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# Supplementary Materials for

## VapBC22 toxin-antitoxin system from *Mycobacterium tuberculosis* is required for pathogenesis and modulation of host immune response

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**The PDF file includes:**

Table S1
Figs. S1 to S4

**Other Supplementary Material for this manuscript includes the following:**

(available at advances.sciencemag.org/cgi/content/full/6/23/eaba6944/DC1)

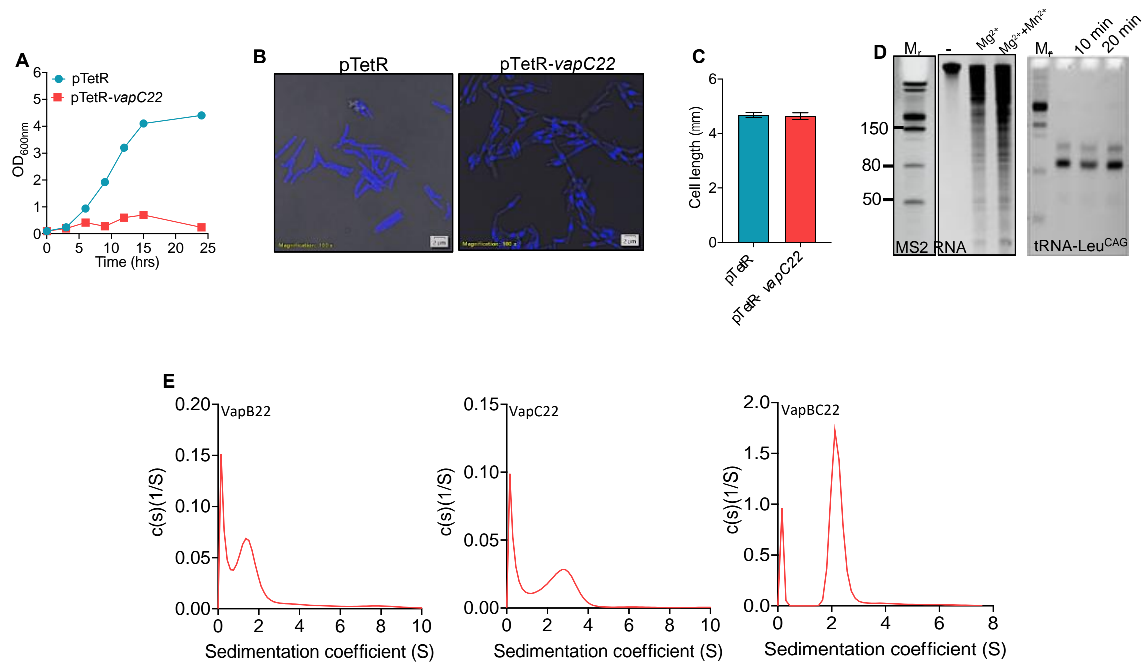
Tables S2 to S6

**Table S1: List of strains, plasmids and primers used in the study.**

| **List of strains used in the present study** | | |
|---|---|---|
| **Bacterial strains** | **Description** | **Reference** |
| *E.coli* XL-1 Blue | *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F´ proAB lacIq ZΔM15 Tn10 (Tetr)]* | Stratagene |
| *E.coli* HB101 | *F⁻, thi$^{-1}$, hsdS20 ($r_B$- $m_B$), supE44, recA13, ara-14, leuB6, proA2, lacY1, galK2, rpsL20 (strr), xyl-5, mtl-1* | Promega |
| *M. smegmatis* mc$^2$155 | *M. smegmatis* parental strain | A kind gift from Prof. Anil Tyagi |
| *M. bovis* BCG Danish | *M. bovis* BCG parental strain | A kind gift from Prof. Anil Tyagi |
| *M. tuberculosis* $H_{37}Rv$ | *M. tuberculosis* parental strain | ATCC |
| *ΔvapC22* | Rv2829c mutant strain of *M. tuberculosis* | This study |
| *ΔvapC22-CT* | Rv2829 complemented strain of *M. tuberculosis* | This study |
| *VapB22-OE* | *M. tuberculosis* strain overexpressing VapB22 | This study |
| **List of plasmids used in the present study.** | | |
| **Plasmids** | **Description** | **Reference** |
| pGEM-T Easy | T/A cloning vector, Ampicillin resistance gene | Promega |
| pTetR | Anhydrotetracycline inducible *E. coli* mycobacterial expression vector | *Agarwal et al.*, 2018 |
| pTetR-*vapC$_x$* | pTetR derivative harboring VapC$_x$ (where x denotes VapC homolog from *M. tuberculosis*) | *Agarwal et al.*, 2018 |
| pTetR-*vapC22*$^{D8A}$ | pTetR harboring D8A mutant Vap22 protein | This study |
| pYUB854 | Cloning vector | *Bardarov et al., 2002* |
| pYUB854*ΔvapC22::hyg$^r$* | pYUB854 with Rv2829c region amplicons flanking the hygromycin resistance gene | This study |
| phAE87 | Temperature sensitive mycobacteriophages | *Bardarov et al., 2002* |
| phAE87*ΔvapC22::hyg$^r$* | phAE87 derivative to replace Rv2829c with hygromycin resistance gene in *M. tuberculosis* | This study |
| pVV16 | *E. coli* mycobacterial shuttle episomal plasmid containing constitutive *hsp65* promoter | A kind gift from BEI resources |
| pVV16-*vapB22* | pVV16 derivative harboring *vapB22* | This study |
| pMV306K | Mycobacterial shuttle integrative vector | A kind gift from Dr. William Jacobs |
| pMV306K-*vapC22*-CT | pMV306K harboring VapBC22 along with the flanking region. | This study |
| pET-Duet | *E. coli* expression vector | *Deep et al., 2017* |
| pET-Duet-*vapBC22* | pET-Duet harboring Rv2830c and Rv2829c | This study |

| pET28b | *E. coli* expression vector | Merck |
|---|---|---|
| pET28b-*vapB22* | pET28b-harboring Rv2830c | This study |
| **List of primers used in the study for cloning purpose.** | | |
| **Primer name** | **Forward primer (5'---- 3')** | **Reverse primer (5'---- 3')** |
| *vapC22* upstream | gggaggcctgtgggcagcagctggtcgtgggc | gggtctagagaccaccagtaggccacatgcg |
| *vapC22* downstream | gggccatggcggcacccacgaccggtcaccgtc | gggactagtggcgtcaccggcagctgcacccagc |
| *vapC22* complemented | gggtctagatcatccgccgggccaggccgatg | gggacgcgtctaccagacggtgaccggtcgtggg |
| pVV16-*vapB22* | gggcatatgaccgctacggaggtgaaggcg | gggaagctttgaaacgttccacgaaaccccggtg |
| pET-Duet-*vapB22* | gggcatatgaccgctacggaggtgaaggcg | ctcactcgagtgaaacgttccacgaaaccccg |
| pET-Duet-*vapC22* | atgcgctagcacgacggtgctgctcgactcgactcgcat | gaagcttctaccagacggtgaccggtcgtggg |
| pET28b-*vapB22* | gggcatatgaccgctacggaggtgaaggcg | ctcactcgagtgaaacgttccacgaaaccccg |
| **List of primers used in the study for quantitative PCR.** | | |
| *sigA* | acgaagaccacgaagacctcgaa | gtaggcgcgaaccgagtcggcgg |
| *vapC22* | ggcttggcttgccgaacaggaacgc | ccttggtcaccagccgccagccg |
| *vapB22* | accgctacggaggtgaaggcg | tgaaacgttccacgaaaccccggtg |
| *mazF6* | gtacaacgcaagtcgccttgcc | ccccaactcggtcggtgaggtc |
| *higB1* | ggagttgcgatggcatgaggcg | tcagatcggtggggtgtcgccg |
| *vapC15* | gaaccgctggccccggtccgcgacg | tcagaacaacggctcggtgcgtag |
| *furA* | gcgcatccacacgccgacacggaaac | caagaccggcagacgatgtgatgg |
| *Rv2660c* | gtgatagcgggcgtcgaccaggcgc | ctagtgaaactggttcaatcccag |
| *Rv2661c* | gtgccctcgttgataatccgcagg | gcgaacggctgcaaacggtcgttg |
| *whib7* | ttgccggttttgccgtgccacgtcg | cgcgcggacgcttgtgactcacg |
| *Eis* | cggctacgggcccgctaccacc | gcacctgcgggcgtagcagcccg |
| *Rv3290c* | gccctggcggtggagaacgcgctc | gcgtggccagtcgaatttcgggaac |
| *rpmI* | atgcccaaggccaagacccacagc | tcagccgttcagcaacgacgtgacc |
| *rplT* | cgcaaagccaaagagcagcagctgc | cgcaatgtcggcgaggtttttccgg |
| *rplN* | gtgattcagcaggaatcgcggctg | tacaacacctccggggccagcg |
| *rplX* | gtattggtcgagggtgtcaaccgg | cgcttggagatacggacgcgcttg |
| *rplE* | gcgctgatcaccgggcagaagccg | cccacaccgtcgaactgtttgggcg |
| **Primers used in the study for *in vitro* transcription** | | |
| Rv2830c | taatacgactcactatagatgaccgctacggaggtgaag | tcatgaaacgttccacgaaac |
| Rv2831 | taatacgactcactatagatgaccgacgacatcctgctg atc | ctaacgcacctgcgcgcggccgcgctg |

## Supplementary Figure Legends.

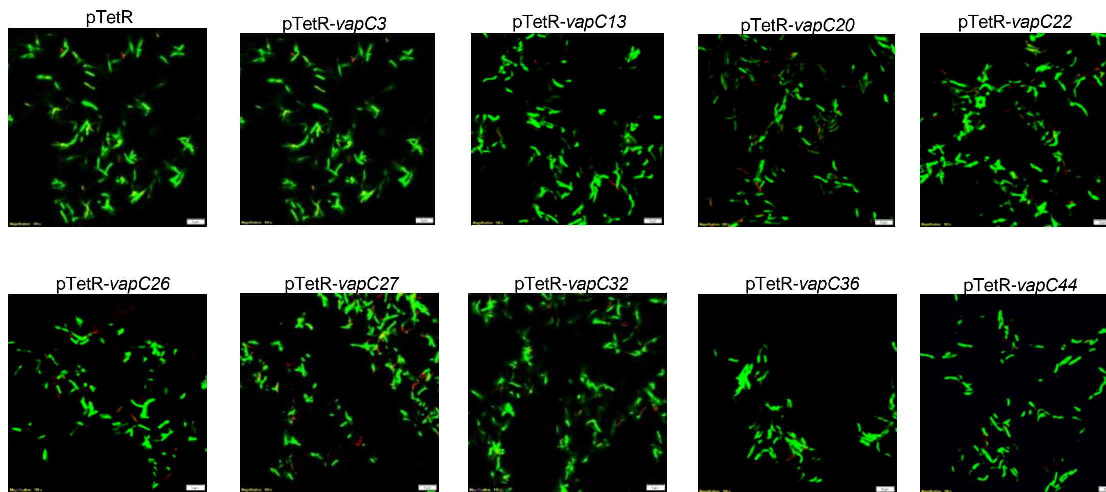



*Agarwal et al., Figure S1*

**Supplementary Figure 1: Functional and biochemical characterization of VapBC22 TA system from *M. tuberculosis*. (A-C) Effect of VapC22 overexpression on the growth, nucleoid morphology and cell length of *M. smegmatis*. (A)** The growth of the recombinant *M. smegmatis* $mc^2155$ harbouring an inducible expression vector, pTetR or pTetR-*vapC22* was monitored by measuring absorbance at 600nm at regular intervals. The data shown in this panel is representative of three independent experiments. **(B)** The 9 hrs induced cultures of *M. smegmatis* harboring either pTetR or pTetR-*vapC22* were stained with DAPI. Representative bright/DAPI overlaid images of parental and VapC22 overexpressing *M. smegmatis* strain is shown. Scale bar, 2 µm. **(C)** The cell length of recombinant *M. smegmatis* harbouring either pTetR or pTetR-*vapC22* was determined after 9 hrs post-Atc induction. The confocal images were captured using 100X objective and cell length of at least 150 bacilli was determined.

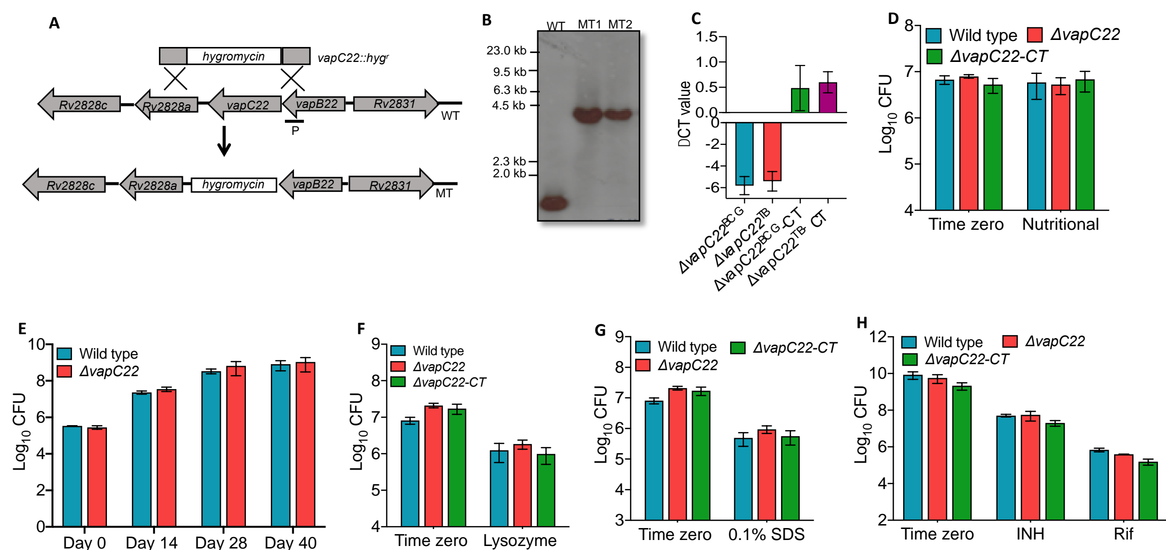
**(D) VapC22 is a functional ribonuclease.** Ribonuclease activity of the purified VapC22 was performed using either MS2 RNA or tRNA-$Leu^{CAG}$ for different time points. The reactions were resolved on 6% UREA-PAGE (for MS2 RNA) or 10% UREA-PAGE (for tRNA-$Leu^{CAG}$) and stained using ethidium bromide.

**(E) AUC analysis of VapB22, VapC22 and VapBC22.** Sedimentation coefficient distribution analysis suggests that VapB22 and VapC22 forms a predominant homodimeric species with observed molecular weight of 21.1 kDa and 29.5 kDa, respectively. Sedimentation coefficient distribution analysis of VapBC22 suggests that these proteins interact to form a predominant heterotetrameric species with observed molecular weight of 48 kDa.



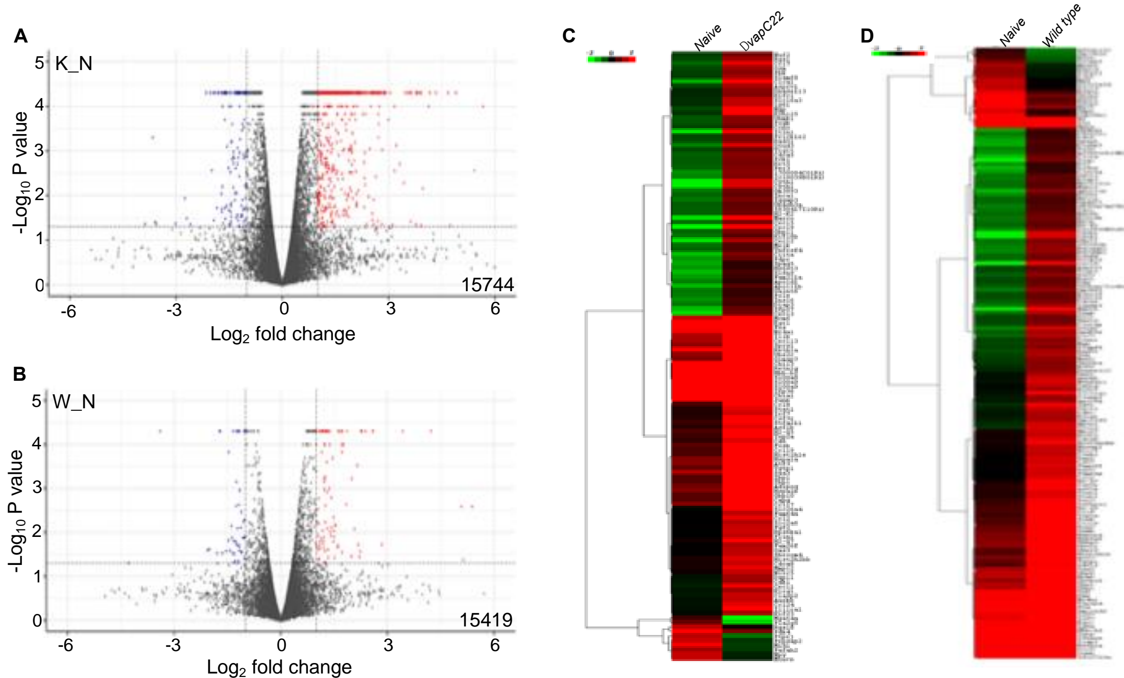

*Agarwal et al., Figure S2*

**Supplementary Figure 2: Overexpression of VapC toxins in *M. bovis* BCG inhibits growth in a bacteriostatic manner.** The expression of various toxins in *M. bovis* BCG was induced by the addition of Atc for 2 days. The induced cultures were harvested, washed, labelled and stained bacilli were visualised by confocal microscopy using 100X objective. The data shown in this panel is representative of two independent experiments. Scale bar, 5 µm.




*Agarwal et al., Figure S3*

**Supplementary Figure 3: Characterization of *ΔvapC22* mutant strain of *M. tuberculosis* and *M. bovis* BCG. (A)** Schematic representation of *vapC22* locus in parental and mutant strain of *M. tuberculosis*. **(B)** For Southern blot analysis, the genomic DNA isolated from wild type (WT) and *ΔvapC22* (MT1, MT2) strains of *M. tuberculosis* was *Pvu II* digested and transferred to nylon membrane. The DIG-labelled probe hybridized with 3.4 kb and 1.8 kb, respectively in genomic DNA isolated from the wild type and mutant strain, respectively. **(C)** The transcript levels of *vapC22* were measured by qPCR using mRNA isolated from parental, mutant and complemented strains of *M. tuberculosis* and *M. bovis* BCG. The data shown is mean $\pm$ S.E. of fold change in the mutant and complemented strain relative to the parental strain obtained from two independent experiments. **(D-H) The effect of deletion of VapC22 on growth of *M. tuberculosis* in different conditions. (D-G)** The susceptibility of different strains was also determined after exposure of early-log phase cultures to either nutritional stress (D) or low oxygen growth conditions (E) or 2.5 mg/ml lysozyme (F) or 0.1 % SDS (G) as described in methods. **(H)** For drug-tolerance experiments, mid-log phase cultures of various strains were exposed to either isoniazid or rifampicin for 14 days as described in materials and methods. For bacterial enumeration, 10.0 fold serial dilutions were prepared and plated on Middlebrook 7H11 medium at 37 $^{o}$C for 3-4 weeks. The data shown in these panels is mean $\pm$ S.E. of $\log_{10}$ cfu obtained from three independent experiments.




*Agarwal et al., Figure S4*

**Supplementary Figure 4: Global transcriptome profile of lung tissues from uninfected and mice infected with wild type and *ΔvapC22* strain. (A and B)** These panels are the volcano plot displaying gene expression profiles in uninfected mice and mice infected with wild type and *ΔvapC22* strain for 4 weeks**.** The y-axis and x-axis depict *P value* and fold change for each gene, respectively. The significant differentially upregulated and downregulated genes between uninfected versus *ΔvapC22* infected mice (A) and uninfected versus wild type infected (B) mice are shown as blue dots and red dots, respectively. **(C and D)** Heat map representation of differentially expressed transcripts between different groups as analysed by RNA-seq. The transcripts with fold change more than 4-fold have been shown. The gene names are indicated on the right of heat map and bacterial growth conditions at the top. The data shown in obtained from three biological replicates. The genes highlighted in red and green means upregulated and downregulated, respectively.