

## **Supplementary data for**

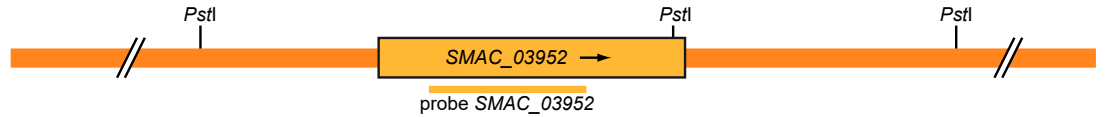
### **Functional characterization of the developmental genes *asm2*, *asm3*, and *spt3* required for fruiting body formation in the filamentous ascomycete *Sordaria macrospora***

by Ramona Lütkenhaus, Jan Breuer, and Minou Nowrousian

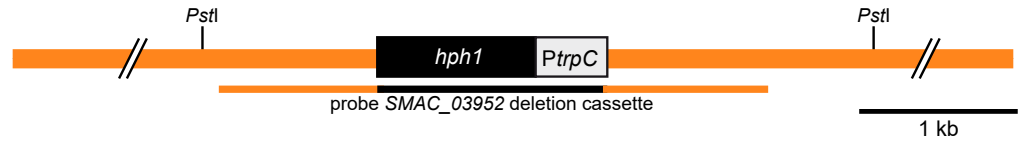
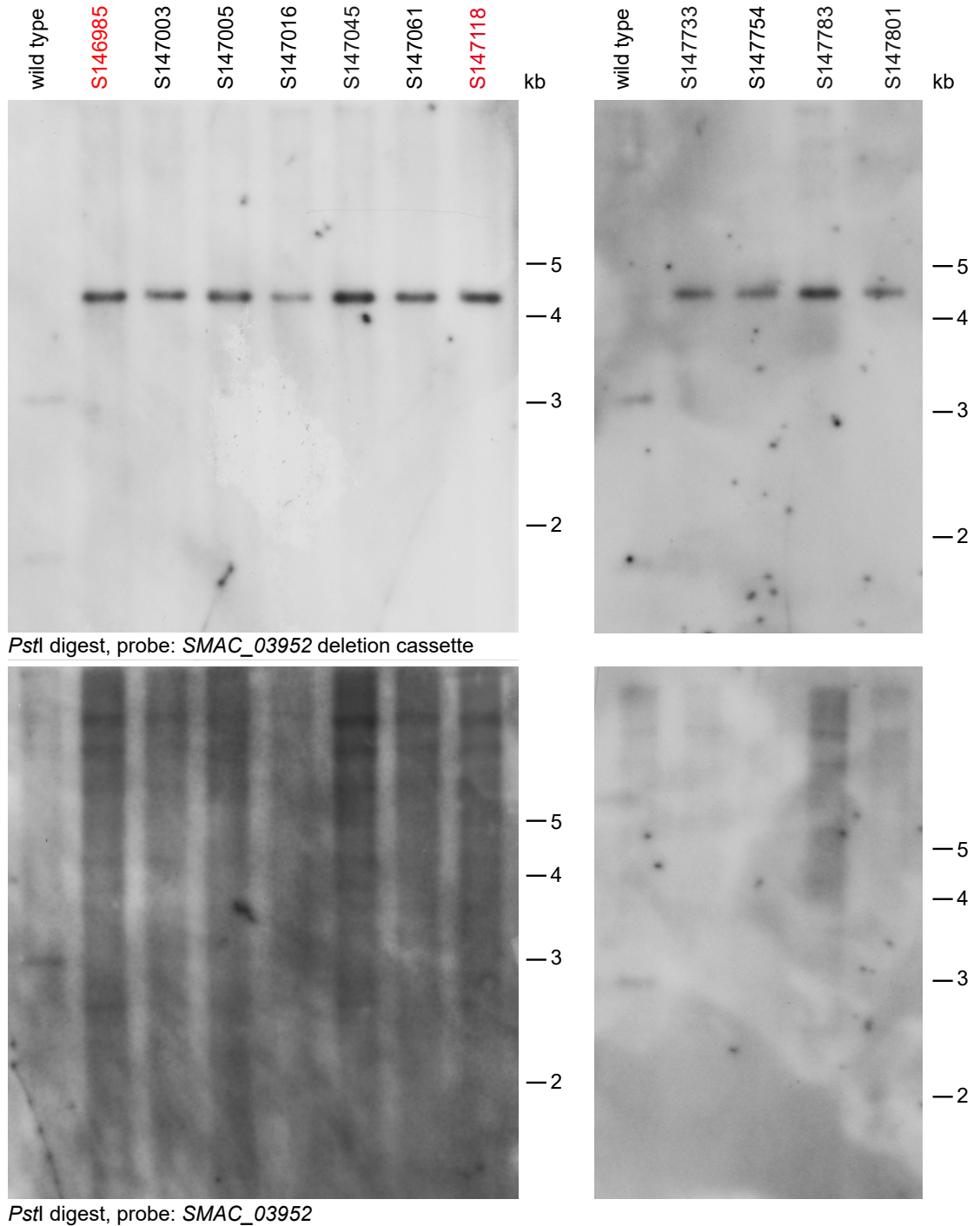
This file contains supplementary figures S1-S9 and supplementary tables S1, S2, and S4.  
Supplementary tables S3 and S5 are given as separate excel files.

**A**

*SMAC\_03952* locus  
in wild type



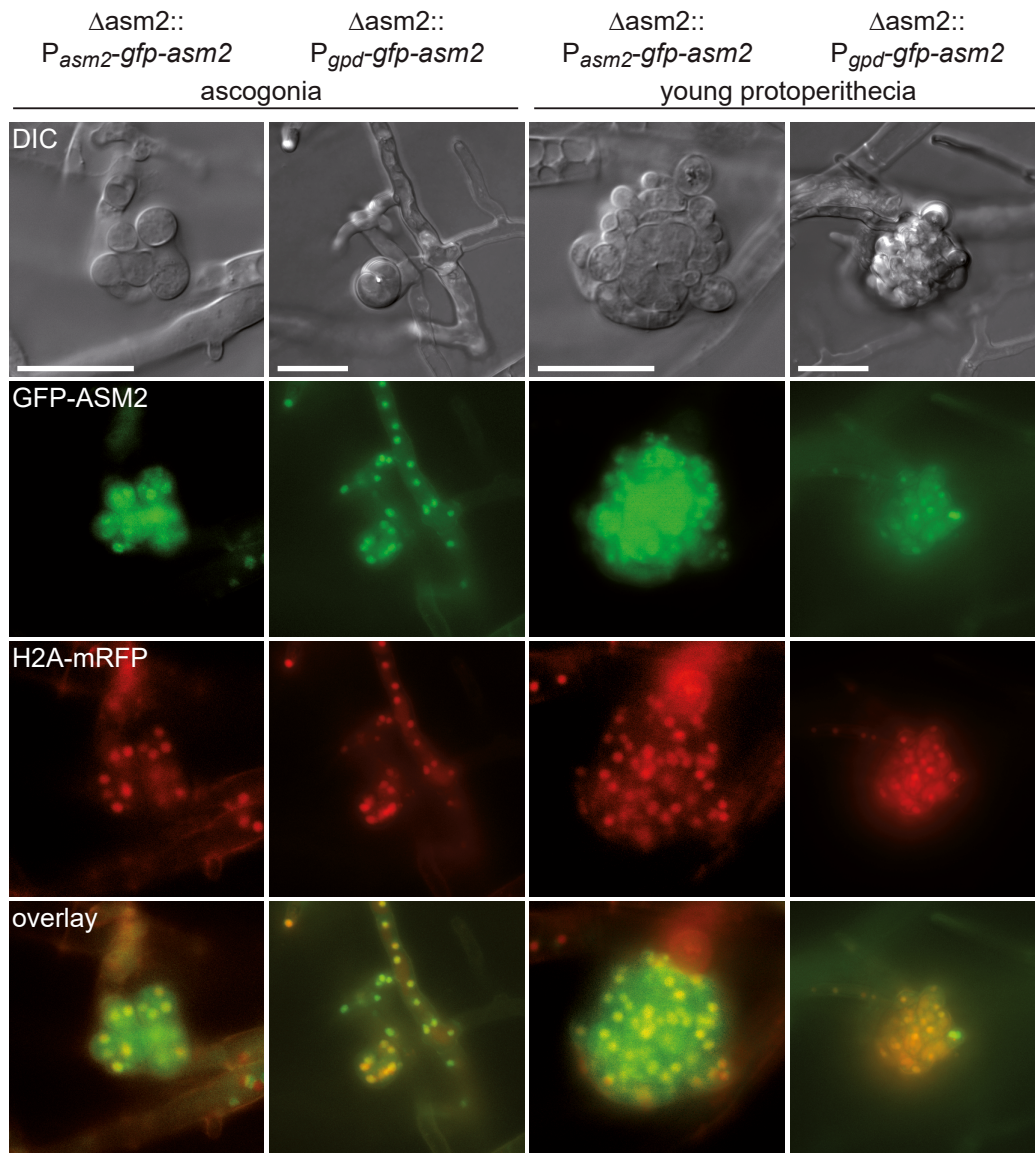
*SMAC\_3952* locus  
in  $\Delta$ *SMAC\_03952*

**B**

**Figure S1.** Southern blot analysis of  $\Delta$ *asm3* (=  $\Delta$ *SMAC\_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 *PstI*. 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.

<i>S. macrospora</i>	1	MREVNFSIPNVNKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKEGR-----SKDMMMDM	81
<i>N. crassa</i>	1	MREVNFSIPNVNKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKEGR-----SKDMMMDM	81
<i>F. graminearum</i>	1	MREVNFSIPNVNKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKQGR-----SKDMMMDM	81
<i>S. sclerotiorum</i>	1	MREVNFSIPNVNKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGVVERLVCILKAGR-----SKDMMMDM	81
<i>A. nidulans</i>	1	MREVNFSIPNVNKASVNITTTLYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKEGR-----SNNLMEM	81
<i>P. nodorum</i>	1	MREVNFSIPNANKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKEGR-----SKDMMMDM	81
<i>A. nigricans</i>	1	MREVNFSIPNVNKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKEGR-----SKDMMMDM	81
<i>P. confluens</i>	1	MREVNFSIPNANKASVGITTALYDRRALDCTSTLPLINSLNHLAYLTTSSARIRDILTVDGGIERLVCILKEGR-----SKELMSM	81
<i>S. cerevisiae</i>	1	MRDSNHRSLTSNKPVIITTTSTVYDRRALINSLPLINSNLTYLTLDSNKGVRETAVNDGALERLVLSTLRSCHLISLFEILLDDLENFNEHENIKDLWEKRLALCA	107
<i>Y. lipolytica</i>	1	MRESNFRSVPSNRSASVTITTTLYDRRALDCTSDKPLINSLNHLTYLTLSSARVREITCTDGGGLERLVAIMKTCQE-----SKHDDKHSDNLLVA	90
<i>S. macrospora</i>	82	WKWNLAFCQVNVNIGVRGTENVTRVVEADMVPIATILDNLIKVIERVREKAEAKHK--SDPHHRH-----VGGSSRVHKSASFSSSRPTN-----	166
<i>N. crassa</i>	82	WKWNLAFCQVNVNIGVRGTENVTRVVEADMVPIATILDNLIKVIERVREKAEAKHK--SDPHHRH-----ISGSNRVHKSASFSSSRPTN-----	166
<i>F. graminearum</i>	82	WKWNLAFCQVNVNIGVRGTENVTRVVEADMVPIATILDNLIKAVDKCKREKAEAKQKQYNDNHRHGHVYTQNPKPSASNSASANASASASSAAVGTSAFGHRSG	188
<i>S. sclerotiorum</i>	82	WKWNLAFCQVNVNIGVRGTENVTRVVEADMVPIATILDNLIKVVDRCKREKAEAKQKALLEHRRHQTGR-----SGDHRSTHKGPSFGNRASR-----	169
<i>A. nidulans</i>	82	WKWSLAFCQVNVNIGVRGSENVRTRVVEADMVPIATILDNLIKVMKDKVARSDEAQRRHRHQLHHK-----ITPTASDSTSRSSFSASSN-----	168
<i>P. nodorum</i>	82	WKWNLAFCQIFNIGVRGSENVRTRVVEADMVPIATILDNLIKVVDKCKARADLHRSARLGASSRHH-----SSRSGEMASGSGSHSHSHS-----	170
<i>A. nigricans</i>	82	WKWNLAFCQVNVNIGVRGTENVTRVVEADMVPIATILDNLIKVVDRCKREKAEAKQKALLEHRRHQTGR-----NAKKAQAGHNNVGNRRARAL-----	155
<i>P. confluens</i>	82	WKWNLAFCQVNVNIGVRGSENVRTRVVEADMVPIATILDNLIKQVMEKVRQR-----NAKPQPRDTRTSEF-----	146
<i>S. cerevisiae</i>	108	KKWILTLTFCQLTCTRGTEQIRKKVVMGVLVSLVTLVDNLYLHKNYDFIKDQT-----MTDFDKGITITETMYKFMRKD-----	182
<i>Y. lipolytica</i>	91	WKWLAFCQCLVFTCTRGTEAIRKRIVEAGLLVLATVLDNLYLLMDEDAKEQELQRRRAEVEQEQMQRQ-----ADDKDRSDAVTASNFGISLCGSTREVSADKE	191
<i>S. macrospora</i>	167	--VDVDQRAFRRQAAFPIDVSAT---LAGPSTEVAAPDTETPTTASRFSG--QTLGERTLFSAHRSHHHHHGGRTQ--ESQSAVPSPTRHEIQPPTSAAGPSSVENI	264
<i>N. crassa</i>	167	--IDVDQRAFRRQAAFPIDVSIT---LAGPSTEAAAPDTETPTTASRFSG--QILGERTLFSAHRSHHHHHGGVRTQ--DSQSAVPSPTRHESQPPAPAGPSSVENV	264
<i>F. graminearum</i>	189	PRVDATQRQRSHRGPSPSIDVSAS---YAGPSTAAPTQHPQQQSLDTAMGGSQWQSQSATERSTSWGSARHQPLRVRGLEDHHAATTSAPRQTMQPLATAVP--ATDAV	290
<i>S. sclerotiorum</i>	169	--FESEFASRRRQPPPSIDIPSN---FSGAPLTAGSDAMDITPTGPQFAL--TSPPERTTFSAHRRHHNRNQDARQH-----FLSPRHNIQPLATAVPSMDAAD	261
<i>A. nidulans</i>	169	-----EORTSRRRQPPPTHEIPFF---FHDTRAVESNAADVSPPRAPMTS---PPERSTGQDTYAHRSAPLRH-----RAIQPLATAVPSMDAAD	250
<i>P. nodorum</i>	171	-----HRSERSSRAIPPEIEIPA---FAQQAQAQAESQLGDATQTPAFSLSSPERTTNNRGRPPHHHHRSHEG-----RAQLFGAAPTTRTG--	254
<i>A. nigricans</i>	156	-----SGVRSRQRPATPNFIDIPSQNEPLATHTHALAPGHRPNASQLPVDHPAFPLAQQARTVGVPDRHQLGLRPIF-----PHQVPAERHMAAL	241
<i>P. confluens</i>	147	-----TRRTGHEASDIPNN---LNTTDHILTLPRPQIQDIQIPAFNRNAQQQVAGDSRQFPQLTN--TREQS-----GLTNLQIPNDL	223
<i>S. cerevisiae</i>	183	-----ENETYQOYEFITG---QDKLKLSTDKNFLNERLVAPSMITPTDFSDIWGRFADLASNFEPDQE-----	243
<i>Y. lipolytica</i>	192	RENTPGQSQSQGQAARRDCRLHSAISSAVSSVSLRCRTQSAVPDEPQAGSSRESSLQSSFSSSMIDSASSTSTTTTIMN-----SSTTTITPSLRQLLSE	287
<i>S. macrospora</i>	265	DGFSRAVREADRLRNMAPFAHANLTSQPASPTTFLP-----TNVRPPSILAPNGRSRRRPSIRHQHSTAETDDMNT--DSVASDENQDAEMS--GTGDVQN--DVEI	361
<i>N. crassa</i>	265	DSFARVRETRDLRLNMGFFAHTNLTSQPASPTTFLP-----TSVRPPSILAPNGRSRRRPSIRHQHSTAGETDDMNT--DSVASDENQDAEMS--GTADVQN--EVGI	361
<i>F. graminearum</i>	291	EGFVRPVRDRLRIASMAPGSDLVSQPTSPPTPLPPQMRSPVTPRPSALPGSGRSRRRPSIRHQNSTA--DADDING--DSMPSPESPEAEMT--GTDNLQS--AVGI	392
<i>S. sclerotiorum</i>	267	GENIRPVRDVRRLPSVMVPGQGLTSQESPTTFLP--AQIRSPVVRTSMVGTTPRSRRRPSIRHQASTAADTEDMNA--DSMASDESSEGPEDITGVSTAEIQSTNVNI	366
<i>A. nidulans</i>	251	GSGLRPPVRDTERLPSMLPAAFNELASQDPSPTTFSG-----AGHIRSNVHVPIGTHARPLPSQHSTSGSDSDANGE--DSIMADDTGSGQSRRIPIGLQSRMIDD	350
<i>P. nodorum</i>	255	-----LGQPLVTALPSMDRKPPTESR-----CGPSPTRQOLSVSGESDLEAQDEIVADNAAVAGPVNEPIVGIQNEINM	325
<i>A. nigricans</i>	242	VGSQAARDDVRMPAMVQAGGASLPVSPPTTNP-----LGGTFNSRTAP--RNGR--LSRRQRSVSGESDDPDS-----DMSNAPVS--MVTENESILDV	307
<i>P. confluens</i>	224	TG--LQPVRDADRPLPSMLQPGVPSQPTSPATGAPPV-----NAGTYQDRTQTTRTGRIYAIRHQRSISGESFDATN-----DINHQTQP-----NEPIVDI	327
<i>S. cerevisiae</i>	244	-----RHDDIDIDSEVESENFDAHKNFSSQDIN-----RPTISITPREFFLGRIVPKQ-----DDVIWSL	299
<i>Y. lipolytica</i>	288	SAILRSGASVESFDTATQNSSSQSDNDQAMVDTDTG-----LALFAERDADGDVEMGSDDFPRRLQASQPQQQQQQQQQPAQPGQPTVTGTATPRPGPGV	385
<i>S. macrospora</i>	362	QQVSVEDGDGIMTG--AALDLATPTASEA--QEAGTFNITHQGAVDASMEN--NTTPTQVTNLHLSPNRPTIANTAQPTMPNNSVPRLYLLDRHVTNPNQMLAAMPREE	463
<i>N. crassa</i>	362	QQVSVEDGDGIMTG--AALDLVTPPTASEA--QEAGTFNITHQGAVDTSMEN--NATPTQVTNLGLSPNRPETIANTAQPSMNSRVPHYLLDRHVTNPNQMLAAMPREE	463
<i>F. graminearum</i>	393	QDITMEDGDTMLGG--TALDLATPTVSETH--QEASTFNINHRSPLDGSLTN--NNAPTVPVPAIGLSPNRPAMATPPQPPLAATTVPRLYLLDRNFVNPQMVAAMPREE	495
<i>S. sclerotiorum</i>	367	QDVMNDENSMLTAVETPIGLTTPPTVSEA--QDAGTFNITHRSADIGSMIN--DATPTGTPTIGLSAQPATVNTP--PTMLPTTIPRYFLDRPTPPQGGVLAAMPREE	469
<i>A. nidulans</i>	351	DADRQTVIDSVTDS--SHDLTVTDTTSDG--QESETFNITHRSADVSGIIT--NDN-----AQAVNNANSPIVPSYSLYFRDRTNIATQNFINTMPREE	440
<i>P. nodorum</i>	326	SDIVDN--AEMLDAGTTPVPIAASEGTD--ENNETFNITHRALDGLSLINPTTPTNPITGFSPMQFAINVVNTNPPP-----AWYPRADRNVAAQYSAAMPREE	424
<i>A. nigricans</i>	328	S--GVVVDQD--SVADPMTLDEGHDPER--QDADFNTIHRSPLDGSLAN--GRTOANNALS-----PNPPTINPGLAIAPALQPSRFPMENASSATLPTMPREE	421
<i>P. confluens</i>	308	QSGVDMERDSMMESVDEPMALPLTHTEAQ--DIAETFNITHRSPIDGSLINPAGPVNPIVGFS-----PNPPTINPTFSIGSLQMRN---PAESPSVVPSSMPREE	404
<i>S. cerevisiae</i>	300	QLLAFVSKYTYMKSTLQNLVESLSFRS---MAYKKQRISEENDLEE--QERDVTVKLSSLYLYLSKNPENNKSIGVLDTSKMDPFFKELEELSNCRCQEEQNE	400
<i>Y. lipolytica</i>	386	APVTAQAQAISIQSSIASMPRIFFENGILMPRDDVWALEILAYVSYSYSTKBEICTCHFVDPDLRLDRKNLPLPLRRAAVQPLMRSKFGSHGEGDVVKTVLDVDEDD	492
<i>S. macrospora</i>	464	DVLMSQLLLAYVSKYCNLRKYFQKCHLVPKLIGKEIKLLDG-----IDPAELEAELEAEDECEEEYLLPNDQNIFFLVEKFTVRYH--STDMQYWAGVVMRNL	562
<i>N. crassa</i>	464	DVLMSQLLLAYVSKYCNLRKYFQKCHLVPKLIGKEIKLLDG-----TDPTLEAELEAEDECEEEYLLPNDQNIFFLVEKFTVRYH--STDMQYWAGVVMRNL	562
<i>F. graminearum</i>	496	DVLMSQLLLAYVSKYCGLSRYFQKSHLVPLKIGKELAAIDKDI--T--AMDEEDVPEVYDEADDEEYLLTNDFNLFPLVEKFTVRYH--STDMQYWAGVVMRNL	597
<i>S. sclerotiorum</i>	470	DVLMSQLLLAYVSKYCNLRSYFQQSHLVPLKIGSELHLLDVEENA---SSNANPSTACSSSHSHDDEEYLLPDDFNIFFLVEKFTVRRH--SQDMQYWAGVVMRNL	572
<i>A. nidulans</i>	441	DVLMSQLLLAYVSKYCNLSYFQNSHFVPLKIDRELRMLDEG-----ASPVELIEEEDYLLPDDVNIFFLVEKFTVRRH--SKDMSYWACVVMRNL	532
<i>P. nodorum</i>	425	DVLMSQLLLAYVSKYCNLRSYFQKSHLVPLKIGSDLQFTGDE-----DVAAAEAKQDEDEELEEYLLPDDFNIFFLVEKFTVRRH--YWASVVMRNL	511
<i>A. nigricans</i>	422	DVLMSQLLLAYVSKYCELRTYFQRSHLVPSLSIKNELYLLDDES-----ERVPAACVKDCQEID--EYLLPDDYNIFFLVEKFTVRRH--PPDMQYWAGVVMRNL	520
<i>P. confluens</i>	405	DVLMSQLLLAYVSKYCNLRTYFQRSHLVPLSTIEPELYLLDDEH-----RHNAASAAAHHKHEEYLLPDYNIFFLVEKFTVRRH--PQDMQYWAGVVMRNL	504
<i>S. cerevisiae</i>	401	ICNNHCPVNLNLFERYRVPKPSDDNAYGDKKERINLRKKISDNFERR-----WSYDKMKKELTNIVYKNKVLTNVNNIFFLVEKYTVSAENTHDVIYSSVIMRNSC	501
<i>Y. lipolytica</i>	493	YKGKICVDETADVGVDDQLDDRTQKQREITAFHKRVTRKSHRKKTDWEKAYDITYDFESEDICAEFLGETINVFPIVEKFTIREY--SNEIQYWAGVIMRNSC	598
<i>S. macrospora</i>	563	RKDDTRGGIRQCAYYQCGKWEETRQFAKRCRCRRTKYCSKECQKSAWAFHRHWCIAATP-----	622
<i>N. crassa</i>	563	RKDDTRGGIRQCAYYQCGKWEETRQFAKRCRCRRTKYCSKECQKSAWAFHRHWCIAATP-----	622
<i>F. graminearum</i>	598	RKDDTRGGIRQCAYYQCGKWEETRQFAKRCRCRRTKYCSKECQKSAWAFHRHWCIAATQ-----	657
<i>S. sclerotiorum</i>	573	RKDDARGGIRQCAYYQCGKWEETRQFAKRCRCRRTKYCSKECQKSAWVHRHWCVAASQ-----	632
<i>A. nidulans</i>	533	RKDESRRGIRQCANIKCGKWEETRQFAKRCRCRRTKYCSKDCQKAWLHYRHWCAATP-----	590
<i>P. nodorum</i>	512	RKDDSRGGIRQCAYYQCGRWEETRQFAKRCRCRRTKYCSKECQKSAWVHRHWCVAATS-----	571
<i>A. nigricans</i>	521	RKDDSRGGIRQCAYYECGKWEETRQFAKRCRCRRTKYCSKDCQKRAWVYHRHWCVAASQ-----	580
<i>P. confluens</i>	505	RKDDSKGGIRQCAYYECGKWEETRQFAKRCRCRRTKYCSKECQKSAWVYHRHWCVAAP-----	564
<i>S. cerevisiae</i>	502	RKNEIL--VRQCANFSCGWEDFPQFAKRCRCRRTKYCSRKQQLKAWGYHYRYWCEHVGSSSHMRSTNTTTGVNTPNEPSSLNATATAADVSNSTSTFTPNISTTVP	607
<i>Y. lipolytica</i>	599	RKNEARGGIRQCAHFDCGKWEETFPQFAKRCRCRRTKYCSRECKLAWNYHKKHWCSTNPPNVASSRSGSSAVTSSAASVSSVSTASTVATATVATVNRMASGGTVA	705
<i>S. macrospora</i>		-----	
<i>N. crassa</i>		-----	
<i>F. graminearum</i>		-----	
<i>S. sclerotiorum</i>		-----	
<i>A. nidulans</i>		-----	
<i>P. nodorum</i>		-----	
<i>A. nigricans</i>		-----	
<i>P. confluens</i>		-----	
<i>S. cerevisiae</i>	608	DEISNRDENSIPE-----	620
<i>Y. lipolytica</i>	706	SGTEGFNPSPNNAASASSASTATVAGGSQSTLSNSRQSMFRSTR	749

**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), *Parasitagonospora 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.

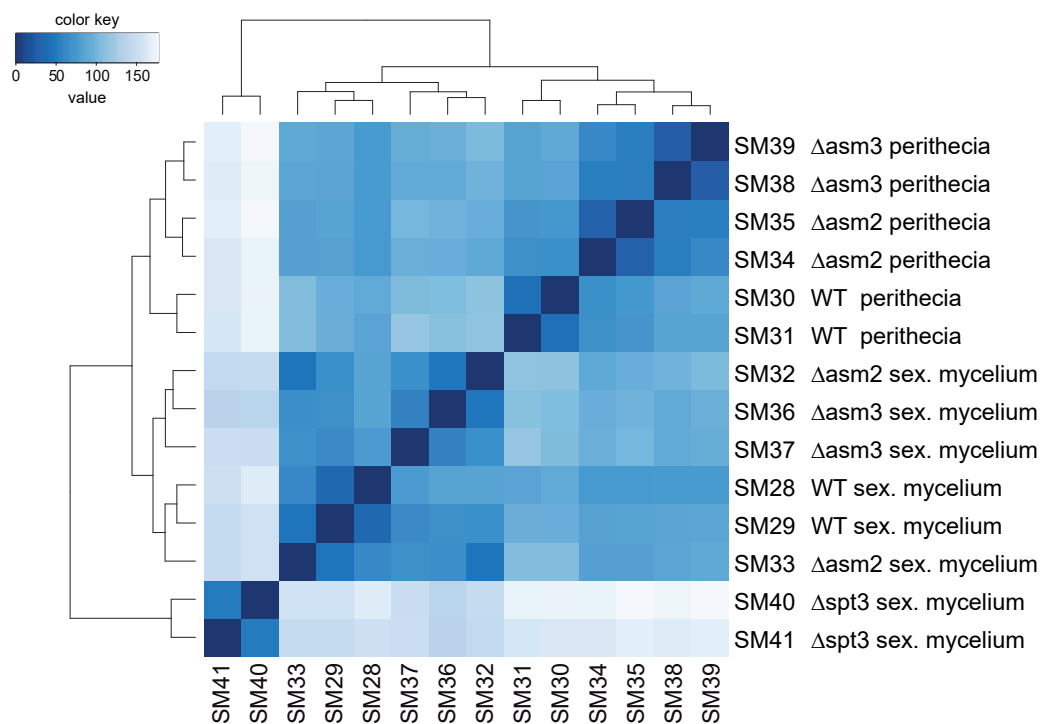


**Figure S3.** Localization of N-terminal GFP-tagged ASM2. Co-localization studies with histone H2A-mRFP revealed the nuclear localization of ASM2 ascogonia and young protoperithecia. Scale bars 20  $\mu\text{m}$



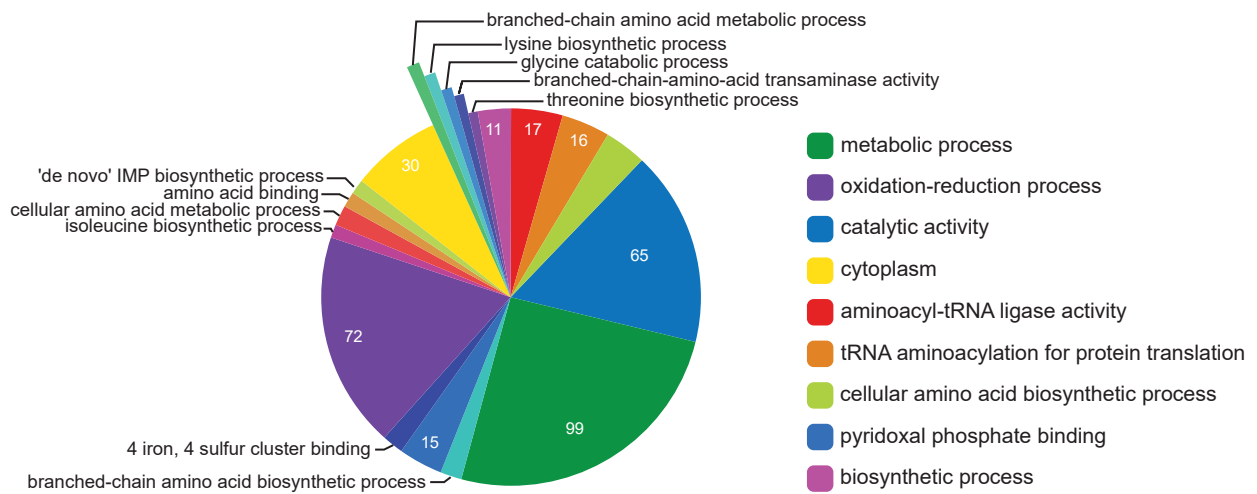


**Figure S4.** RNA-seq results of *asm2*, *asm3*, and *spt3* expression. **A-C.** RNA-seq reads do not map to deleted regions of *asm2*, *asm3* and *spt3* in the corresponding deletion mutants. The figure shows the gene regions of *spt3* (A), *asm3* (B) and *asm2* (C) in the genome browser Artemis (Carver 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<sub>2</sub>-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.

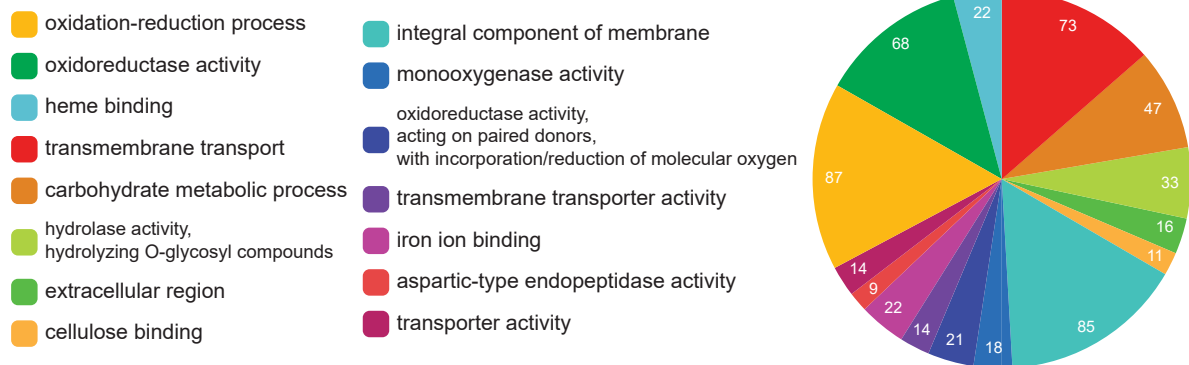


**Figure S5.** Heatmap of Euclidean distance between normalized read counts from RNA-seq samples (variance-stabilized data, analysis was performed with DESeq2). There are two independent biological replicates per condition. Except for the two replicates of sexual mycelium from  $\Delta$ asm2 (samples SM32 and SM33), the two independent samples from each condition cluster together. The two samples from  $\Delta$ spt3 cluster separately from all other samples.

### up-regulated - 20 most significantly enriched categories (GO)

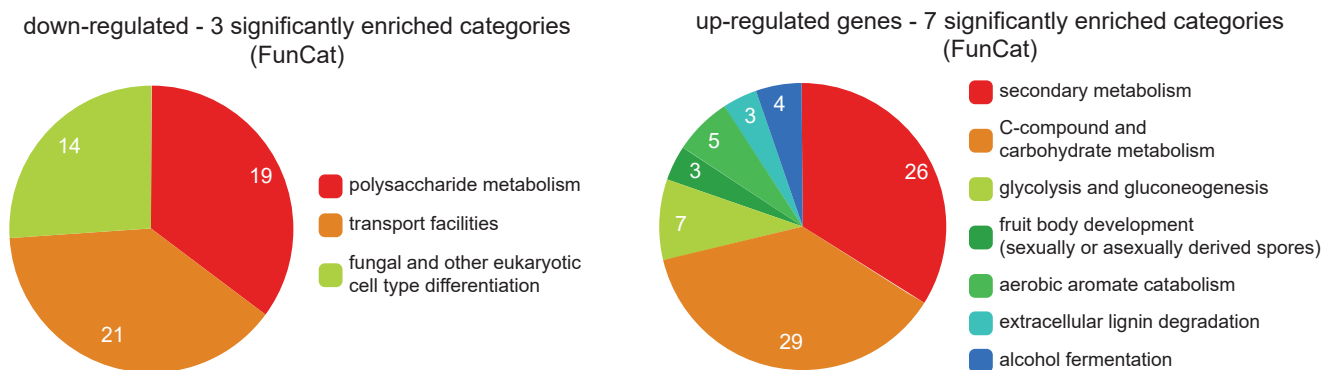


### down-regulated - 15 significantly enriched categories (GO)

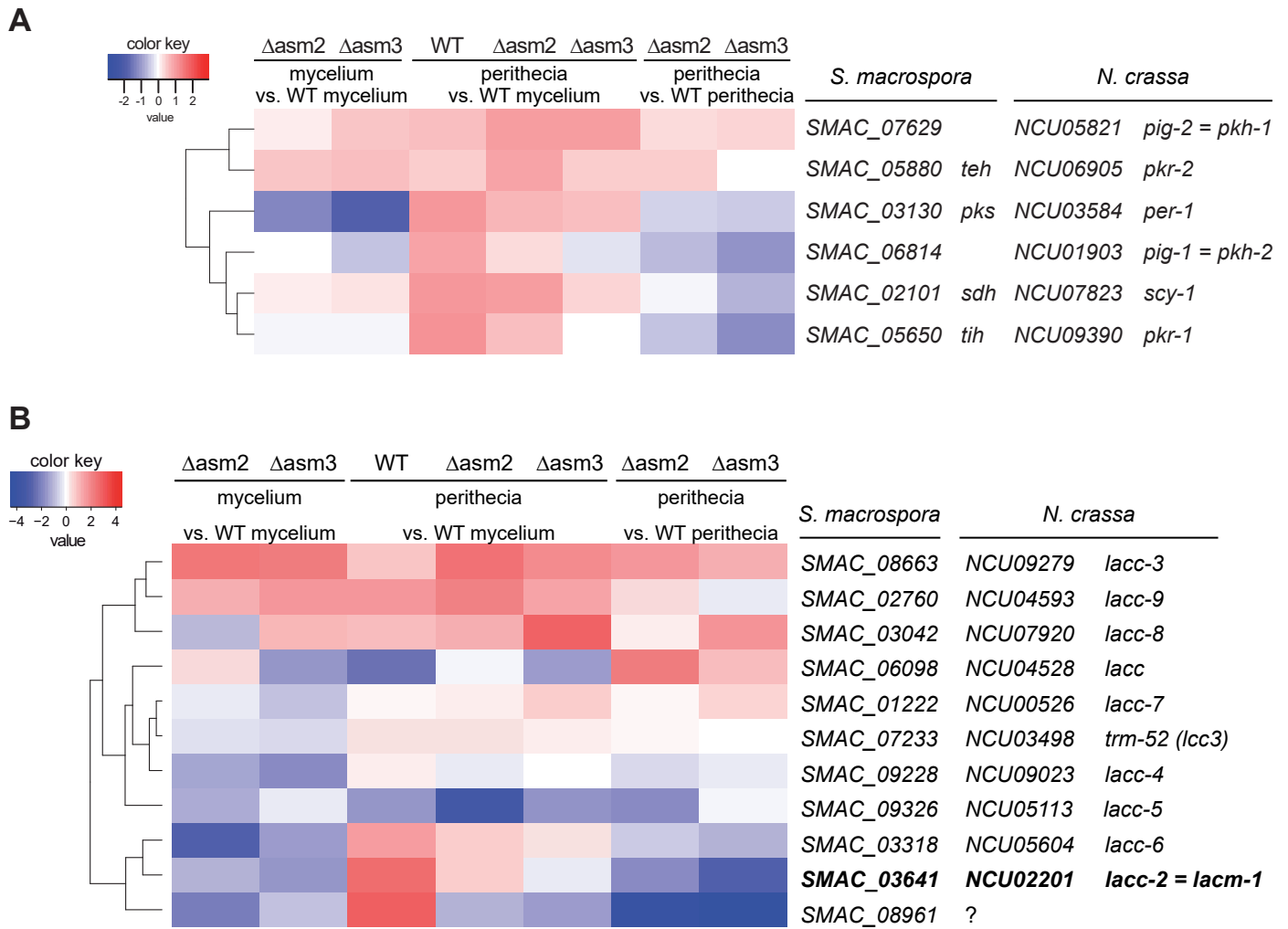


**Figure S6.** Pie charts representing results of gene ontology (GO) analysis of genes that are differentially regulated in  $\Delta$ spt3. Differentially up- and down-regulated genes were significantly enriched in 20 and 15 different categories, respectively. In both cases, mainly categories of primary 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).

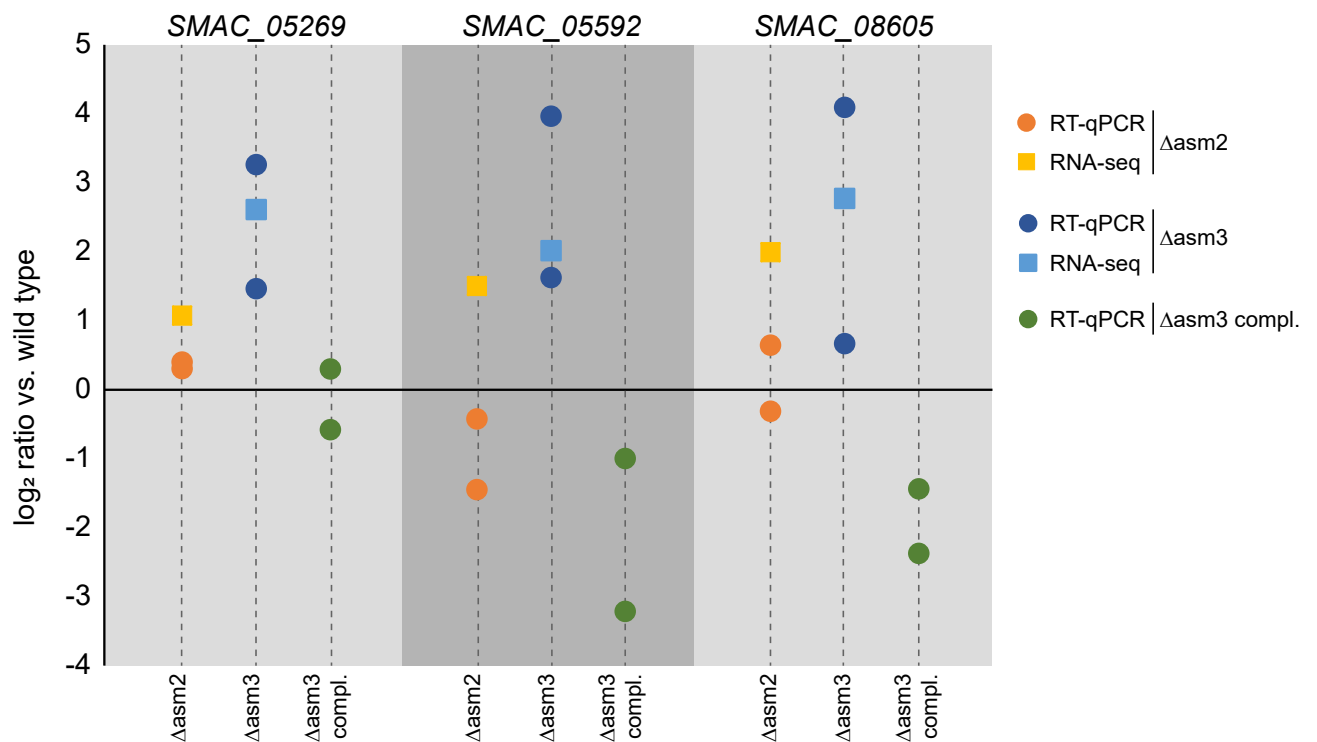




**Figure S7.** Pie charts of significantly enriched categories for differentially regulated genes in wild type perithecia. Functional category (FunCat) analysis was performed with the corresponding proteins of down- and up-regulated genes, respectively, in wild type perithecia. Numbers in pie chart sections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



**Figure S8.** Heatmaps of expression of melanin biosynthesis genes **(A)** and laccase genes **(B)** in  $\Delta$ asm2 and  $\Delta$ asm3 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  $\Delta\text{asm2}$  and  $\Delta\text{asm3}$  mutants as well as a complemented  $\Delta\text{asm3}$  strain expressing *asm3* under a constitutive promoter ( $\Delta\text{asm3 compl.}$ ) 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 ( $\Delta\text{asm2}$ ), S146985 ( $\Delta\text{asm3}$ ) and 6.3 AS2 ( $\Delta\text{asm3 compl.}$ ). For *SMAC\_05269* and *SMAC\_05592*, mean ratios are significantly larger (t-test at  $p < 0.05$ ) in  $\Delta\text{asm3}$  compared to the wild type as well as compared to  $\Delta\text{asm2}$  and the complemented  $\Delta\text{asm3}$  transformant. For *SMAC\_08605*, the difference is statistically significant between  $\Delta\text{asm3}$  and the complemented transformant, but not between  $\Delta\text{asm3}$  and  $\Delta\text{asm2}$ .

**Table S1.** Oligonucleotides used in this study.

Name	Sequence	Remarks
SMAC_03952-ko1	gtaacgccagggttttcccagtcac gaagggatcccatcagatttgaccc gagggcggtc	amplification of upstream region of <i>asm3</i> for cloning of deletion vector
SMAC_03952-ko2	cgagggcaaaggaatagggttcggt tgaggcttggttgccaccacgagt gtcga	amplification of upstream region of <i>asm3</i> for cloning of deletion vector
SMAC_03952-ko3	gccccaaaatgctccttcaatatca gttgctttggacgtttacggaacga tatcc	amplification of downstream region of <i>asm3</i> for cloning of deletion vector
SMAC_03952-ko4	gcggataacaatttcacacaggaaa cagcgatccttggtggtcctccaac attggttctc	amplification of downstream region of <i>asm3</i> for cloning of deletion vector
HR-P3952_fw	tgagcgcgcgtaatacgaactcacta tagcttcgatggctggatagcaaac gtcc	cloning of <i>asm3</i> complementation vector
HR-T3952_rv	catgattacgccaagcgcgcaatta acggacttgctagagccaagtcgag tcg	cloning of <i>asm3</i> complementation vector
SMAC_03952-ORF2	tgtttgatgatttcagtaacgttaa gttcatggcgtggcggcaatgcacc agtgg	cloning of <i>asm3</i> complementation vector
SMAC_03952-ORF3	cgcagcttgactaacagctacagat ctatgaggggaagtcaacttcagcat accca	cloning of <i>asm3</i> complementation vector
HR-3952.3_int_rv_1	acgagtgtcgaggtaggcaccagac g	sequencing of <i>asm3</i> complementation vector
HR-3952.3_int_rv_2	atgccgctccaaccactttaagctc c	sequencing of <i>asm3</i> complementation vector
SMAC_03952-ver1	ccccctctggcctctggcctctcc	verification of homologous integration of <i>asm3</i> deletion vector or sequencing
SMAC_03952-ver2	ctgttcgtgtcgatggttgagttg	verification of homologous integration of <i>asm3</i> deletion vector
SMAC_03952-ver3	cgtggtcaacattggtgtaagagg	verification of homologous integration of <i>asm3</i> deletion vector or sequencing
SMAC_03952-ver4	gatgaaggttggtgacttgagtgg	verification of homologous integration of <i>asm3</i> deletion vector or sequencing
pN03952seq1	accgaagattggcgattgg	sequencing of <i>asm3</i> complementation vector
pN03952seq2	cgtgtcacggtaacgaccac	sequencing of <i>asm3</i> complementation vector
pN03952seq3	gcttggggacaaggtggcatt	sequencing of <i>asm3</i> complementation vector
pN03952seq4	attgccgccacgccaatgatt	sequencing of <i>asm3</i> complementation vector
pN03952seq5	gcatcatcggttggtggttc	sequencing of <i>asm3</i> complementation vector

Name	Sequence	Remarks
09436-N-GFP_fw	catggacgagctgtacaagagcggc cgcatgtcgtcggtcgttaggtc	cloning of <i>asm2</i> complementation vector
09436-N-GFP_bw	ggatccactagttctagagcggccg ctatacaccaccgcccatgaa	cloning of <i>asm2</i> complementation vector
HR-9436_-FT_fw	atcaacaagaccctcccagcatgcg tcgcccctgttgctggttagaaccat tggtggcgcc	cloning of <i>asm2</i> complementation vector with partially deleted fungal specific TF domain
HR-9436_-FT_rv	cgccaccaatggttctaacgacaac aggggcgacgcatgctgggagggtc ttgttgat	cloning of <i>asm2</i> complementation vector with partially deleted fungal specific TF domain
Sm-9436.3_int2fw	ctgctccaacggtagttgc	sequencing of <i>asm2</i> complementation vector
Seq_9436-i_fw	tagcctggaaaactttgc	sequencing of <i>asm2</i> complementation vector
Seq_9436-i_rv	aagctggcaatgtcaatgtg	sequencing of <i>asm2</i> complementation vector
n9346_rv	ggcgaagatacgatcgacgg	sequencing of <i>asm2</i> 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-qPCR
SMAC_05269_rev	TGGGCTCTCAGACTGCGAAG	for RT-qPCR
SMAC_05592_for	acgggcatgtaccatcctcg	for RT-qPCR
SMAC_05592_rev	cccgtacgatgaagtgggct	for RT-qPCR
SMAC_08605_for	TGGCAAGCAACAGCCTGATG	for RT-qPCR
SMAC_08605_rev	AGCGGCCCAAGATTAGACCC	for RT-qPCR
SMAC_08534_for	ATTGACCAGATCCGCGTCGT	for RT-qPCR
SMAC_08534_rev	TGTCTCCGAACAGCCAACCA	for RT-qPCR
SSU1	ATCCAAGGAAGGCAGCAGGC	for RT-qPCR
SSU2	TGGAGCTGGAATTACCGCG	for RT-qPCR

**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	<i>S. cerevisiae</i> <sup>1</sup>	<i>A. nidulans</i> <sup>2</sup>	e-value	<i>S. macrospora</i>	e-value	identity
<b>HAT</b>	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%)
<b>DUB</b>	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%)
<b>Spt</b>	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%)
<b>Taf</b>	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%)

<sup>1</sup> Setiaputra et al. (2015) <sup>2</sup> 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  $\Delta$ sp<sup>t</sup>3 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	<i>ada-10</i> <sup>1</sup>	mat a	normal	smaller size	NF	NA	NF	NA	SMAC_08362	GAL4 TF; fungal_TF_MHR
NCU01706	<i>vsd-6</i> <sup>1</sup>	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	<i>pre-2</i> <sup>2</sup>	mat A	normal	normal	normal	normal	normal	normal	SMAC_08994	PRE2
		mat a	increased	normal	NF	NA	NF	NA		
NCU07617	<i>acon-3</i> <sup>3</sup>	N/A	normal	normal	NF	NA	NF	NA	SMAC_07309	putative ACON3
NCU09915	<i>fsd-1</i> <sup>1</sup>	N/A	normal	normal	NF	NA	NF	NA	SMAC_01666	putative NDT80; TF
NCU00097	<i>bek-1</i> <sup>1</sup>	mat a	normal	normal	normal	no beaks	NF	NA	SMAC_08102	HOX TF
NCU00499	<i>ada-1</i> <sup>4</sup>	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	<i>trm-35</i> <sup>*</sup>	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	<i>div-5</i> <sup>*</sup>	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 <i>superfamily</i> domain
NCU08634	<i>vsd-1</i> <sup>1</sup>	mat a	normal	normal	reduced	normal	NF	NA	SMAC_09090	Forkhead TF
NCU09064	<i>stk-53</i> <sup>5</sup>	N/A	normal	normal	normal	normal	NF	NA	SMAC_05225	putative STK53, kinase
NCU01093	<i>stp-3</i> <sup>*</sup>	N/A	normal	smaller size	normal	normal	normal	normal	SMAC_03168	putative S/T phosphatase
NCU16491	<i>tcf-20</i> <sup>1</sup>	mat a	normal	normal	normal	normal	normal	normal		
		mat A	normal	smaller size	normal	normal	normal	normal	SMAC_04819	GAL4 TF, fungal_TF_MHR
NCU00786	<i>gpr-1</i> <sup>2</sup>	mat a	normal	normal	normal	abnormal beaks	normal	normal	SMAC_01468	G-Protein coupled receptor
NCU09427	<i>gpr-3</i> <sup>2</sup>	mat a	normal	normal	normal	abnormal beaks	normal	normal	SMAC_05685	G-Protein coupled receptor

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

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