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Supplemental information

A dual-function phage regulator controls the response of cohabiting phage elements via regulation of the bacterial SOS response

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Figure S1

A

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                          10        20        30        40        50        60
                           |         |         |         |         |         |
AriS_10403S       MSNLQVIANDMLPVMENEKGEKFVNARELHQSLQVGKKFATWITDKFNNYGFSKDEDYFP
Gp42_A118         MSNLQVIANEMLPVLENEKGEKFVNARTLHEKLMTTTKFADWIKRRIRQYGFVENEDFFS
Lin2418           MTNLKLFNFEGSKVRTVILDEEPFFVGIDVASILGYSNPQKAMRDHCKKPAESLVNDSLG
                  *:**:::  :   *     .*: . .     .:   .:    :  : .: .     :* :

                          70        80        90       100       110       120
                           |         |         |         |         |         |
AriS_10403S       IL---GESTFGRPRTEYLLTLDTAKELAMVQNNEMGRSIRKYFIEVEKQARKLATEYPTF
Gp42_A118         LLKNEKRAIGGTTSIDYIFTLDSGKELAMVENTEQGRAIRKYFIEVEKQARKLATEYPAF
Lin2418           ---RPRRTLVLSESDVYRLVLRS--DLPSAEKFEN-WLMDEVLPSIRKHGAYMTDDTIEK
                        .:         * :.* :  :*. .:: *     : : : .:.*:.  :: :

                         130       140       150       160       170       180
                           |         |         |         |         |         |
AriS_10403S       SYMIEDPVARAKKWIEEQQEKQ-EALKQLEEQKPKVVFAEAVQTSENTILVKDLATILKQ
Gp42_A118         SYMIDDPVARAKKWIEEQQEKQ-EALNQIEEQKPKVIFADAVQTSENTVLVKDLATILKQ
Lin2418           AITDPDFLIRLATNLKEEKSKRLEAEQKIEAQRPKVLFADAVSDTEGTILIRDLAKLIQQ
                  :    * : *  . ::*::.*: ** :::* *:***:**:**. :*.*:*::***.:::*

                         190       200       210       220       230       240
                           |         |         |         |         |         |
AriS_10403S       KGLDIGQNRLFEWLRCSGYLLN-KGAYYNKPSQKAMNLGLFEQKTHIHTDRNGLMITTYT
Gp42_A118         NGLDIGQNRLFEWLRGSGYLLN-KGTYYNKPSQKAMNLGLFEQKTHIHTDRNGLMVTTYT
Lin2418           NGVDIGEKRLFEWLRQNGYLISRRGTDYNRPTQKSMELGLFKIKETAIMRSSGA-HTAIT
                  :*:***::******* .***:. :*: **:*:**:*:****: *        .*   *: *

                         250       260
                           |         |
AriS_10403S       PRVTGKGQIYLLNKLLEEHNQVII  259 aa
Gp42_A118         PRVTGKGQVYLLNKLLEEHGLVLS  262 aa
Lin2418           AKVTGKGQLYFVNKFLE-QSLVAN  256 aa
                  .:******:*::**:** :. *
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B

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                          10        20        30        40        50        60
                           |         |         |         |         |         |
AriS_10403S       MSNLQVIANDMLPVMENEKGEKFVNARELHQSLQVGKKFATWITDKFNNYGFSKDEDYFP
Gp42_A118         MSNLQVIANEMLPVLENEKGEKFVNARTLHEKLMTTTKFADWIKRRIRQYGFVENEDFFS
                  *********:****:************ **:.* . .*** **. ::.:*** ::**:*.

                          70        80        90       100       110       120
                           |         |         |         |         |         |
AriS_10403S       ILGE---STFGRPRTEYLLTLDTAKELAMVQNNEMGRSIRKYFIEVEKQARKLATEYPTF
Gp42_A118         LLKNEKRAIGGTTSIDYIFTLDSGKELAMVENTEQGRAIRKYFIEVEKQARKLATEYPAF
                  :* :   :  * .  :*::***:.******:*.* **:*******************:*

                         130       140       150       160       170       180
                           |         |         |         |         |         |
AriS_10403S       SYMIEDPVARAKKWIEEQQEKQEALKQLEEQKPKVVFAEAVQTSENTILVKDLATILKQK
Gp42_A118         SYMIDDPVARAKKWIEEQQEKQEALNQIEEQKPKVIFADAVQTSENTVLVKDLATILKQN
                  ****:*******************:*:*******:**:********:***********:

                         190       200       210       220       230       240
                           |         |         |         |         |         |
AriS_10403S       GLDIGQNRLFEWLRCSGYLLNKGAYYNKPSQKAMNLGLFEQKTHIHTDRNGLMITTYTPR
Gp42_A118         GLDIGQNRLFEWLRGSGYLLNKGTYYNKPSQKAMNLGLFEQKTHIHTDRNGLMVTTYTPR
                  ************** ********:*****************************:******

                         250       260
                           |         |
AriS_10403S       VTGKGQIYLLNKLLEEHNQVII  259 aa
Gp42_A118         VTGKGQVYLLNKLLEEHGLVLS  262 aa
                  ******:**********. *:
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C

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                          10        20        30        40        50        60
                           |         |         |         |         |         |
AriS_10403S       MSNLQVIANDMLPVMENEKGEKFVNARELHQSLQVGKKFATWITDKFNNYGFSKDEDYFP
Lin2418           MTNLKLFN-----FEGSKVRTVILDEEPFFVGIDVASILGYSNPQKAMRDHCKKPAE---
                  *:**:::      .  .:    ::: . :. .::*.. :.   .:*  .   .*   :

                          70        80        90       100       110       120
                           |         |         |         |         |         |
AriS_10403S       ILGESTFGRPRTEYLLTLDTAKELAMVQNNEMGRSIRKYFIEVEKQARKLATEYP---TF
Lin2418           SLVNDSLGRPRRTLVLSESDVYRLVLRSDLPSAEKFENWLMDEVLPSIRKHGAYMTDDTI
                   * :.::****   :*: . . .*.: .:   ...:.::::    : :    *    *:

                         130       140       150       160       170       180
                           |         |         |         |         |         |
AriS_10403S       SYMIEDP---VARAKKWIEEQQEKQEALKQLEEQKPKVVFAEAVQTSENTILVKDLATIL
Lin2418           EKAITDPDFLIRLATNLKEEKSKRLEAEQKIEAQRPKVLFADAVSDTEGTILIRDLAKLI
                  .   * **    :  *.:  **:.:: ** :::* *:***:**:**. :*.***::***.::

                         190       200       210       220       230       240
                           |         |         |         |         |         |
AriS_10403S       KQKGLDIGQNRLFEWLRCSGYLLN-KGAYYNKPSQKAMNLGLFEQKTHIHTDRNGLMITT
Lin2418           QQNGVDIGEKRLFEWLRQNGYLISRRGTDYNRPTQKSMELGLFKIKETAIMRSSG-AHTA
                  :*:*:***::******* .***:. :*: **:*:**:*:****: *        .*   *:

                         250       260
                           |         |
AriS_10403S       YTPRVTGKGQIYLLNKLLEEHNQVII  259 aa
Lin2418           ITAKVTGKGQLYFVNKFLE-QSLVAN  256 aa
                   *.:******:*::**:** :. *
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D

```
                          10        20        30        40        50        60
                           |         |         |         |         |         |
AriS_10403S       MSNLQVIANDMLPVMENEKGEKFVNARELHQSLQVGKKFATWITDKFNNYGFSKDEDYFP
Lmo2324           MSNLQVIANEMLPVLENEQGEKFVDARMLHEKLLVNTRFNDWIYRMIGNYGYENGLDFYS
                  *********:****:***:*****:** **:.* *..:*  **    :.***:.:. *::.

                          70        80        90       100       110       120
                           |         |         |         |         |         |
AriS_10403S       ILGESTFGRPRTEYLLTLDTAKELAMVQNNEMGRSIRKYFIEVEKQARKLATEYPTFSYM
Lmo2324           TLSK-TNGRPSTNYFLTLDTAKELAMVQNNEMGRAIRKYFIEVEKQARKLATEYPAFSYM
                   *.: * *** *:*:******************:*******************:****

                         130       140       150       160       170       180
                           |         |         |         |         |         |
AriS_10403S       IEDPVARAKKWIEEQQEKQEALKQLEEQKPKVVFAEAVQTSENTILVKDLATILKQKGLD
Lmo2324           IEDPVARAKKWIEEQQEKQEVLKKLEEQKPKVVFAEAVQTSENTILVKDLATILKQKGLD
                  ********************.**:***********************************

                         190       200       210       220       230       240
                           |         |         |         |         |         |
AriS_10403S       IGQNRLFEWLRCSGYLLNKGAYYNKPSQKAMNLGLFEQKTHIHTDRNGLMITTYTPRVTG
Lmo2324           IGQNRLFEWLRGSGYLLSKGAYYNKPSQKAMNLGLFEQKTHIHTDRNGLMKTTYTPQVTG
                  *********** *****.****************************** *****:***

                         250
                           |
AriS_10403S       KGQIYLLNKLLEEHNQVII  259 aa
Lmo2324           KGQVYLLNKLLEEHNQVII  258 aa
                  ***:**************
```

Figure S1. Sequence alignments of AriS homologs of different *comK*-associated phages. A. Clustal W alignment of deduced amino acid sequences of AriS (LMRG_02920; *comK*-prophage, *L. monocytogenes* strain 10403S), Gp42 (*Listeria* phage A118), and Lin2418 (*comK*-prophage, *L. innocua* strain CLIP 11262). Alignment length: 264 residues. Identical amino acids (28.03%) are shown in red and marked with asterisks, highly similar residues (25.00%) in green and marked with colons, and weakly similar residues (9.09%) in blue and marked with dots; different residues (37.88%) in black and unmarked. Tyrosine residues of AriS and Gp42 (Y99 and Y102, respectively) that were predicted to be phosphorylated are shown in bold and underlined. The glutamine Q144 of AriS (in bold and yellow highlighted) was the last residue of the recombinant amino-terminal domain expressed by pPL2-*antA/B*. The sequence alignment was performed using the public server of the PRABI Rhone-Alpes Bioinformatics Center (France) running the Clustal W program (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html), and then manually curated. **B.** Clustal W alignment of deduced amino acid sequences of AriS (LMRG_02920; *comK*-prophage, *L. monocytogenes* strain 10403S) and Gp42 (Listeria *comK* phage A118). Alignment length: 262 residues. Identical amino acids (77.48%) are shown in red and marked with asterisks, highly similar residues (11.45%) in green and marked with colons, and weakly similar residues (3.82%) in blue and marked with dots; different residues (7.25%) in black and unmarked. Tyrosine residues of AriS and Gp42 (Y99 and Y102, respectively) that were predicted to be phosphorylated are shown in bold and underlined. The glutamine Q144 of AriS (in bold and yellow highlighted) is the last residue of the recombinant amino-terminal domain expressed in pPL2-*antA/B*. The basic sequence alignment was performed using the public server of the PRABI Rhone-Alpes Bioinformatics Center (France) running the Clustal W program (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html), and then manually curated.

C. Clustal W alignment of deduced amino acid sequences of AriS (LMRG_02920; *comK*-prophage, *L. monocytogenes* strain 10403S) and Lin2418 (*comK*-prophage, *L. innocua* strain CLIP 11262). Alignment length: 266 residues. Identical amino acids (30.45%) are shown in red and marked with asterisks, highly similar residues (24.81%) in green and marked with colons, and weakly similar residues (12.03%) in blue and marked with dots; different residues (32.71%) in black and unmarked. A tyrosine residue (Y99) of AriS that was predicted to be phosphorylated is shown in bold and underlined. The glutamine Q144 of AriS (in bold and yellow highlighted) is the last residue of the recombinant amino-terminal domain expressed by pPL2-*antA/B*. The basic sequence alignment was performed using the public server of the PRABI Rhone-Alpes Bioinformatics Center (France) running the Clustal W program (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html), and then manually curated. **D.** Clustal W alignment of deduced amino acid sequences of AriS (LMRG_02920; *comK*-prophage, *L. monocytogenes* strain 10403S) and Lmo2324 (*comK*-prophage, *L. monocytogenes* strain EGD-e). Alignment length: 259 residues. Identical amino acids (83.40%) are shown in red and marked with asterisks, highly similar residues (7.34%) in green and marked with colons, and weakly similar residues (3.86%) in blue and marked with dots; different residues (5.41%) in black and unmarked. Tyrosine residues of AriS and Lmo2324 (Y99 and Y98, respectively) that were predicted to be phosphorylated are shown in bold and underlined. The glutamine Q144 of AriS (in bold and yellow highlighted) is the last residue of the recombinant amino-terminal domain expressed by pPL2-*antA/B*. The basic sequence alignment was performed using the public server of the PRABI Rhone-Alpes Bioinformatics Center (France) running the Clustal W program (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html), and then manually curated.
Related to Figure 4.

Figure S2

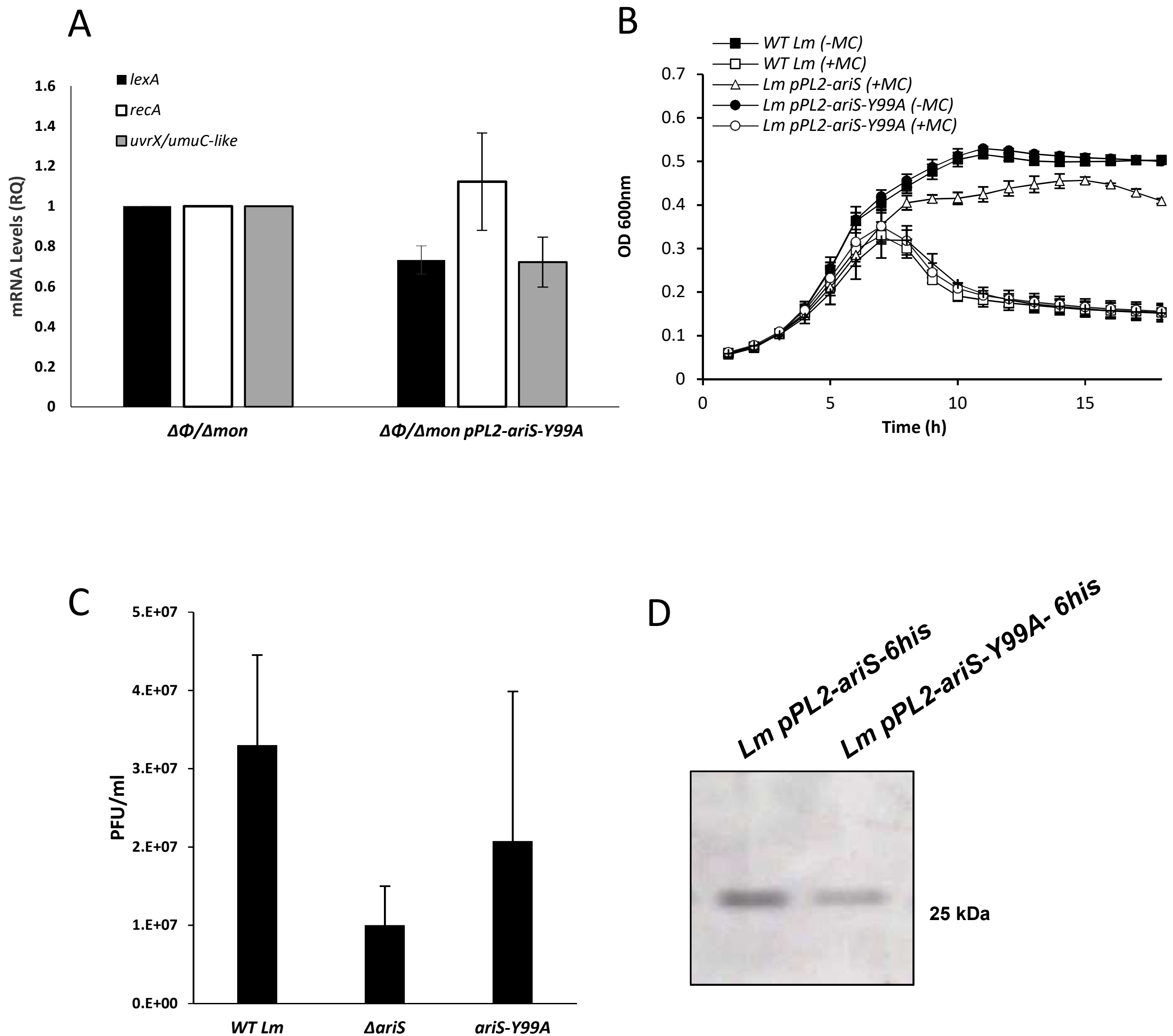

Figure S2. Substitution of AriS tyrosine 99 with alanine abolishes its function.
A. RT-qPCR analysis of representative SOS genes (*recA, lexA* and *uvrX*) in *Δϕ/Δmon* and *Δϕ/Δmon* bacteria expressing AriS-Y99A (using pPL2-*ariS-Y99A*). Indicated strains were grown in BHI medium with MC treatment, at 30 ˚C, for 45 min. mRNA levels are presented as relative quantity (RQ), relative to their levels in WT bacteria. The error bars represent the standard deviation of three independent experiments. **B**. Growth analysis of WT *Lm* with and without overexpression of AriS-Y99A (*Lm* pPL2-*ariS-Y99A*) in the presence (+) or absence (-) of MC, at 30 °C. Overexpression of *ariS* (*Lm* pPL2-*ariS*) was used as a control. The experiment was performed three times and the figure shows a representative result. The error bars represent the standard deviation of three independent experiments, and are sometimes hidden by the symbols. **C.** Virions obtained from MC-treated cultures (6 h after MC treatment) of WT *Lm,* a deletion mutant of the *ariS* gene (*ΔariS*) or *Lm* bacteria possessing the *ariS-Y99A* mutation in the prophage genome (i.e., in the *Lm* genome) were tested on an indicator strain for plaque formation (numerated as plaque-forming units, PFUs). The error bars represent the standard deviation of three independent experiments. **D.** Western blot analysis comparing the protein levels of His-tagged AriS and AriS-Y99A proteins expressed using the pPL2 plasmid. Bacteria were grown in BHI medium for 6 h. Equal amounts of total proteins were separated on 15% SDS-PAGE, blotted and probed with anti-His tag antibody. Related to Figure 7.