Supplementary Figures

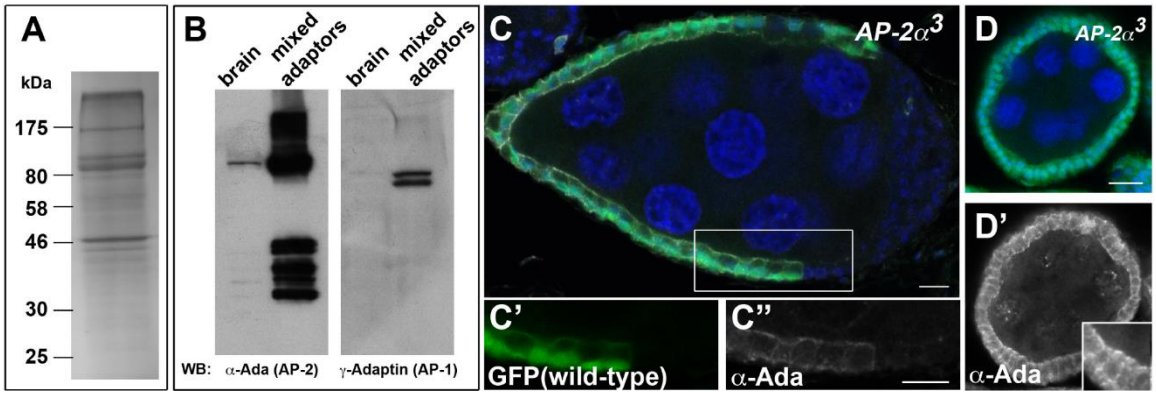

Fig. S1. (A) Coomassie blue-stained gel of mixed adaptors purified from pig brain. (B) Western blot of mixed adaptors, probed for α-Ada (represents AP-2) or γ-adaptin (represents AP-1). AP-2 is predominant in the mixed adaptors. (C-D') Follicles containing *AP-2α*^{3} clones (lacking GFP) in the germ line and part of the follicle epithelium (C-C'') and in the germ line only (D, D'), stained for DAPI (blue) and α-Ada (C'', D', white). Egg chambers were at stage 7 (D) and stage 4 (E). α-Ada is localized at the apical membrane in wild-type follicle cells facing mutant germ line cells. Scale bars represent 10 μm.

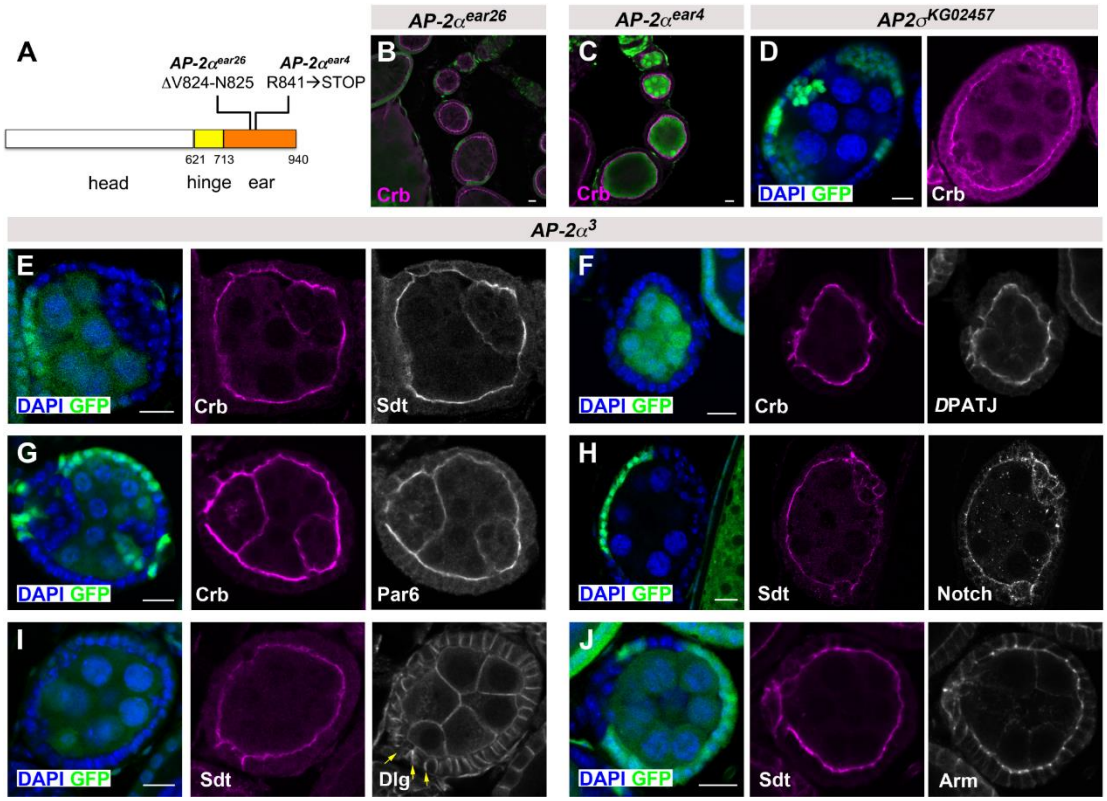

Fig. S2. (A) The scheme depicts the molecular defects of *AP-2α* mutants. Follicles mutant for *AP-2α*^{ear26} (B) and *AP-2α*^{ear4} (C) (lacking GFP) do not show any polarity defects in the follicle epithelium. (D) *AP-2σ*^{KG02457} follicle cell clones (labeled with GFP) are round and develop multi-layered tissues. Punctate staining of Crb around the cell outline is observed in some clones. In *AP-2α*^{3} mutant follicle cells (lacking GFP), Sdt (E) and *D*PATJ (F) are mis-localized and redistributed together with Crb. Par6 (G) and Notch (H) are mis-localized in *AP-2α*^{3} mutant follicle cells. (I) Dlg retracts when Crb expands to the lateral membrane (yellow arrow). (J) Arm is redistributed in *AP-2α*^{3} mutant follicle cells. Scale bars represent 10 μm.

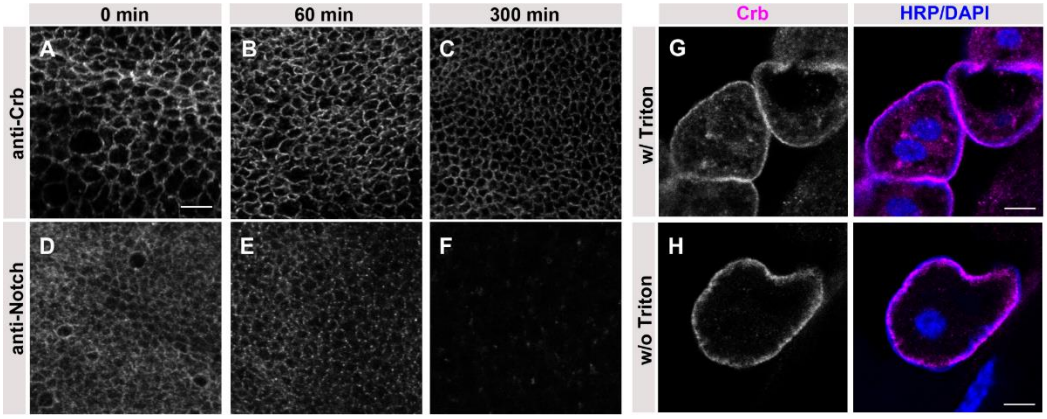

Fig. S3. (A-F) Antibody uptake assay in wing discs. Wing discs were pulsed with anti-Crb (A-C) or anti-Notch antibodies (D-F), and then chased for the indicated time periods. After 60 min chase, Notch appeared mostly in punctae, while Crb was still associated with the plasma membrane. After 300 min, Notch punctae were almost gone, while most Crb protein remained at the plasma membrane. Scale bar represents 5 μm. (G-H) Garland cells of 3rd instar larvae were fixed and stained for Crb (magenta) and membrane marker, HRP (blue) in the presence (w/) or absence (w/o) of Triton. (G) Upon permeabilization, Crb is detected at the plasma membrane and intracellularly. (H) Surface staining of Crb in garland cell. Scale bars represent 10 μm.

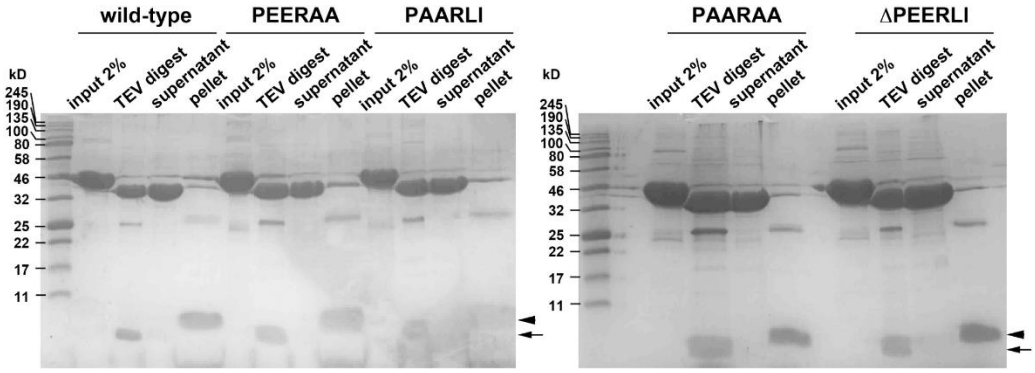

Fig. S4. Coomassie blue-stained gel of purified mCrb2 tail (input), after cleavage of the tag (TEV digest), and after coupling to liposomes and high-speed centrifugation (supernatant, pellet). The mCrb2 tail displays modified migration behavior before (arrow) and after (arrowhead) coupling, probably caused by the covalently attached lipid. The purity and coupling efficiency is shown for the wild-type and the different mutant variants.