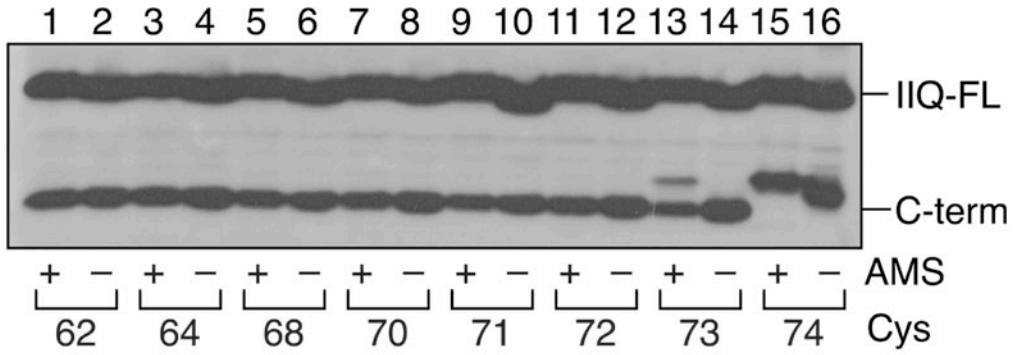
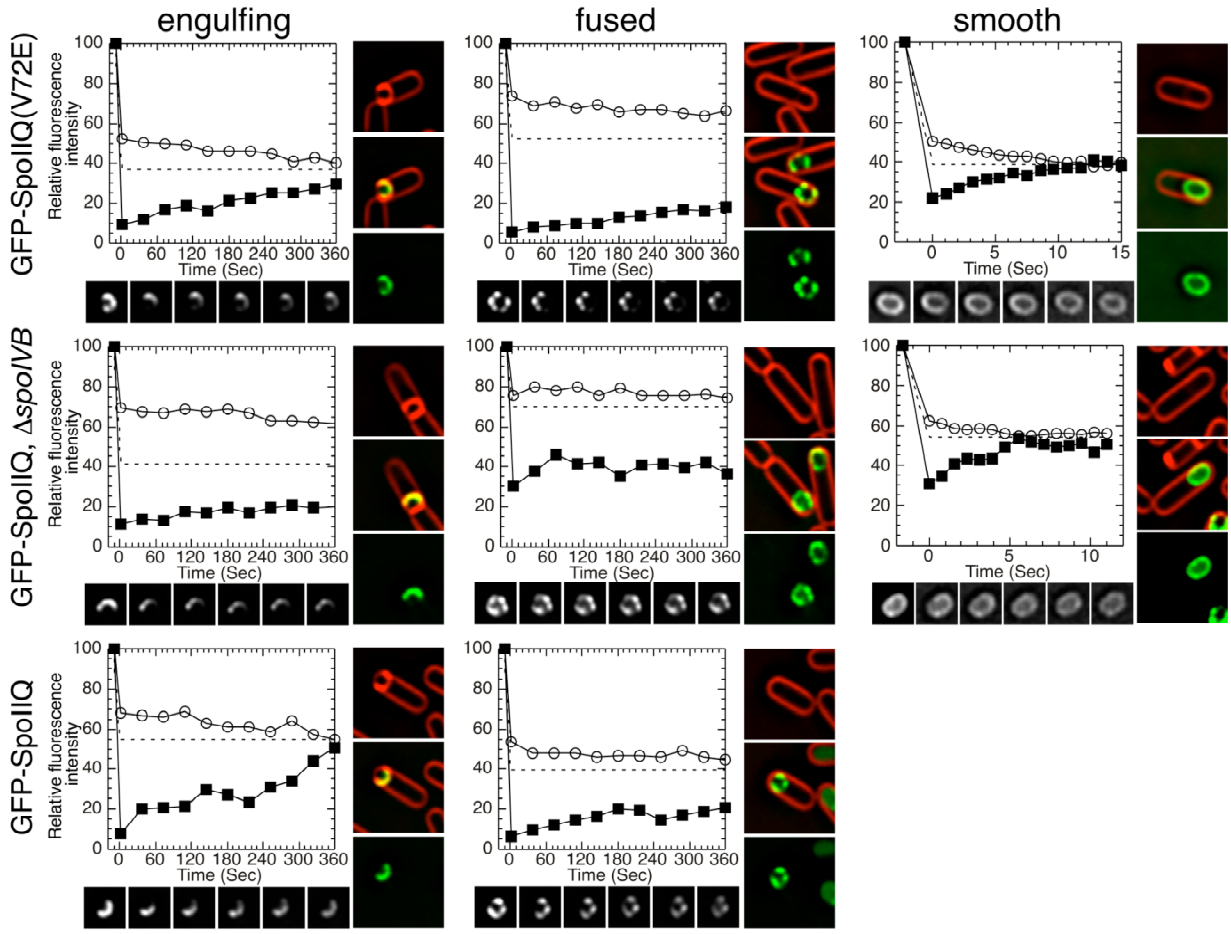
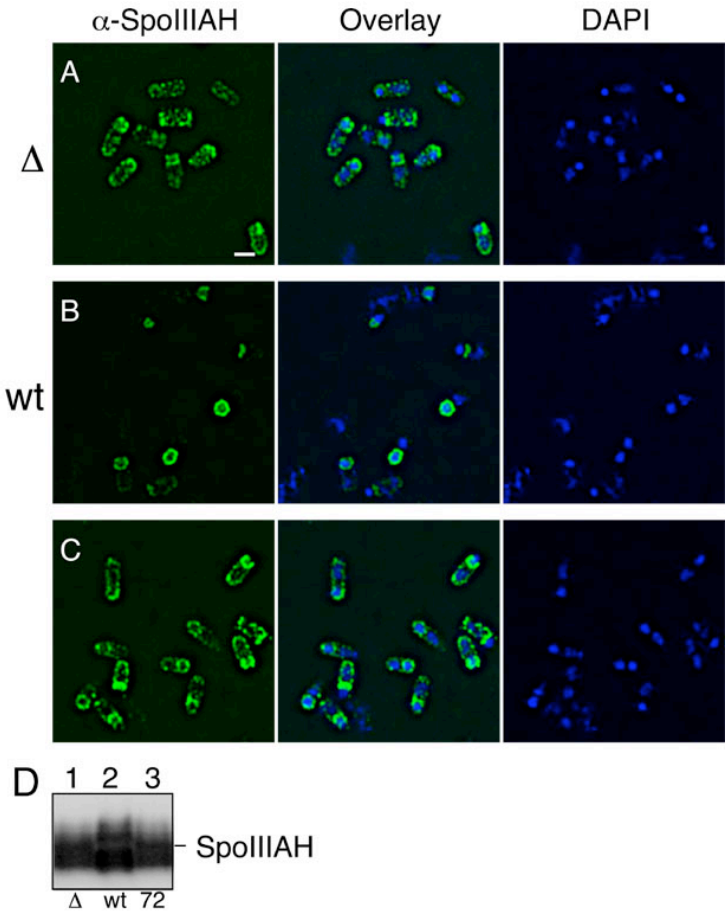
Supplemental Figures and Tables



Supplemental Fig 1. Identification of cleavage site by AMS modification of mono-Cys derivatives of SpoIIQ. Numbers at the bottom indicate position where cysteine was introduced. Whole cell lysates from t_4 of sporulation were incubated with (odd numbered lanes) or without (even numbered lanes) AMS at 37°C for 1 hour, and analyzed by Western blotting using anti-SpoIIQ antiserum. The size shift in the C-terminal fragment following AMS treatment (lane 13 and lane 15) indicates that the cysteine residue is within this fragment.



Supplemental Fig 2. FRAP analysis of GFP-SpoIIQ in the presence and the absence of *spoIVB* and cleavage-defectiveV72Y mutant in engulfing and fused septa performed and quantified as described in Methods. Images of GFP (green) and FM 4-64 stained membrane (red) of bleached cells are to the right of each graph. Images below each plot show the GFP fluorescence during the experiment; the second panel shows the cell just after bleaching. Recovery kinetics were quantified and plotted to show the mean pixel intensity of the bleached (filled square) and unbleached (empty circle) regions and the theoretical pixel intensity value following equilibration between these regions (dashed line).



Supplemental Fig 3. Localization and accumulation level of SpoIIIAH with *spoIIQ*$^{1\text{-}72}$. **(A-C)** Subcellular localization of SpoIIIAH was examined by immunofluorescence using anti-SpoIIIAH polyclonal antiserum. SCB3 (*ΔspoIIQ*; A), SCB4 (*spoIIQ*$^{+}$; B) and SCB74 (*spoIIQ*$^{1\text{-}72}$; C) was sampled at $t_{2.5}$ and probed by anti-SpoIIIAH antiserum (green). **(D)** Cellular accumulation level of SpoIIIAH (at $t_{2.5}$) was examined by Western blotting by probing whole cell lysate from SCB3 (*ΔspoIIQ*; lane 1), SCB4 (*spoIIQ*$^{+}$*;* lane 2) and SCB74 (*spoIIQ*$^{1\text{-}72}$; lane 3) with anti-SpoIIIAH antiserum.

Table S1: Plasmids used in these studies

Plasmid	Insert	Reference
pCH505	*amyE*::P_{spoIIQ}*spoIIQΩcat*	(1)
pCH507	*amyE*::P_{spoIIQ}*gfp(Δ2-6)-spoIIQΩcat*	This study
pCH510	*amyE*::P_{spoIIQ}*spoIIQ-his*$_6$*Ωcat*	This study
pCH528	*amyE*::P_{spoIIQ}*spoIIQA62CΩcat*	This study
pCH530	*amyE*::P_{spoIIQ}*spoIIQD64CΩcat*	This study
pCH531	*amyE*::P_{spoIIQ}*spoIIQD68CΩcat*	This study
pCH532	*amyE*::P_{spoIIQ}*spoIIQV70CΩcat*	This study
pCH533	*amyE*::P_{spoIIQ}*spoIIQE71CΩcat*	This study
pCH534	*amyE*::P_{spoIIQ}*spoIIQV72CΩcat*	This study
pCH535	*amyE*::P_{spoIIQ}*spoIIQG73CΩcat*	This study
pCH538	*amyE*::P_{spoIIQ}*spoIIQV72RΩcat*	This study
pCH539	*amyE*::P_{spoIIQ}*spoIIQV72YΩcat*	This study
pCH540	*amyE*::P_{spoIIQ}*spoIIQV72NΩcat*	This study
pCH541	*amyE*::P_{spoIIQ}*spoIIQV72SΩcat*	This study
pCH542	*amyE*::P_{spoIIQ}*spoIIQV72AΩcat*	This study
pCH543	*amyE*::P_{spoIIQ}*spoIIQV72IΩcat*	This study
pCH544	*amyE*::P_{spoIIQ}*spoIIQV72HΩcat*	This study
pCH545	*amyE*::P_{spoIIQ}*spoIIQV72KΩcat*	This study
pCH546	*amyE*::P_{spoIIQ}*spoIIQV72EΩcat*	This study
pCH547	*amyE*::P_{spoIIQ}*spoIIQV72MΩcat*	This study
pCH548	*amyE*::P_{spoIIQ}*spoIIQG73VΩcat*	This study
pCH549	*amyE*::P_{spoIIQ}*spoIIQG73EΩcat*	This study
pCH550	*amyE*::P_{spoIIQ}*spoIIQ1-72Ωcat*	This study
pCH551	*amyE*::P_{spoIIQ}*spoIIQG73LΩcat*	This study
pCH552	*amyE*::P_{spoIIQ}*spoIIQG73PΩcat*	This study
pCH553	*amyE*::P_{spoIIQ}*spoIIQG73TΩcat*	This study
pCH554	*amyE*::P_{spoIIQ}*spoIIQG73IΩcat*	This study
pCH555	*amyE*::P_{spoIIQ}*spoIIQG73SΩcat*	This study
pCH556	*amyE*::P_{spoIIQ}*spoIIQK74EΩcat*	This study
pCH557	*amyE*::P_{spoIIQ}*spoIIQK74IΩcat*	This study
pCH559	*amyE*::P_{spoIIQ}*spoIIQK74RΩcat*	This study
pCH560	*amyE*::P_{spoIIQ}*spoIIQK74AΩcat*	This study
pCH561	*amyE*::P_{spoIIQ}*spoIIQK74NΩcat*	This study
pCH562	*amyE*::P_{spoIIQ}*spoIIQK74SΩcat*	This study
pCH563	*amyE*::P_{spoIIQ}*spoIIQK74PΩcat*	This study
pCH564	*amyE*::P_{spoIIQ}*spoIIQK74DΩcat*	This study
pCH565	*amyE*::P_{spoIIQ}*spoIIQK74GΩcat*	This study
pCH574	*amyE*::P_{spoIIQ}*gfp(Δ2-6)-spoIIQV72YΩcat*	This study
pCH579	*amyE*::P_{spoIIQ}*gfp(Δ2-6)-spoIIQV72EΩcat*	This study
pCH687	*gst-spoIVFA-flag*	This study
pCH689	*gst-spoIIQV72A*	This study
pCH690	*gst-spoIIQV72M*	This study
pGST-SpoIIQ	*gst-spoIIQ*	(2)
pZR53	*spoIVB-His*$_6$	(3)

Table S2: Primers used in plasmid construction and mutagenesis	
Name	**Sequence**
SP1	5'-GCTGAGGTGATGAAACAATGGGATCCAAGCTTACTAGTAG-3'
SP2	5'-CAGAAGACACTGAACAGTCTCATCACCATCACCATCACTAA-TGAAGAAAACGTCTATC-3'
SP3	5'-CAACGACGATGCAGTTGAANNNGGAAAGTCAATGGAAAAT-3'
SP4	5'-CGACGATGCAGTTGAAGTANNNAAGTCAATGGAAAATGTC-3'
SP5	5'-CGATGCAGTTGAAGTAGGANNNTCAATGGAAAATGTCGCA-3'
SP6	5'-ATGTCATGGATCCATTAAGTCATTTTTCGGAGTA-3'
SP7	5'-CGTACGTGGATCCTTATTCAAATGAAATCACCTGA-3'
SP8	5'-TTCAGGTGATTTCATTTGAAGACTATAAAGACGACGACGAC-AAATAAGGATCCGATCAGACCAG-3'
SP9	5'-ATTGGACCCGTCAGTCAGGGATCCATTAAACCCGCCGTAGCC-3'
SP10	5'-TGATAACGGCGGAAACTCCTGTTATGACAACAACGACGAT-3'
SP11	5'-CGGCGGAAACTCCGCATATTGCAACAACGACGATGCAGTT-3'
SP12	5'-CGCATATGACAACAACGACTGTGCAGTTGAAGTAGGAAAG-3'
SP13	5'-TGACAACAACGACGATGCATGTGAAGTAGGAAAGTCAATG-3'
SP14	5'-CAACAACGACGATGCAGTTTGTGTAGGAAAGTCAATGGAA-3'
SP15	5'-CAACGACGATGCAGTTGAATGTGGAAAGTCAATGGAAAAT-3'
SP16	5'-CGACGATGCAGTTGAAGTATGTAAGTCAATGGAAAATGTC-3'
SP17	5'-CGATGCAGTTGAAGTAGGATGTTCAATGGAAAATGTCGCA-3'

Either A, T, G or C appears randomly at the position shown by N.

Supplemental references

1. Jiang, X., Rubio, A., Chiba, S., and Pogliano, K. (2005) *Mol Microbiol* **58**, 102-115
2. Blaylock, B., Jiang, X., Rubio, A., Moran, C. P., Jr., and Pogliano, K. (2004) *Genes Dev* **18**, 2916-2928
3. Zhou, R., and Kroos, L. (2005) *Mol Microbiol* **58**, 835-846