Supplementary Information for:

The DedA superfamily member PetA is required for the transbilayer distribution of phosphatidylethanolamine in bacterial membranes

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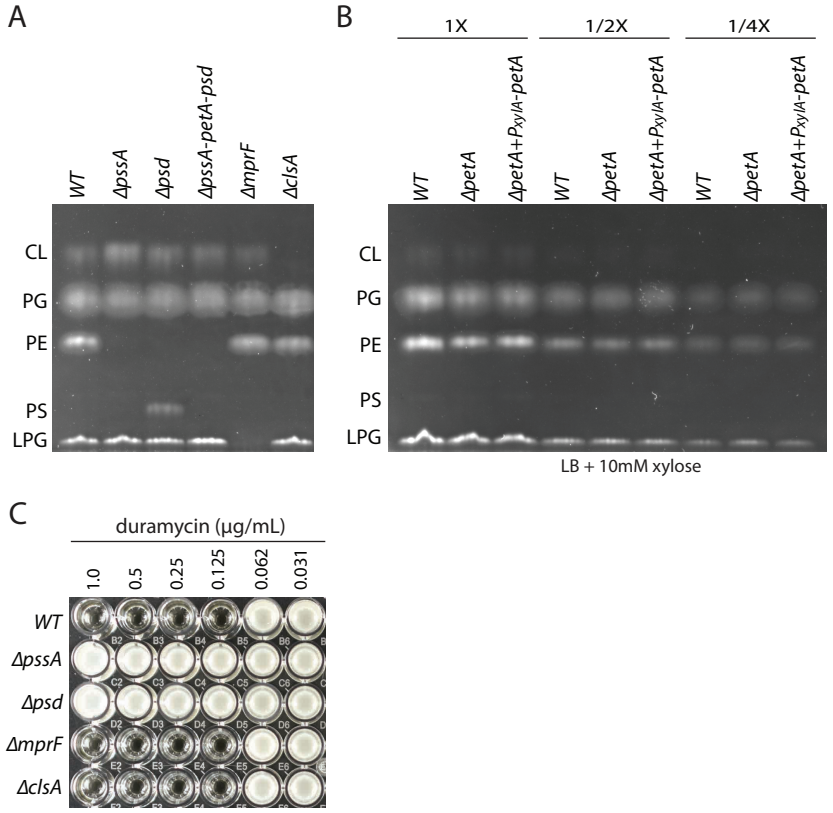
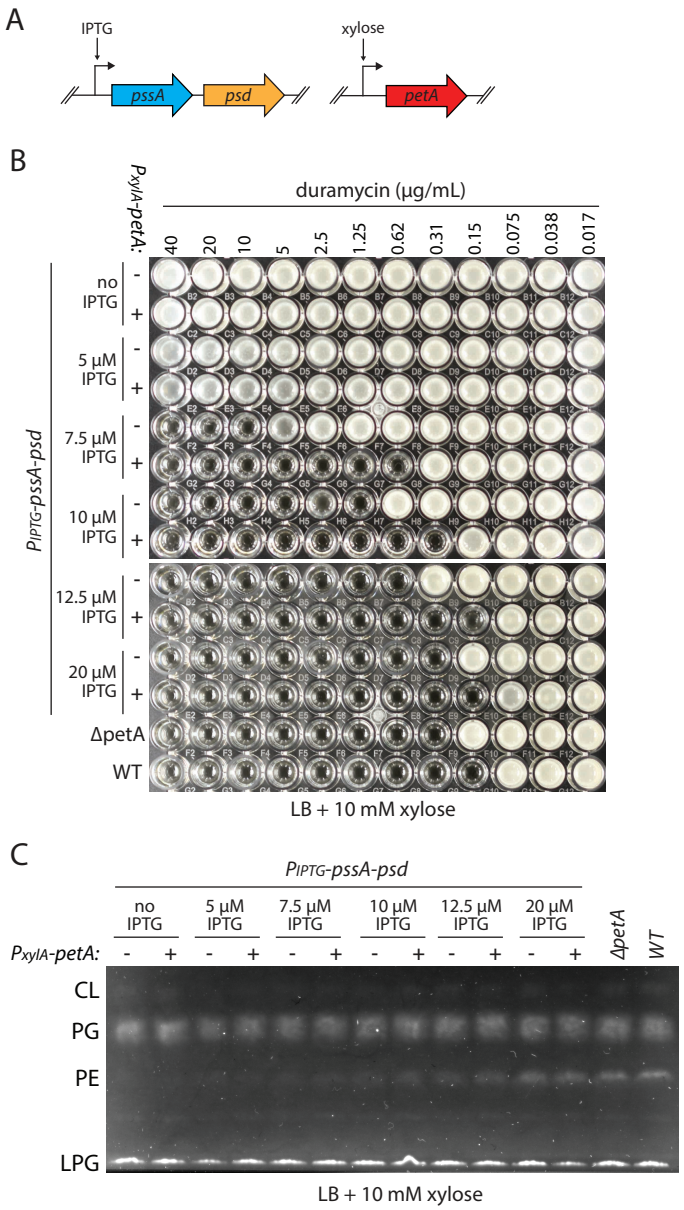
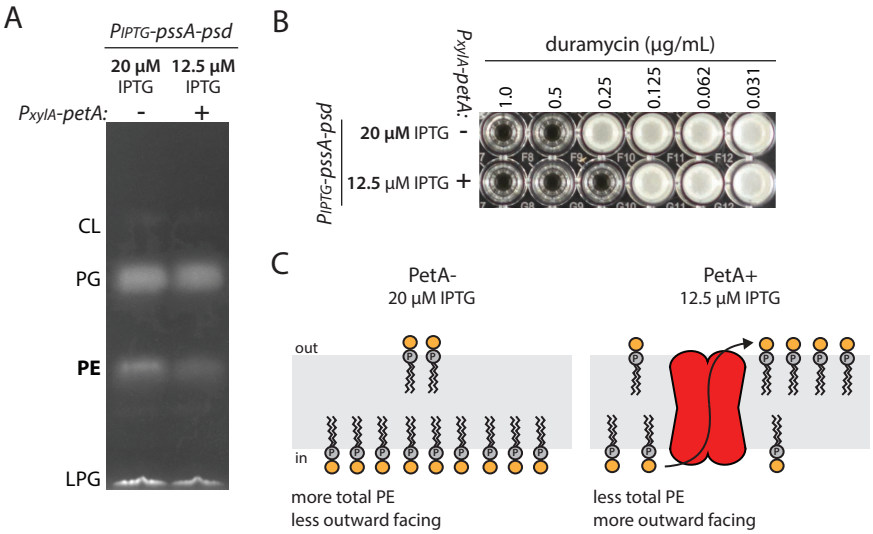
This PDF file includes:
Figures S1 to S7
Table S1 to S6
SI Materials and Methods
Source Data for Figure 3, S5
SI References



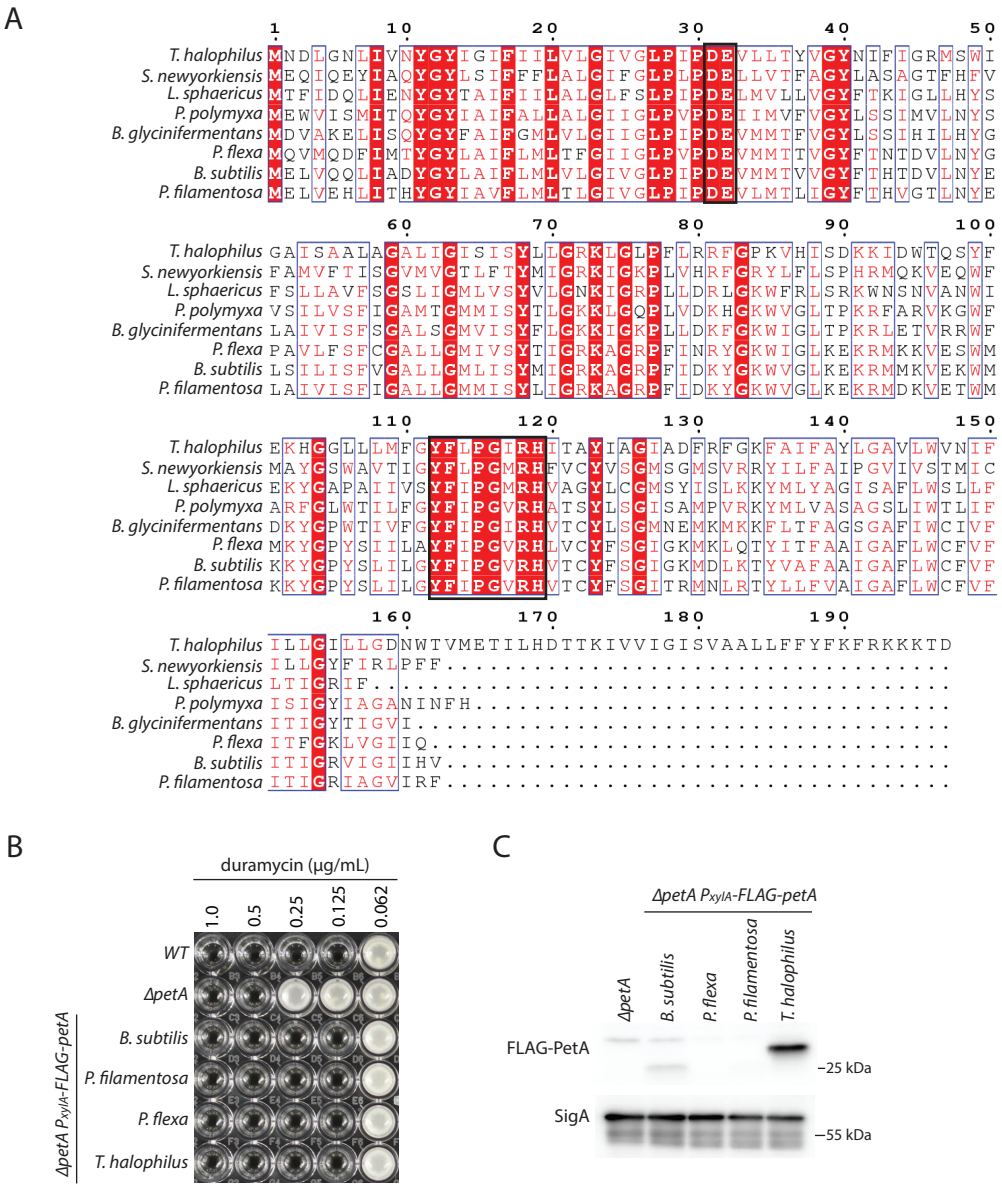
Supplemental Figure 1. PetA expression does not affect PE levels. (A) Validation of TLC assay. All major *B. subtilis* phospholipids can be identified by TLC analysis. Lipid extractions from *B. subtilis* strains with indicated mutations of non-essential phospholipid synthase genes were analyzed to define each species under the TLC conditions used thoughout this paper. **(B)** Two-fold serial dilutions of lipid extractions from wild-type, Δ*petA* and Δ*petA*+P*xylA-petA B. subtilis* strains were analyzed. All three strains have comparable levels of PE. **(C)** Representative duramycin MIC assay with the indicated *B. subtilis* strains confirming that lysylphosphatidylglycerol and cardiolipin do not affect the sensitivty to duramycin.



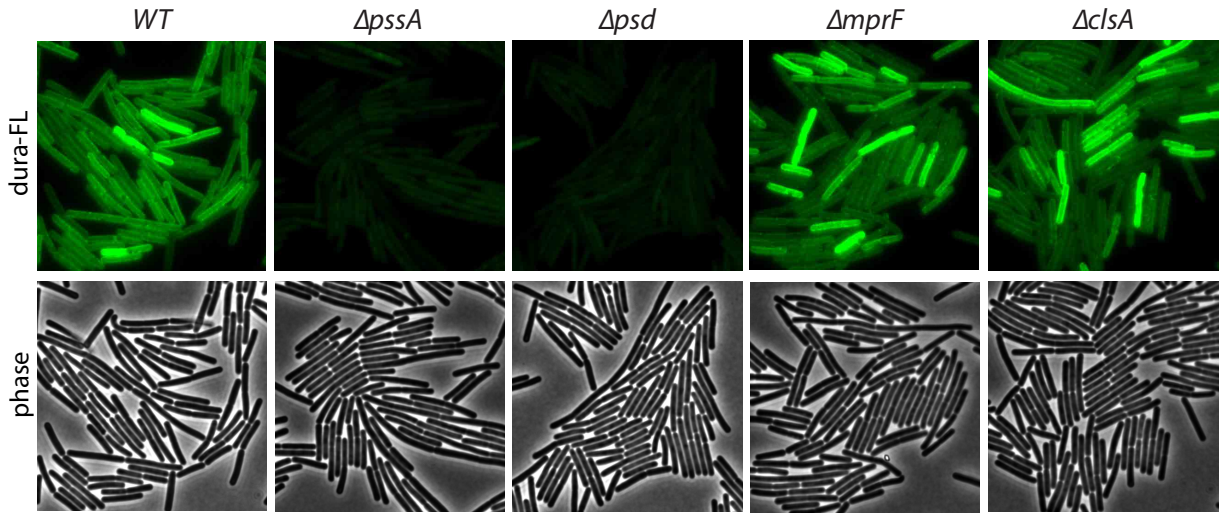
Supplemental Figure 2. Engineered strains with the *pssA-petA-psd* operon reconstructed at two neutral genomic loci confirms that PetA affects duramycin sensitivity without affecting PE levels. (A) Schematic of the engineered *B. subtilis* strains. The *pssA* and *psd* genes were reconstructed as an operon fused to an IPTG-regulated promoter. The *petA* gene was placed under a xylose-regulated promoter (PxylA). **(B)** MIC assay of the indicated *B. subtilis* strains with the indicated concentrations of IPTG and xylose. **(C)** TLC analysis of phospholipids extracted from the strains in (B).



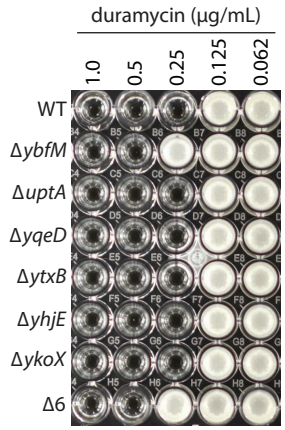
Supplemental Figure 3. A *petA*+ strain with less PE than a *petA*- strain is more sensitive to duramycin. **(A)** TLC analysis of phospholipids extracted from the indicated strains. **(B)** MIC analysis of the strains and conditions in (A). **(C)** Schematic interpretation of the results. The *petA*+ strain produces less total PE but has more on its outer leaflet than the *petA*- strain. The *petA*- strain produces more total PE but has less PE on its outer leaflet.



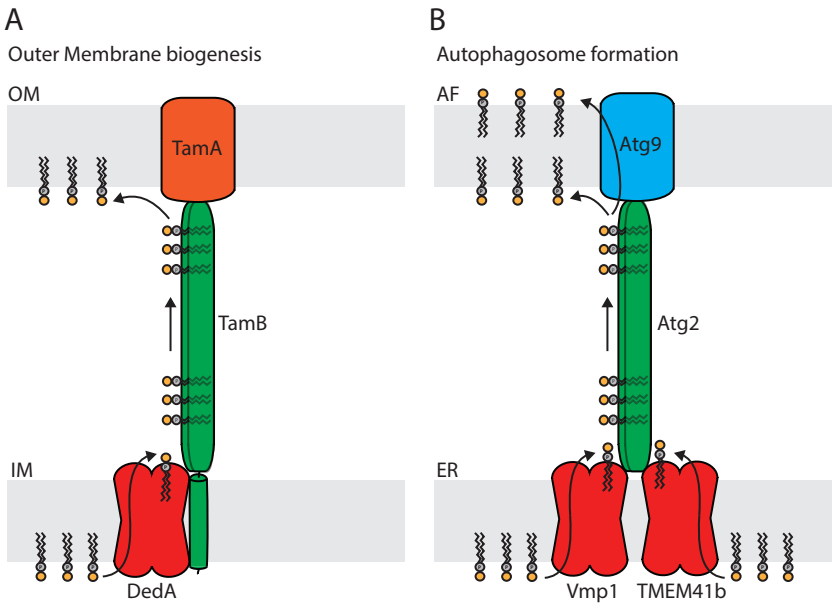
Supplemental Figure 4. Comparison of PetA homologs that can restore duramycin sensitivity to the *B. subtilis* Δ*petA* mutant. (A) Multiple sequence alignment of PetA homologues that can restore duramycin sensitivity to a Δ*petA* mutant. Boxed, conserved residues that form a predicted hydrophilic pocket. **(B)** MIC analysis of the indicated *B. subtilis* strains. N-terminal FLAG tagged PetA proteins were tested for their ability to complement a ΔpetA mutant. **(C)** Immunoblot analysis of strains in (B). The PetA homologue from *T. halophilus* was the only FLAG-tagged protein that was detectable by immunoblot. SigA controls for loading.



Supplemental Figure 5. Duramycin-FL specifically labels cells producing phosphatidylethanolamine. Representative micrographs of the indicated *B. subtilis* strains stained with fluorescent duramycin (dura-FL). Top, dura-FL staining. Bottom, phase-contrast images.



Supplemental Figure 6. PetA is the sole DedA paralog in *B. subtilis* that increases duramycin sensitivity. MIC analysis of the indicated *B. subtilis* strains. A strain lacking all six DedA paralogs Δ6 phenocopies the Δ*petA* mutant.



Supplemental Figure 7. Model for coupling phospholipid flipping by DedA proteins with transport across the periplasmic space by the TamB bridge in gram-negative bacteria. (A) Schematic model of a DedA phospholipid flippase in the inner membrane (IM) working with the TamB bridge that transports phospholipids across the periplasmic space. TamA is proposed to incorporate these lipids into the inner leaflet of the outer membrane (OM). **(B)** Schematic of the Atg2 bridge in eukaryotic cells that is required for phospholipid transport from the endoplasmic reticulum (ER) to the developing autophagosome (AF). The structure of Atg2 and the structural model of TamB are similar. DedA domain-containing proteins, Vmp1 and TMEM41b, make physical contact with the Atg2 protein and are required for autophagosome formation. Atg9 is present in the autophagosome and is required for the incorporation of lipids into both leaflets of the membrane.

Table S1 Uniprot IDs for the proteins included in gene neighborhood analyses

Neighborhood analysis	Organism	Uniprot ID
Figure 2	*B. subtilis*	O31453
	B. amyloliquefaciens	A0A1Y0X3G7
	B. glycinifermentans	A0A0T6BRD7
	T. halophilus	A0A2S0K2U0
	S. newyorkiensis	A0A1X7E785
	V. chiguensis	A0A5B8J2Z1
	L. sphaericus	A0A1G6L866
	P. polymyxa	F9DVW0
	P. filamentosa	A0A1M5VB81
Figure 5a	*A. bacterium*	A0A2V8NTM2
	F. australicus	A0A495IKP1
	A. sp. SGAIR0287	A0A4P8RB10
	P. flavus	A0A1S7B4Y9
	P. cousiniae	A0A1T5IGM6
	P. spAY1F1	A0A2S5UZT2
	S. boreus	A0A3E0VGG3
	P. flavus	A0A3N2C7P8
Figure 5b	*F. sp. MCBA15_019*	A0A1S2IE76
	F. sp. PAMC28766	A0A126YVP3
	M. sp. MYb43	A0A2S8UVD6
	F. sp. PhB24	A0A3N2HQZ3
	S. sp. 101FD-1	A0A506XXA5
	F. australicus	A0A495IM84
	P. flavus	A0A1S7BCN7
	F. sp. Leaf304	A0A0Q5CLJ2
Figure 5c	*H. sp. LS2*	A0A191ZKL5
	M. aggregate	A0A1M5IV87
	C. bisanense	A0A286GHL4
	H. bacterium	A0A2E3W5G8
	M. sp	A0A2G2EWL7
	B. bacterium CG10	A0A2M6X7Q5
	C. sp. WN38	A0A317ZJ87
	P. sp. GY_H	A0A371BD60

Table S2 MIC values

Experiment	Strain	Replicate 1	Replicate 2	Replicate 3	Mean	SD
Figure 2a.	Wildtype	0.13	0.13	0.25	0.17	0.07
	ΔpssA	>2	>2	>2	>2	N/A
	ΔybfM	0.50	0.50	0.50	0.50	0.00
	ΔybfM + ybfM	0.13	0.13	0.13	0.13	0.00
	Δpsd	>2	>2	>2	>2	N/A
	ΔybfM-psd	>2	>2	>2	>2	N/A
	ΔpssA-ybfM-psd	>2	>2	>2	>2	N/A
Figure 2c.	ΔpetA	0.50	0.50	0.50	0.50	0.00
	ΔpetA+uptA	0.50	0.50	0.50	0.50	0.00
	ΔpetA+petA(Bs)	0.13	0.13	0.13	0.13	0.00
	ΔpetA+petA(Bg)	0.13	0.13	0.13	0.13	0.00
	ΔpetA+petA(Th)	0.13	0.13	0.13	0.13	0.00
	ΔpetA+petA(Sn)	0.13	0.13	0.13	0.13	0.00
	ΔpetA+petA(Ls)	0.13	0.13	0.13	0.13	0.00
	ΔpetA+petA(Pp)	0.25	0.25	0.25	0.25	0.00
	ΔpetA+petA(Pfila)	0.13	0.13	0.13	0.13	0.00
	ΔpetA+petA(Pflexa)	0.13	0.13	0.13	0.13	0.00
Figure 3b.	WT	0.13	0.25	0.25	0.21	0.07
	ΔpetA	0.50	0.50	0.50	0.50	0.00
	ΔpetA+FLAG-petA	0.13	0.13	0.13	0.13	0.00
	ΔpetA+FLAG-petA(D31A)	0.25	0.25	0.25	0.25	0.00
	ΔpetA+FLAG-petA(E32A)	0.25	0.25	0.50	0.33	0.14
	ΔpetA+FLAG-petA(D31AE32A)	0.50	0.25	0.25	0.33	0.14
	ΔpetA+FLAG-petA(Y112A)	0.13	0.13	0.25	0.17	0.07
	ΔpetA+FLAG-petA(R118A)	0.50	0.50	0.50	0.50	0.00
	ΔpetA+FLAG-petA(Y112AR118A)	0.50	0.25	0.50	0.42	0.14
	ΔpetA+FLAG-petA(H119A)	0.25	0.25	0.25	0.25	0.00
Figure S1c.	WT	0.25	0.25	0.25	0.25	0
	ΔpssA	>2	>2	>2	>2	N/A
	Δpsd	>2	>2	>2	>2	N/A
	ΔmprF	0.25	0.25	0.25	0.25	0
	ΔclsA	0.25	0.25	0.25	0.25	0
Figure S2b.	-ybfM (0μM IPTG)	>40	>40	>40	>40	N/A
	+ybfM (0μM IPTG)	>40	>40	>40	>40	N/A
	-ybfM (5μM IPTG)	>40	>40	>40	>40	N/A
	+ybfM (5μM IPTG)	>40	>40	>40	>40	N/A
	-ybfM (7.5μM IPTG)	10.00	10.00	10.00	10.00	0.00
	+ybfM (7.5μM IPTG)	0.63	0.63	0.63	0.63	0.00
	-ybfM (10μM IPTG)	1.25	1.25	0.63	1.04	0.36
	+ybfM (10μM IPTG)	0.16	0.31	0.16	0.21	0.09
	-ybfM (12.5μM IPTG)	0.63	0.63	2.50	1.25	1.08
	+ybfM (12.5μM IPTG)	0.16	0.16	0.16	0.16	0.00
	-ybfM (20μM IPTG)	0.31	0.31	0.31	0.31	0.00
	+ybfM (20μM IPTG)	0.08	0.08	0.08	0.08	0.00
	ΔybfM	0.31	0.16	0.16	0.21	0.09
	wt	0.08	0.08	0.08	0.08	0.00
Figure S3b.	-ybfM (20μM IPTG)	0.5	0.5	0.5	0.5	0
	+ybfM (12.5μM IPTG)	0.25	0.25	0.25	0.25	0

Figure S4.	WT	0.25	0.25	0.25	0.25	0.00
	ΔybfM	0.50	0.25	0.50	0.42	0.14
	ΔuptA	0.25	0.25	0.25	0.25	0.00
	ΔyqeD	0.25	0.25	0.25	0.25	0.00
	ΔytxB	0.25	0.25	0.25	0.25	0.00
	ΔyhjE	0.25	0.25	0.25	0.25	0.00
	ΔykoX	0.25	0.25	0.25	0.25	0.00
	Δ6	0.50	0.50	0.50	0.50	0.00
Figure S5b.	WT	0.13	0.25	0.25	0.21	0.07
	ΔpetA	0.50	0.50	0.50	0.50	0.00
	ΔpetA+petA(Bs)	0.13	0.13	0.25	0.17	0.07
	ΔpetA+petA(Pfila)	0.13	0.13	0.25	0.17	0.07
	ΔpetA+petA(Pflexa)	0.13	0.13	0.25	0.17	0.07
	ΔpetA+petA(Th)	0.13	0.13	0.25	0.17	0.07

Table S3 Strains used in this study

Strain	Background	Genotype	Source	Figures
BIR003	*B. subtilis PY79*	*wild-type*	Lab stock(1)	2ab, 3b, SF1ab, SF4, SF5, SF6
BIR1137	*B. subtilis PY79*	Δ(*pssA-ybfM-psd*)*::tet*	This study	2ab, SF1a
BIR1183	*B. subtilis PY79*	Δ*pssA::tet*	This study	2ab, SF1a, SF5
BIR1184	*B. subtilis PY79*	Δ*ybfM::tet*	This study	2abc, 3b, SF1b, SF4bc, SF6
BIR1185	*B. subtilis PY79*	Δ*psd::tet*	This study	2ab, SF1a, SF5
BIR1200	*B. subtilis PY79*	Δ*yngC::tet*	This study	SF6
BIR1201	*B. subtilis PY79*	Δ(*pssA-ybfM-psd*)*::tet, ycgO::Pspank-pssA-psd (spec)*	This study	2d, 4ab, SF2abc, SF3ab
BIR1206	*B. subtilis PY79*	Δ(*pssA-ybfM-psd*)*::tet, ycgO::Pspank-pssA-psd (spec), yvbJ::PxylA-ybfM(kan)*	This study	2d, 4ab, SF2abc, SF3ab
BIR1319	*B. subtilis PY79*	Δ*yqeD::erm*	This study	SF6
BIR1320	*B. subtilis PY79*	Δ*ytxB::tet*	This study	SF6
BIR1321	*B. subtilis PY79*	Δ*yhjE::kan*	This study	SF6
BIR1322	*B. subtilis PY79*	Δ*ykoX::kan*	This study	SF6

BIR1365	*B. subtilis PY79*	Δ(*ybfM-psd*)*::tet*	This study	2ab
BIR1399	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-yngC (kan)*	This study	2c
BIR1400	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-ybfM (kan)*	This study	2c, SF1b
BIR1401	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA(D31A)(Th) (kan)*	This study	3bc
BIR1402	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA(E32A)(Th) (kan)*	This study	3bc
BIR1403	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA(D31AE32A)(Th) (kan)*	This study	3bc
BIR1404	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA(H119A)(Th) (kan)*	This study	3bc
BIR1406	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (B. glycinifermentans) (kan)*	This study	2c
BIR1407	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (L. sphaericus) (kan)*	This study	2c
BIR1408	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (P. fila) (kan)*	This study	2c
BIR1409	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (P. flexa) (kan)*	This study	2c
BIR1410	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (P. polymyxa) (kan)*	This study	2c
BIR1411	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (S.newyorkiensis) (kan)*	This study	2c
BIR1412	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-petA (T. halophilus) (kan)*	This study	2c
BIR1413	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA (kan)*	This study	SF4bc
BIR1415	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA (P.flexa) (kan)*	This study	SF4bc
BIR1416	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA (P.fila) (kan)*	This study	SF4bc
BIR1417	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA (Th) (kan)*	This study	3bc, SF4bc
BIR1418	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA (Y112A) (Th) (kan)*	This study	3bc
BIR1419	*B. subtilis PY79*	Δ*ybfM::tet, yvbJ::PxylA-FLAG-linker-petA (R118A) (T. halophilus) (kan)*	This study	3bc

BIR1420	*B. subtilis PY79*	*ΔybfM::tet, yvbJ::PxylA-FLAG-linker-petA (Y112A R118A) (Th) (kan)*	This study	3bc
BIR1519	*B. subtilis PY79*	*ΔmprF::erm*	This study	SF1a, SF5
BIR1520	*B. subtilis PY79*	*ΔclsA::erm*	This study	SF1a, SF5
BIR1592	*B. subtilis PY79*	*sacA::Pveg-mTagBFP(phleo), amyE::Pamj-yfp(cat), ΔyngC::lox72, ΔykoX::lox72, ΔybfM::tetR, ΔyhjE::lox72, ΔyqeD::lox72, ΔytxB::lox72*	This study	SF6

Table S4 Plasmids used in this study

Plasmid	Description	Source
pIR356	ycgO::Pspank-pssA-psd(spec)(amp)	This study
pIR358	yvbJ::PxylA-ybfM(kan)(amp)	This study
pIR382	yvbJ::PxylA-yngC(kan)(amp)	This study
pIR420	yvbJ::PxylA-ybfM(B. glycinifermentans)(kan)(amp)	This study
pIR421	yvbJ::PxylA-ybfM(L. sphaericus)(kan)(amp)	This study
pIR422	yvbJ::PxylA-ybfM(P. filamentosa)(kan)(amp)	This study
pIR423	yvbJ::PxylA-ybfM(P. flexa)(kan)(amp)	This study
pIR424	yvbJ::PxylA-ybfM(P. polymyxa)(kan)(amp)	This study
pIR425	yvbJ::PxylA-ybfM(S. newyorkiensis)(kan)(amp)	This study
pIR426	yvbJ::PxylA-ybfM(T. halophilus)(kan)(amp)	This study
pIR429	yvbJ::PxylA-FLAG-linker-ybfM(P. flexa)(kan)(amp)	This study
pIR430	yvbJ::PxylA-FLAG-linker-ybfM(P. fila)(kan)(amp)	This study
pIR431	yvbJ::PxylA-FLAG-linker-ybfM(T. halophilus)(kan)(amp)	This study

Table S5 Oligonucleotides used in this study

Name	Sequence
oIR0483	gcagctcaataataaaactagaatcc
oIR0484	CGGTACTGAGCGAGGGAGCAGAAattcttcacaacctgtcctaatc
oIR0485	CGGTAGTTGACCAGTGCTCCCTGctagcggatatgcataggggtgac
oIR0486	cctgtcggcattgttgcaaac
oIR0487	cagaaagatcatagcctttgtcatg
oIR0488	CGGTACTGAGCGAGGGAGCAGAAacatttttctcttttcggaaaacatc
oIR0489	CGGTAGTTGACCAGTGCTCCCTGcaaatcactgcgctgcgcatattattg
oIR0490	gtgcagcacccctttaagataatc
oIR0491	gaaacgactattcctgtcactcttc
oIR0492	CGGTACTGAGCGAGGGAGCAGAATTAgtaatccgctatgagctgctgaa
oIR0493	CGGTAGTTGACCAGTGCTCCCTGcggaagggttatagggattattc
oIR0494	ccagcatatatgtttccattacg
oIR0549	gatacttgctgttctgtcgtgc
oIR0550	CGGTACTGAGCGAGGGAGCAGAAttatactccttaccgggtttg
oIR0551	CGGTAGTTGACCAGTGCTCCCTGgtgagcatgatggagggagac

oIR0552	ctgcctcaatgacagcaaaacg
oIR0553	ctgcgccatacatgtgaagc
oIR0554	CGGTACTGAGCGAGGGAGCAGAAgtctatggtcactcccaacttag
oIR0555	CGGTAGTTGACCAGTGCTCCCTGataaggaggcttgttggccc
oIR0556	ccttctgataatcgcggcgc
oIR0557	ttgaagacggaatgctgaatg
oIR0558	CGGTACTGAGCGAGGGAGCAGAAcctttgaaccacagcaattatgg
oIR0559	CGGTAGTTGACCAGTGCTCCCTGgatttgccaatgacctttacg
oIR0560	gaagaaagctgtaccatatacgc
oIR0929	CAATCATTACGATGGTTCTTTTCAG
oIR0930	GTTCTGGTGAAACTGAAGACAGCAC
oIR1019	GAATTGTGAGCGGATAACAATTAAGCTTacataaggaggaactactATGAATTACATCCCCTGTATGATTACG
oIR1020	CTAATTCCATCTCCCAGACTC
oIR1021	CCTGGAGTCTGGGAGATGGAATTAGacataaggaggaactactATGTTTAATACGGCTGTAAAGATTC
oIR1022	GCgaGCTAGCatCTGCAGttACTAGTTTATTCTTCGTAACCTATCAGTTCTC
oIR1024	TAGCatCTCGAGacataaggaggaactactATGGAATTAGTTCAGCAGCTCATAG
oIR1025	tcgctggGATCCTTAAACATGAATAATCCCTATAACCC
oIR1026	GATTCCCATGTGACAAAACCTTTGG
oIR1027	CGGTACTGAGCGAGGGAGCAGAACAGTGTAACCAAACCTCCAATTG
oIR1028	CGGTAGTTGACCAGTGCTCCCTGAAAGAGGAGCTTGCATAAACGC
oIR1029	CATAATACATTGCACGCTCCAATC
oIR1067	CGGTAGTTGACCAGTGCTCCCTGCATGCGGCAGAAAACCTGGAGTC
oIR1068	GGTCATTCTCACTATCATTCGTTTCAATTC
oIR1069	GGACTTGAGACGGCACATCTG
oIR1070	CGGTACTGAGCGAGGGAGCAGAATTAAACATGAATAATCCCTATAACCCTTC
oIR1103	TAGCatCTCGAGacataaggaggaactactATGGGCAGTTTGATAAGCGAAATTTTAAC
oIR1104	tcgctggGATCCCTATCGTTTAAATATGGCTGTACG
oIR1273	tttgaatgAAGCTTgaGCTAGCatC
oIR1274	cctatcacctcaaatggttcgctg
oIR1281	atCTCGAGacataaggaggaactactAtgGACTATAAGGATGATGATGATAAGGGAAGTggcAGCGGCTCTATGCAAGTGATGCAGGATTTCATC
oIR1282	atCTCGAGacataaggaggaactactAtgGACTATAAGGATGATGATGATAAGGGAAGTggcAGCGGCTCTATGGAACTGGTCGAGCATCTTATC
oIR1283	atCTCGAGacataaggaggaactactAtgGACTATAAGGATGATGATGATAAGGGAAGTggcAGCGGCTCTATGAACGACTTAGGTAACTTAATCG
oIR1305	TGGGGCATTCTTGCCAGGGATTGCACATATCACGGCATATATTGCGG
oIR1306	GTGCAATCCCTGGCAAGAATGCCCCAAACATCAGTAACAAACCTC
oIR1307	GTTACTGATGTTTGGGGCATTCTTGCCAGGGATTCGCCATATC
oIR1308	GAATCCCTGGCAAGAATGCCCCAAACATCAGTAACAAACCTC
oIR1309	GGGTATTTCTTGCCAGGGATTGCACATATCACGGCATATATTGCGG
oIR1310	CAATATATGCCGTGATATGTGCAATCCCTGGCAAGAAATACCC
oIR1311	GGATCGTGGGGCTTCCTATTCCAGCAGAAGTGTTGCTCACCTACGTCGG
oIR1312	GGATCGTGGGGCTTCCTATTCCAGACGCAGTGTTGCTCACCTACGTCGG
oIR1313	GGATCGTGGGGCTTCCTATTCCAGCAGCAGTGTTGCTCACCTACGTCGG
oIR1314	TGGAATAGGAAGCCCCACGATCC

oIR1315	GGGTATTTCTTGCCAGGGATTCGCGCAATCACGGCATATATTGCGGG
oIR1316	GCGAATCCCTGGCAAGAAATACC
oIR1373	gccgcttctgatacaaggacg
oIR1374	CGGTACTGAGCGAGGGAGCAGAAtggtctctccaatcatattctagttcgatc
oIR1375	CGGTAGTTGACCAGTGCTCCCTGtaagacggagtctttttttatttcg
oIR1376	gtcctttgccacatcttctccc
oIR1377	gacatacagagtcacgtccgctc
oIR1378	CGGTACTGAGCGAGGGAGCAGAAtttgtaaccccgctcattcatatttatc
oIR1379	CGGTAGTTGACCAGTGCTCCCTGgatgcgtaaacccccggcctttac
oIR1380	gttgaagcaggaagagcactcatc

Table S6 gBlocks used in this study

gBlock	Sequence
B. glycinifermentans	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGGATGTCGCAAAAGA GCTCATTAGTCAGTACGGATACTTCGCCATTTTTGGAATGTTAGTGTTGGGAATC ATTGGGCTTCCGATCCCGGACGAGGTGATGATGACTTTTGTAGGATACCTGAGC TCTATTCACATTCTCCACTACGGTTTGGCTATTGTAATTTCCTTCTCCGGGGCGTT ATCCGGGATGGTAATTAGTTACTTTCTGGGTAAAAAGATCGGAAAACCGCTTTT GGATAAATTTGGTAAGTGGATTGGTTTGACTCCAAAACGGCTGGAGACAGTCCG CCGTTGGTTCGATAAATACGGACCGTGGACCATAGTCTTTGGTTATTTCATACCT GGCATTAGACACGTAACGTGCTACCTGAGTGGGATGAACGAAATGAAGATGAA AAAGTTCCTCACCTTTGCCGGCTCTGGGGCGTTCATCTGGTGTATTGTGTTCATT ACGATTGGATATACAATTGGTGTGATTTAAGGATCccagcgaaccatttgaggtgatagg
L. sphaericus	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGACTTTTATAGATCAG TTGATAGAGAACTATGGCTACACAGCTATCTTCATAATACTTGCTTTGGGACTGT TCTCACTGCCTATCCCGGATGAGCTGATGGTATTACTCGTAGGATATTTTACCAA AATTGGCTTACTGCACTACTCCTTCTCCTTGCTGGCAGTCTTCTCAGGATCTCTC ATTGGCATGTTGGTTAGTTATGTTCTCGGTAACAAGATAGGCCGGCCTCTTCTGG ATCGGCTGGGTAAGTGGTTCAGACTGTCCCGCAAGTGGAACAGTAATGTAGCTA ACTGGATAGAGAAATACGGGGCACCGGCCATAATAGTTTCATATTTTATTCCTG GGATGAGACATGTTGCCGGTTATTTGTGCGGGATGTCCTACATTAGCTTGAAGA AATATATGCTTTACGCCGGGATCAGCGCATTTCTTTGGTCCCTTCTTTTTCTGAC GATCGGACGGATATTCTAAGGATCccagcgaaccatttgaggtgatagg
P. filamentosa	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGGAACTGGTCGAGCAT CTTATCACACATTATGGATATATCGCCGTGTTTTTAATGCTGACCCTTGGGATTG TAGGGCTTCCGATACCGGACGAGGTTCTTATGACGCTCATAGGATATTTCACGC ACGTTGGTACCTTGAATTATGAGCTGGCTATTGTAATTTCTTTTATAGGAGCCCT GCTGGGTATGATGATAAGCTACTTAATCGGTCGCAAAGCTGGACGCCCTTTCAT CGATAAATATGGGAAGTGGGTTGGCCTTAAAGAGAAAAGAATGGATAAAGTGG AGACCTGGATGAAAAAGTACGGTCCGTACTCTTTGATCTTGGGGTACTTTATTCC AGGCGTTCGTCATGTGACCTGCTACTTCAGCGGTATAACTCGTATGAACCTCAG AACCTACCTTCTTTTTGTAGCAATCGGTGCTTTTCTCTGGTGTTTCGTGTTCATCA CTATCGGGCGCATTGCAGGCGTCATCCGGTTCTAAGGATCccagcgaaccatttgaggtgata gg

P. flexa	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGCAAGTGATGCAGGAT TTCATCATGACGTATGGGTATTTAGCCATCTTCCTGATGCTGACATTCGGCATTA TTGGACTGCCTGTACCTGATGAAGTGATGATGACGACTGTGGGATACTTCACGA ATACCGACGTGCTTAATTATGGTCCGGCCGTACTCTTTTCATTCTGTGGAGCACT TCTGGGGATGATTGTATCATACACCATCGGTAGAAAGGCAGGACGGCCTTTCAT TAACAGATATGGCAAATGGATTGGATTGAAGGAGAAGCGCATGAAAAAGGTGG AATCTTGGATGATGAAGTATGGCCCATATTCAATCATACTTGCTTATTTTATACC GGGCGTTCGTCACCTGGTCTGTTACTTCTCCGGCATAGGTAAGATGAAACTGCA AACATATATTACTTTTGCGGCGATTGGGGCCTTTCTTTGGTGCTTTGTATTTATC ACTTTCGGAAAGCTCGTAGGTATTATACAATAAGGATCccagcgaaccatttgaggtgatagg
P. polymyxa	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGGAGTGGGTAATCTCC ATGATTACCCAATATGGATACATCGCCATCTTTGCCCTTCTGGCGCTCGGCATCA TTGGCTTACCAGTACCAGATGAAATAATTATGGTTTTTGTAGGCTATCTGAGCA GTATCATGGTATTAAACTATAGTGTGAGTATCTTAGTTAGTTTCATTGGTGCAAT GACAGGAATGATGATAAGCTACACGCTCGGGAAAAAGCTGGGGCAACCACTCG TGGATAAGCACGGAAAGTGGGTCGGTTTAACTCCGAAAAGATTTGCCCGGGTA AAGGGCTGGTTCGCGCGTTTTGGTCTGTGGACAATCTTATTCGGCTACTTCATCC CTGGTGTACGCCACGCTACGAGTTACCTTTCTGGTATTAGTGCGATGCCGGTGC GCAAGTACATGCTGGTGGCCTCCGCGGGCAGTCTGATTTGGACCTTGATTTTTAT ATCTATCGGGTATATCGCTGGCGCAAATATCAATTTTCATTAAGGATCccagcgaacc atttgaggtgatagg
S. newyorkiensis	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGGAGCAAATTCAAGA GTATATCGCCCAGTACGGTTATCTGAGTATCTTTTTCTTCTTAGCACTTGGGATT TTTGGACTTCCACTCCCAGATGAGCTCCTTGTGACATTCGCAGGGTATTTAGCAT CTGCAGGAACATTCCATTTTGTATTTGCGATGGTCTTCACCATTAGTGGGGTAAT GGTGGGTACGTTATTCACCTACATGATAGGGCGGAAAATCGGCAAACCATTGGT ACATCGTTTTGGTCGGTATCTCTTTTTATCCCCACACCGTATGCAGAAGGTAGAA CAATGGTTCATGGCATACGGATCATGGGCGGTAACGATAGGGTATTTTTTACCT GGAATGCGTCATTTTGTCTGTTATGTATCCGGCATGTCAGGTATGTCTGTCCGGC GCTACATCTTATTTGCTATCCCTGGTGTTATAGTATCTACCATGATCTGTATATT GCTGGGCTATTTCATACGGTTGCCATTCTTTTAAGGATCccagcgaaccatttgaggtgatagg
T. halophilus	tttgaatgAAGCTTgaGCTAGCatCTCGAGacataaggaggaactactATGAACGACTTAGGTAAC TTAATCGTCAATTATGGCTATATAGGGATTTTTATCATACTTGTTCTTGGGATCG TGGGGCTTCCTATTCCAGACGAAGTGTTGCTCACCTACGTCGGATACAACATTTT TATCGGTCGCATGTCCTGGATAGGAGCTATTAGCGCGGCATTGGCTGGGGCTTT AATTGGAATATCCATTTCTTACCTGCTTGGACGTAAACTCGGTCTCCCGTTTCTC AGACGTTTCGGTCCGAAAGTTCATATCAGCGACAAGAAAATCGACTGGACTCAG TCTTACTTTGAGAAACACGGAGGTTTGTTACTGATGTTTGGGTATTTCTTGCCAG GGATTCGCCATATCACGGCATATATTGCGGGGATTGCGGATTTCAGATTCGGTA AGTTTGCGATATTTGCGTACTTAGGGGCTGTACTCTGGGTGAACATATTCATCTT ACTGGGCATATTGCTCGGAGATAACTGGACAGTGATGGAAACAATACTGCATG ACACTACTAAGATCGTTGTCATCGGTATTAGTGTCGCTGCTTTGCTTTTCTTCTA CTTCAAGTTTCGCAAAAAGAAAACCGATTAAGGATCccagcgaaccatttgaggtgatagg

Supplemental Methods

Strain constructions

B. subtilis deletion mutants

All *B. subtilis* deletion mutants were made by isothermal assembly(2) followed by direct transformation. The assembly reactions contained three PCR products: two of the products contained ~1500 base pairs upstream and downstream of the gene to be deleted, and the third product contained an antibiotic resistance cassette. Antibiotic resistance cassettes with surrounding lox66/lox71 sites were amplified from pWX465(cat), pWX466(spec), pWX467(erm), pWX469(tet) and pWX470(kan) using the primers oJM028 and oJM029. The flanking regions for the respective deletions were amplified using PY79 genomic DNA as template and the following primer sets: *yngC*(oIR483-486);*ykoX*(oIR487-490); *ybfM*(oIR491-494);*yhjE*(oIR549-552);*yqeD*(oIR553-556);*ytxB*(oIR557-560);*pssA(oIR1026-1027,oIR1067-1068);psd(oIR1069-1070,oIR1028-1029);pssA-ybfM-psd(*oIR1026-1029*);mprF*(oIR1373-1376); *clsA*(oIR1377-1380); *ybfM-psd*(oIR491-492,oIR1028-1029).

Construction of *FLAG-petA(Th)* point mutations

Point mutations in FLAG-petA(*Th*) were made by isothermal assembly and direct transformation into *B. subtilis*. Two DNA fragments were amplified using the genomic DNA of BIR1417 [ybfM::tet, yvbJ::PxylA-FLAG-linker-petA(*Th*)(kan)] as a template using oligos flanking the upstream and downstream homology arms (oIR929,oIR930) and mutation specific primers D31A(oIR1311,oIR1314); E32A(oIR1312,oIR1314); D31AE32A(oIR1313,oIR1314); Y112A(oIR1307,oIR1308); R118A (oIR1309,oIR1310); Y112A,R118A(oIR1305,oIR1306); H119A(oIR1315,oIR1316). The two resulting amplification products were purified and added to the isothermal assembly reaction followed by direct transformation into BIR1184 (ybfM::tet). All mutants were confirmed by sequencing.

Plasmid Constructions

All plasmids were confirmed by sequencing.

pIR356[ycgO::Pspank-pssA-psd(spec)(amp)]

pIR356 was generated in a three-piece isothermal assembly reaction with PCR product containing the pssA gene (amplified from PY79 gDNA with oIR1019 and oIR1020), the psd gene (amplified from PY79 gDNA with oIR1021 and oIR1022) and pCB084[ycgO::Pspank-MCS(spec)] digested with HindIII and SpeI.

pIR358[yvbJ::PxylA-ybfM(kan)(amp)]

pIR358 generated in a two-piece ligation with PCR product containing the ybfM gene (amplified from PY79 gDNA with oIR1024 and oIR1025) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR382[yvbJ::PxylA-uptA(kan)(amp)]

pIR382 generated in a two-piece ligation with PCR product containing the uptA gene (amplified from PY79 gDNA with oIR1103 and oIR1104) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR420[yvbJ::PxylA-petA(B.glycinifermentans)(kan)(amp)]

pIR420 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(B.glycinifermentans) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR421[yvbJ::PxylA-petA(L.sphaericus)(kan)(amp)]

pIR421 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(L.spahericus) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR422[yvbJ::PxylA-petA(P.filamentosa)(kan)(amp)]

pIR422 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(P.filamentosa) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR423[yvbJ::PxylA-petA(P.flexa)(kan)(amp)]

pIR423 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(P.flexa) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR424[yvbJ::PxylA-petA(P.polymyxa)(kan)(amp)]

pIR424 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(P.polymyxa) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR425[yvbJ::PxylA-petA(S.newyorkiensis)(kan)(amp)]

pIR425 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(S.newyorkiensis) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR426[yvbJ::PxylA-petA(T.halophilus)(kan)(amp)]

pIR426 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(T.halophilus) with oIR1273 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR429[yvbJ::PxylA-FLAG-linker-petA(P.flexa)(kan)(amp)]

pIR429 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(P.flexa) with oIR1281 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR430[yvbJ::PxylA-FLAG-linker-petA(P.filamentosa)(kan)(amp)]

pIR430 was generated in a two-piece isothermal assembly reaction with PCR product containing the petA gene (amplified from gBlock(Pfilamentosa) with oIR1282 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

pIR431[yvbJ::PxylA-FLAG-linker-petA(T.halophilus)(kan)(amp)]

pIR431 generated in a two-piece ligation with PCR product containing the petA(T.halo) gene (amplified from gBlock(T.halophilus) with oIR1283 and oIR1274) and pCB133 [yvbJ::PxylA-MCS(kan)] digested with XhoI and BamHI.

Source Data

Uncropped immunoblots with molecular weight markers

Figure 3

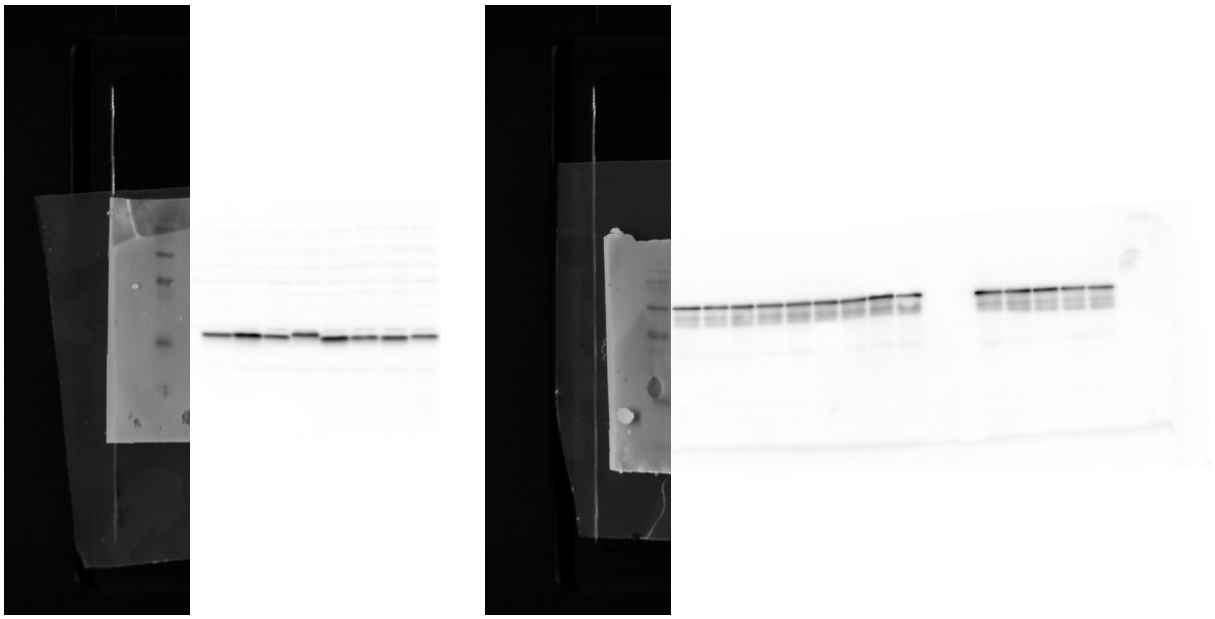
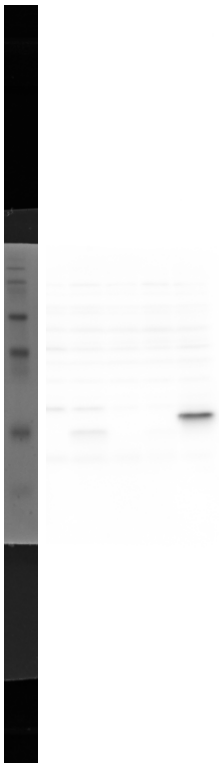


Figure S5



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