Support Information

Metabolic Labeling of Peptidoglycan Enabled Optical Analysis of Probiotic Vitality

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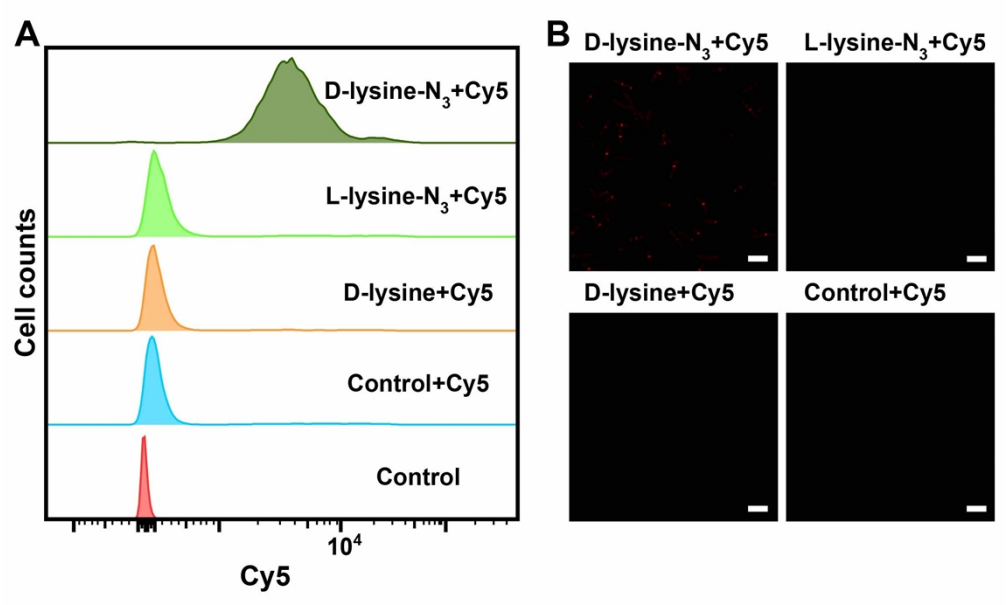

Figure S1. (A) Representative flow cytometry results of metabolic labeling on *L. paracasei* with the addition of different structures of amino acids. (B) Representative confocal images of *L. paracasei* via metabolic labeling with different conditions. Red, DBCO-Sulfo-Cy5. Scale bar, 5 μm.

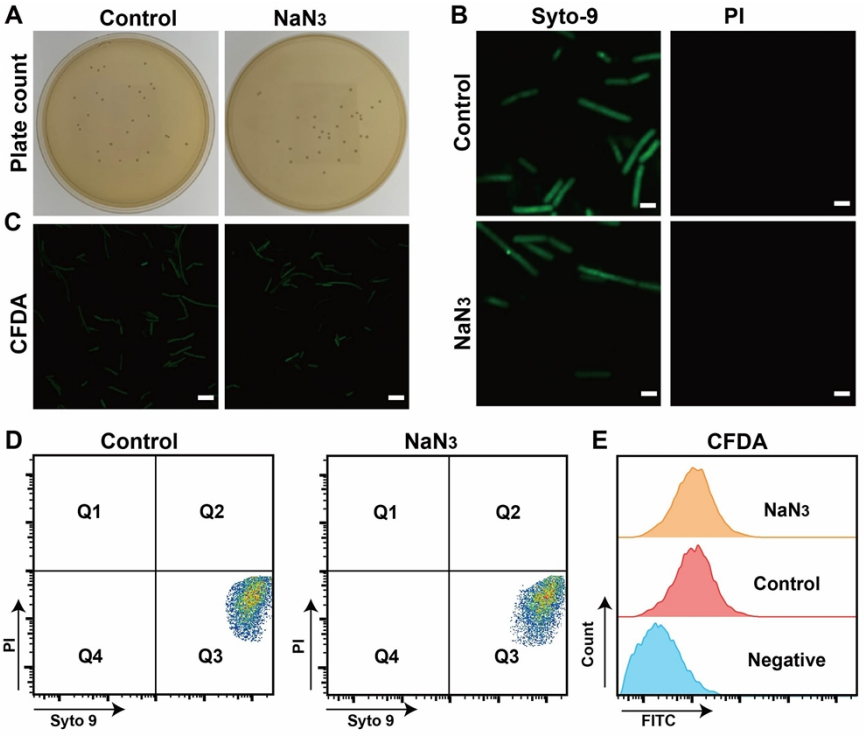

Figure S2. (A) Representative standard plate count results of *L. paracasei* after NaN_3 treatment or not. (B) Representative confocal images of *L. paracasei* with live and dead staining after NaN_3 treatment or not. Green, Syto-9; Red, Propidium iodide. Scale bar, 2

μm. (C) Representative confocal images of *L. paracasei* with CFDA staining after NaN_3 treatment or not. Green, CF (carboxyfluorescein). Scale bar, 5 μm. (D) Representative flow cytometry results showing bacterial live and dead staining of *L.paracasei* after NaN_3 treatment or not. (E) Representative flow cytometry results showing CFDA staining of *L.paracasei* with NaN_3 treatment or not. Negative refer to control without the addition of CFDA.

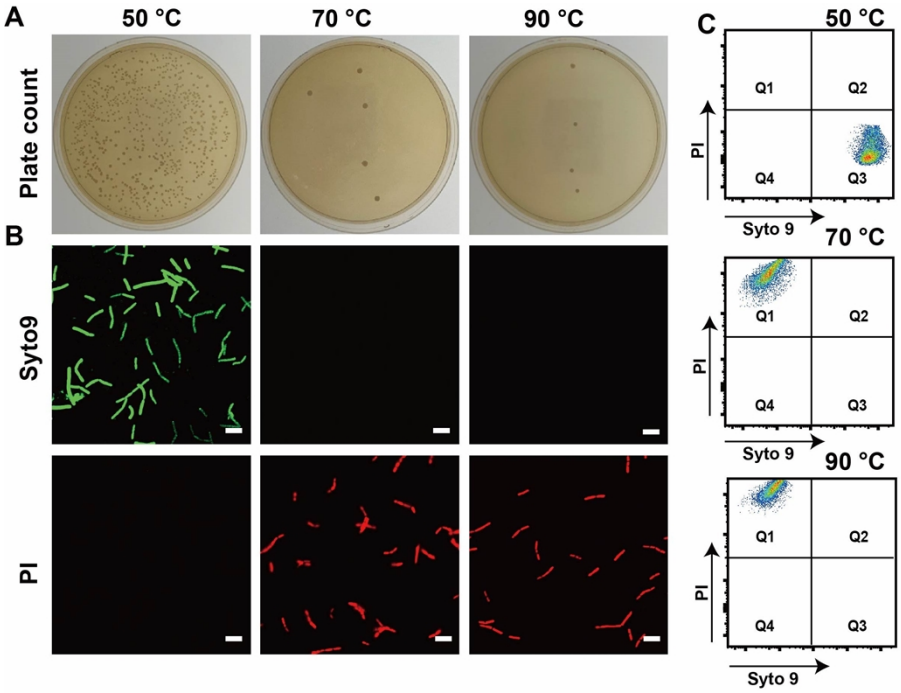

Figure S3. (A) Representative standard plate count results of *L. paracasei* after treatment at different temperatures. (B) Representative confocal images of *L. paracasei* with live and dead staining after treatment at different temperatures. Green, Syto-9; Red, Propidium iodide. Scale bar, 5 μm. (C) Representative flow cytometry results showing bacterial live and dead staining of *L.paracasei* after treatment at different temperatures.

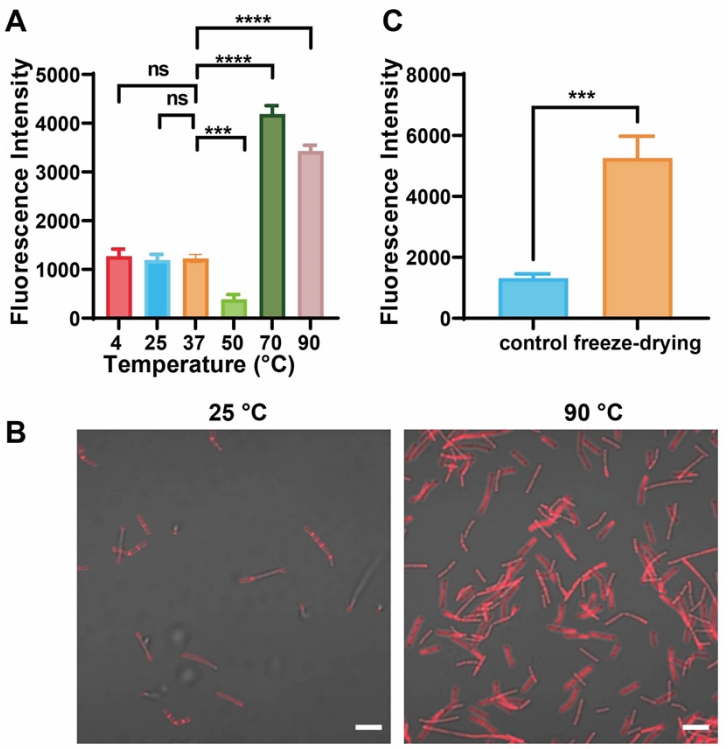

Figure S4. (A) Fluorescence intensity of bacterial solutions via metabolic labeling after treatment of different temperatures (n=3). ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (B) Representative confocal images of *L. paracasei* via metabolic labeling after treatment at 25 °C and 90 °C. Red, DBCO-Sulfo-Cy5.Scale bar, 5 μm. (C) Fluorescence intensity of bacterial solutions via metabolic labeling after the operation of lyophilization or not (n=3). ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

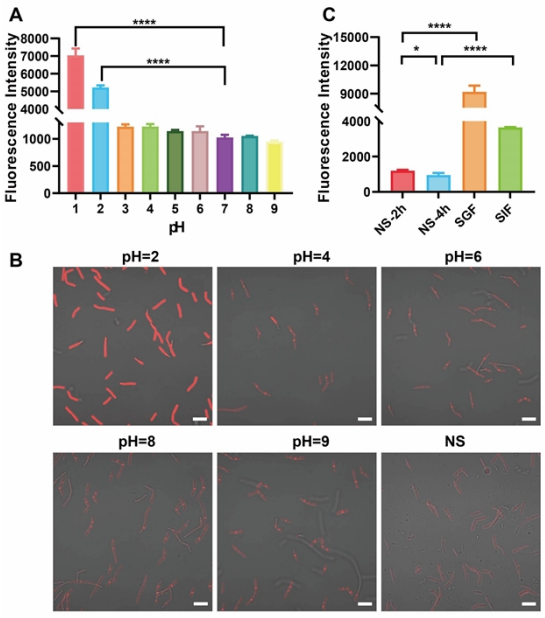

Figure S5. (A) Fluorescence intensity of bacterial solutions through metabolic labeling after treatment of different pH values (n=3). ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001,

****p<0.0001. (B) Representative confocal images of *L. paracasei* via metabolic labeling after treatment at different pH values and culture medium. Red, DBCO-Sulfo-Cy5.Scale bar, 5 μm. (C) Fluorescence intensity of bacterial solutions via metabolic labeling after the treatment of SGF or SIF (n=3). ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

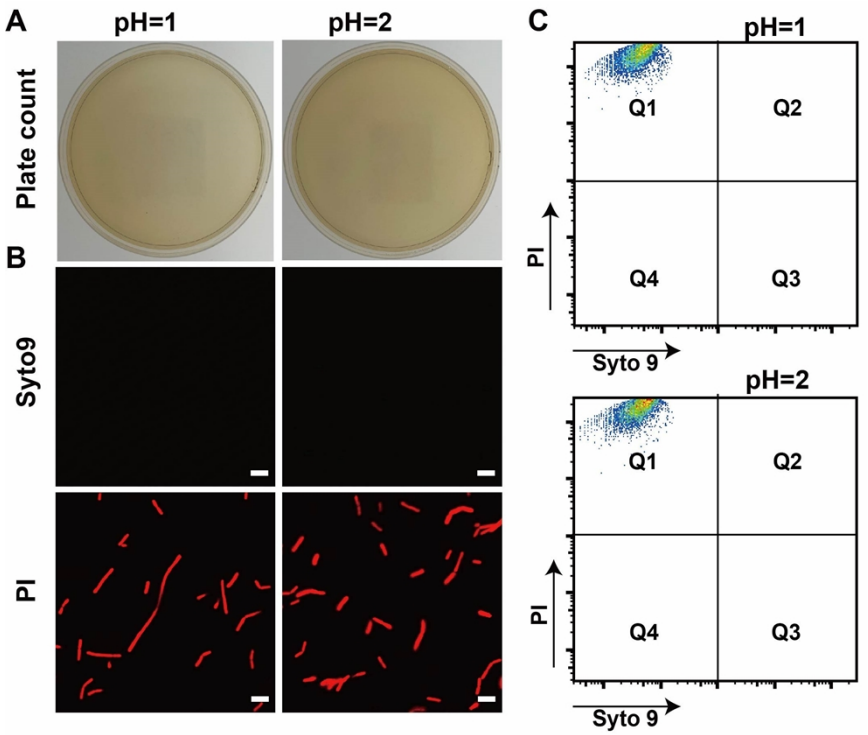

Figure S6. (A) Representative standard plate count results of *L. paracasei* after treatment at different pH values. (B) Representative confocal images of *L. paracasei* with live and dead staining after the treatment of pH=1 and 2. Green, Syto-9; Red, Propidium iodide. Scale bar, 5 μm. (C) Representative flow cytometry results showing bacterial live and dead staining of *L.paracasei* after the treatment of pH=1 and 2.

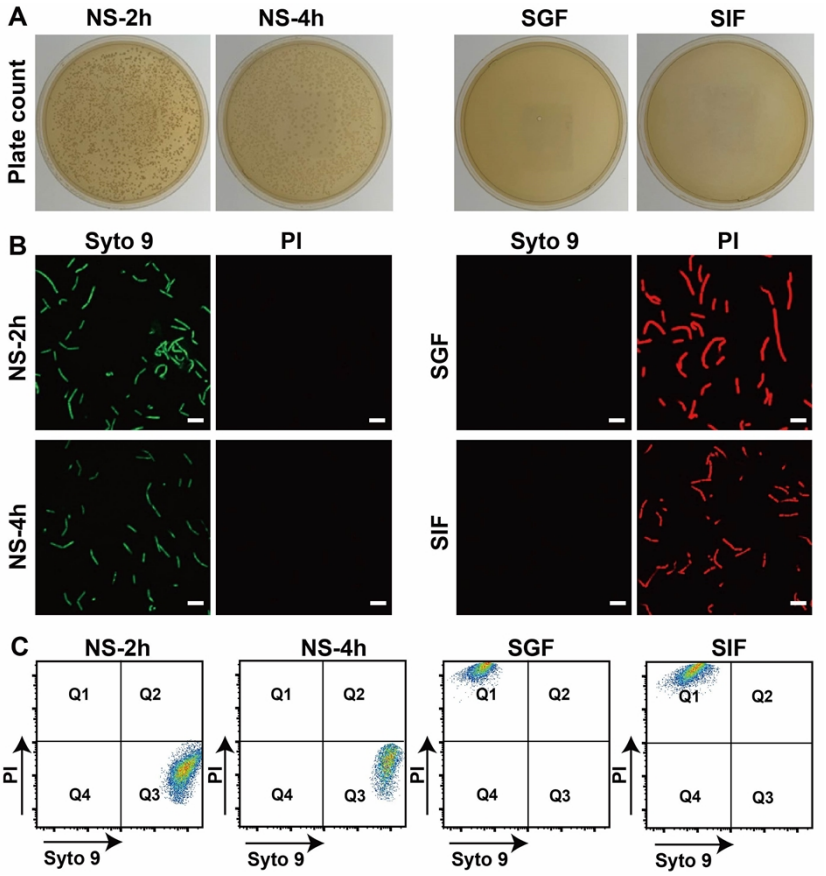

Figure S7. (A) Representative standard plate count results of *L. paracasei* after treatment in normal saline for 2h (NS-2h), SGF for 2h, normal saline for 4h (NS-4h), and SIF for 4 h. (B) Representative confocal images of *L. paracasei* with live and dead staining after being cultured in normal saline for 2h (NS-2h), SGF for 2h, normal saline for 4h (NS-4h), and SIF for 4 h. Green, Syto-9; Red, Propidium iodide. Scale bar, 5 μm. (C) Representative flow cytometry results showing bacterial live and dead staining of *L.paracasei* after the treatment in normal saline for 2h (NS-2h), SGF for 2h, normal saline for 4h (NS-4h), and SIF for 4 h.

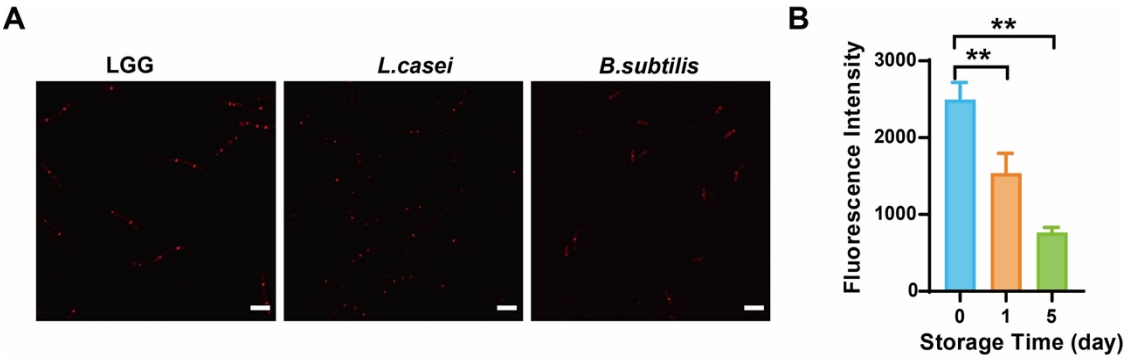

Figure S8. (A) Representative confocal images of *L. rhamnosus* GG, *L. casei* and *B. subtilis* via metabolic labeling under normal culturing conditions. Red, DBCO-Sulfo-Cy5.

Scale bar, 5 μm. (B) Fluorescence intensity of the bacterial solutions via metabolic labeling with different storage time (n=3). $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.