Figure S1. A) Residues 945-963 do not affect the kinesin-1 tail's affinity for microtubules. Dissociation constants of Tail944 R907C-F or Tail963 R907C-F for microtubules were measured by microtubule co-sedimentation and fluorescence anisotropy, and both constructs were found to have similar affinities for microtubules. K_d values from both assays are reported in Table 1. B) The kinesin-1 tail binds to microtubules in a salt-dependent manner. Dissociation constants of Tail944 R907C-F for microtubules were measured by fluorescence anisotropy in the presence of 100 mM NaCl (K_d = 0.093 ± 0.012 μM), 150 mM NaCl (K_d = 0.144 ± 0.016 μM), 200 mM NaCl (K_d = 0.460 ± 0.020 μM), 250 mM NaCl (K_d = 1.286 ± 0.083 μM), or 300 mM NaCl (K_d > 8 μM). Dissociation constants for all data are reported as the mean ± SEM for three experiments.

Figure S2. A) The secondary structures of Tail944 and Tail944 A905C Mutant A+B do not vary significantly. Circular dichroism spectra of Tail944 and Tail944 A905C Mutant A+B in the 200-240 nm range show an identical α-helical content, suggesting that the tail coiled-coil is not significantly disrupted by the alanine substitutions introduced into the Tail944 A905C Mutant A+B construct. Spectra are the mean ± SEM of three measurements performed on three separate samples. B) The basic residues in the 892-914 region of the kinesin-1 tail are important for binding to microtubules. Anisotropy and microtubule co-sedimentation data for determining dissociation constants of Tail944 A905C, Tail944 A905C Mutant A, Tail944 A905C Mutant B, and Tail944 A905C Mutant A+B for microtubules are shown (gel fragments show the amount of free (S) or bound (P) tail in the presence of 10 μM microtubules). Fluorescence anisotropy was not applicable to the measurement of K_d values for the low-affinity interactions (Mutant A, B and A+B) due to sub-saturation binding as discussed in the text. Dissociation constants for all data are reported as the mean ± SEM for three experiments.

Figure S3. The acidic E-hooks of tubulin are important for binding to kinesin-1 tails. A gel of microtubules and microtubules treated with subtilisin shows the complete digestion of both α- and β-tubulin (S3A). Dissociation constants of Tail944 R907C-F for microtubules or subtilisin-treated microtubules were measured by microtubule co-sedimentation and subsequent gel analysis of the amount of free (S) and bound (P) tail (S3B), as well as fluorescence anisotropy (S3C). ($\alpha\beta$ = wild-type microtubules, $\alpha_s\beta_s$ = subtilisin-treated microtubules). K_d values from both assays are reported in Table 1. Dissociation constants for all data are reported as the mean ± SEM for three experiments.

Figure S4. hTau-40 binds to microtubules with a moderate affinity. The dissociation constant of hTau-40 for microtubules was estimated to be ~5 μM in 100 mM NaCl by microtubule co-sedimentation.

Figure S5. Kinesin-1 heads and tails bind to distinct sites on microtubules. The amount of Tail944 bound to a fixed concentration of microtubules in the presence of increasing concentrations of kinesin K349 CLM G234A heads did not change as shown by microtubule co-sedimentation in S5A. The amount of K349 CLM G234A bound to a fixed concentration of microtubules in the presence of increasing concentrations of Tail944 did not change as shown by a microtubule co-sedimentation assay in S5B.

Figure S1

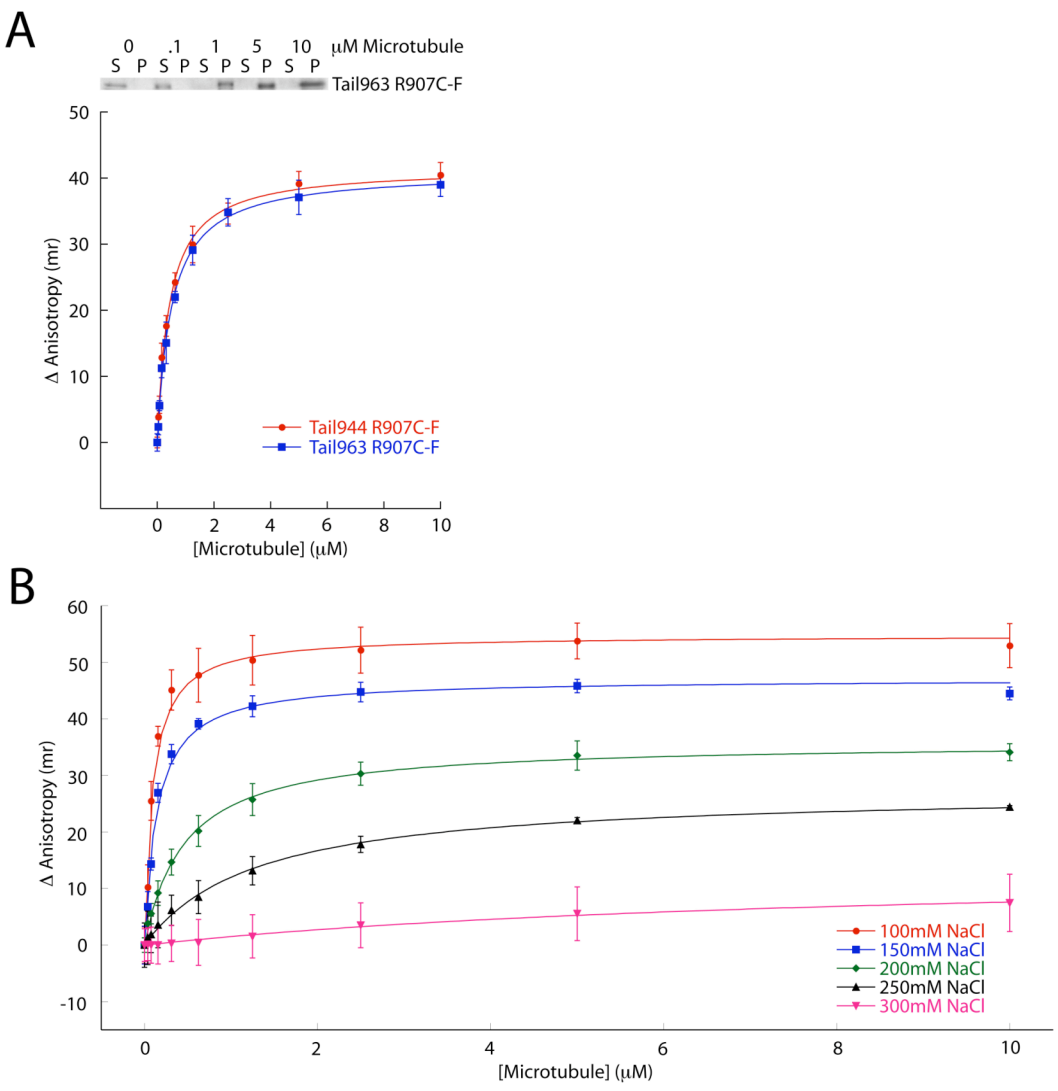

A
0 .1 1 5 10 µM Microtubule
S P S P S P S P S P
Tail963 R907C-F
Δ Anisotropy (mr)
[Microtubule] (µM)
Tail944 R907C-F
Tail963 R907C-F
B
Δ Anisotropy (mr)
[Microtubule] (µM)
100mM NaCl
150mM NaCl
200mM NaCl
250mM NaCl
300mM NaCl

Figure S2

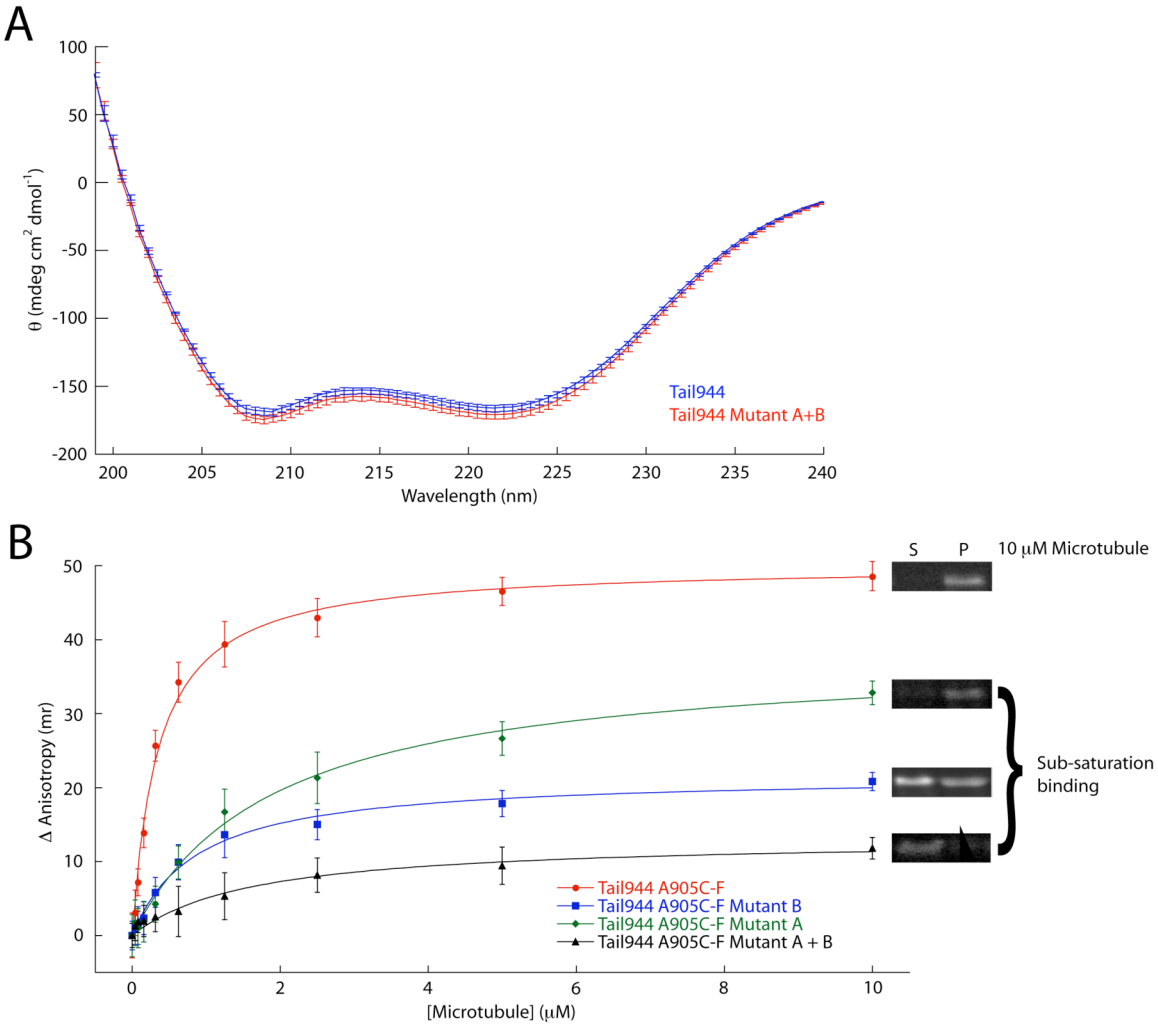

A
100
50
0
-50
-100
-150
-200
θ (mdeg cm^2 dmol^{-1})
200
205
210
215
220
225
230
235
240
Wavelength (nm)
Tail944
Tail944 Mutant A+B
B
50
40
30
20
10
0
Δ Anisotropy (mr)
0
2
4
6
8
10
[Microtubule] (μM)
Tail944 A905C-F
Tail944 A905C-F Mutant B
Tail944 A905C-F Mutant A
Tail944 A905C-F Mutant A + B
S
P
10 μM Microtubule
Sub-saturation
binding

Figure S3

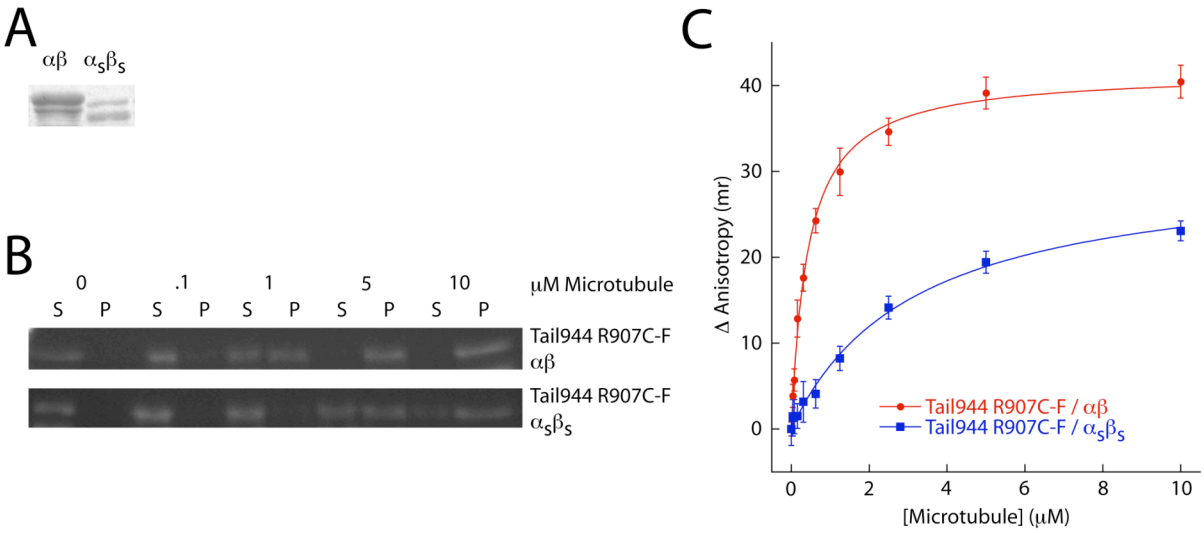

A
αβ
αsβs
B
0
.1
1
5
10
μM Microtubule
S P S P S P S P S P
Tail944 R907C-F
αβ
Tail944 R907C-F
αsβs
C
Δ Anisotropy (mr)
40
30
20
10
0
0
2
4
6
8
10
[Microtubule] (μM)
Tail944 R907C-F / αβ
Tail944 R907C-F / αsβs

Figure S4

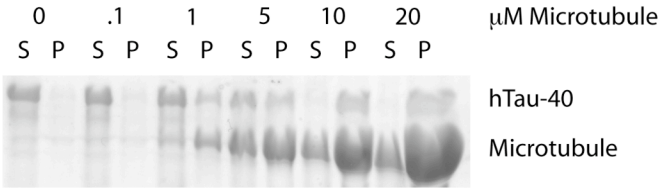
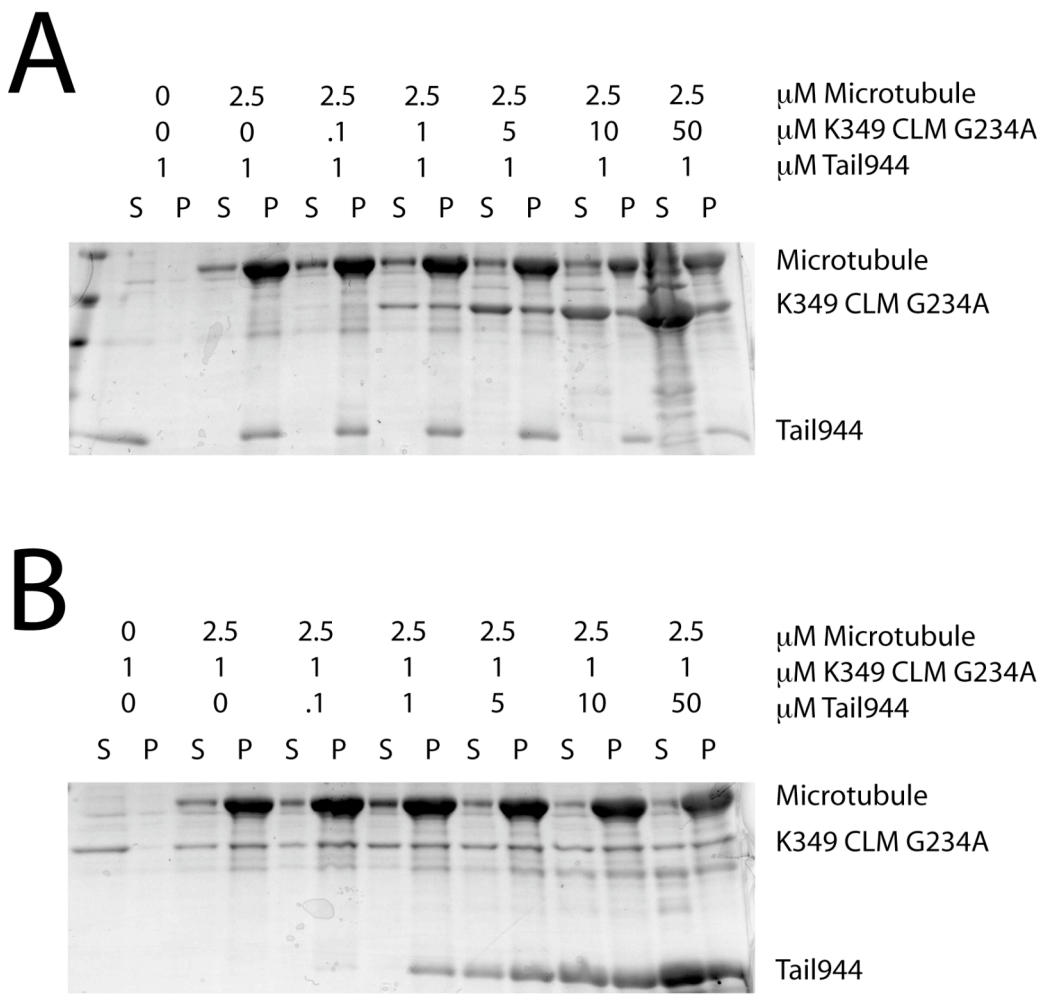

0 .1 1 5 10 20 μM Microtubule
S P S P S P S P S P S P
hTau-40
Microtubule

Figure S5


A
0 2.5 2.5 2.5 2.5 2.5 2.5 μM Microtubule
0 0 .1 1 5 10 50 μM K349 CLM G234A
1 1 1 1 1 1 1 μM Tail944
S P S P S P S P S P S P S P
Microtubule
K349 CLM G234A
Tail944
B
0 2.5 2.5 2.5 2.5 2.5 2.5 μM Microtubule
1 1 1 1 1 1 1 μM K349 CLM G234A
0 0 .1 1 5 10 50 μM Tail944
S P S P S P S P S P S P S P
Microtubule
K349 CLM G234A
Tail944