TABLE A1. Scoring of engulfment

Strain	Time	Total cells	% Migration	% Fusion
KP718 (WT)	T3	67	7.4	64
	T4	121	5.6	76
KP1072 (E88A)	T3	370	4.2	0
	T4	213	0	0
KP1079 (R106A)	T3	400	19.8	0.8
	T4	302	22.2	2
KP1099 (K203A)	T3	319	1.3	0
	T4	331	3.6	0
KP1102 (D210A)	T3	569	40.2	4.4
	T4	351	39.6	13.4

Quantification of engulfment in the various mutants studied here. Membrane fusion assays were scored for the percent of cells showing exclusion of FM 4-64 from the forespore membrane, indicating the completion of engulfment (% complete). The same images were scored to determine the percent of cells showing significant membrane migration, as indicated by the curving of the septal membrane more than half of the way around the forespore (% migration). This shows that SpoIID^{E88A} and SpoIID^{K203A} have a defect in septal thinning, with no membrane migration or complete engulfment observed at any timepoint. These numbers also demonstrate that SpoIID^{D210A} has a decreased rate of membrane migration, since only 44.6% of cells have migrated more than half of the distance around the forespore or completed engulfment at T3. If this strain was blocked after membrane migration, then one would expect a similar percent of cells with membrane migration as in wildtype, or 71.4% of cells should show migration at least halfway at T3. SpoIID^{R106A} supports membrane migration at an even slower rate than SpoIID^{D210A}.

TABLE A2. Primers used in this study.

Primer Name	Sequence	Purpose
JGP58	ACA CAG ATG TTC GCA GTG TAT AAA AGC	Q134A
JGP59	ATG TTC CAG GTG GCA AAA AGC AAA GCG	Y136A
JGP70	ACA AGC AAC GGC TAC GCA GAG AATG CAG AAG CT	T188A
RMSIIDM1	GAA AAC ATT CCG CTT GCG GAG TAT GTG ATT GGA G	E78A
RMSIIDM2	C TCC AAT CAC ATA CTC CGC AAG CGG AAT GTT TTC	E78A
RMSIIDM3	CCT GAA GCG CTG GCG GCC CAG GCG CTT GCC	K99A
RMSIIDM4	GGC AAG CGC CTG GGC CGC CAG CGC TTC AGG	K99A
RMSIIDM5	GCG CTG AAA GCC GCG GCG CTT GCC GCC	Q101A
RMSIIDM6	GGC GGC AAG CGC CGC GGC TTT CAG CGC	Q101A
RMSIIDM7	GCG CTT GCC GCC GCA ACA TTT ATT GTC AG	R106A
RMSIIDM8	CT GAC AAT AAA TGT TGC GGC GGC AAG CGC	R106A
RMSIIDM9	GCC GCC AGA ACA GCG ATT GTC AGA CTG ATG G	F108A
RMSIIDM10	C CAT CAG TCT GAC AAT CGC TGT TCT GGC GGC	F108A
RMSIIDM11	GGC AAA ATC TTA ACG GCC AAC AAC CAG CCG	Y171A
RMSIIDM12	CGG CTG GTT GTT GGC CGT TAA GAT TTT GCC	Y171A
RMSIIDM13	C CCA TAT TTA GCA AGC GTC AAA AGC CC	K203A
RMSIIDM14	GG GCT TTT GAC GCT TGC TAA ATA TGG G	K203A
RMSIIDM15	C CCA TAT TTA AAA GCC GTC AAA AGC CC	S204A
RMSIIDM16	GG GCT TTT GAC GGC TTT TAA ATA TGG G	S204A
RMSIIDM17	GC CCA TGG GCT AAA AAG TCT CCG	D210A
RMSIIDM18	GG GCT TTT GAC GGC TTT TAA ATA TGG G	D210A
RMSIIDM19	C GTC GCC TCC GCA ATG CCG GCA ACC	E88A
RMSIIDM20	GGT TGC CGG CAT TGC GGA GGC GAC G	E88A
RMSIIDM21	GC CGC TGT AAA GGT TTT CGC TGC CTT ATA TTT CGG	T219A
RMSIIDM22	GG GCT TTT GAC GGC TTT TAA ATA TGG G	T219A
JGP71	TTG ATT TGC GGT TCG TGC GAC GGG A	Y65A
JGP72	TTT CAG CGC TTC AGG TGC AAA GGT	K94A
JGP73	CTG GGC TTT CAG CGC TGC AGG TTT	E96A
JGP74	CAG TCT GAC AAT AAA TGC TCT GGC G	T107A
JGP75	TGG GCT TTT GAC GCT TGC TAA ATA T	K203A
JGP76	CCA TGG GCT TTT GAC TGC TTT TAA A	S204A
JGP77	CTT TTT ATC CCA TGG TGC TTT GAC	S207A
JGP78	TGC CTT ATA TTT CGG TGC CTT TTT	S213A
JGP79	TTT CGT TGC CTT ATA TGC CGG AGA	K215A
JGP80	CTG AAA TTC TGC CGC TGC AAA GGT	T223A
JGP81	AAG CTT TTG CTG AAA TGC TGC CGC T	E226A
JGP82	GAC GCC AAG CTT TTG TGC AAA TTC T	Q228A
JGP83	CAG CTT GAC GCC AAG TGC TTG CTG A	K230A
JGP84	GCT AGA TCC ATC CAG TGC GAC GCC A	K234A
JGP85	GGCTCCACTGGTGGCAGATACACAGATG	D128A

JGP86	TCA CTG GTG GAT GCA ACA CAG ATG TTC	D129A
JGP87	CTG GTG GAT GAT GCA CAG ATG TTC CAG	T130A
JGP88	GCG GTA GCC AGT GCA CAA GGC AAA ATC	T164A
JGP89	GGC AAA ATC TTA GCA TAC AAC AAC CAG	T170A
JGP90	AAG GCA ACG AAA GCA TTT ACA GCG GCA	T221A
JGP91	CTG GAT GGA TCT GCA GCA GTA GGG AAG	S239A
JGP92	ATT AAC GGC AAG GCA CTG AAA GGA AGA	T262A
JGP93	AAG CGA AAT GGA GCA AGA ATT ACA GTC	D286A
JGP94	GAT TTT GAA TGG GCA CGA AAT GGA GAC	K282A
JGP95	TTT GAA TGG AAG CGA AAT GGA GAC ACA	R283A
JGP96	ATT CCC GTC TAT GCA ACC GCA AAT CAA	R66A
JGP97	TTC CAG GTG TAT GCA AGC AAA GCG GAG	K137A
JGP98	ACA GAG AAT GCA GCA GCT TAT TGG ACA	E192A
JGP99	TAT AAG GCA ACG GCA ACC TTT ACA GCG	K220A
JGP100	GCA GAA TTT CAG GCA AAG CT GGC GTC	Q229A
JGP101	TCC AAT CAC ATA TGC TTC AAG CGG AAT	E79A
JGP102	GAC TCC AAT CAC TGC CTC TTC AAG CGG	T80A
JGP103	AAA GGT TGC CGG TGC TTC GGA GGC GAC	M89A
JGP104	TTT AAA GGT TGC TGC CAT TTC GGA GGC	P90A
JGP105	GCC GTT GCT TGT TGC GAA AAA GGA TGC	S182A
JGP106	GTA GCC GTT GCT TGC GGA GAA AAA GGA	T183A
JGP107	GAC GCT TTT TAA TGC TGG GAT AGC ACT	Y201A
JGP108	CAT CCC CAC ACC TGC GCC AAA TCC TCT	H297A
JGP109	TTG GCT CAT CCC TGC ACC GTG GCC AAA	V299A
JGP110	TCC GTA TTG GCT TGC CCC CAC ACC GTG	M301A
JGP111	ATT CGC TCC GTA TGC GCT CAT CCC CAC	Q303A
JGP112	AAG CGG AAT GTT TGC TAC GGA TTG ATT	E73A
JGP113	GAA GCA TCC TTT GCA TCC ACA AGC AAC	F181A
JGP116	GGT TGC CGG CAT TTC TGC GGC GAC GAC	S87A
JGP117	CAC ACC GTG GCC TGC TCC TCT CGT CG	F295A
JGPD358	GAAGCTCCTAAAGGCTCACTG	sequencing
JGP2dp	GCTTGTCCCTGCCCATAGACT	sequencing
RMSP10IID	GGCATGGATGAACTATACAAAATGAAACAATTCGCAATCACAC	GFP-IID
RMSP11IID	GTGTGATTGCGAATTGTTTCATTTTGTATAGTTCATCCATGCC	GFP-IID
RMSP12IID	G CAT AAT AAG GAA GCG GGG GCC AGC G	GFP-IID
RMSP13IID	C GCT GGC CCC CGC TTC CTT ATT ATG C	GFP-IID

MOVIE A1. GFP-SpoIIP localization in wild type. Cells were sporulated at 30°C in the presence of FM4-64 and placed on a 1.2% agarose/A+B pad containing FM4-64 at t_2 after sporulation initiation. Cells were imaged on the pad inside a climate-controlled chamber at 30°C. Membranes were stained with FM 4-64. Images were collected five minutes apart for 1 hr. Membrane migration is seen in the majority of cells in this strain and engulfment takes approximately 50 minutes to complete. During the early stages of migration, GFP-SpoIIP forms bright foci at the leading edges of the membranes. When the membranes reach halfway, GFP-SpoIIP becomes an elongated focus, forming a gradient that stretches from the leading edge backwards. After completion of migration, GFP-SpoIIP forms a diffuse cap around the top half of the forespore.

Membrane migration is usually symmetric in this strain with the leading edges meeting directly at the top of the cell. However, in some cells, the membranes appear to inchworm up, with the left side moving slightly ahead in one frame and the right side moving slightly ahead in the next. This suggests that the membranes don’t move as a single sheet up and around the forespore.

MOVIE A2. GFP-SpoIIP localization in a SpoIID^{D210A} background. Cells prepared as described for Movie A1. Membrane migration is seen in few cells in this strain. Often, open bulges form early and become closed in later frames. The rate of membrane migration is hard to assess due to the failure of many sporangia to complete engulfment during the data collection period, but is likely to be >60 minutes. GFP-SpoIIP is seen in three distinct patterns corresponding with different membrane migration events. In large blebs, GFP-SpoIIP is diffuse throughout the bleb, sometimes forming a focus at the center of the septum. In evenly migrating membranes, GFP-SpoIIP forms two, equally bright foci, one at each leading edge. In asymmetrically migrating cells, GFP-SpoIIP forms a bright foci at the leading edge that is that migrating more quickly (more advanced) and a weak, or no, focus at the lagging edge. GFP-SpoIIP tends to be dynamic in its localization in this strain, with lower intensity than the wildtype background and with changing intensities in many foci and throughout blebs. Together, these results indicate that the SpoIID^{D210A} mutation is partially defective in the stabilization of SpoIIP to the leading edge and that SpoIIP localization is associated with an increased rate of membrane migration.