

Engineered membrane vesicle production via *oprF* or *oprI* deletion has distinct phenotypic effects in *Pseudomonas putida*

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Table S1. Putative gene knockout targets in *P. putida* KT2440 to enhance vesiculation.

Candidate protein target	Locus tag/s in <i>P. putida</i> KT2440	Cellular location (GO term)	Proposed mechanism	Precedence	Reference
TolA	PP_1221	Outer membrane	Deletion of <i>tolA</i> will disrupt the Tol cell envelope complex causing the destabilization of the membrane by disrupting the outer membrane to peptidoglycan to inner membrane linkage.	<i>Salmonella typhimurium</i>	(¹)
				<i>Escherichia coli</i>	(^{2,3})
TolR	PP_1220	Plasma membrane	Deletion of <i>tolR</i> will disrupt the Tol cell envelope complex causing the destabilization of the membrane by disrupting the outer membrane to peptidoglycan to inner membrane linkage.	<i>Escherichia coli</i>	(²)
				<i>Salmonella enterica</i> serovar Typhi	(⁴)
TolB	PP_1222	Periplasmic space	Deletion of <i>tolB</i> will disrupt the Tol cell envelope complex causing the destabilization of the membrane by disrupting the outer membrane to peptidoglycan to inner membrane linkage.	<i>Salmonella typhimurium</i>	(¹)
				<i>Escherichia coli</i>	(³)
OprL (Pal)	PP_1223	Outer membrane	Deletion of <i>oprL</i> will destabilize the membrane by disrupting the outer membrane to peptidoglycan linkages.	<i>Salmonella typhimurium</i>	(¹)
				<i>Escherichia coli</i>	(³)
OmpA	PP_1121	Outer membrane	Deletion of <i>ompA</i> will destabilize the outer membrane by disrupting the outer membrane tethering to the peptidoglycan layer.	<i>Acinetobacter baumannii</i>	(5)
	PP_4669				
	PP_1122			<i>Salmonella typhimurium</i>	(1)
	PP_1502				
	PP_4198				
OprF	PP_2089	Outer membrane	Deletion of <i>oprF</i> will destabilize the outer membrane by disrupting the outer membrane tethering to the peptidoglycan layer.	<i>Pseudomonas aeruginosa</i>	(⁶)
OprI	PP_2322	Outer membrane	Deletion of <i>oprI</i> will destabilize the outer membrane by disrupting the outer membrane tethering to the peptidoglycan layer.	<i>Pseudomonas aeruginosa</i>	(⁶)
				<i>Pseudomonas putida</i>	(⁷)
MlaA (VacJ)	PP_2163	Outer membrane	Deletion of <i>mlaA</i> will disrupt the Mla pathway causing phospholipid accumulation in the outer leaflet and increase the outer membrane curvature.	<i>Escherichia coli</i>	(⁸)
				<i>Haemophilus influenzae</i> <i>Vibrio cholerae</i>	(⁹)
MlaD	PP_0960	Extracellular region	Deletion of <i>mldD</i> will disrupt the Mla pathway causing phospholipid accumulation in the outer leaflet and increase the outer membrane curvature.	<i>Veillonella parvula</i>	(¹⁰)

Table S2. Protein sequence identity of OmpA from *E. coli* K12 to *P. putida* KT2440 genes

<i>E. coli</i> K12		<i>P. putida</i> KT2440			
Protein reference	UniProt Accession No.	Gene name	Protein name	UniProt Accession No.	Identity (%)
OmpA	P0A910	PP_1122	OmpA family protein	Q88NT2	42.1
		PP_1121	OmpA family protein	Q88NT3	38.5
		PP_1502	OmpA family protein	Q88MR7	38.9
		PP_4669	OmpA family protein	Q88DZ9	39.0
		PP_4198	OmpA family protein	Q88FA0	34.5
		<i>oprF</i> (PP_2089)	Outer membrane porin F	Q88L46	34.2

Table S3. Strains utilized in this study and corresponding construction details.

Strain	Genotype	Construction details	Reference
KT2440	Wild-type <i>Pseudomonas putida</i> KT2440	n/a	ATCC® 47054
RW29	<i>P. putida</i> KT2440 $\Delta oprF$	pRW007 was transformed into KT2440. Deletion of <i>oprF</i> (PP_2089) was confirmed by colony PCR with oRW037 and oRW038 (Tm=68°C, 2.3 kB) followed by Sanger sequencing.	This study
RW30	<i>P. putida</i> KT2440 $\Delta oprI$	pRW008 was transformed into KT2440. Deletion of <i>oprI</i> (PP_2322) was confirmed by colony PCR with oRW041 and oRW042 (Tm=67°C, 2.3 kB) followed by Sanger sequencing.	This study
RW25	<i>P. putida</i> KT2440 ΔPP_{1121}	pRW005 was transformed into KT2440. Deletion of PP_1121 was confirmed by colony PCR with oRW029 and oRW030 (Tm=61°C, 2.3 kB) followed by Sanger sequencing.	This study
RW27	<i>P. putida</i> KT2440 ΔPP_{4669}	pRW006 was transformed into KT2440. Deletion of PP_4669 was confirmed by colony PCR with oRW033 and oRW034 (Tm=63°C, 2.5 kB) followed by Sanger sequencing.	This study
RW95	<i>P. putida</i> KT2440 ΔPP_{1502}	pRW039 was transformed into KT2440. Deletion of PP_1502 was confirmed by colony PCR with oRW127 and oRW128 (Tm=67°C, 2.5 kB) followed by Sanger sequencing.	This study
RW02	<i>P. putida</i> KT2440 PP_1122::Tc1 ₁₂₈₅₀₆₈ (Km) ^b	Strain was obtained directly from the arrayed transposon mutant library. The barcode was PCR amplified with Barseq_P1 and Barseq_P2 (Tm=55°C, <0.2 kB) and sequence confirmed with BarSeq_SangerSeq.	(¹¹)
RW03	<i>P. putida</i> KT2440 PP_1502::Tc1 ₁₇₀₇₅₄₈ (Km) ^b	Strain was obtained directly from the arrayed transposon mutant library. The barcode was PCR amplified with Barseq_P1 and Barseq_P2 (Tm=55°C, <0.2 kB) and sequence confirmed with BarSeq_SangerSeq.	(¹¹)
RW04	<i>P. putida</i> KT2440 PP_4198::Tc1 ₄₇₄₄₂₄₄ (Km) ^b	Strain was obtained directly from the arrayed transposon mutant library. The barcode was PCR amplified with Barseq_P1 and Barseq_P2 (Tm=55°C, <0.2 kB) and sequence confirmed with BarSeq_SangerSeq.	(¹¹)
RW05	<i>P. putida</i> KT2440 <i>mlaA</i> ::Tc1 ₂₄₆₉₆₃₅ (Km) ^b	Strain was obtained directly from the arrayed transposon mutant library. The barcode for PP_2163 was PCR amplified with Barseq_P1 and Barseq_P2 (Tm=55°C, <0.2 kB) and sequence confirmed with BarSeq_SangerSeq.	(¹¹)
RW09	<i>P. putida</i> KT2440 <i>mlaD</i> ::Tc1 ₁₁₀₁₅₃₃ (Km) ^b	Strain was obtained directly from the arrayed transposon mutant library. The barcode for PP_0960 was PCR amplified with Barseq_P1 and Barseq_P2 (Tm=55°C, <0.2 kB) and sequence confirmed with BarSeq_SangerSeq.	(¹¹)
RW217	<i>P. putida</i> KT2440 + pTM007	pTM007 was transformed into KT2440. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study

RW218	<i>P. putida</i> KT2440 $\Delta oprF$ + pTM007	pTM007 was transformed into RW29. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW219	<i>P. putida</i> KT2440 $\Delta oprI$ + pTM007	pTM007 was transformed into RW30. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW220	<i>P. putida</i> KT2440 + pRW075	pRW075 was transformed into KT2440. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW221	<i>P. putida</i> KT2440 $\Delta oprF$ + pRW075	pRW075 was transformed into RW29. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW222	<i>P. putida</i> KT2440 $\Delta oprI$ + pRW075	pRW075 was transformed into RW30. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW223	<i>P. putida</i> KT2440 + pTM008	pTM008 was transformed into KT2440. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW224	<i>P. putida</i> KT2440 $\Delta oprF$ + pTM008	pTM008 was transformed into RW29. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW225	<i>P. putida</i> KT2440 $\Delta oprI$ + pTM008	pTM008 was transformed into RW30. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW226	<i>P. putida</i> KT2440 + pBTL-2 (Empty vector)	pBTL-2 empty vector was transformed into KT2440. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW227	<i>P. putida</i> KT2440 $\Delta oprF$ + pBTL-2 (Empty vector)	pBTL-2 empty vector was transformed into RW29. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
RW228	<i>P. putida</i> KT2440 $\Delta oprI$ + pBTL-2 (Empty vector)	pBTL-2 empty vector was transformed into RW30. All cultures were grown with 50 mg/L of kanamycin to maintain plasmid.	This study
AG5577	<i>P. putida</i> KT2440 $\Delta PP_{4740::BxB1_RV_phi370_attB}$ cassette $\Delta PP_{4217/4218}$ intergenic::TG1_BL3_A11 8_attB cassette $\Delta PP_{2876::R4_phiBT1_MR11_attB}$ cassette	Strain was obtained courtesy of Adam Guss' group at Oak Ridge National Laboratory. The strain contains a total of nine attB attachment sites (3 in each of 3 loci) for use with serine integrase helper plasmids. See reference for more details.	(12)
TM115	AG5577 $\Delta oprF$	pRW007 was transformed into AG5577. Deletion of <i>oprF</i> (PP_2089) was confirmed by colony PCR with oRW037 and oRW038 (Tm=68°C, 2.3 kbp) followed by sequencing by Oxford Nanopore.	This study
TM117	TM115 with edits at the <i>Bxb1</i> site $attL^{BxB1}::bla::aadA::P_{tac}:plsBC_{Pp}cdsA_{Pp}::attR^{BxB1}$	TM115 transformed with pTM018 and the corresponding helper plasmid pGW31 targeting the Bxb1 site. Transformants were selected using 100 mg/L spectinomycin. Insertion of the plasmid containing <i>bla</i> (amp ^r), <i>aad</i> (spec ^r), and $P_{tac}:plsBC_{Pp}cdsA_{Pp}$ was sequenced confirmed using oTM064_int and oTM65_210 for colony PCR (Tm = 69 °C, 7.75 kbp) followed by sequencing by Oxford Nanopore.	This study

TM118	<i>TM115 with edits at the Bxb1 site</i> <i>attL^{Bxb1}::bla::aadA:P_{tac}:pls</i> <i>BC_{Ec}cdsA_{Ec}::attR^{Bxb1}</i>	TM115 was transformed with pTM017 and the corresponding helper plasmid pGW31 targeting the Bxb1 site. Transformants were selected using 100 mg/L spectinomycin. Insertion of the of the plasmid containing <i>bla</i> (amp ^r), <i>aad</i> (spec ^r), and <i>P_{tac}:plsBC_{Ec}cdsA_{Ec}</i> was sequenced confirmed using oTM064_int and oTM65_210 for colony PCR (Tm=69°C, 7.75 kB) followed by sequencing by Oxford Nanopore.	This study
TM121	<i>TM115 with edits to the TG1 site</i> <i>attL^{TG1}::nptII:P_{em7}:</i> <i>accACDB_{Ec}::attR^{TG1}</i>	TM115 was transformed with pTM015 and the corresponding helper plasmid pGW38 targeting the TG1 site. Transformants were selected using 50 mg/L of kanamycin. Insertion of the plasmid containing <i>nptII</i> (kan ^r) and <i>P_{em7}:accACDB_{Ec}</i> was sequenced confirmed using oTM064_int and oTM67_419 for colony PCR (Tm=68°C, 6.56 kB) followed by sequencing by Oxford Nanopore.	This study
TM122	<i>TM115 with edits to the TG1 site</i> <i>attL^{TG1}::nptII:P_{tac}:gpsA_{Pp}::</i> <i>attR^{TG1}</i>	TM115 transformed with pTM045 and the corresponding helper plasmid pGW38 targeting the TG1 site. Transformants were selected using 50 mg/L of kanamycin. Insertion of the plasmid containing <i>nptII</i> (kan ^r) and <i>P_{tac}:gpsA_{Pp}</i> was sequenced confirmed using oTM064_int and oTM67_419 for colony PCR (Tm=68°C, 3.8 kB) followed by sequencing by Oxford Nanopore.	This study
TM123	<i>TM115 with edits to the TG1 site</i> <i>attL^{TG1}::nptII:P_{tac}:gpsA_{Ec}::</i> <i>attR^{TG1}</i>	TM115 was transformed with pTM044 and the corresponding helper plasmid pGW38 targeting the TG1 site. Transformants were selected using 50 mg/L of kanamycin. Insertion of the plasmid containing <i>nptII</i> (kan ^r) and <i>P_{tac}:gpsA_{Ec}</i> was sequenced confirmed using oTM064_int and oTM67_419 for colony PCR (Tm=68°C, 3.8 kB) followed by sequencing by Oxford Nanopore.	This study
TM124	<i>TM117 with edits to the TG1 site</i> <i>attL^{TG1}::nptII:P_{tac}:</i> <i>gpsA_{Pp}::attR^{TG1}</i>	TM117 was transformed with pTM045 and the corresponding helper plasmid pGW38 targeting the TG1 site. Transformants were selected using 50 mg/L of kanamycin. Insertion of the plasmid containing <i>nptII</i> (kan ^r) and <i>P_{tac}:gpsA_{Pp}</i> was sequenced confirmed using oTM064_int and oTM67_419 for colony PCR (Tm=68°C, 3.8 kB) followed by sequencing by Oxford Nanopore.	This study
TM125	<i>TM117 with edits to the TG1 site</i> <i>attL^{TG1}::nptII:P_{tac}:gpsA_{Ec}::</i> <i>attR^{TG1}</i>	TM118 transformed with pTM045 and the corresponding helper plasmid pGW38 targeting the TG1 site. Transformants were selected using 50 mg/L of kanamycin. Insertion of the plasmid containing <i>nptII</i> (kan ^r) and <i>P_{tac}:gpsA_{Ec}</i> was sequenced confirmed using oTM064_int and oTM67_419 for colony PCR (Tm=68°C, 3.8 kB) followed by sequencing by Oxford Nanopore.	This study

^aNumbers following “Tc1” indicate the location of the transposon insertion site (in bp) in the KT2440 genome.

Table S4. Oligonucleotides utilized in this study.

Name	Sequence (5'→3')	Purpose
oRW029	GCACTGCTGAATCTGATGTAG	Colony PCR of pRW005 integration
oRW030	GGACATGACATGGAAGATTC	
oRW033	ATTGTTGTCGGGACGAGATC	Colony PCR of pRW006 integration
oRW034	GAAGTCAATCTCGACTCGG	
oRW037	ATCGGCCTGGAATATTCGGC	Colony PCR of pRW007 integration
oRW038	GACCGGACACTACCCGTAC	
oRW041	GCTTGCAACGTGCCAATGC	Colony PCR of pRW008 integration
oRW042	GGCCAACATCATGGTCGAC	
oRW127	AGCTGGAATACCGCGATGTC	Colony PCR of pRW039 integration
oRW128	CTCCGTTGCATCCCTATCAG	
Barseq_P1	AATGATACGGCGACCACCGAGATCTACACTCTT TCCCTACACGACGCTCTTCCGATCTNNNNNGTC GACCTGCAGCGTACG	Verification of barcode from arrayed transposon library
Barseq_P2	CAAGCAGAAGACGGCATACGAGATCGTGATGT GACTGGAGTTCAGACGTGTGCTCTTCCGATCTG ATGTCCACGAGGTCTCT	
BarSeq_SangerSeq	GACCACCGAGATCTACAC	
oTM002	TATCTAGATGGTGAGCAAGGGCGAGG	mNeonGreen was PCR extracted from Addgene Plasmid #182388 and assembled in to pBTL2 using EcoRV and xbaI used to generate pTM002
oTM003	ATGATATCTTAATGATGATGATGATGATGGGTAC C	
oTM020	GATATCATTACAGGACGAGCCTCAG	Primers used to PCR pBTL-2 backbone for assembly of pTM007 and pTM008 (T _m 56°C, 2.6 kB).
oTM024	TCCTTAAAATTGAAATAGATTACGGGTGTGAAAT TGTTATCCG	
oTM019	GCTCGTCTGAATGATATCTTAATGATGATGATG ATGATGGGTACCTCC	Used to generate VNp_mNeonGreen and mNeonGreen fragments for Gibson assembly into pBTL-2.
oTM022	TTTCAATTTTAAGGAGGTTTTAATAATGGATGTA TTCATGAAAGG	
oTM027	TTTCAATTTTAAGGAGGTTTTAATAATGGTGAGC AAGGGCGAGG	
oTM035	CTTCTCTTATAGAGGTTCTAGTGTGAAATTGTTA TCCGCTCACAATTCC	Primers used to generate parts for the assembly of pTM018 from <i>P. putida</i> KT2440 genome.
oTM036	AGGTAGAAGACAACCTGGTCTAGACTCGAGG	
oTM037	CAATTTACACTAGAACCTCTATAAGAGAAGTTA GCATGACCCGTTCCCCCTGCACC	
oTM038	AACCTCTTATGTTTTGATGATTAGAGGGTCGAA GTTTCCAGC	
oTM039	GACCCTCTAATCATCAAAACATAAGAGGTTTTTT AATGCTTTATTTCATTGCGTATGTTCC	

oTM042	CTCGAGTCTAGACCAGTTGTCTTCTACCTAGTC AAAAGCCTCCGGTCGGAGGCTTTTGACTTCACA TCACACCCCATTCGGCAGC	
oTM043	AAAAAACCTCCTTTAGAGAGAAAGTCAGGCCAG TTCTTTTTCCATGCGGTCAATGC	
oTM044	CCTGACTTTCTCTCTAAAGGAGGTTTTTTCATGC TTAAACAACGCATCATTACTGC	
oTM064_int	TGTTTCGTCCTCGAGTCTAGACC	Colony PCR of sage integrase plasmids
oTM065_210	TTCCACTGCCATCAGCGC	
oTM067_419	AGAAGTACTACCAGGGCATTGC	

Table S5. Plasmids utilized in this study.

Name	Description	Construction details	Reference
pRW005	pK18sB-based plasmid for deletion of PP_1121 in <i>P. putida</i> KT2440-derived strains	1 kb homology regions upstream and downstream of PP_1121 were designed. An XbaI site was inserted between the two homology regions, and the insert was cloned into the pK18sb backbone at the EcoRI and HindIII sites. The plasmid was synthesized and sequence-verified by Twist Biosciences.	This study
pRW006	pK18sB-based plasmid for deletion of PP_4669 in <i>P. putida</i> KT2440-derived strains	1 kb homology regions upstream and downstream of PP_4669 were designed. An XbaI site was inserted between the two homology regions, and the insert was cloned into the pK18sb backbone at the EcoRI and HindIII sites. The plasmid was synthesized and sequence-verified by Twist Biosciences.	This study
pRW007	pK18sB-based plasmid for deletion of PP_2089 (<i>oprF</i>) in <i>P. putida</i> KT2440-derived strains	1 kb homology regions upstream and downstream of PP_2089 (<i>oprF</i>) were designed. An XbaI site was inserted between the two homology regions, and the insert was cloned into the pK18sb backbone at the EcoRI and HindIII sites. The plasmid was synthesized and sequence-verified by Twist Biosciences.	This study
pRW008	pK18sB-based plasmid for deletion of PP_2322 (<i>oprI</i>) in <i>P. putida</i> KT2440-derived strains	1 kb homology regions upstream and downstream of PP_2322 (<i>oprI</i>) were designed. An XbaI site was inserted between the two homology regions, and the insert was cloned into the pK18sb backbone at the EcoRI and HindIII sites. The plasmid was synthesized and sequence-verified by Twist Biosciences.	This study
pRW039	pK18sB-based plasmid for deletion of PP_1502 in <i>P. putida</i> KT2440-derived strains	1 kb homology regions upstream and downstream of PP_1502 were designed. An XbaI site was inserted between the two homology regions, and the insert was cloned into the pK18sb backbone at the EcoRI and HindIII sites. The plasmid was synthesized and sequence-verified by Twist Biosciences.	This study
Addgene Plasmid #182390	pRSFDuet-1 based plasmid containing VNP-mNeonGreen_OmpA-mCherry	pRSFDuet-1_VNP-mNeonGreen_OmpA-mCherry was a gift from Dan Mulvihill (Addgene plasmid # 182390 ; http://n2t.net/addgene:182390 ; RRID:Addgene_182390)	(13)
Addgene Plasmid #182388	pRSFDuet-1 based plasmid containing VNP6-mNeonGreen	pRSFDuet-1_VNP6-mNeonGreen was a gift from Dan Mulvihill (Addgene plasmid # 182388 ; http://n2t.net/addgene:182388 ; RRID:Addgene_182388)	(13)
pRW075	pBTL-2-based plasmid for the expression of spycatcher003-	The expression cassette included P _{lac} , ribosome binding site [RBS; translation initiation rate (TIR) = 10277.53], <i>ompA_{EC}</i> , linker, spycatcher003, RBS (TIR:	This study

	<i>ompA_{EC}</i> :spytag003-mNeonGreen	193180.16), mNeonGreen (originally from Addgene Plasmid #182388), linker, spytag003, and <i>soxR</i> terminator. The <i>ompA_{EC}</i> , linkers, spycatcher003, and spytag003 were codon optimized using Integrated DNA Technologies Codon Optimization Tool. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid was synthesized and sequence-verified by Twist Biosciences. The sequence of the expression cassette is listed below.	
pTM001	pRSFDuet based plasmid containing VNp-mNeonGreen with his tag.	Using BglII and NdeI the VNp from Addgene Plasmid #182390 was cloned into the place where VNp was in frame with mNeonGreen in the pRSFDuet vector Addgene Plasmid #182388. Plasmid was sequenced verified by Oxford Nanopore Sequencing.	This study
pTM007	pBTL-2-based plasmid for the expression of mNeonGreen	The expression cassette contained the P _{lac} , RBS (TIR: 193180.16), and mNeonGreen (originally from Addgene Plasmid #182388). This plasmid was assembled by Gibson assembly and fragments were generated using primers oTM020, oTM024, oTM019, and oTM027. The RBS was designed using the Salis optimization tool as described in the methods. The plasmid was sequenced by Oxford Nanopore Sequencing. The sequence of the expression cassette is listed below.	This study
pTM008	pBTL-2-based plasmid for the expression of mNeonGreen-vesicle nucleating peptide (vNP)	The expression cassette contained P _{lac} , RBS (same as above TIR: 305719.82), VNp fused to mNeonGreen (originally from Addgene Plasmid #182388). This plasmid was assembled by Gibson assembly and fragments were generated using primers oTM020, oTM024, oTM019, and oTM022. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid was sequenced by Oxford Nanopore Sequencing. The sequence of the expression cassette is listed below.	This study
Addgene Plasmid #22806	pBTL-2 plasmid with no insert containing the <i>lac</i> promoter	pBTL-2 was obtained courtesy of Ryan Gill (Addgene plasmid # 22806 ; http://n2t.net/addgene:22806 ; RRID:Addgene_22806).	(¹⁴)
pJH0419	Sage integrase plasmid for integration in the BxbI site conferring kanamycin resistance	Plasmid obtained courtesy of Adam Guss' group at Oak Ridge National Laboratory.	(¹²)
pJH0210	Sage integrase plasmid with the TG1 integration site in the TG1 integration site	Plasmid obtained courtesy of Adam Guss' group at Oak Ridge National Laboratory.	(¹²)

	conferring ampicillin, spectinomycin, and streptomycin resistance		
pGW31	Integrase Expression Suicide Vector for integration at the Bxb1 site (used for pJH0419)	Plasmid obtained courtesy of Adam Guss' group at Oak Ridge National Laboratory.	(¹²)
pGW38	Integrase Expression Suicide Vector for integration at the TG1 site (used for pJH0210)	Plasmid obtained courtesy of Adam Guss' group at Oak Ridge National Laboratory.	(¹²)
pTM015	pJH0210-based plasmid for the expression of <i>accACDB_{Ec}</i>	The expression cassette included an operon containing P_{em7} , RBS (TIR: 9830.55), <i>accA_{Ec}</i> , RBS (TIR: 11644.13), <i>accC_{Ec}</i> , RBS (TIR: 10352.73), <i>accD_{Ec}</i> , RBS (TIR: 9318.34), <i>accB_{Ec}</i> , and T_{tonB} . This will integrate into the TGI site. The <i>E. coli accACDB</i> was codon optimized using Basebuddy with the <i>P. putida</i> KT2440 codon usage. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid was synthesized and sequence-verified by Twist Biosciences. The sequence of the expression cassette is listed below.	This study
pTM017	pJH0419-based plasmid for the expression of <i>plsBC_{Ec}cdsA_{Ec}</i>	The expression cassette included an operon containing P_{tac} , RBS (TIR: 12736.66), <i>plsB_{Ec}</i> , RBS (TIR: 12571.37), <i>plsC_{Ec}</i> , RBS (TIR: 12845.03), <i>cdsA_{Ec}</i> , and T_{tonB} . This will integrate into the Bxb1 site. The <i>E. coli plsBC</i> and <i>cdsA</i> were codon optimized using Basebuddy with the <i>P. putida</i> KT2440 codon usage. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid was synthesized and sequence-verified by Twist Biosciences. The sequence of the expression cassette is listed below.	This study
pTM018	pJH0419-based plasmid for the expression of <i>plsBC_{Pp}cdsA_{Pp}</i>	The expression cassette included an operon containing P_{tac} , RBS (TIR: 12373.93), <i>plsB_{Pp}</i> , RBS (TIR: 14370.80), <i>plsC_{Pp}</i> , RBS (TIR: 13749.52), <i>cdsA_{Pp}</i> , and T_{tonB} . This will integrate into the Bxb1 site. Genes encoding <i>plsBC</i> and <i>cdsA</i> were amplified from the <i>P. putida</i> KT2440 genome using the primers listed in Table S2. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid backbone was amplified with oTM035 and oTM036 from pJH419. The plasmid was sequenced verified with Oxford Nanopore. The sequence of the expression cassette is listed below.	This study

pTM044	pJH0210-based plasmid for the expression of <i>gpsA_{Ec}</i>	The expression cassette included an operon containing P _{tac} , RBS (TIR: 11274.39), <i>gpsA_{Ec}</i> , and T _{tonB} . This will integrate into the TG1 site. The <i>E. coli</i> <i>gpsA_{Ec}</i> was codon optimized using Basebuddy with the <i>P. putida</i> KT2440 codon usage. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid was synthesized and sequence-verified by Twist Biosciences. The sequence of the expression cassette is listed below.	This study
pTM045	pJH0210-based plasmid for the expression of <i>gpsA_{Pp}</i>	The expression cassette included an operon containing P _{tac} , RBS (TIR: 12388.56), <i>gpsA_{Pp}</i> , and T _{tonB} . This will integrate into the TG1 site. The native sequence of the <i>P. putida</i> <i>gpsA_{Ec}</i> was utilized. The RBS was optimized using the Salis optimization tool as described in the methods. The plasmid was synthesized and sequence-verified by Twist Biosciences. The sequence of the expression cassette is listed below.	This study

Table S6. Sequences for mNeonGreen, tags, and codon-optimized genes. Key: **Promoter**, **RBS**, gene, **linker**, **tag**, and **terminator**

Plasmid expression cassette	Sequence
pRW75 - <i>P_{lac}:ompA_{EC}</i> - Spycatcher003:m NeonGreen- Spytag003	<p> TTGCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT GTGGAATTGTGAGCGGATAACAATTTACACCGCACGATTATTAAGGAGCA AATTTTTATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTC GCTACCGTAGCGCAGGCCGCTCCGAAAGATAACACCTGGTACACTGGTGCT AAACTGGGCTGGTCCCAGTACCATGACACTGGTTTCATCAACAACAATGGCC CGACCCATGAAAACCAACTGGGCGCTGGTGCTTTTGGTGGTTACCAGGTTA ACCCGTATGTTGGCTTTGAAATGGGTACGACTGGTTAGGTCGTATGCCGTA CAAAGGCAGCGTTGAAAACGGTGCATACAAAGCTCAGGGCGTTCAACTGAC CGCTAAACTGGGTTACCCAATCACTGACGACCTGGACATCTACACTCGTCTG GGTGGTATGGTATGGCGTGCAGACACTAAATCCAACGTTTATGGTAAAAACC ACGACACCGGCGTTTCTCCGGTCTTCGCTGGCGGTGTTGAGTACGCGATCA CTCCTGAAATCGCTACCCGTCTGGAATACCAGTGGACCAACAACATCGGTGA CGCACACACCATCGGCACTCGTCCGGACAACGGCATGCTGAGCCTGGGTGT TTCCTACCGTTTTCGGTGGCGGTGGGGTTCGTAACCACCTTATCAGGTTTA TCAGGTGAGCAAGGTCCGTCCGGTGATATGACAACTGAAGAAGATAGTGCT ACCCATATTAAATTCTCAAACGTGATGAGGACGGCCGTGAGTTAGCTGGTG CAACTATGGAGTTGCGTGATTCATCTGGTAAAACTATTAGTACATGGATTTC GATGGACATGTGAAGGATTTCTACCTGTATCCAGGAAAATATACATTTGTCGA AACCGCAGCACCAGACGGTTATGAGGTAGCAACTCCAATTGAATTTACAGTT AATGAGGACGGTCAGGTTACTGTAGATGGTGAAGCAACTGAAGGTGACGCT CATACTGGTCACCATCACCATCACCATAAGATATCTTTCACACCCGTAATC TATTTCAATTTTAAGGAGGTTTAATATGGTGAGCAAGGGCGAGGAGGATA ACATGGCCTCTCTCCAGCGACACATGAGTTACACATCTTTGGCTCCATCAA CGGTGTGGACTTTGACATGGTGGGTGAGGGCACCAGGCAATCCAAATGATGG TTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCC TGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACC CTGACGGGATGTCGCCTTTCCAGGCCGCGATGGTAGATGGCTCCGGATACC AAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTAACTA CCGCTACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCAGGTGAAGG GGACTGGTTTCCCTGCTGACGGTCTGTGATGACCAACTCGCTGACCGCTG CGGACTGGTGACGGTCAAGAAAGACTTACCCCAACGACAAAACCATCATCA GTACCTTTAAGTGGAGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCA CTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGA AGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGA CCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATGTGATGGGCA TGGACGAGCTGTACAAGGCTAGCGGAGGAAGCGGAGGTACCCGTGGCGTT CCTCATATTGTTATGGTGGACGCCTACAAACGCTATAAATAAATTCAGGACG AGCCTCAGACTCCAGCGTAACTGGACTGAAAACAACTAAAGCGCCCTTG GCGCTTTAGTTTT </p>
pTM007 - <i>P_{lac}:mNeonGreen</i>	<p> TTGCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT GTGGAATTGTGAGCGGATAACAATTTACACCGTAATCTATTTCAATTTTAA GGAGGTTTTAATAATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCT CCCAGCGACACATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTT GACATGGTGGGTCAGGGCACCGGCAATCCAAATGATGGTTATGAGGAGTTA </p>

	AACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTC CCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGT CGCCTTTCCAGGCCGCGATGGTAGATGGCTCCGGATACCAAGTCCATCGCA CAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTAACTACCGCTACACCTA CGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGGACTGGTTTCC CTGCTGACGGTCCTGTGATGACCAACTCGCTGACCGCTGCGGACTGGTGCA GGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGTG GAGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCACTGCGCGGACCAC CTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACCAGCCGATG TACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGACCGAGCTCAACTTCA AGGAGTGGCAAAAGGCCTTTACCGATGTGATGGGCATGGACGAGCTGTACA AGGCTAGCGGAGGAAGCGGAGGTACCCATCATCATCATCATTAAGATAT CATTGAGGACGAGCCTCAGACTCCAGCGTAACTGGACTGAAAACAACTAAAGCGCCCTTGTGGCGCTTTAGTTTT
pTM008 - P _{lac} :VNp- mNeonGreen	TTGCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT GTGGAATTGTGAGCGGATAACAATTTACACCGGTAATGATTTCAATTTAA GGAGGTTTTAATAATGGATGTATTCATGAAAGGACTTTCAAAGGCCAAGGAG GGAGTTGTGGCAGCTGCTGAGAAAACCAAACAGGGTGTGGCAGAAGCAGCA GGAAAGACAAAAGAGGGTGTCTCAGATCTGGAGGAAGCGGAATGGTGAGC AAGGGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACAC ATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACC GGCAATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTG ACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCA TCAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCGATGGT AGATGGCTCCGGATACCAAGTCCATCGCACAAATGCAGTTTGAAGATGGTGC CTCCCTTACTGTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGA GAGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCTGTGATGACC AACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAAC GACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGCA AGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGG CGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGC TCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTAC CGATGTGATGGGCATGGACGAGCTGTACAAGGCTAGCGGAGGAAGCGGAG GTACCCATCATCATCATCATTAAGATATCATTGAGGACGAGCCTCAGACT CCAGCGTAACTGGACTGAAAACAACTAAAGCGCCCTTGTGGCGCTTTAGTTT
pTM044 - P _{lac:gpsA_{Ec}}	GAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGG ATAACAATTTACACGATTAAGATATTTAAGGAAGCTACAAAATGAACCAAC GGAACGCCTCAATGACTGTGATCGGTGCAGGCTCGTATGGCACCGCTCTTG CCATCACCTGGCAAGAAATGGCCACGAGGTTGCTCTGCGGGCCATGACC CTGAGCATATCGCAACCCTTGAACGCGACCGCTGTAACGCCGCCTTTCTTCC CGATGTGCCTTTCCCGGATACCCTCCACCTTGAAGCGACCTGGCCACTGC CTTAGCTGCCAGCCGTAATATCCTCGTCGTACCCAGCCATGTCTTTGGG GAGGTGCTGCGCCAGATTAAGCCCTTGATGCGTCCTGATGCGCGCTGCGGT TGGGCCACAAAAGGGCTAGAAGCAGAAACCGGCCGTTTGTACAGGACGTG GCCCGAGAGGCCTTAGGCGATCAAATTCCGCTGGCGGTAATCTCTGGCCCA ACGTTTGCGAAAGAACTGGCCGAGGTTTACCGACAGCCATTTGCTGGCC TCGACCGATCAGACCTTTGCCGATGATCTGCAGCAGCTGCTCCACTGCGGC AAGAGTTTCCGCGTTTACAGCAATCCGATTTTATTGGCGTGCAATTGGGCG GCGCGGTGAAAACGTTATTGCCATTGGTGCGGGGATGTCCGACGGTATAG GTTTTGGTGCCAATGCGAGGACGGCCCTGATAACCCGTGGATTGGCCGAAA

	<p>TGTCCAGACTTGGTGCGGCGCTTGGTGCCGATCCAGCTACCTTTATGGGCA TGGCTGGACTTGGCGATCTGGTGCTGACCTGTACCGACAACCAGTCGCGTA ACCGCCGTTTTGGCATGATGCTGGGTGAGGGCATGGATGTACAGAGCGCGC AGGAGAAGATTGGCCAGGTGGTGGAAGGCTACCGCAATACGAAGGAAGTCC GCGAACTGGCGCACCGCTTTGGCGTTGAGATGCCAATCACCGAGGAGATCT ACCAAGTGTGTACTGCGGGAAGAACGCTCGCGAAGCAGCATTGACCTTAC TGGGTCGAGCACGCAAGGACGAGCGCAGCAGCCACTGAAGTCAAAGCCT CCGACCGGAGGCTTTTGA</p>
pTM045 - P _{tac:gpsA_{Pp}}	<p>GAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGG ATAACAATTTACACTAAGCGAGTCAACATAAGGGGGTACGGGATGACTGA ACAGCAACCTGTTGCGGTTCTGGGCGGCGGCAGCTTCGGCACCGCCGTGG CAAACCTGCTGGCGGAAAACGGTGTGCCGGTGCGCCAATGGATGCGCGAC CCGGCGCAGGCCGAGGCCATGCGTGTGAATCGCGAGAATCCGCGCTATCT CAAGGGTATCCGCCTGCACGATGGGGTTGAGCCGGTCAACGACTTGCTGGC CACCCTGCAGGCCAGCGAGCTGATCTTCGTTGCCCTGCCATCGAGTGCCTT GCGCAGCGTGCTGGCACCGCATGCCGAGCTGCTGCGCGGCAAGGGCCTG GTCAGCCTGACCAAGGGCATCGAAGCGCAAAGCTTCAAGCTGATGAGCCAG ATCCTCGAAGAGATCGCCCCGAGGCGCGCATCGGCGTTTTGTGCGGGCC CAACCTGGCGCGCGAAATCGCCGAACATGCGCTGACTGCCACAGTGGTGG CCAGTGAGCACGAAGACCTGTGCCAGCAGGTACAGGCCGTACTGCACGGG CGCACCTTCCGCGTCTACGCCAGTGCCGACCGTTTCGGCGTCAACTGGGC GGTGCACTGAAGAACGTCTACGCGATCATCGCCGGCATGGCGGTGGCCTTG GGGATGGGCGAGAACACCAAGAGCATGCTGATTACCCGGGCTTGCCGA GATGACCCGTTTCGCCGTGAGCCAGGGCGCCAACCCCATGACGTTCTCGG CCTGGCCGGTGTGCGGTGACCTGATCGTCACCTGCTCCTCGCCCAAGAGCCG CAACTATCAGGTTGGCTACGCACTGGGGCAGGGCCAAAGCCTGGAAGAGG CGGTCAACCGCCTGGGCGAAGTGGCCGAGGGGGTCAACACGCTCAAGGTG CTCAAGACCAAGGCCAGCAAGTGCAGGTGTACATGCCGCTGGTGGCTGG CCTGCATGCCATCCTGTTTGAAGGGCGCACGCTGAACCAGGTGATCGAGCA CCTGATGCGCGCCGAGCCCAAGACCGATGTCGATTTTCAATTCATCAGCGG TTTCAACTGAAGTCAAAGCCTCCGACCGGAGGCTTTTGA</p>
pTM017 - P _{tac:plsBC_{Ec}cdsA_{Ec}}	<p>TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACA ATTTACACTCAACCTTTTAAGGAGGCTTTGATGTCGGGCTGGCCACGAAT TTATTACAAATTGCTGAATCTGCCATTGTCCATCCTGGTCAAAGCAAGTCGA TTCCGGCCGATCCAGCCCCGGAAGTGGGGTTGGACACCTCACGTCCAATCA TGTACGTCTTGCCGTACAACCTCGAAAGCCGACCTCTTGACGTTGCGCGCCC AGTGCTTGGCGCATGACTTGCCAGACCCGTTAGAGCCGTTGGAATCGATG GCACGCTCTTGCCCTCGGTACGTGTTTATCCACGGCGGGCCGCGTGTGTTCA CCTATTACACGCCGAAAGAAGAGAGTATCAAAGTGTTCACGACTATTTGGA CTTGACCGTAGCAACCCAAATCTGGATGTGCAGATGGTGCCAGTGTGCGG GATGTTTGGTAGGGCTCCTGGGCGTGAGAAAGGCGAAGTGAACCCGCCGC TGCGAATGTTGAACGGCGTCCAGAAGTTTTTCGCGGTCTTGTTGGGTC GCGACTCGTTCTGCGGTTCTCGCCGTGAGTTTCGCTGCGCCGTATGGCCG ACGAACACGGCACGGACAAAACGATAGCACAGAACTGGCCCGAGTGGCC CGTATGCACTTTGCCCCGCAACGCTTAGCAGCCGTAGGCCCAAGGTTGCCT GCTCGTCAGGACCTGTTCAATAAGCTGCTAGCCTCCAGGGCCATTGCCAAA GCGGTAGAAGATGAAGCGCGCTCCAAAAAATCTCGCATGAAAAAGCCCAA CAGAACGCGATCGCACTGATGGAAGAAATCGCGGCGAATTTCTCCTACGAA ATGATCCGCTTGACTGACCGTATTCTGGGCTTACCTGGAACCGACTTTACC AGGGCATAAACGTCCATAACGCAGAGCGGTTGCGCCAGTTGGCCACGACG GGCATGAGCTGGTCTATGTGCCATGCCACCGCTCCACATGGACTACCTGC TGTTGTCGTACGTGCTATACCACAGGGTTTGGTGCTCCGCATATCGCAGC CGGTATCAACCTCAATTTCTGGCCTGCGGGGCGGATTTTCCGCCGCTGGG TGCTTCTTCAATAGGCGTACGTTCAAAGGCAACAACTCTATTGACCGTTT</p>

	<p> TCCGGAATACCTCGGCGAACTGTTTCAGCCGCGGTTACTCGGTTCGAGTACT TCGTGGAAGGCGGGCGGTCCAGGACTGGTCGACTACTGGACCCGAAAAC GGTACGTTGTCGATGACCATCCAGGCCATGTTGCGTGGCGGTACGCGCCCG ATTACGTTGATCCCGATCTACATCGGCTACGAACATGTCATGGAAGTGGGCA CTTACGCCAAAGAACTGCGAGGCGCCACCAAAGAGAAGGAAAGCCTGCCGC AGATGCTGCGCGGCTTAAGCAAACCTGCGTAACTTGGGTACGGGTACGTCA ACTTCGGTGAACCAATGCCGCTTATGACGTAATGAACCAAGCATGTCCAGCA CTGGCGCGAATCAATCGACCCTATCGAAGCCGTCCGTCTGCATGGTTGAC GCCGACGGTCAACAATATTGCAGCCGATCTCATGGTCCGCATTAACAACGCA GGAGCGGCAAACGCCATGAACCTGTGCTGCACTGCGCTTCTGGCATCACGT CAGCGCTCATTGACCCGCGAGCAATTGACCGAACAACCTTAAGTGTACCTG GATCTGATGCGAAATGTGCCTTACTCCACGGAATCCACCGTCCCATCCGCCT CAGCCAGCGAACTTATCGACCACGCCCTTCAAATGAACAAGTTCGAAGTCGA AAAAGACACGATCGGCGACATCATCTTCTGCCTAGGGAACAAGCCGTGCT CATGACCTACTACCGCAACAACATCGCGCACATGTTGGTGTCTTCTTCGTA ATGGCGGCAATCGTGACCCAGCACCGGCACATCTCGCGCGACGTCTTATG GAACACGTCAATGTGTTGTACCAATGCTGAAAGCGGAGCTGTTCTTCGAT GGGATCGCGACGAATTGCCGGACGTGATTGATGCGCTGGCAAATGAGATGC AACGGCAGGGTCTGATCACCTGCAAGATGACGAGTTGCATATTAACCCGG CCCATAGTAGGACGCTTCAGCTGTTGGCCGAGGTGCGCGCGAAACGCTG CAACGCTATGCCATCACCTTCTGGTTGTTGAGTGCCAACCCGTCGATCAACA GGGGGACGCTGGAGAAAGAAAGCAGAACCGTCGCCCAACGTCTCTCGGTG TTGCACGGCATCAACGCGCCGAGTTCTTCGACAAGGCGGTGTTCTCGTCG CTGGTGCTGACGCTGCGTGACGAGGGTTATATCAGCGATAGCGGCGATGCC GAACCGGCAGAAACGATGAAAGTCTATCAGTTGCTGGCCGAATTGATCACTT CCGACGTGCGTTTGACGATCGAATCCGCGACGCAAGGCGAAGGTTGATCG CCACTACCTAGGAGGTGCCAAATGCTTTACATCTTCCGCTTGATCATCACC GTGATCTACTCGATCTTGGTCTGCGTATTTCGGCTCGATTTACTGCTTGTTCA GCCCCGCGCAACCCGAAACACGTGGCCACCTTTGGTCACATGTTTCGGTCGCT TGGCCCCGCTGTTTCGGCCTGAAAGTGAATGCCGCAAACCAACGGACGCG GAAAGCTACGGCAACGCGATCTACATCGCGAACCACCAGAACAACTACGAC ATGGTGACTGCGTCGAACATCGTGCAACCGCCGACGGTGACGGTCGGCAAA AAGAGCTTGCTGTGGATACCTTTCTTCGGTCAGTTGTACTGGTTGACCGGCA ACTTGTTGATCGACAGGAACAACCGCACGAAAGCGCACGGCACCATCGCGG AAGTCGTGAACCACTTCAAAAAACGCCGCATCTCCATCTGGATGTTCCCGGA AGGTACCCGCGAGCCGTGGTCGCGGTCTGCTTCCGTTCAAACGGGTGCATT CCACGCGGCAATTGCGGCCGGCGTCCCGATTATTCCTGTGTGCGTCTCGAC TACGTCGAATAAAATCAACTTGAACCGACTGCACAACGGCCTGGTGATCGTC GAAATGCTGCCGCCAATCGACGTCTCGCAGTATGGCAAAGACCAGGTCCGC GAACTGGCGGCCCACTGCCGTTTCGATAATGGAACAAAAAATCGCCGAATTG GACAAAGAAGTCGCAGAACGCGAAGCCGCCGGGAAAGTCTGCAACGTT CCTGAGGAGTCCCAAACATGCTGAAATACCGCCTGATATCGGCGTTCGTGT TGATACCTGTCGTCATCGCCGCCTTGTTTCTGCTCCCGCCGGTGGGTTTCG CCATTGTCACGCTCGTGGTCTGCATGTTGGCAGCCTGGGAATGGGGGCACT TGAGCGGTTTTCAGACGCGCTCGCAGAGGGTATGGTTGGCCGTGTTGTGCG GTTTATTGTTGGCCTTGATGCTCTTCCTGTTGCCGGAATATCACCGAAACATC CATCAACCGCTGGTCGAAATCTCCTTGTTGGGCATCGCTGGGCTGGTGGATC GTCGCCCTTTTGTGGTGTGTTCTACCCAGGTTCCGGCGGCAATCTGGCGT AACTCGAAAACGTTGCGCTTGATCTTCGGCGTGCTTACCATCGTCCCTTTCT TCTGGGGCATGCTGGCCCTCCGGGCCTGGCACTATGACGAAAACCACTACT CGGGCGCGATATGGCTGTTGTACGTCATGATCCTGGTATGGGGCGCGGACT CCGGCGCGTACATGTTTCGGCAAATTGTTTCGGTAAACACAACTGGCACCGA </p>
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	AGGTGTCGCCGGGCAAACCTGGCAAGGCTTCATCGGTGGGCTCGCAACT GCAGCGGTAATCTCGTGGGGCTACGGCATGTGGGCCAATTTGGACGTGCA CCTGTCACCTTGTTGATCTGCTCGATTGTCGCGGCCTTGGCCTCCGTGCTC GGCGATCTGACCGAATCGATGTTCAAACGCGAAGCGGGGATCAAAGACAGC GGCCACTTGATTCCAGGGCACGGTGGCATCCTCGACCGCATTGATAGCCTG ACGGCTGCCGTACCGGTCTTTGCATGCTTGTGCTTCTGGTATTACAGGACGT TGTGAAGTCAAAGCCTCCGACCGGAGGCTTTTGA
pTM018 - <i>P_{tac}:pIsBC_{Pp}cdsA_{Pp}</i>	TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACA ATTTACACTAGAACCTCTATAAGAGAAGTTAGCATGACCCGTTCCCCCT GCACCGCTGATATTCGGCGGCCTGCGTCGCCTGTTGTACCTGTGGGTGCG CTCCGAGACCATCAACCAGTCTTCCATGACCCTCAACCTCGACCGCAGCCG GCCGGTGTCTATGCGTTGCCCTCGCCGCGCTCACCGACCTGGCCGTGCT CGACCACGAGTGACCAAGGCGGGGCTGCCGCGCCCGGTGCTGCCGGTA GCCGTGCGCCCACTGCAGGAACCCGCGCGTTCTTCTACCTGACCCCCGAC CCGGA CTGGCTCGGCCGCCAGGACAAAAGCGGCGCCCGCCACGCTCGA GCGCCTGGTGGCTGCCGTCAGCCAGCATGCCGAAGAAGATGCGCAGATCA TTCCTGTCAGCGTGTCTGGGGCCAGACCCGGCCAGCGAGTCCAGCCCCT GGAAGCTGCTGTTGCGCCGACAGCTGGGCGGTAACCGGGCGCCTGCGCCGG CTGCTGACCGTACTGATCCTGGGGCGCAAGACCCGGGTACAGTTCTCCGCG CCAATCCACCTGCGCGAACTGGTGCAGCACAACAAAGGCCACGAGCGCACC GTGCGCATGGCCCAGCGCCTGATGCGCGTGCACTTTGCAACCTCAAGACT GCCGTATCGGCCCGGACATCTCGCACCGGCGCACCCCTGGTCAAAGGCCT GGTCCATGCCCCGCAAGTGCGCCAGGCGATTGCCGACGAAGCTCAGCGCG AGAACCTGCCGCTGGCCAAAGCCGAGGCCAGGCACTGCGCTATGGCAAC GAGATAGCCTCGGACTACACCTATACCGCCATCCGCTTCTC GAAGTGGTG CTCAGCTGGTTCTGGAACAAGATCTACGACGGCATCAAGGTCAACCACATTG AACAGGTGCAGGGTATCGCCCCGGCCATGAAGTGATCTACGTGCCATGCC ACCGCAGCCACATCGACTACCTGCTGCTGTCGTACCTGCTGTTCCGCAACG GCCTCACCCCGCCGCACGTGGCTGCAGGCATCAACCTCAACATGCCGGTG GTCGGTAACCTGCTACGCCGTGGTGGCGCCTTCTTCATGCGCCGCACGTT AAGGGCAACCCGCTTTACACGGCAGTGTTCAACGAGTACCTGCACACCCTG TACACCAAGGGCTTCCCGGTGCGAGTACTTCGTGGAAGGCGGCCGCTCGCG CACCGGGCGCATGTTGCAGCCGCGCACCGGGATGCTGGCCATTACCCTGC GCAGCTTCTGCGTTGTCGCGTACGCCAATCGTGTTGTCGTCGGGTGTACA TCGGCTACGAGCGCGTGCTCGAAGGCCGTACCTACCTGGGCGAACTGCGC GGCGCCAGCAAGAAGAAGGAGTCGGTGTGGACATCTCAAGGTGTTGCGC GCACTCAAGCAGCGCTTTGGCCAGGTCTACGTCAACTTCGGCGAACCCATC CGCCTGGCTGGTTTCTCGACCAGCAGCAGCCCGGCTGGCGAGAACAGGA TCACGGTCCGCAGTACCGACCGGAATGGCTCAACGCCACCACCGCCCGCC TGGGAGAAACCGTGGCCCGCCACCTCAACGAGGCGGCCGCCATCAACCCG GTCAACCTGGTGGCCCTGGCACTGCTGTCCACCAGCCGCCTTGCCCTGGAC GAGCGCGCCCTGACCCGCGTACTCGACCTGTACCTGGCGCTGCTGCGCCA AGTGCCCTACTCGCCGCACACCACCCTGCCCGAGGGCGACGGCCAGGCAC TGATCGAACATGTGCGCAGCATGAACCTGGTGGCCGAGCAGAAGGACGCC CTGGGGCCGCATCCTCTACCTGGATGAAGGCAACGCGGTGCTGATGACCTAC TACCGCAACAACGTGCTGCACATCTTCGCCCTGCCGGCGCTGCTGGCCAGC TTCTTCTCAGCAGTTCCCGCATGAGCCGCCAACTGCTGGGCCAGTATGTG CATGCGCTTTATCCCTATCTGCAGGCCGAAGTGTTCCTGCGCTGGACGCCA GAACAGCTGGACGAGGTCATCGACCAATGGCTGGTTCGCGCTGGTGGAACA GGGCCTGCTGCGCCAGGACAACGACCTGTACGTGCGCCCGGCGCCAGCT CGCGGCAGTTCGTGTTGCTGACCCTGCTCGCCCGCACCATCACCCAGACCC

	<p> TGCAGCGCTTCTACATGGCAACCTCGCTGCTGATCAACAGCGGGCAGAACA GCCTGAGCGCCGAAGCGCTGGAAGACCTGTGCGTGATGATGGCCCAGCGC CTGTGATCCTGCATGGCCTGAACGCACCGGAGTTCTTCGACAAGACGCTG TTCCGCCACTTCATTACAGACCTTGCTTCAGCAAGGCGTGCTGCACGCCGAC GCGCAAGGCAAGCTGAGTTATCACGACAAGCTCGGCGAGCTGGCCGAGGG CGTGGCCAAGCGAGTACTGTCGGCCGAGCTGCGCCTGTCGATCCGCCAGG TGGCCCTGCACCGTGACGACGGGCTGGAACTTCGACCCTCTAATCATCAA AACATAAGAGGTTTTTAATGCTTTATTTCATTGCGTATGTTCTGCTGGGGC TGCATTTTCTTGCCGTTGGCGCCGTGGGCCTGCTCATTGGCCTGTGCCGCC CCTTCAACCCTGACAACAGCCGCGTTTTTCGCCCGGCTCTACAGCTTGCCGG CCACCTGGCTGATGCGCATCGAGGTCAAGGCCGAAGTCGGCCCATTGTGG GACCACCCGCCCGGCTGCGTGATCGTGGCCAACCACAGTCCAACCTTCGAT CTGTTCTGCTGGGCCAAGTGGTGCCGCAGCGCACCGTCGCCATCGGCAA GAAGAGCCTGGGCTGGATCCCGCTGTTCCGCCAACTGTTCTGGCTGGGCG GCAACGTGCTGGTCGACCGCAAGAATGCCTATCAGGCGCGCAGGGCCTTAC AGAAGACCACCCGGGTTCTGCAGGACGACACCTCGATCTGGATTTTCCCG AAGGCACCCGCAATCCCGGCGAGCATCTGCTGGCGTTCAAGAAAGGCGCAT TTCACATGGCCATCGAGGCCGGTGTGCCGATCGTGCCGGTCTGCGTCAGCC GCTATGCCAGGCGCCTGAGCCTGAACAGCTGGCGCCAGCGCACGGTGATT GTGCGCTCGCTGCCGCCATTGCCACGACGGGCATGACGCTGCAGGACCT GCCAGCGCTGATCGAGCAATGCCGTGGGCAATGCAGCAGTGCAATGACCG CATGGAAAAGAACTGGCCTGATTTCTCTAAAGGAGGTTTTTTCATGCT TAAACAACGCATCATTACTGCGCTGATCCTGCTGCCGGTCGCGCTGGGTGG TTTCTTCTGCTCAATGGCGGGGATTTCGCCCTGTTTCATCGGCTTCGTAGTG ACCCTCGGTGCCTGGGAGTGGGCGCGCCTTGCCGGGCTGATGGCCCAGCC GCTGCGCATTGCCTATGCAGCGGTGGTCGCGGGGGCGCTGATGCTGCTGC ACATCCTTCCGGAAGTGGCGCCCTGGGTGCTGGGCGCTGCCGTGATCTGGT GGGGGCTGGCCACCTGGCTGGTGCTTACCTACCCGCGCAGCAGCGACCTG TGGGCCAGTGCGGCCTGTCGGTTGTTGATCGGCCTGCTGGTGTTGCTGCCG GCCTGGCAAGGGCTGGTGCTGCTCAAGCACTGGCCCTTGGGCAACTGGCT GATCCTGTCGGTCATGGTGCTGGTGTTGGGCCGCCGACATCGGTGCGTACTT CTCTGGCCGGGCATTGGCAAGCGCAAGCTGGCTCCGCAGGTCAGCCCAG GCAAGAGCTGGGAAGGCGGTACGGTGGTCTGGCGGTGAGCCTGTTGATTA CCCTGGGTGTCGGCATCAGCCGCGACTGGGGCTTTGGTCAGATCCTGCTG GGCCTGTTGGGCGCTGCGTTGCTGGTGATGTCCTCGGTGGTGGTGACCT GACCGAGAGCATGTTCAAGCGTCGCTCCGGCATCAAGGACAGCAGCAATCT GCTGCCCGGGCATGGGGGTGTGCTCGATCGCATTGACAGCCTGACTGCCG CAATCCCGATCTTCGCCGTGCTGTTGTGGGCTGCCGAATGGGGTGTGATGT GAAGTCAAAGCCTCCGACCGGAGGCTTTTGA </p>
pTM015 - <i>P_{em7}:accACDB_{Ec}</i>	<p> TTAATTAATTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATA CGACAAGGTGAGGAATAAACCCCTAGGGGTCCTCACCTAAGGAGGTCCC CAAATGTCGCTGAACTTCTTGGAATTCGAACAGCCGATCGCGGAAGTGGAA GCCAAAATCGACTCGCTGACGGCCGTGAGCCGCCAGGACGAAAAACTGGA CATCAACATCGACGAAGAAGTGACCCGCTGCGCGAAAAAAGCGTCGAACT GACGCGCAAAATCTTCGCCGACCTTGCGCGCTGGCAGATTGCCAACTGGC ACGCCATCCACAGCGCCCATACACCCTGGACTACGTCCGCCTGGCGTTTGA CGAATTCGACGAACTGGCAGGCGACCGCGCCTACGCGGACGACAAAGCAA TCGTGCGGTGGTATCGCCCGCTTGACGGTCCGCCGGTGATGATCATCGGCC ACCAAAAAGGCCGCGAAACCAAAGAAAAATCCGCCGCAACTTCGGCATGC CAGCCCCAGAAGGCTACCGCAAAGCACTGCGTCTGATGCAAATGGCGGAAC GCTTCAAAATGCCAATCATCACCTTCATCGACACCCCGGGTGCGTACCCAG </p>

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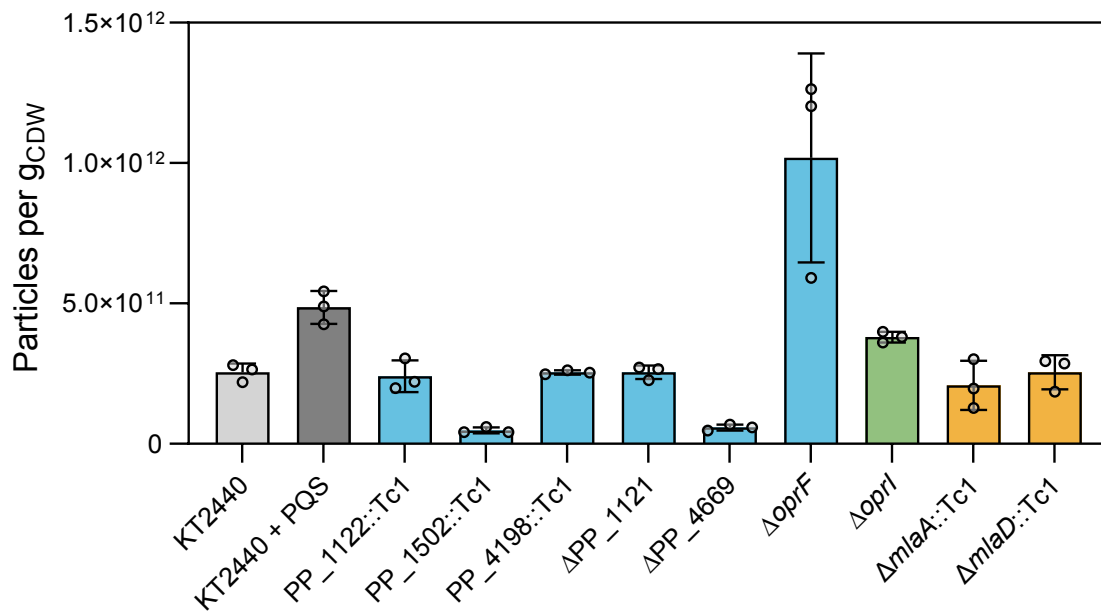


Figure S1. Particle count per g_{CDW} for KT2440 and knockout strains corresponding to data presented in **Fig. 1B**. The absolute counts are provided in **Excel file 1**. The data represent the mean ± the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate.

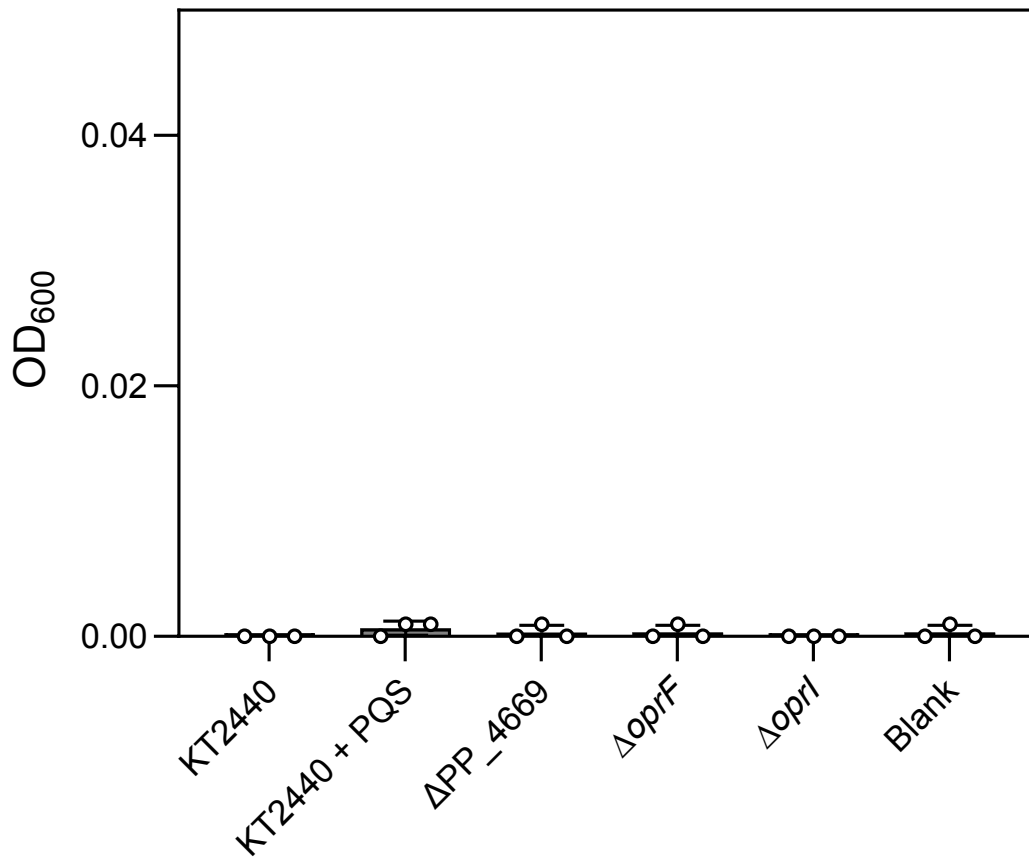


Figure S2. OD₆₀₀ measurements of extracted MVs from KT2440 and knockout strains. The blank consisted of ultrapure MiliQ water and was not significantly different than any of the MV extracts. The data represent the mean \pm the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate. Significant differences ($p < 0.05$) were determined with an unpaired two-tailed t -test between the strains and the blank.

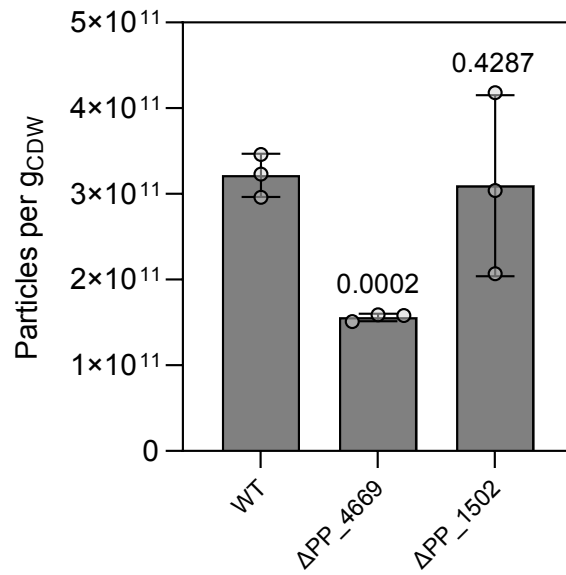


Figure S3. Particle count per g_{CDW} for WT, ΔPP_4669, and ΔPP_1502 grown on 20 mM glucose. The absolute counts are provided in **Excel file 1**. Significant differences ($p < 0.05$) were calculated using unpaired one-tailed *t*-test to test the hypothesis that the deletion strains decreased MVs production compared to WT. The data represent the mean \pm the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate.

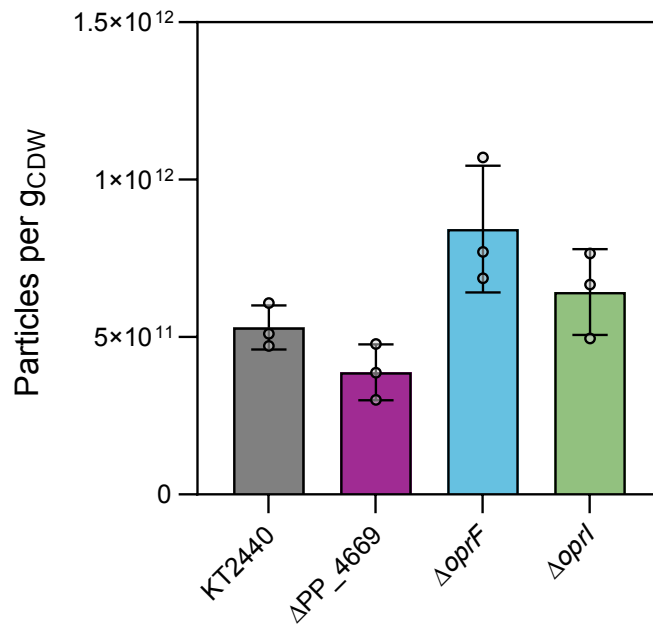


Figure S4. Particle count per g_{CDW} for KT2440 and knockout strains corresponding to data presented in **Fig. 3C**. The absolute counts are provided in **Excel file 1**. The data represent the mean \pm the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate.

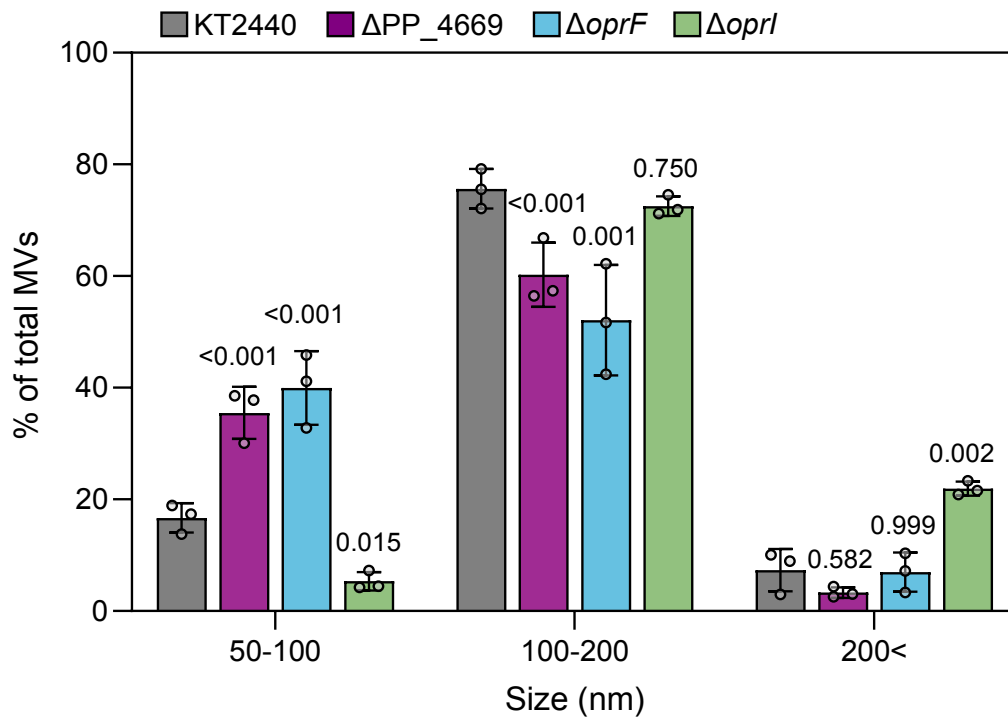


Figure S5. Sizes of MVs corresponding to particle counts in **Fig. S3** were binned into three size ranges and presented as a percentage of the total MV population. The data represent the mean \pm the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate. Exact p -values were determined by two-way ANOVA followed by Dunnett's multiple comparisons test to determine significant differences between the deletion strains and KT2440 in each size range.

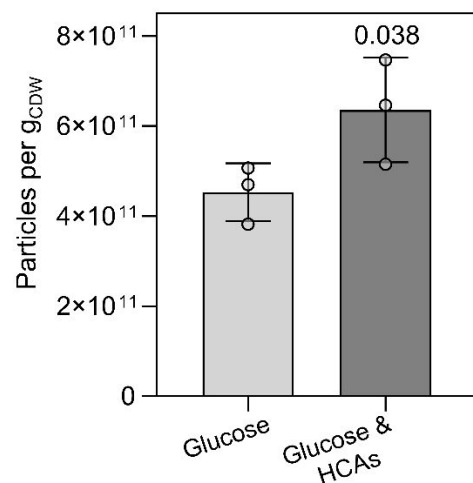


Figure S6. Particle count per g_{CDW} for KT2440 grown on 20 mM glucose alone or 20 mM glucose plus 12.5 mM p-coumarate and 12.5 mM ferulate. The absolute counts are provided in **Excel file 1**. The data represent the mean \pm the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate.

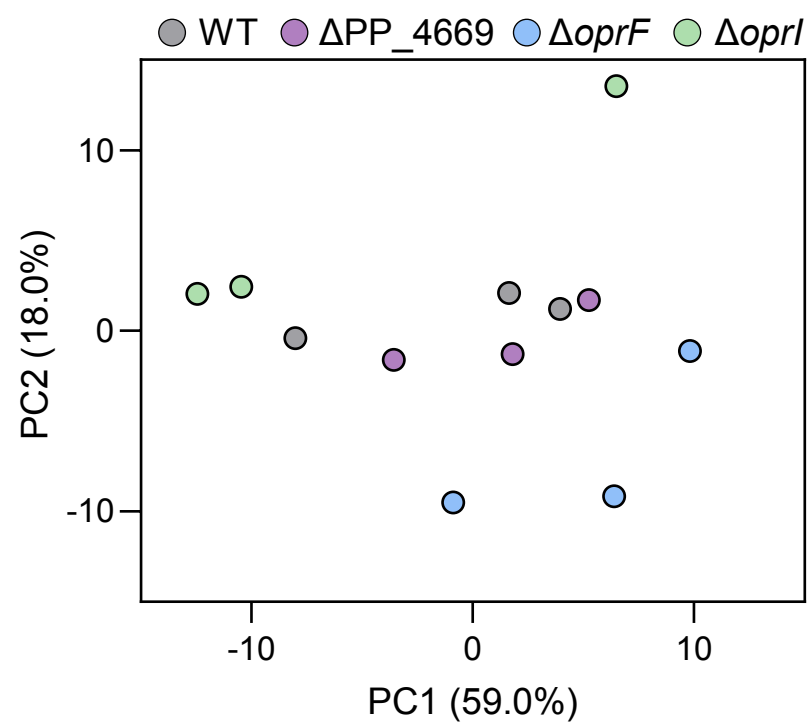


Figure S7. Principal component analysis of the cellular fractions.

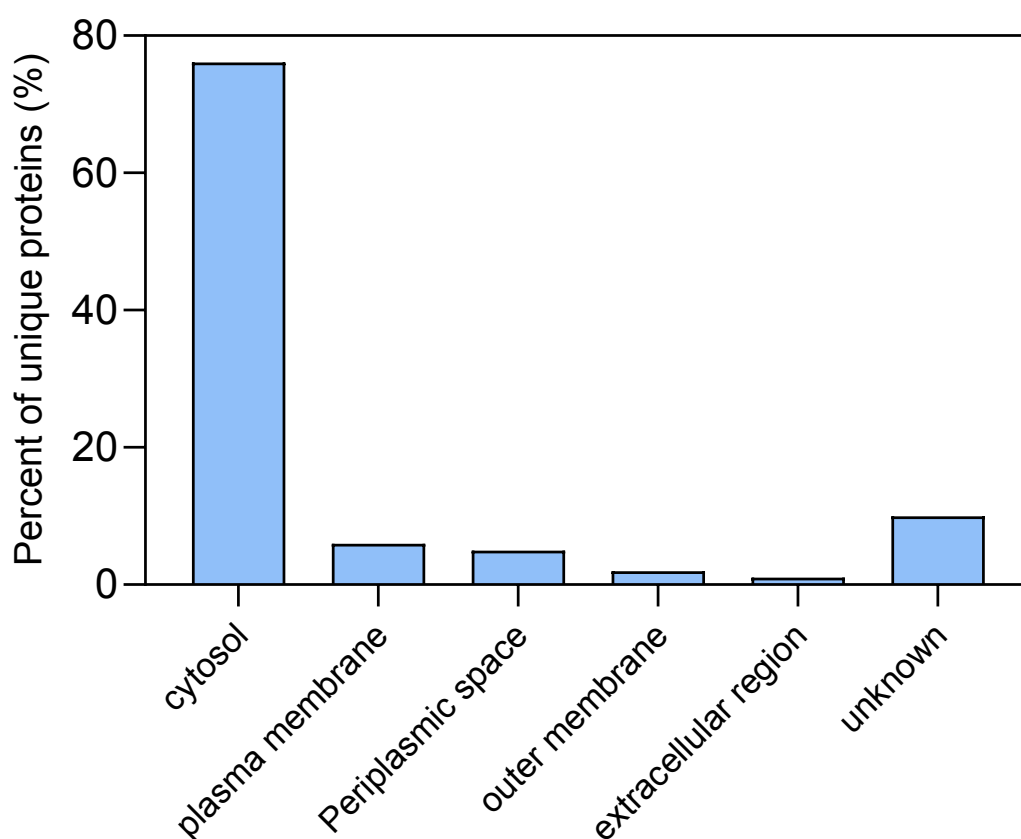


Figure S8. The percentage of proteins classified in a specific cellular location relative to the total number (201) of unique proteins found in the MV fraction for $\Delta oprF$ compared to the KT2440, $\Delta oprI$, and ΔPP_4669 . These proteins correspond to the 201 unique proteins found only in the MV fraction for $\Delta oprF$ illustrated in **Fig. 4E**.

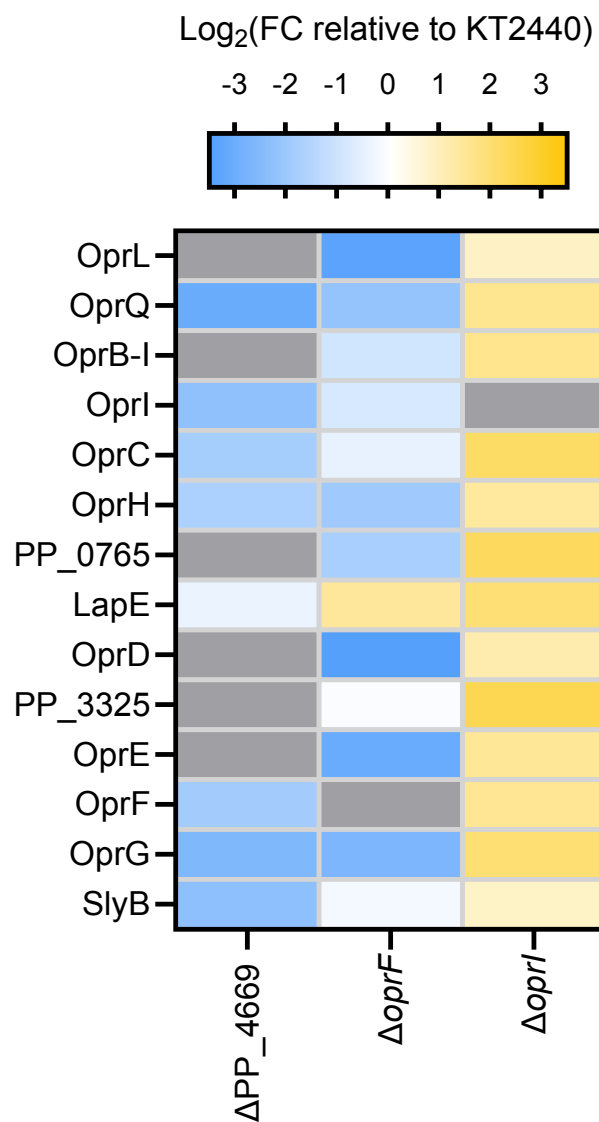


Figure S9. Heatmap of outer membrane proteins in MV fraction with differential abundance, or fold change (FC), for the knockout strains relative to KT2440. Data represents the mean of three biological replicates.

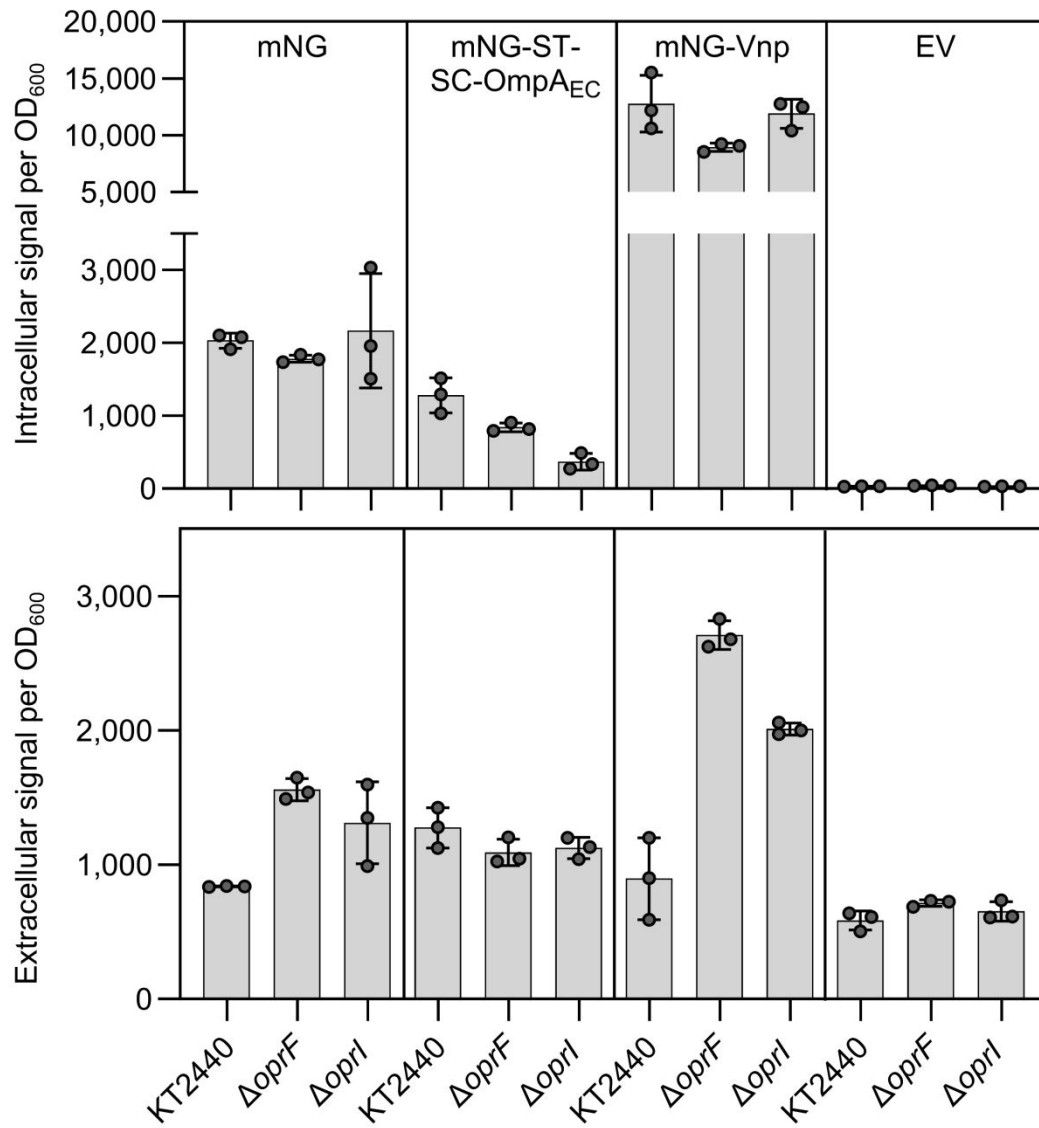


Figure S10. mNeonGreen (mNG) fluorescence signal for the cellular fraction and the extracellular fraction normalized by the OD₆₀₀ of the cell suspension. The data represent the mean \pm the standard deviation determined from three biological replicates. Individual points are illustrated for each biological replicate. Abbreviations include: ST, Spytag; SC, Spycatcher; EV, empty vector.

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