Supplementary data for

Functional characterization of the developmental genes *asm2, asm3,* and *spt3* required for fruiting body formation in the filamentous ascomycete *Sordaria macrospora*

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This file contains supplementary figures S1-S9 and supplementary tables S1, S2, and S4. Supplementary tables S3 and S5 are given as separate excel files.

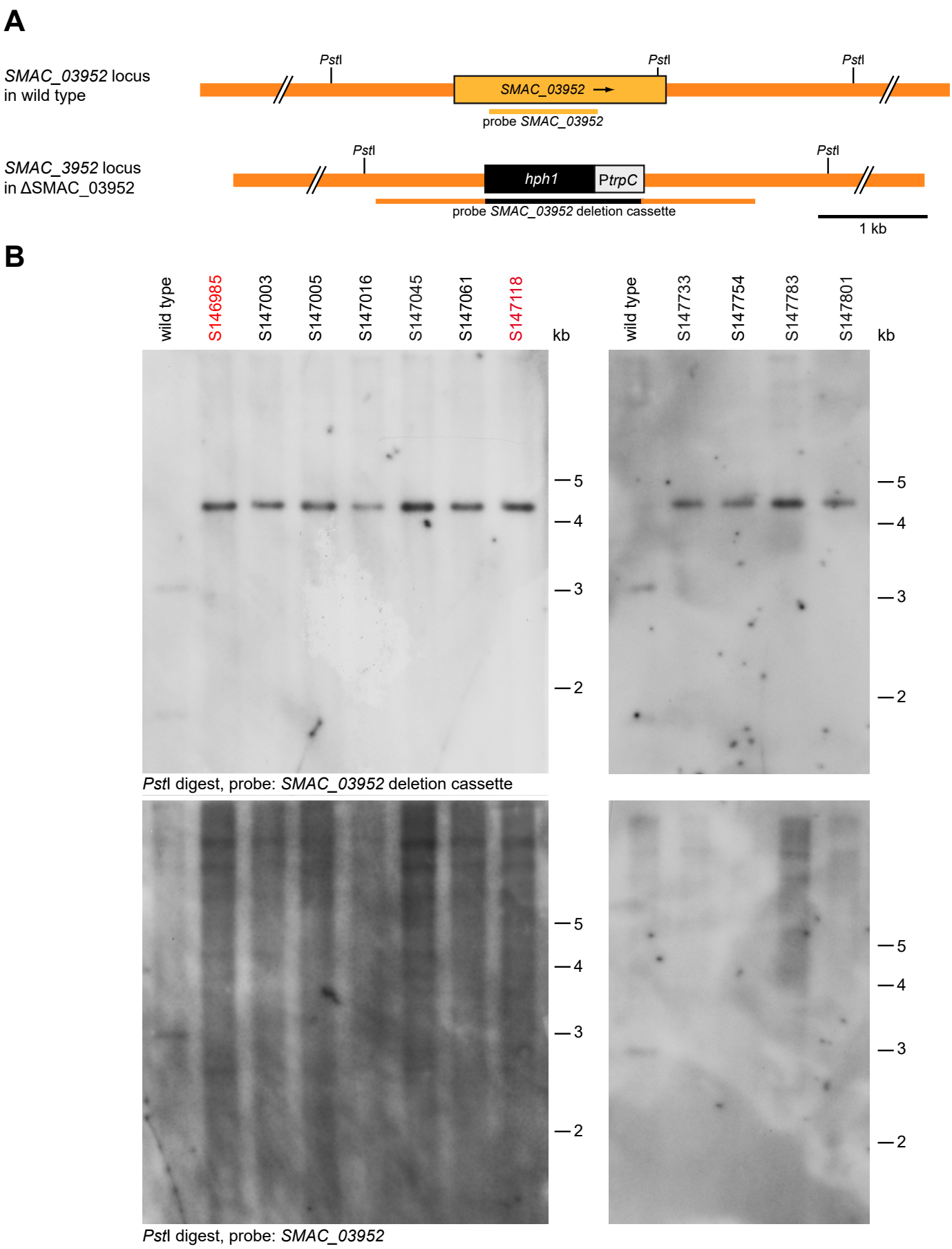


Figure S1. Southern blot analysis of Δasm3 (= ΔSMAC_03952) deletion strains. **A.** Overview of the *SMAC_03952* (*asm3*) genomic locus in the wild type and the corresponding deletion mutant, with the probes for the Southern blot indicated. **B.** Southern blot analysis of the wild type and eleven single spore isolates of two different independent primary transformants after digestion of genomic DNA with *Pst*I. The blots were probed with the indicated probes. The resulting signals are as expected for the *SMAC_03952* deletion for the single spore isolates (4.4 kb band when probed with the deletion cassette, and no signal when probed with the gene-specific probe, whereas the wild type gives bands of 1.8 and 3.0 kb when probed with the deletion cassette, and a 3.0 kb band with the gene-specific probe). Deletion strains that were used in this study are labelled in red.

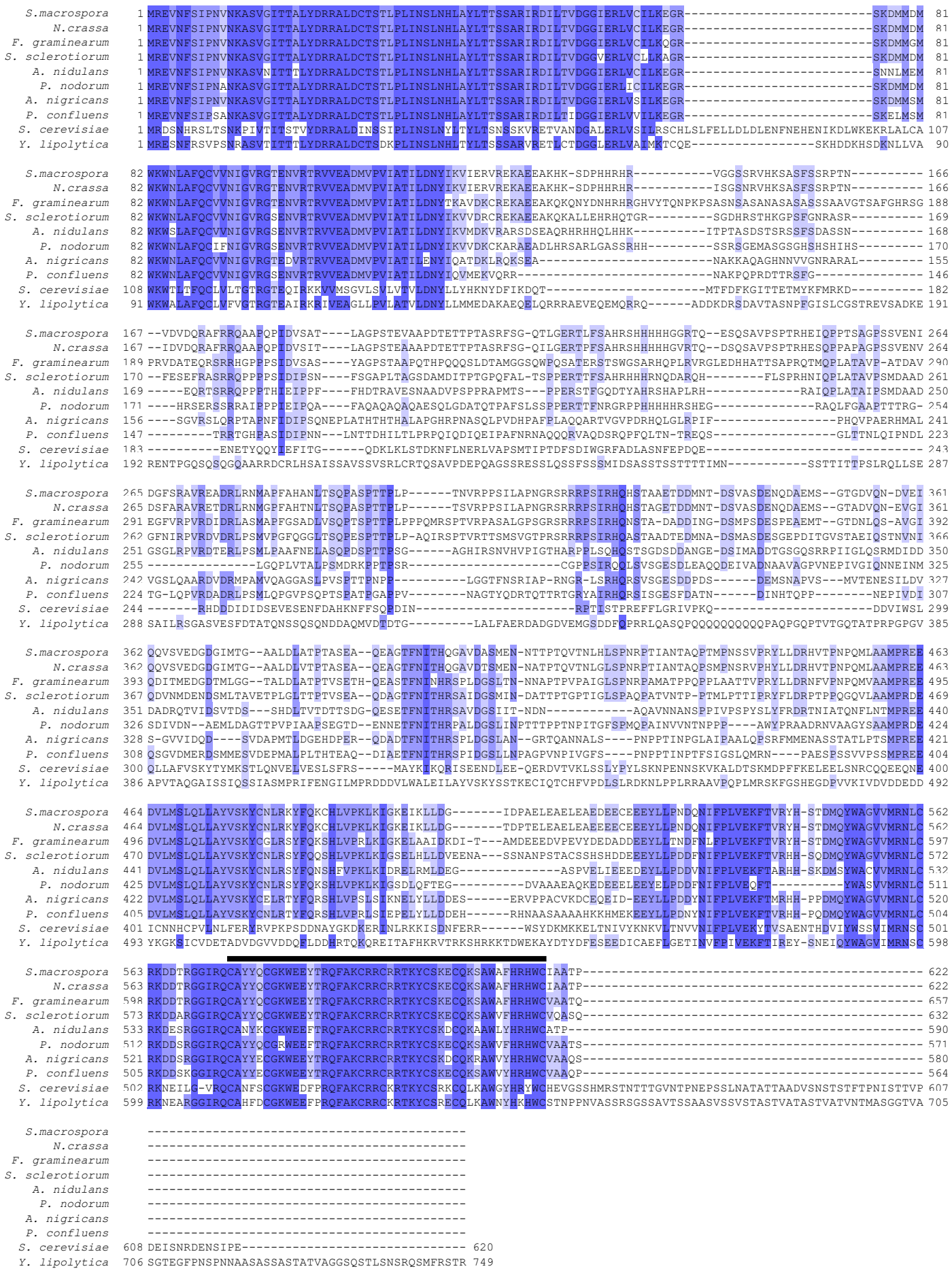


Figure S2. Multiple alignment of ASM3 orthologs from several ascomycetes. Orthologs were determined by bidirectional BLASTP analyses. The following proteins were used (in the order they appear in the alignment): *Sordaria macrospora* ASM3 (SMAC_03952.3, XP_003348106.1), *Neurospora crassa* (XP_960655.1), *Fusarium graminearum* (XP_011327228.1), *Sclerotinia sclerotiorum* (XP_001587555.1), *Aspergillus nidulans* (SamB, XP_657682.1), *Parastagonospora nodorum* (XP_001806167.1), *Ascodesmis nigricans* (TGZ80500.1), *Pyronema confluens* (CCX09978.1), *Saccharomyces cerevisiae* (Mub1p, NP_013818.1), *Yarrowia lipolytica* (XP_503528.1). The zf-MYND (MYND finger) domain is indicated by a black bar above the sequences.

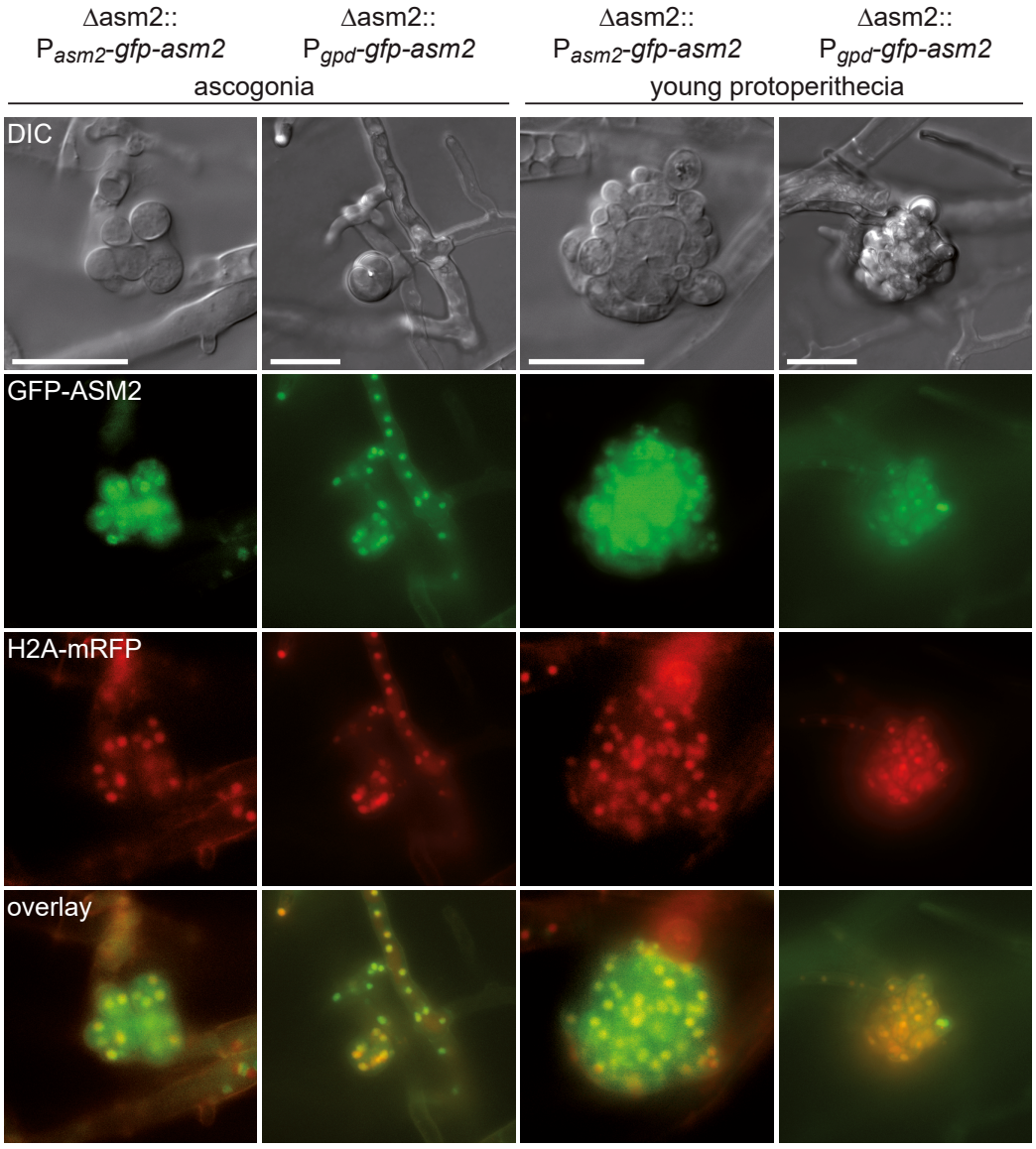
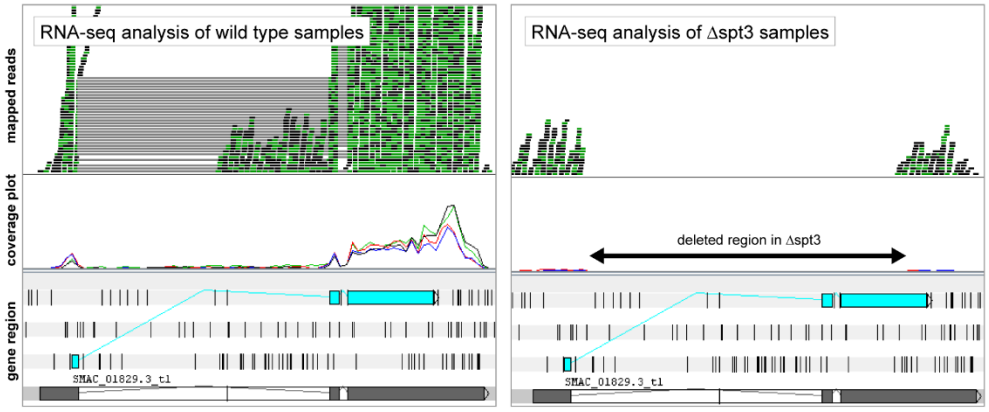
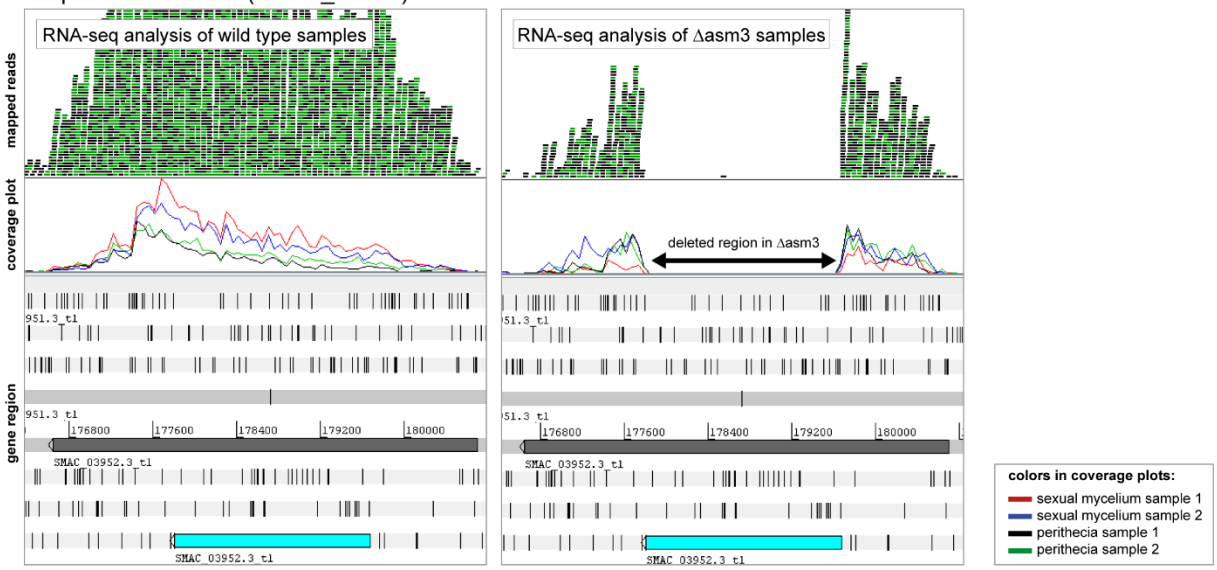


Figure S3. Localization of N-terminal GFP-tagged ASM2. Co-localization studies with histone H2A-mRFP revealed the nuclear localization of ASM2 ascogonia and young protoperithecia. Scale bars 20 µm

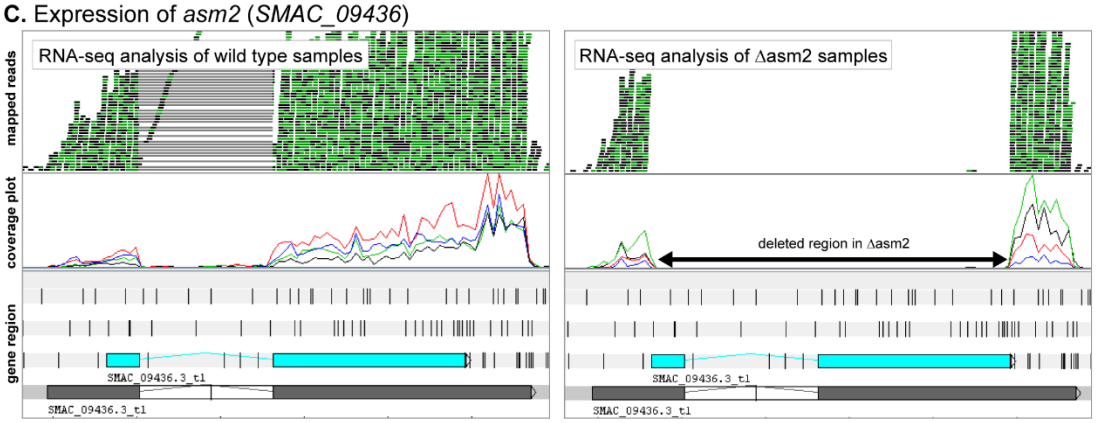
A. Expression of *spt3* (*SMAC_01829*)



B. Expression of *asm3* (*SMAC_03952*)



C. Expression of *asm2* (*SMAC_09436*)



D. Expression of *asm2*, *asm3*, and *spt3* in different strains and conditions.

	Δasm2	Δasm3	Δspt3	WT	Δasm2	Δasm3	Δasm2	Δasm3
	mycelium			perithecia			perithecia	
	vs. WT mycelium			vs. WT mycelium			vs. WT perithecia	
asm2	-2.90	**-1.12**	**-0.50**	**-0.20**	-1.54	**0.10**	-1.33	**0.31**
asm3	**0.06**	-3.37	**-0.97**	**-0.48**	**0.04**	-2.57	**0.51**	-2.09
spt3	**0.15**	**0.10**	-3.48	**0.89**	**0.19**	**-0.04**	**-0.70**	**-0.93**

Figure S4. RNA-seq results of *asm2*, *asm3*, and *spt3* expression. **A-C.** RNA-seq reads do not map to deleted regions of *asm2*, *asm3* and *spt3* in the corresponding deletion mutants. The figure shows the gene regions of *spt3* (A), *asm3* (B) and *asm2* (C) in the genome browser Artemis (Carver et al. Bioinformatics 2012, 28:464-9). Within the gene region, the forward DNA strand and the three forward open reading frames (A, C) or both DNA strands and all six open reading frames (B) are shown with vertical black lines indicating stop codons within the open reading frames. The annotated coding sequences are given in blue, the annotated mRNAs are given in grey. The coverage plot shows the coverage for all samples derived from the indicated strain. Within the mapped reads, split reads (indicating intronic regions) are interrupted by grey horizontal bars. The region that was deleted in the deletion mutants is indicated by a double arrow. Please note that in case of *spt3*, the coding region was not deleted completely (Lütkenhaus et al. 2019, Genetics 213: 1545-1563). As expected, read coverage of the deleted regions is lost in the corresponding mutants. However, RNA-seq reads can still be derived from the 5' and 3' regions that were not deleted in the mutant strains. **D.** Expression of *asm2*, *asm3*, and *spt3* in different strains and conditions. Log_2-fold changes in gene expression for the indicated comparisons are given. Numbers in grey indicate expression values for a gene in the corresponding deletion mutant, which are derived from reads mapping to the remaining 5' and 3' regions of the transcript as indicated in A-C and do not actually indicate expression of a functional gene. None of the genes was significantly differentially expressed in any of the analyzed conditions.

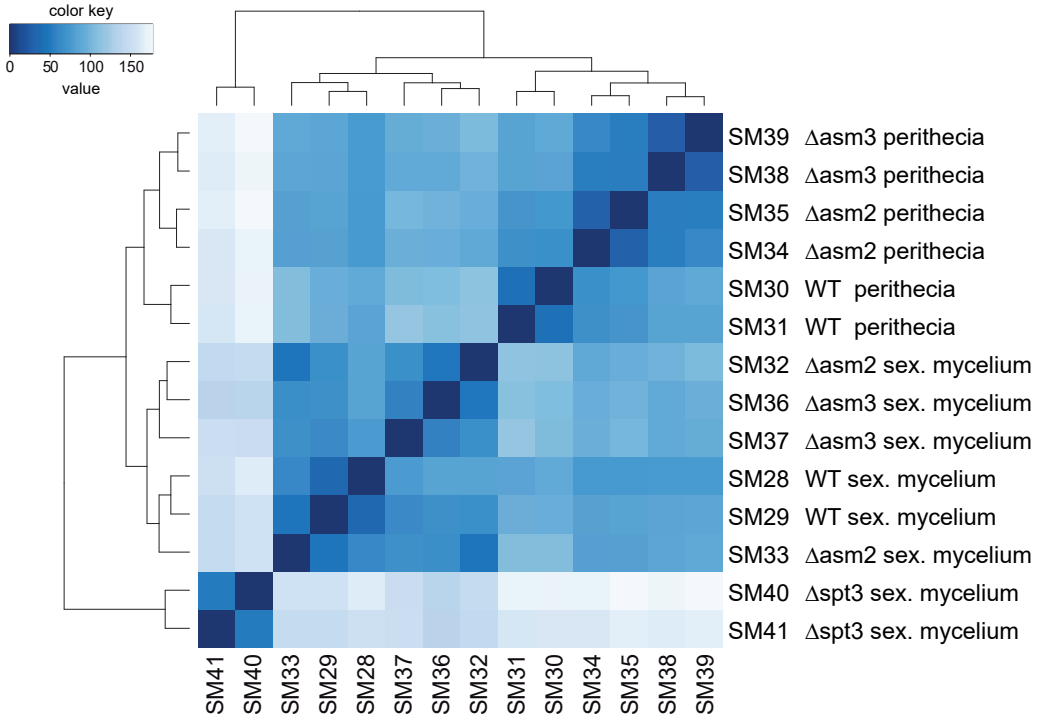


Figure S5. Heatmap of Euclidean distance between normalized read counts from RNA-seq samples (variance-stabilized data, analysis was performed with DESeq2). There are two independent biological replicates per condition. Except for the two replicates of sexual mycelium from Δasm2 (samples SM32 and SM33), the two independent samples from each condition cluster together. The two samples from Δspt3 cluster separately from all other samples.

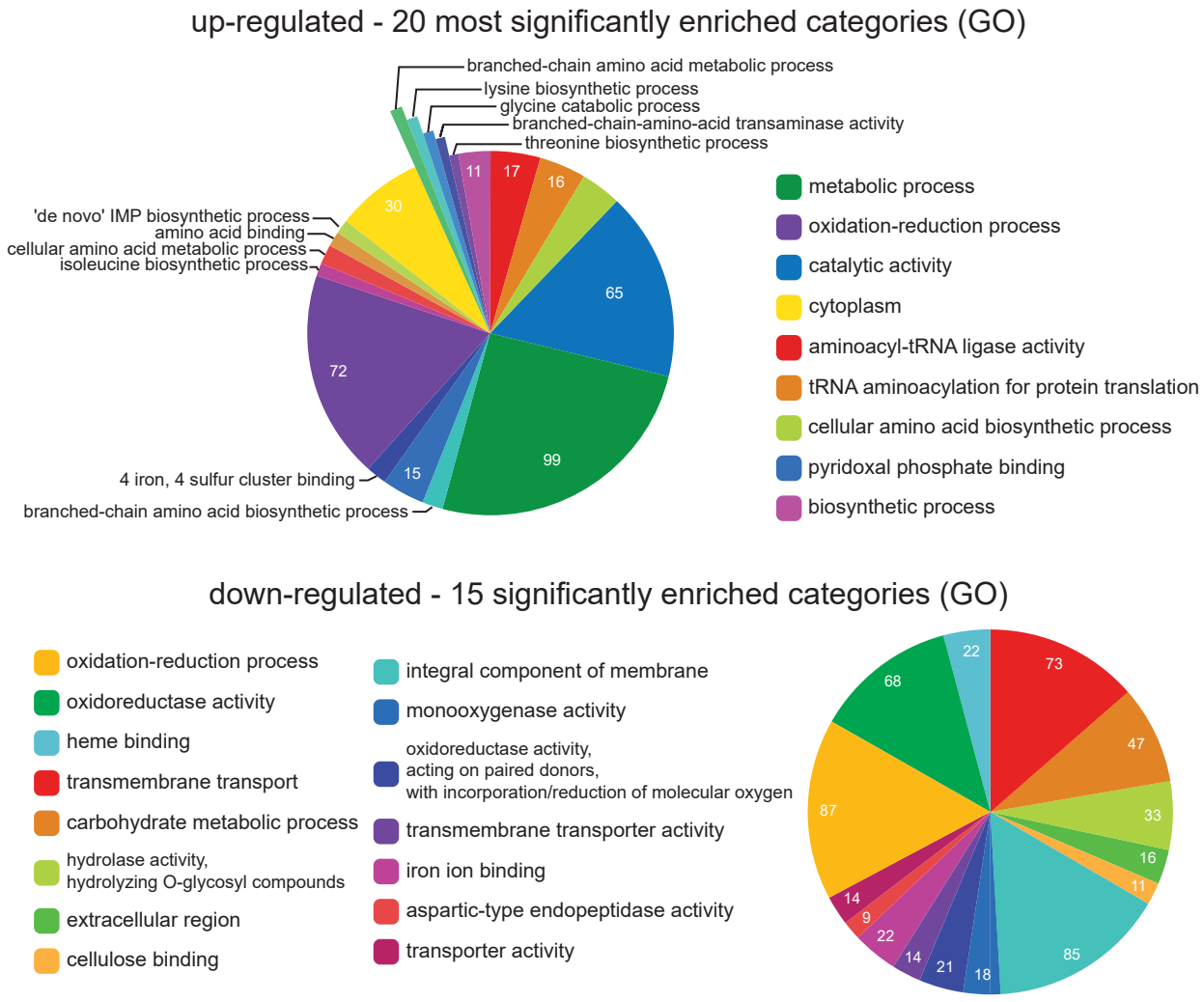


Figure S6. Pie charts representing results of gene ontology (GO) analysis of genes that are differentially regulated in Δspt3. Differentially up- and down-regulated genes were significantly enriched in 20 and 15 different categories, respectively. In both cases, mainly categories of primary metabolism were enriched-.Numbers in pie chart sections give the number of genes that belong to each category (a gene can belong to more than one category).

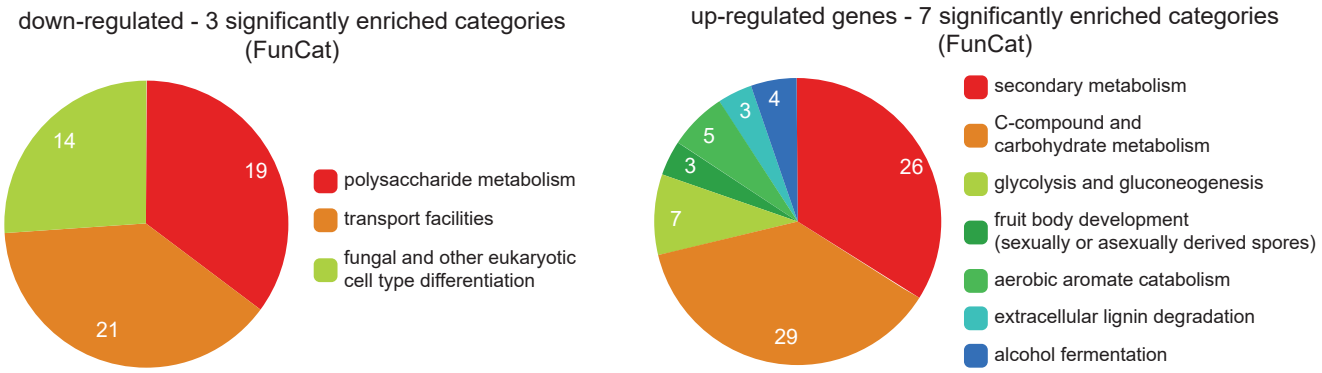
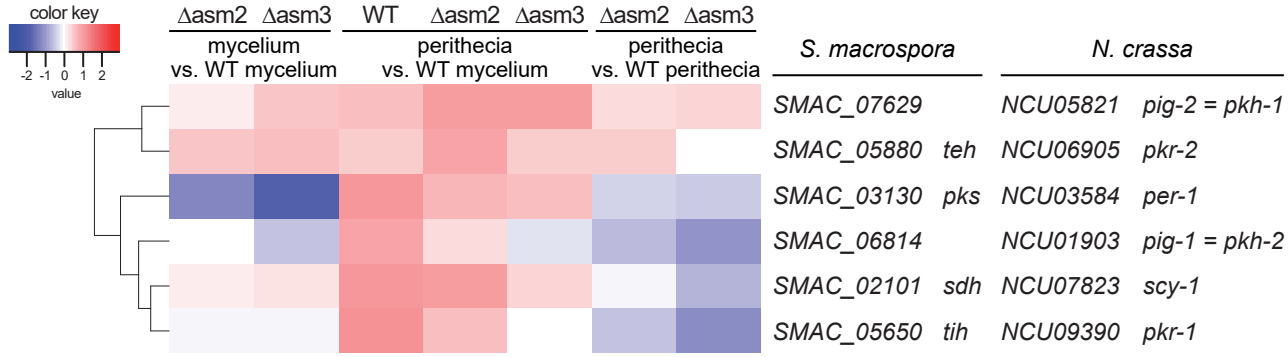


Figure S7. Pie charts of significantly enriched categories for differentially regulated genes in wild type perithecia. Functional category (FunCat) analysis was performed with the corresponding proteins of down- and up-regulated genes, respectivley, in wild type perithecia. Numbers in pie chart sections give the number of genes that belong to each category (a gene can belong to more than one category).

A



B

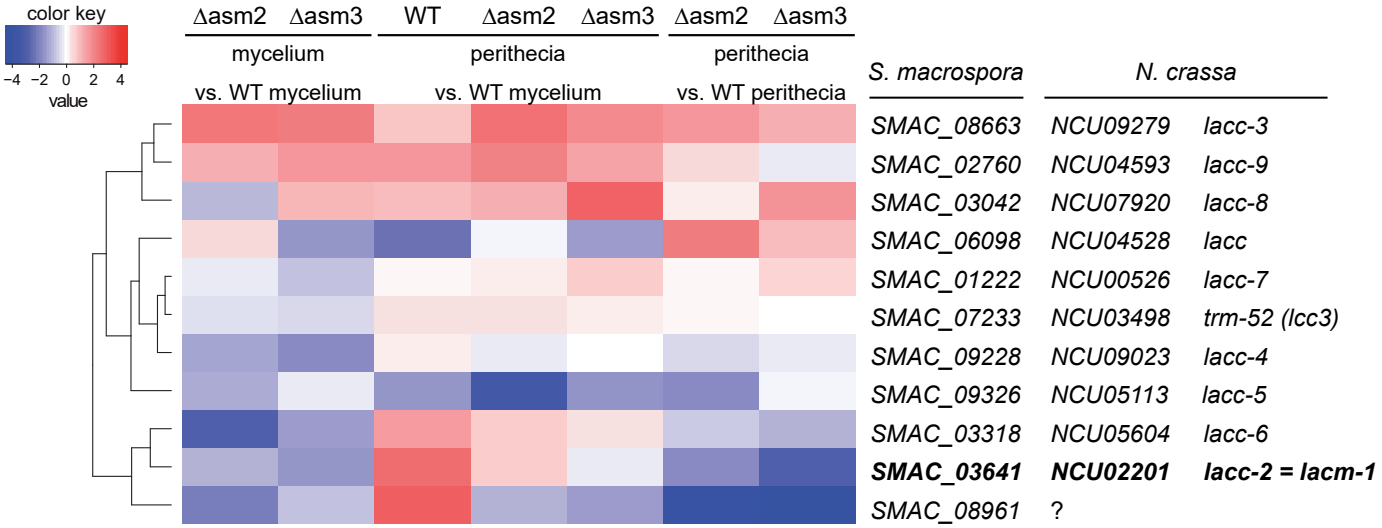


Figure S8. Heatmaps of expression of melanin biosynthesis genes **(A)** and laccase genes **(B)** in Δasm2 and Δasm3 mutants. *S. macrospora* locus tags and gene names (if available) as well as locus tags and gene names of *N. crassa* orthologs are given on the right side. The DESeq2 comparisons are listed above the heatmaps. The putative laccase gene *SMAC_03641*, whose ortholog *NCU02201* is involved in melanin biosynthesis (Ao et al. 2019, Fungal Biol. 123:1-9) is indicated in bold in B.

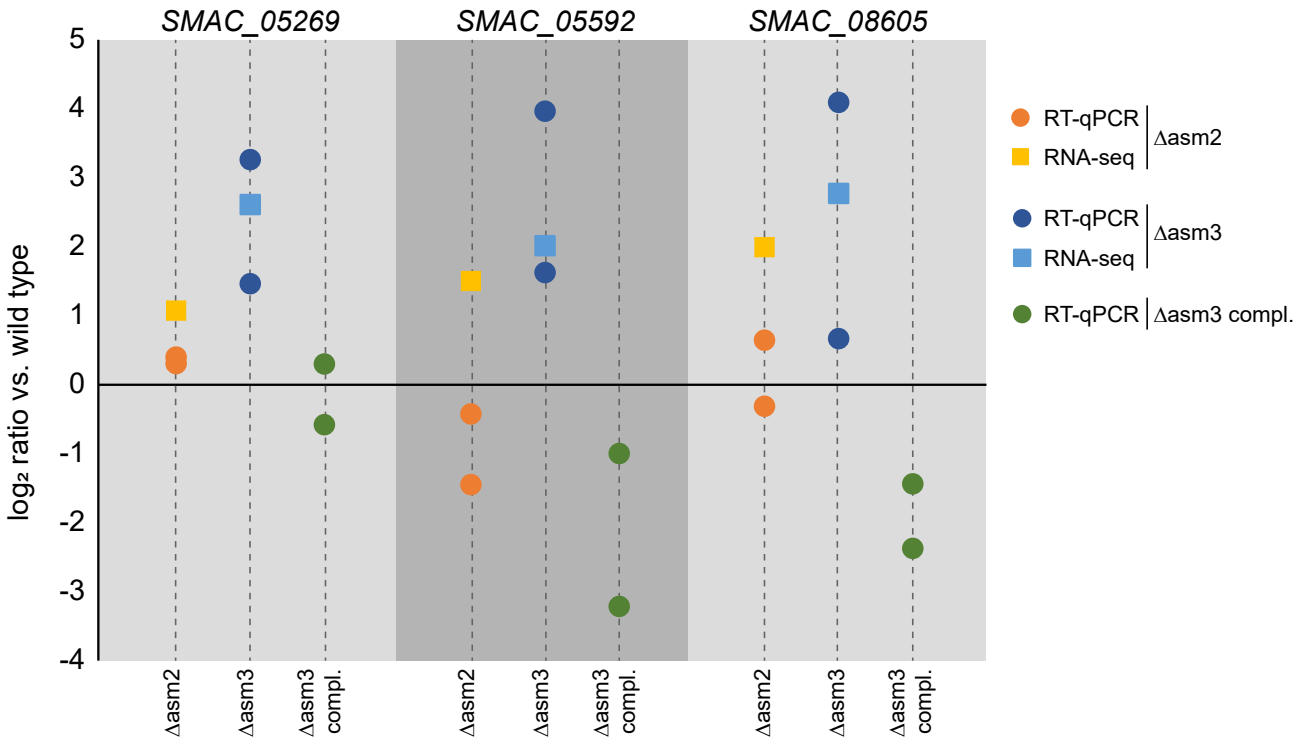


Figure S9. Expression of orthologs to *N. crassa* MSUD genes *sad-1*, *sad-2*, and *sms-2* in perithecia of *S. macrospora*. Transcript levels in perithecia of the Δasm2 and Δasm3 mutants as well as a complemented Δasm3 strain expressing *asm3* under a constitutive promoter (Δasm3 compl.) were compared to transcript levels in wild type perithecia by RNA-seq or RT-qPCR for the three genes *SMAC_05269* (*sad-1* ortholog), *SMAC_05592* (*sad-2* ortholog), and *SMAC_08605* (*sms-2* ortholog). Data points give results of two biologically independent RT-qPCR experiments as well as RNA-seq data for the deletion mutants. Strains used in the experiments were S148734 (Δasm2), S146985 (Δasm3) and 6.3 AS2 (Δasm3 compl.). For *SMAC_05269* and *SMAC_05592*, mean ratios are significantly larger (t-test at $p<0.05$) in Δasm3 compared to the wild type as well as compared to Δasm2 and the complemented Δasm3 transformant. For *SMAC_08605*, the difference is statistically significant between Δasm3 and the complemented transformant, but not between Δasm3 and Δasm2.

Table S1. Oligonucleotides used in this study.

Name	Sequence	Remarks
SMAC_03952-ko1	gtaacgccagggttttcccagtcacgacgggatcccatcagatttgacccgagggcggtc	amplification of upstream region of *asm3* for cloning of deletion vector
SMAC_03952-ko2	cgagggcaaaggaatagggttccgttgaggcttggcttgccaccacgagtgtcga	amplification of upstream region of *asm3* for cloning of deletion vector
SMAC_03952-ko3	gcccaaaaatgctccttcaatatcagttgctttggacgtttacggaacgatatcc	amplification of downstream region of *asm3* for cloning of deletion vector
SMAC_03952-ko4	gcggataacaatttcacacaggaaacagcggatccttgtggtcctccaacattggttctc	amplification of downstream region of *asm3* for cloning of deletion vector
HR-P3952_fw	tgagcgcgcgtaatacgactcactatagcttcgatggctggatagcaaacgtcc	cloning of *asm3* complementation vector
HR-T3952_rv	catgattacgccaagcgcgcaattaacggacttgctagagccaagtcgagtcg	cloning of *asm3* complementation vector
SMAC_03952-ORF2	tgtttgatgatttcagtaacgttaagttcatggcgtggcggcaatgcaccagtgg	cloning of *asm3* complementation vector
SMAC_03952-ORF3	cgcagcttgactaacagctacagatctatgagggaagtcaacttcagcataccca	cloning of *asm3* complementation vector
HR-3952.3_int_rv_1	acgagtgtcgaggtaggcaccagacg	sequencing of *asm3* complementation vector
HR-3952.3_int_rv_2	atgccgctccaaccactttaagctcc	sequencing of *asm3* complementation vector
SMAC_03952-ver1	cccctcctggcctctggcctctcc	verification of homologous integration of *asm3* deletion vector or sequencing
SMAC_03952-ver2	ctgttcgtgtcgatggttgagttg	verification of homologous integration of *asm3* deletion vector
SMAC_03952-ver3	cgtggtcaacattggtgtaagagg	verification of homologous integration of *asm3* deletion vector or sequencing
SMAC_03952-ver4	gatgaaggttggtgacttgagtgg	verification of homologous integration of *asm3* deletion vector or sequencing
pN03952seq1	accgaagattggcgattgg	sequencing of *asm3* complementation vector
pN03952seq2	cgtgtcacggtacgaccac	sequencing of *asm3* complementation vector
pN03952seq3	gcttggggacaaggtggcatt	sequencing of *asm3* complementation vector
pN03952seq4	attgccgccacgccatgatt	sequencing of *asm3* complementation vector
pN03952seq5	gcatcatcgggttggcgttc	sequencing of *asm3* complementation vector

Name	Sequence	Remarks
09436-N-GFP_fw	catggacgagctgtacaagagcggc cgcatgtcgtcggtcgttaggtc	cloning of *asm2* complementation vector
09436-N-GFP_bw	ggatccactagttctagagcggccg ctatacaccaccgcccatgaa	cloning of *asm2* complementation vector
HR-9436_-FT_fw	atcaacaagaccctcccagcatgcg tcgcccctgttgtcgttagaaccat tggtggcgcc	cloning of *asm2* complementation vector with partially deleted fungal specific TF domain
HR-9436_-FT_rv	cgccaccaatggttctaacgacaac aggggcgacgcatgctgggagggtc ttgttgat	cloning of *asm2* complementation vector with partially deleted fungal specific TF domain
Sm-9436.3_int2fw	ctgctccaacggtagttgc	sequencing of *asm2* complementation vector
Seq_9436-i_fw	tagcctggaaaactttgc	sequencing of *asm2* complementation vector
Seq_9436-i_rv	aagctggcaatgtcaatgtg	sequencing of *asm2* complementation vector
n9346_rv	ggcgaagatacgatcgacgg	sequencing of *asm2* complementation vector
dSMU_1	cgatggctgtgtagaagtactcgc	sequencing of deletion vector or verification of homologous integration
dSMU_5	gtgttgacctccactagctccagc	sequencing of deletion vector or verification of homologous integration
1751	gccatattttcctgctctcc	sequencing of complementation vectors
1757	agctgacatcgacaccaacg	sequencing of complementation vectors
egfp-fw	ggtgaacttcaagatccg	sequencing of complementation vectors
426-14	ttaagttgggtaacgccagg	sequencing of complementation and deletion vectors
426-15	ttgtgtggaattgtgagcgg	sequencing of complementation and deletion vectors
SMAC_05269_for	TTCGGCGGCTGATAGTGGTG	for RT-qPRC
SMAC_05269_rev	TGGGCTCTCAGACTGCGAAG	for RT-qPRC
SMAC_05592_for	acgggcatgtaccatcctcg	for RT-qPRC
SMAC_05592_rev	cccgtacgatgaagtgggct	for RT-qPRC
SMAC_08605_for	TGGCAAGCAACAGCCTGATG	for RT-qPRC
SMAC_08605_rev	AGCGGCCCAAGATTAGACCC	for RT-qPRC
SMAC_08534_for	ATTGACCAGATCCGCGTCGT	for RT-qPRC
SMAC_08534_rev	TGTCTCCGAACAGCCAACCA	for RT-qPRC
SSU1	ATCCAAGGAAGGCAGCAGGC	for RT-qPRC
SSU2	TGGAGCTGGAATTACCGCG	for RT-qPRC

Table S2. SAGA complex subunits in *S. macrospora* determined by bi-directional BLASTp analysis. Published SAGA complex subunits of *S. cerevisiae* and *A. nidulans* were used to identify orthologous proteins in *S. macrospora* by BLASTp. Rows highlighted in grey represent results of DELTA-BLASTp, since BLASTp did not reveal an ortholog. The homolog for SMAC_01263 was found only in a BLAST search with the corresponding *A. nidulans* protein. The identity values in the last column give the identity of the *S. macrospora* and *S. cerevisiae* proteins except for SMAC_01263, where the identity to the *A. nidulans* protein is given. No *S. macrospora* homolog was identified for the *S. cerevisiae* Sus1p protein. However, the Sus1p protein is a rather small protein of 96 amino acids in yeast, therefore it is possible that a possible homolog was not annotated during the automated annotation of the *S. macrospora* genome. A TBLASTN search of the *S. macrospora* genome sequence using the *S. cerevisiae* Sus1p and *A. nidulans* AN7253 proteins as query also did not give any results, although for short proteins it is possible that the search might have been confounded by introns in the encoding gene (if one exists in *S. macrospora*).

Modul	*S. cerevisiae*[1]		*A. nidulans*[2]	e-value	*S. macrospora*	e-value	identity
HAT	Ada2p	YDR448W	AN10763 (AdaB)	1E-106	SMAC_01149	4.00E-109	210/504 (41%)
	Ada3p	YDR176W	AN0440	1E-46	SMAC_00331	2.00E-40	136/460 (29%)
	Gcn5p	YGR252W	AN3621 (GcnE)	2,00E-135	SMAC_00218	0	232/352 (65%)
	Sgf29p	YCL010C	AN0668	1,00E-23	SMAC_06921	2.00E-07	41/120 (34%)
DUB	Sgf11p	YPL047W	AN8685	9,20E-02	SMAC_01263	4,00E-54	97/246(39%)
	Sgf73p	YGL066W	AN11747	1,00E-17	SMAC_01972	5.00E-16	37/60 (61%)
	Sus1p	YBR111W-A	AN7253	3,80E-02	no homolog	-	-
	Ubp8p	YMR223W	AN3711	1,00E-56	SMAC_03509	2.00E-48	115/364 (31%)
Spt	Ada1p	YPL254W	AN10953	2,00E-17	SMAC_05567	7.00E-17	2267/250 (26%)
	Spt3p	YDR392W	AN0719 (SptC)	2,00E-75	SMAC_01829	1.00E-71	154/346 (44%)
	Spt7p	YBR081C	AN4894	1,00E-89	SMAC_03720	2.00E-51	107/255 (41%)
	Spt8p	YLR055C	AN4670 (AcdX)	4,00E-46	SMAC_00178	9.00E-48	85/199 (42%)
	Spt20p	YOL148C	AN0976 (RefE)	1,00E-08	SMAC_07789	2,00E-18	57/332(17%)
	Tra1p	YHR099W	AN8000	0	SMAC_04066	0	1184/3292 (35%)
Taf	Taf5p	YBR198C	AN0292	8,00E-115	SMAC_04450	6.00E-106	212/702 (30%)
	Taf6pp	YGL112C	AN8232	1,00E-90	SMAC_05148	6.00E-93	163/405 (40%)
	Taf9p	YMR236W	AN0794	3,00E-30	SMAC_02869	9.00E-24	60/143 (41%)
	Taf10p	YDR167W	AN0154	4,00E-14	SMAC_00049	2.00E-21	60/156 (38%)
	Taf12p	YDR145W	AN2769	1,00E-28	SMAC_03663	3.00E-12	67/189 (35%)
	Chd1p	YER164W	AN1255	0	SMAC_02791	0	484/1124 (43%)

[1] Setiaputra et al. (2015) [2] Georgakopoulos et al. (2013)

Georgakopoulos, P., R.A. Lockington, and J.M. Kelly, 2013 The Spt-Ada-Gcn5 Acetyltransferase (SAGA) Complex in *Aspergillus nidulans*. *PLoS One* 8 (6):e65221.

Setiaputra, D., J.D. Ross, S. Lu, D.T. Cheng, M.Q. Dong *et al.*, 2015 Conformational flexibility and subunit arrangement of the modular yeast Spt-Ada-Gcn5 acetyltransferase complex. *The Journal of biological chemistry* 290 (16):10057-10070.

Table S4. *N. crassa* orthologs of 19 down-regulated genes in Δspt3 leading to a developmentally relevant phenotype upon deletion. The table gives the *N. crassa* gene ID followed by its name, and resulting phenotype regarding the sexual development. Gene IDs labelled in green indicate that the corresponding mutants do not form perithecia, gene IDs in orange indicate that no ascospores are formed, gene IDs in orange indicate other developmental phenotypes. The last two columns give the *S. macrospora* gene locus tag of the corresponding ortholog (determined by BLASTp) as well as its (putative) product or conserved domain.
NA = not applicable; NF = not formed

gene ID	gene name	mating type	Proto. no.	proto. morphology	perith. no.	perith. morphology	ascospore no.	ascospore morphology	locus tag	product description / domain
NCU01386	*ada-10*[1]	mat a	normal	smaller size	**NF**	NA	**NF**	NA	*SMAC_08362*	GAL4 TF; fungal_TF_MHR
NCU01706	*vsd-6*[1]	mat a	normal	normal	**NF**	NA	**NF**	NA	*SMAC_02413*	PRO46; MYB DNA binding TF
NCU03868		N/A	normal	normal	**NF**	NA	**NF**	NA	*SMAC_06291*	NADPH oxidase (NOX)
NCU05758	*pre-2*[2]	mat A	normal	normal	normal	normal	normal	normal	*SMAC_08994*	PRE2
		mat a	increased	normal	**NF**	NA	**NF**	NA		
NCU07617	*acon-3*[3]	N/A	normal	normal	**NF**	NA	**NF**	NA	*SMAC_07309*	putative ACON3
NCU09915	*fsd-1*[1]	N/A	normal	normal	**NF**	NA	**NF**	NA	*SMAC_01666*	putative NDT80; TF
NCU00097	*bek-1*[1]	mat a	normal	normal	normal	no beaks	**NF**	NA	*SMAC_08102*	HOX TF
NCU00499	*ada-1*[4]	mat a	reduced	normal	reduced	no beaks	**NF**	NA	*SMAC_00439*	bZIP TF
NCU01451		mat a	normal	normal	normal	abnormal beaks	**NF**	NA	*SMAC_07019*	hypothetic protein
NCU07530	*trm-35*[*]	N/A	normal	normal	normal	normal	**NF**	NA	*SMAC_09388*	putative transporter SMF2
NCU07874		N/A	reduced	normal	normal	abnormal beaks	**NF**	NA	*SMAC_08416*	RRM1_PUB1 domain
NCU01269	*div-5*[*]	N/A	normal	normal	reduced	normal	**NF**	NA	*SMAC_05745*	CDC20/HCT1 protein
NCU06390		N/A	normal	normal	normal	normal	**NF**	NA	*SMAC_00965*	PAS *superfamily* domain
NCU08634	*vsd-1*[1]	mat a	normal	normal	reduced	normal	**NF**	NA	*SMAC_09090*	Forkhead TF
NCU09064	*stk-53*[5]	N/A	normal	normal	normal	normal	**NF**	NA	*SMAC_05225*	putative STK53, kinase
NCU01093	*stp-3*[*]	N/A	normal	smaller size	normal	normal	normal	normal	*SMAC_03168*	putative S/T phosphatase
NCU16491	*tcf-20*[1]	mat a	normal	normal	normal	normal	normal	normal	*SMAC_04819*	GAL4 TF, fungal_TF_MHR
		mat A	normal	smaller size	normal	normal	normal	normal		
NCU00786	*gpr-1*[2]	mat a	normal	normal	normal	abnormal beaks	normal	normal	*SMAC_01468*	G-Protein coupled receptor
NCU09427	*gpr-3*[2]	mat a	normal	normal	normal	abnormal beaks	normal	normal	*SMAC_05685*	G-Protein coupled receptor

[*] https://fungidb.org/fungidb/; [1] Carrillo et al. (2017); [2] Cabrera et al. (2015); [3] Chung et al. (2011); [4] Tian et al. (2011); [5] Park et al. (2011)

Cabrera, I.E., I.V. Pacentine, A. Lim, N. Guerrero, S. Krystofova *et al.*, 2015 Global analysis of predicted G protein-coupled receptor genes in the filamentous fungus, *Neurospora crassa*. *G3 (Bethesda)* 5 (12):2729-2743.

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