Developmental Cell, Volume *37*

Supplemental Information

Membrane Supply and Demand Regulates F-Actin in a Cell Surface Reservoir

Lauren Figard, Mengyu Wang, Liuliu Zheng, Ido Golding, and Anna Marie Sokac

Inventory of Supplemental Information

Figure S1: Related to Figure 1, this figure shows (A) images of Golgi disappearance after DMSO (Control) or BFA injection, (B) full microvillar membrane depletion profiles for DMSO- (Control) and BFA-injected embryos, and (C, D) quantification and images of Spider-GFP microvillar membrane fluorescence changes after DMSO (Control) or BFA injection.

Figure S2: Related to Figure 4, this figure shows (A,C) the wild-type microvillar membrane depletion profiles for two membrane probes, (B) control furrow ingression kinetics used to generate the converted plots in Figure 4D, and (D) a converted plot showing F-actin versus membrane for the additional membrane marker, Spider-GFP.

Figure S3: Related to Figure 5, this figure shows quantifications of microvillar F-actin fluorescence and furrow length versus distance from the injection site in individual (A) buffer- (Control) and (B) WGA^{Ax488}-injected embryos.

Figure S4: Related to Figure 6, this figure shows quantifications of Golgi fluorescence versus distance from the injection site in individual (A) buffer- (Control) and (B) WGA^{Ax594}-injected GalT-GFP embryos.

Figure S5: Related to Figure 6, this figure shows (A, B) quantifications of vesicular Spider-GFP fluorescence versus distance from the injection site in (A) buffer- (Control) and (B) WGA^{Ax594}-injected Spider-GFP embryos, (C) images of DMSO- (Control) or BFA-injected embryos, stained with $Phalloidin^{Ax488}$ to show F-actin, (D) full microvillar F-actin depletion profiles for DMSO- (Control) and BFA-injected embryos.

Movie S1: Related to Figure 2, this movie shows extension and retraction of individual microvilli in both the “head-on” and “lying-down” orientations.

Supplemental Experimental Procedures: this section contains additional information regarding: generation of Utrophin-mCherry fly stock, embryo injections, embryo fixation and staining, 4D normalization and MATLAB codes.

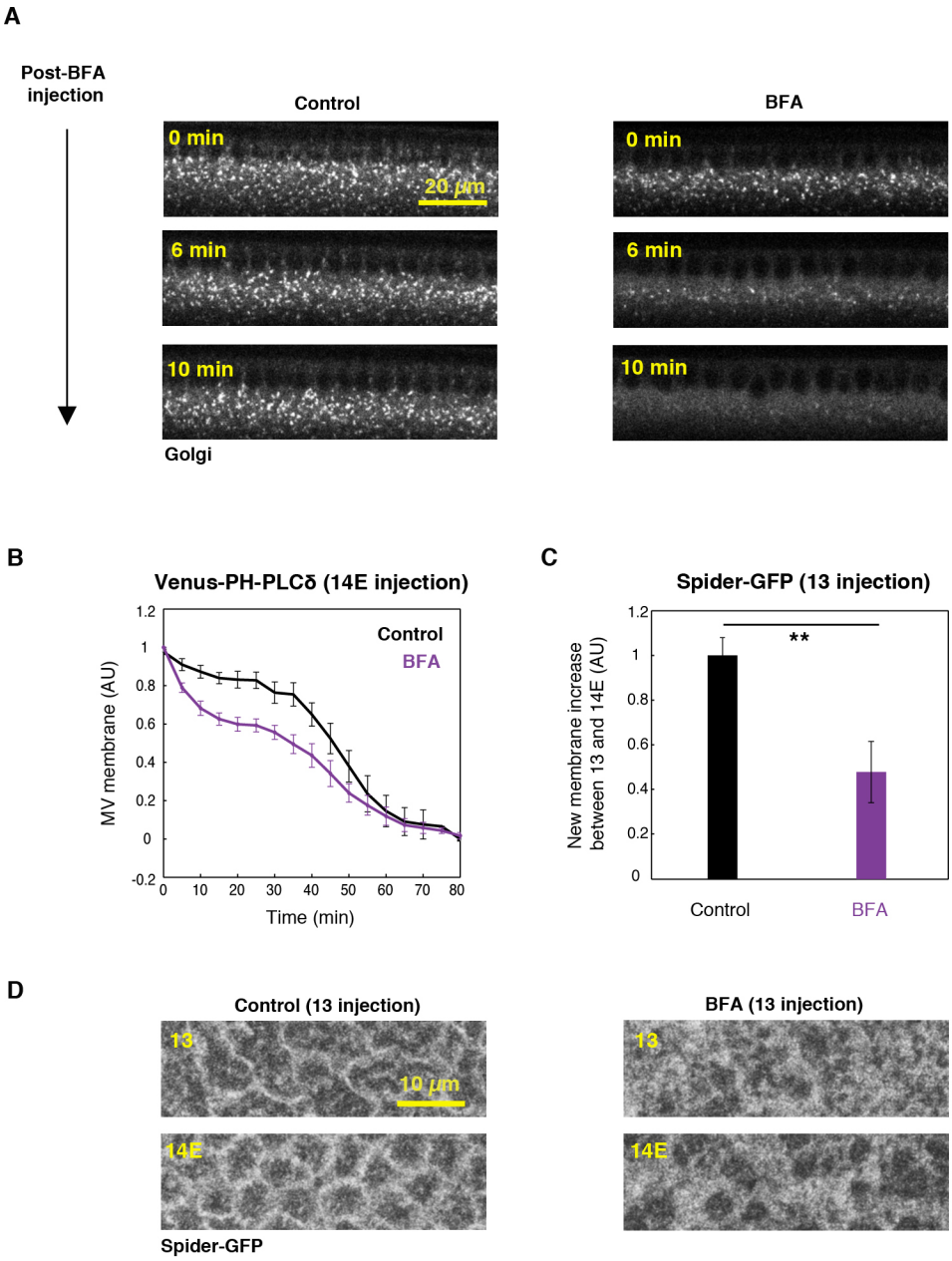

Figure S1: Exocytosis delivers membrane that is stored in a microvillar reservoir; related to Figure 1.

(A) Confocal cross-sections of GalT-GFP in DMSO- (Control) and BFA-injected embryos show BFA induces Golgi body collapse. 0 minutes is onset of imaging.

(B) MV membrane fluorescence versus time from DMSO- (Control) or BFA-injected Venus-PH-PLCδ embryos (N≥7 embryos per condition; mean ± SE). Injection time 14E.

(C) Percent increase in new MV membrane fluorescence between cycle 13 and 14E from DMSO- (Control) or BFA-injected Spider-GFP embryos (N≥7 embryos per condition; mean ± SE; **p<0.01, Student's t-test). Injection time 13.

(D) Z-projections of Spider-GFP in DMSO- (Control) or BFA-injected embryos show MV membrane at the cycle 13 to 14E transition. Injection time 13.

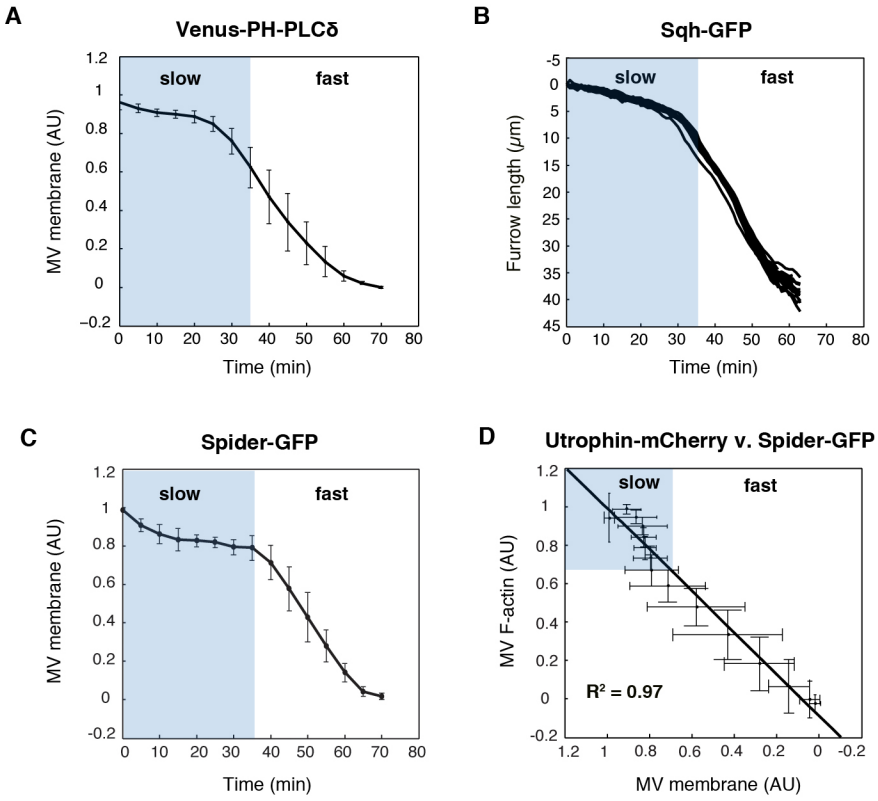

Figure S2: Microvillar F-actin is depleted *in sync* with furrow ingression, related to Figure 4.

(A) MV membrane fluorescence versus time for Venus-PH-PLCδ embryos (N=4 embryos; mean ± SE).

(B) Furrow length versus time for a typical Sqh-GFP embryo (N=16 trajectories from 1 embryo).

(C) MV membrane fluorescence versus time for Spider-GFP embryos (N=4 embryos; mean ± SE).

(D) Average normalized MV F-actin fluorescence (N=12 embryos; mean ± SD) versus average normalized MV membrane fluorescence (Spider-GFP; N=4 embryos; mean ± SD). Line is a linear least-square fit.
For (A)-(D), slow ingression/depletion phase shaded in blue. Shading is based on transition from slow to fast phase as defined in Figard, et al. 2013.

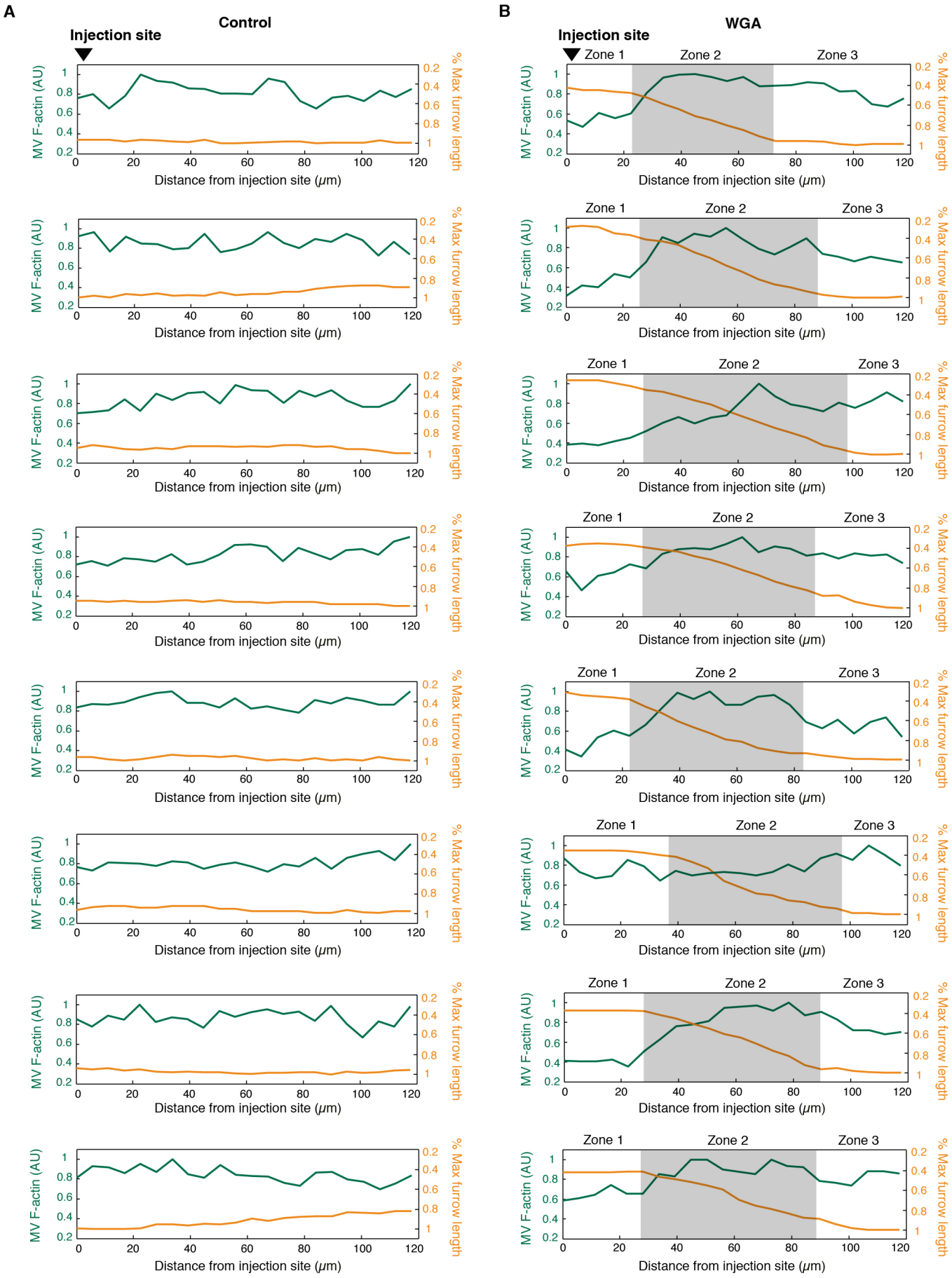

Figure S3: Microvillar F-actin depletion is coupled to furrow ingression; related to Figure 5.
(A, B) MV F-actin fluorescence (green) and furrow length (orange) versus distance from injection site in individual (A) buffer- (Control) or (B) WGA^{Ax488}-injected Utrophin-mCherry embryos.
For (B), zones are as described in the Results.

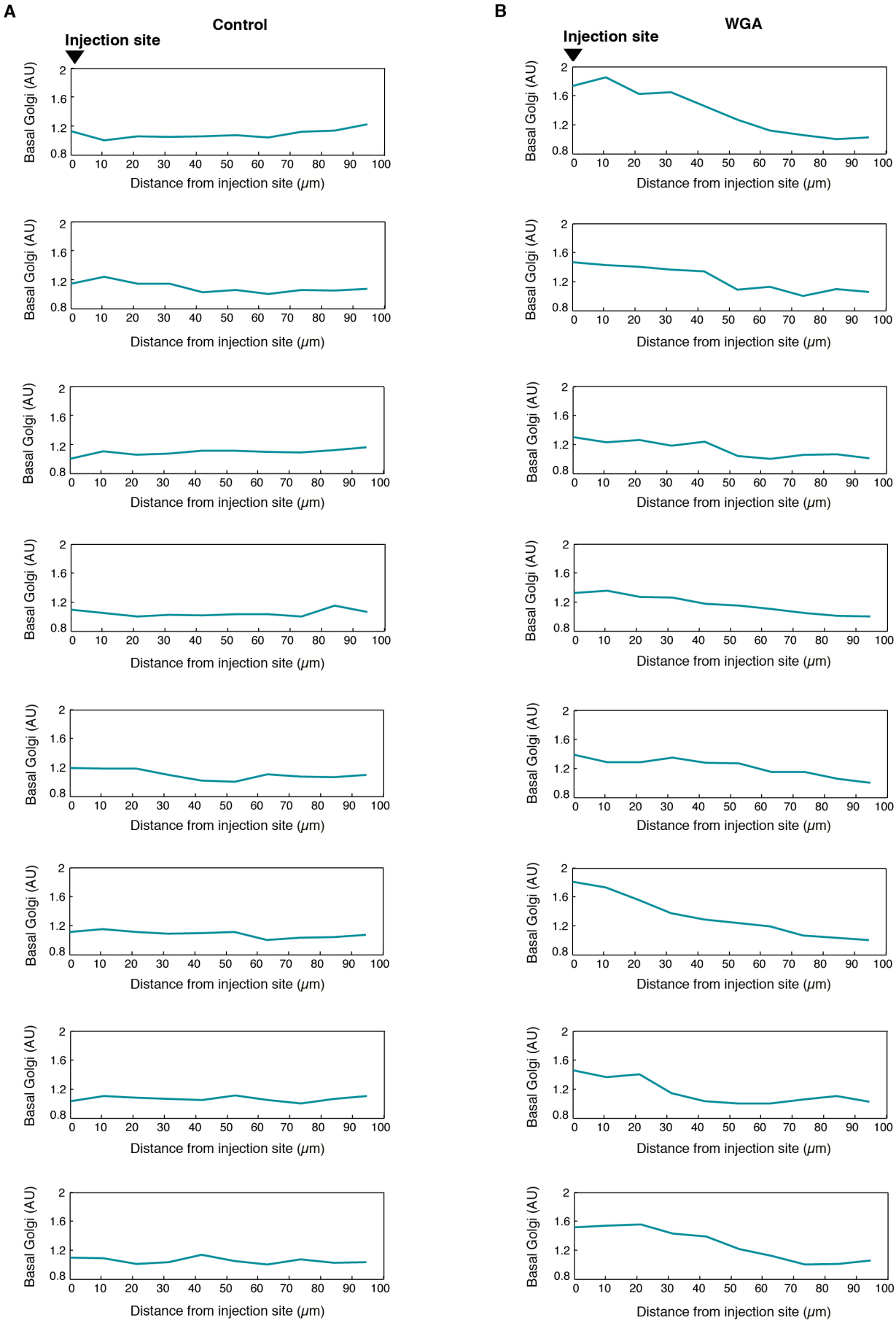

Figure S4: Exocytosis promotes microvillar F-actin assembly; related to Figure 6.
(A, B) Golgi fluorescence versus distance from the injection site in individual (A) buffer- (Control) or (B) WGA^{Ax594}-injected GalT-GFP embryos.

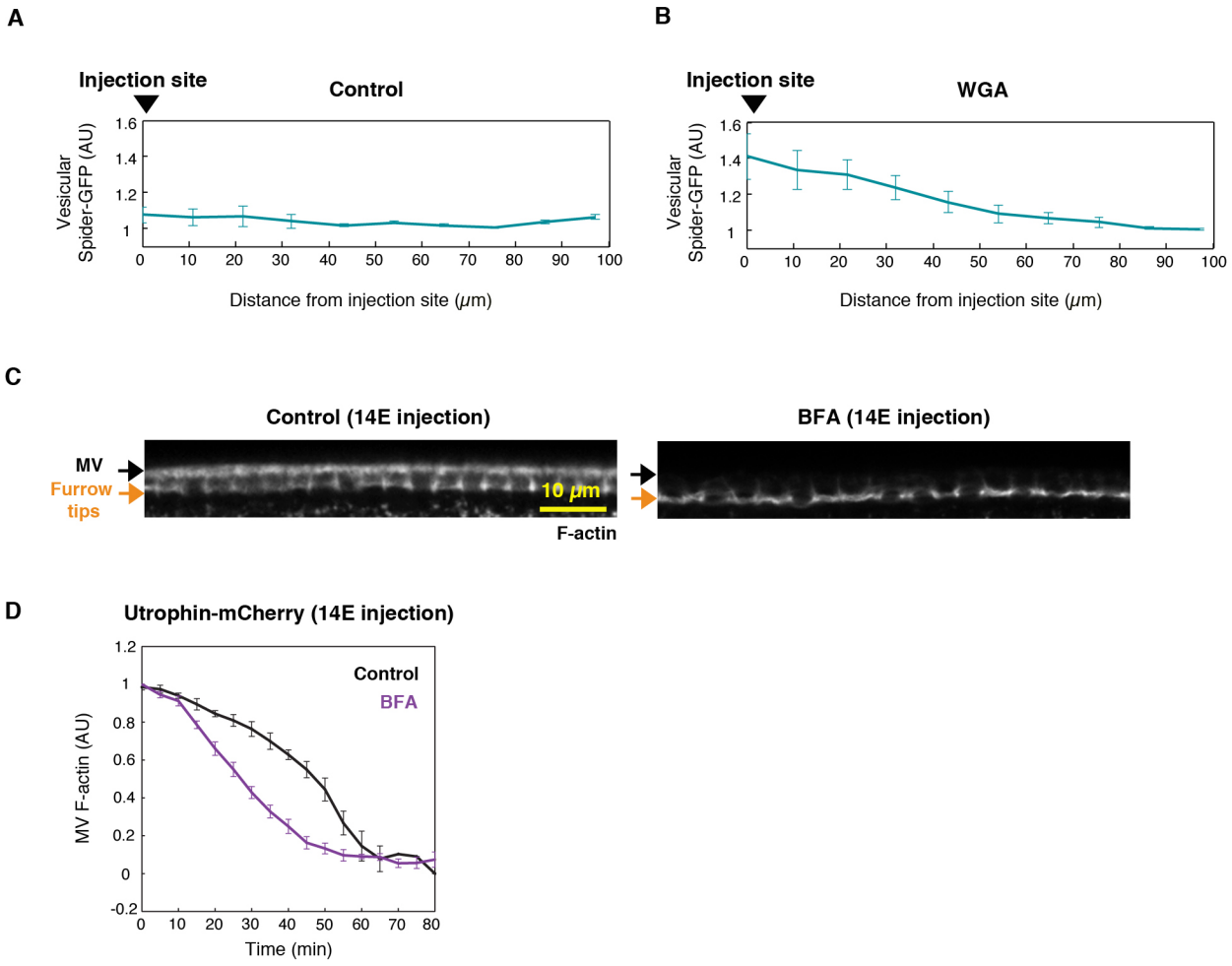

Figure S5: Exocytosis promotes microvillar F-actin assembly; related to Figure 6.

(A, B) Vesicular fluorescence versus distance from the injection site in (A) buffer- (Control) or (B) WGA^{Ax594}-injected Spider-GFP embryos (N=3 embryos per condition; mean ± SE).

(C) Confocal cross-sections of F-actin ($Phalloidin^{Ax488}$) in DMSO- (Control) or BFA-injected embryos. Injection time 14E.

(D) MV F-actin fluorescence versus time in DMSO- (Control) or BFA-injected Utrophin-mCherry embryos (N≥4 embryos per condition; mean ± SE). Injection time 14E.

Supplemental Movie:

Movie S1: Extension of a microvillus in the "Head-on" or "Lying-down" orientation; related to Figure 2.

Concatenated time-lapse TIRF movies of microvilli from Utrophin-mCherry embryos extending and retracting in either the "head-on" or "lying-down" orientation. Timestamp shown as minutes:seconds; "00:00" is the onset of extension. Scale bar is 0.5μm or 1μm, respectively.

Supplemental Experimental Procedures:

Utrophin-mCherry transgenesis and characterization

The Calponin Homology Domain (first 783 base pairs) of human *utrophin* (Burkel et al., 2007) was cloned into a modified pBluescript vector containing mCherry and a multiple cloning site sandwiched between the 5' and 3' untranslated regions of *sqh*. mCherry was C terminal to the *utrophin* sequence. The construct was inserted into pCasper4, and transgenesis and mapping followed standard methods (BestGene, Inc.).

Embryo injections

For RNAi, 50pl of *slam* dsRNA (~1mg/ml) in injection buffer (5mM KCl; 0.1mM sodium phosphate buffer, pH 7) was injected into embryos 30 minutes post-laying (Lecuit et al., 2002). Injection buffer was injected as a control. For WGA injections, WGA^{Ax488} or WGA^{Ax594} (Life Technologies) in phosphate-buffered saline (PBS) was centrifuged for 10 minutes at 14,000 rpm. After a pre-spin, 50pl was injected at 0.5mg/ml concentration near the nuclei on the dorsal side during early cellularization. PBS was injected as a control. For G-actin injections, 50pl of rhodamine non-muscle actin (Cao et al., 2008; 2mg/ml in G-buffer prepared as per the manufacturer's instructions; Cytoskeleton, Inc.) was injected ~15 minutes before cellularization. For Phalloidin injections, Phalloidin (EMD Millipore) was injected at 1.3mM in injection buffer supplemented with 2% DMSO (Planques et al., 1991) during early cellularization. Injection buffer supplemented with 2% DMSO was injected as a control. For BFA injections, BFA (Cell Signaling Technology) was injected at either 100mM or 75mM

concentration in DMSO (Sisson et al., 2000) at the indicated developmental times. 75mM BFA was used for the more sensitive 4D imaging technique because 75mM gave more consistent phenotypes than 100mM BFA. For LatA injection, LatA (AdipoGen) was injected at 0.5mM concentration in DMSO (Cao et al., 2008) near the nuclei on the dorsal side of the embryo. For both BFA and LatA, DMSO was injected as a control.

Fixation and phalloidin staining

OreR embryos were fixed for 25 minutes at the interface of 1:1 heptane and 8% paraformaldehyde in sodium phosphate buffer (pH 7.4). The vitelline membrane was hand-peeled with a needle. Embryos were stained with either Alexa546 or Alexa488-Phalloidin (5U/ml; LifeTechnologies).

4D normalization and MATLAB codes

To normalize 4D data from wild-type, control, and BFA-injected embryos, fluorescence values were normalized between minimum and maximum for each embryo as: $F_{norm}=(F-F_{min})/(F_{max}-F_{min})$. *Slam*^{RNAi} embryos were normalized for each embryo between the maximum and a "theoretical minimum", calculated from expected depletion in control embryos (Figard et al., 2013). Briefly, a theoretical value for F_{min} was first calculated per *slam*^{RNAi} embryo as $F_{mintheor} = F_{maxslam} (1-\Delta^{WT})$, and data was normalized as $F_{normslam}=(F_{slam}-F_{mintheor})/(F_{maxslam}-F_{mintheor})$. For both normalization methods, normalized values per time point were averaged between embryos (Figard et al., 2013).

The F-actin versus membrane depletion plot (Figure 4C, S2D) was generated with a custom MATLAB code that uses the time parameter to relate average normalized

fluorescence values from Utrophin-mCherry 4D data and Spider-GFP or Venus-PH-PLCδ 4D data to one another. The F-actin versus furrow length plot (Figure 4D) was generated with MATLAB code that uses the time parameter to relate average normalized fluorescence values from Utrophin-mCherry 4D data to furrow length using furrow ingression trajectories from a Sqh-GFP embryo. Furrow ingression trajectories (Figure 7B, S2B) were generated using code that locates the embryo's edge, creates a series of lines normal to the surface, and tracks the max fluorescence intensity (furrow tip) along each line over time (Figard et al., 2013).

Supplemental References

Burkel, B.M., von Dassow, G., Bement, W.M., 2007. Versatile fluorescent probes for actin filaments based on the actin-binding domain of utrophin. Cell. Motil. Cytoskeleton *64*, 822–832.

Cao, J., Albertson, R., Riggs, B., Field, C.M., Sullivan, W., 2008. Nuf, a Rab11 effector, maintains cytokinetic furrow integrity by promoting local actin polymerization. J. Cell. Biol.*182*, 301–313.

Figard, L., Xu, H., Garcia, H.G., Golding, I., Sokac, A.M., 2013. The plasma membrane flattens out to fuel cell-surface growth during *Drosophila* cellularization. Dev. Cell *27*, 648-655.

Lecuit, T., Samanta, R., Wieschaus, E., 2002. *slam* encodes a developmental regulator of polarized membrane growth during cleavage of the *Drosophila* embryo. Dev. Cell *2*, 425–436.

Planques, V., Warn, A., Warn, R.M., 1991. The effects of microinjection of rhodamine phalloidin on mitosis and cytokinesis in early stage *Drosophila* embryos. Exp. Cell. Res. *192*, 557–566.

Sisson, J.C., Field, C., Ventura, R., Royou, A., Sullivan, W., 2000. Lava lamp, a novel peripheral golgi protein, is required for *Drosophila melanogaster* cellularization. J. Cell. Biol. *151*, 905–918.