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Supplementary Note

Details of Cryo-EM methods

Sample preparation for single particle cryo-EM

Bovine brain tubulin (Cytoskeleton Inc, Denver, CO) was polymerized at a final concentration of 5 mg/ml in MES MT polymerization buffer (100 mM MES, pH 6.5, 1 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 5 mM GTP) for 1 hour at 37°C. 1 mM paclitaxel in DMSO (Calbiochem) was then added and MTs incubated for a further hour at 37°C. Paclitaxel-stabilized MTs were diluted to 0.25 mg/ml (2.5 μM) in BRB20 (20 mM PIPES, 2 mM MgCl₂, 1 mM EGTA, 2 mM DTT, pH 6.8) and 4 μl were applied to glow discharged C-flat holey carbon grids (Protochips, Raleigh, NC). Excess solution was blotted away and either 4 μl of 2 mg/ml (110 μM) CAMSAP1/3-CKK or 1 mg/ml of CAMSAP1-CKK N1492A mutant in BRB20 with an additional 80mM KCl was immediately added. The grid was then incubated for 30 seconds in a Vitrobot (FEI Co.) operating at 23°C and 100% humidity, then blotted and vitrified in liquid ethane. Although full MT binding was observed at 0.2 mg/ml of CAMSAP1-CKK N1492A, 1 mg/ml (55 μM) was used during grid preparation to facilitate subsequent CTF determination.

Sample preparation for cryo-electron tomography and MT end analysis

Double-cycled bovine brain tubulin GMPCPP MTs were polymerized at a final concentration of 5 mg/ml in BRB80 (80 mM PIPES, 2 mM MgCl₂, 1 mM EGTA, 2 mM DTT, pH 6.8) with 2 mM GMPCPP for 30 minutes at 37°C. MTs were pelleted for 7 minutes at 18,000 × g in a benchtop centrifuge, the supernatant removed and the MTs depolymerized by resuspension to 5 mg/ml tubulin in 4°C BRB80 and incubation on ice for 5 minutes. GMPCPP was then added to 2 mM and MTs polymerized by incubation at 37°C for 45 minutes. GMPCPP stabilized MTs were pelleted as previously and resuspended to 0.25 mg/ml in 30°C BRB80. 4 μl of 10 nm nanogold fiducial-BSA solution (Sigma) was added to glow discharged C-flat holey carbon grids then blotted prior to addition of MTs. 4 μl MTs was then applied to grids, which was incubated for 30 seconds in a Vitrobot (FEI Co.) operating at 30°C and 100% humidity before blotting and vitrification in liquid ethane. For 2D end analysis, GMPCPP MTs were prepared for cryo-EM in the same way but without addition of nanogold. For CKK-GMPCPP MTs 1.5 mg/ml CAMSAP3 CKK domain was added to GMPCPP MTs sequentially prior to blotting as in preparation for single particle cryo-EM.

Data Collection for single particle cryo-EM

Images of MT-CAMSAP3 CKK domain complexes were collected on a Tecnai G2 Polara (FEI) operating at 300kV with a calibrated final sampling of 1.53Å/pixel and a defocus range of 0.4-3.5µm using a DE20 direct electron detector (Direct Electron). A total electron dose of $\sim 50e^{-}/\text{Å}^2$ over a 1.5 seconds exposure and a frame rate of 15 frames/second was used, giving a total of 23 frames at $\sim 2.2e^{-}/\text{Å}^2/\text{frame}$. Images of MT-CAMSAP1 CKK domain complexes were collected on a Tecnai F20 (FEI) operating at 200kV with a calibrated final sampling of 1.54Å/pixel and a similar defocus range using a DE20 direct electron detector (Direct Electron). A total electron dose of $\sim 30e^{-}/\text{Å}^2$ over a 1 seconds exposure and a frame rate of 25 frames/second was used, giving a total of 25 frames at $\sim 1.2e^{-}/\text{Å}^2/\text{frame}$.

Images of MT-CAMSAP1 N1492A mutant CKK domain complexes were collected on a Tecnai G2 Polara operating at 300kV with a calibrated final sampling of 1.39Å/pixel and a similar defocus range using a K2 direct electron detector (Gatan) operating in counting mode at 7e-/pixel/second, with a quantum post-column energy-filter (Gatan), operated in zero-loss imaging mode with a 20-eV energy-selecting slit. The total exposure was $35e^{-}/\text{Å}^2$ over 10 seconds at 4 frames/sec, giving 40 frames at $\sim 0.875e^{-}/\text{Å}^2/\text{frame}$.

Data Collection for MT cryo-electron tomography and MT end analysis

Single-axis tilt series at ~ 3 or 5 µm defocus were collected on a Tecnai G2 Polara operating at 300kV with a calibrated final sampling of 5.39Å/pixel on a K2 summit direct electron detector (Gatan) operating in counting mode at 5e-/pixel/second with a quantum post-column energy-filter (Gatan), operated in zero-loss imaging mode with a 20-eV energy-selecting slit. Whole tilt series used a total dose of either 60 or 80e-/Å² total dose, corresponding to a 12 or 16 second total exposure time. The total dose was divided into bidirectional tilt images from 0° to +60° and 0° to -60° in 3° increments with higher tilts receiving compensatory higher electron doses. Each tilt image was divided into 4 sub-frames, which were aligned on collection with Digital Micrograph prior to creating the final tilt stack. For 2D MT end analysis, images were collected with a total dose of $\sim 25e^{-}/\text{Å}^2$ on a FEI Tecnai T12 operating at 120 kV using a 4096 × 4096-pixel CCD camera (Gatan Inc.). Axial lengths of curved regions in MT ends were quantified with the straight line and 'measure' tools in FIJI¹.

Sub-frame processing of single particle data for 3D reconstruction

For wild type CAMSAP1 and 3 CKK data collected on the DE20 detector individual frames were globally aligned using Imod scripts² then locally aligned using the Optical Flow approach³, implemented in Xmipp⁴. Sums of all frames were used for particle picking and CTF determination in CTFFind3⁵), whereas the first $\sim 25e^{-}/\text{\AA}^2$ (for CAMSAP3 data obtained at 300kV) or $\sim 20e^{-}/\text{\AA}^2$ (for CAMSAP1 data obtained at 200kV) was used in particle processing and generation of the final reconstructions. For mutant CAMSAP1 N1492A CKK data collected on the K2 detector, individual frames were globally aligned using Motioncorr⁶. The total dose of $\sim 35e^{-}/\text{\AA}^2$ was used for particle picking and CTF determination in CTFFind3⁵, whereas $\sim 25e^{-}/\text{\AA}^2$ was used in particle processing and generation of the final reconstructions.

Processing of single particle data

Straight CAMSAP CKK domain-decorated 13pf taxol-stabilized MTs were boxed manually in EMAN boxer, and were input for a set of custom-designed semi-automated single-particle processing scripts utilizing Spider and Frealign as described previously^{7, 8}, with minor modifications. These custom-designed scripts provide diagnostics for MT architecture and decoration, accurately determine seam location in 13 and 14pf MTs and apply pseudo-symmetry between MT protofilaments during reconstruction and refinement. Eight rounds of refinement are performed in Frealign v8, using information at increasing spatial frequency up to a resolution of 8\AA in the final round to generate the final reconstruction. The procedure was modified to avoid using information at the seam in application of symmetry, which was found to be absent of CKK domains in C1 reconstructions.

A kinesin-1-decorated 13pf MT low-pass filtered to 15\AA was used as a reference for initial alignment, yielding $\sim 9\text{\AA}$ reconstructions of both wild type and N1492A mutant CAMSAP1 and CAMSAP3 CKK domains and allowing initial docking of the CAMSAP-3 CKK domain (PDB ID 1UGJ; unpublished) into density. This model was filtered to 15\AA and used as a reference for further rounds of alignment. This greatly improved the seam-finding process in particular, improving the resolution of the reconstructions. The final dataset sizes and resolutions are reported in Table 2.

Tilt series processing, tomogram generation and analysis

Tilt series were processed and tomograms generated using IMOD's Etomo graphical user interface (v4.7.15). Tilt series were aligned with 10nm gold fiducials, which were selected manually based on minimal observed beam induced movement. Final tomograms were binned by 3 on output to reduce noise. Tomogram slices were analyzed using 3DMOD's 'slicer' viewer with 'high resolution' interpolation mode rendering.

14pf MTs were identified by their moire patterns^{9, 10} and confirmed in tomographic volume cross-sections. MT polarity was determined using a combination of previously reported methods that use i) change of moire pattern appearance with tilt angle and ii) directionality of 'arrowhead' moire patterns in 2D projections¹¹ (see Supplementary Fig. 6a,b). Only MTs where polarity was unambiguously determined were analyzed. Quantitative characterization of MT plus and minus ends was performed in 3DMOD's 'slicer' viewer. Protofilament trajectories were traced manually by plotting their three-dimensional center of mass in successive 1.6nm cross-sections along the MT axis from lattice to end. Coordinates of protofilament positions in 3D space were used to calculate distances both within single protofilaments and between adjacent protofilaments. Protofilament curvatures were calculated as monomer rotation (i.e the rotation needed to superimpose one tubulin monomer onto the next), comparable to e.g.¹² and¹³).

References

1. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9, 676-682 (2012).
2. Kremer, J.R., Mastronarde, D.N. & McIntosh, J.R. Computer visualization of three-dimensional image data using IMOD. *Journal of structural biology* 116, 71-76 (1996).
3. Abrishami, V. et al. Alignment of direct detection device micrographs using a robust Optical Flow approach. *Journal of structural biology* 189, 163-176 (2015).
4. de la Rosa-Trevin, J.M. et al. Xmipp 3.0: an improved software suite for image processing in electron microscopy. *Journal of structural biology* 184, 321-328 (2013).
5. Mindell, J.A. & Grigorieff, N. Accurate determination of local defocus and specimen tilt in electron microscopy. *Journal of structural biology* 142, 334-347 (2003).
6. Li, X. et al. Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM. *Nat Methods* 10, 584-590 (2013).
7. Sindelar, C.V. & Downing, K.H. The beginning of kinesin's force-generating cycle visualized at 9-A resolution. *The Journal of cell biology* 177, 377-385 (2007).
8. Sindelar, C.V. & Downing, K.H. An atomic-level mechanism for activation of the kinesin molecular motors. *Proceedings of the National Academy of Sciences of the United States of America* 107, 4111-4116 (2010).
9. Wade, R.H., Chretien, D. & Job, D. Characterization of microtubule protofilament numbers. How does the surface lattice accommodate? *Journal of molecular biology* 212, 775-786 (1990).
10. Chretien, D. & Wade, R.H. New data on the microtubule surface lattice. *Biology of the cell / under the auspices of the European Cell Biology Organization* 71, 161-174 (1991).
11. Chretien, D., Kenney, J.M., Fuller, S.D. & Wade, R.H. Determination of microtubule polarity by cryo-electron microscopy. *Structure* 4, 1031-1040 (1996).

12. Nawrotek, A., Knossow, M. & Gigant, B. The determinants that govern microtubule assembly from the atomic structure of GTP-tubulin. *Journal of molecular biology* 412, 35-42 (2011).
13. Brouhard, G.J. & Rice, L.M. The contribution of alphabeta-tubulin curvature to microtubule dynamics. *The Journal of cell biology* 207, 323-334 (2014).

Supplementary Table 1. List of screened genomes and their sources.

Scientific name	Taxonomy ID	Download (m/d/y)	Assembly version	Reference/source
Aureococcus anophagefferens CCMP1984	44056	6/13/2013	v.1.0 (September 27, 2007)	Gobler CJ, <i>et al.</i> (2011) Niche of harmful alga <i>Aureococcus anophagefferens</i> revealed through ecogenomics. <i>Proceedings of the National Academy of Sciences of the United States of America</i> 108(11):4352-4357
Acanthamoeba castellanii str. Neff	1257118	6/12/2013	Acastellanii.strNEFF v1 (January 9, 2013)	Clarke M, <i>et al.</i> (2013) Genome of <i>Acanthamoeba castellanii</i> highlights extensive lateral gene transfer and early evolution of tyrosine kinase signaling. <i>Genome biology</i> 14(2):R11.
Aquilegia coerulea Goldsmith	218851	17/7/2013	Release of the initial 8X unmapped <i>Aquilegia coerulea</i> Goldsmith genome	<i>Aquilegia coerulea</i> Genome Sequencing Project, http://phytozome.jgi.doe.gov/
Anopheles gambiae str. PEST	180454	7/1/2013	AgamP3.7	Holt RA, <i>et al.</i> (2002) The genome sequence of the malaria mosquito <i>Anopheles gambiae</i> . <i>Science</i> 298(5591):129-149.
Aplanochytrium kerguelense PBS07	702273	4/11/2013	V1	These sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community.
Albugo laibachii Nc14	890382	7/1/2013	ENA 1 (2011-08-ENA)	Kemen E, <i>et al.</i> (2011) Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of <i>Arabidopsis thaliana</i> . <i>PLoS biology</i> 9(7):e1001094.
Aurantiochytrium limacinum ATCC MYA-1381	717989	4/11/2013	V1	These sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community.
Allomyces macrogynus ATCC 38327	578462	6/10/2013	V1, March 1, 2012	Origins of Multicellularity Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Amphimedon queenslandica	400682	6/12/2013	V1.0, (May 28, 2010)	Srivastava M, <i>et al.</i> (2010) The <i>Amphimedon queenslandica</i> genome and the evolution of animal complexity. <i>Nature</i> 466(7307):720-726.
Arabidopsis thaliana	3702	7/3/2013	TAIR10, November 17, 2010	Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant <i>Arabidopsis thaliana</i> . <i>Nature</i> 408(6814):796-815.
Amborella trichopoda	13333	5/1/2013	Unknown	Amborella Genome Project (2013) The <i>Amborella</i> genome and the evolution of flowering plants. <i>Science</i> 342(6165):1241089.
Batrachochytrium dendrobatidis JAM81	684364	6/3/2013	V1	These sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community

Branchiostoma floridae	7739	7/1/2013	Unknown	Putnam NH, <i>et al.</i> (2008) The amphioxus genome and the evolution of the chordate karyotype. <i>Nature</i> 453(7198):1064-1071.
Blastocystis hominis	12968	6/13/2013	V1	Denoeud F, <i>et al.</i> (2011) Genome sequence of the stramenopile Blastocystis, a human anaerobic parasite. <i>Genome biology</i> 12(3):R29.
Brugia malayi	6279	6/27/2013	WS238	Ghedini E, <i>et al.</i> (2007) Draft genome of the filarial nematode parasite Brugia malayi. <i>Science</i> 317(5845):1756-1760. Yook K, <i>et al.</i> (2012) WormBase 2012: more genomes, more data, new website. <i>Nucleic acids research</i> 40(Database issue):D735-741.
Bigeloviella natans CCMP2755	753081	6/13/2013	V1	Curtis BA, <i>et al.</i> (2012) Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. <i>Nature</i> 492(7427):59-65.
Bathycoccus prasinos RCC1005	1075084	5/30/2013	V1	Moreau H, <i>et al.</i> (2012) Gene functionalities and genome structure in Bathycoccus prasinos reflect cellular specializations at the base of the green lineage. <i>Genome biology</i> 13(8):R74.
Catenaria anguillulae PL171	765915	6/10/2013	V1	These sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community.
Conidiobolus coronatus NRRL28638	796925	6/10/2013	V1	Chang Y, <i>et al.</i> (2015) Phylogenomic Analyses Indicate that Early Fungi Evolved Digesting Cell Walls of Algal Ancestors of Land Plants. <i>Genome Biol Evol</i> 7(6):1590-1601.
Caenorhabditis elegans	6239	7/1/2013	WBcel235	C. elegans Sequencing Consortium (1998) Genome sequence of the nematode C. elegans: a platform for investigating biology. <i>Science</i> 282(5396):2012-2018.
Candida glabrata CBS138	284593	6/13/2013	7/12/2012	Dujon B, <i>et al.</i> (2004) Genome evolution in yeasts. <i>Nature</i> 430(6995):35-44. Inglis DO, <i>et al.</i> (2012) The Candida genome database incorporates multiple Candida species: multispecies search and analysis tools with curated gene and protein information for Candida albicans and Candida glabrata. <i>Nucleic acids research</i> 40(Database issue):D667-674.
Ciona intestinalis	7719	6/27/2013	KH (April 29, 2011)	Dehal P, <i>et al.</i> (2002) The draft genome of Ciona intestinalis: insights into chordate and vertebrate origins. <i>Science</i> 298(5601):2157-2167.
Cyanidioschyzon merolae 10D	280699	6/13/2013	V1	Matsuzaki M, <i>et al.</i> (2004) Genome sequence of the ultrasmall unicellular red alga Cyanidioschyzon merolae 10D. <i>Nature</i> 428(6983):653-657.
Cryptococcus neoformans	235443	6/12/2013	V4	Cryptococcus neoformans var. grubii H99 Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/) Loftus BJ, <i>et al.</i> (2005) The genome of the basidiomycetous yeast and human pathogen Cryptococcus neoformans.

				<i>Science</i> 307(5713):1321-1324.
Capsaspora owczarzaki ATCC 30864	595528	5/31/2013	V2	Origins of Multicellularity Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Cryptosporidium parvum Iowa II	414452	5/29/2013	2/23/2007	Abrahamsen MS, <i>et al.</i> (2004) Complete genome sequence of the apicomplexan, <i>Cryptosporidium parvum</i> . <i>Science</i> 304(5669):441-445.
Cyanophora paradoxa	2762	6/13/2013	V1, November 2010	Price DC, <i>et al.</i> (2012) <i>Cyanophora paradoxa</i> genome elucidates origin of photosynthesis in algae and plants. <i>Science</i> 335(6070):843-847.
Chlamydomonas reinhardtii	3055	7/2/2013	Unknown	Merchant SS, <i>et al.</i> (2007) The <i>Chlamydomonas</i> genome reveals the evolution of key animal and plant functions. <i>Science</i> 318(5848):245-250.
Coemansia reversa NRRL 1564	763665	6/10/2013	V1	Chang Y, <i>et al.</i> (2015) Phylogenomic Analyses Indicate that Early Fungi Evolved Digesting Cell Walls of Algal Ancestors of Land Plants. <i>Genome Biol Evol</i> 7(6):1590-1601.
Coccomyxa subellipsoidea C-169	574566	6/14/2013	V2.0 (April 13, 2012)	Blanc G, <i>et al.</i> (2012) The genome of the polar eukaryotic microalga <i>Coccomyxa subellipsoidea</i> reveals traits of cold adaptation. <i>Genome biology</i> 13(5):R39.
Chlorella variabilis NC64A	554065	5/30/2013	V1	Blanc G, <i>et al.</i> (2010) The <i>Chlorella variabilis</i> NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. <i>The Plant cell</i> 22(9):2943-2955.
Dictyostelium discoideum AX4	352472	5/30/2013	Unknown	Eichinger L, <i>et al.</i> (2005) The genome of the social amoeba <i>Dictyostelium discoideum</i> . <i>Nature</i> 435(7038):43-57.
Debaryomyces hansenii CBS767	284592	6/12/2013	V2, February 9, 2012	Dujon B, <i>et al.</i> (2004) Genome evolution in yeasts. <i>Nature</i> 430(6995):35-44.
Drosophila melanogaster	7227	6/26/2013	5.51, May 7, 2013	Adams MD, <i>et al.</i> (2000) The genome sequence of <i>Drosophila melanogaster</i> . <i>Science</i> 287(5461):2185-2195. Attrill H, <i>et al.</i> (2015) FlyBase: establishing a Gene Group resource for <i>Drosophila melanogaster</i> . <i>Nucleic acids research</i> .
Danio rerio	7955	7/1/2013	Zv9	Howe K, <i>et al.</i> (2013) The zebrafish reference genome sequence and its relationship to the human genome. <i>Nature</i> 496(7446):498-503.
Edhazardia aedis USNM 41457	1003232	5/27/2013	4/30/2013	Microsporidia Comparative Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Entamoeba histolytica HM-1:IMSS	294381	5/31/2013	11/27/2009	Loftus B, <i>et al.</i> (2005) The genome of the protist parasite <i>Entamoeba histolytica</i> . <i>Nature</i> 433(7028):865-868.
Emiliania huxleyi CCMP1516	280463	6/26/2013	V1	Read BA, <i>et al.</i> (2013) Pan genome of the phytoplankton <i>Emiliania</i> underpins its global distribution. <i>Nature</i> 499(7457):209-213.
Encephalitozoon intestinalis ATCC 50506	876142	5/31/2013	10/18/2010	Corradi N, Pombert JF, Farinelli L, Didier ES, & Keeling PJ (2010) The complete sequence of the smallest known nuclear genome from the microsporidian <i>Encephalitozoon intestinalis</i> . <i>Nature communications</i> 1:77.

Ectocarpus siliculosus	2880	6/13/2013	Unknown	Cock JM, <i>et al.</i> (2010) The Ectocarpus genome and the independent evolution of multicellularity in brown algae. <i>Nature</i> 465(7298):617-621.
Fonticula alba	691883	7/21/2014	V2	Origins of Multicellularity Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Giardia intestinalis assemblage A	941442	5/29/2013	2/8/2013	Morrison HG, <i>et al.</i> (2007) Genomic minimalism in the early diverging intestinal parasite Giardia lamblia. <i>Science</i> 317(5846):1921-1926.
Galdieria sulphuraria	130081	6/13/2013	ASM34128v1 (February 25, 2013)	Schonknecht G, <i>et al.</i> (2013) Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. <i>Science</i> 339(6124):1207-1210.
Guillardia theta CCMP2712	905079	7/1/2013	Guith1 (2012-12-EnsemblProtist)	Curtis BA, <i>et al.</i> (2012) Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. <i>Nature</i> 492(7427):59-65.
Hyaloperonospora parasitica	272952	7/5/2013	Unknown	Saprolegnia genome Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Homo sapiens	9606	7/1/2013	GRCh37.p10	Lander ES, <i>et al.</i> (2001) Initial sequencing and analysis of the human genome. <i>Nature</i> 409(6822):860-921.
Klebsormidium flaccidum	3175	7/21/2014	V1.0	Hori K, <i>et al.</i> (2014) Klebsormidium flaccidum genome reveals primary factors for plant terrestrial adaptation. <i>Nature communications</i> 5
Kluyveromyces lactis NRRL Y-1140	284590	6/12/2013	ASM251v1, (July 2, 2004)	Dujon B, <i>et al.</i> (2004) Genome evolution in yeasts. <i>Nature</i> 430(6995):35-44.
Leishmania major strain Friedlin	347515	5/29/2013	10/20/2010	Ivens AC, <i>et al.</i> (2005) The genome of the kinetoplastid parasite, Leishmania major. <i>Science</i> 309(5733):436-442.
Monosiga brevicollis MX1 / ATCC 50154	431895 / 487148	6/12/2013	V1.0, (December 20, 2007)	King N, <i>et al.</i> (2008) The genome of the choanoflagellate Monosiga brevicollis and the origin of metazoans. <i>Nature</i> 451(7180):783-788.
Mucor circinelloides 1006PhL	1220926	6/28/2013	V1	Lee SC, <i>et al.</i> (2014) Analysis of a food-borne fungal pathogen outbreak: virulence and genome of a Mucor circinelloides isolate from yogurt. <i>mBio</i> 5(4):e01390-01314.
Mortierella elongata	310910	6/10/2013	V1	These sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community.
Mnemiopsis leidyi	27923	5/29/2013	Initial release	http://research.nhgri.nih.gov/mnemiopsis/
Mus musculus	10090	7/1/2013	GRCm38.p1	Waterston RH, <i>et al.</i> (2002) Initial sequencing and comparative analysis of the mouse genome. <i>Nature</i> 420(6915):520-562.
Micromonas species RCC299	296587	6/17/2013	ASM9098v1 (April 10, 2009)	Worden AZ, <i>et al.</i> (2009) Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes Micromonas. <i>Science</i> 324(5924):268-272.
Mortierella verticillata	1069443	6/10/2013	2/17/2011	Origins of Multicellularity Sequencing

NRRL 6337				Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Neurospora crassa OR74A	367110	6/12/2013	3/11/2013	Neurospora crassa Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/) Galagan JE, <i>et al.</i> (2003) The genome sequence of the filamentous fungus <i>Neurospora crassa</i> . <i>Nature</i> 422(6934):859-868.
Nannochloropsis gaditana CCMP526	1093141	6/13/2013	1.1	Radakovits R, <i>et al.</i> (2012) Draft genome sequence and genetic transformation of the oleaginous alga <i>Nannochloropsis gaditana</i> . <i>Nature communications</i> 3:686.
Naegleria gruberi strain NEG-M	744533	6/19/2013	1	Fritz-Laylin LK, <i>et al.</i> (2010) The genome of <i>Naegleria gruberi</i> illuminates early eukaryotic versatility. <i>Cell</i> 140(5):631-642.
Nematostella vectensis	45351	5/29/2013	1	Putnam NH, <i>et al.</i> (2007) Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. <i>Science</i> 317(5834):86-94.
Ostreococcus lucimarinus CCE9901	436017	7/3/2013	Unknown	Palenik B, <i>et al.</i> (2007) The tiny eukaryote <i>Ostreococcus</i> provides genomic insights into the paradox of plankton speciation. <i>Proceedings of the National Academy of Sciences of the United States of America</i> 104(18):7705-7710.
Oryza sativa japonica	39947	7/1/2013	MSU6 (2009-01-MSU6)	International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. <i>Nature</i> 436(7052):793-800.
Oxytricha trifallax	1172189	5/29/2013	2/12/2012	Swart EC, <i>et al.</i> (2013) The <i>Oxytricha trifallax</i> macronuclear genome: a complex eukaryotic genome with 16,000 tiny chromosomes. <i>PLoS biology</i> 11(1):e1001473.
Phycomyces blakesleeanus NRRL1555	763407	6/10/2013	V2	These sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community.
Plasmodiophora brassicae	37360	12/30/2015	V1	Schwelm A, <i>et al.</i> (2015) The <i>Plasmodiophora brassicae</i> genome reveals insights in its life cycle and ancestry of chitin synthases. <i>Scientific Reports</i> 5:11153.
Plasmodium falciparum 3D7	36329	5/29/2013	3	Gardner MJ, <i>et al.</i> (2002) Genome sequence of the human malaria parasite <i>Plasmodium falciparum</i> . <i>Nature</i> 419(6906):498-511.
Phytophthora infestans T30-4	403677	6/13/2013	ASM14294v1, (June 17, 2009)	Phytophthora infestans Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/) Haas BJ, <i>et al.</i> (2009) Genome sequence and analysis of the Irish potato famine pathogen <i>Phytophthora infestans</i> . <i>Nature</i> 461(7262):393-398.
Perkinsus marinus ATCC 50983	423536	5/29/2013	Unknown	
Polysphondylium pallidum PN500	670386	5/31/2013	PolPal_Dec2009, (January 29, 2010)	Heidel AJ, <i>et al.</i> (2011) Phylogeny-wide analysis of social amoeba genomes

				highlights ancient origins for complex intercellular communication. <i>Genome research</i> 21(11):1882-1891.
Physcomitrella patens subsp. patens	145481	7/4/2013	V1.6	Rensing SA, <i>et al.</i> (2008) The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants. <i>Science</i> 319(5859):64-69. Zimmer AD, <i>et al.</i> (2013) Reannotation and extended community resources for the genome of the non-seed plant Physcomitrella patens provide insights into the evolution of plant gene structures and functions. <i>BMC genomics</i> 14:498.
Paramecium tetraurelia	5888	5/29/2013	v1.78	Arnaiz O & Sperling L (2011) ParameciumDB in 2011: new tools and new data for functional and comparative genomics of the model ciliate Paramecium tetraurelia. <i>Nucleic acids research</i> 39(Database issue):D632-636.
Phaeodactylum tricornutum CCAP1055/1	556484	6/13/2013	ASM15095v2 (December 12, 2008)	Bowler C, <i>et al.</i> (2008) The Phaeodactylum genome reveals the evolutionary history of diatom genomes. <i>Nature</i> 456(7219):239-244.
Piromyces species	45796	7/21/2014	v1 (March 2011)	http://genome.jgi.doe.gov
Rozella allomyces	281847	04/08/2014	GCA_000442015.1	Chang Y, <i>et al.</i> (2015) Phylogenomic Analyses Indicate that Early Fungi Evolved Digesting Cell Walls of Algal Ancestors of Land Plants. <i>Genome Biol Evol</i> 7(6):1590-1601.
Rhizophagus irregularis DAOM 197198	588596	21/07/2014	v1 (March 2012)	Tisserant E, <i>et al.</i> (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. <i>Proceedings of the National Academy of Sciences</i> 110(50):20117-20122.
Saccharomyces cerevisiae S288C	559292	6/13/2013	Release R64-1-1	Goffeau A, <i>et al.</i> (1996) Life with 6000 genes. <i>Science</i> 274(5287):546, 563-547.
Saccoglossus kowalevskii	10224	7/1/2013	Unknown	Simakov O, <i>et al.</i> (2015) Hemichordate genomes and deuterostome origins. <i>Nature</i> 527(7579):459-465.
Schistosoma mansoni	6183	5/29/2013	5	Berriman M, <i>et al.</i> (2009) The genome of the blood fluke Schistosoma mansoni. <i>Nature</i> 460(7253):352-358.
Spironucleus salmonicida	348837	Genome not downloaded, but only accessed online via the nr database of NCBI.		
Symbiodinium minutum	1202447	17/7/13	v1.0	Shoguchi E, <i>et al.</i> (2013) Draft assembly of the Symbiodinium minutum nuclear genome reveals dinoflagellate gene structure. <i>Current biology : CB</i> 23(15):1399-1408.
Selaginella moellendorffii	88036	7/1/2013	v1.0 (2011-05-ENA)	Banks JA, <i>et al.</i> (2011) The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. <i>Science</i> 332(6032):960-963.
Strongylocentrotus purpuratus	7668	28/07/2013	Spur_3.1	Sodergren E, <i>et al.</i> (2006) The Genome of the Sea Urchin Strongylocentrotus purpuratus. <i>Science</i> 314(5801):941-952.
Schizosaccharomyces pombe (strain 972 / ATCC 24843)	4896	7/1/2013	ASM294v1 (2012-03-PomBase)	Wood V, <i>et al.</i> (2002) The genome sequence of Schizosaccharomyces pombe. <i>Nature</i> 415(6874):871-880.

Spizellomyces punctatus DAOM BR117	645134	6/3/2013 / 6/11/2013	3/1/2012	Origins of Multicellularity Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Salpingoeca rosetta	946362	5/31/2013	V1	Origins of Multicellularity Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Trichoplax adhaerens	10228	6/12/2013	V1.0, (June 17, 2008)	Srivastava M, <i>et al.</i> (2008) The Trichoplax genome and the nature of placozoans. <i>Nature</i> 454(7207):955-960.
Trypanosoma brucei TREU 927	185431	6/19/2013	2013-01-16	Berriman M, <i>et al.</i> (2005) The genome of the African trypanosome <i>Trypanosoma brucei</i> . <i>Science</i> 309(5733):416-422.
Toxoplasma gondii ME49	508771	5/29/2013	7/23/2012	Lis Caler, J. Craig Venter Institute
Thalassiosira pseudonana	296543	6/13/2013	ASM14940v1 (January 16, 2009)	Armbrust EV, <i>et al.</i> (2004) The genome of the diatom <i>Thalassiosira pseudonana</i> : ecology, evolution, and metabolism. <i>Science</i> 306(5693):79-86.
Takifugu rubripes	31033	7/1/2013	FUGU5 (October 13, 2011)	Aparicio S, <i>et al.</i> (2002) Whole-genome shotgun assembly and analysis of the genome of <i>Fugu rubripes</i> . <i>Science</i> 297(5585):1301-1310.
Tetrahymena thermophila	5911	5/29/2013	Oct 2008	Eisen JA, <i>et al.</i> (2006) Macronuclear genome sequence of the ciliate <i>Tetrahymena thermophila</i> , a model eukaryote. <i>PLoS biology</i> 4(9):e286. Stover NA, <i>et al.</i> (2006) <i>Tetrahymena</i> Genome Database (TGD): a new genomic resource for <i>Tetrahymena thermophila</i> research. <i>Nucleic acids research</i> 34(Database issue):D500-503.
Tetraselmis sp. GSL018	582737	Genome not downloaded, but only accessed online via the nr database of NCBI.		
Thecamonas trahens ATCC 50062	461836	6/13/2013	10/28/2011	Origins of Multicellularity Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Trichomonas vaginalis G3	412133	5/29/2013	1/11/2007	Carlton JM, <i>et al.</i> (2007) Draft genome sequence of the sexually transmitted pathogen <i>Trichomonas vaginalis</i> . <i>Science</i> 315(5809):207-212.
Ustilago maydis	237631	6/12/2013	ASM32847v1 (March 5, 2007)	Kamper J, <i>et al.</i> (2006) Insights from the genome of the biotrophic fungal plant pathogen <i>Ustilago maydis</i> . <i>Nature</i> 444(7115):97-101.
Volvox carteri	3068	7/3/2013	Unknown	Prochnik SE, <i>et al.</i> (2010) Genomic analysis of organismal complexity in the multicellular green alga <i>Volvox carteri</i> . <i>Science</i> 329(5988):223-226.
Vavraia culicis floridensis	948595	5/27/2013	4/30/2013	Microsporidia Comparative Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)
Xenopus tropicalis	8364	7/1/2013	Xtropicalis_v7 (December 19, 2012)	Hellsten U, <i>et al.</i> (2010) The genome of the Western clawed frog <i>Xenopus tropicalis</i> . <i>Science</i> 328(5978):633-636.
Yarrowia lipolytica CLIB 122	284591	7/1/2013	ASM252v1 (2012-05-EnsemblFungi)	Dujon B, <i>et al.</i> (2004) Genome evolution in yeasts. <i>Nature</i> 430(6995):35-44.