Supplemental material

Diversity of O-antigen repeat-unit structures can account for the substantial sequence variation of Wzx translocases

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Table S1. The oligonucleotides used for clonings in this study.

Primer	DNA sequence 5' to 3' *	Description
6827	TAT **CCA TGG** TAT ATA TAA TAA TC	Forward primer for *wbbL* cloning, consists of 3b dummy sequence, the **NcoI site**, and 18b priming sequence homologous to the start of *wbbL*.
6828	CTG **GGA TCC** TTA TAT TAC GGG TGA AAA	Reverse primer for *wbbL* cloning, consists of 3b dummy sequence, the **BamHI** site, and 18b priming sequence homologous to the end of *wbbL*.
6851	TAT **CCATGG** AA ATG AAT ACG AAT AAA TTA TCT	Forward primer for wzx_{O16} cloning, consists of 3b dummy sequence, the **NcoI** site, and 18b priming sequence homologous to the start of wzx_{O16}.
6966	GTA AGT **CTG CAG** CAA CAA TGA TAT AAT CGT	Reverse primer for wzx_{O16} cloning, consists of 6b dummy sequence, the **PstI** site, and 18b priming sequence homologous to the end of wzx_{O16}.
* Note that the **restriction sites** are in **Bold** and the homology regions are Underlined.		

Table S2. The oligonucleotides used for strain constructions in this study.

Primer	DNA sequence 5' to 3'	Description
6872F	AAG ATC CCC TCA CGC TGC CGC	Primer pair designed to amplify the *kan* gene from pKD4, 6873R has **42b** homologous to sequence just after wzx_{O16} in the O16 gene cluster
6873R	**AAC AAA CCA GAA CCA ACA ATG ATA TAA TCG TAC ATA AAA TCC** TCA GAA GAA CTC GTC AAG AAG	
6900F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* TGA ATA CGA ATA AAT TAT CTT TAA GAA GAA	Primer pair designed to amplify the wzx_{O16} gene from *E.coli* K-12 MG1655; 6900F has *42b* homologous to sequence just before wzx_{O16} in the O16 gene cluster; 6874R has 21b homologous to the 6872F primer. 6900F/6873R are used to generate the wzx_{O16}-*kan* control knock in cassette using products of 6900F/6874R and 6872F/6873R.
6874R	GCG GCA GCG TGA GGG GAT CTT TCA GCA AAC CAG TAA TTT ATT	
6901F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* TGG TAT TAA CAG TGA AAA AAA TTT TAG CGT TT	Primer pair designed to amplify the wzx_{O111} gene from *E.coli* O111; 6901F has *42b* homologous to sequence just before wzx_{O16} in the O16 gene cluster; 6877R has 21b homologous to the 6872F primer. 6901F/6873R are used to generate the wzx_{O111}-*kan* knock in cassette using products of 6901F/6874R and 6872F/6873R.
6877R	GCG GCA GCG TGA GGG GAT CTT TCA ATA GAC ATT TTT CGC TCT	
6787F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* TGA ATA ACA AAC TAG CCA GGC A	Primer pair designed to amplify the wzx_{O7} gene from *E.coli* O7; 6787F has *42b* homologous to sequence just before wzx_{O16} in the O16 gene cluster; 6875R has 21b homologous to the 6872F primer. 6787F/6873R are used to generate the wzx_{O7}-*kan* knock in cassette using products of 6787F/6875R and 6872F/6873R.
6875R	GCG GCA GCG TGA GGG GAT CTT TCA GTC CTT ATT TTT TAT GAG	
6840F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* TGA GCA TAA TAA AAA ATA GTG T	Primer pair designed to amplify the wzx_{SF2a} gene from *Shi. flexneri* 2a; 6840F has *42b* homologous to sequence just before wzx_{O16} in the O16 gene cluster; 6878R has 21b homologous to the 6872F primer. 6840F/6873R are used to generate the wzx_{Sf2a}-*kan* knock in cassette using products of 6840F/6878R and 6872F/6873R.
6878R	GCG GCA GCG TGA GGG GAT CTT TTA GTT TTC ATA TAC AGA ACA	
6781F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* TGA TAA TAA ATA AAA TCA AAA A	Primer pair designed to amplify the wzx_{O157} gene from *E.coli* O157; 6781F has *42b* homologous to sequence just before wzx_{O16} in the O16 gene cluster; 6876R has 21b homologous to the 6872F primer. 6781F/6873R are used to generate the wzx_{O157}-*kan* knock in cassette using products of 6781F/6876R and 6872F/6873R.
6876R	GCG GCA GCG TGA GGG GAT CTT TCA TCC TCT TAT ATT TAA CTG	
6841F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* TGA AAG TTC AAT TGT TAA AAA T	Primer pair designed to amplify the wzx_{SeLT2} gene from *S. enterica* LT2; 6841F has *42b* homologous to sequence just before wzx_{O16} in the O16 gene cluster; 6879R has 21b homologous to the 6872F primer. 6841F/6873R are used to generate the wzx_{SeLT2}-*kan* knock in cassette using products of 6841F/6879R and 6872F/6873R.
6879R	GCG GCA GCG TGA GGG GAT CTT TTA TCC CTT ATT TGC CTT AAT	
6797F	*TTT ACG TTA GAT GAG CTT ATC AGA TTA AAA TTA ATT GCA TGA* GTG TAG GCT GGA GCT GCT TC	Primer pair designed to generate the recombination cassette for the replacement of the wzx_{O16} gene from *E.coli* K-12 MG1655 O-antigen gene cluster; 6797F has *42b* homologous to sequence just before wzx_{O16} in the
6873R	**AAC AAA CCA GAA CCA ACA ATG ATA TAA TCG TAC ATA AAA TCC** TCA GAA GAA CTC	

	GTC AAG AAG	O16 gene cluster; 6873R has **42b** homologous to sequence just after wzx_{O16} in the O16 gene cluster
6937F	CCT TTA ATG GGA ATT AGC CAT GGT CC	Primer pair designed to amplify the *cat* gene from pKD3; 6937F has 6b identical (in reverse direction) to the first 6b of 6935F; 6873R has **42b** homologous to sequence just after wzx_{O111} in the *E. coli* O111 gene cluster in pPR2105.
6938R	**AAT AGA CAT TTT TCG CTC TAT TAA AAT AGA CAA AAG CTA CAT** GTG TAG GCT GGA GCT GCT TCG AAG	
6935F	TGG TTT GGT TTT TCC TTC GTT ATA AAG GAG	Primer pair designed to amplify ~ 250bp sequence just upstream of the wzx_{O111} gene in *E. coli* O111 (pPR2105); 6936R has **42b** homologous to sequence just after wzx_{O16} in the O16 gene cluster. 6935F/6938R are used to generate a wzx_{O111} replacement cassette using products of 6935F/6936R and 6937F/6938R.
6936R	**ATA TTC ATA TGG ACC ATG GCT AAT TCC CAT TAA AGG CAC TCT** ATT CGA AAT AGA GTG C	

Table S3. Strains and plasmids used in this supplemental material.

Strains & plasmids	**Parent strain**	**Strain genotype or plasmid description**[A]	**Source/reference**
Strains			
P5930	P5928	*S. enterica* group B serovar Typhimurium strain LT2,*hsdL trp32 nml flaA66, rpsLxylT404 llvE452 metE551 metA22 hsdA* Δ*galE* Δ*wbaV*	(1)
P9546	P5930	P5930, pPR2190	This study
P5932	MG1655	MG1655, wzx_{O16} gene replaced by a *kan* gene	This study
P5946	P5937	MG1655, wzx_{O16} gene replaced by wzx_{O16}-*kan*, pPR2191	This study
P5944	P5933	MG1655, wzx_{O16} gene replaced by wzx_{O7}-*kan*, pPR2191	This study
P5947	P5938	MG1655, wzx_{O16} gene replaced by wzx_{O111}-*kan*, pPR2191	This study
P5943	P5934	MG1655, wzx_{O16} gene replaced by wzx_{Sf2a}-*kan*, pPR2191	This study
P5953	P5942	MG1655, wzx_{O16} gene replaced by a *kan* gene, pPR2191, pPR2193	This study
P5954	P5943	MG1655, wzx_{O16} gene replaced by wzx_{Sf2a}-*kan*, pPR2191, pPR2193	This study
P5955	P5944	MG1655, wzx_{O16} gene replaced by wzx_{O7}-*kan*, pPR2191, pPR2193	This study
P5956	P5936	MG1655, wzx_{O16} gene replaced by wzx_{SeLT2}-*kan*, pPR2191, pPR2193	This study
P5957	P5947	MG1655, wzx_{O16} gene replaced by wzx_{O111}-*kan*, pPR2191, pPR2193	This study
P5958	P5952	MG1655, wzx_{O16} gene replaced by wzx_{O157}-*kan*, pPR2191, pPR2193	This study
P5948	P5938	MG1655, wzx_{O16} gene replaced by wzx_{O111}-*kan*, pPR2192	This study
P5949	P5937	MG1655, wzx_{O16} gene replaced by wzx_{O16}-*kan*, pPR2192	This study
P5950	P5933	MG1655, wzx_{O16} gene replaced by wzx_{O7}-*kan*, pPR2192	This study
P5951	P5934	MG1655, wzx_{O16} gene replaced by wzx_{Sf2a}-*kan*, pPR2192	This study
M2893	M388	*S. enterica* group D2 serovar Strasbourg, *rpsL*, Δ*galE*	(1)
M2897	M2893	M2893, wzx_{D} gene replaced by *rpsL-kan*	(1)
M2944	M2897	M2897, pPR2190	This study
M2896	M2893	M2893, *wbaV* gene replaced by *rpsL-kan*	(1)
M2950	M2896	M2896, pPR2190	This study
M2820	M126	*S. enterica* group D1 serovar Dublin, Δ*galE* Δ*wbaV*	This study
M2951	M2820	M2820, pPR2190	This study
Plasmids			
pPR2190		pTrc99A (NcoI & BamHI) carrying the wzx_{D} gene from *S. enterica* group D2 serovar Strasbourg, IPTG inducible, ampicilin resistant	This study
pPR2191		pWQ572 (NcoI & BamHI) carrying the *wbbL* gene from *E. coli* K-12 strain WG1, which is controlled by tetracycline inducible P_{Tet} promoter chloramphenicol resistant	This study
pPR2192		pPR691 carrying the *E. coli* O111 gene cluster with the wzx_{O111} gene replaced by a *cat* gene. Kanamycin and chloramphenicol resistance	This study
pPR2193		wzx_{O16} from *E. coli* K-12 cloned into pWQ552 (NcoI & PstI), controlled by tetracycline inducible P_{Tet} promoter, ampicillin resistance	This study
A. Genetic difference from the parent is underlined, including gene replacements and plasmid additions.			

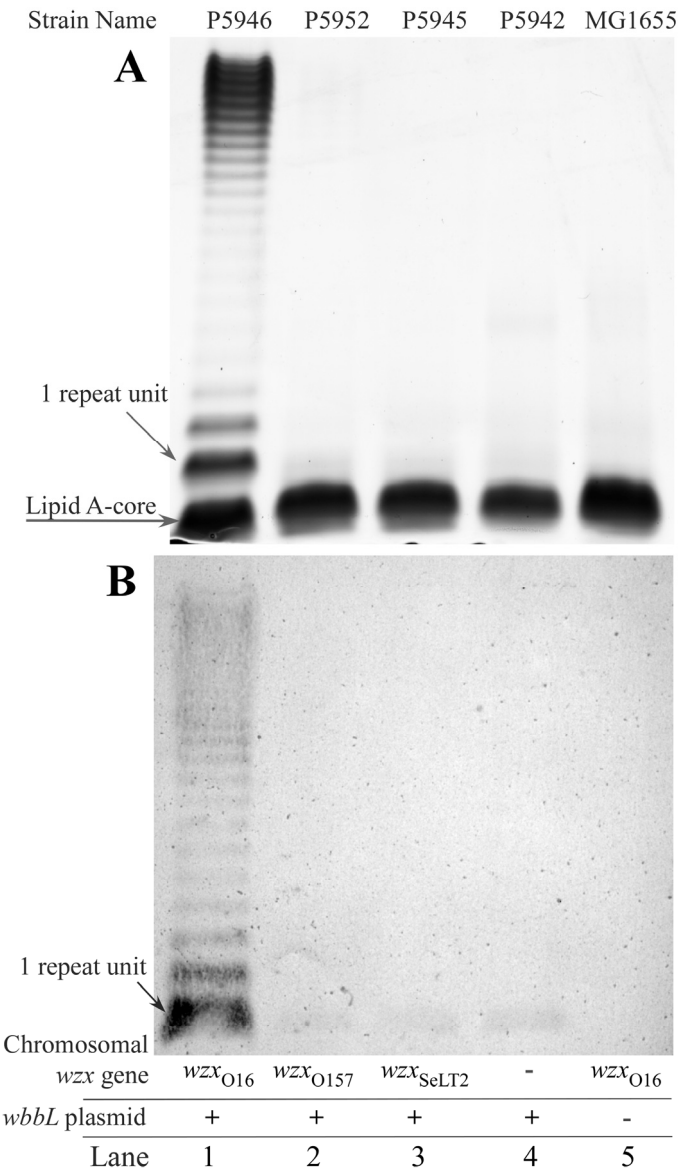

Fig. S1. The *E. coli* strain O16 variants with either a *wzx* gene for a non GlcNAc-initiated O antigen (wzx_{O157} and wzx_{SeLT2}) or with no O-antigen *wzx* gene, produce small amounts of LPS with a single O16 repeat unit. **A**. Silver stained SDS-PAGE. **B**. The same set of LPS, with three times the loading for **A**, were run on a SDS-PAGE, and transferred to nitrocellulose membrane for immunoblotting with monospecific anti-*E. coli* O16 antibodies as described in (1). The immunoblot shows that the small amounts of material detected above the lipid A-core in strains with either a *wzx* gene for a non GlcNAc-initiated O antigen (lanes 2 and 3) or with no O-antigen *wzx* gene (lane 4), are LPS carrying a single O16 repeat unit, whereas the negative control LPS from MG1655 lacking the *wbbL* clone and so unable to produce O16 repeat units, gave no reaction to the anti-O16 antiserum (lane 5). It should be noted that the only O16 antiserum that is commercially available to us had been diluted for slide agglutination and gave a much weaker immunoblotting signal than expected, despite that almost all of the material was used in the single experiment.

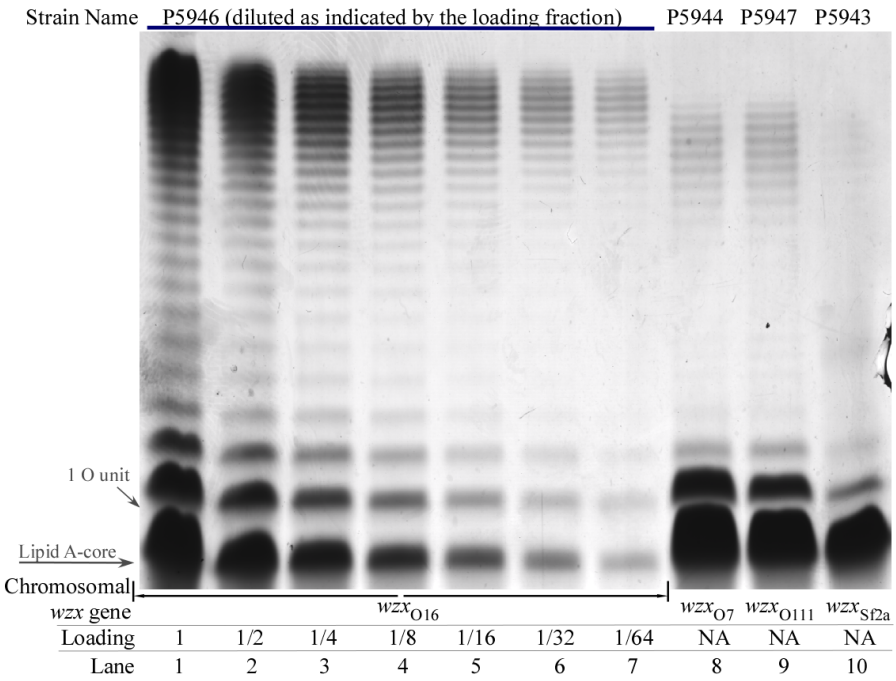

Fig. S2. Comparison of Wzx_{O16} activity with those of Wzx_{O7}, Wzx_{O111}, and Wzx_{Sf2a}, in translocation of the *E. coli* O16 repeat unit. The strains all have the pPR2191 (*wbbL*) plasmid for initiation of O16 antigen production. The extracted LPS samples were separated in 13% Tricine-SDS-PAGE gel and silver stained. For genetic details of strains used in this figure, see Table S3.

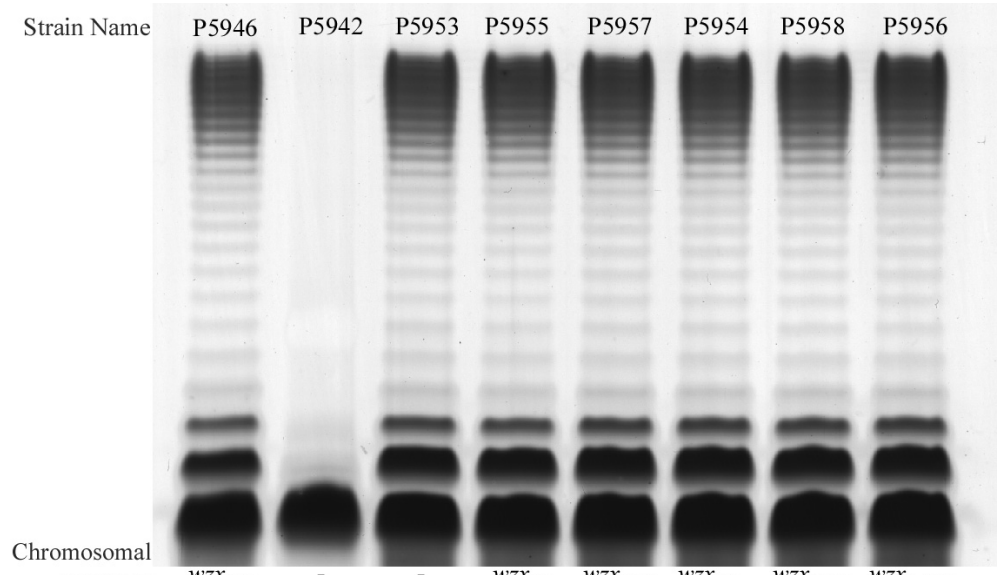

Fig. S3. The LPS profile of *E. coli* strain K-12 variants with different *wzx* genes after addition of the wzx_{O16} clone (pPR2193) to test for complementation. The extracted LPS samples were separated in 13% tricine SDS-PAGE and silver stained. For the genetic detail of strains used in this figure, see Table S3.

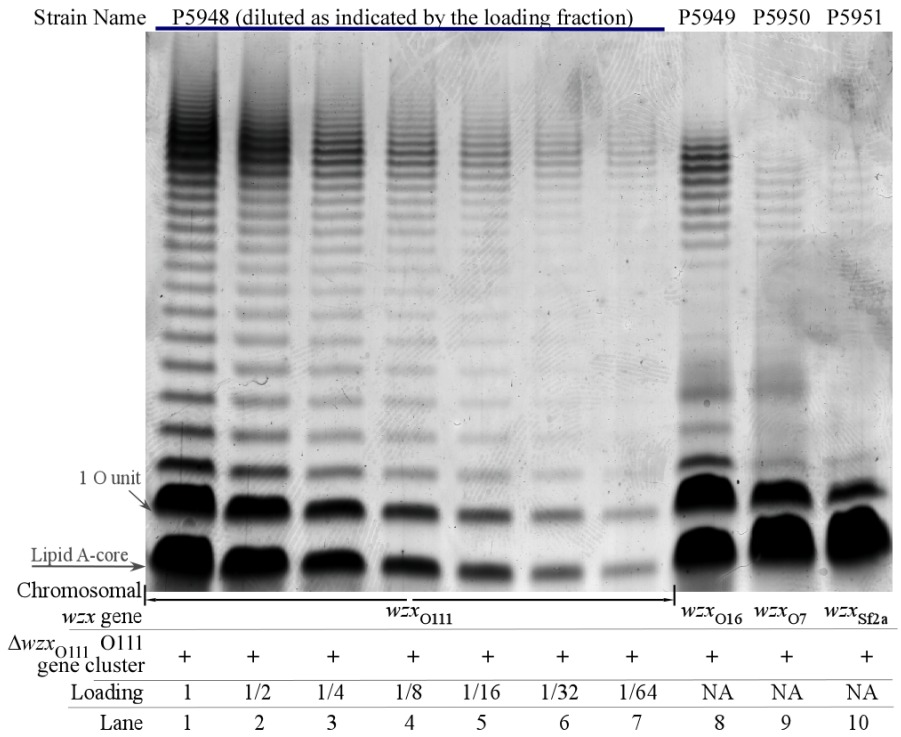

Fig. S4. A comparison of Wzx_{O111} activity with those of Wzx_{O16}, Wzx_{O7}, and Wzx_{Sf2a}, for translocation of the *E. coli* O111 repeat unit. The plasmid, pPR2192 carrying the O111 gene cluster lacking the wzx_{O111} gene, is present in the strains examined for synthesis of O111 repeat units. The extracted LPS samples were separated in 13% Tricine-SDS-PAGE gel and stained by silver nitrate. For the genetic details of strains used in this figure, see Table S3.

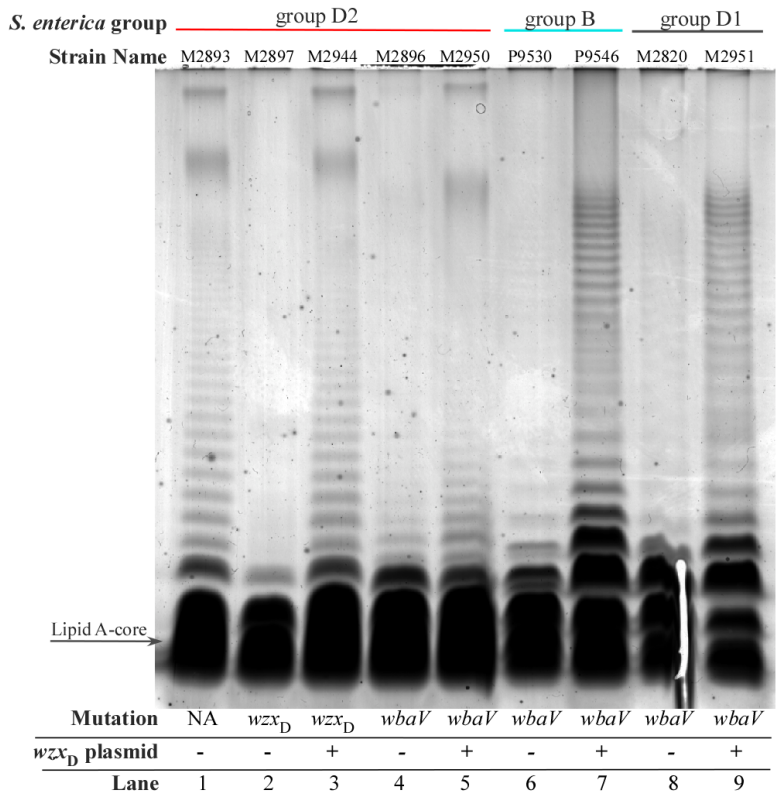

Fig. S5. Effect of over-expression of the *wzx*$_D$ gene in Δ*wbaV* mutants of *Salmonella enterica* groups D2, B1 and D1, that produce repeat units lacking the side-branch tyvelose or abequose. B1, D2 and D1 Δ*wbaV* strains lack the side-branch dideoxyhexose that is normally present (1) and make very liitle long-chain O antigen as shown previously for groups B1 and D2 (1) (lanes 4, 6 and 8 respectively). Addition of a cloned *wzx*$_D$ gene (lanes 5, 7 and 9 respectively) restored wild-type O-antigen levels (lane 1). Note that the Wzx translocases from groups B1 and D2 are known not distinguish between abequose and tyvlose under these experimental conditions (1).

The strains were grown according to Hong *et al.* (1), with IPTG (1mM final concentration) added at the same time as galactose addition. The extracted LPS samples were separated in 13% Tricine-SDS-PAGE gel and silver stained. For genetic details of strains used in this figure, see Table S3.

Reference

1. **Hong Y, Cunneen MM, Reeves PR.** 2012. The Wzx translocases for *Salmonella enterica* O-antigen processing have unexpected serotype specificity. Mol. Microbiol. **84:**620-630.