

Supplemental data for:

**Spatial focalization of pheromone/MAPK signaling triggers commitment to cell-cell fusion**

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**Figure S1 (related to Figure 1): Myo51 and Myo52 co-localize in committed mating pairs.**

Homothallic h90 *myo52-tdTomato myo51-3YFP* cell pairs were washed with fresh media in microfluidics chambers as in Figure 1. Committed mating pairs, as defined by maintenance of their Myo52-tdTomato signal showed strong co-localization of Myo51-3YFP. By contrast, uncommitted cell pairs, which disassembled their fusion focus, showed no or very weak Myo51 signal prior to focus disassembly. Bars = 2  $\mu$ m.

**Figure S2 (related to Figure 2): Polarity factors localize normally in exponentially growing autocrine M-cells.**

(A, B, C) Cell length at division, cell width and septum placement of wt and autocrine M-cells during exponential growth in presence of nitrogen (n = 50). (D) 10-fold serial dilutions of wt and autocrine M-cells on minimal (EMM) and rich (YE) medium. (E) Localization of Myo52-tdTomato or Scd2-GFP in wt and autocrine M-cells during exponential growth in presence of nitrogen. The top images show calcofluor-stained cells. Bars = 2  $\mu$ m. (F) Whole-cell GFP fluorescence intensity as a proxy for gene expression of Mam1-sfGFP, Fus1-sfGFP and Map3-sfGFP in autocrine M-cells grown in media with or without

nitrogen after 16h ( $n > 50$ ).  $t$  test \*\*\*,  $P < 2 \times 10^{-14}$ . (G) Calcofluor-stained wt and autocrine M-cells after 12 hours in MSL-N. Bar is 2  $\mu$ m.

**Figure S3 (related to Figure 3): Pheromone and MAPK signaling are not focalized in heterothallic strains treated with high dose of synthetic pheromones.**

Imaging of heterothallic  $h-$  *sxa2* $\Delta$  (top) or  $h+$  *sxa1* $\Delta$  (bottom) strains treated with synthetic pheromones as indicated, expressing the indicated tagged proteins. Bars = 2  $\mu$ m.

**Figure S4 (related to Figure 6): Lack of focalization and fusion defects upon constitutive activation of Byr1.**

(A) Fusion efficiency of wt and *byr1<sup>DD</sup>* homothallic pairs ( $n > 500$ ).  $t$  test, \*\*\*,  $P < 2 \times 10^{-5}$ . (B) Localization of Byr1-sfGFP and Byr1<sup>DD</sup>-sfGFP in wildtype and *byr1<sup>DD</sup>* cell pairs (top panels; no tag on Myo52), and Myo52-GBP-mCherry in wildtype and *byr1<sup>DD</sup>* cell pairs (bottom panels; no tag on Byr1). Note that the presence of the GBD has no influence in these cells, as these do not express GFP. (C) Quantification of the width of the Byr1-sfGFP and Byr1<sup>DD</sup>-sfGFP fluorescence signals in cells as in (B, top row) ( $n = 10$ ).  $t$  test, \*\*\*,  $P < 7 \times 10^{-9}$ .

**Supplemental Movie 1 (related to Figure 1): Committed and uncommitted cell pairs.**

Time-lapse of a committed and uncommitted mating pair of homothallic *h90* cells expressing Myo52-tdTomato and *p<sup>map3</sup>:GFP* in CellASIC microfluidic chamber. Fresh media is flown at 13 $\mu$ l/h (5psi) for 10 minutes.

**Supplemental Movie 2 (related to Figure 2): Polarized growth and lysis of autocrine M-cells.**

Deconvolved single z-plane DIC and epifluorescence timelapse of autocrine M-cells expressing Myo52-tdTomato. Note that the Myo52 signal localizes at sites of growth, as well as at the site of lysis in the top cell of the bottom panel.

**Supplemental Movie 3 (related to Figure 2): Fusion of autocrine M-cells.**

Deconvolved single z-plane DIC timelapse of autocrine M-cells lysing (two on the left) or fusing together (pair on the right), as in Figure 2H.

**Supplemental Movie 4 (related to Figure 4): Unstable fusion focus in *map3<sup>dn9</sup>* mutant.**

Deconvolved single z-plane epifluorescence timelapse of homothallic *h90* cells expressing Myo52-tdTomato and Map3<sup>dn9</sup>-GFP. Note the mobile Myo52 signal in the *h+* cell marked by the expression of map3<sup>dn9</sup>-GFP, compared to the stable signal in the *h-* cell, which expresses unmarked Mam2.

**Supplemental Movie 5 (related to Figure 4): Unstable fusion focus in *rgs1 $\Delta$*  mutant.**

Deconvolved single z-plane DIC and epifluorescence timelapse of homothallic *h90 rgs1Δ* cells expressing Myo52-tdTomato. Note the multiple cycles of formation and disappearance of the Myo52 focus at the contact site between the pair of cells that fails to fuse on the right. The timelapse also shows one successful fusion event.

**Supplemental Movie 6 (related to Figure 5): Rescue of fusion by forced recruitment of Map3<sup>dn9</sup> to the fusion focus.**

Deconvolved single z-plane DIC and epifluorescence timelapse of homothallic *h90* cells expressing Myo52-GBP-mCherry and Map3<sup>dn9</sup>-GFP. Map3<sup>dn9</sup>-GFP focalization stabilizes the Myo52 focus and cells successfully fuse.

**Supplemental Movie 7 (related to Figure 5): Examples of fragmented and broad Map3<sup>dn9</sup> signal.**

Deconvolved single z-plane DIC and epifluorescence timelapse of homothallic *h90* cells expressing Myo52-GBP-mCherry and Map3<sup>dn9</sup>-GFP. In these instances where Map3<sup>dn9</sup>-GFP signal is fragmented or broad, the cells fail to fuse.

**Supplemental Movie 8 (related to Figure 6): Forced recruitment of MAP2K Byr2 stabilizes the fusion focus and leads to cell fusion in *map3<sup>dn9</sup>* mutant.**

Deconvolved single z-plane DIC and epifluorescence timelapse of homothallic *h90 map3<sup>dn9</sup>* cells expressing Myo52-GBP-mCherry and Byr1-GFP. Byr1-GFP focalization stabilizes the Myo52 focus and cells successfully fuse.

**Supplemental Movie 9 (related to Figure 6): Forced recruitment of MAP2K Byr2 stabilizes the fusion focus and leads to cell lysis in *rgs1*Δ cells.**

Deconvolved single z-plane DIC and epifluorescence timelapse of homothallic *h90 rgs1*Δ cells expressing Myo52-GBP-mCherry and Byr1-GFP. Byr1-GFP focalization stabilizes the Myo52 focus, leading to cell lysis in absence of a paired partner.

**Table S1. Strains used in this study**

Figure 1		
YSM2535	<i>h90 myo52-tdtomato-natMX pmap3-GFP-ura4+</i>	(Dudin et al., 2015)
Figure 2		
YSM952	<i>h- myo52-tdtomato-natMX</i>	Lab Stock
YSM2787	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX</i>	This Study
YSM2788	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX fus1-sfGFP-kanMX</i>	This Study
YSM2791	<i>h- mam2::map3-hphMX fus1::LEU2</i>	This Study
YSM2792	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX exg3-sfGFP-kanMX</i>	This Study
YSM2793	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX eng2-sfGFP-kanMX</i>	This Study
Figure 3		
YSM2787	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX</i>	This Study
YSM2794	<i>h+ sxa1::kanMX myo52-tdtomato-natMX</i>	This Study
YSM2795	<i>h- sxa2::kanMX myo52-tdtomato-natMX</i>	This Study
YSM2789	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX mam1-sfGFP-kanMX</i>	This Study
YSM2790	<i>h- mam2::map3-sfGFP-kanMX myo52-tdtomato-natMX</i>	This Study
YSM2796	<i>h- mam2::map3-hphMX nmt41::GFP-chd gpa1-mCherry<sup>sw</sup>-kanMX</i>	This Study
YSM2797	<i>h- mam2::map3-hphMX fus1::LEU2 mam1-sfGFP-kanMX</i>	This Study
YSM2798	<i>h- mam2::map3-hphMX fus1::LEU2 gpa1-mCherry<sup>sw</sup>-kanMX</i>	This Study
YSM2799	<i>h- mam2::map3-sfGFP-kanMX myo52-tdtomato-natMX fus1::hphMX</i>	This Study
YSM2800	<i>h90 myo52-tdtomato-natMX mam1-GFP-natMX</i>	This Study
YSM2801	<i>h90 myo52-tdtomato-natMX map3-GFP-kanMX</i>	This Study
YSM2802	<i>h90 gpa1-mCherry<sup>sw</sup>-kanMX</i>	This Study

YSM2803	<i>h90 myo52-tdtomato-natMX map3-GFP-kanMX fus1::LEU2</i>	This Study
YSM2804	<i>h90 fus1::LEU2 mam1-GFP-kanMX</i>	This Study
YSM2805	<i>h90 fus1::LEU2 gpa1-mCherry<sup>sw</sup>-kanMX</i>	This Study

#### Figure 4

YSM2126	<i>h90 map3-GFP-kanMX rgs1::hphMX</i>	Lab Stock
YSM2572	<i>h90 eng2-sfGFP-kanMX myo52-tdtomato-natMX</i>	(Dudin et al., 2015)
YSM2573	<i>h90 exg3-sfGFP-kanMX myo52-tdtomato-natMX</i>	(Dudin et al., 2015)
YSM2787	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX</i>	This Study
YSM2791	<i>h- mam2::map3-hphMX fus1::LEU2</i>	This Study
YSM2806	<i>h90 myo52-tdtomato-natMX map3<sup>dn9</sup>-GFP-kanMX</i>	This Study
YSM2807	<i>h90 myo52-tdtomato-natMX rgs1::hphMX</i>	This Study
YSM2808	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX rgs1::kanMX</i>	This Study
YSM2809	<i>h- mam2::map3<sup>dn9</sup>-sfGFP-kanMX myo52-tdtomato-natMX</i>	This Study
YSM2810	<i>h- mam2::map3-sfGFP-kanMX myo52-tdtomato-natMX rgs1::hphMX</i>	This Study
YSM2811	<i>h90 myo52-tdtomato-natMX rgs1::hphMX mam1-sfGFP-kanMX</i>	This Study
YSM2812	<i>h90 gpa1-mCherry<sup>sw</sup>-kanMX rgs1::hphMX</i>	This Study
YSM2813	<i>h90 exg3-sfGFP-kanMX rgs1::hphMX</i>	This Study
YSM2814	<i>h90 eng2-sfGFP-kanMX rgs1::hphMX</i>	This Study
YSM2815	<i>h90 ura4-294-Pmap3-map3<sup>dn9</sup>-ura4+ eng2-sfGFP-kanMX</i>	This Study
YSM2816	<i>h90 ura4-294-Pmap3-map3<sup>dn9</sup>-ura4+ exg3-sfGFP-kanMX</i>	This Study

#### Figure 5

YSM2806	<i>h90 myo52-tdtomato-natMX map3<sup>dn9</sup>-GFP-kanMX</i>	This Study
YSM2817	<i>h90 myo52-GBP-mCherry-bleMX map3<sup>dn9</sup>-GFP-kanMX</i>	This Study

YSM2818	<i>h90 myo52-GBP-mCherry-bleMX map3<sup>dn9</sup>-GFP-kanMX fus1::hphMX</i>	This Study
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Figure 6

YSM2819	<i>h90 myo52-tdtomato-natMX byr2-GFP-kanMX</i>	This Study
YSM2820	<i>h90 myo52-tdtomato-natMX spk1-sfGFP-kanMX</i>	This Study
YSM2821	<i>h90 myo52-tdtomato-natMX byr1-sfGFP-kanMX</i>	This Study
YSM2822	<i>h90 myo52-tdtomato-natMX byr1-sfGFP-kanMX fus1::LEU2</i>	This Study
YSM2823	<i>h90 myo52-tdtomato-natMX byr1-sfGFP-kanMX rgs1::hphMX</i>	This Study
YSM2824	<i>h90 ura4-294-Pmap3-map3<sup>dn9</sup>-ura4+ byr1-sfGFP-kanMX</i>	This Study
YSM2825	<i>h90 ura4-294-Pmap3-map3<sup>dn9</sup>-ura4+ myo52-GBP-mCherry-bleMX byr1-GFP-kanMX</i>	This Study
YSM2826	<i>h90 myo52-GBP-mCherry-bleMX byr1-GFP-kanMX rgs1::hphMX</i>	This Study
YSM2827	<i>h- sxa2::kanMX myo52-GBP-mCherry-bleMX</i>	This Study
YSM2828	<i>h- sxa2::kanMX myo52-GBP-mCherry-bleMX byr1-GFP-natMX</i>	This Study
YSM2829	<i>h90 myo52-GBP-mCherry-bleMX byr1-GFP-kanMX rgs1::hphMX fus1::natMX</i>	This Study
YSM2836	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX byr1-sfGFP-kanMX</i>	This Study
YSM2837	<i>h- mam2::map3-hphMX byr1-sfGFP-kanMX fus1::LEU2</i>	This Study

Figure S1

YSM2529	<i>h90 myo52-tdtomato-NatMX myo51-3YFP-KanMX</i>	(Dudin et al., 2015)
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Figure S2

YSM952	<i>h- myo52-tdtomato-natMX</i>	(Dudin et al., 2015)
YSM2787	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX</i>	This Study
YSM2788	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX fus1-sfGFP-kanMX</i>	This Study
YSM2789	<i>h- mam2::map3-hphMX myo52-tdtomato-natMX mam1-sfGFP-kanMX</i>	This Study



YSM2790	<i>h-</i> <i>mam2::map3-sfGFP-kanMX myo52-tdtomato-natMX</i>	This Study
YSM2830	<i>h-</i> <i>scd2-GFP-kanMX</i>	This Study
YSM2831	<i>h-</i> <i>mam2::map3-hphMX scd2-GFP-kanMX</i>	This Study

#### Figure S3

YSM2794	<i>h+</i> <i>sxa1::kanMX myo52-tdtomato-natMX</i>	This Study
YSM2795	<i>h-</i> <i>sxa2::kanMX myo52-tdtomato-natMX</i>	This Study
YSM2832	<i>h-</i> <i>sxa2::kanMX mam2-sfGFP-kanMX</i>	This Study
YSM2833	<i>h-</i> <i>sxa2::hphMX byr1-sfGFP-kanMX myo52-tdtomato-natMX</i>	This Study
YSM2834	<i>h+</i> <i>sxa1::hphMX map3-sfGFP-kanMX myo52-tdtomato-natMX</i>	This Study

#### Figure S4

YSM2821	<i>h90 myo52-tdtomato-natMX byr1-sfGFP-kanMX</i>	This Study
YSM2851	<i>h90 byr1-DD-sfGFP-kanMX (S214D T218D)</i>	This Study
YSM2852	<i>h90 myo52-GBP-mCherry-bleMX</i>	This Study
YSM2853	<i>h90 byr1-DD (S214D T218D) myo52-GBP-mCherry-bleMX</i>	This Study

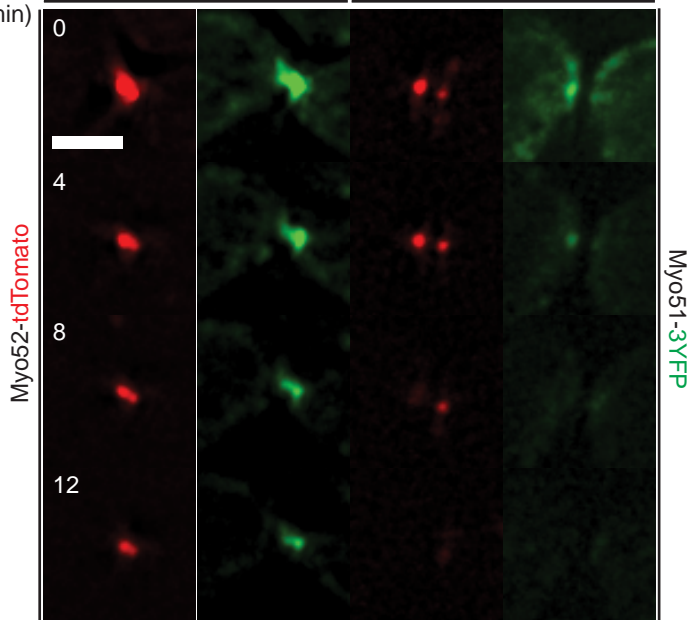
# Figure S1

## A

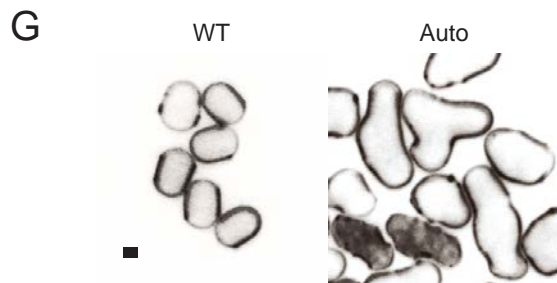
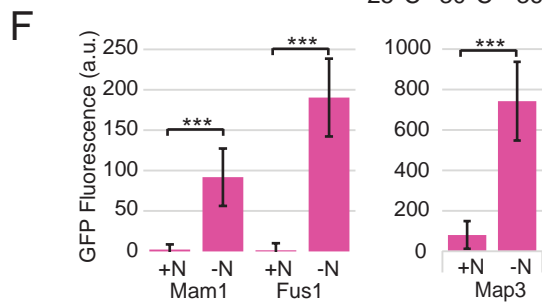
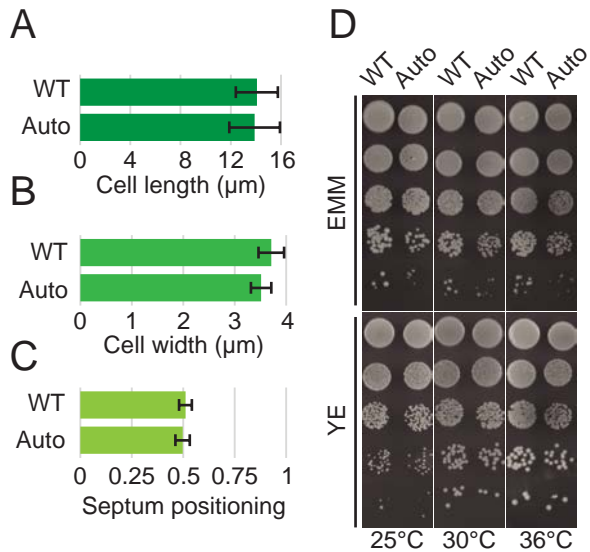
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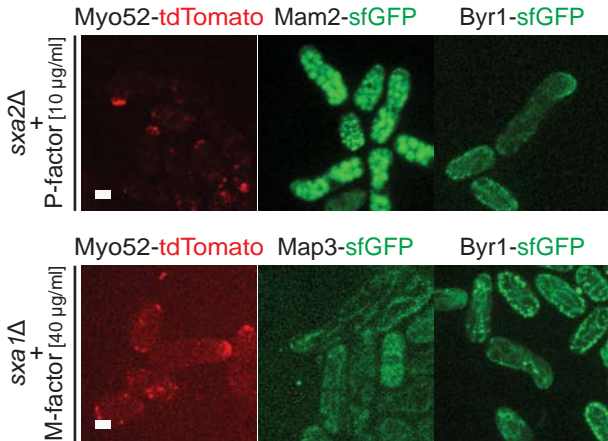
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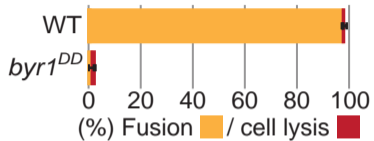
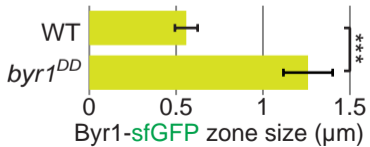


# Figure S2



# Figure S3



**A****B****C**

Myo52-GBP-mCherry Byr1-sfGFP

