

Expanded View Figures

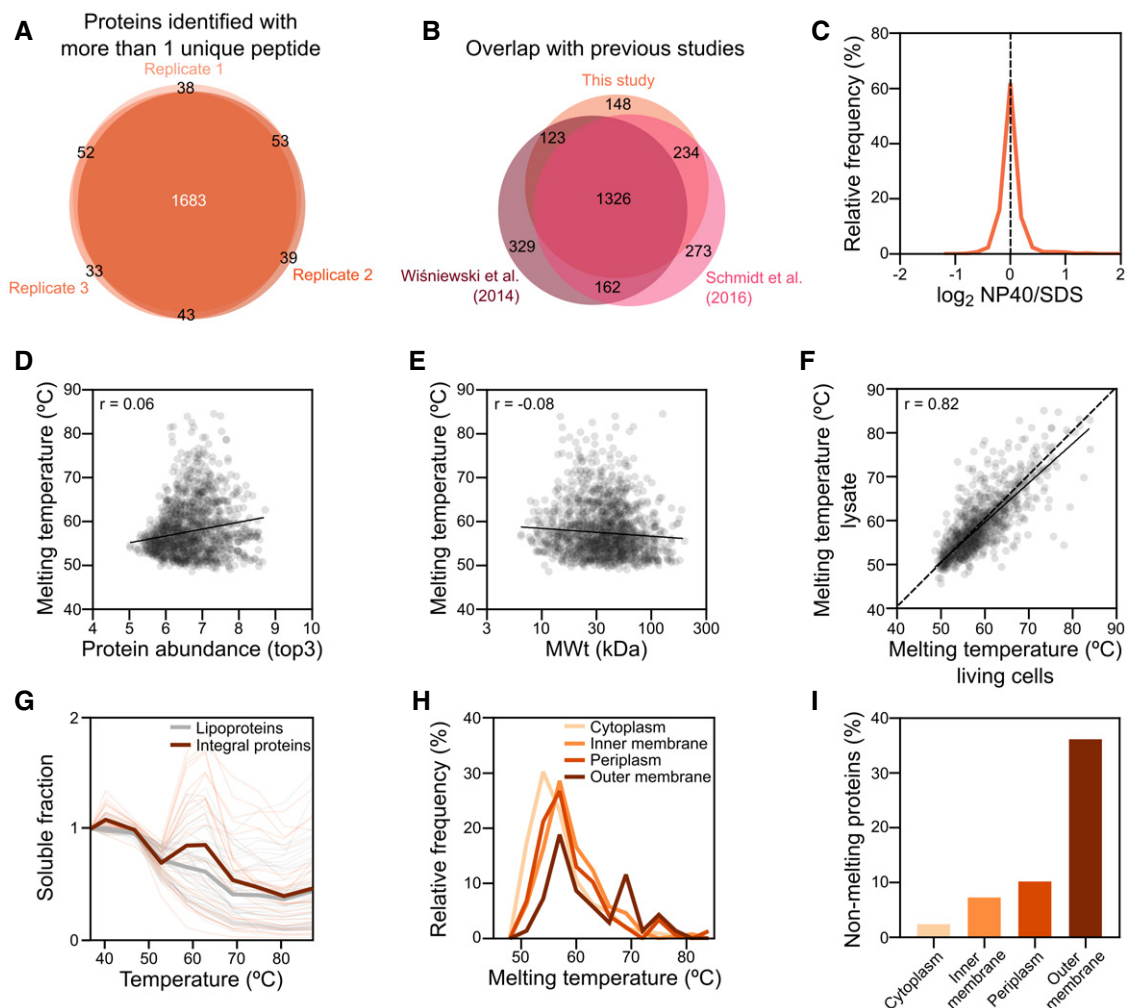


Figure EV1. Melting behavior of proteins identified in the *Escherichia coli* meltome and their properties.

- A Reproducibility of identified proteins in each replicate of *E. coli* meltome analysis.
- B Overlap of identified proteins with previously published proteomics datasets obtained from *E. coli*.
- C Distribution of differences between protein abundance after being extracted with NP-40 or with SDS.
- D, E Correlation of melting point with (D) protein abundance ($r = 0.06$, $P = 0.015$, as measured by the top3 intensity corresponding to the lowest temperature) and (E) molecular weight ($r = -0.08$, $P = 0.0009$).
- F Correlation of melting point in living cells with melting point in lysate—both from TPP ($r = 0.82$, $P < 0.0001$).
- G Melting curves for *E. coli* outer membrane proteins. The average melting curve for each class of outer membrane proteins is shown.
- H Distribution of melting temperatures (T_m) of the *E. coli* proteome according to their cellular compartment.
- I Fraction of proteins with $T_m > 87^\circ\text{C}$ (the highest temperature tested) in each cellular compartment.

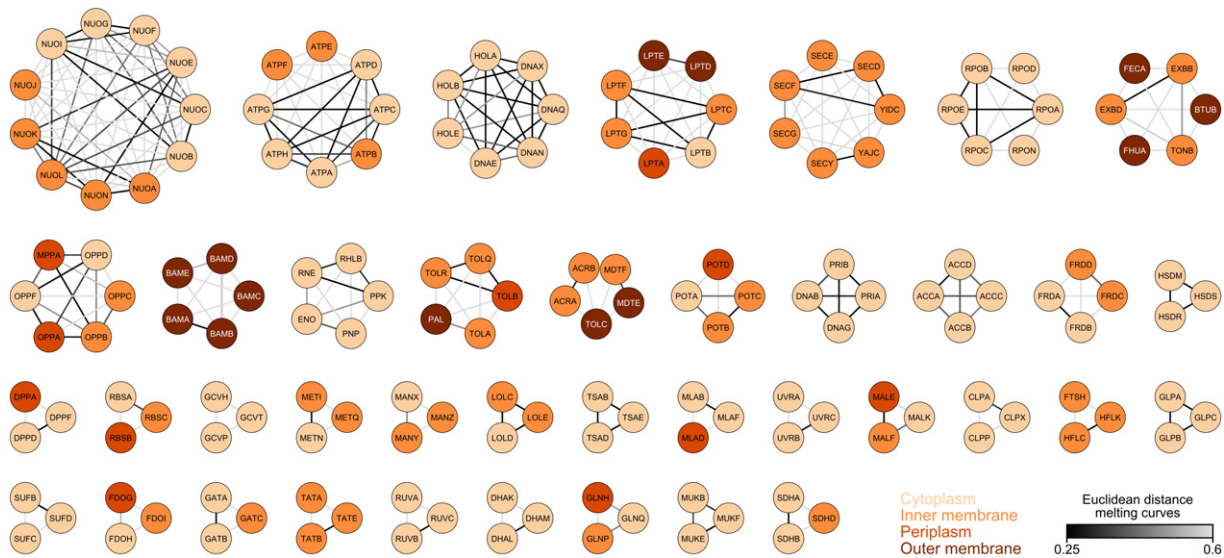


Figure EV2. Melting behavior of protein complexes.

Euclidean distance between all the pairs of melting curves of proteins from each complex—collected from EcoCyc v.21.1 (<https://ecocyc.org/>; Keseler *et al.*, 2017). Only proteins detected with at least two unique peptides in at least two replicates are shown. Node color represents protein location, and edge color represents the average Euclidean distance between the melting curves of complex members.

Figure EV3. Effects of $\Delta tolC$ on protein thermostability and abundance.

- Interaction network of TolC color-coded by the (A) abundance score or (B) stability score. Network was obtained from STRING database by querying only the statistically significant hits (in both abundance and stability) and their interactions with a confidence score of > 0.4 .
- Growth of $\Delta surA \Delta tolC$ in the absence or presence of 1 mM $MgSO_4$ and 0.1 mM $CaCl_2$ in LB medium containing 4 mM sodium citrate with or without 30 $\mu g/ml$ kanamycin. Viability was determined by spotting serial dilutions (10^0 – 10^{-6}) of overnight cultures.
- Cell growth (as measured by OD_{595}) in LB supplemented with 600 mM NaCl of WT, $\Delta tolC$, $\Delta surA$, and $\Delta surA \Delta tolC$ from a starting culture at $OD_{595} = 0.2$.
- Growth of WT, $\Delta tolC$, $\Delta surA$, and $\Delta surA \Delta tolC$ in increasing concentrations of NaCl in LB. Viability was determined by spotting serial dilutions (10^0 – 10^{-6}) of overnight cultures.
- Cell growth (as measured by OD_{595}) after 8 h in the presence of aztreonam in WT, $\Delta tolC$, $\Delta ompF$ and $\Delta ompF \Delta tolC$ cells ($n = 4$; error bars represent standard deviation).
- Cell growth (as measured by OD_{595}) after 8 h in the presence of aztreonam in WT, $\Delta tolC$, $\Delta micF$, and $\Delta micF \Delta tolC$ cells ($n = 4$; error bars represent standard deviation).
- Target engagement affinity of aztreonam in WT and $\Delta tolC$ cells, measured by thermal proteome profiling compound concentration range (TPP-CCR). Stabilization of the secondary known target of aztreonam (MrcA) is shown.

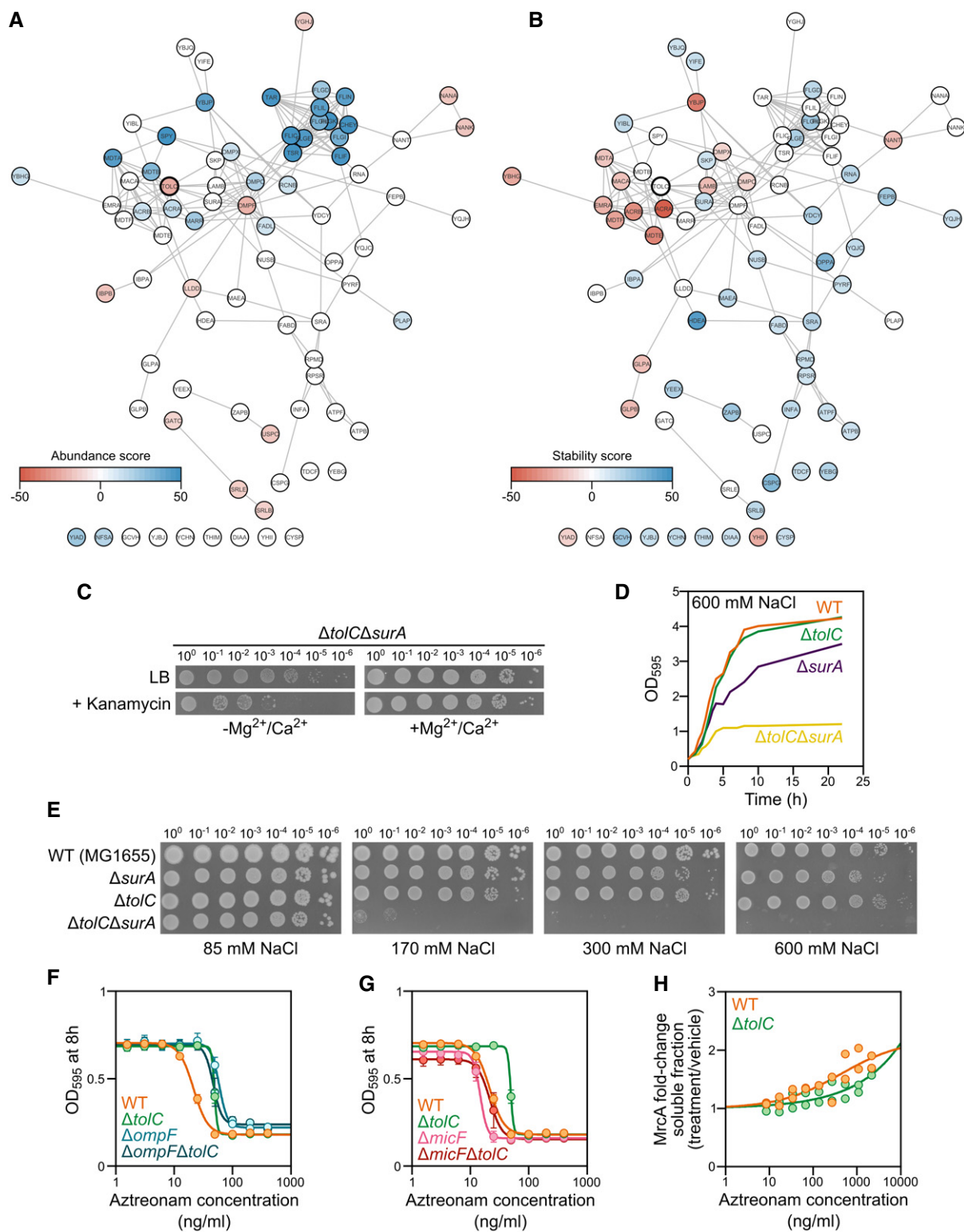


Figure EV3.

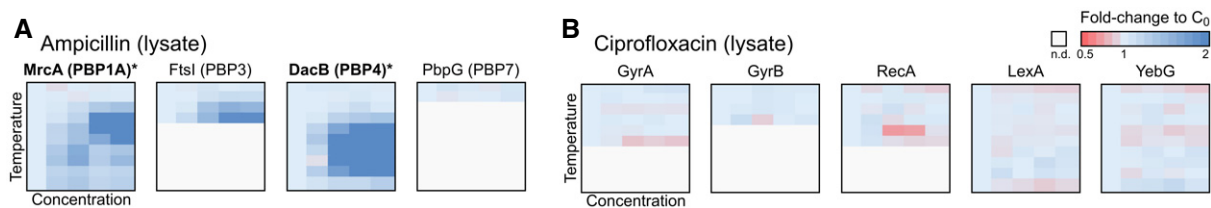


Figure EV4. Identification of direct targets of ampicillin and ciprofloxacin.

A, B Heatmaps of effects on thermostability of proteins after treatment with (A) ampicillin and (B) ciprofloxacin in lysate, with coloring according to what is described in Fig 5A. *FDR controlled at 1% using a bootstrapped permutation approach.