Supplemental Material for:

IreB, A Ser/Thr kinase substrate influences antimicrobial resistance in *Enterococcus faecalis*

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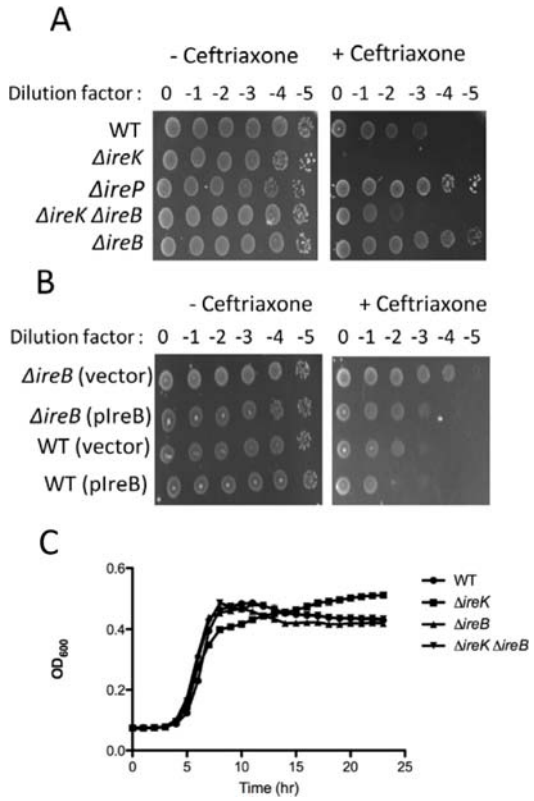

Figure S1. IreB negatively regulates expression of cephalosporin resistance in *E. faecalis*. A. Overnight cultures of *E. faecalis* strains were subjected to 10-fold serial dilution and inoculated (left to right) on MHB agar plates containing either 0 or 20 μg/mL ceftriaxone with incubation at 37˚C overnight. Strains: WT, OG1RF; Δ*ireK,* CK119; Δ*ireP,* CK121; Δ*ireK* Δ*ireB,* CK167; Δ*ireB,* CK164. **B.** Overnight cultures of plasmid-bearing *E. faecalis* strains were subjected to 10-fold serial dilution and inoculated on MHB agar containing chloramphenicol and either 0 μg/mL or 50 μg/mL ceftriaxone with incubation at 37˚C overnight. Strains are in panel (A). Plasmids: vector, pCI3340; pIreB, pCJK187. **C**. Analysis of growth kinetics in hBHI was performed with overnight cultures diluted 10,000 fold and growth was monitored by optical density at 600 nm measured using Bioscreen C plate reader (Oy Growth Curves Ab, Ltd) under static conditions. Strains were as in panel (A).

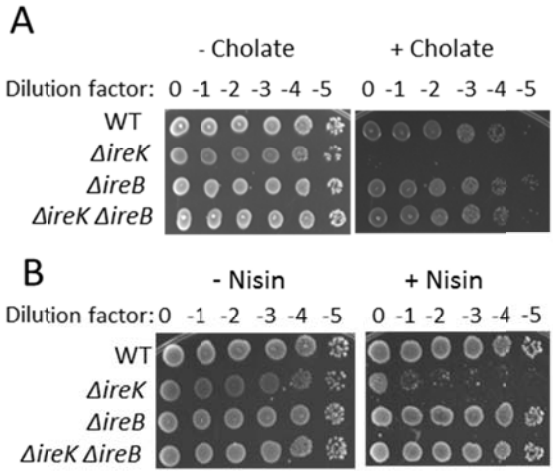

Figure S2 IreB has a negative regulatory role in IreK-mediated responses to additional stresses that act on the cell envelope in *E. faecalis*.

Overnight cultures were subjected to 10-fold serial dilutions and inoculated on MHB agar plates (left to right) with or without 5% sodium cholate from sheep bile (A) or 15 μg/mL nisin (B). Plates were incubated overnight at 37˚C. Strains: WT, OG1RF; Δ*ireK*, CK119; Δ*ireK* Δ*ireB*, CK167; Δ*ireB*, CK164.

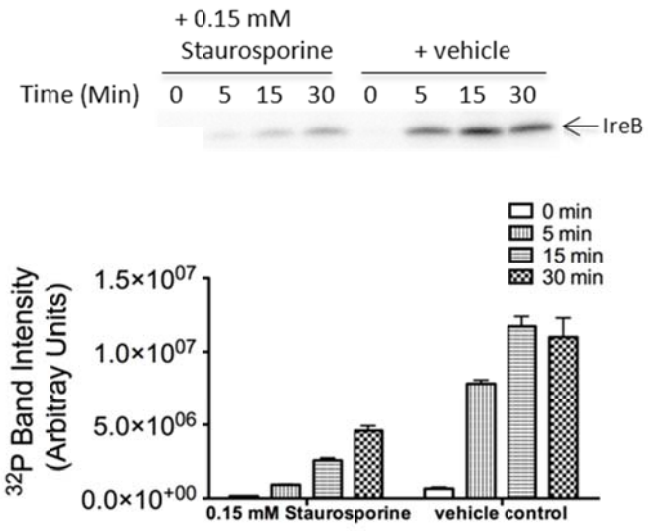

Figure S3 Staurosporine inhibits IreK-n kinase activity *in vitro.* Kinase assays were performed with 33 nM IreK-N, 14.2 μM IreB in kinase buffer containing 0.5 mM 1 μCi [γ-^{32}P]ATP and either 0.15 mM staurosporine or equivalent volume of vehicle (DMSO). Reactions were incubated at 37˚C and aliquots were removed at indicated times. SDS sample buffer was added to stop reactions before analysis on SDS-PAGE. Radiolabelled proteins were visualized by autoradiography. The gel image is representative of three independent experiments, while the graph represents the mean and SEM of three independent experiments.