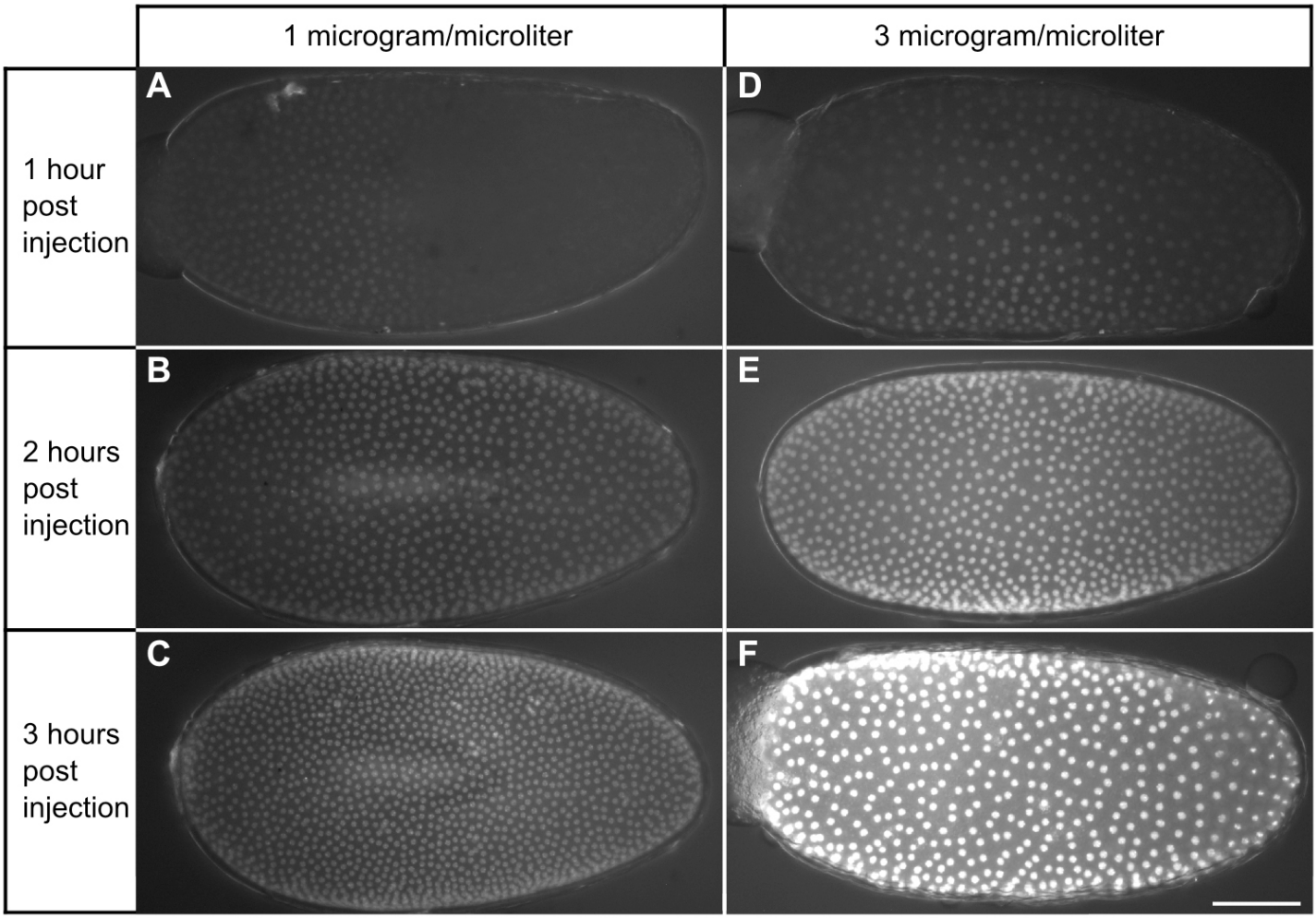


Fig. S1. Fluorescent protein expression in transiently labeled *Tribolium* embryos. (A-F) Embryos injected with mRNA encoding H2B-RFP (A-C) at a concentration of 1 µg/µl, or (D-F) at a concentration of 3 µg/µl, shown at different times after injection. Representative embryos for each condition and time-point were imaged on an epifluorescence microscope using identical settings. Injection of the high mRNA concentration consistently produces a stronger fluorescent signal than the low concentration. (**A**,**D**) One hour post injection, weak fluorescence is detected in a fraction of preblastoderm nuclei. (**B**,**E**) A more homogeneous and stronger fluorescence is detected 2 hours post injection. (**C**,**F**) Three hours post injection, ubiquitous, uniform and strong fluorescence is detected in all nuclei across the entire embryo. All images were captured at multiple focal planes that were combined into a single focused image using the Helicon Focus software. Anterior is towards the left. Scale bar: 100 µm.

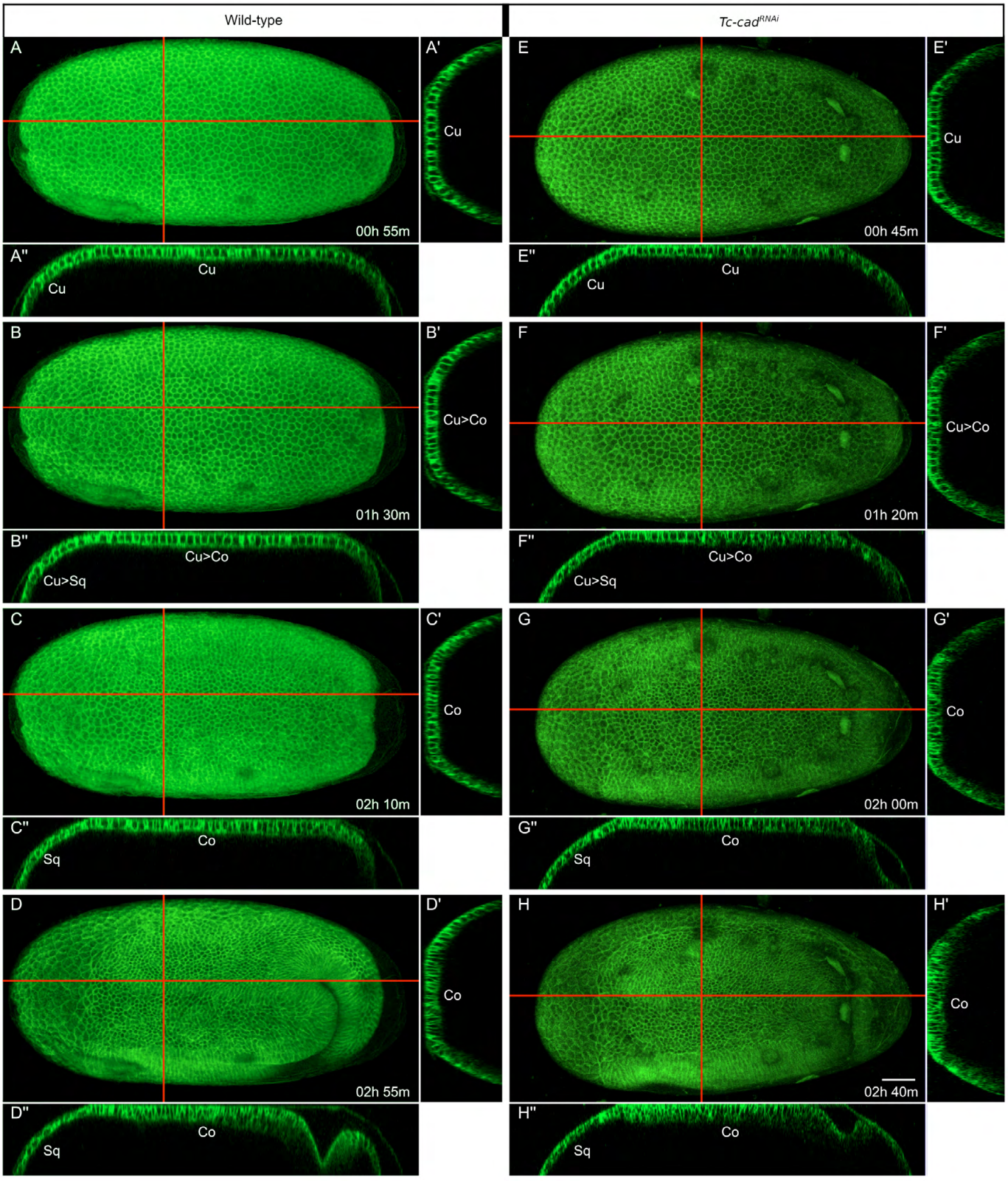


Fig. S2. Cell shape changes during *Tribolium* embryogenesis. (A-H″) Ventrolateral views and cross-sections of GAP43-YFP-labeled (A-D″) wild-type embryo also shown in Fig. 4A-E, and (E-H″) *Tc-cad*^{RNAi} embryo also shown in Fig. 4K-O. (A-H) Average intensity projections. (A′-H′) *YZ* transverse sections. (A″-H″) *XZ* sagittal sections at representative time-points spanning, from top to bottom, the first three stages of *Tribolium* embryogenesis. At each time-point, cells exhibit very similar morphologies in wild-type and *Tc-cad*^{RNAi} embryos. (**A-A″**,**E-E″**) At the end of stage 1, the uniform blastoderm is composed of cuboidal cells (Cu). (**B-B″**,**F-F″**) In the differentiated blastoderm at the end of stage 2, the serosa cells anteriorly start adopting a squamous shape (Cu>Sq) and the rest of the embryonic cells adopt a columnar shape (Cu>Co). (**C-D″**,**G-H″**) During stage 3, the squamous shape of serosa cells (Sq) and the columnar shape of embryonic cells (Co) become progressively more pronounced. In the average intensity projections, anterior is towards the left; in the *YZ* sections, apical is towards the left; in the *XZ* sections, anterior is towards the left and ventral towards the top. Scale bar: 50 µm.

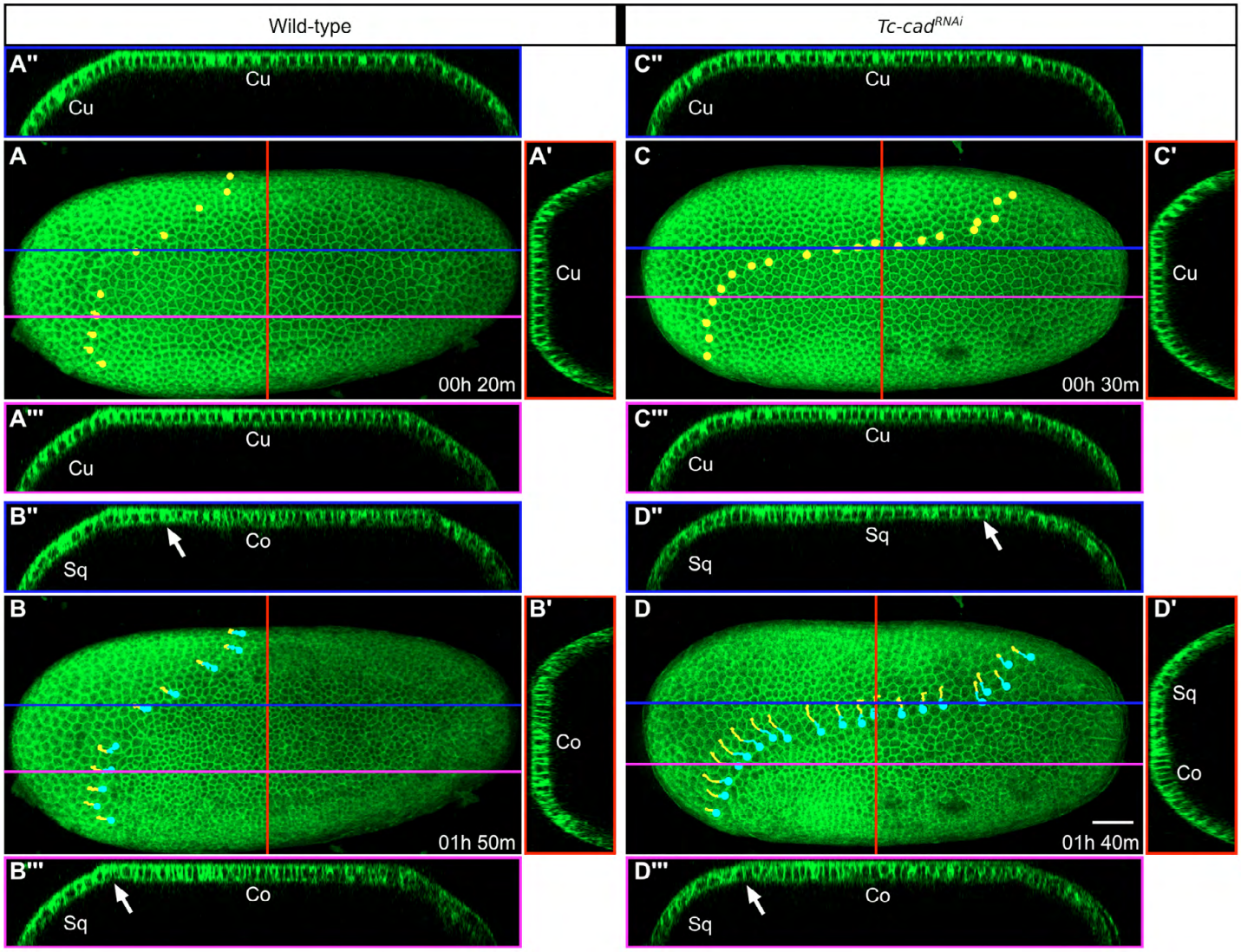


Fig. S3. Differential distribution of serosal and embryonic cells in wild-type and *Tc-cad*^{RNAi} differentiated blastoderms. (A-D‴) Lateral views and cross-sections of GAP43-YFP-labeled (A-B‴) wild-type embryo (also shown in Fig. 4F-J) and (C-D‴) *Tc-cad*^{RNAi} embryo (also shown in Fig. 4P-T). (A-D) Average intensity projections. (A′-D′) *YZ* transverse sections. (A″-D″) *XZ* frontal sections in the dorsal half. (A‴-D‴) *XZ* frontal sections in the ventral half of the blastoderm. The top panels show uniform blastoderm stage embryos at the beginning of stage 1; bottom panels show differentiated blastoderm stage embryos at the end of stage 2. Yellow and cyan dots in the average intensity projections mark the serosa cells at the border between the serosa and embryonic primordium. (**A-A‴**) All cells appear uniform and cuboidal (Cu) in shape. (**B-B‴**) The anterior dorsally tilted cap of serosa cells start adopting a squamous shape (Sq) and the more posterior embryonic cells a columnar shape (Co). The border between flattening serosa and elongating embryonic cells (arrows in B″ and B‴) is slightly more posterior in the dorsal half compared with the ventral half. The transverse section in the middle of the embryo in B′ shows that all cells along the dorsal-ventral axis exhibit a uniform shape (Co). The leading serosa cells move primarily posteriorly. (**C-C‴**) After *Tc-cad* knock-down, the uniform blastoderm is composed of cuboidal (Cu) cells, as in wild-type controls. (**D-D‴**) The serosa primordium covers most of the dorsal half of the differentiated blastoderm and the embryonic primordium is restricted to the ventral half. The border between flattening serosa and elongating embryonic cells (arrows in D″ and D‴) is much more posterior in the dorsal half compared with the ventral half. The transverse section in the middle of the embryo in D′ shows a marked difference in shape between serosa cells dorsally (Sq) and embryonic cells ventrally (Co). The most anterior serosa cells move posteriorly, but all other serosa cells move primarily ventrally. Anterior is towards the left in the average intensity projections and in the *XZ* sections; apical is towards the left in the *YZ* sections. Scale bar: 50 µm.

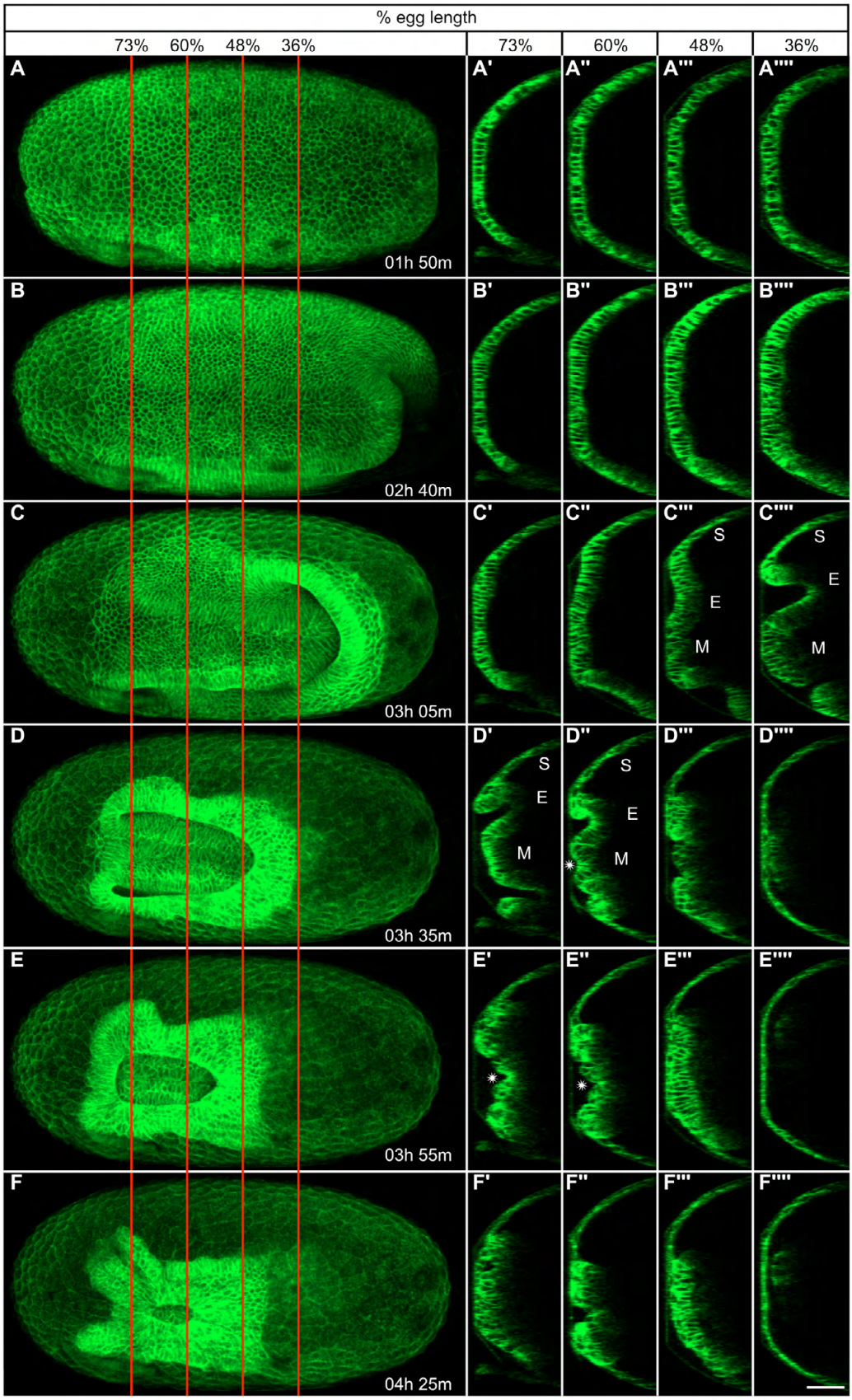


Fig. S4. Germband condensation and gastrulation in *Tribolium* wild-type embryo. (A-F‴′) Ventrolateral views and transverse *YZ* sections of a GAP43-YFP-labeled wild-type embryo (also shown in Fig. 4A-E). (**A-F**) Average intensity projections. (**A′-F′**) Transverse sections at 73% EL. (**A″-F″**) Transverse sections at 60% EL. (**A‴-F‴**) Transverse sections at 48% EL. (**A‴′-F‴′**) Transverse sections at 36% EL at representative time-points spanning, from top to bottom, stages 2 to 5 of *Tribolium* embryogenesis. The characteristic bottle shape of apically constricting mesodermal cells and ventral furrow formation are visible in anterior but not in posterior germband regions (compare C‴′ with D″). M, internalizing mesoderm; E, ectoderm; S, serosa; *, ventral furrow. Scale bar: 50 µm.

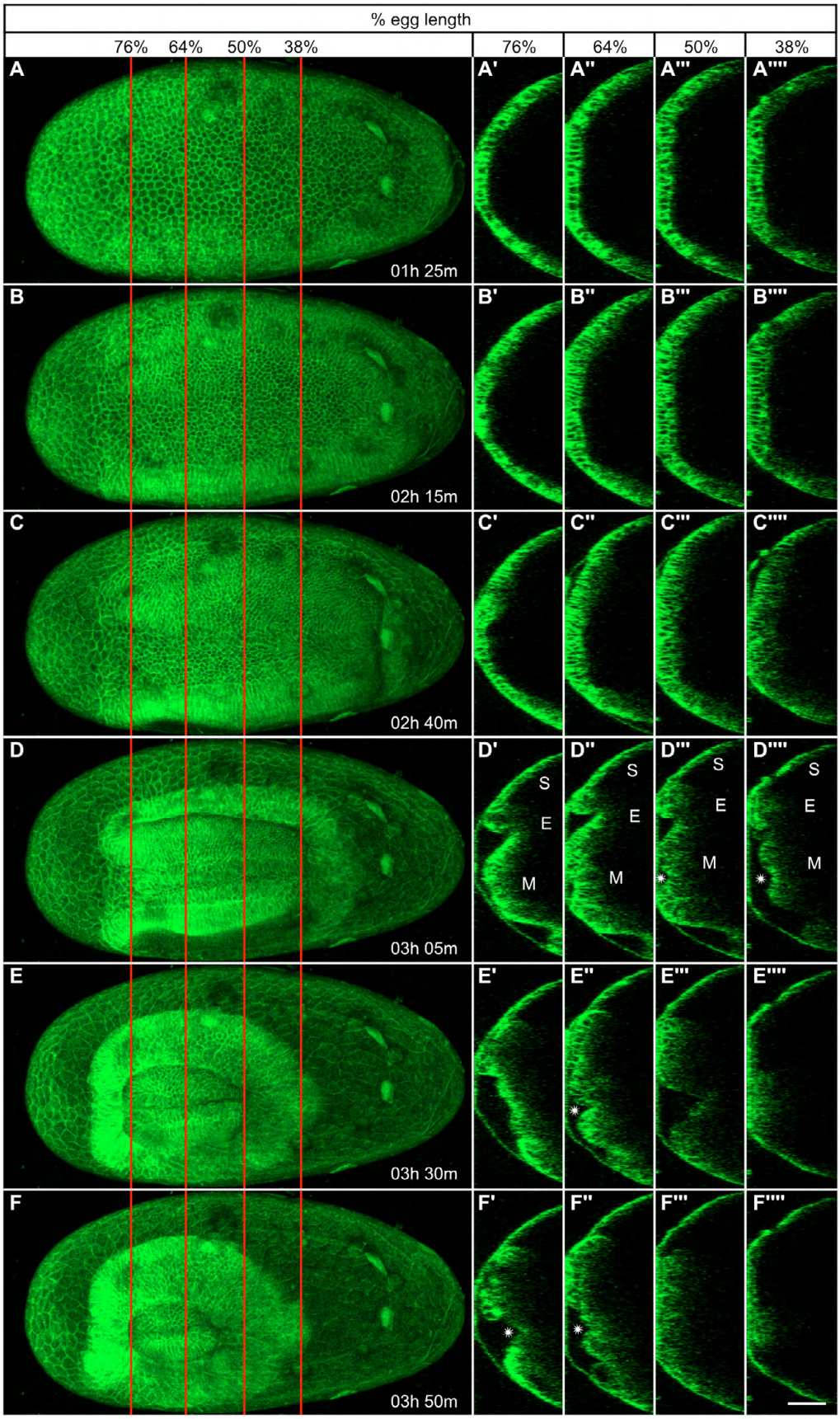


Fig. S5. Germband condensation and gastrulation in *Tribolium Tc-cad*^{RNAi} embryo. (A-F‴′) Ventrolateral views and transverse *YZ* sections of a GAP43-YFP-labeled *Tc-cad*^{RNAi} embryo also shown in Fig. 4K-O. (**A-F**) Average intensity projections. (**A′-F′**) Transverse sections at 76% EL. (**A″-F″**) Transverse sections at 64% EL. (**A‴-F‴**) Transverse sections at 50% EL. (**A‴′-F‴′**) Transverse sections at 38% EL at representative time-points spanning, from top to bottom, stages 2 to 5 of *Tribolium* embryogenesis. The apically constricting mesodermal cells and the ventral furrow are visible along the entire length of the truncated germband (see D′-D‴′). M, internalizing mesoderm; E, ectoderm; S, serosa; *, ventral furrow. Scale bar: 50 µm.

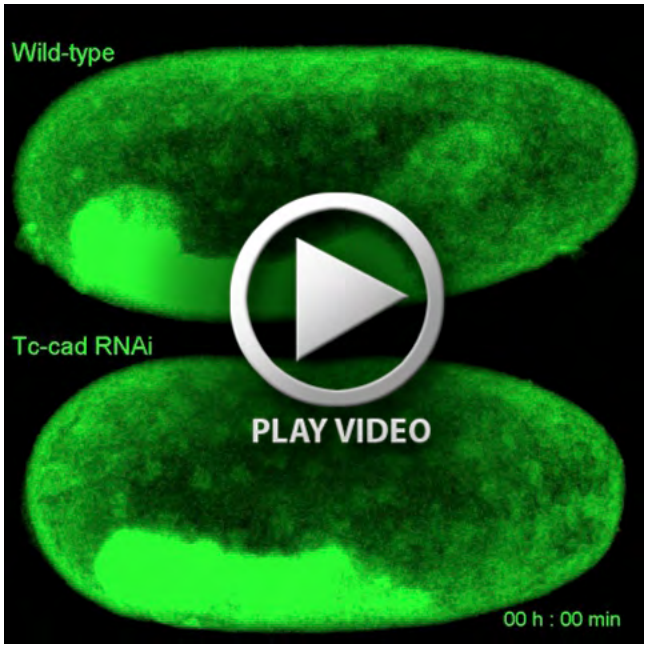
Movie 1. Confocal imaging of the 13th round of cell divisions during *Tribolium* blastoderm differentiation. Fluorescence time-lapse recording of a *Tribolium* embryo labeled with H2B-RFP also shown in Fig. 3E-E‴. The movie covers 85 minutes of *Tribolium* embryogenesis (approximately 9.5-11 hours AEL at 32°C) recorded at 5-minute intervals on a laser scanning confocal microscope using a 20× objective. In each time-point, the movie shows an average intensity projection of 44 focal planes scanned every 3 µm. Lateral view, anterior is towards the left and dorsal towards the top.

Movie 2. Confocal imaging of wild-type *Tribolium* embryogenesis (ventrolateral view). Fluorescence time-lapse recording of a *Tribolium* wild-type embryo labeled with GAP43-YFP (also shown in Fig. 4A-E). The embryo was recorded at 5-minute intervals on a laser scanning confocal microscope using a 20× objective. The movie covers 5 hours 35 minutes of *Tribolium* embryogenesis (~8.5-14 hours AEL at 32°C) spanning the six stages described in the text. In each time-point, the movie shows an average intensity projection of 43 focal planes scanned every 3 µm. The position of tracked cells is indicated with yellow dots; cell tracks are not included to allow visualization of the embryo. Ventrolateral view, anterior is towards the left.

Movie 3. Confocal imaging of wild-type *Tribolium* embryogenesis (lateral view). Fluorescence time-lapse recording of a *Tribolium* wild-type embryo labeled with GAP43-YFP (also shown in Fig. 4F-J). The embryo was recorded at 5-minute intervals on a laser scanning confocal microscope using a 20× objective. The movie covers 5 hours 40 minutes of *Tribolium* embryogenesis (~8.5-14 hours AEL at 32°C) spanning the six stages described in the text. In each time-point, the movie shows an average intensity projection of 38 focal planes scanned every 3 µm. The position of tracked serosa cells is indicated with yellow dots; cell tracks are not included to allow visualization of the embryo. Lateral view, anterior is towards the left and dorsal towards the top.

Movie 4. Confocal imaging of *Tribolium* embryogenesis after *Tc-cad* RNAi (ventrolateral view). Fluorescence time-lapse recording of a *Tribolium Tc-cad*^{RNAi} embryo labeled with GAP43-YFP (also shown in Fig. 4K-O). The embryo was recorded at 5-minute intervals on a laser scanning confocal microscope using a 20× objective. The movie covers 3 hours 50 minutes of *Tribolium* embryogenesis (~8.5-12.5 hours AEL at 32°C) spanning stages 1 to 5 described in the text. In each time-point, the movie shows an average intensity projection of 40 focal planes scanned every 3 µm. The position of tracked cells is indicated with yellow dots; cell tracks are not included to allow visualization of the embryo. Ventrolateral view, anterior is towards the left.

Movie 5. Confocal imaging of *Tribolium* embryogenesis after *Tc-cad* RNAi (lateral view). Fluorescence time-lapse recording of a *Tribolium Tc-cad*^{RNAi} embryo labeled with GAP43-YFP (also shown in Fig. 4P-T). The embryo was recorded at 5-minute intervals on a laser scanning confocal microscope using a 20× objective. The movie covers 5 hours 20 minutes of *Tribolium* embryogenesis (~8.5-14 hours AEL at 32°C) spanning the 6 stages described in the text. At each time-point, the movie shows an average intensity projection of 41 focal planes scanned every 3 µm. The position of tracked serosa cells is indicated with yellow dots; cell tracks are not included to allow visualization of the embryo. Lateral view, anterior is towards the left and dorsal to the top.



Movie 6. Confocal imaging of *Tribolium* germband extension in wild-type and *Tc-cad*^{RNAi} embryos. Combination of two fluorescence time-lapse recordings of *Tribolium* wild-type (top) and *Tc-cad*^{RNAi} (bottom) embryos labeled with GAP43-YFP also shown in Fig. 5. The embryos were recorded at 5-minute intervals on a laser scanning confocal microscope using a 20x objective. The movie covers 2 hours 40 minutes of *Tribolium* embryogenesis (~14.5-17 hours AEL at 32°C) starting at the beginning of the sixth stage described in the text. At each time-point, the movie shows average intensity projections with enhanced brightness/contrast to show the germbands and the membrane-bound yolk spheres. Lateral views, anterior is towards the left and dorsal towards the top.

Movie 7. DIC/confocal imaging of *Tribolium* germband condensation and elongation relative to yolksac dynamics. Combination of differential interference contrast microscopy with fluorescence confocal microscopy for time-lapse recording of a *Tribolium* embryo labeled with H2B-RFP (also shown in Fig. 7). The posterior half of the embryo was recorded at 2.5-minute intervals using a 40× objective. The movie covers 8 hours 40 minutes of *Tribolium* embryogenesis (~8.5-17 hours AEL at 32°C) spanning the six stages described in the text up to the extended germband stage. In each time-point, the movie shows the DIC image of the embryo (left panel), as well as the DIC image overlaid with the corresponding H2B-RFP fluorescence signal in blue (right panel). The dot indicates the position of the leading edge of the posterior yolk-fold. The white track marks the early ventral extension of the yolk-fold and the grey track marks its dorsal retraction up to the corresponding time-point. Lateral views, anterior is towards the left and dorsal to the top.

Table S1. Oligos used in this study

Oligo name	Oligo Sequence (5′>3′)
Dmel_H2B_F_NcoI	TTAACCATGGCTCCGAAAACTAGTGGAAAG
Dmel_H2B_R_XhoI	ACTTCTCGAGTTTAGAGCTGGTGTACTTGG
mRFPruby_F_XhoI	ACAACTCGAGATGGGCAAGCTTACC
mRFPruby_R_PspMOI	TATTGGGCCCTTAGGATCCAGCGCCTGTGC
EGFP_F_BamHI	GTCAGGATCCTCGCCACCAGATCCATGGTGAGCAAGGGCGA
EGFP_R_NotI	ATATGCGGCCGCTTACTTGTACAGCTCGTCC
Tcas_Cad_F	ACTACAACTCGACCAACA
Tcas_Cad_R	GAAGAAGCAACAAGAAGGCA

Table S2. Overview of *Tribolium* embryo injection and transient fluorescence labeling

Treatment	Number of embryos injected	Number of embryos survived 24 hpi	Number of fluorescent embryos			Number of embryos with uniform fluorescence	
			1 hpi	2 hpi	3 hpi	3 hpi	6 hpi
Non-injected	200	156	0	0	0	0	0
Buffer-injected	200	159	0	0	0	0	0
H2B-RFP	200	153	103	186	196	121	N/A
GAP43-YFP	200	122	192	196	197	92	N/A
H2B-RFP/ GAP43-YFP	200	148	N/A	N/A	N/A	N/A	N/A
LA-GFP	200	126	N/A	N/A	140	N/A	140
ABP-tdEosFP[2]	200	119	N/A	N/A	128	N/A	0*

*ABP-tdEosFP produced heterogeneous fluorescent patterns, probably due to the binding properties of the protein in *Tribolium*. A bright spot of fluorescence was always visible near the injection site (either in the yolk or cortical layer) that diminished over time.
hpi, hours post injection.

Table S3. Relationship of our staging system to previously published staging systems of *Tribolium* embryogenesis

***Tribolium* embryogenesis staging systems**	**Authors' nomenclature**	**Relationship to our staging system**
Handel et al., 2000	7-9.5 hrs AEL	Stage 1
	9-11.5 hrs AEL	Stage 2
	11-12.5 hrs AEL	Stage 3
	12-13.5 hrs AEL	Stage 4
Sarrazin et al., 2012	3.II	Stage 3
	3.III	Stage 4
	4.I to 5.I	Stage 5
El-Sherif et al., 2012	B0	N/A
	B1 to B6	Stage 1
	B7 to B8	Stage 2
	B9	Stage 3
	G1 to G4	Stages 4 to 5

AEL, time after egg lay
Study carried out with embryos developing at 30°C.