SUPPLEMENTARY MATERIALS AND METHODS:

Fly Stocks: In addition to knock-in alleles listed in Table 1, the following stocks were used: *w; His2Av::mRFP (“Histone::RFP”, BL#23651); hs-FLP* w^{1118}*; adv*^{1}*/CyO* (BL#6); *hs-FLP* w^{1118}*; FRT-G13 His2Av::mRFP DE-Cad::GFP*; *ovo-FLP w (BL#8727); FRT-G13 ovoD*$^{1\text{-}18}$*/Dp(?;2)bw*^{D} *S*^{1} *wg*$^{Sp\text{-}1}$ *Ms(2)M*^{1} *bw*^{D}*/CyO* (BL#2125); *y*^{1} *w N*^{1}*/FM7c, twi-Gal4 UAS-2xGFP (“twi-GFP”* on X chromosome, BL#6873)*;* w^{1118}*; In(2LR)Gla, wg*$^{Gla\text{-}1}$*/Cyo, twi-Gal4 UAS-2xGFP (“twi-GFP”* on 2nd chromosome, BL#6662)*;* w^{1118}*; Dr*^{Mio}*/TM3, twi-Gal4 UAS-2xGFP (“twi-GFP”* on 3rd chromosome, BL#6663); *cn*^{1} *shg*^{2} *bw*^{1} *sp*^{1}*/CyO* (BL#3085); *y w sdt*^{XP1} *FRT-9-2/FM7c* (Hong et al., 2001); *y w; crb*^{11e22} */ TM3 Sb e* (gift from Dr. Knust (Klebes and Knust, 2000)).

Generation of larval mitotic recombinant clones and germline clones: For generating larval disc clones, virgin females of *hs-FLP w; FRT-G13 His2Av::RFP DE-Cad::GFP* were crossed with males of *y w/Y; FRT-G13 DE-Cad**. Parental flies were transferred every two days under 25 °C. At 72 and 96 hours after egg laying vials containing the first and second instar larvae after the transfer were heat-shocked in a 37°C water bath for 1.5 hours per day. Five to six days after egg laying heat-shocked third instar larvae were dissected for wing discs which were fixed for 1 hour in 4% formaldehyde for immunostaining. Maternal and zygotic mutant embryos were generated by germline clones (GLC) according to the published protocol (Chou and Perrimon, 1996) except that *ovo-FLP* was used in lieu of *hs-FLP*.

Antibodies: Primary antibodies: chicken anti-GFP (Aves Lab, cat# GFP-1010) 1:5000; home-made rabbit anti-GFP (Huang et al., 2009) 1:1500; mouse anti-RFP (Thermo Fisher Scientific, MA5-15257) 1:500; mouse anti-β-catenin (DSHB, N2 7A1) 1:100; guinea pig anti-Baz (Huang et al., 2009); mouse anti-Crb (DSHB, cq4-c) 1:10; rabbit anti-aPKC (Santa Cruz, Sc-216) 1:1000. Secondary antibodies: Cy2- , Cy3 or Cy5-conjugated goat anti-rabbit IgG, anti-mouse IgG, and anti-guinea pig IgG (The Jackson ImmunoResearch Lab, 111-225-003, 115-165-003, and 106-175-003), all at 1:400.

REFERENCES

Chou, T. B. and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in Drosophila melanogaster. *Genetics* **144**, 1673-1679.

Klebes, A. and Knust, E. (2000). A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in Drosophila. *Curr. Biol.* **10**, 76-85.

Genotypes of *Drosophila* Samples Presented in Figures:

Figure 1C:

"WT": *w^{1118};*

"S4D": *y w; FRT-G13 DE-CadS4D::GFP;*

"S4A-αCat": *y w; FRT-G13 DE-CadS4A::α-Cat::GFP;*

"ASSA": *y w; FRT-G13 DE-CadASSA::GFP;*

"WT-αCat": *y w; FRT-G13 DE-Cad::α-Cat::GFP;*

"ΔS-αCat": *y w; FRT-G13 DE-CadΔS::α-Cat::GFP;*

"S4A": *y w; FRT-G13 DE-CadS4A::GFP;*

"S5A": *y w; FRT-G13 DE-CadS5A::GFP;*

"ΔβS-αCat": *y w; FRT-G13 DE-CadΔβS::α-Cat::GFP;*

"KO": *y w; FRT-G13 DE-Cad^{KO}::GFP;*

"ΔβS": *y w; FRT-G13 DE-CadΔβS::GFP;*

"ΔS": *y w; FRT-G13 DE-CadΔS::GFP;*

Figure 1D:

hs-FLP w / y w (or *Y); FRT-G13 DE-Cad$^{\Delta\beta S}$-α-Cat::GFP / FRT-G13 His2Av::RFP; +/+.*

<u>*Cross:*</u> *hs-FLP w; FRT-G13 His2Av::RFP / CyO (X) y w / Y; FRT-G13 DE-CadΔβS::α-Cat::GFP / CyO twi-GFP (X)*

Figure 2A:

"WT": *hs-FLP w / y w* (or *Y); FRT-G13 DE-Cad::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"ASSA": *hs-FLP w / y w* (or *Y); FRT-G13 DE-CadASSA::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"S4D": *hs-FLP w / y w* (or *Y); FRT-G13 DE-CadS4D::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"S4A": *hs-FLP w / y w* (or *Y); FRT-G13 DE-CadS4A::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"KO": *hs-FLP w / y w* (or *Y); FRT-G13 DE-Cad^{KO}::GFP/ FRT-G1 His2Av::RFP DE-Cad::GFP;*

"ΔβS": *hs-FLP w / y w* (or *Y); FRT-G13 DE-CadΔβS::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"ΔS": *hs-FLP w / y w* (or *Y); FRT-G13 DE-CadΔS::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"ΔβS+LK": *hs-FLP w / y w* (or *Y); FRT-G13 DE-CadΔβS+LK::α-Cat::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

"2DN": *hs-FLP w / y w* (or *Y); FRT-G13 DE-Cad2DN::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

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Figure 2B:

"WT-αCat": *hs-FLP w / y w* (or *Y*)*; FRT-G13 DE-Cad::α-Cat::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*
"S4A-αCat": *hs-FLP w / y w* (or *Y*)*; FRT-G13 DE-CadS4A::α-Cat::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*
"ΔβS-αCat": *hs-FLP w / y w* (or *Y*)*; FRT-G13 DE-CadΔβS::α-Cat::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*
"ΔS-αCat": *hs-FLP w / y w* (or *Y*)*; FRT-G13 DE-CadΔS::α-Cat::GFP / FRT-G13 His2Av::RFP DE-Cad::GFP;*

Figure 2D: embryos were collected from the following stocks:
"WT": *y w; FRT-G13 DE-Cad::GFP*
"ASSA": *y w; FRT-G13 DE-CadASSA::GFP*
"S4A": *y w; FRT-G13 DE-CadS4A::GFP / CyO;*
"SDDS": *y w; FRT-G13 DE-CadSDDS::GFP*
"S4D": *y w; FRT-G13 DE-CadS4A::GFP*

Figure 3A and Figure S1A,B:
"WT": w^{1118};
"ASSA": *y w; FRT-G13 DE-CadASSA::GFP ;*
"S4D": *y w; FRT-G13 DE-CadS4D::GFP ;*
"S4A": *y w; FRT-G13 DE-CadS4A::*GFP^{GLC} *;(maternal and zygotic mutant embryo)*
"WT-αCat": *y w; FRT-G13 DE-Cad::α-Cat::GFP ;*
"S4A-αCat": *y w; FRT-G13 DE-CadS4A::α-Cat::GFP ;*

Figure 3B:
"WT": w^{1118};
"ASSA": *y w; FRT-G13 DE-CadASSA::GFP ;*
"WT-αCat": *y w; FRT-G13 DE-Cad::α-Cat::GFP ;*
"S4A-αCat": *y w; FRT-G13 DE-CadS4A::α-Cat::GFP ;*

Figure 3C:
"*WT +* crb^{KO}": (stage 12 embryo)
y w; FRT-G13 DE-Cad::GFP; crb^{KO}
Stock: *y w; FRT-G13 DE-Cad::GFP;* crb^{KO}*/TM3 twi-GFP*

"*ASSA* + *crb*^{KO}": (stage 10 embryo)
y w; FRT-G13 DE-CadASSA::GFP; crb^{KO}
Stock: *y w; FRT-G13 DE-CadASSA::GFP; crb*^{KO}*/TM3 twi-GFP*

"*ASSA* + *sdt*^{KO}": (stage 11 embryo)
w sdt^{KO}*/ Y; FRT-G13 DE-CadASSA::GFP*
Stock: *w sdt*^{KO}*/FM7c twi-GFP; DE-CadASSA::GFP*

"*WT-αCat* + *crb*^{KO}": (late stage 11 embryo)
y w; FRT-G13 DE-Cad-α-Cat::GFP; crb^{KO}
Stock: *y w; FRT-G13 DE-Cad-α-Cat::GFP; crb*^{KO} */ TM3 twi-GFP*

"*S4A-αCat* + *sdt*^{KO}": (late stage 11 embryo)
w sdt^{KO}*/ Y; FRT-G13 DE-CadS4A::α-Cat::GFP ;*
Stock: *w sdt*^{KO} */ FM7c twi-GFP; DE-Cad S4A-αCat::GFP*

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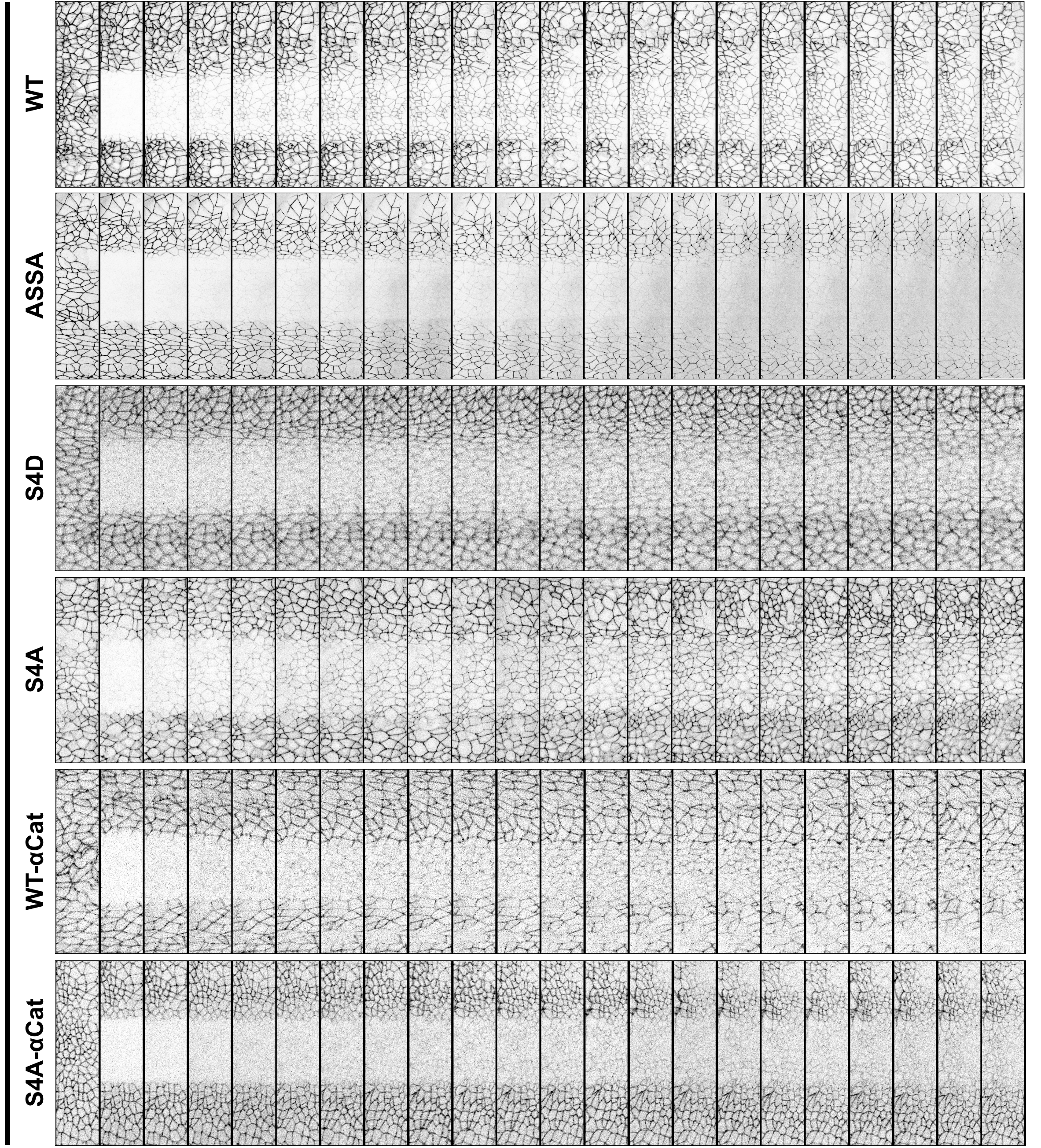
A
Polarizing Cells (st.9-11 embryos)
WT
ASSA
S4D
S4A
WT-αCat
S4A-αCat
2 min

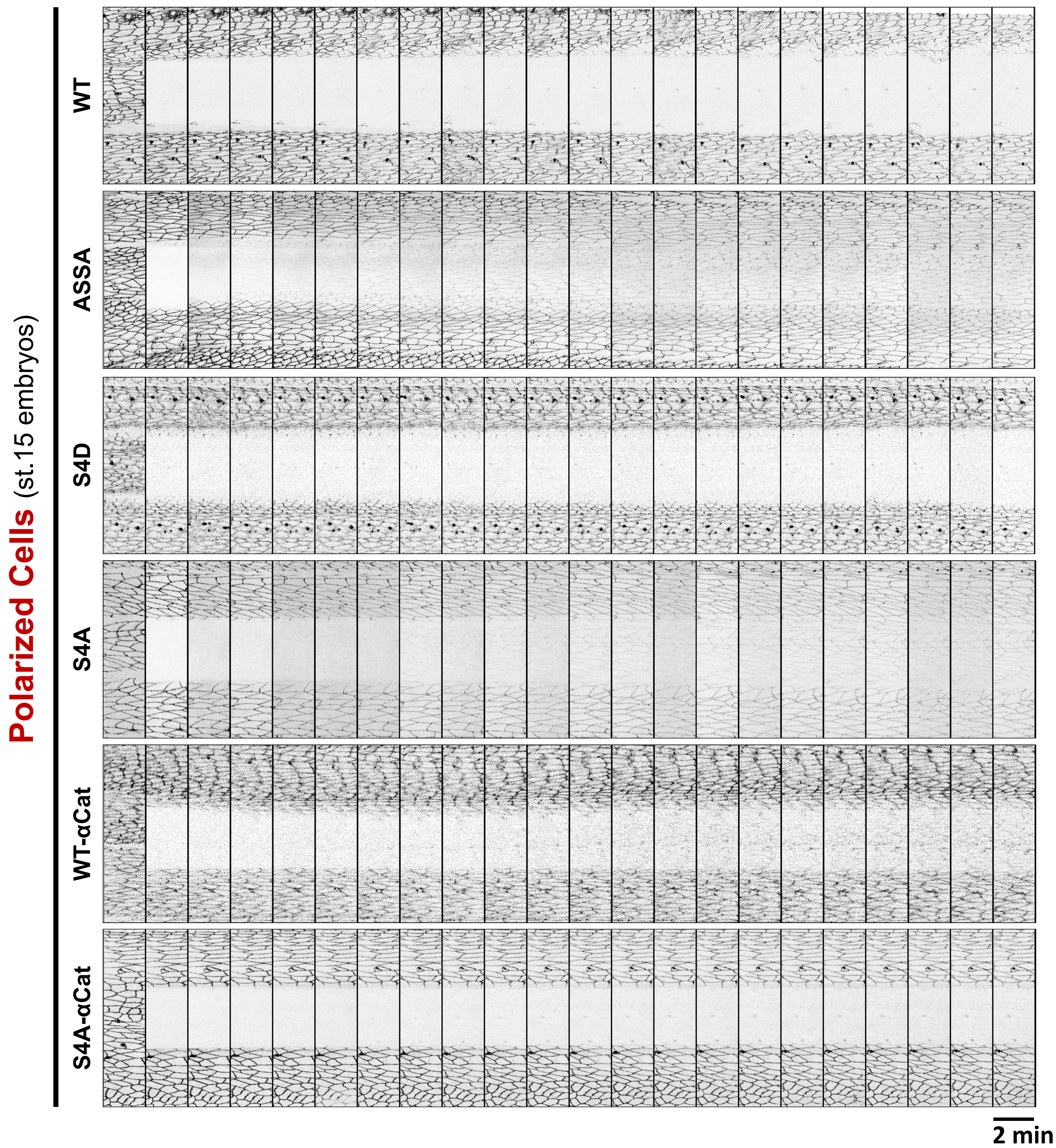

Figure S1. Biosynthetic turnover of DE-Cad* mutants in embryonic epithelia measured by whole-cell FRAP assays.

(**A-B**) Representative whole-cell FRAP samples of selected DE-Cad* mutants in stage 9-11 early embryos (A, for measuring in polarizing embryonic epithelial cells) and stage 15 late embryos (B, for measuring in polarized embryonic epithelial cells). In each whole-cell FRAP sample, GFP in a patch of lateral embryonic epithelium was completely bleached therefore the GFP can only recover from de novo synthesis of DE-Cad. For presentation purpose images were processed in Photoshop to achieve enhanced contrast. All quantifications were done in original unadjusted images.

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Table S1. DE-Cad knock-in mutants. All *DE-Cad* knock-in mutants are tagged with GFP at the C-terminus. Point mutations of phospho-serines in each allele are marked in an "S-SSSS" format abbreviating for Ser-1454, 1457, 1459, 1460 and 1463 in DE-Cad (see Fig. 1A). Each lethal allele was also confirmed by their lethality over the shg^2 null allele, to exclude the possibility of background lethal mutations. Lethal phase of each allele was determined in zygotic homozygotes. DE-Cad*::GFP and β-Catenin levels in mutant AJs were normalized against measurements in AJs formed by wild type DE-Cad::GFP in neighboring twin clones. In parentheses are the numbers of FRAP assays (one assay per embryo, 3rd and 4th columns) or clones quantitatively measured in immunostaining assays (the last three columns). Due to slow recovery of many whole-cell FRAP samples it is impractical to record FRAP long enough to calculate the $t_{1/2}$ and mobile/immobile fractions, therefore we calculated recovery rates as %/min linear rate based on the recovery within the first five to ten minutes (Huang et al., 2011). V: viable. "\": not done. Quantitative data are presented as mean±s.d.

Click here to Download Table S1

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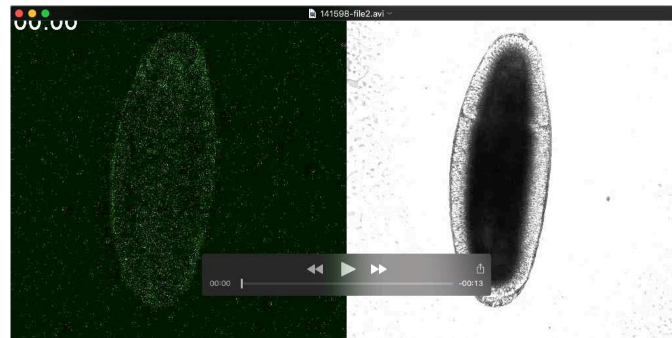
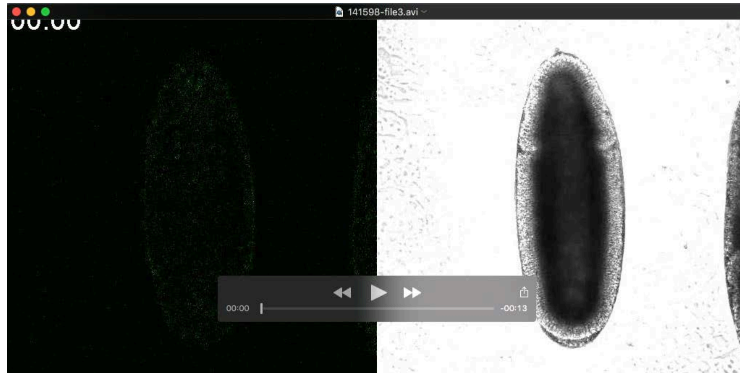
SUPPLEMENTARY MOVIES



Movie 1. Embryogenesis of maternal mutant embryo of *DE-CadS4A::GFP*. The movie is recorded by time-laps from starting stage 7 at 5min interval. The total recording time is 9 hours, with GFP channel at left and DIC channel at right. The genotype the embryo (*ovo-FLP w / +; FRT-G13 DE-CadS4A::GFP*) was confirmed by the absence of *twi-Gal4 UAS-GFP* expression. DE-CadS4A::GFP is too weak to be seen in this recording. Note the failed germ-band retraction starting 06:30 in DIC channel. Time stamp in “hh:mm” format.



Movie 2. Embryogenesis of zygotically rescued maternal mutant embryo of *DE-CadS4A::GFP*. The sample embryo was recorded simultaneously with the embryo in Movie S1. Wild type genotype (*ovo-FLP w / +; FRT-G13 DE-CadS4A::GFP / CyO twi-GFP*) was confirmed by *twi-GFP* expression starting 06:00. Time stamp in “hh:mm” format.