



Regulation of Gonad Morphogenesis and Gametogenesis by the BTB Protein Ribbon

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Abstract:

Cell adhesion and cell-cell signaling are critical for the establishment and maintenance of organ structure and function. In the case of the gonad, defects in organ formation or gametogenesis can result in sterility. The *Drosophila* gonad has proven an excellent model for identifying genetic mechanisms underlying organogenesis. The gonad is formed when the somatic gonadal cells and germ cells migrate and coalesce during embryogenesis. Subsequent development results in the establishment of the germline stem cell niche and stem cell populations that will be maintained throughout the lifetime of the organism. In previous studies, the BTB transcription factor Ribbon (Rib) was identified as a gene required for embryonic gonad formation. Further study has revealed that Rib continues to be expressed during larval gonad development and in the adult ovaries and testes. These results suggest that Rib may regulate significant morphological changes that occur in the larval gonad, as well as gametogenesis in the adult. We have found that overexpression of *rib* in somatic cells throughout development causes significant defects in ovary and testis development. In females overexpressing *rib*, niche structures fail to form, resulting in ovaries with a blob-like appearance and a failure to produce eggs. In males overexpressing *rib*, niche structures form, but testes appear truncated and sperm do not progress through meiosis. The effect of clonal loss of *rib* are being examined in males and females. In order to specifically examine the role of Rib in gametogenesis, *rib* overexpression was limited to adult tissues. When *rib* is overexpressed in somatic cells of the adult ovary, defects in oogenesis occur and a reduction in the number of follicle cells surrounding the egg chamber is observed. *rib* overexpression in somatic cells in the adult testis results in a failure of cells to transition to meiosis and abnormalities in somatic cell gene expression. Given that oogenesis in *rib* overexpression ovaries arrests at a key transition regulated by the Notch signaling pathway, and Notch and Rib have been found to regulate a common target gene, we are currently examining the relationship of Rib to the Notch signaling pathway in the ovary and testis.

Ribbon and Embryonic gonad development

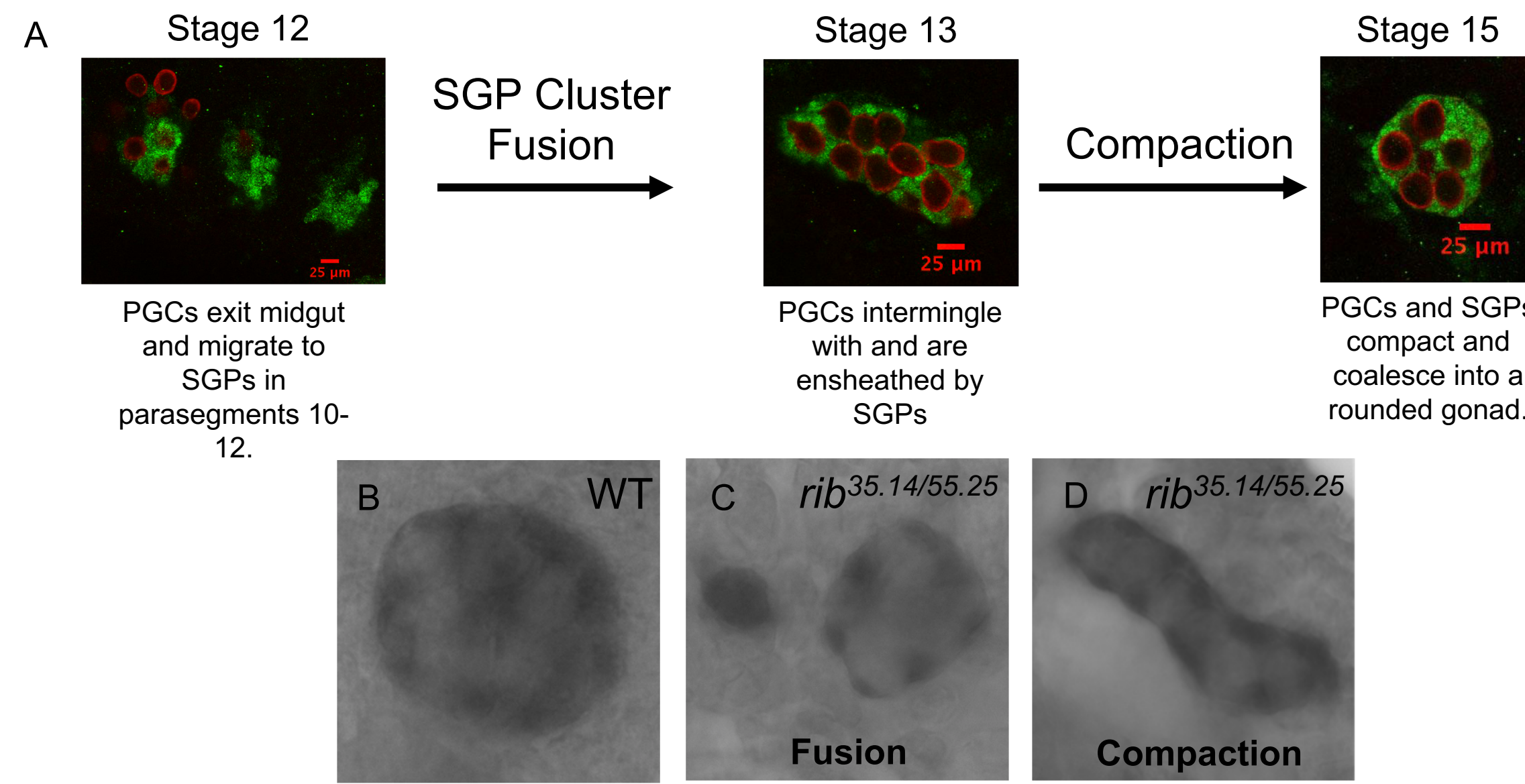


Figure 1. Embryonic Gonad Development. Embryonic gonads at stage 12, 13, and 15 of embryonic development. PGCs visualized with anti-Vasa (red), and SGPs with anti-Traffic jam (green). (B) In control gonads, SGP clusters fuse and compact with PGCs to form a spherical gonad. (C, D) *rib* mutants exhibit fusion and compaction defects. SGPs are visualized using enhancer-trap line (68-77). Published in Silva et al., 2016.

Rib may regulate *bowl* expression in the embryo

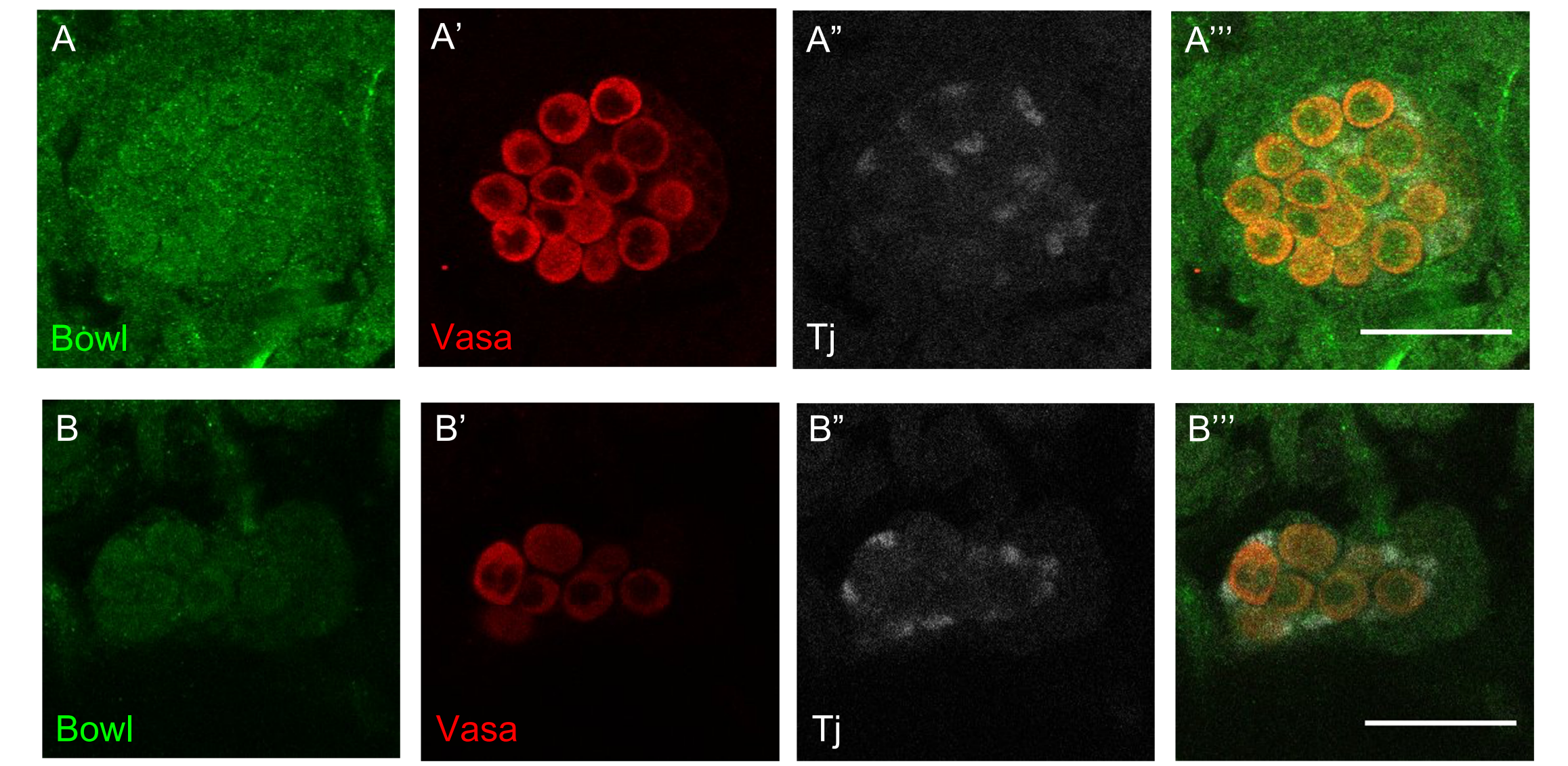


Figure 2. Bowl protein levels are reduced upon *rib* mutation. Protein is present in stage 15 embryonic gonads. Previous studies have demonstrated Bowl is required for hub formation, suggesting Rib may also be required. (A) Control. (B) *rib* mutant. (A', B') Bowl alone. (A'', B'') Vasa alone. (A''', B''') Composite. Scale bar is 25 μm.

Ribbon expression throughout gonad development

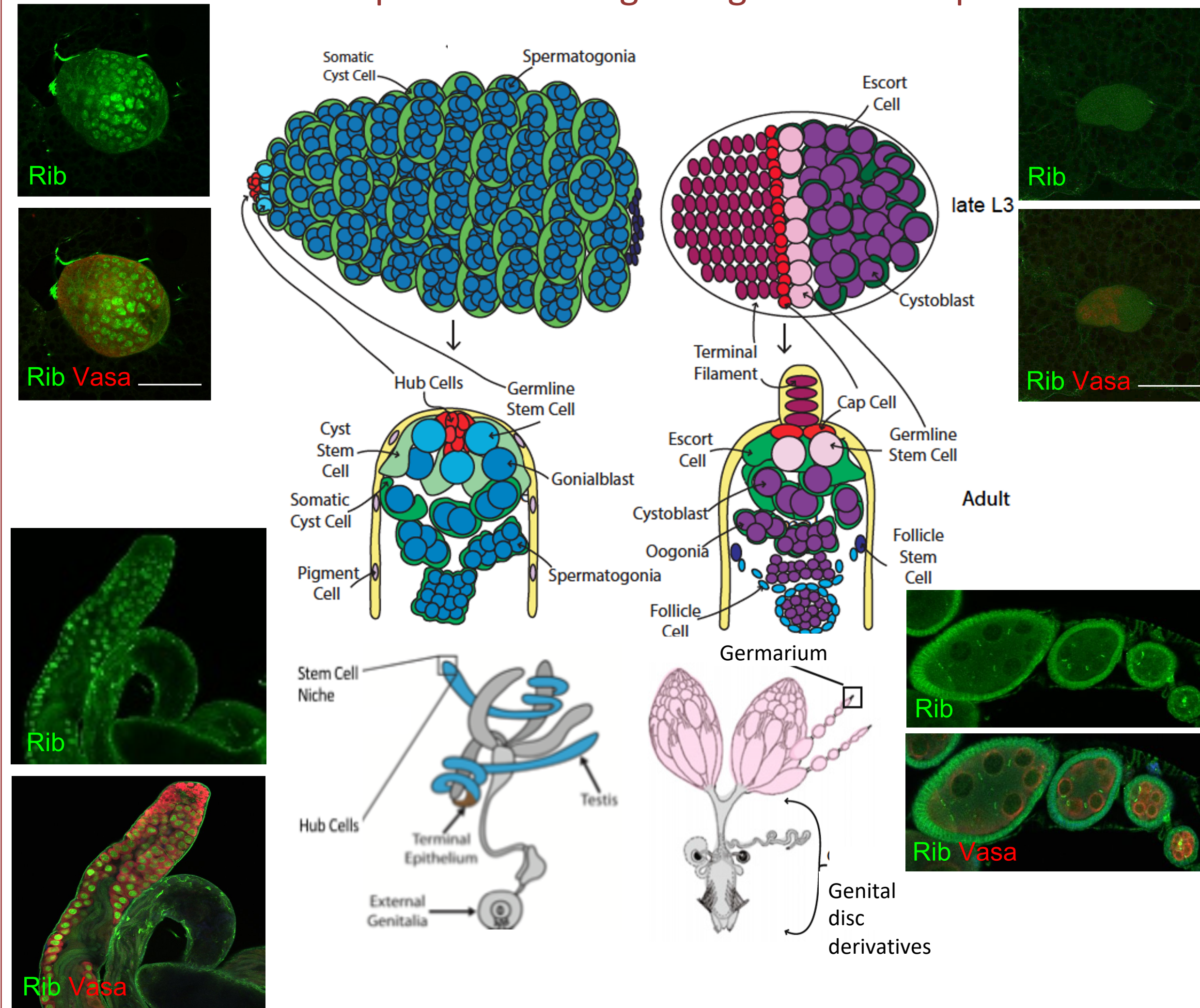


Figure 3. Expression of Ribbon in the larval and adult gonads. Scale bar is 100 μm. Adult schematics have been adapted from Jemc, 2011 and Camara et al., 2008.

Effect of *ribbon* overexpression on the ovary

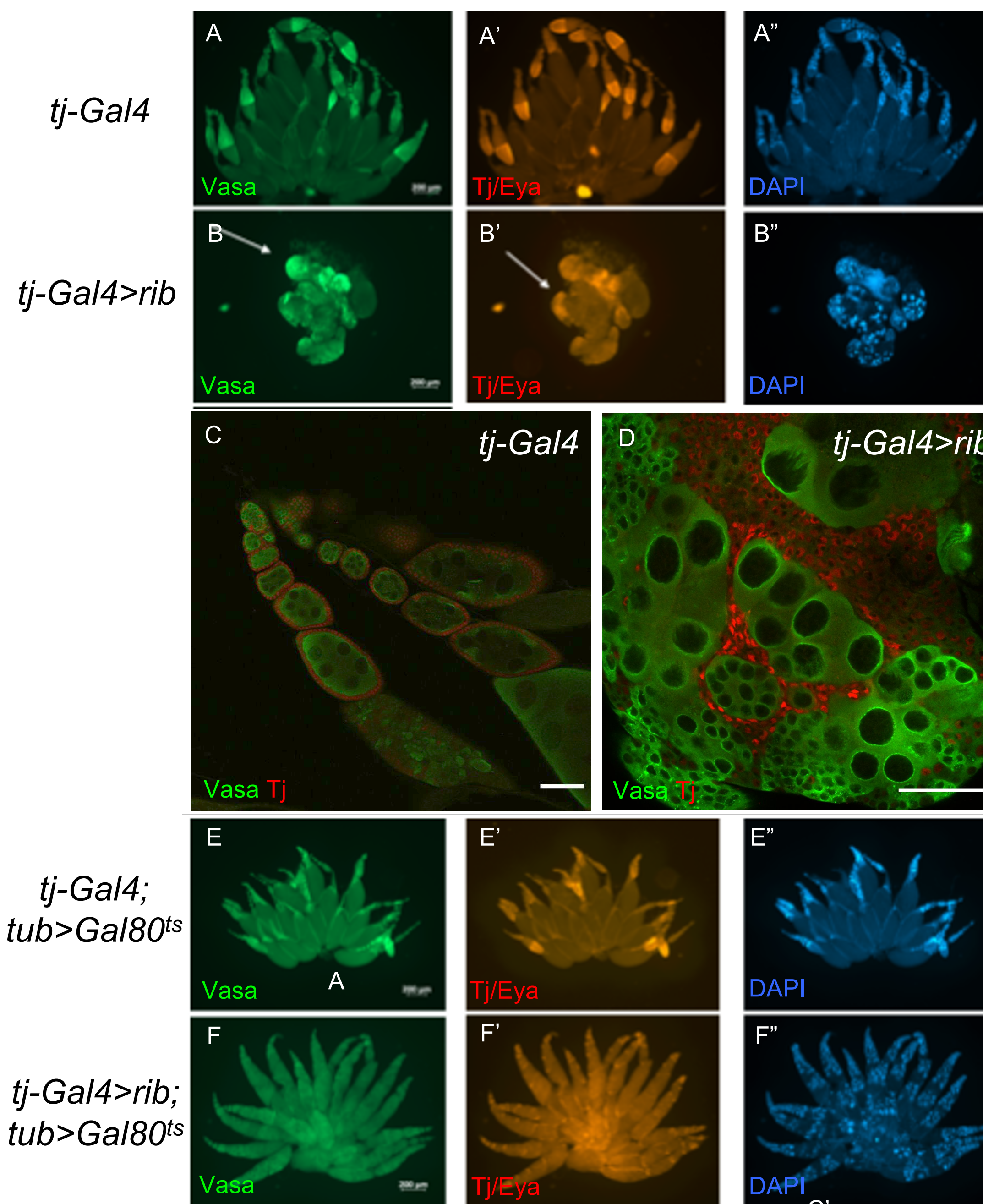


Figure 4. Overexpression of *rib* in somatic cells of the ovaries. (A-D) Expression in somatic cells using *traffic jam* (*tj*)-Gal4 throughout development. (A, C) Gal4 controls, n=29. (C) Scale bar is 100 μm. (B, D) Overexpression of *rib*, n=20. (D) Scale bar is 50 μm. (E-F) Overexpression of *rib* in somatic cells of adult ovaries. (E) Gal4 controls, n=20. (F) Overexpression of *rib* for 1-3 days, n=26. Germ cells (anti-Vasa, green), somatic cells (anti-Tj/Eya, red), and nuclei (DAPI, blue).

Overexpression of *ribbon* may affects follicle cell proliferation

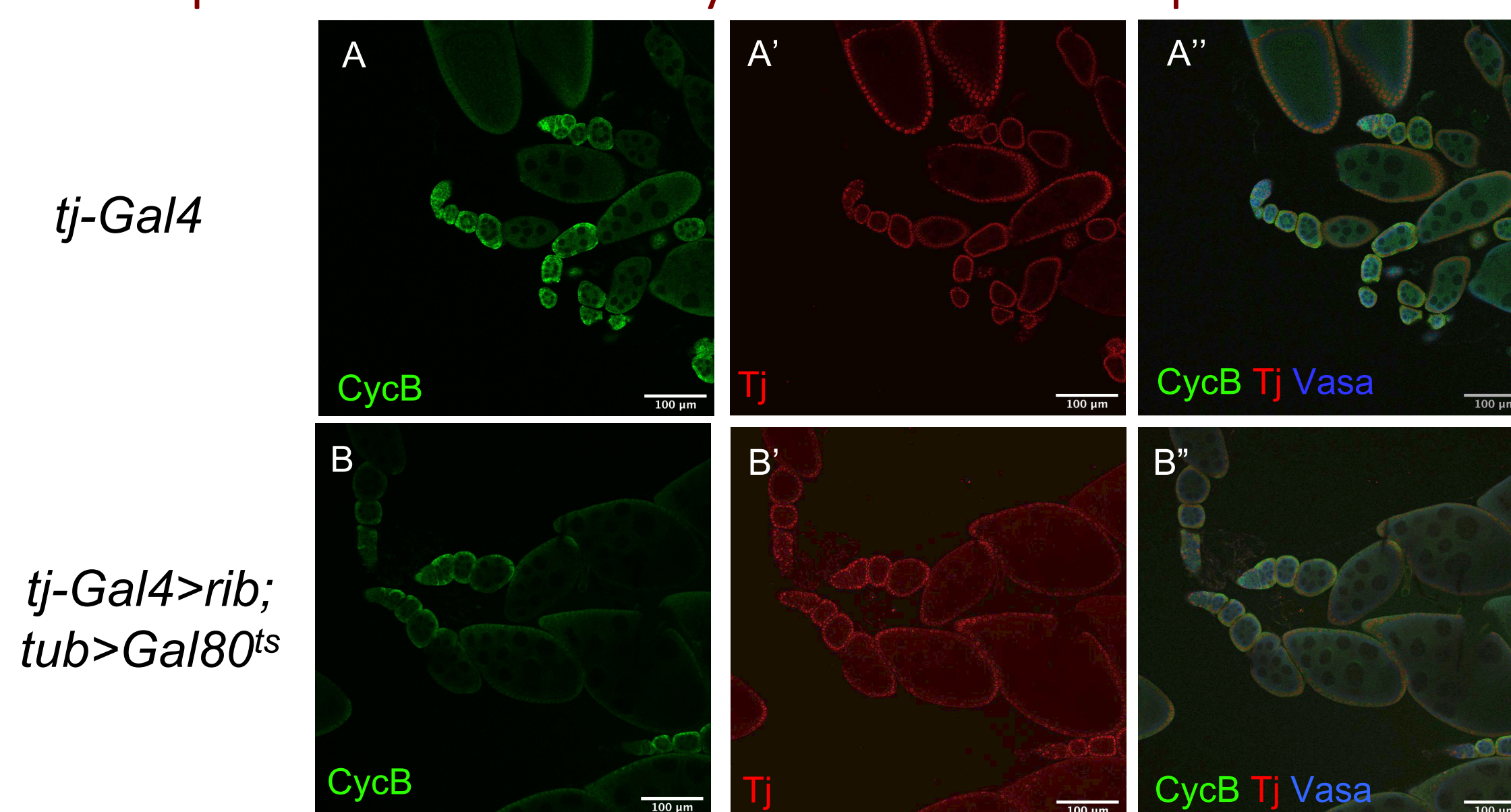


Figure 7. Overexpression of *rib* in somatic cells of adult testes. (A) Gal4 controls. (B) Overexpression of *rib*. Germ cells (anti-Vasa, blue), somatic cells (anti-Tj, red), and anti-Cyclin B (green). Scale bar is 100 μm.

Effect of *ribbon* overexpression on the testis

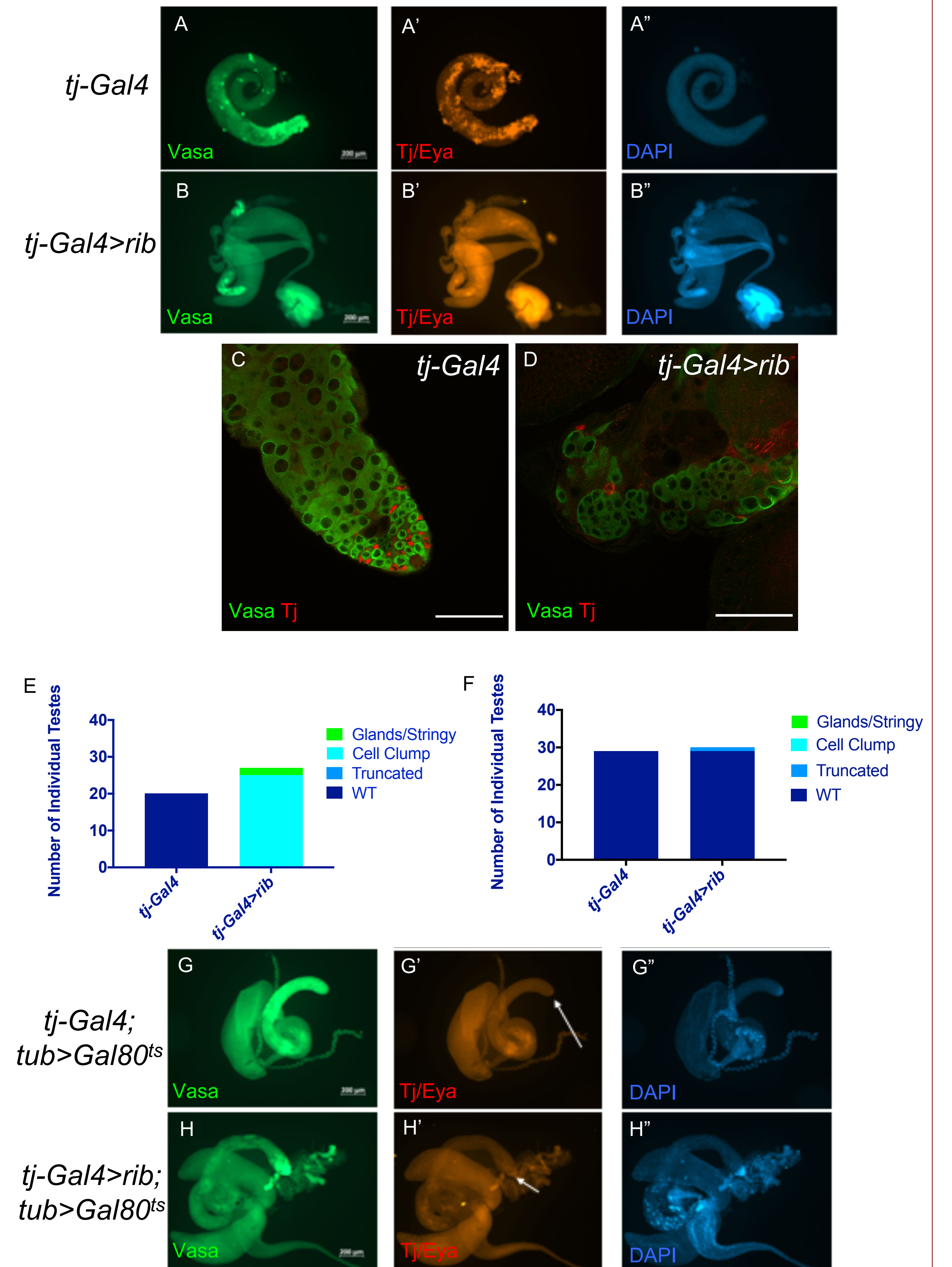


Figure 5. Overexpression of *rib* in somatic cells of the testes. (A-D) Expression in somatic cells using *traffic jam* (*tj*)-Gal4 throughout development. (A, C) Gal4 controls. (C) Scale bar is 50 μm. (B, D) Overexpression of *rib*. (D) Scale bar is 50 μm. (E) Quantitation of testis defects from A-B. (F) Quantitation of testis defects from G-H. (G-H) Overexpression of *rib* in somatic cells of adult testes for 1-3 days. (G) Gal4 controls. (H) Overexpression of *rib*. Germ cells (Vasa, green), somatic cells (Tj/Eya, red) and DAPI (blue).

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Overexpression of *ribbon* affects follicle cell gene expression

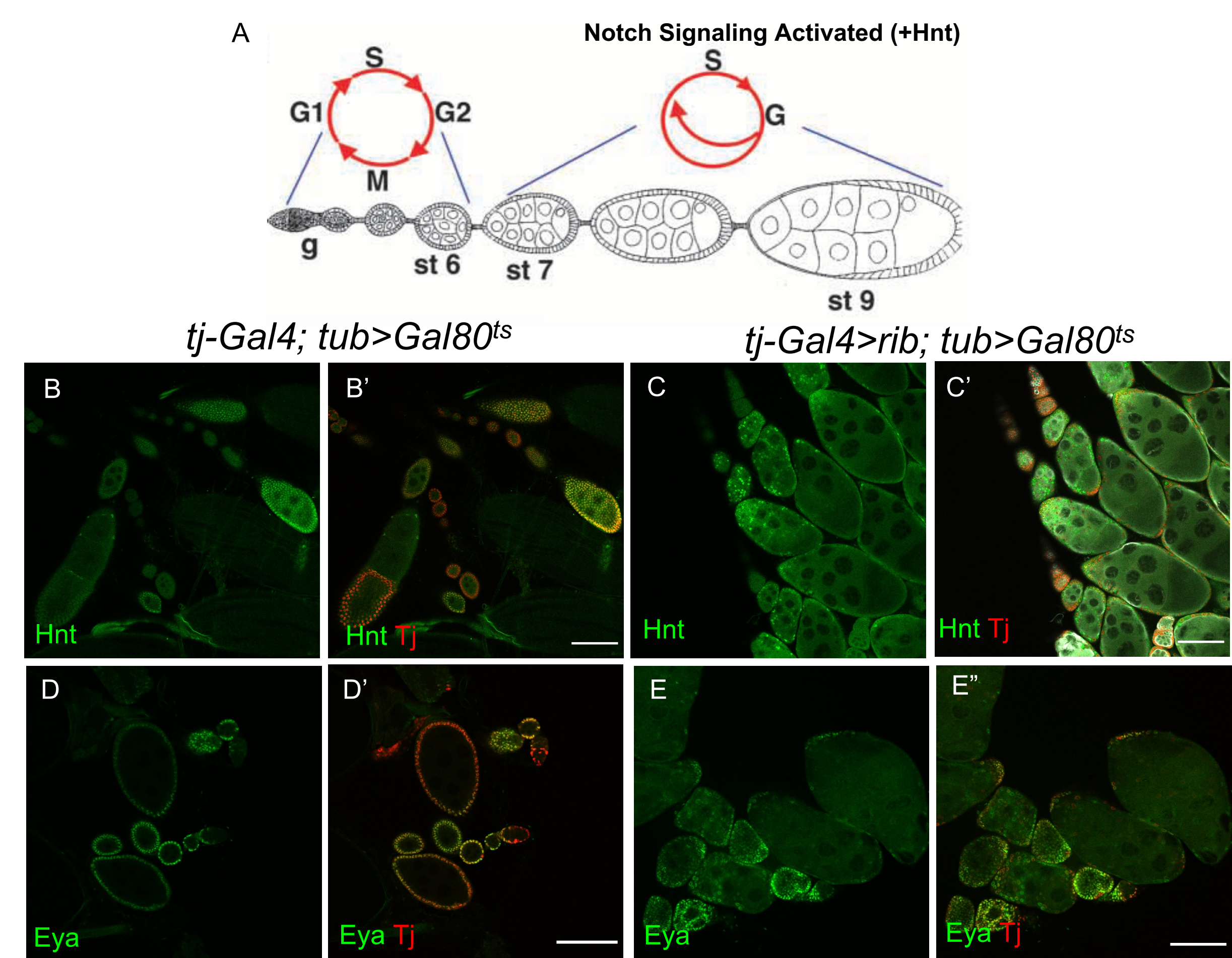


Figure 6. Overexpression of *rib* in somatic cells of adult ovaries. (A) Schematic from Deng et al., 2001. (B, D) Gal4 controls. (C, E) Overexpression of *rib* for 1-3 days. Scale bar is 100 μm.