Supporting Information

Escherichia coli Colonization of Intestinal Epithelial Layers In Vitro in the Presence of Encapsulated *Bifidobacterium breve* for its Protection Against Gastro-intestinal Fluids and Antibiotics

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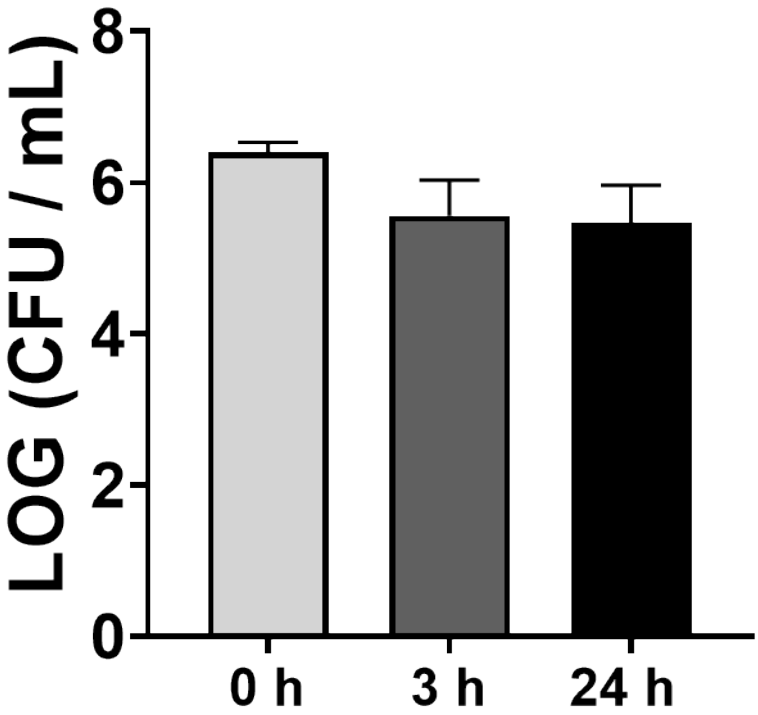


Figure S1. Log (CFU/mL) of *E. coli* Hu734 (3 x 10^6 /mL) exposed to tetracycline (10 μg/mL) for 3 and 24 h in optimized medium, demonstrating tetracycline-resistance. Error bars represent standard deviation over three experiments with separately grown bacteria.

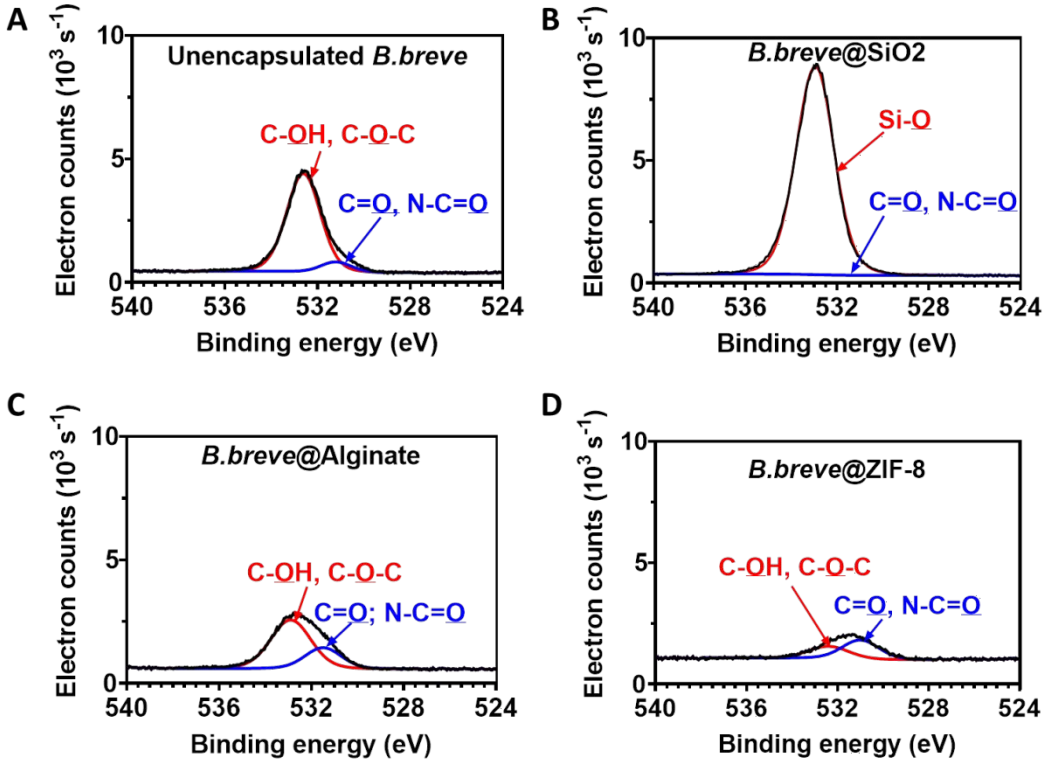


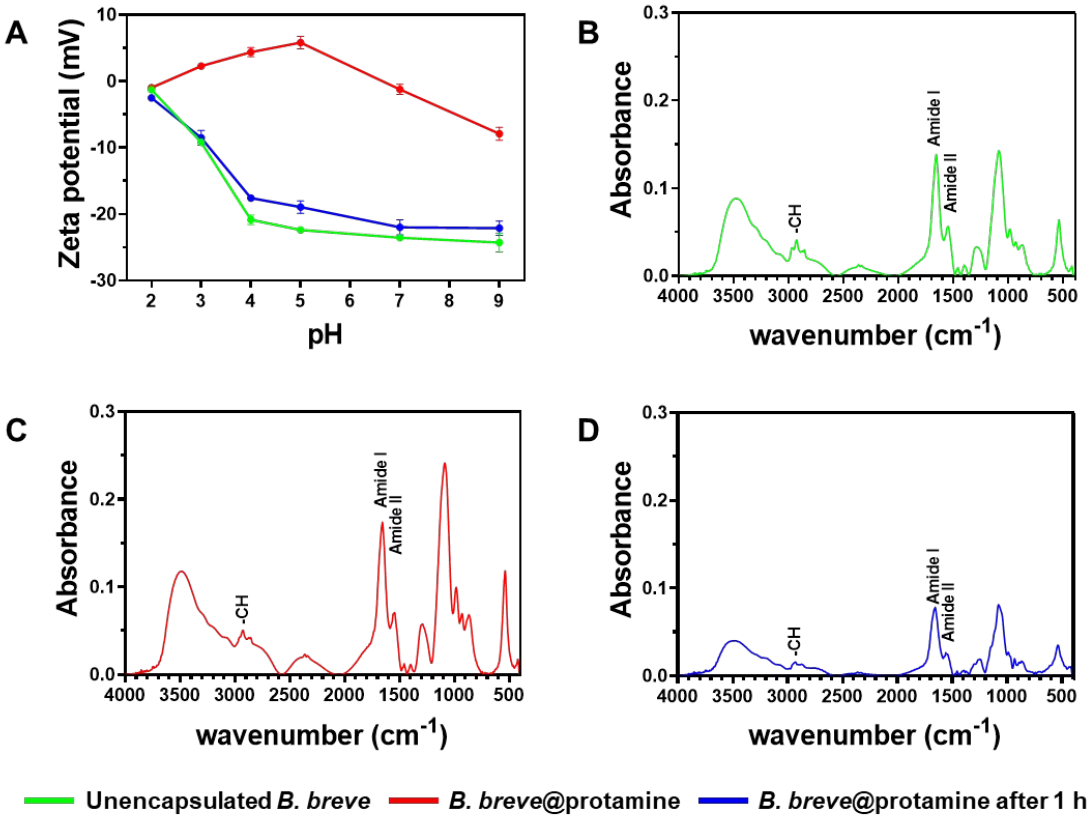
Figure S2. Narrow-scan O_{1s} photoelectron binding energy spectra of unencapsulated and differently encapsulated *B. breve,* decomposed into two components for oxygen containing functionalities at 531.5 and 533.0 eV for =O and -O, respectively.

(A) Unencapsulated *B. breve*.

(B) *B. breve*@SiO_2.

(C) *B. breve*@Alginate. 20 µL alginate droplets were used for encapsulation.

(D) *B. breve*@ZIF-8.



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Samples	AmI/CH	AmII/CH
B. breve	3.8	0.8
B. breve@protamine	6.2	1.3
B. breve@protamine after 1 h	7.6	1.0

Figure S3. Zeta potentials and FTIR spectra of *B. breve* ATCC15700 prior to and after protamine adsorption and internalization.

(A) Zeta potentials of *B. breve* prior to and after protamine adsorption and internalization measured in phosphate buffer (5 mM K_2HPO_4, 5 mM KH_2PO_4) as a function of pH. Error bars indicate standard deviations over triplicate experiments with separately cultured bacteria.

(B) FTIR absorption spectrum of unencapsulated *B. breve* prior to protamine adsorption.

(C) FTIR absorption spectrum of *B. breve* taken immediately after protamine adsorption (*B. breve*@protamine).

(D) FTIR absorption spectrum of *B. breve* taken 1 h after protamine internalization (*B. breve*@protamine after 1 h).

(E) FTIR absorption band ratios of unencapsulated *B. breve* and *B. breve* immediately after protamine adsorption (*B. breve*@protamine) and 1 h after protamine adsorption (*B. breve*@protamine after 1 h). Absorption band ratios were normalized with respect to the CH stretching region around 2930 cm^{-1}. -CH, $-CH_2$, $-CH_3$ band at 2930 cm^{-1}; amide I band at 1653 cm^{-1}; amide II band at 1541 cm^{-1} after scaling the spectra to similar base-line levels using SpectraGryph 1.2.

Method: 3×10^8 mL^{-1} *B. breve* suspended in 1 mg/mL protamine solution were vortexed (1 min) and kept at room temperature for 15 min. FTIR spectra were taken of unencapsulated *B. breve*, *B. breve* immediately after protamine adsorption and 1 h after protamine adsorption while keeping bacteria with adsorbed protamine in RCM medium under anaerobic conditions (85% N_2, 5% CO_2, and 10% H_2) at 37°C for 1 h. FTIR was conducted on freeze-dried bacteria (see Section "2.4 Elemental surface composition and zeta potentials of encapsulated bacteria" in the main manuscript) using an Agilent Cary 600 series FTIR spectrometer (Agilent Technologies, USA). Freeze-dried bacteria

were mixed with KBr powder and pressed into a tablet and spectra were recorded between 4000 and 400 cm^{-1} at a spectral resolution of 4 cm^{-1}. 32 scans were measured and averaged for each sample and resulting spectra were processed with the SpectraGryph 1.2 software. The areas of the absorption bands were calculated and normalized with respect to the integrated intensity of the entire CH stretching region around 2930 cm^{-1} after scaling the spectra to similar base-line levels.

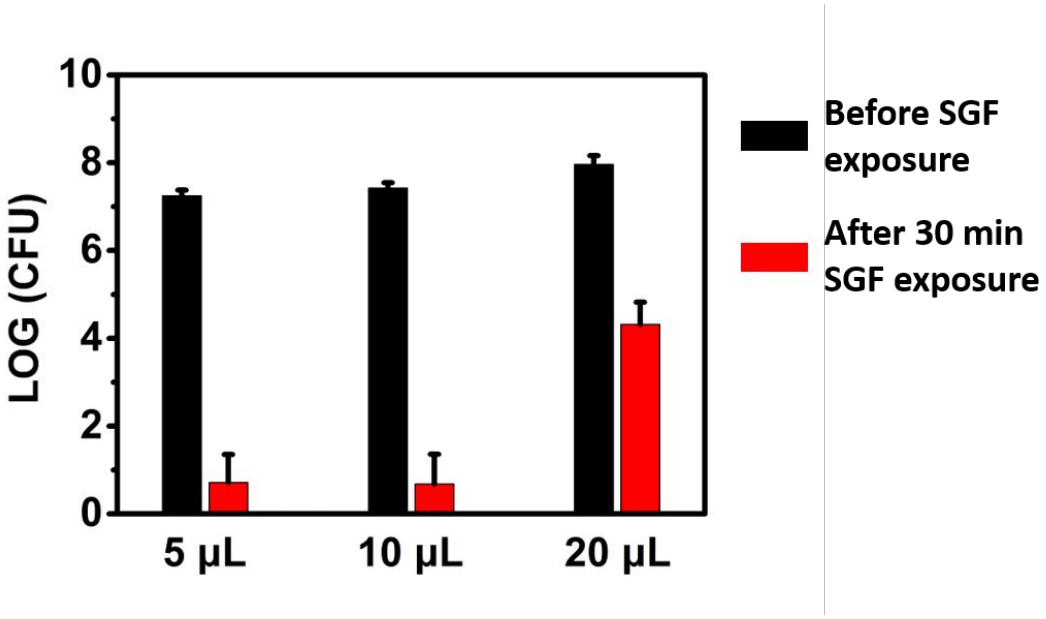

Figure S4. Survival of *B. breve* encapsulated in alginate-hydrogel shells using alginate droplets with different volumes (5 µL, 10 µL and 20 µL) for encapsulation during 30 min exposure to simulated gastric fluid at pH 2. Error bars represent standard errors of mean over three experiments with separately grown bacteria.

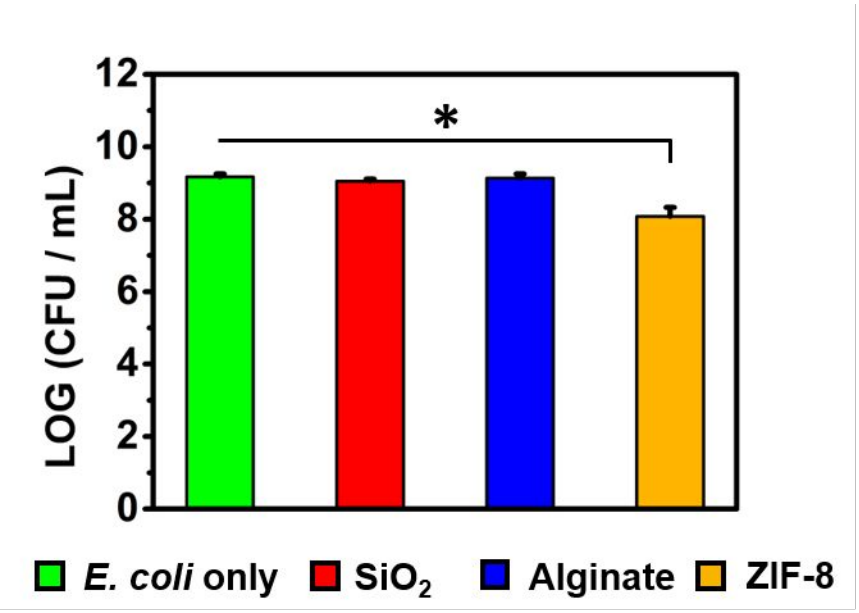

Figure S5. Survival of *E. coli* after culturing with different shell components for 24 h. Error bars represent standard errors of mean over three experiments with separately grown bacteria. * indicate statistically significant differences ($p < 0.05$, Student t-test) with respect to *E. coli* cultured in absence of any shell components.

Method: Pure alginate beads were prepared by dropping 20 μL alginate solution (25 mg/mL) into 0.1 M $CaCl_2$ and stirring for 30 min at room temperature. ZIF-8 suspension was synthesized by mixing 5 mL 0.16 M 2-methylimidazole solution and 5 mL 0.04 M zinc acetate solution. The mixture was shake at 37 ℃, 150 rpm for 10 min. *E. coli* was suspended in modified growth medium (70% cell culture medium supplemented with 30% RCM) to a final concentration of 3×10^6 mL^{-1}. To culture *E. coli* with different shell components, 50 μL 10 mg/mL SiO_2, alginate beads and ZIF-8 suspension were added into bacterial

suspension, respectively and cultured at 37 ℃, 5% CO_2 for 24 h. For *E. coli* only group, 50 μL of PBS was added. After the culture, *E. coli* was collected by centrifugation (6500 g at 10 °C for 5 min) and re-suspended in 10 mM potassium phosphate buffer. Serial dilutions of resulting *E. coli* suspensions were made in phosphate buffer and plated on LB agar. After aerobic culturing of the LB agar plates, the numbers of colony forming units (CFU) were enumerated.

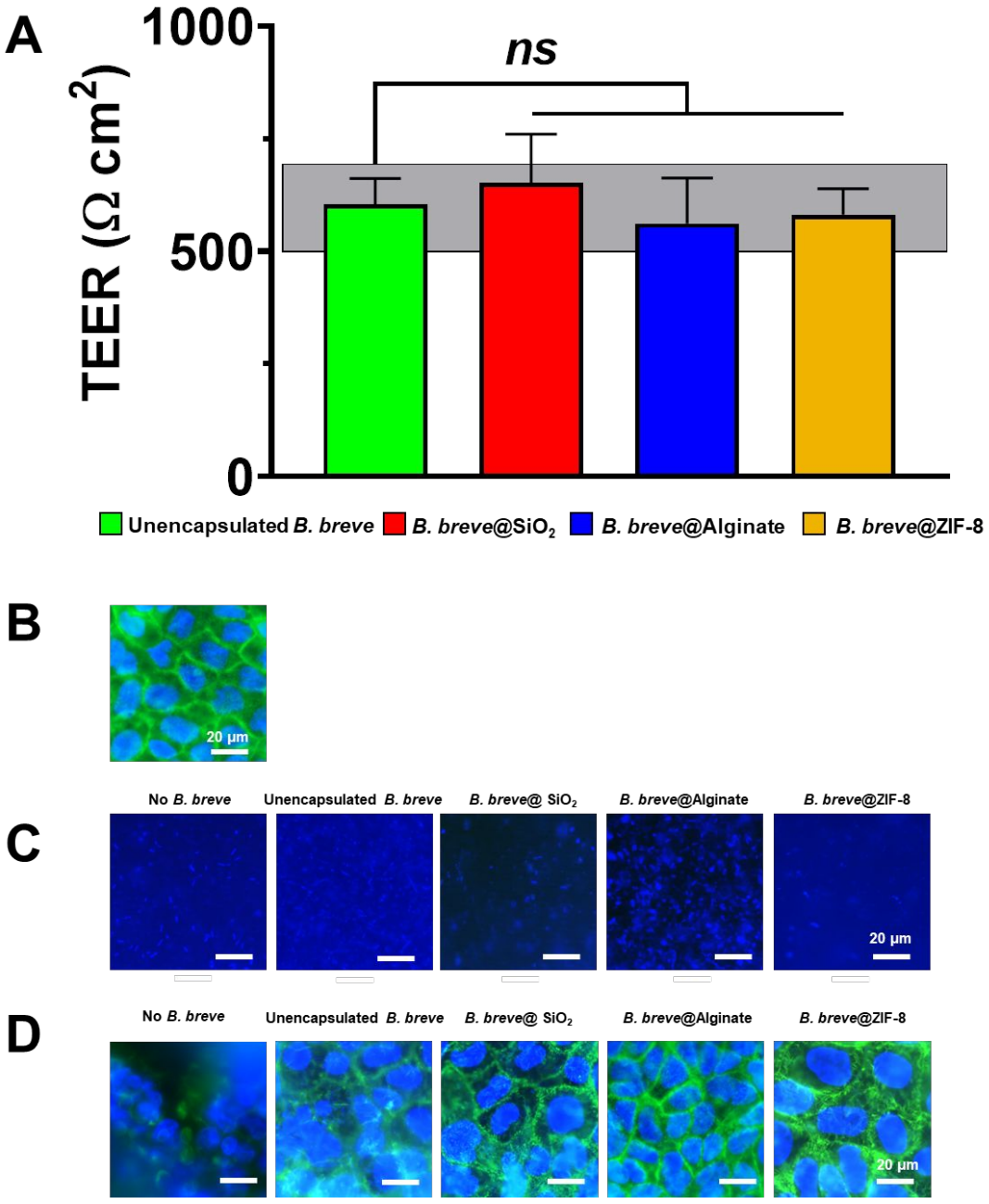

Figure S6. Effects of unencapsulated and differently encapsulated *B. breve* ATCC15700 adhering on intestinal epithelial layers on TEER and membrane surface coverage of the epithelial layers grown on transwell-membranes.

(A) TEER values of epithelial cell layers 24 h after 24 h adhesion of unencapsulated and differently encapsulated *B. breve* in absence of an E. coli

challenge. The horizontal band represents the TEER value of epithelial cell layer in absence of adhering *B. breve*. Error bars represent standard deviation over three experiments with separately grown cell layers and bacteria.

(B) Fluorescence micrograph of intestinal cell layers on a transwell-membrane with or without adhering *B. breve* in absence of an *E. coli* challenge.

(C) Fluorescence micrographs of intestinal epithelial cells stained with phalloidin (F-actin, green) and DAPI (DNA, blue) with adhering *B. breve* and challenged by *E. coli* in absence of tetracycline exposure.

(D) Same as panel (C), but now representing surface coverage by intestinal epithelial cells of the transwell-membrane in presence of tetracycline exposure.