

# Communication between viruses guides lysis–lysogeny decisions

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**Temperate viruses can become dormant in their host cells, a process called lysogeny. In every infection, such viruses decide between the lytic and the lysogenic cycles, that is, whether to replicate and lyse their host or to lysogenize and keep the host viable. Here we show that viruses (phages) of the SPbeta group use a small-molecule communication system to coordinate lysis–lysogeny decisions. During infection of its *Bacillus* host cell, the phage produces a six amino-acids-long communication peptide that is released into the medium. In subsequent infections, progeny phages measure the concentration of this peptide and lysogenize if the concentration is sufficiently high. We found that different phages encode different versions of the communication peptide, demonstrating a phage-specific peptide communication code for lysogeny decisions. We term this communication system the ‘arbitrium’ system, and further show that it is encoded by three phage genes: *aimP*, which produces the peptide; *aimR*, the intracellular peptide receptor; and *aimX*, a negative regulator of lysogeny. The arbitrium system enables a descendant phage to ‘communicate’ with its predecessors, that is, to estimate the amount of recent previous infections and hence decide whether to employ the lytic or lysogenic cycle.**

Temperate phages may choose to infect either through the lytic or the lysogenic cycles<sup>1</sup>. Whereas the lytic cycle leads to lysis of the bacterial cell, in the lysogenic cycle the phage genome integrates into the bacterial genome, and the lysogenized bacterium becomes immune to further infection by the same phage<sup>1</sup>. In accordance, growth dynamics of bacteria infected by temperate phages presents partial, but not full, lysis of the culture, followed by culture recovery due to growth of lysogenized bacteria.

The factors influencing lysis–lysogeny decisions have so far been studied in depth mainly for the bacteriophage Lambda, which infects *Escherichia coli*<sup>2</sup>. The decision was shown to be probabilistic in nature, but influenced by the nutritional state of the infected bacterium as well as by the number of co-infecting phage particles<sup>2,3</sup>. In the current study we report that phages that infect *Bacillus* species can rely on small-molecule communication to execute lysis–lysogeny decisions.

## Conditioned media promote phi3T lysogeny

We initiated this study by attempting to test the hypothesis, which was consequently not verified, that bacteria secrete communication molecules to alert other bacteria of phage infection. For this, we prepared conditioned media from cultures of *Bacillus subtilis* strain 168 infected by four different phages: phi29, phi105, rho14 and phi3T (Fig. 1a). To prepare these conditioned media, we infected bacteria growing at mid-log phase with the phage at a multiplicity of infection (MOI) of 1, and collected the conditioned media three hours after infection (or without infection, for control media). The collected media were finely filtered to retrieve small molecules and eliminate remaining bacteria and phages, and a fresh batch of bacteria was added to the conditioned and control media and subsequently infected by the same phage (Fig. 1a).

For phi3T, the infection dynamics in the conditioned medium, as inferred from the bacterial growth curve, was markedly different to the dynamics in the control medium. Whereas a substantial fraction of the infected bacterial culture had lysed in the control medium two

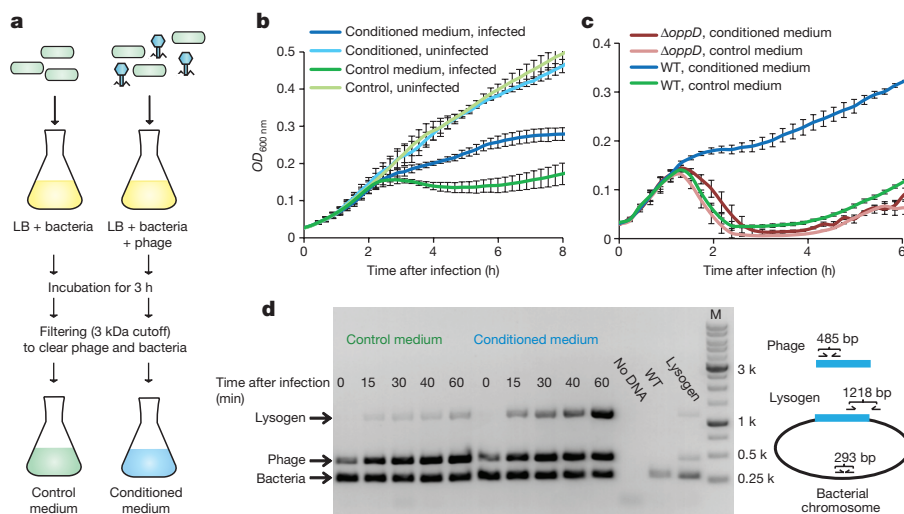
hours after infection, the culture grown in the conditioned medium appeared to be more protected from lysis (Fig. 1b). This effect was not detected for any of the other three phages we tested, for which we did not observe a difference in infection dynamics between the control and conditioned media (Extended Data Fig. 1). Moreover, the conditioned medium prepared from phi3T infection did not affect the infection dynamics of other phages, and conditioned media prepared from other phages did not affect the infection dynamics of phi3T (Extended Data Fig. 1). These results imply that a small molecule is released to the medium during infection of *B. subtilis* by phi3T and this molecule can affect infection dynamics of downstream infections by this phage.

Quorum sensing in Bacilli and other Firmicutes is typically based on short peptides that are secreted into the medium and sensed by intra-cellular or membrane-bound receptors<sup>4–6</sup>. We therefore examined whether the active substance in the medium is proteinaceous by treating the conditioned medium with proteinase K. Infection dynamics in the proteinase-treated conditioned medium were similar to the dynamics in the control medium, suggesting that the active component in the medium is indeed proteinaceous (Extended Data Fig. 2). As communication peptides in *Bacillus* quorum sensing systems are frequently imported into the cell by the oligopeptide permease transporter (OPP), we tested phage infection dynamics in bacteria in which an essential subunit of the OPP transporter *oppD* was deleted (Fig. 1c). The phage-derived conditioned medium lost its effect when the bacteria lacking the functional OPP were infected by phi3T, suggesting that the active substance in the conditioned medium is a peptide sized 3–20 amino acids (aa), which is the size range of peptides that can be imported by the OPP transporter of Gram-positive bacteria<sup>7</sup>.

Examination of the phage infection dynamics in the *oppD* mutant showed increased culture lysis in both control and conditioned media, as compared to infection of wild-type bacteria (Fig. 1c). As phage phi3T is a temperate phage<sup>1,8</sup>, the increased lysis observed in the infection dynamics curve of the *oppD* mutant suggested that the active peptide

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**Figure 1 | Effect of conditioned media on the infection dynamics of phage phi3T.** **a**, Preparation protocol of control and conditioned media. **b**, Growth curves of *B. subtilis* 168 infected with phi3T at MOI = 0.1, in control and conditioned media. **c**, Growth curves of *B. subtilis* strain 3610 (wild type (WT)) and its *oppD* mutant derivative DS4979 (*oppD::kan*)

released to the medium may promote lysogeny of the phage. To assess this hypothesis, we examined phage phi3T integration into the *B. subtilis* genome during infection using a semiquantitative PCR assay. Indeed, increased lysogeny was observed when the bacterial culture was infected in the conditioned medium (Fig. 1d). Combined, these results imply that during phi3T infection a short peptide is released to the medium and, as this peptide accumulates, it acts as a communication agent that affects the lysis–lysogeny decision of later generations of the phage progeny. We named the putative communication molecule ‘arbitrium’ (after the Latin word *arbitrium*, meaning ‘decision’).

### Phage-produced peptide induces lysogeny

Phi3T was isolated four decades ago and was characterized as belonging to the SPbeta group of phages, although to date its genome has not been sequenced<sup>8</sup>. To search for the possible genetic system encoding the arbitrium peptide, we sequenced and analysed the genome of phi3T, which assembled into a single 128-kilobase-pair (kbp) contig containing 201 predicted genes, 128 (64%) of which show substantial homology to SPbeta. We found three open reading frames (ORFs) that were predicted to have an N-terminal signal peptide, suggesting that they are secreted or membrane-localized. Although two of these genes seemed irrelevant (an integral membrane protein and a large nuclease), the third gene exhibited features reminiscent of *Bacillus* quorum sensing peptides (Fig. 2a). Peptides belonging to the Phr family of quorum sensing systems in *B. subtilis* are typically processed from a pre-pro-peptide that contains an N-terminal signal sequence, which is recognized by the secretion (Sec) system and cleaved upon secretion<sup>4</sup>. Once outside the cell, the pro-peptide is further processed by *B. subtilis* extracellular proteases to produce the mature short (5–6 aa) peptide that is typically found on the C-terminal end of the pro-peptide<sup>4</sup>. Our candidate gene encoded a short ORF (43 aa), and displayed both an N-terminal signal sequence and the consensus cleavage site for peptide maturation at its C terminus (Fig. 2a, Extended Data Table 1). If this phi3T-encoded protein is secreted and matured extracellularly, then the predicted mature communication peptide after pro-peptide cleavage would be Ser-Ala-Ile-Arg-Gly-Ala (SAIRGA). Indeed, mass spectrometry analysis confirmed the presence of the SAIRGA peptide in the conditioned medium, but not in the control medium or in medium derived from phi3T in which the gene encoding the peptide was silenced (Extended Data Fig. 3).

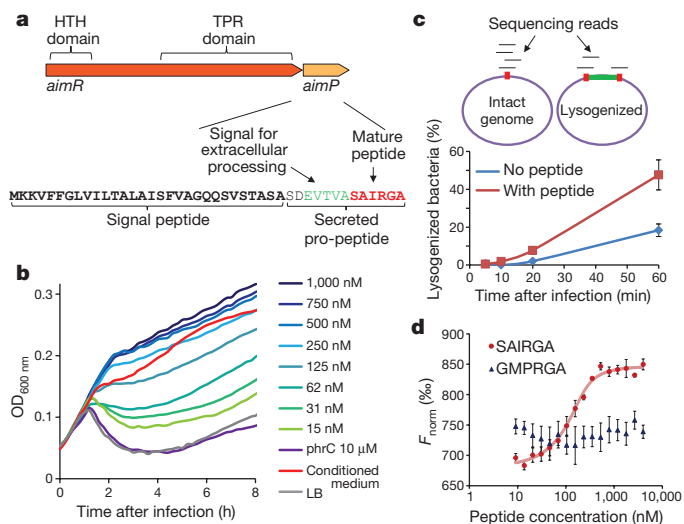
infected by phi3T at MOI = 0.1. In **b** and **c**, data represent an average of 3 biological replicates, each with 3 technical replicates; error bars represent s.e. **d**, Semi-quantitative PCR assay for phage lysogeny during an infection time course of *B. subtilis* 168 with phi3T. No DNA, control without DNA; WT, DNA from uninfected culture; lysogen, genomic DNA of a phi3T lysogen.

To test whether the predicted mature peptide is indeed the arbitrium molecule that influences the phage lysogeny decision, we infected bacteria with phi3T in Luria–Bertani (LB) medium supplemented with increasing amounts of synthesized SAIRGA peptide. A clear concentration-dependent effect on the phage infection dynamics was observed, such that reduced culture lysis was apparent when the medium contained higher concentrations of the synthesized peptide (Fig. 2b). These effects were specific to that peptide, and were not observed for shorter versions of the peptide (SAIRG or AIRGA), or for PhrC, a known quorum sensing peptide of *B. subtilis* (Fig. 2b, Extended Data Fig. 4). The maximal effect on the culture growth curve was observed at SAIRGA peptide concentration of 500 nM, above which the effect seemed to be saturated (Fig. 2b).

To verify that the observed effect of the SAIRGA peptide on the dynamics of the infected culture was the result of increased lysogeny, we directly sequenced the total DNA of bacteria collected from a time course experiment during infection by phi3T with and without the peptide. By comparing the fraction of sequencing reads passing through the intact phage integration site in the bacterial genome to reads demonstrating phage integration at that site, we directly quantified the fraction of lysogenized bacteria at each time point. We found consistently elevated lysogeny in the presence of the SAIRGA peptide, such that 48% (±7.9%) of the bacteria were lysogenized at 60 min after infection, as compared to 18% (±3.3%) of bacteria grown without the addition of the synthesized peptide (Fig. 2c). These results suggest that the phage-encoded gene that we identified is secreted and processed into the mature arbitrium communication peptide that further affects the phage lysis–lysogeny decision. We denoted this gene *aimP*.

To examine the dynamics of arbitrium peptide accumulation during the course of phage infection, we infected bacteria with phi3T at a low MOI (1:1,000 phage:bacteria), and recorded the peptide concentrations in the medium using targeted mass spectrometry during several cycles of phage replication, concurrently recording phage and bacterial counts. Our results show that the peptide accumulates to detectable quantities after 3 cycles of phage infection (time (*t*) = 120), and significantly accumulates after 4 infection cycles when the phage:bacteria ratios reach around 1:1 (Fig. 3). After 150–180 min, the peptide reaches concentrations that are effective in lysogeny promotion as measured in Fig. 2b.

The *aimP* gene is located immediately downstream of a gene encoding a 378-aa-long protein, suggesting that these two genes may

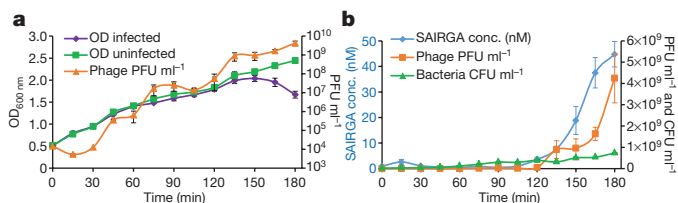


**Figure 2 | The arbitrium peptide and its receptor.** **a**, The arbitrium locus in the phage genome. **b**, Growth curves of *B. subtilis* 168 infected by phi3T at MOI = 0.1, in LB media supplemented with synthesized SAIRGA peptide. Numbers represent peptide concentrations. Average of 3 biological replicates, each with 3 technical replicates. **c**, Sequencing-based quantitative determination of the fraction of lysogenized bacteria. Top, Schematics of the analysis. Per cent of lysogeny was calculated as the fraction of reads spanning the phage–bacteria integration junction out of total reads covering this junction. Integration junction is red; integrated phage is green. Bottom, the percentage of lysogenized bacteria during infection of *B. subtilis* 168 with phi3T at MOI = 2. Average of three biological replicates, error bars denote s.e. Synthesized SAIRGA peptide was added at 1 µM. **d**, Microscale thermophoresis analysis of the binding between purified AimR and synthesized SAIRGA or GMPRGA peptides. Average and s.e. of three replicates.  $F_{\text{norm}}$ , normalized fluorescence.

be functionally linked. This upstream gene encodes a predicted tetratricopeptide repeat (TPR) domain, typical of intracellular peptide receptors of the RRNPP family in quorum sensing systems of Gram-positive bacteria<sup>9–11</sup> (Fig. 2a). We therefore hypothesized that this upstream gene, which we denoted *aimR*, is the receptor of the AimP-derived arbitrium peptide. To test this hypothesis, we purified C-terminal His-tagged AimR, and used microscale thermophoresis to measure the binding between the purified receptor and the synthesized arbitrium peptide. This analysis showed high-affinity binding, at an effective peptide concentration of  $EC_{50} = 138$  nM (118–162 nM at confidence interval of 95%), between the phi3T AimR receptor and the cognate SAIRGA peptide (Fig. 2d), confirming that AimR probably functions as the intracellular receptor of the arbitrium SAIRGA peptide.

### Phage-specific communication code

To appreciate the abundance of this system in nature, we used a homology search to find homologues of the *aimR* gene in available sequenced genomes. We found 112 instances of AimR homologues, virtually all of them in *Bacillus* phages or in prophages found integrated within Bacilli genomes, suggesting that this gene primarily fulfils a phage-related function (Fig. 4a; Supplementary Table 1). In all cases, *aimR* homologues were found upstream of *aimP* candidate genes, that is, short polypeptides encoding an N-terminal signal peptide, followed by a pro-peptide conforming with the processing maturation signal of the *Bacillus* extracellular proteases (Supplementary Table 1; Extended Data Table 1). Although the sequences of the predicted mature peptides were diverse, all of them maintained strict rules for their sequence composition, with an obligatory glycine residue at the fifth position, glycine or alanine at the sixth position, and a preference for positively charged residue at the fourth position (Fig. 4b, c). Whereas most (72%) of the phages harbouring homologues of the arbitrium system belonged to



**Figure 3 | Accumulation of arbitrium peptide during an infection time course.** *B. subtilis* 168 culture was grown to optical density (OD) = 0.5 and then infected at  $t = 0$  by phi3T at MOI = 0.001. PFU, plaque-forming units; CFU, colony-forming units, sampled from the uninfected control. For peptide, PFU and OD shown is average of triplicate (except for  $t = 30$ , where it is duplicate), with error bars representing s.e. CFU was measured in duplicates, and error bars represent the two measured points around the average. **a**, Growth of infected and uninfected bacteria, and phage, during the infection time course. **b**, Accumulation of peptide during the infection course as compared to bacteria and phage.

the SPbeta group, arbitrium was also found in other types of phages, including phi105-like and Mu-like phages (Supplementary Table 1).

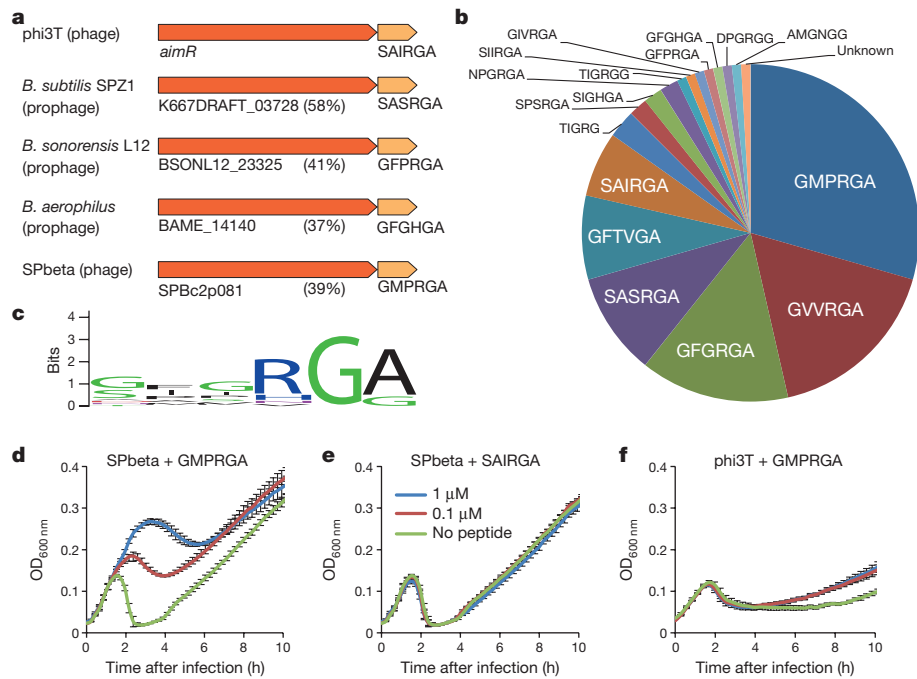
To test the hypothesis that the phage-encoded communication peptides guide phage lysogeny in a sequence-specific manner, we examined the infection dynamics of the SPbeta phage, which is heteroimmune relative to phi3T, and in which we identified a homologue of the AimR–AimP system. The predicted mature AimP-derived arbitrium peptide of SPbeta was GMPRGA, a sequence that differs by the 3 N-terminal amino acids from the SAIRGA peptide of phi3T. Whereas the GMPRGA peptide promoted lysogeny of SPbeta, it did not affect the lysogeny profile of phi3T; and similarly, the phi3T-derived SAIRGA peptide had no effect on the infection dynamics of SPbeta (Fig. 4d–f). In accordance, the SPbeta-derived GMPRGA peptide did not show specific binding to the phi3T AimR receptor (Fig. 2d). These results demonstrate a sequence-specific peptide code that guides phage lysogeny in a phage-specific manner.

### Mechanism of the arbitrium system

In communication systems of Gram-positive bacteria, the binding of the communication peptide to its receptor usually leads to reprogramming of the transcriptional response. This can occur either directly, when the receptor is a transcription regulator, such as in the cases of the PrgX<sup>12,13</sup> in Enterococci, the PlcR<sup>14–16</sup> of the *Bacillus cereus* group, and in other systems<sup>17,18</sup>, or indirectly, as in the case of Rap–Phr systems of Bacilli, in which the receptor is a phosphatase that regulates downstream transcriptional regulators by dephosphorylation<sup>19,20</sup>, or steric interference<sup>21</sup>. The presence of a predicted helix–turn–helix (HTH) motif in the N terminus of AimR suggests that the receptor of the arbitrium system directly binds DNA. To examine whether AimR binds the phage DNA *in vivo*, we engineered a His-tagged *aimR* gene into a *B. subtilis* 168 strain in which CRISPR interference technology<sup>22</sup> was used to silence the expression of the phage AimR gene, but not the cloned His-tagged AimR (Methods). We then performed a ChIP–seq assay 15 min after phage infection with and without the presence of the arbitrium peptide. Sequencing of the DNA bound to AimR clearly showed that AimR binds a single site in the phage genome, directly downstream of the *aimP* gene (Fig. 5a, b). Moreover, this binding only occurred when the arbitrium peptide was lacking from the medium, suggesting that binding of the arbitrium peptide to its AimR receptor leads to dissociation of the receptor from its binding site on the phage DNA.

During the process of AimR purification, we noticed that the protein migrates as homodimer in a gel filtration column. Upon addition of the phi3T-derived SAIRGA peptide, however, the protein strictly migrated as a monomer (Fig. 5c). This observation was corroborated using a crosslinking assay (Extended Data Fig. 5). These results suggest that the arbitrium peptide transfers the signal through alteration of the oligomeric state of its AimR receptor from a DNA-binding dimer





**Figure 4 | A peptide communication code guiding lysogeny in *Bacillus* phages.** **a**, Selected instances of AimR homologues in sequenced genomes. Locus tags are indicated for AimR homologues, along with the percentage of amino acid sequence identity to the phi3T AimR. Mature arbitrium peptide is indicated below the AimP homologue. **b**, Distribution of arbitrium peptides among 112 homologues of AimP. **c**, Amino acid profile of arbitrium peptide types. Bits denote information content. **d**, Growth curves of *B. subtilis* BEST7003 infected by SPbeta at MOI = 0.1, in LB

media supplemented with synthesized GMPRGA peptide. The BEST7003 strain was used for SPbeta infection as the *B. subtilis* 168 strain is naturally immune to SPbeta. **e**, Growth curves of *B. subtilis* BEST7003 infected by SPbeta at MOI = 0.1, in LB media supplemented with synthesized SAIRGA peptide. **f**, Growth curves of *B. subtilis* 168 infected by phi3T at MOI = 0.1, in LB media supplemented with synthesized GMPRGA peptide. Data in **d–f** represent average of 3 biological replicates, each with 3 technical replicates; error bars represent s.e.

to a peptide-bound, dissociated monomer. Addition of the SPbeta GMPRGA peptide did not lead to a change in the AimR oligomeric state, again pointing to the high specificity between the peptide and its receptor in the arbitrium system (Fig. 5c, Extended Data Fig. 5).

To examine whether binding of the arbitrium peptide to its AimR receptor leads to a transcriptional response in the phage genome, we applied RNA-seq to RNA extracted from bacteria during a time course of infection with and without the peptide. The most notable change in the expression was observed for a single transcript, which we denoted *aimX*, that was immediately downstream to the AimR DNA-binding site (Fig. 5d–g, Extended Data Fig. 6). This transcript showed substantial expression in the absence of the arbitrium peptide, starting 10 min after infection; however, its expression was reduced more than 20-fold when the medium was supplemented by 1 μM of the SAIRGA peptide (Fig. 5d–g).

The above results suggest that AimR, when bound to the phage DNA as a dimer in the absence of the arbitrium peptide, is a transcriptional activator of AimX. Indeed, when AimR was silenced using dCas9, the expression of AimX was markedly reduced (Fig. 5d). Moreover, silencing of AimR resulted in increased lysogeny, suggesting that binding of AimR to the phage DNA inhibits lysogeny (or promotes lysis), possibly by activating the expression of AimX (Fig. 5h).

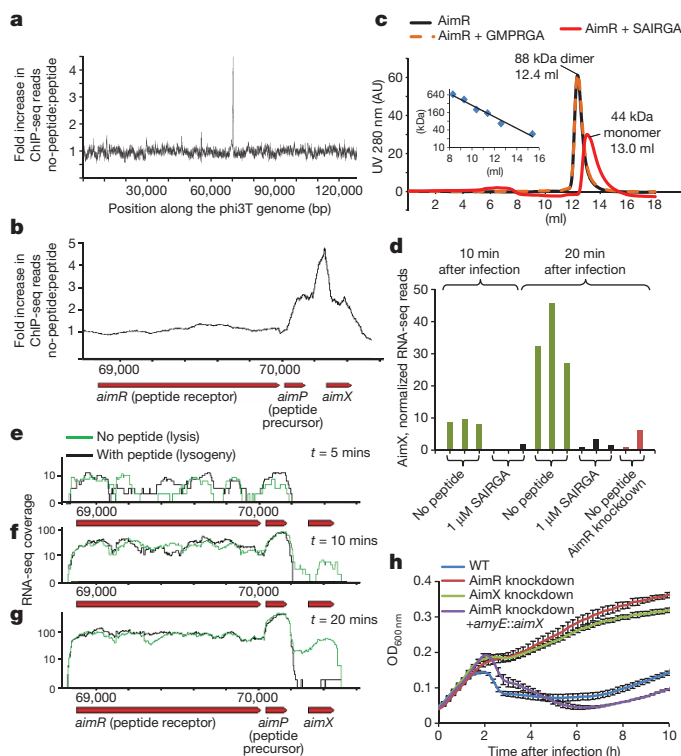
As AimR knockdown did not lead to a marked transcriptional effect for any transcript except AimX at 20 min after infection (Extended Data Fig. 6), we hypothesized that the main function of AimR is to control the expression of *aimX*, and that the expressed AimX works downstream of the AimR–AimP communication system to execute the lysis–lysogeny decision. Consistent with this hypothesis, knockdown of AimX using dCas9 resulted in increased lysogeny (Fig. 5h). Moreover, complementing with an ectopic AimX on the background of AimR knockdown (in which AimX expression is naturally silenced, Fig. 5d) resulted in culture lysis, demonstrating that AimX functions downstream of AimR, and works as the inhibitor of the lysogenic or the promoter of the lytic cycle (Fig. 5h).

The *aimX* transcript encodes a short (51 aa) ORF, followed by an unusually long stretch of additional 62 bases of non-coding RNA region that terminates in a stem-loop structure. A homologue of this ORF follows the *aimR–aimP* operon in only 17 of the 112 phages in which the arbitrium system was identified (Supplementary Table 1). We therefore examined the expression of the arbitrium locus in the SPbeta phage, in which the AimX ORF is absent. We found that although no detectable ORF was present in the region downstream *aimP*, a non-coding RNA was expressed from that intergenic region in SPbeta (Extended Data Fig. 7). Moreover, this non-coding RNA was responsive to the arbitrium peptide such that in the presence of the SPbeta arbitrium, its transcription was abolished, akin to AimX in phage phi3T (Extended Data Fig. 7). These results imply that AimX possibly exerts its function as a regulatory non-coding RNA in a manner that remains to be clarified in future studies.

Together, our results point to the following model: on initial infection of a bacterial culture, phi3T expresses the early genes AimR and AimP. AimR, as a dimer, activates the expression of AimX, which, in turn, blocks the pathway to lysogeny and/or promotes the lytic cycle in a manner that is as yet unknown. At the same time, AimP is secreted into the medium and processed into the mature arbitrium peptide. After several cycles of infection, arbitrium peptides will accumulate in the medium. Thereafter, when a phage particle infects an as-yet-uninfected bacterium, the concentration of the arbitrium peptide, which is internalized into the bacteria by the OPP transporter, will be high enough to bind the AimR receptor. Upon binding, the AimR receptor changes its oligomeric state from the active dimer to the inactive monomer, silencing the AimX lysogeny-inhibitor and leading to lysogeny (Fig. 6).

## Discussion

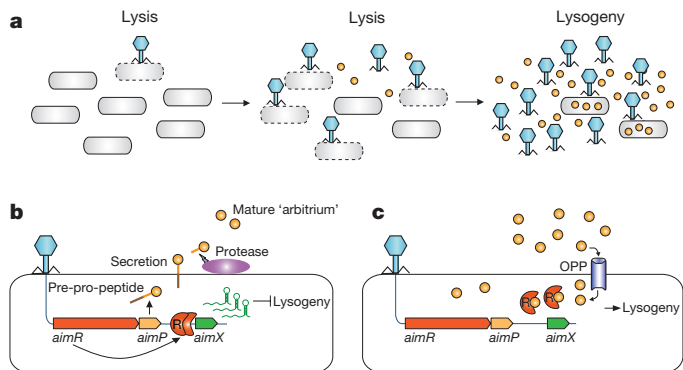
We have shown that phages belonging to the SPbeta group of phages use communication peptides to decide whether to enter a lytic cycle



**Figure 5 | DNA binding and transcription regulation in the arbitrium system.** **a**, ChIP-seq of His-tagged AimR 15 min after infection with or without 1  $\mu$ M of SAIRGA peptide. Shown is the ratio, along the phage genome, between sequenced pulled-down DNA during infection without the peptide and DNA pulled-down when the peptide was present in the medium. **b**, Same as **a**; shown is a zoomed-in region in the phage genome. **c**, Gel-filtration results of purified AimR with or without the presence of either SAIRGA or GMPRGA peptide. Inset presents a calibration curve for the gel filtration using proteins of known sizes. **d**, Expression of the AimX gene during infection. Data presented for individual biological replicates. **e–g**, RNA-seq coverage of the arbitrium locus at 5 min (**e**), 10 min (**f**) and 20 min (**g**) after infection. **h**, Growth curves of wild-type and dCas9-silenced bacterial strains during phi3T-infection. Strains were infected at  $t = 0$  at MOI = 0.1. Shown is average of 3 biological replicates, each with 3 technical replicates; error bars represent s.e.

or lysogenize the infected bacterium. In a sense, the communication mechanism we describe allows a descendant phage to ‘communicate’ with its ancestors, that is, to measure the number of predecessor phages that completed successful infections in previous cycles. The biological logic behind this strategy is clear: when a single phage encounters a bacterial colony, there is ample prey for the progeny phages that are produced from the first cycles of infection, and hence a lytic cycle is preferred. In later stages of the infection dynamics, the number of bacterial cells is reduced to a point that progeny phages are at risk of no longer having a new host to infect. Then, it is logical for the phage to switch into lysogeny to preserve chances for viable reproduction. The arbitrium system provides an elegant mechanism for a phage particle to estimate the amount of recent previous infections and hence decide whether to employ the lytic or lysogenic cycle.

Although the presence of the arbitrium peptide in the medium has a notable effect on phage lysogeny (Fig. 2b, c), this effect is not absolute: even at maximal peptide concentrations, only around 50% of the cells become lysogenized after 60 min, indicating that the probability of lysogeny significantly rises, but is still a stochastic event (Fig. 2c). Similarly, in the absence of the peptide, the phage does not become obligatorily lytic, as observed from an experiment with phi3T in which *aimP* was deleted (Extended Data Fig. 8). Therefore, there seems to be a basal, possibly stochastic, tendency of the phage to lysogenize



**Figure 6 | Mechanistic model for communication-based lysis-lysogeny decisions.** **a**, Dynamics of arbitrium accumulation during infection of a bacterial culture by phage. **b**, At the first encounter of a phage with a bacterial population, the early genes *aimR* and *aimP* are expressed immediately upon infection. AimR, as a dimer, activates AimX expression. AimX is an inhibitor of lysogeny, possibly as a regulatory non-coding RNA, directing the phage to a lytic cycle. At the same time AimP is expressed, secreted and processed extracellularly to produce the mature peptide. **c**, At later stages of the infection dynamics, the arbitrium peptide accumulates in the medium and is internalized into the bacteria by the OPP transporter. Now, when the phage infects the bacterium, the expressed AimR receptor binds the arbitrium molecules and cannot activate the expression of AimX, leading to lysogeny preference.

independently, which is supplemented by the arbitrium system that allows reference to previous infections.

Peptide communication systems are known to exist on conjugative plasmids both in *Bacilli*<sup>23,24</sup> and in *Enterococci*<sup>12,13</sup>, where they can regulate the plasmids horizontal transfer. The presence of bacterial quorum sensing systems on phage genomes has been noted previously<sup>4,25</sup>, but their roles were either unknown or linked to interference with the host bacterial communication<sup>25</sup>. To our knowledge, this study is the first demonstration of actual small-molecule communication between viruses. We found this communication to be manifested through the arbitrium system in a large group of *Bacillus* phages; we envision that such a strategy will also be discovered for other phages using different communication systems. Moreover, this strategy may not be limited to phages, and possibly also guide dormancy and/or lysis decisions in viruses infecting eukaryotes.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** Z.E. directly performed or was involved in all experiments unless otherwise stated. I.S.L. performed conditioned media and proteinase K assays. S.D. annotated phi3T genome. A.S. analysed the mass spectrometry results. Y.P. and S.A. expressed and purified AimR–6×His. A.S.A., A.L. and S.M. constructed strains. G.A. performed microscale thermophoresis, crosslinking and ChIP–seq experiments. M.S. performed RNA-seq experiments. R.S. supervised the project.

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## METHODS

**Data reporting.** No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

**Phages and strains in this study.** *Bacillus subtilis* str. 168 was obtained from the Bacillus Genetic Stock Center (BGSC). *Bacillus subtilis* BEST7003 was obtained from I. Mitsuhiro at Keio University, Japan. The DS4979 strain (*oppD::kan*)<sup>26</sup> was obtained from A. Eldar at Tel Aviv University. The 3610 strain was obtained from I. Kolodkin-Gal at the Weizmann Institute. *Bacillus* phages phi3T, SPbeta, phi105, rho14 and phi29 were all obtained from the BGSC.

**Oligonucleotides and reagents.** All oligonucleotides used in this study were purchased from either Sigma or Integrated DNA Technologies. Native synthetic peptides were purchased from Peptide 2.0 Inc., at 98% purity, desalted. Isotopically labelled peptide was purchased from GenScript, purified using HPLC to 95% purity.

**Phi3T genome sequencing.** Phage DNA was extracted using the Qiagen DNeasy blood and tissue kit (cat. 69504) and subjected to Illumina sequencing on a MiSeq machine with single read length of 50 bases. Genome was assembled using the Velvet software package<sup>27</sup> with hash length = 31. Genome was assembled into 3 contigs that were further manually assembled on the basis of end-homologies. Assembled genome was permuted to conform with the SPbeta phage genome coordinates (accession NC001884). ORFs were predicted using Glimmer<sup>28</sup> with options -o50 -g110 -t30, and products were assigned to the ORFs using rpsblast against the Conserved Domain Database version 3.12 with *e*-value of  $1 \times 10^{-5}$ . Intergenic regions were searched for short ORFs using blastx against the nr database (*e*-value  $\leq 1 \times 10^{-5}$ ), and the ORFs were adjusted accordingly. ORFs were also annotated using blastp against the SPbeta phage proteins (*e*-value  $\leq 1 \times 10^{-5}$ ) (Supplementary Table 2). The annotated genome was deposited in GenBank under accession KY030782.

**Preparation of conditioned and control media.** Overnight cultures of *B. subtilis* 168 were diluted 1:50 in LB media supplemented with 0.1 mM MnCl<sub>2</sub> and 5 mM MgCl<sub>2</sub>, and incubated at 37°C with shaking until reaching OD<sub>600</sub> = 0.5. For the conditioned medium only, phages were added to the bacterial culture at MOI = 1. Cultures were then incubated for 3 h at 37°C with shaking. The media were centrifuged at 4,000 r.p.m. for 10 min at 4°C and the supernatant was filtered with 0.2-µm filter (GE Healthcare Life Sciences, Whatman, cat. 10462200). Phages and large molecules were further filtered out from the media by using Amicon Ultra centrifugal filters at a cutoff of 3,000 nominal molecular weight limit (3 kDa) (Milipore, cat. UFC900324). A plaque assay was performed in order to verify that no phages were left in the medium. The medium was kept at 4°C for up to two months.

For the proteinase K assay, 7.5 mg (per reaction) of proteinase-K-Agarose from *Tritirachium album* (Sigma, cat. P9290) was washed twice with 750 µl of sterile water and then resuspended with 750 µl of LB supplemented with 0.1 mM MnCl<sub>2</sub> and 5 mM MgCl<sub>2</sub>. Next the tubes were centrifuged again, and the supernatant was discarded. 1.5 ml of phi3T-derived conditioned medium or control medium was added to a tube containing the washed proteinase K. The media were incubated for 2 h at 37°C with the proteinase K. The media were centrifuged and the supernatants were collected for the infection assay.

**Growth dynamics of phage-infected cultures.** Overnight cultures of bacteria were diluted 1:100 in LB media and incubated at 37°C with shaking until reaching OD<sub>600</sub> = 0.1. The bacterial culture was centrifuged at 4,000 r.p.m. for 10 min at room temperature. The supernatant was discarded and the pellet was resuspended in LB medium supplemented with 0.1 mM MnCl<sub>2</sub> and 5 mM MgCl<sub>2</sub> at 10% of the initial volume. The concentrated bacterial culture was added to control medium, conditioned medium or medium supplemented with synthesized arbitrium peptide in a ratio of 1:9 (bacteria to medium). The culture was incubated for 1 h at room temperature. The culture was then infected with phages at MOI = 0.1. Optical density measurements at a wavelength of 600 nm were performed using a TECAN Infinite 200 plate reader in a 96-well plate.

For infection experiments that did not include conditioned medium or addition of a synthesized peptide, the diluted overnight culture was grown to early-logarithmic phase and then infected as above.

**Infection dynamics.** For the infection dynamics experiments described in Fig. 3, an overnight culture of *B. subtilis* 168 was diluted 1 in 100 and incubated at 37°C with shaking until reaching OD<sub>600</sub> = 0.5. Next, 0.1 mM MnCl<sub>2</sub> and 5 mM MgCl<sub>2</sub> were supplemented to the medium, and the bacterial culture was split into two flasks. One flask was supplemented with phage phi3T at MOI = 0.001, the second was not infected and used as control. Samples for measuring PFU ml<sup>-1</sup>, CFU ml<sup>-1</sup> and peptide concentration were taken every 15 min during the time course of 180 min. OD<sub>600</sub> was also measured every 15 min. For CFU measurements, samples were taken from the uninfected culture, diluted in 1 × PBS, and plated on LB agar plates. For the PFU measurements, samples were taken from the infected

culture, centrifuged for 5 min at 4,000 r.p.m. and in 4°C. Next, the supernatant was filtered using a 0.2 µm filter (GE Healthcare Life Sciences, Whatman, cat. 10462200). Phage concentrations from these media were measured using a drop assay in serial dilution. For peptide concentration, the media that were filtered previously with the 0.2 µm filter were filtered twice using Amicon Ultra centrifugal filters at a cutoff of 3,000 nominal molecule weight limit (3 kDa) (Milipore, cat. UFC900324). The media were sent to targeted mass spectrometry analysis. Infection dynamics experiments were performed in biological triplicates.

**Semi-quantitative PCR assay for lysogeny.** An overnight culture of bacteria was diluted 1 in 100 until reaching OD<sub>600</sub> = 0.1. Medium was replaced (with conditioned medium or control medium) as described above, and incubated for 1 h at room temperature. Bacteria were infected by phi3T at MOI = 5. Cell pellets were collected at times 0, 15, 30, 40 and 60 min after infection in the presence of conditioned or control medium. DNA was extracted using Qiagen DNeasy blood and tissue kit (cat. 69504). Multiplex PCR assays to detect phage phi3T DNA (both as free-phage DNA and as a lysogen), *B. subtilis* DNA, and the junction between integrated phage and bacterial genome were performed as previously described ref. 29. As controls, genomic DNA from uninfected *B. subtilis* 168 bacteria, as well as a phi3T lysogen (1L1 clone obtained from BGSC), were subject to the same multiplex PCR procedure.

**Mass spectrometry.** Conditioned media was filtered using 3 kDa molecular weight cutoff filters (Millipore). The low molecular weight fraction was spiked with isotopically labelled internal standard peptide (SA(Ile(13C6,15N))RGA) at a final concentration of 50 pg µl<sup>-1</sup>, and then subjected to desalting using the Oasis HLB uElution plates (Waters Corp.). Samples were dried and stored at -80°C until analysis. Ultra liquid chromatography/mass spectrometry grade solvents were used for all chromatographic steps. Each sample was loaded using split-less nano-Ultra Performance Liquid Chromatography (10 kpsi nanoAcquity; Waters). The mobile phase was: (1) H<sub>2</sub>O and 0.1% formic acid and (2) acetonitrile and 0.1% formic acid. Desalting of the samples was performed on-line using a reversed-phase C18 trapping column (180 µm internal diameter, 20 mm length, 5 µm particle size; Waters). The peptides were then separated using a T3 HSS nano-column (75 µm internal diameter, 250 mm length, 1.8 µm particle size; Waters) at 0.35 µl min<sup>-1</sup>. For the experiments that are presented in Fig. 3 and Extended Data Fig. 3d, e, peptides were separated on the T3 HSS nano-column at 45°C and eluted from the column into the mass spectrometer using the following gradient: 4% to 15% B in 15 min, 15% to 90% B in 5 min, maintained at 90% for 5 min and then back to initial conditions. For the experiments that are presented in Extended Data Fig. 3a-c, peptides were separated on the T3 HSS nano-column at 55°C and eluted from the column into the mass spectrometer using the following gradient: 4% to 35% B in 50 min, 35% to 90% B in 5 min, maintained at 90% for 5 min and then back to initial conditions.

The nanoUPLC was coupled online through a nanoESI emitter (10 µm tip; New Objective) to a quadrupole orbitrap mass spectrometer (Q Exactive Plus, Thermo Scientific) using a FlexIon nanospray apparatus (Proxeon). Data was acquired in parallel reaction monitoring mode, targeting precursor masses 287.6690 and 291.1776, the doubly charged form of native and isotopically labelled SAIRGA peptide, respectively. MS2 resolution was set to 35,000 and the maximum injection time set to 200 ms, automatic gain control was set to  $2 \times 10^5$ . Raw data was imported to Skyline software<sup>30</sup> version 3.5. Product ion intensities were extracted and the total area under the curve (AUC) was calculated. Peptide concentration was determined on the basis of the ratio of the AUC from the native versus isotopically labelled peptide, multiplied by the concentration of the standard. A calibration curve was run to verify that the measurements are within the linear dynamic range. As the isotopically labelled peptide was added to the filtered conditioned media, we performed a recovery experiment to calculate the recovery factor in order to obtain the concentration in the unfiltered conditioned medium. We calculated recovery of 40%, thus all concentration measurements were multiplied by 2.5 to yield actual concentration in the pre-filtered medium.

**AimR expression and purification.** AimR was cloned into the expression vector pET28a (Novagen) using Transfer-PCR (TPCR)<sup>31</sup>. Cloning was performed using the forward primer 5'-TTTGTTTAACTTTAAGAAGGAGATATACCATGATTAAGAATGCGAAAAGG-3' and the reverse primer 5'-CTTTGTTAGCAGCCGGATCTTAGTGGTGGTGGTGGTGAATAGAGATAAGGTTTAATAATTCAAG-3'. The integrity of the newly constructed clone, designated pET28-AimR-cHis, was verified by Sanger sequencing. The AimR clone was expressed in *E. coli* BL21 (DE3) cells. Freshly transformed BL21 (DE3) cells harbouring pET28-AimR-cHis were inoculated as a starter culture into 250 ml non-baffled flask containing 100 ml of LB medium supplemented with 30 µg ml<sup>-1</sup> kanamycin. Growth was performed at 37°C and 250 r.p.m. agitation for around 16 h. Following overnight growth, 12.5 ml from the starter culture were inoculated into 51 non-baffled flasks containing 1.25 l of LB medium (1:100 dilution of the starter culture, starting OD<sub>600</sub> was about 0.05) supplemented with 30 µg ml<sup>-1</sup> kanamycin. Large-scale growth

and protein expression was performed using a constant shaking of 200 r.p.m. Cell growth was performed at 37 °C, until OD<sub>600</sub> reached around 0.7. Protein expression was performed at 15 °C for about 18 h using 200 μM IPTG (isopropyl β-D-1-thiogalactopyranoside) as an inducer.

For protein purification, the cell pellet was resuspended in lysis buffer (50 mM Tris (pH 8), 0.3 M NaCl, 20 mM Imidazole, 2 mM DTT, 0.2 mg ml<sup>-1</sup> lysozyme, 1 μg ml<sup>-1</sup> DNase, protease inhibitor cocktail (Calbiochem)), disrupted by a cell disrupter at 4 °C and clarified at 15,000g for 30 min. The clarified lysate was loaded onto a HisTrap FF 5 ml column (GE Healthcare) and washed with buffer containing 50 mM Tris (pH 8), 0.3 M NaCl, 20 mM imidazole and 2 mM DTT. AimR was eluted from the column in one step with the same buffer containing 0.5 M imidazole. Fractions containing AimR were pooled and injected to a size exclusion column (HiLoad 16/60 Superdex 200 prepgrade, GE Healthcare) equilibrated with 20 mM Tris (pH 8), 0.3 M NaCl, 2 mM TCEP. Fractions containing pure AimR were pooled and flash-frozen in aliquots using liquid nitrogen. Purity was verified by the protein running as a single band on SDS PAGE gel stained with Coomassie blue (10 μl at 2 mg ml<sup>-1</sup> loaded per lane), and migration as a single peak on an analytical size-exclusion column.

Pure AimR was injected to an analytical gel-filtration column (Superdex 200 Increase 10/30 GL, GE Healthcare) equilibrated with buffer containing 20 mM Tris (pH 8), 0.3 M NaCl, 2 mM TCEP. The migration position of pure AimR was compared to that of AimR-peptide mixtures at the following molar ratios: AimR and SAIRGA peptide (1:2), AimR and GMPRGA peptide (1:1). The column was calibrated (inset of Fig. 5c) by monitoring the migration positions of the following known proteins/polymers: blue dextran (2,000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), beta-amylase (200 kDa), alcohol dehydrogenase (150 kDa), albumin (66 kDa), carbonic anhydrase (29 kDa).

**Crosslinking assays.** Crosslinking was generated by reacting the amine-reactive reagent bis(sulfosuccinimidyl) suberate (BS3) with the AimR protein in the presence or absence of the 6 amino acid peptide SAIRGA. Purified AimR (1.37 mg ml<sup>-1</sup>) was eluted from a gel-filtration column in 20 mM Tris (pH 8), 300 mM NaCl, 2 mM TCEP. The protein was then dialysed against 20 mM Hepes (pH 7.5), 150 mM NaCl, 2 mM DTT in a 10 kDa cutoff/3 ml G2 Dialysis Cassette (TermoFisher scientific) according to the manufacturer instructions. The dialysed protein was centrifuged at 21,000g at 4 °C for 5 min yielding 0.5 mg ml<sup>-1</sup> (11.1 μM). The protein was then mixed with SAIRGA peptide (final concentration, 100 μM) dissolved in water or with equal amount of volume without peptide and incubated at room temperature for 5 min. The protein samples were then mixed for 30 min with five different BS3 concentrations (0.25, 0.5, 1.0, 2.0, 4.0 mM). BS3 was prepared by dissolving 1 mg in 70 μl water yielding a 25 mM stock concentration. After the incubation period, samples were quenched for 15 min (25 °C) with Tris-HCl (pH 7.5) in a final concentration of 50 mM. Results were analysed by electrophoresis in protein Screen Tape using a TapeStation (Agilent Technologies). Sizes of dimer and monomer were verified using a Novex Bolt 4–12% Bis-Tris gel using an SDS-PAGE apparatus (Novex Bolt).

**Microscale thermophoresis.** Two-step purified 6×His-tagged AimR stored in Tris-NaCl buffer (50 mM Tris (pH 8.0), 150 mM NaCl, 2 mM TCEP) at –80 °C was thawed on ice and centrifuged at 21,000g for 10 min at 4 °C before the analysis. Peptides (SAIRGA and GMPRGA) were solubilized in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl to a final concentration of 100 μM. AimR was diluted to 200 nM and was incubated with 16 different peptide concentrations varying between 9–4,000 nM, which were prepared in Tris-NaCl buffer containing 0.1% [v/v] pluronic acid (NanoTemper). Roughly 3 μl were loaded into NT.LabelFree Zero-Background Premium Coated Capillaries (NanoTemper) and inserted into a Monolith NT.LabelFree device (NanoTemper). Microscale thermophoresis experiments were performed at 60% microscale thermophoresis power (infrared laser) and 20% LED power at 23 °C using the Monolith NT.LabelFree instrument (Nanotemper). Ratios between normalized initial fluorescence and post-temperature-jump and thermophoresis were calculated and averaged from three independent runs (runs were incubated for 20 min at room temperature before the measurement). A plot of fluorescence ratios versus peptide concentration was used to assess the binding capacity of the phage protein and its cognate peptide ligand.

**ChIP-seq.** For the ChIP-seq experiments, cell cultures of *B. subtilis* 168 AimR-His tag complementation strain (described in CRISPRi section of the methods) were grown in 100 ml to OD<sub>600</sub> of 0.1 in LB at 37 °C. Then, 50 ml of culture was centrifuged at 4,000 r.p.m. for 10 min at room temperature. The pellets were then suspended with 25 ml LB either containing or lacking the peptide (SAIRGA) at a final concentration of 1 μM. The cultures were then placed for an additional hour in incubation at room temperature with shaking. After 1 h, the cultures were infected with phage (MOI = 0.5). After 15 min of phage infection, the cultures were centrifuged for 5 min at 4 °C at 3,000g. The supernatant was discarded and the pellets were resuspended with 1 ml of ice-cold 1×PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl (pH 7.4)).

For the formaldehyde fixation of protein to DNA, the 1 ml PBS resuspended pellets were then mixed with 62.5 μl formaldehyde (Thermoscientific; 16% formaldehyde solution (w/v) methanol-free ampule) yielding a final formaldehyde concentration of 1% (w/v) within the solution. The formaldehyde-containing cell suspension was incubated at room temperature for 10 min with mild agitation. After 10 min, 75 μl 2 M glycine (final concentration, 150 mM) was added to quench residual formaldehyde. Glycine-containing samples were kept on ice for an additional 10 min followed by centrifugation at 5500g, 4 °C for 1 min. Supernatant was discarded and pellets were washed with a 1 ml of ice-cold 1×PBS. Centrifugation and wash were repeated three times for each sample.

The cell pellets were then suspended with 600 μl lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl and a protease inhibitor mix (cComplete ULTRA Tablets Roche). The lysis-buffer-containing cells were applied on a lysing matrix B (MP Biomedicals) 0.1 mm silica beads. The mixture and beads were placed in a FastPrep-24 (MP Biomedicals) apparatus and shaken aggressively for 20 s at 6 m s<sup>-1</sup>, 4 °C. The beads were then separated from lysed cells by centrifugation of 10,000g for 1 min at 4 °C according to the manufacturer instructions. Then, 300 μl of the supernatant, containing the lysed cell mixture, was transferred into a 1.5 ml Bioruptor Plus TPX microtubes (diagenode) and kept on ice for 10 min (according to manufacturer's instructions). The sample was then sonicated at 4 °C with full power for 15 min (30 s off/on cycles) using the Bioruptor plus (Diagenode) apparatus. Sonication sheared the DNA to an average size of around 500 bp. Samples were then centrifuged at 20,000g for 10 min at 4 °C.

For the immunoprecipitation experiments, supernatant containing the lysis buffer and cellular content was then mixed with Triton X-100 and deoxycholate yielding a final IP-buffer composition containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% (vol/vol) Triton X-100, 0.1% (wt/vol) sodium deoxycholate supplemented with proteases inhibitors (cComplete ULTRA Tablets Roche). Anti-6×His tag ChIP-grade antibody (abcam (ab9108)) was then added to sonicated samples and gently mixed over night at 4 °C. In parallel, protein G Dynabeads (100.04D; Invitrogen) were washed three times with immunoprecipitation buffer.

DNA-protein-antibody complexes (300 μl) were captured with a 100 μl Dynabeads protein G by mixing them for 1 h at room temperature with rotation. 0.72 μl of 0.5 M EDTA (final concentration, 1 mM) was added to that mixture to prevent DNase activity at room temperature. Beads were applied to a magnetic stand (Qiagen) and washed three times with immunoprecipitation buffer (200 μl) at room temperature. Two elution steps were applied with 100 μl and 50 μl of elution buffer (50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1% (wt/vol) SDS) for 15 min at 65 °C on a rocking platform. Eluate (100 μl) was incubated with 5 μl of proteinase K (20 mg ml<sup>-1</sup>) for 1 h at 50 °C and then for 6 h at 65 °C. Immunoprecipitated DNA was recovered using a QIAquick PCR Purification kit (Qiagen). Immunoprecipitated DNA was then converted into NGS libraries using an existing protocol<sup>32</sup> and was sequenced on a NextSeq500 Illumina machine generating 75-nt-long reads.

**Sequencing-based assay for lysogeny.** An overnight culture of bacteria was diluted 1:100 until reaching OD<sub>600</sub> = 0.1. Medium was replaced as described above (LB supplemented with 0.1 mM MnCl<sub>2</sub> and 5 mM MgCl<sub>2</sub> with or without SAIRGA), and incubated for 1 h at room temperature. Bacteria were infected by phi3T at MOI = 2. Cell pellets were collected at times 5, 10, 20 and 60 min after infection in the presence or absence of SAIRGA peptide at 1 μM final concentration in the medium. DNA was extracted using Qiagen DNeasy blood and tissue kit (cat. 69504) and subjected to Illumina-based whole-genome sequencing on NextSeq500. The relative abundance of lysogens in each sample was estimated using the number of reads mapped to the uninterrupted integration site versus reads mapped to the integration junction spanning the prophage DNA on one end and the bacterial DNA on the other end.

**CRISPRi experiments.** Construction of strains silencing phage genes was done by inserting a dCas9 construct controlled by a xylose promoter<sup>22</sup> (a gift from the Carol Gross laboratory, UCSF), into the *lacA* region in *B. subtilis* 168 genome, and sgRNA with spacers targeting the gene of choice under constitutive promoter to *thrC* region (Spacer targeting *aimR*: 5'-ACCATTACTTTTCATAAC-3', spacer targeting *aimX*: 5'-TTTCCGCTTCATTCTCAAGA-3', spacer targeting *aimP*: 5'-TGTCCTTACTGATTGTTGGC-3'). Infection assays in CRISPRi strains were performed in LB supplemented with 0.1 mM MnCl<sub>2</sub>, 5 mM MgCl<sub>2</sub> and 0.2% xylose.

For complementation assays of *aimR* on the background of *aimR*-silenced CRISPRi strain, *aimR* was amplified from the phi3T genome using the forward primer 5'-AAGAATTCCTCATTTGTGTTTAGGTAAAATAAGAATTC-3' and the reverse primer 5'-AACTGCAGTTAGTGGTGGTGGTGGTG AATAGAGATAAGGTTTAATAATTCAAG-3' (that includes a 6×His tag). These primers amplify *aimR* together with 158 bases from its upstream region, with a 6×His at its C terminus. The amplified fragment was cloned into the pBS1C plasmid (received from BGSC). Then, the native protospacer adjacent motif in the complemented *aimR* gene was changed by a synonymous point



mutation (C>A at codon 20 of the *aimR* gene) using a primer set containing the point mutation and Gibson assembly. The modified gene was then integrated into the *amyE* locus in the *B. subtilis* genome. The *aimX* complementation was constructed on the background of *aimR*-silenced CRISPRi strain. For this, *aimX* was amplified from the  $\phi$ 3T genome using the forward primer 5'-AACTAGTTTAAAGGGAAAGTTCAGAAATTC-3' and reverse primer 5'-AACTGCAGTCCGTTGCCAATAGATTATGC-3'. These primers amplify *aimX* together with 60 bases from its upstream region and 107 bases from its downstream region (containing the gene terminator). The amplified *aimX* was cloned into a pBS1C plasmid modified to contain a xylose promoter, and was then integrated into the *amyE* locus in the *B. subtilis* genome.

**RNA-seq.** For determining the difference in gene expression with and without the peptide, bacteria (*B. subtilis* 168 or *B. subtilis* BEST7003) were incubated for 1 h in LB medium supplemented with 0.1 mM MnCl<sub>2</sub> and 5 mM MgCl<sub>2</sub> and in the presence or absence of 1  $\mu$ M of synthesized SAIRGA peptide. The bacteria were then infected with  $\phi$ 3T (MOI = 0.1). Cell pellets were then collected at 0, 5, 10 and 20 min after infection.

RNA extraction and RNA-seq was performed as described in ref. 33. Briefly, pellets were lysed using the Fastprep homogenizer (MP Biomedicals), and RNA was extracted with the FastRNA PRO blue kit (MP Biomedicals, 116025050) according to the manufacturer's instructions. RNA samples were treated with TURBO DNase (DNase) (Life Technologies, AM2238) and fragmented with fragmentation buffer (Ambion) in 72 °C for 1 min 45 s. The reactions were cleaned by adding  $\times$  2.5 SPRI beads. The beads were washed twice with 80% EtOH, and air dried for 5 min. The RNA was eluted using H<sub>2</sub>O. Ribosomal RNA was depleted by using the Ribo-Zero rRNA Removal Kit (epicentre, MRZB12424). Strand-specific RNA-seq was performed using the NEBNext Ultra Directional RNA Library Prep Kit (NEB, E7420) with the following adjustments: all cleanup stages were performed using  $\times$  1.8 SPRI beads, and only one cleanup step was performed after the end repair step.

For determining the effect of CRISPRi silencing of the *aimR* gene, bacteria at early logarithmic stage were infected with  $\phi$ 3T (MOI = 0.1), and cell pellets were collected 20 min after infection. RNA-seq libraries were prepared as described above.

RNA-seq libraries were sequenced using the Illumina NextSeq500 platform. Sequenced reads were demultiplexed and adapters were trimmed using 'fastx clipper' with default parameters. Reads were mapped to the reference genomes (gene annotation and sequences were downloaded from GenBank: NC\_000964 for *Bacillus subtilis* str. 168, AP012496 for *Bacillus subtilis* BEST7003, NC\_001884 for SPbeta and KY030782 for  $\phi$ 3T) using NovoAlign (Novocraft) V3.02.02 with default parameters. All downstream analyses and normalized genome-wide RNA-seq coverage maps were generated as described in ref. 33.

**Differential expression analysis.** Reads per gene were calculated for each biological replicate of time-point 20 min post infection, with and without the synthetic peptide, and normalized relative to the total mapped reads hitting the phage genome in each replicate. log<sub>10</sub> transformation of the average of 3 replicates per gene in each condition was used to plot Extended Data Fig. 6 and calculate the fold change of gene *aimX*.

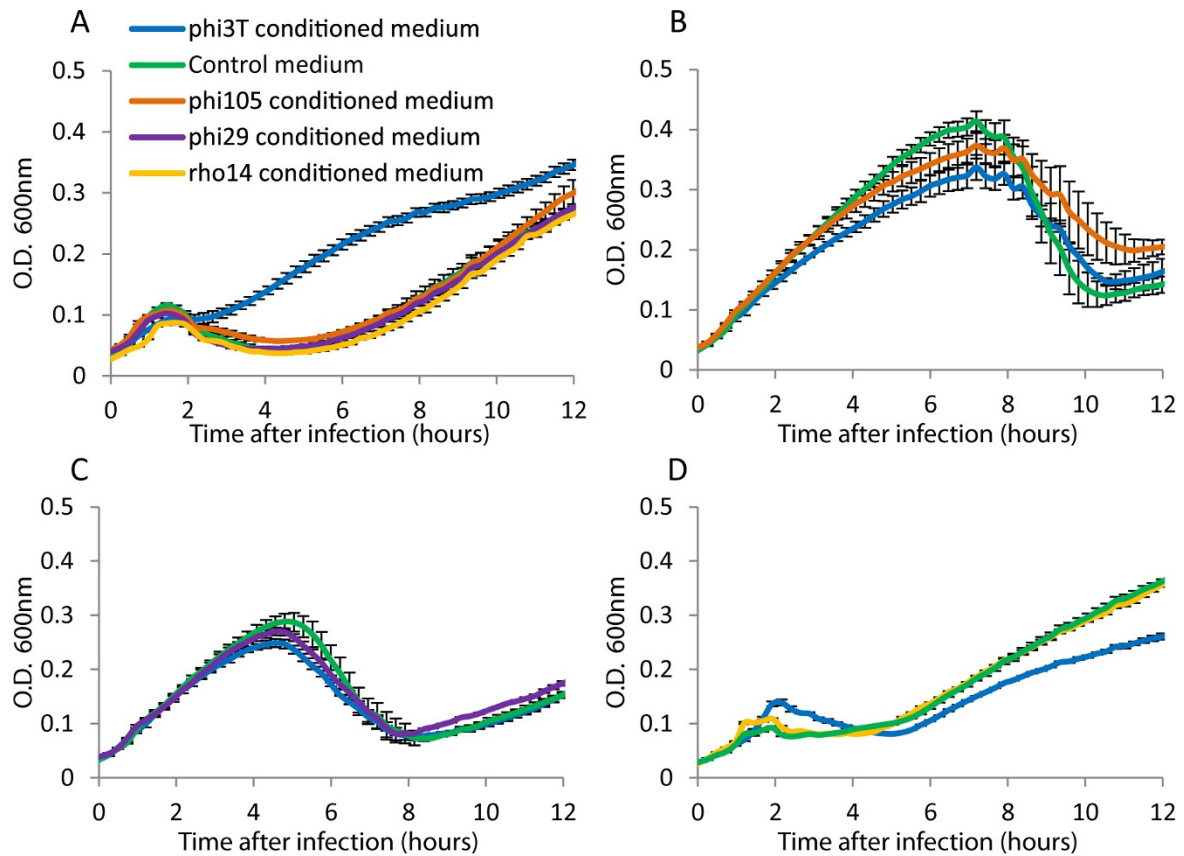
**Identification of AimR homologues and the arbitrium peptide code.** Homologues for the  $\phi$ 3T AimR receptor were searched for using the BLAST option in the Integrated Microbial Genomes (IMG) web server (<https://img.jgi.doe.gov/cgi-bin/mer/main.cgi>). The  $\phi$ 3T AimR was provided as a query sequence and was searched against all isolate genomes with an e-value threshold of  $1 \times 10^{-35}$ . The gene neighbourhood for each AimR homologue was visually inspected using the IMG 'gene neighborhood' representation, and genes found located next to proteins that were annotated as phage proteins were considered as found in a prophage. The immediate downstream gene for each AimR homologue was considered the respective *aimP* gene if it contained a signal peptide, as predicted by the IMG web server. If no immediate downstream gene was annotated, the intergenic region

immediately downstream to the AimR homologue was translated using the Expsy Translate Tool (<http://web.expsy.org/translate/>), and short translated ORFs were inspected for the AimP signature. Phages with the arbitrium system were typed according to the following parameters: prophage genome length, prophage gene order, and Phaster<sup>34</sup> and Virfam<sup>35</sup> classifications. Results of this analysis are presented in Supplementary Table 1.

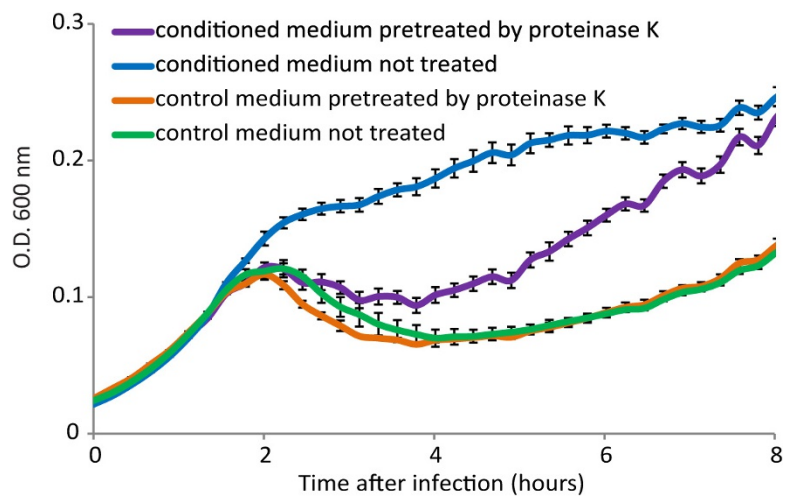
**Construction of aimP phage deletion strain.** In order to exchange *aimP* with an antibiotic cassette, a plasmid composed of four fragments was constructed as follows: (1) the upstream region flanking *aimP* (1,000 bp upstream to *aimP* start codon) was amplified using the forward primer 5'-TGAAGAGCAACTTTAAGTG-3' and the reverse primer 5'-TATTCTCACCTCCTTTCAAAT-3'. (2) The Spectinomycin resistance cassette was amplified from plasmid p15a-pDR110 using the forward primer 5'-GTTTGACAAATTTGAAAGGAGGTGAGAATCGAATGGCGA TTTTCGTTTC-3' and the reverse primer 5'-GTATTATGTATTACCTATTCAA TTATTTAACCCCTATGCAAGGGTTTATT-3'. (3) The downstream region flanking *aimP* (1001 bp downstream to *aimP* stop codon) was amplified using the forward primer 5'-TTAATAATTAAGTGGTAATACATAATAC-3' and the reverse primer 5'-CAGATTCTATTGCTTCATTAAG-3'. (4) The origin of replication and ampicillin resistance was amplified from the plasmid pBS1C using the forward primer 5'-TTATGACTTAATGAAGCAAATAGAAATCTGC GGGACTTACCGAAAGAAAC-3' and the reverse primer 5'-AAGAAGTATT CACTTAAAGTTGCTCTTCATCTTGACACTCCTTATTTGA-3'. The four fragments were assembled together using NEBuilder HiFi DNA Assembly Master Mix (NEB, E2621S). The plasmid was transformed into NEB 10-beta Competent *E. coli* (NEB, C30191) for amplification of the plasmid, and then transformed into *B. subtilis* BEST7003 harbouring a  $\phi$ 3T prophage. The deletion of *aimP* was verified using Illumina sequencing. The phages were then induced using Mitomycin C (Sigma, M0503). Conditioned media preparation and infection dynamics assays with this phage were performed as described above.

**Data availability.** The  $\phi$ 3T genome was deposited in GenBank under accession number KY030782. Phage gene annotations are in Supplementary Table 2. AimR homologues list and the corresponding peptides for each one are in Supplementary Table 1. RNA-seq and ChIP-seq data were deposited in the European Nucleotide Database (ENA), accession number PRJEB18541.

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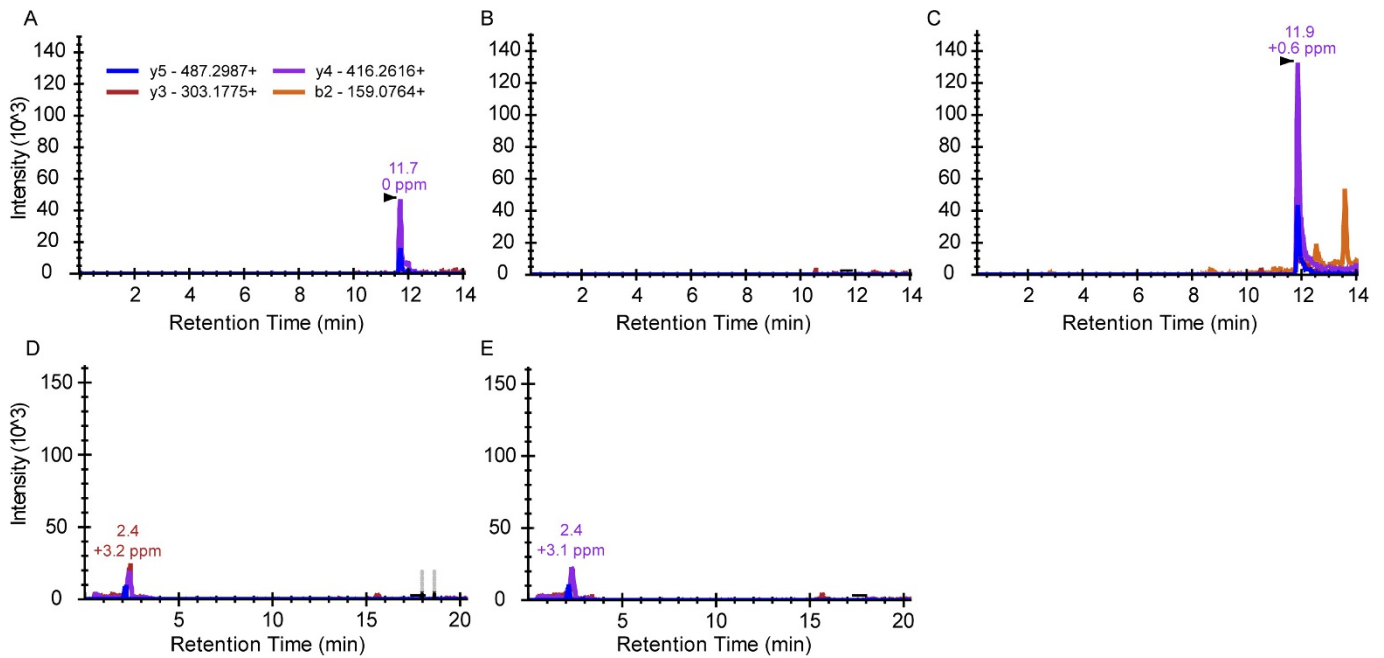


**Extended Data Figure 1 | Specificity of conditioned media.** Conditioned media were prepared using initial infection of *B. subtilis* 168 by four phages: phi3T, phi105, phi29 and rho14. Presented are growth curves of *B. subtilis* 168 in the different media, infected with each phage at MOI = 0.1: phi3T (a), phi105 (b), phi29 (c) and rho14 (d). Data represent average of 3 replicates, and error bars represent s.e.



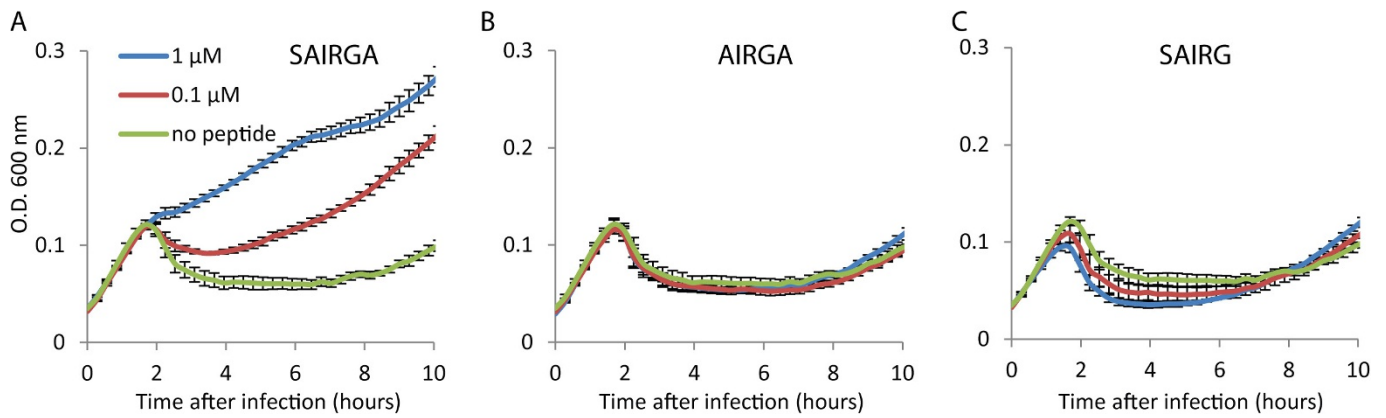
**Extended Data Figure 2 | Proteinase K treatment reduces the effect of conditioned medium.** Growth curves of *B. subtilis* 168 infected by phi3T at MOI = 0.1, in control and conditioned media, with and without pre-treatment with proteinase K. Data represent average of 3 technical replicates, and error bars represent s.e.



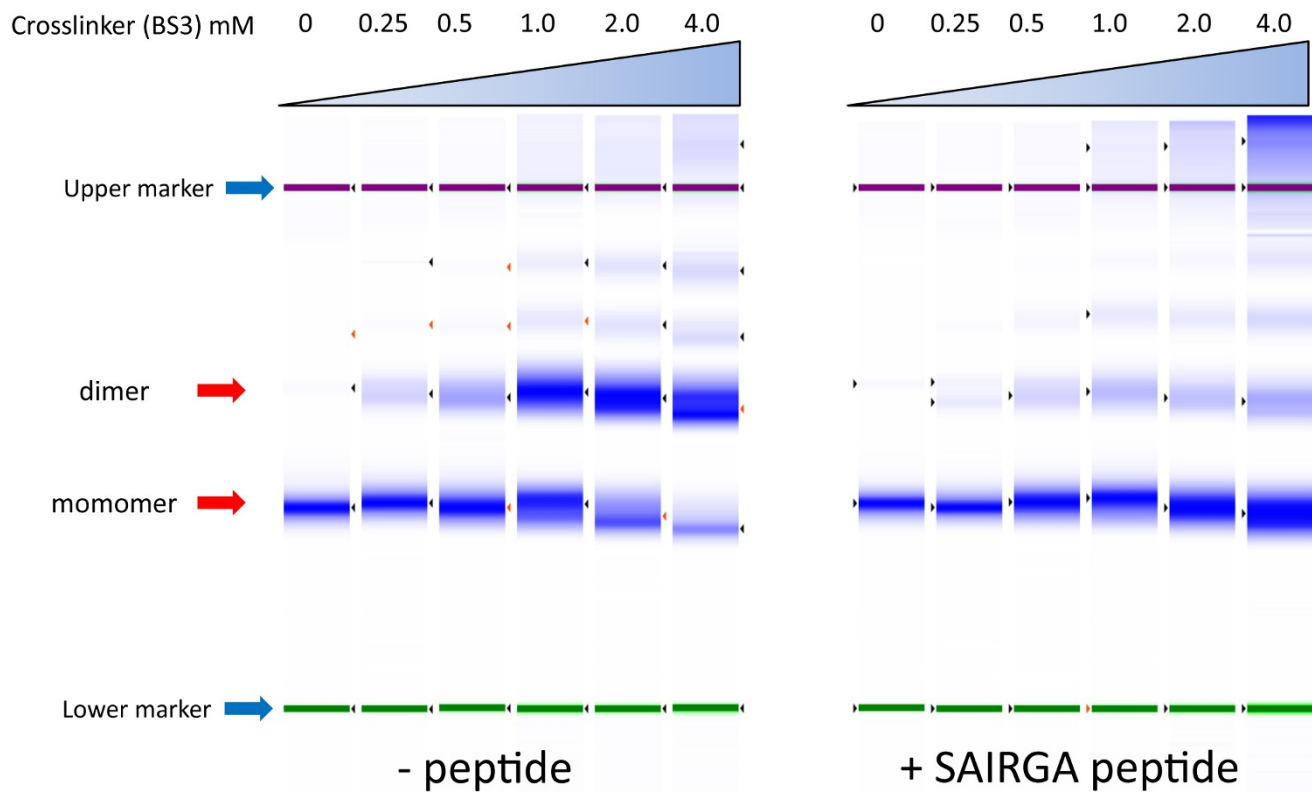


**Extended Data Figure 3 | Mass spectrometry verifies the presence of the SAIRGA peptide in conditioned medium.** Presented are extracted ion chromatograms of targeted mass spectrometry analysis experiment. B2, Y3, Y4 and Y5 refer to fragmentation products of the peptides made by the instrument in the tandem mass spectrometry process, with the expected  $m/z$  ion masses indicated. The  $y$  axis represents the ion intensity of each

fragment ion (arbitrary units). **a**, Reference synthesized SAIRGA peptide at 100nM concentration in LB. **b**, Control medium from *B. subtilis* 168. **c**, phi3T-infected *B. subtilis* 168. **d**, *B. subtilis* 168 expressing dCas9 with a spacer targeting *aimP*. **e**, phi3T-infected *B. subtilis* 168 expressing dCas9 with a spacer targeting *aimP*. Arrowhead depicts the expected retention time of the SAIRGA peptide (**a**, **c**).



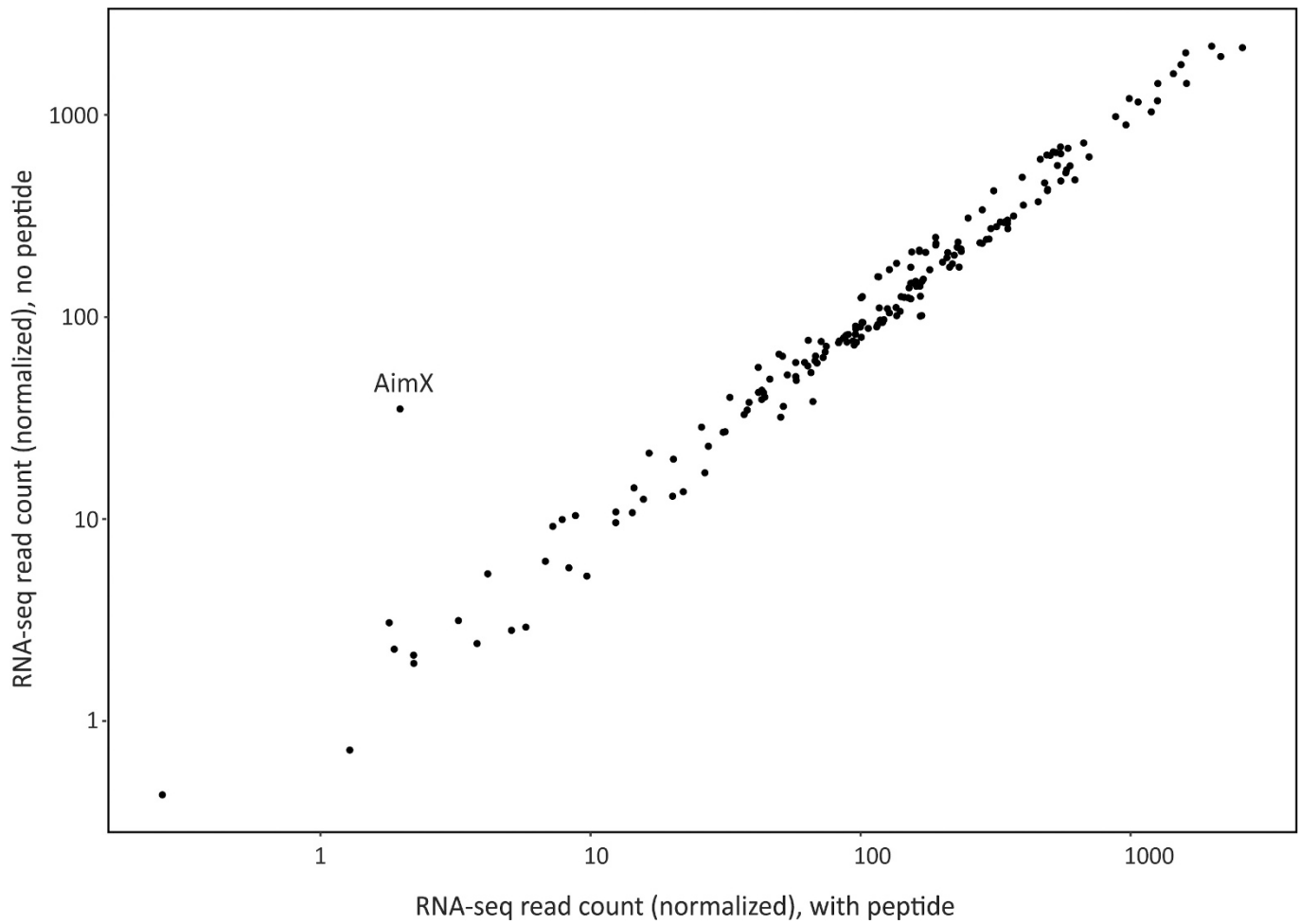
**Extended Data Figure 4 | 5 aa versions of the 6 aa arbitrary peptide do not guide lysogeny.** Growth curves of *B. subtilis* 168 infected by phi3T at MOI = 0.1, in LB media supplemented with synthesized SAIRGA, AIRGA or SAIRG peptide. Shown is average of 3 biological replicates, each with 3 technical replicates. Error bars represent s.e.



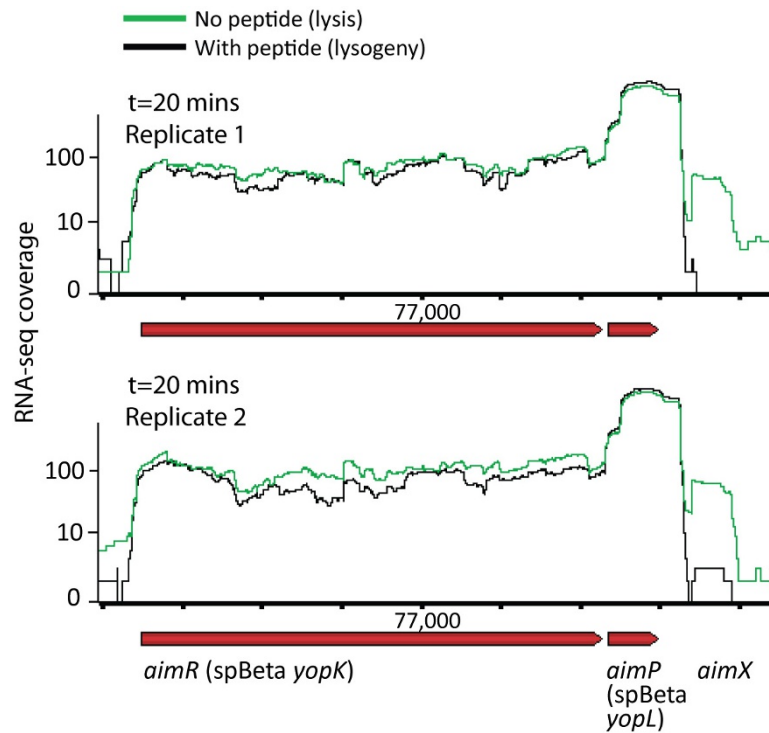
**Extended Data Figure 5 | Exposure of AimR to SAIRGA peptide reduces propensity for dimerization.** Purified AimR was eluted from a gel-filtration column, dialysed, then mixed with SAIRGA peptide (final concentration,  $100\mu\text{M}$ ) dissolved in water or with equal amount of volume without peptide and incubated at room temperature for 5 min. The protein samples were then mixed for 30 min with different concentrations

of the crosslinker BS3 bis(sulfosuccinimidyl)suberate. Presented are electrophoresis results analysed using the TapeStation instrument (Agilent Technologies), showing that in the absence of peptide, purified AimR tends to preferentially be crosslinked into dimers, whereas in the presence of the SAIRGA peptide, dimerization is substantially reduced.



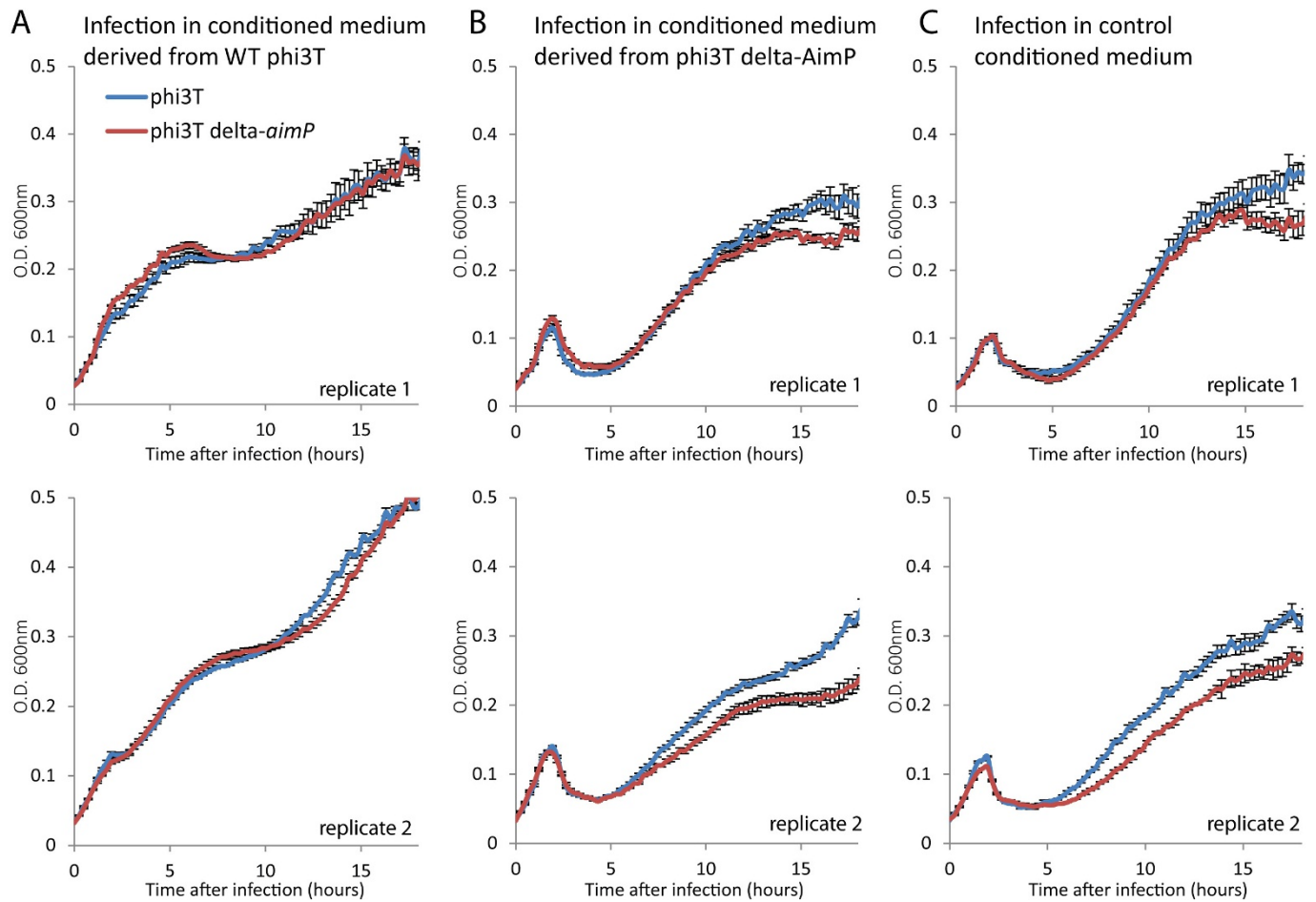


**Extended Data Figure 6 | Phage gene expression 20 min after infection.** Each dot represents a single phage gene. Axes represent average RNA-seq read count per gene from 3 replicates, after normalization to control for RNA-seq library size. *x* axis, expression when phage infection was in the presence of 1  $\mu$ M of SAIRGA peptide; *y* axis, no peptide.



**Extended Data Figure 7 | Expression of the arbitrium locus in phage SPbeta during infection.** RNA-seq coverage of the arbitrium locus at 20 min after infection, with (black) or without (green) 1  $\mu$ M of synthesized GMPRGA peptide in the medium. RNA-seq coverage was normalized

to the number of reads mapped to the phage genome in each RNA-seq library. Shown are two replicates of the experiment. The *x* axis represents the position (in bp) on the phage genome.



**Extended Data Figure 8 | Infection experiments with  $\phi$ 3T  $\Delta$ *aimP* and derived conditioned media.** a–c, Growth curves of *B. subtilis* 168 infected with  $\phi$ 3T or  $\phi$ 3T  $\Delta$ *aimP* in conditioned medium derived from  $\phi$ 3T (a); conditioned medium derived from  $\phi$ 3T  $\Delta$ *aimP* (b); and control

medium (c). Each medium was generated in biological replicate, and the two replicates are presented separately. Each curve represents the average of three technical replicates, and error bars represent s.e.



Extended Data Table 1 | Signal for cleavage by extracellular proteases in the AimP and Phr pre-pro-peptide

	Signal peptide	Pro-peptide	Mature peptide
<b>PhrA</b>	MKSKWMSGLLLVAVGFSFTQVMVHA	GETANTEGK <b>TFHIA</b>	ARNQT
<b>PhrC</b>	MKLKSKLFVICLAAAIFTAAGVASANA	EAL <b>DFHVT</b>	ERGMT
<b>PhrF</b>	MKLKSKLLLSCLALSTVGVATTIANA	PTH <b>QIEVA</b>	QRGMI
<b>PhrG</b>	MKRFLIGAGVAAVILSGWFIA	DHQTHSQ <b>EMKVA</b>	EKMIG
<b>Phr-pTA1060</b>	MKFKGLFSAVLIVSLLVGAGYSFV	HHDE <b>VSVA</b>	SRNAT
<b>AimP</b>	MKKVFFGLVILTALAI SFVAGQOSVSTASA	SDE <b>VTVA</b>	SAIRGA

Shown are the amino acid sequences of *B. subtilis* quorum sensing Phr genes divided to their domains<sup>4</sup>. Recognition signal for *B. subtilis* extracellular proteases is in green. Signal peptide in the phi3T AimP protein was predicted by the SignalP 4.1 web server<sup>36</sup>.