

## Supplementary Information

### Rapid lateral flow test for *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria differentiation

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P9WQP1|A85B_MYCTU   MTDVSRKIR-AWGRRLMIGTAAAVVLPGLVGLAGGAATAGAFSRPGLPVEYLQVPSPSMG 59
P0C2T2|A85B_MYCBO   MTDVSRKIR-AWGRRLMIGTAAAVVLPGLVGLAGGAATAGAFSRPGLPVEYLQVPSPSMG 59
Q49575|A85B_MYCIA    MTDLSEKVR-AWGRRLVVGAAAAATLPGGLIGIAGGAATANAFSRPGLPVEYLQVPSAGMG 59
A1KJU9|A85B_MYCBP    MTDVSRKIR-AWGRRLMIGTAAAVVLPGLVGLAGGAATAGAFSRPGLPVEYLQVPSPSMG 59
P21160|A85B_MYCKA     MTDVSGKIR-AWGRRLLVGAAAAAALPGLVGLAGGAATAGAFSRPGLPVEYLQVPSAAMG 59
Q06947|A85B_MYCAV    MTDLSEKVR-AWGRRLLVGAAAAAVTLPGLIGLAGGAATANAFSRPGLPVEYLQVPSAGMG 59
B1MMV2|B1MMV2_MYCA9 -MEFLQKMRGATARLAAVA AVAAAILPGLIGVTGGSATAGAFSRPGLPVEYLQVPSAMG 59
      :.  *:* * . *   :.:.**, *****:;:*:* * .***** ,**

P9WQP1|A85B_MYCTU   RDIKVQFQSGGNNSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSIVMPVGGQSSFY 119
P0C2T2|A85B_MYCBO   RDIKVQFQSGGNNSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSIVMPVGGQSSFY 119
Q49575|A85B_MYCIA    RDIKVQFQSGGNGSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSIVMPVGGQSSFY 119
A1KJU9|A85B_MYCBP    RDIKVQFQSGGNNSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSIVMPVGGQSSFY 119
P21160|A85B_MYCKA     RSIKVQFQSGGDNSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSIVMPVGGQSSFY 119
Q06947|A85B_MYCAV    RDIKVQFQSGGNGSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSIVMPVGGQSSFY 119
B1MMV2|B1MMV2_MYCA9 RDIVVEFQPGGP--HAVYLLDQQRAREDYNGWDIETAFEDYYQSGISVMPVGGQSSNY 117
      *,* *:* **   ***** *;:*****;* *** *****:;:***** *

P9WQP1|A85B_MYCTU   SDWYSPACGKAGCQTYKWETFLTSELPQWLSANRAVKPTGSAAGLSMAGSSAMILAAYH 179
P0C2T2|A85B_MYCBO   SDWYSPACGKAGCQTYKWETFLTSELPQWLSANRAVKPTGSAAGLSMAGSSAMILAAYH 179
Q49575|A85B_MYCIA    ADWYQPACGKAGCSTYKWETFLTSELPQYLASNKGVKSTGSAAVGISMSGSSAMILAVNH 179
A1KJU9|A85B_MYCBP    SDWYSPACGKAGCQTYKWETFLTSELPQWLSANRAVKPTGSAAGLSMAGSSAMILAAYH 179
P21160|A85B_MYCKA     SDWYSPACGKAGCTTYKWETFLTSELPQWLSANRSVKPTGSAAVGISMAGSSALILSVYH 179
Q06947|A85B_MYCAV    ADWYQPACGKAGCSTYKWETFLTSELPQYLASNKGVKRTGNAAVGISMSGSSAMILAVNH 179
B1MMV2|B1MMV2_MYCA9 TDWYNPAKGDGVWYTYKWETFLTTELPQWLGVNKGISPAANAVVGLSMAGPSALT LAIYH 177
      :***,** ** * *****:;:***,;*, *;:, ,...,:*:*:* **; *; *

P9WQP1|A85B_MYCTU   PQQFIYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKAADMWGPSSDPAWERNDPTQQIPK 239
P0C2T2|A85B_MYCBO   PQQFIYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKAADMWGPSSDPAWERNDPTQQIPK 239
Q49575|A85B_MYCIA    PNQFVYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKADAMWGPSSDPAWQRNDPQLIPA 239
A1KJU9|A85B_MYCBP    PQQFIYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKAADMWGPSSDPAWERNDPTQQIPK 239
P21160|A85B_MYCKA     PQQFIYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKASDMWGPSSDPAWQRNDPQLHIPE 239
Q06947|A85B_MYCAV    PDQFIYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKADAMWGPSSDPAWQRNDPQLHIPE 239
B1MMV2|B1MMV2_MYCA9 PQQFVYAGALSAPLHPSDQK--WQIRVAMSADAGGFNADDMWGPSSDPAWARNDPFLHIDQ 235
      *;*:***:*** :,*;   * ;*,*****;* ***** ***** :*

P9WQP1|A85B_MYCTU   LVANNTRLWVYCGNGTPNELGGAN-----IPAEFLENFVRSSNLKFQDAYNAAGGHNAVF 294
P0C2T2|A85B_MYCBO   LVANNTRLWVYCGNGTPNELGGAN-----IPAEFLENFVRSSNLKFQDAYNAAGGHNAVF 294
Q49575|A85B_MYCIA    LVGNTRLWVYCGNGTPSELGGAN-----MPAEFLENFVRSSNLKFQDAYNAAGGHNAVF 294
A1KJU9|A85B_MYCBP    LVANNTRLWVYCGNGTPNELGGAN-----IPAEFLENFVRSSNLKFQDAYNAAGGHNAVF 294
P21160|A85B_MYCKA     LVANNTRLWIYCGNGTPSELGGAN-----VPAEFLENFVRSSNLKFQDAYNAAGGHNAVF 294
Q06947|A85B_MYCAV    LVGHNTRLWLYCGNGTPSELGGAN-----MPAEFLENFVRSSNLKFQDAYNAGAGGHNAVF 294
B1MMV2|B1MMV2_MYCA9 LIANNTRLWIYCGSGDATDLDKDRNGLEVISGGVIEGQVIDSDKKFAEAYSAGGANAHF 295
      :;:*****:***,* .;*, .   ; , .;*, * .;: ** :;*,*** ** *

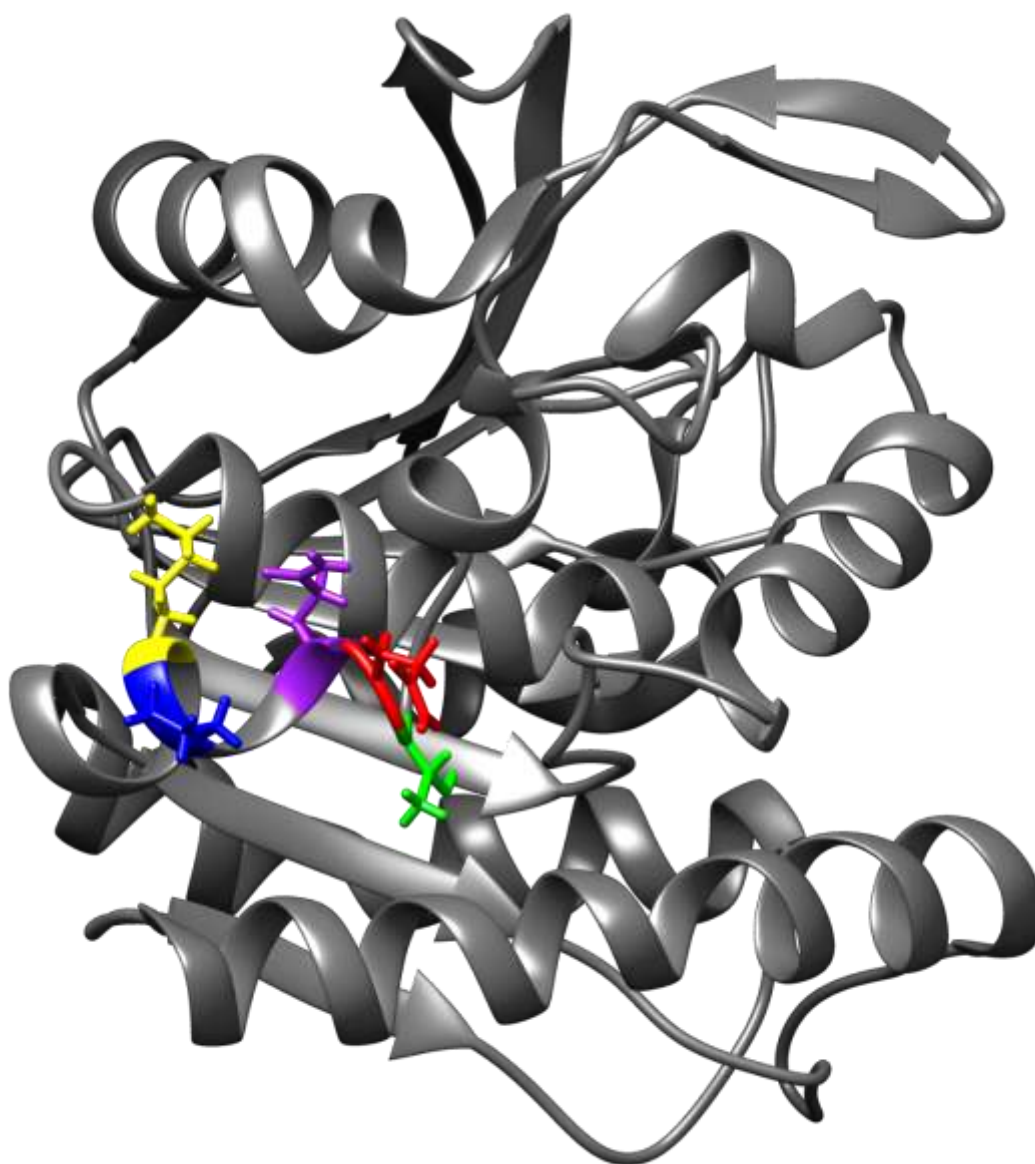
P9WQP1|A85B_MYCTU   NFPPNGTHSWEYWGAQLNAMKGDLQSSLGAG----- 325
P0C2T2|A85B_MYCBO   NFPPNGTHSWEYWGAQLNAMKGDLQSSLGAG----- 325
Q49575|A85B_MYCIA    NFNANGTHSWEYWGAQLNAMKPDQLSALGASSGGGG 330
A1KJU9|A85B_MYCBP    NFPPNGTHSWEYWGAQLNAMKGDLQSSLGAG----- 325
P21160|A85B_MYCKA     NLDANGTHSWEYWGAQLNAMKGDLQASLGAR----- 325
Q06947|A85B_MYCAV    NFNANGTHSWEYWGAQLNAMKPDQLGTLGASPGGGG 330
B1MMV2|B1MMV2_MYCA9 EFPKGGIHNWYWGQNQLRAMKADLVGYLTKEPGTAAV 331
      :; ,* *,* *** **,* ** , *

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## Supplementary Figure S1

### Alignment of MTB and NTM Ag85 protein sequences.

A Clustal Omega multiple sequence alignment [citation] of Ag85 protein sequences from various *Mycobacterium* strains is shown. The MTB strains include P9WQP1 (*M. tuberculosis*), P0C2T2 (*M. bovis* ATCC), and A1KJU9 (*M. bovis* BCG). The NTM strains include Q49575 (*M. intracellulare*), P21160 (*M. kansasii*), Q06947 (*M. avium*), and B1MMV2 (*M. abscessus*). The alignment highlights a unique amino acid sequence (TQQIPK) at positions 234-239 (red underline) present in the MTB strains but absent in the NTM strains.



### Supplementary Figure S2

#### Prediction of MTB-Ag85 (PDB 1F0N) epitope by BCEP.

A surface-accessible epitope including T<sup>234</sup> (green), Q<sup>235</sup> (red), Q<sup>236</sup> (purple), P<sup>238</sup> (blue), and K<sup>239</sup> (yellow) is depicted. The conserved I<sup>236</sup> was buried within the structure.