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Supplementary Materials for

Bioinspired oral delivery of gut microbiota by self-coating with biofilms

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Figs. S1 to S10



Figure S1 Zeta potential characterization. Zeta potential was analyzed by dynamic light scattering (Malvern Zetasizer nano ZS, UK). Error bars represent standard deviation (n = 3). Significance was assessed using Student's t-test, giving p values, p<0.05, *.



Figure S2 Resistance of the bacteria against environmental assaults. **a**, **b** TEM images of BS, FCBS and BCBS after culture in (**a**) SGF (pH 1.2) and (**b**) bile salts (0.3 mg/ml) at 37 °C for the indicated time points. Scale bar: (**a**) 2 μ m, (**b**) 5 μ m. **c** Equal amounts of BS (circle), FCBS (square) and BCBS (triangle) were separately exposed to ampicillin (10 mg/ml). After incubation at 37 °C for the indicated time points, 50 μ l of each sample was washed twice with PBS, spread onto LB agar plates and incubated at 37 °C for 24 hours before bacterial counting. Error bars represent standard deviation (*n* = 3). Significance was assessed using Student's t-test, giving p values, p<0.05, *; p<0.01, **; p<0.001.



Figure S3 Mucoadhesion of BCBS in mouse intestines. Representative microscopic images of Gram staining of the intestines harvested from mice orally delivered with 1 \times 10⁷ CFUs of bacteria at 24 hours post-administration. Scale bar: 200 µm.



Figure S4 Bacterial viability. TEM images of BS, FCBS and BCBS after culture with SIF at 37 $^{\circ}$ C for the indicated time points. Scale bar: 5 μ m.



Figure S5 Biosafety in mice. Representative H&E staining images of mouse intestines harvested from mice orally administrated with 1×10^7 CFUs of bacteria at 120 hours post-administration. Scale bar: 200 µm.



Figure S6 Biosafety in swine. Representative H&E staining images of intestines harvested from swine orally administrated with 3.5×10^8 CFUs of bacteria at day 8 post-administration. Scale bar: 200 µm.



Figure S7 Cytokine assays. Liver function tests including ALT, AST and r-GT. Kidney function tests containing D-BIL, SCR, BUN and GLU. The blood samples were withdrawn from swine orally administrated with 3.5×10^8 CFUs of bacteria at day 8 post-administration. Circle, square and triangle represent BS, FCBS and BCBS, respectively.



Figure S8 Intestinal colonization of *S. aureus* **in mice. a** Quantities of the colonized *S. aureus* in feces separately collected from BALB/c (tetragon) and C57BL-6 (hexagon) at the indicated time points. **b** Colonization of *S. aureus* in mouse intestines harvested at day 7 post-administration of 1×10^9 CFUs of *S. aureus*.



Figure S9 Treatment of intestinal colonization of *S. aureus*. Typical microscopic images of *S. aureus* colonized in the bowel wall 7 days post-treatment. Green fluorescence represents *S. aureus* colonized in the intestines. Scale bar: 200 µm.



Figure S10 Reservation of BCBS in mice colonized with *S. aureus.* a-c Numbers of the colonized probiotics in (a) feces collected at the indicated time points as well as intestinal (b) contents and (c) tissues sampled 7 days post-treatment. Circle, square and triangle represent BS, FCBS and BCBS, respectively. d Amounts of *S. aureus* invaded in blood sampled from the mice 7 days post-treatment. Error bars represent standard deviation (n = 5). Significance was assessed using Student's t-test, giving p values, p<0.05, *; p<0.01, **; p<0.001, ***.