SUPPLEMENTARY INFORMATION

New AMP-forming acid:CoA ligases from *Streptomyces lividans*, some of which are posttranslationally regulated by reversible lysine acetylation.

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Running title: insights into RLA control of CoA ligases in *S. lividans*

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SUPPLEMENTAL FIGURES

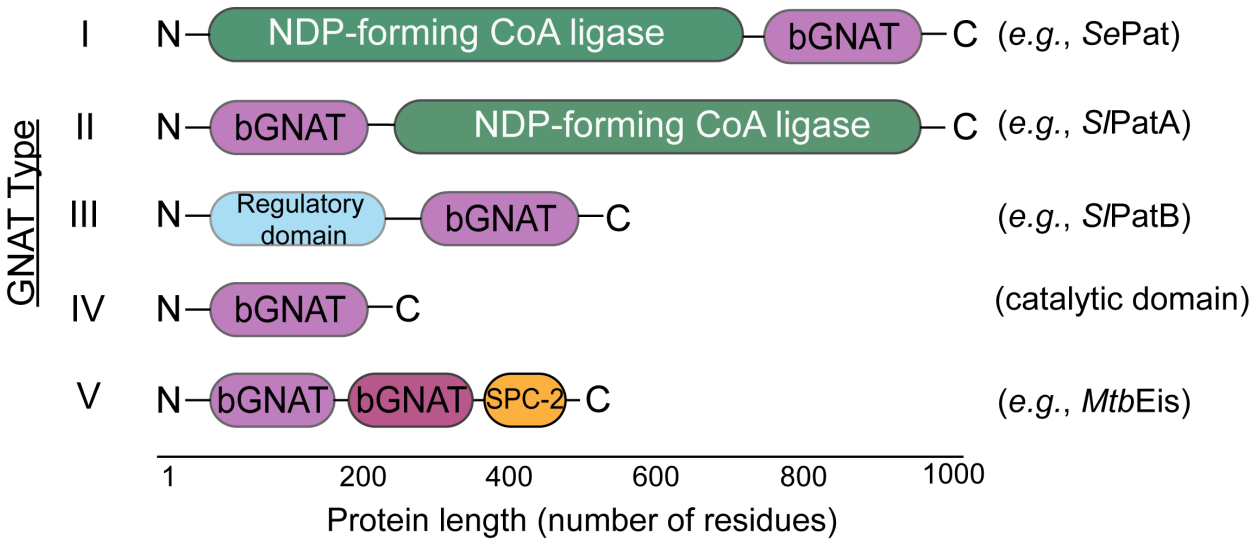

Figure S1. Domain organization of bacterial GNATs(bGNAT). Cartoon representation of the five classes (I-V) of bacterial GNATs (bGNATs), with the bGNAT domain shown in purple. *Sl*PatA is a type II bGNAT while *Sl*PatB is a type III bGNAT. This figure was modified from a figure published in (Burckhardt *et al.*, 2019). The SPC-2 C-terminal domain of *M. tuberculosis* Eis acetyltransferase stands for 'sterol carrier protein-2'. The function of the second bGNAT domain of *Mtb*Eis (darker purple) is unknown. The sequence of the bGNATs is not identical, but it is the site of catalysis.

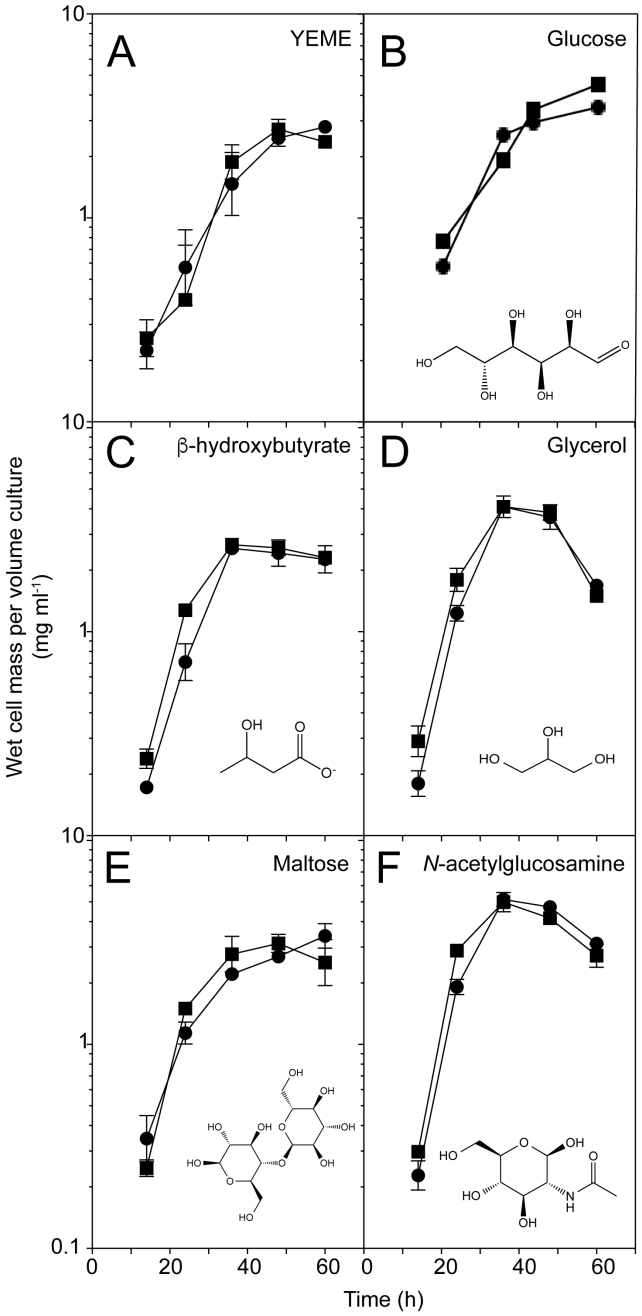

Figure S2. Growth of *S. lividans* wild-type and Δ*patA* strains. *S. lividans* wild-type (circles) or Δ*patA* (squares) strains were grown in rich (YEME) medium (A) or minimal liquid media supplemented with glucose (10 mM, B), β-hydroxybutyrate (30 mM, C), glycerol (30 mM, D), maltose (30 mM, E), or *N*-acetylglucosamine (30 mM, F). Cells were harvested at regular intervals by centrifugation. Excess media was removed by pipetting and cell mass was weighed. Growth is reported as wet cell mass per ml of culture. Growth is reported as the mean ± S.D. in (A) and (C-F) or as single value (B).

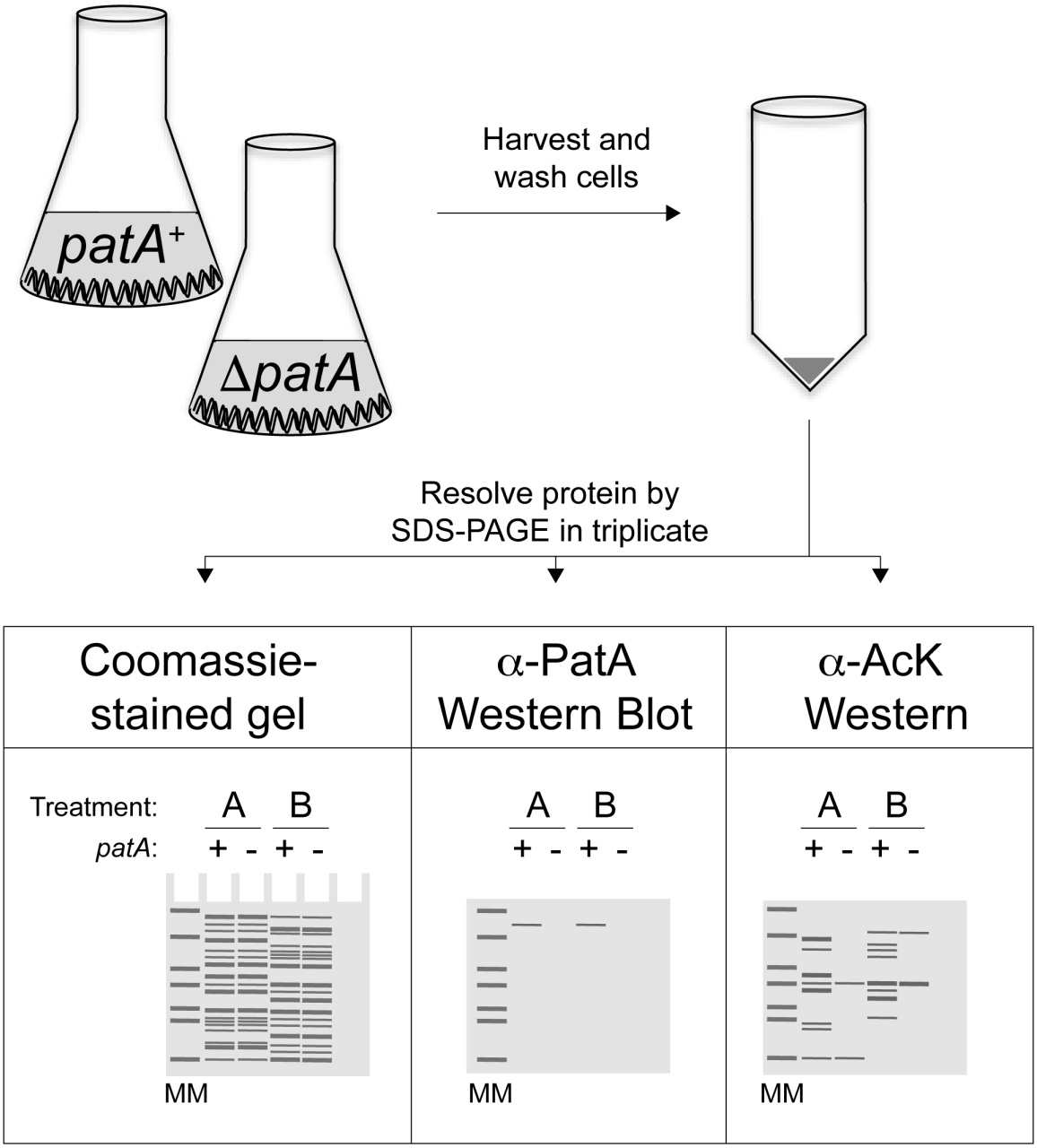

Figure S3. Schematic of *S. lividans* cell isolation and Western blot analysis. *S. lividans* was grown in liquid media described in the *Materials and Methods*. *S. lividans* was cultured in flasks equipped with marine-grade stainless steel springs to aid in cell dispersal. Cells were harvested by centrifugation and washed before lysis. Cell lysates were clarified by centrifugation and quantified before resolving on triplicate SDS-PAGE gels. One gel was stained with Coomassie Blue to observe proteins and ensure equal loading of lanes. Proteins in the two additional replicate gels were transferred to PVDF membranes. Lysates on one membrane were probed for the presence of PatA using rabbit antiserum. Lysates on the remaining membrane were probed for acetyllysine using anti-acetyllysine antibodies.

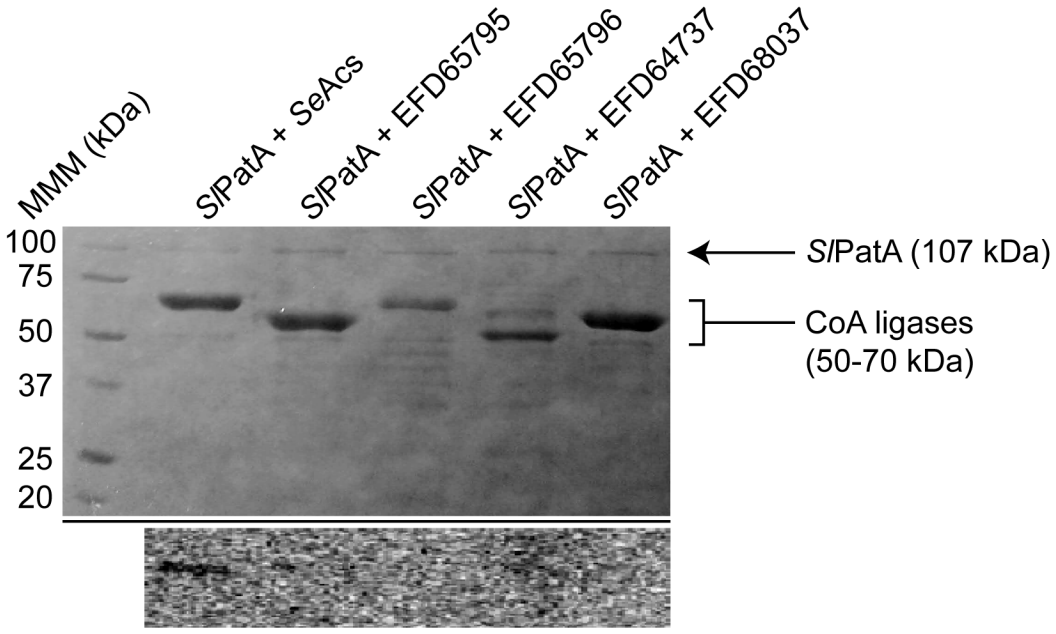

Figure S4. Lack of acetylation of CoA ligases by *Sl*PatA. Purified CoA ligases and *Sl*PatA were incubated with [1-^{14}C]-AcCoA. Reactions were resolved on an SDS-PAGE gel and label distribution was visualized by phosphor imaging. Top panel shows the SDS-PAGE and the bottom panel shows the phosphor image of the above gel. MMM, (kDa), molecular mass markers. *Se*Acs, was used as *bona fide* substrate of *Sl*Acs (Tucker & Escalante-Semerena, 2013).

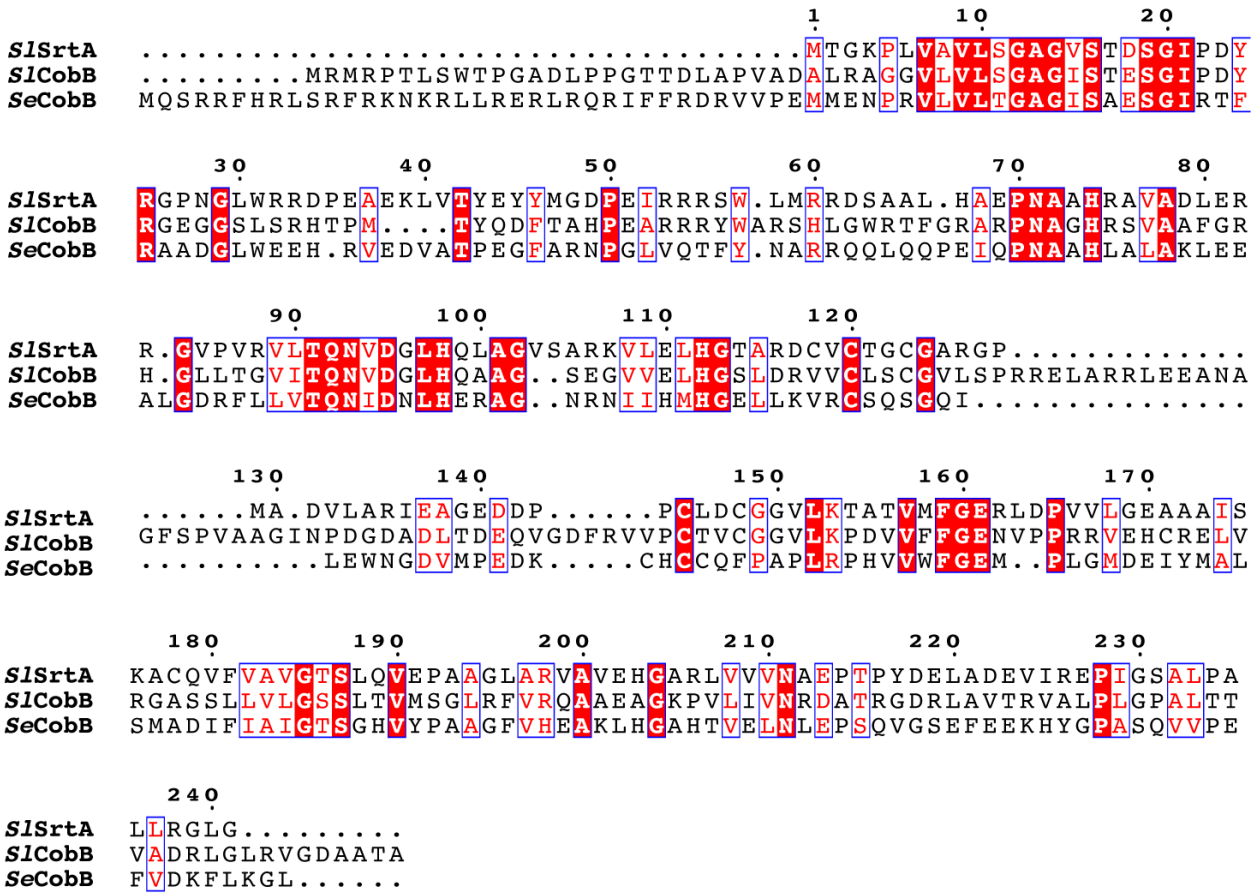

Figure S5. Alignment of sirtuins from *Streptomyces lividans* and *Salmonella enterica*. Protein sequence of *Se*CobB, *Sl*SrtA, and *Sl*CobB were aligned using Geneious software (Kearse *et al.*, 2012). Conserved regions are highlighted in red and similar areas are outlined in blue. *Streptomyces* sirtuins share ~20% identity with *Se*CobB (34% with *Sl*SrtA and 21% with *Sl*CobB) and ~50% similarity (60% with *Sl*SrtN and 49% with *Sl*CobB). *Se*CobB has been shown to deacetylate *Streptomyces* CoA ligases (Tucker & Escalante-Semerena, 2013).

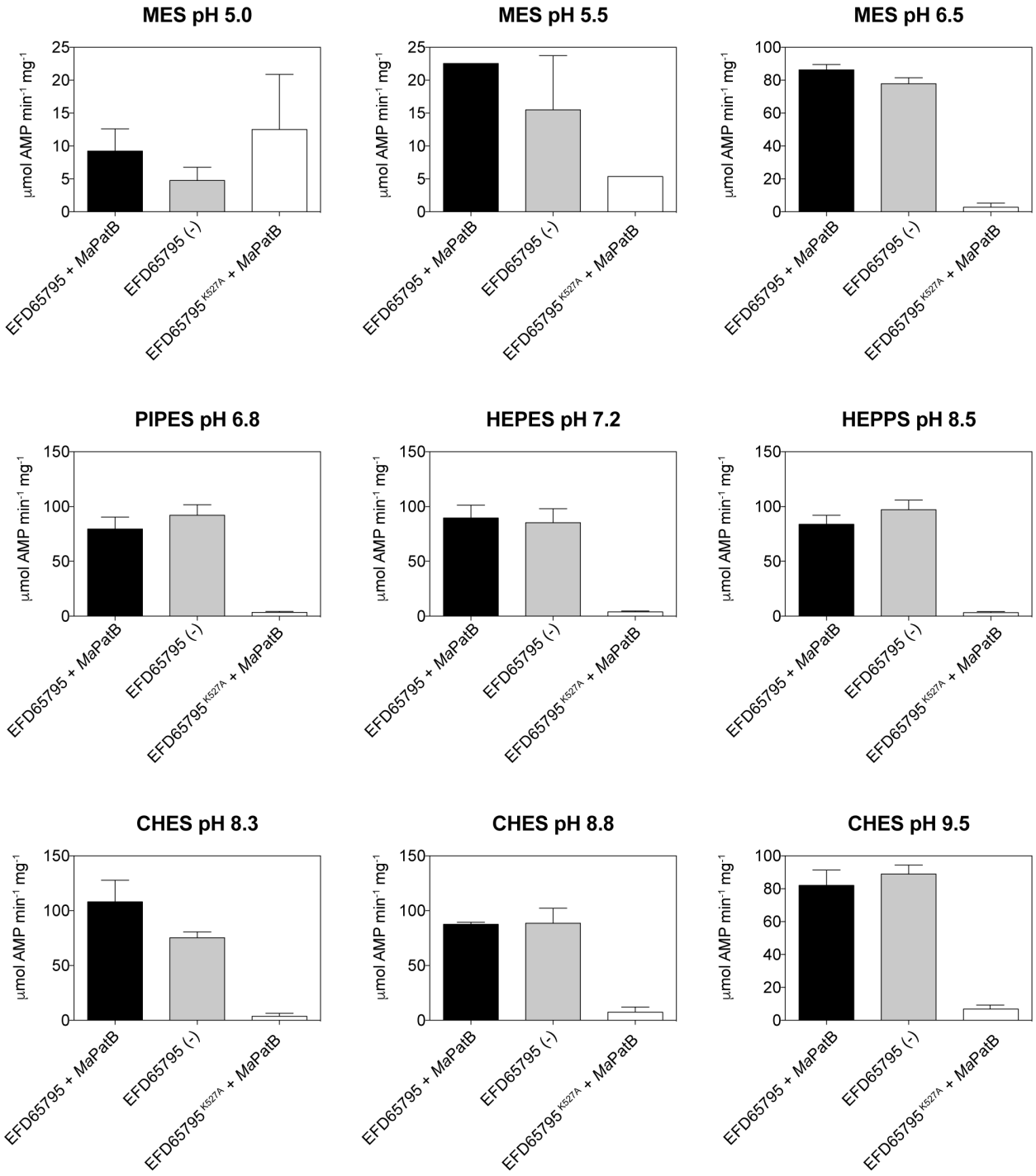

Figure S6. **LbuL CoA ligase activity after acetylation by *Ma*PatB in a range of buffers.** AMP-forming CoA ligase EFD65795 was incubated with *Ma*PatB with or without acetyl-CoA (see Materials and methods) in MES, PIPES, HEPES, HEPPS, and CHES. EFD65795 represents incubation without acetyl-CoA and EFD65795^{K527A} is an inactive variant of this protein. After incubation, CoA ligase activity was determined for acetylated and unacetylated proteins using a CoA ligase activity assay that monitors NADH consumption. Data represent mean ± S.D. of triplicate experiments.

References

Burckhardt, R.M., Buckner, B.A., and Escalante-Semerena, J.C. (2019) *Staphylococcus aureus* modulates the activity of acetyl-Coenzyme A synthetase (Acs) by sirtuin-dependent reversible lysine acetylation. *Mol. Microbiol.* **112**: 588-604.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., and Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647-1649.

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