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Supplemental Information

C-Terminal Tail Polyglycylation and Polyglutamylation Alter Microtubule Mechanical Properties

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Supplementary Materials

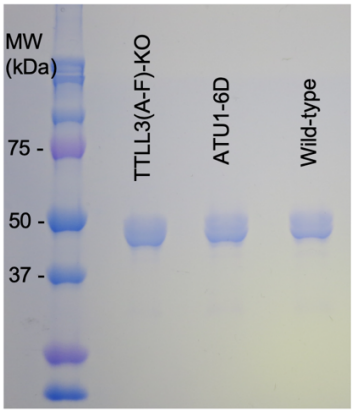


***Figure S1. SDS PAGE gel of tubulin* purified from different T. thermophila strains.**

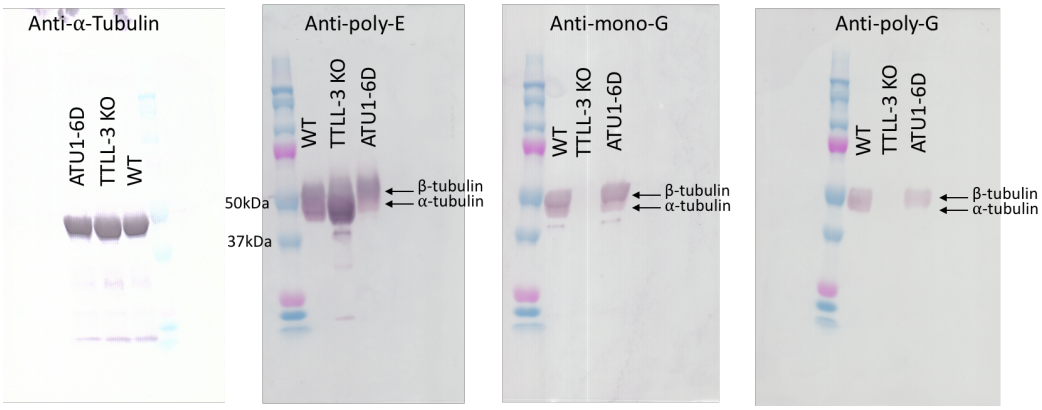


Figure S2. Western blot of tubulin purified from different T. thermophila strains. A qualitative western blot comparing the bulk modifications on each of the three tubulin pools purified from different strains of T. thermophila. The wild-type tubulin has all types of modifications detected. As expected and previously shown (Wloga et al., 2008, 2009), the TTLL3(A-F)-KO tubulin has increased glutamylation when compared to the wild-type tubulin and no apparent glycylation (either mono- or poly-). The ATU1-6D tubulin has limited modifications on the α-subunit. There is a low degree of polyglutamylation and monoglycylation on α-tubulin, but no polyglycylation detected.

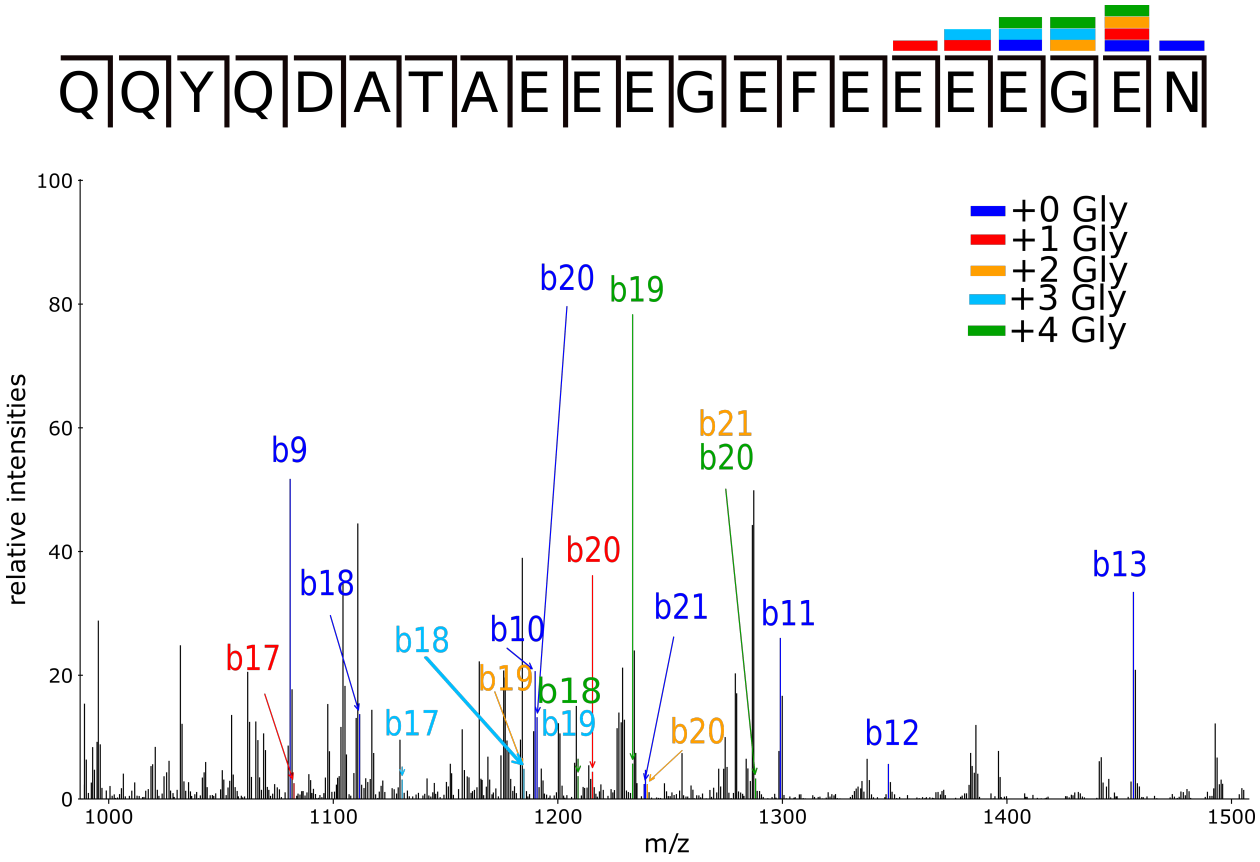


Figure S*3*. Heterogeneous MS/MS mapping of purified cell body wild-type tubulin β-C-terminal tail peptide. MS/MS spectra of the parent ion corresponding to mass of four additional glycine residues. The modification additions are heterogeneous with no clear single arrangement of glycine additions on this peptide. For simplicity, only the b-ions are labeled.

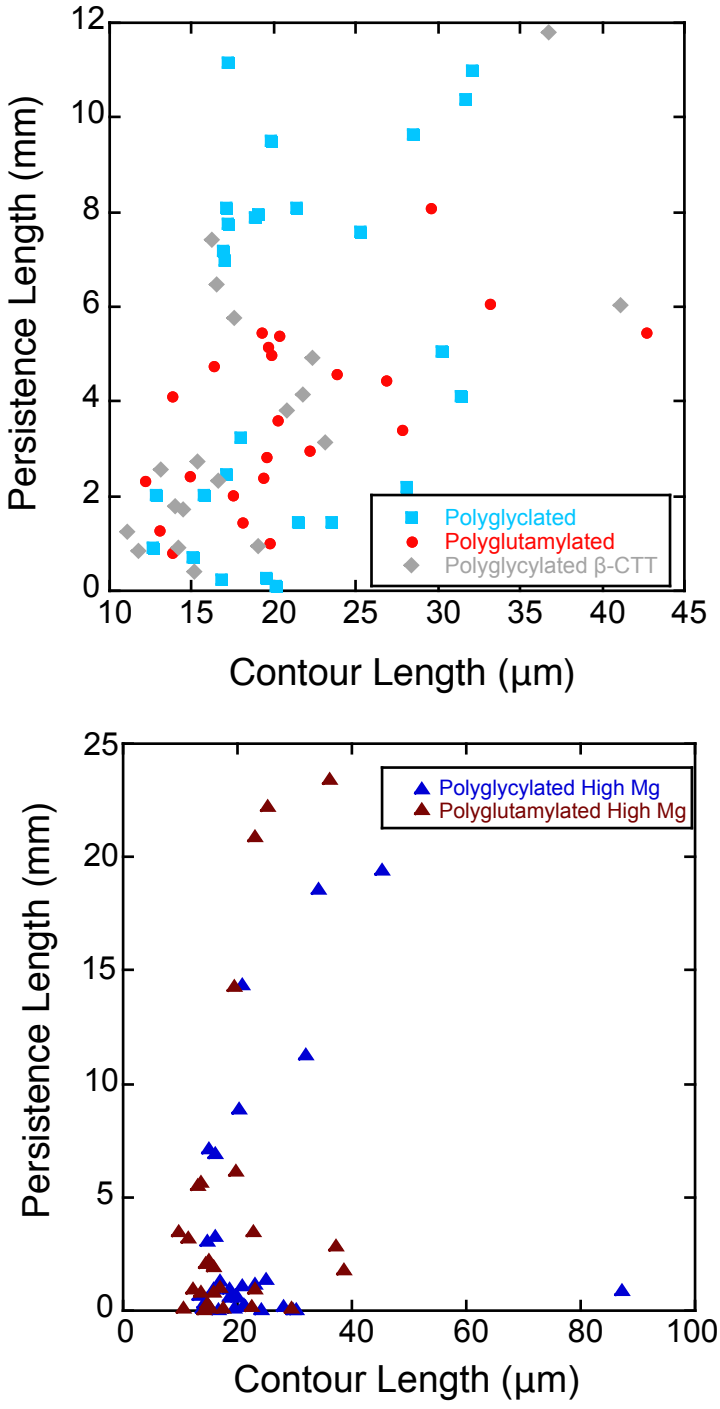
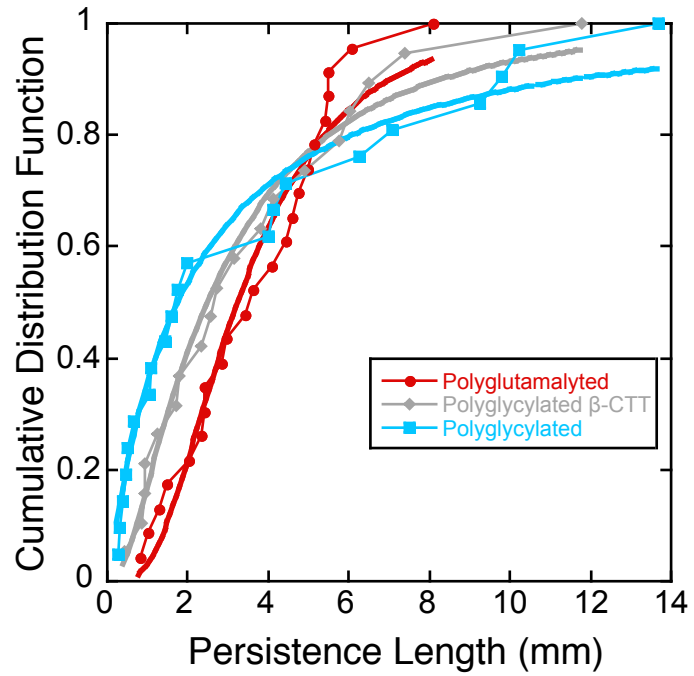


Figure S*4*. Comparison of contour length versus persistence length. (top) Scatter plot of contour length versus persistence length of microtubules in BRB80. (bottom) Scatter plot of contour length versus persistence length of microtubules in high magnesium conditions.



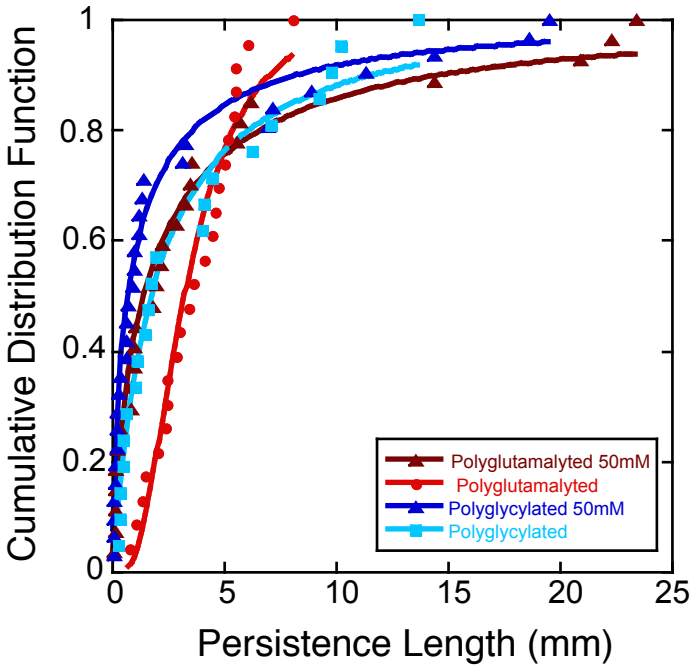


Figure S5. Cumulative Distribution Functions with appropriate fits. Function for CDF fit to $f(x) = 0.5\left\{1 + \mathrm{erf}\left[\frac{ln(x)-\mu}{\sqrt{2}\sigma}\right]\right\}$. Microtubule persistence length distributions are normal or lognormal, so data is displayed as cumulative distribution functions (CDF). When compare to probability distribution function (PDF), CDFs require fewer fit parameters, produce less uncertainties in their fits, and do not involve user to binning the data.

TABLE S1. Persistence length and fit parameters for cumulative distribution function of differentially-modified pools of Tetrahymena tubulin. Function for CDF fit to $f(x) = 0.5\left\{1 + \text{erf}\left[\frac{\ln(x)-\mu}{\sqrt{2}\sigma}\right]\right\}$.

	MgCl2 [mM]	*n*	*Lp (mean,mm)*	μ	σ	R^2
WT (polyglycylated)	1	21	2.00 ± 0.19	0.588±0.047	1.472± 0.073	0.98
WT	5	48	2.92 ±1.10	1.137± 0.021	1.107± 0.035	0.98
WT	10	28	4.43 ± 1.16	1.631± 0.068	1.351± 0.113	0.94
WT	50	31	0.90 ± 0.29	-0.283± 0.042	1.889± 0.073	0.98
TTLL3(A-F)KO (polyglutamylated)	1	23	3.18 ± 0.41	1.19± 0.026	0.604± 0.043	0.96
TTLL3KO	5	29	2.28 ± 0.59	0.843± 0.018	1.142± 0.032	0.99
TTLL3KO	10	22	2.36± 0.58	0.882± 0.033	1.105± 0.0545	0.98
TTLL3KO	50	27	1.49 ± 0.50	0.377± 0.052	1.817± 0.091	0.98
ATU16D (polyglycylated β–CTT, unmodified β–CTT)	1	19	2.63 ± 0.53	0.931± 0.027	0.938± 0.044	0.99

Table S2. Pairwise significance values using a Kolmogorov–Smirnov test for differentially-modified pools of Tetrahymena tubulin. Shaded boxes have p<0.1 (blue), p<0.05 (green) or p<0.01 (orange). The table is symmetric about the diagonal.

cell strain		WT	WT	WT	WT	TTLL3	TTLL3	TTLL3	TTLL3	ATU16D
	magnesium concentration	1	5	10	50	1	5	10	50	1
WT	1	1	0.241	0.0592	0.1055	0.0449	0.7705	0.7019	0.5481	0.5899
WT	5	0.241	1	0.0159	0.0009	0.1831	0.6293	0.7571	0.1586	0.8138
WT	10	0.0592	0.0159	1	0.0012	0.0003	0.0146	0.0212	0.0038	0.004
WT	50	0.1055	0.0009	0.0012	1	0.0001	0.0092	0.0152	0.2229	0.0119
TTLL3	1	0.0449	0.1831	0.0003	0.0001	1	0.2452	0.2799	0.0446	0.7306
TTLL3	5	0.7705	0.6293	0.0146	0.0092	0.2452	1	0.9994	0.3608	0.9386
TTLL3	10	0.7019	0.7571	0.0212	0.0152	0.2799	0.9994	1	0.5125	0.9902
TTLL3	50	0.5481	0.1586	0.0038	0.2229	0.0446	0.3608	0.5125	1	0.2917
ATU16D	1	0.5899	0.8138	0.004	0.0119	0.7306	0.9386	0.9902	0.2917	1

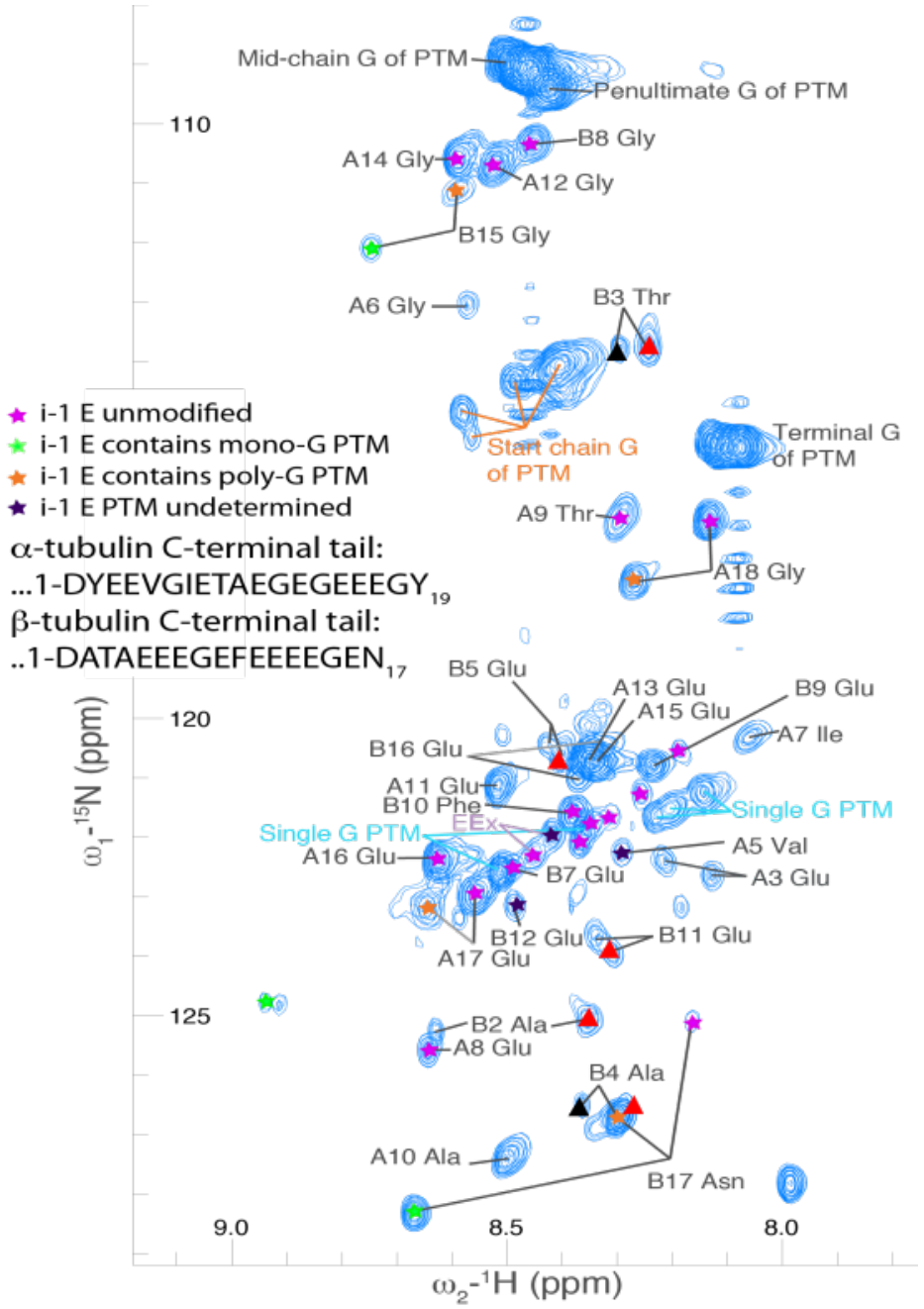


Figure S*6*. Assignment of C-terminal tails of tubulin. HNCO of C-terminal tails of tubulin annotated with the assigned peaks and corresponding modifications (Wall 2016). Added are the split chains from the beginning of the β-C-terminal tail (brighter peaks from the relaxation plots are labeled with red triangles, dimmer peaks are labeled with black triangles).

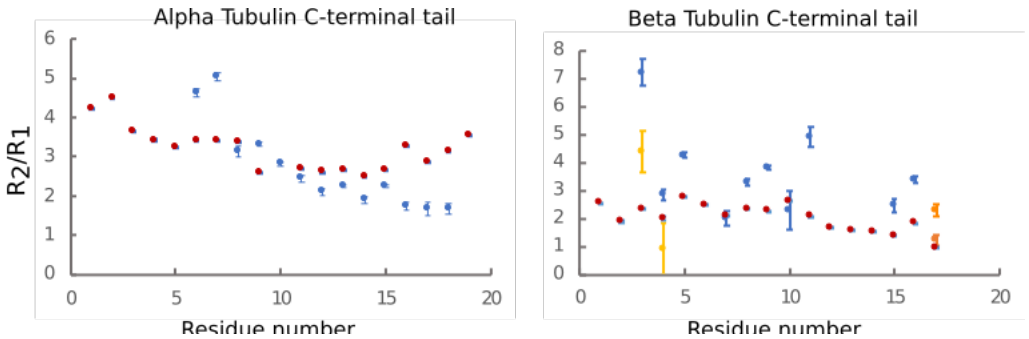


Figure S7. R_2/R_1 Relaxation. Ratios of R_2/R_1 plotted by residue number for the α and β-C-terminal tails.