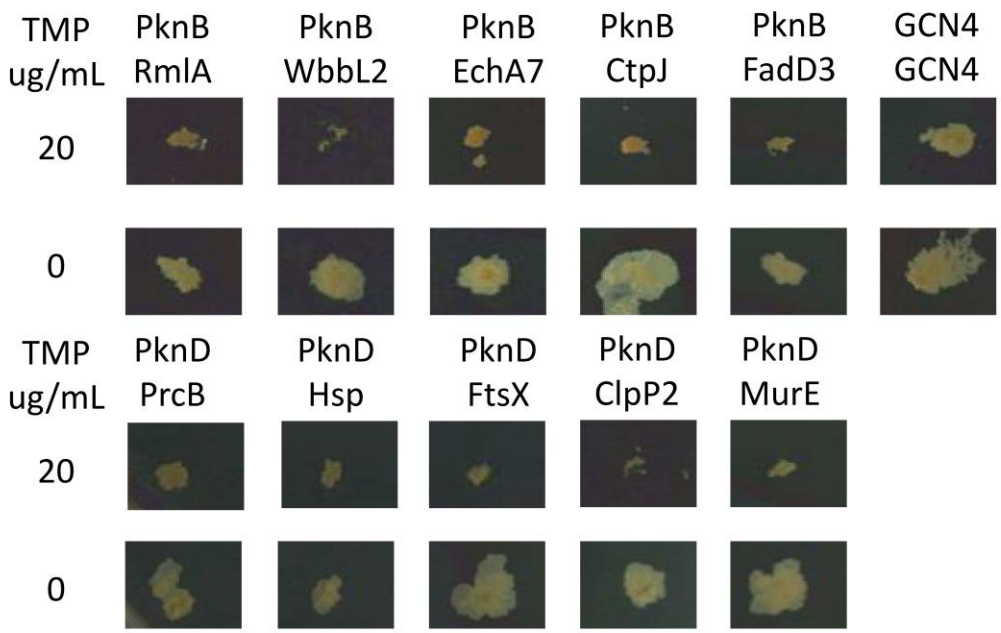
PknH



	Y2H	Interaction
1	PknH+pGADT7	−
2	PknH+Rv3743c	−
3	PknH+Rv1423	−
4	PknH+Rv0286	−
5	PknH+Rv0971c	−
6	PknH+Rv3220c	+
7	PknH+Rv0280	+
8	PknH+Rv0685	+

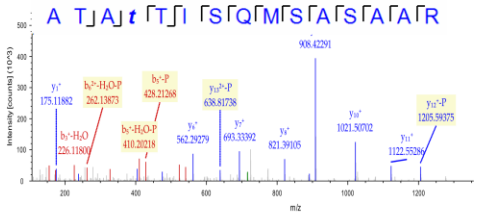
	Y2H	Interaction
1	pknH+pGADT7	−
2	pknH+Rv3025c	−
3	pknH+Rv3248c	−
4	pknH+Rv2247	+
5	pknH+Rv3339c	−
6	pknH+Rv1816	+
7	pknH+Rv0971c	+
8	pknH+Rv0704	+

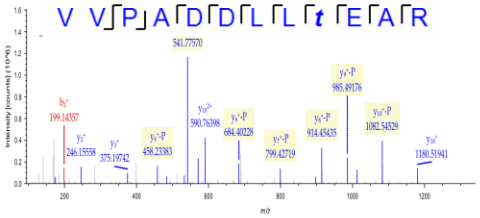
Wu et. al., Figure S4.



Wu et. al., Figure S5.

EchA8





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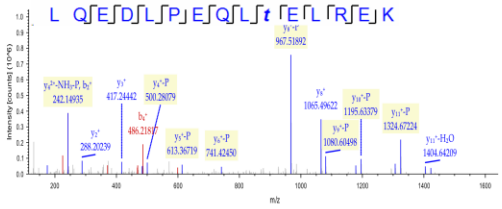

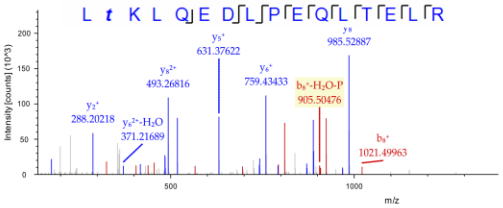

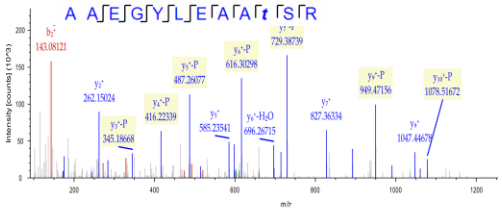

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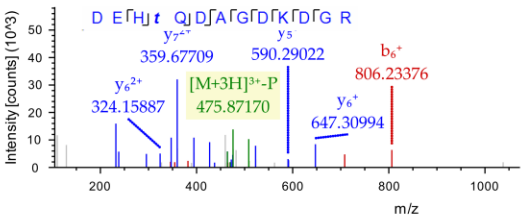

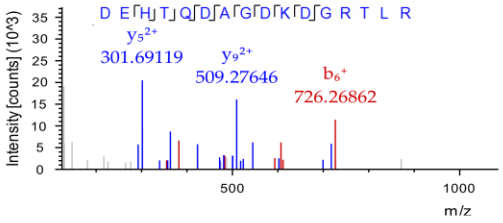

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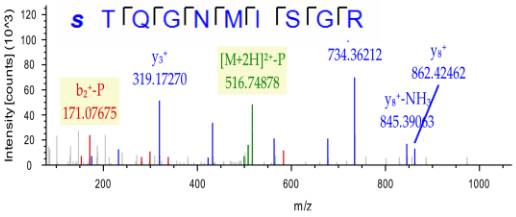

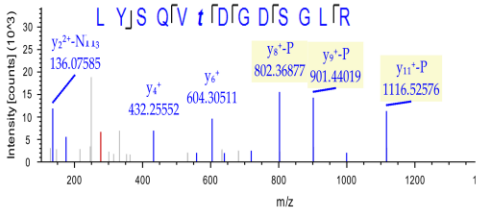

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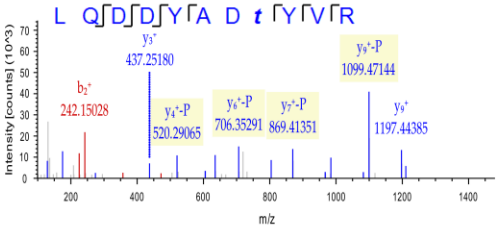

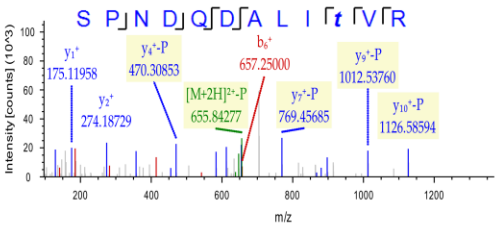

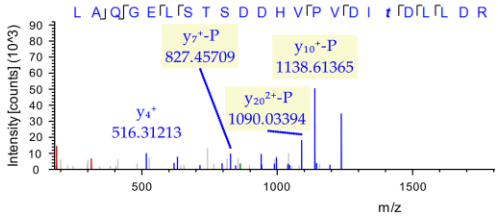

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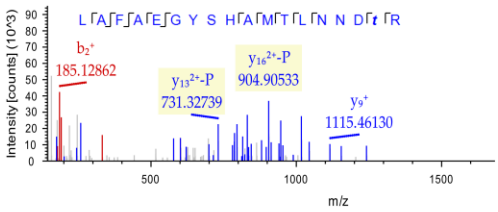

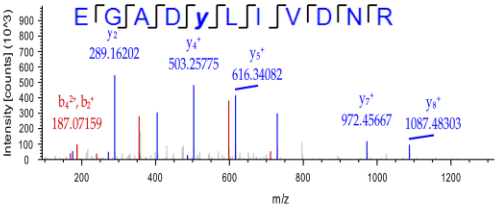

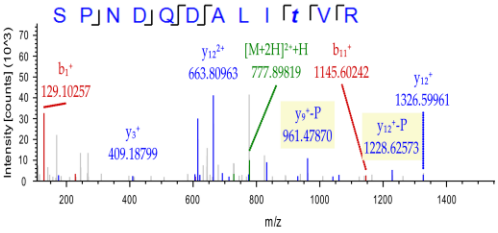

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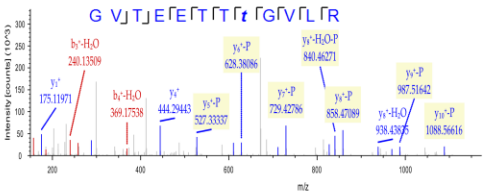
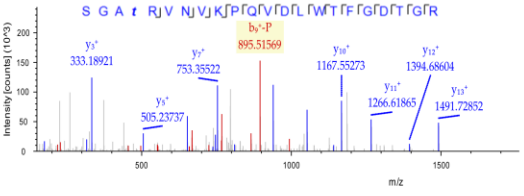
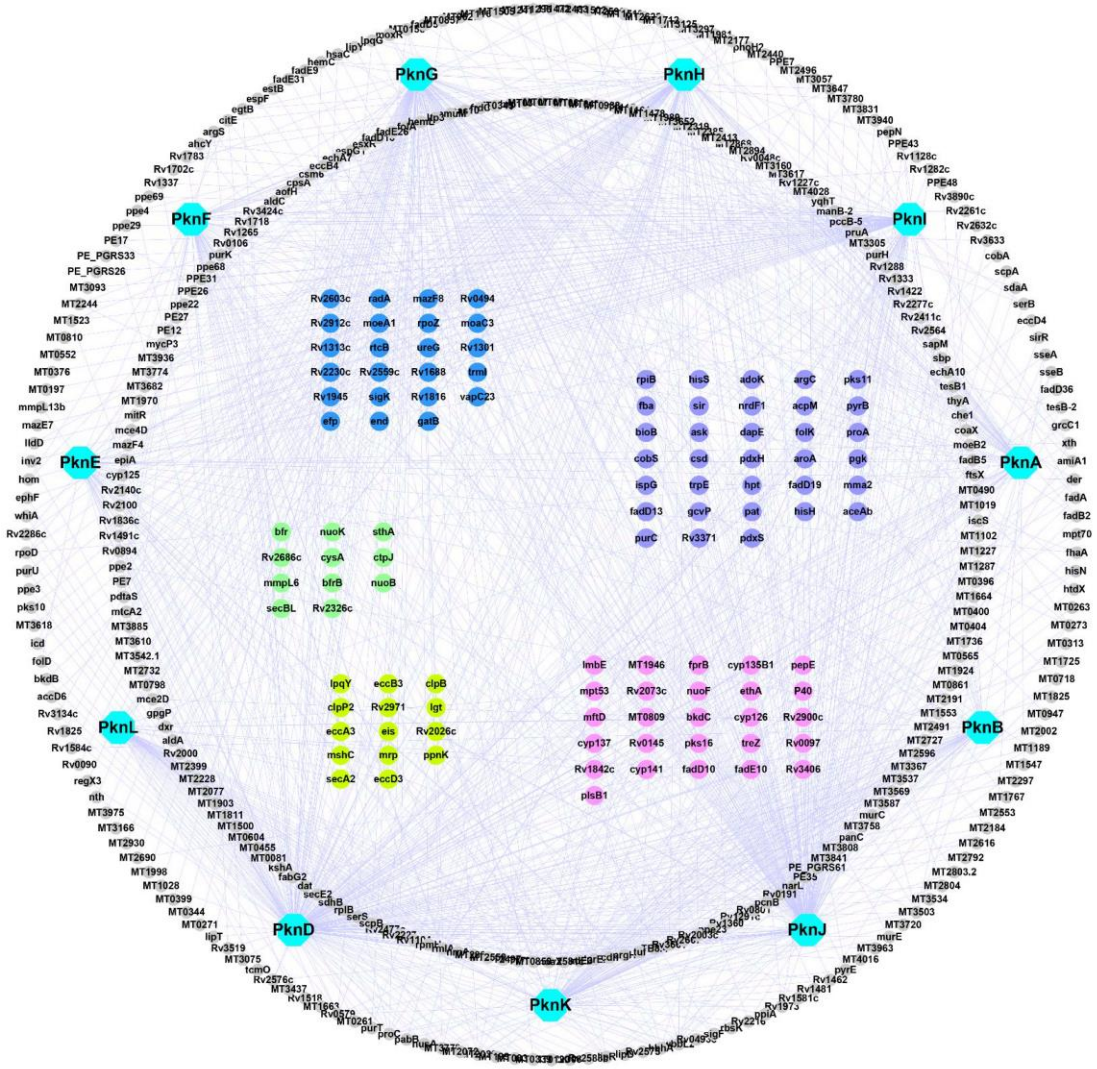




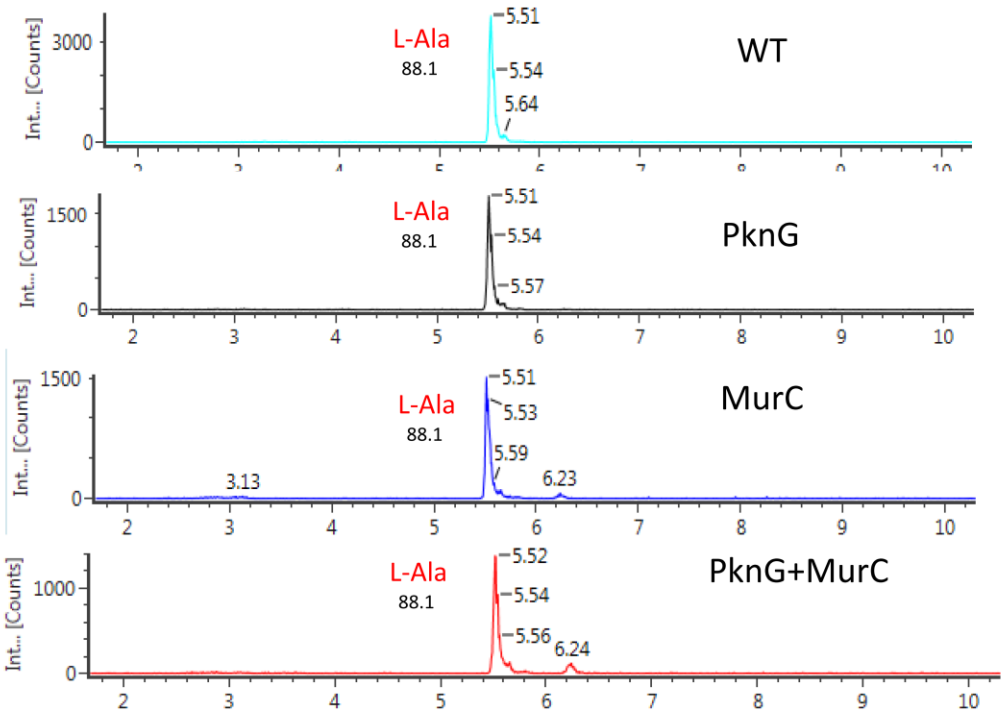
Wu et. al., Figure S6.



Biological process

- Nitrogen biosynthetic
- Cation transport
- Growth
- Cell metabolic
- Nucleotide biosynthetic
- Unknown function

Wu et. al., Figure S7.



Wu et. al., Figure S8.