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Supplementary Material for

A secreted bacterial peptidoglycan hydrolase enhances tolerance to enteric pathogens

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Published 23 September 2016, *Science* **353**, 1434 (2016)
DOI: 10.1126/science.aaf3552

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Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemag.org/content/353/6306/1434/suppl/DC1)

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Materials and Methods

Strains.

C. elegans were maintained as described (*1*). *C. elegans* strains *npr-1(ad609)*, *daf-16(mu86)*, *daf-2(e1370)*; *daf-16(mgDf47), pmk-1(km25), dbl-1(nk3),* and *tol-1(nr2033)* were all provided by the *Caenorhabditis* Genetics Center.

E. coli OP50, *E. coli* DH5α, *E. coli* BL21-RIL(DE3) (Agilent Technologies), and *Salmonella enterica* serovar Typhimurium strain IR715 or 14028 were grown in LB (BD Difco). *Enterococcus faecium* strains NCTC 7171 (ATCC 19434), Com15, Com12, and TX0016 (DO, ATCC BAA-472), *Enterococcus faecalis* strains OG1RF (ATCC 47077) and V583 (ATCC 700802), and *Bacillus subtilis* strain 168 were grown in BHI (BD BBL). *Lactobacillus plantarum* WCFS1 (ATCC BAA-793) was grown in MRS (BD BBL). *E. faecium* strains Com15 and Com12 were a gift from Michael Gilmore. *S. typhimurium* strain Δ*invA* was a gift from Andreas Baumler (*2*). When necessary, ampicillin was used at 100 μg/mL, gentamicin was used at 125 μg/mL, and chloramphenicol was used at 25 μg/mL for *E. coli*, 10 μg/mL for *E. faecium*, and 8 μg/mL for *E. faecalis* and *L. plantarum.*

Plasmids described in this study.

SagA hydrolase domain prediction was determined using Phyre 2 software (*3*). Coiled coil domain prediction were determined using COILS software (*4*). Signal sequence prediction was determined using SignalP v4.1 software (*5*).

Name	**Backbone**	**Description**
pET21a-SagA	pET21a (Novagen)	Amp^R, SagA-His_6
pET21a-AS	pET21a	Amp^R, AS-His_6; C443A, H494A, H506A
pET21a-ΔCterm	pET21a	Amp^R, ΔCterm-His_6; Δ390-529
pET21a-SagA-SS	pET21a	Amp^R, SagA-SS-His_6; Δ2-27
pAM401-SagA	pAM401 (ATCC 37429)	Cam^R, psagA:SagA-His_6
pAM401-psagA:mcherry	pAM401	Cam^R, psagA:mcherry
pTEX5501ts-OG1RF-sagA	pTEX5501ts (Nallapareddy et al)(*6*)	Cam^R, Gen^R, *see below
p67MC1 (Singer et al 2010)(*7*)		Amp^R, mcherry

Cloning strategies:
All cloning was done in *E. coli* DH5α. pET21a constructs were transformed into *E. coli* BL21-RIL (DE3) for expression. pAM401 constructs were transformed into *E. faecium* or *L. plantarum* for expression.

pET21a-SagA:
sagA was PCR amplified from the *E. faecium* Com15 genome using the following primers:

FW: GGACATATGAAAAAGAGTTTAATATCAGCAGTAATGG
RV: GGACTCGAGCATGCTGACAGCAAAGTCAGGTGCAAAC

Restriction sites for Nde1 and Xho1 are underlined. The PCR product was gel purified and subcloned into pGEMTeasy (Promega) for sequencing. Then, an internal Nde1 site was mutated using the Quikchange multi-site directed mutagenesis kit (Stratagene) using the following primer:

AAATATATCGGTACTCCtTATGTTTGGGGCGG

The site mutated is indicated in lowercase, resulting in a synonymous mutation in the SagA protein sequence. *sagA* was then excised from pGEMTeasy with Nde1 and Xho1 and was ligated into cut pET21a (Novagen).

pET21a-AS:
pET21a-SagA was mutagenized with the following primers to generate the indicated cysteine to alanine and histidine to alanine mutations in the SagA protein sequence:

C443A: CCAAGTGGATTTGACgcCTCAGGATTCACACG
H494A: TCACCAGGCGGAACTTACgcCGTAGCGATTGC
H506A: GGAGGACAATATATCgcTGCTCCTCAACCAGG

The mutated sites are indicated in lowercase.

pET21a-ΔCterm:
pet21a-SagA was mutagenized with the following primers to insert BamH1 sites flanking the hydrolase domain:

AACAGATCAAAGTGTgGATCCTGGGAACAGTACTGG
TGCACCTGACTTTGCgGatccCATGCTCGAGCACCAC

The BamH1 sites are underlined. The mutated sites are indicated in lowercase. The plasmid was then cut with BamH1 and re-ligated to form pET21a-ΔCterm, resulting in an in-frame excision of residues 390 through 529 in the SagA protein sequence.

pET21a-SagA-SS:
SagA-SS was PCR amplified from pET21a-SagA_BamH1 (see pAM401-SagA section below) using the following primers:

FW: CATCACCATATGGACGATTTTGATTCTCAGATA
RV: CATCACGGATCCTTTCGGGCTTTGTTA

The Nde1 and BamH1 sites are underlined. This PCR product was cut and ligated back into cut pET21a-SagA_BamH1, resulting in an in-frame excision of residues 2 through 27 in the SagA protein sequence.

pAM401-psagA:mcherry:
The promoter region of *sagA* (*psagA*) was PCR amplified from the *E. faecium* Com15 genome using following primers:

FW: AAAGTCGACACGATGGTGGTCCAATTGAT
RV: TTTCATATGTCATTCCTCCGACTGGCTTA

Restriction sites for Sal1 and Nde1 are underlined. The PCR product was gel purified and subcloned into pGEMTeasy for sequencing. *psagA* was excised with Sal1 and Nde1, and ligated into cut pAM401-padd9:mcherry (**see below) to replace *paad9* with *psagA*.

pAM401-SagA:
pET21a-SagA was mutagenized to insert a BamH1 site after the 6X-His using the following primer to generate pET21a-SagA_BamH1:

TAACAAAGCCCGAAAGGAtccTGAGTTGGCTGCTGC

The BamH1 site is underlined. The mutated sites are indicated in lowercase. *sagA-his6* was then excised with Nde1 and BamH1, and ligated into cut pAM401-psagA:mcherry to replace mcherry with SagA-His_6. pAM401-SagA was transformed into *E. faecium* Com15 or *L. plantarum* as described below.

**Generating E. faecalis-sagA:*

pTEX5501ts-OG1RF-sagA:
pTEX5501ts was a gift from Barbara Murray. 986 bp of sequence "upstream" (on the minus strand) of the intended SagA insertion site was PCR amplified using the following primers:

FW: AAACGGCCGAGTGGGGCGTGTTATTGAAG
RV: TTTGTCGACGGGTAAGCTTCTCATCGTTTTG

The Eag1 and Sal1 sites are underlined. 1013 bp of sequence "downstream" (on the minus strand) of the intended SagA insertion site was PCR amplified using the following primers:

FW: AAACTGCAGTGGAGCCTTGAAGAAAGTTG
RV: TTTGGTACCATTGGCTGCTTTTGTTGCTT

The Pst1 and Kpn1 sites are underlined. The upstream and downstream PCR products were subcloned into pGEMTeasy and sequenced, then were excised and ligated into cut pTEX5501ts sequentially. (Note: For the Eag1/Sal1 double digest of pTEX4401ts, Sal1 was added first, then Eag1 because the restriction sites overlap in the vector, and Eag1 can cut more efficiently at the end of a linear DNA fragment). *psagA:sagA-his$_6$* was excised from pAM401-SagA with Sal1 and BamH1 and inserted into the cut vector, generating pTEX5501ts-OG1RF-sagA. OG1RF-*sagA-his$_6$* was generated using the described protocol (*6*). Briefly, OG1RF was transformed with pTEX5501ts-OG1RF-sagA and single recombinants were selected after plasmid curing at 37 °C. Then colonies were passaged at 37 °C and screened for gentamicin sensitivity and chloramphenicol resistance until such a clone was identified. Chromosomal insertion was verified by PCR and sequencing.

***pAM401-paad9:mcherry:*
paad9:mcherry was designed by us and synthesized by Genewiz into pUC57. It encodes the synthetic promoter to *aad9* (*8*) flanked by Sal1 and Nde1 restriction sites, driving mcherry flanked by Nde1 and BamH1 restriction sites. *paad9:mcherry* was excised from pUC57 with Sal1 and BamH1 and ligated into cut pAM401, inserting into the Tet^R gene.

Generation of L. plantarum-sagA:
pAM401-SagA was transformed into *L. plantarum* WCFS1 by electroporation as described above. A vector control strain was generated by transforming pAM401 into *L. plantarum.*

Electroporation of *Enterococcus* and *Lactobacillus*:
Protocol for preparation of electrocompetent cells and electroporation was adapted from (*9*). Briefly, *Enterococcus* or *Lactobacillus* were grown for 18 hours in M9YE (M9 media, 0.1% casamino acids, 0.3% yeast extract) + 2% glycine. Cultures were diluted in half with M9YE + 3% glycine, and grown for an additional 3 hours. Cultures were chilled, pelleted, and washed 3 times in sucrose wash buffer (0.625 M sucrose, 1 mM $MgCl_2$, pH 4 with HCl), reducing the original culture volume by 1/2, 1/10, then 1/100 successively with each wash. Finally, cells were aliquoted, flash frozen in liquid nitrogen, and stored at -80 °C. Cells were electroporated in 2 mm cuvettes, 25 μF, 400 ohm, 2.5 kV. Cells were allowed to recover for 2 hours at room temperature without shaking in Todd-Hewitt Broth (BD Bacto) or MRS broth (BD Bacto) for *Lactobacillus,* then with shaking at 37 °C for an additional 2 hours before plating on selective media.

Protein purification:
For *E. faecium* Com15 protein expression, plasmid-carrying strains were grown in BHI with appropriate antibiotics overnight. For *E. coli* BL21-RIL (DE3), LB cultures were inoculated with overnight cultures with appropriate antibiotics, grown for 2 hours or until $OD_{600} \sim 0.4$, induced with 1 mM IPTG, then grown for an additional 2 hours. His_6-tagged proteins were purified from culture supernatants using Ni-NTA agarose (Qiagen). Native

purifications were performed as recommended in the manufacturer's protocol. Purified protein was dialyzed into PBS at 4 °C overnight using 5 kDa or 7 kDa MWCO Slide-a-lyzers (Pierce Protein Biology). Protein concentration was estimated by BCA assay (Pierce Protein Biology) and protein was stored at -20 °C.

Protein gel methods and Western blotting:

Proteins were separated by SDS-PAGE on 4-20% Criterion Tris-HCl or Criterion TGX precast gels (Bio-Rad). Protein was visualized either by Coomassie blue (Bio-Rad) staining or stain-free imaging on a ChemiDoc MP system (Bio-Rad). Glycoproteins were visualized using the Pierce Glycoprotein staining kit (Thermo Scientific) as per the manufacturer's protocol. For Western blotting, proteins were transferred to nitrocellulose membrane. HRP conjugated polyclonal anti-His_6 (abcam ab1187) and monoclonal anti-actin (abcam ab14128) were used for His_6 and actin Western blots respectively. Western blots were visualized either by developing film or imaging on a ChemiDoc MP system.

Bacterial growth curves:

Growth of E. coli expressing SagA, AS, or SagA-SS:

Cultures were grown as described for protein purification, except that OD_{600} was monitored at given time points using a Spectromax M2e spectrophotometer. For measurements of OD clearing after protein induction, OD_{600} of *E. coli* BL21-RIL(DE3) expressing SagA, the active site mutant, or SagA-SS under an IPTG-inducible promoter were taken 1 hour post-induction and normalized to the OD_{600} of a mock-induced non-expressing BL21-RIL(DE3) culture.

Growth of E. coli and S. Typhimurium in the presence of SagA:

LB media was inoculated with overnight cultures 1:100. Protein was added at 10 µg/mL and volumes were normalized across all conditions with PBS. OD_{600} was measured at given time points.

Growth of E. faecalis OG1RF-sagA:

BHI media was inoculated with overnight cultures 1:100. Chloramphenicol was added at 10 µg/mL as indicated. OD_{600} was measured at given time points.

Salmonella protein secretion assay:

For each condition, 2 mL of LB was inoculated with overnight cultures 1:30. Protein was added to media at 10 µg/mL and volumes were normalized across all conditions with PBS. The Δ*invA* (*10*) strain was a gift from the Baumler lab. Cultures were grown for 4 hours, shaking at 37 °C, the pelleted at 7000 g for 5 minutes. 1 mL of culture supernatant was precipitated with trichloro acetic acid (TCA) at a final concentration of 10% overnight at 4 °C. Precipitated proteins were pelleted at 20,800 g for 30 minutes, rinsed 2 times in acetone, then air-dried. Proteins were then separated by SDS-PAGE and visualized by Coomassie staining.

C. elegans infection assays:

E. faecium refers to strain Com15 unless otherwise noted in the figure legends. Worms were handled using a Leica M60 microscope and imaged using a Leica IC80 HD camera. No antibiotics were used in any of the growth plates.

Statistical Analysis:

For *C. elegans* survival curves, 3 plates of 30 worms each (total 90 worms) were scored, unless otherwise noted. Mean percent survival and standard deviation of the triplicate plates for a representative experiment are shown for supplementary figures. Mean percent survival and statistical comparisons by log rank test after Bonferroni correction for multiple comparisons using OASIS software (*11*) are shown for main text figures.

Pulsed infection assay:

Worms were synchronized as described (*12*). Synchronized young adult worms were washed in S buffer (129 mL 0.05 M K_2HPO_4, 871 mL 0.05 M KH_2PO_4, 5.85 g NaCl) and transferred to bacterial lawns grown on 2% agar BHI plates for colonization for 1 day. Then, worms were washed and transferred to lawns of OP50 or IR715 grown on BHI plates for infection for another day. Finally, worms were washed and transferred to lawns of OP50 grown on NGM plates (Day 0). Worms were maintained on OP50-NGM plates and survival was scored as described (*12*).

Liquid-treatment pulsed infection assay:

Synchronized young adult worms were transferred to 96-well plate wells containing 32% BHI media, culture supernatant, or live cultures. For protein treatments, 10-20 μg/mL of protein in PBS was included in each well. For treatment with peptidoglycan digests, 100 μL of each digest was added to each well. For treatment with defined peptidoglycan fragments, 50 μM of each fragment in a mixture of PBS and water was included in each well. MDP, GlcNAc, and MurNAc were purchased from Sigma-Aldrich. MurNAc-L-Ala was synthesized as described below in Scheme S1. After 2 hours, worms were washed and transferred to lawns of IR715 grown on BHI plates for 1 day. Then, worms were washed and transferred to lawns of OP50 grown on NGM plates (Day 0). Worms were maintained on OP50-NGM plates and survival was scored.

Continuous infection assay:

Synchronized young adult worms were washed and transferred to bacterial lawns grown on 2% agar BHI plates for colonization for 1 day. Then, worms were washed and transferred to lawns of OP50 or IR715 grown on NGM plates (Day 0). Worms were maintained on OP50- or IR715- NGM plates and survival was scored.

E. faecalis OG1RF pathogenesis assay:

This assay was performed as essentially as described (*13*). Briefly, synchronized young adult worms were washed and transferred to Com15, OG1RF, or OG1RF-*sagA* bacterial lawns grown on BHI plates (Day 0). Worms were maintained on Com15, OG1RF, or OG1RF-*sagA* BHI plates, and survival was scored.

Worm CFU measurements:
Protocol for CFU measurements was adapted from previous protocol (*13*). Briefly, 5-20 worms were rinsed in drops of S buffer, and allowed to crawl free of bacteria on a sterile plate. These worms were then mechanically lysed in PBS using microtubes and pestles (Kimble-Chase). Serial dilutions of worm homogenate were plated on *Salmonella-Shigella* agar (BD BBL) or Enterococcosel (BD BBL) agar plates, and plates were incubated at 37 °C overnight.

Epifluorescence:
For imaging of mcherry-*Salmonella*, worms were treated as described for the pulsed infection assays except worms were infected with *S.* Typhimurium carrying the plasmid p67MC1. For imaging of *E. faecium psagA:mcherry*, worms were fed *E. faecium* carrying the plasmid pAM401-mcherry for 1 day on BHI plates. Worms were mounted as described (*14*), except that worms were paralyzed in 1 mM tetramisole (Sigma) instead of sodium azide. Worms were imaged on a Nikon Eclipse TS100 and pictures were taken using a Nikon Digital Sight DS-Fi1.

Electron Microscopy:
Animals were prepared for electron microscopy using standard methods (*15*). Ultrathin serial sections (70 nm) were collected by using a Leica Ultracut UCT Ultramicrotome. Sections at two regions, 120 µm and 200 µm away from the head region, were examined for each condition. EM images were acquired using an FEI Tecnai G2 Spirit BioTwin transmission electron microscope operating at 80 kV with a Gatan 4K x 4K digital camera.

Treatment of culture supernatants:
Proteinase K treatment: Culture supernatant or media was digested with 0.1 mg/mL of proteinase K in the presence of 1 mM $CaCl_2$ for 2 hours at 37 °C. Then, 1 mM EGTA was added, and digestions were used in the liquid-treatment pulsed infection assay.

Trichloroacetic acid precipitation: Culture supernatant or media was precipitated with TCA (final concentration of 10%) overnight at 4 °C. Precipitated proteins were pelleted and rinsed 2 times in acetone, then air-dried and resuspended in BHI media before use in the liquid-treatment pulsed infection assay.

10-kDa MWCO column filtration: E. faecium culture supernatant or media was filtered through 10-kDa MWCO columns (Vivaspin GE Healthcare), and the flow-thru was used in the liquid-treatment pulsed infection assay.

5-kDa MWCO column filtration of E. coli culture supernatants: E. coli BL21-RIL(DE3) expressing SagA-His or AS-His were induced in BHI media instead of LB as described above. Portions of the culture supernatants were filtered through 5-kDa MWCO columns (Vivaspin GE Healthcare), and the unfiltered supernatants, column concentrate, and column flow-thru were used in the liquid-treatment pulsed infection assay.

Proteomics:

In-gel digestion:

E. faecium was grown overnight at 37 °C with shaking in BHI. 1 mL of culture supernatant was precipitated with TCA (final concentration of 10%) overnight at 4 °C. Precipitated proteins were pelleted, rinsed 2 times in acetone, then air-dried. Proteins were then resuspended in loading buffer and separated by SDS-PAGE. Proteins were processed for in-gel digestion with sequencing grade trypsin (Promega) as previously described (*16*) except that dried samples were resuspended in 5% acetonitrile, 2% formic acid in water for analysis by LC-MS/MS.

In-solution digestion:

E. faecium, *E. faecalis*, or *E. faecalis-sagA* was grown overnight at 37 °C with shaking in BHI (For *E. faecalis-sagA*, 10 µg/mL chloramphenicol was included). 1 mL of culture supernatant was precipitated with TCA as described above. Protein was digested with 0.2 µg of trypsin in 50 mM ammonium bicarbonate buffer overnight at 37 °C. Samples were dried by speed-vac, then resuspended in 5% acetonitrile, 2% formic acid in water. An additional 1 ml of culture supernatant was TCA precipitated and analyzed by SDS-PAGE and anti-His_6 WB to check protein profile and SagA-His_6 expression.

LC/MS-MS was performed on digested peptides by The Rockefeller Proteomics Facility and peptide spectra were analyzed against the *E. faecium* Com15 or *E. faecalis* OG1RF proteome using Mascot v2.3 and Proteome Discoverer software.

Peptidoglycan purification:

Bacteria were grown in fresh BHI medium with shaking at 37 °C to stationary-phase (OD_{600} of 2). Peptidoglycan was extracted by resuspending the bacterial cell pellet in 0.25% SDS solution in 0.1 M Tris / HCl, pH 6.8 and boiling the suspension for 20 minutes at 100 °C in a heating block as previously described (*17*). The resulting insoluble cell wall preparation was washed with distilled water six times until free of SDS. The cell wall was purified by treatment with benzonase and followed by trypsin digestion. Then, insoluble cell wall was recovered by centrifugation (16,000 x *g*, 10 min, 4 °C), and washed once in distilled water. To obtain pure peptidoglycan, cell wall was then suspended in 1 M HCl and incubated for 4 h at 37 °C in a shaker to remove wall teichoic acid. The insoluble material was collected by centrifugation (16,000 x *g*, 10 min, 4 °C) and washed with distilled water repeatedly until the pH was 5–6. The final peptidoglycan was lyophilized and stored at -20 °C. For muropeptide analysis, purified peptidoglycan was digested with mutanolysin from *Streptomyces globisporus* (Sigma, 10 KU/ml of mutanolysin in ddH_2O) in 20 mM Tris-HCl buffer for 16 hr at 37 °C. The enzyme reaction was stopped by incubating at 100 °C for 3 min. The resulting soluble muropeptide mixture was then analyzed by ANTS labeling described below.

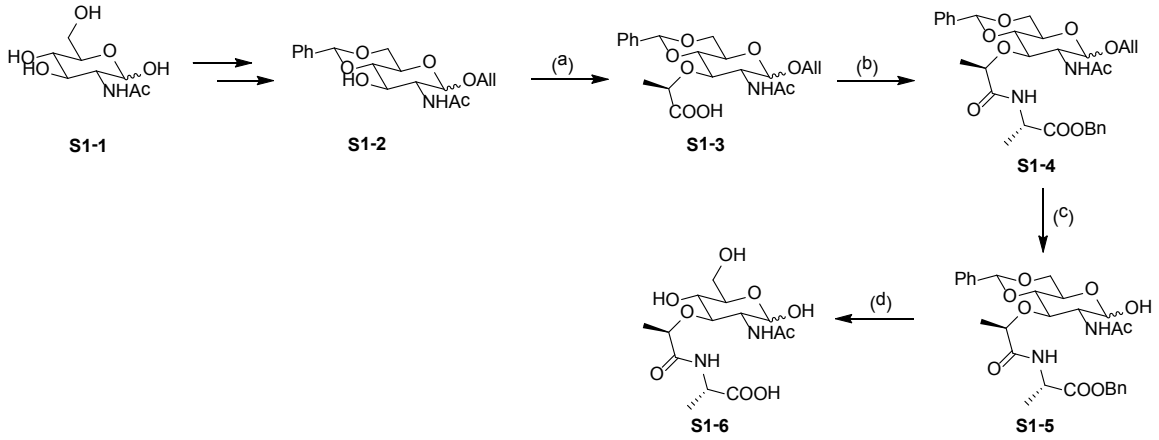
Peptidoglycan digests:

100 µg of *E. coli* peptidoglycan (Invivogen) was digested with 20 µg lysozyme (Sigma) and 20 µg of SagA-His or AS-His overnight in a mixture of PBS and water at 37 °C. Digests were then filtered through 5-kDa MWCO columns (Vivaspin GE Healthcare), and the flow-thru was used in the liquid-treatment pulsed infection assay.

8-aminonaphthalene 1,3,6 trisulfonic acid (ANTS) labeling assay:

E. coli supernatants were prepared by inoculating cultures 1:50 with an overnight culture of *E. coli* BL21-RIL(DE3) expressing SagA-His6 or AS-His6. Cultures were grown for 2 hours, then induced with 1 mM IPTG, and grown for an additional 2 hours. *Enterococcus* supernatants were prepared by growing cultures overnight. Cultures were pelleted, and supernatant was filtered through 10 kDa MWCO columns (Millipore Microcon). For peptidoglycan digests, 100 µg of *E. coli* peptidoglycan was digested with 20 µg lysozyme and 20 µg of SagA-His or AS-His overnight in a mixture of PBS and water at 37 °C. Digests were filtered through 10 kDa MWCO columns. Culture supernatants, peptidoglycan digests, and defined peptidoglycan fragments were dried by speed-vac before ANTS labeling. ANTS labeling was performed as described (*18*). 10 µl of ANTS reaction mix was added to each tube of dried material (1:1 mixture of 0.2 M ANTS (in 3:17 acetic acid:water): 1 M $NaCNBH_3$ (in DMSO)). Reactions were incubated overnight at 37 °C. 0.5-3.5 µL of the ANTS labeled mixtures were mixed 1:1 with 40% glycerol and samples were separated by native PAGE on a hand-cast 37-40% Tris-glycine acrylamide gel (19:1 polyacrylamide:bisacrylamide, with a 20% acrylamide stack) at 150 V for ~ 3 hours or 80 V for ~ 6 hours. ANTS-labeled synthetic fragments MDP, GlcNAc, MurNAc, and MurNAc-L-Ala were run for comparison. A sugar-less pentapeptide Ala-D-γ-Glu-Lys-D-Ala-D-Ala (Sigma) was run to show specificity of the UV signal and empty lanes adjacent to sample lanes were loaded with samples of ANTS labeled Ala-D-γ-Glu-Lys-D-Ala-D-Ala to prevent lane warping. Remaining lanes were loaded with 20% glycerol. Gels were imaged on the ChemiDoc MP system (Bio-Rad) using the Sybr-safe UV imaging setting.

Synthesis of N-acetylmuramic acid-L-alanine:



Scheme S1. Synthesis of the N-acetylmuramic acid-L-alanine. Reagents and conditions: (a) (S)-2-chloropropionic acid, NaH, 45 °C, 16 h. (b) Ala-OBn, DIEA, Cl-HOBt, DIC, CH_2Cl_2/DMF, RT, 16 h. (c) $Pd(PPh_3)_4$, AcOH, 40 °C, 16 h. (d) (i) 60% AcOH, 40-50 °C, 1.5 h; (ii) Pd/C, H_2, MeOH, RT, 5 h.

Compound S1-2. S1-2 was synthesized from commercially available N-acetylglucosamine (**S1-1**) by following and adapting published protocols (*19, 20*).

Compound S1-3. NaH (0.16 g, 6.67 mmol) was added to a suspension of compound **S1-2** (0.358 g, 1.02 mmol) in 1,4-dioxane (9 mL). The mixture was stirred at 40 ^{o}C for 1 h. (S)-2-chloropropionic acid (0.27 mL, 2.95 mmol) in 1,4-dioxane (3 mL) was added to the reaction mixture dropwise. The mixture was stirred at 45 ^{o}C for 16 h. The solvent was removed under vacuum. H_2O (20 mL) and 1M $HCl_{(aq)}$ (20 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 25 mL) and the combined organic layers were dried with $MgSO_4$ and concentrated by rotary evaporation. The resulting solid was washed with cold Et_2O several times and dried in vacuum to yield **S1-3** as a white solid (95 mg, 22%). Crude compound (**S1-3**) was used without further purification. ^{1}H NMR (400 MHz, CD_3OD): δ 7.47 (d, *J* = 4.1 Hz, 2H), 7.37 (d, *J* = 5.0 Hz, 3H), 5.88 (m, 1H), 5.64 (s, 1H), 5.28 (d, *J* = 17.2 Hz, 1H), 5.15 (d, *J* = 10.4 Hz, 1H), 4.63 (d, *J* = 7.7 Hz, 1H), 4.40 (q, *J* = 6.9 Hz, 1H), 4.31 (d, *J* = 4.9 Hz, 1H), 4.28 (t, *J* = 4.6 Hz, 1H), 4.07 (m, 1H), 3.76 (m, 3H), 3.67 (t, *J* = 4.2 Hz, 1H), 3.49 (m, 3H), 1.99 (s, 3H), 1.34 (d, *J* = 6.9 Hz, 3H). ESI-MS [M+H$^+$]: m/z calc. for $C_{21}H_{28}NO_8^+$: 422.1809; found: 422.1807.

Compound S1-4. L-alanine benzyl ester hydrochloride (68 mg, 0.315 mmol) and N,N-diisopropylethylamine (0.118 mL, 0.677 mmol) were added to a suspension of compound **S1-2** (0.95 mg, 0.225 mmol) in CH_2Cl_2. 6-Chloro-1-hydroxybenzotriazole (57 mg, 0.336 mmol) in DMF (0.7 mL) was added to the reaction mixture. N,N'-diisopropylcarbodiimide (45 uL, 0.291 mmol) was added to the reaction mixture. The mixture was stirred at room temperature for 16 h. 1M $HCl_{(aq)}$ (40 mL) was added to quench the reaction. The mixture was extracted with CH_2Cl_2 (2 × 25 mL) and the combined organic layers were dried with $MgSO_4$ and concentrated by rotary evaporation. The resulting solid was washed with cold MeOH several times and dried in vacuum to yield **S1-4** as a white solid (93 mg, 71%). Crude compound (**S1-4**) was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.41 (m, 10H), 5.84 (m, 1H), 5.69 (s, 1H), 5.25 (dd, *J* = 17.4, 1.8 Hz, 1H), 5.15 (d, *J* = 12.8 Hz, 2H), 5.09 (d, 12.6 Hz, 1H), 4.53 (d, *J* = 8.4 Hz, 1H), 4.31 (t, 7.4 Hz, 1H), 4.23 (m, 2H), 4.04 (m, 2H), 3.80 (m, 2H), 3.65 (t, 8.7 Hz, 2H), 3.42 (m, 1H), 1.79 (s, 3H), 1.34 (d, 7.8 Hz, 3H), 1.21 (d, 6.6 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d_6): δ 172.56, 172.40, 170.27, 138.01, 136.37, 134.93, 129.27, 128.87, 128.61, 128.49, 128.20, 126.32, 116.76, 101.25, 100.58, 80.45, 79.71, 77.70, 69.55, 68.23, 66.41, 66.09, 55.20, 47.97, 23.77, 23.47, 19.32, 17.28. ESI-MS [M+H$^+$]: m/z calc. for $C_{31}H_{39}N_2O_9^+$: 583.2650; found: 583.2650.

Compound S1-5. Tetrakis(triphenylphosphine)palladium(0) (95 mg, 0.082 mmol) was added to a suspension of compound **S1-4** (93 mg, 0.160 mmol) in AcOH (1.5 mL). The mixture was stirred at 40 ^{o}C for 16 h. The solution was purified by silica gel column chromatography (MeOH/EtOAc/hexanes = 0.5/7/7)) to yield **S1-5** as a light yellow solid (57 mg, 66%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.50 (d, *J* = 4.2 Hz, 2H), 7.34 (m, 8H), 5.65 (s, 1H), 5.21 (d, *J* = 3.6 Hz, 1H), 5.19 (s, 2H), 4.44 (q, *J* = 7.2 Hz, 1H), 4.27 (q, *J* = 6.6 Hz, 1H), 4.21 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.04 (m, 2H), 3.82 (m, 2H), 3.69 (t, *J* = 9.6 Hz, 1H), 1.97 (s, 3H), 1.42 (d, *J* = 7.2 Hz, 3H), 1.31 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (600 MHz, CD_3OD): δ 174.50, 172.34, 172.20, 137.79, 135.81, 128.52, 128.20, 127.93, 127.86, 127.74, 125.92, 101.40, 101.25, 91.73, 82.08, 77.04, 76.16, 68.73, 66.61, 62.35,

54.21, 21.49, 18.34, 15.91. ESI-MS [M+H$^+$]: m/z calc. for $C_{28}H_{35}N_2O_9^+$: 543.2337; found: 543.2338.

Compound S1-6. Compound **S1-5** was suspended in AcOH (0.6 mL) and H_2O (0.4 mL). The mixture was stirred at 50 ^oC for 1 h and 40 ^oC for 30 min. The solvent was removed under vacuum. The resulting solid was dissolved in MeOH (2 mL). 10% palladium on carbon (21 mg) was added to the solution. The mixture was stirred under 1 atm. of H_2 at room temperature for 5 h. After filtration, the solution was concentrated by rotary evaporation. The resulting oil was purified by silica gel column chromatography (MeOH/$CHCl_3$ = 3:7 to 4:6) to yield compound **S1-6** (α-anomer). ^{1}H NMR (600 MHz, CD_3OD): δ 5.13 (d, *J* = 3.6 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 1H), 4.34 (q, *J* = 6.6 Hz, 1H), 3.94 (dd, *J* = 10.2, 3.0Hz, 1H), 3.82 (m, 2H), 3.73 (m, 1H), 3.68 (m, 1H), 3.50 (m, 1H), 1.97 (s, 3H), 1.45 (d, *J* = 7.6 Hz, 3H), 1.41 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (600 MHz, CD_3OD): δ 174.55, 174.36, 172.07, 95.88, 91.15, 79.38, 77.14, 71.83, 70.10, 61.27, 53.96, 21.46, 18.10, 16.27. ESI-MS [M+H$^+$]: m/z calc. for $C_{14}H_{25}N_2O_9^+$: 365.1555; found: 365.1555.

Mouse infections:

C57BL/6J (000664) mice were purchased from Jackson Laboratories and maintained in our facilities. Genotyping was performed according to the protocols established for the respective strains by Jackson Laboratories. Mice were maintained at the Rockefeller University animal facilities under specific pathogen-free (SPF) or germ-free (GF) conditions. Germ-free C57BL/6J mice were obtained from Sarkis Mazmanian and bred and maintained in germ-free isolators in our facilities. Germ-free status was confirmed by plating feces as well as by qPCR analysis (16S rRNA). Mice were used at 8-10 weeks of age for most experiments. Animal care and experimentation were consistent with the NIH guidelines and were approved by the Institutional Animal Care and Use Committee at the Rockefeller University.

Colonization and S. Typhimurium infection of mice:

To ensure effective colonization and induce infection susceptibility, specific pathogen-free (SPF) mice were gavaged with a daily dose of AMNV antibiotic cocktail (4mg ampicillin, 2mg metronidazole, 4mg neomycin, and 2mg vancomycin) for 14 days. AMNV treatment was ceased 2 days prior to colonization. Germ-free (GF) mice were exported from isolators into autoclaved cages containing autoclaved food, water and bedding. Bacterial cultures were washed and resuspended in sterile PBS at 10^9/mL for *E. faecium*, *E. faecalis*, and *L. plantarum* or at 10^7/mL (SPF) or 10^3/mL (GF) for *S.* Typhimurium. Mice were colonized by gavage with 100 μL of the indicated bacterial suspension 7 days before infection. Colonization was followed by infection with *S.* Typhimurium by oral gavage at the doses indicated above and in the figure legends. Weight loss was monitored from just before infection, and mice were euthanized when they reached 80% baseline weight or when they appeared hunched, moribund or exhibited a visibly-distended abdomen (indicative of peritoneal effusion), whichever occurred first. Death was not used as an end-point. Colony-forming units (CFU) in the feces were determined by plating 5 serial dilutions of feces suspended in sterile PBS on selective agars: Salmonella Shigella Agar (BD 211597) for *S.* Typhimurium, Enterococcosel Agar (BD 212205) for *E. faecium* and *E. faecalis*, and MRS Agar with

8ug/mL chloramphenicol for *L. plantarum*-vector and *L. plantarum–sagA*. Resulting quantities were normalized to feces weight. Experiments in which putative probiotics did not colonize or colonize equally well by day 2 post-colonization were terminated and not included in our analyses.

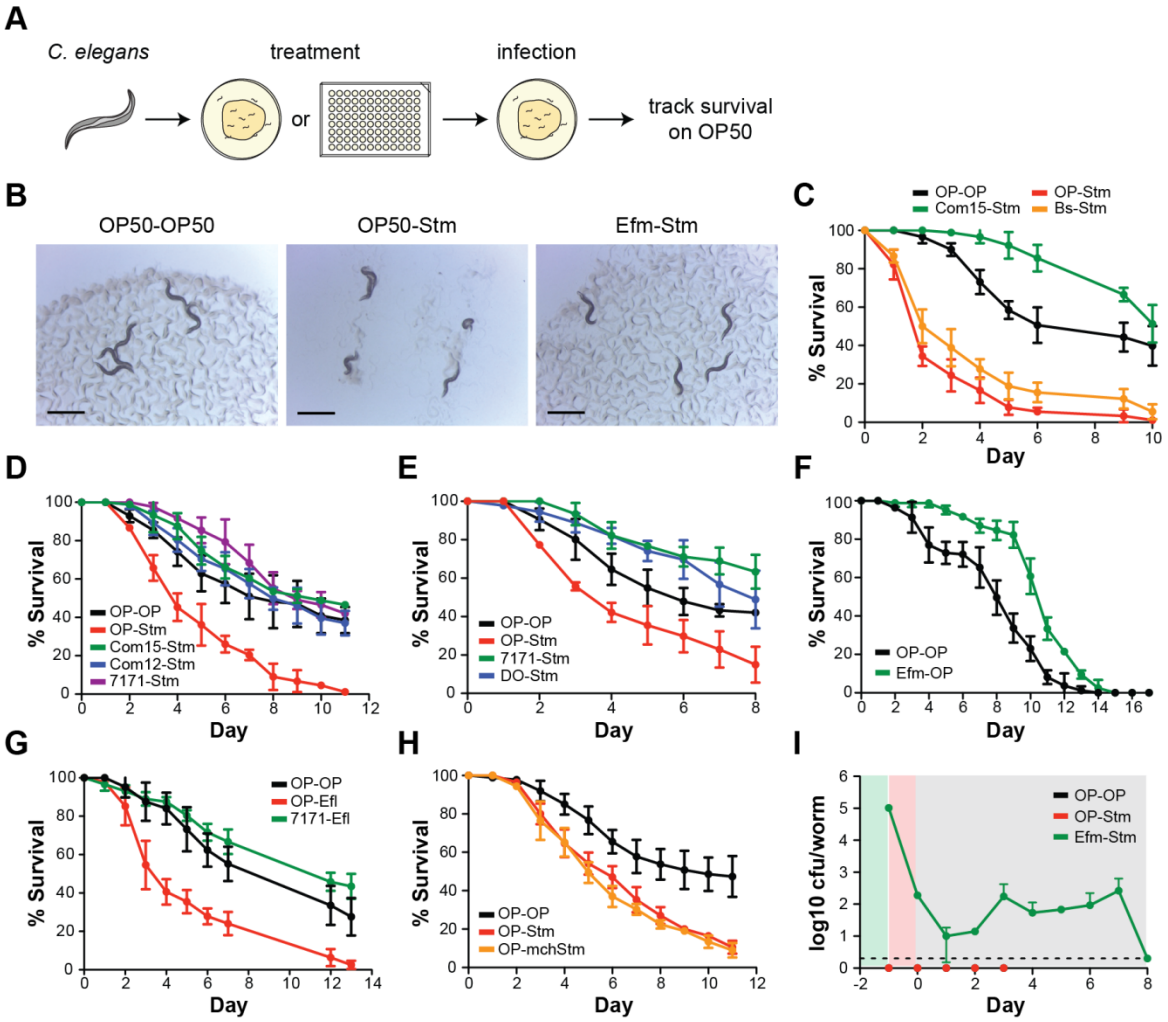


Figure S1. Characterization of *E. faecium*-mediated protection. (A) Schematic of pulsed infection assay. Worms were treated on bacterial lawns for 1 day or in liquid wells for 2 hours before infection with *Salmonella* for 1 day. Worms were then transferred onto *E. coli* OP50 plates for the remainder of the assay, and survival was scored. **(B)** Images of *C. elegans* 1 day post-infection. Scale bar = 1mm. **(C)** Survival curve showing no activity of *B. subtilis* 168 (Bs) as compared to *E. faecium* Com15. **(D)** Survival curve showing comparable activity of *E. faecium* strains NCTC 7171, Com12, and Com15. **(E)** Survival curve showing comparable activity of Efm strains NCTC 7171 and DO. **(F)** Pulsed treatment-infection assay showing *E. faecium* treatment inhibits *E. coli* OP50 pathogenesis. **(G)** Survival curve showing that Efm NCTC 7171 can protect *C. elegans* from *E. faecalis* V583 (Efl) pathogenesis. **(H)** Survival curve showing comparable pathogenicity of mcherry-*S.* Typhimurium (mchStm) to wild-type *S.* Typhimurium (Stm). **(I)** *Enterococcus* CFUs measured in *C. elegans* throughout the pulsed treatment-infection assay. Background shading represents stage of the pulsed treatment-infection assay. Green indicated treatment, red indicates infection, and grey indicates *E. coli* OP50 feeding. The data points represent average CFUs from 5 worms ± standard deviation from two independent experiments. The dotted line indicates detection limit. For (C) through (H), data points represent mean ± s.d. for 3 plates of 30 worms each.

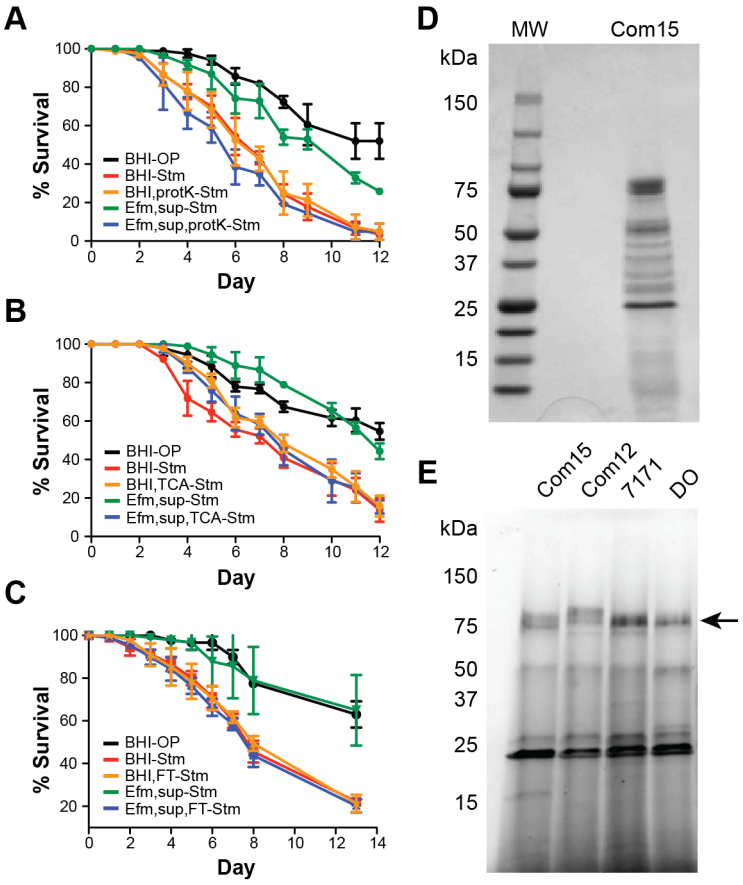


Figure S2. Protein from *E. faecium* culture supernatant is protective. (A) Proteinase-K-treated *E. faecium* culture supernatant (Efm,sup,protK) loses activity compared to untreated *E. faecium* culture supernatant (Efm,sup). Untreated media (BHI) and proteinase-K-treated media (BHI,protK) are inactive. **(B)** Trichloroacetic acid (TCA) precipitated *E. faecium* culture supernatant (Efm,sup,TCA) loses activity. Untreated media (BHI) and TCA precipitated media (BHI,TCA) are inactive. **(C)** 10 kDa MWCO column filtered *E. faecium* culture supernatant (Efm,sup,FT) is inactive. Untreated media (BHI) and 10 kDa MWCO column filtered media (BHI,FT) are inactive. **(D)** Coomassie stained SDS-PAGE of *E. faecium* Com15 culture supernatant subsequently digested for mass spectrometry analysis presented in Table S1 and Fig. 2B. **(E)** Stain-free imaging of *E. faecium* culture supernatants from various *E. faecium* strains separated by SDS-PAGE. Arrow indicates protein band corresponding to typical SagA migration. For (A) through (C), data points represent mean ± s.d. for 3 plates of 30 worms each. Abbreviations: *E. coli* OP50 (OP); *S. typhimurium* (Stm); *E. faecium* (Efm).

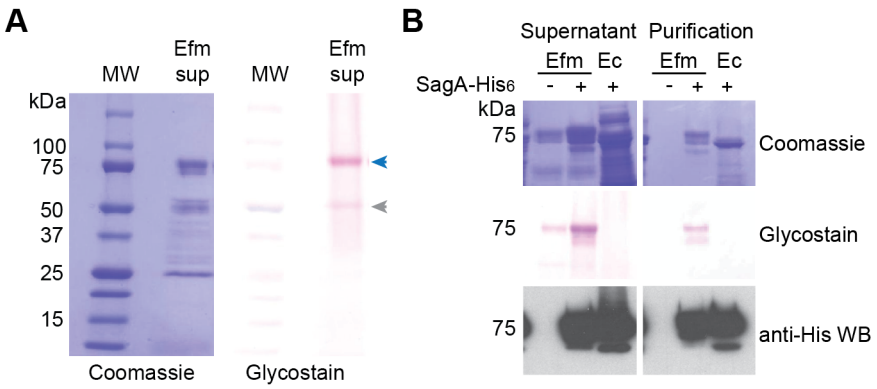


Figure S3. Characterization of SagA from *E. faecium* and *E. coli.* (A) Coomassie staining and glycoprotein-staining of *E. faecium* Com15 culture supernatant. The arrows indicate protein bands excised for mass spectrometry analysis presented in Table S2. The blue arrow indicates a protein band corresponding to typical SagA migration. **(B)** SDS-PAGE of culture supernatants and SagA-His_6 purifications from *E. faecium* Com15 (Efm) and *E. coli* BL21-RIL(DE3) (Ec). Samples were visualized by Coomassie staining, glycoprotein-staining, and anti-His_6 Western blot (WB) as indicated. SagA is glycosylated in *E. faecium* but not *E. coli.*

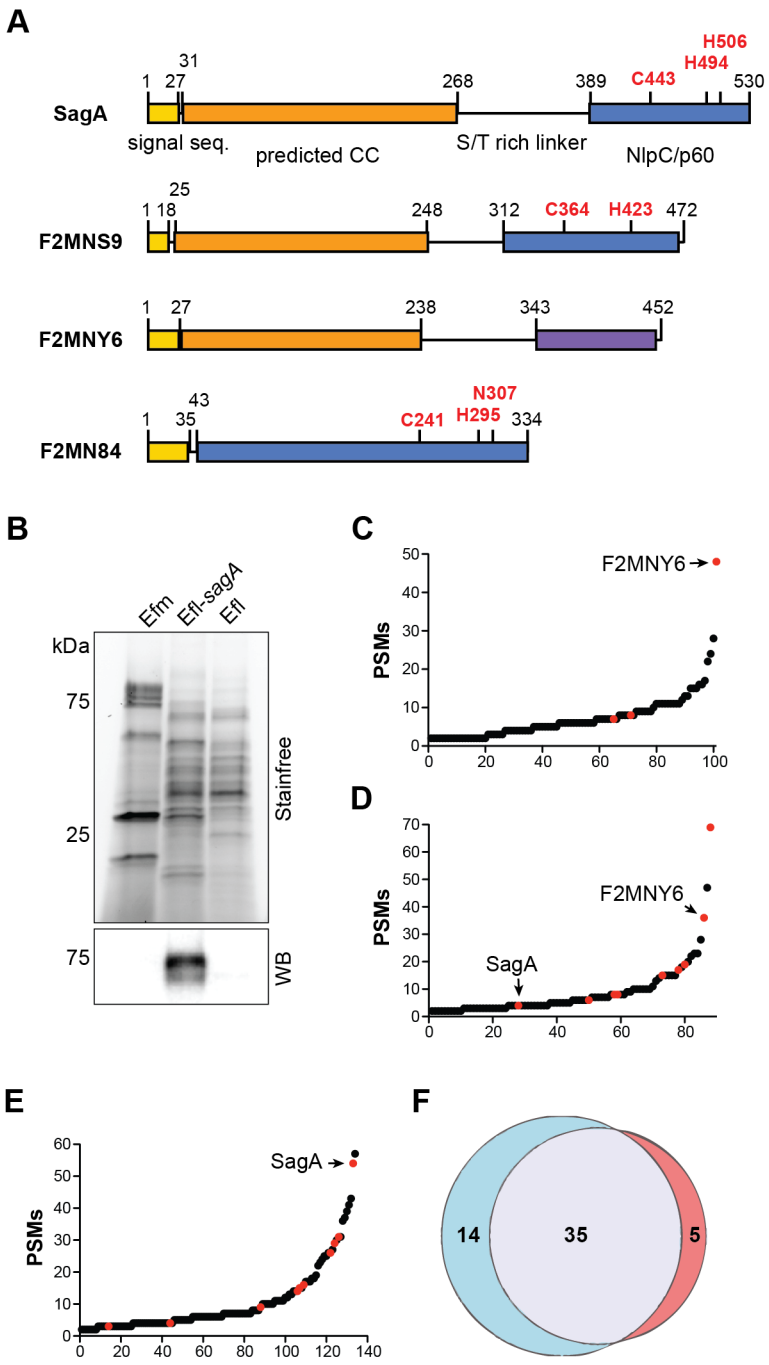


Figure S4. Secretome profiling of *E. faecium*, *E. faecalis*, and *E. faecalis-sagA*. (A) Predicted domain architecture of three proteins in the *E. faecalis* OG1RF genome with significant alignment to SagA. By Needleman-Wunsch global alignment, F2MNS9 has 35.1% sequence identity and 50.6% sequence similarity to SagA; F2MNY6 has 35.6% identity and 54.5% similarity, and F2MN84 has 14.5% identity and 24.2% similarity. Amino acid number is as indicated, and putative catalytic residues are indicated in red bold type. F2MNY6 lacks any putative catalytic residues in its NlpC/p60 like domain, and F2MN84 lacks a predicted coiled-coil domain. As indicated in Fig. S5A, both SagA and F2MNS9 are located after the mreCD operon in the chromosome. **(B)** Stain-free

imaging and anti-His_6 Western blot (WB) of culture supernatants from *E. faecium* Com15 (Efm), *E. faecalis* OG1RF (Efl), and *E. faecalis-sagA* (Efl*sagA*). Parallel samples were trypsin digested in-solution for mass spectrometry analysis presented in (C) through (E), and Tables S3 and S4. **(C)** Summary of proteins identified by mass spectrometry from *E. faecalis* OG1RF culture supernatant. Proteins shown were identified with at least 2 unique peptides. Proteins involved in cell-wall remodeling are highlighted in red. on the y-axis is peptide spectrum matches (PSMs); on the x-axis is arbitrary protein number. For the list of proteins identified, see Table S3. Of the three genetically-encoded putative NlpC/p60-like hydrolases, we detected only F2MNY6 from *E. faecalis* OG1RF culture supernatant (fig. S4C), suggesting that, unlike *E. faecium*, *E. faecalis* does not secrete high levels of active NlpC/p60-type peptidoglycan hydrolases. **(D)** Summary of proteins identified by mass spectrometry from *E. faecalis-sagA* culture supernatant. Proteins shown were identified with at least 2 unique peptides. Proteins involved in cell-wall remodeling are highlighted in red. For the list of proteins identified, see Table S3. *E. faecalis-sagA* culture supernatant contained less relative secreted SagA in as compared to *E. faecium* culture supernatant. **(E)** Summary of proteins identified by mass spectrometry from *E. faecium* Com15 culture supernatant. Proteins shown were identified with at least 2 unique peptides. Proteins involved in cell-wall remodeling are highlighted in red. For the list of proteins identified, see Table S4. **(F)** Venn diagram comparing the proteins identified by mass spectrometry after in-gel trypsin digestion (blue) (See Fig. 2B, Table S1) versus in-solution trypsin digestion (red) (See Fig. S5E, Table S4). Proteins identified in both samples are indicated in the overlap (grey). Proteins included in this graph were identified with at least 2 unique peptides, and at least 10 PSMs.

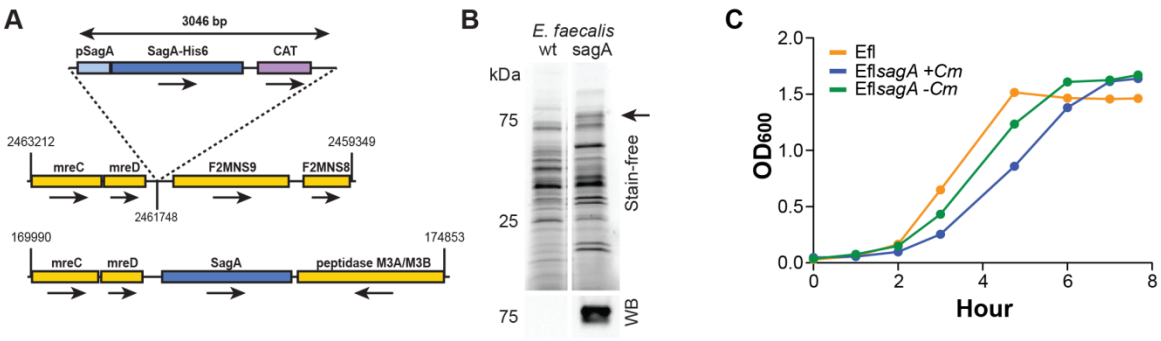


Figure S5. Generation and characterization of *E. faecalis-sagA*. (A) Schematic showing insertion of SagA-His_6 into the *E. faecalis* OG1RF chromosome to generate *E. faecalis-sagA*. CAT = chloramphenicol resistance gene. For comparison, a schematic of the SagA locus in *E. faecium* Com15 is shown underneath. **(B)** Stain-free imaging and anti-His_6 Western blot (WB) of *E. faecalis* and *E. faecalis-sagA* culture supernatants. Arrow indicates protein band corresponding to SagA mobility in SDS-PAGE. **(C)** Growth curves of *E. faecalis* (Efl) and *E. faecalis-sagA* (Efl*sagA*). Cm+ and Cm- indicate ± 10 µg/mL chloramphenicol.

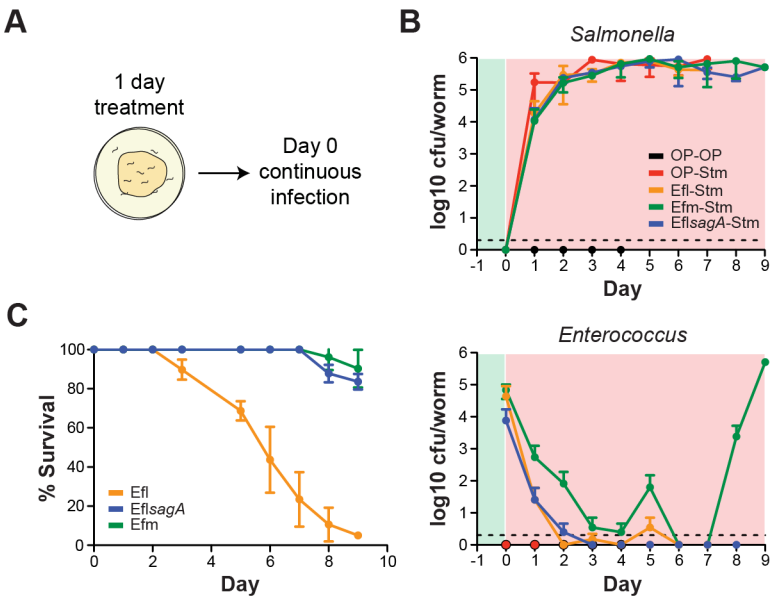


Figure S6. Characterization of *E. faecalis-sagA*. (A) Schematic of a treatment-continuous *Salmonella* infection assay. Animals were treated for 1 day on BHI agar with *E. faecium*, *E. faecalis*, or *E. faecalis-sagA*, then transferred to NGM agar plates for infection with *Salmonella* for the remainder of the assay and survival was scored. Control animals were fed *E. coli* OP50 throughout the assay. **(B)** *Salmonella* and *Enterococcus* CFUs measured in *C. elegans* throughout the continuous infection assay. Data points represent average CFUs from 5 worms ± standard deviation of two independent experiments. The dotted line indicates detection limit. The background shading indicates stages of the treatment-continuous infection. Green indicates treatment and red indicates infection. **(C)** Survival curve showing that *E. faecalis-sagA* is less pathogenic than *E. faecalis* in a continuous *E. faecalis* infection assay. Data points represent mean ± standard deviation for 3 plates of 30 worms each. Abbreviations: *E. coli* OP50 (OP); *S. typhimurium* (Stm); *E. faecium* (Efm); *E. faecalis* (Efl); *E. faecalis-sagA* (Efl*sagA*).

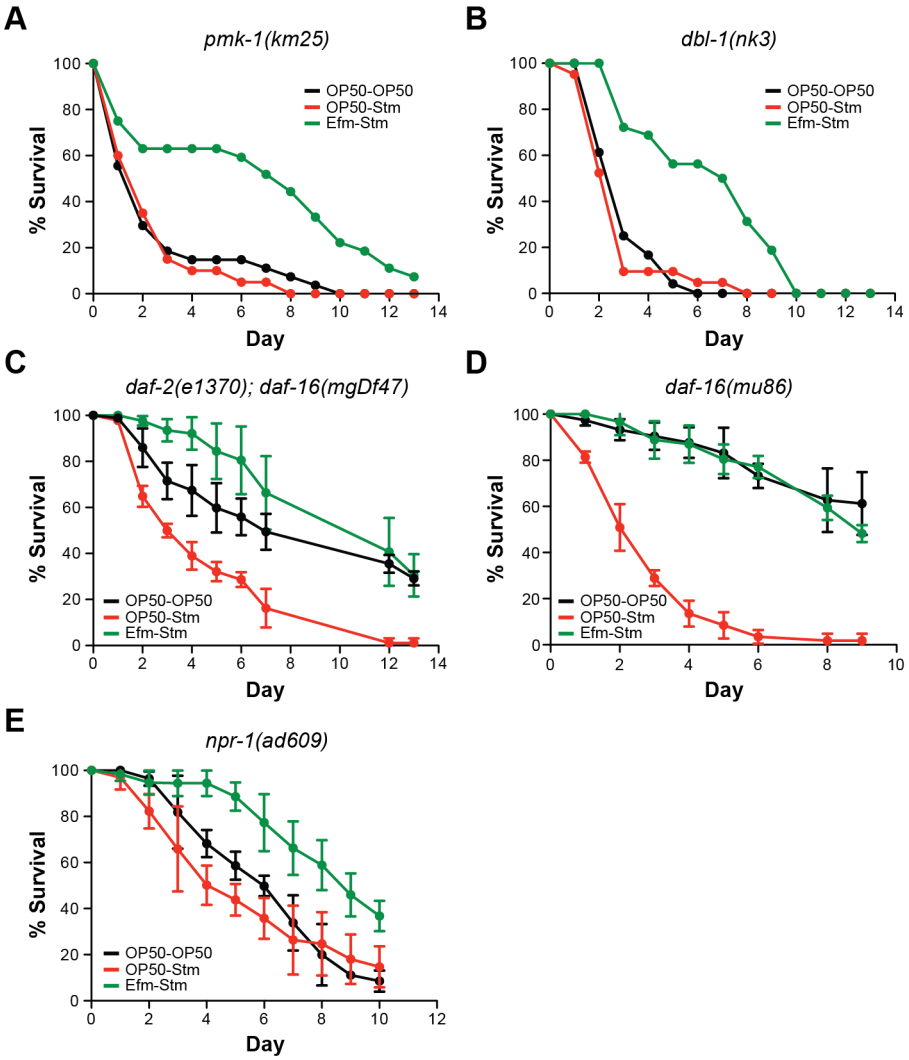


Figure S7. *E. faecium* protection in *C. elegans* mutants. Survival curves assaying *E. faecium*-mediated protection in **(A)** *pmk-1(km25)* **(B)** *dbl-1(nk3)* **(C)** *daf-2(e1370);daf-16(mgDf47)* **(D)** *daf-16(mu86)* and **(E)** *npr-1(ad609)*. For survival curves with error bars, each condition was tested with 3 plates of 30 worms each, and data points represent mean ± standard deviation. For curves without error bars, each condition was tested with one plate of 30 worms. Abbreviations: *E. coli* OP50 (OP); *S. typhimurium* (Stm); *E. faecium* (Efm).

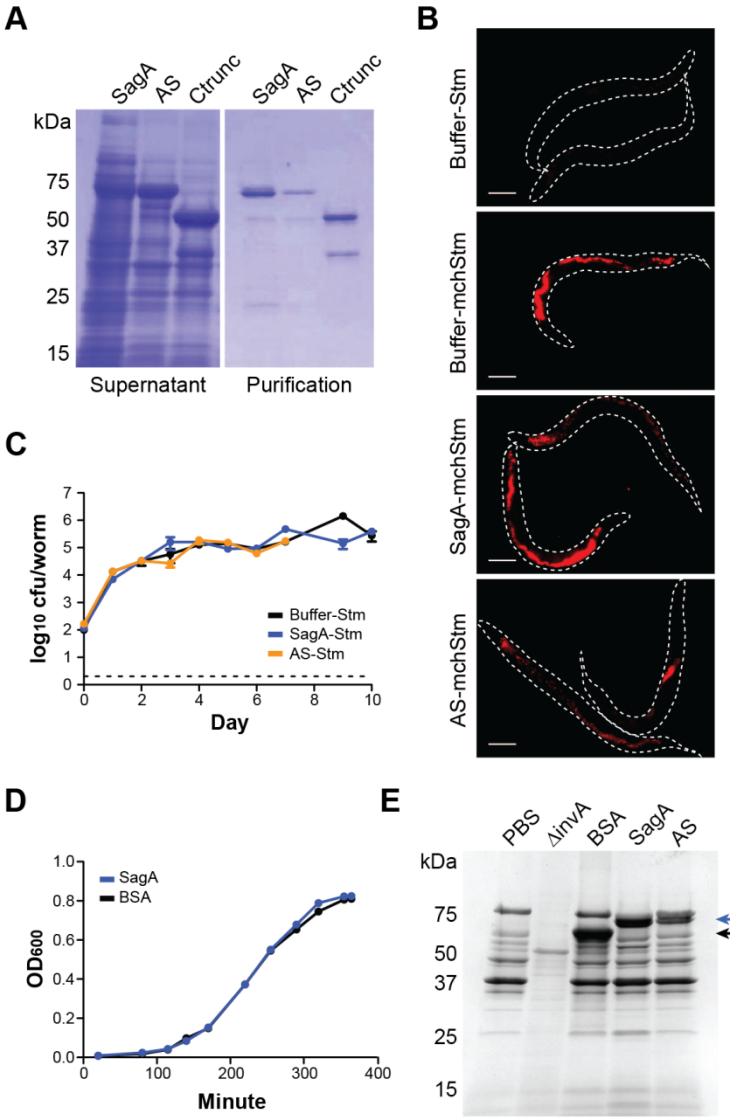


Figure S8. SagA does not affect SPI-1 type III protein secretion in *S. typhimurium*. **(A)** Coomassie stained SDS-PAGE of culture supernatants and His_6 purifications of SagA-His_6, SagA-AS, and SagA-Ctrunc from *E. coli* BL21-RIL(DE3). **(B)** Fluorescence imaging of *C. elegans* treated with SagA or the active site mutant, then infected with *Salmonella* expressing mcherry (mchStm) at day 5 post-infection. The dotted lines indicate an outline of the worm body. Scale bar = 200 µm. **(C)** *Salmonella* CFUs measured in *C. elegans* at indicated time points from an average of 10-20 worms ± standard deviation of three technical replicates. The dotted line indicates detection limit. **(D)** Growth curves of *Salmonella* in LB media + 10 µg/ml SagA or BSA. **(E)** SagA does not affect SPI-1 secretion in culture. *Salmonella* cultures were grown in LB + 10 µg/ml BSA, SagA, or the active site mutant. PBS indicates control treatment. Δ*invA* is a *Salmonella* mutant that does not secrete effectors in culture. The blue arrow indicates typical migration of SagA or SagA-AS, and the black arrow indicates typical migration of BSA. Abbreviations: *E. coli* OP50 (OP); *S. typhimurium* (Stm).

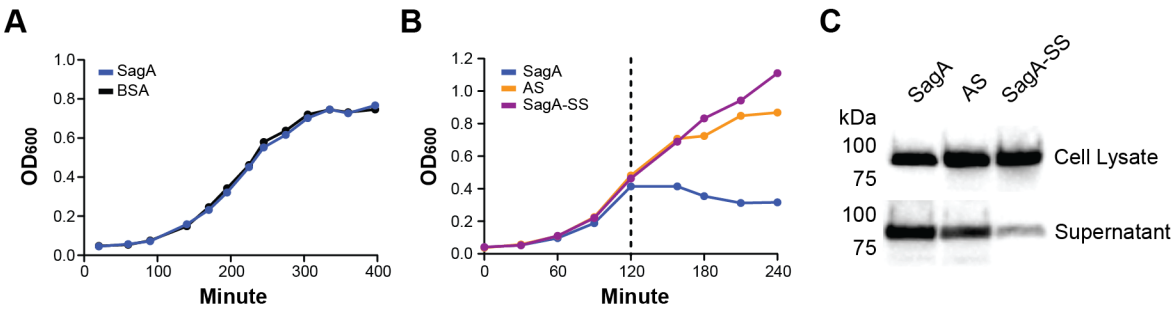


Figure S9. Characterization of SagA enzymatic activity in *E. coli*. (A) Growth curves of *E. coli* BL21-RIL(DE3) in LB media + 10 µg/ml SagA or BSA. **(B)** Growth curves of *E. coli* BL21-RIL(DE3) expressing SagA, the active site mutant (AS), or SagA lacking a signal sequence (Sag-SS) under an IPTG-inducible promoter. The dotted line indicates time of IPTG induction. **(C)** anti-His_6 Western blots of cell lysate or culture supernatant from *E. coli* BL21-RIL(DE3) expressing SagA, the active site mutant, or SagA lacking a signal sequence (Sag-SS) after IPTG induction. SagA-SS is mostly retained in the cell lysates, while SagA and the active site mutant are secreted.

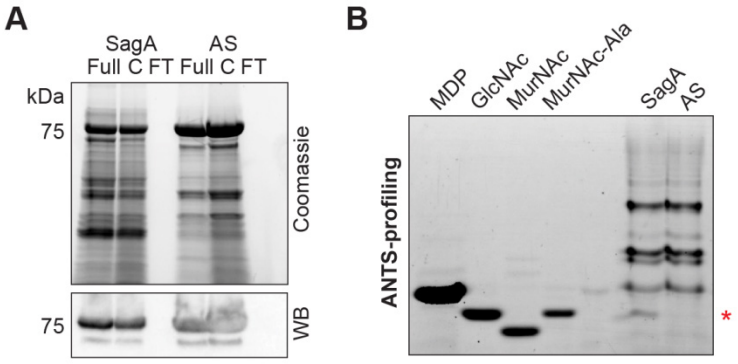


Figure S10. Characterization of SagA peptidoglycan hydrolase activity. (A) Removal of SagA-His_6 from column filtrate. Coomassie staining and anti-His_6 WB of *E. coli* culture supernatants expressing SagA-His_6 or the active site mutant. Supernatant was filtered through a 5-kDa MWCO column. Unfiltered supernatant (Full), column concentrate (C), and column flow-through (FT) are as indicated. **(B)** ANTS visualization of purified *E. coli* peptidoglycan (PG) digested with lysozyme and either SagA or the active site mutant.

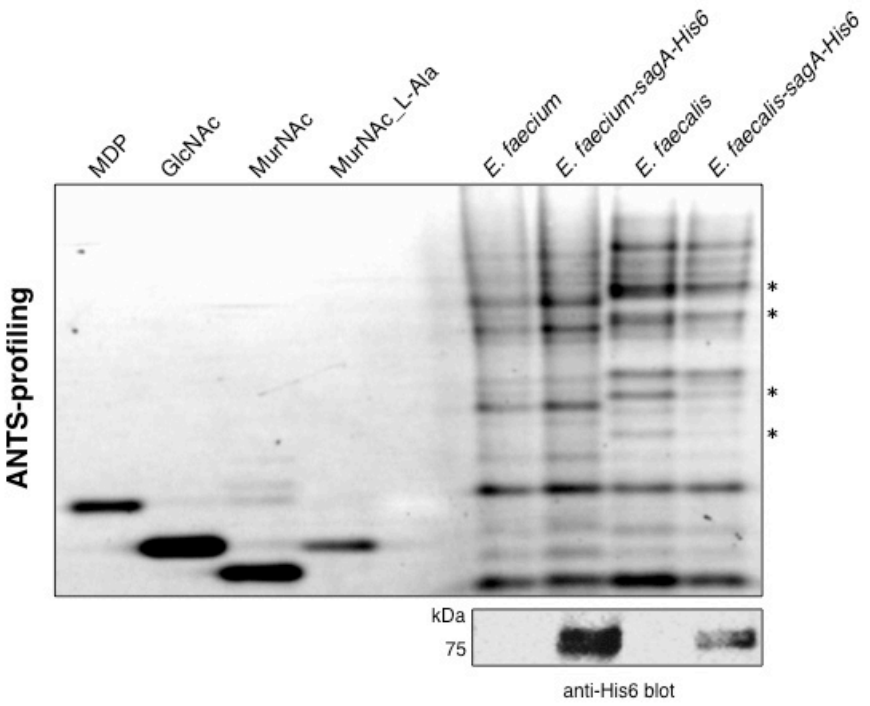


Fig. S11. ANTS profiling of peptidoglycan from *Enterococcus* strains. Analysis of purified peptidoglycan isolated from *E. faecium* (Com15)*, E. faecium-sagA* (Com15 transformed with SagA expression plasmid*), E. faecalis* (OG1RF)*, and E. faecalis-sagA* by ANTS profiling. Muropeptides were isolated as previously described (*17*), dried, labeled with ANTS, separated by native PAGE and then visualized by UV. ANTS-labeled synthetic fragments MDP, GlcNAc, MurNAc, and MurNAc-L-Ala were analyzed in parallel for comparison. Several muropeptide fragments that are altered in *E. faecalis-sagA* are highlighted *. Western blot analysis of SagA-His6 expression in *Enterococcus* strains is included below.

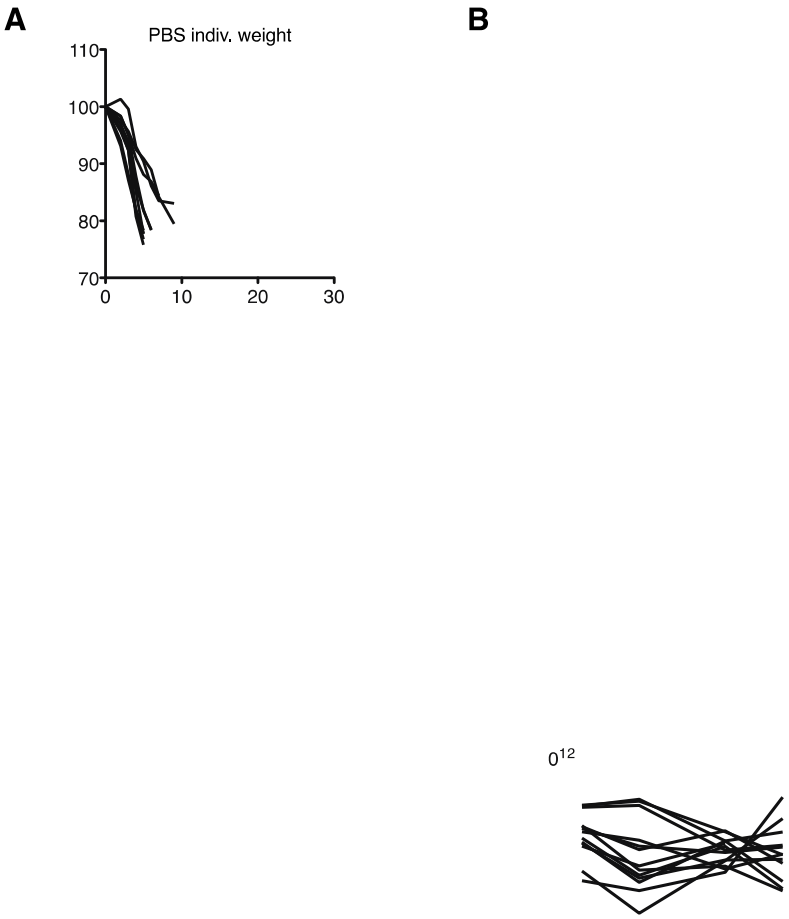


Figure S12. Individual weight loss and colonization of germ-free B6 mice. Germ-free (GF) C57BL/6 mice were orally gavaged with 10^8 CFU *E. faecalis* (*E. fs*), *E. faecalis* expressing *sagA* (*E. fs-sagA*) or *E. fm* 7d before oral infection with 10^2 CFU *S.* Tm. **(A)** Weight loss and **(B)** *E. fs* or *E. fm* CFU in feces are shown for each mouse included in Fig. 4A-C. Pooled data from 4 independent experiments, n=10-14 mice/group. PBS-treated control mice did not exhibit any detectable *E. fs* or *E. fm* in feces.

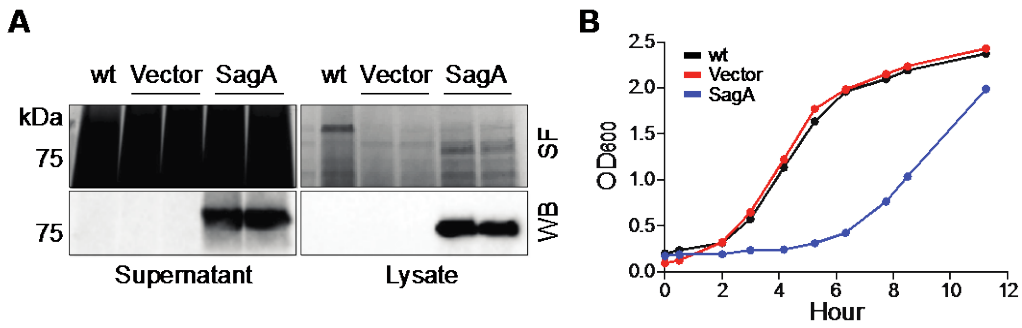


Figure S13. *Lactobacillus plantarum-sagA* produces and secretes SagA. (A) Stain-free imaging (SF) and anti-His6 Western blot (WB) of culture supernatants and cell lysates of *L. plantarum* WCSF1 carrying plasmid-encoded SagA-His6 (75kD) or empty vector. 2 clones each for SagA-His6 and empty vector are shown. Wild-type *L. plantarum* WCSF1 (wt) is shown as a control. The black smear in the stain-free imaging of the supernatant lanes is due to growing bacteria in MRS broth. **(B)** Growth curves of *L. plantarum-sagA* or *L. plantarum*-vector as compared to wild-type *L. plantarum*. SagA expression causes a ~ 2 hour extension of lag phase.

Figure S14. Individual weight loss and colonization of mice with *L. plantarum* expressing *sagA*. C57BL/6 mice were given AMNV for 14d and colonized with 10^8 CFU *L. plantarum* harboring an empty plasmid vector (*L. pl*-vector) or a *sagA* plasmid (*L. pl-sagA*) or 10^8 CFU *E. fm* prior to oral infection with 10^6 *S.* Tm. **(A)** Weight loss and **(B)** *L. pl* or *E. fm* CFU in feces are shown for each mouse included in Fig. 4I-K. Pooled data from 2 independent experiments, n=2-5 mice/group. PBS-treated control mice did not exhibit any detectable *L. pl* (that was chloramphenicol-resistant) or *E. fm* in feces.

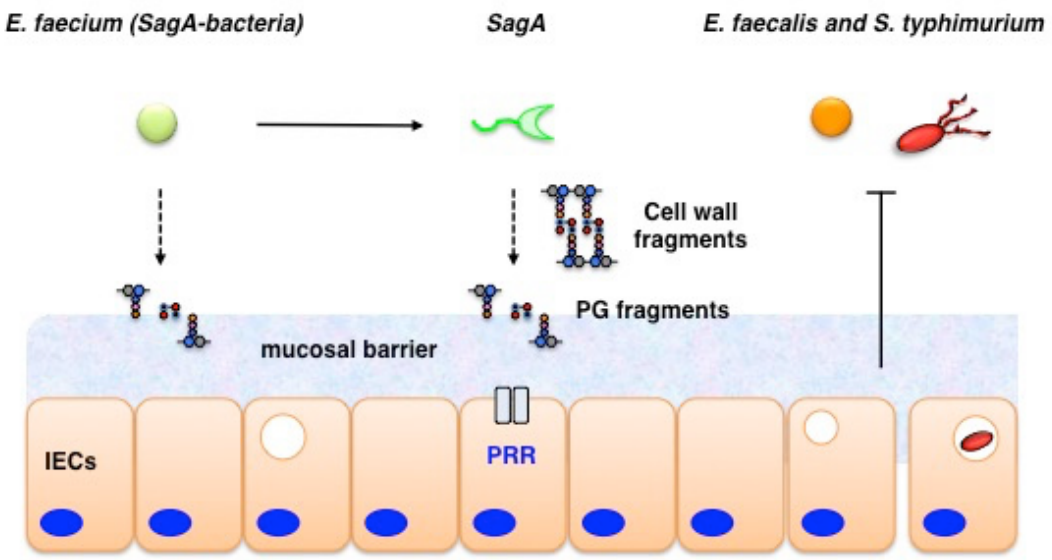


Figure S15. Model of SagA protective activity. SagA expression and secretion in *E. faecium* and other engineered bacteria may remodel peptidoglycan fragments within the bacterium and also cleave extracellular sources of peptidoglycan, both of which may be responsible for activating pattern recognition receptors (PRRs) in intestinal epithelial cells to improve barrier integrity.

Additional data tables S1 through S4 are included in a separate Excel (.xls) file.

Table S1. Proteins identified from *E. faecium* Com15 culture supernatant by mass spectrometry, after in-gel digestion. Proteins listed have at least 2 unique peptides identified. Proteins involved in peptidoglycan remodeling are highlighted in yellow. PSM = peptide spectrum match.

Table S2. Proteins identified by mass spectrometry from the top and bottom bands as indicated in Figure 3A.

Table S3. Proteins in *E. faecalis* OG1RF and *E. faecalis-sagA* culture supernatants identified by mass spectrometry, after in-solution digestion. Proteins listed have at least 2 unique peptides. Proteins involved in cell wall remodeling are highlighted in yellow. F2MNY6 and SagA-His6 are highlighted in orange. PSM = peptide spectrum match.

Table S4. Proteins in *E. faecium* Com15 culture supernatant identified by mass spectrometry, after in-solution digestion. Proteins listed have at least 2 unique peptides. Proteins involved in cell wall remodeling are highlighted in yellow. PSM = peptide spectrum match.

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