Text S1. Bacterial strains, plasmids, or primers referenced here are listed in Tables S1, S2, or S3, respectively. General cloning techniques are described in the main text.

Mouse model of ascending UTI. Mouse ascending UTI experiments were carried out in compliance with protocols approved by the Washington University in St. Louis Animal Studies Committee. Experiments were performed essentially as described previously (1) (modifications noted here) with 7 - 8 week-old C57BL/6 female mice that had been acclimated in a Washington University School of Medicine animal facility for at least one week. Briefly, bacteria grown overnight were diluted 1:10 into BHI alone or BHI supplemented with 40% horse serum (Sigma-Aldrich) and grown 2 h. Cells were harvested by centrifugation, washed, and resuspended in PBS. Anesthetized animals were inoculated transurethrally with ~1×10^7 bacterial CFU in a 200 µL volume. At 6 h or 48 h p.i., mice were sacrificed. Bladders and kidney pairs were harvested and homogenized in PBS and bacterial burdens were determined by viable plating. If no colonies were recovered from a plated sample, its CFU value was set to the limit of detection.

DNA and amino acid sequence and analysis. The *E. faecalis* OG1RF *ebpA*, *ebpB*, *ebpC*, *srtC*, and *srtA* coding sequences and any relevant non-coding regions were retrieved from the OG1RF genome (2) (Accesion: NC_017316.1) using BLAST (3) with the nucleotide sequences of the corresponding *E. faecalis* V583 loci (EF_1091, EF_1092, EF_1093, EF_1094, and EF_3056, respectively, available at http://cmr.jcvi.org) as queries. As suggested by Nallapareddy *et al.* (4), throughout this study we supposed that the translational start codon for EbpA was the Iso residue encoded 120 bp upstream of EF_1091's annotated Met translational start codon (considered EbpA's M41 in this report). Signal peptides were predicted with Signal IP (5): residues 1-28 of EbpA, 1-27 of EbpB, and 1-26 of EbpC. CWSS were identified manually as residues 1108 – 1143 for EbpA (with predicted sortase cleavage between T1111 and G1112),

residues 439 – 476 for EbpB (predicted cleavage between T442 and N443), and residues 591-625 for EbpC (predicted cleavage between T594 and G595). Molecular weights were predicted with the Compute pI/Mw tool (average resolution; http://expasy.org/) (6). Protein domain prediction for EbpA (residues 29-1111) was performed using the Phyre 2 server (7).

Construction of the gram-positive, temperature-sensitive shuttle vector pGCP213. The gram-positive, temperature-sensitive shuttle vector pJRS233 was modified in the following three ways to generate pGCP213: 1) the plasmid size was decreased from 6 kb to 4.3 kb, 2) the gram-negative pSC101 low copy ori was replaced with a pUC high-copy ori to increase plasmid yields, and 3) a full-length *lacZ* gene containing a multiple cloning site (MCS) was introduced to allow for blue/white screening of insertions via α-complementation. pGCP213 was generated as follows: first, linear pCR2.1 plasmid (Invitrogen) was circularized by digesting with EcoRI followed by ligation. A unique ClaI site was introduced into the 5' end of the *lacZ* gene by inside-out PCR using the 5'-phosphorylated primers oJoy80 and oJoy81 followed by ligation to generate pCR2.2. Finally, a unique StuI site was added in the middle of the MCS via SDM using primers GP240 and GP241 to generate pCR2.3. A 1407 bp PCR product containing the pUC ori elements and *lacZ* (containing the MCS) was amplified from pCR2.3 using primers GP224 and GP225 containing the restriction sites MluI and BglII, respectively. Next, a 2873 bp PCR product containing the pJRS233 gram-positive ori elements (originally derived from the pWV01 temperature-sensitive plasmid pG+host4) including the temperature-sensitive allele of *repA* (S54N, D56N, V57I, R59Q as previously described (8)), *orfB*, *orfC*, and *orfD*, and the Erm resistance cassette *ermC* was amplified from pJRS233 using primers GP226 and GP227 containing the restriction sites MluI and BglII, respectively. These two PCR products were digested with BglII and SphI and ligated together to generate the 4286 bp plasmid pGCP213.

Construction of the gram-positive, expression vector pGCP123. To increase the number of available restriction sites and decrease the size of the parental vector (from 7.9 kb to 3 kb), the gram-positive expression vector pABG5 (9) was modified as follows: a 2810 bp PCR product containing the pABG5 ori elements (originally derived from pWV01) including *repA*, *orfB*, *orfC*, and *orfD*, and the Kan resistance cassette *aph(3')-IIIa* was amplified from pABG5 using primers GP133 and GP134 containing the restriction sites SphI and BglII, respectively. A 235 bp PCR product containing the MCS from pJRS233 was amplified from pJRS233 using primers GP135 and GP136 containing the restriction sites BglII and SphI, respectively. This MCS derived from pJRS233 contains a truncated *lacZ* gene which does not work for blue/white screening via α-complementation, but does contain binding sites for the universal sequencing primers M13F (-21) and M13R. These two PCR products were digested with BglII and SphI and ligated together to generate the 3045 bp plasmid pGCP123.

Purification of recombinant, RGS-6×His-tagged EbpA and EbpB. One liter each of LB broth supplemented with Amp (100 μg μL^{-1}) and Kan (25 μg μL^{-1}) was inoculated with 20 mL of an overnight culture of SJH1987 or SJH1988. Once the cultures reached an optical density (600 nm) of ~0.6, expression of recombinant, RGS-6×His-tagged EbpA or EbpB was induced with 0.4 mM isopropyl-d-1-thiogalactopyranoside (IPTG). Induced cultures were grown an additional 2-3 h. After centrifugation, cells were resuspended in 10 mM Tris-HCl/100 mM NaCl (pH 7.9) and stored at -80°C. Cells were disrupted by two passages through a French press with a gauge pressure setting at 1100 p.s.i. to give an estimated cell pressure of 18,000 p.s.i. The lysates were centrifuged at 165,000 × g, and the supernatant was passed through a 0.45 μm filter. Filtrate volume was adjusted to 15 mL with 10 mM Tris-HCL/100 mM NaCL (pH 7.9). Imidazole was then added to a concentration of 6.5 mM to minimize non-specific binding. The samples were

loaded onto a nickel-charged iminodiacetic acid-Sepharose chromatography column (HiTrap chelating HP; Amersham Biosciences Corp., Piscataway, NJ) that had previously been equilibrated with 10 mM Tris-HCl/100 mM NaCl (pH 7.9) and connected to an FPLC system (Pharmacia). Bound protein was eluted with a linear gradient of 0 – 100 mM imidazole in 10 mM Tris-HCl/100 mM NaCl (pH 7.9) over 100 – 200 mL. Protein-containing fractions were analysed by SDS-PAGE, appropriately pooled, and dialysed against 25 mM Tris-HCl/1 mM EDTA (pH 6.5 - 9.0 depending on the calculated pI of the protein purified) before applying the samples to an anion-exchange Sepharose column (HiTrap Q HP; Amersham Biosciences Corp.) for further purification. Bound protein was eluted with a linear gradient of 0 – 0.5 M NaCl in 25 mM Tris-HCl/1 mM EDTA (pH 6.5 – 9.0) over 100 mL. Finally, protein samples were dialysed extensively against PBS (pH 7.4), and stored at 4°C.

Purification of recombinant, RGS-6×His-tagged EbpC. Four liters of LB broth supplemented with Amp (100 μg μL^{-1}) and Kan (25 μg μL^{-1}) were inoculated with 100 mL of an overnight culture of SJH1985. Once the culture reached an optical density (600 nm) of 0.5 - 0.6, expression of recombinant, RGS-6×His-tagged EbpC was induced with 1 mM IPTG. The induced culture was grown an additional 12 h. After centrifugation (4000×g, 20 min), cells were resuspended in lysis buffer (50 mM Tris–HCl, 2 mM EDTA), disrupted by two passages through a French press at room temperature, and centrifuged at 17,000 × g for 45 min at 4°C. The cell lysate was loaded onto a 100 mL DEAE-Sepharose column (GE Healthcare, Waukesha, WI). The column was washed with two column volumes of lysis buffer followed by a linear gradient of potassium chloride (0–1.0 M). The fractions containing EbpC were pooled and the concentration of ammonium sulfate in the pooled fractions was slowly adjusted to 1 M. The pooled sample was applied onto a 20 mL Phenyl-Sepharose column (GE Healthcare)

preliminarily equilibrated with buffer A (50 mM Tris–HCl, 2 mM EDTA, 1 M ammonium sulfate). The column was washed with two column volumes of buffer A followed by a linear gradient of lysis buffer in order to decrease the concentration of ammonium sulfate from 1.0 to 0 M. Fractions containing EbpC were pooled, concentrated to 10 mg mL^{-1} using an Amicon Ultra filter unit with a cut-off of 10 kDa (Millipore, Billerica, MA), and finally applied to a Superdex 75 column (isocratic gradient with 50 mM Tris–HCl, 2 mM EDTA). The DEAE Sepharose, Phenyl-Sepharose, and Superdex 75 columns were connected to an ÄKTA FPLC (Amersham Biosciences Corp.). Purity was checked by SDS–PAGE using 10% polyacrylamide gels.

Bacterial cell fractionation. *E. faecalis* strains grown overnight were diluted 1:100 into TSBG and grown 8 h. Equivalent cell numbers (determined by optical density at 600 nm) were harvested by centrifugation at 5000× g for 10 minutes. Supernatants were passed through 0.2 μm-pore filters (EMD Millipore, Ballerica, MA), and proteins were concentrated 50× by trichloroacetic acid precipitation; the resultant material is referred to as "supernatants" in the text. Cell pellets were washed once in PBS and digested for at least 30 min with 10 mg mL^{-1} lysozyme from chicken egg white (Sigma-Aldrich, St. Louis, MO) and 250 U ml^{-1} mutanolysin (product number M9901; Sigma-Aldrich, St. Louis, MO) in 20% sucrose/10 mM Tris-Cl/50 mM sodium chloride (NaCl)/1 mM EDTA, pH 8.0. The digestion reaction volume was ~$1/25^{th}$ of the original culture volume. Digested pellet material was considered "cell lysates." Alternatively, washed cell pellets were digested as above in a reaction volume ~$1/50^{th}$ of the original culture volume. After digestion, protoplasts and whole cells were removed by centrifugation at 20,000× g for 10 minutes. Supernatants containing material liberated from the cell wall by enzymatic digestion were designated "cell wall fractions." Fractions were stored at -20°C.

Quantification of EbpC-expressing bacterial cells using IFM. Bacterial strains were labeled and imaged by IFM as described in the Material and Methods of the main text. Images of 5-10 fields were captured for each bacterial strain in each experiment using a 100× objective. Exposure times within each experiment were constant. Hoescht staining of bacterial DNA revealed bacterial cells in DAPI channel images. EbpC expression was evident in Texas Red channel images. For quantification, DAPI and Texas Red channel images were separately inverted, and an upper and lower threshold was applied using ImageJ (National Institutes of Health, Bethesda, MD). Threshold values were constant for each channel within each experiment. The Analyze Particles function (pixels: 0-∞; circularity: 0-1) was used on DAPI channel images to generate a binary image of outlines of Hoescht staining (bacterial cells) which were stored as regions of interest (ROIs). ROIs were overlaid on thresholded Texas Red channel images. Bacterial cells were counted manually within the ROIs using the Cell Counter plugin for ImageJ and categorized as either EbpC- or EbpC+ (EbpC-expressing) if signal from the Texas Red channel was observed in close proximity. For each image, a percentage of EbpC+ cells was determined. The percentage of EbpC+ cells for an image = (number EbpC+ cells) / [(number of EbpC+ cells) + (number of EbpC- cells)] × 100.

REFERENCES

1. **Kau AL, et al**. 2005. Infect Immun. **73**:2461-2468.
2. **Bourgogne A, et al**. 2008. Genome Biol. **9**:R110.
3. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ**. 1990. J. Mol. Biol. **215**:403-410.
4. **Nallapareddy SR, et al**. 2006. J. Clin. Invest. **116**:2799-2807.
5. **Petersen TN, Brunak S, von Heijne G, Nielsen H**. 2011. Nat. Methods. **8**:785-786.
6. **Wilkins MR, et al**. 1999. Methods in Mol. Biol. **112**:531-552.
7. **Kelley LA, Sternberg MJ**. 2009. Nat. Protoc. **4**:363-371.
8. **Maguin E, Duwat P, Hege T, D. Ehrlich D, Gruss A**. 1992. J. Bacteriol. **174**:5633-5638.
9. **Granok AB, Parsonage D, Ross RP, Caparon MG**. 2000. J. Bacteriol. **182**:1529-1540.