

SUPPLEMENTARY MATERIAL

Rifabutin acts in synergy and is bactericidal with frontline *Mycobacterium abscessus* antibiotics clarithromycin and tigecycline, suggesting a potent treatment combination

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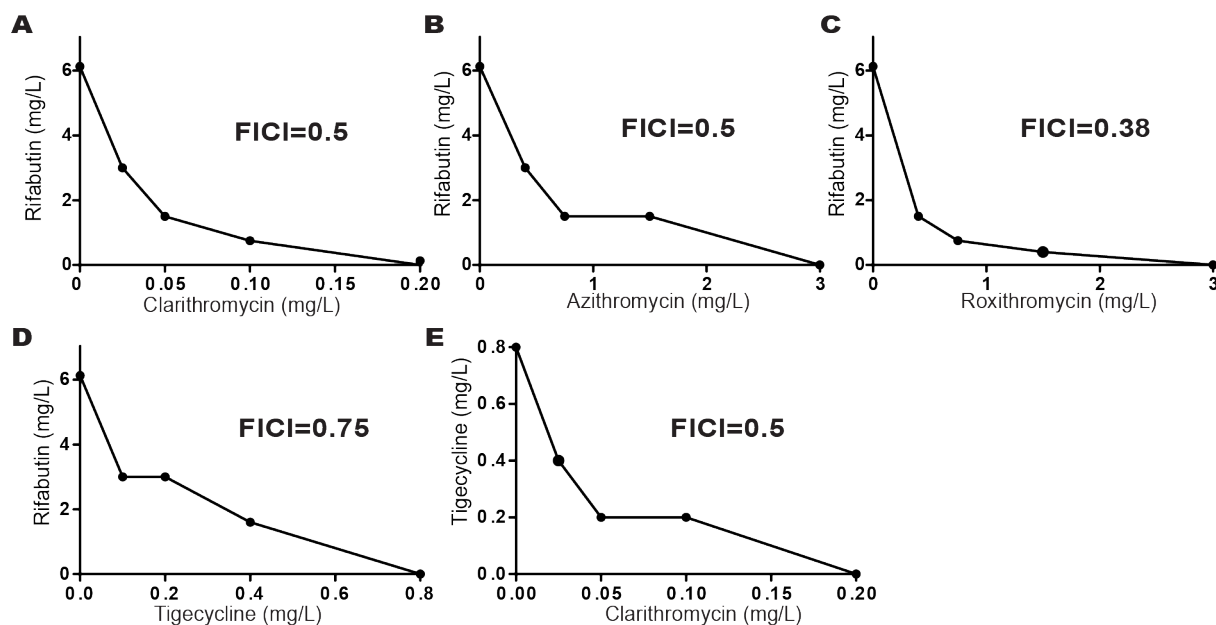


Figure S1. *Mycobacterium abscessus* checkerboard plots of rifabutin in combination with various antibiotics. MAB ATCC19977 MICs to rifabutin were measured in the presence of sub-MIC concentrations of **A)** clarithromycin, **B)** azithromycin, **C)** roxithromycin or **D)** tigecycline. Alternatively, **E)** the tigecycline MIC was measured in the presence of sub-MIC concentrations of clarithromycin. Concave curves represent positive interactions between two antibiotics approaching synergy. FICI values for antibiotic combinations are shown. FICI \leq 0.75 represents synergy.

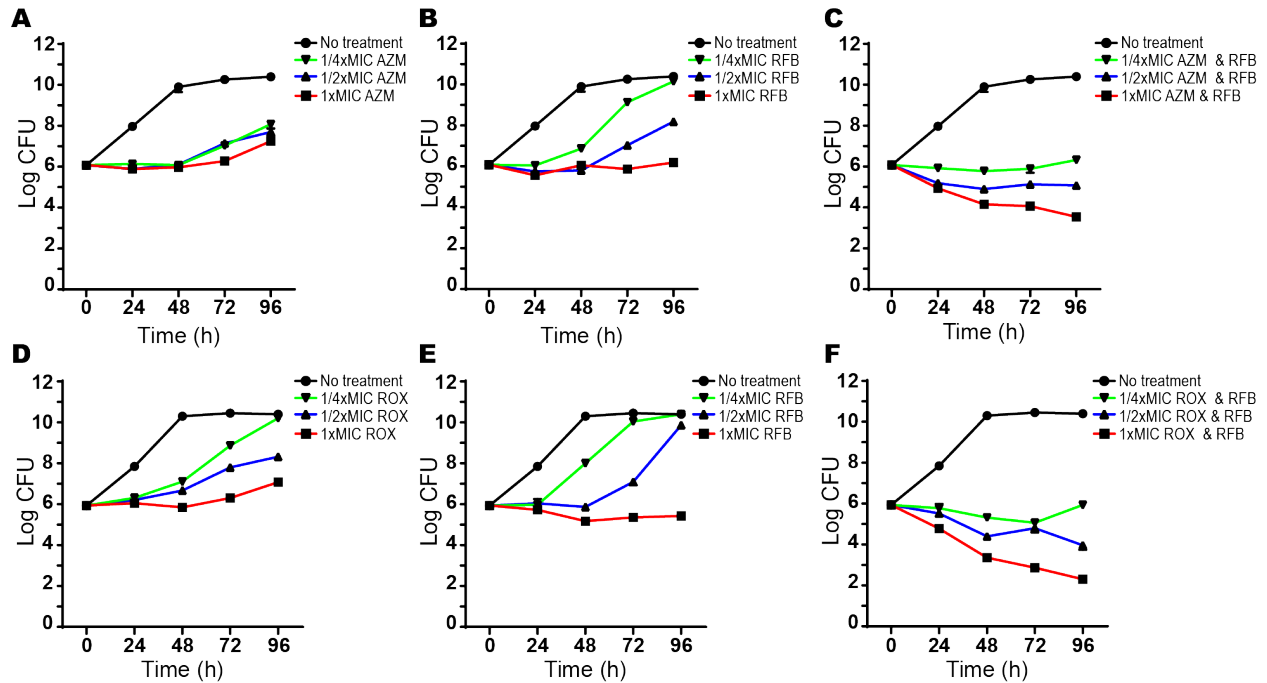


Figure S2. Effect of rifabutin and azithromycin or roxithromycin combinations on *Mycobacterium abscessus* viability. *MAB* ATCC19977 cultures at OD600=0.005 were exposed to **A)** azithromycin at 1x (3.1 mg/L), 1/2x or 1/4xMIC, **B&E)** Rifabutin at 1x (6.3 mg/L), 1/2x or 1/4xMIC, **C)** a combination of azithromycin and rifabutin at 1x, 1/2x, and 1/4xMIC of each antibiotic, **D)** roxithromycin at 1x (3.1 mg/L), 1/2x or 1/4xMIC, or **F)** roxithromycin and rifabutin at 1x, 1/2x or 1/4xMIC of each antibiotic. CFUs were determined at 24 h intervals post antibiotic addition. The dashed line represents the limit of detection. Data points are the means of three replicates with standard deviation presented as error bars.

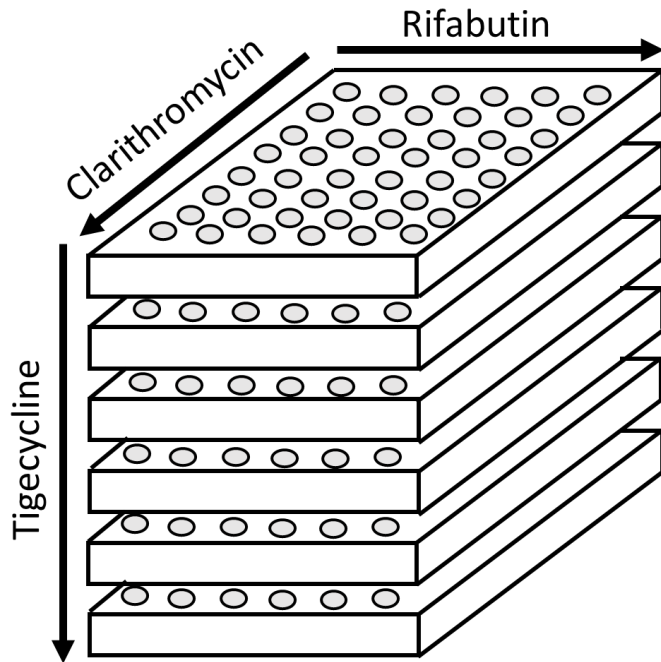


Figure S3. Schematic representation of 3D checkerboard. Arrows describe descending concentrations of the respective antibiotic along the axis indicated.

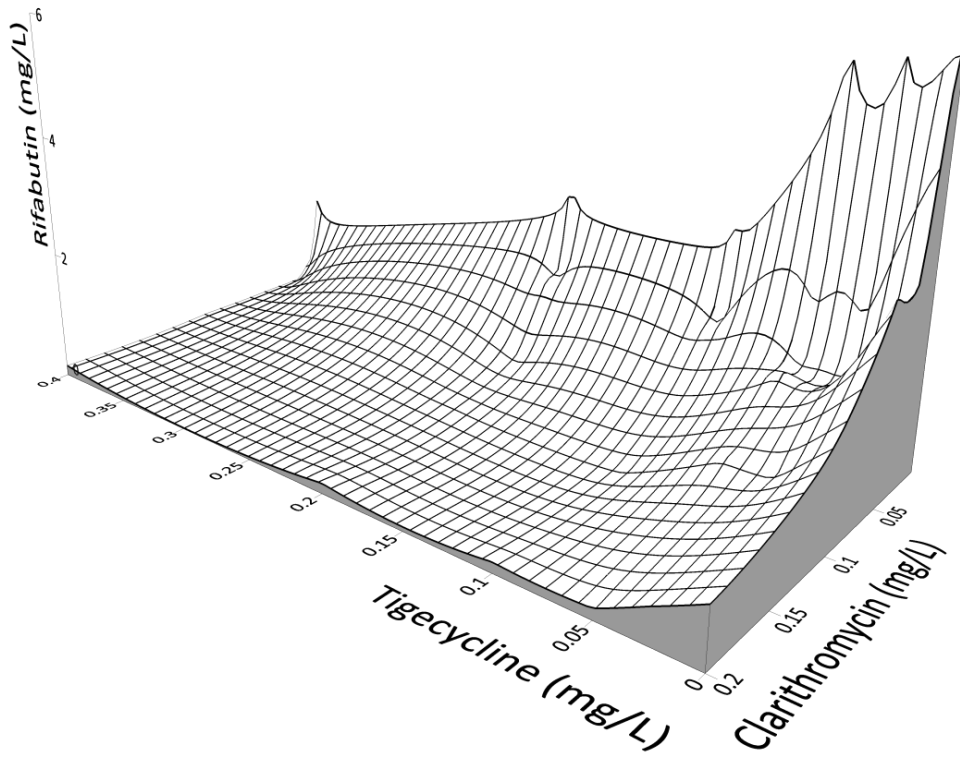


Figure S4. Isobologram showing interaction of clarithromycin, tigecycline and rifabutin against *Mycobacterium abscessus*. Concave areas represent synergistic drug interactions.