Supplementary Information

The cryo-EM structure of a γ-tubulin small complex provides insights into the architecture and regulation of a minimal microtubule nucleation system

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Supplementary Fig. 1. Purification of *C. albicans* wild-type γ -TuSC. a, Anion-exchange chromatography result of wild-type γ -TuSC after Ni-NTA purification. Data points of the absorption curve are included in the Source Data. b, SDS-PAGE (CBB) analysis of wild-type γ -TuSC peak fractions. An uncropped raw image of the SDS-PAGE gel is included in the Source Data. The experiment was repeated four times with the same outcome.



Supplementary Fig. 2. Cryo-EM single particle analysis workflow. a, Representative micrograph of the purified γ -TuSC. 1399 micrographs were acquired in two imaging sessions on two different cryo-EM grids. Three selected particles, aligned on the right side to the micrograph, are shown at higher magnification. Scale bars correspond to 30 nm and 10 nm, respectively. b, Particles were extracted from the micrographs based on a regular grid with 10 nm spacing and computationally sorted via several consecutive steps of 3D classification. Due to the large number of particles, the initial round of 3D classification was performed on four subsets in parallel. Classes are shown for one of the subsets. Classes retained for further processing are highlighted. c, Initial consensus refinement of the retained particles produced a cryo-EM density (grey) at 4.1 Å global resolution. Local resolution is highest at the interface between the two spokes and ranges from 3.7 to ~ 5 Å. Local resolution is color-coded as indicated. d, The γ -TuSC particles were subjected to multibody refinement using the depicted

masks, comprising one spoke each. The output density segments were combined into one composite density at 3.6 Å global resolution. Notably, local resolution increased overall and highest local resolution is achieved in the center of each spoke. Local resolution is color-coded as indicated. Please note that the color-coded range is different from (c). The angular distribution of particle views is shown in the same orientation. **e**, Mask-corrected Fourier shell correlation between the two independently refined half set reconstructions before (red) and after (purple) multibody refinement and Fourier shell correlation between the full reconstruction and the atomic model for the γ -TuSC. The FSC = 0.143 threshold is indicated by a dashed line. Data points for all plotted FSC curves are included in the Source Data.



Supplementary Fig. 3. Molecular interactions mediating Spc97/98- γ -tubulin heterodimer formation. a, Overall arrangement of α -helices in Spc proteins. Spc97 is shown as a representative. b, The Spc core fold is stabilized by hydrophobic interactions within helical bundles and between the GRIP1 and GRIP2 domains. Hydrophobic amino acid residues are shown in brown. Zoomed views as indicated in (a). c, d Complementary charged patches on the Spc GRIP2 domain (left) and γ -tubulin (right) mediate electrostatic interactions between γ -tubulin and Spc97 (c) or Spc98 (d), respectively. Surface representation of the atomic model, colored according to charge (red: negative, blue: positive, white: no charge).



Supplementary Fig. 4. Molecular interactions mediating GRIP1 domain interaction in *C. albicans* and human γ -TuSC units. a, Interface between GRIP1 domains in human GCP2 and GCP3 (left) and *C. albicans* Spc97 and Spc98 (right). The models are shown in surface representation and color-coded according to hydrophobicity (brown: hydrophobic; purple: hydrophilic). Interacting hydrophobic patches are indicated. b, Interface between GRIP1 domains in human GCP2 and GCP3 (left) and *C. albicans* Spc97 and Spc97 and Spc98 (right). The models are shown in surface representation and color-coded according to charge (right). The models are shown in surface representation and color-coded according to charge (red: negative, blue: positive, white: no charge). Complementary charged patches on the two surfaces are indicated.

a Spc97 Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	250 260 270 LIIRELLET LLGHEGHYIOYSKR LVVKDLLTVLLGLEGTYIRYFND AVVEDLLYVLVGVDGRYVSAQPL	280 DPT SQISRIEGPDYK EPSDPETPIEFK GRQSRTFL	290 300 IAKNLDISLKVITKKL IAKKMDPSFKTFSRRI VDPNLDLSIRELVHRI	310 VKFGKF <mark>YSG</mark> LKSFI VRYGKQYMILTRAY LPVAAS <mark>YSAVTR</mark> FI	320 330 QVFDNNKFGKIVQKFC EKWSDTSFGMVLQRFA EEKSSFEYGQVNHALA	340 SEVRKFLSSYQQV YEIRRFLEDVYLKTL AAMRTLVKEHLIL
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	350 360 370 LINVEHEFKFNK-NFNLNMLDSL VERLERDFNKVP-NFSIRELEQI VSQLEQLHRQGLLSLQKLWFY 450 460 470	HQ E- ISNEMTHLYQI NETEVNKQMELLYNI - QP- AMRTMDILASL	400 G I E I SR I T E E RQ KM SQ Y E E I F R E I E E R RT NQ S AT SV DKG	410 AEIMGNFEPTT SQE-DFNNFMDS	420 430 LANT SMNG I NSEPNLY MKNESSLHLRLMVAFD	YGKFDCCKGGLLLQV TTVYPVPKGGAILKI ECLGGSTLSL
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	IQERMVYYKGDPTSLDFLTQLFD FQQKILENLGDRSSVMFLKKLLNN LHDRSFSYTGDSQAQELCLYLLNK	VSSDYIGMLNOWLLE IISQDYCTMLYEWLTO ASAPYFEVLEKWIYR	GVINDPFDEFMIREKR GILNDPYQEFMTYDDL GILHDPYSEFMVEEHE	VPDSFMEIFQSKSE EGK TDNIFDTRD - LRK- ERIQEDYND	YYWNELFLIKIDGLLN RAWDTQYFIRKDVLLR KYWDQRYTIVQQQIPS	QFQNSTIQSKIL DCDSEEDKNLLFKML FLQKMADKIL
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	NTGKYLNIFKRCTGLHNFESLK RTGILLKVVRASLQIPTIPSNSSI STGKYLNVVRECGHDVTCP	EKLTTITSLAA DITIQEINDFADLMEG VAKEIIYTLKE	PDLELKIDEFYHRANK SNLELYVDKCYSRANE RAYVEQIEKAFNYASK	MLMKLLFDGYNFPS IFLKLFFQGYDLIN VLLDFLMEEKELVA	VVNIFQRLFLF-ADSF VLKHLQQIFLGYQSGH HLRSIKRYFLM-DQGD	QIDNFIDSTFSELKR NVLKFLTKNMGELTK FFVHFMDLAEEELRK
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	650 660 670 GKLKISVSRLQKQYDDIFKEKIE HYRNDNNANYDKLLQNFELERQS PVEDITPPRLEALLELALRMSTA	680 IKVGVRP SVY DV LKKN NPNNLMRQ L NT DP FK DD	690 700 QKLSVTSESLYKVVEE LMIQFDTETLPQVLSH LKIDLMPHDLITQLLR	710 LMEKNS YLQIYPEVPENNSA VLAIET	720 730 DY L I S- DNNLRGI FHR NDD SDP LMHA- NNFKN KQEKA	740 VASLRDDSRLTI MNAILFDELSKER-T
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	STADSATENVKDEPTITSVDLTI GAYHGSNLELYTPKSAIYHLKFDI MAHADPTELALSGLEAFSFDY	PLPFPLNLVLNQQLS NIPYPLNIIISRTCM IVKWPLSLIINRKAL	YQYEIMFKLLINIKFI IKYQIILRYQLVLQYH TRYQMLFRHMFYCKHV	SKYNSSNWQEMNYS SRLLDETWMDLNKT ERQLCSVWISNKTA	830 KIWTNSHFNSSV-KKW PSWKYRGYSHTVKRRI KQHSLHSAQW	ILRCRVLHSRICSFI VRATRVLHAKMNHFI FAGAFTLRQRMLNFV
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	850 860 870 HELENYIVHDVIEHNFEEIKNLII KTIMEYFNQNVIDKEVYSLEKCY QNIQYYMMFEVMEPTWHILEKNL	880 ITTATNLATSELGSDI NPTL SASN	890 900 NDEGDNIFNGSLIRGT	910 FNNNSIFDSKVHKH	920 930 RTTTYVEGISTVEQLI AVAIQ IDDVL	940 QKFLDYSTLLNDSL NELEGGLTNIMTNRC GHHTGFLDTCLKDCM
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	950 960 970 LTREESLRQLRKMLDFIFHFNNY LSDLIPLQLQIFDIVYKFCKF LTNPELLKVFSKLMSVCVMFTNCM	980 VQVKKVLVLLNHELF KSMRAKLCQLDPVLY QKFTQSMKLDGELGG	990 1000 NEYSKEFPTKF EKHKSGMMKTLNE QTLEHSTVLGLPAG	1010 EK AEERARKELARK-H	1020 1030 PMDQ E S I D K R F A YRTNNGGQ EDVGYQ ED LAEHADTV Q LV S	1040 N L S DT F L MQY E K F G E A A L E L I Q K L I EY I S N G F E A T I N K F D K N F S A
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	1050 1060 1070 NLVT FLAT I KQVGER EN-QGLLEL ASSIFRKCLINFTQELS-TEKFDF HLLDLLARLSIYSTSDCEHGMASV	1080 SNRLELC FP Y DSSSVDAAGIERVLY I SRLD FNG-FY	1090 1100 E	1110 	1120 	
b Spc98 Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	260 270 280 ILTY LPYTMLGSDSKI FTFSNNY ILKYVSYTLLATTSALFPFDHE LVRDILYVFQGIDGKNIKMNNTE	290 	300 310 SLLREVFEFAILYKQL GLLHLIFEAGLLYQSL DTAVRLSELGWLHNKI	320 A I VVDRYKG-TLVS GYKVEKFRM-LNIS RRYTDQRSLDRSFG	330 340 AIKTAYIAILEAQLNK PMKKALIIEISEELQN LVGQSFCAALHQELRE	350 YVNDINNIFNN YTAFVNNLV <mark>S</mark> SGT YYRLLSVLH <mark>S</mark> QLQLE
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	360 370 380 	390 WISILRFLYRVSNRL NIIRLRIVCRFTEHL PKIRLKTLAALVDHC	400 NRLDGYEFLTFIYSFT EELSGDTFLIELNIFK QGRKGGELASAVHAYT	420 NHGDPKIRGIAVTA SHGDLTIRKIATNL KTGDPYMRSLVQHI	440 FTEVVKPYYNIVEHWI FNSMISLYYEYLMNWL LSLVSHPVLSFLYRWI	450 VKGELIDNNNEFFII TKGLLRATYGEFFIA YDGELEDTYHEFFVA
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	FDQ EQNE-FNSIIKLI ENTDTNGTDDDFIYHIPIEF SDPTVKTDRL-WHDKYTLF	490 PKKIPAFIKSSDK IQERVPAFIPKELAYK KSMIPSFMTMDQSRK	IFQIGKTLIFLNKYCR IFMIGKSYIFLEKYCK VLLIGKSINFLHQVCH	ELKWVNQYNVKYSA EVQWTNEFSKKYHV DQTPTTKMIAVTKS	I- LENNHQGLASMTTN L- YQSNSYRGIST AESPQDAADLFTDLEN	EMIK LIDSOYNEILT NFFEIINDOYSEIVN AFQGKIDAAYFETSK
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	500 570 580 FLTQIIQGNNKLFTHVYNFKRYF HTNQILNQKFHYRDVVFALKNILL YLLDVLNKKYSLLDHMQAMRRYLI	S90 METNDFIDAIMVKGK MGKSDFMDALIEKAN LGQGDFIRHLMDLLK	DVFNESSVNISSTYLR DILATPSDSLPNYKLT PELVRPATTLYQHNLT	620 KVLQDAIQISSVK- RVLQEAVQLSSLRH GILETAVRATNAQF	630 640 NFEYVDRLDS LMNSPRNSSVINGLDA D SPEILRRLDV	RVLNPQHGNLGWESF RVLDLGHGSVGWDVF RLLEVSPGDTGWDVF
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	TIEYKIDDLPMSYLFEGHOHI TLDYILYP-PLSLVLNVNRPFGRK SLDYHVDG-PIATVFTRECM	OY L KM FH FLWK L ROL EY L R I FN FLWR FK K N HY L R V FN FLWR AK RM	NNLLNWHFEMFNELNH NY FYQKEMLKSNDIIR EY ILT DIRKGHMCNAK	NVTKLS SRNR SFKKIRGYNPLI LLRNM	RPLAKSLŠIITSIRFH RDIINKLSRISILRTQ PEFSGVLHQCHILASE	FTOFLNELIAYLSYD FOOFNSKMESYYLNC MVHFIHQMQYYITFE
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	760 770 780 VIEENFQQHIVRKLFYNKNDQDLI IIEENFKEMTRKLQRTEN-KSQNC VLECSWDELWNKVQQA	790 LNKSFMNLSEID IFDLIRLNNGTIELNG	800 810	820 PKFNVNLLTIDE PQKHAIEKTLNIDE QDLDH	830 840 LVELHGTYIDSIINSS LESVHNTFLTNILSHK IIAAHEVFLDTIISRC	850 LLNEKLKGNET LFATNTSEISVGDYS LLDSD
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	860 870 880 NISYIDQIFNILQTIFNFINTSQE GQPYPTSLVLLLNSVYEFVKVYCN SRALLNQLRAVFDQIIELQNAQDA	890 FYSLVVTFGLLVRSD ILNDIGYEIFIKMNLN IYRAALEELQRRLQF	900 910 SNANK I	920 ELEQDQED EASNG AAEEEEENK- RIGE	930 940 LEFQLH <mark>K</mark> IK <mark>R</mark> KIYKDI LL <mark>G</mark> KFNTNLKEIVS FKESIP <mark>K</mark> MCSQLRI	YQHDYKRQLNDLKND QYKNFKDRLYIFRAD LTHFYQGIVQQFLVL
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	980 970 980 LNR DYNLKDLSKLL LKNDGDEELFLLSKSLR LTTSSDESLRFLSFRLDFNEHYKA	990 REPRLRVSLGTRGRRS	1000 S S H T			
c γ-tubulin Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	10 20 MPGETITLOVGOCGNOVGLOVWO MGGEIITLOAGOCGNHVGKFLWS MPREIITLOLGOCGNOIGFEFWK	30 LAT EHGIQSDGSSTP LAKEHAIGTDGLS LCAEHGISPEGIV	40 50 YPKDINDLQLQELNN	60 5G 5 S P <mark>Q S Y P Q Q</mark> T K P I Q L <mark>P D S S T E</mark> E E F A T E	70 80 NGKYRNDH <mark>PELFFTLS</mark> RDDDTK <mark>PFFREN</mark> GTDRKDVFFYQA	90 D S N T Y T P R S I L I D M E S R N K F T P R A I MM D S E D D E H Y I P R A V L L D L E
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	100 110 120 P SVIA K ST SALPMFNPRNVHL P SVIADV - ENT FRGFFDPRNTWVA P RVIHSILN SPYAKLYNPENIYL	130 NOGNGAANNWINGYK SDGASAGNSWANGYD EHGGGAGNNWASGFS	140 150 YGTEEEETLLNLIDRE IGTRNQDDILNKIDKE QGEKIHEDIFDIIDRE	160 VDKCDNLSNFQLFH IDSTDNFEGFQLLH ADGSDSLEGFVLCH	170 180 SVAGGTGSGVGSKMLE SVAGGTGSGLGSNLLE SIAGGTGSGLGSVLLE	190 VISDRYGHKKLLNTF ALCDRY-PKKILTTY RLNDRY-PKKLVQTY
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	200 210 220 SIFPSNEDTSDVVVQPYNTILTL SVFPARSSEVVVQSYNTILAL SVFPNQDEMSDVVVQPYNSLLTL	230 R LIDYSDATFVFHND R LIEDSDATVVFDNA R LIEDSDATVVFDNA R LTQNADCVVVLDNT	240 250 SLNRIENILFNNNSNI SLLNISGKVF ALNRIATRL	260 QHDDNDLFLGANKL RNP-NIDLQHTNQL HIQ-NPSFSQINQL	270 280 I A L V S A S V S N P L R F P G I S T I I S S V T N S I R F P S V S T I M S A S T T L R Y P G	290 YMY SSMESIVSNLIP YMY SSMSSIYSTLIP YMNNDLIGLIASLIP
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	JUU 310 320 TPDLKFLTSSIAPFS TOP SPELHFLSPSFTPFTSDYIHDDIA TPRLHFLMTGYTPLTTD-OSVAS	330 HNYLNEYDMLLELSN HKGHSSYDVMLDLLD VRKTTVLDVMRRLLQ	350 DRYKTNRVGGD PSNSLVSTAMNN PKNVMVSTGRDRQT-N	360 T SY I SMLNYLIGYN PTYFNVYN-TIIGN HCYIAILN-IIQGE	380 LDQREIRKGILKSQQR VEPRQISRAMTKLQQR VDPTQVHKSLQRIRER	390 I SFVPWVAR S VLV I KFPSWSSSAMHV KLANFIPWGPA S IQV
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	400 410 420 VHGKK SPYLKNTNLEGIQV NIGRR SPYLPLQPNENEV SGMML ALSRK SPYLPSAHRV SGLMM	430 NNT SMIDVFTKILKQ NMSTVVNVFENACNT NHTSISSLFERTCRQ	440 450 FDLLIKRKAYLNRYS FDKVFAKGAFLNNYNV YDKLRKREAFLEQFRK	460 SV EEENEVMEMF GDLFQSMQNVQDEF EDMFKDNFD EM	470 480 NESRESVKSIIDEYKA AESREVVQSLMEDYVA DTSREIVQQLIDEYHA	490 CKEITYLDDDDEDDL AEQDSYLDDVLVDDE ATRPDYISW
Candida_albicans Saccharomyces_cerevisiae Homo_sapiens	200 510 520 EDGDGGGGGG NGNNIDDADMO NMVGELEEDLDADGDHKLV- GT QEQ	- - -				

Supplementary Fig. 5. Sequence alignment of *C. albicans*, *S. cerevisiae* and human γ -TuSC subunits. Sequences of Spc97 (a), Spc98 (b) and γ -tubulin (c) are shown. Fungi-specific insertions that were modelled (completely or partially) are highlighted by black boxes. The

same insertions have been mapped back to the structure in Supplementary Fig. 6. Numbering is according to the consensus sequence alignment.

Candida albicans y-TuSC insertions



Supplementary Fig. 6. Location of modelled fungi-specific insertions in γ -TuSC subunits. Modelled fungi-specific insertions (red) of Spc97 (black boxes and numbers), Spc98 (green box) and γ -tubulin (purple boxes) are shown in context of the full model. Insertions in Spc97 are numbered from N- to C-terminus. The corresponding sequence alignments are shown in Supplementary Fig. 5. Coloring as in Fig. 1a.

Mutant γ -TuSC (Spc98 $^{\Delta D627-K650}$)

а



Supplementary Fig. 7. Purification of mutant *C. albicans* γ -TuSC with deleted insertions in either Spc98 or Tub4. a, Anion-exchange chromatography result of the Spc98 mutant of γ -TuSC (Spc98^{AD627-K650}) after Ni-NTA purification. Data points of the absorption curve are included in the Source Data. b, SDS-PAGE (CBB) analysis of peak fractions from (a). An uncropped raw image of the SDS-PAGE gel is included in the Source Data. The experiment was repeated two times with the same outcome. c, Anion-exchange chromatography result of the Tub4 mutant of γ -TuSC (Tub4^{AT38-K71}) after Ni-NTA purification. Data points of the peak fractions from (c). An uncropped raw image of the SDS-PAGE gel is included in the Source Data. The experiment was repeated two times with the same outcome.

b



Supplementary Fig. 8. Negative stain EM analysis of wild-type and mutant γ -TuSC. One representative micrograph is shown together with five selected particles at higher magnification for the wild-type γ -TuSC (**a**), the Spc98 mutant (Spc98^{Δ D627-K650}) γ -TuSC (**b**) and the Tub4 mutant (Tub4^{Δ T38-K71}) γ -TuSC (**c**). Scale bars correspond to 50 nm and 20 nm for the micrographs and particle images, respectively. Data for each γ -TuSC construct were acquired in one imaging session. The numbers of micrographs used were 997 for the wild-type γ -TuSC, 821 for the Spc98^{Δ D627-K650} mutant γ -TuSC and 1147 for the Tub4^{Δ T38-K71} mutant γ -TuSC.



Supplementary Fig. 9. S. cerevisiae mutant Spc98^{Δ K674-H713} causes defects in mitosis. a, Strain ESM243-2 (*MATa ura3-52 lys2-801 ade2-101::pRS402-GFP-TUB1 trp1\Delta63 his3\Delta200 leu2\Delta1 \Deltaspc98::HIS3 pRS316-SPC98 SPC42-mCherry-natNT2) was transformed with pRS425-SPC98, pRS425-spc98^{\DeltaK674-H713} and pRS425 (vector). Transformants were tested for growth after serial dilution at the indicated temperatures on SC-Leu and 5-FOA plates for 2 - 3 days. The growth behavior is identical to strain ESM243-1 shown in Fig. 3d. b-c, Cells of spc98^{\DeltaK674-H713} (b) or SPC98 (c) expressing TUB1-yeGFP (green) and SPC42-mCherry (red) were cultured at 37°C for 3 hours. The Tub1-yeGFP (green) and Spc42-mCherry (red) fluorescent signals and the merged signals with phase contrast are shown. Scale bars, 10 µm. The experiments were repeated two times with the same outcome.*



Conservation of the Spc98 insertion

	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270
Candida_albicans	LFYNKN	4	DQDLLLNKS	FMNLSEID				P N N D L	PKFNVNL	TID
Saccharomyces cerevisiae	QRTEN-		K SQ NQ F D L I	RLNNGTIELN	GILTPK		A	EVLTKSSSK	PQKH-AIEKTL	NID
Clavispora lusitaniae	FREDCL	LGPDFITGKSK	KERKLPILN	DEFAA	- KCTDN		G	ILIGLEKTKO	WNHNGSER	TID
Ogataea polymorpha	AKTDE-		- HDIKVKRN	AKGLKVLES-	GRIKID		K	TYLPLPDNAH	ILEL KYRAY	NLD
Wickerhamomyces ciferrii	KKSDP-		- Q SK L ST TK	T G N A F K V A D -	DVLIPS		K	SYMSHINKSP	STRNDQLFKEQ	SMD
Lachancea fermentati	M SQKD-		- TGKKQKII	KLKNGLNVVE	G I L K P N		T	KMLENFGKAK	SSDRGTNTF	TID
Ascoidea rubescens	VKIGEO	GNGDKIGS	SSGEGKNLI	KRYNQVILP-	KRKFMEDNKR	EFEKHEDERFD	DKFNSIIRNKR	NYIKMPKEEM	IEENLIKEN	SID
Schizosaccharomyces pombe	ΕΚ <mark>Ρ</mark> Ν								A	TLD
Dictvostelium discoideum	DQEA								T	DLD
Coprinopsis cinerea okavama	DKKE								G	DLD
Neurospora crassa	HKEN								C	TLD
Arabidopsis thaliana	EAA								К	DLD
Tetrahymena thermophila	DKT								К	DFA
Chlamvdomonas reinhardtii	A S C								A	DLD
Tricahomonas vaginalis	DQ A								D	NID
Giardia intestinalis	EAA								E	тік
Amphimedon queenslandica	DGA								V	DLD
Dendronephthya aiaantea	H E S								T	NLD
Strongylocentrotus purpuratus	T E A								A	DLD
Ciona intestinalis	EQ S								к	DLD
Clonorchis sinensis	Q A A								T	DLD
Lingula anatina	К Е <mark>А</mark>								К	DLD
Crassostrea gigas	К Е <mark>А</mark>								E	DFD
Trichinella spiralis	HNA								V	DLD
Drosophila melanogaster	Q K A								T	TLD
Danio rerio	QQA								Q	DLD
Podarcis muralis	QQA								Q	DLD
Calvpte anna	QQA								Q	DLD
Homo saniens	00A								o	DLD

251253

326 327

С

Conservation of key residues for the interaction of γ -tubulins in the human γ -TuSC

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Candida_albicans	MFNPRNVHL	EEETLLNL	NNNSNIQHD	LLELSNDRYK
Saccharomyces_cerevisiae	FFDPRNTWV	RNODDILNK	GKVFRNP	MLDLLDPSNS
Clavispora_lusitaniae	MENPRNIHM	EHEEELINL	G S N P N R G S	IVLELLNNKYK
Lachancea_fermentati	LFNERNIWV	ENQDEFINM	SKVFKDS	ILDLFDKNNS
Wickerhamomyces_ciferrii	F F G	RVREPSVKI	NNAENLSLE	NAEDAEPLSI
Ogataea_polymorpha	LFNPKNIYV	RHQEEFLDM	STNLQVS	ILEVLDKKLR
Ascoidea_rubescens	LFNPKNYYS	QHNEELLDV		ILLELLNKDNK
Schizosaccharomyces_pombe	LYNPENILI	RIFEDIMDM	A DR LHTQ	MRRLLLPKNQ
Dictyostelium_discoideum	LYNQENIFV	SFYDDIFDM		MRRLLQPQNI
Coprinopsis_cinerea	LYNPENIFV	RIYEDIMEM		MRRLLQPKNR
Neurospora_crassa	IYNPENFYV	QVHEEIMEM		MRRLLQPKNR
Arabidopsis_thaliana	LYNHENIFV	GVEEEIMDM		MRRLLQTKNI
Tetrahymena_thermophila	LYNPENIFY	KIQDDLLDM		MRRLLQTKNI
Chlamydomonas_reinhardtii	LFNPENIFI	AVQETLLDM		MRRLLQPKNI
Trichomonas_vaginalis	FFNAENMYI	AHY EAFSEI	GGVSKKRTE	MNRLLDKKNI
Giardia_intestinalis	LINPENVYI	AGFEKIVEI	TNHIPNE	VKRLLHPTNG
Amphimedon_queenslandica	LYNPENIYI	K L S E S I F D I		MRRLLQPKNI
Dendronephthya_gigantea	LYNPENIFT	RLHEEIFDI	SDRLHIP	MRRLLQPKNV
Strongylocentrotus_purpuratus	LYNPENIFI	RLYEEVFDI		MRRLLQPKNI
Ciona_intestinalis	LYNPENIYL	K I H E E L F D I		MRRLLQPKNM
Clonorchis_sinensis	LYNRENVYL	K L E E E I F D I	A ERLHIE	MRRLLQPKNM
Lingula_anatina	LYNPENVFT	K LY E EV F D I		MRRLLQPKNM
Crassostrea_gigas	LYNPENIYL	K LY EEIFDI		MRRLLQPKNM
Trichinella_spiralis				MRRLLQPENM
Drosophila_melanogaster	LYNPENVYL	K LQ EEV FDI		MRRLLQPKNM
Danio_rerio	LYNPENIYL	K I H E D I F D I		MRRLLQPKNV
Podarcis_muralis	LYNPENIYL	KIHEDIFDI		MRRLLQPKNV
Calypte_anna	LYNPENIYL	KIHEDIFDI		MRRLLQPKNV
Homo_sapiens	LYNPENIYL		T DRLHIQ	M <mark>R R</mark> L L Q P K N V

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Supplementary Fig. 10. The fungi-specific Spc98 insertion may replace direct interaction of neighboring γ -tubulins during evolution. a, Left: Superposition of atomic models for the two γ -tubulin molecules as arranged in the *C. albicans* (yellow, orange) and human (pink) γ -TuSC units. The γ -tubulin interaction area in the human γ -TuSC is indicated. Right: Complementary charged patches on the two γ -tubulin molecules mediate electrostatic interactions in the human γ -TuSC. Surface representation of the atomic model, colored according to charge (red: negative, blue: positive, white: no charge). b, Spc98 sequences were aligned for a broad range of organisms and the region of the fungi-specific Spc98 insertion is shown. The full multiple sequence alignment is included in the Source Data. Numbering is according to the consensus sequence alignment. **c**, γ -tubulin sequences were aligned for the organisms in (b) and the regions including key residues (boxed) for the electrostatic interaction of γ -tubulins in the human γ -TuSC are shown. The full multiple sequence alignment is included in the Source Data.



Supplementary Fig. 11. The γ -TuSC system in *Saccharomycetes* emerged from the γ -TuRC system by compositional simplification. Phylogenetic tree of life for the major branches of eukaryotes based on genomic similarities. Representative organisms of green branches possess homologs for all five GCP proteins known to be required for formation of the γ -TuRC. *Saccharomycetes* (red branch) possess only homologs for GCP2/Spc97 and GCP3/Spc98. Sequence identifiers for the GCP homologs of all analyzed organisms are included in Supplementary Tables 1 and 2.

Organism	Phylum	GCP2	GCP3	GCP4	GCP5	GCP6
Giardia Intestinalis	Metamonada	A8BD62	A8BFK8	KAE8304190.1	ESU36978.1	A8BUR1
Trichomonas vaginalis	Metamonada	A2E313	A2E3S1	XP_001325680.1	XP_001323803.1	>XP_001328726.1
Angomonas deanei	Euglenozoa	EPY32211.1	EPY37939.1	EPY31448.1	EPY34001.1	EPY30063.1
Tetrahymena thermophila	Alveolates	Q23AE3	Q22ZA9	XP_001025759.3	XP_001014477.2	XP_001015474.3
Phytophthora infestans	Stramenopiles	KAF4031510.1	KAF4046598.1	KAF4031595.1	KAF4137761.1	KAF4030965.1
Gracilariopsis chorda	Rhodophyta	PXF41098.1	PXF45651.1	PXF41306.1	-	PXF43115.1
Chlamydomonas reinhardtii	Chlorophyta	A8J5J8	A8JBY6	XP_001689495.1	XP_001699475.1	PNW77310.1
Arabidopsis thaliana	Spermatophyta	Q9C5H9	Q9FG37	NP_190944.2	NP_565235.3	NP_189947.2
Candida albicans	Ascomycota	Q59PZ2	A0A1D8PS42	-	-	-
Homo sapiens	Chordata	Q9BSJ2	Q96CW5	Q9UGJ1	Q96RT8	Q96RT7

Supplementary Table 1: Sequence identifiers of GCP subunits identified in various eukaryotes

Organism	Class	GCP2	GCP3	GCP4	GCP5	GCP6
Batrachochytrium dendrobatidis	Chytridiomycetes	XP_006676384.1	OAJ42679.1	OAJ44291.1	OAJ37135.1	XP_006681750.1
Ustilago maydis	Ustilaginomycetes	XP_011388094.1	XP_011386949.1	XP_011388935.1	XP_011386225.1	XP_011387192.1
Pneumocystis jirovecii	Pneumocystis	CCJ30183.1	XP_018229083.1	XP_018230983.1	XP_018228508.1	CCJ29560.1
Schizosaccharomyces pombe	Schizosaccharomycetales	Q9Y705	Q9USQ2	Q9P7R5	Q9UT52	P87244
Candida albicans	Saccharomycetes	Q59PZ2	A0A1D8PS42	-	-	-
Saccharomyces cerevisiae	Saccharomycetes	P38863	P53540	-	-	-
Clavispora lusitaniae	Saccharomycetes	XP_002617032.1	OVF11036.1	-	-	-
Ogataea polymorpha	Saccharomycetes	XP_018211750.1	XP_018213099.1	-	-	-
Wickerhamomyces ciferrii	Saccharomycetes	XP_011273399.1	XP_011277192.1	-	-	-
Lachancea fermentati]	Saccharomycetes	SCW03932.1	SCW02410.1	-	-	-
Ascoidea rubescens	Saccharomycetes	XP_020046155.1	XP_020044979.1	-	-	-

Supplementary Table 2: Sequence identifiers of GCP subunits identified in various fungi

Supplementary Table 3: List of primers

Construct	Primer name	Primer sequence
C. albicans tub4 $^{\Delta T38-K71}$	cTub4-pFB-F0	gtttcggtctccacgcatcg
C. albicans tub4 $^{\Delta T38-K71}$	cTub4-pFB-R	tattccggattattcataccgtcccac
C. albicans tub4 $^{\Delta T38-K71}$	cTub4-pFB-R	ctttgaattccgcgcgcttcg
C. albicans tub4 $^{\Delta T38-K71}$	cTub4-T38-K71-F	gtccgacgggagctcatatagaaatgatcatc
C. albicans tub4 $^{\Delta T38-K71}$	cTub4-T38-K71-R	gatgatcatttctatatgagctcccgtcggac
<i>C. albicans</i> spc98 ^{ΔD627-K650}	cSpc98-pFBHTA-F	gattacgatatcccaacgac
\dot{C} . albicans spc98 ^{ΔD627-K650}	cSpc98-pFBHTA-R	gtaggcctttgaattccggatc
C. albicans spc98 ^{ΔD627-K650}	cSpc98-D627-K650-F	gcttttttacaataaaaattttaatgttaatctgttg
C. albicans spc98 ^{ΔD627-K650}	cSpc98-D627-K650-R	caacagattaacattaaaatttttattgtaaaaaagc
<i>S. cerevisiae</i> spc98 ^{∆K674-H713}	Spc98-D674-H713-F	cgccagggttttcccagtc
<i>S. cerevisiae</i> spc98 ^{∆K674-H713}	Spc98-D674-H713-F1	ctgcaacgcacagagaatgcaatcgaaaagacgctg
S. cerevisiae spc98 ^{∆K674-H713}	Spc98-D674-H713-R1	cagcgtcttttcgattgcattctctgtgcgttgcag
<i>S. cerevisiae</i> spc98 ^{∆K674-H713}	Spc98-D674-H713-R	caggaaacagctatgac