APPENDIX

Bacteria elicit a phage tolerance response subsequent to infection of their neighbors

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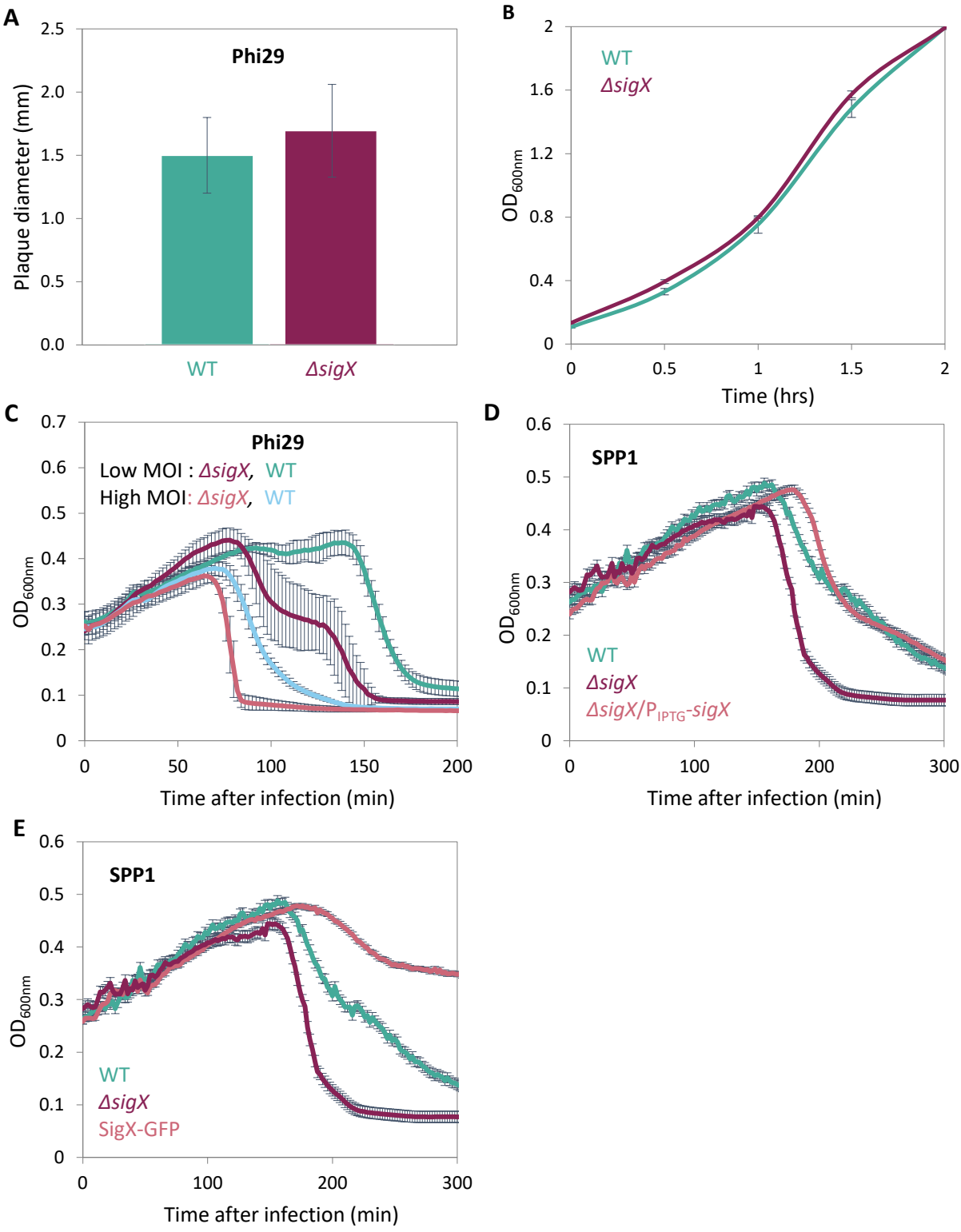
Appendix Figure S1



Appendix Figure S1. Δ*sigX* cells are highly sensitive to infection

(A) PY79 (WT) and ET19 (Δ*sigX*) cells were infected with Phi29 (10^{-6} PFU/ml), spread over MB agar plates, and plaque diameter was monitored after 20 hrs of incubation. Shown is plaque diameter distribution for each strain ($n \geq 120$).

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

(C) PY79 (WT) and ET19 (Δ*sigX*) cells were infected with Phi29 at either high (phages:bacteria 1:1) or low (phages:bacteria 1:20) MOI, and OD_{600nm} was followed at 5 min intervals. Shown is a representative experiment out of 3 biological repeats, and average values and SD of 6 technical repeats.

(D) PY79 (WT), ET19 (Δ*sigX*) and OF211 (Δ*sigX*, P_{IPTG}-*sigX*) cells were infected with SPP1 at low (phages:bacteria 1:20) MOI, and OD_{600nm} was followed at 2 min intervals. Complementation was apparent even without IPTG addition, due to low promoter activity. Shown is a representative experiment out of 2 biological repeats, and the average values and SD of 3 technical repeats.

(E) PY79 (WT), ET19 (Δ*sigX*) and ET26 (P_{sigX}-*sigX-gfp*) cells were infected with SPP1 at low (phages:bacteria 1:20) MOI, and OD_{600nm} was followed at 2 min intervals. SigX-GFP is functional and appears more stable than the native protein as in provides higher phage tolerance in comparison to the WT. Shown is a representative experiment out of 2 biological repeats, and the average values and SD of 3 technical repeats.

Appendix Figure S2

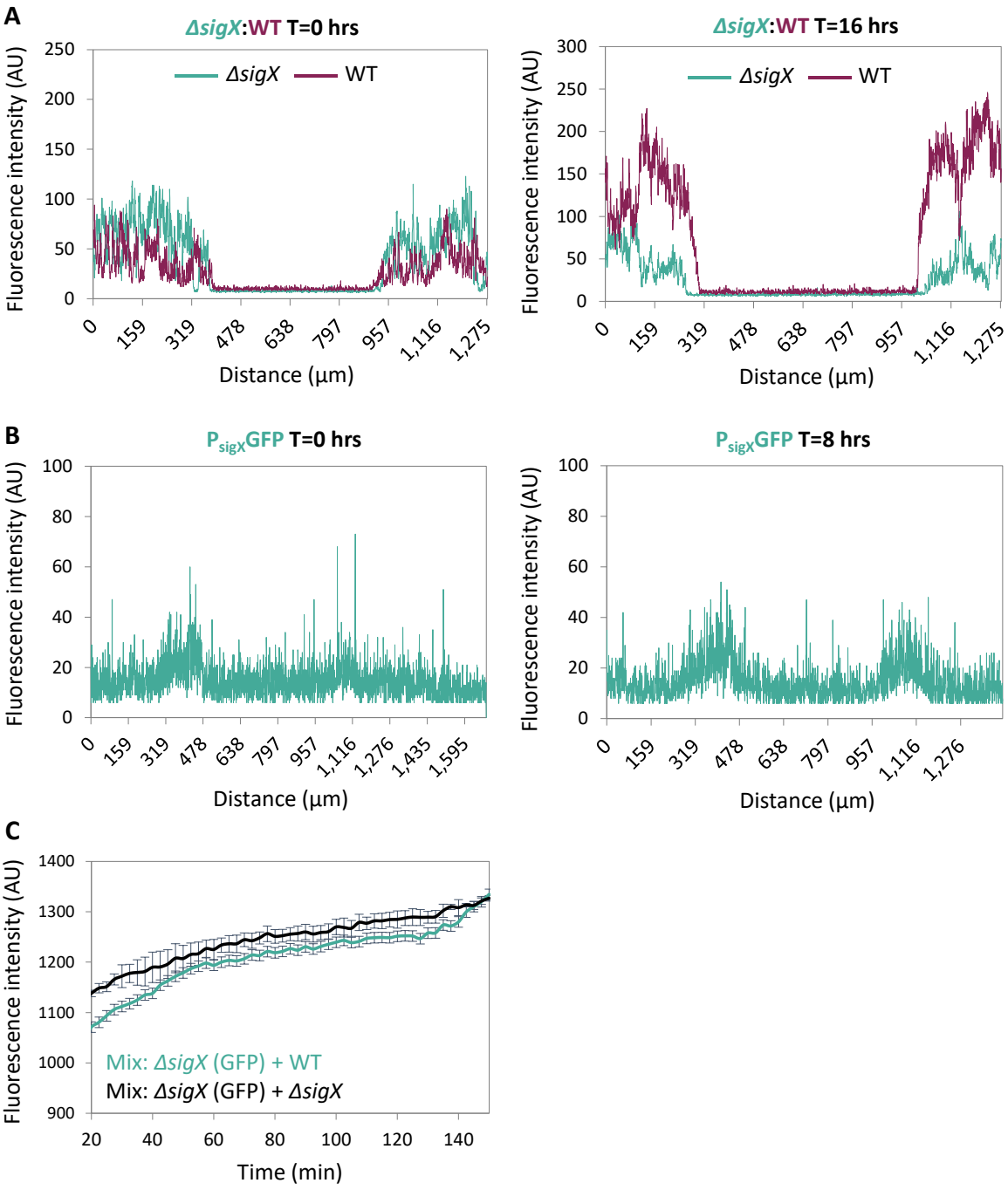


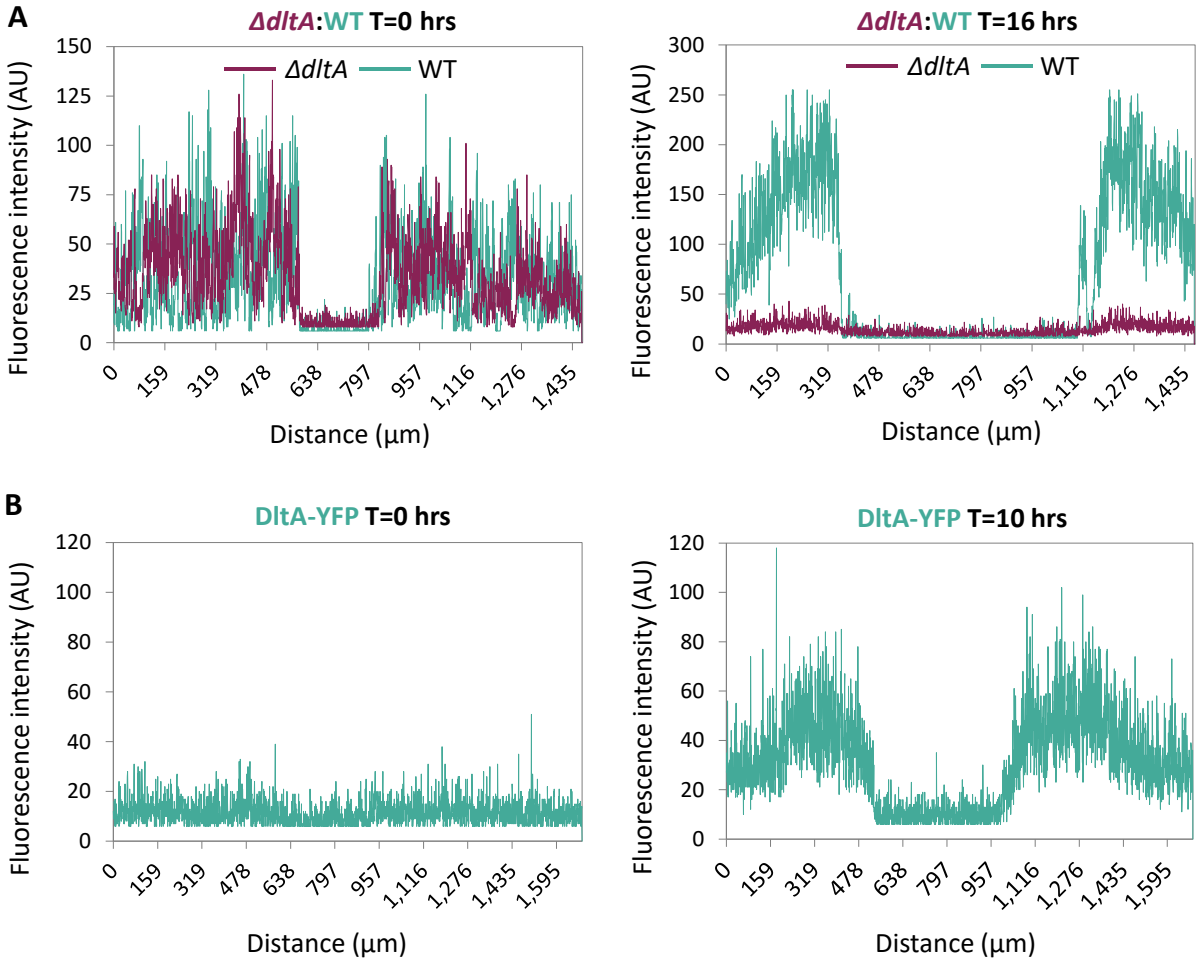
Figure S2. SigX is activated in response to phage infection

(A) Quantification of the images presented in Fig 2E at the indicated time points. Fluorescence intensity (AU) of the plaque formed by phages infecting a mixture of BDR2637 (P_{veg}-*mCherry*) (WT, purple) and ET191 (P_{rrnE}-*gfp*, Δ*sigX*) (Δ*sigX*, cyan) cells. Fluorescence from Z sections that include the plaque region and flanking area was measured.

(B) Quantification of the images presented in Fig 2F at the indicated time points. Fluorescence intensity (AU) of the plaque formed by phages infecting ET27 (P_{sigX}-*gfp*) cells. Fluorescence from Z sections that include the plaque region and flanking area was measured.

(C) ET191 (P_{rrnE}-*gfp*, *ΔsigX*) cells were mixed with PY79 (WT) or with ET19 (*ΔsigX*), and fluorescence intensity from P_{rrnE}-*gfp* (AU) was followed at 2.5 min intervals. Shown is a representative experiment out of 3 biological repeats, and the average values and SD of 3 technical repeats.

Appendix Figure S3



Appendix Figure S3. Efficient plaque constriction requires DltA

(A) Quantification of the images presented in Fig 5F at the indicated time points. Fluorescence intensity (AU) of the plaque formed by phages infecting a mixture of AR16 (P_{rrnE}-*gfp*) (WT, cyan) and ET411 (P_{veg}-*mCherry,* Δ*dltA*) (Δ*dltA*, purple) cells. Fluorescence from Z sections that include the plaque region and flanking area was measured.

(B) Quantification of the images presented in Fig 5G at the indicated time points. Fluorescence intensity (AU) of the plaque formed by phages infecting ET43 (*dltA-yfp)* cells. Fluorescence from Z sections that include the plaque region and surrounding area was measured.

Appendix Table S1. List of bacterial strains and phages used in this study

Strain Name	Genotype	Source
E. coli		
pET2	*amyE::promoter less gfp-cm*	Constructed by amplifying the *gfpmut2* gene from pKL147 (Lemon & Grossman, 1998), by PCR using primers *BamH1-Not1-gfp-U* and *gfp-EcoRI-L*. The PCR-amplified DNA was digested with *BamHI* and *EcoRI* and was cloned into pDG364 (BGSC) digested with the same enzymes.
pET26	*amyE::*P_{sigX}*-sigX-gfp-cm*	Constructed by amplifying the *sigX* ORF with 200 bp upstream of the start codon from gDNA of *B. subtilis* strain (PY79), using primers *PsigX*-U-*BamH*I and *sigX-L-Not*I. The PCR-amplified DNA was digested with *BamH*I and *Not*I and was cloned into pET2 digested with the same enzymes.
pET27	*amyE::*P_{sigX}*-gfp-cm*	Constructed by amplifying 200 bp upstream of the start codon of *sigX* from gDNA of *B. subtilis* strain (PY79), using primers *PsigX*-U-*BamH*I and *PsigX-L-Not*I. The PCR-amplified DNA was digested with *BamH*I and *Not*I and was cloned into pET2 digested with the same enzymes.
pET28	*amyE::*$P_{hyper\text{-}spank}$*-sigX-spc*	Constructed by amplifying the *sigX* from gDNA of *B. subtilis* strain (PY79), using primers *sigX*-U-*Sal*I and *sigX-L-Nhe*I. The PCR-amplified DNA was digested with *Sal*I and *Nhe*I and was cloned into pDR111 digested with the same enzymes.
pET29	*thrC::*$P_{hyper\text{-}spank}$*-sigX-mls*	Constructed by amplifying the *sigX* from gDNA of *B. subtilis* strain (PY79), using primers *sigX*-U-*Sal*I and *sigX-L-Nhe*I. The PCR-amplified DNA was digested with *Sal*I and *Nhe*I and was cloned into pDG1743 digested with the same enzymes.
pET43	*dltA::dltA-yfp-spc*	Constructed by amplifying the *C- terminus* of *dltA* from gDNA of *B. subtilis* strain (PY79), using primers *dltA-Mfe*I-U and *dltA-Xho*I-*L*. The PCR-amplified DNA was digested with *Mfe*I and *Xho*I and was cloned into pKL147 digested with *EcoR*I and *Xho*I
pET72	*amyE::*P_{xyl}*-dltABCDE-spc*	Constructed by amplifying the dlt operon from gDNA of B. subtilis strain (PY79), using primers dltA-U-SalI and dltE-L-NheI. The PCR-amplified DNA was digested with SalI and NheI and was cloned into pDR160 digested with the same enzymes.

B. subtilis		
PY79	*B. subtilis*	Wild type (Youngman et al., 1984).
AR16	*amyE::*P$_{rrnE}$*-gfp-spc*	Lab stock (Rosenberg et al., 2012).
BDR2637	*sacA::*P$_{veg}$*-mCherry-phleo*	Kindly provided by Prof. David Rudner (Harvard university)
BS1	*thrC::*P$_{hyper}$*-spank-sigX-mls*	Constructed by transforming gDNA from ET29 into PY79.
BS4	*amyE::*P$_{sigX}$*-gfp-cm*	Constructed by transforming the gDNA of ET27 into PY79.
BS12	*amyE::*P$_{sigX}$*-gfp-cm ΔyueB::tet*	Constructed by transforming the gDNA of BS4 into ET6.
OF83	*thrC::*P$_{pen}$*-lacIΔ11-cfp-mls*	Constructed by transforming gDNA from GSY1000 (Jakutyte et al., 2011) (Kindly provided by Prof. Paulo Tavares, CNRS, Gif-sur-Yvette) into PY79.
OF132	*Δspx::cm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *spx::cm*-P1-P4 and transforming into PY79.
OF211	*ΔsigX::kan* *amyE::*P$_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from ET19 into OF28.
ET9	*amyE::*P$_{xyl}$*-gfp-gp8(phi29)-cm*	Lab stock (Tzipilevich et al., 2017).
ET18	*ΔsigW::kan*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *sigW::kan*-P1-P4 and transforming into PY79.
ET19	*ΔsigX::kan*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *sigX::kan*-P1-P4 and transforming into PY79.
ET20	*ΔsigY::mls*	Constructed by transforming gDNA from HB10108 (Mascher et al., 2007) (Kindly provided by Prof. John Helmann, Cornell University) into PY79.
ET21	*ΔsigZ::kan*	Constructed by transforming gDNA from HB10109 (Mascher et al., 2007) (Kindly provided by Prof. John Helmann, Cornell University) into PY79.
ET22	*ΔsigV::cm sacA::*P$_{veg}$*-mCherry-phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *sigV::cm*-P1-P4 and transforming into BDR2637.
ET23	*ΔsigM::kan sacA::*P$_{veg}$*-mCherry-phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *sigM::kan*-P1-P4 and transforming into BDR2637.

ET24	*ΔylaC::spec*	Constructed by transforming gDNA from HB10233 (Mascher et al., 2007) (Kindly provided by Prof. John Helmann, Cornell University) into PY79.
ET26	*amyE::*P$_{sigX}$*-sigX-gfp-cm*	Constructed by transforming pET26 into PY79.
ET27	*amyE::*P$_{sigX}$*-gfp-cm, sacA::*P$_{veg}$*-mCherry-phleo*	Constructed by transforming pET27 into BDR2637.
ET28	*amyE::*P$_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming pET28 into PY79.
ET29	*thrC::*P$_{hyper\text{-}spank}$*-sigX-mls sacA::*P$_{veg}$*-mCherry-phleo*	Constructed by transforming pET29 into BDR2637.
ET40	*thrC::*P$_{pen}$*-lacIΔ11-cfp-mls, amyE::*P$_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from ET28 into OF83.
ET41	*ΔdltA::cm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *dltA::cm*-P1-P4 and transforming into PY79.
ET42	*ΔdltA::cm, amyE::*P$_{hyper\text{-}spank}$*-sigX-spc.*	Constructed by transforming gDNA from ET41 into ET28.
ET43	*dltA::dltA-yfp-spc, sacA::*P$_{veg}$*-mCherry-phleo*	Constructed by transforming pET43 into BDR2637.
ET44	*amyE::*P$_{xyl}$*-gfp-gp8(phi29)-cm, thrC::*P$_{hyper\text{-}spank}$*-sigX-mls, sacA::*P$_{veg}$*-mCherry-Phleo*	Constructed by sequential transformation of gDNA from ET9 into ET29.
ET47	*yceCDEFGH::cm, sacA::*P$_{veg}$*-mCherry-phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *yceCDEFGH::cm*-P1-P4 and transforming into BDR2637.
ET48	*yrhH::cm, sacA::*P$_{veg}$*-mCherry-phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *yrhH::cm*-P1-P4 and transforming into BDR2637.
ET49	*yabMNOPQ::cm. sacA::*P$_{veg}$*-mCherry-phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *yabMNOPQ::cm*-P1-P4 and transforming into BDR2637.
ET50	*ywbO::cm, sacA::*P$_{veg}$*-mCherry-phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *ywbO::cm*-P1-P4 and transforming into BDR2637.
ET52	*csbB::kan*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *csbB::kan*-P1-P4 and transforming into PY79.
ET53	*pssA::mls sacA::*P$_{veg}$*-mCherry-Phleo*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *pssA::mls*-P1-P4, and transforming into BDR2637.

ET54	*bcrC::cm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *bcrC::cm*-P1-P4, and transforming into PY79.
ET55	*ywnJ::cm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *ywnJ::cm*-P1-P4, and transforming into PY79.
ET56	*tagU::cm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *tagU::mls*-P1-P4, and transforming into PY79.
ET58	*abh::mls sacA::*P_{veg}*-mCherry-Phleo*	Constructed using Gibson assembly kit (NEB, FUSA) utilizing primers *abh::mls*-P1-P4 , and transforming into BDR2637.
ET59	*fatR-yrhJ::mls*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *fatR-yrhJ::mls* -P1-P4 and transforming into PY79.
ET191	*ΔsigX::kan, amyE::*P_{rrnE}*-gfp-spc.*	Constructed by transforming gDNA from ET19 into AR16.
ET261	*ΔyueB::tet, amyE::*P_{sigX}*-sigX-gfp-cm.*	Constructed by transforming gDNA from ET26 into ET6 (Tzipilevich et al., 2017).
ET411	*ΔdltA::cm, sacA::*P_{veg}*-mCherry-phleo*	Constructed by transforming gDNA from ET41 into BDR2637.
ET471	*yceCDEFGH::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc,*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *yceCDEFGH::cm*-P1-P4 and transforming into ET28.
ET481	*yrhH::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc, sacA::*P_{veg}*-mCherry-phleo*	Constructed by transforming gDNA from ET48 into ET28.
ET491	*yabMNOPQ::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc, sacA::*P_{veg}*-mCherry-phleo*	Constructed by transforming gDNA from ET49 into ET28.
ET501	*ywbO::mls, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc, sacA::*P_{veg}*-mCherry-phleo*	Constructed by transforming gDNA from ET50 into ET28.
ET521	*csbB::kan, amyE::*$P_{hyper\text{-}spank}$*- sigX-spc*	Constructed by transforming gDNA from ET52 into ET28.
ET531	*pssA::mls, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from ET53 into ET28.
ET541	*bcrC::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from ET54 into ET28.

ET551	*ywnJ::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from ET55 into ET28.
ET561	*tagU::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from ET56 into ET28.
ET571	*spx::cm, amyE::*$P_{hyper\text{-}spank}$*-sigX-spc*	Constructed by transforming gDNA from OF132 into ET28.
ET581	*abh::mls , amyE::*$P_{hyper\text{-}spank}$*-sigX-spc, sacA::*$P_{veg\text{-}mCherry}$*-Phleo*	Constructed by transforming gDNA from ET58 into ET28.
ET591	*fatR-yrhJ::mls, amyE::*$P_{hyper\text{-}spank}$*-sigX – spc*	Constructed by transforming gDNA from ET59 into ET28.
ET72	*amyE::*P_{xyl}*-dltABCDE-spc*	Constructed by transforming pET72 into PY79.
Phages		
SPP1	Wild type *B. subtilis* phage	Kindly provided by the Bacillus Genetic Stock Center, BGCID 1P7 .
SPP1-*lysin-yfp*	*gp51::gp51-yfp*	Described previously (Tzipilevich et al., 2017).
SPP1-*delX110lacO64*	*delX110lacO64*	Kindly provided by Prof. Paulo Tavares (Gif-sur-Yvette, France).
Phi29	Wild type *B. subtilis* phage	Kindly provided by the Bacillus Genetic Stock Center, BGCID 1P19.

Appendix Table S2. List of primers used in this study

Primer Name	Primer Sequence (5'-3')
ΔsigW::kan-P1	GCCAGTGCCTCCTATGATTTCTGCG
ΔsigW:kan-P2	CTGAGCGAGGGAGCAGAAATTTATCTAACCTCTGCCTTCACCGG
ΔsigW::kan-P3	GTTGACCAGTGCTCCCTGGTGGGGTGATGAAATGAGCTG
ΔsigW::kan-P4	TCCATCAGCCATTGAAGTGTGCTGAG
ΔsigX::kan-P1	TGACGCTTCAAGGCAGATCATGGGT
ΔsigX::kan-P2	CTGAGCGAGGGAGCAGAATTGAAACCCCTCCGTTCACTTTTTTGTCG
ΔsigX::kan-P3	GTTGACCAGTGCTCCCTGAGACCATCGTTCGCCTCAGGATATT TACAA
ΔsigX:kan-P4	TGCATCTGATATGTCATATTTGCCGA
ΔsigM::kan-P1	GATCCATCTGACAGCCAAGTGAATCAC
ΔsigM::kan-P2	CTGAGCGAGGGAGCAGAATGCCTTTTCTCCCCTCTATGTTATAC
ΔsigM::kan-P3	GTTGACCAGTGCTCCCTG GATCCATCTG ACAGCCAAGT GAATCAC
ΔsigM::kan-P4	CTGGATTTCCTTGGTTGGCCCTGTAAC
ΔsigV::cm-P1	ACCTCAATTTTACAGAATATGAATCGAAAGC
ΔsigV::cm-P2	CTGAGCGAGGGAGCAGAATGCAATAAAGGGCTCCTTTTTGAATATACG
ΔsigV::cm-P3	GTTGACCAGTGCTCCCTGTGGATAAGAGATTACAGCA ATTAAGAGAAGAAT
ΔsigV::cm-P4	ATGTATCGATGATGCTGTGTCTTGTGATAC
*sigX-U-Sal*I	TAGGTCGACAAAGGAGGTGAAATGTACAC ATGGAAGAAACCTTTCAATTATTATATG
*sigX-L-Nhe*I	TAGGCTAGCTTAACTGCCGGAAGTTGACTTAACAAC
ΔdltA::cm-P1	CCAGCTGCTGCTGGCACAAATATAGACA
ΔdltA::cm-P2	CTGAGCGAGGGAGCAGAAAGTTATTCTCTCTCCAATTAGAAATCGTTA
ΔdltA::cm-P3	GTTGACCAGTGCTCCCTGATAAAGAATAAAAACGAGCTGTAAGGCG
ΔdltA::cm-P4	ATATATTGCGGCGCGAGCCCGTGC
*dltA-Mfe*I-*U*	TAGCAATTGCATGCGGACACATTTATGTTTTGCGGA
*dltA-Xho*I-*L*	TAGCTCGAGTACAAGAACCTCTTCGCCAATGCG
ΔyceC::cm-P1	CTGCCTCTTCATCTGTTTTTGCGC
ΔyceC::cm-P2	CTGAGCGAGGGAGCAGAAACGATTCACTCCTACTCATCAAAATGTGAA
ΔyceH::cm-P3	GTTGACCAGTGCTCCCTGTAAAAACCCCGCTTGTGGAACATAAGCG
ΔyceH::cm-P4	GCTTCCTGCTGTGCCAATCAAGTAA
ΔyrhH::cm-P1	ATTTTTAGAAAAAGATGTTTACAAAATGGA

ΔyrhH::cm-P2	CTGAGCGAGGGAGCAGAATTTAAACACCTGCTTTTGGTTTCCTATGTT
ΔyrhH::cm-P3	GTTGACCAGTGCTCCCTGAAAATTTCTCGGGAAATATATCCAAGATC
ΔyrhH::cm-P4	GTGGCCGGACACAAAAACACTGGATA
ΔyabM::cm-P1	AGACAAATCGGACTGGGAATGGAAGGG
ΔyabM::cm-P2	CTGAGCGAGGGAGCAGAACCAAAAGCTCCTTCCCAACGTCAGGGGC
ΔyabQ::cm-P3	GTTGACCAGTGCTCCCTGAAGGAGGACCGTCTGGTTTGAA
ΔyabQ::cm-P4	CAAAGGTTTGTCTCTTTCGTCAACAG
ΔywbO::cm-P1	TGAAAAGCAGGGAGGCGGGGACATCTGC
ΔywbO::cm-P2	CTGAGCGAGGGAGCAGAAGATAATCTCCTTTCATACTAAATTG
ΔywbO::cm-P3	GTTGACCAGTGCTCCCTGGGAAAAAGCTCTCGATAAAGAGAGCTT
ΔywbO::cm-P4	GATTTCGATTATCGTGTTTTCGAAATTC
ΔcsbB::kan-P1	CTCCATGGATTTCCTGAGTTTTGGTACGG
ΔcsbB::kan-P2	CTGAGCGAGGGAGCAGAATAAGGCACCTTCTTTTTATTATTCTTTTTAAG
ΔcsbB::kan-P3	GTTGACCAGTGCTCCCTGAAATACCAAGCGCCATTGGCAGTGCTTTTT TTGC
ΔcsbB::kan-P4	CACACGGTTTTTGTAAAAAGAAATAATAAAC
ΔpssA::mls-P1	GATTGAGTCGGAAGCATTCTATGTTTC
ΔpssA::mls-P2	CTGAGCGAGGGAGCAGAAAGTGTAACCAAACCTCCAATTG
ΔpssA::mls-P3	GTTGACCAGTGCTCCCTGTTCAGCAGCTCATAGCGGATTACGGT
ΔpssA::mls-P4	CATCTTTCCCGTATTTTAAGCC
ΔbcrC::cm-P1	TGCTCGGAGCGGTATCTGGGGCAATCGCC
ΔbcrC::cm-P2	CTGAGCGAGGGAGCAGAAATAATCACCTTTTACATTTTTATATTTAG
ΔbcrC::cm-P3	GTTGACCAGTGCTCCCTGGAAAGACAAAAGCCGGCGTCTGAAATCA AGAC
ΔbcrC::cm-P4	TGTTAAAGCTGAAAAAGATCCAGTGCTGGGAG
ΔywnJ::cm-P1	CAGAAAAGTGTTCAGAATGTTGCTGAGG
ΔywnJ::cm-P2	CTGAGCGAGGGAGCAGAAGACATAACCTCCTTTATAACGTAC
ΔywnJA::cm-P3	GTTGACCAGTGCTCCCTGAGCCGGCTGTCTTGATTTCAGACGCCG
ΔywnJ::cm-P4	GGGGTAAAAATAGAAAGCAATGT
ΔtagU::cm-P1	CTTTTCACAGCGCCATATGATTTCAGCTTG
ΔtagU::cm-P2	CTGAGCGAGGGAGCAGAACCTTTGCACCTCGTCTGTTAAATTAC
ΔtagU::cm-P3	GTTGACCAGTGCTCCCTGAACAAAAAGAAGCTTCGCACAATGTGC
ΔtagU::cm-P4	GAACATTATGAAGGAGCGGCAGA
Δabh::mls-P1	GGTTTTTGGAAGACCTGTCACGAGGTCAC

Δabh::mls-P2	CTGAGCGAGGGAGCAGAAAAAAACCCTTCTTCCTTTAAATGTTTC
Δabh::mls-P3	GTTGACCAGTGCTCCCTGAATTATGCTAAAAAAGGCGGAGTGATATCA
Δabh::mls-P4	GCAGCAGCAGCTCGCTGAGCACTAAATC
ΔfatR::mls-P1	CAATGCAGCAGCAAAGGCTGTATGTATTCC
ΔfatR::mls-P2	CTGAGCGAGGGAGCAGAAGTAATCTGACAACCTCCGATTCGTTTACGG
ΔyrhJ::mls-P3	GTTGACCAGTGCTCCCTGAATATAAAATCCCGCCAATCTGATT GG
ΔyrhJ::mls-P4	GGGAATACAAGTCTTTTAATCAG
Δspx::mls-P1	GATACTGATACAGAAAAACGCCTGAGGTTT
Δspx::mls-P2	CTGAGCGAGGGAGCAGAATCATCTTCACTCCTCTAATTAGTAGGATGAACATCTATTT
Δspx::mls-P3	GTTGACCAGTGCTCCCTGTAGATCGTATCATCAAAAGAAGGCTGAGTCAT
Δspx::mls-P4	ATTTCTCTGCTCGTCATTGTTGGGATT
BamH1-Not1-gfp-U	TAGggatccTAGGCGGCCGCcAGTAAAGGAGAAGAACTTTTCACTGGAGTTG
gfp-EcoRI-L	TAGgaattcTTATTTGTATAGTTCATCCATGCCATGTGTAATCC
psigX-U-*BamH*I	TAGggatccCGAGTCTGAATTTGCCGAAGAATC
*sigX-L-Not*I	TAGGCGGCCGCACTGCCGGAAGTTGACTTAACA
*PsigX-L-Not*I	TAGGCGGCCGCGTATCATATAATAATTGAAAGGTTTCTTCCATTTG

References

Jakutyte L, Baptista C, Sao-Jose C, Daugelavicius R, Carballido-Lopez R, Tavares P (2011) Bacteriophage infection in rod-shaped gram-positive bacteria: evidence for a preferential polar route for phage SPP1 entry in *Bacillus subtilis*. *J Bacteriol* 193: 4893-903

Lemon KP, Grossman AD (1998) Localization of bacterial DNA polymerase: evidence for a factory model of replication. *Science* 282: 1516-9

Mascher T, Hachmann AB, Helmann JD (2007) Regulatory overlap and functional redundancy among *Bacillus subtilis* extracytoplasmic function sigma factors. *J Bacteriol* 189: 6919-27

Rosenberg A, Sinai L, Smith Y, Ben-Yehuda S (2012) Dynamic expression of the translational machinery during *Bacillus subtilis* life cycle at a single cell level. *PLoS One* 7: e41921

Tzipilevich E, Habusha M, Ben-Yehuda S (2017) Acquisition of phage sensitivity by bacteria through exchange of phage receptors. *Cell* 168: 186-199 e12

Youngman P, Perkins JB, Losick R (1984) A novel method for the rapid cloning in *Escherichia coli* of *Bacillus subtilis* chromosomal DNA adjacent to Tn917 insertions. *Mol Gen Genet* 195: 424-33