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Title

In vitro activity of bedaquiline and imipenem against actively growing, nutrient-starved, and intracellular *Mycobacterium abscessus*

Running Title

Bedaquiline-imipenem activity against *M.abscessus*

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Abstract

Mycobacterium abscessus lung disease is difficult to treat due to intrinsic drug resistance and the persistence of drug-tolerant bacteria. Currently, the standard of care is a multi-drug regimen with at least 3 active drugs, preferably including a β-lactam (imipenem or cefoxitin). These regimens are lengthy, toxic, and have limited efficacy. The search for more efficacious regimens led us to evaluate bedaquiline, a diarylquinoline licensed for treatment of multidrug-resistant tuberculosis. We performed *in vitro* time-kill experiments to evaluate the activity of bedaquiline alone and in combination with the first-line drug imipenem against *M. abscessus* under various conditions. Against actively growing bacteria, bedaquiline was largely bacteriostatic and antagonized the bactericidal activity of imipenem. Contrarily, against nutrient-starved persisters, bedaquiline was bactericidal, while imipenem was not, and bedaquiline drove the activity of the combination. In an intracellular infection model, bedaquiline and imipenem had additive bactericidal effects. Correlations between ATP levels and the bactericidal activity of imipenem and its antagonism by bedaquiline were observed. Interestingly, the presence of Tween 80 in the media affected the activity of both drugs, enhancing the activity of imipenem and reducing that of bedaquiline. Overall, these results show that bedaquiline and imipenem interact differently depending on culture conditions. Previously reported antagonistic effects of bedaquiline on imipenem were limited to conditions with actively multiplying bacteria and/or the presence of Tween 80, whereas the combination was additive or indifferent against nutrient-starved and intracellular *M. abscessus*, where promising bactericidal activity of the combination suggests it may have a role in future treatment regimens.

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Introduction

Lung disease caused by *Mycobacterium abscessus* infection is difficult to treat. Currently, the recommended treatment for *M. abscessus* lung infections is a multi-drug regimen comprised of at least 3 drugs with *in vitro* activity and preferably including imipenem or cefoxitin (1). This regimen, which can be administered for months to years, results in cure in only about 50% of patients and is plagued by problems with drug toxicity and poor tolerability (2). Thus, there is an urgent need to improve treatment of *M. abscessus* lung infections. One approach to identify new treatment options is to evaluate drugs that are effective for other mycobacterial infections, with bedaquiline being a good candidate for repurposing against *M. abscessus*.

Traditional treatment regimens for multidrug-resistant tuberculosis (MDR-TB) were 18-24 months in duration and associated with about 50% cure rates (4), a situation comparable to the current standard of care for *M. abscessus* lung disease. Bedaquiline -- a diarylquinoline approved for treatment of MDR-TB-- is a key component of a new, 6-month MDR-TB regimen (bedaquiline-pretomanid-linezolid or “BPaL”) with a demonstrated 90% cure rate (3). Using mouse models, each individual drug in BPaL was shown to contribute to bacterial killing of the regimen; and bedaquiline specifically was found to contribute significant treatment-shortening activity (5). As several studies have demonstrated that bedaquiline has activity against *M. abscessus*, including previous work by our group showing that bedaquiline was highly bactericidal against non-replicating *M. abscessus* populations (6, 7), we considered that bedaquiline has potential for both improving and shortening treatment of *M. abscessus* lung disease.

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Understanding how bedaquiline combines with current first-line drugs is an important step in characterizing bedaquiline's potential as part of a shorter, curative regimen for *M. abscessus* lung disease. To that end, the objective of this study was to evaluate the activity of bedaquiline-imipenem combinations against *M. abscessus.* Interestingly, Lindman and Dick recently reported that bedaquiline antagonized imipenem's *in vitro* bactericidal activity against actively growing *M. abscessus* (8). However, Le Moigne *et al.* did not observe antagonism between these drugs in a mouse model of *M. abscessus* lung disease (9). To achieve our objectives, and in light of these recent findings, we conducted a series of *in vitro* studies focused on evaluating the activity of bedaquiline-imipenem combinations across different *M. abscessus* populations, namely actively growing, nutrient-starved non-replicating, and intracellular bacteria. Additionally, we generated resistant mutants to address the impact of bedaquiline resistance on the combined drug activity across these different conditions. This systematic evaluation has produced novel datasets that highlight how experimental conditions can impact drug activity and also provides insight into how bedaquiline and imipenem may be used together for treatment of *M. abscessus* lung infections.

Results

Selection and characterization of bedaquiline-resistant *M. abscessus* isolates. We selected and characterized three unique isolates with decreased *in vitro* susceptibility to clofazimine in the *M. abscessus* strain ATCC 19977 (wild-type [WT]) background (**Table S1**). Each of these isolates contained a mutation in *MAB_2299c*, a gene encoding a TetR-family

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transcriptional repressor of MmpS-MmpL efflux pump systems that confers cross-resistance to clofazimine and bedaquiline when inactivated (10). Indeed, each of our *MAB_2299c* mutant isolates had reduced susceptibility to bedaquiline. The two isolates with *MAB_2299c* mutations that were analyzed (OM4 and OM7) for concurrent mutations in *atpE*, which encodes the ATP synthase subunit targeted by bedaquiline (11), had no such mutations. None of the isolates had mutations in *MAB_4384*, another gene reported to be associated with bedaquiline resistance in *M. abscessus* (12). In cation-adjusted Mueller-Hinton broth (CAMHB), the minimum inhibitory concentration (MIC) of bedaquiline increased 4- or 8-fold to 0.25-0.5 µg/mL against the *MAB_2299c* mutants compared to the wild-type parent strain (MIC 0.0625 µg/mL). Although neither the Clinical & Laboratory Standards Institute (CLSI) nor the manufacturer have defined testing standards for bedaquiline against *M. abscessus* (13, 14), MICs in this range were previously characterized as “bedaquiline-resistant” for *M. abscessus* isolates (10, 15). Therefore, we refer to our *MAB_2299c* mutants as bedaquiline-resistant strains. Isolate OM7, which contained a 150 bp deletion starting at position -28 upstream of the *MAB_2299c* start codon (**Fig. S1**), was utilized in experiments described in this report.

Assessment of bedaquiline and imipenem/avibactam against *M. abscessus* WT and OM7 mutant in nutrient-rich conditions with Tween 80. We first evaluated the activity of bedaquiline and imipenem alone against actively multiplying *M. abscessus* ATCC 19977 WT parent and OM7 mutant populations in CAMHB supplemented with 0.05% (vol/vol) Tween 80, a surfactant included to reduce the impact of mycobacterial clumping on quantification of colony-forming units (CFUs)(16, 17). Bedaquiline alone exerted only bacteriostatic activity, limiting growth at concentrations ≥0.0625 and ≥2 µg/mL against WT and OM7, respectively

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(**Fig. 1A-B**; see **Table S2** for all CFU data). In contrast, imipenem alone limited growth at 1 µg/mL and had strong bactericidal activity at concentrations ≥2 µg/mL against both strains after 3 days of drug exposure (**Fig. 1C-D**). We also evaluated the activity of imipenem in combination with avibactam, a β-lactamase inhibitor that enhances the susceptibility of *M. abscessus* to β-lactams, including imipenem (18, 19). In combination with avibactam at 2 µg/mL, the imipenem concentration needed to inhibit growth decreased 2-fold to 1 µg/mL for both strains (**Fig. 1E-F**).

Next, we evaluated the activity of bedaquiline and imipenem/avibactam combinations against actively growing *M. abscessus* WT and OM7 mutant strains. As previously observed**,** bedaquiline alone at 0.5 and 1 µg/mL prevented growth of the WT strain and permitted growth of the OM7 mutant, and imipenem/avibactam combinations of 2/2 and 4/2 µg/mL were bactericidal against both bacterial strains (**Fig. 2**; **Table S3**). However, the addition of bedaquiline to the imipenem/avibactam combinations abolished this bactericidal activity. Against both strains, the activity of bedaquiline alone appeared concentration-dependent, but this relationship was less clear when combined with imipenem/avibactam. Therefore, we next examined the concentration-ranging activity of bedaquiline when added to imipenem/avibactam combinations against *M. abscessus* WT and OM7. Against both strains, bedaquiline/imipenem/avibactam combinations were more bactericidal when bedaquiline was included at concentrations ≤0.0156 µg/mL, and this bactericidal activity was bedaquiline concentration-dependent (**Fig. S2**; **Table S4**), with bactericidal activity declining with increasing bedaquiline concentration. Overall, the magnitude of killing associated with

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bedaquiline/imipenem/avibactam combinations was greater against the OM7 mutant than against the WT strain.

Assessment of bedaquiline combined with other cell wall synthesis inhibitors (and non cell wall synthesis inhibitors) **against *M. abscessus* WT in nutrient-rich conditions with Tween 80.** Our data indicated that bedaquiline antagonized the bactericidal activity of imipenem/avibactam against both WT parent and OM7 mutant strains and suggested that this antagonism might only occur at concentrations at which bedaquiline alone was able to limit bacterial growth. Other groups have reported antagonism between bedaquiline and cell wall synthesis inhibitors, including imipenem as well as non-β-lactam inhibitors, against *M. abscessus* and other mycobacteria (8, 20-22), but these previous studies did not include bedaquiline at sub-inhibitory concentrations. Therefore, we examined the concentration-ranging impact of bedaquiline on the bactericidal activity of two additional cell wall synthesis inhibitors: the β-lactam meropenem and N-4S-methylcyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide, a non-β-lactam MmpL3 inhibitor with demonstrated *in vitro* activity against *M. abscessus* (23). We also evaluated the impact of bedaquiline on the bactericidal activity of clarithromycin, a protein synthesis inhibitor. Meropenem, the MmpL3 inhibitor, and clarithromycin were used at concentrations expected to have activity against *M. abscessus* WT: 16, 2, and 4 µg/mL, respectively (6, 23, 24), and meropenem was used without avibactam to allow for more direct comparisons between the different groups. Because these agents are relatively more stable in aqueous media than imipenem, this experiment was extended out to 8 days. Again, bedaquiline alone had limited activity against actively growing WT bacteria, resulting in bacteriostasis at 0.125 µg/mL and a weak bactericidal effect at concentrations of 1-

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2 µg/mL (**Fig. 3A**; see **Table S5** for all CFU data from this figure). When combined with meropenem, these bedaquiline concentrations antagonized the bactericidal activity of meropenem over 8 days, while the lower bedaquiline concentrations of 0.0039 and 0.0156 µg/mL that were largely inactive on their own were not antagonistic but rather contributed additional killing compared to meropenem alone at Day 8 (**Fig. 3B**). When combined with the MmpL3 inhibitor, bedaquiline at concentrations ≥0.0156 µg/mL abrogated the bactericidal activity compared to the MmpL3 inhibitor alone, while bedaquiline added at 0.0039 µg/mL remained highly bactericidal, although to a lesser magnitude than the MmpL3 inhibitor alone (**Fig. 3C**). In contrast, bedaquiline did not diminish the bactericidal activity of clarithromycin and added activity at concentrations ≥0.125 µg/mL at Day 8 (**Fig. 3D**).

Correlation of ATP levels with bedaquiline/imipenem bactericidal activity against *M. abscessus* WT in nutrient-rich conditions with Tween 80. Previous work by Lindman and Dick demonstrated that the bactericidal activity of imipenem correlated with increased *M. abscessus* ATP production, which was also abrogated with co-exposure to bedaquiline (8). Therefore, we next evaluated the relative bacterial ATP levels associated with bedaquiline and imipenem against actively growing WT bacteria in CAMHB with Tween 80. To reduce study variables, avibactam was omitted. In both biological replicates, we observed that imipenem's potent bactericidal activity was unchanged or even increased when bedaquiline was added at concentrations of 0.0039 or 0.0078 µg/mL, yet adding bedaquiline at concentrations ≥0.0625 µg/mL nearly eliminated imipenem's bactericidal activity (**Fig. 4A,B**; **Table S6**). The relative levels of bacterial ATP, measured as relative light units (RLU) adjusted by CFU count, correlated with the bactericidal effect; ATP levels increased when bacteria were exposed to

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bactericidal imipenem/bedaquiline concentrations, and ATP levels decreased as the bedaquiline concentration increased (**Fig. 4C,D**). RLU data not adjusted by CFU counts are presented in **Fig. S3**.

Assessment of bedaquiline and imipenem activity against *M. abscessus* ATCC 19977 WT and OM7 in nutrient-rich conditions without Tween 80. Thus far, the surfactant Tween 80 was present in all experimental conditions. As noted earlier, this was done in an effort to reduce bacterial clumping. However, as bacterial clumping was not always prevented (**Fig. 1**; **Fig. 3**; **Table S3**; **Table S4**), and as surfactants such as Tween 80 can influence cell wall permeability and drug susceptibility (25, 26), we also evaluated the activity of bedaquiline and imipenem against *M. abscessus* in CAMHB without Tween 80. After 3 days in these assay conditions, bedaquiline completely inhibited the growth of WT bacteria starting at 0.0312 µg/mL and exhibited bactericidal activity (reducing CFU by ~0.5-0.9 $\log_{10}$ CFU/mL) at concentrations ≥0.0625 µg/mL (**Fig. 5A**; see all CFU data in **Table S7**). Bedaquiline only limited growth of the OM7 mutant at concentrations ≥0.5 µg/mL (**Fig. 5B**). Therefore, against both WT and OM7 populations, bedaquiline was more active (on a µg/mL basis) in CAMHB without Tween 80 than that with Tween 80 (**Fig. 1A,B**). In contrast, the activity of imipenem was reduced when Tween 80 was omitted from the media. After 3 days of exposure, imipenem inhibited growth of WT and OM7 at 16 and 4 µg/mL, respectively (**Fig. 5C,D**). Although imipenem was tested up to 256 µg/mL, the magnitude of killing never exceeded 2 $\log_{10}$ CFU/mL against either strain, while in CAMHB with 0.05% Tween 80, CFU reductions exceeded 2 $\log_{10}$ CFU/mL at 2-4 µg/mL imipenem (**Fig. 1C,D**).

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We next assessed the activity of imipenem/bedaquiline combinations in CAMHB without Tween 80. Again, we observed that, relative to activity in Tween 80-containing media, bedaquiline alone had increased killing and imipenem alone had decreased killing in the absence of Tween 80 (**Fig. 6A,B** (replicate 1); **Fig. S4A** (replicate 2); see all CFU data in **Tables S8-S9**). For both WT and OM7 strains, the addition of bedaquiline decreased imipenem's bactericidal activity, and lower concentrations of bedaquiline combined with imipenem did not increase bactericidal activity, as was observed in Tween 80-containing media (**Fig. 4**; **Fig. S2**). In these assay conditions, relative bacterial ATP levels also correlated with bacterial killing (**Fig. 6C,D**; **Fig. S4B**). ATP levels, not adjusted by CFU counts, indicate that ATP levels in the samples that overgrew and clumped (precluding CFU quantification) were similar to the ATP levels of the no drug control samples for both WT and OM7 populations (**Fig. S4C**; **Fig. S5**; see all RLU data in **Tables S8-S9**).

Evaluation of bedaquiline and imipenem activity against nutrient-starved *M. abscessus* ATCC 19977 WT and OM7 in PBS without Tween 80. Regardless of the presence or absence of Tween 80, imipenem alone was clearly more active than BDQ alone against actively growing *M. abscessus* strains in nutrient-rich media, a finding that aligns with previously published work (6-8). However, we previously reported the converse against nutrient-starved *M. abscessus*; namely, that bedaquiline is highly bactericidal while imipenem has no observable activity (6). Therefore, we next evaluated the bactericidal activity of imipenem/bedaquiline combinations against *M. abscessus* WT and OM7 populations that had been nutrient-starved in PBS for 14 days prior to drug exposure. After nutrient starvation, drug activity assays were performed in PBS without Tween 80. As expected, bedaquiline alone had

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potent, concentration-dependent bactericidal activity against nutrient-starved *M. abscessus* WT (**Fig. 7A,** Day 3; **Fig. S6A**, Day 7). Bedaquiline also exerted concentration-dependent killing against the nutrient-starved OM7 mutant (**Fig. 7B**; **Fig. S6B**), although with a much lower magnitude of killing compared to the WT parent strain (see all CFU data in **Table S10**). Imipenem alone at 4 or 16 µg/mL had no bactericidal activity against either strain, and the bactericidal activity of bedaquiline was unchanged by the addition of imipenem. In these nutrient starvation conditions, bacterial ATP levels adjusted by CFU counts correlated with bactericidal activity for both strains (**Fig. 7C,D**; **Figs. S6C,D**). For both WT and OM7, the total ATP levels, not adjusted by CFUs, decreased with increasing bedaquiline concentration (**Fig. S7**; **Fig. S8**), similar to what was observed in CAMHB without Tween 80 (**Fig. S5**), although with overall lower bacterial ATP levels in nutrient starvation conditions.

Comparison of bedaquiline and imipenem activity against nutrient-starved *M. abscessus* ATCC 19977 WT in PBS with or without Tween 80. Because the presence of Tween 80 positively impacted the activity of imipenem and negatively impacted the activity of bedaquiline against actively growing *M. abscessus*, we also evaluated the impact of Tween 80 on the bactericidal activity of these drugs, alone and together, against nutrient-starved bacteria in PBS with 0.05% Tween 80. For logistical reasons, bacteria were nutrient starved in PBS for 20 days prior to drug exposure in this experiment, and bacteria were exposed to drugs for up to 4 days in Tween-containing PBS. Although bedaquiline alone still exerted concentration-dependent bactericidal activity in these conditions (**Fig. 8**; **Fig. S9**; see all CFU data in **Table S11**), the magnitude of the killing was greatly reduced compared to its activity in PBS with Tween 80. In PBS with Tween 80, bedaquiline alone at 1 and 4 µg/mL reduced CFU counts by

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approximately 1 and 1.5 $\log_{10}$ CFU/mL, respectively, after 4 days of exposure, while bedaquiline alone at 1 and 4 µg/mL reduced them by approximately 2 and 4 $\log_{10}$ CFU/mL, respectively, in 3 days against nutrient-starved WT in PBS without Tween 80 (**Fig. 7A**). Conversely, imipenem alone reduced CFU counts by >3 $\log_{10}$ CFU/mL against nutrient-starved bacteria in PBS with Tween 80, which was similar to the magnitude of killing observed in CAMHB with Tween 80 (**Fig. 1C**). When imipenem and bedaquiline were combined, bactericidal activity was reduced compared to imipenem alone; however, the combined killing of imipenem/bedaquiline at higher concentrations of bedaquiline aligned with the bactericidal activity of bedaquiline alone. A head-to-head-comparison in PBS with or without Tween against WT bacteria that were nutrient-starved for 14 days prior to drug exposure confirmed these findings (**Fig. S10**; **Table S12**).

Investigation of bedaquiline and imipenem activity against intracellular *M. abscessus* ATCC 19977 WT and OM7 mutant. Our *in vitro* results highlighted the impact that experimental conditions can have on the assessment of drug activity against *M. abscessus*. In order to better understand which assay conditions, if any, may be predictive of activity in models of higher biological complexity, we next evaluated the activity of bedaquiline and imipenem against intracellular *M. abscessus* WT during infection of THP-1 monocytic cells. Exposure to bedaquiline alone at concentrations ranging from 0.25 to 16 µg/mL resulted in concentration-dependent activity, ranging from growth-limiting to bacteriostatic to bactericidal, against intracellular *M. abscessus* (**Fig. S11A-C**; see all CFU data in **Table S13**). In contrast, concentration-ranging activity was not clearly observed with imipenem at concentrations ranging from 16 to 64 µg/mL; imipenem at 16-32 µg/mL had similar activity (static or cidal,

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269 depending on the replicate) against intracellular *M. abscessus*, while imipenem at 8 µg/mL was
270 consistently less active and only limited intracellular bacterial growth relative to the no drug
271 control (**Fig. S11D-F**). The combination of bedaquiline at 0.5 or 1 µg/mL with imipenem at 32-
272 64 µg/mL exerted bactericidal activity, killing ~1-2 $\log_{10}$ intracellular CFU/mL over 5 days (**Fig.**
273 **9**). The 2-drug combinations demonstrated equivalent or better activity against intracellular *M.*
274 *abscessus* compared to either drug alone, and antagonism was not observed in these assay
275 conditions.

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Discussion

In this series of experiments, we systematically evaluated the *in vitro* activity of bedaquiline and imipenem against the ATCC 19977 wild type strain of *M. abscessus* and an isogenic *MAB_2299c* mutant in different culture conditions: actively growing bacteria in nutrient-rich media; net non-replicating bacteria under nutrient starvation in PBS; and actively growing intracellular bacteria in THP-1 cells. Overall, we found that the activity of bedaquiline and imipenem, either alone or in combination, was significantly influenced by assay conditions, highlighting current knowledge gaps and limiting broad generalizations with respect to translating *in vitro* data into predictions of *in vivo* efficacy.

One consistent finding was that bedaquiline alone had limited or no bactericidal activity against actively multiplying *M. abscessus* in nutrient-rich media, a finding that aligns with previous reports (6-8, 11). However, even within this limited activity, we observed that the antibacterial effects of bedaquiline were greater (on a µg/mL basis) against bacteria in CAMHB without the surfactant Tween 80 than in CAMHB supplemented with 0.05% Tween 80. We also consistently observed that bedaquiline had strong bactericidal activity against nutrient-starved *M. abscessus* in the absence of Tween 80, in agreement with our previous report (6), but the magnitude of killing decreased in the presence of Tween 80. Bedaquiline's activity against intracellular *M. abscessus*, which was mainly bacteriostatic with limited bacterial killing, aligned best with the activity in CAMHB, with the magnitude of effect (based on the µg/mL bedaquiline added to assay media) falling somewhere between the activity observed in CAMHB with and without Tween 80.

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The activity of imipenem was affected by Tween 80 to an even greater extent. In CAMHB without Tween 80, imipenem alone had limited bactericidal activity, a finding that aligns with previous reports (6, 27). In contrast, imipenem alone was highly bactericidal in CAMHB with Tween 80. In agreement with our previous study (6), imipenem alone had no bactericidal activity against nutrient-starved bacteria in the absence of Tween 80; but, in the presence of Tween 80, imipenem had striking bactericidal activity against nutrient-starved *M. abscessus*, with killing of a similar magnitude as in CAMHB with Tween 80. The largely bacteriostatic or limited bactericidal activity of imipenem against intracellular bacteria, a finding also reported by others (28, 29), aligned best with the activity in CAMHB without Tween 80.

The activity of imipenem-bedaquiline combinations was also highly dependent on assay conditions. Against actively multiplying *M. abscessus* in CAMHB with Tween 80, the potent bactericidal activity of imipenem was enhanced when bedaquiline was added at low concentrations, ≤0.0078 µg/mL. However, as the concentration of bedaquiline increased above 0.0078 µg/mL, the addition of bedaquiline decreased the bactericidal activity of imipenem until it was no longer evident, although the activity of the imipenem-bedaquiline combination was better than the activity of bedaquiline alone. Lindman and Dick previously reported that inhibitory concentrations of bedaquiline antagonize the *in vitro* bactericidal activity of imipenem against actively multiplying *M. abscessus*; however, the media used in their assay was Middlebrook 7H9 broth (BD Difco) supplemented with 0.5% albumin, 0.2% glucose, 0.085% sodium chloride, 0.0003% catalase, 0.2% glycerol, and 0.05% Tween 80 (verified by written correspondence) (8). In CAMHB without Tween 80, activity of the two-drug combination was driven by the activity of bedaquiline. Any antagonism of imipenem by bedaquiline could not be

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ascertained, as the activity of imipenem alone was more limited in the absence of Tween. Similarly, the bactericidal activity of imipenem-bedaquiline combinations against nutrient-starved *M. abscessus* in the absence of Tween also appeared to be driven by the activity of bedaquiline. However, in the presence of Tween 80, the addition of bedaquiline drastically antagonized the bactericidal activity of imipenem against nutrient-starved bacteria. Additive activity was observed between imipenem and bedaquiline against intracellular bacteria.

Consideration of the clinical relevance of assay conditions is always important when evaluating antibacterial drugs and regimens *in vitro*. Unfortunately, for treatment of *M. abscessus* lung infections, there are no established or validated *in vitro* assays known to predict *in vivo* activity, and the situation is further complicated by the lack of data regarding clinical treatment outcomes. However, inclusion of Tween 80 in drug activity assays may lead to conclusions that are clinically misleading. Tween is a non-ionic surfactant used as an emulsifier in drugs and food. It has been known for decades that Tween 80 can impact drug activity against bacteria, and for agents acting on the cell wall, such as imipenem, Tween 80 may increase susceptibility by increasing membrane permeability (25, 26). Interestingly, Tween 80 had the opposite impact on bedaquiline activity against *M. abscessus*, decreasing bedaquiline's activity in CAMHB and in nutrient starvation assays. Lounis *et al.* reported similar findings for bedaquiline against *M. tuberculosis*, and suggested that Tween 80 may be directly interacting with bedaquiline, thus limiting the amount of free drug in solution (30). We initially included Tween 80 in our assay to reduce bacterial clumping, without appreciating how drastic its impact would be on assay outcomes. In this study, the most biologically complex system used was the intracellular infection assay. Overall, the drug activity against intracellular bacteria

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seemed to be best predicted by activity in CAMHB without Tween 80, providing further weight to the data from *in vitro* assays performed without Tween 80.

The impact of Tween 80 on nutrient-starved drug activity assays is further complicated by the presence of oleic acid, a breakdown product of Tween 80 in aqueous solutions and hydrolysis by mycobacterial enzymes (17, 31-33). Oleic acid can serve as a nutrient source for mycobacteria. Due to its known ability to enhance the *in vitro* growth of *M. tuberculosis* (17, 31), it is routinely used as a supplement in liquid and solid media for isolating and cultivating mycobacteria. Including Tween 80 in the nutrient-starvation samples *during* drug exposure likely introduced the nutrient oleic acid into the PBS. Although we did not observe net bacterial growth in these conditions, it is possible that the presence of oleic acid impacted bacterial metabolism such that the bacteria became more susceptible to killing by imipenem. This concept is further supported by data from Berube *et al.*, who reported that imipenem alone had no bactericidal activity against nutrient-starved *M. abscessus* in PBS with 0.05% tyloxapol, a surfactant that does not provide a nutrient source for the bacteria (34). In this study, we did not have an intracellular or other more biologically complex model of net non-replicating *M. abscessus* to aid in our translation of drug activity in the presence or absence of Tween 80.

The *in vivo* activity of imipenem, bedaquiline, and/or imipenem-bedaquiline have been evaluated in different mouse models of *M. abscessus* lung infection. Story-Roller *et al.* reported that imipenem (100 mg/kg twice daily) had modest *in vivo* bactericidal activity against an actively multiplying *M. abscessus* lung infection in a C3HeB/FeJ mouse model with dexamethasone-induced immunosuppression, with imipenem killing just over 2 $\log_{10}$ CFU/lung

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368 over 4 weeks of treatment (35). Le Moigne *et al.* also evaluated imipenem activity in the lungs
369 of *M. abscessus* -infected C3HeB/FeJ mice, but without inducing immune suppression, and
370 thus in their model, the bacterial burden in untreated mice decreased over time. The lung
371 bacterial burden in mice that received 2 weeks of imipenem alone (100 mg/kg twice daily) was
372 no different from the lung burden in untreated mice (9). Le Moigne *et al.* also evaluated the
373 activity of bedaquiline and bedaquiline-imipenem combinations in this mouse model. After 2
374 weeks of treatment, bedaquiline alone (30 mg/kg once daily) contributed modest bactericidal
375 activity resulting in an approximately 1 $\log_{10}$ CFU/lung reduction compared to the decline
376 observed in untreated mice, and the activity of imipenem-bedaquiline was like that of
377 bedaquiline alone, indicating that the activity of the 2-drug combination was driven more by
378 bedaquiline's activity than imipenem's activity (9). Lerat *et al.* also evaluated bedaquiline alone
379 (25 mg/kg) in an athymic nude mouse model of *M. abscessus* infection in which the lung
380 bacterial burden in untreated mice decreased over time. After 1 month of treatment, there was
381 no difference in lung bacterial burden between untreated and bedaquiline-treated mice but,
382 after 2 months of treatment, there was a modest decline in lung CFU counts in bedaquiline-
383 treated mice (36). In contrast, Obregón-Henao *et al.* reported that dosing bedaquiline alone (30
384 mg/kg once daily for 9 days) resulted in strong bactericidal activity against *M. abscessus* in the
385 lungs of $GKO^{-/-}$ mice, in which the bacterial counts decreased in untreated mice, and also in
386 the lungs of SCID mice, in which the lung bacterial burden increased in untreated mice (37).
387 The mouse models utilized by Story-Roller *et al*., Le Moigne *et al.*, and Lerat *et al.* used *M.*
388 *abscessus* strain ATCC 19977, while Obregón-Henao *et al.* used a different strain in their
389 studies.
390

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How well do the imipenem and bedaquiline activity profiles from our different *in vitro* and intracellular assays align with the observed *in vivo* activity in these mouse models of *M. abscessus* lung infection? Direct comparison between the *in vitro* and *in vivo* studies is confounded by several factors. The duration of our *in vitro* studies, which ranged from 3-7 days, was much shorter than most of the treatment durations in mouse models. In addition, understanding the drug exposures in mice is more complicated than in *in vitro* assays. The imipenem dose administered to mice in the studies described, 100 mg/kg, has been shown to result a maximum plasma concentration of about 85 µg/mL, but the drug is rapidly metabolized, with a plasma half-life of about 18 minutes, which leads to the requirement for multiple doses per day to achieve suitable exposures (38). Further, the imipenem concentrations that we utilized for our *in vitro* combination studies do not fluctuate over time in the manner that they do in mice. Overall, if the goal is to have the range of concentrations tested be representative of exposures in mice, and the imipenem activity data in the mouse models of actively multiplying and declining *M. abscessus* lung infections would have been best predicted by the *in vitro* activity observed in assays without Tween 80 and by the intracellular assays.

For bedaquiline, comparison between *in vitro* and *in vivo* findings is limited by the nature of bedaquiline metabolism in mice. Compared to humans, mice more rapidly convert bedaquiline into its M2 metabolite, *N*-desmethyl bedaquiline, which is the dominant species in mouse plasma and lungs (39). Without understanding the activity of the M2 metabolite against *M. abscessus*, it is impossible to compare *in vitro* activity to the activity observed in bedaquiline-treated mice. However, as bedaquiline treatment resulted in bactericidal activity in several

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mouse models (9, 37), it is reasonable to hypothesize that the M2 metabolite does indeed have activity against *M. abscessus.* It is known that the M2 metabolite contributes approximately 50% of the *in vivo* activity of bedaquiline in mice in infected with *M. tuberculosis* (39). Keeping all of this in mind, and assuming average plasma concentrations in mice of around 0.2 and 1 µg/mL of BDQ and M2, respectively (39), the nature of the bedaquiline and imipenem-bedaquiline activity in the mouse model reported by Le Moigne *et al.* seems to align best with *in vitro* activity observed in the nutrient starvation assay without Tween 80.

Another aspect of the present study was the assessment of bacterial ATP levels in association with imipenem and bedaquiline exposure and bacterial cell death. Similar to Lindman and Dick (8), we found that exposure to bactericidal concentrations of imipenem in CAMHB with Tween 80 was associated with an increase in bacterial ATP levels. This relationship also correlated with the activity of imipenem-bedaquiline combinations in these assay conditions. As observed by Lindman and Dick, exposure to inhibitory concentrations of bedaquiline alone was associated with a modest decline in ATP levels, consistent with bedaquiline's mechanism of action, and ATP levels decreased in association with the observed antagonism of imipenem's bactericidal effects when these inhibitory concentrations of bedaquiline were added to imipenem. However, we also expanded on the observations of Lindman and Dick by evaluating the addition of lower, subinhibitory concentrations of bedaquiline to imipenem, which proved to increase, rather than antagonize, the bactericidal effects of imipenem in association with increases in ATP levels. These concentration-dependent effects of bedaquiline when combined with imipenem may shed further light on the relationship between intrabacterial ATP levels and killing by imipenem and other β-lactams described by Lindman

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and Dick. That the bacteria may respond to sub-inhibitory concentrations of bedaquiline by, at least transiently, increasing ATP production and that this may augment a similar response to imipenem exposure and result in additive effects on both ATP levels and killing further supports their conclusion that increased ATP levels are at least a surrogate for bactericidal effects of imipenem and may be part of the causal pathway. Interestingly, in CAMHB without Tween 80, a condition in which imipenem was not as bactericidal, bacterial ATP levels in imipenem-exposed *M. abscessus* were not higher than those in the no drug control samples (**Figs. S4**, **S5**). Just as the bactericidal activity of imipenem-bedaquiline combinations seemed to be driven by bedaquiline in this condition, ATP levels in bacteria exposed to imipenem-bedaquiline decreased as the bedaquiline concentration increased, and overall the ATP levels in bacteria exposed to imipenem-bedaquiline were not different than in bacteria exposed to bedaquiline alone. A similar relationship was observed during drug exposure in nutrient starvation conditions without Tween 80. Because bedaquiline was bactericidal in these conditions, when adjusting the RLU by CFU counts, it may appear that ATP levels are increasing as the bedaquiline concentration increases (**Fig. 7**). However, the non-CFU-adjusted RLU data indicate that total ATP levels decreased with increasing BDQ concentration (**Figs. S7**, **S8**).

Again, we must ask, what is the biological and/or clinical relevance of these data? Overall, we lean towards the conclusion that assay conditions without Tween 80 are more relevant to drug activity in intracellular and mouse infection models. Lindman and Dick reported that the bactericidal activity of imipenem and cefoxitin against actively multiplying *M. abscessus* was associated with bacterial ATP bursts, and that the addition of bedaquiline antagonized this

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activity; however, the assay media used was not clearly stated (8). Shetty and Dick demonstrated similar bacterial ATP bursts in *Mycobacterium bovis* BCG exposed to cell wall synthesis inhibitors, including isoniazid, and that both bactericidal activity and ATP levels were decreased when the samples were co-exposed to bedaquiline or other agents affecting ATP production (21); and Zeng *et al.* confirmed these findings (20). These assays were conducted in Middlebrook 7H9 broth supplemented with 0.05% Tween 80 and DTA medium supplemented with 0.02% Tween 80. There are intriguing biological processes occurring in mycobacterial cells exposed to cell wall synthesis inhibitors and agents that target ATP production, and further dissection of the impact of media conditions on these relationships is needed to understand their impact during *in vivo* treatment.

The overarching purpose of our work is to provide data which will help inform the design of improved treatment regimens for patients with *M. abscessus* lung disease. A key issue we sought to address in this work was whether imipenem and bedaquiline could be used together in a treatment regimen. The data from our *in vitro* (without Tween) and intracellular assays, as well as from the *in vivo* combination study reported by Le Moigne *et al.* (9), indicate that the activity of imipenem-bedaquiline combinations may be driven largely by the activity of bedaquiline, without marked antagonism of imipenem. However, even if antagonism of imipenem by bedaquiline does have clinical relevance, it may not necessarily rule out using these drugs together. For example, in tuberculosis treatment, the activity of isoniazid, which is highly bactericidal against actively multiplying *M. tuberculosis*, is antagonized both in mouse models and in humans, by pyrazinamide, a drug more active against non-replicating bacteria; however, regimens including this combination of drugs have been used successfully for

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483 decades (40-43). Furthermore, if we consider that imipenem has a much shorter half-life than
484 bedaquiline and reaches steady state much sooner than bedaquiline, imipenem could exert its
485 activity against actively replicating bacilli without interference or may be augmented by sub-
486 inhibitory concentrations of bedaquiline during the initial stages of treatment. Finally, our
487 results show that mutational disruption of *MAB 2299c* reduces bacterial susceptibility to
488 bedaquiline across multiple assay conditions but may increase susceptibility to imipenem. If
489 bedaquiline proves to be a drug with treatment-shortening potential in *M. abscessus* infections,
490 imipenem may be a particularly effective companion agent for preventing emergence of
491 bedaquiline resistance. Ultimately, an imipenem-bedaquiline combination would be part of a
492 larger multi-drug regimen, and other optimized companion agents may further enhance the
493 utility of imipenem-bedaquiline for treatment of *M. abscessus* lung disease.
494
495 **Materials and Methods**
496 **Bacteria.** *M. abscessus* subsp. *abscessus* strain ATCC 19977 was obtained from the
497 American Type Culture Collection (ATCC) and used in all experiments.
498
499 **Media.** Bacterial cultures were initiated in standard growth media: Middlebrook 7H9 broth
500 supplemented with 10% (v/v) Middlebrook OADC supplement, 0.1% (v/v) glycerol, and 0.05%
501 (v/v) Tween 80. Drug activity assays in nutrient-rich media were performed using either
502 Middlebrook 7H9 broth with 10% (v/v) OADC and 0.1% (v/v) glycerol but without Tween; or
503 CAMHB with or without 0.05% Tween 80. Polystyrene petri-dishes (100 mm × 15 mm)
504 containing 20 mL 7H11 agar supplemented with 10% (v/v) OADC and 0.1% (v/v) glycerol were
505 used to determine CFU counts. Difco BBL Mueller Hinton II broth (cation-adjusted) powder

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(i.e., CAMHB powder), Difco Middlebrook 7H9 broth powder, Difco Mycobacteria 7H11 agar powder, and BBL Middlebrook OADC enrichment were manufactured by Becton, Dickinson and Company. Glycerol and Tween 80 were purchased from Fisher Scientific.

Drugs. Imipenem powder was purchased from Biosynth Carbosynth. Bedaquiline and N-4S-methylcyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide were provided by the TB Alliance. Clofazimine, meropenem and clarithromycin were purchased from Sigma. Drugs were dissolved in either PBS (imipenem and meropenem) or dimethyl sulfoxide (bedaquiline, N-4S-methylcyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide, clofazimine, and clarithromycin), and drug solutions were filter-sterilized.

Selection and characterization of *MAB_2299c* mutants. Frozen stock of *M. abscessus* ATCC 19977 WT was cultured in standard growth media until an optical density at 600 nm (OD_{600}) >1.5 was achieved. Ten-fold dilutions (in PBS) of the bacterial suspension were cultured on 7H11 agar containing the following concentrations of clofazimine: 8µg/mL, 16µg/mL, 32µg/mL, and 64µg/mL. Individual colonies growing on 7H11 agar containing 8 µg/mL clofazimine were selected, expanded, and stored in growth media at -80°C; these isolates were also subjected to a second round of selection on agar containing clofazimine at 32µg/mL and 64µg/mL. Individual colonies were selected, expanded, and stored in growth media. The MICs of both clofazimine and bedaquiline were determined for selected isolates using the broth microdilution method in round-bottom, polystyrene 96-well plates as previously described (44), with assays conducted in CAMHB and Middlebrook 7H9 with 10% (v/v) OADC and 0.1% (v/v) glycerol, without Tween 80 in either media. The concentration ranges tested

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were clofazimine 128-0.125 µg/mL and bedaquiline 4-0.0038 µg/mL in CAMHB and 16-0.125 µg/mL in 7H9. MIC was defined as the lowest concentration without visible growth. Isolates with increased MICs at least 4 times higher than the WT parent strain were evaluated by PCR and sequencing for mutations in the following genes: *MAB_2299c*, *MAB_4384*, and *atpE* (*MAB_1448*). Genomic DNA was prepared from each isolate as previously described (45). Primer sequences and expected amplicon sizes were as follows: *MAB_2299c* forward: 5' CGC GTT TCA TCA GGA TCT TT 3', reverse: 5' CCT ACG TGG ATG CCA AGG 3', 862bp; *MAB_4384* forward: 5' GGC AGG GTC AGC AGA AAT 3', reverse: 5' ATG TTG TGT GCG GGG TCT 3', 840 bp; *atpE* forward: 5' TGG ACG AGG ACC ATC ACT AA 3', reverse: 5' GAC GGC AGA AGC GAC AC 3', 375 bp. Purified amplicons were analyzed by Sanger sequencing at GENEWIZ. Sequencing results were compared against the *M. abscessus* ATCC 19977 published genome, NCBI accession number NC_010397.

Drug activity assays in nutrient-rich media. Frozen stocks of *M. abscessus* were cultured in standard growth media until the bacterial suspension reached OD_{600} ~1 (approximately 10^7-10^8 CFU/mL), at which point the assays were initiated ("Day 0"). Assays were conducted with the indicated media in a total volume of 2.5 mL in 14 mL, round-bottom, polystyrene tubes as previously described (6).

Drug activity assays in nutrient starvation conditions. Frozen stocks of *M. abscessus* were cultured in growth media from frozen stock until the bacterial suspension reached OD_{600} ~1. The bacterial suspension was subsequently spun (1900 rcf for 10 minutes), washed, and resuspended three times to the original volume in PBS with 0.05% (v/v) Tween 80, as

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552 previously described(6). After the third wash, the bacteria were resuspended in PBS with
553 0.05% (v/v) Tween 80 and were incubated at 37˚C for the indicated duration (14 or 20 days).
554 Assays were initiated after the indicated duration of nutrient starvation; the bacterial
555 suspensions were diluted in PBS with or without Tween 80 to an OD_{600} of 0.6 on Day 0 of the
556 assay. Drugs stocks were added to achieve the indicated concentrations. Assays were
557 conducted in a total volume of 2.5 mL in 14 mL, round-bottom, polystyrene tubes as previously
558 described (6).
559
560 **Relative ATP measurements.** The Promega BacTiter-Glo™ Microbial Cell Viability Assay
561 was used to measure the relative ATP in assay samples. At each indicated timepoint, 20 µL of
562 assay sample was removed and incubated with 20µL of the BacTiter-Glo™ reagent in clear
563 1.5 mL snap-cap tubes at room temperature for 5 minutes. After the incubation, RLU was
564 recorded for each sample using Turner TD-20/20 luminometer.
565
566 **Intracellular infections and drug activity assays.** Frozen stocks of *M. abscessus* were
567 cultured in standard growth media. After reaching OD_{600} ~1, the culture was spun at 1100 rpm
568 for 7 minutes to remove any cellular debris or dead cells. For infection, the bacterial
569 suspension was subsequently diluted to reach an OD_{600} of 0.0005 in Roswell Park Memorial
570 Institute (RPMI) medium with L-glutamine, supplemented with 10% (v/v) heat-inactivated fetal
571 bovine serum (FBS) to generate a bacterial suspension of ~$5x10^4$ cells/mL. THP-1 cells
572 (obtained from ATCC, product number TIB-202) were grown in supplemented RPMI medium to
573 a density of 10^5 cells/mL, aliquoted into 24-well polystyrene plates (tissue culture-treated, 1mL
574 cell suspension per well), and activated by incubation with 50nM phorbol 12-myristate 13-

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acetate for 24 hours at 37˚C. After activation, the adherent THP-1 cells were washed with PBS and then infected with 1mL of the adjusted bacterial suspension at OD_{600} 0.05, 0.005, 0.0005 to achieve an MOI of 10:1, 1:1, and 1:10 (bacteria:THP-1 cells), respectively. All drug activity assays were conducted at an MOI of 1:10. After 3 hours of infection at 37°C, each well was washed three times with 1mL PBS to remove extracellular bacteria. After the final wash, 1 mL RPMI media containing bedaquiline or imipenem at the indicated concentrations (or no drug) was added to the appropriate wells. Drug-containing media was replaced daily. At each indicated timepoint, each well was washed three times with PBS, and then the THP-1 cells were lysed with sterile deionized water, and lysates were collected for CFU determination. In each biological replicate, three wells were used for each condition (*i.e.*, triplicate technical replicates). All THP-1 cells were incubated at 37˚C and 5% CO_2.

Quantitative cultures, CFU counting, and analysis. In all assays, CFU counts were determined by culturing serial 10-fold dilutions of bacterial suspensions on 7H11 agar. CFU counts were not determined for samples where the bacteria had overgrown, clumped, and fallen out of suspension. Serial dilutions were prepared in PBS, and 0.5 mL of each dilution was cultured on 7H11 agar. After all liquid was absorbed into the agar, the plates were sealed in plastic bags and incubated at 37°C for 5-7 days before reading and recording CFU counts. The dilution that yielded CFU counts between 10-120 and closest to 50 was used to determine CFU/mL. The CFU/mL value (x) was log transformed as $\log_{10}(x + 1)$ prior to analysis. All analyses were conducted using GraphPad Prism version 9.

Acknowledgements.

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598 This work was funded by the National Institutes of Health (R21-AI137814 to ELN), the Cystic
599 Fibrosis Foundation (ELN), and by Genentech (research fellowship grant to OM). KED is
600 supported by National Institutes of Health (K24AI150349).

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Figures

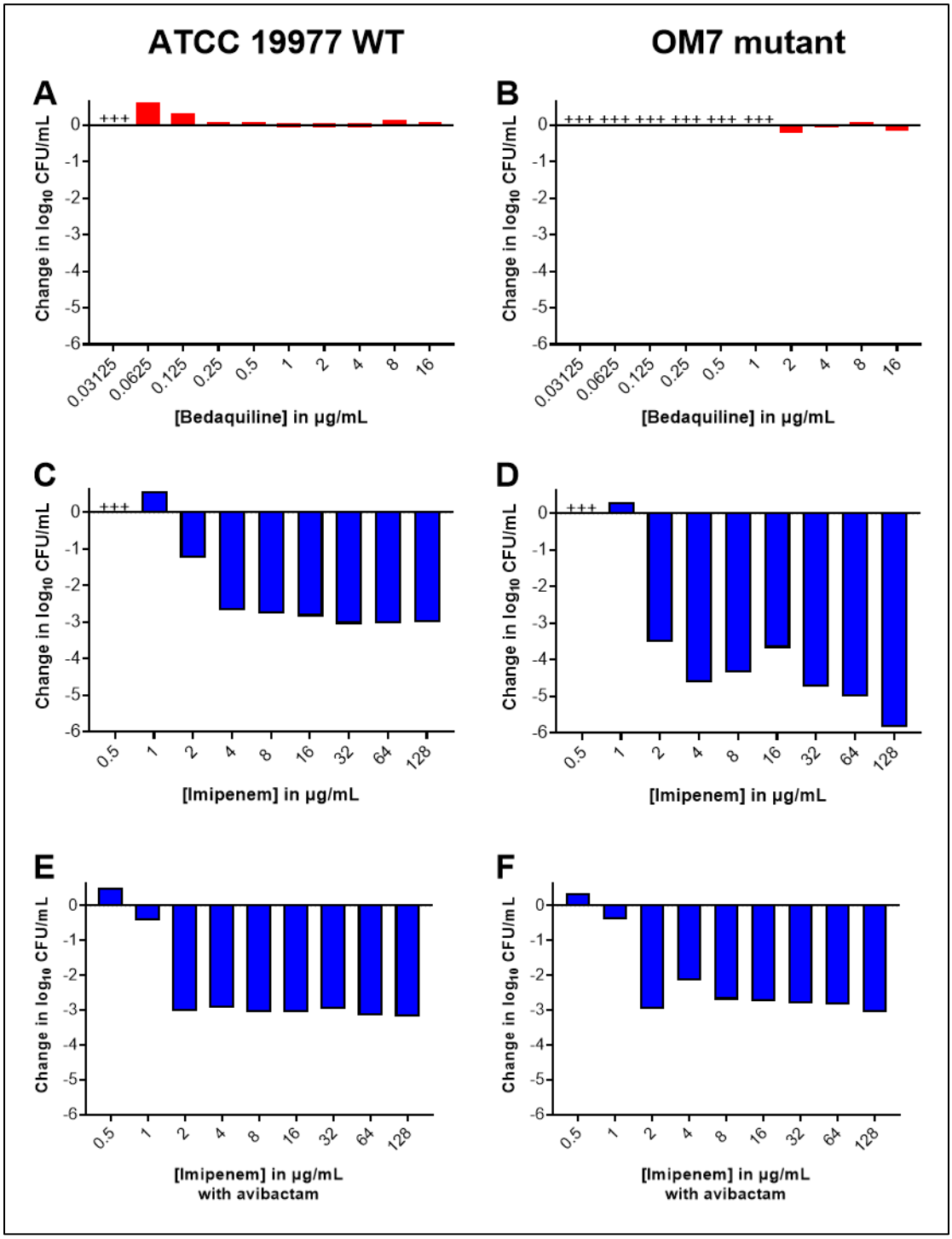

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Fig. 1. Activity of bedaquiline (A-B), imipenem (C-D), and imipenem with avibactam (E-F) against *M. abscessus* ATCC 19977 WT (A, C, E) and OM7 mutant (B, D, F) in CAMHB with 0.05% Tween 80. The change in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 is presented for each drug/strain set. Avibactam concentration in panels E-F was 2 µg/mL. +++ indicates bacterial overgrowth and clumping that precluded CFU determination. Avibactam alone at concentrations from 0.5 to 64 µg/mL had no anti-*M. abscessus* activity; bacteria grew/clumped at all avibactam concentrations, precluding CFU determination. The Day 0 bacterial concentrations (in $\log_{10}$ CFU/mL) for each panel were as follows: A) 5.96; B) 6.11; C) 5.85; D) 5.86; E) 5.85; F) 5.86. CFU values are provided in **Table S2**.

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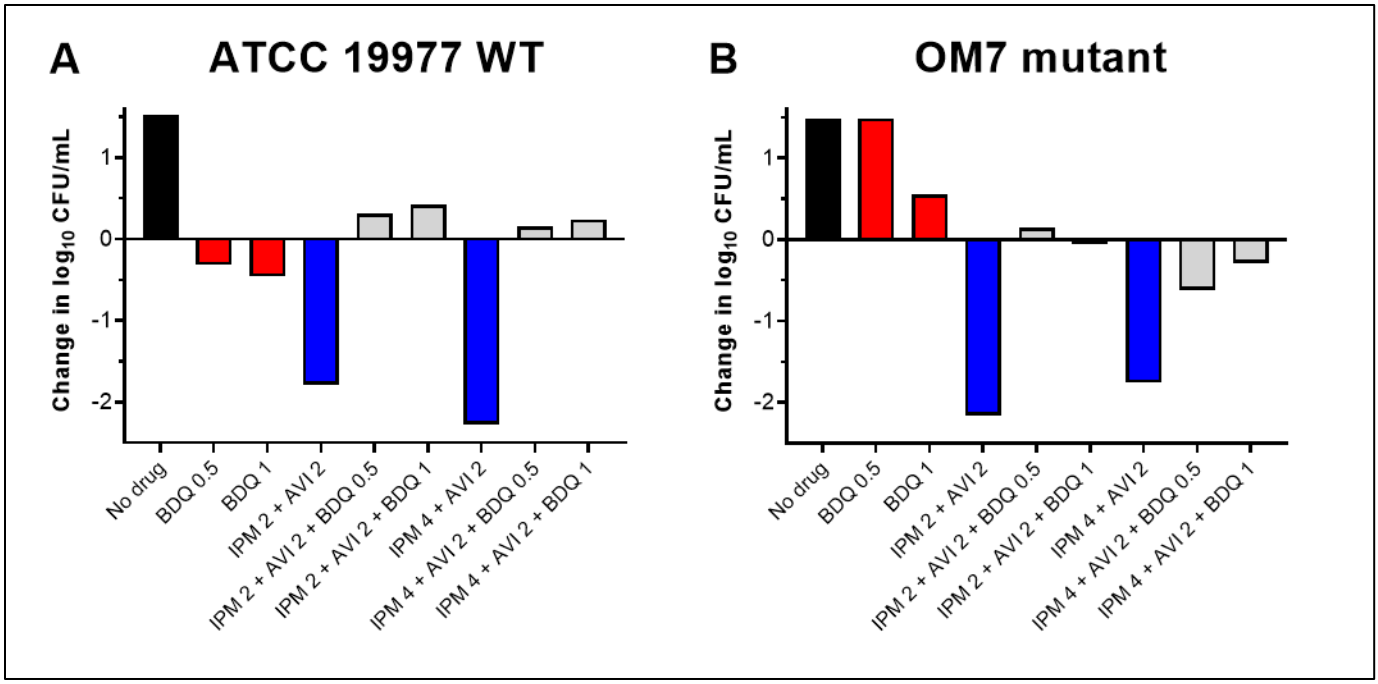

762

763 **Fig. 2. Activity of bedaquiline (BDQ) and imipenem (IPM)/avibactam (AVI) combinations**
764 **against *M. abscessus* ATCC 19977 WT (A) and OM7 mutant (B) strains in CAMHB with**
765 **0.05% Tween 80.** The change in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0
766 is presented for each drug/strain set. Black bars represent the no drug control; red bars
767 indicate BDQ only samples; blue bars represent IPM/AVI only samples, and gray bars indicate
768 IPM/AVI plus BDQ samples. The number after each drug abbreviation represents the
769 concentration in µg/mL. The Day 0 bacterial concentrations (in $\log_{10}$ CFU/mL) were 5.57 and
770 5.76 for WT and OM7 mutant strains, respectively. CFU values are provided in **Table S3**.

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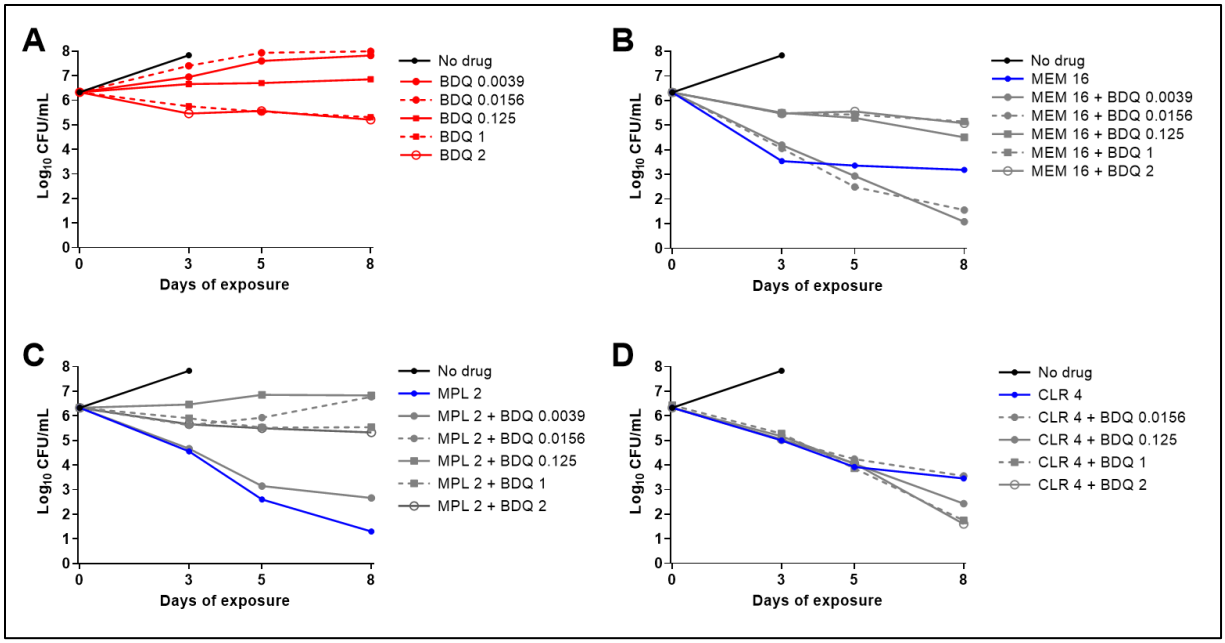

Fig. 3. Activity of bedaquiline (BDQ) alone (A), BDQ plus meropenem (MEM) (B), BDQ plus MmpL3 inhibitor (MPL) (C), and BDQ plus clarithromycin (CLR) (D) against *M. abscessus* ATCC 19977 WT in CAMHB with 0.05% Tween 80. The number after each drug abbreviation represents the concentration in µg/mL. MPL is N-4S-methylcyclohexyl-4,6-dimethyl-1H-indole-2- carboxamide. The bacteria in the no drug control overgrew and clumped after Day 3, precluding CFU quantification. CFU values are provided in **Table S5**.

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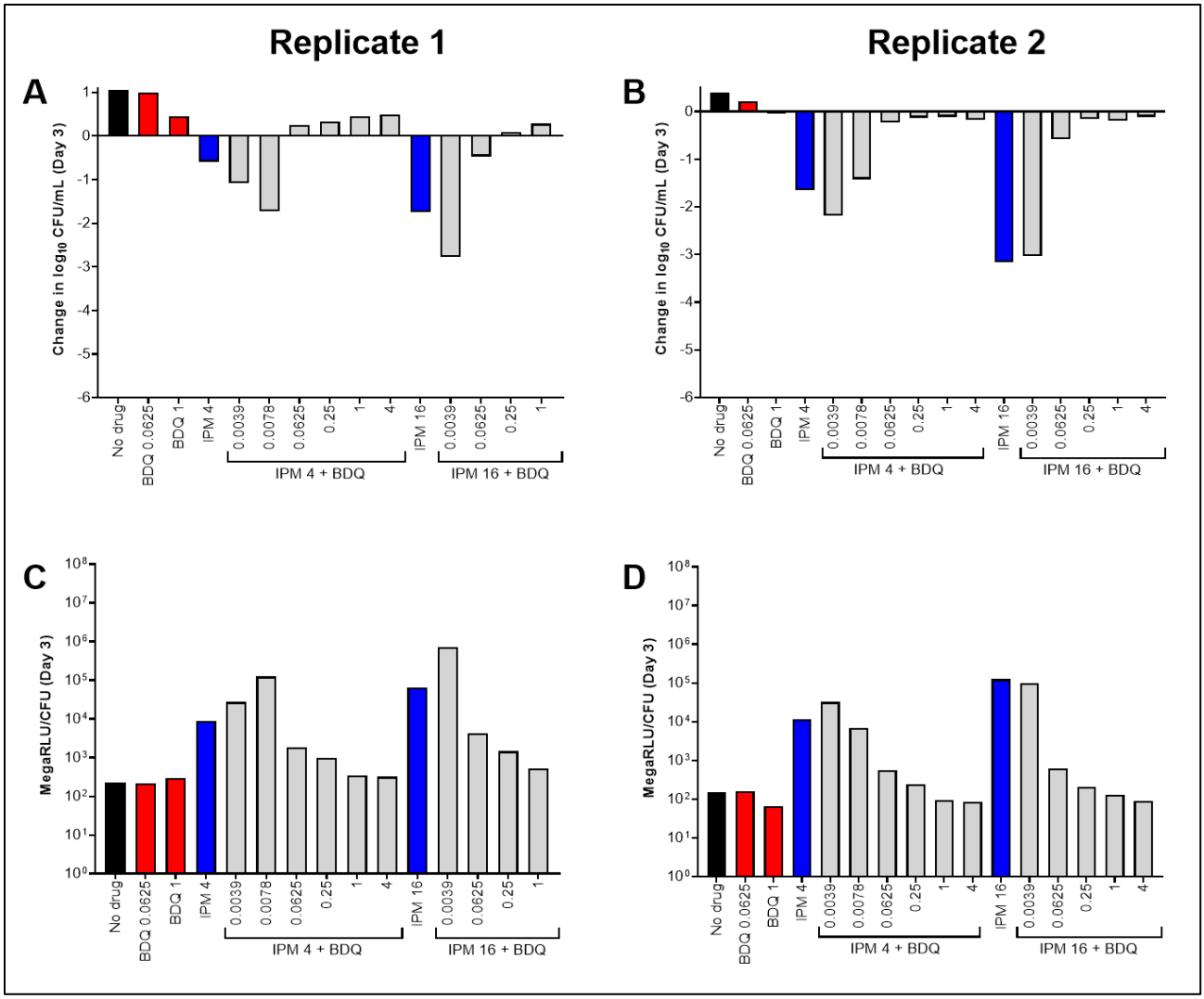

Fig. 4. Activity of bedaquiline (BDQ) and imipenem (IPM) against *M. abscessus* ATCC 19977 WT (A,B) and relative bacterial ATP levels (C,D), in CAMHB with 0.05% Tween 80. The change in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 is presented for each biological replicate in panels A and B. CFU-adjusted ATP levels, indicated by relative light units (RLU)/CFU from samples after 3 days of drug exposure, are presented for each biological replicate in panels C and D. Black bars represent the no drug control; red bars indicate BDQ only samples; blue bars represent

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789 IPM only samples, and gray bars indicate IPM plus BDQ samples. The number after
790 each drug abbreviation represents the concentration in µg/mL (for gray bars the BDQ
791 concentration in µg/mL is presented under each bar). RLU/mL for all samples (not
792 adjusted for CFUs) are presented in **Fig. S3**. The Day 0 bacterial concentration for
793 panels A and B was 6.25 and 7.03 $\log_{10}$ CFU/mL, respectively. CFU and RLU values
794 are provided in **Table S6**.

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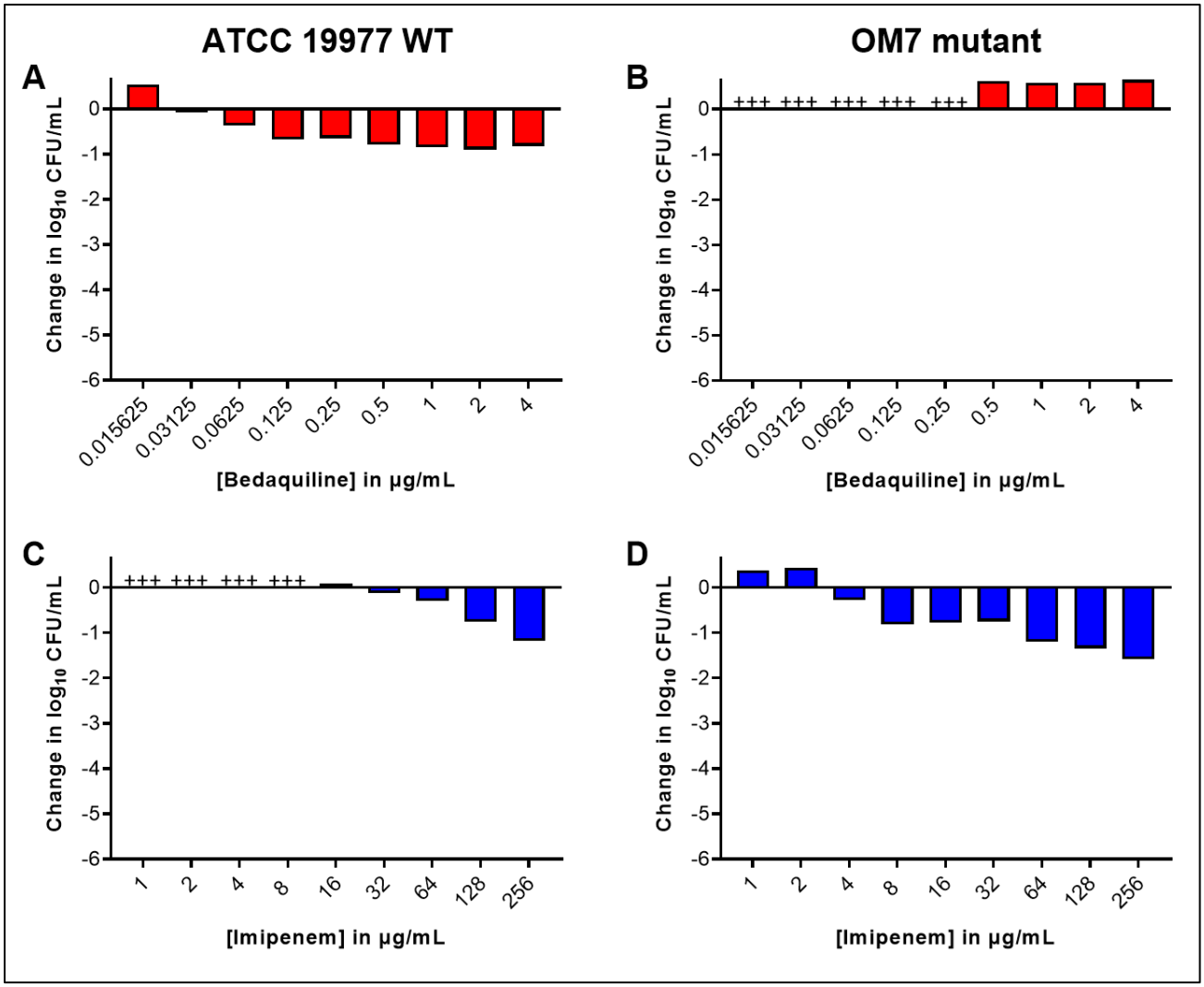

Fig. 5. Activity of bedaquiline (A,B) and imipenem (C,D) against *M. abscessus* ATCC 19977 WT (A,C) and OM7 mutant (B,D) in CAMHB without Tween 80. The change in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 is presented for each drug/strain set. +++ indicates bacterial growth and clumping that precluded CFU determination. The Day 0 bacterial concentrations (in $\log_{10}$ CFU/mL) for each panel were as follows: A) 5.90; B) 5.08; C) 5.91; D) 6.20. CFU values are provided in **Table S7.**

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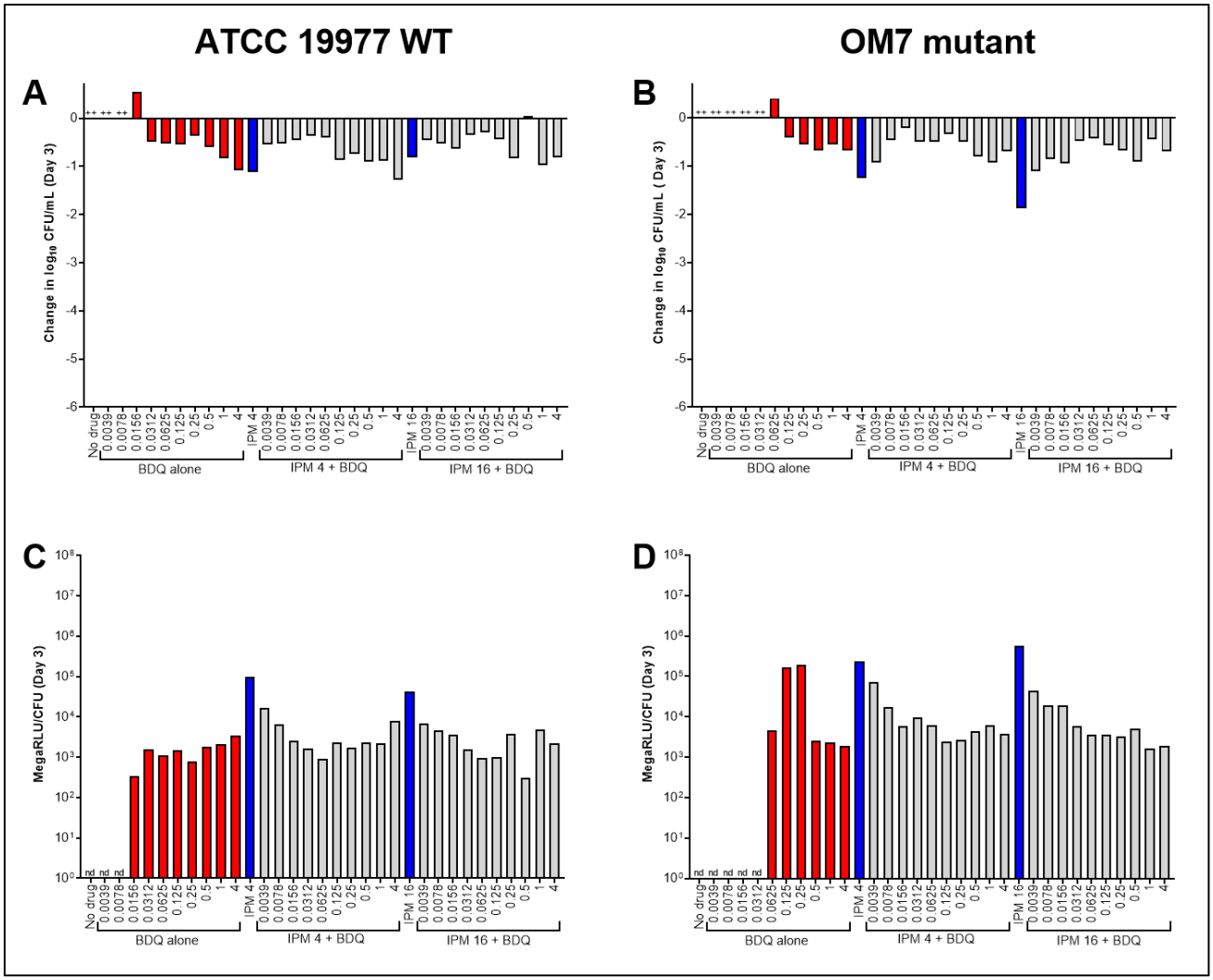

Fig. 6. Activity of bedaquiline (BDQ) and imipenem (IPM) against *M. abscessus* ATCC 19977 WT (A) and OM7 mutant (B), with relative bacterial ATP levels (C,D), in CAMHB without Tween 80. The changes in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 are presented in panels A and B; CFU-adjusted ATP levels, indicated by relative light units (RLU)/CFU from samples after 3 days of drug exposure, are presented in panels C and D. Red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars,

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814 the BDQ concentration in µg/mL is under each bar. ++ indicates that the bacterial
815 overgrew and clumped, precluding CFU quantification. nd indicates not determined (due
816 to lack of CFU count data). RLU/mL for all samples (not adjusted for CFUs) are
817 presented in **Fig. S5**. The Day 0 bacterial concentration for panels A and B was 5.62
818 and 5.70 $\log_{10}$ CFU/mL, respectively. CFU and RLU values are provided in **Table S8**.

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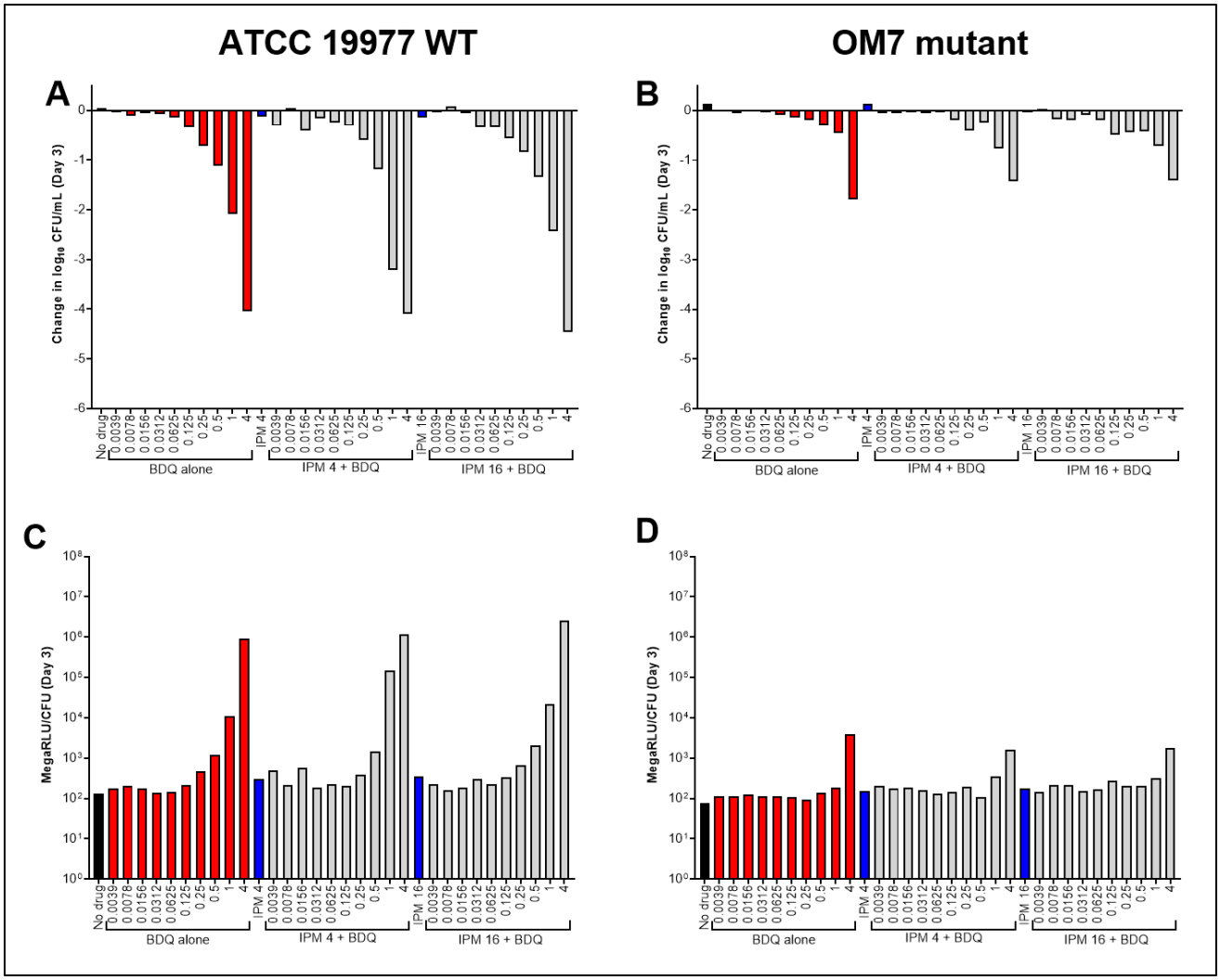

Fig. 7. Activity of bedaquiline (BDQ) and imipenem (IPM) against nutrient-starved *M. abscessus* ATCC 19977 WT (A) and OM7 mutant (B), with relative bacterial ATP levels (C,D), in PBS without Tween 80. Bacteria were nutrient-starved in PBS for 14 days prior to drug exposure. The changes in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 are presented in panels A and B; CFU-adjusted ATP levels, indicated by relative light units (RLU)/CFU from samples after 3 days of drug exposure, are presented in panels C and D. Red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number

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after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars, the BDQ concentration in µg/mL is under each bar. Data after 7 days of drug exposure are presented in **Fig. S7**. RLU/mL (not adjusted for CFUs) for WT and OM7 are presented in **Fig. S8** and **Fig. S9**, respectively. The Day 0 bacterial concentration for panels A and B was 6.25 and 6.29 $\log_{10}$ CFU/mL, respectively. CFU and RLU values are provided in **Table S9**.

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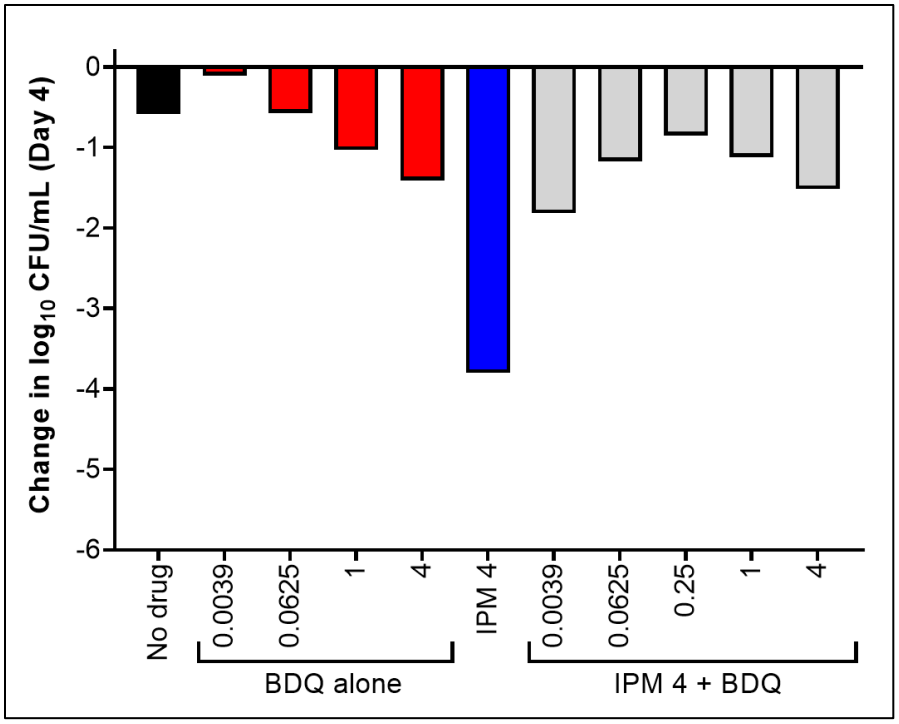

Fig. 8. Activity of bedaquiline (BDQ) and imipenem (IPM) against nutrient-starved *M. abscessus* ATCC 19977 WT in PBS with 0.05% Tween 80. Bacteria were nutrient-starved in PBS for 20 days prior to drug exposure. The changes in $\log_{10}$ CFU/mL after 4 days of drug exposure relative to Day 0 are presented. Red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars, the BDQ concentration in µg/mL is under each bar. Data after 1 day of drug exposure, as well as data from a biological replicate, are presented in **Fig. S13**. The Day 0 bacterial concentration was 6.07 $\log_{10}$ CFU/mL. CFU values are provided in **Table S11**.

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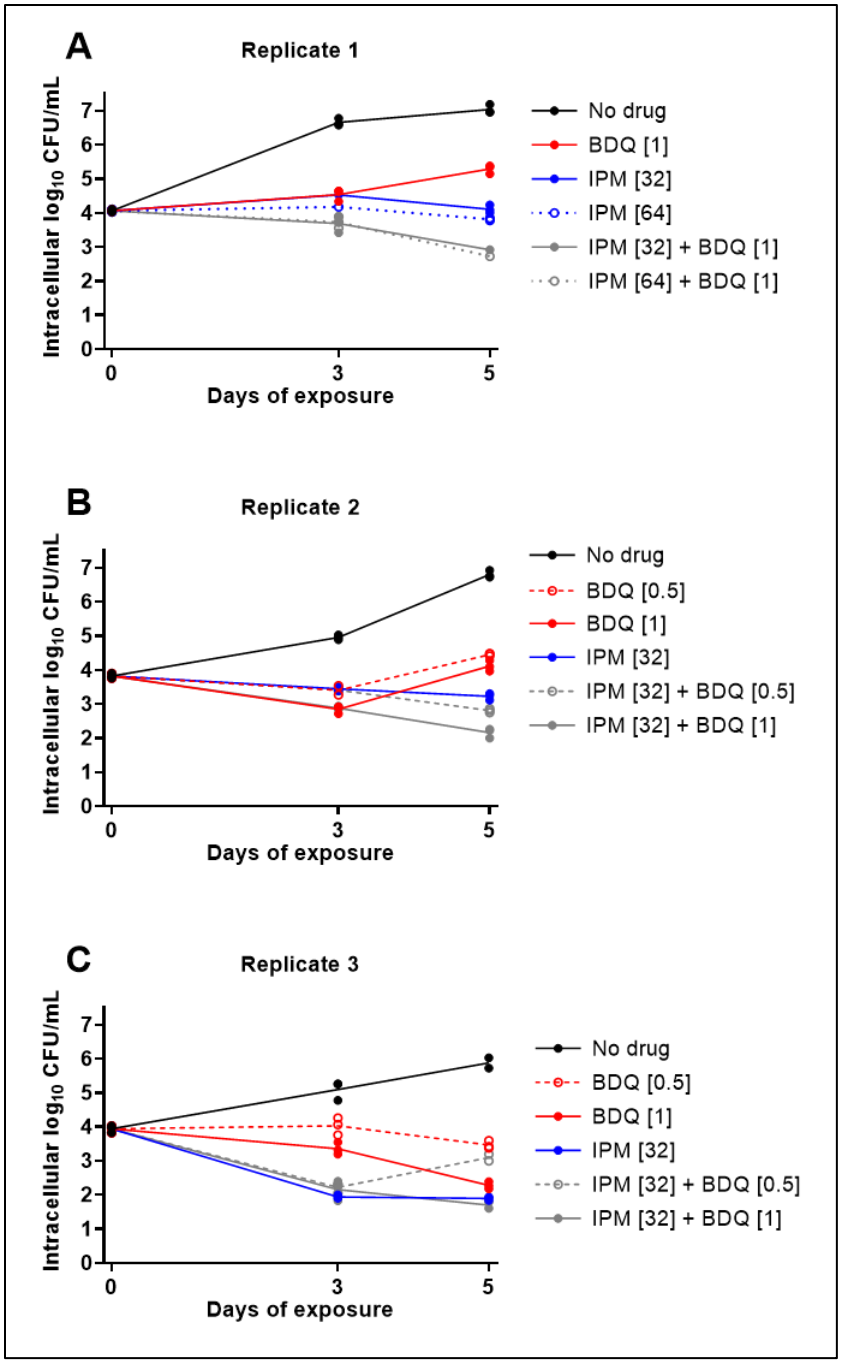

Fig. 9 Activity of bedaquiline (BDQ) and imipenem (IPM) combinations against intracellular (THP-1 cells) *M. abscessus* ATCC 19977 WT. Data are presented for 3 biological replicates: replicate 1, panel A; replicate 2, panel B; replicate 3, panel C. Each data point represents a technical replicate, and the connecting lines pass through the

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854 mean values. The number in brackets after each drug abbreviation represents the
855 concentration in µg/mL. All CFU values, including data from additional BDQ+IPM
856 combinations and a fourth biological replicate, are provided in **Table S13**.

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SUPPLEMENTAL MATERIAL

Supplemental figures

- **Fig. S1.** *MAB_2299c* PCR amplicons from *M. abscessus* isolates with reduced susceptibility to clofazimine and bedaquiline.
- **Fig. S2.** Concentration-ranging bactericidal activity of bedaquiline with imipenem/avibactam combinations against *M. abscessus* ATCC 19977 WT parent and OM7 mutant strains.
- **Fig. S3.** Relative ATP levels, not adjusted by CFU counts, associated with exposure of *M. abscessus* ATCC 19977 WT to bedaquiline and imipenem for 1 day or 3 days in CAMHB with 0.05% Tween 80.
- **Fig. S4.** Activity of bedaquiline and imipenem against *M. abscessus* ATCC 19977 WT, with CFU-adjusted and unadjusted relative bacterial ATP levels, in CAMHB without Tween 80.
- **Fig. S5.** Relative ATP levels, not adjusted by CFU counts, associated with exposure of *M. abscessus* ATCC 19977 WT and OM7 mutant to bedaquiline and imipenem for 1 day or 3 days in CAMHB without Tween 80.
- **Fig. S6.** Activity of bedaquiline and imipenem against nutrient-starved *M. abscessus* ATCC 19977 WT and OM7 mutant, with relative bacterial ATP levels, in PBS without Tween 80.
- **Fig. S7.** Relative ATP levels, not adjusted by CFU counts, associated with exposure of nutrient-starved *M. abscessus* ATCC 19977 WT to bedaquiline and imipenem for 4 hours, 1 day, 3 days, or 7 days in PBS without Tween 80.
- **Fig. S8.** Relative ATP levels, not adjusted by CFU counts, associated with exposure of nutrient-starved *M. abscessus* OM7 mutant to bedaquiline and imipenem for 4 hours, 1 day, 3 days, or 7 days in PBS without Tween 80.
- **Fig. S9.** Activity of bedaquiline and imipenem against nutrient-starved *M. abscessus* ATCC 19977 WT in PBS with 0.05% Tween 80.
- **Fig. S10.** Activity of bedaquiline and imipenem against nutrient-starved *M. abscessus* ATCC 19977 WT in PBS without Tween 80 and with 0.05% Tween 80.
- **Fig. S11.** Concentration-ranging activity of bedaquiline and imipenem alone against intracellular (THP-1 cells) *M. abscessus* ATCC 19977 WT.

Supplemental tables

- **Table S1.** Bedaquiline-resistant *M. abscessus* laboratory isolates.
- **Table S2.** CFU data from samples presented in Fig. 1.
- **Table S3.** CFU data from samples presented in Fig. 2.
- **Table S4.** CFU data from samples presented in Fig. S2.
- **Table S5.** CFU data from samples presented in Fig. 3.
- **Table S6.** CFU and relative light unit data from samples presented in Fig. 4 and Fig. S3.
- **Table S7.** CFU data from samples presented in Fig. 5.
- **Table S8.** CFU and relative light unit data from samples presented in Fig. 6 and Fig. S5.
- **Table S9.** CFU and relative light unit data from samples presented in Fig. S4.
- **Table S10.** CFU and relative light unit data from samples presented in Fig. 7, Fig. S6, Fig. S7, and Fig. S8.
- **Table S11.** CFU data from samples presented in Fig. 8 and Fig. S9.
- **Table S12.** CFU data from samples presented in Fig. S10.
- **Table S13.** CFU data from intracellular samples presented in Fig. 9 and Fig. S11.

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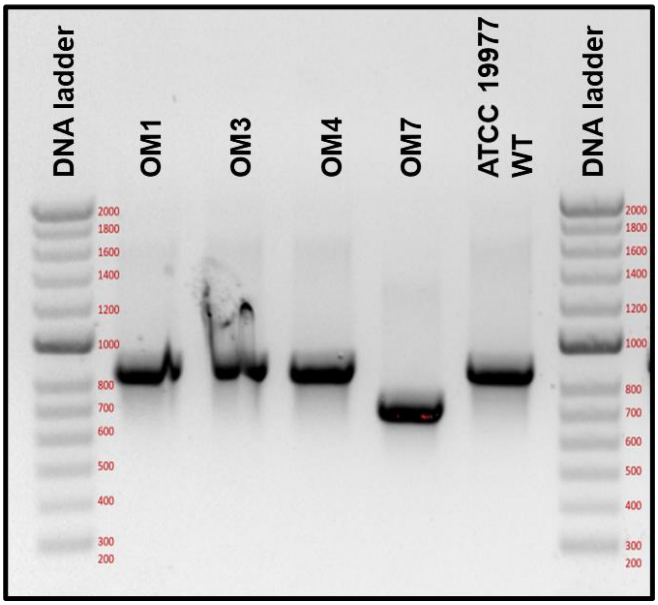

Fig. S1. *MAB_2299c* PCR amplicons from *M. abscessus* isolates with reduced susceptibility to clofazimine and bedaquiline. The expected amplicon size for the *M. abscessus* ATCC 19977 WT parent strain was 796 bp.

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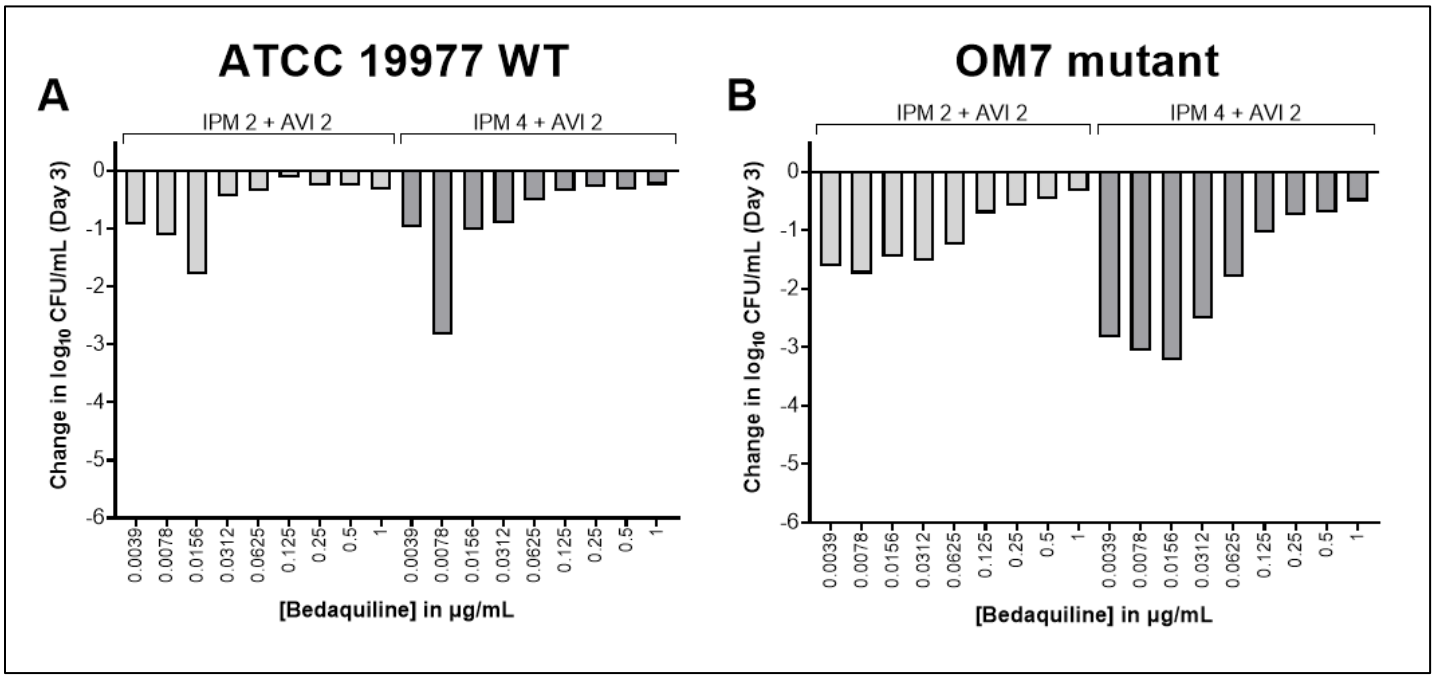

Fig. S2. Concentration-ranging bactericidal activity of bedaquiline with imipenem (IPM)/avibactam (AVI) combinations against *M. abscessus* ATCC 19977 WT parent (A) and OM7 mutant (B) strains. The change in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 is presented for each drug/strain set. Assay medium was CAMHB with 0.05% Tween 80. The number after each IPM and AVI abbreviation represents the concentration µg/mL. The Day 0 bacterial concentrations were 6.13 and 6.22 $\log_{10}$ CFU/mL for WT and OM7 mutant strains, respectively. CFU values are provided in **Table S4**.

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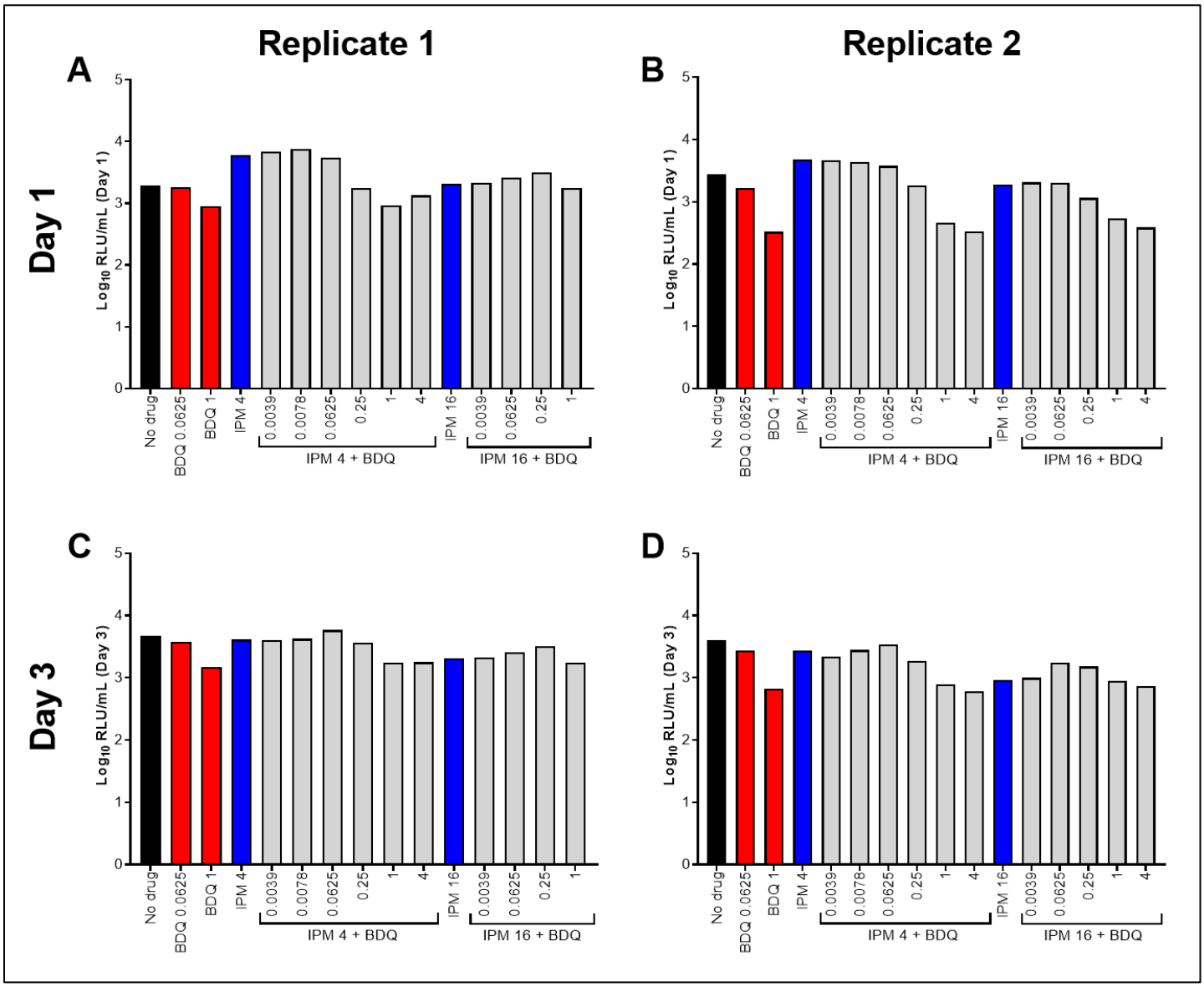

Fig. S3. Relative ATP levels, not adjusted by CFU counts, associated with exposure of *M. abscessus* ATCC 19977 WT to bedaquiline (BDQ) and imipenem (IPM) for 1 day (A,B) or 3 days (C,D) in CAMHB with 0.05% Tween 80. Replicate 1 (A,C) is the experiment presented in **Fig. 4A,C**. Replicate 2 (B,D) is the experiment presented in **Fig. 4B,D**. ATP levels are indicated by relative light units (RLU)/CFU from samples. Black bars represent the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM plus BDQ samples. The number after each drug abbreviation represents the concentration in µg/mL (for gray bars the BDQ concentration in µg/mL is presented under each bar). RLU values are provided in **Table S6**.

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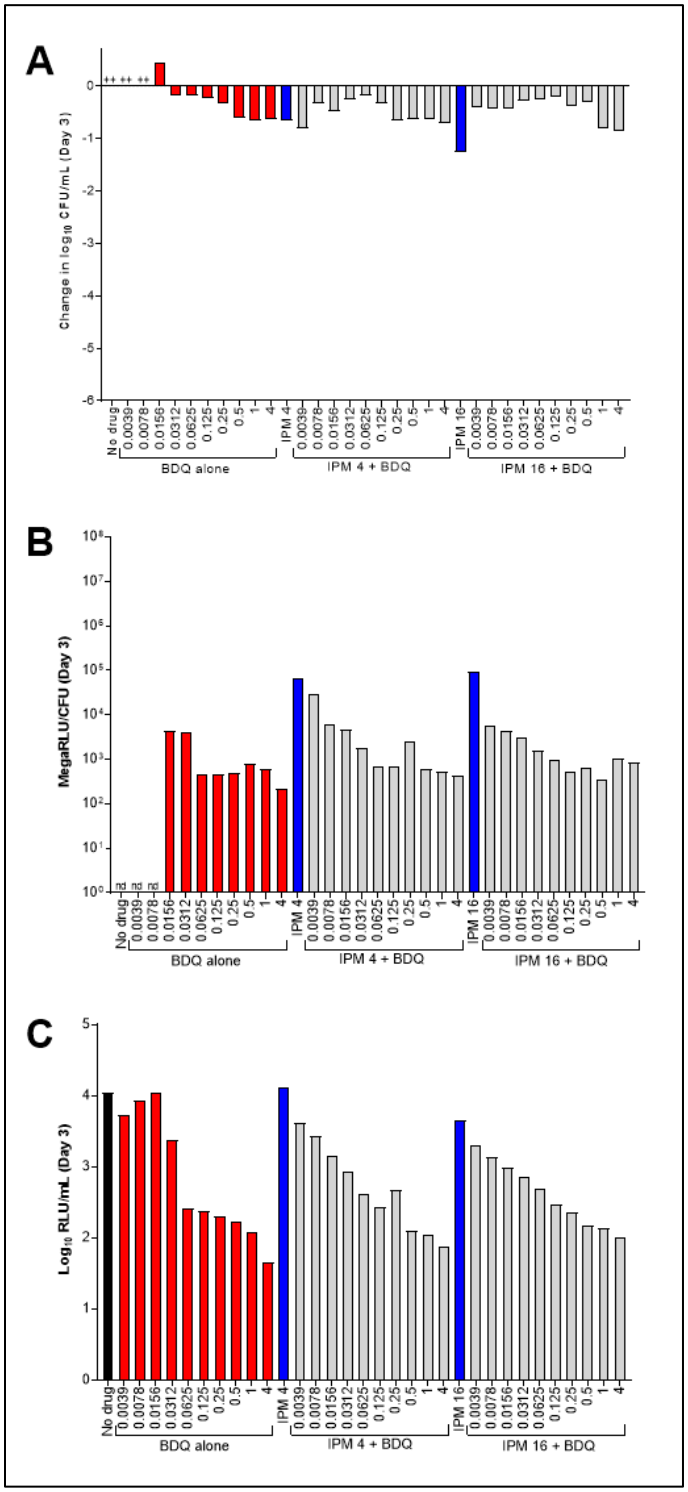

Fig. S4. Activity of bedaquiline (BDQ) and imipenem (IPM) against *M. abscessus* ATCC 19977 WT (A), with CFU-adjusted (B) and unadjusted (C) relative bacterial ATP levels, in CAMHB without Tween 80. These data are from a second biological replicate of the experiment in **Fig. 6**. The changes in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 are presented in panel A. CFU-adjusted ATP levels, indicated by relative light units (RLU)/CFU from samples after 3 days of drug exposure, are presented in panel B; relative ATP levels, not adjusted by CFU counts, are presented in panel C. Black bars indicate the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars, the BDQ concentration in µg/mL is under each bar. The Day 0 bacterial concentration for panel A was 5.95 $\log_{10}$ CFU/mL. CFU and RLU values are provided in **Table S9**.

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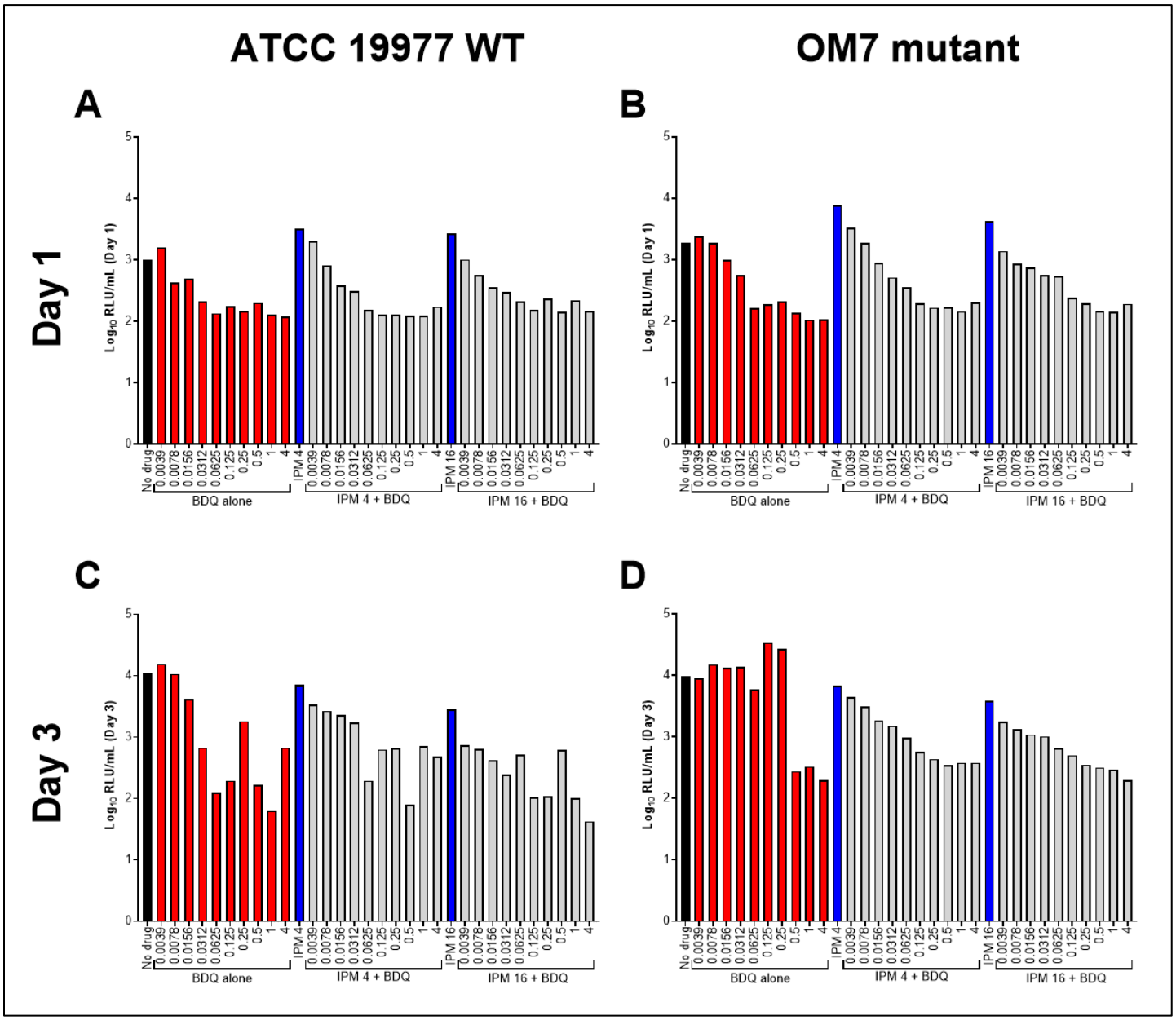

Fig. S5. Relative ATP levels, not adjusted by CFU counts, associated with exposure of *M. abscessus* ATCC 19977 WT (A,C) and OM7 mutant (B,D) to bedaquiline (BDQ) and imipenem (IPM) for 1 day (A,B) or 3 days (C,D) in CAMHB without Tween 80. ATP levels are indicated by relative light units (RLU)/CFU from samples. Black bars represent the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM plus BDQ samples. The number after each drug abbreviation represents the concentration in µg/mL (for gray bars the BDQ concentration in µg/mL is presented under each bar). RLU values are provided in **Table S8**.

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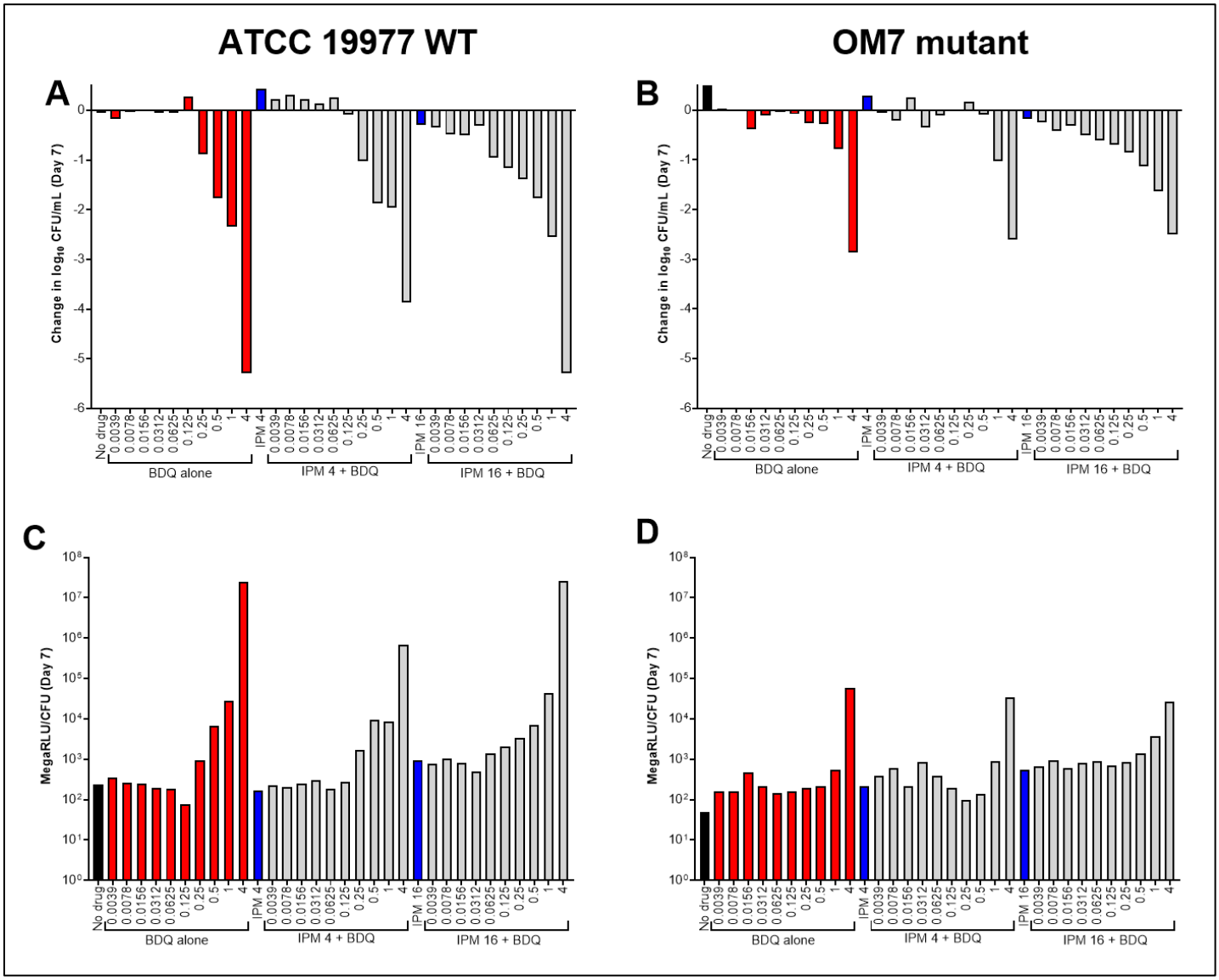

Fig. S6. Activity of bedaquiline (BDQ) and imipenem (IPM) against nutrient-starved *M. abscessus* ATCC 19977 WT (A) and OM7 mutant (B), with relative bacterial ATP levels (C,D), in PBS without Tween 80. Bacteria were nutrient-starved in PBS for 14 days prior to drug exposure. The changes in $\log_{10}$ CFU/mL after 7 days of drug exposure relative to Day 0 are presented in panels A and B; CFU-adjusted ATP levels, indicated by relative fluorescence units (RLU)/CFU from samples, after 7 days of drug exposure are presented in panels C and D. Black bars indicate the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars, the BDQ concentration in µg/mL is under each bar. Data after 3 days of drug exposure are presented in **Fig. 7**. RLU/mL (not adjusted for CFUs) for WT and OM7 are presented in **Fig. S7** and **Fig. S8**, respectively. The Day 0 bacterial concentration for panels A and B was 6.25 and 6.29 $\log_{10}$ CFU/mL, respectively. CFU and RLU values are provided in **Table S10**.

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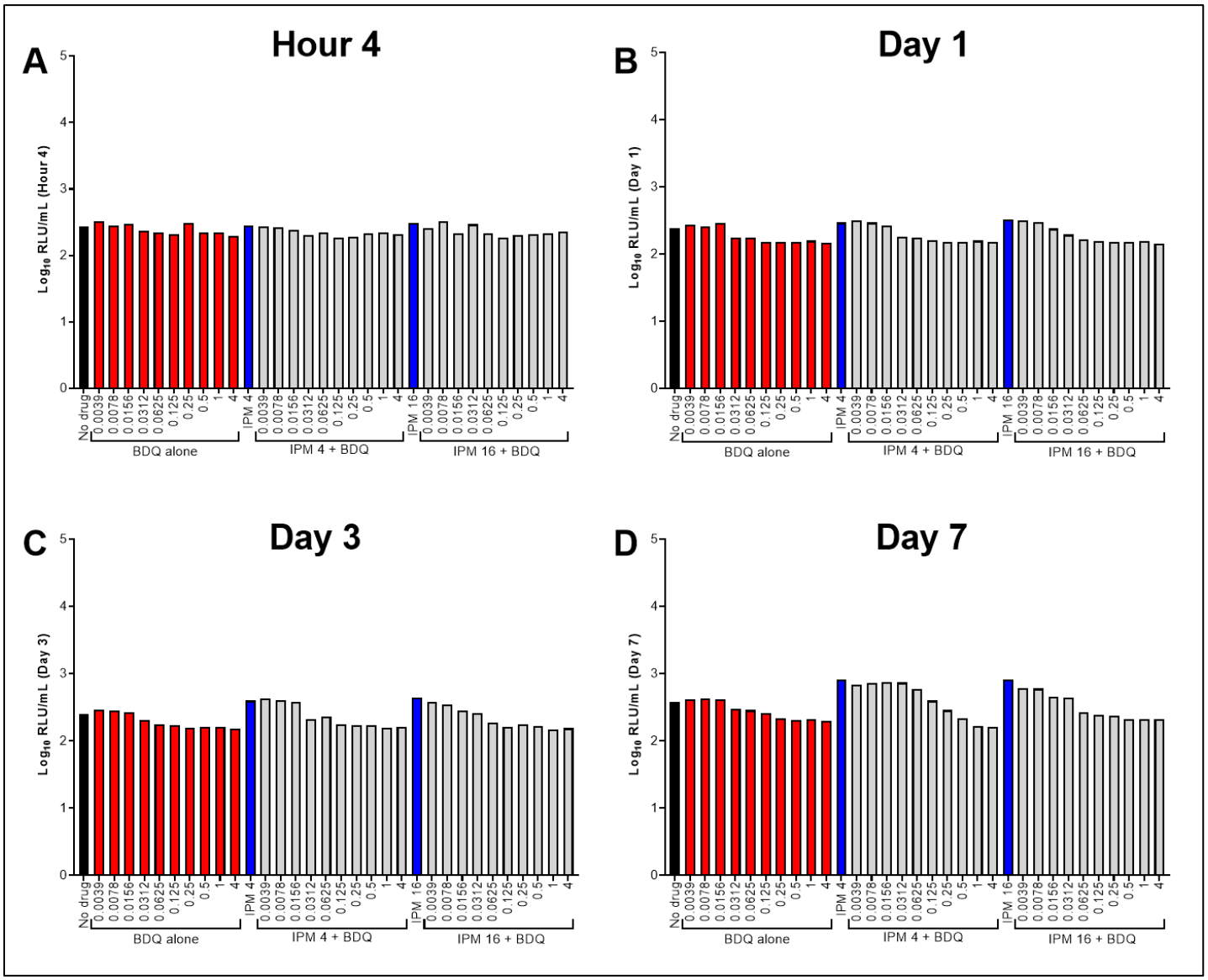

Fig. S7. Relative ATP levels, not adjusted by CFU counts, associated with exposure of nutrient-starved *M. abscessus* ATCC 19977 WT to bedaquiline (BDQ) and imipenem (IPM) for 4 hours (A), 1 day (B), 3 days (C), or 7 days (D) in PBS without Tween 80. Bacteria were nutrient-starved in PBS for 14 days prior to drug exposure. ATP levels are indicated by relative light units (RLU)/CFU from samples. Black bars represent the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM plus BDQ samples. The number after each drug abbreviation represents the concentration in µg/mL (for gray bars the BDQ concentration in µg/mL is presented under each bar). RLU values are provided in **Table S10**.

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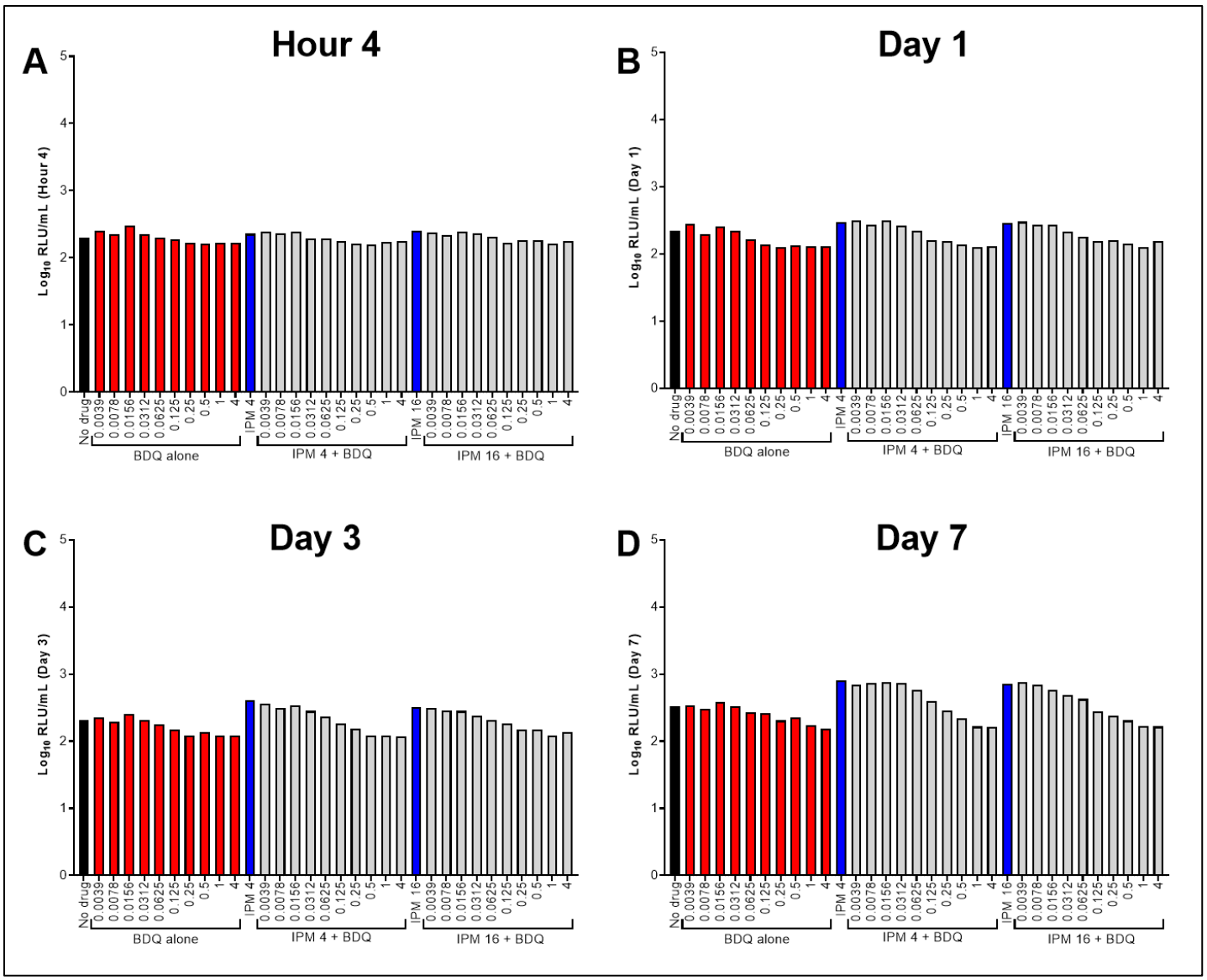

Fig. S8. Relative ATP levels, not adjusted by CFU counts, associated with exposure of nutrient-starved *M. abscessus* OM7 mutant to bedaquiline (BDQ) and imipenem (IPM) for 4 hours (A), 1 day (B), 3 days (C), or 7 days (D) in PBS without Tween 80. Bacteria were nutrient-starved in PBS for 14 days prior to drug exposure. ATP levels are indicated by relative fluorescence units (RLU)/CFU from samples. Black bars represent the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM plus BDQ samples. The number after each drug abbreviation represents the concentration in μg/mL (for gray bars the BDQ concentration in μg/mL is presented under each bar). RLU values are provided in **Table S10**.

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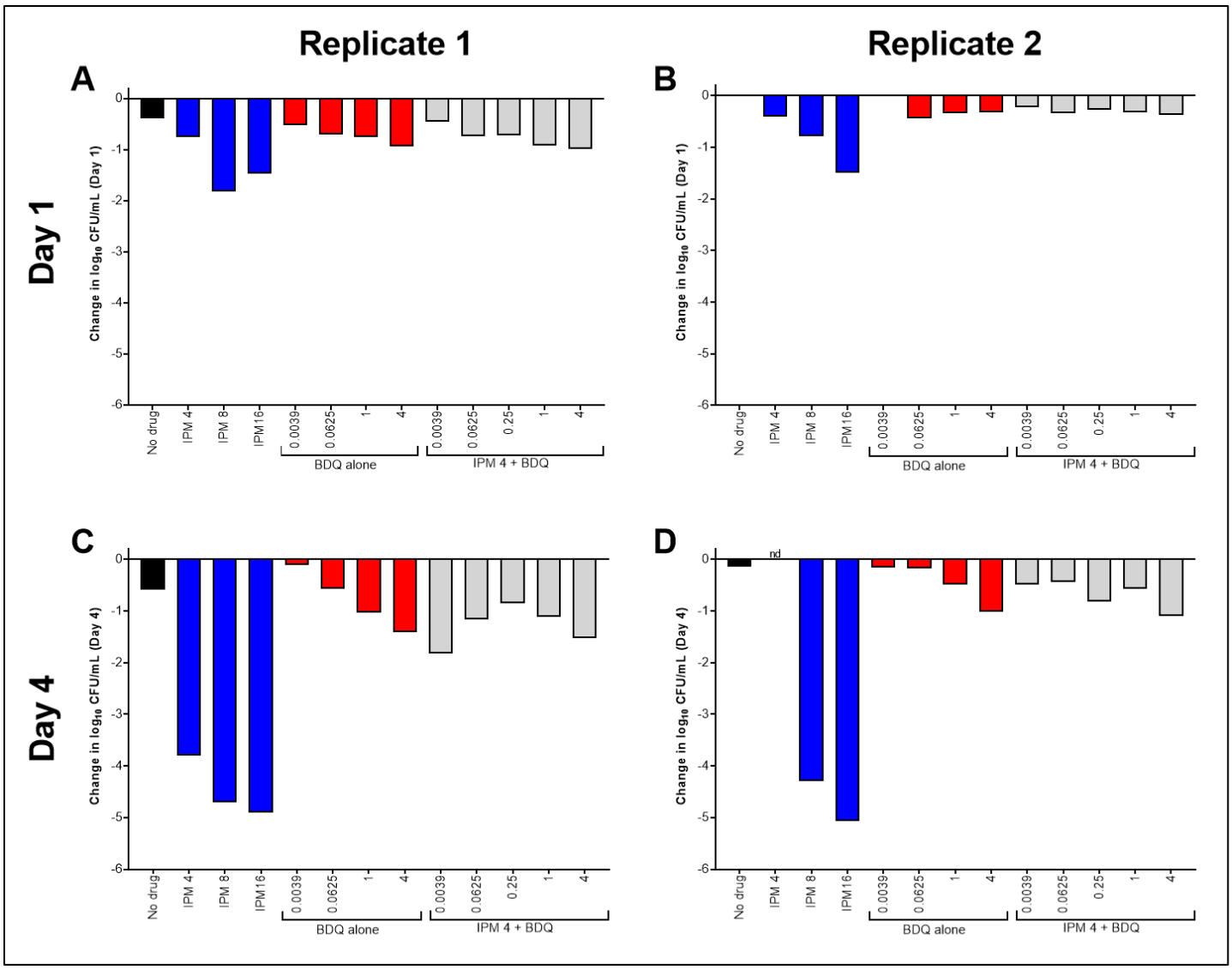

Fig. S9. Activity of bedaquiline (BDQ) and imipenem (IPM) against nutrient-starved *M. abscessus* ATCC 19977 WT in PBS with 0.05% Tween 80. Bacteria were nutrient-starved in PBS for 20 days prior to drug exposure. Data are presented for two biological replicates (replicate 1 in panels A and C; and replicate 2 in panels B and D). The changes in $\log_{10}$ CFU/mL after 1 and 4 days of drug exposure relative to Day 0 are presented in panels A/B and C/D, respectively. Black bars indicate the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars, the BDQ concentration in µg/mL is under each bar. Partial data from replicate 1 after 4 days of drug exposure are presented in **Fig. 8**. The Day 0 bacterial concentration for replicate 1 and 2 was 6.07 and 6.38 $\log_{10}$ CFU/mL, respectively. CFU values are provided in **Table S11**.

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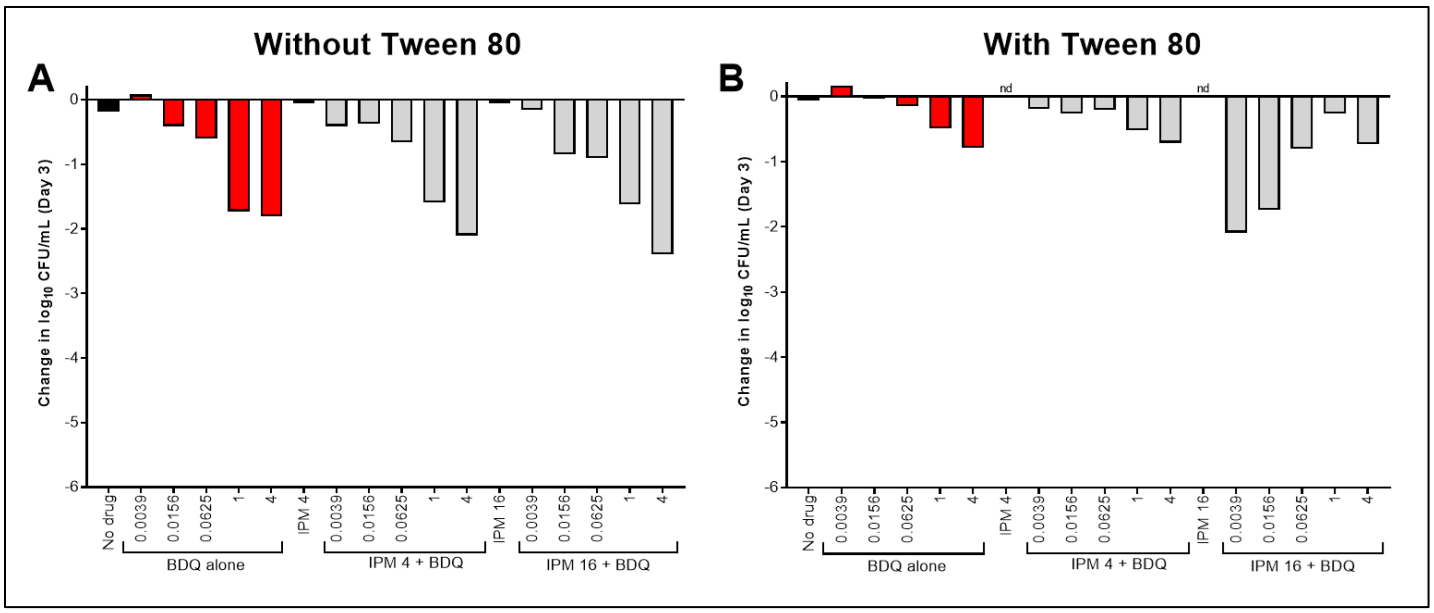

Fig. S10. Activity of bedaquiline (BDQ) and imipenem (IPM) against nutrient-starved *M. abscessus* ATCC 19977 WT in PBS without Tween 80 (A) and with 0.05% Tween 80 (B). Bacteria were nutrient-starved in PBS for 20 days prior to drug exposure. Data are presented for two biological replicates (replicate 1 in panels A and C; and replicate 2 in panels B and D). The changes in $\log_{10}$ CFU/mL after 3 days of drug exposure relative to Day 0 are presented. Black bars indicate the no drug control; red bars indicate BDQ only samples; blue bars represent IPM only samples, and gray bars indicate IPM + BDQ samples. The number after the IPM abbreviation represents the concentration in µg/mL; for red and gray bars, the BDQ concentration in µg/mL is under each bar. The Day 0 bacterial concentration for panel A and B was 6.48 and 6.51 $\log_{10}$ CFU/mL, respectively. nd, not determined (samples lost). CFU values are provided in **Table S12**.

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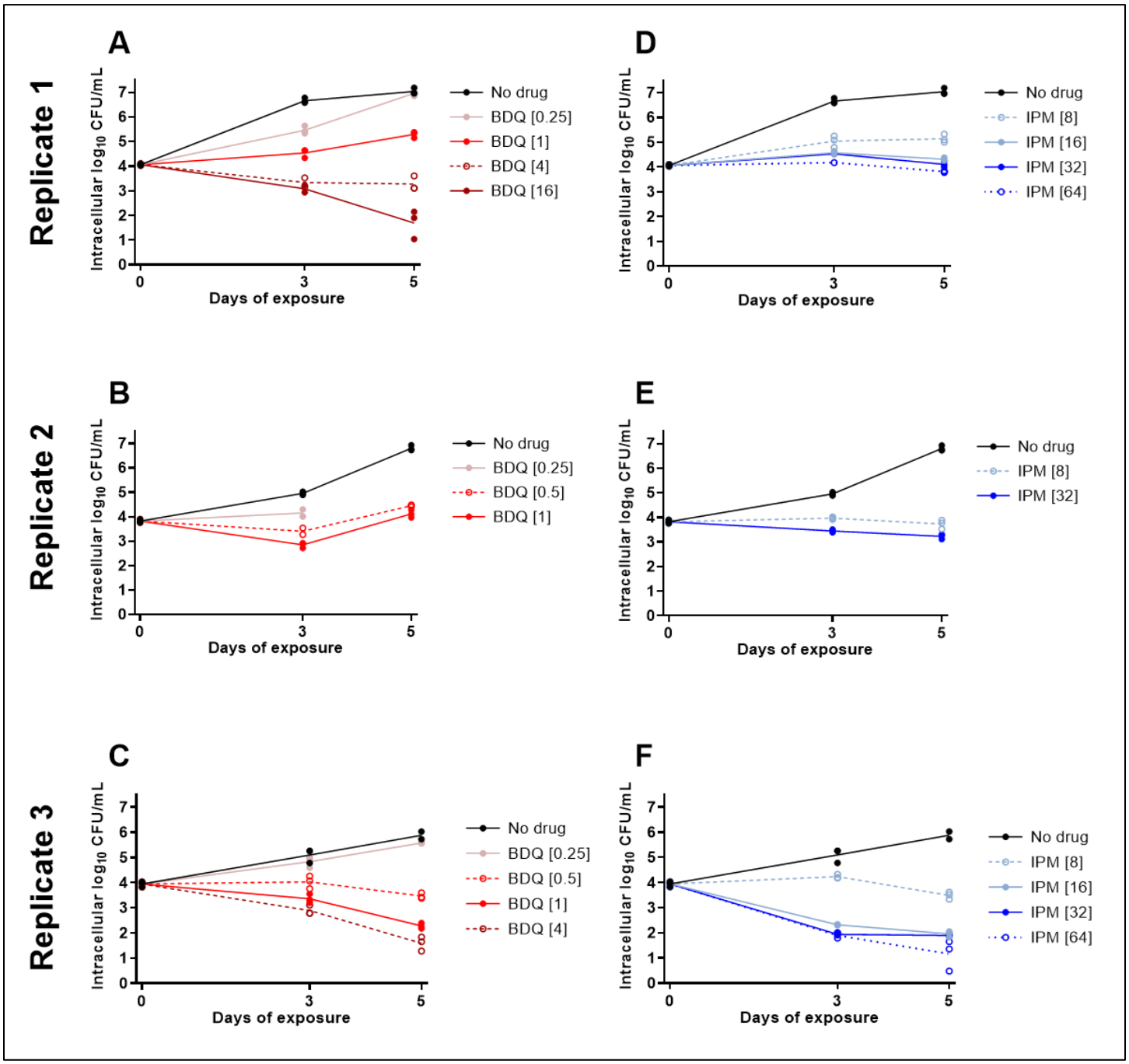

Fig. S11. Concentration-ranging activity of bedaquiline (BDQ) (A-C) and imipenem (IPM) (D-F) alone against intracellular (THP-1 cells) *M. abscessus* ATCC 19977 WT. Data are presented for 3 biological replicates: replicate 1, panels A,D; replicate 2, panels B,E; replicate 3, panels C,F. Each data point represents a technical replicate, and the connecting lines pass through the mean values. The [number] after each drug abbreviation represents the concentration in µg/mL. All CFU values are provided in **Table S13**.

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Table S1. Bedaquiline-resistant *M. abscessus* laboratory isolates.

***M. abscessus* isolate**	**Mor-pho-type**	**CFZ MIC (µg/ml)**		**BDQ MIC (µg/ml)**		***MAB_2299c* mutation**	**MAB_2299c AA change**	***MAB_4384* mutation**
		CAMHB	**7H9**	**CAMHB**	**7H9**			
ATCC 19977 WT	WT	0.25	8	0.0625	1	None	None	None
OM1	WT	4	16	0.25	4	19C>T	Q7I	None
OM3	WT	2	16	0.5	4	19C>T	Q7I	None
OM4	Rough	nd	16	nd	4	566G>del	Frameshift	None
OM7	WT	2	16	0.5	4	-18_122del	N-terminal truncation	None

WT, wild type. CFZ, clofazimine. BDQ, bedaquiline. MIC: minimum inhibitory concentration, determined by broth microdilution. CAMHB: cation-adjusted Mueller-Hinton broth; 7H9: Middlebrook 7H9 broth with 10% (v/v) Middlebrook oleic acid-albumin-dextrose-catalase supplement; AA, amino acid; nd, not determined. Tween 80 was not included in either assay medium.

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Table S2. CFU data from samples presented in Fig. 1. This table also includes data from replicate assays that are not shown in Fig. 1. Assay medium was CAMHB with 0.05% Tween 80. Red shading indicates bedaquiline (BDQ) samples, and blue shading indicates imipenem (IPM) or IMP with avibactam (AVI) samples.

Strain, condition, Day 0 bacterial burden, figure number	**Drug(s) and concentration(s) in µg/mL**	**Log_{10} CFU/mL at the following time points:**	
		Day 3	**Day 7**
ATCC 19977 WT, CAMHB + Tween, Day 0 = 5.96 log_{10} CFU/mL, **Fig. 1A** (Day 3 data); Day 7 data not in a figure	No drug	Clump	Clump
	BDQ 0.015625	Clump	Clump
	BDQ 0.03125	Clump	Clump
	BDQ 0.0625	6.58	6.56
	BDQ 0.125	6.27	6.41
	BDQ 0.25	6.02	6.08
	BDQ 0.5	6.04	5.78
	BDQ 1	5.93	5.91
	BDQ 2	5.95	5.30
	BDQ 4	5.92	5.53
	BDQ 8	6.09	5.53
	BDQ 16	6.03	5.30
OM7 mutant, CAMHB + Tween, Day 0 = 6.11 log_{10} CFU/mL, **Fig. 1B** (Day 3 data); Day 7 data not in a figure	No drug	Clump	Clump
	BDQ 0.015625	Clump	Clump
	BDQ 0.03125	Clump	Clump
	BDQ 0.0625	Clump	Clump
	BDQ 0.125	Clump	Clump
	BDQ 0.25	Clump	Clump
	BDQ 0.5	Clump	Clump
	BDQ 1	Clump	Clump
	BDQ 2	5.90	5.95
	BDQ 4	6.10	6.11
	BDQ 8	6.15	5.72
	BDQ 16	5.96	5.72
ATCC 19977 WT, CAMHB + Tween, Day 0 = 5.81 log_{10} CFU/mL, **Fig. 1C**	No drug	Clump	nd
	IPM 0.5	Clump	nd
	IPM 1	6.45	nd
	IPM 2	4.62	nd
	IPM 4	3.17	nd
	IPM 8	3.09	nd
	IPM 16	3.02	nd
	IPM 32	2.81	nd
	IPM 64	2.82	nd
	IPM 128	2.86	nd
ATCC 19977 WT, CAMHB + Tween, Day 0 = 6.20 log_{10} CFU/mL, Replicate not in a figure	No drug	Clump	Clump
	IPM 1	6.64	Clump
	IPM 2	6.48	Clump
	IPM 4	6.00	Clump
	IPM 8	4.90	Clump
	IPM 16	4.41	6.85
	IPM 32	3.10	6.03
	IPM 64	3.32	5.41
	IPM 128	3.48	2.51
	IPM 256	3.87	1.54

Table continued on next page.

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Table S2, continued.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:	
		Day 3	Day 7
OM7 mutant, CAMHB + Tween, Day 0 = 5.85 log_{10} CFU/mL, **Fig. 1D**	No drug	Clump	nd
	IPM 0.5	Clump	nd
	IPM 1	6.16	nd
	IPM 2	2.33	nd
	IPM 4	1.23	nd
	IPM 8	1.49	nd
	IPM 16	2.17	nd
	IPM 32	1.11	nd
	IPM 64	0.85	nd
	IPM 128	0*	nd
OM7 mutant, CAMHB + Tween, Day 0 = 5.96 log_{10} CFU/mL, Replicate not in a figure	No drug	Clump	Clump
	IPM 1	5.60	Clump
	IPM 2	3.00	Clump
	IPM 4	3.00	6.31
	IPM 8	2.30	2.79
	IPM 16	3.58	2.42
	IPM 32	3.14	2.66
	IPM 64	3.35	2.68
	IPM 128	4.15	2.75
	IPM 256	0.85	2.41
ATCC 19977 WT, CAMHB + Tween, Day 0 = 5.81 log_{10} CFU/mL, **Fig. 1E**	No drug	Clump	nd
	AVI 2	Cump	nd
	IPM 0.5 + AVI 2	6.37	nd
	IPM 1 + AVI 2	5.45	nd
	IPM 2 + AVI 2	2.83	nd
	IPM 4 + AVI 2	2.90	nd
	IPM 8 + AVI 2	2.81	nd
	IPM 16 + AVI 2	2.81	nd
	IPM 32 + AVI 2	2.89	nd
	IPM 64 + AVI 2	2.72	nd
	IPM 128 + AVI 2	2.66	nd
OM7 mutant, CAMHB + Tween, Day 0 = 5.85 log_{10} CFU/mL, **Fig. 1F**	No drug	Clump	nd
	AVI 2	Cump	nd
	IPM 0.5 + AVI 2	6.22	nd
	IPM 1 + AVI 2	5.45	nd
	IPM 2 + AVI 2	2.87	nd
	IPM 4 + AVI 2	3.72	nd
	IPM 8 + AVI 2	3.15	nd
	IPM 16 + AVI 2	3.10	nd
	IPM 32 + AVI 2	3.04	nd
	IPM 64 + AVI 2	3.02	nd
	IPM 128 + AVI 2	2.78	nd

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification. nd, not determined.

*Lower limit of detection: 0.48 log_{10} CFU/mL

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Table S3. CFU data from samples presented in Fig. 2. Assay medium was CAMHB with 0.05% Tween 80. Red shading indicates bedaquiline (BDQ) samples, blue shading indicates imipenem (IPM) or IMP with avibactam (AVI) samples, and gray shading indicates IPM + AVI + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:	
		Day 3	Day 5
ATCC 19977 WT, CAMHB + Tween, Day 0 = 5.57 log_{10} CFU/mL, **Fig. 2A** (Day 3 data); Day 5 data and data from additional combinations not included in a figure	No drug	Clump	Clump
	BDQ 0.5	5.45	Clump
	BDQ 1	5.30	Clump
	AVI 2	Clump	Clump
	IPM 2	Clump	Clump
	IPM 4	5.26	Clump
	IPM 2 + AVI 2	3.78	Clump
	IPM 4 + AVI 2	3.30	Clump
	IPM 2 + AVI 2 + BDQ 0.00390625	3.60	<3.30*
	IPM 2 + AVI 2 + BDQ 0.015625	5.14	5.56
	IPM 2 + AVI 2 + BDQ 0.0625	5.26	5.03
	IPM 2 + AVI 2 + BDQ 0.25	5.82	5.45
	IPM 2 + AVI 2 + BDQ 0.5	5.88	5.73
	IPM 2 + AVI 2 + BDQ 1	5.99	5.81
	IPM 4 + AVI 2 + BDQ 0.00390625	3.26	Clump
	IPM 4 + AVI 2 + BDQ 0.015625	4.13	Clump
	IPM 4 + AVI 2 + BDQ 0.0625	5.26	Clump
	IPM 4 + AVI 2 + BDQ 0.25	5.66	Clump
	IPM 4 + AVI 2 + BDQ 0.5	5.72	Clump
	IPM 4 + AVI 2 + BDQ 1	5.81	Clump
OM7 mutant, CAMHB + Tween, Day 0 = 5.76 log_{10} CFU/mL, **Fig. 2B** (Day 3 data); Day 5 data and data from additional combinations not included in a figure	No drug	Clump	Clump
	BDQ 0.5	7.06	Clump
	BDQ 1	6.12	Clump
	AVI 2	Clump	Clump
	IPM 2	6.18	Clump
	IPM 4	5.08	Clump
	IPM 2 + AVI 2	3.60	Clump
	IPM 4 + AVI 2	4.00	Clump
	IPM 2 + AVI 2 + BDQ 0.00390625	2.78	5.58
	IPM 2 + AVI 2 + BDQ 0.015625	3.20	5.56
	IPM 2 + AVI 2 + BDQ 0.0625	4.89	Clump
	IPM 2 + AVI 2 + BDQ 0.25	5.45	Clump
	IPM 2 + AVI 2 + BDQ 0.5	5.90	Clump
	IPM 2 + AVI 2 + BDQ 1	5.75	Clump
	IPM 4 + AVI 2 + BDQ 0.00390625	4.87	4.82
	IPM 4 + AVI 2 + BDQ 0.015625	2.78	4.99
	IPM 4 + AVI 2 + BDQ 0.0625	4.25	6.24
	IPM 4 + AVI 2 + BDQ 0.25	5.30	Clump
	IPM 4 + AVI 2 + BDQ 0.5	5.15	5.64
	IPM 4 + AVI 2 + BDQ 1	5.48	6.66

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification.

*The lower limit of detection for this sample was 3.30 log_{10} CFU/mL.

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Table S4. CFU data from samples presented in Fig. S2. Assay medium was CAMHB with 0.05% Tween 80. Gray shading indicates imipenem (IPM) + avibactam (AVI) + bedaquiline (BDQ) samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:	
		Day 3	Day 5
ATCC 19977 WT, CAMHB + Tween, Day 0 = 6.13 log_{10} CFU/mL, **Fig. S2A** (Day 3 data); Day 5 data not included in a figure	No drug	Clump	Clump
	IPM 2 + AVI 2 + BDQ 0.00390625	5.20	7.15
	IPM 2 + AVI 2 + BDQ 0.0078125	5.03	6.45
	IPM 2 + AVI 2 + BDQ 0.015625	4.34	6.79
	IPM 2 + AVI 2 + BDQ 0.03125	5.70	6.56
	IPM 2 + AVI 2 + BDQ 0.0625	5.79	6.30
	IPM 2 + AVI 2 + BDQ 0.125	6.01	6.20
	IPM 2 + AVI 2 + BDQ 0.25	5.89	6.58
	IPM 2 + AVI 2 + BDQ 0.5	5.87	5.76
	IPM 2 + AVI 2 + BDQ 1	5.81	6.34
	IPM 4 + AVI 2 + BDQ 0.00390625	5.16	7.20
	IPM 4 + AVI 2 + BDQ 0.0078125	<3.30*	5.82
	IPM 4 + AVI 2 + BDQ 0.015625	5.11	6.51
	IPM 4 + AVI 2 + BDQ 0.03125	5.23	6.45
	IPM 4 + AVI 2 + BDQ 0.0625	5.62	6.29
	IPM 4 + AVI 2 + BDQ 0.125	5.79	6.87
	IPM 4 + AVI 2 + BDQ 0.25	5.86	5.82
	IPM 4 + AVI 2 + BDQ 0.5	5.81	5.87
	IPM 4 + AVI 2 + BDQ 1	5.89	5.97
OM7 mutant, CAMHB + Tween, Day 0 = 6.22 log_{10} CFU/mL, **Fig. S2B** (Day 3 data); Day 5 data not included in a figure	No drug	Clump	Clump
	IPM 2 + AVI 2 + BDQ 0.00390625	4.60	6.72
	IPM 2 + AVI 2 + BDQ 0.0078125	4.48	6.56
	IPM 2 + AVI 2 + BDQ 0.015625	4.76	6.56
	IPM 2 + AVI 2 + BDQ 0.03125	4.70	6.64
	IPM 2 + AVI 2 + BDQ 0.0625	4.97	6.53
	IPM 2 + AVI 2 + BDQ 0.125	5.51	6.70
	IPM 2 + AVI 2 + BDQ 0.25	5.64	5.94
	IPM 2 + AVI 2 + BDQ 0.5	5.76	6.15
	IPM 2 + AVI 2 + BDQ 1	5.89	5.72
	IPM 4 + AVI 2 + BDQ 0.00390625	3.38	6.70
	IPM 4 + AVI 2 + BDQ 0.0078125	3.15	5.30
	IPM 4 + AVI 2 + BDQ 0.015625	3.00	5.09
	IPM 4 + AVI 2 + BDQ 0.03125	3.70	6.19
	IPM 4 + AVI 2 + BDQ 0.0625	4.41	5.03
	IPM 4 + AVI 2 + BDQ 0.125	5.18	5.27
	IPM 4 + AVI 2 + BDQ 0.25	5.48	5.96
	IPM 4 + AVI 2 + BDQ 0.5	5.53	6.45
	IPM 4 + AVI 2 + BDQ 1	5.72	5.57

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification.
*The lower limit of detection for this sample was 3.30 log_{10} CFU/mL. The value of 3.30 log_{10} CFU/mL was used to calculate the change in CFU from Day 0, presented in **Fig. S2**.

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Table S5. CFU data from samples presented in Fig. 3. Assay medium was CAMHB with 0.05% Tween 80. Red shading indicates bedaquiline (BDQ) samples; blue shading indicates meropenem (MEM), MmpL3 inhibitor (MPL), or clarithromycin (CLR) samples; and gray shading indicates BDQ combination samples. MPL is N-4S-methylcyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide.

Strain, condition, Day 0 bacterial burden, figure number	**Drug(s)/compound(s) and concentration(s) in µg/mL**	**Log_{10} CFU/mL at the following time points:**		
		Day 3	**Day 5**	**Day 8**
ATCC 19977 WT, CAMHB + Tween, Day 0 = 6.40 log_{10} CFU/mL, **Fig. 3**	No drug	7.59	Clump	Clump
	BDQ 0.00390625	6.77	7.20	6.43
	BDQ 0.015625	7.15	7.67	6.59
	BDQ 0.125	6.41	6.48	5.40
	BDQ 1	5.34	5.36	5.04
	BDQ 4	5.20	5.26	4.85
	MEM 16	3.23	2.48	2.73
	MEM 16 + BDQ 0.00390625	3.98	2.66	4.78
	MEM 16 + BDQ 0.015625	3.76	2.32	4.85
	MEM 16 + BDQ 0.125	5.34	4.95	4.20
	MEM 16 + BDQ 1	3.76	5.15	4.85
	MEM 16 + BDQ 4	5.20	5.00	4.78
	MPL 2	4.11	2.30	1.04
	MPL 2 + BDQ 0.00390625	5.26	2.85	2.38
	MPL 2 + BDQ 0.015625	6.34	5.70	6.48
	MPL 2 + BDQ 0.125	6.15	6.56	6.53
	MPL 2 + BDQ 1	5.40	5.34	5.39
	MPL 2 + BDQ 4	5.43	5.26	5.05
	CLR 4	4.78	3.52	3.27
	CLR 4 + BDQ 0.015625	4.65	3.51	3.20
	CLR 4 + BDQ 0.125	4.90	3.43	2.23
	CLR 4 + BDQ 1	4.91	3.45	1.61
	CLR 4 + BDQ 4	4.79	3.40	1.32

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification.

Table S6. CFU and relative light unit (RLU) data from samples presented in Fig. 4 and Fig. S3. Assay medium was CAMHB with 0.05% Tween 80. Red shading indicates bedaquiline (BDQ) samples, blue shading indicates imipenem (IPM) samples, and gray shading indicates IPM + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:		Log_{10} RLU/mL at the following time points:		MegaRLU/CFU at the following time points:	
		Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
ATCC 19977 WT, CAMHB + Tween, Day 0 = 6.25 log_{10} CFU/mL, **Fig. 4A,C; Fig. S3A,C** (Replicate 1)	No drug	6.73	7.31	3.28	3.67	356	228
	BDQ 0.0625	6.72	7.24	3.27	3.58	355	217
	BDQ 1	6.68	6.70	2.95	3.17	185	296
	IPM 4	6.12	5.66	3.78	3.61	4576	8945
	IPM 16	4.34	4.51	3.32	3.32	95273	64547
	IPM 4 + BDQ 0.00390625	6.06	5.16	3.84	3.60	5966	27435
	IPM 4 + BDQ 0.0078125	6.32	4.53	3.88	3.63	3648	124618
	IPM 4 + BDQ 0.0625	6.62	6.51	3.74	3.77	1311	1830
	IPM 4 + BDQ 0.25	6.58	6.58	3.24	3.57	461	971
	IPM 4 + BDQ 1	6.78	6.70	2.97	3.24	156	351
	IPM 4 + BDQ 4	6.76	6.75	3.13	3.25	231	319
	IPM 16 + BDQ 0.00390625	4.56	3.48	3.33	3.33	59389	712667
	IPM 16 + BDQ 0.0625	6.11	5.78	3.41	3.41	2026	4322
	IPM 16 + BDQ 0.25	6.66	6.34	3.51	3.51	697	1457
	IPM 16 + BDQ 1	6.62	6.53	3.25	3.25	421	520
ATCC 19977 WT, CAMHB + Tween, Day 0 = 7.03 log_{10} CFU/mL, **Fig. 4B,D; Fig. S3B,D** (Replicate 2)	No drug	7.06	7.42	3.44	3.60	242	152
	BDQ 0.0625	6.79	7.23	3.23	3.44	273	162
	BDQ 1	7.09	7.01	2.52	2.82	27	65
	IPM 4	6.40	5.38	3.69	3.44	1910	11632
	IPM 16	6.21	3.87	3.28	2.97	1169	125338
	IPM 4 + BDQ 0.00390625	6.60	4.83	3.67	3.34	1170	32228
	IPM 4 + BDQ 0.0078125	6.26	5.61	3.64	3.45	2433	6934
	IPM 4 + BDQ 0.0625	6.89	6.79	3.58	3.54	485	556
	IPM 4 + BDQ 0.25	6.89	6.89	3.26	3.27	236	241
	IPM 4 + BDQ 1	7.02	6.91	2.66	2.90	44	96
	IPM 4 + BDQ 4	6.86	6.86	2.53	2.79	47	85
	IPM 16 + BDQ 0.00390625	6.26	4.00	3.32	3.00	1125	100050
	IPM 16 + BDQ 0.0625	6.82	6.45	3.30	3.25	304	630
	IPM 16 + BDQ 0.25	6.97	6.87	3.06	3.18	124	205
	IPM 16 + BDQ 1	7.00	6.83	2.73	2.95	54	131
	IPM 16 + BDQ 4	6.92	6.91	2.59	2.87	46	91

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Table S7. CFU data from samples presented in Fig. 5. This table also includes data from replicate assays that are not shown in Fig. 5. Assay medium was CAMHB without Tween 80. Red shading indicates bedaquiline (BDQ) samples, and blue shading indicates imipenem (IPM) samples.

Strain, condition, Day 0 bacterial burden, figure number	**Drug(s) and concentration(s) in µg/mL**	**Log_{10} CFU/mL at Day 3**
ATCC 19977 WT, CAMHB (no Tween), Day 0 = 5.90 log_{10} CFU/mL, **Fig. 5A**, with additional concentrations not in the figure	No drug	Clump
	BDQ 0.0078125	Clump
	BDQ 0.015625	6.44
	BDQ 0.03125	5.83
	BDQ 0.0625	5.53
	BDQ 0.125	5.23
	BDQ 0.25	5.25
	BDQ 0.5	5.11
	BDQ 1	5.05
	BDQ 2	5.00
	BDQ 4	5.08
	BDQ 8	5.29
	BDQ 16	5.23
OM7 mutant, CAMHB (no Tween), Day 0 = 5.08 log_{10} CFU/mL, **Fig. 5B**, with additional concentrations not in the figure	No drug	Clump
	BDQ 0.0078125	Clump
	BDQ 0.015625	Clump
	BDQ 0.03125	Clump
	BDQ 0.0625	Clump
	BDQ 0.125	Clump
	BDQ 0.25	Clump
	BDQ 0.5	5.70
	BDQ 1	5.66
	BDQ 2	5.66
	BDQ 4	5.79
	BDQ 8	5.85
	BDQ 16	5.95
ATCC 19977 WT, CAMHB (no Tween), Day 0 = 5.91 log_{10} CFU/mL, **Fig. 5C**	No drug	Clump
	IPM 1	Clump
	IPM 2	Clump
	IPM 4	Clump
	IPM 8	Clump
	IPM 16	6.01
	IPM 32	5.79
	IPM 64	5.62
	IPM 128	5.15
	IPM 256	4.73
ATCC 19977 WT, CAMHB (no Tween), Day 0 = 5.28 log_{10} CFU/mL, Replicate not in a figure	No drug	Clump
	IPM 1	Clump
	IPM 2	Clump
	IPM 4	Clump
	IPM 8	Clump
	IPM 16	6.06
	IPM 32	6.12
	IPM 64	5.00
	IPM 128	4.78
	IPM 256	4.33

Table continued on next page.

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Table S7, continued.

Strain, condition, Day 0 bacterial burden, figure number	**Drug(s) and concentration(s) in µg/mL**	**Log_{10} CFU/mL at Day 3**
OM7 mutant, CAMHB (no Tween), Day 0 = 6.20 log_{10} CFU/mL, **Fig. 5D**	No drug	Clump
	IPM 1	6.58
	IPM 2	6.64
	IPM 4	5.91
	IPM 8	5.38
	IPM 16	5.41
	IPM 32	5.45
	IPM 64	5.00
	IPM 128	4.86
	IPM 256	4.62
OM7 mutant, CAMHB (no Tween), Day 0 = 5.72 log_{10} CFU/mL, Replicate not in a figure	No drug	Clump
	IPM 1	5.66
	IPM 2	5.22
	IPM 4	5.13
	IPM 8	5.05
	IPM 16	5.21
	IPM 32	3.94
	IPM 64	4.97
	IPM 128	4.51
	IPM 256	4.40

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification.

Table S8. CFU and relative light unit (RLU) data from samples presented in Fig. 6 and Fig. S5. Assay medium was CAMHB without Tween 80. Red shading indicates bedaquiline (BDQ) samples, blue shading indicates imipenem (IPM) samples, and gray shading indicates IPM + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at Day 3	Log_{10} RLU/mL at the following time points:		MegaRLU/CFU at Day 3
			Day 1	Day 3	
ATCC 19977 WT, CAMHB (no Tween), Day 0 = 5.62 log_{10} CFU/mL, **Fig. 6A,C**; **Fig. S5A,C** (replicate 1)	No drug	Clump	3.00	4.04	nd
	IPM 4	4.51	3.51	3.85	100211
	IPM 16	4.81	3.43	3.45	42488
	BDQ 0.00390625	Clump	3.20	4.20	nd
	BDQ 0.0078125	Clump	2.63	4.03	nd
	BDQ 0.015625	6.16	2.69	3.62	339
	BDQ 0.03125	5.12	2.32	2.83	1569
	BDQ 0.0625	5.09	2.13	2.10	1092
	BDQ 0.125	5.07	2.25	2.29	1509
	BDQ 0.25	5.26	2.17	3.26	813
	BDQ 0.5	5.03	2.30	2.22	1846
	BDQ 1	4.78	2.11	1.80	2151
	BDQ 4	4.53	2.08	2.83	3506
	IPM 4 + BDQ 0.00390625	5.08	3.31	3.53	16902
	IPM 4 + BDQ 0.0078125	5.09	2.91	3.43	6482
	IPM 4 + BDQ 0.015625	5.16	2.58	3.36	2630
	IPM 4 + BDQ 0.03125	5.26	2.49	3.24	1673
	IPM 4 + BDQ 0.0625	5.22	2.19	2.29	932
	IPM 4 + BDQ 0.125	4.75	2.11	2.80	2307
	IPM 4 + BDQ 0.25	4.87	2.11	2.82	1736
	IPM 4 + BDQ 0.5	4.72	2.09	1.90	2322
	IPM 4 + BDQ 1	4.73	2.09	2.85	2263
	IPM 4 + BDQ 4	4.34	2.24	2.68	7870
	IPM 16 + BDQ 0.00390625	5.16	3.01	2.87	7015
	IPM 16 + BDQ 0.0078125	5.09	2.76	2.81	4658
	IPM 16 + BDQ 0.015625	4.99	2.56	2.63	3720
	IPM 16 + BDQ 0.03125	5.28	2.48	2.39	1562
	IPM 16 + BDQ 0.0625	5.33	2.32	2.71	971
	IPM 16 + BDQ 0.125	5.19	2.19	2.02	991
	IPM 16 + BDQ 0.25	4.79	2.37	2.04	3800
	IPM 16 + BDQ 0.5	5.66	2.16	2.79	313
	IPM 16 + BDQ 1	4.64	2.34	2.01	4980
	IPM 16 + BDQ 4	4.81	2.17	1.63	2272

Table continued on next page.

Table S8, continued.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at Day 3	Log_{10} RLU/mL at the following time points:		MegaRLU/CFU at Day 3
			Day 1	Day 3	
OM7 mutant, CAMHB (no Tween), Day 0 = 5.70 log_{10} CFU/mL, **Fig. 6B,D; Fig. S5B,D**	No drug	Clump	3.27	3.98	nd
	IPM 4	4.45	3.89	3.83	239354
	IPM 16	3.83	3.63	3.59	566382
	BDQ 0.00390625	Clump	3.38	3.95	nd
	BDQ 0.0078125	Clump	3.27	4.18	nd
	BDQ 0.015625	Clump	2.99	4.12	nd
	BDQ 0.03125	Clump	2.75	4.14	nd
	BDQ 0.0625	6.11	2.21	3.77	4576
	BDQ 0.125	5.30	2.27	4.53	169485
	BDQ 0.25	5.15	2.32	4.43	192156
	BDQ 0.5	5.03	2.14	2.44	2592
	BDQ 1	5.16	2.02	2.52	2289
	BDQ 4	5.02	2.03	2.30	1915
	IPM 4 + BDQ 0.00390625	4.78	3.52	3.65	73657
	IPM 4 + BDQ 0.0078125	5.24	3.27	3.49	17571
	IPM 4 + BDQ 0.015625	5.49	2.95	3.27	6024
	IPM 4 + BDQ 0.03125	5.20	2.71	3.18	9487
	IPM 4 + BDQ 0.0625	5.20	2.55	2.99	6118
	IPM 4 + BDQ 0.125	5.36	2.29	2.75	2417
	IPM 4 + BDQ 0.25	5.20	2.22	2.64	2699
	IPM 4 + BDQ 0.5	4.90	2.23	2.54	4303
	IPM 4 + BDQ 1	4.78	2.16	2.58	6301
	IPM 4 + BDQ 4	5.00	2.30	2.58	3773
	IPM 16 + BDQ 0.00390625	4.60	3.14	3.25	44835
	IPM 16 + BDQ 0.0078125	4.85	2.93	3.12	19020
	IPM 16 + BDQ 0.015625	4.76	2.87	3.04	19084
	IPM 16 + BDQ 0.03125	5.23	2.75	3.01	6047
	IPM 16 + BDQ 0.0625	5.27	2.73	2.82	3577
	IPM 16 + BDQ 0.125	5.13	2.38	2.70	3709
	IPM 16 + BDQ 0.25	5.03	2.29	2.55	3275
	IPM 16 + BDQ 0.5	4.79	2.17	2.50	5090
	IPM 16 + BDQ 1	5.26	2.15	2.47	1616
	IPM 16 + BDQ 4	5.01	2.28	2.29	1898

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification. nd indicates not determined (due to lack of CFU count data).

Table S9. CFU and relative light unit (RLU) data from samples presented in Fig. S4. Assay medium was CAMHB without Tween 80. Red shading indicates bedaquiline (BDQ) samples, blue shading indicates imipenem (IPM) samples, and gray shading indicates IPM + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:		Log_{10} RLU/mL at the following time points:			MegaRLU/CFU at the following time points:	
		Day 1	Day 3	Hour 4	Day 1	Day 3	Day 1	Day 3
ATCC 19977 WT, CAMHB (no Tween), Day 0 = 5.95 log_{10} CFU/mL, **Fig. S4** (replicate 2)	No drug	7.01	Clump	1.87	2.90	4.04	77	nd
	IPM 4	5.68	5.29	2.72	3.78	4.11	12490	65650
	IPM 16	5.02	4.68	3.09	3.65	3.66	43291	94145
	BDQ 0.00390625	6.66	Clump	2.38	3.17	3.74	323	nd
	BDQ 0.0078125	6.35	Clump	2.41	3.00	3.93	443	nd
	BDQ 0.015625	5.98	6.41	2.17	2.51	4.05	341	4341
	BDQ 0.03125	5.81	5.78	1.28	2.19	3.38	243	4007
	BDQ 0.0625	5.76	5.76	1.50	2.24	2.42	296	449
	BDQ 0.125	5.70	5.72	1.56	2.06	2.38	231	459
	BDQ 0.25	5.70	5.60	1.95	1.89	2.30	154	502
	BDQ 0.5	5.66	5.34	not detected	1.91	2.24	176	789
	BDQ 1	5.75	5.29	not detected	1.87	2.08	131	613
	BDQ 4	5.58	5.32	not detected	1.64	1.66	114	217
	IPM 4 + BDQ 0.00390625	5.70	5.13	2.53	3.47	3.62	5864	30496
	IPM 4 + BDQ 0.0078125	5.48	5.62	2.69	3.41	3.43	8651	6400
	IPM 4 + BDQ 0.015625	5.76	5.48	2.10	3.01	3.16	1781	4850
	IPM 4 + BDQ 0.03125	5.92	5.68	1.64	2.59	2.94	464	1813
	IPM 4 + BDQ 0.0625	5.66	5.78	2.14	2.32	2.61	455	682
	IPM 4 + BDQ 0.125	5.70	5.60	2.10	2.20	2.44	321	684
	IPM 4 + BDQ 0.25	5.75	5.28	2.21	2.04	2.68	194	2479
	IPM 4 + BDQ 0.5	5.70	5.32	1.73	2.14	2.10	276	606
	IPM 4 + BDQ 1	5.87	5.30	1.70	1.85	2.04	96	545
	IPM 4 + BDQ 4	5.48	5.24	1.30	1.72	1.87	173	427
	IPM 16 + BDQ 0.00390625	5.68	5.53	2.87	3.27	3.30	3859	5841
	IPM 16 + BDQ 0.0078125	5.75	5.51	2.85	3.20	3.14	2839	4351
	IPM 16 + BDQ 0.015625	5.75	5.51	2.54	2.95	2.99	1604	3050
	IPM 16 + BDQ 0.03125	5.81	5.66	1.78	2.73	2.86	833	1558
	IPM 16 + BDQ 0.0625	5.78	5.68	2.05	2.29	2.69	322	1011
	IPM 16 + BDQ 0.125	5.64	5.73	1.81	2.02	2.47	240	549
	IPM 16 + BDQ 0.25	5.78	5.56	2.13	2.10	2.36	212	642
	IPM 16 + BDQ 0.5	5.68	5.64	1.73	2.10	2.18	260	347
	IPM 16 + BDQ 1	5.82	5.14	1.61	1.98	2.15	145	1020
	IPM 16 + BDQ 4	5.64	5.09	1.68	1.66	2.02	103	838

"Clump" indicates that the bacteria had overgrown and formed clumps, precluding CFU quantification. nd indicates not determined (due to lack of CFU count data).

Table S10. CFU and relative fluorescence unit (RLU) data from samples presented in Fig. 7, Fig. S6, Fig. S7, and Fig. S8. Bacteria were nutrient-starved in PBS for 14 days (NS-14) prior to drug exposure. Drug activity assays were performed in PBS without Tween 80. Red shading indicates bedaquiline (BDQ) samples, blue shading indicates imipenem (IPM) samples, and gray shading indicates IPM + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:		Log_{10} RLU/mL at the following time points:				MegaRLU/CFU at the following time points:	
		Day 3	Day 7	Hour 4	Day 1	Day 3	Day 7	Day 3	Day 7
ATCC 19977 WT, NS-14 (no Tween), Day 0 = 6.25 log_{10} CFU/mL, **Fig. 7A,C; Fig. S6A,C; Fig. S7**	No drug	6.26	6.20	2.44	2.39	2.40	2.58	135	241
	IPM 4	6.12	6.68	2.45	2.47	2.60	2.91	302	170
	IPM 16	6.09	5.95	2.49	2.52	2.65	2.92	357	918
	BDQ 0.00390625	6.23	6.07	2.52	2.44	2.47	2.62	178	355
	BDQ 0.0078125	6.13	6.20	2.45	2.41	2.45	2.63	208	264
	BDQ 0.015625	6.18	6.23	2.48	2.46	2.43	2.62	178	250
	BDQ 0.03125	6.17	6.18	2.37	2.25	2.31	2.48	138	200
	BDQ 0.0625	6.09	6.18	2.34	2.24	2.25	2.46	145	188
	BDQ 0.125	5.90	6.53	2.32	2.18	2.23	2.42	213	78
	BDQ 0.25	5.53	5.36	2.49	2.18	2.20	2.34	471	953
	BDQ 0.5	5.13	4.48	2.34	2.18	2.21	2.31	1182	6752
	BDQ 1	4.16	3.89	2.34	2.20	2.21	2.33	11142	27340
	BDQ 4	2.21	0.95	2.29	2.17	2.18	2.30	938750	25137500
	IPM 4 + BDQ 0.00390625	5.93	6.48	2.44	2.50	2.63	2.84	499	229
	IPM 4 + BDQ 0.0078125	6.28	6.56	2.42	2.47	2.61	2.86	213	203
	IPM 4 + BDQ 0.015625	5.83	6.48	2.38	2.43	2.58	2.88	565	255
	IPM 4 + BDQ 0.03125	6.07	6.38	2.31	2.26	2.33	2.87	183	307
	IPM 4 + BDQ 0.0625	6.00	6.51	2.34	2.25	2.36	2.77	230	186
	IPM 4 + BDQ 0.125	5.93	6.17	2.27	2.21	2.25	2.60	206	270
	IPM 4 + BDQ 0.25	5.64	5.22	2.28	2.18	2.23	2.46	382	1730
	IPM 4 + BDQ 0.5	5.06	4.37	2.33	2.18	2.23	2.34	1450	9322
	IPM 4 + BDQ 1	3.03	4.27	2.35	2.20	2.20	2.22	147685	8750
	IPM 4 + BDQ 4	2.15	2.38	2.32	2.18	2.21	2.21	1158214	675208
	IPM 16 + BDQ 0.00390625	6.24	5.89	2.41	2.50	2.58	2.79	223	782
	IPM 16 + BDQ 0.0078125	6.33	5.76	2.52	2.48	2.54	2.78	163	1051
	IPM 16 + BDQ 0.015625	6.18	5.75	2.33	2.38	2.45	2.66	186	808
	IPM 16 + BDQ 0.03125	5.91	5.93	2.47	2.29	2.41	2.64	311	503
	IPM 16 + BDQ 0.0625	5.91	5.28	2.33	2.22	2.27	2.43	228	1412
	IPM 16 + BDQ 0.125	5.68	5.07	2.27	2.19	2.21	2.39	336	2059
	IPM 16 + BDQ 0.25	5.41	4.86	2.31	2.18	2.25	2.38	679	3347
	IPM 16 + BDQ 0.5	4.90	4.48	2.32	2.18	2.22	2.33	2096	7125
	IPM 16 + BDQ 1	3.82	3.70	2.33	2.19	2.17	2.33	22515	42660
	IPM 16 + BDQ 4	1.79	0.95	2.36	2.15	2.19	2.32	2594167	25987500

Table continued on next page.

Table S10, continued.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:		Log_{10} RLU/mL at the following time points:				MegaRLU/CFU at the following time points:	
		Day 3	Day 7	Hour 4	Day 1	Day 3	Day 7	Day 3	Day 7
OM7 mutant, NS-14 (no Tween), Day 0 = 6.29 log_{10} CFU/mL, **Fig. 7B,D; Fig. S6B,D; Fig. S8**	No drug	6.42	6.82	2.30	2.34	2.31	2.52	78	50
	IPM 4	6.43	6.58	2.36	2.47	2.61	2.91	150	215
	IPM 16	6.26	6.12	2.41	2.46	2.51	2.85	174	536
	BDQ 0.00390625	6.31	6.32	2.40	2.45	2.36	2.53	113	162
	BDQ 0.0078125	6.23	6.27	2.35	2.29	2.29	2.48	115	159
	BDQ 0.015625	6.30	5.91	2.48	2.41	2.41	2.58	128	468
	BDQ 0.03125	6.26	6.19	2.35	2.35	2.32	2.52	113	213
	BDQ 0.0625	6.20	6.26	2.30	2.22	2.25	2.43	112	148
	BDQ 0.125	6.15	6.21	2.28	2.14	2.18	2.42	107	161
	BDQ 0.25	6.11	6.03	2.22	2.10	2.09	2.31	95	192
	BDQ 0.5	6.00	6.02	2.21	2.13	2.14	2.35	138	215
	BDQ 1	5.83	5.51	2.22	2.12	2.09	2.24	182	544
	BDQ 4	4.51	3.43	2.23	2.12	2.09	2.19	3838	57854
	IPM 4 + BDQ 0.00390625	6.25	6.25	2.39	2.50	2.56	2.84	206	390
	IPM 4 + BDQ 0.0078125	6.25	6.08	2.37	2.43	2.49	2.87	175	612
	IPM 4 + BDQ 0.015625	6.26	6.56	2.39	2.50	2.53	2.88	188	212
	IPM 4 + BDQ 0.03125	6.25	5.94	2.29	2.42	2.45	2.87	161	838
	IPM 4 + BDQ 0.0625	6.26	6.19	2.29	2.34	2.37	2.77	131	386
	IPM 4 + BDQ 0.125	6.11	6.30	2.25	2.21	2.26	2.60	143	200
	IPM 4 + BDQ 0.25	5.89	6.47	2.21	2.19	2.19	2.46	200	98
	IPM 4 + BDQ 0.5	6.06	6.20	2.20	2.14	2.09	2.34	109	138
	IPM 4 + BDQ 1	5.53	5.26	2.24	2.10	2.09	2.22	359	894
	IPM 4 + BDQ 4	4.86	3.68	2.25	2.11	2.07	2.21	1644	33760
	IPM 16 + BDQ 0.00390625	6.33	6.05	2.38	2.48	2.49	2.88	143	673
	IPM 16 + BDQ 0.0078125	6.12	5.87	2.34	2.44	2.46	2.84	219	936
	IPM 16 + BDQ 0.015625	6.11	5.97	2.39	2.43	2.45	2.76	220	606
	IPM 16 + BDQ 0.03125	6.20	5.79	2.37	2.33	2.38	2.69	153	799
	IPM 16 + BDQ 0.0625	6.09	5.68	2.31	2.26	2.32	2.63	170	880
	IPM 16 + BDQ 0.125	5.81	5.60	2.23	2.19	2.26	2.44	281	693
	IPM 16 + BDQ 0.25	5.86	5.45	2.26	2.21	2.17	2.38	205	851
	IPM 16 + BDQ 0.5	5.87	5.16	2.26	2.15	2.18	2.31	202	1401
	IPM 16 + BDQ 1	5.58	4.66	2.21	2.10	2.09	2.23	327	3682
	IPM 16 + BDQ 4	4.89	3.79	2.25	2.19	2.13	2.22	1748	26782

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Table S11. CFU data from samples presented in Fig. 8 and Fig. S9. Bacteria were nutrient-starved in PBS for 20 days (NS-20) prior to drug exposure. Drug activity assays were performed in PBS with 0.05% Tween 80. Red shading indicates bedaquiline (BDQ) samples, blue shading indicates imipenem (IPM) samples, and gray shading indicates IPM + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	Drug(s) and concentration(s) in µg/mL	Log_{10} CFU/mL at the following time points:	
		Day 1	Day 4
ATCC 19977 WT, NS-20 + Tween, Day 0 = 6.07 log_{10} CFU/mL, **Fig. 8; Fig. S9A,C** (replicate 1)	No drug	5.68	5.48
	IPM 4	5.33	2.27
	IPM 8	4.26	1.36
	IPM 16	4.60	1.18
	BDQ 0.00390625	5.56	5.96
	BDQ 0.0625	5.37	5.51
	BDQ 1	5.32	5.04
	BDQ 4	5.13	4.66
	IPM 4 + BDQ 0.00390625	5.62	4.26
	IPM 4 + BDQ 0.0625	5.33	4.90
	IPM 4 + BDQ 0.25	5.35	5.23
	IPM 4 + BDQ 1	5.15	4.95
	IPM 4 + BDQ 4	5.08	4.56
ATCC 19977 WT, NS-20 + Tween, Day 0 = 6.38 log_{10} CFU/mL, **Fig. S9B,D** (replicate 2)	No drug	6.38	6.23
	IPM 4	5.97	Contam.
	IPM 8	5.60	2.08
	IPM 16	4.88	0.00
	BDQ 0.00390625	6.37	6.22
	BDQ 0.0625	5.94	6.21
	BDQ 1	6.04	5.89
	BDQ 4	6.05	5.37
	IPM 4 + BDQ 0.00390625	6.15	5.89
	IPM 4 + BDQ 0.0625	6.03	5.94
	IPM 4 + BDQ 0.25	6.11	5.56
	IPM 4 + BDQ 1	6.06	5.81
	IPM 4 + BDQ 4	6.01	5.29

"Contam." indicates that bacterial or fungal contamination precluded assessment of this sample.

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Table S12. CFU data from samples presented in Fig. S10. Bacteria were nutrient-starved in PBS for 20 days (NS-20) prior to drug exposure. Drug activity assays were performed in PBS with or without 0.05% Tween 80. Red shading indicates bedaquiline (BDQ) samples; blue shading indicates imipenem (IPM) samples; gray shading indicates IPM + BDQ samples.

Strain, condition, Day 0 bacterial burden, figure number	**Drug(s) and concentration(s) in µg/mL**	**Log_{10} CFU/mL at Day 3**
ATCC 19977 WT, NS-20 (no Tween), Day 0 = 6.48 log_{10} CFU/mL, **Fig. S10A**	No drug	6.29
	IPM 2	6.32
	IPM 4	6.47
	IPM 8	6.43
	IPM 16	6.48
	IPM 64	6.02
	BDQ 0.00390625	6.56
	BDQ 0.015625	6.07
	BDQ 0.0625	5.88
	BDQ 1	4.75
	BDQ 4	4.66
	IPM 4 + BDQ 0.00390625	6.06
	IPM 4 + BDQ 0.0625	6.11
	IPM 4 + BDQ 0.25	5.82
	IPM 4 + BDQ 1	4.89
	IPM 4 + BDQ 4	4.38
	IPM 16 + BDQ 0.00390625	6.32
	IPM 16 + BDQ 0.0625	5.62
	IPM 16 + BDQ 0.25	5.58
	IPM 16 + BDQ 1	4.86
	IPM 16 + BDQ 4	4.08
ATCC 19977 WT, NS-20 + Tween, Day 0 = 6.51 log_{10} CFU/mL, **Fig. S10B**	No drug	6.45
	IPM 4	nd
	IPM 16	nd
	BDQ 0.00390625	6.66
	BDQ 0.015625	6.48
	BDQ 0.0625	6.36
	BDQ 1	6.02
	BDQ 4	5.72
	IPM 4 + BDQ 0.00390625	6.32
	IPM 4 + BDQ 0.0625	6.23
	IPM 4 + BDQ 0.25	6.29
	IPM 4 + BDQ 1	5.98
	IPM 4 + BDQ 4	5.79
	IPM 16 + BDQ 0.00390625	4.41
	IPM 16 + BDQ 0.0625	4.76
	IPM 16 + BDQ 0.25	5.70
	IPM 16 + BDQ 1	6.24
	IPM 16 + BDQ 4	5.78

nd, not determined (samples lost).

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Table S13. CFU data from intracellular samples presented in Fig. 9 and Fig. S11. Within each biological replicate, data from up to 3 technical replicates were acquired for each group at each time point. Red shading indicates bedaquiline (BDQ) samples; blue shading indicates imipenem (IPM) samples; gray shading indicates IPM + BDQ samples.

Strain, condition, replicate, figure number	Drug(s) and concentration(s) in µg/mL	Technical replicate	Intracellular log_{10} CFU/mL at		
			Day 0	Day 3	Day 5
ATCC 19977 WT, Intracelluar (THP-1 cells) assay with MOI 1:10, Biological replicate 1, **Fig. 9A; Fig. S11A,D**	No drug	1	4.06	6.58	7.19
		2	4.03	6.62	6.97
		3	4.11	6.78	6.96
	BDQ 0.25	1	---	5.34	6.93
		2	---	5.41	6.86
		3	---	5.64	7.10
	BDQ 1	1	---	4.64	5.15
		2	---	4.34	5.38
		3	---	4.64	5.35
	BDQ 4	1	---	---	3.11
		2	---	3.53	3.61
		3	---	3.15	3.11
	BDQ 16	1	---	3.23	2.15
		2	---	2.94	1.90
		3	---	---	1.04
	IPM 8	1	---	4.79	5.00
		2	---	5.25	5.33
		3	---	5.09	5.10
	IPM 16	1	---	4.51	4.38
		2	---	4.64	4.30
		3	---	---	4.26
	IPM 32	1	---	---	4.00
		2	---	4.53	4.24
		3	---	---	4.09
	IPM 64	1	---	---	3.85
		2	---	4.18	3.82
		3	---	---	3.78
	IPM 32 + BDQ 1	1	---	3.90	---
		2	---	3.75	2.92
		3	---	3.42	---
	IPM 64 + BDQ 1	1	---	3.73	---
		2	---	3.56	---
		3	---	3.89	2.73
ATCC 19977 WT, Intracelluar (THP-1 cells) assay with MOI 1:10, Biological replicate 2, **Fig. 9B; Fig. S11B,E; plus additional combination data not in a figure**	No drug	1	3.82	4.96	6.93
		2	3.90	4.89	6.73
		3	3.76	5.03	6.76
	BDQ 0.25	1	---	4.01	---
		2	---	4.30	---
		3	---	---	---
	BDQ 0.5	1	---	3.27	4.41
		2	---	3.54	4.49
		3	---	---	4.47
	BDQ 1	1	---	2.72	3.97
		2	---	2.92	4.10
		3	---	2.90	4.28
	IPM 8	1	---	4.02	3.78
		2	---	3.98	3.89
		3	---	3.92	3.53
	IPM 32	1	---	3.51	3.26
		2	---	3.39	3.31
		3	---	3.45	3.12

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Table S13, continued

Strain, condition, replicate, figure number	Drug(s) and concentration(s) in µg/mL	Technical replicate	Intracellular log_{10} CFU/mL at		
			Day 0	Day 3	Day 5
ATCC 19977 WT, Intracelluar (THP-1 cells) assay with MOI 1:10, Biological replicate 2, **Fig. 9B; Fig. S11B,E; plus additional combination data not in a figure**	IPM 32 + BDQ 0.25	1	---	2.79	---
		2	---	3.01	---
		3	---	2.79	---
	IPM 32 + BDQ 0.5	1	---	3.40	2.75
		2	---	3.45	2.81
		3	---	3.39	2.87
	IPM 32 + BDQ 1	1	---	2.85	2.26
		2	---	2.94	2.23
		3	---	2.86	2.00
ATCC 19977 WT, Intracelluar (THP-1 cells) assay with MOI 1:10, Biological replicate 3, **Fig. 9c; Fig. S11C,F**; **plus additional combination data not in a figure**	No drug	1	4.03	5.26	5.73
		2	3.97	4.78	6.03
		3	3.83	5.26	---
	BDQ 0.25	1	---	4.90	5.56
		2	---	5.00	5.60
		3	---	4.60	5.58
	BDQ 0.5	1	---	3.76	3.60
		2	---	4.07	3.38
		3	---	4.26	3.42
	BDQ 1	1	---	3.32	2.18
		2	---	3.55	2.27
		3	---	3.20	2.39
	BDQ 4	1	---	2.78	1.28
		2	---	3.11	1.84
		3	---	2.79	1.65
	IPM 8	1	---	4.33	3.34
		2	---	4.20	3.51
		3	---	4.18	3.62
	IPM 16	1	---	2.30	1.83
		2	---	2.34	2.00
		3	---	---	2.05
	IPM 32	1	---	2.00	1.91
		2	---	1.91	1.94
		3	---	1.91	1.84
	IPM 64	1	---	2.00	0.48
		2	---	1.79	1.36
		3	---	---	1.65
	IPM 32 + BDQ 0.25	1	---	2.33	1.74
		2	---	2.54	2.52
		3	---	2.38	1.65
	IPM 64 + BDQ 0.5	1	---	2.31	3.20
		2	---	2.05	---
		3	---	2.31	3.00
	IPM 32 + BDQ 1	1	---	2.24	---
		2	---	2.40	1.61
		3	---	1.83	1.79
	IPM 64 + BDQ 4	1	---	1.83	1.46
		2	---	1.88	0.85
		3	---	1.92	1.11

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Table S13, continued

Strain, condition, replicate, figure number	Drug(s) and concentration(s) in µg/mL	Technical replicate	Intracellular log_{10} CFU/mL at		
			Day 0	Day 3	Day 5
ATCC 19977 WT, Intracelluar (THP-1 cells) assay with MOI 1:1, Biological replicate 4, **Data not presented in a figure**	No drug	1	5.04	6.79	nd
		2	4.96	6.53	nd
	BDQ 0.0625	1	---	6.62	nd
		2	---	6.56	nd
	BDQ 1	1	---	4.89	nd
		2	---	4.93	nd
	BDQ 2	1	---	4.60	nd
		2	---	4.56	nd
	IPM 8	1	---	5.06	nd
		2	---	5.30	nd
	IPM 16	1	---	5.15	nd
		2	---	5.15	nd
	IPM 32	1	---	4.96	nd
		2	---	5.10	nd
	IPM 32 + BDQ 1	1	---	4.20	nd
		2	---	4.38	nd
		3	---	4.48	nd

--- indicates not applicable (Day 0) or sample lost (Days 3 or 5). nd, not determined.