TABLE S1. *E. coli* strains and plasmids

E. coli Strains	Genotype	Source or Reference
DH5α	*Φ80ΔlacZΔM15 Δ(lacZYA-argF)*U*169 deoR recA1 endA hsdR17(rk-,mk+) phoA supE44 thi-1gyrA96 relA1*	Lab collection
MG1655	F− λ− *ilvG rfb-50 rph-1*	Lab collection
BMKM111-3	*thr-1 leuB6 proA2 thr-1 argE3 lacY1 galK2 ara-14 xyl-5 thi-1 rpsL31 mtl-1 tsx-33 supE44 D(recC ptr recB recD)::Plac-bet exo cmr ΔyrfF307::res-npt-res (KanR)*	Lab collection
DR1	MG1655 *rcsB11*::ΔTn*10*	(1)
DR5	MG1655 Δ*rcsF::frt*	(1)
DR6	MG1655 *rcsA72*::ΔTn*10*	(1)
DR5-PF	DR5 / pFtsAZ	This work
DR5-PL	DR5 / pLolA	This work
DR20	MG1655 Δ*osmB::kan*	This work
JW5239-4	BW25113 Δ*bdm-774::kan*	Kieo collection (2)
DR21	MG1655 Δ*bdm-774::kan*	MG1655 + P1 (JW5239-4)
JW1477-1	BW25113 Δ*osmC775::kan*	Kieo collection (2)
DR22	MG1655 Δ*osmC775::kan*	MG1655 + P1 (JW1477-1)
JW1488-7	MG1655 Δ*gadB786::kan*	Kieo collection (2)
DR23	MG1655 Δ*gadB786::kan*	MG1655 + P1 (JW1488-7)
DR24	MG1655 Δ*safA::kan*	This work
JW1495-1	BW25113 Δ*ydeP722::kan*	Kieo collection (2)
DR25	MG1655 Δ*ydeP722::kan*	MG1655 + P1 (JW1495-1)
DR26	MG1655 Δ*rprA::kan*	This work
JW3478-1	BW25113 Δ*hdeA765::kan*	Kieo collection (2)
DR27	MG1655 Δ*hdeA765::kan*	MG1655 + P1 (JW3478-1)
JW3479-1	BW25113 Δ*hdeD766::kan*	Kieo collection (2)
DR28	MG1655 Δ*hdeD766::kan*	MG1655 + P1 (JW3479-1)
DR29	MG1655 Δ*gadY::kan*	This work
JW3485-1	BW25113 Δ*gadA772:kan*	Kieo collection (2)
DR30	MG1655 Δ*gadA772::kan*	MG1655 + P1 (JW3485-1)
JW1023-1	BW25113 Δ*csgD781::kan*	Kieo collection (2)
KE27	MG1655 Δ*csgD781::kan*	MG1655 + P1 (JW1023-1)
DR31	DR5 Δ*csgD::kan*	DR5 + P1 (JW1023)
JW1881-1	BW25113 Δ*flhD745::kan*	Kieo collection (2)
DR32	MG1655 Δ*flhD745::kan*	MG1655 + P1 (JW1881-1)
DR33	DR6 Δ*flhD745::kan*	DR6 + P1 (DR32)
DR34	MG1655 Δ*yjbE::kan*	This work
DR35	MG1655 Δ*wza::kan*	This work
DR36	MG1655 Δ*wza::frt*	This work
DR37	DR36 / pWza	This work

DR38	MG1655 Δ[*wza-wcaM*]*::frt*	This work
DR39	MG1655 Δ*wcaD::frt*	This work
DR40	MG1655 Δ*wxzC::frt*	This work
DR41	MG1655 Δ*cpsB::frt*	This work
DR42	MG1655 Δ*cpsG::frt*	This work
DR43	DR42 / pCpsG	This work
DR44	MG1655 Δ*ugd::frt*	This work
DR45	DR44 / pUgd	This work
DR46	MG1655 Δ*wcaJ::frt*	This work
DR47	MG1655 Δ*wcaJ* Δ*wzxC:: frt*	This work
DR48	MG1655 Δ*cpsG* Δ*wcaJ::kan*	This work
DR49	MG1655 Δ*ugd::frt* Δ*wcaJ::kan*	This work
DR50	MG1655 / pWcaJ	This work
DR51	DR38 / pWcaJ	This work
DR52	DR36 / pUppS	This work
DR53	DR44 / pUppS	This work
DR54	DR36 / pUppP	This work
DR55	DR44 / pUppP	This work
DR56	MG1655 Δ*uppP::kan*	This work
DR57	DR38 *rcsB*::Tn10	DR38 + P1 (DR1)

Plasmids		
pDEV	*colE1 lacI*^{q} P_{lac} *(ΔlacZ) kan*	(1)
pDKR2	pDEV- P_{lac}*::dsbAss-sfgfp*	(1)
pFtsAZ	pDEV- P_{lac}*::ftsAZ*	This work
pLolA	pDEV- P_{lac}*::lolA*	This work
pWza	pDEV- P_{lac}*::wza*	This work
pCpsG	pDEV- P_{lac}*::cpsG*	This work
pUgd	pDEV- P_{lac}*::ugd*	This work
pWcaJ	pDEV- P_{lac}*::wcaJ*	This work
pUppS	pDEV- P_{lac}*::uppS*	This work
pUppP	pDEV- P_{lac}*::uppP*	This work

TABLE S2. Primers

To construct	Primer Name	Forward, F or Reverse, R	Sequences
pFtsAZ	DP132	F	CGCGGATCCTGATTAACTTTATAAGGAGGAAAAACAT ATGATCAAGGCGACGGACAG
	DP55	R	GCGCTGCAGTTAATCAGCTTGCTTACGCA
pLolA	DP152	F	CGCGAATTCGGGAGTGACGTAATTTGAGGAATAATG
	DP150	R	CGCGGATCCCTACTTACGTTGATCATCTACCG
pWza	DP364	F	CGCGGATCCTGATTAACTTTATAAGGAGGAAAAACAT ATGATGAAATCCAAAATGAAATT
	DP365	R	CGCAAGCTTTTACCAGTTATGAATGTCGC
pCpsG	DP277	F	CCGGGATCCTGATTAACTTTATAAGGAGGAAAAACAT ATGAAAAAATTAACCTGCTTTAA
	DP278	R	CGCAAGCTTTTACTCGTTCAGCAACGTC
pUgd	DP330	F	CGCGGATCCTGATTAACTTTATAAGGAGGAAAAACAT ATGAAAATCACCATTTCCGGTAC
	DP331	R	CGCAAGCTTGCCCTGATAACAAGATGTTA
pWcaJ	DP316	F	CGCGAATTCTGATTAACTTTATAAGGAGGAAAAACAT GTGATGTTGTCTGCTACTCA
	DP317	R	CGCAAGCTTTCAATATGCCGCTTTGTTAACG
pUppS	DP314	F	CGCGAATTCCCTGTCAGGGAATAAAAAACGCGTG
	DP315	R	CGCAAGCTTTCAGGCTGTTTCATCACCG
pUppP	DP326	F	CGCGAATTCATAATTTAGGGGTTTATTG
	DP327	R	CGCAAGCTTTTAAAAGAACACGACATACA
DR20 (Δ*osmB*)	DP141	F	TTAGCTATTATAGTTATAGAGAGCTTACTTCCGTGAAT CAATTCCGGGGATCCGTCGACC
	DP142	R	TAATCGACATTTATTTACGATTATTTACCGACCTGGTG ACTGTAGGCTGGAGCTGCTTCG
DR24 (Δ*safA*)	DP211	F	AACGATTTTTAACGTTATCCGCTAAATAAACATATTTG AAATTCCGGGGATCCGTCGACC
	DP212	R	ACTTTTTTAACATTTCATATTTATAATTTGCTGTTTGTT TTGTAGGCTGGAGCTGCTTCG
DR26 (Δ*rprA*)	DP130	F	TCTGATCGACGCAAAAAGTCCGTATGCCTACTATTAGC TCATTCCGGGGATCCGTCGACC
	DP131	R	GCGAGGTAGCGAAGCGGAAAAATGTTAAAAAAAAGC CCATTGTAGGCTGGAGCTGCTTCG
DR29 (Δ*gadY*)	DP207	F	AATGGCTGATCTTATTTCCAGTAAAAGTTATATTTAAC TTATTCCGGGGATCCGTCGACC
	DP208	R	CTGCGGAAGGAATAAGATTATAGAGTTTTACTCAGAC ATATGTAGGCTGGAGCTGCTTCG
DR34 (Δ*yjbE*)	DP309	F	TTTGGGTAAATCTCCATTCATTCAATGAAGGGAAATTG TTATTCCGGGGATCCGTCGACC
	DP310	R	TGCAATGACTCGAATTATTTTGGGGATACATACTTTAT TATGTAGGCTGGAGCTGCTTCG
DR35 (Δ*wza*)	DP279	F	TTGCACAGTACTTCCGGGCAGCAATATGTCGACGATGG GCTGTGTAGGCTGGAGCTGCTT

	DP280	R	GGCAAATATTGCCGACACAGACAACTAAGATGTTGTT AAACATATGAATATCCTCCTTAG
DR38 [Δ*wza-wcaM*]	DP263	R	TACTGACACCAATAATGGCATCAATTTCATTTTGGATT TTGTAGGCTGGAGCTGCTTCG
	DP273	R	TCCTAAGTATGACTCCATTTTTCCAGGAATGGTCGCAA ATTGTAGGCTGGAGCTGCTTCG
DR39 (Δ*wcaD*)	DP351	F	CTACCTGTTGCTGCCGCTGATTTATCTGCTGGTTAACGT CATTCCGGGGATCCGTCGACC
	DP352	R	TTACCTCGTAATATTTAACGCTTTTCGCAAAATAAACG GATGTAGGCTGGAGCTGCTTCG
DR40 (Δ*wzxC*)	DP350	F	TATGAGCTTACGTGAAAAAACCATCAGCGGCGCGAAG TGGATTCCGGGGATCCGTCGACC
	DP298	R	AACCGAAAACAGCAACTCACCCCGCCCGTAAAAGCAT TTTTGTAGGCTGGAGCTGCTTCG
DR41 (Δ*cpsB*)	DP265	F	ACGTGTTACGTCAATTTATAAATGATATTCGGGGATAA TTATTCCGGGGATCCGTCGACC
	DP266	R	TACACCGCGGTTTCGCATTCATTGCCTGATGCGACGTT TATGTAGGCTGGAGCTGCTTCG
DR42 (Δ*cpsG*)	DP267	F	TTCGGTCAGGGCCAACTATTGCCTGAAAAAGGGTAAC GATATTCCGGGGATCCGTCGACC
	DP268	R	GCCCCTTACCCGCAGTGGGGTAAGGGAAGATCCGACA TTATGTAGGCTGGAGCTGCTTCG
DR44 (Δ*ugd*)	DP321	F	ACATCATGATTCACAGTTAAGTTAATTCTGAGAGCATG AAATTCCGGGGATCCGTCGACC
	DP322	R	GGGCGTAAATAGCCCTGATAACAAGATGTTAGTCGCT GCCTGTAGGCTGGAGCTGCTTCG
DR46 (Δ*wcaJ*)	DP283	F	GGATCTTCCCTTACCCCACTGCGGGTAAGGGGCTAATA ACATTCCGGGGATCCGTCGACC
	DP284	R	CGACAATCGACATCCCCCCGGTCAGCACATTGATAAA CTGTGTAGGCTGGAGCTGCTTCG
DR56 (Δ*uppP*)	DP322	F	ACGCAGTAGTTCGGACAAGCGGTACATTTTAATAATTT AGATTCCGGGGATCCGTCGACC
	DP333	R	CAATCGTTGACAACGCCAAGCATCCGACACTATTCCTC AATGTAGGCTGGAGCTGCTTCG

BamHI, GGATTC; PstI, CTGCAG; EcoRI, GAATTC; HindIII, AAGCTT

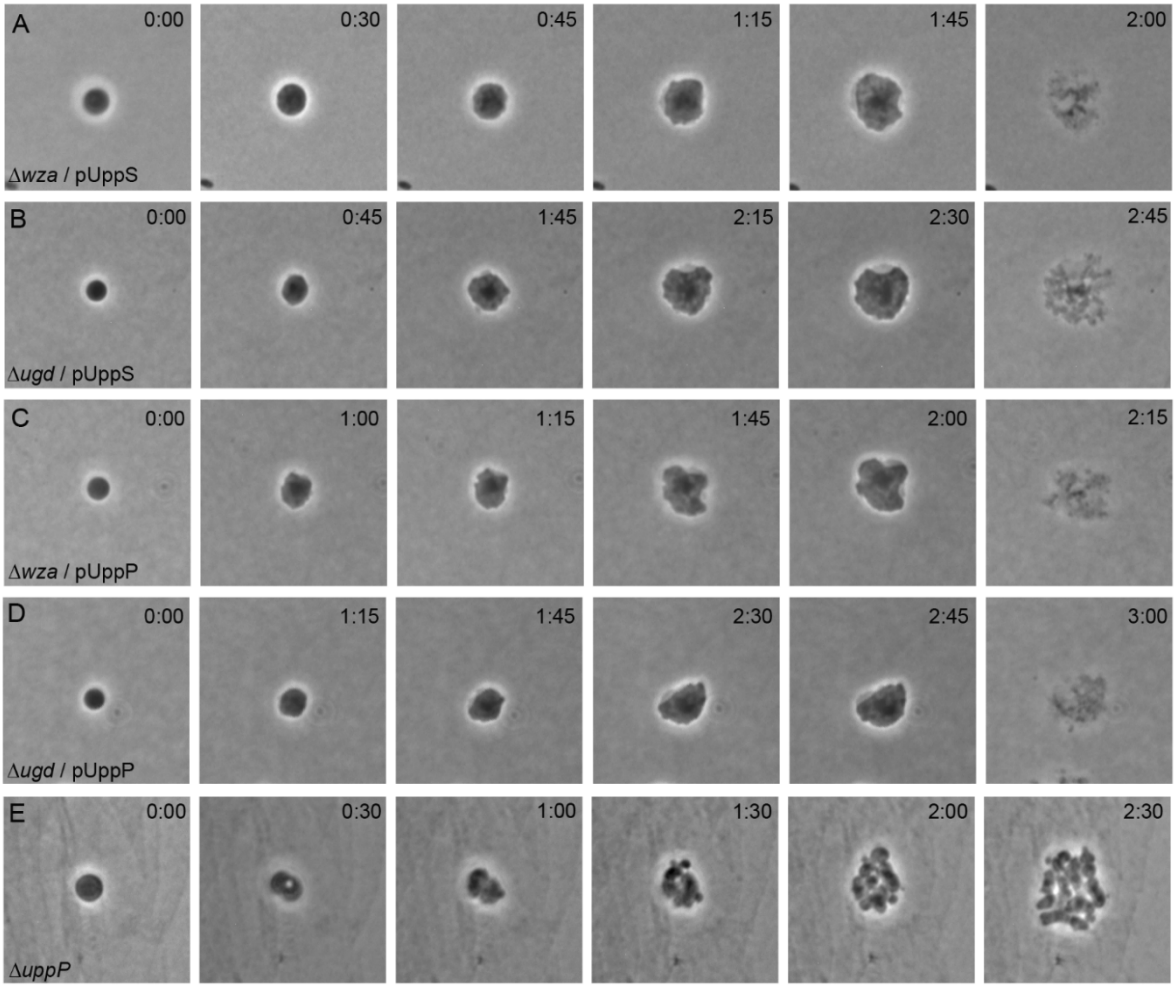

Fig. S1. Undecaprenyl phosphate (Und-P) levels did not suppress WcaJ-generated toxicity and failed to rescue spheroplast recovery. Spheroplasts from the indicated strains were grown on osmotically protected sucrose recovery medium and the recovery process was monitored by time-lapse microscopy. Time after plating is displayed in the upper right-hand corner of each panel (h:min). The images are representative results. A. *E. coli* DR52 (N = 9), B. *E. coli* DR53 (N=16), C. *E. coli* DR54 (N = 9), D. *E. coli* DR55 (N = 4), E. *E.coli* DR56 (N = 8).

Supplemental References

1. **Ranjit, D. K., and K. D. Young.** 2013. The Rcs stress response and accessory envelope proteins are required for *de novo* generation of cell shape in *Escherichia coli*. J Bacteriol **195:**2452-2462.
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