Supplementary information

Structural basis of lipopolysaccharide maturation by the O-antigen ligase

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Structural basis of Lipopolysaccharide Maturation by O-Antigen Ligase WaaL

Khuram U. Ashraf[1], Rie Nygaard[1], Owen N. Vickery[2,3,#], Satchal K. Erramilli[4,#], Carmen M. Herrera[5,#], Thomas H. McConville[6,#], Vasileios I. Petrou[7,8], Sabrina I. Giacometti[1], Meagan Belcher Dufrisne[1], Kamil Nosol[4], Allen P. Zinkle[1], Chris L.B. Graham[2], Michael Loukeris[9], Brian Kloss[9], Karolina Skorupinska-Tudek[10], Ewa Swiezewska[10], David I. Roper[2,1], Oliver B. Clarke[1,11], Anne-Catrin Uhlemann[6], Anthony A. Kossiakoff[4], M. Stephen Trent[5*], Phillip J. Stansfeld[2,3*] and Filippo Mancia[1*]

[1]Department of Physiology and Cellular Biophysics, Columbia University Irving Medical Center, New York, NY 10032, USA.

[2]School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK.

[3]Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK.

[4]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, 60637, USA.

[5]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602, USA.

[6]Department of Medicine, Division of Infectious Diseases, Columbia University Medical Center, New York, New York, 10032, USA.

[7]Department of Microbiology, Biochemistry, and Molecular Genetics, New Jersey Medical School, Rutgers Biomedical Health Sciences, Newark, NJ, 07103, USA.

[8]Center for Immunity and Inflammation, New Jersey Medical School, Rutgers Biomedical Health Sciences, Newark, NJ, 07103, USA.

[9]New York Consortium on Membrane Protein Structure, New York Structural Biology Center, 89 Convent Avenue, New York, NY, 10027, USA.

[10]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland.

[11]Department of Anesthesiology, Columbia University Irving Medical Center, New York, NY, 10032, USA.

[#]These authors contributed equally.

*Correspondence to be addressed to: strent@uga.edu (M.S.T.),

Phillip.Stansfeld@warwick.ac.uk (P.J.S.), fm123@cumc.columbia.edu (F.M.)

Table of Content:

*Red, dashed, boxes indicate how gels where cropped.

SI Figure 1 | Uncropped LPS assay gels from Main Figures. a, LPS assay gel from Fig. 2c showing analysis of *Cm*WaaL ligase activity in whole cells. LPS gel showing O-antigen extension in *C. metallidurans* expressing *Cm*WaaL variants. With and empty plasmid as a control. **b,** Functional analysis gel from Fig. 4c of *Ec*WaaL ligase variants in whole cells by LPS gel analysis. WaaL proteins were expressed from plasmid pWSK29 in the W3110 ΔwaaL strain. With and empty plasmid as a control.

SI Figure 2 | Uncropped LPS assay gels from Extended Data Figures. a, LPS assay gel from Extended Data Fig. 1c showing functional analysis of CmWaaL ligase activity in whole cells, showing O-antigen ligase activity is abolished when the gene encoding WaaL is deleted and activity is restored by plasmid complementation. **b,** Functional analysis of *Ec*WaaL ligase activity from Extended Data Fig. 7a carried out in whole cells by LPS gel analysis. W3110 ΔwaaL containing either empty vector pWSK29 (Ø), pWSK29::*Ec*WaaL (WT) as controls and pWSK19::*Ec*WaaL- mutant variants to test for O-antigen ligase activity. **c,** Functional analysis gel from Extended Data Fig. 7e showing *Cm*WaaL ligase activity in whole cells. *C. metallidurans ΔwaaL* containing either empty vector pBBR1(Ø), pBBR1:*Cm*WaaL (WT) as controls for evaluating *Cm*WaaL-variants for O-antigen extension.

SI Figure 3 | Uncropped SDS-page gels from Extended Data Figures. a, SDS-PAGE gel from Extended Data Fig. 1e depicting *Cm*WaaL purified in detergent, *Cm*WaaL reconstituted into nanodiscs (MSP1E3D1 and POPG), and CmWaaL reconstituted into nanodiscs (MSP1E3D1 and POPG) with Fab (WaB10) bound. Molecular weight markers can also be observed and are labelled. **b,** SDS-PAGE gels from Extended Data Fig. 7c of all *Ec*WaaL mutants used for LPS ligase analysis, that were purified to verify expression.

SI Figure 4 | Uncropped TLC and Western Blot analysis from Extended Data Figures. a, TLC analysis from <u>Extended Data Fig. 5d</u> of detergent purified CmWaaL along with suitable controls: POPG, Und-P, Und-PP and an unrelated control protein expressed in *E. coli* were run in separate lanes. **b,** Western blot analysis, from <u>Extended Data Fig. 7d</u>, using a mouse monoclonal anti-Flag antibody, of Flag purified WT *Cm*WaaL and mutants, grown in *C. metallidurans,* for testing in the O-antigen ligase assay. Red markers indicate molecular weight markers.

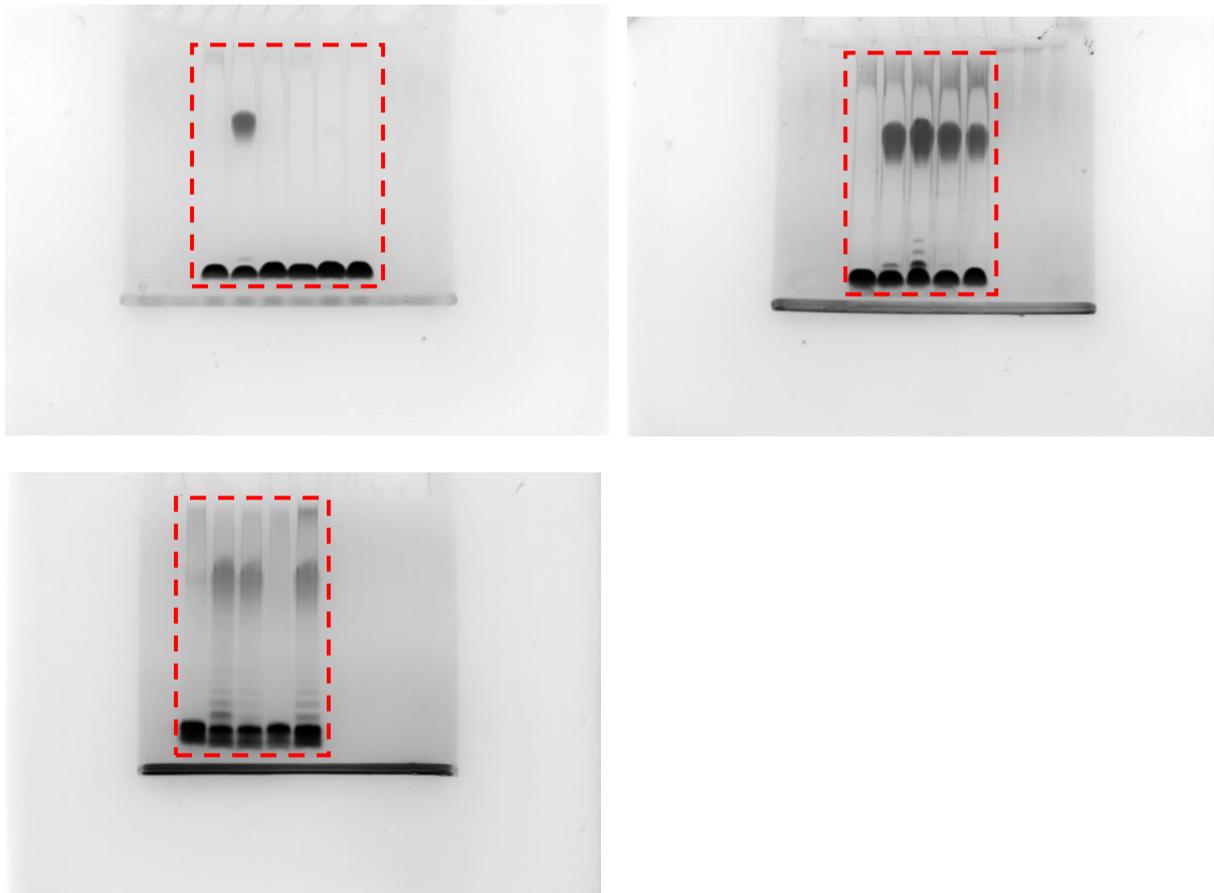**Supplementary Table 1 |** Raw Single-point ELISA and Multi-point sAB ELISA data
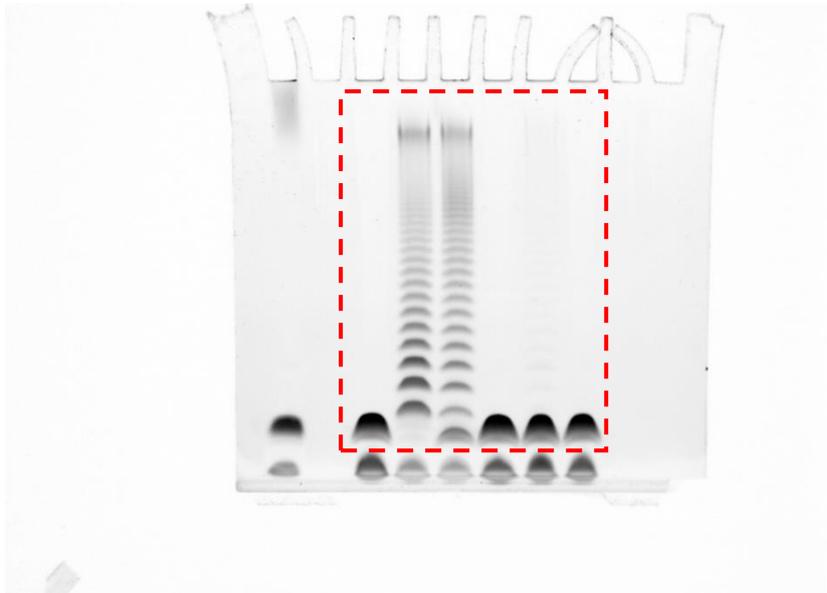
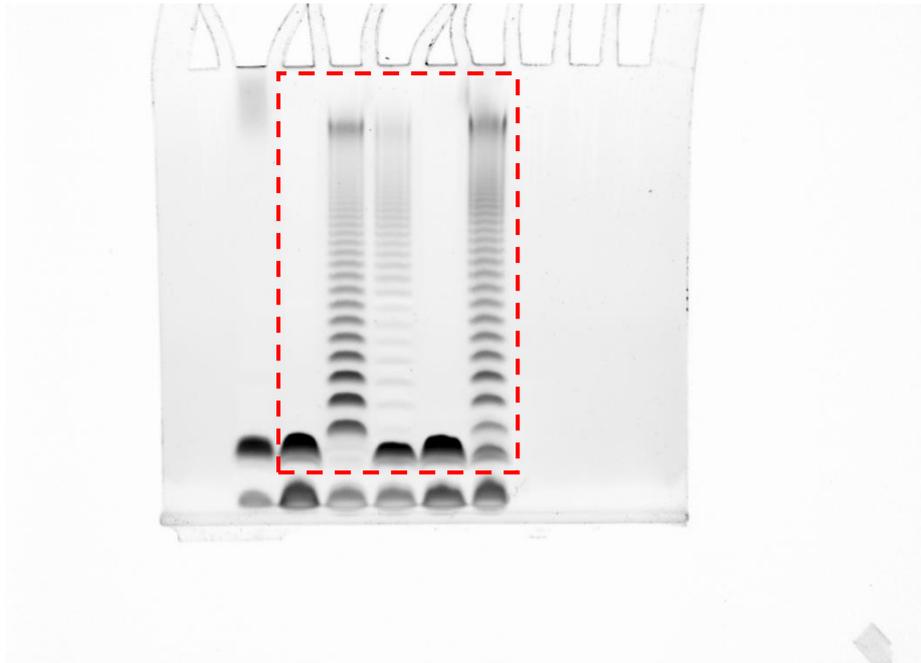**Supplementary Figure 1a | Uncropped gel from Figure 2c.**
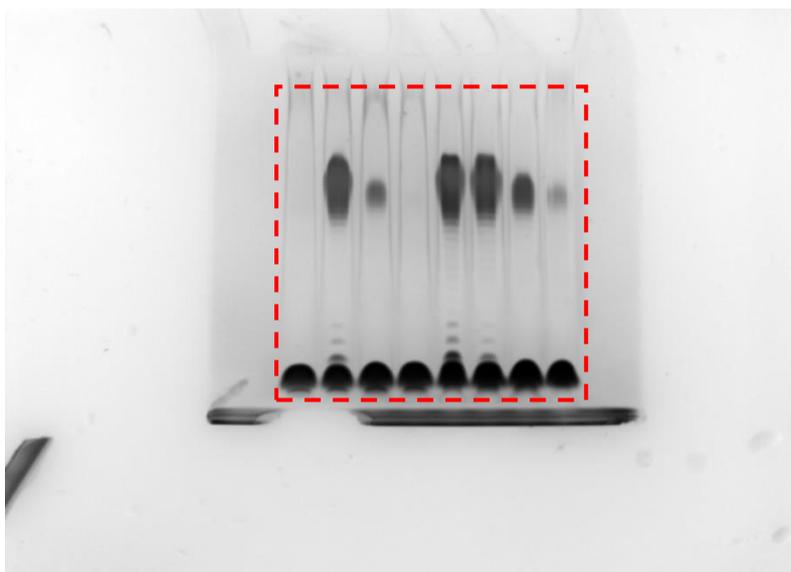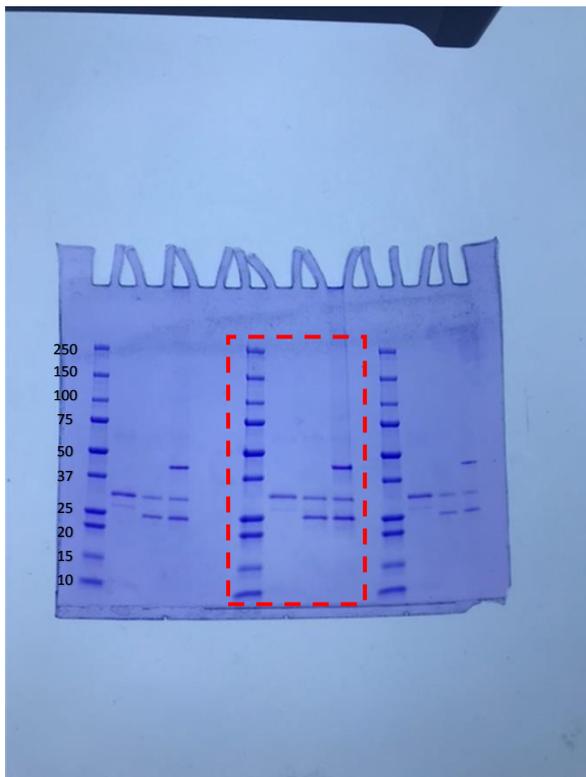

Supplementary Figure 1b | Uncropped gel from Figure 4c.
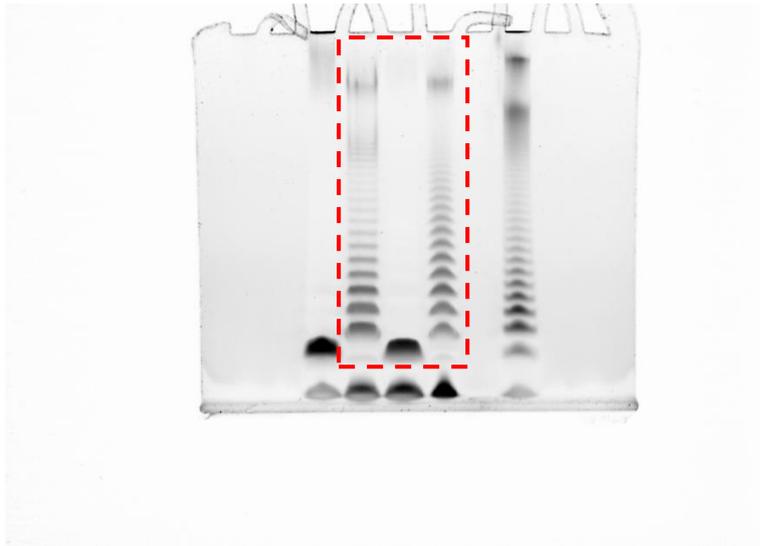
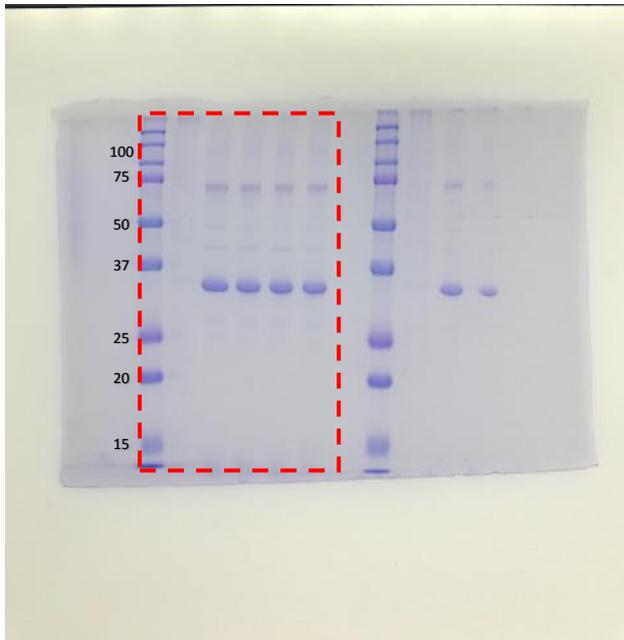**Supplementary Figure 2a | Uncropped gel from Extended Data Fig. 1c**
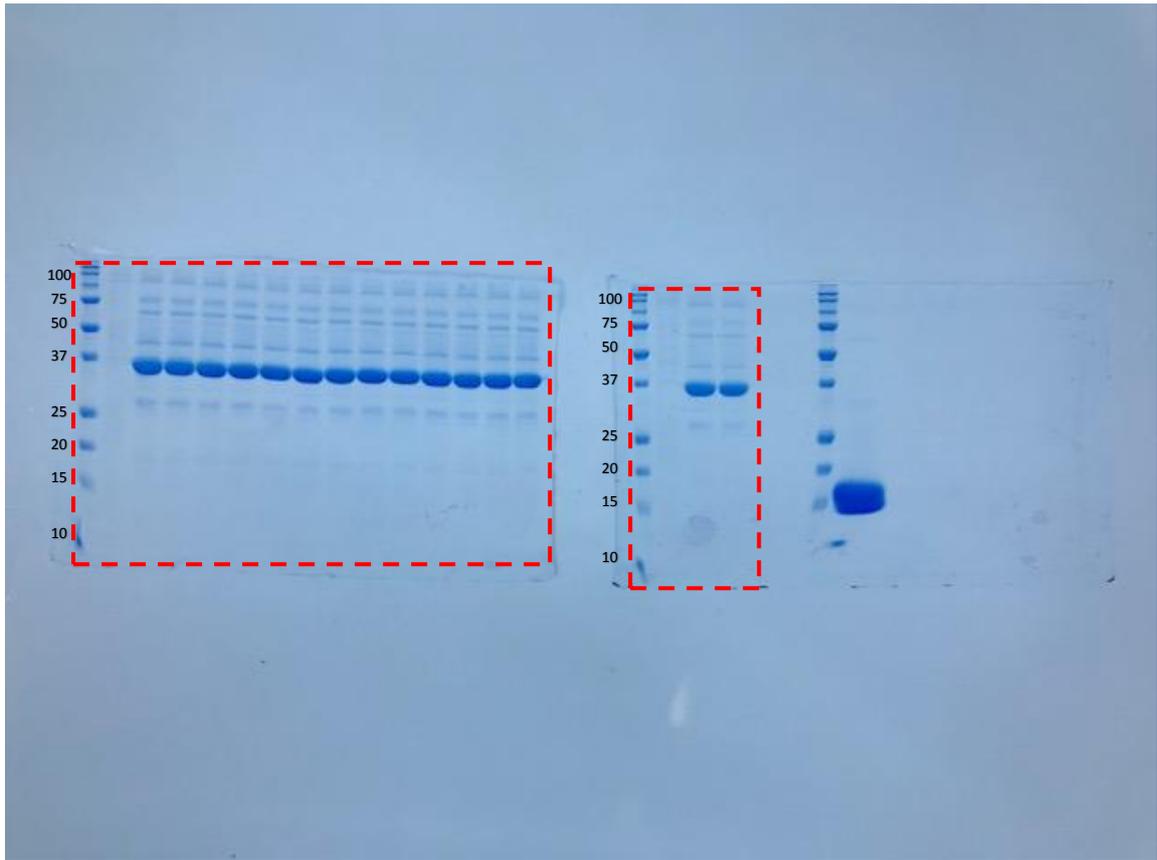

Supplementary Figure 2b | Uncropped gels from Extended Data Fig. 7a

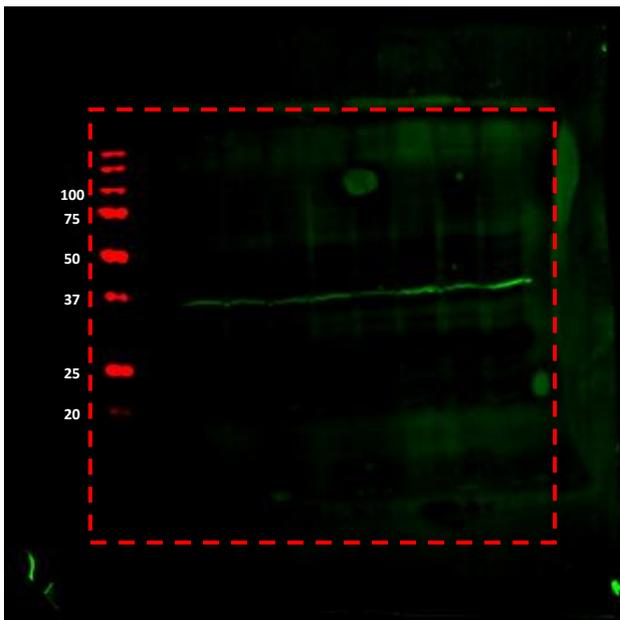**Supplementary Figure 2c | Uncropped gels from Extended Data Fig. 7e**

Supplementary Figure 3a | Uncropped gel from Extended Data Fig. 1e

Supplementary Figure 3b | Uncropped gel from Extended Data Fig. 7c

Supplementary Figure 4a | Uncropped gel from Extended Data Fig. 5d

Supplementary Figure 4b | Uncropped gel from Extended Data Fig. 7d