Supporting information:

Wall teichoic acids prevent antibody binding to epitopes within the cell wall of *Staphylococcus aureus*

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Supplemental figures:

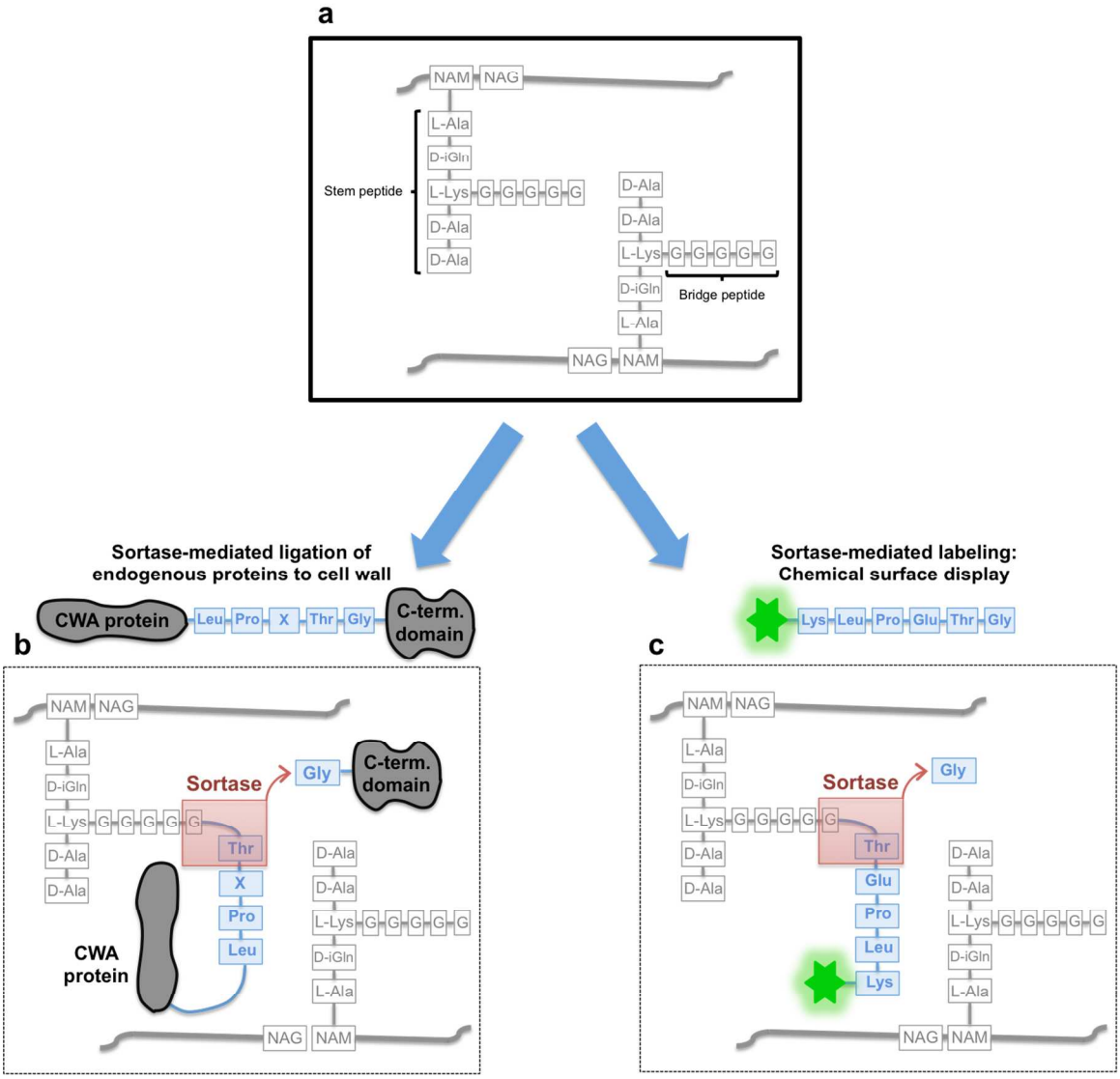

Fig. S1 | Schematic representation of endogenous sortase activity and sortase-mediated chemical display within the cell wall of *S. aureus*. (a) Structures of the stem peptide and bridge peptide in *S. aureus* peptidoglycan are shown. **(b)** The endogenous function of sortase: recognition of the LPxTG peptide motif, cleavage of the glycine together with the C-terminal domain, and formation of a covalent bond between the threonine of the cell wall-associated protein and the N-terminal glycine of the bridge peptide. **(c)** Sortase-mediated incorporation of exogenous small molecules: recognition of the LPETG peptide motif, cleavage of the C-terminal glycine, and formation of a covalent bond between the threonine of the probe and the N-terminal glycine of the bridge peptide. Abbreviations: CWA protein, cell wall-associated protein; D-Ala, D-alanine; G, glycine; D-iGln, D-iso-glutamine; Gly, glycine; L-Ala, L-alanine; Leu, L-leucine; Lys, L-lysine; NAM, N-acetylmuramic acid; NAG, N-acetyglucosamine; Pro, L-proline; X, any amino acid.

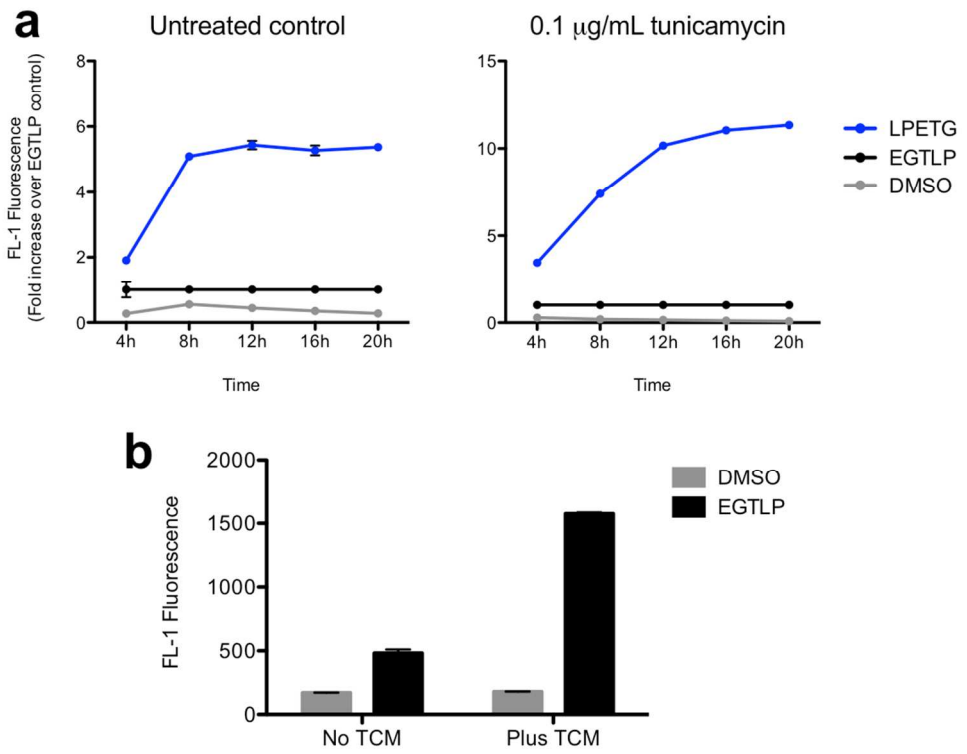

Fig. S2 | Sortase-mediated labeling in Δ*spa S. aureus* in the presence and absence of tunicamycin. (a) Δ*spa S. aureus* Newman was labeled with K(FITC)-LPETG, K(FITC)-EGTLP, or DMSO vehicle control with or without 0.1 μg mL^{-1} tunicamycin. At indicated times, samples were analyzed for fluorophore incorporation by measuring total cell FL-1 fluorescence on a flow cytometer. Data shown is expressed as fold increase over EGTLP control. **(b)** Δ*spa S. aureus* Newman was labeled with K(FITC)-EGTLP or DMSO vehicle control with or without 0.1 μg mL^{-1} tunicamycin for 16h and analyzed for fluorophore incorporation by measuring total cell FL-1 fluorescence on a flow cytometer. Data shown is representative of multiple independent trials. Experiments performed in technical duplicate; error bars indicate SEM. Abbreviations: TCM, tunicamycin.

Movie S1 | 3-Dimensional SIM imaging reveals that antibodies are recruited superficial sortase-incorporated cell wall epitopes. Δ*spa S. aureus* Newman was labeled with K(A488)-LPETG with 0.1 μg mL^{-1} tunicamycin, and then incubated with anti-Alexa 488 primary antibodies, followed by Alexa 647-conjugated secondary antibodies. Structured illumination microscopy (SIM) imaging analysis was performed to visualize epitope and antibody localization at super-resolution. The movie represents a rotation of the maximal 3-D projection of the cell shown in Figure 2c around its Y-axis (a cell with no surface-bound antibodies is also seen below).

Supplemental methods:

Bacterial strains and culture: *S. aureus* Newman WT and Δ*srtA* mutant SrtA– strain were kindly donated by O. Schneewind (University of Chicago).[1] *S. aureus* strain *spa::kan*, a protein A deletion mutant, was obtained from T. J. Foster (Trinity College, Dublin, Ireland).[2] *S. aureus* Newman Δ*tagO* mutant strain was a kind gift from S. Walker (Harvard Medical School).[3] *S. aureus* Reynolds WT and CP- mutant strain were generously provided by J.C. Lee (Harvard Medical School).[4] *S. aureus* was routinely cultured in LB broth (Difco, Sparks, MD) at 37°C with agitation at 200 RPM. 5 mL liquid cultures were inoculated with single colonies and grown overnight to stationary phase before dilution and addition of peptides

Chemical synthesis: Probes were synthesized as reported in reference [5].

Peptide labeling: Stationary phase cultures were diluted 1:100 K(FITC)-LPETG, K(FITC)-EGTLP, K(biotin)-LPETG, K(biotin)-EGTLP, and K(A488)-LPETG probes were added at 1 mM from 100 mM stocks in DMSO and incubated for indicated lengths of times. Cultures were collected by centrifugation at 12,000 x g for 2 minutes and then washed 3X in ice-cold PBS. Finally, cells were washed with acetone to fix and more effectively remove non-specifically adsorbed probe, and resuspended in PBS for analysis by flow cytometry.

Flow cytometry: For experiments involving bacteria, samples were diluted in PBS to a concentration of <5,000 cells μL^{-1} and 10,000 events per sample were analyzed on an Accuri C6 flow cytometer (BD, Franklin Lakes, NJ) on medium speed fluidics with a minimum threshold of 40,000 FSC-H. Maximum FSC and SSC gates were set to exclude multi-cell aggregates (e.g. 50,000 FSC-A and 50,000 SSC-A for *S. aureus*).

Protein recruiting: For recruiting experiments, stationary phase cultures were diluted 1:100 and K(FITC)-LPETG, K(FITC)-EGTLP, K(biotin)-LPETG, K(biotin)-EGTLP, and K(A488)-LPETG probes were added at 1 mM and incubated for 16 hours. Cultures were collected by centrifugation at 12,000 x g for 2 minutes and then washed 3X in PBS. Cells were then resuspended in PBS to a volume of 100 µl with antibody or streptavidin, incubated 30 minutes at 4°C, washed with PBS, and analyzed by flow cytometry. Streptavidin recruiting experiments were conducted using streptavidin-Alexa 647 at 2.5 $\mu g\ mL^{-1}$ (Invitrogen, Carlsbad, CA, Cat # S-21374**)**. For antibody recruiting to FITC, a DyLight 649 conjugated goat anti-FITC antibody (Rockland, Gilbertsville, PA, Cat # 600-143-096) was used at a final concentration of 5 $\mu g\ mL^{-1}$. For high-resolution imaging experiments, rabbit Anti-Alexa 488 antibody (Invitrogen, Cat # A-11094) was used at 5 $\mu g\ mL^{-1}$ and a goat secondary antibody conjugated to Alexa-568 directed against rabbit IgG was used at 5 $\mu g\ mL^{-1}$ (Invitrogen**)**. After recruitment of antibodies, bacteria were prepared for microscopy analysis by fixation in 4% paraformaldehyde (diluted from fresh ampoules of 16% solution, Electron Microscopy Sciences, Hatfield, PA), and washing 2X with PBS. Cells were then resuspended in PBS, dropped onto 1.5 mm glass coverslips coated with poly-L-lysine (Santa Cruz Biotechnology, Santa Cruz, CA), and air-dried.

Coverslips were mounted in ProLong Gold antifade reagent (Invitrogen) and cured for at least 24 hours before imaging.

Microscopy: Both conventional fluorescence (widefield) and three dimensional-structured illumination microscopy (3D-SIM) images were acquired using a U-PLANAPO 60X/1.42 PSF, oil immersion objective lens (Olympus, Center Valley, PA) and CoolSNAP HQ2 CCD cameras with a pixel size of 0.080µm (Photometrics, Tucson, AZ) on the OMX version 3 system (Applied Precision, Issaquah, WA) equipped with 488-, and 561-nm solid-state lasers (Coherent, Santa Clara, CA and MPB Communications, Montréal, Quebec). Select widefield Z-stacks were subjected to deconvolution processing using Softworx software (Applied Precision). For 3D-SIM imaging, samples were illuminated by a coherent scrambled laser light source that had passed through a diffraction grating to generate the structured illumination by interference of light orders in the image plane to create a 3D sinusoidal pattern, with lateral stripes approximately 0.270 nm apart. The pattern was shifted laterally through five phases and through three angular rotations of 60º for each Z-section, separated by 0.125 nm. Exposure times were typically between 200 and 500 ms, and the power of each laser was adjusted to achieve optimal intensities of between 2,000 and 4,000 counts in a raw image of 16-bit dynamic range, at the lowest possible laser power to minimize photobleaching. Raw images were processed and reconstructed to reveal structures with 100-125 nm resolution using Softworx software (Applied Precision). The channels were then aligned in x, y, and rotationally using predetermined shifts as measured using a target lens and the Softworx alignment tool (Applied Precision). For clarity of display, small changes to brightness and contrast were performed on 3D reconstructions.

References:

(1) Mazmanian, S. K.; Liu, G.; Jensen, E. R.; Lenoy, E.; Schneewind, O. *Proc Natl Acad Sci U S A* **2000**, *97*, 5510.

(2) Patel, A. H.; Foster, T. J.; Pattee, P. A. *Journal of general microbiology* **1989**, *135*, 1799.

(3) Suzuki, T.; Swoboda, J. G.; Campbell, J.; Walker, S.; Gilmore, M. S. *Antimicrobial agents and chemotherapy* **2011**, *55*, 767.

(4) Watts, A.; Ke, D.; Wang, Q.; Pillay, A.; Nicholson-Weller, A.; Lee, J. C. *Infect Immun* **2005**, *73*, 3502.

(5) Nelson, J. W.; Chamessian, A. G.; McEnaney, P. J.; Murelli, R. P.; Kazmierczak, B. I.; Spiegel, D. A. *ACS chemical biology* **2010**, *5*, 1147.