

Large-Scale Chemical-Genetic Strategy Enables the Design of Antimicrobial Combination Chemotherapy in Mycobacteria

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Supporting information

Supplemental Figures

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Supplemental Figures

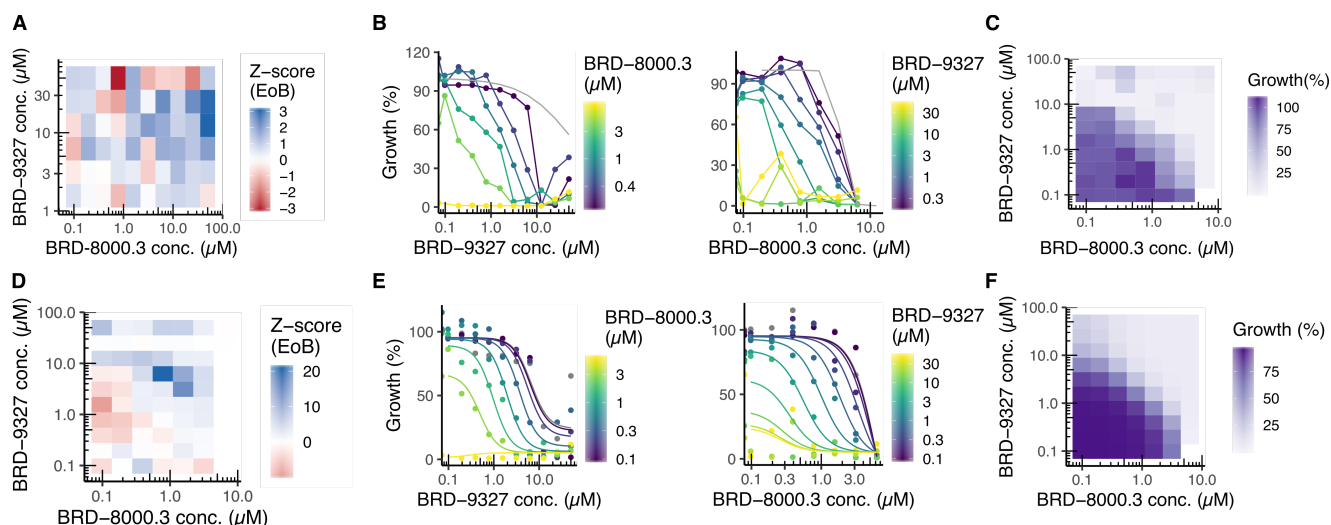


Figure S1. Synergy between EfpA inhibitors BRD-8000.3 and BRD-9327.

(A) Excess-over-Bliss (EoB) of initial EtBr efflux rate inhibition in Msm at varying combined concentrations of BRD-9327 and BRD-8000.3, demonstrating synergy between the two EfpA inhibitors.

(B) Growth inhibition from checkerboard broth microdilution assay of Mmar at varying combined concentrations of BRD-8000.3 and BRD-9327. Filled circles indicate the mean ($n = 4$).

(C) The data in (B) represented as a heatmap.

(D) Excess-over-Bliss (EoB) calculated for the data in (B), demonstrating synergy between the two EfpA inhibitors. The best fit parameter (\pm standard deviation) $\alpha = 30 \pm 10$ indicates synergy in potency while $\beta = -0.5 \pm 0.1$ indicates modest synergy in efficacy.

(E) Global best fit MuSyC model for the data in (B). Filled circles indicate the mean ($n = 4$).

(F) The best fit MuSyC surface for the data in (B) and (C).

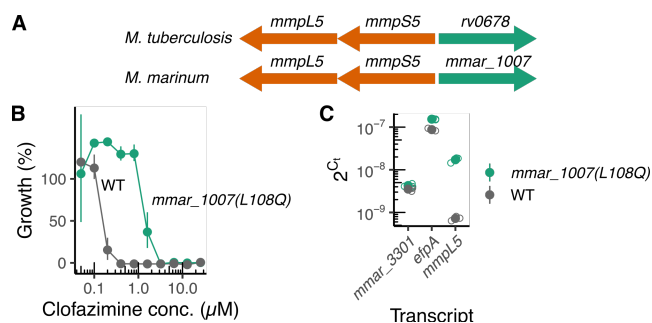


Figure S2. Low-level cross resistance between BRD-9327 and BRD-8000 is mediated by mutation in the efflux pump MmpL5 regulator *mmr_1007*.
 (A) Chromosomal organization of the MmpL5 pump regulator Rv0679 (Mtb) and MMAR_1007 (Mmar), demonstrating synteny.
 (B) Broth microdilution assay of wild-type Mmar and Mmar *mmr_1007*(L108Q) against clofazimine, demonstrating resistance.
 (C) Results of a qRT-PCR assay of *mmpL5* and *efpA* transcripts in an *mmr_1007* mutant compared to wild-type Mmar, demonstrating 19-fold overexpression of the multidrug efflux pump MmpL5 in *mmr_1007* mutants.

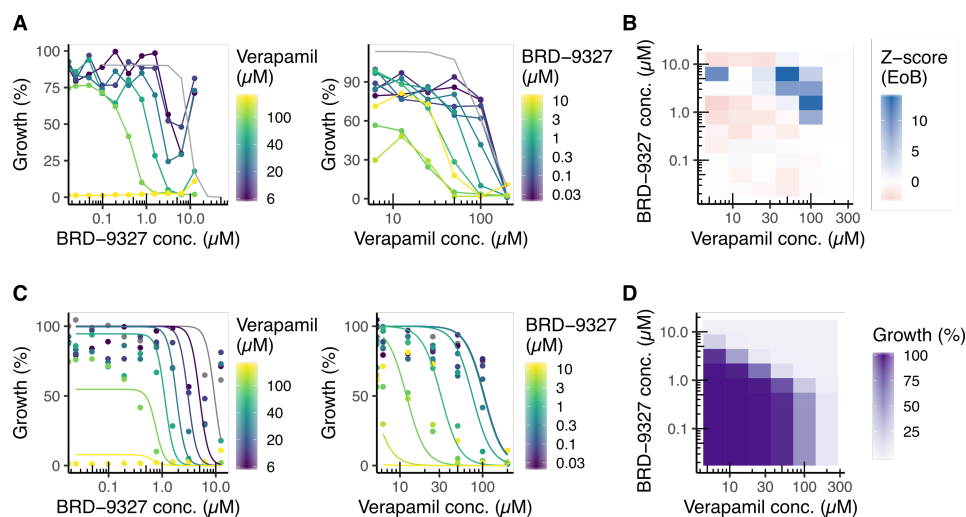


Figure S3. Synergy between BRD-9327 and verapamil.

(A) Growth inhibition from checkerboard broth microdilution assay of Mmar at varying combined concentrations of Verapamil and BRD-9327. Filled circles indicate the mean ($n = 4$). (B) Excess-over-Bliss (EoB) calculated for the data in (A), demonstrating synergy between the two EfpA inhibitors. The best fit parameter (\pm standard deviation) $\alpha = 5 \pm 1$ indicates synergy in potency while $\beta = -0.01 \pm 0.01$ indicates no interaction in efficacy. (C) Global best fit MuSyC model for the data in (A). Filled circles indicate the mean ($n = 4$). (D) The best fit MuSyC surface for the data in (A).