Supplemental Material

Table S1: **Oligonucleotides used in cloning.** Restriction sites used are underlined. Stop codons are shown in bold. The oligonucleotides are written 5' to 3'.

Primer	*Sequence*	*Description*
pECG28F	GCGCGCCATATGATCGTGGCGTTTTGTTTATAT	Forward primer for pECG28; NdeI site
pECG28R	GCGCGCGGATCCTCAACCATCTAAACCACCTGT	Reverse primer for pECG28; BamHI site
pECB21F	GGGACCTCCATATGGTGAGTTTTACTC	Forward primer for pECB21; NdeI site
pECB21R	CTATTTCCCGGAGGAAACTCGAGAAACAAAG	Reverse primer for pECB21; XhoI site
pECI21F	GGGACCTCGCTAGCCAGCAGGTGTTTTTCCAG	Forward primer for pECI21; NheI site
pECI21R	CTTTGTTTCTCGAGATGCTTTATCTTTTCAATAAA	Reverse primer for pECI21; XhoI site
pSTI28F	GGGACCTCGCTAGCATGAGCAGAAAATATTTTGAAG	Forward primer for pSTI28; NheI site
pSTI28R	CTTTGTTTCTCGAGTTATTCAAGAAGTTTACGTTTAAAG	Reverse primer for pSTI28; XhoI site
pSTJ28F	GGGACCTCCATATGGATTCATTTCCTGAGATAGAAATAG	Forward primer for pSTJ28; NdeI site
pSTJ28R	CTTTGTTTCTCGAGTTATTTGTGGAAAAGTTTACGATAA AG	Reverse primer for pSTJ28; XhoI site
pSTK28F	GGGACCTCCATATGATTAAAAAAATCATATTTACTGTTA CTC	Forward primer for pSTK28; NdeI site
pSTK28R	CTTTGTTTCTCGAGTCACTTATCAAACCAGCTTTTCATT TGTTC	Reverse primer for pSTK28; XhoI site

Table S2: **Abbreviations used for lipid A-oligosaccharides**

	Abbreviation	**Full Name**	**Label on Images**
	Hep_2-1-deP-KLA	$Heptose_2$-1-dephospho-Kdo_2-Lipid A	A
1 core sugar added	Glc-Hep_2-1-deP-KLA	Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	B
2 cores sugars added	Gal-Glc-Hep_2-1-deP-KLA	Galactose α(1→6)-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	C
	Glc_2-Hep_2-1-deP-KLA	Glucose α(1→3)-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	C_O
	Gal*-Glc- Hep_2-1-deP-KLA	Galactose α(1→3)-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	C_I
3 core sugars added	Glc-[Gal]-Glc-Hep_2-1-deP-KLA	Glucose α(1→3)-[Galactose α(1→6)]-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	D
	Gal-[Gal]-Glc-Hep_2-1-deP-KLA	Galactose α(1→3)-[Galactose α(1→6)]-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	D_s
4 core sugars added	Glc-Gal-[Gal]-Glc-Hep_2-1-deP-KLA	Glucose α(1→2)-Galactose α(1→3)-[Galactose α(1→6)]-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	E_s
5 core sugars added	GlcNAc-Glc-Gal-[Gal]-Glc-Hep_2-1-deP-KLA	*N*-Acetylglucosamine α(1→2)-Glucose α(1→2)-Galactose α(1→3)-[Galactose α(1→6)]-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	F_s
Multiple glucoses added	Glc_n-Hep_2-1-deP-KLA	(Glucose α(1→3))$_n$-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	C_O*
	Glc_n-[Gal]-Glc-Hep_2-1-deP-KLA	(Glucose α(1→3))$_n$-[Galactose α(1→6)]-Glucose α(1→3)-$Heptose_2$-1-dephospho-Kdo_2-Lipid A	D*

Figure S1: **UDP-Glucose was the only sugar that WaaG transferred to the Hep_2-1-deP-KLA.** Panel A: WaaG (0.01 mg/ml) was reacted for 1 min with 0.01 mM Hep_2-1-deP-KLA, 50 mM Hepes, pH 7.5, 0.1 % Triton X-100 and <1 μM ^{14}C-UDP-Glucose or ^{14}C-GDP-mannose and then displayed on a TLC plate as described in Materials and Methods. Panel B: WaaG (0.01 mg/ml) was reacted for 1 min with 10 μM [^{32}P] Hep_2-1-deP-KLA in the presence of various sugar nucleotides (1 mM), 50 mM Hepes, pH 7.5, 0.01 % Triton-X-100.

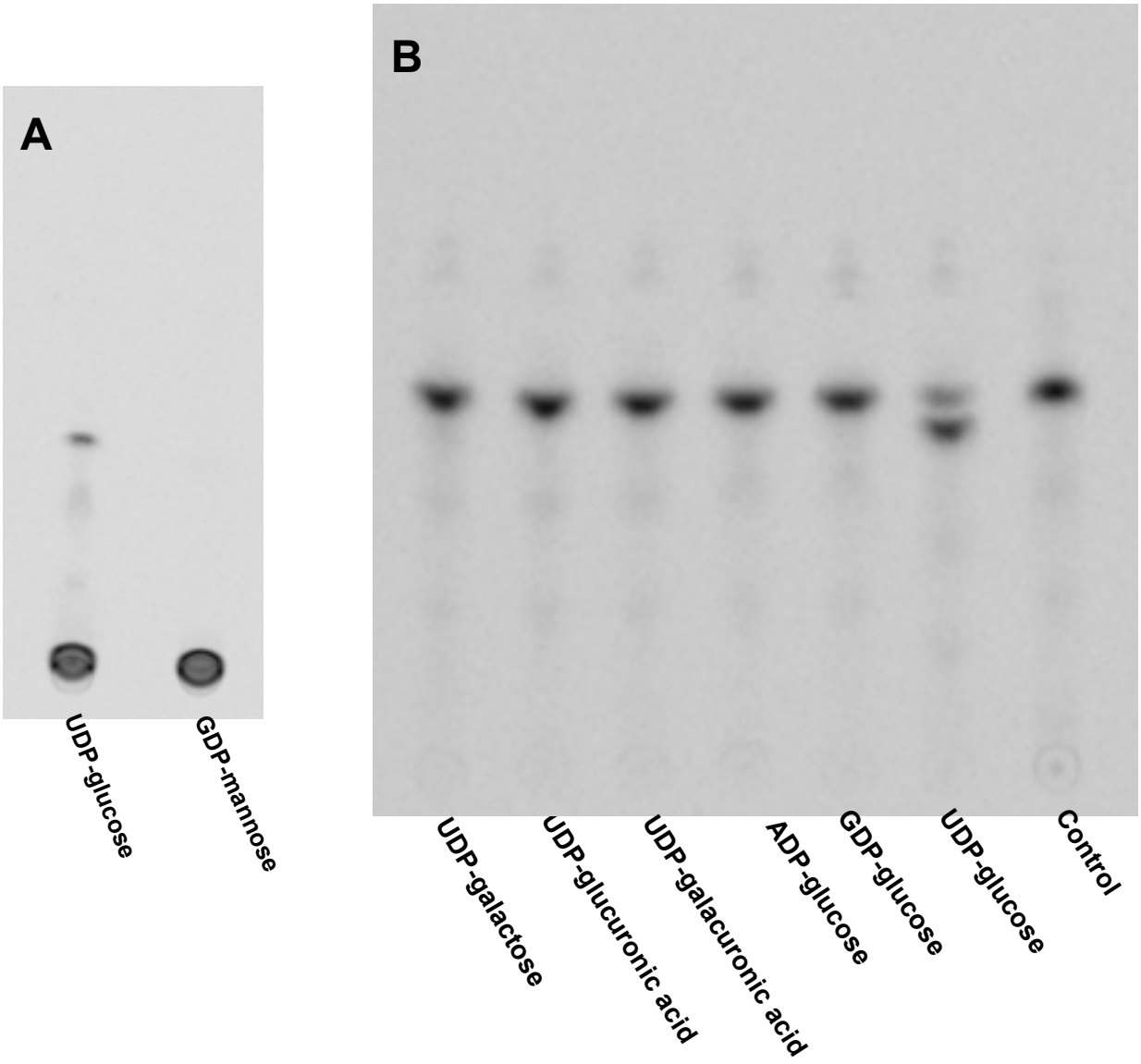

Figure S2: **Triton Dependence of WaaG reaction.** The Triton X-100 concentration was varied from 0 to 2 % in the in vitro assay described in the Materials and Methods.

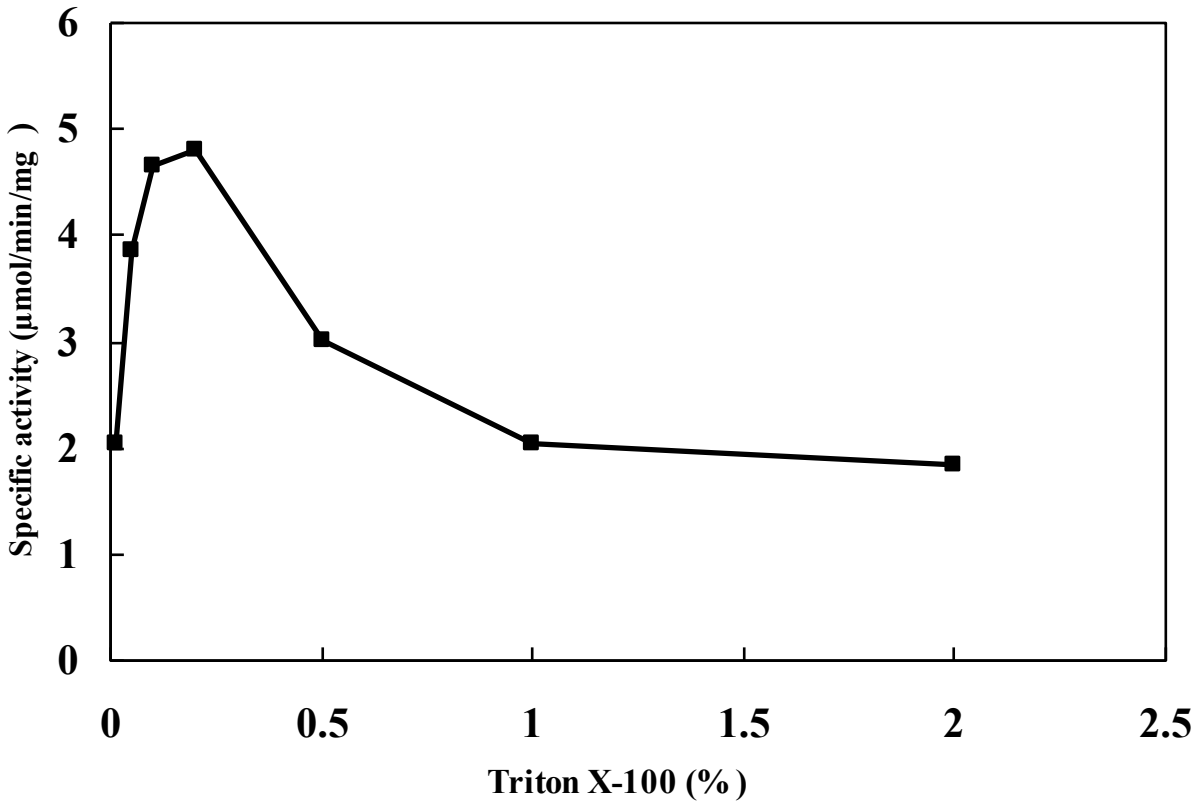

Figure S3: **$MgCl_2$ dependence of the WaaG reaction.** $MgCl_2$ concentration was varied from 0 to 5 mM in the WaaG *in vitro* reaction described in the Materials and Methods.

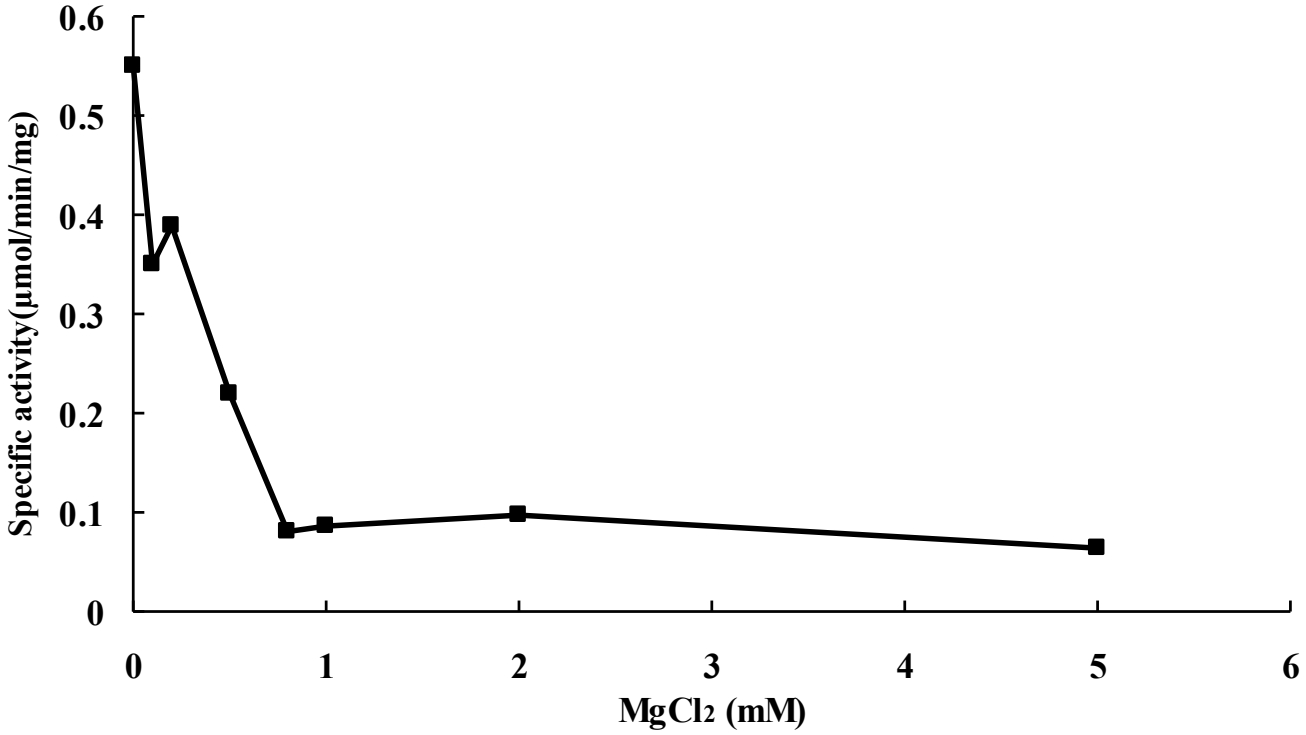

Figure S4: **WaaG is inhibited by $MgCl_2$ and other divalent cations.** WaaG (0.01 mg/ml) was reacted for 30 min with 0.5 mM UDP-Glc, 0.01 mM [^{32}P]-Hep_2-1-deP-KLA, 50 mM Hepes, pH 7.5, 0.1 % Triton X-100, and 1 mM of the indicated cation and then spotted to a TLC plate as described in Materials and Methods.

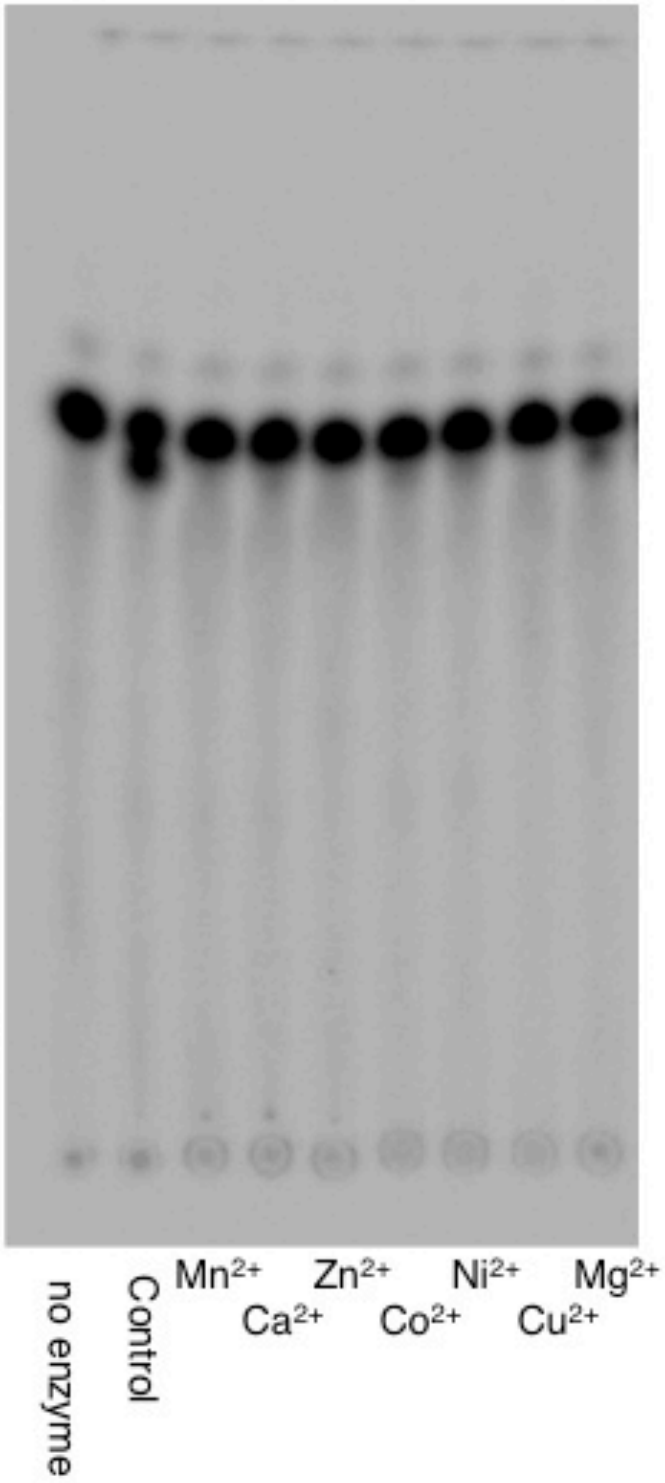

Figure S5: **WaaB hydrolyzes UDP-Gal but WaaG and WaaI hydrolyze UDP-Glc.** WaaG (0.25 mg/ml), WaaB (0.2 mg/ml), or WaaI (0.2 mg/ml) were reacted for 30 min with [^{14}C]-UDP-galactose or [^{14}C]-UDP-glucose, 0.1 % Triton X-100, 50 mM Hepes, pH 7.5 and displayed on TLC as described in Materials and Methods.

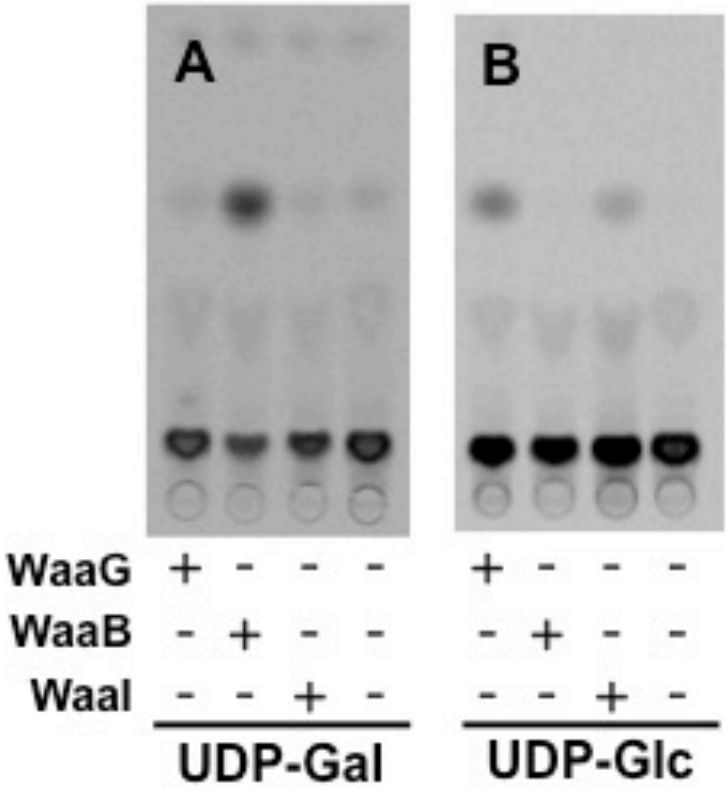

Figure S6: ***S. typhimurium* WaaI uses UDP-galactose as its sugar donor.** Panel A, left side: WaaG (0.01 mg/ml) was incubated with 10 μM [^{32}P]-Hep$_2$-1-deP-KLA (10 μM), 0.1 % Triton X-100, 50 mM Hepes, pH 7.5, 1 mM UDP-Glc, and 1 mM UDP-Gal as indicated and resolved on TLC as described in Materials and Methods. Panel A, right side: WaaG (0.01 mg/ml) was incubated with 10 μM [^{32}P]-Hep$_2$-1-deP-KLA (10 μM), 0.1 % Triton X-100, 50 mM Hepes, pH 7.5, and 1 mM UDP-Glc for 15 minutes and then 1 mM $MgCl_2$, 1 mM UDP-Gal and WaaI (0.18 mg/ml) were added, incubated as indicated and resolved on TLC as described in Materials and Methods. Panel B: The exact same reactions as Panel A except no UDP-Gal was added. "A" indicates the location of Hep$_2$-1-deP-KLA, "B" indicates the location of Glc-Hep$_2$-1-deP-KLA, and "D_s" indicates the location of Gal*-Glc-Hep$_2$-1-deP-KLA (* indicates this Gal is added in a different linkage than the Gal added by WaaB).