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# SUPPLEMENTARY MATERIAL

**corresponding to:**

# Cyto-architecture constrains the spread of photoactivated tubulin in the syncytial *Drosophila* embryo

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**Movie S1. Cytoplasmic GFP.** *GFP expressed under the* ubiquitin *promoter is imaged across the syncytial division cycles. Note that GFP enters the nuclei in interphase.*

**Movie S2. mCherry-Tubulin.** *mCherry-Tubulin expressed with* mat-*Gal4 is imaged in the syncytial division cycles. Note mCherry-Tubulin incorporation into centrosome, spindle and cortical microtubules.*

**Movie S3. PA-GFP anterior photoactivation.** *Region of interest at the anterior is photoactivated to create a source of PA-GFP. Note that PA-GFP enters the nuclei in interphase.*

**Movie S4. PA-GFP-Tubulin anterior photoactivation.** *Region of interest at the anterior is photoactivated to create a source of PA-GFP-Tubulin. Note PA-GFP-Tubulin incorporation into centrosome, spindle and cortical microtubules.*

**Movie S5. PA-GFP middle photoactivation.** *Region of interest in the middle of the embryo is photoactivated to create a source of PA-GFP.*

**Movie S6. PA-GFP-Tubulin middle photoactivation.** *Region of interest in the middle of the embryo is photoactivated to create a source of PA-GFP-Tubulin.*

**Movie S7. PA-GFP anterior photoactivation in RhoGEF2-OE embryos.** *Region of interest at the anterior is photoactivated to create a source of PA-GFP in RhoGEF2-OE embryos.*

**Movie S8. PA-GFP-Tubulin anterior photoactivation in RhoGEF2 mutants.** *Region of interest at the anterior is photoactivated to create a source of PA-GFP-Tubulin in RhoGEF2-OE embryos.*

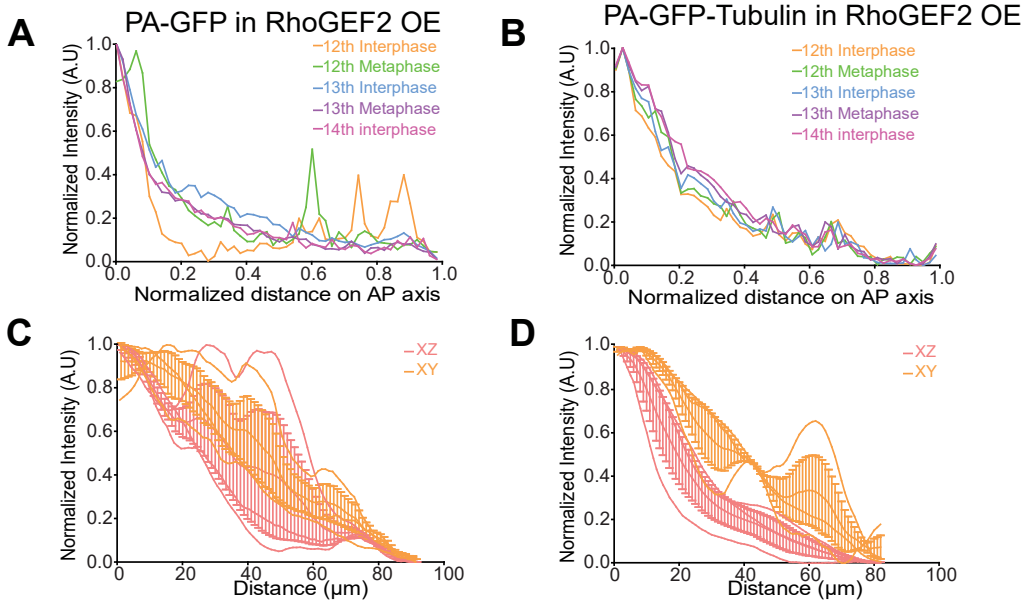
**Movie S9. mCherry-Tubulin in control embryos.** *Live imaging of mCherry-Tubulin embryos showing a single optical plane. Note the mitotic cycles and regular spacing of nuclei and spindles.*

**Movie S10. mCherry-Tubulin in *eb1* RNAi expressing embryos.** *Live imaging of mCherry-Tubulin in EB1 mutant embryo showing a single optical plane. Note the presence of aberrant spindles and non-uniform nuclear spacing.*

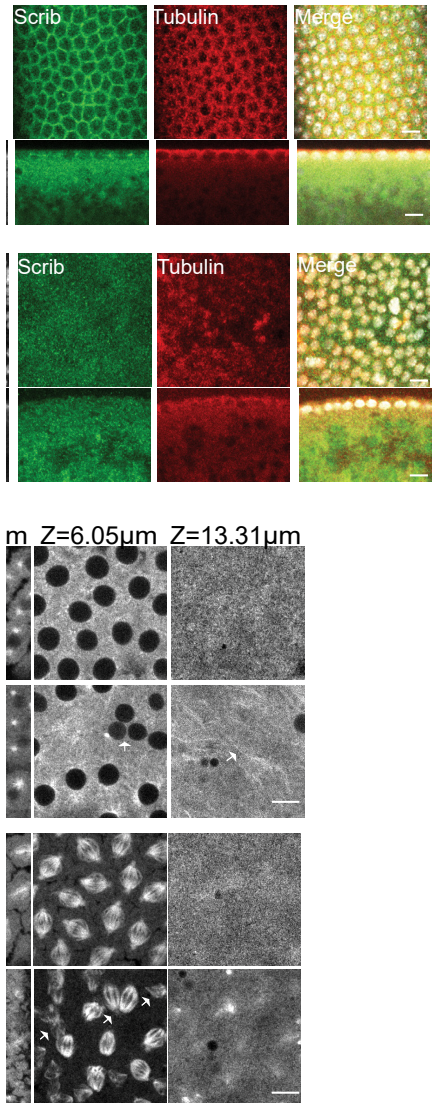
**Movie S11. PA-GFP anterior photoactivation in *eb1* mutant embryos.** *Region of interest at the anterior is photoactivated to create a source of PA-GFP in* eb1 *RNAi expressing embryos.*

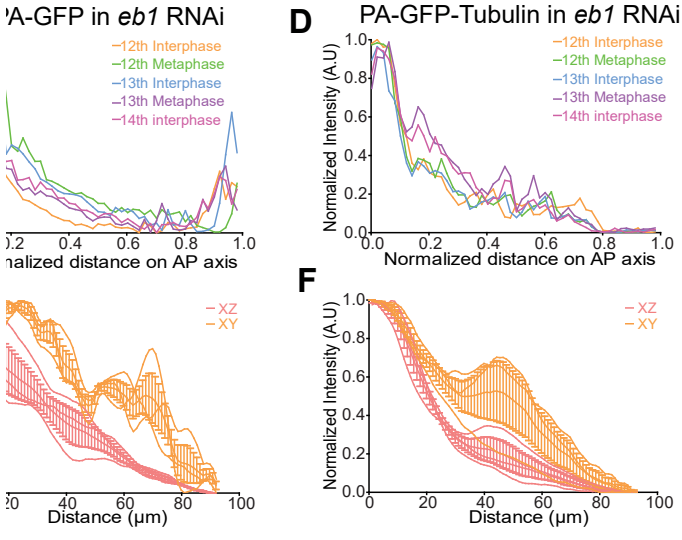
**Movie S12. PA-GFP-Tubulin anterior photoactivation in *eb1* mutant embryos.** *Region of interest at the anterior is photoactivated to create a source of PA-GFP-Tubulin in* eb1 *RNAi expressing embryos. Note the undulations caused by yolk contractions and that the cytoplasm remains peripheral, without mixing with the embryo yolk region.*

All movies are in shown in 16 color intensity rainbow scale where blue represents the lowest intensity and red represents the highest intensity. Scale bar, 10 μm or 50 μm as mentioned.




**Supp. Fig. S1. Analysis of photoactivation in embryos overexpressing RhoGEF2. (A,B)** *Quantification of evolution of the photoactivation across nuclear cycles in embryos overexpressing RhoGEF2. A line profile was drawn in syncytial cycles is plotted for PA-GFP* **(A)** *and PA-GFP-Tubulin* **(B)** *for one embryo. Similar profiles were observed in multiple embryos (n=3 for each).* **(C,D)** *Quantification of extent of photoactivation in XY vs XZ direction for PA-GFP* **(C)** *and PA-GFP-Tubulin* **(D)** *in embryos overexpressing RhoGEF2. The raw data is shown in a lighter color and the averaged data is shown in a darker color, error bars represent standard error on means. (n=3 embryos for PA-GFP and PA-GFP-Tubulin each).*




**Supp. Fig. S2. Analysis of photoactivation and morphological defects in spindles in *eb1* RNAi expressing embryos. (A)** eb1 *RNAi expressing embryos show tubulin cytoskeletal defects: Surface and sagittal views of fixed control or* eb1 *RNAi embryos in interphase, stained with Tubulin (Red), Scribbled (Green) and DNA (Grey), show lowered interphase tubulin and scrib staining (100%, n=25 embryos). Scale bar, 10 µm.* **(B)** *Interphase images are shown from different Z-stacks of embryos expressing maternally expressing mCherry-Tubulin in a control or an* eb1 *RNAi background (similar trends were observed for n=3 movies). Regular spacing of nuclei is disturbed and fused nuclei can be seen. Long tubulin fibers are also observed below the region containing nuclei. Scale bar=10µm. Metaphase images are shown from different Z-stacks of embryos expressing maternally expressing mCherry-Tubulin in a control or an* eb1 *RNAi background (similar trends were observed for n=3 movies). Weakly fluorescent spindles (left of the image) and tripolar spindles (right of the image) can be observed in this stage. Scale bar=10µm.* **(C,D)** *Quantification of evolution of photoactivated signal across nuclear cycles in* eb1 *RNAi embryos. Graph depicts intensity change in PA-GFP* **(C)** *and PA-GFP-Tubulin* **(D)** *for one embryo. Similar profiles were observed in multiple embryos (n=3 for each).* **(E,F)** *Quantification of the extent of photoactivated probe spread in XY vs XZ direction for PA-GFP* **(E)** *and PA-GFP-Tubulin* **(F)** *in* eb1 *RNAi embryos. The raw data is shown in a lighter color and the averaged data is shown in a darker color, error bars represent standard error on means (n=3 embryos for PA-GFP and PA-GFP-Tubulin each).*