

Table S1. RFB susceptibility profile of the *M. abscessus* 104536^T S and R strains in different broth media at 30 °C. Results are representative of 3 independent experiments.

| Medium | MIC (µg/mL) | |
|------------|---------------|---------------|
| | CIP104536 (S) | CIP104536 (R) |
| CaMHB | 100 | 12.5 |
| 7H9 | 25 | 6.25 |
| 7H9 + OADC | 50 | 50 |
| Sauton | 25 | 25 |

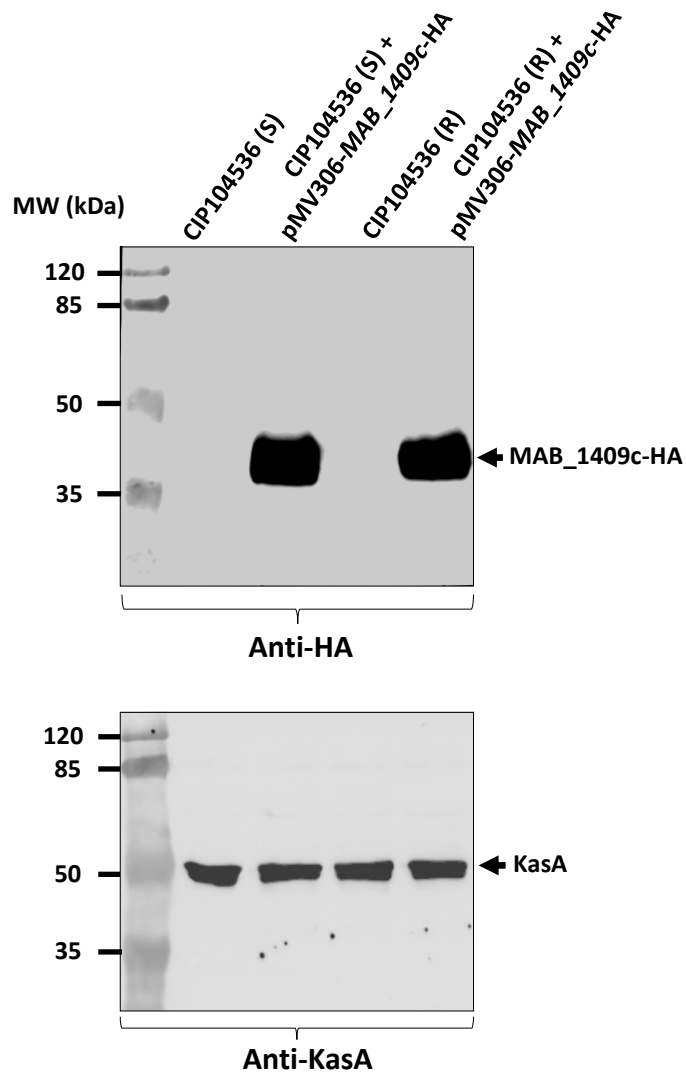


Figure S1. Overexpression of MAB_1409c-HA in *M. abscessus*. The *MAB_1409c* gene was fused to HA and expressed under the control of *hsp60* promoter. Western blot analysis of MAB_1409c overexpression in the CIP104536 S and R variants using anti-HA antibodies (upper panel). The KasA protein (probed with anti-KasA antibodies) was used as a loading control (lower panel).

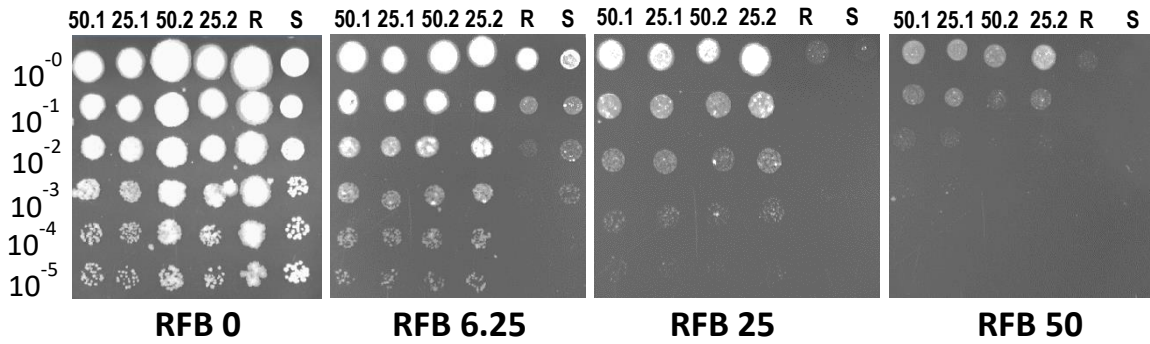


Figure S2. Drug susceptibility phenotypes of *M. abscessus* to RFB. Four microliter of 10-fold serially diluted bacterial suspension of exponentially growing cultures of S and R wild-type strains and resistant strains (50.1, 25.1, 50.2 and 25.2) derived from the R variant were spotted on Middlebrook 7H10 plates supplemented with OADC enrichment in the absence or presence of RFB (6.25 to 50 $\mu\text{g}/\text{mL}$). Plates were incubated at 37°C for 4 days.

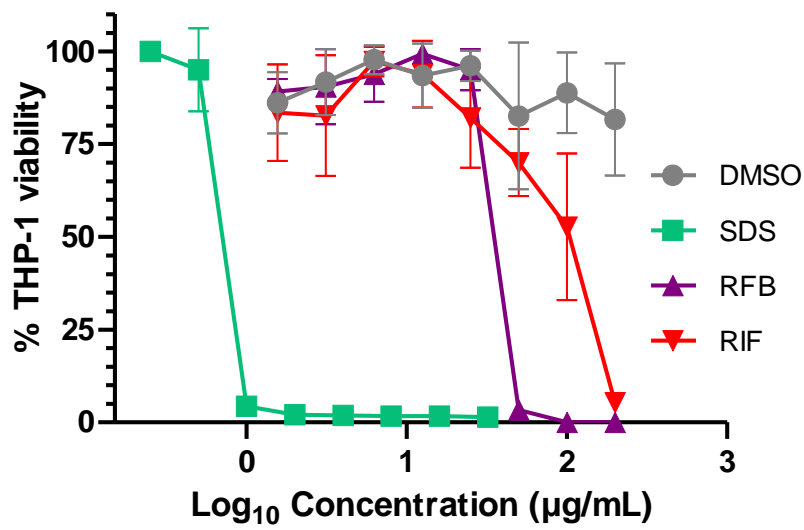


Figure S3. Cytotoxicity assay of rifabutin (RFB) and Rifampicin (RIF) on THP-1 differentiated macrophages. Cells were differentiated with PMA for 48 hrs and exposed to increasing concentration of either RFB or RIF (starting at 200 µg/mL) for an additional 72 hrs at 37°C with 5% CO₂. SDS was used as a positive control and DMSO as negative control. Results are representative of 3 independent experiments done in duplicate. CC₅₀ SDS is 0.6 g/mL, CC₅₀ RFB is 33.6 µg/mL and CC₅₀ RIF is 82.6 µg/mL.