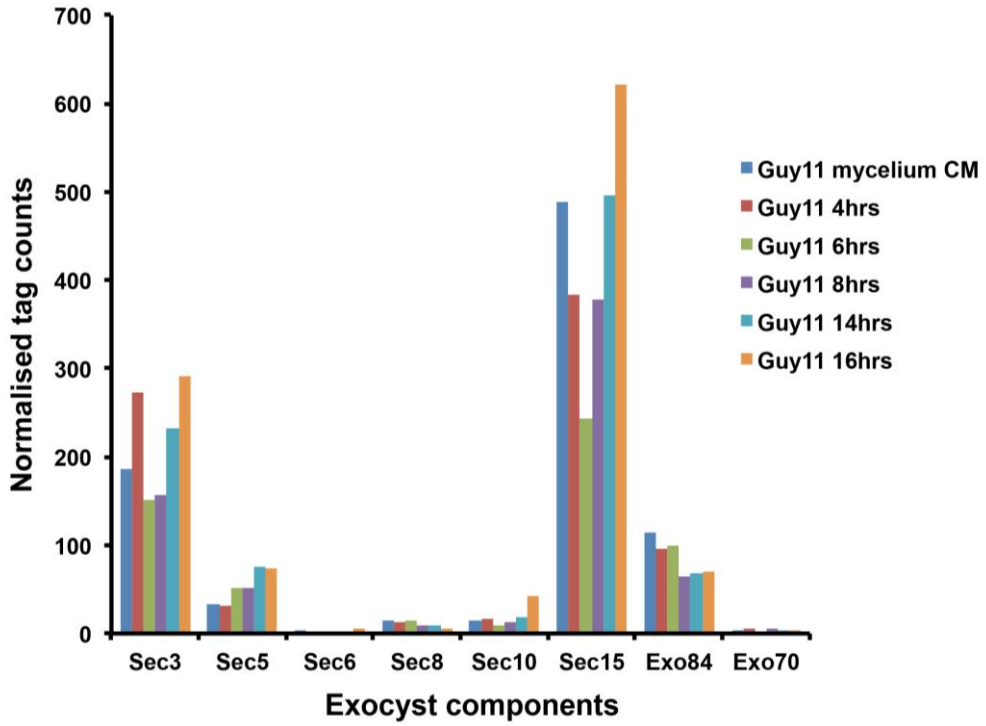
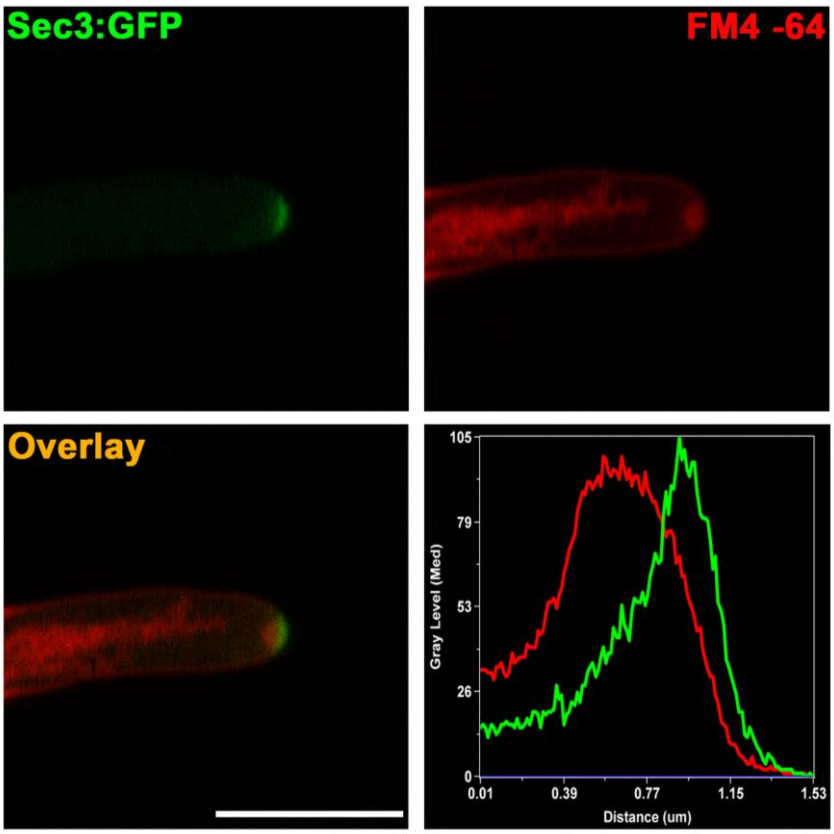


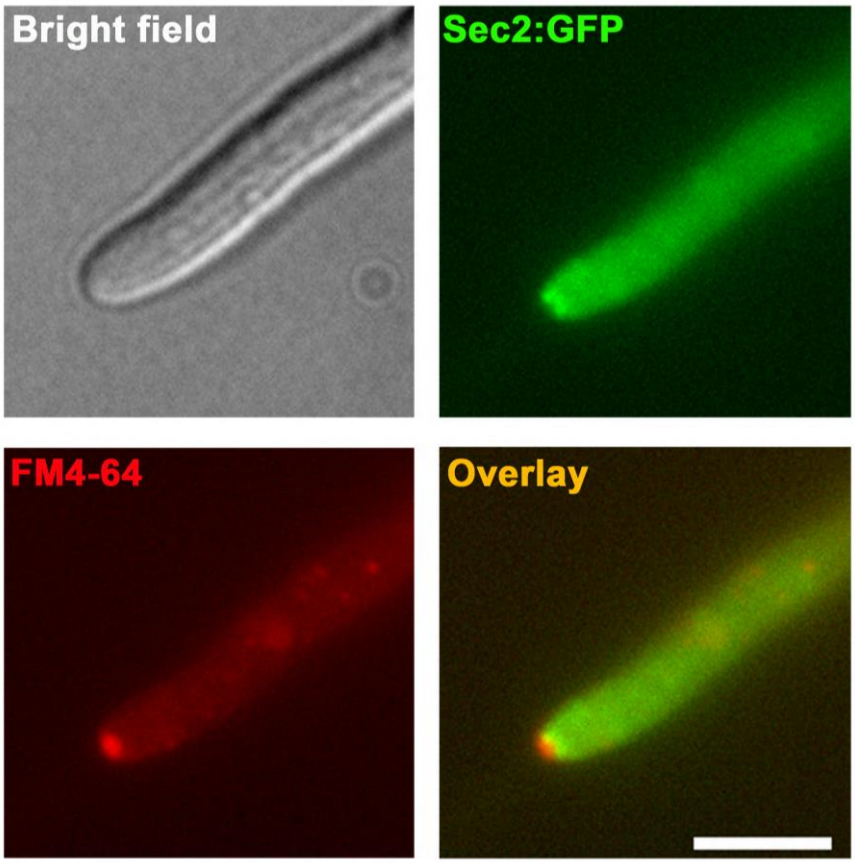
Supplemental Figure 1. Relative transcript abundance of the exocyst subunit-encoding genes in mycelium and during appressorium development.

High-throughput (HT)-SuperSAGE analysis was carried out to observed relative expression levels of exocyst genes (from the public data-set of Soanes et al, 2012) (http://cogeme.ex.ac.uk/supersage). The bar chart represents relative transcript abundance for each exocyst gene expressed in mycelium and during a time course of appressorium development at 4, 6, 8, 14 and 16 h.



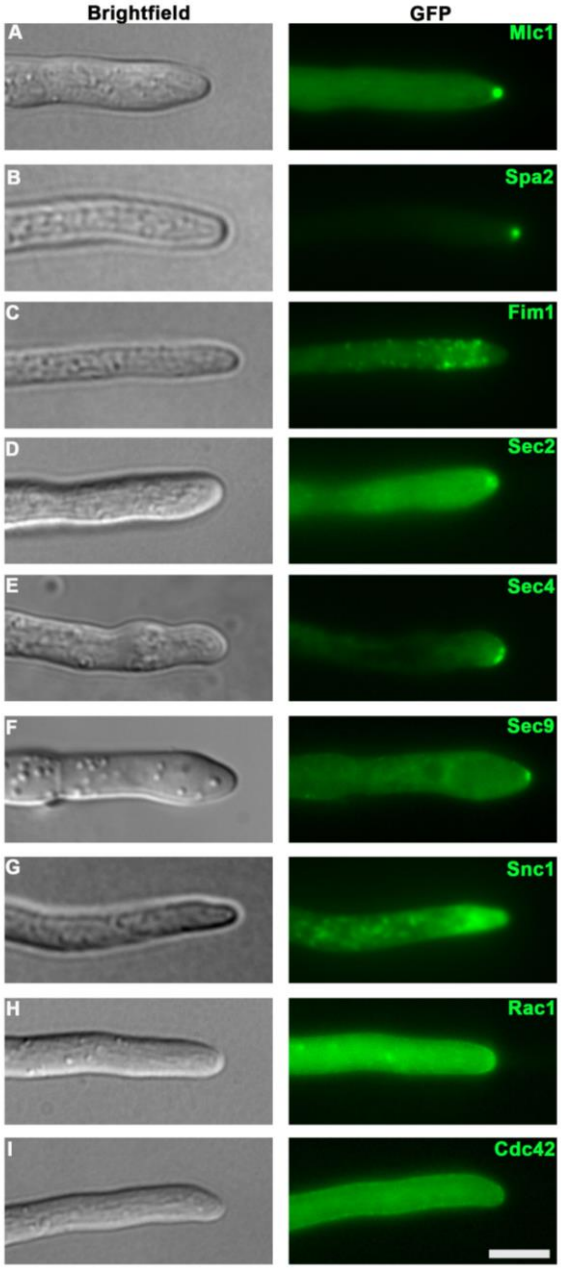
Supplemental Figure 2. High-resolution epifluorescence micrograph of Sec3-GFP and FM4-64-labelled Spitzenkörper in vegetative hyphae of *Magnaporthe oryzae*.

Micrograph and linescan graph to show relative localisation of Sec3-GFP and the Spitzenkörper in growing hyphae of *M. oryzae*, labelled with FM4-64. Epifluorescence micrographs were overlaid to observe relative localisation patterns and a line scan graph was generated. Scale bar=10µm.



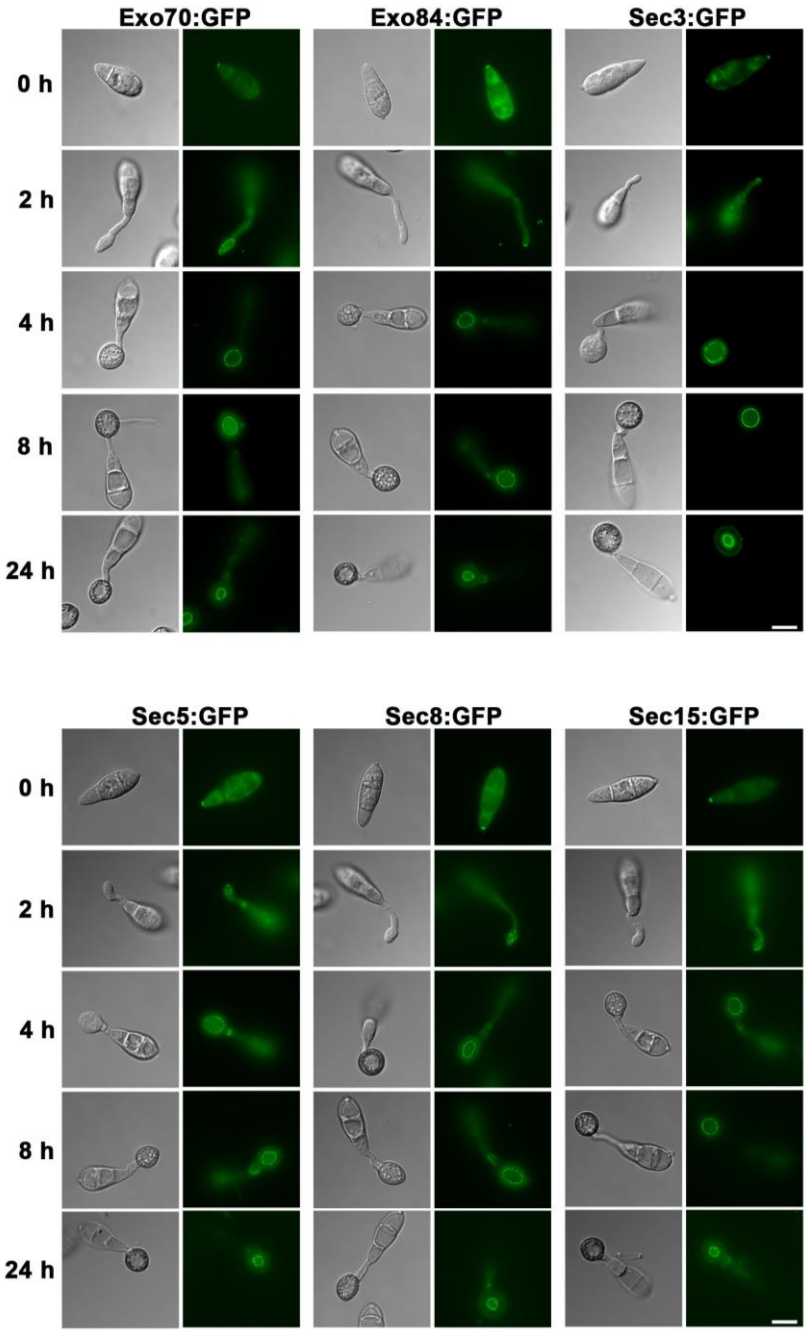
Supplemental Figure 3. Localisation of Sec2-GFP and FM4-64-labelled Spitzenkörper in vegetative hyphae of *Magnaporthe oryzae*.

Micrograph and linescan graph to show relative localisation of Sec2-GFP and the Spitzenkörper in growing hyphae of *M. oryzae*, labelled with FM4-64. Epifluorescence micrographs were overlaid to observe relative localisation patterns and a line scan graph was generated. Scale bar=10μm.



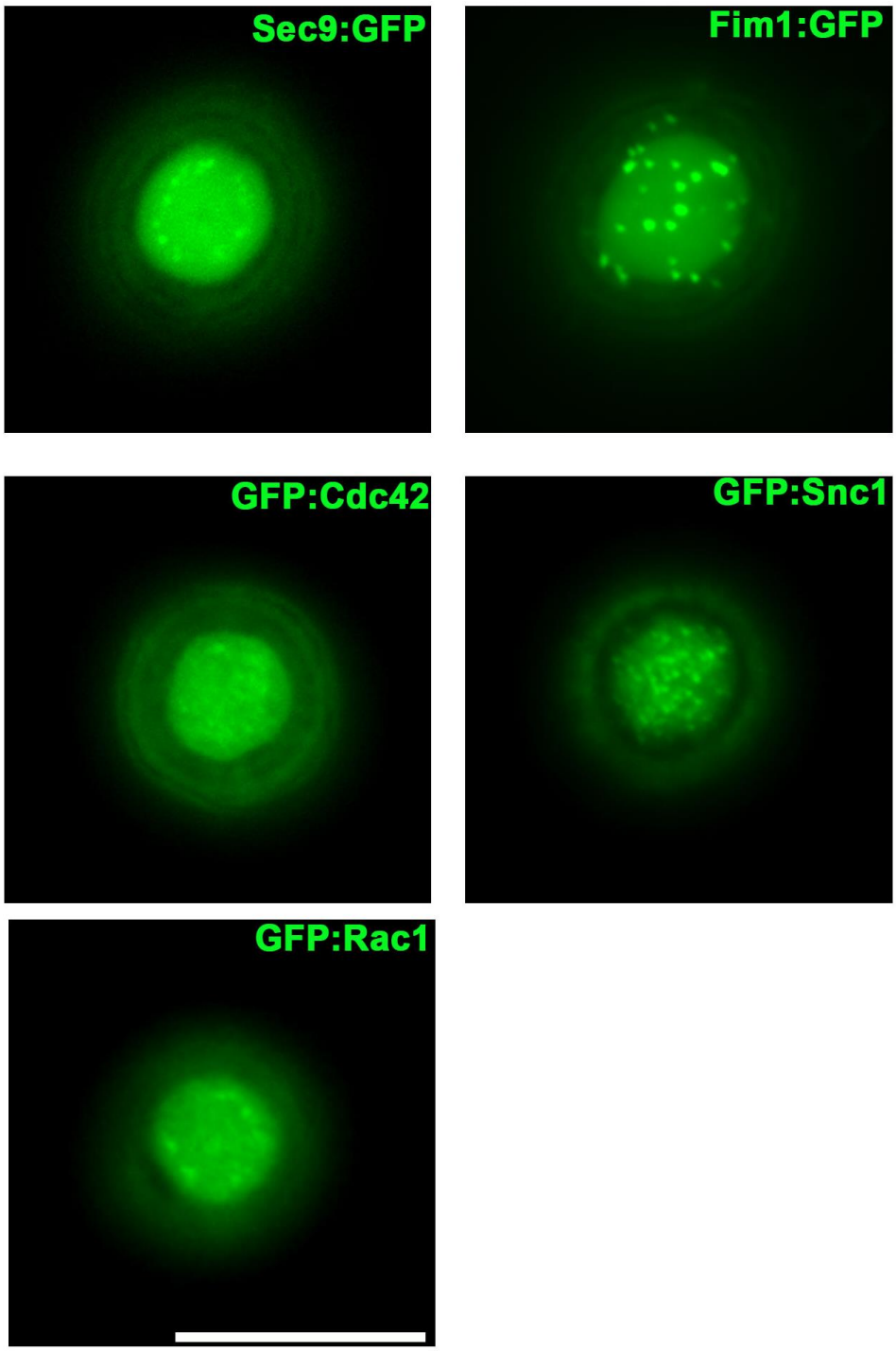
Supplemental Figure 4. Localization of polarized secretory apparatus in growing vegetative hyphae.

A range of polarity determinant and secretory protein-encoding genes were tagged with GFP, expressed as single insertions under control of their native promoter and visualized in growing vegetative hyphae (A-I). All images are representative of 3 biological replications of the experiment. **(A)** Myosin light chain protein Mlc1:GFP accumulated as a bright spot at the hyphal tip. **(B)** Polarisome component Spa2:GFP localised to the tip of the hyphae. **(C)** Fimbrin-GFP (Fim1) was expressed in the sub-apical cortical region of hyphae. **(D)** Sec2:GFP, the GEF for Sec4 (**E**), both localised to the hyphal tip. **(F) the** t-SNARE Sec9:GFP localised to the tips of hyphae. **(G)** The v-SNARE GFP:Snc1 localised in a gradient of vesicles which were concentrated at the hyphal tip. **(H)** The Rho GTPase, GFP:Rac1 localised as a crescent to the hyphal tip. **(I)** The Rho GTPase, GFP:Cdc42 localised the plasma-membrane in a punctate distribution, with increasing concentration at the hyphal tip. Scale bar=10μm.



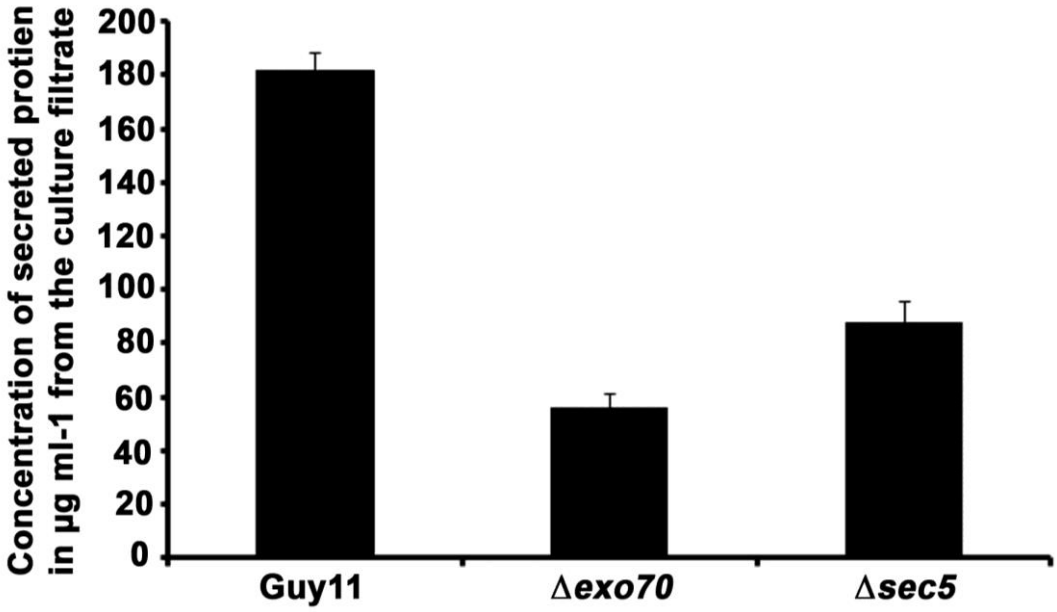
Supplemental Figure 5. Exocyst localization during a time course of appressorium development in *M. oryzae*.

Exocyst subunits, Exo70, Exo84, Sec3, Sec5, Sec8 and Sec15 were tagged with C-terminal translational gene fusions expressed under the control of their respective native promoters. Conidia were harvested with each GFP tagged exocyst strains and inoculated on hydrophobic coverslips, and observed by epifluorescence microscopy. All the exocyst subunits shows similar pattern of expression. Early stage of conidia germination all the exocyst are localized the apex or tip of the germ tube. In appressorium from 4 h to 8h all the exocyst subunits localized just under the plasma-membrane. In mature appressorium at 24 h all the exocyst subunits localized at the pore of the appressoria. Scale bar=10μm.



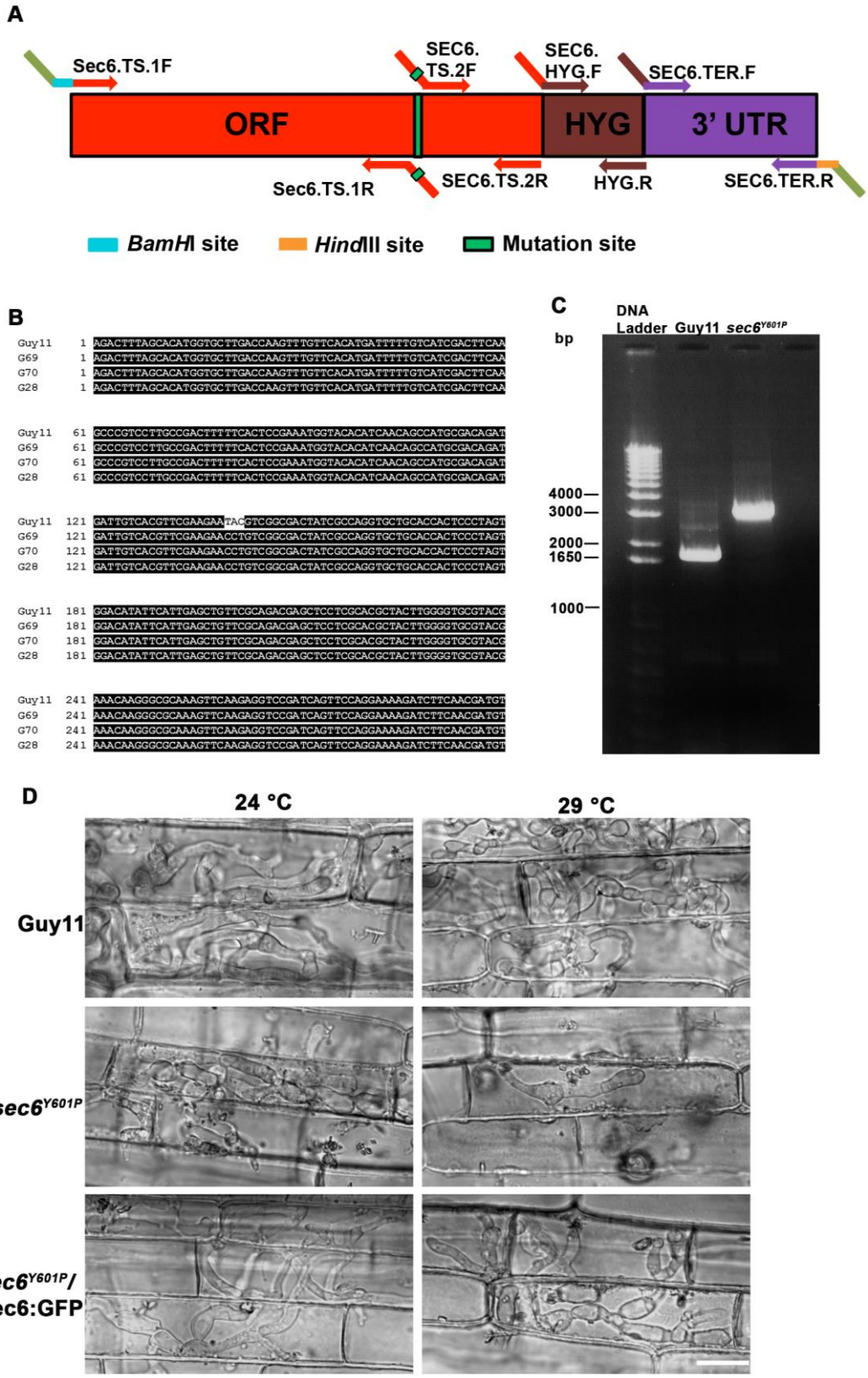
Supplemental Figure 6. Localization of polarity determinants in the *M. oryzae* appressorium.

Epifluorescence micrographs of mature appressoria in strains expressing Sec9-GFP, Fim1-GFP, GFP-Cdc42, GFP-Snc1, and GFP-Rac1 gene fusions under their native promoters. The t-SNARE Sec9 shows punctate distribution around the appressorial pore while v-SNARE Snc1 localize in the pore region. Fim1, Cdc42 and Rac1 were also observed in centre of the appressorium pore. Scale bar=10μm.



Supplemental Figure 7. Quantification of secreted protein from culture filtrates of exocyst mutants.

The wild type strain Guy11, Δ*sec5* and the Δ*exo70* mutant strains were grown in liquid CM for 48 h. Mycelium was recovered and transferred to liquid GMM for a further 24 h. Culture filtrates were collected and lyophilised. Total secreted protein was measure by the Bradford method (Bradford, 1976). Values are mean ± S.D. for three biological repetitions of the experiment.


A
Sec6.TS.1F
SEC6. TS.2F
SEC6. HYG.F
SEC6.TER.F
ORF
HYG
3' UTR
Sec6.TS.1R
SEC6.TS.2R
HYG.R
SEC6.TER.R
BamHI site
HindIII site
Mutation site
B
Guy11 1 AGACTTTAGCACATGGTGCTTGACCAAGTTTGTTCACATGATTTTTGTCATCGACTTCAA
G69 1 AGACTTTAGCACATGGTGCTTGACCAAGTTTGTTCACATGATTTTTGTCATCGACTTCAA
G70 1 AGACTTTAGCACATGGTGCTTGACCAAGTTTGTTCACATGATTTTTGTCATCGACTTCAA
G28 1 AGACTTTAGCACATGGTGCTTGACCAAGTTTGTTCACATGATTTTTGTCATCGACTTCAA
Guy11 61 GCCCGTCCTTGCCGACTTTTTCACTCCGAAATGGTACACATCAACAGCCATGCGACAGAT
G69 61 GCCCGTCCTTGCCGACTTTTTCACTCCGAAATGGTACACATCAACAGCCATGCGACAGAT
G70 61 GCCCGTCCTTGCCGACTTTTTCACTCCGAAATGGTACACATCAACAGCCATGCGACAGAT
G28 61 GCCCGTCCTTGCCGACTTTTTCACTCCGAAATGGTACACATCAACAGCCATGCGACAGAT
Guy11 121 GATTGTCACGTTCGAAGAATACGTCGGCGACTATCGCCAGGTGCTGCACCACTCCCTAGT
G69 121 GATTGTCACGTTCGAAGAACCTGTCGGCGACTATCGCCAGGTGCTGCACCACTCCCTAGT
G70 121 GATTGTCACGTTCGAAGAACCTGTCGGCGACTATCGCCAGGTGCTGCACCACTCCCTAGT
G28 121 GATTGTCACGTTCGAAGAACCTGTCGGCGACTATCGCCAGGTGCTGCACCACTCCCTAGT
Guy11 181 GGACATATTCATTGAGCTGTTCGCAGACGAGCTCCTCGCACGCTACTTGGGGTGCGTACG
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Guy11 241 AAACAAGGGCGCAAAGTTCAAGAGGTCCGATCAGTTCCAGGAAAAGATCTTCAACGATGT
G69 241 AAACAAGGGCGCAAAGTTCAAGAGGTCCGATCAGTTCCAGGAAAAGATCTTCAACGATGT
G70 241 AAACAAGGGCGCAAAGTTCAAGAGGTCCGATCAGTTCCAGGAAAAGATCTTCAACGATGT
G28 241 AAACAAGGGCGCAAAGTTCAAGAGGTCCGATCAGTTCCAGGAAAAGATCTTCAACGATGT
C
DNA Ladder Guy11 sec6Y601P
bp
4000
3000
2000
1650
1000
D
24 °C
29 °C
Guy11
sec6Y601P
sec6Y601P/ Sec6:GFP

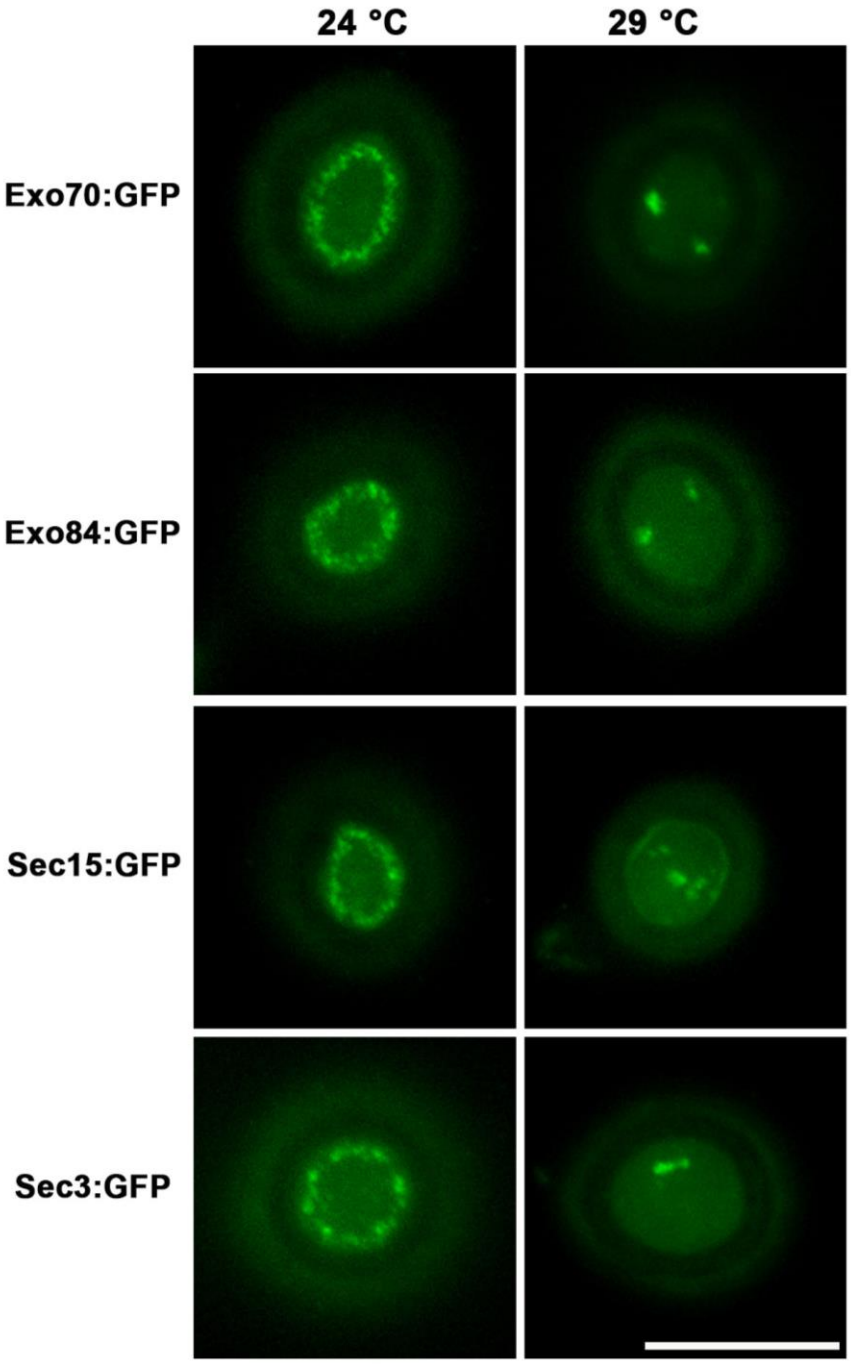
Supplemental Figure 8. Generation of the *sec6*^{Y601P} temperature-sensitive mutant.

(A) Schematic representation of the construction of the *sec6*^{Y601P} temperature-sensitive mutant. The PYC1 vector was used for cloning and primers were designed with 30 bp over-hangs complementary to the region and a 3 bp mutation introduced in SEC6.TS.1R and SEC6.TS.2F primers. The Hygromycin resistance gene cassette was cloned between the coding region and terminator of the *SEC6* gene. *BamH*I and *Hind*III sites were introduced into the Sec6.TS.1F and Sec6.TER.R primers respectively. the *BamH*I and *Hind*III digested fragment was used for transformation of the wild type strain Guy11, leading to allelic replacement of the native *SEC6* locus.

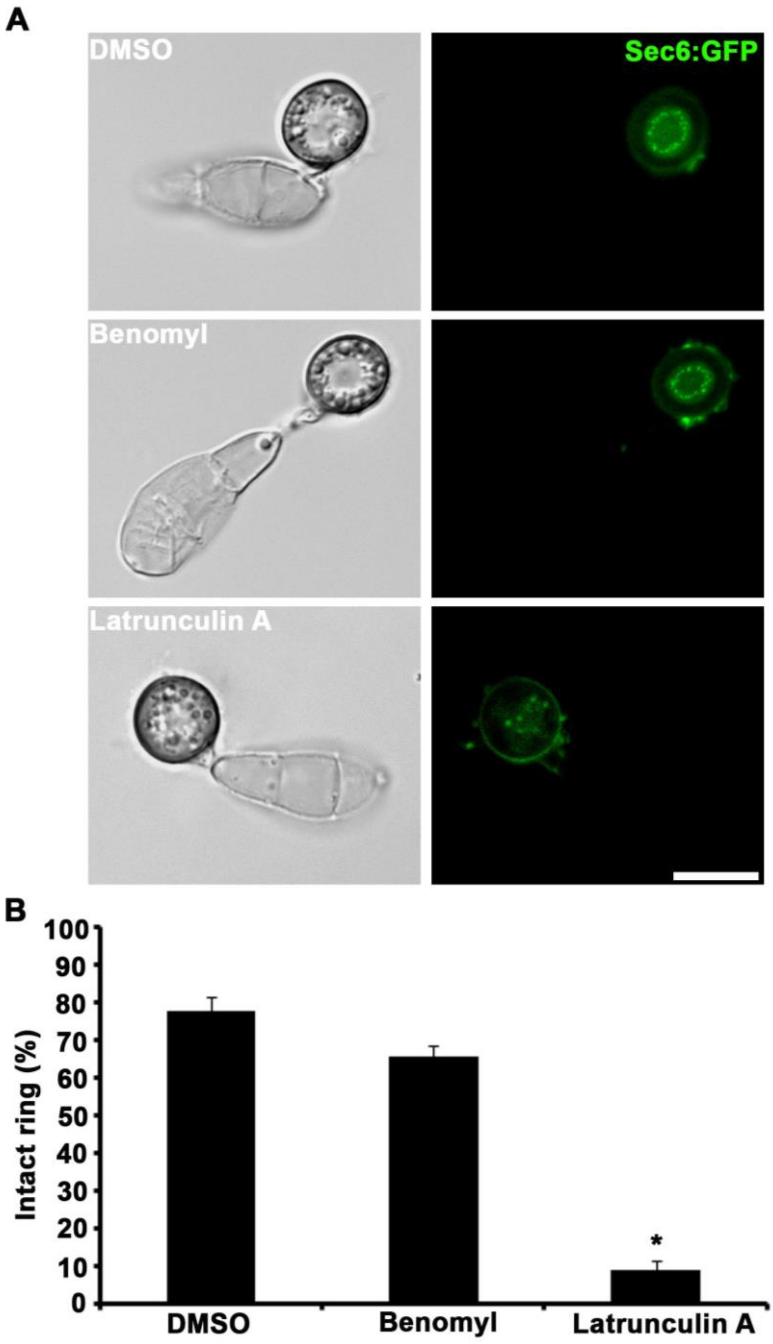
(B) Sequencing of 3 independent transformants and the isogenic wild type strain Guy11 was performed to confirm mutations.

(C) PCR confirmation to show the integration of selectable maker hygromycin (1.4 kb) between the ORF and terminator, using primers Sec6.TS.2F and Sec6.30.1.

(D) Functional complementation of *sec6*^{Y601P} mutant with Sec6:GFP. A leaf sheath infection assay was performed to observe host tissue invasion of susceptible rice cultivar Co-39. Conidia were harvested from each strain and inoculated onto leaf sheath for 45 h. Inoculations were performed at 24 °C and 29 °C. At the permissive temperature, 24 °C, the wild type strain Guy11, *sec6*^{Y601P} mutant and complemented strain (*sec6*^{Y601P} mutant::Sec6:GFP) all infected rice epidermis normally. At the semi-restrictive temperature, 29 °C, the *sec6*^{Y601P} mutant formed infection hyphae that remained in the first rice cell while the complemented strain and Guy11 infection hyphae proceeded to proliferate normally. Scale bar=10µm.



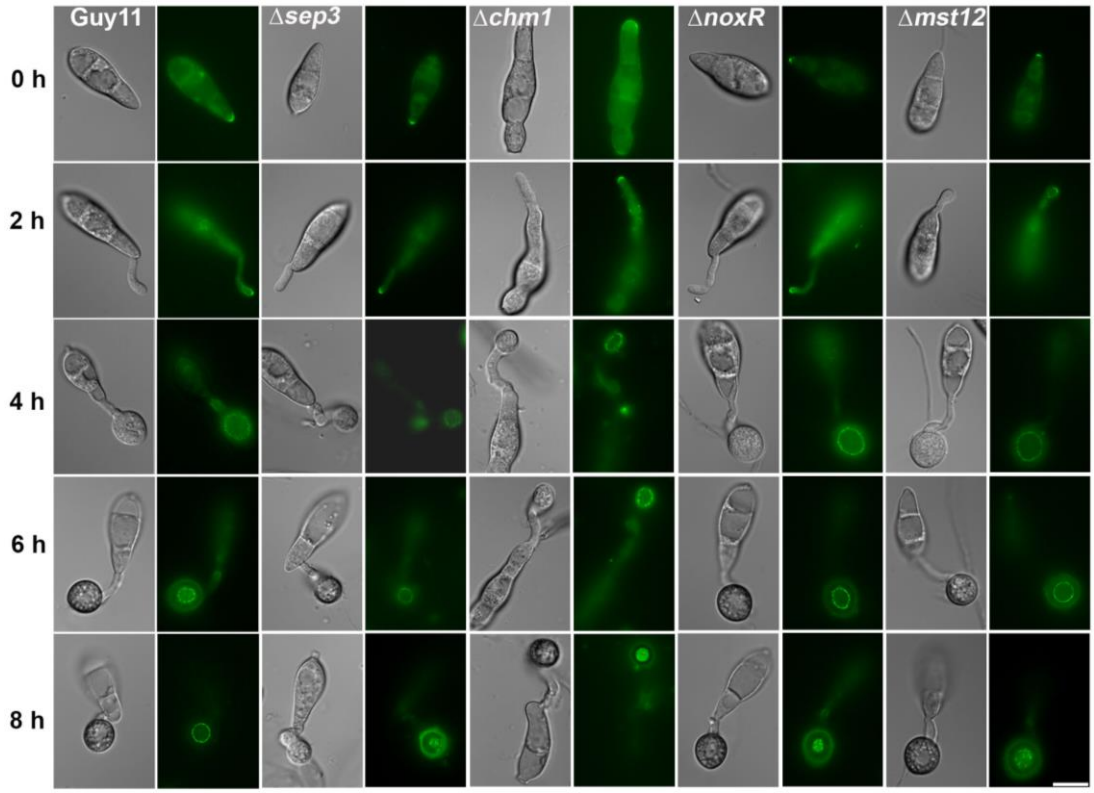
Supplemental Figure 9. Expression and localization of Exo70:GFP, Exo84:GFP, Sec15:GFP and Sec3:GFP in the *sec6*^{Y601P} mutant. Independent transformants were selected, inoculated onto glass coverslips, and observed by epifluorescence microscopy. At the permissive temperature, 24 °C, Exo70-GFP, Exo84-GFP, Sec15-GFP and Sec3-GFP were all observed in a ring conformation at the appressorial pore, but at the semi-restrictive temperature they were mis-localized. Scale bar=10μm.



Supplemental Figure 10. The F-actin cytoskeleton is required for exocyst ring formation at the appressorium pore.

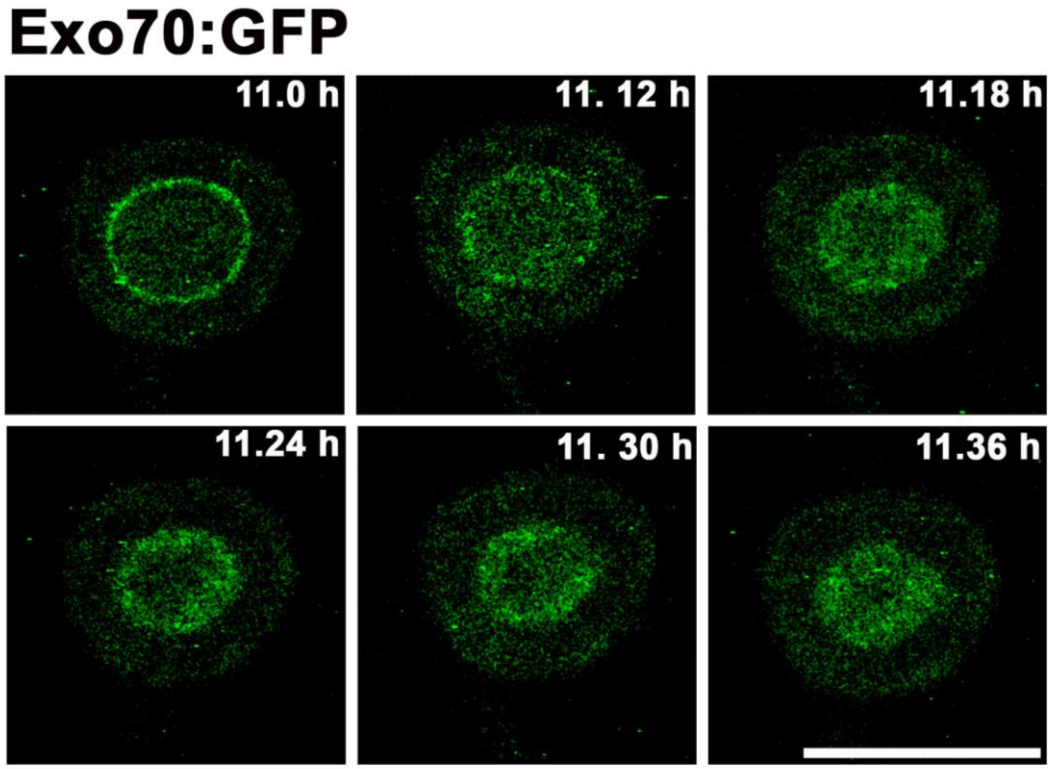
(A) In mature appressoria Sec6:GFP localized at the appressorium pore. Exposure to 10 µM latrunculin A led to disorganization of the exocyst ring after 24 h, while there was no effect following exposure to 30 µM benomyl or 0.1 % DMSO, which was used as a negative control. Scale bar=10µm.

(B) Bar chart to show the proportion of intacts exocyst rings following exposure to latrunculin A compared with benomyl and DMSO treatments. Results were significant ($P<0.01$) (Values are mean ± S.D., three experiments, n=100).



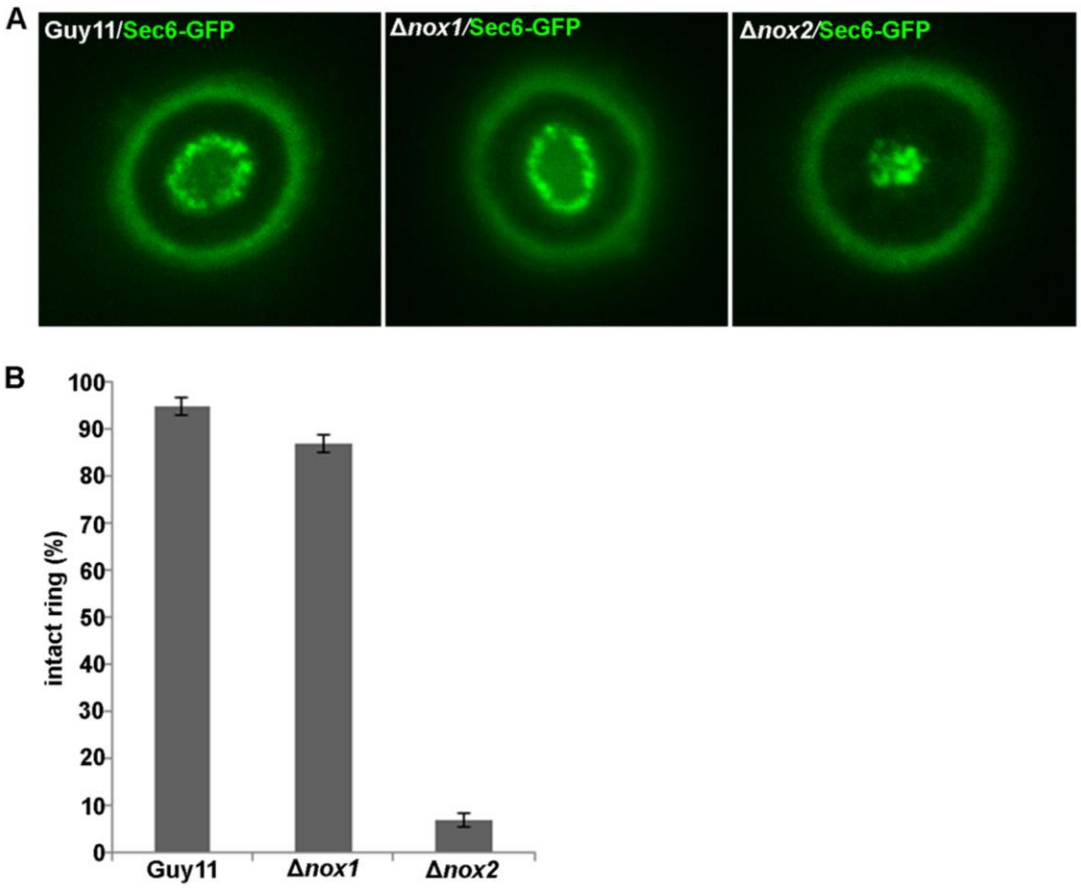
Supplemental Figure 11. Sec6:GFP localization in Δ*sep3*, Δ*noxR* and Δ*mst12* mutants of *M. oryzae.*

Micrograph to show the expression of Sec6:GFP in Δ*sep3*, Δ*chm1,* Δ*noxR* and Δ*mst12* mutants during a time course of appressorium development. Conidia were harvested from each strain and inoculated on glass coverslips and observed by epifluorescence microscopy. Localization of Sec6:GFP was identical in all strains before 6 h. After 8 h there a ring conformation was present in wild type strains but completely mis-localised in Δ*sep3*, Δ*chm1,* Δ*noxR* and Δ*mst12* mutants. Scale bar=10μm.



Supplemental Figure 12. Transition of the exocyst from the cortex of the appressorium to the appressorial pore during maturation.

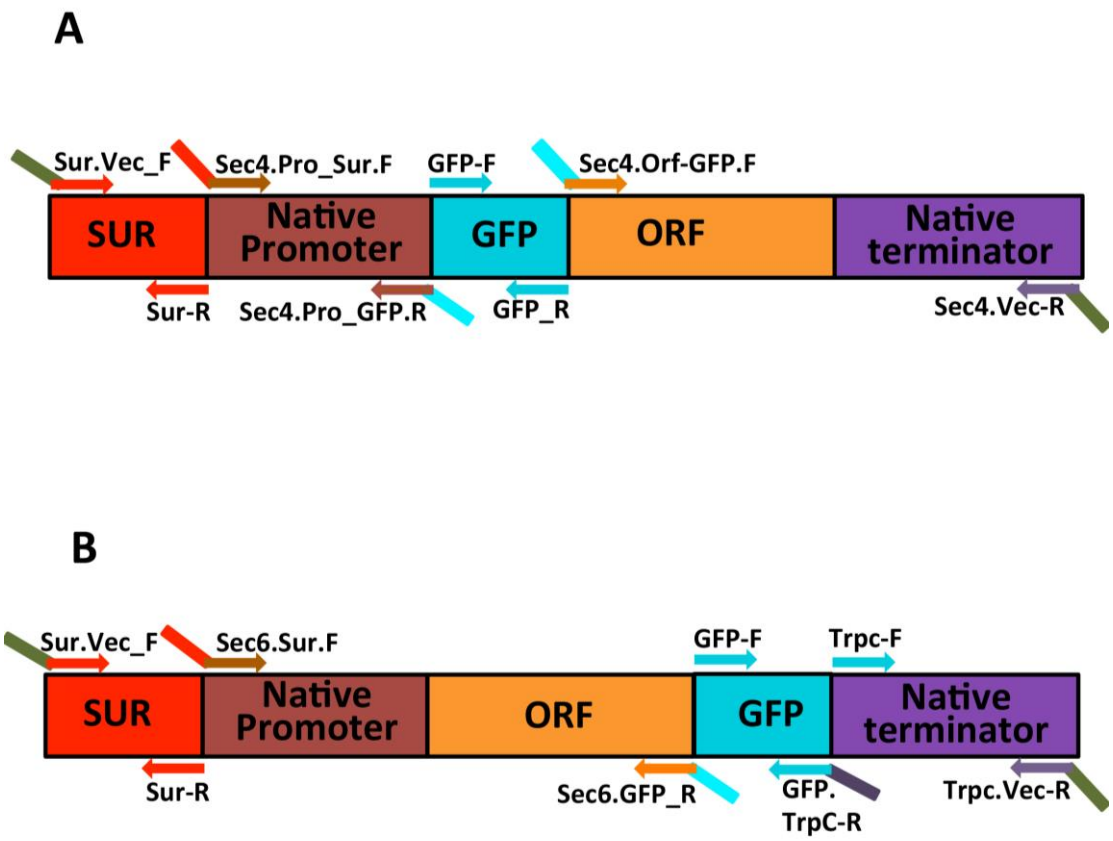
Conidia were harvested from a strain expressing Exo70:GFP inoculated onto glass coverslip and observed by laser confocal microscopy. After 8 h of appressorium development, Exo70:GFP expression was observed and images recorded every 6 min throughout the 8h time course. Transition of Exo70:GFP from the cortex to the appressorium pore occurred after 11 h and the time of observation is shown in every image at the top corner. Scale bar = 10 µm.



Supplemental Figure 13. Sec6:GFP localization in Δ*nox1* and Δ*nox2* mutants of *M. oryzae.*

(A) Epifluorescence microscopy to show localisation of the exocyst sub-unit Sec6:GFP expressed in Guy11, Δ*nox1* and *Δnox2* mutants of *M. oryzae*. Conidial suspensions at 5 × 10^4 ml^{-1} were inoculated onto glass coverslips and allowed to germinate and form appressoria. Expression of Sec6:GFP was observed 24 h after inoculation. Scale bar = 10 µm.

(B) Bar chart showing the percentage of appressoria expressing Sec6:GFP 24 h after inoculation. Values are mean ± S.D. for three repetitions of the experiment, n = 300.



Supplemental Figure 14. Schematic diagram to show cloning methodology for each GFP fusion construct.

(A) N-terminal translational fusion constructs were designed with each primer having 30 bp of overlap with the adjoining fragment. The Sulphonylurea resistance gene cassette (*ILV1*) (Sweigard *et al*, 1997), a 2 kb fragment of the native promoter of each gene, the *GFP* gene, the gene coding sequence, and 1kb downstream of the stop codon (3' UTR and terminator) regions were separately amplified and cloned into the PYC1 vector.

(B) C-terminal translational fusions were constructed by amplifying the *ILV1* resistance cassette, 2 kb native promoter, the gene coding region, GFP and the TrpC terminator and cloning the fragments into the PYC1 vector. All constructs were made using recombination-mediated, gap repair.

Supplementary Table 1. List of genes characterised in this study.

Name	*S. cerevisiae* protein	*M. oryzae* protein	Size (aa)	Function	Blastp e-value
SPA2	YLL021W	MGG_03703	951	Polarisome component	1.9e-23
MLC1	YGL106W	MGG_09470	147	Mysoin light chain regulator	1.3e-23
FIM1	YDR129C	MGG_04478	651	actin-bundling protein, maintenance of the actin cytoskeleton and involved in endocytosis	1.3e-222
SEC2	YNL272C	MGG_02923	670	Guanyl-nucleotide exchange factor Rab-GTPase Sec4p	6.9e-23
SEC4	YFL005W	MGG_06135	206	Rab-GTPase, involved in exocytosis and autophagy	2.2e-60
SEC3	YER008C	MGG_03323	1434	Exocyst subunit	4.9e-29
SEC5	YDR166C	MGG_07150	1055	Exocyst subunit	1e-22
SEC6	YIL068C	MGG_03235	755	Exocyst subunit	9.2e-37
SEC8	YPR055W	MGG_03985	1101	Exocyst subunit	1.3e-41
SEC10	YLR166C	MGG_04559	851	Exocyst subunit	4.7e-31
SEC15	YGL233W	MGG_00471	775	Exocyst subunit	1.3e-43
EXO84	YBR102C	MGG_06098	681	Exocyst subunit	5.4e-30
EXO70	YJL085W	MGG_01760	633	Exocyst subunit	5.4e-30
SEC9	YGR009C	MGG_00522	465	t-SNARE required for secretory vesicle-plasma-membrane fusion	4.2e-09
SNC1	YAL030W	MGG_12614	126	v-SNARE, involved in exocytosis and endocytosis	2.3e-24
CDC42	YLR229C	MGG_00466	194	Rho-like GTPase, important for establishment and maintenance of cell polarity	1e-85
RAC1	-	MGG_02731	199	small GTPase involved in actin cytoskeleton organization and polarized cell growth	

Supplementary Table 2. Primers used in this study

Primer Name	DNA Sequence (5`-3`)
Sec6.SUR_F	GATTATTGCACGGGAATTGCATGCTCTCACCCGTCTTTTTGTTGTCGTCTCT
Sec6.GFP_R	GGTGAACAGCTCCTCGCCCTTGCTCACCATCTTGACTCTACTCATAATAG
SEC6.TS.1F	AACTGTTGGGAAGGGCGATCGGTGCGGGCCGGATCCAGCAGGAGTTCACACGCAAG
SEC6.TS.1R	TCGCCGACAGGTTCTTCGAACGTGACAATCATCTGTCGCATGGCTGTTGA
SEC6.TS.2F	GATTGTCACGTTCGAAGAACCTGTCGGCGACTATCGCCAGGTGCTGCACCACTC
SEC6.TS.2R	GCCAAGCCCAAAAATGCTCCTTCAATATCATCACTTGACTCTACTCATAATAGT
SEC6.HYG.F	CCCGAGACTATTATGAGTAGAGTCAAGTGATGATATTGAAGGAGCATTTTT
Sec6.30.1	CTGGCGTTGGTTTTGAGTTTGTCG
HygR	GGTCGGCATCTACTCTATTCC
SEC6.TER.F	GAGGGCAAAGGAATAGAGTAGATGCCGACCGAGATACCAACCAAAGGCACAATC
SEC6.TER.R	TTCACACAGGAAACAGCTATGACCATGATTAAGCTTCTATATACAGATGGGCGCTGAGGT
Sur_vec.F	AACTGTTGGGAAGGGCGATCGGTGCGGGCCGTCGACGTGCCAACGCCACAGTGC
SurR	GTCGACGTGAGAGCATGCAATTCC
Cdc42.Pro_SUR	GATTATTGCACGGGAATTGCATGCTCTCACTCGTCATACTGGCTGCTTCC
Cdc42.Pro_GFP	GGTGAACAGCTCCTCGCCCTTGCTCACCATTGTTTAGAGCTGAGCGGGAG
Cdc42.Orf-GFP	ATCACTCACGGCATGGACGAGCTGTACAAGATGGTGGTTGCAACGATTAA
Cdc42.Ter-R	TTCACACAGGAAACAGCTATGACCATGATTCATCCAAACTTTACCTGCCC
GFP-F	ATGGTGAGCAAGGGCGAGGA
GFP-R	CTTGTACAGCTCGTCCATGC
Exo70.Sur_F	GATTATTGCACGGGAATTGCATGCTCTCACTTCTTCTCCACACCTCCCAGCA
EXO70.GFP_R	GGTGAACAGCTCCTCGCCCTTGCTCACCATGTAAAGGCTGGCGAAAACGGCA
Exo70 50.1	GCTTCGGGCATTTTGGTCATCTGA
Exo70 M13f	GTCGTGACTGGGAAAACCCTGGCGAGACCCCAGATGATTGTAGCTCGT
Exo70 M13r	TCCTGTGTGAAATTGTTATCCGCTCAAGGGCAAGGGCAAGTATGTCAA
EX0 30.1	ATTACTTACCACGCTGCACATGGG
Exo70.5.1	CACTACATACCGCATTTTAACCAA
Exo70.3.1	TTCTTGATACTTTCCTTGTCCTTG
SEC5-SUR-F	GATTATTGCACGGGAATTGCATGCTCTCACAGGAGTGGCCCAGTTAGAATGA
Sec5.GFP_R	GGTGAACAGCTCCTCGCCCTTGCTCACCATTACGGAATCCTTGCGCTCCGTT
Sec5.50.1	TTGAACTTTGAAGCGATCTCGTCC
Sec5.M13F	GTCGTGACTGGGAAAACCCTGGCGGAACTAATCAACGAGCAGCCAAGA
Sec5.M13R	TCCTGTGTGAAATTGTTATCCGCTAAAAAGGTCAAGCGGTCAAACTCG
Sec5.30.1	CTTACGGTTTTGAATGCTTGTGTG
Sec5.ORF.F	GACTCCAATGACAAAAAGGCAA
Sec5.ORF.R	CTGCGTATGACTGAATCTTTCC
EXO84.SUR_F	GATTATTGCACGGGAATTGCATGCTCTCACAGAGGGTGGATAGAAAAGGA
EXO84.GFP_R	GGTGAACAGCTCCTCGCCCTTGCTCACCATAGAGAGGCCGAGGCCAAGCG
Sec3.SUR_F	GATTATTGCACGGGAATTGCATGCTCTCACAATACAGGACTGGGAAACGC
Sec3.GFP_R	GGTGAACAGCTCCTCGCCCTTGCTCACCATCCCTCTCCCAAGGTTCGAAA
Sec4.Pro-Sur.F	GATTATTGCACGGGAATTGCATGCTCTCACTTTGGGTTCGTCGGGCTT
Sec 4.Pro-FP.R	GGTGAACAGCTCCTCGCCCTTGCTCACCATGGTGCTGCGTTTGGAGCA
Sec4.Orf-GFP.F	ATCACTCACGGCATGGACGAGCTGTACAAGATGGCCAACAGGAATTACGATG
Sec4.Ter.R	TTCACACAGGAAACAGCTATGACCATGATTAACGGACAACGACGCACTCTAT
Sec9.Sur-F	GATTATTGCACGGGAATTGCATGCTCTCACAATGATGCGGTGTCTTGAGT
Sec9.GFP-R	GGTGAACAGCTCCTCGCCCTTGCTCACCATACCCTTCTTGTAGATGCGGT
Sec15.SUR_F	GATTATTGCACGGGAATTGCATGCTCTCACGGCATCCATAAAGCGAAACT
Sec15.GFP_R	GGTGAACAGCTCCTCGCCCTTGCTCACCATGCTGAAACCAAAACGAGATG
SEC8-SUR-F	GATTATTGCACGGGAATTGCATGCTCTCACCCAGAGGGCATTACACAAAA
SEC8-GFP-R	GGTGAACAGCTCCTCGCCCTTGCTCACCATAACCGTCCCCAAACCTTCAG
Spa2-Sur-F	GATAATGGGAATTGATTATTGCACGGGAATGCAATACGCACAAACATGGAGC
Spa2-GFP-R	CAGCTCCTCGCCCTTGCTCACCATTGAAAAGTCATCACCACCACC
1F.FIM	TGACTTCGATGAGCTGGATGG
2R.FIM	ACATGGAAGGTAGGAGCGGAA
3R.FIM.SpeI	ACTAGTAGCCATCTTTTCATGCGTCGC
4F.FIM.NcoI	GGTACCATGAACGTTCTCAAACTTCAGAG
5F.FIM.XbaI	TATCTAGAATGAACGTTCTCAAACTTCAGAG
1F.SNC1.GFP	CACGGCATGGACGAGCTGTACAAGATGCCCGAAGACGCTCCCTAC
2R.SNC1.GFP	CAGCTCCTCGCCCTTGCTCACCATGTTTGCGGTTGCGGCTCTTTC
3F.SNC1	ATTTCCCATACGCTGCCGACCAT
4R.SNC1	TTGCGTGCTGATGTTGTGTATTG
SNC1.F	CGTATCACATCGTCAATTCTCA
SNC1.R	GACAACGCACTACAAAGGAGG
Mlc1SUR	GATTATTGCACGGGAATTGCATGCTCTCACGTACCATTCGCAACGTGTTTGGAG
Mlc1GFP	GGTGAACAGCTCCTCGCCCTTGCTCACCATGTTGGCCAGAATGGTCCTAACAAG