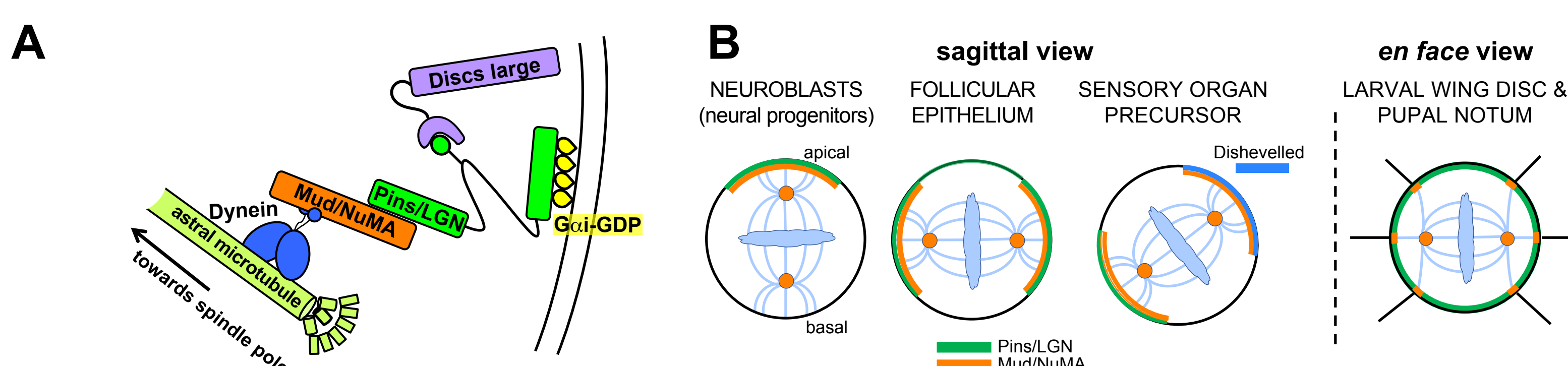


# Slogging through Mud: Isoform Expression and Function

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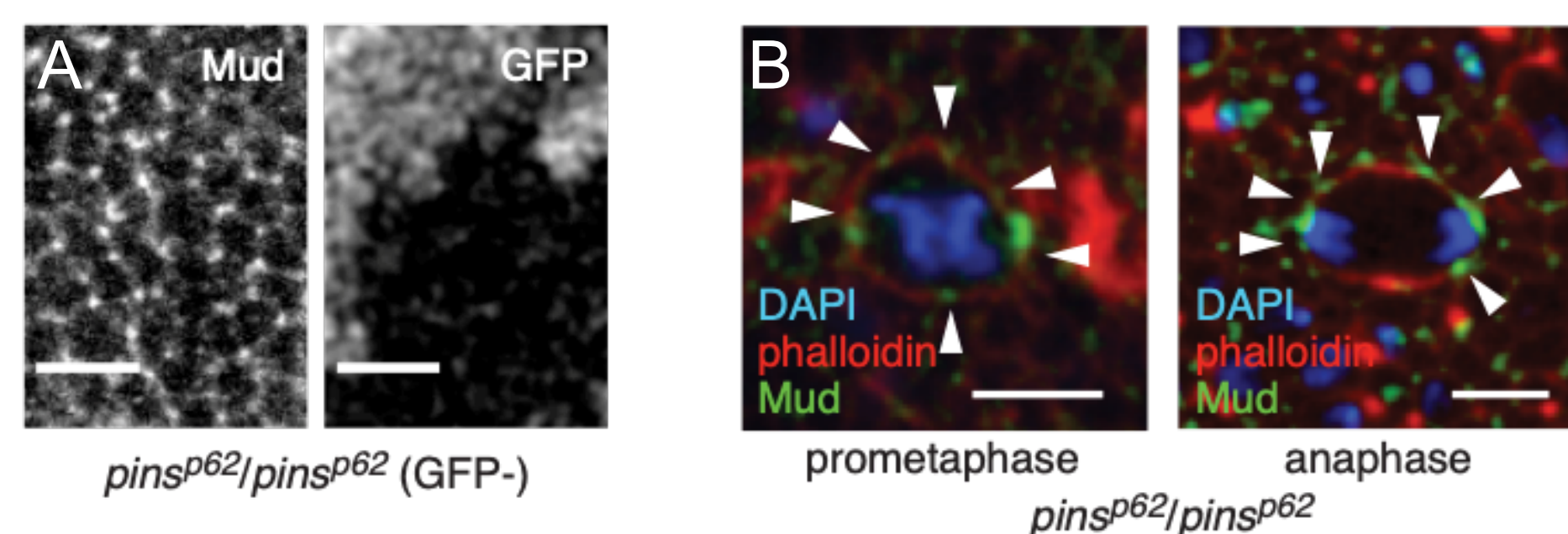
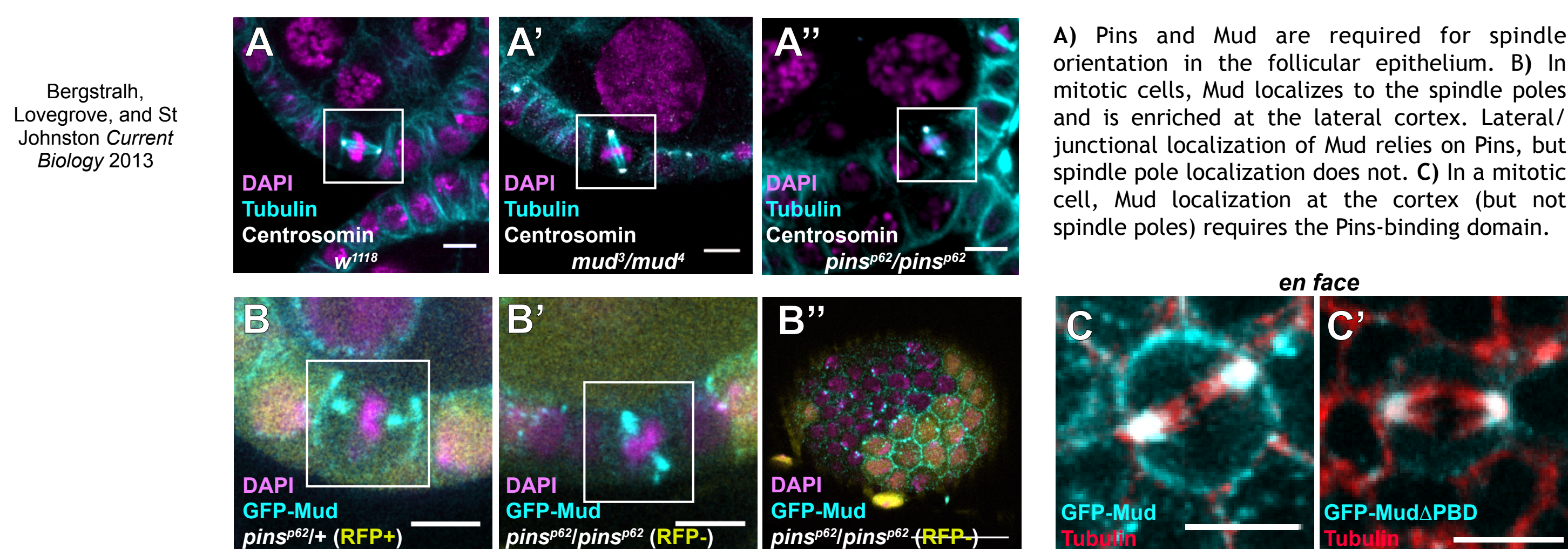
**Abstract:** The conserved protein Mud/NuMA/Lin-5 (flies, vertebrates, worms) has long been implicated in the orientation of cell division. Together with Dynein, Mud acts to produce a pulling force that reels astral microtubules, and therefore the metaphase spindle, into alignment. This force is anchored by another conserved factor, Pins/LGN/GPR1-2 (flies, vertebrates, worms), in most cell types examined. We and others have found that multiple isoforms of Mud do not include the Pins-binding domain. Mud is less well-studied outside of spindle orientation, but is implicated in diverse processes, including migration of the oocyte nucleus and spindle cohesion at meiosis II. We are currently investigating the expression and function of Mud isoforms, and in particular whether they participate in Mud function outside of spindle orientation.

Localization of the spindle-orienting machinery is cell-type specific.



A) The direction of cell division is determined by the orientation of the mitotic spindle at metaphase. Spindle orientation relies on a highly conserved complex that includes the adapter protein Mud and microtubule motor protein Dynein. These proteins act together to exert a pulling force on astral microtubules, which draws the spindle into alignment. B) The spindle-orienting complex is restricted to distinct regions of the cell cortex, depending on the cell type.

In the *Drosophila* follicular epithelium, Pins is required to localize Mud to the cortex, but not to spindle poles.

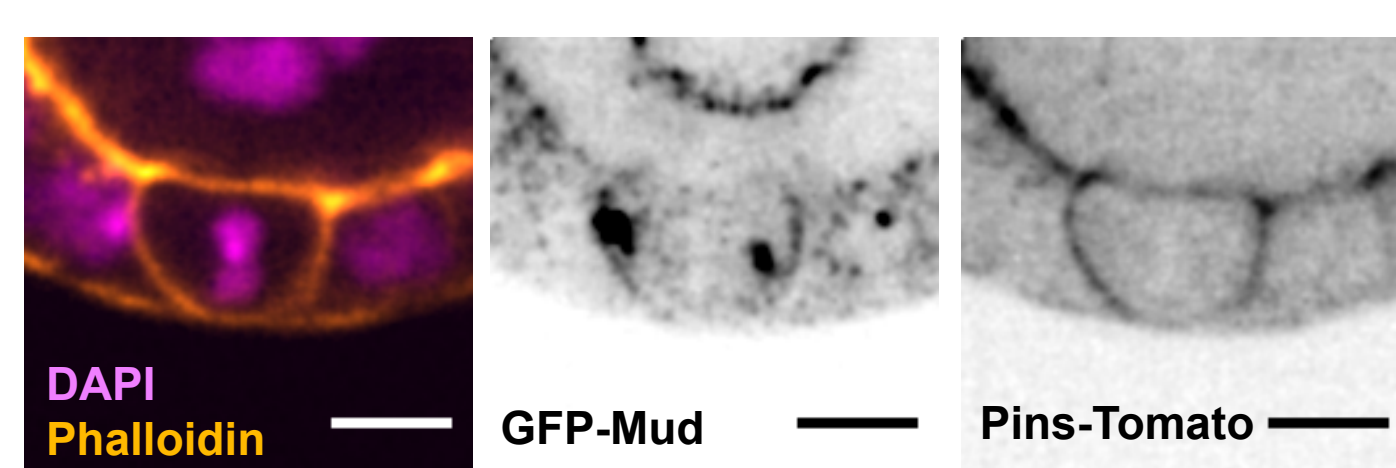
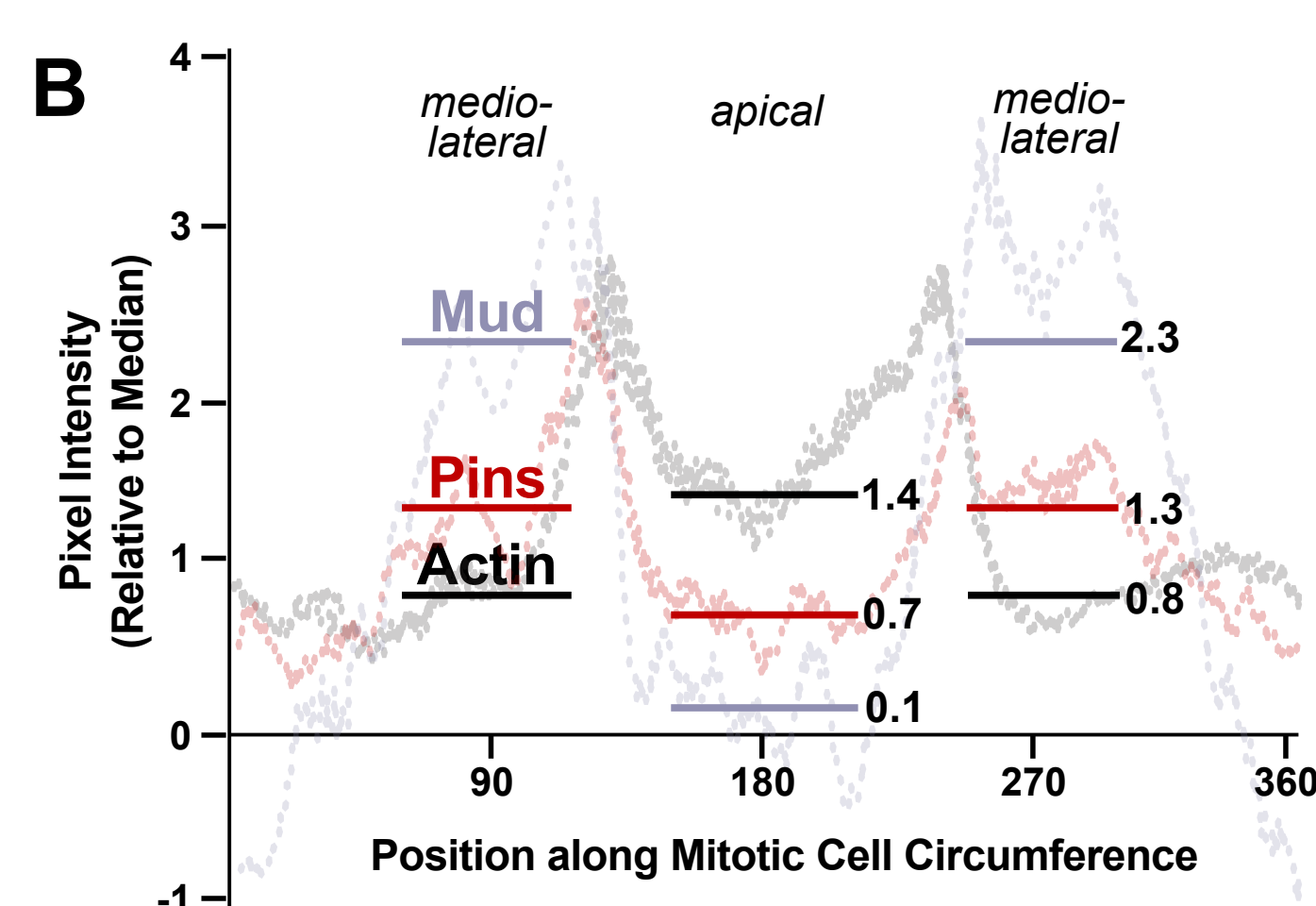
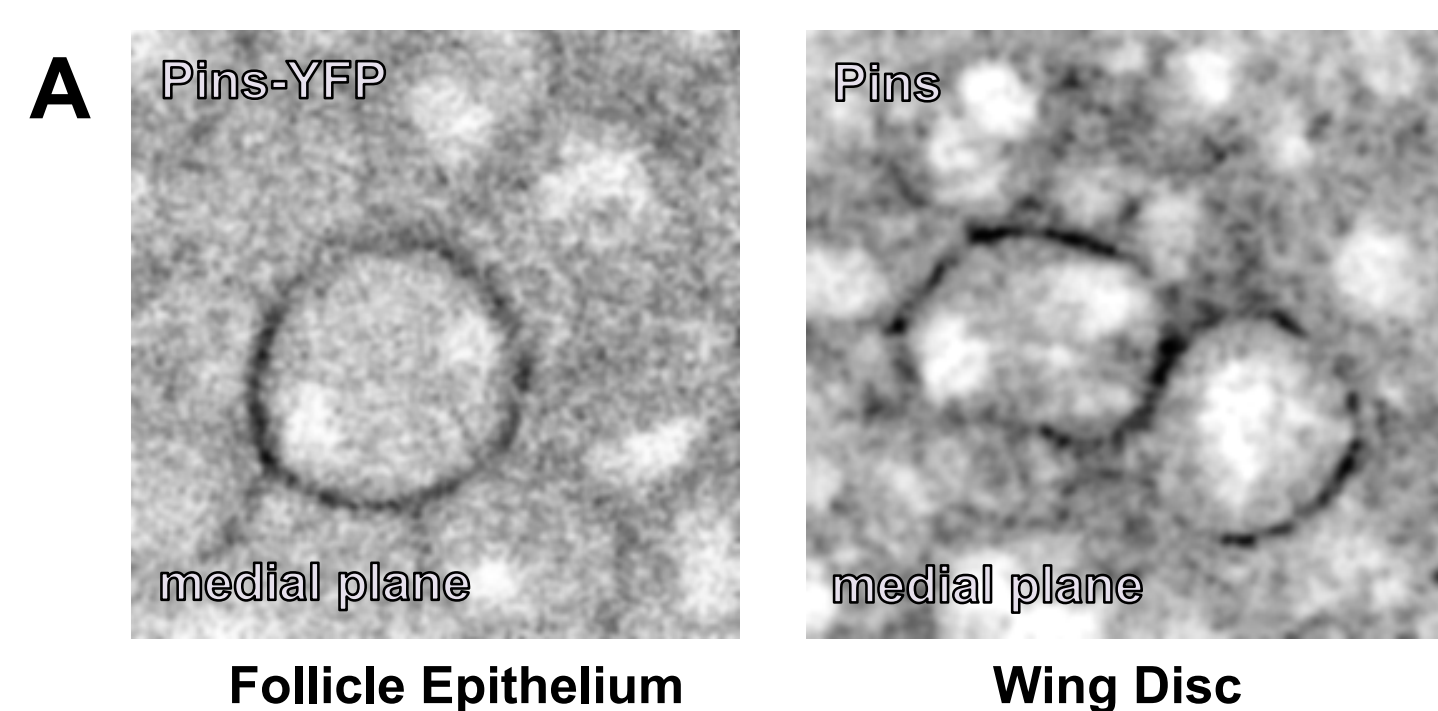


Mud localization is Pins-independent in the wing disc.

A and B) In the wing disc, Mud localizes to tricellular junctions and spindle poles in the absence of Pins.

Bosveld et al. Nature 2016  
Bergstrahl et al. Development 2016

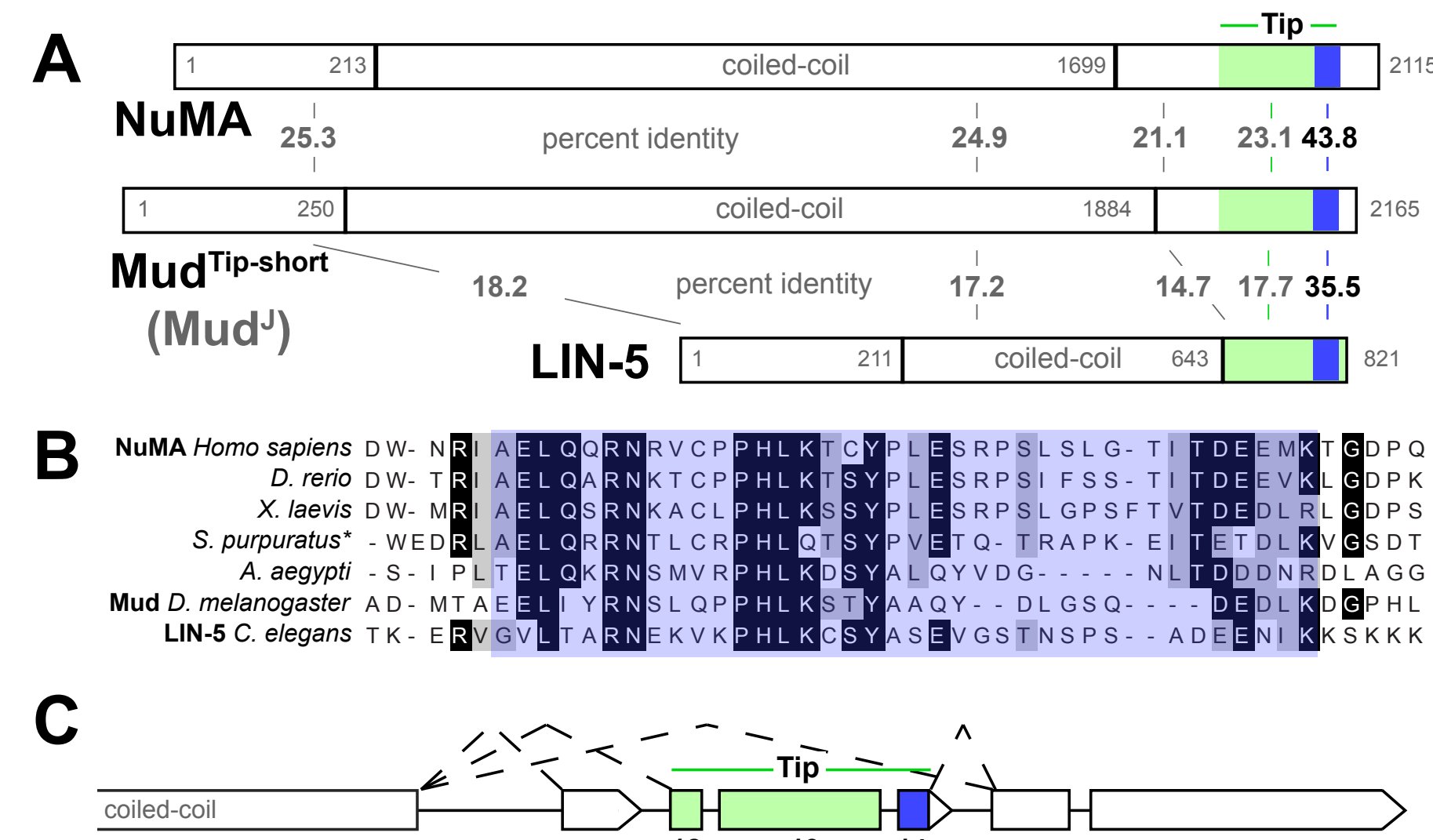
Pins is not sufficient to localize Mud.



A) Pins localizes circumferentially in both the follicular epithelium and wing disc. B) Pins is observed at the apical surface of mitotic follicle cells, but Mud is not.

## How Does Mud Work?

We have identified the Mud<sup>TIP</sup> region

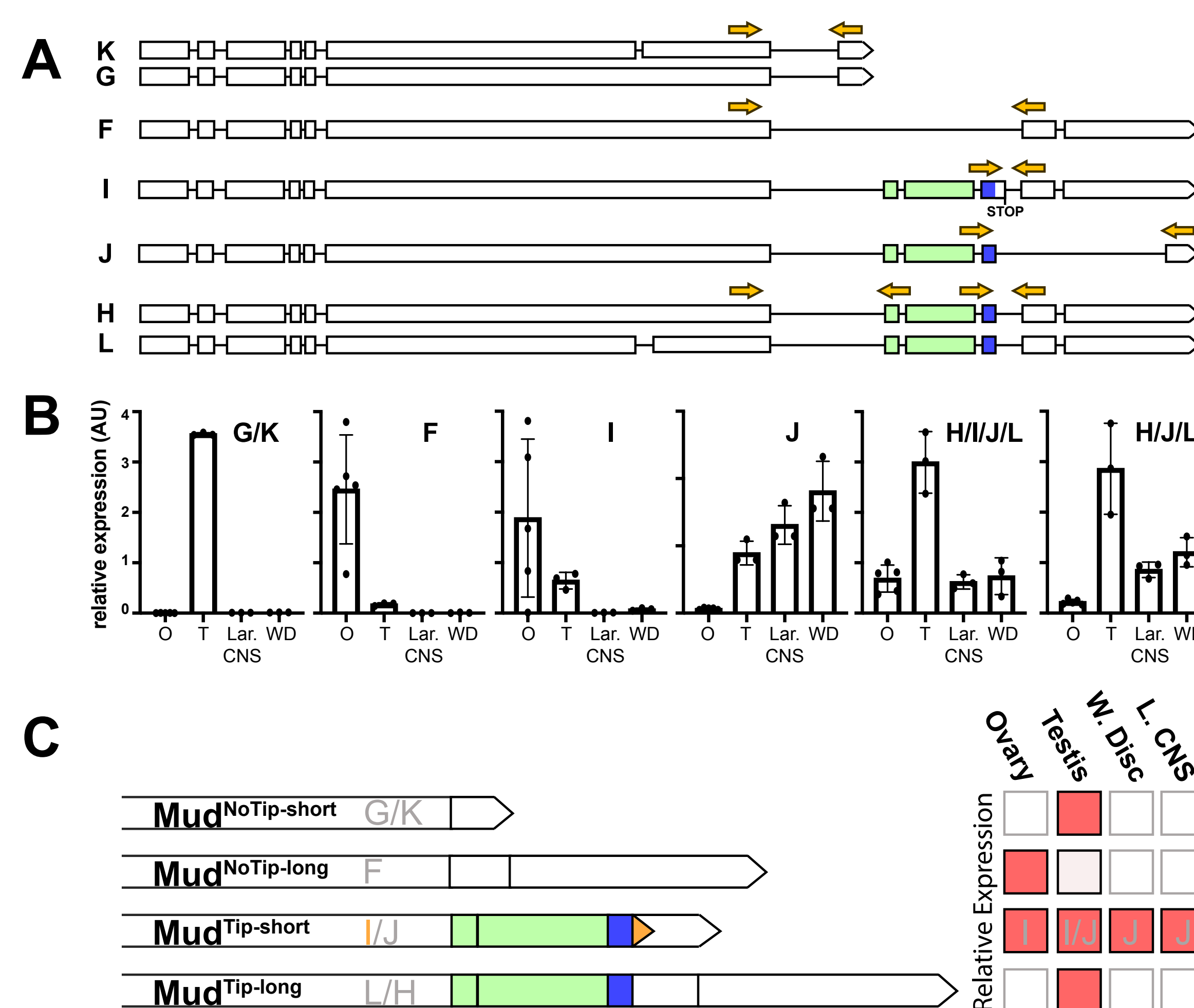


A microtubule binding domain (MTBD) and LGN/Pins binding domain (PBD) have been roughly mapped to the Mud C-terminus. These domains are in close proximity (or overlap) within a region identified as the “NuMA-TIP” (Seldin *et al.*).

A and B) The TIP regions (green) of NuMA, Mud, and LIN-5, as identified by homologous position at the C-terminus. The TIPs included a short highly conserved subsequence (blue). C) The Mud-TIP is encoded by three exons. The highly conserved sequence is encoded by the third of these.

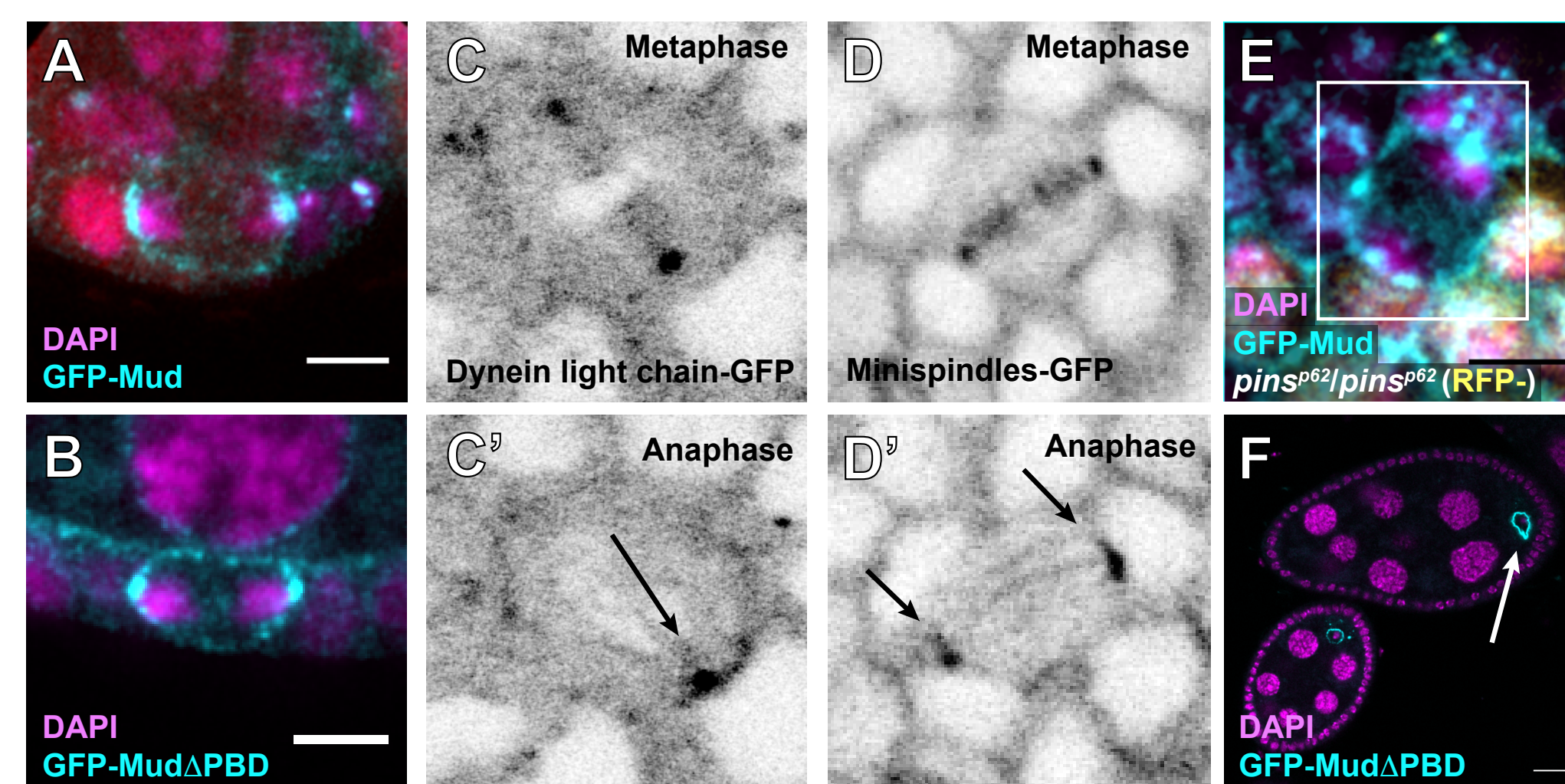
Haren L, Merdes A *Journal of Cell Science* 2002  
Hauschen C, Kenny S, Xu K, Dumont S *eLife* 2017  
Seldin L, Muroyama A, Lechler T. *eLife*. 2016

Mud<sup>NoTip</sup> isoforms are expressed in the ovary and testis



(A) Relative protein expression results of Mud isoforms in different tissues from qPCR. Isoforms G, K, and F are expressed in gametic tissue. (B) Mud Isoforms split into categories based on size and inclusion of Mud<sup>TIP</sup>. Some are cross-listed due to very similar gene products.

Preliminary work suggests Pins-independent functions for Mud.



A and B) Mud is cortically enriched at anaphase, even if its Pins-binding domain is deleted (B). C) At metaphase, Dynein light chain is observed at spindle poles, and weakly decorates the spindle. At anaphase, it can be clearly observed at the pole and cortex. Only one of two poles is seen in this image, as the other one has dropped below the plane of focus. D) Minispindles, a centrosome-associated protein, decorates the spindle and is strongly observed at poles. Localization spreads slightly along the cortex at anaphase. E) Mud enrichment can be seen at the anaphase cortex in a pins null cell. F) Like Mud (not shown), MudΔPBD is observed around the oocyte nucleus.