Supplementary information

Selective enrichment of mycobacterial proteins from infected host macrophages

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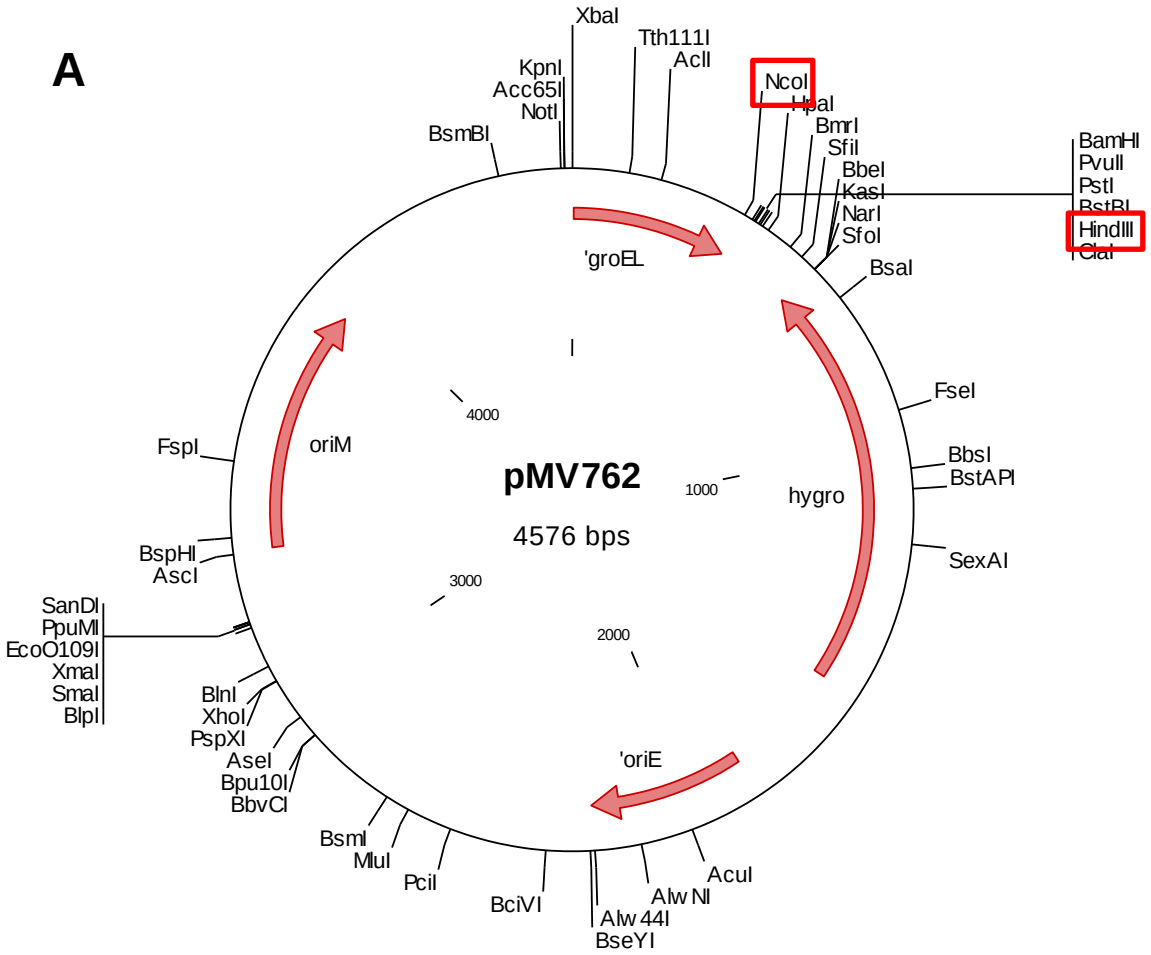
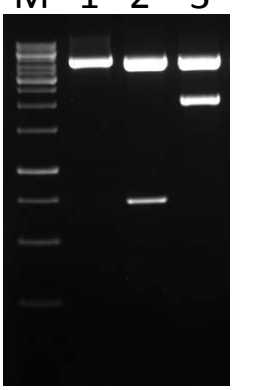
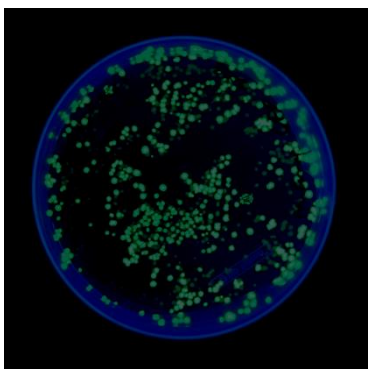
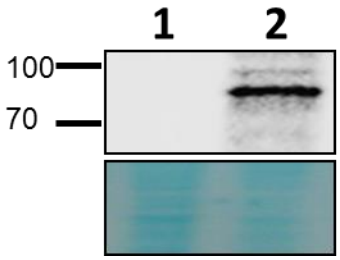
Supplementary Fig. 1









1: pMV762

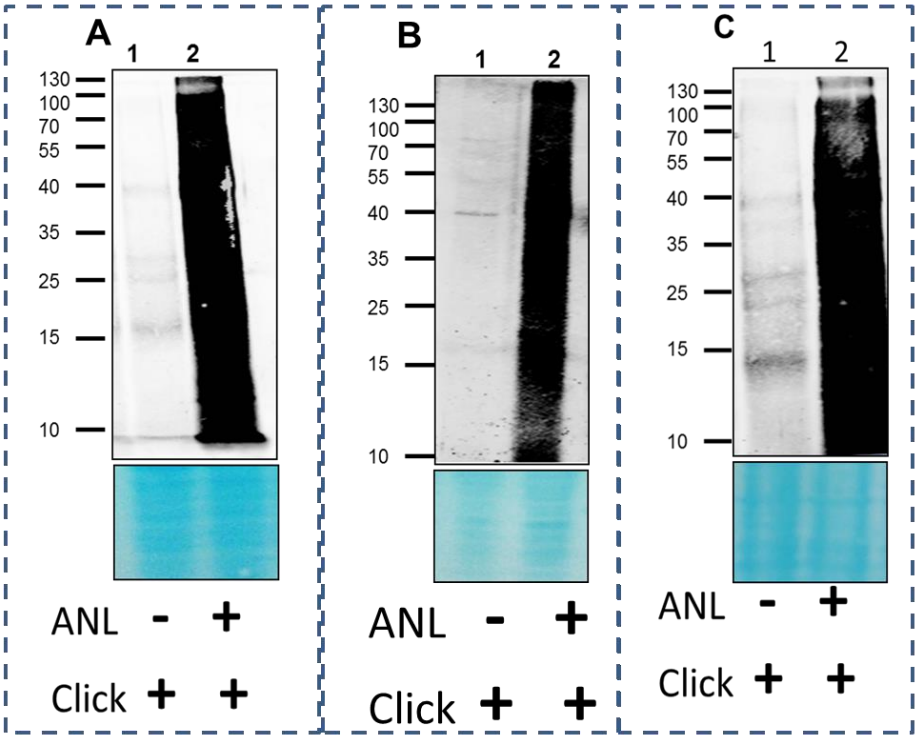
2: pMV-GFP

3: pMV-Met-HA

M: 1kb DNA marker

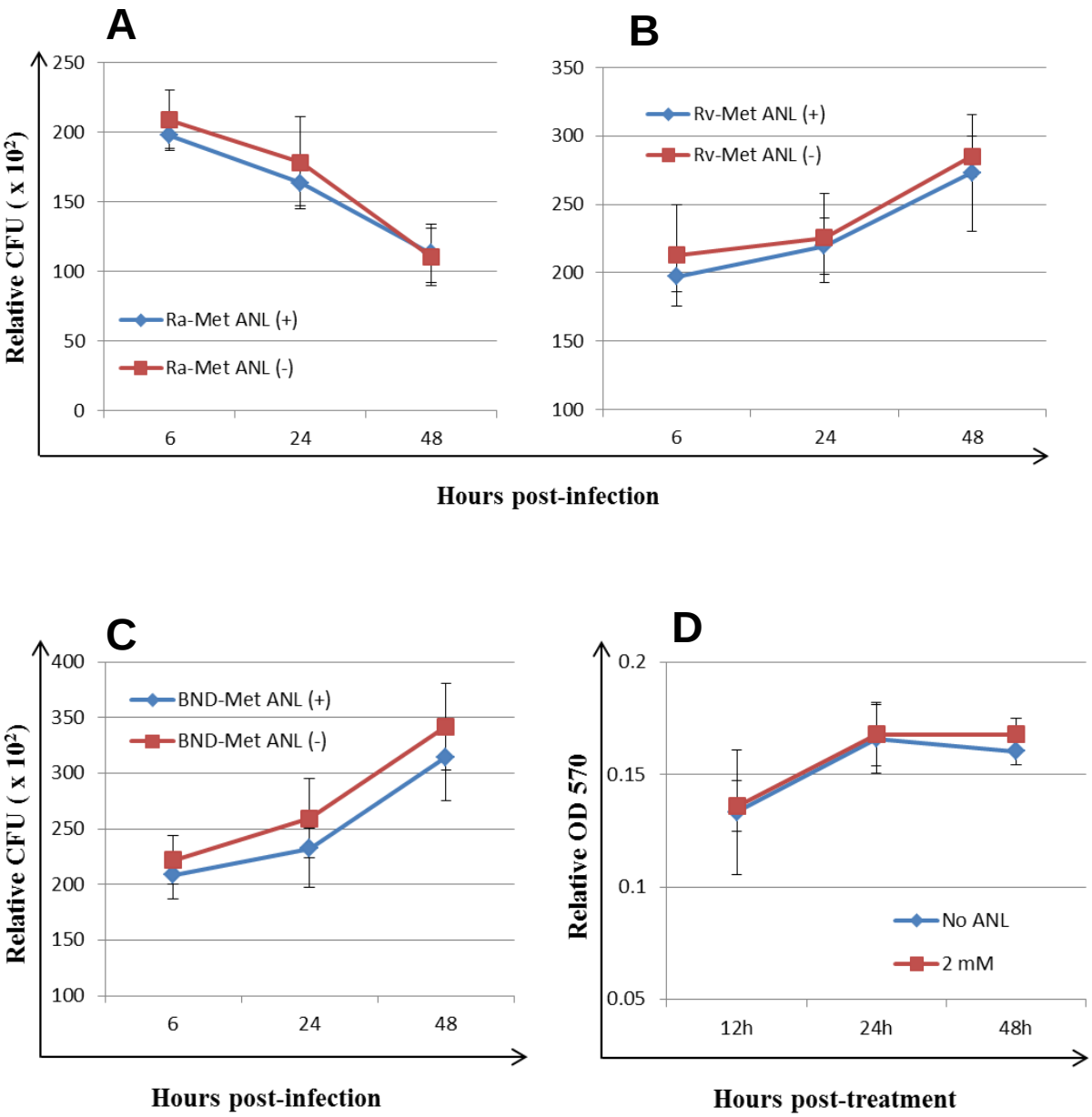
Suppl. Fig-1. Generation of mycobacterial constructs for expression of the target protein and metabolic labeling: (A) pMV762 vector map showing the NcoI/HindIII sites downstream to the GroEL promoter; (B) The GFP and HA-tagged Myco-Met encoding nucleotides were cloned in pMV762 at NcoI/HindIII sites. The gel picture shows the fragment release upon digestion with the said enzymes; (C) GroEL promoter driven GFP expression in M. smeg colonies: pMV762-GFP vector transformed colonies were selected on hygromycin and checked for the expression of GFP under a UV trans-illuminator; (D) HA tagged Myco-MetRS expression from the lysates of empty vector transformed (1) and pMV-Myco-MetRS-transformed (2) M. smeg strain was assessed by immunoblotting using mouse monoclonal anti-HA antibody (1:4000 dilution). The signal was detected using IR-labeled anti-mouse secondary antibody. Stained membrane served as a loading control.

Supplementary Fig. 2



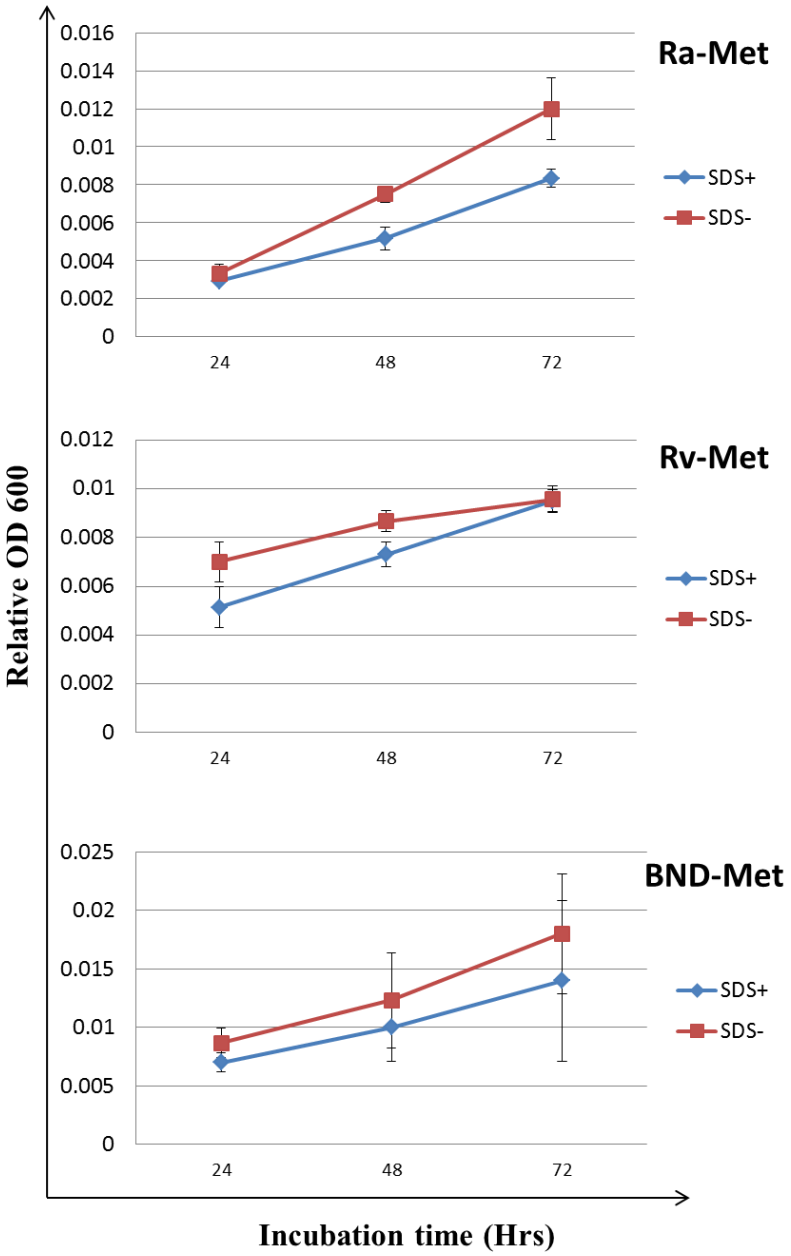
Suppl. Fig-2. Metabolic incorporation of ANL into proteins of H37Rv (A), H37Ra (B) and BND433 (C): Cell were grown in the presence (+) or absence (-) of ANL, and all the cell lysates were treated with biotin-alkyne for covalent incorporation using click reaction. Streptavidin –IR800CW dye signal was captured using Odyssey infra-red scanning system. Stained membrane (bottom) served as a loading control. The signal indicates streptavidin interaction with the covalently captured biotin-alkyne to the ANL incorporated into cellular proteins.

Supplementary Fig. 3



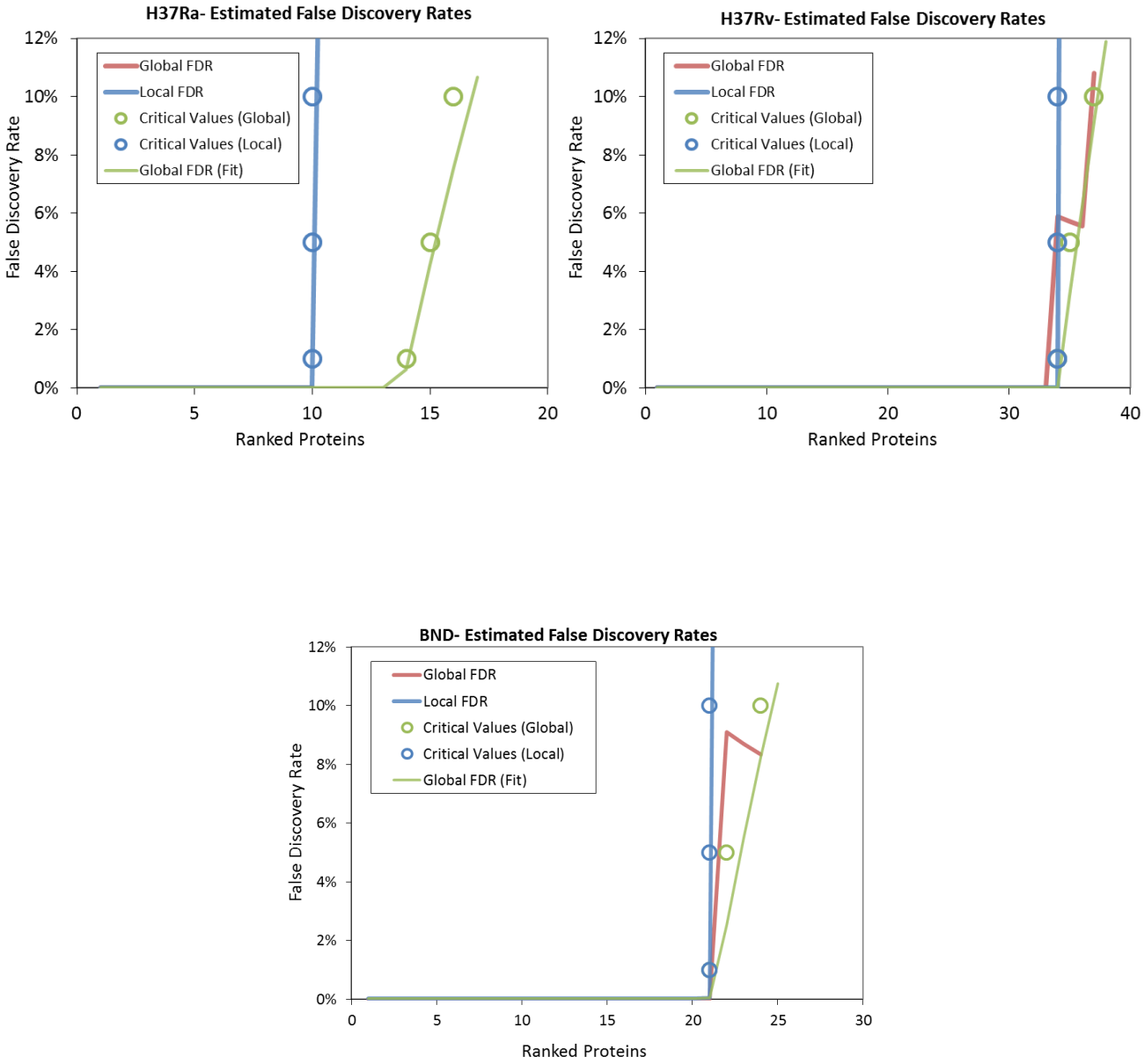
Suppl. Fig-3. ANL toxicity: growth properties of the individual isolates (A-C) and the THP1 host macrophages (D) in presence or absence of ANL. For the mycobacterial isolates CFU values, expressed as a function of viable mycobacterial cells obtained at the indicated times after infection, are shown. Whereas the effect of ANL on the host was examined by MTT assay. (n=3, ±SD)

Supplementary Fig. 4



Suppl. Fig-4. Extracellular growth properties of the individual isolates: Mycobacteria were recovered from the infected host by lysis with 1% SDS followed by centrifugation. Recovered bacteria were washed with PBS three times and then inoculated in the middlebrook 7H9 medium. The optical density (OD;600) obtained at the indicated times is shown. Untreated 7H9 broth cultures (SDS-) served as the respective controls for each of the strain under study. (n=3, ±SD)

Supplementary Fig. 5



Suppl. Fig-5. Measured False Discovery Rates for MTb Strains: Threshold of 5% accepted Global False discovery rate (G-FDR-fit) proteins criteria were selected for identification of Mtb secreted proteins.

Sequence of Myco-MetRS:

ATGACCCAGGTGGCCAAGAAAATCCTGGTGACCTGCGCCAACCCGTACGCC
AACGGCTCGATCCACCTGGGCCACATGCTGGAACACATCCAGGCCGACGTG
TGGGTGCGCTACCAGCGCATGCGTGGCCACGAGGTGAACTTCATCTGTGCC
GACGACGCCCACGGCACCCCGATCATGCTGAAGGCCCAGCAGCTGGGCAT
CACCCCCGAGCAGATGATCGGCGAGATGTCGCAAGAGCACCAGACCGACT
TCGCCGGTTTCAACATCTCGTACGACAACTACCACTCGACCCACTCGGAGG
AAAACCGCCAGCTGTCGGAGCTGATCTACTCGCGCTTGAAAGAGAACGGC
TTCATCAAGAACCGCACCATCTCGCAGCTGTACGACCCGGAAAAGGGCATG
TTCCTGCCGGACCGCTTCGTGAAGGGCACGTGCCCGAAGTGCAAGTCGCC
GGACCAGTACGGCGACAACTGCGAGGTGTGCGGTGCCACCTACTCGCCGA
CCGAGCTGATCGAGCCGAAGTCGGTGGTGTCGGGTGCCACCCCGGTGATG
CGCGACTCGGAGCACTTCTTCTTCGACCTGCCGTCGTTCTCGGAGATGCTG
CAGGCCTGGACCCGCTCGGGTGCCCTGCAAGAACAGGTGGCCAACAAGAT
GCAAGAGTGGTTCGAGTCGGGCTTGCAGCAGTGGGACATCTCGCGTGATG
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CAAGCGTGGCGACTCGGTGTCGTTCGACGAGTACTGGAAGAAGGACTCGA
CCGCCGAGCTGTACCACTTCATCGGCAAGGATATCGTGTACTTCCTGTCGCT
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GTTCGTGCACGGCTACGTGACCGTGAACGGTGCCAAGATGTCGAAGTCGC
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GACGGCGTGCTGGCCTCGGAATTGGCCGACCCGCAGCTGTACAAGACCTTC
ACCGATGCCGCCGAGGTGATCGGCGAGGCCTGGGAGTCGCGTGAGTTCGG
TAAGGCCGTGCGCGAGATCATGGCCCTGGCCGATCTGGCCAACCGCTACGT
GGACGAGCAAGCCCCGTGGGTGGTGGCCAAGCAAGAAGGTCGCGACGCC
GACCTGCAGGCCATCTGCTCGATGGGCATCAACCTGTTCCGCGTGCTGATG
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CCTGAACACCGAGCTGACCTGGGACGGCATCCAGCAGCCGCTGCTGGGCC
ACAAGGTGAACCCGTTCAAGGCCCTGTACAACCGCATCGACATGCGCCAG
GTCGAGGCCCTGGTCGAAGCCTCGAAGGAAGAGGTGAAAGCCGCCGCCGC
CCCGGTCACCGGTCCGTTGGCCGATGATCCGATCCAAGAAACCATCACCTT
CGACGACTTCGCCAAGGTGGACCTGCGCGTGGCCCT<u>GATCGAGAACGCCG</u>
<u>AGTTC</u>GTCGAGGGCTCGGACAAGCTGCTGCGCCTGACCCTGGACCTGGGT
GGCGAGAAGCGCAACGTGTTCTCGGGCATCCGCTCGGCCTACCCAGATCCG
CAGGCCTTGATCGGTCGCCACACCATCATGGTGGCCAACCTGGCCCCGCGT
AAGATGCGCTTCGGCATCTCGGAGGGCATGGTGATGGCCGCCGGTCCGGGT
GGCAAGGA<u>CATCTTCCTGCTGTCGCCG</u>GATGCCGGTGCCAAACCGGGTCAC
CAGGTGAAGTGA

Note: underlined nucleotides were changed following the Mycobacterial codon usage.