SUPPLEMENTARY METHODS

Non-denaturing RNA-FISH

To confirm the specificity of the dH1598-AF647 probe, we knocked down *drongo* gene expression in the germline by performing crosses between *drongo*^{iHMJ}/CyO virgin females and MTD-GAL4 males. RNA-FISH with dH1598-AF647 molecular beacon probe was performed as described in the main text using ovaries isolated from CyO (wild type) and non-CyO (*drongo*^{iHMJ}) first generation female flies. Samples were mounted as described and images were acquired and processed using the parameters optimal for the wild type sample. Heat map images were generated using the HeatMap Histogram ImageJ plugin.

smFISH with *drongo*-specific Stellaris® probes

Stellaris® probes for *drongo*-RH were designed using the Bioresearch Technologies website (Petaluma, CA), and were then synthesized, labeled with Cy5, and purified as previously described[1]; their sequences are available upon request. Denaturing smFISH with Stellaris® probes was performed as previously described.[2] Briefly, fixed egg chambers were incubated for 10 min at room temperature with hybridization buffer (10% dextran sulfate, 1 μg/μl Escherichia coli tRNA, 2 mM vanadyl ribonucleoside complex, 0.02% RNase free BSA, 10% formamide, 2x SSC), then for 4 h to overnight incubation at 37 ˚C with 1-2 ng/μl Stellaris® probe solution in hybridization buffer, washed three times, 10 min each at room temperature, with hybridization wash buffer (10% formamide, 2x SSC) and rinsed with 2x SCC. Egg chambers were mounted in ProLong® Gold (Life Technologies; P36931), or glycerol based (90% glycerol, 1x PBS, 1% diazobicyclo[2.2.2]octane) media.

FM 4-64x assay

Endocytosis assays were performed using a fixable form of FM 4-64, as previously described.[3] Briefly, ovaries teased from 4-6 flies were placed in Schneider's *Drosophila* medium supplemented with 10 μM FM 4-64x, or water (negative control), and incubated at room temperature with gentle rocking. After 5 min, the ovaries were washed for 10 min with Schneider's *Drosophila* medium at room temperature, followed by fixation for 5 min in 4% paraformaldehyde in PBS. After two washes, ovarioles were tweezed apart directly in mounting medium (ProLong® Gold, or glycerol based: 90% glycerol, 1x PBS, 1% diazobicyclo[2.2.2]octane), mounted and sealed, and imaged on the same day.

Immunofluorescence

Immunofluorescence experiments to determine if anti-Drongo recognizes Drongo-EGFP (Supplementary information Fig. S2C) were performed as described in the main text, with the exception that the incubation with the primary antibody was extended to 2 days at room temperature. To compare Drongo protein levels in a defective *drongo*^{iHMJ} oocyte with the ones in a wild type oocyte, the images were acquired and processed using the optimal parameters for the wild type sample. Heat map images were generated using the HeatMap Histogram ImageJ plugin.

REFERENCES

1. Batish M, Raj A, Tyagi S. Single molecule imaging of RNA in situ. Methods in molecular biology 2011; 714:3-13.

2. Bayer LV, Batish M, Formel S, Bratu DP. Single Molecule RNA in situ Hybridization (smFISH) and Immunofluorescence (IF) in the Drosophila Egg Chamber. Methods in molecular biology 2015:In press.

3. Sanghavi P, Lu S, Gonsalvez GB. A functional link between localized Oskar, dynamic microtubules, and endocytosis. Developmental biology 2012; 367:66-77.

SUPPLEMENTARY TABLE

Table S1. Molecular beacon sequences, labels and target regions.

Name	Sequence* (5' > 3')	mRNA Target Region
dH1111	(Cy3 or Cy5)-*caGG*UCAUGUUCACGUAGGUUGGU*CCUg*-BHQ-2	*drongo* CDS: RF; RH; RI
dH1598	AF647-*gcugc*UUGUUUUGGCUGUUGUUCUGGUGAU*gcagc*-BHQ-2	*drongo* CDS: all
o2209	Cy5-*gcugc*AAAAGCGGAAAAGUUUGAAGAGAA*Gcagc*- BHQ-2	*osk* 3'-UTR: RA; RC

* 2'O-Methyl RNA backbone; *italic nucleotides* = stem sequence; UPPER CASE nucleotides = probe sequence; Cy3 and Cy5 = cyanine 3 and 5 dyes; AF647 = Alexa Fluor 647; BHQ-2 = Black Hole Quencher 2.

SUPPLEMENTARY FIGURES

Figure S1

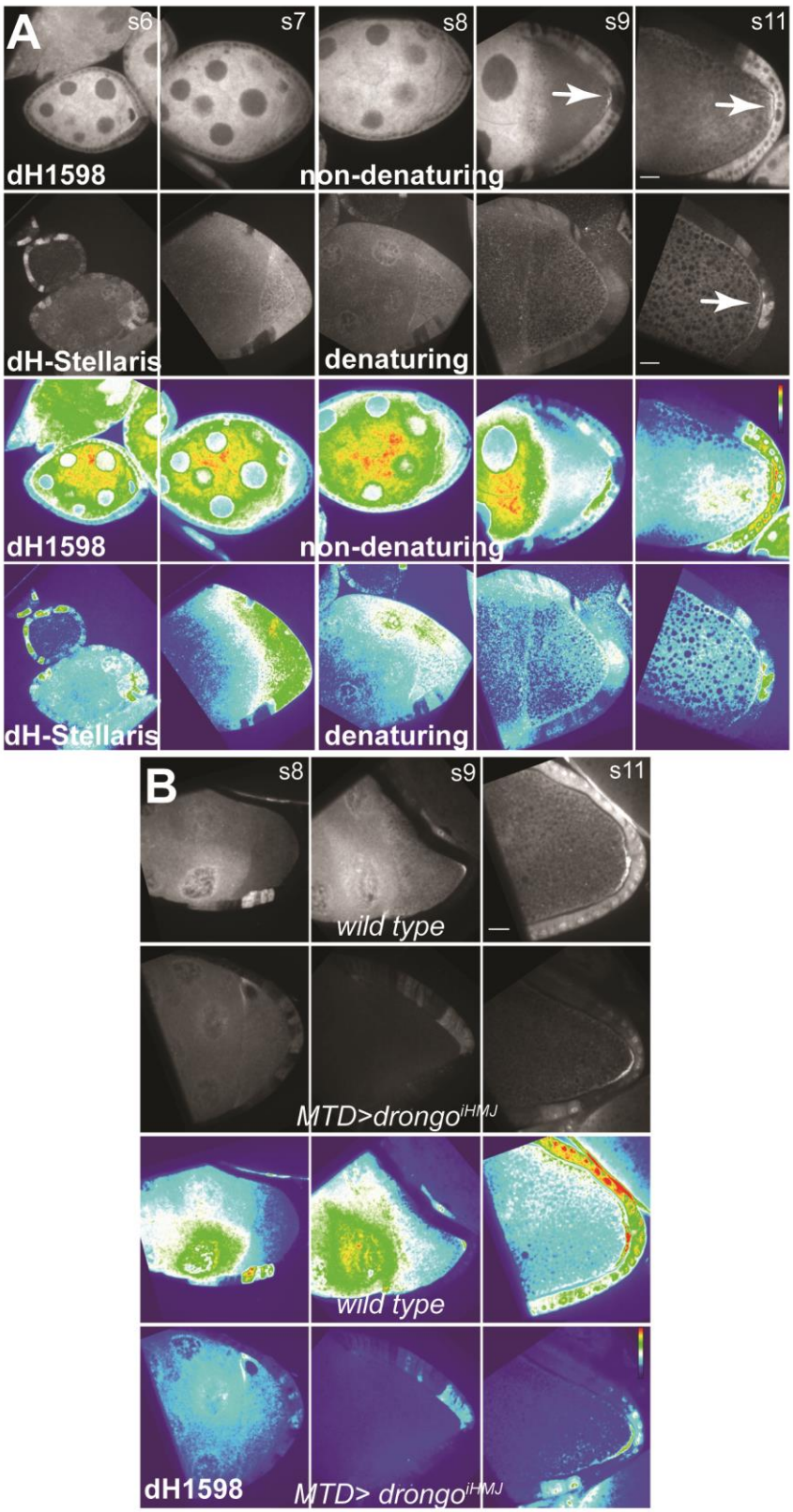


Figure S1. Localization of endogenous *drongo* mRNA in fixed egg chambers. (A) RNA-FISH using a non-denaturing (*drongo*-specific molecular beacon) and a denaturing (Cy5-labeled Stellaris® probes) protocols, in wild type egg chambers, at the indicated stages. Arrows indicate *drongo* mRNA localization at the oocyte's cortex. **(B)** *drongo* mRNA levels and localization in fixed wild type and $drongo^{iHMJ}$ egg chambers as determined using non-denaturing FISH with dH1598-AF647, at the indicated stages. s=stage, A=anterior, P=posterior. Bars, 20 μm.

Figure S2

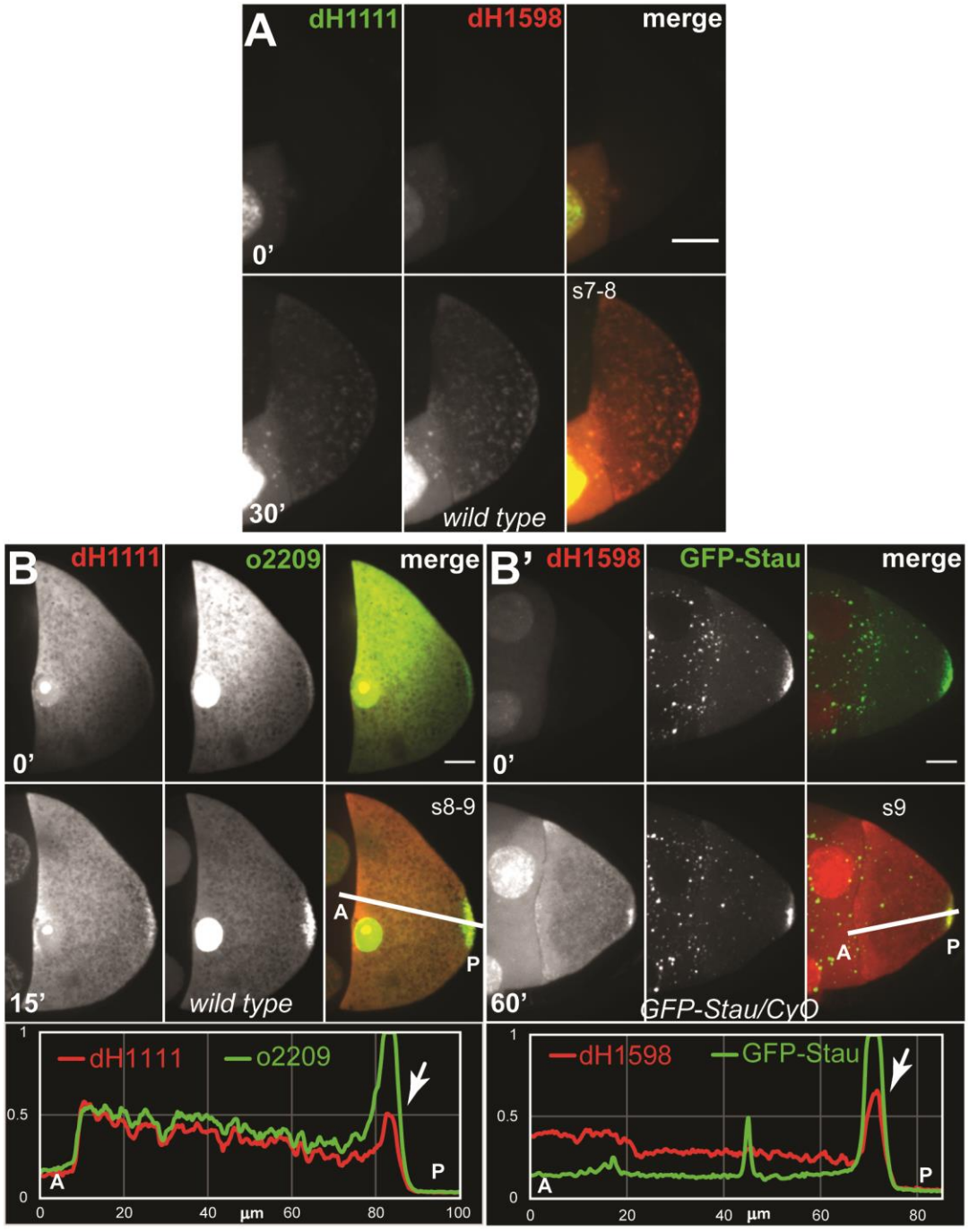


Figure S2. Localization of endogenous *drongo* mRNA in live egg chambers. (A) Co-injection of dH1111-Cy3 and dH1598-AF647 molecular beacons in a nurse cell of a wild type egg chamber (XY-projection of 7 Z-slices/1 µm each). **(B)** Colocalization of endogenous *drongo* and *osk* mRNAs from microinjection of a *drongo*-specific and *osk*-specific molecular beacon cocktail

into the oocyte of a stage 8-9 wild type egg chamber, at the indicated time points (XY-projection of 9 Z-slices/1 μm each). **(B')** Colocalization of endogenous *drongo* mRNA and GFP-Stau from microinjection of dH1598-AF647 molecular beacon into a nurse cell of a stage 9 GFP-Stau egg chamber, at the indicated time points (XY-projection of 12 Z-slices/1 μm each). Normalized intensity line plot profiles measured from the lines (1 pixel, 385 nm/pixel) drawn as shown in the merged images. Arrows indicate colocalization at the oocyte's posterior. s=stage, A=anterior, P=posterior. Bars, 20 μm.

Figure S3

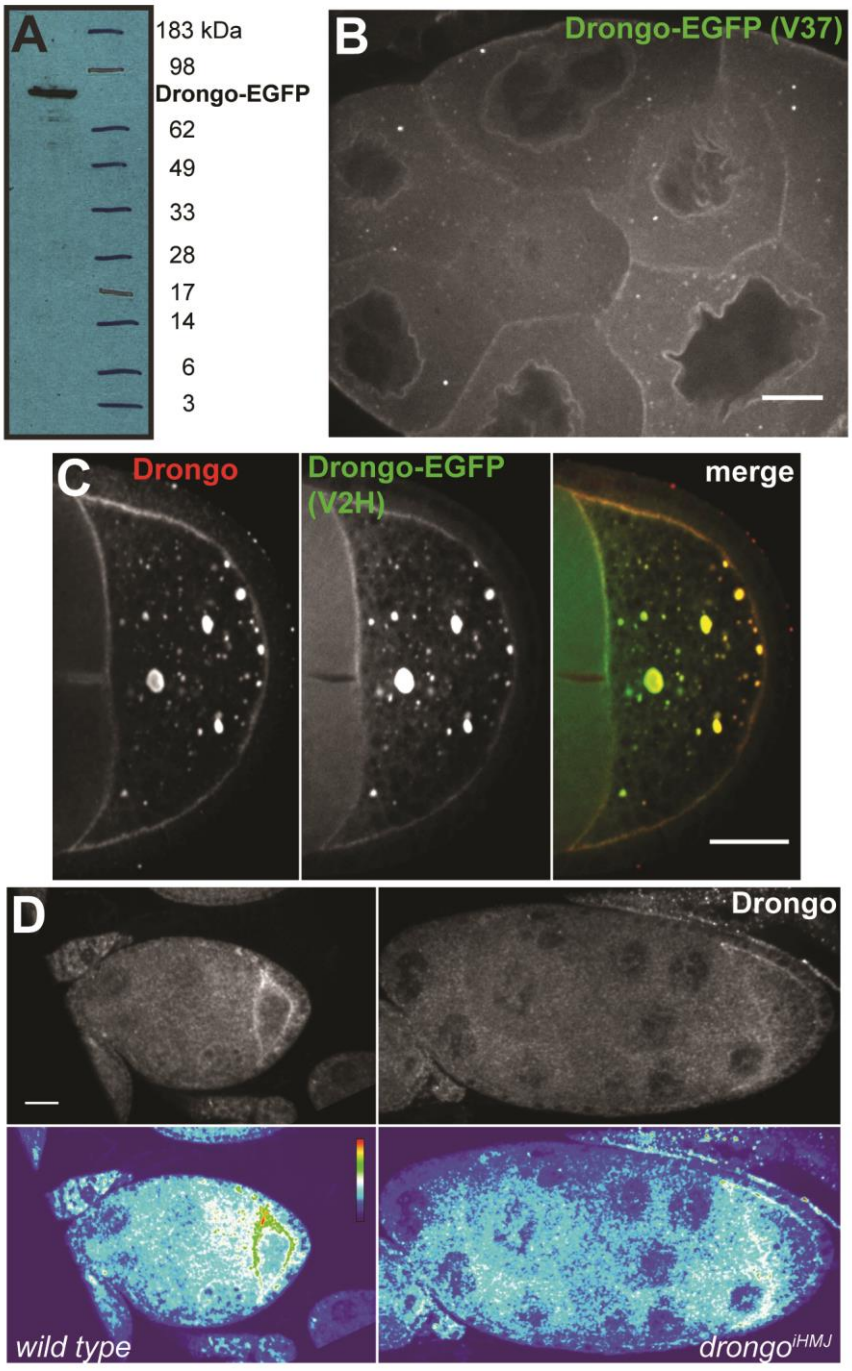


Figure S3. Drongo-EGFP protein overexpression in the germline. (A) Western blot analysis of *MTD>drongo-EGFP* ovary lysates using anti-GFP antibody. **(B)** Drongo-EGFP localization in the nurse cells of a stage 9-10 egg chamber. **(C)** Localization of endogenous Drongo and Drongo-EGFP using the Drongo peptide antibody in fixed *V2H>Drongo-EGFP* egg chambers.

(D) Comparison of levels of endogenous Drongo protein in stage 8, wild type and *drongo*^{iHMJ} egg chambers using the Drongo peptide antibody. Bars, 20 μm.

Figure S4

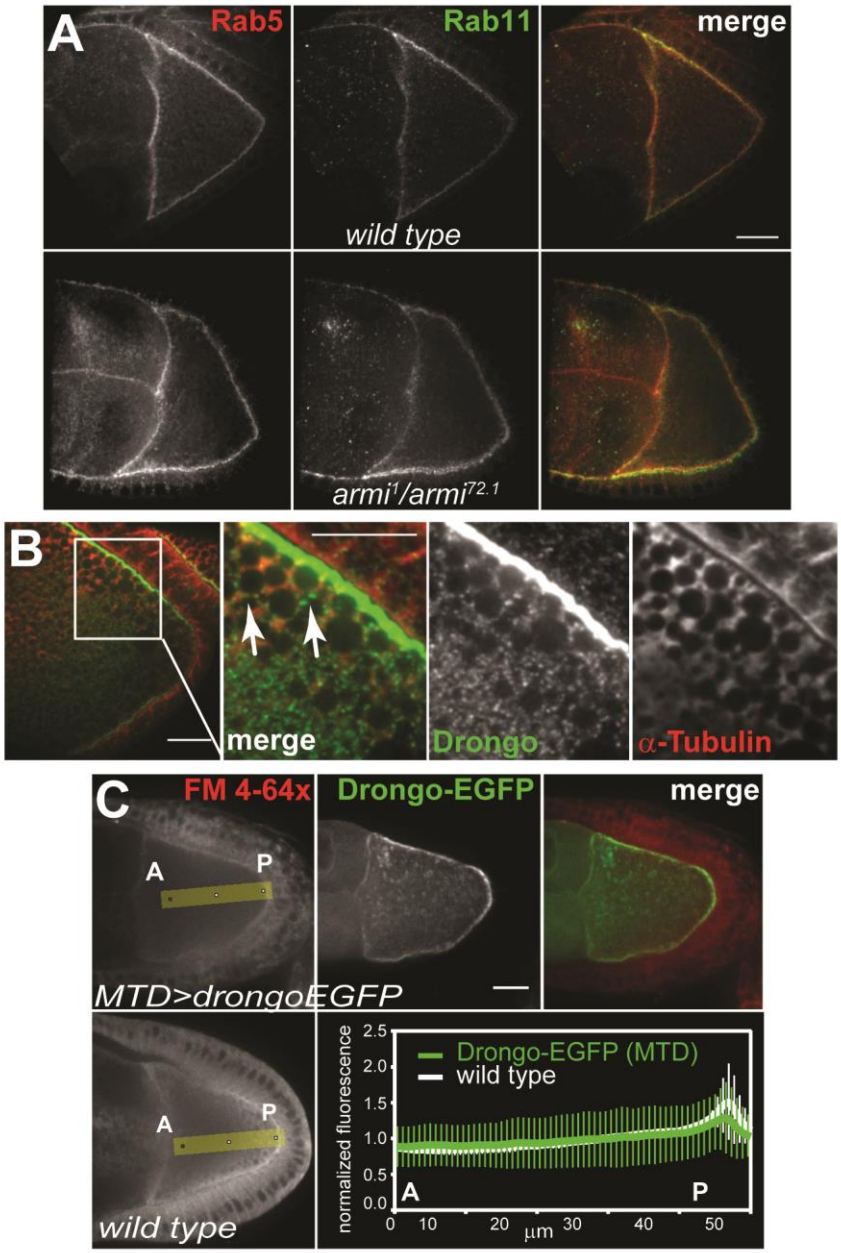
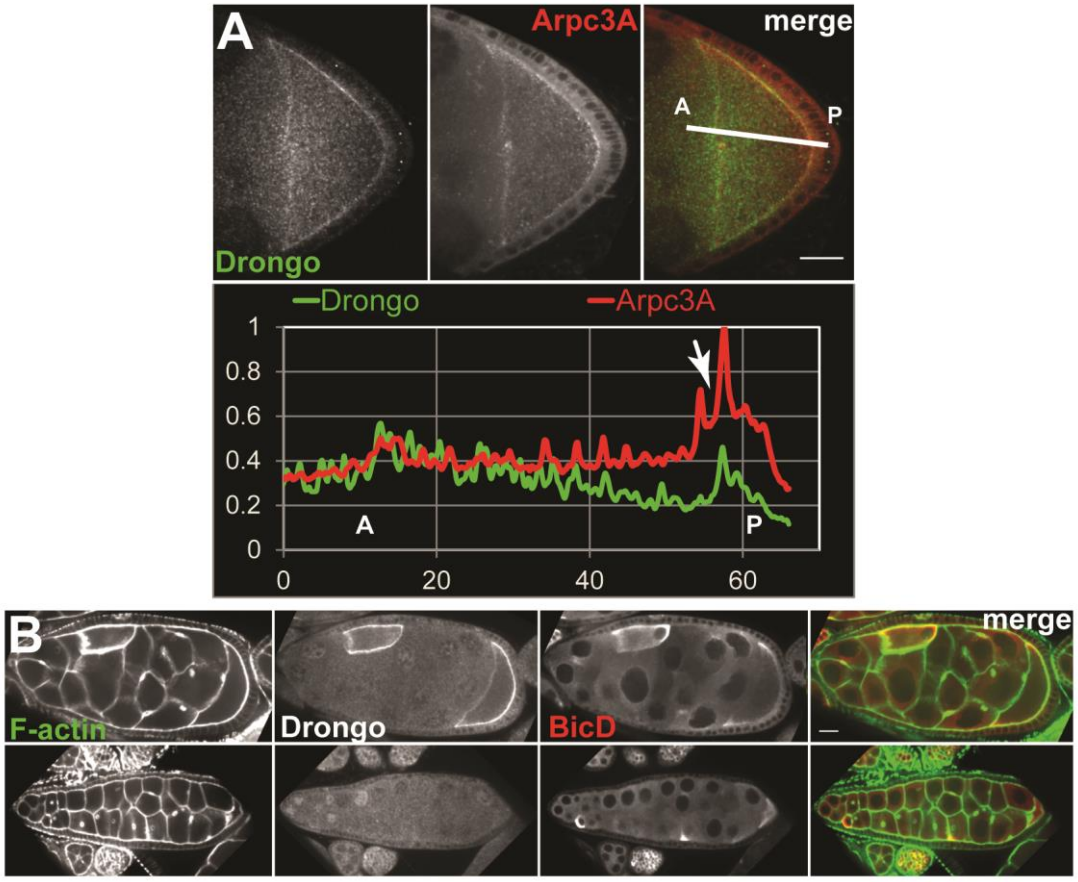


Figure S4. **Distribution of Rab5 and Rab11 in *armi* mutant, and uptake of FM 4-64x in live egg chambers. (A)** Immunofluorescence studies of stage 8-9, wild type and *armi* mutant egg chambers, using anti-Rab5, and anti-Rab11 antibodies. **(B)** Endogenous Drongo localization in the ooplasm of a fixed egg chamber (> stage 9). Arrows indicate Drongo particles in close proximity to yolk granules, which were observed using an anti-α-Tubulin stain. **(C)** FM 4-64x uptake in stage 9, wild type and Drongo-EGFP oocytes. The fluorescence intensity line plot

profile was measured for a 25 pixels-thick line (385 nm/pixels), as drawn in the FM 4-64x panels. A=anterior, P=posterior. Bars, 20 μm.

Figure S5



92

93 **Figure S5**. **Drongo and Arpc3A localization in wild type, and Drongo distribution in *arpc4*^{i}**
94 **egg chambers. (A)** Colocalization of endogenous Drongo and Arpc3A at the oocyte's cortex of
95 a stage8-9 wild type egg chamber using normalized fluorescence intensity line plot profile along
96 the line indicated in the merge panel. **(B)** Defects in egg chambers where knockdown of *arpc4*
97 expression was initiated in the germarium (MTD-GAL4). Two Z-stacks were stitched for the top
98 image, one representative Z-slice is shown. The oocyte was highlighted with Bic-D as a marker.
99 Bars, 20 μm.

Figure S6

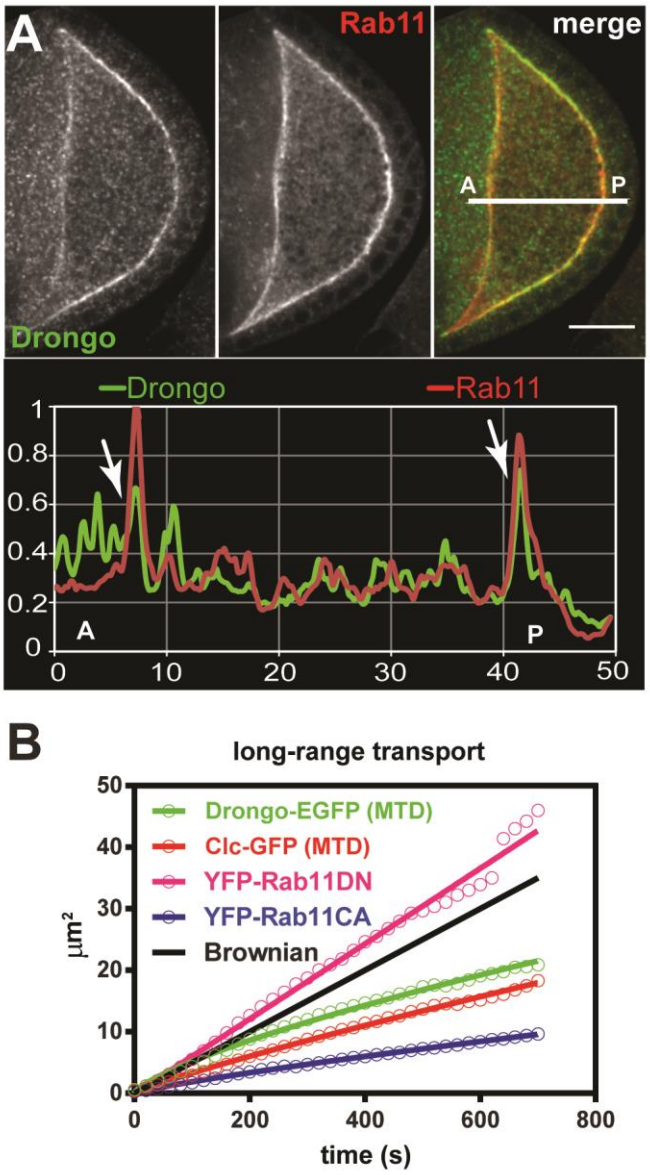


Figure S6. Localization of endogenous Drongo and Rab11, and analysis of Drongo transport in live egg chambers. (A) Colocalization of Drongo and Rab11 at the oocyte's cortex using normalized fluorescence intensity line plot profile along the line indicated in the merge panel. Object-based colocalization for endogenous Drongo with Rab11 within the oocyte of this wild type egg chamber is 19%. **(B)** MSD analysis for long-range of Drongo-EGFP (green) or Clc-GFP (red) particles in the wild type background; and Drongo-EGFP particles in Rab11DN (magenta) and Rab11CA (blue) backgrounds.

SUPPLEMENTARY MOVIE LEGENDS

MovieS1. Transport of endogenous *drongo* mRNA in a wild type oocyte. A mix of dH1111-Cy3 (green) and dH1598-AF647 (red) *drongo*-specific molecular beacons was microinjected in one of the nurse cells adjacent to the oocyte of a stage 8 egg chamber. Images were analyzed by time-lapse confocal microscopy using a spinning disc confocal microscope. Frames were acquired every minute for at least 49 min and are shown as a projection of seven 1 μm Z-slices at 6 frames/sec. Bar, 20 μm.

MovieS2. Colocalization of *drongo* mRNA and Me31B-YFP. dH1598-AF647 (red) *drongo*-specific molecular beacon was microinjected in a nurse cell of a stage 8 Me31B-YFP (green) egg chamber. Images were analyzed by time-lapse confocal microscopy using a spinning disc confocal microscope. Frames were acquired every 20 sec for at least 20 min and are shown as a projection of five 1 μm Z-slices at 18 frames/sec. Bar, 20 μm.

MovieS3. Colocalization of *drongo* and *osk* mRNA in a wild type oocyte. A cocktail of dH1111-Cy3 (*drongo*-specific) (red) and o2209-Cy5 (*osk*-specific) (green) molecular beacons was microinjected in the oocyte of a stage 8-9 wild type egg chamber. Images were analyzed by time-lapse confocal microscopy using a spinning disc confocal microscope. Frames were acquired every minute for at least 18 min and are shown as a projection of nine 1 μm Z-slices at 6 frames/sec. Bar, 20 μm.

MovieS4. Colocalization of *drongo* mRNA and Stau in live GFP-Stau oocytes. dH1598-AF647 (red) *drongo*-specific molecular beacon was microinjected in the nurse cell adjacent to the oocyte of a *GFP-Stau/Cyo* (green) egg chamber. Images were analyzed by time-lapse confocal microscopy using a spinning disc confocal microscope. Frames were acquired every minute for at least 65 min and are shown as a projection of twelve 1 μm Z-slices at 6 frames/sec. Bar, 20 μm.

MovieS5. Localization of *drongo* mRNA in an *armi* mutant oocyte. dH1598-AF647 *drongo*-specific molecular beacon was microinjected in the nurse cell adjacent to the oocyte of an *armi* mutant egg chamber. Images were analyzed by time-lapse confocal microscopy using a spinning

disc confocal microscope. Frames were acquired every minute for at least 53 min and are shown as a projection of ten 1 μm Z-slices at 6 frames/sec. Bar, 20 μm.

MovieS6. Transport and localization of Drongo-EGFP in YFP-Rab11 backgrounds. Drongo-EGFP particles were detected in wild type, YFP-Rab11DN and YFP-Rab11CA mutant oocytes. Images were acquired by time-lapse confocal microscopy using a spinning disc confocal microscope. Frames were acquired every 20 sec for at least 30 min and are shown as a projection of thirteen 0.5 μm Z-slices at 18 frames/sec. Bar, 20 μm.

MovieS7. Morphology of egg chambers coexpressing Drongo-EGFP and YFP-Rab11DN. Drongo-EGFP (green) and YFP-Rab11DN were coexpressed with the V2H-GAL4 driver. Images are the result of stitching of three Z-stacks with 46 individual slices of 0.5 μm each and are shown as 3 Z-stacks/sec. Z-stacks were acquired using a spinning disc confocal microscope. F-actin (red) was highlighted using fluorescently labeled phalloidin. Inset shows one Z-slice of an F-actin stain using egg chambers expressing YFP-Rab11DN alone using the same GAL4 driver. s=stage. Bar, 20 μm.