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Supplemental information

Developmental regulation of oocyte lipid intake through `patent' follicular epithelium in *Drosophila melanogaster*

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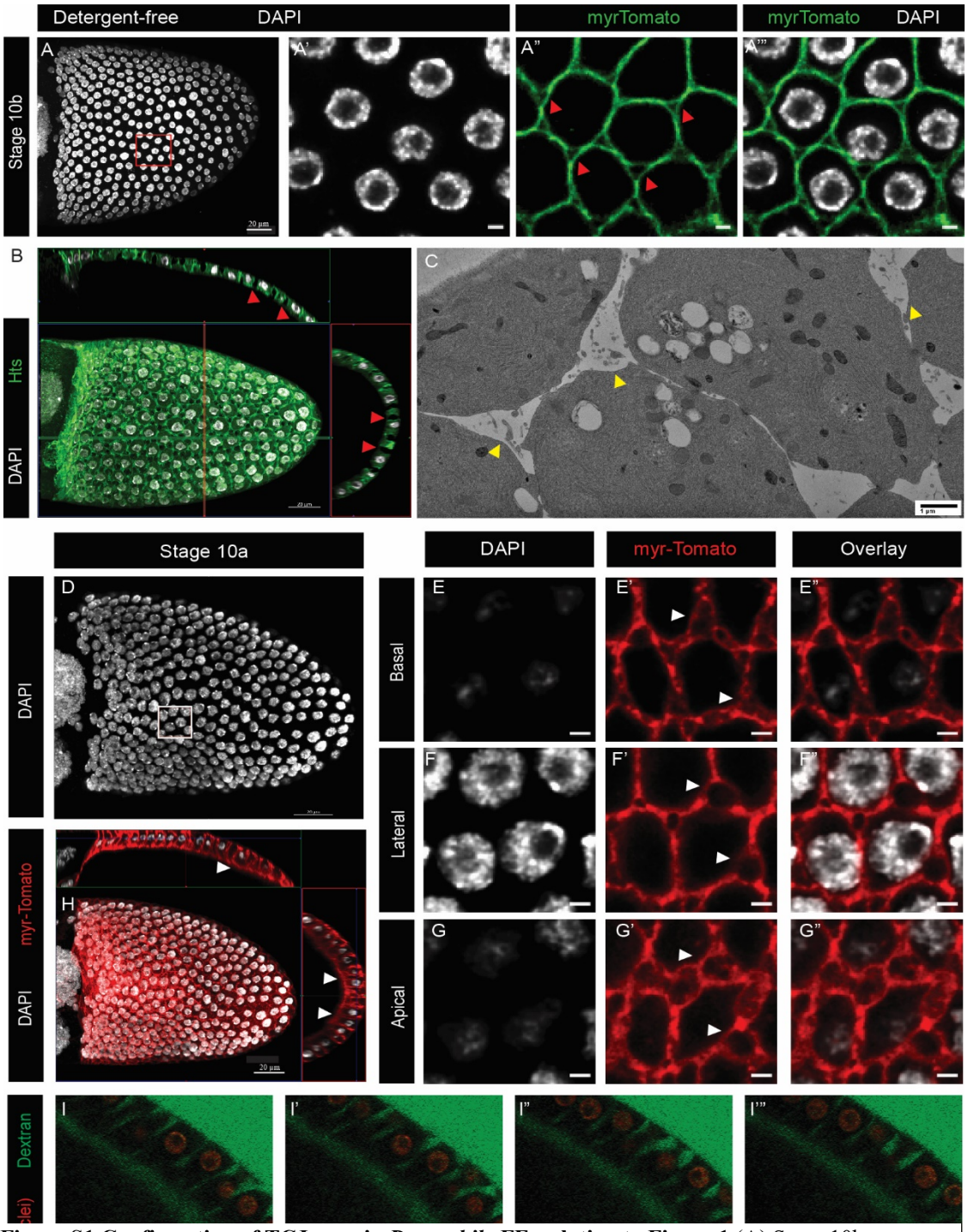

Figure S1 Confirmation of TCJ gaps in *Drosophila* FE, relating to Figure 1 (A) Stage 10b egg chamber expressing *Tj-Gal4, 10XUAS-myr-Td-Tomato*, fixed and mounted using a detergent-free protocol. Red box is enlarged in (A'-A"') showing patent TCs. (B) Ortho view of a projection of a St10a egg chamber with gaps across the FE (red arrowheads) Hts marks the membrane. (C) TEM image of a St10a egg chamber with material in the gaps (yellow arrowheads). (D-G") Stage 10a oocyte-associated FE expressing *10XUAS-myr-Td-Tomato*. White box is expanded in (E-G"). (E-E", F-F", G-G") Basal, lateral, and apical view, respectively. (H) Ortho view of a projection of a St10a *10XUAS-myr-Td-Tomato* expressing egg chamber, with gaps across the FE (white arrowheads). DAPI marks nuclei. (I-I"') Snapshots from live imaging of Stage 10a egg chamber expressing His2Av (marking nuclei), incubated in Dextran (green).

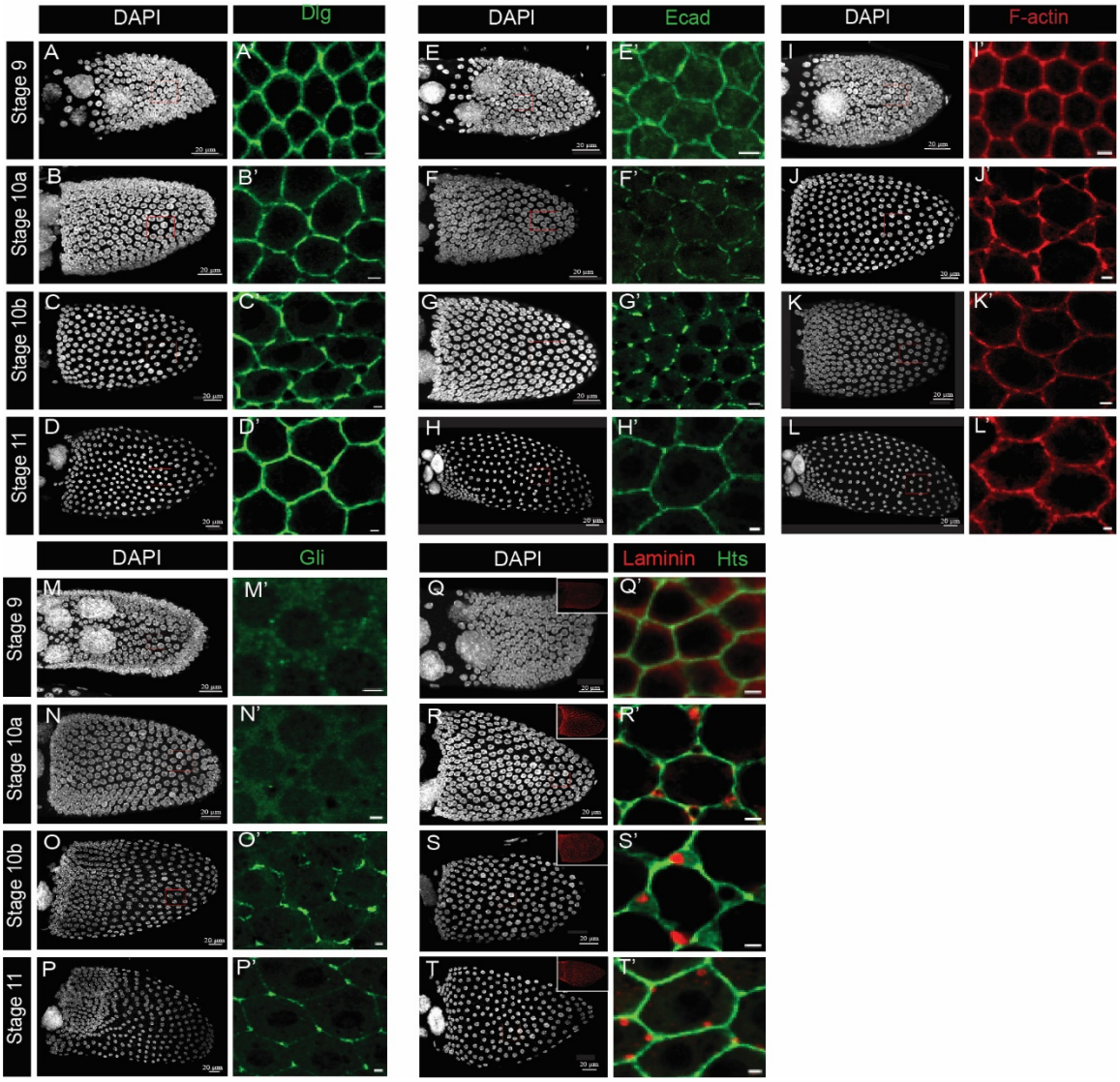

Figure S2 Characterization of adhesion and junction proteins over patency between stage 9 and 11, relating to Figure 2 Distribution of Septate junction protein Dlg (A-D'), adherens junction protein E-cadherin (E-H'), cortical F-actin (Phalloidin) (I-L'), tricellular septate junction protein, Gliotactin (Gli) (M-P'), and basement membrane component Laminin (Q-T'). Insets in (Q-T') Laminin in the full FE.

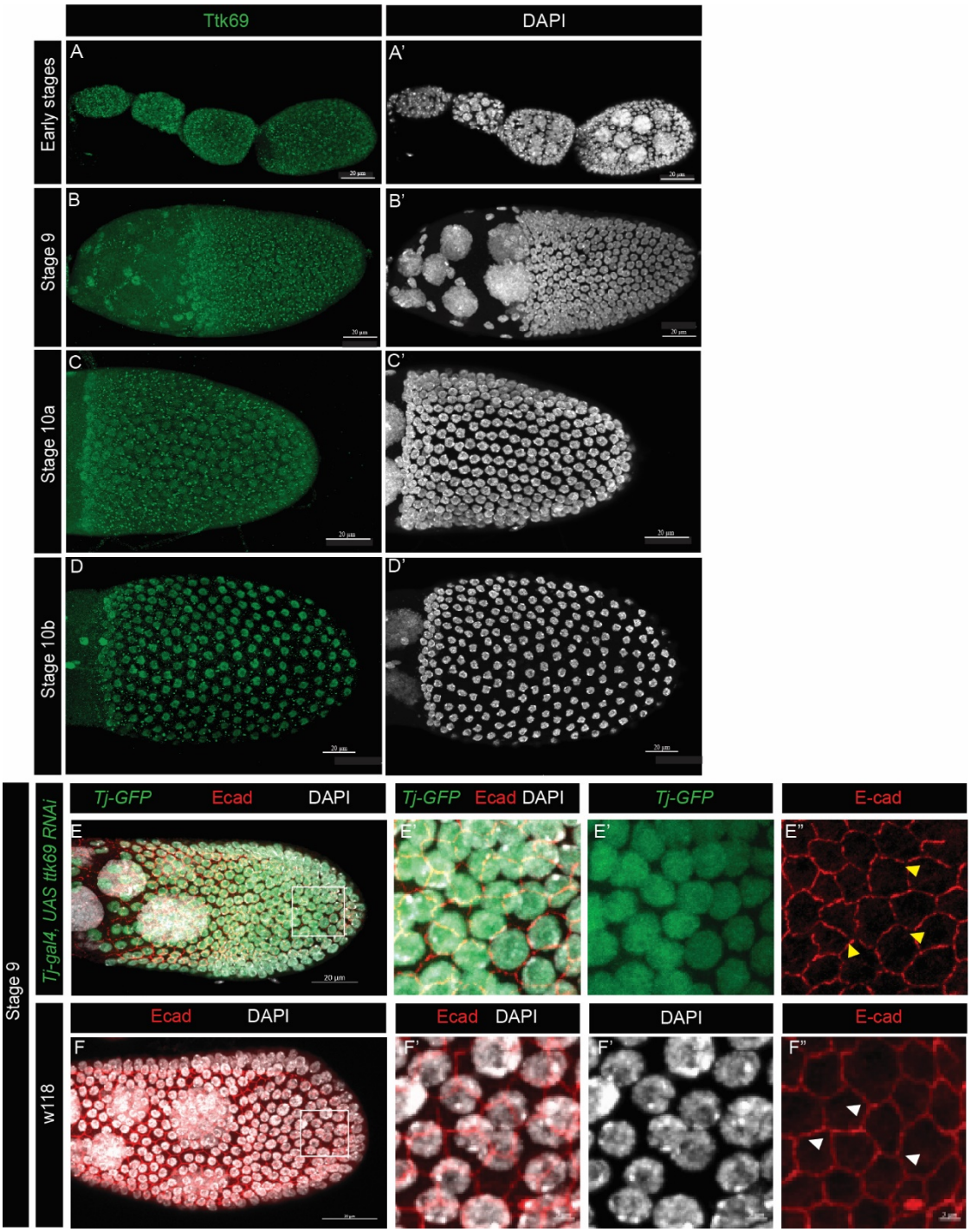

Figure S3 Ttk69 pattern in WT FE and *UAS-ttk-RNAi* expressing FE, related to Figure 3 (A-D') Egg chambers immunostained for Ttk69 protein. Punctate signals is background noise. (E-E") Stage 9 egg chamber expressing *UAS-ttk69-RNAi* under *Tj-Gal4*. White box is expanded in (E'-E"). E-cad is not present at TCs (yellow arrowheads). (F-F") Stage 9 wildtype egg chamber White box is expanded in (F'-F"). E-cad present at TCs (white arrowheads). DAPI marks the nucleus.

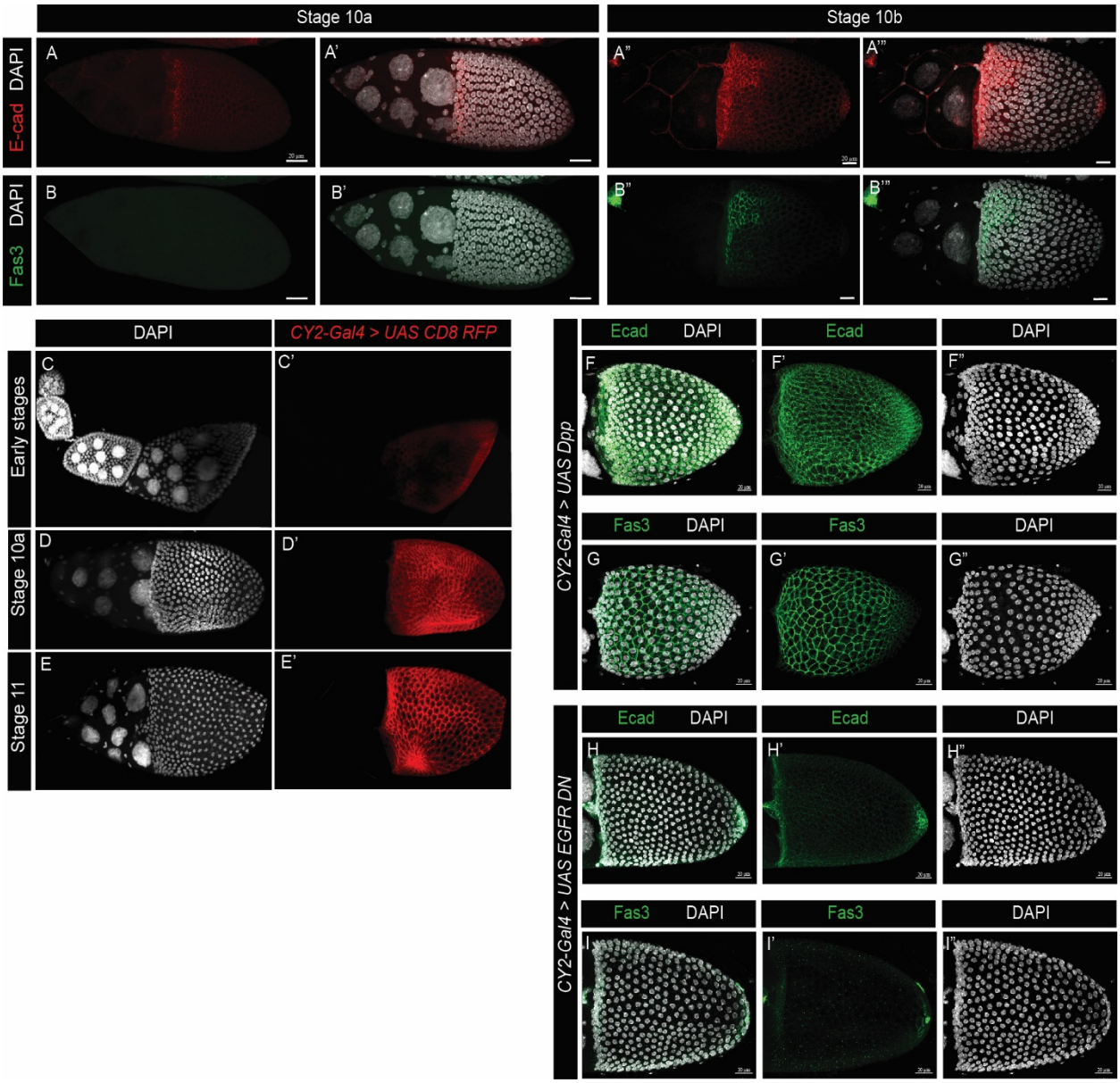

Figure S4 Dorsal pattern of adhesion proteins E-cad and Fas3, related to Figure 4 (A-B'") WT egg chambers with E-cad and Fas3 from St10a (A-A', B-B' respectively) to St10b (A"-A'", B"-B"', respectively). (C-E") *CY2-Gal4* expression pattern in *Drosophila* ovaries marked by *UAS-CD8:RFP* on the membrane. (F-G") Egg chambers expressing *UAS-Dpp* under *CY2-Gal4* driver. (H-I") Egg chambers expressing a dominant negative form of EGFR under UAS control using the *CY2-Gal4* driver.

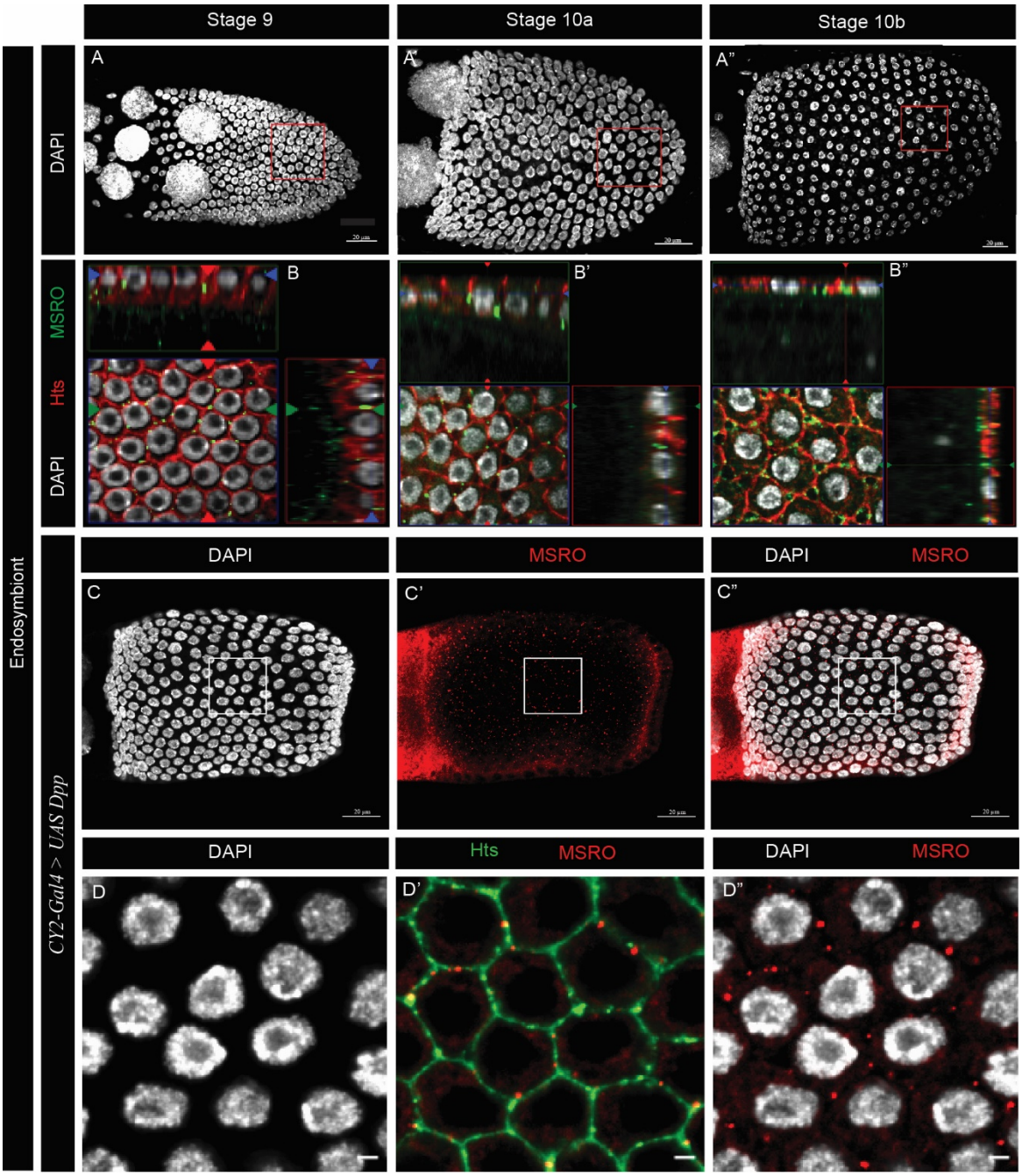

Figure S5 Vertical transfer of the endosymbiont *Spiroplasma pulsonii*, related to Figure 5 (A-A") Projections of DAPI stained Stage 9 – 11 egg chambers. Red boxed are enlarged in (B-B"), and ortho views are provided for top and cross-sectional views. (C-D") MSRO staining in *UAS-Dpp* expressing FE. MSRO marks the bacteria, Hts marks the membrane, and DAPI stains the nuclei.

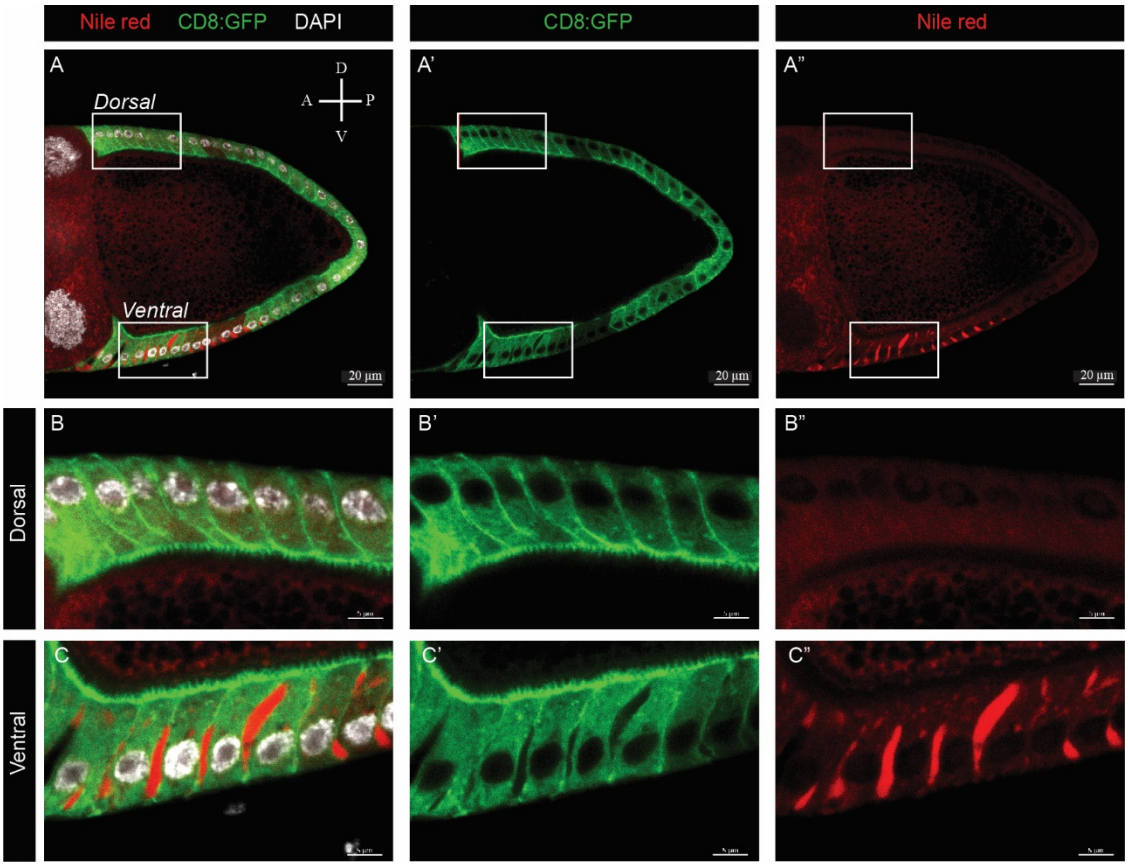

Figure S6. Spatial pattern of trans-epithelial transport of lipids across the FE, related to Figure 5. (A-A") Cross section of a *UAS-CD8:GFP* expressing egg chamber. White box labelled Dorsal is expanded in (B-B"); white box labelled Ventral is expanded in (C-C") showing TC gaps and lipids present between the cells in the patent TCs. Nile red marks lipids, DAPI marks nuclei.

Transparent methods

Drosophila strains and culture

All Drosophila stocks were maintained and crossed at 21–22°, unless otherwise indicated. The w[118] strain was used as the wild-type control. To control the temporal and regional gene expression targeting (TARGET) system, temperature-sensitive Gal80 (Gal80TS) under the ubiquitous tubulin driver was used to regulate the upstream activating sequence (UAS)-transgene expression by altering temperatures. Tj-Gal4 and CY2-Gal4 were used to drive UAS transgene expression in combination with Gal80TS. The crosses involving Gal80TS were crossed and maintained at 18˚C, and progeny were kept at 29˚C for 48hrs prior to dissection.

Tj-Gal4 is used to drive transgenes under UAS control in all the follicle cells across oogenesis. Transgenes that disrupt vitellogenic progression were used with CY2-Gal4, that drives transgenes under UAS control in only the oocyte associated follicle cells starting at low levels at St 9, and at high levels from St 10a [Fig. S4.(C-E')].

Fluorescence Microscopy and Image Analysis

Ovaries were dissected, fixed, and stained with antibodies using the protocol outlined below: (Adopted from Deng *et al*., 2001; Sun and Deng, 2005)
Ovaries were dissected in 1×PBS buffer and fixed for 10 minutes in 500µl of 4% paraformaldehyde. They were rinsed for 5 minutes with 1XPBT buffer and blocked in 20% goat serum (in PBT) for 2 hours at room temperature. The ovaries were then incubated in primary antibodies diluted in blocking buffer overnight at 4°C, followed by 4 10-minute PBT washes. They were then incubated in secondary antibodies for 2 hours at room temperature, and stained with DAPI (1µg/ml in PBT), washed three times with PBT (10 minutes each), and transferred onto slides in mounting solution (70% glycerol, 2%NPG, 1×PBS).

The following primary antibodies were used: Hu li tai shao (Hts) (1:5, 1B1), E-cadherin (Ecad) (1:20, DCAD2), Discs large (Dlg) (1:50, 4F3), and Fasciclin3 (Fas3) (1:15, 7G10) all from the Developmental Studies Hybridoma bank (DSHB), Laminin (gamma 1) (1:200, ab47651, Abcam), Gliotactin (1:50, a gift from Dr. Auld), MSRO (1:200, a gift from Dr. Lemaitre), Ttk69 (1:200, a gift from Dr. Xi). Alexa Fluor 488- or 546-conjugated goat anti-mouse and anti-rabbit secondary antibodies (1:400; Molecular Probes, Eugene, OR) were used. Phalloidin-546 (Invitrogen) was used to stain F-actin. Nuclei were labeled with DAPI (1:1000). Images were captured with Zeiss LSM 800 confocal microscope. Zeiss and ImageJ software were used for image analyses and processing.

Detergent-free protocol was modified from the standard protocol above, with all the same reagents prepared without Tween-20.

Lipid Staining

Ovaries were dissected in PBS and fixed in 4% PFA for 15 minutes. Following 2 washes with PBT, the ovaries were incubated in Nile red (TCI America, 7385-67-3) (0.002% dye diluted in PBT, adopted from 29) or BODIPY 493/503 (ThermoFisher Scientific, Cat No. D3922) (Stock: 1mg/ml BODIPY in absolute ethanol, Working: 1:500 in PBS) in dark for 30 minutes. Following 2 PBT washes, the ovaries were either stained with antibodies, or with DAPI, and then mounted onto slides.

Live imaging

Flies with RFP-tagged His2Av (BL 23650) were incubated in 25˚C with yeast paste for 2 days. Ovaries were dissected in the Schneider Drosophila Medium (Genesee Scientific, #25-512), then st9-st11 egg chambers were mounted in 1% low-melting agarose and cultured in freshly prepared imaging medium (Schneider Drosophila Medium with 15% fly extract, 0.5% penicillin/streptomycin, and 20μg/ml insulin) on cell imaging dishes (145 μm cover glass bottom, Eppendorf #30740009). Dextran-FITC (12.5ug/ml, Sigma # 46944) was added right before imaging started. Live images were taken using a Zeiss LSM 980 Confocal Microscope with Airyscan 2, and set 1hr duration. The imaging raw data were processed and prepared by the ZEN 3.0 software.

Transmission Electron Microscopy

The samples for TEM were prepared using the protocol outlined below: (adopted from Tamori et al., 2016, Matsumoto et al., 1988):
Tissue samples were fixed with Karnovsky's Fixative. After three rinses with 0.1 M sodium cacodylate buffer, samples were embedded in 3% agarose and sliced into small blocks (1mm3), rinsed with the same buffer three times and post-fixed with 1% osmium tetroxide and 0.8 % Potassium Ferricyanide in 0.1 M sodium cacodylate buffer for one and a half hours at room temperature. Samples were rinsed with water and en bloc stained with 4% uranyl acetate in 50% ethanol for two hours. They were then dehydrated with increasing concentration of ethanol, transitioned into propylene oxide, infiltrated with Embed-812 resin and polymerized in a 60°C oven overnight. Blocks were sectioned with a diamond knife (Diatome) on a Leica Ultracut 7 ultramicrotome (Leica Microsystems) and collected onto copper grids, post stained with 2% aqueous Uranyl acetate and lead citrate. Images were acquired on a JEM-1400 Plus transmission electron microscope equipped with a LaB6 source operated at 120 kV using an AMT-BioSprint 16M CCD camera.

Quantification and Statistical Analysis

All quantification data was analyzed by two-tailed Student's T-test. P-values were calculated using GraphPad Prism 8. Graphs were prepared in Excel. Sample sizes and p-values where appropriate are described in the main text, figures, or figure legends.

Supplementary references

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