Supplemental data

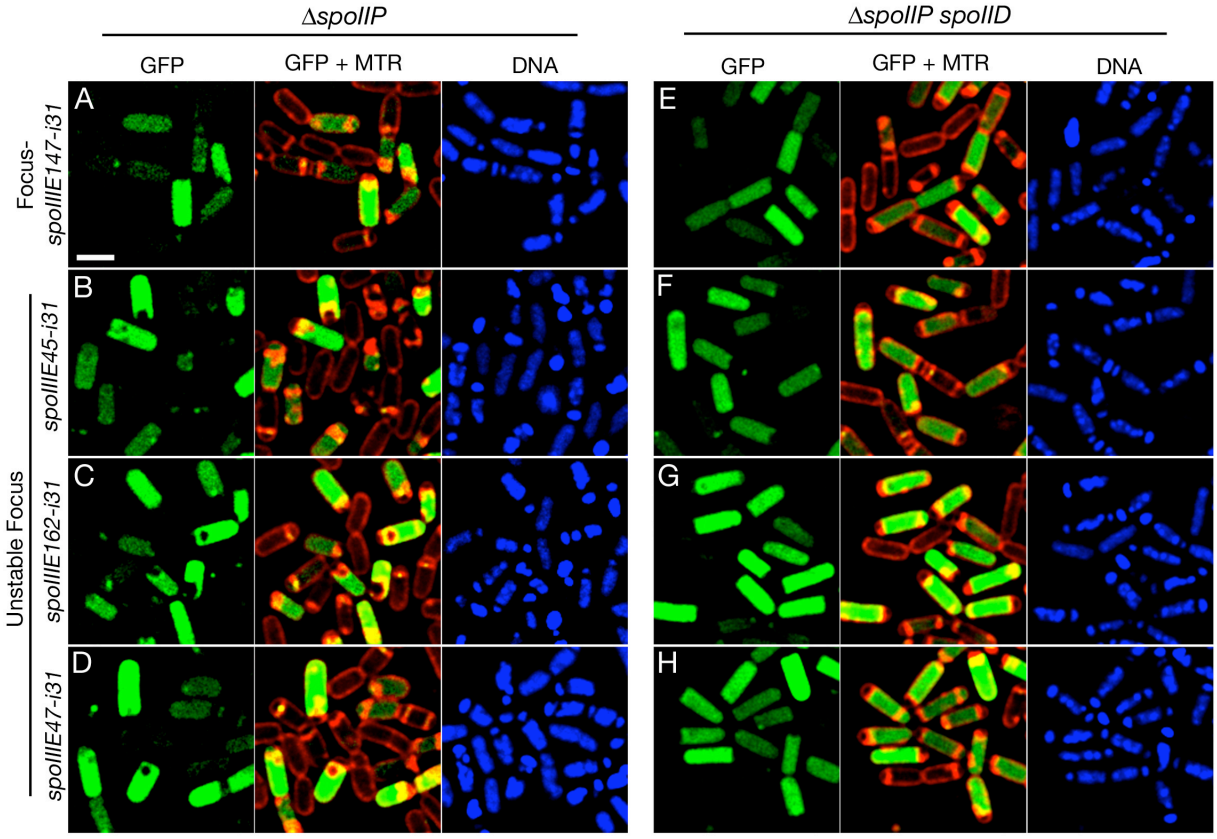

Figure S1: Engulfment mutants rescue the GFP diffusion defect in SpoIIIE assembly mutants. Images were collected at t_3, with membranes stained with Mitotracker Red and DNA with DAPI (blue). (**A)** Focus defective insertion *spoIIIE147-i31* in *spoIIP* (KP6149). (**B**) Unstable focus insertion *spoIIIE45-i31* in *spoIIP* (KP6151). (**C**) Unstable focus insertion *spoIIIE162-i31* in *spoIIP* (KP6152). (**D**) Unstable focus insertion *spoIIIE47-i31* in *spoIIP* (KP6153). (**E**) Focus defective *spoIIIE147-i31* in *spoIIP spoIID* (KP6176). (**F**) Unstable focus insertion *spoIIIE45-i31* in *spoIIP spoIID* (KP6177). (**G**) Unstable focus insertion *spoIIIE162-i31* in *spoIIP spoIID* (KP6178). (**H**) Unstable focus insertion *spoIIIE47-i31* in *spoIIP spoIID* (KP6179). For each of these mutant proteins at t_3, the *spoIIP* mutation improved the ability of the protein to block GFP diffusion (by about 50%), while introducing both *spoIIP* and *spoIID* mutations almost completely rescued the diffusion defect (by about 97%, Table S1). Thus, septal peptidoglycan is sufficient to prevent GFP diffusion in *spoIIIE* mutants that are completely or partially defective in focus assembly. Scale bar in A is 2 μm.

Table S1: GFP diffusion in engulfment mutant backgrounds

	Percent sporangia with GFP confined to mother cell			
***spoIIIE* mutant**			***+ spoIIP::tet***	***+ spoIIP::tet, spoIID::Tn917***
wild type	t_2	100	99	99
	t_3	100	98	98
spoIIIE36	t_2	97	97	99
	t_3	100	98	98
Δ*spoIIIE*	t_2	0	62	97
	t_3	0	48	95
Focus – *spoIIIE147-i31*	t_2	37	68	93
	t_3	7	46	95
Unstable Focus *spoIIIE45-i31*	t_2	69	81	97
	t_3	55	74	98
Unstable Focus *spoIIIE162-i31*	t_2	67	91	98
	t_3	35	73	97
Unstable Focus *spoIIIE47- i31*	t_2	40	73	96
	t_3	18	56	97

An average of 297 sporangia was scored for each strain at each time point.

Table S2: Strains used in this study

Strain	Genotype	Source
KP92	*spoIIIE36*	Wu and Errington, 1994
KP141	*ΔspoIIIE::spc*	Pogliano *et al.* 1997
KP514	*cotE-gfpΩkan, spoIIP::tet*	This study
KP723	*spoIIIE36, cotE-gfpΩkan*	Sharp and Pogliano, 1999
KP725	*spoIIIE::spec, cotE-gfpΩkan*	Sharp and Pogliano, 1999
KP6149	*amyE::spoIIIE147-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet*	This study
KP6151	*amyE::spoIIIE45-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet*	This study
KP6152	*amyE::spoIIIE162-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet*	This study
KP6153	*amyE::spoIIIE47-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet*	This study
KP6174	*spoIIIE36, cotE-gfpΩkan, spoIIP::tet*	This study
KP6175	*spoIIIE::spec, cotE-gfpΩkan, spoIIP::tet*	This study
KP6176	*amyE::spoIIIE147-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study
KP6177	*amyE::spoIIIE45-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study
KP6178	*amyE::spoIIIE162-i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study
KP6179	*amyE::spoIIIE47- i31-'spoIIIE-gfpΩcat, ΔspoIIIE::spc, cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study
KP6180	*cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study
KP6181	*spoIIIE36, cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study
KP6182	*ΔspoIIIE::spec, cotE-gfpΩkan, spoIIP::tet, spoIID::Tn917Ωmls*	This study