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Supplemental Material to:

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Linkage of autophagy to fungal development, lipid storage and virulence in *Metarhizium robertsii*

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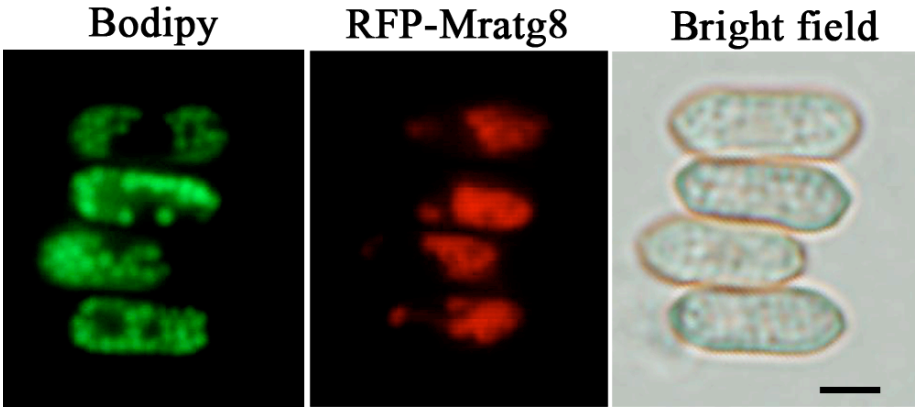

Figure S1. MrAtg8 is not localized on the lipid droplets (LDs). The conidia of RFP-MrAtg8 (wild-type strain transformed with an *RFP-MrATG8* fused gene) were stained with the LD-specific fluorescent dye, Bodipy. The stained LDs and RFP-labeled MrAtg8 showed different patterns. Bar, 5 μm.

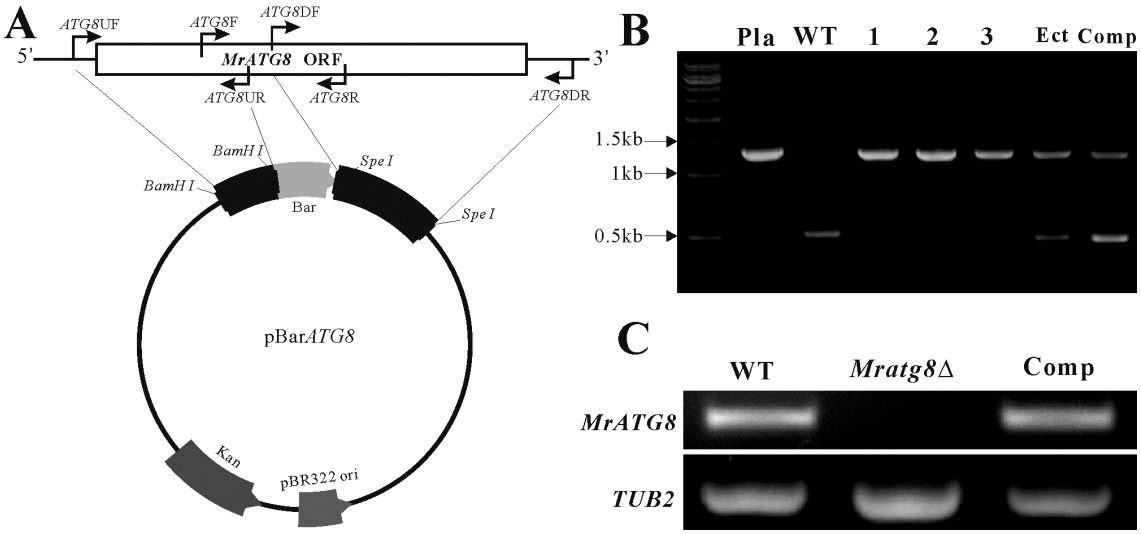

Figure S2. *MrATG8* gene disruption and verification. (A) Construction of the vector pBar*ATG8* for homologous gene disruption. (B) Transformants were verified by PCR with primers *ATG8*F and *ATG8*R. Pla, plasmid pBar*ATG8* DNA used as a template; WT, wild type; Lanes 1, 2 and 3 amplified from three independent null mutants; Ect, ectopically integrated transformant with two bands; Comp, complemented mutant acquired by transforming *Mratg8*Δ with the *MrATG8* gene. (C) RT-PCR verification of *MrATG8* transcripts. Lane 1, wild type (WT); Lane 2, *Mratg8*Δ; Lane 3, *Mratg8*Δ complemented transformant with the *MrATG8* gene. *TUB2*, tubulin beta-2 gene used as a reference.

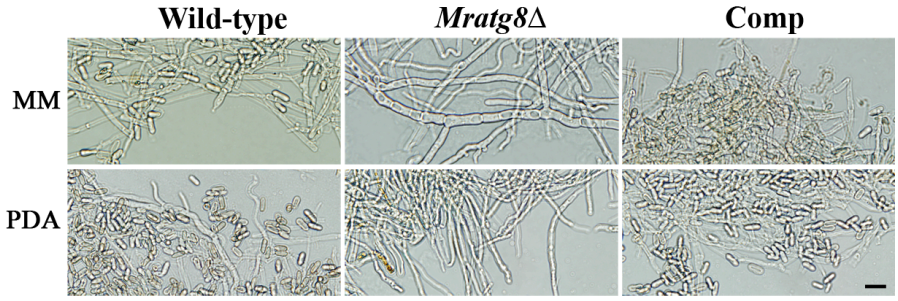

Figure S3. Differences of conidial formation. In contrast to the wild-type and complemented mutant (Comp), *Mratg8*Δ failed to produce conidia either on the nutrient-rich potato dextrose agar (PDA) or the nutrient-poor minimal medium (MM). Bar, 15 μm.

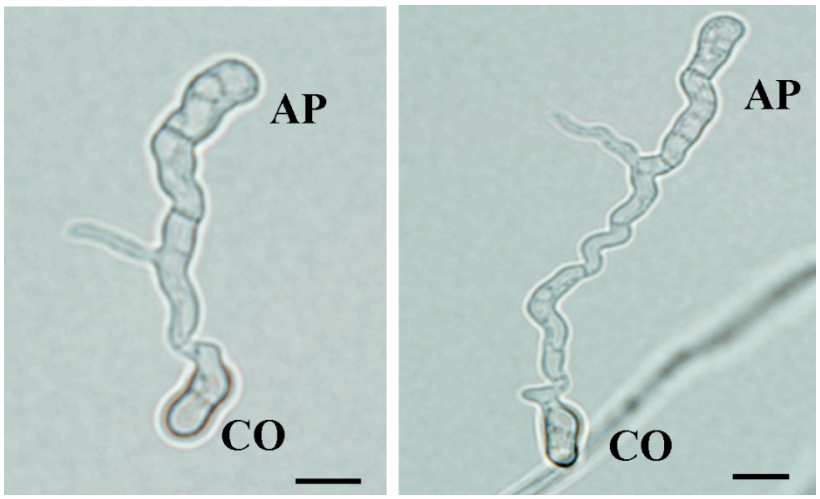

Figure S4. Appressorium production by the M8-3 mutant on a hydrophobic surface. When compared to the wild type (Left, a differentiation rate at 88.4%), the mutant could similarly form appressoria (Right, 82.22%) on a hydrophobic surface. CO, conidium; AP, appressorium. Bar, 5 μm.

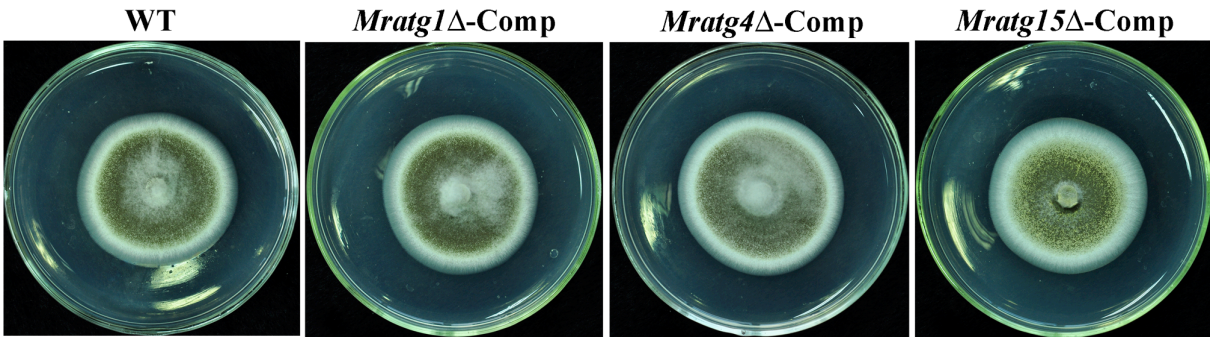

Figure S5. Phenotyping of *Mratg1*Δ, *Mratg4*Δ and *Mratg15*Δ complemented with corresponding genes grown on PDA medium. The cultures were grown on the medium for 10 days.

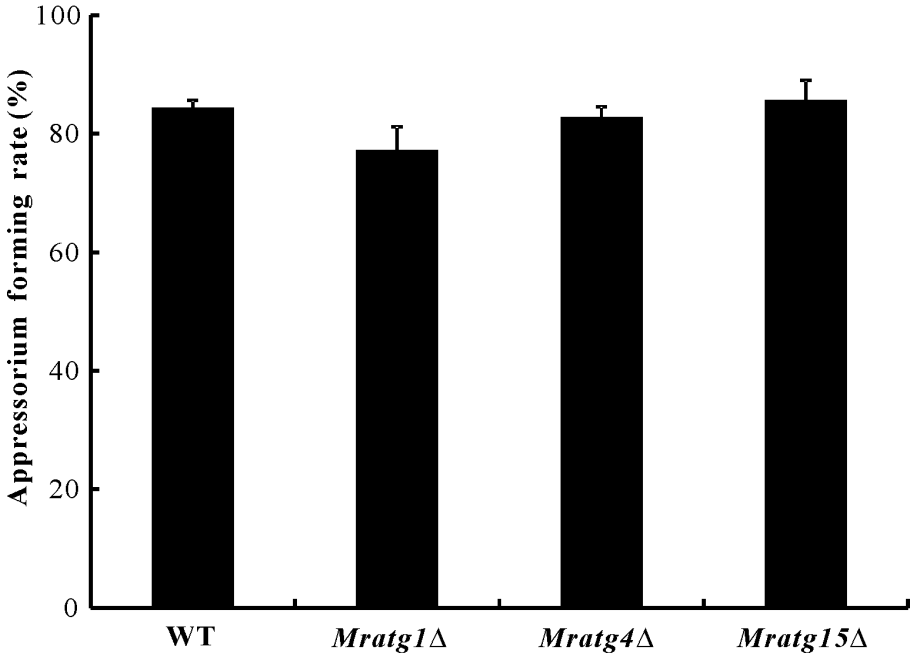

Figure S6. Appressorium production by the wild type and different gene deletion mutants. Conidia of the wild type (WT), *Mratg1*Δ, *Mratg4*Δ and *Mratg15*Δ harvested after incubation for 20 days on CM plates were subjected to appressorial induction for 16 h on a hydrophobic surface (sterile plastic petri dishes of six cm diameter containing 3 ml of 0.0125% yeast extract). No significant differences were detected between different strains ($P > 0.05$).

Table S1. List of oligonucleotide primers used in the study.

Primer	Sequence (5'- 3')	Purpose of use
*ATG8*UF	CGGGATCCGTGACTACAGAGTAGCAGGCTGTC	For deletion and verification of *MrATG8*
*ATG8*UR	CGGGATCCGATGTCGCTCTTCTCTACCTT	
*ATG8*LF	GGACTAGTCAGCATCTATGAGGAGCACAAG	
*ATG8*LR	GGACTAGTGCAGTCTGGTAGAGTCCGGTAT	
*ATG8*F	TGAAAAGCGTAAGGCTGAGG	
*ATG8*R	CCGAGTAGGTGATGTAGAGGAATC	
*TUB*F	GATCTTGAACCTGGCACCAT	For RT-PCR internal control
*TUB*R	CCATGAAGAAGTGCAGACGA	
A8XbU	GCTCTAGACCGAGCATTCAGTCATCATGCGAAG	Mratg8 C-terminal amino acid mutations
A8XbD	GCTCTAGATTACGCGGTGCCAAAAGTGTTTTCG	
A8XbD1	GCTCTAGATTACGCGGTGGCAAAAGTGTTTTCG	
A8XbD2	GCTCTAGATTACGCGGTAAAAGTGTTTTCG	
A8XbD3	GCTCTAGATTAGCCAAAAGTGTTTTCGCCC	
A8XbD4	GCTCTAGA TTAGGCAAAAGTGTTTTCGCCC	
A8XbD5	GCTCTAGA TTAAAAAGTGTTTTCGCCCGAG	
RfpXbF	GCTCTAGAGCAGGAATTCATGGCCTCCTCCGAG	For RFP-Mratg8 fusion
RfA81	CGTCCTTGAACTTGCTTCGCATGGCGCCGGTGGAGTGGCGGC	
RfA82	GCCGCCACTCCACCGGCGCCATGCGAAGCAAGTTCAAGGACG	
A8Rf1	GACGTCCTCGGAGGAGGCCATCGCGGTGCCAAAAGTGTTTTCG	For Mratg8-RFP fusion
A8Rf2	CGAAAACACTTTTGGCACCGCGATGGCCTCCTCCGAGGACGTC	
RfpXbR	GCTCTAGATTAGGCGCCGGTGGAGTGGCG	
*ATG1*UF	AACTGCAGACTCATTTCACAGACGCATCC	For deletion, verification and complementation of *MrATG1*
*ATG1*UR	AACTGCAGTCAGTACTGGTAACGGTAAGAGG	
*ATG1*LF	GGACTAGTGAGATCCCTGGATTAGTGGAAGAC	
*ATG1*LR	GGACTAGTCTGTCTAGCTCACTGCTAGCTTCT	
*ATG1*F	GCGAACTAGGAGACCTGTCG	
*ATG1*R	GGAGTTCAACGTGGTTTCGT	
*ATG1*CF	GGACTAGTCCCTTATCACGCTTCCTC	
*ATG1*CR	GGACTAGTGTTCCCTATCACAAGTCCAA	
*ATG4*UF	CGGGATCCTGGAATGGACCATGTAGTGAG	For deletion, verification and complementation of *MrATG4*
*ATG4*UR	CGGGATCCGACAGAGACAAAGCTGATGCTG	
*ATG4*LF	GGACTAGTCGCTACTGCTCGTTGTATCCAGTA	
*ATG4*LR	GGACTAGTCCTAAGCTAAGCAGCCAGATTC	
*ATG4*F	CAGCATCAGCTTTGTCTCTGTC	
*ATG4*R	ATGCCCAGTCTCGTTCCTACTA	
*ATG4*CF	GGACTAGTTTCCTGCTGCCCTGTTGG	
*ATG4*CR	GGACTAGTCCTGAAGGCTGCGATGTT	
*ATG15*UF	CGGAATTCCGCTCGCTCTTTGAATGT	For deletion, verification and complementation of *MrATG15*
*ATG15*UR	CGGGATCCTTGCAGGTGCCAATGTAG	
*ATG15*LF	GCTCTAGATACAGGAGTCACGGCTACG	
*ATG15*LR	CGAGCTCAACCAACATCACAGAGGGA	
*ATG15*F	TTCTGATCCCAAAGCTCCT	
*ATG15*R	ATCCGAGGTTGTAGTGATA	
*ATG15*CF	GCTCTAGAATGAGGGTGTCGTAGAAGAGG	
*ATG15*CR	GCTCTAGAAACCAACATCACAGAGGGAGT	
*MPL1*F	GAACCCTCCCTTCTTTCACC	For RT-PCR analysis of *MPL1* gene
*MPL1*R	CCTTGGCATTGGCATAGACT	

Table S2. Comparison of autophagy-related genes encoded in the genomes of *S. cerevisiae*, *M. oryzae* and *M. robertsii*.

Function*	Gene symbols	*S. cerevisiae*	*M. oryzae*	*M. robertsii*
Atg1 kinase and its regulators	ATG1	NP_011335.1	MGG_06393	**MAA_03501**
	ATG13	NP_015511.1	MGG_00454	MAA_02276
	ATG17	NP_013527.1	MGG_07667	MAA_07123
	ATG29	NP_015159.1	MGG_02790	MAA_08445
	ATG31	NP_010305.1	None	None
PtdIns 3-kinase complex	ATG6	NP_015205.1	MGG_03694	MAA_00746
	ATG14	NP_009686.1	None	None
Atg12 conjugation system	ATG5	NP_015176.1	MGG_09262	MAA_02911
	ATG7	NP_012041.1	MGG_07297	MAA_03074
	ATG10	NP_013058.1	None	None
	ATG12	NP_009776.1	MGG_00598	MAA_02068
	ATG16	NP_013882.1	MGG_05255	MAA_08906
Atg8 conjugation system	ATG3	NP_014404.1	MGG_02959	MAA_03709
	ATG4	NP_014176.2	MGG_03580	**MAA_04475**
	ATG7	NP_012041.1	MGG_07297	MAA_03074
	ATG8	NP_009475.1	MGG_01062	**MAA_02674**
Atg2-Atg18 complex	ATG2	NP_014157.1	MGG_05998	MAA_03175
	ATG18	NP_444297.1	MGG_03139	MAA_05300
Cytoplasm to vacuole targeting (Cvt) pathway	ATG9	NP_010132.1	MGG_09559	MAA_04484
	ATG11	NP_015374.1	MGG_04486	MAA_04312
	ATG19	NP_014559.1	None	None
	ATG20	NP_010170.1	MGG_12832	MAA_05815
	ATG21	NP_015225.1	MGG_03088	MAA_010306
	ATG23	NP_013535.1	None	None
	ATG24	NP_012498.1	MGG_03638	MAA_05264
	ATG27	NP_012357.2	MGG_02386	MAA_01720
Pexophagy	ATG11	NP_015374.1	MGG_04486	MAA_04312
	ATG25	None	None	None
	ATG26	NP_013290.1	MGG_03459	MAA_06719
	ATG28	None	None	None
	ATG30	None	None	None
Mitophagy	ATG32	NP_012120.1	None	None
	ATG33	NP_013460.1	None	None
Autophagic degradation and recycle	ATG15	NP_009994.2	MGG_12828	**MAA_00506**
	ATG22	NP_009892.1	MGG_09904	MAA_08468
Others	ATG34	NP_014558.1	None	None

*, functional grouping is based on the information acquired from the autophagy database (http://tp-apg.genes.nig.ac.jp/autophagy/index.html). The genes of *M. robertsii* highlighted in bold were deleted in this study for functional studies.