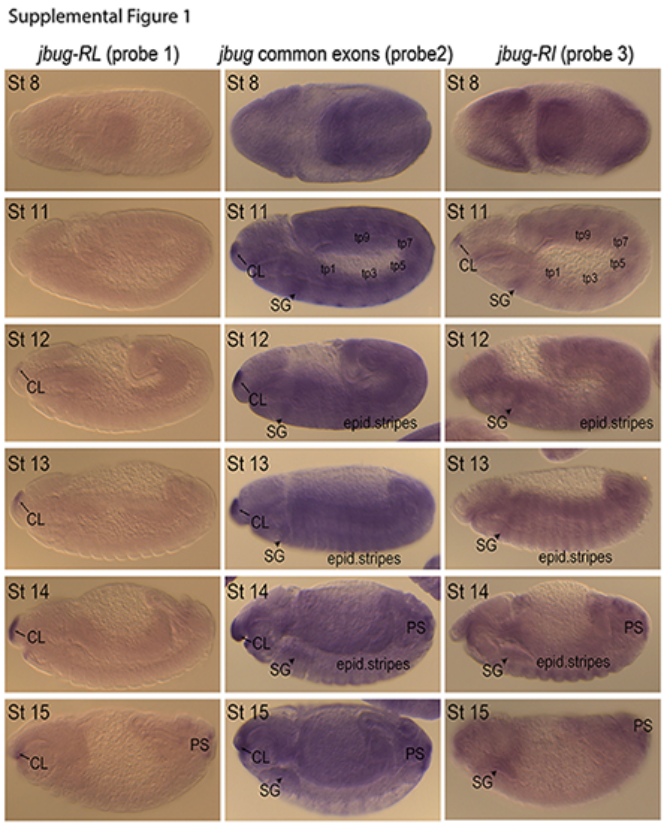
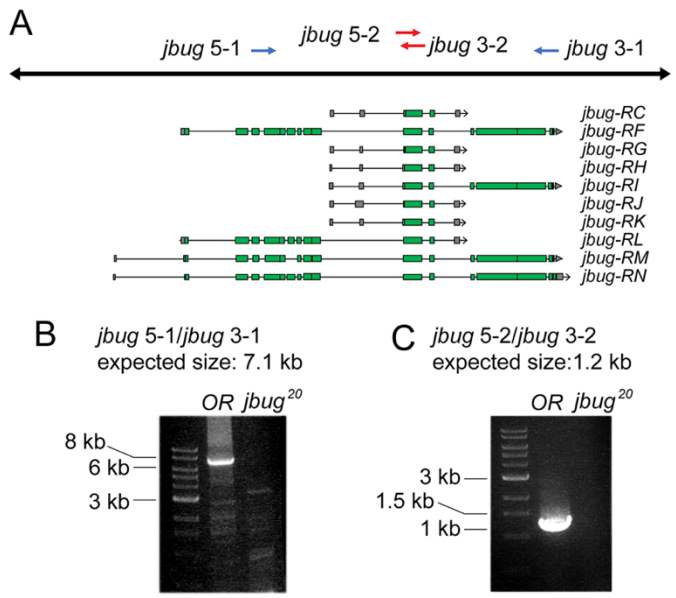


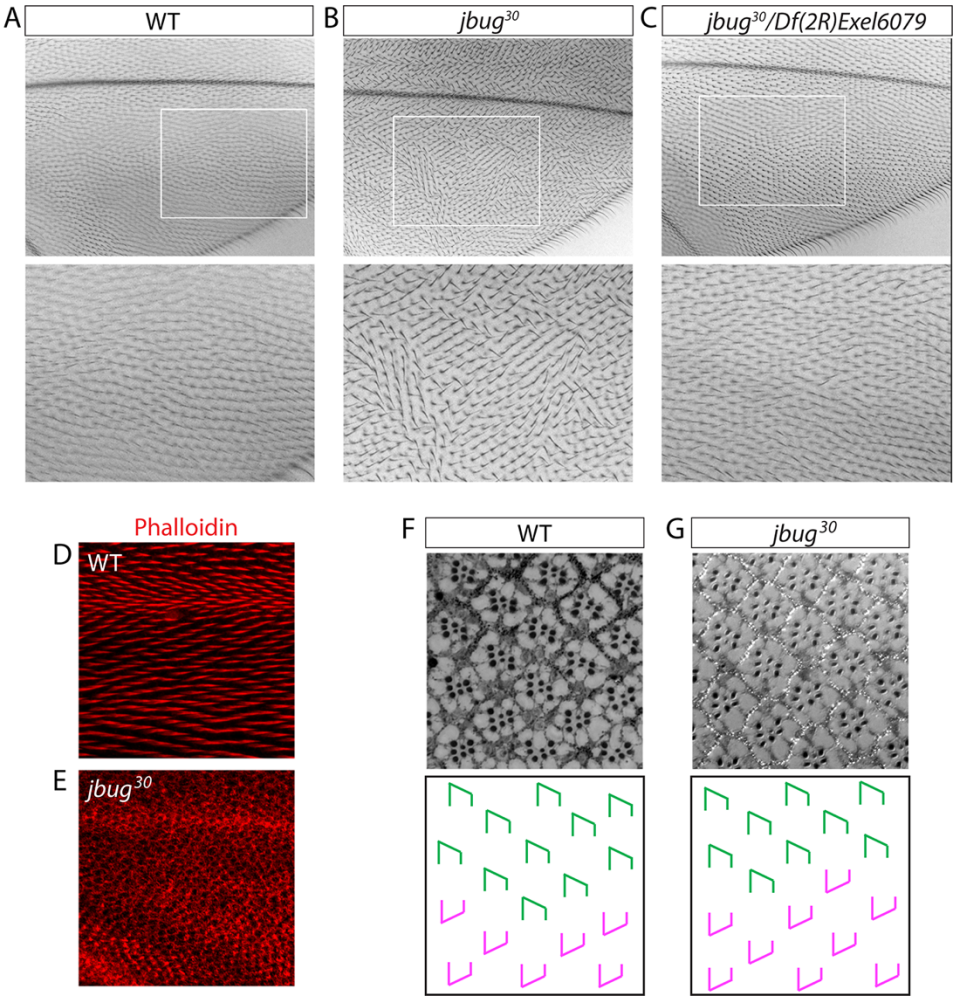
Supplemental Figure 1. In situ hybridization. In situ hybridization of wild-type embryos using probes specific for different *jbug* splice forms as shown in Figure 1A. CL, clypeolabrum. SG, salivary gland. tp, tracheal pits. PS, posterior spiracles.

Supplemental Figure 2

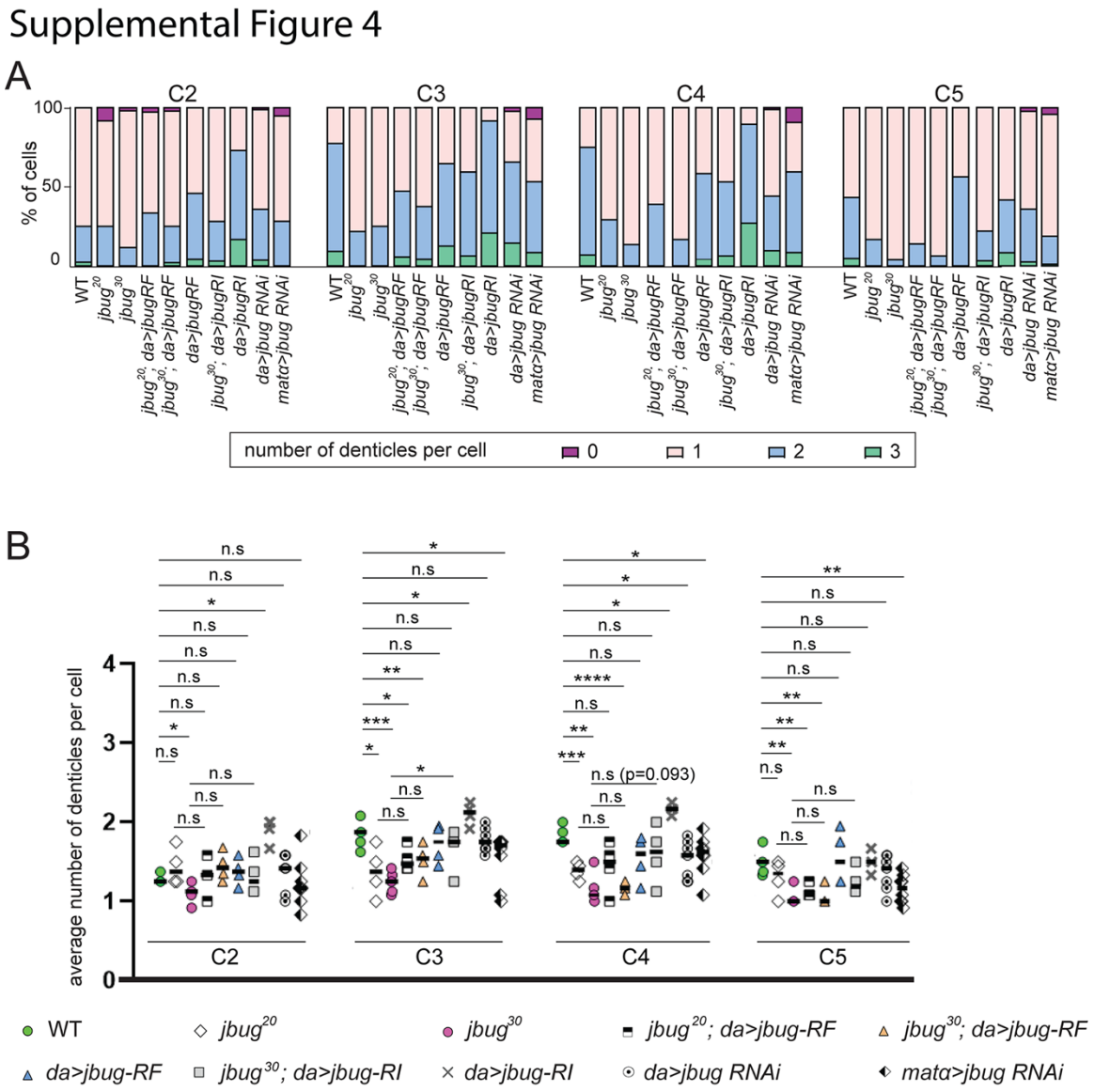


Supplemental Figure 2. Reverse transcriptase-PCR (RT-PCR) reveals a loss of *jbug* mRNA in *jbug*^{20} null mutants. (A) Genomic structures for the jbug locus. Blue and red arrows represent primers used for RT-PCR. (B and C) RT-PCR using cDNAs made from wild-type (OR) and *jbug*^{20} homozygous embryos. Whereas RT-PCR using wild-type cDNA produces a PCR product of expected sizes (left lanes in B and C), no bands are detected in *jbug*^{20} (right lanes in B and C).

Supplemental Figure 3

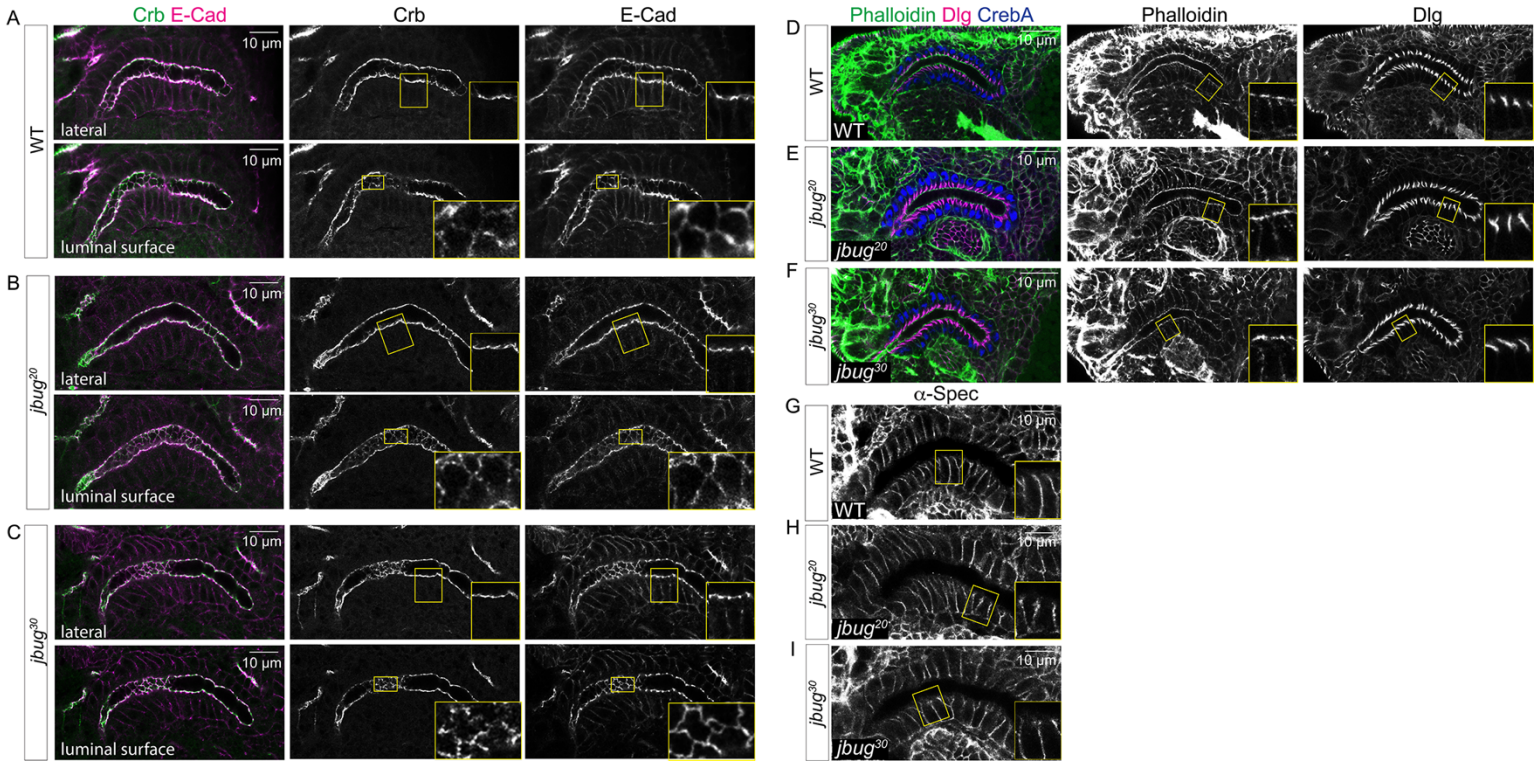


Supplemental Figure 3. Mutations in *jbug* cause mild planar cell polarity defects in adult wing hair orientation. (A-C) Adult wings. Compared to the largely distal orientation of wing hairs in the wild-type wing (A), wings in *jbug*^{30} mutants (B) show mild swirling patterns of wing hairs. (C) *jbug*^{30}*/Exel6079* flies do not show defects in the wing hair orientation. White boxed regions are shown in higher magnification in the bottom. (D, E) Wild-type (D) and *jbug*^{30} mutant (E) pupal wings (32 hours APF) stained for phalloidin. Prehair formation is delayed in the pupal wing in *jbug*^{30}. (F, G) Adult ommatidia of wild type (F) and *jbug*^{30} (G) near the dorsal/ventral boundary, the equator. Schematic drawings are shown in the panels below the actual images. Green and magenta shapes indicate the orientation of ommatidia normally found in the dorsal and ventral hemisphere of the eye, respectively. *jbug*^{30} flies do not show planar cell polarity defects in the orientation of ommatidia.



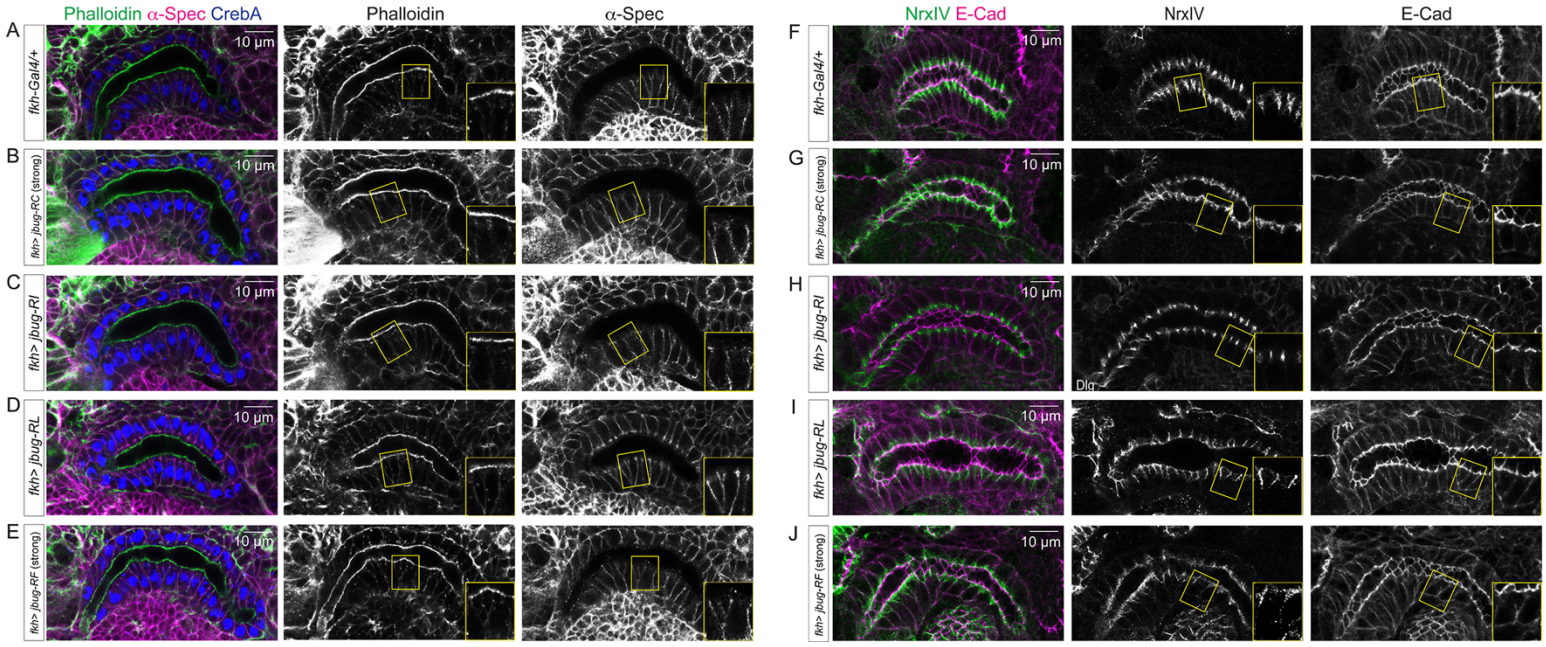
Supplemental Figure 4. Jbug regulates the formation of denticle precursors in the ventral epidermis of the embryo. (A) Percentage of cells that have different numbers of denticles in rows C2-C5. (B) Quantification of the average number of denticles per cell in C2-C5. Student's t-test with Welch's correction is used for statistics calculation. The same four cells as shown in Figure 5K were chosen for quantification for each genotype.

Supplemental Figure 5



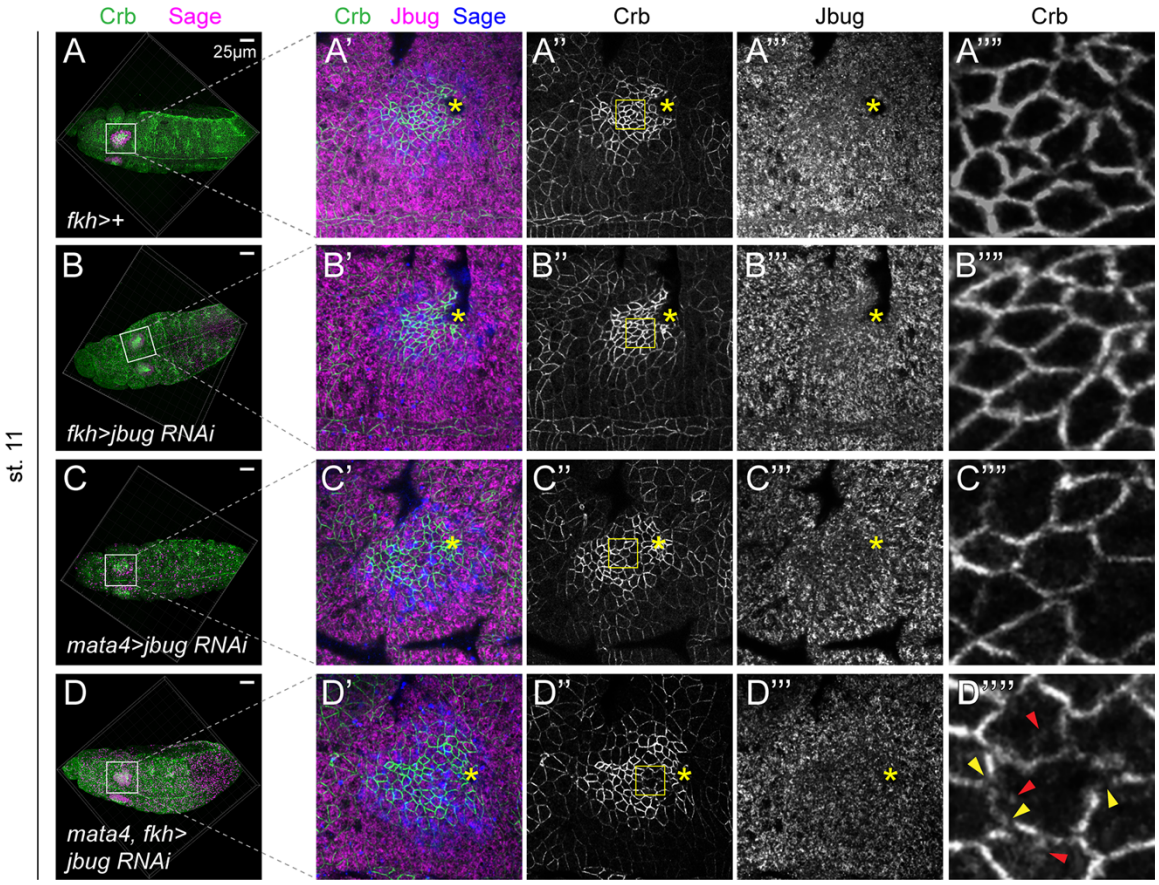
Supplemental Figure 5. Epithelial polarity markers and F-actin and are not affected in salivary glands in *jbug* mutants. (A-I) Confocal images of stage 16 salivary glands. (A-C) Wild-type (A), *jbug*^{20} (B) and *jbug*^{30} (C) salivary glands stained for Crb (green) and E-Cad (magenta). (D-F) Wild-type (E), *jbug*^{20} (F) and *jbug*^{30} (G) salivary glands stained for phalloidin (green) and Dlg (magenta). (G-I) Wild-type (G), *jbug*^{20} (H) and *jbug*^{30} (I) salivary glands stained for α-Spec. Insets, higher magnification of the boxed region.

Supplemental Figure 6



Supplemental Figure 6. F-actin, epithelial polarity markers and junction markers are not significantly altered in salivary glands overexpressing different Jbug isoforms. (A-J) Confocal images of stage 16 control (*fkh-Gal4/+*, A and F) and salivary glands overexpressing Jbug-RC (B and G), Jbug-RI (C and H), Jbug-RL (D and I) and Jbug-RF (E and J). (A-E) Salivary gland stained for phalloidin (green), α-Spec (magenta) and a salivary gland nuclear marker CrebA (blue). (F-J) Salivary glands stained for Nrx-IV (green) and E-Cad (magenta). Insets, higher magnification of the boxed region.

Supplemental Figure 7



Supplemental Figure 7. (A-D) Confocal images of stage 11 embryos stained for Crb (green), and Sage (blue). Control (*fkh-Gal4/+*; A), zygotic (*fkh>jbug RNAi*; B), maternal (*matα>jbug RNAi*; C), and both maternal and zygotic knockdown of *jbug* (*matα, fkh>jbug RNAi*; D). (A'-D''') Higher magnification of the salivary glands in A-D. Crb (green), Jbug (magenta) and Sage (blue) signals are shown. (A''-D''') Crb (A''-D'') and Jbug (A'''-D''') only. Jbug signals are slightly reduced in *jbug* knockdown, with a more significant reduction in maternal (C''') and maternal/zygotic knockdown (D'''). (A''''-D'''') Higher magnification of the boxed region in A''-D''. Maternal/zygotic knockdown of *jbug* results in gaps (yellow arrowheads) and mislocalized Crb signals to the apical surface (red arrowheads).