UNIVERSITY OF CALIFORNIA

Los Angeles

Methods and Systems Developed for Characterization of Mammalian Katanin

A dissertation

submitted in partial satisfaction of the requirements

for the degree Doctor of Philosophy

in Biochemistry, Molecular and Structural Biology

by

Nicole A. Lynn

2023

ABSTRACT OF THE DISSERTATION

Methods and Systems Developed for Characterization of Mammalian Katanin

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Nicole A. Lynn

Doctor of Philosophy in Biochemistry, Molecular and Structural Biology

University of California, Los Angeles, 2023

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The katanin family of microtubule-severing enzymes present themselves as important characters in the story of cell homeostasis, division, proliferation, and migration. These enzymes are critical in promoting the microtubule density or stability required for the aforementioned functions. While it is well-known these enzymes function in the role of microtubule-severing, it is unclear why so many isoforms of A- and B- katanin exist in eukaryotes. The primary purpose of this work was to identify the redundancies, and/or specialized functions of the katanin superfamily using a series of knockout and rescue experimentation. The secondary purpose of this work was to clarify inter-subunit interactions, in particular those between AL2 and B1 katanin, as well as understand the structural and post-translational mechanisms of these enzymes. Developing a better understanding of how these enzymes function in the above cellular roles, as well as why there is a need for multiple isoforms with high sequence similarity in multiple mammalian species can promote the use of these proteins as diagnostic tools or use as targets for cancer and disease therapeutics.

The dissertation of Nicole A. Lynn is approved.

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2023

Dedication

I would like to dedicate this thesis to my loving husband Damon Martinez for his relentless support of all my journeys in life, including the arts and my Ph.D., which we both did not anticipate being as traumatic or disheartening as it became, with the onslaught of a global pandemic, the increased demands of caring for a severely ill grandparent with dementia, and the harassment and discrimination I faced in the Ph.D.; No matter how difficult life got, you stood strongly by my side. You are my rock. Thank you.

I also would like to dedicate this thesis to my two cats, one who will have been with me for 18 years when this is published, putting her paws and tail on all my assignments, rubbing your face on my computer screen, and keeping me company during all-nighters that got me into grad school. Thank you lil' G, my baby cat, for being a loving and studious companion all these years. I also am thankful for my second cat that I inherited from grandma. My Candy-man, my Candy-Mandy. Thank you for letting us be your family when grandma could no longer care for you, and for thriving despite your diabetes, thyroid, heart, and medical conditions. May you now rest in power, little guy. Thank you kitties, for letting us love you and give you happy and long winters.

I also want to thank my in-laws, my husband's family, and my friends inside and outside of STEM. You are my chosen family. You are where I found love and acceptance. Thank you for supporting and loving me for who I am.

I want to thank my mentors and friends from Cal Poly Pomona, who have been an abundance of light as I pursued my Ph.D. at UCLA. Thank you for supporting me and for giving me a community when I felt abandoned and alone at my home institution. Dr. Buckley, Jeyashree, Isis, Ana, and more, thank you for reminding me that my merit and

hard work are things to be proud of. Thank you for reminding me there are good people in science: people who care. Thank you for making sure I am still a person who cares.

Lastly, I want to dedicate this degree to all of my peers who were unable to pursue their Ph.D. due to issues like familial obligation, finance, personal responsibility, or unsupportive mentors. There is a place for you in science, do not give up. Stay true to yourself, always be kind, and harbor gratitude. In the words of Jason Mraz, “Living your dream is hard work, it’s never too late to be what you might have been”.

Table of Contents

Acknowledgements

I would like to acknowledge my new committee members Dr. Joseph Loo, Dr. Carla Koehler, Dr. Margot Quinlan, and Dr. Samantha Butler. Thank you for working with me to finish my thesis and degree. Thank you for supporting me when the need arose. I will forever be thankful for this. I also would like to thank the Title IX office, who helped ensure the committee reconstitution.

I would like to acknowledge Dr. Mark Arbing who worked diligently to produce Sf9 recombinant insect proteins for the work on post-translational analysis as well as the possibility of the structure work for AL2 and A1-B1 katanin. I appreciate all of your kindness, support, and your willingness to help.

I would like to acknowledge Dr. Joseph Ong, a previous BMSB student, who served as a surrogate thesis advisor and mentor, offering experimental and professional support when I did not have it on campus. Your aid and influence have been immeasurable.

I want to acknowledge and thank Penny Jennings who acted as one of the most inclusive people in our department, recruiting my work on the Chemistry and Biochemistry Department Annual Newsletter in 2022 and 2023. I also want to acknowledge the building managers, administrators, and custodians in Young Hall and Boyer Hall, including Nati Alcaraz, Janette Kropat, and BMSB staff Marla Gonzales, among others, who continuously made me feel welcome and safe despite my rank as grad student, my heritage/ethnicity, or my gender. I am thankful for the community and kindness you provided while I worked toward my Ph.D.

I want to acknowledge and thank Comfort Dorn and Angela Hopp at ASBMB Today for allowing me to volunteer and supporting my endeavors in science communication and giving me the opportunity to write over 15 articles with their science news source.

I lastly want to acknowledge my multiple funding sources, including UCLA Eugene V. Cote Robles, UCLA CMB Training Grant, and the NSF GRFP.

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Publications

1) Lynn, N. A Pioneer in Lethal Pathogen Research. ASBMB Today. In press. 2023.

2) Lynn, Nicole. A., Spotlighting Faculty: Dr. Margot Quinlan. June 2023. UCLA 2023 Chemistry and Biochemistry Annual Report (print, online).
3) Lynn, Nicole. A., Spotlighting Faculty: Dr. Jorge Torres and Dr. Anastassia Alexandrova. June 2022. UCLA 2022 Chemistry and Biochemistry Annual Report (print, online).
4) Lynn, et al., The Mammalian Family of Katanin Microtubule-Severing Enzymes. Frontiers Cell Dev Biol 9, 692040 (2021), (print, online).
5) Lynn, N. Microbes and Minerals. ASBMB Today: print, online. October 2022.
6) Lynn, N. Conference inspires a shift in undergrad's career goals. ASBMB Today: print, online. October 2022.
7) Lynn, N. In Memoriam: Michael Stitleman. ASBMB Today: print, online. April May 2022.
8) Lynn, N. Pivoting to Transform a Pandemic Classroom. ASBMB Today: print, online. April 2022.
9) Lynn, N. Deciphering Antiviral Mechanisms During the Pandemic. ASBMB Today: print, online. March 2022.
10) Lynn, N. Improving Disease Detection for Personalized Vaccines. ASBMB Today: print, online. February 2022.
11) Lynn, N. Telomerase Studies led Collins toward Discoveries in Genetic Elements. ASBMB Today: print, online. December 2021.
12) Lynn, N. Breastfeeding Awareness Month 2021. ASBMB Today: print, online. August 2021.
13) Lynn, N. Starved to Death: Can Dietary Methionine Combat Cancer. ASBMB Today: print, online. July 2021.
14) Lynn, N. The Arginine Tango. ASBMB Today: print, online. April 2021.
15) Lynn, N. From the Journals: MCP. ASBMB Today: print, online. March 2021.
16) Lynn, N. I Smell a Winner: Linking Plant Olfactory Stimuli to Genetic Regulation. ASBMB Today: print, online. March 2021.
17) Lynn, N. Meet Ahna Skop. ASBMB Today: print, online. August 2021.
18) Lynn, N. How a Virus Can Affect Memory: The Role of HIV in HAND. ASBMB Today: print, online. Dec 2020.
19) Lynn, N. World Pneumonia Day: 2020. ASBMB Today: print, online. November 2020.
20) Lynn, N. Decoding a Protein's Role in Connective Tissue Disorders. ASBMB Today: print, online. August 2020.
21) Lynn, N. National Women's Health Week 2020. ASBMB Today: print, online. May 2020.

Introduction

The eukaryotic cell is composed of three main structural elements: intermediate filaments, actin, and microtubules.[1] While intermediate filaments are critical for resisting cellular stress and mechanical strength, and actin is important for cell shape/locomotion, microtubules are critical to creating filamentous networks used for intracellular transport, cellular structure, and homeostasis;[2] microtubules are also important for maintaining the specific position of the organelles within the cell, as well as the connection to the chromosome during interphase and cell division.[3] Microtubules utilize a large number of different proteins to modulate their growth/dynamics, and abundance; these proteins are known as microtubule-associated proteins, or MAPs.[2] One such protein is the enzyme katanin.

Katanin is a microtubule-severing enzyme originally discovered in the sea urchin, and named after the Japanese sword Katana for its role in cutting microtubules.[4] A severase, katanin cuts the microtubule in a longitudinal fashion (along the center of the protofilament), creating smaller fragments that harbor both plus and minus ends.[5] Producing microtubule fragments with plus and minus ends allows for two outcomes: first, the promotion of microtubule nucleation and growth, observed during mitosis,[4, 6] and second, recruitment of stabilizing MAPs to reduce or stabilize of microtubule growth, as observed with MAP2/tau in neurons (Figure 1).[2, 7]

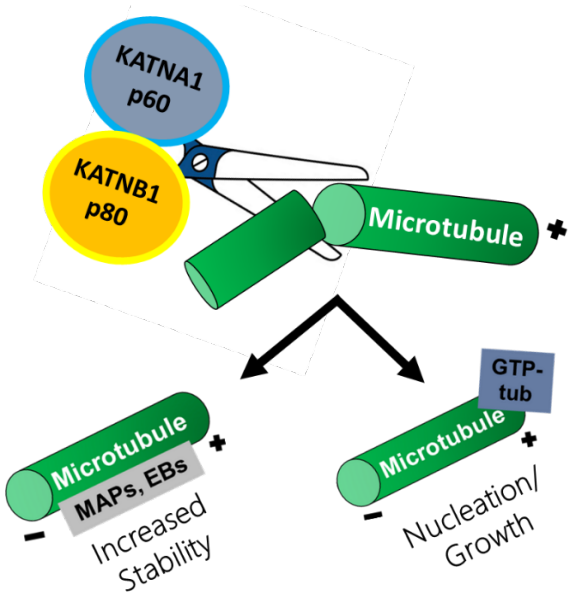

Figure 1. Schematic of Canonical katanin Severing. A-subunit p60 katanin (blue) forms heterodimer with B-subunit p80 katanin (yellow) and severs microtubule (green). The severed microtubule fragments can be decorated with stabilizing or destabilizing proteins.

The katanin protein is composed of a catalytic AAA+ domain containing p60 A-subunit and regulatory p80 B-subunit.[5, 8] The A-subunit is unique in that it can sever microtubules without the B-subunit,[9-11] however the B-subunit is required for A-subunit localization and regulation of activity.[8, 12] The subunits are known to form a heterodimer,[10, 13] similarly, the A- subunit has been shown to form stable higher order hexameric structures, with crystal structures of the A-subunit *C. elegans* homologue MEI-1 available.[14, 15] The A- and B-katanin subunits are evolutionarily conserved among Eukaryotes.

While the current research on katanin proteins continues to grow, it is established that the eukaryotic katanin family plays key roles in multiple cellular processes, including cellular homeostasis, meiosis, mitosis, neurological development, mammalian development, and

cancer.[16-20] What remains unknown is the need for so many katanin subunits, and if each contains a specified function. In eukaryotes, up to five total katanin subunits have been observed: A1, AL1, and AL2 subunit isoforms for the canonical A-subunit, and B1 and BL1 isoforms for the B-subunit.[12] How does the regulation vary between them, for example, the current debate that stands regarding AL2 and B1 interaction and regulation? Protein-protein interactions with critical regulators like TP53 were also to be assessed. Lastly, the structure and modifications of these enzymes were to be evaluated.

Chapter 1 of this dissertation contains the review publication of the mammalian katanins I produced in 2021. Its purpose was to address the current understanding of mammalian katanin and the research available describing functions and structure, then to make connections between modifications, dysregulations, and disease.

Chapter 2 of this dissertation is meant to investigate the question of katanin subunit redundancy: are there unique functions for each katanin A- and B- subunit? Currently, the literature has shown that the knockdown or reduction of katanin A- and B- subunits leads to overlapping phenotypes.[21-25] As such, there is a lack of multiple and/or combinatorial knockouts to evaluate the potential for overlapping or nuanced function among A- and B-subunits in any given cell type. Tools were set up to knock out katanin, such as the monoclonal iCas9 RPE1 cell line that would pair with the knockout sgRNA viral constructs.

Chapter 3 of this dissertation is meant to evaluate the connections between the katanin catalytic A-subunits (A1, AL1, AL2) and the tumor suppressor protein TP53. The literature demonstrates that at this time katana1 binds to TP53 via the AAA+ domain of A1 and the DNA binding domain of TP53.[19] Furthermore, TP53 has been shown to bind to the

promoter of katanin A1 and up-regulate mRNA expression.[26] With this information and the fact that the katanin A1 subunit shares high homology to the AL1 and AL2 subunits, it was a feasible hypothesis that AL1 and AL2 may both bind and be regulated similarly. The preliminary data demonstrated: (a) Upon reduction of TP53 Katanin AL2 protein levels were reduced, and (b) Co-immunoprecipitation experiments suggested a possible binding event between AL2 and TP53.

Chapter 4 of this dissertation evaluates the inter-subunit interactions of katanin. Specifically, the purpose of this chapter is to determine the propensity for heterodimerization among the A-subunits. The rationale for this hypothesis is the propensity for the canonical A1 subunit to form higher order structures through the AAA+ domain.[5, 13, 15] Due to the high conservation of the AAA+ domain seen in A1, AL1, and AL2,[27] it seemed feasible the A-subunits would be able to form higher order hetero-structures with each other.

The second goal of Chapter 4 was to resolve the current conflict with the reporting in 2016 by which A1 and AL1 are interactors with B1, but AL2 is not.[12] Currently there is data that supports a hypothesis where AL2 and B1 do in fact interact, as was shown in 2017, and appears to be a master regulator of the A-subunits, from a report in 2021.[11, 28] The conflicting data may be due to a difference in cell types (cancerous vs non-cancerous, or somatic vs germ cell), and needed resolving. The creation of multiple tagged protein constructs was established, with the aim to perform *in vitro* and *in vivo* co-immunoprecipitation.

Chapter 5 of this dissertation was meant to assess the higher order structures of the katanin subunits as well as the post-translational modifications. First, the A-B katanin

heterodimer was to be purified using recombinant techniques: from *E. coli* and later *Sf9* cells. The purpose was to use the purified protein for cryo-EM structure imaging. The recombinant *Sf9* cells were created with the help of Mark Arbing in the Department of Chemistry and Biochemistry. Next, AL2 was to be assessed for higher order structures and post-translational modifications. This would have been, to my knowledge, the first profiling of katanin AL2 for PTMs. Unfortunately, there was contamination with the *Sf9* cells, which caused delays. The AL2 work made some progress, including obtaining a preliminary profile of binding partners in an overexpressed tagged AL2, with the help of Eileen Olivares, a graduate student in the Loo lab.

References

1. Pollard, T. D.; Goldman, R. D., Overview of the Cytoskeleton from an Evolutionary Perspective. *Cold Spring Harb Perspect Biol* **2018,** *10* (7).

2. Goodson, H. V.; Jonasson, E. M., Microtubules and Microtubule-Associated Proteins. *Cold Spring Harb Perspect Biol* **2018,** *10* (6).

3. Alberts, B., *Molecular biology of the cell*. 4th ed.; Garland Science: New York, 2002; p xxxiv, 1463, 86 p.

4. Vale, R. D., Severing of stable microtubules by a mitotically activated protein in Xenopus egg extracts. *Cell* **1991,** *64* (4), 827-39.

5. McNally, F. J.; Vale, R. D., Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **1993,** *75* (3), 419-29.

6. McNally, K.; Audhya, A.; Oegema, K.; McNally, F. J., Katanin controls mitotic and meiotic spindle length. *J Cell Biol* **2006,** *175* (6), 881-91.

7. Dehmelt, L.; Halpain, S., The MAP2/Tau family of microtubule-associated proteins. *Genome Biol* **2005,** *6* (1), 204.

8. Hartman, J. J.; Mahr, J.; McNally, K.; Okawa, K.; Iwamatsu, A.; Thomas, S.; Cheesman, S.; Heuser, J.; Vale, R. D.; McNally, F. J., Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. *Cell* **1998,** *93* (2), 277-87.

9. Hartman, J. J.; Vale, R. D., Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* **1999,** *286* (5440), 782-5.

10. Rezabkova, L.; Jiang, K.; Capitani, G.; Prota, A. E.; Akhmanova, A.; Steinmetz, M. O.; Kammerer, R. A., Structural basis of katanin p60:p80 complex formation. *Sci Rep* **2017,** *7* (1), 14893.

11. Dunleavy, J. E. M.; Okuda, H.; O'Connor, A. E.; Merriner, D. J.; O'Donnell, L.; Jamsai, D.; Bergmann, M.; O'Bryan, M. K., Katanin-like 2 (KATNAL2) functions in multiple aspects of haploid male germ cell development in the mouse. *PLoS Genet* **2017,** *13* (11), e1007078.

12. Cheung, K.; Senese, S.; Kuang, J.; Bui, N.; Ongpipattanakul, C.; Gholkar, A.; Cohn, W.; Capri, J.; Whitelegge, J. P.; Torres, J. Z., Proteomic Analysis of the Mammalian Katanin Family of Microtubule-severing Enzymes Defines Katanin p80 subunit B-like 1 (KATNBL1) as a Regulator of Mammalian Katanin Microtubule-severing. *Mol Cell Proteomics* **2016,** *15* (5), 1658-69.

13. Faltova, L.; Jiang, K.; Frey, D.; Wu, Y.; Capitani, G.; Prota, A. E.; Akhmanova, A.; Steinmetz, M. O.; Kammerer, R. A., Crystal Structure of a Heterotetrameric Katanin p60:p80 Complex. *Structure* **2019,** *27* (9), 1375-1383.e3.

14. Nithianantham, S.; McNally, F. J.; Al-Bassam, J., Structural basis for disassembly of katanin heterododecamers. *J Biol Chem* **2018,** *293* (27), 10590-10605.

15. Shin, S. C.; Im, S. K.; Jang, E. H.; Jin, K. S.; Hur, E. M.; Kim, E. E., Structural and Molecular Basis for Katanin-Mediated Severing of Glutamylated Microtubules. *Cell Rep* **2019,** *26* (5), 1357-1367.e5.

16. Willsey, H. R.; Walentek, P.; Exner, C. R. T.; Xu, Y.; Lane, A. B.; Harland, R. M.; Heald, R.; Santama, N., Katanin-like protein Katnal2 is required for ciliogenesis and brain development in Xenopus embryos. *Dev Biol* **2018,** *442* (2), 276-287.

17. Banks, G.; Lassi, G.; Hoerder-Suabedissen, A.; Tinarelli, F.; Simon, M. M.; Wilcox, A.; Lau, P.; Lawson, T. N.; Johnson, S.; Rutman, A.; Sweeting, M.; Chesham, J. E.; Barnard, A. R.; Horner, N.; Westerberg, H.; Smith, L. B.; Molnár, Z.; Hastings, M. H.; Hirst, R. A.; Tucci, V.; Nolan, P. M., A missense mutation in Katnal1 underlies behavioural, neurological and ciliary anomalies. *Mol Psychiatry* **2018,** *23* (3), 713-722.

18. Ye, Q.; Zhang, M.; Yin, Y., Katanin P80 correlates with larger tumor size, lymph node metastasis, and advanced TNM stage and predicts poor prognosis in non-small-cell lung cancer patients. *J Clin Lab Anal* **2020,** *34* (4), e23141.

19. Korulu, S.; Yildiz, A., p60-katanin: a novel interacting partner for p53. *Mol Biol Rep* **2020,** *47* (6), 4295-4301.

20. Hu, W. F.; Pomp, O.; Ben-Omran, T.; Kodani, A.; Henke, K.; Mochida, G. H.; Yu, T. W.; Woodworth, M. B.; Bonnard, C.; Raj, G. S.; Tan, T. T.; Hamamy, H.; Masri, A.; Shboul, M.; Al Saffar, M.; Partlow, J. N.; Al-Dosari, M.; Alazami, A.; Alowain, M.; Alkuraya, F. S.; Reiter, J. F.; Harris, M. P.; Reversade, B.; Walsh, C. A., Katanin p80 regulates human cortical development by limiting centriole and cilia number. *Neuron* **2014,** *84* (6), 1240-57.

21. Matsuo, M.; Shimodaira, T.; Kasama, T.; Hata, Y.; Echigo, A.; Okabe, M.; Arai, K.; Makino, Y.; Niwa, S.; Saya, H.; Kishimoto, T., Katanin p60 contributes to microtubule

instability around the midbody and facilitates cytokinesis in rat cells. *PLoS One* **2013,** *8* (11), e80392.

22. Ververis, A.; Christodoulou, A.; Christoforou, M.; Kamilari, C.; Lederer, C. W.; Santama, N., A novel family of katanin-like 2 protein isoforms (KATNAL2), interacting with nucleotide-binding proteins Nubp1 and Nubp2, are key regulators of different MT-based processes in mammalian cells. *Cell Mol Life Sci* **2016,** *73* (1), 163-84.

23. Gao, L. L.; Xu, F.; Jin, Z.; Ying, X. Y.; Liu, J. W., Microtubule-severing protein Katanin p60 ATPase-containing subunit A-like 1 is involved in pole-based spindle organization during mouse oocyte meiosis. *Mol Med Rep* **2019,** *20* (4), 3573-3582.

24. Loughlin, R.; Wilbur, J. D.; McNally, F. J.; Nédélec, F. J.; Heald, R., Katanin contributes to interspecies spindle length scaling in Xenopus. *Cell* **2011,** *147* (6), 1397-407.

25. Sonbuchner, T. M.; Rath, U.; Sharp, D. J., KL1 is a novel microtubule severing enzyme that regulates mitotic spindle architecture. *Cell Cycle* **2010,** *9* (12), 2403-11.

26. Kırımtay, K.; Selçuk, E.; Kelle, D.; Erman, B.; Karabay, A., p53 regulates katanin-p60 promoter in HCT 116 cells. *Gene* **2020,** *727*, 144241.

27. Lynn, N. A.; Martinez, E.; Nguyen, H.; Torres, J. Z., The Mammalian Family of Katanin Microtubule-Severing Enzymes. *Front Cell Dev Biol* **2021,** *9*, 692040.

28. Dunleavy, J. E. M.; O'Connor, A. E.; Okuda, H.; Merriner, D. J.; O'Bryan, M. K., KATNB1 is a master regulator of multiple katanin enzymes in male meiosis and haploid germ cell development. *Development* **2021,** *148* (24).

Chapter 1:

The Mammalian Family of Katanin Microtubule-Severing Enzymes

Published in *Frontiers in Cell and Developmental Biology*:
Lynn NA, Martinez E, Nguyen H and Torres JZ (2021) The Mammalian Family of Katanin Microtubule-Severing Enzymes. *Front. Cell Dev. Biol.* 9:692040. doi: 10.3389/fcell.2021.692040

The Mammalian Family of Katanin Microtubule Severing Enzymes

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Abstract

The katanin family of microtubule-severing enzymes is critical for cytoskeletal rearrangements that affect key cellular processes like division, migration, signaling, and homeostasis. In humans, aberrant expression, or dysfunction of the katanins, is linked to developmental, proliferative, and neurodegenerative disorders. Here, we review current knowledge on the mammalian family of katanins, including an overview of evolutionary conservation, functional domain organization, and the mechanisms that regulate katanin activity. We assess the function of katanins in dividing and non-dividing cells and how their dysregulation promotes impaired ciliary signaling and defects in developmental programs (corticogenesis, gametogenesis, and neurodevelopment) and contributes to neurodegeneration and cancer. We conclude with perspectives on future katanin research that will advance our understanding of this exciting and dynamic class of disease-associated enzymes.

Introduction

Discovered in sea urchin eggs in 1993 and named after the Japanese expression for sword (katana),the katanins are a family of microtubule-severing enzymes (McNally and Vale, 1993) belonging to the ATPases Associated with diverse cellular Activities (AAAC) protein superfamily (Snider et al., 2008). Katanins function by harnessing the energy produced from ATP hydrolysis to drive microtubule-severing events (McNally and Vale, 1993; McNally and Thomas, 1998). Due to their role in facilitating cytoskeletal rearrangements, the katanins have become the subject of intense research in the fields of neuroscience (Banks et al., 2018), cancer (Wang et al., 2020), and cell and structural biology (Willsey et al., 2018; Faltova et al., 2019; Korulu and Yildiz, 2020). Katanin is a heterodimeric complex composed of the catalytic ATPase containing A-subunit (p60, KATNA1) and regulatory B-subunit (p80, KATNB1) (McNally and Vale, 1993; Hartman et al., 1998). While the p60 (A) and p80 (B) katanin subunits are conserved in eukaryotes with regard to protein sequence and function (McNally and Thomas, 1998; Srayko et al., 2000), the genomes of vertebrates encode multiple A- and B-subunits. As a testament to the importance of katanins in cellular homeostasis, dysregulation of katanin A- or B-subunit function is associated with developmental and proliferative disorders in vertebrates (Mishra-Gorur et al., 2015; Willsey et al., 2018) and mammals (Bartholdi et al., 2014; Stessman et al., 2017; Banks et al., 2018). For example, perturbation or loss of katanin subunit expression contributes to ciliopathies (Hu et al., 2014; Willsey et al., 2018), defective corticogenesis (Lombino et al., 2019), defective spermiogenesis (Smith et al., 2012; Pleuger et al., 2016), and cancer pathogenesis (Fu et al., 2018; Ye et al., 2020). In this review, we focus on the mammalian family of katanin proteins: how they

function, how they are regulated, and how their dysregulation can lead to an array of human diseases. We examine the function of katanin in shaping diverse microtubule-based structures during interphase, cell division, and ciliation in post-mitotic (terminally differentiated) cells and in post-meiotic cells during spermiogenesis. Furthermore, we contextualize the importance of katanins' diverse functions with its dysregulation in altered cellular states, disease pathologies, and developmental disorders. Similarly, the structural studies that have informed on the catalytic activity and function of katanins and the mechanisms by which katanins are regulated (genetic, transcriptional, and posttranslational) will be discussed. We conclude with future directions and perspectives on this exciting class of disease-associated microtubule-severing enzymes.

Katanin Subunit Conservation and Domain Organization

Katanin is a heterodimeric protein composed of a catalytic p60 A-subunit and a regulatory p80 B-subunit. While the A-subunit is capable of severing microtubules alone (Hartman and Vale, 1999; Dunleavy et al., 2017; Rezabkova et al., 2017), the binding of the B-subunit to the A-subunit regulates the intracellular localization and activity of the A-subunit (Hartman et al., 1998; Cheung et al., 2016). The mammalian katanin A-subunit KATNA1 (A1) is evolutionarily conserved in unicellular eukaryotes, invertebrates such as Caenorhabditis elegans and Drosophila melanogaster, and vertebrates including Danio rerio, Xenopus laevis, Mus musculus, Rattus norvegicus, and Homo sapiens (Figure 1). Additional katanin A-like subunits KATNAL1 (AL1) and KATNAL2 (AL2) are also present in unicellular eukaryotes, Drosophila, and vertebrates (Figure 1). While the katanin B-subunit KATNB1 (B1) is conserved from C. elegans to H. sapiens, the katanin B-like subunit KATNBL1 (BL1) is not as widely conserved (Cheung et al., 2016; Figure 1).

Interestingly, nematodes also contain two variants of the canonical B-subunit where the N-terminal WD40 domain is removed (McNally and Roll-Mecak, 2018; Figure 1). The presence of additional A-like katanin subunits among eukaryotes and the additional B-like subunits observed in vertebrates alludes to an increased complexity in microtubule severing that is required for cellular homeostasis.

Katanin subunits share several important domains that are critical to their heterodimerization (A–B dimers), homo-oligomerization (A–A oligomers), binding to microtubules and other proteins, and microtubule-severing function (Figure 1). For example, katanin A-subunits share a catalytic AAA+ domain that contains the conserved Walker A and B motifs (hereafter referred to as pore loops 1 and 2), which coordinate ATP binding and hydrolysis, respectively (Snider et al., 2008; Zehr et al., 2017; Nithianantham et al., 2018). Structural models indicate that upon A-subunit hexamerization, the pore loop 1 X-R-G motif creates a positively charged surface at the entrance to the catalytic site that functions to recognize and remodel tubulin (Zehr et al., 2017; Shin et al., 2019).

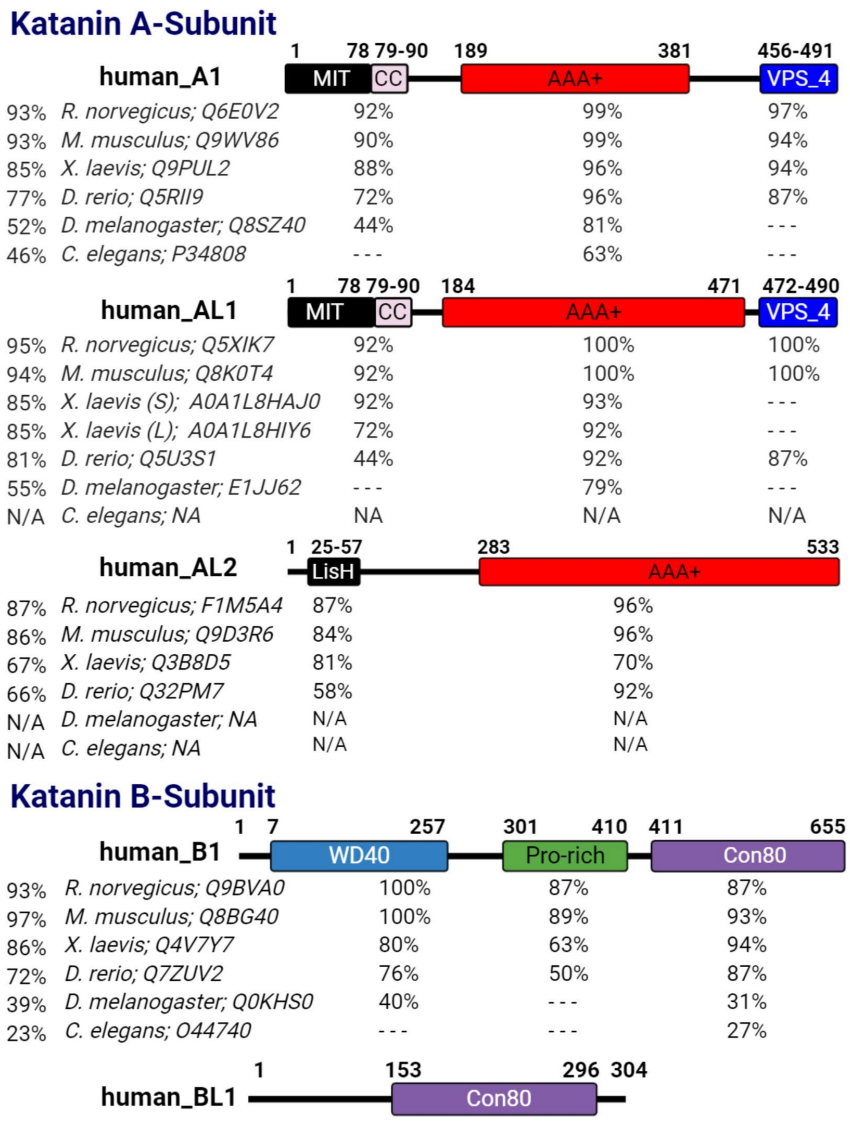

FIGURE 1 | Katanin subunit conservation. The five human katanin subunits—A1 (UniProt ID: O75449), AL1 (UniProt ID: Q9BW62), AL2 (UniProt ID: Q8IYT4), B1 (UniProt ID: Q9BVA0), and BL1 [UniProt ID: Q9H079 (human)]—and their corresponding domains are displayed. For each human katanin subunit, the relative percent identity to full-length (FL) and to each domain therein is indicated for its orthologs in other organisms. Percent identity was determined using NCBI protein blast with BLOSUM62 matrix. Dashed lines indicate no percent identity for that region; N/A indicates that the species lacks that

homolog. For Xenopus laevis AL1, S and L indicate short and long isoforms, respectively. For Caenorhabditis elegans B1 homologs F47G4.4 and F47G4.5 are WormBase IDs and a UniProt ID was used for MEI-2.

Katanins first discovered functions during meiosis placed them into the meiotic clade of AAA+ proteins, which includes microtubule-severing enzymes such as fidgetin and spastin, as well as VPS4, an ESCRT disassembly and membrane remodeling protein (Monroe and Hill, 2016). Outside of the AAA+ domain, KATNA1 and KATNAL1 are nearly identical, sharing the N-terminal microtubule interacting and trafficking (MIT) and C-terminal VPS4_C domains, which are both absent in KATNAL2. The importance of the VPS4_C domain to KATNA1 and KATNAL1 in microtubule severing has yet to be assessed; however, eukaryotic proteins with VPS4_C domains exhibit roles in mediating membrane remodeling/fission and protein degradation (McCullough et al., 2018). In addition to binding to the microtubule lattice, the MIT domain of KATNA1 and KATNAL1 mediates their binding to KATNB1 (Hartman and Vale, 1999; McNally et al., 2000; Cheung et al., 2016). Interestingly in KATNAL2, the MIT domain of KATNA1 and KATNAL1 is replaced by a lissencephaly homology (LisH) domain (Figure 1; Cheung et al., 2016; Ververis et al., 2016). In the ciliate Tetrahymena thermophila, the LisH domain of the katanin homolog Kat2 is important for its stability, self-dimerization, and localization to the basal body and ciliary axoneme (Joachimiak et al., 2020). Although the functional importance of the KATNAL2 LisH domain has yet to be determined in vertebrates, it is likely to regulate KATNAL2 localization, stabilization, self-association, and association with other proteins.

The archetypal regulatory katanin B-subunit contains an N-terminal WD40 domain followed by a proline-rich region and a conserved C-terminal domain (denoted con80 or KATNB1-CTD) (Figure 1). The WD40 domain directs A-subunit localization to the spindle poles of the cell (McNally et al., 2000). The proline-rich region of KATNB1 has yet to be assessed; however, eukaryotic proteins with proline-rich regions have roles in transcription, cytoskeletal rearrangements, and intracellular signaling (Kay et al., 2000). The con80 domain of KATNB1 is required for binding to the A-subunit and regulation of its microtubule-severing activity (Hartman et al., 1998; McNally et al., 2000). Recent co-immunoprecipitation studies in HEK293 cells and in elongating spermatids revealed that KATNAL2 was capable of interacting with KATNB1 (Dunleavy et al., 2017). This interaction is intriguing as previous studies in HeLa cells failed to detect an interaction between KATNAL2 and KATNB1 (Cheung et al., 2016), suggesting that this interaction may be cell type specific. How this KATNAL2–KATNB1 interaction is mediated is still unknown, as KATNAL2 lacks the canonical MIT domain. It is possible that this association is mediated through the KATNAL2 LisH domain in the same manner that the KATNA1 MIT and KATNB1 con80 domains interact, as LisH replaces the MIT (McNally et al., 2000). Alternatively, the KATNAL2 LisH domain may interact with the WD40 domain of KATNB1, as WD40 domains can mediate protein–protein interactions (Xu and Min, 2011). In support of this idea, previous studies with LisH and WD40 domain containing proteins showed that the LisH–WD40 interaction promoted oligomerization (Choi et al., 2008). In this regard, the KATNAL2 LisH domain may function to facilitate higher-order oligomerization with the katanin B-subunit. Therefore, defining the nature of the KATNAL2–KATNB1 interaction will inform on the ability of KATNB1 to modulate

KATNAL2 activity and/or affect its localization. Interestingly, KATNBL1 lacks the N-terminal WD40 and proline-rich domains found in KATNB1 and only maintains the C-terminal con80 domain, which is necessary and sufficient for binding to and regulating KATNA1 and KATNAL1 microtubule-severing activity (Figure 1; Cheung et al., 2016).

Katanin Higher-Order Structures

Homohexameric KATNA1 was first observed in the sea urchin Strongylocentrotus purpuratus by electron microscopy (EM), where it displayed ring-like structures 14–16 nm in diameter (Hartman et al., 1998). Hexamerization occurs via the AAA+ domain, and further characterization of KATNA1 led to the discovery that oligomerization stimulates catalytic activity and increases microtubule affinity (Hartman and Vale, 1999). Subsequent studies using X-ray diffraction, solution small-angle X-ray scattering, and cryo-EM structures of full-length C. elegans, human KATNA1, and human KATNAL1 have confirmed their homohexameric assembly (Zehr et al., 2017, 2020; Nithianantham et al., 2018). Stable KATNA1 oligomerization occurs in an ATP- and microtubule-dependent manner. Here, the microtubule acts as a scaffold that promotes interactions necessary for higher-order assembly, while binding to ATP is proposed to enhance hexamer stability (Hartman and Vale, 1999). Recent studies have shown that oligomerization is also dependent upon KATNA1 concentration: at low levels (< 24 μM) KATNA1 is largely monomeric; however, at increased concentrations (> 25 μM and higher), hexamerization is observed (Zehr et al., 2017; Shin et al., 2019). Although these structural studies have advanced our understanding of the structure and function of KATNA1 and KATNAL1, especially the critical and conserved AAA+ domain, there are currently no structures available for KATNAL2. The KATNAL2 AAA+ domain shares about 50% identity with that

of KATNA1 and KATNAL1; therefore, determining its structure will inform on the functional conservation or divergence of this poorly characterized A-like subunit.

In addition to the available homohexameric structures, various structures of the heterodimeric katanin A–B complex have been solved and have defined the KATNA1:MIT and KATNB1:CTD (aka con80) interaction. For example, the mouse KATNA1-MIT:KATNB1-CTD structure (PDB ID: 5NBT) showed the formation of a tight heterodimeric complex relying on interactions between residues R516 and Y519 of the KATNB1-CTD with S75 and K77 of the KATNA1-MIT, respectively (Rezabkova et al., 2017). When overexpressed in cells, the KATNA1-MIT:KATNB1-CTD heterodimeric complex decorates microtubule ends, causing bending and breakage; disrupting the formation of this complex prevents KATNB1-CTD from recruiting KATNA1-MIT to microtubules (Rezabkova et al., 2017). Another mouse-derived KATNA1-MIT:KATNB1-CTD structure (PDB: 6GZC) revealed the formation of a heterotetramer (dimer of heterodimers) (Faltova et al., 2019). Here, heterotetramer formation was shown to limit the accessibility of key residues required for microtubule end-binding compared with the heterodimeric complex. As a heterotetramer KATNA1-MIT:KATNB1-CTD lacks the ability to bind to microtubule plus ends; however, when tested on stable microtubules, its lattice binding and microtubule-severing activities were enhanced upwards of 17-fold compared with heterodimeric katanin (Faltova et al., 2019). These studies suggest that katanin can exist in multiple conformations within the cell and that these conformations are reflective of katanin's multiple functions. Lastly, despite the increased progress on solving various heterodimeric structures of the KATNA1-MIT:KATNB1-CTD, a structure of the katanin holoenzyme in complex with the microtubule has not been resolved, nor has any single

subunit been observed in complex with a microtubule. These structures could further inform on katanin assembly and function on the lattice and at other microtubule regions, such as the plus and minus ends.

Katanin Function in Cycling Cells

The mammalian cell cycle is composed of interphase (G1, S, and G2 phases), where a cell grows and duplicates its DNA, and mitosis (M phase), where a cell equally segregates its DNA into two daughter cells (Wenzel and Singh, 2018). During interphase, cytoplasmic microtubules extend radially from the centrosome, an organelle that acts as the microtubule organizing center, which is composed of two microtubule-based centrioles surrounded by a matrix of proteins (pericentriolar matrix) (Cooper, 2000; Goodson and Jonasson, 2018). The microtubule cytoskeleton network is essential for shaping the cell, intracellular trafficking, and cell motility (Fletcher and Mullins, 2010). The katanins are critical for regulating the length and dynamics of cytoskeletal microtubules through their microtubule-severing activity, and katanin dysregulation can lead to errors in cell shape, cell migration, cell cycle progression, and cell proliferation (Buster et al., 2002; O'Donnell et al., 2012; Smith et al., 2012; Lombino et al., 2019). KATNA1, KATNAL1, KATNAL2, and KATNB1 localize diffusely to cytoplasmic microtubules and concentrate at the centrosomes (Hartman et al., 1998; McNally and Thomas, 1998; Cheung et al., 2016; Ververis et al., 2016; Willsey et al., 2018). In contrast, KATNBL1 localizes to the nucleus in interphase via an N-terminal nuclear localization signal (Cheung et al., 2016), which suggests that KATNBL1 may have important roles independent of its microtubule-severing activity and/or that its sequestration in the nucleus is a regulatory mechanism to keep cytoplasmic microtubule severing in balance. In support of this idea, overexpression

of KATNA1 or KATNAL1 increases cytoplasmic microtubule severing (Figure 2A; McNally et al., 2000; Sonbuchner et al., 2010), and KATNBL1 has been shown to modulate KATNA1 and KATNAL1 activity in vitro (Cheung et al., 2016), similar to the observed regulation by KATNB1 (McNally et al., 2000). However, KATNBL1-based regulation is concentration dependent: enhancing activity at low concentrations while inhibiting activity at increasing or equimolar concentrations (Cheung et al., 2016).

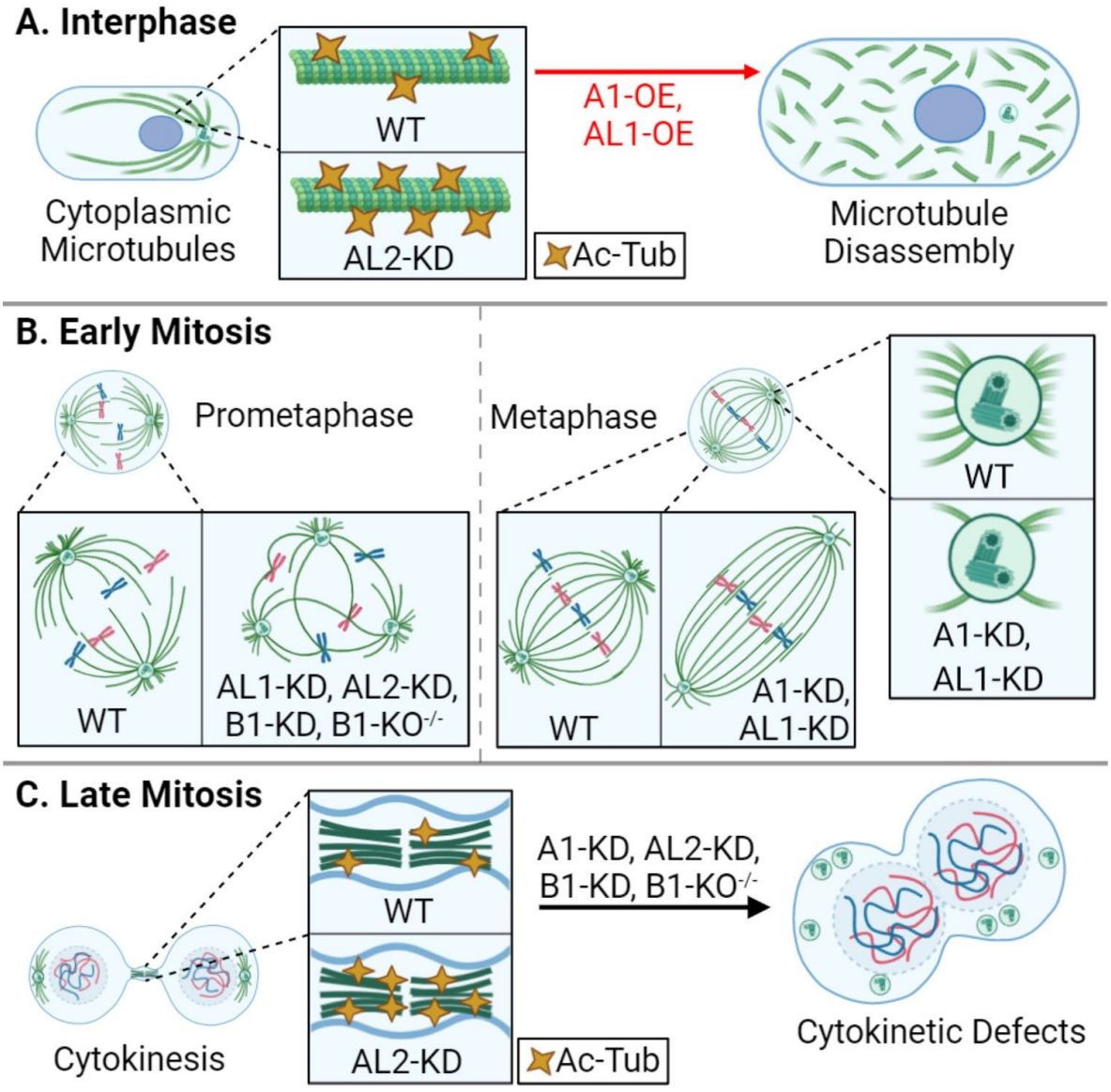

FIGURE 2 | Katanin function in the cell cycle. During the cell division cycle, the katanins are important for cytoplasmic microtubule rearrangements, mitotic spindle assembly, and proper cytokinesis. (A) In interphase, overexpression (OE) of A1 or AL1 leads to rapid disassembly of the microtubule lattice, and AL2 knockdown (KD) leads to hyper-acetylated tubulin (Ac-Tub). (B) In early mitosis (prometaphase), KD of AL1, AL2, or B1, or homozygous knockout of B1 (B1-KO–/–) leads to multipolar spindles; KD of A1 or AL1 leads to an elongated spindle; KD of A1 or AL1 also leads to a reduction of aster microtubule density at the spindle poles. (C) In late mitosis, KD of A1, AL2, or B1, or homozygous knockout of B1 (B1-KO–/–) leads to cytokinesis failure, enlarged cells with large nuclei, and supernumerary centrioles. KD of AL2 also leads to hyper-Ac-Tub at intercellular bridge microtubules.

In addition to their effect on cytoplasmic microtubules, modulating the expression of the katanin A-subunits also influences cell cycle progression. For example, cells depleted of KATNA1 or KATNAL2 accumulate in G2/M phase with enlarged cytoplasmic volumes (Matsuo et al., 2013; Ververis et al., 2016). KATNAL2 depletion also leads to an increase in acetylated microtubules, which is a hallmark of microtubule stability (Figure 2A; Matsuo et al., 2013; Ververis et al., 2016). The G2/M checkpoint is critical for ensuring that DNA and cellular damage is repaired prior to mitotic entry (Chao et al., 2017). Furthermore, the G2–M transition marks the end of cell growth and the beginning phase of mitosis (prophase). This transition is marked by an acute decrease in microtubule polymer and an increase in microtubule dynamics to initiate the necessary structural changes, for example, the breakdown of the nuclear envelope (Zhai et al., 1996). Therefore, it is possible that the perturbation of microtubule dynamics through a decrease in microtubule

severing and/or an increase microtubule stabilization via acetylation, seen upon depletion of KATNA1 or KATNAL2, are sensed as damage by the G2/M checkpoint.

The active process of cell division involves mitosis, where a cell's DNA is equally distributed to two nascent daughter cells, and cytokinesis, where the cytoplasm is bisected to generate two distinct daughter cells (Poon, 2016). During mitosis, katanins are primarily responsible for regulating the size and shape of the mitotic spindle through their functions at the centrosome (McNally and Thomas, 1998) and spindle (Loughlin et al., 2011). At the centrosome, KATNA1 recruits γ-tubulin, which is required for nucleating microtubules and generating microtubule density; inhibition of KATNA1 significantly reduces spindle density in prometaphase, concomitant with the loss of γ-tubulin (Figure 2B; Buster et al., 2002). In X. laevis and Xenopus tropicalis, KATNA1 regulates the length of mitotic and meiotic spindles as well as the length of k-fibers, which attach to chromosomes (Loughlin et al., 2011). Depletion of KATNA1 or KATNAL1 leads to similar spindle defects, including reduced spindle pole density and an increase in spindle length (Figure 2B; Sonbuchner et al., 2010). In contrast to KATNA1 and KATNAL1, KATNAL2 is more abundant along the mitotic spindle (Cheung et al., 2016; Willsey et al., 2018). Despite slight differences in localization, knockdown of KATNAL2 in mammalian cells phenocopies that of KATNAL1 and KATNB1, leading to the increased production of multipolar spindles (Figure 2B; Hu et al., 2014; Ververis et al., 2016; Gao et al., 2019). The different domain composition of KATNAL2 compared with KATNA1 and KATNAL1 suggests that KATNAL2 localization and effect on the mitotic spindle may be driven by other factors, which could include LisH-mediated protein–protein interactions.

As mitosis progresses, the katanins are dynamically redistributed from the spindle and spindle poles to other microtubule-based structures. For example, during telophase, KATNA1 localizes to the gap between the contractile ring and central spindle bundle, and at microtubules flanking the midbody; this distribution is independent of the B-subunit (Matsuo et al., 2013). Furthermore, KATNA1 knockdown leads to cytokinesis failure and an increase in binucleate cells (Figure 2C; Matsuo et al., 2013). While proteins like ASPM can complex with KATNA1 and KATNB1 to regulate microtubule-severing activity at the spindle poles and on the microtubule lattice (Jiang et al., 2017), little is known about the protein interactions that regulate KATNAL1 and KATNAL2. For example, KATNAL2 localizes to the midbody during cytokinesis, and its depletion leads to an increase in microtubule acetylation at the midbody, as well as the production of chromosome bridges, multinucleated cells, and apoptosis (Figure 2C; Ververis et al., 2016; Willsey et al., 2018), but little is known about how this occurs. KATNB1 also has a dynamic cell cycle phase-dependent subcellular localization, localizing to the cytoplasm and nucleus during interphase, the spindle midzone in anaphase, and to sister chromatids in cytokinesis (Suko and Maru, 2007; Jin et al., 2017). The variable and dynamic localization of katanin A- and B-subunits in the later stages of mitosis highlight a potential for subunit-specific functions. Similarly, variable A- and B-subunit localization has been observed in ciliates such as Tetrahymena, which may be explained by differences in A–B-subunit expression (Waclawek et al., 2017). It is also possible that changes in localization are driven by KATNB1-specific interactions; for example, in mammals, KATNB1 binds to Lis1, a protein known to localize to the kinetochore microtubules, which does not interact with KATNA1

(Toyo-Oka et al., 2005). Alternatively, the differences in localization of the katanin A- and B-subunits could be due to improperly validated reagents, such as the antibodies used.

Outside of their mitotic functions, katanins are also critical for meiotic cell divisions. During C. elegans meiosis I, the katanin A-subunit (MEI-1) and B-subunit (MEI-2) are critical for spindle organization, assembly, and shortening (Srayko et al., 2000, 2006; McNally et al., 2006). Interestingly, unlike the A-subunits of sea urchins and vertebrates, which can sever microtubules independent of the B-subunit, the activity of C. elegans MEI-1 is contingent upon MEI-2 (Hartman et al., 1998; McNally et al., 2006; Joly et al., 2016). During meiosis II, the MEI-1/MEI-2 complex is essential for central spindle disassembly, where microtubules between polar bodies and the female pronucleus are severed (McNally et al., 2006; Gomes et al., 2013). Prior to fertilization, C. elegans oocytes arrest in meiosis; fertilization triggers the rapid completion of meiosis and the transition to mitotic divisions (DeRenzo and Seydoux, 2004). During this oocyte-to-embryo transition, elimination of MEI-1 and MEI-2 is required to progress from the last meiotic division to the first mitotic division; failure to do so results in spindle defects and embryonic lethality (Srayko et al., 2000; Lu and Mains, 2007). In mouse spermatocytes, mutations that render KATNB1 dysfunctional promote stalls in anaphase I and an increase in the frequency of binucleate cells (O'Donnell et al., 2012). In mouse oocytes, KATNAL1 is essential for ensuring spindle pole integrity during meiosis I and II; modulating KATNAL1 influences oocyte maturation and fertility (Gao et al., 2019). However, the roles (if any) of the other mammalian A/B katanin subunits in meiosis remain to be determined. The varied spatial and functional requirement of specific katanin subunits for regulating diverse microtubule-

based structures in specialized settings highlights their capabilities for sub-specializations during mitotic and meiotic cell divisions.

Katanin in Ciliary Homeostasis and Development

Cilia are microtubule-based organelles that protrude from most mammalian cells and are either non-motile (primary) or motile (Ishikawa and Marshall, 2011; Malicki and Johnson, 2017). The assembly of primary cilia initiates in G1 phase, and growth continues as cells exit into G0 (Malicki and Johnson, 2017). G0 encompasses a phase of the cell cycle where cells are not actively dividing (quiescent) but have the potential to re-enter division; this phase is also used to describe cells that have become terminally differentiated (TD), such as neurons (Schafer, 1998). During cell cycle re-entry, primary cilia resorption occurs before the G1/S transition, although some cells exhibit cilia of minimal length during S phase (Plotnikova et al., 2009). Primary cilia are essential for signaling processes like chemosensation, osmosensation, and phototransduction (Waters and Beales, 2011; Figure 3A). Motile cilia, on the other hand, are important during mammalian development to establish unidirectional flow of extraembryonic fluid, which is subsequently required for left–right patterning (Hirokawa et al., 2009). Furthermore, specific populations of cells in the lungs, oviducts, and brain ventricles are multiciliated and can form up to 300 motile cilia (Meunier and Azimzadeh, 2016). Due to the importance of cilia during development and in organismal homeostasis, ciliary dysfunction is associated with a broad array of diseases that affect development, reproduction, and organ function, which are collectively known as ciliopathies (Waters and Beales, 2011).

The function of katanin on the cilia has been widely studied using unicellular eukaryotes; for example, research investigating Tetrahymena and Chlamydomonas A- and B-subunit

homologs has informed on katanin activity and localization on ciliary structures (Waclawek et al., 2017; Joachimiak et al., 2020), as well as the biogenesis of motile cilia (Sharma et al., 2007). Several recent studies have implicated katanin in the assembly, disassembly, and function of cilia in vertebrates (Hu et al., 2014; Willsey et al., 2018; Mirvis et al., 2019). For example, in mouse kidney cells, KATNA1 localizes to the base of the primary cilium, and its overexpression induces both rapid and gradual loss of cilia, but rapid deciliation is favored (Figure 3B; Mirvis et al., 2019). These findings are intriguing as Tetrahymena does not require the A- or A-like subunits for stress-induced deciliation compared with vertebrates (Sharma et al., 2007), while Chlamydomonas requires the katanin A-subunit to induce deciliation, specifically severing the axoneme (Lohret et al., 1998), suggesting that the katanins in vertebrate deciliation may be more similar to that of Chlamydomonas.

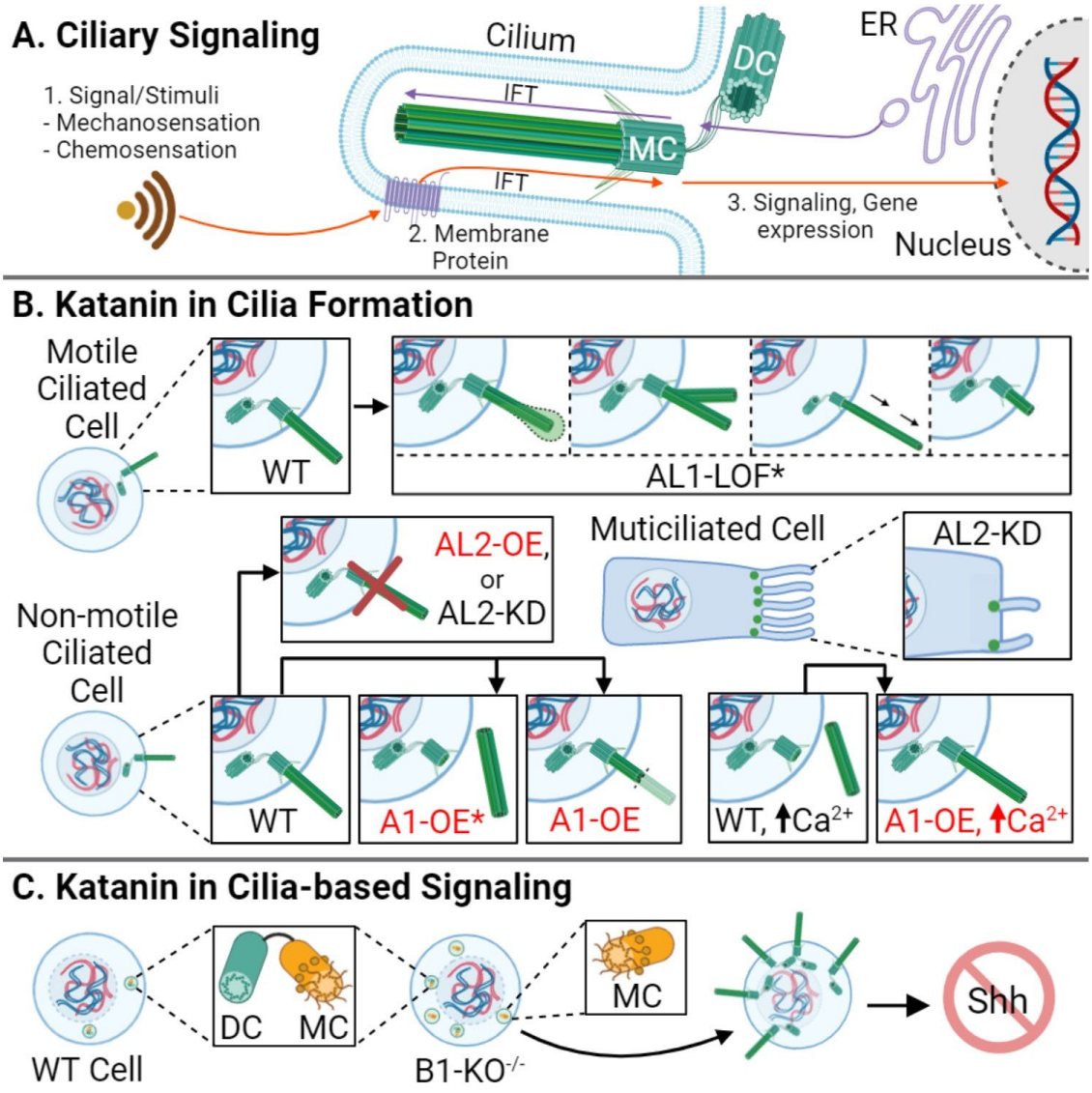

FIGURE 3 | Katanin function in ciliogenesis and cilia resorption. (A) Cilia are important microtubule-based organelles important for cell signaling during development.(B) In motile cilia, AL1-LOF* (asterisk denoting the mutation AL11H/1H) causes a variety of defects, including swelling at the ciliary tip, ciliary bifurcation, elongation, and shortening, causing defective ciliary movement. In non-motile ciliated cells, A1 overexpression (OE) promotes cilia disassembly, with rapid deciliation as the favored method (indicated by asterisk). In cells with high levels of Ca2+, which signals

deciliation, A1-OE prevents the loss of cilia. Knockdown or overexpression of AL2 in non-motile cilia causes a reduction in ciliogenesis, while in multiciliated cells, AL2 KD reduces cilia number and reduces cilia length. KD of AL2 to levels below 50% or AL2 OE triggers apoptosis (not shown). (C) Homozygous knockout of B1 (B1-KO–/–) leads to the generation of supernumerary centrioles, excess mother centrioles, aberrant ciliation, and disrupted cellular signaling, including sonic hedgehog (Shh) signaling. DC indicates daughter centriole, and MC indicates mother centriole.

In comparison with KATNA1, KATNAL2 localizes to the ciliary axoneme, basal body, and daughter centriole; furthermore, its knockdown leads to a 50% reduction in ciliated cells in mice (Ververis et al., 2016). In the multiciliated embryonic epithelial cells of X. tropicalis, KATNAL2 is required for ciliogenesis, and knockdown results in ciliary shortening and a reduction of cilia number (Figure 3B; Willsey et al., 2018). In motile cilia, such as those observed in mouse ependymal cells, KATNAL1 is important in cilia maintenance and function. For example, a KATNAL1 loss-of-function (LOF) mutation within the AAA+ domain (L286V, designated KATNAL11H/1H) leads to cilia bifurcation, bending, increases and decreases in ciliary length, and swollen ciliary tips (Figure 3B), abnormalities that affect beat frequency (Banks et al., 2018). These studies indicate that the A-subunits are critical players in ciliogenesis and cilia maintenance and function. However, the role of the B-subunits in these ciliary processes remains to be explored further among vertebrates; such exploration should inform on what katanin A–B complexes are the most important within a particular ciliary context.

The Hedgehog (Hh) signaling pathway is critical for embryo axial body patterning, limb patterning, and organogenesis (Corbit et al., 2005; Komiya and Habas, 2008; Jia et al.,

2015; Bangs and Anderson, 2017). Hh signaling is reliant on functional cilia to signal key cellular events like differentiation, growth, and tissue patterning (Huangfu and Anderson, 2005; Nikonova and Golemis, 2015). For example, Hh proteins interact with membrane receptors on the primary cilia such as Patched1 (PTCH1) or Patched 2 (PTCH2), which trigger an accumulation of downstream activating proteins like Smoothened (SMO) that recruits GLI family zinc finger 1 (GLI1) (Raleigh and Reiter, 2019) and influence gene expression programs (Kalderon, 2002). Due to the importance of katanin in ciliogenesis and ciliary maintenance, katanin A- and B-subunits have been implicated in a variety of cilia-dependent signaling pathways including Hh (Hu et al., 2014), Wnt (Willsey et al., 2018), and left–right signaling (Furtado et al., 2017). For example, the complete loss of KATNB1 (KATNB1–/–) in mouse embryonic fibroblasts (MEFs) leads to centriole overduplication, an increased presence of mother centrioles, aberrant ciliogenesis, and defective Hh signaling (Hu et al., 2014; Figure 3C). These ciliary perturbations result in reduced expression of downstream sonic hedgehog (Shh) pathway targets GLI1 and Patched; KATNB1–/– mice undergo lethality by embryonic day 15.5 (E15.5), which is the final week of prenatal growth where cardiovascular, palate, and musculoskeletal development occur (Hu et al., 2014; Mishra-Gorur et al., 2015). This outcome is phenotypically similar to that of mice lacking C2CD3, a gene encoding a regulatory Hh protein required for GLI3 processing during embryonic development (Hoover et al., 2008). Although it is apparent that katanin's role in ciliary maintenance affects signaling, it remains unknown whether katanin loss/defects influences intracellular crosstalk between signaling pathways, for example, crosstalk between Wnt and Hh. Similar to their roles in Hh developmental pathways, the katanins are also important for the Wnt/Planar cell

polarity (PCP) pathway. For example, KATNAL2 is enriched in multiciliated cells of the developing brain and organs in X. laevis; here, LOF or depletion of KATNAL2 disrupts ciliogenesis and cilia maintenance and promotes defects in neural crest migration, blastopore closure, and the disorganization of apical actin (Willsey et al., 2018). Further studies are required to determine if the associated outcomes are due to KATNAL2 dysfunction in ciliary homeostasis/maintenance, the effect of KATNAL2 loss on the Wnt/PCP pathway independent of its role on the cilium, or both. KATNB1 is also ubiquitously expressed throughout embryonic development, with a strong presence in the node pit and crown cells of the developing embryo (Furtado et al., 2017). Upon homozygous loss (KATNB1–/–), mice display left–right cardiac malformations (atrial and ventricular septal defects, heterotaxy, and axial distortions) during embryonic development (Furtado et al., 2017). The KATNB1-specific cardiac anomalies were phenotypically similar to those observed upon the loss of Pixt2, which is a critical transcription factor in left–right and Wnt signaling pathways (Chinchilla et al., 2011). Intriguingly, the Wnt pathway has been demonstrated to regulate LR patterning in the node of mouse embryos (Kitajima et al., 2013), which suggests that the observed KATNB1 cardiac abnormalities may be more related to the Wnt pathway. Whether KATNB1, KATNBL1, or the katanin A-subunits have direct roles in regulating Wnt signaling and LR patterning, or if their effect on these pathways are a result of their function in ciliogenesis, remains to be determined.

Katanin Dysfunction in Corticogenesis

Mature TD cells are classified as post-mitotic, remain in G0, and can no longer divide; a classic example of TD cells is neural cells (Ahmad et al., 1999). During corticogenesis,

neurons migrate out of the germinal layers to distinct regions within the developing central nervous system (CNS) and become differentiated; neural migration is critical to CNS development, as it provides a mean for cells to interact spatially and reach their final destinations (Figure 4A; Rahimi-Balaei et al., 2018). Katanin expression correlates with axonal development; here, KATNA1 levels increase during axon growth and drop when the axons reach their target or when growth ceases (Karabay et al., 2004). In neurons, katanins have been implicated in generating microtubule fragments, which are trafficked to the cell's leading edge and neural branches (Ahmad et al., 1999). This function could explain katanins' varied contributions to neural development, including proliferation (Lombino et al., 2019), migration (Mishra-Gorur et al., 2015), and process elongation (Hatakeyama and Hayashi, 2018). Perturbation of katanin expression levels disrupts these processes. For example, overexpression of KATNA1 in rat neurons inhibits neural migration (Figure 4A), generates defective nucleus–centrosome coupling (Figure 4B), and reduces axon growth (Figure 4B; Toyo-Oka et al., 2005). Similarly, overexpression of dominant-negative KATNA1 leads to a reduction in process elongation (Toyo-Oka et al., 2005; Figure 4B). In contrast to what is observed with KATNA1 overexpression, KATNAL1 knockdown enhances process elongation in Neuro2a cells (Hatakeyama and Hayashi, 2018). Furthermore, a LOF mutation that renders KATNAL1 inactive in mice (KATNAL11H/1H) leads to an increase in neural body (soma) size and shortened thin axons with few dendritic spines (Figure 4B; Banks et al., 2018). Intriguingly, in mice neuronal cells, KATNAL1 is expressed at higher levels and is more stable compared with KATNA1 (Hatakeyama and Hayashi, 2018). Together, these studies suggest that both

KATNA1 and KATNAL1 severing activities are important for corticogenesis and neuronal maintenance.

KATNB1 is also highly expressed in neurons (Mishra-Gorur et al., 2015). KATNB1 depletion studies in MEFs and samples derived from patients who harbor mutations that reduce KATNB1 expression showed impaired neuronal migration, reduced neural progenitors, and a reduction of neurons at the cortical plate (Figure 4A; Hu et al., 2014; Mishra-Gorur et al., 2015; Jin et al., 2017). Intriguingly, KATNB1 overexpression in rats increases the number of branches that extend from the neuron, which is in opposition to what is observed when KATNA1 is overexpressed; KATNB1 overexpression instead leads to similar outcomes observed when KATNAL1 is overexpressed (Figure 4B; Yu et al., 2005). This suggests that KATNB1 may preferentially pair with KATNAL1 in neurons, which remains to be tested.

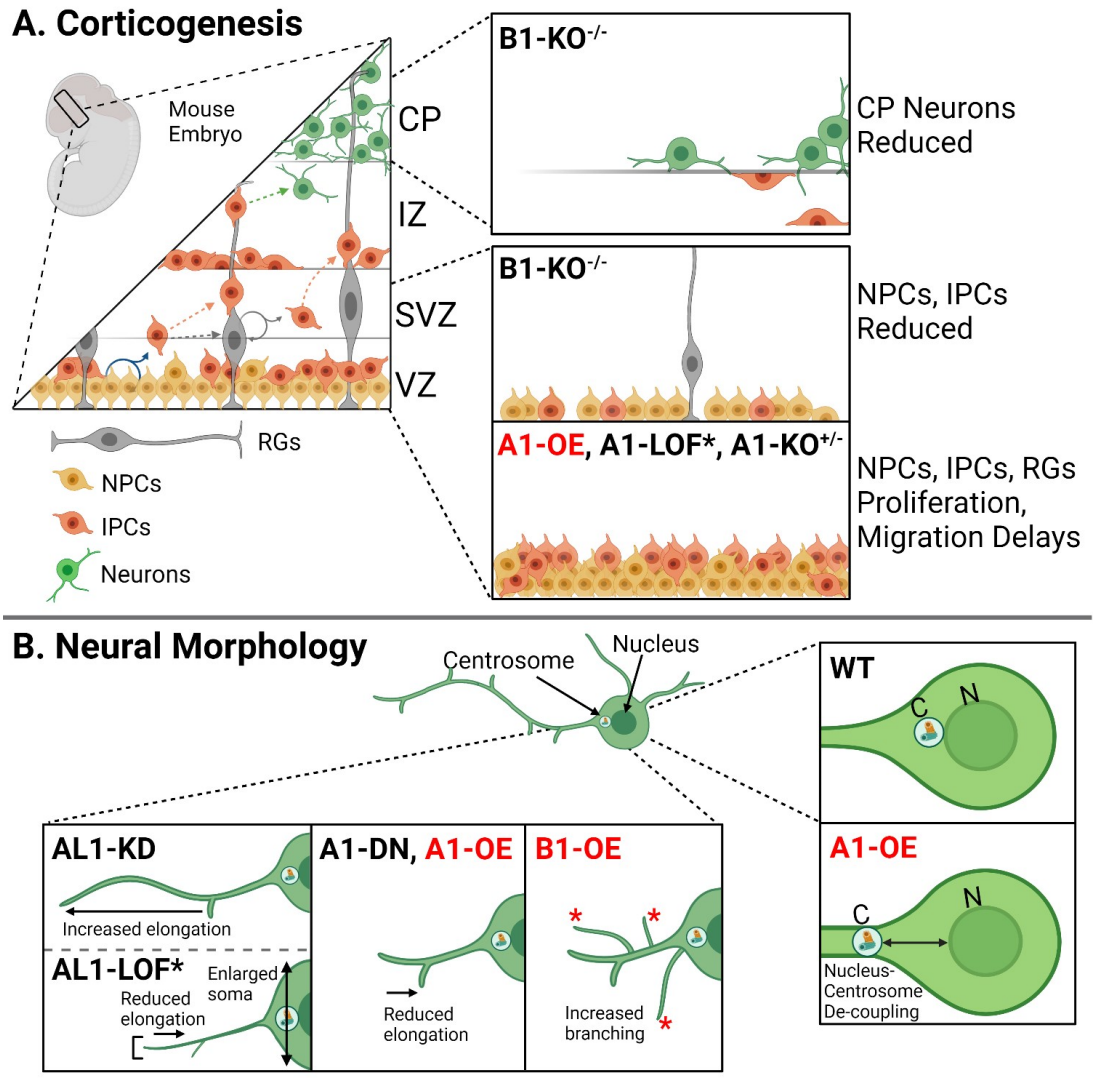

FIGURE 4 | Katanin function during corticogenesis and in neural morphology. (A) During mouse corticogenesis, the katanins regulate asymmetrical proliferation, cellular migration, and neuron development. Dysregulation of katanin subunit levels by heterozygous knockout (KO+/−), homozygous knockout (KO–/–), loss of function (LOF), and/or overexpression (OE) leads to disruptions at different cortical layers (VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; MZ, marginal zone). The affected cells include neural progenitor cells (NPCs), intermediate progenitor cells (IPCs), radial glia, and developed neurons.(B) Katanin A1, AL1, and B1 are involved

in neural morphology, contributing to process elongation and branching. Knockdown of AL1 enhances process length (process elongation), while AL1 loss of function by means of a recessive mutation AL11H/1H in mice (indicated by asterisk; see Figure 3 for this mutant) contributes to shorter and thinned axons, reduced process elongation, and enlarged soma. Dominant negative A1 leads to reduced process elongation as does A1 overexpression. Additionally, A1 overexpression increases the distance in nucleus–centrosome coupling in neurons. Lastly, B1 overexpression promotes increased branching in neurons.

Katanin Dysfunction in Neurodevelopment

Neurodevelopmental disorders (NDDs) consist of several conditions that often co-occur to impair an individual's personal, social, and academic functions; these disorders include autism spectrum disorder (ASD), intellectual disabilities (IDs), attention deficit/hyperactivity disorder (ADHD), and communication disorders (Morris-Rosendahl and Crocq, 2020). In humans, haploinsufficiency of KATNAL1 has been correlated with disorders like ID and microcephaly (O'Roak et al., 2012; Bartholdi et al., 2014). Furthermore, KATNAL1 and KATNAL2 were both recently identified as candidate genes in ID and ASD, respectively (O'Roak et al., 2012; Bartholdi et al., 2014; Stessman et al., 2017). In mice, homozygous loss of KATNA1 (KATNAL1–/–) compromises neural migration and ventricle size, resulting in ID, impaired learning, memory, and vocalization (Banks et al., 2018). In mice and zebrafish, mutagenesis of KATNB1 at exons 2 and 6, which leads to full deletion and/or the production of a dysfunctional N-terminal truncated protein, respectively, results in gastrulation and forebrain defects during development (anencephaly, microcephaly, and holoprosencephaly) (Hu et al., 2014). Unlike mice and

zebrafish, humans with homozygous KATNB1 mutations do not exhibit embryonic lethality (Bartholdi et al., 2014; Hu et al., 2014), suggesting that in humans, KATNB1 mutations may retain partial function or that KATNBL1 may compensate for dysfunctional KATNB1. Together, these studies indicate that the observed perturbations to embryonic development are reflective of the dominant katanin subunits used in a given species and suggest a reliance for specific katanin subunits in specific cell types and/or during specific stages of development.

Neurodegenerative disorders, like Alzheimer's Disease (AD), are characterized by changes in personality, impaired/decreased judgment, mood disturbances, and progressive dementia (decline in language, memory, and ability to perform basic functions) (Weller and Budson, 2018). Neuropathological hallmarks of AD include the presence of β-amyloid plaques and toxic neurofibrillary tangles (NFTs); in the neurons of AD patients, NFTs are primarily composed of the hyperphosphorylated protein Tau (Lane et al., 2018). Tau is a microtubule-associated protein responsible for stabilizing microtubules along the lattice and growing ends; phosphorylation reduces Tau's affinity for microtubules, and hyperphosphorylation is associated with neurodegenerative disease (Barbier et al., 2019). Tau-bound microtubules are protected from severing by katanin even in conditions where katanin is overexpressed, while microtubules that lack Tau demonstrate increased sensitivity to katanin-based severing (Qiang et al., 2006). This increased sensitivity to severing by katanin has been proposed as a basis for microtubule loss in tauopathies (Sudo and Baas, 2010, 2011). After Tau loss, the microtubule network rapidly disintegrates, likely as a result of katanin-mediated severing. Other microtubule-severing enzymes are also important for neural cell homeostasis and

function, such as Spastin, whose mutations lead to defects in axonal transport and degeneration, and diseases like hereditary spastic paraplegia (HSP) (Leo et al., 2017). Intriguingly, with respect to the protection of microtubules, Tau offers less protection for spastin microtubule severing when compared with katanin (Yu et al., 2008). One example of Tau-based katanin regulation is with KATNAL1, which is overexpressed in the absence of Tau in both MEFs and human mammary epithelial cells (HMECs) (Sudo, 2018). The overexpression of KATNAL1 in Tau's absence suggests that Tau may modulate katanin expression while also providing protection to microtubules.

The dysregulation of brainstem nuclei has previously been postulated as a primary mechanism for AD pathogenesis (Iatrou et al., 2017). In nuclei derived from the brainstem of AD patients, KATNB1 expression is reduced (Andrés-Benito et al., 2018). This same study showed interactions between KATNB1 and a protein encoded by KIAA0556, a gene implicated in AD pathology (Andrés-Benito et al., 2018). These nuclei often contain hyperphosphorylated Tau, which is less protective against katanin severing due to its diminished capacity to interact with microtubules (Brandt et al., 2005; Andrés-Benito et al., 2018). The combination of Tau hyperphosphorylation in conjunction with reduced levels of KATNB1 may therefore create an environment in the cell where the katanin A-subunits can sever microtubules uncontrollably. The mechanism linking KATNB1 dysregulation to AD disease pathology, however, has yet to be explored. Nonetheless, these findings support the idea that an imbalance between Tau and the katanin subunits could contribute to neurological disease progression.

Katanin Dysfunction in Gametogenesis

Gametogenesis is the process by which mature sex (germ) cells are formed (Figure 5A). For males, this occurs in two phases and involves the rapid remodeling of complex microtubule structures in meiosis and mitosis to form the spermatogonia and spermatocytes, respectively (Larose et al., 2019). Here, katanin is expressed at high levels and is important for germ cell production (O'Donnell et al., 2012; Pleuger et al., 2016). In Sertoli cells (SCs), testicular nurse cells that aid in spermatogenic development, KATNAL1 regulates microtubule dynamics involved in spermatid adhesion and release, and KATNAL1 LOF leads to male-specific sterility (Figure 5B; Smith et al., 2012; Hatakeyama and Hayashi, 2018). KATNAL2 is similarly involved in SC function (spermatid adhesion/release, acrosome attachment), in addition to directing morphology in spermiogenesis (head shape, tail growth) (Dunleavy et al., 2017). Like the effects of KATNAL1, KATNAL2 knockdown in SCs results in germ cell remodeling defects that lead to male sterility in mice (Dunleavy et al., 2017; Smith et al., 2017; Figure 5B). Of interest, previous studies using mice identified multiple KATNAL2 isoforms that were required for proper ciliogenesis (Ververis et al., 2016) and were expressed during different stages of spermatogenesis and spermiogenesis (Dunleavy et al., 2017). The presence of multiple KATNAL2 isoforms in mice with overlapping ciliary and spermiogenic roles suggests that there may be a need for specific KATNAL2 isoforms during differing stages of these processes.

KATNB1 is also essential for mammalian spermatogenesis (O'Donnell et al., 2012; Pleuger et al., 2016). In mice, KATNB1 functions in shaping the sperm head and developing the flagella during spermiogenesis. A missense mutation in the KATNB1

WD40 domain resulting in the conversion of V to F at position 234 (denoted KATNB1Taily/Taily) results in reduced protein levels and a loss of KATNB1 function (O'Donnell et al., 2012). Male KATNB1Taily/Taily mice are infertile, likely due to the production of abnormal meiotic spindles during spermatogenesis (elongated spindles and binucleate spermatids), as well as formation of defective cell structures during spermiogenesis (nuclear distortion, abnormally long microtubules, and axoneme defects in sperm tails) (Figure 5B; O'Donnell et al., 2012). In humans, KATNB1 exhibits variable expression during the different stages of gametogenesis and functions in spermatogonium spindle assembly and in shaping the manchette and flagellum during spermiogenesis (Pleuger et al., 2016). These findings indicate that the katanin holoenzyme is important for establishing male gamete architecture, and this architecture translates to proper function. As such, the katanin holoenzyme has the potential to serve as a novel target for research on male-specific infertility.

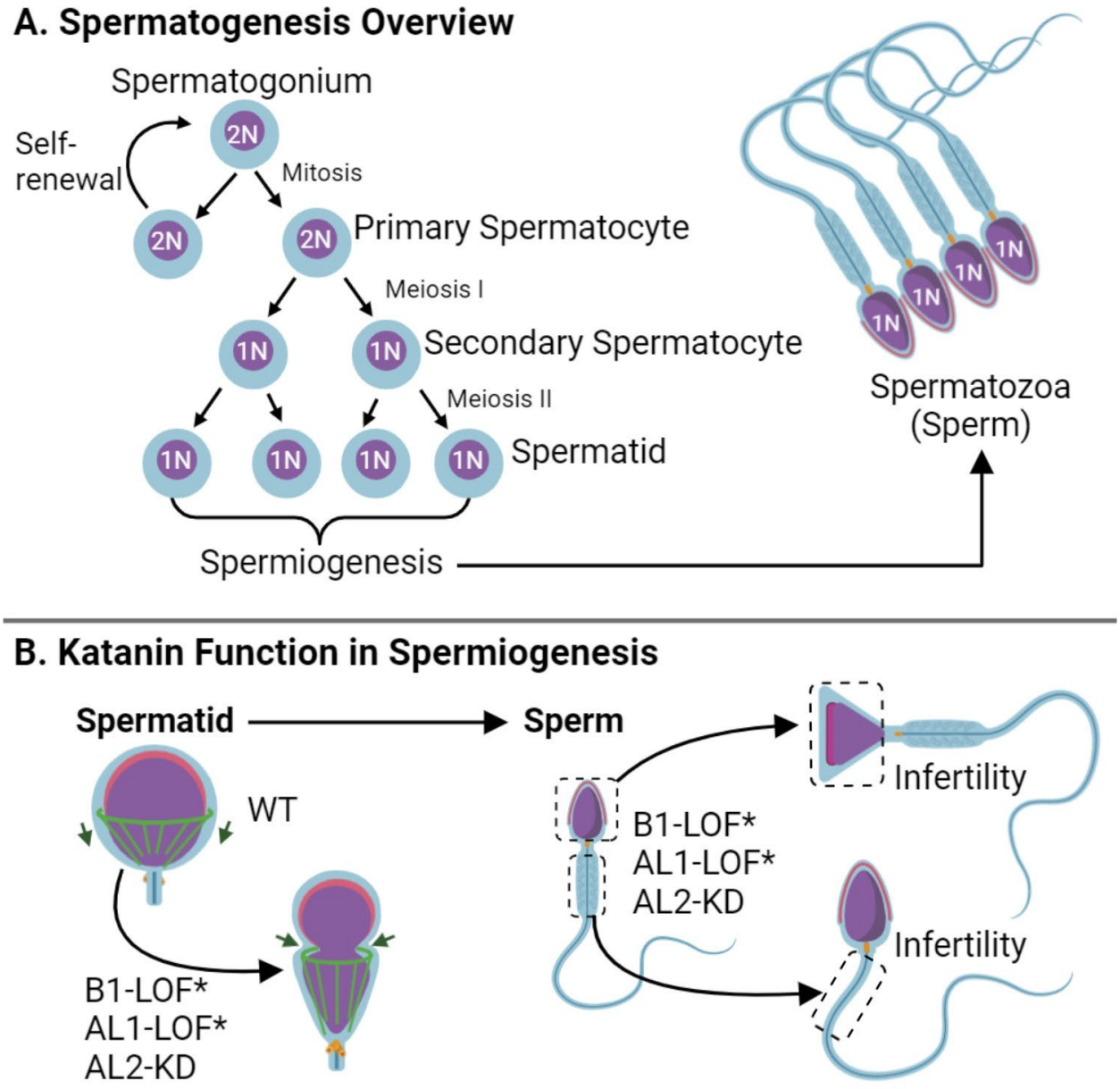

FIGURE 5 | Katanin function in gametogenesis. (A) Spermatogenesis overview. During spermatogenesis, the spermatogonium either experiences self-renewal or undergoes two rounds of meiosis to become a spermatid; spermiogenesis occurs after the second meiotic completion and consists of the shaping of the sperm head and tail. (B) During spermiogenesis, katanin B1 and AL2 subunits work in shaping the manchette (green) for purposes of head shaping and tail formation. Knockdown (KD) of either AL1

or AL2 as well as B1 loss of function (LOF) by means of the Taily mutation in mice (KATNB1Taily/Taily) leads to sperm head and tail shaping defects that cause infertility.

Katanin Dysfunction in Cancer

During carcinogenesis, the signaling pathways required during early development often become aberrantly re-activated, granting cells a proliferative advantage, favored survival, and invasive abilities (Fouad and Aanei, 2017; Nwabo Kamdje et al., 2017). The katanins are expressed during development and throughout life but are aberrantly expressed in multiple types of cancers (Ye et al., 2012; Li et al., 2018). It is well-established that katanin-based microtubule severing is important for proper spindle assembly during cell division and for microtubule rearrangements required for cell migration (McNally and Thomas, 1998; Zhang et al., 2007, 2011). The dysregulation of katanin correlates with errors in these processes and contribute to tumorigenesis and metastasis (Ye et al., 2012, 2020). For example, elevated KATNA1 expression is detectable in metastatic breast and prostate cancers and correlates with enhanced cell migration and reduced proliferation (Table 1; Ye et al., 2012; Fu et al., 2018). Consistently, ectopic KATNA1 overexpression in breast and prostate cancer cells leads to reduced proliferation and enhanced migration, while a reduction in endogenous KATNA1 expression in breast cancer cells enhances proliferation and decreases migration (Table 1; Ye et al., 2012; Fu et al., 2018). The human KATNAL1 mutant L123V identified in breast cancer cells resides in the flexible linker region at the N-terminus of the protein and promotes microtubule-severing activity even in the presence of Tau (Sudo and Nakajima, 2016). The excessive microtubule severing driven by KATNAL1-L123V leads to chromosome bridge formation during cell division and the subsequent formation of micronuclei and aneuploidy, which are

associated with breast carcinoma pathogenesis (Sudo and Nakajima, 2016). The elevated expression of KATNB1 shares a similar positive correlation with advanced breast cancer staging, lymph node metastasis, and reduced rates of overall survival in patients (Table 1; Li et al., 2018). Additionally, in non-small cell lung cancer (NSCLC), the elevated expression of KATNA1 and KATNB1 correlates with lymph node metastasis and advanced tumor progression (Table 1; Wang et al., 2020; Ye et al., 2020). Katanin expression is also dysregulated in other types of cancers like papillary thyroid carcinoma (PTC), where both KATNA1 and KATNB1 are highly expressed in tumors and tumor-adjacent tissues; furthermore, elevated katanin expression correlates with advanced PTC staging and worse disease-free survival in patients (Chen et al., 2020). The overexpression of katanin subunits in various cancers and findings that activating mutations in katanin contribute to cancer pathogenesis indicate that katanin dysregulation is an important step in tumorigenesis. As such, advancing our understanding of the connection between katanin holoenzyme dysregulation and cancer pathology could highlight the importance of the katanin enzymes as novel targets for the development of future cancer therapeutics.

Table 1. Katanin Involvement in Cancer Development

Katanin	Cancer Type	Information	Reference
KATNA1	NSCLC	Increased KATNA1 expression correlates with lymph node metastasis, advanced TNM stages	Wang et al, 2020; PMID: 32631334
	Breast Cancer	KATNA1 overexpression promotes cell migration, inhibits cell proliferation; silencing promotes proliferation, inhibits cell migration. KATNA1 expression is significantly increased in primary breast cancer tissue compared to non-cancerous tissue.	Fu et al., 2018; PMID: 29552132
	Prostate Cancer	Elevated KATNA1 expression enhances migratory capacity and inhibits cell proliferation. KATNA1 expression in metastatic cells was associated with the re-emergence of basal-cell like phenotype.	Ye et al., 2012; PMID: 21681775
	Papillary Thyroid Carcinoma (PTC)	KATNA1 is highly expressed in tumor and tumor-adjacent tissue. Elevated expression correlates with larger tumor size, extrathyroidal invasion and advanced cancer staging (pT-, pN-, and TNM-stages), as well as worse disease-free survival (DFS) in patients.	Chen et al., 2020; PMID: 33274499
KATNB1	NSCLC	Elevated KATNB1 expression is associated with larger tumor size, lymph node metastasis, advanced cancer staging (TNM), and decreased rate of disease-free (DFS) and overall patient (OS) survival.	Ye et al., 2020; PMID: 31944409
	Breast Cancer	Elevated KATNB1 expression positively correlates with lymph node metastasis, advanced cancer staging (pN-, TNM-stages), and reduced overall survival (OS).	Li et al., 2018; PMID: 30223388
	Papillary Thyroid Carcinoma (PTC)	KATNB1 is highly expressed in tumor and tumor-adjacent tissue. Elevated expression correlates with advanced cancer staging (pN-stage, TNM-stage), and worse disease-free survival (DFS) in patients.	Chen et al., 2020; PMID: 33274499

TNM is the clinical cancer staging: I, II, III, or IV. T = size, direct extent of tumor; N = degree of spread to lymph; M = presence of distant metastasis; pT or pN = pathological cancer staging.

TP53 is an important regulator of the cell cycle, proliferation, and apoptosis and is the most widely mutated cancer gene (Fouad and Aanei, 2017). Intriguingly, the TP53 DNA-binding domain (DBD), which functions as a sequence-specific transcription factor (Harms and Chen, 2006), was recently found to bind to the KATNA1 C-terminal region (Korulu and Yildiz, 2020). Furthermore, TP53 is an activator of KATNA1 gene expression. In mammalian HCT116 cells, TP53 was found to bind to the KATNA1 promoter, within the

–117 to –95 region, and to upregulate KATNA1 transcription (Kırımtay et al., 2020). Because TP53 is critical for regulating the cell cycle and proliferation, further analysis into the importance of the KATNA1–TP53 interaction will be paramount to understanding tumorigenesis.

Katanin Regulatory Mechanisms

As is the case with most proteins, katanin abundance, activity, and function is regulated at the transcriptional (Selçuk et al., 2013; Kelle et al., 2019) and posttranslational levels (Cummings et al., 2009; Loughlin et al., 2011; Whitehead et al., 2013). In addition to KATNA1 transcriptional regulation by TP53, KATNA1 and KATNB1 are differentially regulated by the Elk-1 transcription factor. Elk-1 belongs to a family of oncogene transcription factors that activate/repress genes involved in diverse processes like growth, survival, differentiation, proliferation, development, apoptosis, and cancer (Besnard et al., 2011). Elk-1 binds to the 5′ UTR of KATNA1 in a methylation-dependent manner, resulting in reduced KATNA1 expression; this mechanism of regulation is unique to katanin when compared with other severases (Kelle et al., 2019). In contrast, the binding of Elk-1 to the KATNB1 promoter (near but excluding the 5′ UTR) leads to an increase in KATNB1 mRNA and protein levels (Selçuk et al., 2013). The transcriptional regulation of other katanin subunits by Elk-1 has not been explored, and their analysis is likely to advance our understanding of katanin regulation in various developmental and disease contexts.

Protein posttranslational modifications can function as molecular switches to regulate protein localization, activity, interactions, and abundance (Lee and Yaffe, 2016). In particular, phosphorylation and ubiquitylation are important for regulating katanin localization, microtubule binding, microtubule-severing activity, and levels (Cummings et

al., 2009; Loughlin et al., 2011). For example, in humans, phosphorylation of KATNA1 at S42, S109, and T133 by the dual specificity tyrosine-regulated kinase 2 (DYRK2) targets KATNA1 for degradation via the DYRK2-EDD-DDB1/VPRBP (DYRK2-EDVP) E3 ubiquitin ligase complex (Maddika and Chen, 2009). These phosphorylation and ubiquitination events are required for proper cell cycle progression and mitotic transition (Table 2; Maddika and Chen, 2009).

Table 2. Post Translational Regulation of Katanin						
Katanin	**Organism**	**Modified Residue(s)**	**Kinase or Phosphatase**	**Ubiquitin Ligase or Ligase Adapter**	**Information**	**Reference**
KATNA1 (p60)	*H. sapiens*	S131	Aurora B Kinase*		Regulates KATNA1 activity at kinetochores	Advani et al, 2018; PMID: 30176123
KATNA1 (p60)	*H. sapiens*	S42, S109, T133	DYRK2		Regulates KATNA1 levels for cell cycle progression and mitotic function	Maddika et al., 2009; PMID: 19287380
KATNA1 (p60)	*X. laevis*	S131	Aurora B Kinase*		Inhibits KATNA1 in a concentration-dependent manner during mitosis by disrupting the ATPase cycle	Whitehead et al 2013; PMID: 23178168
KATNA1 (p60)	*X. laevis*	S131	Aurora B Kinase*		Regulates KATNA1 activity during scaling of mitotic spindle	Loughlin et al 2011;PMID: 22153081
MEI-1 (p60)	*C. elegans*	S90, S92, S113, S137	MBK-2		MEI-1 S92 phosphorylation promotes its binding to MEL-26, signals degradation by $CRL3^{MEL-26}$ after meiosis; single phosphorylation at S90, S92, S113, S137 renders MEI-1 insensitive to MTs	Joly et al, 2020; PMID:32412594
MEI-1 (p60)	*C. elegans*	S92	$PP4^{PPFR-1}$		Dephosphorylation by PP4 enhances MEI-1 activity during meiosis	Gomes et al, 2013; PMID: 23918937
MEI-1 (p60)	*C. elegans*	unknown	$PP4^{PPFR-1}$		PP4 phosphatase stimulates MEI-1 activity during meiosis	Xue Han et al 2009; PMID:19087961
MEI-1 (p60)	*C. elegans*	S92	MBK-2		Regulates MEI-1 abundance at meiotic exit by increasing affinity for $CRL3^{MEL-26}$	Stitzel et al, 2006; PMID:16338136
MEI-2 (p80)	*C. elegans*	T32, S68 (if MEI-1 is present)	MBK-2		not reported	Joly et al, 2020; PMID:32412594
MEI-1 (p60)	*C. elegans*	unknown		Cul3	COP9/signalosome is required for the degradation of MEI-1 after meiosis, likely through regulation of Cul3.	Pintard et al, 2003; PMID: 12781129 Kurtz et al, 2000; PMID: 11847342
MEI-1 (p60)	*C. elegans*	unknown		Cul2, RFL-1, Cul3, MEL-26	MEL-26 levels are kept low in meiosis by Cul2 and RFL-1. In meiosis, MEL-26 regulates MEI-1 activity/abundance; MEI-1regulation is essential for meiotic cell viability. Following meiosis, MEL-26 eliminates MEI-1 activity. Following meiosis, MEL-26 interacts with Cul3 and MEI-1 to control MEI-1 degradation, *in vitro* and *in vivo*.	F.A. Johnson, et al., 2009; PMID: 19361490 Pintard et al, 2003; PMID: 13679921

***Hypothesized kinase to act on katanin but has not been shown experimentally.**

In C. elegans, Minibrain kinase 2 (MBK-2) phosphorylates the KATNA1 homolog MEI-1 at multiple serines to inhibit its activity (Table 2). More specifically, MBK-2-mediated phosphorylation of MEI-1 at S92 is necessary and sufficient to target MEI-1 for

degradation during the oocyte-to-embryo transition (Table 2; Joly et al., 2020). MBK-2 can also phosphorylate the KATNB1 homolog MEI-2, but the significance of this modification has not been determined (Joly et al., 2020). The ubiquitin ligase adaptor MEL-26 functions in parallel to MBK-2 and is required for MEI-1 degradation (Lu and Mains, 2007). Here, MEL-26 interacts with Cul3 and MEI-1 to promote MEI-1 degradation after meiosis and is important in meiotic cell viability (Table 2; Johnson et al., 2009). In mammalian cells, the Cul3 ubiquitin ligase substrate adaptor Ctb9/KLHDC5 similarly targets KATNA1 for ubiquitin-mediated degradation, likely recruited in response to KATNA1 phosphorylation (Cummings et al., 2009). The exact mechanism of Ctb9/KLHDC5 substrate recognition is still unknown; however, this interaction is important for controlling the abundance of KATNA1 during mitosis and for promoting normal mitotic progression (Cummings et al., 2009).

Direct phosphorylation of KATNA1 also plays an important role in regulating microtubule-severing activity at the kinetochores (Loughlin et al., 2011; Whitehead et al., 2013). In X. laevis, phospho-inhibition of KATNA1 at the predicted Aurora B kinase consensus site S131 reduces microtubule-severing activity and leads to an increase in spindle length (Table 2); S131 is within a region that contains multiple predicted phosphorylation sites for mitotic kinases like Polo-like kinase 1, Cyclin-dependent kinase 1, and Aurora A and B kinases (Loughlin et al., 2011; Whitehead et al., 2013); therefore, future studies should address which kinases are directly involved in phosphorylating this region. Future detailed analyses of the transcriptional, posttranscriptional, and posttranslational regulation of each katanin subunit in varied developmental and stress-induced conditions will advance

our understanding of the dynamic role(s) that they have in specialized contexts and will inform on how their dysregulation contributes to human disease.

In addition to posttranslational modifications of katanin subunits, posttranslational modifications of tubulin, the building blocks of microtubules, are also known to affect katanin activity. For example, preincubation of microtubules with the X. laevis Polo-like kinase 1 homolog (Plx1) increased the rate of KATNA1 microtubule severing five-fold in vitro, indicating that microtubule phosphorylation was facilitating severing (McNally et al., 2002). Tubulin acetylation also affects microtubule severing. For example, in rat hippocampal neurons and fibroblasts, elevated or reduced levels of acetylated tubulin render microtubules more or less sensitive to katanin-based severing, respectively (Sudo and Baas, 2010). Although tubulin C-terminal tails (CTTs) are key sites of posttranslational modifications (Gadadhar et al., 2017) and these modifications are known to regulate katanin-mediated severing (Bailey et al., 2015; Zehr et al., 2020), KATNA1 was recently found to interact with microtubules lacking the CTT in vitro (Belonogov et al., 2019). Here, KATNA1 depolymerized tubulin polymers by removing mass from the ends in a concentration-dependent but ATP-independent manner (although ATP enhances depolymerization) (Belonogov et al., 2019). These findings present a novel concentration-dependent katanin mechanism by which katanin is able to bind to CTT-deficient tubulin, potentially loosening the bonds between α- and β-tubulin, regardless of the presence of ATP or higher-order assembly.

Protein–protein interactions also regulate katanin subcellular localization. In neurons, phosphorylation of the nuclear distribution protein nudE-like 1 (NDEL-1) by CDK5 or CDK2 is necessary for KATNA1 centrosomal localization (Toyo-Oka et al., 2005).

Additionally, the nuclear mitotic apparatus (NuMa) and the putative tumor suppressor LAPSER proteins interact with KATNB1 and are thought to direct the localization of KATNB1 to sister chromatids (Jin et al., 2017). LAPSER is also important for the translocation of KATNB1 to the midbody during cell division (Suko and Maru, 2007). In vitro and structural studies of the KATNA1 N-terminus, KATNB1 C-terminus, and the abnormal spindle-like microcephaly associated (ASPM) protein showed that they can form a complex (Jiang et al., 2017). Intriguingly, ASPM and katanin stimulated each other's activity. Here, ASPM is critical for katanin spindle localization, while the ASPM–katanin complex was important for maintaining proper spindle microtubule dynamics (Jiang et al., 2017). Although the protein–protein interaction networks for the five mammalian katanin subunits have been defined through mass proteomic approaches (Cheung et al., 2016), there is still much to learn about how katanin interacting proteins regulate katanin localization and activity, and the importance of these interactions within various disease contexts.

Conclusion and Future Perspectives

Microtubule polymers and their dynamic rearrangements are paramount to cell architecture, cell motility, cell polarity, cell division, and cell signaling (Muroyama and Lechler, 2017; Goodson and Jonasson, 2018). As such, the growth, maintenance, and function of microtubules require a complex interplay of proteins that interact with and modify the microtubule network. The katanin family of microtubule-severing enzymes has emerged as important factors for regulating microtubule rearrangements. Intriguingly, as evolution has produced more complex organisms, the diversity of katanin subunits in these organisms has expanded. The greater diversity of katanin A-like and B-like subunits

in higher organisms raises important questions with regard to their function. For example, are these subunits redundant? Do they have specialized functions? Or do they have both redundant and specialized functions depending on the context or cell type? Recent studies point to the latter, where these subunits share overlapping functions in some cell types and have specific functions in other specialized cell types and contexts, such as during development, during neurogenesis, in mature neurons, and in germ cells (Smith et al., 2012; Hu et al., 2014; Dunleavy et al., 2017; Hatakeyama and Hayashi, 2018; Gao et al., 2019; Lombino et al., 2019). Defining these overlapping and specific functions for each katanin subunit (and their isoforms) is imperative to understanding their function, how they are regulated, and how their dysregulation promotes disease progression.

While transcriptional regulation and posttranslational modifications are known to affect the levels of KATNA1 and its activity, our understanding of how the remaining mammalian subunits (KATNAL1, KATNAL2, KATNB1, and KATNBL1) are regulated is still lacking. Obtaining a systematic profile of degradation and/or activation mechanisms (via phosphorylation, ubiquitination, etc.) for all katanin subunits (in vertebrates and invertebrates) in diverse cell types across phyla and contexts will provide a more comprehensive understanding of how these enzymes are regulated. Phosphorylation, for example, has been postulated to alter the capacity of katanins to oligomerize and to activate microtubule-severing activity (Whitehead et al., 2013). Therefore, resolving the structures of phosphorylated katanin subunits would provide a valuable insight into the role that phosphates, kinases, and phosphatases play in regulating katanin function and whether phosphorylation states can promote observable structural differences in katanins across phyla. Furthermore, the full-length A–B katanin complex has yet to be solved;

outside of the canonical MIT:Con80 domain interactions, much remains to be understood regarding the formation of the entire complex. Similarly, full-length structures of the vertebrate B-subunit remain unresolved. The verified interactions between the katanin A- and B-subunits in vertebrates (KATNA1, KATNAL1, and KATNBL1) (Cheung et al., 2016; Willsey et al., 2018) and the propensity for A- and B-katanin heterodimeric formation (Hartman and Vale, 1999; Faltova et al., 2019) raise questions regarding the possibility of mixed hetero-oligomerized katanin complexes and whether mixed hetero-oligomers serve cell- and or context-specific functions. Moreover, there is also a great need for a microtubule-bound katanin structure, as it could inform on its mechanism of action and resolve microtubule-severing models.

In conclusion, the mammalian family of katanins is an intriguing subject for research, as it intersects with cellular pathways including those critical for development that are dysregulated in human diseases. Further research into katanin subunit redundancies and specificities, protein interactions, holoenzyme structure determination, and mechanisms of regulation will further define their roles in human disease and their potential as therapeutic targets.

Author Contributions

NL and JT conceptualized the project. NL wrote the original draft. NL and JT wrote, reviewed, and edited the manuscript. NL prepared the figures. EM and HN prepared the tables and data for the figures. All authors contributed to the article and approved the submitted version.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Acknowledgments

We apologize to colleagues whose work could not be cited to keep this review to a reasonable length. Figures were created with BioRender.com and exported using a paid subscription.

Footnotes

Funding. This material was based upon work supported by the National Institutes of Health NIGMS Grant Numbers R35GM139539 and R01GM117475 to JT. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Institutes of Health. This work was also supported by an NIH-NIGMS Ruth L. Kirschstein National Research Service Award GM007185 and a National Science Foundation Graduate Research Fellowship DGE-1650604 and DGE-2034835 to NL. EM was supported by the NSF California Alliance for Minority Participation (CAMP) Program. HN was supported by a Beckman Scholar Scholarship from the Arnold and Mabel Beckman Foundation.

References

1. Advani S., Maresca T. J., Ross J. L. (2018). Creation and testing of a new, local microtubule-disruption tool based on the microtubule-severing enzyme, katanin p60. Cytoskeleton (Hoboken) 75 531–544. 10.1002/cm.21482 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
2. Ahmad F. J., Yu W., McNally F. J., Baas P. W. (1999). An essential role for katanin in severing microtubules in the neuron. J. Cell Biol. 145 305–315. 10.1083/jcb.145.2.305 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
3. Andrés-Benito P., Delgado-Morales R., Ferrer I. (2018). Altered regulation of KIAA0566, and katanin signaling expression in the locus coeruleus with neurofibrillary tangle pathology. Front. Cell. Neurosci. 12:131. 10.3389/fncel.2018.00131 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
4. Bailey M. E., Sackett D. L., Ross J. L. (2015). Katanin severing and binding microtubules are inhibited by tubulin carboxy tails. Biophys. J. 109 2546–2561. 10.1016/j.bpj.2015.11.011 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
5. Bangs F., Anderson K. V. (2017). Primary cilia and mammalian hedgehog signaling. Cold Spring Harb. Perspect. Biol. 9:a028175. 10.1101/cshperspect.a028175 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
6. Banks G., Lassi G., Hoerder-Suabedissen A., Tinarelli F., Simon M. M., Wilcox A., et al. (2018). A missense mutation in Katnal1 underlies behavioural, neurological

and ciliary anomalies. Mol. Psychiatry 23 713–722. 10.1038/mp.2017.54 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

7. Barbier P., Zejneli O., Martinho M., Lasorsa A., Belle V., Smet-Nocca C., et al. (2019). Role of Tau as a microtubule-associated protein: structural and functional aspects. Front. Aging Neurosci. 11:204. 10.3389/fnagi.2019.00204 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
8. Bartholdi D., Stray-Pedersen A., Azzarello-Burri S., Kibaek M., Kirchhoff M., Oneda B., et al. (2014). A newly recognized 13q12.3 microdeletion syndrome characterized by intellectual disability, microcephaly, and eczema/atopic dermatitis encompassing the HMGB1 and KATNAL1 genes. Am. J. Med. Genet. A 164A 1277–1283. 10.1002/ajmg.a.36439 [PubMed] [CrossRef] [Google Scholar]
9. Belonogov L., Bailey M. E., Tyler M. A., Kazemi A., Ross J. L. (2019). Katanin catalyzes microtubule depolymerization independently of tubulin C-terminal tails. Cytoskeleton 76 254–268. 10.1002/cm.21522 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
10. Besnard A., Galan-Rodriguez B., Vanhoutte P., Caboche J. (2011). Elk-1 a transcription factor with multiple facets in the brain. Front. Neurosci. 5:35. 10.3389/fnins.2011.00035 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
11. Brandt R., Hundelt M., Shahani N. (2005). Tau alteration and neuronal degeneration in tauopathies: mechanisms and models. Biochim. Biophys. Acta 1739 331–354. 10.1016/j.bbadis.2004.06.018 [PubMed] [CrossRef] [Google Scholar]

12. Buster D., McNally K., McNally F. J. (2002). Katanin inhibition prevents the redistribution of gamma-tubulin at mitosis. J. Cell Sci. 115 1083–1092. 10.1242/jcs.115.5.1083 [PubMed] [CrossRef] [Google Scholar]
13. Chao H. X., Poovey C. E., Privette A. A., Grant G. D., Chao H. Y., Cook J. G., et al. (2017). Orchestration of DNA damage checkpoint dynamics across the human cell cycle. Cell Syst. 5 445–459.e5. [PMC free article] [PubMed] [Google Scholar]
14. Chen Q., Lin F., Lin E., Huang Q., Wu G. (2020). Katanin P60 and P80 in papillary thyroid carcinoma patients: indicators for exacerbated tumor features and worse disease-free survival. J. Clin. Lab. Anal. 34:e23502. [PMC free article] [PubMed] [Google Scholar]
15. Cheung K., Senese S., Kuang J., Bui N., Ongpipattanakul C., Gholkar A., et al. (2016). Proteomic analysis of the mammalian katanin family of microtubule-severing enzymes defines katanin p80 subunit B-like 1 (KATNBL1) as a regulator of mammalian katanin microtubule-severing. Mol. Cell Proteomics 15 1658–1669. 10.1074/mcp.m115.056465 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
16. Chinchilla A., Daimi H., Lozano-Velasco E., Dominguez J. N., Caballero R., Delpón E., et al. (2011). PITX2 insufficiency leads to atrial electrical and structural remodeling linked to arrhythmogenesis. Circ. Cardiovasc. Genet. 4 269–279. 10.1161/circgenetics.110.958116 [PubMed] [CrossRef] [Google Scholar]
17. Choi H. K., Choi K. C., Kang H. B., Kim H. C., Lee Y. H., Haam S., et al. (2008). Function of multiple Lis-Homology domain/WD-40 repeat-containing proteins in feed-forward transcriptional repression by silencing mediator for retinoic and

thyroid receptor/nuclear receptor corepressor complexes. Mol. Endocrinol. 22 1093–1104. 10.1210/me.2007-0396 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

18. Cooper G. M. (2000). Microtubules, The Cell: A Molecular Approach. Sunderland, MA: Sinauer Associates. [Google Scholar]

19. Corbit K. C., Aanstad P., Singla V., Norman A. R., Stainier D. Y., Reiter J. F. (2005). Vertebrate smoothened functions at the primary cilium. Nature 437 1018–1021. 10.1038/nature04117 [PubMed] [CrossRef] [Google Scholar]

20. Cummings C. M., Bentley C. A., Perdue S. A., Baas P. W., Singer J. D. (2009). The Cul3/Klhdc5 E3 ligase regulates p60/katanin and is required for normal mitosis in mammalian cells. J. Biol. Chem. 284 11663–11675. 10.1074/jbc.m809374200 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

21. DeRenzo C., Seydoux G. (2004). A clean start: degradation of maternal proteins at the oocyte-to-embryo transition. Trends Cell Biol. 14 420–426. 10.1016/j.tcb.2004.07.005 [PubMed] [CrossRef] [Google Scholar]

22. Dunleavy J. E. M., Okuda H., O'Connor A. E., Merriner D. J., O'Donnell L., Jamsai D., et al. (2017). Katanin-like 2 (KATNAL2) functions in multiple aspects of haploid male germ cell development in the mouse. PLoS Genet. 13:e1007078. 10.1371/journal.pgen.1007078 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

23. Faltova L., Jiang K., Frey D., Wu Y., Capitani G., Prota A. E., et al. (2019). Crystal structure of a heterotetrameric katanin p60:p80 complex. Structure 27 1375–1383.e3. [PubMed] [Google Scholar]

24. Fletcher D. A., Mullins R. D. (2010). Cell mechanics and the cytoskeleton. Nature 463 485–492. [PMC free article] [PubMed] [Google Scholar]

25. Fouad Y. A., Aanei C. (2017). Revisiting the hallmarks of cancer. Am. J. Cancer Res. 7 1016–1036. [PMC free article] [PubMed] [Google Scholar]

26. Fu W., Wu H., Cheng Z., Huang S., Rao H. (2018). The role of katanin p60 in breast cancer bone metastasis. Oncol. Lett. 15 4963–4969. [PMC free article] [PubMed] [Google Scholar]

27. Furtado M. B., Merriner D. J., Berger S., Rhodes D., Jamsai D., O'Bryan M. K. (2017). Mutations in the Katnb1 gene cause left-right asymmetry and heart defects. Dev. Dyn. 246 1027–1035. 10.1002/dvdy.24564 [PubMed] [CrossRef] [Google Scholar]

28. Gadadhar S., Bodakuntla S., Natarajan K., Janke C. (2017). The tubulin code at a glance. J. Cell Sci. 130 1347–1353. [PubMed] [Google Scholar]

29. Gao L. L., Xu F., Jin Z., Ying X. Y., Liu J. W. (2019). Microtubule-severing protein Katanin p60 ATPase-containing subunit A-like 1 is involved in pole-based spindle organization during mouse oocyte meiosis. Mol. Med. Rep. 20 3573–3582. [PubMed] [Google Scholar]

30. Gomes J. E., Tavernier N., Richaudeau B., Formstecher E., Boulin T., Mains P. E., et al. (2013). Microtubule severing by the katanin complex is activated by PPFR-1-dependent MEI-1 dephosphorylation. J. Cell Biol. 202 431–439. 10.1083/jcb.201304174 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

31. Goodson H. V., Jonasson E. M. (2018). Microtubules and microtubule-associated proteins. Cold Spring Harb. Perspect. Biol. 10:a022608. [PMC free article] [PubMed] [Google Scholar]

32. Han X., Gomes J. E., Birmingham C. L., Pintard L., Sugimoto A., Mains P. E. (2009). The role of protein phosphatase 4 in regulating microtubule severing in the Caenorhabditis elegans embryo. Genetics 181 933–943. 10.1534/genetics.108.096016 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

33. Harms K. L., Chen X. (2006). The functional domains in p53 family proteins exhibit both common and distinct properties. Cell Death Differ. 13 890–897. 10.1038/sj.cdd.4401904 [PubMed] [CrossRef] [Google Scholar]

34. Hartman J. J., Vale R. D. (1999). Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. Science 286 782–785. 10.1126/science.286.5440.782 [PubMed] [CrossRef] [Google Scholar]

35. Hartman J. J., Mahr J., McNally K., Okawa K., Iwamatsu A., Thomas S., et al. (1998). Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. Cell 93 277–287. 10.1016/s0092-8674(00)81578-0 [PubMed] [CrossRef] [Google Scholar]

36. Hatakeyama E., Hayashi K. (2018). KATNAL1 is a more active and stable isoform of katanin, and is expressed dominantly in neurons. Biochem. Biophys. Res. Commun. 507 389–394. 10.1016/j.bbrc.2018.11.048 [PubMed] [CrossRef] [Google Scholar]

37. Hirokawa N., Tanaka Y., Okada Y. (2009). Left-right determination: involvement of molecular motor KIF3, cilia, and nodal flow. Cold Spring Harb. Perspect. Biol. 1:a000802. 10.1101/cshperspect.a000802 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

38. Hoover A. N., Wynkoop A., Zeng H., Jia J., Niswander L. A., Liu A. (2008). C2cd3 is required for cilia formation and Hedgehog signaling in mouse. Development 135 4049–4058. 10.1242/dev.029835 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

39. Hu W. F., Pomp O., Ben-Omran T., Kodani A., Henke K., Mochida G. H., et al. (2014). Katanin p80 regulates human cortical development by limiting centriole and cilia number. Neuron 84 1240–1257. 10.1016/j.neuron.2014.12.017 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

40. Huangfu D., Anderson K. V. (2005). Cilia and Hedgehog responsiveness in the mouse. Proc. Natl. Acad. Sci. U.S.A. 102 11325–11330. 10.1073/pnas.0505328102 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

41. Iatrou A., Kenis G., Rutten B. P., Lunnon K., van den Hove D. L. (2017). Epigenetic dysregulation of brainstem nuclei in the pathogenesis of Alzheimer's disease: looking in the correct place at the right time? Cell. Mol. Life Sci. 74 509–523. 10.1007/s00018-016-2361-4 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

42. Ishikawa H., Marshall W. F. (2011). Ciliogenesis: building the cell's antenna. Nat. Rev. Mol. Cell Biol. 12 222–234. 10.1038/nrm3085 [PubMed] [CrossRef] [Google Scholar]

43. Jia Y., Wang Y., Xie J. (2015). The Hedgehog pathway: role in cell differentiation, polarity and proliferation. Arch. Toxicol. 89 179–191. 10.1007/s00204-014-1433-1 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

44. Jiang K., Rezabkova L., Hua S., Liu Q., Capitani G., Altelaar A. F. M., et al. (2017). Microtubule minus-end regulation at spindle poles by an ASPM-katanin complex. Nat. Cell Biol. 19 480–492. 10.1038/ncb3511 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

45. Jin M., Pomp O., Shinoda T., Toba S., Torisawa T., Furuta K., et al. (2017). Katanin p80, NuMA and cytoplasmic dynein cooperate to control microtubule dynamics. Sci. Rep. 7:39902. [PMC free article] [PubMed] [Google Scholar]

46. Joachimiak E., Waclawek E., Niziolek M., Osinka A., Fabczak H., Gaertig J., et al. (2020). The LisH domain-containing N-terminal fragment is important for the localization, dimerization, and stability of katnal2 in Tetrahymena. Cells 9:292. 10.3390/cells9020292 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

47. Johnson J. L., Lu C., Raharjo E., McNally K., McNally F. J., Mains P. E. (2009). Levels of the ubiquitin ligase substrate adaptor MEL-26 are inversely correlated with MEI-1/katanin microtubule-severing activity during both meiosis and mitosis. Dev. Biol. 330 349–357. 10.1016/j.ydbio.2009.04.004 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

48. Joly N., Beaumale E., Van Hove L., Martino L., Pintard L. (2020). Phosphorylation of the microtubule-severing AAA+ enzyme Katanin regulates C. elegans embryo development. J. Cell Biol. 219:e201912037. [PMC free article] [PubMed] [Google Scholar]

49. Joly N., Martino L., Gigant E., Dumont J., Pintard L. (2016). Microtubule-severing activity of the AAA+ ATPase Katanin is essential for female meiotic spindle assembly. Development 143 3604–3614. [PubMed] [Google Scholar]

50. Kalderon D. (2002). Similarities between the Hedgehog and Wnt signaling pathways. Trends Cell Biol. 12 523–531. 10.1016/s0962-8924(02)02388-7 [PubMed] [CrossRef] [Google Scholar]

51. Karabay A., Yu W., Solowska J. M., Baird D. H., Baas P. W. (2004). Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. J. Neurosci. 24 5778–5788. 10.1523/jneurosci.1382-04.2004 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

52. Kay B. K., Williamson M. P., Sudol M. (2000). The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains. FASEB J. 14 231–241. 10.1096/fasebj.14.2.231 [PubMed] [CrossRef] [Google Scholar]

53. Kelle D., Kırımtay K., Selçuk E., Karabay A. (2019). Elk1 affects katanin and spastin proteins via differential transcriptional and post-transcriptional regulations. PLoS One 14:e0212518. 10.1371/journal.pone.0212518 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

54. Kırımtay K., Selçuk E., Kelle D., Erman B., Karabay A. (2020). p53 regulates katanin-p60 promoter in HCT 116 cells. Gene 727:144241. 10.1016/j.gene.2019.144241 [PubMed] [CrossRef] [Google Scholar]

55. Kitajima K., Oki S., Ohkawa Y., Sumi T., Meno C. (2013). Wnt signaling regulates left-right axis formation in the node of mouse embryos. Dev. Biol. 380 222–232. 10.1016/j.ydbio.2013.05.011 [PubMed] [CrossRef] [Google Scholar]

56. Komiya Y., Habas R. (2008). Wnt signal transduction pathways. Organogenesis 4 68–75. 10.4161/org.4.2.5851 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

57. Korulu S., Yildiz A. (2020). p60-katanin: a novel interacting partner for p53. Mol. Biol. Rep. 47 4295–4301. 10.1007/s11033-020-05557-6 [PubMed] [CrossRef] [Google Scholar]

58. Kurz T., Pintard L., Willis J. H., Hamill D. R., Gönczy P., Peter M., et al. (2002). Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. Science 295 1294–1298. 10.1126/science.1067765 [PubMed] [CrossRef] [Google Scholar]

59. Lane C. A., Hardy J., Schott J. M. (2018). Alzheimer's disease. Eur. J. Neurol. 25 59–70. [PubMed] [Google Scholar]

60. Larose H., Shami A. N., Abbott H., Manske G., Lei L., Hammoud S. S. (2019). Gametogenesis: a journey from inception to conception. Curr. Top. Dev. Biol. 132 257–310. 10.1016/bs.ctdb.2018.12.006 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

61. Lee M. J., Yaffe M. B. (2016). Protein regulation in signal transduction. Cold Spring Harb. Perspect. Biol. 8:a005918. [PMC free article] [PubMed] [Google Scholar]

62. Leo L., Weissmann C., Burns M., Kang M., Song Y., Qiang L., et al. (2017). Mutant spastin proteins promote deficits in axonal transport through an isoform-specific mechanism involving casein kinase 2 activation. Hum. Mol. Genet. 26 2321–2334. 10.1093/hmg/ddx125 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

63. Li X., Liu J., Shi P. F., Fu P. (2018). Katanin P80 expression correlates with lymph node metastasis and worse overall survival in patients with breast cancer. Cancer Biomark. 23 363–371. 10.3233/cbm-181369 [PubMed] [CrossRef] [Google Scholar]

64. Lohret T. A., McNally F. J., Quarmby L. M. (1998). A role for katanin-mediated axonemal severing during Chlamydomonas deflagellation. Mol. Biol. Cell 9 1195–1207. 10.1091/mbc.9.5.1195 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

65. Lombino F. L., Muhia M., Lopez-Rojas J., Brill M. S., Thies E., Ruschkies L., et al. (2019). The microtubule severing protein katanin regulates proliferation of neuronal progenitors in embryonic and adult neurogenesis. Sci. Rep. 9:15940. [PMC free article] [PubMed] [Google Scholar]

66. Loughlin R., Wilbur J. D., McNally F. J., Nédélec F. J., Heald R. (2011). Katanin contributes to interspecies spindle length scaling in Xenopus. Cell 147 1397–1407. 10.1016/j.cell.2011.11.014 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

67. Lu C., Mains P. E. (2007). The C. elegans anaphase promoting complex and MBK-2/DYRK kinase act redundantly with CUL-3/MEL-26 ubiquitin ligase to degrade MEI-1 microtubule-severing activity after meiosis. Dev. Biol. 302 438–447. 10.1016/j.ydbio.2006.09.053 [PubMed] [CrossRef] [Google Scholar]

68. Maddika S., Chen J. (2009). Protein kinase DYRK2 is a scaffold that facilitates assembly of an E3 ligase. Nat. Cell Biol. 11 409–419. 10.1038/ncb1848 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

69. Malicki J. J., Johnson C. A. (2017). The cilium: cellular antenna and central processing unit. Trends Cell Biol. 27 126–140. 10.1016/j.tcb.2016.08.002 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

70. Matsuo M., Shimodaira T., Kasama T., Hata Y., Echigo A., Okabe M., et al. (2013). Katanin p60 contributes to microtubule instability around the midbody and facilitates cytokinesis in rat cells. PLoS One 8:e80392. 10.1371/journal.pone.0080392 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

71. McCullough J., Frost A., Sundquist W. I. (2018). Structures, functions, and dynamics of ESCRT-III/Vps4 membrane remodeling and fission complexes. Annu. Rev. Cell Dev. Biol. 34 85–109. 10.1146/annurev-cellbio-100616-060600 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

72. McNally F. J., Roll-Mecak A. (2018). Microtubule-severing enzymes: from cellular functions to molecular mechanism. J. Cell Biol. 217 4057–4069. 10.1083/jcb.201612104 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

73. McNally F. J., Thomas S. (1998). Katanin is responsible for the M-phase microtubule-severing activity in Xenopus eggs. Mol. Biol. Cell 9 1847–1861. 10.1091/mbc.9.7.1847 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

74. McNally F. J., Vale R. D. (1993). Identification of katanin, an ATPase that severs and disassembles stable microtubules. Cell 75 419–429. 10.1016/0092-8674(93)90377-3 [PubMed] [CrossRef] [Google Scholar]

75. McNally K. P., Bazirgan O. A., McNally F. J. (2000). Two domains of p80 katanin regulate microtubule severing and spindle pole targeting by p60 katanin. J. Cell Sci. 113(Pt 9) 1623–1633. 10.1242/jcs.113.9.1623 [PubMed] [CrossRef] [Google Scholar]

76. McNally K. P., Buster D., McNally F. J. (2002). Katanin-mediated microtubule severing can be regulated by multiple mechanisms. Cell Motil. Cytoskeleton 53 337–349. 10.1002/cm.10080 [PubMed] [CrossRef] [Google Scholar]

77. McNally K., Audhya A., Oegema K., McNally F. J. (2006). Katanin controls mitotic and meiotic spindle length. J. Cell Biol. 175 881–891. 10.1083/jcb.200608117 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

78. Meunier A., Azimzadeh J. (2016). Multiciliated cells in animals. Cold Spring Harb. Perspect. Biol. 8:a028233. 10.1101/cshperspect.a028233 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

79. Mirvis M., Siemers K. A., Nelson W. J., Stearns T. P. (2019). Primary cilium loss in mammalian cells occurs predominantly by whole-cilium shedding. PLoS Biol. 17:e3000381. 10.1371/journal.pbio.3000381 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

80. Mishra-Gorur K., Çağlayan A. O., Schaffer A. E., Chabu C., Henegariu O., Vonhoff F., et al. (2015). Mutations in KATNB1 cause complex cerebral malformations by disrupting asymmetrically dividing neural progenitors. Neuron 85:228. 10.1016/j.neuron.2014.12.046 [PubMed] [CrossRef] [Google Scholar]

81. Monroe N., Hill C. P. (2016). Meiotic clade AAA ATPases: protein polymer disassembly machines. J. Mol. Biol. 428 1897–1911. 10.1016/j.jmb.2015.11.004 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

82. Morris-Rosendahl D. J., Crocq M. A. (2020). Neurodevelopmental disorders-the history and future of a diagnostic concept. Dialogues Clin. Neurosci. 22 65–72. 10.31887/DCNS.2020.22.1/macrocq [PMC free article] [PubMed] [CrossRef] [Google Scholar]

83. Muroyama A., Lechler T. (2017). Microtubule organization, dynamics and functions in differentiated cells. Development 144 3012–3021. 10.1242/dev.153171 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

84. Nikonova A. S., Golemis E. A. (2015). Control of ciliation in embryogenesis. Nat. Cell Biol. 17 109–111. 10.1038/ncb3103 [PubMed] [CrossRef] [Google Scholar]

85. Nithianantham S., McNally F. J., Al-Bassam J. (2018). Structural basis for disassembly of katanin heterododecamers. J. Biol. Chem. 293 10590–10605. 10.1074/jbc.ra117.001215 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

86. Nwabo Kamdje A. H., Takam Kamga P., Tagne Simo R., Vecchio L., Seke Etet P. F., Muller J. M., et al. (2017). Developmental pathways associated with cancer metastasis: Notch, Wnt, and Hedgehog. Cancer Biol. Med. 14 109–120.

10.20892/j.issn.2095-3941.2016.0032 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

87. O'Donnell L., Rhodes D., Smith S. J., Merriner D. J., Clark B. J., Borg C., et al. (2012). An essential role for katanin p80 and microtubule severing in male gamete production. PLoS Genet. 8:e1002698. 10.1371/journal.pgen.1002698 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

88. O'Roak B. J., Vives L., Girirajan S., Karakoc E., Krumm N., Coe B. P., et al. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485 246–250. 10.1038/nature10989 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

89. Pintard L., Kurz T., Glaser S., Willis J. H., Peter M., Bowerman B. (2003). Neddylation and deneddylation of CUL-3 is required to target MEI-1/Katanin for degradation at the meiosis-to-mitosis transition in C. elegans. Curr. Biol. 13 911–921. 10.1016/s0960-9822(03)00336-1 [PubMed] [CrossRef] [Google Scholar]

90. Pleuger C., Fietz D., Hartmann K., Weidner W., Kliesch S., O'Bryan M. K., et al. (2016). Expression of katanin p80 in human spermatogenesis. Fertil. Steril. 106 1683–1690.e1. [PubMed] [Google Scholar]

91. Plotnikova O. V., Pugacheva E. N., Golemis E. A. (2009). Primary cilia and the cell cycle. Methods Cell Biol. 94 137–160. 10.1016/s0091-679x(08)94007-3 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

92. Poon R. Y. (2016). Cell cycle control: a system of interlinking oscillators. Methods Mol. Biol. 1342 3–19. 10.1007/978-1-4939-2957-3_1 [PubMed] [CrossRef] [Google Scholar]

93. Qiang L., Yu W., Andreadis A., Luo M., Baas P. W. (2006). Tau protects microtubules in the axon from severing by katanin. J. Neurosci. 26 3120–3129. 10.1523/jneurosci.5392-05.2006 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

94. Rahimi-Balaei M., Bergen H., Kong J., Marzban H. (2018). Neuronal migration during development of the cerebellum. Front. Cell. Neurosci. 12:484. 10.3389/fncel.2018.00484 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

95. Raleigh D. R., Reiter J. F. (2019). Misactivation of Hedgehog signaling causes inherited and sporadic cancers. J. Clin. Invest. 129 465–475. 10.1172/jci120850 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

96. Rezabkova L., Jiang K., Capitani G., Prota A. E., Akhmanova A., Steinmetz M. O., et al. (2017). Structural basis of katanin p60:p80 complex formation. Sci. Rep. 7:14893. [PMC free article] [PubMed] [Google Scholar]

97. Schafer K. A. (1998). The cell cycle: a review. Vet. Pathol. 35 461–478. [PubMed] [Google Scholar]

98. Selçuk E., Kırımtay K., Canbaz D., Cesur G. I., Korulu S., Karabay A. (2013). Katanin-p80 gene promoter characterization and regulation via Elk1. PLoS One 8:e69423. 10.1371/journal.pone.0069423 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

99. Sharma N., Bryant J., Wloga D., Donaldson R., Davis R. C., Jerka-Dziadosz M., et al. (2007). Katanin regulates dynamics of microtubules and biogenesis of motile

cilia. J. Cell Biol. 178 1065–1079. 10.1083/jcb.200704021 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

100. Shin S. C., Im S. K., Jang E. H., Jin K. S., Hur E. M., Kim E. E. (2019). Structural and Molecular basis for katanin-mediated severing of glutamylated microtubules. Cell Rep. 26 1357–1367.e5. [PubMed] [Google Scholar]

101. Smith I., Greenside P. G., Natoli T., Lahr D. L., Wadden D., Tirosh I., et al. (2017). Evaluation of RNAi and CRISPR technologies by large-scale gene expression profiling in the Connectivity Map. PLoS Biol. 15:e2003213. 10.1371/journal.pbio.2003213 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

102. Smith L. B., Milne L., Nelson N., Eddie S., Brown P., Atanassova N., et al. (2012). KATNAL1 regulation of sertoli cell microtubule dynamics is essential for spermiogenesis and male fertility. PLoS Genet. 8:e1002697. 10.1371/journal.pgen.1002697 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

103. Snider J., Thibault G., Houry W. A. (2008). The AAA+ superfamily of functionally diverse proteins. Genome Biol. 9:216. 10.1186/gb-2008-9-4-216 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

104. Sonbuchner T. M., Rath U., Sharp D. J. (2010). KL1 is a novel microtubule severing enzyme that regulates mitotic spindle architecture. Cell Cycle 9 2403–2411. 10.4161/cc.9.12.11916 [PubMed] [CrossRef] [Google Scholar]

105. Srayko M., Buster D. W., Bazirgan O. A., McNally F. J., Mains P. E. (2000). MEI-1/MEI-2 katanin-like microtubule severing activity is required for

Caenorhabditis elegans meiosis. Genes Dev. 14 1072–1084. [PMC free article] [PubMed] [Google Scholar]

106. Srayko M., O'toole E. T., Hyman A. A., Müller-Reichert T. (2006). Katanin disrupts the microtubule lattice and increases polymer number in C. elegans meiosis. Curr. Biol. 16 1944–1949. 10.1016/j.cub.2006.08.029 [PubMed] [CrossRef] [Google Scholar]

107. Stessman H. A., Xiong B., Coe B. P., Wang T., Hoekzema K., Fenckova M., et al. (2017). Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. Nat. Genet. 49 515–526. 10.1038/ng.3792 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

108. Stitzel M. L., Pellettieri J., Seydoux G. (2006). The C. elegans DYRK kinase MBK-2 marks oocyte proteins for degradation in response to meiotic maturation. Curr. Biol. 16 56–62. 10.1016/j.cub.2005.11.063 [PubMed] [CrossRef] [Google Scholar]

109. Sudo H. (2018). Microtubule hyperacetylation enhances KL1-dependent micronucleation under a Tau deficiency in mammary epithelial cells. Int. J. Mol. Sci. 19:2488. 10.3390/ijms19092488 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

110. Sudo H., Baas P. W. (2010). Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. J. Neurosci. 30 7215–7226. 10.1523/jneurosci.0048-10.2010 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

111. Sudo H., Baas P. W. (2011). Strategies for diminishing katanin-based loss of microtubules in tauopathic neurodegenerative diseases. Hum. Mol. Genet. 20 763–778. 10.1093/hmg/ddq521 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

112. Sudo H., Nakajima K. (2016). The mitotic tensegrity guardian tau protects mammary epithelia from katanin-like1-induced aneuploidy. Oncotarget 7 53712–53734. 10.18632/oncotarget.10728 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

113. Suko H., Maru Y. (2007). LAPSER1 is a putative cytokinetic tumor suppressor that shows the same centrosome and midbody subcellular localization pattern as p80 katanin. Fed. Am. Sci. Exp. Biol. 21 2086–2100. 10.1096/fj.06-7254com [PubMed] [CrossRef] [Google Scholar]

114. Toyo-Oka K., Sasaki S., Yano Y., Mori D., Kobayashi T., Toyoshima Y. Y., et al. (2005). Recruitment of katanin p60 by phosphorylated NDEL1, an LIS1 interacting protein, is essential for mitotic cell division and neuronal migration. Hum. Mol. Genet. 14 3113–3128. 10.1093/hmg/ddi339 [PubMed] [CrossRef] [Google Scholar]

115. Ververis A., Christodoulou A., Christoforou M., Kamilari C., Lederer C. W., Santama N. (2016). A novel family of katanin-like 2 protein isoforms (KATNAL2), interacting with nucleotide-binding proteins Nubp1 and Nubp2, are key regulators of different MT-based processes in mammalian cells. Cell Mol. Life Sci. 73 163–184. 10.1007/s00018-015-1980-5 [PubMed] [CrossRef] [Google Scholar]

116. Waclawek E., Joachimiak E., Hall M. H., Fabczak H., Wloga D. (2017). Regulation of katanin activity in the ciliate Tetrahymena thermophila. Mol. Microbiol. 103 134–150. 10.1111/mmi.13547 [PubMed] [CrossRef] [Google Scholar]

117. Wang L., Tantai J., Zhu X. (2020). Katanin P60: a potential biomarker for lymph node metastasis and prognosis for non-small cell lung cancer. World J. Surg. Oncol. 18:157. [PMC free article] [PubMed] [Google Scholar]

118. Waters A. M., Beales P. L. (2011). Ciliopathies: an expanding disease spectrum. Pediatr. Nephrol. 26 1039–1056. 10.1007/s00467-010-1731-7 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

119. Weller J., Budson A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. F1000Res. 7:F1000FacultyRev–1161. [Google Scholar]

120. Wenzel E. S., Singh A. T. K. (2018). Cell-cycle checkpoints and aneuploidy on the path to cancer. In Vivo 32 1–5. [PMC free article] [PubMed] [Google Scholar]

121. Whitehead E., Heald R., Wilbur J. D. (2013). N-terminal phosphorylation of p60 katanin directly regulates microtubule severing. J. Mol. Biol. 425 214–221. 10.1016/j.jmb.2012.11.022 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

122. Willsey H. R., Walentek P., Exner C. R. T., Xu Y., Lane A. B., Harland R. M., et al. (2018). Katanin-like protein Katnal2 is required for ciliogenesis and brain development in Xenopus embryos. Dev. Biol. 442 276–287. 10.1016/j.ydbio.2018.08.002 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

123. Xu C., Min J. (2011). Structure and function of WD40 domain proteins. Protein Cell 2 202–214. 10.1007/s13238-011-1018-1 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

124. Ye Q., Zhang M., Yin Y. (2020). Katanin P80 correlates with larger tumor size, lymph node metastasis, and advanced TNM stage and predicts poor prognosis in non-small-cell lung cancer patients. J. Clin. Lab. Anal. 34:e23141. [PMC free article] [PubMed] [Google Scholar]

125. Ye X., Lee Y. C., Choueiri M., Chu K., Huang C. F., Tsai W. W., et al. (2012). Aberrant expression of katanin p60 in prostate cancer bone metastasis. Prostate 72 291–300. 10.1002/pros.21431 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

126. Yu W., Qiang L., Solowska J. M., Karabay A., Korulu S., Baas P. W. (2008). The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. Mol. Biol. Cell 19 1485–1498. 10.1091/mbc.e07-09-0878 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

127. Yu W., Solowska J. M., Qiang L., Karabay A., Baird D., Baas P. W. (2005). Regulation of microtubule severing by katanin subunits during neuronal development. J. Neurosci. 25 5573–5583. 10.1523/jneurosci.0834-05.2005 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

128. Zehr E. A., Szyk A., Szczesna E., Roll-Mecak A. (2020). Katanin grips the β-tubulin tail through an electropositive double spiral to sever microtubules. Dev. Cell 52 118.e–131.e. [PMC free article] [PubMed] [Google Scholar]

129. Zehr E., Szyk A., Piszczek G., Szczesna E., Zuo X., Roll-Mecak A. (2017). Katanin spiral and ring structures shed light on power stroke for microtubule severing. Nat. Struct. Mol. Biol. 24 717–725. 10.1038/nsmb.3448 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

130. Zhai Y., Kronebusch P. J., Simon P. M., Borisy G. G. (1996). Microtubule dynamics at the G2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis. J. Cell Biol. 135 201–214. 10.1083/jcb.135.1.201 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

131. Zhang D., Grode K. D., Stewman S. F., Diaz-Valencia J. D., Liebling E., Rath U., et al. (2011). Drosophila katanin is a microtubule depolymerase that regulates cortical-microtubule plus-end interactions and cell migration. Nat. Cell Biol. 13 361–370. 10.1038/ncb2206 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

132. Zhang D., Rogers G. C., Buster D. W., Sharp D. J. (2007). Three microtubule severing enzymes contribute to the “Pacman-flux” machinery that moves chromosomes. J. Cell Biol. 177 231–242. 10.1083/jcb.200612011 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Chapter 2

Establishing Inducible CRISPR-Cas9 Cell Lines for Katanin KO-Rescue

Chapter 2- Introduction

The Katanin proteins have been established as critical players in cell homeostasis, proliferation, development, and cancer.[1-5] Previous attempts to knock-out or knock-down these proteins promoted delays in cell cycle progression (A1, AL2),[6, 7] multipolar spindles (observed among katanin AL1, AL2, and B1),[1, 6, 8] elongation of spindles, and reduced aster microtubules at the spindle poles (A1, AL1).[9, 10] Reduction of AL1, AL2 or B1 (KD or KO) leads to cytokinetic defects, including improper division, enlarged nucleus, and multiple centrioles.[4, 6, 11, 12,13] These defects, however, have always been reported as mild in the total cell population, meaning the cells are still able to function despite the loss, raising questions about subunit redundancy.

The overlapping nature of the katanin subunit phenotypes (post knock-out or knock-down) makes determining a true phenotype for each individual katanin subunit difficult. This is compounded by the fact that each katanin A- and B- subunit are ubiquitously expressed, with high domain conservation.[6, 14-16] Because of this, I hypothesized it was possible, due to the high conservation, for the katanin A- and B- subunits to potentially compensate for one another, upon the loss of the “like” subunit. If true, the compensation can mask the true phenotypes for each katanin. As such, developing a strategy for single, double, and triple subunit knock-out for the A- and B- katanin would be useful for obtaining a systematic profile of phenotype and function for each subunit.

The purpose of the following experimental strategy was to create a series of inducible single and combinatorial knockouts targeting each A- and B- subunit using two sgRNAs per gene. The goal was to have the following sets of knockouts to study: ΔA1, ΔAL1, ΔAL2, ΔB1 and ΔBL1. Once the initial knockouts were established, the second goal was

to create combinatorial knockouts to assess the localization, function, and phenotype upon induced loss of each individual subunit. For example: ΔA1, ΔAL1, and ΔAL2, for a total loss of the A-subunit, then to evaluate A1, creating a cell line with ΔAL1, and ΔAL2. This would have ensured a strict evaluation on the localization and made possible *in vitro* co-immunoprecipitation to identify specific protein interactions. This would be done for each A-subunit. Similarly, I planned to develop a ΔB1 and ΔBL1 total knockout and assess them in a similar manner, as described with A. Finally, I would have assessed combinations, for example, ΔA1, ΔAL1, and ΔAL2, and observe what happens to the B-subunit, e.g., is it destabilized? Similarly, what is the effect on each A-subunit upon depletion of all B-subunits?

Chapter 2- Methods

Cheeseman cell line transduction, selection, and induction.

The Cheeseman HeLa iCas9 cell line was obtained from the Cheeseman lab at MIT. Transduction began with plating cells at 50% confluency in a 6-well dish (500,000-600,000 cells/well). I used 10 µl of viral supernatant (produced by a graduate student in our lab) containing the viral vector with the sgRNA targeting each katanin, and polybrene (Millipore-Sigma cat#C788D57) with a final concentration of 7.5 µg/ml in 2 ml of complete media. Cells were cultured with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12, Thermo Fisher cat# 11320033) supplemented with tetracycline-approved fetal bovine serum (FBS, cat# A4736401) for one overnight with viral supernatant mixture; media was replaced on day two. One Day three of transduction, cells were expanded to a 10 cm plate. On Day four, selection began by adding 2 µg/ml of puromycin. Selection lasted 5 days. Cells were then frozen or passaged for continued use. Surviving colonies

were picked and grown in larger dishes using the same media. To test sgRNA, each colony was seeded in a 6-well dish at approximately 150K cells/well using two conditions: non-induced and induced. The non-induced well contained media alone and the induced well received 1 µg/ml of doxycycline (Thermo Fisher Scientific, cat# J60422-03). The cells were allowed to grow for 48h with the doxycycline, and then were harvested using scraping. The harvested cells were lysed using LAP 200 buffer (200mg/ml KCl, 0.5mM DTT, 0.3% NP40) and lysed as previously described in Bradley et al..[17] Cell lysates were then run on a protein gel and immunoblotted using anti-katanin antibodies: A1, abcam cat# 111881 1:500 anti-rabbit; AL1 Proteintech cat# 20166-1-AP, 1:1000 anti-rabbit; AL2 abcam cat#184829 1:500 anti-rabbit; B1 Proteintech cat#14969-1-AP 1:1000 anti-rabbit; BL1 Proteintech 27495-1-AP 1:500 anti-rabbit.

AIO mCherry cell line creation and immunoblotting

Wildy type HeLa Cells were cultured using Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12, Thermo Fisher cat# 11320033) supplemented with fetal bovine serum (FBS, cat# 26140079). The cells were transduced with the AIO-mCherry plasmid containing sgRNA targeting katanin B1 (TTGGTCACCTTCAGCTTGG) and BL1 (TAAGAAATCTCCAAAACAGT) using the FuGENE HD system from Promega (cat # E2311). On Day 0 of transfection, cells were seeded at approximately 300K cells/well with the aim to have approximately 500-600K/well on transfection day. On Day 1 of transfection, cells were first incubated for 30 minutes with Opti-Mem; while incubating, the cell transfection mixture was prepared using a 1:4 ratio of DNA. Briefly, 2 µg of DNA (AIO empty plasmid and sgRNA) was used with 8 µl of FuGENE HD and Opti-Mem media (Thermo Fisher cat # 31985062). The prepared mixture was incubated for 15 minutes

total then added to the HeLa cells in a dropwise and slow swirling fashion. Five hours later, the media was replaced with the original growth media. On Day 2-3 of transfection, the cells were allowed to grow/expand. On Day 4, cells were taken to the Terasaki Core for sorting using the mCherry fluorescent marker (cells were fluorescing red post-transfection). The cell sorting buffer was composed of 1.5% Bovine Serum Albumin, 5mM Hepes, 100mg/ml DNAse1. Cells were first lifted using 750 µl Trypsin (Fisher Scientific cat# 25-510) per sample well of the 6-well plate and incubated for 2.5 minutes. The lifted cells were neutralized from Trypsin treatment using 500 µl of media and transferred to a 1.5 ml centrifuge tube and spun at 0.8xg for 10 minutes at 4ºC to pellet the cells. The cell media was then aspirated, and the cells were resuspended in the sorting buffer and transferred to a flow cytometry tube. Three conditions were used: non-transfected (to equilibrate the instrument), and transfected condition 1 (KATNB1 targeting sample) and 2 (KATNBL1 targeting sample). Single cells were sorted into three 24-well plates and allowed to grow. Expanded cells were first tested for knockdown of each subunit, then frozen if successful.

Creating iCas9 cell lines in RPE-1 and LNCaP

The RPE-1 cells were cultured similar to the HeLa (described in the previous section) and LNCaP cells. They were grown using RPMI 1640 media (Thermo Fisher Scientific cat#11875093) and supplemented with the same FBS as the RPE-1 cell line. To make the viral supernatant containing the inducible Cas9 plasmid (Addgene Cat. 85400), HeK293T cells were used. These cells were incubated using the same media conditions as described for the HeLa cells in the previous section, however, the FBS used was tet-tested, as described in the first CRISPR section.

Viral packaging

The HEK293T cells were plated with 300K cells/well on Day 0 such that they would be at ~60% confluency on Day 1. On Day 1, HEK293T cells were first incubated with Opti-Mem similar to the AIO-mCherry experiment. Viral packaging components rev (addgene 12253, pSRV-REV), envelope plasmid (addgene 12259, pMD2.G), packaging plasmid (addgene 12260 PSPAX2), and transfer plasmid (addgene 85400, for iCas9 expression), were used at a ratio of 4:2:1:1 with a total DNA concentration of 2 µg. These components were combined into one tube with 100 µl Opti-Mem, and in a second tube 8 µl of FuGENE was combined with 100 µl Opti-Mem. The Fugene Ratio 1:4 (DNA to FuGENE) was used. The tubes were mixed together and allowed to incubate 15 minutes, then added to the HEK293T cells in a slow swirling dropwise manner. The cells were incubated with the mixture for 24h. Media was harvested at 24h, 48h and 72h. Viral titer was confirmed using Takara Lenti-X glo Stix (cat# 631280).

Cellular transduction

The 48h and 72h viral supernatants had high titer compared to 24h (no titer on Lenti-X glo); the total supernatant volume per date was approximately 1.5ml. The same protocol as the viral transduction described in the first iCas9 section was used, but adjusted for the change in volume of virus (7.5mg/ml [C_f] Polybrene, 1.5 ml viral supernatant, and 0.5ml complete media). Briefly, the RPE-1 and LNCaP cells were seeded on Day 0 at 300-350K cells per well. On Day 1 the cells were transduced by swapping the media for the viral supernatant-Polybrene mixture. Cells were incubated overnight. On Day 2, the media was changed back to the original culture media (transduction mixture was aspirated and new media was added). Day 3-4 cells were allowed to grow/expand. On

Day 5, G418 selection began using 500 µg/ml per plate. Cells were selected for 7 days. Colonies that remained were picked and expanded in a 6-well plate, then split for freezing and testing. To test the cells, samples were plated at 150K in a 6-well plate using non-induced and induced conditions, where non-induced corresponded to media alone and induced corresponded to media plus 1µg/ml of Doxycycline (as previously described).

Chapter 2-Results

Dual-guide Inducible Cas9, Borrowed Systems

My first effort to produce a knockout utilized a system we had inherited from the Cheeseman lab at MIT. Here, we had obtained a HeLa cell line that could be induced to express Cas9 using the chemical doxycycline[18]. In combination with this borrowed system, a partnership with Robert Damoiseaux in the Molecular Shared Screening Resource (MSSR) of UCLA CNSI allowed us to borrow a large library of sgRNA targets. These two systems were combined in the cell using transduction methods to virally infect and obtain stable integrants for antibiotic selection. The resulting cells were lysed; I attempted to identify if induction of CRISPR led to a reduction in the protein of interest (POI), in this case each katanin subunit, for the sgRNA provided (Figure 2-1).

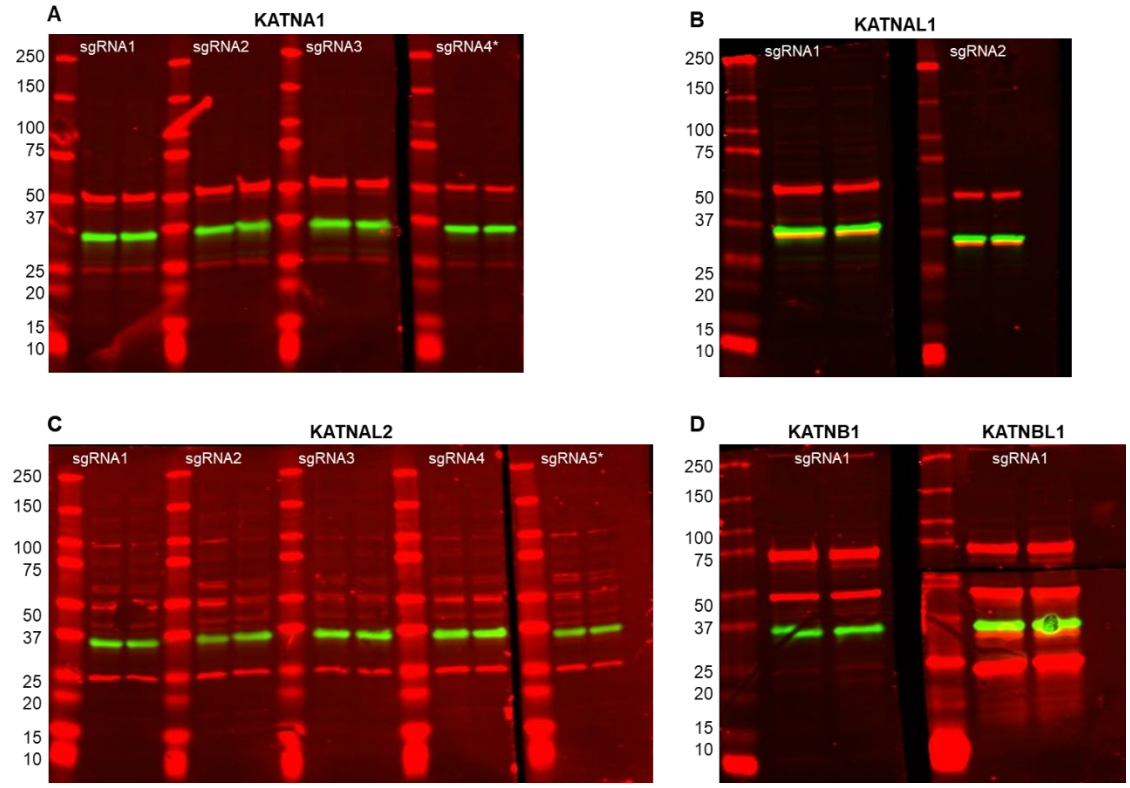

Figure 2-1 Lysates of HeLa Cells Transduced with CNSI borrowed Katanin sgRNA. Cheeseman iCas9 HeLa cells paired with sgRNA targeting katanin A1, AL1, AL2, B1,and BL1. For each lane, left corresponds to WT lysate, right to CRISPR induced to promote protein loss. Green bands show loading control using GAPDH. A. KATNA1 (red channel) sgRNA 1-3 did not promote KO; sgRNA4 asterisk lists sample that was mislabeled KATNA7. B. KATNAL1, shown in red band. Again, no changes between WT and CRISPR induction for sgRNA1 or 2. C. KATNAL2 sgRNA1-4 showed no changes in protein levels; sgRNA5 asterisk corresponds to testing against mislabeled sample (KATNA7). D. KATNB1 and BL1 sgRNA1 show no changes in protein levels.

Unfortunately, our lab was not given information on the guides other than the labels indicating which katanin subunit was targeted. A few guides were mislabeled upon receipt of the samples. For example, the sgRNA targeting the A-subunit contained a sample

labeled “KATNA7”, and the B-subunits contained sgRNA called “KATNBN1”. Without information, I made a calculated judgement that the “7” in the A-sample could possibly correspond to a 1 or a 2 if written incorrectly by hand, and therefore included this sample in panels A and C of Figure 1. Neither corresponded to a reduction in protein. The sample labeled “BN1” was used in panel D, when testing against both B1 and BL1 antibodies. This was done because it was unclear if “BN1” corresponded to B1 or BL1.

AIO-mCherry non-inducible Cas9 System

Pivoting to a constitutive Cas9 expression system was a decision that was not taken lightly, given the negative effects that can arise with constitutive Cas9 expression: cell toxicity, and increased likelihood of off-target knockouts.[19] The all-in-one (AIO) expression system we chose to implement, however, takes advantage of the d10 nickase activity of the Cas9 enzyme family, promoting higher specificity and less off-target effects.[20] The alternative benefits of this system included the ability to control and know which sgRNAs we were targeting compared to borrowed systems with proprietary data. By controlling where in the genome, we are targeting the sgRNA, we can also learn which regions may more effectively produce a stable knock-out.

The targets I chose to use for the AIO-mcherry constitutive Cas9 system were katanin B1 and BL1. I chose these proteins because I wanted to perform the first comparison between the two and also because this could fulfil the goal of producing a full B-subunit knock-out (part of the initial goal listed above). For each subunit (B1 and BL1) I chose to target exon 2, which had previously been shown to produce effective knock-outs among mice and zebrafish.[1] Wild type mammalian cervical cancer cells (HeLa) were transfected transiently with AIO-B1 or AIO-BL1 plasmids, then sorted using the fluorescent mCherry

marker using FACS via collaboration with the UCLA Terasaki core. The resulting colonies were grown until they produced large populations that could be used to validate for loss and freeze for later use. Cell lysates were immunoblotted against katanin B1 and BL1 specific antibodies, and in the case of B1, a knockdown was produced (Figure 2-2).

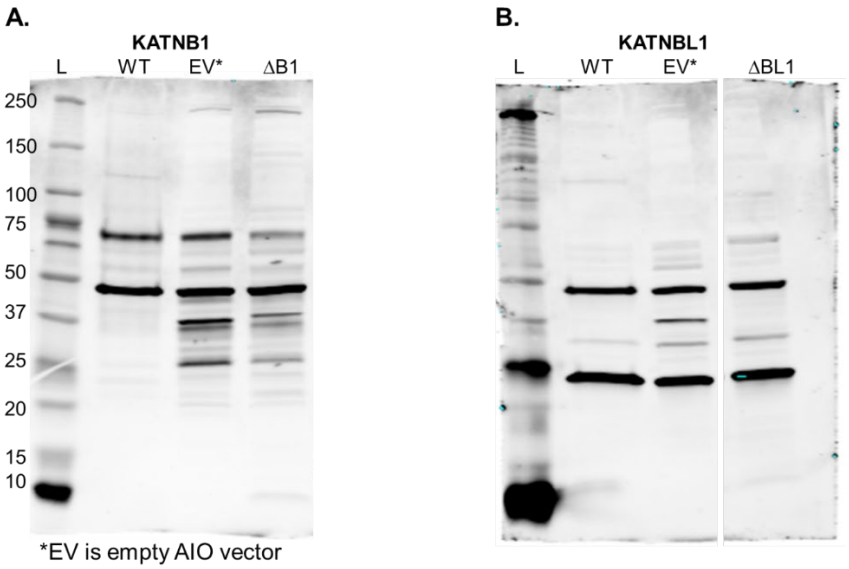

Figure 2-2 Katanin B1 and BL1 HeLa Cell Lysates after AIO-mcherry transfection and colony selection. Wild type cell is non-transfected, EV corresponds to empty vector transfection. A. A significant reduction can be seen around the 72kDa mark (faint band in lane 4) demonstrates reduction of B1 protein levels after transfection and selection. B. The band that corresponds to BL1 around the 32kDa mark does not show a reduction in protein levels. There is reduced protein in lane 2, indicating possible issues with the wild type control, however the empty vector control is comparable to the protein levels seen in delta BL1. GAPDH was used for loading control and can be seen at 37kDa. Panel A is missing GAPDH.

Chapter 2- Inducible Cas9 System (iCas9, non-borrowed)

An attempt was made to pivot back to the inducible Cas9 system. In this leg of the experiment, I was able to establish iCas9 containing non-cancerous retinal pigmented epithelial RPE-1

cells and cancerous prostate LNCaP cells using stable viral transduction and antibiotic colony selection.

Similar to the initial system we tested, which was borrowed from the Cheeseman Lab at MIT and borrowed sgRNA from UCLA CNSI, the inducible Cas9 system would use two parts: first the creation of a stable Cas9 inducible cell line, and second, the production of paired sgRNA vectors that would work in conjunction with the stable cell line. The benefit of this system would be the control over (1) Cell line production (quality control) and (2) sgRNA production. In this sense, we would not have to worry about any unknowns as we had in the initial phases of experimentation with the borrowed materials. Using both the stable cell line and paired sgRNA vector, one can virally transduce the sgRNA into the already stable cell lines containing iCas9.

I chose two cell lines to produce for the iCas9 system because (1) I wanted to have both a cancer and non-cancer cell line approach, and (2) because I wanted to see if I could assess the katanin subunit knockouts in a prostate cell line. Katanin has been shown to be heavily expressed in reproductive organs, and I thought it would be interesting to understand their function in the prostate.

Viral transduction, or the process of infecting a host cell (in this case RPE-1 and LNCAP cell lines) with your DNA of interest[21] (here, the katanin gene-specific sgRNA coupled plasmid), was used to produce stable integrants with the DNA of interest. After the viral transduction, cells were selected using antibiotics; the RPE-1 and LNCAP cells were later induced to express Cas9 (which also induced EGFP fluorescence) and sorted using FACS in partnership with the UCLA Terasaki core. The LNCaP cells did not survive the FACS selection process and were frozen as a polyclonal population. The RPE-1 cells proved robust during the FACS selection process and yielded many colonies, which were then validated. The resulting

colonies were grown to a larger population (no longer induced during cell expansion to prevent Cas9 toxicity or abnormalities), which were subsequently divided for freezing and testing. A portion of each colony was used to test doxycycline induction of Cas9 expression. These populations were assessed for their green fluorescence under the microscope to confirm effective transduction and then the cells were collected and lysed (non-induced and induced for each colony sample). Samples were tested using immunoblotting with Cas9 specific antibodies (Figure 2-3). Results showed that the Cas9 induction did in fact work for RPE-1 monoclonal colony D10 without leaky expression (Cas9 expression in non-induced lane) where the C11, colony demonstrated leaky expression.

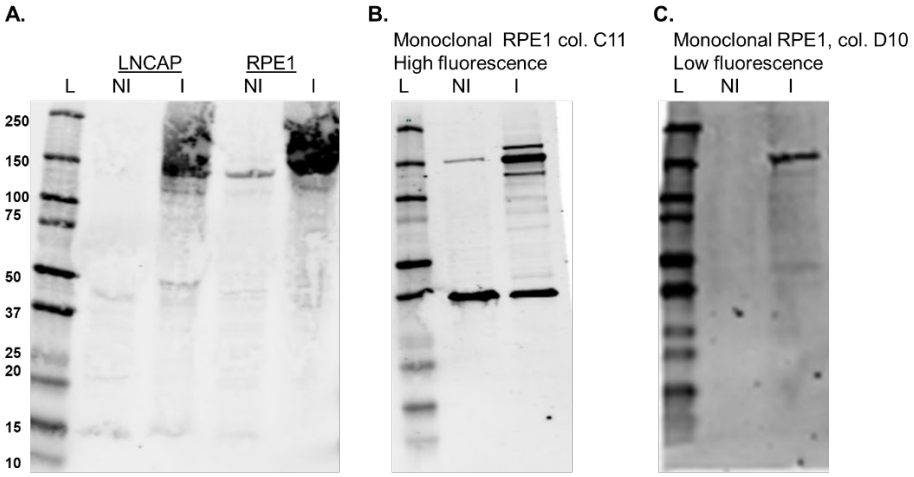

Figure 2-3 Creating iCas9 LNCaP and RPE-1 polyclonal and monoclonal cell lines. A. Immunoblot against Cas9 antibody (abcam 191468, anti-mouse 1:1000) at 150kDa in polyclonal iCas9 populations of LNCaP and RPE-1 cells after viral transduction and geneticin (G418) selection. B. Colony C11 from monoclonal RPE-1 selection using FACS. Band at 150kDa represents Cas9 specific antibody and the band at 37kDa corresponds to a GAPDH loading control. Potential leaky expression is observed in the presence of a faint 150kDa band observed in the non-induced lane (NI). C. Colony D10 from monoclonal

iCas9 RPE1 selection. Band at 150kDa corresponds to Cas9. No apparent leaky expression in NI lane.

Chapter 2- Discussion

The early phases of this research encompassed approaches using borrowed cellular systems, including the Cheeseman iCas9 HeLa cell line as well as CNSI borrowed viral vectors containing sgRNA targeting the individual katanin subunits. It was the goal of my original advisor to only move forward with a 100% KO, despite obtaining a partial KO in the AIO system, therefore this project was terminated. The purpose of this continued work was to find an appropriate combination of sgRNA targets that would produce a knock-out or knock-down with a significant loss of the protein of interest (POI).

Because it was and still is unknown what sequence in the gene of the katanin subunits targeted (the sgRNA guide sequences were not provided by the CNSI collaborators), it is difficult to interpret the data or make conclusions as to why the experiment did not yield a reduction in the protein. After cellular transduction, selection, and Cas9 induction for each stable cell line corresponding to the sgRNA target, the data demonstrated that for some reason the sgRNA guides were not working (Figure 2-1).

It may have been useful to test the sgRNA guides in a non-cancerous iCas9 system, such as the one I was able to develop in the third portion of this experiment. As noted previously, the ploidy of the cell line is important when factoring how to knock out a gene, especially considering the unique ploidy of HeLa cells.[19, 22, 23] HeLa cells are hypertriploid (3n+) and can contain up to 76-80 chromosomes per cell due to their cancerous nature.[22, 23] I was discouraged from moving into a non-cancerous cell line. Future experiments should repeat testing on the sgRNA targets, replacing the aneuploid HeLa cell line with

the monoclonal iCas9 RPE-1 cell line I established, as the RPE-1 cells do not exhibit aneuploidy.

While the AIO-mcherry system produced a viable B1 knock-down, it still existed in a system where Cas9 was constitutively expressed (always on), which can lead to off-target effects and cellular toxicity over time.[19] Similarly, it was the hope of my director to obtain a full 100% knock-out species for the katanin subunits. There are many reasons the sgRNA targets for B1 and BL1 may not have worked as effectively to reduce or remove the Katanin subunits in totality.

I hypothesize that one main reason the system did not work as efficiently is the number of chromosomes present in cancer cells, similar to the initial iCas9 approaches using the borrowed systems. The increase in chromosome number alone can make targeting by CRISPR-based systems incredibly difficult, given the number of targets significantly increase per cell. Because the system worked partially for B1, it is my conclusion that the chromosome number does matter when using CRISPR-based systems, it may also contribute to the inconsistency in replicating CRISPR-based work.

The sgRNA targeting katanin BL1 likely failed to produce a knock-down or knock-out because of the region on the gene that it targeted. After performing a second assessment of the KATNBL1 gene through a CRISPR sgRNA algorithm (CRISPOR, http://crispor.tefor.net/) and aligning each sequence with the corresponding exon region, I found there are no regions of the KATNBL1 gene where viable sgRNA targets can be created until exon 4. In this experiment I chose to target exon 2 in both B1 and BL1 based off of previous experiments using B1.[1] Keeping this in mind for the future will allow for better sgRNA design with regard to Katanin BL1, and hopefully lead to a successful knock-out or knock-down species. Future

adjustment would include modifying the targeting region for katanin B1, in addition to using a cell line that does not exhibit aneuploidy, for example RPE-1. However, there are potential concerns regarding constitutive Cas9 systems.

While the newly adopted and established iCas9 system, which was modeled after work done at Yale University,[24] proved effective in producing a monoclonal RPE-1 inducible Cas9 cell line, and a polyclonal LNCaP inducible Cas9 (which can eventually be sorted), progress was stopped shortly after the completion of these cell lines. I was able to create the complementary sgRNA plasmid with the katanin protein AL1 that paired with these stable inducible Cas9 cell lines that I made (not listed) using basic cloning techniques. To test this sgRNA vector, I completed a transient transfection of the AL1 sgRNA containing plasmid, but the cells perished during the puromycin selection process (data unavailable). With the available cell lines and the one available paired vector, future work to move forward could include creating virus containing the sgRNA containing plasmid (for example, using the AL1 sgRNA plasmid), followed by a viral transduction into the RPE-1 colony d10 stable iCas9 cell line (shown in (Figure 2-3)), cell expansion, puromycin selection, and then testing the final colonies for Cas9 expression and katanin subunit loss, in this case AL1. It would be interesting to see what can come from this project moving forward.

Chapter 2- References

(1) Hu, W. F.; Pomp, O.; Ben-Omran, T.; Kodani, A.; Henke, K.; Mochida, G. H.; Yu, T. W.; Woodworth, M. B.; Bonnard, C.; Raj, G. S.; et al. Katanin p80 regulates human cortical development by limiting centriole and cilia number. *Neuron* **2014**, *84* (6), 1240-1257. DOI: 10.1016/j.neuron.2014.12.017.

(2) Zhang, D.; Rogers, G. C.; Buster, D. W.; Sharp, D. J. Three microtubule severing enzymes contribute to the "Pacman-flux" machinery that moves chromosomes. *J Cell Biol* **2007**, *177* (2), 231-242. DOI: 10.1083/jcb.200612011.

(3) Ye, X.; Lee, Y. C.; Choueiri, M.; Chu, K.; Huang, C. F.; Tsai, W. W.; Kobayashi, R.; Logothetis, C. J.; Yu-Lee, L. Y.; Lin, S. H. Aberrant expression of katanin p60 in prostate cancer bone metastasis. *Prostate* **2012**, *72* (3), 291-300. DOI: 10.1002/pros.21431.

(4) Willsey, H. R.; Walentek, P.; Exner, C. R. T.; Xu, Y.; Lane, A. B.; Harland, R. M.; Heald, R.; Santama, N. Katanin-like protein Katnal2 is required for ciliogenesis and brain development in Xenopus embryos. *Dev Biol* **2018**, *442* (2), 276-287. DOI: 10.1016/j.ydbio.2018.08.002.

(5) Toyo-Oka, K.; Sasaki, S.; Yano, Y.; Mori, D.; Kobayashi, T.; Toyoshima, Y. Y.; Tokuoka, S. M.; Ishii, S.; Shimizu, T.; Muramatsu, M.; et al. Recruitment of katanin p60 by phosphorylated NDEL1, an LIS1 interacting protein, is essential for mitotic cell division and neuronal migration. *Hum Mol Genet* **2005**, *14* (21), 3113-3128. DOI: 10.1093/hmg/ddi339.

(6) Ververis, A.; Christodoulou, A.; Christoforou, M.; Kamilari, C.; Lederer, C. W.; Santama, N. A novel family of katanin-like 2 protein isoforms (KATNAL2), interacting with nucleotide-binding proteins Nubp1 and Nubp2, are key regulators of different MT-based processes in mammalian cells. *Cell Mol Life Sci* **2016**, *73* (1), 163-184. DOI: 10.1007/s00018-015-1980-5.

(7) Matsuo, M.; Shimodaira, T.; Kasama, T.; Hata, Y.; Echigo, A.; Okabe, M.; Arai, K.; Makino, Y.; Niwa, S.; Saya, H.; et al. Katanin p60 contributes to microtubule instability around the midbody and facilitates cytokinesis in rat cells. *PLoS One* **2013**, *8* (11), e80392. DOI: 10.1371/journal.pone.0080392.

(8) Gao, L. L.; Xu, F.; Jin, Z.; Ying, X. Y.; Liu, J. W. Microtubule-severing protein Katanin p60 ATPase-containing subunit A-like 1 is involved in pole-based spindle organization during mouse oocyte meiosis. *Mol Med Rep* **2019**, *20* (4), 3573-3582. DOI: 10.3892/mmr.2019.10605.

(9) Sonbuchner, T. M.; Rath, U.; Sharp, D. J. KL1 is a novel microtubule severing enzyme that regulates mitotic spindle architecture. *Cell Cycle* **2010**, *9* (12), 2403-2411. DOI: 10.4161/cc.9.12.11916.

(10) Loughlin, R.; Wilbur, J. D.; McNally, F. J.; Nédélec, F. J.; Heald, R. Katanin contributes to interspecies spindle length scaling in Xenopus. *Cell* **2011**, *147* (6), 1397-1407. DOI: 10.1016/j.cell.2011.11.014.

(11) Suko, H.; Maru, Y. LAPSER1 is a putative cytokinetic tumor suppressor that shows the same centrosome and midbody subcellular localization pattern as p80 katanin. *Federation of American Sciences for Experimental Biology* **2007**, *21* (9), 2086-2100.

(12) Jin, M.; Pomp, O.; Shinoda, T.; Toba, S.; Torisawa, T.; Furuta, K.; Oiwa, K.; Yasunaga, T.; Kitagawa, D.; Matsumura, S.; et al. Katanin p80, NuMA and cytoplasmic dynein cooperate to control microtubule dynamics. *Sci Rep* **2017**, *7*, 39902. DOI: 10.1038/srep39902.

(13) Lynn, N. A.; Martinez, E.; Nguyen, H.; Torres, J. Z. The Mammalian Family of Katanin Microtubule-Severing Enzymes. *Front Cell Dev Biol* **2021**, *9*, 692040. DOI: 10.3389/fcell.2021.692040.

(14) McNally, F. J.; Vale, R. D. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **1993**, *75* (3), 419-429.

(15) Hatakeyama, E.; Hayashi, K. KATNAL1 is a more active and stable isoform of katanin, and is expressed dominantly in neurons. *Biochem Biophys Res Commun* **2018**, *507* (1-4), 389-394. DOI: 10.1016/j.bbrc.2018.11.048.

(16) McNally, F. J.; Roll-Mecak, A. Microtubule-severing enzymes: From cellular functions to molecular mechanism. *J Cell Biol* **2018**, *217* (12), 4057-4069. DOI: 10.1083/jcb.201612104.

(17) Bradley, M.; Ramirez, I.; Cheung, K.; Gholkar, A. A.; Torres, J. Z. Inducible LAP-tagged Stable Cell Lines for Investigating Protein Function, Spatiotemporal Localization and Protein Interaction Networks. *J Vis Exp* **2016**, (118). DOI: 10.3791/54870.

(18) Peterson, D.; Lee, J.; Lei, X. C.; Forrest, W. F.; Davis, D. P.; Jackson, P. K.; Belmont, L. D. A chemosensitization screen identifies TP53RK, a kinase that restrains apoptosis after mitotic stress. *Cancer Res* **2010**, *70* (15), 6325-6335. DOI: 10.1158/0008-5472.CAN-10-0015.

(19) Erard, N.; Knott, S. R. V.; Hannon, G. J. A CRISPR Resource for Individual, Combinatorial, or Multiplexed Gene Knockout. *Mol Cell* **2017**, *67* (6), 1080. DOI: 10.1016/j.molcel.2017.08.027.

(20) Chiang, T. W.; le Sage, C.; Larrieu, D.; Demir, M.; Jackson, S. P. CRISPR-Cas9(D10A) nickase-based genotypic and phenotypic screening to enhance genome editing. *Sci Rep* **2016**, *6*, 24356. DOI: 10.1038/srep24356.

(21) Bulcha, J. T.; Wang, Y.; Ma, H.; Tai, P. W. L.; Gao, G. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther* **2021**, *6* (1), 53. DOI: 10.1038/s41392-021-00487-6.

(22) Chen, T. R. Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. *Cytogenet Cell Genet* **1988**, *48* (1), 19-24. DOI: 10.1159/000132579.

(23) Macville, M.; Schröck, E.; Padilla-Nash, H.; Keck, C.; Ghadimi, B. M.; Zimonjic, D.; Popescu, N.; Ried, T. Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping. *Cancer Res* **1999**, *59* (1), 141-150.

(24) Cao, J.; Wu, L.; Zhang, S. M.; Lu, M.; Cheung, W. K.; Cai, W.; Gale, M.; Xu, Q.; Yan, Q. An easy and efficient inducible CRISPR/Cas9 platform with improved specificity for multiple gene targeting. *Nucleic Acids Res* **2016**, *44* (19), e149. DOI: 10.1093/nar/gkw660.

Chapter 3

Exploring the Relationship Between Katanin and TP53

Chapter 3- Introduction

While there is a robust set of data on the role of katanin on the microtubule, which has led to an increase in our understanding of katanin functions,[1-7] protein interaction dynamics,[8-10] severing mechanisms,[11-13] and structure,[11, 14-17] the role of katanin in relationship to cancer remains undercharacterized. Only in recent literature has katanin been investigated in relation to cancer mechanisms, with evidence linking katanin subunits to cancer cell proliferation, metastasis, and tumor progression.[18-22] Of interest, the literature also recently showed that at minimum, the A1 subunit is capable of binding to tumor suppressor protein TP53 on the protein level, increased levels of TP53 lead to an increase in activation of A1 katanin, and that TP53 can activate A1 expression by binding to the promoter on the mRNA level.[23, 24]

Given the significant homology shared in the AAA+ region of the katanin A-subunits,[25] I hypothesized that like A1 katanin, AL1 and AL2 may harbor the potential to bind and be modulated by TP53. A1 katanin has also largely been the focus with regard to the recent uptick in katanin cancer research (noted above). The increased expression in A1 katanin is correlated with increased lymph node metastasis,[22] breast cancer cell migration/metastasis,[19] prostate cell migration/metastasis,[18] as well as advanced tumor sizes and a reduction in survival rates in papillary thyroid carcinomas.[26] Due to the high homology shared among the A-subunits and the overlapping phenotypes previously described, it was a reasonable hypothesis to investigate the relationship with TP53 and AL1 and AL2. Similarly to A1, increases in katanin B1 expression are also correlated with advanced tumor stages and tumor size in non-small cell lung cancers,[18] as well as metastasis, staging and survival with breast cancer and papillary thyroid carcinoma.[21, 26]

With this information in mind, I designed a set of experiments to first assess the role that TP53 may play in modulating katanin subunit expression, followed by co-immunoprecipitation binding experiments that would determine potential direct binding between TP53 and katanin subunits. The second stage of this experiment was to determine if the katanin A- and B- protein subunits could, in fact, bind to the TP53 protein in the same manner as was demonstrated for A1 by Korulu and Kirimtay.[23, 24]

Chapter 3- Methods

Inducing RPE-1 knock-down using doxycycline

The RPE-1 cell line was previously established,[28] and uses short hairpin RNA (shRNA) to target the protein of interest upon the addition of doxycycline. The RPE-1 cells were seeded in 6-well plates at approximately 350-450K cells/well with the aim for 50-60% confluency on Day 1. Cells were maintained in DMEM-F12 media (Thermo Fisher cat# 11320033) supplemented with tetracycline-approved fetal bovine serum (FBS, Thermo Fisher cat# A4736401) to prevent activation of the tetracycline-dependent shRNA system. Day 1 cells were treated with (induced) or without (non-induced) 1µg/ml of doxycycline (Thermo Fisher Scientific, cat# J60422-03), and incubated for 48h to ensure adequate loss of TP53 protein.

RPE1 TP53(+/-) induction cell harvest and immunoblot analysis

Cells were harvested by first aspirating away the original media, incubation with 750µl of Trypsin (Fisher Scientific cat# 25-510) for 2.5 minutes in the incubator, then neutralized with 750µl of culture media and transferred to a 1.5ml tube. The cells were spun at 0.8xg for 10 minutes at 4°C to pellet the cells. The media was removed using aspiration, and replaced with LAP200 buffer as described in Chapter 2. The cells lysates were then used

to run on an SDS-PAGE followed by western blot (immunoblot) using the katanin specific antibodies described in Chapter 2.

SP6 TNT in-vitro transcription, translation, co-immunoprecipitation, and imaging

The Promega SP6 TNT Quick Coupled in-vitro system (cat# L2080) was implemented for a rapid and effective transcription and translation of the katanin subunits for use in immunoprecipitation analysis. Each reaction used 10µl total volume, with 0.5µl of methionine, 150-200ng of DNA from the protein of interest, 8µl of master mix, and PCR grade water (all included in the Promega system). Once combined, each reaction was set to incubate at 30°C for 1h and 30m. After the reaction time elapsed, the samples were incubated with Flag-tagged beads and the bait protein (Flag TP53). A small volume of the initial expressed proteins were saved for use as inputs in the final gel prior to adding the beads. The samples incubating with Flag beads and Flag-TP53 were allowed to incubate for 2h, then the beads were racked against a metal rack to stabilize them during the wash steps (to prevent loss during aspiration). After the washes were complete, the beads were cooked in 6X sample loading buffer and used as the bead portion of the gel. Samples were immunoblotted against Flag (Rockland cat# 200-345-383, 1:1000 anti-mouse) and HA (Proteintech cat# 51064-2-AP, 1:1000 anti-rabbit) antibodies, and imaged.

Chapter 3- RESULTS

The first experiment utilized a RPE-1 cell line established in our lab that could be induced to lose TP53 protein using the addition of doxycycline. After inducing TP53 loss, the cells were then blotted for each katanin to evaluate if the loss of TP53 had an effect on katanin protein levels (Figure 3-1).

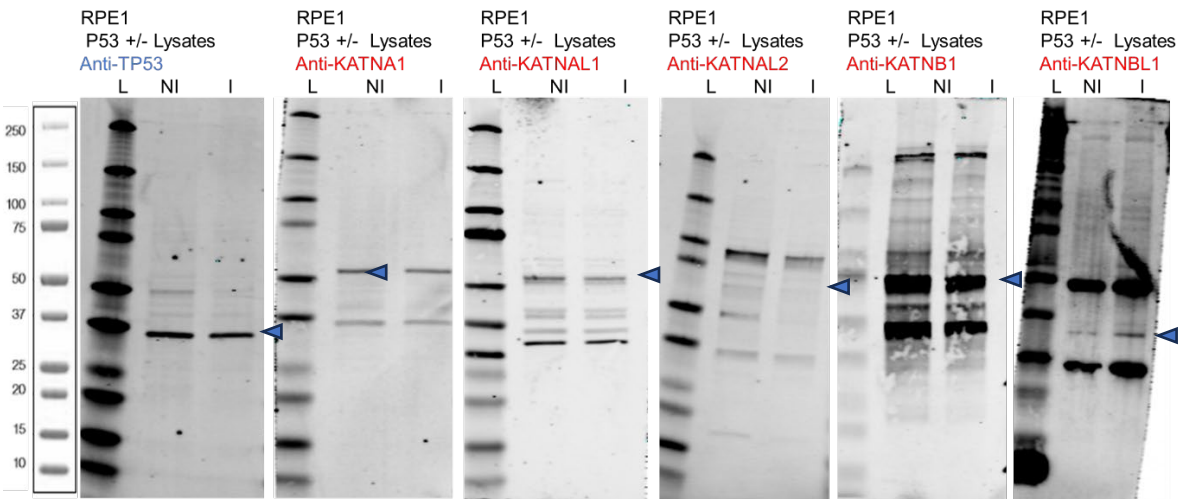

Figure 3-1 Immunoblots of RPE-1 +/- TP53 using non-induced (NI) and induced (I) conditions, which correspond to the lack or presence of 1ug/ml doxycycline. TP53 reduction after doxycycline induction is seen in the panel with TP53 labeled in blue. The Katanin A1, AL1, and B1 proteins do not show significant decrease in protein levels, where katanin AL2 shows a slight decrease in protein levels at approximately 50-75kDa, however it is difficult to place the protein as it normally runs at 60kDa and the bands in this region are hard to read. BL1 shows a possible increase in protein levels at 32kDa.

The second stage of this experiment was to determine if the katanin A- and B- protein subunits could, in fact, bind to the TP53 protein directly, as was demonstrated for A1 by Korulu and Kirimtay.[23, 24] To do this, I took advantage of the gateway cloning system using the pDONR221 constructs available in my lab, and shuttled the katanin subunits into destination vectors that were either N-terminally tagged with the HA or FLAG peptides (Table 3-1). Katanin AL1 and AL2 needed to be re-cloned from the original pDONR221 vector to remove N-terminal mutations, or truncations, respectively.

Protein of Interest	PDEST Plasmids Created
TP53	pDest HA-tagged and pDest Flag-tagged vectors
Katanin A1	pDest HA-tagged and pDest Flag-tagged vectors
Katanin AL1	pDest HA-tagged and pDest Flag-tagged vectors
Katanin AL2	pDest HA-tagged and pDest Flag-tagged vectors
Katanin B1	pDest HA-tagged and pDest Flag-tagged vectors
Katanin BL1	pDest HA-tagged and pDest Flag-tagged vectors

Table 3-1. A list of vectors constructed for use in co-Immunoprecipitation experiments between katanin subunits and TP53. All have been sequence verified.

I repeated this process for the TP53 protein, establishing pDONR and pDEST constructs, which were not previously available in our lab. I then performed *in-vitro* transcription and translation followed by co-immunoprecipitation to determine TP53 bound to any other katanin subunit as it does with A1. After co-Immunoprecipitation, the samples were run on a gel and blotted against the tag-specific antibodies (Figure 3-2).

The immunoblots of the co-Immunoprecipitation experiment using Flag-tagged TP53 and HA-tagged katanin subunits depict a possible strong binding event between katanin AL2 and TP53, as well as less strong binding events with katanin A1 and AL1.

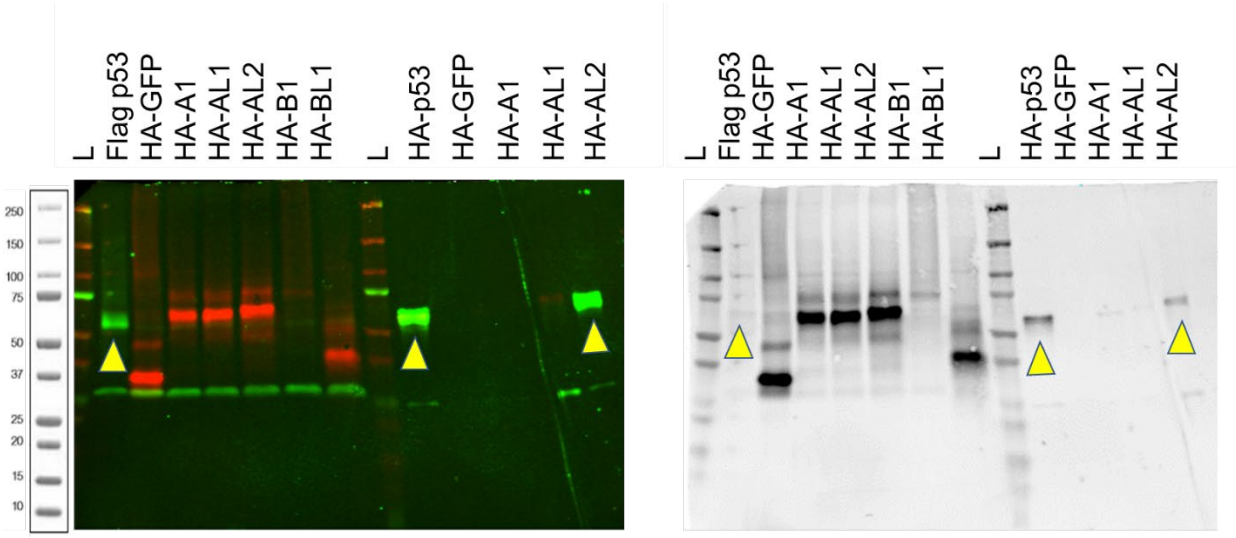

Figure 3-2 Immunoblots of co-Immunoprecipitation (binding) experiments with TP53 and Katanin proteins. Left shows the dual overlay of the channels capturing Flag-

tag and HA-tag fluorescent markers. Right shows only the overlay for the HA-tag input and bead fractions, with the input corresponding to solo protein expression (red) and beads corresponding to proteins bound to TP53 (green). The left is included to demonstrate TP53 expression was abundant (seen in green). What is notable is the pull down of HA-tagged p53, which acts as a positive control for TP53 binding, in addition to the presence of banding at HA-tagged AL2. There are faint bands that may also correspond to HA-tagged A1 and AL1.

The positive control, HA-tagged TP53 proves useful in demonstrating the capacity of TP53 to bind to itself, which has been previously validated in the literature.[27] As such, the positive control in this experiment serves its purpose. The HA-tagged GFP protein served as a negative control and did not bind to Flag-TP53.

Chapter 3- Discussion

The initial phase of the experiment was meant to evaluate the effect that the loss of TP53 may have on the katanin subunits, given its previously demonstrated effects on the A1 subunit. What was surprising to see was that the loss of TP53 did not seem to have a great effect on A1, AL1, or B1, but it did seem to decrease AL2 levels while also potentially increasing BL1 (Figure 3-1). Opposite to the manner that overexpression can activate and increase levels of A1, the loss of TP53 seems to have a reductive effect on AL2. Intriguingly, it could also have a stimulatory effect on BL1 based on the available data. The next stages of this research would be to assess the mRNA levels of these proteins in the samples lacking TP53, in order to determine if the protein levels are truly affected by the loss.

The tag-based binding experiment between TP53 and the katanin proteins led to intriguing data that indicated potential binding with AL2. What remains minimally concerning is the lack of strong binding between katanin A1 and TP53, which should have been observable based on the previous literature demonstrating the strong interaction between these proteins. Proceeding with caution with data interpretation, a promising aspect of this preliminary result suggests that katanin AL2 is a TP53 interactor. The data in Figure 3-1 may support this putative interaction, given AL2 protein levels are seemingly affected by TP53 loss; thus repeating these experiments is strongly encouraged. These data currently serves as a glimpse into what can be possible within the realm of katanin and TP53 interactions.

Chapter 3- References

(1) McNally, F. J.; Vale, R. D. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **1993**, *75* (3), 419-429.

(2) Roll-Mecak, A.; McNally, F. J. Microtubule-severing enzymes. *Curr Opin Cell Biol* **2010**, *22* (1), 96-103. DOI: 10.1016/j.ceb.2009.11.001.

(3) Zhang, D.; Rogers, G. C.; Buster, D. W.; Sharp, D. J. Three microtubule severing enzymes contribute to the "Pacman-flux" machinery that moves chromosomes. *J Cell Biol* **2007**, *177* (2), 231-242. DOI: 10.1083/jcb.200612011.

(4) Willsey, H. R.; Walentek, P.; Exner, C. R. T.; Xu, Y.; Lane, A. B.; Harland, R. M.; Heald, R.; Santama, N. Katanin-like protein Katnal2 is required for ciliogenesis and brain development in Xenopus embryos. *Dev Biol* **2018**, *442* (2), 276-287. DOI: 10.1016/j.ydbio.2018.08.002.

(5) Sonbuchner, T. M.; Rath, U.; Sharp, D. J. KL1 is a novel microtubule severing enzyme that regulates mitotic spindle architecture. *Cell Cycle* **2010**, *9* (12), 2403-2411. DOI: 10.4161/cc.9.12.11916.

(6) O'Donnell, L.; Rhodes, D.; Smith, S. J.; Merriner, D. J.; Clark, B. J.; Borg, C.; Whittle, B.; O'Connor, A. E.; Smith, L. B.; McNally, F. J.; et al. An essential role for katanin p80 and microtubule severing in male gamete production. *PLoS Genet* **2012**, *8* (5), e1002698. DOI: 10.1371/journal.pgen.1002698.

(7) McNally, K.; Audhya, A.; Oegema, K.; McNally, F. J. Katanin controls mitotic and meiotic spindle length. *J Cell Biol* **2006**, *175* (6), 881-891. DOI: 10.1083/jcb.200608117.

(8) Ververis, A.; Christodoulou, A.; Christoforou, M.; Kamilari, C.; Lederer, C. W.; Santama, N. A novel family of katanin-like 2 protein isoforms (KATNAL2), interacting with nucleotide-binding proteins Nubp1 and Nubp2, are key regulators of different MT-based processes in mammalian cells. *Cell Mol Life Sci* **2016**, *73* (1), 163-184. DOI: 10.1007/s00018-015-1980-5.

(9) Toyo-Oka, K.; Sasaki, S.; Yano, Y.; Mori, D.; Kobayashi, T.; Toyoshima, Y. Y.; Tokuoka, S. M.; Ishii, S.; Shimizu, T.; Muramatsu, M.; et al. Recruitment of katanin p60 by phosphorylated NDEL1, an LIS1 interacting protein, is essential for mitotic cell division and neuronal migration. *Hum Mol Genet* **2005**, *14* (21), 3113-3128. DOI: 10.1093/hmg/ddi339.

(10) Cheung, K.; Senese, S.; Kuang, J.; Bui, N.; Ongpipattanakul, C.; Gholkar, A.; Cohn, W.; Capri, J.; Whitelegge, J. P.; Torres, J. Z. Proteomic Analysis of the Mammalian Katanin Family of Microtubule-severing Enzymes Defines Katanin p80 subunit B-like 1 (KATNBL1) as a Regulator of Mammalian Katanin Microtubule-severing. *Mol Cell Proteomics* **2016**, *15* (5), 1658-1669. DOI: 10.1074/mcp.M115.056465.

(11) Zehr, E. A.; Szyk, A.; Szczesna, E.; Roll-Mecak, A. Katanin Grips the β-Tubulin Tail through an Electropositive Double Spiral to Sever Microtubules. *Dev Cell* **2020**, *52* (1), 118-131.e116. DOI: 10.1016/j.devcel.2019.10.010.

(12) Belonogov, L.; Bailey, M. E.; Tyler, M. A.; Kazemi, A.; Ross, J. L. Katanin catalyzes microtubule depolymerization independently of tubulin C-terminal tails. *Cytoskeleton (Hoboken)* **2019**, *76* (3), 254-268. DOI: 10.1002/cm.21522.

(13) Bailey, M. E.; Sackett, D. L.; Ross, J. L. Katanin Severing and Binding Microtubules Are Inhibited by Tubulin Carboxy Tails. *Biophys J* **2015**, *109* (12), 2546-2561. DOI: 10.1016/j.bpj.2015.11.011.

(14) Rezabkova, L.; Jiang, K.; Capitani, G.; Prota, A. E.; Akhmanova, A.; Steinmetz, M. O.; Kammerer, R. A. Structural basis of katanin p60:p80 complex formation. *Sci Rep* **2017**, *7* (1), 14893. DOI: 10.1038/s41598-017-14194-2.

(15) Nithianantham, S.; McNally, F. J.; Al-Bassam, J. Structural basis for disassembly of katanin heterododecamers. *J Biol Chem* **2018**, *293* (27), 10590-10605. DOI: 10.1074/jbc.RA117.001215.

(16) Faltova, L.; Jiang, K.; Frey, D.; Wu, Y.; Capitani, G.; Prota, A. E.; Akhmanova, A.; Steinmetz, M. O.; Kammerer, R. A. Crystal Structure of a Heterotetrameric Katanin p60:p80 Complex. *Structure* **2019**, *27* (9), 1375-1383.e1373. DOI: 10.1016/j.str.2019.07.002.

(17) Shin, S. C.; Im, S. K.; Jang, E. H.; Jin, K. S.; Hur, E. M.; Kim, E. E. Structural and Molecular Basis for Katanin-Mediated Severing of Glutamylated Microtubules. *Cell Rep* **2019**, *26* (5), 1357-1367.e1355. DOI: 10.1016/j.celrep.2019.01.020.

(18) Ye, Q.; Zhang, M.; Yin, Y. Katanin P80 correlates with larger tumor size, lymph node metastasis, and advanced TNM stage and predicts poor prognosis in non-small-cell lung cancer patients. *J Clin Lab Anal* **2020**, *34* (4), e23141. DOI: 10.1002/jcla.23141.

(19) Ye, X.; Lee, Y. C.; Choueiri, M.; Chu, K.; Huang, C. F.; Tsai, W. W.; Kobayashi, R.; Logothetis, C. J.; Yu-Lee, L. Y.; Lin, S. H. Aberrant expression of katanin p60 in prostate cancer bone metastasis. *Prostate* **2012**, *72* (3), 291-300. DOI: 10.1002/pros.21431.

(20) Wang, L.; Tantai, J.; Zhu, X. Katanin P60: a potential biomarker for lymph node metastasis and prognosis for non-small cell lung cancer. *World J Surg Oncol* **2020**, *18* (1), 157. DOI: 10.1186/s12957-020-01939-z.

(21) Li, X.; Liu, J.; Shi, P. F.; Fu, P. Katanin P80 expression correlates with lymph node metastasis and worse overall survival in patients with breast cancer. *Cancer Biomark* **2018**, *23* (3), 363-371. DOI: 10.3233/CBM-181369.

(22) Fu, W.; Wu, H.; Cheng, Z.; Huang, S.; Rao, H. The role of katanin p60 in breast cancer bone metastasis. *Oncol Lett* **2018**, *15* (4), 4963-4969. DOI: 10.3892/ol.2018.7942.

(23) Korulu, S.; Yildiz, A. p60-katanin: a novel interacting partner for p53. *Mol Biol Rep* **2020**, *47* (6), 4295-4301. DOI: 10.1007/s11033-020-05557-6.

(24) Kırımtay, K.; Selçuk, E.; Kelle, D.; Erman, B.; Karabay, A. p53 regulates katanin-p60 promoter in HCT 116 cells. *Gene* **2020**, *727*, 144241. DOI: 10.1016/j.gene.2019.144241.

(25) Lynn, N. A.; Martinez, E.; Nguyen, H.; Torres, J. Z. The Mammalian Family of Katanin Microtubule-Severing Enzymes. *Front Cell Dev Biol* **2021**, *9*, 692040. DOI: 10.3389/fcell.2021.692040.

(26) Chen, Q.; Lin, F.; Lin, E.; Huang, Q.; Wu, G. Katanin P60 and P80 in papillary thyroid carcinoma patients: Indicators for exacerbated tumor features and worse disease-free survival. *J Clin Lab Anal* **2020**, e23502. DOI: 10.1002/jcla.23502.

(27) Harms, K. L.; Chen, X. The functional domains in p53 family proteins exhibit both common and distinct properties. *Cell Death Differ* **2006**, *13* (6), 890-897. DOI: 10.1038/sj.cdd.4401904.

(28) Peterson, D.; Lee, J.; Lei, X. C.; Forrest, W. F.; Davis, D. P.; Jackson, P. K.; Belmont, L. D. A chemosensitization screen identifies TP53RK, a kinase that restrains apoptosis after mitotic stress. *Cancer Res* **2010**, *70* (15), 6325-6335. DOI: 10.1158/0008-5472.CAN-10-0015.

Chapter 4
Katanin AB and AL2 Recombinant Expression and Analysis

Chapter 4- Introduction

The structural understanding of the katanin subunits has gained much traction over the last few decades, beginning with Hartman's observations of the p60 and p80 sea urchin subunits under rotary-shadowing electron microscopy, to the recent cryo-electron microscopy (cryo-EM) images of p60 katanin depicting the p60:p80 complex or interactions and mechanism on the microtubule.[1-3] (Note: The literature interchanges p60 and p80 with A and B katanin, respectively. In the sea urchin literature, the katanins were first referred to as p60 and p80 based on their approximate molecular weights.)

The purpose of the work described in this chapter was to recombinantly express and purify A- and B- katanin for the use in structural analysis via cryo-EM, and also for use in analyzing the post-translational modifications these proteins encounter in mammalian cells. Developing an understanding of the katanin structure alone as well as in relation to other katanin proteins is important in elucidating their mechanism of action and paves the way for developing katanin-specific inhibitors or small molecule katanin targets for therapeutic use.

Chapter 4- Methods

pET Duet-1 A1-B1 construct creation, and E. coli induction

The pET Duet-1 vector (Sigma Aldrich #71146) was first digested using BamHI restriction enzyme (NEB #R3136S) to open up MCS1. The Gibson cloning method was used for cloning, as such the Katanin A1 was amplified using primers A1_For: GCAGCCATCA CCATCATCACCACAGCCAGGATCCAaTGAGTCTTCTTATGATTAGTGAGAATGTAAAA TTG and A1_rev: GCTTGTCGACCTGCAGGCGCGCCGAGCTCGAATTCTTAGCATGA TCCAAACTCAAATATCCATTTCTC; modeled for Gibson assembly, primers included homology with the katanin A1 protein backbone and 30 nt regions of overlap with the pET Duet-1 backbone. The pET-Duet-1 digested backbone and amplified A1 PCR product were ligated together using the traditional Gibson buffer system and protocols (NEB #E2611). Briefly the backbone and PCR product were combined with the buffer components of Gibson and incubated at 50°C for 1h. The reaction was then transformed into DH5α competent cells (Thermo Fisher #18265017) using the Thermo Fisher protocol. Colonies were picked and sequenced using Genewiz sequencing. Half of the sequence-verified A1_MCS1 pET Duet-1 clones were frozen for long-term storage and the second half was used for cloning B1 into MCS2. Katanin B1 was cloned into MCS2 using the same methods described above using the A1_MCS1 pET Duet-1 backbone, digesting it with KpnI (NEB #R3142S) to open MCS2. B1 primers included B1_for: AATTTCGCAGCAGCGGTTTCTTTACCAGACTCGAGGGTACCGTCCAGACTGGCCAT GAG and B1_rev: AATTTCGCAGCAGCGGTTTCTTTACCAGACTCGAGGGTACCGT CCAGACTGGCCATGAG. Katanin B1 primers were designed in the same manner as A1, with the same nucleotide number for Gibson overhang. The final construct was

transformed into DH5α cells as described above, and a portion of it was frozen for storage. The remainder was grown and transformed into BL21 cells for use in the IPTG induced overexpression experiments. A portion of this sample was also frozen and stored.

Sf9 cell viral transduction and protein induction

This work was done by our collaborator Dr. Mark Arbing and is similar to methods previously described.[4, 5]

Mammalian HA-tag AL2 overexpression

Cells were treated in the same manner as described in Chapter 2; however, instead of using AIO-mcherry, the cells were transfected with HA-tagged AL2 mammalian vector. Harvest of transfected cells was performed in a similar manner as cell harvest described in Chapters 2 and 3.

Mass spectrometry analysis

Excised gel bands were washed with 50:50 acetonitrile: 100mM ammonium bicarbonate. Each wash cycle was followed with a 15-minute incubation at 37°C. Proteins in gels were reduced with 100 mM TCEP for an hour with shaking at 56°C and then were alkylated with 100 mM iodoacetamide for 30 minutes in the dark at room temperature. Gel bands were washed again with 50:50 acetonitrile: 100mM ammonium bicarbonate and then covered in 100% acetonitrile for 10 minutes. Acetonitrile was removed and the gel bands were air-dried and digested with 0.4µg of trypsin (Pierce™) in 100 mM ammonium bicarbonate at 37°C overnight.

For each gel band, peptides were extracted with 47.5:47.5:5% water: acetonitrile: formic acid. Peptides were air dried and then reconstituted in 0.5% formic acid. Peptides were further concentrated and desalted with a C18 StageTip50 and then dried. Peptides were

dissolved in 1% acetonitrile with 0.1% formic acid and were separated on an EASY-Spray HPLC column (25cm x 75μM internal diameter, PepMAP RSLC C18, 2μM, Thermo-Fisher) connected to the UltiMate™ 3000 RSLCnano HPLC (ThermoFisher). Peptides were eluted using a 3%-35% acetonitrile in 0.1% formic acid elution gradient and a flow rate of 300nL/min. An Orbitrap Exploris 480 mass spectrometer (ThermoFisher) interfaced to a nano-ESI source (ThermoFisher) was used to collect tandem mass spectra in a data-dependent acquisition manner. The data was searched against the human proteome by Proteome Discoverer version 2.4 using the Mascot software. The precursor mass tolerance was ±10 ppm and the fragment mass tolerance was ±0.02 Th. For the peptide sequence search parameters, oxidation, phosphorylation, propionamide, acetylation, formylation, methylation and pyroglutamine were added as variable modifications and cysteine carba-midomethylation was added as a static modification.

Chapter 4- RESULTS

pET Duet-1 Recombinant Expression System

To obtain a recombinant protein with the katanin subunits of interest, I used a system known as pET Duet, which can express two proteins under the same promoter system in *E. coli*. I was able to produce a recombinant construct with A1 in cloning site 1 and B1 in cloning site 2. I optimized induction using trials at different temperatures and incubation times, and found that a 5h incubation at 37°C and an overnight incubation at 18°C were comparable; furthermore, the optimal IPTG induction concentration was determined to be 0.5 mM. Expression of the A1 subunit was clear while the B1 subunit was expressed at lower levels, if at all (Figure 4-1).

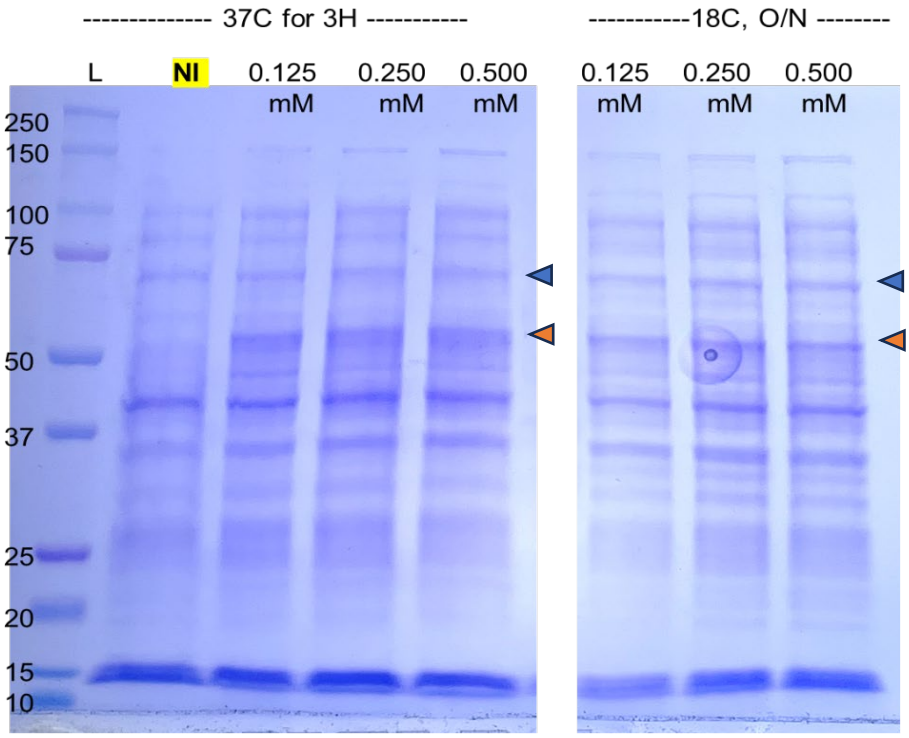

Figure 4-1 Trial Induction Conditions for pET Duet-1 containing A1 and B1 katanin. The pETDuet-1 plasmid containing A1 and B1 katanin was induced using 0.125mM-0.5mM of IPTG at 37°C for 5h and 18°C overnight. Samples were lysed then ran on a SDS-PAGE gel and stained with Coomassie Brilliant Blue (Thermo Fisher cat# 20279) for protein induction analysis. In the non-induced lane, no strong banding is seen at approximately 55kDa (orange arrow), where the A1 katanin weight is observed. At 72kDa (blue arrow) it is difficult to see the banding for katanin B1, however a slight increase can be seen around 72kDa when comparing the non-induced fraction to the 18°C overnight 0.5m MIPTG fraction.

After determining optimal induction conditions, I repeated the experiment using 0.5mM IPTG and incubated samples at 18°C overnight as this appeared to be the best conditions for both A1 and B1 katanin overexpression. The SDS-PAGE gel was transferred to a western membrane and immunoblotted with katanin A1 and B1 antibodies to determine if

the minimal overexpression observed on using Coomassie was true for the A1 and B1 proteins. The results demonstrated that indeed there was significant expression of both A1 and B1 proteins after IPTG induction (Figure 4-2).

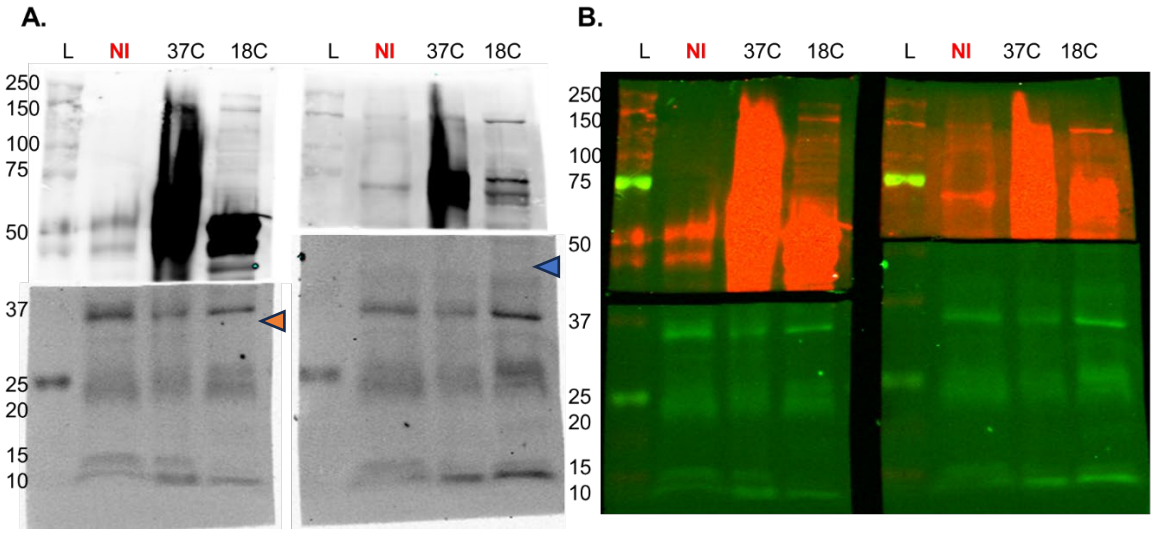

Figure 4-2 Immunoblot analysis of pET Duet-1 containing A1 and B1 katanin post IPTG induction. Samples were induced with 0.5mM IPTG and allowed to incubate at 37°C for 5h or 18°C overnight while shaking. Lysates were Immunoblotted against katanin specific antibodies. A. Black and white image captures the channel (wavelength 680 nm) each katanin is imaged using anti-rabbit katanin specific antibodies. Orange arrow shows A1 increase observed at both 37°C and 18°C conditions. Blue arrow shows a B1 increase only during the 37°C incubation window. B. Overlay of both channels (680 nm in red and 700 nm in green) capturing anti-rabbit katanin specific antibodies (red) and anti-mouse GAPDH specific antibodies for loading control (green).

Sf9 Insect Cell Expression System

While I was trying to get the recombinant *E. coli* system to work, the same protein purification using insect cells was attempted to obtain a purified protein that contained post-translational modifications that were observed in higher systems. It was our

hypothesis that with post-translational modifications, the protein stability may increase, and we would be able to obtain a structure that reflected that of higher order systems. I collaborated with Dr. Mark Arbing (Protein Expression Lab, UCLA-DOE Institute), who used molecular cloning to create three constructs: An N-terminal 6X histidine tag was added to the N-terminus of A1, while the N-terminus of B1 contained a SUMO and streptavidin tag; the AL2 was also designed to contain an N-terminal 6X histidine tag. These constructs were used to virally infect Sf9 insect cells for recombinant protein production.

Cells obtained from Dr. Arbing were harvested at Day 3 and Day 6 after protein expression was induced. Non-induced samples were also provided. The lysates were run via SDS-PAGE and underwent two types of expression confirmation: 1. General protein induction observed using Coomassie Stain and 2. Katanin-specific protein detection using immunoblotting (Figure 4-3). These tests confirmed at minimum the Sf9 insect cell induction of A1 and B1 katanin expression was successful for A1 at the Day 3 and Day 6 sample collection (Figure 4-3 B), while the katanin B1 expression struggled, suffering from either poor expression or possible sample degradation (Figure 4-3 C).

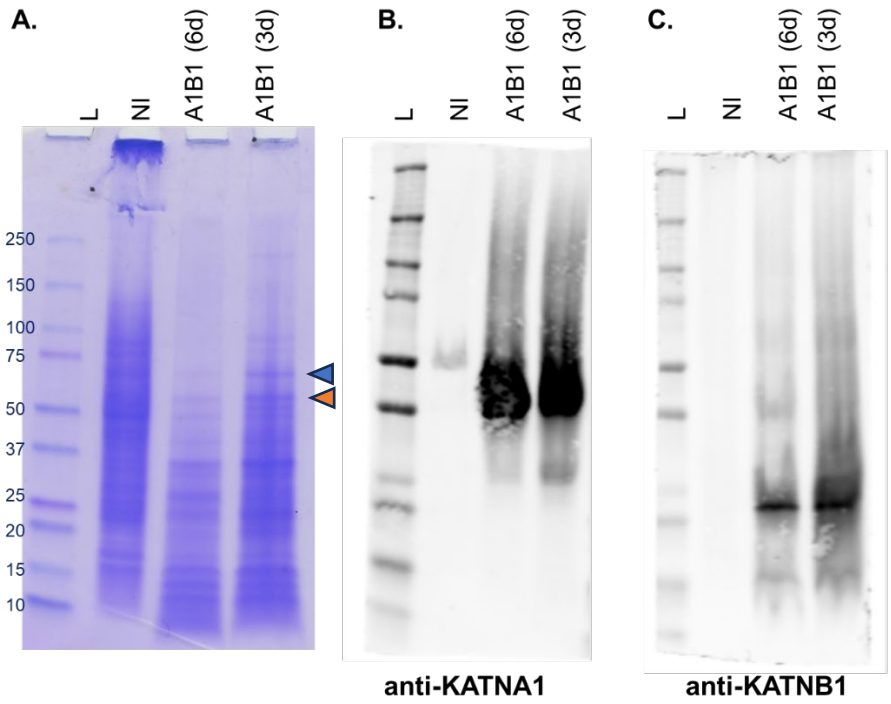

Figure 4-3 Coomassie Stain Gel of Sf9 Insect Cells Overexpressing A1-B1 co-expression. A. Left to Right shows lysed Sf9 containing A1-B1 co-expressed proteins for non-induced condition, then induced for 6d and 3d, respectively. The 3d co-expression exhibits better induction of protein in comparison to the 6d sample with a significant band pattern showing at ~50kDa (orange arrow) and ~72kDa (blue arrow), corresponding to the A1 and B1 katanin. B. Immunoblot using KATNA1 specific antibody confirms induction of katanin A1 subunit with high expression at 3d and 6d. C. Immunoblot using KATNB1 specific antibodies shows a lack of stable B1 protein at 72kDa, and instead banding around 20kDa.

Similar to the A1 and B1 viral Sf9 infection and protein induction, a construct containing the katanin AL2 protein was also used to infect Sf9 insect cells and underwent protein induction. Cells were collected in a similar manner as A1 and B1, at 3 and 6 day intervals. The lysates were run and tested in the manner described above for A1 and B1

(Coomassie stain and immunoblot) (Figure 4-4). Interestingly, unlike the A1 and B1 viral co-infection, AL2 demonstrated a clear induction by eye on the Coomassie stain (Figure 4-4 A)

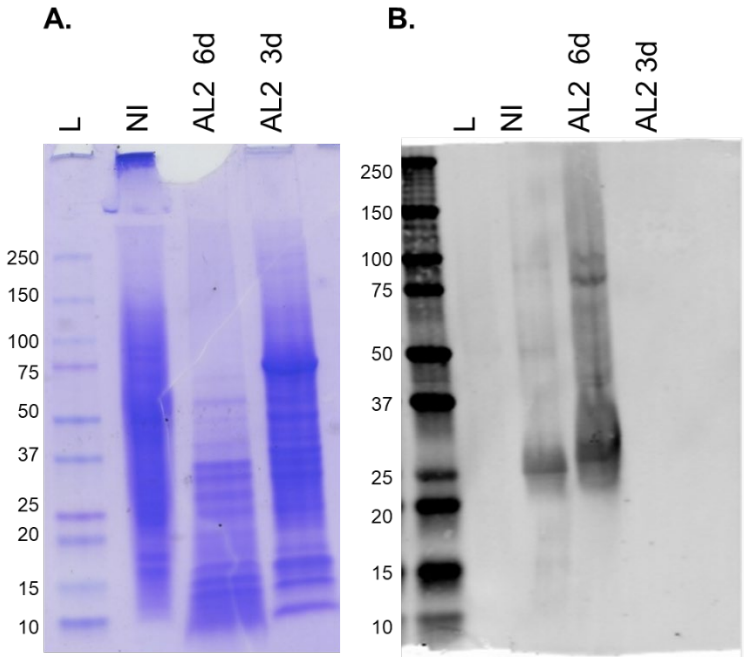

Figure 4-4 Coomassie Stain Gel of Sf9 Insect Cells Overexpressing A1-B1 co-expression. A. Coomassie stain of Sf9 insect cell lysates induced to overexpress katanin AL2. Left to Right shows lysed Sf9 containing AL2 proteins for non-induced condition, then induced for 6d and 3d, respectively. AL2 shows best induction at the 3d but appears to run at a higher size (~75kDa). B. Immunoblot using katanin AL2 specific antibodies confirms katanin AL2 overexpression, detecting AL2 again at a higher size (75kDa) for the 3-day induction condition.

Post-Translational Analysis of AL2 Katanin using HeLa Cells

While I waited for the Sf9 insect cells, I decided to pivot to a mammalian cell-based system. I chose this system because the purpose of these experiments was to obtain purified proteins that were overexpressed in systems that would implement post-

translational modifications that were comparable to mammals. Due to the stall in Sf9 cell growth, I chose to pivot into the next best system: mammalian cells.

Here, I chose to over-express an N-terminal HA-tagged AL2 in HeLa wild-type cells using the Fugene HD transient expression described in Chapter 2, iCas9 section 1. After transient transfection, the cells were collected and lysed. Lysates were analyzed using SDS-PAGE and then stained with Coomassie and Immunoblotted. After verifying the presence of AL2 in the immunoblot, I excised the corresponding bands from the SDS-PAGE Coomassie-stained gel for subsequent mass spectrometry measurements. Intriguingly, both the Coomassie stained gel and the immunoblot for HA-tagged katanin AL2 mammalian overexpression showed banding patterns at both 60kDa and 75kDa (Figure 4-5 A-B).

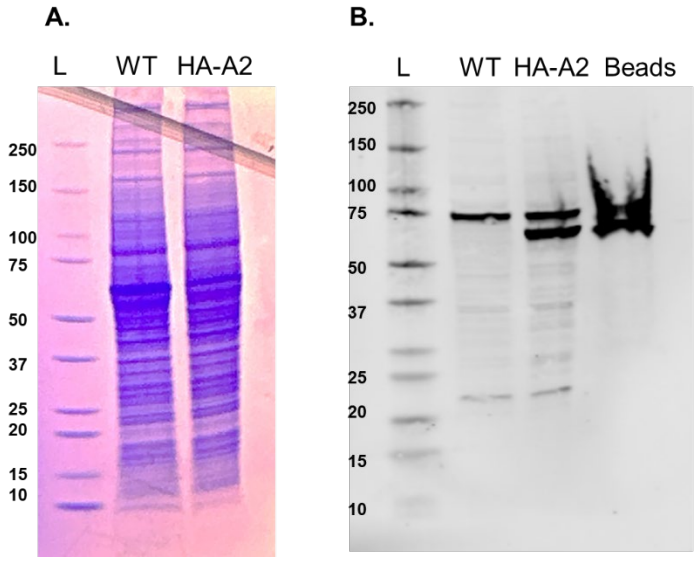

Figure 4-5. Overexpression of N-terminally HA-tagged katanin AL2. A. Coomassie stain of the non-transfected (WT) and transfected (HA-tagged) for AL2 overexpression to determine if overexpression worked. B. Immunoblot using katanin AL2 specific antibodies to detect overexpressed protein. Bands observed at 75kDa in both wild-type and

overexpressed conditions. In HA-A2 and bead conditions, a second band is observed around 60kDa.

I became curious as to what was happening, for example, was this a post-translational shift or is there a second protein (binding partner?) present A similar pattern was observed with the Sf9 cells, which also showed a band at 75kDa for the AL2 protein. This sparked the question about post-translational modifications and AL2 binding partners. To understand what was going on, I sent the gel bands to a collaborator in the Loo lab at UCLA. Collaborators in the Loo lab digested the bands in the gel observed at 60kDa and 75kDa and analyzed them using mass spectrometry and were able to produce a table of proteins that corresponded to each band (Table 4-1).

KATNAL2 Sample	MS Accession No.	MS Accession Description
WT	P02769	SWISS-PROT:P02769 (Bos taurus) Bovine serum albumin precursor
	P12763	SWISS-PROT:P12763 (Bos taurus) Alpha-2-HS-glycoprotein precursor
	P61978	Heterogeneous nuclear ribonucleoprotein K, Homo Sapiens
	P34955	SWISS-PROT:P34955 (Bos taurus) Alpha-1-antiproteinase precursor
	P11142	Heat shock cognate 71 kDa protein Homo sapiens
	P13797	Plastin-3, Homo sapiens
	P35527	SWISS-PROT:P35527 Keratin, type I cytoskeletal 9
	P14866	Heterogeneous nuclear ribonucleoprotein L Homo sapiens
	P17844	Probable ATP-dependent RNA helicase DDX5 Homo sapiens
	Q9Y262	Eukaryotic translation initiation factor 3 subunit L Homo sapiens
	P0DMV8	Heat shock 70 kDa protein 1A Homo sapiens
	Q07065	Cytoskeleton-associated protein 4 Homo sapiens
	P02768	Serum albumin Homo sapiens
	O43776	Asparagine--tRNA ligase, cytoplasmic Homo sapiens
	P29401	Transketolase Homo sapiens
HA-tagged	P02769	SWISS-PROT:P02769 (Bos taurus) Bovine serum albumin precursor
	P12763	SWISS-PROT:P12763 (Bos taurus) Alpha-2-HS-glycoprotein precursor
	P61978	Heterogeneous nuclear ribonucleoprotein Homo sapiens
	P11142	Heat shock cognate 71 kDa protein Homo sapiens
	P34955	SWISS-PROT:P34955 (Bos taurus) Alpha-1-antiproteinase precursor
	P10809	60 kDa heat shock protein, mitochondrial Homo sapiens
	P17987	T-complex protein 1 subunit alpha Homo sapiens
Unknown 60kDa	P07900	Heat shock protein HSP 90-alpha Homo sapiens
	P08238	Heat shock protein HSP 90-beta Homo sapiens
	P29966	Myristoylated alanine-rich C-kinase substrate Homo sapiens
	Q58FG1	Putative heat shock protein HSP 90-alpha A4 Homo sapiens
	Q92945	Far upstream element-binding protein 2 Homo sapiens
	P13639	Elongation factor 2 Homo sapiens
	Q14974	Importin subunit beta-1 Homo sapiens
	Q16891	MICOS complex subunit MIC60 Homo sapiens

Table 4-1. List of Mass-Spec Identified proteins in AL2 gel. Proteins detected in gel slices corresponding to Wild-Type, 75kDa (HA-overexpressed), and 60kDa (unknown)

bands from a SDS-PAGE gel stained with Coomassie Brilliant Blue. The grey highlighted regions demonstrate regions of overlap between WT and HA-tagged overexpressed AL2.

Chapter 4- Discussion

To determine whether or not the A1B1 pET Duet-1 protein induction (observed initially with the Coomassie stained gel (Figure 4-1)) was a true representation of the A1 and B1 proteins, immunoblot methods using antibodies specific to the A1 and B1 katanins were performed (Figure 4-2). The banding pattern on the immunoblot demonstrates that despite the minimal induction observed on the Coomassie gel, there is indeed a significant presence of katanin A1 and B1 proteins in the sample. There are notable inconsistencies observed via the loading control (GAPDH, green), with the 37°C induction showing a small dip in the protein values (Figure 4-2B). The variation in GAPDH can be due to loading errors and is likely masked due to the significant increase in katanin protein levels. It would be best practice to repeat these experiments and obtain clean and replicable data for future use of the recombinant pET Duet A1-B1 katanin system to confirm these data demonstrating positive induction for both A1 and B1.

The next stage of the pETDuet-1 A1-B1 katanin system was to scale up the reaction to produce and purify larger quantities of the A1-B1 protein. The pET Duet-1 system includes an N-terminal 6XHis tag in cloning site 1 and a C-terminal S-tag at cloning site 2. The N-terminal His-tag was associated with the A1 subunit and would have been used in column purification to obtain A1-B1 heterodimers, which were to be used in structural analysis of the katanin protein.

Because the pET Duet system exhibited unequal bicystronic expression (katanin A1 subunit was more heavily expressed in the Coomassie stain and in the immunoblot

compared to B1), it was suggested to me to stop using the A1-B1 vector for recombinant *E. coli* expression and purification, and in the future to instead re-clone the pET Duet-1 vector with the A1 and B1 katanin subunits in reverse order, with the B1 in cloning site 1 and A1 in cloning site 2.

The next stage of the protein expression trials took advantage of the Sf9 insect cell expression system. The purpose was to produce recombinant proteins of katanin A1, A1-B1, and AL1, in a system that allowed post-translational modifications appropriate for higher order systems. I was to use these proteins for structural and post-translational analysis using mass spectrometry to observe and understand more about higher order katanin structure as well as the post-translational modifications specific to the A-B heterodimer and AL2 individual subunit, which is undercharacterized compared to A1 and AL1[6]. The early inductions produced by Dr. Mark Arbing proved successful in inducing the A1 protein (Figure 4-3 A,B), however, the same could not be said for katanin B1, which showed minimal induction on the Coomassie stain (Figure 4-3 A) and possible degradation on the immunoblot (Figure 4-3 C). Unfortunately, the samples were exhausted during these trials as well as trials attempting mass spectrometry/proteomics measurements (data unavailable due to lack of sufficient sample). Our collaborator ran into a few months of contamination problems, preventing new Sf9 growth or transduction, and as a result prevented our ability to gather new samples. By the time our collaborator resolved the contamination issue, the project was terminated.

The final stage of this project was attempts toward mass spectrometry analysis of AL2 katanin using HA-overexpression in mammalian cells. The goal here was to learn more about post-translational modifications of AL2 as well as their interactors. The data in this

section represent the latter, examining the gel bands of non-induced and induced HA-AL2 overexpression, using a non-transfected wilt-type fraction as a control. The mass spectrometry data revealed a list of proteins that were identified in the gel bands from the AL2 overexpression experiment (Table 4-1). The grey highlights represent the proteins shared in wild type and HA-AL2 overexpression samples. Outside of the proteins shared with the control, the overexpressed HA-AL2 fraction was observed to associate with heat shock and molecular chaperone TCPA. The unknown band, which also was detected using the AL2 specific antibodies, associated with a wide range of proteins, including heat shock proteins, elongation proteins, and mitochondrial associated proteins. It would be interesting to follow up on this experiment with a larger fraction of overexpressed AL2 in order to determine if these interactions are real, or artifacts of the antibody used.

Acknowledgements

This work was made possible with the aid of our collaborator Dr. Mark Arbing at UCLA as well as Eileen Olivares in the Loo lab at UCLA. I am thankful for the work they put into this project.

References

(1) Hartman, J. J.; Mahr, J.; McNally, K.; Okawa, K.; Iwamatsu, A.; Thomas, S.; Cheesman, S.; Heuser, J.; Vale, R. D.; McNally, F. J. Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. *Cell* **1998**, *93* (2), 277-287.

(2) Shin, S. C.; Im, S. K.; Jang, E. H.; Jin, K. S.; Hur, E. M.; Kim, E. E. Structural and Molecular Basis for Katanin-Mediated Severing of Glutamylated Microtubules. *Cell Rep* **2019**, *26* (5), 1357-1367.e1355. DOI: 10.1016/j.celrep.2019.01.020.

(3) Faltova, L.; Jiang, K.; Frey, D.; Wu, Y.; Capitani, G.; Prota, A. E.; Akhmanova, A.; Steinmetz, M. O.; Kammerer, R. A. Crystal Structure of a Heterotetrameric Katanin p60:p80 Complex. *Structure* **2019**, *27* (9), 1375-1383.e1373. DOI: 10.1016/j.str.2019.07.002.

(4) Nika, L.; Wallner, J.; Palmberger, D.; Koczka, K.; Vorauer-Uhl, K.; Grabherr, R. Expression of full-length HER2 protein in Sf9 insect cells and its presentation on the surface of budded virus-like particles. *Protein Expr Purif* **2017**, *136*, 27-38. DOI: 10.1016/j.pep.2017.06.005.

(5) Jensen, I. S.; Inui, K.; Drakulic, S.; Jayaprakash, S.; Sander, B.; Golas, M. M. Expression of Flp Protein in a Baculovirus/Insect Cell System for Biotechnological Applications. *Protein J* **2017**, *36* (4), 332-342. DOI: 10.1007/s10930-017-9724-z.

(6) Lynn, N. A.; Martinez, E.; Nguyen, H.; Torres, J. Z. The Mammalian Family of Katanin Microtubule-Severing Enzymes. *Front Cell Dev Biol* **2021**, *9*, 692040. DOI: 10.3389/fcell.2021.692040.

Chapter 5

Conclusions and Future Directions

Chapter 5- Introduction

The katanin enzyme family are incredibly dynamic and important players within the cell, contributing to multiple events including cell homeostasis, signaling, division/proliferation, cell migration, and stages of cell development.[1-6] [7-11] Because the katanin protein family exhibits multifaceted roles within eukaryotic organisms, developing a better understanding of their holistic and individual subunit functions is critical. Expanding our knowledge on this family of proteins creates an opportunity to research their potential as targets for therapeutic disease intervention.

One such way I aimed to study the katanin family was by creating a system where I can knock-out each subunit in a targeted and sequential manner. I aimed to produce full A- and B- subunit knock-outs by targeting all A1 and B1 subunits within the cell (ΔA1, ΔAL1, ΔAL2, and ΔB1, ΔBL1). The purpose was to assess what happens to the cell upon full-subunit loss (cell death? excessive proliferation?), and also evaluate the stability of the B-subunit when A is completely missing (destabilization without a partner?). The second approach for knockout analysis was to create combinatorial knock-outs to assess one protein at a time. For example, ΔAL1, ΔAL2 to assess A1 localization, stability and eventually loss, and vice versa for AL1 and AL2. I planned to do the same with the katanin B-subunits. The katanin A-subunits have been known to exhibit overlapping phenotypes upon knockdown;[11-13] performing knock-out analysis as described above could help to elucidate their true localization, resolve individual function, and either confirm redundancy or provide insights on individual subunit mechanisms on the microtubule.

Chapter 5- Katanin Knockout Approaches

The inducible Cas9 approach that worked for the purposes of establishing a system for katanin knock-out was the iCas9 RPE-1 system that I derived. The benefit of this system is that the RPE-1 cells do not exhibit aneuploidy and therefore should ameliorate system issues with targeting multiple chromosomes. Another benefit to this system is that after transfection, the cells can be selected for colonies containing the sgRNA plasmid using puromycin. Future work could utilize the complimentary plasmid and use molecular cloning to insert the POI sgRNA, followed by selection and testing the colonies for knock-out.

The other systems implemented for the inducible Cas9 knock-out of katanin proteins largely did not work and could not be repeated, unfortunately (Chapter 2). Repeating these experiments in a cell line that does not exhibit aneuploidy (here HeLa was used) so as to determine if the lack of knock-out was due to poor sgRNA design, or due to an overabundance of chromosomes within the sample.

Chapter 5- Katanin Interactions with TP53

It was previously demonstrated that katanin A1 interacts with the DNA binding domain of TP53, and also is regulated at the level of the promoter by this protein.[14, 15] The katanin A-subunits exhibit a high homology in the catalytic AAA+ region, which was the region shown to bind to the TP53 DNA binding domain.[5, 13] Because of their high homology, and the overlapping functions previously demonstrated with the A-subunits, I hypothesized it was possible for the A-like subunits (AL1 and AL2) could also interact with and possibly are modulated by TP53. To investigate this, I first used a RPE-1 TP53 knock-out cell line to determine if the loss of TP53 had any effect on katanin protein levels within the cell.

The results indicated that upon loss of TP53, katanin AL2 protein levels decreased while the katanin BL1 levels increased minimally (Figure 3-1). This experiment was followed by a TP53 co-immunoprecipitation experiment where an N-terminal Flag-tagged TP53 was used to pull on/bind with katanin HA-tagged constructs in an *in-vitro* mammalian transcription and translation system. The results showed the TP53 may in fact interact with katanin AL2. The positive control (HA-TP53) showed binding while the negative control (HA-GAPDH did not bind); however, it was difficult to verify binding with A1 and AL2 (Figure 3-2). Future experiments should be repeated to verify repeatability for the binding. The next stages of this experiment would encompass assessment of protein localization and assessing the possibility for TP53 to act on the promoters of AL1 and AL2 to modulate expression. I also performed immunofluorescence analysis, but data is not included here.

Chapter 5- Katanin Protein Expression Analysis

The next stage of this project was to assess the protein structure and modifications of the katanin family members, specifically focusing on katanin A1, B1, and AL2. The reason to investigate A1 and B1 was to determine if we could obtain (1) a complete structure of mammalian A1B1 katanin, which has been difficult due to instability of the B-subunit,[16-19] and (2) an understanding of the post-translational modifications of the katanin A-B heterodimer as well as among AL2, which is undercharacterized compared to A1 and AL1. This portion of the project used recombinant protein expression methods to obtain species that overexpressed A1, AL2 and B1 katanin.

The pET Duet-1 strategy was successful in that I was able to induce protein overexpression for A1 and B1 katanin; the best conditions for overexpression were best

18°C overnight growth with 0.5mM IPTG (Figure 4-1). The induction itself was a weak example of overexpression; it is more common for the induced condition to have a thick band in the gel compared to the non-induced, and this was not the case for the pET Duet-1 system. Because it was unclear if the banding was truly an overexpression of the katanin A-B proteins, immunoblotting was implemented. The results from the immunoblot demonstrated that a viable induction (overexpression of A1 and B1) could be observed both in the 18°C and 37°C conditions (Figure 4-2). It was confirmed that the pET Duet-1 system containing A1 and B1 katanin successfully induced protein overexpression for both subunits, however the amount of B1 expression remained relatively low. To address this, swapping the orientation of the A-B subunits such that B1 was in the first cloning site and succeeded by A1 was recommended. Placing B1 in the first cloning site would theoretically increase the protein production because it is the first in the set of proteins under the same promoter. An ultimate goal would be to partner with a structural biologist to obtain a crystal or cryo-EM image of the A-B or B-A heterodimer and its higher order structures.

The next attempts for protein purification involved production of Sf9 cell purified katanin A1, B1, and AL2. I also worked in mammalian cells to transiently overexpress and observe the interactors for katanin AL2. The first project made it as far as confirming the protein presence after induction (Figure 4-3 and Figure 4-4) but contamination of the Sf9 cells was problematic. The mammalian cell overexpression was the most successful in obtaining data on protein interactions for AL2 (Table 4-1). It is difficult to interpret the data due to a large overlap between the overexpressed and wild-type samples, as well as the fact that neither sample detected katanin AL2. This is concerning, as the sample should

at minimum contain the protein of interest, as the goal includes understanding the interactions of this protein. Therefore, this data should be taken with some caution. The unknown band, which was also detected on the immunoblot for AL2, contained a variety of heat shock, elongation and other proteins. It would be interesting to evaluate the potential for interaction with these proteins and AL2 katanin.

The katanin family of microtubule-severing enzymes are important in many cellular activities and have implications in different diseases and cancer.[3, 20-22] Developing a better understanding of these proteins not only would be helpful for the greater understanding of science, but it also can aid with the potential future use of katanin as a therapeutic maker or target in the context of disease and cancer. Unlike the canonical A1 and B1 katanin family members, the other “like” family members are relatively understudied.[13, 23] As such, studies focused on these like-proteins are important for the greater understanding of the katanin family function.

Much of the work reported was stopped early, and was taken to a point where another student can use these samples and move forward. For example, the iCas9 RPE-1 cell lines can be used in conjunction with the original CNSI sgRNA constructs for tests in a system that does not exhibit aneuploidy (unlike HeLa). This is one jumping point for immediate data collection and confirmation of the validity of the borrowed sgRNA.

Outside of the iCas9 work, the construction of pET Duet-1 A1-B1 was validated for induced expression of each subunit. If one were not too selective about cloning site orientation, they can move forward immediately with protein overexpression and His-column purification (pET Duet-1 MCS1 includes a 6X His tag). Here, the histrionically expressed A1-B1 can be tested downstream for protein abundance, then utilized for

structure and post-translational analysis. The other option would be to move forward with re-cloning to obtain a B1-A1 orientation, and then move forward similarly.

The last stage of this research used Sf9 and mammalian overexpression systems to understand more about AL2 interactions and structures. The Sf9 cells encountered contamination, however I was able to overexpress AL2 with an HA N-terminal tag in HeLa cells. An immunoblot of the overexpressed fraction demonstrated a second band, either corresponding to degradation or to possible binding partners. With regard to katanin, it would be advantageous to repeat these experiments and also develop other *in vivo* methods (i.e., the knockouts, in cell binding) to get a better understanding of each subunit and resolve the inconsistencies among reported interacting partners.[24-26]

Chapter 5- References

(1) Lohret, T. A.; McNally, F. J.; Quarmby, L. M. A role for katanin-mediated axonemal severing during Chlamydomonas deflagellation. *Mol Biol Cell* **1998**, *9* (5), 1195-1207. DOI: 10.1091/mbc.9.5.1195.

(2) Hartman, J. J.; Mahr, J.; McNally, K.; Okawa, K.; Iwamatsu, A.; Thomas, S.; Cheesman, S.; Heuser, J.; Vale, R. D.; McNally, F. J. Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. *Cell* **1998**, *93* (2), 277-287.

(3) Willsey, H. R.; Walentek, P.; Exner, C. R. T.; Xu, Y.; Lane, A. B.; Harland, R. M.; Heald, R.; Santama, N. Katanin-like protein Katnal2 is required for ciliogenesis and brain development in Xenopus embryos. *Dev Biol* **2018**, *442* (2), 276-287. DOI: 10.1016/j.ydbio.2018.08.002.

(4) McNally, F. J.; Thomas, S. Katanin is responsible for the M-phase microtubule-severing activity in Xenopus eggs. *Mol Biol Cell* **1998**, *9* (7), 1847-1861. DOI: 10.1091/mbc.9.7.1847.

(5) McNally, F. J.; Vale, R. D. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **1993**, *75* (3), 419-429. DOI: 10.1016/0092-8674(93)90377-3.

(6) Loughlin, R.; Wilbur, J. D.; McNally, F. J.; Nédélec, F. J.; Heald, R. Katanin contributes to interspecies spindle length scaling in Xenopus. *Cell* **2011**, *147* (6), 1397-1407. DOI: 10.1016/j.cell.2011.11.014.

(7) Srayko, M.; O'toole, E. T.; Hyman, A. A.; Müller-Reichert, T. Katanin disrupts the microtubule lattice and increases polymer number in C. elegans meiosis. *Curr Biol* **2006**, *16* (19), 1944-1949. DOI: 10.1016/j.cub.2006.08.029.

(8) Smith, L. B.; Milne, L.; Nelson, N.; Eddie, S.; Brown, P.; Atanassova, N.; O'Bryan, M. K.; O'Donnell, L.; Rhodes, D.; Wells, S.; et al. KATNAL1 regulation of sertoli cell microtubule dynamics is essential for spermiogenesis and male fertility. *PLoS Genet* **2012**, *8* (5), e1002697. DOI: 10.1371/journal.pgen.1002697.

(9) Lombino, F. L.; Muhia, M.; Lopez-Rojas, J.; Brill, M. S.; Thies, E.; Ruschkies, L.; Lutz, D.; Richter, M.; Hausrat, T. J.; Lopes, A. T.; et al. The Microtubule Severing Protein Katanin Regulates Proliferation of Neuronal Progenitors in Embryonic and Adult Neurogenesis. *Sci Rep* **2019**, *9* (1), 15940. DOI: 10.1038/s41598-019-52367-3.

(10) Joly, N.; Martino, L.; Gigant, E.; Dumont, J.; Pintard, L. Microtubule-severing activity of the AAA+ ATPase Katanin is essential for female meiotic spindle assembly. *Development* **2016**, *143* (19), 3604-3614. DOI: 10.1242/dev.140830.

(11) Hatakeyama, E.; Hayashi, K. KATNAL1 is a more active and stable isoform of katanin, and is expressed dominantly in neurons. *Biochem Biophys Res Commun* **2018**, *507* (1-4), 389-394. DOI: 10.1016/j.bbrc.2018.11.048.

(12) McNally, K. P.; Buster, D.; McNally, F. J. Katanin-mediated microtubule severing can be regulated by multiple mechanisms. *Cell Motil Cytoskeleton* **2002**, *53* (4), 337-349. DOI: 10.1002/cm.10080.

(13) Lynn, N. A.; Martinez, E.; Nguyen, H.; Torres, J. Z. The Mammalian Family of Katanin Microtubule-Severing Enzymes. *Front Cell Dev Biol* **2021**, *9*, 692040. DOI: 10.3389/fcell.2021.692040.

(14) Korulu, S.; Yildiz, A. p60-katanin: a novel interacting partner for p53. *Mol Biol Rep* **2020**, *47* (6), 4295-4301. DOI: 10.1007/s11033-020-05557-6.

(15) Kırımtay, K.; Selçuk, E.; Kelle, D.; Erman, B.; Karabay, A. p53 regulates katanin-p60 promoter in HCT 116 cells. *Gene* **2020**, *727*, 144241. DOI: 10.1016/j.gene.2019.144241.

(16) Rezabkova, L.; Jiang, K.; Capitani, G.; Prota, A. E.; Akhmanova, A.; Steinmetz, M. O.; Kammerer, R. A. Structural basis of katanin p60:p80 complex formation. *Sci Rep* **2017**, *7* (1), 14893. DOI: 10.1038/s41598-017-14194-2.

(17) Jin, M.; Pomp, O.; Shinoda, T.; Toba, S.; Torisawa, T.; Furuta, K.; Oiwa, K.; Yasunaga, T.; Kitagawa, D.; Matsumura, S.; et al. Katanin p80, NuMA and cytoplasmic dynein cooperate to control microtubule dynamics. *Sci Rep* **2017**, *7*, 39902. DOI: 10.1038/srep39902.

(18) Jiang, K.; Rezabkova, L.; Hua, S.; Liu, Q.; Capitani, G.; Altelaar, A. F. M.; Heck, A. J. R.; Kammerer, R. A.; Steinmetz, M. O.; Akhmanova, A. Microtubule minus-end regulation at spindle poles by an ASPM-katanin complex. *Nat Cell Biol* **2017**, *19* (5), 480-492. DOI: 10.1038/ncb3511.

(19) Nithianantham, S.; McNally, F. J.; Al-Bassam, J. Structural basis for disassembly of katanin heterododecamers. *J Biol Chem* **2018**, *293* (27), 10590-10605. DOI: 10.1074/jbc.RA117.001215.

(20) Ye, Q.; Zhang, M.; Yin, Y. Katanin P80 correlates with larger tumor size, lymph node metastasis, and advanced TNM stage and predicts poor prognosis in non-small-cell lung cancer patients. *J Clin Lab Anal* **2020**, *34* (4), e23141. DOI: 10.1002/jcla.23141.

(21) Li, X.; Liu, J.; Shi, P. F.; Fu, P. Katanin P80 expression correlates with lymph node metastasis and worse overall survival in patients with breast cancer. *Cancer Biomark* **2018**, *23* (3), 363-371. DOI: 10.3233/CBM-181369.

(22) Hu, W. F.; Pomp, O.; Ben-Omran, T.; Kodani, A.; Henke, K.; Mochida, G. H.; Yu, T. W.; Woodworth, M. B.; Bonnard, C.; Raj, G. S.; et al. Katanin p80 regulates human cortical development by limiting centriole and cilia number. *Neuron* **2014**, *84* (6), 1240-1257. DOI: 10.1016/j.neuron.2014.12.017.

(23) McNally, F. J.; Roll-Mecak, A. Microtubule-severing enzymes: From cellular functions to molecular mechanism. *J Cell Biol* **2018**, *217* (12), 4057-4069. DOI: 10.1083/jcb.201612104.

(24) Dunleavy, J. E. M.; O'Connor, A. E.; Okuda, H.; Merriner, D. J.; O'Bryan, M. K. KATNB1 is a master regulator of multiple katanin enzymes in male meiosis and haploid germ cell development. *Development* **2021**, *148* (24). DOI: 10.1242/dev.199922.

(25) Dunleavy, J. E. M.; Okuda, H.; O'Connor, A. E.; Merriner, D. J.; O'Donnell, L.; Jamsai, D.; Bergmann, M.; O'Bryan, M. K. Katanin-like 2 (KATNAL2) functions in multiple aspects of haploid male germ cell development in the mouse. *PLoS Genet* **2017**, *13* (11), e1007078. DOI: 10.1371/journal.pgen.1007078.

(26) Cheung, K.; Senese, S.; Kuang, J.; Bui, N.; Ongpipattanakul, C.; Gholkar, A.; Cohn, W.; Capri, J.; Whitelegge, J. P.; Torres, J. Z. Proteomic Analysis of the Mammalian Katanin

Family of Microtubule-severing Enzymes Defines Katanin p80 subunit B-like 1 (KATNBL1) as a Regulator of Mammalian Katanin Microtubule-severing. *Mol Cell Proteomics* **2016**, *15* (5), 1658-1669. DOI: 10.1074/mcp.M115.056465.