Title:

Imidazoles induce reactive oxygen species in *Mycobacterium tuberculosis* which is not associated with cell death.

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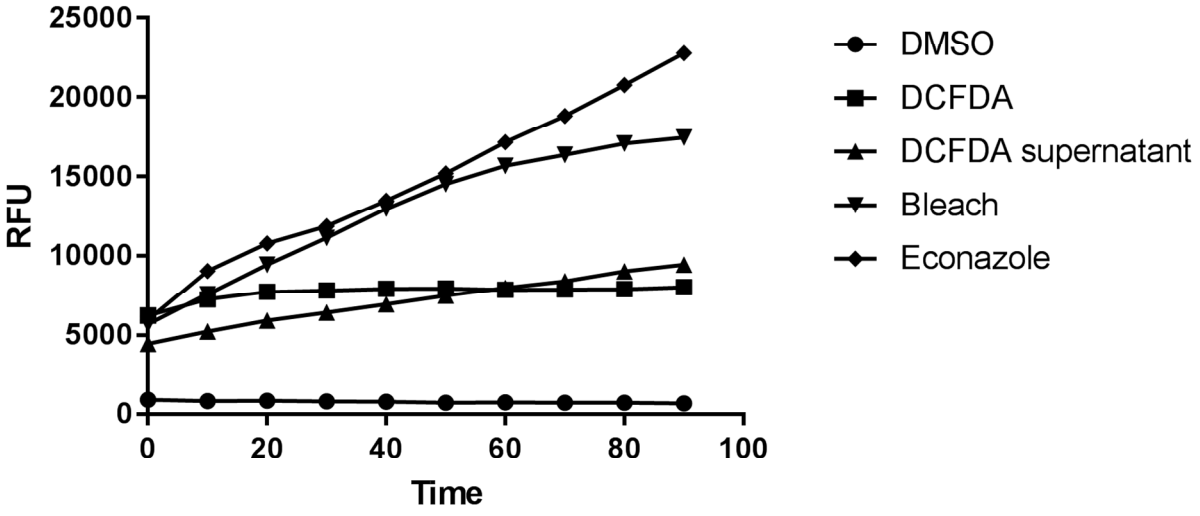

Figure S1. Validation of DCFDA fluorescent probe for detection of ROS. *M. tuberculosis* was loaded with DCFDA and incubated with 100 µM bleach or econazole for 90 minutes and fluorescence measured. DMF was used as a negative control. Fluorescence of the supernatant of cells loaded with DCFDA was also measured to evaluate efflux of the probe from the cells.

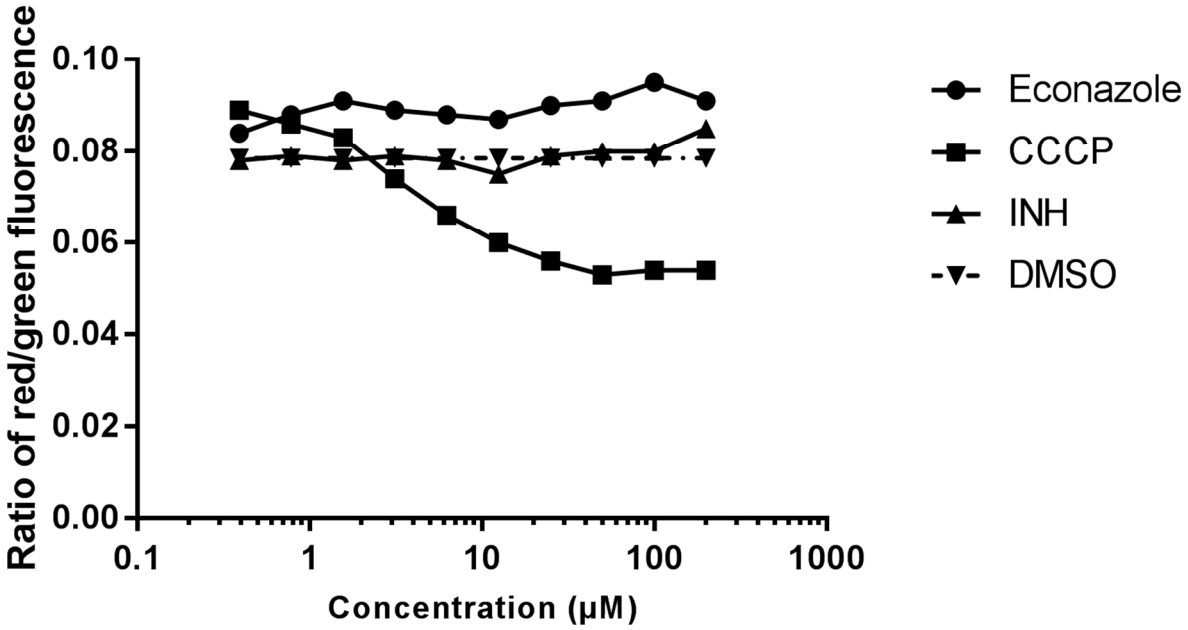

Figure S2. Membrane potential of *M. tuberculosis* is unaffected by exposure to econazole. A mid-log culture of M. tuberculosis was adjusted to an OD_{590} = 0.5, harvested by centrifugation, washed with 7H9-Tw and resuspended in 7H9-Tw containing 15 µM 3',3'-diethyloxacarbocyanine iodide ($DiOC_2$). Bacteria were incubated at room temperature for 20 min, washed, and dispensed into black-walled clear bottom 96-well plates. Bacteria were exposed to econazole for 30 min and fluorescence measured at Ex485/Em530 and Ex485/Em610530. The ratio of red (Em610) to green (Em530) fluorescence was calculated. Mtb cells fluoresce green, but fluorescence shifts toward red emission as the dye molecules self-associate due to higher cytosolic concentrations caused by larger membrane potentials. CCCP was used as a positive control for disruption of membrane potential.

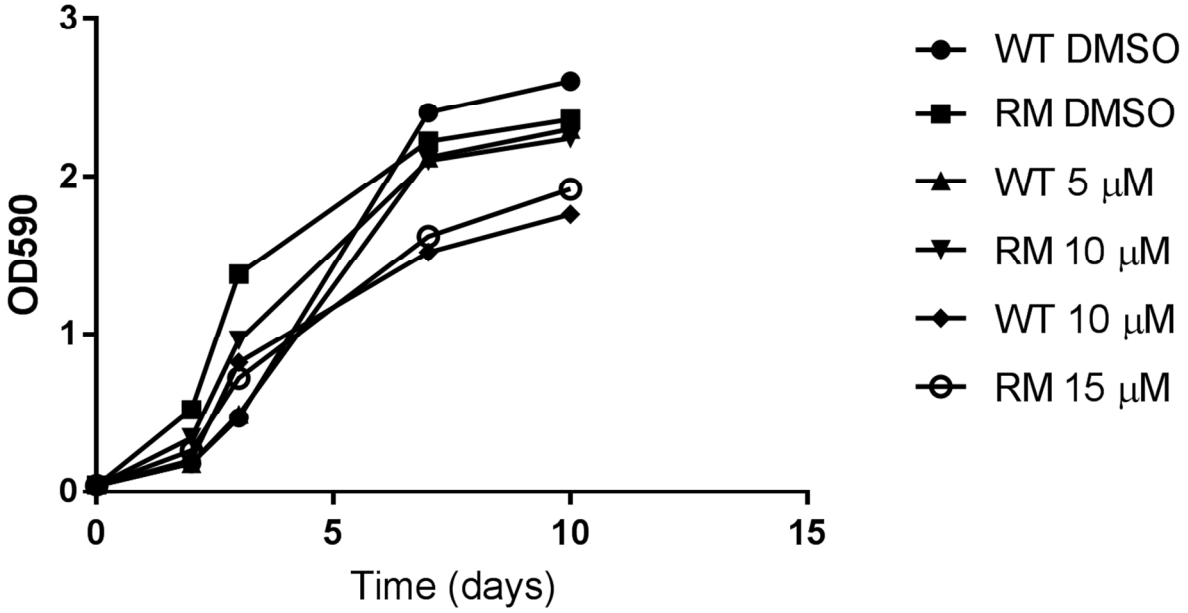

Figure S3. Growth curves in presence of econazole in aerobic roller culture. *M. tuberculosis* H37Rv wild-type and econazole-resistant mutant strains were grown in roller bottles for 10 days with the indicated concentrations of econazole. Concentrations were chosen for metabolomics analysis that resulted in a similar effect on growth of the cells over time (5 μM for WT vs. 10 μM for RM and 10 μM for WT vs. 15 μM RM).