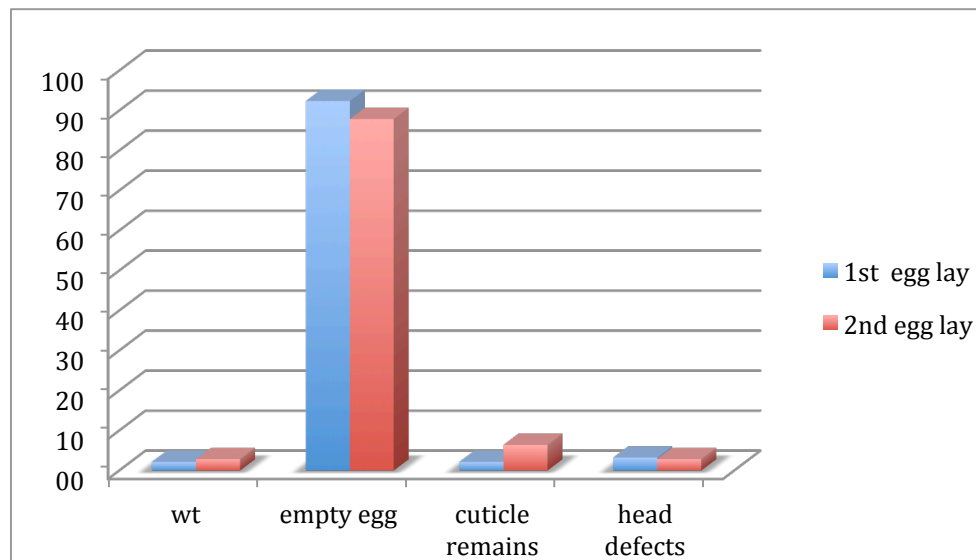


## Supplementary Figures

**Figure S1: phenotypic analysis of *Tc-cic* RNAi experiments**

A)



RNAi phenotypes (%)	1st egg lay	2nd egg lay
wt	2,2	2,9
empty egg	92,4	87,9
cuticle remains	2,2	6,4
head defects	3,3	2,9

B)

RNAi (n)	1st egg lay	2nd egg lay
wt	2	4
empty egg	85	123
cuticle remains	2	9
head defects	3	4
total	92	140

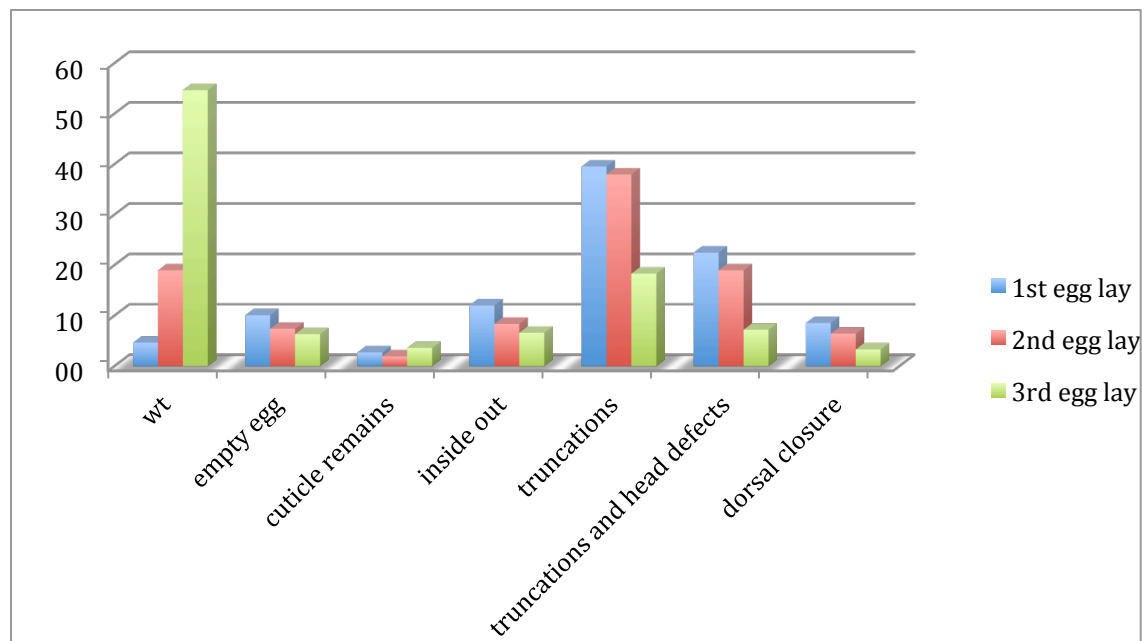
Summary of RNAi experiments. Percentages (A) and numbers (B) of the observed phenotypic defects obtained with RNAi treatments using *Tc-cic* dsRNA (1µg /µl).

Figure S1: Phenotypic analysis of *Tc-cic* RNAi experiments

Summary of RNAi experiments. Percentages (A) and numbers (B) of the observed phenotypic defects obtained with RNAi treatments using *Tc-cic* dsRNA (1µg /µl). Eggs were collected between the 10th and 13th day (1. egg lay) and 13th and 16th (2. egg lay) day post injection.

**Figure S2: phenotypic analysis of *Tc-mael* RNAi experiments**

A)



RNAi phenotypes (%)	1st egg lay	2nd egg lay	3rd egg lay
wt	4,7	19,0	54,7
empty egg	10,1	7,4	6,3
cuticle remains	2,7	1,9	3,6
inside out	12,0	8,4	6,6
truncations	39,5	37,9	18,3
truncations and head defects	22,5	19,0	7,2
dorsal closure	8,5	6,4	3,3

B)

RNAi Phenotypes (n)	1st egg lay	2nd egg lay	3rd egg lay
wt	12	59	182
empty egg	26	23	21
cuticle remains	7	6	12
inside out	31	26	22
truncations	102	118	61
truncations and head defects	58	59	24
dorsal closure	22	20	11
total	258	311	333

Summary of RNAi experiments. Percentages (A) and numbers (B) of the observed phenotypic defects obtained with RNAi treatments using *Tc-mael* dsRNA (1µg/µl).

**Figure S2: phenotypic analysis of *Tc-mael* RNAi**

Summary of RNAi experiments. Percentages (A) and numbers (B) of the observed phenotypic defects obtained with RNAi treatments using *Tc-mael* dsRNA (1µg/µl). Eggs were collected between the 10th and 13th day (1. egg lay) and 13th and 16th (2. egg lay) day post injection.

## Table S1: Differential expression screen

Relative expression values for wildtype (T1), tsl RNAi (T2), and cic RNAi (T3) as revealed by SOLiD Next Generation Sequencing. In total, 12,795 genes showed differences in mRNA expression levels. All genes were categorized according to their expression values. We found 1801 genes to be up-regulated in Tc-tsl RNAi and down-regulated in Tc-cic RNAi (class: +/-) and 2790 genes that were up-regulated in Tc-cic RNAi but down-regulated in Tc-tsl RNAi (class: -/+). In addition, 2157 genes were up-regulated (class: ++) in both, Tc-cic and Tc-tsl RNAi and 4589 genes down-regulated (class: --) in both, Tc-cic and Tc-tsl RNAi. Some genes that were not expressed in wildtype became expressed after Tc-tsl (class: +0) or Tc-cic RNAi (class: 0+).

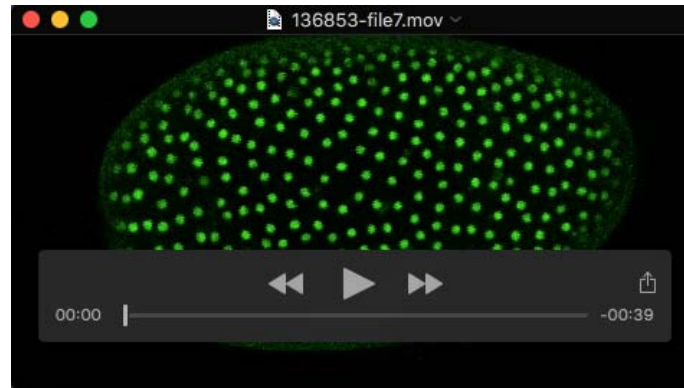
[Click here to Download Table S1](#)

## Table S2: Summary of the candidate-gene screen

50 genes were selected for closer analysis. Relative fold change (T2 or T3 divided by T1) in tsl RNAi (T2) or cic RNAi (T3) is shown in comparison to wildtype (T1) (tsl RNAi vs wt: T2:T1, cic RNAi vs wt: T3:T1) Larvae were scored for RNAi phenotypes. Hoechst and  $\beta$ -Tubulin staining were used to visualize early embryonic phenotypes ("early defects"). Expression of candidate genes was monitored by whole-mount in-situ hybridisation.

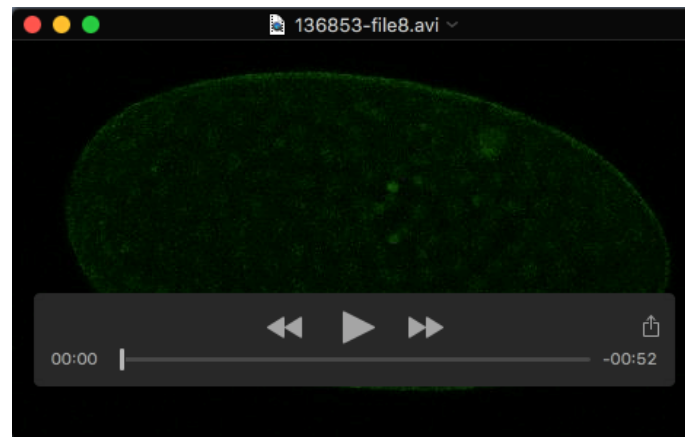
[Click here to Download Table S2](#)

## Supplementary Movies



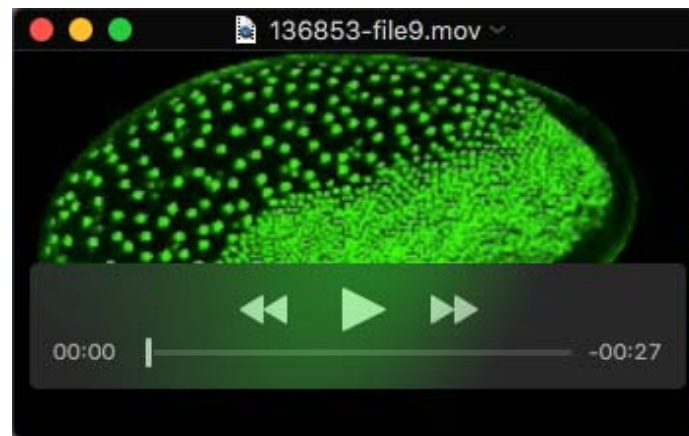
### Movie S1: Live imaging of early wildtype embryo

12 hours Time-lapse fluorescence recording of a *Tribolium* embryo expressing nuclear-localized GFP under a ubiquitous promoter (transgenic line EFA-nGFP). Stacks were recorded every 12 minutes at 10x magnification and 20°C. After synchronous cell divisions, the embryonic anlagen condense and give rise to germ-rudiment that progressively becomes covered by the extending serosa. The germ-band extends posteriorly, bending around the posterior pole. Anterior to the left.



### Movie S2: Live imaging of early *Tc-capicua* RNAi embryo

12 hours Time-lapse fluorescence recording of a *Tribolium* embryo expressing nuclear-localized GFP under a ubiquitous promoter (transgenic line EFA-nGFP). Stacks were recorded every 12 minutes at 10x magnification and 20°C. Upon *Tc-cic* depletion, the serosa anlagen expands at the expense of the anterior head anlagen. Eventually extraembryonic membranes cover the entire egg. Embryonic tissue is restricted to the posterior pole of the egg and becomes internalized completely. Anterior to the left.



### Movie S3: Live imaging of early *Tc-maelstrom* RNAi embryo

12 hours Time-lapse fluorescence recording of a *Tribolium* embryo expressing nuclear-localized GFP under a ubiquitous promoter (transgenic line EFA-nGFP). Stacks were recorded every 12 minutes at 10x magnification and 20°C. Upon *Tc-mael* RNAi, head anlagen are expanded and less condensed as compared to wildtype. Serosa window formation remains incomplete to some degree and RNAi posterior elongation fails. Eventually, the serosa ruptures premature. Anterior to the left.