SUPPLEMENTARY INFORMATION FOR:

Mycobacterium tuberculosis nitrogen assimilation and host colonization require aspartate

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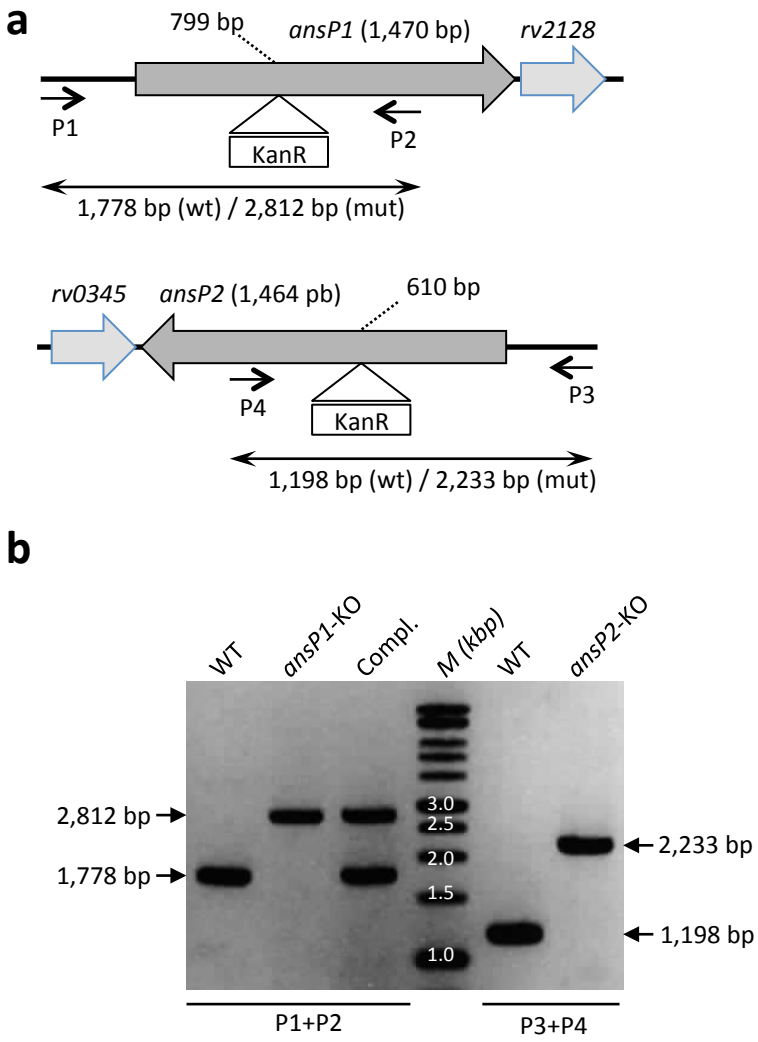
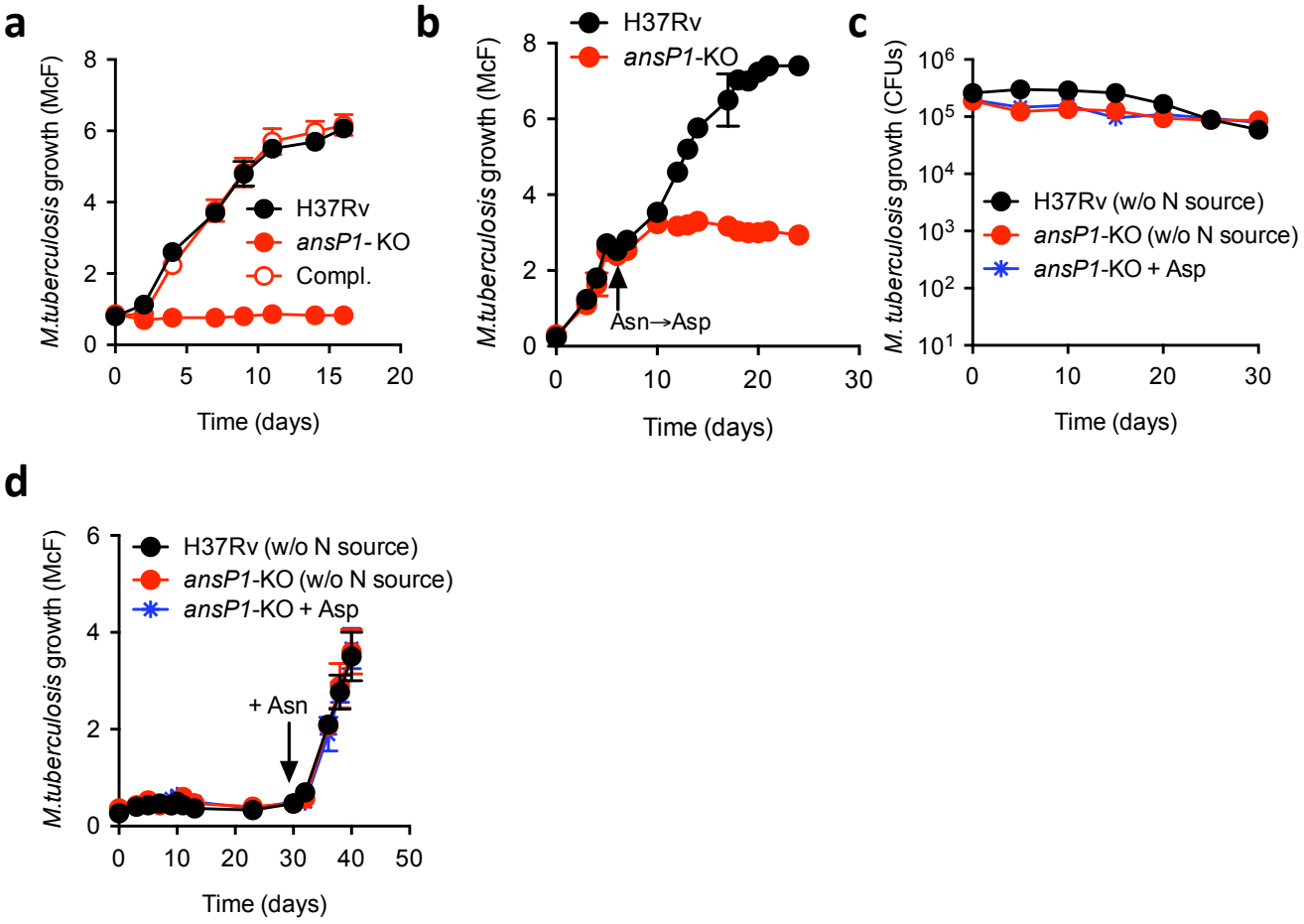
SUPPLEMENTARY RESULTS

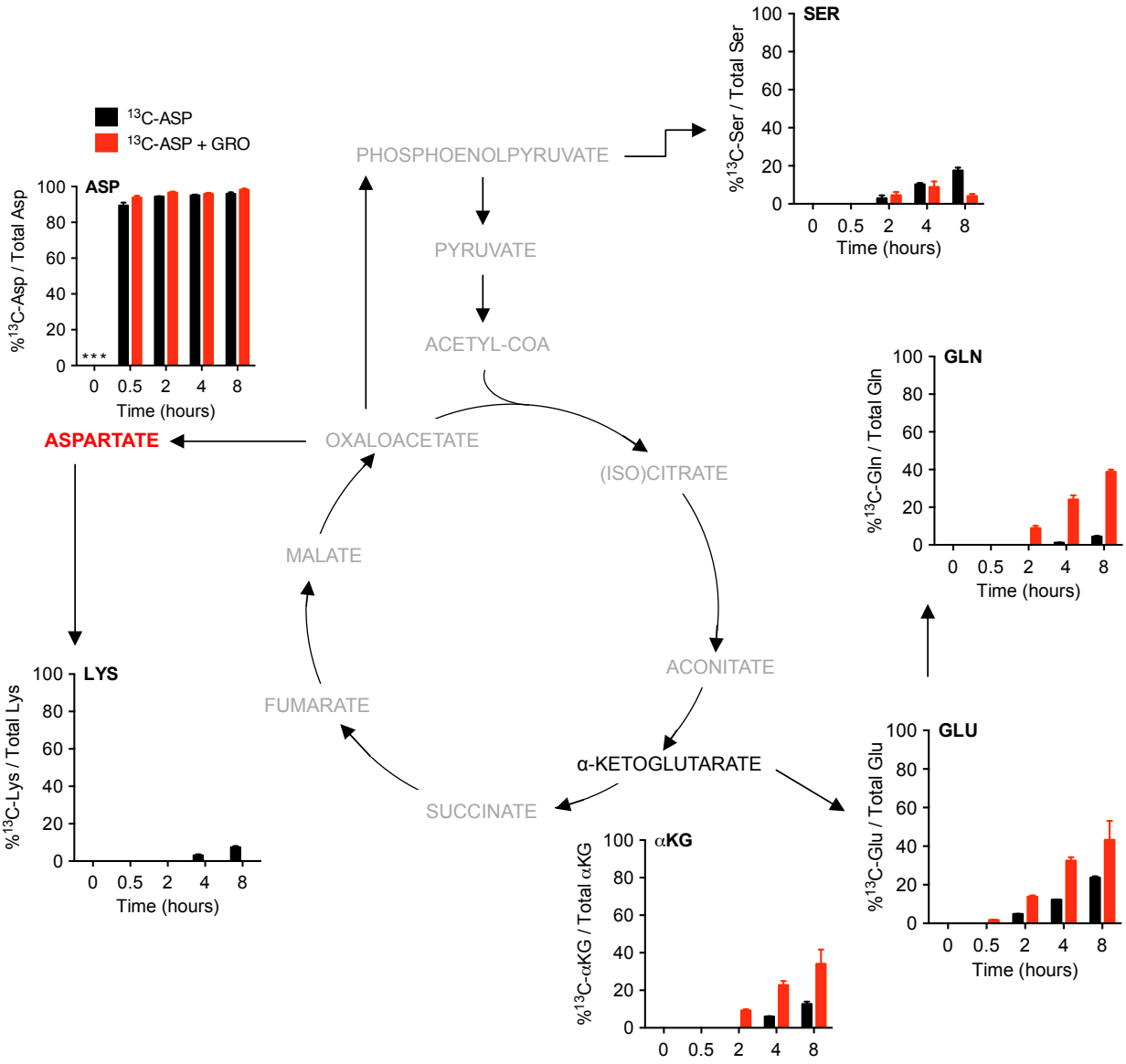


Supplementary Figure 1: Generation and characterization of *ansP1*-KO and *ansP2*-KO mutants. **(a)** A schematic representation of the genetic organization of *ansP1* and *ansP2* genes. The position of the oligonucleotides (P1-4) used for PCR validation and the expected size of the resulting amplicons are shown. **(b)** PCR analysis of the *ansP1*-KO and *ansP2*-KO mutants with the primers shown in (a).

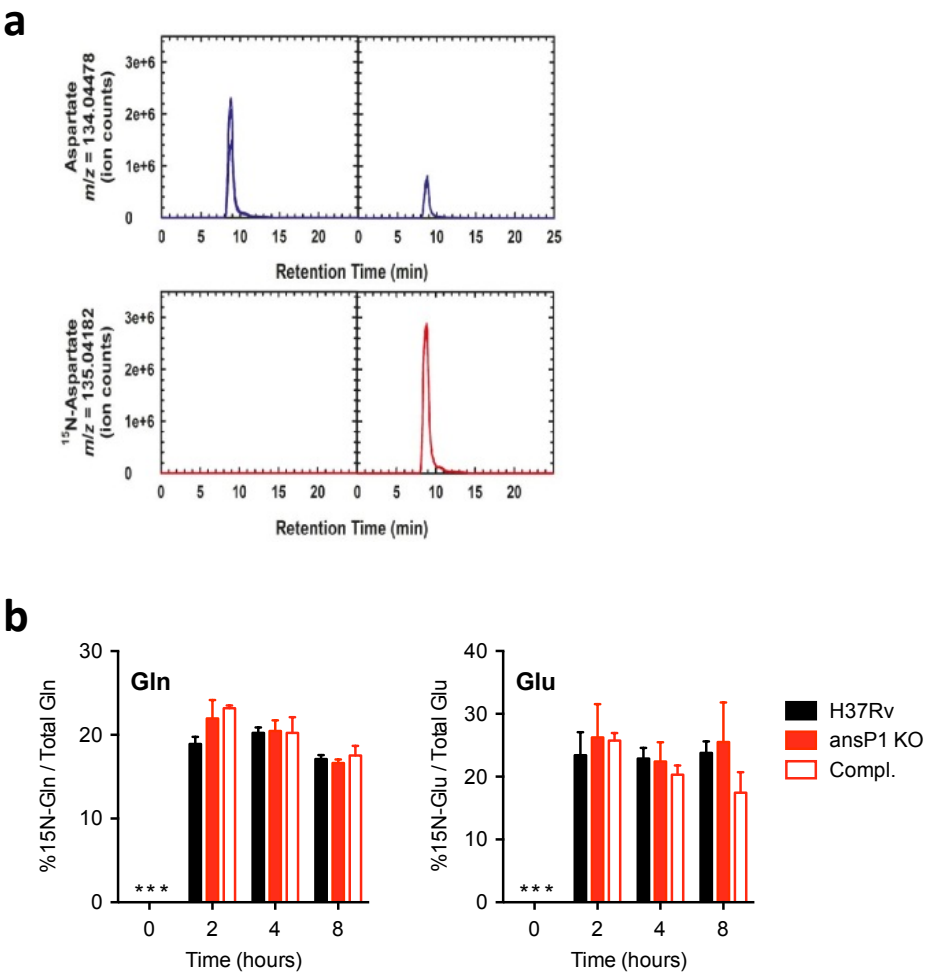


Supplementary Figure 2: AnsP1 is essential for *M. tuberculosis* growth on aspartate as sole nitrogen source.

Growth of *M. tuberculosis* H37Rv, the *ansP1*-KO mutant and the *ansP1*-KO complemented (Compl.) strains in various conditions. Growth was measured by monitoring turbidity (a, b, d) or CFU scoring (c); data represent mean ±s.d. of triplicate samples and are representative of at least three independent experiments. (a) Bacteria were grown in minimal medium containing 5 mM aspartate as sole nitrogen source. (b) Bacteria were grown for 5 days in minimal medium containing 5 mM asparagine (Asn) as sole nitrogen source, after what asparagine was replaced by 5 mM aspartate (Asp) for the rest of the experiment. (c) Bacteria were grown for 30 days in minimal medium void of any nitrogen source (w/o N source) or supplemented with 5 mM aspartate (Asp) as sole nitrogen source. (d) Bacteria were grown as in (c), and asparagine was added to the culture medium to a final concentration of 5 mM after 30 days of bacterial growth.

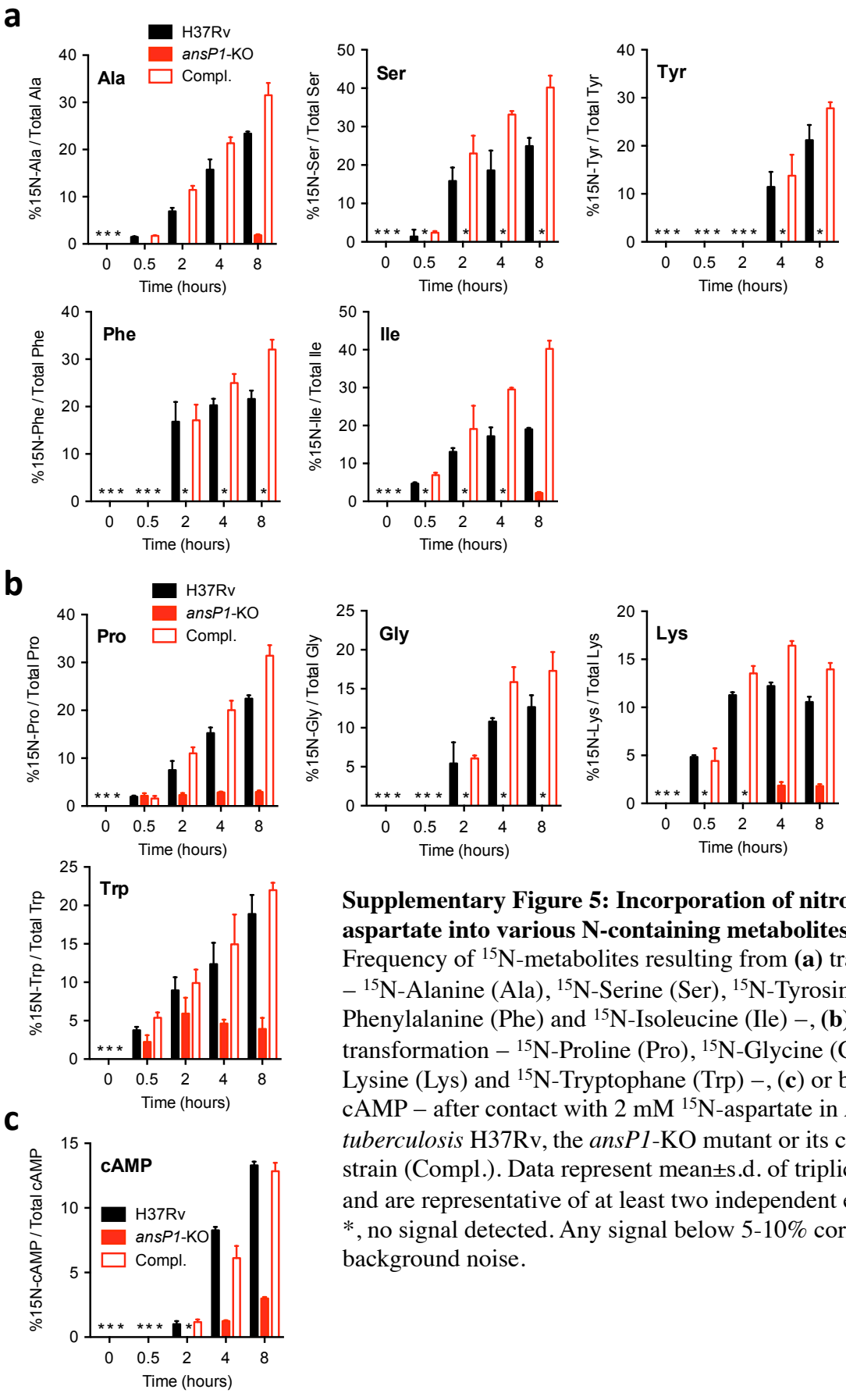


Supplementary Figure 3: Assimilation of U-^{13}C isotopic-labeled aspartate.
The figure shows isotopic incorporation of U-^{13}C aspartate carbon into intracellular pool of selected metabolites (aspartate, lysine, α-ketoglutarate, glutamate, glutamine and serine), in the absence or presence of unlabeled glycerol. Isotopic labeling is indicated on the Y-axis as percentage of ^{13}C incorporated at the indicated time points. U-^{13}C-aspartate was provided at 2 mM and unlabeled glycerol at 10 g/L. Data represent mean±s.d. of three biological replicates.

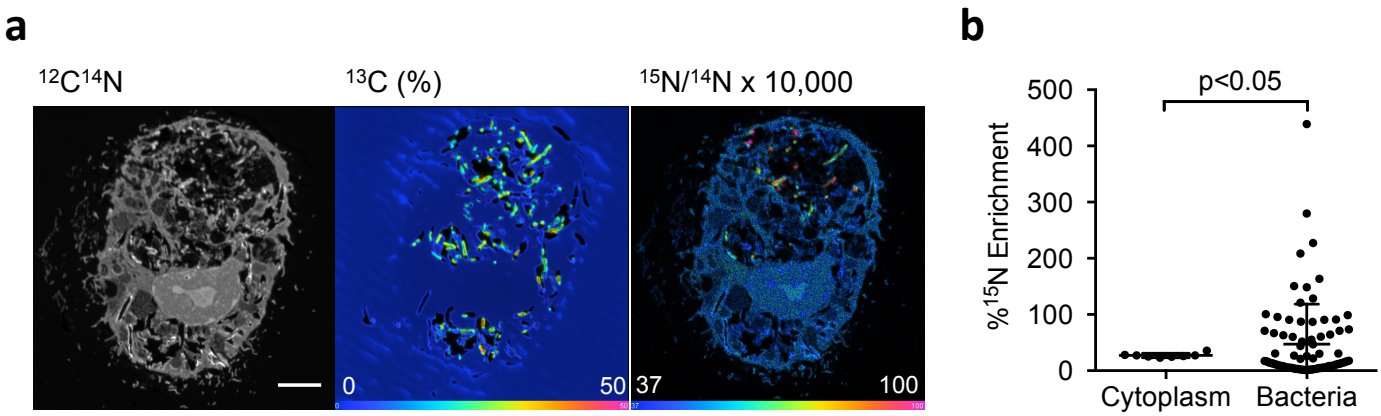


Supplementary Figure 4: Incorporation of nitrogen from asparagine into glutamine and glutamate.

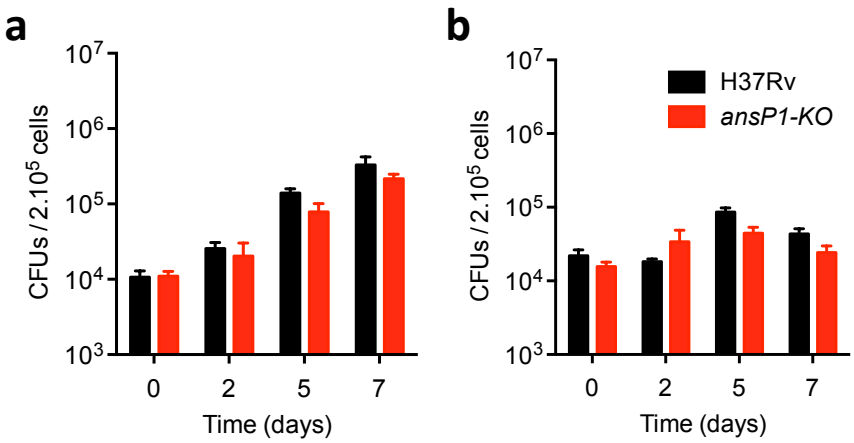
(a) Illustrative plot showing representative chromatograms of natural and ^{15}N-labelled aspartate from *M. tuberculosis* metabolome. Nitrogen labelling is easily detected by accurate mass TOF. Extracted ion chromatograms of unlabelled aspartate (top panel) and [^{15}N]-aspartate (bottom panel). Left panels show time zero (no label in the media), and right panels show data for 3 hours exposure to media containing labeled aspartate. **(b)** Frequency of ^{15}N-glutamine and ^{15}N-glutamate after contact with 2 mM $^{15}N_2$-asparagine in *M. tuberculosis* H37Rv, the *ansP1*-KO mutant, or its complemented strain (Compl.). Data represent mean ±s.d. of triplicate samples and are representative of three independent experiments. *, no signal detected. Any signal below 5-10% corresponds to background noise.



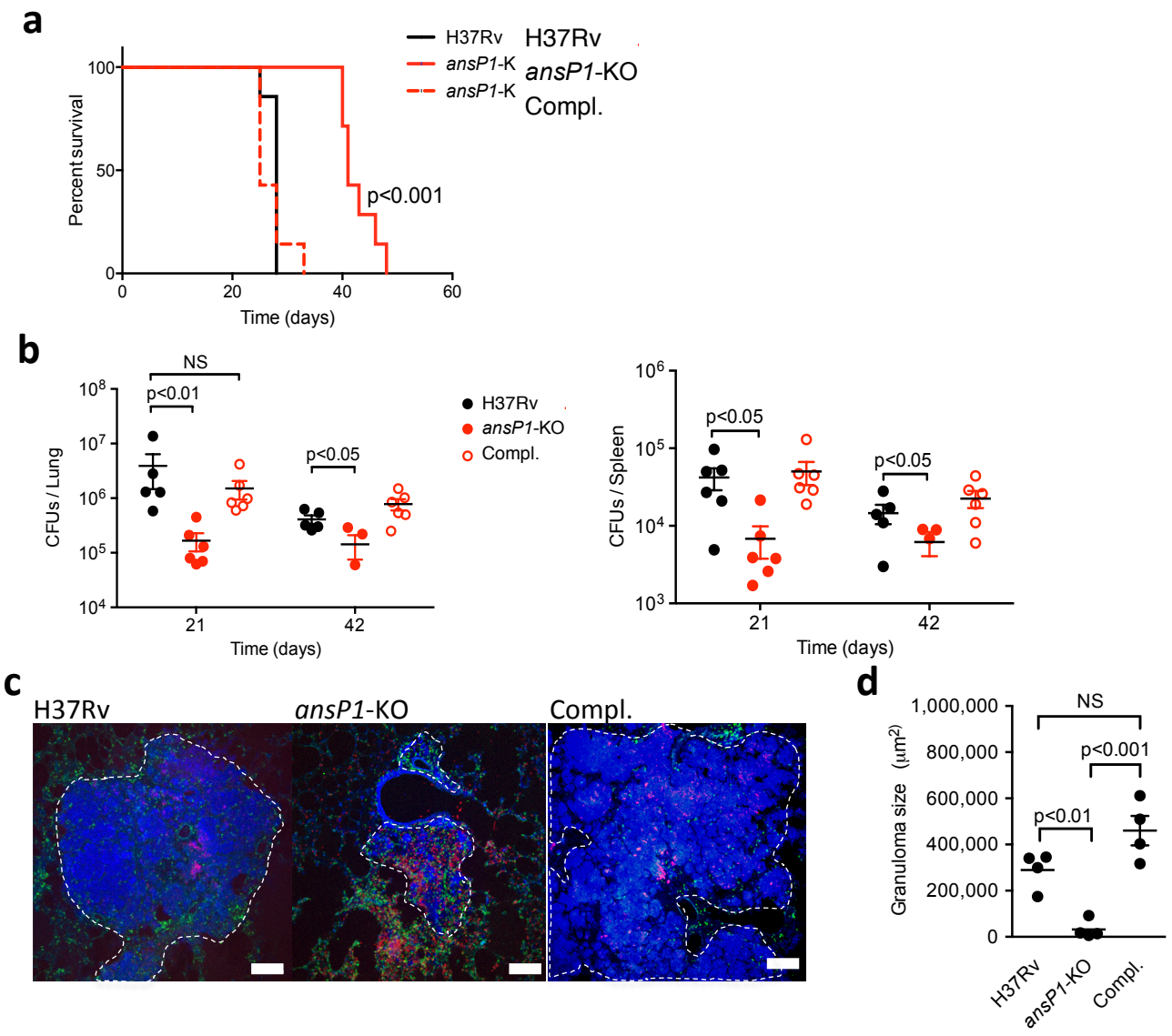
Supplementary Figure 5: Incorporation of nitrogen from aspartate into various N-containing metabolites. Frequency of ^{15}N-metabolites resulting from **(a)** transamination – ^{15}N-Alanine (Ala), ^{15}N-Serine (Ser), ^{15}N-Tyrosine (Tyr), ^{15}N-Phenylalanine (Phe) and ^{15}N-Isoleucine (Ile) –, **(b)** direct transformation – ^{15}N-Proline (Pro), ^{15}N-Glycine (Gly), ^{15}N-Lysine (Lys) and ^{15}N-Tryptophane (Trp) –, (**c**) or both – ^{15}N-cAMP – after contact with 2 mM ^{15}N-aspartate in *M. tuberculosis* H37Rv, the *ansP1*-KO mutant or its complemented strain (Compl.). Data represent mean±s.d. of triplicate samples and are representative of at least two independent experiments. *, no signal detected. Any signal below 5-10% corresponds to background noise.



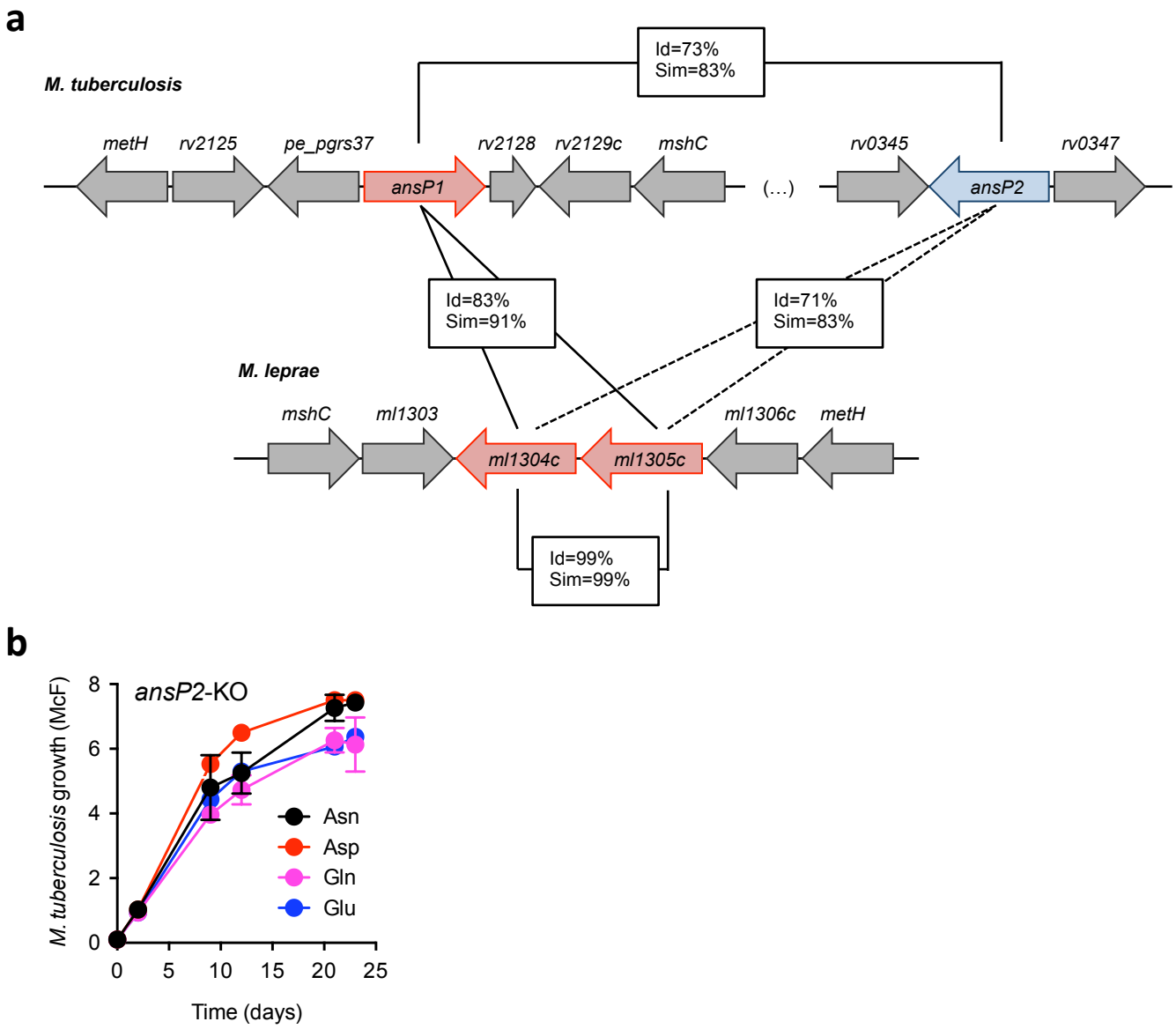
Supplementary Figure 6: Aspartate can access the mycobacterial phagosome in murine macrophages. (a) ^{13}C-labeled bacteria were used to infect mouse bone marrow-derived macrophages at a multiplicity of infection of 10 bacteria per cell for 4 hrs, after which cells were washed and pulsed with fresh medium. At 20 hrs post-infection, cells were pulsed for 4 hrs with 2 mM ^{15}N-aspartate, and ^{13}C and ^{15}N isotope compositions were analyzed by dynamic secondary ion mass spectrometry (NanoSIMS). Images display a representative infected cell, as derived from NanoSIMS. Left panel is the as recorded $^{12}C^{14}N^-$ image showing the histological aspect of the cell (scale bar represents 5 μm). The central panel represents the ^{13}C atomic fraction map (in %) of the corresponding area, which allows the identification of the mycobacteria within the cell. The image is displayed in HSI (Hue-Saturation-Intensity) color scale with linear scale bar. Highly labeled bacteria appear purple, those moderately labeled in yellowish-green, all contrast with the natural background level in blue. The right panel, also displayed in HIS color scale, shows the $^{15}N/^{14}N$ ratio image indicating the ^{15}N-aspartate uptake. For enhanced visibility, the ratio was multiplied by $1x10^4$. The $^{15}N/^{14}N$ ratio at natural ^{15}N abundance appears blue. **(b)** Quantification of ^{15}N isotope enrichment (compared to the resin) in surface areas chosen in the cell cytoplasm (n=8), and intracellular ^{13}C labeled bacteria (n=82). Data represent mean ±s.d. and were analyzed using the Student's *t* test. The ^{15}N-enriched phagosomes, arbitrarily defined as those vacuoles with a ^{15}N enrichment above mean +3s.d. of that observed in the host cell cytoplasm, represent 35.4% of all phagosomes.



Supplementary Figure 7: The *ansP1*-KO mutant is not attenuated in murine macrophages. Resting (left panel) or LPS- and IFNγ-activated (right panel) mouse bone marrow-derived macrophages were infected at a MOI of 0.1 bacterium/cell for 4 hrs, washed and chased with fresh medium for the indicated time. Cells were then lysed, and cell lysates were plated onto agar for CFU scoring. Data show mean ±s.d., are representative of two independent experiments and were analyzed using the Student's *t* test. No significant difference was observed between the wild-type and the mutant strains under any conditions or time-points tested.



Supplementary Figure 8: AnsP1 is involved in *Mycobacterium tuberculosis* virulence.
(a) Immuno-deficient SCID mice (n=7) were infected intranasally with 500 CFUs of *M. tuberculosis* wild-type (H37Rv), the *ansP1*-KO mutant or its complemented strain (Compl.) , and survival was monitored over time. Data are representative of two independent experiments, and were analyzed using the Kaplan-Meier method. **(b)** C57BL/6 mice were infected intranasally with 500 CFUs of *M. tuberculosis* wild-type (H37Rv), *ansP1*-KO or its complemented strain (Compl.). At day 21 and 42 after infection, the mice were sacrificed, and their lung and spleen homogenates were plated onto agar for CFU scoring. Each dot corresponds to one animal. Data were analyzed using the Student's *t* test and are representative of three independent experiments. **(c)** Immuno-histological analysis of the lungs of C57BL/6 mice infected for 21 days with *M. tuberculosis* H37Rv (left panel), the *ansP1*-KO mutant (middle panel) or the complemented mutant (right panel). Images represent Z-stacks of 30 µm sections showing F4/80 in blue (macrophages), CD4 (T cells) in green and Gr1 in red (neutrophils, monocytes). Bar represents 100 µm **(d)** Granuloma size was calculated as the surface area occupied by F4/80 positive cells (dotted white lines in (c)) for 4 independent granulomas. Data were analyzed using the Student's *t* test. In (c) and (d), data are representative of two independent experiments. NS, not significant.



Supplementary Figure 9. Genetic organization of the *ansP* loci in *M. tuberculosis* and *M. leprae* and growth of an *ansP2*-KO mutant on various nitrogen sources.
(**a**) Genetic loci were retrieved from the Tuberculist (http://genolist.pasteur.fr/TubercuList/) and Leproma(http://genolist.pasteur.fr/Leproma/) servers. Gene homology was analyzed using the Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). (b) Growth of *M. tuberculosis* H37Rv and the *ansP2*-KO mutant in minimal medium containing 5 mM asparagine (Asn), aspartate (Asp), glutamine (Gln) or glutamate (Glu) as sole nitrogen sources. Growth was measured by monitoring turbidity; data represent mean±s.d. of triplicate samples and are representative of at least three independent experiments.