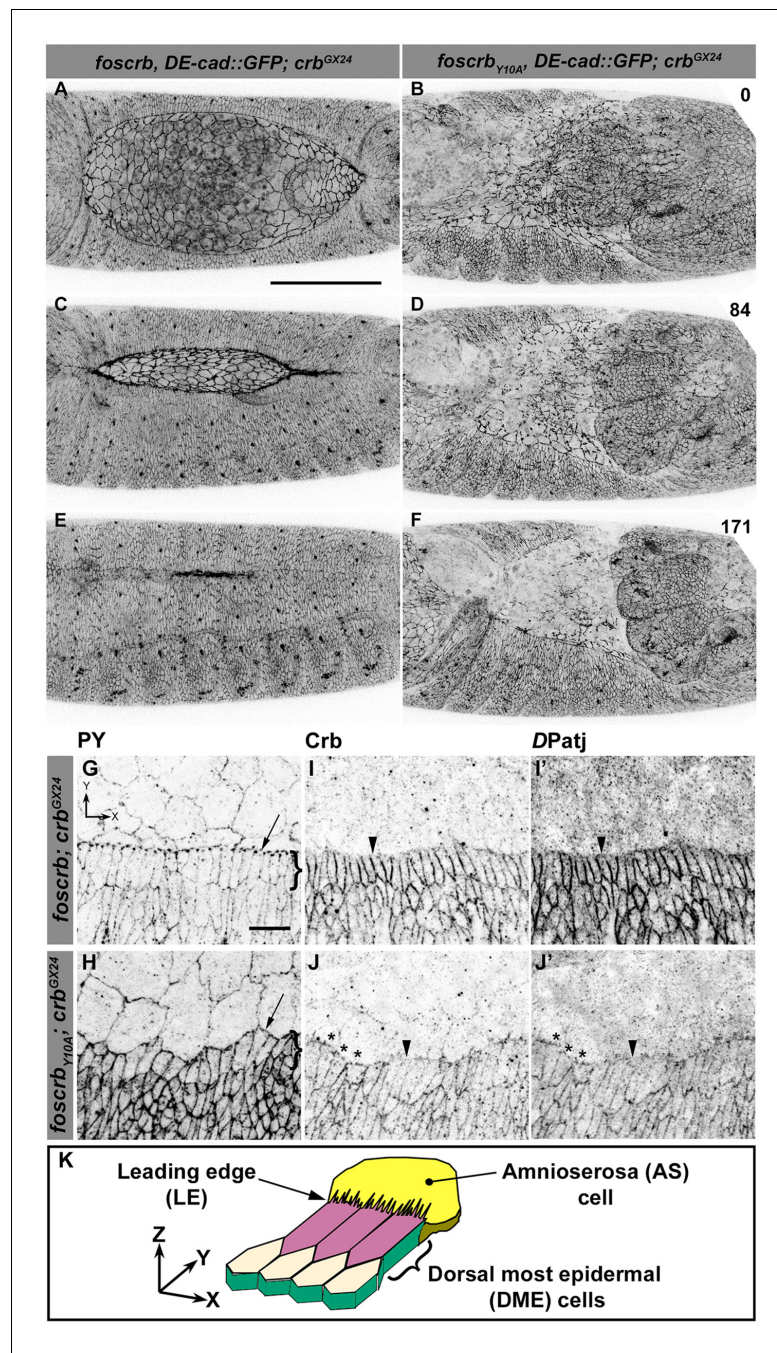


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## Figures and figure supplements

Crumbs is an essential regulator of cytoskeletal dynamics and cell-cell adhesion during dorsal closure in *Drosophila*

**David Flores-Benitez and Elisabeth Knust**



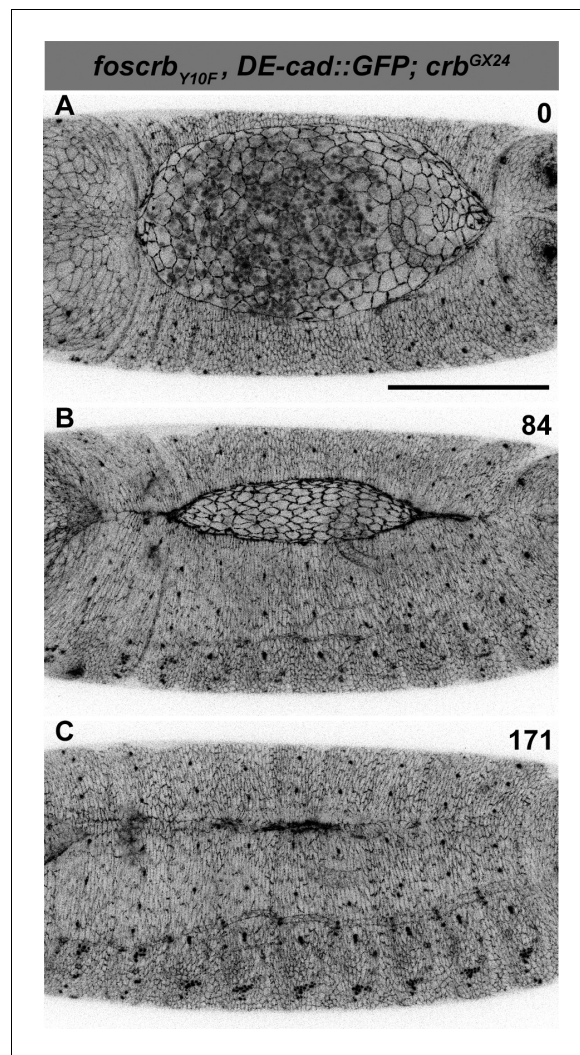
**Figure 1.** The FERM-binding domain motif (FBM) of Crb is essential for dorsal closure (DC). (A-F) Stills from dorsal views of live imaging of embryos expressing *DE-cad::GFP*. In all images the anterior part is towards the left. A, C and E, *w;foscrb,DE-cad::GFP;crb<sup>GX24</sup>* (Video 1). B, D and F, *w;foscrb<sup>Y10A</sup>,DE-cad::GFP;crb<sup>GX24</sup>* (Video 2). All embryos were collected at the same time (1 hr collection), incubated at 28°C for 7 hr and imaged together. Numbers in (B,D and F) indicate the time in minutes for the corresponding row. While DC is completed in *foscrb* embryos (E), in *foscrb<sup>Y10A</sup>* embryos, the amnioserosa (AS) is disorganised and progressively lost (F). Scale bar: 100  $\mu$ m. (G-J') Localisation of phosphotyrosine (PY), Crb and *DPatj* in the dorsal epidermis at the beginning of DC. In all images the AS is at the top (see reference axis in G and in the scheme K). (G, I, I') *w;foscrb;crb<sup>GX24</sup>*. (H, J, J') *w;foscrb<sup>Y10A</sup>;crb<sup>GX24</sup>*. (K) Schematic representation of the dorsal epidermis at the beginning of DC indicating that the leading edge (LE) of the dorsal most epidermal (DME) cells is in contact with the AS. Arrows in (G,H) indicate LE of the DME (row of cells marked by brackets). The arrowheads indicate where the corresponding protein is absent

Figure 1 continued on next page

*Figure 1 continued*

from the LE (I-J'). The asterisks mark LE membranes positive for Crb (J) and DPatj (J') in *foscrb<sub>Y10A</sub>* mutant. Scale bar: 10  $\mu$ m. Representative images from 8–12 different embryos for each genotype.

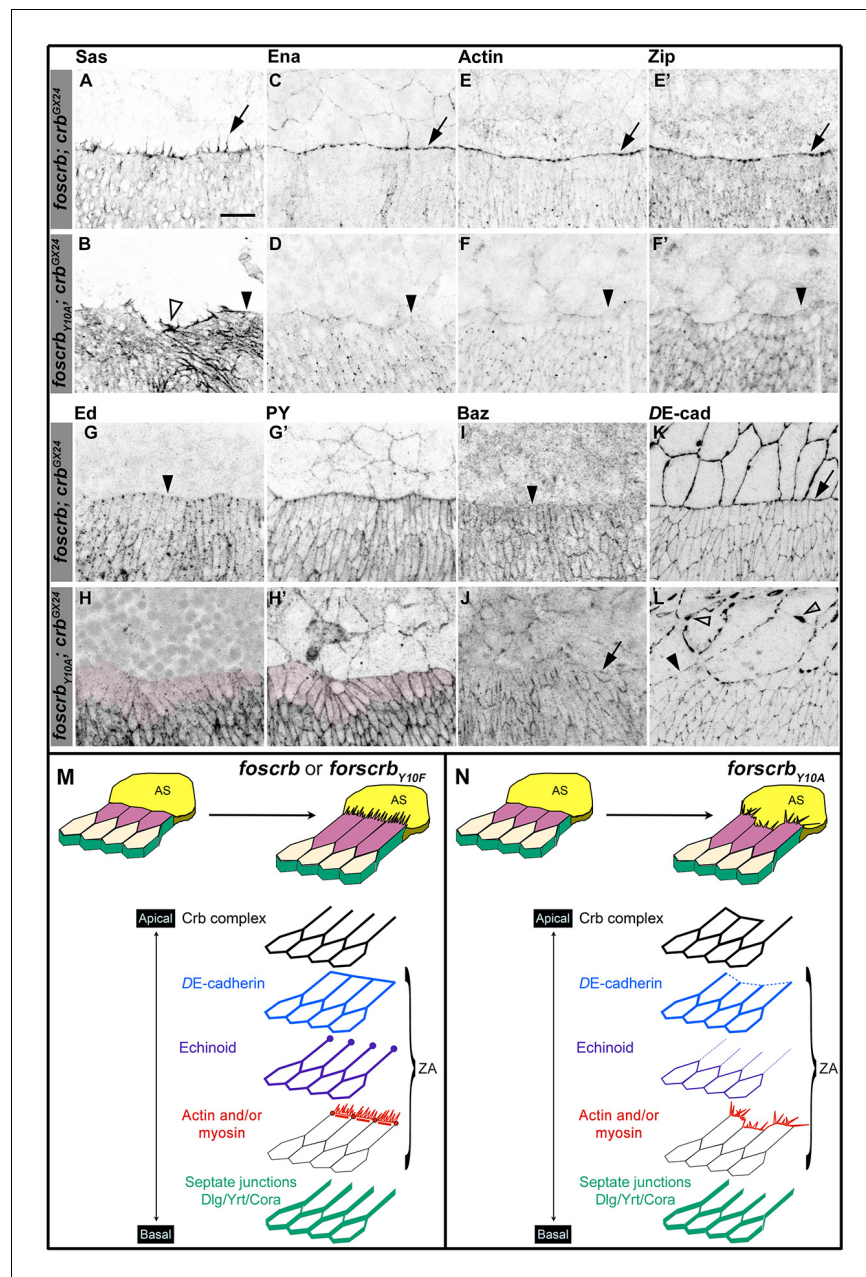
DOI: <http://dx.doi.org/10.7554/eLife.07398.003>



**Figure 1—figure supplement 1.** DC in *foscrb<sub>Y10F</sub>* embryos. (A–C) Stills from dorsal views of live imaging of embryos expressing *DE-cad::GFP* in *w;foscrb<sub>Y10F</sub>,DE-cad::GFP;crb<sup>GX24</sup>*. Embryos collected and imaged as described in **Figure 1**. Numbers indicate the time in minutes for the corresponding row. DC proceeds as in *foscrb* embryos. Scale bar: 100  $\mu$ m.

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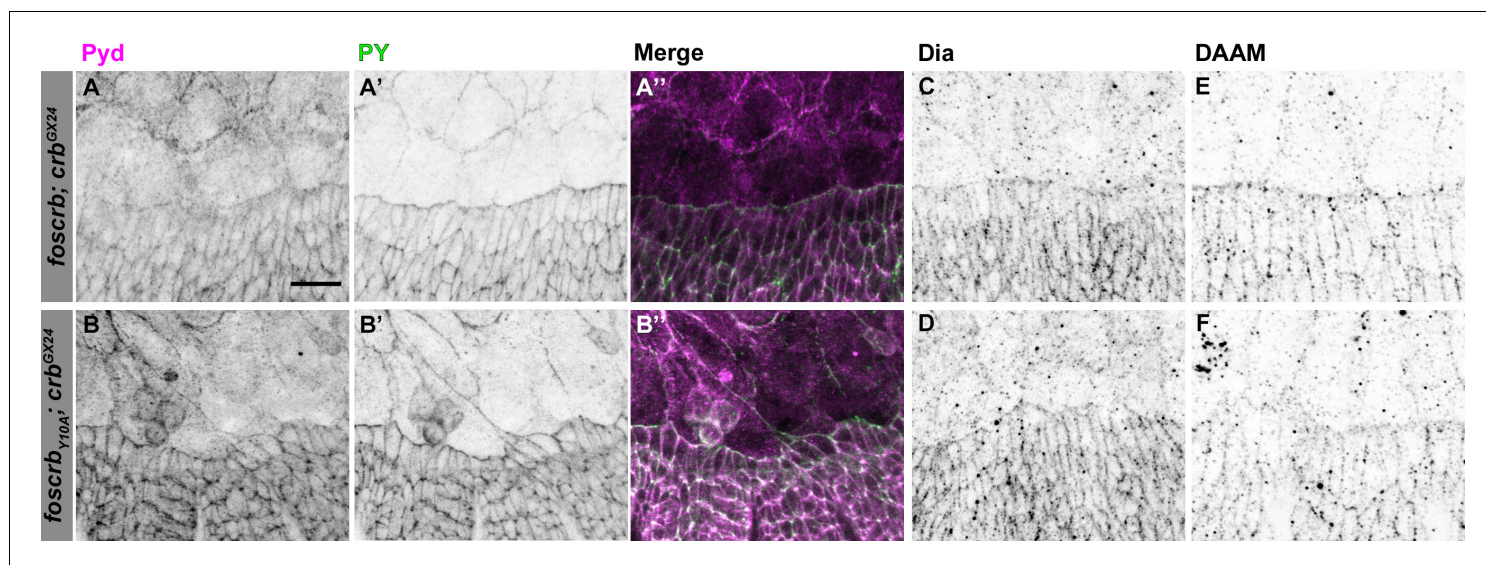


**Figure 2.** The FBM of Crb is important the establishment of the supracellular actomyosin cable at the LE of the DME cells during DC. (A–L) Localisation of Stranded at second (Sas, A,B), Enabled (Ena, C,D), Actin (E,F), Zipper (Zip, E',F'), Echinoid (Ed, G,H), phosphotyrosine (PY, G',H'), Bazooka (Baz, I,J), and DE-cadherin (DE-cad, K,L) at the beginning of stage 14. In all images the AS is at the top half, for the genotypes *w;foscrb;crb<sup>GX24</sup>* and *w;foscrb<sup>Y10A</sup>;crb<sup>GX24</sup>*. Filopodia extend dorsally in *foscrb* embryos (A, arrow), but in *foscrb<sup>Y10A</sup>* embryos filopodia are absent (B, arrowhead) or disorganised (B, empty arrowhead). Ena, Actin and Zip concentrate at the LE in *foscrb* embryos (C,E and E', arrows), but these proteins are almost absent from the LE in *foscrb<sup>Y10A</sup>* embryos (D,F and F', arrowheads). Ed is absent from the LE of *foscrb* embryos (G, arrowhead), but the DME cells of *foscrb<sup>Y10A</sup>* embryos show an important decrease of the protein (H, magenta overlay) though the PY staining is still clearly associated with the ZA in the same cells (H', magenta overlay). Similarly, Baz decreases at the LE of *foscrb* embryos (I, arrowhead), but in *foscrb<sup>Y10A</sup>* embryos, the cells that do not elongate keep Baz at the LE (J, arrow), while other DME cells show a reduction of Baz (J, and **Figure 2—figure supplement 3**). DE-cad (mTomato signal) localises at all cell-cell contacts in *foscrb* embryos (K). However, in *foscrb<sup>Y10A</sup>*, the DE-cad localisation is affected in both the dorsal epidermis (L, solid arrowhead) and the AS (L, empty arrowheads). Scale bar: 10  $\mu$ m. (M) Schematic representation of the changes in DME cells at the beginning of DC in embryos expressing either *foscrb* or *foscrb<sup>Y10A</sup>*. **Figure 2 continued on next page**

*Figure 2 continued*

*fosCrb<sub>Y10F</sub>*. The elongation of the DME cells is accompanied by the removal of the Crb protein complex, Ed, Baz and the septate junction components from the LE. At the LE a supracellular actomyosin cable is established and filopodia extend dorsally and attach to the AS cells. Representative images from 8–12 different embryos for each genotype. **(N)** Schematic representation of the defects in the DME cells of embryos expressing the *fosCrb<sub>Y10A</sub>* variant. At the beginning of DC, the DME cells do not elongate uniformly. In the cells that do not elongate, the Crb protein complex and Baz remain at the LE. Reduced *DE-cad* suggest defects in the ZA function. Ed is dramatically reduced in DME cells, probably contributing to the absence of the supracellular actomyosin cable. Also, the DME cells exhibit disorganised filopodia. Nevertheless, the septate junction components are properly removed from the LE. The Crb protein complex is apical to the ZA, but Ed and the actomyosin cable are associated with the ZA.

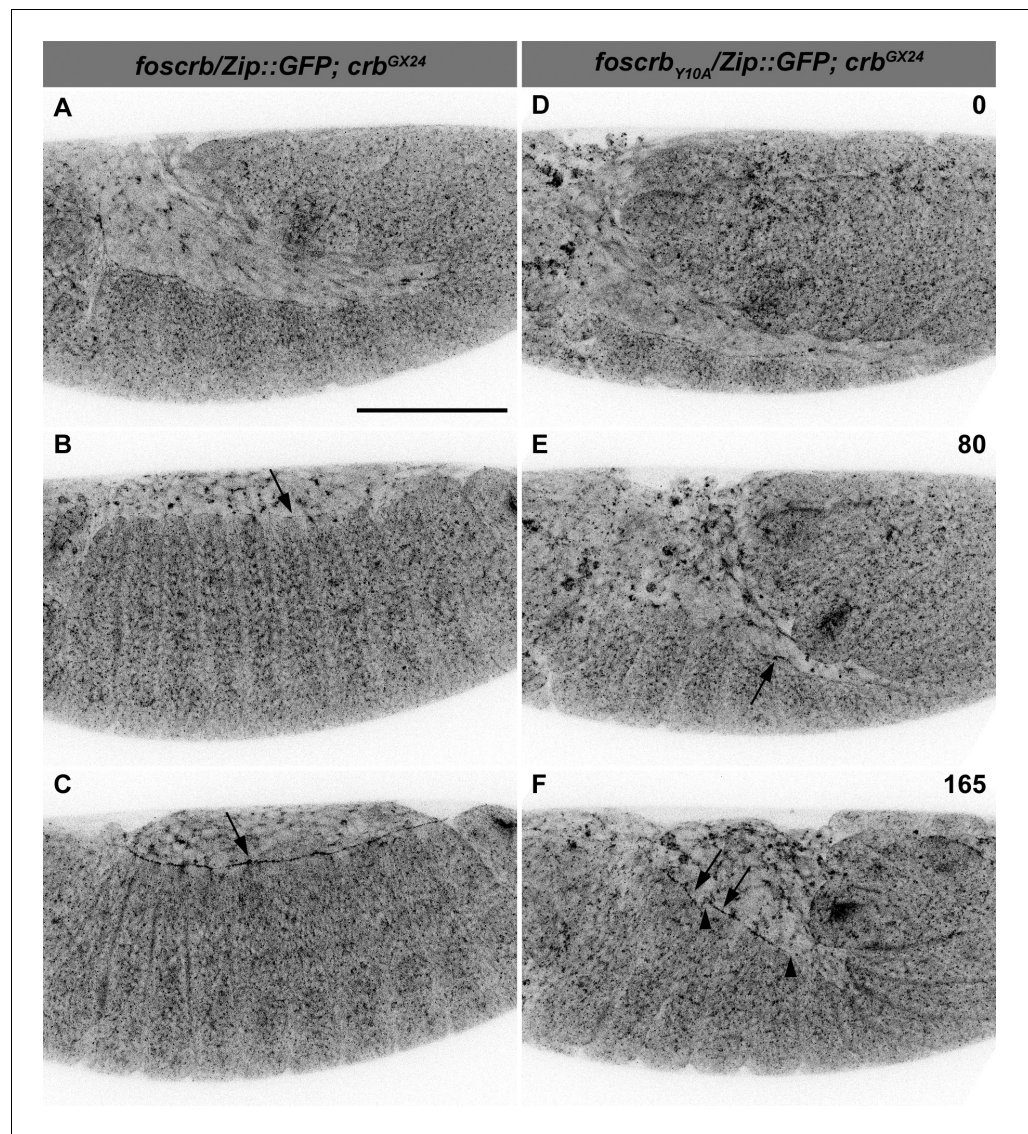
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**Figure 2—figure supplement 1.** Localisation of Pyl, Dia and DAAM in *foscrl* and *foscrl<sub>Y10F</sub>* embryos. Localisation of Polychaetoid (Pyl, A,B), Phosphotyrosine (PY, A',B'), Diaphanous (Dia, C,D), and Dishevelled Associated Activator of Morphogenesis (DAAM, E,F) in embryos at the beginning of stage 14. In all images the AS is at the top, for the genotypes *w;foscrl;crb<sup>GX24</sup>*, and *w;foscrl<sub>Y10A</sub>;crb<sup>GX24</sup>*. The localisation of Pyl (A,B'') is comparable between the different genotypes, despite the irregularly extended DME cells in *w;foscrl<sub>Y10A</sub>;crb<sup>GX24</sup>* embryos (B,B',B''). The PY staining (A', B') marks the ZA. The localisation of Dia (C,D) and DAAM (E,F) is similar in the different genotypes. Scale bar: 10  $\mu$ m. Representative images from 8–12 different embryos for each genotype.

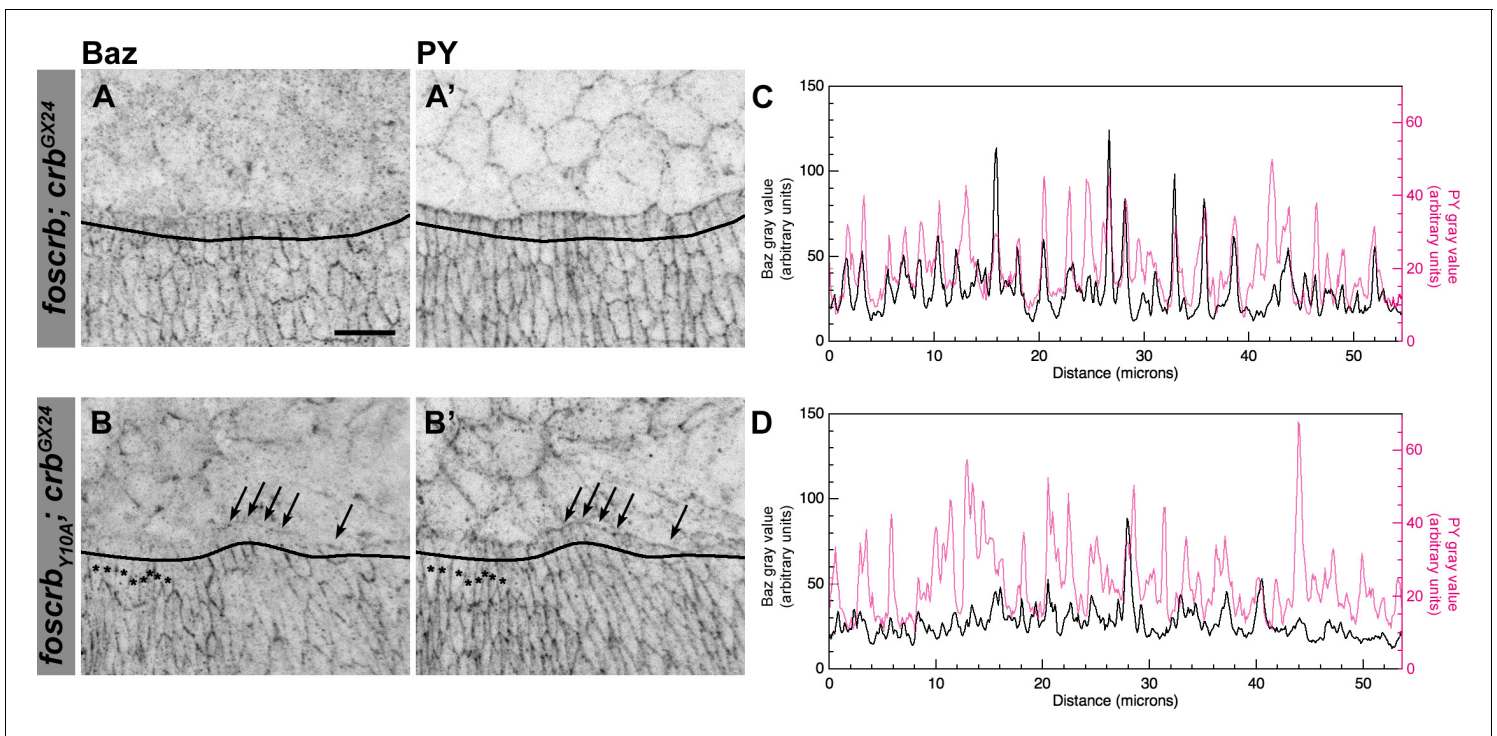
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**Figure 2—figure supplement 2.** The FBM of Crb is important for the establishment of the supracellular actomyosin cable. Stills from live imaging of embryos expressing Zip::GFP. In all images the anterior part is to the left. (A–C) *w;foscrl/Zip::GFP; crb<sup>GX24</sup>* and (D–F) *w;foscrl<sub>Y10A</sub>/Zip::GFP; crb<sup>GX24</sup>* embryos were followed during GB retraction. Numbers in (D–F) indicate the time in minutes for the corresponding row. Arrow in (B) marks the incipient formation of the supracellular actomyosin cable in a *foscrl* embryo. The supracellular actomyosin cable is continuous at later time points (C, arrow). In *foscrl<sub>Y10A</sub>* embryos, some segments of the DME cells concentrate Zip::GFP at the LE (E, arrow). At the time when GB retraction should be completed and thereafter, the actomyosin cable forms randomly at the LE (F, arrows), and several discontinuities are present (F, arrowheads). Scale bar: 100  $\mu$ m. Representative images from 6–8 different embryos for each genotype.

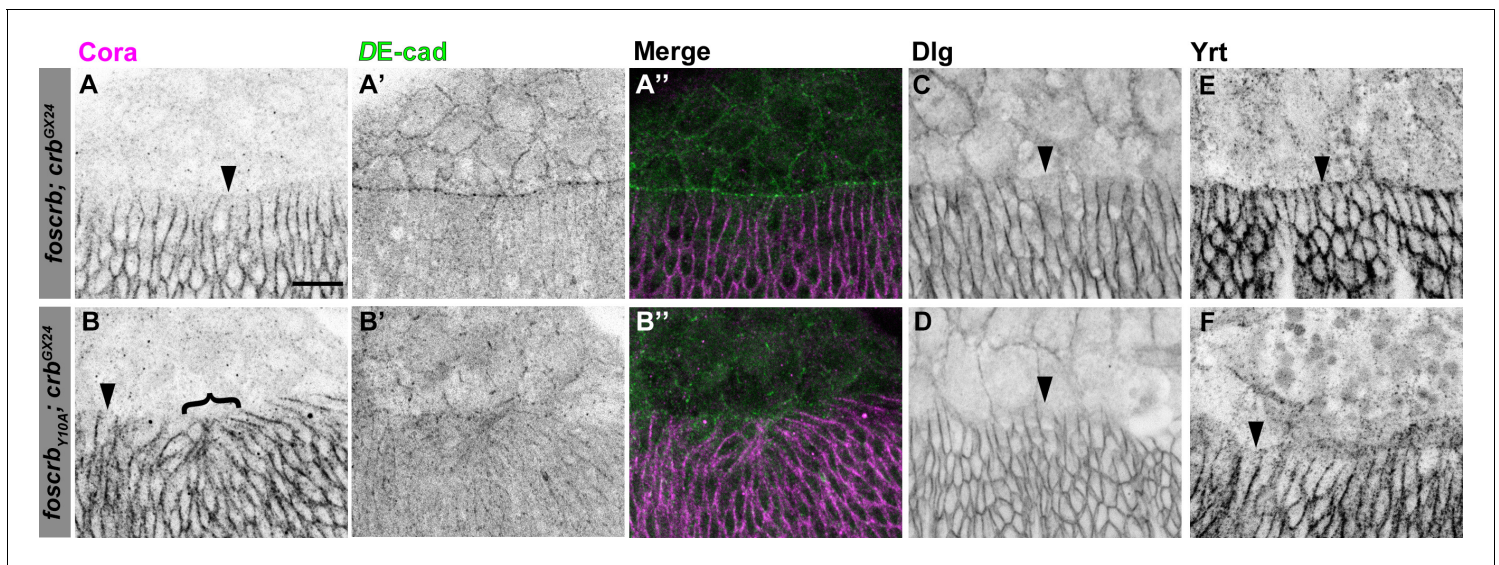
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**Figure 2—figure supplement 3.** Reduction of Baz in DME cells of *foscrlb<sup>Y10A</sup>* embryos. Localisation of Bazooka (Baz, A,B), and phosphotyrosine (PY, A', B') at the beginning of stage 14 in *w;foscrlb;crb<sup>GX24</sup>* and *w;foscrlb<sup>Y10A</sup>;crb<sup>GX24</sup>* embryos. The black lines in A-B' mark the position for the plot profile (C,D) of the Baz signal (C,D, black line) and the PY signal (C,D, magenta line) in the DME cells. Maxima intensities overlap for both markers, but note that the intensity of Baz in *foscrlb<sup>Y10A</sup>* embryos is lower than in *foscrlb* embryos. The arrows indicate where Baz is preserved at the LE of those cells that do not elongate properly, while the asterisks mark the DME cells that extend normally, and have a reduction of Baz signal in the junctions. Scale bar: 10  $\mu$ m.

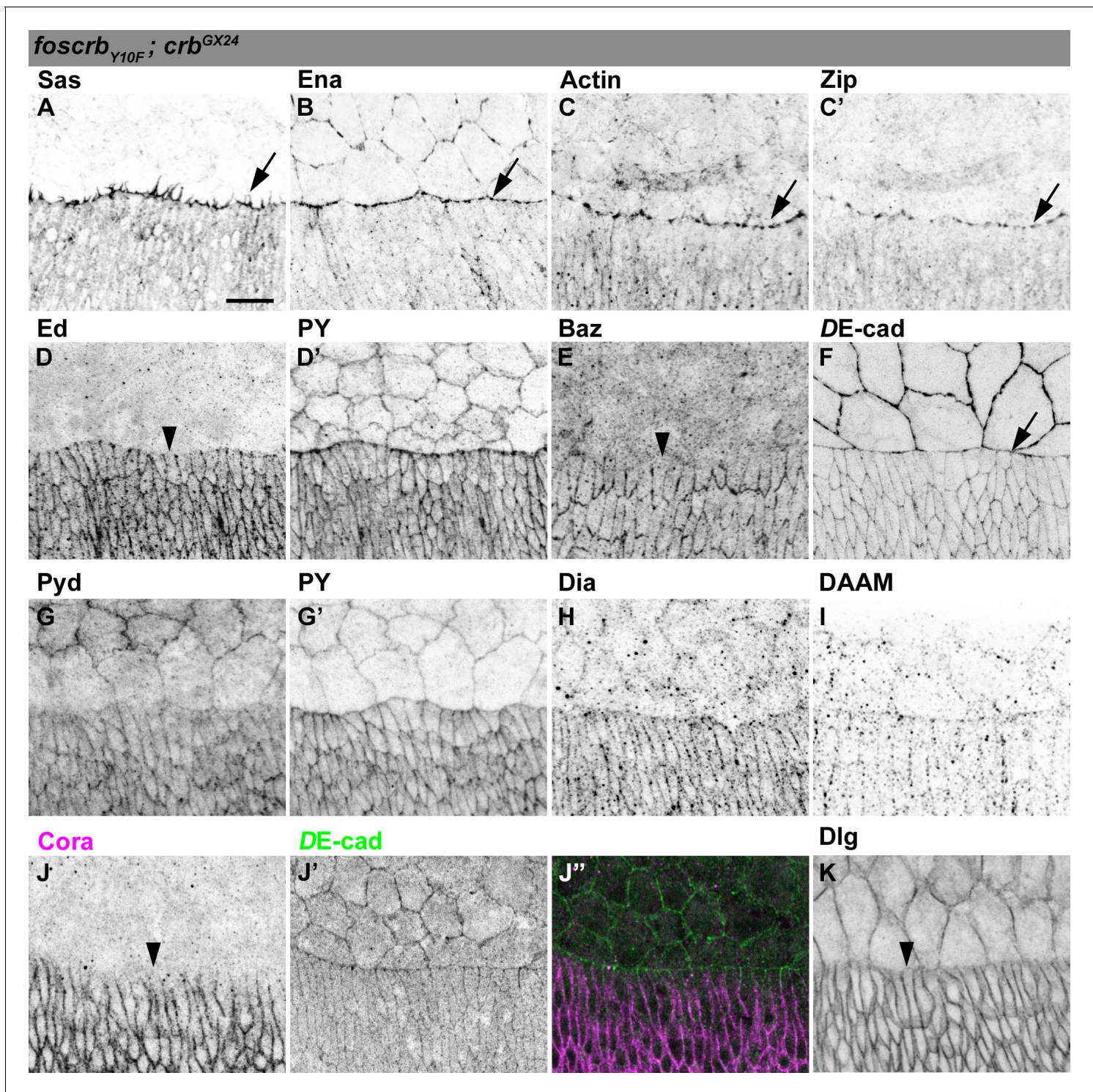
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**Figure 2—figure supplement 4.** Distribution of septate junction components in DME cells. Localisation of Coracle (Cora, **A,B**), DE-cad (**A',B'**), Disc large (Dlg, **C,D**) and Yurt (Yrt, **E,F**) in embryos at the beginning of stage 14. In all images the AS is at the top, for *w;foscrb;crb<sup>GX24</sup>* and *w;foscrb<sub>Y10A</sub>;crb<sup>GX24</sup>* embryos. The septate junction proteins Cora (**A,B**), Dlg (**C,D**) and Yrt (**E,F**) are absent from the LE in all genotypes (arrowheads). Bracket in (**B**) marks bunching of dorsal epidermis observed in *foscrb<sub>Y10A</sub>* embryos. The DE-cad staining (**A',B'**), is a maximal projection of the first ~1.5 μm from the surface of the embryo, while the Cora staining is a maximal projection of the whole Z-stack. The merge of these projections (**A'',B''**) shows that Cora is mainly present in the epidermis. Scale bar: 10 μm. Representative images from 8–12 different embryos for each genotype.

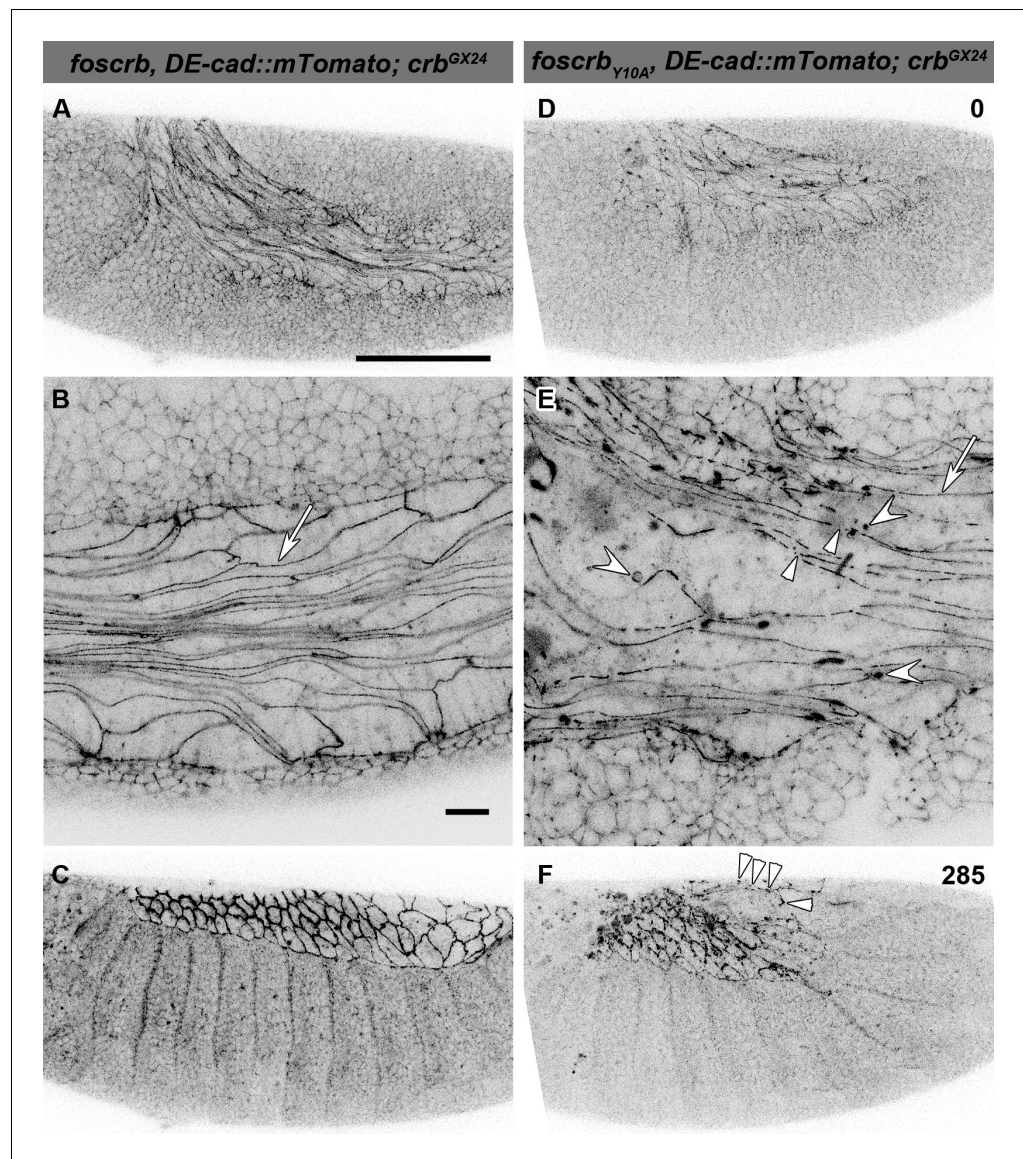
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**Figure 2—figure supplement 5.** Distribution of actomyosin and junctional components in DME cells of *foscrb*<sub>Y10F</sub> embryos. (A–K) Localisation of Sas at the filopodia (A, arrow). Ena (B), Actin (C), and Zip (C') concentrate at the LE (arrows). Ed (D, and PY, D'), and Baz (E) are absent from the LE (arrowheads). DE-cad::mTomato (F) and Pyd (G, and PY, G') localise at all cell-cell contacts. Localisation of Dia (H) and DAAM (I). The septate junction components Cora (J, the corresponding DE-cad, J' and the merge, J''), and Dlg (K) are absent from the LE (J,K, arrowheads). The localisation of all these proteins is similar to the one observed in *foscrb* embryos. Scale bar: 10  $\mu$ m. Representative images from 8–12 different embryos for each genotype.

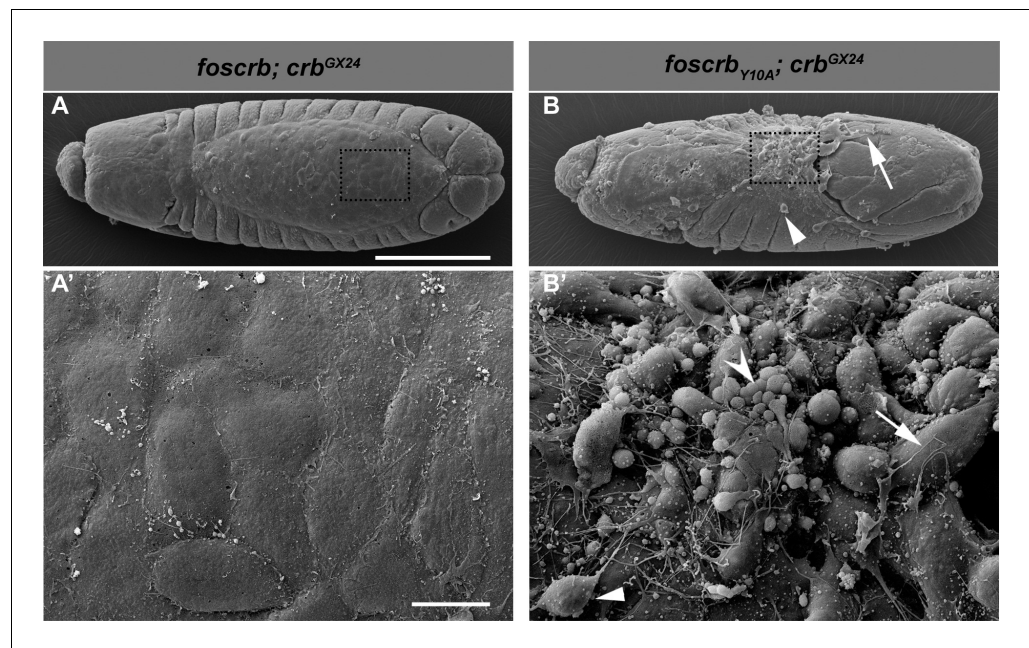
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**Figure 3.** The FBM of Crb is important for the maintenance of the AS. (A-F) Stills from lateral views of live imaging of *DE-cad::mTomato* knock-in at the beginning of germ band (GB) retraction (**Video 4**). In all images the anterior part is towards the left, for the genotypes *w;foscrb,DE-cad::mTomato;crb<sup>GX24</sup>* and *w;foscrb<sub>Y10A</sub>,DE-cad::mTomato;crb<sup>GX24</sup>*. All embryos were collected at the same time (1 hr collection), incubated at 28°C for 5 hr and imaged together. The numbers in (D,F) indicate the time in min. for the corresponding row. At stage 11 (A,B,D,E), the AS cells are elongated along the AP-axis, and *DE-cad::mTomato* localises along the ZA (B,E, arrows); in *foscrb<sub>Y10A</sub>* mutant, the continuity of *DE-cad::mTomato* along the ZA is lost (E, arrowhead) and *DE-cad::mTomato* is also found in large clusters (E, white concave arrowhead). At the end of GB retraction the AS covers the dorsal aspect of *foscrb* embryos (E), but in *foscrb<sub>Y10A</sub>* (F), GB retraction is impaired and *DE-cad::mTomato* signal is fragmented in the AS (F, arrowheads). Scale bar: 100  $\mu$ m, except for (B,E) 10  $\mu$ m.

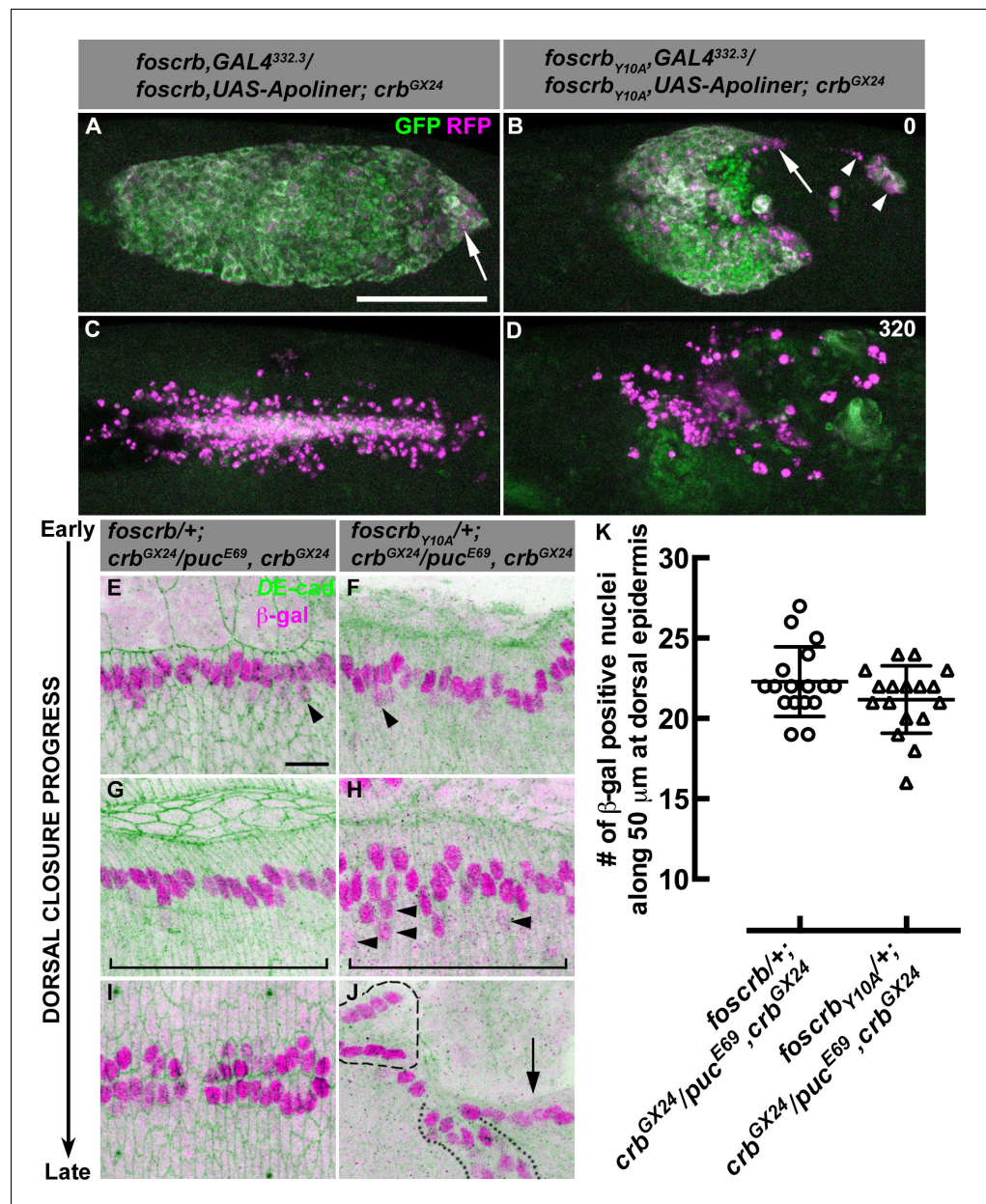
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**Figure 3—figure supplement 1.** The FBM of Crb is important for the integrity of the AS. (A–B') Scanning electron micrographs of dorsal views of embryos incubated for 8 hr at 28°C after egg collection (1 hr collection) for the genotypes *w;foscrb;crb<sup>GX24</sup>* and *w;foscrb<sub>Y10A</sub>;crb<sup>GX24</sup>*. The boxed area in (A,B) is shown in (A',B') respectively. In *foscrb* embryos (A') the AS appears as a flat continuous monolayer, while in *foscrb<sub>Y10A</sub>* embryos (B'), the AS is disorganised and some cells exhibit large filopodia (B,B', arrow). Other cells are completely detached and may be AS cells or haemocytes (B,B', arrowheads), and some cells have the appearance of apoptotic cells (B', concave arrowhead). Scale bars: 100 μm (A,B) and 10 μm (A',B'). Representative images from 17–37 embryos for each genotype.

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**Figure 4.** AS detachment in *foscrcb<sub>Y10A</sub>* embryos is accompanied by premature apoptosis. (A-D) Stills from dorsal views of live imaging of embryos in which the apoptotic reporter Apoliner is driven in the AS with the line *GAL4<sup>332.3</sup>* (Video 6). Apoptotic cells in magenta appear more intense than their neighbours. In all images the anterior part is towards the left for the genotypes *w; foscrcb, GAL4<sup>332.3</sup>/foscrcb, UAS-Apoliner; crb<sup>GX24</sup>*, and *w; foscrcb<sub>Y10A</sub>, GAL4<sup>332.3</sup>/foscrcb<sub>Y10A</sub>, UAS-Apoliner; crb<sup>GX24</sup>*. All embryos were collected at the same time (1 hr collection), incubated at 28°C for 7 hr and imaged together. The numbers in (B,D) indicate the time in minutes for the corresponding row. After GB retraction in *foscrcb* embryos (A), some apoptotic cells are found mainly at the posterior canthus (A, arrow). In comparison, in *foscrcb<sub>Y10A</sub>* embryos, some of the cells that have detached from the AS (B, arrowheads), as well as those in the posterior edge of the AS (B, arrow), are apoptotic. As DC is completed in *foscrcb* embryos (C), a significant portion of the internalised AS cells are apoptotic, while the remaining internalised cells are still localised in a rod-like structure along the dorsal part of the embryo. In contrast, in *foscrcb<sub>Y10A</sub>* embryos (D) all the remaining AS cells are apoptotic cells (the GFP signal in (D) does not belong to the AS). Scale bar: 100  $\mu$ m. Representative images from 8–12 different embryos for each genotype. (E-K) Activation of the JNK pathway in the DME cells analysed with the enhancer trap *puc<sup>E69</sup>* ( $\beta$ -galactosidase staining). DE-cad staining is in green. In all images anterior is to the left for the genotypes *w; foscrcb/+; crb<sup>GX24</sup>/puc<sup>E69</sup>, crb<sup>GX24</sup>* and *w; foscrcb<sub>Y10A</sub>/+; crb<sup>GX24</sup>/puc<sup>E69</sup>, crb<sup>GX24</sup>*. From the beginning to the end of DC, Puc expression is normally induced on

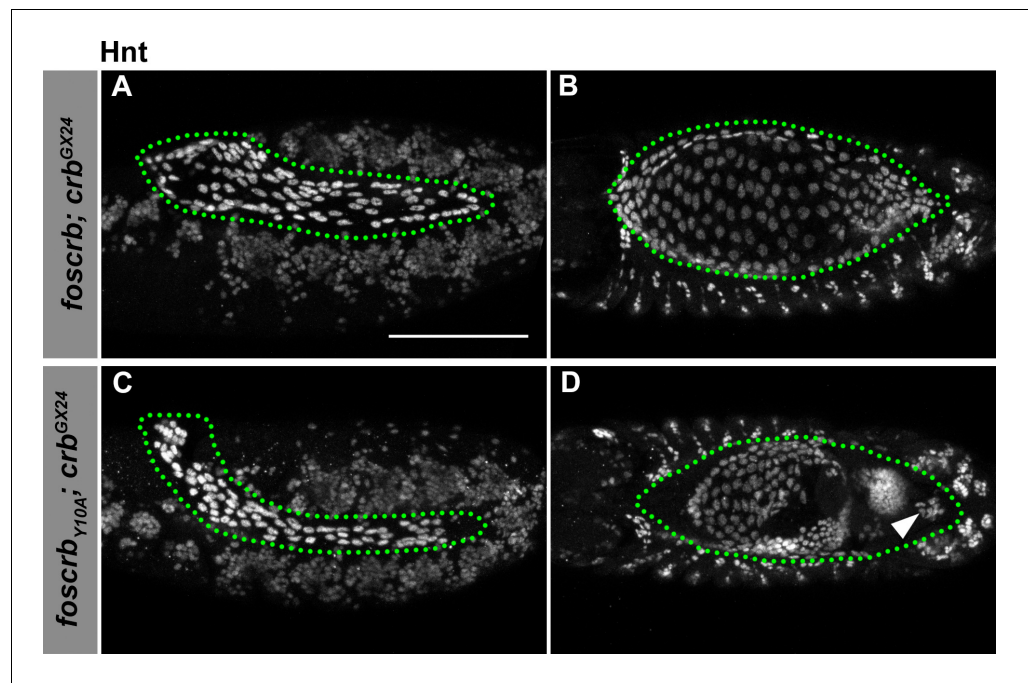
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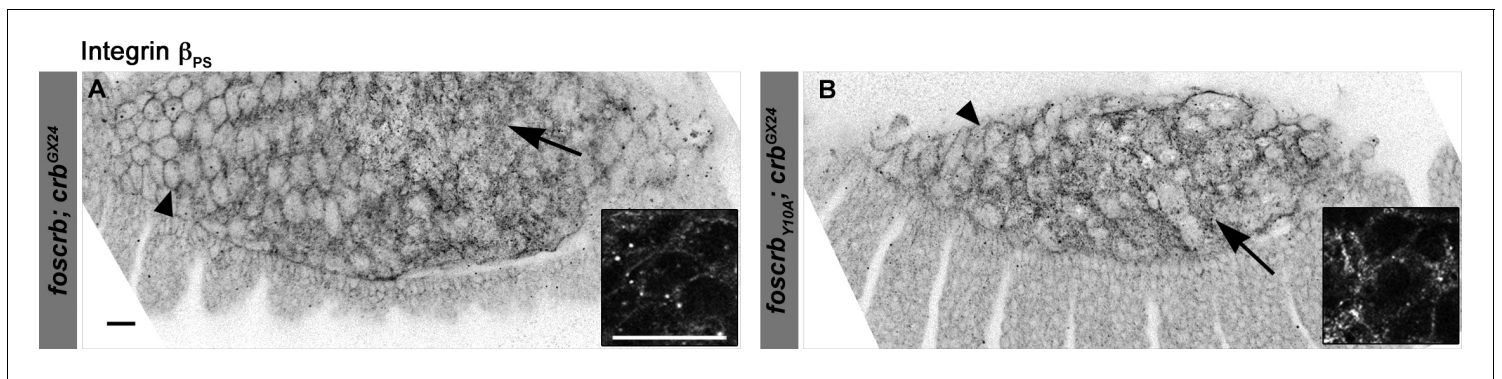
each side of the embryo in the single row of DME cells in both genotypes, and few positive  $\beta$ -gal nuclei appear below the row of DME cells (**E,F**, arrowheads). In *foscrb*<sub>Y10A</sub> embryos at middle DC some  $\beta$ -gal positive cells appear below the DME cells (**H**, arrowheads). When DC is completed in *foscrb* embryos (**I**), a single row of cells on each side of the embryo is  $\beta$ -gal positive, even in *foscrb*<sub>Y10A</sub> embryos, independently of whether the epidermis contacted the corresponding segment of the epidermis on the dorsal midline (**J**, dashed line), bunched on the same side of the embryo (**J**, dotted line) or fail to touch the complementing segment (**J**, arrow). Scale bar: 10  $\mu$ m. (**K**) No significant difference in the number of  $\beta$ -gal positive nuclei at middle DC along 50  $\mu$ m at the dorsal epidermis (indicated by the brackets in **G,H**), mean  $\pm$  SD, n= 17 embryos per genotype.

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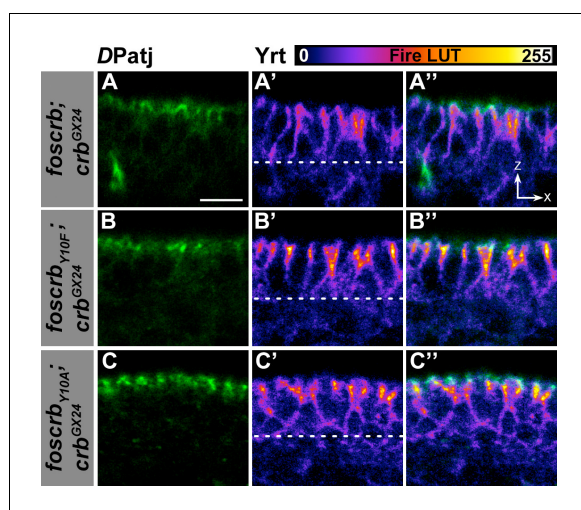


**Figure 4—figure supplement 1.** Hindsight expression in *foscrb* and *foscrb<sup>Y10A</sup>* embryos. (A-D) Expression of Hindsight (Hnt) at stage 12 (A,C, lateral view) and stage 14 (B,D, dorsal view). In all images the AS is inside the green dotted line. Note that the AS is properly specified in *foscrb* and *foscrb<sup>Y10A</sup>* embryos, and at stage 14, Hnt staining is comparable between the two genotypes (B,D), and Hnt is present even in the cells that have detached from the AS in the *foscrb<sup>Y10A</sup>* embryos (D, arrowhead). Scale bar: 100  $\mu$ m.

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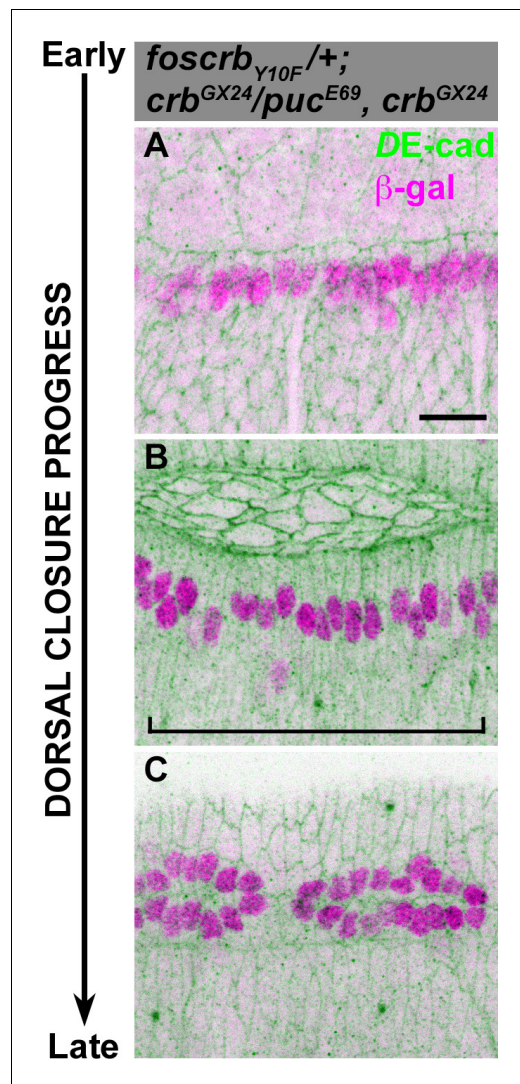


**Figure 4—figure supplement 2.** Localisation of integrin  $\beta_{PS}$  in the AS of *foscrb* and *foscrb<sup>Y10A</sup>* embryos. (A,B) The localisation of the integrin- $\beta_{PS}$  is similar in *foscrb* and *foscrb<sup>Y10A</sup>* embryos. The images are projections of  $\sim 1 \mu\text{m}$  thickness; thus, in some cells it is possible to see the localisation of the integrin- $\beta_{PS}$  at the basal membrane (arrows), while in other cells it is possible to see the protein localisation at the lateral membrane (arrowheads). The inserts are magnification of a single confocal plane ( $0.45 \mu\text{m}$ ) through the middle part of the AS cells in the respective genotypes. Scale bars:  $10 \mu\text{m}$ . DOI: <http://dx.doi.org/10.7554/eLife.07398.021>



**Figure 4—figure supplement 3.** Localisation of DPatj and Yrt in the dorsal epidermis. (A–C'') Cross section (ZX view –see reference axis in **Figure 1K**) of the dorsal epidermis of embryos at stage 14 stained for DPatj (green) and Yrt (fire LUT-pseudocolor). In all images the apical aspect of the cells is at the top and the dotted line marks the basal aspect. (A–A'') *w;foscrb; crb<sup>GX24</sup>*. (B–B'') *w;foscrb<sup>Y10F</sup>; crb<sup>GX24</sup>*. (C–C'') *w; foscrb<sup>Y10A</sup>; crb<sup>GX24</sup>*. Note that Yrt is concentrated toward the apical aspect of the cells in all genotypes. Scale bar: 5  $\mu$ m. Representative images from 8–12 different embryos for each genotype.

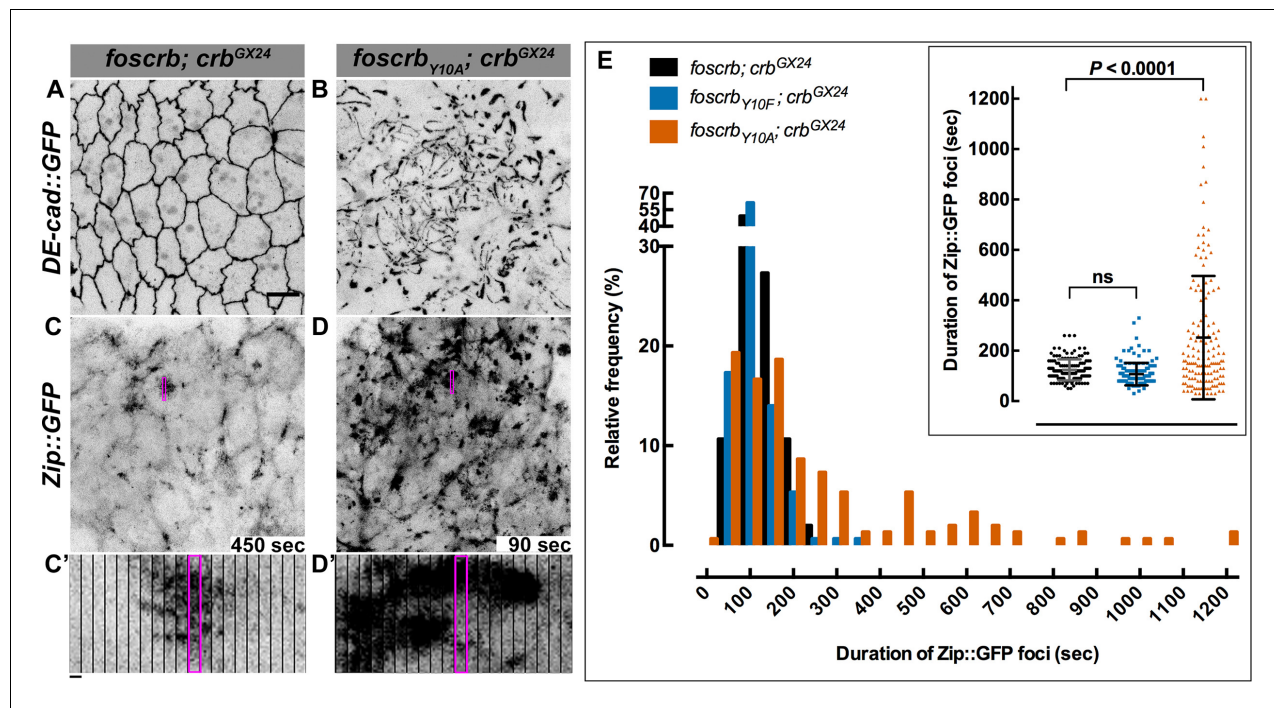
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**Figure 4—figure supplement 4.** JNK signalling is normal in *foscrb*<sub>Y10F</sub> embryos. (A–C) Activation of the JNK pathway in the DME cells analysed with the enhancer trap *puc*<sup>E69</sup> (β-galactosidase staining). DE-cad staining is in green. In all images anterior is to the left. From the beginning to the end of DC, Puc expression is normally induced on each side of the embryo in the single row of DME cells. When DC is completed, a single row of cells on each side of the embryo is β-gal positive (C). Scale bar: 10 μm.

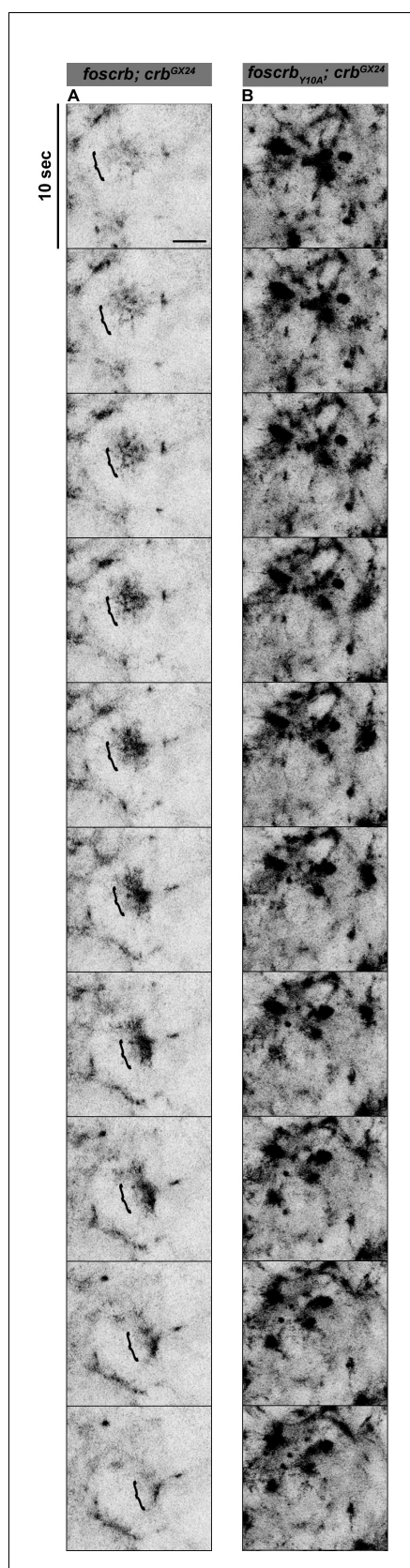
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**Figure 5.** The FBM of Crb is essential for the regulation of actomyosin activity in the AS. Stills from views of the AS in live imaging of embryos expressing *DE-cad::GFP* knock-in (A,B, **Video 8**) or *Zip::GFP* (C-D', **Video 9**). In all images the anterior part is towards the left. Scale bar: 10  $\mu$ m. (A) *w; foscrb, DE-cad::GFP; crb<sup>GX24</sup>*. (B) *w; foscrb<sup>Y10A</sup>, DE-cad::GFP; crb<sup>GX24</sup>*. (C) *w; foscrb/Zip::GFP; crb<sup>GX24</sup>*. (D) *w; foscrb<sup>Y10A</sup>/Zip::GFP; crb<sup>GX24</sup>*. The embryos were collected during 30 min, incubated at 28°C for 7 hr and imaged under the same conditions. The numbers in (C,D) indicate the time in seconds for the corresponding frame in **Video 9**. In *foscrb* embryos (A), *DE-cad::GFP* is localised at cell-cell junctions; but in *foscrb<sup>Y10A</sup>* (B) embryos *DE-cad::GFP* continuity is strongly disturbed. (C,D') Kymographs of the *Zip::GFP* foci in the magenta box in (C,D). Scale bar in (C') 10 sec. (E) Histogram of the relative frequency of *Zip::GFP* foci duration during the pulsed contractions of the AS in *w; foscrb/Zip::GFP; crb<sup>GX24</sup>*, *w; foscrb<sup>Y10F</sup>/Zip::GFP; crb<sup>GX24</sup>* and *w; foscrb<sup>Y10A</sup>/Zip::GFP; crb<sup>GX24</sup>* embryos. The graph in the insert shows all data points collected, and indicates the mean  $\pm$  SD. ANOVA test followed by a Dunnett's multiple-comparison test; ns-not significant difference.  $n = 150$  foci collected from each of the three different embryos.

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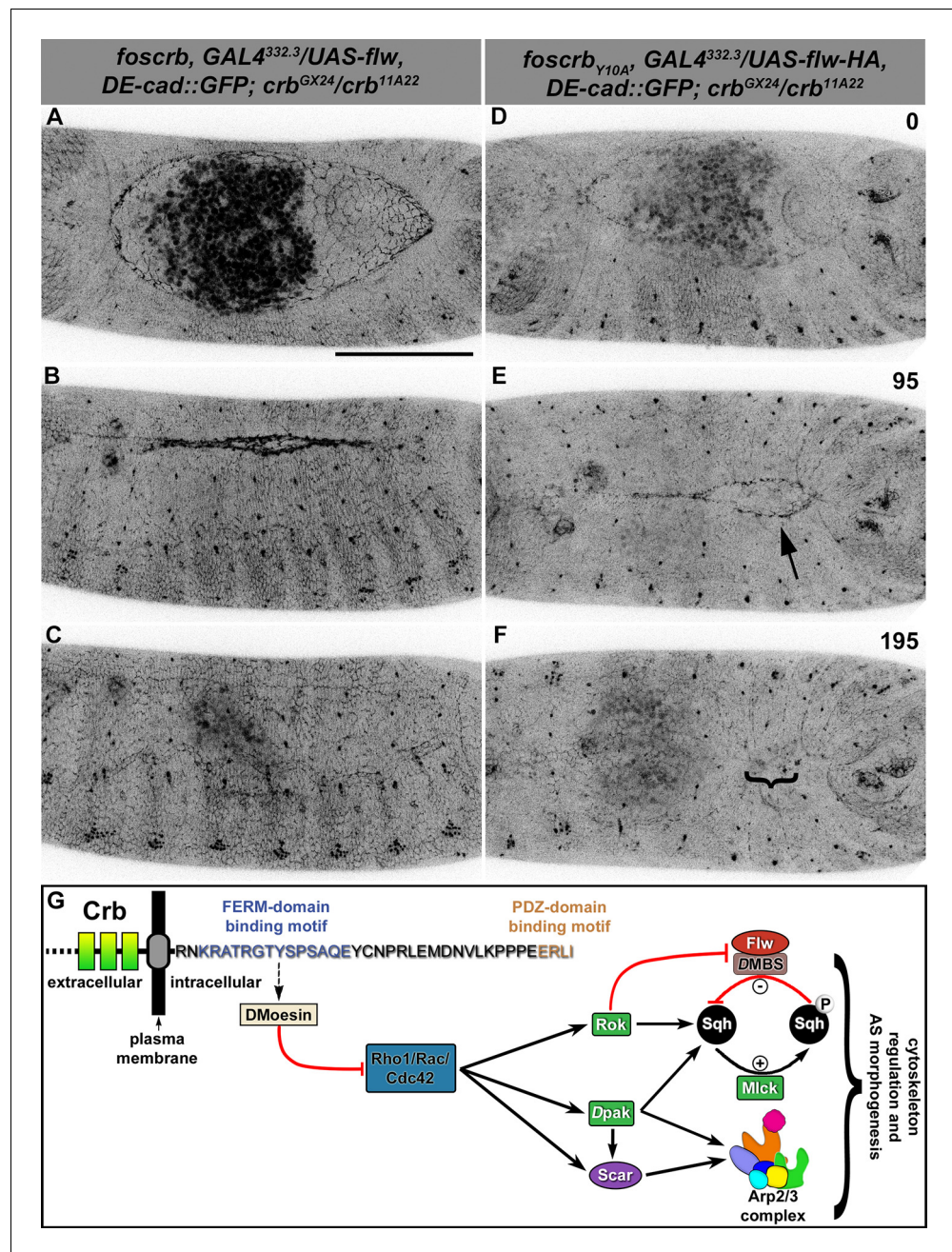
**Figure 5—figure supplement 1.** The FBM of Crb

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regulates the actomyosin activity in the AS. Stills from **Video 10** where a Zip::GFP cluster forms and disappears (followed by the bracket) in an AS cell during the pulsed contraction in a *w;foscrb/Zip::GFP; crb<sup>GX24</sup>* embryo (A). In contrast, several Zip::GFP foci are present and do not disappear in the *w;foscrb<sup>Y10A</sup>/Zip::GFP;crb<sup>GX24</sup>* embryo (B). Scale bar: 5  $\mu$ m.

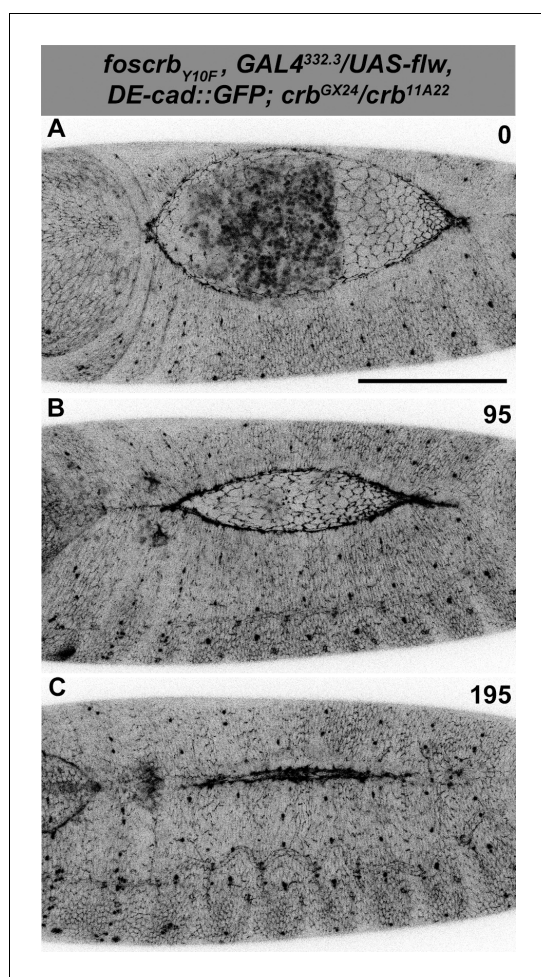
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**Figure 6.** Expression of the myosin phosphatase Flapwing in the AS of *foscrcb<sub>Y10A</sub>* embryos suppresses the DC defects. (A-F) Stills from dorsal views of live imaging of embryos expressing *DE-cad::GFP* knock-in and *Flw-HA* in the AS cells under the control of the *GAL4<sup>332.3</sup>* driver (**Video 11**), for the genotypes *w;foscrcb*, *GAL4<sup>332.3</sup>/UAS-flw-HA*, *DE-cad::GFP*; *crb<sup>GX24</sup>/crb<sup>11A22</sup>*, *UAS-Act::RFP* and *w;foscrcb<sub>Y10A</sub>*, *GAL4<sup>332.3</sup>/UAS-flw-HA*, *DE-cad::GFP*; *crb<sup>GX24</sup>/crb<sup>11A22</sup>*, *UAS-Act::RFP*. All embryos were collected at the same time (1 hr collection), incubated at 28°C for 7 hr and imaged together. The numbers on (D-F) indicate the time in minutes for the corresponding row. The over-expression of *Flw-HA* in the AS cells does not produce any obvious phenotype in *foscrcb* (A-C) embryos, and it suppresses the DC defects in *foscrcb<sub>Y10A</sub>* (D-F) embryos; some defects found include an irregular zippering at the posterior canthus (E, arrow) as well as bunching of the dorsal epidermal (F, bracket). Scale bar: 100  $\mu$ m. Representative images from 6–9 different embryos for each genotype. (G) Scheme of the possible pathways regulated by the FBM of Crb in the AS. Crb: Crumbs; Rok: Rho-kinase; Dpak: *Drosophila* p21-activated kinase; Flw: Flapwing; DMBS: *Drosophila* myosin-binding-subunit; Sqn: spaghetti-squash; Mlck: myosin-light chain kinase.

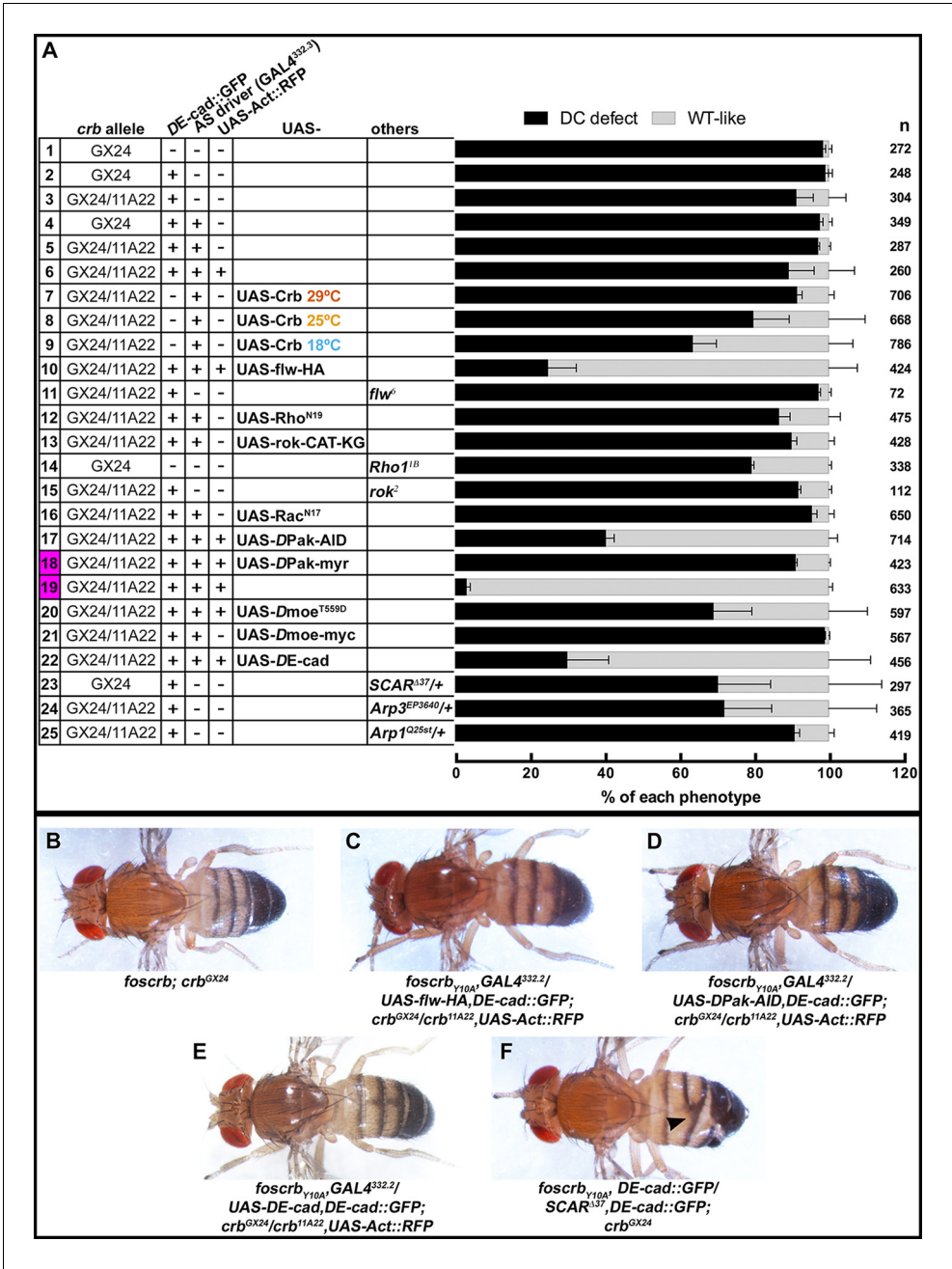
DOI: <http://dx.doi.org/10.7554/eLife.07398.031>



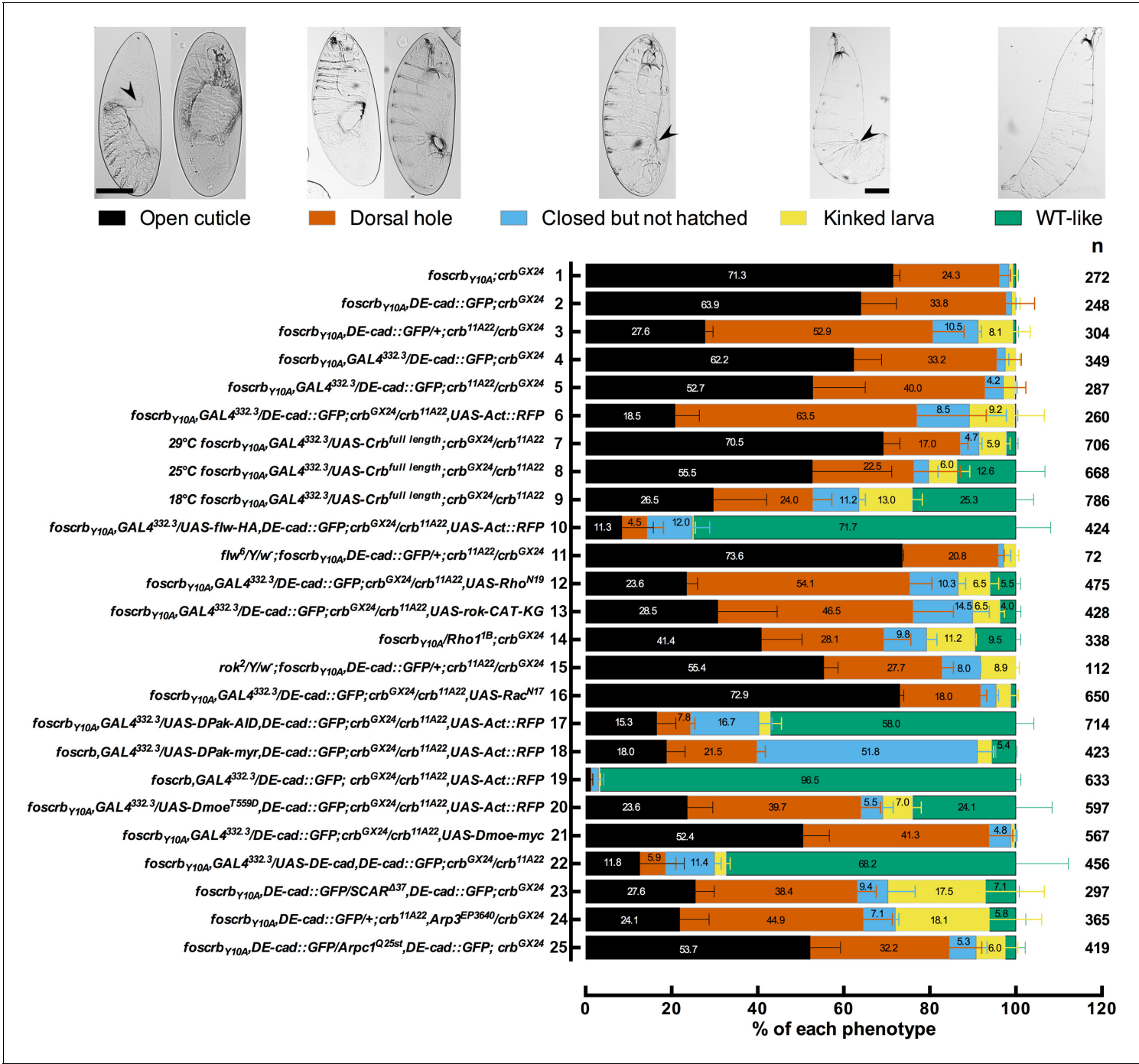


**Figure 6—figure supplement 1.** Normal DC after Flapwing expression in the AS of *foscrb<sub>Y10F</sub>* embryos. (A–C) Stills from dorsal views of live imaging of embryos expressing *DE-cad::GFP* knock-in and *Flw-HA* in the AS cells under the control of the *GAL4<sup>332.3</sup>* driver, for the genotype *w;foscrb<sub>Y10F</sub>, GAL4<sup>332.3</sup>/UAS-flw-HA, DE-cad::GFP;crb<sup>GX24</sup>/crb<sup>11A22</sup>, UAS-Act::RFP*. Embryo collection, incubation and imaging as described in **Figure 6**. The numbers on (A–C) indicate the time in minutes for the corresponding row. The over-expression of *Flw-HA* in the AS cells does not produce any obvious phenotype. Scale bar: 100  $\mu$ m. Representative images from 7 different embryos. DOI: <http://dx.doi.org/10.7554/eLife.07398.032>

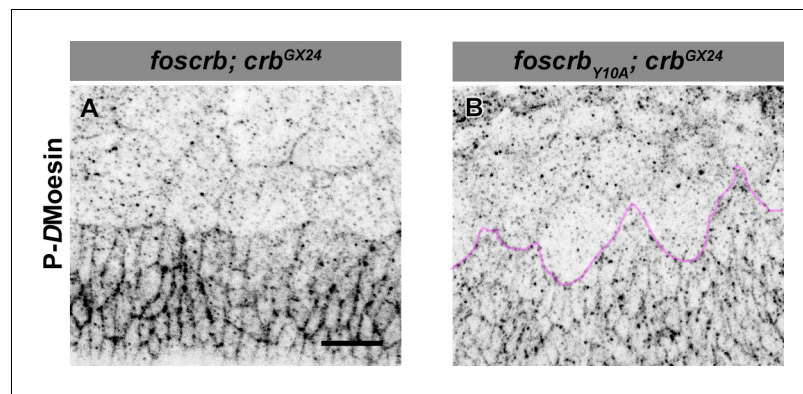




**Figure 7.** Reduction in actomyosin activity suppresses the DC defects in embryos expressing the *foscrb*<sup>Y10A</sup> variant. (A) Quantification of the defects observed in cuticle preparations from the genotypes indicated in the graph. For the complete genotype see **Figure 7—figure supplement 1**. The category “DC defect” includes a range of defects ranging from cuticles of embryos that completed DC but do not hatch, to cuticles with large DC openings. The category “WT-like” includes all larvae that hatch. For details about the classifications see **Figure 7—figure supplement 1**. Note that all the genotypes have the *foscrb*<sup>Y10A</sup> background, except the ones highlighted in magenta, numbers 18 and 19, that have the *foscrb* background. mean ± SD from 2–4 independent crosses. n = total number of cuticles counted for the indicated genotype. Note that suppression of the DC phenotype in *foscrb*<sup>Y10A</sup> embryos is particularly evident upon expression of Flw-HA (10), Pak-AID (17), and DE-cad (22). (B–F) Adult flies of the indicated genotypes. In (F), the arrowhead marks the defects in the dorsal abdomen. DOI: <http://dx.doi.org/10.7554/eLife.07398.034>



**Figure 7—figure supplement 1.** Reduction in the actomyosin activity suppresses the DC defects in embryos expressing the *foscrb*<sub>Y10A</sub> variant. Quantification of the defects observed in cuticle preparations from the genotypes indicated in the graph. In the category “Open cuticle”, the dorsal opening is so prominent that in some cases the mouthparts are exposed (arrowhead). Category “Dorsal hole” corresponds to those cuticles in which a medium (left picture) or small (right picture) dorsal hole is present, but the anterior part is closed. In the category “Closed but not hatched”, the closure is complete, the puckering of the epidermis is noticeable (arrowhead), but the larvae fail to hatch. In the category “Kinked larvae”, the puckering of the epidermis (arrowhead) results in larvae with the tail pointing upwards, so the larvae seem to have a kink. In the category “WT-like”, no defects are evident so the larvae are alike to wild type. mean ± SD from 2–4 independent crosses. n = total number of cuticles counted for the indicated genotype. For the statistical analysis see **Table 1**. DOI: <http://dx.doi.org/10.7554/eLife.07398.035>



**Figure 7—figure supplement 2.** Phosphorylated *DMoesin* levels are reduced in embryos expressing the *foscrlb<sub>Y10A</sub>* variant. Localisation of phospho-*DMoesin* (P-DMoe, **A,B**) in embryos at the beginning of stage 14. In all images the AS is at the top, for the genotypes *w;foscrlb;crb<sup>GX24</sup>* and *w;foscrlb<sub>Y10A</sub>;crb<sup>GX24</sup>*. The LE of *foscrlb<sub>Y10A</sub>* embryo is marked with a magenta line (**B**). Scale bar: 10  $\mu$ m. Representative images from 9 different embryos for each genotype.

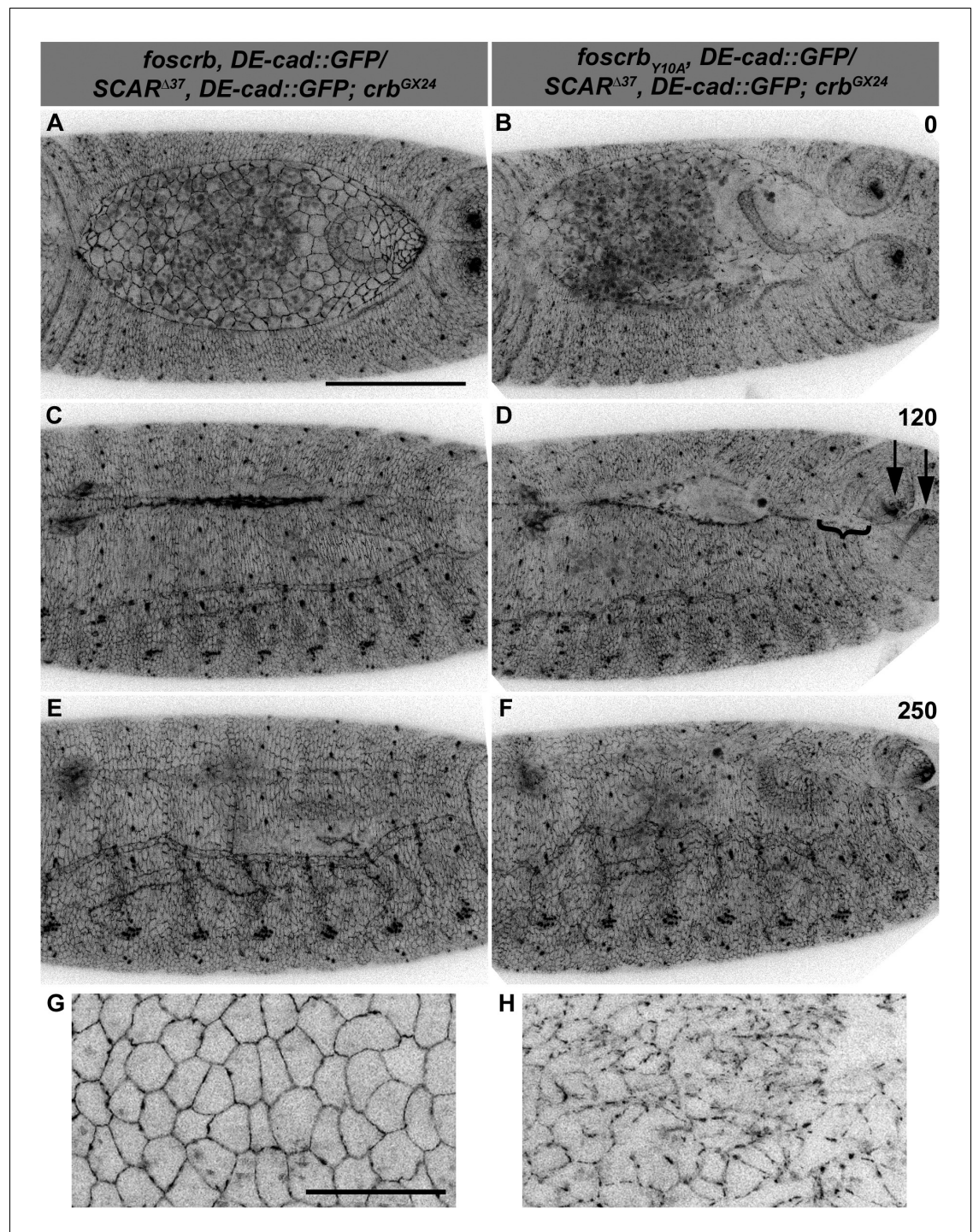
DOI: <http://dx.doi.org/10.7554/eLife.07398.036>



**Figure 7—figure supplement 3.** Weak head phenotype of embryos expressing the *foscrb*<sub>Y10A</sub> variant. Examples of cuticles with a weak head phenotype: the arrows mark an opening in the anterior part.

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**Figure 8.** Reduction of the SCAR-Arp complex activity suppresses the DC defects and ameliorates the loss of DE-cadherin in the AS of embryos expressing the *foscrb<sup>Y10A</sup>* variant. (A-F) Stills from dorsal views of live imaging of embryos expressing *DE-cad::GFP* knock-in and heterozygous for the *SCAR<sup>Δ37</sup>* loss of function allele (Video 13). In all images the anterior is to the left, for the genotypes *w;foscrb,DE-cad::GFP/SCAR<sup>Δ37</sup>,DE-cad::GFP;crb<sup>GX24</sup>* and *w;foscrb<sup>Y10A</sup>,DE-cad::GFP/SCAR<sup>Δ37</sup>,DE-cad::GFP;crb<sup>GX24</sup>*. All embryos were collected at the same time (1 hr collection), incubated at 28°C for 7 hr and imaged together. The numbers in (B,D,F) indicate the time in minutes for the corresponding row. DC occurs normally in *foscrb* (A,C,D) embryos heterozygous for the *SCAR<sup>Δ37</sup>* allele, and DC defects are suppressed in *foscrb<sup>Y10A</sup>* (B,D,F) embryos; some defects still visible include the impaired GB retraction (compare B with A), asymmetric position of the posterior spiracles (D, arrows), and bunching of the

Figure 8 continued on next page

*Figure 8 continued*

dorsal epidermis (**D**, bracket). Scale bar: 100  $\mu\text{m}$ . (**G,H**) Magnified views of AS from (**A,B**, respectively). Note that, in order to make the localisation of *DE-cad::GFP* more perceptible, the autofluorescence of the yolk (visible in **A,B**) was removed from the original stack by hand using Fiji. Scale bar: 100  $\mu\text{m}$ . Representative images from 6–9 different embryos for each genotype.

DOI: <http://dx.doi.org/10.7554/eLife.07398.039>