

**Figure S1.** Motility testing of *V. fischeri* strain ES114 *waaL*::Tn*erm* demonstrates a diminished flagellar-dependent migration in soft agar. Strains of ES114 wild-type, ES114 *waaL*::Tn*erm* (MB06859) and ES114 Ig::Tn*erm* (control) were inoculated through LBS 0.3% agar using round toothpicks. Strain ES114 Ig::Tn*erm* harbors a similar antibiotic resistance cassette as the *waaL* strain and was used as a control to demonstrate that the motility defect was not related to the presence of the cassette. The migration distance was measured as the diameter of the outer ring during chemotaxis in soft agar (A), and plates were imaged at 24 h (B). Plotted are the mean and standard error for three replicates in a typical experiment.



**Figure S2.** Box-whisker plot of luminescence, in relative luminescence units (RLU), of animals colonized with different bacterial strains, 44 h into exposure. N = 10 animals for each treatment except N=20 for MB06859. Statistical comparison of treatments is by Mann-Whitney U test, following Kruskal-Wallis test for all data. Significantly different treatments are marked with \* (p < .05).



**Figure S3.** Box-whisker plot of *V. fischeri* CFU per light organ, determined by homogenizing and plating animals frozen 24 h into exposure. Animals were colonized with either wild-type or *waaL*-mutant bacteria, N = 16 animals for each treatment. Statistical comparison of treatments is by Mann-Whitney U test, p < .001 (indicated by \*\*\*). Logarithmic scale.

Figure S4



**Figure S4.** GC-MS analyses of the TMS derivatives of LPS isolated from (A) ES114 wild-type and (B) ES114 *waaL V. fischeri*. Mannitol was added as internal standard. The peak labeled with an \* indicates an impurity peak in the sample.

Figure S5



**Figure S5.** GC-MS analyses of the TMS derivatives of *O*-LPS isolated from (A) ES114 wild-type and (B) ES114 *waaL V. fischeri*.

Figure S6



**Figure S6.** GC-MS analyses of the TMS derivatives of PS isolated from (A) ES114 wild-type and (B) ES114 *waaL V. fischeri*. These data demonstrate that the wild-type sample has FucNAc and Yersiniose whereas the PS isolated from ES114 *waaL* does not contain those sugars.

Figure S7



**Figure S7.** GC-MS spectra of TMS derivatives of HF-treated PS from (A) ES114 wild-type and (B) ES114 *waaL V. fischeri*. The HF treatment removed any phosphate groups from the structure. The appearance of DD-Hep in the HF treated PS, but not in the untreated PS demonstrates that a phosphate group is linked to this sugar. The assignment of LD-Hep versus DD-Hep was done by matching the GC-MS fragmentation pattern with previous reports of LD- and DD-Hep on similar phase columns.



**Figure S8.** Negative ion vMALDI-LIT mass spectra of the *O*-deacylated LPS (*O*-LPS) from (A) ES114 wild-type, (B) ES114 waaL (MB06859) (C) ES114 waaL vector only control (BK111) and (D) ES114 waaL complement (BK110) strains. Masses labeled in bold correspond to the predominant glycoforms present in the samples. OS prompt fragments are labeled in italics. The <sup>a-f</sup> letters correlate the OS prompt fragments with their intact *O*-LPS masses. Masses labeled with an \* designate major masses minus water or CO<sub>2</sub>. The neutral loss of phosphoric acid, H<sub>3</sub>PO<sub>4</sub>, from major masses are indicated with a ^ symbol. The addition of 123 Da corresponds to the addition of one PEA group. The differences seen in the masses of the ES114 waaL vector only control glycoforms compared to the other strains are due to differences in the heterogeneity in the lipid A components of these structures.

Figure S9



**Figure S9**. MALDI-MS spectra of HF treated ES114 *waaL* PS run in (A) negative ion mode and (B) positive ion mode. HF treatment removes any phosphate groups from the LPS and cleaves the PS from the lipid A. Ions circled indicate the dephosphorylated PS liberated from the *O*-LPS. Peaks labeled with a ^ indicate major ions which have lost  $CO_2$ .



**Figure S10.** MALDI-MS spectra of HF treated ES114 wild-type PS run in (A) negative ion mode and (B) positive ion mode. HF treatment removes any phosphate groups from the LPS and cleaves the PS from the lipid A. Ions circled indicate the dephosphorylated PS liberated from the *O*-LPS. Peaks labeled with a ^ or with an \*indicate major ions which have lost  $CO_2$  or  $H_2O$  respectively.



**Figure S11**. <sup>31</sup>P 1D NMR spectra of the ES114 wild-type and ES114 *waaL* mutant *V. fischeri* PS samples.



**Figure S12**. <sup>31</sup>P/<sup>1</sup>H COSY spectra of the *V. fischeri* ES114 wild-type (A) and the ES114 *waaL* mutant (B) PS samples. The resonances derived from the PEA and the core components are indicated.