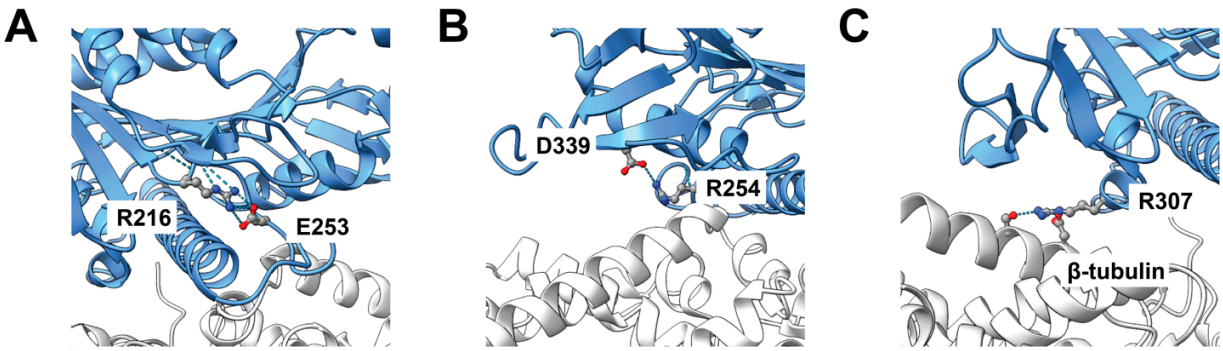
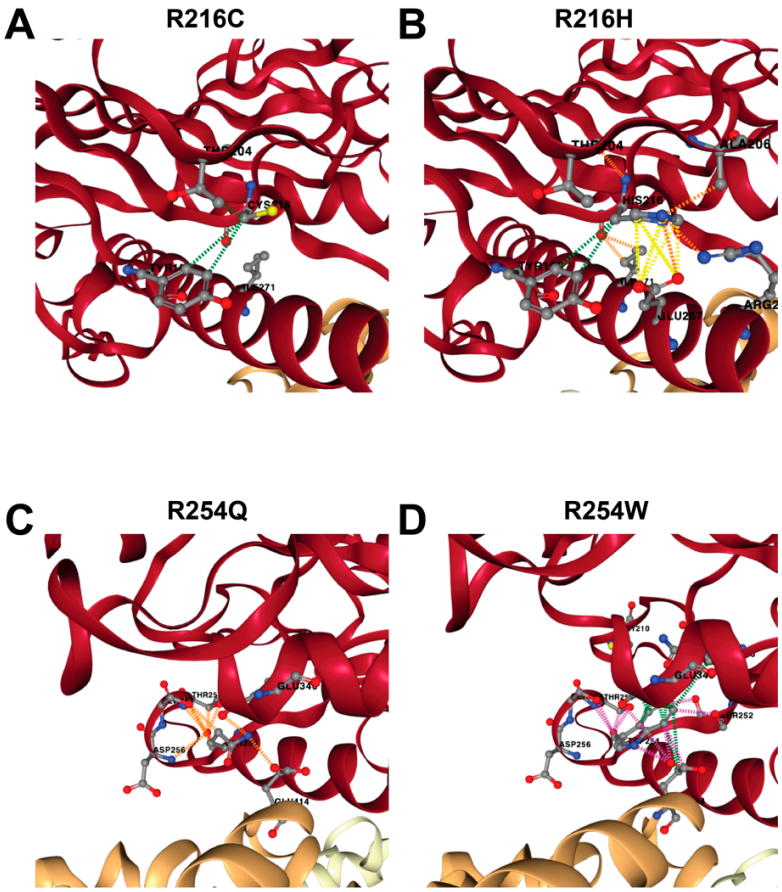


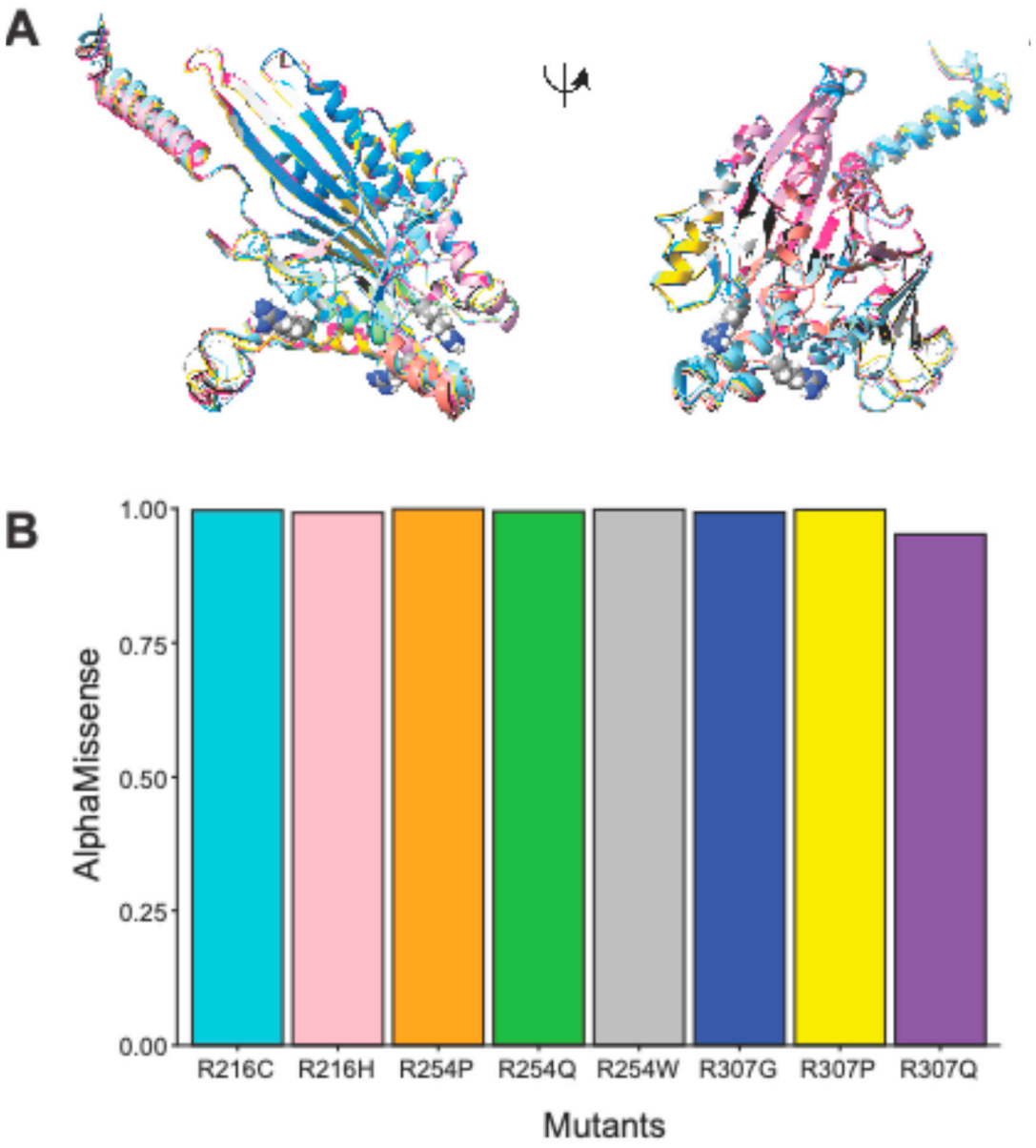
Supplemental Figure S1. Multiple sequence alignment of selected kinesins generated using Clustal Omega [1]. KIF1A residues R216, R254, and R307 are highlighted in red.



Supplemental Figure S2. Local interactions of KIF1A residues R216, R254, and R307 in the ADP state when bound to microtubules (PDB 8UTR) [2]. **(A)** R216 interacts with E253. **(B)** R254 interacts with D339. **(C)** R307 interacts with S413 and D417 in β-tubulin.



Supplemental Figure S3. Structural prediction of KIF1A mutants using graph-based deep learning.



Supplemental Figure S4. Structural and Functional Predictions for Disease-Associated KIF1A Mutations. **(A)** Overlay of AlphaFold2-predicted structures of wild-type and mutant KIF1A motor domains. Residues R216, R254, and R307 are shown in space-filling representation. Color coding: black, wild-type; cyan, R216C; pink, R216H; green, R254Q; orange, R254P; gray, R254W; purple, R307Q; yellow, R307P; and blue, R307G. **(B)** AlphaMissense score for the KIF1A mutations analyzed in this study. Higher scores indicate a greater predicted impact on protein function.

[1] F. Madeira *et al.*, "The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024," *Nucleic Acids Res,* vol. 52, no. W1, pp. W521-W525, Jul 5 2024, doi: 10.1093/nar/gkae241.

[2] M. Benoit, L. Rao, A. B. Asenjo, A. Gennerich, and H. Sosa, "Cryo-EM unveils kinesin KIF1A's processivity mechanism and the impact of its pathogenic variant P305L," *Nat Commun,* vol. 15, no. 1, p. 5530, Jul 2 2024, doi: 10.1038/s41467-024-48720-4.