Developmental Cell *14*

Supplemental Data

Local Actin-Dependent Endocytosis Is Zygotically Controlled to Initiate *Drosophila* Cellularization

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Supplemental Experimental Procedures

Flies and Genetics.

The *nulloX* (X) embryos were collected from the C(1)DX, *ywf* stock (Wieschaus and Sweeton, 1988). The *nullo* gain-of-function embryos and imaginal wing discs were generated by crossing males homozygous for *UASnullo-HA3A* (Hunter and Wieschaus, 2000) with females homozygous for *matα4-GAL-VP16* (II and III) or females homozygous for *ptc559.1-GAL* (II), respectively. The Dynamin perturbation used shi^{ts1} (Sweitzer and Hinshaw, 1998). Fixed *nulloX* embryos were genotyped by absence of Runt expression. The *nullo* gain-of-function embryos were genotyped by presence of HA expression. Fluorescent protein-tagged stocks were UASGFP-Clathrin light chain (Chang et al., 2002), UASVenus-GAP43 (Mavrakis and Lippincott-Schwartz, unpublished data), Histone-GFP and UASGFP-2xFYVE (Wucherpfennig et al., 2003).

Table S1. Primary and Secondary Antibody Concentrations

Antibody	Fixation	Concentration	Source
M anti-Nullo	Heat	1:5	Postner and Wieschaus
R anti-Myosin-2 (Zipper)	Heat	1:1000	Sokac and Wieschaus
Rt anti-HA	Heat	1:50	Roche Molecular Biochemicals
GP anti-Runt	Heat	1:500	Gift of C. Alonso and J. Reinitz
M anti-Septin 4C9H4 (Peanut)	Form.	1:5	Developmental Study Hybridoma Bank (DSHB)
R anti-Amph	Form.	1:1400	Gift of G. Boulianne and H. McMahon
M anti-DPATJ	Form.	1:1000	Gift of M. Bhat
R anti-GFP	Form.	1:2000	Torrey Pines Labs
M anti-Dynamin	Form.	1:500	BD Transduction Laboratories
M anti-Phospho Tyrosine (PY20)	Form.	1:5	Santa Cruz Biotechnology
G anti-M, R, Rt, or GP Alexa-488	Heat or Form.	1:1000	Invitrogen-Molecular Probes
G anti-M, R, Rt, or GP Alexa-546	Heat or Form.	1:1000	Invitrogen-Molecular Probes

M, mouse; R, rabbit; Rt, rat; GP, guinea pig; G, goat; Form., 4% formaldehyde

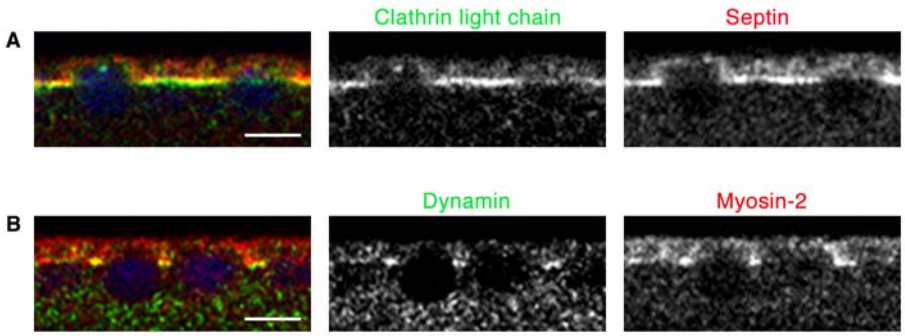

Figure S1. Endocytic Machinery Localizes to Somatic Bud Margins at Early Mitotic Cycles

(A-B) Cross-sections from embryos at mitotic cycle 13. (A) Clathrin light chain (green) concentrates with Septin (red) at somatic bud margins. (B) Dynamin (green) concentrates with Myosin-2 (red) at somatic bud margins. Bars are 5 μm.

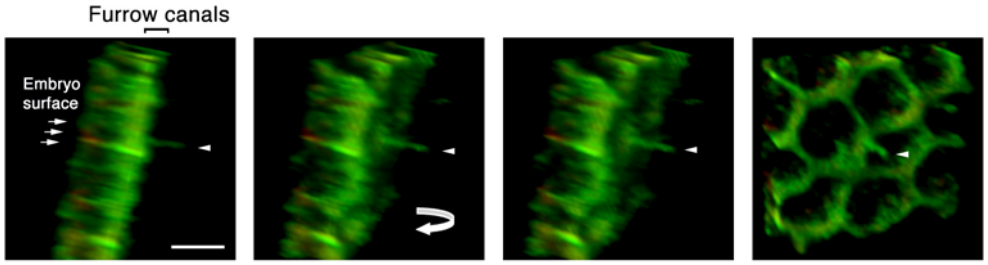

Figure S2. Amph Structures Are Tubules, Not Sheets

Rotation of a volumetric rendering shows an Amph structure (green; arrowhead) hanging below the dense plane of the furrow canal network. The structure is a tubule. This Amph tubule is the same one shown in Figure 3B and 3D. See Movie S1. Bar is 5 μm.

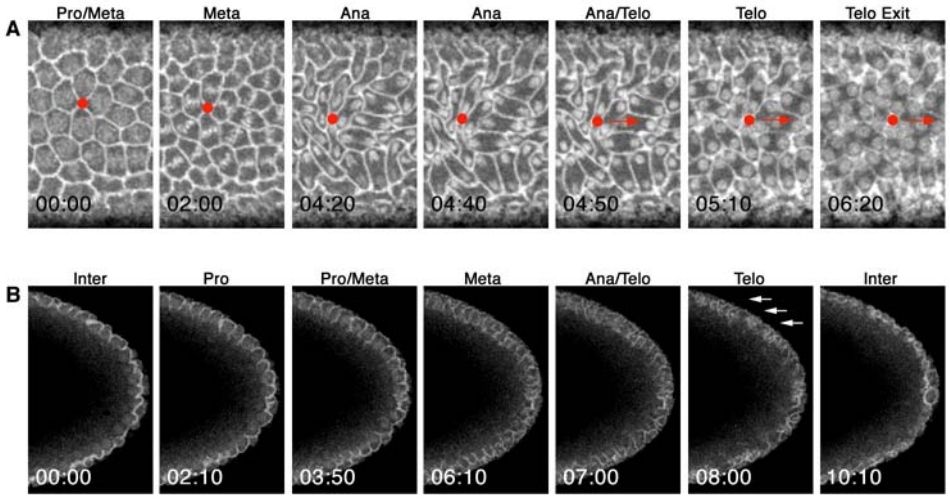

Figure S3. Maximum Cortical Displacement Coincides with Metaphase Furrow Regression at Late Anaphase/Telophase

(A, B) Time-lapse of cycle 13 embryos expressing histone-GFP and Venus-GAP43 to detect DNA and PM dynamics, respectively. The 00:00 time point was set arbitrarily (min:sec). (A) In single-plane *en face* images at mitosis, a cortical spot is followed (red dot), which marks the vertices of four "cells". This spot undergoes maximum displacement (red arrow) along the anterior/posterior axis during late anaphase/telophase when metaphase furrows regress. After regression, furrows are no longer visible at this plane. See Movie S5. (B) Cross-sections show metaphase furrows ingress at prophase/metaphase then regress at late anaphase/telophase. Furrow regression coincides with maximum cortical displacement along the anterior/posterior axis (arrows). See Movie S6.

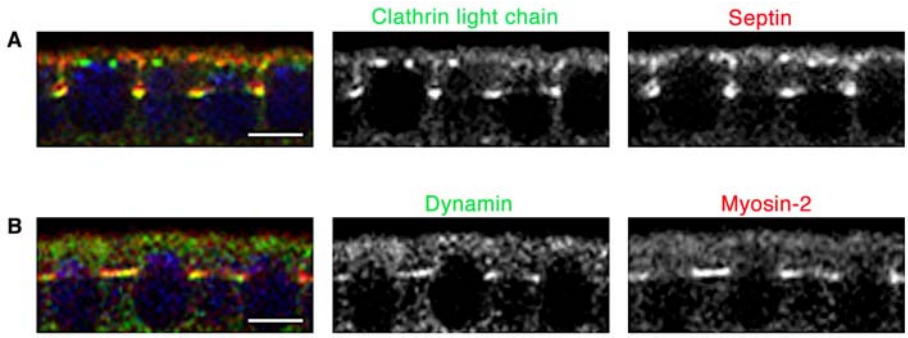

Figure S4. Endocytic Machinery Localizes to Furrows Tips at Early Cellularization

(A-B) Cross-sections from embryos at early cellularization. (A) Clathrin light chain (green) concentrates with Septin (red) at furrow tips. The Clathrin light chain/GFP fusion also localizes to centrosomes, which sit above the nuclei (blue). (B) Dynamin (green) concentrates with Myosin-2 (red) at furrow tips. Bars are 5 μm.

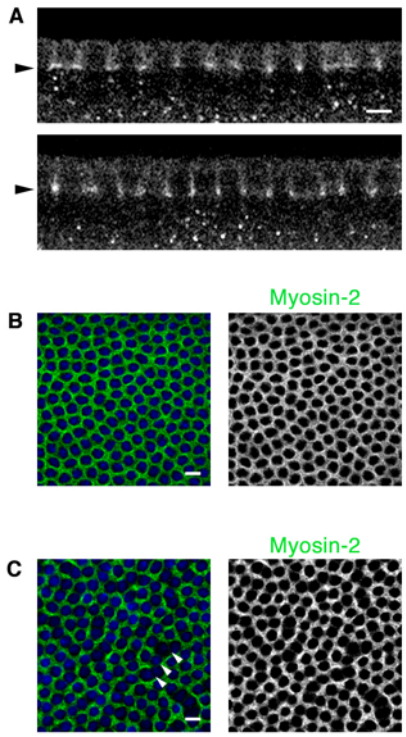

Figure S5. Nullo Localizes to Furrows Tips at Early Cellularization and Is Required for Furrow Canal Assembly

(A) Cross-sections show Nullo concentrates at furrow tips (level indicated by arrowheads). (B) En face images of wild-type furrow canals show Myosin-2 (green) accumulates between every nucleus (blue) to form a continuous network. (C) En face images of *nulloX* furrow canals show Myosin-2 (green) depleted between some nuclei (blue), creating gaps in the network (arrowheads). Bars are 5 μm.

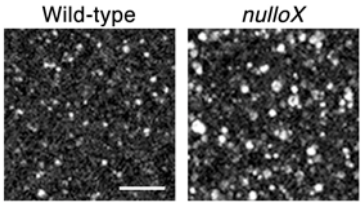

Figure S6. Early Endosomes Are Enlarged in *nulloX* Embryos

En face images show larger early endosomes (FYVE-GFP; green) in *nulloX* embryos compared to wild-type. Bar is 5 μm.

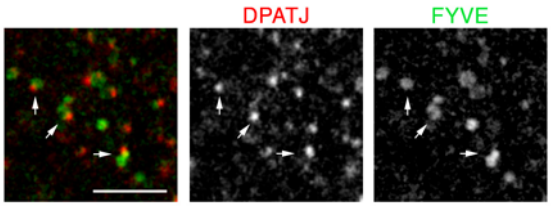

Figure S7. DPATJ Associates with Early Endosomes

En face images from *nulloX* embryos show DPATJ (red) at early endosomes (FYVE-GFP; green) in *nulloX* embryos. Bar is 5 μm.