# SUPPLEMENTAL MATERIAL

**Suppressor mutations in LptF bypass essentiality of LptC by forming a six-protein trans-envelope bridge that efficiently transports lipopolysaccharide**

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Running Head: Control of LPS transport in Gram-negative bacteria

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# SUPPLEMENTAL METHODS

***In vivo* site-specific UV-photocrosslinking and whole-cell lysate analysis for LptF-LptA interaction.** Amber codons were introduced into pGS445 and pGS451 plasmids, harbouring *lptFGAB* and *lptF$^{R212G}$GAB*, respectively, pEVOL-Spn vector (1, 2) was used to incorporate *p*-benzoyl-L-phenylalanine (*p*Bpa) into LptF or LptF$^{R212G}$. Plasmids carrying amber mutations were used to transform AM604 strain. When indicated, cells were also transformed with pBAD/HisA-LptC plasmid to overexpress LptC. Diploid strains were used for all experiments and the protocol was modified from Simpson *et al*. (3). Briefly, overnight cultures were diluted in 10 mL of LB medium containing 0.45 mM *p*BPA (Bachem) and 0.2% arabinose to an optical density at 600 nm ($OD_{600}$) ~ 0.1. Strains were grown to $OD_{600}$ ~ 0.5 – 0.7. A total amount of 1 OD of cells was transferred to a 6-well flat-bottom cell culture plate (Greiner bio-one) and irradiated for 10 min on ice with a UV lamp (365 nm). An additional sample corresponding to 1 OD of cells was used as UV-untreated control. Samples were then TCA precipitated (10% w/v trichloroacetic acid final), resuspended in 100 µl of sample buffer SDS sample buffer for SDS−PAGE and analysed by western blotting using anti-LptA and anti-LptF antibodies.

**ATPase activity assay on DDM-solubilized complexes.** The ATPase activity of 0.2 µM $LptB_2FG$ and $LptB_2F^{R212G}G$ purified complexes, or 0.1 µM $LptB_2FGC$ and $LptB_2F^{R212G}GC$ purified complexes, was measured in 50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 10% glycerol, 0.01% DDM. The reaction was started at room temperature by the addition of 5 mM ATP/$MgCl_2$. Aliquots (15 µL) were taken at 0, 5, 10, 20, 40, and 60 min and mixed with the same volume of 12% SDS to stop the reaction. The amounts of inorganic phosphate were determined according to the method reported in (4). Absorbance was read at 850 nm using the EnSpire Multimode Plate Reader (PerkinElmer). The assay was repeated three times for each condition and the results were analysed with GraphPad Prism

(GraphPad Software, Inc., San Diego, CA, USA). Plotted data represents the mean value of 3 replicates, error bars indicate the standard deviation.

**Surface Plasmon Resonance (SPR)-based binding assay.** $LptB_2FG$ and $LptB_2F^{R212G}G$ purified in DDM were immobilized onto a CM5 chip in a T200 instrument (GE Healthcare) through standard EDC/NHS method in 10 mM MES pH 6.0, 300 µM DDM at 21°C. About 4000 RU of immobilized protein was achieved. LptAm was injected at different concentrations in 20 mM Tris, 150 mM NaCl, 300 µM DDM, pH 8.0 buffer. The sensorchip was regenerated between two injections with 10 s NaOH 10 mM, 300 µM DDM pulses. Sensorgrams accounting from buffer alone and from a control sensorchip were subtracted prior to analysis. Kd was determined with the BiaEval software considering steady state binding prior to dissociation.

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