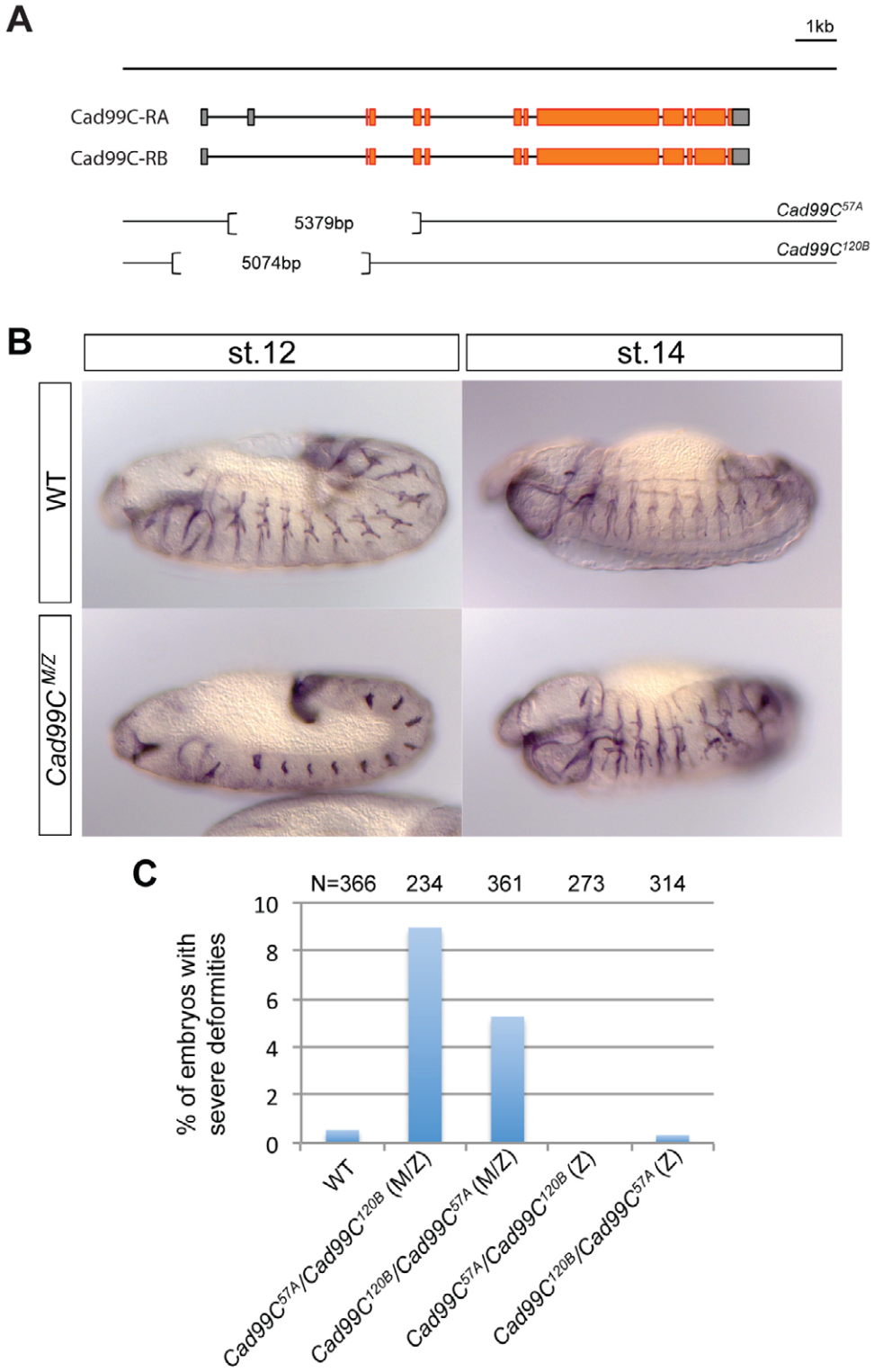

Fig. S1. Molecular map of the *Cad99C* mutant alleles used in this study. (A) The gene structure of *Cad99C* and the two alleles used in this study, *Cad99C*^{57A} and *Cad99C*^{120B}. The starting codon is deleted in both alleles and both are protein null (Schlichting et al., 2005; Schlichting et al., 2006). The exons encoding the open reading frame are shown in orange. (B) A small subset of the *Cad99C*$^{M/Z}$ embryos that develop have severe morphological defects revealed by staining with Crb. (C) Quantification of the number of *Cad99C*$^{M/Z}$ embryos with severe defects out of the total number that develop is shown.

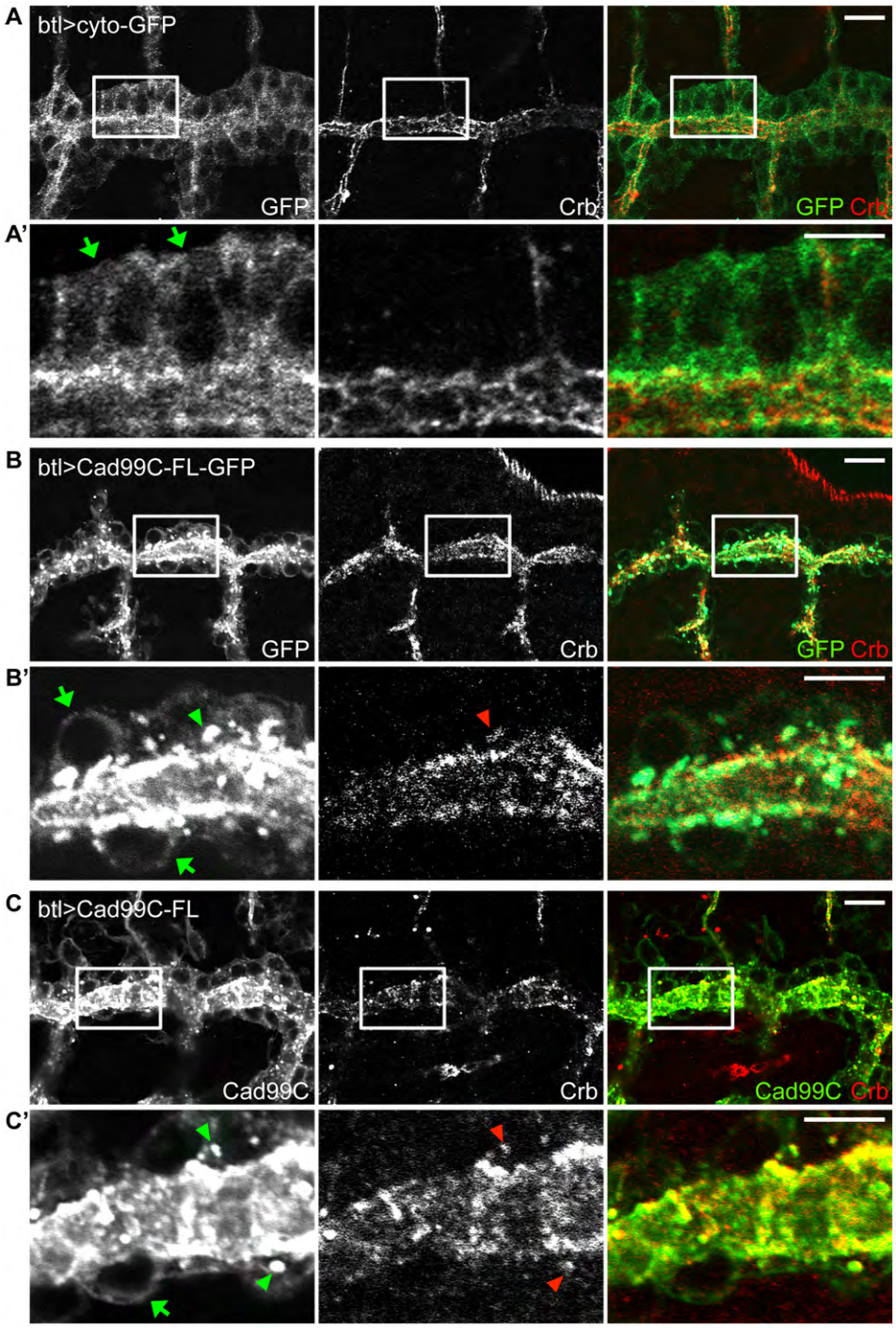

Fig. S2. Cad99C overexpression in the trachea also results in round epithelial cells. (A-C) Single sections of confocal images of stage 15 (A, B) and stage 16 trachea (C) (metameres 4-5 in A, C; metameres 3-5 in B). A'-C' are higher magnifications of the boxed regions in A-C. (A) Control embryo is shown expressing cytoplasmic-GFP in tracheal cells under the control of *breathless* (*btl*)-Gal4. Arrows indicate typical cuboidal cell shapes. (B, C) GFP-tagged (B) or untagged (C) Cad99C-FL overexpression in the trachea with *btl*-Gal4 causes expansion of the apical surface and rounding of tracheal cells (green arrows). Crb marks apical membranes (red). Note that Cad99C overexpression resulted in a number of both Cad99C- and Crb-positive large vesicular structures near the apical surface (green and red arrowheads). Scale bars: 10 ㎜.

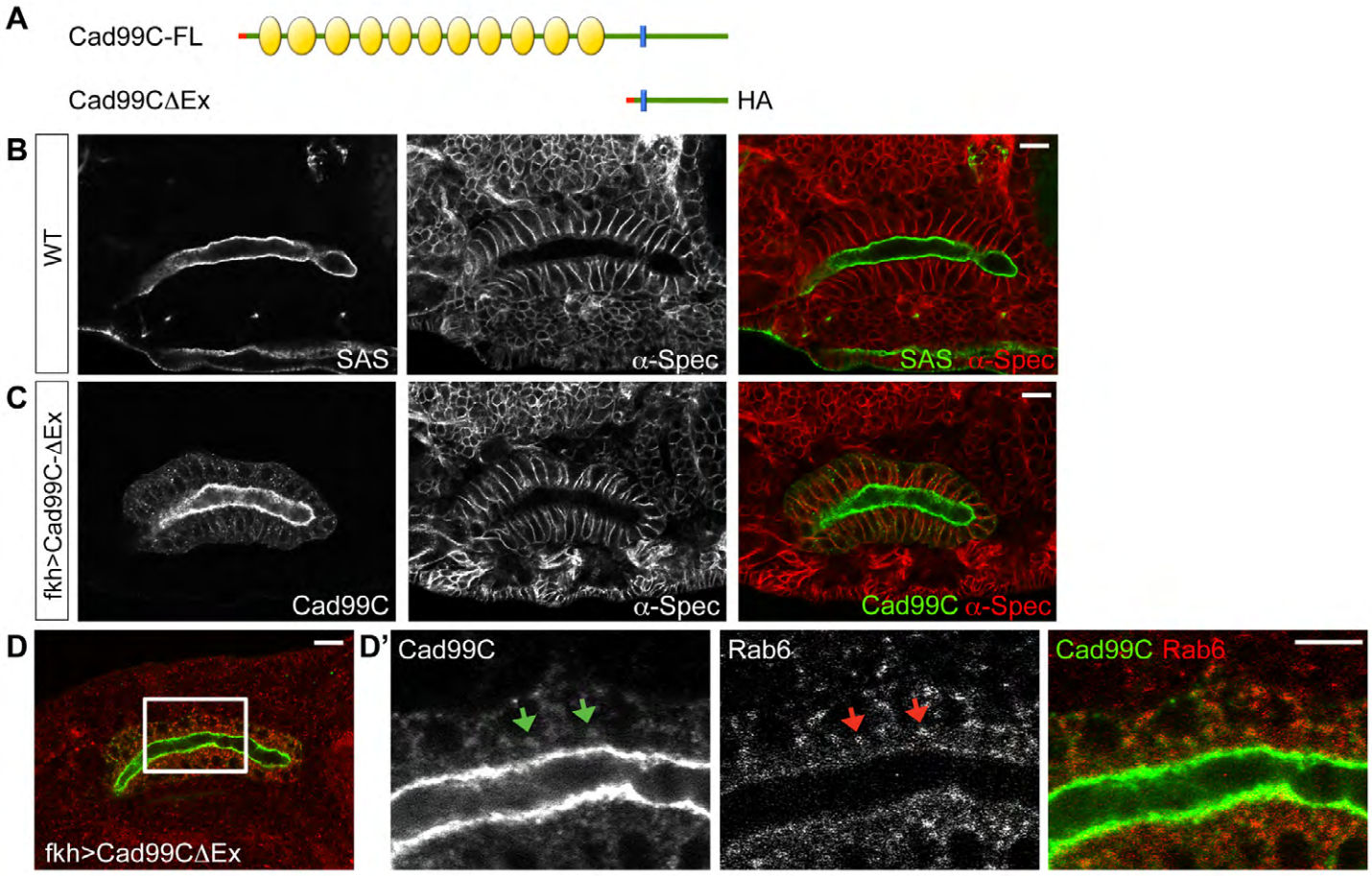

Fig. S3. Extracellular region-deleted Cad99C does not cause overt phenotypes in tubular organs. (A) Schematic drawings of full-length (FL) and extracellular region-deleted (ΔEx) Cad99C proteins. The Cad99CΔEx construct has an HA tag at the C terminus. The red bar, the signal peptide; the yellow ovals, the cadherin repeats; the blue rectangle, the transmembrane domain. (B, C) Stage 15 SGs of WT (B) or Cad99CΔEx-HA (C) are morphologically quite similar. Note that Cad99CΔEx protein is mostly detected in the apical membrane, showing similar patterns to SAS in WT and being complimentary to α-Spec, a lateral membrane marker. (D) The cytoplasmic punctate signals of Cad99CΔEx co-localize with Rab6. Scale bars: 10 ㎜.

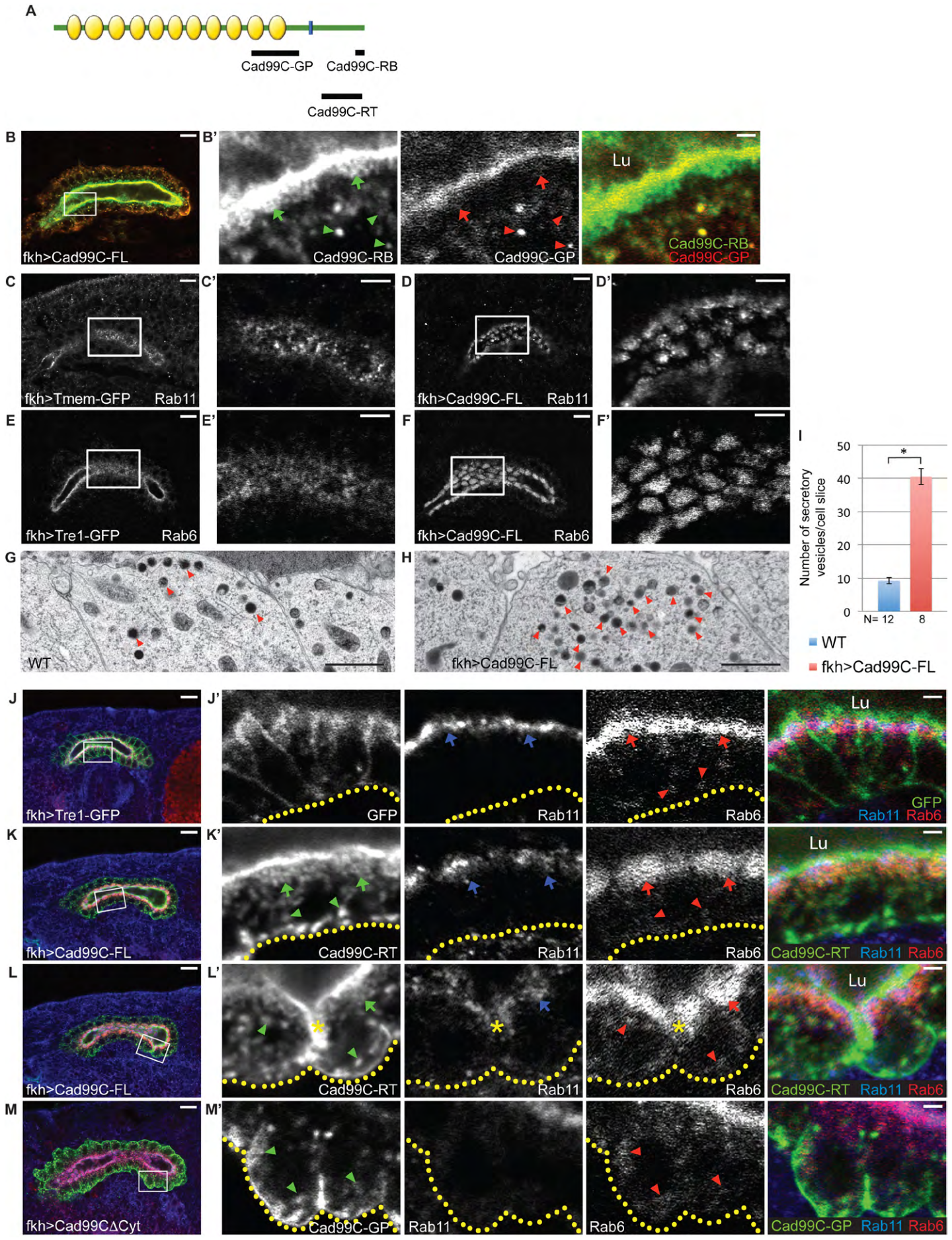
A
Cad99C-GP
Cad99C-RB
Cad99C-RT
B
fkh>Cad99C-FL
B'
Cad99C-RB
Cad99C-GP
Lu
Cad99C-RB
Cad99C-GP
C
fkh>Tmem-GFP
Rab11
C'
D
fkh>Cad99C-FL
Rab11
D'
E
fkh>Tre1-GFP
Rab6
E'
F
fkh>Cad99C-FL
Rab6
F'
G
WT
H
fkh>Cad99C-FL
I
Number of secretory vesicles/cell slice
50
40
30
20
10
0
*
N= 12
8
WT
fkh>Cad99C-FL
J
fkh>Tre1-GFP
J'
GFP
Rab11
Rab6
Lu
GFP
Rab11
Rab6
K
fkh>Cad99C-FL
K'
Cad99C-RT
Rab11
Rab6
Lu
Cad99C-RT
Rab11
Rab6
L
fkh>Cad99C-FL
L'
Cad99C-RT
Rab11
Rab6
Lu
Cad99C-RT
Rab11
Rab6
M
fkh>Cad99CΔCyt
M'
Cad99C-GP
Rab11
Rab6
Cad99C-GP
Rab11
Rab6

Fig. S4. Vesicular Cad99C co-localizes with Rab6. (A) A schematic drawing of the Cad99C protein structure shows regions to which antisera were generated indicated with black bars. Yellow oval, cadherin repeats; blue rectangle, the transmembrane domain. (B) A Cad99C-overexpressing SG stained with Cad99C antibodies specific for the intracellular (Cad99C-RB, green) and the extracellular (Cad99C-GP, red) region. B' shows higher magnification of the boxed region. The apical membrane and vesicular structures near the apical membrane are recognized by both Cad99C-RB (green arrows) and Cad99C-GP (red arrows) antibodies, with stronger signals with Cad99C-RB antibody. Cad99C-positive punctate structures are also observed throughout the cells (arrowheads). (C-F) Images of Rab11 or RFP-Rab6 signals in the SGs of control or Cad99C-overexpressing SGs. C'-F' are higher magnifications of C-F. Compared to control glands (C, E), Cad99C-overexpressing SGs have a dramatic increase of Rab11- and Rab6-positive vesicles near the apical surface (D, F). (G, H) TEM analysis shows an increase in the number of apical vesicles in the Cad99C-overexpressing SGs (arrowheads). (I) A histogram of the quantification of the vesicle numbers per cell slice is shown. Error bars: s.e.m. *, $p<10^{-11}$. (J-M) Images of Rab11 (cyan) and RFP-Rab6 (red) signals in the Tre1-GFP, Cad99C-FL or Cad99CΔCyt overexpressing stage 16 SGs (green). K and L are different focal planes of the same gland. J'-M' are higher magnifications of J-M. Rab11-positive vesicles localize in a narrower domain near the apical membrane, both in control (J') and Cad99C-overexpressing SGs (K'-M'). Cad99C-positive vesicular structures overlap more with Rab6-positive vesicles than with Rab11-positive vesicles (arrows in K'-M'). Cad99C-positive punctate structures found throughout the cytoplasm (green arrowheads in K'-M') co-localize with Rab6 signals (red arrowheads in K'-M') but not with Rab11. All confocal images shown here are single sections. Lu, lumen. Scale bars: 10 mm in B-F, J-M and C'-F'; 2 mm in G, H, B', J'-M'.

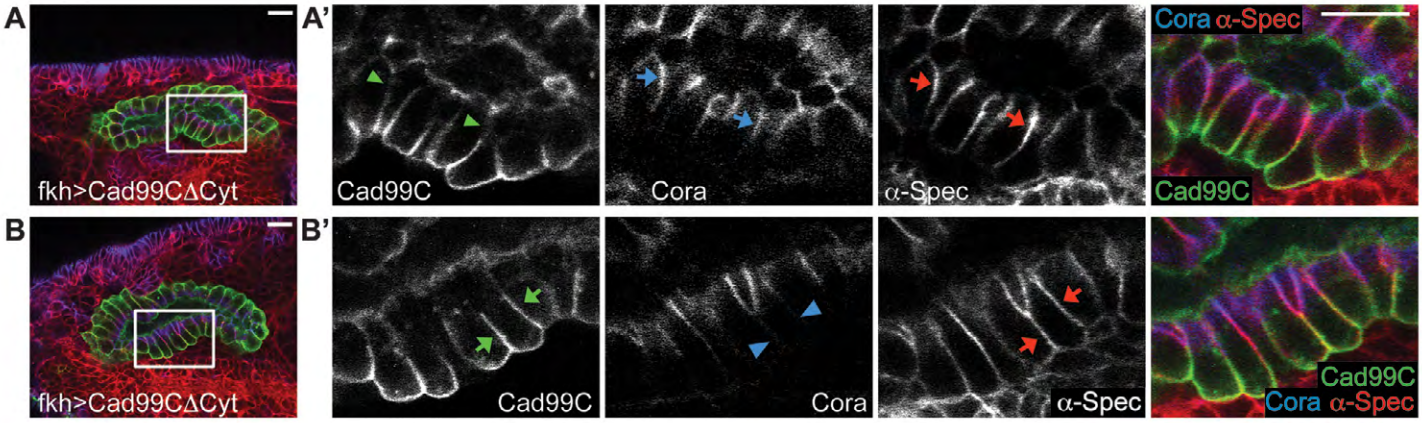

Fig. S5. Mislocalized Cad99C signals are mutually exclusive with Cora and α-Spec in rounded cells. (A, B) A single section of a confocal image of stage 16 SG overexpressing Cad99CΔCyt. Higher magnification of the boxed region is shown in A', B'. (A) In many cases, Cora (SJ marker) and α-Spec (lateral membrane marker) show the same localization pattern (cyan and red arrows), which is mutually exclusive with mislocalized Cad99C (green arrowheads). (B) In some cases, however, α-Spec signals overlap mislocalized Cad99C (green and red arrows), where Cora is absent (cyan arrowheads). Scale bars: 10 mm.

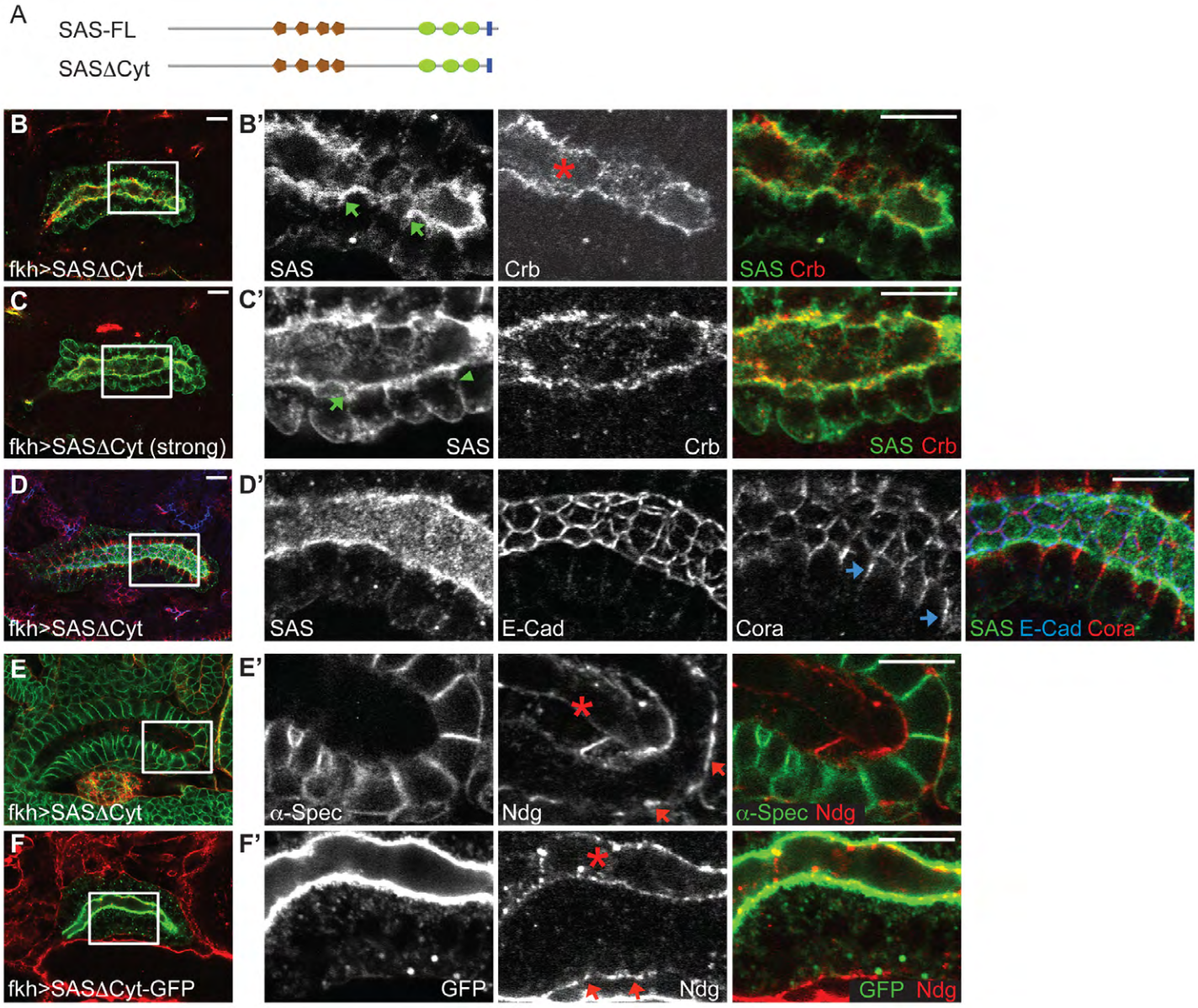

Fig. S6. SASΔCyt expression almost completely phenocopies SAS-FL overexpression. (A) Schematic drawings of full-length (FL) and cytoplasmic region-deleted (ΔCyt) SAS proteins. The brown pentagons and the green ovals represent von Willebrand factor (vWF) type C domains and Fibronectin type 3 domains, respectively. The blue rectangle indicates the transmembrane domain. (B) SASΔCyt-overexpressing SGs have expanded apical membranes protruding into the lateral domain (green arrows). Note that the Crb signals are more dispersed and less enriched in the SAR (red asterisk). (C) Strong expression of SASΔCyt causes mislocalization of SAS to the basal domain and cell roundness. The expanded apical membranes protrude into the lateral domain (green arrow). SAS signals are absent in the putative SJ domain (green arrowhead). (D) SAS overexpression does not affect AJs, showing intact E-Cad signals, but causes elongated SJs (cyan arrows). (E, F) SASΔCyt expression causes mislocalization of Ndg in the apical matrix (red asterisks). The basal Ndg sheet appears normal (red arrows). Scale bars: 10 ㎜.

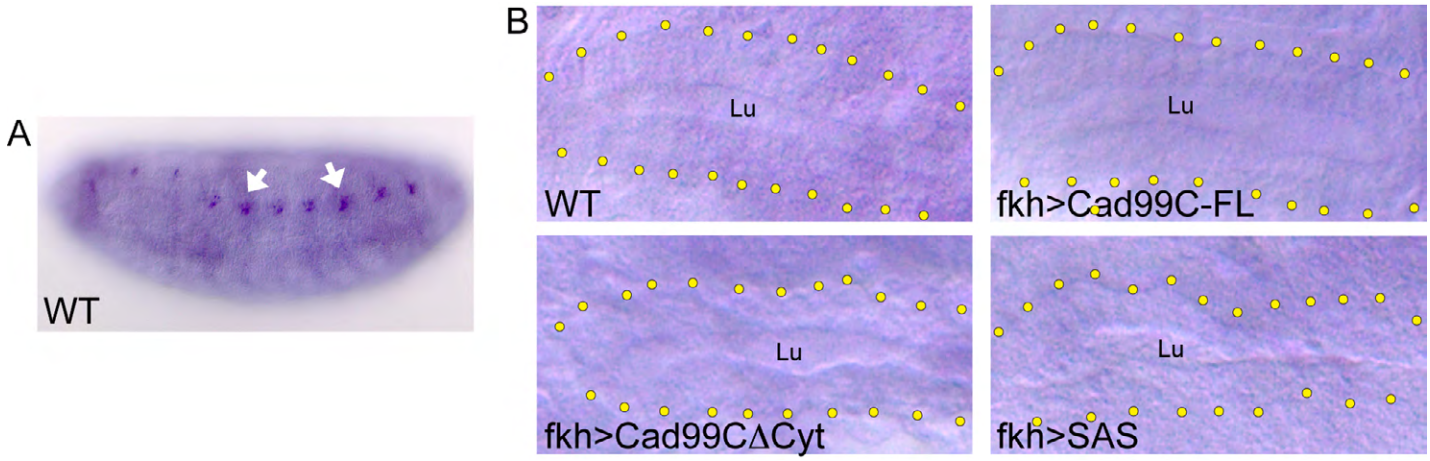

Fig. S7. *ndg* mRNA localization is not affected by Cad99C or SAS overexpression. (A) In situ hybridization of *ndg* against WT embryos. *ndg* is expressed in a subset of mesodermal cells (arrows). (B) Higher magnification of *ndg* in situs in the WT, Cad99C-FL-, Cad99CΔCyt- and SAS-overexpressing SGs. Yellow dots mark the gland. Lu, the lumen.