Supplementary Material

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

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This document contains:

- Supplementary Figures (S1 – S5)
- Supplementary Table S1
**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.
### Table A: FTIR analysis of MLP

| Expected region (cm⁻¹) | Observed values (cm⁻¹) | IR Stretching frequency (cm⁻¹) |
|------------------------|------------------------|-------------------------------|
| 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   |

### Table B: FTIR analysis of NRP-II

| Expected region (cm⁻¹) | Observed values (cm⁻¹) | IR Stretching frequency (cm⁻¹) |
|------------------------|------------------------|-------------------------------|
| 3650-3200              | 3276                   | Broad peak Hydrogen-bonded    |
| 2926                   | 2935                   | CH₂ 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.
| Name            | Oligonucleotide sequence                        | Purpose       |
|-----------------|------------------------------------------------|---------------|
| Mtb-otsB1-RT-f  | 5’ – attgtcgggcacagttgat – 3’                 | qRT-PCR       |
| Mtb-otsB1-RT-r  | 5’ – gaccttatctcgccgcggg – 3’                 | qRT-PCR       |
| Mtb-galE2-RT-f  | 5’ – gatgttcaccgagcagca – 3’                 | qRT-PCR       |
| Mtb-galE2-RT-r  | 5’ – caacccgacagacacact – 3’                 | qRT-PCR       |
| Mtb-pimB-RT-f   | 5’ – gatgttcaccgagcagca – 3’                 | qRT-PCR       |
| Mtb-pimB-RT-r   | 5’ – caacccgacagacacact – 3’                 | qRT-PCR       |
| Mtb-LdtA-RT-f   | 5’ – agtgggtcgctagcttcgct – 3’               | qRT-PCR       |
| Mtb-LdtA-RT-r   | 5’ – agtgggtcgctagcttcgct – 3’               | qRT-PCR       |
| Mtb-glgB-RT-f   | 5’ – caacccgacagacacact – 3’                 | qRT-PCR       |
| Mtb-glgB-RT-r   | 5’ – caacccgacagacacact – 3’                 | qRT-PCR       |
| Mtb-malQ-RT-f   | 5’ – gtttgtcgtcggtgagta – 3’                 | qRT-PCR       |
| Mtb-malQ-RT-r   | 5’ – gtttgtcgtcggtgagta – 3’                 | qRT-PCR       |
| Mtb-udgA-RT-f   | 5’ – accgtatcgtctcttggtta – 3’               | qRT-PCR       |
| Mtb-udgA-RT-r   | 5’ – accgtatcgtctcttggtta – 3’               | qRT-PCR       |
| Mtb-LdtB-RT-f   | 5’ – cgccgacagacacact – 3’                   | qRT-PCR       |
| Mtb-LdtB-RT-r   | 5’ – cgccgacagacacact – 3’                   | qRT-PCR       |
| Mtb-rpiB-RT-f   | 5’ – ccaattgatcggcatcggc – 3’                | qRT-PCR       |
| Mtb-rpiB-RT-r   | 5’ – ccaattgatcggcatcggc – 3’                | qRT-PCR       |
| Mtb-1635-RT-f   | 5’ – ggagttgctgtgggccatact – 3’              | qRT-PCR       |
| Mtb-1635-RT-r   | 5’ – ggagttgctgtgggccatact – 3’              | qRT-PCR       |
| Mtb-ponA2-RT-f  | 5’ – ggatcttagagccggccgaa – 3’               | qRT-PCR       |
| Mtb-ponA2-RT-r  | 5’ – ggatcttagagccggccgaa – 3’               | qRT-PCR       |
| Mtb-0648-RT-f   | 5’ – ccggccttggtgctgct – 3’                  | qRT-PCR       |
| Mtb-0648-RT-r   | 5’ – ccggccttggtgctgct – 3’                  | qRT-PCR       |
| Mtb-ald-RT-f    | 5’ – cggatccacactgcactct – 3’                | qRT-PCR       |
| Mtb-ald-RT-r    | 5’ – cggatccacactgcactct – 3’                | qRT-PCR       |

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