

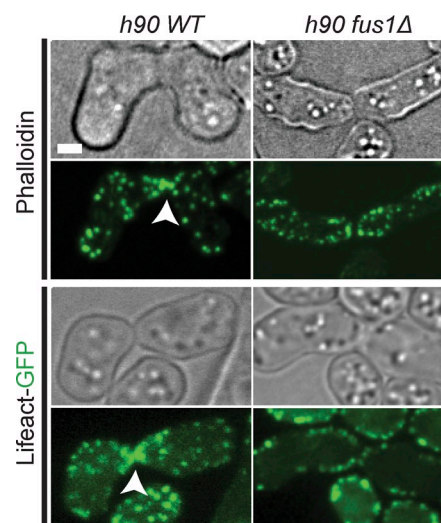
Dudin et al., <http://www.jcb.org/cgi/content/full/jcb.201411124/DC1>

Figure S1. **Fus1-dependent actin accumulation at the prospective fusion site.** (Related to Fig. 1.) Images of homothallic wild-type (WT) *h90* strains (left) fixed and stained with Alex Fluor-phalloidin (top) or live expressing LifeAct-GFP. The arrowheads indicate actin accumulation at the fusion site. (right) In contrast, no actin accumulation is observed in *h90 fus1Δ* strains. Bar, 1  $\mu$ m.

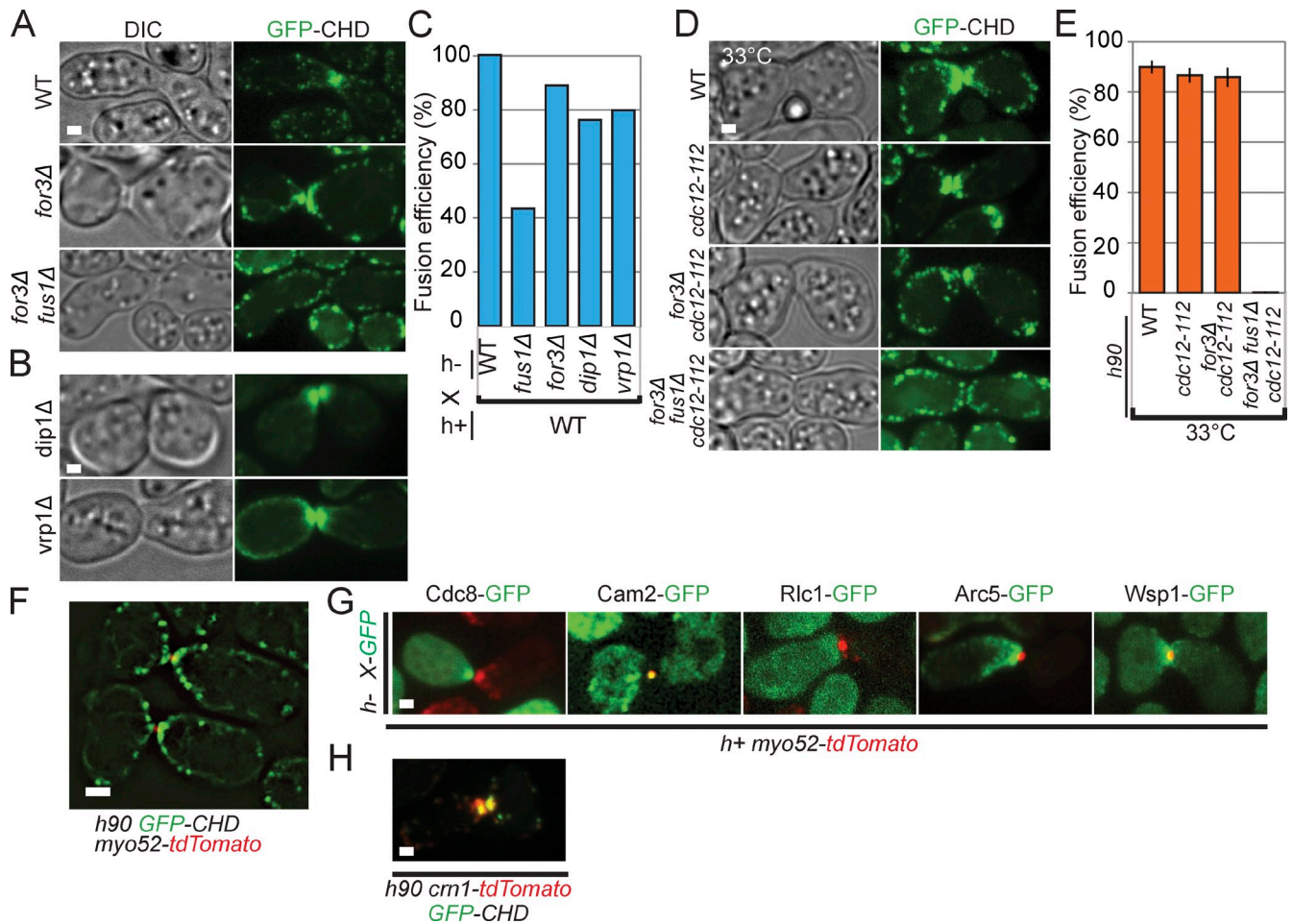


Figure S2. **Fusion focus formation is independent of formins For3 and Cdc12 and of actin patch components.** (Related to Fig. 2.) (A) Homothallic *h90* wild-type (WT), *for3Δ*, and *for3Δ fus1Δ* strains expressing GFP-CHD. An actin focus forms in *for3Δ* but not in *for3Δ fus1Δ*. (B) Homothallic *h90 dip1Δ* and *vrp1Δ* strains expressing GFP-CHD, in which the fusion focus forms normally. (C) Fusion efficiency of indicated heterothallic crosses,  $n > 90$ . (D) Homothallic *h90* wild-type, *cdc12-112*, *cdc12-112 for3Δ*, and *cdc12-112 for3Δ fus1Δ* strains expressing GFP-CHD imaged at 33°C. An actin focus forms in all cases, except upon *fus1* deletion. Note the presence of some aberrant actin structures in the *cdc12-112 for3Δ* double mutant. (E) Fusion efficiency of homothallic *h90* wild-type, *cdc12-112*, *cdc12-112 for3Δ*, and *cdc12-112 for3Δ fus1Δ* mutants at 33°C. For3 and Cdc12 do not contribute during cell-cell fusion. Error bars are standard deviations. (F) 3D SIM of GFP-CHD and Myo52-tdTomato colocalization in homothallic *h90* wild type. This image represents the same cell pair as shown in Fig. 2 F, after which time-lapse imaging of only the GFP channel was acquired. (G) Heterothallic *h-* strains expressing indicated GFP fusions crossed to *h+* *myo52-tdTomato* cells. Cdc8 tropomyosin and Cam2 calmodulin form a dot at the fusion site, but not Rlc1, Arc5, or Wsp1. (H) Homothallic *h90 crn1-tdTomato* GFP-CHD. Crn1 coronin decorates the fusion focus. DIC, differential interference contrast. Bars, 1  $\mu$ m.

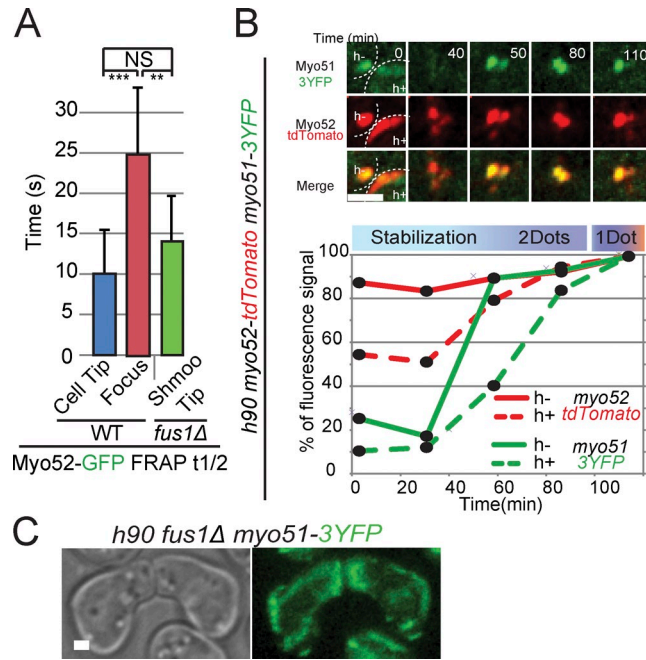


Figure S3. **Type V myosin localization and dynamics define multiple steps in the formation of the fusion focus.** (Related to Fig. 4.) (A) Mean FRAP recovery half-times of Myo52-GFP at the cell tip of vegetative growing cells or during cell-cell fusion in presence or absence of Fus1. Myo52 lower mobility at the fusion focus depends on Fus1. Note that the half-time values measured with Myo52-GFP are somewhat larger than those measured using Myo52-tdTomato (see Fig. 3 C), but the differences between focus and cell tip are similar. *t* test, \*\*\*,  $P = 6.9 \times 10^{-10}$ ; \*\*,  $P = 1.3 \times 10^{-4}$ ;  $n > 12$  for each category. Error bars are standard deviations. WT, wild type. (B, top) Detail of the contact zone of a homothallic *h90 myo52-tdTomato myo51-3YFP* mating pair. (bottom) The graph shows Myo52 and Myo51 fluorescence intensities normalized to the maximum of each fluorophore. Myo51 and Myo52 focalize first in the  $h^-$  cell. This is one representative example out of five such measured mating pairs. Cell contours are shown with dotted lines. (C) Homothallic *h90 fus1Δ myo51-3YFP* strain. Myo51 accumulates as a crescent at the shmoo tip in absence of Fus1. Bars, 1  $\mu$ m.

Table S1. Strains used in this study

Strain number	Genotype	Source
<b>Fig. 1</b>		
2514	<i>h90 p<sup>map3</sup>-tdTomato-ura4+ nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2515	<i>h90 myo52-tdTomato-natMX nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2516	<i>h90 fus1::LEU2 p<sup>map3</sup>-tdTomato-ura4+ nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
<b>Fig. 2</b>		
2515	<i>h90 myo52-tdTomato-natMX nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2517	<i>h90 fus1-sfGFP-kanMX p<sup>map3</sup>-tdTomato-ura4+ ade6<sup>+</sup> leu1+ ura4-D18</i>	This study
2518	<i>h<sup>+</sup> fus1-sfGFP-kanMX myo52-tdTomato-natMX</i>	This study
2519	<i>h<sup>-</sup> fus1-sfGFP-kanMX myo52-tdTomato-natMX</i>	This study
2520	<i>h90 myo52-GFP-kanMX leu1-32</i>	This study
2521	<i>h90 fus1::LEU2 myo52-GFP-kanMX leu1-32</i>	This study
740	<i>h<sup>+</sup> myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
2522	<i>h90 for3::kanMX nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2516	<i>h90 fus1::LEU2 p<sup>map3</sup>-tdTomato-ura4+ nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2587	<i>h90 for3::kanMX fus1::LEU2 nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2523	<i>h<sup>-</sup> cdc12-3GFP-kanMX myo52-tomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2524	<i>h<sup>+</sup> cdc12-3GFP-kanMX myo52-tomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2525	<i>h<sup>-</sup> p<sup>cdc15</sup>-mEGFP-cdc15:: kanMX myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2526	<i>h<sup>+</sup> p<sup>cdc15</sup>-mEGFP-cdc15:: kanMX myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2527	<i>h<sup>-</sup> for3-3GFP-kanMX myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2528	<i>h<sup>+</sup> for3-3GFP-kanMX myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2529	<i>h90 myo52-tdTomato-natMX myo51-3YFP-kanMX leu1-32 ura4-D18</i>	This study
2530	<i>h<sup>-</sup> kanMX-P<sup>myo1</sup>-mGFP-my01 myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2531	<i>h<sup>+</sup> kanMX-P<sup>myo1</sup>-mGFP-my01 myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2532	<i>h<sup>-</sup> myo52-tdTomato-natMX dip1-GFP-kanMX ade6-M216 leu1-32 ura4-D18</i>	This study
2533	<i>h<sup>+</sup> myo52-tdTomato-natMX dip1-GFP-kanMX ade6-M216 leu1-32 ura4-D18</i>	This study
<b>Fig. 3</b>		
2515	<i>h90 myo52-tdTomato-NatMX nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2534	<i>h<sup>-</sup> myo52-GFP-kanMX leu1-32</i>	This study
2535	<i>h90 myo52-tdTomato-natMX p<sup>map3</sup>-GFP-ura4+ ade6-M216 leu1-32 ura4-D18</i>	This study
952	<i>h<sup>-</sup> myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
740	<i>h<sup>+</sup> myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
1273	<i>h<sup>-</sup> nmt41::GFP-CHD-leu<sup>+</sup> ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
2594	<i>h<sup>+</sup> nmt41::GFP-CHD-leu<sup>+</sup> ade6-M216 leu1-32 ura4-D18</i>	This study
2529	<i>h90 myo52-tdTomato-natMX myo51-3YFP-kanMX leu1-32 ura4-D18</i>	This study
2518	<i>h<sup>+</sup> fus1-sfGFP-kanMX myo52-tdTomato-natMX</i>	This study
2519	<i>h<sup>-</sup> fus1-sfGFP-kanMX myo52-tdTomato-natMX</i>	This study
<b>Fig. 4</b>		
1372	<i>h<sup>-</sup> WT (972)</i>	Laboratory stock
1371	<i>h<sup>+</sup> WT (975)</i>	Laboratory stock
2536	<i>h<sup>-</sup> myo52::ura4+ leu1<sup>-</sup> ura4-</i>	This study
1923	<i>h<sup>+</sup> myo52::ura4+ leu1<sup>-</sup> ura4-</i>	Laboratory stock
1532	<i>h<sup>-</sup> myo51::ura4+ ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
2537	<i>h<sup>+</sup> myo51::ura4+ leu1-32 ura4-D18</i>	This study
2538	<i>h<sup>-</sup> myo52::ura4+ myo51::ura4+ ade6<sup>-</sup> ura4-</i>	This study
2539	<i>h<sup>+</sup> myo52::ura4+ myo51::ura4+ leu1<sup>-</sup> ura4-</i>	This study
1024	<i>h<sup>-</sup> fus1::LEU2 ade6-</i>	Laboratory stock
1025	<i>h<sup>+</sup> fus1::LEU2 ade6-</i>	Laboratory stock
1273	<i>h<sup>-</sup> nmt41::GFP-CHD-leu<sup>+</sup> ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
663	<i>h<sup>+</sup> myo51::ura4+ myo52::ura4+ nmt41::GFP-CHD-leu<sup>+</sup> ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
2517	<i>h90 fus1-sfGFP-kanMX p<sup>map3</sup>-tdTomato-ura4+ ade6<sup>+</sup> leu1+ ura4-D18</i>	This study
2541	<i>h90 myo52::ura4+ fus1-sfGFP leu1<sup>-</sup> ura4-</i>	This study
2542	<i>h90 myo51::ura4+ fus1-sfGFP leu1<sup>-</sup> ura4-</i>	This study
2543	<i>h90 myo52::ura4+ myo51::ura4+ fus1-sfGFP leu1<sup>-</sup> ura4-</i>	This study
2544	<i>h90 myo52::ura4+ leu1<sup>-</sup> ura4-</i>	This study
2545	<i>h90 myo51::ura4+ leu1<sup>-</sup> ura4-</i>	This study
1396	<i>h90 WT (968)</i>	Laboratory stock
2546	<i>h<sup>-</sup> myo52<sup>tail</sup>-tdTomato-kanMX ade6-M210 leu1-32 ura4-D18</i>	Lo Presti et al., 2012

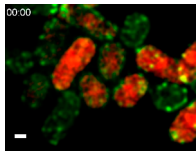
Table S1. Strains used in this study (Continued)

Strain number	Genotype	Source
2005	<i>h<sup>-</sup> myo51<sup>Δtail</sup>-3GFP-ura4<sup>+</sup> ade6-M216 leu1<sup>-</sup> ura4-</i>	Lo Presti et al., 2012
<b>Fig. 5</b>		
2547	<i>h<sup>-</sup> agn1-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2548	<i>h<sup>+</sup> agn1-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2549	<i>h<sup>-</sup> agn2-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2550	<i>h<sup>+</sup> agn2-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2551	<i>h<sup>-</sup> eng1-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2552	<i>h<sup>+</sup> eng1-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2553	<i>h<sup>-</sup> eng2-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2554	<i>h<sup>+</sup> eng2-GFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2555	<i>h<sup>-</sup> exg1-GFP::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2556	<i>h<sup>+</sup> exg1-GFP::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2557	<i>h<sup>-</sup> exg2-GFP::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2558	<i>h<sup>+</sup> exg2-GFP::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2559	<i>h<sup>-</sup> exg3-GFP::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2560	<i>h<sup>+</sup> exg3-GFP::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2561	<i>h<sup>-</sup> agn1::ura4<sup>+</sup> myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2562	<i>h<sup>-</sup> agn2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2563	<i>h<sup>-</sup> eng1::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2564	<i>h<sup>-</sup> eng2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2195	<i>h<sup>-</sup> exg1::kanMX leu1-32</i>	Dueñas-Santero et al., 2010
2565	<i>h<sup>-</sup> exg2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2566	<i>h<sup>-</sup> exg3::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2567	<i>h<sup>-</sup> eng2::kanMX agn2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2595	<i>h<sup>+</sup> exg3::kanMX agn2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2568	<i>h<sup>-</sup> exg3::kanMX agn2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2569	<i>h<sup>-</sup> exg3::kanMX eng2::kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2570	<i>h<sup>-</sup> exg3::kanMX eng2::kanMX agn2::kanMX ade6-M216 leu1-32 ura4-D18</i>	This study
1088	<i>h<sup>-</sup> bgs1::ura4<sup>+</sup> P<sup>bgs1</sup>:GFP-bgs1-leu1<sup>+</sup> leu1-32 ura4-D18 his3-D1</i>	Cortés et al., 2002
806	<i>h<sup>-</sup> bgs4::ura4<sup>+</sup> P<sup>bgs4</sup>:GFP-bgs4-leu1<sup>+</sup> leu1<sup>-</sup> ura4-D18</i>	Cortés et al., 2005
2571	<i>h90 agn2-sfGFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2572	<i>h90 eng2-sfGFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2573	<i>h90 exg3-sfGFP-kanMX myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	This study
2574	<i>h90 agn2-sfGFP-kanMX myo52-tdTomato-natMX fus1Δ::LEU2 ade6<sup>+</sup> leu1-32 ura4-294</i>	This study
2575	<i>h90 eng2-sfGFP-kanMX myo52-tdTomato-natMX fus1Δ::LEU2 ade6<sup>+</sup> leu1-32 ura4-294</i>	This study
2576	<i>h90 exg3-sfGFP-kanMX myo52-tdTomato-natMX fus1Δ::LEU2 ade6<sup>+</sup> leu1-32 ura4-294</i>	This study
2577	<i>h90 myo52::ura4<sup>+</sup> myo51::ura4<sup>+</sup> agn2-sfGFP-kanMX leu1<sup>-</sup> ura4-</i>	This study
2578	<i>h90 myo52::ura4<sup>+</sup> myo51::ura4<sup>+</sup> eng2-sfGFP-kanMX leu1<sup>-</sup> ura4-</i>	This study
2579	<i>h90 myo52::ura4<sup>+</sup> myo51::ura4<sup>+</sup> exg3-sfGFP-kanMX leu1<sup>-</sup> ura4-</i>	This study
952	<i>h<sup>-</sup> myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
2580	<i>h<sup>+</sup> myo51<sup>Δtail</sup>-12myc-ura4<sup>+</sup> agn2-sfGFP-kanMX ade6<sup>+</sup> leu1-32 ura4-D18</i>	This study
2581	<i>h<sup>+</sup> myo52<sup>Δtail</sup>-tdTomato-kanMX agn2-sfGFP-kanMX ade6-M216 leu1-32 ura4-D18</i>	This study
2582	<i>h<sup>+</sup> myo51<sup>Δtail</sup>-12myc-ura4<sup>+</sup> eng2-sfGFP-kanMX ade6<sup>+</sup> leu1-32 ura4-D18</i>	This study
2583	<i>h<sup>+</sup> myo52<sup>Δtail</sup>-tdTomato-kanMX eng2-sfGFP-kanMX ade6-M216 leu1-32 ura4-D18</i>	This study
2584	<i>h<sup>+</sup> myo51<sup>Δtail</sup>-12myc-ura4<sup>+</sup> exg3-sfGFP-kanMX ade6<sup>+</sup> leu1-32 ura4-294</i>	This study
2585	<i>h<sup>+</sup> myo52<sup>Δtail</sup>-tdTomato-kanMX exg3-sfGFP-kanMX ade6-M216 leu1-32 ura4-D18</i>	This study
<b>Fig. S1</b>		
1396	<i>h90 WT (968)</i>	Laboratory stock
1442	<i>h90 fus1::LEU2 leu1<sup>-</sup> ura4-D18</i>	Laboratory stock
2596	<i>h90 nmt41::lifeact-GFP-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2586	<i>h90 fus1::LEU2 nmt41::lifeact-GFP ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
<b>Fig. S2</b>		
2514	<i>h90 p<sup>map3</sup>-tdTomato-ura4<sup>+</sup> nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2522	<i>h90 for3::kanMX nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2587	<i>h90 for3::kanMX fus1::LEU2 nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2597	<i>h90 dip1::natMX nmt41::GFP-CHD leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2598	<i>h90 vrp1::kanMX nmt41::GFP-CHD ade6-M216 leu1-32 ura4-D18 his3-D1</i>	This study
2599	<i>h<sup>-</sup> vrp1::kanMX myo52-tdTomato-natMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
1944	<i>h<sup>-</sup> dip1::natMX ade6-M216 leu1-32 ura4-D18</i>	Basu and Chang, 2011

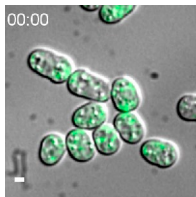
Table S1. **Strains used in this study** (Continued)

Strain number	Genotype	Source
1055	<i>h<sup>-</sup> for3::kanMX ade6<sup>-</sup> leu1-32 ura4-D18</i>	Laboratory stock
2588	<i>h90 cdc12-112 nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2589	<i>h90 for3::kanMX cdc12-112 nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1<sup>-</sup> ura4-D18</i>	This study
2590	<i>h90 for3::kanMX cdc12-112 fus1::LEU2 nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
2173	<i>h<sup>-</sup> leu1::nmt41-GFP-cdc8-ura4<sup>+</sup> ura4-D18</i>	Skau et al., 2011
2591	<i>h<sup>-</sup> rlc1-GFP-kanMX leu1<sup>-</sup> ura4-</i>	This study
2163	<i>h<sup>-</sup> arc5-mGFP-kanMX ade6-M216 leu1-32 ura4-D18 his3-D1</i>	Arasada and Pollard, 2011
2194	<i>h90 cam2::ura4<sup>+</sup> ade6&lt;&lt;cam2-GFP leu1-32 ura4-D18</i>	Itadani et al., 2006
2161	<i>h<sup>-</sup> kanMX-P<sup>wsp1</sup>-mGFP-wsp1 ade6-M216 leu1-32 ura4-D18 his3-D1</i>	Arasada and Pollard, 2011
2592	<i>h90 crn1-tdTomato-natMX nmt41::GFP-CHD-leu<sup>+</sup> ade6<sup>-</sup> leu1-32 ura4-D18</i>	This study
740	<i>h<sup>+</sup> myo52-tdTomato-natMX ade6-M216 leu1-32 ura4-D18</i>	Laboratory stock
<b>Fig. S3</b>		
2520	<i>h90 myo52-GFP-kanMX leu1-32</i>	This study
2521	<i>h90 fus1::LEU2 myo52-GFP-kanMX leu1-32</i>	This study
2529	<i>h90 myo52-tdTomato-natMX myo51-3YFP-kanMX leu1-32 ura4-D18</i>	This study
2593	<i>h90 myo51-3YFP-kanMX fus1::LEU1 ade6-M216 leu1-32 ura4-D18</i>	This study

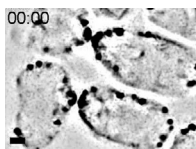
All tagging constructs are in frame after the last codon of the gene or before the first one and integrated at the endogenous genomic locus. All deletions are replacements of the complete gene ORF with the selectable marker. The *p<sup>map3</sup>*-driven transgenes are integrated in the *map3* promoter locus. WT, wild type.



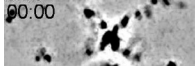
Video 1. **An actin fusion focus forms before cell fusion.** (Related to Fig. 1.) Time lapse of homothallic *h90 p<sup>map3</sup>::tdTomato GFP-CHD* strain obtained by wide field microscopy and deconvolved using the DeltaVision platform (Olympus IX-71; Applied Precision). CHD-GFP is shown in green; tdTomato in red. Time interval is 2 min. The video is sped up sevenfold. Three distinct mating pairs can be seen fusing during the course of the video. Note formation of the fusion focus before fusion, entry of the red signal in the *h<sup>+</sup>* cell at fusion time and focus disassembly after fusion. Bar, 1  $\mu$ m.



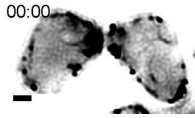
Video 2. **Absence of actin fusion focus in *fus1*Δ.** (Related to Fig. 1.) Time-lapse of homothallic *h90 fus1*Δ *GFP-CHD* strain obtained by wide field microscopy and deconvolved using the DeltaVision platform (Olympus IX-71; Applied Precision). CHD-GFP is shown in green; differential interference contrast image in gray. Time interval is 2 min. The video is sped up sevenfold. Four distinct mating pairs can be seen, all of which fail to fuse. Instead, mating partners keep growing in a polarized fashion toward each other. Actin structures, likely actin patches can be seen dynamically localizing at the shmoo tip, but no long-lasting actin focus is observed. Note the GFP-CHD channel has been contrasted to allow visibility on the differential interference contrast image. Bar, 1  $\mu$ m.



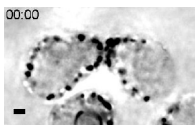
Video 3. **The actin fusion focus visualized by 3D SIM.** (Related to Fig. 2.) Time-lapse of GFP-CHD in homothallic *h90 WT GFP-CHD myo52-tdTomato* cells. Only the GFP-CHD channel is shown. Inverted images are shown obtained using the SIM setup (Eclipse T1; Nikon). Time interval is 6 s. The video is sped up sevenfold. Long linear dynamic actin cables are observed originating from the zone of cell-cell contact. Contrast was adjusted to optimize cable visualization. Bar, 1  $\mu$ m.



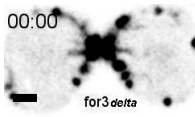
Video 4. **The actin fusion focus visualized by 3D SIM in *for3Δ*.** (Related to Fig. 2.) Time-lapse of homothallic *h90 for3Δ* GFP-CHD cells. Inverted images are shown obtained using the SIM setup (Eclipse T1; Nikon). Time interval is 3 s. The video is sped up sevenfold. Short linear dynamic actin cables are observed originating from the zone of cell–cell contact. Contrast was adjusted to optimize cable visualization. Bar, 1  $\mu$ m.



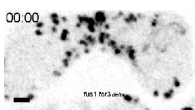
Video 5. **Actin at the zone of cell–cell contact visualized by 3D SIM in *fus1Δ*.** (Related to Fig. 2.) Time-lapse of homothallic *h90 fus1Δ* GFP-CHD cells. Inverted images are shown obtained using the SIM setup (Eclipse T1; Nikon). Time interval is 3 s. The video is sped up sevenfold. Long dynamic actin cables are observed emanating from a broad region at the cell–cell contact site. Contrast was adjusted to optimize cable visualization. Bar, 1  $\mu$ m.



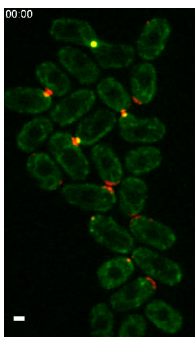
Video 6. **Actin at the zone of cell–cell contact visualized by 3D SIM in *fus1Δ for3Δ*.** (Related to Fig. 2.) Time-lapse of homothallic *h90 fus1Δ for3Δ* GFP-CHD cells obtained using the SIM setup (Eclipse T1; Nikon). Inverted images are shown. Time interval is 3 s. The video is sped up sevenfold. No actin cables were observed; however, a perinuclear actin ring is seen. Note that only part of this ring is visible because only a single focal plane is shown. Bar, 1  $\mu$ m.



Video 7. **The actin fusion focus visualized by scanning confocal microscopy in WT and *for3Δ*.** (Related to Fig. 2.) Images of homothallic *h90 WT GFP-CHD myo52-tdTomato* (left) and *h90 for3Δ GFP-CHD* (right) cells obtained by laser-scanning confocal microscopy (LSM 710; Carl Zeiss). Only the GFP-CHD signal was imaged to minimize the time interval (2 s). The video is sped up sevenfold. Long and short actin cables are observed originating from the zone of cell–cell contact in WT cells, and short linear actin cables are observed in *for3Δ* cells. Contrast was adjusted to optimize cable visualization. Bar, 1  $\mu$ m.



Video 8. **Actin at the zone of cell–cell contact visualized by scanning confocal microscopy in *fus1Δ* and *fus1Δ for3Δ*.** (Related to Fig. 2.) Images of homothallic *h90 fus1Δ GFP-CHD* (left) and *h90 fus1Δfor3Δ GFP-CHD* (right) cells obtained by laser-scanning confocal microscopy (LSM 710; Carl Zeiss). Time interval is 2 s. The video is sped up sevenfold. Long dynamic, unfocalized actin cables are observed in *fus1Δ*. No actin cables were observed in *fus1Δ for3Δ*. Contrast was adjusted to optimize cable visualization. Bar, 1  $\mu$ m.



Video 9. **Asymmetric maturation of the fusion focus.** (Related to Fig. 3.) Time-lapse of homothallic *h90 WT myo52-tdTomato myo51-3YFP* obtained by spinning disk microscopy (UltraVIEW system [PerkinElmer] on DMI4000B [Leica]). Myo52-tdTomato is shown in red; Myo51-3YFP is shown in green. Time interval is 1.3 s. The video is sped up sevenfold. The video shows five mating pairs at distinct stages of the fusion process. Pair 1 shows Myo52-tdTomato crescent in both partner cells, with the bottom cell structure focalizing in the course of the video. Pairs 2, 3, and 4 show asymmetric structures, with Myo51-3YFP strongly focalized with Myo52-tdTomato in only one of the two mating partners. Note how the Myo52-tdTomato structure in the other cell moves at the contact zone relative to that stable focus. In the course of the video, Myo51-3YFP accumulates in the focus of the bottom cell of pair 4. Finally, pair 5 shows a single Myo51-Myo52 focus at the fusion site, which disassembles in the course of the video. Bar, 1  $\mu$ m.

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