SUPPORTING INFORMATION

Title: Polyglutamylation of tubulin's C-terminal tail controls pausing and motility of kinesin-3 family member KIF1A

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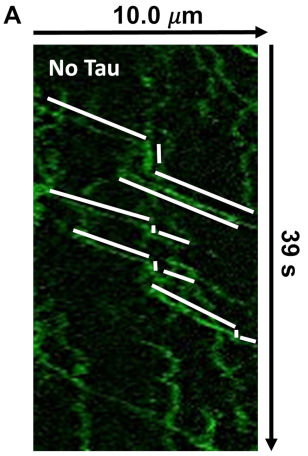
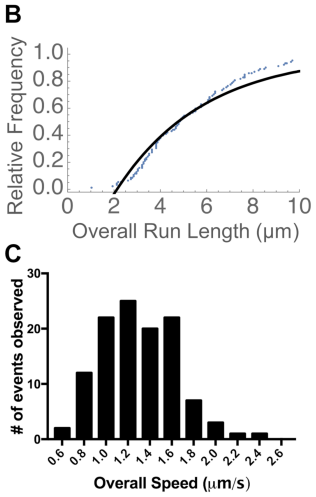
Materials Included:
Supplemental Movie 1 (uploaded separately)
Supplemental Movie 1 Legend
Supplemental Figures and Legends 1-7
Supplemental References

SI FIGURES

Movie S1. Sample motility of KIF1A on taxol-stabilized microtubules. TIRF microscopy was used to observe the motility of KIF1A-LZ-3xmCitrine on rhodamine labeled, taxol-stabilized microtubules. 500 frames were recorded at 5 fps, shown here at 25 fps. Data was analyzed as described in the Methods section.

Figure S1.





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	Taxol MTs
Overall Run Length (μm)	5.30 ± 2.32
Overall Speed (μm/s)	1.30 ± 0.35
Pauses per Distance (# pauses/μm)	0.17

Figure S1. **The LZ does not influence KIF1A pausing behavior.** **A)** Representative kymograph of the non-leucine zipper KIF1A construct (KIF1A[1-396]-GFP) on taxol-stabilized microtubules. KIF1A-GFP undergoes extensive pausing behavior during a single overall run (outlined in white). There are also many purely diffusive events of KIF1A molecules not stabilized by the leucine zipper, presumably monomeric motors [1]. **B)** Quantification of overall run length displayed as cumulative frequency plot. C) Histogram of overall speed. **D)** Summary of KIF1A-GFP motility and behavior on taxol-stabilized microtubules. KIF1A-GFP exhibits an overall run length (ORL) of 5.30 ± 2.32 μm [n=123]), an overall speed of 1.30 ± 0.35 μm/s [n=123] and exhibits 0.17 pauses/μm. Run length values and standard deviations were calculated as previously reported [2]. Each condition is representative of at least four independent experiments. Mean ± SD reported.

Figure S2.

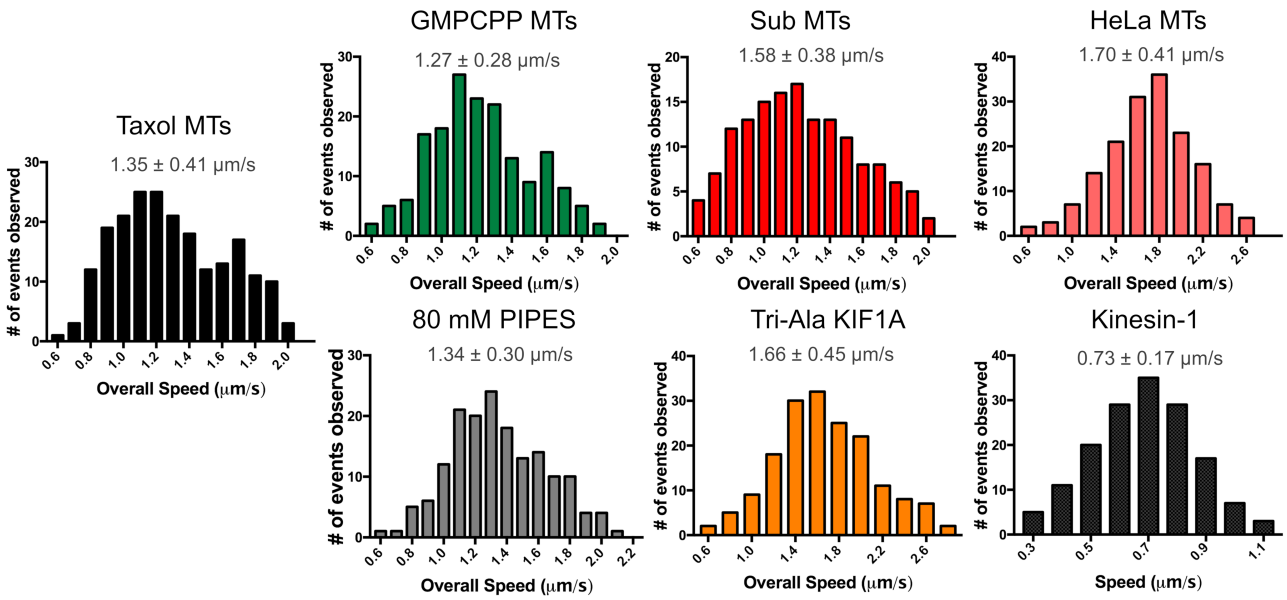


Figure S2. Histograms of overall speed across all conditions The Mean ± SD for each condition is presented above each graph.

Figure S3.

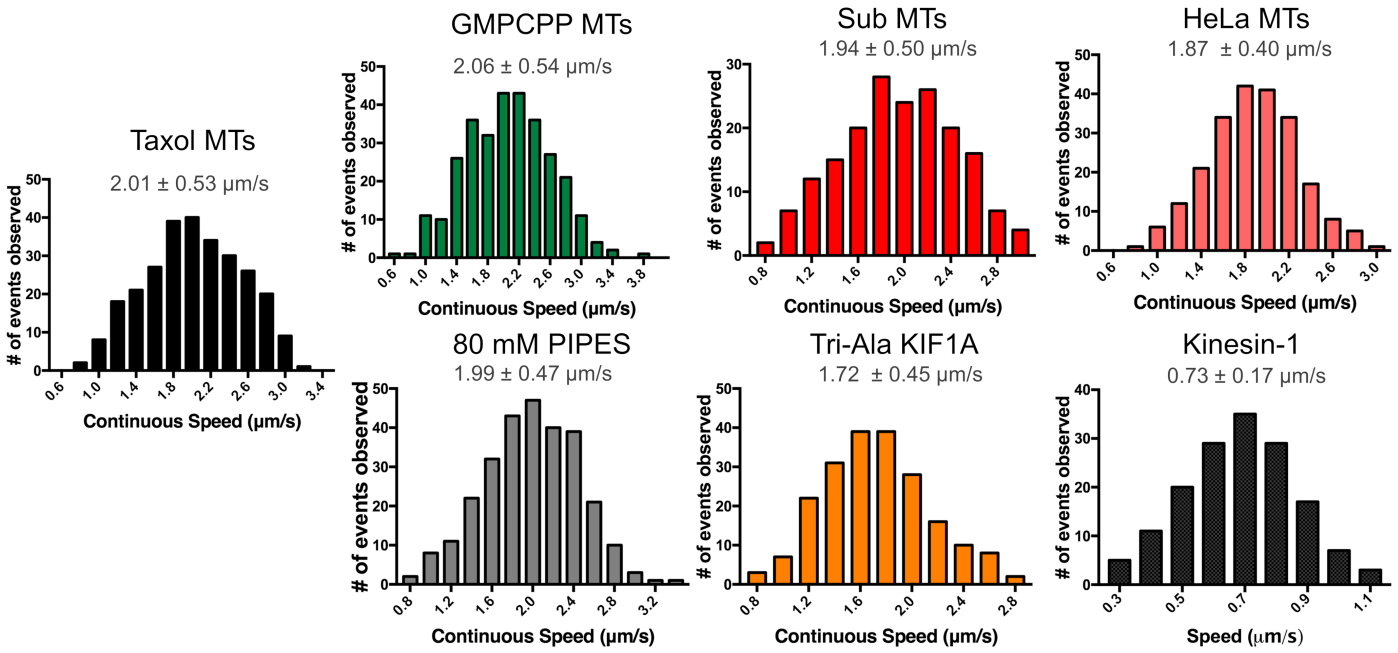


Figure S3. Histograms of continuous speed across all conditions The Mean ± SD for each condition is presented above each graph.

Figure S4.

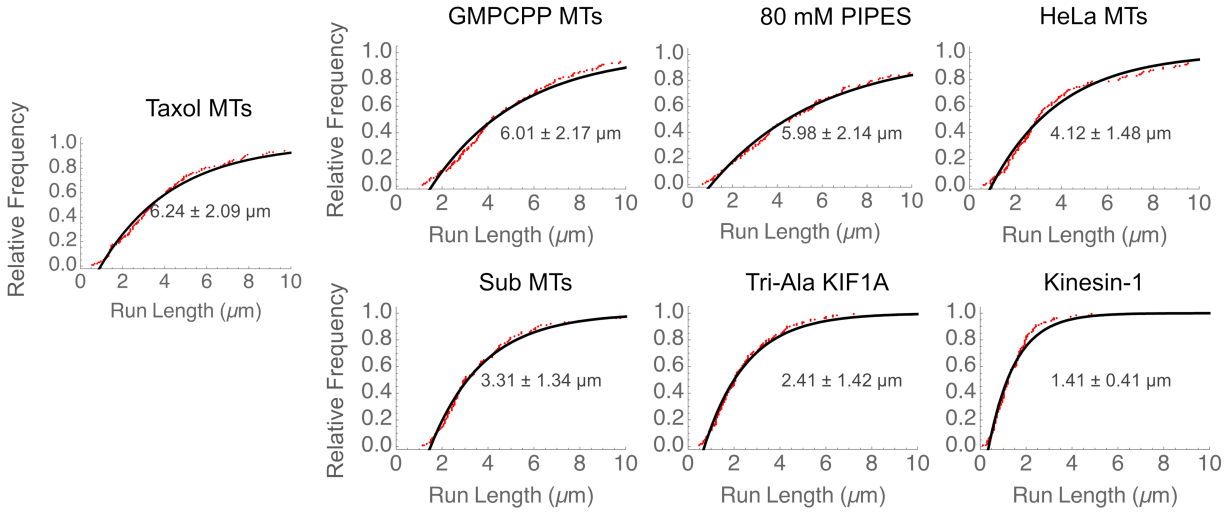


Figure S4. Cumulative frequency plots of overall run length across all conditions. Black dots represent the raw run length and red dots represent the observed cumulative frequency. Kinesin-1 was used as an experimental control; run length of kinesin-1 is reported. The mean overall run length was calculated as previously reported [2] and is shown within each graph (Mean ± SD).

Figure S5.

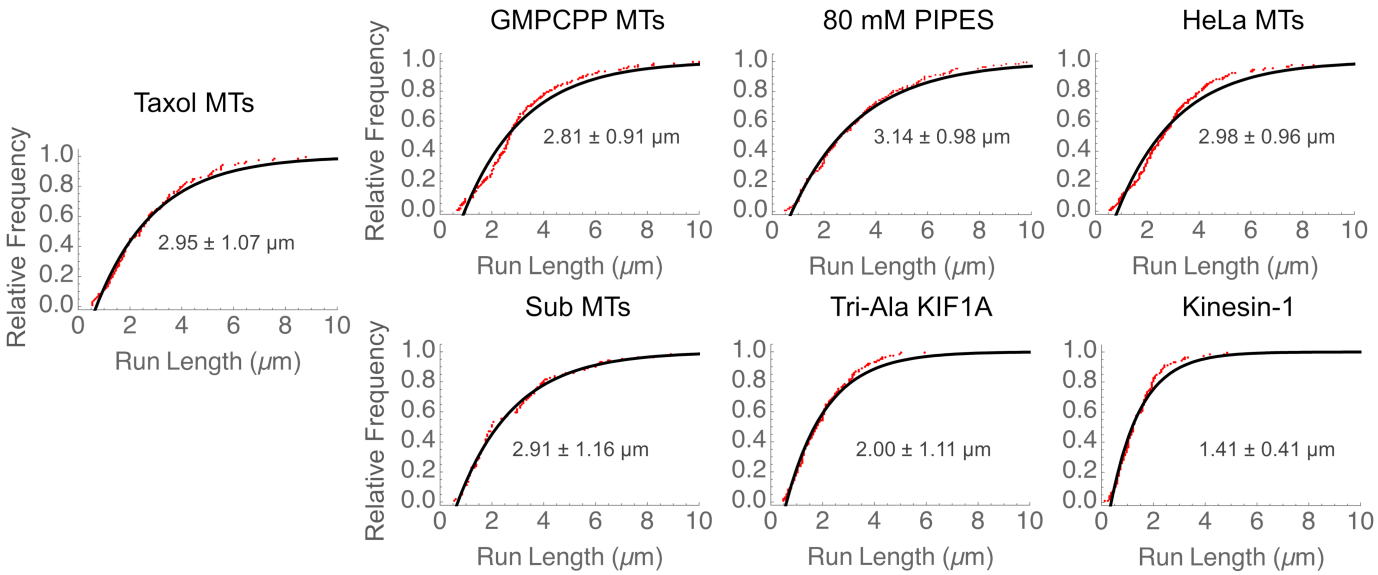


Figure S5. Cumulative frequency plots of continuous run length across all conditions. Black dots represent the raw run length and red dots represent the observed cumulative frequency. Kinesin-1 was used as an experimental control; run length of kinesin-1 is reported. A continuous run length was calculated as previously reported [2] and is shown within each graph (Mean ± SD).

Figure S6.

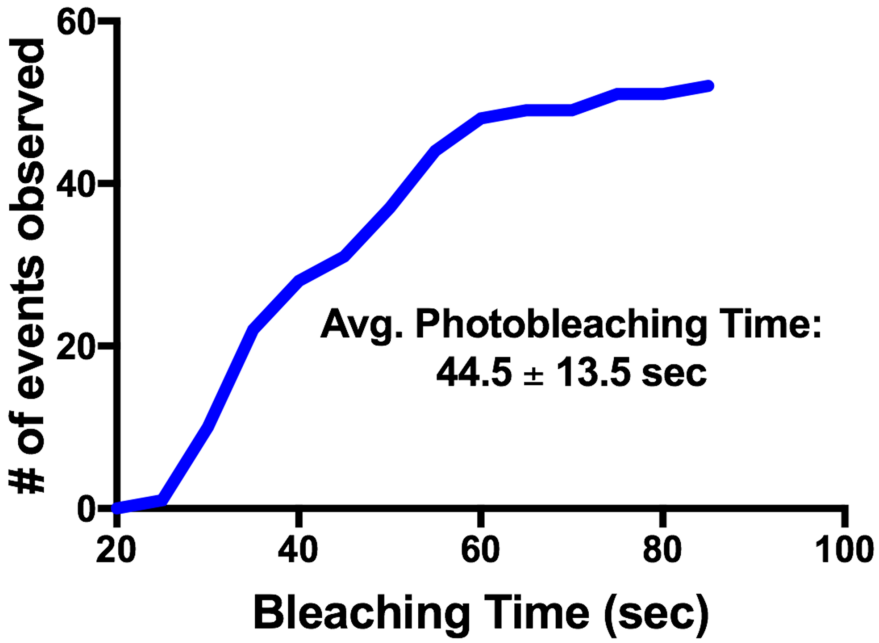
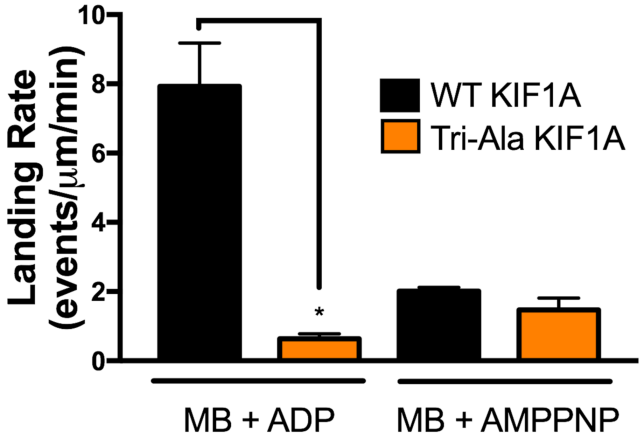


Figure S6. Photobleaching assay measuring average KIF1A-LZ-3xmCitrine bleaching time. KIF1A motors in an AMPPNP nucleotide state were imaged using TIRF microscopy. Fluorescence intensity over time of individual motors was measured and corrected for channel background noise. Mean ± SD reported.

Figure S7.



	MB + ADP	MB + AMPPNP
WT KIF1A	7.93 ± 1.25	2.01 ± 0.49
Tri-Ala KIF1A	0.64 ± 0.14*	1.47 ± 0.34*

Figure S7. Comparison of WT and Tri-Ala KIF1A landing rates in the ADP vs AMPPNP state. In motility buffer supplemented with ADP as the available nucleotide (MB + ADP), WT KIF1A had a landing rate of 7.93 ± 1.25 events/μm/min (n=1552) while Tri-Ala KIF1A had a landing rate of 0.64 ± 0.14 events/μm/min (n=527). In motility buffer supplemented with AMPPNP (MB + AMPPNP), WT KIF1A had a landing rate of 2.01 ± 0.49 events/μm/min (n=917) while Tri-Ala KIF1A had a landing rate of 1.47 ± 0.34 events/μm/min (n=574). Mean ± SD reported. * = $p<0.001$ relative to WT KIF1A under corresponding condition.

SUPPLEMENTAL REFERENCES

1. Soppina, V., et al., *Dimerization of mammalian kinesin-3 motors results in superprocessive motion.* Proc Natl Acad Sci U S A, 2014. **111**(15): p. 5562-7.
2. Thompson, A.R., G.J. Hoeprich, and C.L. Berger, *Single-molecule motility: statistical analysis and the effects of track length on quantification of processive motion.* Biophys J, 2013. **104**(12): p. 2651-61.