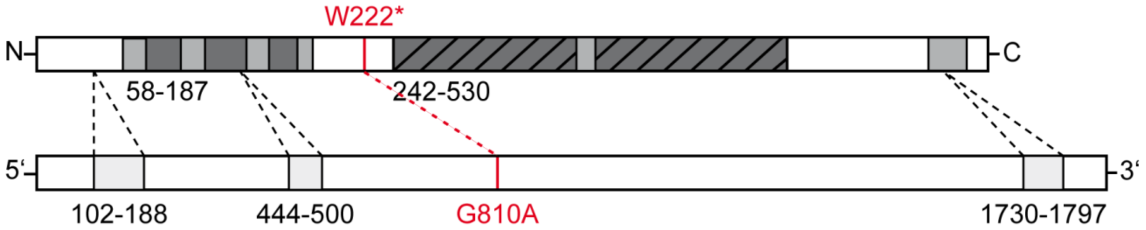

Figure S1 Structure of the *nox1* gene and derived protein. The 1874 bp gene contains three introns (light grey boxes) and the predicted mature mRNA encodes a protein of 645 aa, containing a highly conserved ferric recuctase-like domain at the N-terminus (dark grey boxes), a ferredoxin reductase like domain (striped dark grew boxes) and six predicted transmembrane domains (light grey boxes). A G810A mutation in sterile mutant pro32 leads to a translational stop at position 222 of the NOX1 protein, resulting in a severely truncated NOX1 lacking the ferredoxin reductase-like domain.

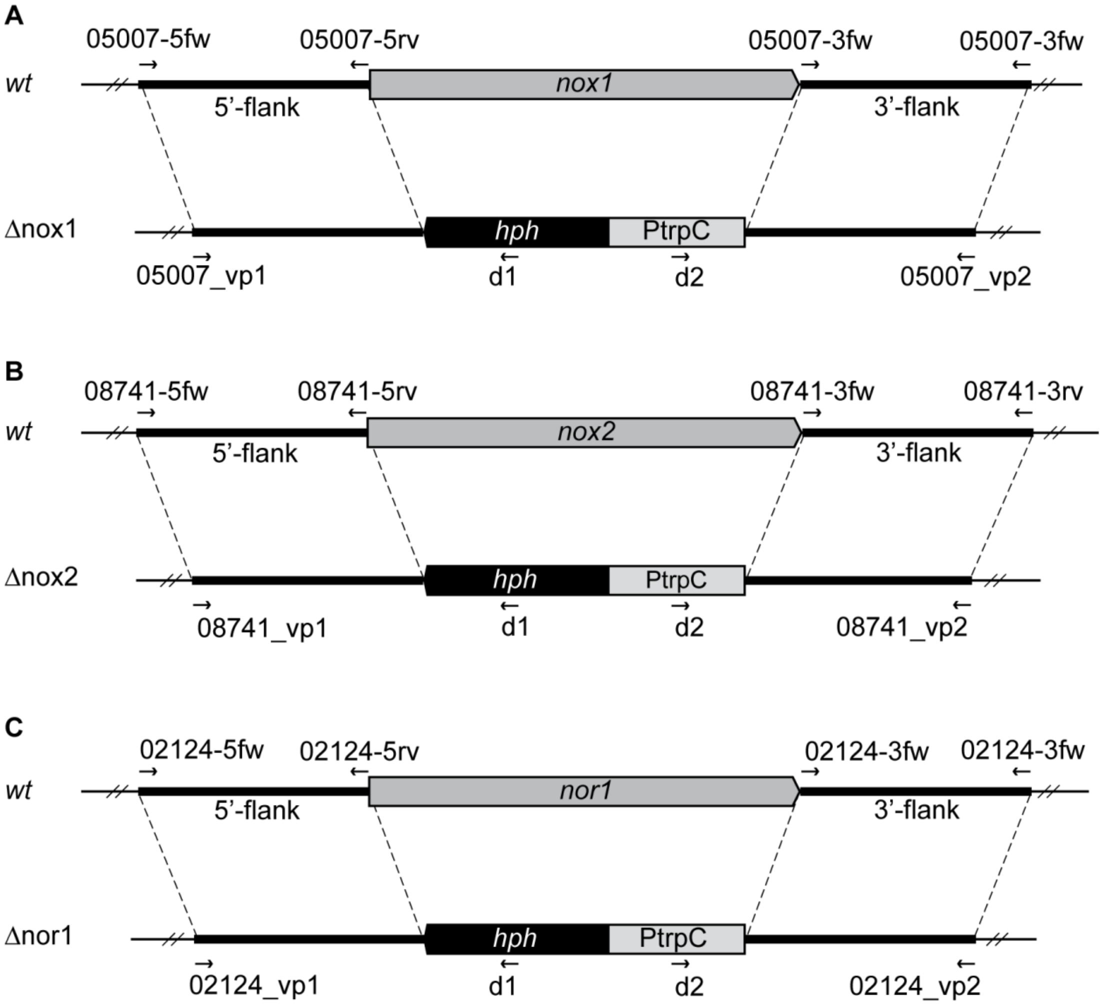

Figure S2 Genomic organization of *nox1* (A), *nox2* (B) and *nor1* (C) loci in wild type and deletion mutants. Primers used for PCR (Table 2) are indicated with small arrows.

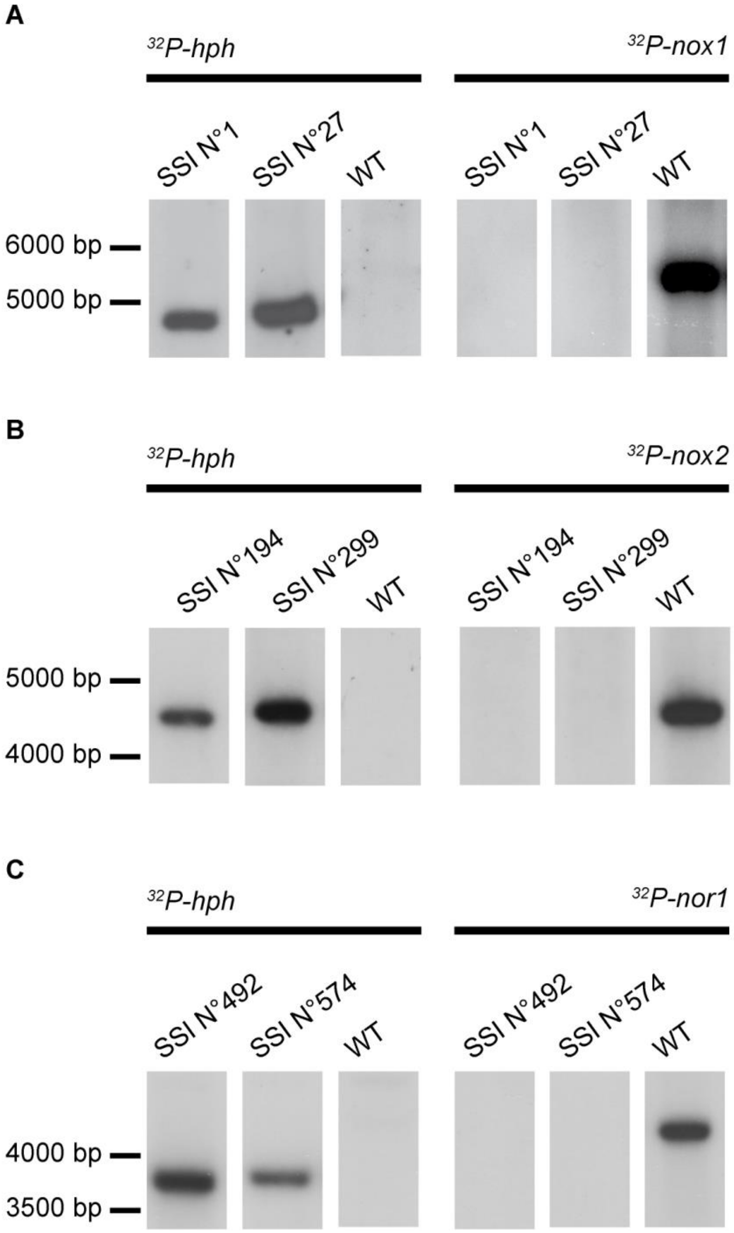

Figure S3 Southern hybridisation of single spore isolates (SSI) to verify the deletion of *nox1* (A), *nox2* (B) and *nor1* (C) mutants. Samples were hybridized with *hph* or gene-specific probes as indicated.

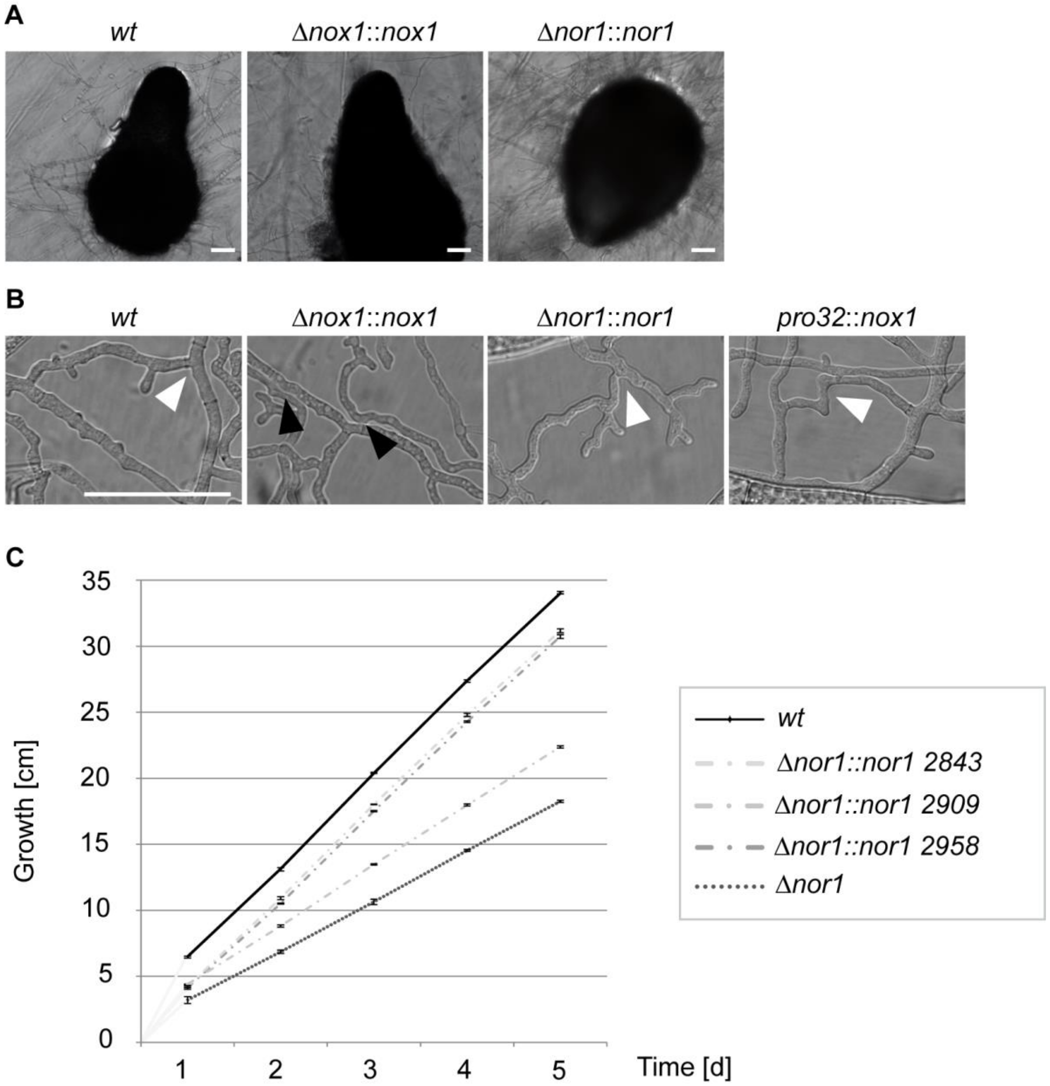

Figure S4 Phenotypes of complemented Δnox1, pro32 and Δnor1 mutants. (A) Complementation of Δnox1 and Δnor1 with the corresponding genes results in normal sexual development. The bar is 50 µm. (B) Hyphal fusion ability of pro32 and Δnor1 mutants was restored in Δnor1::*nor1* and pro32::*nox1* but not in fertile Δnox1::*nox1* complemented strains. Hyphal fusion is indicated by white arrowheads; the lack of hyphal fusion is marked by black arrowheads. The bar is 50 µm. (C) Normal growth was restored in Δnor1::*nor1* as it was in Δnox1::*nox1* complemented strains. Growth was followed in race tubes for 6 days in three replicates.

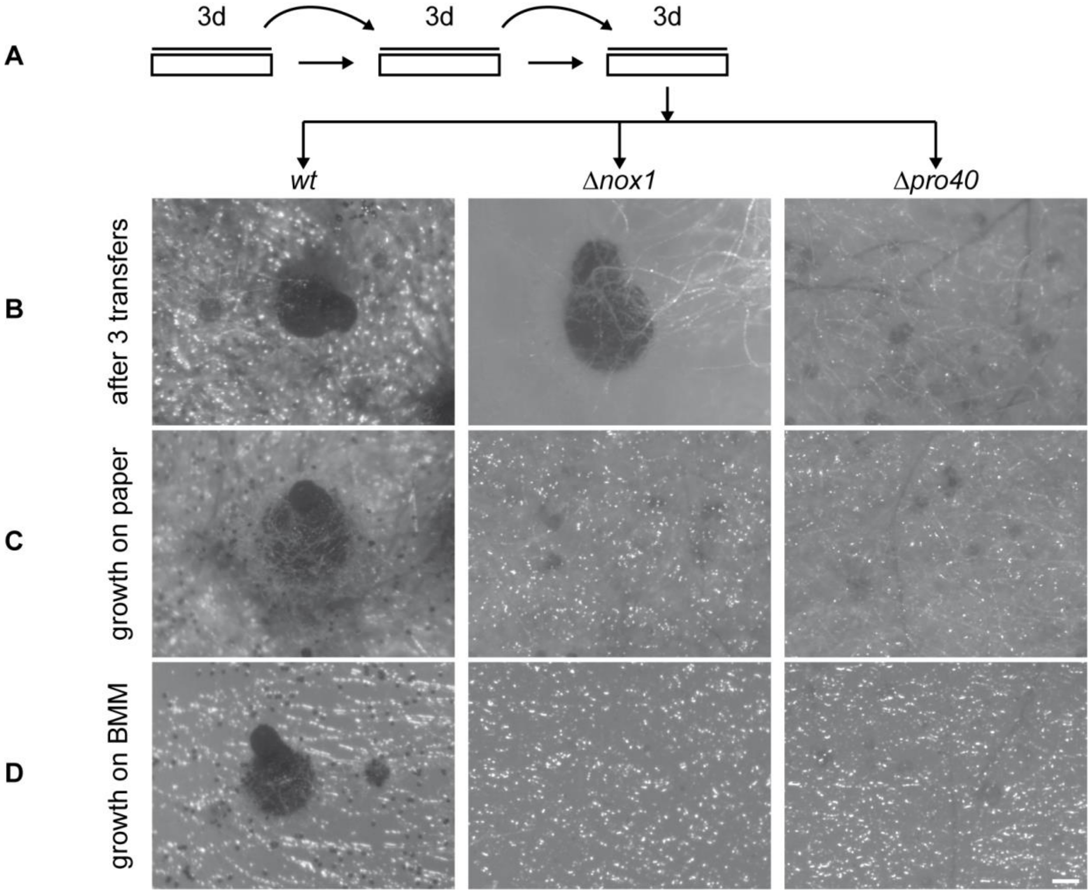

Figure S5 Restoration of Δnox1 fertility by a serial transfer to BMM medium. (A) Sceme to demonstrate the serial transfer of surface cultures on BMM medium. Strains were grown for 3 d on filter papers and subsequently transferred to fresh BMM media; (B) Growth of strains as indicated after 9 d. Serial transfers were done as depicted in (A); (C) Strains were grown for 9 d on filter papers layered on BMM medium; (D) Same as (C) without filter papers

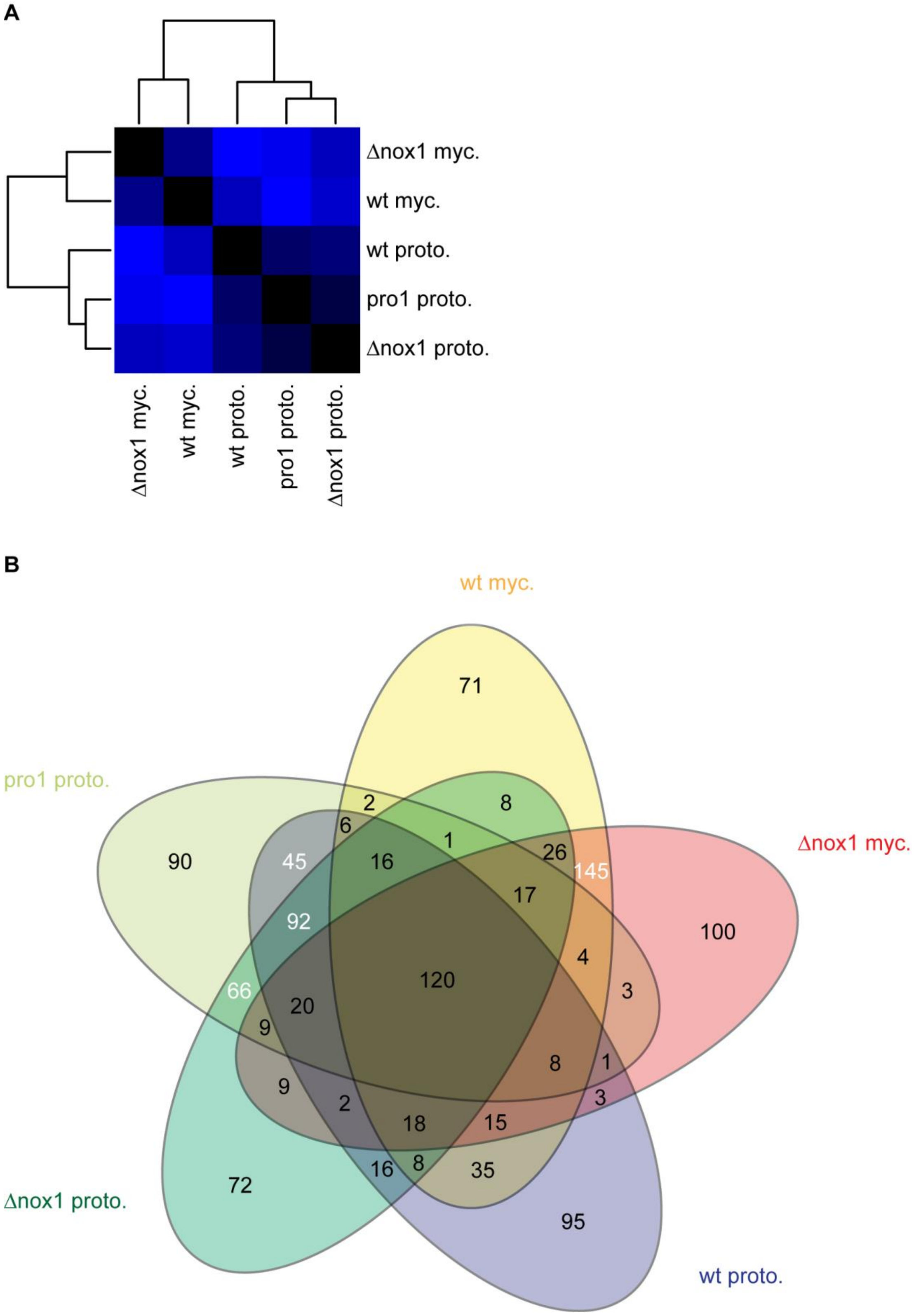

Figure S6 Expression patterns of protoperithecia are distinct from total mycelial samples. (A) Heatmap of correlation coefficients (Spearman) calculated from normalized read counts for classical analysis from protoperithecial (proto.) and mycelial (myc.) samples. Clustering and heatmap were done in R. (B) Venn diagrams of top 500 genes in different samples. Numbers of genes that are in the top 500 group for one or more or the five samples are given. In this analysis, only reads that map within 100 to 400 bases from the 3’ end of the mRNA were used to account for the 3’ bias in the microdissection samples and different mRNA lengths. Numbers for the four intersections containing the highest numbers of genes are indicated in white (not counting fields that represent genes occuring in all or only in one group). These intersections are wt mycelia and Δnox1 mycelia (145), wt protoperithecia and Δnox1 protoperithecia and pro1 protoperithecia (92), Δnox1 protoperithecia and pro1 protoperithecia (66), and wt protoperithecia and pro1 protoperithecia (45).

Files S1-S4

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.159368/-/DC1

File S1 Analysis of raw read counts of *S. macrospora* genes obtained from RNA-seq analysis of mycelia and protoperithecia of Δnox1, pro1 and wild type.

File S2 Movie 1 Germination of fus spores. Ascospores were inoculated on BMM-Ac at 27°C. Ascospore germination was imaged with 5 min intervals for 60 min and analyzed with MetaMorph (version 7.7.5.0, Universal Imaging) software. Display rate, 1 frame /12 s. Related to Figure 5C.

File S3 Movie 2 Germination of Δnox2/fus spores. Ascospores were inoculated on BMM-Ac at 27°C. Ascospore germination was imaged with 5 min intervals for 60 min and analyzed with MetaMorph (version 7.7.5.0, Universal Imaging) software. Display rate, 1 frame /12 s. Related to Figure 5C.

File S4 Movie 3 Germination of Δgsa3Δnox2/fus spores. Ascospores were inoculated on BMM-Ac at 27°C. Ascospore germination was imaged with 5 min intervals for 60 min and analyzed with MetaMorph (version 7.7.5.0, Universal Imaging) software. Display rate, 1 frame /12 s. Related to Figure 5C.

Table S1 Summary of sequence reads generated from mutant and wild type samples

Sample	Total no. of reads	Read length in bases	Total MB	Coverage	No. of reads mapped to reference genome	% of reads mapped to reference genome
wt_3	108,513,967	51	5534	134x	105,047,645	96.8
pro32/fus	91,404,127	51	4662	114x	89,264,279	97.7

Table S2 Summary of sequence reads generated with RNA-seq analysis in this study

Condition	Sample	No. of reads	No. of reads mapped to reference genome	% of reads mapped to reference genome
wild type sexual mycelium	SM10	43,457,800	40,739,279	93.7
Δnox1 sexual mycelium	SM12	48,567,244	46,076,199	95.1
	SM15	58,330,616	55,736,653	95.6
Δnox1 protoperithecia	SM16	71,689,682	65,226,730	91.0
	SM17	65,178,132	60,057,518	92.1

Table S3 Cellular functions of differentially regulated genes in Δnox1 protoperithecia compared to wild type protoperithecia

Direction of regulation	*S. macrospora* locus tag	Gene designation	Cellular function of the corresponding protein in several organisms
Genes involved in cytoskeleton remodeling and hyphal fusion			
Upregulated	SMAC_00609	*ham-10*	Hyphal fusion[a]
	SMAC_00958	*profilin*	Profilin binds actin monomers[b]
	SMAC_02216	*cdc42*	Regulation of septin[c], STE20[d], NoxA localization[e], leads to actin assembly and polarized growth in response to pheromones[f]
	SMAC_02227	*crn-1*	Establishment of polarity, growth and stable Spitzenkörper[g]
	SMAC_02633	*rts-1*	Bud growth, accumulation of G1 cyclin[h]
	SMAC_04679	*rdi1*	Recycling of CDC42[i]
	SMAC_05207	*las17*	Stabilization of actin patches (endocytosis)[b]
	SMAC_07118	*dynactin 6*	Active transport along the microtubules[j]
	SMAC_04212	*kinesin*	Active transport along the microtubules[j]
	SMAC_06372	*kinesin*	Active transport along the microtubules[j]
	SMAC_07150	*kinesin*	Active transport along the microtubules[j]
	SMAC_07711	*kinesin*	Active transport along the microtubules[j]
Downregulated	SMAC_01612	*rax1*	Bipolar budding of dipoloid cells[k]
	SMAC_02720	*rsr1/bud1*	Localization[l] and regulation[m] CDC42
	SMAC_02963	*cbk1*	Septum disruption after cell division[n]
	SMAC_05949	*bem3*	GAP of CDC42[o]
	SMAC_09273	*cofilin*	De-polimerization of actin filaments[p]
Genes involved in ubiquitin mediated protein degradation or autophagy			
Upregulated	SMAC_01774	*ubiquitin-activating E1 1*	Ubiquitin binding[q]
	SMAC_03099	*ubiquitin-conjugating E*	Ubiquitin binding[q]
	SMAC_05013	*ubiquitin-conjugating E2 13*	Ubiquitin binding[q]
	SMAC_05407	*apc5*	Protein degradation[r]
	SMAC_05726	*pex4*	Ubiquitin binding[q]
	SMAC_06684	*cul-4*	Protein degradation[r]
	SMAC_06747	*ubiquitin-protein ligase gene*	Ubiquitin binding[q]
Downregulated	SMAC_06998	*atg12*	Autophagosome formation[s]
Genes involved in sexual development			
Upregulated	SMAC_00047	*fl*	Transcription factor[t]
	SMAC_02283	*pre-1*	Pheromone receptor[u]
	SMAC_05401	*SmtA-1*	Mating type factor[v]

	SMAC_05403	*SmtA-3*	Mating type factor[v]
Downregulated	SMAC_06479	*ste12*	Ascospore germination[w]
Genes involved in mitochondrial respiratory chain			
Upregulated	SMAC_01349	*nuo9.5*	Subunit of complex I (mitochondrial respiration)[x]
	SMAC_02450	*nuo78*	Subunit of complex I (mitochondrial respiration) [x]
	SMAC_04043	*nuo11.5*	Subunit of complex I (mitochondrial respiration[x]
	SMAC_04093	*cytochrome-c oxidase chain VIIc*	Cytochrome c oxidase of complex III (mitochondrial respiration)[y]
	SMAC_05824	*nuo49*	Subunit of complex I (mitochondrial respiration) [x]
	SMAC_07036	*nuo10.4*	Subunit of complex I (mitochondrial respiration) [x]
	SMAC_07180	*nuo21*	Subunit of complex I (mitochondrial respiration) [x]
	SMAC_08634	*nuo14*	Subunit of complex I (mitochondrial respiration) [x]
Downregulated	SMAC_12686	*ATP synthase subunit 6*	Mitochondrial ATPase[z]
	SMAC_12688	*ATP synthase subunit 9*	Mitochondrial ATPase[z]

[a] (Fu *et al.* 2011); [b] (Berepiki *et al.* 2011); [c] (Dagdas *et al.* 2012); [d] (Chen and Thorner 2007); [e] (Semighini and Harris 2008); [f] (Jones and Bennett 2011); [g] (Echauri-Espinosa *et al.* 2012); [h] (Artiles *et al.* 2009); [i] (Das *et al.* 2013); [j] (Rank and Rayment 2013); [k] (Krappmann *et al.* 2007); [l] (Pulver *et al.* 2013); [m] (Park *et al.* 1997); [n] (Brace *et al.* 2011); [o] (Knaus *et al.* 2007); [p] (Berepiki and Read 2013); [q] (Strieter and Korasick 2012); [r] (van der Veen and Ploegh 2012); [s] (Iino and Noji 2013); [t] (Bailey and Ebbole 1998); [u] (Mayrhofer *et al.* 2006); [v] (Klix *et al.* 2010); [w] (Nolting and Pöggeler 2006); [x] (Tanida 2011); [y] (Duarte and Videira 2000); [z] (Mavridou *et al.* 2013)

Table S4 Differentially regulated genes in Δnox1 protoperithecia compared to wild type protoperithecia with an impact on sexual development in *N. crassa*

S. macrospora locus tag	*N.crassa* locus tag	Gene designation	Gene product	Phenotype of *N. crassa* deletion mutant
upregulated in Δnox1 protoperithecia compared to wild type protoperithecia				
SMAC_00047	NCU_08726	*fl*	transcription factor fluffy	sterile[a]
SMAC_00190	NCU00911	*cps1*	Polysaccharide synthase Cps1p	sterile, reduced growth[a]
SMAC_00609	NCU02833	*ham-10*	Hyphal anastomosis-10	sterile, reduced growth, cell fusion deficient[b]
SMAC_02227	NCU00202	*crn*	Coronin-1	sterile, reduced growth, no polarity during germination[c]
SMAc_02283	NCU00138	*pre-1*	Pheromone receptor-1	female sterile[d]
SMAC_02450	NCU01765	*nuo78*	NADH:ubiquinone oxidoreductase 78	Reduced perithecia formation, no ascospore production[e]
SMAC_04395	NCU04198	*cac-1*	Chromatin assembly-1	Sterile, reduced growth[a]
SMAC_05401	NCU01958	*matA-1*	Mating type protein A-1	sterile, heterokaryon compatible[f]
SMAC_05403	NCU01960	*matA-3*	Mating type protein A-3	few ascospores formed[g]
SMAC_05824	NCU02534	*nuo49*	NADH:ubiquinone oxidoreductase 49	Sterile, reduced growth[e]
SMAC_06177	NCU04001	*ff-7*	Transcription factor female fertility-7	sterile[a]
SMAC_06684	NCU00272	*cul-4*	Cullin-4	sterile, reduced growth[a]
SMAC_07314	NCU07622		Putative GTPase activating protein	sterile[a]
SMAC_08994	NCU05758	*pre-2*	Pheromone receptor-2	sterile, no ascospore formation[a]
downregulated in Δnox1 protoperithecia compared to wild type protoperithecia				
SMAC_00010	NCU10142		Putative heterokaryon incompatibility protein	sterile[a]
SMAC_00177	NCU09211	*sad-3*	Suppressor of ascus dominance-3	no ascospore production[h]
SMAC_01666	NCU09915	*fsd-1*	Female sexual development-1	sterile, defect in ascospore

				maturation[a]
SMAC_02093	NCU07816		Putative magnesium and cobalt transporter CorA	sterile[a]
SMAC_02094	NCU07817	*ncw-3*	Non-anchored cell wall protein-3	sterile, reduced growth[a]
SMAC_06479	NCU00340	*pp-1*	protoperithecium-1	sterile, reduced growth, ascsospore germination defect[i]
SMAC_07309	NCU07617	*acon-3*	Aconidiate-3	sterile[a]
SMAC_07994	NCU08227		Putative glycosyl hydrolase	sterile[a]
SMAC_08793	NCU08739		endothiapepsin	sterile, reduced growth[a]
SMAC_12613	NCU07172	*stk-8*	Serine/threonine protein kinase-8	sterile, reduced growth, defective in conidia development[j]

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[a]*Neurospora crassa* Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/); [b](Fu *et al.* 2011); [c](Echauri-Espinosa *et al.* 2012); [d](Kim and Borkovich 2004); [e](Tanida 2011); [f](Glass and Lee 1992); [g](Ferreira *et al.* 1998); [h](Hammond *et al.* 2012); [i](Li *et al.* 2005); [j](Park *et al.* 2011)

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