

## Supplementary Data

### **Trapped lipopolysaccharide and LptD intermediates reveal lipopolysaccharide translocation steps across the *Escherichia coli* outer membrane**

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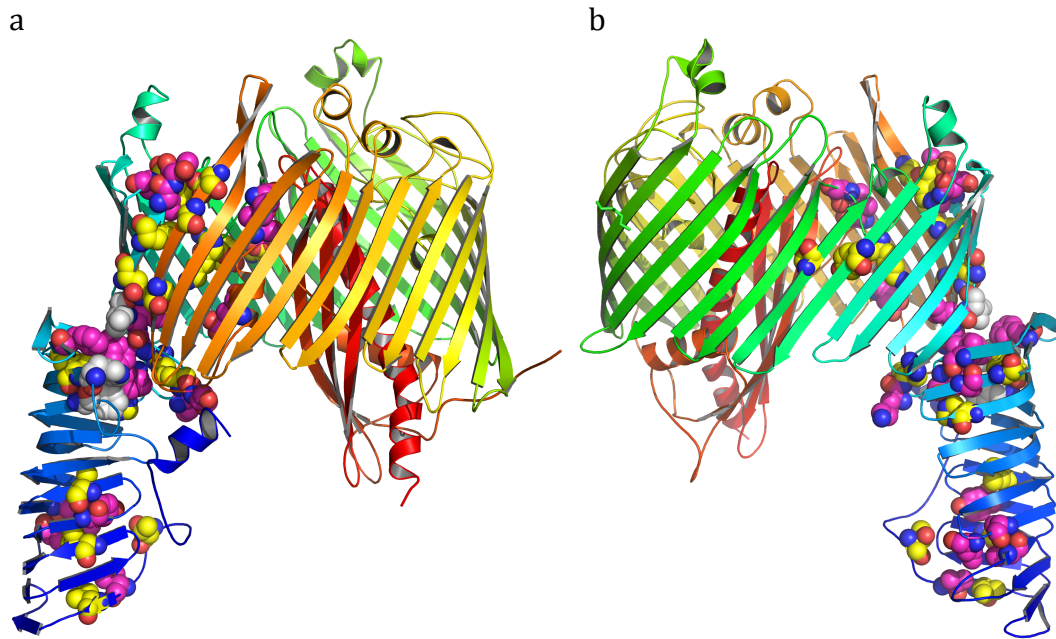
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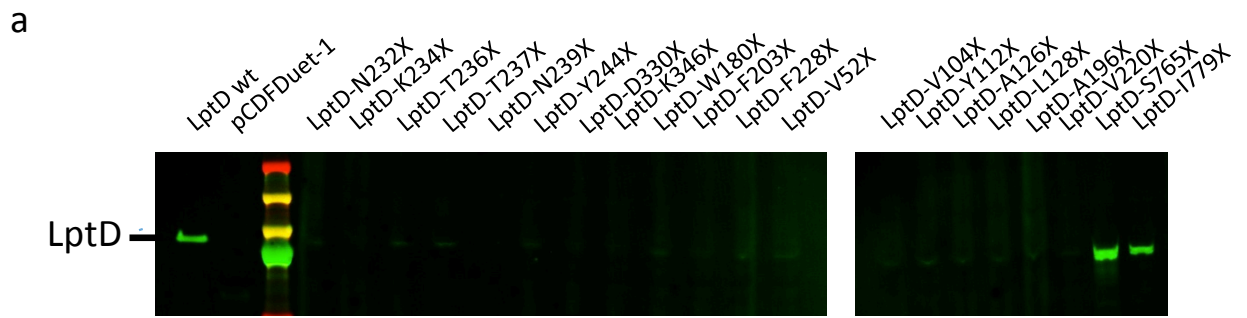
**Table S1: oligonucleotides used in this study**

Names	Sequences
T47Xfor	GGCGATTAGAACGATCTGCCGGTTACTATCAATG
T47Xbac	GATCGTTCTAATCGCCTTTTACCAGAGGACGATCGTAGC
V52Xfor	CTGCCGTAGACTATCAATGCCGATAACGCTAAAG
V52Xbac	TTGATAGTCTACGGCAGATCGTTGGTATCGCCT
I54Xfor	GGTTACTTAGAATGCCGATAACGCTAAAGGTAAGTAC
I54Xbac	CGGCATTCTAAGTAACCGGCAGATCGTTGGTATCG
L82Xfor	AGCCGCTAGCAAGCGGATGAAGTGCAGCTTCATCAGA
L82Xbac	CGCTTGCTAGCGGCTATTCCCCTGCATAATGTCCA
V104Xfor	CGCACCCTAGGATGCGCTGGGTAATGTGCATTATGA
V104Xbac	CGCATCTCTAGGTGCGTACAGGTTCCGGCTGAC
Y112Xfor	ATGTGCATCTAGGATGACAATCAGGTCATCCTTAAAGGG
Y112Xbac	TTGTATCTCTAATGCACATTACCCAGCGCATCGACG
L119Xfor	GGTCATCTAGAAAGGGCCGAAGGGCTGGGCGAA
L119Xbac	GCCCTTTCTAGATGACCTGATTGTCATCATAATGCAC
A126Xfor	GGCTGGTAGAACCTGAACACCAAAGACACGAACG
A126Xbac	TTCAGGTTCTACCAGCCCTTCGGCCCTTTAAGGA
L128Xfor	GCGAACCTAGAACACCAAAGACACGAACGTCTGGGA
L128Xbac	TTGGTGTCTAGTTTCGCCAGCCCTTCGGCCCTTTA
F170Xfor	GGCAGCTAGACCTCCTGTCTGCCTGGCTCCGAT
F170Xbac	GGAGGTCTAGCTGCCGTTTTCCAGAATGGTATAGCG
W180Xfor	CGATACCTAGAGCGTGGTGGGGAGTGAAGTCAT
W180Xbac	CACGCTCTAGGTATCGGAGCCAGGCAGACAGGA
V182Xfor	CTGGAGCTAGGTGGGGAGTGAAGTCATCCATGACCG
V182Xbac	CCCCACCTAGCTCCAGGTATCGGAGCCAGGCAG
H189Xfor	AGTCATCTAGGACCGCGAAGAACAGGTTGCGGAG
H189Xbac	GCGGTCCTAGCTAACTTCACTCCCCACCACGCTC
A196Xfor	ACAGGTTTAGGAGATCTGGAACGCCCGGTTTAAAG
A196Xbac	AGATCTCTAAACCTGTTCTTCGCGGTCATGGAT
I198Xfor	GCGGAGTAGTGAACGCCCGGTTTAAAGTAGGTC
I198Xbac	CGTTCCACTACTCCGCAACCTGTTCTTCGCGGTC
F203Xfor	GCCCGGTAGAAAGTAGGTCCGGTTCCGATCTTTTA
F203Xbac	CCTACTTTCTACCGGGCGTTCAGATCTCCGCAA
L218Xfor	ATTTACAGTAGCCCGTCGGTGACAAGCGTCGCTC
L218Xbac	GACGGGCTACTGTAAATAGGGGCTATAAAAGATCG
V220Xfor	GCTACCCCTAGGGTGACAAGCGTCGCTCAGGTT
V220Xbac	TGTCACCCTAGGGTAGCTGTAAATAGGGGCTATAAA
K223Xfor	CGGTGACTAGCGTCGCTCAGGTTTCTTGATCC
K223Xbac	GCGACGCTAGTCACCGACGGGTAGCTGTAAATA
F228for	CTCAGGTTAGCTGATCCCGAACGCGAAATACACG
F228bac	GGATCAGCTAACCTGAGCGACGCTTGTACCGACG
L229for	CAGGTTTCTAGATCCCGAACGCGAAATACACGACCA
L229bac	CGGGATCTAGAAACCTGAGCGACGCTTGTACCC
I230for	GTTTCCTGTAGCCGAACGCGAAATACACGACCAAGAA
I230bac	GTTTCGGCTACAGGAAACCTGAGCGACGCTTGTCA
N232for	GATCCCGTAGCGGAAATACACGACCAAGAAGTATTTTCG

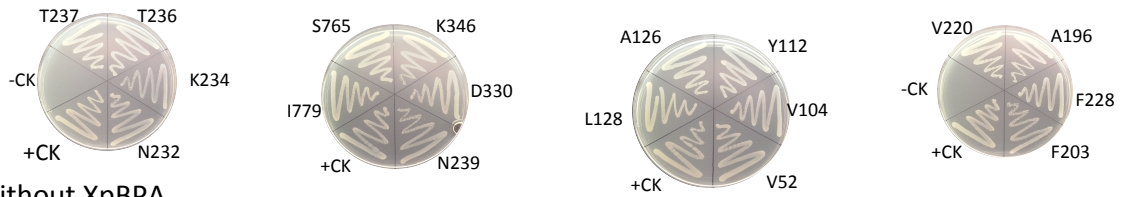
N232bac	TATTTTCGCCTACGGGATCAGGAAACCTGAGCGAC
K234for	AACGCGTAGTACACGACCAAGAACTATTTTCGAGTTCTA
K234bac	TCGTGTACTACGCGTTTCGGGATCAGGAAACCTG
T236for	CGAAATACTAGACCAAGAACTATTTTCGAGTTCTACTTA
T236bac	TCTTGGTCTAGTATTTTCGCGTTTCGGGATCAGGAA
T237for	AATACACGTAGAAGAACTATTTTCGAGTTCTACTTACCG
T237bac	ATAGTTCTTCTACGTGTATTTTCGCGTTTCGGGATCA
K238for	ACGACCAGAACTATTTTCGAGTTCTACTTACCGTATTA
K238bac	GAAATAGTTCTAGGTCGTGTATTTTCGCGTTTCGGGA
N239for	ACCAAGTAGTATTTTCGAGTTCTACTTACCGTATTACTG
N239bac	CTCGAAATACTACTTGGTTCGTGTATTTTCGCGTTC
Y244for	CGAGTTCAGTTTACCGTATTACTGGAACATCGCGCC
Y244bac	ACGGTAACTAGAACTCGAAATAGTTCTTGGTTCGTGTA
Y314Xfor	GGTTATTCCTAGTGGCAGCACTCAGGCGTGATGGA
Y314Xbac	GCTGCCACTAGAATAACCAGCGGTGCTTATCGCCC
D330for	TTAACGTCTAGTACACCAAAGTCAGCGACTCCAGC
D330bac	TTGGTGTACTAGACGTTAAAACGCCACACCTGATCC
K346for	CGACAGTTAGTACGGTTCAGTACCGACGGCTACG
K346bac	GAACCGTACTAAGTGTGAAATCGTTAAAGTAGCTGGAG
T351Xfor	TTCCAGTTAGGACGGCTACGCAACGCAGAAATTCA
T351Xbac	GCCGTCCTAAGTGAACCGTACTTACTGTGCGAAA
D751for	CGATTTATTAGAACGCGATTGGCTTCAACATTGAG
D751bac	TCGCGTTCTAATAAATCGCGTGTGTTTATCGTTATC
A753for	TTATGATAACTAGATTGGCTTCAACATTGAGCTGCG
A753bac	AGCCAATCTAGTTATCATAAATCGCGTGTGTTTATC
N757for	GGCTTCCTAGATTGAGCTGCGCGGTTTGAGCTC
N757bac	AGCTCAATCTAGAAGCCAATCGCGTTATCATAAATCG
S765Xfor	TTTGAGCTAGAACTACGGCCTCGGCACGCAAGAAA
S765Xbac	CCGTAGTTCTAGCTCAAACCGCGCAGCTCAATGT
E773for	CACGCAATAGATGTTGCGTTTGAACATTCTGCC
E773bac	CGCAACATCTATTGCGTGCCGAGGCCGTAGTTA
I779Xfor	CGAACCTAGCTGCCGTACCAAAGCTCTATGTAATC
I779Xbac	TACGGCAGCTAGTTTGAACGCAACATTTCTTGCCTG



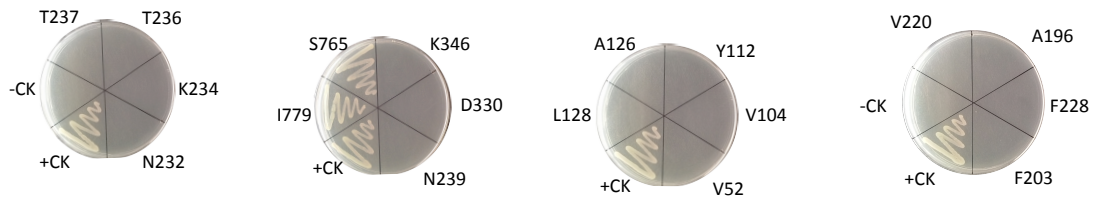
**Figure S1| Residues of *S. typhimurium* LptD are selected for XpBPA incorporation and UV cross-linking.** Residues in magenta can cross-link with LPS. Figure a rotates 180 degree along y-axis to become figure b.



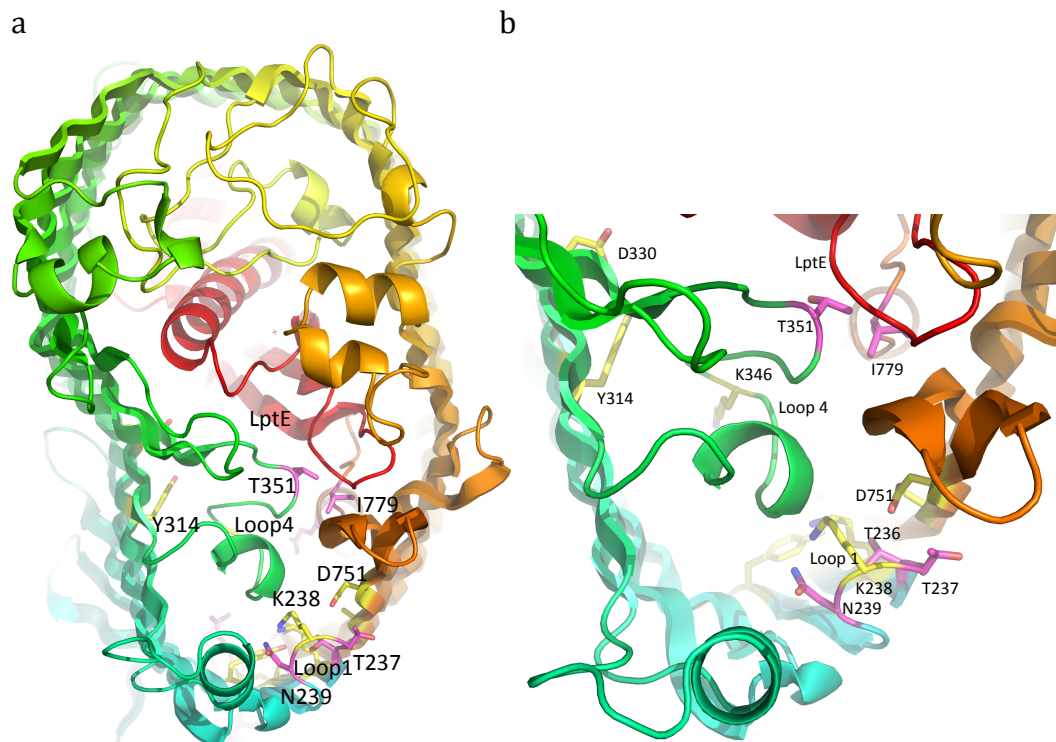
b, with XpBPA



c, without XpBPA



**Figure S2 | Incorporation of pBPA into mutant LptDs could produce a functional LptD** (only some examples are shown here). a, Total cell lysate from BL21(DE3) expressing His-tagged wild type or mutant LptDs were detected with anti-his antibody. Without pBPA, full-length LptDs couldn't be produced (in contrast to Fig2c, 2d, 3c and 3d, where full length LptD variants could be produced in the presence of pBPA). b, LptD depleted AM661 strain expressing mutant LptDs could grow similarly well to that expressing wild type LptD in the presence of pBPA. c, Except for S765 and I779, mutations at other positions resulted in the death of the host AM661 in the absence of pBPA. +CK, positive control (AM661 harbouring plasmid expressing wild type LptD); -CK, negative control (AM661 containing empty plasmid).



**Figure S3| Extracellular view of LptD/E translocon.** Residues in magenta can cross-link with LPS. Extracellular loop 1 and 4 open the pore for the core oligosaccharide and O-antigen exit from the LptD channel. Figure b is part of figure a.