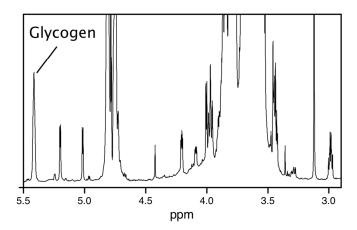
Supporting online information for:

Free glucosylglycerate is a novel marker of nitrogen stress in *Mycobacterium smegmatis*

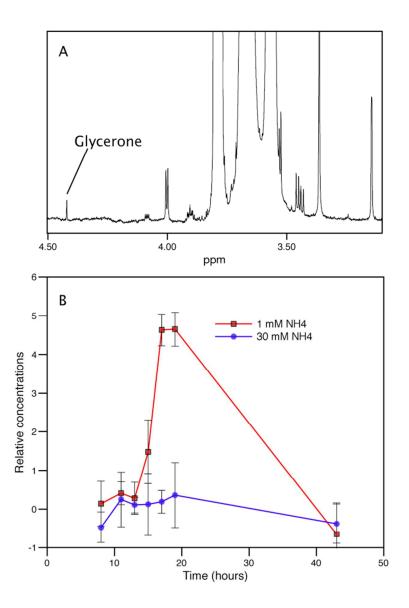
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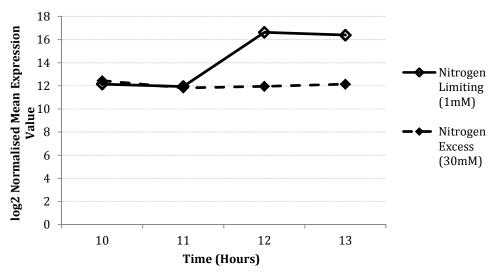
^{*}These authors contributed equally to the study.



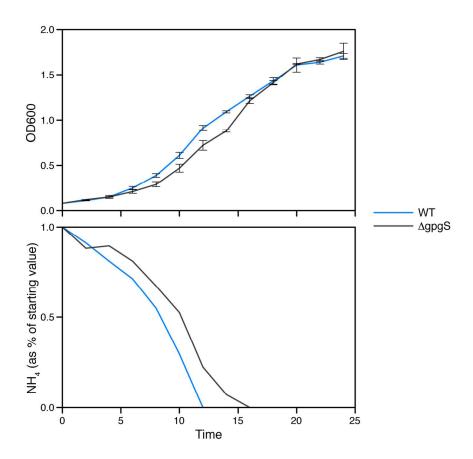
Supporting Figure S1. Characteristic glycogen resonance annotated on ¹H NMR spectrum.



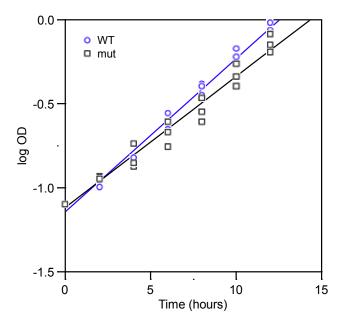
Supporting Figure S2. Detection and response of extracellular glycerone in *Mycobacterium smegmatis* cultures. A: glycerone annotated on ${}^{1}H$ NMR spectrum. B: increase in extracellular glycerone in low-nitrogen cultures following nitrogen run-out. Error bars represent +/- SD (n = 3).



Supporting Figure S3. Expression of gpgS (glucosyl-3-phosphoglycerate synthase) is increased following nitrogen run out at 12 hours in nitrogen limiting cultures in M. smegmatis. Expression values are the mean of three biological replicates)



Supporting Figure S4. A glucosyl-3-phosphoglycerate synthase mutant ($\Delta gpgS$) of M. *smegmatis* (A) has reduced growth rate in nitrogen-limiting conditions compared to wild type and (B) has reduced ammonium uptake rates from the growth medium compared to wild-type. Error bars +/- SEM (n = 3).



Supporting Figure S5. The reduction in growth rate of the $\Delta gpgS$ mutant compared to wild-type is statistically significant. Analysis of covariance (ANCOVA) of log-transformed OD₆₀₀ data up to the point of ammonium runout for the wild-type (12 hours); P = 0.0017, test for homogeneity of regression.