Expanded View Figures

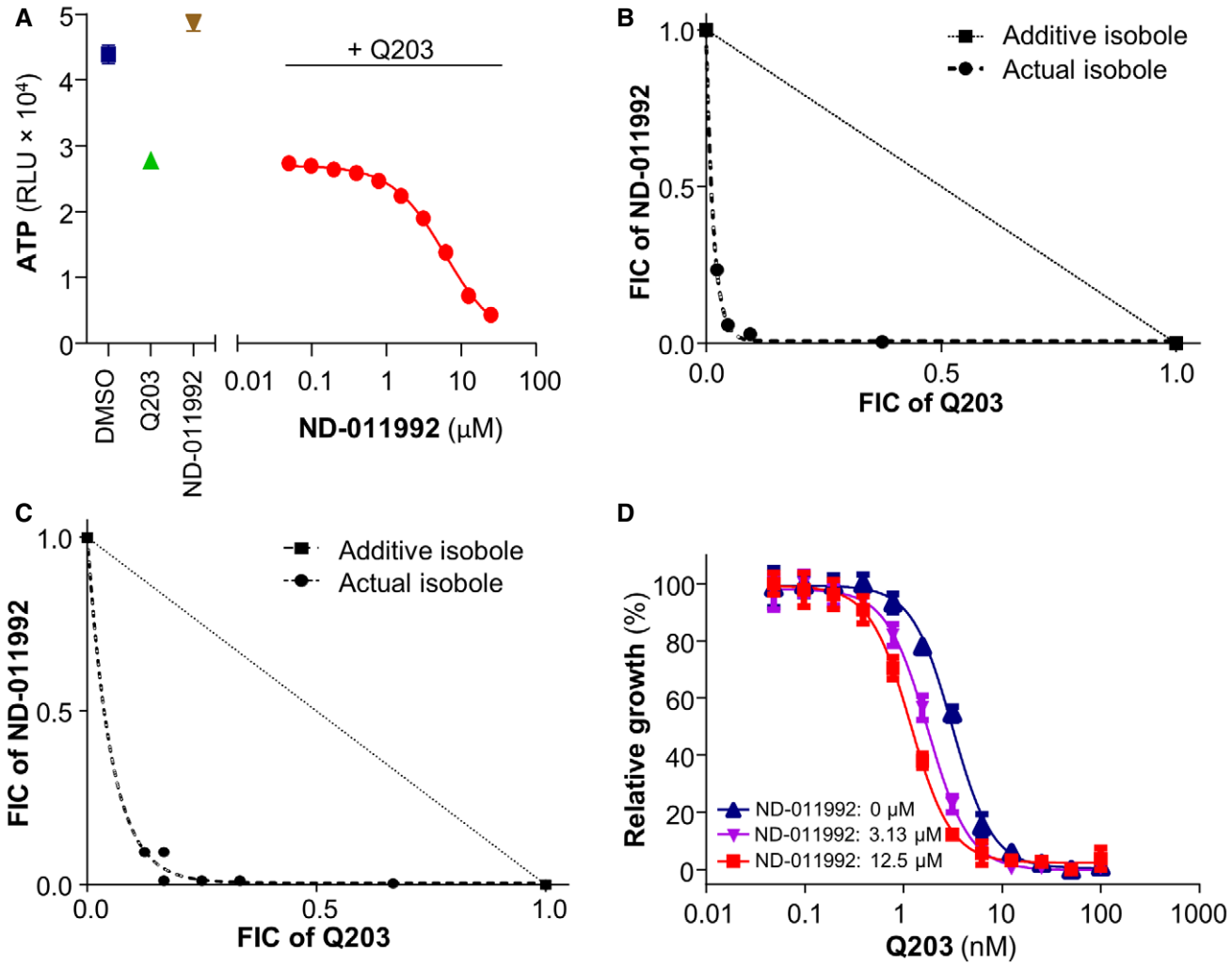

Figure EV1. ND-011992 synergizes with Q203 to inhibit ATP production and growth in mycobacteria.

A Effect of ND-011992 on the intracellular ATP level in *M. tuberculosis* H37Rv. The bacteria were treated with DMSO (blue square), 100 nM Q203 (green triangle), 25 μM ND-011992 (brown triangle), and varying concentrations of ND-011992 in the presence of 100 nM Q203 (red circles) for 15 h.

B Isobologram analysis reflecting the interactions between ND-011992 and Q203 in an intracellular ATP depletion checkerboard assay in *M. bovis* BCG.

C Isobologram analysis reflecting the interactions between ND-011992 and Q203 in an intracellular ATP depletion checkerboard assay in *M. tuberculosis* H37Rv.

D Effect of ND-011992 on the dose-dependent growth inhibition curve of Q203 in *M. tuberculosis* H37Rv. Blue triangles: Q203 alone; purple triangles: with 3.13 μM ND-011992; red squares: with 12.5 μM ND-011992. Data are expressed as the mean ± SD of triplicates for each condition.

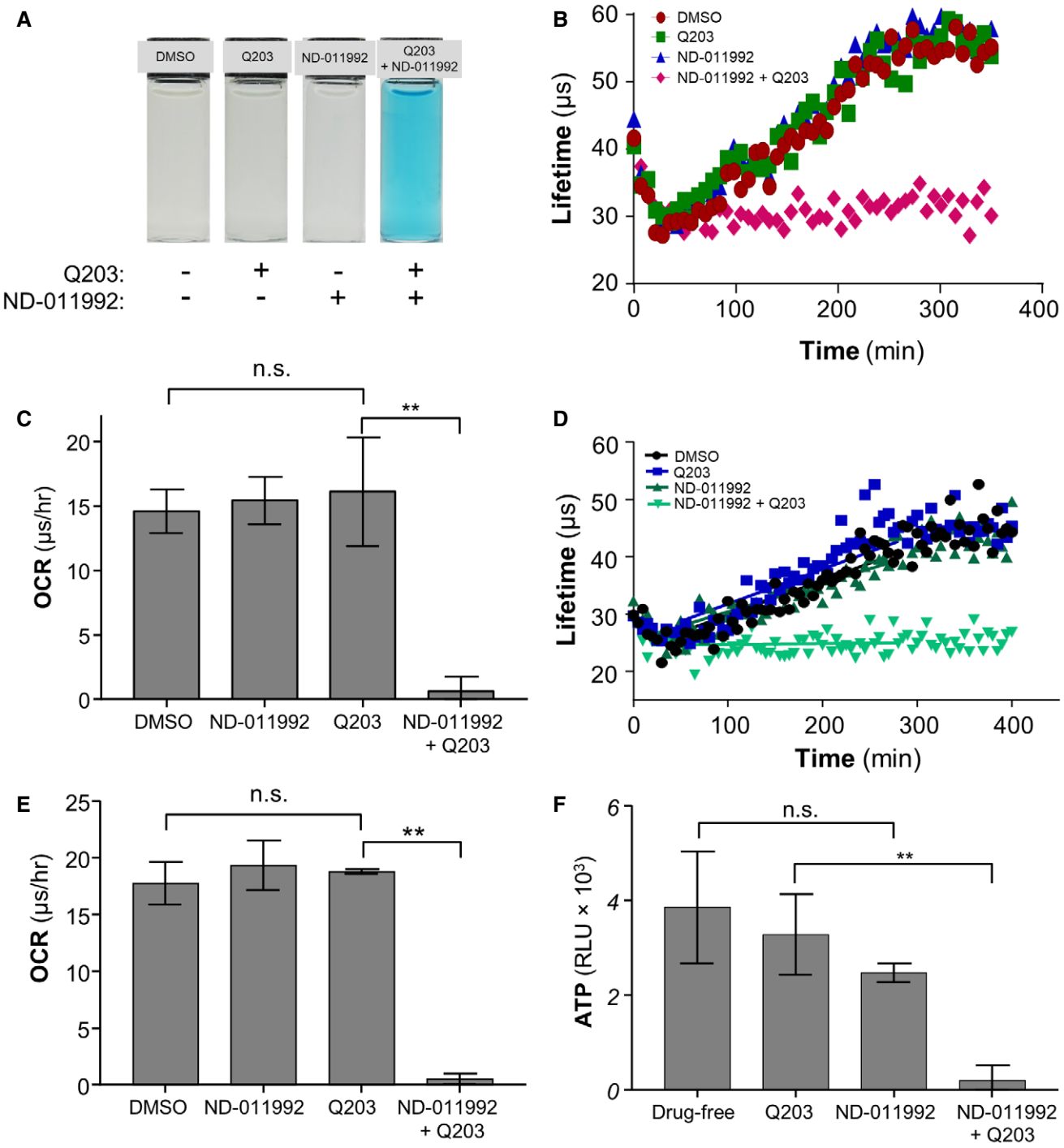

Figure EV2.

Figure EV2. ND-011992-Q203 combination inhibits oxygen respiration in mycobacteria.

A Oxygen consumption assay in *M. tuberculosis* H37Rv using methylene blue as an oxygen sensor.

B Oxygen consumption assay in *M. tuberculosis* H37Rv using the MitoXpress Xtra® probe. H37Rv was incubated with the oxygen probe in the presence of 1% DMSO (red circles), 100 nM Q203 (green squares), 25 μM ND-011992 (blue triangles), or 25 μM ND-011992 + 100 nM Q203 (pink diamonds).

C Oxygen consumption rate of *M. tuberculosis* H37Rv derived from the MitoXpress Xtra® assay (panel B). $^{**}P = 0.0036$, unpaired Student's t-test; two-tailed; $n = 3$.

D Oxygen consumption assay in *M. bovis* BCG using the MitoXpress Xtra® probe. *M. bovis* BCG was incubated with the oxygen probe in the presence of 1% DMSO (black circles), 100 nM Q203 (blue squares), 25 μM ND-011992 (dark green triangles), or 25 μM ND-011992 + 100 nM Q203 (light green triangles).

E Oxygen consumption rate of *M. tuberculosis* H37Rv derived from the MitoXpress Xtra® assay (panel D). $^{**}P = 0.00000042$, unpaired Student's t-test; two-tailed; $n = 3$.

F Effect of ND-011992 on the ATP synthesis of *M. tuberculosis* H37Rv $mc^{2}6230$ inverted-membrane vesicles (IMV) using the Roche Bioluminescence Assay Kit CL II. Q203 was used at 100 nM, and ND-011992 was used at 40 μM. The figure shows averages and standard deviations of triplicate repeats with the blank subtracted from all conditions. *P*-value between drug-free and ND-011992: 0.1159; $^{**}P = 0.0020$, unpaired Student's *t*-test; two-tailed; $n = 3$.

Data information: Data are expressed as the mean ± SD of triplicates for each condition.

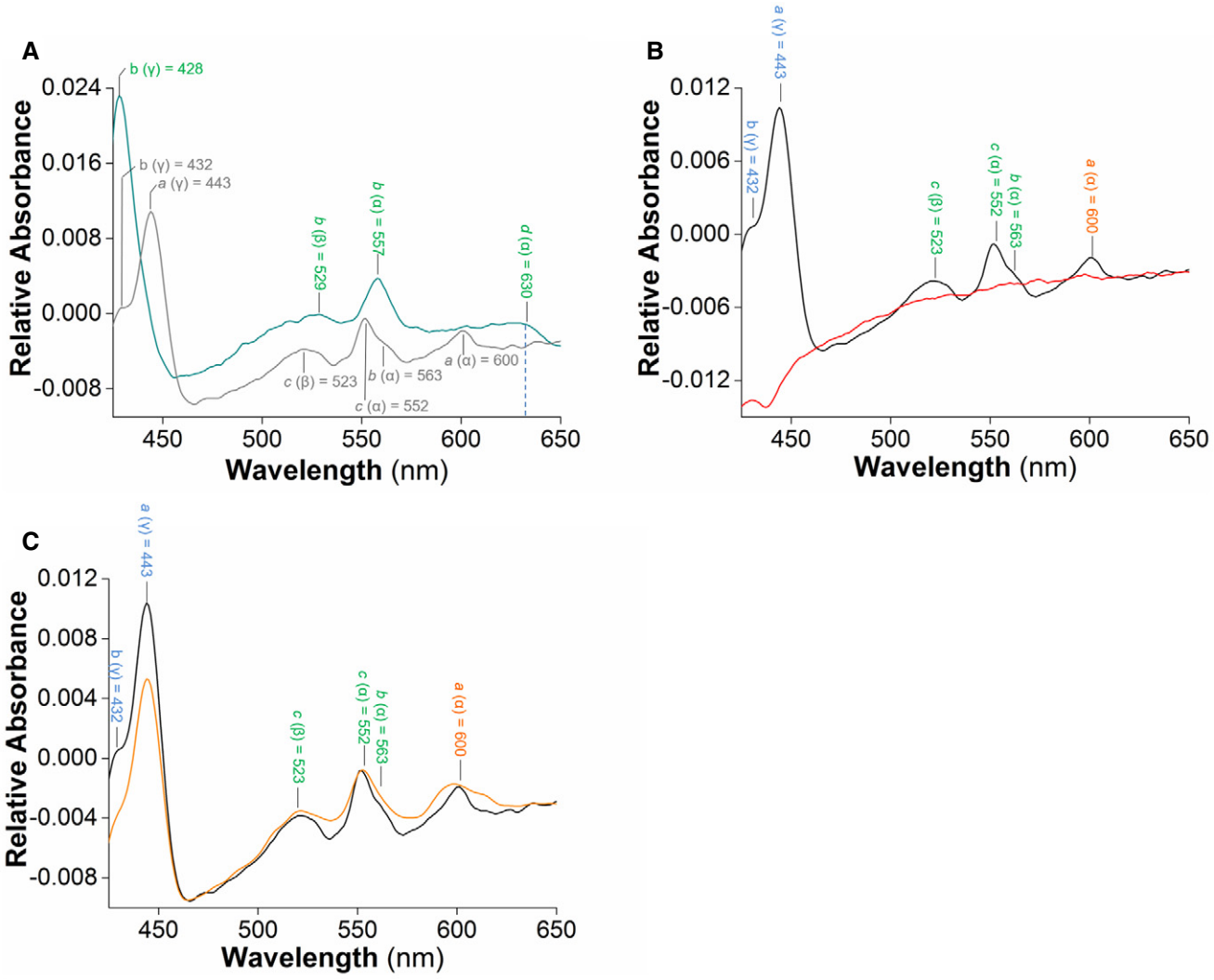

Figure EV3. ND-011992 does not affect electron transfer within the cytochrome *bcc:aa₃* supercomplex.

A NADH-driven electron transfer within plasma membrane vesicles (IMVs) of the wild-type (gray) and Δ*qcrCAB M. smegmatis (green)*. The difference spectrum of IMVs from WT *M. smegmatis* after addition of the electron donor NADH shows the major absorbance peaks for heme *a*, *b*, and *c* of the cytochrome *bcc-aa₃* supercomplex. The characteristic peaks of Cyt-*bd* are displayed after reduction by 2 mM NADH in the Δ*qcrCAB* background.

B, C Effect of Q203 and ND-011992 on NADH-driven electron transfer in wild-type *M. smegmatis* IMVs. (B) The NADH-based difference spectra in the absence (black) and presence of 7.5 μM Q203 (red) reflect the effect of Q203 on the reduction of hemes in the cytochrome *bcc-aa₃*. (C) The NADH-based difference spectra in the absence (black) and the presence of 10 μM ND-011992 (orange).

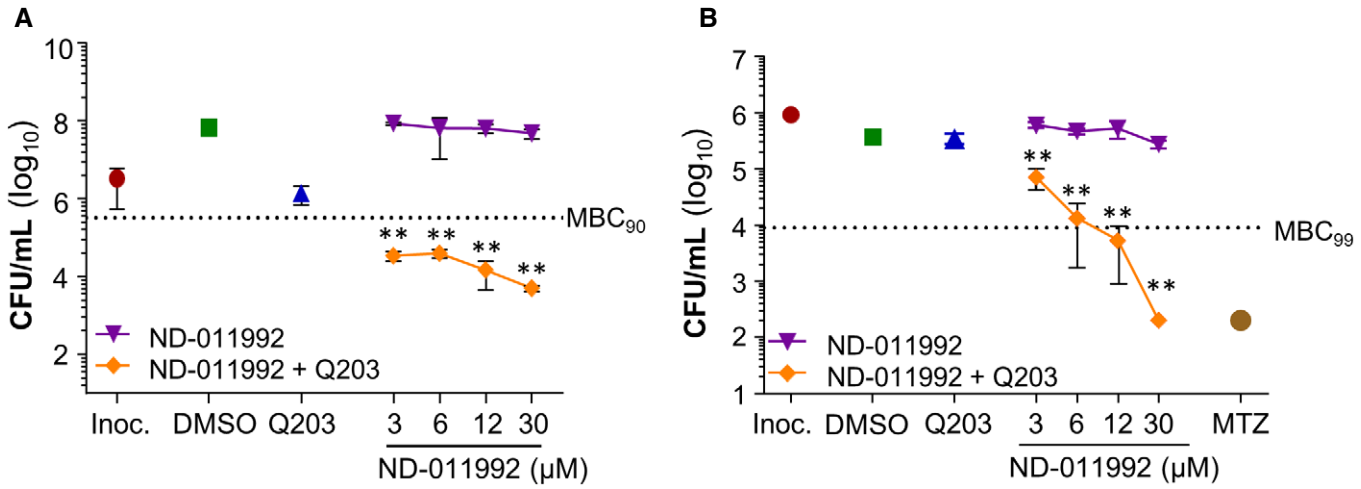

Figure EV4. The drug combination ND-011992-Q203 is bactericidal against *Mycobacterium bovis* BCG.

A Bactericidal activity of ND-011992 and ND-011992-Q203 combination in replicating *M. bovis* BCG. Q203 was used at 100 nM, and ND-011992 used at 3, 6, 12, and 30 μM alone or in combination with Q203. Dotted line represents 90% reduction in CFU/ml compared with the initial inoculum at time 0. Exact *P*-values (from left to right): 0.0002, 0.0002, 0.0003, 0.00002.

B Bactericidal activity of ND-011992 and ND-011992-Q203 combination in *M. bovis* BCG under oxygen-starved condition. Metronidazole (MTZ) was used at 200 μM, Q203 was used at 100 nM, and ND-011992 was used at 3, 6, 12, and 30 μM. Bacterial viability was determined via CFU enumeration after 10 days of treatment. Dotted line represents 99% reduction in CFU/mL compared with the initial inoculum at time 0. Exact *P*-values (from left to right): 0.0024, 0.0075, 0.0029, 0.0000003.

Data information: $^{**}P < 0.01$, unpaired Student's t-test, one-tailed; $n = 3$; comparing Q203 alone *vs* Q203 + ND-011992. Data are expressed as the mean ± SD of triplicates for each condition.