Supporting Information

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SI Materials and Methods

Plasmids and Recombinant DNA. A list of the plasmids used in these studies can be found in Table S2. *Enterococcus faecalis* genomic DNA was isolated using a DNeasy Blood and Tissue kit (Qiagen). PCR fragments were amplified using KOD Hot Start DNA Polymerase (Novagen) according to the manufacturer's instructions. *E. faecalis* V583 genomic DNA served as template for PCR reactions. Primer sequences are available on request. All plasmids generated for allelic exchange in *E. faecalis* are derivations of the temperature-sensitive plasmid pLT06 (1). Electroporation of pLT06-based constructs was done using *Escherichia coli* ElectroTen-Blue cells (Agilent Technologies). To construct the markerless exchange vector pLTPH07 used for the deletion of the ϕV7 phage element, a two-step PCR strategy was used to generate a ϕV7 deletion (deletion of EF2936–EF2953). Two PCR fragments were initially generated. The first PCR product extended from +1,048 to +89 bp in reference to the predicted stop codon of EF2936. The second PCR product extended from −166 to −1,412 bp in reference to the predicted start codon of EF2953. These two products had complementary terminal sequences and were mixed for a second PCR that fused the products together. This PCR generated an ∼2.2 kb ϕV7 deletion product that was subsequently cloned into *EcoRI-SphI*–digested pLT06, creating pLTPH07. To generate the EF0339 capsid deletion plasmid pLT0339, PCR was used to amplify two products, one extending from −986 to +9 bp with respect to the EF0339 start codon and one extending from +6 to −1,064 bp with respect to the EF0339 stop codon. These products were used in a second PCR to generate an ∼2.1 kb in frame deletion of EF0339. This PCR product is deleted for the nucleotides representing aa 4–286 of the EF0339 capsid polypeptide sequence. The EF0339 deletion product was then cloned into *BamHI-NcoI*–digested pLT06, generating pLT0339. The in-frame deletion construct used to mutagenize the ϕV7 DNA primase gene EF2948 was created as described above. PCR amplified a sequence extending from +9 to −1,063 bp in reference to the EF2948 start codon and a second product extending from +6 to −1,069 bp in relation to the EF2948 stop codon. These products were united using PCR, and the ∼2-kb DNA fragment was cloned into *EcoRI-BamHI*–digested pLT06, generating the pLT2948 plasmid. The EF2948 deletion construct in pLT2948 is devoid of coding sequence corresponding to aa 4–793 of the EF2948 DNA primase. The inserts from all allelic exchange vectors were verified by DNA sequencing at the University of Texas Southwestern DNA Sequencing Core Facility before their use for mutagenesis in *E. faecalis*. Prophage DNA standards were constructed by PCR amplifying a 200- to 300-bp DNA fragment from each of the seven *E. faecalis* V583 prophages. These PCR products were then cloned into pCR2.1 using a topoisomerase (TOPO) TA Cloning Kit (Invitrogen) and transformed into chemically competent *E. coli* DH5α (Invitrogen). Recombinant clones were selected on LB agar containing ampicillin. These individual plasmids each carry a unique V583 prophage sequence (Table S2) that was used for phage DNA quantification experiments.

Generation of *E. faecalis* Mutant Strains. Growth of *E. faecalis* during mutagenesis procedures was done using Todd Hewitt broth (THB) or THB agar (Becton Dickinson). Isogenic mutants of *E. faecalis* V583 were created by transforming *E. faecalis* with variations of pLT06 carrying specified deletion constructs that shared homology with the *E. faecalis* chromosome (see above) using electroporation (2). Transformed *E. faecalis* cells were selected on THB agar containing chloramphenicol at 30 °C. The clones were then serially passaged at 42 °C in THB under chloramphenicol selection to force plasmid integration followed by serial passage at 30 °C in THB in the absence of selection to remove the plasmid from the chromosome. The clones were then grown on MM9YEG agar containing 10 mM *p*-chloro-phenylalanine (Sigma) to select against merodiploids (1, 3). *E. faecalis* chromosomal deletions were verified by PCR.

Generation of ϕV1/7 Lysogens. The ϕV1/7 double lysogens CHLys3.2 and ATCCLys4.2 were isolated by streaking CH188 or ATCC 29212 cells from semiconfluent lysis plates after overnight exposure to ϕV1/7 onto Brain Heart Infusion (BHI) agar. Putative lysogenic bacterial strains were passaged onto fresh BHI agar two times, after which individual colonies were picked and grown to mid-logarithmic phase (OD_{600} of ∼0.5). The culture supernatant from these strains was tested for the ability to form plaques on the parental background strain. Phage DNA was isolated as previously described and analyzed by Southern blot.

Propagation of ϕV1/7 for Transmission EM. To obtain a high titer of ϕV1/7 for transmission EM, 5×10^6 ϕV1/7 particles were mixed with 1×10^6 cfu *E. faecalis* ATCC 29212 and added to 5 mL BHI broth supplemented with 5 mM $CaCl_2$ and 10 mM $MgSO_4$. Duplicate cultures were prepared and incubated at 37 °C for 2 h. Each culture was added to 5 mL fresh BHI and incubated for an additional 4 h at 30 °C, after which the cultures were pooled, centrifuged at 3,220 × *g* for 10 min, and filtered through a 0.45-μm syringe filter. The phages were then precipitated by the addition of NaCl to a final concentration of 1 M and PEG-8000 to a final concentration of 10% wt/vol. The mixture was incubated on ice overnight at 4 °C. The precipitated phages were collected by centrifugation at 7,025 × *g* for 20 min and resuspended in 1 mL SM buffer. The phage particles were concentrated 10-fold using a 30 K MWCO Amicon Ultra conical filter (Millipore); 50 μL phage were extracted with an equal volume of chloroform before preparation for transmission EM.

Negative Staining EM. For the microscopy experiments, high-titer ϕV1/7 preparations were generated (*SI Materials and Methods*). Copper grids (400 mesh; Ted Pella Inc.) were coated with an ∼5-nm layer of carbon film followed by hydrophilic rendering by glow discharge in air (40 mA current for 90 s); 2 μL chloroform-treated ϕV1/7 ($\sim 2 \times 10^6$ pfu) were added to the grid for 1 min, and excess liquid was blotted from the grid surface. The grids were floated in 0.5% uranyl acetate for 1 min and air-dried. The grids were imaged using a JEOL 2200FS FEG transmission electron microscope. Images were taken in low-dose mode (∼20 electron/$Å^2$) at 50,000× with defocus level varying between −1.2 and −2.0 μm. Images were captured using a 2Kx2K Tietz slow-scan CCD camera (with a 1.69 postcolumn magnification factor).

Protein Sequencing. Total protein from $\sim 1 \times 10^8$ pfu ϕV1/7 particles was separated on a 4–12% gradient polyacrylamide Bis·Tris gel (Bio-Rad) and transferred to a PVDF membrane. Proteins were detected with imido black staining, excised from the membrane, and subjected to N-terminal sequencing at the University of Texas Southwestern Protein Technology Center.

ϕV1/7 Lysis Assay. To determine if ϕV1/7 was capable of lysing different *E. faecalis* strains, ϕV1/7 was mixed with various *E. faecalis* strains at multiplicities of infection of 5 (for ATCC 29212

and ATCC Lys4.2) and 25 (for CH188 and CHLys3.2) in BHI supplemented with 5 mM $CaCl_2$ and 10 mM $MgSO_4$. The mixtures were incubated at 37 °C for 2 h; 200 μL each culture were added to 5 μM DNA binding dye Sytox green (Invitrogen) and incubated at room temperature for 10 min. Because Sytox is excluded from intact bacterial cells, the binding of Sytox to DNA in the culture provided a readout of DNA release and thus, cell permeabilization of *E. faecalis* cells after exposure to ϕV1/7. Fluorescence intensity was measured using a SpectraMax M5^e plate reader (Molecular Devices) at 485-nm (excitation) and 525-nm (emission) wavelengths.

Quantitative Standard Curves. Standard curves were used to quantify the absolute copy number of phage DNA from *E. faecalis* culture supernatant and mouse intestinal samples. Standards were generated using V583 prophage gene-specific primers. The primers (0.3 μM each) were mixed with plasmid standards (Table S2) ranging from 2.5×10^7 to 2.5×10^2 copies of plasmid. Quantitative PCR was used to amplify prophage DNA target sequences from plasmid DNA in triplicate as described previously. Standard curves were calculated as total target DNA copies vs. threshold C_t values. Purified phage DNA samples were diluted 1:50, and 2 μL were used in triplicate quantitative PCR reactions. A comparison of the average C_t value was plotted against the appropriate standard curve to calculate the absolute copy number of each prophage within a sample.

Human Microbiome Searches. Taxonomic distribution of *Enterococcus* 16S rRNA gene sequences was performed on publicly available PCR-amplified libraries of human feces within the MG-RAST database (4). Searches had a maximum identity cutoff value of 1×10^{-5} and a minimum length of 50 nt using the Ribosomal Database Project as the annotation source. Human fecal metagenomes (5) within the CAMERA portal (6, 7) were searched for prophage ϕV1- and ϕV7-selected genes with a search query set for default BLASTN parameters.

1. Thurlow LR, Thomas VC, Hancock LE (2009) Capsular polysaccharide production in *Enterococcus faecalis* and contribution of CpsF to capsule serospecificity. *J Bacteriol* 191:6203–6210.
2. Shepard BD, Gilmore MS (1995) Electroporation and efficient transformation of *Enterococcus faecalis* grown in high concentrations of glycine. *Methods Mol Biol* 47: 217–226.
3. Kristich CJ, Chandler JR, Dunny GM (2007) Development of a host-genotype-independent counterselectable marker and a high-frequency conjugative delivery system and their use in genetic analysis of *Enterococcus faecalis*. *Plasmid* 57:131–144.
4. Meyer F, et al. (2008) The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9:386.
5. Gill SR, et al. (2006) Metagenomic analysis of the human distal gut microbiome. *Science* 312:1355–1359.
6. Sun S, et al. (2011) Community cyberinfrastructure for Advanced Microbial Ecology Research and Analysis: The CAMERA resource. *Nucleic Acids Res* 39:D546–D551.
7. Turnbaugh PJ, et al. (2009) A core gut microbiome in obese and lean twins. *Nature* 457: 480–484.

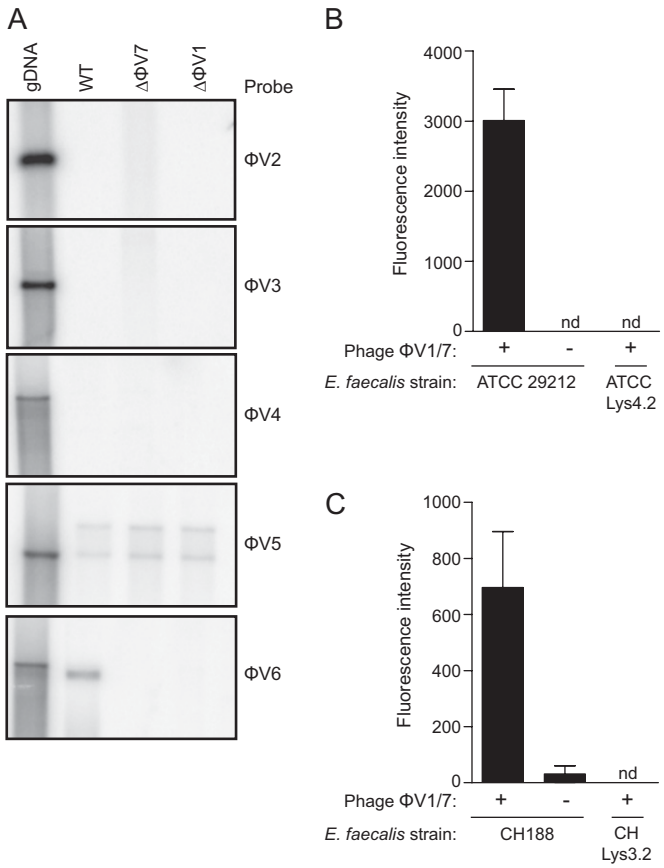

Fig. S1. Analysis of phage production and lysis. (*A*) *E. faecalis* V583 genomic DNA (gDNA) or phage DNA isolated from the culture supernatant of WT, Δϕ V7, or Δϕ V1 (ΔEF0339 capsid) *E. faecalis* was probed for five different prophage elements (ϕV2, ϕV3, ϕV4, ϕV5, and ϕV6) by Southern blot. (*B* and *C*) To assess whether ϕV1/7 lyses *E. faecalis* target cells, we used the DNA-binding fluorescent dye Sytox green to quantify DNA release from cells in culture. Lysis of *E. faecalis* ATCC 29212 (*B*) and CH188 (*C*) was quantified in the presence and absence of ϕV1/7. Lysis of ATCC 29212 and CH188 was compared with the ϕV1/7 lysogenized strains ATCCLys4.2 and CHLys3.2 in the presence of an equal quantity of ϕV1/7 particles. The absolute fluorescence intensity was calculated by subtracting the background fluorescence intensity of uninoculated medium from each sample. *n* = 3 experiments performed in duplicate. nd, not detected.

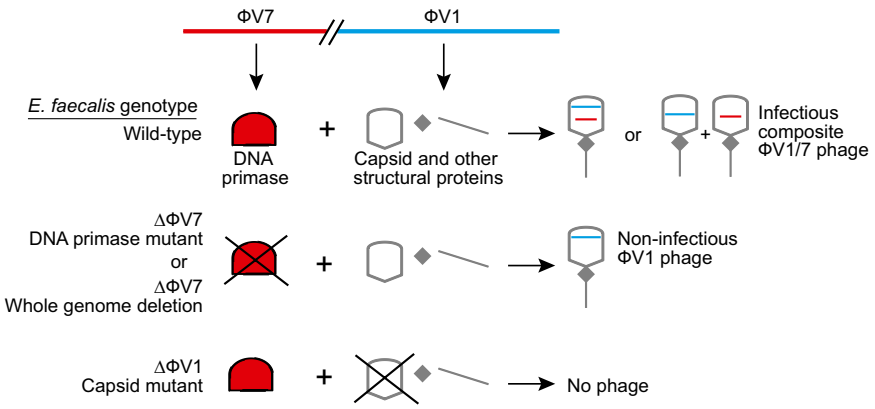

Fig. S2. ϕV1/7 composite phage model. The ϕV1 and ϕV7 prophages are indicated by blue and red lines, respectively. ϕV7 encodes a DNA primase required for the replication of ϕV7 DNA. ϕV1 encodes genes responsible for the phage particle structure, including a phage head capsid gene. Both ϕV1 and ϕV7 DNAs must be packaged into newly synthesized phage particles to generate the composite phage ϕV1/7, which can infect *E. faecalis* CH188 or ATCC 29212. Our data are consistent with both a copackaging model, in which both phage genomes are packaged in the same phage head, and a model in which the genomes are packed in distinct phage heads. In the absence of the DNA primase or when the entire ϕV7 genome is deleted from the *E. faecalis* V583 chromosome, noninfectious phage particles containing only ϕV1 DNA are produced. In the absence of the ϕV1 capsid gene (EF0339) no infectious ϕV1/7 particles are produced, presumably because of a deficiency in capsid assembly.

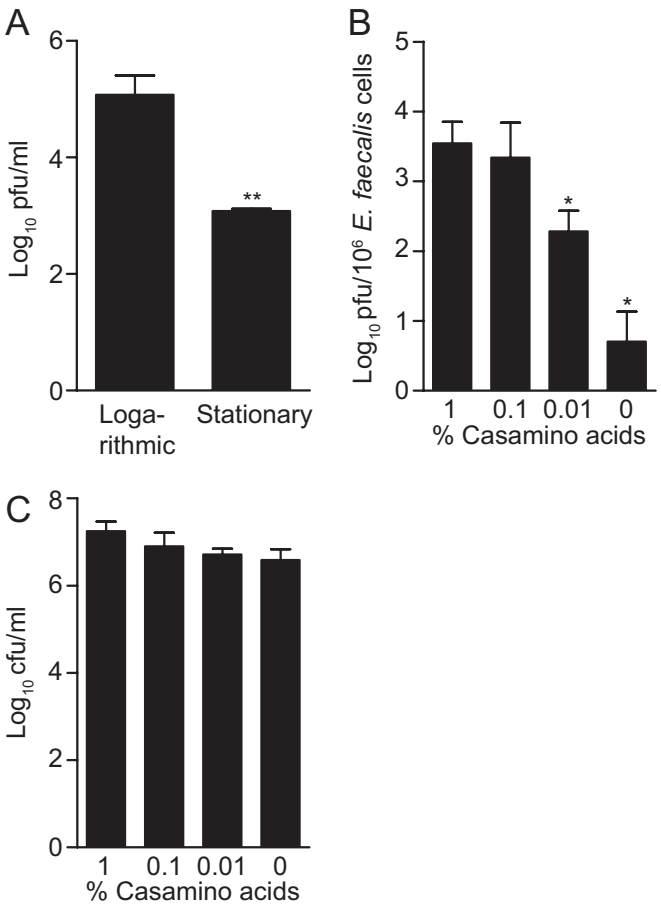

Fig. S3. Growth phase measurement and amino acid induction of ϕV1/7 particles. (*A*) The ϕV1/7 titers from culture supernatant of *E. faecalis* cells grown to logarithmic (OD_{600} = 0.5) or stationary phase (OD_{600} = 2.5). The ϕV1/7 titer was determined by plaque assay using *E. faecalis* ATCC 29212 as an indicator strain. $^{**}P = 0.001$ (two-tailed Student *t* test; $n = 3$). (*B* and *C*) *E. faecalis* V583 was grown in BHI medium to an OD_{600} = 0.5, after which the cells were centrifuged and washed two times with defined medium (50 mM $Na_2HPO_4{\cdot}7H_2O$, 20 mM $NaH_2PO_4{\cdot}H_2O$, 1 mM Tris·HCl, pH 7.4, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM NaCl, 0.5% glucose). These cells were used to seed 10 mL volumes (starting OD_{600} = 0.025) of defined medium containing various concentrations of casamino acids. (*B*) ϕV1/7 titer was measured after 4 h of incubation, and the total bacterial cell density (*C*) from each culture was determined concurrently. This experiment supports the results in Fig. 3 by showing that addition of amino acids to a defined medium also results in enhanced ϕV1/7 particle production. $^{*}P < 0.05$ (two-tailed Student *t* test with Mann–Whitney correction; $n = 3$).

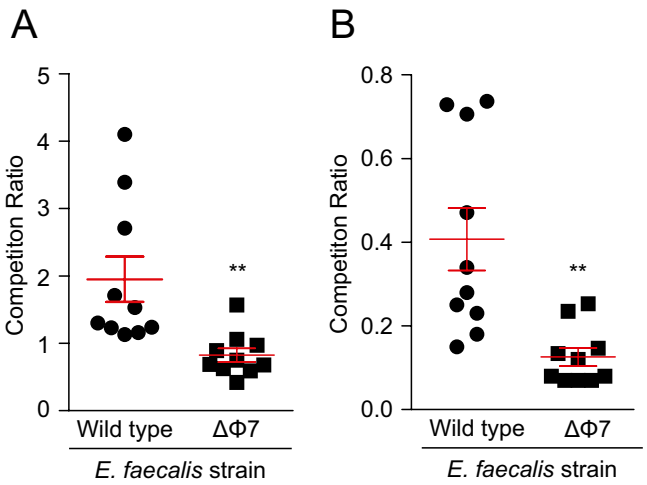

Fig. S4. In vitro competition assays. (*A*) *E. faecalis* V583 or the isogenic mutant ΔϕV7 were mixed with an equal number of *E. faecalis* ATCC 29212 in BHI broth. (*B*) *E. faecalis* V583 or the isogenic mutant ΔϕV7 were mixed with an equal number of *E. faecalis* CH188 in BHI broth supplemented with 1% casamino acids. After 3 h of growth at 37 °C, the abundance of each strain was enumerated by selective plating. The competition ratio was calculated as the ratio of the parental (V583 background) to the susceptible (ATCC 29212 or CH188) after normalization to the starting inoculum for each strain. $^{**}P < 0.005$ (two-tailed Student *t* test; $n = 10$).

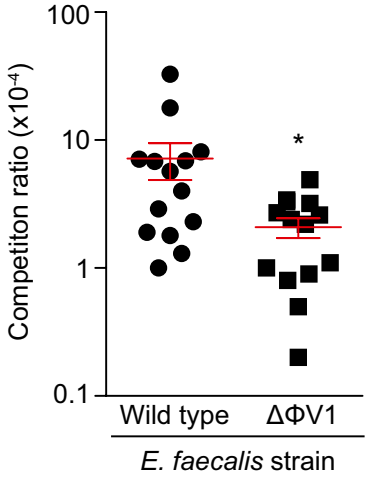

Fig. S5. In vivo competition between *E. faecalis* ATCC 29212 and ϕV1/7-producing (WT) or -deficient (ΔϕV1) *E. faecalis* V583. Germ-free C57BL/6 mice were precolonized by oral inoculation ($\sim 1 \times 10^9$ cfu) with either WT *E. faecalis* V583 or the isogenic ϕV1 capsid mutant strain (ΔEF0339) for 48 h before cocolonization with $\sim 1 \times 10^5$ cfu *E. faecalis* ATCC 29212, a ϕV1/7-susceptible strain. After 10 h of cocolonization, fecal pellets were collected and the total number of each bacterial strain within the feces was enumerated by plating onto antibiotic-selective medium (Enterococcosel agar containing either gentamicin or chloramphenicol), which differentiates the *E. faecalis* V583 background from the background of ATCC 29212. The data are plotted as the ratio of the V583 background strain relative to the ATCC 29212 strain (competition ratio). $^{*}P < 0.05$ (two-tailed Student *t* test with Mann–Whitney correction; $n = 14$).

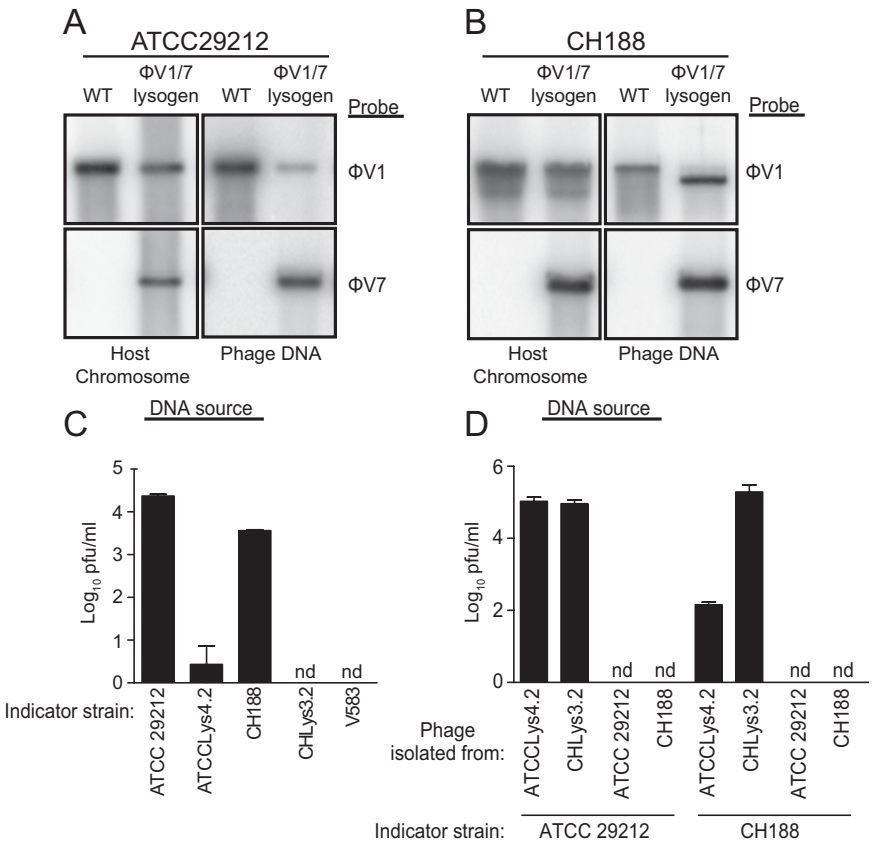

Fig. S6. Characterization of ϕV1/7 lysogens. (*A* and *B*) Southern blot analysis of ϕV1 and ϕV7 DNA within host chromosomal DNA and phage particle DNA isolated from WT *E. faecalis* ATCC 29212 and the ϕV1/7 lysogen ATCCLys4.2 (*A*) or host chromosomal DNA and phage particle DNA isolated from WT *E. faecalis* CH188 and the ϕV1/7 lysogen CHLys3.2 (*B*). (*C*) Plaque assay showing that the ϕV1/7 lysogenic strains ATCCLys4.2 and CHLys3.2 resist superinfection by ϕV1/7 particles. (*D*) Plaque assay quantifying ϕV1/7 particles produced by the ϕV1/7 lysogenized *E. faecalis* strains ATCCLys4.2 and CHLys3.2 compared with the parental WT *E. faecalis* strains ATCC 29212 and CH188, respectively.

A

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READ_18248    ATTGACTGGTATAAGGAAGGCGGTATCATGACGAAACCGACCATCTTTGGCATGAATGG
V583_EF0348   ATAGAATGGTACAAAGACGGCGGTATCATGACGCAACCTACCATATTTGGCATGAACGG
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B

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READ_10541089   TGCCAAAGTTTAGAAATAAGTCAAATGGAACATACAGACGTTTGCTTATCGTGCCTTTTG
V583_EF2948     TGCCTAAAATTCGTAATAAATCTAATGGAACGTATCGCCGTTTGCTTATTGTACCTTTTA
                **** **  ** * ***** ** ******** **  * ********** ** ******

READ_10541089   AAAAGTCTTTTACCGCAGATAATGACGATTGGAAAATAAAGGATGATTATATTAAACGCA
V583_EF2948     ATGCTTCATTTGAAGGTGCTAAAGATGATTGGAGAATAAAAGAAGAGTATATCAACCGAA
                *    ** ***   *  * *** ** ******* ****** ** ** ***** ** ** *

READ_10541089   AAGA
V583_EF2948     AAGA
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Fig. S7. Prophage ϕV1/7 DNA sequences identified from human fecal metagenomes. (*A*) Alignments of DNA from the ϕV1 phage tail gene (EF0348) and (*B*) the ϕV7 DNA primase gene (EF2948) with sequences from human fecal metagenomic DNA (1, 2). The EF0348 and EF2948 alignments are 85% ($E = 6 \times 10^{-9}$) and 73% ($E = 2 \times 10^{-9}$) identical, respectively, over the coverage sequence.

1. Gill SR, et al. (2006) Metagenomic analysis of the human distal gut microbiome. *Science* 312:1355–1359.
2. Turnbaugh PJ, et al. (2009) A core gut microbiome in obese and lean twins. *Nature* 457:480–484.

Table S1. Abundance of enterococcal 16S rDNA sequences from human feces

Fecal sample	Total 16S rDNA sequences	Enterococcal 16S rDNA sequences	Genus and species	Percent of total
4455435.3	2,908	1	***E. faecalis***	**0.07**
4455456.3	4,145	17	***E. faecalis***	**0.41**
4455456.3		25	*E. faecium*	0.60
4455456.3		1	*E. casseliflavus*	0.02
4455493.3	518,951	1	*E. casseliflavus*	0.0002
4455493.3		6	*E. durans*	0.001
4455493.3		20	***E. faecalis***	**0.003**
4455493.3		15	*E. faecium*	0.003
4455493.3		53	*E. hirae*	0.01
4455493.3		3	*E. villorum*	0.0006
4455620.3	2,690	15	***E. faecalis***	**0.56**
4455620.3		24	*E. faecium*	0.89

Bold indicates samples where *E. faecalis* 16S rDNA sequences were detected.

Table S2. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant properties	Source
E. faecalis		
V583	Human blood isolate, Vm^r, Gm^r, Tc^s, Cm^s	1
CH188	Human liver abscess isolate, Tc^r, Vm^s	2
ATCC 29212	Human urine isolate, Cm^r, Gm^s	ATCC culture collection
BDU-12	ΔϕV7 mutant of V583	This study
BDU-20	ΔEF0339 capsid mutant of V583	This study
BDU-43	ΔEF2948 primase mutant of V583	This study
ATCCLys4.2	ATCC 29212 strain background lysogenized with ϕV7, resists infection by ϕV1/7	This study
CHLys3.2	CH188 strain background lysogenized with ϕV7, resists infection by ϕV1/7	This study
E. coli		
DH5α	F^- ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(rk^-, mk^+) *pho*A*sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-	Invitrogen
ElectroTen-Blue	Δ(*mcrA*)*183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1gyrA96 relA1 lac* Km^r [F′ *proAB lacIqZΔM15* Tn*10* (Tc^r)]	Agilent Technologies
Plasmids		
pLT06	*E. faecalis* allelic exchange vector, Cm^r	3
pLTPH07	pLT06 with Δϕ7 encompassing deletion of EF2936 through EF2953, Cm^r	This study
pLT0339	pLT06 with ΔEF0339 extending from +10 to +858 with respect to the predicted start site, Cm^r	This study
pLT2948	pLT06 with ΔEF2948 extending from +10 to +2,379 with respect to the predicted start site, Cm^r	This study
pCR2.1	TOPO TA cloning vector, Ap^r	Invitrogen
pCR-PH01	pCR2.1 with a fragment from EF0339, used for ϕV1 quantification, Ap^r	This study
pCR-PH02	pCR2.1 with a fragment from EF1289, used for ϕV2 quantification, Ap^r	This study
pCR-PH03	pCR2.1 with a fragment from EF1440, used for ϕV3 quantification, Ap^r	This study
pCR-PH04	pCR2.1 with a fragment from EF1884, used for ϕV4 quantification, Ap^r	This study
pCR-PH05	pCR2.1 with a fragment from EF2145, used for ϕV5 quantification, Ap^r	This study
pCR-PH06	pCR2.1 with a fragment from EF2823, used for ϕV6 quantification, Ap^r	This study
pCR-PH07	pCR2.1 with a fragment from EF2943, used for ϕV7 quantification, Ap^r	This study

Ap^r, ampicillin-resistant; Cm^r, chloramphenicol-resistant; Cm^s, chloramphenicol-sensitive; Gm^r, gentamicin-resistant; Gm^s, gentamicin-sensitive; Km^r, kanamycin-resistant; Tc^r, tetracycline-resistant; Tc^s, tetracycline-sensitive; Vm^r, vancomycin-resistant; Vm^s, vancomycin-sensitive.

1. Paulsen IT, et al. (2003) Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. *Science* 299:2071–2074.
2. Murray BE, et al. (1991) Evidence for clonal spread of a single strain of beta-lactamase-producing *Enterococcus* (*Streptococcus*) *faecalis* to six hospitals in five states. *J Infect Dis* 163: 780–785.
3. Thurlow LR, Thomas VC, Hancock LE (2009) Capsular polysaccharide production in *Enterococcus faecalis* and contribution of CpsF to capsule serospecificity. *J Bacteriol* 191:6203–6210.