



Figure S1 *ric8* gene structure, mutant verification, and expression RIC8-GFP. A) The *ric8* genomic region from *Neurospora crassa*. Intron locations (black triangles) and restriction enzyme sites (black lines) are shown. B) Complementation of $\Delta ric8$ by expression of a *ric8-gfp* allele. A VM plate culture is shown for wild-type (2489), $\Delta ric8$ (R81a), and $\Delta ric8, ric8^+::gfp^+::his-3^+$ (R8GFP). C) RT-PCR analysis of *ric8* mRNA levels. Samples containing 2 μ g of total RNA isolated from conidia of strains used in (B), with a duplicate R81a reaction, were subjected to RT-PCR with primers designed to flank the first predicted intron of *ric8* (R811fw and R811rv, TABLE S1). The predicted intron size is 56 bp, making the size of the RT-PCR fragment about 354 bp. For the control and probe DNA, a 410 bp fragment of genomic DNA was amplified by PCR from plasmid pSM2 using the same primers. Expression of the 18S rRNA gene was assessed under identical RT-PCR conditions (See methods). D) Western analysis. Protein from a whole cell extract isolated from a 16 hr submerged culture of strain R8GFP was subjected to Western analysis using anti-GFP antiserum (See methods). The approximately 93 kDa protein is indicated by the black arrow.

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Nc -----MASTGVSQPAKLVAVTTLHKLTEDLKSTSLSP-----ERDKALELKVYGRDPRNADPI-----FTKCGIETLTKHAFDS
Pa -----MNAKAKIPVAKLDVAKVQLLGSLEDELETSSLAPQRWFIFPAALBIASNKFEIGRESLLELKIYGRDPNACDPI-----FTKEGITLVRHAFDS
Mg -----MLNNTLTKGTDKLLDLKHHNLSQ-----ERDAALEQLKVYGRDPRDAPPI-----FTKEGISTLAHSEDFG
Fg -----MALPAPAGLPTGTDKLEYIKELINDLEDLNELELDP-----DRAAVLDQKHLTRDPIINADPL-----YTBEGISTLLRHAYDK
Ss MSISSTRTSLSQCLTGEAKLADVTNIVBSLKADELNISLLPH-----ORDALLEQLKVYGRDPRADPI-----FTREGIETLSKHAFNS
An -----MDLRILQSGEKLQOVTKLLNSLEKDLKNSLNSA-----ORTQILQLRQYGTSPDNAGPI-----YSRKGIEITLSKVCIDG
Hc -----MATPALVVEVTSCLNAEKTAAQFGSRGS-----WEQNINLEINFLGLOFQONNSR-----IRGEGKTRG
Bc MSISARTSLSQELTGAKLTDVTKIVBSLKEDELEKISLLEPH-----ORDALLEQLKVYGRDPRNADPI-----FTKEGIEITLSKHAFNS
Sj -----MPDSKSTVDLLSFERDNGSFVPTICSKINLLKPTAWELSAN-----LIEIAVSLKELRKKSRPKDACQKISNALDWTLVLAERAFVSKLRNSA
Yl -----MIGHLHLYDITYTISLLEHNPVQYHFSPPKMSKKTAYEDFQS-----EPQDPLICLALKMGLTTACAWTCIG-----GISAADDIATHVLDG

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Nc PSETTSRNALRVLGNAMLLIPETQRQRFVDLGYEAKACEKLN--DNWDEFLAARVIFSTYGTIVDLAKLIDBHHLAESVANLARHARSRISEHAKNKIKPDPME
Pa TSVNTRSRGALRILCNLILLPEPQRQRFVDLGYAAKASEKLE--DNSDDEFLVARLLISTYNTNIDLPKLIHQHGLADSIANHLARHAKRISSTNR-STTANPMD
Mg SDDTTSRNALRCLANAMLLPSSRQELVDLGYHIKAKALAS--DSWDEFLVSRVLMLANARNINHEILITQHGLAELIARLESMMRFLP----DEDSSPNTME
Fg PSTKSAADARRVLANAMVLTVDRIFNKGCAPNAOQGLNG--GSFDDEFLNSRILFLSTYGTIKVDLKKLIDDEKLADRISENLARHAK----ESKAKSDPMQ
Ss PSKFTSRNALRCLANALLKPDTRQTLVTLGYDAKACTLLKDRNGSVVDEFLSRIIFLITYGTTININLIDHCHLADATSHNLANHARLHNAKQKVKQLDPM
An ETTDVRHAALRCVANALLDSNMROLFVDTCGKGRLEAMLKC--DSSHEMVISRILFLSTYDININFDLNNHSLGDNINYOILRHSKQPKSGR--PLSQVD
Hc -----CHAIKIKITCOQYKNSSSFSKSVNTECDLE--BNSDDEFLASRLFLFLATYDIDLEFKLIDNNNLGEYINNHYRHSKRFKARKK--KLDQMD
Bc PSKFTSRNALRCLANALLKPDTRQTLVGSYDERACALLKDRIASVDEFLSRIIFLITYGTTININLIDQCHLADITSHNLANHDKLHSAKQKKTROLDPME
Sj HSLELIRLIVNVLVNPMSQRIASLFSNSAFDEESFQNSACQIALRLFLLCVGENDEDRWTFRITPEGLQALGIVVNNLSTEPVSASVLDLRLRAY
Yl SLEASTLNLAATVSNQYFLLRQYITTSATKAPKLVNWAATTPDTERGKMCRRILELCSSEIRIEGLNVATLAKGLEBLGLTDLQDLQLEKQKQKQLLLOHREIL

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Nc LMAIGETLRLLFNVTSKCPKSLDCFTAAVPHIVTLLSLDIPPKKTPPLESPLSPLVNALMNLKLDSEARSCLYPKDAPSSLAELKITLLELSIKAYS-DOELD
Pa PMALBETLKLITFNVSCHSPNHLASHEPAAPHIITILCSIDLPSHTKTPGPFPGPAVNALNLDLSPTAKYLYLDPSSPSSFDRLIKLISLSTIKAYK-NPDLE
Mg GMALSETLKMFPVAVLRFQGOSTRBACAGHILTLICQRPLE--AESVLDPPFGPLINALCTLDLEDEAITNIFPERDPSIVTSRLISILDRALSEKNGMDD
Fg DMALVESAKLLFNVTYCPDKSSFSATAPHLVALLLKQDIS--QTKPLDPPVGFIFINALNLDVGSDCQKSIHPEDEPKVVSRLISILDPAAMKNVP-DNQLD
Ss DMALAESLKLFLNITFCPCRNSSFSQALPHILSILDKREI--PNTPLESPISQLINLNLPLEDEPCNIVLFRANPHSHVYCKFLELLEHSHINEYK-DEELE
An ELAFTDITLKLFNIAKLFQDLAPITFSPSTPYIFKIIIRIDILPLKPLDGLGLTLLNCLSTLLENKKNKPYDGNPIFFPTFNQCNVDKLNILDQAVSAYE-PSELE
Hc ELALSETLKLNFNITNBYHIVDAPSPSPHIIKILSRIEITPEPLQAPVNYLINSLLNLDLEAKKSKHFCTNPLPKFDQNCNVDKLNILDQAVAMHK-POHLE
Bc DMALGS-----PNNVVEKFNILLDHSINKYK-DEELE
Sj AVGSKDKPKSSTAQLSPEFVIQLWKETGTRDPSYTSNTSWHIVNALVLPLQNLTSSSLYQITDIALTALDVLPLKGIQLLEAGNASFKVFNFAVVELTAKELE
Yl SGFVCLITVYKTVREDQEKQTSSELKQIVPHVLTIAQNP-----BLLYPCPIISCRPMAVESQKLFVYLETLEVOIKQVLCATYPTDIALA---QLL

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Nc ATVIPLVCLISSIYENAPASPVRDFIRKSLLPSEERHNKVLGKGDTPAKLLANMTPNPLAPBFARAVSHLLFNVSQDKANKFVENIGYGYASGFLFQNNIPV--
Pa QIVIPLYCALSTVYEHAPAS--VKQSIKCALLPTEKDRTEALGKADTLQGHLLKNWNSPEAPELCKATAHLYFDLSNRDPHKFVKNVGYGYASGFLFQNNISFTPE
Mg PTMTVLAGSLCIIIEAEP--VRISMCAQLLPTDEDRKEVLGSTELESPLSKLLKMTNMAPKSRKAIHLLFLGLSDRDAGKFVEKVGYGYASGFLFEENIPVPOS
Fg ATVSPLLGVISSVYKHAADS--SKKYIREKLLPTEEDRKEVLGKGDALSAKLLQNFNPLAPSIVGTVITQHLLYDLSENDANKFVENVGYGFASGFLFQNNIPVPS
Ss QLVSPLLTLIRRYEYEGPSE--VHKFLQIRLLPSDKD-----HASTFVQNLGYGFASGFLFQRNIPIPEN
An TKATPLFHTLVITHEVADPG--PRKYMOWLLLEDNDRDRPTGQSDTSSKLLKLSMTHYAN-LKVAISELMFVLSCKNABSLTNIGYGFAGLLASRGMDIPKS
Hc SLAVPLLTLLRKLVSFAPEG--PKKYMFWLLLEDNDHDLPIGKSNLTLSSRLRLSSTPVAPSLREGISALMFELSAGSDAIDFVRVNVGYGFAGFLSHDMPVPE
Bc QLVSPLLTLIRKANEIGPSE--VHOFMCIQLLPSDKDREQPVGRGESLSARLQOLSTSPMAPOVRESISALLFELSDKDASTFVENLGYGFASGFLFQHNVPIPEN
Sj VRISPLLALLQNVKINDTO--TCRLLESRLPSEADRSASLAFSGSLPSRLRLVLPFCFLOPOQAHLIYSLCRENAKVLDTIGYGFASGILRVCCBETDST
Yl YGLAPLVPVTTVDGABPAR---EILCRVFLPSDEDRKEALGKTKSIPSRLLKVIIEPEFQDCFLVNEAQVLCENKDKFTDAILGAYAGYLTFGAASSAS

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Nc EGLCGDAEKGESSQACQSS--RRAVNPITGQFLDTEFTFDPPEMTEEEKEREAERLFLVLFERAKLGIIVNVENPVAKAQEGRFEELPDDYEDSD-----
Pa ELNKGGETVECEDEGVREI--KRFINPITGQFLDTERVSELPEMTEDEKEREERLFLVLFERLQOTGIIDVONPVECAMREGRFEELPDDK-----
Mg ALAS-----EGASCSTMQ--GRAVNPITGQFLDAEKLSLIPMPSOEBEKEEAERLFLVLFERLKKITGVVDVONPVEKAFQEGRFEELDDDEDGDNENKVKVEQK
Fg ASDFG-----S--QKRVNPTGQHVDAKPVDEPEMTEEEKEREAERLFLVLFERLKNITGVVDVONPVEBAMREGRFEELKDDVEEIE-----
Ss ALEAWSKENDNDGKARSSHDSGKAVNPITGQFLHAEKIDMPMPSOEBEKEERAEKLFVLK---KNGIITAKNPEBALQGRFEELDDADSD-----
An ACEAFATN-----SCLNPNINPTGQFWAAEKEDTGPMTKEEKEREAERLFLVLFERAKANGILGVENPVQALREGRFEELPDSSE-----
Hc AKEAFSTS-----AGGLDPMNPTGQFWDAEPQDGGPEMTEEEKEREAERLFLVLFERLKATGVVDVNPVSAALQTCRFEELG-----
Bc AFEAWSTENDKNGKARPSQDSGKAVNPITGQFLHAEKIDMPMPSOEBEKEEREAERLFLVLFERLKNNGIITANPNPEBALQGRFEELDDADSD-----
Sj VSKDSLFSG-----LSTATADNVNPTGQFTSEKPP--VPEMSEEEKEREAERLFLVLFERLKNNGIIVENPVKKAVERSCLFENSNNDNA-----
Yl GPASNISGFG-----YDKVITGQKLTPELQELKQEQEIS--AMSPBEKEREAELMYLNLNRMRELGIIVNFHPHRAAQESGRFEELD-----

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Figure S2 RIC8 homologs in other fungi. ClustalW (<http://www.ch.embnet.org/software/ClustalW.html>) was used to align RIC8 sequences from *Neurospora crassa* (Nc; NCU02788.3), *Podospora anserina* (Pa; Pa_1_3900), *Magnaporthe grisea* (Mg; MGG_14008), *Fusarium graminearum* (Fg; FSG_01511), *Sclerotinia sclerotiorum* (Ss; SS1G_02787.1), *Aspergillus nidulans* (An; AN1661.3), *Histoplasma capsulatum* (Hc; HCAG_03335.1), *Botrytis cinerea* (Bc; BC1G_02317.1), *Schizosaccharomyces japonicus* (Sj; SJAG_02027), *Yarrowia lipolytica* (Yl; ACCESSION# XP_505953). Shading indicates identical (black) and similar (grey) amino acid residues (http://www.ch.embnet.org/software/BOX_form.html).

Table S1 Oligonucleotides used in this study

Name	Sequence (5'-3')
R8 GFP fw	TATCTAGAATGGCCTCAATAGGAGTTTCTGGGCCAGGTC
R8 GFP rv	GGGGATCCAGTCCGAATCCTCTTCGTAGTCGTCGGG
R8I1fw	CACCATGGCCTCAATAGGAGTTTCTGGG
R8I1rv	CAGTTGTCGTTTTGAGCTTTTCGCAGGCC
R8YR11fw	GTAACGCCAGGGTTTTCCAGTCACGACCGCCTGAGATGTGTTGTATCGAGAG
R8YR11rv	CAGCTTATGAATGAGAGTGGTGACAGCTTGCAACTGGCTGGCCAGAACTCCTATTGAGG
R8YR12fw	CCAAGTTGCAAGCTGTCACTCTCATTATAAGCTGACCGAGGACCTCAAGAGCAC
R8YR12rv	GCGGATAACAATTTACACAGGAAACAGCCGGCAAGACTGGAGGGTGCCTTGGGG
R8YR21fw	GTAACGCCAGGGTTTTCCAGTCACGACCGTCCGGTCCCGCATATAGTG
R8YR21rv	CAGGGTTCTCAACATTAACGATTCCAGCTTCTCGCCCTCTCAAACAAGACAAACAGTCTCTC
R8YR22fw	GGCGAGGAAGCTGGGAATCGTTAATGTTGAGAACCCTGTTGCCAAGGCGGTTTCAGGAG
R8YR22rv	GCGGATAACAATTTACACAGGAAACAGCGGGAGTCGTATACCTCCGCGGAGGAAGGCC
R8Y2Hfw	AATTGAATTCATGGCCTCAATAGGAGTTTCTGGG
R8Y2Hrv	AATTGGATCCTCAGTCCGAATCCTCTTCGTAGTC
NΔ13G1fw	TTAACATATGGCCCGAACGAGGAGATTGAGAAC
NΔ13G1rv	TTAAAGATCTTCAAATCAAACCGCAGAGACGCAGG
NΔ13G2fw	TTAACATATGGCCTCCCGGTCGCGCGACGTAG
NΔ13G2rv	TTAAGGATCCTCACAGGATAAGTTGTTTCAGGTTTCGCTGG
NΔ12G3fw	TTAACATATGGAGCAGAAGAAGAGGAGCCAGAAGATC
NΔ12G3rv	TTAAGGATCCTCATAGAATACCGGAGTCTTTAAGGGCG
R8 NdeI fw	AATTCATATGGCCTCAATAGGAGTTTCTGGGC
R8 BamHI rv	AATTGGATCCTCAGTCCGAATCCTCTTCGTAGTCG