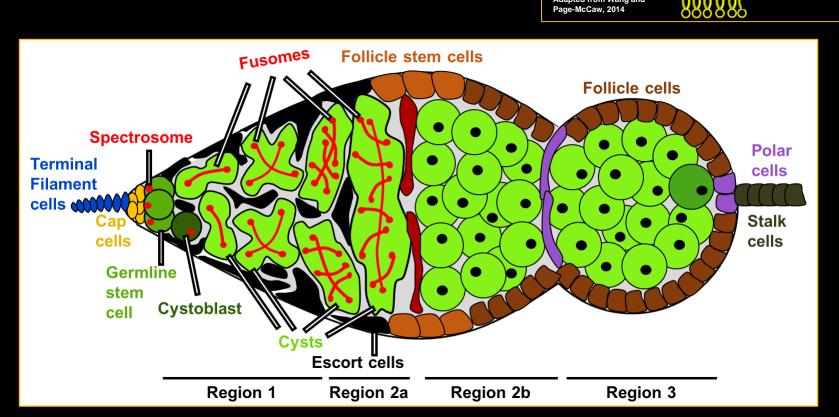


Dally-like protein sequesters and functionally inhibits multiple Wnt ligands in the *Drosophila* germarium

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1) Introduction

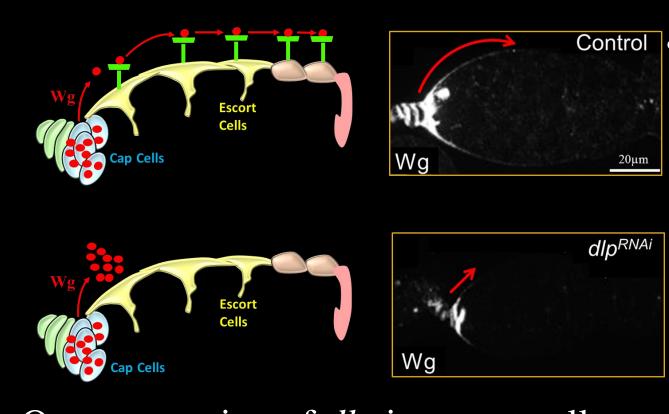
- Dally-like protein (Dlp) is a cell surface heparan sulfate proteoglycan that binds to multiple secreted ligands and can play an important role in regulating signaling specificity in target cells.
- Several aspects of oogenesis are regulated by Wnt ligands.
- Our aim is to understand the role of Dlp in spreading of secreted Wnt ligands in the germarium.



Oogenesis in *Drosophila* occurs in germarium (above)

- Four Wnt ligands are expressed in the germarium. wg and Wnt6 are expressed in cap cells. Wnt2 and Wnt4 are expressed in escort cells. They all activate Wnt signaling in escort cells.
- Loss of *Wnt2/Wnt4* in escort cells results in loss of germline differentiation and escort cells.
- Loss *Wnt6* results in cap cells results in loss of germline stem cells.

2) Dlp (Dally-like) promotes Wg spreading to follicle stem cells in the germarium

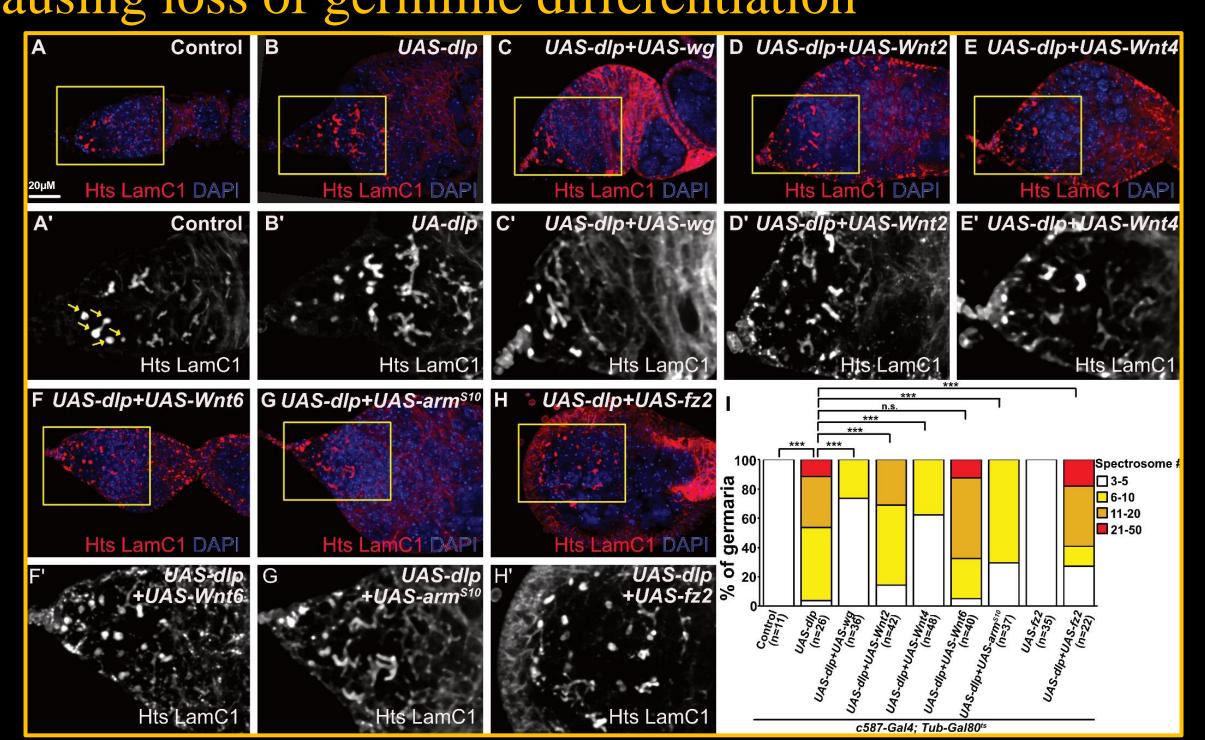


• Downregulation of *dlp* in escort cells results in attenuation of Wg spreading from cap cells to follicle stem cells as seen by extracellular anti-Wg staining (left). This results in decreased proliferation of follicle stem cells

(Wang and Page –McCaw, 2014)

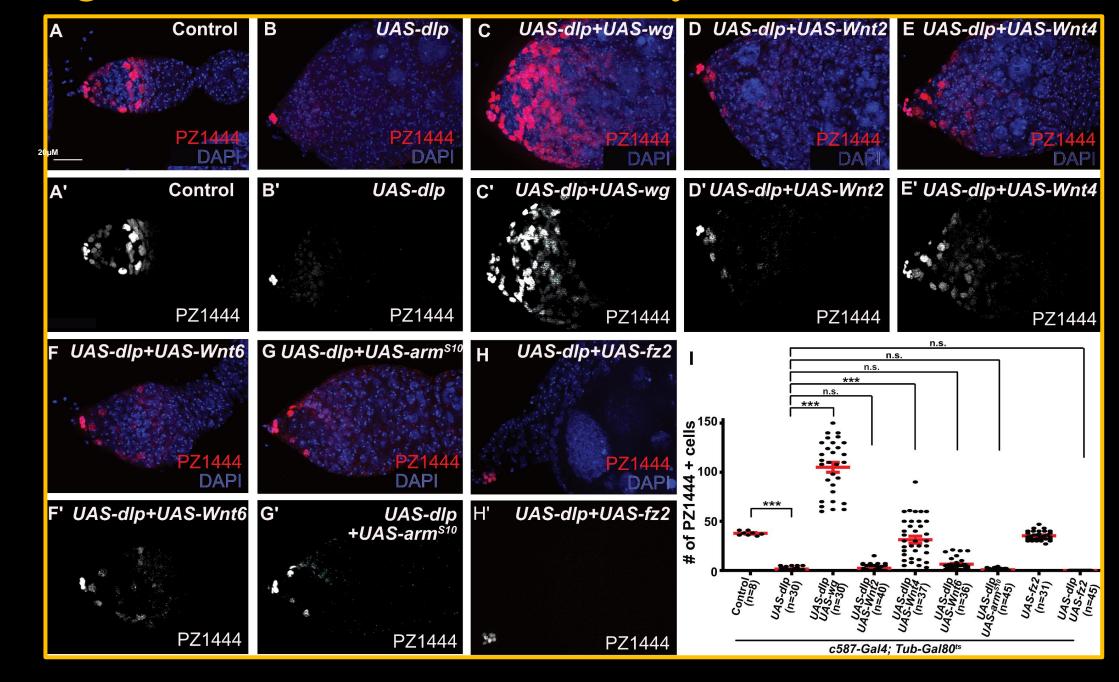
- Overexpression of *dlp* in escort cells results in overgrowth and dramatic disruption of germarium structure—phenotypes that have not been previously attributed to the disruption of Wg spreading and function but associated with disruption of functions of other Wnt ligands in the germarium.
- *dlp* overexpression phenotypes include:
 - . Loss of germline differentiation
 - 2. Loss of escort-cell identity
- 3. Loss of germline stem cells
- 4. Accumulation of germline cysts
- 5. Increase in germline nuclear size

3) Dlp inhibits Wg/Wnt4 signaling in escort cells, causing loss of germline differentiation



- Overexpression of dlp results in loss of germline differentiation.
- Overexpression of wg or Wnt4 suppresses the loss of germline differentiation caused by dlp overexpression.

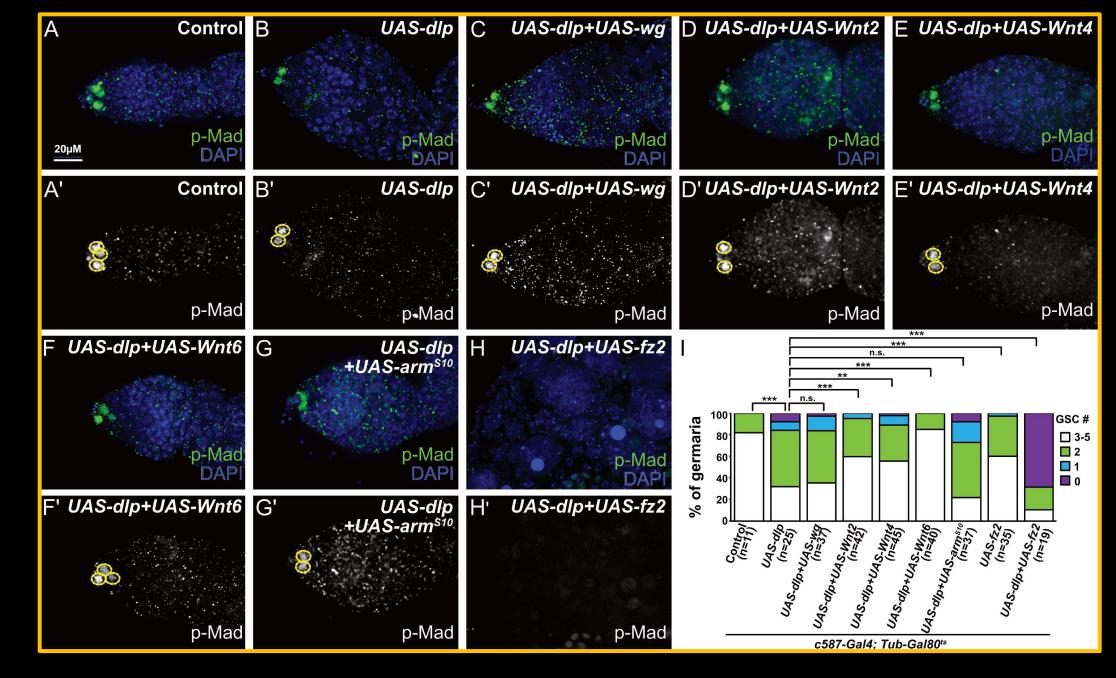
4) Dlp inhibits Wg/Wnt4 signaling in escort cells, causing loss of escort-cell identity



PZ1444: Escort cell marker

- Overexpression of *dlp* results in loss of escort-cell identity.
- Overexpression of wg or Wnt4 strongly suppresses the loss escort-cell identity caused by dlp overexpression.

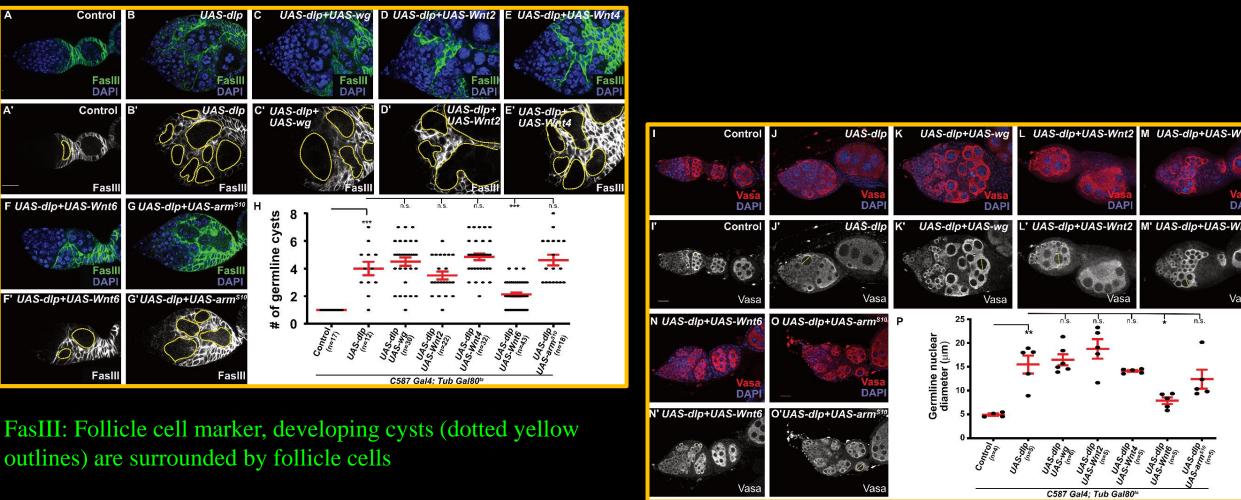
5) Dlp inhibits Wnt6 signaling in escort cells, causing loss of germline stem cells



p-Mad: Germline stem cell (dotted yellow circle) marker

- Overexpression of *dlp* results in loss of germline stem cells.
- Overexpression of *Wnt6* strongly suppresses the loss of germline stem cells caused by *dlp* overexpression.

6) *dlp* overexpression in escort cells causes accumulation of germline cysts

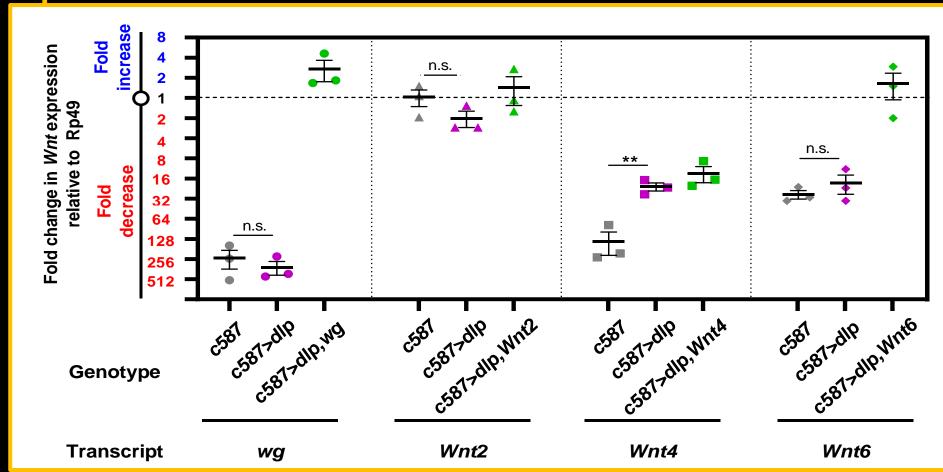


Vasa: germline marker

- Overexpression of *dlp* results in accumulation of germline cysts with increased germline nuclear size.
- Overexpression of *Wnt6* suppresses the accumulation of germline cysts and increased germline nuclear size caused by *dlp* overexpression.

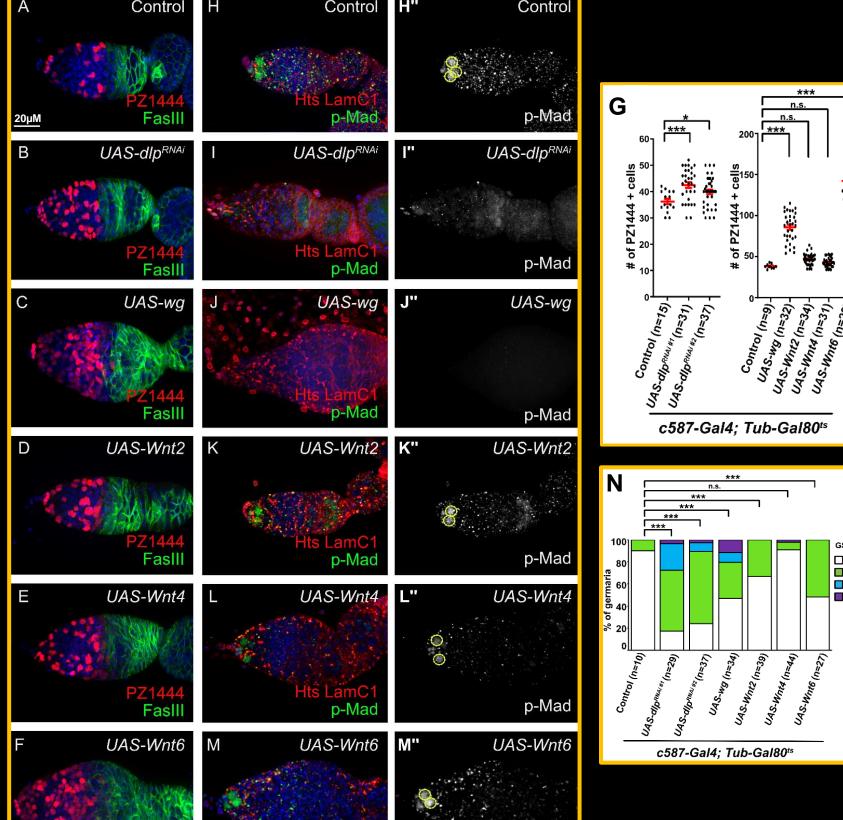
Note: Accumulation of germline cysts and increase in germline nuclear size are not *Wnt6* lof phenotypes, and yet they are suppressed by *Wnt6* coexpressing in *dlp* overexpressing germaria (see section 10).

7) Suppression by different *Wnts* is not related to their expression levels



- Normalized to *rp49*, a housekeeping gene, *wg*, *Wnt2*, and *Wnt6* expression levels are with two-fold range of each other, and *Wnt4* expression level is twenty-fold lower.
- wg, Wnt2, and Wnt6 each suppress different aspects of dlp overexpression phenotype despite being overexpressed at comparable levels.
- Overexpression of *dlp* does not affect transcription of *wg*, *Wnt2*, and *Wnt6*. However, *Wnt4* expression is subject to homeostatic regulation in *dlp* overexpression background.

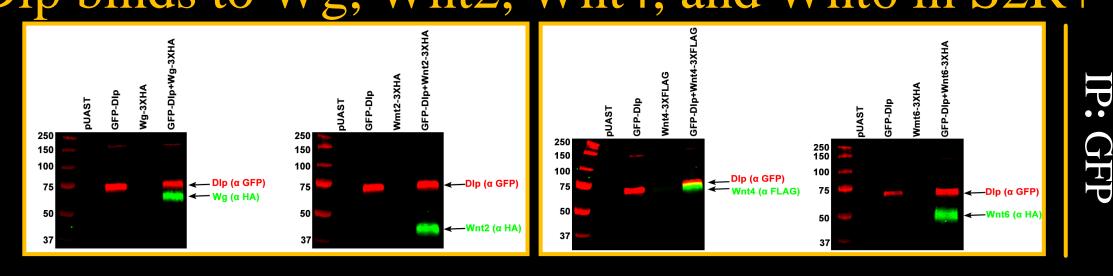
8) *dlp* loss of function (lof) phenocopies Wnt gain of function (gof) in escort cells



•Downregulation of *dlp* or overexpression of *wg/Wnt6* causes increase in escort cell number (A-F, G)

•Downregulation of *dlp* or overexpression of *wg/Wnt2/Wnt6* causes loss of germline stem cells (H-N)

9) Dlp binds to Wg, Wnt2, Wnt4, and Wnt6 in S2R+ cells



• Wg, Wnt2, Wnt4, and Wnt6 can be pulled down with Dlp in S2R+ cells

10) Conclusions

- *dlp* lof disrupts long-range Wg function in follicle stem cells (section 2, top)
- *dlp* lof phenocopies Wnt gof in escort cells (section 8).
- *dlp* gof phenocopies canonical and non-canonical Wnt signaling lof in escort cells (sections 3,4,5).
- *dlp* gof may also inhibit other secreted ligands that function redundantly with Wnt6 (section 6).
- Wnt ligands have functional specificity in the germarium (section 7)
- Dlp binds to Wnt ligands in S2R+ cells (section 9).

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