Development of an *in vitro* assay for detection of Resuscitation-promoting factor dependent mycobacteria induced by treatment with antimicrobial agents

Jessica Loraine[$], Feifei Pu[&], Obolbek Turapov, Galina V. Mukamolova

Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK

[$] Current address: UCL School of Pharmacy, University College London, London, UK

[&] Current address: Department of Clinical Training Center Diagnostics Division, Siemens China, Shanghai, China

*To whom correspondence should be addressed. E-mail: gvm4@le.ac.uk

Running Title: Drug-induced Rpf-dependency

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**Table S1. Primers used in the study**

| Primer | Sequence 5'-3' | Description |
|---|---|---|
| RpfAF | AGTGGATCCATGTTGCGCCTGGTAGTCGGTGCG | *Mtb rpfA* cloning in pMind |
| RpfAR | ATAACTAGTTCAACGCGTGCGCGCACCCGCTCGT GCAGC | *Mtb rpfA* cloning in pMind |
| RpfBF | AGTGGATCCATGTTGCGCCTGGTAGTCGGTGCG | *Mtb rpfB* cloning in pMind |
| RpfBR | ATAACTAGTTCAACGCGTGCGCGCACCCGCTCGT GCAGC | *Mtb rpfB* cloning in pMind |
| RpfCF | AGTGGATCCGTGCATCCTTTGCCGGCCGACCAC | *Mtb rpfC* cloning in pMind |
| RpfCR | TATACTAGTTCACATATGGCGCGGAATACTTGCCT GAAT | *Mtb rpfC* cloning in pMind |
| RpfDF | AGTGGATCCCAGCAAGGTGGAGCTGCTATG | *Mtb rpfD* cloning in pMind |
| RpfDR | CATACTAGTTCAACGCGTATCGTCCCTGCTCCCC GAACA | *Mtb rpfD* cloning in pMind |
| RpfEF | TCGGGATCCGCGAAAGGAACAACGTTGAAGAAC | *Mtb rpfE* cloning in pMind |
| RpfER | TGCACTAGTTCACGCGTGCCGCGGCGGCCGCAG | *Mtb rpfE* cloning in pMind |
| RpfF | TGCCGGATCCGCCGATCAGCGAGGA | *rpf* cloning in pMind |
| RpfR | GTCACTAGTTCAGGCCTGCGGCAG | *rpf* cloning in pMind |
| RT-RpfAF | CGG*ACTAGT*CTA GCCAACGATGATGAT | qRT-PCR *Mtb rpfA* |
| RT-RpfAR | TCAGAACGGAATCATCCACCGTGA | qRT-PCR *Mtb rpfA* |
| RT-RpfBF | GCGATGCCGAAATCCATCACCTTT | qRT-PCR *Mtb rpfB* |

| | | |
|---|---|---|
| RT-RpfBR | AGAACCTCAACGTCTACGGCTTCA | qRT-PCR *Mtb rpfB* |
| RT-RpfCF | AGCTGCCTCTCGGGAACAA | qRT-PCR *Mtb rpfC* |
| RT-RpfCR | GACCACAGTGCGATCGGAAGG | qRT-PCR *Mtb rpfC* |
| RT-RpfDF | GCAACAGATCGAGGTCGCAG | qRT-PCR *Mtb rpfD* |
| RT-RpfDR | CGAGGAACGTCAGGATGTGG | qRT-PCR *Mtb rpfD* |
| RT-RpfEF | T GGCCTACAGCGTGAACTGG | qRT-PCR *Mtb rpfE* |
| RT-RpfER | GAACGCAGCACGTTCTCCAGC | qRT-PCR *Mtb rpfE* |
| 16srRNAF | TCCGGGCCTTGTACACA | qRT-PCR *16s rRNA* |
| 16srRNAR | TAACACCCGAAGCCAGTGG | qRT-PCR *16s rRNA* |
| MindF | TGAGTCATAGTTGCACTTTATCAT | Primer for pMind |
| MindR | TCCGAATCAATACGGTCTAGAGA | Primer for pMind |

*Restriction sites are outlined

**Table S2. Viable counts for mycobacterial cultures prior to drug exposure**

| Strain | Log10 CFU ±STDV | Log10 MPN ±STDV | Log10 MPN_SN ±STDV | Figures for treated samples |
|---|---|---|---|---|
| *M. tuberculosis* H37Rv | 7.65±0.3 | 7.68±0.41 | 7.8 | Figure 1 A, B; S1 |
| *M. smegmatis* | 7.54±0.21 | 7.48±0.27 | 7.6±0.19 | Figure 1 C, D; S2 |
| *M. smegmatis* pMind | 7.42±0.23 | 7.37±0.21 | N/D | Figure 4B, C; S5 |
| *M. smegmatis* pMind::*rpfD* | 7.21±0.03 | 7.17±0.05 | N/D | Figure 4B, C; S5 |

N/D – not determined

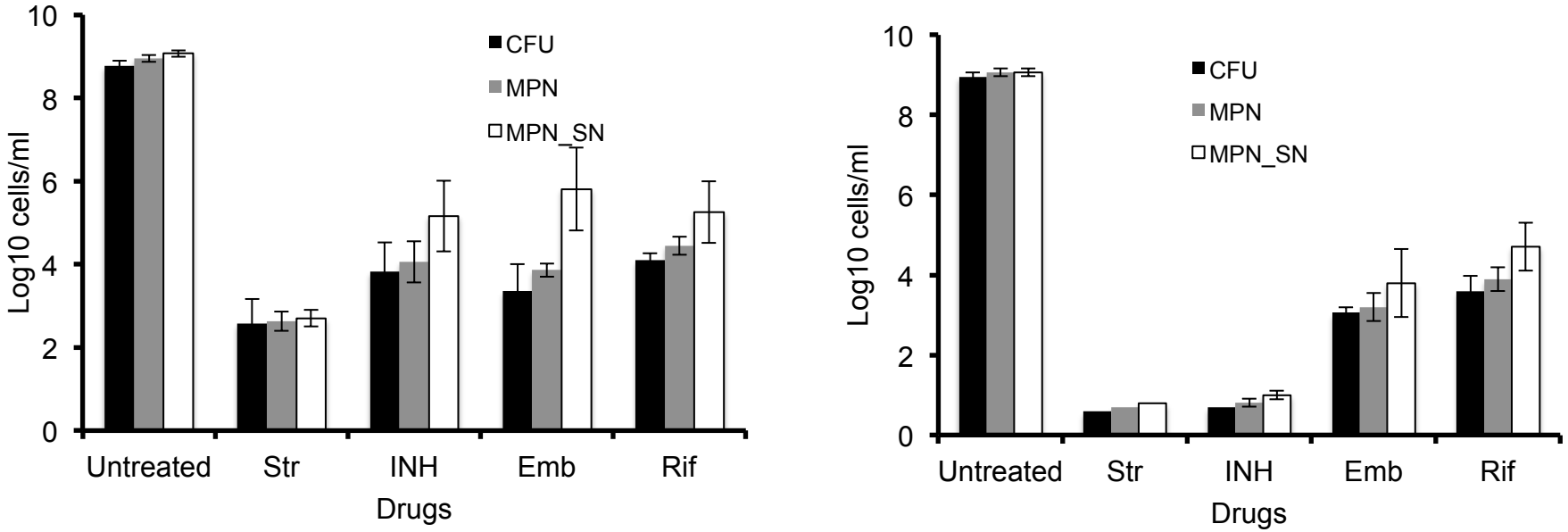


**Figure S1. Effect of drug treatment on viability of *M. tuberculosis***. Log phase cultures were inoculated in 7H9 medium without (untreated control) or with drugs and incubated at 37°C with shaking for 3 days (left panel) or 7 days (right panel). Cells were pelleted and washed with 7H9 medium before determination of CFU counts on 7H10 agar or MPN counts in 7H9 medium (MPN) or in 7H9 supplemented with 50% (v/v) culture supernatant. Average of three independent experiments (done in duplicates) are shown. Error bars indicate standard deviations. Drugs were added at the following concentrations (µg/ml): streptomycin (Str) 20, rifampin (RIF) 5, isoniazid (INH) 10, ethambutol (Emb) 20.

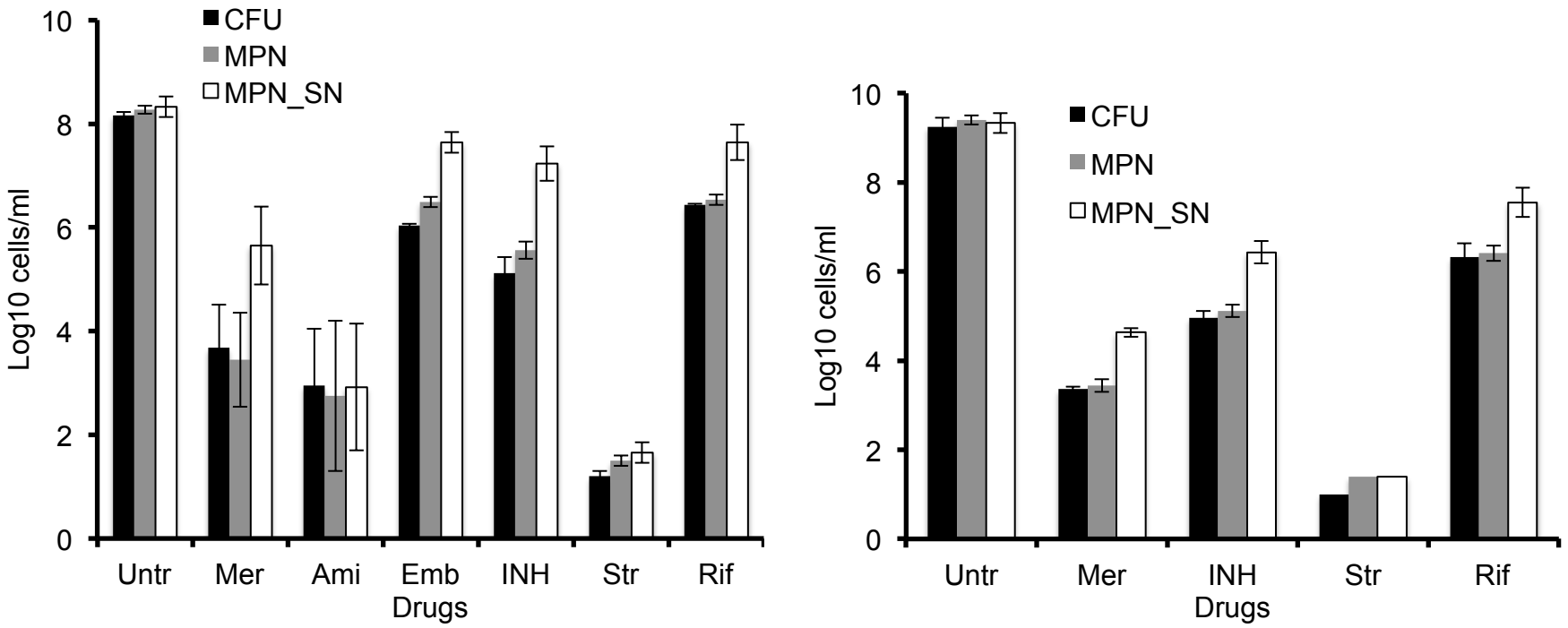


**Figure S2. Effect of drug treatment on viability of *M. smegmatis.*** Log phase cultures were inoculated in 7H9 medium without (untreated control) or with drugs and incubated at 37°C with shaking for 24 hours (left panel) or 48 hours (right panel). Cells were pelleted and washed with 7H9 medium before determination of CFU counts on 7H10 agar or MPN counts in 7H9 medium (MPN) or in 7H9 supplemented with 50% (v/v) culture supernatant. Average of three independent experiments (done in duplicates) are shown. Error bars indicate standard deviations. Drugs were added at the following concentrations (µg/ml): streptomycin (Str) 20, rifampin (RIF) 100, isoniazid (INH) 10, ethambutol (Emb) 20, meropenem (Mer) 50, amikacin (Ami) 100.

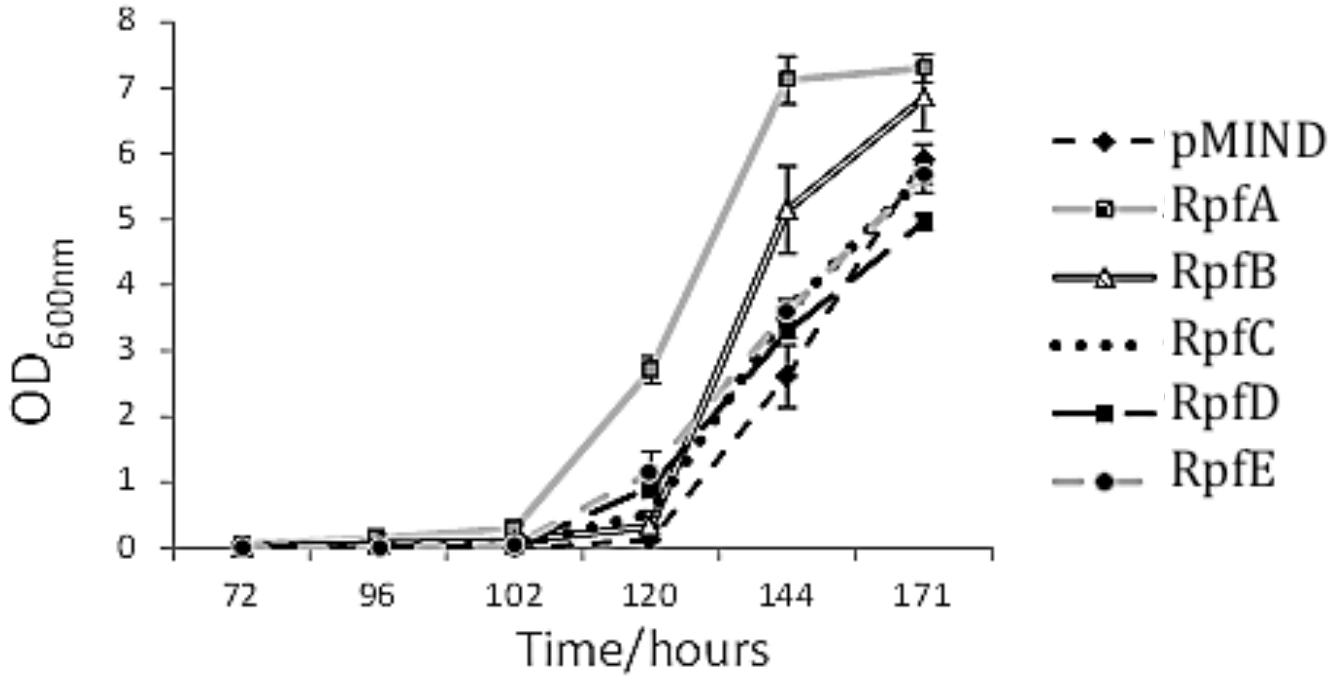


**Figure S3. Effect of Rpf overexpression on *M. smegmatis* growth in Sauton's medium.** 10 ml *M. smegmatis* cultures were grown in Sauton's medium supplemented with 50 µg/ml kanamycin and 20 ng/ml tetracycline. Average $OD_{600}$ values of three independent experiments are shown, error bars indicate standard deviation.

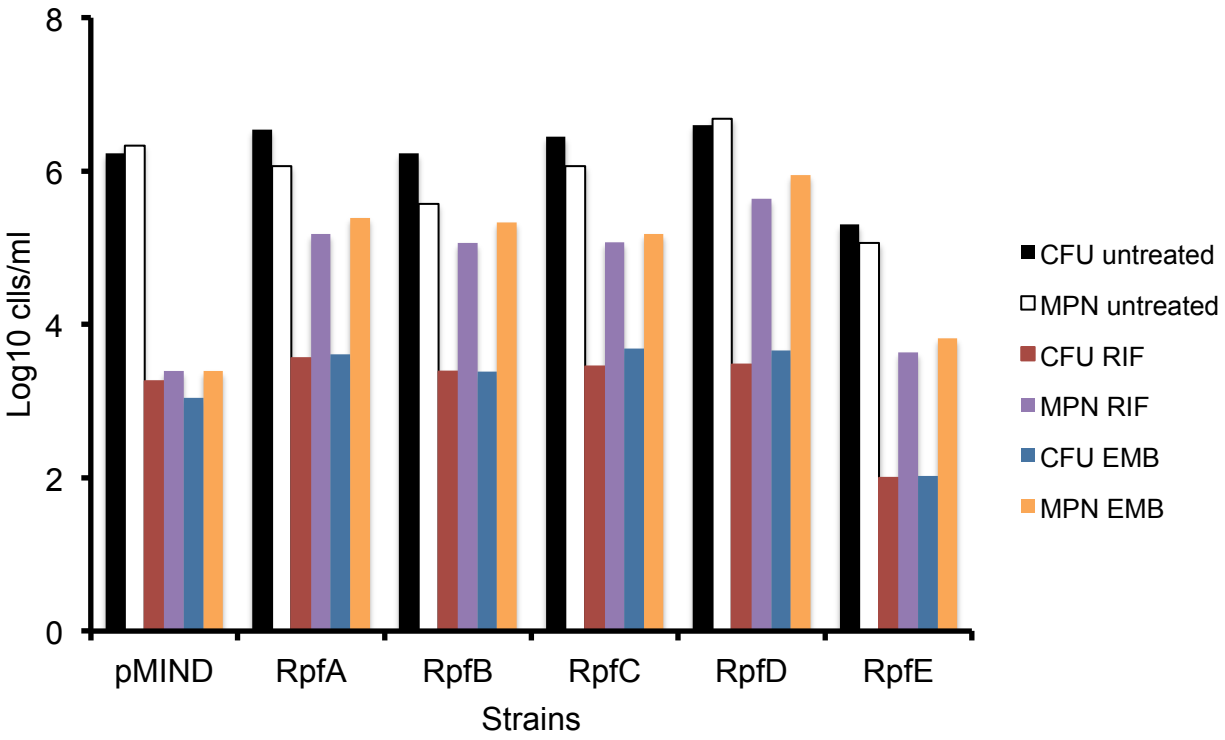


**Figure S4. Viable counts of rifampin-, ethambutol-treated and untreated *M.smegmatis*.** Mycobacteria from logarithmic growth phase were treated with antimicrobials and their viable counts determined as described in Methods. Drugs were added at the following concentrations (µg/ml): rifampin (RIF) 100 and ethambutol (Emb) 20.

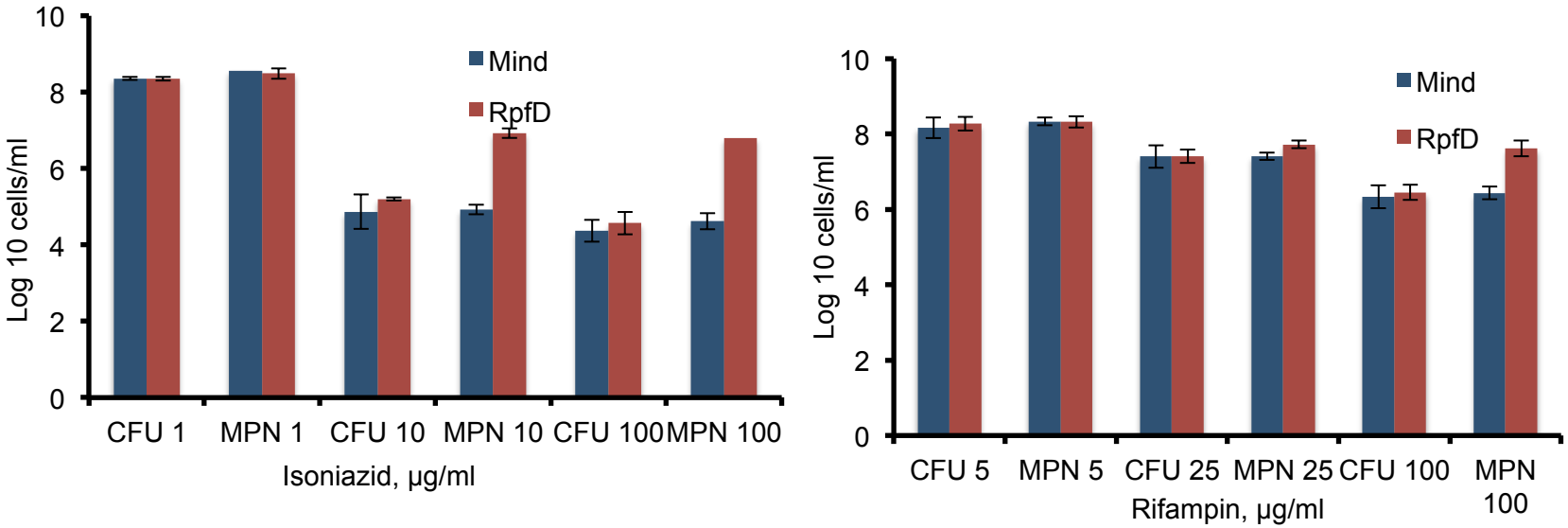


**Figure S5. Viable counts of *M. smegmatis* strains exposed to different concentrations of isoniazid (left panel) and rifampin (right panel).** Mycobacteria from logarithmic growth phase were treated with antimicrobials for 24 hours and their viable counts determined as described in Methods. Drugs were added at the following concentrations (µg/ml): isoniazid 1, 10 and 100; rifampin 5, 25 and 100.