SUPPORTING INFORMATION

Table S1. Cortex peptidoglycan analysis of WT and Δ*spoVK* strains. Extracted peptidoglycan from developing forespores of the wild type and Δ*spoVK* strains harvested 5 h after the onset of sporulation was digested with mutanolysin and separated using HPLC. Muropeptides were identified based on previous studies of elution times[1,2]. Peaks were then integrated, and peptidoglycan structural parameters were calculated. Errors are S.D.

PG species	WT	Δ*spoVK*
% muramic acid as lactam	33.3 ± 1.5	6.5 ± 3.0
% muramic acid with alanine	20.6 ± 0.6	19.5 ± 0.9
% muramic acid with tripeptide	15.9 ±1.9	42.9 ± 3.7
% muramic acid with tetrapeptide	30.2 ± 0.4	31.2 ± 1.4
% muramic acid with pentapeptide	0.0 ± 0.0	0.0 ± 0.0
% peptide as tetrapeptide	65.6 ± 3.0	42.2 ± 2.5
% peptide as tripeptide	34.4 ± 3.0	57.8 ± 2.5
% peptide in crosslinks	19.0 ± 0.6	20.4 ± 2.2
% peptide in effective crosslinks	19.0 ± 0.6	20.4 ± 2.2
% disaccharide units in disaccharides	36.4 ± 3.0	87.1 ± 6.0
% disaccharide units in tetrasaccharides	54.7 ± 2.8	12.9 ± 6.0
% disaccharide units in hexasaccharides	9.0 ± 0.5	0.0 ± 0.0
% lactam in regular distribution	82.1 0.9	100.0 ± 0.0
% disaccharide peptide crosslinked	23.8 ± 0.3	20.4 ± 2.2
% tetrasaccharide peptide crosslinked	5.0 ± 0.2	0.0 ± 0.0
% lactam reduced	0.2 ± 0.0	0.2 ± 0.0
% tripeptide crosslinked	39.6 ± 0.8	31.1 ± 1.7
% tetrapeptide crosslinked	8.2 ± 0.5	5.7 ± 4.7
% disaccharide per effective crosslink	11.5 ± 0.7	6.6 ± 0.4
% muramic acid with crosslink	8.8 ± 0.5	15.1 ± 0.9

[1]DOI: 10.1128/JB.182.16.4491-4499.2000
[2]DOI: 10.1128/jb.178.22.6451-6458.1996

Table S2. Bacterial Strains.

Bacillus subtilis strains used in this study		
Strain	Genotype or description	Reference
PY79	Prototrophic derivative of *B. subtilis* 168	Youngman et al., 1984
KP73	*ΔspoIVA::neo*	Price and Losick, 1999
KR160	*thrC::gfp-spoIVA spec*	Ramamurthi and Losick, 2008
TD549	*ΔspoVK::erm thrC::gfp-spoIVA spec*	This study
TD520	*ΔspoVK::erm*	This study
TD513	*ΔspoVK::erm amyE::spoVK cat*	This study
TD514	*ΔspoVK::erm amyE::spoVK*^{A5V} *cat*	This study
JPC221	*ΔspoIVA::neo thrC::spoIVA*^{T70A,T71A} *spec*	Castaing et al., 2013
TD524	*ΔspoIVA::neo thrC:: spoIVA*^{T70A,T71A} *spec ΔspoVK::erm*	This study
TD530	*ΔspoIVA::neo thrC:: spoIVA*^{T70A,T71A} *spec ΔspoVK::erm amyE::spoVK cat*	This study
TD531	*ΔspoIVA::neo thrC:: spoIVA*^{T70A,T71A} *spec ΔspoVK::erm amyE::spoVK*^{A5V} *cat*	This study
TD523	*ΔspoIVA::neo thrC::spoIVA*^{D97A} *spec ΔspoVK::erm*	This study
TD528	*ΔspoIVA::neo thrC::spoIVA*^{D97A} *spec ΔspoVK::erm amyE::spoVK cat*	This study
TD529	*ΔspoIVA::neo thrC::spoIVA*^{D97A} *spec ΔspoVK::erm amyE::spoVK*^{A5V} *cat*	This study
KR438	*ΔspoIVA::neo thrC::spoIVA*^{K30E} *spec*	Ramamurthi and Losick, 2008
TD817	*ΔspoIVA::neo thrC::spoIVA*^{K30E} *spec ΔspoVK::erm*	This study
TD818	*ΔspoIVA::neo thrC::spoIVA*^{K30E} *spec ΔspoVK::erm amyE::spoVK cat*	This study
TD819	*ΔspoIVA::neo thrC::spoIVA*^{K30E} *spec ΔspoVK::erm amyE::spoVK*^{A5V} *cat*	This study
TD845	*ΔspoIVA::neo amyE::spoIVA*^{D97A} *cat thrC::GFP-spoIVA*^{D97A} *spec ΔspoVK::erm pyrD::spoVK cat::tet*	This study
TD846	*ΔspoIVA::neo amyE::spoIVA*^{T70A,T71A} *cat thrC::GFP-spoIVA*^{T70A,T71A} *spec ΔspoVK::erm pyrD::spoVK cat::tet*	This study
TD854	*ΔspoIVA::neo amyE::spoIVA*^{K30E} *cat thrC::GFP-spoIVA*^{K30E} *spec ΔspoVK::erm pyrD::spoVK cat::tet*	This study
TD848	*ΔspoIVA::neo amyE::spoIVA*^{D97A} *cat thrC::GFP-spoIVA*^{D97A} *spec ΔspoVK::erm pyrD::spoVK*^{A5V} *cat::tet*	This study
TD849	*ΔspoIVA::neo amyE::spoIVA*^{T70A,T71A} *cat thrC::GFP-spoIVA*^{T70A,T71A} *spec ΔspoVK::erm pyrD::spoVK*^{A5V} *cat::tet*	This study
TD855	*ΔspoIVA::neo amyE::spoIVA*^{K30E} *cat thrC::GFP-spoIVA*^{K30E} *spec ΔspoVK::erm pyrD::spoVK*^{A5V} *cat::tet*	This study
JB103	*ΔspoIVA::neo thrC::spoIVA*^{D97A} *spec*	This study
TD604	*amyE::spoVK-linker-GFP cat*	This study
TD652	*ΔspoIVA::neo amyE::spoVK-linker-GFP cat*	This study
TD675	*ΔspoVK::erm amyE::spoVK cat pyrD::spoVK-linker-GFP cat::tet*	This study
TD682	*ΔspoIVA::neo thrC::spoIVA*^{D97A} *ΔspoVK::erm amyE::spoVK cat pyrD::spoVK-linker-GFP cat::tet*	This study
TD684	*ΔspoIVA::neo thrC::spoIVA*^{T70A,T71A} *ΔspoVK::erm amyE::spoVK cat pyrD::spoVK-linker-GFP cat::tet*	This study

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TD836	ΔspoIVA::neo thrC::spoIVA^{K30E} ΔspoVK::erm amyE::spoVK cat pyrD::spoVK-linker-GFP cat::tet	This study
TD557	ΔspoIVA::neo thrC::spoIVA^{D97A} spec amyE::spoVK cat	This study
TD558	ΔspoIVA::neo thrC::spoIVA^{D97A} spec amyE::spoVK^{A5V} cat	This study
TD563	ΔspoIVA::neo thrC::spoIVA^{T70A,T71A} spec amyE::spoVK cat	This study
TD564	ΔspoIVA::neo thrC::spoIVA^{T70A,T71A} spec amyE:: spoVK^{A5V} cat	This study
TD859	ΔspoIVA::neo thrC::spoIVA^{K30E} spec amyE::spoVK cat	This study
TD860	ΔspoIVA::neo thrC::spoIVA^{K30E} spec amyE:: spoVK^{A5V} cat	This study
TD574	ΔspoVK::erm amyE::spoVK$^{Δ2-42}$ cat	This study
TD575	ΔspoVK::erm amyE::spoVK^{K105A,T106A} cat	This study
TD576	ΔspoVK::erm amyE::spoVK^{D162A,E163A} cat	This study
TD578	ΔspoVK::erm amyE::spoVK^{R218A,R276A} cat	This study
TD597	amyE::spoVK cat	This study
TD598	amyE::spoVK$^{Δ2-42}$ cat	This study
TD599	amyE::spoVK^{K105A,T106A} cat	This study
TD600	amyE::spoVK^{D162A,E163A} cat	This study
TD602	amyE::spoVK^{R218A,R276A} cat	This study
TD883	amyE::P$_{spoVID}$-spoVK^{D162A,E163A}-His$_6$ cat	This study
TD884	amyE::P$_{spoVID}$-spoVK^{D162A,E163A}-FLAG cat	This study
TD1193	amyE::P$_{spoVID}$-spoVK^{A5V,D162A,E163A}-FLAG cat	This study
TD651	ΔspoVID::neo amyE::spoVK-linker-GFP cat	This study
TD695	ΔspoVID::neo amyE::spoVID^{T532G} cat pyrD::spoVK-linker-GFP cat::tet	This study
TD1257	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE::spoVID$^{501-575}$ pyrD::spoVK-linker-GFP cat::tet	This study
KR394	ΔspoIVA::neo thrC::spoIVA spec	Ramamurthi et al., 2006
JB171	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec	This study
JB174	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE::spoVID cat	This study
TD892	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE::spoVID$^{501-575}$	This study
TD893	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE::spoVID$^{526-575}$	This study
TD1268	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE::P$_{spoVID}$-spoVK$^{D162A-E163A}$-FLAG cat	This study
TD1281	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE:: P$_{spoVID}$-spoVK$^{D162A-E163A}$-FLAG cat sacA::GFP-spoVID$^{501-575}$ cat::tet	This study
TD1267	ΔspoIVA::neo thrC::spoIVA spec amyE:: P$_{spoVID}$-spoVK$^{D162A-E163A}$-FLAG cat	This study
TD1277	ΔspoIVA::neo thrC::spoIVA spec amyE:: P$_{spoVID}$-spoVK$^{D162A-E163A,Δ2-6}$-FLAG cat	This study
TD1283	ΔspoIVA::neo thrC::spoIVA spec amyE:: P$_{spoVID}$-spoVK$^{D162A-E163A,A5V}$-FLAG cat	This study
TD1278	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE::P$_{spoVID}$-spoVK$^{D162A-E163A,Δ2-6}$-FLAG cat	This study
TD1284	ΔspoIVA::erm ΔspoVID::neo thrC::spoIVA spec amyE:: P$_{spoVID}$-spoVK$^{D162A-E163A,A5V}$-FLAG cat	This study
SC765	amyE::P$_{spoVM}$-SpoVM-Iphluorin-cat	This study
SC766	amyE::P$_{spoIIQ}$-Iphluorin-cat	This study
SC767	amyE::P$_{spoVM}$-Iphluorin-cat	This study
SC777	thrC::P$_{hyperspank}$-Iphluorin-erm	This study

Escherichia coli strains used in this study		
Strain	Genotype and description	Reference
BL21(DE3)	*pTD211 (pET28a* backbone, P_{T7}-*spoVK-His*$_6$)	This study
BL21(DE3)	*pTD308* (*pET28a* backbone, P_{T7}- *spoVK*^{D162A,E163A}-*His*$_6$)	This study

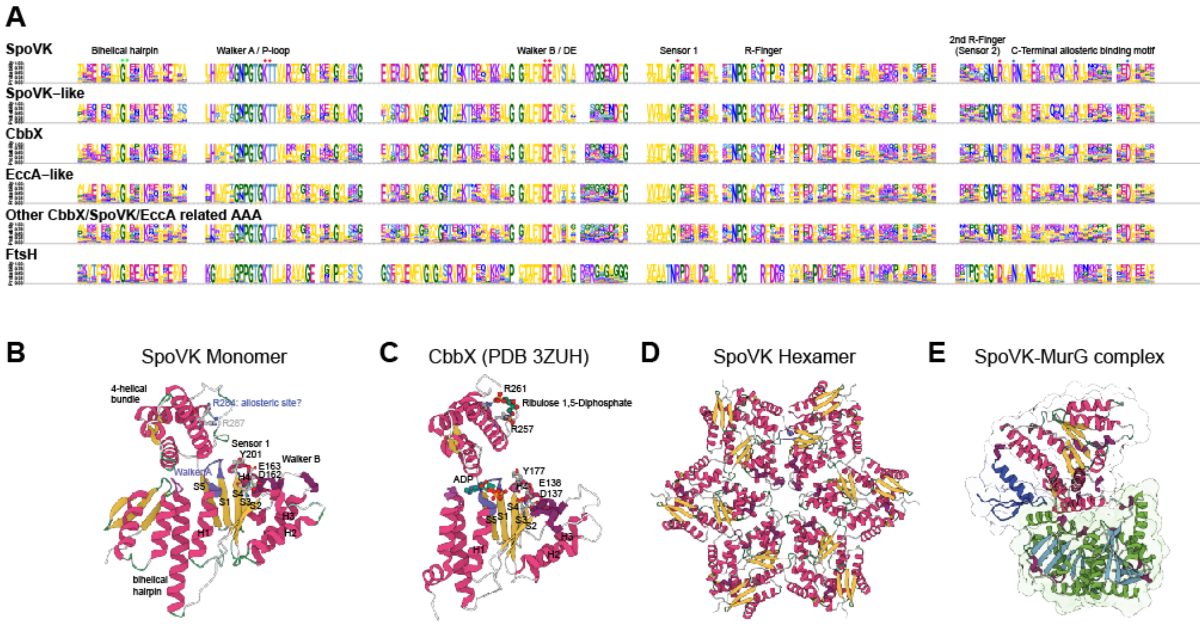

Figure S1. Sequence and structural comparison between SpoVK and members of the SpoVK-EccA-like AAA+ ATPase clade. (A) Sequence logo displaying conservation of amino acid residues in the SpoVK-EccA-like AAA+ ATPase clade. Letters represent amino acid abbreviations; height of each letter represents the probability of conservation among orthologs of the family. The first and second arginine fingers are marked with red dots. Blue dots are conserved residues contacting the allosteric ligand ribulose bisphosphate. (B-E) Cartoon depictions of (B) AlphaFold2 prediction of SpoVK structure, (C) CbbX from *Cereibacter sphaeroides* (PDB 3ZUH), (D) AlphaFold-Multimer prediction of SpoVK hexamer, and (E) AlphaFold-Multimer prediction of SpoVK-MurG complex.

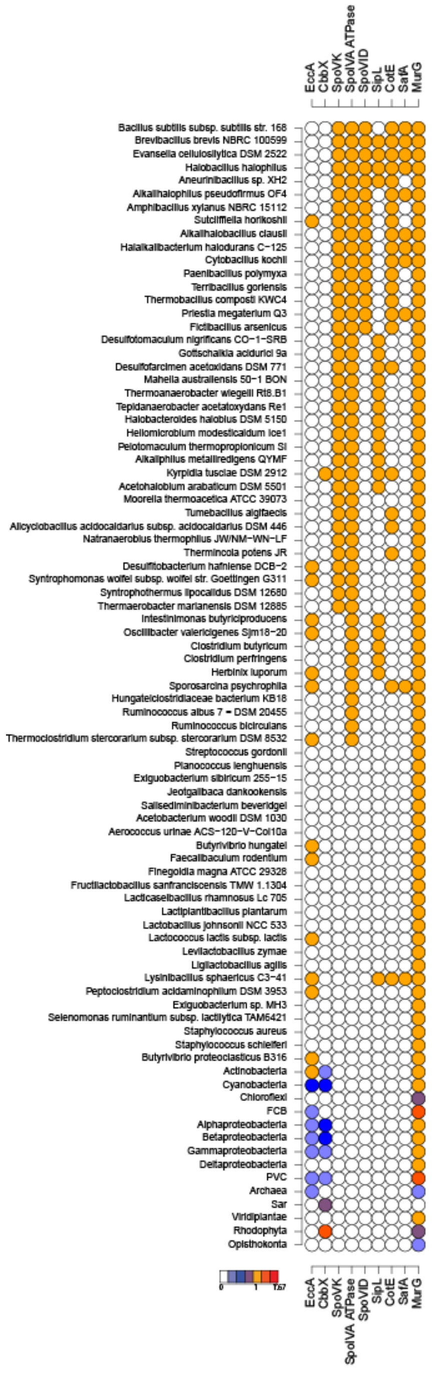

Figure S2. Phyletic pattern vectors of EccA, CbbX, SpoVK, SpoIVA ATPase, SpoVID, SipL, CotE, SafA, and MurG domain proteins. The presence or absence of the protein in the clade or organism (Firmicutes) are shown. The legend shows the color gradient for normalized counts that were scaled by square root.

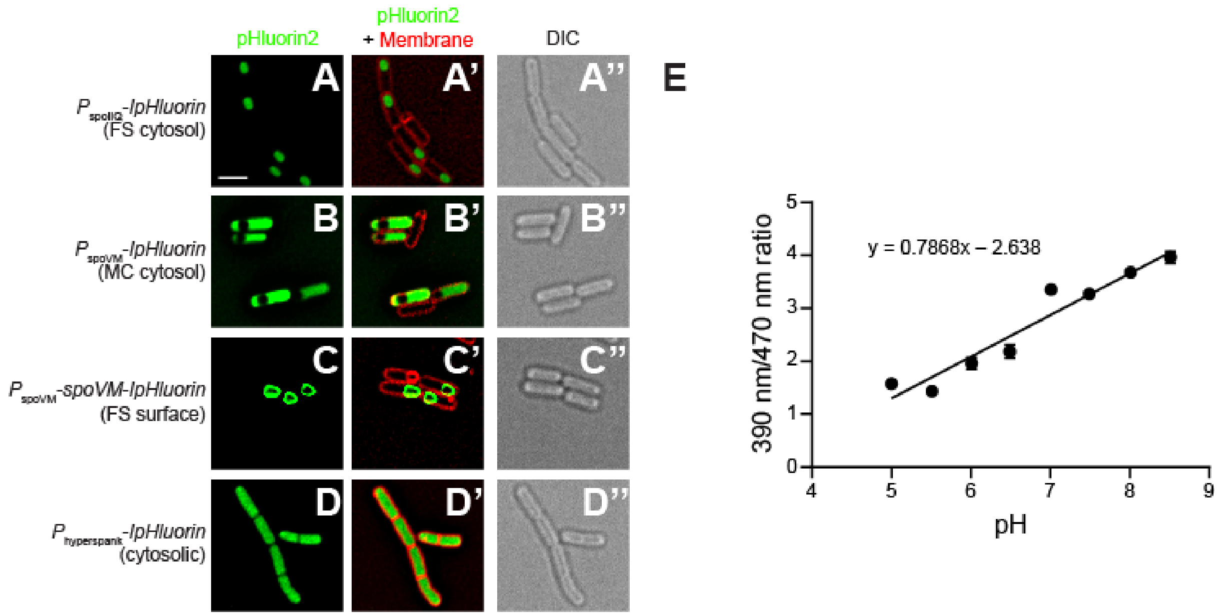

Figure S3. Subcellular localization of various IpHluorin constructs during sporulation. Fluorescence micrographs of *B. subtilis* (A-C'') at t = 4 h after induction of sporulation producing (A-A'') IpHluorin in the forespore, expressed under control of the *spoIIQ* promoter, (B-B'') IpHluorin in the mother cell, expressed under control of the *spoVM* promoter, or (C-C'') SpoVM-IpHluorin at the forespore surface, expressed in the mother cell under control of the *spoVM* promoter; or (D-D'') during vegetative growth producing IpHluorin, expressed under control of an IPTG-inducible promoter at t = 2 h after IPTG induction. (A-D) fluorescence from IpHluorin; (A'-D') overlay, IpHluorin and membranes visualized using FM4-64; (A''-D'') differential interference contrast. Strains: SC765, SC766, SC767, and SC777. Scale bar: 2 μm. (E) Calibration curve of ratio of fluorescence emission at 510 nm when excited at either 390 nm or 470 nm ratio as a function of media pH.

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