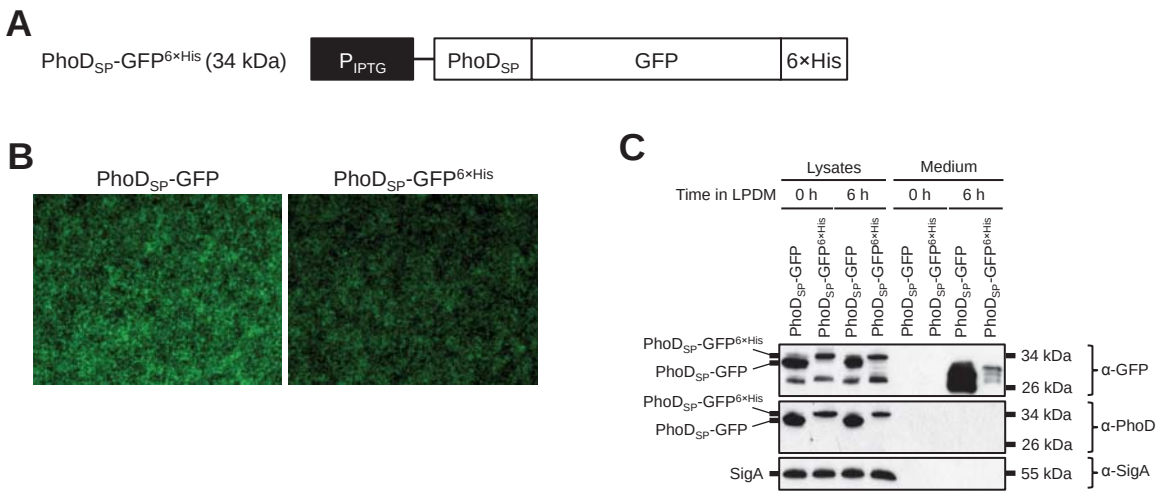

FIG S1 PhoD-GFP$^{6\times His}$ expression and secretion from *B. subtilis*. (A) PhoD$_{SP}$-GFP$^{6\times His}$ construct design. The predicted molecular weight is indicated in parenthesis. (B) Intracellular expression of folded GFP$^{6\times His}$. The indicated strains were grown overnight in HPDM supplemented with 1 mM IPTG. The next day, the cells were examined for folded GFP expression by fluorescence microscopy. (C) Secretion of total GFP$^{6\times His}$. The indicated strains were grown overnight in HPDM supplemented with 1 mM IPTG. The next day, the cells were washed and resuspended in LPDM supplemented with 1 mM IPTG. After 6 h in LPDM, the cells were lysed and the medium was precipitated with TCA. The cell lysates and precipitated medium were examined by Western blot analysis probing with α-GFP, α-PhoD, and α-SigA antibodies. Migration of PhoD$_{SP}$-GFP, PhoD$_{SP}$-GFP$^{6\times His}$, and SigA bands is indicated to the left of each blot. Migration of molecular weight standards is indicated to the right of each blot.

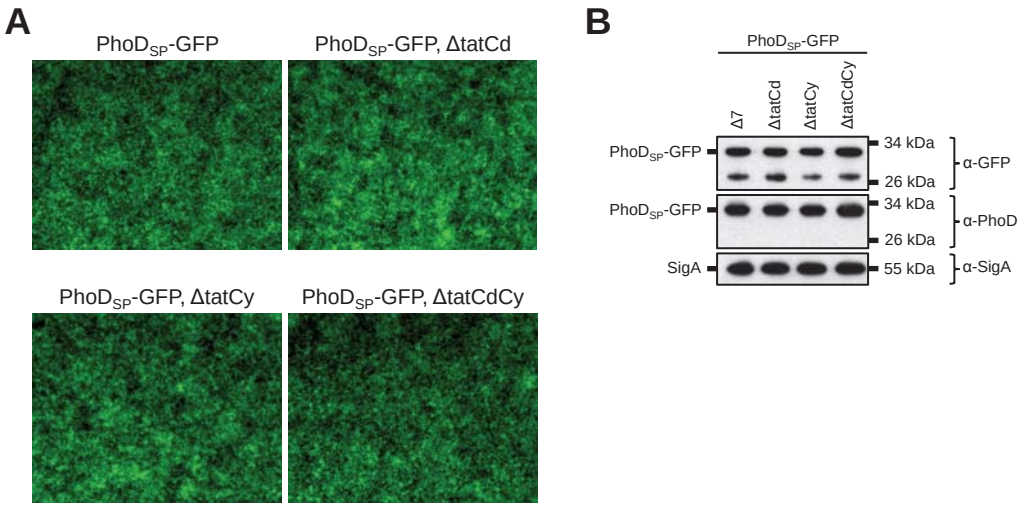

FIG S2 Characterization of intracellular PhoD$_{SP}$-GFP expression in *B. subtilis* single and double Tat-deletion strains. The indicated strains were grown overnight in HPDM supplemented with 1 mM IPTG. The next day, the cells were either (A) examined for folded GFP expression by fluorescence microscopy or (B) lysed and examined for total GFP expression by Western blot analysis probing with α-GFP, α-PhoD, and α-SigA antibodies. In panel B, migration of PhoD$_{SP}$-GFP and SigA bands is indicated to the left of each blot. Migration of molecular weight standards is indicated to the right of each blot.

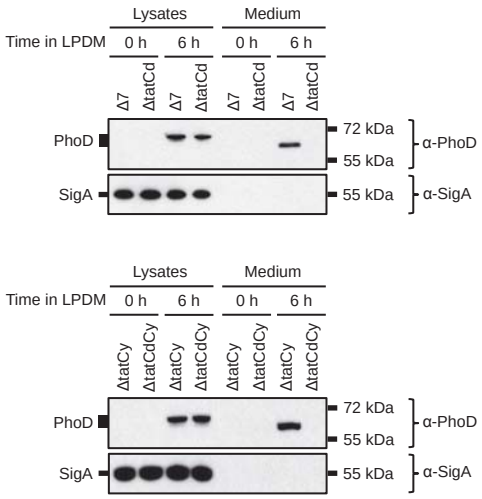

FIG S3 Secretion of endogenous alkaline phosphatase D (PhoD) from *B. subtilis* single and double Tat-deletion strains. The indicated strains were grown overnight in HPDM. The next day, the cells were washed and resuspended in LPDM. After 6 h in LPDM, the cells were lysed and the medium was precipitated with TCA. The cell lysates and precipitated medium were examined by Western blot analysis probing with α-PhoD and α-SigA antibodies. Migration of PhoD and SigA bands is indicated to the left of each blot. Migration of molecular weight standards is indicated to the right of each blot.

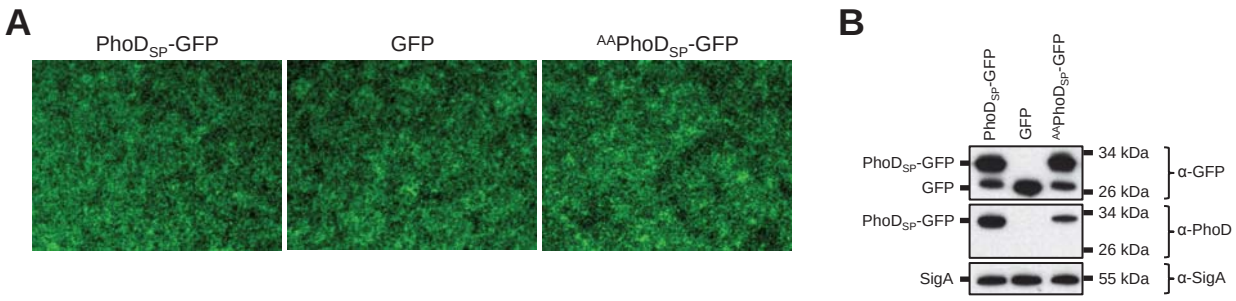

FIG S4 Characterization of intracellular ^{AA}PhoD$_{SP}$-GFP expression in *B. subtilis*. The indicated strains were grown overnight in HPDM supplemented with 1 mM IPTG. The next day, the cells were either (A) examined for folded GFP expression by fluorescence microscopy or (B) lysed and examined for total GFP expression by Western blot analysis probing with α-GFP, α-PhoD, and α-SigA antibodies. In panel B, migration of PhoD$_{SP}$-GFP, GFP, and SigA bands is indicated to the left of each blot. Migration of molecular weight standards is indicated to the right of each blot.

TABLE S1 Plasmids used in this study

Plasmid	Genotype	
pDP311	*Ωbpr mls*	
pDP312	*Ωvpr mls*	
pDP313	*ΩnprE mls*	
pDP314	*ΩaprE mls*	
pDP315	*Ωepr mls*	
pDP319	*ΩwprA mls*	
pDP320	*Ωmpr mls*	
pDR111	*amyE::$P_{hyspank}$ spec amp*	(Kearns and Losick 2005)
pMiniMAD2	*ori^{BsTs} amp mls*	(Patrick and Kearns, 2008)
pTM1	*amyE::$P_{hvspank}$-$PhoD_{SP}$ spec amp*	
pTM2	*amyE::$P_{hyspank}$-$PhoD_{SP}$-GFP spec amp*	
pTM7	*amyE::$P_{hvspank}$-GFP spec amp*	
pTM8	*amyE::$P_{hyspank}$-$^{AA}PhoD_{SP}$-GFP spec amp*	
pTM9	*amyE::$P_{hyspank}$-$PhoD_{SP}$-GFP^{6xHis} spec amp*	
pTM14	*amyE::$P_{hvspank}$-$AmyE_{SP}$-GFP spec amp*	

[1]Kearns, DB, Losick, R. (2005) Cell population heterogeneity during growth of Bacillus subtilis. *Genes Dev.* **19**(24):3083-94.
[2]Patrick, JE and Kearns, DB. (2008) MinJ (YvjD) is a topological determinant of cell division in Bacillus subtilis. *Mol Microbiol.* **70**(5):1166-79.

TABLE S2 Primers used in this study

Primer	Sequence
1739	aggaggaattcgtggatacactgatcgtgat
1740	ctcctgtcgactctgtttttcgtttttttcctca
1741	aggaggtcgacaatggaaaacttaatatgaacacagaaaat
1742	ctcctggatccggtttaacgcataataaaaattga
1743	caaagcgaattcgtgttgga
1744	ctcctgtcgacgttttgaccgaagaacctttc
1745	aggaggtcgacaagcagaaagcgaatgatccc
1746	ctcctggatcctctctcccgccaatttgctt
1747	aggaggaattcacagaaacacgaatgcaatcg
1748	ctcctgtcgacagcaacagacaatttcttacct
1749	aggaggtcgacgttgaagcagcctggaatgc
1750	ctcctggatccatgatcaacctcgaaaacctg
1751	cctatgaattctccattttcttct
1752	ctcctgtcgaccaagctgatccacaatttttg
1753	aggaggtcgacggaaaagggttaatcaacgtac
1754	ctcctggatccgtatatgaagtgaacatgtcag
1755	aggaggaattcatctgcacaaattcagcgatc
1756	ctcctctcgagtacaacaagtttgcaagacatg
1757	aggagctcgagaaacggctgaacgccgtca
1758	ctcctggatcctaggtatgggttgctgccaa
1855	aggaggaattcatttgccaattggttttcaattg
1856	ctcctctcgagttcctcttctaaatctgacgg
1857	aggagctcgagaatgtcatgaaggctgtcagc
1858	ctcctggatccttttcaaatgacaccgcacaat
1759	aggaggaattccttgtgtcgttaaaccattgat
1760	ctcctctcgagttgttttctgaatcttggaacta
1770	ctcctggatccattctgcaaaatcagctctcg
1919	aggagctcgagacagggcagacagctattgcc
3069	gttggggcctttgaagtaaatgctagtaaaggagaagaacttttcact
3070	ccgaattagcttgcatgcgggttatttgtatagttcatccatgc
3250	acgactcactatagggcgaattg
3251	ctcactaagggaacaaaagctgg
3252	caggactttctcttggattaac
3253	caattcgccctatagtgagtcgtgcgaagctcttctaaatgcc
3254	ccagcttttgttccctttagtgagctgtttgaagtgagtgtcacc
3255	gcaatgattgttgccattatcg
3256	cgcatttccttttccattgct
3257	caattcgccctatagtgagtcgtcaatatgctccagcagcga
3258	ccagcttttgttccctttagtgagagcagtgctgccgatcgg
3259	tgctggctacttcagcaaca
3860	caattcgccctatagtgagtcgtaatgtttgaaaacataatttccac
3861	caattcgccctatagtgagtcgtaggaccgatcggcatatttg
3866	ttcacttacaatcgccgtttg
3867	caattcgccctatagtgagtcgtaagcttaattccatatccttttc
3868	ccagcttttgttccctttagtgaggacaaacaaatgtaggataaatc
3869	tgtaaggtcgaaacgcttcg
3903	ttgtgagcggataacaattatttcctataatggagacgatcaa
3904	ccgaattagcttgcatgcgggtcgacgcggcatttacttcaaaggccccaac
TM858	ggcagagaaccaacaaatgaa
TM859	tccagagataccggctcttga
TM860	catccatgccatgtgtaatcccagc
TM861	ccattacctgtccacacaatctgccc
TM883	aggaaagctttcaaaacaatacgtttgacgcagcaaaatttattcaaggagcggggaagattgc
TM884	gcaatcttccccgctccttgaataaattttgctgcgtcaaacgtattgttttgaaagctttcct
TM885	gaggagagaggggatcttgaatgagtaaaggagaagaacttttc
TM886	gaaaagttcttctcctttactcattcaagatcccctctctcctc
TM887	ggcatggatgaactatacaaacatcaccatcaccatcactaacccgcatgcaagctaattc
TM888	gaattagcttgcatgcgggttagtgatggtgatggtgatgtttgtatagttcatccatgcc
TM1013	gaggagagaggggatcttgaatgtttgcaaaacgattc
TM1014	gtgaaaagttcttctcctttactagcactcgcagccgcc

TABLE S3 Relative expression levels of GFP mRNA in the *B. subtilis* Δ7 ancestral strain[a]

Construct	Fold difference in GFP mRNA levels (± SD[b])
$PhoD_{SP}$-GFP	1.0
$PhoD_{SP}$-$GFP^{6\times His}$	0.28 (± 0.07)
$PhoD_{SP}$-GFP, ΔtatCd	0.95 (± 0.19)
$PhoD_{SP}$-GFP, ΔtatCy	0.91 (± 0.13)
$PhoD_{SP}$-GFP, ΔtatCdCy	0.97 (± 0.21)
$^{AA}PhoD_{SP}$-GFP	0.67 (± 0.15)
GFP	1.8 (± 0.12)

[a]Expression of $PhoD_{SP}$-GFP was standardized to 1.0.
[b]Standard deviation
[c]GFP mRNA not detected

TABLE S4 Mass spectrometry peptide identification of the top band of purified GFP$^{6\times His}$

Peptide	Variable modifications	m/z	Da	Score	Expect
(F)EVNASKGEEL(F)		538.70	0.43	18.9	2.30×10^{-7}
(F)EVNASKGEELF(T)		1222.74	0.14	21.1	2.70×10^{-6}
(F)SVSGEGEGDATY(G)		586.49	0.25	22.5	5.50×10^{-8}
(F)SVSGEGEGDATYGKL(T)		735.55	0.21	23.4	1.20×10^{-8}
(F)SVSGEGEGDATYGKLTL(K)		842.93	0.52	15.6	2.10×10^{-6}
(F)ARYPDHMKQHDFF(K)	Oxidation at position 7	854.53	0.14	17.0	2.50×10^{-7}
(F)ARYPDHMKQHDFF(K)		564.60	0.0036	15.1	2.20×10^{-5}
(F)FKSAMPEGYVQERTIF(F)	Oxidation at position 5	640.38	0.063	23.9	5.40×10^{-7}
(F)FKSAMPEGY(V)		515.49	0.25	19.5	1.10×10^{-7}
(F)KSAMPEGY(V)	Oxidation at position 4	449.91	0.21	19.3	2.50×10^{-7}
(F)KSAMPEGYVQERTIF(F)	Oxidation at position 4	886.69	0.25	18.2	1.90×10^{-8}
(F)KSAMPEGYVQERTIFF(K)	Oxidation at position 4	640.44	0.12	21.8	2.70×10^{-6}
(F)KSAMPEGY(V)		441.65	-0.055	16.9	1.10×10^{-7}
(Y)VQERTIF(F)		446.91	0.16	16.5	3.90×10^{-5}
(Y)VQERTIFF(K)		1039.65	0.093	16.0	7.20×10^{-5}
(Y)VQERTIFFKDDGNY(K)		866.74	0.32	19.2	1.00×10^{-7}
(F)FKDDGNY(K)		429.92	0.23	15.2	1.80×10^{-6}
(Y)KTRAEVKFEGDTL(V)		747.64	0.24	22.9	2.30×10^{-7}
(F)EGDTLVNRIEL(K)		629.66	-0.18	16.4	8.10×10^{-6}
(F)KEDGNILGHKL(E)		612.47	0.13	16.3	1.40×10^{-6}
(F)KEDGNILGHKLEY(N)		506.26	0.33	24.7	5.90×10^{-7}
(F)KEDGNILGHKLEYNYNSHNVY(I)		627.39	-0.16	15.7	4.50×10^{-4}
(L)GHKLEYNYNSHNVY(I)		869.67	0.27	15.9	4.90×10^{-7}
(L)EYNYNSHNVY(I)		651.97	0.20	17.0	3.30×10^{-6}
(Y)NYNSHNVY(I)		505.96	0.24	15.8	8.30×10^{-7}
(Y)IMADKQKNGIKVNF(K)	Oxidation at position 2	541.41	0.11	25.8	1.20×10^{-7}
(Y)IMADKQKNGIKVNF(K)		803.65	0.21	22.5	9.80×10^{-8}
(F)KIRHNIEDGSVQL(A)		755.19	0.28	20.4	5.50×10^{-7}
(F)KIRHNIEDGSVQLADHY(Q)		666.01	0.34	29.9	6.50×10^{-8}
(L)ADHYQQNTPIGDGPVLLPDNHY(L)		822.37	0.31	24.6	1.20×10^{-6}
(Y)QQNTPIGDGPVLLPDNHY(L)	Gln→pyro-Glu at position 1	981.23	0.26	21.4	1.40×10^{-8}
(Y)QQNTPIGDGPVLLPDNHY(L)		989.27	-0.22	19.0	1.60×10^{-7}

TABLE S5 Mass spectrometry peptide identification of the bottom band of purified GFP$^{6\times His}$

Peptide	Variable modifications	m/z	Da	Score	Expect
(F)SVSGEGEGDATY(G)		586.44	0.20	22.1	8.80×10^{-8}
(F)SVSGEGEGDATYGKL(T)		735.57	0.23	25.8	5.60×10^{-10}
(F)ARYPDHMKQHDFF(K)		564.96	0.36	15.5	1.80×10^{-5}
(F)KSAMPEGY(V)	Oxidation at position 4	898.46	0.063	17.4	4.60×10^{-8}
(F)KSAMPEGYVQERTIF(F)	Oxidation at position 4	591.51	0.22	23.0	7.90×10^{-7}
(F)KSAMPEGYVQERTIF(F)		586.35	0.39	23.5	2.10×10^{-7}
(F)KSAMPEGYVQERTIFF(K)		635.37	0.38	19.1	1.30×10^{-5}
(Y)VQERTIFFKDDGNY(K)		866.61	0.19	18.0	5.40×10^{-7}
(F)FKDDGNY(K)		429.94	0.25	15.2	4.30×10^{-7}
(F)EGDTLVNRIEL(K)		1258.72	0.056	17.6	1.50×10^{-5}
(F)KEDGNILGHKLEY(N)		506.34	0.41	22.9	7.20×10^{-7}
(F)KEDGNILGHKLEYNYNSHNVY(I)		836.71	0.31	27.4	2.20×10^{-8}
(Y)NYNSHNVY(I)		505.94	0.22	15.1	1.20×10^{-6}
(Y)IMADKQKNGIKVNF(K)	Oxidation at position 2	541.64	0.34	18.3	2.90×10^{-5}
(Y)IMADKQKNGIKVNF(K)		803.74	0.30	21.0	2.50×10^{-7}
(F)KIRHNIEDGSVQLADHY(Q)		666.12	0.45	31.3	2.20×10^{-8}
(F)KIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHY(L)		989.79	0.55	18.1	1.80×10^{-5}
(L)ADHYQQNTPIGDGPVLLPDNHY(L)		822.52	0.46	23.6	1.10×10^{-6}
(L)ADHYQQNTPIGDGPVLLPDNHYLSTQSAL(S)		1055.73	0.22	16.0	5.60×10^{-6}
(Y)QQNTPIGDGPVLLPDNHY(L)	Gln→pyro-Glu at position 1	981.18	0.21	24.9	5.00×10^{-9}
(Y)QQNTPIGDGPVLLPDNHY(L)		989.62	0.13	17.7	5.50×10^{-7}
(Y)QQNTPIGDGPVLLPDNHYL(S)		697.77	0.082	17.2	5.90×10^{-5}
(Y)QQNTPIGDGPVLLPDNHYLSTQSAL(S)		893.78	0.33	18.7	3.10×10^{-6}