Supplementary Information

# Chemical reaction-mediated covalent localization of bacteria

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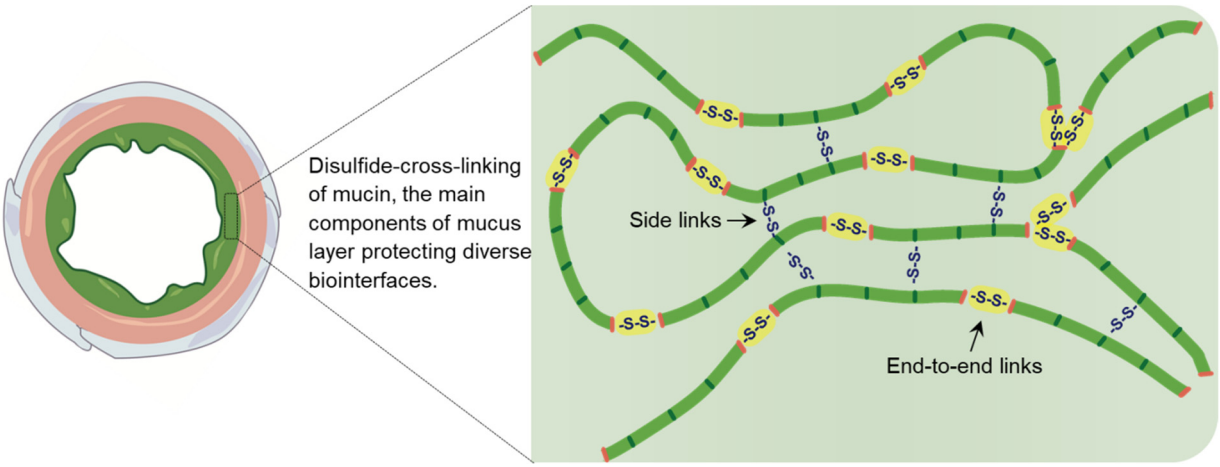
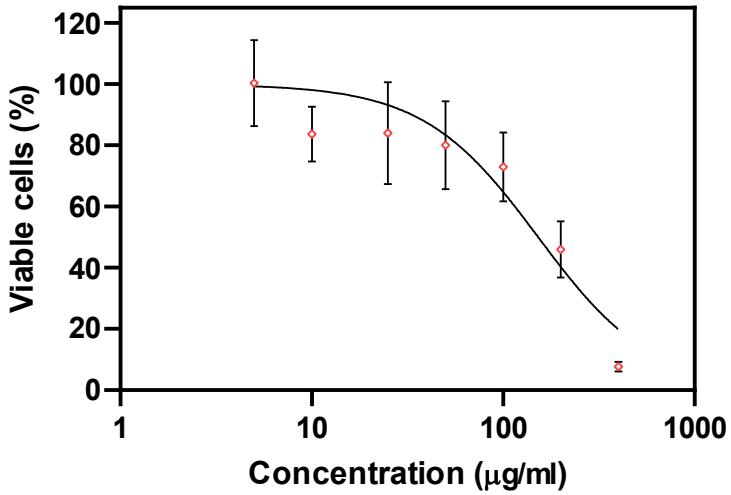
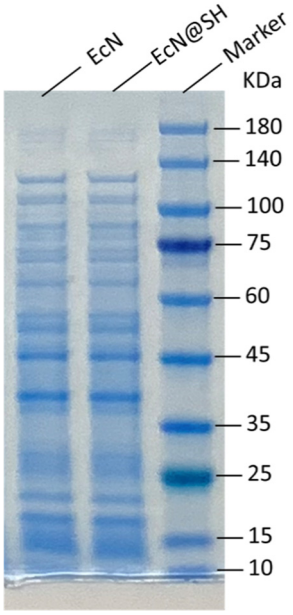


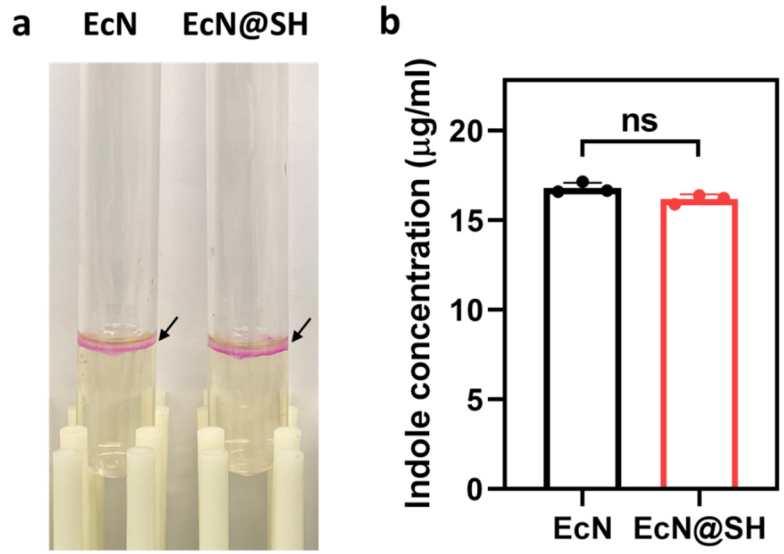

**Supplementary Fig. 1.** Structure of mucins. Mucins are an important category of heavily glycosylated proteins existing at various tissue interfaces, with cysteine-rich domains in their N and C termini that mediate polymer extension by end-to-end disulfide linkage of mucin monomers. Cysteine-rich regions locating in internal domains also contribute to disulfide side links between intermediate cysteine thiols.



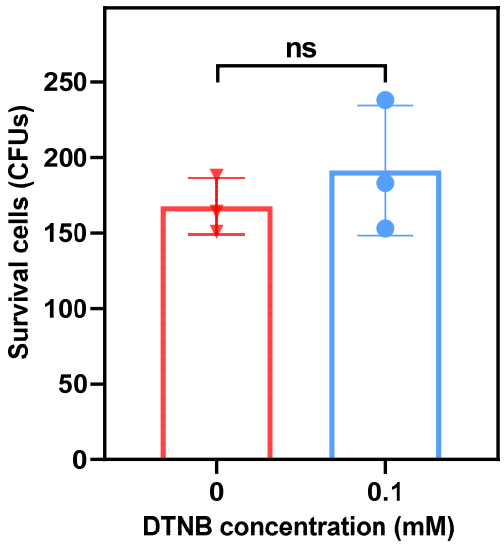

**Supplementary Fig. 2.** Representative curve of bacterial survival after reaction with 2-iminothiolane ($IC_{50}$ 152.3 μg/ml). The number of viable cell was counted by bacterial plating counting. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Source data are provided in the Source Data file.



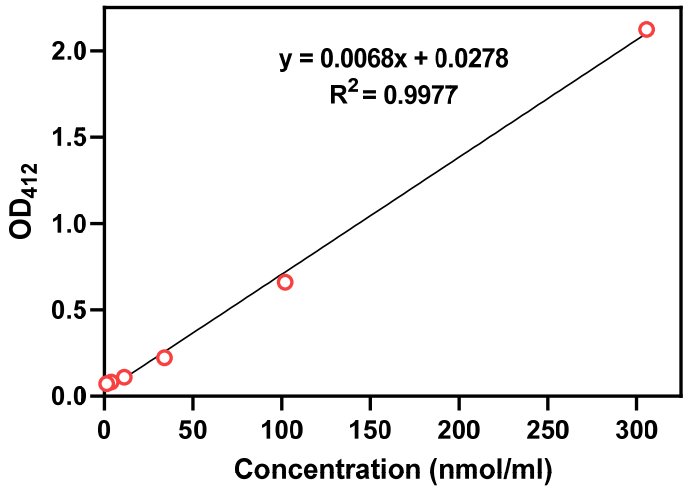

**Supplementary Fig. 3.** Coomassie blue staining of the total proteins extracted from native and thiolated EcN, respectively. Images are representative of three independent biological samples.



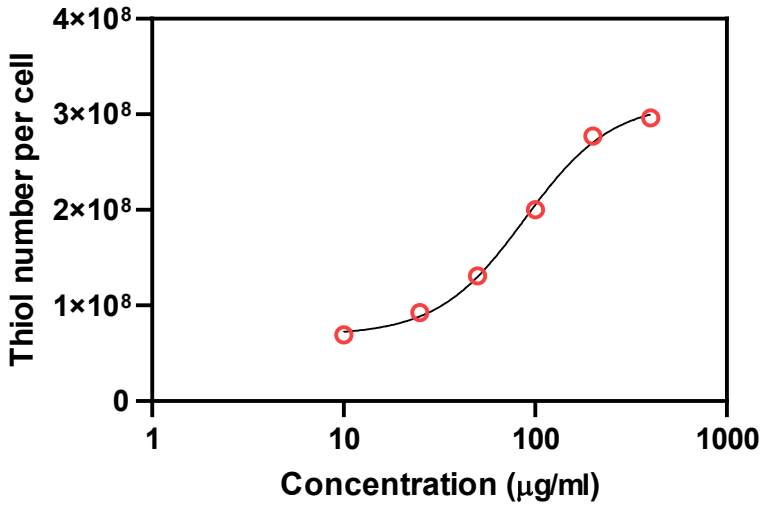

**Supplementary Fig. 4. a** Digital photos of the indole test on the tryptophan metabolism of native and thiolated EcN. Black arrows indicate the purple indole ring of positive results. **b** Quantitative detection of indole production by HPLC. Data are presented as mean values ± SD ($n$ = 3, from independent biological samples). Significance was assessed using Student's $t$-test (two-tailed). ns, no significance. Source data are provided in the Source Data file.



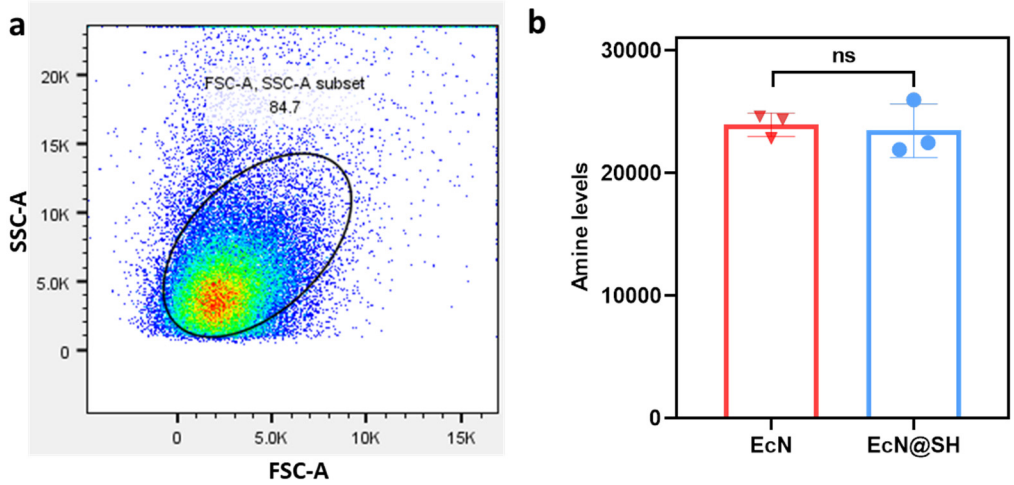

**Supplementary Fig. 5.** Survival cells after treatment with 0.1 mM DTNB for 2 hours. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Significance was assessed using Student's $t$-test (two-tailed). ns, no significance. Source data are provided in the Source Data file.



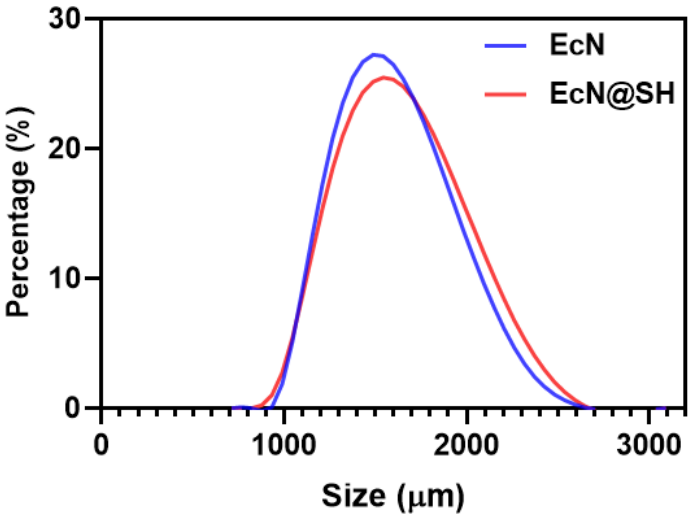

**Supplementary Fig. 6.** Standard curve for quantitatively measuring thiol content in varied concentrations of L-cysteine using Traut's reagent.



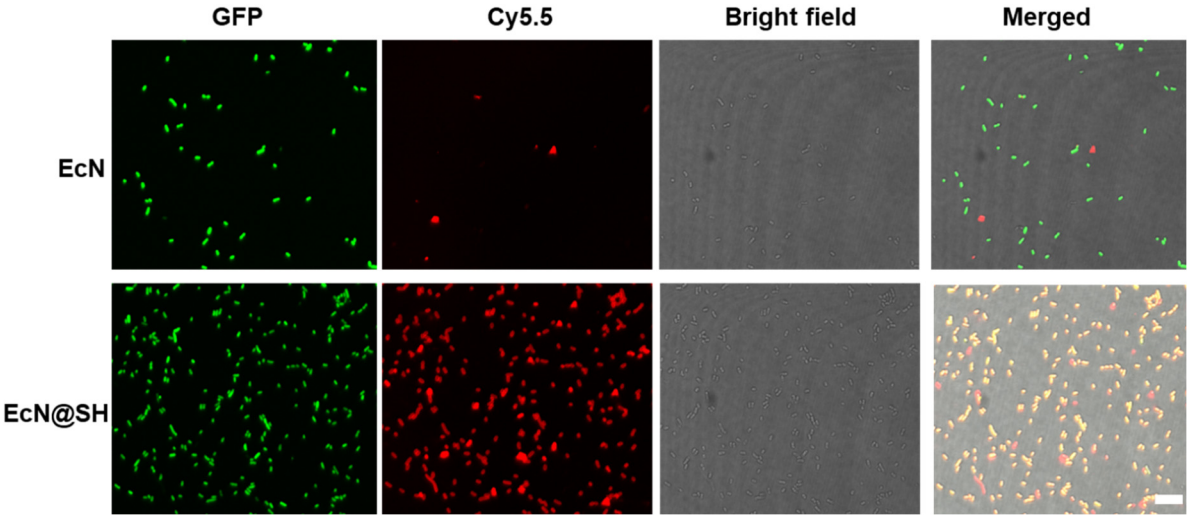

**Supplementary Fig. 7.** Representative curve of thiol number per bacterium after reaction with 2-iminothiolane ranging from 0 to 400 μg/mL for 90 minutes ($EC_{50}$ 88.3 μg/ml). Source data are provided in the Source Data file.



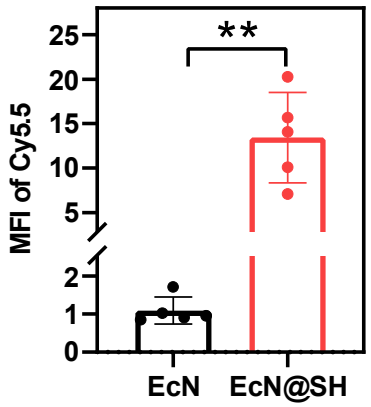

**Supplementary Fig. 8. a** Gate strategy to select bacterial cells for Cy5-NHS labelling using FSC-A and SSC-A. Dead cells were excluded and further analysis were performed on the live cell population. **b** Amine levels of native EcN and EcN@SH measured by flow cytometry using Cy5-NHS labelling. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Significance was assessed using Student's $t$-test (two-tailed). ns, no significance. Source data are provided in the Source Data file.



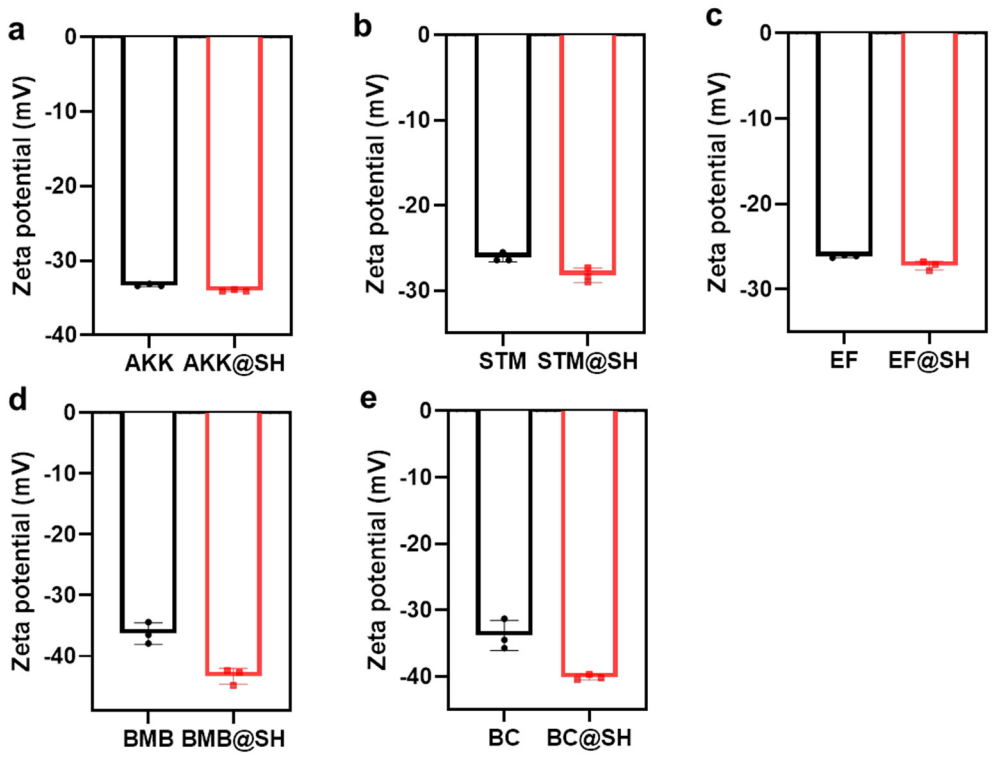

**Supplementary Fig. 9.** Size distributions of native EcN and EcN@SH measured by DLS. Source data are provided in the Source Data file.



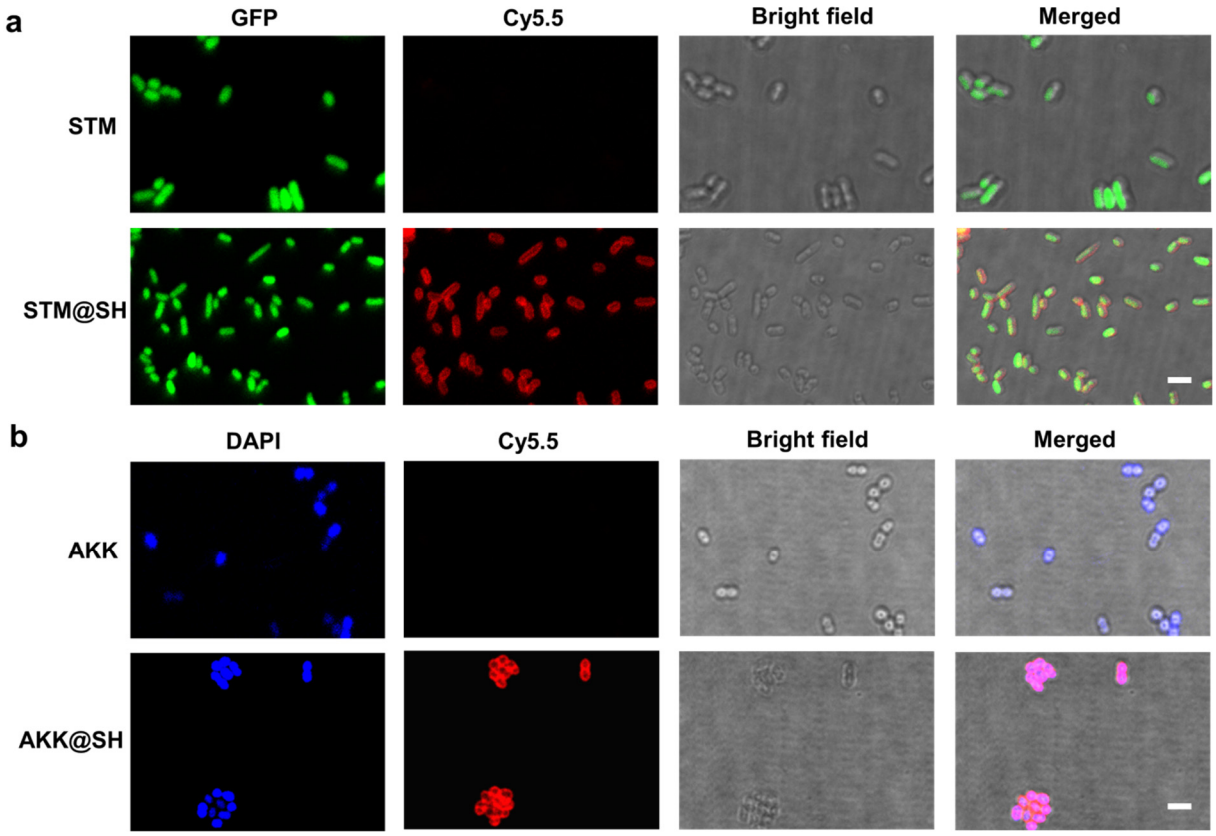

**Supplementary Fig. 10.** LSCM images of unmodified EcN and thiolated EcN@SH post labelling with Cy5.5-maleimide. Images are representative of three independent biological samples. Green and red channels separately show inherent GFP of EcN and Cy5.5-maleimide chemically-stained thiols layer. Scale bar: 10 μm.



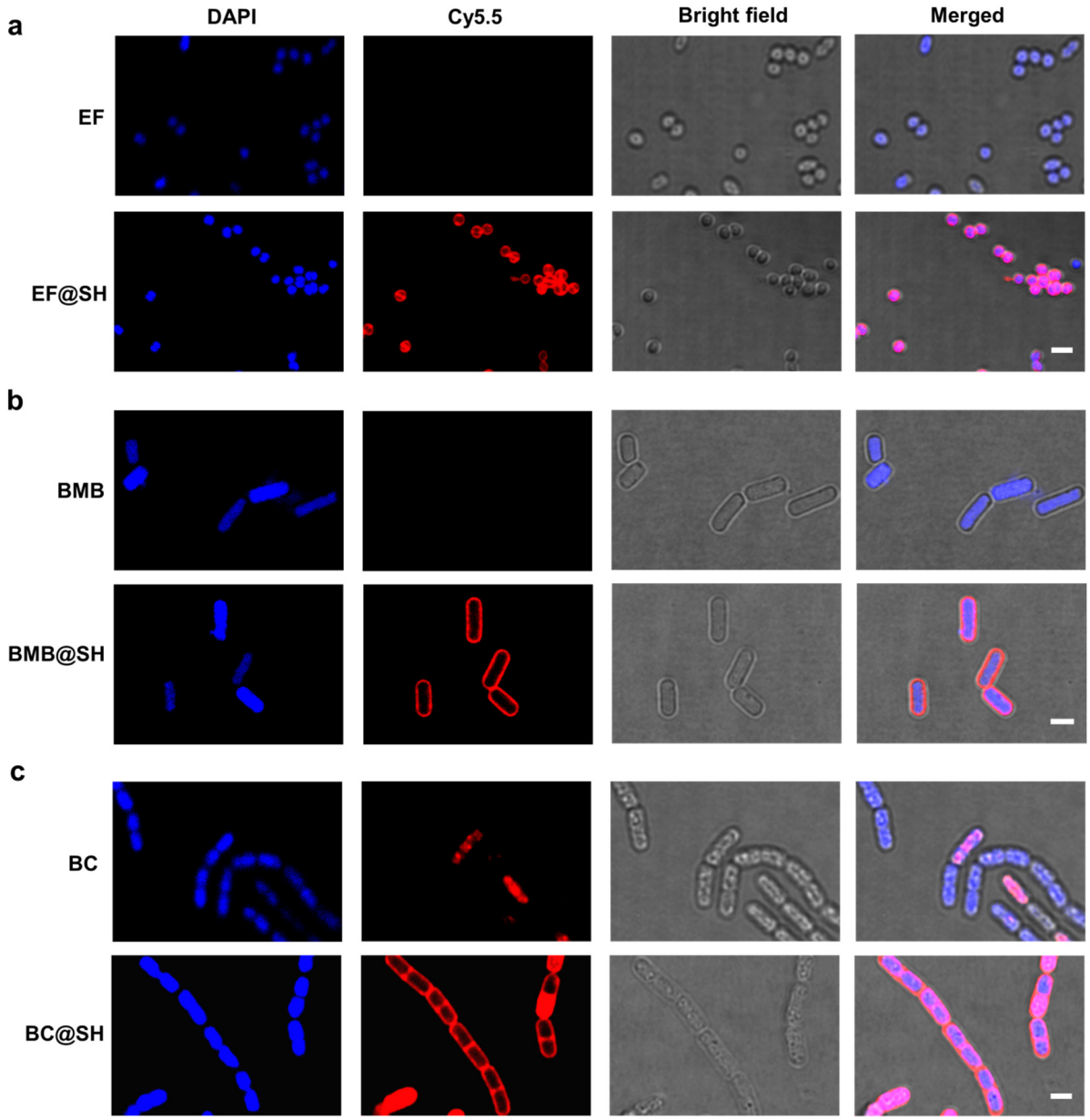

**Supplementary Fig. 11.** Mean fluorescence intensity (MFI) of Cy5.5-maleimide labelled thiol groups on EcN and EcN@SH by confocal analysis. Data are presented as mean values ± SD ($n$ = 5, from independent fields). Significance was assessed using Student's $t$-test (two-tailed), giving $p$ values, $^{**}p < 0.01$. Source data and the exact $p$ values are provided in the Source Data file.



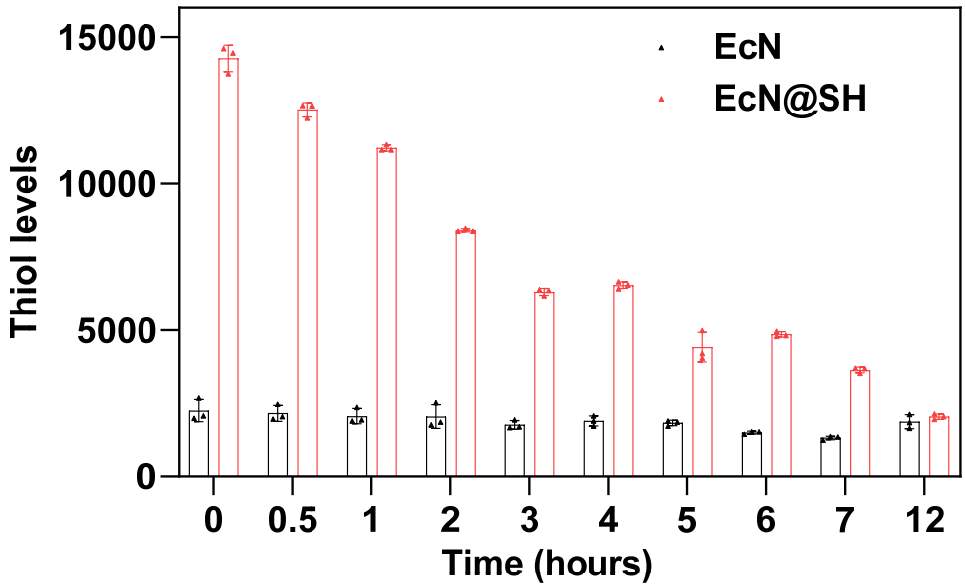

**Supplementary Fig. 12.** Zeta potentials of native and thiolated bacteria including (**a**) AKK, (**b**) STM, (**c**) EF, (**d**) BMB, and (**e**) BC. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Source data are provided in the Source Data file.



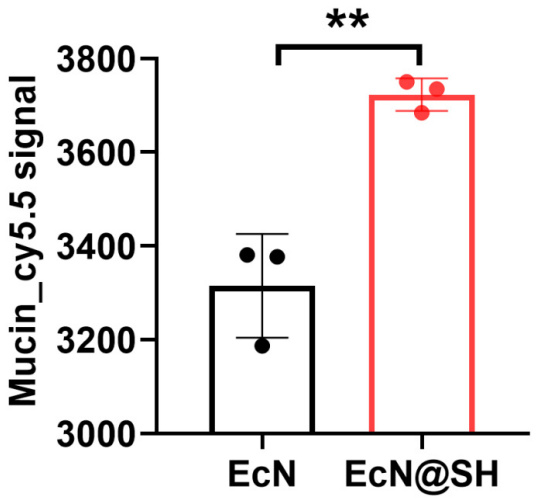

**Supplementary Fig. 13.** Typical LSCM images of unmodified and thiolated Gram negative bacteria after reaction with Cy5.5-maleimide, including **a** STM and **b** AKK. Images are representative of three independent biological samples. Scale bar: 2 μm.



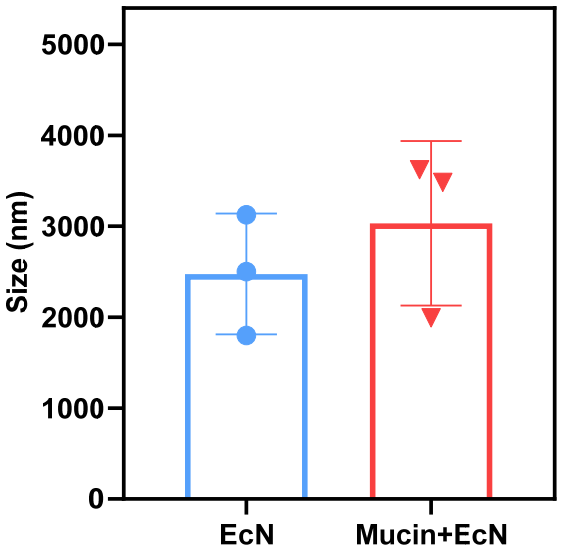

**Supplementary Fig. 14.** Typical LSCM images of unmodified and thiolated Gram positive bacteria after reaction with Cy5.5-maleimide, including **a** STM, **b** BMB, and **c** BC. Images are representative of three independent biological samples. Scale bar: 2 μm.



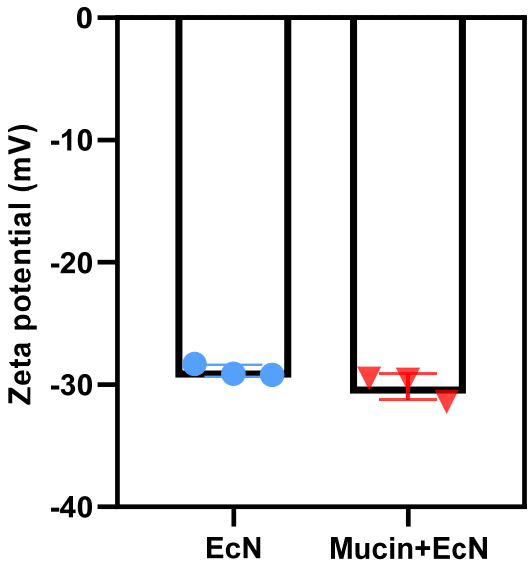

**Supplementary Fig. 15.** Retention of thiol levels on native EcN and EcN@SH over time measured by flow cytometry using SH-sensitive and clickable marker Cy5.5-maleimide. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Source data are provided in the Source Data file.



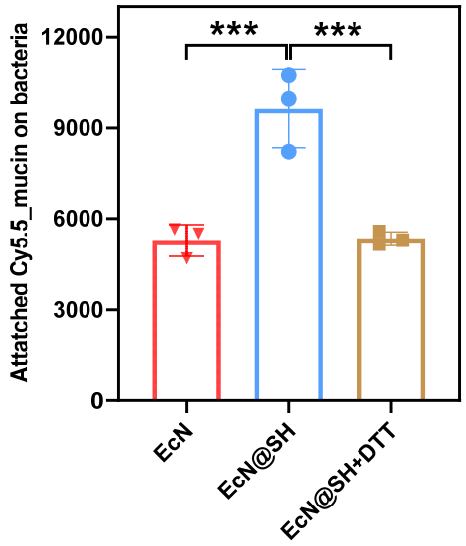

**Supplementary Fig. 16.** Flow cytometric analysis of native EcN and EcN@SH after reaction with Cy5.5_mucin (0.1 mg/ml) for 1 hour. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Significance was assessed using Student's $t$-test (two-tailed), giving $p$ values, $^{**}p < 0.01$. Source data and the exact $p$ values are provided in the Source Data file.



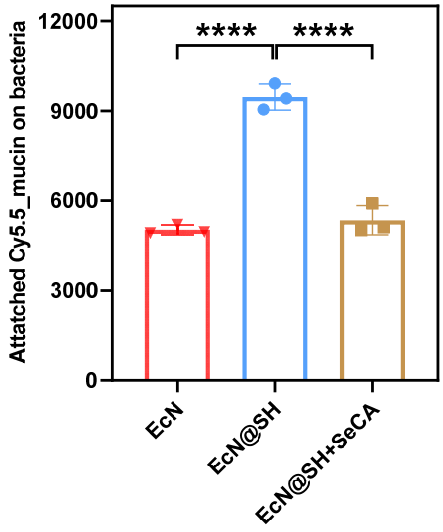

**Supplementary Fig. 17.** Sizes of EcN and mucin-attached EcN measured by DLS. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Source data are provided in the Source Data file.



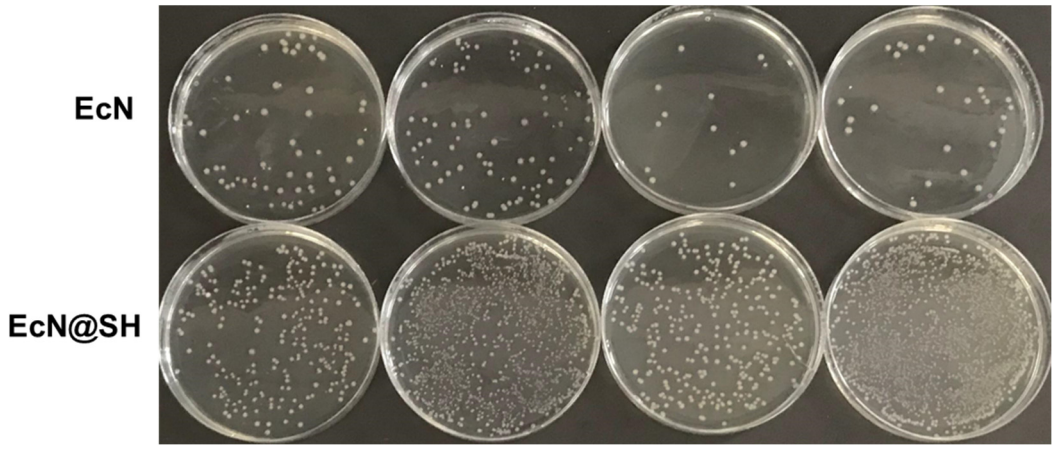

**Supplementary Fig. 18.** Zeta potentials of EcN and mucin-attached EcN measured by DLS. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Source data are provided in the Source Data file.



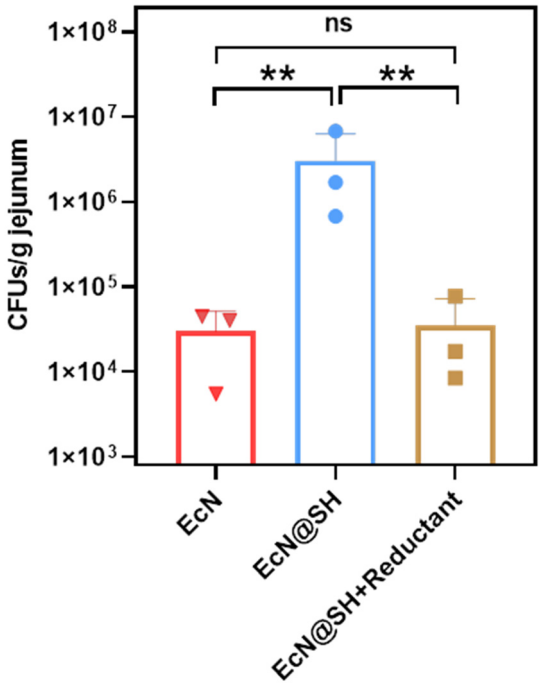

**Supplementary Fig. 19.** MFI values of EcN@SH after reaction with 0.15 mg/ml of Cy5.5_mucin in the presence of 0. 1 mg/ml of DTT. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Significance was assessed using one-way ANOVA analysis followed by Fisher's LSD multiple comparisons, giving $p$ values, ***$p$ < 0.001. Source data and the exact $p$ values are provided in the Source Data file.



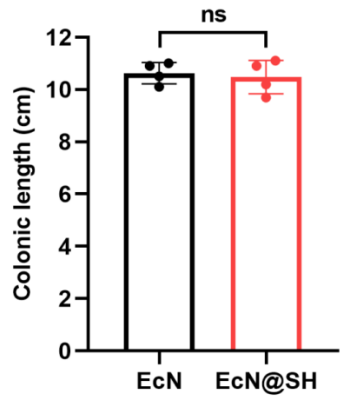

**Supplementary Fig. 20.** MFI values of EcN@SH after reaction with 0.15 mg/ml of Cy5.5_mucin in the presence of 0.3 mg/ml of SeCA. Data are presented as mean values ± SD ($n$ = 3, from independent experiments). Significance was assessed using one-way ANOVA analysis followed by Fisher's LSD multiple comparisons, giving $p$ values, ****$p$ < 0.0001. Source data and the exact $p$ values are provided in the Source Data file.



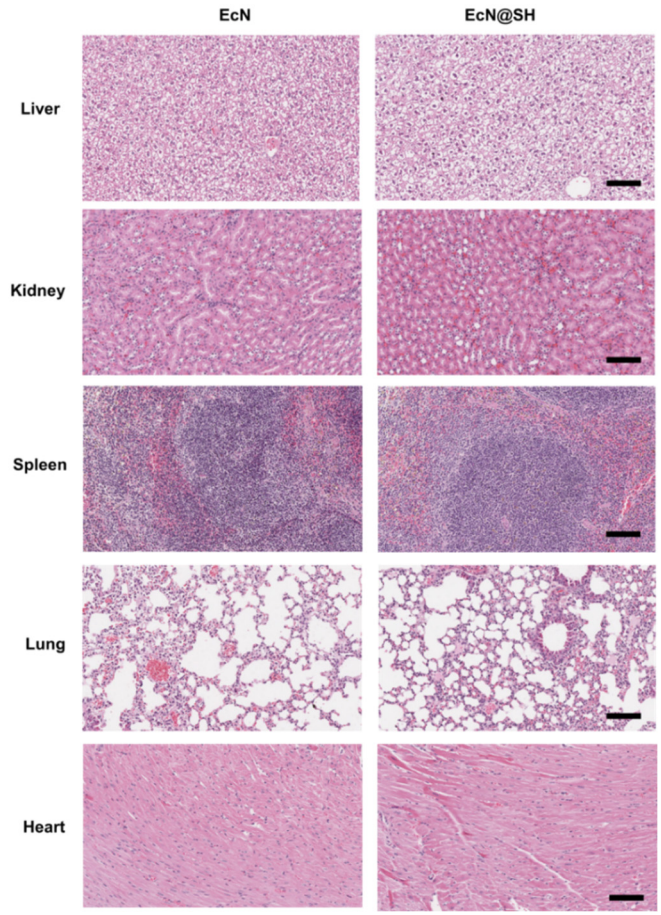

**Supplementary Fig. 21.** Representative images of agar plates containing EcN collected from jejunal mucus 4 hours after oral administration of equivalent EcN and EcN@SH ($1.0 \times 10^8$ CFUs per mouse).



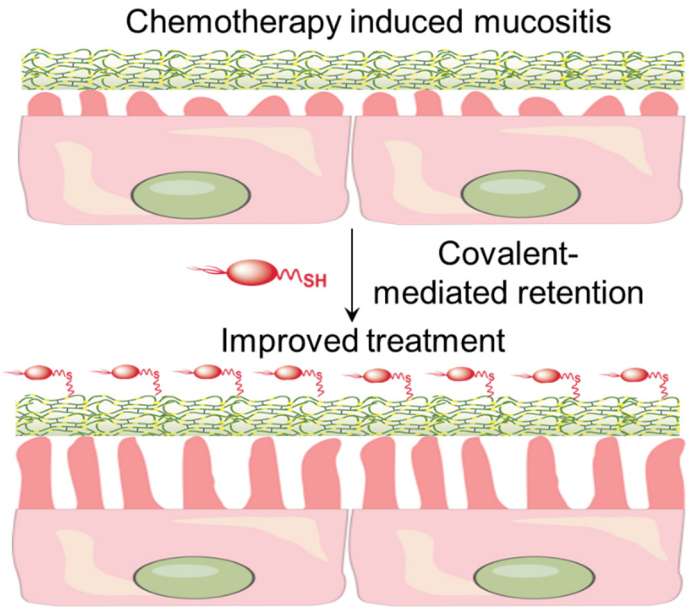

**Supplementary Fig. 22.** Counts of EcN collected from mouse jejunal mucus at 1 hour after oral delivery of unmodified EcN, EcN@SH, or EcN@SH plus 1 mg/ml of L-ascorbic acid. Data are presented as mean values ± SD ($n$ = 3 mice). Significance was assessed using one-way ANOVA analysis followed by Fisher's LSD multiple comparisons, giving $p$ values, $^{**}p < 0.01$. ns, no significance. Source data and the exact $p$ values are provided in the Source Data file.



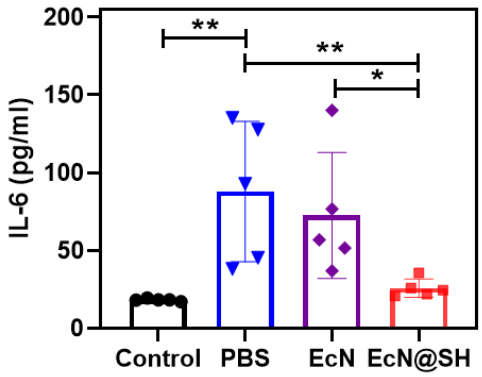

**Supplementary Fig. 23.** Average intestinal length after administration with equivalent EcN or EcN@SH ($1.0 \times 10^8$ CFUs per mouse). Data are presented as mean values ± SD ($n$ = 4 mice). Significance was assessed using Student's $t$-test (two-tailed). ns, no significance. Source data are provided in the Source Data file.



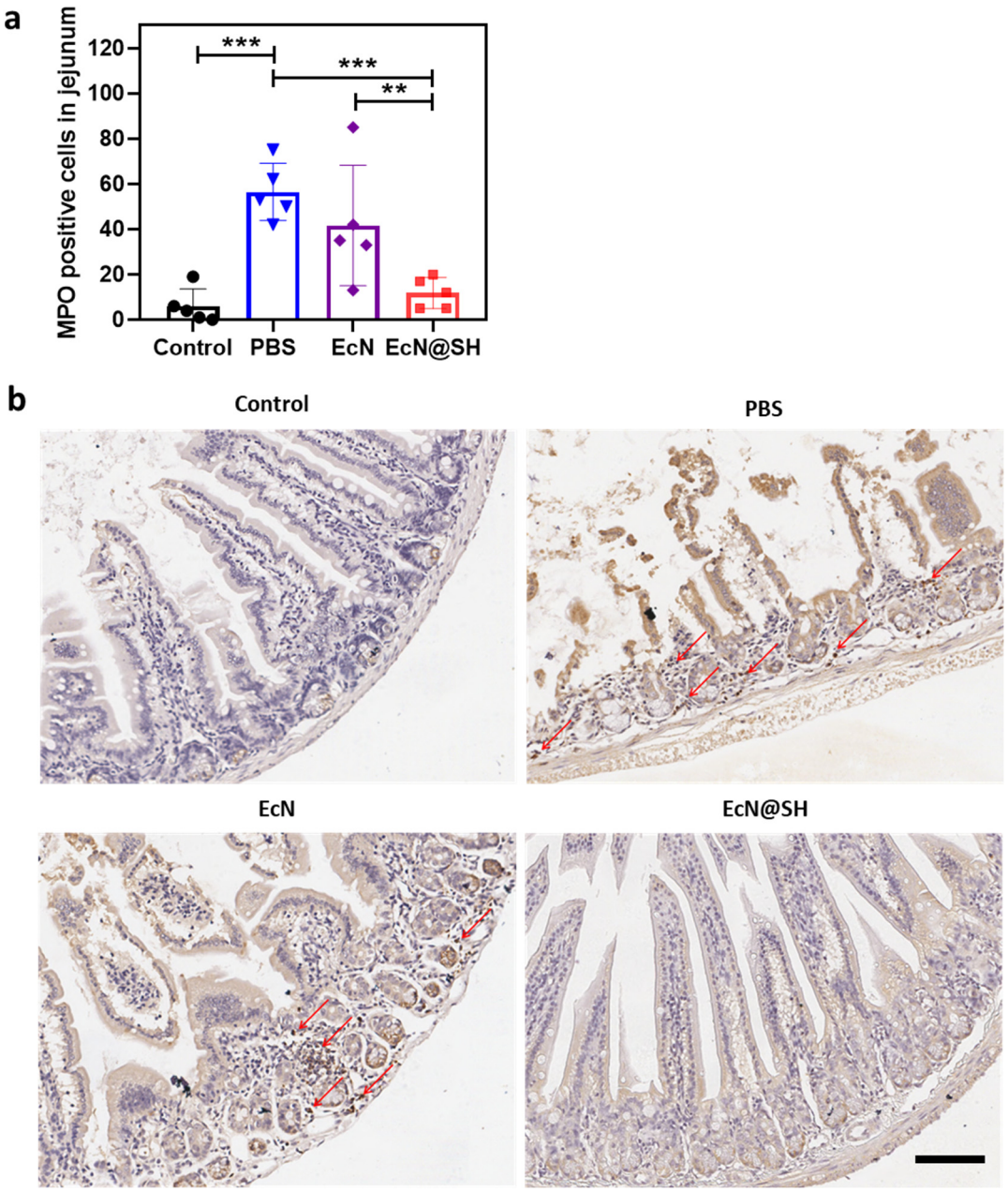

**Supplementary Fig. 24.** H&E staining images of liver, kidney, spleen, lung, and heart tissues sectioned from mice administered with equivalent unmodified EcN or EcN@SH ($1.0 \times 10^8$ CFUs per mouse). Images are representative of four independent biological samples. Scale bar: 100 μm.




**Supplementary Fig. 25.** Schematic illustration of enhanced treatment of jejunal mucositis by covalent bonding mediated localization of thiolated EcN.




**Supplementary Fig. 26.** Level of IL-6 in serum measured by commercially available ELISA kits. Data are presented as mean values ± SD ($n$ = 5 mice). Significance was assessed using one-way ANOVA analysis followed by Fisher's LSD multiple comparisons, giving $p$ values, *$p < 0.05$, **$p < 0.01$. Source data and the exact $p$ values are provided in the Source Data file.




**Supplementary Fig. 27. a** Mean MPO positive cells counted in jejunm. **b** Typical images of MPO staining of jejunum tissues. Red arrows indicate MPO positive cells. Scale bar: 50 μm. Data are presented as mean values ± SD ($n$ = 5 mice). Significance was assessed using one-way ANOVA analysis followed by Fisher's LSD multiple comparisons, giving $p$ values, $^{**}p < 0.01$, $^{***}p < 0.001$. Source data and the exact $p$ values are provided in the Source Data file.