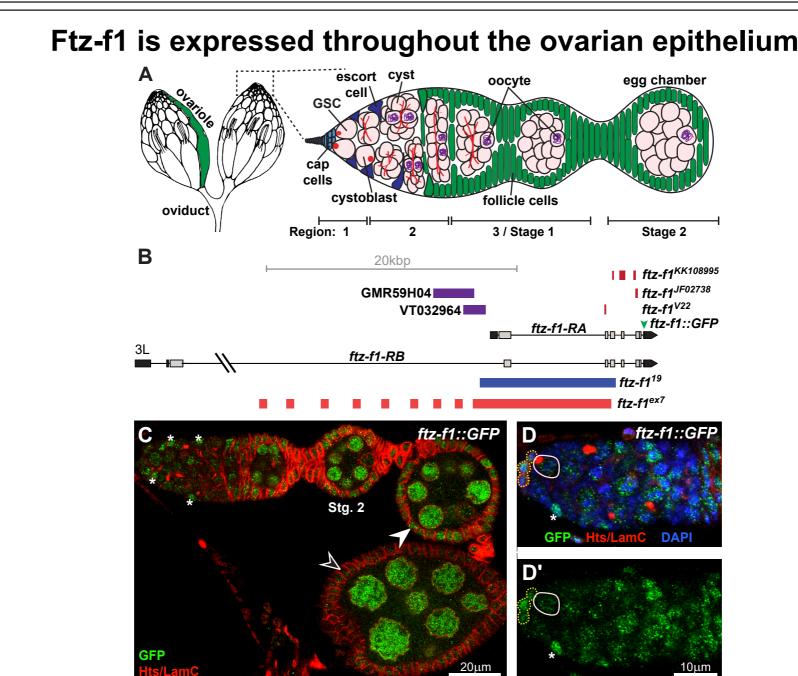
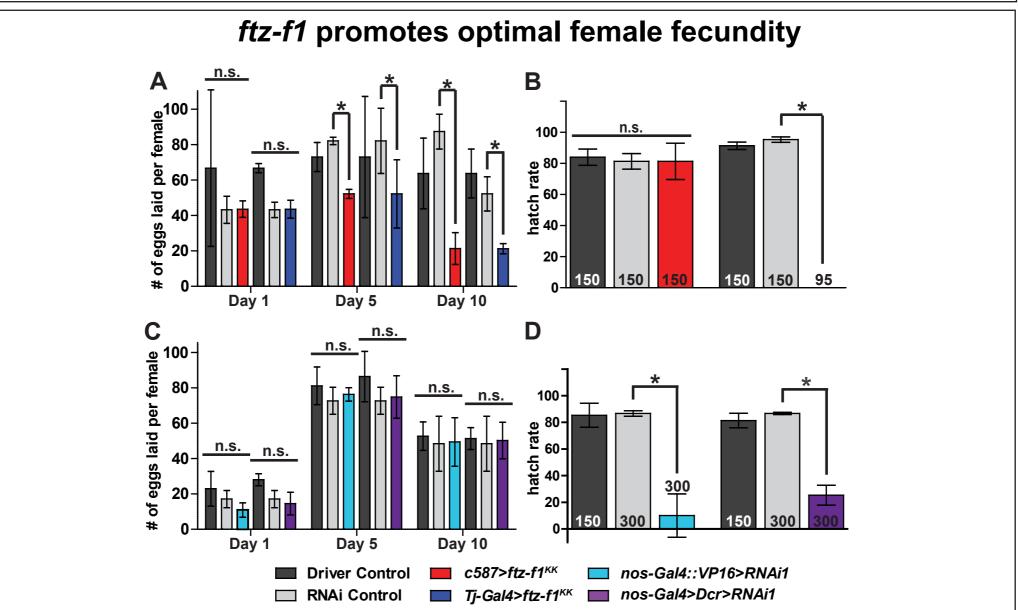


ECU Germ cell division and encapsulatioj by somatic cells during Drosophila oogenesis require the orphan nuclear receptor ftz-f1

Gamete production in mammals and insects is controlled by cell signaling pathways that facilitate communication between germ cells and somatic cells. Nuclear receptor signaling is a key mediator of many aspects of reproduction, including gametogenesis. For example, the NR5A sub-family of nuclear receptors are essential for gonadogenesis and sex steroid production in mammals. Yet despite the original identification of the NR5A sub-family in the model insect Drosophila melanogaster, it has been unclear whether Drosophila NR5A receptors directly control oocyte production. Here, we demonstrate that ftz-f1 (NR5A3) is necessary for multiple aspects of early oocyte development. Ftz-f1 is expressed throughout the ovary, including in germline stem cells (GSCs), germline cysts, and several populations of somatic cells. Ftz-f1 is necessary in GSCs and their dividing daughters for timely mitotic cyst divisions and accumulation of oocyte-specific proteins in the presumptive oocyte which dictate oocyte positioning within the cyst. In parallel, ftz-f1 in somatic escort cells and pre-follicle cells promotes proper cyst division and cyst encapsulation. Interestingly, our data suggest that ftz-f1 promotes escort cell-dependent cyst encapsulation via a complex genetic interaction with the steroid hormone ecdysone. We propose the model that Ftz-f1 and ecdysone signaling via the Ecdysone Receptor (NR1H1) interdependently promote communication between escort cells and germ cells. Taken together, these results demonstrate that the reproductive functions of the NR5A sub-family are largely conserved between insects and mammals. Our data underscore the importance of nuclear receptors in the control of reproduction and highlight the utility of Drosophila oogenesis as a key model for unraveling the complexity of nuclear receptor signaling in gametogenesis.

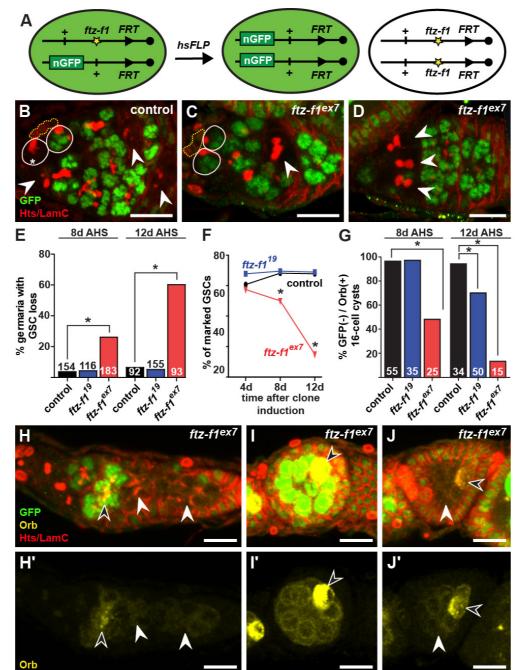


(A) The Drosophila ovary is made of 15-20 ovarioles, each harboring a germarium and progressively older follicles. Germline stem cells (GSCs) are housed in the germarium and divide to form daughter cells. These daughter cells will divide four additional times to form 16-cell germline cysts composed of nurse cells and an oocyte. Escort cells (blue) will navigate the cysts towards the follicle cells (green) that encapsulate each cyst, forming a follicle that pinches off from the germarium and progresses through 14 stages of oogenesis. (B) The locus of the ftz-f1 gene with corresponding mutant alleles and transgenic constructs used in this study. (C–D') Single plane image of ovariole (C) and germarium (D–D') labeled with anti-GFP (green; ftz-f1::GFP), anti-Hts (red; fusomes and follicle cell membranes), anti-LamC (red; nuclear envelopes), and DAPI (blue, nuclei). GSCs are outlined in solid white lines; cap cells are outlined in dotted yellow lines. Asterisks indicate GFP-positive escort cells. D' depicts the green channel only of the image above. Filled arrowhead denotes GFP-positive follicle cells; open arrowhead denote GFP-negative follicle cells. Scale bars, 20 μ m (C) or 10 μ m (D–D').

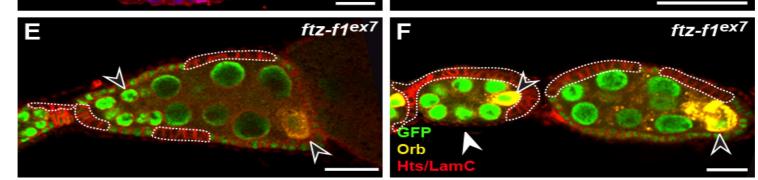


(A, C) Five pairs of control (black and grey bars) and ftz-f1 mutant (A: germline knock-down, teal and purple bars; C: somatic knock-down, red and blue bars) females per bottle (in triplicate) were kept on wet yeast paste beginning one day after eclosion, and the number of eggs laid per female was quantified. (B, D) Fifty eggs laid by control and mutant females were then monitored for hatching. *p < 0.001.

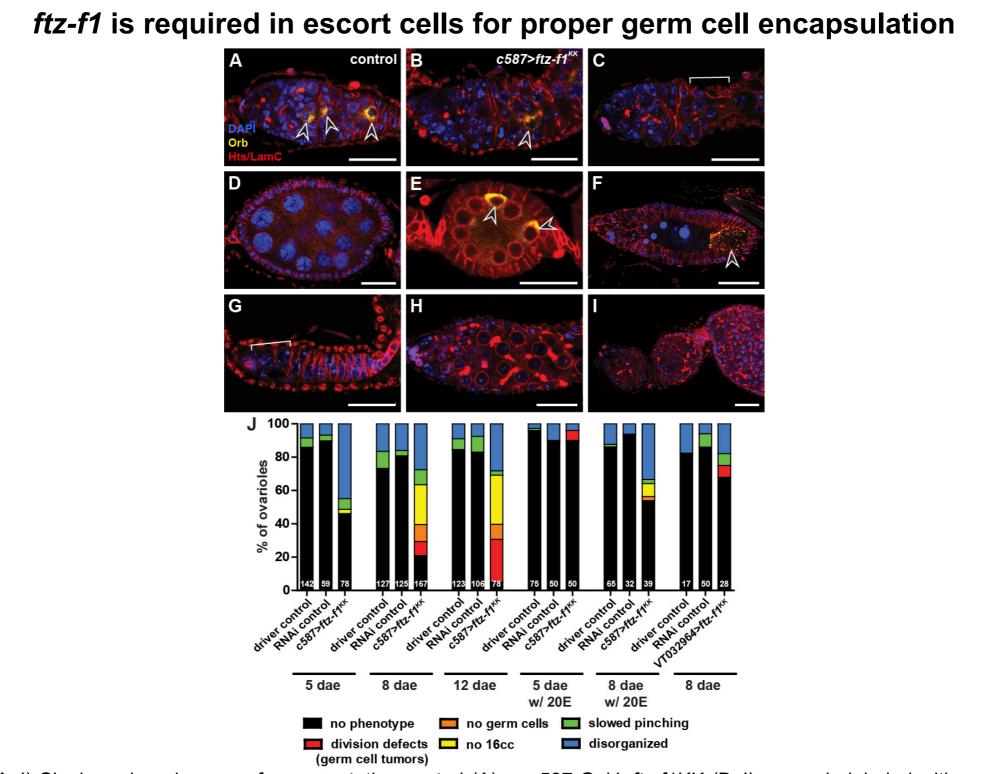
ftz-f1 is necessary for GSC maintenance, timly cyst divisions, and accumulation of oocyte-specific polarity factors



(A) The FLP/FRT technique was used to generate genetic mosaics. Mitotic recombination is mediated by heat-shock-induced expression of flippase (hsFLP). ftz-f1 homozygous mutant cells are identified by the absence of a nuclear GFP marker, which is linked to the wildtype allele. (B–D) Maximum intensity projections (5 µm z-plane) of representative control mosaic (B) or ftz-f1 mutant mosaic (C, D) germaria labeled with anti-GFP (green), anti-Hts (red; fusomes and follicle cell membranes), anti-LamC (red; nuclear envelopes). GSCs are outlined in solid white lines; asterisk indicates a wildtype GFP-negative GSC. Cap cells are outlined in dotted yellow lines. In control mosaic germaria (B), where all cells are genetically wildtype, GFP-negative daughter germ cells (arrows, B) co-exist with GFP-negative GSCs. In ftz-f1 mutant mosaics (C-D), GFP-negative daughter germ cells are frequently observed in the absence of their GFP-negative mother GSC (a GSC loss phenotype). (E–F) Frequencies of control, ftz-f119, and ftz-f1ex7 mosaic germaria with a GSC loss phenotype (E) or with a GFP-negative GSC (F). Number of germline-mosaic germaria analyzed is shown inside or above bars. (G–J) Accumulation of Orb protein is delayed in ftz-f1 mutant 16-cell cysts as compared to adjacent GFP-positive control cysts (G). Maximum intensity projections (10 µm z-plane) of a representative ftz-f1ex7 mutant mosaic germarium (H) or ovariole (I–J) labeled with anti-GFP (green), anti-Hts and anti-LamC (red), and anti-Orb (yellow; presumptive oocytes). Panels I (all cells are GFP-positive) and J (germ cells only are GFP-negative) are adjacent egg chambers from the same ovariole. H'-I' depict the yellow channel only of the images above. Filled arrowheads denote GFP-negative cysts; open arrowheads denote GFP-positive (wildtype) cysts. Scale bars, 10 μ m. *p < 0.01.

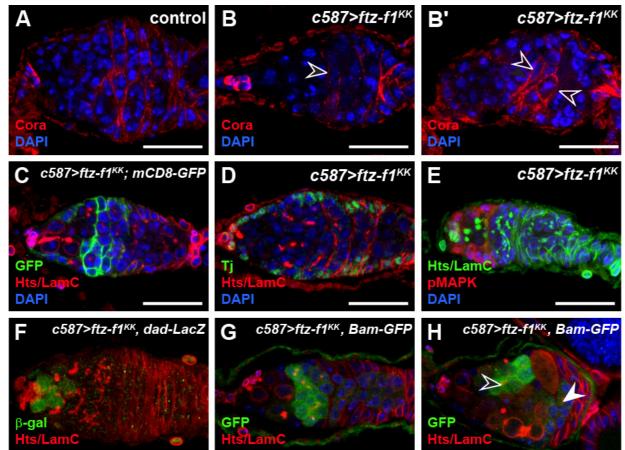


(A–D) Single z-plane images of representative control (A–B) or tj-Gal4>ftz-f1KK (C–D) germaria labeled with anti-Hts (red; fusomes and follicle cell membranes), anti-LamC (red; nuclear envelopes), anti-Orb (yellow; presumptive oocytes), and DAPI (blue; nuclei). Knock-down of ftz-f1 in somatic cells impacts egg chamber formation, ranging in severity from complete block to separation (C) to encapsulation of multiple cysts (and thus, extra oocytes) in a single egg chamber (D). (E–F) Single z-plane images of ftz-f1 mutant mosaic egg chambers with GFP-negative follicle cell clones (outlined in dotted white lines) and wildtype (GFP-positive) germ cells, labeled with anti-GFP (green), anti-Hts and anti-LamC (red), and anti-Orb (yellow). The egg chamber depicted in (F) lacks a complete follicle cell monolayer. Filled arrowheads denote GFP-positive cysts; open arrowheads denote Orb-positive oocytes. Scale bars, 20 μ m (A, C, E–F) or 50 μ m (B, D).



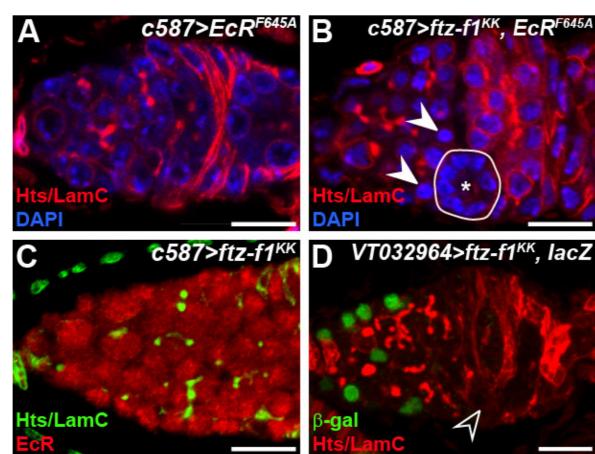
(A–I) Single z-plane images of representative control (A) or c587-Gal4>ftz-f1KK (B–I) germaria labeled with anti-Hts (red; fusomes and follicle cell membranes), anti-LamC (red; nuclear envelopes), anti-Orb (yellow; presumptive oocytes), and DAPI (blue; nuclei). Knock-down of ftz-f1 in escort cells results in a variety of encapsulation defects ranging from disorganized follicle cells (B), slowed pinching of follicle cells around the cyst (C, bracket), cysts with extra germ cells (D), cysts with extra oocytes (E), egg chamber death (F), no germ cells or 16 cell cysts (G), and germ cell division defects (H–I). Penetrance of phenotypes at 5, 8, and 12 days after eclosion (dae) with or without ecdysone (20E) supplemented in the diet is quantified in (J). Scale bars, 20 µm.

Knockdown of *ftz-f1* in escort cells impairs projection stability or pathfinding, but does not appear to disrupt EGF or BMP signaling

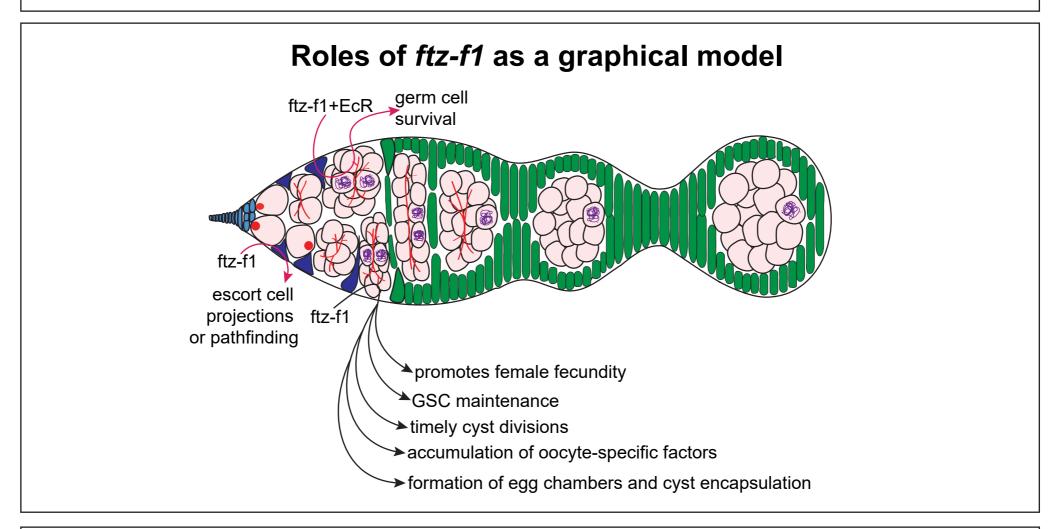


(A–H) Representative control (A) or c587-Gal4>ftz-f1KK mutant (B–H) germaria. In (A–B'), germaria were labeled with anti-Cora (red; escort cell projections) and DAPI (blue; nuclei). In c587-Gal4>ftz-f1KK mutants, escort cells extend projections, but these either fail to connect around germ cells (B) or encapsulate single germ cells (B'). (C) Similar results were obtained by labeling escort cells using a membrane-tethered GFP (green, UAS-mCD8::GFP) and anti-Hts antibodies (red; fusomes and follicle cell membranes). (D–E) Loss of ftz-f1 does not appear to impact the number of escort cells (labeled in D in green with anti-Tj antisera) or EGF signaling (labeled in E with anti-pMAPK antisera). (F–H) Loss of ftz-f1 does not impact BMP signaling in the anterior germarium. c587-Gal4>ftz-f1KK germaria express the BMP transcriptional target dad [F; labeled with anti-Hts (red; fusomes and follicle cell membranes), anti-LamC (red; nuclear envelopes), and anti-β-gal (green; dad-lacZ expression)] and the differentiation factor Bam [G–H; labeled with anti-Hts and anti-LamC (red), GFP (green; Bam-GFP expression) and DAPI (blue; nuclei)]. We occasionally observed (H) 2-cell cysts displaced posteriorly in the germarium, adjacent to 16-cell cysts; however, this did not appear to be the result of altered Bam expression. Images in E and F are maximum intensity projections (10 µm z-plane). Scale bars, 20 µm.

ftz-f1 and EcR synergize in escort cells to support germ cell survival



(A–B) Single z-plane images of representative c587-Gal4>EcRF645A (A) or c587-Gal4>ftz-f1KK, EcRF645A (B) germaria labeled with anti-Hts (red; fusomes and follicle cell membranes), anti-LamC (red; nuclear envelopes) and DAPI (blue; nuclei). A circular cyst with condensed DNA is outlined in white. Solid arrowheads indicate other cells with condensed DNA. (C) Single z-plane image of representative c587-Gal4>ftz-f1KK labeled with anti-Hts and anti-LamC (green) and anti-EcR antisera that recognizes all EcR isoforms (red). Asterisks indicate escort cells. (D) Maximum intensity projection (10 μ m z-plane) of representative VT032964-Gal4>ftz-f1KK germarium labeled with anti-Hts and anti-Hts and anti-Hts and anti-B-gal (green; UAS-nlacZ). Bracket indicates slowed pinching of follicle cells around the cyst. Scale bars, 10 μ m.



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