

Supplementary Information

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

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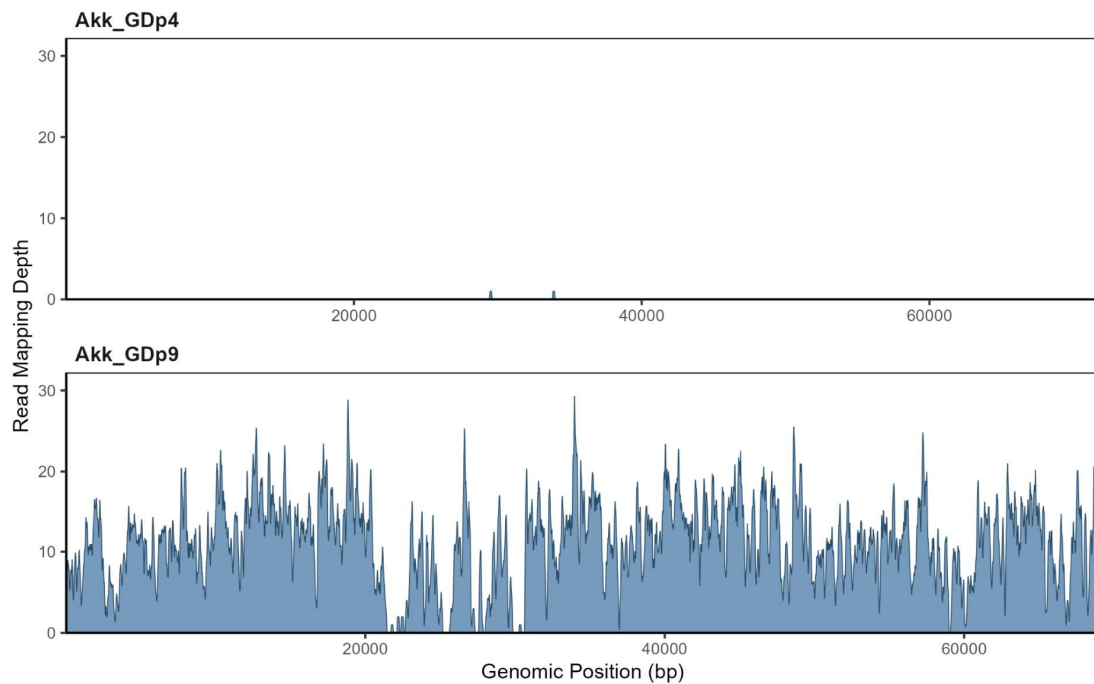
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The PDF file includes:

Supplementary Figures. S1 to S14

a Nanshan Water Purification Plant



b Futian Water Purification Plant

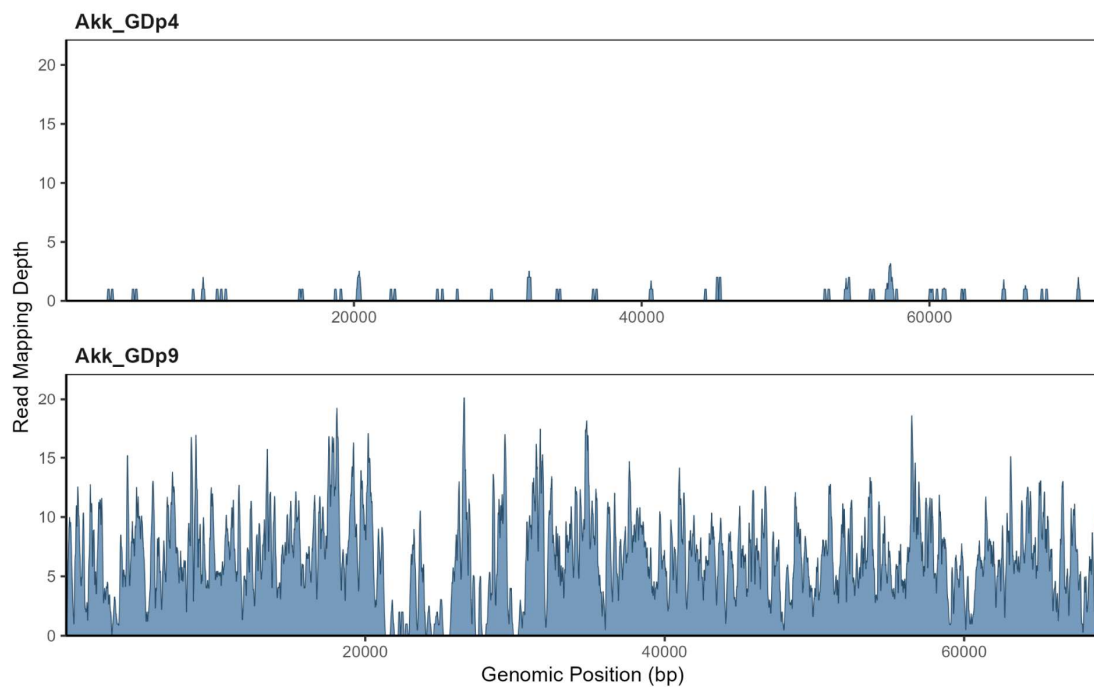


Figure. S1. Genetic evidence for the presence of isolated phages in environmental wastewater. Coverage depth profiles showing the alignment of metagenomic reads from **(a)** Nanshan Water Purification Plant and **(b)** Futian Water Purification Plant against the representative genomes of isolated phage, Akk_GDp4 and Akk_GDp9. The y-axis represents the read depth per base, and the x-axis indicates the genomic position (bp).

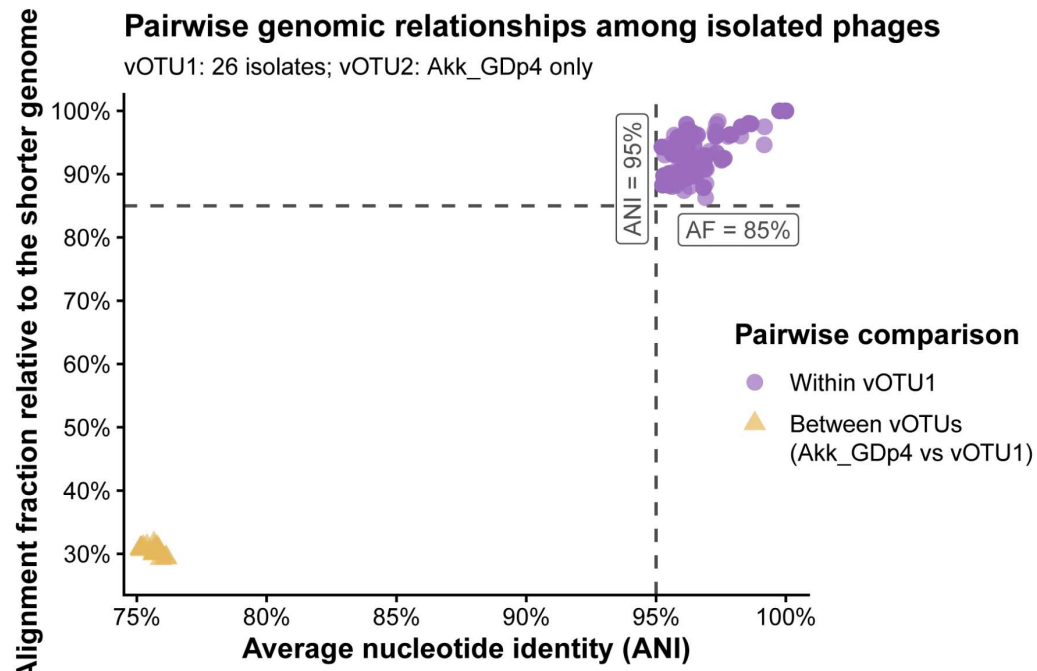
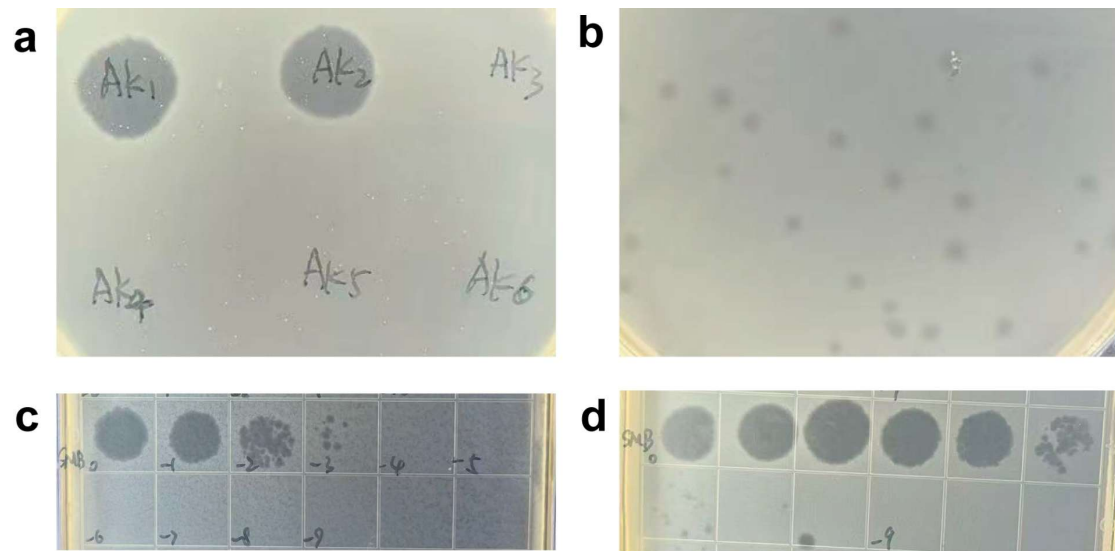


Figure. S2. Pairwise average nucleotide identity (ANI) and alignment fraction (AF) among the 27 cultured *Akkermansia* phage isolates. Each point represents one unordered pair of phage genomes. ANI is shown on the x axis, and AF relative to the shorter genome is shown on the y axis. Purple circles represent comparisons among the 26 isolates assigned to vOTU1, whereas yellow triangles represent comparisons between Akk_GDp4, the sole cultured member of vOTU2, and individual members of vOTU1. Dashed vertical and horizontal lines indicate the operational species-level thresholds of 95% ANI and 85% AF, respectively.

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56 **Figure. S4. Plaque morphologies of isolated *Akkermansia* phages. a** Representative
 57 **plaques observed during phage enrichment. b** Representative plaques obtained after
 58 **plaque purification. c, d** Plaque morphologies of Akk_GDp4 (c) and Akk_GDp9 (d).
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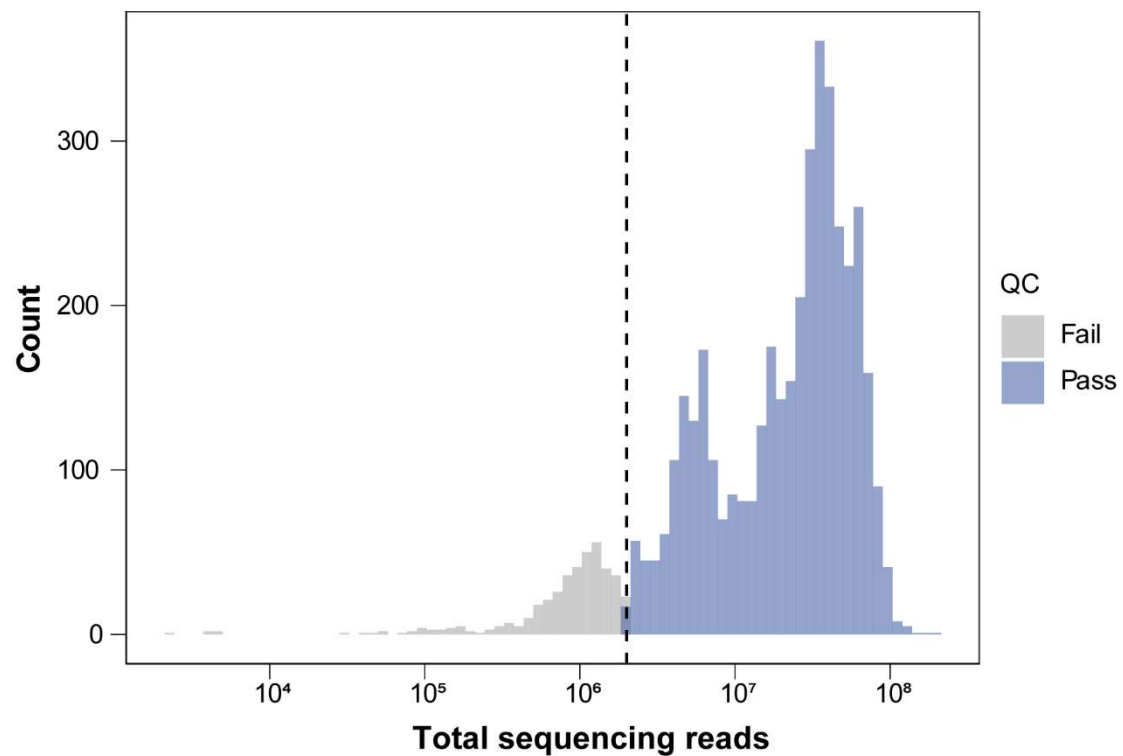


Figure. S5. Read depth distribution across metagenomic samples. Histogram of total sequencing reads per sample on $\log_{10}$ scale, colored by quality control status. Samples with ≥ 2 million reads (blue) passed QC and were retained for analysis; samples below the threshold (gray) were excluded. The vertical dashed line indicates the 2M reads cutoff.

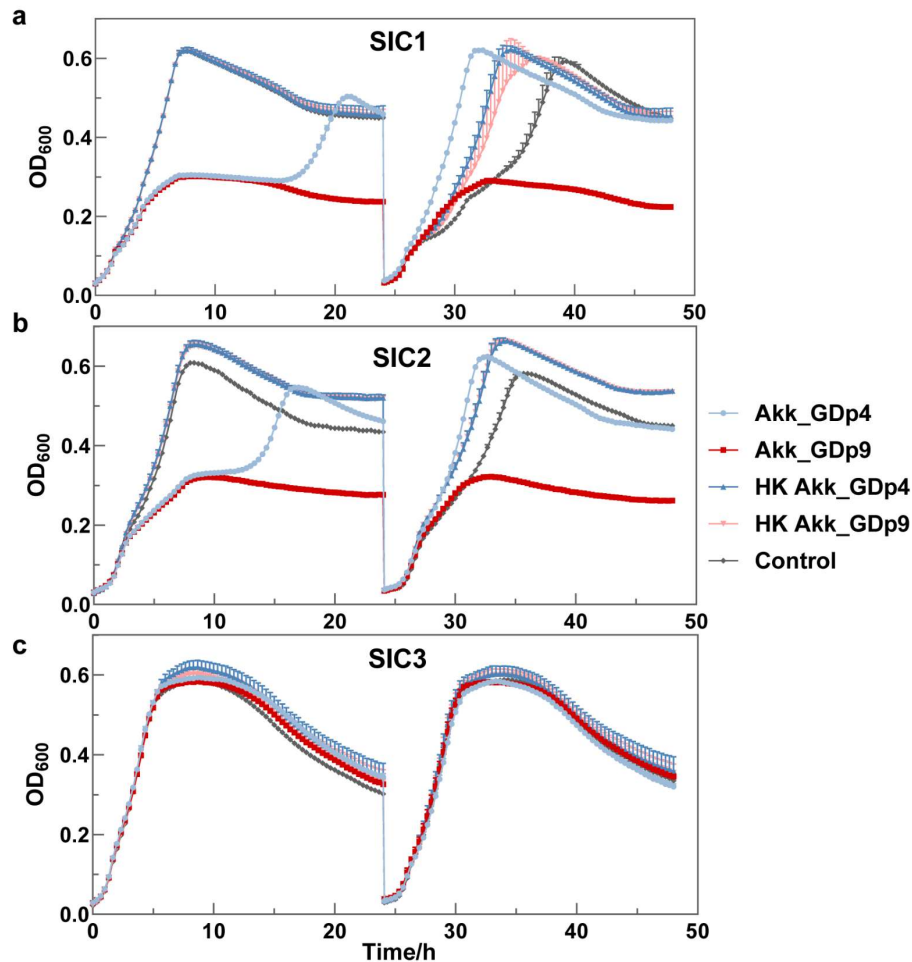


Figure. S6. Growth dynamics of representative stool-derived *in vitro* communities following phage treatment. a, b, c OD₆₀₀ growth curves of three representative communities SIC1(a), SIC2(b), and SIC3(c) over 48 h with 24-h subcultivation. Each community was treated with blank control, Akk_GDP4 (weaker phage), Akk_GDP9 (potent phage), or the corresponding heat-killed controls.

Cultures were grown in anaerobic conditions, with OD₆₀₀ measured at multiple time points. Data points represent mean $\pm$ SD of three technical replicates (independent wells inoculated from the same starting community culture). Subcultivation at 24 h was performed separately for each replicate well to maintain independent lineages. Vertical dashed lines indicate the time of subcultivation. These panels illustrate the differential growth suppression patterns: Akk_GDP9 induces sustained growth inhibition, particularly in high-*A. muciniphila* communities (e.g., SIC1 and SIC2), whereas Akk_GDP4 shows transient suppression followed by recovery, consistent with its weaker and less persistent activity.

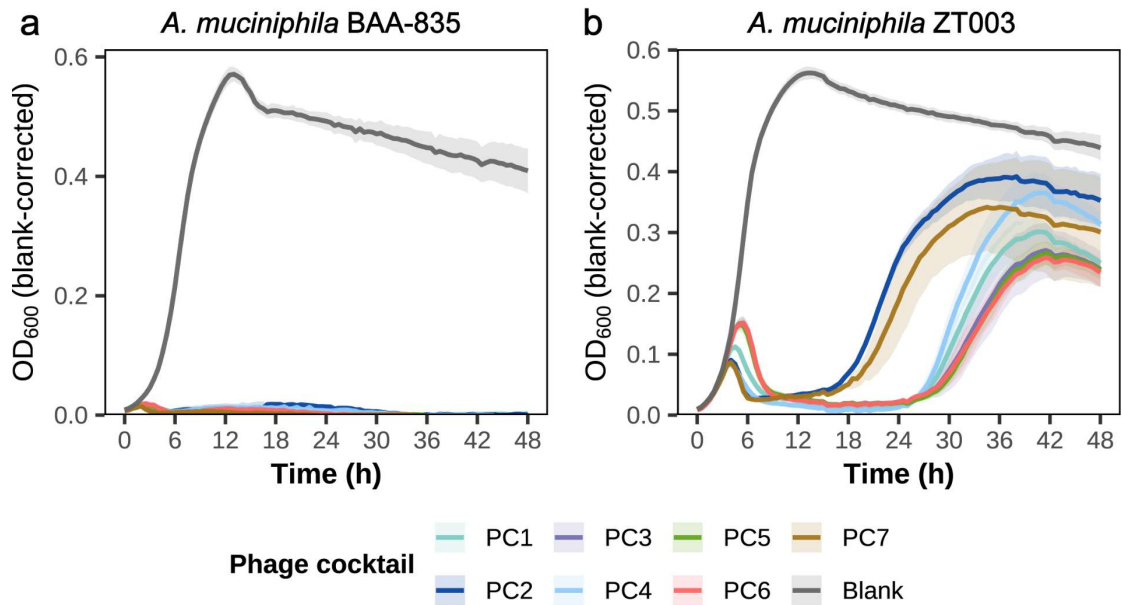


Figure. S7. Phage cocktails differentially inhibit the growth of two *Akkermansia* strains *in vitro*. **a, b** Growth curves of *A. muciniphila* ATCCBAA-835 (**a**) and *A. muciniphila* ZT003 (**b**) treated with seven phage cocktails (PC1–PC7) or blank control. Data represent mean $\pm$ s.d. (n = 3 biological replicates) of blank-corrected optical density at OD₆₀₀. Shaded ribbons indicate standard deviation.

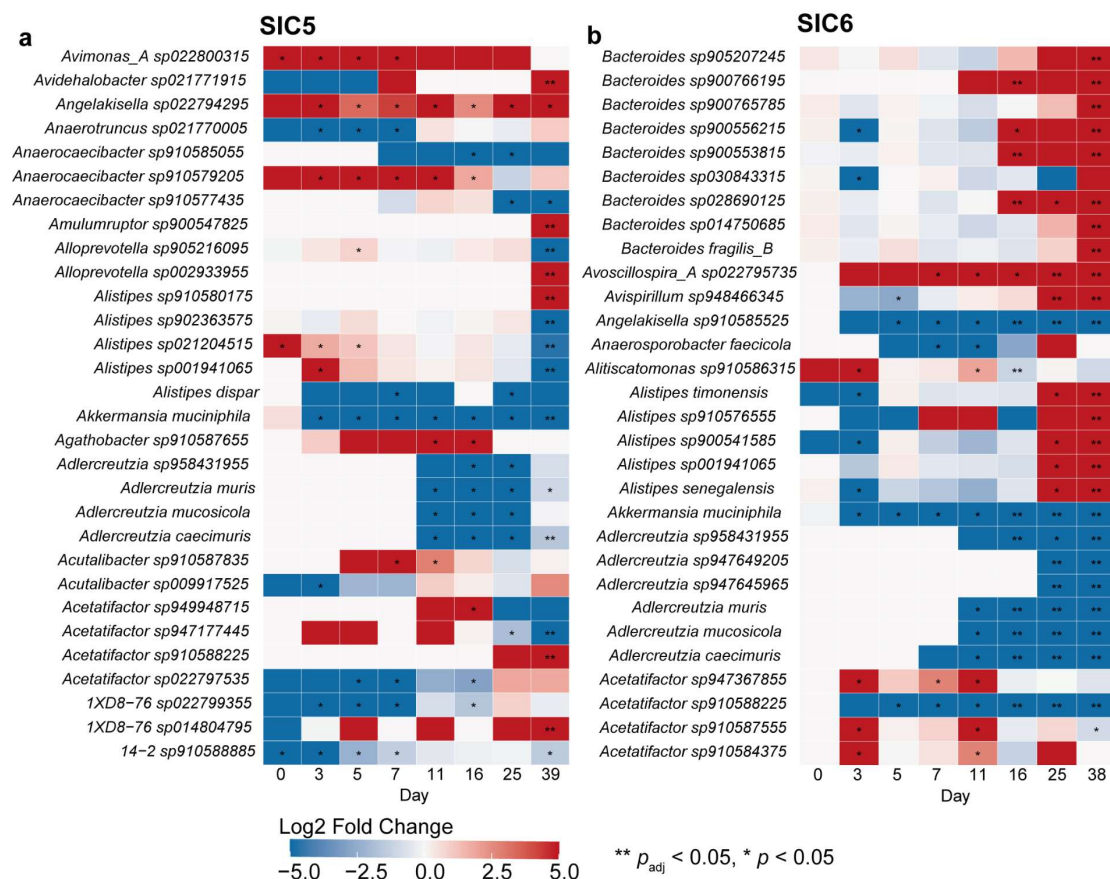


Figure. S8. Temporal dynamics of top differential taxa in response to phage intervention across humanized microbiota donors.

a Heatmap of log2 fold changes (PC6 treatment versus heat-killed control) over time in SIC5-colonized mice. Top 30 taxa were selected based on the minimum p value across all time points. Color scale represents log₂FC; red indicates increased and blue indicates decreased. Significance is indicated by asterisks (** $p_{adj} < 0.05$; * $p < 0.05$; Wilcoxon rank-sum test). **b** Corresponding heatmap for SIC6-colonized mice using the same selection and visualization criteria. Absolute abundance was calculated by multiplying relative abundance with qPCR-based total bacterial load.

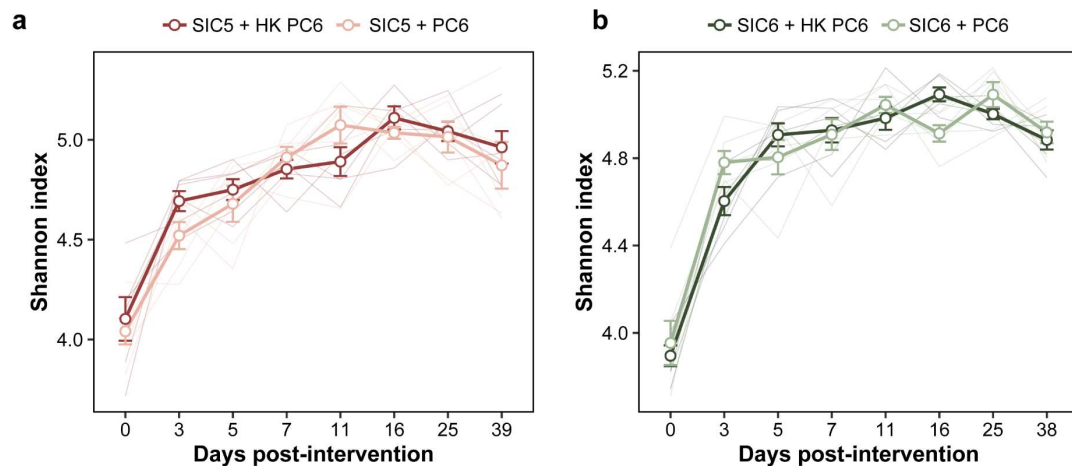


Figure. S9. Shannon diversity dynamics in humanized microbiota mouse models following targeted phage intervention. **a** Shannon index over time in SIC5-colonized mice. Thin lines represent individual animals; thick lines with error bars indicate mean $\pm$ SEM. **b** Shannon index dynamics in SIC6-colonized mice under the same experimental conditions. Diversity was calculated at the species level using the vegan package.

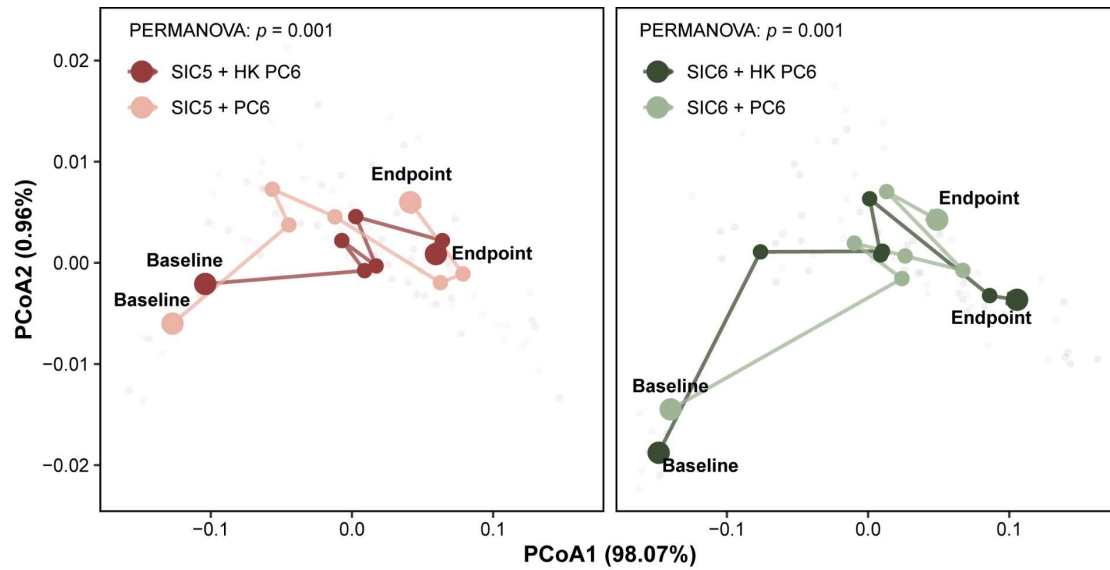


Figure. S10. PCoA of MetaCyc pathway abundance profiles showing functional community trajectories from day 0 to day 25 in SIC5- and SIC6-HMA mice. Individual samples are plotted with low opacity; baseline (D0) and endpoint (D25) are highlighted with larger labels.

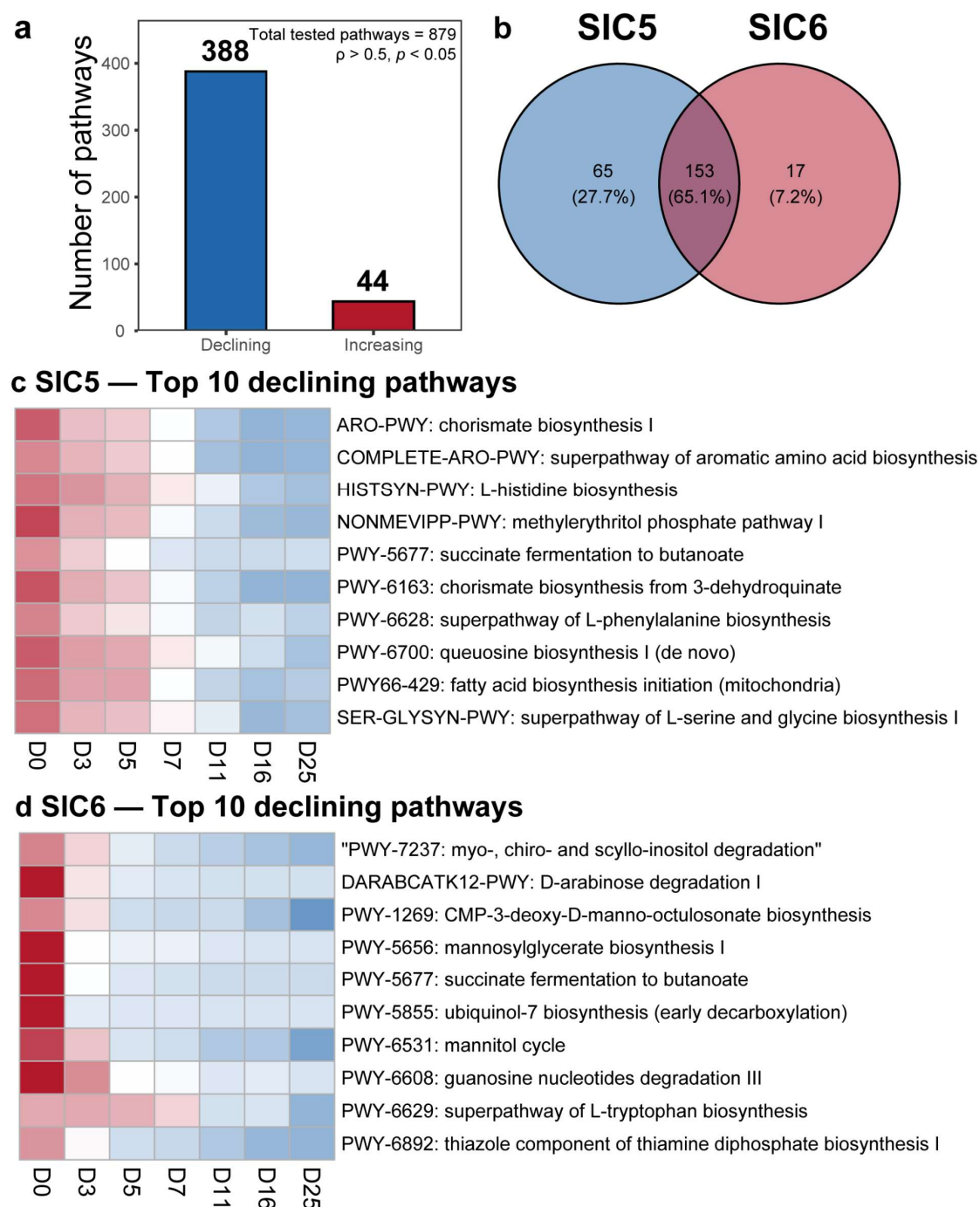


Figure. S11. Significant temporal decline of human-derived metabolic pathways following fecal microbiota transplantation into mice. **a** Proportion of pathways showing significant temporal decline ($n = 388, 44.1\%$) versus increase ($n = 44, 5.0\%$) among 879 pathways with sufficient variation. **b** Overlap of significant declining pathways (Spearman $\rho < -0.5, p < 0.05$) between SIC5- and SIC6-colonized mice. **c, d** Heatmaps of the top 10 most strongly declining pathways in SIC5- (**c**) and SIC6-colonized (**d**) mice. Color scale represents z-score of $\log_{10}(\text{CPM} + 1)$. All displayed pathways meet the significance threshold ($\rho < -0.5, p < 0.05$).

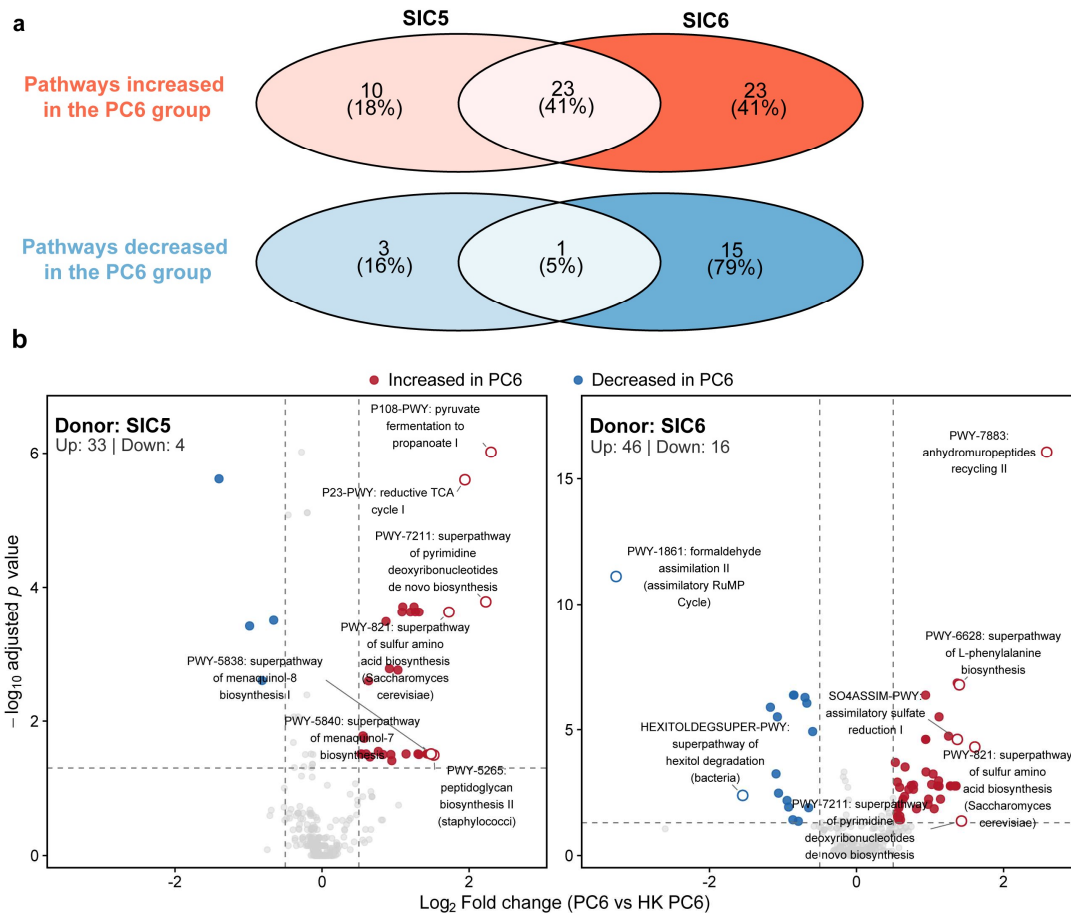


Figure. S12. Overlap of differentially abundant MetaCyc pathways between the SIC5 and SIC6 communities across the stable phase. **a** Venn diagrams show the intersection of pathways significantly altered by PC6 versus heat-killed PC6 in the stable phase (days11, 16, and 25). Top panel: pathways increased in the PC6 group; Bottom panel: pathways decreased in the PC6 group. Numbers represent the count of pathways unique to each donor or shared between both. Analysis was performed using limma with Benjamini–Hochberg correction ($p_{adj} < 0.05$ and $|\log_2 FC| > 0.5$). Only pathways passing these thresholds in at least one donor are included. **b** Volcano plots of differentially abundant MetaCyc pathways across the stable phase (days11, 16, and 25) between PC6 and HK PC6 treatments, faceted by donor. x-axis: $\log_2 FC$ (PC6 vs HK PC6); y-axis: $-\log_{10}$ (Benjamini–Hochberg-adjusted p value). Dashed lines indicate thresholds ($p_{adj} = 0.05$ and $|\log_2 FC| = 0.5$). Points are coloured by significance category. Selected significant pathways with the largest absolute $\log_2$ fold changes, together with predefined biologically relevant significant pathways, are labelled.

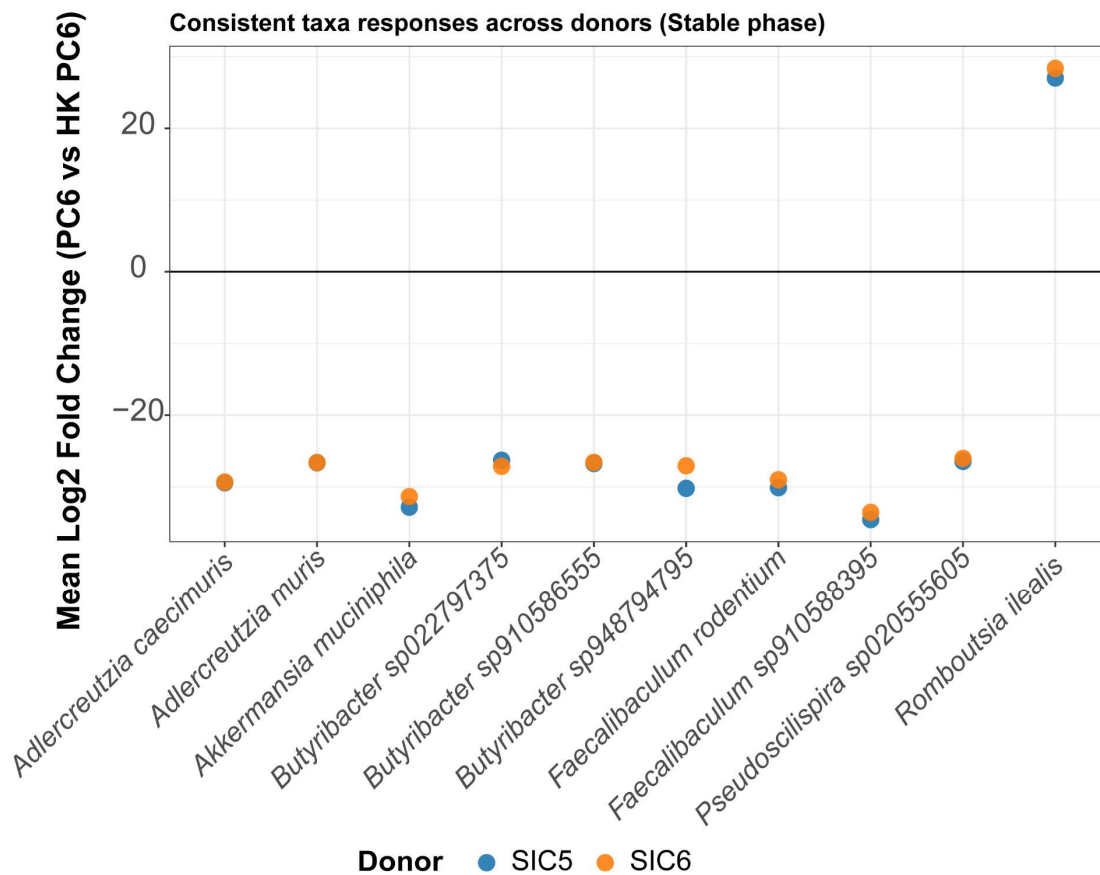


Figure. S13. Cross-donor consistent taxa responses in the stable phase following phage PC6 intervention. Mean $\log_2FC$ (PC6 vs HK PC6) of the top eight taxa exhibiting consistent directional shifts (same sign of $\log_2FC$) in both SIC5- and SIC6-colonized mice across the stable phase (days 11, 16, and 25). Taxa are ranked by the mean absolute $\log_2FC$ across the two donors. This panel highlights the reproducible, donor-independent microbial responses to precise *A. muciniphila* depletion.

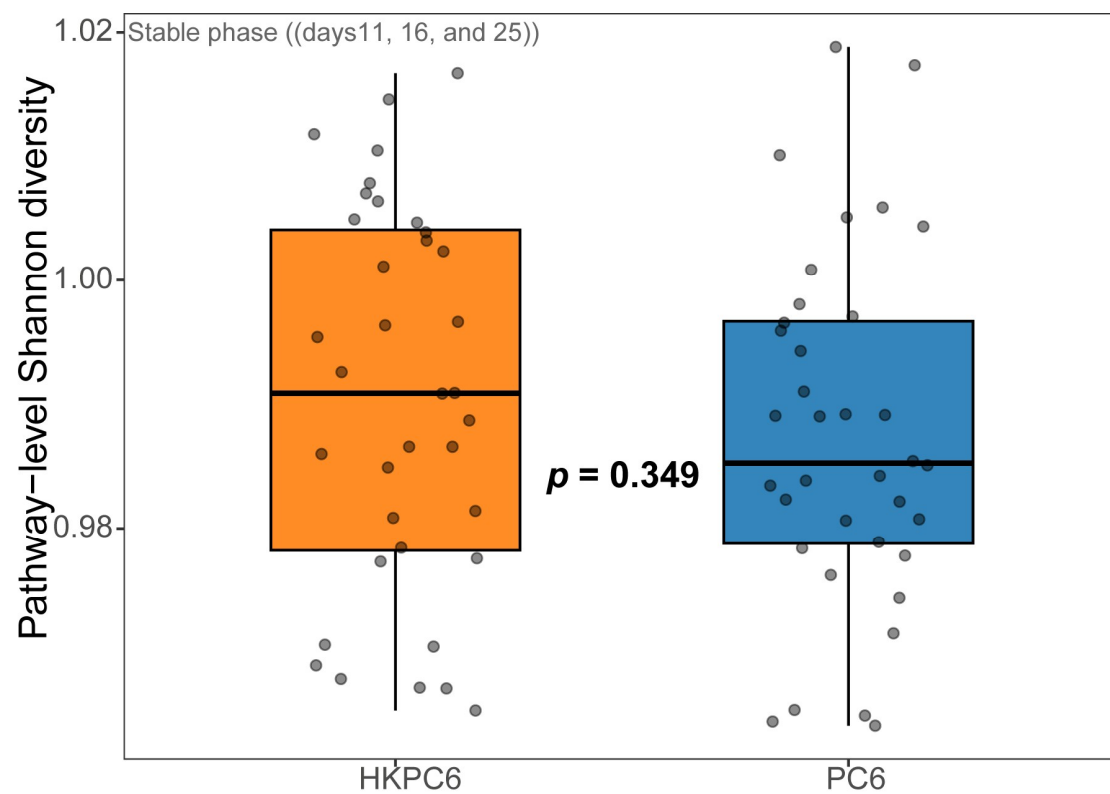


Figure. S14. Shannon diversity of MetaCyc pathway abundance profiles in PC6- and HK PC6-treated mice across the stable phase. Box plots with individual data points (jittered). Wilcoxon rank-sum test p -value is annotated.