SUPPLEMENTAL DATA Bacterial tubulin

INDEX:

Table S1. Results of scanning the *P. dejongeii* genome with protein family profiles (Pfam) of chaperones and other proteins interacting with tubulin or microtubules.

Protein	*Pfam code*	*Description*	*Number of hits found*[a]	*Bit score of hits*	*Trusted score cut-off*[b]	*Number of trusted hits*
CCT chaperonin	PF00118	TCP-1/cpn60 chaperonin family	1	155.1	19.8	1
CRIPT	PF10235	Microtubule-associated protein CRIPT	0			0
Dinein heavy chain N-t 1	PF08385	Dynein heavy chain, N-terminal region 1	0			0
Dinein heavy chain N-t 2	PF08393	Dynein heavy chain, N-terminal region 2	1	11.5	24.9	0
Dinein light chain N-t 2	PF01221	Dynein light chain type 1	0			0
Dynein heavy chain	PF03028	Dynein heavy chain	0			0
Kinesin	PF00225	Kinesin motor domain	0			0
Kinesin light chain	PF00515	Tetratricopeptide repeat	28	9.5-32.9	20.4	13
MAP 1A/1B	PF02991	Microtubule associated protein 1A/1B, light chain 3	0			0
MAP2	PF08377	MAP2/Tau projection domain	0			0
MAP65	PF03999	Microtubule associated protein (MAP65/ASE1 family)	0			0
MAP7	PF05672	MAP7 (E-MAP-115) family	0			0
Microtubule-associated protein	PF07989	Microtubule associated	1	10.6	22.0	0
Prefoldin family 1	PF02996	Prefoldin subunit	0			0

Prefoldin family 2	PF01920	Prefoldin subunit	3	9.4-13.3	21.1	0
Stahmin	PF00836	Stathmin family	0			0
Tau	PF00418	Tau and MAP protein, tubulin-binding repeat	1	11.1	26.6	0
Tubulin cofactor A	PF02970	Tubulin folding cofactor A	0			0
Tubulin cofactor B	PF01302	CAP-Gly domain	0			0
Tubulin cofactor C	PF07986	Tubulin folding cofactor C	0			0
Tubulin cofactor D C-t	PF12612	Tubulin folding cofactor D C-terminal	0			0

[a] Number of hits below an E-value of 1.0
[b] Obtained from http://pfam.sanger.ac.uk/

Table S2. Oligonucleotides employed in this work. Long oligonucleotides were used to introduce multiple substitutions (underscored), insertions (bold) and deletions (stars) from bovine tubulin into Btub. Oligos were designed to minimize the number of changes to favour hybridization with original template.

Name	Sequence (5' to 3')	Location (5'-start position)
A_T147G-F	GCCATCGGTGGCGGTGGAGGGTCGGGTTTTGGGG	424 (BtubA)
A_T147G-R	CCCCAAAACCCGACCCTCCACCGCCACCGATGGC	457 (BtubA)
A1A-F	GTAGCGCCACGTGGGGGCGACGACTCTTTTACCACCTTCTTCTGCGAAACTTCCTCTGGCAGCTAC	127 (BtubA)
A1A-R	GTAGCTGCCAGAGGAAGTTTCGCAGAAGAAGGTGGTAAAAGAGTCGTCGCCCCCACGTGGCGCTAC	192 (BtubA)
AMA-F	TGCGCCTTTGCTCCGGTGATATCGGCAGAGAAGGCTTACCACGAGCAATTGAGCATTGAGGAGATGATC	819 (BtubA)
AMA-R	GATCATCTCCTCAATGCTCAATTGCTCGTGGTAAGCCTTCTCTGCCGATATCACCGGAGCAAAGGCGCA	888 (BtubA)
BMA-F	GTGCGCCTTTGCTCCGTTGACATCGCGAGGCAGCCAACAGTATAGGGCATTGACCATTGAGGAGATGATC	819 (BtubA)
BMA-R	GATCATCTCCTCAATGGTCAATGCCCTATACTGTTGGCTGCCTCGCGATGTCAACGGAGCAAAGGCGCAC	888 (BtubA)
ASA-F	GCTATGTGGAGCAGCCCCCTACTGTCGTCCCAGGAGGAGACCTCGCAAAAGTCCAGAAGAGCATGGTGCTGC	1070 (BtubA)
ASA-R	GCAGCACCATGCTCTTCTGGACTTTTGCGAGGTCTCCTCCTGGGACGACAGTAGGGGGCTGCTCCACATAGC	1116 (BtubA)
B_S144G-F	CTCCATCGGTGGCGGTGGCGGCTCAGGCCTTGGC	414 (BtubB)
B_S144G-R	GCCAAGGCCTGAGCCGCCACCGCCACCGATGGA	447 (BtubB)
B1B-F	GCAGGTACCCTGAAAGGAGACAGC***GATCTGCAACTTGAACGCATGGAAGTCTTCTTT	97 (BtubB)
B1B-R	AAAGAAGACTTCCATGCGTTCAAGTTGCAGATC***GCTGTCTCCTTTCAGGGTACCTGC	156 (BtubB)
BMB-F	GCTAGCTTTGCGCCGTTGACTAGCAGGGGATCGCAACAGTACAGGGCGCTGAATTTCCCAGACCTG	802 (BtubB)
BMB-R	CAGGTCTGGGAAATTCAGCGCCCTGTACTGTTGCGATCCCCTGCTAGTCAACGGCGCAAAGCTAGC	867 (BtubB)
AMB-F	GCTAGCTTTGCGCCGGTGATTTCGGCGGAAAAGGCATACCACGAGCAGTTGAGTTTCCCAGACCTGGC	802 (BtubB)
AMB-R	GCCAGGTCTGGGAAACTCAACTGCTCGTGGTATGCCTTTTCCGCCGAAATCACCGGCGCAAAGCTAGC	869 (BtubB)
BSB-F	AAACTGGGGTATGCGGATATACCGCCGAGAGGTTTAAAAATGAGTGGCCTGGCCCTG	1048 (BtubB)
BSB-R	CAGGGCCAGGCCACTCATTTTTAAACCTCTCGGCGGTATATCCGCATACCCCAGTTT	1104 (BtubB)

Table S3. α- and β-tubulin tree-determinant residues. Tree-determinants were calculated by the Sequence Harmony method (Exp. Procedures) where upper case indicates preferred residue for that position and lower case any other present residue.

Secondary structure element	α-tubulin		β-tubulin		BtubA		BtubB	
	Id[a]	Residues	Id[a]	Residues	Id	Residue[b]	Id	Residue[b]
S1	8	Hn	8	Q	10	S	8	H
H1	12	Ag	12	C	14	A	12	C
H1	14	ICVta	14	N	16	N	14	N
H1	18	N	18	Astcg	20	A	18	D
H1	20	Ca	20	F	22	F	20	F
H1	23	L	23	Vti	25	T	23	L
H1-S2	24	Yf	24	Ivm	26	V	24	A
H1-S2	26	L	26	Dge	28	L	26	R
H1-S2	36	Mlfkv	36	Y	38	Q	36	L
H1-S2	44	Dhr	42	Lim	49	W	46	S
H1-S2	46	Asg	44	R	51	S	47	N
H1-S2	47	F	45	Imalv	52	F	48	M
H1-S2	49	T	47	V	54	S	50	V
H1-S2	54	T	52	Asvgi	59	S	55	V
T2	73	Tns	71	Ga	76	S	72	G
H2	77	E	75	Satv	80	N	76	R
H2-S3	84	Rks	82	G	86	G	83	S
H2-S3	88	Hn	86	Rk	90	N	87	D
H2-S3	90	Eq	88	D	92	A	89	S
H2-S3	92	Lmi	90	Fyv	94	L	91	I
S3	96	K	94	Q	98	T	95	I
T3	97	Ea	95	Stncp	99	E	96	P
T3	98	D	96	G	100	G	97	G
T3	103	Yf	101	W	105	F	102	W
T3	110	IV	108	E	112	A	109	E
H3	112	Kre	110	Avqs	114	R	111	E
H3	121	Rkap	119	V	123	R	120	V
H3	125	Lvmai	123	E	127	E	124	A
S4	136	Lfim	134	Q	138	I	135	L
H4	155	E	153	Sng	157	E	154	E
H4	158	Sa	156	Rh	160	K	157	R
H4	159	Vtild	157	E	161	E	158	Q
H4-S5	162	Gt	160	P	164	G	161	P
H4-S5	163	Kr	161	D	165	E	162	K
H4-S5	164	K	162	R	166	I	163	K
S5	166	K	164	Mil	168	V	165	I
T5	172	Ywt	170	Vfmicl	174	L	171	V
T5	179	Tns	177	Dl	181	S	178	D
T5	180	Asg	178	Tvc	182	V	179	S
H5	193	STag	191	Ql	195	T	192	R
S6	200	CVg	198	Eahkq	202	A	199	G
H6	214	Rknq	212	Fmsy	216	H	213	K
H6-H7	216	Nshlq	214	T	218	K	215	K
H6-H7	218	Dgns	216	Kr	220	N	217	N
H7	226	Nhs	224	D	228	D	224	D
H7	229	Rnq	227	Hgk	231	L	227	N
H7	233	Q	231	Avilmfgst	235	E	231	L
H7	237	S	235	G	239	G	235	S
T7	250	Vl	248	SAc	252	V	248	T
H8	253	Tn	251	R	257	R	251	S
H8	254	Ed	252	K	258	E	252	E

H8	255	Fi	253	Lm	259	L	253	F
H8	256	Qp	254	Ac	260	L	254	V
H8	257	T	255	V	261	T	255	T
S7	268	PMva	266	Fy	272	L	266	L
S7	271	Sta	269	G	275	A	269	S
S7-H9	280	Kr	278	Savnt	284	R	278	Q
S7-H9	283	Hr	281	Yf	287	F	281	Q
S7-H9	284	Eq	282	Rniks	288	E	282	V
H9-S8	304	Kvnr	302	Adl	308	A	302	A
H9-S8	313	M	311	L	317	L	311	L
S8	314	As	312	T	318	S	312	A
S8-H10	322	D	320	RKhpqv	326	I	320	D
H10	329	Nhqt	327	De	333	A	327	D
H10	331	As	329	Qn	335	A	329	N
H10	332	VI	330	Ml	336	A	330	M
H10	336	Kr	334	Qv	340	M	334	R
H10-S9	341	Ivfl	339	Satgnpq	344	L	337	L
H10-S9	347	Csr	345	Il	350	I	343	M
H10-S9	349	Tc	347	Nhs	352	T	345	A
H10-S9	350	Gs	348	Na	353	A	346	S
S9	351	F	349	Vitc	354	F	349	L
S9	354	G	352	ASt	357	G	352	G
S9-S10	358	Qeh	356	Ivs	361	Q	356	T
S9-S10	369	As	359	Rkt	364	I	359	E
S9-S10	370	Kpsenr	360	Gd	365	S	360	G
S10	373	Rk	363	Mi	368	K	363	S
S10	377	M	367	F	372	L	367	A
S10	379	Sa	369	Ge	374	A	369	V
H11	385	Ascv	375	Qhr	380	A	375	A
H11	387	AV	377	Lmict	382	V	377	V
H11	394	Kn	384	Qh	389	N	384	Q
H11	397	Li	387	Avgks	392	K	387	I
H11-H12	405	V	395	Lim	400	A	395	T
H12	424	Dv	414	N	419	S	414	Q
H12	426	Aly	416	Nqhst	421	Q	416	A
H12	427	Ae	417	D	422	E	417	T
H12	429	E	419	Vil	424	V	419	A

[a] Residue numbering taking *B. taurus* tubulin (PDB entry 1JFF) as reference
[b] Color code: α-tubulin-like (red), β-tubulin-like (blue) or none of them (green) according to single amino acid or its biochemical group.

Table S4. Tubulin tree-determinants analyzed by protein zone.

Eukaryotic tubulin zones	Tree determinants conserved in bacterial tubulin					
	BtubA			BtubB		
	α	β	None	α	β	None
Nucleotide binding loops T1-T7	5	3	1	1	7	1
Loops H1-S1, S7-H9, S9-S10	7	3	4	1	4	9
Other loops	6	3	6	8	1	6
Regular secondary structure elements (α-helix and β-strands)	18	15	17	21	14	15
All zones	36	24	28	31	26	31

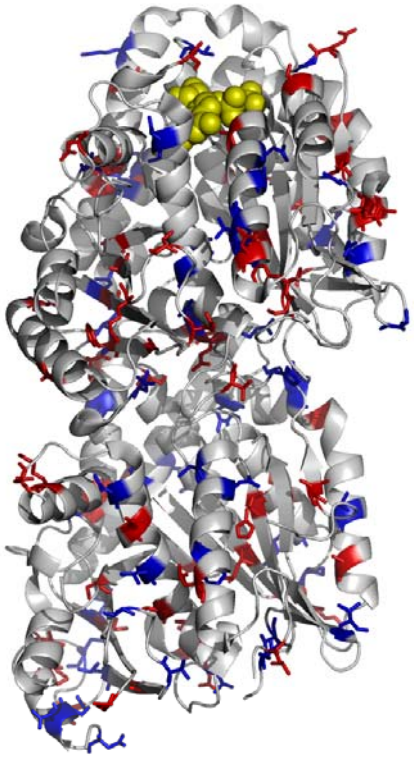

Figure S1. Tree-determinant residues from α- and β-tubulin mapped on the BtubA/B dimer structure (PDB entry 2BTQ). α–tubulin-like residues are marked in red, β-tubulin-like residues in blue (see Tables S3 and S4 for details).

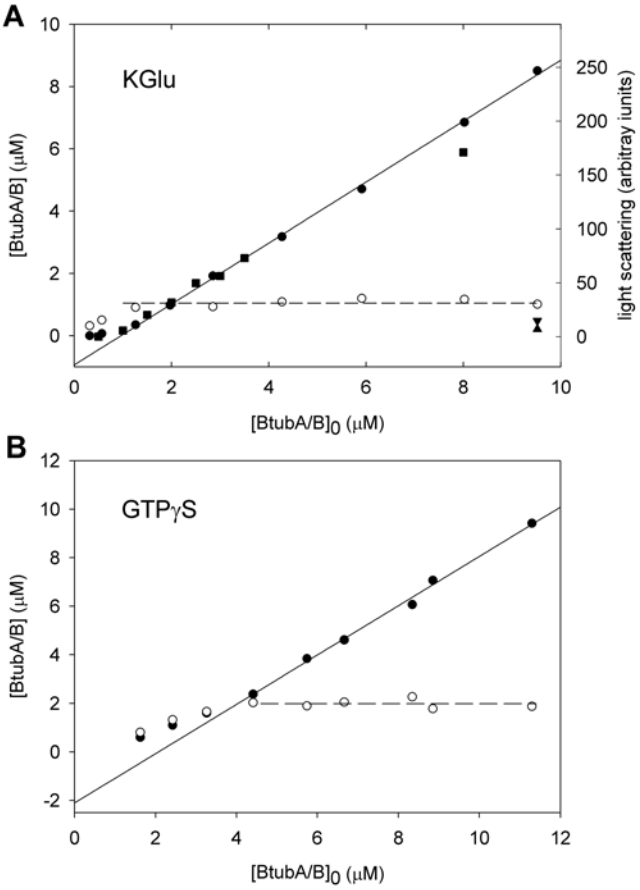

Figure S2. Continuation of Figure 4. A, BtubA/B polymerization in Tris-KGlu buffer with 1 mM GTP. B, BtubA/B polymerization in Tris-KCl buffer with 0.1 mM GTPγS. Polymers assembled with GTPγS from 10 μM BtubA/B (initially with bound GDP and GTP) contained 0.5-0.6 GTPγS plus 0.3-0.4 GDP and 0.3-0.4 GTP per BtubA/B

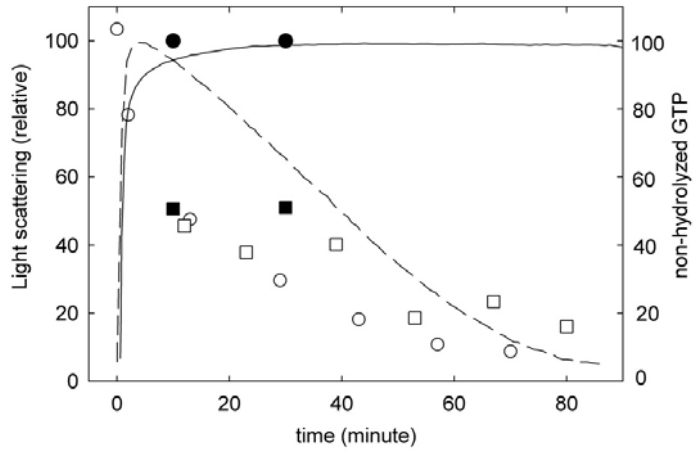

Figure S3. Continuation of Figure 6. Solid line, assembly time course of 3 μM BtubA/B with 50 μM GTP regenerating system in Tris-KGlu buffer with 2 mM $MgCl_2$ at 30 ºC. Solid circles and squares, percent GTP in supernatant and polymer pellet. Dash line, assembly without regenerating system. Void circles and squares, percent non-hydrolyzed GTP in the supernatant and polymer pellet, respectively

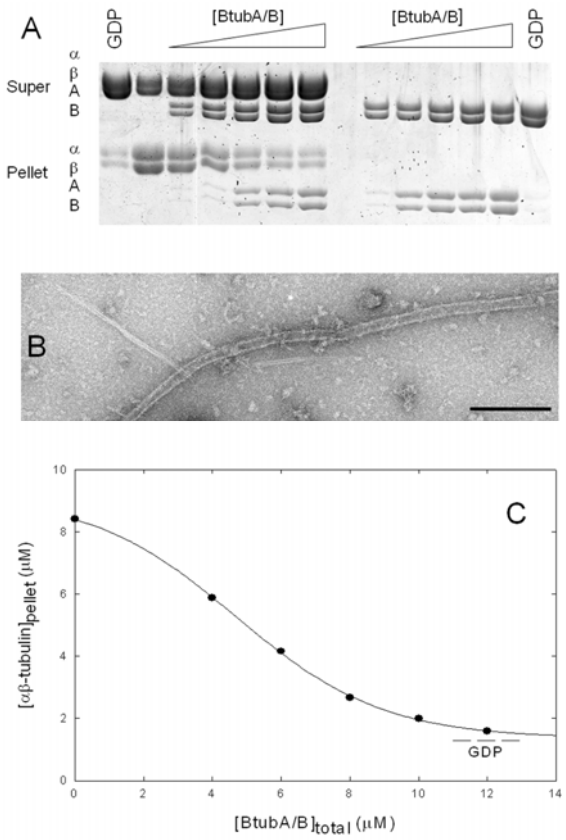

Fig. S4. Inhibition of microtubule assembly by bacterial tubulin. The similarities and differences observed between bacterial and eukaryotic tubulin posed the question of whether or not the two proteins could co-polymerize. We explored this possibility with light scattering, sedimentation assays and electron microscopy, under different solution conditions. BtubA/B (10 μM) did not significantly interfere with the assembly of αβ-tubulin in microtubule buffers GAB-1 mM GTP and Pipes-0.1 mM GMPCPP at 30 °C. However, inhibition of the plateau light scattering values of microtubule assembly was observed in Tris-KGlu and Pipes-D_2O buffers with 1 mM GTP, in which both BtubA/B and αβ-tubulin polymerize. GTP was in excess during these experiments.

The effect of BtubA/B on microtubule assembly in Pipes-D_20 was quantified by pelleting. Panel A, polymerization of αβ-tubulin (18 μM) with increasing concentrations of BtubA/B (0-12 μM) in Pipes-D_2O buffer with 1 mM GDP. Note that αβ-tubulin separates due to its retarded mobility in SDS-PAGE. Pellets and supernatants were loaded in same lanes with a 30 min electrophoretic shift. Panel B, electron micrograph of BtubA/B polymers and one microtubule assembled in the same solution; the bar indicates 100 nm. Panel C, quantified and plotted results from panel A; the line is drawn solely to show the trend of the data. The results show a clear inhibition by BtubA/B at the concentrations above its Cr at which it polymerizes, whereas there was no effect below Cr (panel A). BtubA/B copolymerization into microtubules at the concentrations below its Cr was not detected by this method. The polymers observed in the mixtures of αβ-tubulin and BtubA/B consisted of separate microtubules and characteristic bacterial tubulin filaments (panel B). BtubA/B progressively inhibited αβ-tubulin assembly, reaching the background level of non-specific pelleting (panel C). On the other hand, αβ-tubulin (18 μM) did not reproducibly modify BtubA/B assembly in Pipes-D_2O or Tris-KGlu buffers.

Loop 1 (H1-S2)

```
BtubA       37G Q T A P G V A P R G N W S S F F S K L G E S S S G S Y V65
BtubB       34G T L K E G S*A A - - A N S N M E V F F H K V R D G K Y V61
Alpha-tub   34G Q M P S D K T I G G G D D S F T T F F C E T G A G K H V62
Beta-tub    34G S Y H G D S D L - - Q L E R I N V Y Y N E A T G N K Y V60
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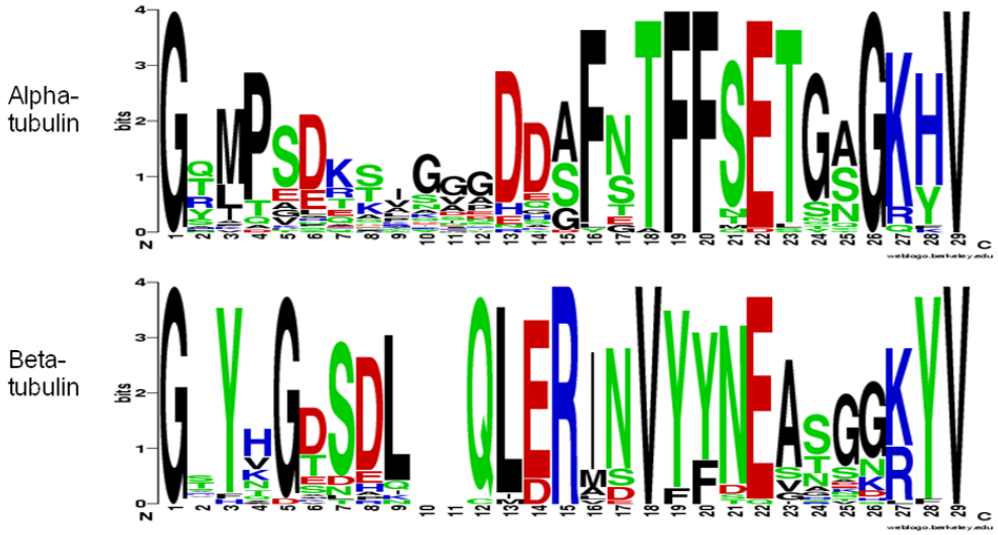

*N (Asn) insertion

Loop S (S9-S10)

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BtubA       358Y V E Q P G - - - - - - - - - I S H R K S369
BtubB       353Y A E T A P - - - - - - - - - E G F A S S364
Alpha-tub   355I N Y Q P P T V V P G G D L A K V Q R A374
Beta-tub    353V C D I P P - - - - - - - - - R G L K M S364
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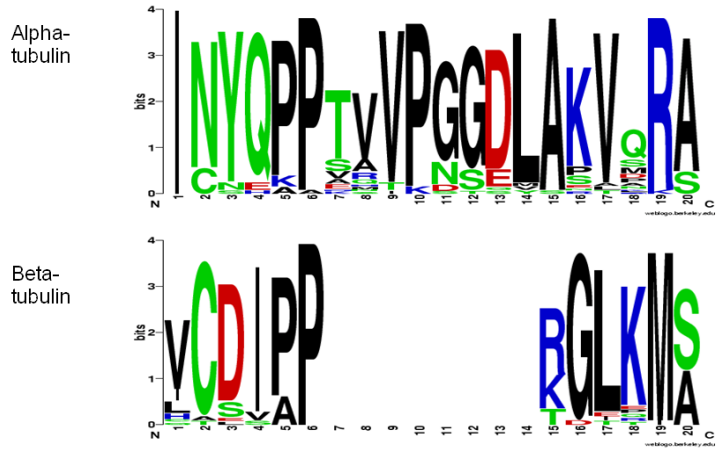

Figure S5. Btub/tubulin sequence alignments and tubulin occurrence logos for loops H1-S2 and S9-S10. Equivalents loops for BtubA and BtubB subunits, aligned with bovine alpha- and beta-tubulin. Logos resulting for the alignment of non-redundant eukaryotic tubulin sequences clusterd at 90% identity are shown. Logos were produced with the WebLogo server (http://weblogo.berkeley.edu).

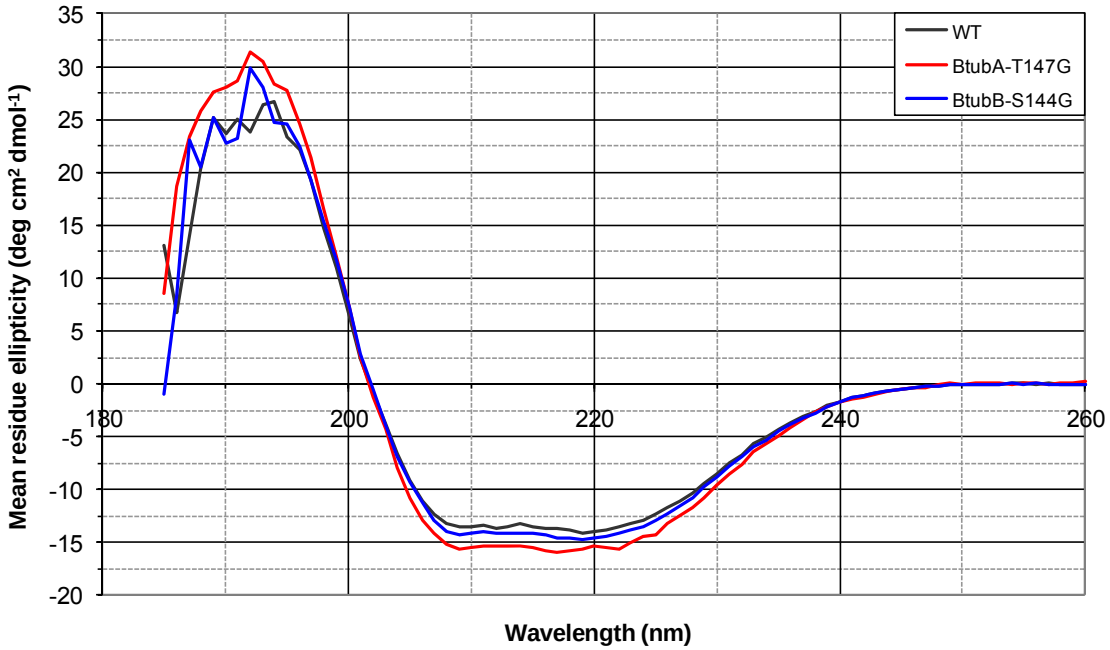

Figure S6 Circular dichroism spectra of BtubA/B mutants compared to wild type

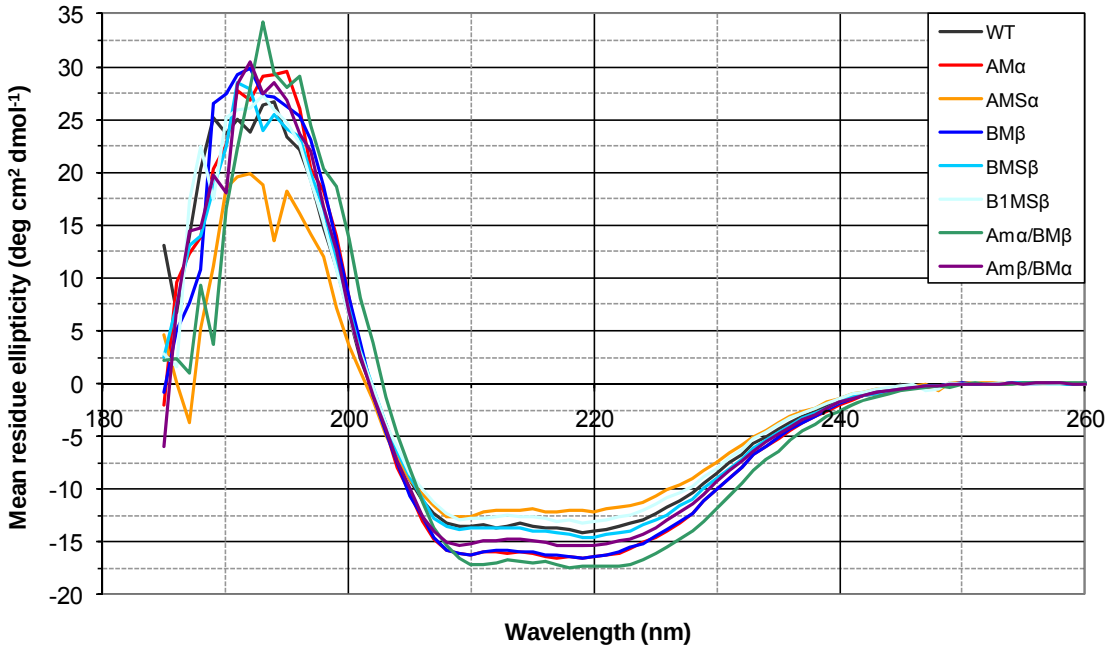

Figure S7 Circular dichroism spectra of BtubA/B chimera with αβ-tubulin loops