

P-type ATPase Apt1 of the fungal pathogen *Cryptococcus neoformans* is a lipid flippase of broad substrate specificity

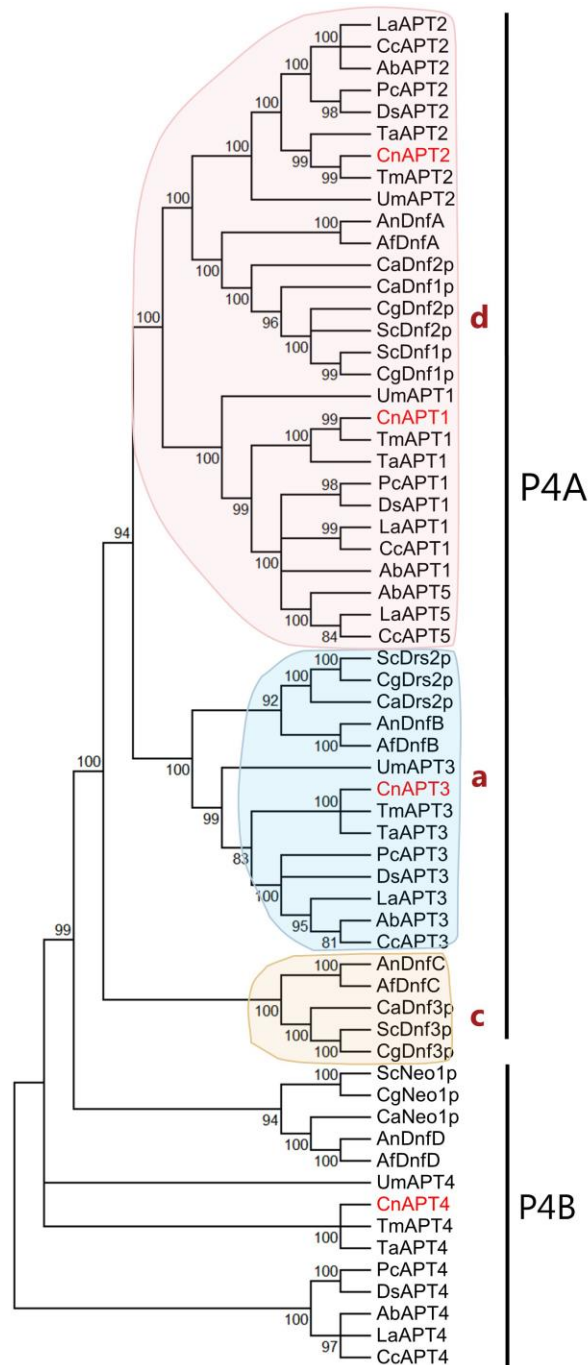
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Supplementary Information

This file includes all supplementary information for the manuscript:

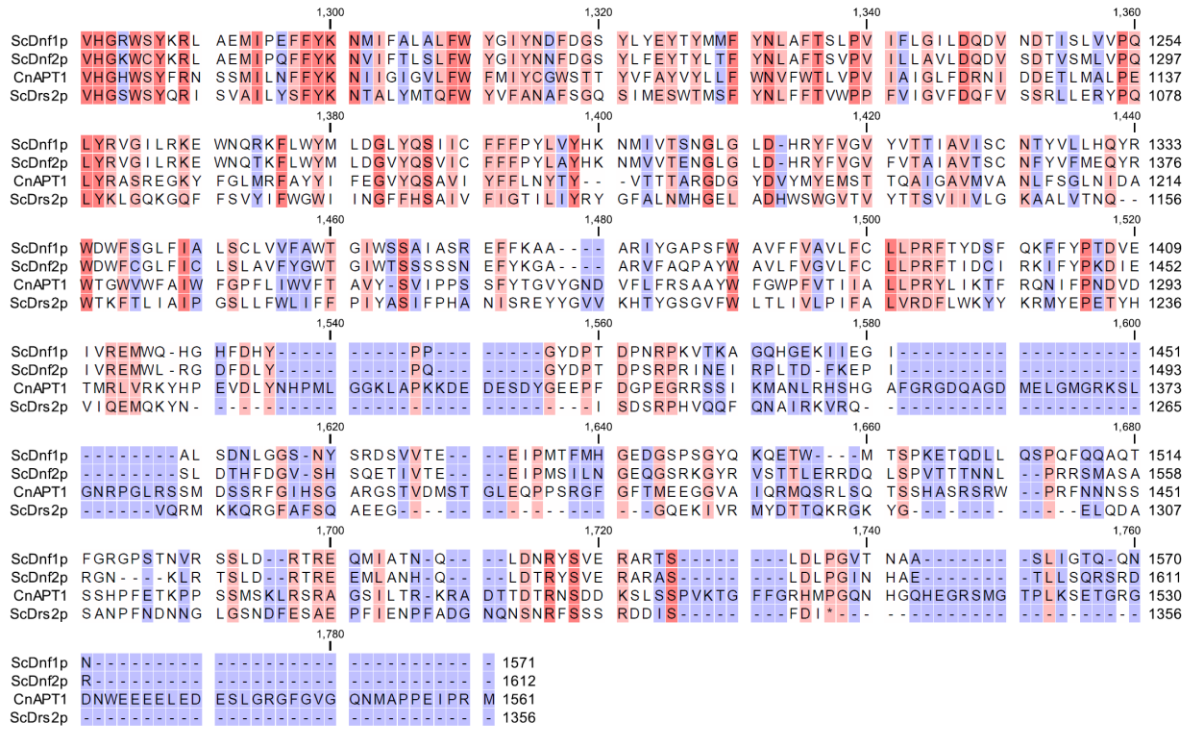
- Figures S1, S2 and S3

- Tables S1

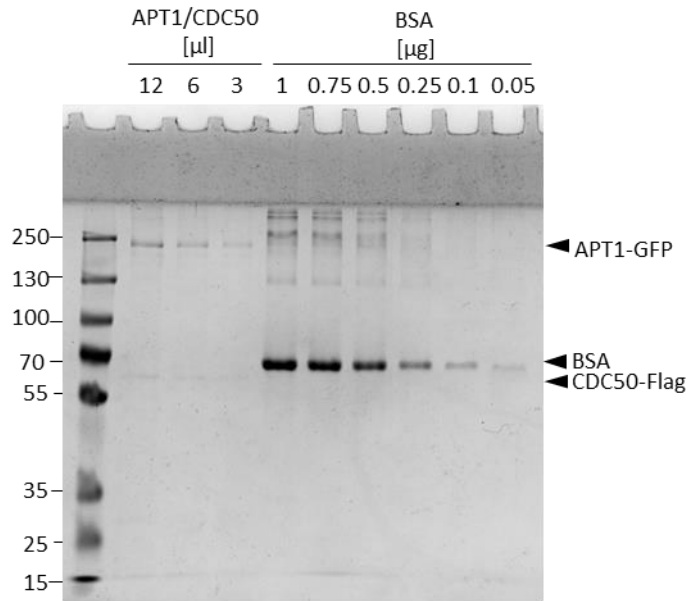


Supplementary Figure S1. Phylogenetic relations amongst fungal P4 ATPases. Phylogenetic tree of sequences representing all annotated P4-ATPases from selected yeast and fungi: *Saccharomyces cerevisiae* (Sc), *Cryptococcus neoformans* (Cn); *Candida albicans* (Ca), *Candida glabrata* (Cg), *Aspergillus nidulans* (An), *Aspergillus fumigatus* (Af), *Tremella mesenterica* (Tm), *Trichosporon asahii* (Ta), *Agaricus bisporus* (Ab), *Ustilago maydis* (Um), *Phanerochaete carnos* (Pc), *Laccaria amethystine* (La), *Coprinopsis cinerea* (Cc), *Dichomitus squalens* (Ds). The phylogenetic tree was inferred from maximum likelihood analysis with 1,000 bootstrap iterations performed using Mega X. Node values represent maximum likelihood statistical values with a maximum of 100%. All nodes with statistical values less than 80% were collapsed into multifurcations. In the phylogenetic tree, proteins from *C. neoformans* are indicated in red. Colored balloons indicate proteins that belong to the same phylogenetic cluster (small dark red letters), as described in [Palmgren et al., 2019, Biochim Biophys Acta Biomembr. 1861:1135-1151]. For accession numbers, see Materials and methods.

ScDnf1p	MSGTF----	-----HGDDG	HAP-----MS	PFEDTFQFED	NSS-NEDTHI	APTHFDDGAT	SNKYSRP--	-----QVS	53
ScDnf2p	MSSPSKPTSP	FVDDIEHESG	SASNGLSSMS	PFDDSFQFEK	PSSAHGNIEV	AKT---GGSV	LKRQSKPMKD	ISTPDL SKVT	77
CnAPT1	MGA-----	-----	-----	-----	-----	-----	-----SKP	-----	6
ScDrs2p	MNDRETTPK	RKPGEDD--	-----T	LFDIDFLDDT	TSHSGSRSKV	TNSHANGYI	PPSHVLPEET	IDL-----	61
ScDnf1p	FN--DETPK	NKREDAEFT	-----FN	DDTEYDNHSF	QPTPKLNNGS	GTFFDDVELDN	DSGEPHTNYD	G-----MKRF	117
ScDnf2p	FDGIDDYSDN	NDINDDDELN	GKKTEIHEHE	NEVDDDLHSF	QATPMPNTGG	--FEDVELDN	NEG---SNND	SQADHKLKRV	152
CnAPT1	-----	-----	-----	-----	-----PL--	-----	-----	-----	8
ScDrs2p	-----DADD	NIENDVHE--	-NLFMSNNHO	DQTSWNANRF	-----	-----DS	DAYQPQSLRA	VKPPGLFARF	115
ScDnf1p	RMGTRKRNKKG	NPIMGRSKTL	KWARKNI PNP	FEDF-TKDD-	IDPGAT---N	RAQEL-RTVY	YNMPLPKDMI	DEEGN-----	186
ScDnf2p	RFGTRRNKSG	RIDINRSKTL	KWAKKNFNHA	IDEFSTKEDS	LENSAL--QN	RSDEL-RTVY	YNLPLPEDML	DEEDGL-----	224
CnAPT1	-----VPRSKKH	NP-----	SWLDRNIVKP	LE-----S	LAPSKLFARR	RSPPVPRSVF	INEPLPSEYY	DKKKG-----	65
ScDrs2p	GNGLKNAATF	K-----RKGGP	ESFEMNHNA	VTN-----NE	LDDNYLDSRN	KFN--IKILF	NRYLRLKNVG	DAENGGEPRV	184
ScDnf1p	-----PIM	Q--YPRNKIR	TTKYTPLTFL	PKNILFQFHN	FANVYFLVLI	ILGAFQIFGV	TNPGLSAVPL	VVIVITAIK	257
ScDnf2p	-----PLA	V--YPRNKIR	TTKYTPLTFF	PKNILFQFHN	FANIYFLILL	ILGAFQIFGV	TNPGFASVPL	IVIVITAIK	295
CnAPT1	-----ILR	AHFATNQNV	TSKYTVITFI	PKNLFEQFSR	VANCFFLAIS	ILQFPKPFST	ISPLGLVILPL	IIVLAITALK	138
ScDrs2p	IHINDSLANS	SFGYSDNHIS	TTKYNFATFL	PKFLFQEFRR	YANLFLFCTS	AIQQVPHVSP	TNRYTITIGTL	LVVLIVSAAK	264
ScDnf1p	DAIEDSRRTV	LDLEVNNTKT	HILEGVENEN	VSTDNISLWR	RFKKANSRLL	FKFIQYCKEH	LTEEGKKKRM	QKRHRELVRQ	337
ScDnf2p	DGIEDSRRTV	LDLEVNNTKT	HILSGVKNEH	VAVDNVSLWR	RFKKANTRAL	IKIFEYFSEN	LTAAGREKKL	VKKREELRRK	375
CnAPT1	DGYEDIKRHQ	ADHRTNHAI	VHVLGGQDYTN	-----Q	NPMASKDKTF	IPAIPLPKR--	-----RSKKA	KKAEAEALN	203
ScDrs2p	ECIEDIKRAN	SKELNNSTI	EIFSEAHDD-	-----	-----	-----	-----	-----	293
ScDnf1p	KTVGTSGPRS	SLDSIDSYRV	SADYGRPSLD	YDNLEQGAGE	AN-----I	VDRSLPPRTD	CKFAKNYWK	VKVGDIIRVIH	410
ScDnf2p	RNSRSGFGRG	SLDSIGSYRM	SADYGRPSLD	YDNLEQGAGE	AN-----I	VDRSLPPRTD	CKFAKNYWK	VKVGDIIRVIH	455
CnAPT1	MQGRSS----	-----STENFAA	EPVPGAEPRG	QDELQRMRSQ	VSNWDEDPEA	GD--SPG-	LGWHRTIWD	VKVGDFVKIY	272
ScDrs2p	-----	-----	-----	-----	-----	-----FVEKRWID	IRVGDIIRVK	311	
ScDnf1p	NNDEIPADII	LLSTSDTGA	CYVETKNLDG	ETNLKVRQSL	KCTNTIRTSK	DIARTKFWIE	SEGPSNLYT	YQG--NMKW	487
ScDnf2p	NNDEIPADMI	LLSTSDTGA	CYVETKNLDG	ETNLKVRQSL	KCSKIIKSSR	DIARTKFWIE	SEGPSNLYS	YQG--NFKW	532
CnAPT1	ENEQFPADIV	ICATSEEDV	AYIETKNLDG	ETNLKSRNGV	PGLSHLNTAE	ACAKAHLCID	LDAPESNMFR	LNGAVINLEE	352
ScDrs2p	SEEPIPADTI	LLSSSEPEGL	CYIETANLDG	ETNLKIKQSR	VETAKFIDVK	TLKNMNGKVV	SEQPNSSLYT	YEGTM-----	386
ScDnf1p	RNLADGEIRN	EPITINNVL	RGCTLRNTKW	AMGVVMFTGG	DTKIMLNSGI	TPTKKSIRSR	ELNFSVINF	VLLFILCFVS	567
ScDnf2p	QDTQNGNIRN	EPVNINNLL	RGCTLRNTKW	AMGVVIFTGD	DTKIMINAGV	TPTKKSIRSR	ELNFSVILNF	VLLFILCFVA	612
CnAPT1	YD-EDEQHPI	HPITLETMTL	RGCVLKNTAW	VIGIIVYTG	DTKII RNAGA	TPSKRSKVEK	QMNQVQIINL	VILAAIVVC	431
ScDrs2p	-TLNDRQI--	-PLSPDMIL	RGATLRNTAW	IFGLVITFGH	ETKLLRNATA	TPIKRTAVEK	IINRQIIRLF	TVLAILILIS	462
ScDnf1p	GIANGVYYDK	KGRSRFSYEF	-GTIAGSAA	TNGFVSFWVA	VILYQSLVPI	SLYISVEIK	TAQAAFIYGD	VLLYNAK--L	643
ScDnf2p	GIVNGVYYKY	KPRSRDYFEF	-GTIIGGSAS	TNGFVSFWVA	VILYQSLVPI	SLYISVEIK	TAQAAFIYTD	VLLYNAK--L	688
CnAPT1	AVVDHVNVEE	WDRQAYWML	FADTSGDNPN	INGLVTANA	FITFQNI VPI	SLYISIEAVR	TIQAAFIYWD	RDIIKYKDG	511
ScDrs2p	SIGN-VIMST	ADAKHLSYLY	LEGTNKAGLF	FKDLFTFW-	-ILFSNLVPI	SLFVTVLEIK	YQAFMIGSD	LDLYYEK--T	536
ScDnf1p	DYPCTPKSWN	ISDDLGGQVEY	IFSDKTGTLT	QNVMEFKKCT	INGVSY-GRA	YTEALAGLRK	RQGDIVETEG	RREKAEIAKD	722
ScDnf2p	DYPCTPKSWN	ISDDLGGQVEY	IFSDKTGTLT	QNVMEFKKCT	INGVSY-GRA	YTEALAGLRK	RQGDIVETEG	RREKAEIAKD	767
CnAPT1	TTRTTARSWN	LSDDLGGQIEY	IFSDKTGTLT	QNAMIFROQS	VGGKIYTDGD	LPPSHPTITH	QHQPVPVHGH	DDQDDPIAKS	591
ScDrs2p	DTPTVVTRSS	LVEELGGQIEY	IFSDKTGTLT	RNIMEFKSK	IAGHCYIDKI	PEDKTATV--	EDGIEV--G	YRKFDLKKK	611
ScDnf1p	RDTMID-ELR	ALSGNSQ---	-FYPEEV--	TFVSKFVRD	LKGASGEVQQ	RCCEHFMLAL	ALCHSVLVEA	NPDNPKKLDL	794
ScDnf2p	RETMIID-ELR	SMSDNTQ---	-FCPEDL--	TFVSKFVRD	LKGSSGDHQQ	KCCHEFLLAL	ALCHSVLVEP	NKDDPKKLDL	839
CnAPT1	ASESDSDPK	KISTEDDPDEI	KVTLPKEVLA	TFHDAELDQ	LEAHDSQSE	RILHGFFAVL	GLCHTVL--A	AETEPGVIEY	668
ScDrs2p	LNDPSDED--	-----	-----	-----	-----S	PIINDFTLL	ATCHTVIPEF	QSD--GSIKY	648
ScDnf1p	KAQSPDEAAL	VATARDVGFS	FVGKTKKGLI	IE--MQGIQK	FEILNILEF	NSSRKRMSCI	VKIPGLNPGD	EPRALLICKG	872
ScDnf2p	KAQSPDEAAL	VSTARQLGYS	FVGSSKSGLI	VE--IQGVQK	EFQVLNVLEF	NSSRKRMSCI	IKIPGSTPKD	EPKALLICKG	917
CnAPT1	KAQSPDEAAL	VQSAADVGFS	FRGRDHNILR	MSTPFSVSD	EYELHNVLEF	NSARKRMSCI	LR---KLDE	DGRIFLLCKG	744
ScDrs2p	QAASPDGAL	VQGGADLGYS	FIIIRKNSVT	VLLLEETGEEK	EYQLNICEF	NSTRKRMSAI	FRFP--	DGSIKLCKG	722
ScDnf1p	ADSI IYSRLS	RQSGSNSEAT	LEKTALHLEQ	YATEGLRTLQ	IAQRELWSWE	YEKWNKVDI	AAASLANRED	ELEVVDVIE	952
ScDnf2p	ADSVIYSRLD	RTQ--NDATL	LEKTALHLEE	YATEGLRTLQ	LAQRELTSWE	YERWVKTYDV	AAASVTNREE	ELDKVTVIE	995
CnAPT1	ADNVIFERLT	KDS--NQREM	REKTDQDLQY	FASEGLRTLQ	LAYRILDPQV	YEQWAKYHN	ATVALQDREE	RIESVSSSI	822
ScDrs2p	ADTVILERLD	DEA--NQY	VEATMRHLED	YASEGLRTLQ	LAMRDISEGE	YEEWNSIYNE	AATTLDNRAE	KLDEAANLI	798
ScDnf1p	RELILLGGTA	IEDRLQDGVF	DCIELLAEAG	IKLWVLTDGK	VETAINIGFS	CNVLNNMEL	LVIK-----	TTGDDVKE--	1024
ScDnf2p	RELILLGGTA	IEDRLQDGVF	DSIALLAEAG	IKLWVLTDGK	VETAINIGFS	CNVLNNMEL	LVVK-----	ASGDDVKE--	1067
CnAPT1	RDILLGGATA	IEDKLQDGVF	DTISDLKRAE	IKVWVATGDK	LETAVAI GYT	TNLLTKDTNL	IIVVREGHRSI	GDQLREALLE	902
ScDrs2p	KNLILIGATA	IEDKLQDGVF	ETIHTQEAG	IKI WVLTDGK	QETAINIGMS	CRLLEDMDNL	LIINE--ET	RDDTE--	870
ScDnf1p	-FGSEPSEIV	DALLSKYLKE	YFNL-----	--TGSEEEI	FEAKKDHEFP	KGNVAVITDG	DALKLALYGE	DIRRKFLLC	1094
ScDnf2p	-FGSDPIQVV	NNLVTKYLRE	KFGM-----	--SGSEEL	KEAKREHGLP	QGNFAVITDG	DALKVALNGE	EMRRKFLLC	1137
CnAPT1	FFGEDAG--L	RTTLRSRIDS	RNSMDPPRLT	RVNTGVRSLV	---GRDNGTR	PGGFSLVIEG	HALAHCFDDE	ETEAALLLS	977
ScDrs2p	-----	RNLLKEI--	-----	--NALNEHQL	---STHDMK	-SLALVIDG	KSLGFALEPE	-LEDYLTV	918
ScDnf1p	KNCRAVLCRR	VSPSQKAAV	KLVKDSL DVM	TLATGDGSDN	VAMIQSADV	IGIAGEEGRQ	AVMCSDYAIG	QFRYLARLVL	1174
ScDnf2p	KNCKAVLCRR	VSPAQAQAAV	KLVKKTL DVM	TLATGDGSDN	VAMIQSADV	VGIAGEEGRQ	AVMCSDYAIG	QFRYVTRVL	1217
CnAPT1	TRCNTVLCRR	VSPQLQAQIV	HLIKNDL DVM	CLAI GDGAND	VAMIQAADVG	VGISGEEGLQ	AVNCSDYAIA	QFRYLKRLLL	1057
ScDrs2p	KLCKAVLCRR	VSPQLKALVV	KMKVKRSSSL	L LAI ASGAND	VSMIQAADV	VGISGMEGMC	AARSADIALG	QKFLKLL	998



Supplementary Figure S2. Sequence alignment of Apt1p and its nearest orthologs in *S. cerevisiae*. The sequences were aligned with Mega X using MUSCLE. Positions are coloured blue to red according to increasing sequence identity. For accession numbers, see Materials and methods.



Supplementary Figure S3. Quantification and visualization of the purified Apt1p-GFP/Cdc50a-FLAG. Purified Apt1p-GFP/Cdc50a-FLAG were subjected to SDS-PAGE followed by Coomassie Brilliant Blue staining. By comparing the band intensity of the purified protein with that of BSA, the concentration of Apt1p-GFP was estimated to be approximately $6.4 \text{ ng } \mu\text{l}^{-1}$. Molecular mass markers (M) and the molecular mass (kDa) are shown on the left. Band intensities were quantified using Image Lab™ Software from Bio-Rad.

Supplementary Table S1. Primers used in this study.

Set	Name	Sequence
#1	<i>APT1_F</i>	5'-AAAAAACCCCGGATCCATGGGCGCCTCCAAACC-3'
	<i>APT1_R</i>	5'-GTCGTATTACGGATCCTTACATCCTCGGTATTTCTGGTGGG -3'
#2	<i>Spacer-myc-for</i>	5'-GGTTCGTGTTCTGAACAGAAGTTGATTTCCG-3'
	<i>Spacer-myc-rev</i>	5'-AGAACCAGAACCCATCCTCGGTATTTCTGG-3'
#3	<i>APT1_Frag. 1. FOR</i>	5'-AAAAAACCCCGGATCCATGGGCGCCTCC-3'
	<i>APT1-spacerGFP-rev</i>	5'-CTCACCATAGAACCAGAACCAGAACCAGAACCC -3'
#4	<i>spacer-eGFP-for</i>	5'-TGGTTCTATGGTGAGCAAGGGCGAG-3'
	<i>eGFP-rev</i>	5'-GTCGTATTACGGATCCTTACTTGTACAGCTCGTCCATGCC -3'
#5	<i>CDC50_F</i>	5'-CACTAAAGGGCGGCCGCATGGCCATATTCAACAGGAAGCC-3'
	<i>CDC50_R</i>	5'-GATACTAGTGCGGCCGCTTATAATCCATTTGCGTTGGGCTGGT-3'
#6	<i>CDC50_F</i>	5'-CACTAAAGGGCGGCCGCATGGCCATATTCAACAGGAAGCC-3'
	<i>CDC50_R without Stop</i>	5'-GATACTAGTGCGGCCGCTAATCCATTTGC-3'
#7	<i>Dead-Mut for</i>	5'-CCTCGACGGTcAAACCAACTT-3'
	<i>Dead-Mut rev</i>	5'-TTTTTGGTTTCGATGTACGC-3'