Table S1: Strains used in this study

STRAIN NAME	ALTERNATE NAME	GENOTYPE	ORIGIN
FGSC 2489	wild-type	*74-OR23-1VA*	FGSC
FGSC 11042	Δ*adv-1*	*adv-1::hph A*	FGSC
FGSC 12550	Δ*amph-1*	*amph-1::hph A*	FGSC
FGSC 22020	Δ*arg-15*	*arg-15::hph A*	FGSC
FGSC 20615	Δ*bem-1*	*bem-1::hph A*	FGSC
FGSC 11404	Δ*cse-1*	*cse-1::hph A*	FGSC
FGSC 20306	Δ*csp-6*	*csp-6::hph a*	FGSC
	Δ*doc-1*	*doc-1::hph A*	(HELLER *et al.* 2016)
FGSC 14644	Δ*doc-2*	*doc-2::hph A*	FGSC
	Δ*doc-1* Δ*doc-2*	*doc-1 doc-2::hph A*	(HELLER *et al.* 2016)
	Δ*doc-1* Δ*ham-11*	*doc-1::hph; ham-11::hph a*	this study
	Δ*doc-2* Δ*ham-11*	*doc-2::hph; ham-11::hph a*	this study
	Δ*doc-1* Δ*doc-2* Δ*ham-11*	*doc-1 doc-2::hph; ham-11::hph a*	this study
FGSC 20086	Δ*gyp-5*	*gyp-5::hph A*	FGSC
FGSC 21396	Δ*ham-10*	*ham-10::hph A*	FGSC
FGSC 17545	Δ*ham-11*	*ham-11::hph A*	FGSC
	Δ*ham-11*	*ham-11::hph a*	this study
FGSC 17234	Δ*ham-12*	*ham-12::hph A*	FGSC
FGSC 14337	Δ*ham-14*	*ham-14::hph A*	FGSC
FGSC 14577	Δ*ham-2*	*ham-2::hph A*	FGSC
FGSC 11299	Δ*ham-3*	*ham-3::hph A*	FGSC
FGSC 12081	Δ*ham-4*	*ham-4::hph A*	FGSC
FGSC 15046	Δ*ham-5*	*ham-5::hph A*	FGSC
FGSC 16993	Δ*ham-6*	*ham-6::hph A*	FGSC
FGSC 13776	Δ*ham-7*	*ham-7::hph A*	FGSC
FGSC 17225	Δ*ham-8*	*ham-8::hph a*	FGSC
FGSC 19549	Δ*ham-9*	*ham-9::hph a*	FGSC
FGSC 13351	Δ*lao-1*	*lao-1::hph A*	FGSC
FGSC 11326	Δ*mik-1*	*mik-1::hph A*	FGSC
FGSC 12362	Δ*mob-3*	*mob-3::hph a*	FGSC
FGSC 11213	Δ*nik-2*	*nik-2::hph A*	FGSC
FGSC 12868	Δ*nox-1*	*nox-1::hph A*	FGSC
FGSC 16574	Δ*pkr-1*	*prk1::hph a*	FGSC
FGSC 11547	Δ*ppg-1*	*ppg-1::hph A*	FGSC
FGSC 12467	Δ*ras-2*	*ras-2::hph a*	FGSC
FGSC 12310	Δ*sec-22*	*sec-22::hph A*	FGSC
FGSC 508	Δ*soft*	*soft::hph A*	FGSC
FGSC 12115	Δ*spr-7*	*spr-7::hph A*	FGSC
FGSC 11324	Δ*ste-20*	*ste-20::hph A*	FGSC
FGSC 21578	Δ*whi-2*	*whi-2::hph A*	FGSC
FGSC 6103	wild-type *his-3-*	*his-3 A*	FGSC
FGSC 9716	wild-type *his-3-*	*his-3 a*	FGSC
	Δ*adv-1 his-3-*	*adv-1::hph; his-3 A*	this study
	Δ*amph-1 his-3-*	*amph-1::hph; his-3 A*	this study
	Δ*arg-15 his-3-*	*arg-15::hph; his-3 a*	this study
	Δ*bem-1 his-3-*	*bem-1::hph; his-3 a*	this study
	Δ*cse-1 his-3-*	*cse-1::hph; his-3 A*	this study
	Δ*csp-6 his-3-*	*csp-6::hph; his-3 A*	this study

	Δ*doc-1 his-3-*	*doc-1::hph; his-3 A*	(HELLER *et al.* 2016)
	Δ*doc-1* Δ*doc-2 his-3-*	*doc-1 doc-2::hph; his-3 A*	(HELLER *et al.* 2016)
	Δ*doc-2 his-3-*	*doc-2::hph; his-3 A*	(HELLER *et al.* 2016)
	Δ*doc-1* Δ*ham-11 his-3-*	*doc-1::hph; ham-11::hph; his-3 A*	this study
	Δ*doc-2* Δ*ham-11 his-3-*	*doc-2::hph; ham-11::hph; his-3 A*	this study
	Δ*doc-1* Δ*doc-2* Δ*ham-11 his-*	*doc-1 doc-2::hph; ham-11::hph; his-3 A*	this study
	Δ*gyp-5 his-3-*	*gyp-5::hph; his-3 A*	this study
	Δ*ham-10 his-3-*	*ham-10::hph; his-3 A*	this study
	Δ*ham-11 his-3-*	*ham-11::hph; his-3 A*	this study
	Δ*ham-12 his-3-*	*ham-12::hph; his-3 A*	this study
	Δ*ham-14 his-3-*	*ham-14::hph; his-3 A*	this study
	Δ*ham-2 his-3-*	*ham-2::hph; his-3 a*	this study
	Δ*ham-3 his-3-*	*ham-3::hph; his-3 A*	this study
	Δ*ham-4 his-3-*	*ham-4::hph; his-3 A*	this study
	Δ*ham-5 his-3-*	*ham-5::hph; his-3 A*	this study
	Δ*ham-6 his-3-*	*ham-6::hph; his-3 A*	this study
	Δ*ham-7 his-3-*	*ham-7::hph; his-3 A*	this study
	Δ*ham-8 his-3-*	*ham-8::hph; his-3 A*	this study
	Δ*ham-9 his-3-*	*ham-9::hph; his-3 A*	this study
	Δ*lao-1 his-3-*	*lao-1::hph; his-3 A*	this study
JPG25	Δ*lfd-1 his-3-*	*lfd-1::hph; his-3 a*	(PALMA-GUERRERO *et al.* 2014)
AF-M621	Δ*mak-2 his-3-*	*mak-2::hph; his-3 A*	(FLEISSNER *et al.* 2009b)
	Δ*mek-2 his-3-*	*mek-2::hph; his-3 A*	(DETTMANN *et al.* 2012)
	Δ*mik-1 his-3-*	*mik-1::hph; his-3 A*	this study
	Δ*mob-3 his-3-*	*mob-3::hph; his-3 A*	this study
	Δ*nik-2 his-3-*	*nik-2::hph ; his-3 a*	this study
	Δ*nox-1 his-3-*	*nox-1::hph; his-3 A*	this study
	Δ*nrc-1 his-3-*	*nrc-1::hph; his-3 A*	(DETTMANN *et al.* 2012)
	Δ*pkr-1 his-3-*	*prk-1::hph; his-3 A*	this study
	Δ*pp-1 his-3-*	*pp1::hph; his-3 A*	(LEEDER *et al.* 2013)
	Δ*ppg-1 his-3-*	*ppg-1::hph; his-3 A*	this study
R16-52	Δ*prm-1 his-3-*	*prm-1::hph; his-3 A*	(FLEISSNER *et al.* 2009a)
	Δ*ras-2 his-3-*	*ras-2::hph; his-3 A*	this study
	Δ*sec-22 his-3-*	*sec-22::hph; his-3 A*	this study
AF-H4	Δ*soft his-3-*	*soft::hph; his-3 A*	(FLEISSNER AND GLASS 2007)
	Δ*spr-7 his-3-*	*spr-7::hph; his-3 a*	this study
	Δ*ste-20 his-3-*	*ste-20::hph; his-3 a*	this study
	Δ*whi-2 his-3-*	*whi-2::hph; his-3 a*	this study
WT-Luc9.2	wild-type *(Pprm1-Luciferase)*	*Pprm1-Luciferase::his-3+ A*	this study
	Δ*adv-1 (Pprm1-Luciferase)*	*adv-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*amph-1 (Pprm1-Luciferase)*	*amph-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*arg-15 (Pprm1-Luciferase)*	*arg-15::hph; Pprm1-Luciferase::his-3+*	this study

	Δ*bem-1 (Pprm1-Luciferase)*	*bem-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*cse-1 (Pprm1-Luciferase)*	*cse-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*csp-6 (Pprm1-Luciferase)*	*csp-6::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*doc-1 (Pprm1-Luciferase)*	*doc-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*doc-1* Δ*doc-2 (Pprm1-Luciferase)*	*doc-1 doc-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*doc-2 (Pprm1-Luciferase)*	*doc-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*doc-1* Δ*ham-11 (Pprm1-Luciferase)*	*doc-1::hph; ham-11::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*doc-2* Δ*ham-11 (Pprm1-Luciferase)*	*doc-2::hph; ham-11::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*doc-1* Δ*doc-2* Δ*ham-11 (Pprm1-Luciferase)*	*doc-1 doc-2::hph; ham-11::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*gyp-5 (Pprm1-Luciferase)*	*gyp-5::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-10 (Pprm1-Luciferase)*	*ham-10::hph; Pprm1-Luciferase::his-3+*	this study
Δham-11-Luc3.14	Δ*ham-11 (Pprm1-Luciferase)*	*ham-11::hph; Pprm1-Luciferase::his-3+ A*	this study
	Δ*ham-12 (Pprm1-Luciferase)*	*ham-12::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-14 (Pprm1-Luciferase)*	*ham-14::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-2 (Pprm1-Luciferase)*	*ham-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-3 (Pprm1-Luciferase)*	*ham-3::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-4 (Pprm1-Luciferase)*	*ham-4::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-5 (Pprm1-Luciferase)*	*ham-5::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-6 (Pprm1-Luciferase)*	*ham-6::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-7 (Pprm1-Luciferase)*	*ham-7::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-8 (Pprm1-Luciferase)*	*ham-8::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-9 (Pprm1-Luciferase)*	*ham-9::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*lao-1 (Pprm1-Luciferase)*	*lao-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*lfd-1 (Pprm1-Luciferase)*	*lfd-1::hph; Pprm1-Luciferase::his-3+*	this study
Δmak-2-Luc1.8	Δ*mak-2 (Pprm1-Luciferase)*	*mak-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*mek-2 (Pprm1-Luciferase)*	*mek-2::hph; Pprm1-Luciferase::his-3+ A*	this study
	Δ*mik-1 (Pprm1-Luciferase)*	*mik-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*mob-3 (Pprm1-Luciferase)*	*mob-3::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*nik-2 (Pprm1-Luciferase)*	*nik-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*nox-1 (Pprm1-Luciferase)*	*nox-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*nrc-1 (Pprm1-Luciferase)*	*nrc-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*pkr-1 (Pprm1-Luciferase)*	*prk-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*pp-1 (Pprm1-Luciferase)*	*pp1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ppg-1 (Pprm1-Luciferase)*	*ppg-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*prm-1 (Pprm1-Luciferase)*	*prm-1::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ras-2 (Pprm1-Luciferase)*	*ras-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*sec-22 (Pprm1-Luciferase)*	*sec-22::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*soft (Pprm1-Luciferase)*	*soft::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*spr-7 (Pprm1-Luciferase)*	*spr-7::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ste-20 (Pprm1-Luciferase)*	*ste-20::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*whi-2 (Pprm1-Luciferase)*	*whi-2::hph; Pprm1-Luciferase::his-3+*	this study
	Δ*ham-11 (Ptef1-ham-11-v5)*	Δ*ham-11; Ptef1-ham-11-v5::his-3+*	this study
	wild-type (Pccg1-gfp)	*Pccg1-gfp::his+*	(JONKERS *et al.* 2014)
	Δ*ham-11 (Pccg1-gfp)*	*ham-11::hph; Pccg1-gfp::his-3+*	this study
	Δ*doc-1* Δ*doc-2 (Pccg1-gfp)*	*doc-1 doc-2::hph; Pccg1-gfp::his-3+*	(HELLER *et al.* 2016)
	wild-type (Pccg1-mak-2-gfp)	*Pccg1-mak-2-gfp::his+*	(LEEDER *et al.* 2013)
	Δ*ham-11 (soft-gfp)*	*ham-11::hph; Pccg1-soft-gfp::his-3+*	this study

	wild-type (sec-9^{GRD2})	*plp-1 plp-1::hph; sec-9^{GRD2}::sec-9*	(HELLER *et al.* 2018)
	Δ*ham-11 (sec-9^{GRD2})*	Δ*ham-11::hph; plp-1 plp-1::hph; sec-9^{GRD2}::sec-9*	this study
	Δ*doc-1 (sec-9^{GRD2})*	Δ*doc-1::hph; plp-1 plp-1::hph; sec-9^{GRD2}::sec-9*	this study
	Δ*doc-2 (sec-9^{GRD2})*	Δ*doc-2::hph; plp-1 plp-1::hph; sec-9^{GRD2}::sec-9*	this study
	Δ*doc-1* Δ*doc-2 (sec-9^{GRD2})*	Δ*doc-1* Δ*doc-2::hph; plp-1 plp-1::hph; sec-9^{GRD2}::sec-9*	this study
	JW258	*N. crassa* wild isolate *a*	(HELLER *et al.* 2016)
	P4471	*N. crassa* wild isolate *a*	(HELLER *et al.* 2016)
	JW220	*N. crassa* wild isolate *A*	(HELLER *et al.* 2016)
	wild-type (nrc-1^{P451S})	*Pccg1-3xFLAG-nrc-1^{P451S}::his-3+ A*	(DETTMANN *et al.* 2012)
	wild-type (nrc-1^{P451S})	*Pccg1-3xFLAG-nrc-1^{P451S}::his-3+ a*	(DETTMANN *et al.* 2012)
	Δ*ham-11 (nrc-1^{P451S})*	Δ*ham-11::hph; Pccg1-3xFLAG-nrc-1^{P451S}::his-3+*	this study
	Δ*ste-20 (nrc-1^{P451S})*	Δ*ste-20::hph; Pccg1-3xFLAG-nrc-1^{P451S}::his-3+*	this study

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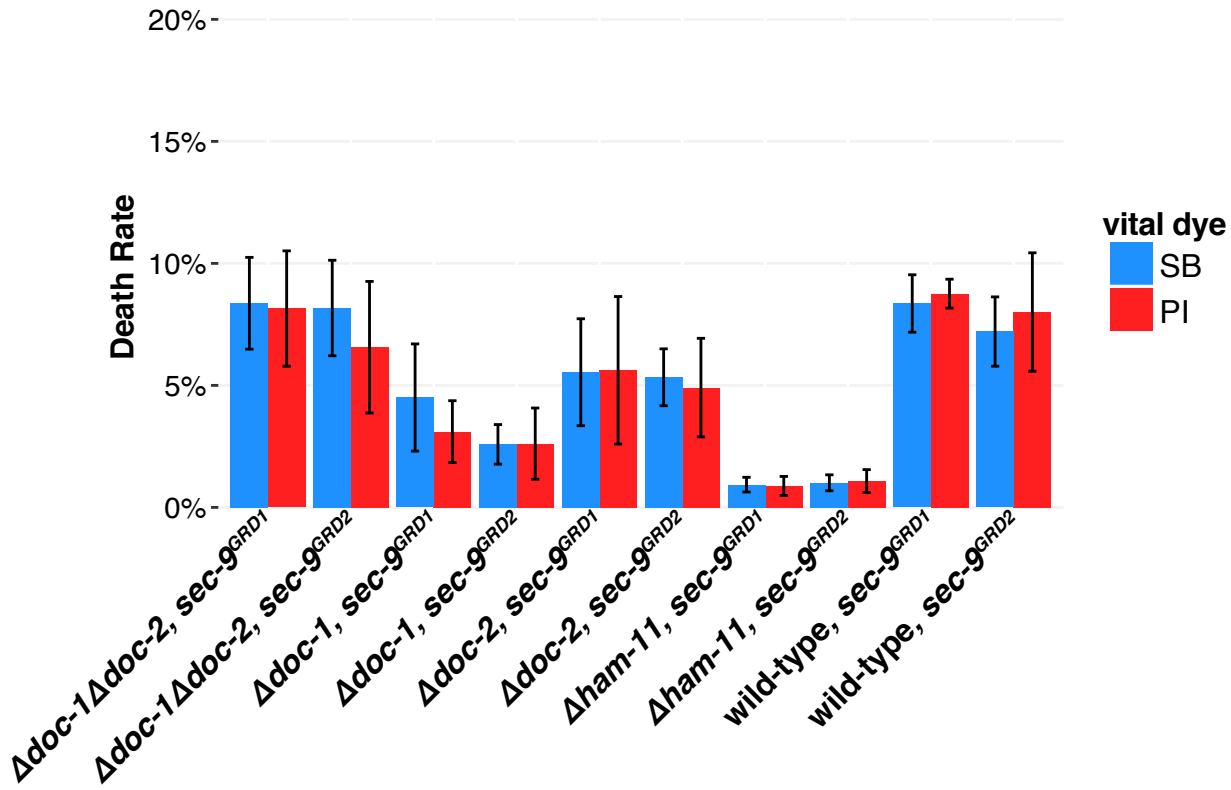
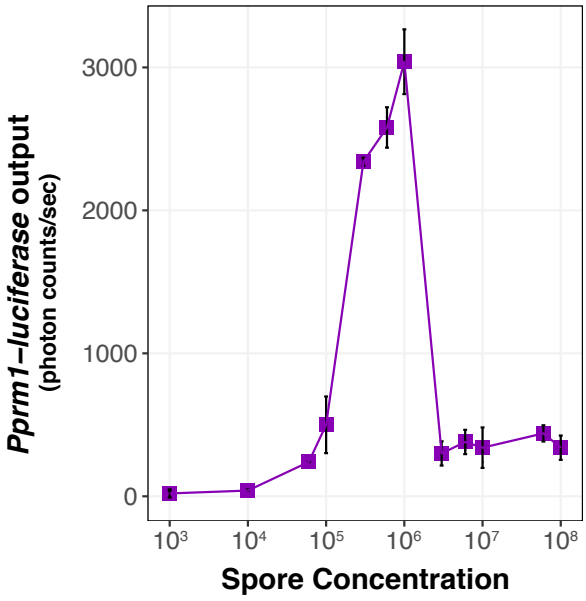
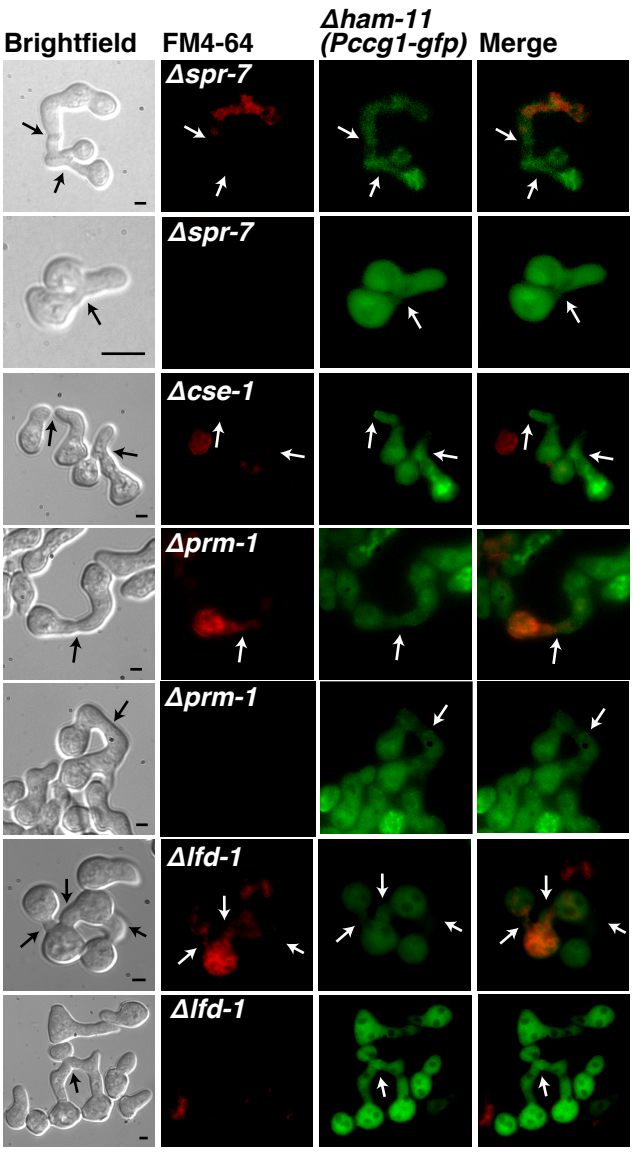


Supplemental Figure 1. Basal death rate of each strain used for flow cytometry germling fusion assay

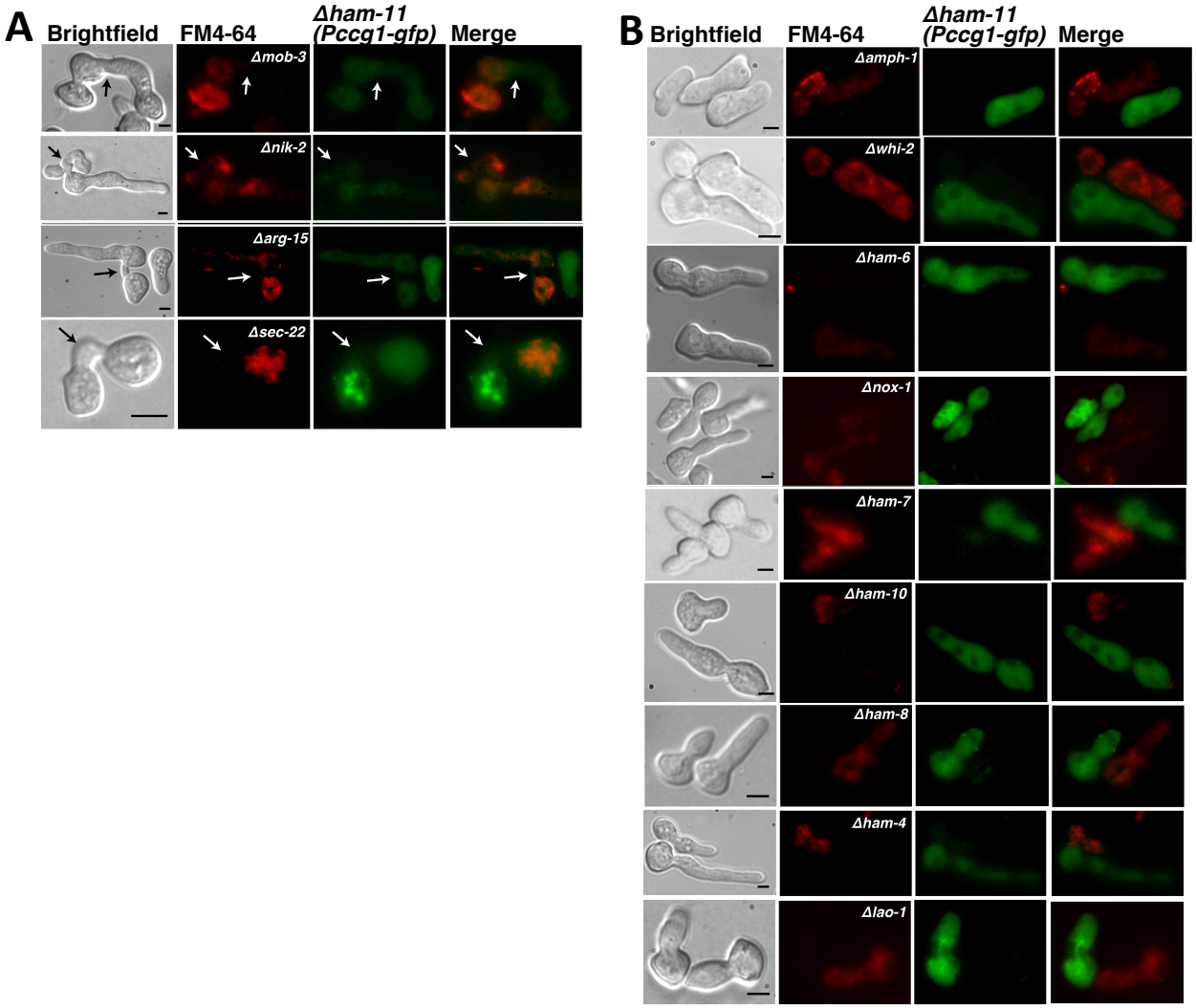
Each strain was grown alone (not in combination with any other strain) and stained with two different vital dyes, SYTOX blue (SB) and Propidium Iodide (PI) and then analyzed via flow cytometry. Conidia were removed from the data analysis and death rate was determined by the proportion of cells for each strain that were stained with a vital dye (dead) versus not stained (alive). Error bars indicate standard deviation (n=5-7).



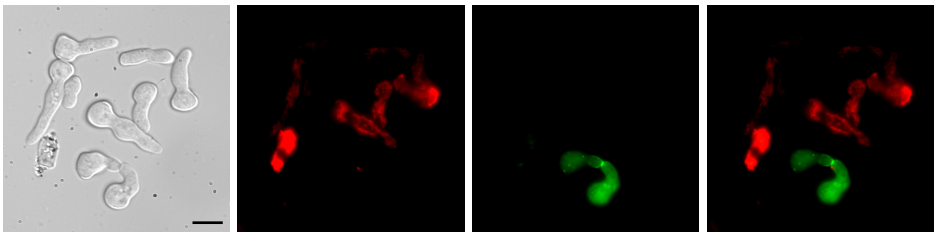
Supplemental Figure 2. Development of P*prm1-luciferase* reporter construct.
Pprm1-luciferase expression in wild-type germlings across a spore inoculum concentration gradient. Error bar indicates standard deviation (n=3).



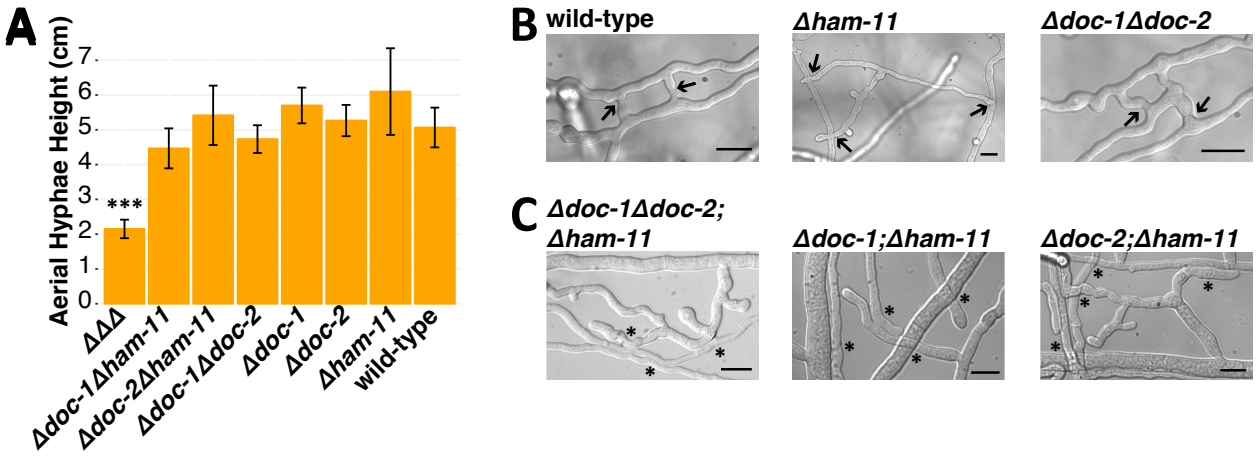
Supplemental Figure 3. Δ*ham-11* self-fusion when co-cultured with *Δspr-7*, *Δcse-1*, *Δprm-1*, and *Δlfd-1* cells. Germling phenotype of *Δham-11 (Pccg1-so-gfp)* cells co-cultured *Δspr-7*, *Δcse-1*, *Δprm-1*, or *Δlfd-1* cells stained with FM4-64. Arrows indicate chemotropic interactions and fusion events. Scale bars = 5μm.



Supplemental Figure 4. Images of *Δham-11 (Pccg1-so-gfp)* germlings co-cultured with FM4-64 stained communication mutants. (A) Arrows indicate chemotropic interactions and fusion events between *Δham-11 (Pccg1-so-gfp)* germlings and FM4-64 stained mutant germlings. **(B)** Absence of chemotropic growth and cell fusion between *Δham-11 (Pccg1-so-gfp)* germlings and representative fusion defective mutants encoding proteins of unknown biochemical/cellular function (*ham-6, ham-7, ham-8 and ham-10*), fusion defective mutants that have characterized orthologs in other species (*amph-1, lao-1, whi-2*), a mutant encoding a subunit of NADPH oxidase (*nox-1*) and a mutant encoding a subunit of the signaling STRIPAK complex (*ham-4*). Scale bars = 5μm.

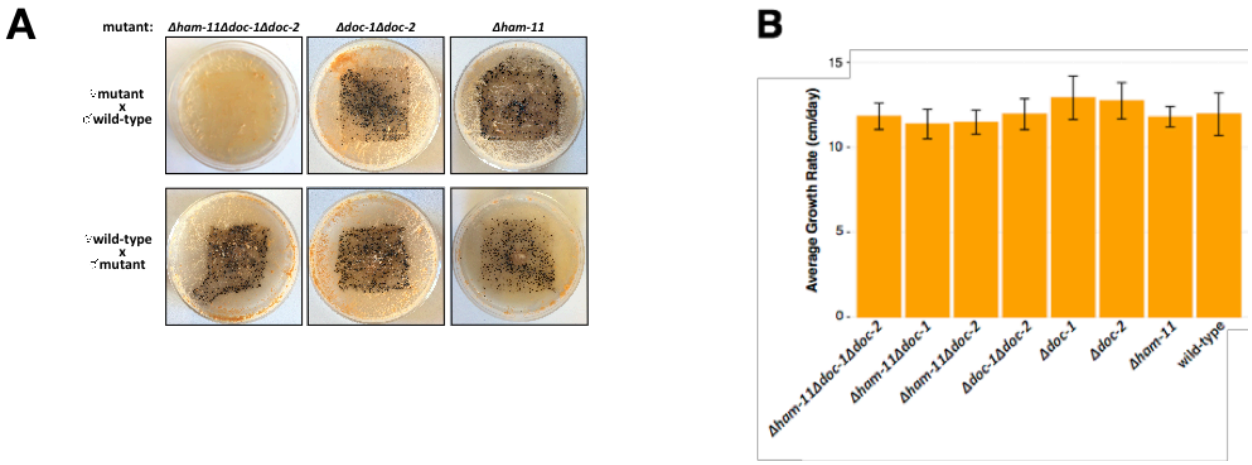


Supplemental Figure 5. Wild-type *(Pccg1-mak-2-gfp)* does not engage in chemotropic interactions with *Δsoft* (stained with FM4-64). scale bar = 10μm



Supplemental Figure 6. The Δdoc-1 Δdoc-2; Δham-11 mutant has a synthetic hyphal fusion and aerial hyphae phenotype.

(A) Aerial hyphae extension in each single, double, and triple mutant and wild-type. 106 conidia were inoculated into 1mL of liquid VMM and incubated at 30C for 4 days before measuring aerial hyphae extension, error bars indicate standard deviation (***p<0.0001, ANOVA+TukeyHSD, n=6). "ΔΔΔ" = Δdoc-1 Δdoc-2; Δham-11. (B) Hyphal phenotype of the Δham-11 mutant and wild-type. Arrows indicate chemotropic interactions and fusion. Scalebar = 30 um. (C) Hyphal phenotypes of each double and triple mutant. Right panel shows the interaction between each mutant stained with FM4-64 and wild-type (Pccg-1-gfp) or Δdoc1 Δdoc2 ("ΔΔ(gfp)" or "Δd1Δd2(gfp)"). Arrows indicate chemotropic interactions and fusion, asterisks indicate areas where hyphae are physically touching, but cell fusion has not occurred. scale bar = 10μm



Supplemental Figure 7. The *Δdoc-1 Δdoc-2; Δham-11* mutant is female sterile but male fertile and has a wild-type-like growth rate

(A) Top panel shows each indicated mutant used as a female in a cross with wild-type males, while the bottom panel shows each indicated mutant used as a male in a cross with wild-type females. Panel on the right shows a typical wild-type x wild-type cross for reference. Male cells were applied in a square pattern on each plate of females. Tiny black perithecia are indicative of successful mating. **(B)** Average growth rate per day at 30C, measured over 4 days, no significant differences between strains (ANOVA+TukeyHSD, n=3). Error bars indicate standard deviation.