

Fig. S1. Determination of the MBC concentration of RIF for *Mtb*. MBC was considered to be the lowest concentration of the drug that can reduce the cfu/ml by 2-log_{10} or more within a period of 6 days (28). The MBC of RIF for *Mtb* was estimated to be 0.1 $\mu\text{g/ml}$ since about 2-log_{10} reduction in the cfu was found to be very close to 0.1 $\mu\text{g/ml}$ of RIF.

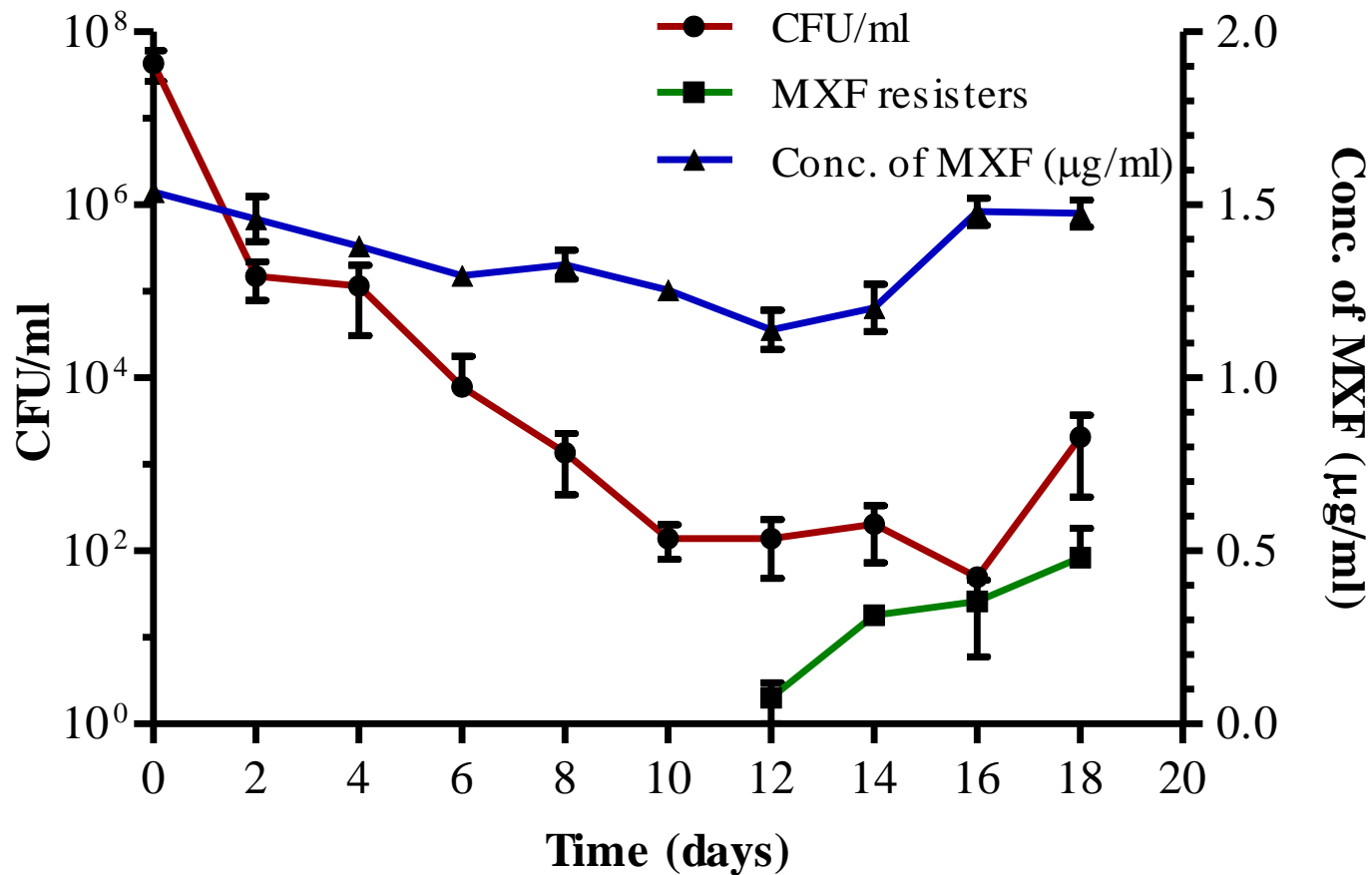


Fig. S2. MXF susceptibility profile of *Mtb* during 1 $\mu\text{g/ml}$ MXF treatment for 18 days showing a triphasic curve with an initial killing phase followed by a persistence phase and a phase of regrowth. MXF resistant colonies were observed from 12th day of exposure onwards. Right Y-axis represent the MXF concentration during the exposure. Although 1 $\mu\text{g/ml}$ of MXF was used, the concentration estimated was found to be not exactly 1 $\mu\text{g/ml}$ due to the sensitivity limits of *Staphylococcus aureus* bioassay for MXF.

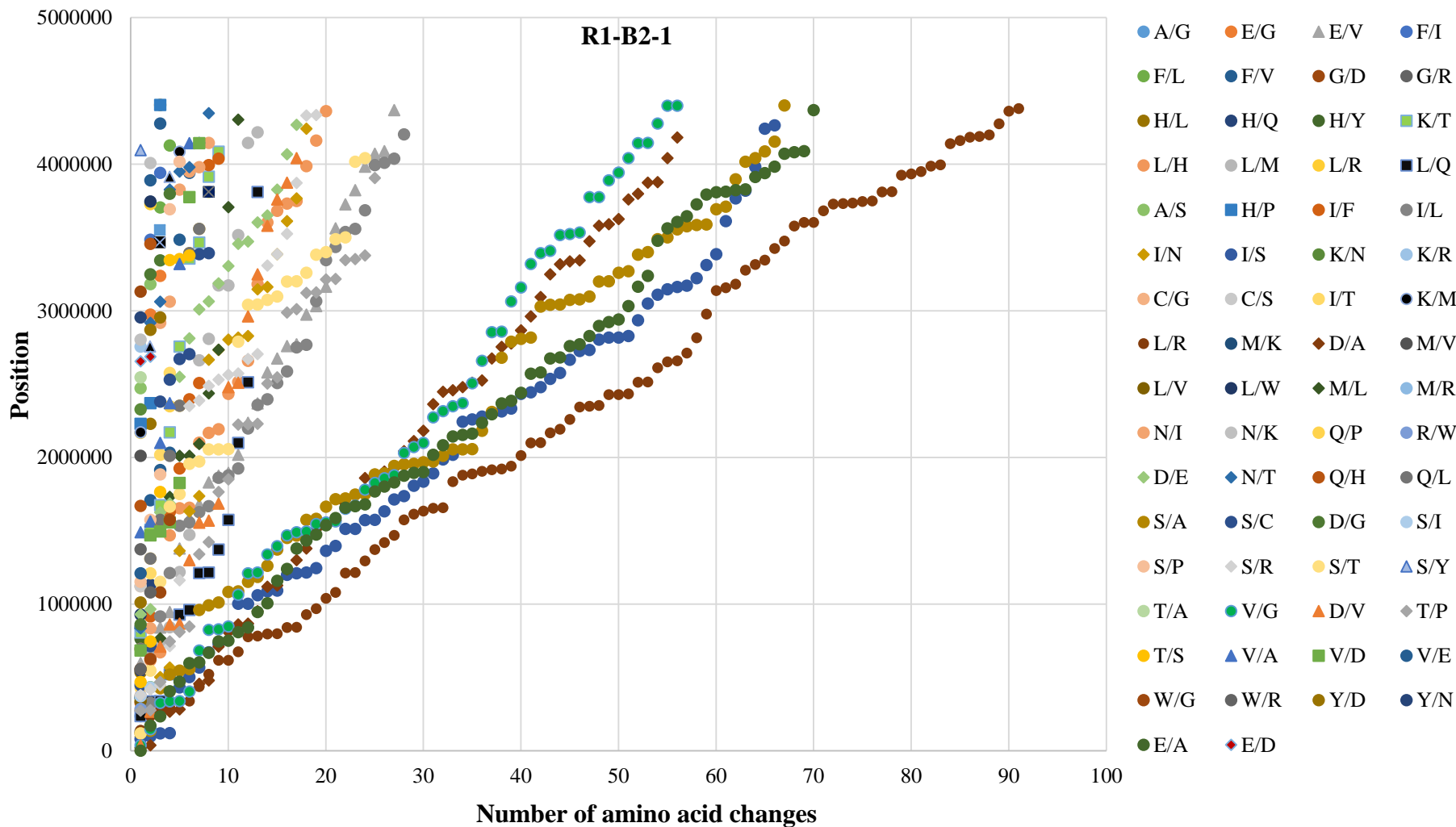


Fig. S3. Scatter plot representing the amino acid changes corresponding to the mutations found in the whole genome sequencing of R1-B2-1 RIF-resistant mutant. The y-axis represents the position of the mutation and the x-axis shows the number of the corresponding amino acid change.

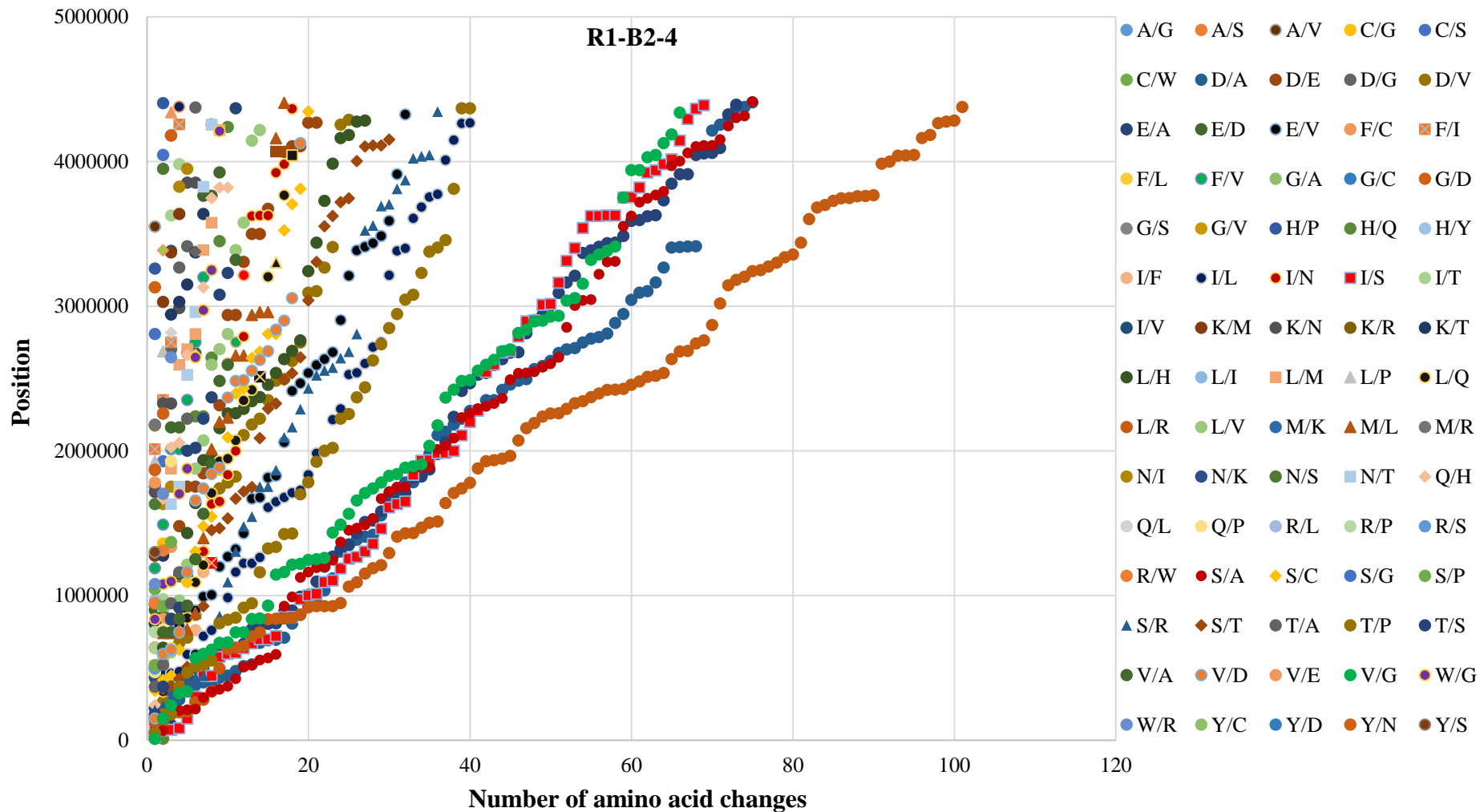


Fig. S4. Scatter plot representing the amino acid changes corresponding to the mutations found in the whole genome sequencing of R1-B2-4 RIF-resistant mutant. The y-axis represents the position of the mutation and the x-axis shows the number of the corresponding amino acid change.

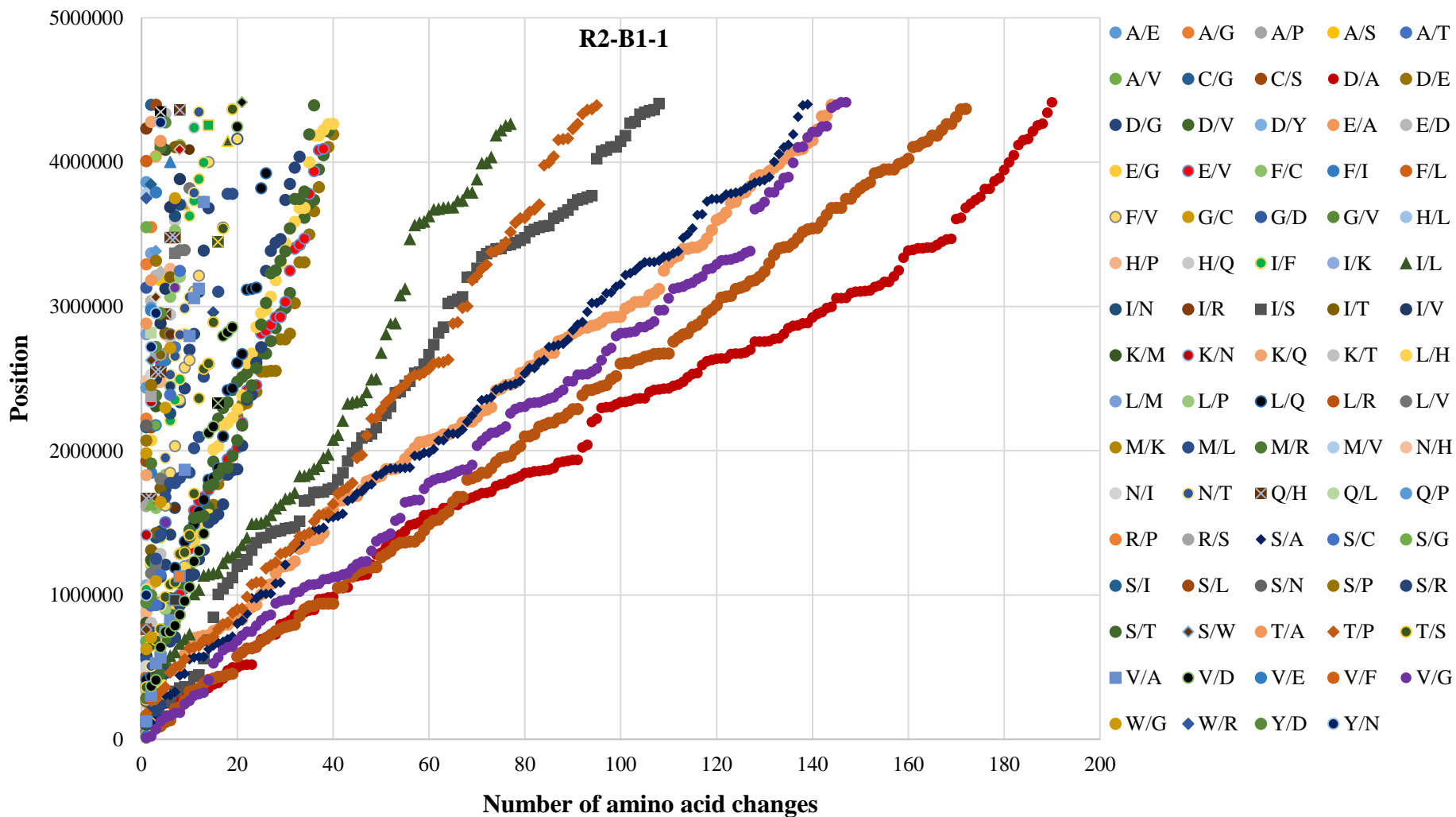


Fig. S5. Scatter plot representing the amino acid changes corresponding to the mutations found in the whole genome sequencing of R2-B1-1 RIF-resistant mutant. The y-axis represents the position of the mutation and the x-axis shows the number of the corresponding amino acid change.

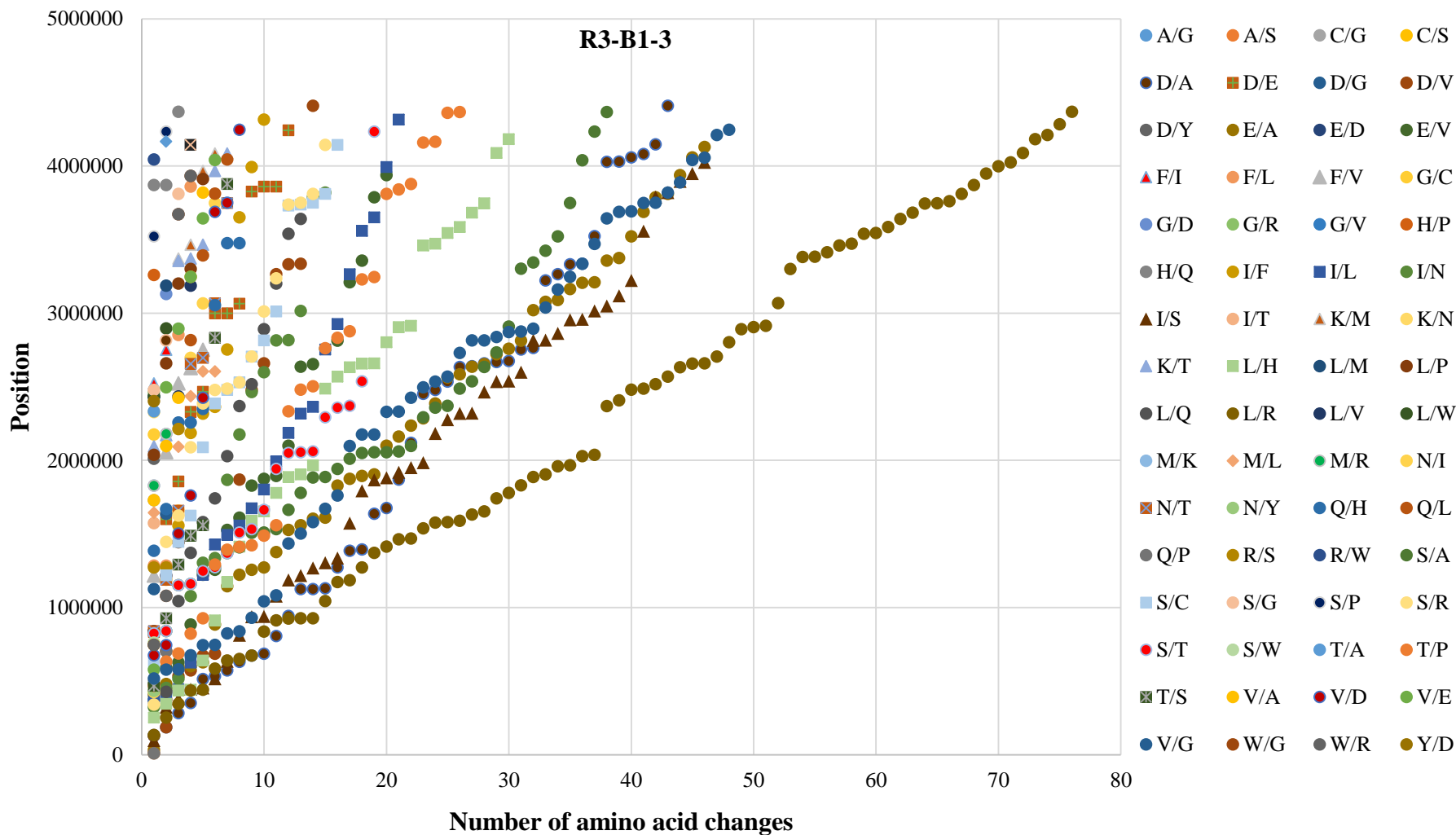


Fig. S6. Scatter plot representing the amino acid changes corresponding to the mutations found in the whole genome sequencing of R3-B1-3 RIF-resistant mutant. The y-axis represents the position of the mutation and the x-axis shows the number of the corresponding amino acid change.

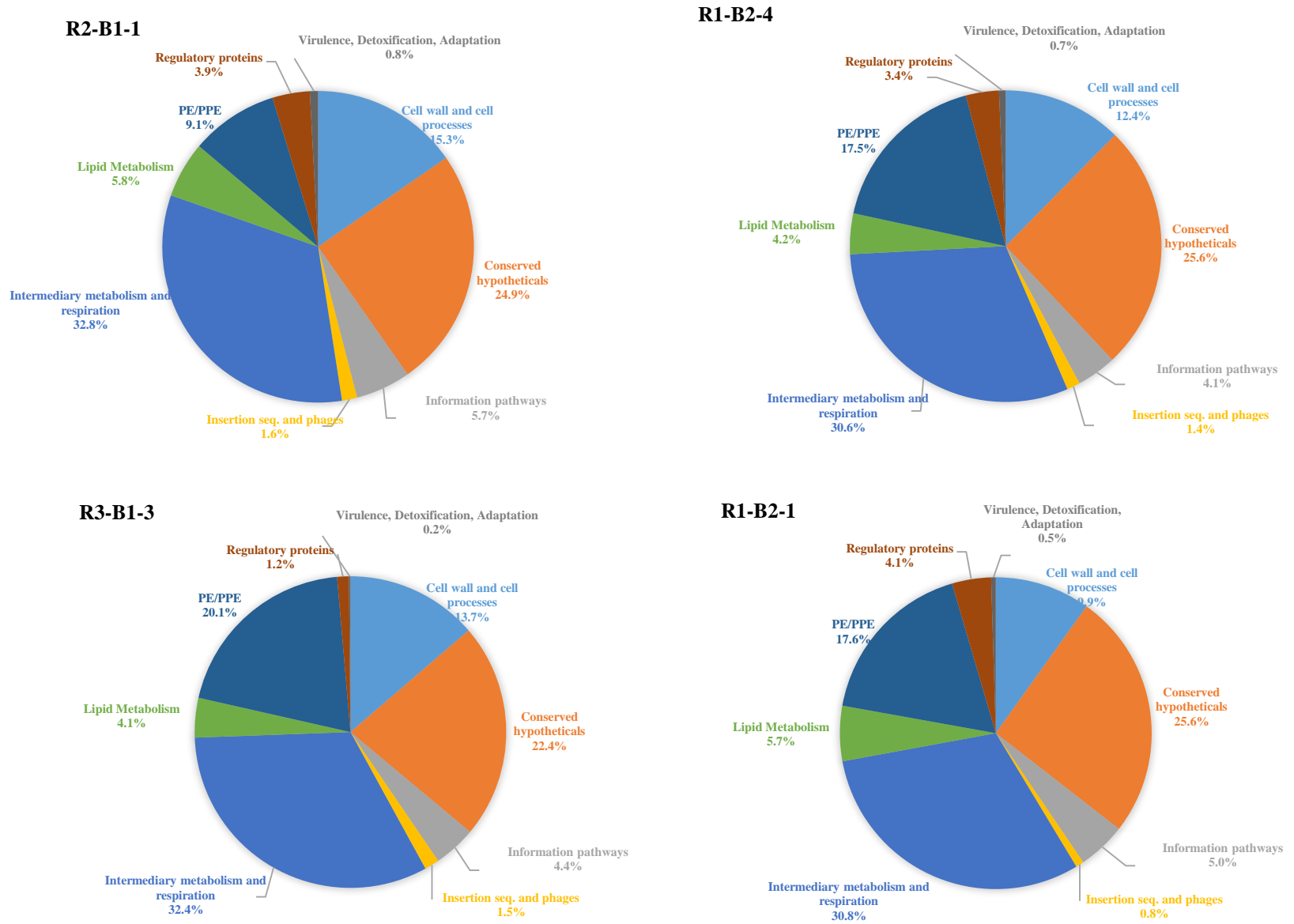


Fig. S7. PIE diagrams of the 4 RIF-resistant mutants showing mutations in the different category of the genes with respect to their parental strain. The genes were categorised based on their functions (source: Tuberculist).

TABLE S1. Bacterial strains and plasmid vectors used in the study

Bacterial strain or plasmid vector	Purpose	References (Supplementary)
Bacterial strains		
<i>Mycobacterium tuberculosis</i> H ₃₇ R _a	Experimental system	1
<i>Escherichia coli</i> JM109	Cloning	2
<i>Staphylococcus aureus</i> ATCC 25923	RIF & MXF bioassay	ATCC
Plasmids/constructs		
pMV762-Mrx1-roGFP2	Redox sensor vector	3
pAKMN2	Genome integration vector	4
pAKMN2-Mrx1-roGFP2	Redox sensor vector for genome integration	This study
pBS-KS	Sequencing vector	5
pBS-KS-Mrx1-roGFP2	Cloning vector	This study

TABLE S2. Oligonucleotide primers used in the study

Name of the Oligonucleotide primer	Sequence ^a
Mrx1-rogfp2-f	5' <u>ccttaatta</u> agatccggaggaatcacttc 3'
Mrx1-rogfp2-r	5' cc <u>atttaaatt</u> tacttgtacagctcgtccatg 3'
Mtu-rpoB-seg2-f	5' <u>gctctag</u> aggaccagcgagcagattg 3'
Mtu-rpoB-seg2-r	5' <u>ggggtacc</u> gcgcgtacaccgacagc 3'
Mtu-gyrA-QRDR-f	5' atgacagacacgacgttgc 3'
Mtu-gyrA-QRDR-r	5' tcgtagttaggatgaaatcg 3'
Mtu-gyrB-QRDR-f	5' agaaggtctgtaacgaacagc 3'
Mtu-gyrB-QRDR-r	5' gttgtgccaaaaacacatg 3'

^a Restriction enzyme sites are underlined.

TABLE S3. Proportion of *Mtb* cells with respect to mid-log phase cells during exposure to 10x MBC RIF in 3 independent cultures (R1, R2, R3)

Time (Days) (Post-exposure)	CFU/ml in RIF-free plate	Proportion (%) of the cells from the respective day's culture post-exposure to 10x MBC RIF
<u>R1 Culture</u>		
0 (Mid-log phase)	3.00 x 10 ⁸	100
10 (Persistence phase)	1.00 x 10 ³	0.00033
11 (Persistence phase)	1.20 x 10 ³	0.00040
12 (Persistence phase)	2.00 x 10 ³	0.00067
13 (Persistence phase)	1.80 x 10 ⁴	0.00600
14 (Persistence phase)	9.80 x 10 ³	0.00327
15 (Persistence phase)	0.00	0.00000
16 (Regrowth phase)	0.00	0.00000
17 (Regrowth phase)	3.00 x 10 ⁴	0.01000
18 (Regrowth phase)	0.00	0.00000
<u>R2 Culture</u>		
0 (Mid-log phase)	6.30 x 10 ⁸	100
10 (Persistence phase)	0.00	0.00000
11 (Persistence phase)	2.00 x 10 ³	0.00032
12 (Persistence phase)	2.00 x 10 ⁴	0.00317
13 (Persistence phase)	2.00 x 10 ⁴	0.00317
14 (Persistence phase)	3.00 x 10 ⁴	0.00476
15 (Persistence phase)	1.00 x 10 ⁴	0.00159
16 (Regrowth phase)	2.10 x 10 ⁵	0.03333
17 (Regrowth phase)	2.80 x 10 ⁵	0.04444
18 (Regrowth phase)	8.90 x 10 ⁵	0.14127
<u>R3 Culture</u>		
0 (MLP)	6.00 x 10 ⁸	100
10 (Persistence)	8.50 x 10 ³	0.00142
11 (Persistence phase)	1.00 x 10 ⁴	0.00167
12 (Persistence phase)	1.00 x 10 ³	0.00017
13 (Persistence phase)	1.00 x 10 ⁴	0.00167
14 (Persistence phase)	4.00 x 10 ³	0.00067
15 (Persistence phase)	0.00	0.00000
16 (Regrowth phase)	0.00	0.00000
17 (Regrowth phase)	1.00 x 10 ⁴	0.00167
18 (Regrowth phase)	0.00	0.00000

Proportion (%) of the cfu/ml is given with respect to the mid-log phase cfu/ml (day 0) of *Mtb* cells of each culture (n = 3). Multiple time points of the persistence phase cultures are given, showing minimal variation in the cfu/ml. The cfu/ml of the killing phase (day 1 to day 9) is not given. The zero values of cfu/ml indicate the absence of any colony in spite of plating aliquots of undiluted culture.

TABLE S4. Proportion of *Mtb* cells with respect to mid-log phase cells during exposure to 20x MBC RIF in 3 independent cultures (R1, R2, R3)

Time (Days) Post-exposure	CFU/ml in RIF-free plate	Proportion (%) of the cells from the respective day's culture post-exposure to 20x MBC RIF
<u>R1 Culture</u>		
0 (Mid-log phase)	4.69 x 10 ⁸	100
12 (Persistence phase)	3.80 x 10 ⁴	0.00810
14 (Persistence phase)	3.10 x 10 ⁴	0.00661
16 (Persistence phase)	0.00	0.00000
18 (Regrowth phase)	2.00 x 10 ⁵	0.04264
20 (Regrowth phase)	4.00 x 10 ⁵	0.08529
<u>R2 Culture</u>		
0 (Mid-log phase)	6.14 x 10 ⁸	100
12 (Persistence phase)	5.80 x 10 ³	0.00094
14 (Persistence phase)	4.40 x 10 ³	0.00072
16 (Persistence phase)	1.30 x 10 ⁴	0.00212
18 (Regrowth phase)	1.10 x 10 ⁵	0.01792
20 (Regrowth phase)	0.00	0.00000
<u>R3 Culture</u>		
0 (Mid-log phase)	5.72 x 10 ⁸	100
12 (Persistence phase)	6.00 x 10 ³	0.00105
14 (Persistence phase)	8.60 x 10 ³	0.00150
16 (Persistence phase)	7.20 x 10 ³	0.00126
18 (Regrowth phase)	4.00 x 10 ³	0.00070
20 (Regrowth phase)	0.00	0.00000

Proportion (%) of the cfu/ml is given with respect to the mid-log phase cfu/ml (day 0) of *Mtb* cells of each culture (n = 3). Multiple time points of the persistence phase cultures are given, showing minimal variation in the cfu/ml. The cfu/ml of the killing phase (day 1 to day 11) is not given. The zero values of cfu/ml indicate the absence of any colony in spite of plating aliquots of undiluted culture.

TABLE S5. Proportion of RIF-resistant mutants of *Mtb* cells that emerged during exposure to 10x MBC RIF in the 3 independent cultures (R1, R2, R3) and selected on 50x MBC RIF plates.

Time (Days) (Post-exposure)	CFU/ml (RIF-free plate)	CFU/ml (RIF-plus plate)	Proportion (%) of the RIF-resistant mutants
<u>R1 Culture</u>			
0 (Mid-log phase)	3.00 x 10 ⁸	0.00	00.00
10 (Persistence phase)	1.00 x 10 ³	4.00 x 10 ¹	04.00
11 (Persistence phase)	1.20 x 10 ³	0.00	00.00
12 (Persistence phase)	2.00 x 10 ³	2.10 x 10 ²	10.50
13 (Persistence phase)	1.80 x 10 ⁴	6.10 x 10 ²	03.39
14 (Persistence phase)	9.80 x 10 ³	2.69 x 10 ³	27.45
15 (Persistence phase)	0.00	0.00	00.00
16 (Regrowth phase)	0.00	1.60 x 10 ³	UND
17 (Regrowth phase)	3.00 x 10 ⁴	2.30 x 10 ³	07.67
18 (Regrowth phase)	0.00	2.10 x 10 ³	UND
<u>R2 Culture</u>			
0 (Mid-log phase)	6.30 x 10 ⁸	0.00	00.00
10 (Persistence phase)	0.00	0.00	00.00
11 (Persistence phase)	2.00 x 10 ³	0.00	00.00
12 (Persistence phase)	2.00 x 10 ⁴	4.20 x 10 ²	02.10
13 (Persistence phase)	2.00 x 10 ⁴	3.00 x 10 ²	01.50
14 (Persistence phase)	3.00 x 10 ⁴	4.74 x 10 ³	15.80
15 (Persistence phase)	1.00 x 10 ⁴	0.00	00.00
16 (Regrowth phase)	2.10 x 10 ⁵	1.42 x 10 ³	00.68
17 (Regrowth phase)	2.80 x 10 ⁵	0.00	00.00
18 (Regrowth phase)	8.90 x 10 ⁵	1.50 x 10 ³	01.69
<u>R3 Culture</u>			
0 (Mid-log phase)	6.00 x 10 ⁸	0.00	00.00
10 (Persistence phase)	8.50 x 10 ³	0.00	00.00
11 (Persistence phase)	1.00 x 10 ⁴	0.00	00.00
12 (Persistence phase)	1.00 x 10 ³	0.00	00.00
13 (Persistence phase)	1.00 x 10 ⁴	2.50 x 10 ²	02.50
14 (Persistence phase)	4.00 x 10 ³	1.57 x 10 ³	39.25
15 (Persistence phase)	0.00	6.40 x 10 ²	UND
16 (Regrowth phase)	0.00	1.80 x 10 ³	UND
17 (Regrowth phase)	1.00 x 10 ⁴	1.21 x 10 ³	12.10
18 (Regrowth phase)	0.00	2.32 x 10 ³	UND

Proportion of the RIF-resistant mutants (from RIF-plus plate) is given with respect to the cfu/ml (from RIF-free plate) of the same day's culture post-exposure to RIF. Different proportions of RIF-resistant mutants could be seen in different day's culture. Colonies could be observed only after 6-7 weeks post-plating, but fresh colonies kept on coming even after 8-10 weeks. The cfu/ml of the killing phase (day 1 to day 9) is not given. The zero values of cfu/ml indicate the absence of any colony in spite of plating aliquots of undiluted culture. UND, undefined since 0 is in the denominator.

TABLE S6. Proportion of *Mtb* cells with respect to mid-log phase cells during exposure to 2x MBC MXF in 3 independent cultures (M1, M2, M3)

Time (Days) (Post-exposure)	CFU/ml in MXF-free plate	Proportion (%) of the cells on the respective days post-exposure
<u>M1 Culture</u>		
0 (MLP)	3.47×10^7	100
10 (Persistence phase)	2.00×10^2	0.00058
11 (Persistence phase)	1.66×10^3	0.00478
12 (Persistence phase)	2.40×10^2	0.00069
13 (Persistence phase)	6.00×10^1	0.00017
14 (Persistence phase)	3.40×10^2	0.00098
15 (Persistence phase)	6.00×10^2	0.00173
16 (Persistence phase)	5.00×10^1	0.00014
17 (Regrowth phase)	1.50×10^3	0.00432
18 (Regrowth phase)	3.21×10^3	0.00925
<u>M2 Culture</u>		
0 (MLP)	6.30×10^7	100
10 (Persistence phase)	1.40×10^2	0.00022
11 (Persistence phase)	7.60×10^2	0.00121
12 (Persistence phase)	1.20×10^2	0.00019
13 (Persistence phase)	4.00×10^1	0.00006
14 (Persistence phase)	8.00×10^1	0.00013
15 (Persistence phase)	1.00×10^2	0.00016
16 (Persistence phase)	0.00	0.00000
17 (Regrowth phase)	1.00×10^2	0.00016
18 (Regrowth phase)	1.79×10^2	0.00028
<u>M3 Culture</u>		
0 (MLP)	3.30×10^7	100
10 (Persistence phase)	8.00×10^1	0.00035
11 (Persistence phase)	5.00×10^1	0.00205
12 (Persistence phase)	6.00×10^1	0.00035
13 (Persistence phase)	1.00×10^1	0.00009
14 (Persistence phase)	1.90×10^2	0.00056
15 (Persistence phase)	3.10×10^2	0.00094
16 (Persistence phase)	0.00	0.00000
17 (Regrowth phase)	2.10×10^3	0.00636
18 (Regrowth phase)	2.81×10^3	0.00852

Proportion (%) of the cfu/ml is given with respect to the mid-log phase cfu/ml (day 0) of *Mtb* cells of each culture (n = 3). Multiple time points of the persistence phase are given, showing minimal variation in the cfu/ml. The cfu/ml of the killing phase (day 1 to day 9) is not given. The zero values of cfu/ml indicate the absence of any colony in spite of plating aliquots of undiluted culture.

TABLE S7. Proportion of MXF-resistant mutants of *Mtb* cells that emerged during exposure to 2x MBC MXF in the 3 independent cultures (M1, M2, M3) and selected on 4x MBC MXF plates.

Time (Days) (Post-exposure)	CFU/ml (MXF-free plate)	CFU/ml (MXF-plus plate)	Proportion (%) of the MXF-resistant mutants
<u>M1 Culture</u>			
0 (MLP)	3.47 x 10 ⁷	0.00	00.00
10 (Persistence phase)	2.00 x 10 ²	0.00	00.00
11 (Persistence phase)	1.66 x 10 ³	0.00	00.00
12 (Persistence phase)	2.40 x 10 ²	1.00 x 10 ¹	04.17
13 (Persistence phase)	6.00 x 10 ¹	0.00	00.00
14 (Persistence phase)	3.40 x 10 ²	0.00	00.00
15 (Persistence phase)	6.00 x 10 ²	1.20 x 10 ²	20.00
16 (Persistence phase)	5.00 x 10 ¹	3.00 x 10 ¹	60.00
17 (Regrowth phase)	1.50 x 10 ³	2.20 x 10 ²	14.67
18 (Regrowth phase)	3.21 x 10 ³	4.70 x 10 ²	14.64
<u>M2 Culture</u>			
0 (MLP)	6.30 x 10 ⁷	0.00	00.00
10 (Persistence phase)	1.40 x 10 ²	0.00	00.00
11 (Persistence phase)	7.60 x 10 ²	0.00	00.00
12 (Persistence phase)	1.20 x 10 ²	0.00	00.00
13 (Persistence phase)	4.00 x 10 ¹	0.00	00.00
14 (Persistence phase)	8.00 x 10 ¹	0.00	00.00
15 (Persistence phase)	1.00 x 10 ²	1.00 x 10 ¹	10.00
16 (Persistence phase)	0.00	4.00 x 10 ¹	UND
17 (Regrowth phase)	1.00 x 10 ²	0.00	00.00
18 (Regrowth phase)	1.79 x 10 ²	7.00 x 10 ¹	39.10
<u>M3 Culture</u>			
0 (MLP)	3.30 x 10 ⁷	0.00	00.00
10 (Persistence phase)	8.00 x 10 ¹	0.00	00.00
11 (Persistence phase)	5.00 x 10 ¹	0.00	00.00
12 (Persistence phase)	6.00 x 10 ¹	3.00 x 10 ¹	50.00
13 (Persistence phase)	1.00 x 10 ¹	2.00 x 10 ¹	200.0
14 (Persistence phase)	1.90 x 10 ²	1.80 x 10 ²	94.74
15 (Persistence phase)	3.10 x 10 ²	2.50 x 10 ²	80.65*
		4.50 x 10 ²	145.16*
16 (Persistence phase)	0.00	4.40 x 10 ²	UND
17 (Regrowth phase)	2.10 x 10 ³	1.12 x 10 ³	53.33
18 (Regrowth phase)	2.81 x 10 ³	1.97 x 10 ³	70.11

Different proportions of MXF-resistant mutants could be seen in different day's culture. Colonies could be observed only after about 3 months post-plating, but fresh colonies kept coming up even after 3 months. *For example, in M3-15 culture, 2.5 x 10² was the cfu after 3 months post-plating and 4.5 x 10² after 4 months because more number of colonies had emerged on the same plates. This was the case where the proportions were unusually high. The cfu/ml of the killing phase (day 1 to day 9) is not given. The zero values of cfu/ml indicate the absence of any colony in spite of plating aliquots of undiluted culture. UND, undefined since 0 is in the denominator.

TABLE S8. List of *gyrA* mutations at the QRDR^a of MXF-resistant mutants of *Mtb* cells isolated from the RIF persistence phase cells and plated on 4x MBC MXF plates in the Luria-Delbruck experiment

Clone Name ^b	Mutation Codon	Nucleotide Position	AA ^c Change	AA ^c Position	Nucleotide Change
01-M2-1	GAC-AAC	280	D-N	94	G-A
12-M3-1	GAC-AAC	280	D-N	94	G-A
14-M2-1	GAC-AAC	280	D-N	94	G-A
14-M2-2	GAC-AAC	280	D-N	94	G-A
14-M2-3	GAC-AAC	280	D-N	94	G-A
18-M1-1	GAC-AAC	280	D-N	94	G-A
18-M1-2	GAC-AAC	280	D-N	94	G-A
19-M1-1	GAC-AAC	280	D-N	94	G-A
19-M3-1	GAC-AAC	280	D-N	94	G-A

^aquinolone resistance determining region. ^bClones were named in the order of the day of post-exposure to RIF, tube number (M1 to M5), and colony number. ^cAmino acid. M4 and M5 tubes did not have any mutant.

Supplementary References

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