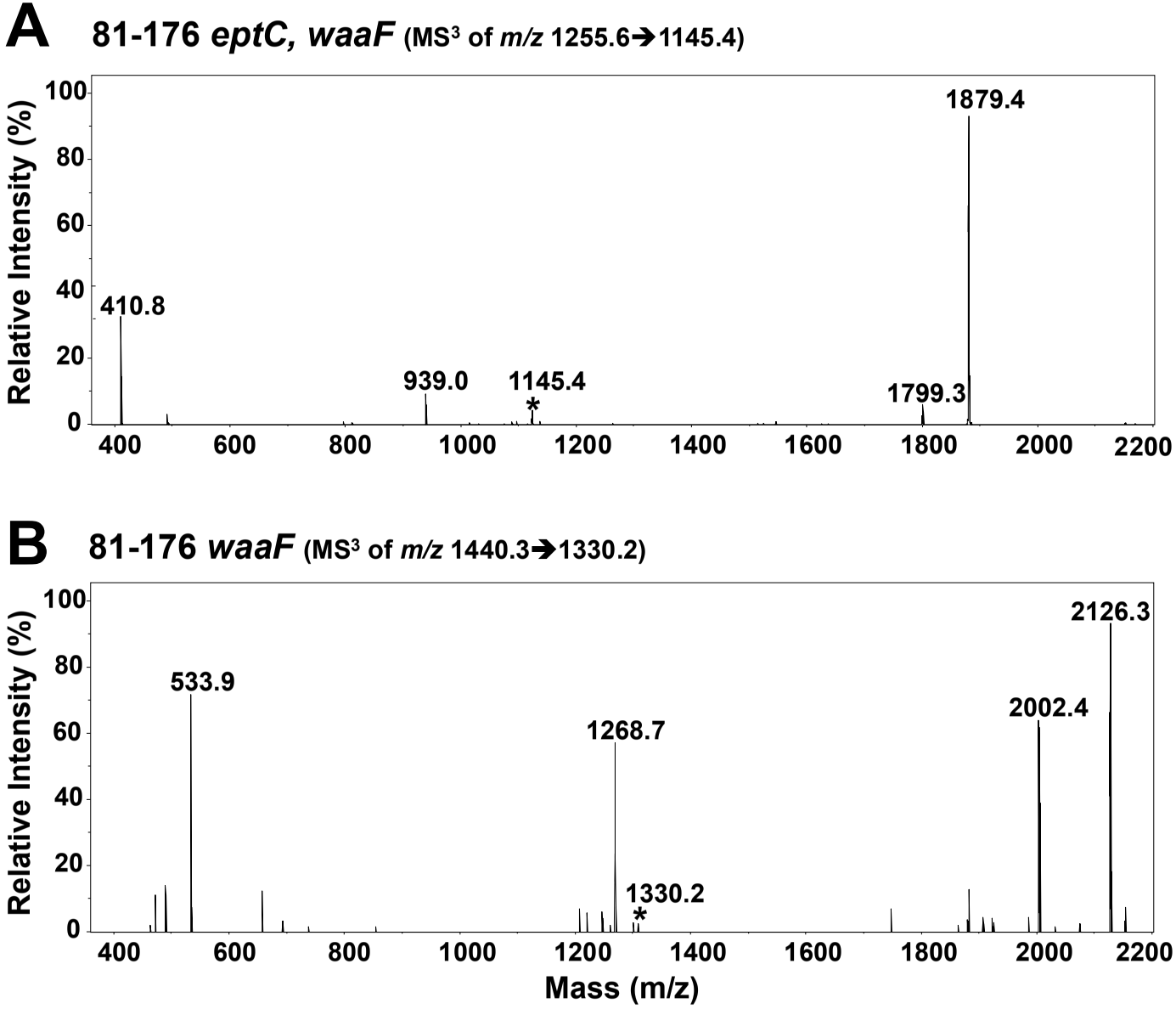
Supplemental Figure 1. LC/ESI-MS^3 of A) precursor ion m/z 1145.4 (origin: MS/MS of m/z 1255.6) from 81-176 *eptC, waaF* and B) precursor ion m/z 1330.2 (origin: MS/MS of m/z 1440.3) from 81-176 *waaF* LOS. The ion of m/z 1330.2 which is postulated to evolve upon the loss of one Kdo group (a pathway illustrated in supplemental figure 3) was isolated and subjected to a second stage of collisional activation, yielding the MS^3 spectrum shown in Supplemental Figure 1B. Two of the key fragment ions, m/z 533.9 and 2126.3, are directly analogous to the complementary pair observed in Supplemental Figure 1A (m/z 410.8 and 1879.4) and correspond to the same glycosidic bond cleavage. Most importantly, the ion of m/z 533.9 confirms that one of the pEtN modifications remains on the (heptose + Kdo sugar) sub-structure of the inner core domain, whereas the other two pEtN groups remain on the lipid A structure. The selected precursor ions are labeled with an asterisk. The results agree between at least two experimental replicates of a single biological sample.

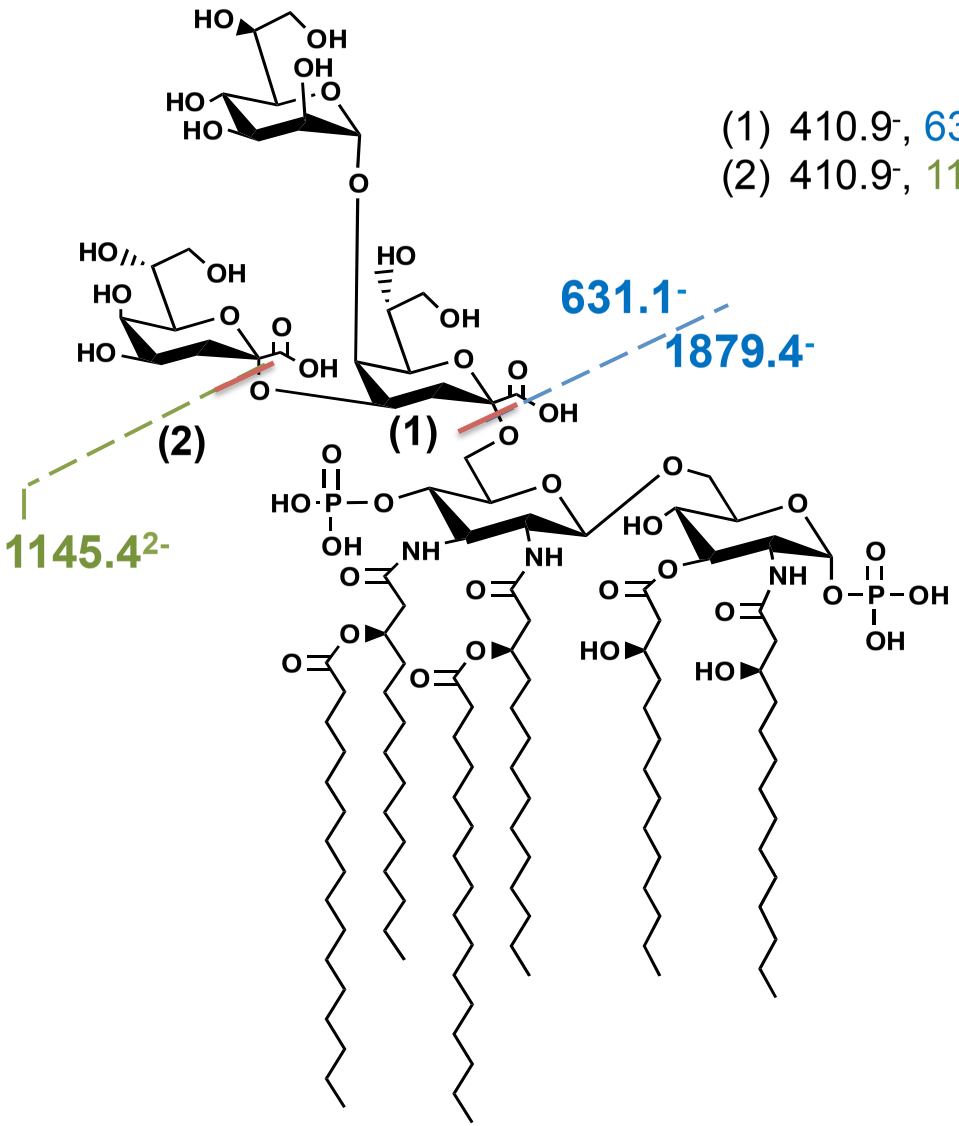
Supplemental Figure 2. Fragmentation map for *C. jejuni* 81-176 *eptC, waaF* molecular ion 1255.6 (2-). The key cleavages are labeled as 1, 2 and 3, and these cleavages result in the ions summarized in the upper right inset. Fragment ions that arise from two cleavages are listed next to each cleavage number.

Supplemental Figure 3. Fragmentation map for *C. jejuni* 81-176 *waaF* molecular ion 1440.3 (2-). The key cleavages are labeled as 1, 2 and 3, and these cleavages results in the ions summarized in the upper right inset. Fragment ions that arise from two cleavages are listed next to two cleavage numbers.

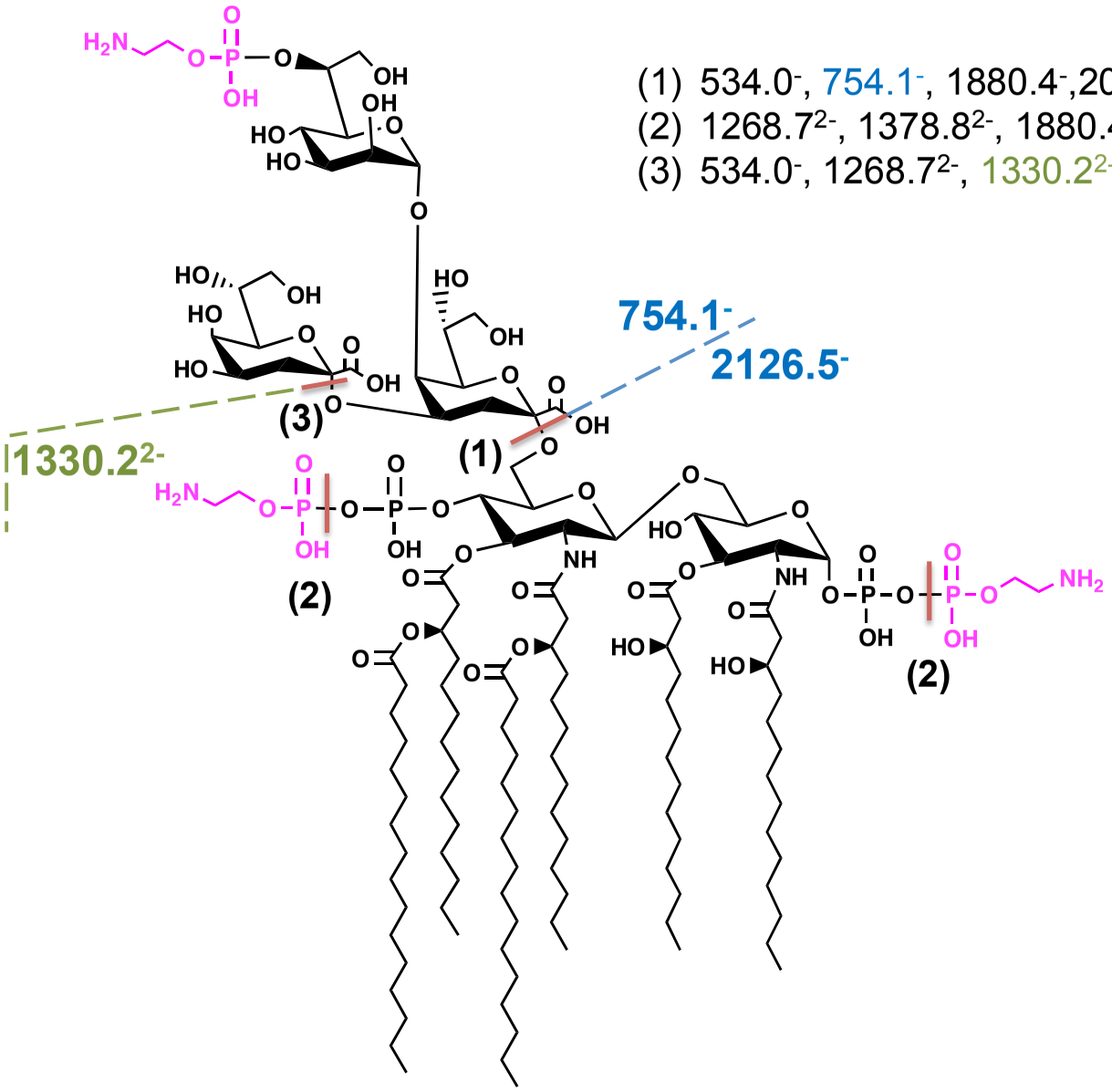
Supplemental Figure 4. Activation of human TLR2 by *C. jejuni* LOS. Activation of hTLR2 was monitored using HEK-293 cells stably transfected with hTLR2. TLR activation was monitored colorimetrically using a secreted alkaline phosphatase (SEAP) reporter gene placed under the control of an NF-κB inducible promoter. HEK-293 cells were stimulated for 24 hours with the indicated ligands. Highly purified LOS from the indicated strains of *C. jejuni* was added to each well in triplicate at the indicated concentrations. Values are the means of results from triplicate wells ± standard deviation. No significant activation of hTLR2 was seen in LPS prepared from all *C. jejuni* strains ($p > 0.05$). For each experiment Pam3CSK4, a synthetic lipopeptide, and LPS from *E. coli* were used as positive and negative controls for activation of hTLR2. Identical results were seen for activation of murine TLR2 (data not shown). Statistical comparisons of mean activation were made between wild type and select mutants LOS/whole cells. Data for this figure was generated from three experimental replicates of a single biological sample. However, all results agreed between at least two biological replicates.



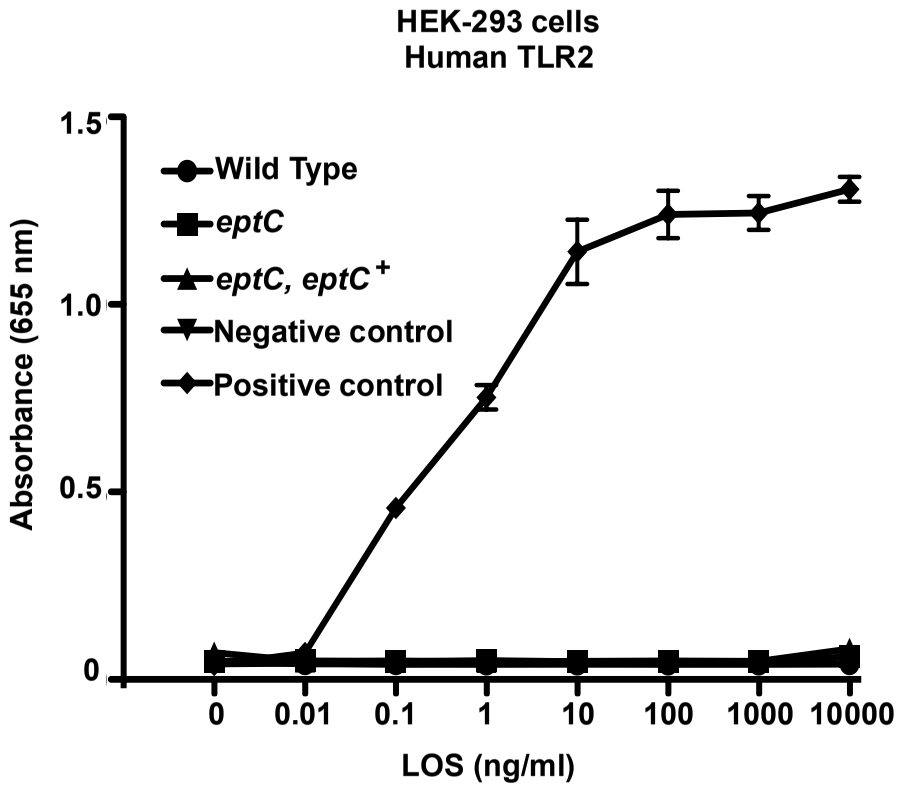
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4