

Supplementary materials

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NFAP2, a novel cysteine-rich anti-yeast protein from *Neosartorya fischeri* NRRL 181:
Isolation and characterization

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Table S1 Putative NFAP2 homologs from annotated filamentous *Ascomycota* genomes

Fungus	Name of the protein	Acc. number or protein ID*	Sequence	Additional information	Database
<i>Acremonium chrysogenum</i> ATCC 11550	hypothetical protein ACRE_009950	KFH48093.1	IATDAYYACNCPNNCGHKEGSSCKFYSGPSDNGIISGHCHYPNGNPMGAKECVP	n.d.	NCBI
<i>Acremonium strictum</i> DS1bioAY4a	gm1.11729_g	1373459	IATDAYYACNCPNNCGHSEGSCKYYGGPSDTHDIIISGTCNYPNGNRFASIECVA	n.d.	JGI
	gm1.6463_g	1368193	IATDPFFACQCPNNCDHSSGSSCKFYGGPSDSSNVVDGHCTDTADGLKCI		
<i>Alternaria brassicicola</i>	AB02612.1	2612	IAPDAINACNCPNNCQHKLGDSCKFYKEGYVVT AICRATDDDDVLECR	n.d.	JGI
<i>Aspergillus fumigatus</i> var. RP-2014	hypothetical protein BA78_8705	KEY83917.1	IATSPYYACNCPNNCKHKKGSSCKYHSGPSDKSKVISGKCEWQGGQLNCIAT	Origin: Brazil. Isolation source: tropical forest floor. Collection date: 2007.	NCBI
<i>Aspergillus fumigatus</i> Z5	hypothetical protein Y699_05481	KMK56566.1	IATSPYYACNCPNNCKHKKGSSCKYHSGPSDKSKVISGKCEWQGGQLNCIAT	Origin: China, Nanjing. Isolation source: compost heaps. Collection date: Mar 2010.	NCBI
<i>Byssothecium circinans</i> CBS 675.92	fgenes1_kg.8_#_209_#_Locus10000v1rpk3.04	489823	IAPDPIAACNCPNNCRHKNKGSSCKFYDNGNTISGECQIEGDHLKCA	Origin: USA, South Dakota. Isolation source: rotten crown. Substrate (including host): <i>Medicago sativa</i> .	JGI
<i>Claviceps purpurea</i> strain 20.1	uncharacterized protein CPUR_03466	CCE29619.1	IATDPFFACNCPNNCKHNEGTSRYYSNYSDSGPVSRGKCGWKNQLYCYA	n.d.	NCBI
	uncharacterized protein CPUR_03467	CCE29620.1	IATDAYHACNCPNNCGHKSMTGCRFFAGPSSNSNVLTKCESVGGYLSIAGPAQ		
	uncharacterized protein CPUR_03481	CCE29634.1	IATDAYHACNCPNNCSYRSGSSCRFRGGPSGNAPVLKGCCEFVGGYLSIAQ		
	uncharacterized protein CPUR_03482	CCE29635.1	IATDPYFACNCPNNCSHKEGSSCRYYKNFSDSGPVAKGKCGWQNGHLNCA		
<i>Coniochaeta ligniaria</i> NRRL 30616	fgenes1_kg.1_#_380_#_Locus11190v1rpk0.72	626667	IATDPVSACNCPNNCSHKSAGSSCKYYSGPSDSSPIDSGKCAWRGSLICVV	n.d.	JGI
<i>Coniochaeta</i> sp. PMI_546	gm1.7183_g	970051	IATDPVSACNCPNNCSHKKGSSCRYYSGPSDSSPVDSGHCEWRGSLICIV	Isolation source: <i>Populus deltoides</i> .	JGI
<i>Daldinia eschscholzii</i> EC12	gm1.7079_g	24471	IATNSVAACNCPNNCSHKENSSCKFYSGPSDTSKVVVSGRCHSQNGVLTICIP	n.d.	JGI
<i>Eutypa lata</i> UCR-EL1	hypothetical protein UCREL1_925	EMR72015.1	IATDPGYACTCPNNCDHHAGSSCKYYSGPSDNSPIIEGTCVDRNGLTCVA	n.d.	NCBI
<i>Hypoxylon</i> sp. CI-4A	gm1.10063_g	16636	IATDAFSACNCPNNCDHSAGSSCKYHAGPSDSSKTVSGKCNKPNPNPYASLECIVTS	n.d.	JGI
<i>Hypoxylon</i> sp. CO27-5	gm1.426_g	26620	IAVDVAACNCPNNCSHNGSSCKYLAGPSTSSVISGKCTPEADGTLICIPK	n.d.	JGI
	gm1.7264_g	33458	IATTAFAACNCPNNCDHKAAGSDCKYYSGPSSKSDVLKGCACAKPNPNPYASIECIPN		
<i>Hypoxylon</i> sp. EC38	gm1.528_g	528	IAVDVAACNCPNNCSHNGSSCKYLAGPSTSSVISGKCTPEADGTLICIPK	n.d.	JGI
	gm1.11636_g	11636	IATTAFAACNCPNNCEHKAAGSDCKFYSGPSSKSDVLKGCACAKPNPNPYASIECIPN		
<i>Karstenula rhodostoma</i> CBS 690.94	gm1.14118_g	492330	IAPDPDAACNCPNNCQHSNGESCSFYRDGNHLDGICQHTGEGGRLLCVA	Origin: Sweden, Uppland, Dalby Parish, NW of farm Viggeby. Substrate (including host): <i>Frangula alnus</i> . Collection date: 25/02/1986.	JGI

<i>Massarina eburnea</i> CBS 473.64	gm1.2277_g	514471	IAADPVAACNCPGNCKHKNGDSCFKFYENGNTLSGQCVDGDHLKCV A	Country: Switzerland, Zürich, Talstrasse, Rehalp Forest. Substrate (including host): <i>Fagus sylvatica</i> .	JGI
<i>Melanomma pulvis-pyrius</i>	fgenesh1_kg.370_#_2_#_Locus74v1rpkml368.98	327945	IAPDPISACNCPNNCQHKVGSCKFYSGQNTLSGECQLNGNAGNLICIA	n.d.	JGI
<i>Myriangium duriae</i> CBS 260.36	gm1.4858_g	293795	IAPDPTSACNCPKNCSHHAGSSCKFYRNGNVITGVCNDVGGHLICQD	Origin: Argentina, Delta del Paraná. Substrate (including host): <i>Chrysomphalus aonidium</i> .	JGI
<i>Niesslia exilis</i> CBS 358.70	estExt_Genemark1.C_1_t10048	854608	IATNPFYACNCPNNCDHGPDSCKYYDGPSTSPVVDGTCQSSGSATGWNCVPN	Origi: Netherlands, Noord Brabant, Kampina Heide. Substrate (including host): <i>Pteridium aquilinum</i> petiole.	JGI
	estExt_Genemark1.C_190067	864148	IATTSY AACNCPNNCSY TQGSSCKYFAGPSD TDTIISGTCQYPNGNQAAEITCIA		
	gm1.8228_g	828052	IATDAYSACNCPNNCSYSGQTGCRYYSTPSNSGPV ASGTCDY PGGNHNSGLTCIPSS		
<i>Paraconiothyrium sporulosum</i> AP3s5-JAC2a	estExt_Genemark1.C_5_t20273	1259469	IAANPDAACNCPNNCQHNNGDSCSFYRDGNQLNGICQHTGEGGRLLCNA	n.d.	JGI
<i>Pseudogymnoascus pannorum</i> VKM F-3808	hypothetical protein O988_03546	KFY00055.1	IATDPYYACNCPNNCSYKPGTGCRFYSGPSDNSKVLK GK CQNKNGLTCIPT	Origin: Russia, Tverskaya oblast. Isolation source: hair.	NCBI
<i>Pseudogymnoascus pannorum</i> VKM F-4513	hypothetical protein V494_01379	KFY44674.1	VATDPYYACNCPNNCSYKEGSGCRFYSGPSDNSRILK GK CHR KD GKLTCVP	Origin: Russia, Kolyma Lowland. Isolation source: permafrost soil.	NCBI
<i>Pseudogymnoascus pannorum</i> VKM F-4514	hypothetical protein V495_02588	KFY46251.1	IATDPYYACNCPNNCSYKPGTGCRFYSGPSDNSKVLK GK CQNKNGLTCIPT		
<i>Pseudogymnoascus pannorum</i> VKM F-4516	hypothetical protein V497_02880	KFY61541.1	IATDPYYACNCPNNCSYKPGTGCRFYSGPSDNSKVLK GK CQNKNGLTCIPT		
<i>Pyrenophora tritici-repentis</i>	PTRT_08027	153706	IAPDATNACNCPNNCRHRVGDSCFKFYQQGYVVTATCQQGGANGALICK	n.d.	JGI
<i>Sordaria macrospora</i> K-hell	hypothetical protein SMAC_09189	XP_003344816.1	IATDPFYACNCPNNCSHRAGSGCKYHSGPSDKSSVVKGKCVVRGGGLTCVSG	Tissue type: mycelium.	NCBI
<i>Thozetella</i> sp. PMI_491	gm1.14407_g	828875	IAPDPVAACNCPNNCKHHLGSSCKFYSGNVLSGVCQHDGSGLKCAV	Origin: Australia. Isolation source: bark.	JGI

Abbreviation of databases: NCBI, National Center for Biotechnology Information; Bethesda, MD, USA; JGI, Joint Genome Institute; Walnut Creek, CA, USA. *: In the cases of NCBI and JGI database the accession number and the protein ID is given, respectively

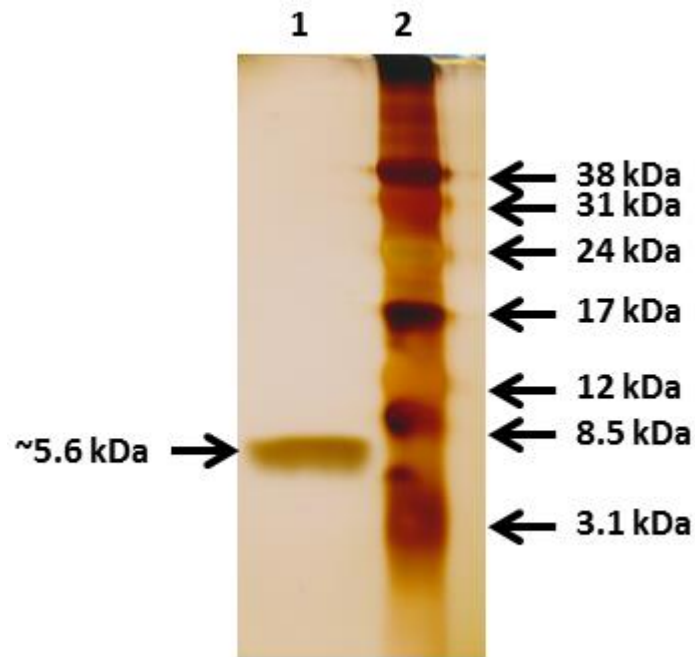


Fig. S1 Purity of the NFAP2 after the ion-exchange chromatography, checked with 18% (w/v) tris-glycine sodium dodecyl sulfate-polyacrylamide gel (Novex™ 18% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well; Thermo Fisher Scientific, Waltham, MA, USA) electrophoresis. Protein bands were detected with silver staining. Lane 1: purified NFAP2 (1.5 μ g), lane 2: Low-range Amersham Rainbow Marker (GE Healthcare Life Sciences, Little Chalfont, UK)

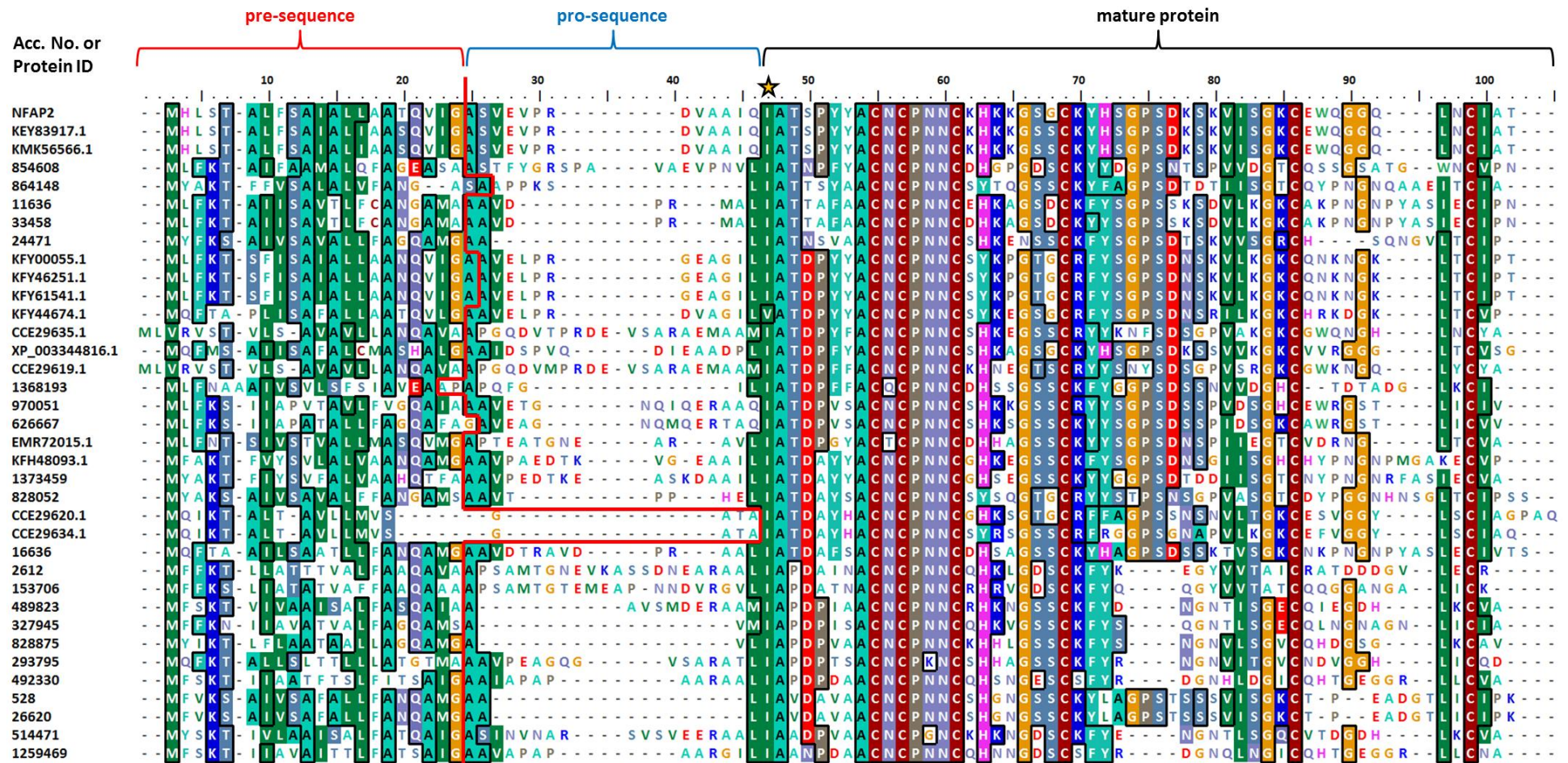


Fig. S2 Alignment of the putative NFAP2 homolog proteins from filamentous ascomycetes. Red line indicates the cleavage site of the predicted signal sequence (SignalP1 4.1 server; Petersen et al. 2011). First amino acid of the mature protein is marked by an asterisk. Accession number or the protein ID is indicated in the figure

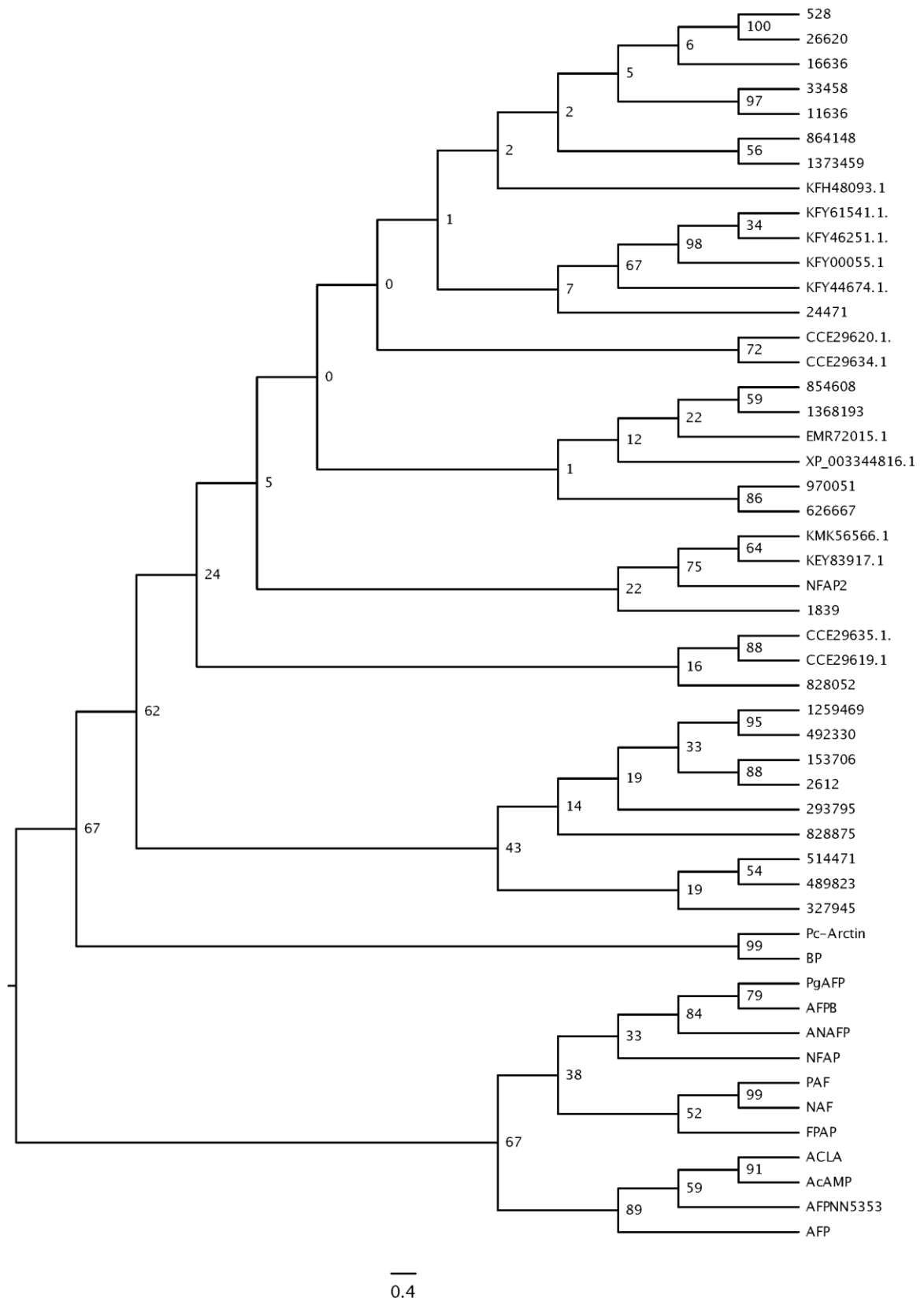


Fig. S3 Bootstrap values of the maximum likelihood tree presented in Fig. 3b

AcAFP, *Aspergillus clavatus* VR1 antifungal protein (Acc. No.: A1CSS4); AcAMP, *Aspergillus clavatus* ES1 antimicrobial peptide (Acc. No.: D3Y2M3); AFP, *Aspergillus giganteus* MDH 18894 antifungal protein (Acc. No.: P17737); AFP_{NN5353}, *Aspergillus giganteus* A3274 antifungal protein (Acc. No.: -); AFPB, *Penicillium digitatum* CECT 20796 antifungal protein (Acc. No.: K9FGI7); ANAFP, *Aspergillus niger* KCTC 2025 antifungal protein (Acc. No.: A2QM98); BP, *Penicillium brevicompactum* Dierckx ‘bubble protein’ (Acc. No.: G5DC88); FPAP, *Fusarium polyphialidicum* SZMC 11042 antifungal protein (Acc. No.: E1UGX4); NAF, *Penicillium nalgiovense* BFE 66, 67, 474 antifungal protein (Acc. No.: -); NFAP, *Neosartorya fischeri* NRRL 181 antifungal protein (Acc. No.: D4YWE1); NFAP2, *Neosartorya fischeri* NRRL 181 antifungal protein 2 (Acc. No.: A1DBL3); PAF, *Penicillium chrysogenum* Q176 antifungal protein (Acc. No.: B6HWK0); Pc-Arctin, *Penicillium chrysogenum* A096 ‘bubble protein’ (Acc. No.: CAP96194); PgAFP, *Penicillium chrysogenum* RP42C antifungal protein (Acc. No.: D0EXD3). The Acc. No. or Protein ID of the putative NFAP2 homolog proteins is indicated. For further information (species name, sequence, etc.) see Table S1