Systematic analysis of F-box proteins reveals
a new branch of the yeast mating pathway

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FIGURE S1

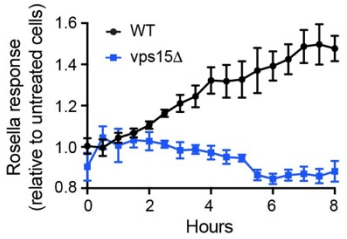

Fig. S1: Pheromone-induced vacuolar targeting requires the regulatory kinase Vps15. Time course of Rosella response upon treatment with by 1 μM α-factor in BY4741 *bar1*Δ cells lacking Vps15, the regulatory subunit of the PI 3-kinase complex. Related to Figure 2A.

FIGURE S2

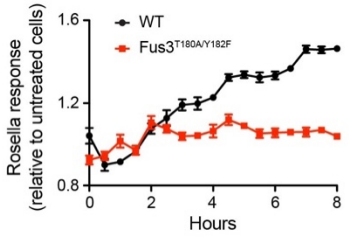

Fig. S2: Pheromone-induced vacuolar targeting requires the phosphorylation sites on the MAP kinase Fus3. Time course of Rosella response upon treatment with 1 μM α-factor in BY4741 *bar1*Δ cells expressing a non-phosphorylatable form of the MAPK ($Fus3^{T180AY182F}$). Related to Figure 2C.

FIGURE S3

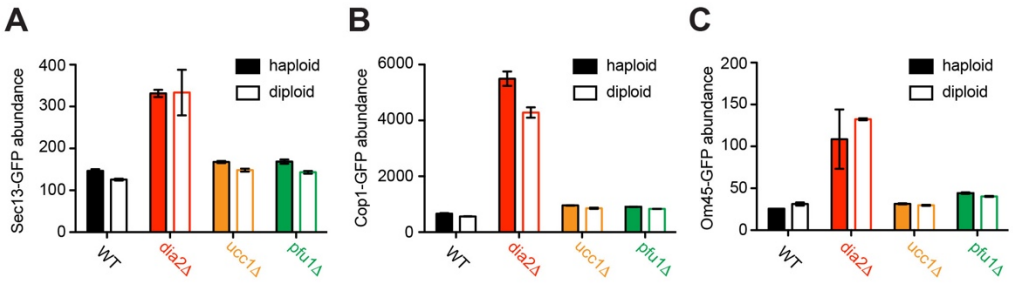

Fig. S3: Dia2 limits abundance of organelle marker proteins under basal conditions. Flow cytometry analysis of the abundance of organelle marker proteins in haploid and diploid BY4741 ("WT"), *dia2*Δ, *ucc1*Δ and *pfu1*Δ cells. Abundance is reported by fluorescence of GFP fused with (A) Sec13 (endoplasmic reticulum-to-Golgi transport vesicles), (B) Cop1 (early Golgi), or (C) Om45 (mitochondria). Flow cytometry data are ± standard deviation for four biological replicates (10,000 cells each).

TABLE S1

Yeast strains used in this study.

Strain	Description	Source
BY4741	*MAT**a** his3Δ leu2Δ met15Δ ura3Δ LYS2*	Yeast Knockout Collection (Invitrogen)
BY4742	*MAT**α** his3Δ leu2Δ lys2Δ ura3Δ MET15*	Invitrogen
bar1Δ	*BY4741 bar1Δ::KanMX*	Slessareva et al 2006
pho8Δ60	*BY4741 pho8Δ::pho8Δ60::KanMX*	This study
vps34Δ bar1Δ	*BY4741 vps34Δ::KanMX bar1Δ::HIS3MX6*	This study
atg14Δ bar1Δ	*BY4741 atg14Δ::KanMX bar1Δ::HIS3MX6*	This study
vps38Δ bar1Δ	*BY4741 vps38Δ::HIS3MX6 bar1Δ::KanMX*	This study
kss1Δ bar1Δ	*BY4741 kss1Δ::KanMX bar1Δ::HIS3MX6*	This study
fus3Δ bar1Δ	*BY4741 fus3Δ::KanMX bar1Δ::HIS3MX6*	This study
Chc1-GFP vps34Δ	*BY4741 Chc1-GFP::HIS3MX6 vps34Δ::HYGRO*	This study
Cop1-GFP vps34Δ	*BY4741 Cop1-GFP::HIS3MX6 vps34Δ::HYGRO*	This study
Sec13-GFP vps34Δ	*BY4741 Sec13-GFP::HIS3MX6 vps34Δ::HYGRO*	This study
Sac6-GFP vps34Δ	*BY4741 Sac6-GFP::HIS3MX6 vps34Δ::HYGRO*	This study
Chc1-RFP vps34Δ	*BY4742 Chc1-RFP::KanMX vps34Δ::HYGRO*	This study
Cop1-RFP vps34Δ	*BY4742 Cop1-RFP::KanMX vps34Δ::HYGRO*	This study
Sec13-RFP vps34Δ	*BY4742 Sec13-RFP::KanMX vps34Δ::HYGRO*	This study
Sac6-RFP vps34Δ	*BY4742 Sac6-RFP::KanMX vps34Δ::HYGRO*	This study
dia2Δ bar1Δ	*BY4741 dia2Δ::KanMX bar1Δ::HIS3MX6*	This study
das1Δ bar1Δ	*BY4741 das1Δ::KanMX bar1Δ::HIS3MX6*	This study
ufo1Δ bar1Δ	*BY4741 ufo1Δ::KanMX bar1Δ::HIS3MX6*	This study
pfu1Δ bar1Δ	*BY4741 ydr306cΔ::KanMX bar1Δ::HIS3MX6*	This study
hrt3Δ bar1Δ	*BY4741 hrt3Δ::KanMX bar1Δ::HIS3MX6*	This study
mfb1Δ bar1Δ	*BY4741 mfb1Δ::KanMX bar1Δ::HIS3MX6*	This study
ucc1Δ bar1Δ	*BY4741 ucc1Δ::KanMX bar1Δ::HIS3MX6*	This study
ylr352wΔ bar1Δ	*BY4741 ylr352w3Δ::KanMX bar1Δ::HIS3MX6*	This study
saf1Δ bar1Δ	*BY4741 saf1Δ::KanMX bar1Δ::HIS3MX6*	This study
ydr131cΔ bar1Δ	*BY4741 ydr131cΔ::KanMX bar1Δ::HIS3MX6*	This study
ynl311cΔ bar1Δ	*BY4741 ynl311cΔ::KanMX bar1Δ::HIS3MX6*	This study
ymr258cΔ bar1Δ	*BY4741 ymr258cΔ::KanMX bar1Δ::HIS3MX6*	This study
mdm30Δ bar1Δ	*BY4741 mdm30Δ::KanMX bar1Δ::HIS3MX6*	This study
grr1Δ bar1Δ	*BY4741 grr1Δ::KanMX bar1Δ::HIS3MX6*	This study
Chc1-GFP dia2Δ	*BY4741 Chc1-GFP::HIS3MX6 dia2Δ::HYGRO*	This study
Cop1-GFP dia2Δ	*BY4741 Cop1-GFP::HIS3MX6 dia2Δ::HYGRO*	This study
Sec13-GFP dia2Δ	*BY4741 Sec13-GFP::HIS3MX6 dia2Δ::HYGRO*	This study
Sac6-GFP dia2Δ	*BY4741 Sac6-GFP::HIS3MX6 dia2Δ::HYGRO*	This study
Chc1-RFP dia2Δ	*BY4742 Chc1-RFP::KanMX dia2Δ::HYGRO*	This study
Cop1-RFP dia2Δ	*BY4742 Cop1-RFP::KanMX dia2Δ::HYGRO*	This study
Sec13-RFP dia2Δ	*BY4742 Sec13-RFP::KanMX dia2Δ::HYGRO*	This study
Sac6-RFP dia2Δ	*BY4742 Sac6-RFP::KanMX dia2Δ::HYGRO*	This study

dia2Δ	*BY4741 dia2Δ::KanMX*	Yeast Knockout Collection (Invitrogen)
ucc1Δ	*BY4741 ucc1Δ::KanMX*	Invitrogen
hrt3Δ	*BY4741 hrt3Δ::KanMX*	Invitrogen
das1Δ	*BY4741 das1Δ::KanMX*	Invitrogen
mfb1Δ	*BY4741 mfb1Δ::KanMX*	Invitrogen
ylr352wΔ	*BY4741 ylr352wΔ::KanMX*	Invitrogen
ydr131cΔ	*BY4741 ydr131cΔ::KanMX*	Invitrogen
ynl311cΔ	*BY4741 ynl311cΔ::KanMX*	Invitrogen
ufo1Δ	*BY4741 ufo1Δ::KanMX*	Invitrogen
pfu1Δ	*BY4741 ydr306cΔ::KanMX*	Invitrogen
saf1Δ	*BY4741 saf1Δ::KanMX*	Invitrogen
ymr258cΔ	*BY4741 ymr258cΔ::KanMX*	Invitrogen
mdm30Δ	*BY4741 mdm30Δ::KanMX*	Invitrogen
grr1Δ	*BY4741 grr1Δ:URA*	This study
Chc1-GFP ucc1Δ	*BY4741 Chc1-GFP::HIS3MX6 ucc1Δ::HYGRO*	This study
Cop1-GFP ucc1Δ	*BY4741 Cop1-GFP::HIS3MX6 ucc1Δ::HYGRO*	This study
Sec13-GFP ucc1Δ	*BY4741 Sec13-GFP::HIS3MX6 ucc1Δ::HYGRO*	This study
Sac6-GFP ucc1Δ	*BY4741 Sac6-GFP::HIS3MX6 ucc1Δ::HYGRO*	This study
Chc1-RFP ucc1Δ	*BY4742 Chc1-RFP::KanMX ucc1Δ::HYGRO*	This study
Cop1-RFP ucc1Δ	*BY4742 Cop1-RFP::KanMX ucc1Δ::HYGRO*	This study
Sec13-RFP ucc1Δ	*BY4742 Sec13-RFP::KanMX ucc1Δ::HYGRO*	This study
Sac6-RFP ucc1Δ	*BY4742 Sac6-RFP::KanMX ucc1Δ::HYGRO*	This study
Chc1-GFP pfu1Δ	*BY4741 Chc1-GFP::HIS3MX6 ydr306cΔ::HYGRO*	This study
Cop1-GFP pfu1Δ	*BY4741 Cop1-GFP::HIS3MX6 ydr306c Δ::HYGRO*	This study
Sec13-GFP pfu1Δ	*BY4741 Sec13-GFP::HIS3MX6 ydr306c Δ::HYGRO*	This study
Sac6-GFP pfu1Δ	*BY4741 Sac6-GFP::HIS3MX6 ydr306c Δ::HYGRO*	This study
Chc1-RFP pfu1Δ	*BY4742 Chc1-RFP::KanMX ydr306c Δ::HYGRO*	This study
Cop1-RFP pfu1Δ	*BY4742 Cop1-RFP::KanMX ydr306c Δ::HYGRO*	This study
Sec13-RFP pfu1Δ	*BY4742 Sec13-RFP::KanMX ydr306c Δ::HYGRO*	This study
Sac6-RFP pfu1Δ	*BY4742 Sac6-RFP::KanMX ydr306c Δ::HYGRO*	This study
Bem1-GFP	*BY4741 Bem1-GFP::His3MX6*	This study
Bem1-GFP pfu1Δ	*BY4741 Bem1-GFP::His3MX6 ydr306c Δ::KanMX*	This study
Om45-GFP	*BY4741 Om45-GFP::His3MX6*	GFP library
Om45-GFP dia2Δ	*BY4741 Om45-GFP::His3MX6 dia2Δ::HYGRO*	This study
Om45-GFP pfu1Δ	*BY4741 Om45-GFP::His3MX6 pfu1Δ::HYGRO*	This study
Om45-GFP ucc1Δ	*BY4741 Om45-GFP::His3MX6 ucc1Δ::HYGRO*	This study