

# Tracking fungal growth: Establishment of dynactin Arp1 as a marker for polarity establishment and active hyphal growth in filamentous ascomycetes

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## Figures

Sc_ARP1	MDQLSDSYALYNQPVVIDNGSGIIK-----AGFSGEERPKALEYCLV	42
Sp_ARP1	---MTVTEIFDNQPICIDNGSGFIK-----AGFAGDDIPKCLFPTCV	39
Ca_ARP1	-----MAEILYNQPVVIDNGSGNLK-----AGFAGEDKPKSYASAI	37
Pc_ARP1	---MEFNDVLTNQPICIDNGSGVIK-----AGFAGEDQPKSFFPSYV	39
Um_ARP1	MAGNEFDDVLTNQPVIDNGSGSIK-----AGFAGQDAPKCFPSFV	42
Ro_ARP1	-MNTEFDDVLTNQPVIDNGSGVIK-----AGFAGEEQPKCFPSMV	41
Ml_ARP1	-MNSEFDDVLTNQPVIDNGSGVIK-----AGFAGEEQPKCFPSMV	41
Cn_ARP1	-MATEFDDVLTNQPVIDNGSGNIK-----AGFAGEEQPSCYIPSFV	41
Cc_ARP1	-MSAEFDDVLTNQPVIDNVGPLSPGTPSPSSTGIWNHKGFCRSRSSQMLLP---	56
Sco_ARP1	-MSAEFDDVLTNQPVIDNGSGTIK-----AGFAGQDHPKCFPSFV	41
An_ARP1	-----MTEATLHNAPIVIDNGSGTIR-----AGFAGEEIPSCYFPSFV	38
Mo_ARP1	-----MAQLLHNSPIVIDNGSGTIR-----AGFAGEDLPKCFPSFV	37
Sm_ARP1	-----MTDSLHNAPIVLDNGSGTIR-----AGFAGDDVPKCHFPSFV	37
Nc_ARP1	-----MTDSLHNAPIVLDNGSGTIR-----AGFAGDDVPKCHFPSFV	37
Cg_ARP1	-----MTDSLHNAPIVLDNGSGTIR-----AGFAGDDLKCYFPSFV	37
Fo_ARP1	-----MADSLHNAPIVLDNGSGTIR-----AGFAGDDLKCYFPSWV	37
	: * *: : ** . . ** . .	
Sc_ARP1	GNTKYDKVMLEGL-----QGDTFIGNNA-QKLRLGLLKLRYPIKHGVVEDWDSMELI	92
Sp_ARP1	GRIKHERVMPSSI-----QKDMFVGSEA-QNLRGLLKIQRPIERGIIQNWSDMEEI	89
Ca_ARP1	GRPKYQKIMAAGSTSLSEQQSHDQLFIGNSA-QDNRGLLKLSPYIEHGIVNNWSDMEKL	96
Pc_ARP1	GRPKHLKIMAGAI-----EGDIFIGNKA-QELRGLLKIYPIEHGIVVDWDDMERI	89
Um_ARP1	GRPKHPRVMAGAI-----EGDLFIGRKA-QELRGLLKIYPMEHGIVTDWDDMERI	92
Ro_ARP1	GRPKHVRIMAGAV-----EGDNFIGRKA-QELRGLLKIYPIEHGIVTDWDDMERI	91
Ml_ARP1	GRPKHVRIMAGAV-----EGDNFIGRKA-QELRGLLKIYPIEHGIVTDWDDMERI	91
Cn_ARP1	GRPKHPRVMAGAI-----QDNLFIGNRA-QEFRGLLKIYPMEHGVVMDWDDMERI	91
Cc_ARP1	--LFHERVMAGAL-----EGDVFIGRKA-QEFRGLLKIYPMEHGIVTNWDDMERI	104
Sco_ARP1	GRPKHVRVMAGAL-----EGDVFIGKRA-QEFRGLLKIYPMEHGIVTDWDDMERI	91
An_ARP1	GRPKHPRVMAGGL-----EGDVFIGQRA-QELRGLMKIRYPLEHGIVTDWDDMEKI	88
Mo_ARP1	GRPKHLKVLALAGAL-----EGDVFIGEKAATELRGLLKIYPLEHGIVTDWDDMEKI	88
Sm_ARP1	GRPKHLRVLALAGAL-----EGEVFIGQKAATELRGLLKIYPLEHGIVTDWDDMEKI	88
Nc_ARP1	GRPKHLRVLALAGAL-----EGEVFIGQKAASELRGLLKIYPLEHGIVTDWDDMEKI	88
Cg_ARP1	GRPKHLRVLALAGAL-----EGEVFIGQKAATELRGLLKIYPLEHGIVTDWDDMEKI	88
Fo_ARP1	GRPKHLRVLALAGAL-----EGEVFIGQKASTELRGLLKIYPLEHGIVTDWDDMERI	88
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Sc_ARP1	WSYVLNEVLQLQNIGEHPLLLITEAPMNP LKNREQMAQVLFETFDVSALYVSNPAVLSLYA	152
Sp_ARP1	WSYIYSDQQLNTLPEEHPLLLTEPPLANIRNKEKIAEYFYETLNVFALSFSLQPV LALYA	149
Ca_ARP1	WYHTYTQ-ELKTQSEDHPLLLITEAPLNPRSNRNKMCQILFENFNIPCIYTSIQAVLSLYA	155
Pc_ARP1	WQFIYTE-ELKTVSEEHPVLLTEAPLNPRTNRDQAAQVFFETFNVPALFTSIQAVLSLYA	148
Um_ARP1	WNHVYSD-ELETLSEEHPVLLTEAPLNPRSNRDMAAQIFFETFNVPALFTSIQAVLSLYS	151
Ro_ARP1	WQYIYTD-ELKTLSEEHPVLLTEAPLNPRANRD TAAQIFFETFNVPALFTSIQAVLSLYS	150
Ml_ARP1	WQYIYTD-ELKTLSEEHPVLLTEAPLNPRANRD TAAQIFFETFNVPALFTSIQAVLSLYS	150
Cn_ARP1	WGWWYGE-GLKALSEEHPVLLTEAPLNPRQN RDIAAQIFFETFNVPAFFTSVQAVLSLYS	150
Cc_ARP1	WNWVYAE-ELGTLSEEHPVLLTEAPLNPR TNRDVAAQIFFDTFNVPALFTSVQAVLSLYS	163
Sco_ARP1	WSWVYAE-ELGTLSEEHPVLLTEAPLNPR SNRDIAAQIFFDTFNVPAMFTSVQAVLSLYS	150
An_ARP1	WHYVYEN-ELKTLPEEHPVLLTEPPLNPRKN RDIAAQIMFETFNVPALYTSIQAVLSLYA	147
Mo_ARP1	WGYVYEE-GLKVLSEEHPVLLTEPPLNPRAN RD TAAQILFETFNVPALYTSIQAVLSLYA	147
Sm_ARP1	WAYVYDE-GLKTLSEEHPVLLTEPPLNPRAN RD TAAQILFETFNVPALYTSIQAVLSLYA	147
Nc_ARP1	WAYVYDE-GLKTLSEEHPVLLTEPPLNPRAN RD TAAQILFETFNVPALYTSIQAVLSLYA	147
Cg_ARP1	WEYVYGE-GLKTLSEEHPVLLTEPPLNPR SNRD TAAQILFETFNVPALYTSIQAVLSLYA	147
Fo_ARP1	WEYVYGE-GLKTLSEEHPVLLTEPPLNPR SNRD TAAQILFETFNVPALHTSIQAVLSLYA	147
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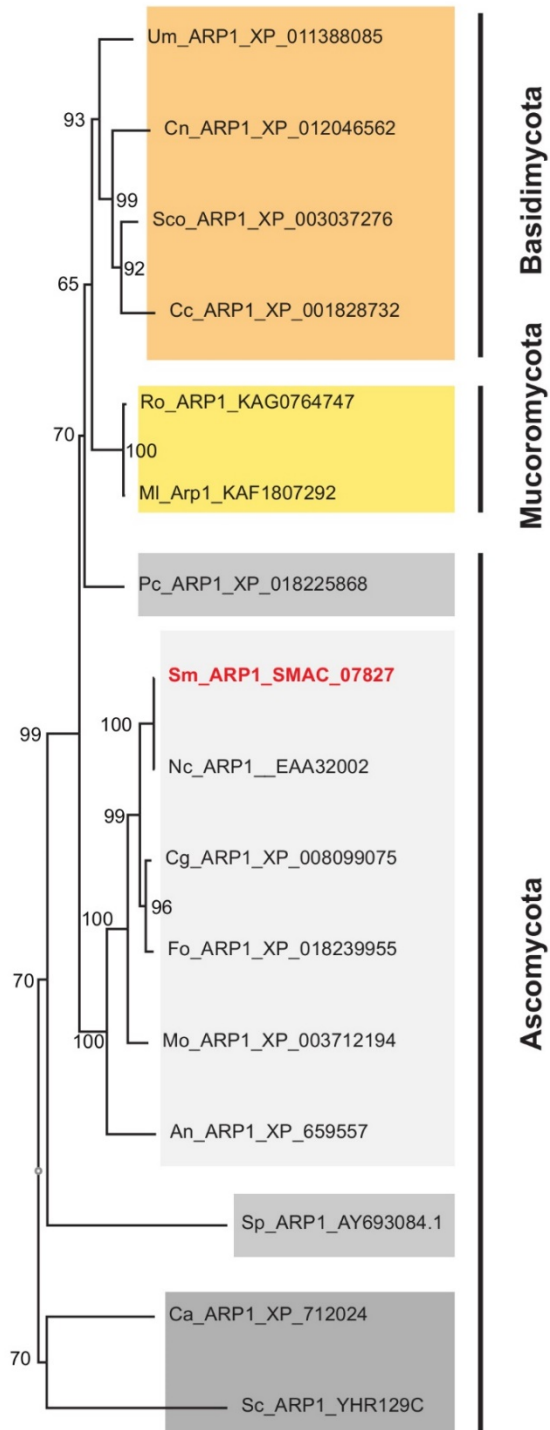
Sc_ARP1	SGRTTGCVVDCGEGYCVSTVPIYDGFALPASM MRMDIGG-----ADITEQLQFQLRKS	204
Sp_ARP1	SARTTGIVLECGDGLTHSVPIYDGF SIPS AIQQEEIGG-----RDVTDYLQLQLR-K	200
Ca_ARP1	SGRTTGVVIDSGDGVTHVPVYEGFALPPS IKRMDIAG-----RDITESLAFNIRRM	207
Pc_ARP1	SGRTTGVVLDSDGDGVTHAVPIYEGFAMP SAIRRIDIAG-----RDVTEYLQLLLR-K	199
Um_ARP1	SGRTTGIVLDSDGDGVTHAVPVYEGFSMP NAIRRVDVAG-----RDVTENLQTHLR-K	202
Ro_ARP1	SGRTTGIVLDSDGDGVTHAVPVYEGFAIP HAIRRVDIAG-----RDVTEYLQLLLR-R	201
Ml_ARP1	SGRTTGIVLDSDGDGVTHAVPVYEGFAIP HAIRRVDVAG-----RDVTEYLQLLLR-K	201
Cn_ARP1	SGRTTGIVLDSDGDGVTHAVPVFEGFSMP HAVRRIDLAG-----RDITDHLQLLLR-K	201
Cc_ARP1	SGRTTGIVLDSDGDGVTHAVPVYEGFSMP HAIRRVDVAGSSEHGPF SRDVTDHLQLLLR-K	222
Sco_ARP1	SGRTTGIVLDSDGDGVTHAVPVFEGFSMP HAIRRVDVAG-----RDVTDHLQLLLR-K	201
An_ARP1	SGRTTGVVLDSDGDGVSHAVPVYEGFAIP NSIRRIDVAG-----RDVTEQLQLLLR-K	198
Mo_ARP1	SGRTTGIVLDSDGDGVSHAVPVYEGFAIT NSIQRIDVAG-----RDVTEHLQTLLR-K	198
Sm_ARP1	SGRTTGVVLDSDGDGVSHAVPVYQGFTVP NSIRRIDVAG-----RDVTEYLQTLLR-K	198
Nc_ARP1	SGRTTGVVLDSDGDGVSHAVPVYQGFTVP NSIRRIDVAG-----RDVTEYLQTLLR-K	198
Cg_ARP1	SGRTTGIVLDSDGDGVSHAVPVYEGFAMP SSIRRIDVAG-----RDVTEYMQMLLR-K	198
Fo_ARP1	SGRTTGIVLDSDGDGVSHAVPVYEGFAMP SSIRRIDVAG-----RDVTEYMQTLLR-K	198
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Sc_ARP1	AGVSLFSSSEREIVRTMKEKVCYLAKNIKKEE KYLQGTQ-----DLISTFKLPDGRCI	258
Sp_ARP1	SGHELVSAAEKEIVREIKEKCCYVASDFR SEIESWTEHK-----PQIHTYQLPDNQTI	253
Ca_ARP1	TGVALQSSSELEIVRLIKEQNCFISKDPVR DEKKYKGSHYSRNNPNNELMSTYKLPDGHEI	267
Pc_ARP1	SGTIFHTSAEKEIVRIIKEKCSYVTLDP RKEEKEWINASISGGKDYTKEEEFKLPDGNVL	259
Um_ARP1	AGYHLHTSAEKELVRIIKEKTCYVALNP TKEEKDTP-----QHEEFKLPDGNTI	251
Ro_ARP1	SGYNFHTTAEKEVVRIIKEKTCYIALNPV KEEKETS-----GKVDDFMLPDGNII	251
Ml_ARP1	SGYNFHTTAEKEVVRIIKEKTCYIALNP KEEKETS-----GKVDDFMLPDGNII	251
Cn_ARP1	AGHNLHTSAEKEVVRTIKEKTCYIALNPV KEEKDQG-----GAWEERFRLPDGKVI	251
Cc_ARP1	AGHHLHTSAEREVVRTIKEKCCYVALNP IKEEKDSL-----GRTEEFRLPDGNIV	272
Sco_ARP1	AGHHLHTSAEREVVRTIKEKCCYIPISPA KEKDTN-----GRTEDFRLPDGNVV	251
An_ARP1	TGHVLTHTSAEKEVVRMIKEKVCSVSLDP KREEKEWMNSYKSES----KHADYVLPDGHKI	254
Mo_ARP1	SGYVFHTSAEKEVVRLIKEATSYVAHD PKKEEKEWAGAKLDPSK---MMSDYTLPDGNKI	255
Sm_ARP1	SGYVFHTSAEKEVVRLIKESVTYVAHD PRKEEKEWAAAKMDPAK---I-AEYVLPDGNKL	254
Nc_ARP1	SGYVFHTSAEKEVVRLIKESVTYVAHD PRKEEKEWAAAKMDPAK---I-AEYVLPDGNKL	254
Cg_ARP1	SGYVFHTSAEKEVVRLIKESVTYVAHD PRKEEKEWANRT-DPTK---G-VEYILPDGHKL	253
Fo_ARP1	SGYVFHTSAEKEVVRLIKESVSYVAHD PRKEERDWWGVKPNESK---F-AEYVLPDGFKL	254
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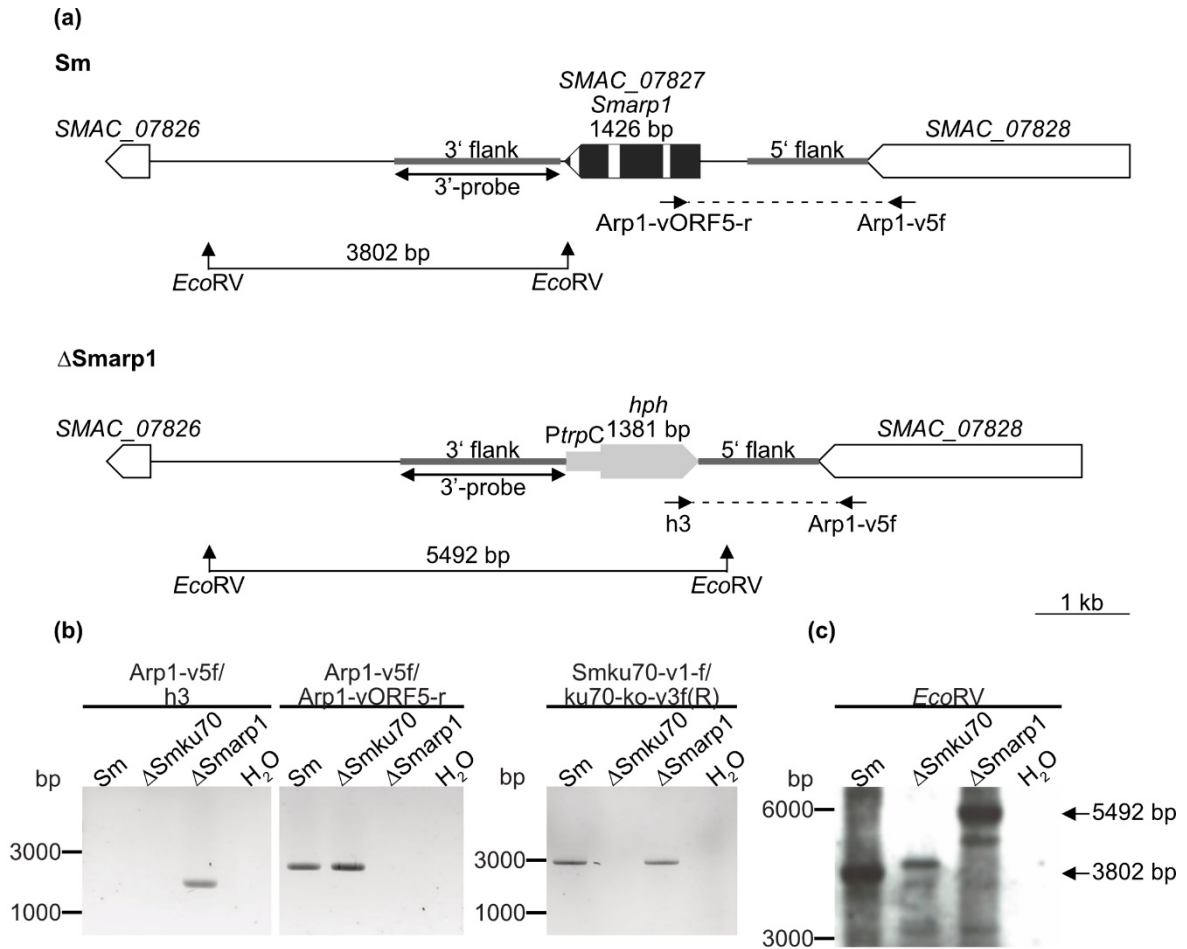
Sc_ARP1	EVGNDRYRAPEILFSPQIIGLGYDGLSDM CMQSIWKVDLDRKPLLSIIILSGGTTLTKG	318
Sp_ARP1	TLGTECFSAPEVLFPNPEMMGSEASGLHI QLFKSILLSDIDLRLSTLYSNIVLSGGSTLLRG	313
Ca_ARP1	QLGVERFRATEILFNPQLIGHESPGIHE LTSIAIAKTDLRLSTLYQNIILSGGNTLLKN	327
Pc_ARP1	RLGAERFRAPEILFDPEIIGSEYSGIHQ VVVD AISRVLDLDRKSIFGNIVLSGGSTLTRG	319
Um_ARP1	RLGAERFRAPEILFNPELIGQEFPGIHQ VIVDS INRTDLDLRKNLFSNIVLSGGSTLTKG	311
Ro_ARP1	KLGAERFRAPEILFQPELIGEEYPGIHQ VIVDC ISRADLDRKSISYSNVLSGGSTLCKG	311
Ml_ARP1	KLGAERFRAPEILFQPELIGEEYPGIHQ VIVDC IGRADLDRKSISYSNVLSGGSTLCKG	311
Cn_ARP1	QLGTERFLAPEILFNPPELVGQEPGVHQ VIVDS INRTDLDLRKSIFSNIVLSGGSTLCTG	311
Cc_ARP1	QLGAERFRAPEILFDPELIGQEYAGVHQ VVVD SINRVLDLDRKSIFSNIVLSGGSTLCRG	332
Sco_ARP1	QLGPERFRAPEILFNPPEIIGQEYAGVHQ VIVDAITRVLDLDRKSISYSNIVLSGGSTLTKG	311
An_ARP1	KIGQERFRAPEILFDPELIGLEYPGVHQ IVD AII RTDLDLRKSILYNIVLSGGSTLCKN	314
Mo_ARP1	KLGAERFRAPEILFDPEIIGLEYPGVHQ MVINAIGKTDLDRKSISYSNIVLSGGSTLTKG	315
Sm_ARP1	KIGAERFRAPEILFDPEIIGLEYPGVHQ IVD SINRTDLDLRDLYSNIVLSGGSTLTKG	314
Nc_ARP1	KIGAERFRAPEILFDPEIIGLEYPGVHQ IVD SINRTDLDLRDLYSNIVLSGGSTLTKG	314
Cg_ARP1	KIGAERFRAPEILFDPEIIGLEYPGVHQ IVD AINRTDLDLRKSISYSNIVLSGGSTLTRG	313
Fo_ARP1	KIGAERFRAPEILFDPEIIGLEYPGVHQ IVD AINRTDLDLRKSISYSNIVLSGGSTLTKG	314
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Sc_ARP1	FGDRMLWDLEALT-----KGTSKIKI IAPSERKYTTWIGGSILT	357
Sp_ARP1	FGERFISELRAIS-----GKKNQVKIYASPERMHNWLGGSSILA	352
Ca_ARP1	FGDRMLKEIKDLQQPLQEDQKMSIWNKNVQNDVYNTKMKVKIYAPPERKYSTWIGGSILA	387
Pc_ARP1	FGDRLLSEIRRLA-----VKDVKIKI FAPPERKYSTWIGGSILA	358
Um_ARP1	FGDRMLAEVKRLA-----VREMKIKI FAPPERKYATWIGGSILA	350
Ro_ARP1	FGERLLSEIKRLA-----LKDIKIKIYAPPERKYSTWIGGSILA	350
MI_Arp1	FGERLLSEIKRLA-----LKDIKIKIYAPPERKYSTWIGGSILA	350
Cn_ARP1	FGDRLLNEVKKLA-----VKDVKLKIYAPPERKYSTWIGGSILA	350
Cc_ARP1	FGDRLLNEVKKLA-----LKDIKIKIYAPPERKYSTWIGGSILA	371
Sco_ARP1	FGDRLLNEVKKLA-----LKDIKIKIYAPPERKYSTWIGGSILA	350
An_ARP1	FPDRMLREIKRLA-----VEDMKIRISAPAERKYTTWIGGGILA	353
Mo_ARP1	FGDRLLTEVQKLA-----VRDMRIKIFAPPERKYSTWIGGSILA	354
Sm_ARP1	FGDRLLTEVQKLA-----VKDMRIKIFAPPERKYSTWIGGSILA	353
Nc_ARP1	FGDRLLTEVQKLA-----VKDMRIKIFAPPERKYSTWIGGSILA	353
Cg_ARP1	FGDRLLTELQRLA-----VKDMRIKIFAPPERKYSTWIGGSILA	352
Fo_ARP1	FGDRLLTELQKLA-----VKDMRIKIFAPPERKYSTWIGGSILA	353
* :*:: :. :		:::* * ** : :*:**.*:
Sc_ARP1	GLSTFQRLWTKKSDWLEDSTRVYSNLM*	384
Sp_ARP1	SLSTFRRLITSEEYKNDQNVIFRRRF-	379
Ca_ARP1	GLSTFKKMWVTSEEYHENPDIVHVKCM-	414
Pc_ARP1	SLSTFRKMWVSAAEYQEDPDI IHRKSI-	385
Um_ARP1	GLSTFKRMWVSAAEYNEDPDVIHKKTF-	377
Ro_ARP1	SLSTFKKMWVSAAEYQEDPDI IHHKTF-	377
MI_Arp1	SLSTFKKMWVSAAEYQEDPDI IHHKTF-	377
Cn_ARP1	GLSTFKKMWVSAD EYKEDPDI IHHKAF-	377
Cc_ARP1	GLSTFKKMWVSAAEYQEDPDI IHHKFGF	399
Sco_ARP1	GLSTFKKMWVSAAEYQEDPDI IHHKTFG	378
An_ARP1	GLSTFRKMWVSAD EWHEDPEI IHRKFA-	380
Mo_ARP1	GLSTFRKMWVSMD DWHENPDI IHTKFN-	381
Sm_ARP1	GLSTFRKMWVSID DWHENPDI IHTKFT*	380
Nc_ARP1	GLSTFRKMWVSID DWHENPDI IHTKFT-	380
Cg_ARP1	GLSTFRKMWVSID DWHENPEI IHTKLT-	379
Fo_ARP1	GLSTFRKMWVSID DWHENPDI IHTKFT-	380
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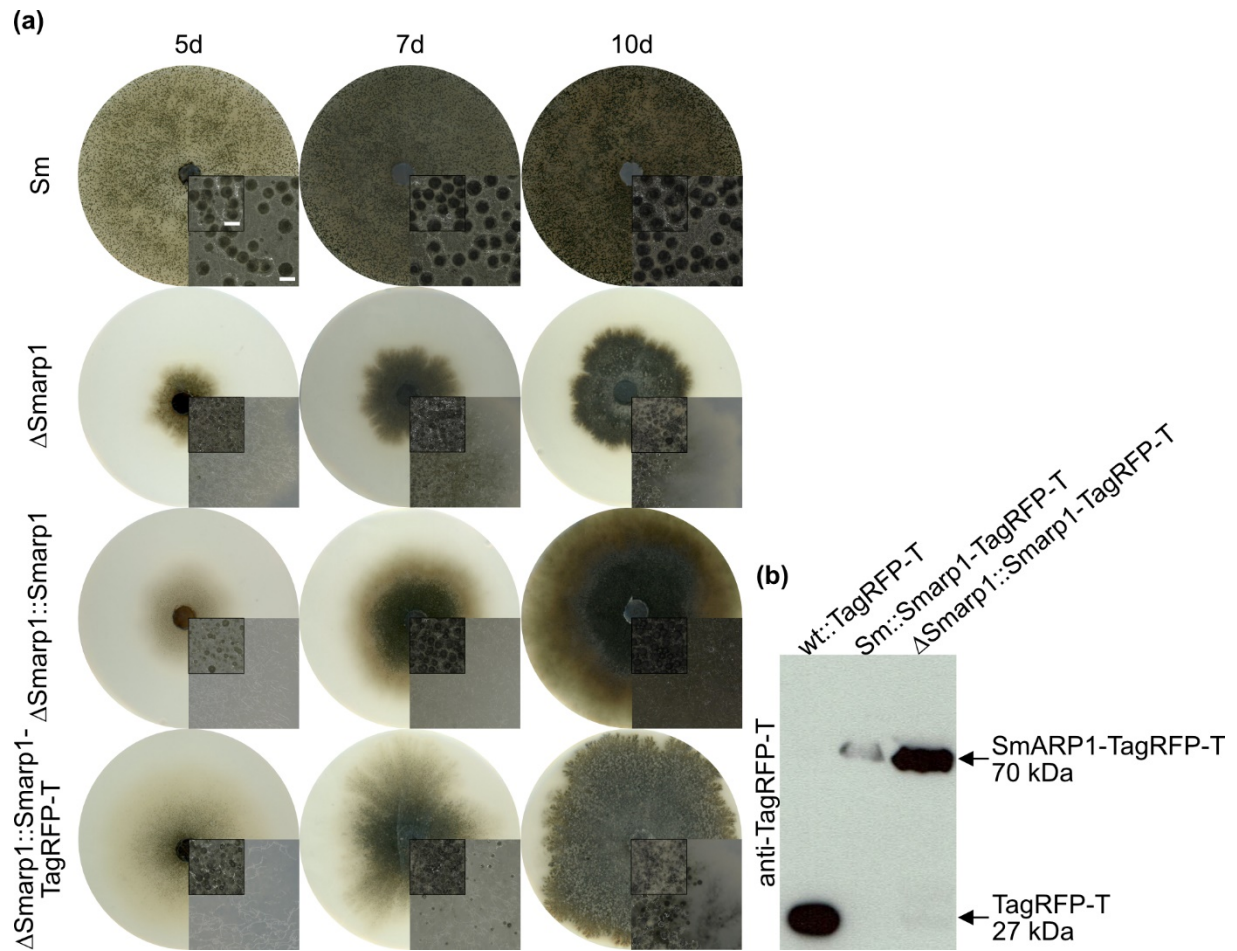
**Figure S1.** CLUSTAL Omega (1.2.4) multiple sequence alignment of fungal ARP1 orthologs. Accession numbers are indicated in Figure S2 (phylogenetic tree). Abbreviations: Sm, *Sordaria macrospora*; Nc, *Neurospora crassa*; Cg, *Colletotrichum graminicola*; Fo, *Fusarium oxysporum* f. sp. *Lycopersici*; Mo, *Magnaporthe oryzae*; An, *Aspergillus nidulans*; Sc, *Saccharomyces cerevisiae*; Ca, *Candida albicans*; Pc, *Pneumocystis carinii*; Sp, *Schizosaccharomyces pombe*; Cn, *Cryptococcus neoformans* var. *grubii*; Um, *Ustilago maydis*; Cc, *Coprinopsis cinerea*; Sco, *Schizophyllum commune*; Ro, *Rhizopus oryzae*; MI, *Mucor lusitanicus*. . In the conservation line, asterisk indicates positions, which have a single, fully conserved residue; colon indicates conservation between groups of strongly similar properties; and period indicates conservation between groups of weakly similar properties.



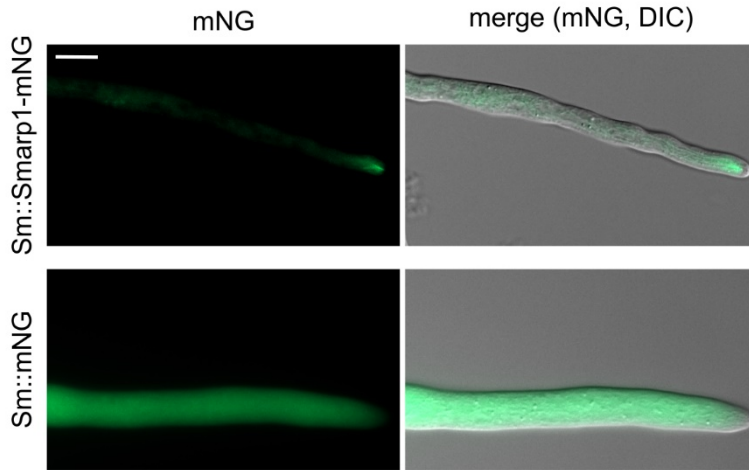
**Figure S2.** Phylogenetic tree of Arp1 orthologs from fungi. The phylogenetic tree of Arp1 was generated with the Neighbor Joining method. Orthologs were identified with BLASTP search using amino acid sequences of the *Sordaria macrospora* SmARP1 (SMAC\_07827) indicated in red. The multiple sequence alignment and phylogenetic analysis was performed with MAFFT version 7 [1]. Bootstrap values based on 1000 replications are rounded to whole numbers and are indicated at the nodes. Accession numbers are indicated. Abbreviations: Sm, *Sordaria macrospora*; Nc, *Neurospora crassa*; Cg, *Colletotrichum graminicola*; Fo, *Fusarium oxysporum* f. sp. *Lycopersici*; Mo, *Magnaporthe oryzae*; An, *Aspergillus nidulans*; Sc, *Saccharomyces cerevisiae*; Ca, *Candida albicans*; Pc, *Pneumocystis carinii*, Sp, *Schizosaccharomyces pombe*; Cn, *Cryptococcus neoformans* var. *grubii*; Um, *Ustilago maydis*; Cc, *Coprinopsis cinerea*; Sco, *Schizophyllum commune*; Ro, *Rhizopus oryzae*; MI, *Mucor lusitanicus*.



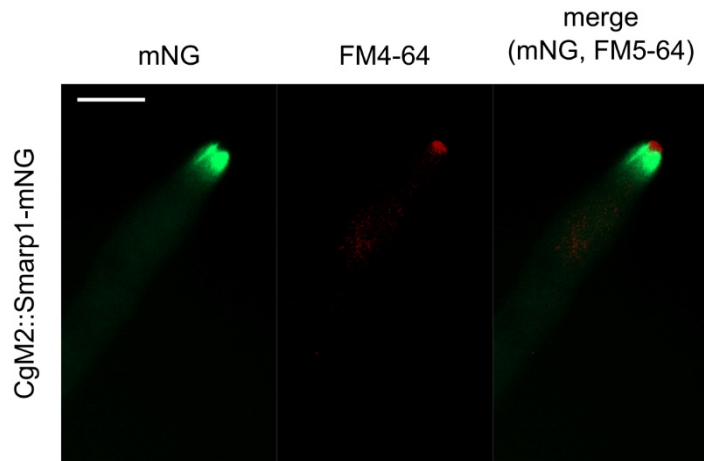
**Figure S3.** Verification of *S. macrospora Smarp1* deletion by PCR and Southern blot analysis. (a) Schematic representation of the *Smarp1* ORF SMAC\_07827 (black arrow) with three introns (white boxes). White arrows indicate adjacent ORFs. After Golden-Gate cloning, the *hph* cassette replaced the *Smarp1* ORF. Primer binding sites for the verification of *hph* cassette integration (Arp1-v5f/ h3), presence of *Smarp1* gene (Arp1-v5f/ Arp1-vORF5-r), and probe amplification for Southern hybridization are indicated. Regions of probe binding and size of the expected DNA fragments generated by *EcoRV* hydrolyzation for Sm and Δ*Smarp1* are plotted. (b) PCR verification of the integration of the *hph* cassette into the *Smarp1* locus and the presence of *Smku70* gene in the *Smarp1* deletion mutant. (c) Confirmation of *Smarp1* deletion by Southern Blot analysis. Genomic DNA was hydrolyzed with *EcoRV*. Signals detected correspond to the fragment size of 3802 bp for Sm and Δ*Smku70* strains and 5492 bp for the Δ*Smarp1* strain (indicated by arrows).



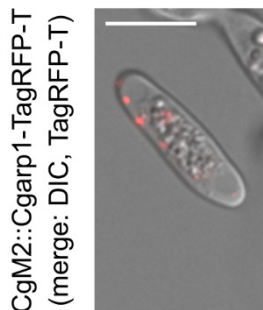
**Figure S4.** Growth of *S. macrospora*  $\Delta$ Smarp1 and complementing strains and corresponding expression controls via Western blot analysis. (a) Phenotypic analysis of *S. macrospora* wt (Sm),  $\Delta$ Smarp1 and the complementation strains  $\Delta$ Smarp1::Smarp1 and  $\Delta$ Smarp1::Smarp1-TagRFP-T. Strains were grown on SWG medium at 27 °C. Pictures of the agar plates and microscopic images were taken after indicated days, scale bar = 0.5 mm. (b) Western blot analysis of *S. macrospora* wt (Sm) and  $\Delta$ Smarp1 ectopically expressing SmARP1-TagRFP-T, wt::TagRFP-T served as control. Expected protein sizes are indicated.



**Figure S5.** Localization of SmArp1-mNG and free mNG in *S. macrospora*. Heterologous expression of pSmarp1-mNG in *S. macrospora* wildtype hyphae. Recording of hyphae from growing strains on BMM-covered glass slides after incubation for 24 h at 27 °C. SmArp1-mNG shows comet-like localization at the hyphal tip. Expression of mNG serves as control, size bar = 10  $\mu$ m.



**Figure S6.** Localization of Arp1 and FM4-64 in *C. graminicola*. Heterologous expression of pSmarp1-mNG in *C. graminicola* wildtype CgM2 hyphae. Recording of hyphae from growing colonies on CM topped with cellophane after incubation for 3 d at 23 °C. Hyphae were stained with 1  $\mu$ g/ ml FM4-64 for 15 min at 37 °C, size bar = 10  $\mu$ m.



**Figure S7.** Localization of Arp1 in unpolarized, non-germinated oval conidia of *C. graminicola*. Oval conidia of CgM2 expressing endogenous Arp1-TagRFP-T were incubated to rate the chemotropic response to 50 mM glucose (glc) for 6 h, 23 °C in the 3D printed device, size bar = 10  $\mu$ m.

## Tables

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

Oligonucleotide	Sequence (5' to 3')
Arp1_promotor_f	TAAAACGACGGCCAGTGAGCGCGCGTAATTCAAAAATGGTTCGTGC ACACAAA
Arp1-Neo_r	ATGTTATCCTCCTCGCCCTTGCTCACCATTGTAAACTTCGTATGAATG ATATCCGGA
Neo_f	ATGGTGAGCAAGGGCGAG
Neo_TtrpC_r	GTTTGATGATTTTCAGTAACGTTAAGTGGACTACTTGTACAGCTCGTCC ATGC
TtrpC_f	TCCACTTAACGTTACTGAAATCATCAAAC
TtrpC_pRS_r	GCGGATAACAATTTACACAGGAAACAGCTCGAGTGAGATGTGGA GTGG
Arp1_5'f	TAAAACGACGGCCAGTGAGCGCGCGTAATTCAAAAATGGTTCGTGC ACA
Arp1_RFP_r	TTAATCAGCTCTTCGCCCTTAGACACCATTGTAAACTTCGTATGAATG A
RFP-f	ATGGTGTCTAAGGGCGAAGAG
RFP-r-trpC	TTTGATGATTTTCAGTAACGTTAAGTGGATTTACTTGTACAGCTCGTCC ATGC
TtrpC_F	GATCCACTTAACGTTACTGAAATCATCAA
pRS426GFPprev	GCGGATAACAATTTACACAGGAAACAGCTCGAGTGAGATGTGGA GTG
5'CgArp1_pJet_fw	GTTTTTCAGCAAGATATCGCCAGATTTCTAGGCCCC
CgArp1_rev	CGTAAGCTTGGTGTGTATGATCT
tagRFP-	CACACCAAGCTTACGATGGTGTCTAAGGGCGAAG
T_CgArp1_fw	TCGAGTGAGATGTGGAGTG
TtrpC_r	CACATCTCCACTCGATATCTCAGAAGAACTCGTCAAGAAGG
nptII_TtrpC_fw	ATCTTCTAGAAAGATCTTGGCTGGAGCTAGTGGA
PttrpC_pJet_rev	TCACTTCGACCCCTTACTAAC
Arp1-v5f	gtactcgccgatagtggaaac
h3	AAACACCTCGCCCTCTAATGC
Arp1-vORF5-r	CATCGAGGTGAGCAAGTCAATG
Smku70-v1-f	GCGCAACTCCAGCGTGACTG
ku70-ko-v3f(R)	TAGGGCGAATTGGGTACCG
GG_KO_fw	GGCCGCTCTAGAACTAGTG
GG_KO_rv	GACTGGTCTCA AGTCCCCTTGGTGCAGTACATGCTC
Arp1-ko-5f	CAGAGGTCTCA GCAGTGGGAGGGAATGTCTTCAAGA
Arp1-ko-5r	GTACGGTCTCG GTCATGATGATGACGACGACGA
Arp1-ko-3f	CTCAGGTCTCC CGTAACGATATGAAGCTATGAAGAC
Arp1-ko-3r	GTAACGCCAGGGTTTTCCCAGTCACGACG
Arp1-nat-5f	CCTTGGTGCAGTACATGCTC
Arp1-nat-3r	GCGGATAACAATTTACACAGGAAACAGC ACGATATGAAGCTATGAAGA



**Table S2.** Plasmids used in this study.

Name of plasmid	Features	Reference
pRS-nat	<i>amp<sup>R</sup>, ura3, nat<sup>R</sup></i>	[2]
pRS-hyg	<i>amp<sup>R</sup>, ura3, hyg<sup>R</sup></i>	[3]
p1783-1	<i>amp<sup>R</sup>, ura3, hyg<sup>R</sup>, Pgpd::egfp::TtrpC</i>	[4]
pxyl-mNG	<i>amp<sup>R</sup>, ura3, nat<sup>R</sup>, Pxyl::mNG::TtrpC</i>	[5]
pTagRFP-T_nat	<i>amp<sup>R</sup>, nat<sup>R</sup>, Pccg1::TagRFP-T::TtrpC</i>	[6]
pDest-Amp	Destination vector for Golden-Gate cloning; <i>bla</i> ( <i>Bsamut</i> ), <i>lacZ</i> gene with two internal <i>BsaI</i> sites: <i>BsaI</i> (4) and <i>BsaI</i> (7)	[7]
pGG-hph	<i>BsaI</i> (6)::trpC(p)::hph::BsaI(5) in pDrive; <i>bla</i> , <i>kan</i>	[7]
pArp1-KO	<i>amp<sup>R</sup>, 5'-flanking region and 3'-flanking region of Smarp1 interrupted by hyg<sup>R</sup> in pDest-Amp</i>	this study
pSmarp1	<i>amp<sup>R</sup>, ura3, nat<sup>R</sup>, Smarp1P::Smarp1::Smarp1T</i>	this study
pSmArp1-TagRFP-T_nat	<i>amp<sup>R</sup>, ura3, nat<sup>R</sup>, Smarp1P::Smarp1::TagRFP-T::TtrpC</i>	this study
pSmArp1-TagRFP-T_hyg	<i>amp<sup>R</sup>, ura3, hyg<sup>R</sup>, Smarp1P::Smarp1::TagRFP-T::TtrpC</i>	this study
pSmArp1-mNG	<i>amp<sup>R</sup>, ura3, nat<sup>R</sup>, Smarp1P::Smarp1::mNG::TtrpC</i>	this study
pII99	5.3-kb <i>nptII</i> , <i>amp<sup>R</sup></i>	[8]
pJet1.2	<i>amp<sup>R</sup></i>	ThermoFisher Scientific
pCgArp1-TagRFP-T	<i>amp<sup>R</sup>, gen<sup>R</sup>, Cgarp1P::Cgarp1::TagRFP-T::TtrpC</i>	this study
pJet_gen	<i>amp<sup>R</sup>, gen<sup>R</sup></i>	this study

*nat<sup>R</sup>*: nourseothricin resistant, *hyg<sup>R</sup>*: hygromycin resistant; *gen<sup>R</sup>*: geneticin disulphate resistant; *amp<sup>R</sup>*: ampicillin resistance; *kan<sup>R</sup>*: kanamycin resistance, *ura3*: Orotidine-5'-phosphate decarboxylase gene of *S. cerevisiae*; *Pgpd*: promoter of the glycerinaldehyd-3-phosphat-dehydrogenase-gene of *A. nidulans*; *Pccg1*: promoter of the clock controlled gene 1 of *N. crassa*; native promoters of genes are named 5'; *TtrpC*: terminator of the anthranilate synthase gene of *A. nidulans*; *mNG*: gene for green fluorescence protein monomeric NeonGreen (mNG) of *Branchiostoma lanceolatum*; *egfp*: gene for enhanced green fluorescent protein (eGFP) of *Aequorea Victoria*; *TagRFP-T*: gene for red fluorescence protein TagRFP-T of *Entacmaea quadricolor*, *nptII*: neomycin phosphotransferase gene

## Videos

**Video 1.** Dynamic localization of SmArp1-mNG to growing hyphal tips. Heterologous expression of pSmarp1-mNG in *S. macrospora* wildtype hyphae. Recording of hyphae from growing strains on BMM-covered glass slides after incubation for 24 h at 27 °C, recording interval = 5 s, size bar = 10 µm.

**Video 2.** Nuclear localization of SmArp1-mNG. Heterologous expression of pSmarp1-mNG in *S. macrospora* wildtype hyphae. Nuclei are labeled by histone 2B with tdTomato (RH2B). Recording of hyphae from growing strains on BMM-covered glass slides after incubation for 24 h at 27 °C, recording interval = 5 s, size bar = 10 µm.

**Video 3.** Dynamic localization of CgArp1-TagRFP-T in growing *C. graminicola* germlings. Heterologous expression of CgArp1-TagRFP-T in *C. graminicola* wildtype CgM2. Tracking of Arp1-TagRFP-T in germlings derived from oval conidia after 2 h of incubation on water agar, 23 °C. Recording interval = 5 min, size bar = 10 µm.

**Video 4.** Dynamic localization of SmArp1-mNG in *S. macrospora* germinating ascospores. Germinating *S. macrospora* ascospores which express Smarp1-mNG and red fluorescent histone 2B (RH2B tdTomato) on BMM-agar supplemented with agarose after incubation for 3-4 h at 27 °C, recording interval = 5 s, size bars = 10 µm.

**Video 5.** Establishment of a polar Arp1-TagRFP-T localization prior to germination of *C. graminicola*. Heterologous expression of CgArp1-TagRFP-T in *C. graminicola* wildtype CgM2. Tracking of Arp1-TagRFP-T in oval conidia after 2 h of incubation on water agar, 23 °C. Recording interval = 5 min, size bar = 10 µm.

**Video 6.** Tracking of Arp1-RFP-T during the germination of *C. graminicola* oval conidia. Heterologous expression of CgArp1-TagRFP-T in *C. graminicola* wildtype CgM2. Tracking of Arp1-TagRFP-T in oval conidia after 2 h of incubation on water agar, 23 °C. Recording interval = 5 min, size bar = 10 µm.

**Video 7.** Visualization of *C. graminicola* penetration hyphae dynamics during onion epidermis perforation. 10<sup>3</sup> oval conidia of *C. graminicola* CgM2::Cgarp1-TagRFP-T were inoculated on heat-inactivated onion epidermis overlaying water agar for 29 h at 23 °C, recording interval = 0.5 µm, size bars = 10 µm.

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