

## *Supplementary Material*

# **Hypoxic Nonreplicating Persistent *Mycobacterium tuberculosis* Develops Thickened Outer Layer that helps in Restricting Rifampicin Entry**

Kishor Jakkala, Parthasarathi Ajitkumar\*

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, Karnataka, India.

\*Corresponding author. Parthasarathi Ajitkumar, Tel: 91-80-2293-2344; E-mail: [ajitkpartha@gmail.com](mailto:ajitkpartha@gmail.com)

This document contains:

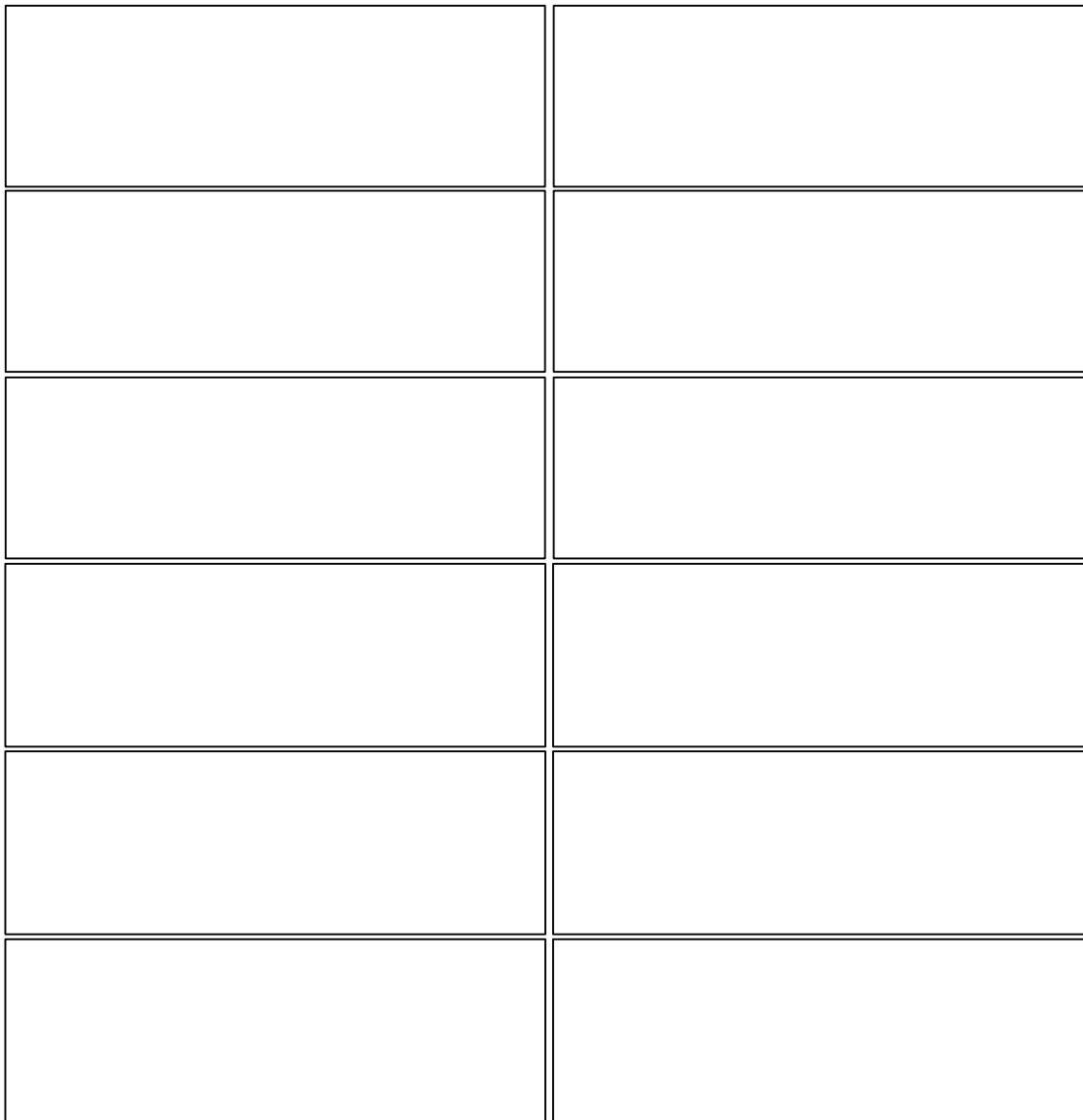
- Supplementary Figures (S1 – S5)
- Supplementary Table S1

**A**

<b>NRP-II</b>	
<b>Cell Size (μm)</b>	<b>Percentage of cells (%)</b>
1.9	0.6
2.3	3.4
2.6	8.9
3.09	14.8
3.5	18.6
<b>4.1</b>	<b>19.6</b>
<b>4.8</b>	<b>18.1</b>
<b>5.5</b>	<b>12.2</b>
<b>6.4</b>	<b>3.8</b>

**B**

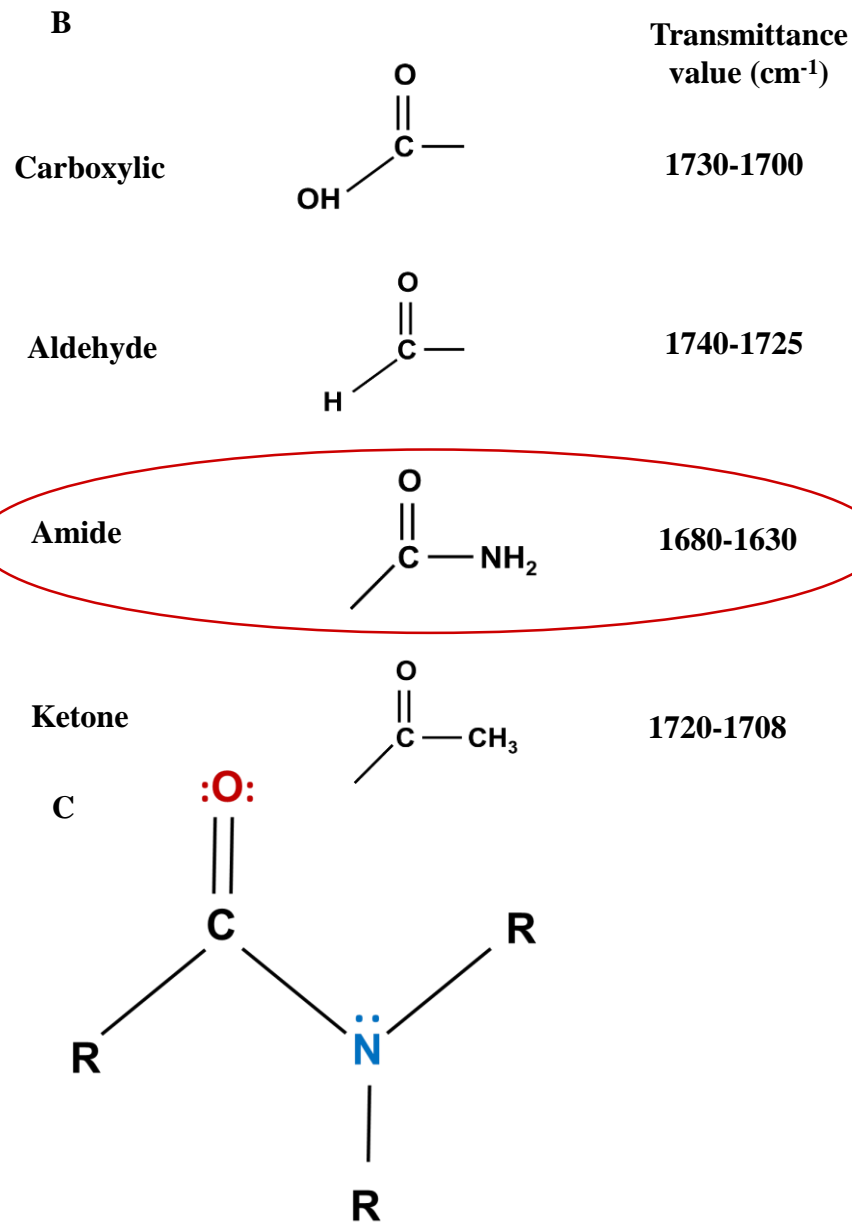
<b>MLP</b>	
<b>Cell Size (μm)</b>	<b>Percentage of cells (%)</b>
1.9	12.5
2.3	27.2
2.6	31.1
3.09	21.7
3.5	7.5

**C****D**

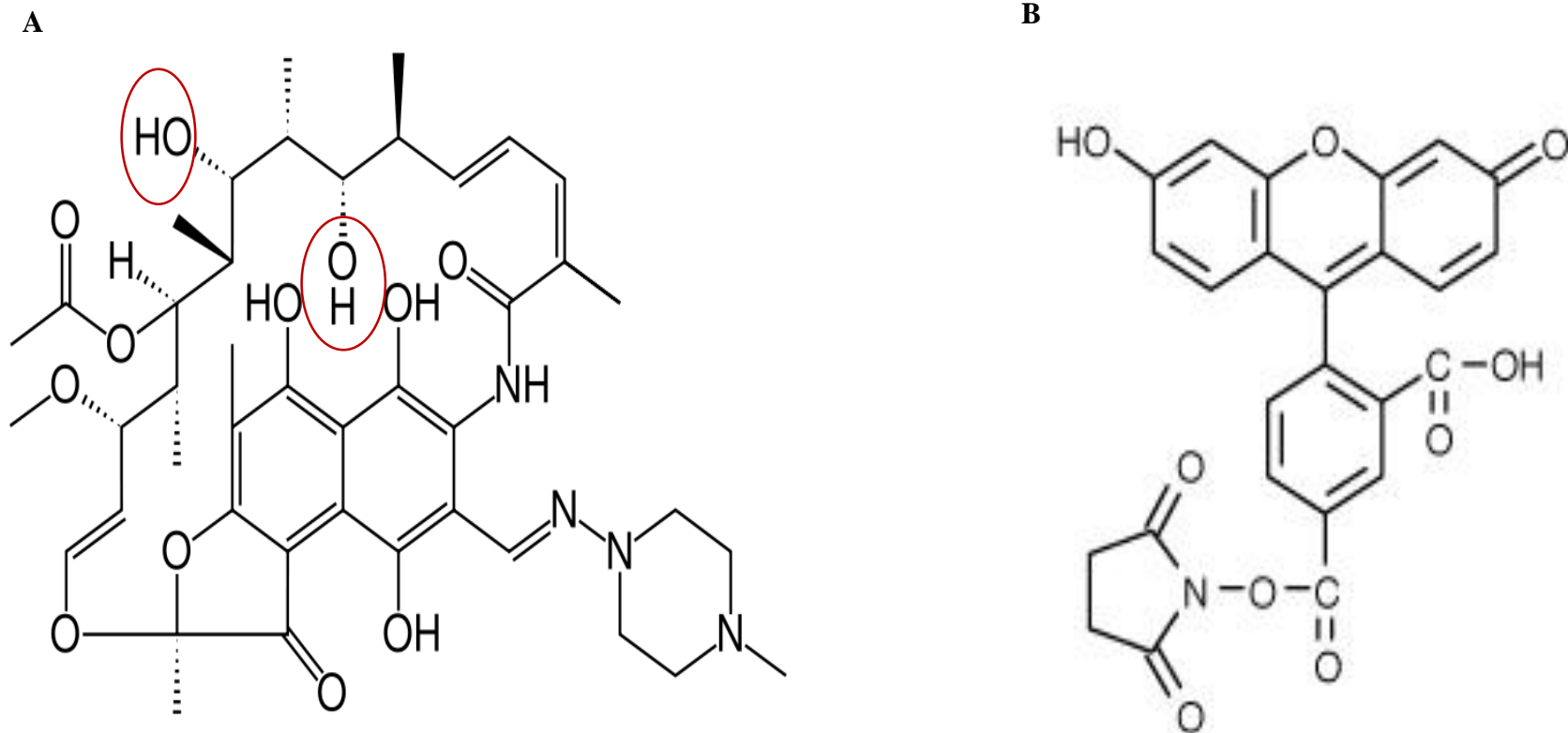
**Figure S1.** Size Measurement using Dynamic Light Scattering (DLS) using the Instrument, Malvern Zetasizer nano ZS (Sensitivity - 0.3 nm - 10 μm). **(A).** Average lengths of NRP stage 2 cells and the proportion of cells of such lengths. **(B).** Average lengths of MLP cells and the proportion of cells of such lengths. **(C & D).** Cell length distribution of MLP and NRP stage 2 cells determined using Zetasizer. Each peak represents cell size (nm) and the percentage of cells at that particular cell size range.

**A**

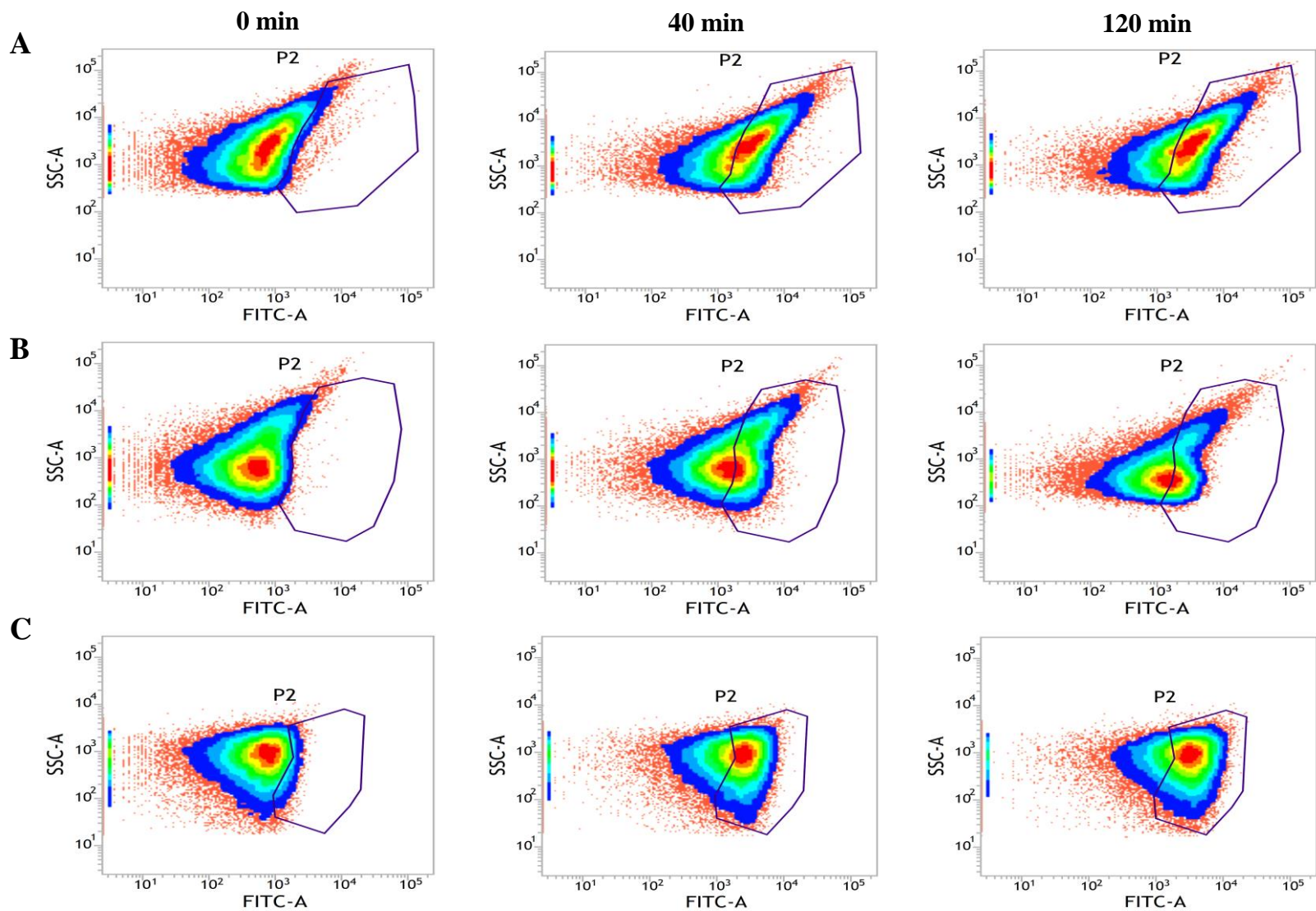
MLP		
Expected region (cm <sup>-1</sup> )	Observed values (cm <sup>-1</sup> )	IR Stretching frequency (cm <sup>-1</sup> )
3650-3200	3229	OH, Broad peak Hydrogen-bond
3400-2400	2357	OH (intermolecular) stretching frequency
1350-1000	1065	C-N frequency
970-700	976	Trans disubstituted alkenes
NRP-II		
Expected region (cm <sup>-1</sup> )	Observed values (cm <sup>-1</sup> )	IR Stretching frequency (cm <sup>-1</sup> )
3650-3200	3276	Broad peak Hydrogen-bonded
2926	2935	CH <sub>2</sub> of methine frequency
1680-1630	1649	C=O of amide frequency
1640-1550	1545	N-H of amide bend frequency
1350-1000	1033	C-N frequency
970-700	976	Trans disubstituted alkenes



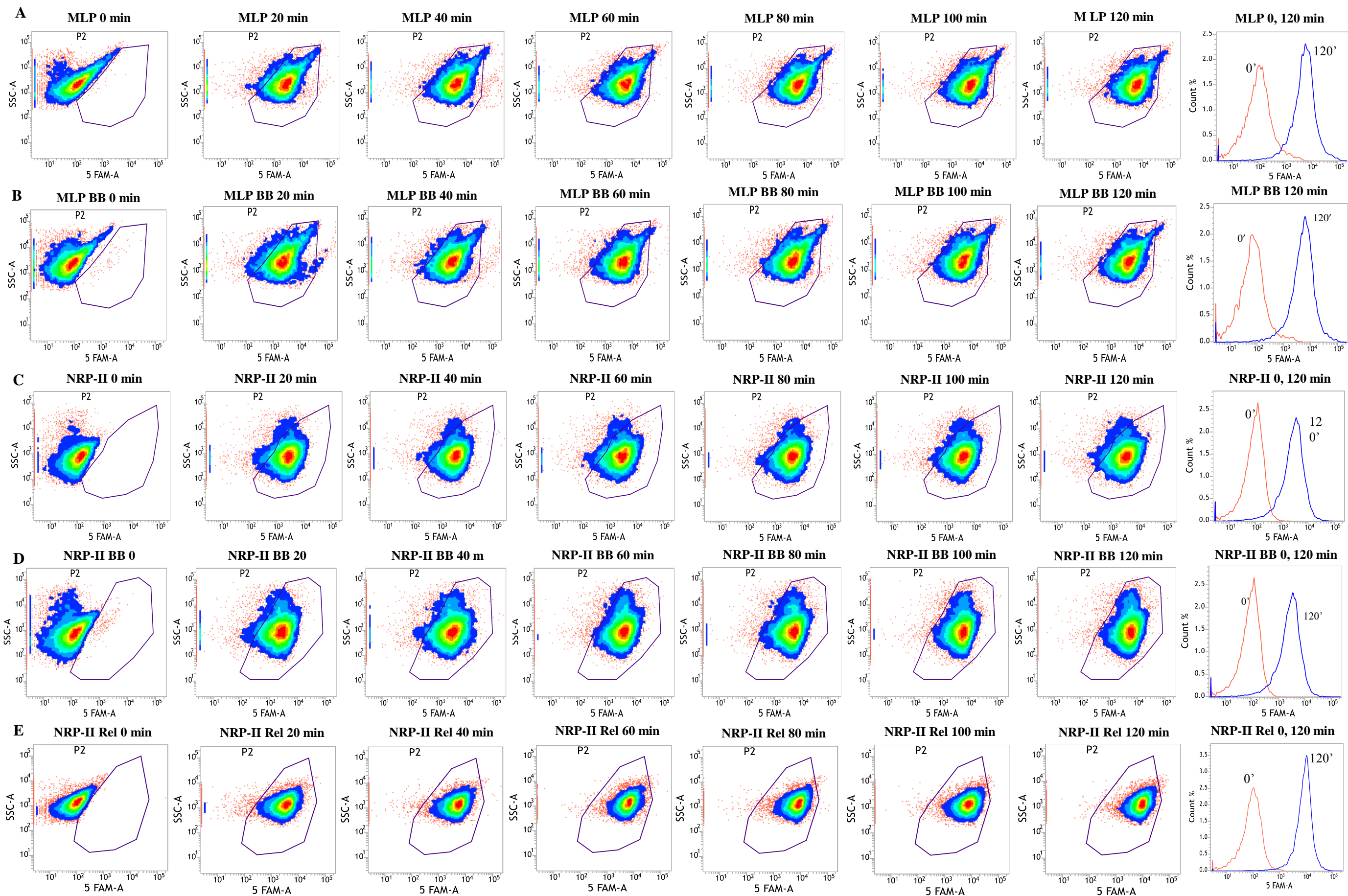
**Figure S2.** (A) Major functional group differences between *Mtb* MLP and NRP stage 2 cells from FTIR analysis. (B) Most probable pairing of carbonyl group with other functional groups. (C) Diagrammatic representation of carbonyl group with amide bend frequency [Pavia et al., 2001].



**Figure S3.** Chemical structures of the antibiotic (Rifampicin) and the fluorophore 5-carboxyfluorescein (5-FAM). (A). Chemical structure of the antibiotic Rifampicin. (B). The conjugate 5-carboxy fluorescein (5-FAM). Conjugation site for 5-FAM on rifampicin is encircled in red (A).



**Figure S4.** Flow cytometry profile of the 5-FAM fluorescence as a measure of the permeability of 5-FAM-RIF into *Mtb* MLP and NRP stage 2 cells over a period of 120 min. (A) MLP cells; (B) NRP stage 2 cells; (C) NRP stage 2 bead-beaten cells.



**Figure S5.** Flow cytometry profile of the 5-FAM fluorescence as a measure of the permeability of 5-FAM-RIF into *Mtb* MLP and NRP stage 2 cells over a period of 120 min. (A) MLP cells; (B) MLP bead-beaten cells; (C) NRP stage 2 cells; (D) NRP stage 2 bead-beaten cells; (E) NRP stage 2 cells post-release from hypoxia into normoxia.

**Table S1. Oligonucleotides used for qRT-PCR in the study**

<u>Name</u>	<u>Oligonucleotide sequence</u>	<u>Purpose</u>
Mtb-otsB1-RT-f	5' – attggtcgggcacagttgat – 3'	qRT-PCR
Mtb-otsB1-RT-r	5' – gacttctatctcggcggtgg – 3'	qRT-PCR
Mtb-galE2-RT-f	5' – gatgttcaccgaggacagca – 3'	qRT-PCR
Mtb-galE2-RT-r	5' – agtcaactgcaatatcggggc – 3'	qRT-PCR
Mtb-pimB-RT-f	5' – gcggttcgttgaataacct – 3'	qRT-PCR
Mtb-pimB-RT-r	5' – caaccgcgacagacacacta – 3'	qRT-PCR
Mtb-LdtA-RT-f	5' – agtgggtcgcctagcaatgctc – 3'	qRT-PCR
Mtb-LdtA-RT-r	5' – aggtatgtgccgagatgctg – 3'	qRT-PCR
Mtb-glgB-RT-f	5' – caacgactccgccaacaatg – 3'	qRT-PCR
Mtb-glgB-RT-r	5' – cagcccagatcgatagtcac – 3'	qRT-PCR
Mtb-malQ-RT-f	5' – gttgtcgtcggtgaggatct – 3'	qRT-PCR
Mtb-malQ-RT-r	5' – cgcaatctcgatcctgctca – 3'	qRT-PCR
Mtb-udgA-RT-f	5' – accgtatcgtccttggggta – 3'	qRT-PCR
Mtb-udgA-RT-r	5' – tggcggataccttgaccaac – 3'	qRT-PCR
Mtb-LdtB-RT-f	5' – gatgtggcggtcaacaccta – 3'	qRT-PCR
Mtb-LdtB-RT-r	5' – cgcacggctcagtatcttgg – 3'	qRT-PCR
Mtb-rpiB-RT-f	5' – ccaattgatcggcatcggc – 3'	qRT-PCR
Mtb-rpiB-RT-r	5' – gtgggtccgttcgtactcg – 3'	qRT-PCR
Mtb-1635-RT-f	5' – ggagtctgctgggccatc – 3'	qRT-PCR
Mtb-1635-RT-r	5' – gaagcccgaccataattcg – 3'	qRT-PCR
Mtb-ponA2-RT-f	5' – ggatctagaagccggcgaaa – 3'	qRT-PCR
Mtb-ponA2-RT-r	5' – acgtcgagttggcggttaac – 3'	qRT-PCR
Mtb-0648-RT-f	5' – cggcctgttgaccattaca – 3'	qRT-PCR
Mtb-0648-RT-r	5' – gtcagcgcgaccttttgag – 3'	qRT-PCR
Mtb-ald-RT-f	5' – cggatccacactcgctactc – 3'	qRT-PCR
Mtb-ald-RT-r	5' – acctggtttcatatgcgcga – 3'	qRT-PCR