

# The Effect of *ribbon* Overexpression on Follicle Cell Proliferation

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## Abstract:

Ribbon (*rib*) is a transcriptional factor which facilitates different aspects of morphogenesis in *Drosophila*. In the embryo, *rib* is found to be a major regulator of gonad coalescence and compaction in addition to regulating somatic cell migration that occurs during development (Weyers et al., 2011; Silva et al., 2016). *rib* is expressed in somatic gonadal precursor cells that migrate and coalesce with primordial germ cells in order to form the premature gonad. Previous work in the Mierisch lab has found that the overexpression of *rib* in somatic follicle cells can cause a premature arrest in oogenesis. This study investigates if the reduction of somatic follicle cells is linked to a decrease in mitosis when levels of *rib* proteins increase. We used the Gal4/UAS system to induce overexpression of *rib* in the female *Drosophila* gonads, and we used fluorescent immunohistochemistry staining to analyze the extent of somatic follicle cell proliferation.

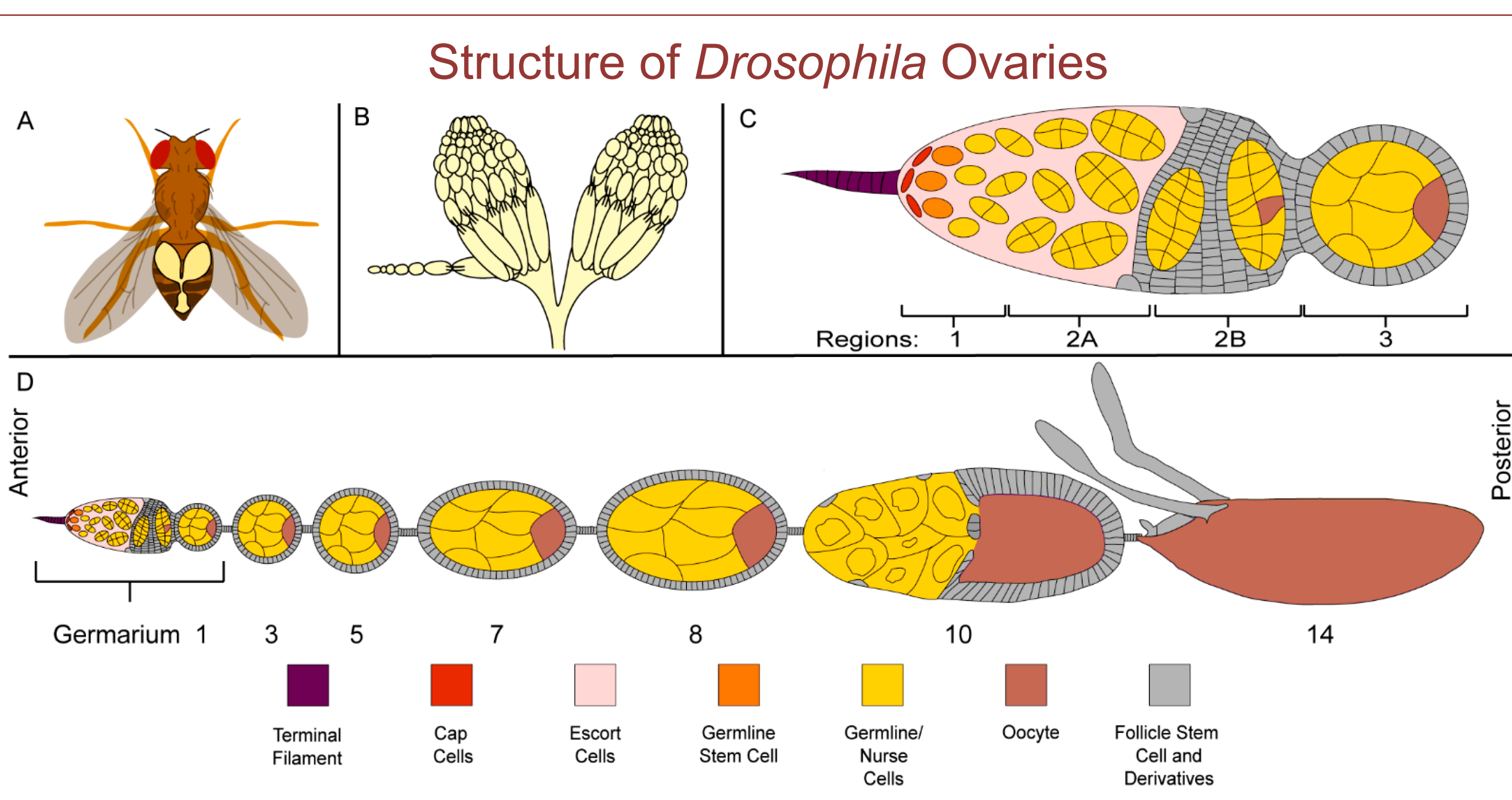


Figure 1. Schematic of *Drosophila* female ovaries (Lebo et al., 2021). Every *Drosophila* female has 2 ovaries, and each ovary contains between 16-20 ovarioles. An ovariole contains 6-7 egg chambers, and each egg chamber is made up of 16 germ cells, including 1 oocyte and 15 nurse cells. The germ cells are enveloped by a layer of somatic follicle cells which assist in the maturation of egg chambers.

## Stages 1-7 of Egg Chamber Development

Stage	1	2	3	4	5	6	7
Identification	reside in the region R3 of germarium (arrow)	karyosome not formed yet, similar sizes of oocyte and nurse cell	Karyosome (arrowhead) and endobody detectable in germinal vesicle	nurse cell nuclei has similar DNA amount and show five-blob phenotype	no five-blob phenotype, posterior nurse cells contain more DNA	follicle cells cease mitosis, equal nurse cell ploidy	more obviously elongated shape of egg chamber, higher nurse cell ploidy at posterior, no oocyte visible
DAPI image							
Area ( $\mu\text{m}^2$ )	N/A	579<	579-970	970-2026	2026-4043	4043-4690	4690-6751
Time of Growth (h)N/A		8	8	6	5	3	6

Figure 2. Image of stages 1-7 in follicle cell development (Jia et al. 2016). Follicle cells progress through 3 phases of development: The mitotic cycle, endocycle, and gene amplification. The mitotic cycle is stages 1-6, where follicle cells go through 8-9 divisions of mitosis. The endocycle is stages 7-10a, where follicle cells undergo growth and DNA synthesis (S), skipping over the second growth(G2) and mitosis(M) stages. The areas of stages 1-6 were used to locate cells in the mitotic phase.

## Gal4/Gal80 UAS System

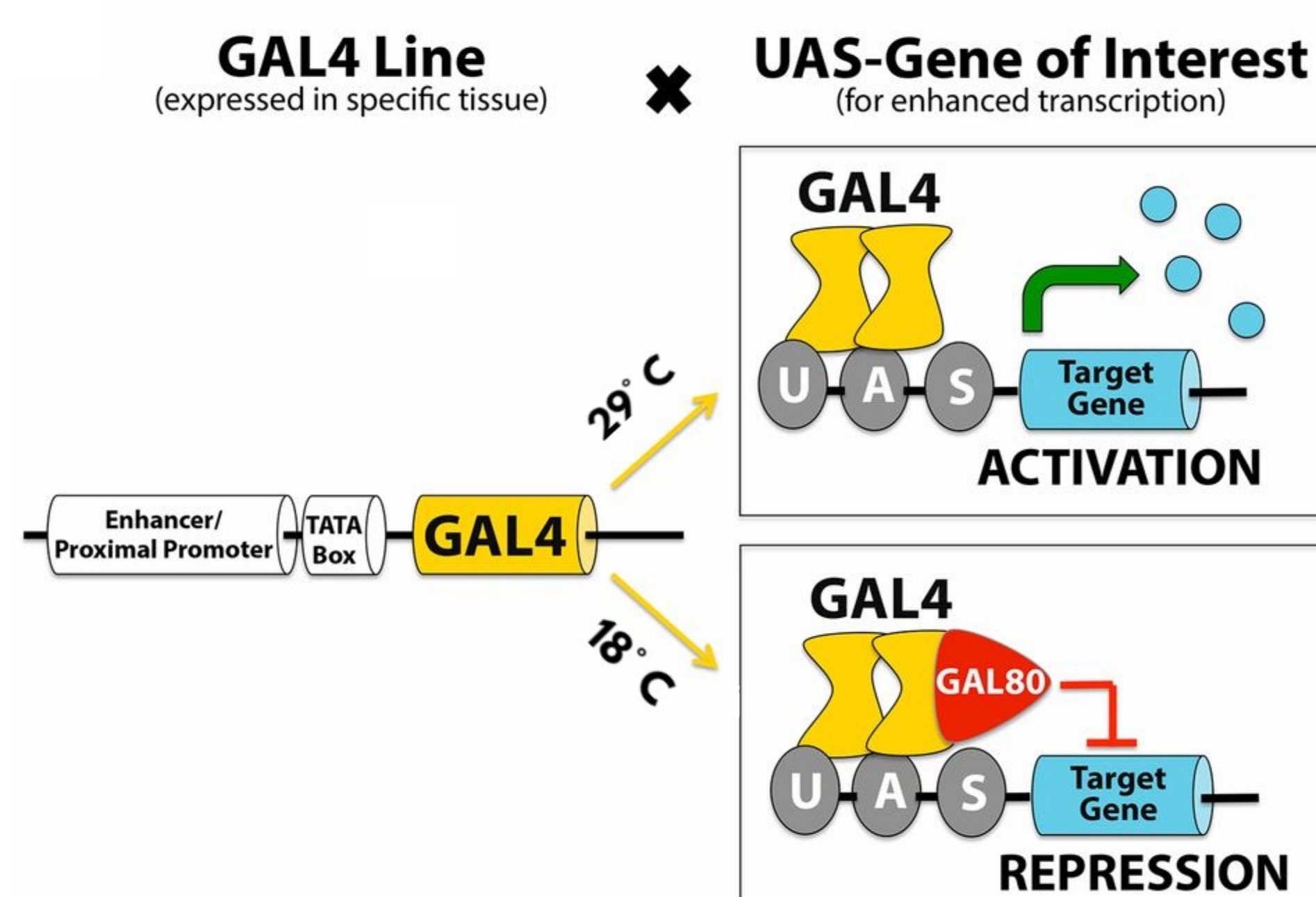


Figure 3. Diagram of Gal4/UAS and Gal80 system of gene regulation (Silva and Jemc, 2015). Gal80 inhibits Gal4 at 18°C, preventing overexpression. Gal4 is activated at 29°C, promoting overexpression of *rib* because Gal80 denatures at higher temperatures.

## Overexpression of *ribbon* Causes Arrest in Oogenesis

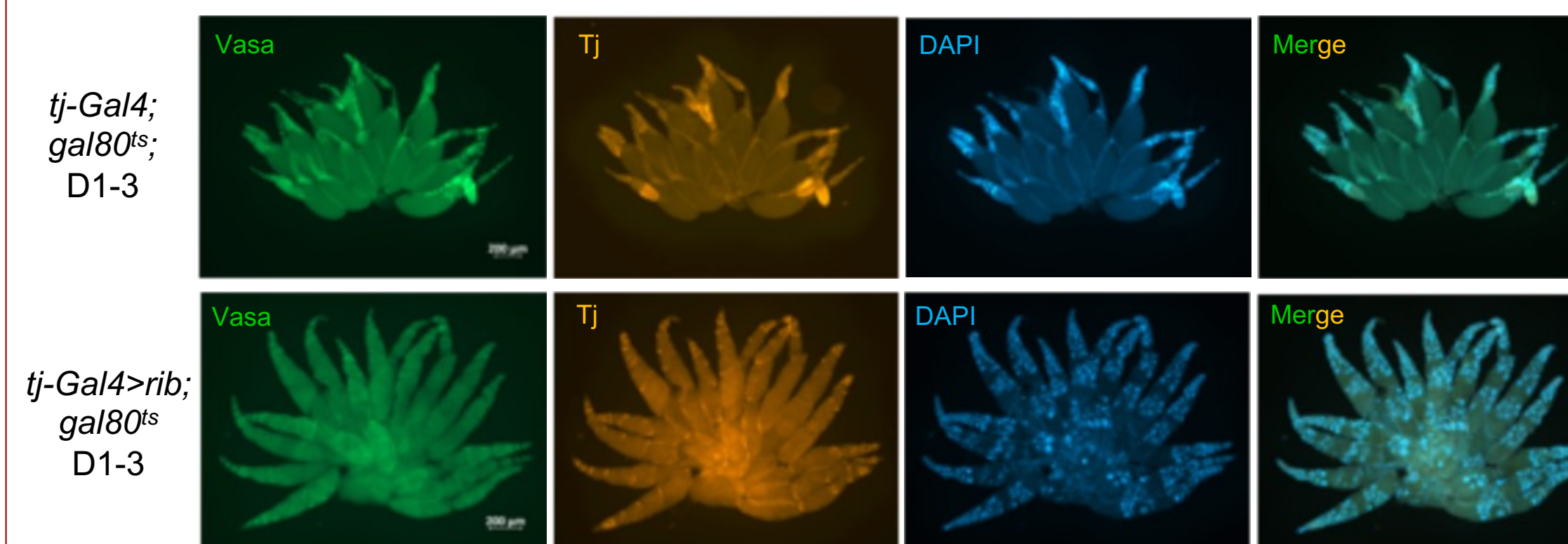


Figure 4. Overexpression of *ribbon* in adult germ and somatic cells affects ovary development (Alvarez 2018). The control ovaries (*tj-Gal4; gal80ts*) demonstrate normal morphology. The mutant ovaries with induced overexpression (*tj-Gal4>rib; gal80ts*) demonstrate a different morphology with varying mutant ovariole shapes and sizes. *Vasa* is a germ cell marker, *Tj* (traffic jam) is a somatic cell marker, and DAPI is a nuclei marker.

## Overexpression of *ribbon* Causes a Reduction in Somatic Follicle Cells

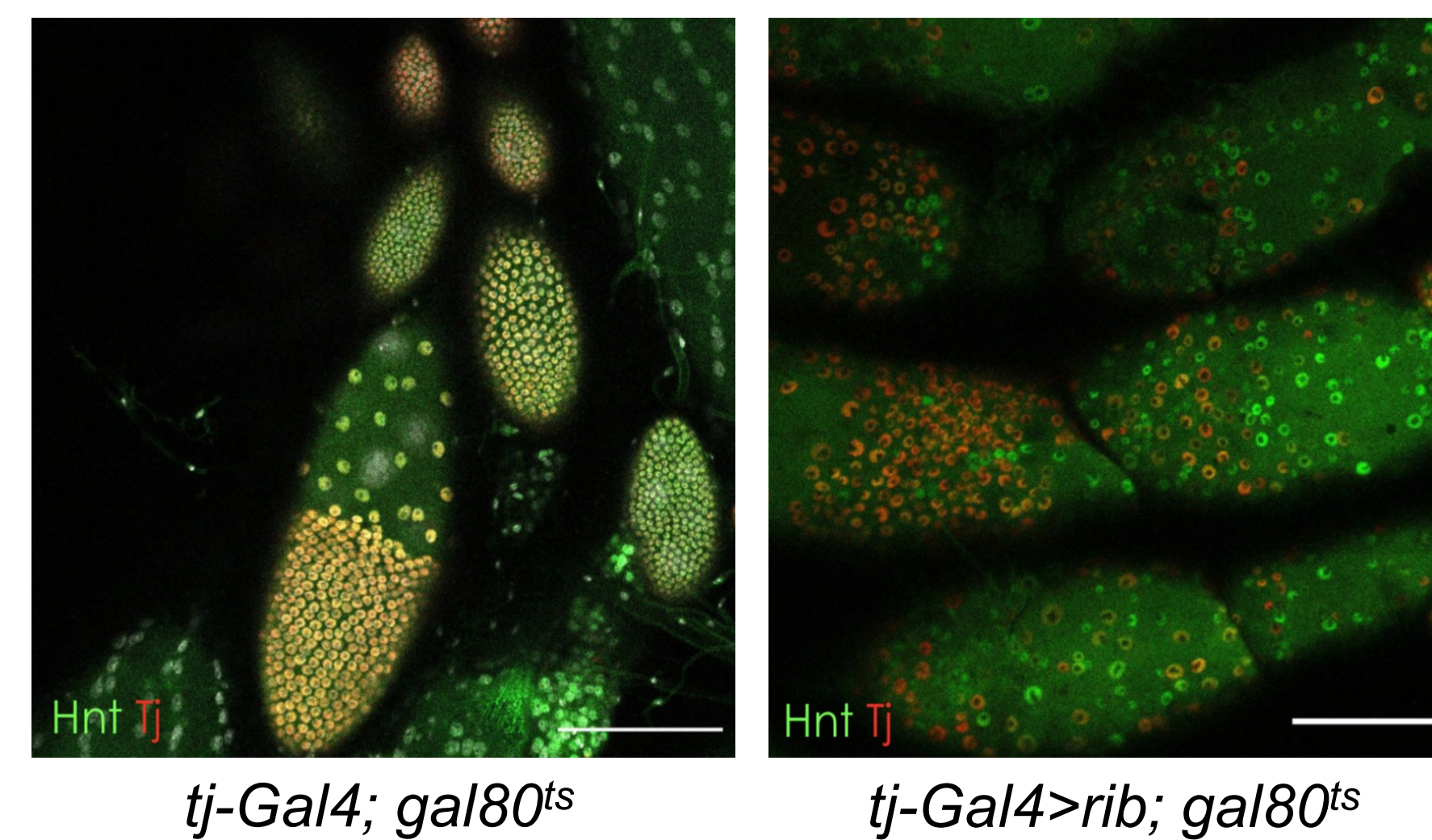


Figure 5. Overexpression of *ribbon* in somatic follicle cells (Ryan 2021). Overexpression of *rib* has demonstrated a decrease in the amount of somatic follicle cells as well as leading to varying sizes of the somatic follicle cells. The average number of follicle cells in normal ovarioles is 656 cells, and the average number of follicle cells in mutant overexpressed ovarioles is 130 cells. *Hnt* labels stage 7-10 follicle cells and *Tj* is a somatic follicle cell marker.

## The Reduction of Somatic Follicle Cells Is Not Caused By Cell Death

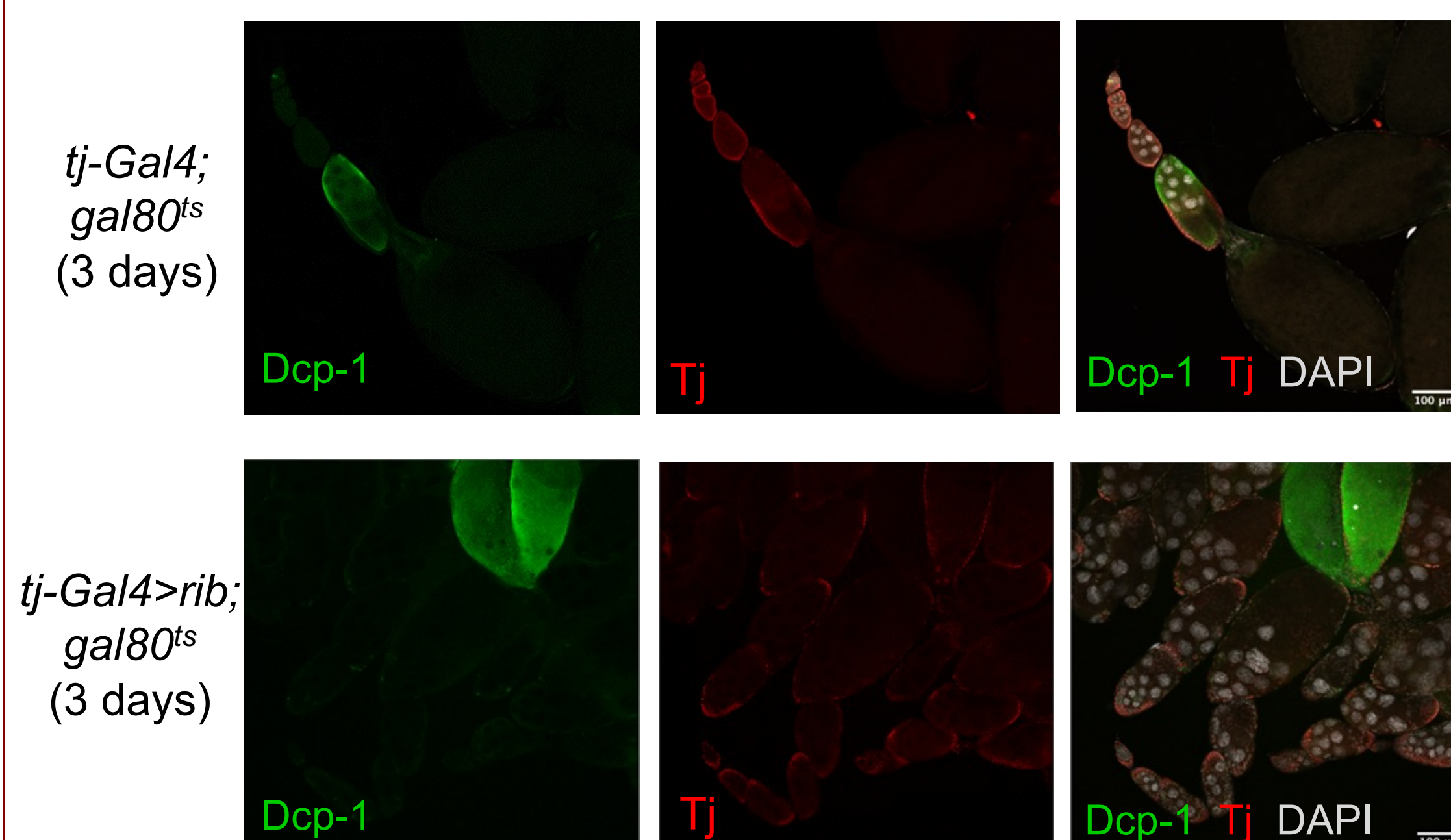


Figure 6. Overexpression of *rib* in *Drosophila* somatic cells (Ryan 2021). Ovarioles of *rib* overexpression show growth reaching to late-stage egg chambers. *Dcp-1* is cell death marker, *Tj* is a somatic follicle cell marker, and DAPI is a nuclei marker.

## Somatic Follicle Cell Proliferation in Ovarioles

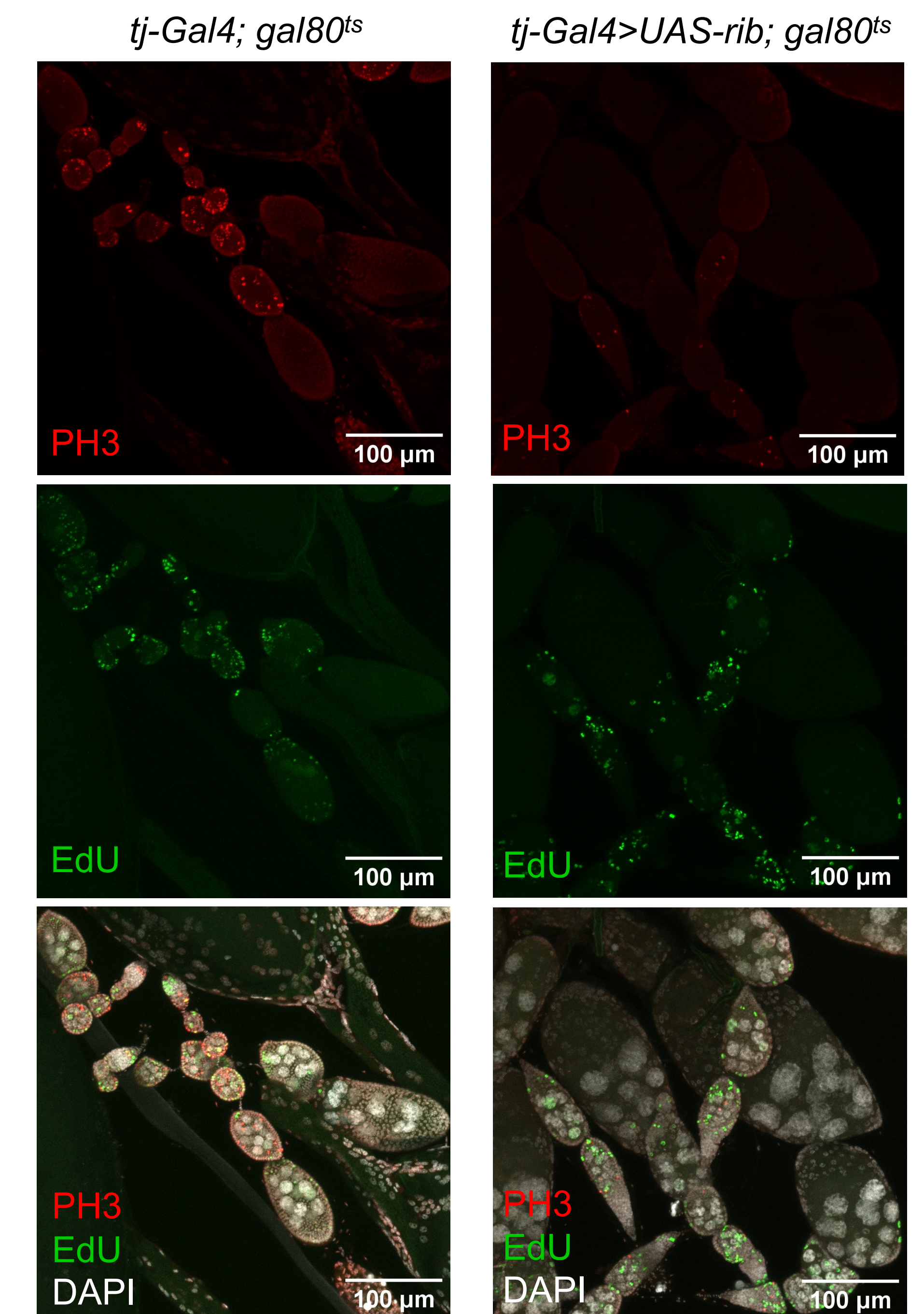


Figure 7. Overexpression of *rib* in somatic follicle cells of the ovaries. PH3 (phosphohistone H3) is a mitotic marker, EdU is a marker for DNA synthesis (S phase), and DAPI is a nucleic marker.

## Preliminary Results of Somatic Cell Proliferation:

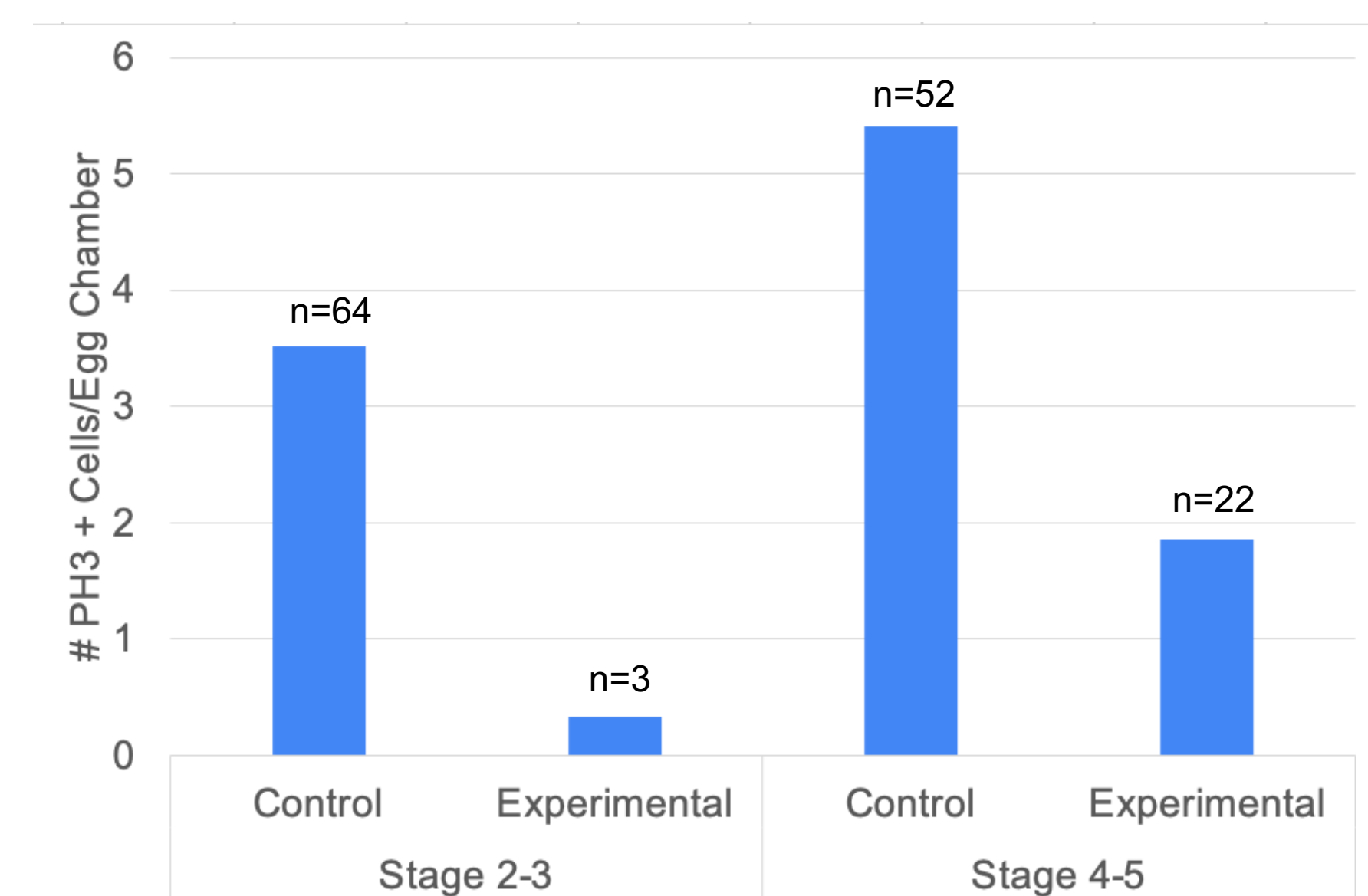


Figure 8. PH3 positive cells (PH3+) in control and *rib* overexpression (experimental) ovarioles in stages 2-3 and stages 4-5. Preliminary results suggest mutant ovarioles in stages 2-3 of ovarioles, the average amount of PH3+ cells in an ovariole is 3.52 cells in the control and 0.33 cells in the experimental. In stages 4-5 of ovarioles, the average amount of PH3+ cells in an ovariole is 5.40 cells in the control and 1.86 cells in the experimental. Grace Flemming and Ashwini Modi assisted with scoring.

## Acknowledgements

Special thanks to Dr. Mierisch, Savannah Ryan, and Manny Alvarez. This work was funded by research funds from Loyola University Chicago through the Provost Summer Fellowship and the Mulcahy Fellowship to A.S. and additional funding to S. R., S. M., U. K., and J. J. M. from Loyola University Chicago.