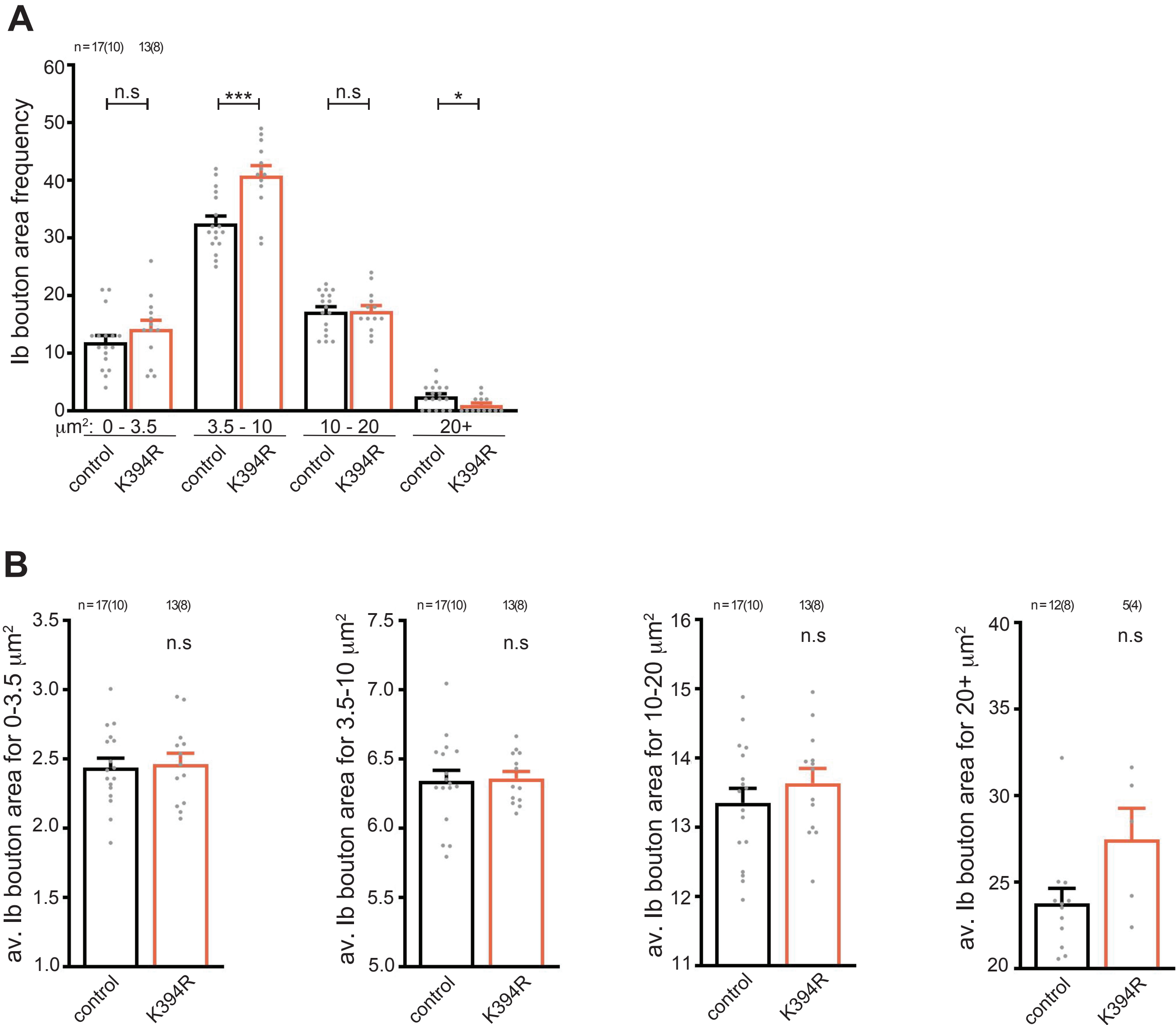

Figure S1. Bouton size is not affected in the K394R mutants, related to Figure 1.

(A and B) Distribution of bouton sizes (A) and average bouton sizes (B) in control and K394R mutants. In control larvae, most boutons have an area of 3.5-10 μm^2, with an average bouton area of 6.34 ± 0.32 μm^2. K394R mutants have more boutons in the 3.5-10 μm^2 area category, reflective of the overall increase in bouton number in the mutants; however, the average area of these boutons is equivalent to controls (6.36 ± 0.18 μm^2). The number of boutons of other sizes and their areas are similar between controls and K394R mutants, although the number of infrequent "extra-large" boutons (>20 μm^2 area) decreases slightly. Overall, the average size of the boutons in K394R mutants is comparable to controls. p-values, distribution of bouton sizes: p=0.25, 0-3.5 μm^2 control v. K394R; p=0.000422, 3.5-10 μm^2 control v. K394R; p=0.96, 10-20 μm^2 control v. K394R; p=0.0363, >20 μm^2 control v. K394R (A). p-values, average bouton sizes: p=0.83, 0-3.5 μm^2 control v. K394R; p=0.86, 3.5-10 μm^2 control v. K394R; p=0.35, 10-20 μm^2 control v. K394R; p=0.054, >20 μm^2 control v. K394R (B).
Quantification: Student's unpaired t-test. All data are mean ± SEM. n.s=non-significant; *p=0.01–0.05; ***p=0.001–0.0001.

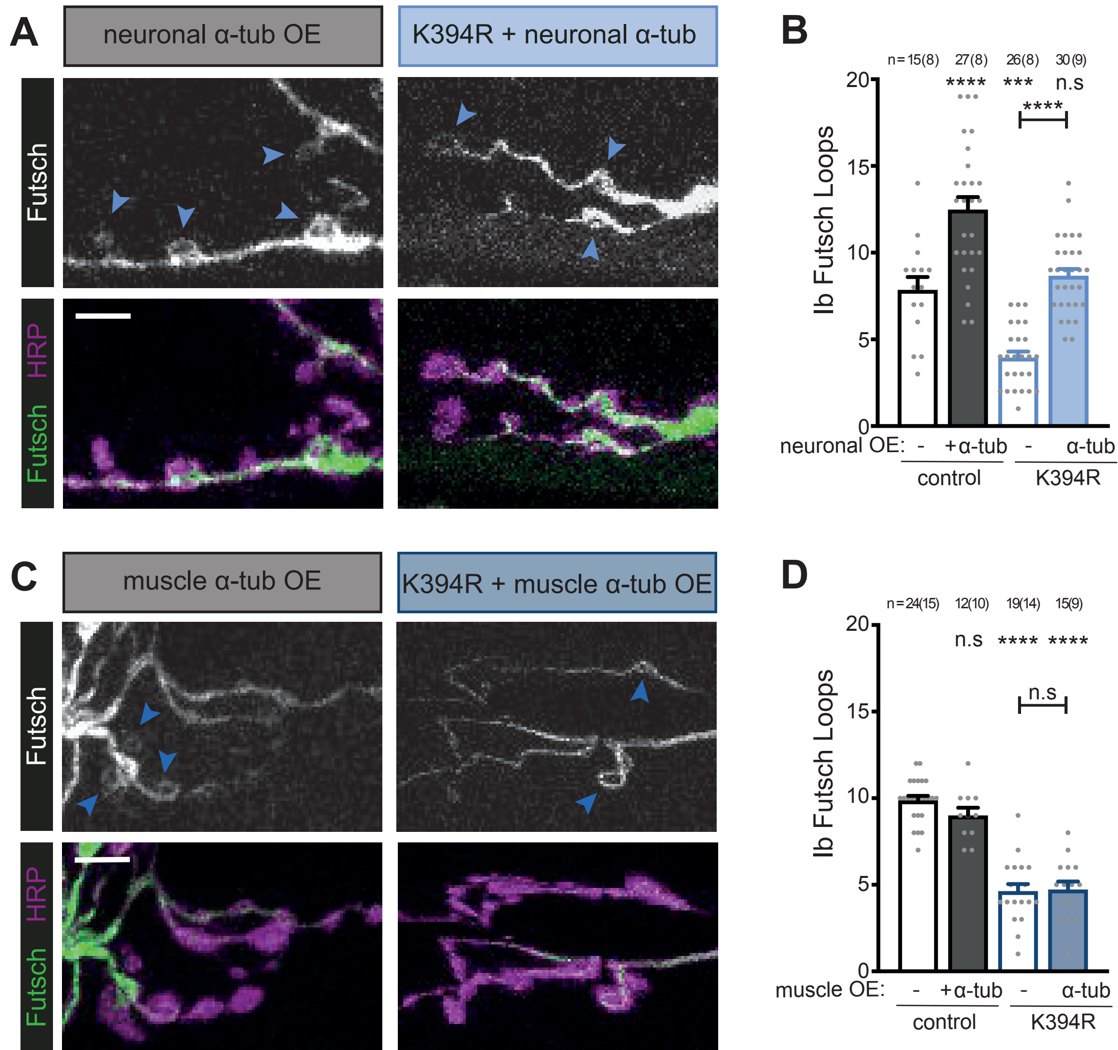

Figure S2. Over-expression of wild-type α-tubulin in neurons, but not muscles, rescues Futsch loops in K394R mutants, related to Figure 2.

(A and B) Representative images (A) and quantification (B) of control and K394R mutants alone and with the over-expression of α-tubulin in neurons. p-values, Ib Futsch loops: $p<0.000001$, control v. +α-tubulin; $p=0.0002$, control v. K394R; $p=0.80$, control v. K394R +α-tubulin; $p<0.000001$, K394R v. K394R +α-tubulin (B).
(C and D) Representative images (C) and quantification (D) of control and K394R mutants with and without the over-expression of α-tubulin in muscles. p-values, Ib Futsch loops: $p=0.44$, control v. +α-tubulin; $p<0.000001$, control v. K394R; $p<0.000001$, control v. K394R +α-tubulin; $p=0.998$, K394R v. K394R +α-tubulin (D).
Quantification: One-way ANOVA with post-hoc Tukey. All data are mean ± SEM. n.s= non-significant; ***p=0.01–0.001; ****p<0.0001.

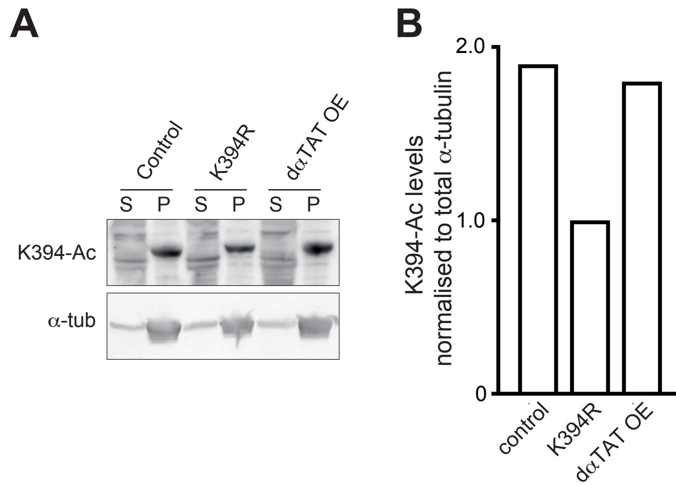

Figure S3. α-tubulin K394 acetylation is not regulated by dαTAT, related to Figure 6.

(A and B) Western blot analysis (A) and quantification (B) of K394 acetylation of microtubules pelleted from control and K394R fly heads and fly heads over-expressing d TAT. elav-Gal4 was used to over-express one copy of UAS-d TAT. The anti-Ac-K394 signal in the tubulin pellet lane is normalised to total α-tubulin. S=supernatant, P=pellet.

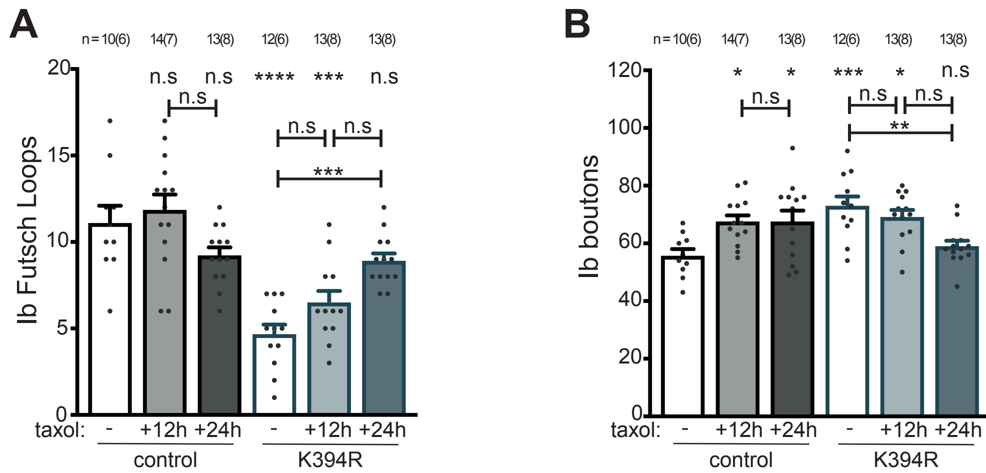

Figure S4. Time-dependent effects of taxol on Futsch loops and bouton numbers in control and K394R mutant animals, related to the STAR Methods.

(A and B) Quantification of Futsch loops (A) and boutons (B) in control and K394R mutant larvae incubated with taxol-free food (control) or food containing 10 µM taxol for 12 or 24 hours before dissection. p-values, Ib Futsch loops: p=0.97, control v. +12 h taxol; p=0.45, control v. +24 h taxol; p=0.066, +12 h taxol v. +24 h taxol; p<0.000001, control v. K394R; p=0.00046, control v. K394R +12 h taxol; p=0.27, control v. K394R +24 h taxol; p=0.44, K394R v. K394R +12 h taxol; p=0.00055, K394R v. K394R +24 h taxol; p=0.14, K394R +12 h taxol v. K394R +24 h taxol (A). p-values, Ib boutons: p=0.040, control v. +12 h taxol; p=0.046, control v. +24 h taxol; p=0.99, +12 h taxol v. +24 h taxol; p=0.000902, control v. K394R; p=0.016019, control v. K394R +12 h taxol; p=0.96, control v. K394R +24 h taxol; p=0.91, K394R v. K394R +12 h taxol; p=0.0058, K394R v. K394R +24 h taxol; p=0.086, K394R +12 h taxol v. K394R +24 h taxol (B).
Quantification: One-way ANOVA with post-hoc Tukey. All data are mean ± SEM. n.s=non-significant; *p=0.01-0.05; **p=0.01-0.001; ***p=0.01–0.001; ****p<0.0001.

Primers used to generate *αTub84B* knock-in strains	
K394A forward	TCTGGACCACGCCTTCGATCTGATGTACGCCAAGCGTGCCTTCGTCC
K394A reverse	CGGGCCCAGGCCTCGGCG
K394R forward	CCCGTCTGGACCACAGGTTCGATCTGATGTACGCC
K394R reverse	GGCGTACATCAGATCGAACCTGTGGTCCAGACGGG
Primers used to generate pIHEU-αTub84B	
αTub84B forward (EcoRI)	CGGCGCTCTGAACGTGGATCTGACTGAG
αTub84B reverse (XbaI)	TCAAAGCGGAGAGAGGCGGTAATCGAG
Primers used to generate pBSK-U63-EB1-gRNA	
EB1 guide forward	CGTCCTCTGGGTTTTAGAGCTAGAAATAGC
EB1 guide reverse	AGGAGTATTACGACGTTAAATTGAAAATAGGTC

Table S1. Oligonucleotide primers used in this study, related to STAR Methods.