SUPPLEMENTARY MATERIAL

Characterization of and lipopolysaccharide binding to the *E. coli* LptC protein dimer

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Figure S1. A) Crystallographic N-terminal dimer structure of LptC G153R (pdb: 4B54²⁰) with studied residues highlighted. Residues 1-58 and 183-191 are missing from the structure. B) Purified LptC Q65C protein in the presence of 50-fold molar excess of DTME (lane 3) or BMOE (lane 5) and in the absence of crosslinker (lane 7) as observed by Coomassie stained SDS-PAGE. Lane 1 contains molecular weight markers. The bands observed in lanes 2, 4, and 6 are the result of sample overloading and are intended to be blank. The LptC monomer (m) and dimer (d) locations are indicated. C) CW X-band EPR spectral overlays for LptC Y182R1 at 2 μ M (black) and 100 μ M (purple) protein concentration. Spectral width is 100 G.



Figure S2. CW X-band EPR spectra of 10 μ M M24R1, F95R1, V121R1, and V139R1 LptC in the absence and presence of 85 μ M WT LptA (black and blue, respectively) or 30% Ficoll (gray). Spectral width is 100 G; spectra are normalized to the same center line height.



Figure S3. Structure of LptC (pdb: 3MY2¹⁸) with the side chain colors representing motional changes due to the addition of LPS. Purple indicates that R1 has decreased motion, pink indicates R1 has increased motion (S76, V117, A172), and gray indicates similar motion (V139) in the presence of excess LPS compared to the apo state. Dashed lines represent the N- and C-terminal regions unresolved in the crystal structure.



Figure S4. Far UV circular dichroism spectra of WT, F78R1, and Y182R1 LptC at a protein concentration of 5 μ M in 50 mM sodium phosphate, pH 7.0, buffer with 0–3 mM NaCl. The F78R1 data are scaled down 4.8-fold.