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The Impact of Cyclin B1 on Tuberin Stabilization

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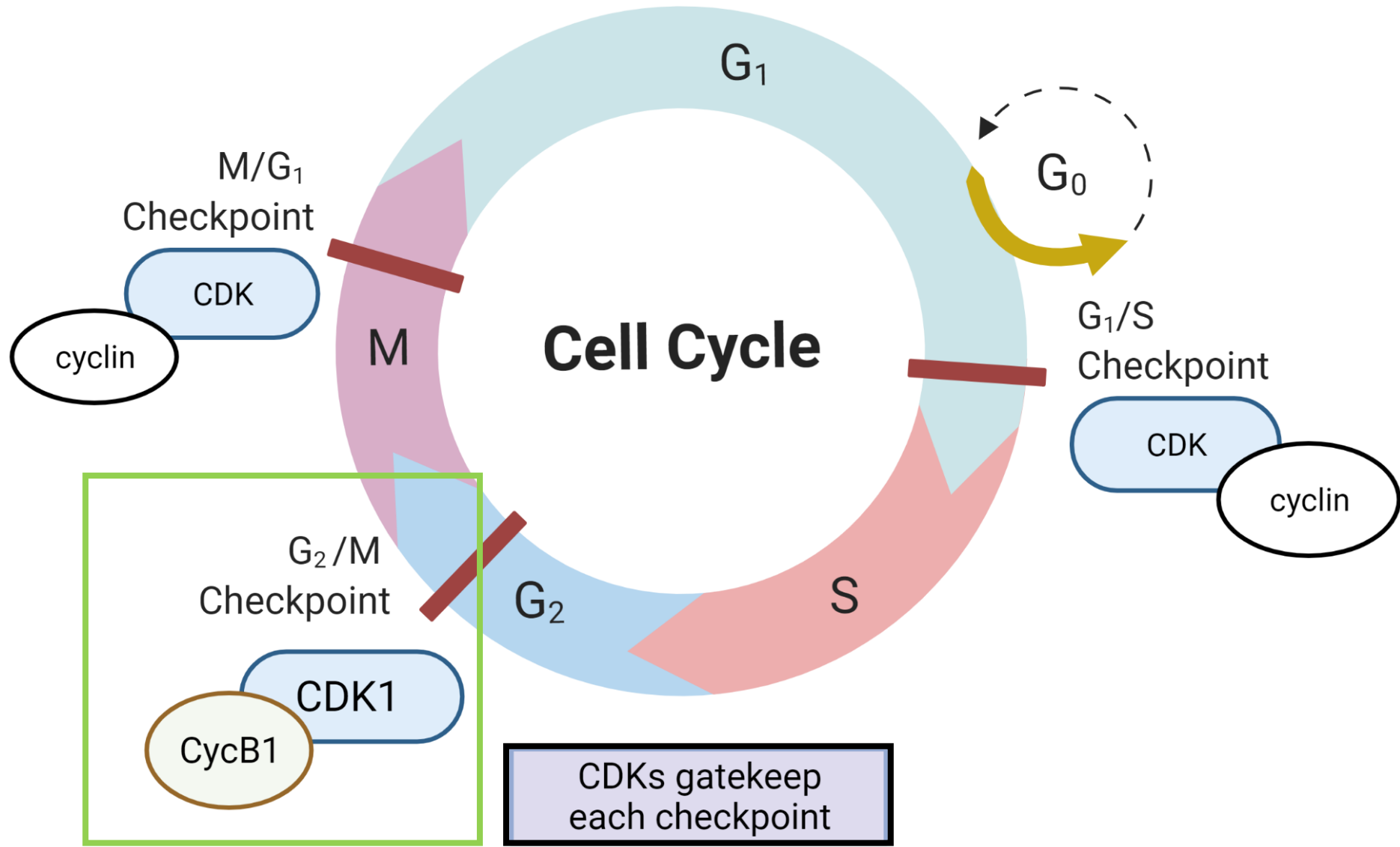
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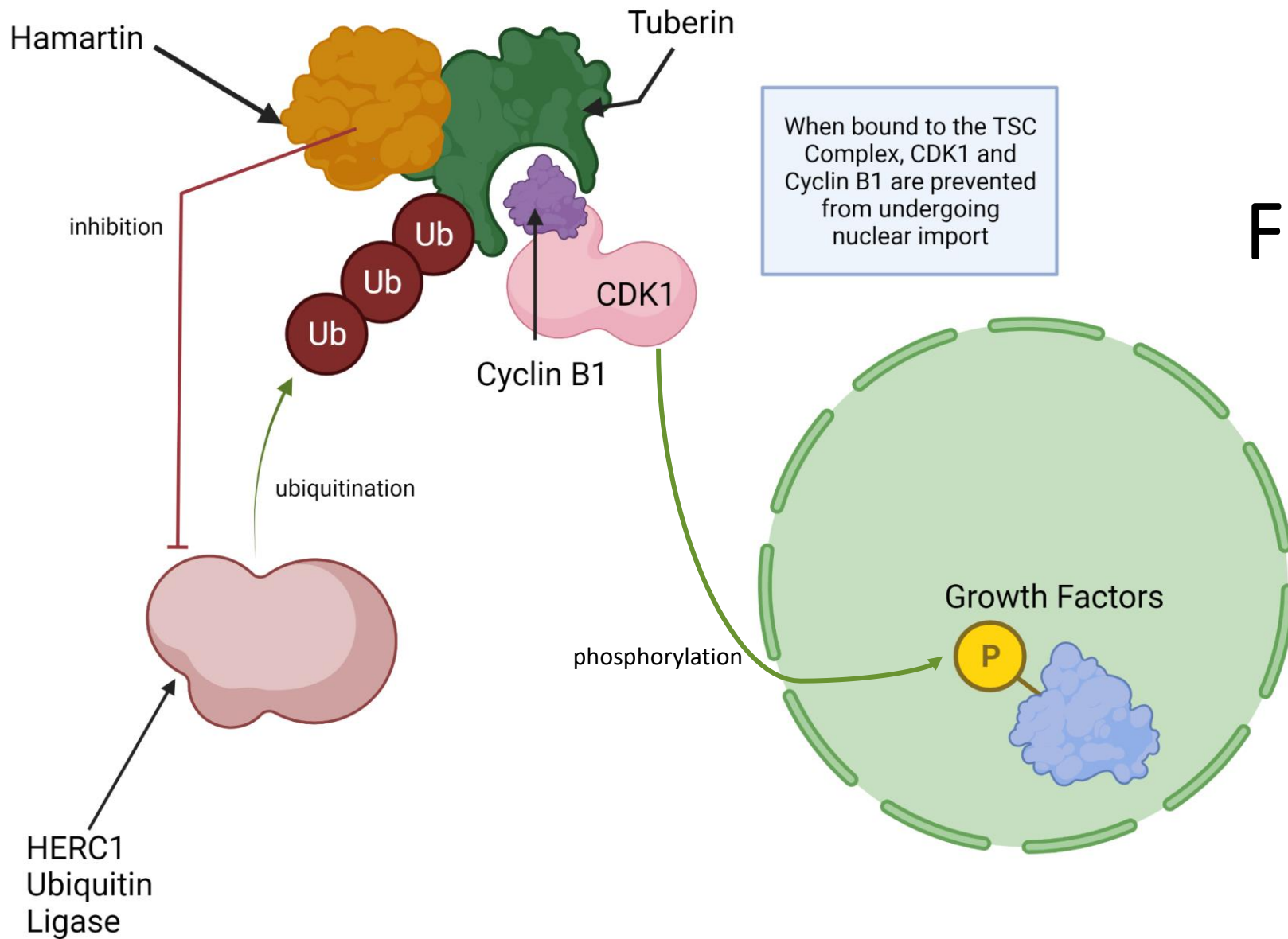
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The Impact of Cyclin B1 on Tuberin Stabilization

By Aiden Mitrevski
Dr. Porter's Laboratory
Tuberin Group







Key Players: Tuberin Function and Stability

- Tuberin prevents CycB1-CDK1 complex from triggering cell division
- Without Hamartin, Tuberin is rapidly degraded
- Hamartin prevents HERC1 from ubiquitinating Tuberin → prevents degradation

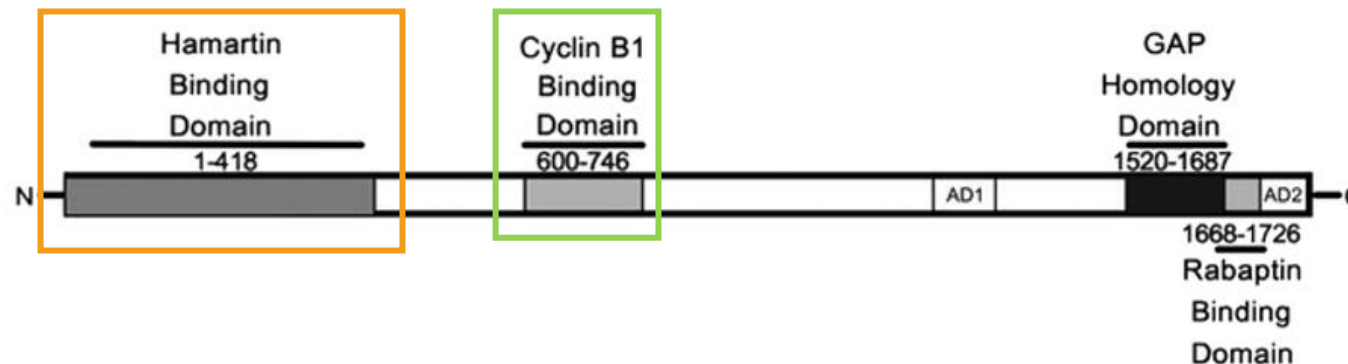
How does Tuberin interact with Hamartin and CycB1?

Essential to Tuberin Stabilization

- Hamartin Binding Domain (Residues 1-418)

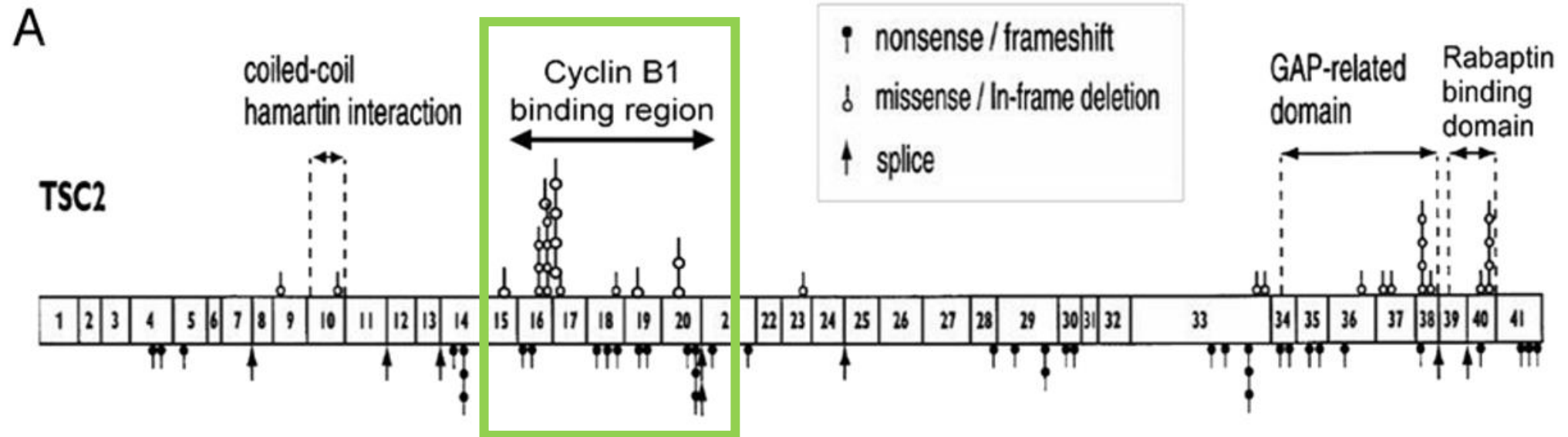
Essential to G2/M Control

- Cyclin B1 Binding Domain (Residues 600-746)



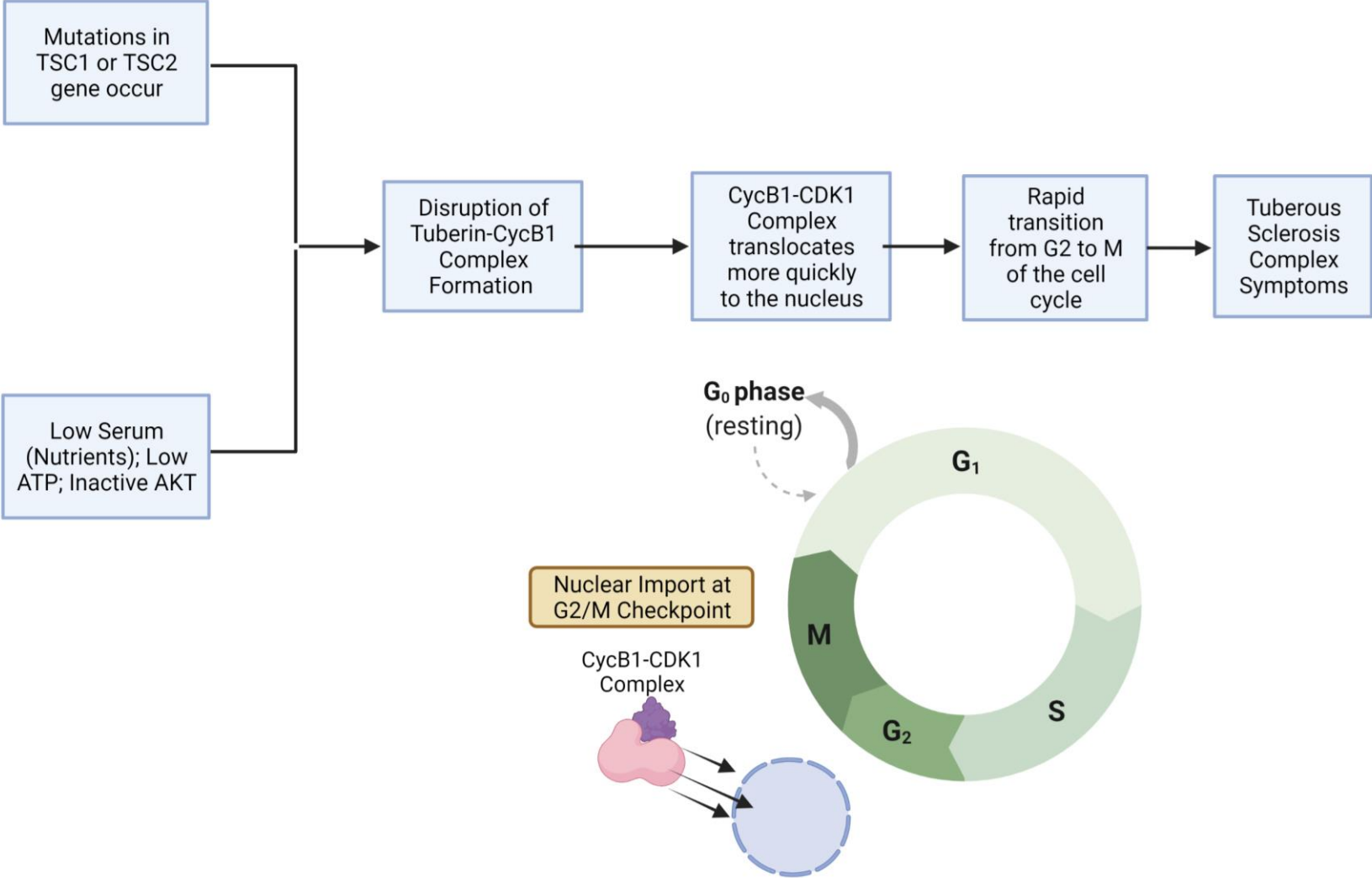
focused on this

Mutational Hotspots in Tuberin Gene (TSC2)



Cell Cycle, 10:18, 3129-3139, DOI: 10.4161/cc.10.18.17296

Dysregulation of the G2/M Checkpoint - Tuberous Sclerosis Complex



Does Cyclin B1
contribute to the
stabilization of
Tuberin?



IF SO, is this effect
independent of
Hamartin's
stabilizing ability?

Preliminary Data

Performed with HEK293 cell lines

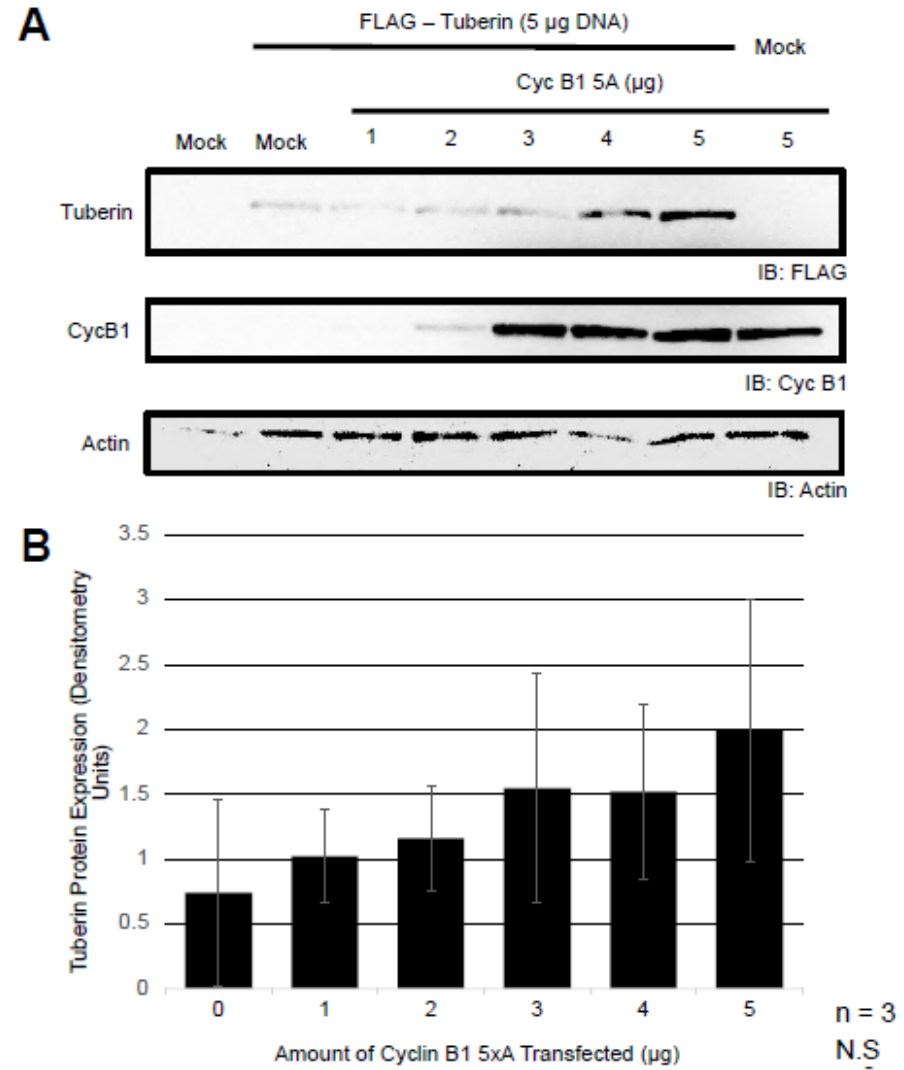
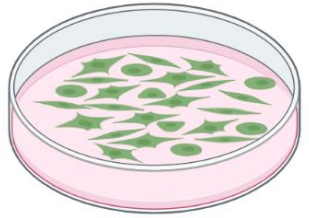


Figure 6: Graded amounts of mutant Cyclin B1 5A does not lead to altered Tuberin protein levels.

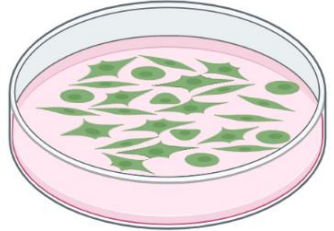
What We Did...

to question whether any CycB1-mediated stabilization observed is dependent on Hamartin

IC2 Cells (Hamartin Absent)



HEK293 Cells (Hamartin Present)

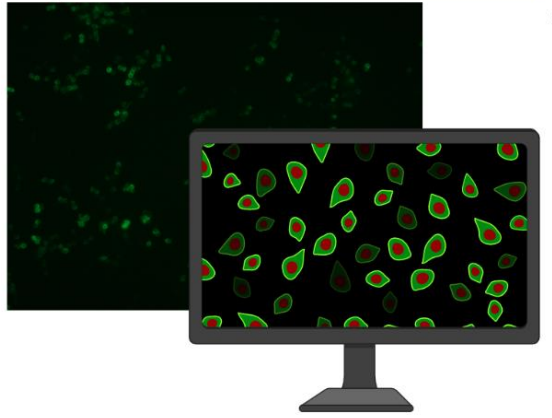


transfection with CycB1 5xA DNA gradient

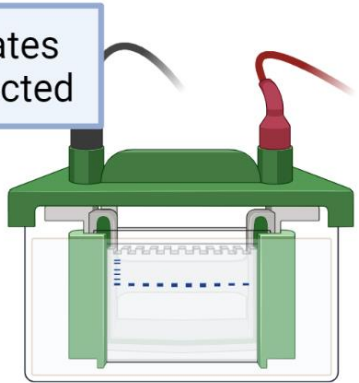


binds 3x stronger to Tuberin than wild-type

transfection success confirmed by GFP fluorescence



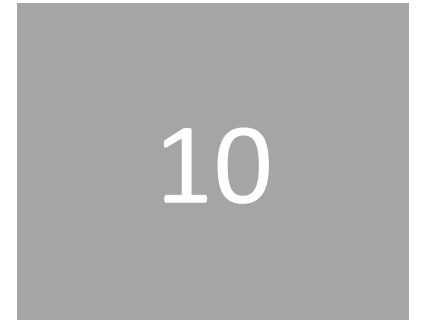
cell lysates are collected



SDS-PAGE is performed followed by Western Blot Analysis

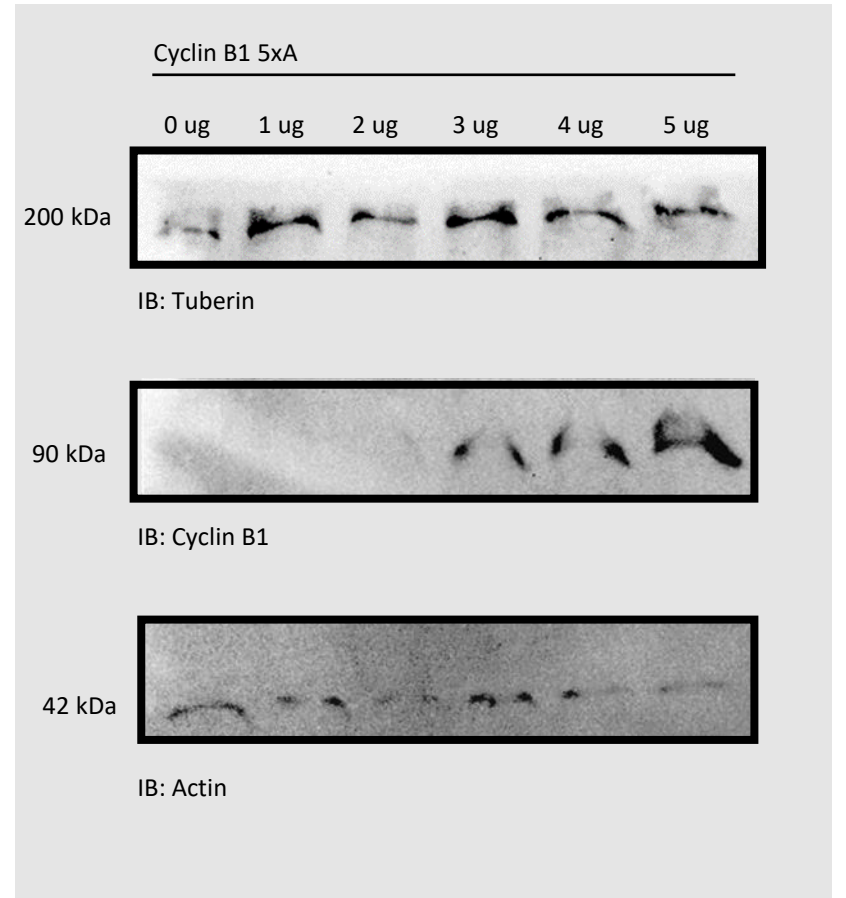
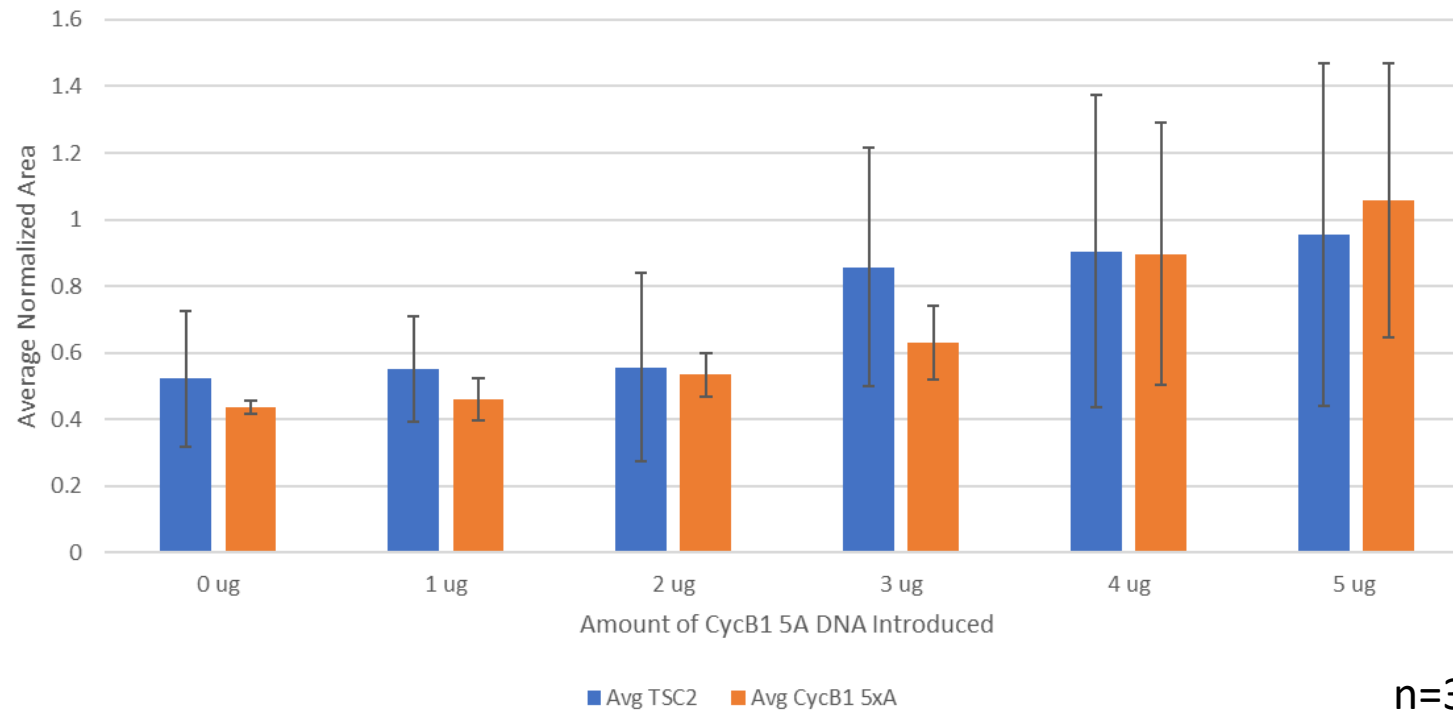


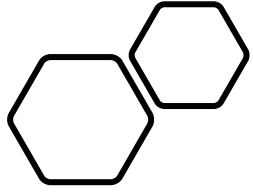
Cyclin B1 5A Might Stabilize Tuberin in the Absence of Hamartin



Cell Line: IC2 (Hamartin -/-)

Densitometric Analysis of TSC2 and CycB1 5A Relative to Actin





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