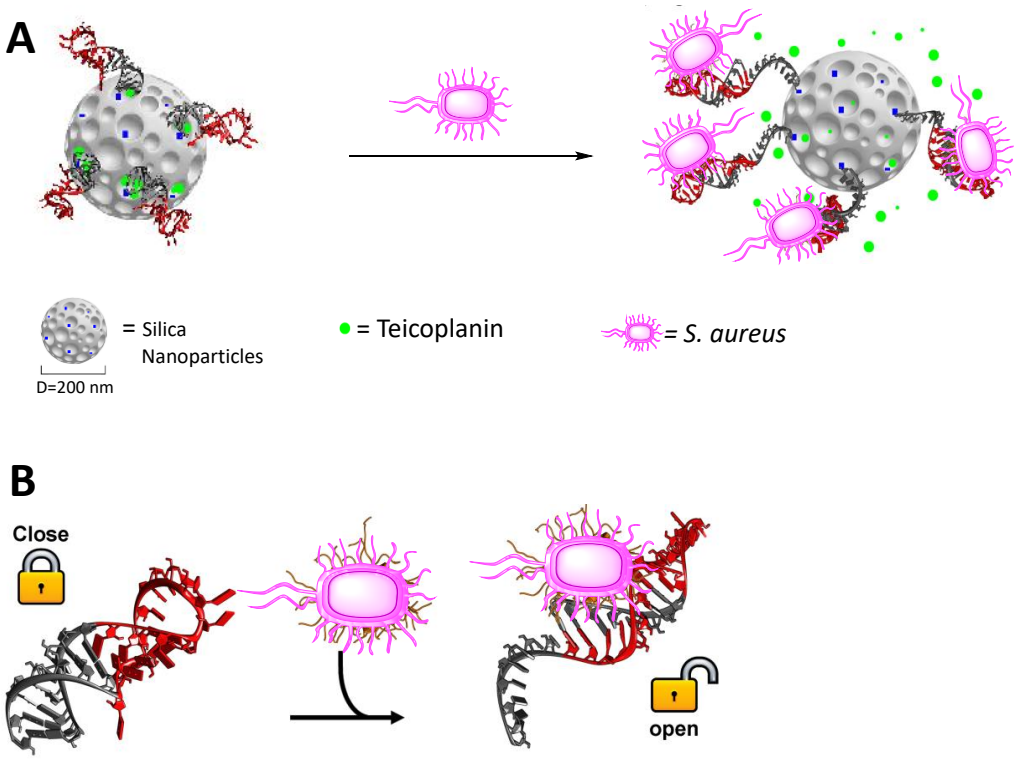
Electronic Supplementary Information (ESI)

Antibiotic administration in targeted nanoparticles protects faecal microbiota of mice

Baris A. Borsa,*[a] Mert Sudagidan,[b] Mehmet E. Aldag,[c] Isik I. Baris,[d] Elif E. Acar,[b] Cagatay Acuner,[e] Murat Kavruk[f] and Veli C. Ozalp *[g]

[a]Nucleic Acid Technologies Lab, Department of Physics, Chemistry and Biology (IFM), Linköping University, SE-58183, Linköping, Sweden., Baris.ata.borsa@liu.se
[b]Kit-Argem Research Centre, Konya Food and Agriculture University, Meram, 42080, Konya, Turkey
[c]Corlu State Hospital, Department of Medical Microbiology, Tekirdag, Turkey.
[d]Cakmak Erdem Hospital, Department of Pathology, Istanbul, Turkey.
[e]Department of Medical Microbiology, Medical Scool, Yeditepe University, Istanbul, Turkey
[f]Test and Calibration Center, Turkish Standards Institution (TSE), 41400, Gebze, Kocaeli, Turkey
[g]Department of Medical Biology, Medical School, Atilim University, Incek, 06830, Ankara, Turkey. cengiz.ozalp@atilim.edu.tr



Scheme S1. Aptamer-gated teicoplanin delivery nanoparticles (T-ASNP). A) Aptamer hairpin structures were covalently conjugated to the surface of mesoporous silica nanoparticles, entrapping previously loaded cargo molecule (teicoplanin). In the presence of *S. aureus* cells, aptamer gates interacts specifically with the target *S. aureus* cell surface antigens, leading to changes in molecular conformation of the aptamer gates and openning of the pore openings, thus *S. aureus*-triggered release of teicoplanin are observed. B) The principle of aptamer gate openning mechanism.

Table S1. Classification statistics.

Taxonomic level	Reads classified to taxonomic level	% total reads classified to taxonomic level
Kingdom	119.673	98.44 %
Phylum	118.483	97.46 %
Class	117.404	96.57 %
Order	116.945	96.20 %
Family	113.368	93.25 %
Genus	100.294	82.50 %
Species	62.725	51.60 %

Table S2. Species level diversity.

Sample ID	Shannon's Species Diversity	Number of Species Identified
PBS	2.663 ± 0.196	547 ± 28.7
TEICP	3.009 ± 0.183	610 ± 30.8
TASNP	2.932 ± 0.171	564 ± 25.2
NI	2.527 ± 0.215	526 ± 28.7
PBS24	2.626 ± 0.174	498 ± 22.2
TEICP24	2.472 ± 0.181	531 ± 26.1
TASNP24	2.830 ± 0.213	450 ± 21.6
NI24	2.836 ± 0.188	650 ± 31.1

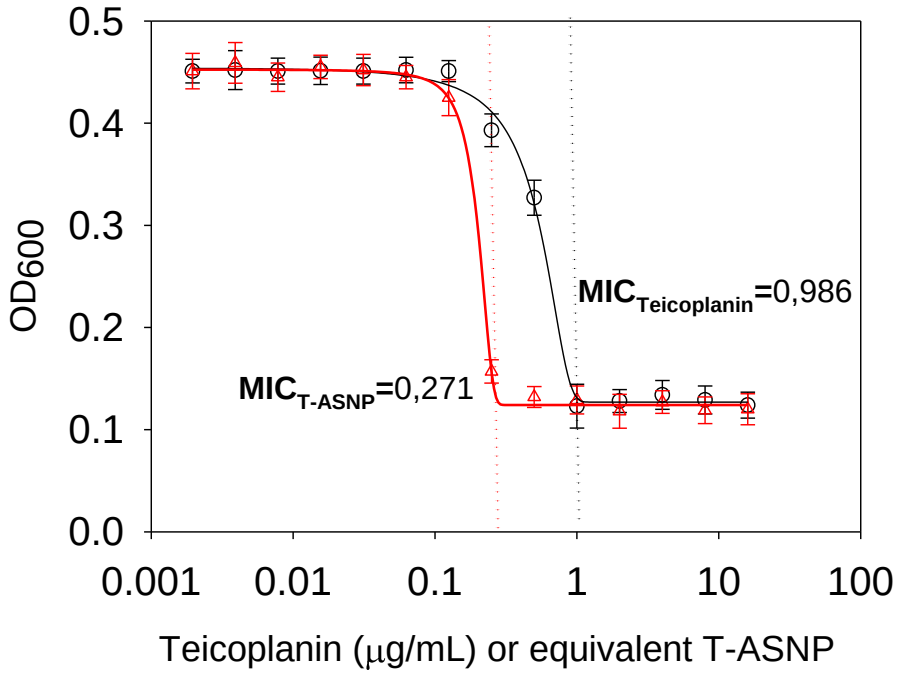


Fig. S1. MIC of *S. aureus* with teicoplanin (black line) or targeted teicoplanin (T-ASNP) (red line). *S. aureus* cultures were treated with 2-fold serial dilutions of teicoplanin and T-ASNP at 37 °C for 24 hours and the viable bacterial cells were quantified by measuring absorbances (OD) at 600 nm. All measurements were performed in triplicate and the error bars indicate the standard deviations.

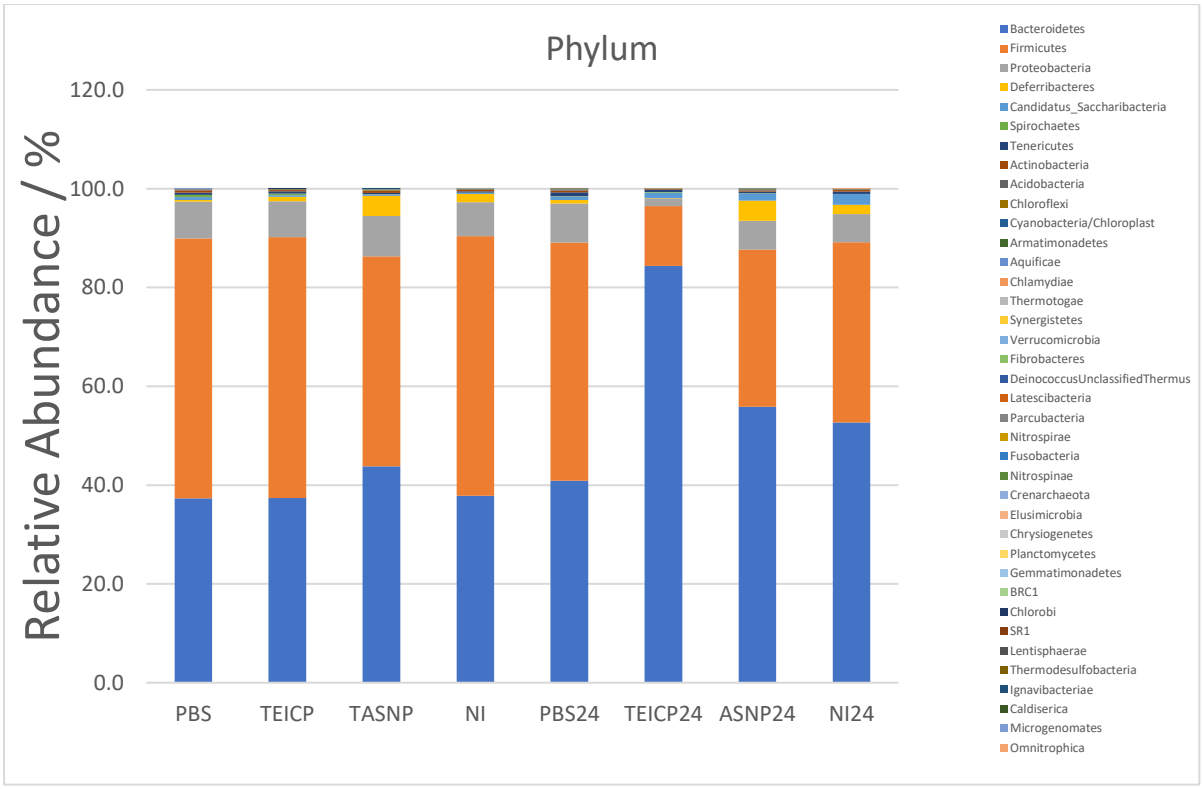


Fig. S2. Metagenomic analysis of targeted teicoplanin and control groups of mice at Phylum level abundancies as relative percentages.

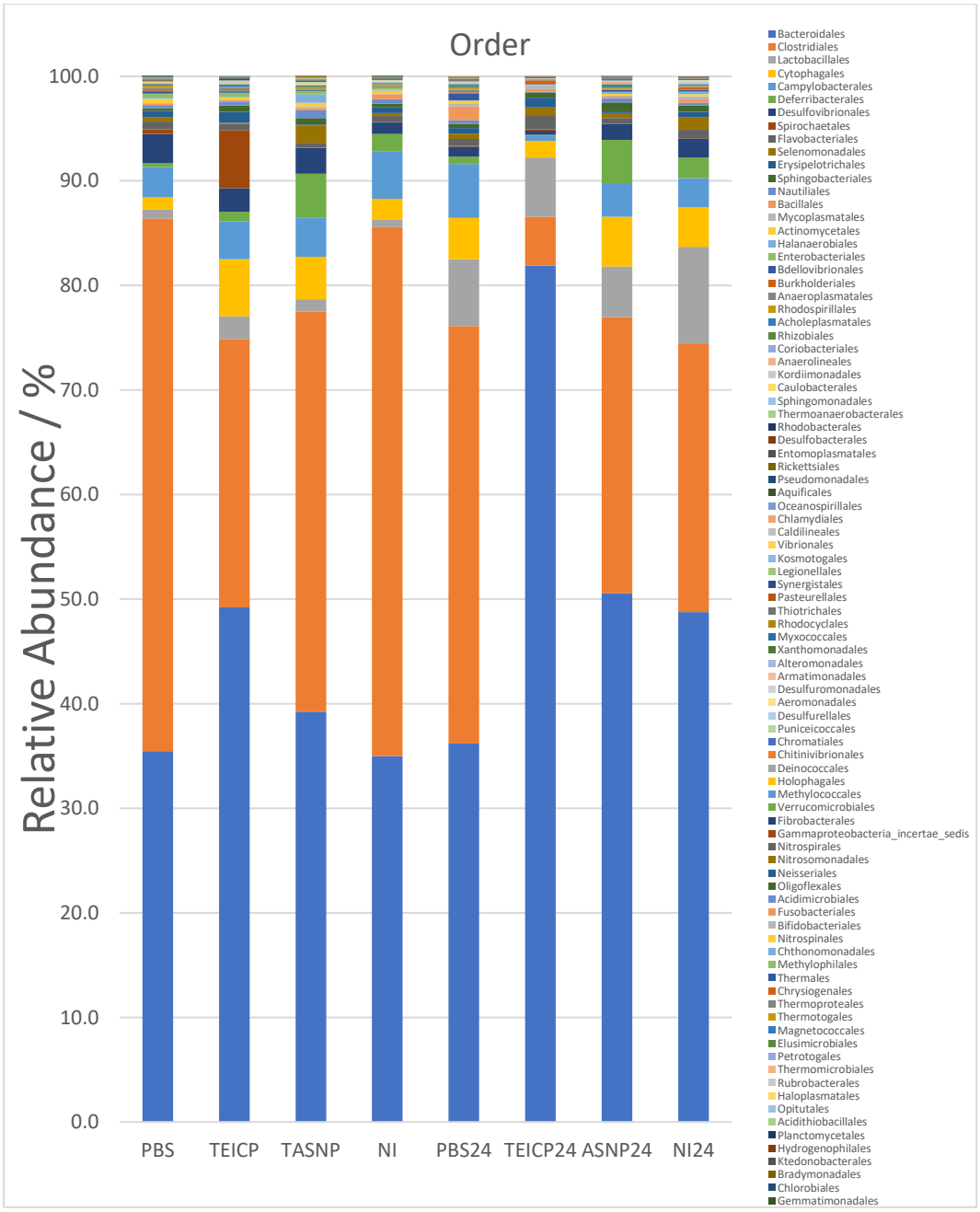


Fig. S3. Metagenomic analysis of targeted teicoplanin and control groups of mice at Order level abundancies as relative percentages.

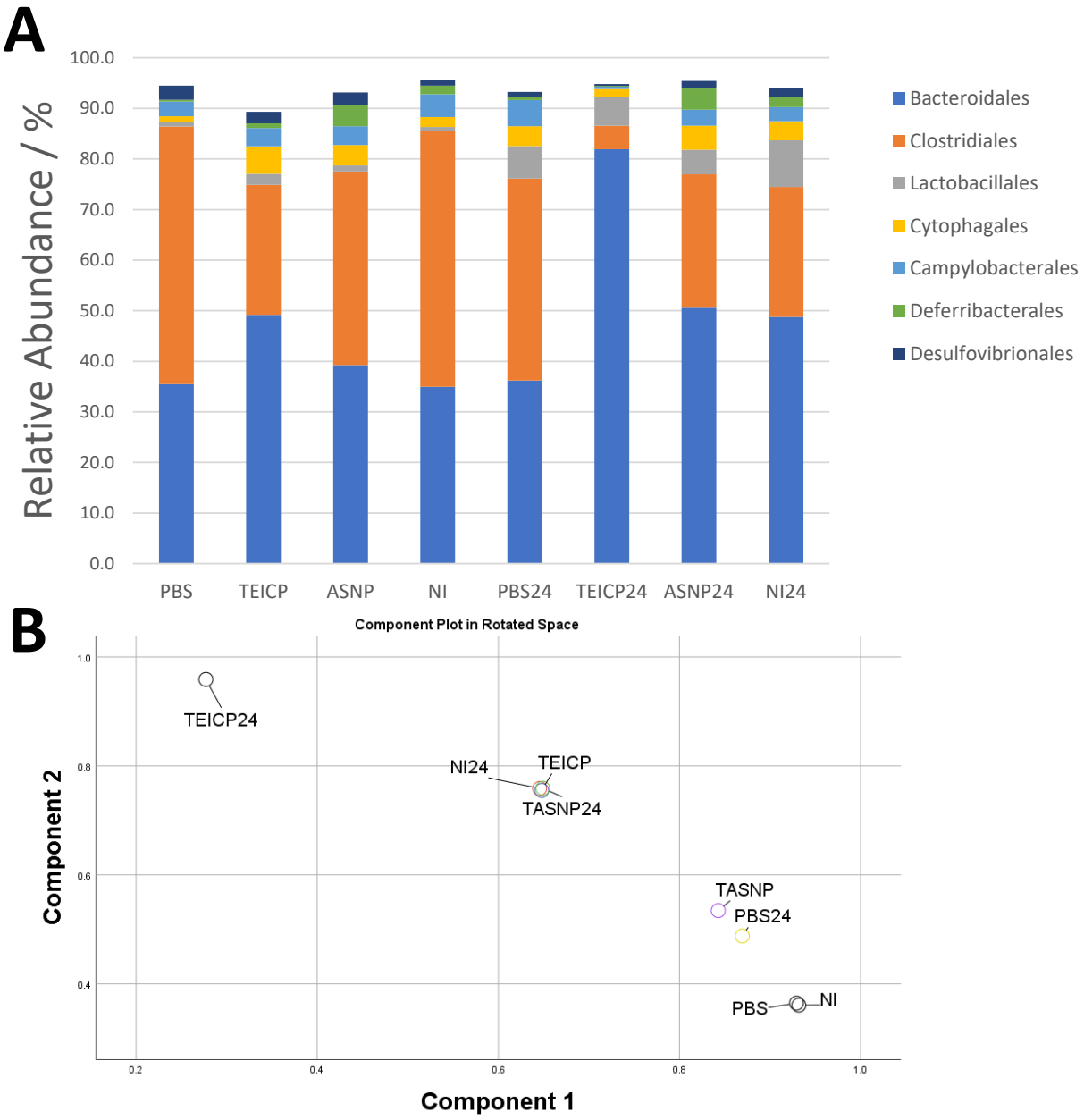


Fig. S4. A) Metagenomic analysis of targeted teicoplanin and control groups of mice at Order level abundancies as relative percentages (>1% abundancies were included). B) Similarity analysis by PCoA of microbiota profiling at order level.

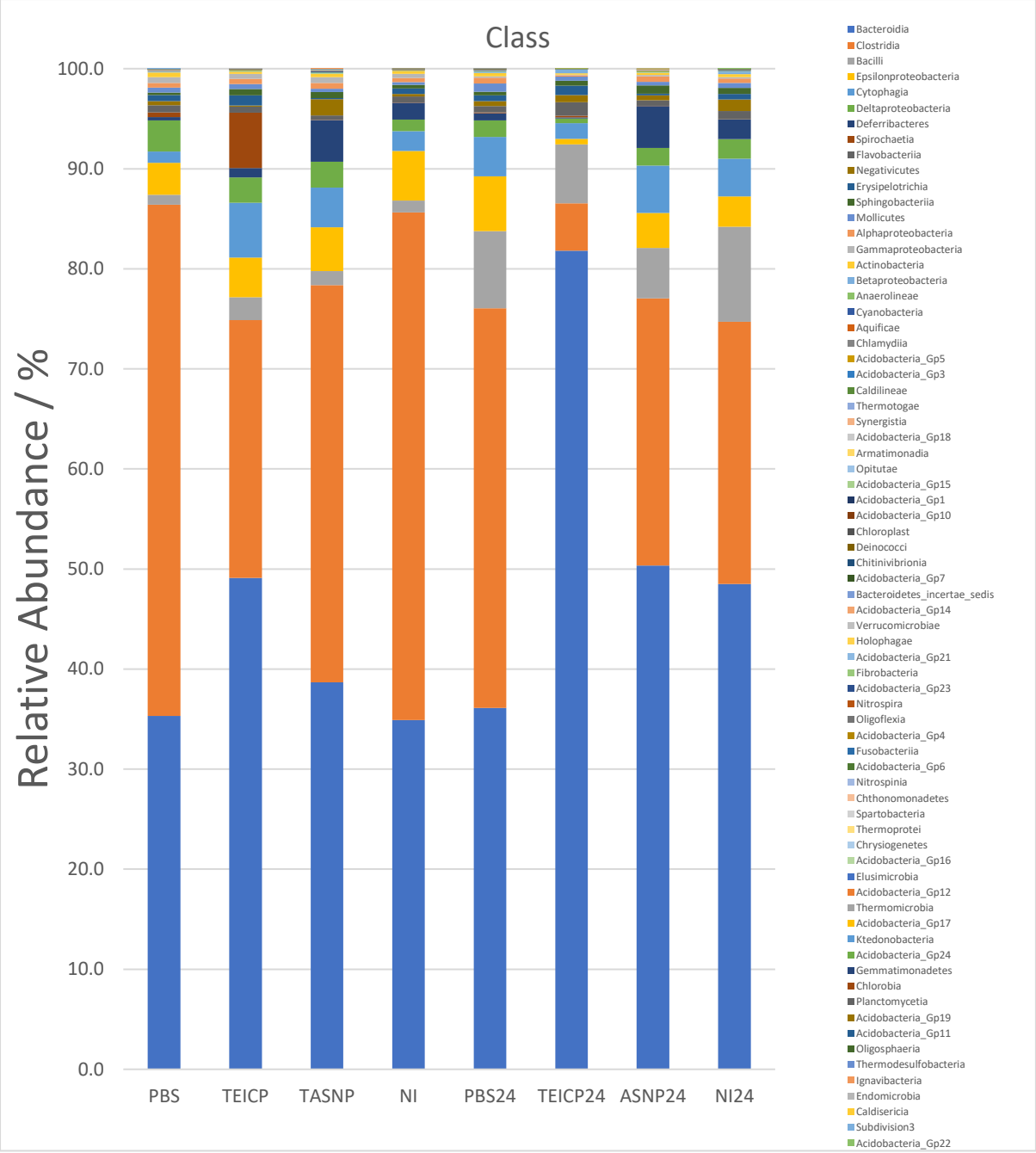


Fig. S5. Metagenomic analysis of targeted teicoplanin and control groups of mice at Class level abundancies as relative percentages.

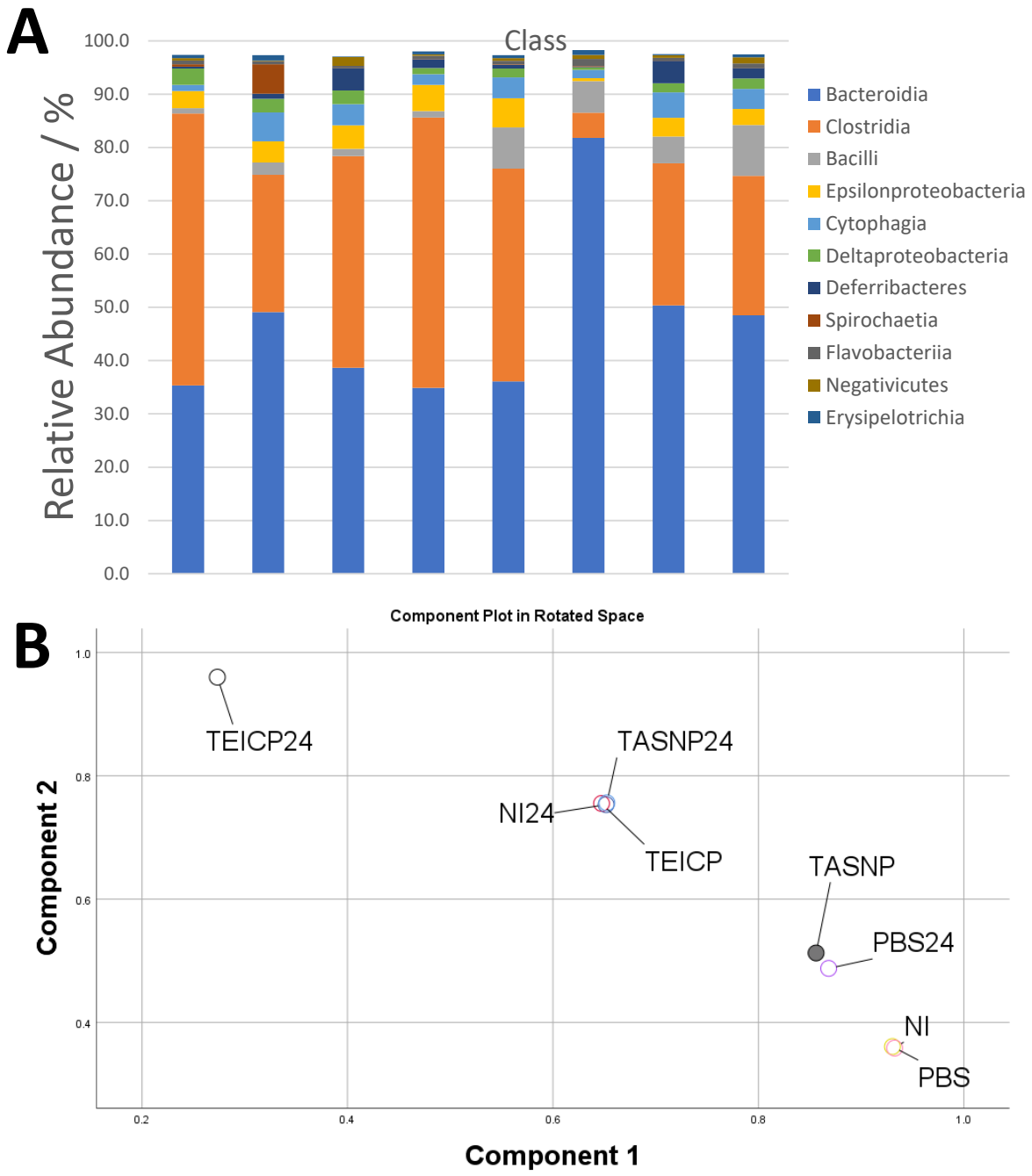


Fig. S6. A) Metagenomic analysis of targeted teicoplanin and control groups of mice at Class level abundancies as relative percentages (>1% abundancies were included). B) Similarity analysis by PCoA of microbiota profiling at Class level.

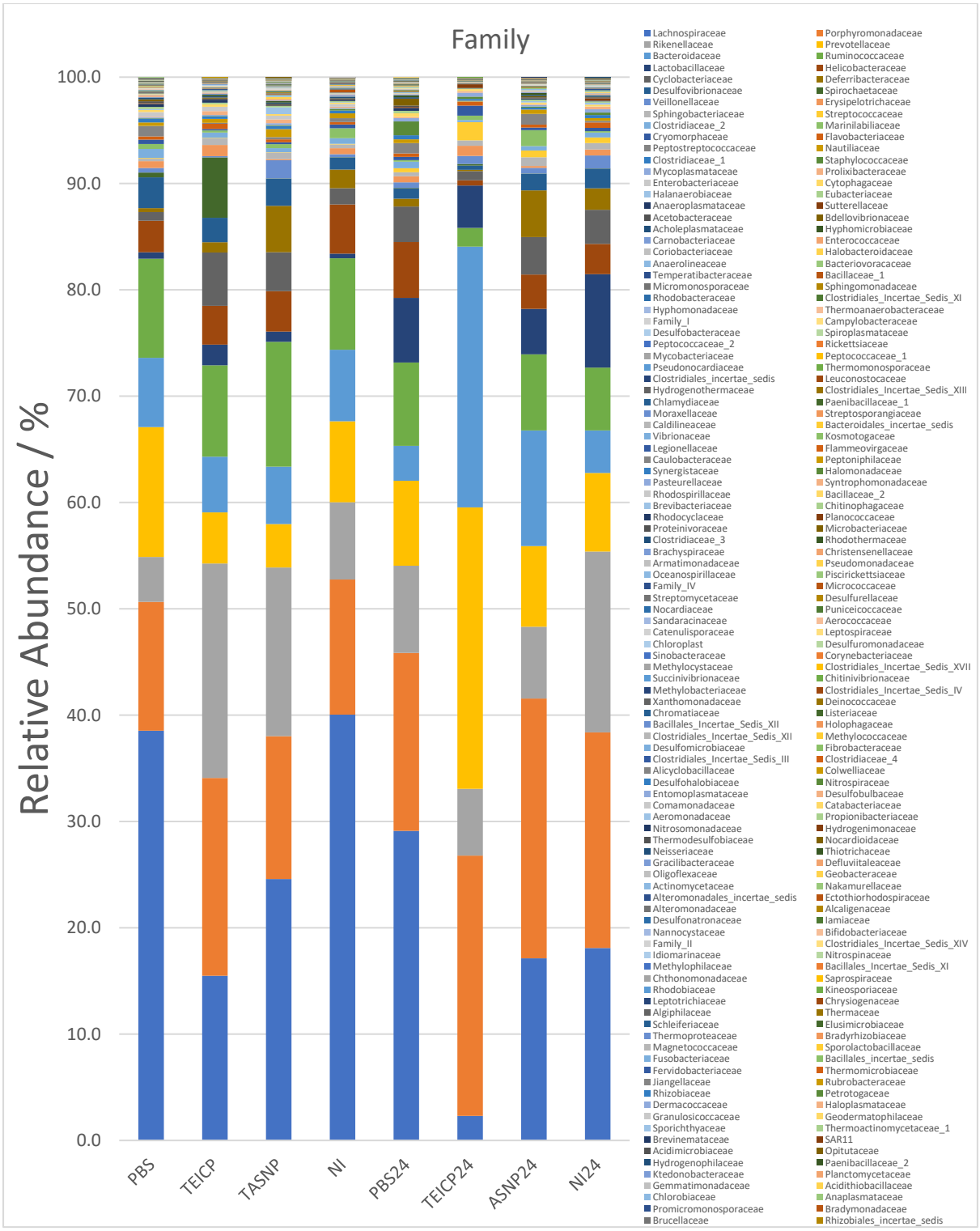


Fig. S7. Metagenomic analysis of targeted teicoplanin and control groups of mice at Family level abundancies as relative percentages.

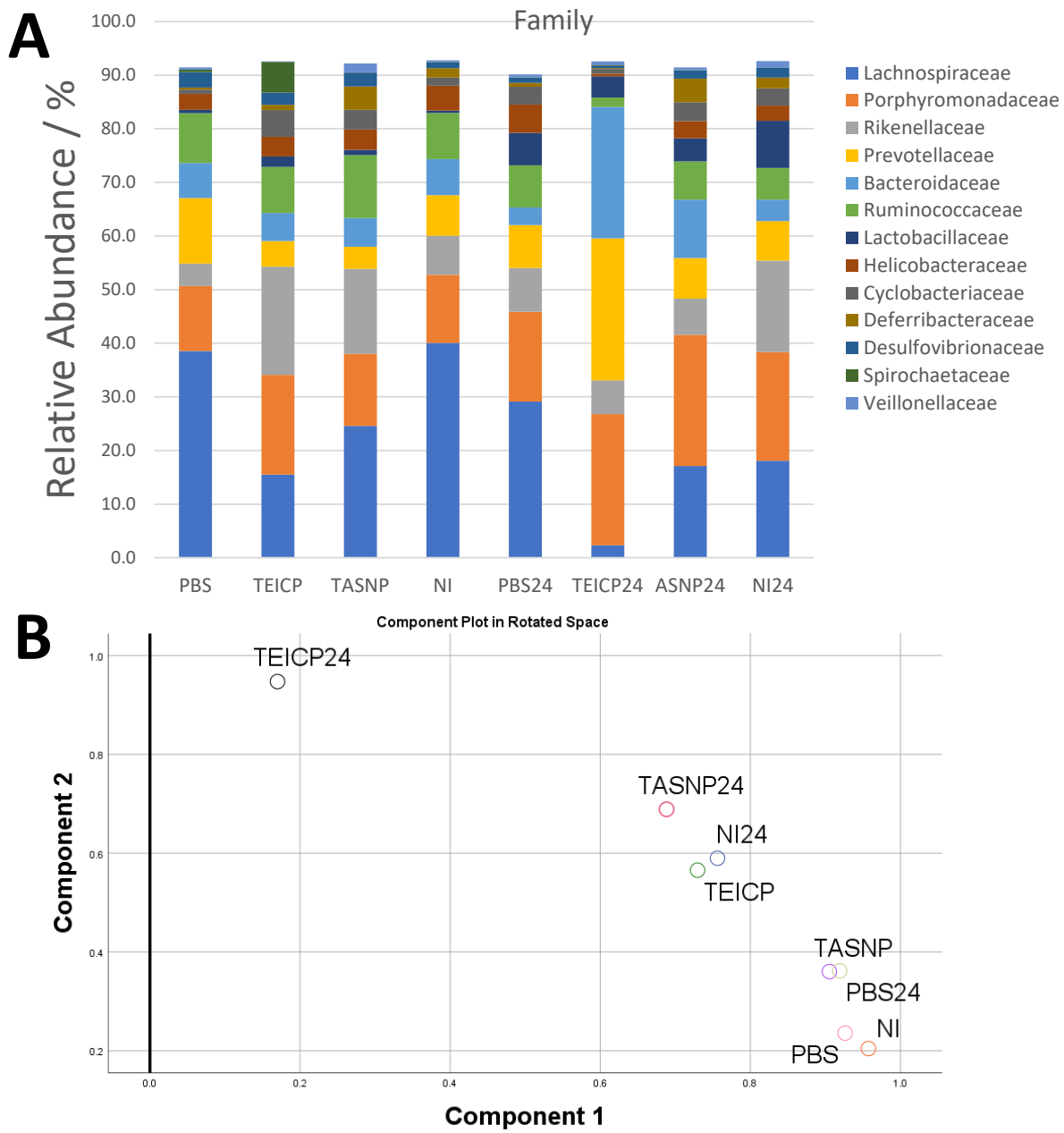


Fig. S8. A) Metagenomic analysis of targeted teicoplanin and control groups of mice at Family level abundancies as relative percentages (>1% abundancies were included). B) Similarity analysis by PCoA of microbiota profiling at Family level.

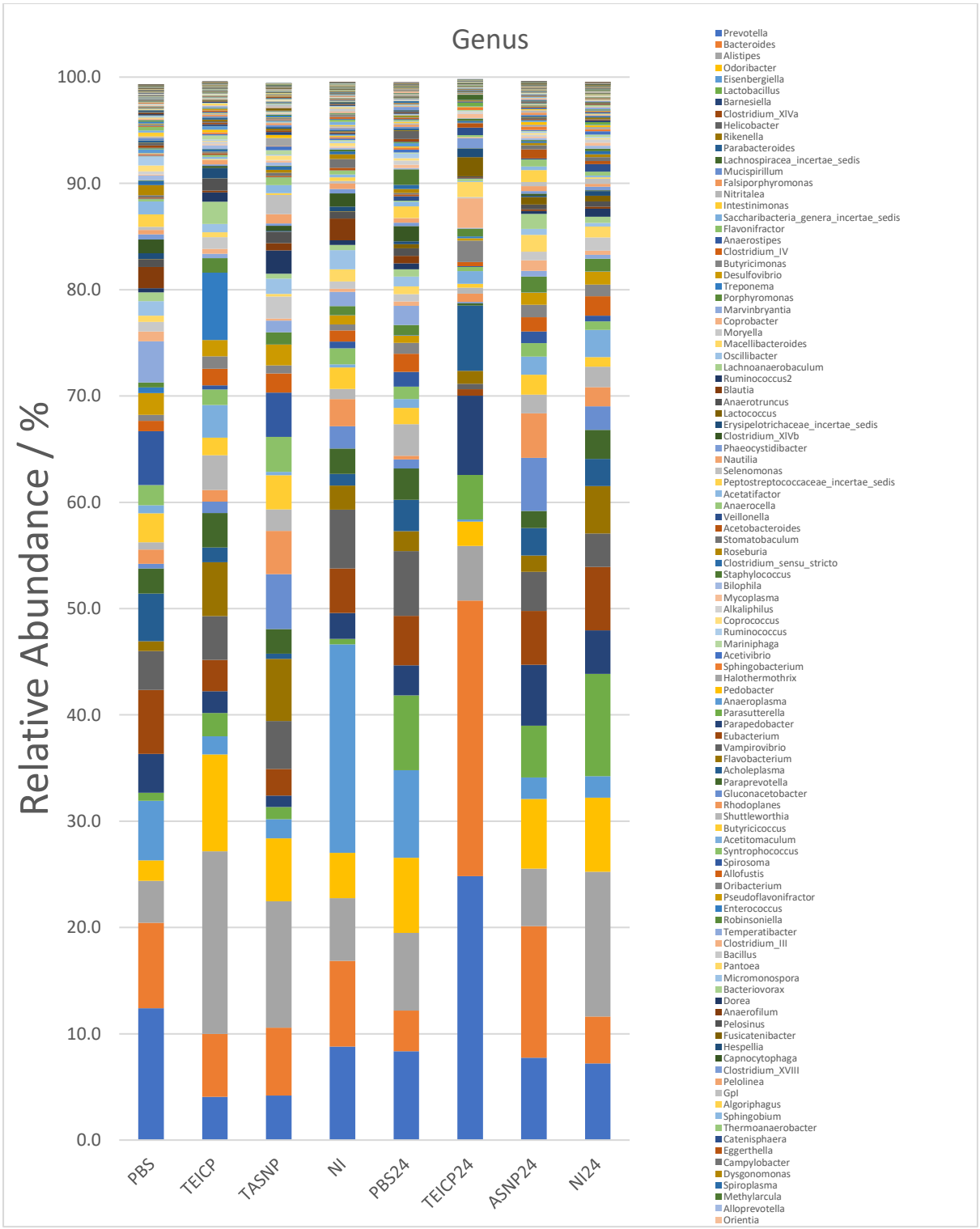


Fig. S9. Metagenomic analysis of targeted teicoplanin and control groups of mice at Genus level abundancies as relative percentages.

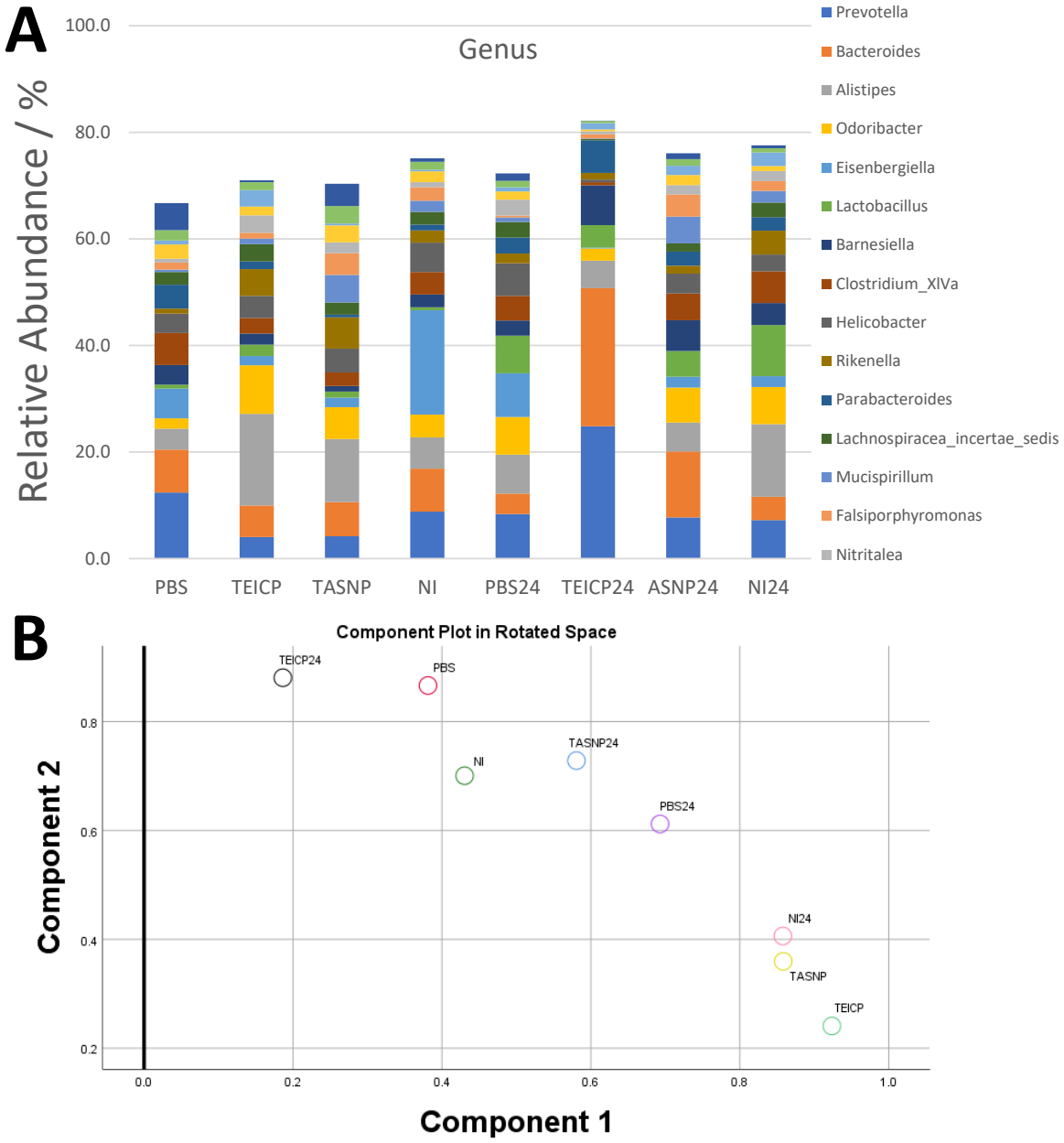


Fig. S10. A) Metagenomic analysis of targeted teicoplanin and control groups of mice at genus level abundancies as relative percentages (most abundant 15 genus were included). B) Similarity analysis by PCoA of microbiota profiling at genus level.

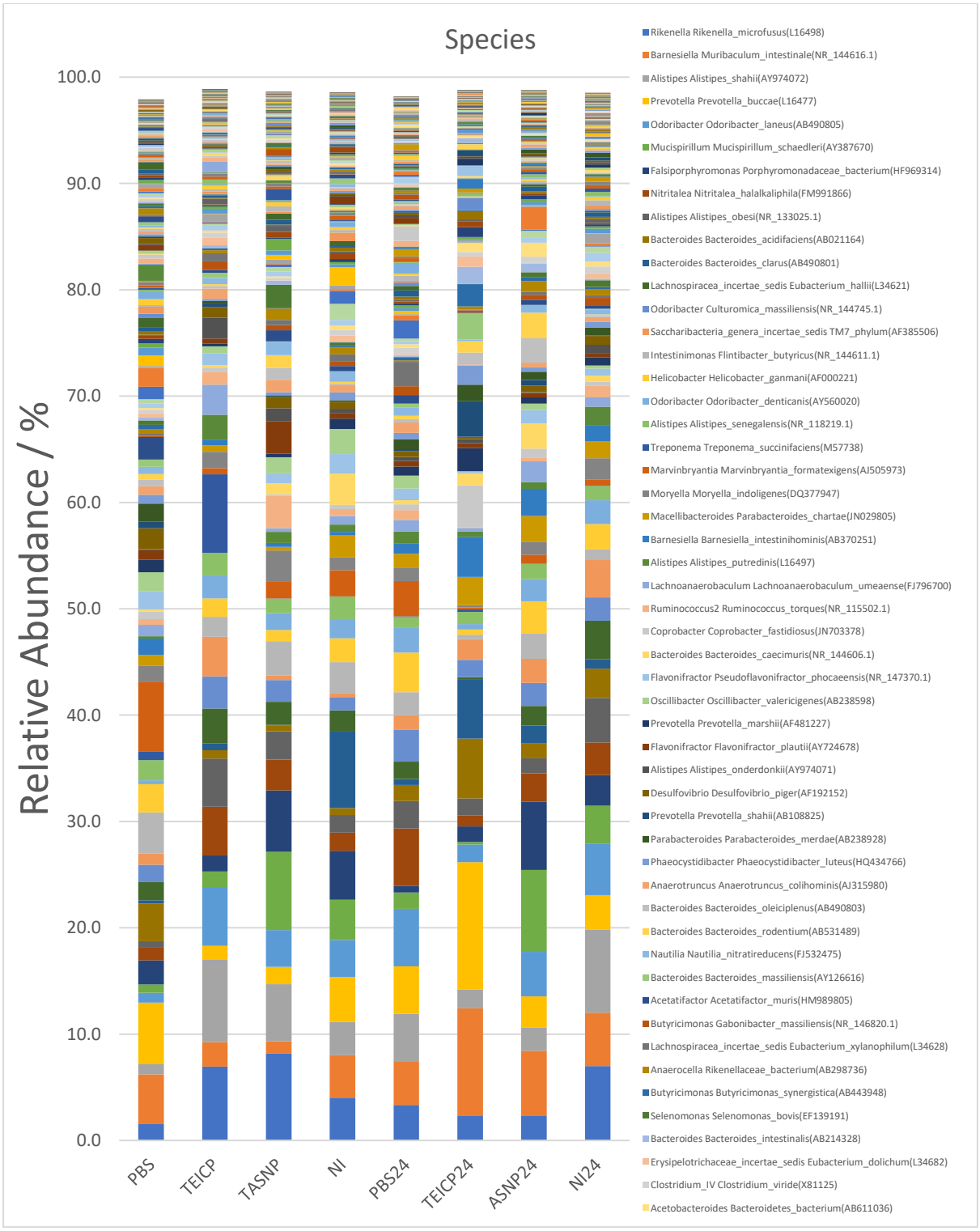


Fig. S11. Metagenomic analysis of targeted teicoplanin and control groups of mice at Species level abundancies as relative percentages.

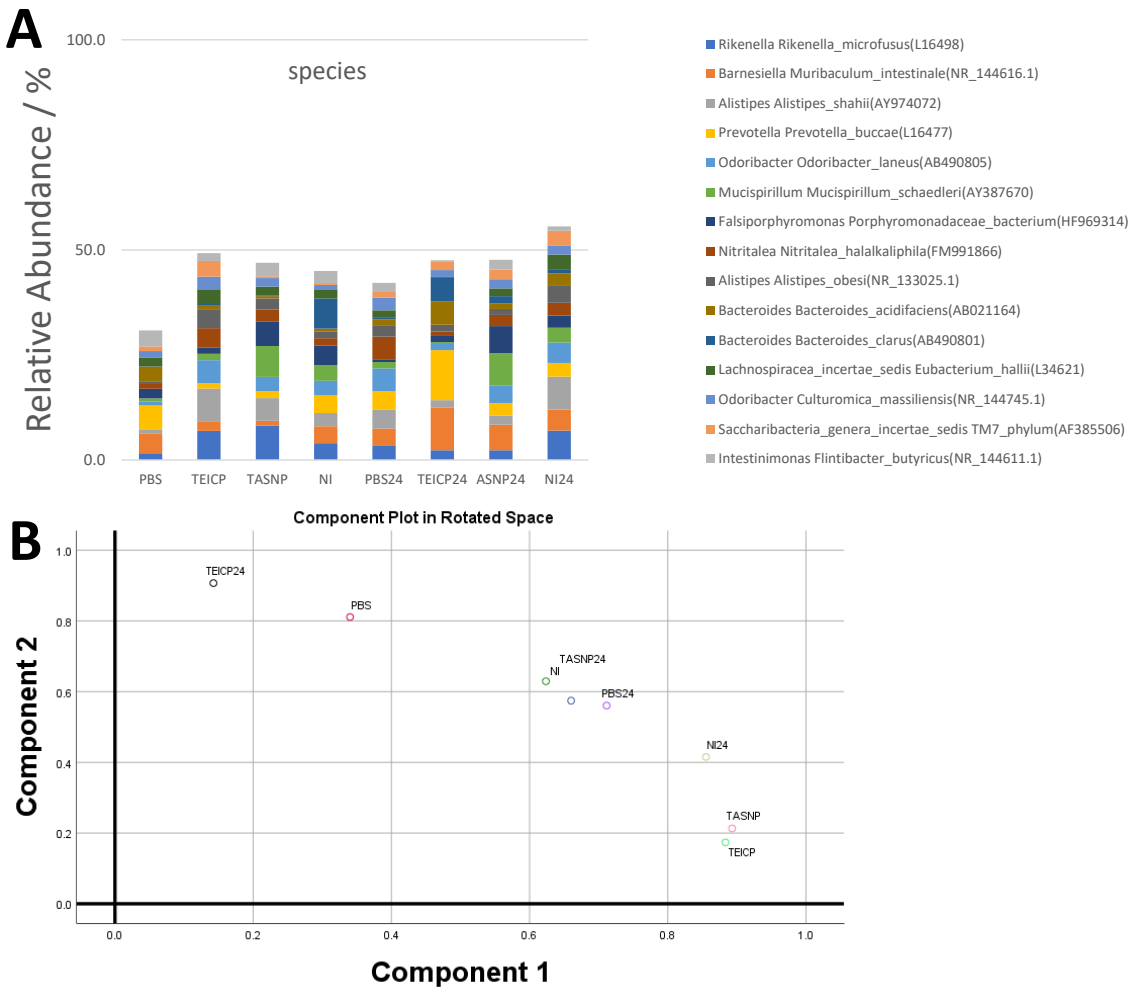


Fig. S12. A) Metagenomic analysis of targeted teicoplanin and control groups of mice at species level abundancies as relative percentages (most abundant 15 species were included). B) Similarity analysis by PCoA of microbiota profiling at species level.

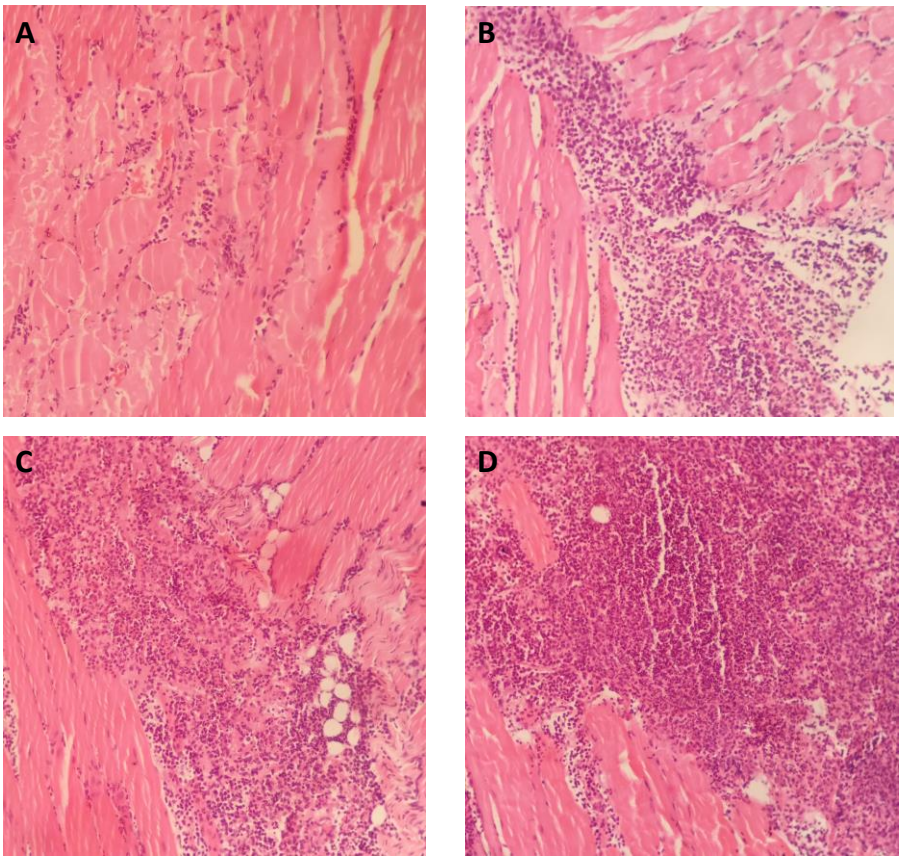


Fig. S13. Represantative images of pathological analysis for each treatment group. Inflammation scores were obtained from images. A) Group 1 (non-infected), B) Group 2 (infected PBS-treated), C) Group 3 (infected teicoplanin-treated) and D) Group 4 (infected T-ASNP treated.)

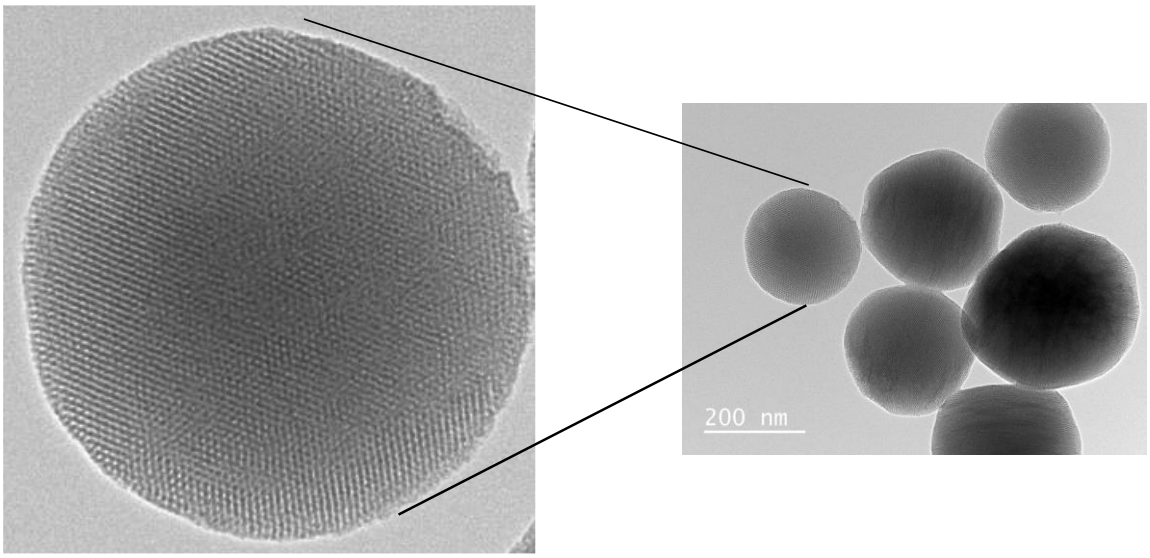


Fig. S14. A blown-up image of one of the particles shown in Fig. 1A. The ordered pore morphology can be observed on the particle surface.

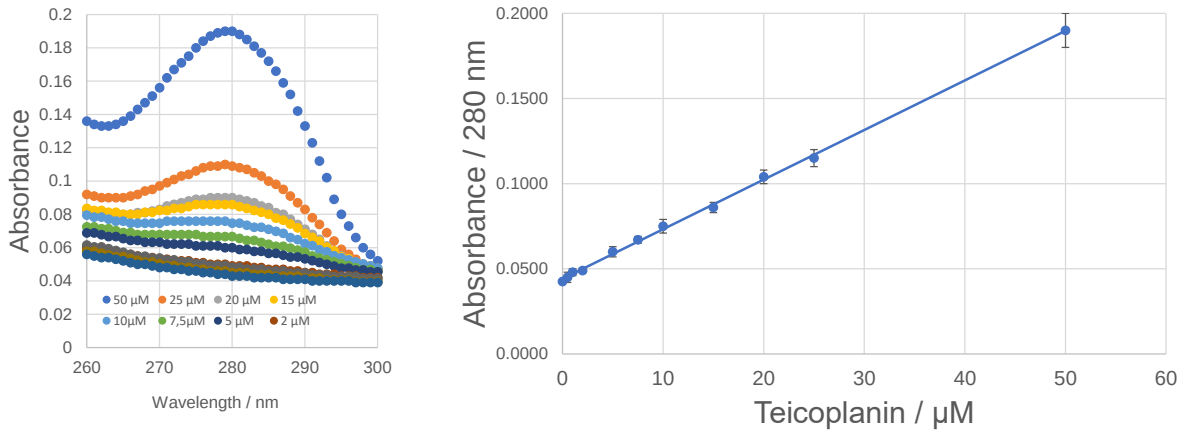


Fig. S15. Calibration curve for teicoplanin determination. The absorbance spectral analysis between 260 and 300 nm showed peak values at 280 nm, which followed a linear relationship up to 50 µM teicoplanin.

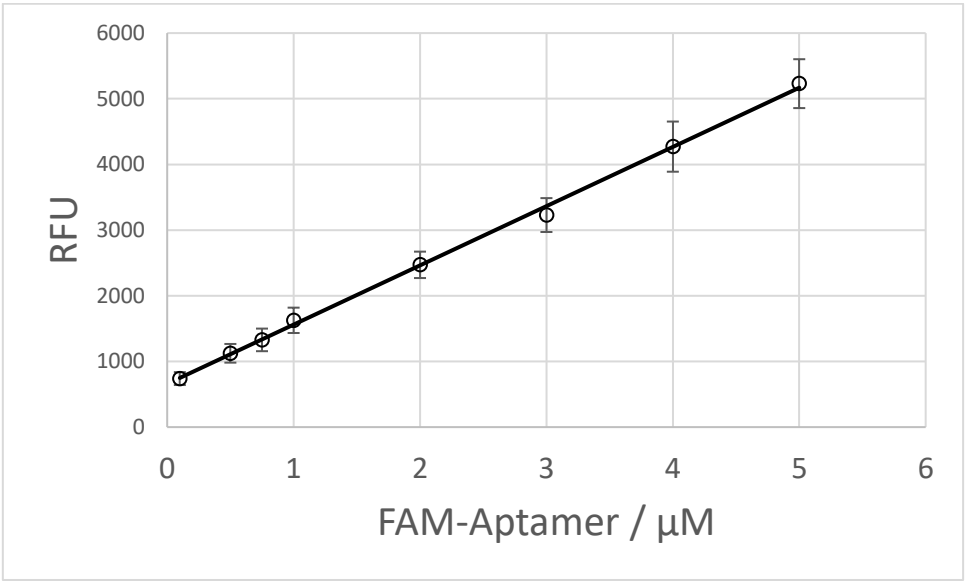


Fig. S16. Calibration curve for FAM-labelled aptamer determination. The 520 nm emmited signals were recoreded after 480 nm excitation.

Experimental Procedures

Bacterial strains and Aptamer gate preparation

S. aureus (ATCC 29213) was inoculated onto Tryptic Soya Agar (TSA, Oxoid, UK) and incubated at 37 ^{o}C for 18-24 hours. A single colony was transferred to Tryptic Soya Broth (TSB, Oxoid, UK) and incubated 12 hours at 37 ^{o}C. A bacteria suspension for thigh infection model was prepared in PBS and the final bacterial concentration was adjusted to 10^{7} CFU/ml.

Synthesis of T-ASNP

The silica particles with *S. aureus* binding aptamer gates (ASNP) were synthesized according to previous procedures. Briefly, mesoporous silica nanoparticles were synthesized by following a sol-gel method.[1] N-cetyltrimethylammonium bromide (CTAB) solution of 2.74 mmol was mixed with 2 M sodium hydroxide. This mixture was heated to 80°C and 5 ml of 22.4 mmol tetraethoxysilane (TEOS) was added into the mixture dropwise within 5 min by mixing. After 2h reaction, white precipitate was collected on a filter, washed with water and methanol several times and dried under air. The surfactant template from 1.5 g synthesized product was removed by refluxing in HCl (1.5 ml, 37%)/methanol (150 ml) solution. After 6h, the product was filtered, washed with methanol and water, finally surfactant free mesoporous silica nanoparticles (SNP) were obtained after drying.

50 mg SNP powder in 20 ml of 95% ethanol containing 1 mM acetic acid and 3% (w/w) of Diethoxy(3-glycidyloxypropyl)methylsilane were stirred for 30 min at room temperature, followed by washing with ethanol by centrifugation (14000x*g*, 1 min) to obtain epoxy grafted SNP. One mg SNP were loaded with teicoplanin cargo by incubation in 1 mM teicoplanin solution in buffered phosphate saline (PBS, 0.01 M phosphate buffered saline; NaCl-0.138 M; KCl-0.0027 M; pH 7.4) overnight and used for coupling with amino-modified oligonucleotides. The particles were then washed thoroughly 3 times with PBS. Loading was calculated from the absorbance of the particles at 280 nm by comparing with a calibration curve (Fig. S15). The amount of aptamer immobilized on T-ASNP was measured by using complementary DNA labeled with FAM. The unloaded aptamer-particles were prepared as above and 0.01 mg of particles mixed with 5 µM FAM-labeled complementary DNA, followed by an incubation of 30 min. The particles were washed 5 times and re-suspended in 2 ml buffer for fluorescence reading. Bare particles were used to normalize the readings and the amount of immobilized aptamers was calculated from a calibration curve (Fig. S16). The samples were analyzed by a spectrofluorometer (Fluostar omega, BMG Labtech) with appropriate filters for Fluorescein (Ex. 480 / Emm. 520). The loading efficiency was calculated as the amount of total teicoplanin divided by nanoparticle weight and encapsulation efficiency was calculated as nonencapsulated teicoplanin subtracted from total teicoplanin in encapsulation solution and divided by total teicoplanin.

Nanoparticle Characterization

The particles of about 100 µg per ml of PBS buffer (10 mM Phosphate, 139 mM NaCl, pH=7.4) were analyzed by dynamic light scattering (DLS) for determining average hydrodynamic size. DLS and zeta potential measurements were performed by Zetasizer Nano ZS (Malvern Instruments Worcestershire, UK) with 633 nm laser and 173 degrees detection optics. Data acquisition and analysis was achieved by Malvern DTS v.6.20 software. Morphological assessment of the particles was analyzed by Transmission Electron Microscopy (TEM, JEOL-Jem 2100). Brunauer-Emmett-Teller (BET) analysis was performed by a BET analyzer (Micromeritics -TriStar II Plus).

Murine thigh infection model

10 weeks old 16 female immunocompetent BALB/c mice were divided into 4 different groups as non-infected, infected PBS-treated, infected 40 mg/kg teicoplanin-treated, and infected 11 mg/kg nano-teicoplanin-treated. 30 microliters of 10^7 CFU/ml bacteria solution were inoculated into both thigh muscles of each mice except non-infected group which has received 30 microliters PBS instead. Each mouse has received single dose 200 microliters intraperitoneal administrations of indicated chemicals 2 hours after bacteria inoculations. All mice were sacrificed 24 hours after initial infection and both thigh muscles were removed. The left thigh muscles of each mice were investigated for bacteriologic response while the right thigh muscles were investigated for inflammatory response. Besides, blood and stool samples were collected before and after experiment from each mouse in order to compare the changes in faecal microbiota and blood count.

Microbiologic analysis

Thigh muscle tissues were homogenized in sterile conditions and three different dilutions of homogenates were prepared using PBS. Then, all samples were inoculated onto 5% sheep blood agar (Oxoid, UK) and incubated at 37 ^{o}C for 18-24 hours. The colony counts were determined and calculated as CFU/per gram tissue. All investigations were performed as triplicates.

Blood analysis

Blood samples were collected using capillary tubes and blood count were analysed using PE-6800VET hematology analyser (Shenzen Prokan Electronics Inc., China) before and after experiment.

Inflammation Scores:

Paraffinized sections (3-4 µm) from each sample (3-10 sections/mouse) were stained with hematoxylin-eosin and slides were examined under a light microscope (Olympus BX51; Olympus, Tokyo, Japan). Level of inflammation was scored as follows: 1=mild inflammation (<5 inflammatory cells in x200 magnification); 2=moderate inflammation (inflammatory cells scattered throughout the tissue but background stromal connective tissue clearly visible); 3=severe inflammation (inflammatory cells densely infiltrating the tissues). [2] Representative images were given in Fig. S13.

DNA Extraction from Faecal Samples

Faecal sample microbial DNA extraction was performed by a microbial extraction kit based on mechanical disruption of bacterial cells with metal beads agitation (15 min, Multi Bio RS-24, Biosan, Latvia) according to supplier's procedures (Qiagen, QIAamp® Power Fecal DNA kit) and concentrations were determined using a fluorescent DNA quantification kit (AccuBlue® NextGen dsDNA Quantitation kit, Biotium Inc., Fremont CA).

16S Microbial Metagenomic Analysis

Microbial identity of faecal DNA extracts was determined by sequencing hypervaiable V3-V4 region of 16S rRNA gene region using Illumina iSeq 100 system. Samples were prepared for 16S metagenomic sequencing library according to Illumina guide (Illumina, Inc., California, USA). Primers with overhang adapter sequences were used in PCR amplification of V3-V4 region of rRNA gene region by KAPA HiFi

HS Mix (Roche). Index-PCR was performed using the Nextera® XT index Kit v2 Set-A (Illumina). Amplicon PCR and index-PCR products were cleaned using AMPure XP beads (Beckman Coulter). Cleaned DNA samples were quantified using AccuBlue® NextGen dsDNA Quantitation kit (Biotium, Inc. USA) as described by manufacturer's instructions using multimode plate reader (Mithras^2 LB943, Berthold, Germany). The concentration of library diluted to 35 pM. The amplicon sequencing was performed on iSeq100 system (Illumina).

Primers used in the PCR analysis:
F-primer 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGG NGGCWGCAG-3′ and
R-primer 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′

The results were analyzed by "basespcae 16S metagenomics" package Version 1.1.0 (Illumina inc.) and processed by SPSS 26 (IBM inc.). Principal Components Analysis (PCoA) was performed by rotation method varimax with Kaiser normalization.

References

1. B. G. Tuna, P. B. Atalay, G. Kuku, E. E. Acar, H. K. Kara, M. D. Yilmaz and V. C. Ozalp, *RSC Advances*, 2019, **9**, 36005-36010.
2. R. F. Wang, M. L. Beggs, L. H. Robertson and C. E. Cerniglia, *FEMS Microbiol Lett*, 2002, **213**, 175-182.