



LARP6/PIH1D3 Complex as a Possible Cause of Primary Ciliary Dyskinesia

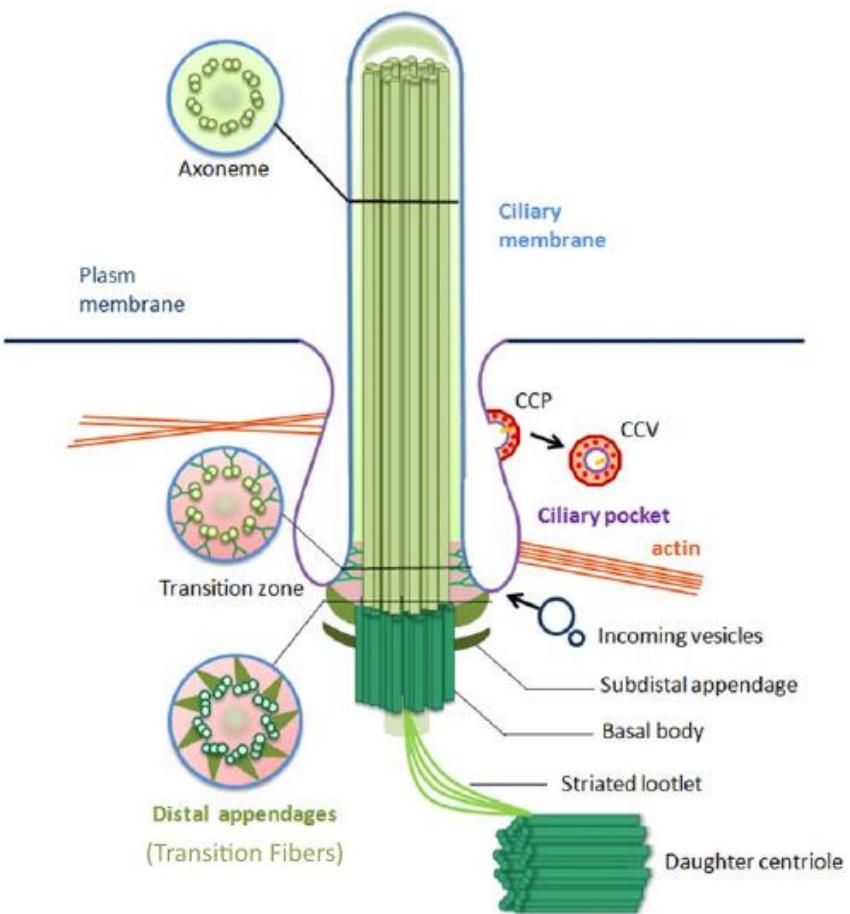
Summer Research Fellowship 2017

Ryan Earwood – Kato Lab

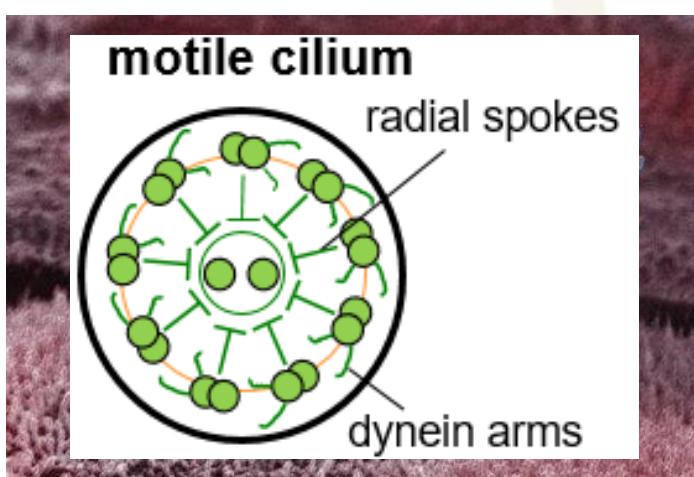
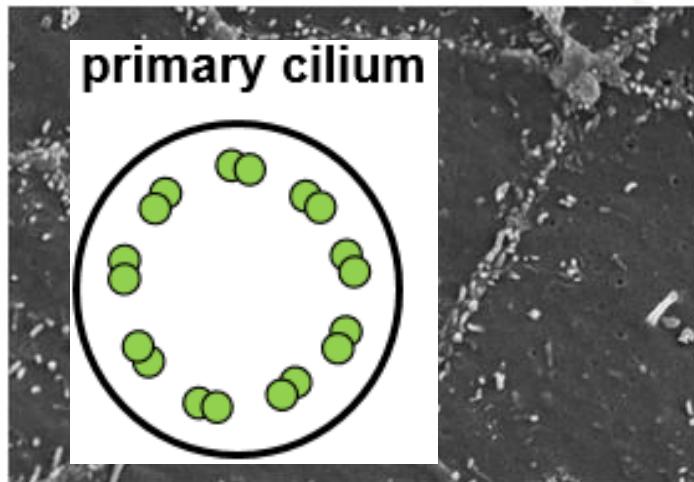


CILIA

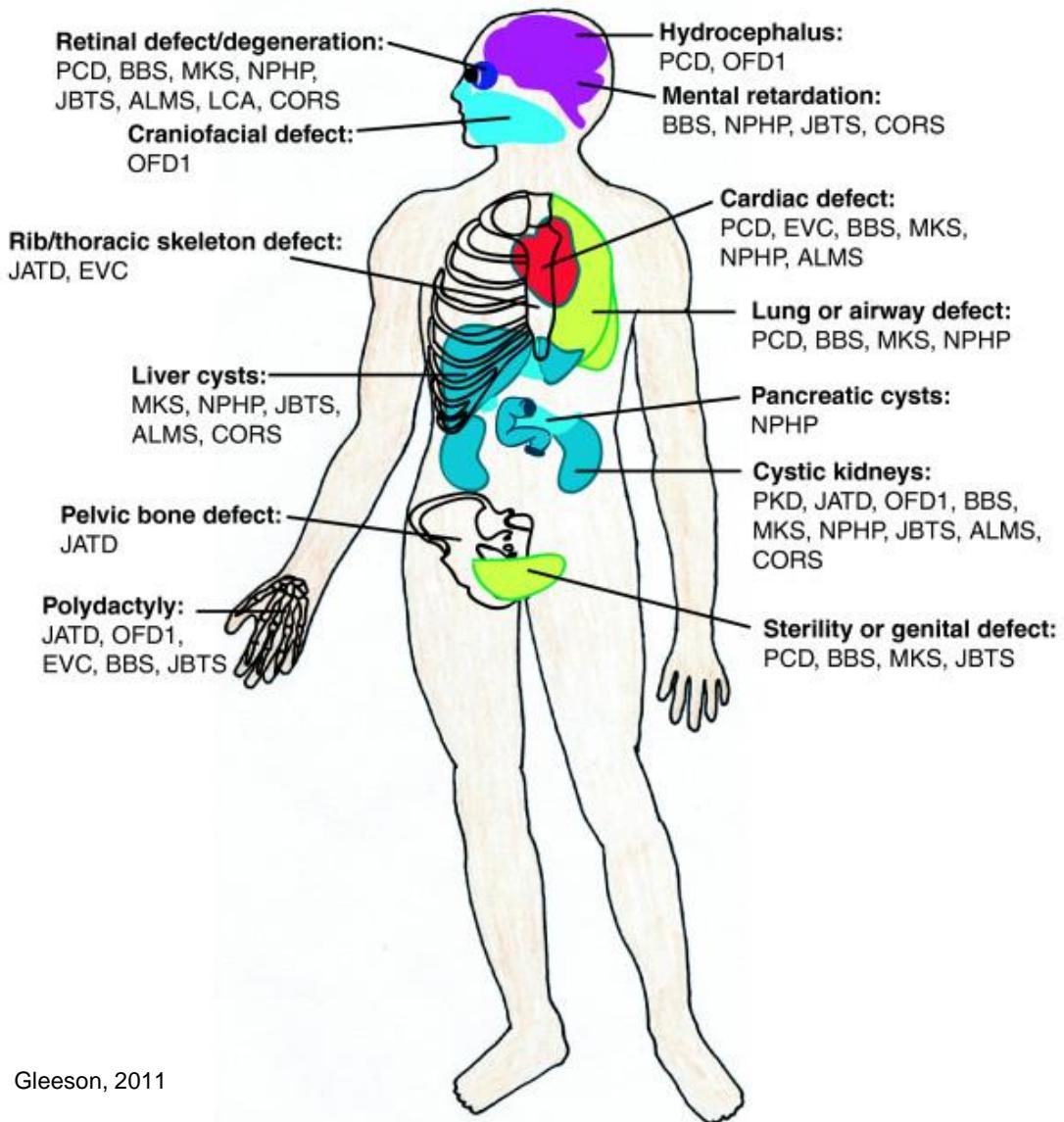
Background



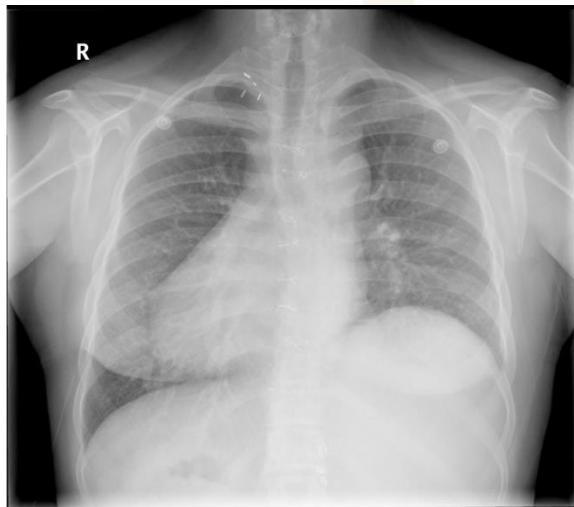
Ke & Yang, 2014



Ciliopathies



Physopaedia

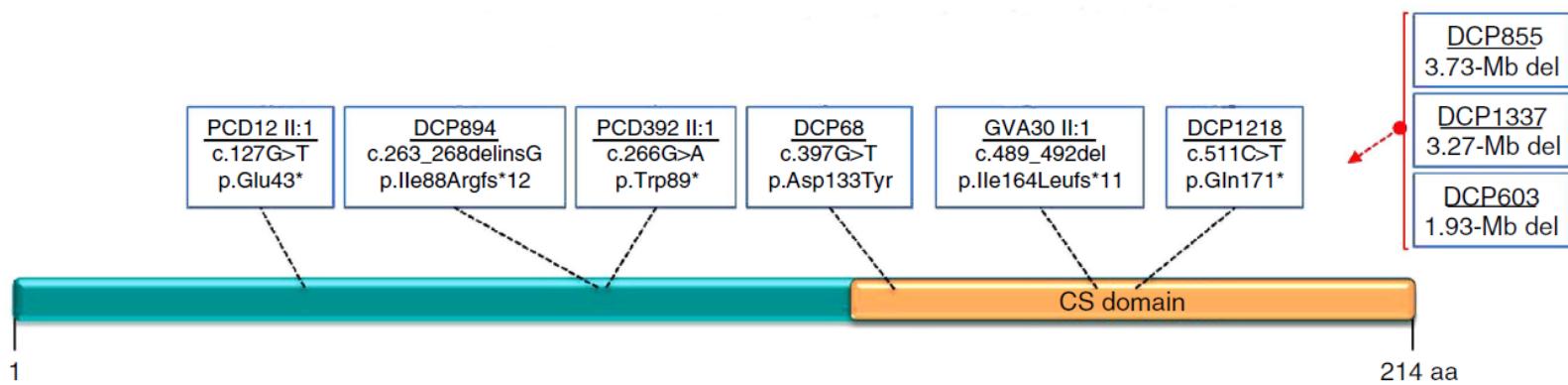


Radiopaedia



PIH1D3

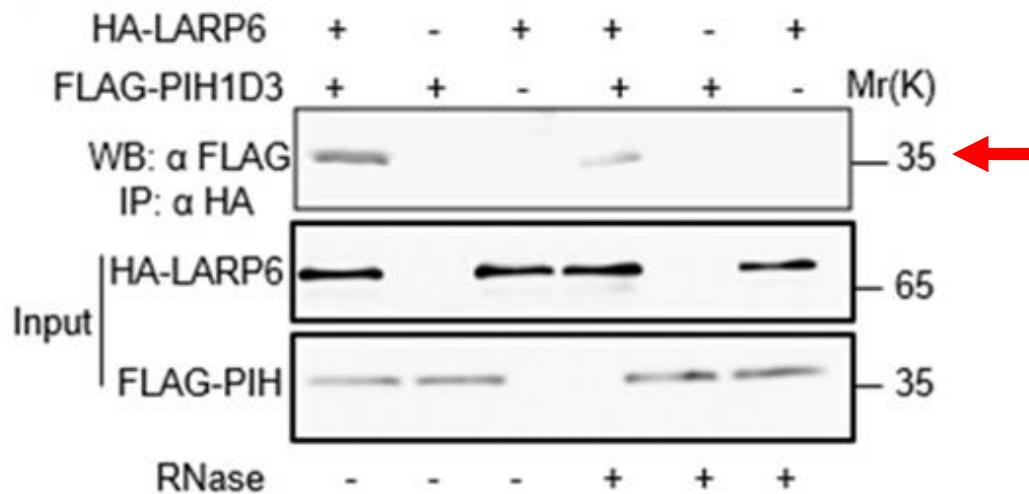
- X-Linked form of human PCD
- Contains CS domain
- Localizes to apical cytoplasm
- Leads to dysfunctional motile cilia



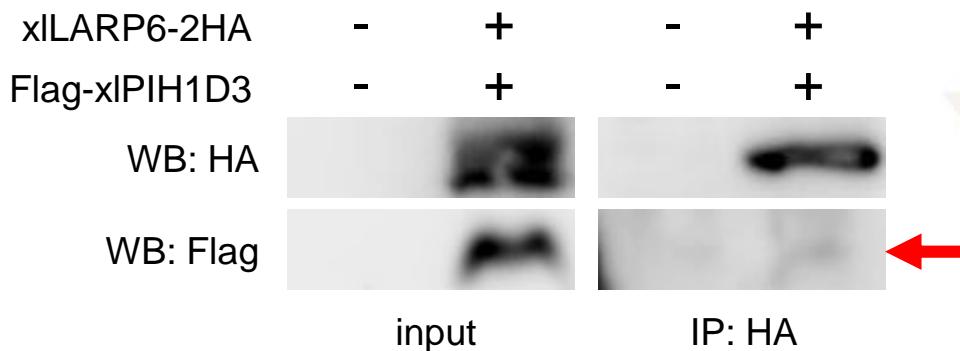


PIH1D3 interacted with LARP6

A. mammalian cells



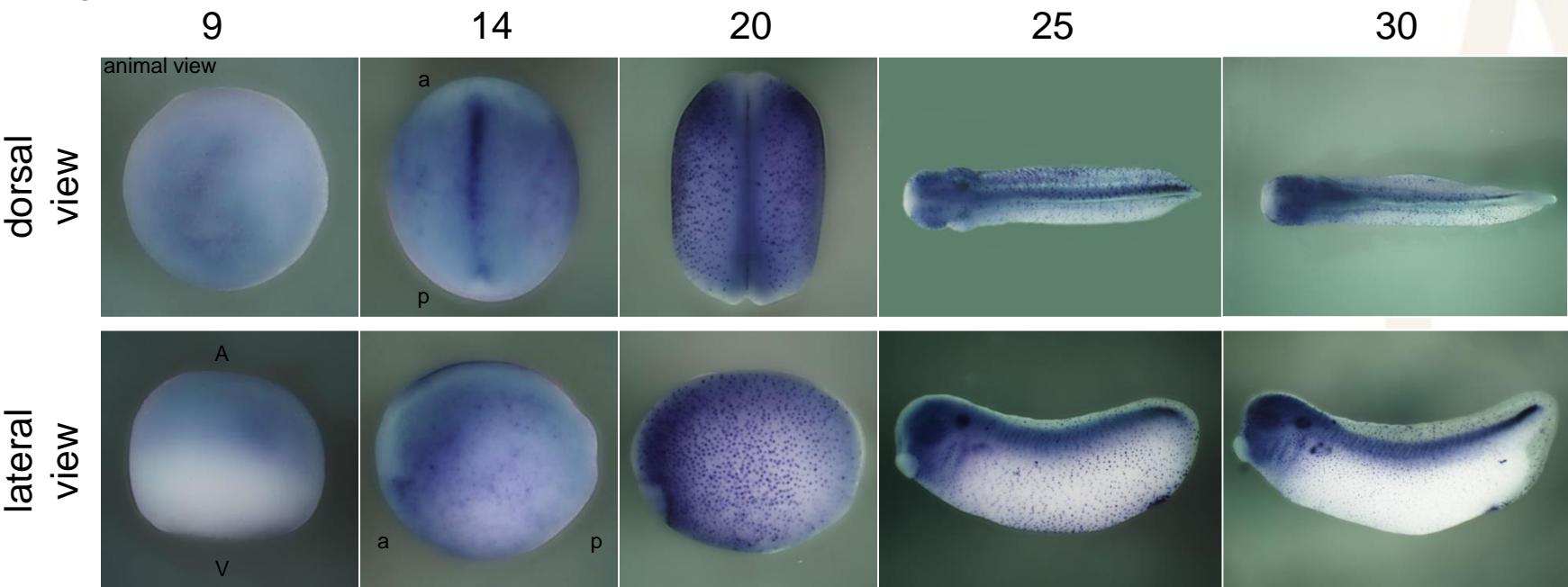
B. *Xenopus* embryos





Expression profiles *PIH1D3*

Stages



A: Animal pole; V: Vegetal pole; a: anterior; p: posterior



PIH1D3 knockdown induced swelling phenotype

Control



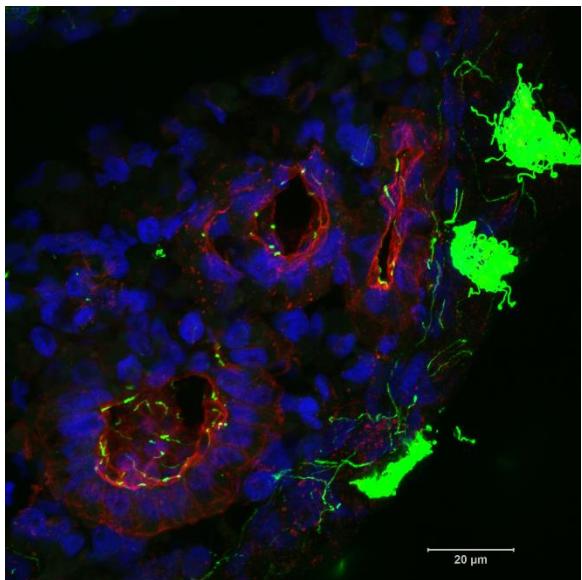
PIH1D3-MO



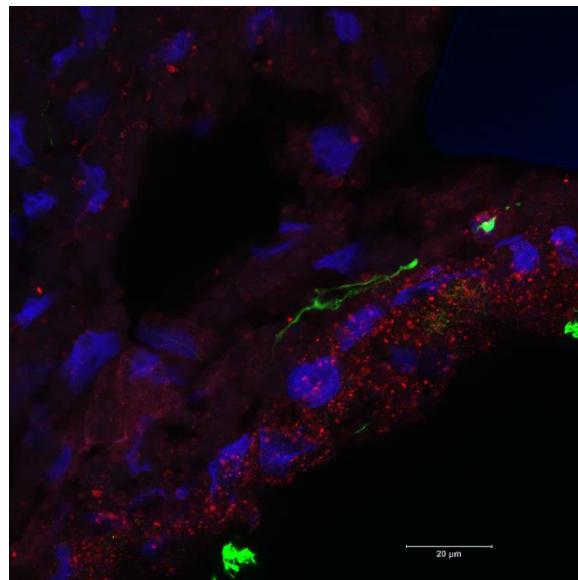


PIH1D3 knockdown compromised the structure of pronephros

Control MO



PIH1D3 MO



α -tubulin: cilia

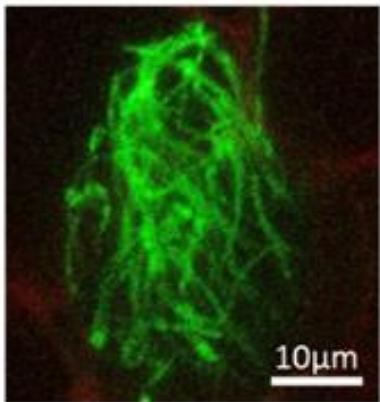
Hoechst: nucleus

Lectin: tubular epithelial cells

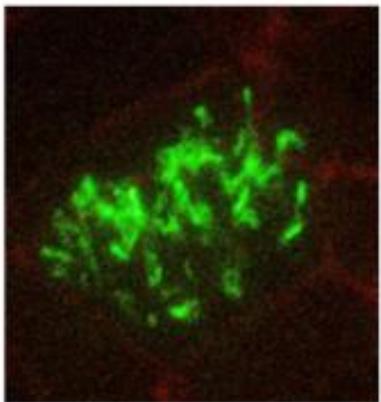
mRFP: plasma membrane

PIH1D3 knockdown induced ciliary defects in the multi-ciliated epidermal cells

A



Control MO

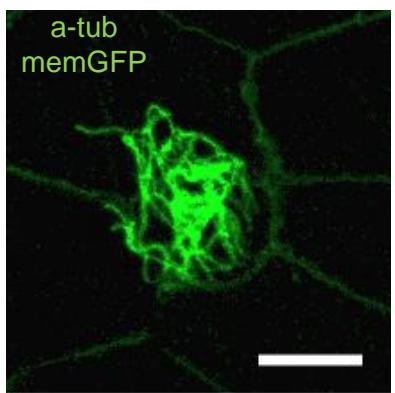


PIH1D3 MO

α -tubulin: cilia

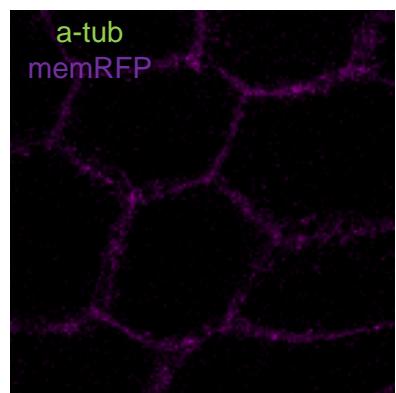
mRFP: plasma membrane

B



α -tub
memGFP

Control MO



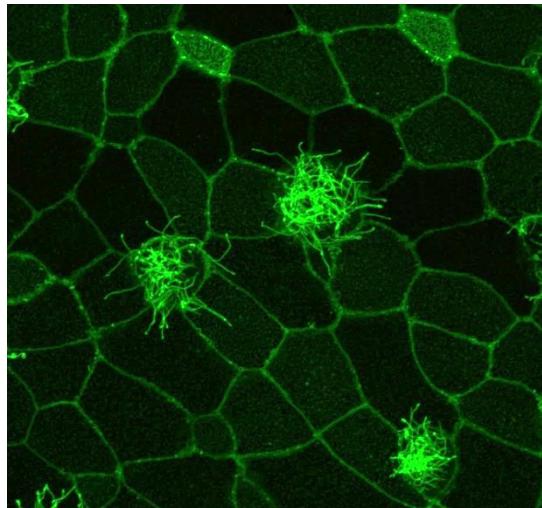
α -tub
memRFP

LARP6 MO

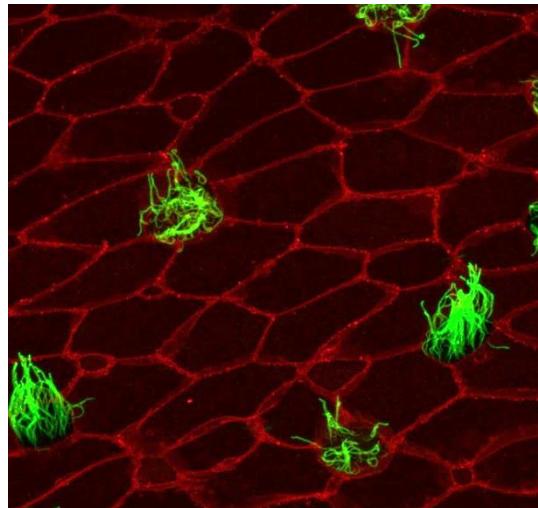
mRFP: plasma membrane

mGFP: plasma membrane

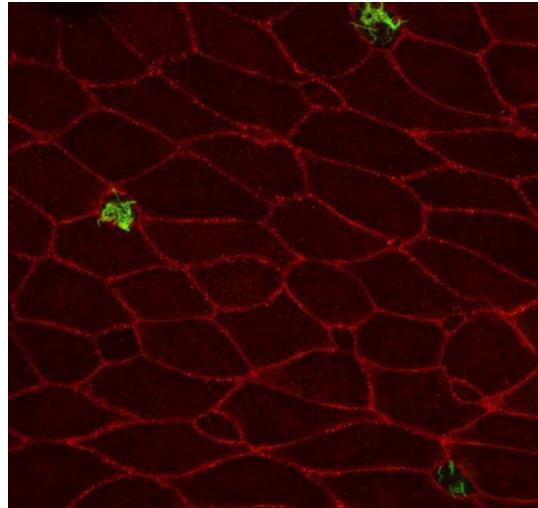
PIH1D3 and LARP6 controls ciliogenesis of the multi-ciliated cells together



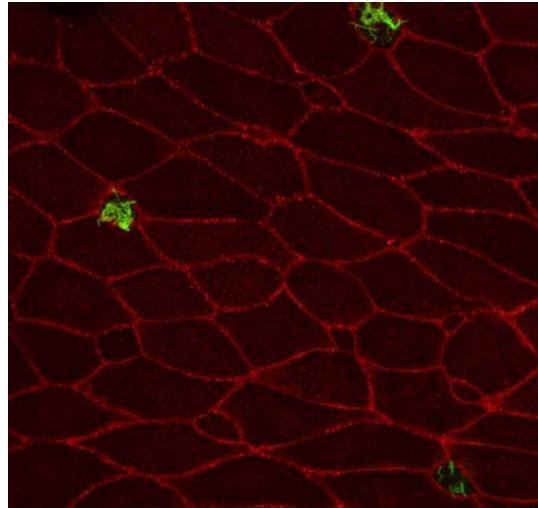
Control MO



LARP6 MO – 7.5ng



PIH1D3 MO – 5ng



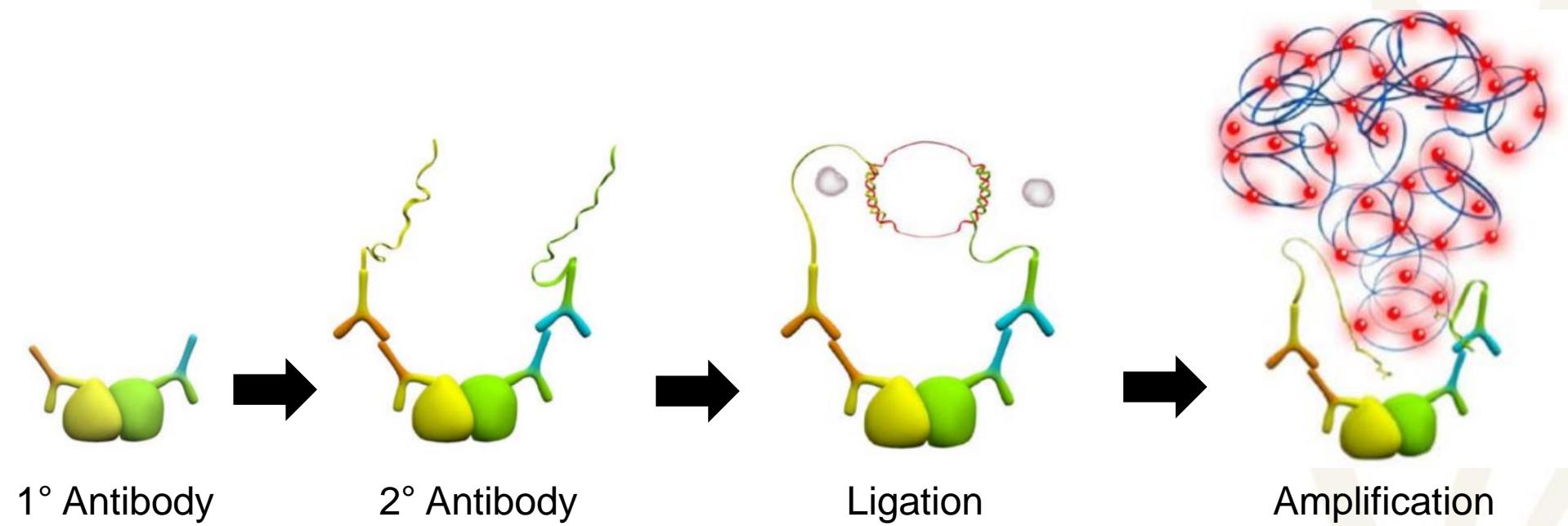
LARP6 MO/PIH1D3 MO

α -tubulin: cilia

mRFP: plasma membrane

mGFP: plasma membrane
(control)

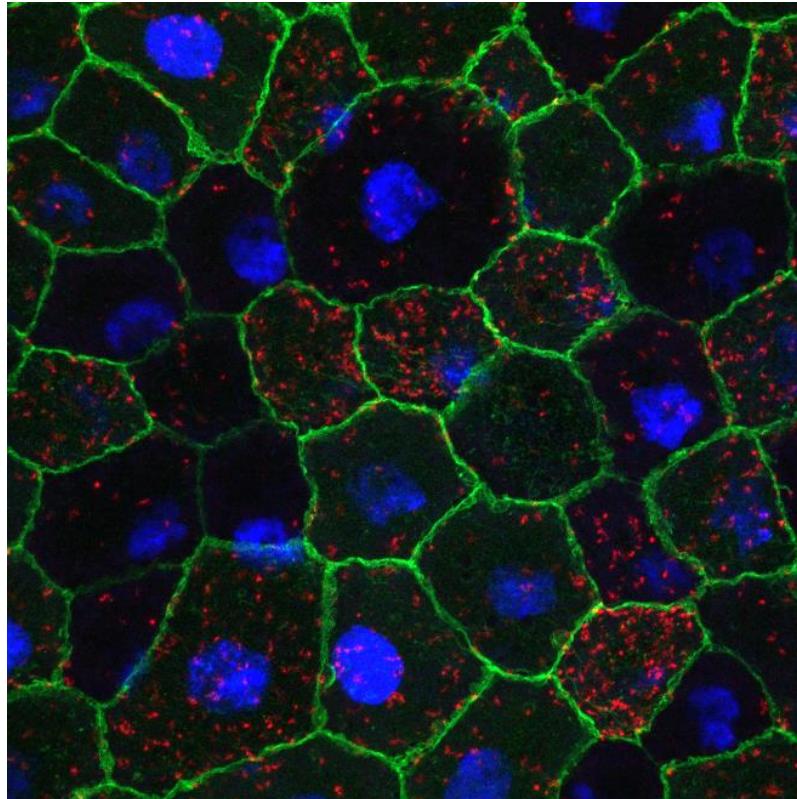
Proximity Ligation Assay



PIH1D3
LARP6

PIH1D3 and Larp6 formed granules in the multi-ciliated cells

HA-Larp6/Flag-PIH1D3



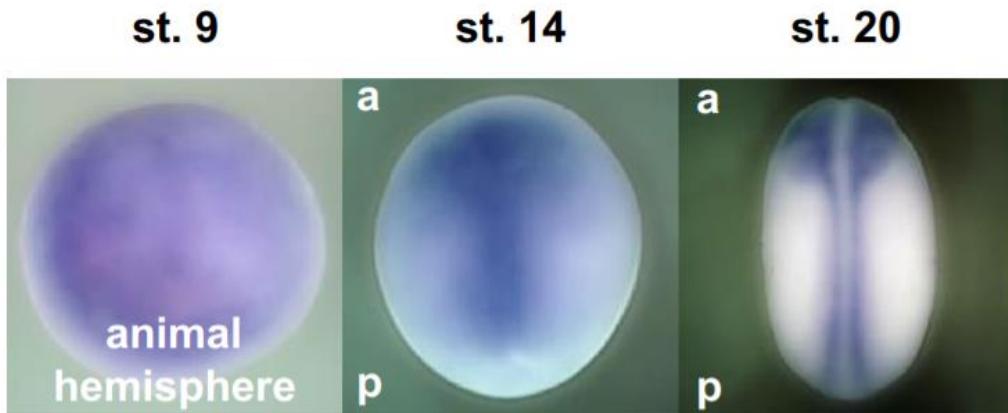
Hoechst: nucleus

mGFP: plasma membrane

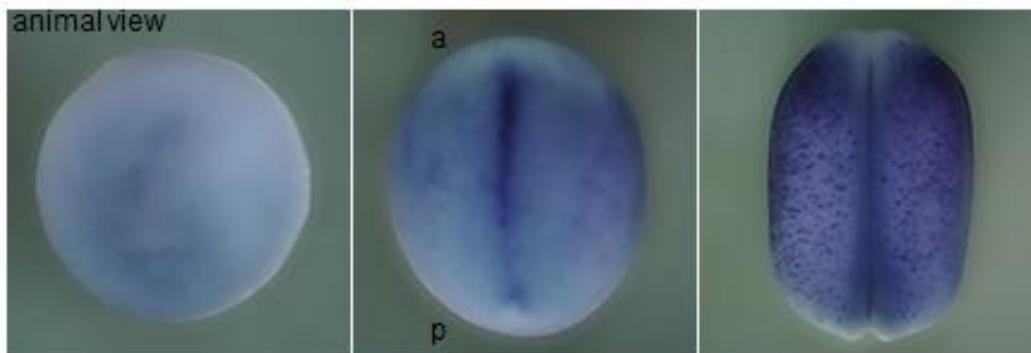
Cy3:
PIH1D3/LARP6
complex

Expression profiles

Larp6

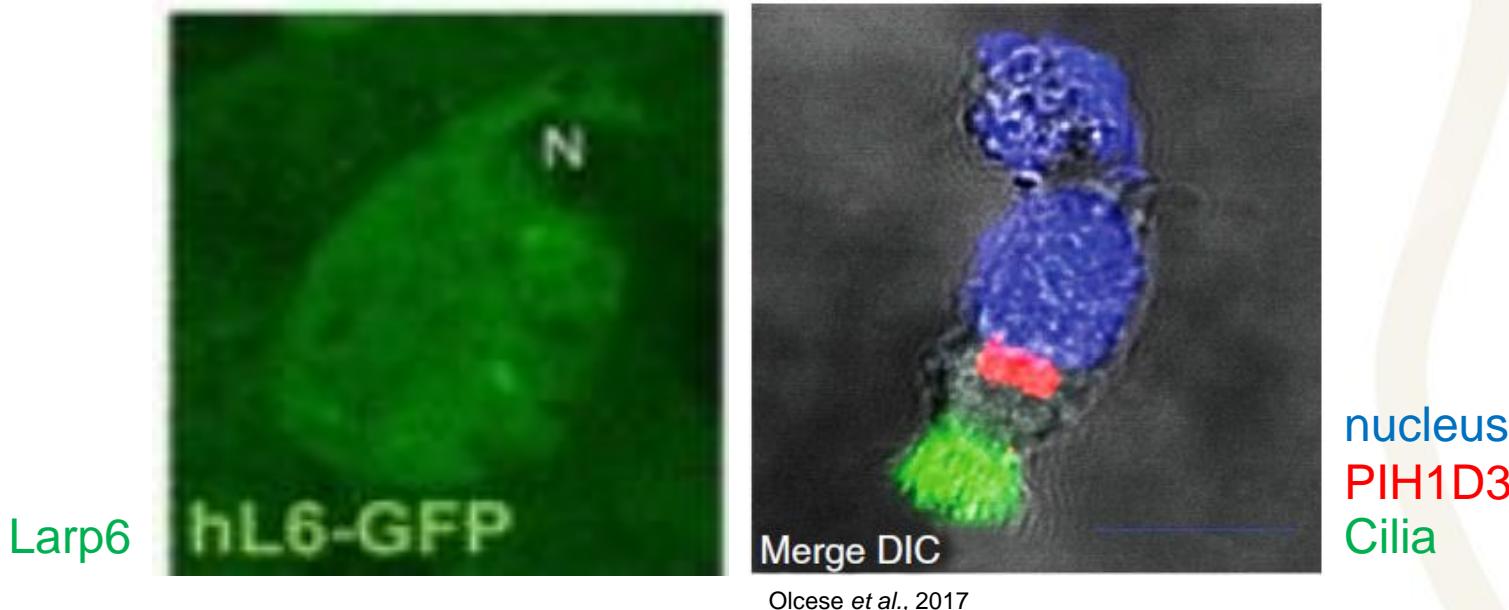


PIH1D3





LARP6/PIH1D3 Localization



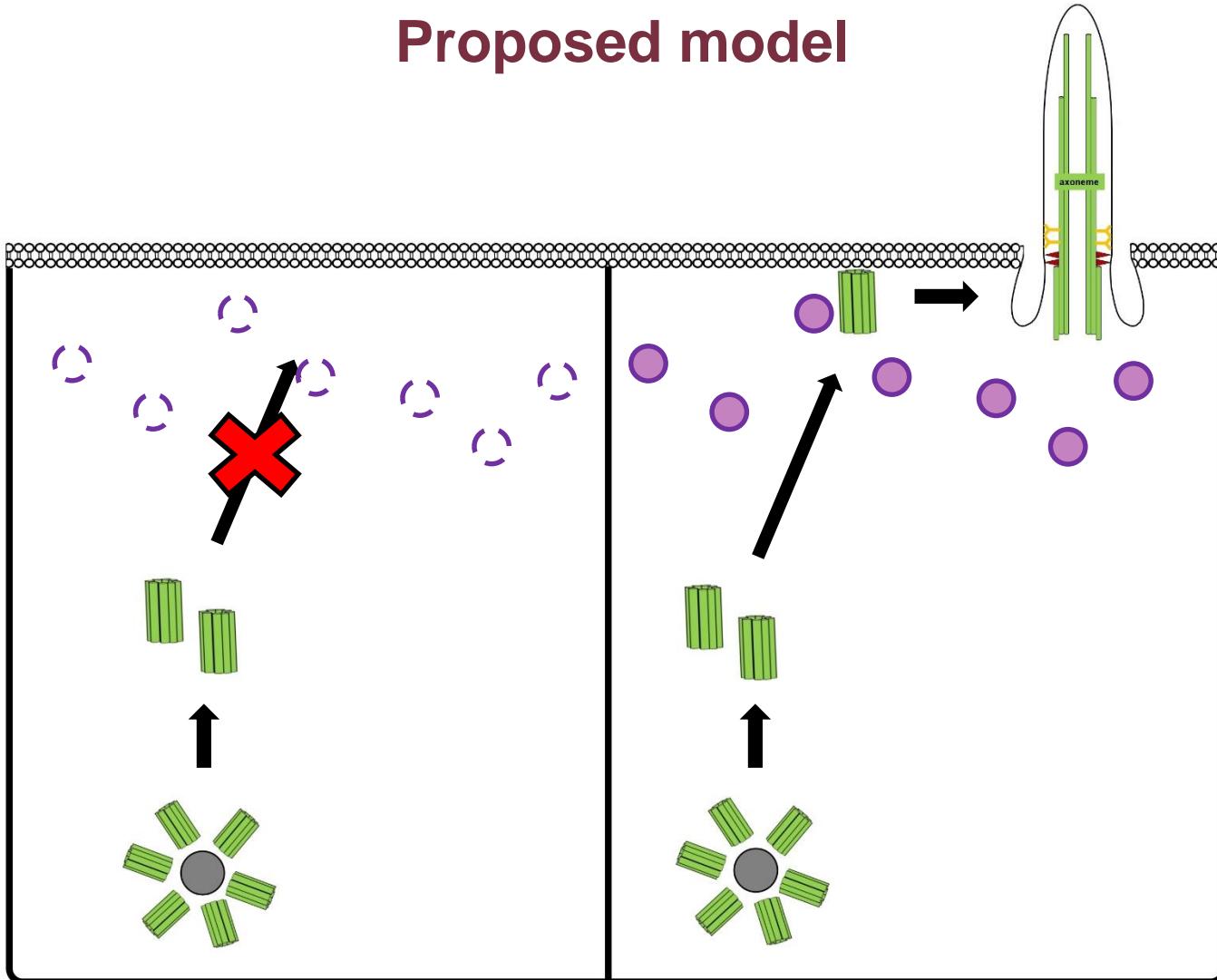
Olcese *et al.*, 2017



Summary

- PIH1D3 is involved in kidney development.
- PIH1D3 synergizes of Larp6 function in MCC ciliogenesis.
- PIH1D3 and LARP6 form distinct granules in MCCs.

Proposed model



● LARP6/PIH1D3 Complex
● Centrosome/Basal Body



Reference List

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- Yi-Ni Ke, Wan-Xi Yang, Primary cilium: an elaborate structure that blocks cell division? *Gene*, Volume 547, Issue 2, 1 September 2014, Pages 175-185, ISSN 0378-1119, <https://doi.org/10.1016/j.gene.2014.06.050>



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Future directions

- Identify the role of granules including LARP6/PIH1D3 complex in ciliogenesis.
- Identify RNAs that interact with LARP6 in ciliogenesis and test if they are included in LARP6/PIH1D3 complex.
- Screen for patients with mutations in LARP6.