

Fig. S1. GG detection in in-vivo centrifuged syncytial embryos. A, A') Glycogenin-YFP expressing embryo. A = bright field image; expected location of organelles indicated on the left, according to (Cermelli et al. 2006) and this study. The least dense fraction (the refractive lipid droplet cap) is at the top; the densest fractions (i.e., the refractive yolk vesicles and clear glycogen) are at the bottom. Scale bar = 50 μ m. A' = merged fluorescent image of the same embryo showing Glycogenin-YFP (yellow) at the very bottom and YV autofluorescence (blue) in the layer above). B,C) Centrifuged wild-type (B) and Jabba mutant (C) embryos and stained for glycogen (fPAS, red) and LDs (BODIPY, cyan). In the wild type, glycogen and LDs are present at opposite poles. In the *Jabba* mutant embryo, LD and glycogen are comingled.

Supplementary Figure 2



Fig. S2. Glycogen distribution at various embryonic stages. A) Glycogenin-YFP at different stages showing the clustering of glycogen granules and exclusion of glycogen from the pole plasm/pole cells (arrowhead). Left: YFP channel; right: corresponding brightfield image. B, C) fPAS staining of wild-type embryos. B) At the onset of germ band extension, fPAS signal is dimming and predominantly present the yolk cell. C) At the end of germ band extension (>7 hrs post fertilization), fPAS staining is undetectable. D) Glycogenin-YFP embryo during dorsal closure (~12 hours post fertilization). YFP signal is very faint and present in the yolk cell. Signal appears brighter at the periphery due to optical sectioning. Images taken by confocal microscopy. E) Time course of fPAS staining in wild-type and *Jabba* mutant embryos imaged using epifluorescence. The middle panels are at later germ band extension like that shown in panel C. We notice no differences between the genotypes. All scale bars = 100 µm.

Supplementary Figure 3



Wild type

Jabba -/-

120s



Fig. S3. Jabba uniquely prevents inappropriate interaction between LDs and GGs

ensuring LD motility. A,B) TEM images of newly laid wild-type (A) and *Jabba* mutant (B) embryos. GGs pseudo colored red; lipid droplets pseudo colored green. Scale bars = 2 μ m. C) Embryos of different genotypes stained for LDs (BODIPY493/503, cyan) and glycogen (fPAS). In *Stur, klar, and LSD2/dPLIN2* null embryos, LDs are not associated with GGs. An embryo with reduced *Jabba* dosage displays partial association. D) Live imaging of Stage 2 wild-type and *Jabba* mutant embryos injected with BODIPY493/503 (cyan) to track LD motion. Arrowheads track selected LDs through time. The direction of the cytoplasmic flow is indicated by the black arrow. E) Stage 2 wild-type and *Jabba* mutant embryos injected with LipidSpot 610 to label LDs (magenta)and co-imaged for YV autofluorescence (blue). In the *Jabba* mutant embryo, the LDs rings are not around YVs. C-E) Scale bars = 10 μ m; images recorded by confocal microscopy. F) cartoon showing the gross directions of the flowing cytoplasm during Stages 1-3.

Supplementary Figure 4

Wild type



Jabba -/-



Fig. S4. Jabba prevents inappropriate interaction between LDs and GGs. A) TEM cross section of < 1hr old wild-type embryo. B) TEM cross section of a < 1-hour old *Jabba* mutant embryo, note the LDs bound to GG and the lack of LDs free in the cytoplasm. Scale bars = $20 \mu m$.



Movie 1. Wild-type embryo injected with BODIPY493/503 (displayed in inverted greyscale) to label LDs. The embryo is imaged in a subcortical plane. The video starts in Stage 1-2 (<1 hour post fertilization) and captures 30 minutes real time. Scale bar is 100 μm.



Movie 2. Wild-type embryo injected with BODIPY 493/503 to label LDs (shown in greyscale). The embryo is imaged at 40 μm below the subcortical plane. The video captures about 90minutes real time. Scale bar is 100 μm.



Movie 3. Glycogenin-YFP embryo with YFP in greyscale. The embryo is imaged in a subcortical plane. The video starts in Stage 1-2 and captures 20 minutes real time. Scale bar is 100 µm.



Movie 4. Glycogenin-YFP embryo with YFP in greyscale. The embryo is imaged in a subcortical plane. The video starts in Stage 1-2 (<1 hour post fertilization) and captures 2 hours real time. Scale bar is 100 µm.



Movie 5. *Jabba* mutant embryo injected with BODIPY 493/503 (displayed in inverted greyscale) to label LDs. The embryo is imaged in a subcortical plane. The video starts in Stage 1-2 (<1 hour post fertilization) and captures 30 minutes real time. Scale bar is 100 μm.



Movie 6. *Jabba* mutant embryo injected with BODIPY 493/503 to label LDs (shown in greyscale). The embryo is imaged at 40 μm below the subcortical plane. The video captures about2 hours real time. Scale bar is 100 μm.