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Arbitrium communication controls phage lysogeny through non-lethal modulation of a host toxin–antitoxin defence system

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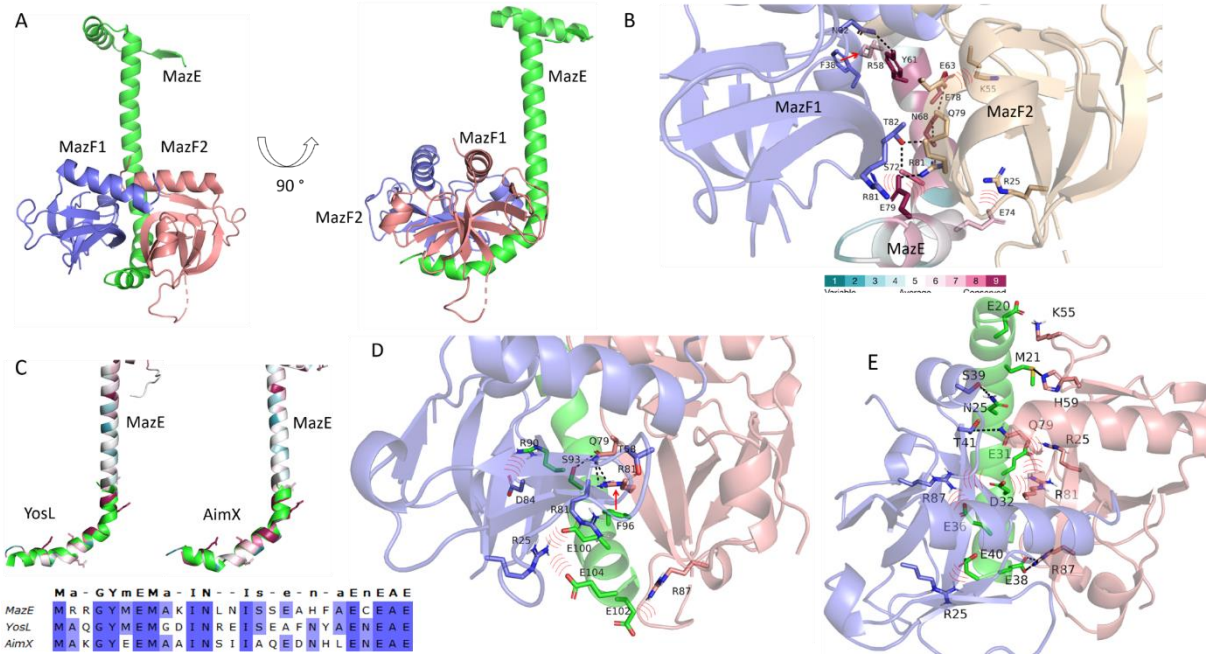
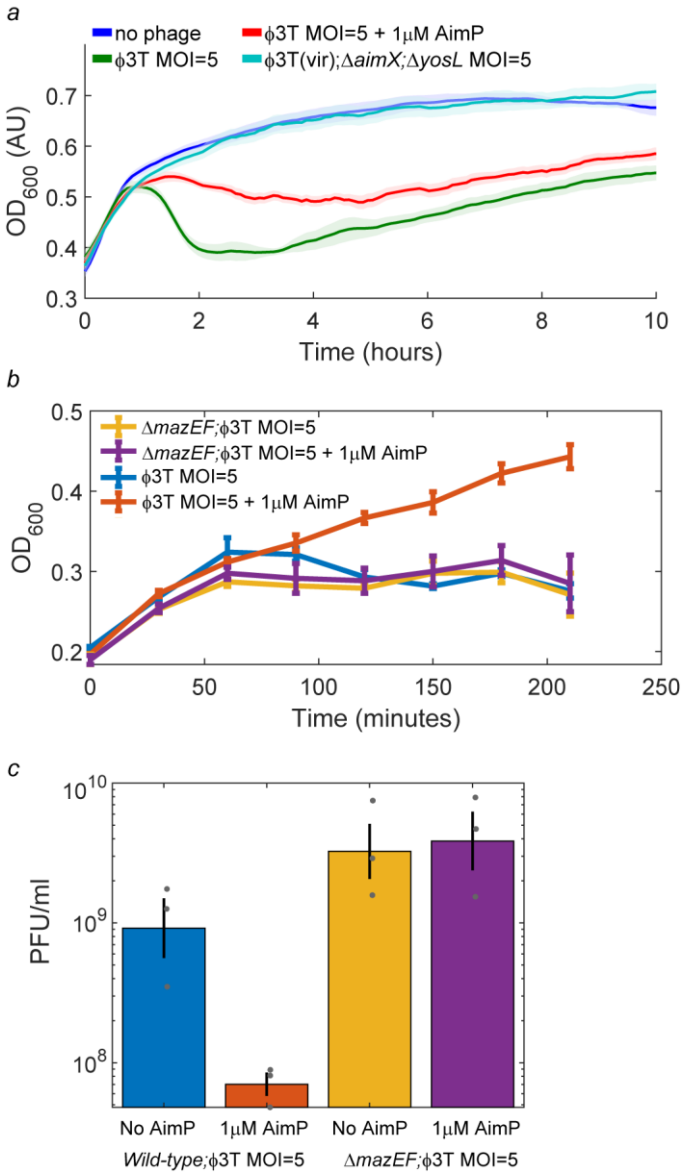
Supplementary Figures

Guler et al, "Arbitrium communication controls phage lysogeny through non-lethal modulation of a host toxin-antitoxin defense system"

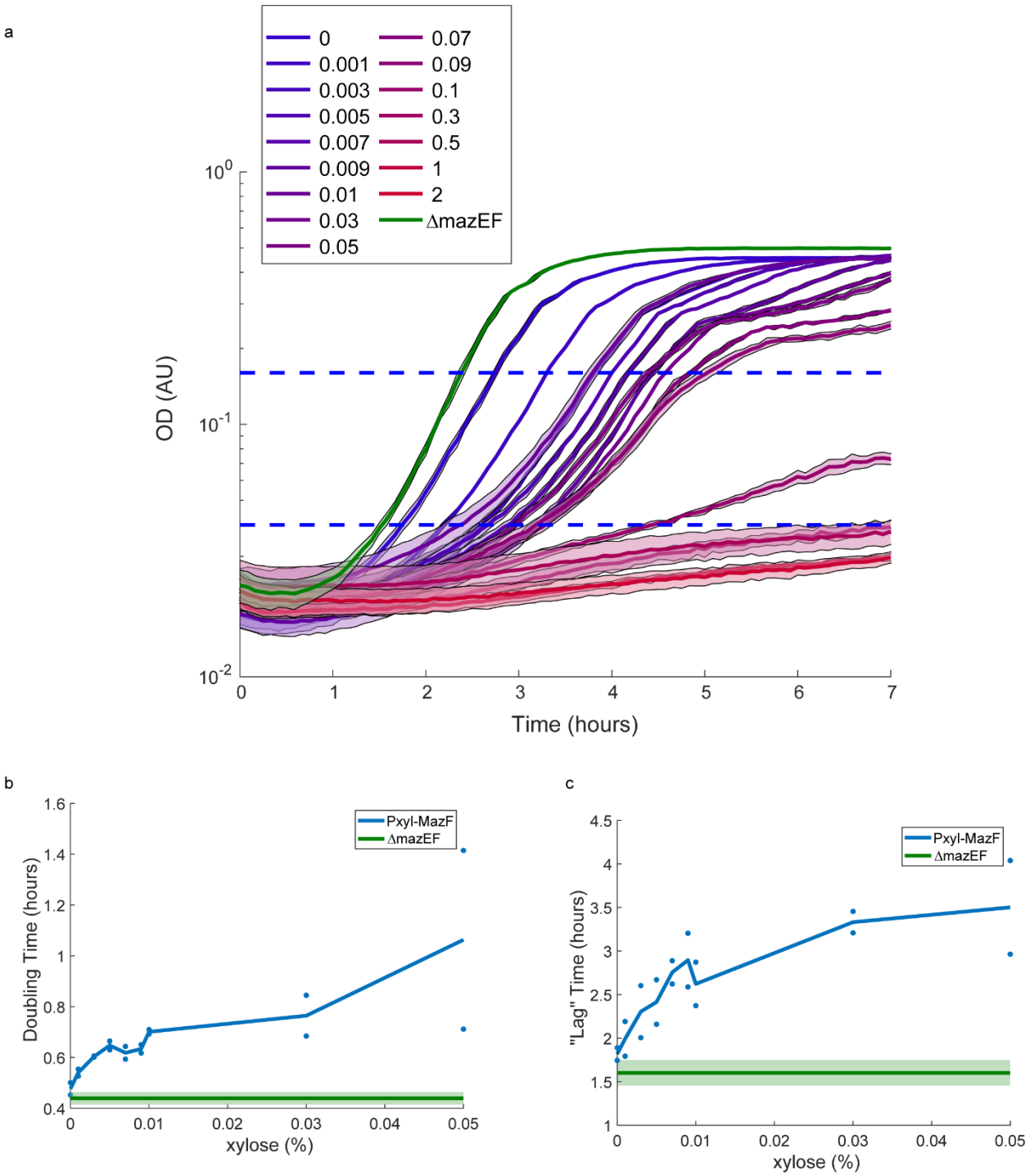


Supplementary Figure 1: Homology modeling of AimX and YosL binding to a MazF dimer. Global (a) and detailed (b) structure of MazE and a mazF dimer, derived from the crystal structure of the proteins (PDB ID: 4me7)[1]. In (A), the global structure is shown from two different angles, where the three chains are shown as ribbons, with MazE colored green and the two MazF chains colored blue and light pink. In (b), MazE is colored by evolutionary conservation (see color code at the bottom of the image), calculated using ConSurf[2], and MazF is shown as in (a). The polar side chain interactions between MazE and MazF are noted as follows: hydrogen bonds are shown as black dashed lines, ionic interactions are shown as red curves, and the cation-π interaction is shown as a red arrow. (c-e) global (c) and detailed (d,e) predicted structures of YosL (c-left, d) and AimX (c-right, e). The structures were predicted by homology modeling, based on the crystal structure of the MazE-MazF$_2$ dimer (Methods). The sequence homology between MazE and the two phage proteins, on which the modeling relied, is also shown (c-bottom). No considerable changes to the structure are observed for YosL and AimX and many of the interactions are preserved. Amino-acid with specific interactions between antitoxin and MazF are shown in (d-f).



Supplementary Figure 2: Addition of 1μM arbitrium peptide during infection does not block phage lysis, but reduces it 10-fold. (a) Plate reader growth curves of different strains and conditions. Growth of wild-type bacteria in the absence of infection (blue), or when infected (at time 0) by ϕ3T at MOI of 5, either in the absence (green) or presence (red) of 1μM ϕ3T arbitrium peptide (SAIRGA). Also shown is infection by the ϕ3T(vir);Δ*aimX;*Δ*yosL* strain at MOI of 5 (cyan). Note the clear difference between infection in the presence of peptide of the wild-type, showing late lytic like behavior and infection with the triple mutant. Shown are mean (line) and SEM (gray region) of three biological repeats. (b) Growth curves of different infected strains. Optical density was measured manually every 30 minutes. Conditions are intended to reproduce Cui et al manuscript[3], due to lack of clear details in the methods, we were unable to reproduce the exact results. cells were infected at optical density of 0.2 and were kept unshaken in room temperature after infection (see methods for further details). Phages were infected at

time zero at a MOI of 5. Strains, phages and conditions are listed. Note the increased growth of the wild-type strain when infected with wild-type ϕ3T in the presence of peptide compared to in its absence. (c) Plaque counts taken 210 minutes post infection (last time point in (b)) for all four strain in (b), as indicated by the color of the bar. Note the 10-fold reduction in PFU/ml when comparing infection of ϕ3T on wild-type cells with or without 1μM arbitrium peptide. Compare this with the 6 orders of magnitude reduction observed for infection the ϕ3T(vir);Δ*aimX;*Δ*yosL* (Fig. 3a). Error bars in b,c mark SEM over three biological repeats.



Supplementary Figure 3: Bacterial growth as a function of MazF induction. (a) growth curve (on a logarithmic scale) as a function of time of a Δ*mazEF* culture (green) and a Δ*mazEF;*P_{xyl}-*mazF* culture (purple – red) induced with different % w/v of xylose as indicated in the legend. Lines and shaded regions reflect mean and standard error due to technical replicates. This is one example out of a duplicate of repeats done on different days. Dashed blue lines mark two optical density thresholds different by a factor of four, from which average doubling time (D) was calculated as indicated in the figure. (b,c) doubling time and 'lag" time (time to reach the low

threshold) as a function of xylose % w/v. Indicated are the $MazF^{+}$ and $MazF^{++}$ conditions shown in Fig. 3 and used for RNAseq analysis (Fig. 4). Green line shows the values for the Δ*mazEF* culture. Error bar show standard error based on two experiments for each value.

1. Simanshu, D. K., Yamaguchi, Y., Park, J.-H., Inouye, M. & Patel, D. J. Structural basis of mRNA recognition and cleavage by toxin MazF and its regulation by antitoxin MazE in Bacillus subtilis. *Molecular cell* **52**, 447–458 (2013).
2. Glaser, F. *et al.* ConSurf: identification of functional regions in proteins by surface-mapping of phylogenetic information. *Bioinformatics* **19**, 163–164 (2003).
3. Cui, Y. *et al.* Bacterial MazF/MazE toxin-antitoxin suppresses lytic propagation of arbitrium-containing phages. *Cell Reports* **41**, 111752 (2022).