Expanded View Figures

Figure EV1. Evidence for the presence of phages at the plaque periphery during constriction.

A PY79 (WT) cells were infected with low concentrations (10^{-8} PFU/ml) of SPP1-$_{lysin\text{-}yfp}$, placed on an agarose pad, and plaque formation was followed by time-lapse confocal microscopy. Shown are overlay images of phase contrast (gray) and signal from Lysin-$_{SPP1}$-YFP (cyan) captured at the indicated time points (h) postinfection (left panels). Corresponding signal from Lysin-$_{SPP1}$-YFP (cyan) is shown separately (right panels). Scale bars, 50 μm. Corresponds to Movie EV3.

B Quantification of the SPP1-$_{lysin\text{-}yfp}$ fluorescence intensity (AU) at the indicated time points. Fluorescence from Z sections that include the plaque region and flanking area was measured. Corresponds to EV1A.

C Quantification of the diameter of the YFP fluorescence (AU) ring, derived from SPP1-$_{lysin\text{-}yfp}$. Corresponds to EV1A.

D Screening for phage-resistant bacteria at the plaque rim. PY79 (WT) cells were infected with SPP1 (10^{-6} PFU/ml) and plated for plaque formation. At t = 18 h, similar numbers of bacteria were collected from 30 "non-plaque" and 30 "plaque rim" regions, and bacterial smears were plated over plates with (10^{-4} PFU/ml) or without phages. No phage-resistant colonies were detected in both populations.

Source data are available online for this figure.

© 2021 The Authors

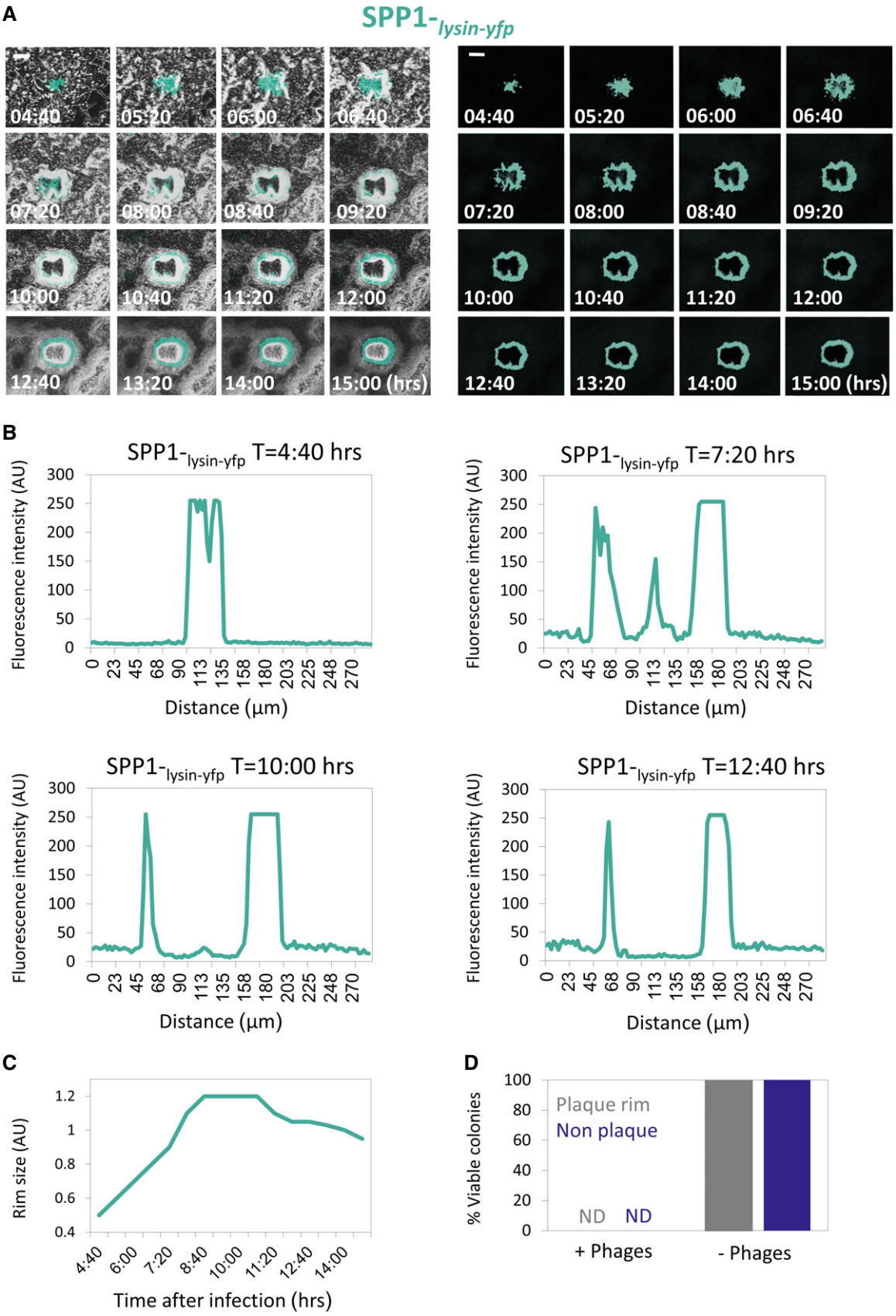

Figure EV1.

Figure EV2. Δ*sigX* cells are excluded from the plaque rim during constriction.

A BDR2637 (P_{veg}-*mCherry*) (WT) (purple) cells were mixed with AR16 (P_{rrnE}-*gfp*) (WT) (cyan) (1) or with ET191 (Δ*sigX*, P_{rrnE}-*gfp*) (cyan) (2-5) cells. The mixtures were infected with low concentrations (10^{-8} PFU/ml) of SPP1, placed on an agarose pad, and plaque formation was followed by time-lapse confocal microscopy. Shown are overlay images of mCherry (purple) and GFP (cyan) signals captured 16 h postinfection. (1-3) show plaque regions, whereas (4-5) show regions remote from any visible plaque site. Scale bar, 100 μm.

B Quantification of images 1, 3, and 4 presented in EV2A. Fluorescence intensity (AU) of the plaques formed by phages infecting the corresponding cells is shown. Fluorescence from Z sections that include the plaque region and flanking area or control areas was measured.

Source data are available online for this figure.

© 2021 The Authors

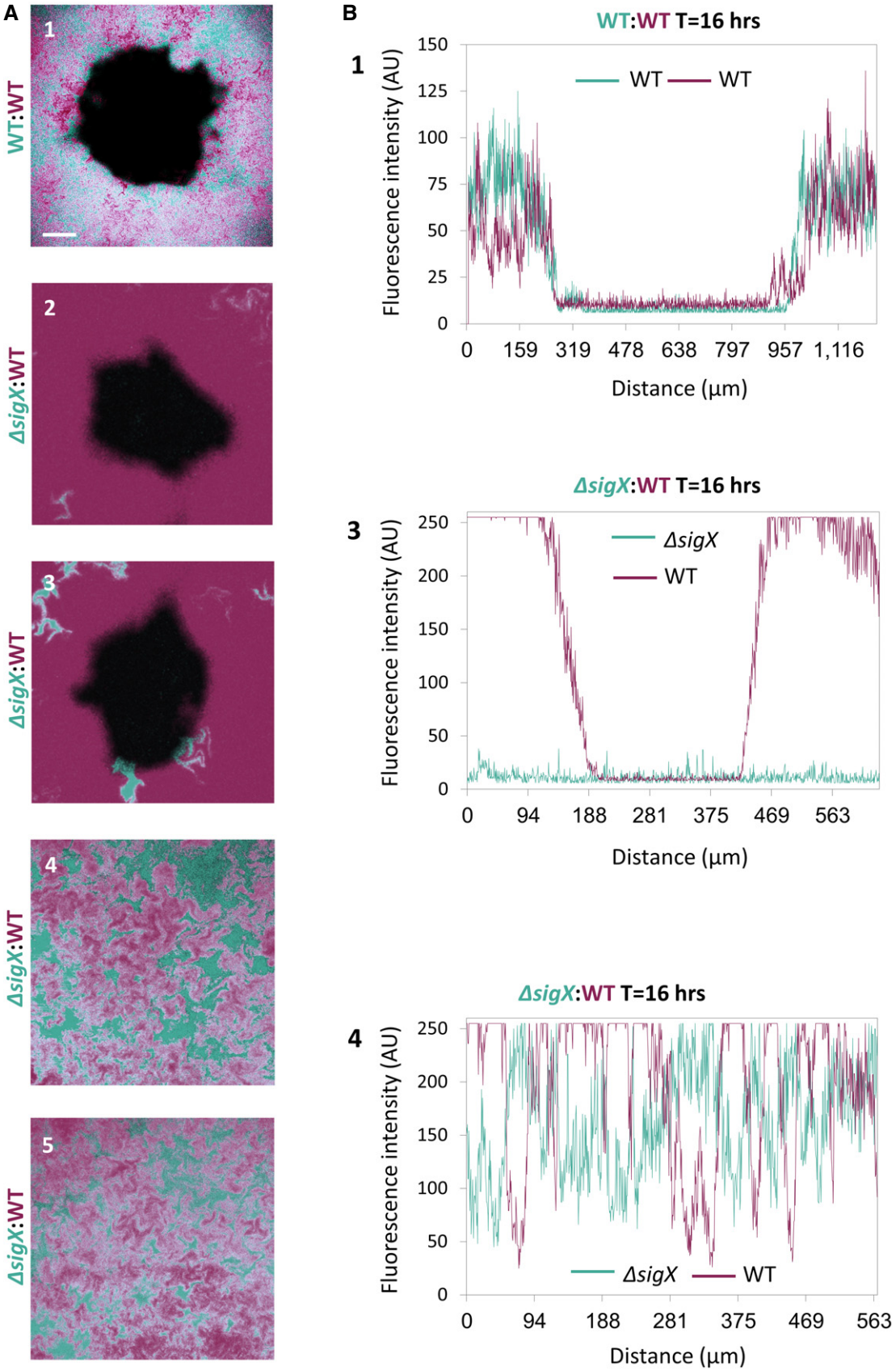

Figure EV2.

Figure EV3. Monitoring SigX activation during phage infection.

A PY79 (WT) and ET19 (Δ*sigX*) cells were infected with SPP1 or Phi29 (10^{-6} PFU/ml), spread over MB agar plates, and incubated at either 37°C or 48°C. Plaque diameter was monitored after 20 h of incubation ($n \geq 50$). Shown are average values and SD of 3 independent repeats.

B PY79 (WT) and ET19 (Δ*sigX*) were grown in liquid LB medium at 48°C and OD_{600nm} monitored. Shown are average values and SD of 3 biological repeats.

C Corresponds to the experiment presented in Fig 3A. Phage-sensitive PY79 (WT) cells were mixed with phage-resistant BS12 (Δ*yueB*, P_{sigX}-*gfp*) cells and the mixture was infected with SPP1 at 2:1 (phages:bacteria) MOI, and OD_{600nm} was followed at 2.5-min intervals. Uninfected mixed population served as a control (-SPP1). Shown is a representative experiment out of 3 biological repeats, and the average values and SD of $n \geq 3$ technical repeats.

D BS12 (P_{sigX}-*gfp*, Δ*yueB*) cells were infected with SPP1 at 2:1 (phages:bacteria) MOI, and fluorescence intensity from P_{sigX}-*gfp* (AU) was followed at 2.5-min intervals. Uninfected BS4 (P_{sigX}-*gfp*) cells served as a control. Shown is a representative experiment out of 3 biological repeats, and the average values and SD of 3 technical repeats.

E ET26 (P_{sigX}-*sigX-gfp*) cells were infected with SPP1 at 5:1 (phages:bacteria) MOI, placed on an agarose pad, and followed by time-lapse fluorescence microscopy. Shown are signal from SigX-GFP (upper panels), and corresponding phase contrast images (lower panels), captured at the indicated time points postinfection. Scale bar, 1 μm.

Source data are available online for this figure.

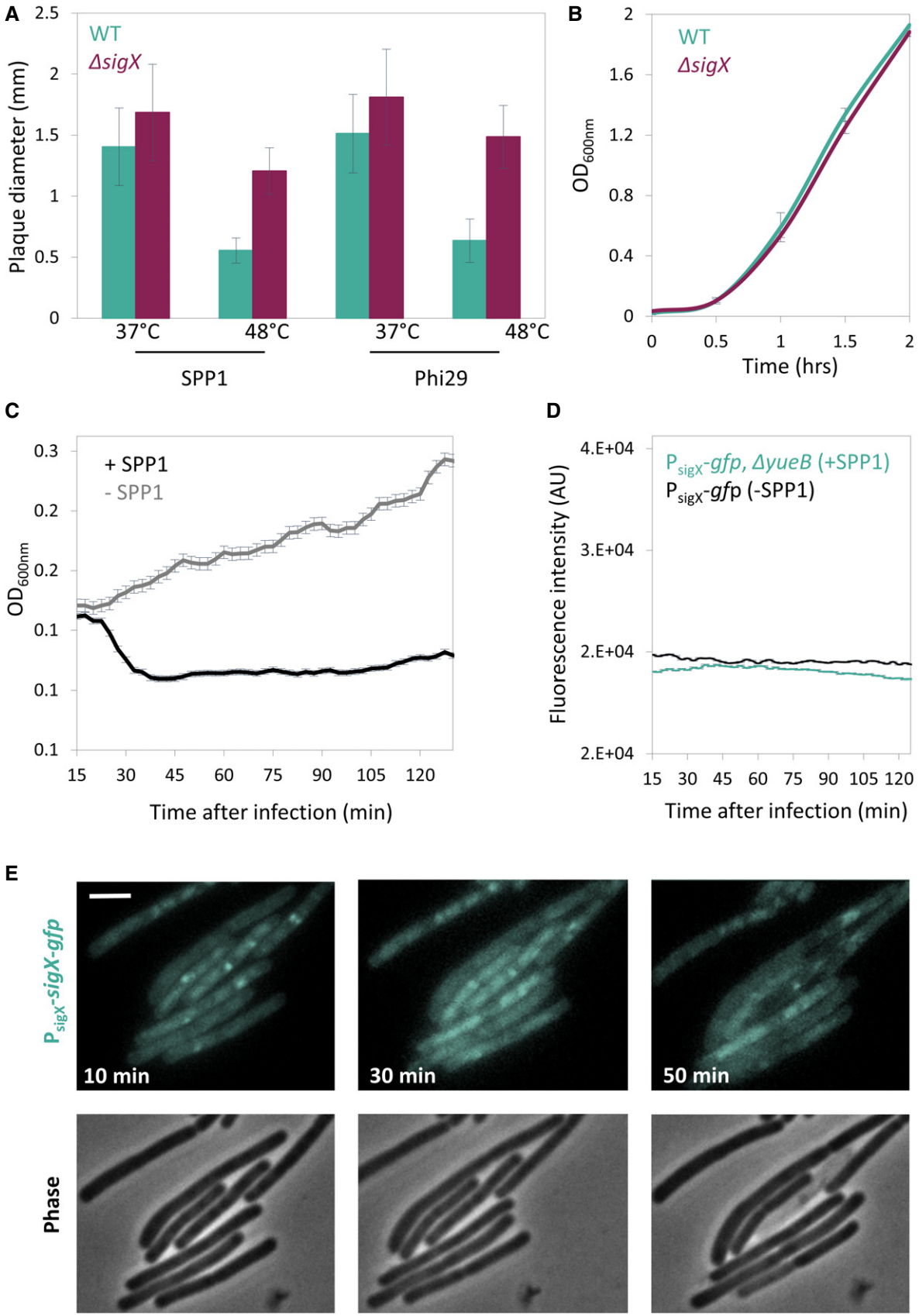

Figure EV3.

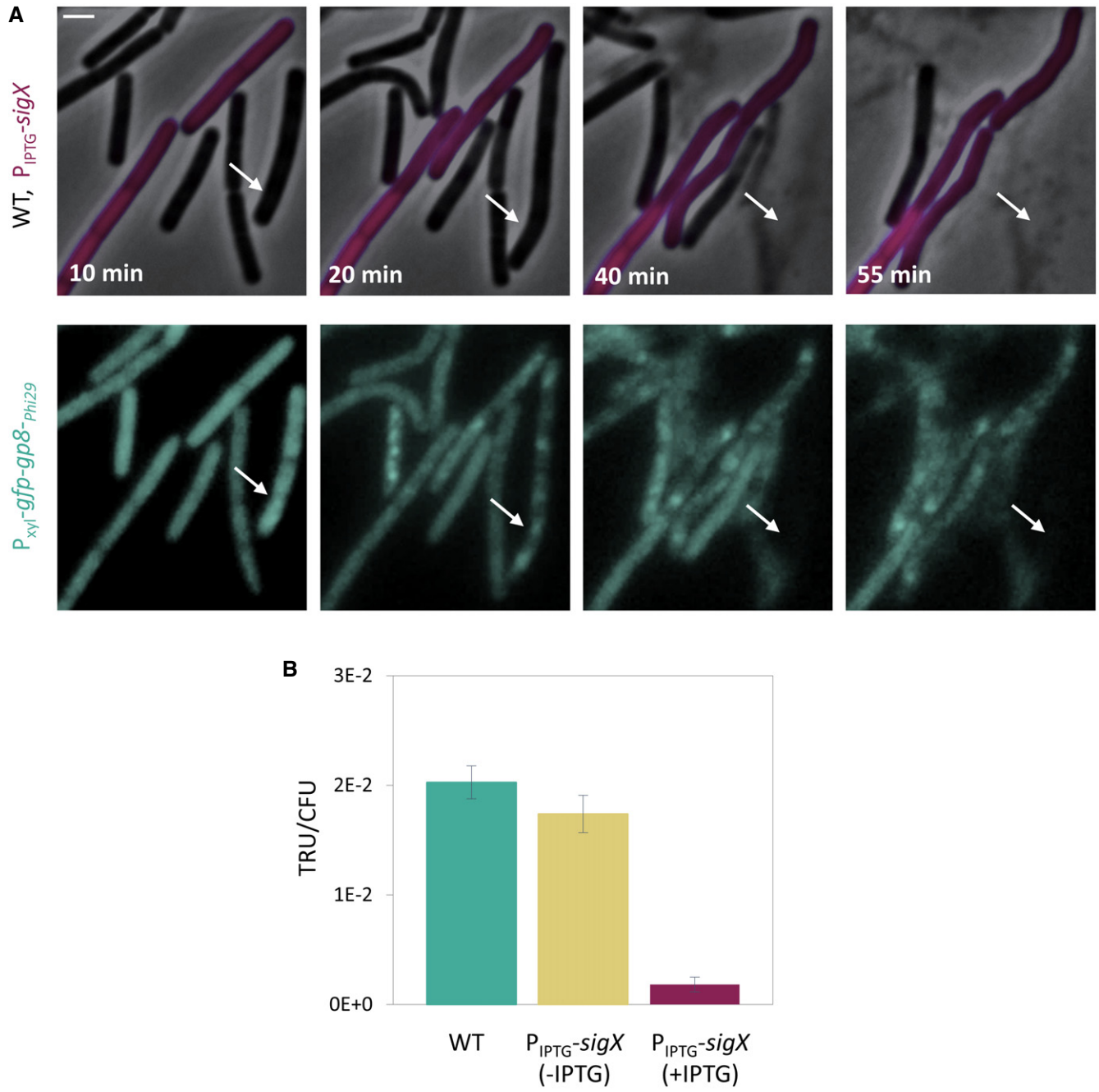

Figure EV4. SigX over-expression interferes with phage infection.

A ET9 (P_{xyl}-*gfp-gp8-*$_{Phi29}$) (WT) and ET44 (P_{veg}-*mCherry*, P_{IPTG}-*sigX*, P_{xyl}-*gfp-gp8-*$_{Phi29}$) (P_{IPTG}-*sigX*, purple) cells were grown in the presence of IPTG and xylose, mixed, and infected with Phi29 at 5:1 (phages:bacteria) MOI. The mixture was placed on an IPTG and xylose-containing agarose pad and followed by time-lapse fluorescence microscopy. Gp8-$_{Phi29}$ is the major Phi29 capsid protein that localizes into discrete foci during Phi29 infection (Tzipilevich *et al*, 2017). Shown are overlay images of phase contrast (gray) and signal from mCherry (purple) (upper panels), and signal from GFP-Gp8-$_{Phi29}$ (cyan) (lower panels), captured at the indicated time points. Arrows highlight GFP-Gp8-$_{Phi29}$ foci appearance in ET9 cells. Scale bar, 1 μm.

B PY79 (WT) and ET28 (P_{IPTG}-*sigX*), grown in the presence or absence of IPTG, were transduced with SPP1-pBT163 lysate, and the number of transductants was monitored by plating the cells on corresponding selective plates. Transduction unit (TRU) was calculated as the number of transductant colonies /total CFU. Shown are average values and SD of 3 biological replicates.

Source data are available online for this figure.

Figure EV5. SigX impact on phage tolerance is Dlt-mediated.

A Bacterial strains harboring P_{IPTG}-*sigX* as well as the indicated gene deletions were grown in the presence of IPTG. At $t = 60$ min, cells were infected with SPP1 at low (phages:bacteria 1:20) MOI, and OD_{600nm} was followed at 2-min intervals. PY79 (WT) was infected in parallel for comparison. Knockout of *ywbO* and the *dlt* operon (*ΔdltA*) largely abolished the tolerance to phage infection conferred by SigX over-expression. Shown is a representative experiment out of 3 independent biological repeats, with average values and SD of 3 technical repeats.

B ET42 (*ΔdltA*, P_{IPTG}-*sigX*) cells were grown in the presence of IPTG and mixed with BDR2637 (P_{veg}-*mCherry*) (WT, purple) cells. The mixture was infected with SPP1-$_{lysin\text{-}yfp}$ at 5:1 (phages:bacteria) MOI, placed on an IPTG-containing agarose pad, and followed by time-lapse fluorescence microscopy. Shown are overlay images of phase contrast (gray) and signal from mCherry-labeled cells (purple) (upper panels), and the corresponding signal from Lysin-$_{SPP1}$-YFP (green) (lower panels), captured at the indicated time points. Yellow arrows denote infected WT cells, whereas white arrows highlight infected ET42 cells that lysed rapidly. Scale bar, 1 μm.

C PY79 (WT), ET41 (Δ*dltA*), and ET72 (P_{xyl}-*dltABCD*) cells, grown with or without xylose as indicated, were infected with SPP1 at low (1:20) MOI, and OD_{600nm} was followed at 2-min intervals. Shown is a representative experiment out of 2 biological repeats, and the average values and SD of 4 technical repeats.

D PY79 (WT) and ET41 (*ΔdltA*) cells were grown in LB liquid medium, and OD_{600nm} was followed. Shown are average values and SD of 3 biological repeats.

Source data are available online for this figure.

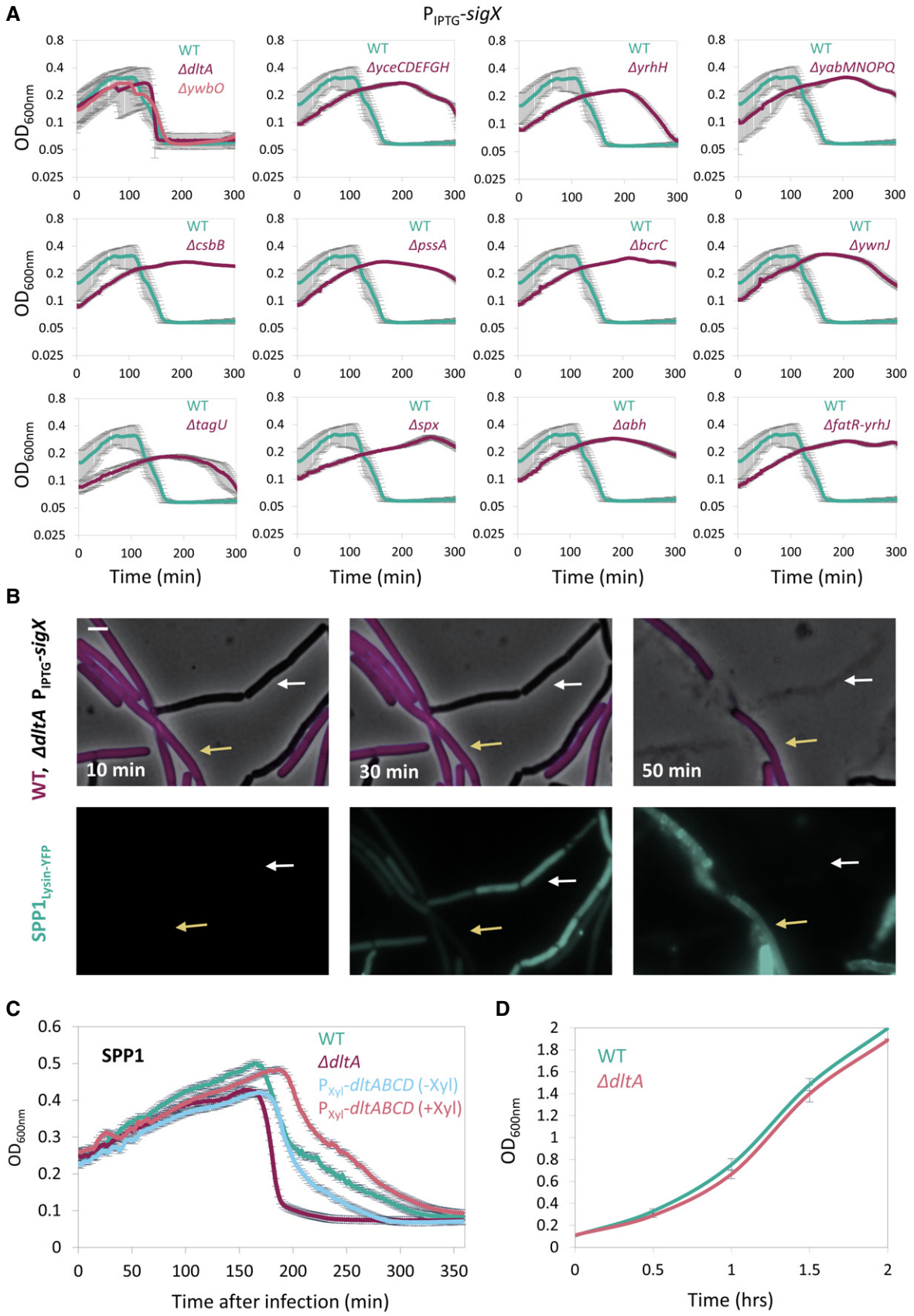
A
P IPTG-sigX
WT
ΔdltA
ΔywbO
ΔyceCDEFGH
ΔyrhH
ΔyabMNOPQ
ΔcsbB
ΔpssA
ΔbcrC
ΔywnJ
ΔtagU
Δspx
Δabh
ΔfatR-yrhJ
OD600nm
Time (min)
B
WT, ΔdltA PIPTG-sigX
SPP1Lysin-YFP
10 min
30 min
50 min
C
SPP1
WT
ΔdltA
PXyl-dltABCD (-Xyl)
PXyl-dltABCD (+Xyl)
OD600nm
Time after infection (min)
D
WT
ΔdltA
OD600nm
Time (hrs)

Figure EV5.