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Figures and figure supplements

A mutant *Escherichia coli* that attaches peptidoglycan to lipopolysaccharide and displays cell wall on its surface

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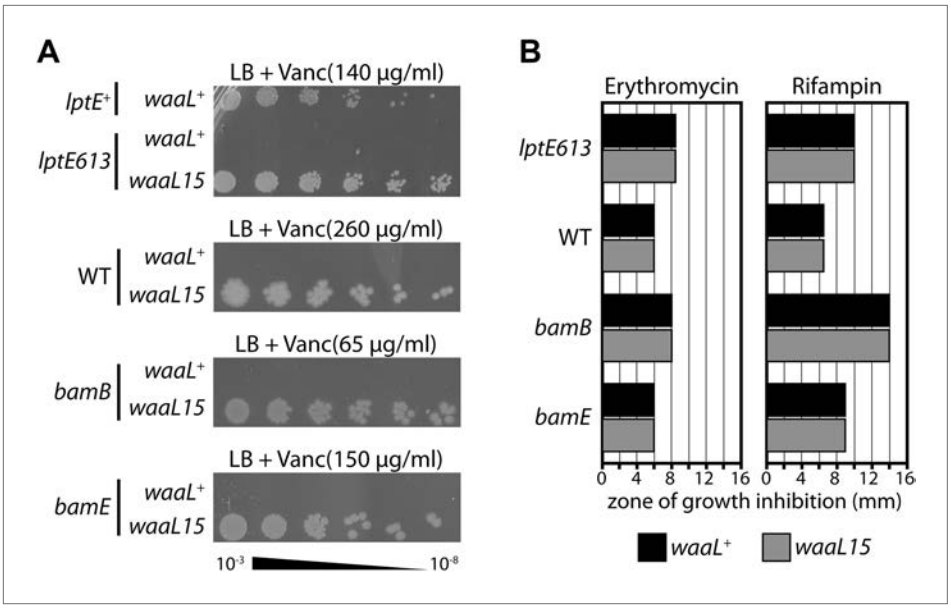

Figure 1. A mutant O-antigen ligase increases vancomycin resistance. (**A**) *waaL15* provides a strain-independent increase in vancomycin resistance. Isogenic strains, differing by a point mutation in *waaL*, were plated by serial dilution on LB agar containing indicated amounts of vancomycin. (**B**) *waaL15* does not improve resistance against other antibiotics. Antibiotic discs containing either 15 µg erythromycin or 5 µg rifampin were placed on LB agar overlays inoculated with the indicated strains. Diametric zones of growth inhibition were measured across the disc. The disc diameter was 6 mm and this value represents growth at the disc.

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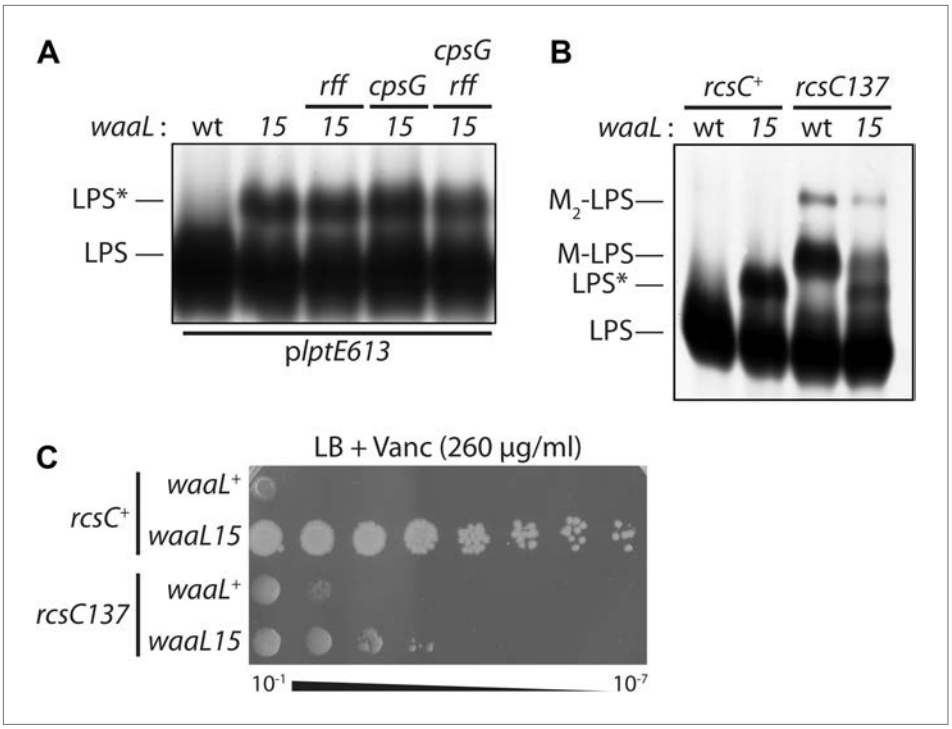

Figure 2. Mutant O-antigen ligase produces a novel form of LPS that is directly responsible for vancomycin resistance. (**A**) WaaL15 uses a novel substrate to produce a new LPS glycoform. Isolated LPS was resolved by SDS-PAGE and detected by silver staining. A higher molecular weight glycoform (LPS*) appears in *waaL15* strains. Mutations that inactivate biosynthesis of ECA (*rff*::Tn*10*-66) or CA (Δ*cpsG*::*kan*) do not abrogate LPS* production. (**B**) Overproduction of CA leads to decreased LPS* abundance. Isogenic strains were constructed to express either wt *rcsC*$^+$ or the *rcsC137* mutant allele that hyper-activates CA biosynthesis. LPS was isolated and visualized as in (**A**). LPS molecules modified with a one- or two- CA repeat units are labeled M-LPS and M_2-LPS, respectively. (**C**) Reduced LPS* levels correlate with reduced vancomycin resistance. Strains were plated by serial dilution onto LB agar supplemented with vancomycin.

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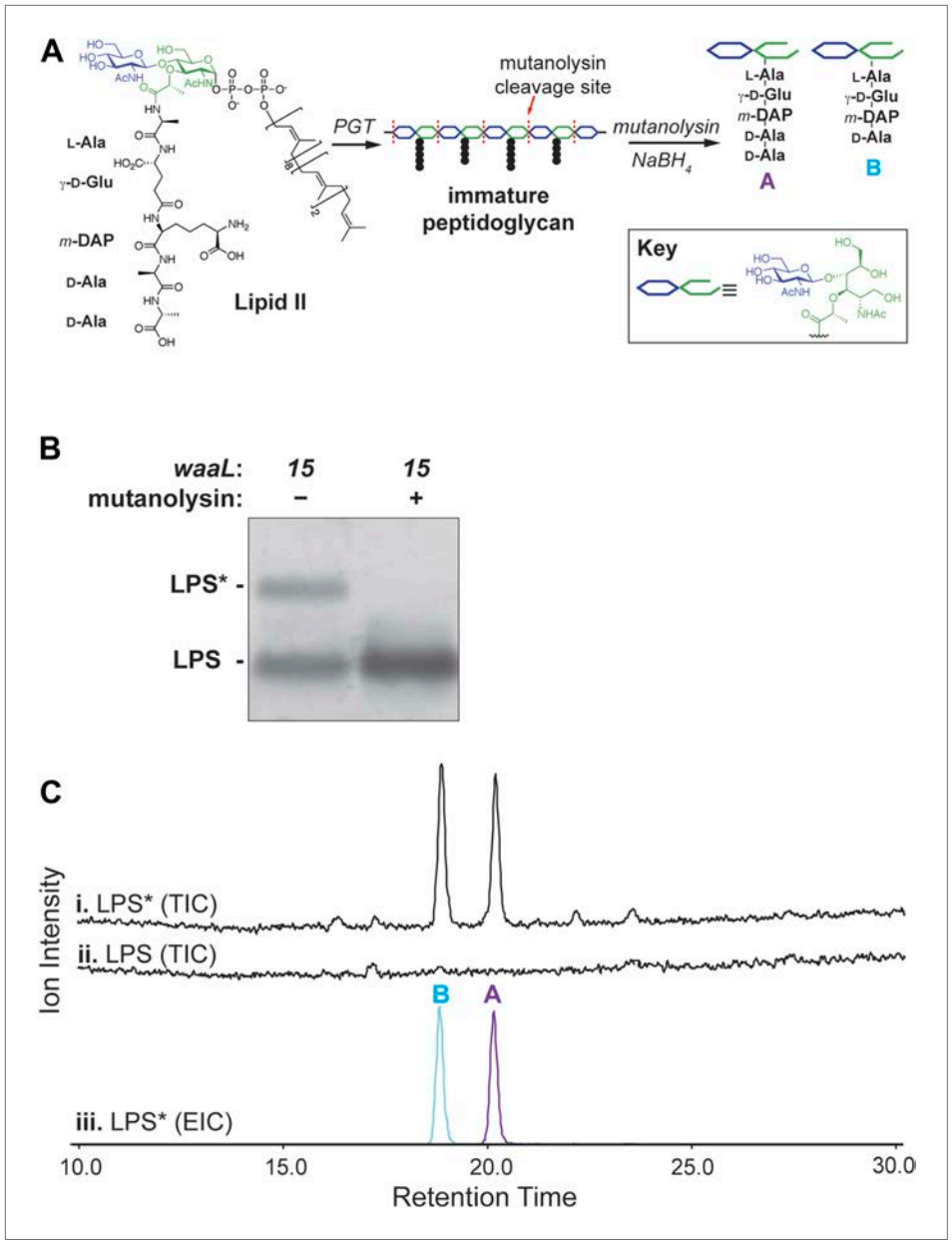

Figure 3. Lipid II is the glycosyl donor for LPS*. (**A**) Structure of lipid II and schematic of peptidoglycan cleavage by mutanolysin that releases disaccharide pentapeptide ('A') and tetrapeptide ('B') species. (**B**) Treatment of *waaL15* isolated LPS with mutanolysin cleaves the LPS* modification. (**C**) LPS* is glycosylated with equivalent amounts of lipid II-sourced disaccharide pentapeptide and tetrapeptide. Isolated and purified LPS* from *waaL15* and LPS from *waaL*$^{+}$ were treated with mutanolysin and analyzed by LC-MS. Total ion chromatogram for degradation products (i and ii), and the extracted ion chromatogram for LPS* degradation (iii) are shown. M+H and (M+2H)/2 ions corresponding to each fragment were extracted (A: 1013.3 + 507.2; B: 942.3 + 471.7).

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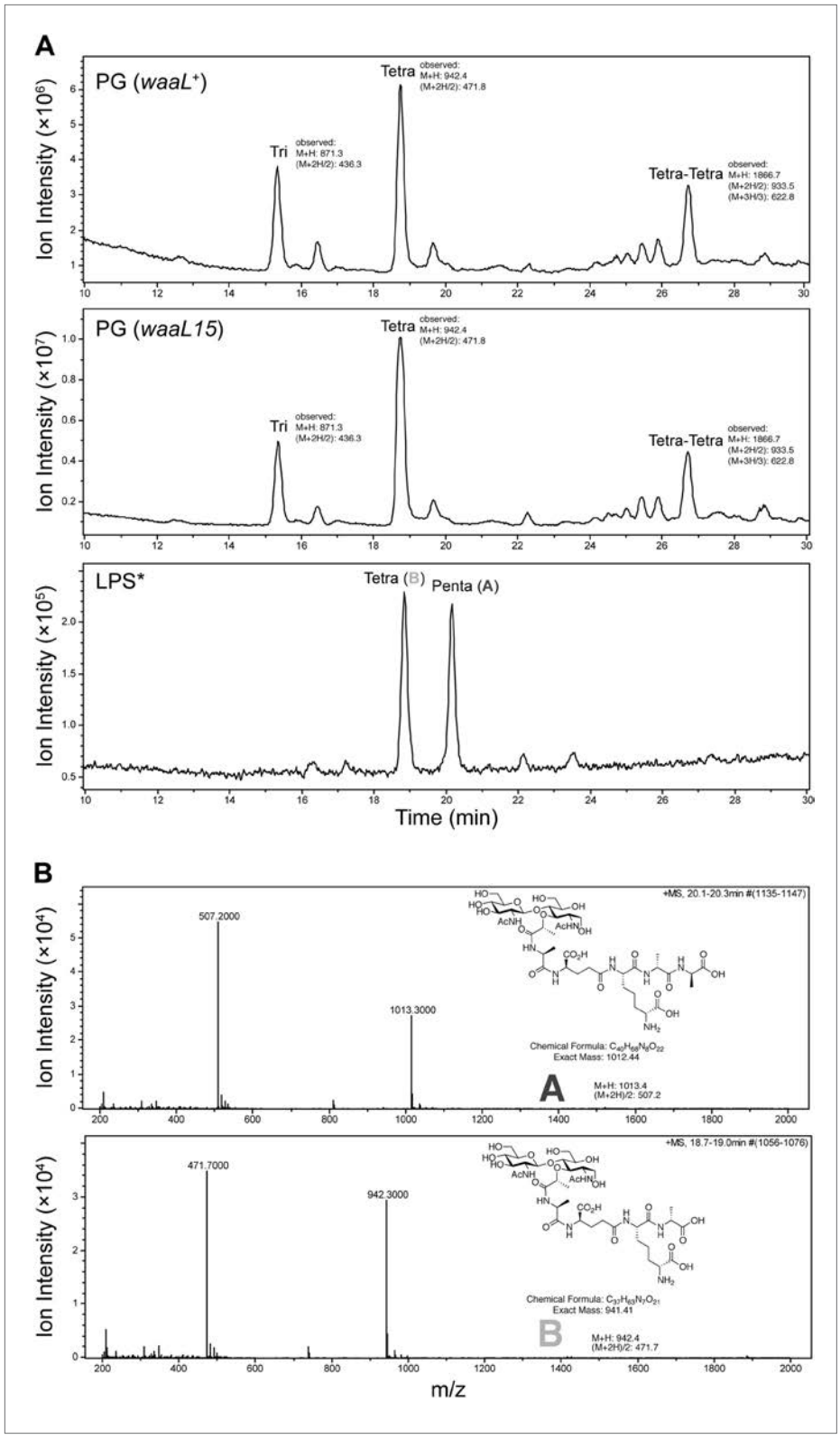

Figure 3—figure supplement 1. The *waaL15* mutation does not affect the PG cell wall. (**A**) The total ion chromatograms (TIC) of mutanolysin digested PG cell wall from *waaL*⁺ strain MG1210, and *waaL15* strains MG1211 are nearly identical. Disaccharide pentapeptide (DPP, fragment A) is present in mutanolysin digested LPS* but not in the PG cell wall samples. (**B**) Mass spectra and structures for reduced DPP (fragment A) and reduced disaccharide tetrapeptide (fragment B) from mutanolysin digested LPS*.

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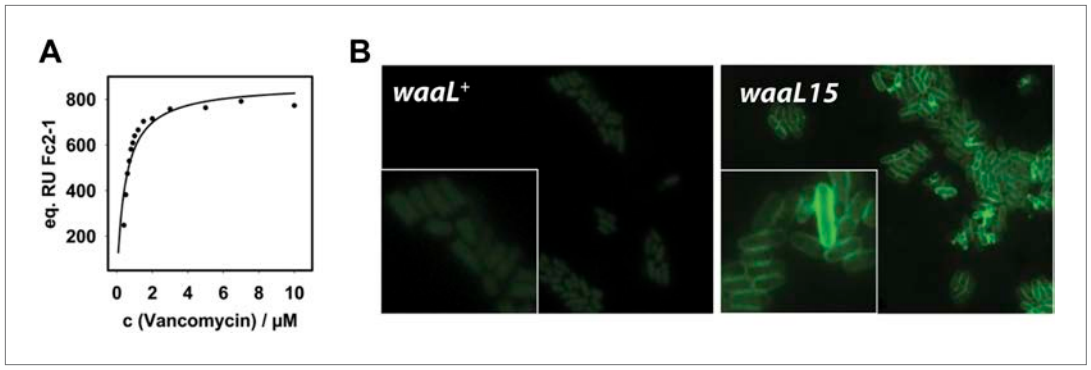

Figure 4. Mutant WaaL attaches peptidoglycan fragments to LPS. (**A**) LPS* specifically binds vancomycin. Purified LPS* was immobilized on a CM3 chip and varying concentrations of vancomycin were applied. Binding was measured at 25°C by surface plasmon resonance. Fitting of equilibrium signal yielded a K_d = 0.48 ± 0.08 µM. Standard deviation was measured for 0.6 µM and 1.2 µM and was ±1 RU. (**B**) Vancomycin binds to LPS* at the cell surface. Live exponential-phase growing cells labeled with 1 µg/ml vancomycin-BODIPY for 10 min. Cells were spotted onto M63 minimal medium agar pads and imaged by fluorescence microscopy.

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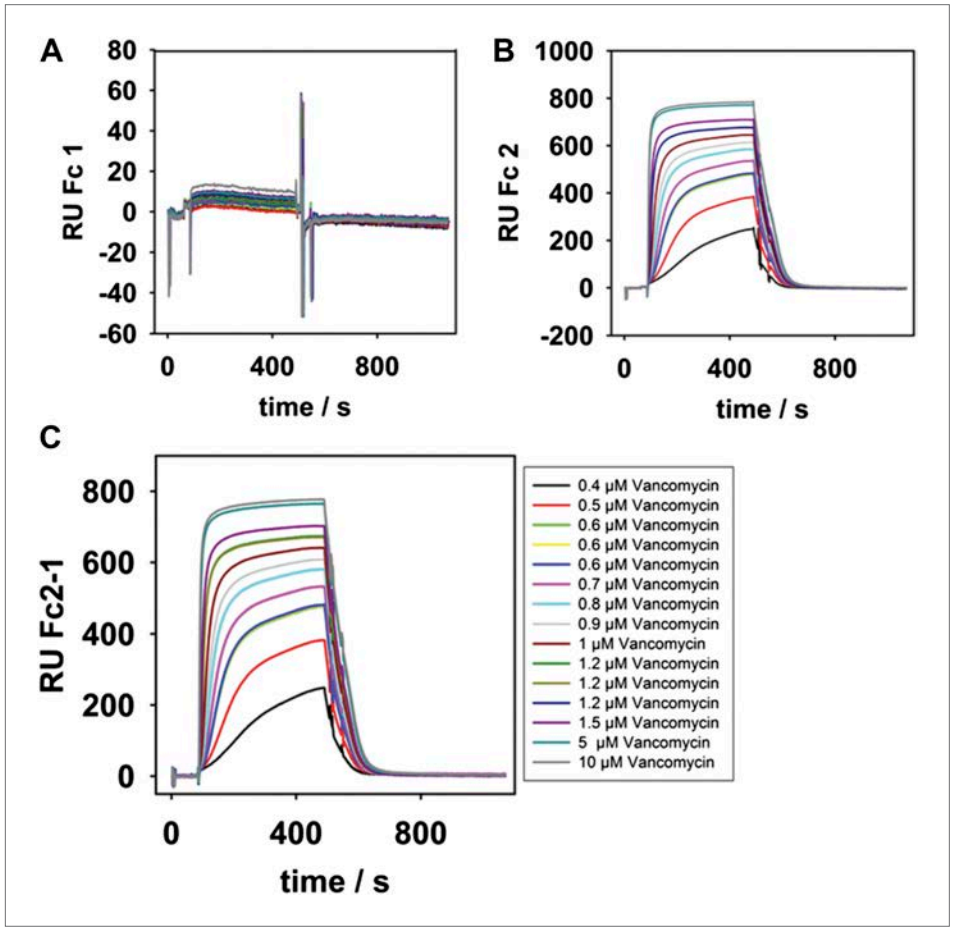

Figure 4—figure supplement 1. SPR binding kinetics at 25°C. (**A** and **B**), Different concentrations (see legend inset in **C**) of vancomycin were passed over surfaces of total isolated LPS from *waaL*+ (strain MG1210) or total isolated LPS from *waaL15* (strain MG1211). (**C**), The reference subtracted kinetics after 400 s of injection. The equilibrium signals were plotted in ***Figure 4A***.

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