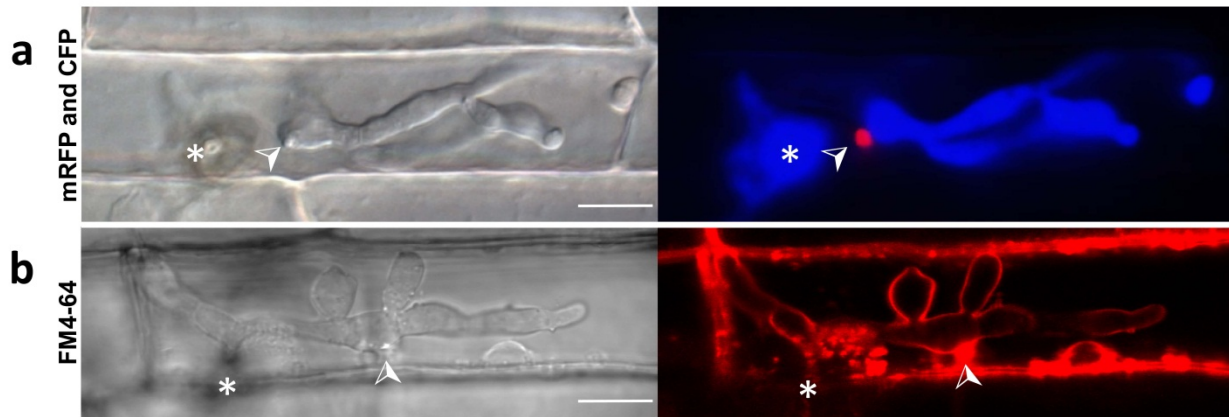


## **Supplementary Information**

**Two distinct secretion systems facilitate tissue invasion by the rice blast fungus**  
*Magnaporthe oryzae*

**Martha C. Giraldo, Yasin F. Dagdas, Yogesh K. Gupta, Thomas A. Mentlak, Mihwa Yi,  
Ana Lilia Martinez-Rocha, Hiromasa Saitoh, Ryohei Terauchi, Nicholas J. Talbot and  
Barbara Valent**



**Supplementary Figure S1. BICs are interfacial structures outside the fungus.** (a) BICs are spatially separated from the fungal cytoplasm. Imaging was performed with fungal transformants expressing a cytoplasmic fluorescent protein, in this case the cyan fluorescent protein (blue) under control of the constitutive *M. oryzae* ribosomal protein P27 promoter. The BIC (➤), labeled with cytoplasmic effector Bas1:mRFP, does not co-localize with the fluorescent marker in the fungal cytoplasm. Images left to right are bright-field; and merged mRFP and CFP channels. (b) BICs contain material derived from plant membranes. The BIC region (➤) is heavily stained by the lipophilic styryl dye FM4-64<sup>37,39,45</sup>, which is internalized by endocytosis in infected rice cells. FM4-64 is excluded from fungal membranes by the surrounding plant-derived EIHM. Here, infected rice sheath cells were exposed to FM4-64 for 4 h before imaging. Lack of staining in fungal membrane is clearly observed by the absence of dye in the hyphal septa, which would have become stained by lateral diffusion of dye that reached the fungal plasma membrane. Additionally, there is no late-stage staining of fungal vacuolar membranes. Note that exclusion of FM4-64 from fungal membranes *in planta* means that this dye is not useful for identifying Spitzenkörper in these hyphae. Images left to right are bright-field alone; and red channel alone. Appressorium penetration sites are labeled with a white asterisk. Scale bars, 10µm.

**a Mlc1 protein sequence alignment**

```

M. oryzae 1 GG---DFDFET-FQRVLNRPGGFRDPGEPEEYCRGFQVFDKDMTGFIVGVGQLKYILTNL
N. crassa 1 GG---DFDFET-FQRVLNRPGGFRDPGEPEEYCRGFQVFDKDMTGFIVGVGQLKYILTNL
A. nidulans 1 GG---DFDFES-FLKVLNRPGGFRFPGEPEEYCRGFQVFDKDMTGVIGKGAVALYLDL
S.cerevisiae 1 RDASSLTLDQITGLIEVNEKELDATTKKTEDEVKAFQVFDKISTGKIVSGVGLRMYMLTGL

M. oryzae 56 112GEKMTDEEVDELKSVDT-SSGQVNYTGKSE-TSCWRLQ 150
N. crassa 56 104GEKMTDEEVDELLKAVDT-SSGQVNYTELVRTILAN--- 139
A. nidulans 56 104GR--EDLR----- 110
S.cerevisiae 61 113GEKMTDAEVDELKGVVDSNGEFDYKKFIEDVLRQ--- 149

```

**b Snc1 protein sequence alignment**

```

M. oryzae 1 GERLDVLDQKTDNLAQSAQGFRRGANVRKQMWKDMKMRVCLIVGIIILLIILPIVF
N. crassa 1 GERLDALQTKTDLAISAQGFRRGANVRKQMWKDMKMRCLIVGIIILLVIVPAAV
A. nidulans 1 GERLDSLDQKTDNLAQSAQGFRRGANVRKQMWKDMKMRVCLITCIILLVIVPAAV
S.cerevisiae 1 GERLTSLEDKADNLAQSAQGFRRGANVRKAMWTKDKMKMCLALVIVILLVIVPAAV

M. oryzae 61 120HFKGN 125
N. crassa 61 113ATR-- 116
A. nidulans 61 113TTR-- 117
S.cerevisiae 61 113HFSR- 117

```

**c Spa2 protein sequence alignment**

```

M. oryzae 1 -----MNAATRNPPLSPISIGNEP
N. crassa 1 -----MN---VRNAP-LSPISQCAFS
A. nidulans 1 -----MN---GSGTMSPISVD--GS
S.cerevisiae 1 TDVSDQLRRIGEDANQPDYLLPKANFHMKRNRQARQKLANLSQTRFNDLLDDLFEIKRR

M. oryzae 23 22SWNSGYSPPRGVDIKDGYYPNGRG-----NPFSPPISSGSIQA
N. crassa 18 17DWN-----HPNDNNG-----QYNTPPDSANPAGA
A. nidulans 17 16DWSG--LNQYQKSAFSPPTFSRS-----NLATPPTSGIPAPP
S.cerevisiae 61 120GFDKLDAPRPLPQPMKQEVSRDSDDTARTSTNSSSVTQVAPNVSVQPSLIPKMP

M. oryzae 62 MNG 64-FPPGERS---TGGSPPPSIGRSSQGTNIYARSESGRSRAPEDSLVLTTHY
N. crassa 44 MNG 46GFSEMPQSP--NGAPSPPPSIRSS--TGYARSKSSQSC--ENQELVLSHY
A. nidulans 54 NSM 56GLPNSSQLSISGNPSPNSIARS-----SDGTLCDORSRQRQVEETLAQHY
S.cerevisiae 117 ASID 180WSSEEEEEQVKEKPNPEPKQISMDEKKEAKPALNPVVTDSDEPDSQVLR

M. oryzae 115 NLLKRF 120LGSSTQ-----NCGQFPPPNKAR
N. crassa 96 ISLKRY 101LSATSR-----DGNPKPPPNKAR
A. nidulans 106 SALKRF 111LYTSYR-----DERSNRKSSKAQ
S.cerevisiae 173 DITSMART 240PTLTHKNYWDVNDSPIIKVDKDI DNEKGPEQLKSPEVQRAENNNPNSE

M. oryzae 138 DKLQRLITV- 146----QFLELSTDVYDELNRRFPPQNSEP-----PKYLLPRDN
N. crassa 120 DKLQRLTEV- 128----QFLELSTDVYDELKRRREQVARAGPNAPPETAPDFLLPQDN
A. nidulans 130 DKLLRLSPT- 138----QHLELSTDVYDELLRRCQ-AMPSPNPPRPVPPFLPERSD
S.cerevisiae 229 MEDKVKELTDLN 300SDLHLQIEDLNKASLTSKKEKKEEKEEKEKKNLKNYTI

M. oryzae 181 ----- 180-----FHPKRNQARQKLSSLP-----
N. crassa 170 ----- 169-----FHPKRNQARQKLSSLG-----
A. nidulans 179 ----- 178-----FHEKRNQARQKLASLQ-----
S.cerevisiae 285 DESFQKELLSLSQIG 360ELSIENENLKQKISEFELHQKKNDDHNDLKITDGFISKYS

M. oryzae 197 ---APRFRDLATDVFCE 210LERRIPRVADGELPRVNS-----
N. crassa 186 ---PRFRDLATDVFCE 199LERRIPRVANLEMLNAS-----
A. nidulans 195 ---HQRFRDLASDVENE 208LERRIPQPTRESRRASP-----
S.cerevisiae 341 SADGLIPAQYLNANNLIQ 420FTTRLSAVPIGDSTAIHQIGEELFQILSLSNLIS

M. oryzae 229 ----- 228-----PASMRRMSRSRQTPVSGMNGYF
N. crassa 218 ----- 217-----PIAMRGEPSRSCTFNNGMGGFP
A. nidulans 227 ----- 226-----APSTR---GRPPNGVPEGGYF
S.cerevisiae 397 QLLSADLLQYKDQVILLKASLSH 480AITSIRYFSVYVPLIKITVQAAVSEVCFAM

M. oryzae 250 PRNPSRARRPSEASSRST----- 268-ALNSAYGIPPSGPLP-----
N. crassa 240 PRGOS-HRRPSEASSRSARSGPPMP 264PMNGGYGIPPSGPLP-----
A. nidulans 246 PPNPS--RRSQRGPPRMG----- 262-RGYPSGGPPGSPMYPERK-----
S.cerevisiae 453 CNLIDSAKIKSISNGESTTSNEGQRVQL 540EYSSPTATPMTPTFPSTSGINMKKGF

```

Supplementary Information (Giraldo *et al.*, 2013)

*M. oryzae* 285 -----PGA---YDRPMAKQSOSN 299TIVPVKSTMVEED-----  
*N. crassa* 282 -----NGD---YGRPMPKQFOSN 296TIVPNKSTMVEED-----  
*A. nidulans* 281 -----MSISGAGMNGGCPMAKSFOSN 301TIVPNKSTMVEED-----  
*S.cerevisiae* 509 NPRKPASFLNDVEEEESPVKPLKITKAINSP 600IRPSISNGVFTTSRKPSGTGLFS

*M. oryzae* 313 -DDSPISPTSP-ENGDDYGVDPGQTRMNNERSSA 344TSEADRQLDDYEKQVRLLQAK  
*N. crassa* 310 -DEGNIMSPG--QGGPMYNGG--RRSMG-----Q 333AFEADRKLQDYENQVRLQEK  
*A. nidulans* 315 -DDAAGTEDDYDSRSDAFALDSFIRSRRTG-TT 346IGDGERKLLAETQSOVSTLQEK  
*S.cerevisiae* 565 LMIDSSIAKNSSHKEDNDKYVSPIKAVTSASNSASS 660NISEIPKLTLPQAKIGTVI

*M. oryzae* 367 VDLLELDLKKKDELEAVQD----- 386----RERLSATRINVDK  
*N. crassa* 356 VDNVEMEMRKKDEELNNMQS----- 375----GDEN-----K  
*A. nidulans* 369 VSKLEELKTKDEEITKYQDRQEVGKLEELLRAKLEE 406LAKYQEDQDKSQISNAER  
*S.cerevisiae* 621 PPSENVVFNKIENTEDNKRSDITN-----EISVK 711PTSSIDKDKQFEQSS

*M. oryzae* 400 KSWDARLDLENQLAQAQDLNDRLQELDRIRDHAADAROM 441REEIDALRONGTRG  
*N. crassa* 381 KDWDETRQSLKTLAQAQELNDSLQRELDRIREHEDETRKL 422REGLDGRQS----  
*A. nidulans* 425 QWDEIKSELENKIHKAEDLNLSLQLELEKVRAEHVMERDL 466QAQLSCTSRH---G  
*S.cerevisiae* 668 EKKSSPKENPIAKEEMDSKPKLNSNKFITSMNDVSTDDSSSGNE 771NDADDDDDFTY

*M. oryzae* 456 GSGGGGAGDAFLQREND-----DLRRLIREQE 482QVTEEVQOE  
*N. crassa* 433 ---GCKGDILARENE-----ELRQSLQEQ 455QTEVRRRA  
*A. nidulans* 479 EDDELQAFADLEIRHQ-----KLAELQEQR 505QVTEEVRRRA  
*S.cerevisiae* 724 MALKQTMKREGSKIEKNNDSKLPANIVELDLHESPESVKIISPESIKE 831ITSEMSS

*M. oryzae* 493 QNLREMRTLQQSGAAWEKQTEERTIELEAEAQEWRSRYTKTKTQLR 542NLKATS  
*N. crassa* 466 QFLREMRTLQQSGAAWEKQTELERTIESLEKEKEWRSRYATQQLR 515SRDSS  
*A. nidulans* 516 AGFLMEMRELSQSHSRLEEERLSEVHRLDEIVTKGRYAKAKQLR 565HLRASS  
*S.cerevisiae* 780 EMPSSSPKRLVEDVEPSEMPEKGASVESVRKKNFQPLGNVESPDMTQVK 891SLGM

*M. oryzae* 549 MSI-TVDD-AKRYMRENGFTT-----ENGLVKDEHVTKFQV 582AI  
*N. crassa* 522 EGV-PLRQNAKRYVREKGFVE-----DSGLVKDEHVTKFQI 556AI  
*A. nidulans* 572 AGIPELRSVNTVAKNEFH-----DGLTKDVHVTKFQL 607SI  
*S.cerevisiae* 836 TGKAVGPESDSIVESPGMTGQIKSLNMAGKVVGPEADSRVSPGMKEQKSLGTMG 951

*M. oryzae* 585 DELLQARSDUPDKIMETMKTIVVVSVRRIKDMDAGNGS---EQRKLGRIASTAN 6  
*N. crassa* 559 DELLQARNDIPERVDSMKAVVVSVRRIKDIDNPONDSILOEAKLKARVSSAN 6  
*A. nidulans* 610 DELLRVARSDDHRHVMQOINAVVIVSRHLQDVQLSKSSDS--AEAKATRVSATAN 6  
*S.cerevisiae* 892 KITAQESIKSPEAARKLASSFVDKIESPRMVRSESLEAVGNTIPSNMTVKMESPNLKG

*M. oryzae* 640 39NLITAAKNFAASAGIS-----  
*N. crassa* 617 16NLITASKNFASSAGIS-----  
*A. nidulans* 666 65NLITASKNFASSAGIS-----  
*S.cerevisiae* 952 1011NTVSEPEIRDIASSEPIENVDPKVLKIVFPKAVNRTGSPKSVKTPSATL

*M. oryzae* 656 -- 655--PVSLIDAAASHLVAIVELLKTVKIRTPAGELE-----  
*N. crassa* 633 -- 632--PVSLLDAAASHLVAIVELLRAAKIRTPAGELE-----  
*A. nidulans* 682 -- 681--PISLLDAAASHLSTAVIELIRMKIRTPADELN-----  
*S.cerevisiae* 1007 KKSGL 1071PEPNSQIVSPELAKNSPLAPKKNVELRETNKPHTEITITSVEPTNKDANT

*M. oryzae* 690 ----- 689--DEDDDDMTPGN-----NSVGFSPRQAPTPLTTKLPAE  
*N. crassa* 667 ----- 666--EEDDGTTPVG-----SASFFSPRNGSQ--TSSVSAH  
*A. nidulans* 716 ----- 715--DDEEQFMQK-----SPDIFSVAPSQSR--LSNGSIY  
*S.cerevisiae* 1062 SWRDADLNR 1131IKRDEEDEDFRVNHNIQITGAYTKTKIDYHKIPVDRKAKSEAE

*M. oryzae* 726 ENLPPPPPPFR 735GEG---NRLSADSSAYS-----  
*N. crassa* 699 EVLAQPPAFQ 708GLGGGGSRLSVDSSAYS-----  
*A. nidulans* 747 SAMSPPP-- 753-----SEHVP-----  
*S.cerevisiae* 1117 VHTSEEDIDESNNVN 1191GKRADAQIHITERKHAFVNPTENSQVKKTSHPFLNSKPV

*M. oryzae* 751 ----- 750---VNSPRESTATDFRFGGGLGINGSNNGINGNMNGSGKAL  
*N. crassa* 727 ----- 726---ANSPRESYAES-----K  
*A. nidulans* 760 ----- 759---NGLKNGYSV-----  
*S.cerevisiae* 1172 QYENSESNGGINNHKIKNT 1251GETTARHDEKHYSDDDDSSYFVPMKHEEQQEQR

*M. oryzae* 790 PPPPSNGYGANGYGGMA 807PQRDNAAEELKIVVEDQTAFLVADTQELVAAIRSDAA  
*N. crassa* 739 ETAPP----- 743PRR---VELKFLYQDKTSVMVEIQNLVQLIRSDAN  
*A. nidulans* 770 ----- 769-QENHELOELRFYVEDQADGLVQSIQSLVASIR-GEES  
*S.cerevisiae* 1227 SEEESEDDDEEEDSDFDVDTFDI 1311ENPDNTLSELLYEHQTMDEVISTIQSLT

*M. oryzae* 845 IITLRDEIARIASVDKIVDET 866S---RSSGGPNAFPN----LKNLOACRERILEAG  
*N. crassa* 778 INQLSGEIALITDYSKVVVAET 799SCLGSSASSTVVVDV----VRLSKCSRINEAG  
*A. nidulans* 806 VTTTRTHVSHIATSVINVSST 827EH--LISRPEIAPAL----RQRAGASIEITLEYQR  
*S. cerevisiae* 1282 SIKKPOVTKENLRGESNAINQVIGQMV DAT 1371SISMEQSRNANLKKHGDWVVQSLRD  
  
*M. oryzae* 894 ERGQHSIDGGIGPPSR-EWRMWTQTL 918PPIAFETARETKELVQSVDRVLVIGGP-GGG  
*N. crassa* 830 QHGIIIDLAQSSSPTSREWRMWAQTL 854PPIAFETARETKELVQSVDRLAAAGMNDGA  
*A. nidulans* 856 SRLVSAAEGBGATDAGQLCVETNQL 881PPIAFETARETKDLVQRIDSTDHGDA--ED  
*S. cerevisiae* 1337 CSRRTILCOITG DGILAKEKSDDYADKNFKQRL 1431AGIAFDVAKCTKELVKTVVEE  
  
*M. oryzae* 948 DDFS- 951  
*N. crassa* 885 DDFA- 888  
*A. nidulans* 910 DDFR- 913  
*S. cerevisiae* 1392 ASLDEINYLNSKLK 1466

## d Sec5 protein sequence alignment

*S. cerevisiae* 1 LKDFKYGNQISIDKESRAYLNDESLSYI--RDPLNGQE-WSKELQH----LPNDS-MRLN  
*A. nidulans* 1 LDRSNRRGPNL-GSMKGNNSRENLVKVDEPDPLGSSVSVINALKRGLPWAFDSRLRNR  
*M. oryzae* 1 LERAVSTRSSVVPVPGSEKTANGTIGNLVQRDEPDPLGTTDSVVRTLKQYGVPLQDDERLRNR  
*N. crassa* 1 LERVVSNRSSVVPVPGSETSAAGCVGNLVQRDEPDPLGTTDSVVRTLKQMGVPLQDDSLRNR  
  
*S. cerevisiae* 53 FLLSSTGFSPALFSLQVHSDASTESLINGLNVLSQSIDQKSASLKVLVENANFERFVRAKA  
*A. nidulans* 60 FLLSSTTFSPALFSLQVHASADINQLMLGLDALSQSIDQKSASLKVLVESNFERFVRAKA  
*M. oryzae* 61 FLLSSTTFSPALFSLQVHADSTEALLNGLEVLNQSIDQKSASLKVLVESNFERFVRAKA  
*N. crassa* 61 EVLRITTFKLFK-----PLMDNYQKILNYQATKFFELNKFYENLPKS  
  
*S. cerevisiae* 113 TIDNVYKEMKYRGAEPAPPTARARAHSRHTSRNSFRGSCNINLTAPLTFQPPDPRKKN  
*A. nidulans* 120 TIDNVYKEMKYRGAEPAP--RARAHSRHSRNSFQSTSAAGGLANSIGP-TIDPRKKN  
*M. oryzae* 121 LKRCLTNDFNEFIIEYSKCLTLR---RRFNQSSDAS-----QSLVTKRIWTQIE  
*N. crassa* 103 LSKSIKQKDYESVFEQYRKARALTQEAKNIADIAGSERPLTDEETVYVLAAGRMWIDVD  
  
*S. cerevisiae* 173 ALRRESEYGVMGVKAPLLDVSAKAEDFWCPALGGREKEEHLKTVGSSDITYKYDVEISAA  
*A. nidulans* 176 NLRVTYKDLIWNLSLI-----NSNFNIDOPQETILSLFSKLLNLENFNINNQRESESGNK  
*M. oryzae* 168 QQTQCFKRDLRRLSEAPSTSTRITTSGLPIEYEMELIGALLE-----  
*N. crassa* 163 EQISAFKRTVWRRLSALHNVSRSID-GPQOPQOHMELISLLEL-----  
  
*S. cerevisiae* 233 NTSSSNENPILRWMSIEMNGFQNELNELSGHMISKIHSQRLLQNNTNQDKSQGCVEL  
*A. nidulans* 230 ---GVDNPIWVWLLSRDYLRKAKIKAFECERKVEI-----EILRRRLASGAEPPTQEV  
*M. oryzae* 211 ---GAEINPIEWWLLSRYDNLKQKIQSMADRAKVEI-----EVLRRRLSSSEPPAPRAI  
*N. crassa* 206 ---GVEINPIWAWLQSRVAYLKSRIQSTAEKSKVEI-----EVLRRRLANVEKPSQSI  
  
*S. cerevisiae* 293 ASYLR-----RTPQDSSTGPAH-----LPDIDQVIE  
*A. nidulans* 281 GSYLR-----ALGRQSIDSRPT-----NWDSGNIE  
*M. oryzae* 262 ASNLR-----GLGRQSIDSTP-----TDSAEVLD  
*N. crassa* 257 NIIESYQKSLILKEEQINEVRLKGEFITSVSQNLISFFTSSQ-SSLPS-----  
  
*S. cerevisiae* 319 LWEKMIEMFSTLLSPO-GILAEVVEFWQTVQGEIDGKIQRSLEPTGYNGESVHHQLSEDW  
*A. nidulans* 307 VWETLVAFNNLNSPO-GILAEVVEFWQTVQGEIMDGNTRILPLGYKCESQGHRLIQOS  
*M. oryzae* 288 -----LKDSTGDI TRSNKDSGSP-LDYGHIPNCNGLSCLRYLPKIVPEFIIKFST  
*N. crassa* 306 VTPTESR---FKLDEKNIFFPTPKRGEPWEDYAEWPEFNSNSLGVNLYGQFI I IIGAAG  
  
*S. cerevisiae* 378 --SCELQRATV---ELVDVIREHVISFFTGPPPEDLSALVSPL-PSTPNTPESTTPGSA  
*A. nidulans* 366 ELAQLNITTNG---ITICRNTHSTIINRCVGAISSTPKLRDISNFYQLENWQVYETVIFS  
*M. oryzae* 337 EMTTLEPVSSSSTSQ-ELLRGLVSTIIRERAVRISCSAWAKDAEVCRLLEDW-----TRD  
*N. crassa* 363 EMATAPVGLGDAQELELLKTLVNSRERCVAICAAWNKDAETIKHVEDW-----NRA  
  
*S. cerevisiae* 432 SKSQDSSKNLTFEYGVTFPEVLSFQEVSKTRRDLLEAYEKIPIINCISVSYPSKQL  
*A. nidulans* 422 PKRRD-----TKMPALFVNFQNALVSGI QKILYMSEAMAKPGTVTVTQPPTKL  
*M. oryzae* 390 PEDRD-----VTKMPACFSAFEGTLLAGMQKILYIPEASAKAGAEDVTPAPTKL  
*N. crassa* 417 PTRRD-----VTKMPASFAAFERALLSGMQKILYVSEAMTKPGAEDVLPAPTKL  
  
*S. cerevisiae* 492 LQMVREFFISSEKALGCVETAEHPTTEENDEWVSEATAVVRNSN-----  
*A. nidulans* 472 LQMVRSQVTTLYKALSGMVENAERS-ITKSDDEWTTDPEVLGATSAANSRTSLRMSGG  
*M. oryzae* 440 LQMVRSQVTTLYKALSGMVENAELP-ITKPDDEWTTDANFVNNP-----SR  
*N. crassa* 467 LSKMESSIFGNLYSDLINRDTLEEKFEINWPMYTSNSFRVGDYIEAMILIVHVS

```

S. cerevisiae 540 TSSTISVGSSTINAGDRNVRLMLLTLNLQRLRSDFVPSLNTQFENAFSVKLTDETKTIRDV
A. nidulans 531 LRVSTIGGATIDAGDSNVRLMLLTLNLQALRSEVVPNLNTQFENAFSVKLTDETKTIRDV
M. oryzae 489 ECFRIGPQ-----LTHKILLETQIFARIYLFEAFKPYVGNLSNDGS
N. crassa 526 ETSITIPSTSSSTSSRSAASSAAGSQSPLEITIVLTHLLTQVCTALVNAFNLRASSYNAL

S. cerevisiae 600 LGQIDARLRFQSYTRPSIESLRRIIRAGVTASWPPPSGQKPREVKPYIYEALLDVVLVHT
A. nidulans 591 LQITVDIEFFQKVMGPLEKDTBATLRAQLQNCFONDIN---RLOKCTNEINPIV SANL
M. oryzae 530 IQATLDTEFFIAQMSQYVSEESAVQSQIYVELLQRITHEARARLQSELGEMRGIIKRLR
N. crassa 585 LQAALDVEFAAQTQHYTTQRAKDIQNQIYQELSRSDRDAVLNMQNELPELRSLLKRLR

S. cerevisiae 660 KRFAIQFAAFS-----
A. nidulans 647 ERKKEFACFRKPKPSGTS-----QKSGAA-----
M. oryzae 590 DASKNEFACFKKVKRSNSAET-----ERKDSV-----
N. crassa 645 EASKSEFACFRKPKKITGPAATAGSSAGSAAMLAAQVGDMSGLERTDTRDTGRSYGTGTS

S. cerevisiae --
A. nidulans --
M. oryzae 617 ER
N. crassa 705

```

## e Exo70 protein sequence alignment

```

S. cerevisiae 1 RNNVLTTLQRNIESTLNS----VASVKDLANEASKYEITQKGINQVGLKQYTVVHKLD
A. nidulans 1 NTQSLQITNNIDRVIEA----IERLRQPLDAKNREEGIRAGEQFNNLPQYLAAMRGVN
M. oryzae 1 ETKKLQVL--DIDNVLSA----IERLRAPADSKNDEEQIIRMGPKEADLPNYLNSIKRLN
N. crassa 1 ETRKLQVLGNSSKSSGESSPYLVHQILRSPADSKNDEEQIIRMGPKEAGLPNYLASIKRLN

S. cerevisiae 57 DALMDITSINLKSNOKA----SFFTSLLGIGNSKLDLILRKLGHVSPTEPLHYLT
A. nidulans 57 KALMDKASNLRSNOQT----LNDLQRLVTLGTLQLATLFDKLLRSETPRSIEPLHYIT
M. oryzae 55 KALMDMKASNLRSNOQT----LADLQRLVKTGNSQLESSEFDKLLRSETPRAIEPLHYLT
N. crassa 61 FLEFFAKEISTAK---NAPYEKSSGMSNYTEALLGFANEKSLMDDLYSQYTEISKPHIL

S. cerevisiae 111 KDKPFPLSRDNVNRGLFYSEV--AGNRQKGVGS--ESTFAEVYSEVRGEPYLAETLA
A. nidulans 112 KNPFPVPLSQEKITRLGLMNSYL--TGvhQQNIGAGSSQESPVIKIYAEVRQYLLSLTG
M. oryzae 110 SQILSPLISAYAKLFGANLKIVRSNLENFQFFSFEIVSINDVKKSLRGKELQYNLLQD
N. crassa 118 QMTCRSALAEYSKTIRENDYIRNLLDCFLAFEIILIVTAKSYDIELKTEELKSLFLE

S. cerevisiae 167 NLAATASTNTAKKK-TPEAVYKAGTNGMGTYAQAMEGLFLAEYDNICSIFMR--EDWGPVF
A. nidulans 170 CTQEVVRQVTSLFRDAIRRIKANSISTIPSNNGVTEATVDTMSRLRFSEYKNGCLGA
M. oryzae 170 ALFPVRETAKYSLAELIETKRFAQAIPMIPPNGAPTPLVDKVMRSLELTGYOKPLASI
N. crassa 178 CLFPVRETAKTSLGELIEDTKRRVANMQSIPADGAPFVIAETMORLQTMVEILRPVSSI

S. cerevisiae 224 MDNITRENWLPNYKEKEY----TLQNEANWEDHVLVLSCEISDCITTLAVNLERLAQ
A. nidulans 230 LITSIGDGNWRSLSASSMNT-----PLDVN-PDSDVLFSHILLDVITLLALEARAR
M. oryzae 230 MISIGNGGWKSLASSRGGG-DALPSLASFVVG-ANGQETFADICSDTILLILLSLDGRAR
N. crassa 238 MISIGDGGWKSAASKGGATDTPSLVSVFVG-ADGQETFAHICADTIEITLLSSLDARAR

S. cerevisiae 279 QL-----HRTKAAQGVFLSNFCLVDRAIRSPELARFLGSPDSVSRID
A. nidulans 281 MV-----NGKKEVVGVEIANSIALTERSIRESDLAFLMET--RLGLE
M. oryzae 288 VLY-----QOKKAVIGVFLANNVTVIERMINES-GLVTLLOS--RLQVLD
N. crassa 297 KEKFRKFNEGFEDLVSKTKQYKLSDPKLVTRKSEIISLVMPMYERFYRYKDSFKLPRK

S. cerevisiae 323 TYKPKAKLYTEPCKDVSMLHFDVITHTSKS---ARPSGQASADSAIILKQLSSKDKKESI
A. nidulans 322 VWRKKATALYTECKEISHLFDVHTNRT---ARPGSQGMVSSASIMKGLSSKDKKIKI
M. oryzae 330 HTIYTPDELTTVINQLVR
N. crassa 357 YTYDKGSLSAQLASLQ-

S. cerevisiae 380 KGMFTAFNSGFEDMVARHKQFTM-EKEVRQMLAQDVQHMLEPLYNRFWDRYHEIDKGGK
A. nidulans 379
M. oryzae
N. crassa

```

## f Sso1 protein sequence alignment

```

A. nidulans 1 -----QDVEMNPVQHPV-----DPSNIFNVQKIKKGIATRNLRNRLA
S. cerevisiae 1 -----SALGAECHDFVG-----FMNKISQINRDLKDYDH-TINQVD
M. oryzae 1 GRDDYSSSQNVEMASITQNGAGFSQOGASGNSDPNIFILNECRSIDDGVGQTEG-NLNQLR
N. crassa 1 -----NNMEMSITTGGLYGG-----DPTAILNECRDIDNGIEQIEA-NLRELR

```

```

A. nidulans      41 VAQNALLESNPREDDTARQALNETQDEISIGYOKLNDTARVKKTEPGSATVQSQLELV-
S. cerevisiae   36 SLHKRLLEEVNEEQASHLRHSLDNFVAQATDLQFKLKNEIKSAQRD-GITDNKQAQAEEN
M. oryzae       60 MLQDRSLNEADSTGSGTQRG-LDSLSSETMAYRALTDNRKVKSSPEASQARNTAQVNR
N. crassa       44 RLQDRCLAEADSSASSSSRQ-LDILNLTETMALYRITIDRVRKIKSSPEGRQPRNQAOVGR

A. nidulans      100 -GRAIRREFEQVQKSOVSFQKRIKEQVRRVQINNTASPEEIDQTVBAVLAGEQTFQV
S. cerevisiae   95 SRQRFLLKIQDYRIVDSNYKEENKEQAKROYMIQPEATEDEVBAATSDV--GGQIIFSG
M. oryzae       119 VDRRLRAATNOYQQLIESGFROKSRDQERQYRIVRPAADDRVIRDAVEDAANGGGQIFQQ
N. crassa       103 VDRRLRCAIQDYQGVESFRKKIQEQARQYRIVRPAATEDEVKAAVEDT-TGNSQIFQQ

A. nidulans      159 A---CARLKRCHDVRDAVAARSFEELRSTEAKIVEVSNLVTTMAQMI-EQQAPVEQITQG
S. cerevisiae   153 ALLNANRRGEARTALAEVQARHQELLKIEKSMAEITQLFNDMEELV-IEQQENVVDVTKN
M. oryzae       179 ALMQSDRRGQARAVLNAVQDRHRCQMKKIEQQMTELAQLFQDMITLXRSARRACPRXCK
N. crassa       162 ALMQNNRVGEARAVLSAVQDRHRALQRIEQQMVELAQLFEQINLTI-VEQDVKIQATQCT

A. nidulans      215 HENVARDIGNANTQLCAVESARKARWKWYALIIIVLIIATIVATAVA-----
S. cerevisiae   212 VEVAQLDVEQGVGHTDKAVKSARKARKNKIRQWLIVFAIIVVVVVVVV-----
M. oryzae       239 GEEIVENLDKGNEEIVVAVEIATKTRKKKWCGLGICIIIVVVAIAVAIYFVVISPPRAN
N. crassa       221 SEEVVDNLDKGNEEIVAVQIARATRKKKWCGLGICIIIVVVIIVVVVVVIVTHPPGCG

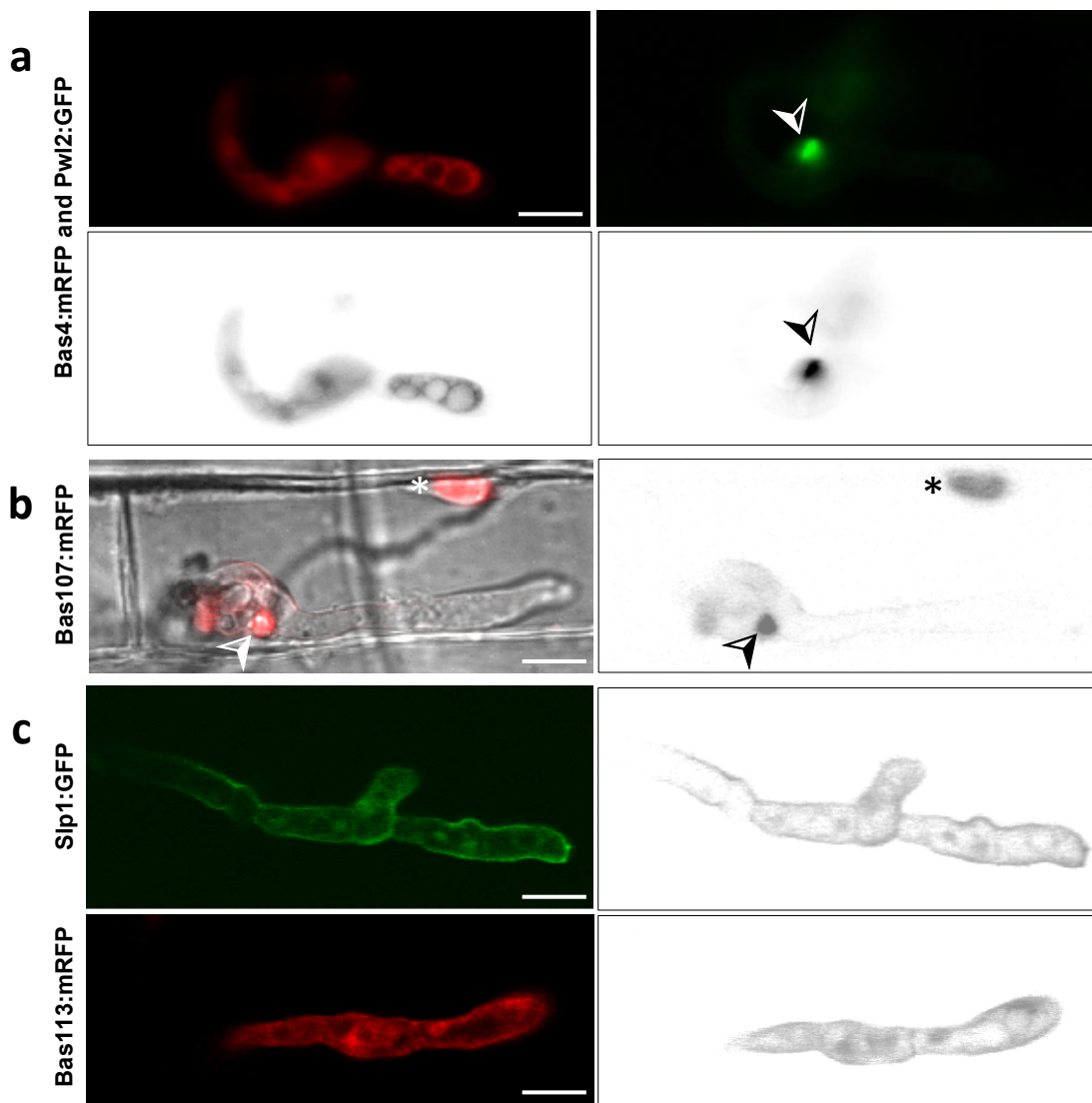
A. nidulans      264 -----
S. cerevisiae   261 -----PA-----
M. oryzae       299 NNNAPAPAATATPAANTNPKRSTGNGLPELHVDVDFDGER-----PVFNIYAQSKI
N. crassa       281 NR-----PAQNTK-RGLIQRSF--DDLQMNNAIAIQIAPMAGPISRIHQSNY

A. nidulans      264 -----VTQTR-----
S. cerevisiae   263 -----VKKTR-----
M. oryzae       349 VITDEFNSKTEETSPLA-----IFAGKSSVMKRYVTEDEVGPSPTGPTGPGGI
N. crassa       328 VVPVSADESSELVKRLSPBAFDRIISPYIQAPAGATNAKREVTEDEILEFMEQNNAVASRG

A. nidulans      -----
S. cerevisiae   -----
M. oryzae       401 PSMHSEGGFEKTSRASGGLRKNKRLALS GCKAFD VDSATLVVATKTE---
N. crassa       388 P--HRACKRFVITDEIILESMR-----GGQVADHKKFVPGHNDAKDGGK

```

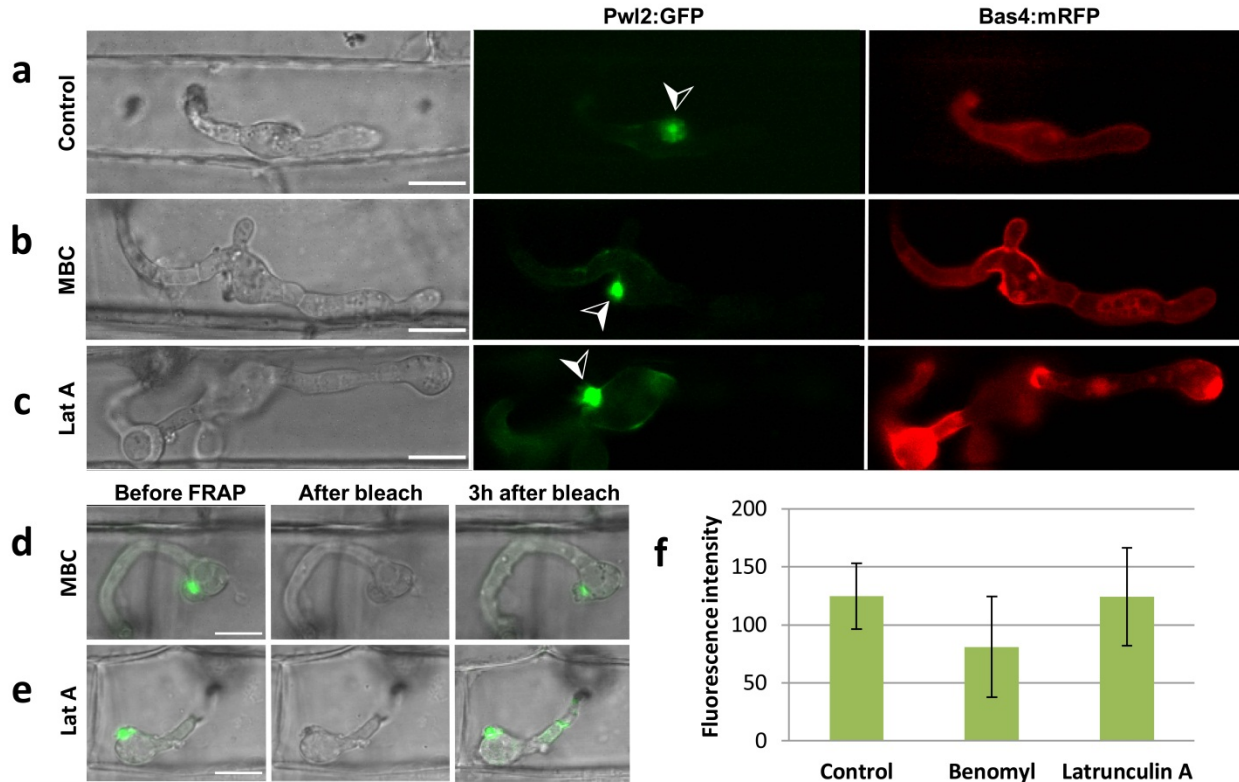
**Supplementary Figure S2. Alignment of the predicted amino acid sequences.** Sequences for protein secretory components in *M. oryzae* and other fungi were aligned using ClustalW<sup>43</sup> and shaded by BoxShade 3.2. Identical amino acids are highlighted on a black background and similar amino acids on a light grey background. The *M. oryzae* amino acid sequence is aligned with the sequences of the putative homologs in *S. cerevisiae*, *A. nidulans* and *N. crassa* respectively. (a) Mlc1 (MGG\_09470.6) is aligned with YGL106W, AN6732 and NCU06617. (b) Snc1 (MGG\_12614.6) is aligned with YAL030W, AN8769 and NCU00566. (c) Spa2 (MGG\_03703.6) is aligned with YLL021W, AN3815 and NCU03115. (d) Sec5 (MGG\_07150.6) is aligned with YDR166C, AN1002 and NCU07698. (e) Exo70 (MGG\_01760.6) is aligned with YJL085W, AN6210 and NCU08012. (f) Sso1 (MGG\_04090.6) is aligned with YPL232W, AN3416 and NCU02460.



**Supplementary Figure S3. Cytoplasmic effector sequences determine BFA insensitivity.** In fungi, a hallmark of Golgi-dependent secretion is sensitivity to the fungal metabolite Brefeldin A (BFA)<sup>40</sup>, which blocks ER-to-Golgi transport by blocking the recruitment of the ADP-ribosylation factor Arf1 to the Golgi. We tested multiple fluorescent effectors, with and without an artificially-added nuclear localization signal (NLS), to confirm that BFA-insensitive secretion is characteristic of BIC-localized cytoplasmic effectors and is independent of the fluorescent protein or added NLS. Fluorescent BICs are labeled with an arrowhead (➤). **(a)** Secretion of cytoplasmic effector Pwl2:GFP, with a C-terminal fusion to GFP instead of mCherry:NLS (Fig. 4b), continues in the presence of BFA. In this case, the Pwl2:GFP fusion protein does not contain

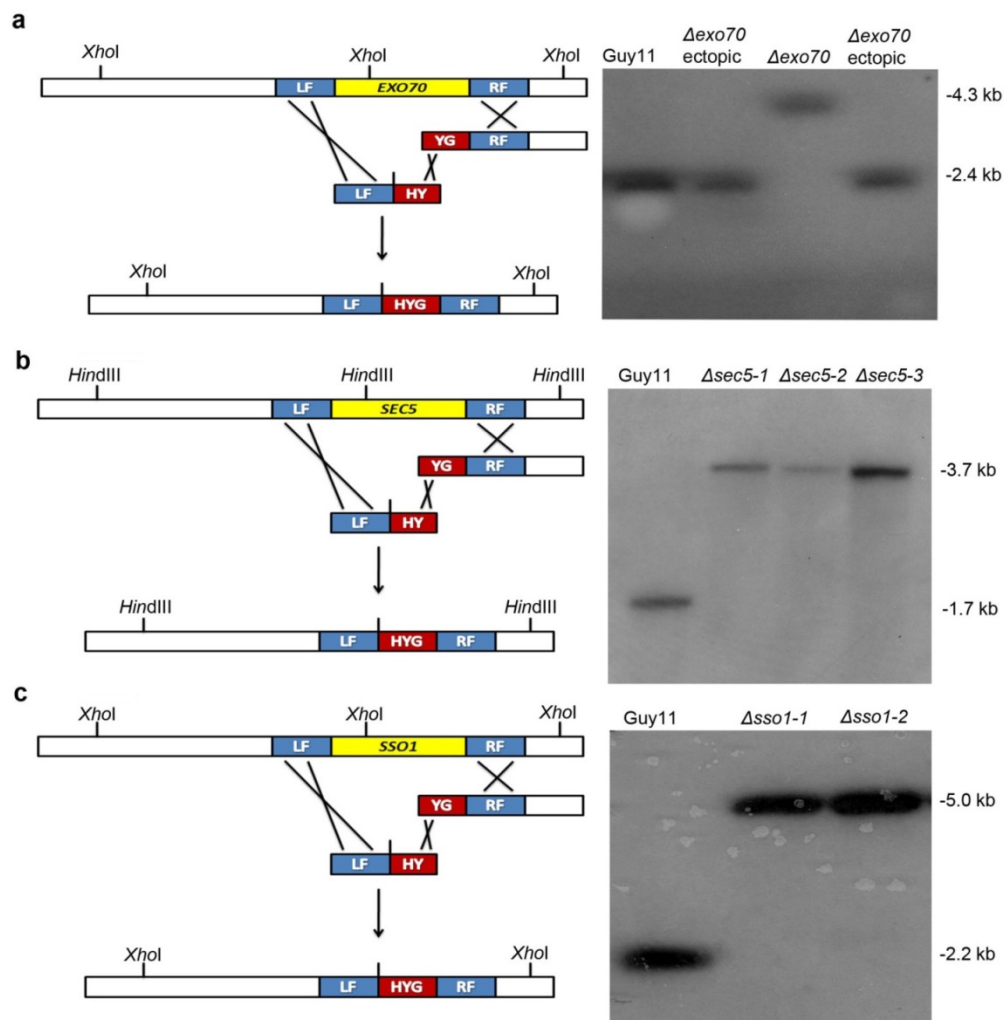


an NLS signal. Even after 6 h and 50 minutes, fluorescent Pwl2:GFP is not retained in the fungal cytoplasm. At the same time, secretion of apoplastic effector Bas4:mRFP is blocked by BFA treatment. The red or green channel in each image in the top panel is shown as a black and white inverse image below, in order to highlight the fluorescence patterns associated with apoplastic and cytoplasmic effectors, respectively, after BFA treatment. **(b)** Secretion of cytoplasmic effector Bas107 is not impaired by BFA treatment. In this image, Bas107:mRFP, which accumulates in the BIC and is translocated to the rice cytoplasm, shows its normal localization pattern 5 h after exposure to BFA. Note that Bas107 fusion proteins naturally accumulate in the rice nucleus (white asterisk) after translocation, without an artificially-added NLS. Images from left to right are merged bright-field and mRFP; and mRFP as a black and white inverse image. **(c)** Additional apoplastic effectors Slp1:GFP and Bas113:mRFP show retention in the fungal ER similarly to Bas4:GFP and Bas4:mRFP. Both infection sites were imaged 5 h after exposure to BFA. Images from left to right: GFP or mRFP alone with corresponding black and white inverse images. Scale bars, 10 $\mu$ m.



**Supplementary Figure S4. Effect of cytoskeleton inhibitors on effector secretion.** (a) In the absence of inhibitors, cytoplasmic effector Pwl2:GFP is secreted and localized in the BIC (➤) and apoplastic Bas4:mRFP outlines the IH. (b) Rice sheath tissue invaded by the fungal transformant in (a) was exposed to MBC (active ingredient in the fungicide benomyl), which inhibits microtubule formation in the fungus, but not in plants. Secretion of apoplastic effector Bas4:mRFP is impaired by 3 h after addition of MBC, but no effect was observed on accumulation of Pwl2:GFP in the BIC (➤). (c) Rice sheath tissue invaded by the transformant in (a) was exposed to Latrunculin A (LatA), which inhibits polymerization of actin in fungi and plants. By 3 h after exposure to LatA, IH depolarization occurs and secretion of apoplastic Bas4:GFP is impaired. No effect was observed on secretion and accumulation of Pwl2:GFP in the BIC (➤). (d-f) FRAP analysis demonstrates continuous secretion of Pwl2:GFP into the BIC with or without chemical treatments. Transformants expressing both Pwl2:GFP and Bas4:mRFP were incubated in MBC or LatA for 3 h, or until impaired secretion of Bas4:mRFP was observed, before photobleaching the BIC. (d) Recovery of pre-bleach Pwl2:GFP fluorescence (green) was seen after 3 additional hours in the presence of MBC. (e) Recovery of Pwl2:GFP fluorescence (green) was seen in the BIC after 3 additional hours in the presence of LatA. (f)

FRAP results were identical in the presence or absence of MBC ( $p=0.014$ ) and in the presence or absence of LatA ( $p=0.015$ ). Bars show mean fluorescence intensity recovery after bleaching (mean $\pm$ SD, four FRAP experiments). Images (a-c) from left to right are bright-field; GFP alone; and mRFP alone; and all images (d-e) are merged bright-field and GFP. Scale bars, 10 $\mu$ m.



### Supplementary Figure S5. Southern blot analyses of targeted gene deletion mutants.

Genomic DNAs were extracted from wild type strain Guy11 and putative transformants. DNAs were digested with the indicated restriction enzymes and gel fractionated. DNA gel blots were probed with Left Flank sequences (1.5 kb upstream of start codon). Fragment size differences in each blot are consistent with replacement of the target gene coding regions with the hygromycin or sulfonyleurea resistance cassettes. **(a)** Targeted deletion of *EXO70*. *XhoI* digested genomic DNAs showed a 1.9 kb difference, consistent with replacement of *EXO70* gene. **(b)** Targeted deletion of *SEC5*. *HindIII* digested DNAs showed a 2.0 kb difference, consistent with replacement of *SEC5* gene. **(c)** Targeted gene deletion of *SSO1*. *XhoI* digested DNAs showed a 2.8 kb difference, consistent with replacement of *SSO1*. Similar analyses confirmed targeted gene replacement mutants in a second wild type strain, O-137.

**Supplementary Table S1. Protein secretion components used.**

<b>Name</b>	<b>SGD ID</b>	<b>Broad Inst. Version 6</b>	<b>Size nt (aa)</b>	<b>Function</b>	<b>Localization</b>	<b>Protein Family</b>	<b>Blastp % e-value</b>
<b>Mlc1</b>	YGL106W	MGG_09470	1018 (147)	Myosin regulatory light chain	Apical body, Spitzenkörper marker	EF-hand superfamily	60% 3.1e-24
<b>Snc1</b>	YAL030W	MGG_12614	1295 (125)	v-SNARE, mediate endocytosis and exocytosis	Transport vesicle membrane	Synaptobrevin/VAMP family of R-type v-SNARE proteins	68% 3.6e-25
<b>Spa2</b>	YLL021W	MGG_03703	3006 (951)	Polarisome component	Polarisome, hyphal tip	GIT_SHD domain family of ADP-ribosylation factor GTP-activating proteins	55% 3.5e-22
<b>Exo70</b>	YJL085W	MGG_01760	1977 (630)	Exocyst complex subunit	Sites of the exocyst in the plasma membrane	Exo7-like	43% 7.9e-23
<b>Sec5</b>	YDR166C	MGG_07150	3227 (1055)	Exocyst complex subunit	Vesicle tethering in exocytosis	Sec5-like	52% 1e-22
<b>Sso1</b>	YPL232W	MGG_04090	1900 (500)	t-SNARE, determine the site of vesicle fusion	Plasma membrane	Syntaxin-related	52% 2.8e-28

**Supplementary Table S2. Plasmids used.**

Clone	Description
pBV14	RP27:ECFP expression binary vector derived from pSM324. Ampicillin and Bialaphos resistance.
pBV176	EGFP expression binary vector derived from pBHt2. Kanamycin and Hygromycin resistance.
pBV317	Pwl2:mRFP: $\beta$ -tubulin-terminator cloned in <i>EcoRI</i> - <i>HindIII</i> sites of pBHt2. Kanamycin and Hygromycin resistance.
pBV367	mRFP expression binary vector derived from pBGt (Seogchan Kang, Pennsylvania State University), consisting of three modules: P27 ( <i>EcoRI</i> - <i>Bam</i> HI fragment), mRFP ( <i>Bam</i> HI- <i>Sph</i> I fragment), and <i>N.crassa</i> $\beta$ -tubulin terminator ( <i>Sph</i> I- <i>HindIII</i> fragment) that were cloned in <i>EcoRI</i> - <i>HindIII</i> sites of pBGt. Kanamycin and Geneticin (G418) resistance.
pBV403	EGFP expression binary vector derived from pBGt (Seogchan Kang, Pennsylvania State University), consisting of 2 kb P27 <i>AVR</i> - <i>Pita</i> SP:GFP ( <i>EcoRI</i> - <i>Bam</i> HI fragment), and <i>N.crassa</i> $\beta$ -tubulin terminator ( <i>Sph</i> I- <i>HindIII</i> fragment) cloned in <i>EcoRI</i> - <i>HindIII</i> sites of pBGt. Kanamycin and Geneticin (G418) resistance.
pBV435	For expression of Pwl2:EGFP:Ter and Bas4:mRFP:Ter, cloned in <i>EcoRI</i> - <i>Bam</i> HI sites of pBV367. Kanamycin and Geneticin (G418) resistance.
pBV591	For expression of Pwl2:mCherry:NLS:Tnos and Bas4:EGFP:Ter, cloned in <i>EcoRI</i> - <i>HindIII</i> sites of pBHt2. Kanamycin and Hygromycin resistance.
pBV912	BAS107:mRFP expression binary vector derived from pFLR. Kanamycin and Hygromycin resistance.
pBV915	BAS113:mRFP expression binary vector derived from pFLR. Kanamycin and Hygromycin resistance.
pBV946	For expression of <i>Mlc1</i> :GFP, 1.76 kb of <i>Mlc1</i> gene, including 1 kb promoter and coding sequence, cloned in <i>EcoRI</i> - <i>Bam</i> HI sites of pBV403. Kanamycin and G418 resistance.
pBV947	For expression of <i>Exo70</i> :GFP, 2.97 kb of <i>Exo70</i> gene, including 1 kb promoter and coding sequence, cloned in <i>EcoRI</i> - <i>Bam</i> HI sites of pBV403. Kanamycin and G418 resistance.
pNJT1	For expression of <i>Sso1</i> :GFP, 2 kb of <i>Sso1</i> gene promoter and coding sequence, cloned in PYC1 vector carrying Sulphonylurea resistance.
pNJT2	For expression of GFP: <i>Snc1</i> , 2 kb of <i>Snc1</i> gene promoter and coding sequence, cloned in PYC1 vector carrying Sulphonylurea resistance.
pNJT3	For expression of <i>Spa2</i> :GFP, 2 kb of <i>Spa2</i> gene promoter and coding sequence, cloned in PYC1 vector carrying Sulphonylurea resistance.

Clone	Description
pNJT4	For expression of Slp1:GFP, 2 kb of <i>Slp1</i> gene promoter and coding sequence, cloned in PYC1 vector carrying Sulphonylurea resistance.
pNJT5	For expression of Pma1:GFP, 2 kb of <i>Slp1</i> gene promoter and coding sequence, cloned in PYC1 vector carrying Sulphonylurea resistance.
pNJT6	Lti6B:GFP, obtained from Kurup <i>et. al.</i> , 2005 <sup>54</sup> .
pNJT7	GFP:HDEL, obtained from Zheng <i>et. al.</i> , 2005 <sup>55</sup> .

**Supplementary Table S3. Fungal and rice transformants.**

Name	Description [background strain; plasmid used]
KV9	Transformant expressing constitutive cyan fluorescent protein [strain O-391; pBV14]. Bialaphos resistance.
KV96	Transformant expressing Bas1 promoter and coding sequence (115 aa) with mRFP reporter gene into KV9 [strain O-391; pBV367]. Bialaphos resistance.
KV105	Transformant expressing both a fusion of the Pwl2 promoter and entire coding sequence with the EGFP reporter gene, and a fusion of the Bas4 promoter and entire coding sequence with the mRFP reporter gene [strain O-137; pBV435]. Hygromycin resistance.
KV112	Transformant expressing Bas1 promoter and coding sequence (115 aa) with mRFP reporter gene [strain O-137; pBV367]. G418 resistance.
KV118	Transformant expressing Pwl2 promoter and coding sequence (145 aa) with mRFP reporter gene [strain O-137; pBV317]. Hygromycin resistance.
KV121	Transformant expressing both a fusion of the Pwl2 promoter and entire coding sequence with the mCherry:NLS reporter gene, and a fusion of the Bas4 promoter and entire coding sequence with the EGFP reporter gene [strain O-137; pBV591]. Hygromycin resistance.
KV125	Transformant expressing Mlc1, promoter and coding sequence with GFP reporter gene [strain CP987; pBV403]. G418 resistance.
KV126	Transformant expressing Mlc1, promoter and coding sequence with GFP reporter gene [strain KV118; pBV403]. G418 resistance.
KV130	Transformant expressing Exo70, promoter and coding sequence with GFP reporter gene [strain CP987; pBV403]. G418 resistance.
KV131	Transformant expressing Exo70, promoter and coding sequence with GFP reporter gene [strain KV118; pBV403]. G418 resistance.
KV132	Transformants expressing BAS107:mRFP [strain O-137; pBV912]. Hygromycin resistance.
KV133	Transformants expressing BAS113:mRFP [strain O-137; pBV915]. Hygromycin resistance.
Ex0149	Transformant expressing GFP:Sncl (2kb promoter and entire coding sequence) [strain Guy11]. Sulfonylurea resistance.
Ex0150	Transformant expressing Spa2 (2kb promoter and entire coding sequence) fused to GFP [strain Guy11]. Sulfonylurea resistance.
Ex0151	$\Delta$ exo70, KO mutant into KV121 [strain O-137; pBV591]. Sulfonylurea resistance.



Supplementary Information (Giraldo *et al.*, 2013)

Name	Description [background strain; plasmid used]
Ex0152	$\Delta sec5$ , KO mutant into KV121 [strain O-137; pBV591]. Sulfonyleurea resistance.
Ex0153	$\Delta sso1$ , KO mutant into KV121 [strain O-137; pBV591]. Sulfonyleurea resistance.
Ex0158	$\Delta exo70$ , KO mutant into KV112 [strain O-137; pBV367]. Hygromycin resistance.
Ex0159	$\Delta sec5$ , KO mutant into KV112 [strain O-137; pBV367]. Hygromycin resistance.
Ex0160	$\Delta sso1$ , KO mutant into KV112 [strain O-137; pBV367]. Hygromycin resistance.
Ex0166	Transformant expressing Slp1 (2kb promoter and entire coding sequence) fused to GFP [strain Guy11]. Sulfonyleurea resistance.
Ex0174	Transformant expressing Spa2 (2kb promoter and entire coding sequence) fused to GFP [strain KV118; pBV317]. Sulfonyleurea resistance.
Ex0175	$\Delta exo70$ , KO mutant into the strain Guy11. Hygromycin resistance.
Ex0176	$\Delta sec5$ , KO mutant into the strain Guy11. Hygromycin resistance.
Ex0177	$\Delta sso1$ , KO mutant into the strain Guy11. Hygromycin resistance.
Ex0178	Transformant expressing Pma1 (2kb promoter and entire coding sequence) fused to GFP, into KV118 [strain O-137; pBV317]. Sulfonyleurea resistance.
Ex0179	Transformant expressing Sso1 (2kb promoter and entire coding sequence) fused to GFP, into KV118 [strain O-137; pBV317]. Sulfonyleurea resistance.
EXR1	<i>Oryza sativa</i> cv. <i>sasanishiki</i> rice line containing Lti6B:GFP vector, Lti6B gene expressed under 35S promoter. [pNJT6].
EXR2	<i>Oryza sativa</i> cv. <i>sasanishiki</i> rice line containing GFP:HDEL vector, HDEL sequence expressed under 35S promoter. [pNJT7].