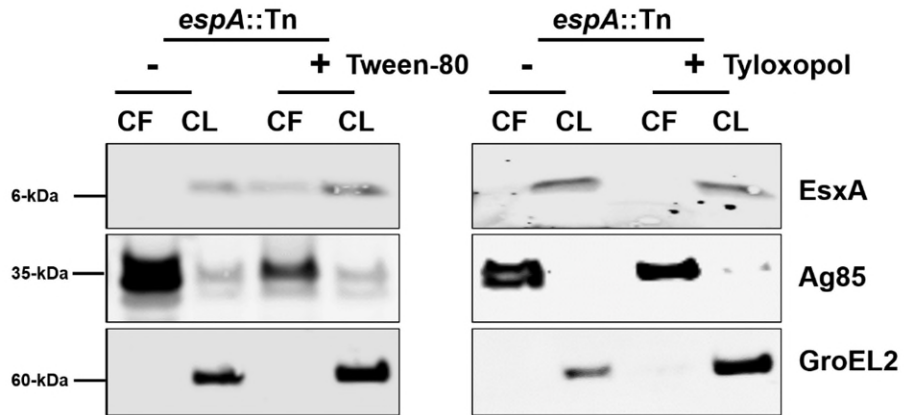


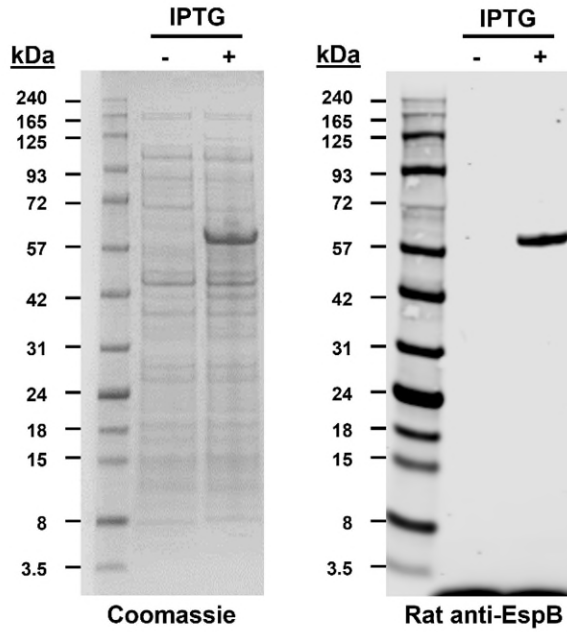
1

2 **Supplementary fig. 1. Cellular localization of EspK in *M. tb* H37Rv.** Immunoblots of
 3 10 µg/well each of CF, CW, CM and cytosol proteins from *M. tb* H37Rv sourced from BEI
 4 Resources (ATCC).



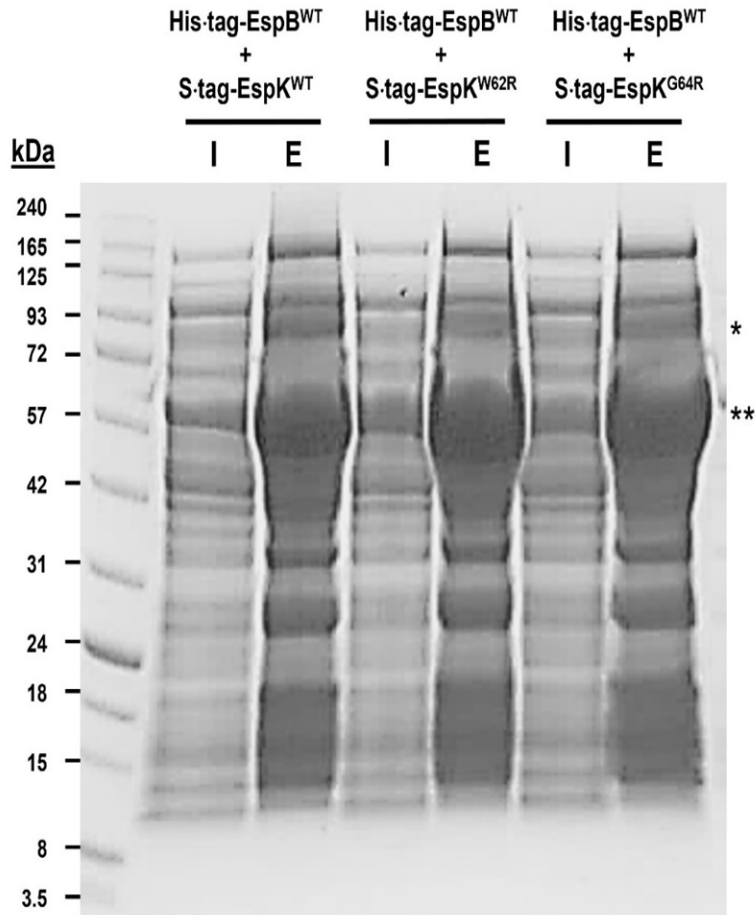
5

6 **Supplementary fig. 2. Secretion and expression of EsxA by *espA::Tn* cultured in**
 7 **liquid media with and without detergent.** Immunoblot analysis of CF (10 µg/well) and
 8 CL (5 µg/well) proteins of *espA::Tn* strain cultured for 3 days in Sauton's medium with
 9 and without Tween-80 (left), and with and without Tyloxapol (right).



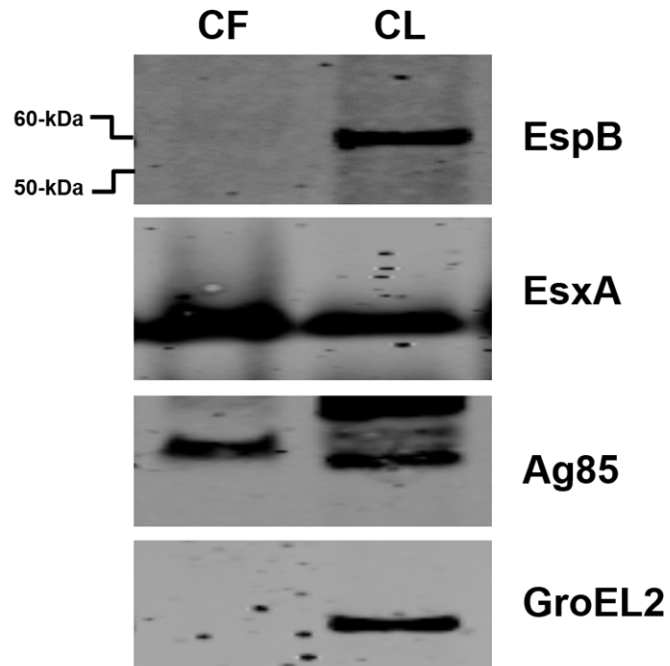
10

11 **Supplementary fig. 3. Specificity of rat anti-EspB antibody.** Coomassie stained SDS-
12 PAGE gel (left) and immunoblot using rat anti-EspB antibodies generated for this study
13 (right) of lysates from non-induced and IPTG-induced *E. coli*/His-tag EspB^{WT} expressing
14 full-length EspB.



15

16 **Supplementary fig. 4. Coomassie stained SDS-PAGE gel of input lysates and**
 17 **proteins eluted from his-tag Dynabeads.** SDS-PAGE gel of input lysates (I) at 30
 18 $\mu\text{g}/\text{well}$ each of *E. coli*/His-tag EspB^{WT} + S-tag EspK^{WT}, *E. coli*/His-tag EspB^{WT} + S-tag
 19 EspK^{W62R} and *E. coli*/His-tag EspB^{WT} + S-tag EspK^{G64R} and corresponding total captured
 20 and eluted proteins (E) in 50 μL of SDS-PAGE loading buffer (the single asterisk indicates
 21 the position of EspK protein band while the double asterisk indicates the position of the
 22 EspB protein band).



28

29 **Supplementary fig. 6. Secretion and expression of EsxA and EspB by *M. africanum*.**
30 Immunoblots of CF (10 μ g/well) and CL (5 μ g/well) proteins from *M. africanum* NR-49251
31 (Type-1 strain) cultured for 4 days in Sauton's medium without Tween-80 (representative
32 of 2 independent experiments).