Supporting Information

Kershaw and Talbot 10.1073/pnas.0901477106

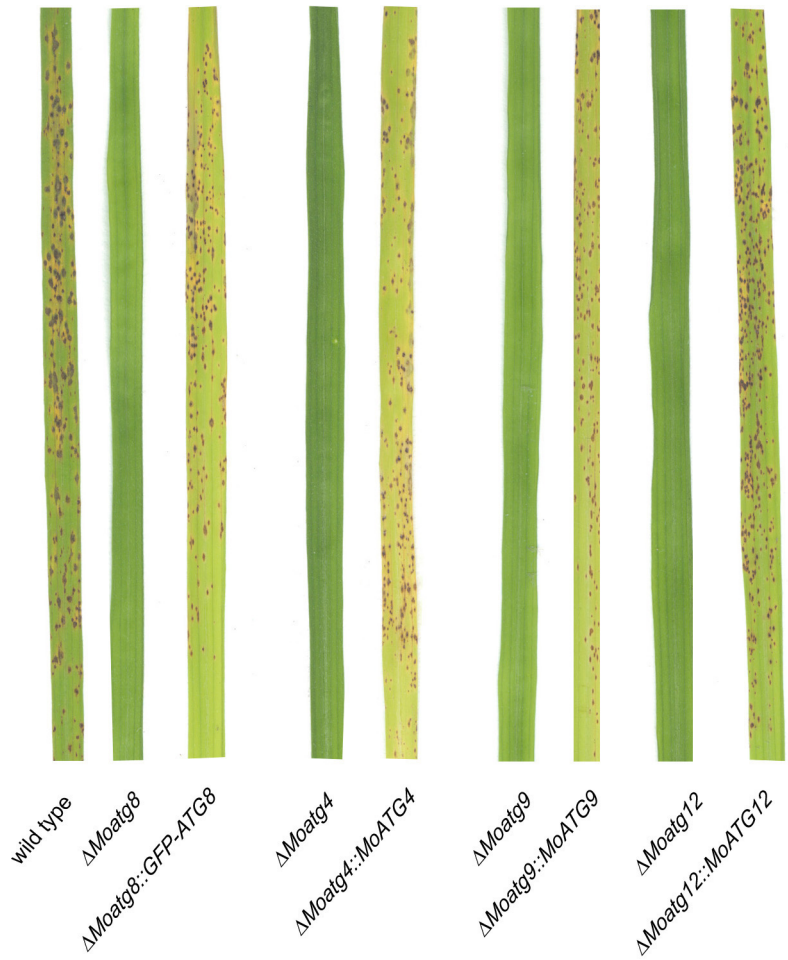

Fig. S1. Complementation of autophagy mutants. Seedlings of rice cultivar CO-39 were inoculated with uniform conidial suspensions harvested from Guy-11 (wt), from Δ*Moatg4*, Δ*Moatg9*, Δ*Moatg8*, and Δ*Moatg12* deletion mutants and from autophagy deletion mutants which had been complemented with the native genes Δ*Moatg4*, Δ*Moatg9*, and Δ*Moatg12* or the construct Δ*Moatg8-gfp*. Seedlings were incubated for 5 days to allow development of disease symptoms. To construct the vectors for complementation, the full length genomic copies of *ATG4*, *ATG9*, and *ATG12* were amplified using primers Atg4prom with ATG4 RF3′, which generated an amplicon of 4.2 kb, ATG9prom with ATG9 RF3′, which generated an amplicon of 5.4 kb and ATG12prom with Atg12 RF3′, which generated an amplicon of 3.5 kb (see Table S1). The primers were designed to include 1.5 kb upstream of the start codon and 1 kb 3′ UTR. The amplicons were cloned into strataclone (Stratagene) to generate pAtg4st, pAtg9st, and pAtg12st. The *ATG4* amplicon was excised from pAtg4st by digestion with KpnI-NotI and cloned into pCB1530, which carries a selectable marker conferring resistance to Basta, to generate pAtg4com. The *ATG12* amplicon was excised from pAtg12st by digesting with SacI-XbaI and cloned into pCB1530 to generate pAtg12com. The *ATG9* complementation vector was generated by amplifying the Bar gene from pCB1530 as a SpeI fragment which was then ligated into pAtg9st to generate pAtg9com. The resulting vectors pAtg4com, pAtg9com and pATG12com were transformed into the *M. oryzae* mutant strains Δ*Moatg4*, Δ*Moatg9*, and Δ*Moatg12*. Transformants were selected in the presence of Basta (50 μg mL-1).

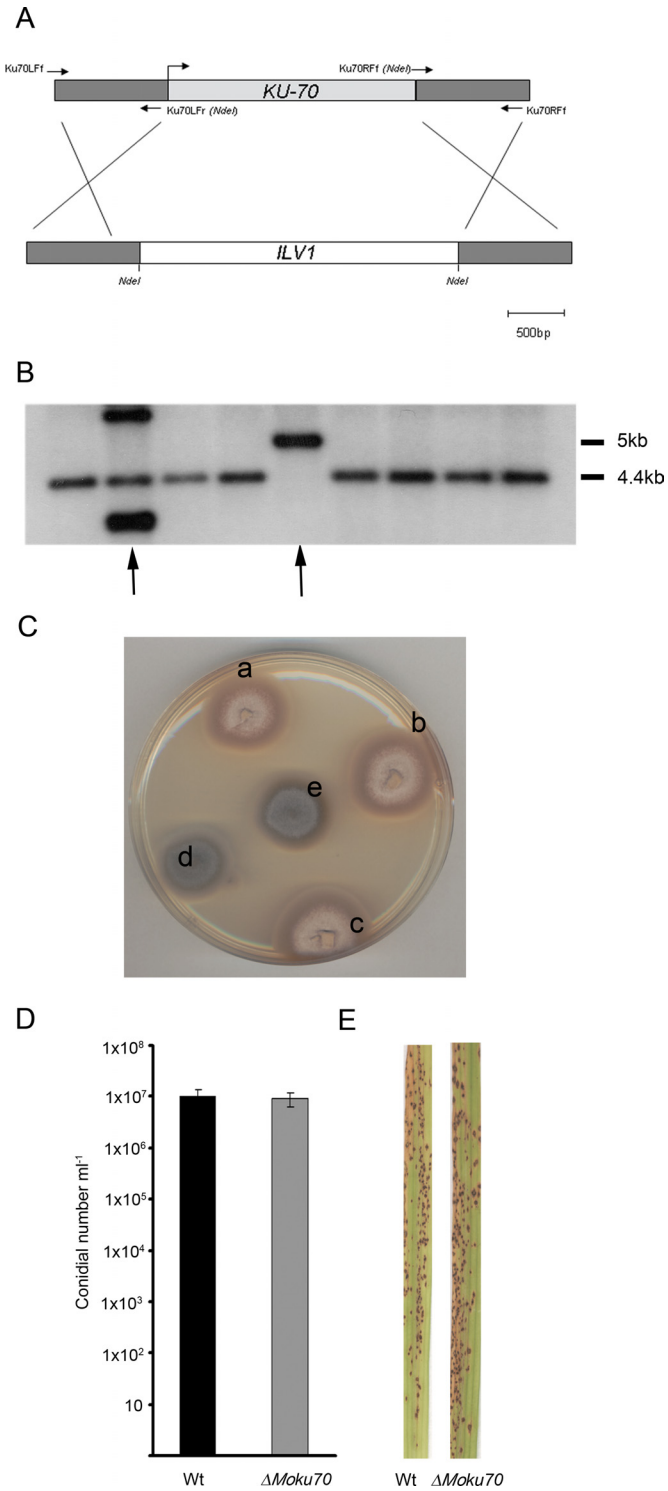

Fig. S2. Targeted gene disruption of the *M. oryzae KU-70* gene. (*A*) A 2.8 kb NdeI fragment containing the *ILV1* resistance cassette was introduced into a vector containing two 1 kb regions flanking the *KU-70* ORF to create pMJG1. (*B*) The resulting 4.8 kb fragment was introduced into *M. oryzae* Guy-11 and deletion mutants identified. Southern blot analysis was carried out. Arrows indicate one ectopic integrant (lane 2) and one deletion mutant (lane 5). (*C*) A deletion cassette for the *M. oryzae BUF1* gene was used to transform a Δ*ku70* deletion mutant. The results indicated a 80% rate of homologous integration. Transformants (*a*–*c*) show melanin deficient phenotype associated with Δ*buf1*, (*d*) is an ectopic transformant and (e) Guy-11. (*D*) Bar graph of numbers of conidia produced from cultures of wild type (Guy-11) and the Δ*Moku70* mutant. Conidia were collected by flooding plate cultures and gently scraping aerial mycelium with a glass rod. Suspensions were filtered to remove mycelial debris and counted. (*E*) Seedlings of rice cultivar CO-39 were inoculated with uniform conidial suspensions of Guy-11 and Δ*Moku70*. Seedlings were incubated for 5 days to allow development of disease symptoms.

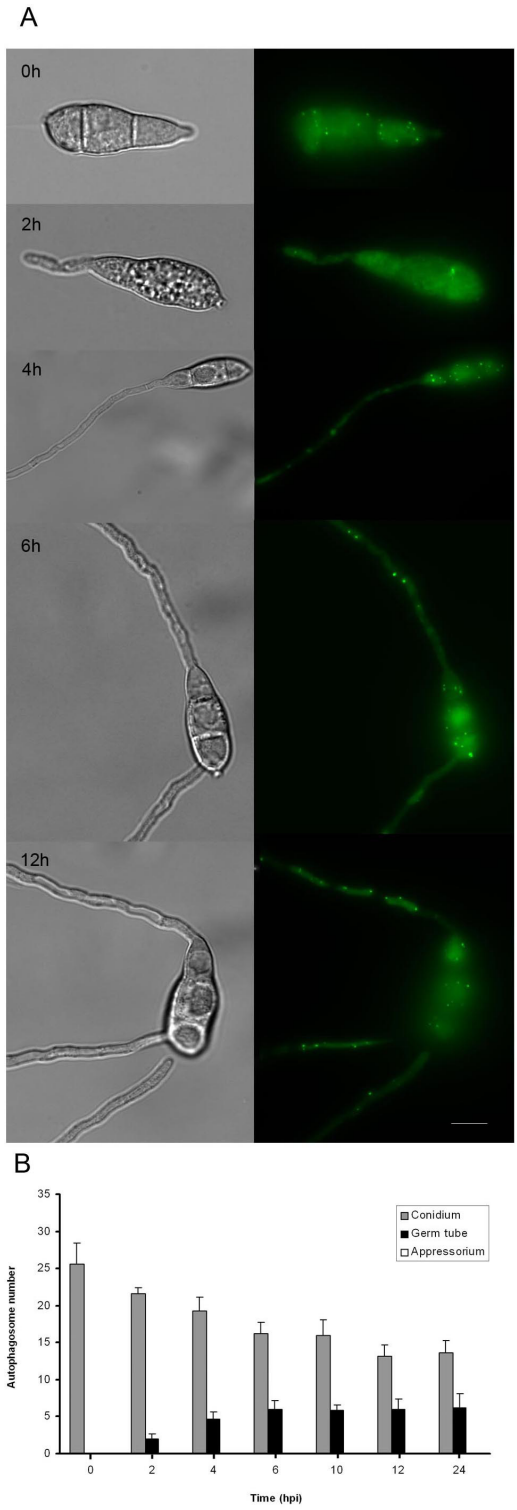

Fig. S3. Cellular localization of autophagosomes during infection-related development of a Δ*pmk1* mutant of *M. oryzae*. (*A*) Conidia were harvested from a Δ*pmk1* transformant expressing a *GFP:MoATG8* gene fusion, inoculated onto glass coverslips and observed by epifluorescence microscopy at the times indicated (Scale bars, 10 μm.). (*B*) Bar chart showing mean autophagosome numbers present in conidium and germ tubes 0 h, 2 h, 4 h, 6 h, 10 h and 12 h after inoculation with Δ*pmk1* mutant expressing a *GFP:MoATG8* (error bars indicate ± 2 SE). The Δ*pmk1* mutants are unable to elaborate an appressorium (1).

1. Sweigard J, Chumley F, Carroll A, Farrall L, Valent B (1997) A series of vectors for fungaltransformation. *Fungal Genet Newsl* 44:52–53.

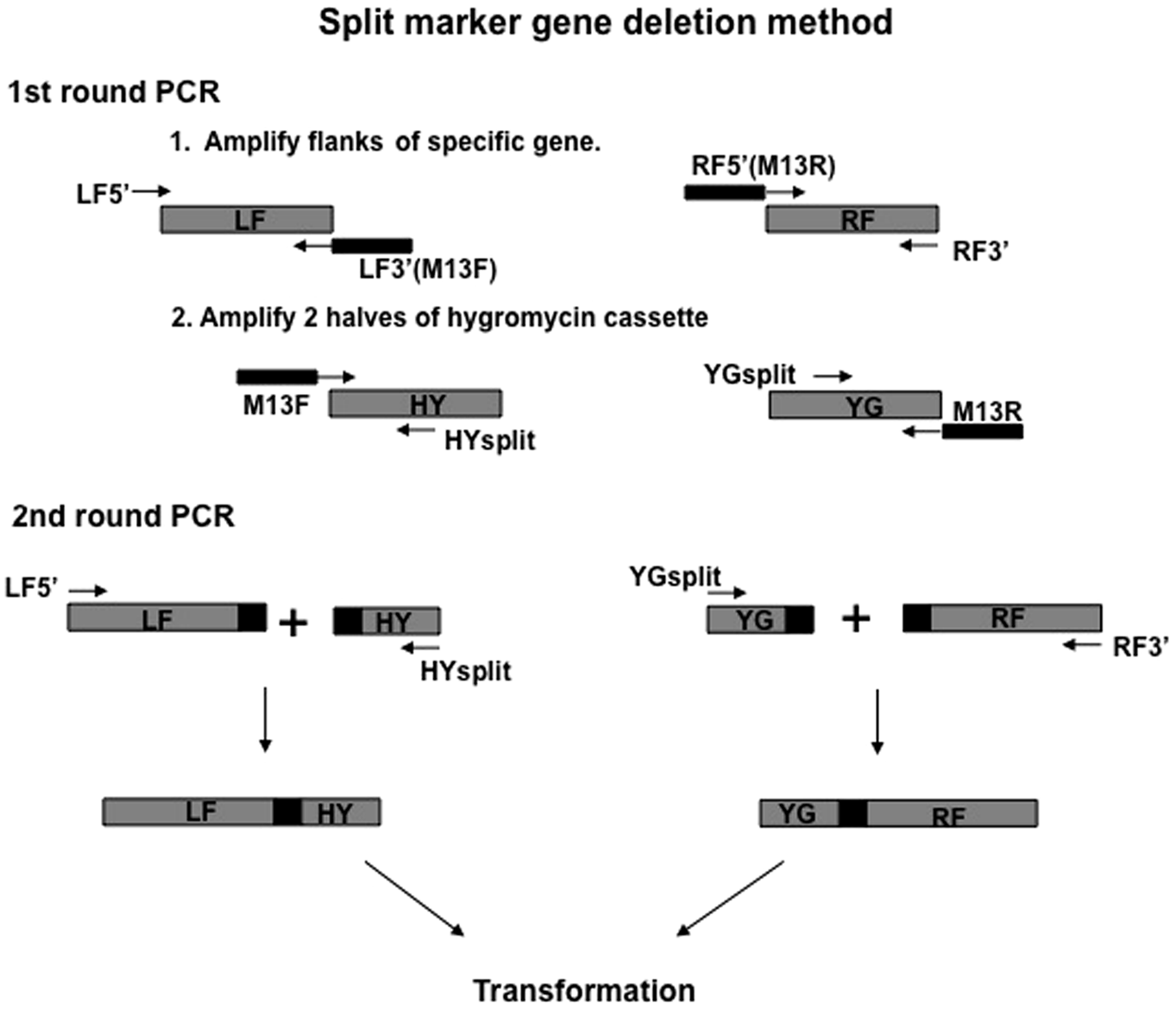

Fig. S4. Schematic representation of the split marker method used to generate the autophagy gene deletion set. The method is described in ref. 2 and the figure can be used as a key to the primers given in Table S3. A first round of PCR is used to amplify flanking sequences on either side of the gene of interest. At the same time overlapping fragments of the 5′ and 3′ end of the hygromycin resistance cassette are amplified. A fusion PCR results in 2 amplicons that are used to transform *M. oryzae*, resulting in homologous recombination and assembly of the selectable marker gene (2).

1. Catlett NL, Lee B-N, Yoder OC, Turgeon BG (2003) Split-marker recombination for efficient targeted deletion of fungal genes. *Fungal Genetics Newslett* 50:9–11.

Table S1. Autophagy genes identified in *M. oryzae*

Yeast Gene*	Function (*Saccharomyces cerevisiae*)	*M. oryzae* hits†
ATG1	Protein serine/threonine kinase, required for autophagy and for the cytoplasm-to-vacuole targeting (Cvt) pathway	MGG_06393
ATG2	Peripheral membrane protein required for the formation of cytosolic sequestering vesicles involved in vacuolar import through both the Cvt pathway and autophagy; interacts with Atg9p and is necessary for its trafficking	MGG_05998
ATG3	Protein involved in autophagy; E2-like enzyme that plays a role in formation of Atg8p-phosphatidylethanolamine conjugates, which are involved in membrane dynamics during autophagy	MGG_02959
ATG4	Cysteine protease required for autophagy; cleaves Atg8p to a form required for autophagosome and Cvt vesicle generation; mediates attachment of autophagosomes to microtubules through interactions with Tub1p and Tub2p	MGG_03580
ATG5	Conserved autophagy-related protein that undergoes conjugation with Atg12p and then associates with Atg16p to form a cytosolic complex essential for autophagosome formation	MGG_09262
ATG6	Protein that forms a membrane-associated complex with Apg14p that is essential for autophagy; involved in a retrieval step of the carboxypeptidase Y receptor, Vps10p, to the late Golgi from the endosome; involved in vacuolar protein sorting	MGG_03694
ATG7	Autophagy-related protein and dual specificity member of the E1 family of ubiquitin-activating enzymes; mediates the conjugation of Atg12p with Atg5p and Atg8p with phosphatidylethanolamine, required steps in autophagosome formation	MGG_07297
ATG8	Protein required for autophagy; modified by the serial action of Atg4p, Atg7p, and Atg3p, and conjugated at the C terminus with phosphatidylethanolamine, to become the form essential for generation of autophagosomes	MGG_01062.5
ATG9	Transmembrane protein involved in formation of Cvt and autophagic vesicles; cycles between the pre-autophagosomal structure and other cytosolic punctate structures, not found in autophagosomes	MGG_09559.5
ATG10	E2-like conjugating enzyme that mediates formation of the Atg12p-Atg5p conjugate, which is a critical step in autophagy	MGG_14737
ATG11	Peripheral membrane protein required for delivery of aminopeptidase I (Lap4p) to the vacuole in the cytoplasm-to-vacuole targeting pathway; also required for peroxisomal degradation (pexophagy	MGG_04486
ATG12	Ubiquitin-like modifier, conjugated via an isopeptide bond to a lysine residue of Atg5p by the E1 enzyme, Atg7p, and the E2 enzyme, Atg10p, a step that is essential for autophagy	MGG_00598
ATG13	Phosphorylated protein that interacts with Vac8p, required for the cytoplasm-to-vacuole targeting (Cvt) pathway and autophagy	MGG_00454
ATG14	Subunit of an autophagy-specific phosphatidylinositol 3-kinase complex (with Vps34p, Vps15p, and Vps30p) required for organization of a pre-autophagosomal structure; ATG14 transcription is activated by Gln3p during nitrogen starvation	None
ATG15	Lipase, required for intravacuolar lysis of autophagic bodies; located in the endoplasmic reticulum membrane and targeted to intravacuolar vesicles during autophagy via the multivesicular body (MVB) pathway	MGG_12828
ATG16	Protein that interacts with the Atg12p-Atg5p conjugate during formation of the pre-autophagosomal structure; essential for autophagy	MGG_05255
ATG17	Scaffold potein responsible for pre-autophagosomal structure organization; interacts with and is required for activation of Apg1p protein kinase; involved in autophagy but not in the Cvt (cytoplasm to vacuole targeting) path	MGG_07667
ATG18	Phosphatidylinositol 3,5-bisphosphate-binding protein of the vacuolar membrane, predicted to fold as a seven-bladed beta-propeller; required for recycling of Atg9p through the pre-autophagosomal structure	MGG_03139
ATG19	Protein involved in the cytoplasm-to-vacuole targeting pathway and in autophagy, recognizes cargo proteins and delivers them to the preautophagosomal structure for eventual engulfment by the autophagosome and degradation	None
ATG20	Protein required for transport of aminopeptidase I (Lap4p) through the cytoplasm-to-vacuole targeting pathway; binds phosphatidylinositol-3-phosphate, involved in localization of membranes to the preautophagosome, potential Cdc28p substrate	None
ATG21	Phosphatidylinositol 3,5-bisphosphate-binding protein required for maturation of pro-aminopeptidase I, predicted to fold as a seven-bladed beta-propeller; displays punctate cytoplasmic localization	None
ATG22	Protein required for the breakdown of autophagic vesicles in the vacuole during autophagy, putative integral membrane protein that localizes to vacuolar membranes and punctate structures attached to the vacuole	MGG_09904
ATG23	Peripheral membrane protein, required for autophagy and for the cytoplasm-to-vacuole targeting (Cvt) pathway	None

Yeast Gene*	Function (*Saccharomyces cerevisiae*)	*M. oryzae* hits†
ATG24	Sorting nexin, involved in the retrieval of late-Golgi SNAREs from the post-Golgi endosome to the trans-Golgi network and in cytoplasm to vacuole transport; contains a PX domain; forms complex with Snx41p and Atg20p	MGG_03638
ATG26	UDP-glucose:sterol glucosyltransferase, conserved enzyme involved in synthesis of sterol glucoside membrane lipids, involved in autophagy	MGG_03459
ATG27	Type II membrane protein involved in autophagy; binds phosphatidylinositol 3-phosphate, required for the cytoplasm-to-vacuole targeting (Cvt) pathway	MGG_02386
ATG28	Involved in degradation of peroxisomes	MGG_08061
ATG29	Protein specifically required for autophagy; may function in autophagosome formation at the pre-autophagosomal structure in collaboration with other autophagy proteins	MGG_02790
ATG31	May form a complex with Atg17p and Atg29p that localizes other proteins to the pre-autophagosomal structure	None

*Source of information; http://yeastgenome.org/.
†Identified as best bidirectional Blast hits at $<$ 1e-5.

Table S2. Sub-type-specific autophagy genes in *M. oryzae*

		Involvement in autophagy	(*S.cerevisiae*)	*M.oryzae* homologues
Gene Name		Cvt pathway	Pexophagy	Non-selective autophagy
ATG11	Yes	Yes	No	MGG_04486
ATG17	No	No	Yes	MGG_05255
ATG19	Yes	No	No	None
ATG20	Yes	No	No	None
ATG21	Yes	Yes	No	None
ATG23	Yes	No	—	None
ATG24	Yes	Yes	No	MGG_03638
ATG26	No	Yes	No	MGG_03459
ATG27	—	No	No	MGG_02386
ATG28	—	Yes	No	MGG_08061
ATG29	No		Yes	MGG_02790

Table S3. Primers used to make gene deletions of *M. oryzae* autophagy loci

Gene	Primers
ATG1	LF5′ TCTCAGTTCAGTCCAAGGCAAGGT
	LF3′ GTCGTGACTGGGAAAACCCTGGCGGTGCTGATCGGTCCGCCATAATTC
	RF5′ AGGTTTTTGGAGTACGGGGAGGTT
	RF3′ TCCTGTGTGAAATTGTTATCCGCTACAGTTCACGATAAGCACCCAGCA
ATG2	LF5′ gatggactgctacaccacacc
	LF3′ GTCGTGACTGGGAAAACCCTGGCGGGGCCTCGCGAGTGTTTGCTAAGC
	RF5′ gatagctccaagcagtcttga
	RF3′ TCCTGTGTGAAATTGTTATCCGCTAAGATAATCTGGGGGACAGTGAGG
ATG3	LF5′ gctgccgccgaggaggatgacgac
	LF3′ gtcgtgactgggaaaaccctggcgcgctacgaactcctctggtgtgat
	RF5′ gctgccgccgaggaggatgacgac
	RF3′ tcctgtgtgaaattgttatccgctcgctacgaactcctctggtgtgat
ATG4	LF5′ tcaacaacgcagacagacacctca
	LF3′ gtcgtgactgggaaaaccctggcgacggcatttggggcaccttgtttg
	RF5′ gcaaagaaggacgacccgccaagt
	RF3′ tcctgtgtgaaattgttatccgcttcagggcaagtccatcattacagt
ATG5	LF5′ acagcaaagtgagaaggggggatg
	LF3′ gtcgtgactgggaaaaccctggcg ccgccgacgtgattcttgatagtg
	RF5′ ttactctcctcatcctcatcagcc
	RF3′ tcctgtgtgaaattgttatccgct gctttgtttaaccgtagtgccatt
ATG6	LF5′ catcctcgtcatcaccatcttc
	LF3′ GTCGTGACTGGGAAAACCCTGGCGttggcaaaacatcatcgacacggc
	RF5′ AGCTAGGCATGTTCATGGTCC
	RF3′ TCCTGTGTGAAATTGTTATCCGCTgacatatgctcgacgtggtgggtg
ATG7	LF5′ tgtgtccgtgaactttgggtatgt
	LF3′ gtcgtgactgggaaaaccctggcgtctatctgtgactcgaatggcgca
	RF5′ cccaaaacatcatgtacggcgtgc
	RF3′ tcctgtgtgaaattgttatccgct TAGGCATAGTTCCTCACCAGGTCC
ATG9	LF5′ cttacacttcagttccaccgacag
	LF3′ gtcgtgactgggaaaaccctggcggatcggttcaaattctcctcgtcg
	RF5′ acgagcccagatattgattgacag
	RF3′ tcctgtgtgaaattgttatccgctgacatagatgaaagtggaggtgcg
ATG10	LF5′ ATCAACAACTACCCGCTCCATGCG
	LF3′ GTCGTGACTGGGAAAACCCTGGCGTCAGCGGTCAGAAATGGATAGCTC
	RF5′ GCGAAGGAGACAAAATGGCGACAG
	RF3′ TCCTGTGTGAAATTGTTATCCGCTCTGCTCCAAAGAAGACTACCTGAT
ATG11	LF5′ tctagcatcatatcgcacacacga
	LF3′ gtcgtgactgggaaaaccctggcgtcggcaggtctatctccagcttga
	RF5′ atgagtcttttccctgtaaccgcg
	LF5′ tcctgtgtgaaattgttatccgctaaccaaggagaagagtgacgatgc
ATG12	LF5′ acctcgaagtactgcaatagcggc
	LF3′ gtcgtgactgggaaaaccctggcgtcggtgagacgacattgtgaaag
	RF5′ tgttggacgagctgttgctgcact
	Rf3′ tcctgtgtgaaattgttatccgct cttactccatgactcctgcctttg
ATG13	LF5′ CTGTTACCGCTGTGCTTGCTGCTT
	LF3′ GTCGTGACTGGGAAAACCCTGGCGCGGAGGATAGCGGGACTGTTGATG
	RF5′ GTCGTGACTGGGAAAACCCTGGCG
	Rf3′ TCCTGTGTGAAATTGTTATCCGCTAAGATTCGGGGTGGTCGTTATGAA
ATG15	LF5′ TGGCTCGTTCAACCTGTTCGTCCC
	LF3′ GTCGTGACTGGGAAAACCCTGGCGTAACGGTTTTCCTCGCGCAATGAC
	RF5′ GCTGAAGCCCTCGTCGTCATCCGC
	RF3′ TCCTGTGTGAAATTGTTATCCGCTGTGAGAAGGGTATGGGTGAGTTAT
ATG16	LF5′ gagcgagaactgggtaaagtcctc
	LF3′ gtcgtgactgggaaaaccctggcggaagacattgggatcgtgattttg
	RF5′ taaatacaatctatgtctcaggga
	RF3′ tcctgtgtgaaattgttatccgctgccaaacgaggatgaatcaaaact
ATG17	LF5′ gttgacattgctggtcagtgtg
	LF3′ GTCGTGACTGGGAAAACCCTGGCGGCTCGGCAATGCTCTCCTTCAGTG
	RF5′ AGGAGAGTGCTGAGACCAAAGGAG
	RF3′ TCCTGTGTGAAATTGTTATCCGCTgcgagggtctcactttgaagaacc
ATG18	LF5′ tttgttgctcttacgcccatgacc
	LF3′ GTCGTGACTGGGAAAACCCTGGCGgatgaagtttagcgttgcagtcgc
	RF5′ aacgaagcgaagcagaagaccaag
	RF3′ TCCTGTGTGAAATTGTTATCCGCTTGGAAACGATAACACGGGTTTCAG

Gene	Primers
ATG22A	LF5′ TGACCACTATGACCTCCTACGAAT
	LF3′ GTCGTGACTGGGAAAACCCTGGCGAAGAATACGAGCGGAAAGACC
	RF5′ GATACTCTGCTGTCGCTCGGATGA
	RF3′ TCCTGTGTGAAATTGTTATCCGCTTTCCCAAGCGATGACGACGATG
ATG22B	LF5′ CTCTCATCCGTCTTTCTGGCCCTG
	LF3′ GTCGTGACTGGGAAAACCCTGGCGGGGCGTTGTTACTGCTGACCATGA
	RF5′ GCATGAGCAGCTTGTGGCCTTTGA
	RF3′ TCCTGTGTGAAATTGTTATCCGCTGACACGGACCAGGCAAAGATTGAC
ATG24	LF5′ atgatggcgagtcggcgaattata
	LF3′ gtcgtgactgggaaaaccctggcggttgtgcgttggctgttccgattc
	RF5′ cctccaagcagtcatcagtcggct
	RF3′ tcctgtgtgaaattgttatccgcttcatcagggaggtgtccgattttg
ATG26	LF5′ GCAAGATCCGCGTTATTGACAACG
	LF3′ GTCGTGACTGGGAAAACCCTGGCG TCAAAGCGGCTGCCTCGTCGTAAT
	RF5′ TTTTTCTTGTCGGGAATTGGGCGG
	RF3′ TCCTGTGTGAAATTGTTATCCGCTCCCTCGAACGAAGCCATGTTGTTT
ATG27	LF5′ GTGAAAGGTGGAGTCGGTGGTATT
	LF3′ GTCGTGACTGGGAAAACCCTGGCGCAGGAGCGAGAGGATCGTCTTTGG
	RF5′ AATATCACATGCTCGGTGGATGGG
	RF3′ TCCTGTGTGAAATTGTTATCCGCTTTCTTCACCCCAGCGTATTCCTCG
ATG28	LF5′ TGCCCGTACCCTTTGTCATTCAAC
	LF3′ GTCGTGACTGGGAAAACCCTGGCGTGGGGGTTTGGTCACGTAGCGGCG
	RF5′ CAGACACGATACGCCAATGAGCGA
	RF3′ TCCTGTGTGAAATTGTTATCCGCTCAAAGGGAGGAGAGCGATAATGAG
ATG29	LF5′ TGAATACCTGGTCTGCTGCTGCGA
	LF3′ GTCGTGACTGGGAAAACCCTGGCGCCTCTTGGGAAAGGGAGACGGATG
	RF5′ CAACCTGCCACAGCATTCTTCCCC
	RF3′ TCCTGTGTGAAATTGTTATCCGCTCAACGGAGAAGAAGTAGGAGAAGC

Table S4. Sequences of primers used in this study

Primer name	Sequence 5′-3′
A. KU70 deletion cassette	
Ku70 LFf	AGATCTTGTCGAGCAGCTTGCACA
Ku70LFr	tacatatgGTGTTTGTCGGCACGATC
Ku70 Rff	ATCATATGTATCATCCCGTCTAAGAGACC
Ku70RFr	TAGCGGCCGCCATCAGGTTTAATGCTCA
SurF	ATCATATGGTCGACGTGCCAACGCCACAG
SurR	aTCATATGGTCGACGTGAGAGCATGCAAT
B. Split Marker Hygromycin cassette	
M13F	cgccagggttttcccagtcacgac
M13R	agcggataacaatttcacacagga
HYsplit	ggatgcctccgctcgaagta
YGsplit	cgttgcaagacctgcctgaa
C. GFP-ATG8 Construct	
ATG8 spe1	gcactagtcaagccacctgcacctgcaattca
ATG8 nco1 rev	gagcccatggcggcggttgattgag
GFP cla1 rev	gtgcatcgatcttgtacagctcgtccatgccgag
ATG8 cla1	gccatcgatcgctccaagttcaaggacgagcac
ATG8 xho1	gcctcgagatgatgtcgatgtctcgcctcagct
D. Complementation	
ATG4prom	TCTAGATGGTACYGGGGGCTTGC
ATG9prom	GTGGGAACTTGGGACCTACATCGG
ATG12prom	GCCTGTCCACATCACCTCGAAGTA