

University of Windsor

Scholarship at UWindsor

UWill Discover Conference

UWill Discover 2022

Applying CRISPR/Cas9 and fluorescent tools to dissect the role of Tuberin in cell cycle regulation

Ali Nadi

University of Windsor, nadi111@uwindsor.ca

Jackie Fong MSc

University of Windsor, Jackie.Fong@uwindsor.ca

Elizabeth Fidalgo Da Silva PhD

University of Windsor, FIDALGO@UWINDSOR.CA

Lisa Porter PhD

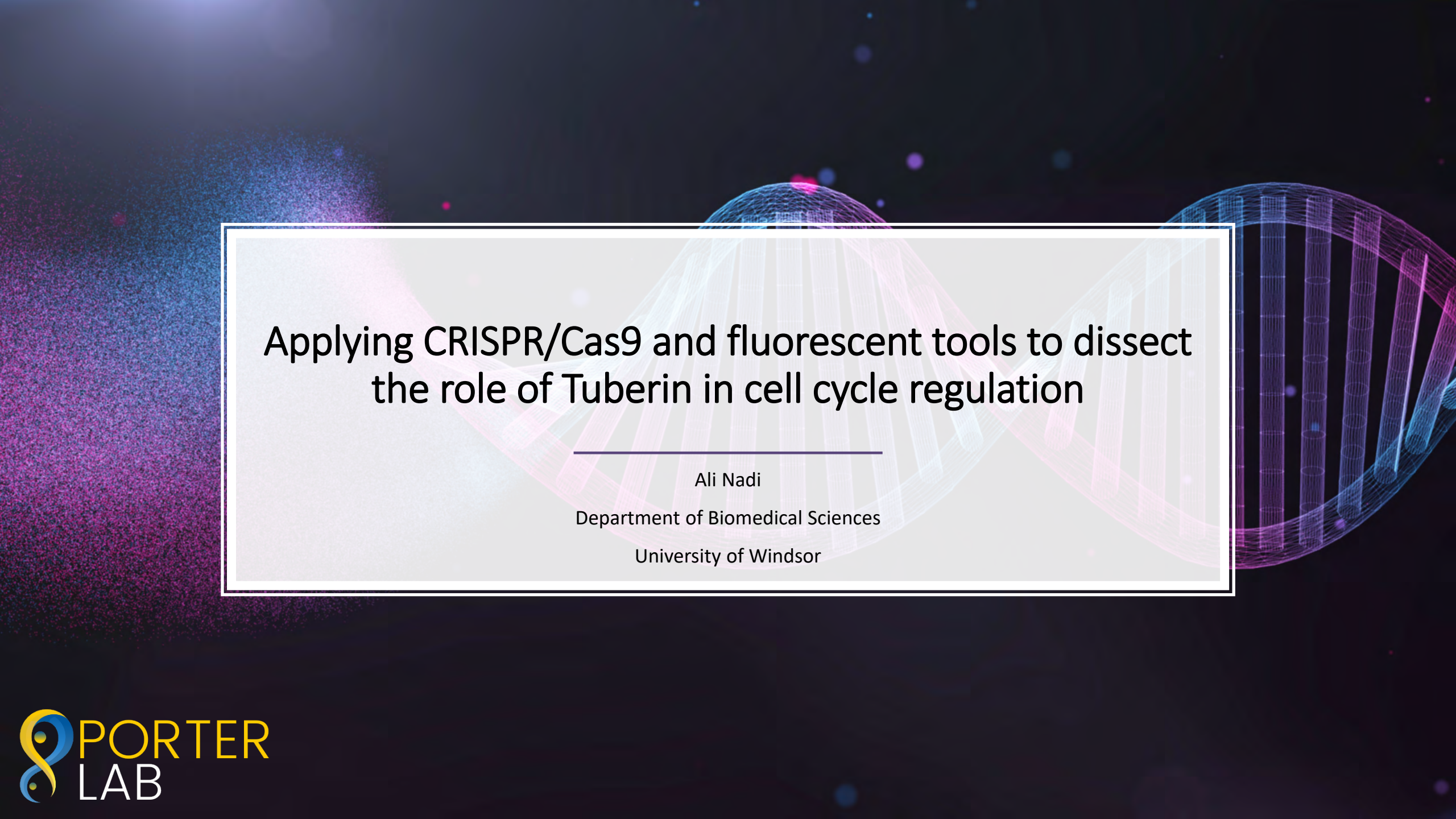
University of Windsor, lporter@uwindsor.ca

Follow this and additional works at: <https://scholar.uwindsor.ca/uwilldiscover>

Nadi, Ali; Fong, Jackie MSc; Fidalgo Da Silva, Elizabeth PhD; and Porter, Lisa PhD, "Applying CRISPR/Cas9 and fluorescent tools to dissect the role of Tuberin in cell cycle regulation" (2022). *UWill Discover Conference*. 14.

<https://scholar.uwindsor.ca/uwilldiscover/2022/2022Day2/14>

This Event is brought to you for free and open access by the Conferences and Conference Proceedings at Scholarship at UWindsor. It has been accepted for inclusion in UWill Discover Conference by an authorized administrator of Scholarship at UWindsor. For more information, please contact scholarship@uwindsor.ca.



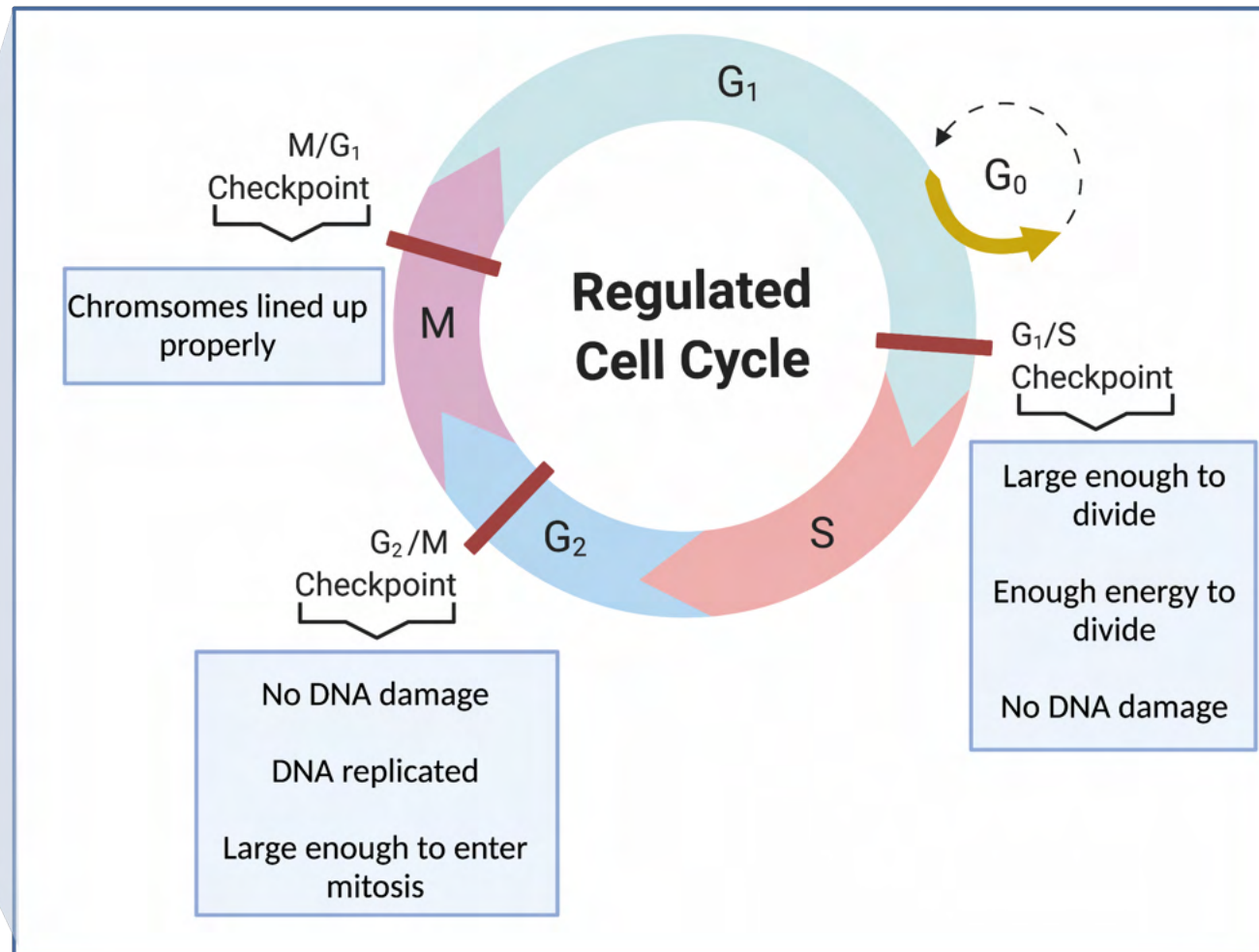
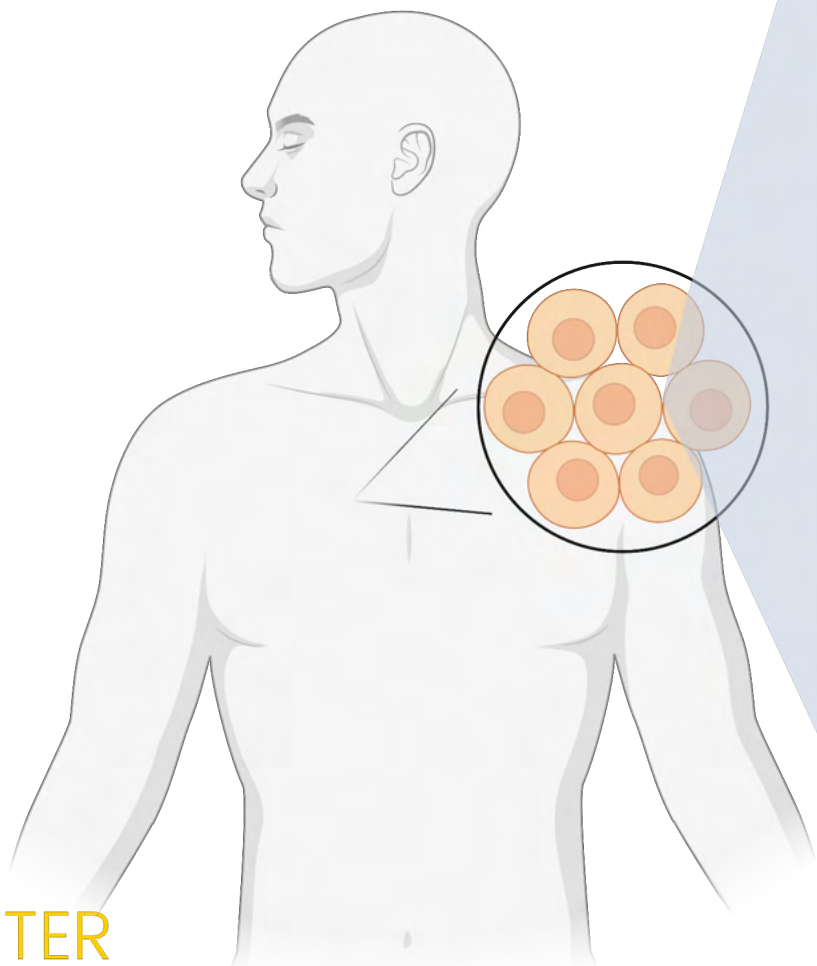
Applying CRISPR/Cas9 and fluorescent tools to dissect the role of Tuberin in cell cycle regulation

Ali Nadi

Department of Biomedical Sciences

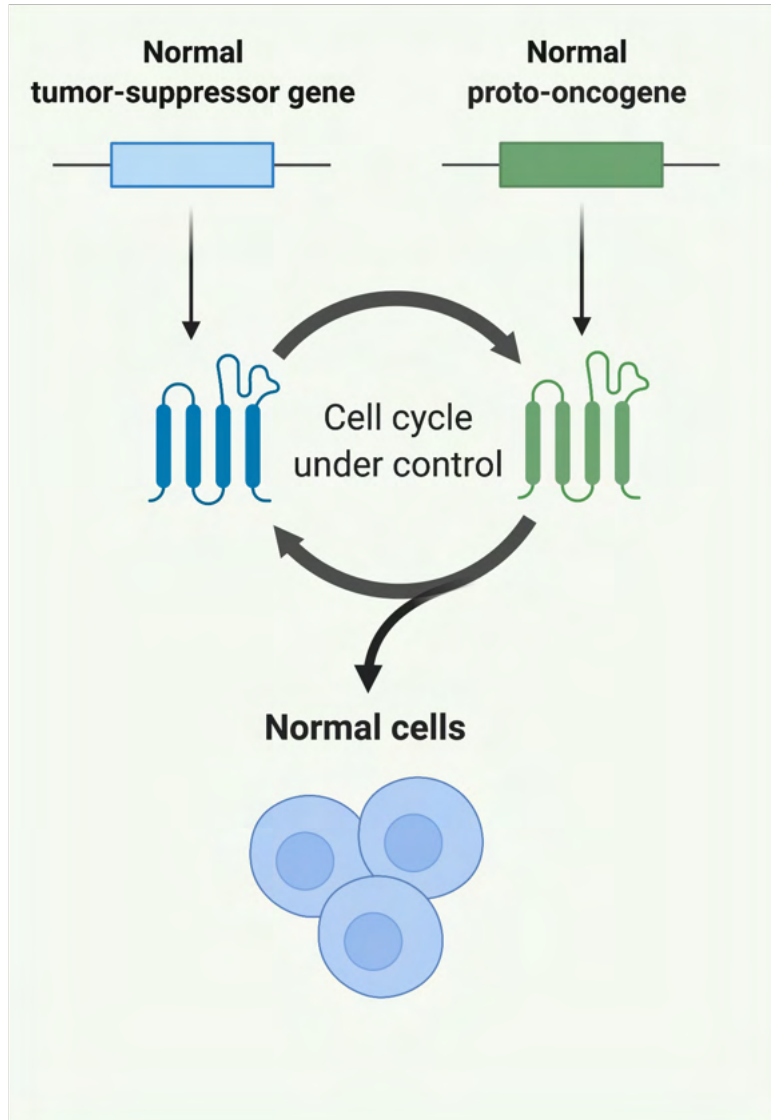
University of Windsor

To proliferate or not to proliferate, that is the question

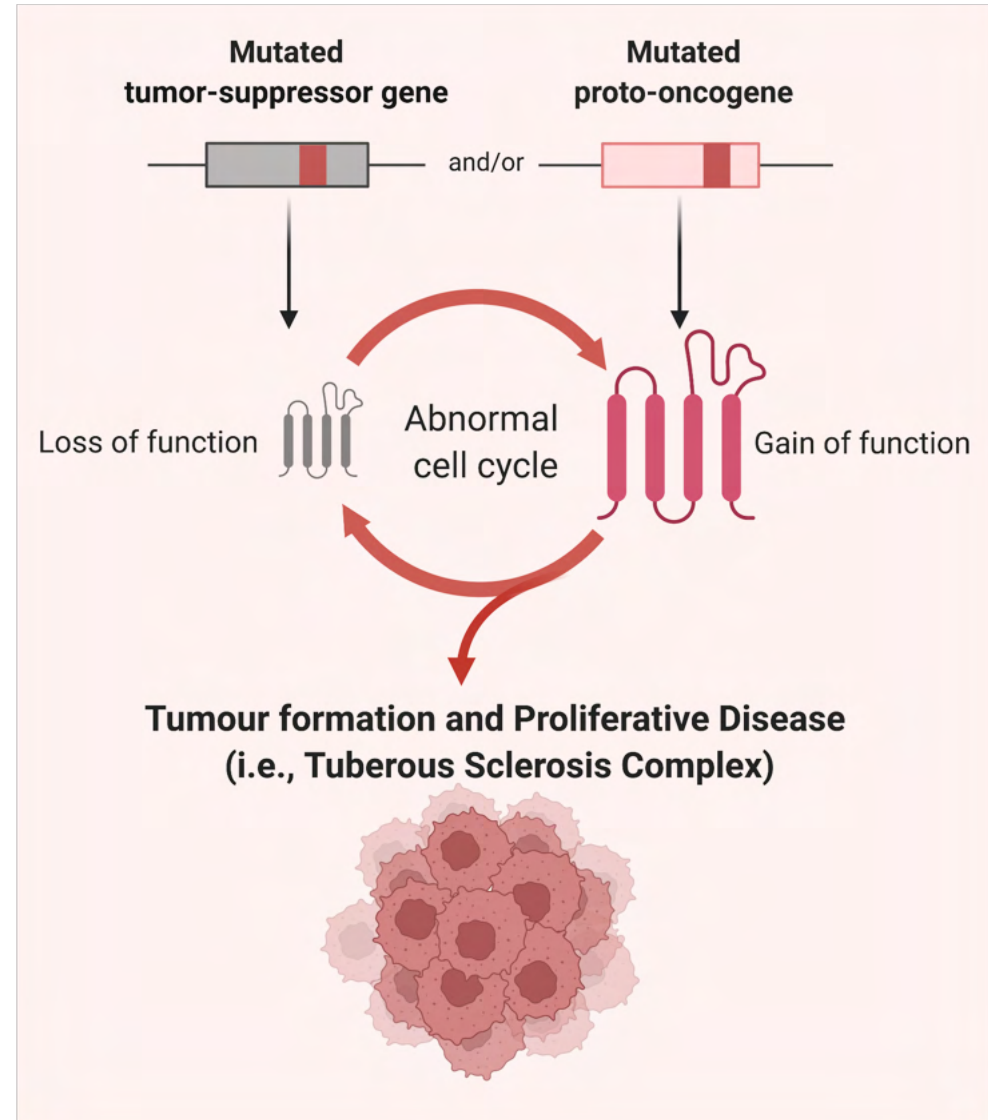


Tumour Suppressors Rein in Cellular Division

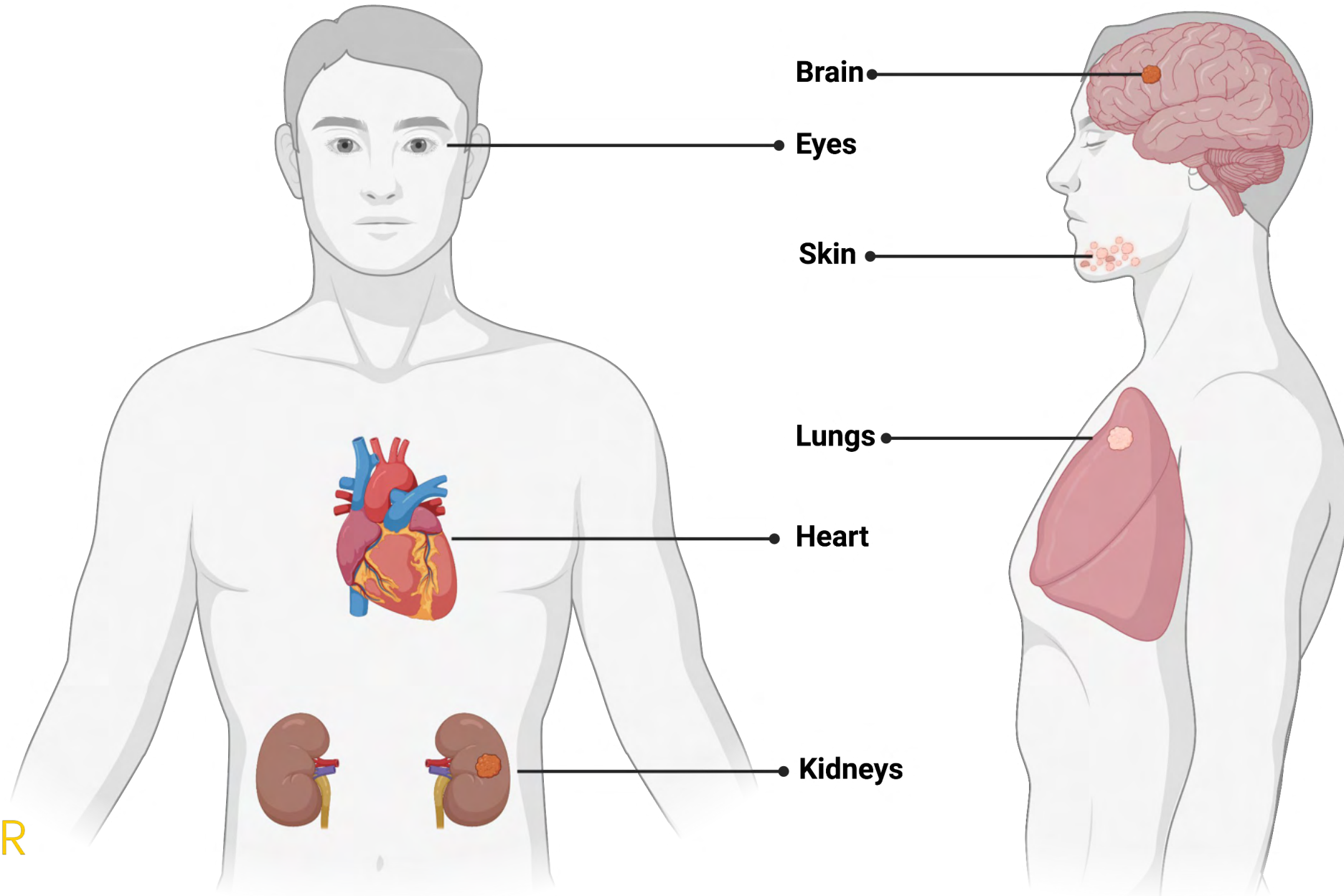
Normal Cell Division



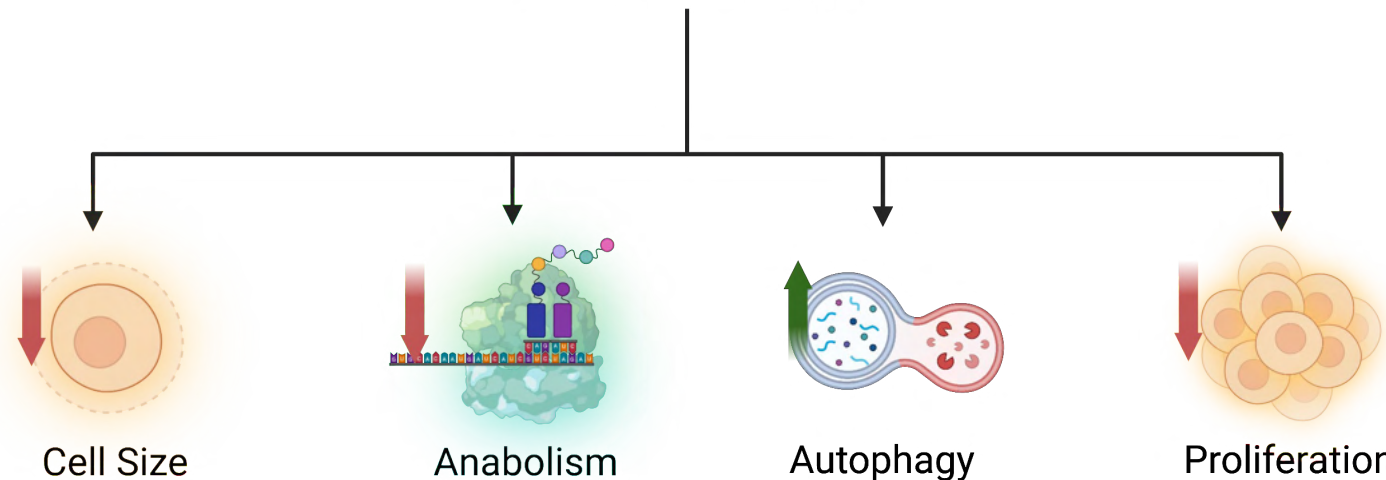
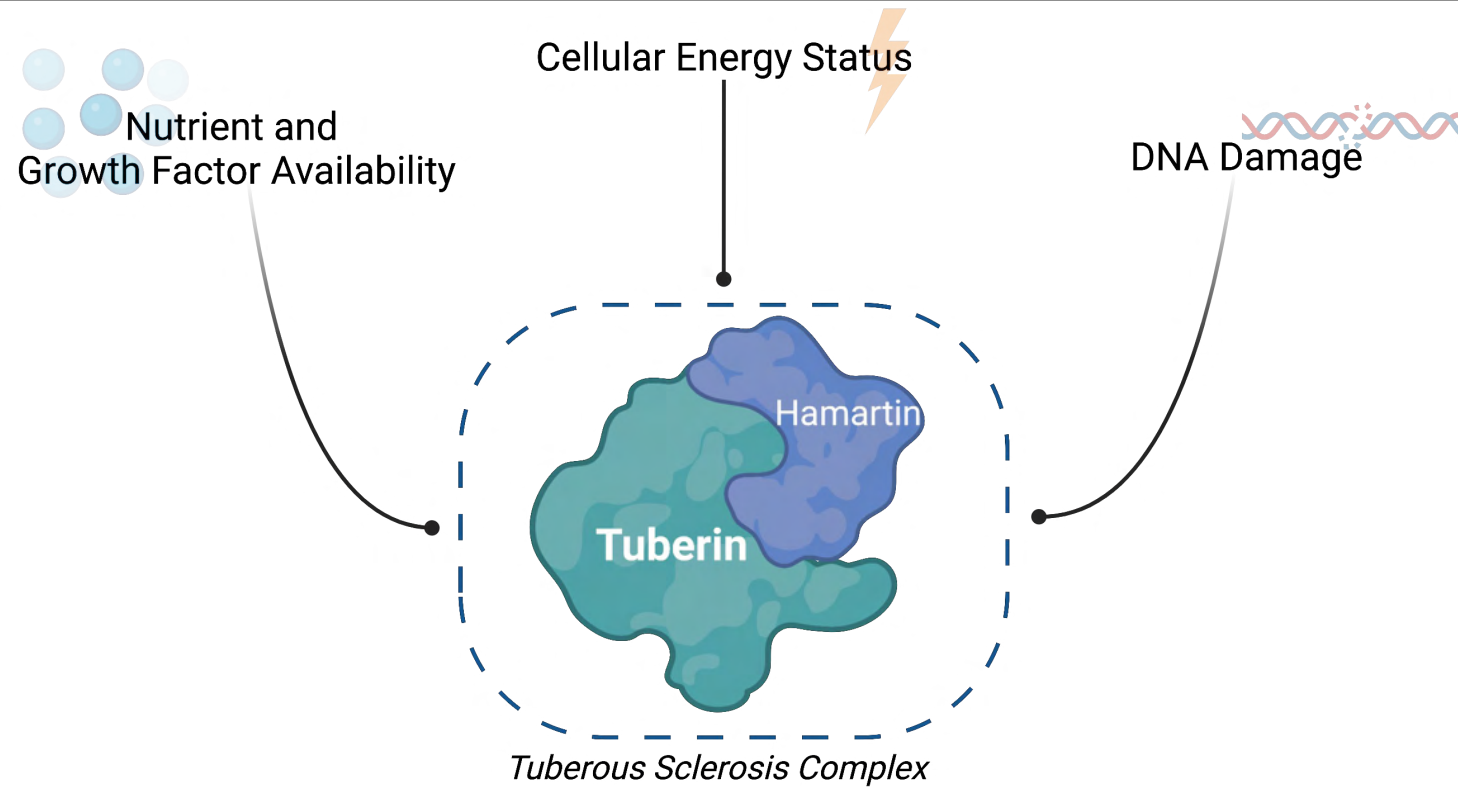
Abnormal Cell Division



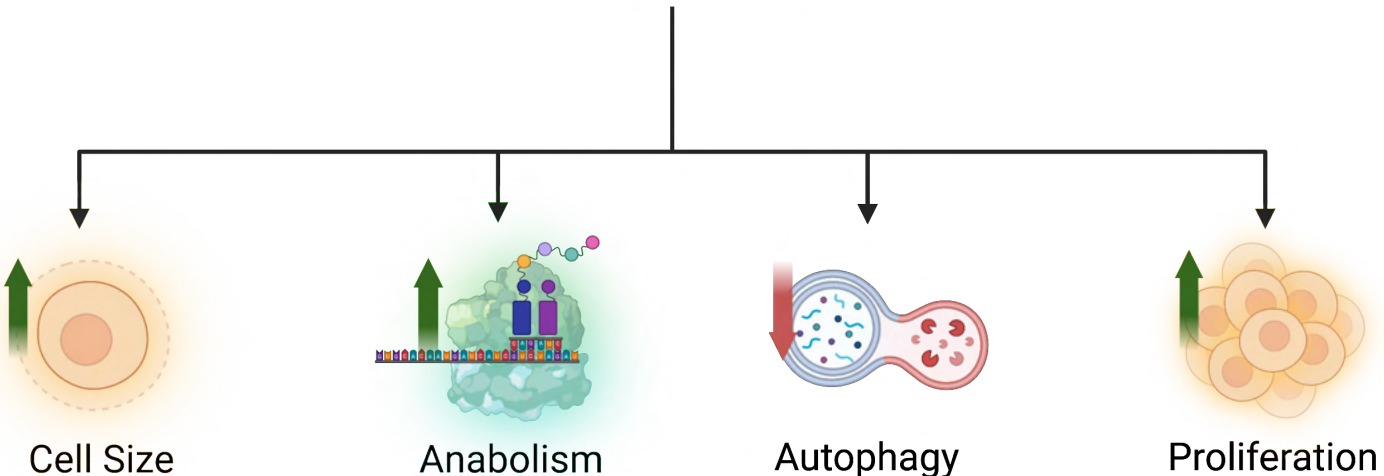
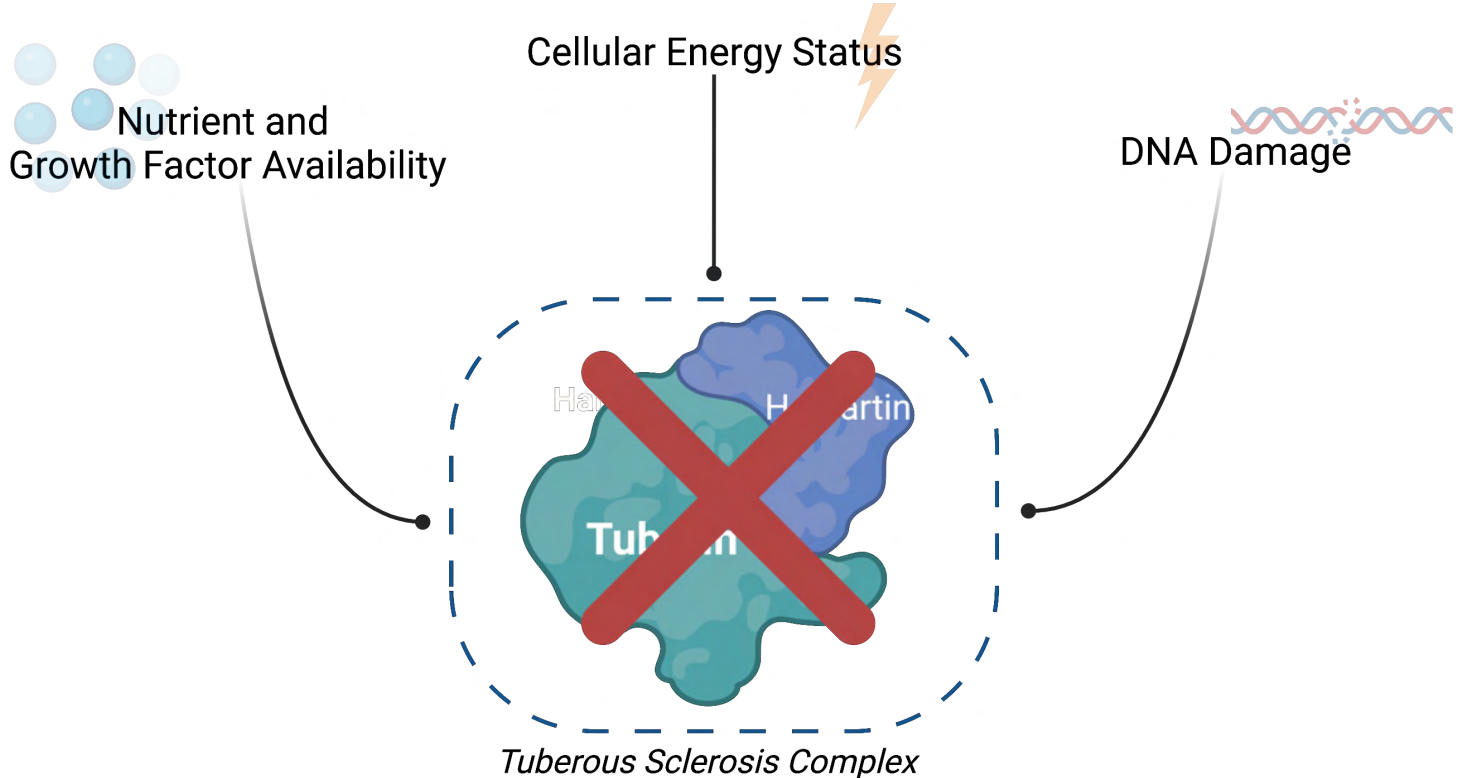
Tuberous Sclerosis Complex



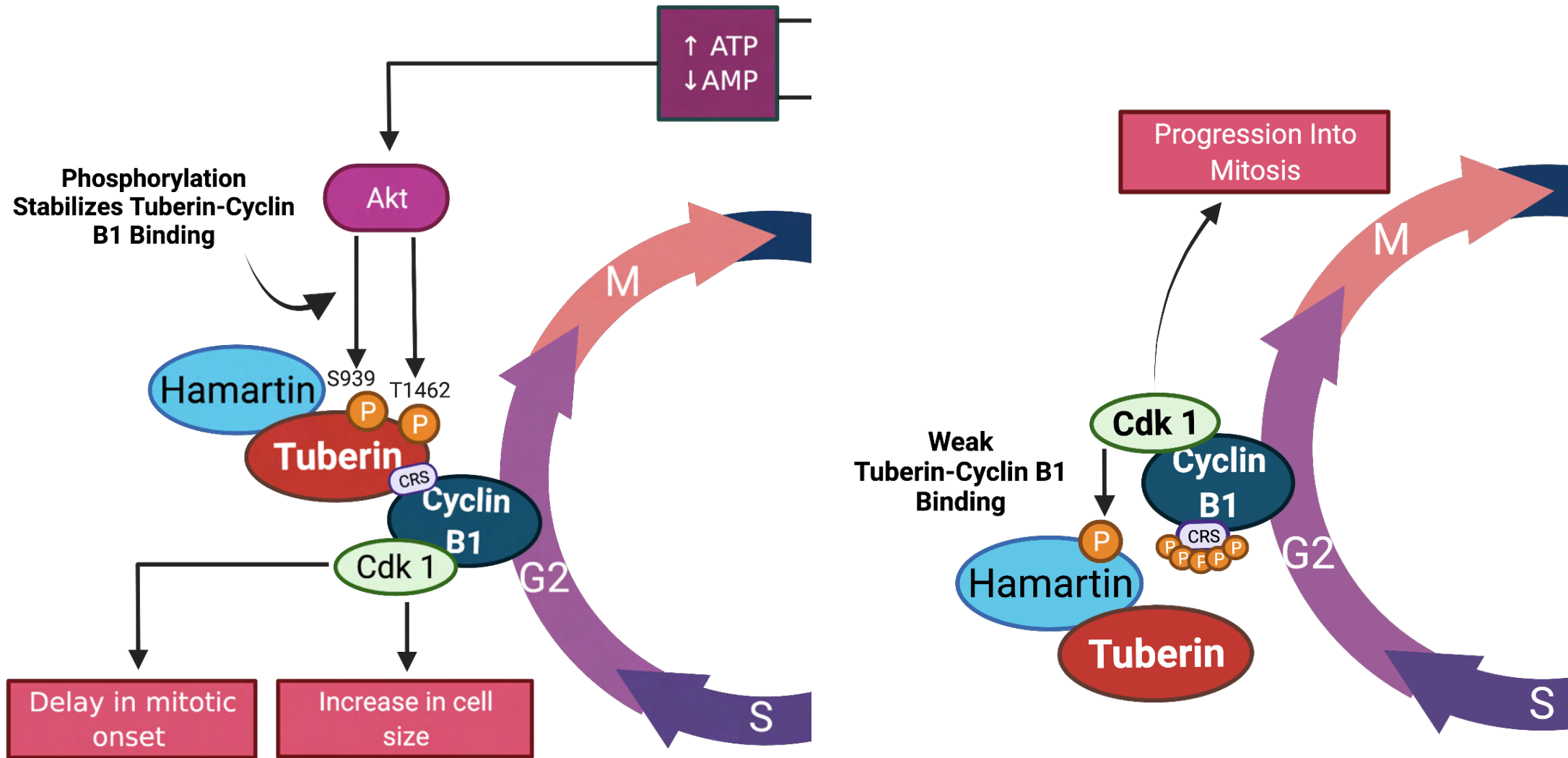
A Functional Tuberous Sclerosis Complex



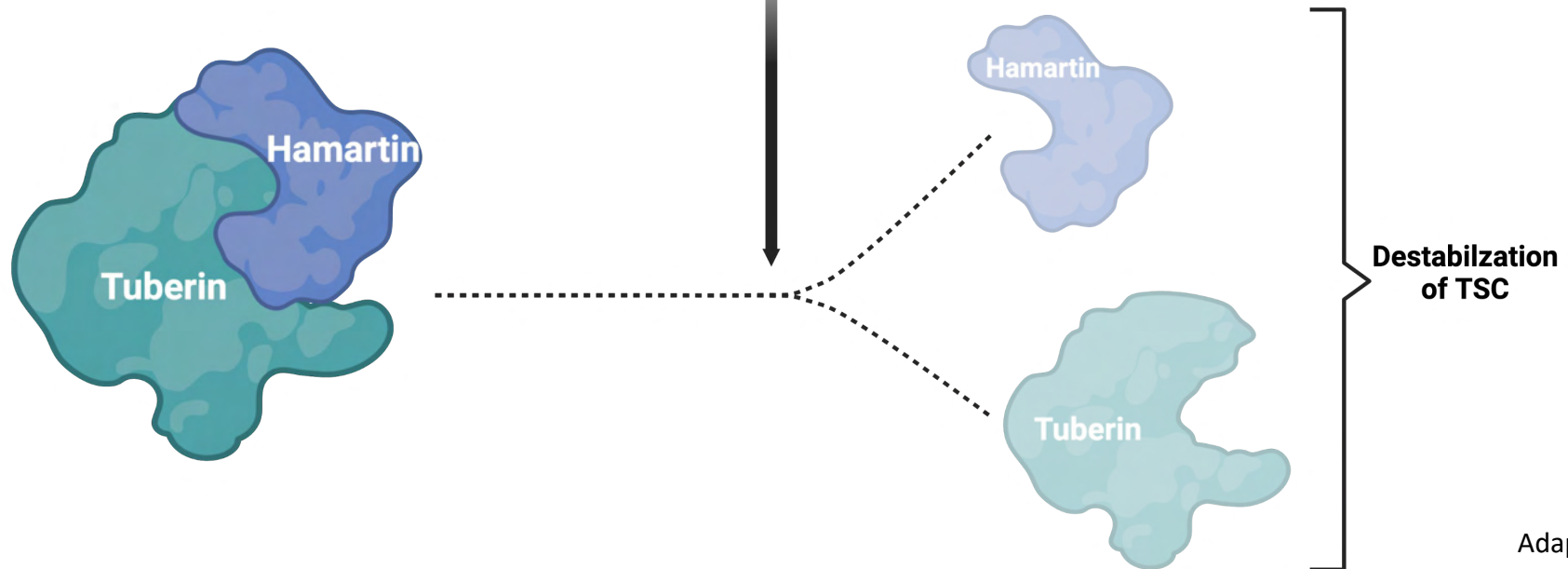
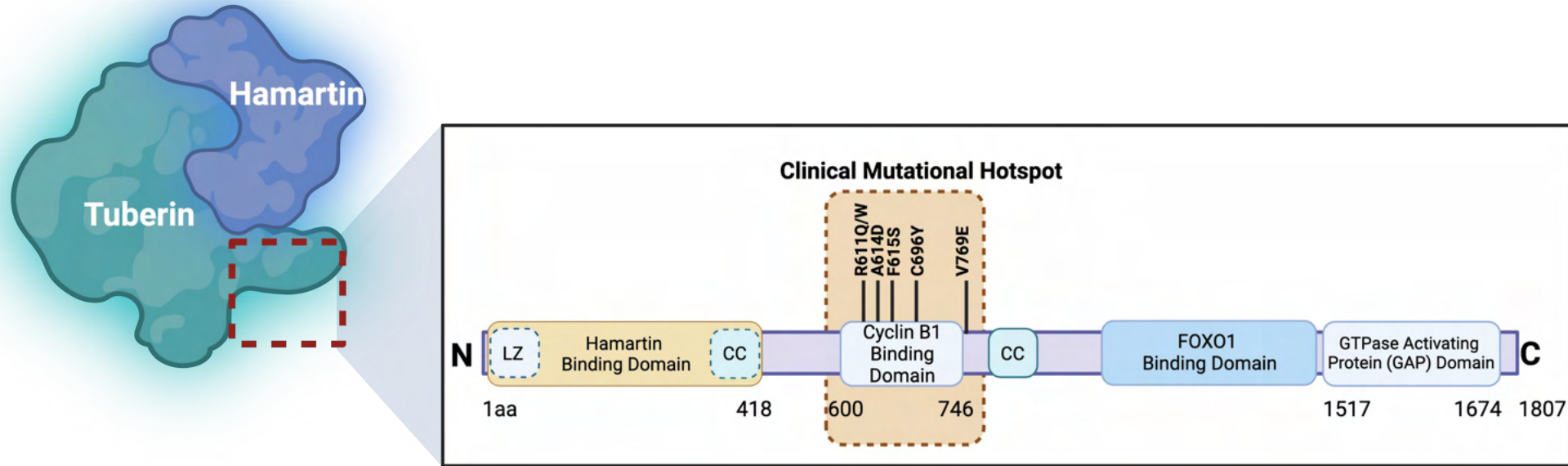
Disruption of the Tuberous Sclerosis Complex



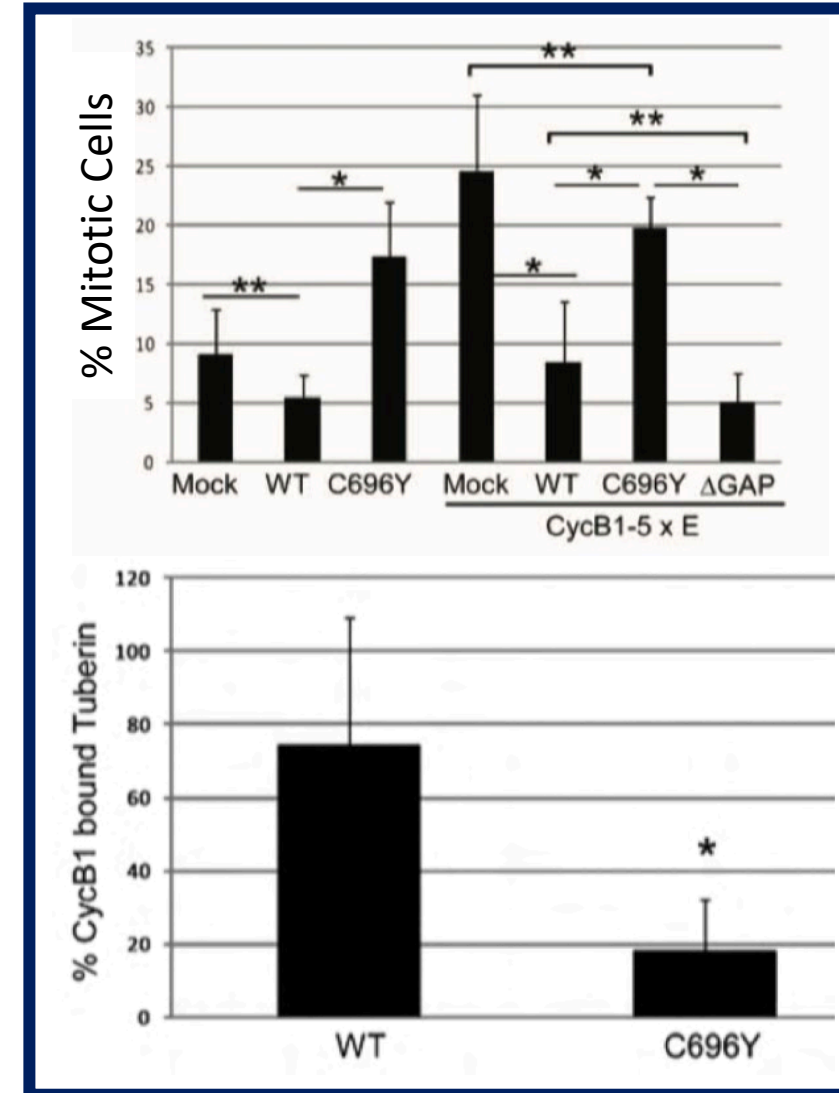
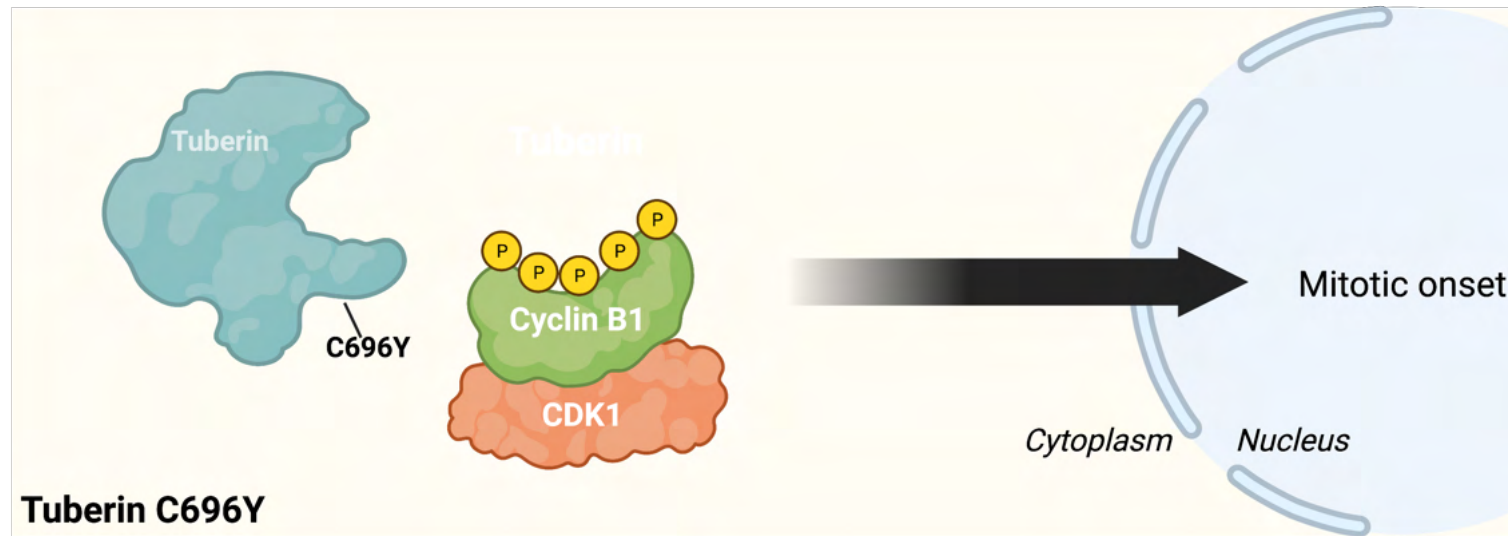
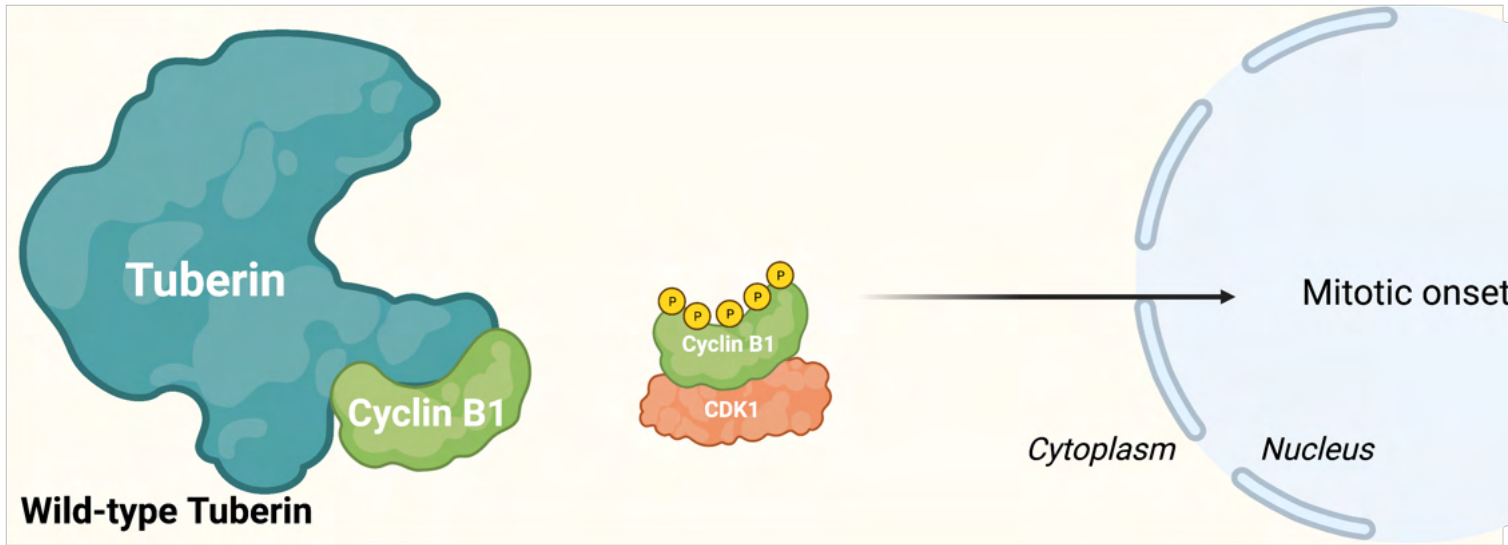
Tuberin: A Key Tumour Suppressor



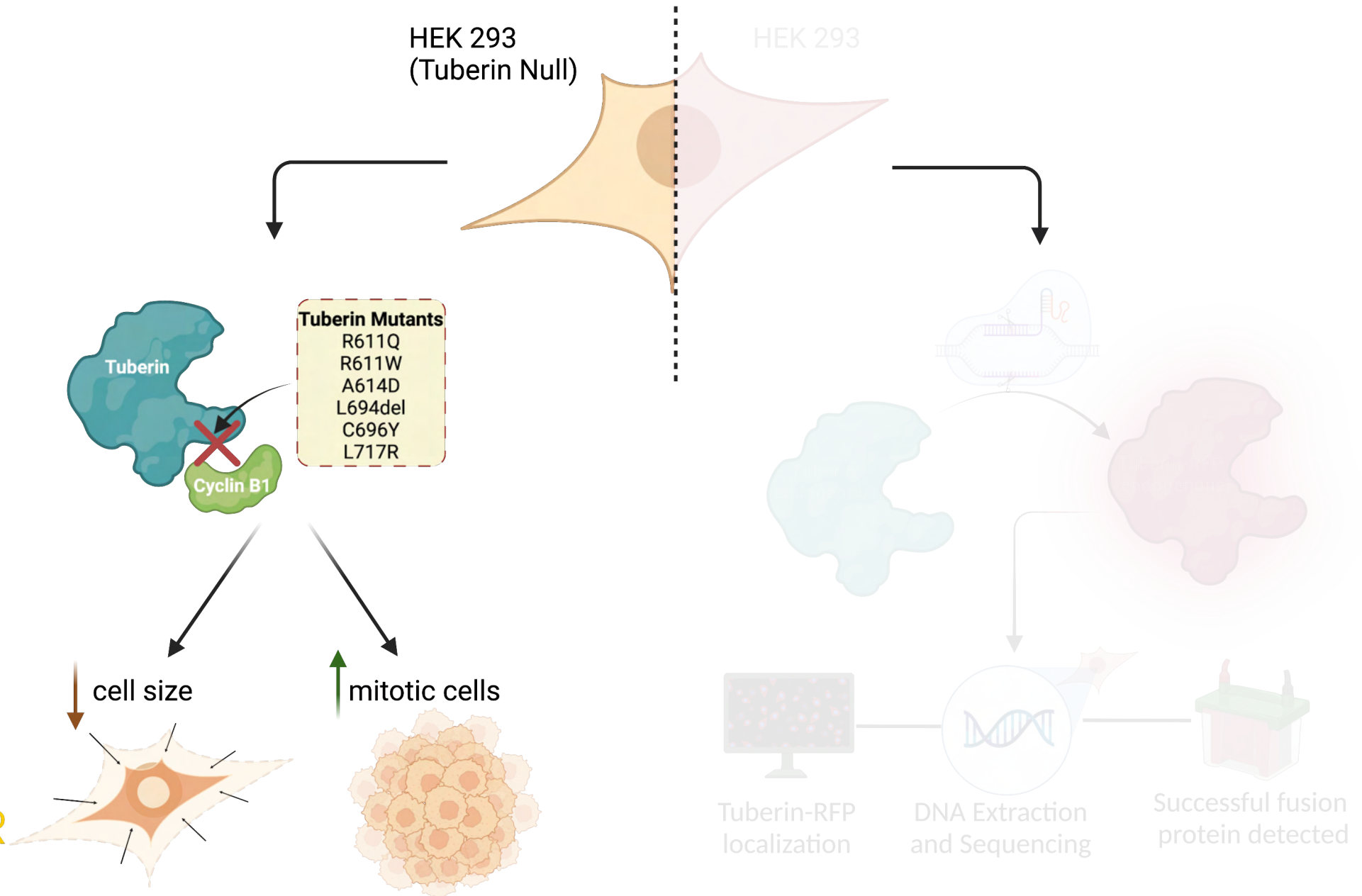
Tuberlin Clinical Mutations Destabilize the TSC



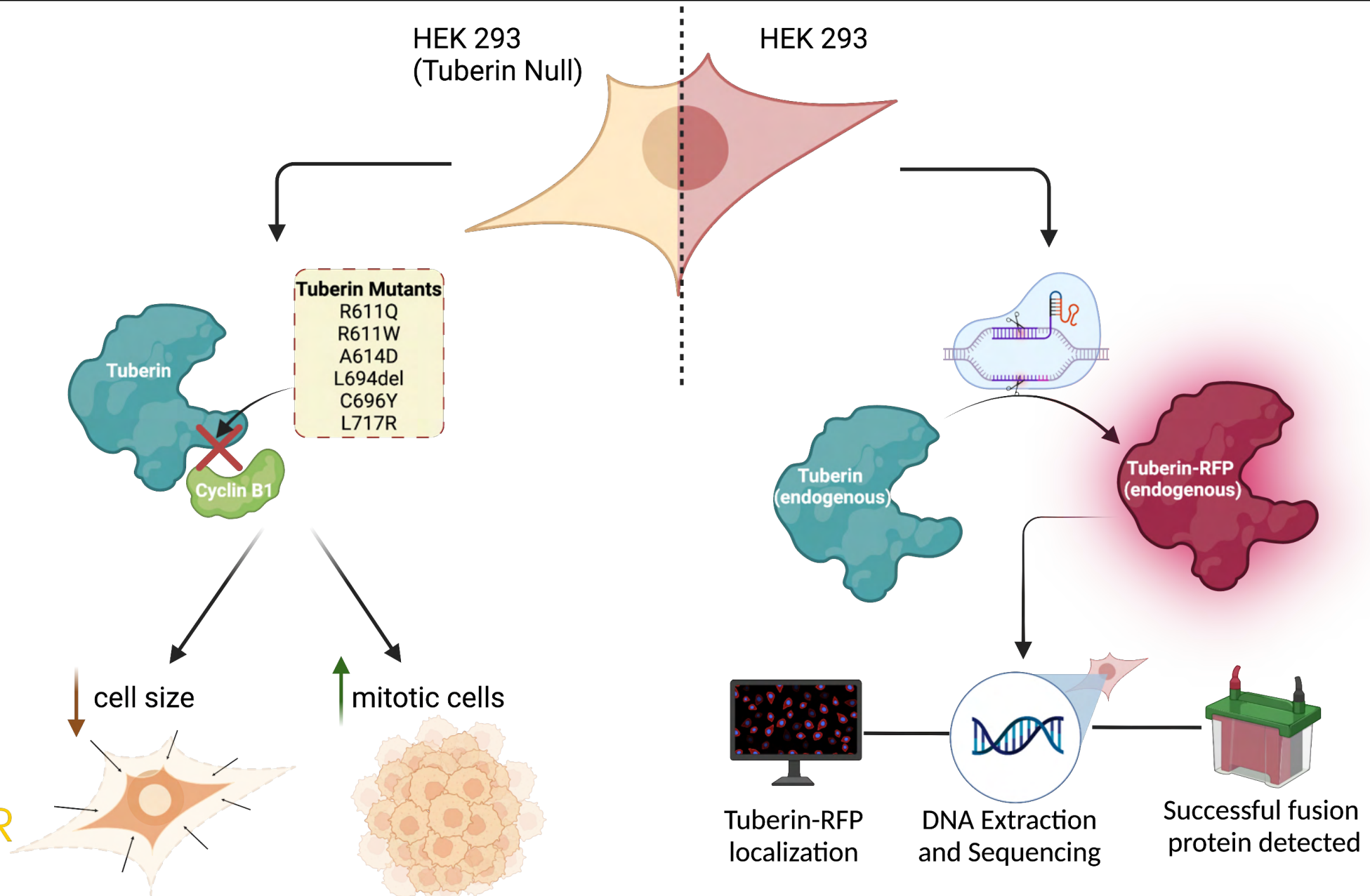
Tuberin-C696Y Results in Earlier Mitotic Onset



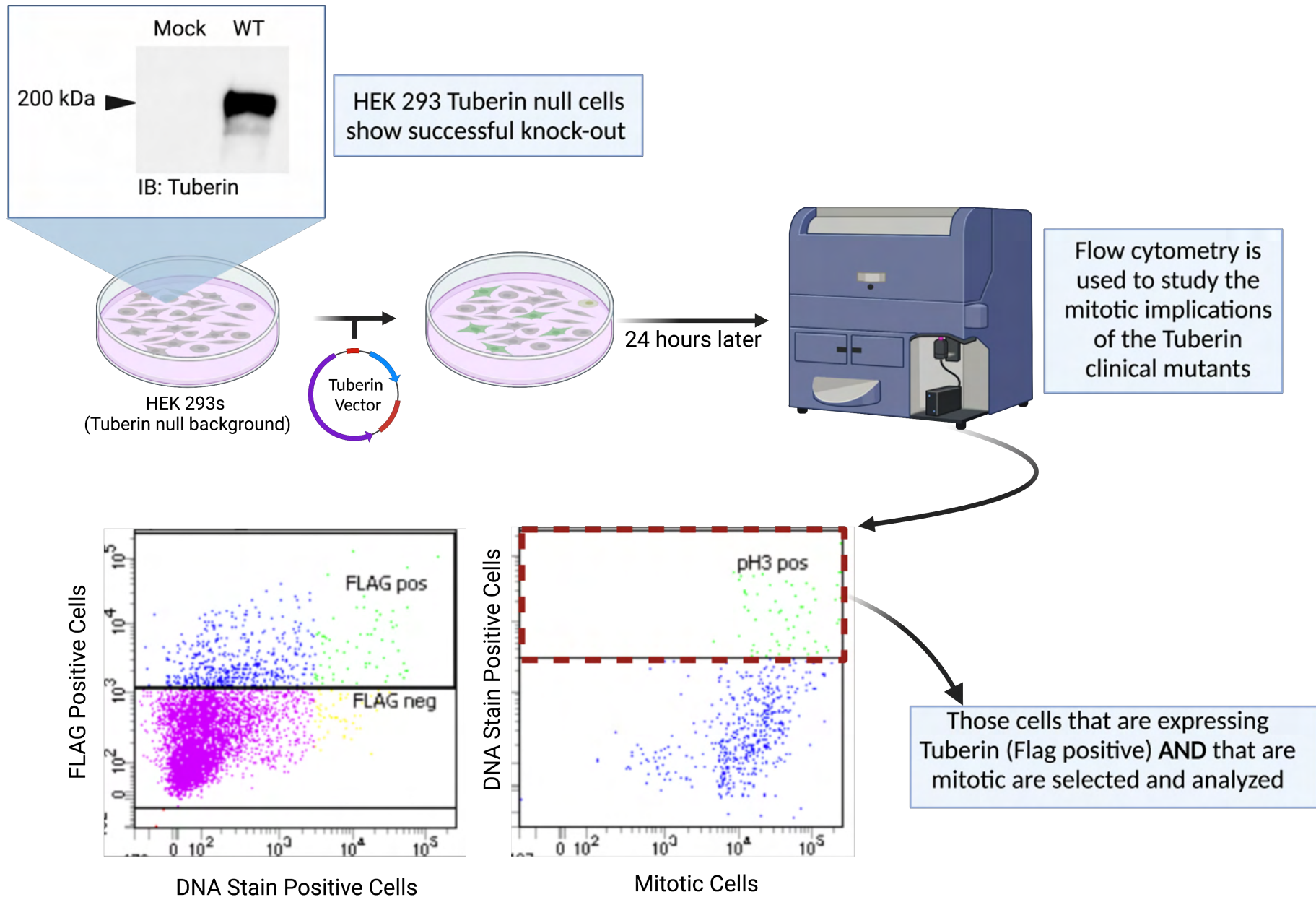
Hypothesis and Objectives



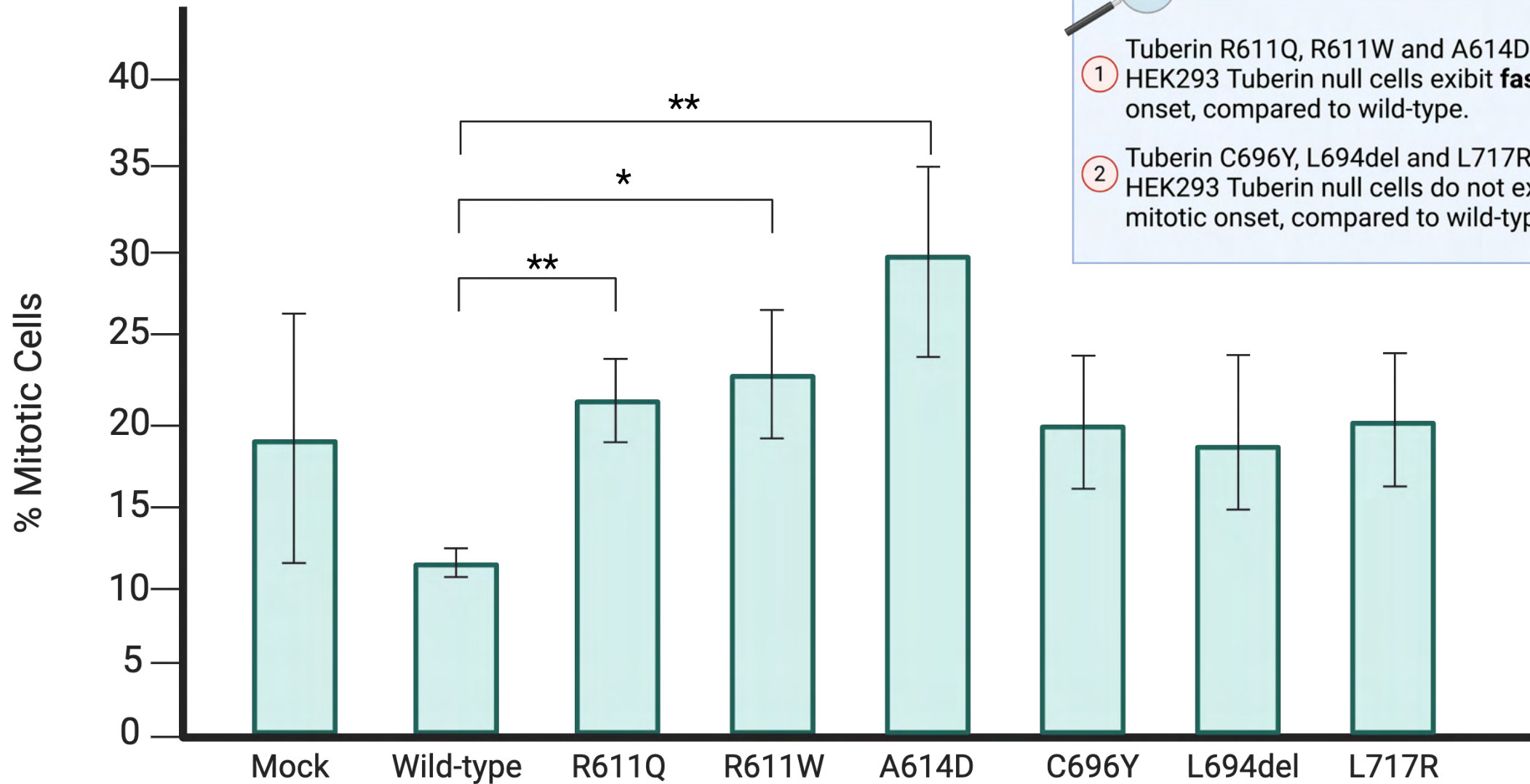
Hypothesis and Objectives



Characterizing Clinical Mutants in Tuberin Null Cells

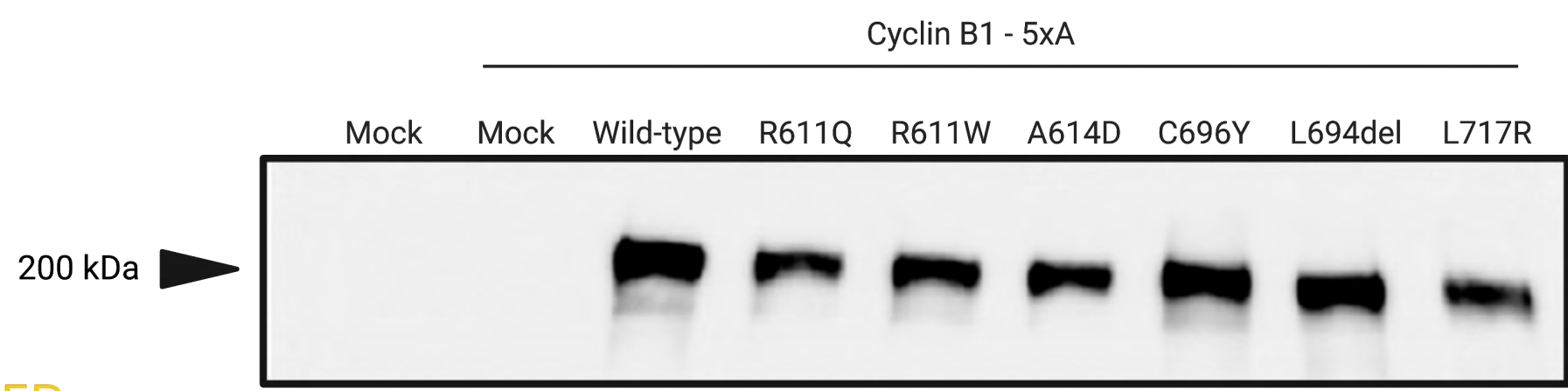
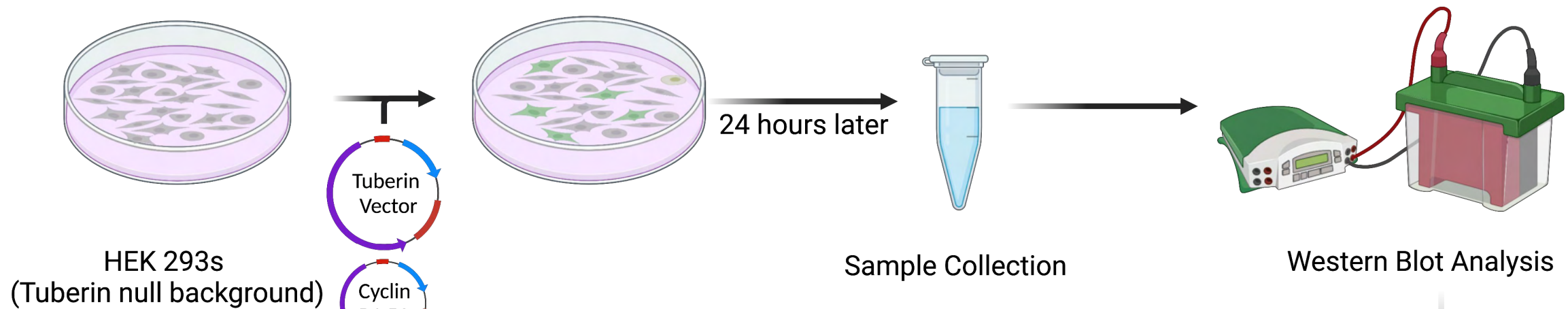


Some Clinical Mutations Exhibit Increased Mitotic Index



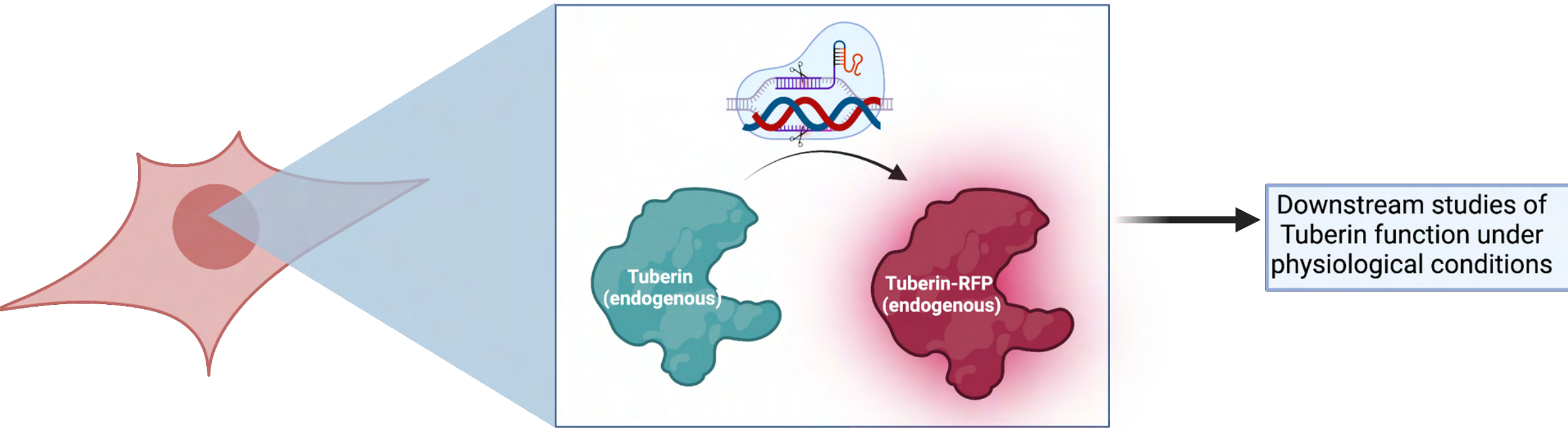
- 1 Tuberin R611Q, R611W and A614D transfected HEK293 Tuberin null cells exhibit **faster** mitotic onset, compared to wild-type.
- 2 Tuberin C696Y, L694del and L717R transfected HEK293 Tuberin null cells do not exhibit faster mitotic onset, compared to wild-type.

Mutants Show Uniform Expression in Tuberin Null Cells

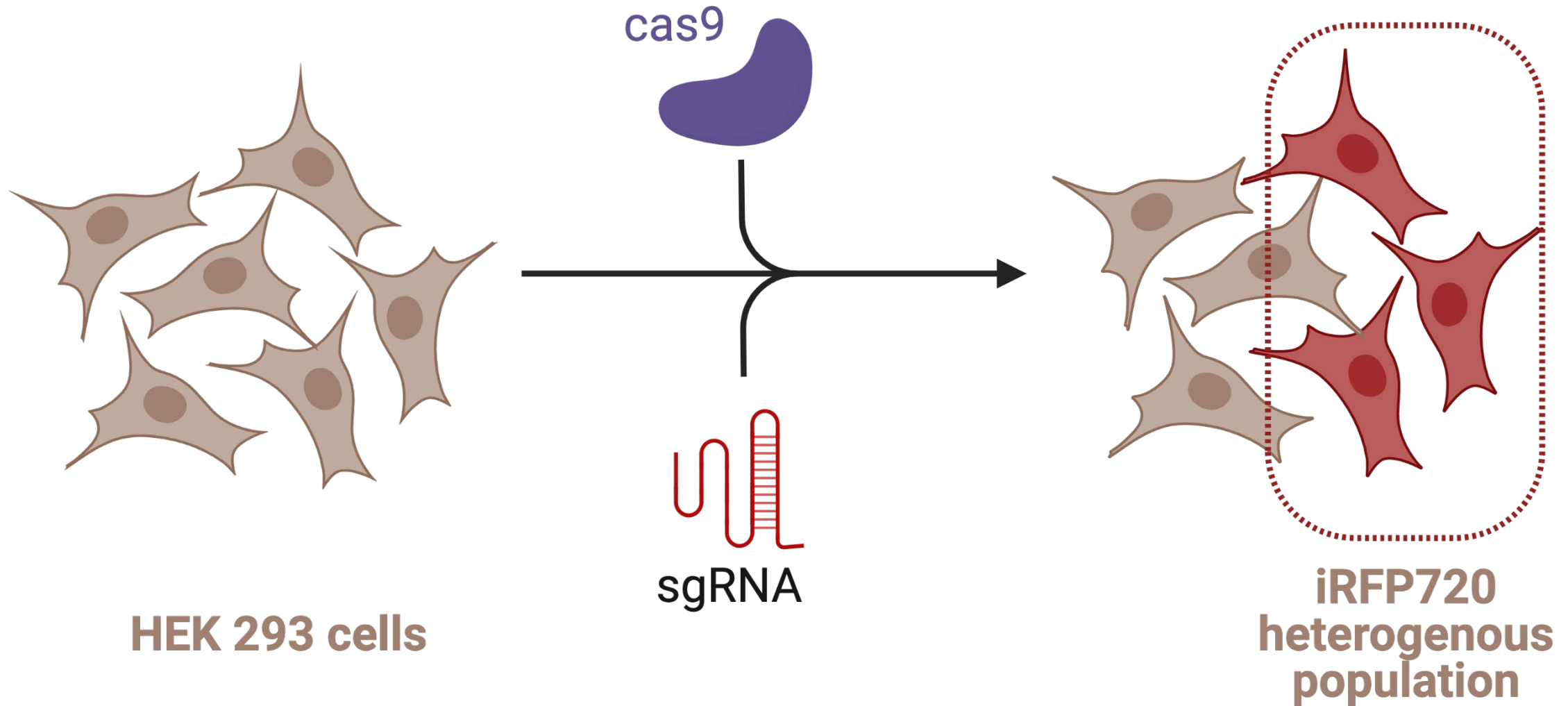


IB: Tuberin

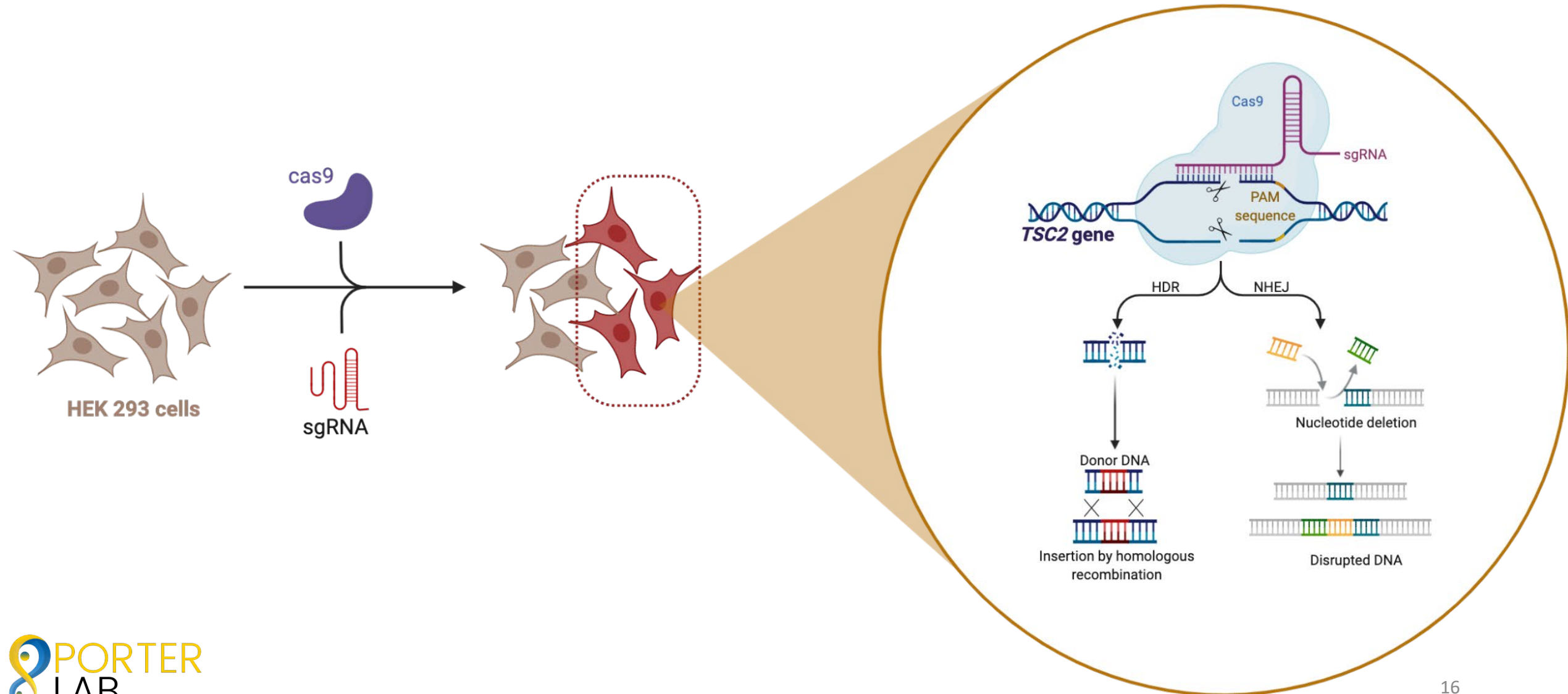
Tagging Endogenous Tuberin in HEK 293 Cells



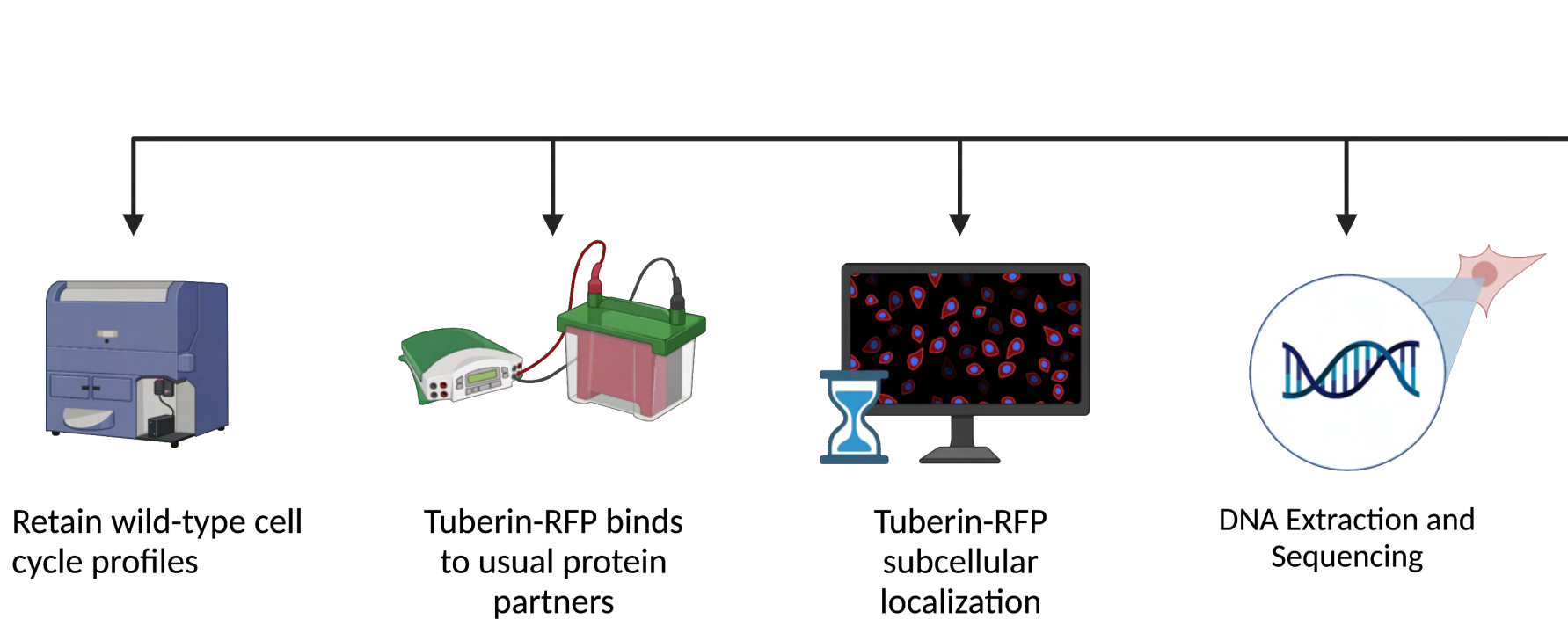
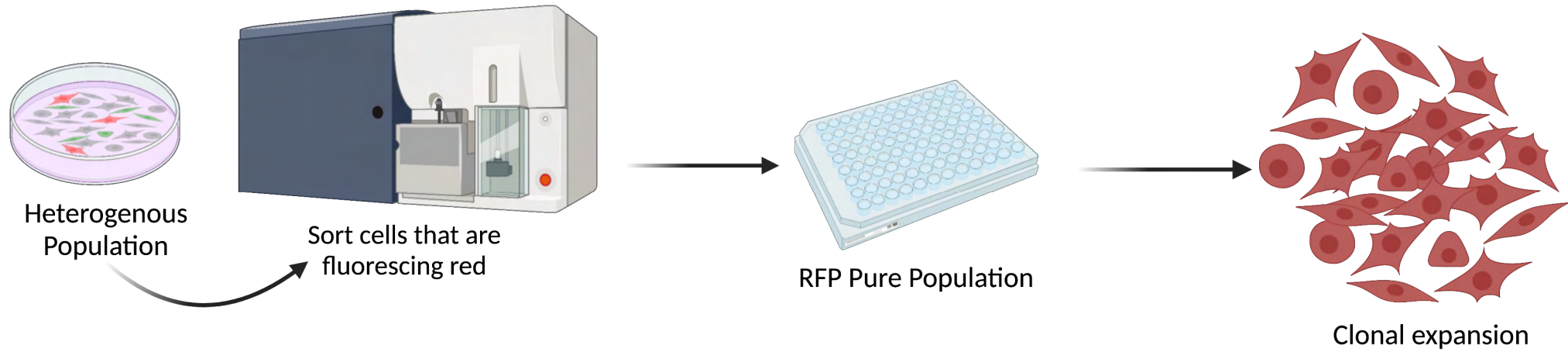
Tagging Endogenous Tuberin in HEK 293 Cells



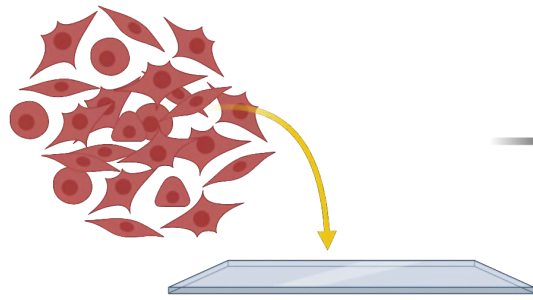
Using Genomic Scissors to insert iRFP tag



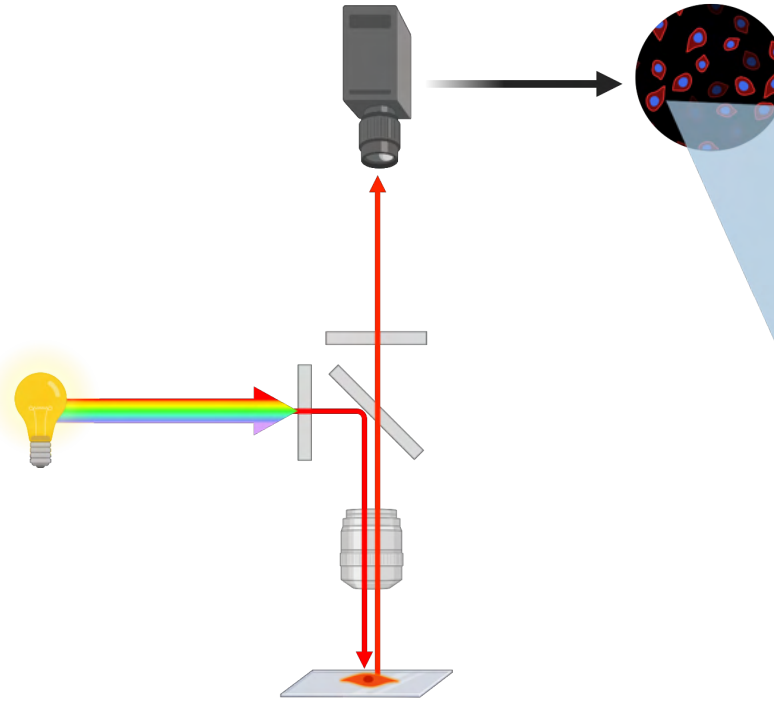
Validating successful iRFP Knock-in



iRFP Tag Insertion is successful



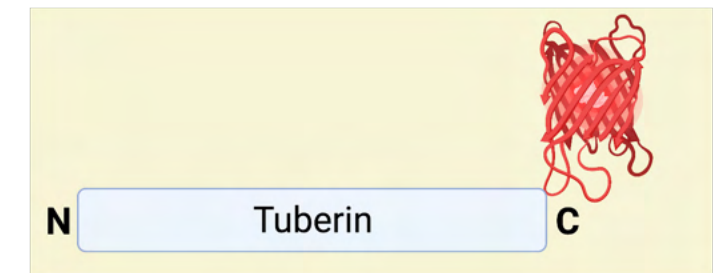
Seeding RFP cells on microscope slide for immunofluorescence



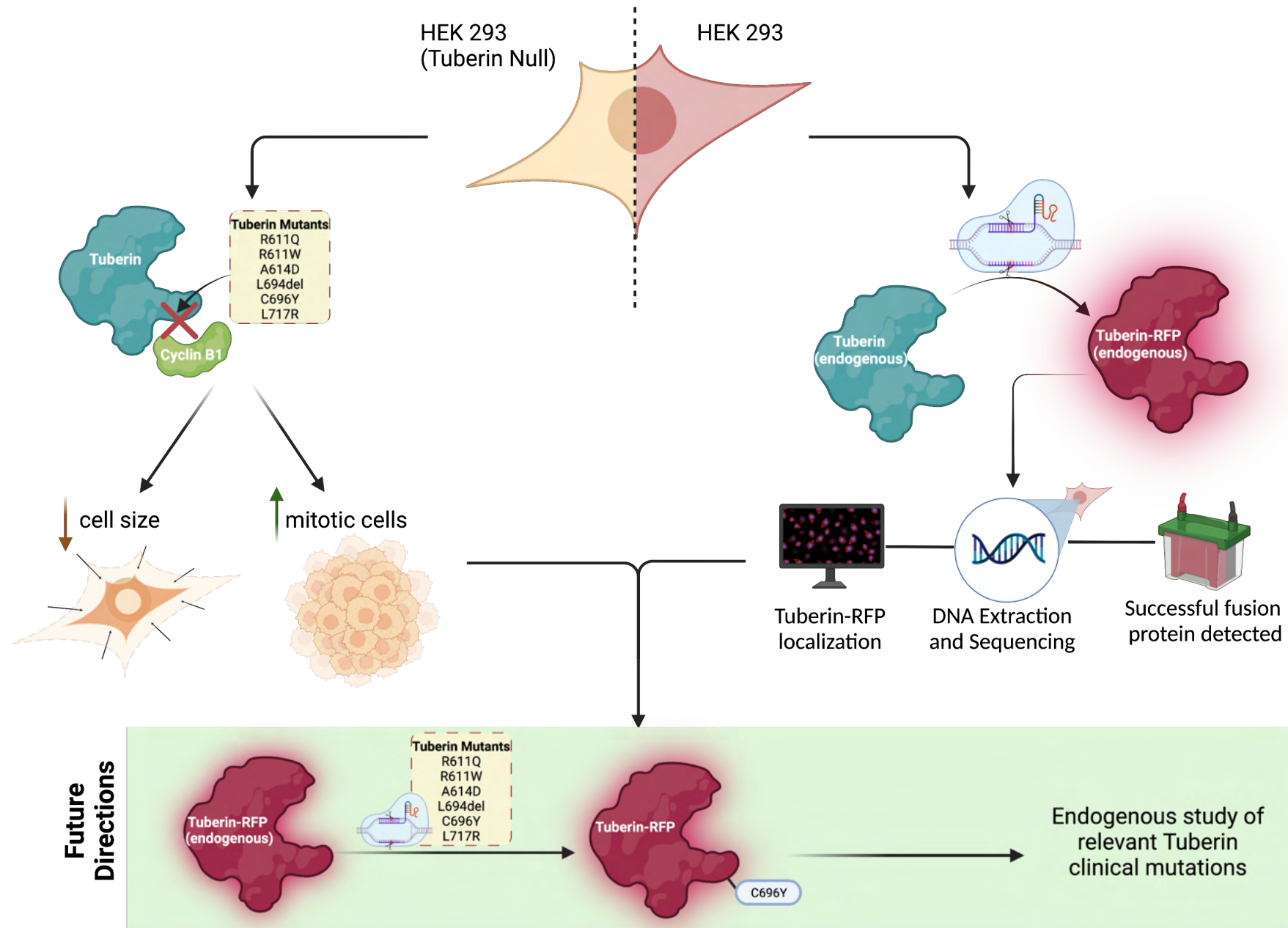
RFP cells are imaged using a fluorescence microscope



Red = Tuberin-RFP (C-terminal insertion)
Blue = DAPI (stains nucleus)



Tuberin Clinical Mutations Disrupt Cell Cycle Regulation



Acknowledgments

Thank you to Dr. Lisa Porter, Dr. Elizabeth Fidalgo da Silva, Adam Pillon (PhD student), and Jackie Fong MSc, for their continuous support throughout the year!

Thank you to the amazing graduate and undergraduate Porter lab rats for the countless memories and amazing friendships made over these past few years!

