Supplemental Materials

Molecular Biology of the Cell

Howes et al.

Figure S1. Modification of tubulin *in vitro*. A) Western blot of tubulin samples used to prepare EM grids after *in vitro* modification with TAT1 probed with an anti-acetylated tubulin antibody (top) and replicated lane stained with SYPRO (middle and bottom). Un = untreated, Ac = acetylated, dAc = deacetylated, Cil = ciliary tubulin. Quantification of Western blot shown below. **B)** Relative signals using different concentrations of TAT1. Western blot and gels same as **A).**

Figure S2. Refinement of helical parameters. Estimations for rise (A) and twist (B) of tubulin dimers in 14 protofilament microtubules shown at each iteration of refinement during the reconstruction process of dynamic microtubules assembled from untreated, acetylated and deacetylated tubulin, both in the presence or absence of kinesin.

Figure S3. Subtilisin digestion cleaves N-terminal loop of -tubulin. Performed microtubules, dynamic (left) and stabilized with taxol (right) were incubated with subtilisin for the time indicated, and then probed with the anti-acetylated tubulin antibody. A decrease in signal at later time points suggests that the N-terminal loop of -tubulin is nonspecifically cleaved along with the C-terminal tails.

Figure S4. TAT1 binding to microtubules decreases with increasing salt. SDS gel (top) and quantitation (bottom) of pelleting assays showing that TAT1 co-sediments less with microtubules in the presence of increasing KCl. Self-pelleting of TAT1 in the absence of microtubules was subtracted when calculating the fraction bound. Data from three independent co-sedimentation assays. Error bars show standard deviation.

Figure S5. TAT1 does not oligomerize. Gel filtration chromatograms at 200 mM and 10 mM KCl showing no change in the elution profile of TAT1.

Supplemental Table 1. Data summary of reconstructions. For each microtubule reconstruction, the final parameters and number of microtubule segments that went into the reconstruction are shown. The total number of segments that were boxed from the micrographs and the total number of microtubules are shown in parentheses (segments, microtubules).

			Rise	Twist	Resolution	Included particles
Dynamic	Acetylated *(8936, 154)*	13pf	9.355	-27.688	8.49	3445
		14pf	8.884	-25.759	10.03	3161
	Deacetylated *(4777, 81)*	13pf	9.359	-27.689	8.56	2047
		14pf	8.715	-25.762	8.34	2066
Dynamic with kinesin	Acetylated *(27933, 1073)*	13pf	9.501	-27.692	8.67	16273
		14pf	8.794	-25.765	8.75	6658
	Deacetylated *(23616, 797)*	13pf	9.489	-27.696	9.23	4346
		14pf	8.801	-25.759	9.15	15717
Taxol with kinesin	Acetylated *(22580,860)*	13pf	9.625	-27.682	8.63	6394
		14pf	9.083	-25.705	9.29	2837
	Deacetylated *(25624, 1287)*	13pf	9.505	-27.685	9.81	8001
		14pf	8.927	-25.703	11.4	3133

Figure S1

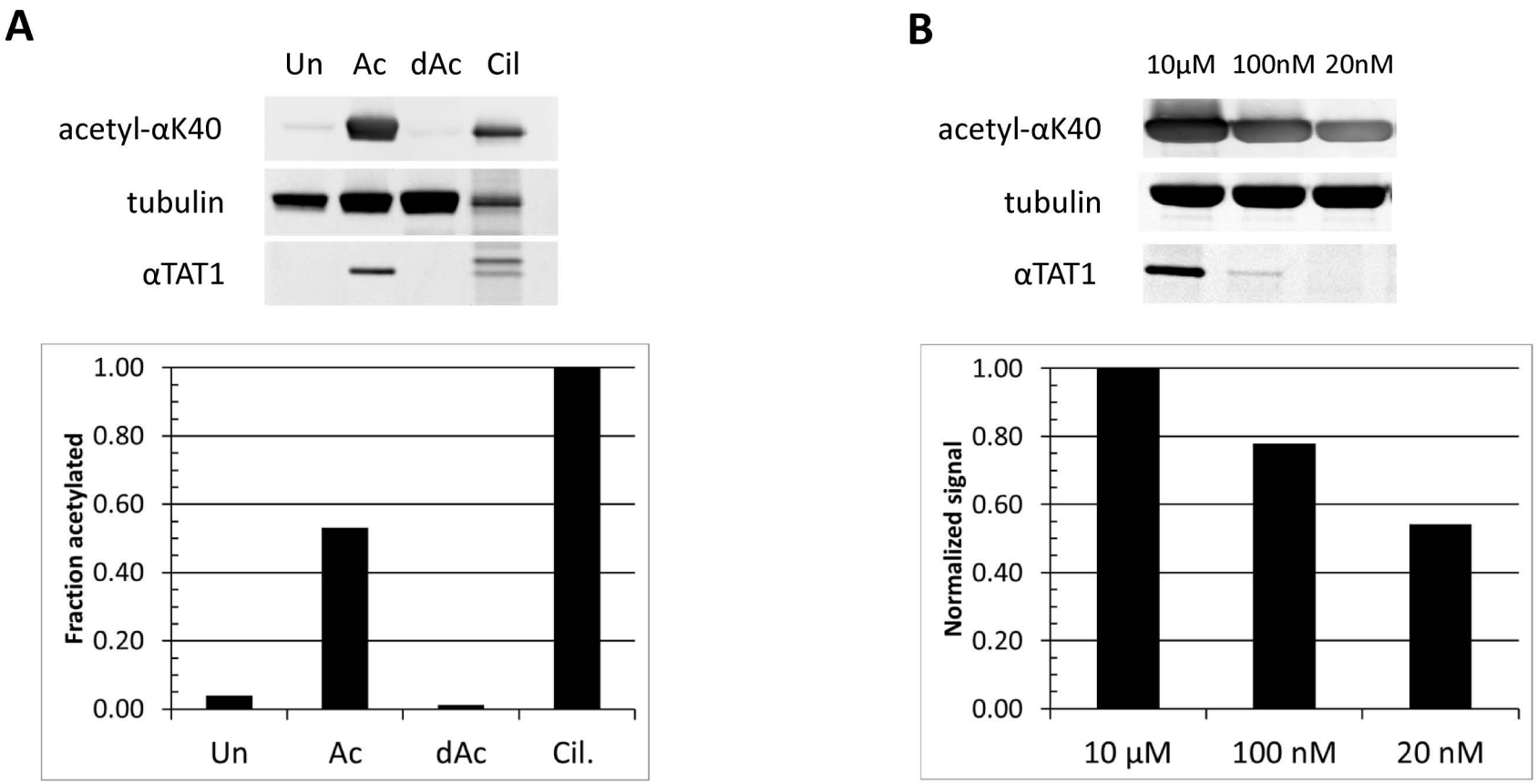

A
Un
Ac
dAc
Cil
acetyl-αK40
tubulin
αTAT1
1.00
0.80
0.60
0.40
0.20
0.00
Fraction acetylated
Un
Ac
dAc
Cil.
B
10µM
100nM
20nM
acetyl-αK40
tubulin
αTAT1
1.00
0.80
0.60
0.40
0.20
0.00
Normalized signal
10 µM
100 nM
20 nM

Figure S2

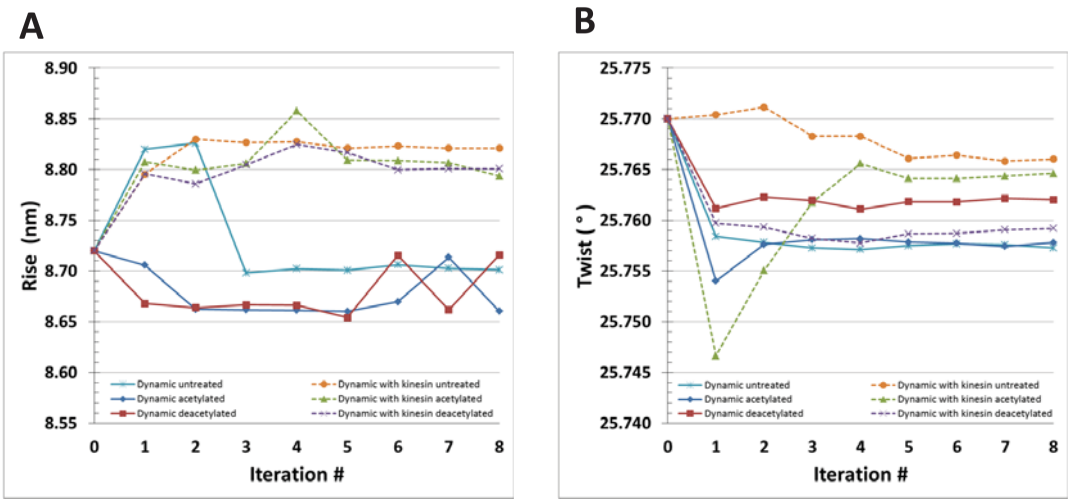

A
8.90
8.85
8.80
8.75
8.70
8.65
8.60
8.55
Rise (nm)
0 1 2 3 4 5 6 7 8
Iteration #
Dynamic untreated
Dynamic with kinesin untreated
Dynamic acetylated
Dynamic with kinesin acetylated
Dynamic deacetylated
Dynamic with kinesin deacetylated
B
25.775
25.770
25.765
25.760
25.755
25.750
25.745
25.740
Twist (°)
0 1 2 3 4 5 6 7 8
Iteration #
Dynamic untreated
Dynamic with kinesin untreated
Dynamic acetylated
Dynamic with kinesin acetylated
Dynamic deacetylated
Dynamic with kinesin deacetylated

Figure S3

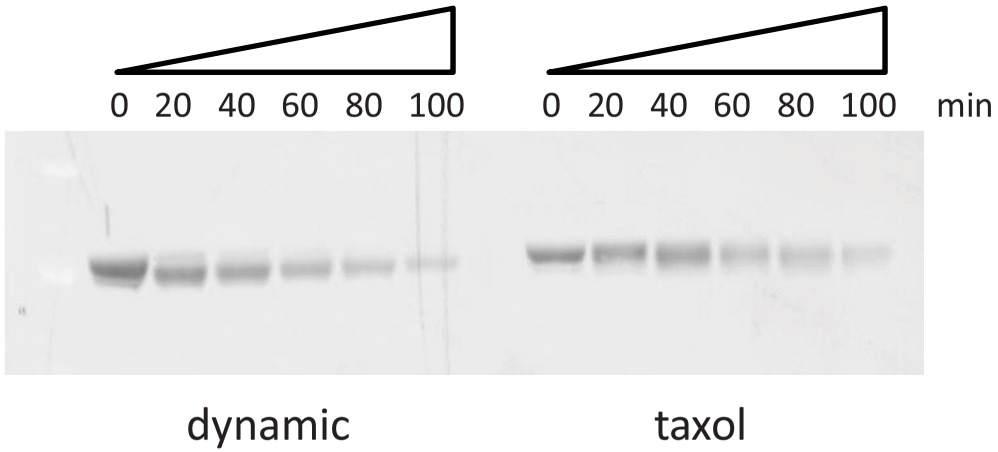
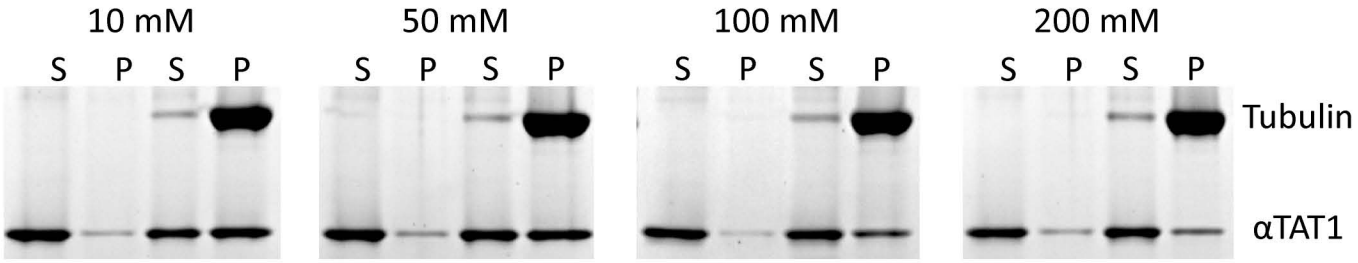


Figure S4


10 mM
50 mM
100 mM
200 mM
S P S P
Tubulin
αTAT1

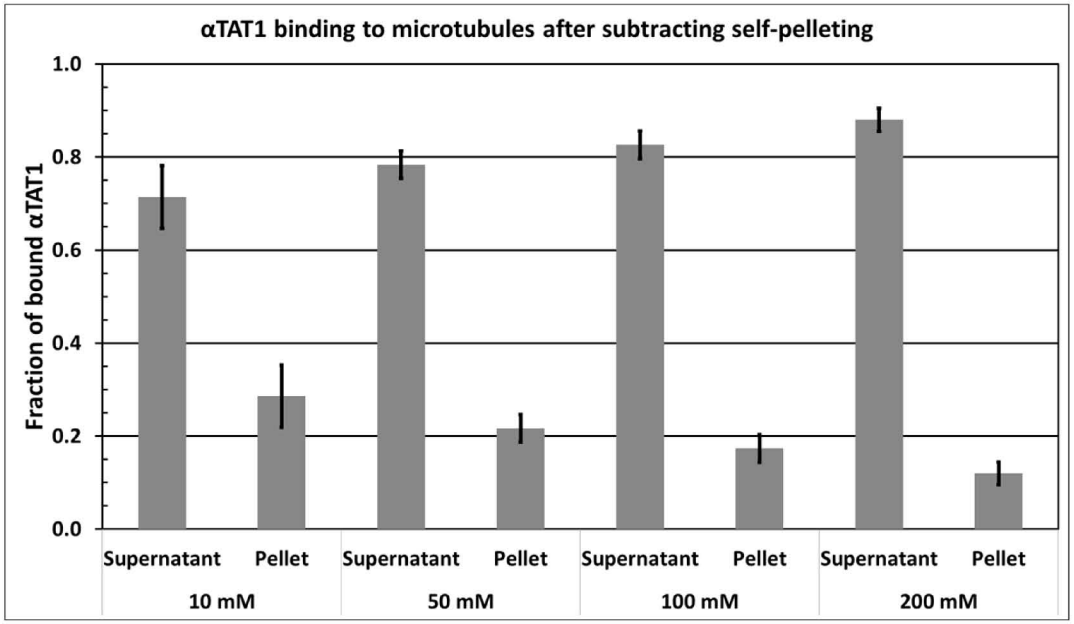
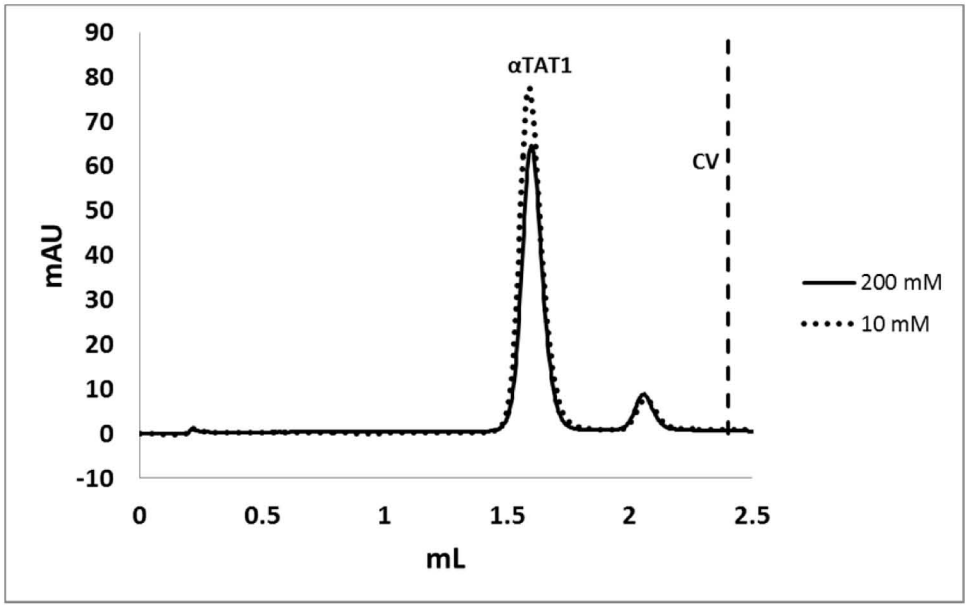

αTAT1 binding to microtubules after subtracting self-pelleting
Fraction of bound αTAT1
1.0
0.8
0.6
0.4
0.2
0.0
Supernatant
Pellet
10 mM
50 mM
100 mM
200 mM

Figure S5


90
80
70
60
50
40
30
20
10
0
-10
mAU
0
0.5
1
1.5
2
2.5
mL
αTAT1
CV
200 mM
10 mM