Table S1. Properties of mycobacterial *vapBC* toxin-antitoxin modules selected for study

Gene	In vitro essentiality[a]	Other properties and/or features
Antitoxin (VapB)		
Rv0550c	No [1]	Induced by heat stress [2], during macrophage infections [3] and high concentrations of vancomycin [4]
Rv0596c	-	Induced in a *sigE* mutant after SDS stress [5]
Rv0626	-	Induced *in vivo* but not *in vitro* [6]; repressed during infection of human macrophage-like cells [7]; and structure of C-terminal region determined in complex with toxin Rv0627 [8]
Rv1952	No [1]	Induced during phosphate starvation [9] and during macrophage infections [7]
Rv2009	No [1]	Induced during human macrophage infections [3,10], in SCID mice [6], after SDS stress [5] and during transition to hypoxia [3]; but repressed during nutrient starvation [11], hypoxia [12], and in wild type H37Rv vs. a *phoP* mutant [13]; Part of a genomic island [3]
Rv2545	No [1]	Repressed at low pH *in vitro* [14]; and a P19L polymorphism was identified in MDR strain of Mtb [15]
Rv2547	No [1]	Induced during hypoxia [3,16] and infection of macrophages [3,17]
Rv2550c	No [1,18]	Induced during macrophage infections [17], in Balb/c mice [6], in the presence of high iron concentrations [19] and SDS stress [5]; but repressed by hypoxia [20] and in a *sigE* mutant after SDS stress [5]
Rv2830c	Yes [1]	Induced during hypoxia [21], SDS stress [5] and during *in vitro* and *in vivo* growth [6]
Rv3321c	No [1]	Induced in SCID mice [6] and during human macrophage infections [10]
MSMEG_1283	-	-
Toxin (VapC)		
Rv0549c	No [1]	Induced by hypoxia [3,16], SDS stress [5], during adaptation to nutrient starvation [22], during infection of human macrophages [3,10], and in the presence of high concentrations of vancomycin [4]. Non-toxic in *E. coli* [23] but toxic in *M. smegmatis* [3]
Rv0595c	No [1]	Required for survival in nonhuman primate lungs [24]; induced during adaptation to nutrient starvation [22], macrophage infection [17] and by SDS stress [5]. Part of a genomic island [3]. Toxic in *E. coli* [23] but not in *M. smegmatis* [3]
Rv0627	Yes [1]	Structure determined in complex with C-terminal part of antitoxin (Rv0626) and biochemical evidence for ribonuclease activity [25]. Non-

		toxic when over-expressed in *E. coli* [23] or *M. smegmatis* [3] but toxic when over-expressed in *M. tuberculosis* [25]
Rv1953	No [1,18]	C-terminally truncated and lacking part of the PIN domain. Induced during adaptation to nutrient starvation [22]. Non-toxic in *E. coli* [23] and *M. smegmatis* [3]
Rv2010	No [1]	Induced during hypoxia [16] and in Balb/c mice [6], but repressed during nutrient starvation [11], adaptation to hypoxia [12] and in wild type H37Rv compared to a *phoP* mutant [13]. Protein identified in 30-d infected guinea pig lungs [26]. Part of a genomic island [3]. Non-toxic in *E. coli* [23] but toxic in *M. smegmatis* [3]
Rv2546	No [1]	Induced in Balb/c mice [6] and during treatment with SRI#967, a compound exhibiting strong anti-mycobacterial properties [27]. Non-toxic in *E. coli* [23] and *M. smegmatis* [3]
Rv2548	No [1]	Induced during hypoxia [16] and macrophage infections [17], but repressed in sputum [28]. Non-toxic in *E. coli* [23] but toxic in *M. smegmatis* [3]
Rv2549c	No [1]	Induced during macrophage infection [17] and in the presence of high iron concentrations [19]. Toxic in *E. coli* [23] but non-toxic in *M. smegmatis* [3]
Rv2829c	-	Induced during macrophage infection [3], hypoxia [3,12] and nutrient starvation [22]. Protein identified in 30-d infected guinea pig lungs [26]. Non-toxic in *E. coli* [23] but toxic in *M. smegmatis*[3]
Rv3320c	No [1]	Repressed during hypoxia [12] and nutrient starvation [11]. Part of a genomic island [3]. Non-toxic in *M. smegmatis* [3]
MSMEG_1284	-	Toxic in *M. smegmatis* [29]

a. Deduced from genome-wide essentiality screens

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