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Supplemental Material to:

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Autophagy genes *Smatg8* and *Smatg4* are required 1 for fruiting-body 2 development, vegetative growth and ascospore germination in the filamentous 3 ascomycete *Sordaria macrospora*

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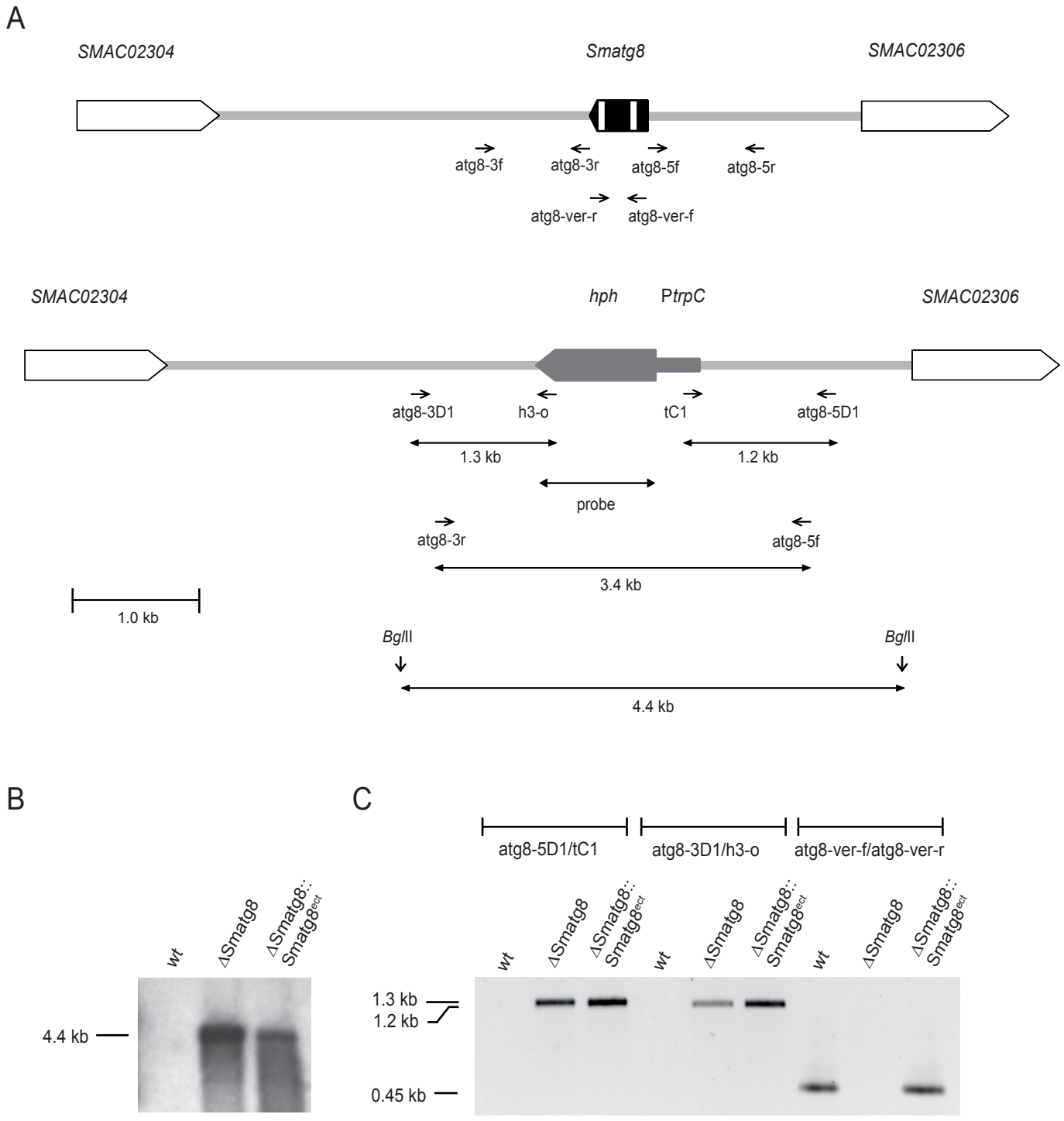

Figure S1. Generation and complementation of a ΔSmatg8 strain. (A) Schematic illustration of the *Smatg8* locus before and after homologous integration of the deletion cassette, primer position used for the verification of the deletion and complementation are indicated by small arrows. Sizes of PCR fragments and fragments obtained after restriction enzyme cleavage are given. Position and size of the probe used for southern hybridization is indicated. (B) Southern hybridization (Sambrook et al., 2001) demonstrating the successful integration of the deletion cassette using an *hph* gene specific probe. The gDNA of wt and ΔSmatg8 and complementation strain ΔSmatg8::Smatg8 was digested with *Bgl*II and hybridized with the *hph*-gene specific probe. An expected signal of 4400 bp was detected in the deletion and the complemented strain. (C) Deletion and complementation was confirmed by PCR.

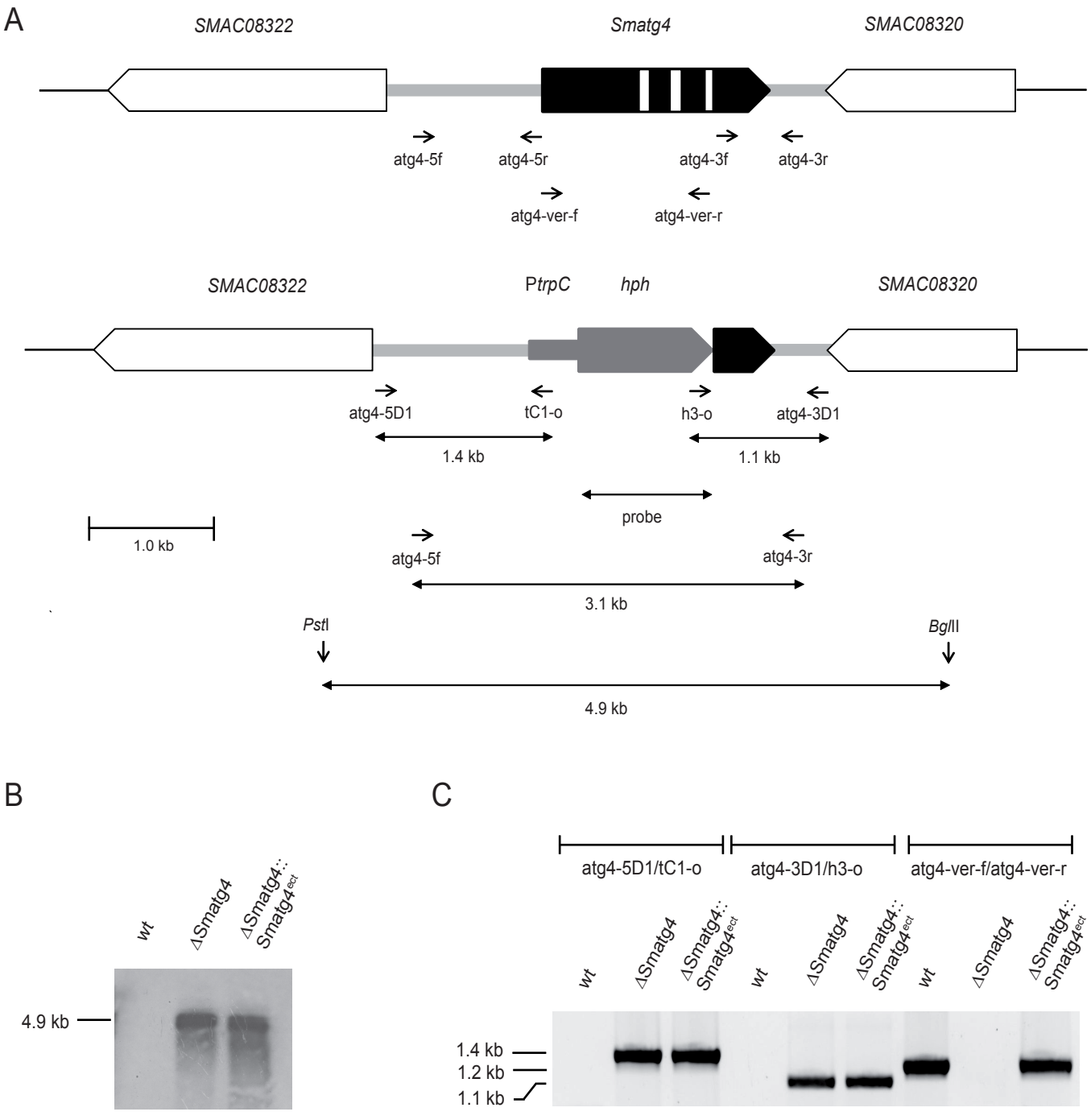

Figure S2. Construction of a *Smatg4* deletion strain. (A) Schematic illustration of the *Smatg4* locus before and after gene replacement. Positions of primers for deletion and complementation analysis are indicated by small arrows. Sizes of PCR fragments and fragments obtained after restriction enzyme cleavage are given. Position and size of the probe used for Southern hybridization is indicated. (B) Southern hybridization with a *hph* probe to verify single copy integration of the *Smatg4* deletion cassette. The gDNA of wt, ΔSmatg4 and the complemented strain ΔSmatg4::Smatg4 was digested with *Pst*I and *Bgl*II and hybridized with the *hph*-gene specific probe. An expected signal of 4900 bp was detected in the deletion and the complemented strain. (C) Deletion and complementation was confirmed by PCR.

Table S1
Plasmids used during this study

Plasmid	Features/Inserts	Source
pRS246	*URA3*	Christianson et al. (1992)
pRSnat	*URA3*, *nat*-cassette	Klix et al. (2010)
pDS23-eGFP	*egfp* under control of *gpd* promoter and *trpC* terminator of *A. nidulans*, *URA3*, *nat*-cassette	Nowrousian unpublished
p1783	*egfp* under control of *gpd* promoter and *trpC* terminator of *A. nidulans*, *hph*-cassette	Pöggeler et al. (2003)
pRS426-met25	*met25* promoter, *URA3*, amp^R	Mumberg et al., 1994
pGBKT7	*TRP1*, *GAL4-BD*, kan^R	Clonetech
pGADT7	*LEU2*, *GAL4-AD*, amp^R	Clonetech
pRSmet-Smatg8	*Smatg8* under control of *met25* promoter, *URA3*, amp^R,	This work
pRSmet-Smatg4	*Smatg4* under control of *met25* promoter, *URA3*, amp^R,	This work
pRS315-EGFP-Atg8	*LEU2*, amp^R, e*gfp-atg8* under control of endogenous promoter	M. Thumm, Göttingen[a]
pRS316-Atg4	*URA3*, amp^R, *atg4* under control of endogenous promoter	M. Thumm, Göttingen[a]
pBD-Smatg8	*TRP1*, *GAL4-BD*, kan^R, *Smatg8*	This work
pBD-Smatg4	*TRP1*, *GAL4-BD*, kan^R, *Smatg4*	This work
pAD-Smatg8	*LEU2*, *GAL4-AD*, amp^R, *Smatg8*	This work
pAD-Smatg4	*LEU2*, *GAL4-AD*, amp^R, *Smatg4*	This work
pAD-RAN-Bpm	*LEU2*, *GAL4-AD*, amp^R, *RanBPM*	Tucker et al. (2009)
pDsRed-SKL	*DsRed-SKL* under control of *gpd* promoter and *trpC* terminator of *A. nidulans*, in pRHN1	Elleuche and Pöggeler (2008)
pRS-ΔSmatg8	1023 bp of the 5' flanking region and 1021 bp of 3' flanking region of *Smatg8* interrupted by the *hph*-cassette in pRSnat	This work
pRS-ΔSmatg4	1031 bp of 5' flanking region and 468 bp of 3' region plus 214 bp flanking region of *Smatg4* disrupted by the *hph*-	This work

	cassette in pRSnat	
pRS-Smatg8-comp	*Smatg8* plus 1023 bp of 5' and 1021 bp of 3' flanking region in pRSnat	This work
pRS-Smatg4-comp	*Smatg4* plus 1031 bp of 5' and 214 bp of 3' flanking region in pRSnat	This work
pRS-egfp-Smatg8	1023 bp of the 5' flanking region of *Smatg8*, *egfp* excluding stop codon, *Smatg8* without start codon and 1021 bp of 3' flanking region of *Smatg8* in pRSnat	This work
pRS-Smatg8-egfp	*Smatg8* excluding stop codon in pDS23-eGFP	This work
pRS-egfp-Smatg8mut	*Smatg8* excluding stop codon in pDS23-eGFP, AA 115-118 substituted with alanine	This work
pRS-Smatg4-egfp	*Smatg4* excluding stop codon in pDS23-eGFP	This work
pRS-egfp-Smatg8^{G116}	*Smatg8* C-terminally truncated (AA 1-116) in pDS23-eGFP	This work
pRS-egfp-Smatg8-DsRedSKL	1023 bp of the 5' flanking region of *Smatg8*, egfp exluding stop codon, *Smatg8* without start codon and 1021 bp of 3' flanking region of *Smatg8* and *DsRed-SKL* under control of *gpd* promoter and *trpC* terminator of *A. nidulans*, in pRSnat	This work

Table S2
Primers applied in this study

Oligo name	Specific sequence (5'-3')[b]
atg8-5f	GTAACGCCAGGGTTTTCCCAGTCACGACGGACGACTTCAC AGTGACATC
atg8-5r	CAAAAAATGCTCCTTCAATATCAGTTAACGATCTCATTTTG GCGGTTTG
atg8-3f	GAGTAGATGCCGACCGGGAACCAGTTAACGATTTCGAGAC TGCGTAATC
atg8-3r	GCGGATAACAATTTCACACAGGAAACAGCGACGCAGCCCT TTGTTTCCC
atg4-5f	GTAACGCCAGGGTTTTCCCAGTCACGACGATATCGTTTGCC GCTTGGTC
atg4-5r	CAAAAAATGCTCCTTCAATATCAGTTAACGAATCTCTTGAA TGCCCAGA
atg4-3f	GAGTAGATGCCGACCGGGAACCAGTTAACAGGTATAGCCG GGTGAGTCC
atg4-3r	GCGGATAACAATTTCACACAGGAAACAGCAGAGTCCTTTG TTCTATCAT
atg8-5D1	CGAACGGAGAAGGCGGACAC
atg8-3D1	CAGTTGCGATACATTACAAC
atg8-ver-f	CAAGTTTAAGGACGAGCACCCCTTC
atg8-ver-r	GAAGGTGTTCTCGCCCGAGTATGTG
atg4-5D1	CTGCATGAAAGGTAAAGAGG
atg4-3D1	CTCTGGCTGACAATATTTCC
atg4-ver-f	ATGACGTCCTCGCGACCTGGTGGCAC
atg4-ver-r	ATCGACTGAGGTAACTGTAGAGTTG
Atg8-cf	*ACTAGT*ATGAGATCCAAGTTTAAGGA
Atg8-cr	*GTCGAC*TTACGCAGTCTCGAAAT
atg4-cf	*ACTAGT*ATGACGTCCTCGCGACCTGG
atg4-cr	*GTCGAC*TTATGCTCCTAGAGCGGTTT
atg8-RT-f	GGCCAGTTCGTTTACGTCAT
atg8-RT-r	GTGTTCTCGCCCGAGTATGT
atg4-RT-f	GTCTTTGGCGTACGAAGAGC
atg4-RT-r	GTTTGCGAGAAGGCTTTGAC
atg8-Hf	*CATATG*AGATCCAAGTTTAAGGACGA
atg8-Hr	*GAATTC*GCTTACGCAGTCTCGAAATCGC
atg4-Hf3	*GAATTCCCC*ATGACGTCCTCGCGACCTGG
atg4-Hr3	*CTCGAGG*GTTATGCTCCTAGAGCGGTTT
atg4-Hr	*GTCGACGC*TTATGCTCCTAGAGCGGTTT
atg8-egfp-5r	GTGAACAGCTCCTCGCCCTTGCTCACCATTTTGGCGGTTTG CTTTGATG
atg8-egfp-3f	TCACTCTCGGCATGGACGAGCTGTACAAGAGATCCAAGTT TAAGGACGA
atg8-egfp-3r	GCGGATAACAATTTCACACAGGAAACAGCGTGTACTACCT ATCATGTAT
atg8-gf	*GCGAGATCT*ATGAGATCCAAGTTTAAGGAC
atg8-gr	*CGCAAGCTT*CGCAGTCTCGAAATCGCCGA
atg8-gr2	*CGCAAGCTT*CGCAGTCTCGGCGGCGGCGGCGGTGTTCTCGC

	CCGAGTAT
atg4-gf	*GCGCCATGGCC*ACGTCCTCGCGACCTGGTGG
atg4-gr	*CGCCCATGGC*TGCTCCTAGAGCGGTTTCTC
atg8pegfp-5r	GCCGAAGGTGTTCTCGCCCG
atg8pegfp-3f	TCACATACTCGGGCGAGAACACCTTCGGCT AATCCATATCCTCTCTACC
atg8-5f3	GTAACGCCAGGGTTTTCCCAGTCACGACGTTTATGTTGTAA TAGACGGG
atg8-org-5r2	GTGTACTACCTATCATGTATTTCTA
atg8-org-3f	AGCTTAGAAATACATGATAGGTAGTACACGTACAGTGACC GGTGACTCT
MiPe-r2	GCGGATAACAATTTCACACAGGAAACAGCTCGAGTGGAGA TGTGGAGTG
hph-f	GTTAACTGATATTGAAGGAGCATTTTTTGG
hph-r	GTTAACTGGTTCCCGGTCGGCATCTACTC
tC1	CACCGCCTGGACGACTAAACC
tc1-o	CCTGGACGACTAAACCAAAA
h3-o	GATGGCTGTGTAGAAGTACT
egfp-f	ATGGTGAGCAAGGGCGAGGAGCTGTTCAC
egfp-r	CTTGTACAGCTCGTCCATGCCGAGAGTGA
ssu-f	ATCCAAGGAAGGCAGCAGGC
ssu-r	TGGAGCTGGAATTACCGCG

[a], Department of Biochemistry II, Georg-August University Göttingen, Germany
[b], Underlined sequences represent 29 bp overhangs for homologous recombination in *S.cerevisiae* and letters in italics indicate generation of restriction sites.