Supplemental material

Table 1. Alignment of α-tubulin C-terminal sequences and relation to classification.

ID	Organism	CNBr C-terminal sequence	
EDO07846	Babesia bovis	EEGEFSEAREDLAALEKDYEEVGLDTT--------YDEEAENY	**Apicomplexans**
44.m02671	Toxoplasma gondii	EEGQLTEARDDLAALERDYDEVASDTKMDADDEEDLNEDFFPN	
583.m00022	Toxoplasma gondii	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGYGDEY	
AA015882	Neospora caninum	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGYGDEY	
P12543	Plasmodium yoelii yoelii	EEGEFSEAREDLAALEKDYEEVGIETNDG---EGEDEGYEADY	
P14642	Plasmodium falciparum	EEGEFSEAREDLAALEKDYEEVGIETNEG---EGEDEGYEAEY	
XP_001351526	Plasmodium falciparum	EEGEFSEAREDLAALEKDYEEVGIETNEG---EGEDEGYE---	
CAA61255	Eimeria acervulina	EEGEFSEAREDLAALEKDYEEVGIETAEG---EAEEEGYGDEF	
AAD20239	Cryptosporidium parvum	EEGEFSEAREDLAALEKDYEEVGIEIADG---EDEEVHYEGDF	
ABV72532	Heterocapsa triquera	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGYGDEF	**Dynoflagellates**
ABV72560	Heterocapsa rotundata	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGYGDEF	
ABV22199	Karlodinium micrum	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGYGDEF	
P41351	Tetrahymena pyriformis	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGY----	**Ciliates**
P10872	Tetrahymena pyriformis	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGY----	
AAT09064	Bigelowiella natans	EEGEFSEAREDLAALEKDYEEVGTESQEGGEGEGEGAEEF---	**Chloroarachyophyta**
Q40832	Pelvetia fastigiata	EEGEFSEAREDLAALEKDYEEVGAETAEG---EGEEEDFGEEY	**Phaeophyta**
Q40831	Pelvetia fastigiata	EEGEFSEAREDLVALEKDYEEVGAETADG---DGEEEEFGEEY	
CAA77810	Oxytricha granulifera	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGME---	**Ciliates**
P09243	Stylonychia lemnae	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGME---	
AAL73386	Euplotes focardii	EEGEFSEAREDLAALEKDYEEVGVETAEG---EGEEE-ME---	
CAI38956	Paramecium tetraurelia	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEA-----	
CAA67848	Paramecium tetraurelia	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEGEA----	
XP_001454509	Paramecium tetraurelia	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEGEG----	
Q08114	Euplotes octocarinatus	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEGME---	
CAA77816	Euplotes vamus	EEGEFSEAREDLAALEKDYEEVGIETAEG---EGEEEDMA---	
ABU93324	Monocercomonoides sp	EEGEFSEAREDLAALEKDYEEVGAESGED---EEEGEGGEEY-	**Oxymonad flagellates**
ABC97356	Streblomastix trix	EEGEFSEAREDLAALEKDYEEVGAESGGG---EEEEE--EEA-	
P11237	Naesteria gruberi	EEGEFSEAREDLAALEKDYEEVGTESQEGDGEEGEDGGDQ---	**Amoeba**
P50258	Physarum polycephalum	EEGEFSEAREDLAALEKDYEEVGAESSEA---GGDEEGEY---	
CAA65329	Reticulomyxa filosa	EEGEFSEAREDLAALEKDYEEVGAESLQN---GVEEDEMEV--	
CAA65330	Reticulomyxa filosa	EEGEFSEAREDLAALEKDYEEVGAESLQH---GAEDEEMEV--	
P33625	Euglena gracilis	EEGEFSEAREDLAALEKDYEEVGAESADV---EGEEDVEEY--	**Euglenozoa**
ABA00480	Trypanosoma danilewskyi	EEGEFSEAREDLAALEKDYEEVGAESGDL---EGEEDVEEY--	
CAJ16362	Trypanosoma brucei	EEGEFSEAREDLAALEKDYEEVGAESADM---DGEEDVEEY--	
AAA58321	Leishmania donovani	EEGEFSEAREDLAALEKDYEEVGAESADD---MGEEDVEEY	

Figure S1. Immunoblot analysis of Tyr-tubulin and polyGlu tubulin posttranslational modification of cytoskeletal tubulins

Immunoblot stained with anti-polyGlu-tubulins (a), anti-β & anti-polyGlu-tubulin (b) and anti-α-tubulin (c). The same immunoblot was used for each antibody. The immunoblot was stripped between antibody incubations using a standard protocol.

Figure S2. Analysis of methylation posttranslational modification of cytoskeletal tubulins using immunofluorescence microscopy and immunoblot.

A. Phase contrast image (a) corresponds to images b through e. Phase contrast image (a') corresponds to images b' through e'. Immunofluorescence analysis of cells imaged with DAPI for DNA (b, b'), YFP-α-tubulin (c, c'), anti-H4K20me3 (d, d'), and the merge of images of c and d (e, e'). The nucleus is indicated by arrows in figure d' and the conoid of daughter cells is indicated in arrows in figure e.

B. 2D immunoblot analysis using anti-α & β-tubulin (1), anti-α-tubulin (2), and anti-H4K20me3 (3) demonstrating reaction of anti-H4K20me3 (and possible methylation) in both α and β tubulin. The same immunoblot was used for each antibody. The immunoblot was stripped between antibody incubations using a standard protocol.

C. Immunoblot analysis using and anti-H4K20me3 (1) anti- β-tubulin (2) and anti-α-tubulin (3) for control (bovine brain tubulin, lane 1), Human Foreskin Fibroblasts (HFF, lane 2), HFF pellet (lane 3) and *T.gondii* tubulins (lane 4) in a 1D gel.

D. Coomassie blue (1) stain of separated α and β tubulin from *T. gondii* and Immunoblot analysis with anti-H4k20me3 (2) and anti-β- and anti-α-tubulin (3) after tubulins were separated on a 1D gel.

Supplemental Text for Figure S2

Detection of methylation on T. gondii tubulin using immunofluorescence microscopy and immunoblot. The H4K20me3 antibody labelled the anterior region of *T. gondii* intensely. This antibody is known to react with the methyl modifications at K20 in H4 (histone). By immunoblot, the H4K20me3 antibody reacted with *T. gondii* tubulins focusing at pIs more basic than the β-tubulin region (Fig. S2-B). Since *T. gondii* were grown in human foreskin fibroblasts (HFF), it was necessary to exclude that the reaction of anti-H4K20me3 originated from the HFF. The soluble fraction of uninfected HFF cells were prepared using the same protocol as cytoskeleton fraction of *T.gondii* and subjected to 1D SDS PAGE and immunoblot analysis in parallel with the cytoskeleton fraction of *T. gondii.* Tubulin was also purified using aTaxol assisted pelleting method [28] and subjected to 1D SDS PAGE and immunoblot analysis. Tubulins from bovine brain (served as the control), HFF cells, *T. gondii* were stained by anti-α & β-tubulins (Fig. S2-C). However, only the tubulins from *T. gondii* were labeled by anti-H4K20me3 (Fig. S2-C), suggesting that the tubulins from *T. gondii*, but not HFF, react with this serum and are likely methylated (Fig. S2-C). There is a band in HFF cells reactive with H4K20me3 antibody with a molecular weight of about 40 kDa, but no evidence for the presence of tubulin was found in this 40 kDa band by mass spectrometry. A band near 76 kDa was strongly labeled with H4K20me3, however mass spectrometry analysis revealed that there was no tubulin in this region. In the narrow pH range (pH 4.5-5.5) 2D gel of cytoskeletal tubulin, H4K20me3 antibody stained a number of ~50 kDa spots (Fig. S2-B). The alignment of these spots with those detected by α- or β-tubulin antibodies (Fig. S2-B) suggested that this antibody and possible methylation occurs on both α- and β-tubulins from *T. gondii.* The H4K20me3 antibody reactive β-tubulins were located at the basic end of the β-tubulin region on the gel, which overlaps with α-tubulin. This is expected, since the pI of these β-tubulins would be shifted to the

basic side if methylation occurs on glutamates and aspartates of their C-termini. Figure S3-C, panel 1 also demonstrates that some spots of approximately 60 kDa and 75 kDa were also recognized by H4K20me3 antibody. These spots did not contain tubulin by mass spectrometry analysis. To further confirm the occurrence of methylation on tubulin, α- and β-tubulins were separated on a 10% mini-gel (Bio-Rad, CA) and eluted from gel slices using an Electro-Eluter (Model 422 Electro-Eluter, Bio-Rad, CA), followed by immunoblot analysis. The separated α- and β-tubulins both demonstrated labelling by H4K20me3 antibody at the tubulin bands as indicated by anti-α and β-tubulin (Fig. S2-D), consistent with methylation on both α- and β-tubulins. While it is possible that the anti-H4K20me3 reaction with *T. gondii* is due to some unrelated cross reaction in this rabbit polyclonal serum, we believe this is unlikely, since staining by IFA and immunoblot clearly demonstrated that this was specific to *T. gondii* tubulin, limited reactivity was seen with anti-H4K20me3 and other proteins in *T. gondii* and independent mass spectrometry data confirmed that methylation occurred on *T. gondii* tubulin. Confirmation of the immunolocalization of *T. gondii* methyl tubulin antibody will require the production of a *T. gondii* methyl tubulin specific antiserum.