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Supplementary Materials for

Gut-targeted nanoparticles deliver specifically targeted antimicrobial peptides against *Clostridium perfringens* infections

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Experimental Section

Bacterial Strains and growth conditions: The strains *E .coli* ATCC25922， *E .coli* O157:H7, *S. aureus* ATCC 25932, *E. faecalis* ATCC 29212, *S. typhimurium* ATCC 14028, *A. baumannii* ATCC 19606, *S. flexneri* ATCC 12022 were obtained from College of Animal Science, Zhejiang University (Hangzhou, China). *C. perfringens* ATCC 13124 was purchased from Guangdong Microbial Culture Collection Center (Guangzhou, China). *S. aureus* USA300 were obtained from Hangzhou Bio Sci co. ltd. The *C. perfringens* ATCC 13124 was cultured in a reinforced clostridium medium (RCM) or Mueller−Hinton Broth (MHB) for 24 hours in anaerobic environment. The other bacterial strains were cultivated in MHB with shaking at 225 rpm. Cultures were synchronized to exponential phase and standardized for each experiment based on OD_{600}. For all assays in this experiment, 100 μL of the test solution was added to the wells of a 96-well plate, and the optical density was measured using a microplate reader.

Materials: MHB, RCM, and phosphate buffered saline (PBS) were obtained from Solarbio (China). Tryptone-Sulfite-Cycloserine Agar (TSCA) was obtained from Hopebio-Technology (China). Bovine serum albumin (BSA), N-phenyl-1-naphthylamine (NPN), Propidium iodide (PI), 3,3-dipropylthiadicarbocyanine ($DiSC_3$-5), Triton X-100, 1-anilino-8-naphthalene sulfonate (ANS), Sodium dodecyl sulfate (SDS), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich (China). Dimethyl formamide (DMF) was purchased from Aladdin (China). The Ph. D-12 Phage Display Peptide Library was purchased from New England Biolabs (USA). Methylthiazolyldiphenyl-tetrazolium bromide (MTT) was purchased form APExBIO (China). D-Cycloserine, Kanamycin, Gentamicin, Colistin, Metronidazole, and Vancomycin were obtained from MACKUN (China). BCA Kit were purchased from Abbkine (China). 2,3-bis(2-methyloxy-4-nitro-5-sulfophenyl)-2H-tertazolium-5-carboxanilide (XTT) Kit was purchased from KeyGEN BioTECH (China). Mouse *C. perfringens* alpha-toxin (CPA), TNF-α, IL-6, and IL-1β enzyme-linked immunosorbent assay (ELISA) Kit were purchased from Shanghai Hengya Biological Technology Co. Ltd (China).

Machine Learning Model: The machine learning (ML) model was established for predicting the

activity levels of AMPs against intestinal infection pathogens. We obtain the minimal inhibitory concentration (MIC) of 1621 special AMPs (against *S. aureus, E. coli, S. typhimurium, A. baumannii, S. flexneri, C. difficile, or C. perfringens, etc.*) from DBAASP (https://dbaasp.org/home). Meanwhile, we computed 93 features according to their sequences, and applied the following MIC thresholds: high activity (0 μg/mL, 32 μg/mL), low activity (32 μg/mL, 128 μg/mL), no activity (128 μg/mL, +∞). BorderlineSMOTE was used for oversampling. The dataset was randomly split into 80% training and 20% test data. AutoGluon was used to establish the ML prediction models, and 26 ML models with 10-fold cross-validation was measured. The code was completely executed according to AutoGluon's reference case (https://auto.gluon.ai/stable/index.html). The relevant dataset, code, and model are available on Dryad (DOI: 10.5061/dryad.31zcrjds7) and GitHub (https://github.com/xubocheng/Anti_Cp.git).

Peptide synthesis and property calculations. The peptides developed in this study were synthesized at GL Biochem Ltd (Shanghai, China) and purified to >95%. The quality of each peptide was indicated by mass spectrometry (MS) and high-performance liquid chromatography (HPLC). The mean hydrophobicity < H> and mean amphipathic moment <μH> were calculated by HeliQuest (https://heliquest.ipmc.cnrs.fr/). The physicochemical parameters were computed with DBAASP (*40*) (https://dbaasp.org/home). The predicted structure for the peptides generated using Iterative Threading ASSEmbly Refinement (I-TASSER) server (https://zhanggroup.org//I-TASSER/).

Circular dichroism measurements: CD spectra of 100 μg/mL peptides were measured in 10 mM PBS (PH 7.4), 50% TFE, and 30 mM SDS micelles on a J-1500 spectropolarimeter (Jasco; Tokyo, Japan), with a quartz cuvette (1.0 mm path length). The spectra were recorded from 195–250 nm at a scanning speed of 10 nm/min. All CD measurements were performed with an average of three technical replicates. The acquired CD spectra were then converted to the mean residue ellipticity using the following equation: $\theta_M = (\theta_{obs} \times 1000)/(c \times l \times n)$, where θ_M is the mean residue ellipticity (deg cm^2 $dmol^{-1}$), θ_{obs} is the observed ellipticity corrected for the buffer at a given wavelength (mdeg), c is the peptide concentration, l is the path length, and n is the number of amino acids. The secondary structure content of the peptides was estimated using K2D3 (http://cbdm-01.zdv.uni-mainz.de/~andrade/k2d3/).

Biopanning of phage-displayed peptides: Phage display technology was used to obtain targeted peptide domain with modifications as described previously (*12*). The screening antigen (*C. perfringens* ATCC 13124) and shielding antigen (*E. coli* O157:H7, *S. typhimurium* ATCC 14028, and *S. flexneri* ATCC 12022) were coupled to magnetic beads respectively, and the 12-peptide library was incubated with the shielding antigen for 1 hour; the supernatant was then magnetically separated by a magnetic frame. The supernatant was incubated with the screening antigen for 2 h. The supernatant was magnetically separated, unbound phages were washed away, and the eluate phage were bound to the original screen. For the first round of biopanning, 96-well plates were coated with 100 μL whole cells of *C. perfringens* ATCC 13124 (50 μg/mL) resuspended in PBS at 4℃ overnight. After plates were washed with TBST thrice and blocked with 5% BSA in TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl), the plates were incubated with 50 μL of phage library at 37 ℃ for 2 h. Unbound phages were removed by six washes with 0.1% PBST and three washes with PBS. Subsequently, the bound phages were eluted by the addition of 100 μL elution buffer (0.2 M glycine-HCl, pH 2.2, 1 mg/mL BSA) at room temperature for 8 min. After neutralization with 15 μL 1 M Tris-HCl, pH 9.1, the eluted phages were amplified in 1 mL E. coli ER2738 ($OD_{600} \approx 0.6$) by an infection method. The amplified phages were titred and used for the second round of biopanning. After four rounds, the eluted phages were identified by ELISA, positive clones were selected and used to prepare phage stocks to isolate phage genomic DNA for nucleotide sequencing (PRJNA884199).

Peptide pull down assays: Total proteins from *C. perfringens* ATCC 13124 were incubated with biotinylated F6 peptide which was combined with streptavidin magnetic beads in PBS at 4℃ overnight. Streptavidin magnetic beads were used as the control group. The beads were magnetically retrieved, washed thrice with PBST, and boiled in Laemmli buffer for 10 min. Subsequently, RIPA Buffer cell lysate and loading buffer were added. The samples were boiled in boiling water for 10 min, and centrifuged at 12,000 rpm for 5 min. The supernatant was collected and resolved by SDS-PAGE; the protein bands were detected with Coomassie blue staining and identified via mass spectrometry.

Antimicrobial activity: The antibacterial activity of peptides was evaluated using a standard broth microdilution protocol with minor modifications. In brief, the bacteria synchronized to mid-log phase and then washed three times with PBS, subsequently standardized to a final bacterial cell solution (0.5-1×10^6 CFU/mL) in MHB or RCM. The MIC assays were performed using 96-well plates with the peptides doubly diluted in bovine serum albumin solution (0.2% BSA 0.01% acetic acid solution), and equal volumes of the bacterial suspension were added to each well. Untreated bacterial culture and medium as positive and negative control wells were implemented. The incubation of plates was at 37°C for 18–24h in anaerobic or aerobic environment. MIC that is the lowest peptide concentration that fully inhibited bacterial growth was observed. In particular, we determined the MIC values against *C. perfringens* under four different conditions (see fig. S3 for the experimental procedure). Specifically, *C. perfringens* was cultured in both MHB and RCM. The synchronized bacterial suspension was then diluted in either RCM or MHB to a concentration of 0.5-1×10^6 CFU/mL for MIC experiment. There were four combinations: MM, MR, RR, and RM, where the first letter represents *C. perfringens* cultured in RCM or MHB, and the second letter represents the medium used for dilution.

Killing kinetics assays: *C. perfringens* ATCC 13124 were cultivated in RCM or MHB until mid-log phase, and then washed and diluted to a final bacterial cell solution in PBS. Aliquots of cultures (~10^5 CFU) treated with different peptides were taken at 3, 5, 10, 30, 60, and 120 min, diluted 100-fold, and plated on TSCA plates. Colonies were counted after overnight incubation in anaerobic environment at 37ºC. Each test was performed on three independent experiments.

Hemolytic assays and eukaryotic cytotoxicity: Human red blood cells (hRBCs) obtained from a healthy donor (R.Y), who has provided written informed consent, were washed thrice with PBS and diluted to a 2% solution (v/v). After peptide treatment, the cells were incubated at 37 ºC for 1 h, and centrifuged at 3500 rpm for 5 min. Aliquots of the supernatant were carefully transferred to a fresh 96-well microplate. The amount of hemoglobin released was measured at 570 nm. The percent lysis was calculated by assuming 100% release when human blood cells were treated with 2% Triton X-100, and 0% release when incubated with PBS buffer. The % hemolysis was calculated as (treated sample− negative control)/ (positive control − negative control) × 100%. The peptide concentration

that caused 10% lysis of hRBCs is defined as HL10. The cell selectivity index (CSI) was calculated as the ratio between HL10 and MIC of the corresponding peptide against a select pathogenic species. We performed the cytotoxicity assay as previously described using CCK8 as a readout for cellular viability, with modifications for Hek293T cells. Briefly, the resuspended cells (1×10^5 cells per well) and peptides with different concentrations were mixed in a 96-well plate and incubated for 24 hours under a humidified atmosphere containing 5% CO2 at 37 °C.

Flow cytometry analysis: We used flow cytometry analysis to investigate the growth model of *C. perfringens* and antibacterial tendency of HAMPs against *C. perfringens* under four culture conditions. Bacteria were cultured in MHB or RCM until reaching the logarithmic growth phase. The cells were then washed three times with PBS to remove metabolites from the RCM medium. 1) The cells were diluted in PBS to achieve an OD_{600} of 0.1, and the diluted samples were analyzed using flow cytometry. 2) The cells were diluted in either MHB or RCM to an OD_{600} of 0.1, followed by anaerobic cultivation at 37°C for 2 hours and 12 hours and the samples were analyzed using flow cytometry. 3) The cells were diluted in either MHB or RCM to an OD_{600} of 0.1 and supplemented with a final concentration of 32 μg/mL of HAMPs. The cells were then cultured under anaerobic conditions at 37°C for 2 hours and 12 hours, and the samples were analyzed using flow cytometry. Vegetative cells, sporulation cells and free spores cannot be quantified in this study, so the former two are collectively referred to as rod-shaped *C. perfringens*. Flow results can only distinguish morphological changes by cell size.

***Cell wall permeabilization*:** Cell wall permeability of *C. perfringens* ATCC 13124 cultivated in MHB was determined by the uptake of NPN. Briefly, logarithmic growing microbial cells were harvested (6000rpm, 3min) and diluted to OD_{600} = 0.2 in 5 mM HEPES buffer (pH=7.4, containing 5 mM glucose). The bacterial suspension was further incubated with 10 μM NPN in the dark for 30 min. Subsequently, the different concentrations (1/2, 1, 2×MIC) of peptides were added, and the NPN fluorescence was detected (excitation λ =350 nm, emission λ = 420 nm). The untreated bacterial suspension served as a negative control, and a 10 μg/mL polymyxin B-treated bacterial suspension served as a positive control.

PI assays: Membrane disruption was determined by PI uptake. The bacteria were cultivated to exponential phase in MHB and diluted to an OD_{600} of 0.02. PI was added at 10 μg/mL along with 0.5, 1, 2×MIC HAMPs and including a no treatment control sample. The samples were incubated in a 37 °C water bath for 20 and 60 minutes. Samples were then analysed using an BD Accuri flow cytometer. Data were graphed using the FlowJo software program.

$DiSC_3$-5 assays: Inner membrane depolarization was measured using $DiSC_3$-5 cationic dye (*9*). *C. perfringens* cultivated in MHB. Mid-log phase bacteria were pelleted and washed with HEPES buffer thrice, and resuspended and diluted to an OD_{600} = 0.05 in 5 mM HEPES buffer (containing 20 mM glucose), and then 0.4 μM $DiSC_3$-5 was added in the dark for 90 min at 37 °C. The suspension was further incubated with 100 mM KCl for 30 min. Subsequently, various MIC-fold peptides were added, and fluorescence was detected (excitation λ = 622 nm, emission λ = 670 nm), until no changes were observed. Background fluorescence was also recorded. Results were normalised to relevant background controls.

TEM of peptdies: The morphologies of the peptides were observed using TEM. The peptide solution was diluted to 256μg/mL in 1×PBS or 50% TFE, and then placed onto carbon-coated surface of a copper grid (300 square mesh). After 1 min, the grid was stained with a 10 μL aliquot of 1% uranyl acetate three times and washed in Milli-Q water three times, the excess dye was absorbed, and the sample was air-dried for at least 15 min before imaging.

ANS fluorescence assay: The critical assembly concentration (CAC) of HAMPs was determined using the ANS fluorescence assay. ANS was dissolved in DMF to a concentration of 1 mM, and then 1 μL of ANS was added to 100 μL of different concentrations of peptides. Then, a microplate reader was used to measure the fluorescence spectrum of the peptide solution under an excitation wavelength of 360 nm, an emission wavelength range of 420–670 nm, and an interval of 5 nm. Finally, the corrected fluorescence intensity at a wavelength of 460 nm and the logarithm of peptide concentration for the plot were used. CAC was determined as the intersection of the two fitted straight lines.

Antibiofilm activity: The inhibitory and clearing effect of HAMPs and EPV@HAMPs on *C. perfringens* biofilm was evaluated using the crystal violet staining method. Briefly, *C. perfringens* cultured in RCM or MHB from the exponential phase was diluted in RCM or MHB to a concentration of 2×10^6 CFU/mL. A 200 μL bacterial inoculum was added to a 96-well plate and incubated anaerobically at 37°C for 24 hours to allow biofilm formation. Subsequently, the culture medium was discarded, and the wells were gently washed three times with PBS to remove planktonic bacteria. Drugs at final concentrations ranging from 8 to 256 μg/mL in PBS were added to the 96-well plate. After further incubation anaerobically at 37°C for 24 hours, the wells were washed three times with PBS, and the biofilms were fixed with 100 μL of methanol for 15 minutes. Then, 100 μL of a 1% crystal violet staining solution was added and incubated at 37°C for 15-30 minutes. After washing the wells three times with PBS, the crystal violet was dissolved in 95% ethanol, and the absorbance at 595 nm was measured using a microplate reader. The quantification of biofilm (%) was calculated using the formula [(A - Ao)/(Auntreated - Ao)] × 100%, where A represents the absorbance of the current well, Ao represents the absorbance of the blank well, and a untreated represents the absorbance of the well with established biofilm treated with PBS. It is important to note that each step before adding the solution should be air-dried at room temperature for 5 minutes. To avoid disrupting the biofilm during the washing steps, the plate was gently inverted and submerged in a glass container filled with PBS, and slight shaking was applied. The anti-biofilm formation assay is similar to the MIC assays, with the only difference being the inoculum size (50 μL 2×10^7 CFU/mL). The subsequent quantification of the biofilm remains consistent with the aforementioned tests.

In addition, we employed the XTT assay to assess the viability of planktonic bacteria and bacteria in biofilm. Consistent with the aforementioned protocol, after 24 hours of treatment with the antimicrobials (without the removal of planktonic bacteria), 10 μL of the XTT solution was added to each well and the plates were further incubated at 37°C for 2-4 h. Due to the significant impact of uneven bacterial distribution on optical density measurements, quantitative measurements at OD450nm were not employed in this experiment.

Localization of FITC-Labeled Peptides: Midlogarithmic growth-phase *C. perfringens* was washed PBS thrice and diluted on an $OD_{600} = 0.3$ in PBS. 64 μg/mL FITC-peptides and 10 μM PI were

incubated with bacteria in the dark for 60 min at 37°C. Post incubation, 1 mL suspension was harvested (6000 rpm, 3 min), washed with PBS thrice, and then resuspended to 80 μL. 3 μL samples were examined with a confocal laser scanning microscope (LSM 880, Germany) and the data were processed using Zen 2010 software.

TEM Observation of the Morphological Changes of Bacteria: Exponential phase bacteria were treated with 2×MIC of peptides, and fixed with glutaraldehyde (2.5%, w v−1) at 4 °C overnight. For TEM observation, the sample was first dehydrated by a graded series of ethanol (30%, 50%, 70%, 80%) for ~15 min at each step, and dehydrated with a graded series of acetone (90%, 95%) for ~15 min at each step. Subsequently, the samples were dehydrated twice using absolute acetone for 20 min. The specimens were placed in 1:1 mixture of absolute acetone and the final Spurr resin mixture for 1 hour at room temperature, transferred to 1:3 mixture of absolute acetone and the final resin mixture for 3 h, and to the final Spurr resin mixture overnight. Specimens were placed in Eppendorf tubes containing Spurr resin and heated at 70 ℃ for > 9 h. The specimens were sectioned with a LEICA EM UC7 ultratome, stained with uranyl acetate and alkaline lead citrate for 5–10 min each, and observed with a Hitachi Model H-7650 TEM.

In Vivo Toxicity Assessment: 35 healthy female C57BL/6 mice (6 weeks old) were randomly assigned to seven cages (5 per cage). Animal cages were kept individually in ventilated cages (IVCs) at a temperature of 20 to 24ºC, humidity of 50 to 60%, 60 air exchanges per hour and a 12/12-hour light/dark cycle. Mice were acclimated in the facility for two days prior to experimentation. The mice were intraperitoneally injected (I.P.) with 100 μL of PBS or different concertation peptide solutions (10, 20, 30 mg/kg) diluted with PBS. During the five days, the toxicity scores and changes in body weight were monitored. At the end of the experiments (on the 5th day), all animals were sacrificed with subsequent cervical dislocation, organ harvesting, colon length measuring. The liver, kidney, lung, and spleen were weighed and fixed with 4% paraformaldehyde for H&E staining. Blood samples were acquired from the orbital vein of mice. The blood biochemical factors including ALT, ALP, TBIL, BUN, and CREA were measured with an automatic biochemical analyzer to reflect hepatic and renal damage.

Biodistribution of HAMPs: Biodistribution of FITC-peptides in mice were measured by in vivo imaging system. For appropriate dose and time, a pre-experiment was conducted. 6 healthy female C57BL/6 mice (6–8 weeks old) were randomized into three experimental groups, namely control group, FITC-F6P1 group, and FITC-F6P6 group. At 2h or 10h after I.P. 20mg/kg FITC-HAMPs, mice and organs was imaged by in vivo imaging system. From 2 to 10 hours, HAMPs were from systemic to peritoneal distribution. Meanwhile, the FITC-HAMPs in the liver and kidney were depleted, and the residence time in the intestine was longer (fig. S23). The above results are consistent with the absorption route of intraperitoneal drug delivery, so we adjusted the experiment as follows. 9 healthy female C57BL/6 mice (6–8 weeks old) were randomized into three experimental groups. At 12h after 5mg/kg I.P. HAMPs, mice were euthanized. Major organs including gut, heart, liver, spleen, kidney, lung, and intestine were isolated. Mice and organs were imaged by *in vivo* imaging system.

***Synthesis of gut-targeted nanoparticles*:** The logarithmic-growing *Microcystis* was cultured and placed in a light incubator at a slow speed (70 rotations/min) for 12 hours, and then 500 mL of cultured medium were centrifuged for collecting gas vesicles (GVs). The *Salmonella typhi* was cultured with medium containing lysozyme (0.1 mg/mL) at 37°C for 1.5 hour, and centrifuged for collceting the cytoderm particles (CPs). The Digalactosyldiacylglycerol (DGDG), Monogalactosyldiacylglycerol (MGDG), phosphatidic acid (PA), biotin, and CPs were dissolved with dichloromethane (DCM) in glass tubes and mixed them in a ratio of 2:1:5:1:1. After that, the mixed solution was transferred to a pear-shaped flask with biotin loading lipid thin film (BLTF) prepared from the raw lipids and CPs, and the flask was sonicated with repeated pipetting. Collection of the BLTFs: The mixed solution was centrifuged after decomposition for 3 hours at 4°C and 9000 rpm. Finally, the GVs, AMPs, and BLTFs were mixed in BTS buffer and sonicated with repeated pipetting for synthesizing engineering particle vaccine (EPV) for 12h at 4°C. Afterward, the EPVs were collected for subsequently experiments.

In Vivo Activity Assessment

Experiment 1: To disrupt colonization resistance and enable infection with *C. perfringens*, 25 healthy female C57BL/6 mice (6 weeks old) were provided with an antibiotic cocktail (0.4 mg/mL

Kanamycin, 0.035 mg/ mL Gentamicin, 850U/ mL Colistin, 0.215 mg/ mL Metronidazole, 0.045 mg/ mL Vancomycin) added in their drinking water (from day -6 to day -3). Mice were then switched to regular drinking water. On day −1, mice were I.P. with clindamycin (30 mg/kg). On day 0, mice were challenged with *C. perfringens* ATCC 13124 inoculation (10^6 spores) cultured in RCM (termed R group) or MHB (termed R group) by oral gavage. At 24 hours post-infection, the R and M groups were randomly divided into three subgroups and received intraperitoneal injections of 100 μL of either 20 mg/kg F6P1, 20 mg/kg F6P6, or PBS. The PBS-treated group served as the control group. The six groups were named as follows: R (n = 5), R+F6P1 (n = 5), R+F6P6 (n = 5), M (n = 5), M+F6P1 (n = 5), and M+F6P6 (n = 5). To monitor *C. perfringens* colonization, fecal samples were collected, weighed, and diluted with 0.1% peptone water. Colonic tissues were weighed and homogenized in 0.1% peptone solution. The CFUs were quantified by plating serial dilutions on TSCA plates supplemented with 0.05% D-cycloserine and incubated anaerobically at 37°C.

Experiment 2: 25 healthy female C57BL/6 mice (6-8 weeks old) were provided with an antibiotic cocktail added in their drinking water (from day -6 to day -3). Mice were then switched to regular drinking water. On day −1, mice were I.P. with clindamycin (30 mg/kg). On day 0, mice were challenged with *C. perfringens* ATCC 13124 inoculation (10^6 spores) cultured in RCM by oral gavage and mice were randomly assigned to five treatment groups: 20 mg/kg F6P1 (n = 5), 20 mg/kg F6P6 (n = 5), 3×10^9 EPV@F6P1 (n = 5), 3×10^9 EPV@F6P6 (n = 5), and 1×PBS (n = 5). Beginning at 24 and 72 hours after *C. perfringens* challenge, mice were I.P. HAMPs, EPV@HAMPs, or PBS. To monitor *C. perfringens* colonization, fecal samples were exclusively collected, weighed and diluted with 0.1% peptone water. Colonic tissues were weighed and homogenized in 0.1% peptone water. CFUs were quantified using TSCA plates supplemented with 0.05% D-cycloserine at 37°C under anaerobic conditions. Five groups of mice were sacrificed at 144 hours post infection. Blood samples and colon samples were obtained for evaluation of cytokine levels (TNF-α, IL-6, and IL-1β) and CPA levels. The tissue samples were fixed in 4% paraformaldehyde for H&E staining and immunofluorescence staining.

Experiment 3: On day −1, mice were I.P. with clindamycin (100 mg/kg). On day 0, mice were infected with *C. perfringens* ATCC 13124 inoculation (10^5 spores) by oral gavage. On day 1, the mice were given an I.P. injection of 100 mg/kg clindamycin. Beginning at 2 hours, 1 day, 3 days and 5 days after antibiotic treatment, the mice were given an I.P. injection of 20 mg/kg F6P6, 3×10^9

EPV@F6P6, or PBS vehicle. Fecal samples were collected and weighed, and CFU/g were enumerated in feces. Three groups of mice were sacrificed at 10 days post infection. Colonic content and tissue were collected and enumerated. The colonic tissue was fixed in 4% paraformaldehyde for H&E staining.

***The effects of HAMPs and EPV@HAMPs on healthy mice*:** 25 female C57BL/6 mice (6-8 weeks old) were divided into five groups after a three-day acclimation period. Over the course of three consecutive days (0, 1, 2 day), each group received a 100 μL intraperitoneal injection of either PBS, 20 mg/kg F6P1, 20 mg/kg F6P6, 3×10^9 EPV@F6P1, or 3×10^9 EPV@F6P6. On the fifth day, blood, organs, and colon tissues were collected from the mice. Serum was separated for ELISA analysis of inflammatory cytokine levels. Organs such as liver, kidney, spleen, and lungs were weighed, and a portion of the colon was fixed in 4% paraformaldehyde for 24 hours to enable immunofluorescence analysis.

Microbiome Analysis: Feces of each mouse were collected separately and stored at −80 °C. Microbial DNA was obtained from fecal homogenates using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, USA). To ensure that no contamination had occurred, the concentration and purity of the DNA samples were measured with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, USA) and examined by electrophoresis using 1% agarose gels. The V4 gene regions of the bacterial 16S rRNA gene were amplified with the primer pairs 515 F (5'-GTGCCAGCMGCCGCGG-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') using the Illumina MiSeq platform at Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China). Thereinto, operational taxonomic units (OTUs) were clustered in UPARSE (version 7.1). Then, the taxonomy of the OTU representative sequence was evaluated by using RDP Classifier. All data were finally analyzed on the Majorbio Biocloud platform and R. The sequence data were then deposited in the Sequence Read Archive under the accession number PRJNA857195.

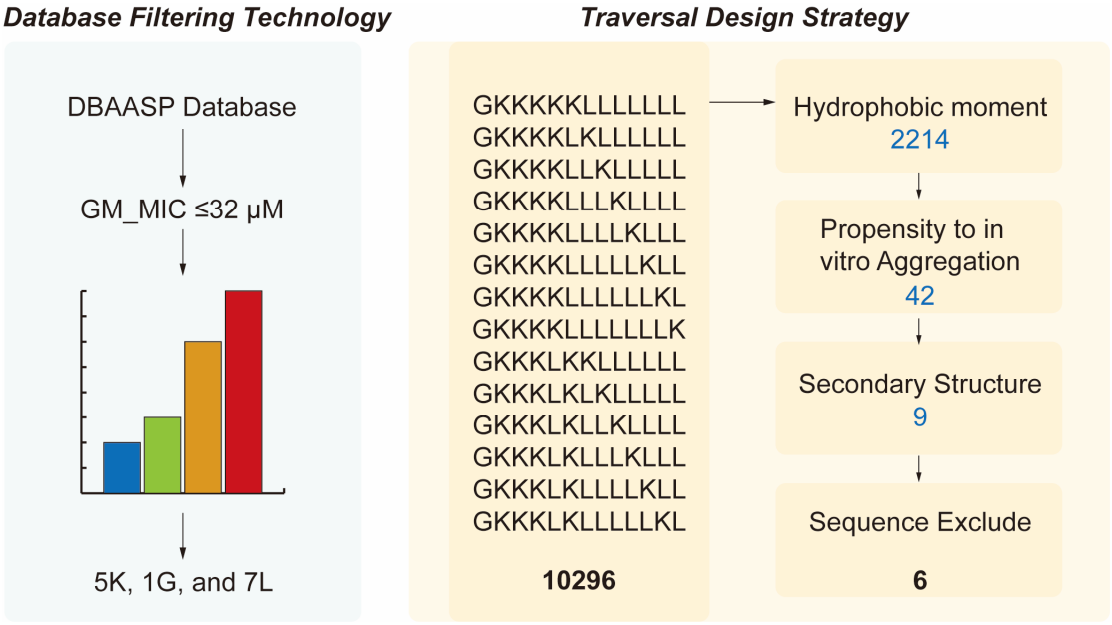

fig. S1 **Sequence screening process of traversal design.** GM_MIC: Geometric mean of MIC.

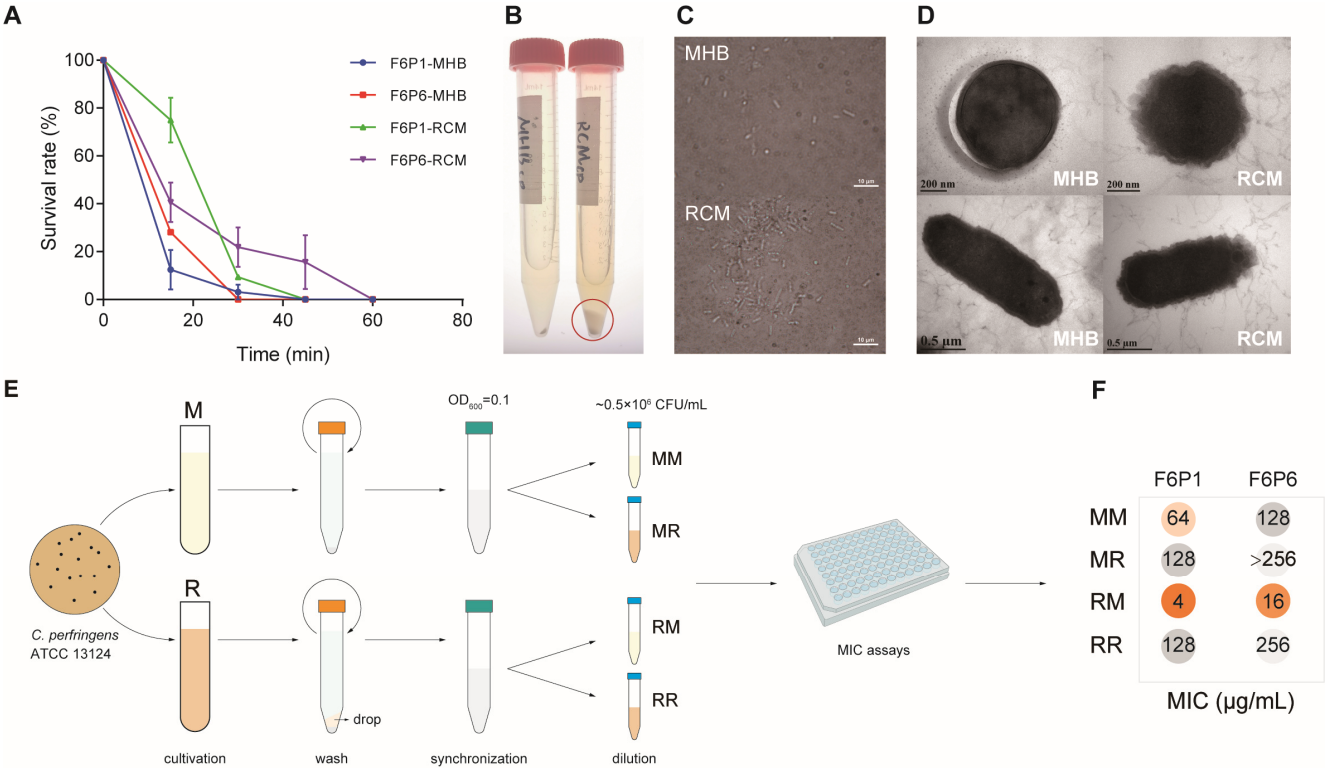

fig. S2 **HAMPs against *C. perfringens* under different conditions. (A)** Bactericidal killing kinetics assay. Data are presented as the mean ± SEM (n = 3, biologically independent samples). **(B)** Differences in centrifugation of *C. perfringens* cultured in MHB and RCM. **(C)** Microscopy imaging of *C. perfringens* cultured in MHB and RCM. India ink staining was used to show the capsule of *C. perfringens*. **(D)** TEM imaging of *C. perfringens* cultured in MHB or RCM. Scale bars, 0.2 or 0.5 μm. (**E**) Experimental design of MIC determination. (**F**) MIC values of F6P1 and F6P6 on *C. perfringens* in different culture conditions.

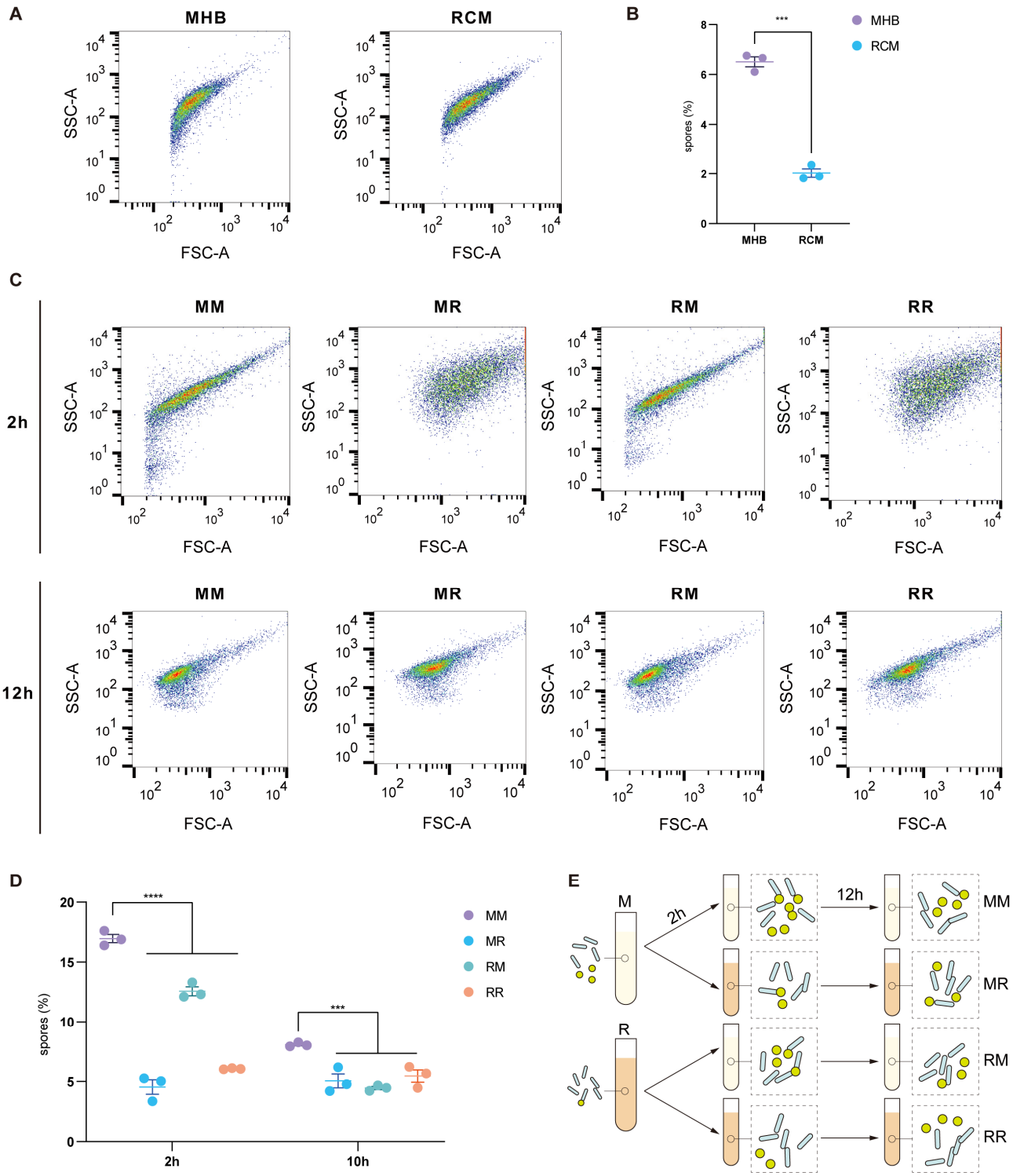

fig. S3 **Flow cytometric analysis of the growth model of *C. perfringens* under different culture conditions.** (**A** and **B**) Flow cytometric analysis (A) and spore ratio statistics (B) of *C. perfringens* overnight cultured in MHB and RCM. (**C** and **D**) Flow cytometric analysis (C) and spore ratio statistics (D) of *C. perfringens* cultured for 2 hours and 12 hours under four different culture conditions. (E) Growth pattern of *C. perfringens* in four conditions (a total of 4 conditions, namely MM, MR, RR, RM).

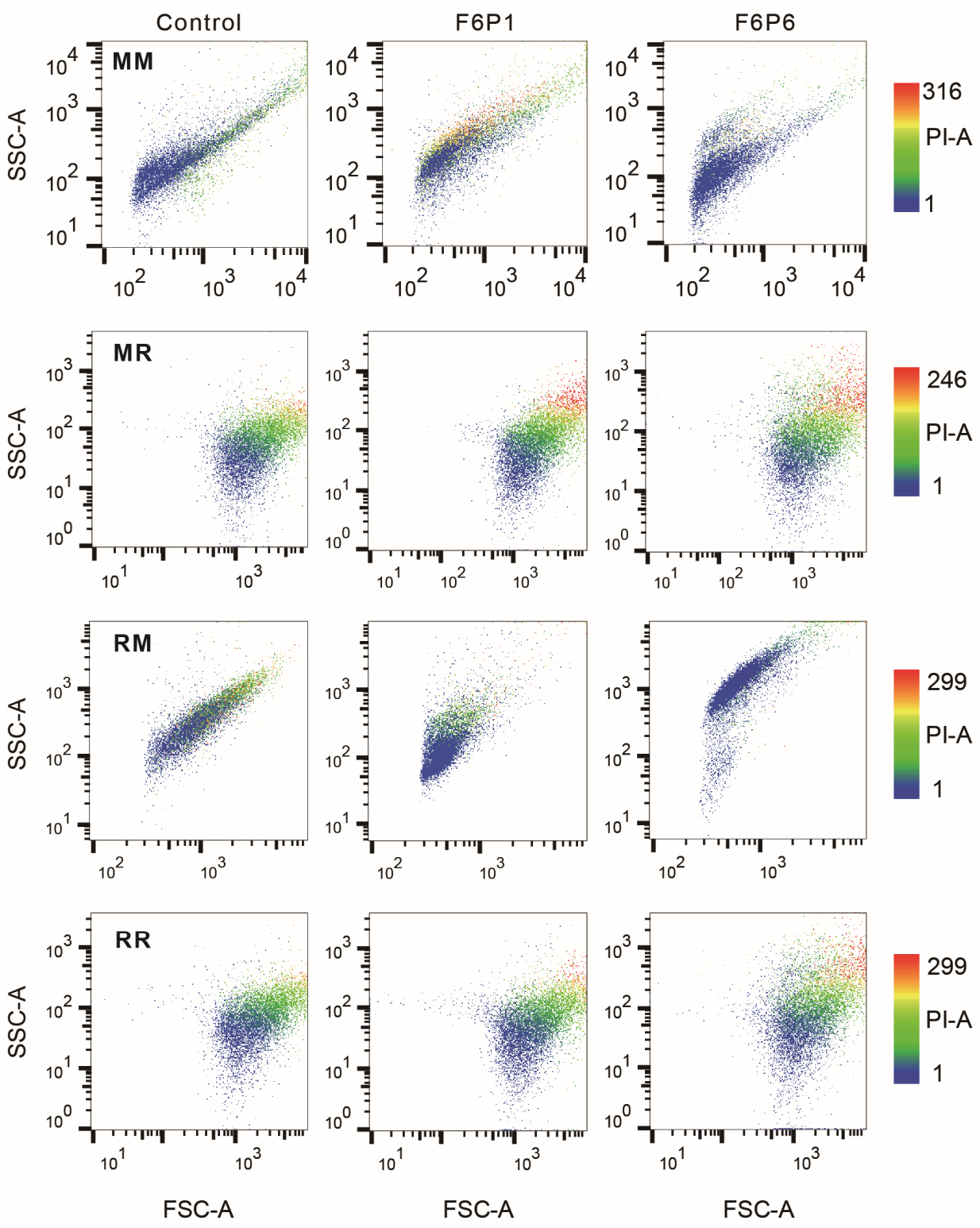

fig. S4 **Antibacterial tendency assay of HAMPs against *C. perfringens* under four conditions.** Inoculate RCM and MHB with 10^7 CFU/mL *C. perfringens* cultured in RCM or MHB. After two hours of cultivation, 32 μg/mL HAMPs treated them for 1 hour, and HAMPs preferentially destroyed rod-shaped *C. perfringens*.

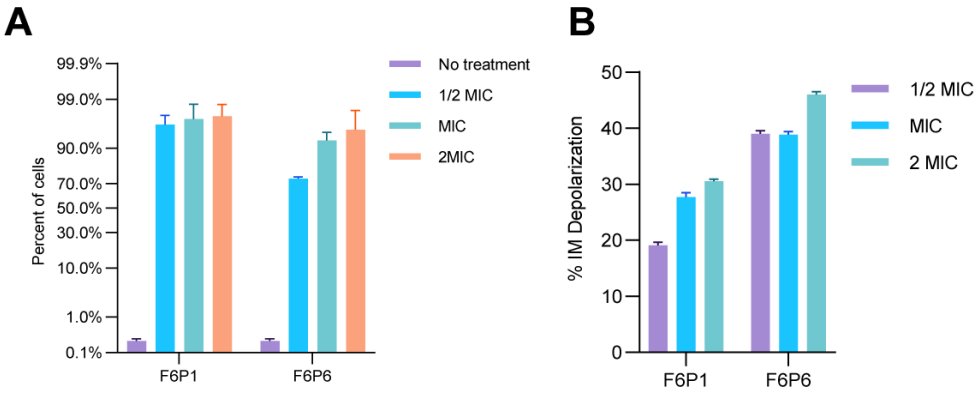

fig. S5 **PI and DiSC$_3$-5 assays.** (**A**) PI-positive cells detected via flow cytometry for *C. perfringens* treated with F6P1 and F6P6. Values are the mean ± SEM (n=3 biological replicates). (**B**) Statistical plot of DiSC$_3$-5 assays. Values are the mean ± SEM (n=4 biological replicates).

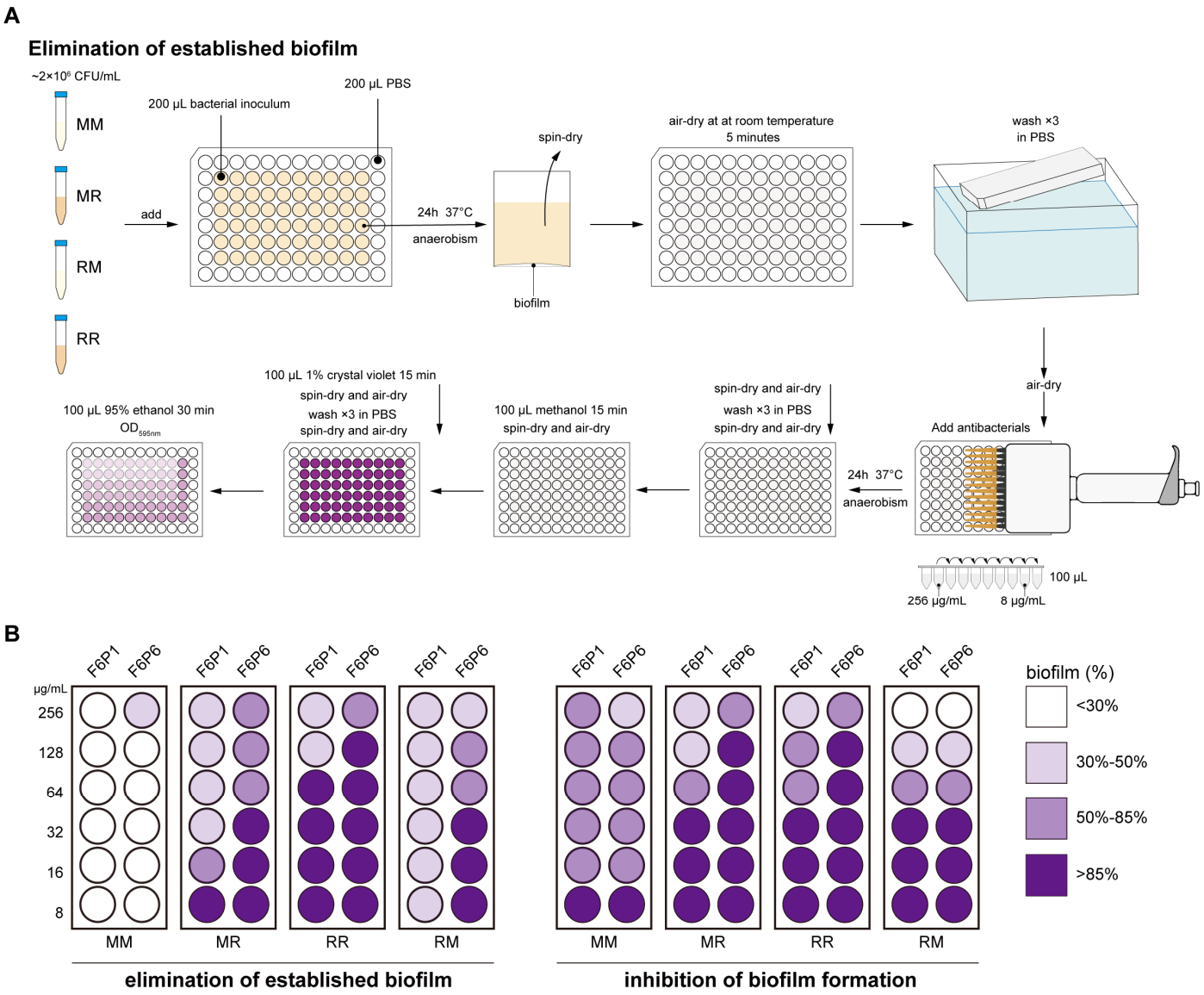

fig. S6 **Anti-biofilm activity of HAMPs.** (A) Experimental procedure for clearing established biofilms by HAMPs. The anti-biofilm formation assay involves rearranging the dosing step from the elimination of established biofilm assays to the initial step. (B) Cleaning of biofilm (left) and inhibition of biofilm formation (right) by HAMPs under four conditions (n=3 biological replicates).

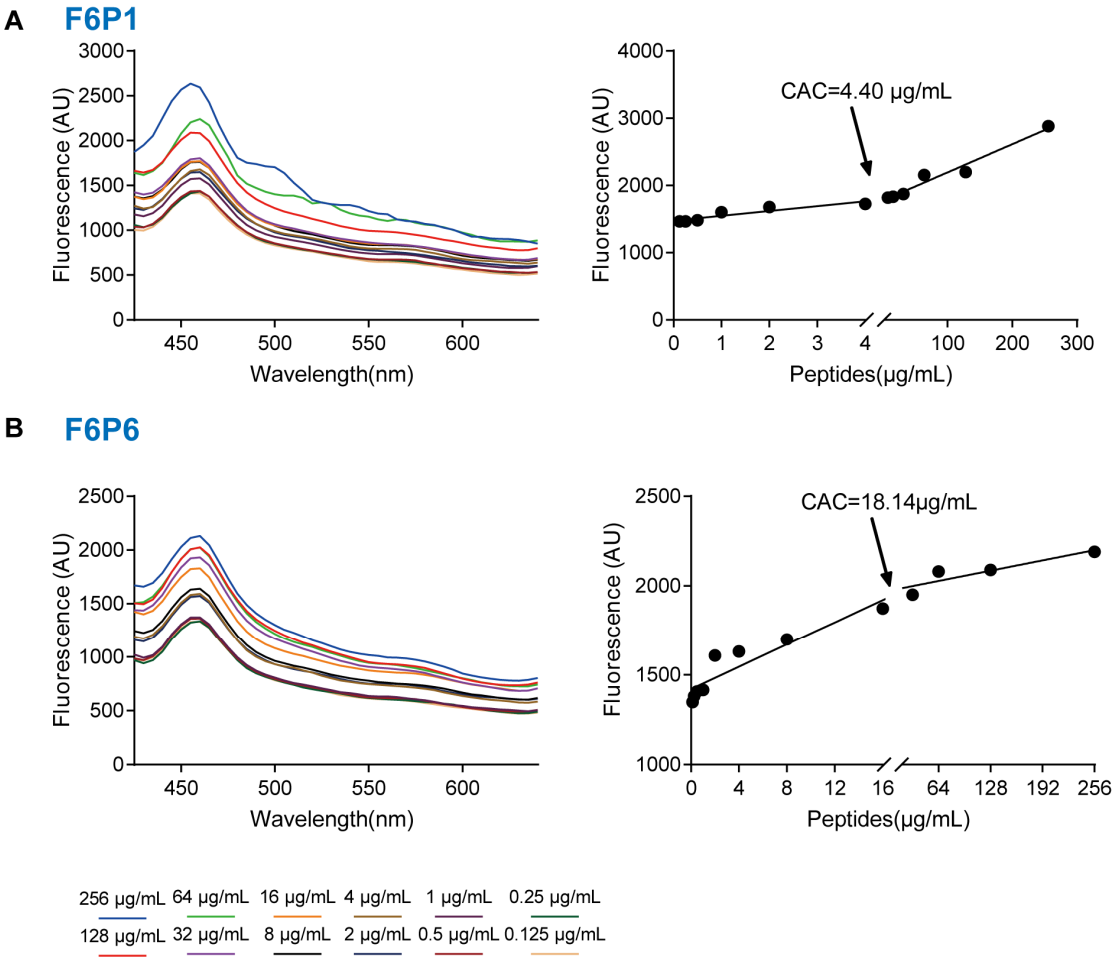

fig. S7 **Self-assembling ability and the critical aggregation concentration of HAMPs in water environment.** All ANS assays performed with an average of three technical replicates.

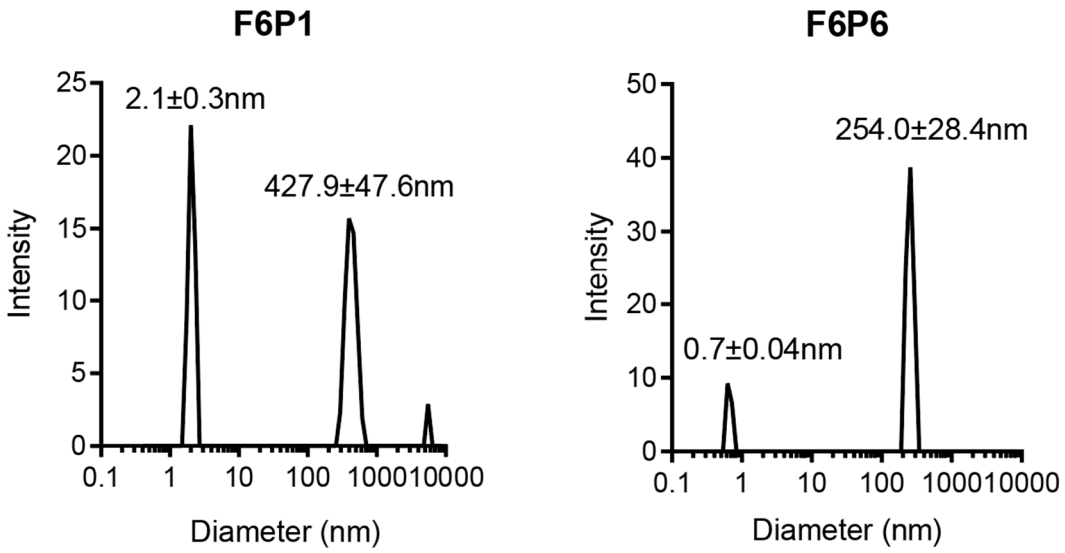

fig. S8 **The diameter of HAMPs.**

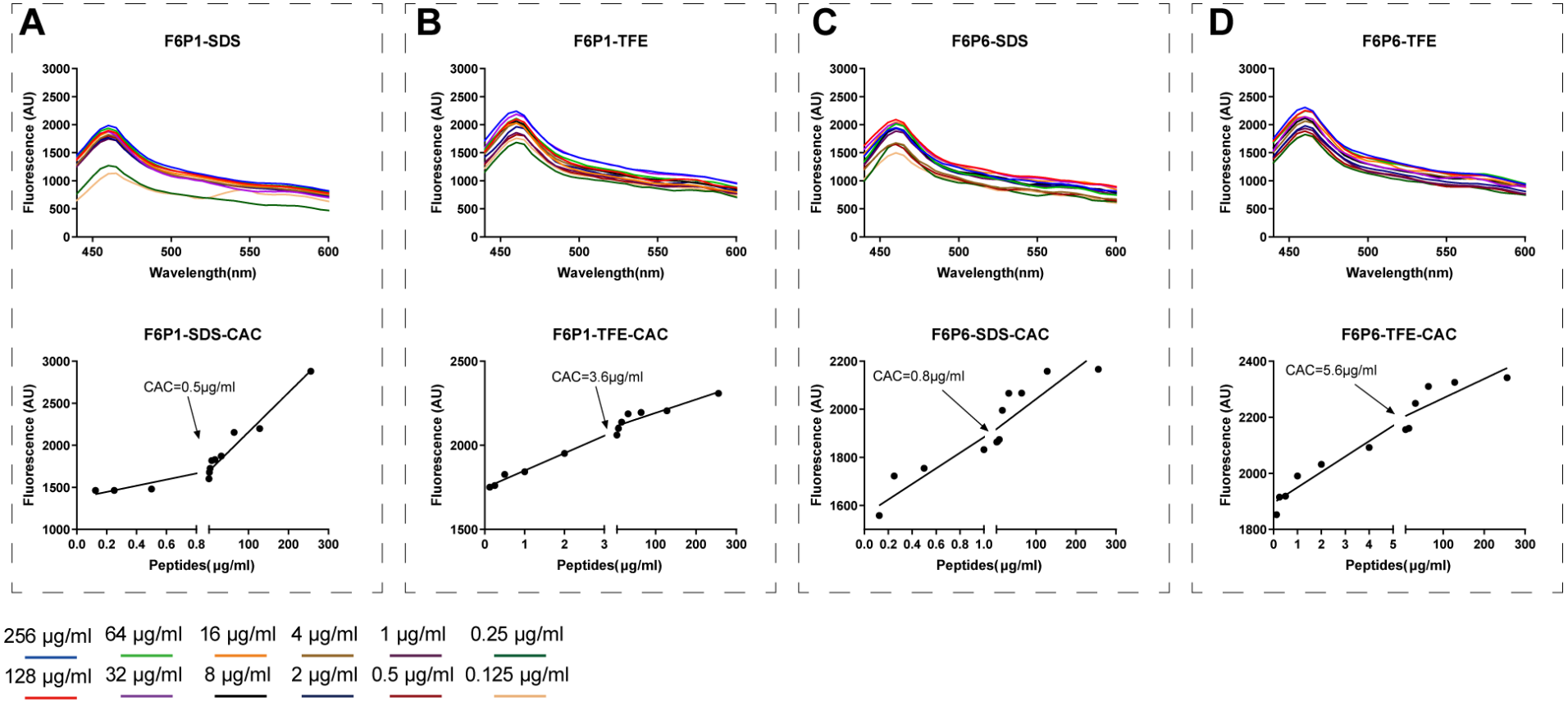

fig. S9 **Self-assembling ability and the critical aggregation concentration of HAMPs in SDS and TFE.** All ANS assays performed with an average of three technical replicates.

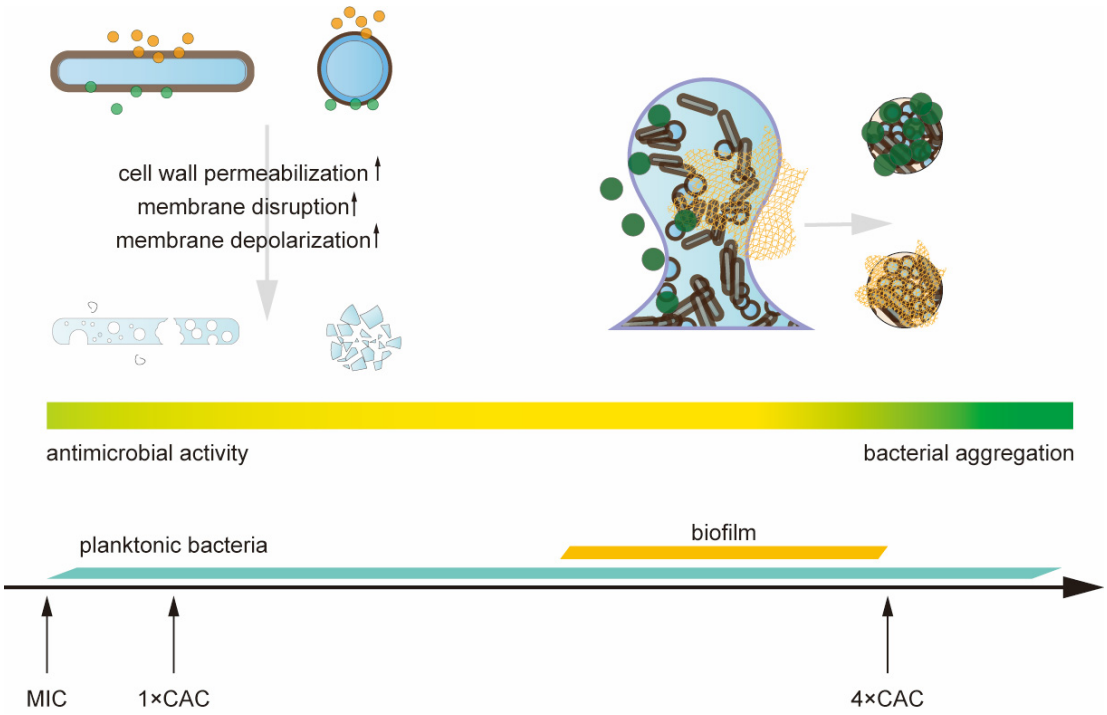

fig. S10 **The model of the relationship of antimicrobial activity and bacterial aggregation.**

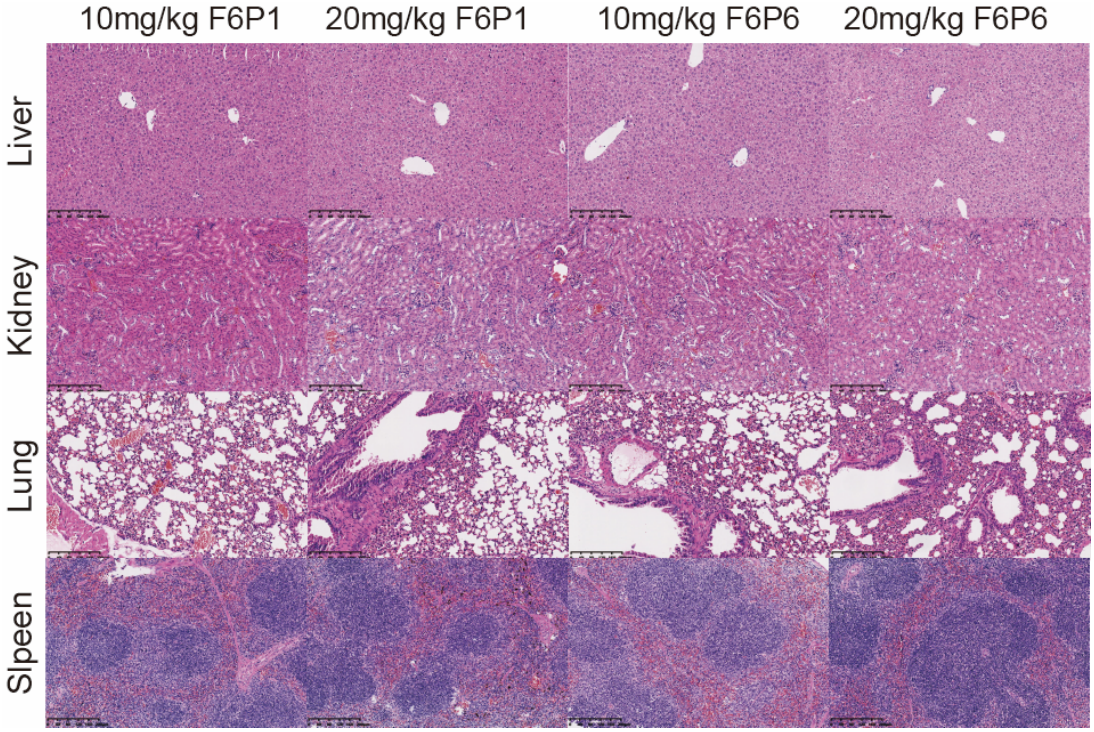

fig. S11 **Histopathological morphology analysis in mice following administration of 10 or 20 mg/kg HAMPs for 5 days.** Scale bar, 200 μm.

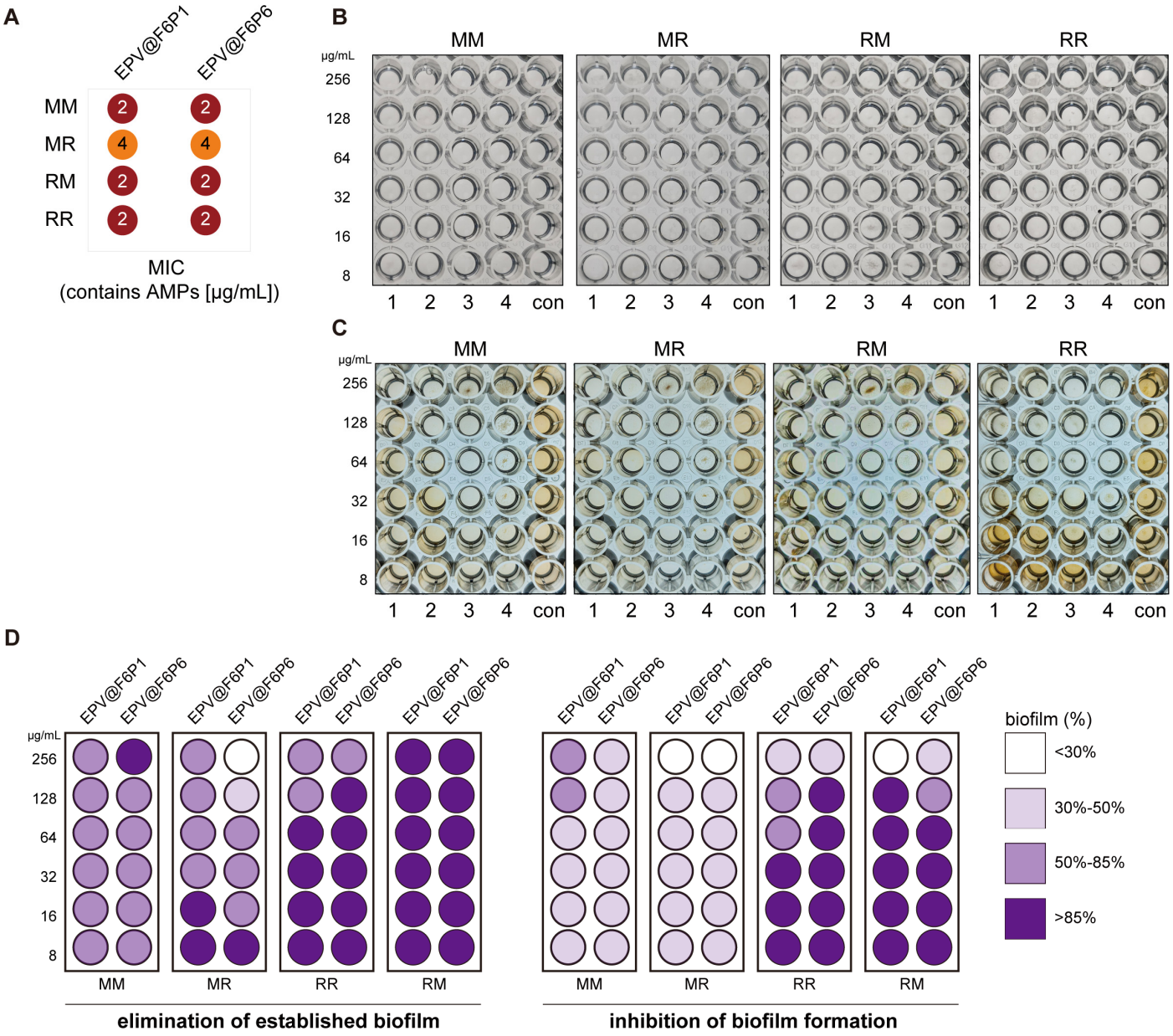

fig. S12 **EPV@HAMPs against *C. perfringens* under four conditions.** (A) MIC assays. (B) Bacterial agglutination was observed in the antibiofilm assays. (C) XTT assays for visualization of biofilm viability. The first to fourth columns represent F6P1, F6P6, EPV@F6P1, and EPV@F6P6, respectively. Bacterial aggregation can be observed in the wells. (D) Cleaning of biofilm (left) and inhibition of biofilm formation (right) by EPV@HAMPs under four conditions (n=3 biological replicates).

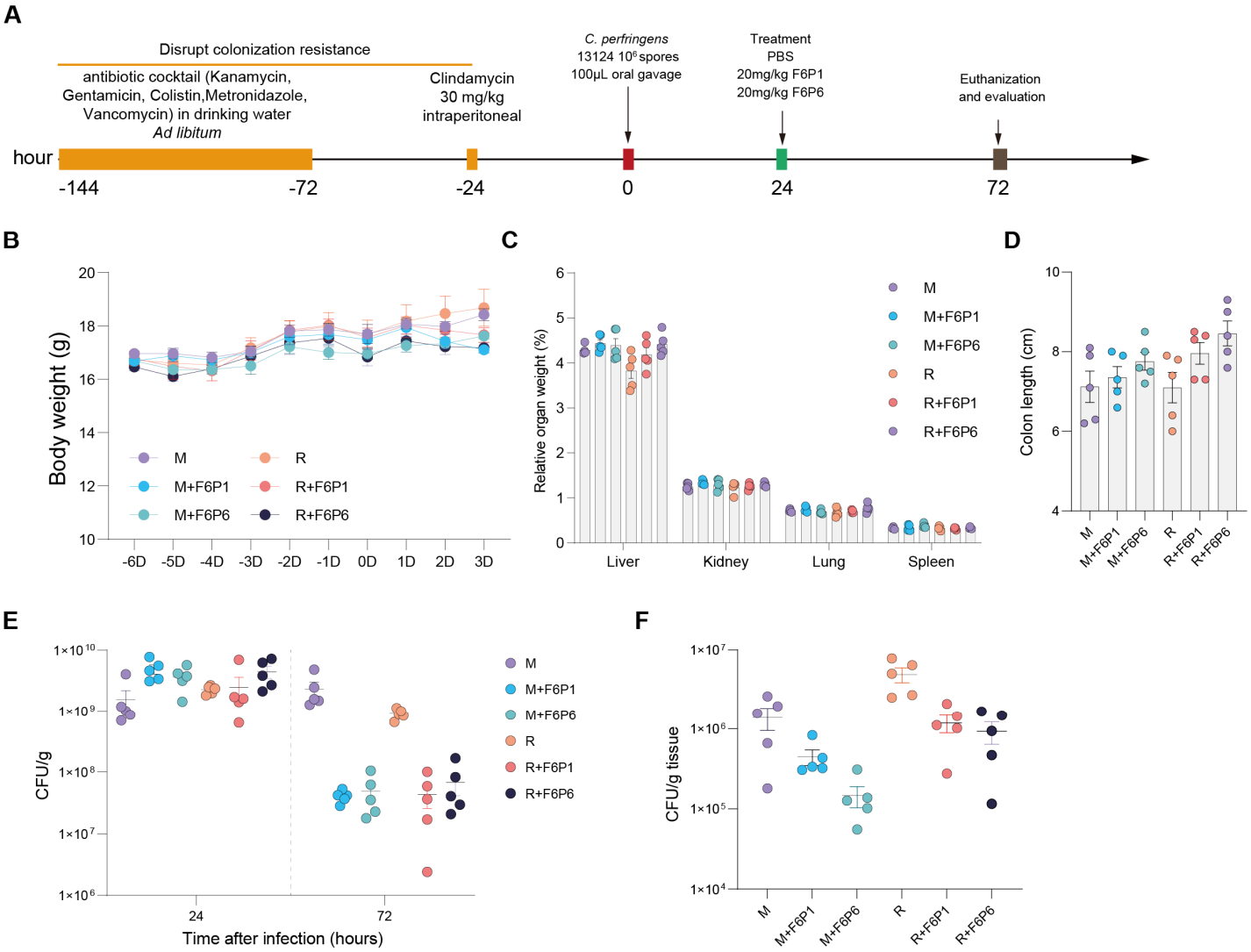

fig. S13 ***In vivo*** **activity test of HMAPs against *C. perfringens* cultured in RCM or MHB.** (**A**) Experimental design for CPI and treatment. (**B** to **D**) Body weight, relative organ weight, and colon length of mice. The initial inoculum of bacteria was ~10^6 CFU. Data are presented as the mean ± SEM; n = 5 mice per group. (**E** and **F**) Bacterial load of *C. perfringens* in feces (**E**) and colon tissues (**F**) of infected mice.

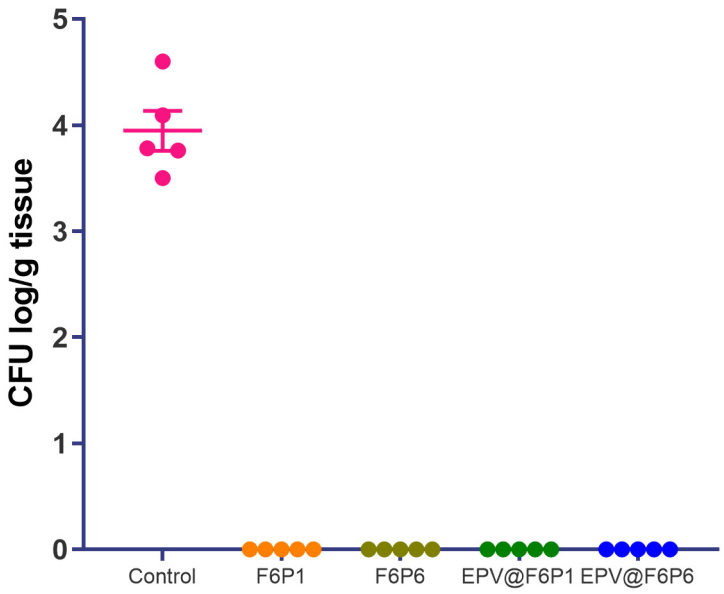

fig. S14 ***C. perfringens* load in liver.** Each sample for which *C. perfringens* was not detected, which was measured in triplicate. Values are the mean ± SEM, n = 5.

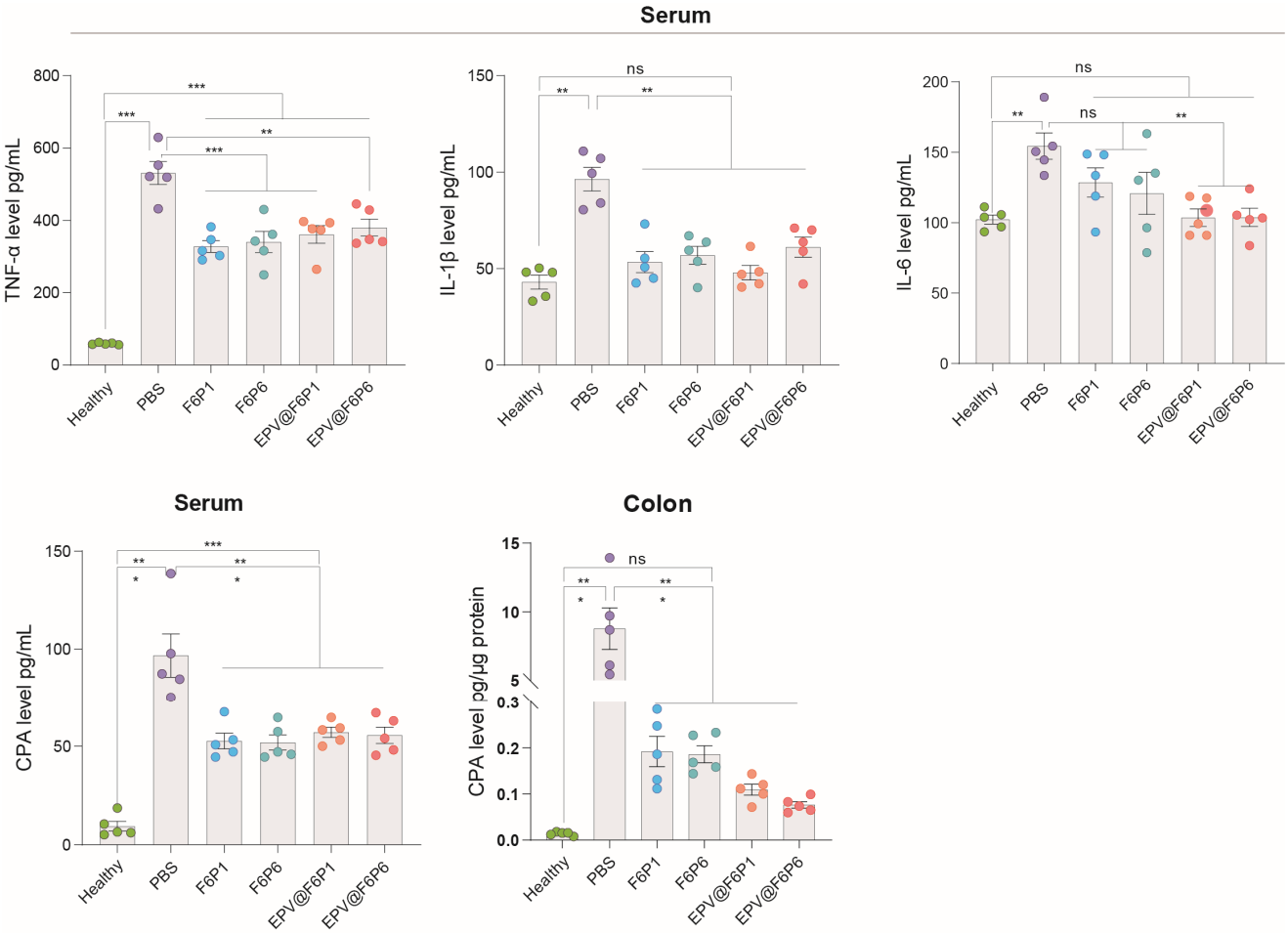

fig. S15 **The levels of TNF-α, IL-1β, IL-6, and CPA toxin of serum or colon in healthy and CPI mice treated with PBS and HAMPs and EPV@HAMPs.** Data are presented as the mean ± SEM; n = 5 mice per group. The differences between the groups were determined using one-way ANOVA followed by Tukey's post hoc analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

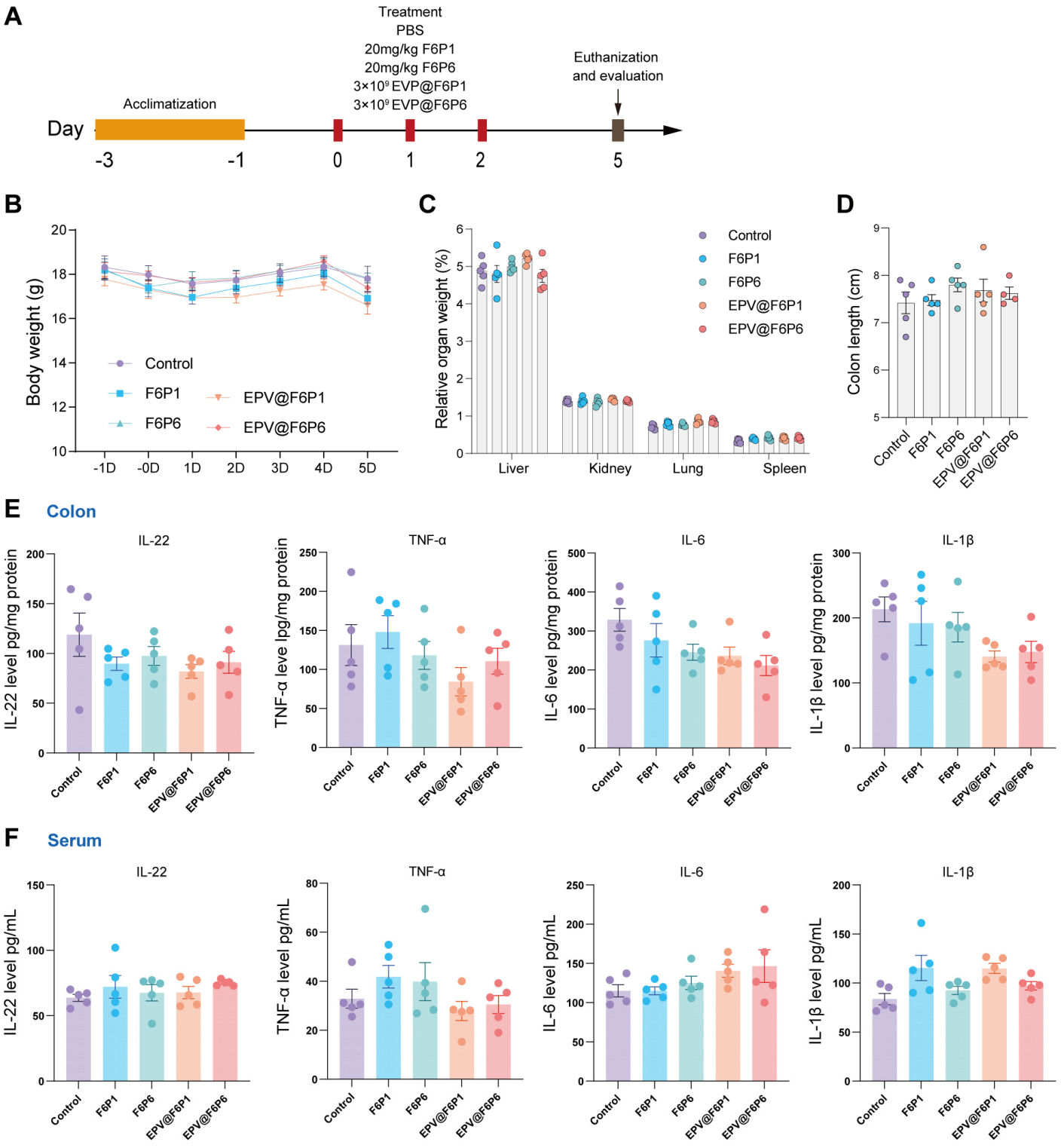

fig. S16 **HAMPs and EPV@HAMPs have no effect on colon length and serum inflammatory cytokine levels in healthy mice.** (**A**) Experimental design. (**B** to **D**) Body weight, colon length, and relative organ weight of mice. Data are presented as the mean ± SEM; n = 5 mice per group. (**E** to **F**) Effects of HAMPs and EPV@HAMPs on the levels of inflammatory cytokine in mice colon (**E**) and serum (**F**).

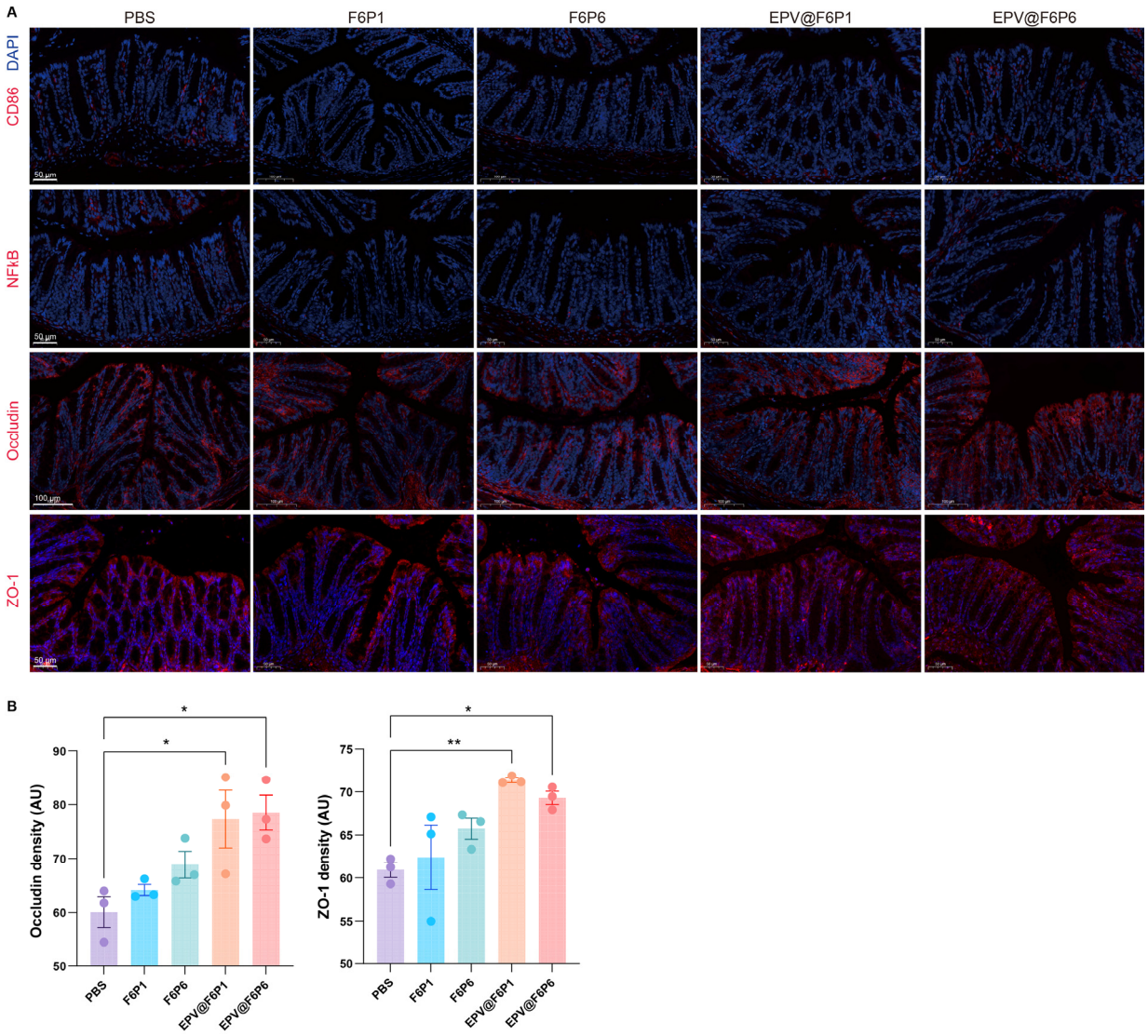

fig. S17 **Immunofluorescence analysis on the colon tissues.** (**A**) Immunofluorescence for CD86, NFκB, Occcludin, and ZO-1 was performed in frozen colon sections from healthy mice treated by PBS, HAMPs and EPV@HAMPs for five days. The PBS treatment group was used as control. Scale bar, 50 or 100 μm. (**B**) The semi-quantitative analysis of immunofluorescence staining of Occludin and ZO-1. Data are presented as the mean ± SEM; n = 3. The differences between the groups were determined using one-way ANOVA followed by Tukey's post hoc analysis. *$p < 0.05$, **$p < 0.01$.

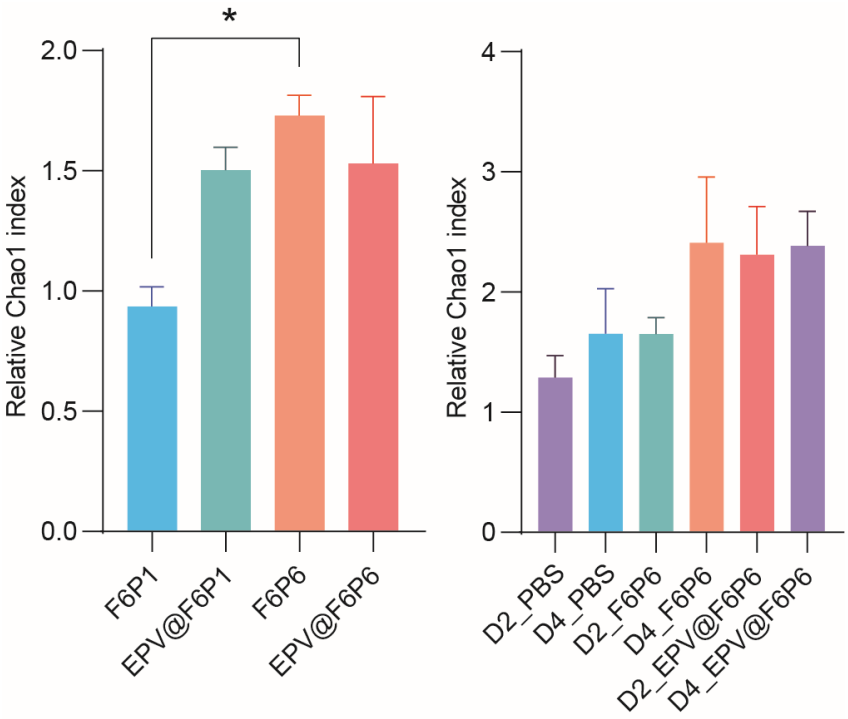

fig. S18 **Relative Chao1 index of observed operational taxonomic units showed the α-diversity of the microbial community.** Relative Chao1 index was adjusted by D1's fecal bacteria chao1 index.

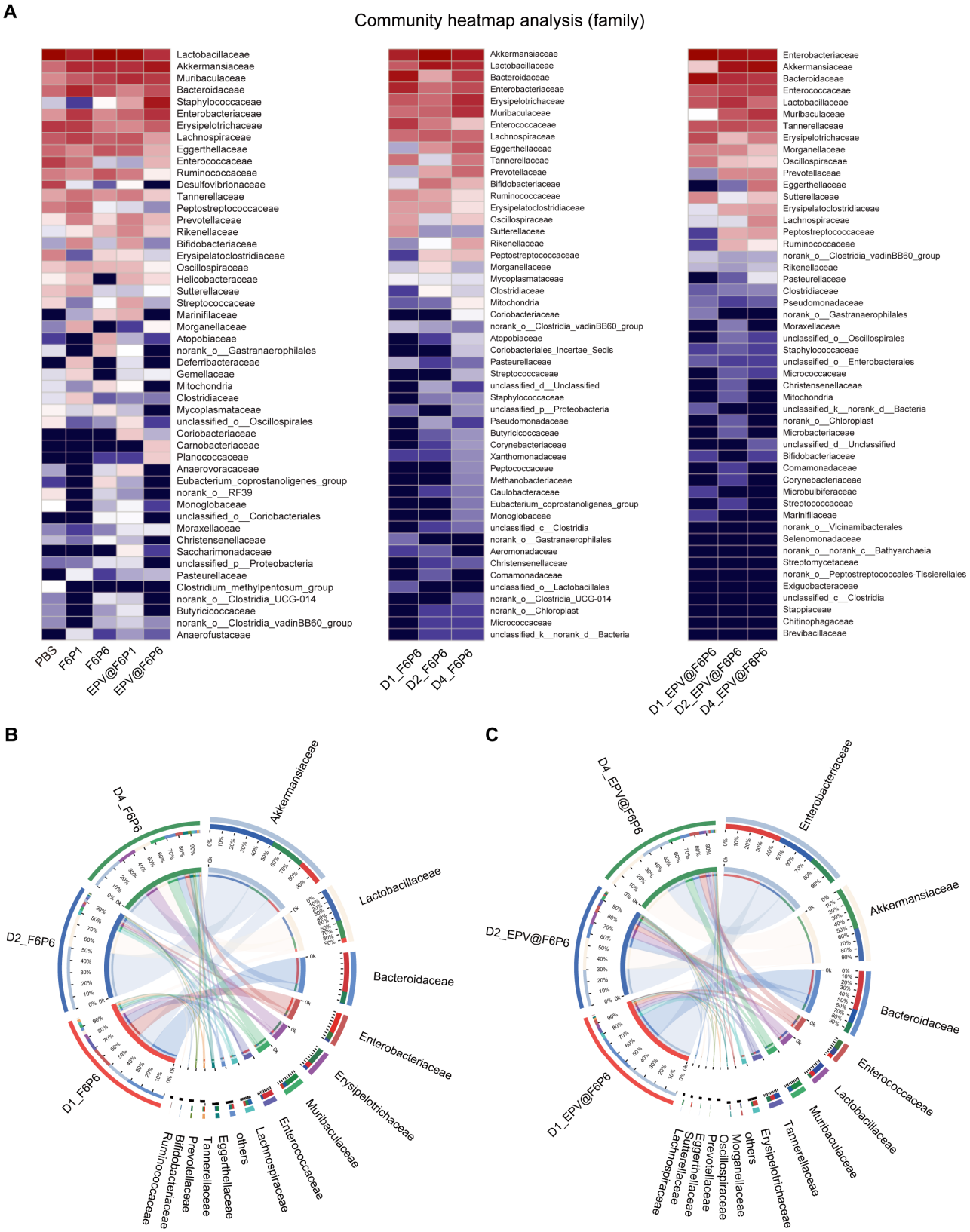

fig. S19 **Heatmap and Circos plots.** (**A**) Heatmap showing the relative abundance of microbial compositional profiling at the family level. (**B** and **C**) Circos plots of the corresponding abundance relationship between samples and bacterial communities at the family level.

fig. S20 **16S rRNA sequencing analysis of microbiota regulated by F6P6 and EPV@F6P6.** (**A**) Random forest analysis on family level. Each point represents each mouse. (**B**) Venn diagram of F6P6 vs Control and EPV@F6P6 vs Control and their important bacteria. (**C** and **D**) Related networks of F6P6 and EPV@F6P6. In analysis of the figure, the "PBS" group represents all fecal and colonic content samples (n=20) with treatment of PBS; the "F6P6" group represents all fecal and colonic content samples (n=15) with treatment of F6P6; the "EPV@F6P6" group represents all fecal and colonic content samples (n=15) with treatment of EPV@F6P6.

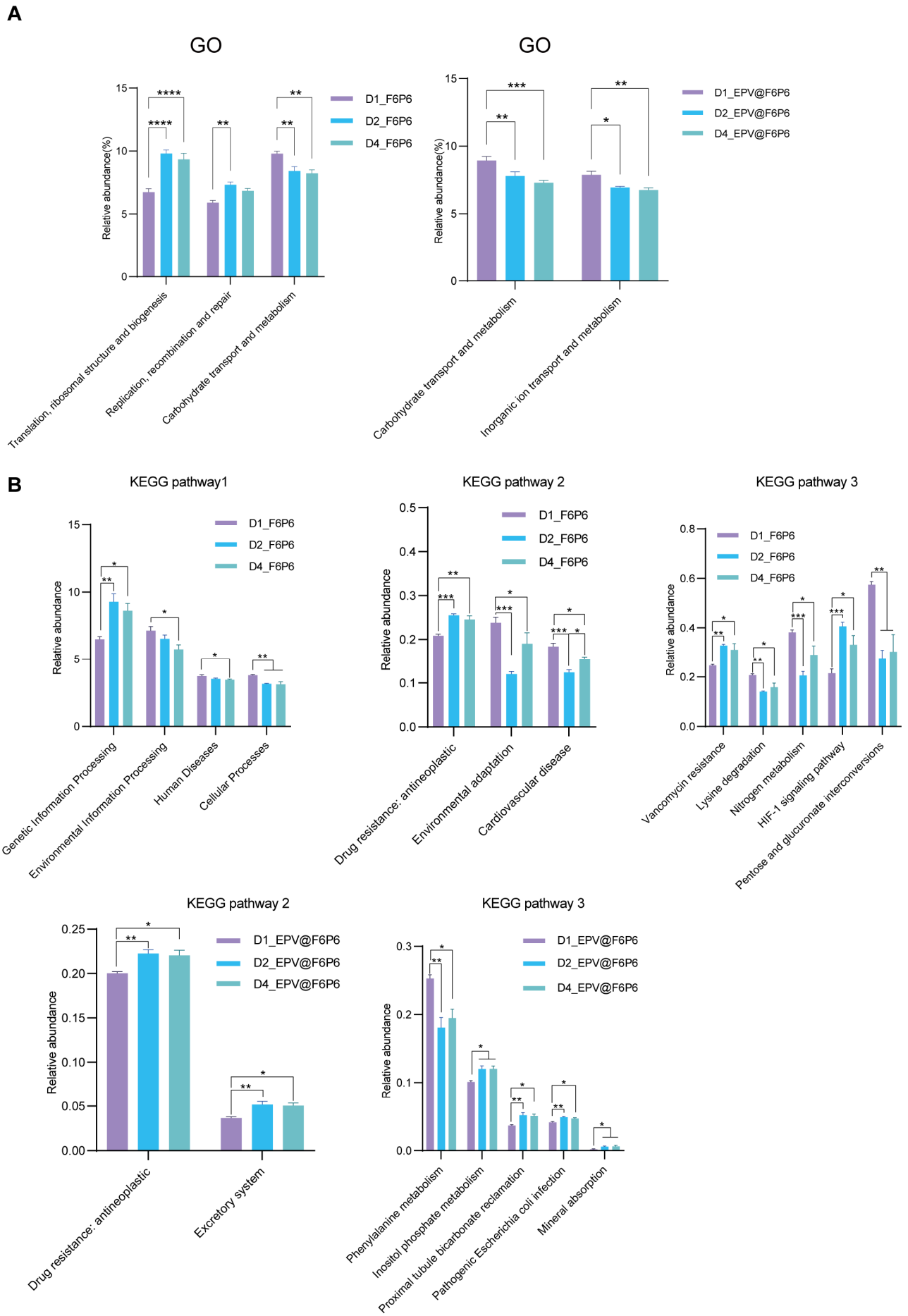

fig. S21 **The GO and KEGG analysis of microbiota functions for F6P6 and EPV@F6P6.** D1, D2, and D4 represent the fecal samples collected after 0, 1, and 2 administrations of F6P6 or EPV@F6P6, respectively. The Kruskal–Wallis test was applied for statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

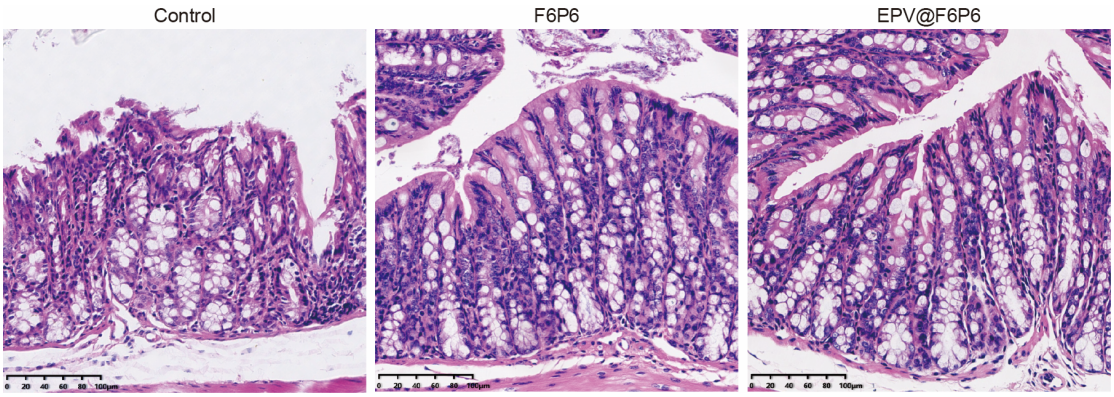

fig. S22 **Histopathological H&E staining of colon tissues of persistent CPI.** Scale bar, 100 μm.

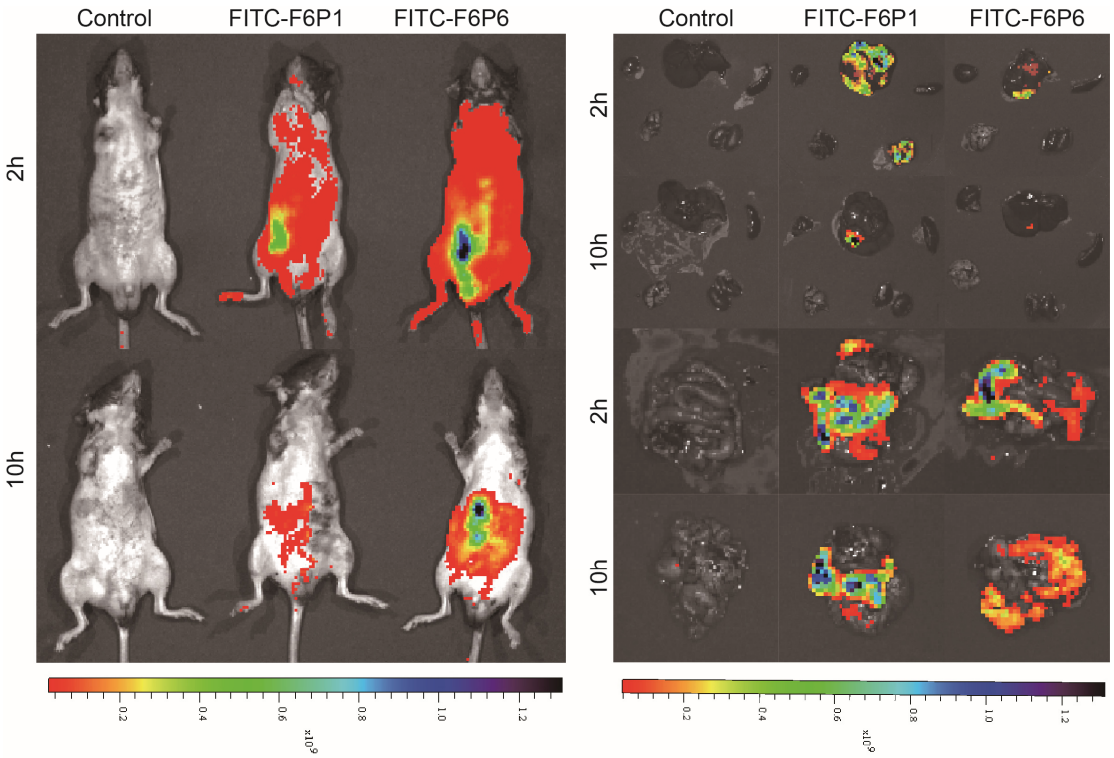

fig. S23 **Mice and organs were imaged by *in vivo* imaging system at 2h or 10h after I.P. 20mg/kg FITC-HAMPs.** The intestine with strong signals compared with other organs.

table S1 Performance of machine learning models

Order	model	roc_auc_ovo_macro	accuracy	f1_macro	f1_micro	precision_macro	precision_micro	recall_macro	recall_micro
1	WeightedEnsemble_L3	0.912	0.768	0.764	0.768	0.764	0.768	0.765	0.768
2	LightGBMXT_BAG_L2	0.912	0.768	0.764	0.768	0.764	0.768	0.765	0.768
3	XGBoost_BAG_L2	0.911	0.780	0.776	0.780	0.776	0.780	0.777	0.780
4	LightGBM_BAG_L2	0.909	0.773	0.768	0.773	0.768	0.773	0.769	0.773
5	CatBoost_BAG_L2	0.907	0.761	0.757	0.761	0.757	0.761	0.758	0.761
6	RandomForestEntr_BAG_L2	0.905	0.749	0.744	0.749	0.744	0.749	0.745	0.749
7	ExtraTreesEntr_BAG_L2	0.905	0.768	0.764	0.768	0.764	0.768	0.764	0.768
8	RandomForestGini_BAG_L2	0.903	0.763	0.759	0.763	0.760	0.763	0.760	0.763
9	NeuralNetFastAI_BAG_L2	0.903	0.761	0.756	0.761	0.757	0.761	0.757	0.761
10	ExtraTreesGini_BAG_L2	0.903	0.756	0.752	0.756	0.752	0.756	0.753	0.756
11	NeuralNetTorch_BAG_L2	0.902	0.756	0.751	0.756	0.752	0.756	0.752	0.756
12	LightGBMLarge_BAG_L2	0.902	0.768	0.763	0.768	0.763	0.768	0.764	0.768
13	ExtraTreesEntr_BAG_L1	0.895	0.742	0.736	0.742	0.738	0.742	0.738	0.742
14	LightGBMXT_BAG_L1	0.889	0.731	0.724	0.731	0.726	0.731	0.726	0.731
15	WeightedEnsemble_L2	0.889	0.731	0.724	0.731	0.726	0.731	0.726	0.731
16	ExtraTreesGini_BAG_L1	0.887	0.724	0.716	0.724	0.719	0.724	0.718	0.724
17	LightGBM_BAG_L1	0.886	0.726	0.720	0.726	0.721	0.726	0.721	0.726
18	RandomForestEntr_BAG_L1	0.882	0.717	0.708	0.717	0.711	0.717	0.711	0.717
19	XGBoost_BAG_L1	0.881	0.714	0.708	0.714	0.709	0.714	0.710	0.714
20	CatBoost_BAG_L1	0.881	0.731	0.725	0.731	0.727	0.731	0.726	0.731
21	RandomForestGini_BAG_L1	0.880	0.707	0.699	0.707	0.701	0.707	0.702	0.707
22	NeuralNetFastAI_BAG_L1	0.879	0.738	0.735	0.738	0.735	0.738	0.735	0.738
23	LightGBMLarge_BAG_L1	0.877	0.705	0.698	0.705	0.698	0.705	0.699	0.705
24	NeuralNetTorch_BAG_L1	0.873	0.719	0.714	0.719	0.714	0.719	0.715	0.719
25	KNeighborsDist_BAG_L1	0.860	0.717	0.709	0.717	0.716	0.717	0.713	0.717
26	KNeighborsUnif_BAG_L1	0.821	0.646	0.643	0.646	0.646	0.646	0.645	0.646

table S2 The importance of features

Features	importance	P value	Features	importance	P value
Tilt Angle	0.046	0.000	Z5	0.001	0.251
Angle Subtended by the Hydrophobic Residues	0.023	0.005	Small_Mole.	0.001	0.213
Propensity to in vitro Aggregation	0.016	0.003	VHSE2	0.001	0.152
Net Charge	0.012	0.030	T3	0.001	0.104
Length	0.010	0.005	T1	0.001	0.152
BLOSUM10	0.010	0.003	Isoelectric Point	0.001	0.341
Basic_Mole.	0.010	0.009	Boman Index	0.001	0.213
ProtFP7	0.009	0.012	F1	0.001	0.311
KF9	0.007	0.008	BLOSUM4	0.001	0.213
Aromatic_Mole.	0.007	0.025	ST6	0.001	0.187
Charged_Mole.	0.007	0.112	BLOSUM8	0.001	0.271
Hp	0.007	0.032	MSWHIM3	0.001	0.294
KF3	0.007	0.001	T4	0.001	0.294
Ep	0.007	0.060	F3	0.001	0.238
VHSE8	0.007	0.016	VHSE1	0.001	0.187
KF10	0.007	0.004	Aliphatic_Mole.	0.000	0.433
MSWHIM2	0.006	0.013	Disordered Conformation Propensity	0.000	0.389
BLOSUM7	0.006	0.050	PP2	0.000	0.311
KF4	0.005	0.095	BLOSUM5	0.000	0.436
Amphiphilicity Index	0.005	0.006	Hydrophobic residue%	0.000	0.399
PP3	0.005	0.051	Propensity to PPII coil	0.000	0.500
ST1	0.004	0.027	BLOSUM9	0.000	0.500
KF5	0.004	0.128	miuH	0.000	0.500
BLOSUM3	0.004	0.080	BLOSUM6	0.000	0.500
MSWHIM1	0.004	0.008	Acidic_Mole.	0.000	0.500
KF6	0.003	0.012	Z2	0.000	0.500
ST5	0.003	0.054	VHSE7	0.000	0.500
F6	0.003	0.080	KF7	0.000	0.500
Normalized Hydrophobic Moment	0.003	0.067	ST4	0.000	0.500
F2	0.003	0.026	ProtFP4	0.000	0.500
ST8	0.003	0.016	Z1	0.000	0.500
Tiny_Mole.	0.003	0.274	Z4	0.000	0.586
VHSE5	0.003	0.072	H	0.000	0.586
ProtFP3	0.002	0.045	ST7	0.000	0.626
F5	0.002	0.243	ST2	0.000	0.611
Z3	0.002	0.017	KF1	-0.001	0.676
ProtFP6	0.002	0.187	ProtFP2	-0.001	0.813
VHSE3	0.002	0.017	F4	-0.001	0.813
NonPolar_Mole.	0.002	0.149	PP1	-0.002	0.950
Penetration Depth	0.002	0.071	VHSE6	-0.002	0.911
ST3	0.002	0.197	Normalized Hydrophobicity	-0.002	0.879
T2	0.002	0.115	BLOSUM1	-0.002	0.813
ProtFP1	0.002	0.121	BLOSUM2	-0.002	0.955
Polar_Mole.	0.002	0.121	KF2	-0.002	0.885
VHSE4	0.002	0.121	ProtFP5	-0.003	0.998
T5	0.002	0.050	ProtFP8	-0.005	0.990
KF8	0.002	0.008	Linear Moment	-0.007	0.997

table S3 The parameters of MLamPs

ID	Sequence	Length	Hydrophobic residue%	Helix (%)	<H>	<μH>	Normalized Hydrophobic Moment	Normalized Hydrophobicity	Net Charge	Isoelectric Point	Penetration Depth	Tilt Angle	Disordered Conformation Propensity	Linear Moment	Propensity to in vitro Aggregation	Angle Subtended by the Hydrophobic Residues	Amphiphilicity Index	Propensity to PPII coil
MLamP1	KLKKLLLGLLLKK	13	53.85%	84.62%	0.535	0.310	0.83	-0.52	5	11.27	14	94	0.02	0.43	282.39	80	1.41	0.81
MLamP2	KLLKLKKLLGLLK	13	53.85%	84.62%	0.535	0.324	0.79	-0.52	5	11.27	14	95	0.02	0.29	33.90	100	1.41	0.81
MLamP3	KKLLLKGLLLLKK	13	53.85%	92.31%	0.535	0.337	1.09	-0.52	5	11.27	14	94	0.02	0.42	14.06	90	1.41	0.81
MLamP4	KKLLLGLLKLKKL	13	53.85%	84.62%	0.535	0.338	1.07	-0.52	5	11.27	14	91	0.02	0.38	26.63	100	1.41	0.81
MLamP5	LKKLGLLLKKKLL	13	53.85%	84.62%	0.535	0.354	1.02	-0.52	5	11.28	14	95	0.02	0.33	1.87	100	1.41	0.81
MLamP6	KKLLGLLLKLKLK	13	53.85%	84.62%	0.535	0.365	1.10	-0.52	5	11.27	14	94	0.02	0.39	25.52	130	1.41	0.81

Physicochemical properties of peptides were calculated by DBAASP.

table S4 In vitro biocompatibility of MLamPs (μg/mL)

Peptides	**HL_{10}**	**CSI (hRBCs)**	**CT_{10}**	**CSI (HEK293 cells)**
MLamP1	256	2	>256	4
MLamP2	>256	32	256	16
MLamP3	>256	128	256	64
MLamP4	>256	8	256	4
MLamP5	>256	4	256	2
MLamP6	>256	2	256	1

HL_{10}: lowest AMPs concentration that induces 10% hemolysis. CT_{10}: lowest AMPs concentration that causes 10% cell death. CSI: cell selectivity index = HL_{10}/MIC or CT_{10}/ MIC. Each test was performed on three independent experiments.

table S5 α-Helical and β-sheet content of AMPs

	MLamP1			**MLamP6**		
	10mM PBS	50% TFE	30mM SDS	10mM PBS	50% TFE	30mM SDS
Helix (%)	3.78%	39.65%	60.27%	5.51%	46.02%	46.90%
Beta (%)	36.24%	6.70%	1.82%	9.38%	4.62%	3.26%

The secondary structure content of the peptides was estimated using K2D3.

table S6 ELISA-based analysis of the binding of 12-residue peptides to the tested microorganisms and sequence of targeting peptides

ID	***C. perfringens* OD_{450}**	***S. flexneri* OD_{450}**	***E. coli* O157:H7 OD_{450}**	***S. typhimurium* OD_{450}**	**Sequence**
G4	1.1849	0.4081	0.2767	0.4756	TQGFTESHTQLF
A6	1.5053	0.0958	0.1037	0.1163	WVRPLPLPSVHN
B1	0.3396	0.0527	0.0458	0.0556	LNVCHNCVWQKT
D10	0.4911	0.0667	0.064	0.089	GAVNGYYGHLIP
F6	4.016	0.2247	0.198	0.2573	MSPSILKHWSRQ
A2	1.3862	0.3562	0.1808	0.7241	FGVLLRGVLLR
B3	1.012	0.1568	0.134	0.3106	QSSMRFASPATL
B10	2.4652	0.1786	0.1675	0.1886	LVPAPPHVHFPA

All ELISA measurements were performed with an average of three technical replicates.

table S7 Proteins pulled down by biotin-F6

Accession	Names	Length	Mass	Subcellular locate
tr|Q8XKT9|Q8XKT9_CLOPE	Glyceraldehyde-3-phosphate dehydrogenase	332	35362	Cytoplasmic
tr|Q8XMK5|Q8XMK5_CLOPE	Amidophosphoribosyltransferase	481	52724.8	Cytoplasmic
tr|Q8XN32|Q8XN32_CLOPE	UTP--glucose-1-phosphate uridylyltransferase	306	34516.6	Cytoplasmic
tr|Q8XL89|Q8XL89_CLOPE	Formate acetyltransferase	744	83321.7	Cytoplasmic
tr|Q8XP82|Q8XP82_CLOPE	Probable phosphoglycerate mutase	207	24677.6	Cytoplasmic
sp|Q8XI54|G6PI_CLOPE	Glucose-6-phosphate isomerase	450	49771	Cytoplasmic
sp|Q8XKK2|CLPX_CLOPE	ATP-dependent Clp protease ATP-binding subunit ClpX	435	48177.8	Cytoplasmic
sp|P26823|DNAK_CLOPE	Chaperone protein DnaK OS=Clostridium perfringens	619	66481.2	Cytoplasmic
tr|Q8XIV6|Q8XIV6_CLOPE	RNA polymerase sigma factor SigA	373	42683.8	Cytoplasmic
sp|P58693|SYN_CLOPE	Asparagine--tRNA ligase	465	53168.1	Cytoplasmic
sp|Q8XID2|ATPA_CLOPE	ATP synthase subunit alpha	502	55265.2	Cytoplasmic
sp|Q8XHT3|RL14_CLOPE	50S ribosomal protein L14	122	13232.4	Cytoplasmic
tr|Q8XKZ5|Q8XKZ5_CLOPE	Phosphatidylglycerol lysyltransferase	851	97828.9	Cytoplasmic Membrane

table S8 The parameters of HAMPs

ID	Sequence	Length	Hydrophobic residue%	<H>	<μH>	Normalized Hydrophobic Moment	Normalized Hydrophobicity	Net Charge	Isoelectric Point	Penetration Depth	Tilt Angle	Disordered Conformation Propensity	Linear Moment	Propensity to in vitro Aggregation	Angle Subtended by the Hydrophobic Residues	Amphiphilicity Index	Propensity to PPII coil
F6P1	MSPSILKHWSRQGGGKLKKLLLGLLLKK	28	42.86 %	0.444	0.177	0.45	0.15	7.00	11.85	15	94	-0.06	0.34	267.87	40.00	1.22	0.91
P1F6	KLKKLLLGLLLKKGGGMSPSILKHWSRQ	28	42.86 %	0.444	0.252	0.52	0.15	7.00	11.85	15	85	-0.06	0.35	119.22	30.00	1.22	0.91
F6P6	MSPSILKHWSRQGGGKKLLGLLLKLKLK	28	42.86 %	0.444	0.158	0.27	0.15	7.00	11.85	15	93	-0.06	0.36	30.90	20.00	1.22	0.91
P6F6	KKLLGLLLKLKLKGGGMSPSILKHWSRQ	28	42.86 %	0.444	0.386	0.96	0.15	7.00	11.85	15	86	-0.06	0.37	21.61	30.00	1.22	0.91

Physicochemical properties of peptides were calculated by DBAASP.

table S9 In vitro biocompatibility of hybrid AMPs (μg/mL)

Peptides	**HL_{10}**	**CSI (hRBCs)**	**CT_{10}**	**CSI (HEK293 cells)**
F6P1	>256	256	64	32
P1F6	>256	128	>256	64
F6P6	>256	64	256	16
P6F6	256	32	256	16

HL_{10}: lowest AMPs concentration that induces 10% hemolysis. CT_{10}: lowest AMPs concentration that causes 10% cell death. CSI: cell selectivity index = HL_{10}/MIC or CT_{10}/ MIC

table S10 α-Helical and β-sheet content of AMPs

	F6P1			F6P6		
	10mM PBS	50% TFE	30mM SDS	10mM PBS	50% TFE	30mM SDS
Helix (%)	11.96%	75.02%	66.53%	6.54%	66.87%	51.87%
Beta (%)	9.88%	0.22%	0.34%	10.99%	0.51%	3.29%

The secondary structure content of the peptides was estimated using K2D3.

Data S1

The data in the pictures are presented in the corresponding tables:

Fig.1
Fig.3
Fig.5
Fig.6
Fig.7
Fig.8
Fig.9
fig. S3
fig. S6
fig. S7
fig. S8
fig. S9
fig. S13
fig. S14
fig. S15
fig. S16
fig. S17