Mutations in the F_{420} biosynthetic pathway and a nitroreductase enzyme are the primary resistance determinants in spontaneous *in vitro* selected PA-824 mutants of *Mycobacterium tuberculosis*

SUPPLEMENTARY ELECTRONIC MATERIAL

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Supplementary Table S1: Target genes and primers.

Gene	Protein	Length (aa)	Function	Pathway	Primers
Rv3547	DDN	151	F_{420} –dependent nitroreductase	F_{420}	sRV3527F- 5' gcaccgaccagagcctcgcgctg 3' sRv3527R- 5' cgccctggcttcacgttcg 3'
Rv0407	FGD1	336	F_{420}-dependent glucose-6-phosphate dehydrogenase	Pentose phosphate pathway (oxidative branch)	sRv0407AF- 5' gcgggtcgcgtttatggc 3' sRv0407AR- 5' cgggcacgtcgtagatcg 3' sRv0407BF- 5' ggtggggctaatgcggcagc 3' sRv0407BR- 5' gcagagagccgcgtaatcggc 3'
Rv3261	FBIA	331	Catalyzes the transfer of the 2-phospholactate moiety from lactyl (2) diphospho-(5')guanosine (LPPG) to 7,8-didemethyl-8-hydroxy-5-deazariboflavin (FO) with the formation of the L-lactyl phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin (F_{420}-0) and GMP	Cofactor biosynthesis; coenzyme F_{420} biosynthesis	sRv3261F1: 5' gcggttctgttgtggttggg 3' sRv3261R1-1: 5' gcatgatgatgtcggcgtcgg 3' sRv3261F1-1: 5' gccaccgacgaccgttgcgaaaccc 3' sRv3261R1: 5' ccgatgacgggcaggatctcgatgg 3'
Rv3562	FBIB	448	Catalyzes the GTP-dependent successive addition of multiple gamma-linked L-glutamates to the L-lactyl phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin (F_{420}-0) to form polyglutamated F_{420} derivatives	Cofactor biosynthesis; coenzyme F_{420} biosynthesis	sFBiBF3: 5' ggtgccgctgctgatgaccgacc 3' sFBiBR1: 5' cgacccagctcgagcgcttcgg 3' sRv3262F1-1: 5' gccgccgacttggtcaaaggc 3' sRv3262R1: 5' cgcggtgaattgatccgtcggg 3'
Rv1173	FBIC	856	Catalyzes the radical-mediated transfer of the hydroxybenzyl group from 4-hydroxyphenylpyruvate (HPP) to 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione to form 7,8-didemethyl-8-hydroxy-5-deazariboflavin (FO)	Cofactor biosynthesis; coenzyme F_{420} biosynthesis	sRv1173FP1- 5' cctctcacggatggtatcgc 3' sRv1173RP1- 5' cgtcgaggatctcgtcgggttcc 3' sRv1173FP2- 5' ggtgtttatcccggtcacccgg 3' sRv1173RP2- 5' ccttggcgcggaagttctgcacg 3' sRv1173FP3- 5' cgcgccgatacgttacatgcg 3' sRv1173RP3- 5' ccgatcgcagggcggcaagc 3' sRv1173FP4- 5' gggaatcgatccgcgagcagg 3' sRv1173RP4- 5' ccagcgaacctcgtcgtccagg 3' sRv1173FP5- 5' gcgtcaccaagagcgggc 3' sRv1173RP5- 5' cgtcgccggggctaggccagattgc 3'

Supplementary Table S2: Mutations on *fbi*ABC genes

Gene Rv1173/ *fbiC*

Nucleotide Change	Amino acid (aa) Change	Freq.	*Conc	Comments
^{148}G>C	^{50}Ala→Pro	1	1	Single nucleotide substitution
$^{155\Delta}$G	^{52}Gly→Val	1	1	Single nucleotide deletion →Frame-shift
$^{160/161}$GA>AT	^{54}Glu→Met	1	5	Dinucleotide substitution.
^{258}T>G	^{86}Tyr→STOP	1	1	Single nucleotide substitution→Early termination codon
^{271}T>G	^{91}Phe→Val	1	1	Single nucleotide substitution
^{313}T>C	^{105}Cys→Arg	1	5	Single nucleotide substitution
^{569}A>G	^{190}His→Arg	1	5	Single nucleotide substitution
^{604}T>C	^{202}Ser→Pro	1	1	Single nucleotide substitution
^{611}T>C	^{204}Leu→Pro	1	1	Single nucleotide substitution
^{628}T>C	^{210}Ser→Pro	1	1	Single nucleotide substitution
^{647}A>C	^{216}Glu→Ala	1	1	Single nucleotide substitution
^{818}C>G	^{273}Thr→Arg	1	1	Single nucleotide substitution
$^{830⌂}$A	^{277}Ile→Asn,	1	1	Single nucleotide insertion → Frame-shift
$^{847⌂}$G	^{283}Glu→Gly	1	5	Single nucleotide insertion → Frame-shift
^{1008}C>G	^{336}Asn→Lys	1	5	Single nucleotide substitution
^{1066}G>T	^{356}Gly→Cys	1	1	Single nucleotide substitution
^{1072}T>G	^{358}Ser→Ala	1	1	Single nucleotide substitution
^{1114}C>T	^{372}Pro→Ser	3	5	Single nucleotide substitution
^{1154}G>T	^{385}Gly→Val	1	1	Single nucleotide substitution
^{1159}G>T	^{387}Asp→Tyr	2	1/5	Single nucleotide substitution
^{1891}C>T	^{631}His→Tyr	1	1	Single nucleotide substitution
^{2124}G>A	^{708}Met→Ile	2	5	Single nucleotide substitution
$^{2127/28\Delta}$GT	^{709}Met→Ile	1	5	Dinucleotide deletion →Frame-shift
^{2131}G>T	^{711}Gly→Trp	1	5	Single nucleotide substitution
$^{2133\Delta}$G	^{711}Gly→Gly	1	1	Single nucleotide deletion →Frame-shift
^{2143}A>C	^{715}Ser→Arg	1	1	Single nucleotide substitution
^{2156}G>T	^{719}Trp→Leu	1	5	Single nucleotide substitution
^{2159}T>G	^{720}Val→Gly	1	1	Single nucleotide substitution
^{2158}G>A	^{720}Val→Ile	3	1	Single nucleotide substitution
^{2165}A>G	^{722}His→Arg	1	5	Single nucleotide substitution
^{2158}G>A	^{720}Val→Ile	1	1	Single nucleotide substitution
$^{2274⌂}$G	^{758}Arg→Arg	1	5	Single nucleotide insertion → Frame-shift
$^{2374⌂}$CAGACCAGCTGGGTGAAACTT	$^{⌂792}$QTSWVKL	1	5	Repeat of preceding 7 aa, in-frame insertions
^{2399}T>G	^{800}Leu→Arg	1	5	Single nucleotide substitution
$^{2549⌂}$C	^{843}Thr→Thr	4	5	Single nucleotide insertion → Frame-shift
$^{2734⌂}$AACTT	$^{912⌂}$AACTT	1	1	Five nucleotide insertion → Frame-shift
$^{491\Delta}$AACGGGG	^{164}Glu→Ala	1	5	Seven nucleotide deletion →Frame-shift
^{2158}G>A	^{720}Val→ Ile	1	5	Single nucleotide substitution
Total number of f*biC* mutants		**47**		

*Conc: concentration used to select mutant.

Gene Rv3261/ *fbiA*

Nucleotide Change	Amino acid Change	Freq.	*Con (μM)	Comments
^{62}A>C	^{21}Gln→Pro	1	1	Single nucleotide substitution
^{145}G>T	^{43}Asp→Tyr	1	1	Single nucleotide substitution
^{167}T>C	^{56}Leu→Pro	1	1	Single nucleotide substitution
^{188}A>G	^{63}Asp→Gly	2	1/5	Single nucleotide substitution
^{195}C>G	^{65}Cys→Trp	1	5	Single nucleotide substitution
$^{211\Delta}$G	^{71}Gly→Ala	2	5	Single nucleotide deletion →Frame-shift
$^{222\Delta}$C	^{74}Asp→Asp	1	5	Single nucleotide deletion →Frame-shift
$^{227\text{⌂}}$C	^{76}Gln→Pro	1	5	Single nucleotide insertion →Frame-shift
^{237}G>A	^{79}Trp→STOP	2	5	Single nucleotide substitution→Early termination codon
$^{242\text{⌂}}$C	^{81}Gln→Pro	1	5	Single nucleotide insertion →Frame-shift
^{263}C>A	^{88}Ala→Asp	1	5	Single nucleotide substitution
$^{337\text{⌂}}$T	^{113}Leu→Ser	1	1	Single nucleotide insertion →Frame-shift
$^{349\Delta}$C	^{117}Gln→Pro	1	1	Single nucleotide deletion →Frame-shift
^{356}T>C	^{119}Leu→Pro	1	1	Single nucleotide substitution
^{376}T>C	^{126}Ser→Pro	1	1	Single nucleotide substitution
^{406}T>C	^{136}Trp→Arg	2	5	Single nucleotide substitution
^{436}A>G	^{146}Thr→Ala	1	1	Single nucleotide substitution
$^{899\Delta}$GGGTGACG	^{164}Gly →Gly	1	5	Single nucleotide deletion →Frame-shift
$^{899\Delta}$G	^{164}Gly →Gly	1	5	Single nucleotide deletion →Frame-shift
^{514}T>C	^{172}Trp→Arg	1	1	Single nucleotide substitution
$^{563\text{⌂}}$T	^{188}Val→Val	2	1	Single nucleotide insertion →Frame-shift
$^{575\text{⌂}}$A	^{192}Lys→Lys	1	1	Single nucleotide insertion →Frame-shift
$^{575\Delta}$A	^{192}Lys→Ser	1	1	Single nucleotide deletion →Frame-shift
^{713}C>A	^{238}Ala→Glu	1	1	Single nucleotide substitution
^{747}AA>GT	^{249}Glu→Glu	1	1	Dinucleotide substitution→Frame-shift
^{775}T>C	^{259}Cys→Arg	2	1	Single nucleotide substitution
^{847}G>A	^{283}Gly→Arg	2	1	Single nucleotide substitution
^{968}G>T	^{323}Gly→Val	1	1	Single nucleotide substitution
Total number of *fbiA* mutants		**35**		

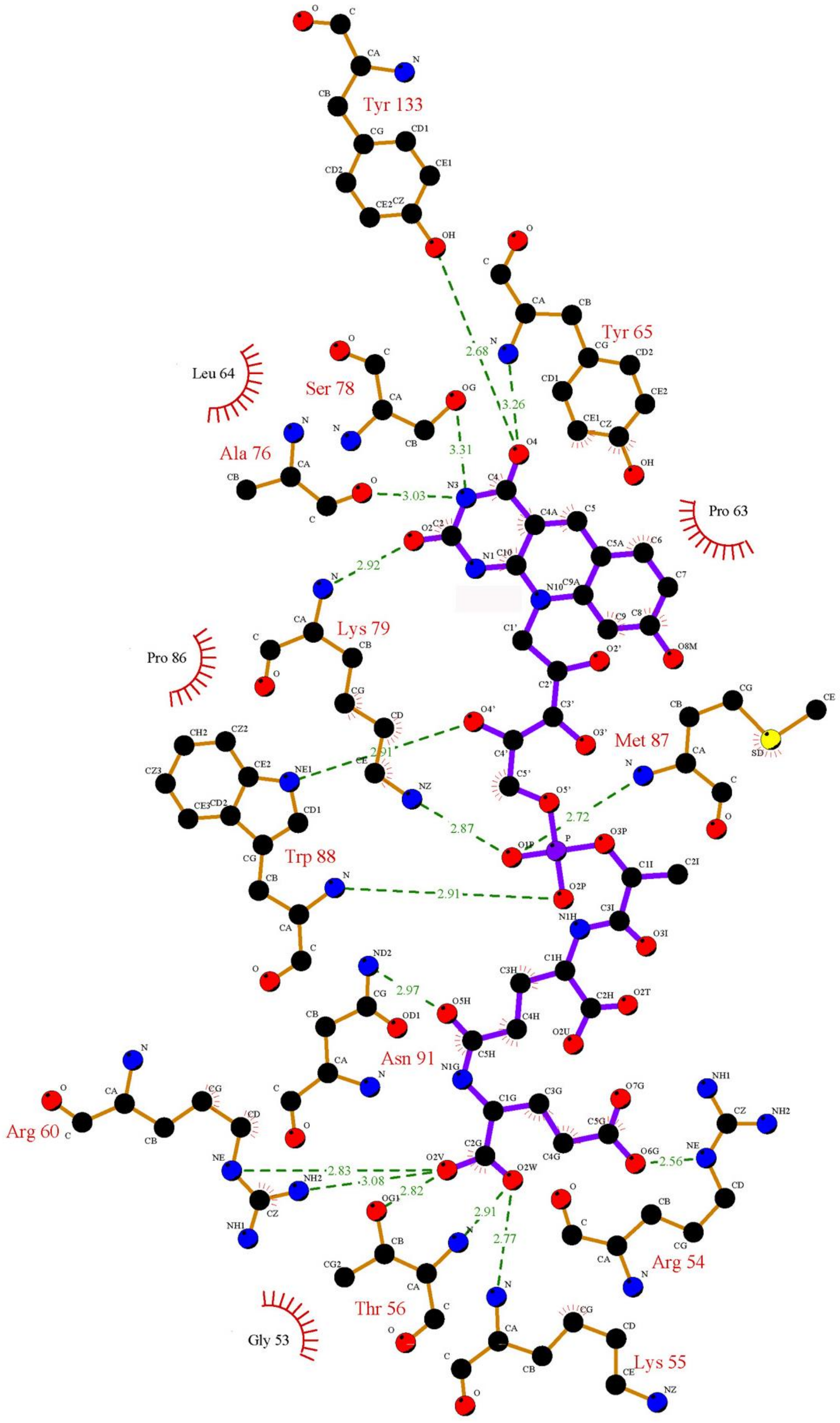
Rv3262/*fbiB*

Nucleotide Change	Amino acid Change	Freq.	*Con (μM)	Comments
$^{\text{⌂}36}$GGCTCCGCCTCGACCAT	Frame-shift	1	5	Seventeen nucleotide insertions → Frame-shift
^{117}G>A	^{39}Trp→STOP	1	5	Single nucleotide substitution→Early termination codon
^{458}G>T	^{153}Gly→Val	1	1	Single nucleotide substitution
^{1081}C>G	^{361}Pro→Ala	1	5	Single nucleotide substitution

Total number of *fbiB* mutants 4

***Conc: concentration used to select mutant.**

Supplementary Figure S1: Schematic 2D interacting plot of F_{420} cofactor in the crystal structure of Ddn. Hydrogen bonds (green dashed lines) and van der Waals contacts (red semi-circles with radiating spokes). Color scheme: carbon in dark, nitrogen in blue, oxygen in red and sulfur in yellow. The image was obtained with LigPlot program [1].



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[1] Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein engineering* 1995; **8**: 127-34.

Supplementary Table S3: Minimum Inhibitory Concentration (MIC_{99})

Gene	Mutation	MIC_{99} (µM)
ddn (3547)	^{11}Ser > Stop (15 samples)	>10
	^{22}Ser > Leu	>1<5
	^{25}Asn >Ile	>10
	^{48}Leu > Pro	>5<10
	^{78}Ser > Pro	>10
	^{88}Trp > Arg	>10
	^{137}Gln > Stop	>10
fgd1 (0407)	^{143}Trp > Stop	>10
	^{169}Gly > Ala	>5<10
	^{230}Glu > Lys	>10
fbiC (1173)	^{52}C-deletion	>10
	^{86}Tyr > Stop	>10
	^{105}Cys > Arg	>10
	^{164}AACGGG-deletion	>10
	^{336}Asn > Lys	>10
	^{708}Met > Ile	>5<10
	^{709}GT-deletion	>10
	^{720}Val > Ile	>10
fbiA (3261)	^{43}Asp > Tyr	>10
	^{56}Leu > Pro	>10
	^{71}G-deletion	>10
	^{74}C-deletion	>10
	^{81}C-insertion	>10
	^{119}Leu > Pro	>10
fbiB (3262)	Nil tested	--
No mutation identified in *ddn, fgd1, fibABC.*	2 samples	>10
Wild Type H37Rv		<1