

1 **Lytic phages lead to targeted and sustained depletion of *Akkermansia*** 2 ***muciniphila* in the gut microbiome**

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Abstract

Natural bacteriophages are major regulators of gut bacterial populations, but their capacity to impose sustained, taxon-selective control over individual commensals in the gut remains unclear. Here, we establish a collection of isolated lytic phages targeting *Akkermansia muciniphila*, a mucosa-associated and context-dependent gut commensal, and demonstrate their utility as precision perturbation tools. We isolated 27 phages representing two distinct vOTUs within the class *Caudoviricetes*, neither of which had close cultured representatives in public databases. The phages lysed susceptible *A. muciniphila* strains at low multiplicities of infection and showed no detectable activity against other tested gut bacteria. Selected phages reproducibly depleted *A. muciniphila* across distinct stool-derived *in vitro* communities, demonstrating that the magnitude of structural disruption scales with the target's baseline abundance. Oral gavage of the optimized cocktail PC6 achieved targeted and sustained suppression in *A. muciniphila*-colonized and human microbiota-associated mouse models, with infective phages recoverable from feces. Shotgun metagenomics revealed changes in a limited subset of taxa and functional pathways, comprising both shared and donor-specific responses, without broad loss of pathway-level diversity. These findings expand the collection of cultured phages that infect *A. muciniphila* and provide a tractable strategy for causally probing the ecological roles of defined gut commensals.

1. Introduction

Elucidating the causal roles of individual gut commensals requires perturbation strategies that selectively remove defined bacteria while preserving the surrounding ecological context. To date, such causal dissection has largely relied on species-dropout experiments in synthetic or defined microbial consortia, which have revealed interspecies interactions, keystone-like effects and metabolic dependencies[1–3]. Extending this framework to natural, host-associated microbiotas remains challenging, because the target organism must be selectively and persistently depleted with minimal direct effects on non-target taxa.

Akkermansia muciniphila is a prominent mucin-degrading commensal whose effects on host physiology appear strongly context-dependent. Its abundance or administration has frequently been associated with improved metabolic regulation, mucosal barrier integrity and immune homeostasis[4–7]. However, adverse effects have also been reported in selected experimental settings, including excessive mucus degradation, increased pathogen susceptibility and exacerbation of inflammatory or disease-associated phenotypes[8–13]. These divergent outcomes may depend on host condition, bacterial strain and the surrounding microbial community. Most mechanistic studies of *A. muciniphila* have relied on supplementation or colonization, whereas complementary loss-of-function approaches remain limited. Selectively removing *A. muciniphila* from an otherwise intact microbiota would therefore provide a direct means of defining its ecological contributions and the conditions that shape its context-dependent effects.

Lytic bacteriophages (phage) offer a natural means of achieving species- or strain-selective bacterial depletion[14]. For *A. muciniphila*, however, experimentally validated cultured lytic phages remain scarce, with previous reports derived largely from metagenomic predictions or the induction of temperate phages[15–18]. This resource gap has limited direct tests of whether *A. muciniphila* can be selectively and persistently depleted from complex gut communities. Moreover, although phages have

been used to modulate gut bacterial populations[2,19–22], activity in pure culture does not necessarily predict efficacy in complex communities or *in vivo*. Target abundance, strain susceptibility, community interactions and phage persistence may all influence the magnitude and duration of depletion, as well as its indirect effects on non-target taxa. It therefore remains unclear whether natural lytic phages can achieve sustained *A. muciniphila* suppression across compositionally distinct microbiotas and how this targeted perturbation propagates through the surrounding community.

Here, we isolated and systematically characterized natural lytic phages targeting *A. muciniphila* from diverse environmental sources. We defined their genomic features, host specificity, antibacterial activity and occurrence in metagenomic datasets, and tested whether they could mediate sustained *A. muciniphila* suppression in stool-derived *in vitro* communities and mouse models. This work expands the biological resources available for studying *A. muciniphila* and establishes a phage-based framework for selectively manipulating a defined gut commensal within complex microbiotas.

2. Results

2.1 Isolation and genomic characterization reveal two species-level vOTUs among cultured lytic *Akkermansia* phages

To isolate novel lytic phages targeting *A. muciniphila*, we collected wastewater samples from diverse environmental sources, including water purification plants, hospital, and industrial facilities, across multiple provinces in China (Supplementary Table 1). Following enrichment and plaque purification (Figure 1a), we obtained 27 lytic phage isolates infecting *A. muciniphila*, each of which was subjected to whole-genome sequencing. All 27 genomes were de novo assembled, and reads were mapped back, achieving coverage of at least 1,000× per genome. The assembled genomes ranged from 67.5 to 72.5 kb in length, with an average GC content of 50.7% (Supplementary Table 2). Their authentic presence in the original wastewater samples was independently confirmed by read mapping to representative genomes (Figure S1). All complete phage genomes were deposited in NCBI, and accession numbers are listed in Supplementary Table 2.

Pairwise average nucleotide identity (ANI) and alignment fraction (AF) analyses separated the 27 phage isolates into two species-level viral operational taxonomic units (vOTUs) (Figure 1b and Figure S2). Twenty-six isolates shared >95% ANI and ≥85% AF[17] with one another, irrespective of their geographical origins, and were therefore assigned to the predominant vOTU1. By contrast, Akk_GDp4 shared only approximately 75% ANI with the vOTU1 isolates and was therefore assigned to a separate singleton vOTU, designated vOTU2. Consistent with this classification, protein-sharing network analysis using vConTACT2 placed the 26 vOTU1 isolates within a common major cluster, whereas Akk_GDp4 remained a weakly connected outlier. However, neither lineage could be assigned to an established taxonomic group represented in the reference network (Figure S3 and Supplementary Tables 4–5). BLASTn searches against the NCBI nucleotide database identified two closely related metagenome-derived viral genomes from the Cenote Human Virome Database

(CHVD)[17], ctdHC2 (BK017422.1) and ctHFD3 (BK017549.1), both annotated as members of the class *Caudoviricetes* (Figure 1c and Supplementary Table 6). The vOTU1 isolates were consistently more similar to ctdHC2 than to ctHFD3, with median ANI and AF values of 94.0% and 87.5%, respectively. Although the AF exceeded the operational threshold of 85%, the ANI remained below 95%, indicating that ctdHC2 represents a closely related but distinct species-level viral genome. In contrast, Akk_GDp4 was more closely related to ctHFD3, with ANI and AF values of 97.2% and 97.6%, respectively, placing the two genomes within the same operationally defined species-level vOTU. Both ctdHC2 and ctHFD3 were recovered from human metagenomes, and no cultured *Akkermansia* phages corresponding to either vOTU were identified in the public databases searched.

Genome analysis of the 27 cultured *Akkermansia* phages identified no antimicrobial resistance genes, known virulence factors, tRNAs or complete lysogeny-associated modules, consistent with a lytic lifestyle (Figure S4 and Supplementary Table 2). We next selected Akk_GDp9 and Akk_GDp4 as representatives of vOTU1 and vOTU2, respectively, for Phold-based annotation. Both genomes encoded conserved modules involved in DNA replication, genome packaging, head morphogenesis, host cell lysis and virion assembly. Tail-associated features were identified in both representatives, including tail fiber proteins and head–tail adaptors, supporting the presence of a tailed virion architecture. In Akk_GDp9, the predicted tail-associated proteins included two tail fiber proteins, a tail-length tape-measure protein and a putative L-shaped tail fiber assembly protein, suggesting a relatively complex tail structure (Figure 1e and Supplementary Tables 7–8). Transmission electron microscopy of Akk_GDp4 and Akk_GDp9 revealed virions with isometric capsids approximately 65–95 nm in diameter and visible tail-like structures (Figure 1d). These morphological features were consistent with the predicted structural proteins. Together with their genomic relationships to metagenome-derived *Caudoviricetes* genomes, these results support the assignment of the cultured *Akkermansia* phages to the class *Caudoviricetes*.

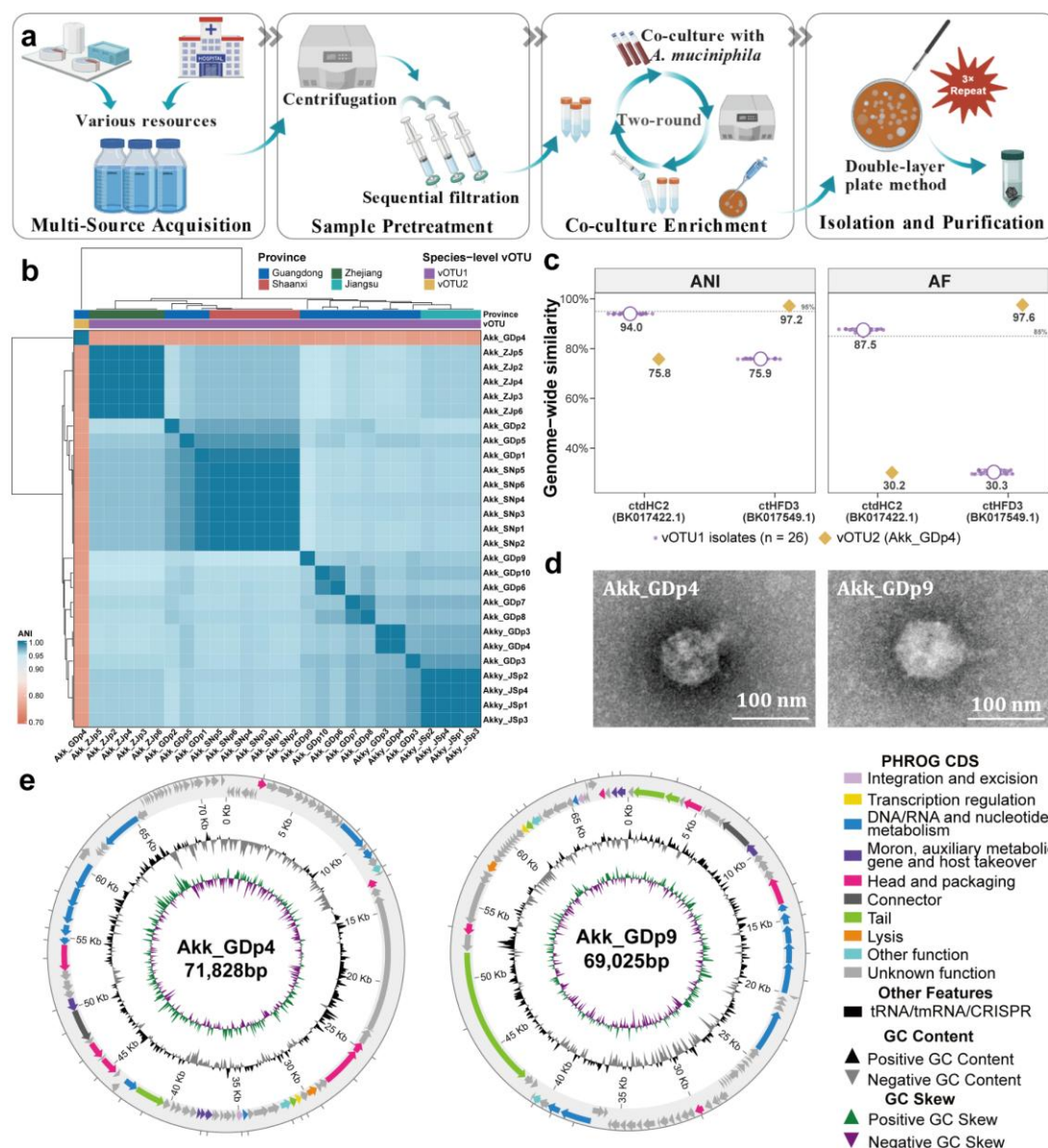


Figure 1 | Geographic isolation, vOTU delineation and genomic relationships of cultured *Akkermansia* phages. **a** Schematic workflow for the isolation of *Akkermansia* phages. **b** Pairwise average nucleotide identity (ANI) among the 27 cultured *Akkermansia* phage isolates. Rows and columns represent individual phage genomes, which were hierarchically clustered using a genome distance of $1 - \text{ANI}$. The color scale indicates pairwise ANI. Colored annotations above the heatmap denote the geographical province of isolation and species-level viral operational taxonomic unit (vOTU) assignment. The collection comprised a predominant vOTU1 containing 26 isolates, and a singleton vOTU2 represented by Akk_GDp4. Species-level vOTUs were operationally delineated using thresholds of $\geq 95\%$ ANI and $\geq 85\%$ alignment fraction (AF) relative to the shorter genome. **c** Genome-wide similarity between the cultured phage isolates and their closest publicly available metagenome-derived viral genomes. The left and right panels show ANI and AF, respectively, for comparisons of the 27 cultured isolates with two NCBI metagenome-assembled viral genomes. Purple points represent the 26 isolates assigned to vOTU1, with the lightly shaded distributions

showing the corresponding value distributions and open circles indicating the median. Yellow diamonds represent Akk_GDp4, the sole cultured member of vOTU2; adjacent values indicate its corresponding ANI or AF. Dashed horizontal lines indicate the operational species-level thresholds of 95% ANI and 85% AF relative to the shorter genome. **d** Transmission electron micrographs of representative isolated *Akkermansia* phages. Scale bars, 100 nm. **e** Genome maps of representative phages. Akk_GDp4 and Akk_GDp9 annotated using Phold. Predicted genes are color-coded by functional categories.

2.2 Genomic relationships and human gut distribution of representative *Akkermansia* phages

We next selected six cultured phages representing both vOTUs and capturing variation in geographical origin and isolation host for comparative genomic and metagenomic analyses. Whole-genome comparisons revealed extensive homology among the cultured isolates, although several homologous regions differed in order or orientation, indicating local genome rearrangements (Figure 2a). By contrast, similarity to the two previously described temperate phages associated with *A. muciniphila* strain YL44[16] was restricted to short local regions, including putative lysis-related genes and a locus adjacent to predicted tail fiber protein. These comparisons indicate that the cultured lytic phages share a conserved but locally rearranged genomic framework that is distinct from currently described temperate *Akkermansia* phages.

We next compared the prevalence and geographic distribution of sequences related to the six representative cultured phages with those related to previously reported temperate *Akkermansia* phages[16] and crAss-like phages[23,24] in human gut metagenomes. Screening 4,408 publicly available metagenomes from 13 countries showed that sequences related to the temperate *Akkermansia* and crAss-like reference phages were widely distributed across geographic cohorts. Although sequences related to the cultured isolates were detected less frequently, they were present in multiple countries (Supplementary Table 9; Figure 2b), indicating that these phage lineages are not restricted to their sites of isolation. We next examined whether phage detection was associated with the relative abundance of *A. muciniphila*. No significant difference was

observed between metagenomes presence or absence for the two crAss-like reference phages (Figure 2c). By contrast, metagenomes containing either of the two previously described temperate *Akkermansia* phages showed significantly higher *A. muciniphila* abundance, consistent with the Piggyback-the-Winner hypothesis[25,26]. Among the cultured isolates, all five vOTU1 representatives showed the same directional pattern, with significantly higher *A. muciniphila* abundance in phage-presence metagenomes. This association was not detected for Akk_GDp4, suggesting that the relationship between cultured-phage occurrence and host abundance may differ between the two vOTUs.

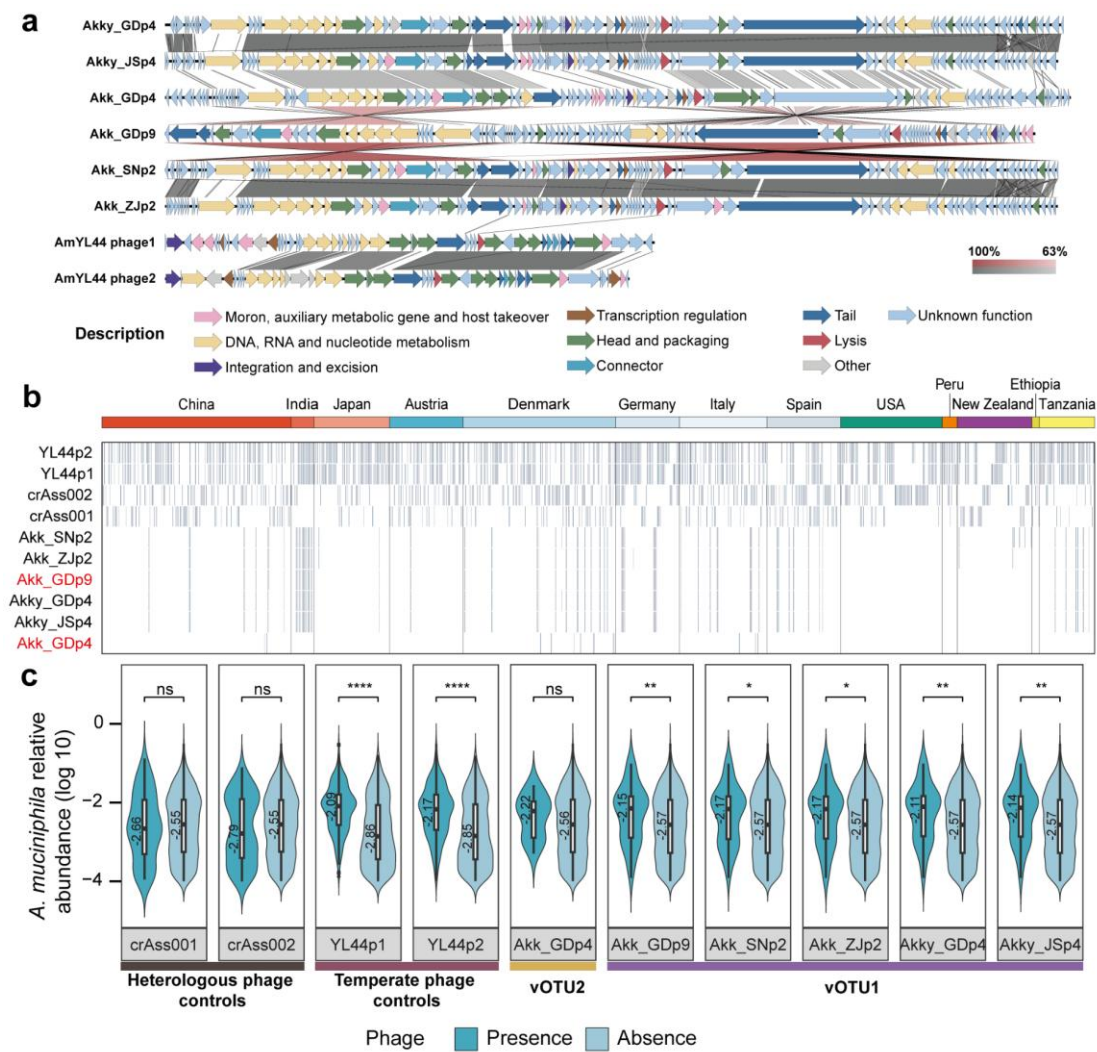


Figure 2 | Sequence conservation and ecological context of *Akkermansia* phages. a

Comparative genome organization of representative cultured *Akkermansia* phages and the two previously reported *A. muciniphila* YL44-associated temperate phages. Coding sequences are coloured according to Phold functional categories. Regions of nucleotide similarity were identified using BLASTn and visualized with Easyfig. Black and red connecting blocks indicate collinear and inverted nucleotide matches, respectively. **b** Prevalence of the isolated phages, alongside reference phages (crAss001, crAss002, *A. muciniphila* YL44 phages 1 and 2) and, across international human gut metagenomic cohorts ($n = 4,408$). Dark blue tiles indicate phage-positive samples. **c** *A. muciniphila* relative abundance by phage status. Box plots show median and interquartile range. Wilcoxon rank-sum test with Benjamini-Hochberg correction was used for statistical comparison; significance markers (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant) are shown above each panel.

2.3 Lytic activity and host specificity of representative *Akkermansia* phages

To compare the antibacterial activity, we monitored the growth of their respective isolation hosts following infection at MOIs ranging from 1 to 0.001 (Figure 3a). All five vOTU1 isolates rapidly suppressed *A. muciniphila* growth across the tested MOIs. For three isolates, OD₆₀₀ values remained below 0.05 throughout the 48-h incubation period, whereas Akk_ZJp2 and Akk_SNP2 showed modest increases in bacterial density after approximately 30 h, with average OD₆₀₀ values remaining below 0.2. By contrast, the sole vOTU2 isolate, Akk_GDp4, produced only transient growth suppression: bacterial growth began to resume at approximately 15 h at all tested MOIs and approached that of the uninfected control by approximately 26 h. Thus, although limited late regrowth was observed for two vOTU1 isolates, growth suppression by the vOTU1 isolates was substantially more sustained than that observed for Akk_GDp4, paralleling their genomic separation.

To assess host-range specificity, we performed spot assays with these four isolates against a panel comprising five *A. muciniphila* strains, one *A. glycaniphila* strain, and

four additional gut bacterial strains—*Bacteroides fragilis* NCTC 9343, *Bacteroides*
uniformis ATCC 8492, *Fusobacterium varium* DA690, and *Anaerostipes hadrus*
DA538—under anaerobic conditions (Figure 3b). Spot assays produced lysis zones for
most phage–*Akkermansia* combinations, although no detectable zones were observed
for several specific phage–host pairs under the assay conditions. For example,
Akky_GDp4 and Akky_JSp4 produced no detectable lysis on either *A. muciniphila*
ZT003 or *A. glycaniphila* DSM 100705. None of the four non-*Akkermansia* gut
bacterial strains showed detectable lysis. Thus, within the tested panel, the activity of
these phages was confined to the genus *Akkermansia*, indicating a restricted apparent
host range under the assay conditions.

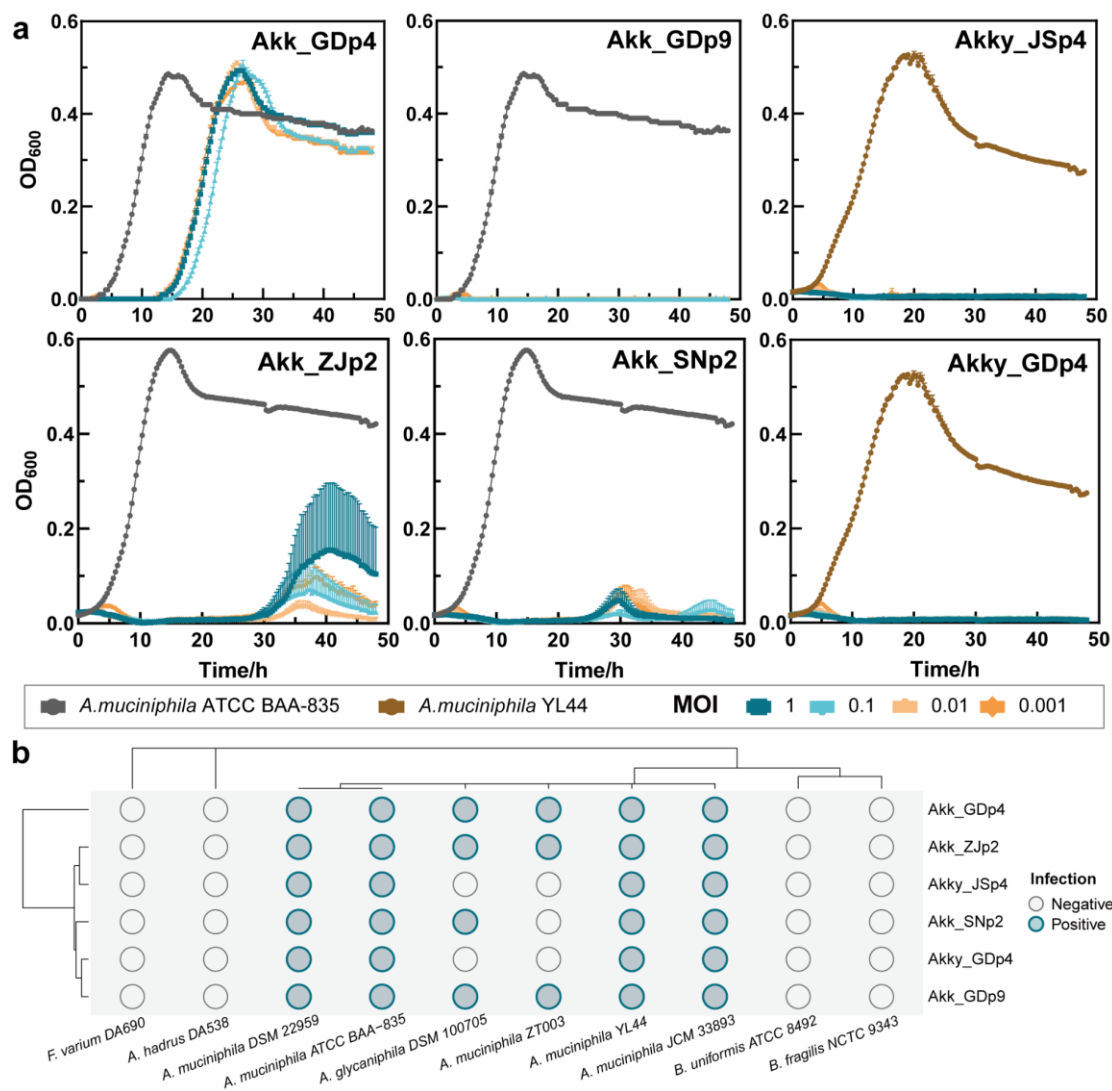


Figure 3 | Lytic activity and host range of representative *Akkermansia* phages.

a Killing curves of selected *Akkermansia* phages against their cognate hosts at multiplicities of infection (MOIs) of 1, 0.1, 0.01, and 0.001. Data are presented as mean $\pm$ s.d. **b** Host range of isolated *Akkermansia* phages determined by infection assay on *Akkermansia* strains and representative anaerobic gut bacteria. Phylogenetic trees of bacterial hosts (left; MashTree) and phages (top; ViPTree) are linked to a binary infection matrix. Blue filled circles indicate visible plaque formation, whereas white open circles indicate no detectable plaques. Strains ATCC BAA-835, DSM 22959, and JCM 33893 are representatives of *A. muciniphila* sourced from distinct culture collections.

2.4 Targeted *A. muciniphila* depletion across stool-derived *in vitro* communities

To evaluate the targeting efficiency and specificity of these phages in complex microbial communities, we established six stool-derived *in vitro* communities[27] spiked with *A. muciniphila* (SIC1-SIC6). Following stabilization through serial passaging, the baseline relative abundance of *A. muciniphila* varied markedly among communities, ranging from 0.7% to 31.1% (Figure 4e). We selected Akk_GDp9 and Akk_GDp4, representing the two vOTUs and contrasting *in vitro* lytic activities, to compare their ability to deplete *A. muciniphila* within these communities. These communities were treated with live phages or their heat-killed (HK) controls and passaged every 24 h for two passages (Figure 4a). Phage treatment shifted community composition, with the greatest displacement observed in Akk_GDp9-treated communities, which also showed the most pronounced reduction in the three detected *A. muciniphila* strains (Figure 4b). Despite this stronger target depletion, Akk_GDp9 did not significantly alter community Shannon diversity relative to baseline. By contrast, Akk_GDp4 treatment was associated with a significant reduction in Shannon diversity ($p < 0.05$; Figure 4c).

Absolute quantification further confirmed the strong depletion of *A. muciniphila* by Akk_GDp9. At 48 h, live Akk_GDp9 reduced the absolute abundance of *A.*

262 *muciniphila* by more than three orders of magnitude relative to its heat-killed control,
 263 whereas no reduction was observed with Akk_GDp4 (Figure 4d). Growth dynamics
 264 further revealed differences in the duration of phage-mediated growth suppression
 265 (Figure S6). In communities with high initial *A. muciniphila* abundance (e.g., SIC2,
 266 31.1%), both phages initially suppressed total community growth. However, while
 267 Akk_GDp9 maintained this suppression across 48 h and subsequent passaging,
 268 Akk_GDp4-treated cultures rebounded to HK control levels at 17.5 h. This rebound
 269 mirrors the *in vitro* monoculture kill-curves and suggests the rapid emergence of phage-
 270 resistant subpopulations against Akk_GDp4. Conversely, in communities with low
 271 baseline *A. muciniphila* abundance (e.g., SIC3, 3.6%), overall growth profiles remained
 272 unperturbed regardless of treatment, indicating that phage-induced growth disruptions
 273 scale with target abundance. Ultimately, species-level profiling confirmed that
 274 Akk_GDp9 overcomes donor-specific microbiome variations to execute precise and
 275 durable target depletion without triggering a broad community collapse (Figure 4e).

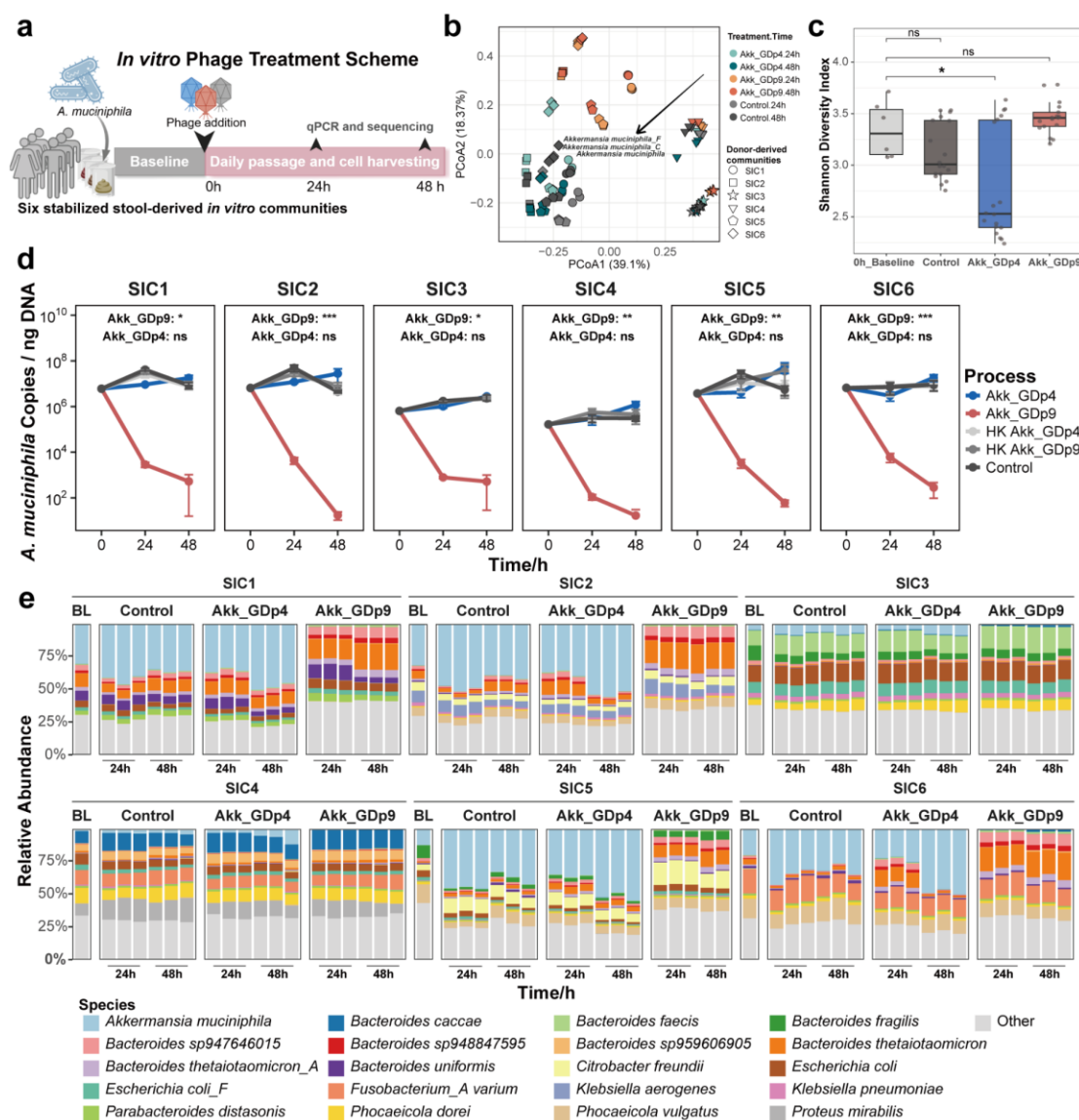


Figure 4 | Efficient depletion of *A. muciniphila* by phages across diverse complex microbial communities. **a** Schematic of the experimental design. Six stool-derived *in vitro* communities with varying initial *A. muciniphila* relative abundances (0.7%–31.1%) were stabilized by serial passaging and treated with Akk_GDp4 (weaker phage), Akk_GDp9 (potent phage), a blank control, or their corresponding heat-killed (HK) controls. Communities were maintained by 24-h subcultivation for 48 h. **b** Principal coordinate analysis (PCoA) of microbial community compositions based on Bray–Curtis dissimilarities, highlighting phage-driven shifts linked to *A. muciniphila* depletion. **c** Shannon alpha diversity at 48 h compared with 0-h baseline. The center line indicates the median, box limits represent the upper and lower quartiles, whiskers extend to 1.5× the interquartile range, and dots represent individual samples. **d** Absolute

abundance of *A. muciniphila* quantified via qPCR across the six distinct communities at 48 h. Data are presented as mean $\pm$ s.e.m. ($n = 3$). **e** Relative abundance profiles of the top 20 bacterial species at 48 h, determined by metagenomic sequencing.

2.5 Sustained *in vivo* *A. muciniphila* depletion by phage cocktail PC6

To select a phage cocktail for *in vivo* evaluation, we formulated candidate cocktails based on phage infectivity and propagation feasibility, and then compared their activity against the reference strain *A. muciniphila* ATCC BAA-835 and mouse-derived *A. muciniphila* ZT003 (Supplementary Table 10 and Figure S7). PC6 maintained bacterial abundance at the lowest level and was therefore advanced for evaluation in two antibiotic-conditioned SPF mouse models: an *A. muciniphila*-colonized model and human microbiota-associated (HMA) models reconstituted with fecal microbiota from human donors.

In the *A. muciniphila*-colonized model, PC6 induced rapid and prolonged depletion of intestinal *A. muciniphila*. Following antibiotic pretreatment and a 3-day stabilization period after *A. muciniphila* gavage, mice received PC6 or heat-killed PC6 (HK PC6) daily for 7 days (Figure 5a). Fecal *A. muciniphila* abundance fell below the predefined limit of reliable quantification by day 3 and remained strongly suppressed through day 11 (Figure 5b). *A. muciniphila* subsequently partially rebounded but remained at approximately 10^4 copies per mg of feces on day 25. Infectious phages were still recoverable from day-25 fecal samples by double-layer agar plating (Figure 5c), consistent with the persistence of viable PC6-derived phages after dosing had ceased.

To determine whether PC6 remained effective in more complex microbiota backgrounds, we next tested the cocktail in HMA mice colonized with SIC5 or SIC6 previously examined *in vitro* (Figure 5d). Following antibiotic pretreatment and microbiota reconstitution, mice received PC6 or HK-PC6 daily for 7 days and were monitored for more than one month. Within each donor background, PCoA indicated

differences in community composition between PC6- and HK-PC6-treated mice (Figure 5e, h). *A. muciniphila* was efficiently suppressed in both SIC5- and SIC6-colonized mice, as independently supported by metagenomic profiling and qPCR, although the subsequent rebound kinetics differed between the two microbiota backgrounds (Figure 5f, g, i, j). In SIC5-colonized mice, *A. muciniphila* loads remained low at 10^4 – 10^5 copies/mg feces throughout the monitoring period, whereas those in SIC6-colonized mice remained near the lower limit of quantification through day 16, followed by a partial rebound by day 25. These results suggest that microbiota background influences the ability of *A. muciniphila* to rebound under sustained phage pressure.

We next assessed whether *A. muciniphila* depletion was accompanied by changes in non-target taxa. Differential abundance analysis across the 25-day follow-up revealed predominantly donor-specific responses (Figure 5k–m; Supplementary Table 11 and Figure S8). A smaller subset of taxa nevertheless changed concordantly in the SIC5 and SIC6 communities. Among the most prominent shared responses, *Romboutsia ilealis* and *Lactobacillus* sp. 948500755 increased, whereas *Faecalibaculum* sp. 910588395 and *Eubacterium_G* sp. 910574895 decreased. These concordant responses identify a limited set of non-target taxa reproducibly associated with PC6-mediated *A. muciniphila* depletion across the two HMA communities, although whether these associations reflect direct or indirect ecological interactions remains unresolved.

Finally, we investigated whether this taxonomic restructuring was accompanied by changes in predicted functional potential. To minimize the influence of early, non-specific functional shifts associated with community adaptation following microbiota engraftment[28], we focused on the stable post-intervention phase from days 11 to 25 (Figure S9–11). Across the two HMA communities, the predicted abundances of several metabolic pathways were significantly associated with taxa showing concordant responses to PC6 treatment, including mixed acid fermentation, TCA cycle V (2-oxoglutarate synthase), and L-glutamine biosynthesis III (Figure 5n and Figs. S12–S13).

These associations coincided primarily with stable-phase changes in *Butyribacter* spp. Moreover, higher *A. muciniphila* abundance was associated with greater representation of gluconeogenesis III and ADP-heptose biosynthesis, whereas lower abundance was associated with greater representation of reductive TCA cycle I and pyruvate fermentation to propanoate I. Despite these pathway-specific associations, pathway-level Shannon diversity did not differ between the PC6 and HK PC6 groups during the stable phase ($p = 0.349$; Figure S14).

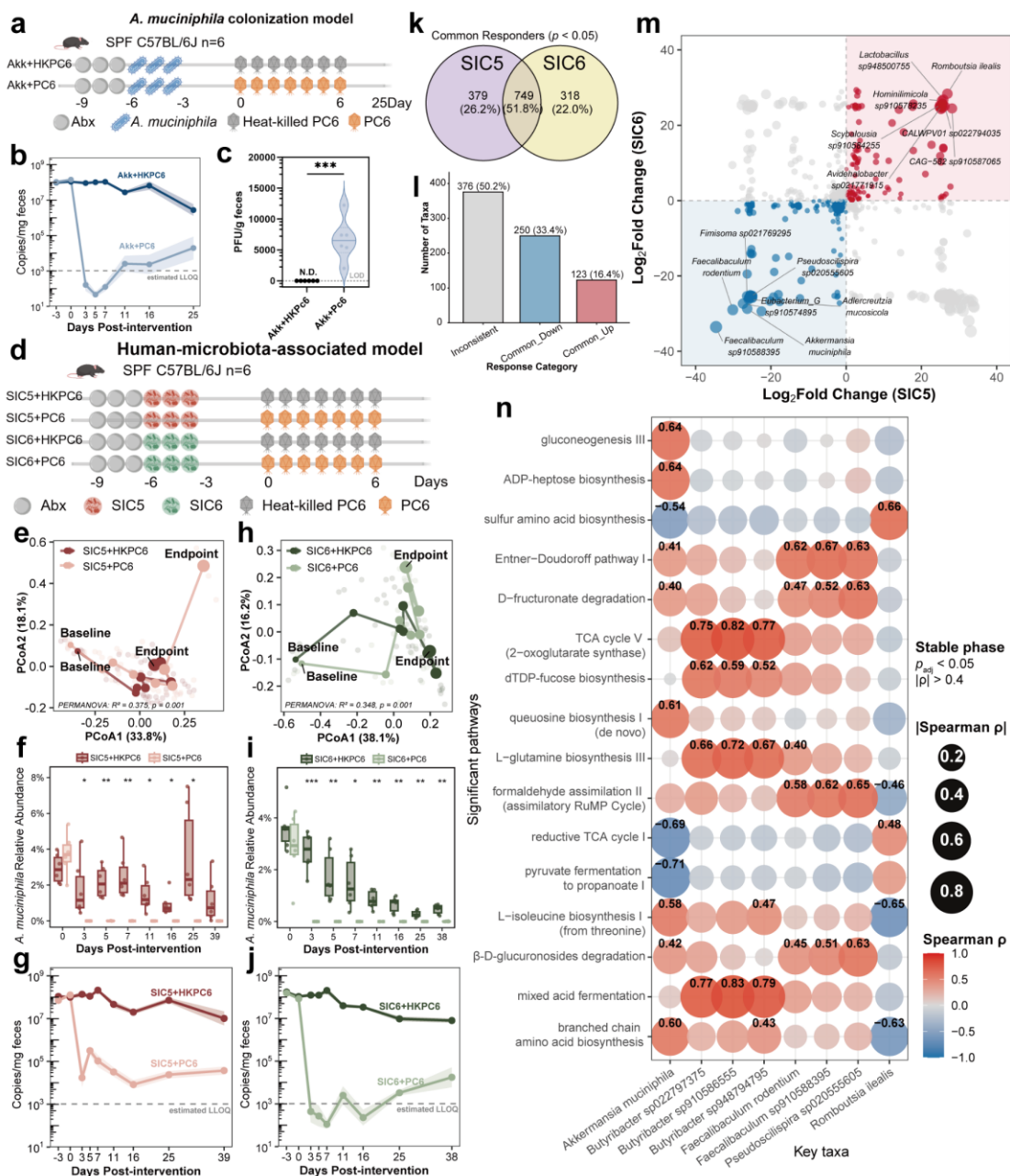


Figure 5 | Phage cocktail PC6 drives durable *A. muciniphila* suppression and donor-dependent ecological remodeling *in vivo*. **a** Schematic of phage-mediated *A.*

muciniphila modulation in *A. muciniphila*-colonized model. **b** Absolute abundance of *A. muciniphila* in *A. muciniphila*-colonized model. **c** Phage titers quantified in *A. muciniphila*-colonized model at day 25 post-infection. **d** Schematic of phage-mediated *A. muciniphila* modulation in human microbiota-associated (HMA) mice. **e–g**, Community restructuring and *A. muciniphila* abundance dynamics in SIC5-colonized mice, shown by PCoA, relative abundance, and absolute abundance. **h–j**, Corresponding analyses in SIC6-colonized mice. **k** Overlap of taxa showing significant PC6-mediated abundance shifts in SIC5- and SIC6-colonized mice across post-intervention time points. **l** Classification of shared responsive taxa according to cross-donor directional consistency, including concordant increases, concordant decreases, and inconsistent responses. **m** Cross-donor consistency of PC6-mediated shifts in species-level absolute abundance. Each point represents a shared responsive taxon, plotted by mean $\log_2FC$ in SIC5- and SIC6-colonized mice; point size indicates the absolute consistency score, defined as $|\log_2FCSIC5 \times \log_2FCSIC6|$. Red and blue points indicate concordant increases and decreases, respectively, whereas grey points indicate inconsistent responses. The top-ranked concordant taxa are labeled. **n** Strongest correlations between key consistent taxa and functional pathways during the stable phase (days 11, 16, and 25). Only associations meeting both Benjamini–Hochberg adjusted $p < 0.05$ and $|\text{Spearman } \rho| > 0.4$ were considered significant. Bubbles represent these top 25 associations ranked by descending $|\rho|$. Numeric labels inside bubbles show the exact Spearman ρ value. Pathway labels in the plot were abbreviated for readability; full MetaCyc pathway IDs and names are provided in the Supplementary Information.

3. Discussion

Here, we describe a collection of lytic phages targeting the mucin-degrading gut commensal *A. muciniphila* and establish phage-mediated depletion as a species-resolved strategy for probing its ecological role in complex gut communities. The isolates comprised two distinct vOTUs within the class *Caudoviricetes*, and an optimized cocktail, PC6, achieved rapid, taxon-selective and sustained suppression of

A. muciniphila in mouse models. Target depletion was accompanied by reproducible as well as donor-specific compositional responses and by selective pathway-level associations, without a broad reduction in pathway diversity. These findings support natural lytic phages as precision perturbation tools for resolving the ecological contributions of defined gut commensals while preserving broad pathway-level diversity.

The isolated phages also expand the currently limited genomic and functional resource for studying *Akkermansia* phages. Unlike previously described inducible or predicted temperate phages associated with *A. muciniphila*, our isolates form a genomically cohesive but underrepresented group of lytic phages[16,29]. The repeated recovery of only two vOTUs from geographically distinct samples may reflect both methodological selection and biological filtering. Enrichment on a limited set of host strains likely constrained the accessible diversity, whereas strain-specific surface structures, phage receptors and defense systems may restrict tropism[30–34]. The conserved mucin-associated niche of *A. muciniphila* could impose an additional ecological filter across geographically separated populations. Broader host panels and alternative cultivation conditions will be needed to determine whether the apparently restricted culturable repertoire reflects true ecological constraint or incomplete sampling[35].

Phenotypically, vOTU1 displayed strong infective capacity, achieving rapid and sustained host suppression even at low MOIs. The modest OD₆₀₀ recovery observed toward 48 h indicates delayed regrowth, although resistant isolates and the mechanisms underlying recovery were not characterized. The data therefore support a prolonged suppression window but do not yet establish a high barrier to resistance[36,37]. At the ecological level, previously reported positive associations between temperate *Akkermansia* phages and *A. muciniphila* abundance have been interpreted within the Piggyback-the-Winner framework[25,26]. The similar positive association observed for our obligately lytic vOTU1 phages indicates that phage–host co-occurrence is not specific to temperate lifestyles, but can also emerge when abundant hosts support

productive infection and increase phage detectability in metagenomic data[38,39]. In cross-sectional cohorts, this pattern represents population-level coupling that may integrate temporally lagged phage–host fluctuations[40].

Across six stool-derived *in vitro* communities, Akk_GDp9 reproducibly depleted *A. muciniphila* despite substantial variation in its initial abundance. The direction of community change was consistent, but its magnitude depended on the baseline prominence of the target. Communities with abundant *A. muciniphila* showed larger reductions in total biomass and greater ordination displacement, whereas communities in which the target was initially rare were less affected. The resulting biomass deficit was not rapidly compensated by the remaining community over the experimental window[41,42]. Thus, *A. muciniphila* depletion produced a directionally reproducible but quantitatively context-dependent perturbation, identifying initial target abundance as a major determinant of the community-level response.

In vivo, PC6 produced rapid and prolonged suppression of *A. muciniphila*. In *A. muciniphila*-colonized model, target abundance fell below the lower limit of quantification by day 3 and remained strongly depleted through day 25, while phages remained detectable in feces, consistent with prolonged phage persistence or activity in the gut. PC6 also efficiently suppressed *A. muciniphila* in two HMA models, although the timing and extent of target rebound differed between the donor communities. Such recovery may reflect reversible changes in phage susceptibility or spatial protection within the gut. Phase-variable surface structures can alter phage susceptibility in gut bacteria[32], and *A. muciniphila* itself possesses a phase-variable capsular polysaccharide system[43], although its role in phage resistance remains unknown. In addition, mucosal refuges can protect susceptible bacteria from phage predation and promote long-term coexistence[44]. The concurrent recovery of *A. muciniphila* and persistence of infective phages is therefore consistent with dynamic phage–host coexistence rather than complete clearance of either population[44,45]. Metagenomic profiling revealed both shared and donor-specific responses. Recurrent relative enrichment of *Romboutsia ilealis* and *Lactobacillus* spp., together with reductions in

Faecalibaculum spp. and *Eubacterium*_G spp., provisionally nominates a limited set of taxa that may respond consistently to *A. muciniphila* depletion. These patterns are compatible with indirect effects mediated by resource competition or cross-feeding, because *A. muciniphila* releases mucin-derived substrates that can support other mucus-associated bacteria[2,46]. Donor-specific responses, in turn, may reflect differences in interaction networks and in the availability of potential cross-feeding partners. These observations generate testable hypotheses about ecological dependencies but do not by themselves establish direct interactions.

During the day 11–25 interval, functional responses remained selective rather than global. The covariation between reduced *Butyribacter* spp. abundance and the mixed acid fermentation, TCA cycle V (2-oxoglutarate synthase) and L-glutamine biosynthesis III pathways is compatible with altered carbon- and nitrogen-metabolic relationships following *A. muciniphila* depletion. However, these associations do not demonstrate direct metabolic dependence. Their biological plausibility is supported by the capacity of *A. muciniphila*-mediated mucin degradation to release substrates used by fermentative and butyrate-producing taxa[42,47–49]. The moderate initial abundance of *A. muciniphila* in both donor communities, approximately 3–4%, may partly explain the limited system-wide response and is consistent with the abundance-dependent effects observed *in vitro*. Functional redundancy may have provided further buffering[50,51]. Accordingly, unchanged pathway-level Shannon diversity should be interpreted as an absence of broad functional collapse, rather than an absence of functionally meaningful change.

More broadly, this study establishes phage-mediated depletion as a controlled, species-resolved approach for investigating the ecology of a defined gut commensal. *A. muciniphila* represents a particularly informative target because its mucin-degrading lifestyle places it at a key interface between the gut microbiota and the host, while its reported associations with metabolic and immune health have stimulated substantial interest in its development as a next-generation probiotic. Despite this interest, the ecological consequences of selectively removing *A. muciniphila* from an intact

microbial community have remained difficult to resolve from observational associations or supplementation-based studies. By enabling sustained and target-specific depletion, our phages provide a complementary loss-of-function strategy to identify candidate ecological dependencies and trace how perturbation of a health-relevant commensal propagates through the surrounding community, consistent with the framework of microbiome ripple effects[52]. Such an approach may also inform the rational evaluation of microbiome-based products and interventions by revealing the broader community consequences of altering a specific bacterial population. It complements direct genetic manipulation, which remains limited in *A. muciniphila* despite the recent development of a transposon-based system[53]. Beyond their immediate use as depletion tools, the newly isolated phages may provide starting materials for future host-specific delivery or genome-editing systems. Although these applications will require dedicated molecular engineering and validation, they extend the potential value of this phage resource from ecological interrogation towards precise, species-specific and mechanism-based manipulation of the microbiome[14,54].

Several limitations should be noted. Phage activity was evaluated against a limited panel of *A. muciniphila* strains, and the HMA experiments included only two donor communities, restricting the generalizability of both host-range and community-level responses. Moreover, the functional responses inferred from metagenomic profiling reflect predicted metabolic potential rather than measured activity and will require validation through targeted metabolomics, while the proposed metabolic interactions warrant direct testing in interaction assays. The mechanisms underlying target rebound also remain unresolved, including the potential contributions of strain variation, spatial refuges and phage resistance. Finally, HMA mouse models do not fully reproduce human gut physiology or long-term host-microbiome interactions. Broader strain panels, additional donor communities and longer-term mechanistic studies will therefore be required to establish the robustness and translational scope of this approach.

4. Methods

4.1 Bacterial strains and culture conditions

A. muciniphila ATCC BAA-835 was purchased from the American Type Culture Collection. Additional *A. muciniphila* strains (YL44, DSM 22959, and JCM 33893) and *A. glycaniphila* DSM 100705 used for host range testing were kindly provided by Prof. Sheng Yang (Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences). *A. muciniphila* ZT003 was shared by Prof. Tao Zuo (Sun Yat-sen University). Other human gut commensal strains used in this study included *Bacteroides fragilis* NCTC 9343, *Bacteroides uniformis* ATCC 8492, *Fusobacterium varium* DA690, and *Anaerostipes hadrus* DA538.

All bacterial strains were cultured anaerobically at 37 °C in an anaerobic chamber (90% N₂, 5% CO₂, 5% H₂). *A. muciniphila* strains were routinely grown in BHIs-mucin (BHI, Brain Heart Infusion) supplemented with (0.4%, w/v) mucin (S12066, Yuanye Bio), (0.05%, w/v) L-cysteine, vitamin K3 (0.5 mg L⁻¹), and hemin (0.5 mg L⁻¹). Other commensal strains were cultured in BHIs. For *in vitro* cultivation of fecal samples, MiPro medium was used. The composition of the MiPro medium was the same as described by Wu et al[27].

4.2 Phage preparation, preservation, and heat inactivation

Phage lysates were freshly propagated and briefly stored at 4 °C. The phage cocktails were formulated by combining select isolates in equal PFU ratios, ensuring that the cumulative phage titer met the target concentration. To freeze the formulation, the phage mixture was blended with the glycerol stock solution to yield a final glycerol concentration of 15% (v/v). For experimental use, the frozen stocks were rapidly thawed in a 37 °C water bath and diluted with SM buffer. HK phage controls were prepared by incubating the diluted lysates at 98 °C for 30 min.

4.3 Wastewater-based isolation and purification of phages

Wastewater samples were collected from five provinces in China between May and

October 2024 (Supplementary Table 1). Samples were transported at 4 °C. Coarse debris was removed by centrifugation at 5,000 × g for 10 min at 4 °C, followed by filtration through qualitative filter paper. The supernatant was sequentially passed through 0.8 µm, 0.45 µm, and 0.22 µm PES membranes to obtain virus-enriched filtrates, which were stored at 4 °C.

Phage enrichment was performed using a two-step procedure in 4× BHIs-mucin. For the first-round enrichment, filtrate (6 mL) was mixed with 4× BHIs-mucin medium (2 mL) containing (2%, v/v) mid-log phase host bacterium culture. The mixture was incubated anaerobically at 37 °C for 24 h, centrifuged at 5,000 × g for 5 min at 4 °C, and the supernatant was sterilized through a 0.22 µm PES filter. For the second-round enrichment, 6 mL of the first-round lysate was mixed with fresh 4× BHIs-mucin medium (2 mL) and (3%, v/v) host bacterium, and the enrichment procedure was repeated.

The second-round lysates were screened for lytic activity using the double-layer agar method. Briefly, host suspension (500 µL) was mixed with 4 mL of molten BHIs-mucin agar (50 °C) and overlaid onto 1.5% BHIs base agar plates. Ten-fold serial dilutions of the enriched lysates in SM buffer were spotted onto the plates. Plates were incubated anaerobically at 37 °C for 24 h. Lysates producing clear plaques were subjected to three rounds of serial dilution and single-plaque purification to ensure clonal purity.

4.4 Phage genome sequencing, assembly and annotation

Phage genomic DNA was extracted using the Takara MiniBEST Viral RNA/DNA Extraction Kit Ver. 5.0. Sequencing libraries were constructed using the Hieff NGS® DNA Lib Prep Primer Mix for MGI® (Yeasten, Shanghai, China) according to the manufacturer's protocols, which involved enzymatic fragmentation, end-repair, 3'-adenylation, and MGI-compatible adaptor ligation. The resulting PCR products were submitted to the China National GeneBank (CNGB) for high-throughput sequencing. At the sequencing facility, the library was circularized to generate single-stranded

circular (ssCir) DNA, followed by rolling circle amplification (RCA) to prepare DNA nanoballs (DNBs). Sequencing was subsequently executed on the BGI DNBSEQ platform with a paired-end 150 bp read configuration. Raw reads were quality-filtered using fastp v0.24.0[55], and *de novo* genome assembly was carried out with Unicycler v0.5.1[56]. Following assembly, contigs with an average sequencing depth below 1,000× (determined by Bowtie 2 v2.5.4[57] mapping) or shorter than 10 kb were discarded. The quality and completeness of the retained phage genomes were then assessed using CheckV v1.0.3[58].

The resulting phage genomes were annotated using Pharokka v1.9.0[59] and Phold v0.2.0[60] to predict CDS and functional domains. Taxonomic classification and gene-sharing network analysis were performed with vConTACT2 v0.11.3[29]. For reference, a subset of 69 sequences from the Gut Phage Database (GPD)[61] was retrieved via Diamond BLASTp (e-value < 1×10^{-5} , top 5 hits), together with sequences from Prokaryotic Viral RefSeq v211. Protein clusters (PCs) were generated using Diamond and MCL, while viral clusters (VCs) were defined using both MCL and ClusterONE algorithms. The resulting network was visualized in Cytoscape v3.10.4[62]. ANI between phage genomes was calculated and genomes were clustered using vclust v1.3.1[63]. Comparative genomic visualization and synteny analysis were conducted with Easyfig v2.2.5[64].

4.5 Transmission Electron Microscopy

High-titer phage suspensions were fixed by mixing with glutaraldehyde to a final concentration of 2.5% (v/v) and incubated overnight at 4 °C. A aliquot (10 µL) of the fixed sample was applied onto a carbon-coated copper grid and allowed to adsorb for 1 min. The grid was then negatively stained with 1% phosphotungstic acid for 1 min and air-dried at room temperature. Phage particles were imaged using a Hitachi HT-7800 transmission electron microscope.

4.6 Phage kill-curve assays

A. muciniphila cultures in mid-logarithmic growth phase ($OD_{600} \approx 0.4\sim0.6$) were

inoculated into BHIs-mucin at 1% (v/v) to standardize the initial baseline OD₆₀₀ to approximately 0.01. The bacterial concentration was estimated based on OD₆₀₀ measurements. Phages were added to the cultures at multiplicities of infection (MOIs) of 1, 0.1, 0.01, and 0.001, calculated according to the estimated bacterial CFU and phage titers. The mixtures were aliquoted into 96-well plates in technical triplicate and incubated anaerobically at 37 °C. Bacterial growth was monitored by measuring OD₆₀₀ using a microplate reader.

4.7 Phage cocktail optimization against *A. muciniphila*

Leveraging the single-phage kill-curve benchmarks, candidate phage cocktails were formulated, centering on the most potent isolate, Akk_GDp9. The lytic efficacy of these formulations was evaluated against two reference hosts, *A. muciniphila* ATCC BAA-835 and *A. muciniphila* ZT003. Briefly, anaerobic *A. muciniphila* cultures in 96-well plates were adjusted to an initial OD₆₀₀ of approximately 0.01. The cultures were then challenged with the cocktails at a MOI of approximately 0.01. To establish infection kinetics, bacterial growth was monitored continuously at 37 °C under anaerobic conditions for 48 h.

4.8 Host range determination

The host range of the isolated phage was determined using the standard spot assay. High-titre phage stocks (~ 10⁸ PFU mL⁻¹) were spotted (3 µL) onto top-agar overlays seeded with target bacterial strains, which had been previously identified by 16S rRNA gene sequencing. After anaerobic incubation at 37 °C for 24 h, the plates were visually inspected, and bacterial strains exhibiting clear lysis zones or distinct plaques were recorded as susceptible.

4.9 Screening of fecal communities supporting high *A. muciniphila* colonization

To obtain stool-derived microbial communities capable of supporting high *A. muciniphila* abundance, candidate donor samples were selected from the frozen baseline community stocks based on historical screening data[27]. The personalized baseline communities were revived and maintained by daily subculture in MiPro

medium under anaerobic conditions at 37 °C. For colonization stabilization, the communities were inoculated with *A. muciniphila* ATCC BAA-835 at 5% biomass and subjected to serial daily passaging (1:200 dilution) for six cycles. Accounting for both historical permissive capacity and real-time revival robustness, six distinct stool-derived *in vitro* communities that achieved stable and robust *A. muciniphila* colonization were ultimately selected for subsequent experiments. High-throughput sampling and microbial pellets collection were executed following the established protocol.

4.10 *In vitro* phage treatment experiments in complex communities

To evaluate the effects of specific phages on the stabilized stool-derived *in vitro* communities[27] (SIC1–SIC6, 1% v/v inoculum) were challenged with two distinct phages, Akk_GDp9 (potent) and Akk_GDp4 (weaker), at a dose of 7×10^6 PFU per well. The experiments were conducted in 96-deep-well plates containing 1 mL of MiPro medium and incubated anaerobically at 37 °C for 24 h. HK phage controls and untreated control were included in parallel. Following the initial treatment, the *A. muciniphila*-containing communities were subcultured daily at a 1:200 dilution. At designated experimental endpoints, cell pellets were collected by centrifugation ($4,000 \times g$, 15 min, 4 °C) and stored at –80 °C for downstream metagenomic sequencing.

4.11 Animals and ethics statement

All female C57BL/6J mice (6–7 weeks old) were purchased from Zhuhai BesTest Bio-Tech Co., Ltd. and acclimated for one week. Mice were housed under SPF conditions in individually ventilated cages (23 ± 3 °C, 40–70% humidity, 12 h light/dark cycle) with free access to a standard sterile diet and water. Prior to experimentation, mice were stratified by body weight and randomly assigned to groups. All procedures were approved by the Institutional Animal Care and Use Committee of Shenzhen Brain Science Infrastructure (license SYXK (Yue) 2023-0351; ethics approval SIAT-BSI-IRB-251105-HCS-DL-A0144).

4.12A. *muciniphila*-colonized model

Mice were randomly assigned to experimental or control groups to establish an *A. muciniphila*-driven model (n = 6 per group). Animals received a five-antibiotic cocktail (ampicillin 0.25 g L⁻¹, vancomycin 0.2 g L⁻¹, neomycin 0.25 g L⁻¹, metronidazole 0.2 g L⁻¹, gentamicin 0.05 g L⁻¹, sucrose 2.5 g L⁻¹) via drinking water for 3 days. Following antibiotic withdrawal, mice were orally gavaged daily with 200 µL of PBS containing 3 × 10⁷ CFU *A. muciniphila* ATCC BAA-835 for 3 consecutive days. After a 3-day stabilization period, the treatment group received 200 µL PC6 (9 × 10⁷ PFU) daily for 7 days, while the control group received an equivalent volume of HK PC6. Fecal samples were collected at predefined intervals, and total genomic DNA was extracted using the QIAamp PowerFecal Pro DNA Kit for subsequent sequencing.

4.13 Human microbiota-associated (HMA) model

To establish the HMA model, mice (n = 6 per group) underwent the identical antibiotic pretreatment, stabilization, phage intervention, and sampling workflows as described for the *A. muciniphila* colonization model. The sole modification was that during the colonization phase, the single *A. muciniphila* gavage was replaced with a PBS suspension (200 µL) of the predefined *A. muciniphila*-containing communities, SIC5 or SIC6. To prepare these inocula, the *A. muciniphila*-containing communities were thawed and subcultured for two generations to the mid-logarithmic phase (OD₆₀₀ ≈ 0.5~0.6). Glycerol was then added to a final concentration of 18% (v/v) before aliquoting and flash-freezing at -80 °C. For experimental colonization, these frozen stocks were rapidly thawed in a 37 °C water bath and resuspended in PBS prior to oral gavage.

4.14 Quantitative PCR (qPCR) Analysis

To quantify the absolute abundances of *A. muciniphila* and total bacteria in both *in vitro* cultures and *in vivo* mouse fecal samples, qPCR was performed using total extracted DNA as the template. Reaction mixtures were prepared with the SYBR Green Premix Pro Taq HS qPCR Kit (High Rox Plus, Accurate Biotechnology, Hunan, China) and target-specific primers (Supplementary Table 3). Amplification was conducted on a

StepOnePlus Real-Time PCR System. Absolute copy numbers were calculated based on standard curves generated from known concentrations of *A. muciniphila* genomic DNA and universal bacterial DNA reference.

4.15 Metagenomic and Bioinformatics Analysis

Metagenomic library preparation and sequencing. Identical protocols were applied for both *in vitro* cultures and *in vivo* mouse samples. Metagenomic DNA was fragmented using a Covaris E220 ultrasonicator, adaptor-ligated, and circularized to generate single-stranded circular DNA libraries. Libraries were amplified via rolling circle amplification into DNA nanoballs and sequenced on the MGI DNBSEQ platform (MGI Tech, Shenzhen, China) following the standard CNGB Meta WGS workflow.

Bioinformatic processing and profiling. Raw sequencing reads were quality-filtered using fastp v0.24.0 (minimum length 18 bp). Clean reads were mapped against the mouse reference genome (GRCm39) using Bowtie 2 v2.5.4 to remove host DNA. The retained microbial reads were classified against the Genome Taxonomy Database (GTDB) using Kraken2 v2.1.2[65], with species- and genus-level abundances subsequently re-estimated by Bracken v3.0.1[66] (150 bp read length, 40-read minimum threshold). For functional profiling, the host-depleted reads were processed using HUMAnN 3 (v3.9[67], incorporating MetaPhlAn) to quantify the abundances of microbial gene families and MetaCyc metabolic pathways. For phage alignment, ten phage reference genomes were used for read alignment. BBSplit (v39.26) was used with minimum identity = 0.80, and all ambiguous mappings were reported ('ambiguous=all'). Coverage statistics were extracted from BBSplit output files, and the "Covered_percent" metric was used to determine phage presence. A sample was classified as phage-positive if Covered_percent > 30%; otherwise, it was classified as phage-negative.

Statistical analysis and visualization. All statistical analyses were performed in R v4.3.2. Alpha diversity (Shannon index) was calculated using the vegan package and compared via two-sided Wilcoxon rank-sum tests. To evaluate longitudinal community

shifts, PCoA was performed based on Bray-Curtis dissimilarities. Differences in trajectory were tested using PERMANOVA (adonis2 function, 999 permutations). Longitudinal divergence from baseline (Day 0) was quantified using linear mixed-effects models (lme4) with individual mice assigned as random intercepts, followed by post-hoc pairwise comparisons via emmeans.

Differential pathway abundance during the stable phase (Days 11–25) was identified from log₂-transformed counts per million (CPM) using empirical Bayes moderated t-tests in the limma package. Pathways were considered significant with a Benjamini-Hochberg (BH) adjusted $p < 0.05$ and $|\log_2FC| > 0.5$. Associations between key differentially abundant taxa and functional pathways were evaluated using Spearman's rank correlation; only correlations with a BH-adjusted $p < 0.05$ and $|r| > 0.4$ were considered significant. Visualizations were generated using ggplot2 and ggrepel, and schematic diagrams were created with the Generic Diagramming Platform (BioGDP, <https://www.biogdp.com>)[68].

Data availability

The raw metagenomic sequencing data generated in this study, after host-sequence depletion, have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1479162. All other data supporting the findings of this study are available within the Article and its Supplementary Information. Source data are provided with this paper.

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Author contributions

L.D. and E.L.L. supervised the study, provided resources and revised the manuscript. X.K. drafted the manuscript and prepared the figures. W.Z., J.S., Z.Q., J.H. and Y. M. commented on data analysis and manuscript revision. X.K. performed the experiments with advice or assistance from J.S., Z.Q., Y.Z., J.H., S.S. and Y. M. X.K. and W.Z. performed the bioinformatic analyses with technical assistance from H.N. All authors approved the final manuscript.

Competing interests

The authors declare no competing interests.

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