Supporting Information to:

Metabolomics reveals D-Alanine:D-Alanine ligase as the target of D-cycloserine in Mycobacterium tuberculosis

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Materials and Methods

MICs

M. tuberculosis H37Rv was cultured at 37 °C in a biosafety level three laboratory in Middlebrook 7H9 media supplemented with 0.2 % glycerol, 0.2 % glucose, 0.04 % tyloxapol, 0.05 % bovine serum albumin and 0.085 % NaCl (w/v). 20 µl of an OD_{600} 0.4-0.6 culture was inoculated into individual wells of a 24 well plate containing chemically equivalent 7H10 media supplemented with specified concentrations of drug and/or amino acid. Plates were incubated at 37 °C for 7 days prior to qualitative analysis. Wells containing visible bacterial growth were scored as positive (green cells in Figure 1). MICs (in absence of alanine supplement) were as follows: DCS, 20 $\mu g.ml^{-1}$; LCS, 5 $\mu g.ml^{-1}$, BCDA, 20 $\mu g.ml^{-1}$; Streptomycin, 0.8 $\mu g.ml^{-1}$.

***M. tuberculosis* culture and metabolite extraction**

M. tuberculosis H37Rv was cultured in 7H9 media as described above. 25 mm 0.22 µm nitrocellulose filters were inoculated with 1 ml of a culture at OD_{600} 1.0 under vacuum pressure and transferred to chemically equivalent 7H10 solid media. Following 5 days incubation at 37 °C filters were transferred to fresh chemically equivalent 7H10 media with or without supplements under investigation. Cellular metabolism was quenched and polar metabolites extracted at specific time points by immersion of biomass (separated from filter using a cell scraper) in pre-cooled (-40 °C) extraction buffer (40 % methanol, 40 % acetonitrile, 20 % ddH_2O) on dry ice. 1 ml of extraction buffer was used per filter. Biomass-extraction buffer mixtures were transferred to 2 ml screw cap vials containing 300-400 µl acid-washed 150 µm glass beads and ribolysed for 30 seconds at speed setting 6.5. Extracts were centrifuged for 10 min at 2 °C and the supernatant spin-filtered through a 0.22 µm cellulose acetate filter. Metabolite extracts were stored at -20 °C. LC-MS analysis of metabolite extracts was performed as previously described.[1]

Results

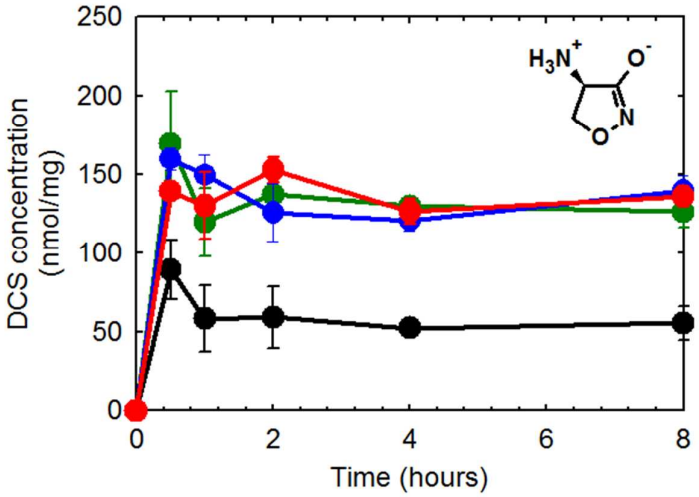

Figure S1. Changes in intracellular pool sizes of DCS over 8 h following transfer of H37Rv-laden filters to 7H10 media containing 80 µg.ml^{-1} DCS (2× MIC) and 0 (black), 50 (green), 100 (blue) or 500 (red) µM D-Ala. *Y*-axis values are relative concentrations of target compounds normalized to the residual protein content of the respective sample (as a surrogate of cellular biomass). Data are the average ± 1 standard deviation of duplicate samples.

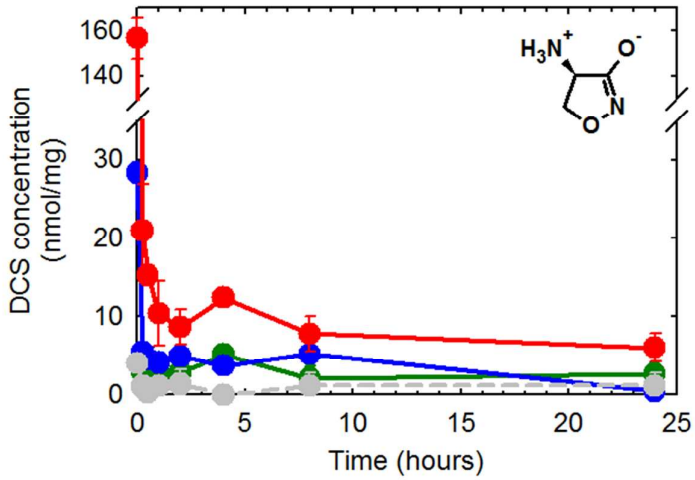

Figure S2. Changes in intracellular pool sizes of DCS over 24 h following transfer of H37Rv-laden filters to 7H10 media lacking DCS after 16 h DCS challenge at 0.25× (green), 1× (blue) or 5× (red) MIC, or 1× MIC of LCS (dashed grey). *Y*-axis values are relative concentrations of target compounds normalized to the residual protein content of the respective sample (as a surrogate of cellular biomass). Data are the average ± 1 standard deviation of duplicate samples.

References

(1) Larrouy-Maumus, G.; Biswas, T.; Hunt, D. M.; Kelly, G.; Tsodikov, O. V.; de Carvalho, L. P. *Proc. Natl. Acad. Sci. U S A* **2013**, *110*, 11320.