

Prophage-encoding engineered bacteria enable prophylactic lytic phage therapy for enteric infection in mice

In the format provided by the
authors and unedited

Table of Contents	Pages
Supplementary Table 1. Bacteria strains, phage strains, and plasmids used in this study.	2 - 3
Supplementary Table 2. Oligonucleotide sequences for StyLT1 <i>mod</i> gene promoters.	4
Supplementary Table 3. Primers used for amplification and validation in this study.	5
Supplementary Table 4. Oligomer sequences for silent mutation insertion.	6
Supplementary Figure 1. Scheme of prophylactic approach.	7
Supplementary Figure 2. Deletion of kanamycin resistance impacts phage production.	8
Supplementary Figure 3. Promoters of increasing strength driving expression of StyLT1 <i>mod</i> .	9
Supplementary Figure 4. <i>E. coli</i> phage P22* against various Salmonella isolates.	10
Supplementary Figure 5. Phage resistant STm isolates from the coculture experiments.	11
Supplementary Figure 6. Absence of streptomycin results in inconsistent STm infection.	12
Supplementary Figure 7. Bacteria concentrations in mice pre-colonized with <i>E. coli</i> .	13
Supplementary Figure 8. STm isolates can develop phage resistance.	14
Supplementary Figure 9. Inflammatory markers in mice before STm infection.	15
Supplementary Figure 10. Inflammatory markers in mice after STm infection.	16
Supplementary Figure 11. Pathological scores of cecal or colonic inflammation.	17
Supplementary Figure 12. Phage resistance of fecal STm isolates.	18

Supplementary Table 1. Bacteria strains, phage strains, and plasmids used in this study.

Name used in this paper	Parental strain	Strain/Genotype	Source
<i>Bacteria</i>			
<i>E. coli</i> K-12 parental	-	<i>E. coli</i> K-12(Δ <i>lacZ</i>)	Pamela Silver, (Harvard Medical School)
<i>E. coli</i> K-12	<i>E. coli</i> K-12 parental	<i>E. coli</i> K-12 (<i>rbsA-rbsR</i> ::(<i>camR</i> -J23119 ^{UP} - <i>StyLT1mod</i>); <i>rpsL</i> ::K43T; Δ <i>lacZ</i>)	This paper
<i>E. coli</i> K-12 (P22-susceptible)	<i>E. coli</i> K-12 parental	<i>E. coli</i> K-12+pPR1347	This paper
<i>E. coli</i> K-12::c2 (P22-susceptible)		<i>E. coli</i> K-12 (<i>araB-araC</i> ::(ProC-c2))+pPR1347	This paper
<i>E. coli</i> K-12(P22)	<i>E. coli</i> K-12 parental	<i>E. coli</i> K-12 (<i>thrW</i> ::P22(Δ <i>orf25-orf80</i>); <i>araB-araC</i> ::(ProC-c2); <i>rbsA-rbsR</i> ::(<i>camR</i> -J23119 ^{UP} <i>StyLT1mod</i>); <i>rpsL</i> ::K43T; Δ <i>lacZ</i>)	This paper
<i>E. coli</i> K-12(P22*)	<i>E. coli</i> K-12 parental	<i>E. coli</i> MG1655 (<i>thrW</i> ::P22(Δ <i>orf25-orf80</i> ; <i>c2</i> ::33_34dupTAA); <i>araB-araC</i> ::(ProC-c2); <i>rbsA-rbsR</i> ::(<i>camR</i> -J23119 ^{UP} <i>StyLT1mod</i>); <i>rpsL</i> ::K43T; Δ <i>lacZ</i>)	This paper
<i>E. coli</i> KP7 parental	-	<i>E. coli</i> KP7(pCAH63(<i>tetR</i> ; <i>camR</i>))	Tracy Raivio (University of Alberta)
<i>E. coli</i> KP7	<i>E. coli</i> KP7 parental	<i>E. coli</i> KP7 (pCAH63(<i>tetR</i> ; <i>camR</i>); <i>rpsL</i> ::K43T)	This paper
<i>E. coli</i> KP7(P22)	<i>E. coli</i> KP7 parental	<i>E. coli</i> KP7 (<i>thwR</i> ::P22(Δ <i>ninB-ninH</i>); <i>araB-araC</i> ::(ProC-c2); <i>rbsA-rbsR</i> ::(<i>camR</i> -J23119 ^{UP} <i>StyLT1mod</i>); <i>rpsL</i> ::K43T; pCAH63(<i>tetR</i> ; Δ <i>camR</i>))	This paper
<i>E. coli</i> KP7(P22*)	<i>E. coli</i> KP7 parental	<i>E. coli</i> KP7 (<i>thwR</i> ::P22(Δ <i>ninB-ninH</i> ; <i>c2</i> ::33_34dupTAA); <i>araB-araC</i> ::(ProC-c2); <i>rbsA-rbsR</i> ::(<i>camR</i> -J23119 ^{UP} <i>StyLT1mod</i>); <i>rpsL</i> ::K43T; pCAH63(<i>tetR</i> ; Δ <i>camR</i>))	This paper
STm wildtype	-	<i>Salmonella enterica</i> sv. Typhimurium 14028S	Michael McClelland (UC Irvine)
STm	STm wildtype	STm(<i>phoN</i> :: <i>tetR</i> ; <i>rpsL</i> ::K43N), or STm(<i>phoN</i> :: <i>kanR</i> ; <i>rpsL</i> ::K43N)	Michael McClelland (UC Irvine)

STm(P22)	STm wildtype	STm(<i>thwR</i> ::P22(Δ <i>ninB-ninH</i>); <i>rpsL</i> ::K43N)	This paper
STm (T3SS mutant)	STm wildtype	STm(<i>invG</i> :: <i>kanR</i> ; <i>ssaV</i> :: <i>tetR</i> ; <i>rpsL</i> ::K88N)	Michael McClelland (UC Irvine)
STm(<i>StySA res</i> ⁻)	STm wildtype	STm(<i>StySA res</i> :: <i>kanR</i>)	Michael McClelland (UC Irvine)
STm(<i>StyLT1 res</i> ⁻)	STm wildtype	STm(<i>StyLT1 res</i> :: <i>camR</i>)	Michael McClelland (UC Irvine)
STm(<i>StySA res</i> ⁻ ; (<i>StyTL1 res</i> ⁻)	STm(<i>StySA res</i> ⁻)	STm(<i>StySA res</i> :: <i>kanR</i> ; <i>StyLT1 res</i> :: <i>camR</i>)	This paper

Phages

P1	-	Generalized transducing phage	Pamela Silver, (Harvard Medical School)
P22	-	P22 WT temperate phage, <i>podoviridae</i>	Pamela Silver, (Harvard Medical School)
P22*	P22*	P22(Δ <i>orf25-orf80</i> ; <i>c2</i> ::33_34dupTAA)	This paper
P22 ^{frt-kanR-frt}	P22	P22(Δ <i>orf25-orf80</i> ::(<i>kanR</i>))	This paper
P22 ^{frt-kanR-frt,*}	P22*	P22(Δ <i>orf25-orf80</i> ::(<i>kanR</i>)); <i>c2</i> ::33_34dupTAA)	This paper
P22-H5	P22	Lytic P22 with a non-synonymous mutation in the phage repressor gene	Kelly Hughes (University of Utah)
P22-HT105/1 <i>int</i> -201	P22	An integration-deficient mutant of P22 used for generalized transduction	Michael McClelland (UC Irvine)

Plasmids

pCP20	-	FLP recombinase expression plasmid, temperature-sensitive ori101 repA101ts replicon; ampR, camR	Pamela Silver, (Harvard Medical School)
pGGA-select	-	Golden Gate Assembly plasmid with multiple cloning sites	New England Biolabs
pKD46	-	λ red recombination plasmid, arabinose-inducible, temperature-sensitive ori101 repA101ts replicon; ampR	Pamela Silver, (Harvard Medical School)
pPR1347	-	<i>rfb</i> gene cluster and <i>rfc</i> gene; kanR	SGSC, University of Calgary

Supplementary Table 2. Oligonucleotide sequences for StyLT1 *mod* gene promoters. Each sequence has the homology regions (blue), the chloramphenicol resistance cassette (underlined), the UP sequence (green, if present), the promoter (in bold), and the RBS (in italics). Relative promoter strength was calculated based on the predicted transcription rates relative to the natural promoter of the StyLT1 *mod* gene in STm. The transcriptional rate of each promoter was predicted as described in the main text.

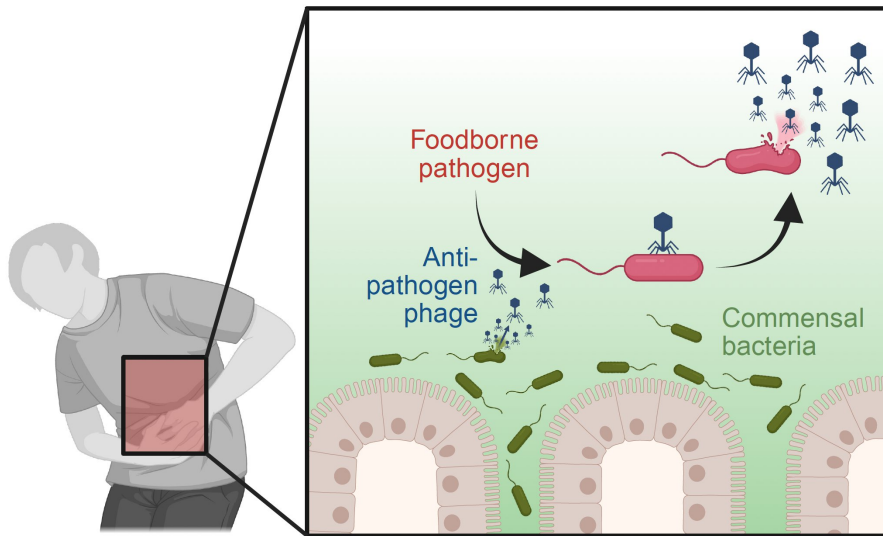
Promoter ID	Promoter strength	G-block sequence
J23119 ^{UP}	78	<u>TGAGCAGGCCACCCAGGAAGTGTTAATGGCTGCCGCTGTGGGCAAGCTTAATCGC</u> <u>GTGAATCAGGAGTAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATT</u> <u>CATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAACCTGAATC</u> <u>GCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAC</u> <u>GGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAACTGGTGAACTCA</u> <u>CCCAGGGATTGGCTGAGACAAAAACATATTCTCAATAAACCCCTTTAGGGAAATAG</u> <u>GCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGTGTAGAACTGCCG</u> <u>GAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGA</u> <u>AAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCCTCTTTCATT</u> <u>GCCATACGAAATTCGGGATGAGCATTATCAGGCGGGCAAGAATGTGAATAAAGG</u> <u>CCGGATAAACTTGTCCTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCA</u> <u>GCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGT</u> <u>TCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTCTCC</u> <u>ATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGT</u> <u>GATCTTATTTTATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCGGAAAAATTTT</u> <u>TTTAAAAAAAAC</u>TTGACAGCTAGCTCAGTCCTAGGTATAATAGATCTGAATTC <u>ATTAAGAGGAGAAAGGTACCATGTTGAAAGATAACCAAAAAACACAACGAGTCTGT</u> <u>TGCCCCGAATAGCGCCTTTCTGTCTGAGTTACAAC</u>
J23119	41	<u>TGAGCAGGCCACCCAGGAAGTGTTAATGGCTGCCGCTGTGGGCAAGCTTAATCGC</u> <u>GTGAATCAGGAGTAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATT</u> <u>CATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAACCTGAATC</u> <u>GCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAC</u> <u>GGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAACTGGTGAACTCA</u> <u>CCCAGGGATTGGCTGAGACAAAAACATATTCTCAATAAACCCCTTTAGGGAAATAG</u> <u>GCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGTGTAGAACTGCCG</u> <u>GAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGA</u> <u>AAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCCTCTTTCATT</u> <u>GCCATACGAAATTCGGGATGAGCATTATCAGGCGGGCAAGAATGTGAATAAAGG</u> <u>CCGGATAAACTTGTCCTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCA</u> <u>GCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGT</u> <u>TCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTCTCC</u> <u>ATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGT</u> <u>GATCTTATTTTATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCTTGACAGCT</u> AGCTCAGTCCTAGGTATAATAGATCTGAATTCATTAAGAGGAGAAAGGTACCATG <u>TTGAAAGATAACCAAAAAACACAACGAGTCTGTTGCCCGAATAGCGCCTTTCTGTC</u> <u>TGAGTTACAAC</u>
J23107 ^{UP}	19	<u>TGAGCAGGCCACCCAGGAAGTGTTAATGGCTGCCGCTGTGGGCAAGCTTAATCGC</u> <u>GTGAATCAGGAGTAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATT</u> <u>CATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAACCTGAATC</u> <u>GCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAC</u> <u>GGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAACTGGTGAACTCA</u> <u>CCCAGGGATTGGCTGAGACAAAAACATATTCTCAATAAACCCCTTTAGGGAAATAG</u> <u>GCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGTGTAGAACTGCCG</u> <u>GAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGA</u> <u>AAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCCTCTTTCATT</u> <u>GCCATACGAAATTCGGGATGAGCATTATCAGGCGGGCAAGAATGTGAATAAAGG</u> <u>CCGGATAAACTTGTCCTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCA</u> <u>GCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGT</u> <u>TCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTCTCC</u> <u>ATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGT</u> <u>GATCTTATTTTATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCGGAAAAATTTT</u> <u>TTTAAAAAAAAC</u>TTTACGGCTAGCTCAGCCCTAGGTATTATAGATCTGAATTC <u>TTAAAGAGGAGAAAGGTACCATGTTGAAAGATAACCAAAAAACACAACGAGTCTGTT</u> <u>GCCCCGAATAGCGCCTTTCTGTCTGAGTTACAAC</u>

Supplementary Table 3. Primers used for amplification and validation of integration in the bacterial genome.

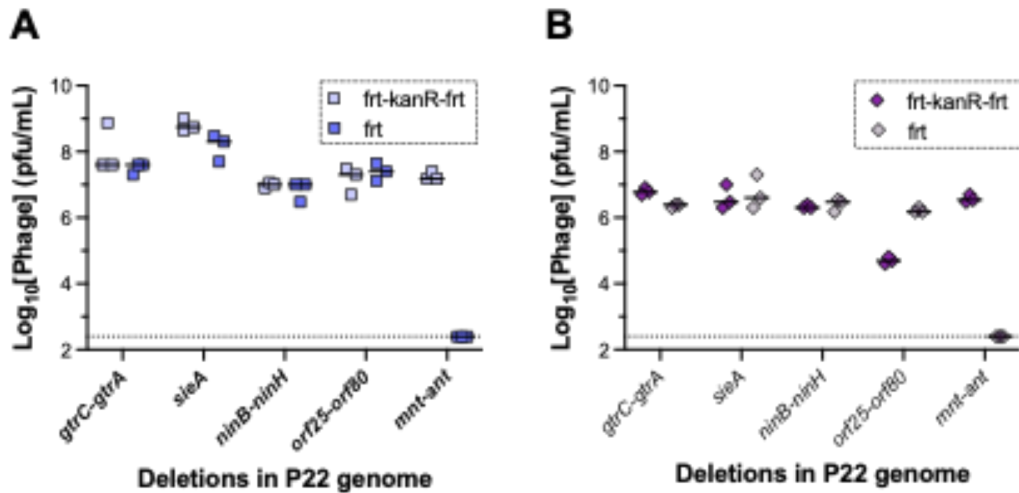
Purpose	Primers ID	Primer sequence
Validate P22 integration downstream of the <i>thrW</i> site in <i>E. coli</i> (P22)	RB01	ACCGGGTGATGCAAAAGTAG
	RB02	CGGACAAACAATCATGCAAC
Validate P22 integration downstream of the <i>attP22</i> site in <i>E. coli</i> (P22)	RB03	GGCAGCAGATAGACATGCAA
	RB04	AGCTCATTAACTCACGTACCG
Validate the transformation of pPR1347 in <i>E. coli</i>	RB05	ATGCGCTGACTTTGTTTCCT
	RB06	CCGTAAGAATTGAGGGGAAAA
Validate the transformation of pKD46 in <i>E. coli</i>	ZB11	GCAAATATTGACGGCCAGTT
	ZB12	AAACCAATTTTCAGCCAGTGC
Amplify gene constructs from the Golden Gate Assembly ligation product (pGGAselect) for homologous recombination in the recipient strain	ZB15	CTGCAGGAAGGTTTAAACGCATTTAGG
	ZB16	TAATACGACTCACTATAGGGGAGACTC
Validate the integration of the phage repressor construct in the arabinose operon in <i>E. coli</i> (P22)	HB142	GGTTATTCGGGGCATCAC
	HB143	CATACTCCCACCATTCAG
Validate the integration of the StyLT1 <i>mod</i> gene construct in the <i>rbsA-rbsR</i> locus in <i>E. coli</i> (P22)	RB85	CTACCCGTGGCGTAGATGTC
	RB86	CTGGGCTATCGCCATACGTT
Validate the integration of StyLT1 promoters in <i>E. coli</i> (P22*)	RB127	AGGCCACCCAGGAAGTGTTA
	RB128	AACTCAGACAGAAAGGCGCT
Validate the mutation in the c2 phage repressor gene in <i>E. coli</i> (P22*)	RB46	TGTGGCCTCGTTTTACCTT
	RB47	AACGCATCAGCGACTTTACG
Validate the point mutation in the <i>rpsL</i> in <i>E. coli</i> and STm	RB48	TGGCCTTACTTAACGGAGAACC
	RB49	TCGGCGTCCTCATATTGTG
Validate transduction of StyLT1 <i>res⁻</i> gene in STm (StyLT1 <i>res⁻</i> , StySA <i>res⁻</i>)	RB153	CCTTCCTCCCGTCGTTTTCA
	RB154	CCGTTAGTTTGCCTTCCCCT

Supplementary Table 4. Antisense oligomer sequence for silent mutation insertion in the *c2* repressor gene in the P22 prophage. Stop codons are indicated in bold.

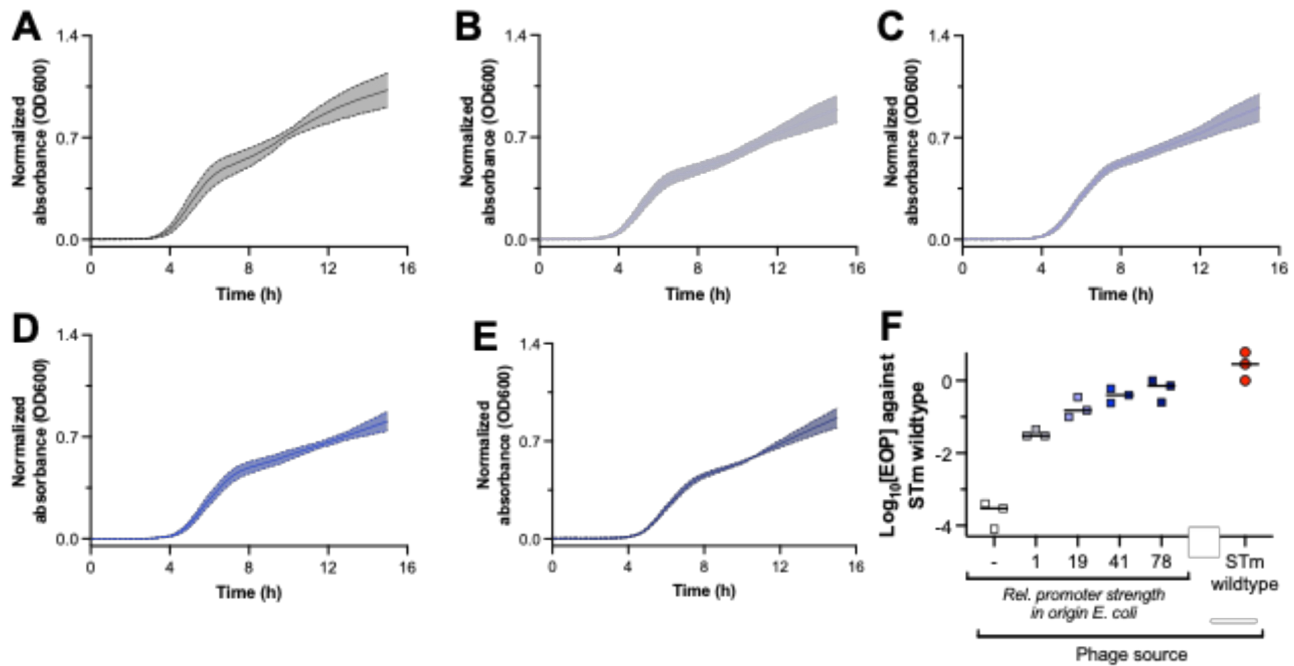
Strain	Oligomer sequence
<i>E. coli</i> (P22*) K-12	ATGAATACACAATTGATGGGTGAGCGTATTCGCT TAATA AGCTCGAAGAAAAAACT CAAGATTAGACAAG
<i>E. coli</i> (P22*) KP7	AACGATCTCTAATCGGTATGCGTCTTTCTCTGGGATAACTTCCTTCCACTGAGAGA CCGCTGCATCGCTAATGCCTAAAGCCTTAGCTACTGCACGCTGGGTTCCGAAGTG GTCGATAACATCTTTCTTGTACATAGACTCGCTCCGAAATTAAAGAACACTTAAATT ATCTACTAAAGGAATCTTTAGTCAAGTTTATTTAAGATGACTTAACTATGAATACACA ATTGATGGGTGAGCGTATTCGCT TAATA AGCTCGAAGAAAAAACTCAAGATTAGAC AAGCCGCTCTTGGTAAGATGGTGGGAGTGTCTAATGTTGCAATATCGCAATGGGA GCGCTCGGAGACTGAGCCAAATGGGGAGAACCTGTTGGCACTTTTCAAGGCTCTT CAGTGCTCCCCTGACTATTTGCTGAAAGGAGATTTAAGCCAGACAAACGTTGCCTA TCATAGTAGGCATGAGCCAAGAGGATCATACCCTCTTATCAGTTGGGTA



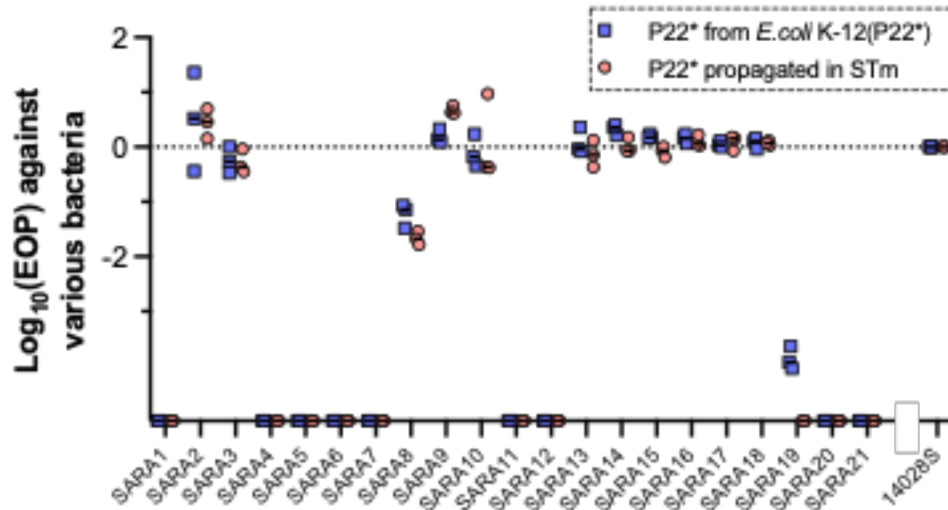
Supplementary Figure 1. Scheme of prophylactic approach to phage therapy using an anti-pathogen prophage. A gut bacterium (green) is engineered to spontaneously produce lytic phages (blue) that target a pathogen of different species (red). Colonization with this engineered bacterium establishes high concentrations of these phages in the gut so that a foodborne pathogen can be killed upon ingestion. Created in BioRender. Hsu, B. (2026)



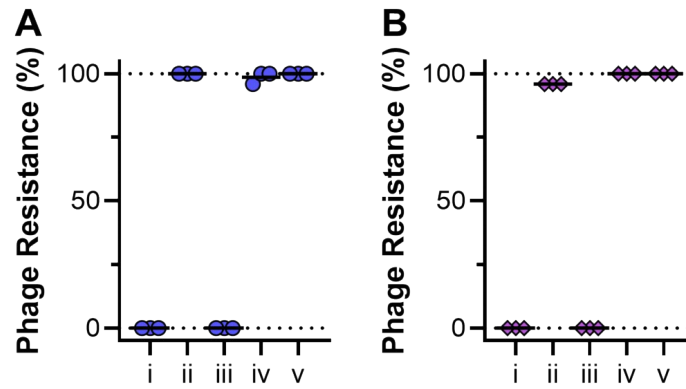
Supplementary Figure 2. Deletion of the kanamycin resistance cassette impacts phage production in *orf25-orf80* mutants in *E. coli* KP7(P22). P22 phage produced from lysogens of (A) *E. coli* K-12(P22) and (B) *E. coli* KP7(P22) with the kanamycin resistance gene inserted into different loci of the P22 prophage (n = 3 independent experiments per group, the solid lines represent the median). Phage was quantified using *E. coli* susceptible to P22. The antibiotic cassette was excised by self-recombination of the FRT sequences flanking the gene, aided by a FLP-recombinase expressed from the temperature-sensitive plasmid pCP20. Of interesting note is that excision of the kanR cassette from the *mnt-ant* region led to an inability to produce active phage. For the work further described the P22 phage in K-12 was modified in the *orf25-orf80* region, whereas the phage in KP7 was modified in the *ninB-ninH* region. Dashed lines represent the lower limit of detection.



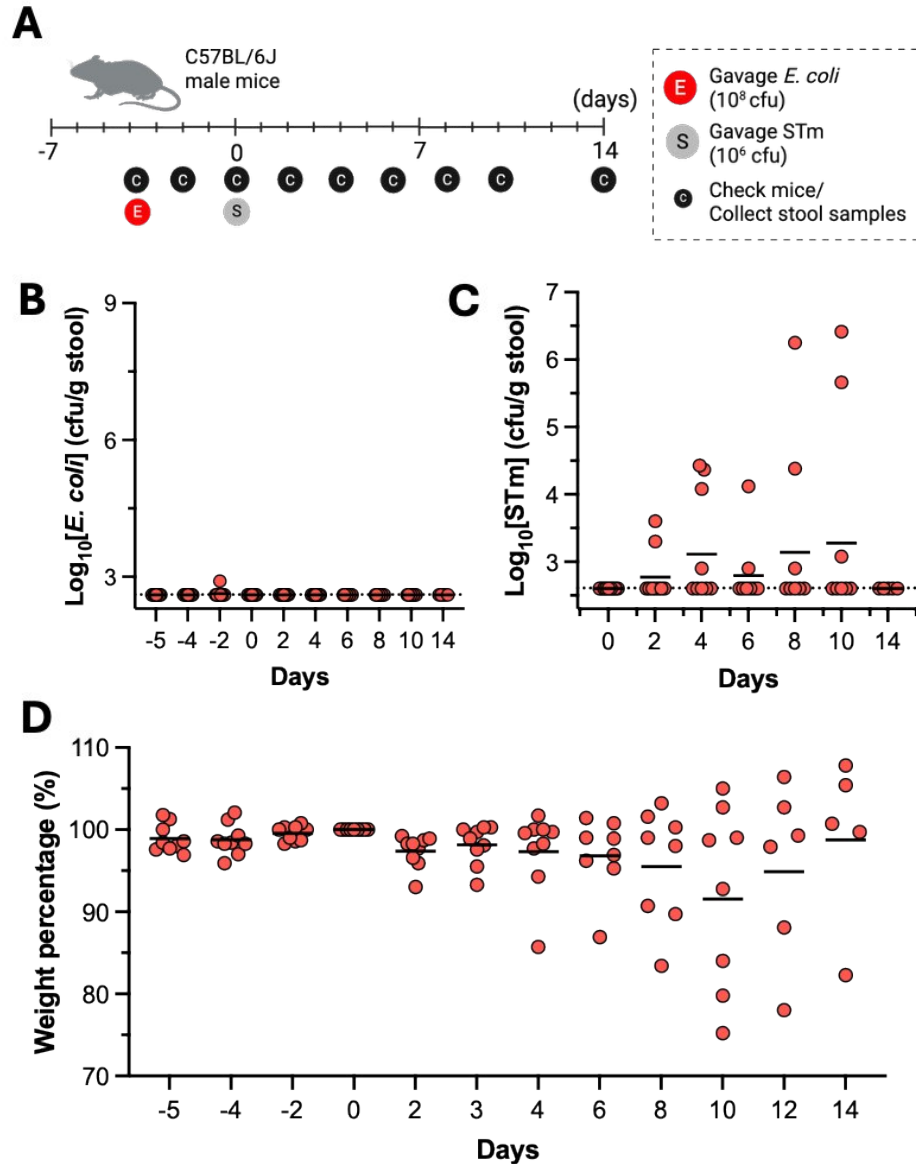
Supplementary Figure 3. Promoters of increasing strength driving expression of *StyLT1 mod* restore EOP of P22 phage against wildtype STm. Growth curves for *E. coli* K-12(P22) (A) without *StyLT1 mod* gene, with one chromosomal copy of the heterologous *StyLT1 mod* gene from STm under promoters with relative strengths of (B) 1, (C) 19, (D) 41, and (E) 78 ($n = 5$ independent experiments, the lines represent the mean, and shaded areas the standard deviation). (F) Promoters of increasing strength driving expression of *StyLT1 mod* gene restore EOP of P22 phage against wildtype STm ($n = 3$ independent experiments per group, the solid lines represent the median). Relative promoter strength was calculated based on predicted transcriptional rate relative to the natural promoter of the *StyLT1 mod* gene in STm. The promoter sequences are described in Supplementary Table 2.



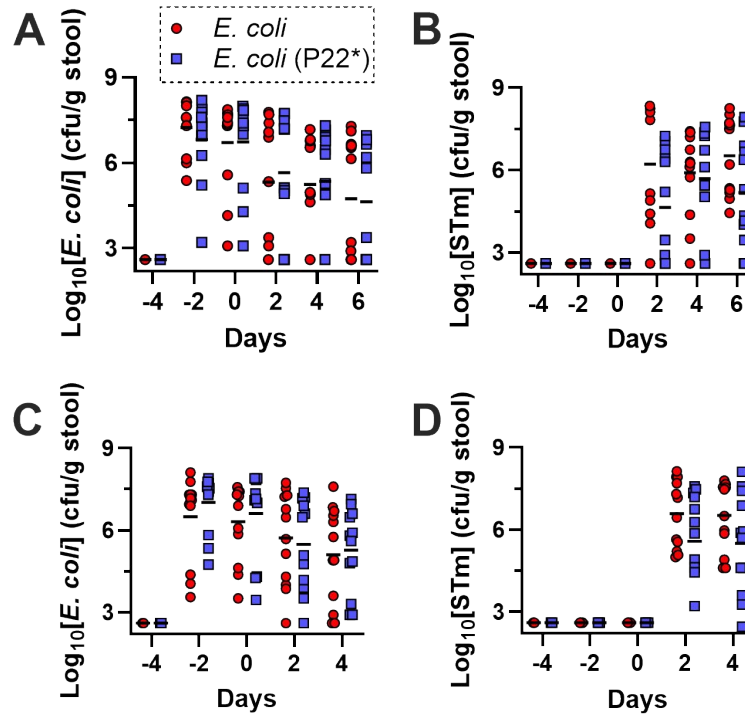
Supplementary Figure 4. *E. coli* phage P22* infects and replicates in various *Salmonella* isolates. Virulent P22 from *E. coli* K-12 (P22*) and P22* propagated in wildtype STm were assessed to infect strains of the *Salmonella* reference collection A (SARA). Phage infection in the STm isolates was carried out using the spot assay method ($n = 3$ independent experiments per group, the solid lines represent the median). The efficiency of plating (EOP) was calculated based on the ratio of the phage titer in one of the SARA strains and the phage titer in the STm wildtype (14028S).



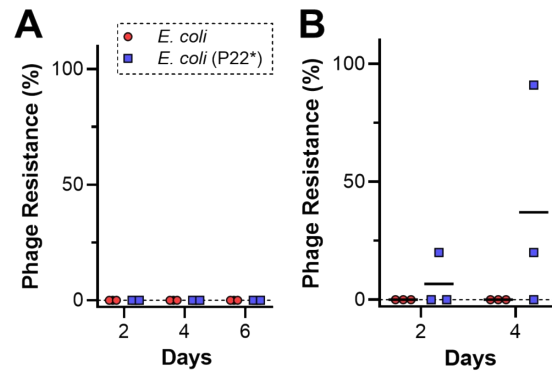
Supplementary Figure 5. Phage resistant STm isolates from the coculture experiments. Colonies of STm from cultures with **(A)** *E. coli* K-12 or **(B)** *E. coli* KP7 were tested for resistance to P22-H5 phage. STm cultures were (i) grown alone or in a mixture with (ii) free P22* phage, (iii) non-lysogenic *E. coli*, or (iv) *E. coli* lysogenized by P22 or (v) P22* for 8 h. For all conditions, at least 25 colonies were tested, except for condition v, in which we tested all the colonies available (1 to 3 colonies) (n = 3 mice per group, the solid line represent the median).



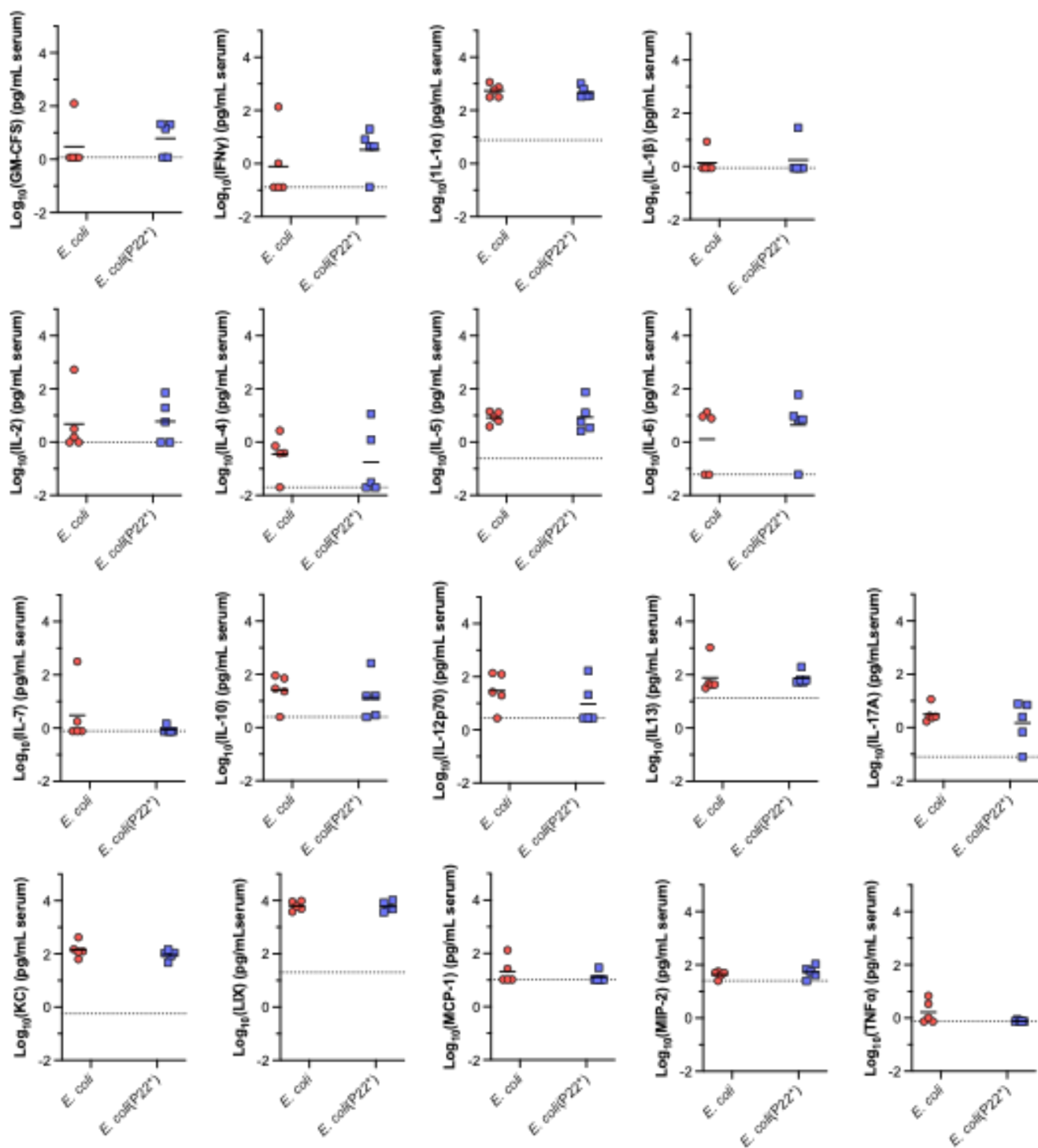
Supplementary Figure 6. The absence of streptomycin results in inconsistent STm infection in the mouse gut. (A) Experimental scheme of the oral infection of mice with STm. Mice were orally gavaged (10^8 cfu) with non-lysogenic *E. coli* K-12 (control group) and, four days later, infected with STm by oral gavage (10^6 cfu). Mouse health and weight were tracked regularly, and stool samples were collected to assess the presence of bacteria. (B) *E. coli* and (C) STm levels in mouse stool, and (D) mice weight variation relative to the weight right before STm infection ($n = 9$ mice, the solid lines represent the geometric mean). Dashed lines represent the lower limit of detection.



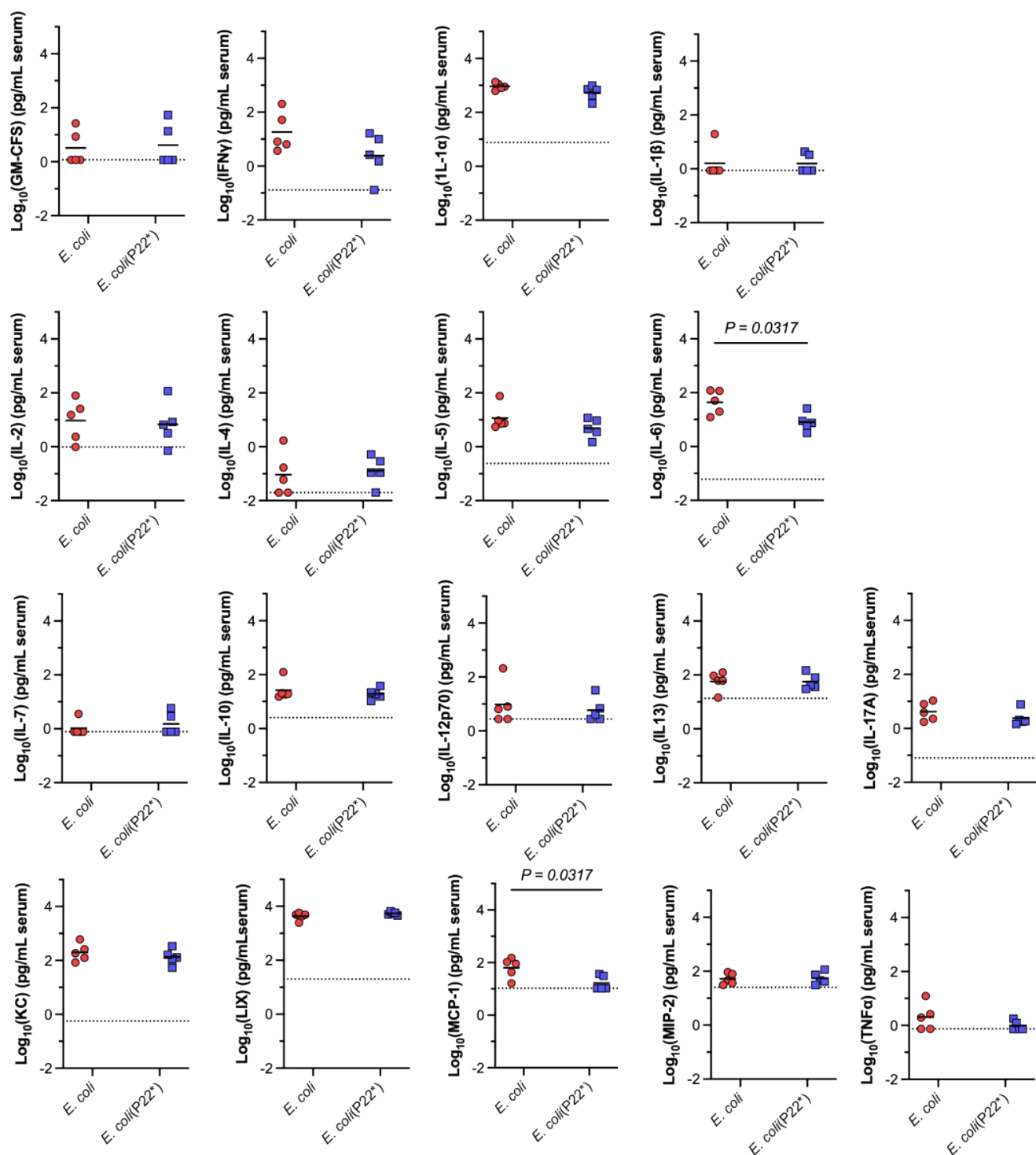
Supplementary Figure 7. Fecal bacteria concentrations in mice pre-colonized with *E. coli* K-12 or *E. coli* K-12(P22*) and infected with STm. The experimental scheme of the oral infection of mice with STm in a streptomycin-treated model of infection is presented in Figure 3A. Results for the *E. coli* K-12 (control) and *E. coli* K-12(P22*) (treated) groups infected with (A-B) low (10^2 cfu) and (C-D) high (10^6 cfu) STm dose. (A and C) *E. coli*, (B and D) STm levels in mouse stool pellets (n = 12 mice per group, the solid lines represent the geometric mean).



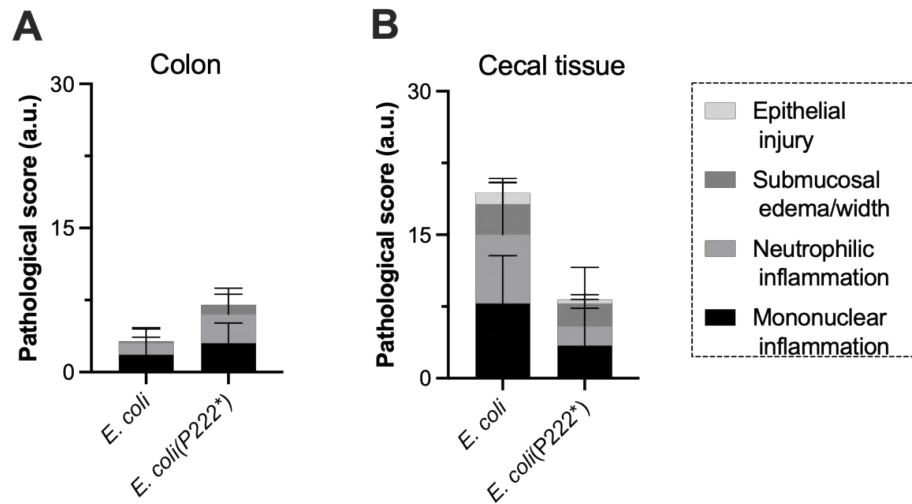
Supplementary Figure 8. Fecal STm isolates develop phage resistance when given at a high dose but not a low dose to *E. coli* K-12(P22*)-treated mice. Phage resistance of STm colonies isolated from stool samples of mice infected with (A) 10² cfu and (B) 10⁶ cfu of STm. Phage resistance was calculated by streaking 10-25 colonies against P22-H5. Each point represents the percentage of resistant colonies in each stool sample (n = 3 mice per group, the solid lines represent the mean). The experimental scheme of the oral infection of mice with STm in a streptomycin-treated model of infection is presented in Figure 3A.



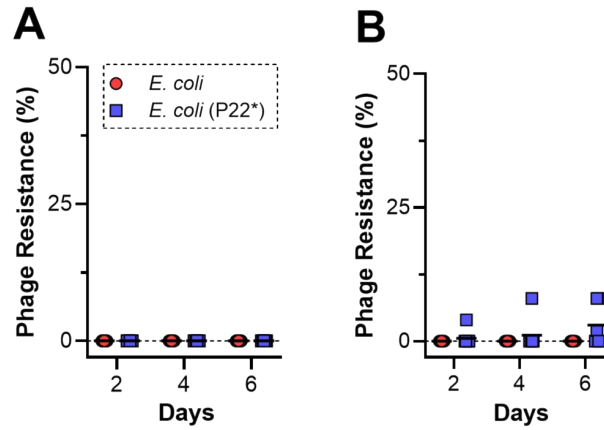
Supplementary Figure 9. Mice treated with *E. coli* K-12 and *E. coli* K-12(P22*) do not show differences in inflammatory markers before STm infection. Cytokine panel of mouse serum samples before STm infection (day 0). Samples with undetected concentrations were set to the limit of detection for each cytokine (n = 5 mice per group, the solid lines represent the mean). Statistical analyses were performed by two-tailed, unpaired Mann-Whitney test, which showed no significant comparisons. Dashed lines represent the lower limit of detection. The schematic representation of the mouse treatment is shown in Figure 3A.



Supplementary Figure 10. Lower levels of IL-6 and MCP-1 inflammatory biomarkers are found in mice treated with *E. coli* K-12(P22*) after STm infection. Cytokine panel of mouse serum samples 4 days after STm infection. Samples with undetected concentrations were set to the limit of detection for each cytokine (n = 5 mice per group, the solid lines represent the mean). Statistical analyses were performed by two-tailed, unpaired Mann-Whitney test. Dashed lines represent the lower limit of detection. The schematic representation of the mouse treatment is shown in Figure 3A.



Supplementary Figure 11. Mice treated with *E. coli* K-12 and *E. coli* K-12(P22*) do not show significant differences in cecal or colonic inflammation. Pathological scores for (A) colon and (B) cecal mouse tissues collected 8 days after STm infection. Fixed hematoxylin- and eosin-stained samples were scored blindly by board-certified veterinary anatomic pathologist. The total pathological score is the sum of the four inflammation markers, each individually assessed for each mouse (n = 5 mice per group, error bars represent the standard deviation). Statistical analyses were performed by two-tailed, unpaired Mann-Whitney test, which showed no significant comparisons.



Supplementary Figure 12. Fecal STm isolates have limited phage resistance against P22-H5 for groups treated with commensal *E. coli* KP7 (P22*). Phage resistance of STm colonies isolated from stool samples of mice treated with *E. coli* or *E. coli*(P22*) and infected with (A) 10² cfu or (B) 10⁶ cfu of STm (n = 7 mice per group, the solid lines represent the mean). Phage resistance was measured by cross-streaking 10-25 colonies against P22-H5 from each stool sample. Dashed lines represent the lower limit of detection. The experimental scheme of this mouse experiment is detailed in Extended Figure 5.