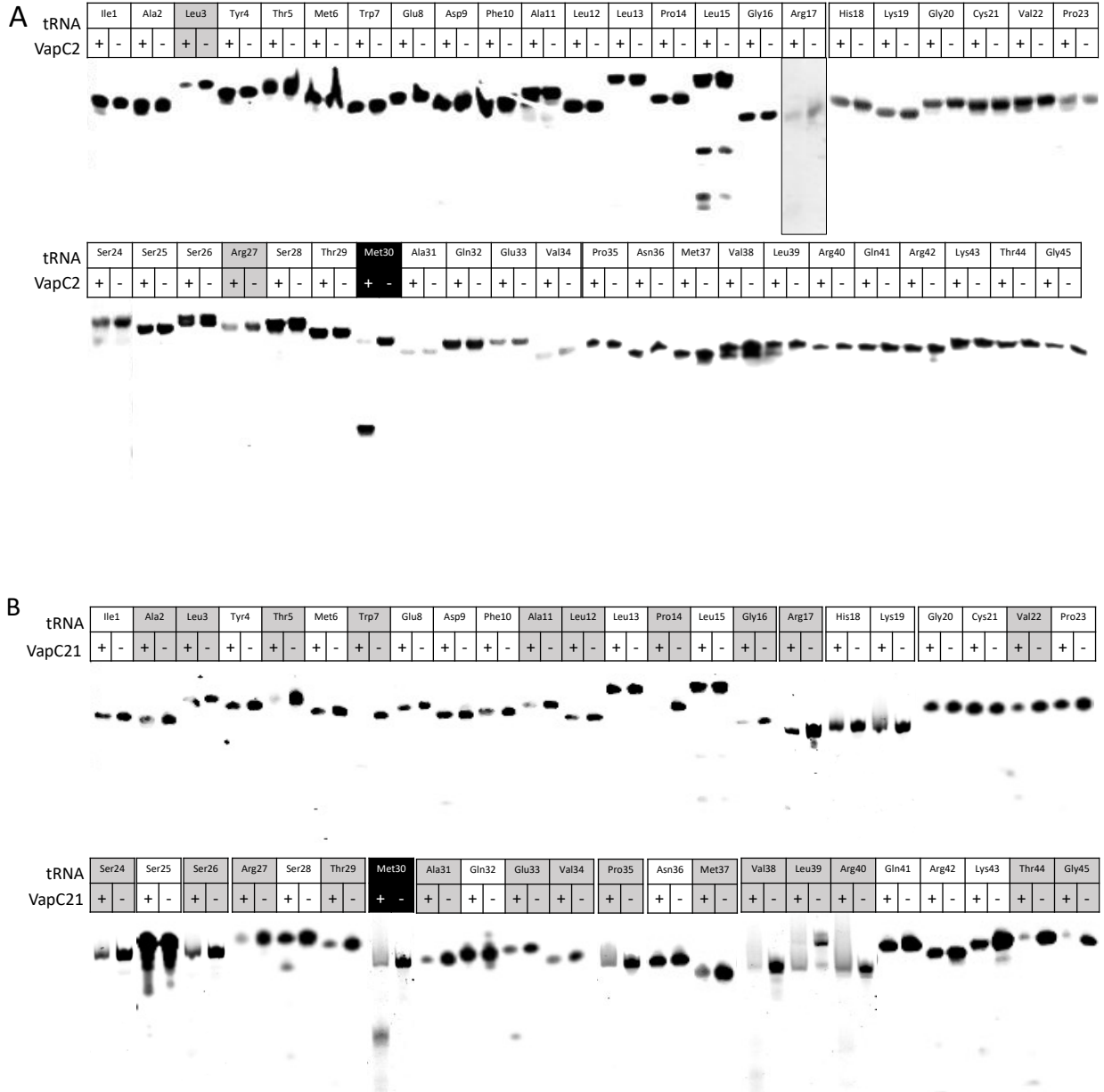
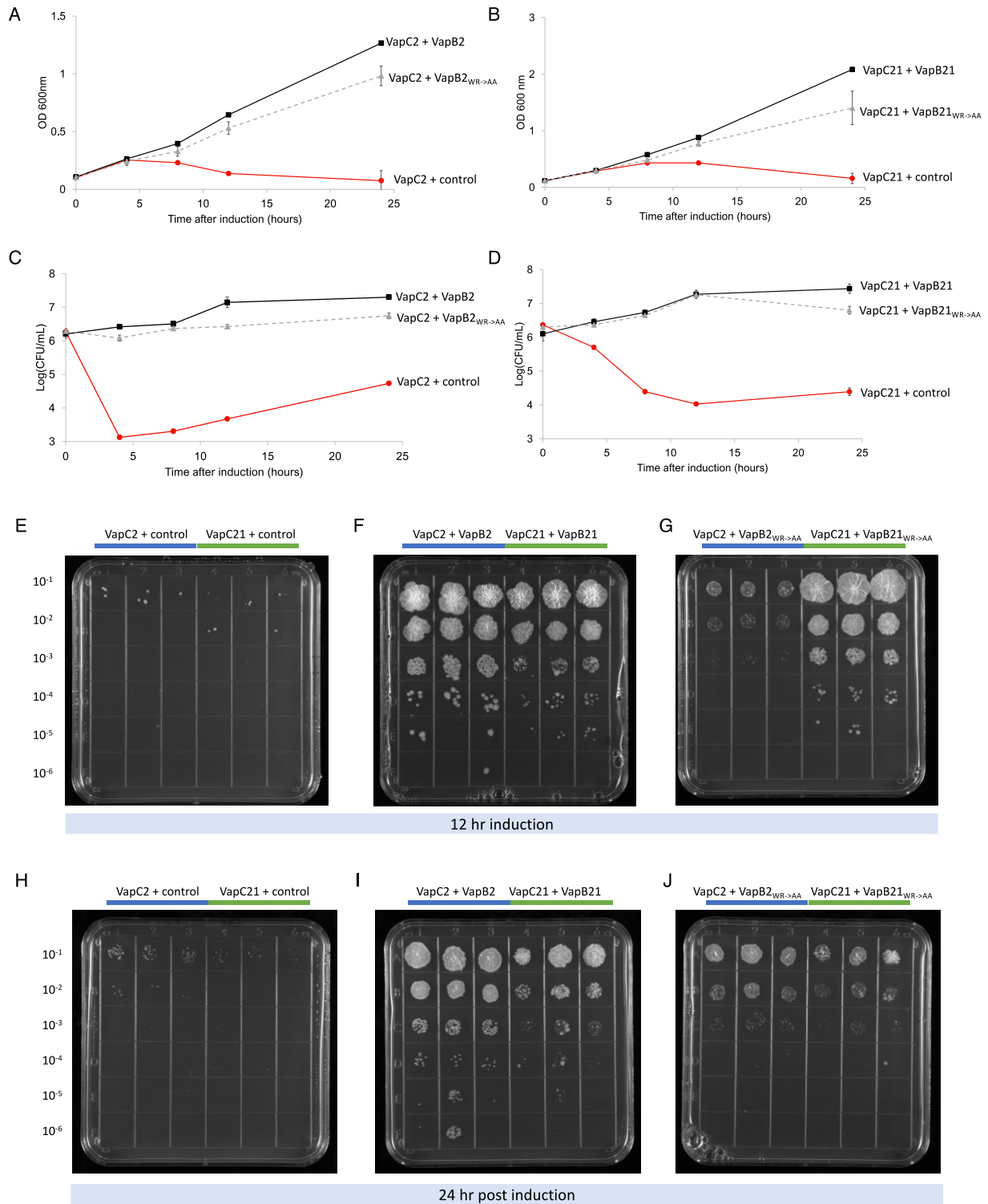


Supplemental Material



Supplemental Fig. 1. VapC2 and VapC21 cleave multiple tRNAs *in vitro*. Cleavage assays with each of the 45 *in vitro* synthesized Mtb tRNAs. Individual tRNAs were incubated with either VapC2 (A) or VapC21 (B) at 37°C for 3 hours. The initiator tRNA^{fMet} (Met30) highlighted in black. Other tRNAs showing substantial cleavage in this assay shown in gray. tRNAs showing some degree of cleavage were repeated to confirm the results shown. Exposure enhancement was required to view Arg17 in panel A. tRNA numbering from the Lowe lab genomic tRNA database <http://gtrnadb.ucsc.edu> (1).



Supplemental Fig. 2. Mutant VapB2 and VapB21 are not able to efficiently rescue toxin-mediated growth defects. (A) Growth profiles of *M. smegmatis* expressing VapC2 toxin from the ATC-inducible pMC1s plasmid plus either the empty isovaleronitrile (IVN)-inducible pNIT plasmid (control, red circle), wild type VapB2 antitoxin in pNIT (black square), or VapB2 W72A/R73A

mutant antitoxin in pNIT (WR→AA, grey triangle). (B) Growth profiles of *M. smegmatis* expressing VapC21 from the ATC-inducible pMC1s plasmid plus either the empty IVN-inducible pNIT plasmid (control, red circle), wild type VapB21 antitoxin in pNIT (VapC21 + VapB21, red circle), or VapB21 W79A/R80A mutant antitoxin in pNIT (WR→AA, grey triangle). Growth curves performed in triplicate; standard deviation indicated. The difference in degree of growth rescue shown in liquid culture here compared to Fig. 5C,D appears to be due to reagent lot variability effecting potency of the inducer (IVN) driving antitoxin expression. (C,D) CFU/ml plots for growth profiles shown in panels A and B, respectively. (E-G) growth observed in 5 μ l aliquots of serial dilutions from the 12 hr time point from panels A and B; sample names match growth profiles (H-J) growth observed in 5 μ l aliquots of serial dilutions from the 24 hr time point from panels A and B; sample names match growth profiles.

Reference

1. Chan PP, Lowe TM. 2016. GtRNADB 2.0: an expanded database of transfer RNA genes identified in complete and draft genomes. *Nucleic Acids Res* 44:D184-9. <https://doi.org/10.1093/nar/gkv1309>