

Supplemental Information

Table S1. Strains used in this study.

Strain Name	Description
NH0132	<i>W303 MAT a leu2-3,112 trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX</i>
NH0615	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-FUS2(54-99)S67A-mCherry</i>
NH0610	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-KTR-mCherry (reporter variant 1)</i>
NH0618	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-reporter variant 2-mCherry</i>
NH0620	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-reporter variant 3-mCherry</i>
NH0621	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-reporter variant 4-mCherry</i>
NH0617	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-reporter variant 5-mCherry</i>
NH0619	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-reporter variant 6-mCherry</i>
NH0639	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-KTR-mCherry, kss1::URA3</i>
NH0611	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-KTR-mCherry, fus3::URA3</i>
NH0613	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-KTR-mCherry, FUS3Q93G</i>
NH0625	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-KTR-mCherry, P_{FUS1}-UBI-YΔkGFP -HIS3</i>
NH0626	<i>W303 MAT a trp1-1 can1-100 ura3-1 ADE2+ his3-11,15, bar1Δ, NHP6a-iRFP-kanMX, leu2-3,112::LEU2-P_{ADHI}-KTR-mCherry, FUS3Q93G, kss1::URA3</i>

A

54 KLNDKKFTN⁶⁷KRPAALDLHSIVESLSN⁸⁴KKIYSPINTEIFQN⁹⁹VVRLNL 99

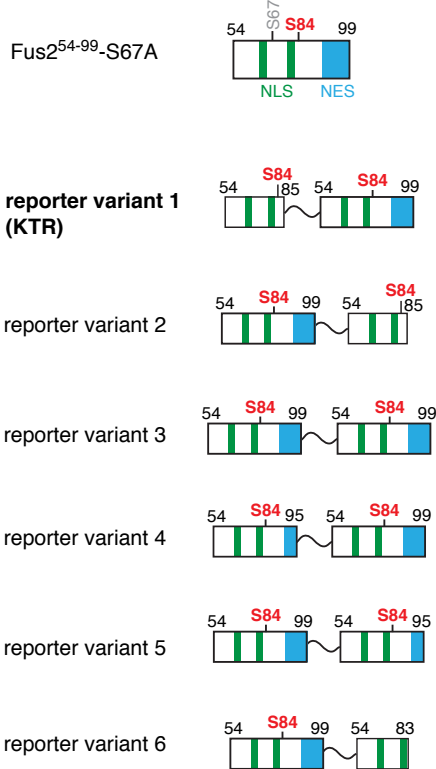
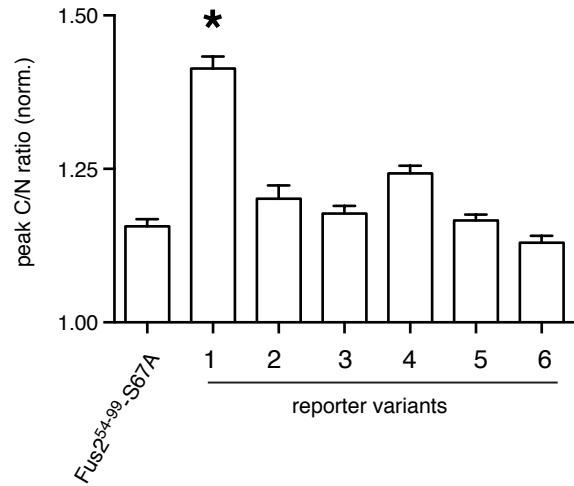
**B**

Fig. S1. Examination of reporter variants. (A) Schematics of reporter variants. Top panel: the amino acid sequence of Fus2⁵⁴⁻⁹⁹-S67A. Green regions: NLS; blue regions: NES. A series of reporter variants were constructed by assembling different truncated forms of Fus2⁵⁴⁻⁹⁹-S67A to the N or C terminus of the original fragment with a flexible linker (Gly-Gly-Ser-Gly-Gly) in between. (B) Bar graphs comparing the dynamic ranges of reporter variants. The translocation responses of different reporter variants were measured in response to a 30-minute pulse of 1 μ M pheromone treatment and quantified as the cytoplasmic over nuclear fluorescence intensities (C/N ratio) normalized by the basal level. The peak values were plotted for Fus2⁵⁴⁻⁹⁹-S67A and each variant. Reporter variant 1 showed the highest peak response and was chosen as the kinase translocation reporter (KTR) for Fus3 activity.

round shaped/vegetative growth

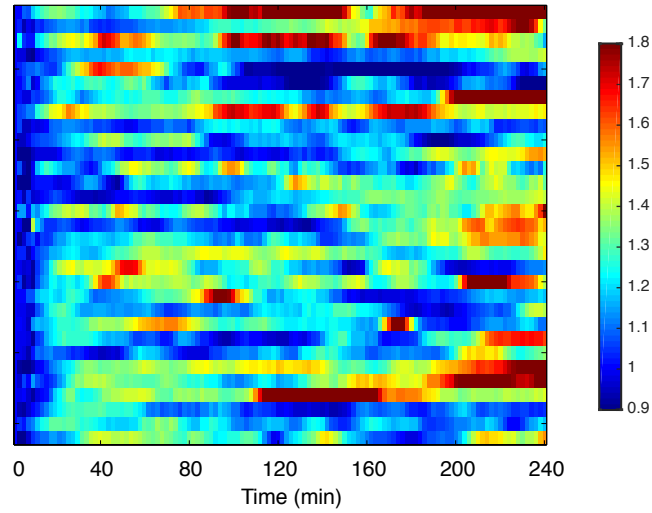


Fig. S2. Fus3 dynamics in cells remaining round shaped or vegetative growth. Single-cell color map trajectories of Fus3 activation for cells that remained round shaped or vegetative growth in response to 0.25 μM pheromone treatment. Each row represents the time trace of a single cell. Color represents the normalized C/N ratio, as indicated in the color bar (identical to that used in Fig. 2).