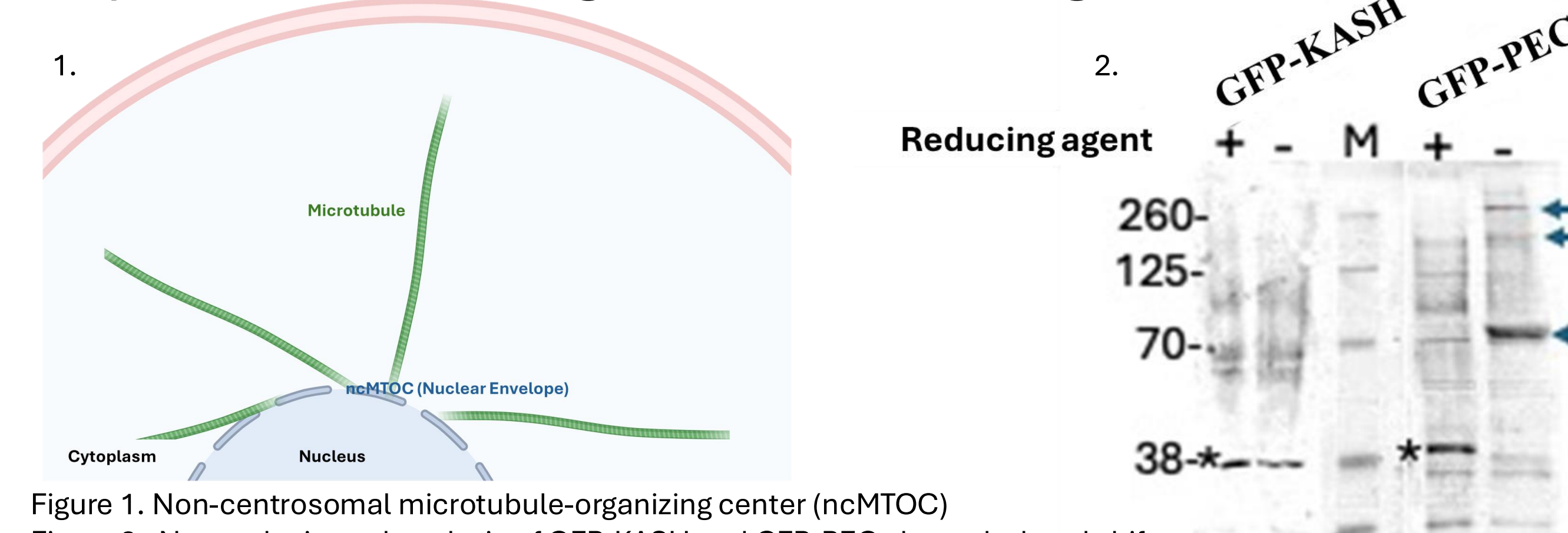


A KASH-less Msp300 isoform has a novel outer nuclear membrane targeting domain and scaffolds the non-centrosomal microtubule organizing center in fat body cells.

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Introduction

In many cells the MTOC, microtubule organizing center, occurs at the centrosome. However, *Drosophila* fat body cells have a noncentrosomal microtubule-organizing center (ncMTOC) located on the nuclear surface. Typically a perinuclear ncMTOC is formed by a LINC complex between a nesprin, KASH and SUN domain. In *Drosophila* fat bodies, the nesprin is Msp300. Msp300 has 11 isoforms. Previous research has shown that only 2 isoforms are needed in order to generate the ncMTOC; Msp300-PE and Msp300-PG. Closer inspection showed that Msp300-PE has an unusual domain structure where it actually lacks a KASH domain. This means that there is an unknown inner nuclear membrane partner that Msp300-PE is binding with to generate the ncMTOC. A Western blot confirmed this, by using a non reducing agent Msp300-PEC has a higher molecular weight than before.



Methods

We maintained *Drosophila* by changing their food every 4 weeks. Flies were raised using standard food made of cornmeal, molasses, and yeast and were maintained at 25°C. *Drosophila* fat body cells used were obtained by dissecting 3rd instar larvae in 1x D-PBS then transferred to PolyK slides. We used immunostaining to highlight certain cellular structures and fluorescence microscopy to capture high resolution images. We used CoinFLP during knockdowns, specifically with GD10317 and GD10416 in order to compare the cellular changes.

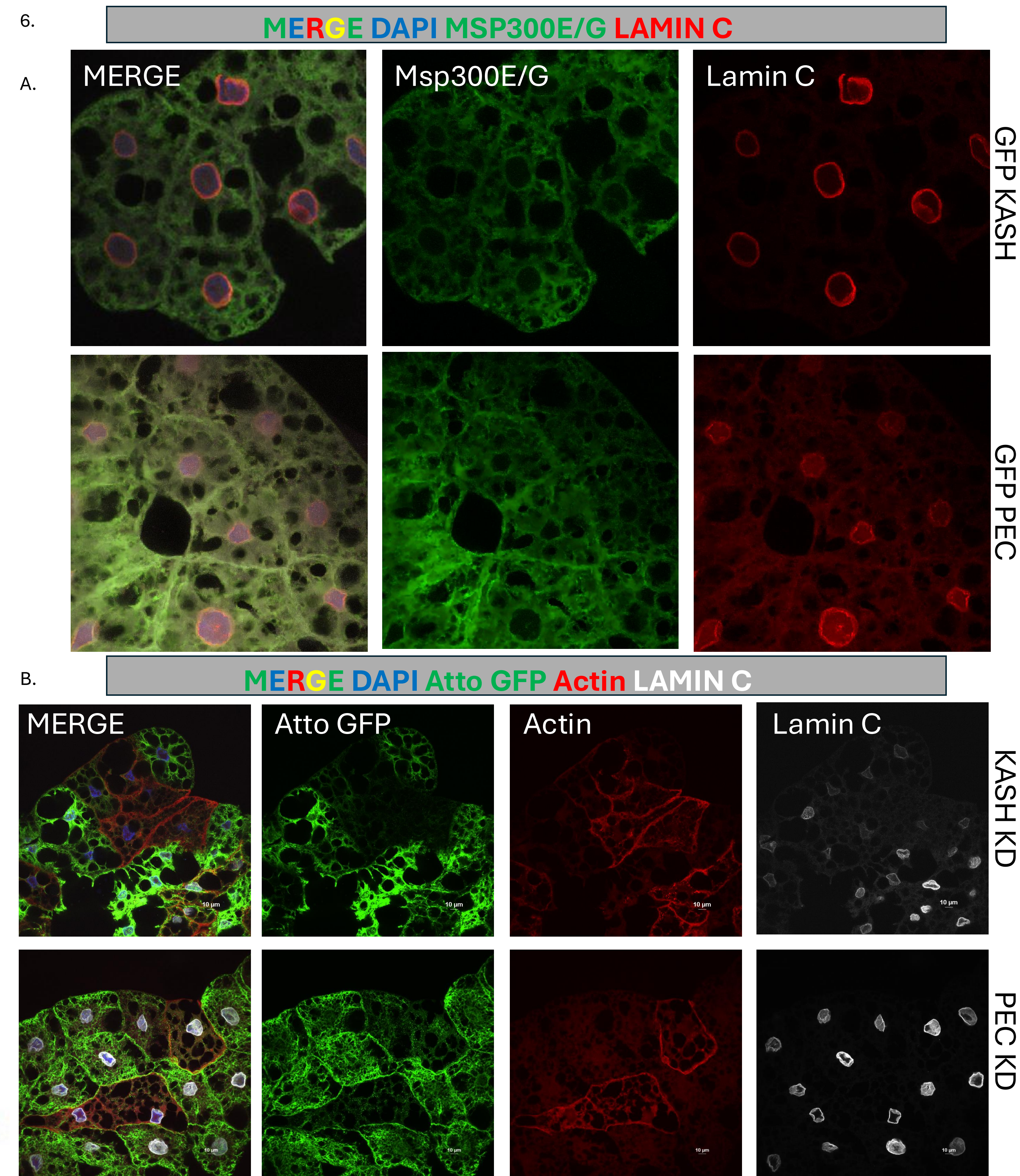
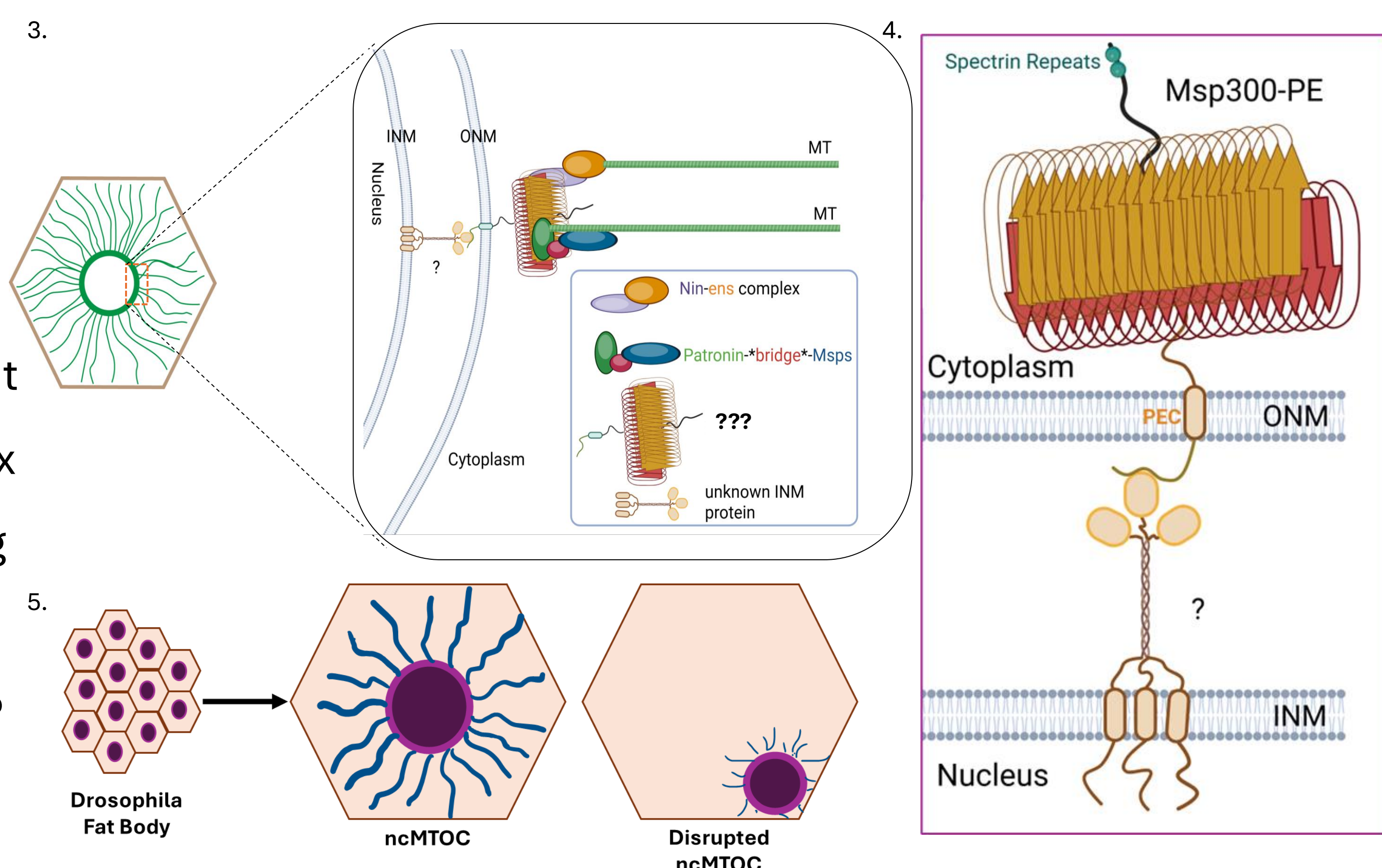


Figure 6. Msp300-PE is sufficient to generate a ncMTOC. A. GFP PEC localizes perinuclearly. B. Knockout of PEC causes disruption of the ncMTOC while knockout of KASH does not.



Results

In GD10317 KASH knockdown was stimulated, from this we can see no nuclear mispositioning or affect on the organization of microtubules. However, in GD10416 where knockdown of -PE and -PG were stimulated instead both nuclear mispositioning and misorganization of the microtubules can be seen. In both GFP KASH and GFP PEC they can be seen localizing around the nucleus, while the signal for GFP KASH is stronger than GFP PEC.

Discussion

In KASH knockdown there is not disruption of the ncMTOC unlike in the knockdown of -PE and -PG. Indicating that the KASH domain in the *Drosophila* fat bodies are not necessary for the generation of the ncMTOC. Msp300 -PE is localized perinuclearly and is sufficient to generate the perinuclear ncMTOC. The stronger signal from GFP KASH indicates that KASH is more prevalent on the nuclear membrane than GFP PEC. Though GFP PEC is weaker than KASH, it localized perinuclearly. Isoform E has a KASH-less C terminal, this unique structure indicates an unknown partner on the inner nuclear membrane. While we are unsure what exactly is the unknown partner on the inner nuclear membrane we are currently working towards finding out.

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Figure 3. Overview of the nucleus-associated non-centrosomal microtubule-organizing center (ncMTOC) in the *Drosophila* fat body mediated by Msp300-PE. Figure 4. Msp300-PE may form LINC-like complex with yet-unknown INM partner. Figure 5. Normal ncMTOC compared to Disruption of ncMTOC.