Phage-encoded sigma factors alter bacterial dormancy

Schwartz DA*, Lehmkuhl BK, Lennon JT*

Department of Biology, Indiana University, Bloomington, IN 47405, USA

* Corresponding authors: danschw@iu.edu and lennonj@iu.edu

ABSTRACT

By entering a reversible state of reduced metabolic activity, dormant microorganisms are able to tolerate suboptimal conditions that would otherwise reduce their fitness. Dormancy may also benefit bacteria by serving as a refuge from parasitic infections. Here we focus on dormancy in the *Firmicutes*, where endospore development is transcriptionally regulated by the expression of sigma factors. A disruption of this process could influence the survivorship and reproduction of phages that infect spore-forming hosts with implications for coevolutionary dynamics. Here, we characterized the distribution and diversity of sigma factors in nearly 3,500 phage genomes. Homologs of sporulation-specific sigma factors were identified in phages that infect spore-forming hosts. Unlike sigma factors required for phage reproduction, the sporulation-like sigma factors were non-essential for lytic infection. However, when expressed in the spore-forming *Bacillus subtilis*, sigma factors from phages activated the bacterial sporulation gene network and reduced spore yield. Our findings suggest that the acquisition of host-like transcriptional regulators may allow phages to manipulate a complex and ancient trait in one of the most abundant cell types on Earth.

INTRODUCTION

Dormancy is a life history strategy that allows individuals to enter a reversible state of reduced metabolic activity. An example of convergent evolution, it has independently arisen throughout the tree of life as a means of coping with fluctuating and unpredictable environments[1]. Dormancy is particularly prevalent among microbial life forms where it contributes to the persistence and fitness of populations in environments where variables like pH, oxygen, and resource availability are suboptimal for growth and reproduction[2]. In addition to buffering populations against abiotic features of the environment, dormancy may be reinforced through dynamics that arise from species interactions. For example, dormancy diminishes the strength of competition, which in turn can promote species coexistence[3]. In addition, dormancy may benefit populations by serving as a refuge against predator consumption or parasite infection[4,5].

Among microorganisms, dormancy can protect hosts from viral parasites in a number of ways. As cells transition into an inactive state, they often undergo morphological changes that affect how viruses physically interact with their host. For example, the formation of dormant cells, such as cysts and spores, often involves the development of a thick exterior coating[6-8] that masks the surface molecules used by viruses for attachment[9-12]. Even if a virus is able to gain entry into a dormant cell, parasite productivity will be low owing to constraints imposed by the host's reduced metabolism[13-16]. Furthermore, viral defense genes are often located in proximity to genes that regulate dormancy and cell suicide, suggesting that dormancy may contribute to multilayered protection against viral infection[17,18]. For example, virus-induced dormancy has been linked to CRISPR-Cas systems in bacteria[20] and archaea[19,20]. As a physiological refuge[21], dormancy can

46 confer herd immunity and diminish the spread of epidemics[22], which may ultimately shape host-
47 virus coevolutionary dynamics.
48
49 A take-home lesson from studies on antagonistic coevolution is that host defenses are prone to
50 being overcome by viruses[23,24]. One general mode of virus adaptation involves the acquisition of
51 host genes. Viral genomes commonly encode homologs of genes that are involved in host
52 metabolism. These so-called "auxiliary" genes can alter cellular processes in ways that affect virus
53 fitness[25]. Originally motivated by the discovery of photosynthesis genes in marine
54 cyanophages[26,27], auxiliary genes have been implicated in host nutrition (e.g., N and P metabolism)
55 and energy acquisition (sulfur oxidation, fermentation, etc.), along with basic cellular functions
56 such as protein translation and bacterial communication via quorum sensing[28-30]. In addition, some
57 virus genomes contain host defense genes, which has led to speculation that auxiliary genes may
58 modify parasite infectivity and reproduction[31,32]. Similarly, some phage genomes have been
59 reported to have genes similar to those required for the development of endospore-forming bacteria
60 [33-42]. Phages might use sporulation homologs to inhibit their host from entering a dormancy refuge,
61 thereby enhancing the reproductive component of parasite fitness. Alternatively, phages might
62 exploit sporulation in a way that extends longevity and thereby enhance the survivorship
63 component of fitness. This could happen through a process known as entrapment whereby a phage
64 genome is translocated into the developing spore resulting in the production of a "virospore"[12,43-
65 46]. Analogous to pseudolysogeny, the phage genome is protected by the endospore from conditions
66 that would otherwise contribute to phage decay without it being integrated into the host
67 chromosome[45]. When environmental conditions improve, the dormant cell undergoes germination,
68 and the phage resumes its lytic reproductive cycle.

69

70 As a complex form of dormancy, sporulation presents phages with many opportunities for
71 intervention. For proper development, sporulation requires the coordinated regulation of a large
72 gene network[47-49]. The central regulatory module of sporulation relies on the activity of sigma
73 factors, the exchangeable subunit of the transcriptional machinery which dictates promoter
74 specificity of RNA polymerase[50]. Among bacteria, a primary sigma factor (*sigA* in *Bacillus*
75 *subtilis)* is essential for growth, reproduction, and other housekeeping processes in a wide range
76 of bacteria[51]. During *B. subtilis* spore development *sigA* is swapped out by a cascade of
77 sporulation-specific sigma factors, each driving the expression of a subset of sporulation genes in
78 distinct cellular compartments at specific times[52]. Following an asymmetrical cell division, gene
79 expression in the maturing spore (i.e., forespore) is driven first by *sigF* and then *sigG*, while
80 expression in the mother cell is driven by *sigE* and then *sigK*. Sigma factors are also encoded by
81 some phage genomes, where they regulate phage gene expression during different stages of lytic
82 development[52,53]. More recently, homologs of sporulation-specific sigma factors have been
83 identified in phage genomes[34,41,42,54], yet their function has not been explored. As central nodes of
84 the sporulation gene network, sigma factors could be coopted by phages to modify the outcome of
85 sporulation.

86

87 Here, we use a combination of bioinformatics and laboratory experiments to test whether
88 homologs of sporulation-specific sigma factors can be used by phages to manipulate host
89 dormancy. Using sequence homology and phylogenetic analyses, we identify and classify
90 hundreds of phage-encoded sigma factors. We find that phages capable of infecting spore-forming
91 hosts preferentially encode sigma factors that are homologous to the forespore-specific sigma

92 factors, *sigF* and *sigG*. When homologs of sporulation-specific sigma factors are expressed in *B.*
93 *subtilis*, we observed that conserved phage-encoded homologs alter host gene expression and
94 reduce spore yield. Together, our findings have implications for understanding dormancy
95 dynamics in environmental, engineered, and host-associated ecosystems where endospores
96 constitute one of the most abundant cell types on Earth[55].
97

98 **RESULTS**

99 **Distribution of sigma factors in phage genomes —** We characterized the diversity and
100 distribution of sigma factors in more than 3,400 phage genomes in the viral orthologous groups
101 (VOG) database. From this, we found that 14% of all the genomes analyzed contained at least one
102 sigma factor gene while some genomes (0.7%) contained up to three different homologs (Fig. 1).
103 The distribution of phage-encoded sigma factors was not random with respect to virus or host
104 taxonomy (Fisher's Exact Test, $P_{simulated} < 0.0001$, Fig. 1). Sigma factor containing phages were
105 most prevalent among those that infect the *Cyanobacteria* (65%), *Proteobacteria* (15%) and
106 *Firmicutes* (19%). Among the tailed phages (*Caudovirales*), sigma factors were recovered in 5 of
107 9 families. Three of these five viral families (*Siphoviridae*, *Podoviridae*, and *Myoviridae*) have
108 members that infect hosts from multiple bacterial phyla. However, only among phages that infect
109 *Firmicutes* do all three of these virus families have members with sigma factor genes (Fig. 1c).
110

111 *Firmicutes* phages were also notable in that many of them encode multiple sigma factors.
112 For phages that infect *Cyanobacteria* and *Proteobacteria*, 95% of the genomes with a sigma factor
113 only contained a single copy of such a gene (Fig. 1d). In contrast, for phages that infect *Firmicutes*,
114 41% of the genomes with a sigma factor possessed two or three gene copies. Sigma factor gene

115 multiplicity was detected in diverse *Firmicutes* phages, predominantly among strains that could
116 infect spore-forming genera (Fig. S1). Most of these phages belonged to the *Herelleviridae*, a
117 family of strictly lytic phages that was recently split from the *Myoviridae* and are thought to only
118 infect bacteria in the *Firmicutes*[56]. Of the 102 *Herelleviridae* phages in our dataset, half possessed
119 multiple sigma factors (n = 51) and only infected *Bacillus* hosts (Fig. S2). In contrast, none of the
120 49 phages that infect non spore-forming genera (*Enterococcus*, *Lactiplantibacillus*, *Lactobacillus*,
121 *Listeria*, *Staphylococcus*) possessed more than a single sigma factor gene. Additionally, a similar
122 trend was found among *Firmicutes* phages belonging to the *Siphoviridae*. Within this group, there
123 were five phages with multiple sigma factors (Fig. S2), four that infect hosts capable of forming
124 endospores (*Bacillus* and *Brevibacillus*), and one whose host range likely evolved from a *Bacillus*
125 to *Staphylococcus* host[57].
126
127 **Sporulation-specific sigma factors are encoded by *Firmicutes* phages —** Many of the sigma
128 factors recovered in *Firmicutes* phages were similar to the bacterial-encoded sigma factors
129 involved in sporulation. To interpret this finding in an evolutionary context, we constructed a
130 phylogeny of the VOG sigma factors together with sigma factors from diverse bacteria.
131 Sporulation-specific sigma factors from bacteria belonged to a monophyletic clade that also
132 contained *sigB*, a gene that *B. subtilis* uses to regulate its stress response (Fig. 2a,b). While some
133 phage-encoded sigma factors were found in phage-specific clades, others clustered with bacterial-
134 encoded sigma factors, including those that are known to regulate sporulation. All phage-encoded
135 sigma factors in the sporulation clade were from *Firmicutes* phages, apart from two cyanophage
136 genes that grouped with the *sigB* sub-clade.
137

138 To complement the phylogenetic analyses, we classified phage-encoded sigma factors by
139 estimating homology to bacterial sigma factor protein families (TIGRFAMs) using hidden Markov
140 models. Phage-encoded homologs of sporulation-specific sigma factors of *Firmicutes*, henceforth
141 "sporulation-like" sigma factors, were found nearly exclusively in phages that infect hosts of this
142 phylum (Fig. 2c). Homologs of three of the four sporulation-specific sigma factors (*sigF*, *sigG* and
143 *sigE*) were only detected in *Firmicutes* phages, with the majority resembling *sigF*. Furthermore,
144 these homologs were only recovered from phages that infect *Bacillus*, *Clostridium*, and
145 *Brevibacillus*, which are genera that commonly engage in endosporulation (Fig. S4). However, we
146 did identify homologs of the sporulation-specific *sigK* in a few phages that infect *Cyanobacteria*
147 and *Actinobacteria.*
148
149 The sporulation-like sigma factors recovered from phage genomes were distinct from phage-
150 encoded sigma factors that regulate expression during lytic replication (Fig. 2a). In our
151 phylogenetic analysis, sigma factors that are essential for phage development clustered in one of
152 three other groups. First, there was a phage-specific clade of genes from phages that infect
153 *Proteobacteria* and *Cyanobacteria*. This clade included gp55 from phage T4, which is known to
154 control transcription during late infection stages[50,53]. Second, we identified a phage-specific clade
155 of genes from *Firmicutes* phages, which included gp34, a sigma factor that controls late gene
156 expression in *B. subtilis* phage SPO1[36]. Last, there was a paraphyletic cluster that included phage-
157 and bacterial-derived sigma factors. This group contained gp28, which regulates the expression of
158 middle infection genes in phage SPO1[36]. In addition, the latter group contained alternative sigma
159 factors that are involved in bacterial regulation of motility (*sigD*), stationary phase (*sigH*), and heat
160 stress response (*sigI*).

161

162 The pattern of gene multiplicity among *Firmicutes* phages (Fig. S1) reflects that these viruses
163 contain sigma factors with divergent functions. They either cluster with genes known to regulate
164 the lytic cycle or with bacterial sporulation-specific sigma factors (Fig. 2b). For example, *B.*
165 *subtilis* phage SPO1 encodes two sigma factors that are essential for phage replication (gp28 and
166 gp34)[36]. Likewise, phage SP10 has two sigma factors that cluster with the essential SPO1 genes
167 which are known to regulate phage genes[58]. Each of these phages also encode a third homolog that
168 clusters with sporulation-specific sigma factors. Similarly, all but two of the 58 *Firmicutes* phages
169 exhibiting gene multiplicity contain one or two sporulation-like sigma factors (Fig. S3).
170 Additionally, in several *Bacillus* phages the sole sigma factor is sporulation-like. This group of
171 phages was enriched with representatives from the *Podoviridae* and the *Siphoviridae*, which
172 includes some temperate phages such as Wβ which infects *B. anthracis*[42]. In sum, our
173 bioinformatic analyses revealed that homologs of sporulation-specific sigma factors are found in
174 diverse phages with contrasting lifestyles (i.e., lytic and temperate), often alongside sigma factors
175 that phages use to regulate lytic reproduction.

176

177 **A sporulation-like sigma factor is non-essential for phage reproduction —** Over time, a host-
178 derived sigma factor could be repurposed by phages for regulating phage genes that are essential
179 for reproductive functions like genome replication and capsid assembly. To test this hypothesis,
180 we used CRISPR-Cas9 to delete the sporulation-like sigma factor g120 from SP10, a phage that
181 infects *B. subtills* (see Figs. 2b and Table S1) and has overall 3 sigma factors (see above). After
182 removing the entire coding sequence of the sporulation-like sigma factor g120, this phage could
183 still productively infect its host, demonstrating that g120 was non-essential for phage reproduction

184 under standard lab conditions. In fact, there was no detectable reduction of virulence when
185 infecting *B. subtilis* with the mutant phage ($t_{12.9} = 1.22$, $P = 0.25$; Fig. S4).
186
187 **Phage-encoded sigma factors alter expression of host sporulation genes —** To evaluate the
188 ability of phage-encoded homologs to activate transcription of host sporulation genes, we cloned
189 four diverse sporulation-like sigma factors (Fig. S5) from three *Bacillus* phages (Table S1) under
190 an IPTG inducible promoter into the chromosome of a spore-forming strain of *B. subtilis*. As a
191 control, we independently cloned the host-encoded sigma factors *sigF* and *sigG* into the same
192 strain of *B. subtilis* in a similar manner. We then induced the expression of each of the cloned
193 sigma factors during exponential growth, a time when native sporulation-specific sigma factors
194 (i.e., *sigF*, *sigG, sigE* and *sigK*) and other sporulation genes are not typically expressed (Fig. 3a).
195 Using RNAseq, we found that there was a strong positive correlation ($\rho = 0.76$) between
196 differential gene expression following induction of the sigma factor g169 derived from phage
197 Eldridge (ELDg169) and host-derived sigma factors (*sigF* and *sigG*) suggesting conservation of
198 gene function (Fig. 3b, S6). Similarly, when ELDg169 was induced, we observed the upregulation
199 of genes involved in sporulation ($P < 0.0001$; Figs. 3c, S7). However, not all phage-derived sigma
200 factors affected host expression equally. For example, induction of the other sporulation-like sigma
201 factor cloned from phage Eldridge (ELDg168) also resulted in differential expression of many host
202 genes. However, the genes affected by ELDg168 were significantly different than those observed
203 in populations where host-derived sigma factors were induced ($P < 0.0001$; Fig. S6), and they were
204 not enriched in sporulation genes (Figs. 3c, S7). Meanwhile, induction of the two other less
205 conserved sporulation-like sigma factors (SP10 g120 and Goe3 g157) had only modest effects on

206 gene expression, with less than 50 genes differentially expressed by each induced gene, and no
207 enrichment of sporulation genes (Figs. 3c, S6, S7, S8).
208
209 **Phage-encoded sigma factors inhibit sporulation —** Induction of the sporulation-like sigma
210 factors altered spore yield in populations of *B. subtills* (Figs. 4, S9). When the host-derived sigma
211 factors were induced in sporulating cultures, we observed a reduction in spore yield, a pattern that
212 likely resulted from the misregulation of the sporulation gene network. Compared to an empty
213 vector control with no cloned sigma factor, induction of *sigF* led to an 85% reduction in spore
214 yield ($t_{8.2}$ = 6.43, $P < 0.001$) while *sigG* reduced spore yield by 50% ($t_{13.9}$ = 6.43, P = 0.015).
215 Expression of phage-derived sigma factors also affected sporulation, in one case to a greater degree
216 than host-derived sigma factors (Fig. 4, Table S2). Induction of ELDg169 reduced the spore yield
217 by 99% compared to the empty vector control (t_7 = 7.8, $P < 0.0001$), while the other Eldridge-
218 derived gene, ELDg168, had a smaller (~33%) and marginal effect on spore yield ($t_{15.1}$ = 2.1, P =
219 0.064). The sigma factor from phage Goe3 reduced spore yield by > 50% ($t_{8.9}$ = 4.39, P = 0.002),
220 while expression of the sigma factor from phage SP10 had no effect on spore yield ($t_{10.4}$ = 0.46, P
221 = 0.65). Because spore yield was calculated as a percentage of the total population, we compared
222 cell counts between induced and non-induced controls (Fig. S10). From this, we concluded that
223 the observed reductions in spore yield were not due to a significant reduction in vegetative cells
224 (Table S3).
225

226 **DISCUSSION**

227 It is well known that some phages encode for sigma factors that regulate transcription during
228 lytic development[50,52,53]. In fact, early investigations of phage-encoded sigma factors elucidated

229 how the swapping of sigma subunits controls gene transcription by RNA polymerase[50]. However,
230 those phage-encoded sigma factors are quite divergent from bacteria-encoded sigma factors[53,59].
231 More recently, genomic data have identified phage-encoded sigma factors that bear a greater
232 resemblance to bacterial sigma factors, especially those involved in the regulation of sporulation
233 in the *Firmicutes*. While the function of these homologs remains unexplored, it is possible that
234 sporulation-like sigma factors have been coopted by phages to manipulate host dormancy in ways
235 that could potentially enhance their reproduction and survival. In support of this hypothesis, our
236 bioinformatic and phylogenetic analyses revealed that sporulation-like sigma factors were found
237 primarily in phages that infect spore-forming hosts, where they could potentially act as analogs of
238 the host's own sporulation-specific sigma factors. We show that these phage-encoded sigma
239 factors are distinct from genes known to be essential for regulating lytic reproduction and that they
240 are non-essential for phage reproduction. When expressed in *Bacillus subtilis,* phage-encoded
241 sigma factors reduced spore yield, likely owing to their ability to alter the expression of host genes,
242 which in one case led to transcriptional activation of the sporulation network. Together our
243 findings highlight novel ways in which dormancy may influence antagonistic coevolution between
244 spore-forming bacteria and their phages.
245
246 **Diversity and distribution of phage-encoded sigma factors —** Phages are able to regulate
247 gene expression in different ways. For example, they can make use of host-encoded sigma factors,
248 encode sigma factor analogs (e.g., gp33 in T4), or use their own RNA polymerases that do not
249 require sigma factors[50,53,60,61]. In addition, some phages encode their own sigma factors. Of the
250 nearly 3,500 genomes examined, we found that 14% of the phages possess a sigma factor. The
251 distribution of sigma factors in phage genomes was strongly influenced by host and viral

252 taxonomy. While common among phages that infect *Cyanobacteria* and *Proteobacteria*, only
253 among the *Firmicutes* phages did we recover sigma factors that were encoded by representatives
254 from all three of the major tailed-phage families (*Siphoviridae*, *Podoviridae* and *Myoviridae*; Fig.
255 1). This suggests that sigma factors may be beneficial to diverse phages that infect *Firmicutes*
256 hosts. Furthermore, we identified a host-specific pattern of gene multiplicity. A diverse set of
257 *Firmicutes* phages carried two sigma factors with host ranges that were limited to spore-forming
258 genera, while phages containing three sigma factors were restricted to just two viral families
259 (*Herelleviridae* and *Siphoviridae*) that could only infect strains of *Bacillus*. This convergent
260 pattern of gene multiplicity is associated with the presence of sporulation-like sigma factors in
261 phages that infect *Firmicutes*. Given the high degree of sequence similarity between sigma factors
262 encoded by phages and those encoded by their hosts, it seems likely that viruses have acquired
263 these genes from their hosts. Furthermore, because the genes are widespread among diverse viral
264 lineages, it seems reasonable to hypothesize that there are functional consequences for phage that
265 encode sporulation-like sigma factors. Nevertheless, in the database used in our study, these genes
266 are not universal in phages that infect spore-forming *Firmicutes*. We expect that future
267 investigations of metagenomes from environmental, engineered, and host-associated ecosystems
268 will shed light on the eco-evolutionary factors that influence the distribution and abundance of
269 dormancy related auxiliary genes, including sporulation-like sigma factors.
270
271 **Sporulation-like sigma factors appear to be non-essential for phages —** In bacteria, sporulation
272 and other specialized functions are controlled by alternative sigma factors that replace the primary
273 housekeeping sigma factor[51]. Because alternative sigma factors are only transiently used, their
274 deletion is typically non-lethal and thus they are considered non-essential. Similarly, multiple lines

275 of evidence suggest that sporulation-like sigma factors are non-essential for fundamental aspects
276 of phage biology. Unlike gp28 and gp34, which are required by phage SPO1, a sporulation-like
277 sigma factor (gp2.21) is known to be non-essential for lytic infection under standard laboratory
278 conditions[36]. While it has been hypothesized that gp2.21 directs transcription of phage genes from
279 *sigK*-like promoters found in its genome[36], this has not been confirmed. In our study, we deleted
280 the sporulation-like sigma factor from phage SP10 (g120) with no observable effect on virulence
281 (Fig. S4). Even though g120 is expressed during SP10 infection of its host[58], available evidence
282 suggests that at least some sporulation-like sigma factors are non-essential for lytic infection. If
283 this pattern is generalizable, then non-essentiality could reflect that phage-encoded sigma factors
284 have alternate function, which could involve the control of genes that are required for processes
285 other than replication.
286
287 **Phage-encoded sigma factors disrupt regulation of the sporulation network —** The prevalence
288 of sporulation-like sigma factors among diverse phages suggests that these genes may have
289 consequences for phage performance. One possibility is that these sigma factors play the same role
290 in phages as they do in their host, that is, they regulate the expression of sporulation genes. In
291 support of this notion, previous experimental studies have documented that phage-encoded
292 sporulation-like sigma factors retain a function that is relevant to host sporulation. For example,
293 in lysogenic *B. anthracis*, expression of a sporulation-like sigma factor encoded by the Wβ
294 prophage is elevated during sporulation[42]. Additionally, *in vitro* reconstitution of RNA polymerase
295 with the sporulation-like sigma factor of *B. anthracis* phage Fah, a close relative of Wβ, revealed
296 patterns of transcriptional activity and inhibition that are similar to that of a bacterial-encoded
297 sigma factor (*sigF*)[54]. Finally, sporulation-like sigma factors cloned from two diverse *B. anthracis*

298 phages, Bcp1 (*Herelleviridae*) and Wip4 (*Siphoviridae*), were associated with phage-dependent
299 inhibition of host sporulation[41].
300
301 In our study, the induction of phage-derived sporulation-like sigma factors altered host gene
302 expression and disrupted sporulation in *B. subtilis*. The magnitude of this effect was contingent
303 upon the identity and phylogenetic distance between the phage- and host-encoded sigma factors
304 (Fig, S11). When we induced ELDg169, the most host-like gene in our experiments, we observed
305 the differential expression of hundreds of bacterial genes, including the upregulation of nearly 400
306 sporulation genes (Fig. S7), which led to a ~99% reduction in spore yield (Fig. 4b). In contrast, we
307 observed a less pronounced reduction in spore yield, and almost no effect on the sporulation gene
308 network when two less conserved phage-derived genes (ELDg168 and Goe3 g157) were induced.
309 Last, induction of g120 from phage SP10 resulted in a very mild transcriptional response and had
310 no effect on spore yield, in accordance with it being the most divergent gene from bacterial *sigF*
311 and *sigG*. Thus, our studies suggest that that the functionality of phage-encoded sigma factors is
312 influenced by the degree of similarity with host-encoded sigma factors. However, this does not
313 rule out that phage-encoded sigma factors may play other roles depending on host and
314 environmental conditions. For example, the manipulation of sporulation seems to be the likely
315 function of these genes in phage Eldridge, possibly also in phage Goe3, but not in phage SP10.
316
317 As is the case with many evolutionary investigations, carefully planned experiments are
318 required to make strong inferences about the adaptive significance of genetic variation in a
319 population. We hypothesize that sporulation-like sigma factors could influence phage fitness in
320 different ways. By controlling the timing of host sporulation, phages may increase their probability

321 of entrapment[46], which would increase the survivorship component of phage fitness. On the other
322 hand, by impeding sporulation, there is more opportunity for virus replication, which would
323 increase the reproductive component of phage fitness. In our control experiments, induced
324 expression of bacterial genes activated the sporulation network during exponential growth.
325 However, when induced during sporulation, these host genes, which are known to promote
326 sporulation, led to a reduction in spore yield. This pattern likely stems from the regulatory genes
327 being expressed in the wrong place and time. As a consequence, it is challenging to conclusively
328 deduce whether the reduction in spore-yield reflects the function of phage proteins in infected cells
329 or other aspects of our experiment. However, results from one particular phage provide some
330 additional insight. The two Eldridge-derived genes, which are genomic neighbors, both reduced
331 sporulation to some degree, even though they had very different transcriptional profiles. If these
332 adjacent genes work toward a common function, it seems likely that this results in the inhibition
333 of host sporulation. This interpretation is consistent with the observation that complete inhibition
334 of sporulation in *B. anthracis* is likely mediated by a pair of sporulation-like sigma factors found
335 in tandem in the genome of phage Bcp1[41]. Additional experiments are needed to understand the
336 evolutionary consequences of sporulation-like sigma factors for phage populations. For example,
337 comparisons between phages in which sigma factors are present or deleted (like the SP10 mutant
338 we constructed) will be useful in determining whether auxiliary dormancy genes enhance phage
339 fitness. Likewise, comparative transcriptomic analysis during infection with phages with and
340 without sigma factors may help identify the regulatory targets of phage-encoded sigma factors.
341 Looking beyond sporulation, studies are needed to better understand how bacterial-like sigma
342 factors may be used by phages to manipulate other survival strategies that are common in non-
343 growing bacteria[62].

344

345 Taken together, our findings reveal a pattern of genomic convergence. Phages from diverse
346 families, with contrasting infection strategies (virulent vs. temperate) that infect spore-forming
347 hosts also contain sporulation-specific sigma factor homologs. Importantly, these sigma factors
348 are phylogenetically distinct from sigma factors that regulate phage genes during lytic replication.
349 At least some of these genes are non-essential for phages, which further supports the view that
350 alternate sigma factors were not acquired for the purpose of regulating lytic programs. Instead, our
351 experiments demonstrate that phage-encoded sigma factors retain features of their ancestral
352 function, which means that they can be viewed as auxiliary genes that can regulate the sporulation
353 network with consequences for spore yield. That being said, our results also reveal that some
354 sporulation-like sigma factors in phages have variable and yet unknown functions, which may
355 reflect neutral or adaptive divergence.

356

357 **Eco-evolutionary implications of auxiliary dormancy genes —** As obligate parasites, phages
358 are unavoidably dependent on the metabolism of their bacterial hosts. Bacteria are capable of
359 responding to their dynamic environments by replacing the sigma subunit of RNA polymerase,
360 which leads to changes in gene expression[50,51]. Some phages use a similar strategy to coordinate
361 expression of their own genes during different stages of infection[53]. Our analysis points to the
362 existence of a second class of sigma factors that phages may use to manipulate host metabolism.
363 Whether phages promote or inhibit sporulation, such manipulation of host dormancy has the
364 potential to modify the environmental conditions under which we should expect to find bacteria in
365 an active vs. dormant state. This has implications for development of novel therapeutic treatments

366 that combine phage therapy with antimicrobials, which tend to target metabolically active
367 bacteria[63].
368
369 Viruses have evolved to utilize, maintain, and rearrange a variety of biochemical pathways in
370 the cells that they take over[25,28,64]. The discovery of auxiliary metabolic genes has revealed that
371 viruses can appropriate the cellular building blocks and protein translation machinery of their
372 hosts. Our findings highlight an additional aspect of phage-host coevolution involving the co-
373 option of gene networks used to coordinate a complex and ancient form of dormancy, which many
374 microorganisms use to contend with harsh and unpredictable environments. Manipulation of the
375 host's response to such conditions through the acquisition of host regulatory genes could represent
376 a strategy which buffers viruses from the dynamic cellular environment on which their survival
377 and reproduction is dependent.
378

379 **METHODS**

380 **Phage sigma factor distribution and classification —** We retrieved sigma factors from the
381 database of viral orthologous groups (VOG release vog202, vogdb.org) based on text searches of
382 the VOG descriptors (Table S4). We matched VOG proteins to host and viral taxonomy using the
383 virus-host database[65]. We classified phage-encoded sigma factors using hmmscan with default
384 parameter settings using HMMER v3.3[66], and queried each protein against hidden Markov model
385 (HMM) profiles of bacterial-encoded sigma factor families that were retrieved from TIGRFAM
386 (Table S5). We used the best hmmscan match (smallest sequence E-value) to classify proteins,
387 unless it was a general TIGRFAM ("sigma70-ECF" or "SigBFG"), in which case the next best
388 match was chosen, if available.

389

390 **Phylogenetic analysis —** For phylogenetic analysis of sigma factors, we aligned VOG proteins
391 from phage genomes along with sigma factor proteins belonging to 24 bacteria from diverse taxa[67].
392 We aligned sigma factor sequences using MAFFT (v.7.475)[68] with the E-INS-I strategy and
393 trimmed the alignment with trimAL (v1.4.rev22)[69] using the gappyout method. From the 180
394 amino acids in the trimmed alignment we then inferred 200 maximum likelihood phylogenetic
395 trees using RAxML-NG (v0.9.0-pthreads)[70] with the LG+G4 substitution model selected using
396 modeltest-NG (v0.1.6)[71] with default settings. We present the best scoring maximum likelihood
397 tree with Transfer Bootstrap Expectation supports[72] from 500 bootstrapped trees. We plotted the
398 tree using the ggtreeExtra R package[73].

399

400 **Strains and media —** Strains used are listed in Table S1. For routine culturing of bacteria, we
401 used LB medium with low salt (5 g/L NaCl). We amended this recipe with agar (15 g/L) for plating
402 and with $CaCl_2$ (10 mM) to facilitate virus adsorption. We used Difco sporulation media (DSM)
403 for sporulation assays[74]. For plaque assays, we used double-layer plating with 0.3% agar
404 overlays[75]. To amplify phages, we collected lysates from plate infections after flooding Petri dishes
405 with phage buffer (10 mM Tris, 10 mM $MgSO_4$, 4 g/L NaCl, 1 mM $CaCl_2$, pH 7.5). We then
406 cleared the phage-containing buffer from bacteria by centrifugation (7,200 Xg, 10 min) and
407 filtration (0.2 μm).

408

409 **Deletion of phage-encoded sigma factor —** We used the CRISPR-Cas9 system and the CutSPR
410 assay design-tool[76] to test whether sigma factors are essential for phage replication. Briefly, we
411 cloned a single-guide RNA and a deletion cassette into plasmid pJOE8999[77] (Tables S6, S7) and

412 transformed the resulting plasmid into *B. subtilis* TS01 (Table S3), which was made competent
413 with D-mannitol induction. We next infected the transformed culture with phage SP10 (Table S1)
414 and conducted a plaque assay with medium containing the Cas9-inducer D-mannose. Using
415 primers SP10_validF+R (Table S7), we screened multiple plaques for the deletion. To isolate the
416 mutant phages, we picked and replated PCR-positive plaques onto host *B. subtilis* Δ6[78] (Table S1).
417 We screened these secondary plaques as above and confirmed the deletion by Sanger sequencing
418 of the locus. We then quantified the virulence of the mutant and wild-type phages[79]. After
419 dispensing *B. subtilis* Δ6 host cultures (OD600 = 1) into microtiter wells, we infected cells with
420 serially diluted lysates of SP10 or SP10 Δg120 that were adjusted to an equal titer. We monitored
421 bacterial density during growth for 16 h by OD600 with a Synergy H1 plate reader (Biotek). From
422 this, we calculated the virulence index[79] based on change in bacterial growth and lysis as a function
423 of the phage:bacteria ratio (i.e., multiplicity of infection; Fig S4).
424
425 **Inducible expression of sigma factors —** We tested the effect of phage-derived sigma factors on
426 bacterial expression by cloning coding sequences under an inducible promoter into an ectopic site
427 (*amyE*) of the *B. subtilis* genome. As a control, we also cloned host-derived sporulation genes
428 (s*igF* and *sigG*) in the same manner, and a gene-less promoter as a negative control.
429
430 *Strain construction.* We amplified coding sequences by PCR from phage lysates or from extracted
431 bacterial genomic DNA as templates using primers adapted with restriction sites, and a ribosome
432 binding site on the forward primer (Table S7). We then cloned the PCR products into plasmid
433 pDR110 (Table S6) by restriction enzyme digestion (Table S6), gel purification, and ligation (T4
434 ligase). We selected for plasmids that were transformed into *E. coli* (One Shot TOP10, Fisher)

435 with ampicillin (100 µg/ml) and verified the insertion by PCR and Sanger sequencing using
436 primers oDAS9+10 (Table S7). We transformed purified plasmids (QIAgen mini prep) into *B.*
437 *subtilis* TS01, as described above, using spectinomycin selection (100 µg/ml). We verified the
438 insertion into the *amyE* locus by PCR and Sanger sequencing, and by the loss of erythromycin
439 resistance carried in the *amyE* locus by strain TS01.
440
441 *Transcriptional response to phage-encoded sigma factor*. We diluted overnight *B. subtilis* cultures
442 (OD600 = 0.1) in fresh LB and grew them (37 °C, 200 RPM) to mid exponential phase (OD600 =
443 0.5). We then split the cultures and added 1mM IPTG (final concentration) to one half to induce
444 expression of the cloned gene. We added an equal volume of water to the other half of the split
445 culture as a non-induced control. After induction, we incubated the cultures for 2 h before
446 harvesting cells. Upon sampling, we immediately treated bacteria with the RNAprotect Bacteria
447 reagent (Qiagen), and stored pellets at -80 °C for <1 week before RNA extraction using RNeasy
448 Protect Bacteria Mini Kit (Qiagen) according to the manufacturer's instructions (protocol #5),
449 including an on-column RNase-free DNase digestion. Library construction, sequencing, and
450 analysis of differential gene expression were all carried out at the Indiana University Center for
451 Genomics and Bioinformatics. Libraries were constructed using the Illumina TruSeq Stranded
452 mRNA HT kit following depletion of rRNA using Illumina Ribo-Zero Plus kit. Libraries were then
453 sequenced on an Illumina NextSeq 500 platform as paired end reads (2 x 38 bp). We trimmed
454 adapters and filtered reads using Trimmomatic 0.38[80] with the cutoff threshold for average base
455 quality score set at 20 over a window of 3 bases. Reads shorter than 20 bases post-trimming were
456 excluded. We mapped the cleaned reads to the reference genome (Deposited with sequencing data
457 to the Gene Expression Omnibus, see below) using bowtie2 version 2.3.2[81], and counted reads

458 mapping concordantly and uniquely to the annotated genes using featureCounts tool ver. 2.0.0 of
459 subread package[82]. Read alignments to antisense strand, or to multiple regions on the genome or
460 those overlapping with multiple genes were ignored (parameters: -s 2 -p -B -C). We performed
461 differential expression analysis using DESeq2 ver. 1.24.0[83] from normalized read counts by
462 comparing samples induced with IPTG to non-induced paired control samples, with multiple-
463 testing correction. We tested for the effects of gene enrichment and overlap of differentially
464 expressed genes using the hypergeometric distribution in R[84].
465
466 *Sporulation of cells expressing cloned sigma factors*. To test for the effects of induced sigma
467 factors on host sporulation, we diluted overnight *B. subtilis* cultures in fresh DSM (OD600 = 0.05)
468 and dispensed each culture into multiple wells of a 96-well plate that was then incubated in a
469 Biotek Synergy H1 plate reader (37 °C, fast and continuous shake setting). Under these conditions,
470 we determined that cells enter stationary phase after approximately 4.5 h, marking the onset of
471 sporulation. At this time, we induced expression of the cloned gene by adding IPTG (final
472 concentration 1 mM) to half the cultures in the plate. We added water to the rest of the wells, which
473 served as non-induced controls. At 24 h, we quantified the number of spores and vegetative cells
474 in each well using a flow-cytometry assay that distinguished spores from vegetative cells (non-
475 spores) based on differential uptake of the nucleic acid stain SYBR green[85]. We diluted each
476 sample in TE buffer (pH 8) and then fixed the cells in 0.5% glutaraldehyde for 15 min at 4 °C. We
477 stained the fixed samples with SYBR green (20,000x dilution of commercial stock, Lonza) for 10
478 min at room temperature in the dark. We then enumerated cells using a volumetric NovoCyte
479 2000R flow cytometer (Acea; ex 488 nm, em 530/30 nm) and an automatic gating pipeline.
480

481 **Code and data availability —** All code and data used in the analyses in this study are available
482 at github.com/LennonLab/sigma-spore-phage and github.com/LennonLab/sigma-spore-phage-
483 flow. RNA sequencing data are available at the Gene Expression Omnibus under accession
484 number GSE187004. In addition, prior to publication, all data and code will be made available
485 on Zenodo.
486

487 **ACKNOWLEDGEMENTS**

488 We acknowledge R Hertel, L Temple, AC Hernandez, M Liu, X Wang, David Rudner, D Zeigler,
489 and DB Kearns for suggestions and strains; E Long and C Chen for technical support; and J Bird
490 for assistance with preliminary computation. An earlier version of the manuscript was improved
491 based on critical feedback from FJ Fishman, C Karakoç, A Magalie, JG McMullen, RZ Moger-
492 Reischer, EA Muller, PG Wall and JS Weitz. Research was supported by the National Science
493 Foundation (DEB-1934554 JTL and DAS, DBI- 2022049 JTL), US Army Research Office Grant
494 (W911NF-14-1-0411 JTL), the National Aeronautics and Space Administration
495 (80NSSC20K0618 JTL). This research was also supported in part by Lilly Endowment, Inc.,
496 through its support for the Indiana University Pervasive Technology Institute.
497

498 **REFERENCES**

499 1. Lennon, J. T., den Hollander, F., Wilke-Berenguer, M. & Blath, J. Principles of seed
500 banks and the emergence of complexity from dormancy. *Nat. Commun.* **12**, 1-16 (2021).
501 2. Lennon, J. T. & Jones, S. E. Microbial seed banks: the ecological and evolutionary
502 implications of dormancy. *Nat. Rev. Microbiol.* **9**, 119-130 (2011).
503 3. Cáceres, C. E. Temporal variation, dormancy, and coexistence: a field test of the storage
504 effect. *Proc. Natl. Acad. Sci. USA* **94**, 9171-9175 (1997).
505 4. Klobutcher, L. A., Ragkousi, K. & Setlow, P. The *Bacillus subtilis* spore coat provides
506 "eat resistance" during phagocytic predation by the protozoan *Tetrahymena thermophila*.
507 *Proc. Natl. Acad. Sci. USA* **103**, 165-170 (2006).

508 5. Verin, M. & Tellier, A. Host-parasite coevolution can promote the evolution of seed
509 banking as a bet-hedging strategy. *Evolution* **72**, 1362-1372 (2018).
510 6. Berleman, J. E. & Bauer, C. E. Characterization of cyst cell formation in the purple
511 photosynthetic bacterium *Rhodospirillum centenum*. *Microbiology* **150**, 383-390 (2004).
512 7. Driks, A. & Eichenberger, P. in *The Bacterial Spore: From Molecules to Systems* 179-
513 200 (American Society of Microbiology, 2016).
514 8. Kaplan-Levy, R. N., Hadas, O., Summers, M. L., Rücker, J. & Sukenik, A. in *Dormancy*
515 *and resistance in harsh environments Topics in Current Genetics* (eds Esther Lubzens,
516 Joan Cerdà, & Melody Clark) 5-27 (Springer, Berlin, Heidelberg, 2010).
517 9. Burroughs, N. J., Marsh, P. & Wellington, E. M. H. Mathematical analysis of growth and
518 interaction dynamics of streptomycetes and a bacteriophage in soil. *Appl. Environ.*
519 *Microbiol.* **66**, 3868-3877 (2000).
520 10. Dowding, J. Characterization of a bacteriophage virulent for *Streptomyces coelicolor* A3
521 (2). *Microbiology* **76**, 163-176 (1973).
522 11. Singh, R. N. & Singh, P. K. Isolation of Cyanophages from India. *Nature* **216**, 1020-1021
523 (1967).
524 12. Gabiatti, N. *et al.* Bacterial endospores as phage genome carriers and protective shells.
525 *Appl. Environ. Microbiol.* **84**, e01186 (2018).
526 13. Hadas, H., Einav, M., Fishov, I. & Zaritsky, A. Bacteriophage T4 development depends
527 on the physiology of its host *Escherichia coli*. *Microbiology* **143 (Pt 1)**, 179-185 (1997).
528 14. Middelboe, M. Bacterial growth rate and marine virus–host dynamics. *Microb. Ecol.* **40**,
529 114-124 (2000).
530 15. Abedon, S. T. & Yin, J. in *Bacteriophages: Methods and Protocols, Volume 1: Isolation,*
531 *Characterization, and Interactions* (eds Martha R. J. Clokie & Andrew M. Kropinski)
532 161-174 (Humana Press, 2009).
533 16. Bryan, D., El-Shibiny, A., Hobbs, Z., Porter, J. & Kutter, E. M. Bacteriophage T4
534 infection of stationary phase *E. coli*: life after log from a phage perspective. *Front*
535 *Microbiol* **7** (2016).
536 17. Makarova, K. S., Anantharaman, V., Aravind, L. & Koonin, E. V. Live virus-free or die:
537 coupling of antivirus immunity and programmed suicide or dormancy in prokaryotes.
538 *Biol Direct* **7**, 7-40 (2012).
539 18. Koonin, E. V. & Zhang, F. Coupling immunity and programmed cell suicide in
540 prokaryotes: Life-or-death choices. *Bioessays* **39**, e201600186 (2017).
541 19. Meeske, A. J., Nakandakari-Higa, S. & Marraffini, L. A. Cas13-induced cellular
542 dormancy prevents the rise of CRISPR-resistant bacteriophage. *Nature* **570**, 241-245
543 (2019).
544 20. Bautista, M. A., Zhang, C. & Whitaker, R. J. Virus-Induced dormancy in the archaeon
545 *Sulfolobus islandicus*. *mBio* **6**, e02565 (2015).
546 21. Lenski, R. E. in *Adv. Microb. Ecol.* Vol. 10 (ed K. C. Marshall) 1-44 (Springer, 1988).
547 22. Lopatina, A., Tal, N. & Sorek, R. Abortive infection: bacterial suicide as an antiviral
548 immune strategy. *Annu Rev Virol* **7**, 371-384 (2020).
549 23. van Houte, S., Buckling, A. & Westra, E. R. Evolutionary ecology of prokaryotic
550 immune mechanisms. *Microbiol. Mol. Biol. Rev.* **80**, 745-763 (2016).
551 24. Ofir, G. & Sorek, R. Contemporary phage biology: from classic models to new insights.
552 *Cell* **172**, 1260-1270 (2018).

553 25. Thompson, L. R. *et al.* Phage auxiliary metabolic genes and the redirection of
554 cyanobacterial host carbon metabolism. *Proc. Natl. Acad. Sci. USA* **108**, E757-E764
555 (2011).
556 26. Lindell, D. *et al.* Transfer of photosynthesis genes to and from *Prochlorococcus* viruses.
557 *Proc. Natl. Acad. Sci. USA* **101**, 11013-11018 (2004).
558 27. Mann, N. H., Cook, A., Millard, A., Bailey, S. & Clokie, M. Bacterial photosynthesis
559 genes in a virus. *Nature* **424**, 741-741 (2003).
560 28. Hargreaves, K. R., Kropinski, A. M. & Clokie, M. R. Bacteriophage behavioral ecology:
561 How phages alter their bacterial host's habits. *Bacteriophage* **4**, e29866-e29866 (2014).
562 29. Silpe, J. E. & Bassler, B. L. Phage-encoded LuxR-type receptors responsive to host-
563 produced bacterial quorum-sensing autoinducers. *mBio* **10**, e00638-00619 (2019).
564 30. Mizuno, C. M. *et al.* Numerous cultivated and uncultivated viruses encode ribosomal
565 proteins. *Nat. Commun.* **10**, 752 (2019).
566 31. Seed, K. D., Lazinski, D. W., Calderwood, S. B. & Camilli, A. A bacteriophage encodes
567 its own CRISPR/Cas adaptive response to evade host innate immunity. *Nature* **494**, 489-
568 491 (2013).
569 32. Murphy, J. *et al.* Methyltransferases acquired by lactococcal 936-type phage provide
570 protection against restriction endonuclease activity. *BMC Genomics* **15**, 1-11 (2014).
571 33. Dragoš, A. *et al.* Pervasive prophage recombination occurs during evolution of spore-
572 forming Bacilli. *ISME J* **15**, 1344-1358 (2021).
573 34. Reveille, A. M., Eldridge, K. A. & Temple, L. M. Complete genome sequence of *Bacillus*
574 *megaterium* bacteriophage Eldridge. *Genome Announc.* **4**, e0172815 (2016).
575 35. Ritz, M. P., Perl, A. L., Colquhoun, J. M., Chamakura, K. R. & Everett, G. F. K.
576 Complete genome of *Bacillus subtilis* myophage CampHawk. *Microbiol Resour Announc*
577 **1**, e00984-00913 (2013).
578 36. Stewart, C. R. *et al.* The genome of *Bacillus subtilis* bacteriophage SPO1. *J. Mol. Biol.*
579 **388**, 48-70 (2009).
580 37. Van Goethem, M. W., Swenson, T. L., Trubl, G., Roux, S. & Northen, T. R.
581 Characteristics of wetting-induced bacteriophage blooms in biological soil crust. *mBio*
582 **10**, e02287-02219 (2019).
583 38. Yuan, Y., Gao, M., Wu, D., Liu, P. & Wu, Y. Genome characteristics of a novel phage
584 from *Bacillus thuringiensis* showing high similarity with phage from *Bacillus cereus*.
585 *PLoS One* **7**, e37557 (2012).
586 39. El-Arabi, T. F. *et al.* Genome sequence and analysis of a broad-host range lytic
587 bacteriophage that infects the *Bacillus cereus* group. *Virol J* **10**, 1-11 (2013).
588 40. Zimmer, M., Scherer, S. & Loessner, M. J. Genomic analysis of *Clostridium perfringens*
589 bacteriophage φ3626, which integrates into guaA and possibly affects sporulation. *J.*
590 *Bacteriol.* **184**, 4359-4368 (2002).
591 41. Schuch, R. & Fischetti, V. A. The secret life of the anthrax agent Bacillus anthracis:
592 bacteriophage-mediated ecological adaptations. *PLoS One* **4** (2009).
593 42. Schuch, R. & Fischetti, V. A. Detailed genomic analysis of the Wβ and γ phages
594 infecting *Bacillus anthracis*: implications for evolution of environmental fitness and
595 antibiotic resistance. *J. Bacteriol.* **188**, 3037-3051 (2006).
596 43. Sonenshein, A. L. Trapping of unreplicated phage DNA into spores of *Bacillus subtilis*
597 and its stabilization against damage by ^{32}P decay. *Virology* **42**, 488-495 (1970).

598 44. Sonenshein, A. L. Bacteriophages: How bacterial spores capture and protect phage DNA.
599 *Curr. Biol.* **16**, R14-R16 (2006).
600 45. Takahashi, I. Incorporation of bacteriophage genome by spores of *Bacillus subtilis*. *J.*
601 *Bacteriol.* **87**, 1499-1502 (1964).
602 46. Sonenshein, A. L. & Roscoe, D. H. The course of phage Øe infection in sporulating cells
603 of *Bacillus subtilis* strain 3610. *Virology* **39**, 265-276 (1969).
604 47. Galperin, M. Y. *et al.* Genomic determinants of sporulation in *Bacilli* and *Clostridia*:
605 towards the minimal set of sporulation-specific genes. *Environ. Microbiol.* **14**, 2870-2890
606 (2012).
607 48. Meeske, A. J. *et al.* High-throughput genetic screens identify a large and diverse
608 collection of new sporulation genes in *Bacillus subtilis*. *PLoS Biol.* **14**, e1002341 (2016).
609 49. Ramos-Silva, P., Serrano, M. & Henriques, A. O. From root to tips: sporulation evolution
610 and specialization in *Bacillus subtilis* and the intestinal pathogen *Clostridioides difficile*.
611 *Mol. Biol. Evol.* **36**, 2714-2736 (2019).
612 50. Helmann, J. D. Where to begin? Sigma factors and the selectivity of transcription
613 initiation in bacteria. *Mol. Microbiol.* **112**, 335-347 (2019).
614 51. Paget, M. S. Bacterial sigma factors and anti-sigma factors: structure, function and
615 distribution. *Biomolecules* **5**, 1245-1265 (2015).
616 52. Losick, R. & Pero, J. Cascades of sigma factors. *Cell* **25**, 582-584 (1981).
617 53. Nechaev, S. & Severinov, K. Bacteriophage-induced modifications of host RNA
618 polymerase. *Annu. Rev. Microbiol.* **57**, 301-322 (2003).
619 54. Minakhin, L. *et al.* Genome sequence and gene expression of *Bacillus anthracis*
620 bacteriophage Fah. *J. Mol. Biol.* **354**, 1-15 (2005).
621 55. Wörmer, L. *et al.* Microbial dormancy in the marine subsurface: Global endospore
622 abundance and response to burial. *Sci Adv* **5**, eaav1024 (2019).
623 56. Barylski, J. *et al.* Analysis of spounaviruses as a case study for the overdue
624 reclassification of tailed phages. *Syst. Biol.* **69**, 110-123 (2020).
625 57. Swanson, M. M. *et al.* Novel bacteriophages containing a genome of another
626 bacteriophage within their genomes. *PLoS One* **7**, e40683 (2012).
627 58. Yee, L. M. *et al.* The genome of *Bacillus subtilis* phage SP10: a comparative analysis
628 with phage SPO1. *Biosci., Biotechnol., Biochem.* **75**, 944-952 (2011).
629 59. Lonetto, M., Gribskov, M. & Gross, C. A. The sigma 70 family: sequence conservation
630 and evolutionary relationships. *J. Bacteriol.* **174**, 3843-3849 (1992).
631 60. Twist, K.-A. F. *et al.* Crystal structure of the bacteriophage T4 late-transcription
632 coactivator gp33 with the β-subunit flap domain of *Escherichia coli* RNA polymerase.
633 *Proc. Natl. Acad. Sci. USA* **108**, 19961-19966 (2011).
634 61. Sokolova, M. *et al.* A non-canonical multisubunit RNA polymerase encoded by the AR9
635 phage recognizes the template strand of its uracil-containing promoters. *Nucleic Acids*
636 *Res.* **45**, 5958-5967 (2017).
637 62. Jaishankar, J. & Srivastava, P. Molecular basis of stationary phase survival and
638 applications. *Front Microbiol* **8**, 2000 (2017).
639 63. Rodriguez-Gonzalez, R. A., Leung, C. Y., Chan, B. K., Turner, P. E. & Weitz, J. S.
640 Quantitative models of phage-antibiotic combination therapy. *mSystems* **5**, e0075619
641 (2020).
642 64. Breitbart, M., Bonnain, C., Malki, K. & Sawaya, N. A. Phage puppet masters of the
643 marine microbial realm. *Nat Microbiol* **3**, 754-766 (2018).

644 65. Mihara, T. *et al.* Linking virus genomes with host taxonomy. *Viruses* **8**, 66 (2016).
645 66. Eddy, S. R. Accelerated profile HMM searches. *PLoS Comp. Biol.* **7**, e1002195 (2011).
646 67. Burton, A. T., DeLoughery, A., Li, G.-W. & Kearns, D. B. Transcriptional regulation and
647 mechanism of SigN (ZpdN), a pBS32-encoded sigma factor in *Bacillus subtilis*. *mBio* **10**,
648 e0189919 (2019).
649 68. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7:
650 improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772-780 (2013).
651 69. Capella-Gutiérrez, S., Silla-Martínez, J. M. & Gabaldón, T. trimAl: a tool for automated
652 alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972-1973
653 (2009).
654 70. Kozlov, A. M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. RAxML-NG: a fast,
655 scalable and user-friendly tool for maximum likelihood phylogenetic inference.
656 *Bioinformatics* **35**, 4453-4455 (2019).
657 71. Darriba, D. *et al.* ModelTest-NG: a new and scalable tool for the selection of DNA and
658 protein evolutionary models. *Mol. Biol. Evol.* **37**, 291-294 (2020).
659 72. Lemoine, F. *et al.* Renewing Felsenstein's phylogenetic bootstrap in the era of big data.
660 *Nature* **556**, 452-456 (2018).
661 73. Xu, S. *et al.* ggtreeExtra: Compact visualization of richly annotated phylogenetic data.
662 *Mol. Biol. Evol.* **38**, 4039-4042 (2021).
663 74. Harwood, C. R. & Cutting, S. M. *Molecular biological methods for Bacillus*. (Wiley,
664 1990).
665 75. Kauffman, K. M. & Polz, M. F. Streamlining standard bacteriophage methods for higher
666 throughput. *MethodsX* **5**, 159-172 (2018).
667 76. Schilling, T., Dietrich, S., Hoppert, M. & Hertel, R. A CRISPR-Cas9-based toolkit for
668 fast and precise in vivo genetic engineering of *Bacillus subtilis* phages. *Viruses* **10**, 241
669 (2018).
670 77. Altenbuchner, J. Editing of the *Bacillus subtilis* genome by the CRISPR-Cas9 system.
671 *Appl. Environ. Microbiol.* **82**, 5421-5427 (2016).
672 78. Westers, H. *et al.* Genome engineering reveals large dispensable regions in *Bacillus*
673 *subtilis*. *Mol. Biol. Evol.* **20**, 2076-2090 (2003).
674 79. Storms, Z. J., Teel, M. R., Mercurio, K. & Sauvageau, D. The virulence index: a metric
675 for quantitative analysis of phage virulence. *Phage (New Rochelle)* **1**, 27-36 (2020).
676 80. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina
677 sequence data. *Bioinformatics* **30**, 2114-2120 (2014).
678 81. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods*
679 **9**, 357-359 (2012).
680 82. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for
681 assigning sequence reads to genomic features. *Bioinformatics* **30**, 923-930 (2014).
682 83. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion
683 for RNA-seq data with DESeq2. *Genome Biol* **15**, 1-21 (2014).
684 84. R: A Language and Environment for Statistical Computing (R Foundation for Statistical
685 Computing, Vienna, Austria, 2021).
686 85. Karava, M., Bracharz, F. & Kabisch, J. Quantification and isolation of *Bacillus subtilis*
687 spores using cell sorting and automated gating. *PLoS One* **14**, e0219892 (2019).

688 86. Zhu, B. & Stülke, J. Subti Wiki in 2018: from genes and proteins to functional network
689 annotation of the model organism *Bacillus subtilis*. *Nucleic Acids Res.* **46**, D743-D748
690 (2018).
691

692 **FIGURE CAPTIONS**

693

694 **Fig 1. Sigma factors in viral genomes. a-c**, The overall number of phage genomes in each
695 taxonomic group is depicted by light grey bars with nested darker bars depicting how many of
696 those genomes contained one or more sigma factors. Taxonomic groups are host phylum (**a**),
697 viral family (**b**), and viral families containing sigma factors separated by host phyla (**c**). **d**,
698 Multiplicity of sigma factors genes in phage genomes. The distribution of sigma factor gene
699 counts in phage genomes is shown for all phages in the Viral Orthologous Groups (VOG)
700 database (left facet), and for each of the host phyla as in **c**. Phage genomes from the VOG
701 database were mapped to hosts using the Virus-Host Database. Viral families are specified for
702 tailed phages (*Caudovirales*), and all non-tailed phages are shown in a single category of non-
703 *Caudovirales*, with an asterisk to indicate that this is not a true viral family. The two
704 *Bacteroidetes* phages with sigma factors indicated in **a**, each from a different viral family are
705 excluded from **c** for clarity.

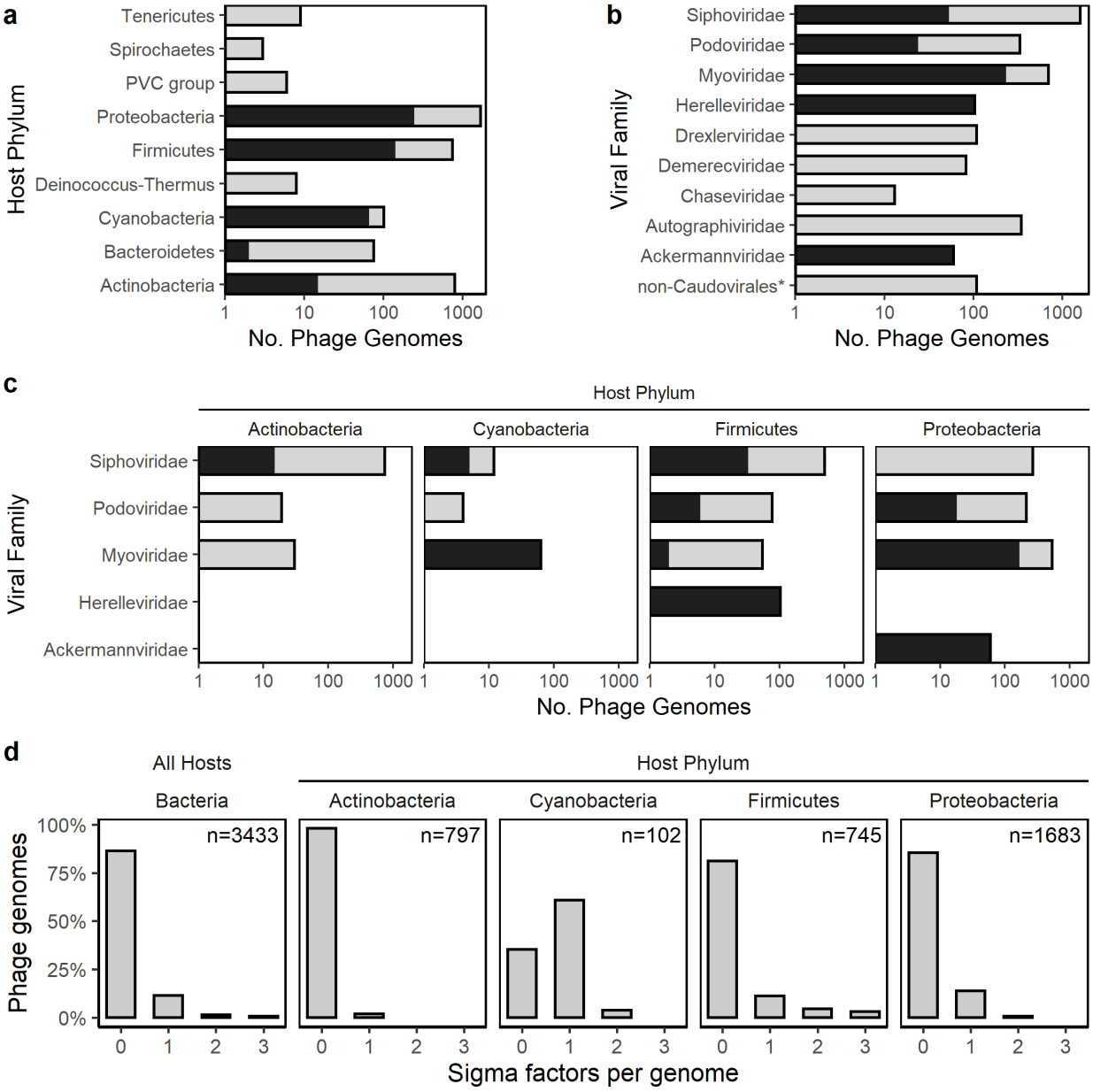
706

707 **Fig 2. Phylogeny and classification of phage-encoded sigma factors. a**, Phylogenetic tree
708 representing proteins of phage-encoded sigma factor from the database of viral orthologous
709 groups. For reference, we included sigma factors from 24 genomes of diverse bacterial species.
710 Blue branches on the tree correspond with monophyletic clades that only contain phage-encoded
711 sigma factors. Black branches show bacterial-encoded sigma factors and the internal branches
712 leading to them. Phage proteins discussed in the text are labeled at branch tips (e.g., ELD
713 gp128). In circle 1 (C1) we identify the source (phage vs. bacteria) of a sigma factor protein. In
714 circle 2 (C2), we designate the taxonomy of the bacterial host (phyla). In circle 3 (C3) we

715 provide the best hit (hmmscan sequence E-value) of the homolog to bacterial-encoded sigma
716 factors families in TIGRfam. Outside of circle 3, we indicate the relative positions of *Bacillus*
717 *subtilis* sigma factor genes and depict the different sigma factor families (grey wedges). Branch
718 nodes are labeled with nonparametric bootstrap support values (n = 500). **b**, Clade of
719 sporulation-specific sigma factors depicted by red arc in **a**. Triangles point to genes cloned and
720 expressed in this study. **c**, Summarized proportions of phage-encoded sigma factors as a function
721 of host taxonomy and protein family as described for circles 2 and 3 of panel **a**, respectively.
722
723 **Fig 3. Bacterial gene expression following induction of phage-encoded sigma factors. a**,
724 Illustration of the experimental design. Phage and host derived sigma factors were cloned in
725 *Bacillus subtilis* under an IPTG-inducible promoter (grey arrow) in a strain (Δ6) that contains a
726 fully functional sporulation gene network, including its native sporulation specific sigma factors
727 (black arrows show representative examples). To estimate differential expression, we compared
728 splits of *Bacillus subtilis* cultures. One half was induced to express a cloned sigma factor during
729 exponential growth, while the other half used as a non-induced control (n = 3). Differentially
730 expressed genes were defined as those where P values < 0.05 (horizonal dashed line in **b**) and
731 |fold change| > 2 (outside of vertical grey bar in **b**). **b**, Correlation of differentially expressed
732 genes for cells induced to express phage-encoded sigma factors and cells induced to express
733 bacteria-encoded sigma factors (*sigF* and *sigG*). Spearman's correlation coefficient (ρ) is
734 displayed. **c**, Sporulation genes[86] were upregulated after induction of a phage-encoded sigma
735 factor (ELDg169) and host-encoded sigma factors that regulate sporulation (*sigF* and *sigG*). The
736 number of significantly upregulated (△) and downregulated (▽) genes are noted.

737 **Fig. 4. Phage-derived sigma factors reduced sporulation. a**, Illustration of the experimental
738 design. Phage and host derived sigma factors were cloned in *Bacillus subtilis* under an IPTG-
739 inducible promoter (grey arrow) in a strain (Δ6) that contains a fully functional sporulation gene
740 network, including its native sporulation specific sigma factors (black arrows show
741 representative examples). We compared sporulation in these strains to an empty-vector negative
742 control strain that also had an IPTG-inducible promoter. For each data point in **b** replicate
743 cultures (n = 6) of a single colony were grown in sporulation medium for 4.5 hours, a time at
744 which sporulation was induced by nutrient exhaustion. We then induced expression of the cloned
745 sigma factor by the addition of IPTG to half of the replicates, while leaving the remainder as
746 controls. Spore and vegetative cells were quantified by flow-cytometry. Spore yield was
747 calculated as the ratio of percent spores in induced cultures and their paired controls. **b**, Bars
748 represent the mean ± SEM of independent clones (n ≥ 8). Shapes represent different
749 experimental batches. Asterisks correspond to significance levels (adjusted for multiple testing)
750 from Welch's *t*-tests used to evaluate the effect of each sigma factor compared to the empty-
751 vector negative control (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). ELD = phage Eldridge.

752 **Fig. 1**


a
Host Phylum
Tenericutes
Spirochaetes
PVC group
Proteobacteria
Firmicutes
Deinococcus-Thermus
Cyanobacteria
Bacteroidetes
Actinobacteria
1
10
100
1000
No. Phage Genomes
b
Viral Family
Siphoviridae
Podoviridae
Myoviridae
Herelleviridae
Drexlerviridae
Demerecviridae
Chaseviridae
Autographiviridae
Ackermannviridae
non-Caudovirales*
No. Phage Genomes
c
Host Phylum
Actinobacteria
Cyanobacteria
Firmicutes
Proteobacteria
Viral Family
Siphoviridae
Podoviridae
Myoviridae
Herelleviridae
Ackermannviridae
No. Phage Genomes
d
All Hosts
Host Phylum
Bacteria
Actinobacteria
Cyanobacteria
Firmicutes
Proteobacteria
Phage genomes
100%
75%
50%
25%
0%
n=3433
n=797
n=102
n=745
n=1683
0
1
2
3
Sigma factors per genome

753

Fig. 2

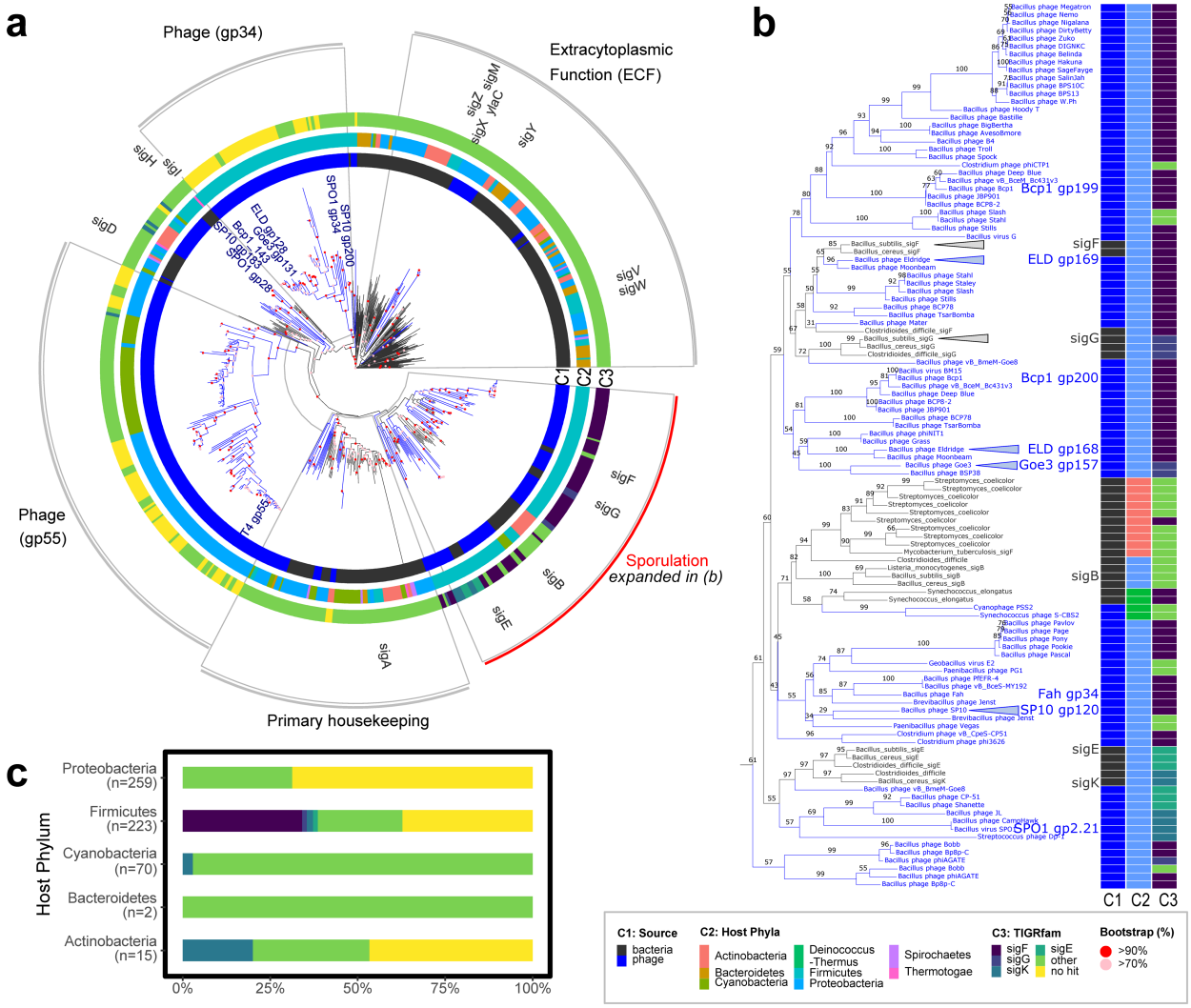
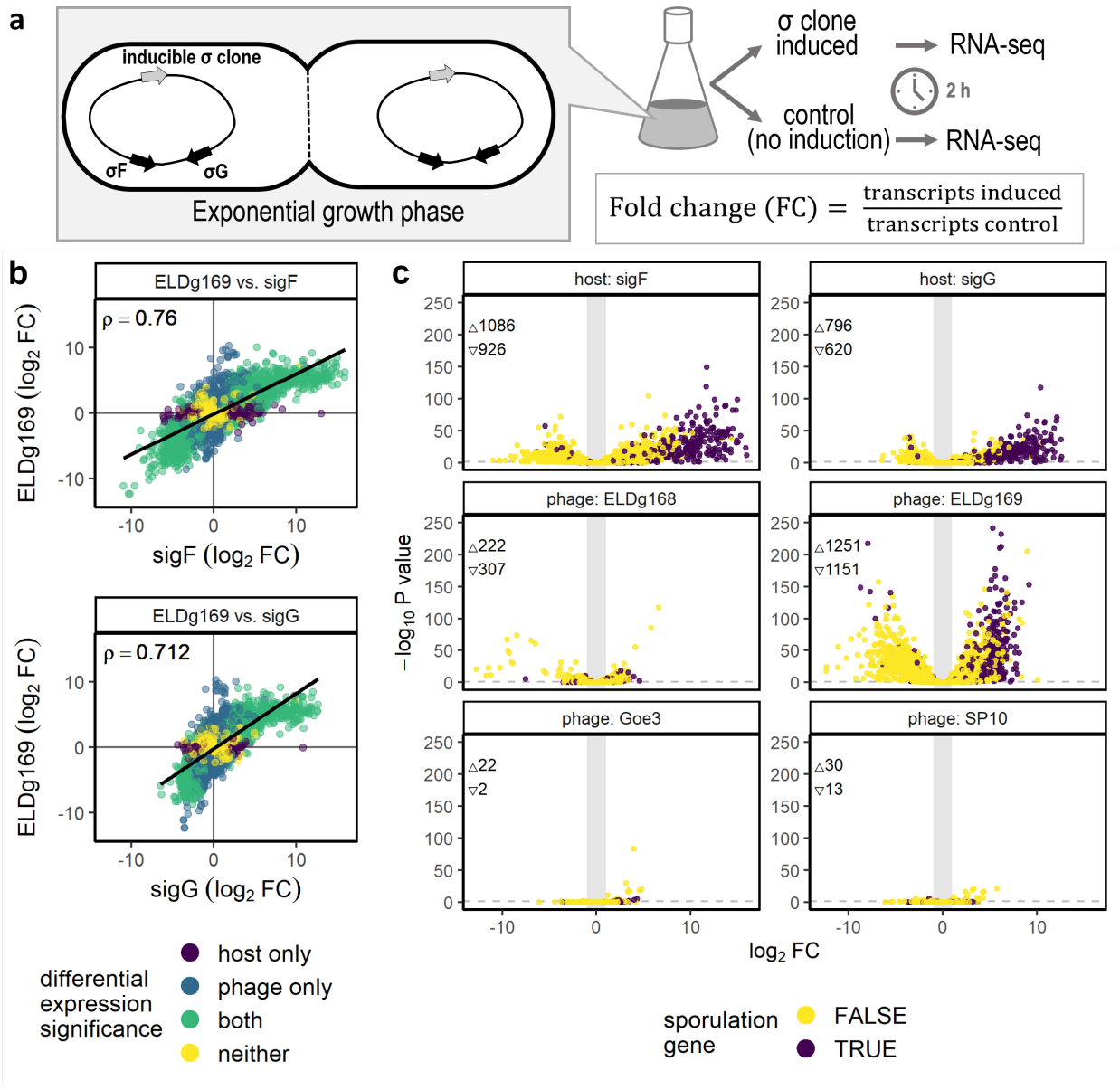
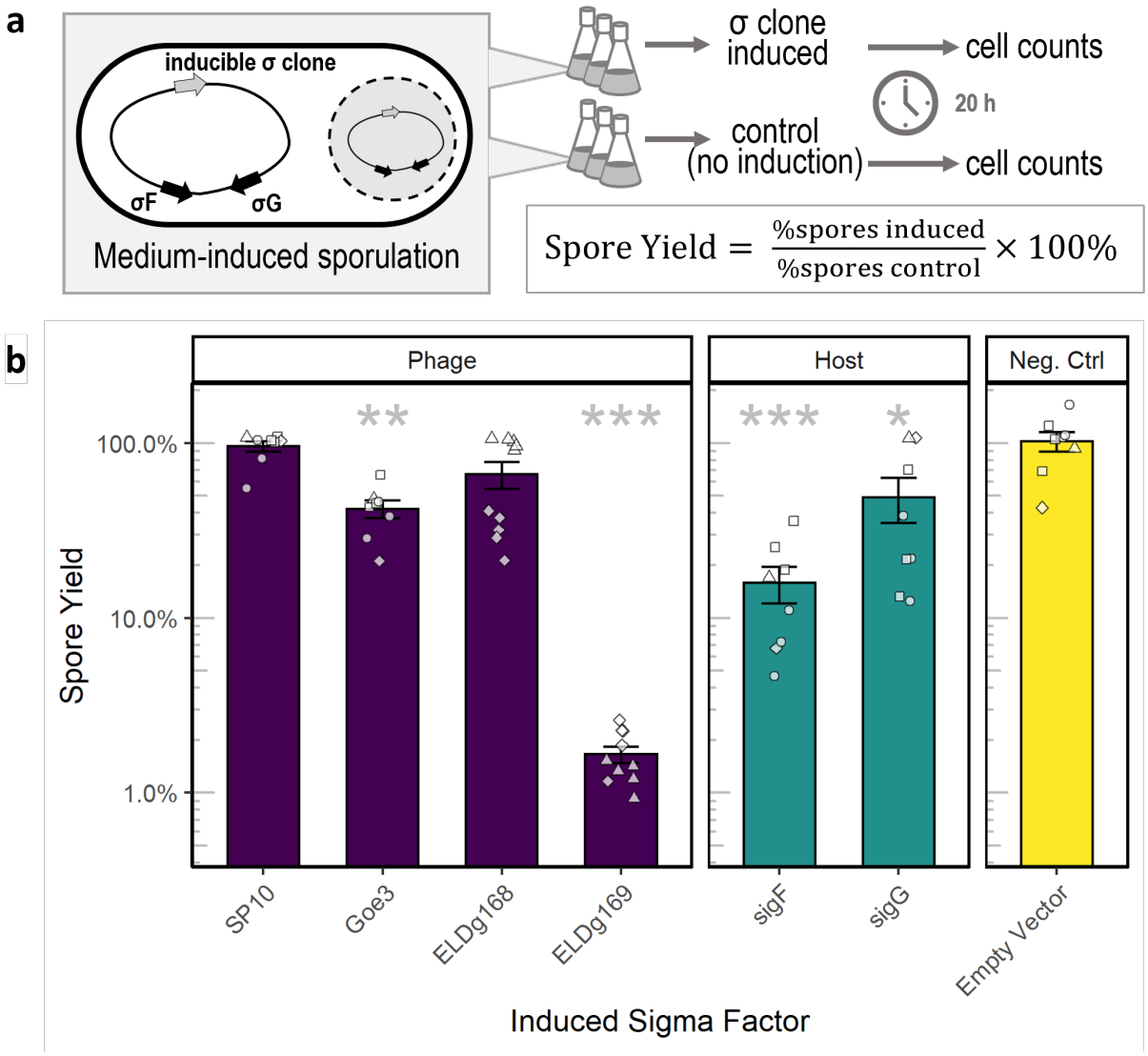

a
Phage (gp34)
Extracytoplasmic Function (ECF)
sigZ sigM
sigX yleC
sigY
sigV
sigW
sigF
sigG
sigB
sigE
sigA
sigD
sigH sigI
Phage (gp55)
Primary housekeeping
Sporulation expanded in (b)
C1
C2
C3
SPO1 gp34
SP10 gp200
ELD gp128
Goe3 gp131
Bcp1 gp143
SP10 gp183
SPO1 gp28
T4 gp55
b
Bcp1 gp199
sigF
ELD gp169
sigG
Bcp1 gp200
ELD gp168
Goe3 gp157
sigB
Fah gp34
SP10 gp120
sigE
sigK
SPO1 gp2.21
C1 C2 C3
C1: Source
bacteria
phage
C2: Host Phyla
Actinobacteria
Bacteroidetes
Cyanobacteria
Deinococcus -Thermus
Firmicutes
Proteobacteria
Spirochaetes
Thermotogae
C3: TIGRfam
sigF
sigG
sigK
sigE
other
no hit
Bootstrap (%)
>90%
>70%
c
Host Phylum
Proteobacteria (n=259)
Firmicutes (n=223)
Cyanobacteria (n=70)
Bacteroidetes (n=2)
Actinobacteria (n=15)
0%
25%
50%
75%
100%

Fig. 3


a
inducible σ clone
σF
σG
Exponential growth phase
σ clone induced
RNA-seq
2 h
control (no induction)
RNA-seq
Fold change (FC) = transcripts induced / transcripts control
b
ELDg169 vs. sigF
ρ = 0.76
ELDg169 (log2 FC)
sigF (log2 FC)
ELDg169 vs. sigG
ρ = 0.712
ELDg169 (log2 FC)
sigG (log2 FC)
differential expression significance
host only
phage only
both
neither
c
host: sigF
△1086
▽926
host: sigG
△796
▽620
phage: ELDg168
△222
▽307
phage: ELDg169
△1251
▽1151
phage: Goe3
△22
▽2
phage: SP10
△30
▽13
−log10 P value
log2 FC
sporulation gene
FALSE
TRUE

758

759 **Fig. 4.**



760