Supplemental Material: Supplemental Figures, Supplemental Tables, and Construction of Strains and Plasmids Introduced in this Study

Supplemental Figure S1. Live cell anti-SLPS antibody staining. Live cells were incubated with anti-SLPS antibody and visualized with a fluorescent secondary antibody. In wild-type cells, the S-layer blocks LPS epitopes; hence, an S-layer-negative (*rsaA*$^{-}$) strain was used as a positive control for staining.

Supplemental Figure S2. LPS-silver stained gel of polysaccharide preparations. Polysaccharides from wild-type CB15N (lane 1), LPS-negative *wbqP*$^{-}$ (CJW1249, lane 2), and *wbqL*$^{-}$ (CJW1090, lane 3) were run on a 12% polyacrylamide gel and silver stained for LPS according to the modified (7) protocol of Tsai and Frasch (6).

Supplemental Figure S3. A faster-migrating O-antigen species appears in inner membrane peak fractions. Top panel, anti-Omp85 (outer membrane marker) and anti-FliF (inner membrane marker) immunoblot of wild-type CB15N membrane fractions collected from a sucrose density gradient. Bottom panel, anti-O-antigen immunoblot of the same fractions.

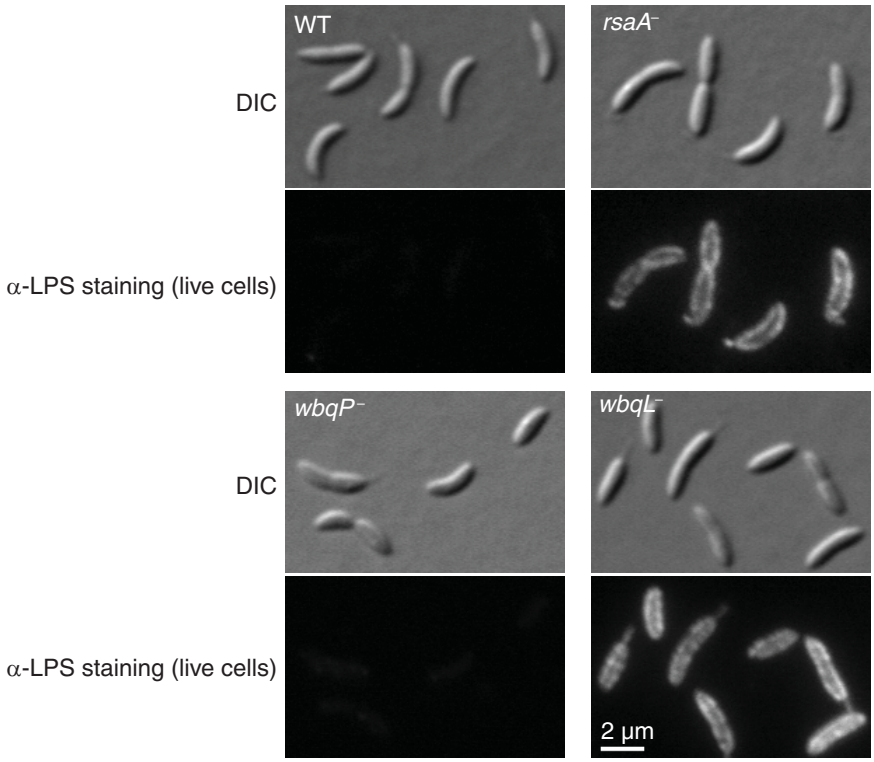
WT
rsaA⁻
DIC
α-LPS staining (live cells)
wbqP⁻
wbqL⁻
DIC
α-LPS staining (live cells)
2 µm

Figure S1

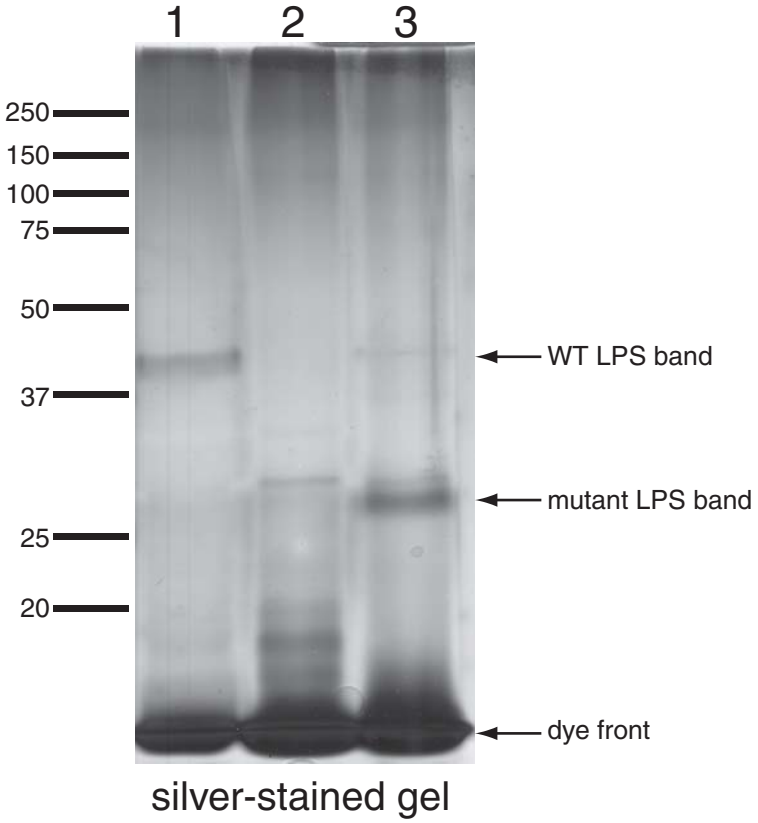
1
2
3
250
150
100
75
50
37
25
20
WT LPS band
mutant LPS band
dye front
silver-stained gel

Figure S2

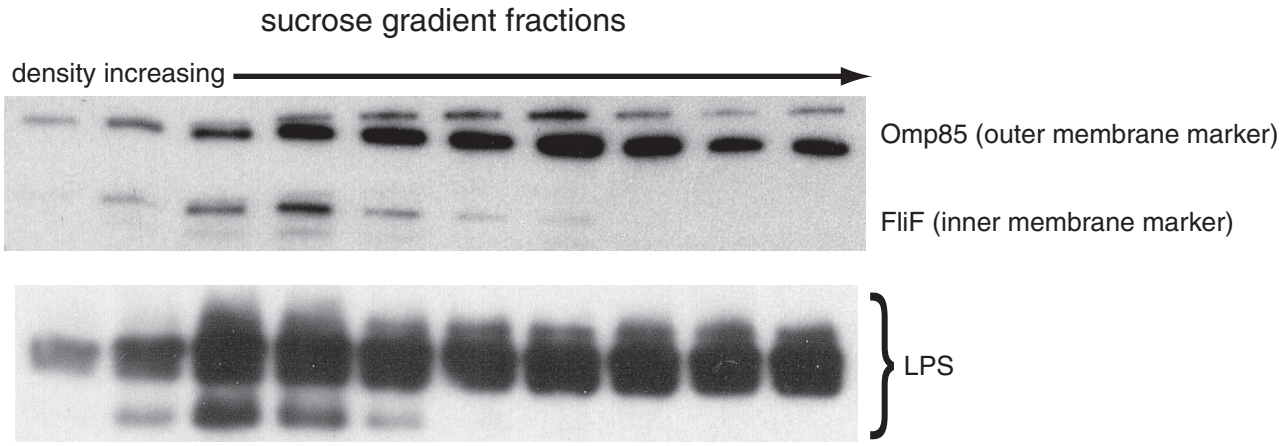
sucrose gradient fractions
density increasing
Omp85 (outer membrane marker)
FliF (inner membrane marker)
LPS

Figure S3

Table S1. Summary of *C. crescentus* PG composition in wild-type, *wbqL*$^-$ and *wbqP*$^-$ strains

Muropeptide species[a]	PG composition (molar % ± SD) of cells		
	Wild-type	*wbqL*$^-$	*wbqP*$^-$
Monomer	41.22 ± 1.26	39.37 ± 1.05	38.96 ± 0.46
Dimer	35.42 ± 0.40	38.97 ± 0.84	39.11 ± 0.69
Trimer	19.16 ± 0.28	18.04 ± 0.24	17.99 ± 0.23
Tetramer	4.21 ± 0.15	3.62 ± 0.08	3.95 ± 0.09
Cross-linkage	38.57 ± 0.34	38.52 ± 0.51	38.72 ± 0.43
Anhydro	12.05 ± 0.15	10.41 ± 0.12	10.09 ± 0.09
Average glycan chain length[b]	8.30 ± 0.17	9.61 ± 0.13	9.91 ± 0.10

a Results from two independent experiments.

b Values represent number of disaccharide subunits per glycan chain.

Table S2. Muropeptide profiles

Muropeptide species	Wild-type (CB15N)		CJW1090 (*wbqL*$^-$)		CJW1249 (*wbqP*$^-$)	
	Composition (molar %)					
	Mean	SD	Mean	SD	Mean	SD
Tri	0.44	0.04	0.39	0.10	0.21	0.05
Tetra + Penta(Gly5)[a]	27.16	1.35	27.20	1.03	26.11	0.06
Penta	12.73	0.51	11.03	0.56	11.90	0.51
TetraTri(D,D)	0.62	0.06	0.79	0.01	0.64	0.02
TriAnh + Tetratri(D,L)[a]	0.24	0.02	0.25	0.03	0.21	0.00
TetraPenta(Gly5)	5.65	0.12	8.10	0.46	7.46	0.76
TetraTetra	11.28	0.32	11.80	0.38	11.92	0.15

TetraPenta	9.36	0.24	10.07	0.69	11.77	0.03
TetraTetraPenta(Gly5)	1.21	0.13	1.73	0.10	1.43	0.10
TetraTetraTetra	3.31	0.06	3.63	0.09	3.62	0.06
TetraTetraPenta	1.99	0.06	2.20	0.17	2.43	0.04
TetraTetraTetraTetra	0.36	0.09	0.42	0.06	0.45	0.04
TetraTetraTetraPenta	0.33	0.12	0.31	0.01	0.38	0.04
PentaAnh	0.88	0.08	0.74	0.03	0.74	0.04
TetraPenta(Gly5)Anh	0.89	0.16	1.07	0.13	0.78	0.04
TetraTetraAnh	3.03	0.10	2.85	0.03	2.70	0.01
TetraPentaAnh	2.78	0.03	2.94	0.17	2.43	0.03
TetraTetraTetraAnh	5.04	0.16	4.20	0.09	4.05	0.10
TetraTetraPentaAnh	5.57	0.22	4.70	0.14	4.86	0.20
TetraTetraTetraPenta(Gly5)Anh	0.62	0.02	0.56	0.04	0.56	0.03
TetraTetraTetraPentaAnh	1.47	0.03	1.26	0.04	1.40	0.05
TetraTetraTetradiAnh	1.11	0.06	0.94	0.00	0.90	0.02
TetraTetraTetraPentadiAnh	1.43	0.07	1.06	0.04	1.15	0.05
TetraTetradiAnh	1.56	0.02	1.11	0.02	1.20	0.04
TetraTetraPentadiAnh	0.94	0.06	0.64	0.00	0.69	0.03

Values are derived from two independent experiments.

a These peak identities are grouped together because the individual peaks could not be resolved in the chromatogram.

Strains and Plasmids Introduced in this Study

***Caulobacter crescentus* strains**

CJW914

Plasmid pJS14PxylcreSΔN27 was introduced into CB15N.

CJW926

CJW1126 was UV-mutagenized by irradiating 100µl drops of overnight culture (stationary phase) on a UV transilluminator for 40 seconds. One of the resulting mutants displayed a straight phenotype. This mutant (CJW898) was then cured of pMR10divK-cfp by growing cells without kanamycin for 4 days, then screening for kanamycin sensitivity, creating CJW926.

CJW940

CB15N *recA* / pKML3001 (LS800; gift of Lucy Shapiro) was mated with CJW734 (S17-1 / pBGENTpleC-tdimer2, creating CJW793. This strain was cured of the unstable plasmid pKML3001 (which contains the *Psuedomonas aeruginosa recA* gene; (4)) and mated with CJW882 (S17-1 / pMR20divJ-myfp-divK-mcfp; (5)).

CJW1034

CB15N *recA* / pKML3001 (LS800; gift of Lucy Shapiro) was mated with CJW1013 (S17-1 / pBGENTcckA-yfp-divJ-tdimer2, creating CJW1022. This strain was then phage ϕCR30 transduced with *cckA*::Ω (from CJW455), creating CJW1023. This strain was then cured of the unstable plasmid pKML3001 (which contains the *Psuedomonas aeruginosa recA* gene; (4)) and mated with CJW1019 (S17-1 / pMR20divL-mcfp).

CJW1084

Cosmid cos1-10-a was introduced to CJW926 by mating it with CJW1080.

CJW1090

Plasmid pBGENT-KO was introduced to CB15N by electroporation.

CJW1117

Plasmid pMR20wbqL was introduced to CJW1090 by mating it with CJW1082.

CJW1126

CB15N was transduced with ϕCR30 lysate carrying *pleC::pleC-yfp* (gift of J.Y. Matroule), creating CJW478. This strain was then mated with S17-1 / pMR10divK-cfp to create CJW1126.

CJW1537

Plasmid pMR20PxylcreSΔN27-tc was introduced to LS3812 (CB15N Δ*creS*; (3)) by mating with CJW1259 (S17-1 / pMR20P$_{xyl}$creS$_{\Delta N27}$-tc).

CJW1908

Plasmid pBGENT-KO was introduced into CJW1249 by mating with CJW1907.

CJW1917

Plasmid pMR20tipN-wbqP was introduced into CJW1908 by mating with CJW3363. The *tipN* gene is immediately upstream of *wbqP* on the *C. crescentus* chromosome.

CJW1930

Plasmid pBGENT-KO was introduced into CJW1243 by mating with CJW1907.

CJW1933

The Himar*1* insertion into *cc_0632* was introduced into CB15N by phage transduction. The ϕCR30 phage lysate was made by infecting strain CJW1932 (CB15N *recA cckA*::Ω *divJ*::pBGENTcckA-yfpdivJ-tdimer2 *cc_0632*::Himar*1*; this strain is identical to CJW1034 but was cured of pMR20divL-cfp).

CJW1935

Plasmid pMR20wbqL was introduced into CJW1933 by conjugation with CJW1082.

CJW2861

Plasmid pHL23creS-mgfp was electroporated into CB15N.

CJW2876

Plasmid pHL23creS-mgfp was electroporated into CJW1090.

CJW3130

A ϕCR30 phage lysate carrying *wbqL*::pBGENT-KO (made by infecting CJW1117) was used to transduce the *wbqL*::pBGENT-KO mutation into CJW3112 (CB15N *ftsZ*::pXMCS7ftsZ *creS*::pHL23creS-mgfp). CJW3112 was made by transducing *creS*::pHL23creS-mgfp by using phage ϕCR30 (transducing lysate made from CJW2861) into CB15N *ftsZ*::pXMCS7ftsZ (CJW2860).

CJW3292

A ϕCR30 phage lysate carrying *wbqL*::pBGENT-KO (made by infecting CJW1117) was used to transduce the *wbqL*::pBGENT-KO mutation into YB1585 (CB15N *ftsZ*::pBJM1) (8).

CJW3295

CJW1090 was conjugated with CJW1446 to create CJW3295.

CJW3329

First, the *wbqL*::pBGENT-KO mutation was transduced into LS3812 (CB15N Δ*creS*) using a ϕCR30 phage lysate (made from CJW1117), thereby making CJW3293 (CB15N Δ*creS wbqL*::pBGENT-KO). pRK415wbqL was introduced into CJW3293 by electroporation, thereby creating CJW3294 (CB15N Δ*creS wbqL*::pBGENT-KO / pRK415wbqL), which is phage-sensitive because *wbqL* is complemented with the unstable plasmid. *Pvan*::pBGENTPvancreS-tc::pHL32PvancreS-gfp was then transduced into CJW3294 using a ϕCR30 phage lysate (made from CJW2207 (2). The resulting transductants were screened for the loss of oxytetracycline resistance (i.e., loss of the pRK415wbqL plasmid), therefore creating CJW3329.

CJW3330

CB15N was mated with CJW1446.

CJW3332

pRK415wbqL was electroporated into CJW3329 to make a strain sensitive to ϕCR30. The resulting strain was then transduced using a ϕCR30 phage lysate made from CJW2860 (CB15N *ftsZ*::pXMCS7ftsZ). Transductants were grown in the absence of oxytetracycline, leading to loss of the pRK415wbqL plasmid.

***Escherichia coli* strains**

CJW1082, CJW1259, CJW1446, CJW1907, CJW3363

S17-1 was transformed with the plasmids listed in the strain table by electroporation.

Plasmids

pBGENT-KO

A 450bp SacI/Kpn internal fragment was excised from a plasmid containing the *wbqL* gene and ligated into pBGENT cleaved with the same enzymes. This plasmid interrupts the *wbqL* gene with the vector sequence when integrated on the chromosome, inactivating it.

pHL23creS-mgfp

The 3' half of *creS* from pKScreS-gfp (CJW944) was cleaved with EcoRI/NcoI, and ligated with a NcoI/NotI fragment encoding monomeric GFP cleaved from pKSmgfp (CJW1180; (1)) into pHL32.A 3' *creS-mgfp* fragment was then cut from pHL32 3'creS-mgfp (CJW3244) with StuI/NotI and ligated with an EcoRI/StuI 5' *creS* fragment including the *creS* promoter into pHL23.

pJS14creS-tc

pBGENTcreS was amplified with primers 268 and 269 to introduce sequence coding for the TC tag and a HindIII site (3') and an EcoRI site (5'). The PCR product was cleaved with EcoRI/HindIII and ligated into pKS cleaved with the same enzymes (making pKScreSTC). The same fragment was then cut out of pKS with EcoRI/HindIII and ligated into pJS14 using the same enzymes.

pJS14PxylcreSΔN27

Primers 121 and 61 were used to PCR out 1.5kb fragment from pKScreSR (CJW894). Fragment was blunt-end cloned directly into the EcoRV site of pKS and sequenced. *creS* was then excised with NdeI/HindIII, and cloned with P_{xyl}, cleaved with EcoRV/NdeI, into pJS14 cut with SmaI/HindIII.

pMR10divK-cfp

A fragment containing *divK* was cut with SacI and SalI from pMR10divK-yfp (CJW580) and ligated with sequence encoding CFP (cleaved from pECFP-N1 plasmid by SalI and NotI) into pKS cleaved with SacI/NotI (CJW477). The *divK-cfp* fragment was then cleaved from pKS with SacI/NotI and ligated into pMR10.

pMR20divL-mcfp

Chromosomal *divL* was PCR-amplified with primers 45 and 46, cloned into pTOPO, and sequenced (there is a silent C>T transition at nt 886 of the coding sequence), creating CJW635. *divL* was cleaved from CJW635 with SpeI/ HindIII and ligated into pKS, creating CJW637. The first half of *divL* was then cleaved from CJW637 with SpeI/BamHI, and the second half of *divL* was obtained from a PCR (primers 45/147) introducing an XhoI site in place of the stop codon using CJW636 as a template. The second half was sequence verified and digested wtih BamHI and XhoI. A triple ligation of the two *divL* fragments was performed into the SpeI and XhoI sites of pKS, creating CJW781. This plasmid was then cleaved with SpeI/XhoI, and ligated with an XhoI/XbaI fragment containing *mcfp* (from CJW647) into pKS cleaved with SpeI/XbaI, creating CJW798. Finally, *divL-mcfp* was cleaved from this plasmid using KpnI/XbaI and ligated into pMR20 cleaved with the same enzymes.

pMR20wbqL

The cosmid cos1-10-A was digested with BamHI, and a 2167 bp BamHI fragment was cloned into BamHI-cleaved pMR20. The insert contains genes *cc_0631* (*wbqL*) and *cc_0630*, which reads in the opposite orientation. The *lac* promoter in the vector sequence reads into *wbqL*.

pMR20tipN-wbqP

pHL32wbqP (containing just *wbqP* sequence) was integrated into CB15N. Genomic DNA from the resulting strain was purified and cut with EcoRI. EcoRI cuts within the vector as well as within the genome just upstream of *CC_1484*. After religating EcoRI ends, electroporating into *E. coli*, and selecting for Kan resistance, an intact plasmid was obtained containing all three genes in their chromosomal order (*CC_1484*, *tipN*, *wbqP*). The three-gene insert was moved into pKS with EcoRI/SpeI. *cc_1484* was then removed by cutting this plasmid with XmnI/PmlI/SacI and ligating a 3.5kb fragment containing *tipN-wbqP* into pHL23 cleaved with SacI/SmaI. *tipN-wbqP* was then cleaved from this plasmid using KpnI/SacI and ligated into pMR20 using these same enzymes, with the genes against the *lac* promoter.

pRK415wbqL

The 2167bp fragment in pMR20wbqL was cleaved from that plasmid with BamHI, cloned into pKS to increase the copy number, then excised from pKS with BamHI and ligated into the BamHI site of pRK415.

Primers

45 5' CAAAGGCCTGCTTGGCCAGGACAGC
46 5' CGGGTGAAAAAAGCTTCCCCGCTCC
61 5' CGATGACCATCGTCCTGGCCGA
121 (NdeI) 5' TCACATATGCAGCACCAGATCGAGTCCA
147 5' GGCTCTCGAGGAAGCCGAGTTCG
268 (EcoRI) 5' ACATGAATTCCTTCGCTTGAATCGGCTCGCCCA
269 (HindIII) 5'
ACAAAAGCTTTTAACAACATCCTGGACAACAGGCGCTCGCGGCCACGTCGCCG

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