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Supplemental Information

Intracellular *Mycobacterium tuberculosis* Exploits Multiple Host Nitrogen Sources during Growth in Human Macrophages

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Figure S1

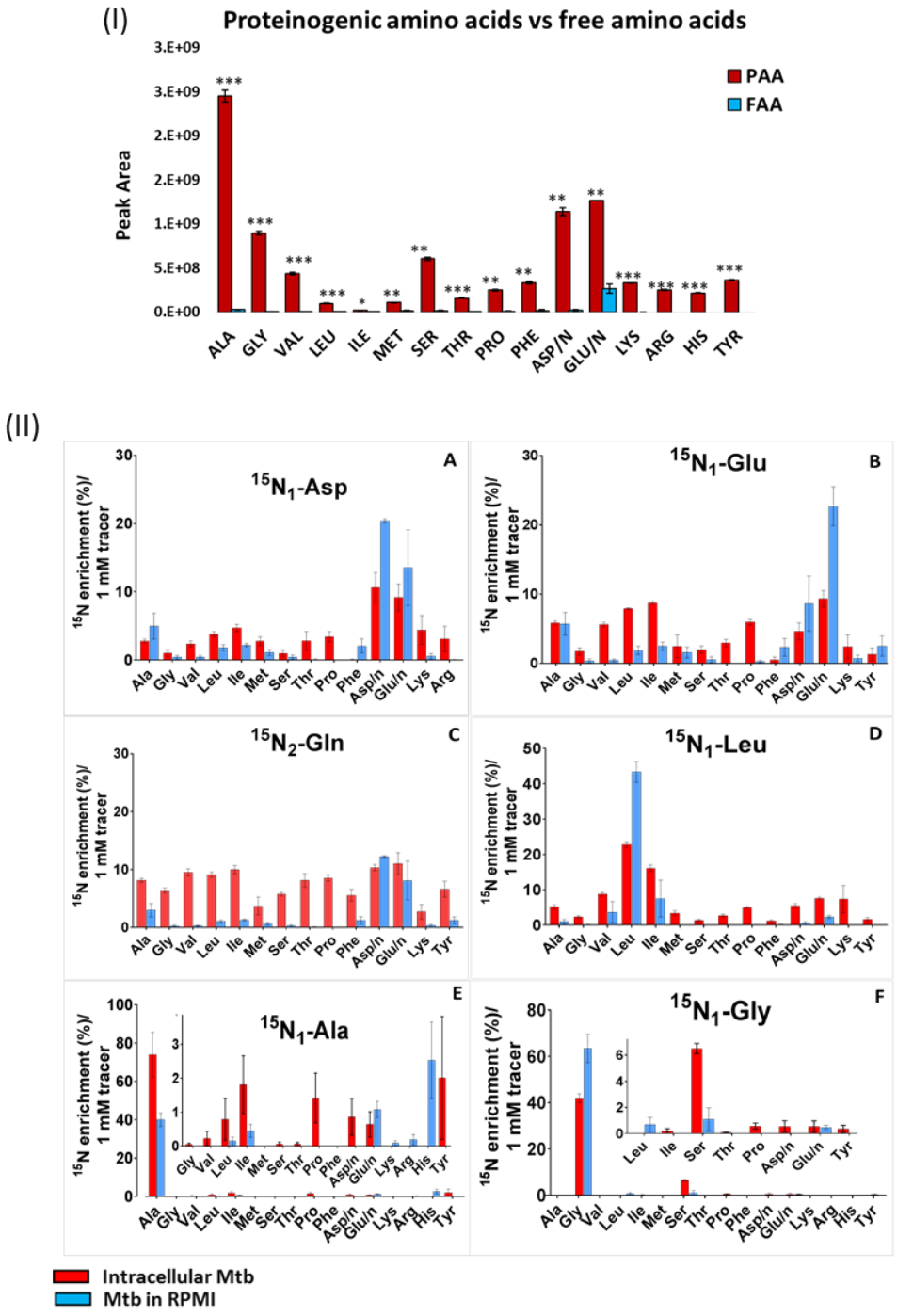

Figure S1 (I) **Relative abundance of free and proteinogenic amino acids in Mtb, Related STAR Methods**. Amino acids- free and proteinogenic were harvested by quenching Mtb cells in methanol:chloroform (2:1), followed by polar/non-polar biphasic separation of free (FAA) amino acids and proteinogenic amino acids (PAA). Free amino acids in the polar phase were dried for GC-MS analysis and proteinogenic amino acids in the non-polar phase were isolated by centrifugation and hydrolysed in 6M HCl, followed by GC-MS analysis. Values are mean ± SEM from 2 technical replicates. Statistical significance, * $P < 0.05$; **, $p < 0.005$; *** $P < 0.0005$. (II) **^{15}N enrichment of amino acids measured in intracellular Mtb and RPMI grown Mtb (control), Related to Figure 1, STAR Methods**. Measurements for intracellular Mtb and RPMI grown Mtb were obtained from experiments using tracers- $^{15}N_1$-Asp (A), $^{15}N_1$-Glu (B), $^{15}N_2$-Gln (C), $^{15}N_1$-Leu (D), $^{15}N_1$-Ala (E) and

$^{15}N_1$-Gly (F). Enrichments were measured ((normalized for each 1mM tracer) in the protein-derived amino acids of intracellular Mtb and RPMI grown Mtb following incubation with the tracers for 48 h. Values are mean ± SEM from 3-8 biological replicates for intracellular Mtb and from 3 biological replicates for RPMI grown Mtb respectively.

Figure S2

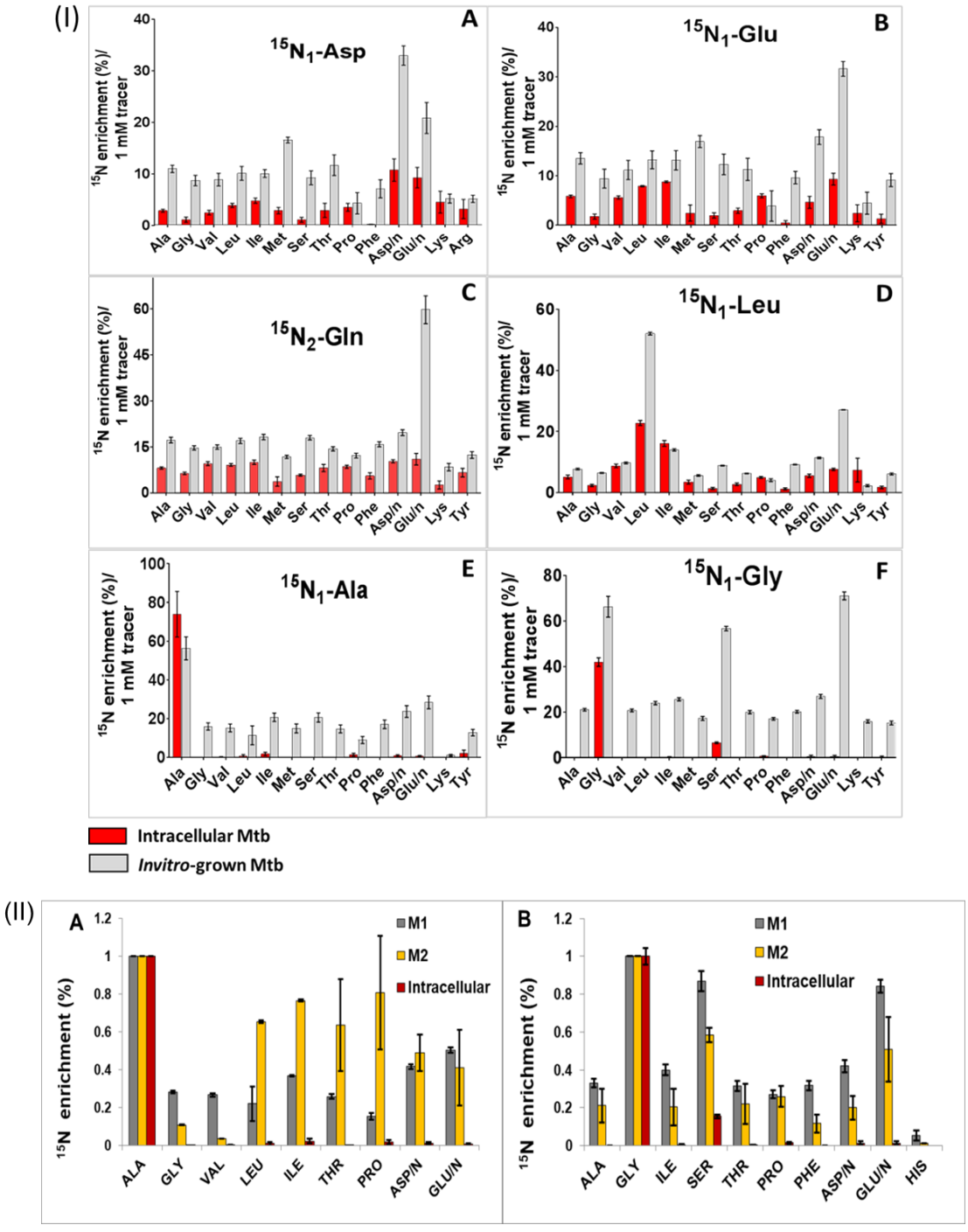

Figure S2. (I) **^{15}N enrichment of amino acids measured in intracellular Mtb and *in vitro*-grown Mtb, Related to Fig. 1**. Data is shown for intracellular Mtb and *in vitro*-grown Mtb in Roisins minimal media following 48 h incubation with each of the tracers $^{15}N_1$-Asp (A), $^{15}N_1$-Glu (B), $^{15}N_2$-Gln (C), $^{15}N_1$-Leu (D), $^{15}N_1$-Ala (E) and $^{15}N_1$-Gly (F). After incubation, enrichments were measured (normalized for

each 1mM tracer) in the protein-derived amino acids of intracellular Mtb isolated from the macrophage and from Mtb incubated in RPMI. Values are mean ± SEM from 3-8 biological replicates for intracellular Mtb and from 3 biological replicates for *in vitro*-grown Mtb respectively. (II) **Comparison of amino acid extraction methods M1 vs M2 from *in vitro*-grown Mtb, Related to Figure 1, STAR Methods**. M1- the original method used in the manuscript to obtain measurements in Fig. 1D and M2 is the method followed exactly like for the amino acid harvest from intracellular Mtb (Fig. 1A) with extensive washing. We performed quantitative comparisons of M1, M2 with the intracellular data (Fig. 1A). Fractional labelling was compared for the two amino acid tracers $^{15}N_1$-ALA (A) and $^{15}N_1$-GLY (B). The enrichments were normalized to the parent amino acid labels for quantitative comparisons across the three methods used for in vitro and intracellular Mtb. Students t-test and Holm-Sidak statistical analysis found no significant differences between the enrichment profiles measured with M1 and M2. Values are mean ± SEM from 3-8 biological replicates for intracellular Mtb and from 3 biological replicates for *in vitro*-grown Mtb respectively.

Figure S3

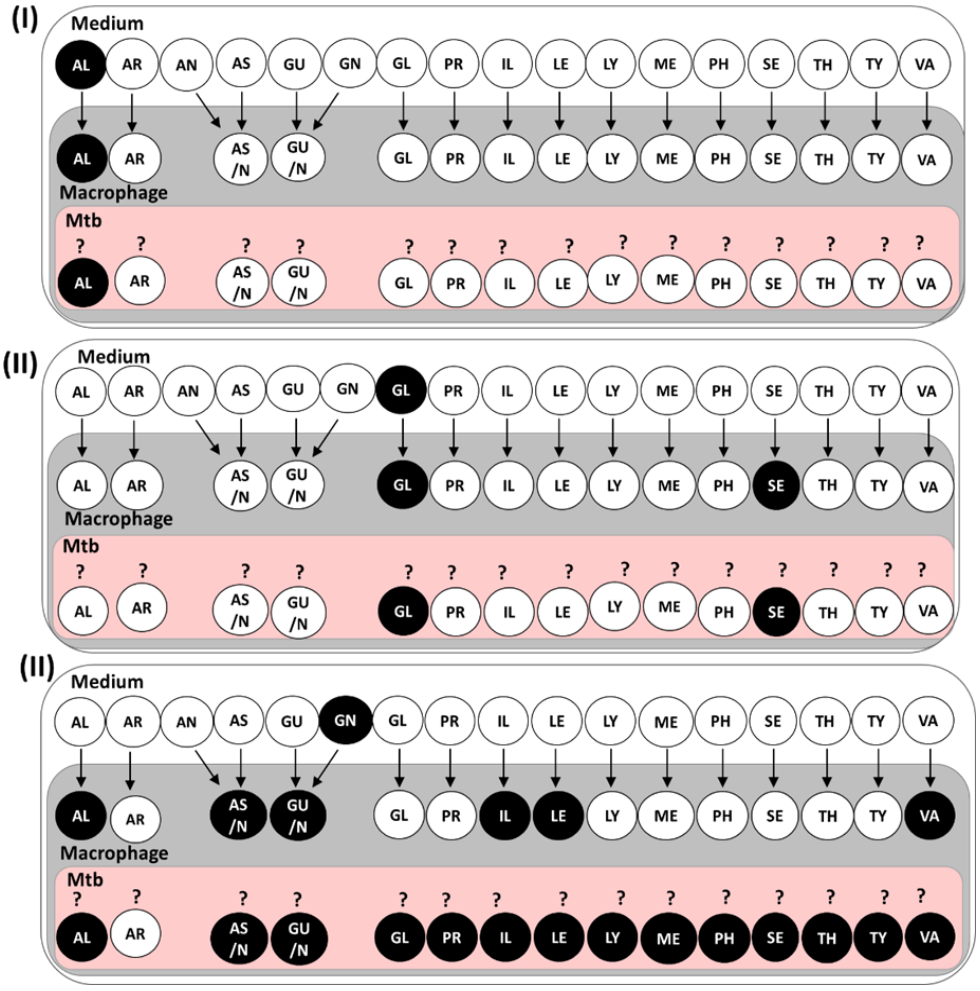

Figure S3. **Qualitative ^{15}N Labelling patterns of intracellular Mtb, infected macrophages and growth medium, Related to STAR Methods**. The pattern is shown for the tracers- $^{15}N_1$-Ala (I), $^{15}N_1$-Gly (II) and $^{15}N_2$-Gln (III). Labelled amino acids are filled in black and the unlabelled amino acids in white. Amino acids acquired directly from the medium by infected macrophage are indicated by arrows. The uncertainty of direct or indirect uptake of amino acids by Mtb from the macrophage is indicated by ?. Abbreviations for amino acids- AL (alanine), AR (arginine), AN (asparagine), AS (aspartate), AS/N (aspartate/asparagine), GU (glutamate), GN (glutamine), GU/N

(glutamate/glutamine), GL (glycine), PR (proline), IL (isoleucine), Le (leucine), LY (lysine), ME (methionine), PH (phenylalanine), SE (serine), TH (threonine), TY (tyrosine) and VA (valine).

Figure S4

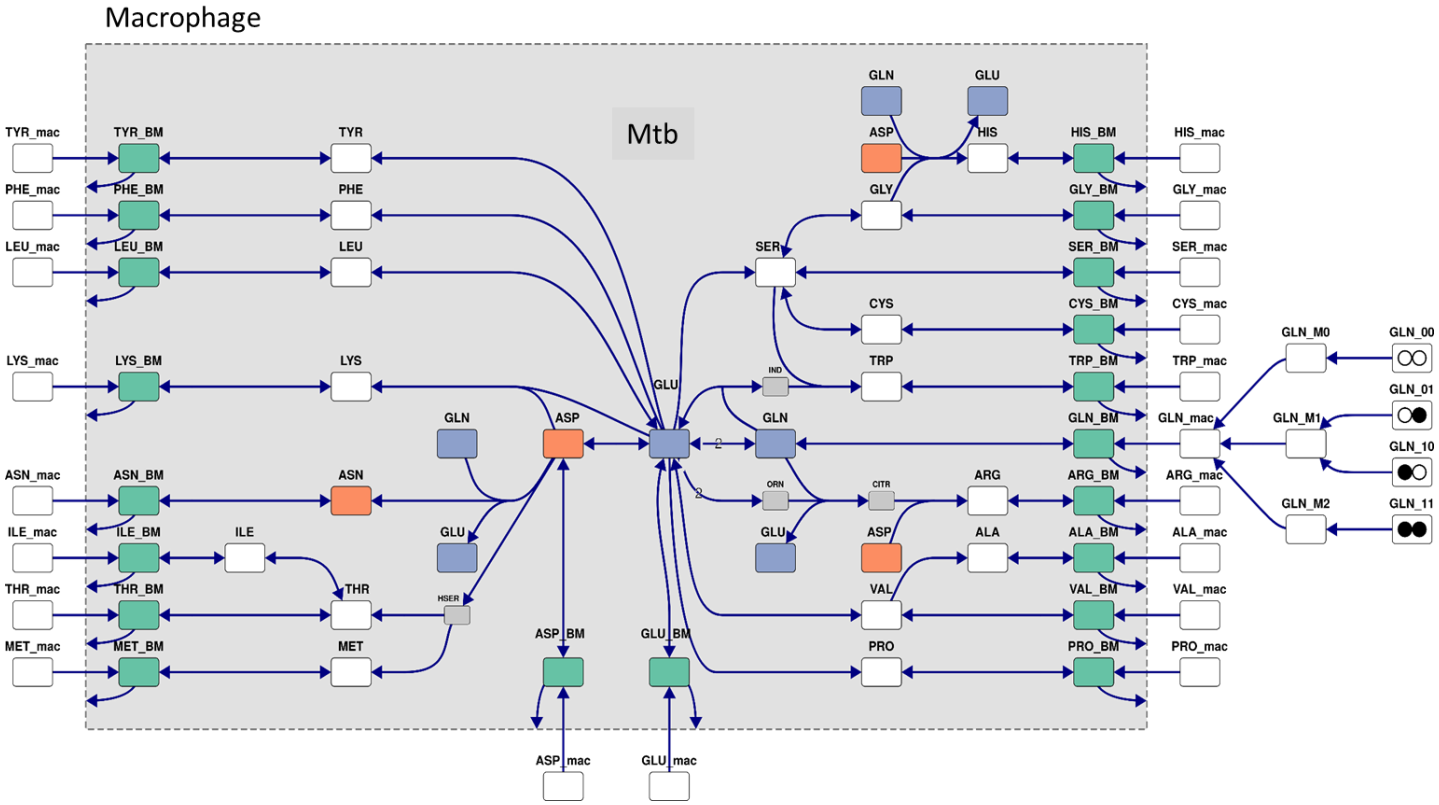

Figure S4. **^{15}N FSRA metabolic network of Mtb, Related to Figure 5 and Table 1.** Network model of nitrogen metabolism. On the right, the input pool deconvolution is shown for GLN (glutamine). Biomass pools are coloured in green. ASP/ASN and GLN/GLU pairs occur several times in the network and are coloured in red and blue, respectively. Grey coloured metabolites indicate intermediate pools. White boxes inside the MTB box code for *de novo* synthesized amino acids. The following abbreviations are used: CITR (citrulline), HSER (homoserine), IND (indole) and ORN (ornithine).

Figure S5

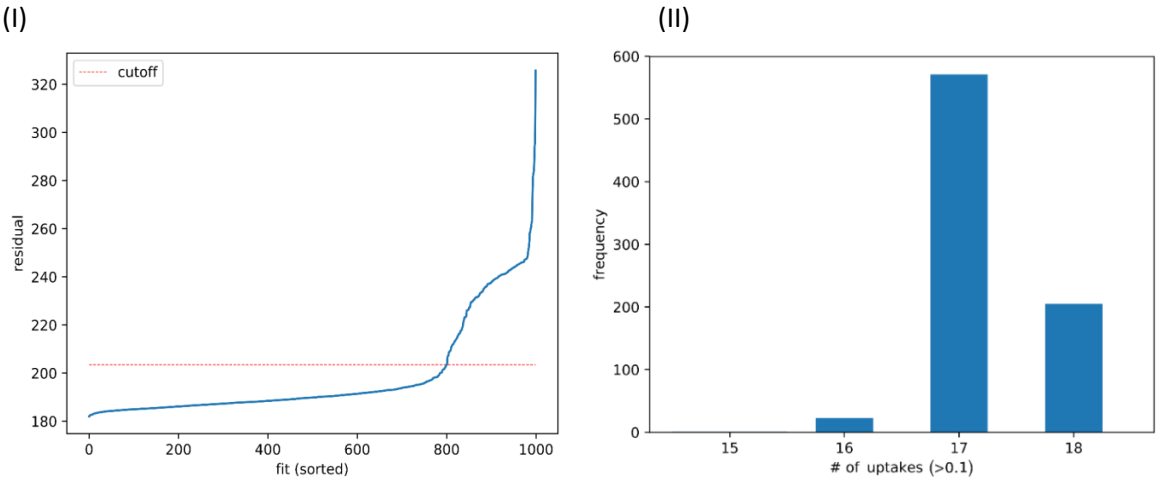

Figure S5. (**I) Residual values, Related to Table 1 and STAR Methods.** Residual values of 1,000 flux estimations sorted by size. Fits with residual values below the red line (cut-off=182.01) were accepted and used for interpretation. (II) **Histogram showing the total number of amino acids taken up from the macrophage, Related to Table 1 and STAR methods.**

Figure S6

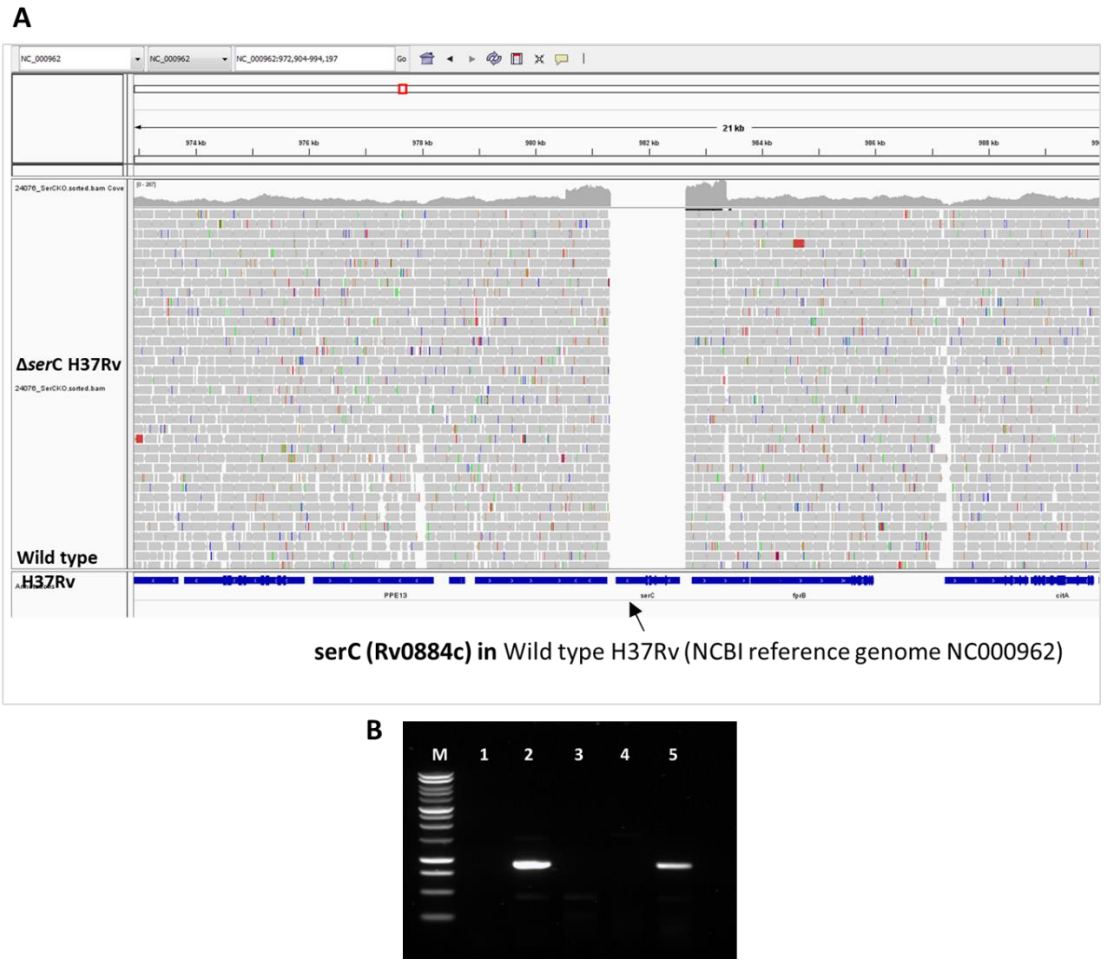

Figure S6. **Confirmation of Δ*serC* mutant and complement Δ*serC*::SERC strains, Related to STAR methods.** (A) Sequence comparisons between the wild type and Δ*serC* H37Rv strains using Artemis. The comparisons show a gap in *ser*C (Rv0884c) gene in the mutant strain confirming the deletion of the gene (Data file S5). (B) PCR analysis to confirm the complement Δ*serC*::SERC. 923kb PCR product of *hsp60* promoter and *serC* gene was amplified from the pMV361*serC* construct (lane 2) and Δ*ser*C::SERC genomic DNA (gDNA) (lane 5) confirming the complement to have the integrated construct pMV361*serC*. Marker-M, 1- water control, 2- pMV361*serC* plasmid, 3- WT H37Rv gDNA , 4- Δ*serC* H37Rv gDNA and 5- Δ*serC*::SERC H37Rv gDNA.

Table S1**. A- Strains and plasmids used for construction of Δ*serC* mutant and complement Δ*serC*::SERC strains, Related to STAR Methods. B- Primers used for construction of Δ*serC* mutant and complement Δ*serC*::SERC strains, Related to STAR methods.**

A

Strain	**Genotype and relevant characteristics**	**Reference or source**
Escherichia coli DH5α	Competent cells	Beste et al., 2013
E. coli HB101	F–, thi-1, hsdS20 (rB–, mB–), supE44, recA13, ara-14, leuB6, proA2, lacY1, galK2, rpsL20 (strr), xyl-5, mtl-1	Invitrogen
E. TOP10	Competent cells, HYG^R	Thermo Fisher Scientific
E. coli DH5α-pMV361*serC*	Strain harbouring pMV361*serC* construct, KAN^R	This work
M. smegmatis mc^2155	Easily transformable strain, used for phage preparation	Baradov et al., 2002
Plasmid		
pYUB854	Cosmid vector, with res sites flanking the HYG^R gene	Bardarov et al. 2002
pYUB854_LF	pYUB854 + 716 bp left flanking sequence of *serC* (Rv0884c)	This work
pYUB854_RF	pYUB854 + 787 bp right flanking sequence of *serC* (Rv0884c)	This work
pYUB854_LFRF-AES	pYUB854 + left and right flanking sequence of *serC* (Rv0884c)	This work
pMV361	*E. coli*-Mycobacteria shuttle vector, groEL2 (*hsp60*) promoter, KAN^R, OriM	Stover et al., 1991
pMV361*serC*	pMV361 containing serC (Rv0884c) gene, KAN^R	This work
Phage		
phAE159	Mycobacterial phage	Bardarov et al. 2002
phAE159:Δ*serC*	phAE159 + pYUB854_LFRF-AES, HYG^R	This work

B

Primer Sequences		
Gene/loci	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
serC (Rv0884c) upstream (left flanking region), allelic exchange	GGTGGTCTTAAGATGATCGGATGCAGCGACTT	GGTGGTTCTAGACTGGTGGCTGGGTCATAGTG
serC (Rv0884c) downstream (right flanking region), allelic exchange	GGTGGTAAGCTTTAGAGTGCGCACGTAACAGG	GGTGGTACTAGTCACATCTTCCCAGGCAGGTA
serC (Rv0884c) PCR to construct pMV361*ser*C	GGTGGTGAATTCATGGCCGACCAGCTCAC	GGTGGTAAGCTTCTAAAGCCGCTCGACCACC
Δ*serC*::SERC confirmation PCR	ACATACTCACCCGGATCGGA	CGAAGTAGTAGGCGTCGGTC