

NORTHWEST

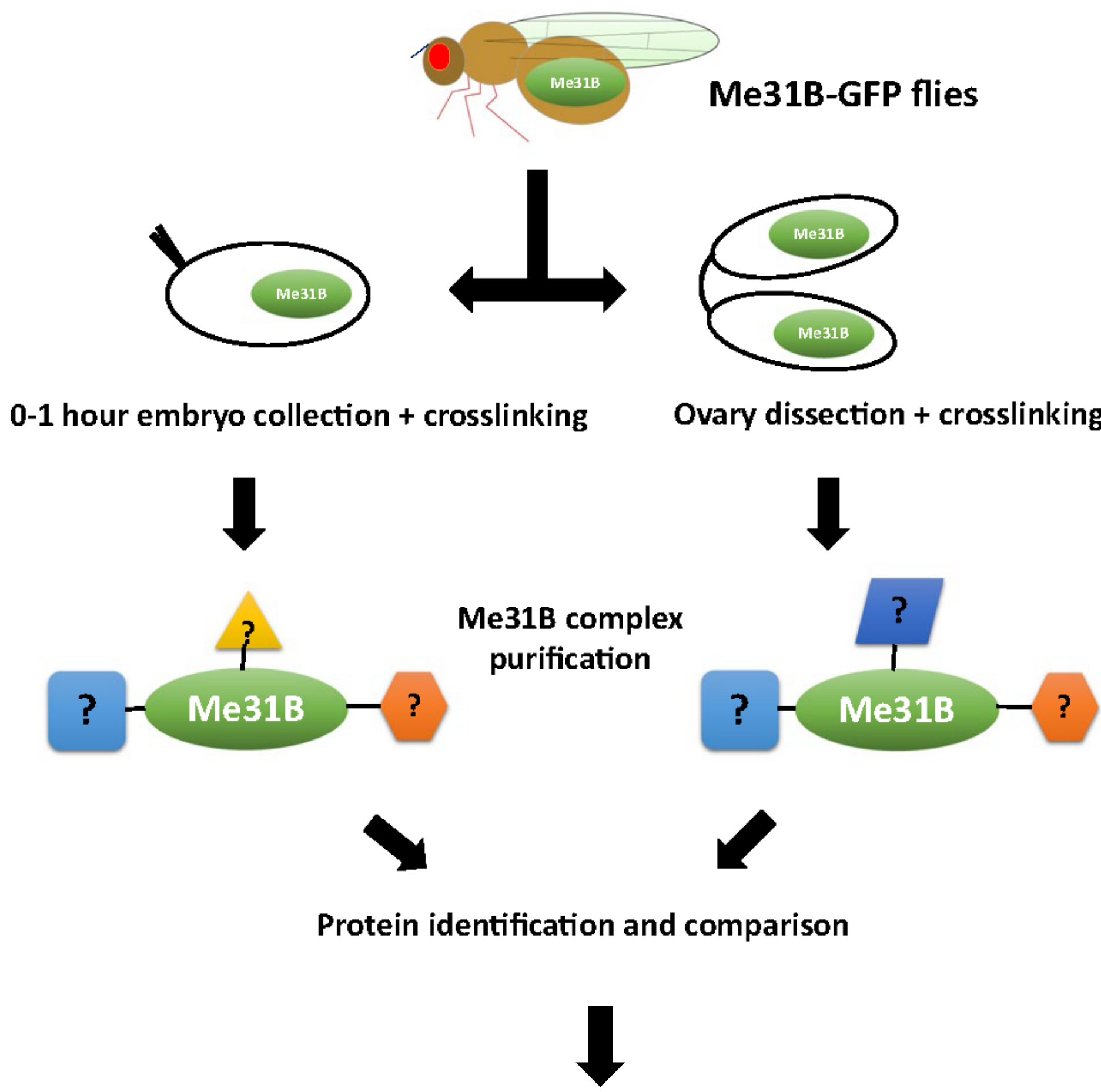
Comparative Proteomics Reveal Me31B’s Interactome Dynamics, Expression Regulation, and Assembly Mechanism into Germ Granules during *Drosophila* Germline Development

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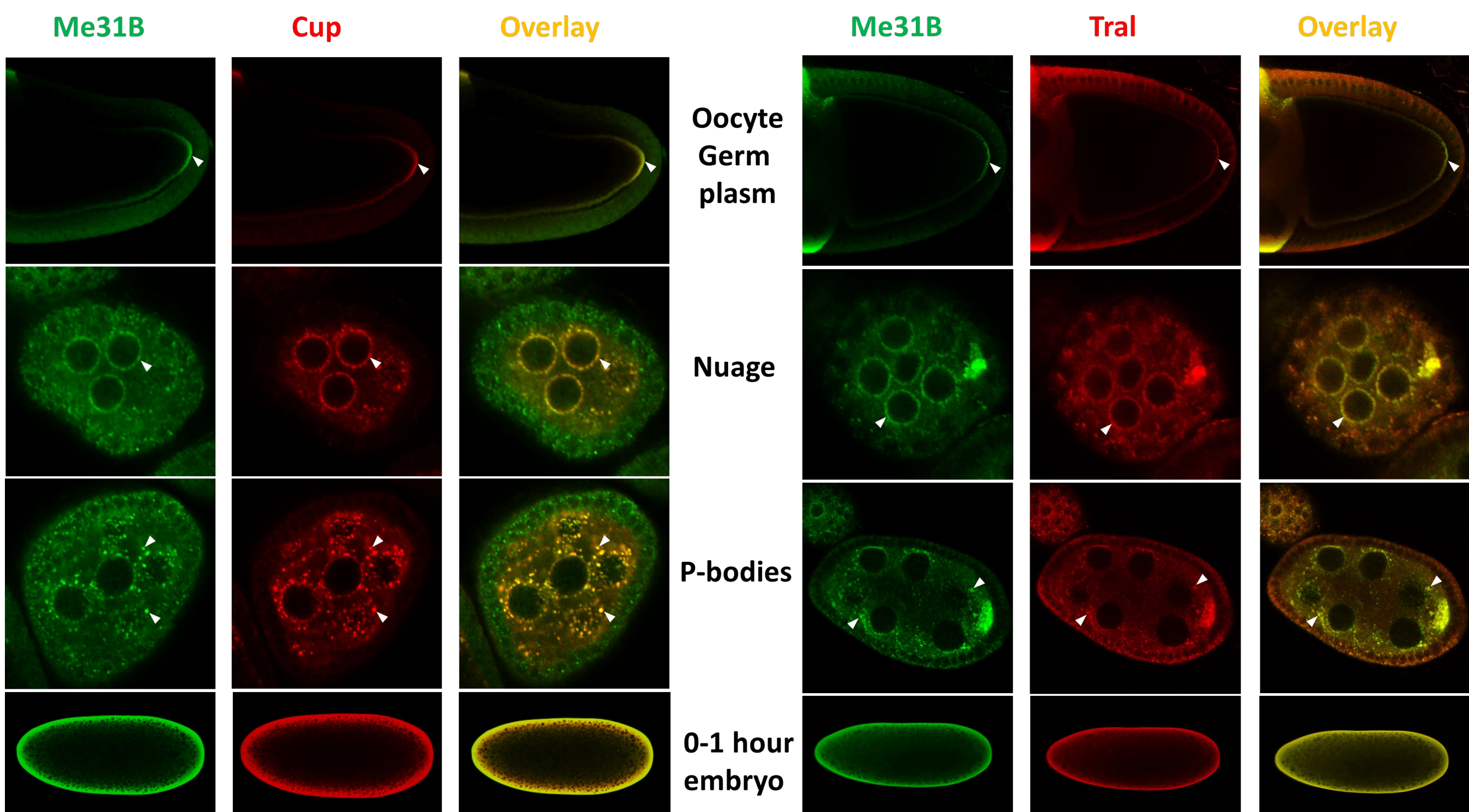
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Abstract: Me31B is a protein component of *Drosophila* germ granules and plays an important role in germline development by interacting with other proteins and RNAs. To understand the dynamic changes that the Me31B interactome undergoes from oogenesis to early embryogenesis, we characterized the early embryo Me31B interactome and compared it to the known ovary interactome. The two interactomes shared RNA regulation proteins, glycolytic enzymes, and cytoskeleton/motor proteins, but the core germ plasm proteins Vas, Tud, and Aub were significantly decreased in the embryo interactome. Our follow-up on two proteins, Tral and Cup, present in both interactomes revealed that they likely colocalize with Me31B in different types of ribonucleoprotein (RNP) granules throughout oogenesis. We further show that Tral and Cup are both needed for maintaining Me31B protein and mRNA stability, with Tral’s effect being more specific. In addition, we provide evidence that Me31B likely colocalizes and interacts with germ plasm marker Vas in the ovaries and early embryo germ granules. Finally, we show that Me31B’s localization in the germ plasm is likely independent of the Osk-Vas-Tud-Aub germ plasm assembly pathway, although its proper enrichment in the germ plasm may still rely on conserved germ plasm proteins such as Aub..

Dynamics of the Me31B Interactome in the Ovary and the Early Embryo

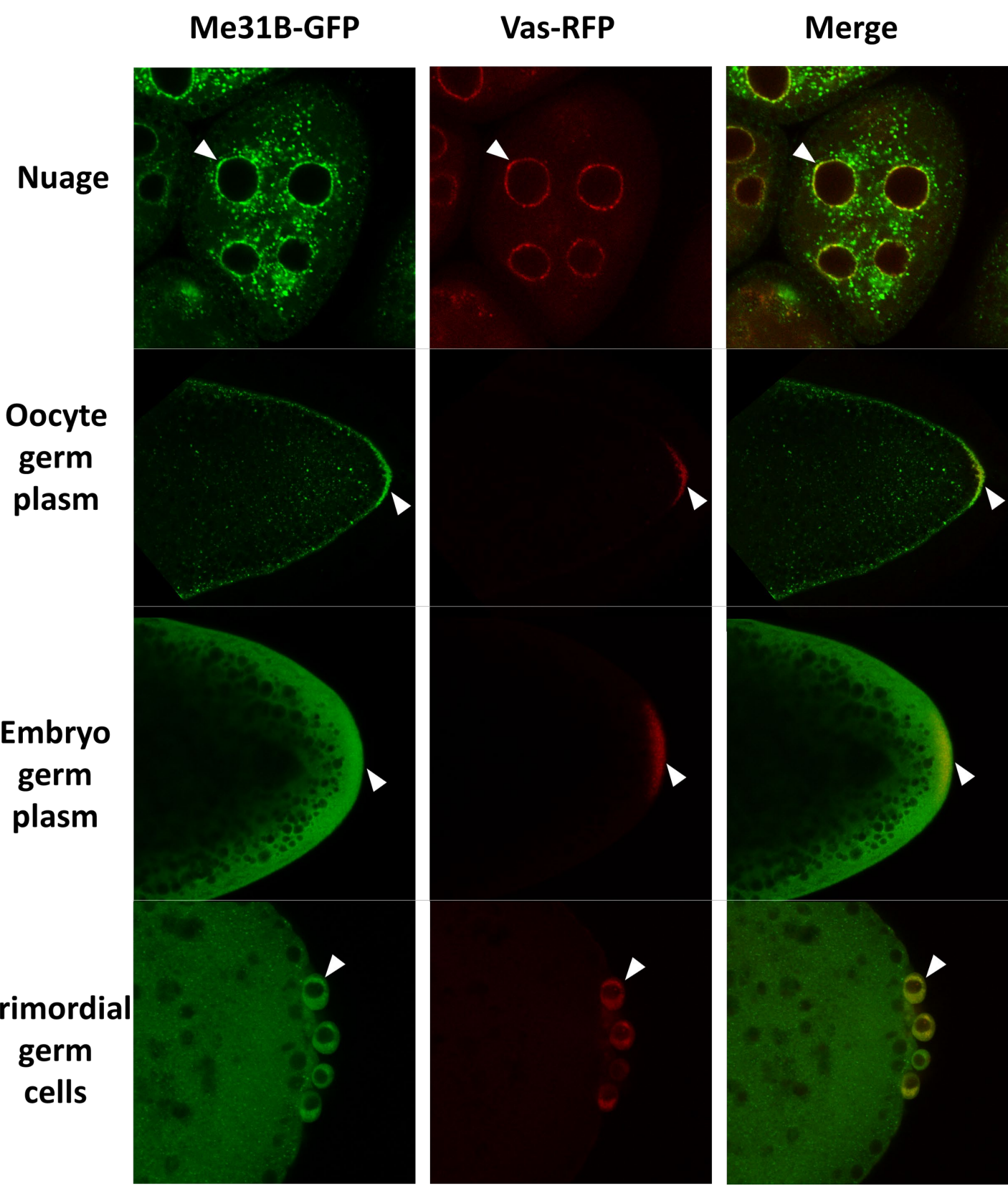


Me31B Colocalizes with Cup and Tral in Different Types of Germ Granules



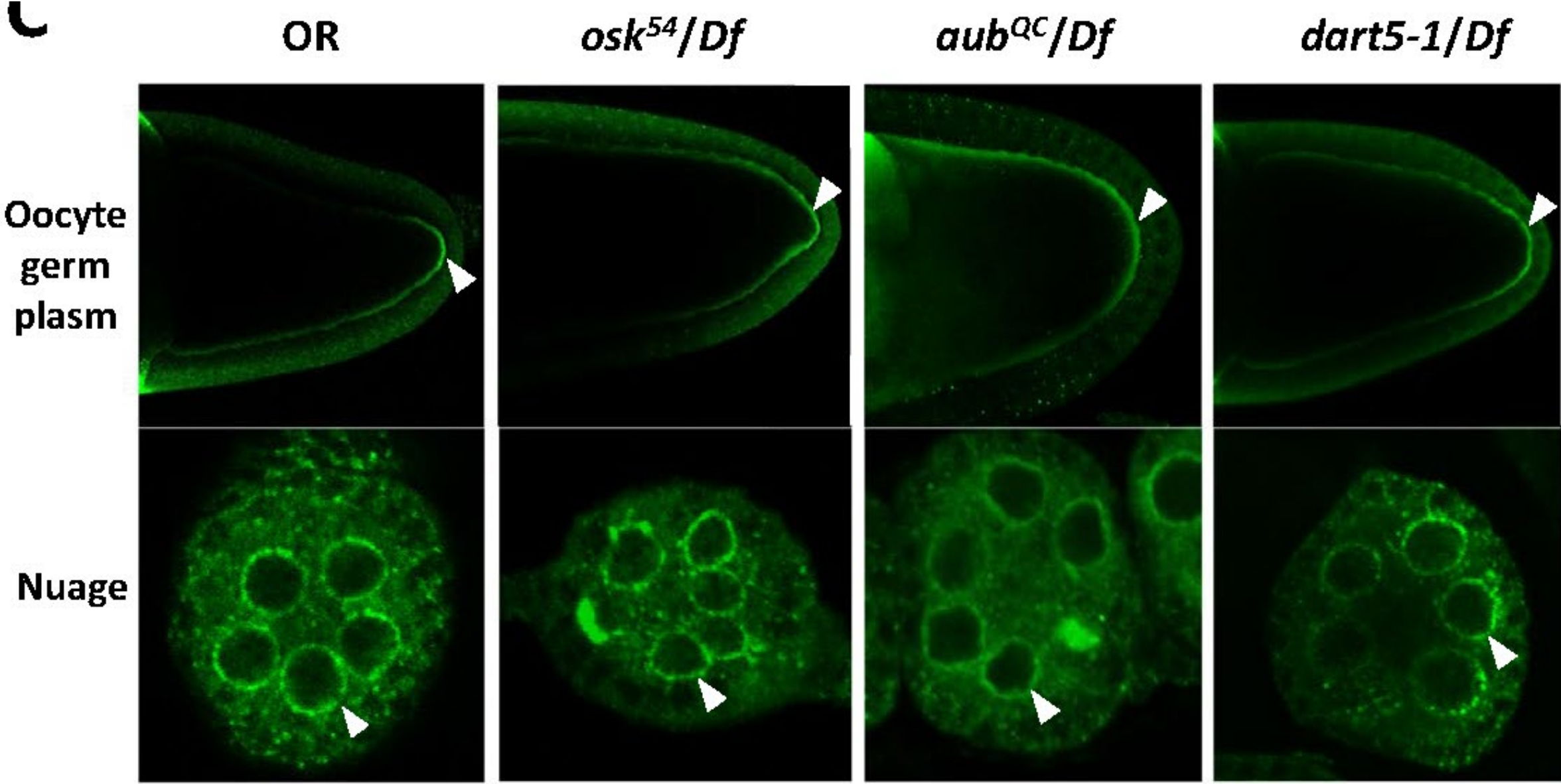
The Me31B-Cup (left panels) and Me31B-Tral (right panels) co-staining show that Me31B colocalizes extensively with both Cup and Tral in the nuage granules of nurse cells, nurse cell cytoplasmic granules that appear to be P-bodies, germ plasm granules in the posterior pole of mid-stage oocytes, and dispersedly in the cytoplasm of early embryos

Me31B and Vas Colocalize During Early Development



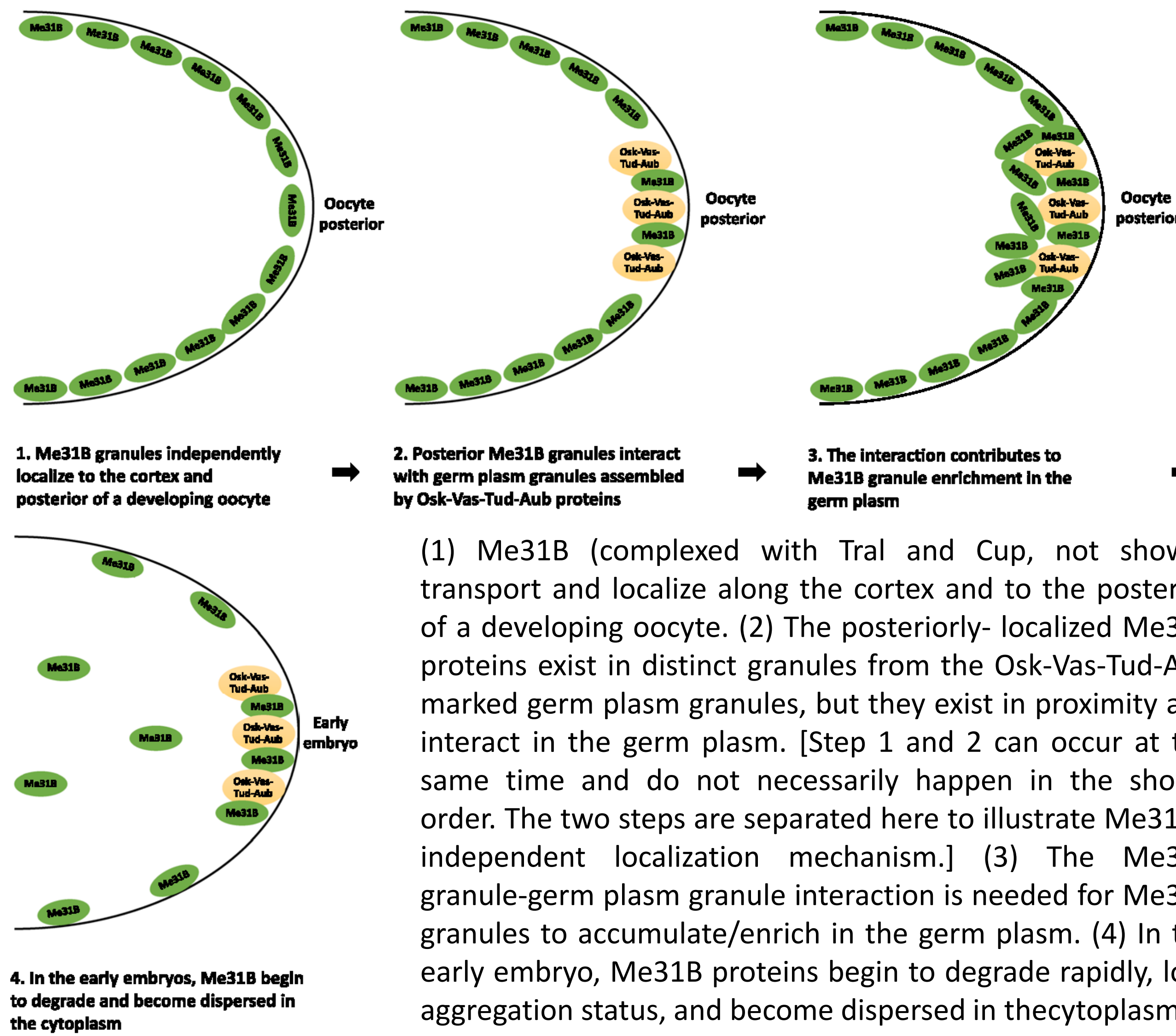
In the ovaries, Me31B-GFP proteins aggregated into granular foci in the nuage of nurse cells and oocyte germ plasm granules, and these foci seemed to colocalize with Vas-RFP granules. However, in the early embryos and primordial germ cells, Me31B-GFP appeared randomly dispersed rather than aggregated. Although the dispersed Me31B-GFP still overlapped with the Vas-RFP, we found it more reasonable to interpret that the Me31B-GFP simply diffused to the Vas-RFP area rather than associated specifically with the Vas-RFP granules

Me31B Recruitment to Germ Granules Is Independent of Conserved Germ Plasm Proteins



Me31B localization to the posterior and cortex of oocytes (arrowheads, top panels) and nuage granules (arrowheads, bottom panels) in *ask54/Df*, *aubQC/Df*, and *dart5-1/Df* ovaries are similar to the OR control. However, the Me31B enrichment levels at the posterior over that along the cortex in *aubQC/Df* is significantly lower than the control.

Hypothetical model of Me31B localization and enrichment into germ plasm



(1) Me31B (complexed with Tral and Cup, not shown) transport and localize along the cortex and to the posterior of a developing oocyte. (2) The posteriorly- localized Me31B proteins exist in distinct granules from the Osk-Vas-Tud-Aub marked germ plasm granules, but they exist in proximity and interact in the germ plasm. [Step 1 and 2 can occur at the same time and do not necessarily happen in the shown order. The two steps are separated here to illustrate Me31B’s independent localization mechanism.] (3) The Me31B granule-germ plasm granule interaction is needed for Me31B granules to accumulate/enrich in the germ plasm. (4) In the early embryo, Me31B proteins begin to degrade rapidly, lose aggregation status, and become dispersed in the cytoplasm.

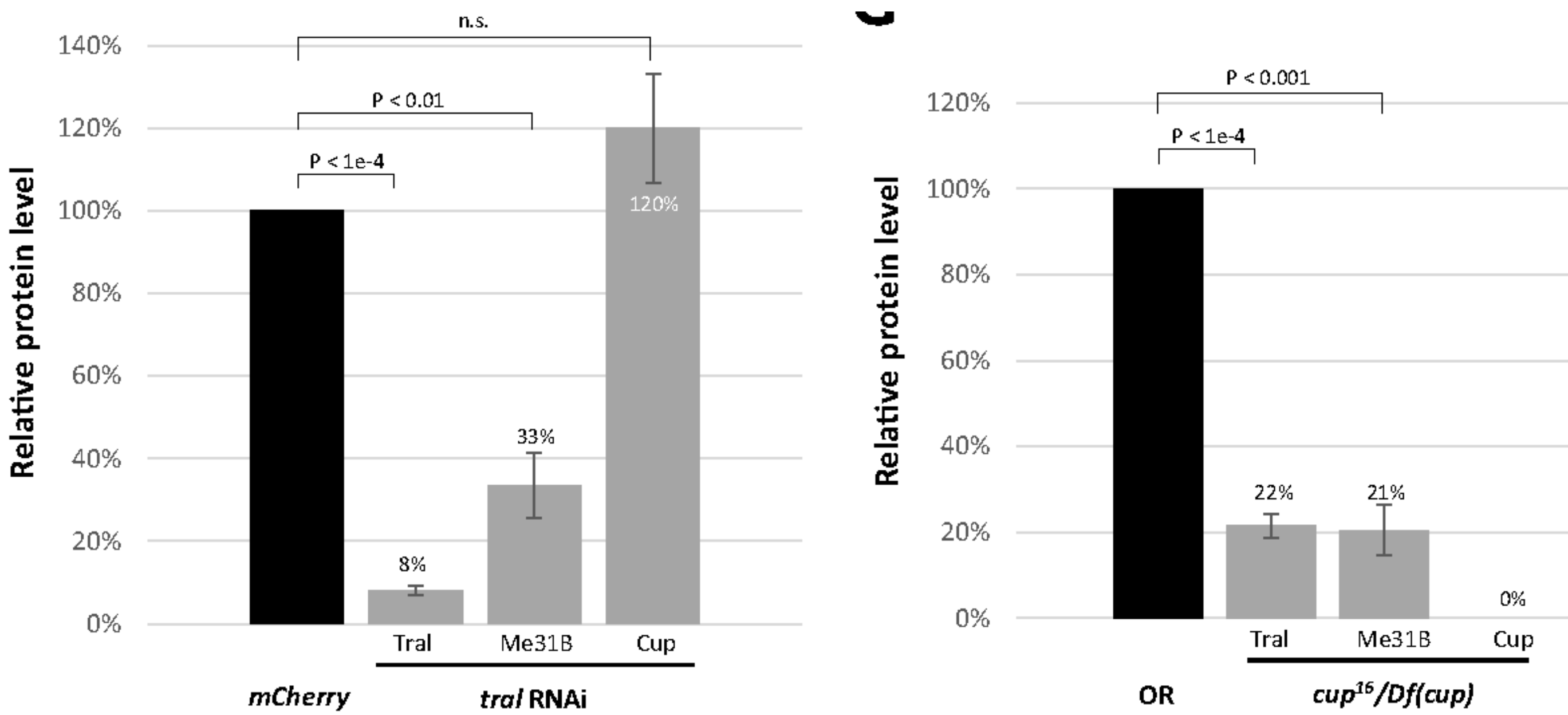
Proteins	Embryo	Ovary*	Granule type
<i>RNA regulation proteins</i>			
Cup (Cup)	•	•	P body, nuage, germ plasm
Trailer hitch (Tral)	•	•	P body, nuage, germ plasm
Belle (Bel)	•	•	Nuage, germ plasm
Bicaudal-C (BicC)	•	•	P body
Edc3 (Edc3)		•	P body
Pacman (Pcm)	•	•	P body
NOT1 (Not1)		•	P body
eIF4G (eIF-4G)		•	
eIF4E (eIF-4E)	•	•	
<i>Cytoskeleton and motor proteins</i>			
Dynein heavy chain (Dhc 64C)	•	•	Germ plasm
Kinesin heavy chain (Khc)	•	•	
Kinesin light chain (Klc)		•	
β-Tubulin (BetaTub 56D)	•	•	
<i>Glycolytic enzymes</i>			
Pyruvate kinase (PyK)	•	•	Germ plasm
Phosphoglycerate kinase (PgK)	•	•	Germ plasm
Enolase (Eno)	•	•	
6-phosphofructokinase (Pfk)	•	•	
<i>Germ plasm proteins</i>			
Tudor (Tud)		•	Nuage, germ plasm
Vasa (Vas)		•	Nuage, germ plasm
Aubergine (Aub)**		•••	Nuage, germ plasm
eIF4A (eIF-4a)	•	•	Germ plasm

“•”Indicates the protein was detected in at least 3 out of the 4 independent repeats and are enriched more than 2-fold over the control

*The ovary Me31B interacting protein data were published previously.

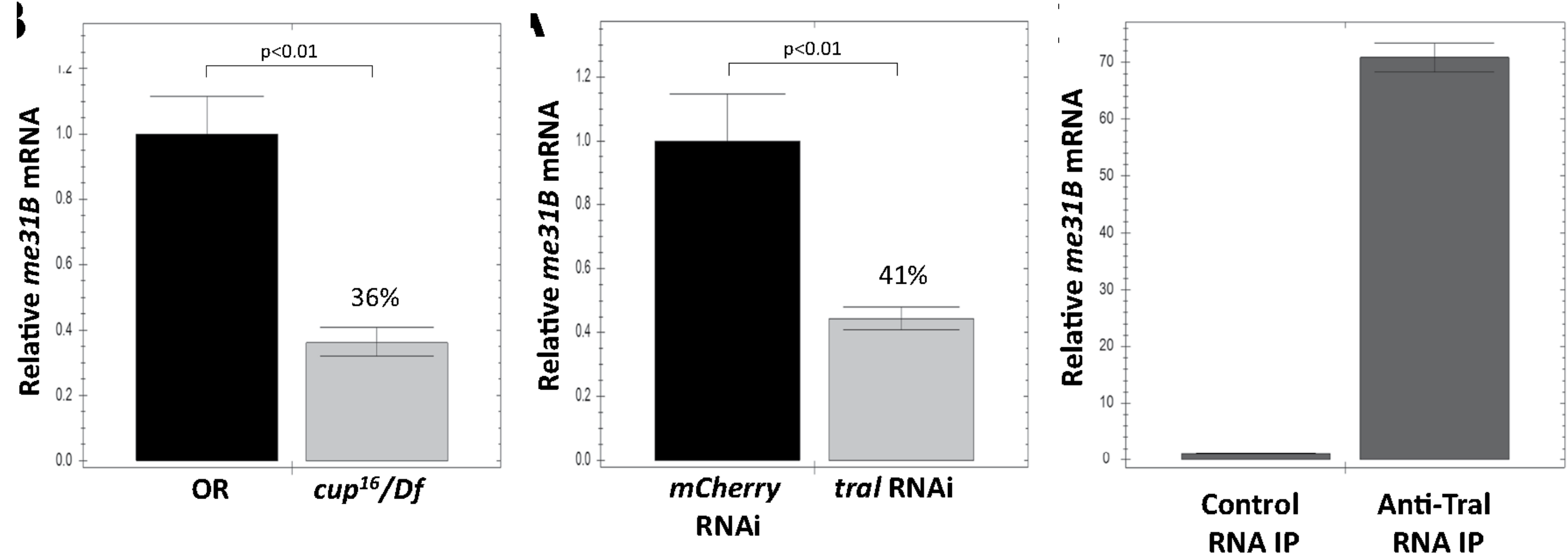
**Aub was reported as a component of Me31B ovary interactome in the previously study, and Aub was detected in 2 out of the 4 independent biological repeats in that study.

Me31B Protein Level Depends on Cup and Tral



Quantification of anti-Me31B western blots were conducted in *cup* mutants, *tral* RNAi, and control fly ovaries. Me31B protein levels decrease in all the *cup* mutants as well as the *tral* RNAi strain. Note that Tral protein level also decreases in all the *cup* mutants.

me31B mRNA Level Depends on cup and tral



RT-PCR quantification *me31B* mRNA levels show a decrease in *cup* mutants (left panel) and *tral* RNAi (central panel) compared to control fly ovaries. RNA IP of *me31B* RNA from Tral indicates specific *me31B*-Tral binding.

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