SUPPLEMENTAL DATA

Fig. S1. Effect of expressing VapC toxins. *E. coli* BW25113Δ6 cells were transformed with pBAD33 or each of the 23 VapC toxins in pBAD33. Expression of VapC-mt4, -mt11, -mt19 and -mt20 inhibited colony formation. The cells were plated on M9-chloramphenicol plates with (+Arab) or without (-Arab) induction and incubated at 37°C. A section of each plate is displayed.

Fig. S2. Schematic representation of the *vapBC-mt4* loci. The *vapBC-mt4* TA system, encoding the VapB-mt4 antitoxin (white arrow) and VapC-mt4 toxin (black arrow), is located in an operon on the *M. tuberculosis* chromosome. In the operon the *vapB-mt4* TGA stop codon (overlined) overlaps the *vapC-mt4* GTG start codon (underlined).

Fig. S3. Purification of recombinant VapBC-mt4 complex. Cell lysates from BL21(DE3)pLysE cells containing *A*. pET28a or pET28a-His_6-VapC-mt4 and pET21c-T7-VapB-mt4 or *B*. pET28a or pET28a-His_6-VapB-mt4 and pET21c-T7-VapC-mt4 were passed over a Ni-NTA affinity column. Purified His-tagged proteins and interacting T7-tagged proteins were subjected to SDS-PAGE followed by Coomassie staining (left) or Western analysis (right) using a polyclonal antibody to *A*. VapB-mt4 (Rv0496c) or *B*. VapC-mt5 (Rv0627). The position of the molecular weight markers (kDa) is indicated on the left. The positions of His-tagged and T7-tagged proteins are indicated on the right.

Fig. S4. Expression of VapC-mt4 in *M. smegmatis* leads to growth arrest. Growth profile for pMC1s (□), pMC1s-VapBC-mt4 (◆) and pMC1s-VapC-mt4 (●) in *M. smegmatis* mc^2155 grown at 37°C in 7H9-TW80-ADN medium containing 30% spent culture supernatant and 200 ng/ml anhydrotetracycline. Cultures were inoculated with single colonies. Data represent the average ± SD of three independent experiments.

Fig. S5. Effect of VapC-mt4 on DNA replication and transcription. *A*. [^{3}H]thymidine and *B*. [^{3}H]uridine incorporation into *E. coli* BW25113Δ6 cells with (●) or without (□) VapC-mt4 expression. Expression of VapC-mt4 decreased DNA replication (*A*.) and increased RNA synthesis (*B*.) after 1 hour. Chloramphenicol (▲) was added to a final concentration of 25 µg/ml in the absence of VapC-mt4 expression as a control. Data represent the average ± SD of two independent experiments.

Fig. S6. Expression of VapC-mt4 leads to stabilization of polysomes. *A*. -VapC-mt4, -Clm; ribosomes were prepared from *E. coli* BW25113Δ6 cells containing pBAD18-VapC-mt4 under non-inducing conditions without adding chloramphenicol. *B*. -VapC-mt4, +Clm; ribosomes were prepared from *E. coli* BW25113Δ6 cells containing pBAD18-VapC-mt4 to which chloramphenicol was added to a final concentration of 0.1 mg/ml to stabilize polysomes. *C*. +VapC-mt4, -Clm; ribosomes were prepared from *E. coli* BW25113Δ6 cells containing pBAD18-VapC-mt4 induced with 0.2% arabinose for 1 hour. Expression of VapC-mt4 results in stabilization of polysomes comparable to that achieved with 0.1 mg/ml chloramphenicol. The fractions corresponding to the polysomes, 70S, 50S, 30S and tRNAs/RNAs not associated with ribosomes or ribosomal subunits were concentrated by centrifugation at 45,000 rpm for 20 hours and subjected to Western analysis using an antibody against VapC-mt5 (Rv0627). VapC-mt4 was found in the fraction containing tRNA and other RNA species not associated with ribosomes or ribosomal subunits.

Fig. S7. VapC-mt4 does not cleave $tRNA^{fMet}$. *A*. 20 pmoles of *E. coli* $tRNA^{fMet}$ was incubated with 2, 25 or 50 pmoles of VapC-mt4 or *B*. 2 pmol of *M. tuberculosis* $tRNA^{fMet}$ was incubated with 0.2, 2.5, or 5 pmoles of VapC-mt4 at 37°C for 15 min to achieve VapC-mt4:$tRNA^{fMet}$ ratios of 0.1:1, 1.25:1 and 2.5:1, respectively. The position of the RNA markers (nt) is indicated on the left. RNA was visualized by staining with *A*. EtBr or *B*. SYBR gold.

Fig. S8. VapC-mt4 contains a PIN domain. *A*. The protein sequences of PIN and 5' exonuclease domains were aligned using the program T-Coffee (29). The four conserved acidic residues (D or E) and a fifth invariant hydroxyl residue (S or T) comprising the catalytic site of PIN domain proteins are highlighted in black. Gaps introduced to maximize alignment are indicated by dashes. Numbers in parentheses within the sequences indicate insertions. Numbers at the C-termini of the sequences indicate the amino acids in the full-length proteins used in the alignment. *B*. Mutating any of the five conserved residues that form the active site in PIN domain containing proteins abrogates VapC-mt4 toxicity in *E. coli* BW25113Δ6 cells on solid medium. A section of each plate is displayed. *C*. The VapC-mt4 mutants were able to form protein complexes with VapB-mt4 suggesting that the structure of the protein was retained. Cell lysates from BL21(DE3)pLysE cells containing pET21c-T7-VapB-mt4 and pET28a (Lane 1), pET28a-His_6-VapC-mt4 (Lane 2), pET28a-His_6-VapC-mt4-D9A (Lane 3), pET28a-His_6-VapC-mt4-E40A (Lane 4), pET28a-His_6-VapC-mt4-D98A (Lane 5), pET28a-His_6-VapC-mt4-T114A (Lane 6) or pET28a-His_6-VapC-mt4-D116A (Lane 7) were passed over a Ni-NTA affinity column. Purified His-tagged VapC-mt4 toxins and the interacting T7-tagged VapB-mt4 antitoxin were subjected to SDS-PAGE followed by Western analysis using a polyclonal antibody to VapC-mt5 (Rv0627) and VapB-mt4 (Rv0496c). The position of the molecular weight markers (kDa) is indicated on the left. The positions of the His-tagged VapC-mt4 toxins and T7-tagged VapB-mt4 antitoxin are indicated on the right.

Fig. S9. VapC-mt4 mediated RNA cleavage requires GC nucleotides at the 3' end. A radioactively labeled synthetic 20 nucleotide RNA containing an internal ACGC consensus site was cleaved by VapC-mt4 in the presence of Mg^{+2} (Lane 4). Substitution of either the G or C within the internal ACGC sequence prevented VapC-mt4 cleavage (Lanes 6 and 8). Lanes labeled "-" represent control reactions to which no protein was added. In lanes labeled "+" purified His_6-VapC-mt4 was added. The cleavage site/product is indicated by a black arrow on the right side of the gel and in the relevant RNA sequence below the gel. T1 and AH denote RNA digested with RNase T1 and partially hydrolyzed with alkali, respectively. Numbers indicate the position of G nucleotides.

Fig. S10. VapC-mt4 expression leads to accumulation of total RNA. RNA was extracted from *E. coli* BW25113Δ6 cells with (■) or without (□) expression of *A*. VapC-mt4 or *B*. MazF-mt1. The normalized RNA concentration was plotted against time. Data represent the average ± SD of three independent experiments.