Table S1. Bacterial strains, plasmids and primers used in this work.

E. coli:	Relevant genotype:	Source or reference:
DH5α	F-, θ80Δ*lacZ*M15 Δ(*lacZYA-argF*)U169 *deoR recA1 endA1* $hsdR17$ (r$_k$-m$_k$+) *phoA supE44* λ- *thi-1 gyrA96 relA1*	Gibco, BRL
ER2566	F- λ- *fhuA2* [lon] *ompT lacZ::T7 geneI gal sulA11* Δ(*mcrC-mrr*)114::IS10 R(*mcr-73*::miniTn10)2 R(*zgb-210*::Tn10)1 (Tets) *endA1* [dcm]	(Chong & Garcia, 1994)

M. smegmatis:		
mc^{2}155	wild type	(Snapper *et al.*, 1990)
SMR5	mc^{2}155Δ*rpsL*	(Sander *et al.*, 1995)
SMR5*dop*	SMR5*dop*::aph; Kan^r	(Imkamp *et al.*, 2009)
MsHD396	SMR5, pMN-NL-His$_6$-*pup*-GGQ; Hyg^r	This work
MsHD397	SMR5, pMN-NL-His$_6$-*pup*-GGE; Hyg^r	This work
MsHD398	SMR5*dop*, pMN-NL-His$_6$*pup*-GGQ; Hyg^r, Kan^r	This work
MsHD399	SMR5*dop*, pMN-NL-His$_6$*pup*-GGE; Hyg^r, Kan^r	This work
MsHD206	mc^{2}155, pMN-NL-His$_6$-*pup*-GGQ; Hyg^r	This work

MsHD207	mc^{2}155, pMN-NL-*pup*-His$_6$; Hyg^r	This work
MsHD209	mc^{2}155, pMN-NL-His$_6$-*pup*-G; Hyg^r	This work
MsHD208	mc^{2}155, pMN-NL-His$_6$-*pup*-GG; Hyg^r	This work
MsHD212	mc^{2}155, pMN-NL-His$_6$-*pup*-GGA; Hyg^r	This work
MsHD219	mc^{2}155, pMN-NL-His$_6$-*pup*-AGQ; Hyg^r	This work
MsHD217	mc^{2}155, pMN-NL-His$_6$-*pup*-GAQ; Hyg^r	This work
MsHD213	mc^{2}155, pMN-NL-His$_6$-*pup*-AAQ; Hyg^r	This work
MsHD214	mc^{2}155, pMN-NL-His$_6$-*pup*-AAE; Hyg^r	This work
MsHD215	mc^{2}155, pMN-NL-His$_6$-*pup*-GGE; Hyg^r	This work
MsHD220	mc^{2}155, pMN-NL-His$_6$-*pup*-GGN; Hyg^r	This work
MsHD221	mc^{2}155, pMN-NL-His$_6$-*pup*-GGD; Hyg^r	This work
MsHD218	mc^{2}155, pMN-NL-His$_6$-*pup*-GGA5Q; Hyg^r	This work
MsHD216	mc^{2}155, pMN-NL-His$_6$-*pup*-GFQ; Hyg^r	This work
MsHD223	mc^{2}155, pMN-NL-His$_6$-*pup*-GGQG; Hyg^r	This work
MsHD222	mc^{2}155, pMN-NL-His$_6$-*pup*-GGQA; Hyg^r	This work
MsHD225	mc^{2}155, pMN-NL-His$_6$-*pup*-GGEG; Hyg^r	This work
MsHD224	mc^{2}155, pMN-NL-His$_6$-*pup*-GGEA; Hyg^r	This work

M. tuberculosis:

H37Rv	wild type American Type Culture Collection 25618	ATCC
CDC1551	wild type	W. Bishai collection
MHD2	*pafA282*::ΦMycoMarT7; Kan^r	(Darwin *et al.*, 2003)
MHD6	*pafA420*::ΦMycoMarT7; Kan^r	(Darwin et al., 2003)
MHD58	CDC1551 *dop*::ΦMycoMarT7; Kan^r	(Lamichhane *et al.*, 2003)
MHD18	H37Rv, pMV306; Hyg^r, Kan^r	(Darwin et al., 2003)
MHD525	H37Rv pMN-NL-His$_6$-*pup*-GGQ; Hyg^r	This work
MHD531	H37Rv pMN-NL-His$_6$-*pup*-GGE; Hyg^r	This work
MHD537	MHD2 pMN-NL-His$_6$-*pup*-GGQ; Hyg^r, Kan^r	This work
MHD535	MHD2 pMN-NL-His$_6$-*pup*-GGE; Hyg^r, Kan^r	This work
MHD586	CDC1551 pMN402; Hyg^r	This work
MHD585	CDC1551 pMN-NL-His$_6$-*pup*-GGQ; Hyg^r	This work
MHD584	CDC1551 pMN-NL-His$_6$-*pup*-GGE; Hyg^r	This work
MHD62	MHD2, pMV306; Hyg^r, Kan^r	(Festa *et al.*, 2007)
MHD63	MHD2, pMV-*pafA*-His$_6$; Hyg^r, Kan^r	This work

MHD65	MHD6, pMV-*pafA*-His$_6$; Hyg^r, Kan^r	This work
MHD273	MHD2, pMV-*pafA*(1-414)-His$_6$; Hyg^r, Kan^r	This work
MHD259	MHD2, pMV-*pafA*(E9A)-His$_6$; Hyg^r, Kan^r	This work
MHD261	MHD2, pMV-*pafA*(E11A)-His$_6$; Hyg^r, Kan^r	This work
MHD578	MHD2, pMV-*pafA*(R53E)-His$_6$; Hyg^r, Kan^r	This work
MHD579	MHD2, pMV-*pafA*(Y55K)-His$_6$; Hyg^r, Kan^r	This work
MHD620	MHD2, pMV-*pafA*(D57N)-His$_6$; Hyg^r, Kan^r	This work
MHD263	MHD2, pMV-*pafA*(E63A)-His$_6$; Hyg^r, Kan^r	This work
MHD580	MHD2, pMV-*pafA*(H123A)-His$_6$; Hyg^r, Kan^r	This work
MHD269	MHD2, pMV-*pafA*(R171A)-His$_6$; Hyg^r, Kan^r	This work
MHD581	MHD2, pMV-*pafA*(R187A)-His$_6$; Hyg^r, Kan^r	This work
MHD582	MHD2 pMV-*pafA*(H207E)-His$_6$; Hyg^r, Kan^r	This work
MHD583	CDC1551 pMV306; Hyg^r	This work
MHD375	MHD58 pMV306; Hyg^r, Kan^r	This work
MHD376	MHD58 pMV-*dop*-His$_6$; Hyg^r, Kan^r	This work
MHD559	MHD58 pMV-*dop*(E8A)-His$_6$; Hyg^r, Kan^r	This work
MHD560	MHD58 pMV-*dop*(E10A)-His$_6$; Hyg^r, Kan^r	This work

MHD607	MHD58 pMV-*dop*(D95N)-His$_6$; Hyg^r, Kan^r	This work
MHD561	MHD58 pMV-*dop*(E100A)-His$_6$; Hyg^r, Kan^r	This work
MHD629	MHD58 pMV-*dop*(R222A)-His$_6$; Hyg^r, Kan^r	This work
MHD608	MHD58 pMV-*dop*(H96V)-His$_6$; Hyg^r, Kan^r	This work
MHD563	MHD58 pMV-*dop*(R206A)-His$_6$; Hyg^r, Kan^r	This work
MHD539	MHD58 pMN-NL-His$_6$-*pup*-GGQ; Hyg^r, Kan^r	This work
MHD541	MHD58 pMN-NL-His$_6$-*pup*-GGE; Hyg^r, Kan^r	This work

Plasmids and primers

(sequences are 5' to 3'):

pET24b(+)	For production of C-terminal His$_6$ epitope-tagged protein; Kan^r	Novagen
pMN402	Hyg^r; shuttle plasmid with *gfp* under the control of the BCG *hsp60* promoter	(Scholz *et al*., 2000)
pMV306	Hyg^r; Mycobacterial plasmid that integrates at attB site on the Mtb chromosome	(Stover *et al*., 1991)
pET24b(+)-*pafA(1-414)*-His$_6$	Rv2098c-f1 GCCTGTTCCAGGTAGGTAGT	
	PafA(1-414)r CGAAGCTTATCACGTCCCGCCTCCTGCG	

pET24b(+)-His$_6$-*pup*	Used as template for making *pup* mutant plasmids.	(Pearce *et al*., 2008)
pMV-*pafA*-His$_6$	Used as template for making pafA plasmids.	(Festa et al., 2007)
pMV-*pafA(1-414)*-His$_6$	pMV306F	CGGTTCCTGGCCTTTTGCTGGCC
	414PafA-ClaIr	CGATCGATATCACGTCCCGCCTCCTGCGCGGCGCTG
pMV-*pafA*(E9A)-His$_6$	PafA-E9Af	AGCGTCGAATCATGGGCATCGCCACCGAGTTCGGTGTCACCTG
	PafA-E9Ar	CAGGTGACACCGAACTCGGTGGCGATGCCCATGATTCGACG
pMV-*pafA*(E11A)-His$_6$	PafA-E11Af	GAATCATGGGCATCGAAACCGCCTTCGGTGTCACCTGCACATT
	PafA-E11Ar	AATGTGCAGGTGACACCGAAGGCGGTTTCGATGCCCATGATTC
pMV-*pafA*(R53E)-His$_6$	PafA-R53Ef	CGTCTTCCTGCGTAACGGTGCGGAACTGTATCTCGACGTGGGCAG
	PafA-R53Er	CTGCCCACGTCGAGATACAGTTCCGCACCGTTACGCAGGAAGACG
pMV-*pafA*(Y55K)-His$_6$	PafA-Y55Kf	CTGCGTAACGGTGCGCGCCTGAAGCTCGACGTGGGCAGTCATCCCG
	PafA-Y55Kr	CGGGATGACTGCCCACGTCGAGCTTCAGGCGCGCACCGTTACGCAG
pMV-*pafA*(D57N)-His$_6$	PafA-D57Nf	CGGTGCGCGCCTGTATCTCAACGTGGGCAGTCATCCCGAGTACG
	PafA-D57Nr	CGTACTCGGGATGACTGCCCACGTTGAGATACAGGCGCGCACCG
pMV-*pafA*(E63A)-His$_6$	PafA-E63Af	TCGACGTGGGCAGTCATCCCGCCTACGCCACCGCCGAATGCG
	PafA-E63Ar	CGCATTCGGCGGTGGCGTAGGCGGGATGACTGCCCACGTCG
pMV-*pafA*(H123A)-His$_6$	PafA-R123Af	CGGGCAACTCCTACGGCTGCGCCGAAAACTACCTGATCGTGCG

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	PafA-R123Ar	CGCACGATCAGGTAGTTTTCGGCGCAGCCGTAGGAGTTGCCCG
pMV-*pafA*(R171A)-His$_6$	PafA-R171Af	CCACCTACTGCTTAAGCCAAGCCGCCGAACACATTTGGGAGGG
	PafA-R171Ar	CCCTCCCAAATGTGTTCGGCGGCTTGGCTTAAGCAGTAGGTGG
pMV-*pafA*(R187A)-His$_6$	PafA-R187Af	CTCTAGCGCCACGACCCGATCCGCCCCCATTATCAACACCCGCGACG
	PafA-R187Ar	CGTCGCGGGTGTTGATAATGGGGGCGGATCGGGTCGTGGCGCTAGAG
pMV-*pafA*(H207E)-His$_6$	PafA-R207Ef	CGAGAAGTACCGGCGGCTGGAAGTCATCGTCGGCGACTCCAACATG
	PafA-R207Er	CATGTTGGAGTCGCCGACGATGACTTCCAGCCGCCGGTACTTCTCG
pMV-*dop*-His$_6$	XbaI-pafDf1	GATCGTCTAGAACCACCGCCCAGGCGGCCAACATCGGAGCC
	HindIII-pafDr1	GATCGAAGCTTTTAGCGAGGCTCAGCGGTCAGTTGCTCTAC
pMV-*dop*(E8A)-His$_6$	PafD-E8Af	CAGCGGATTATCGGAACGGCCGTCGAGTACGGCATTTCCTCG
	PafD-E8Ar	CGAGGAAATGCCGTACTCGACGGCCGTTCCGATAATCCGCTG
pMV-*dop*(E10A)-His$_6$	PafD-E10Af	GGATTATCGGAACGGAGGTCGCCTACGGCATTTCCTCGCCGTCGG
	PafD-E59Ar	CCGACGGCGAGGAAATGCCGTAGGCGACCTCCGTTCCGATAATCC
pMV-*dop*(D95N)-His$_6$	PafD-D95Nf	CGGGGCGCGGCTGTATGTCAACCACGCGCACCCGGAATACTCCG
	PafD-D95Nr	CGGAGTATTCCGGGTGCGCGTGGTTGACATACAGCCGCGCCCCG
pMV-*dop*(E100A)-His$_6$	PafD-E100Af	ATGTCGACCACGCGCACCCGGCCTACTCCGCGCCCGAATGCACCG
	PafD-E100Ar	CGGTGCATTCGGGCGCGGAGTAGGCCGGGTGCGCGTGGTCGACAT

pMV-*dop*(R222A)-His$_6$ PafD-R222Af   CTGGAAACAACGCTCAAGGCCGGCATCATCAACACCCGCGACG

PafD-R222Ar   CGTCGCGGGTGTTGATGATGCCGGCCTTGAGCGTTGTTTCCAG

pMV-*dop*(H96V)-His$_6$ PafD-H96Vf   CGGGGCGCGGCTGTATGTCGACGTCGCGCACCCGGAATACTCCG

PafD-H96Vr   CGGAGTATTCCGGGTGCGCGACGTCGACATACAGCCGCGCCCCG

pMV-*dop*(R206A)-His$_6$ PafD-R206Af   CCGGCTTCCAGCTATCCCAGGCCTCGGACTACATCGAGGTCGAG

PafD-R206Ar   CTCGACCTCGATGTAGTCCGAGGCCTGGGATAGCTGGAAGCCGG

All plasmids listed below were made with PCR products using the same forward primer, His-prcC-Nde_F2:

pMN-NL-His$_6$-*pup*-GGQ   His-*prcC*-Nde_F2   GGGAATTCCATATGCACCACCACCACCACCACGCGCAAGAGCAGACCAAGC

HIII_*prcC*-p300_R   CCCAAGCTTTCACTGTCCGCCCTTTTGGA

pMN-NL-*pup*-His$_6$ HindIII-His6   CCCAAGCTTTCAGTGGTGGTGGTGGTGGTGGTG

pMN-NL-His$_6$-*pup*-G   prcC_NoG_RV_R   CCGGATATCTCAGCCCTTTTGGACGTATGCGC

pMN-NL-His$_6$-*pup*-GG   *prcC*_NoQ_RV_R   CCGGATATCTCATCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGA   prcC_QtoA_RV_R   CCGGATATCTCACGCTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-AGQ   prcC_AGQ_RV_R   CCGGATATCTCACTGTCCGGCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GAQ   prcC_GtoAQ_RV_R   CCGGATATCTCACTGTGCGCCCTTTTGGACGTATGCGC

pMN-NL-His$_6$-*pup*-AAQ   prcC_AAQ_RV_R   CCGGATATCTCACTGTGCTGCCTTTTGGACGTATGCGCGGA

pMN-NL-His$_6$-*pup*-AAE   prcC_AAE_RV_R   CCGGATATCTCACTCTGCTGCCTTTTGGACGTATGCGC

pMN-NL-His$_6$-*pup*-GGE   prcC_QtoE_RV_R   CCGGATATCTCACTCTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGN   prcC_QtoN_RV_R   CCGGATATCTCAGTTTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGD   prcC_QtoD_RV_R   CCGGATATCTCAGTCTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGA5Q   prcC_A5_RV_R   CCGGATATCTCACTGTGCTGCTGCTGCTGCTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GFQ   prcC_GtoF_RV_R   CCGGATATCTCACTGAAAGCCCTTTTGGACGTATGCGC

pMN-NL-His$_6$-*pup*-GGQG   prcC_GGQG_RV_R CCGGATATCTCATCCCTGTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGQA   prcC_GGQA_RV_R CCGGATATCTCATGCCTGTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGEG   prcC_GGEG_RV_R CCGGATATCTCATCCCTCTCCGCCCTTTTGGACGTATG

pMN-NL-His$_6$-*pup*-GGEA   prcC_GGEA_RV_R CCGGATATCTCATGCCTCTCCGCCCTTTTGGACGTATG

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