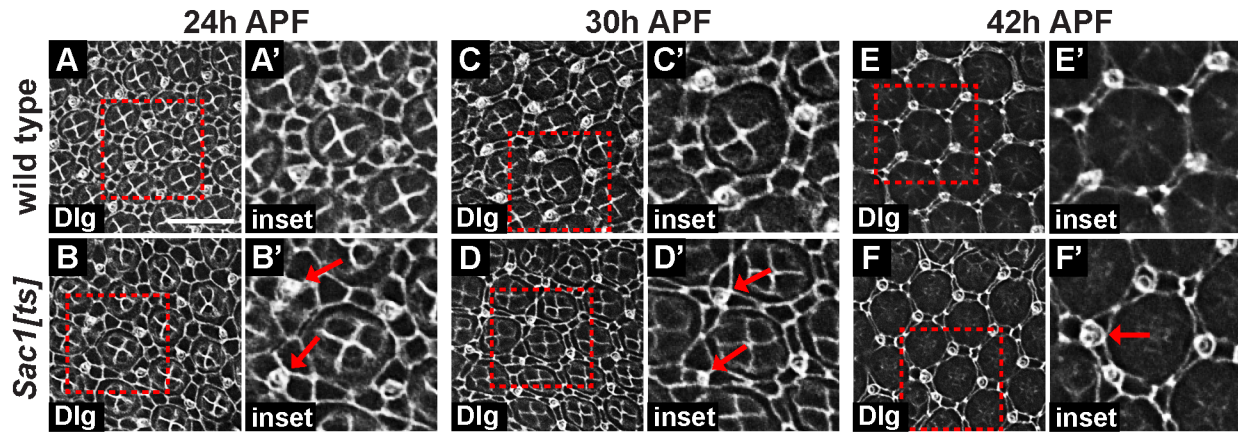
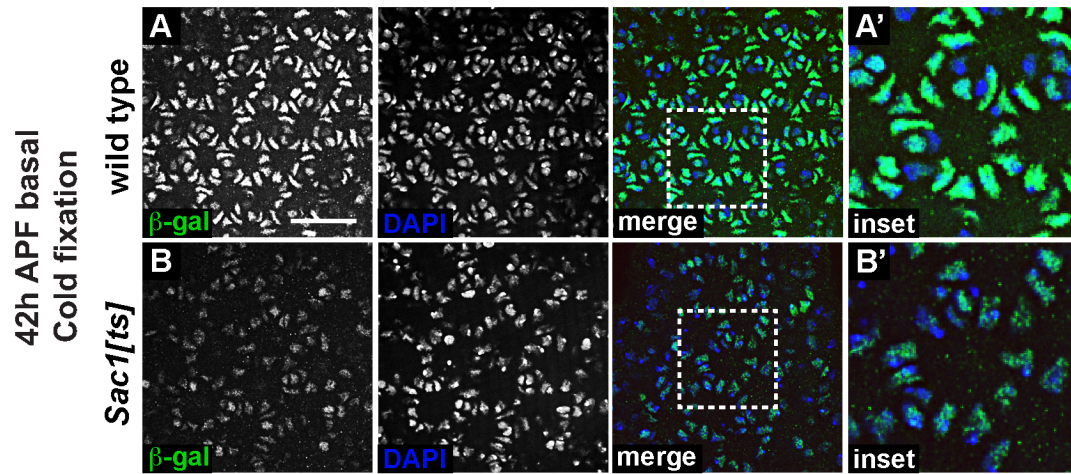


**Figure S1. Sac1(PR) has reduced phosphatase activity compared to Sac1(WT).** (A)

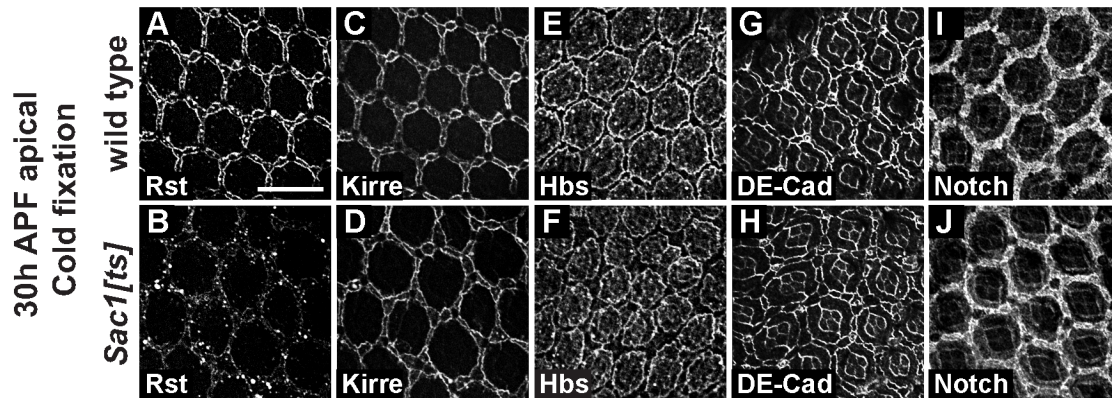
Phosphatase assays performed on Flag-tagged hSac1, dSac1, hSac1(PD) and dSac1(PR) affinity purified from HeLa cells. Incubation time in minutes is indicated. (B) Immunoblot confirming expression of Flag-tagged constructs used in (A).



**Figure S2. *Sac1* is required for proper IOC patterning.** (A-F') Confocal micrographs of pupal retinas stained for Discs large (Dlg, white) at 24h, 30h and 42h APF. Red-boxed regions in A-F are magnified 2-fold in A'-F'. Note defects in IOC organization and shape, as well as misplaced or extra bristle cells in *Sac1<sup>ts</sup>* (B', D', F' arrows). Scale bar: 15µm. Flies were raised at 25°C.

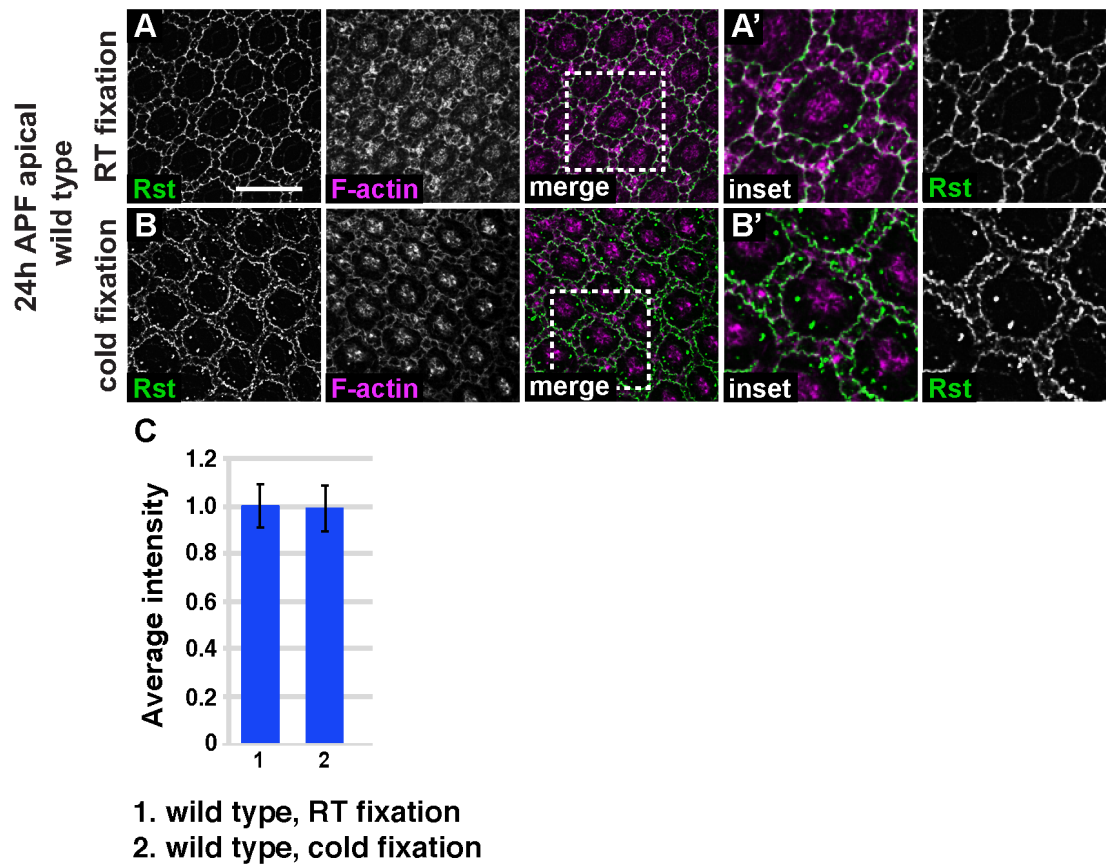


**Figure S3. *Sac1<sup>ts</sup>* mutants 2°/3°pc exhibit defects in cell fate.** (A-B') Confocal images showing basal regions of WT and *Sac1<sup>ts</sup>* retinas expressing the cell specification marker BA12-lacZ at 42h AFP. Retinas were stained for β-gal (green) and DNA (blue). Boxed regions in A,B are magnified 2.5-fold in A',B'. Scale bar: 15μm. Flies were raised at 25°C.



**Figure S4. Defect in Rst localization is not due to a developmental delay in *Sac1<sup>ts</sup>* mutants.**

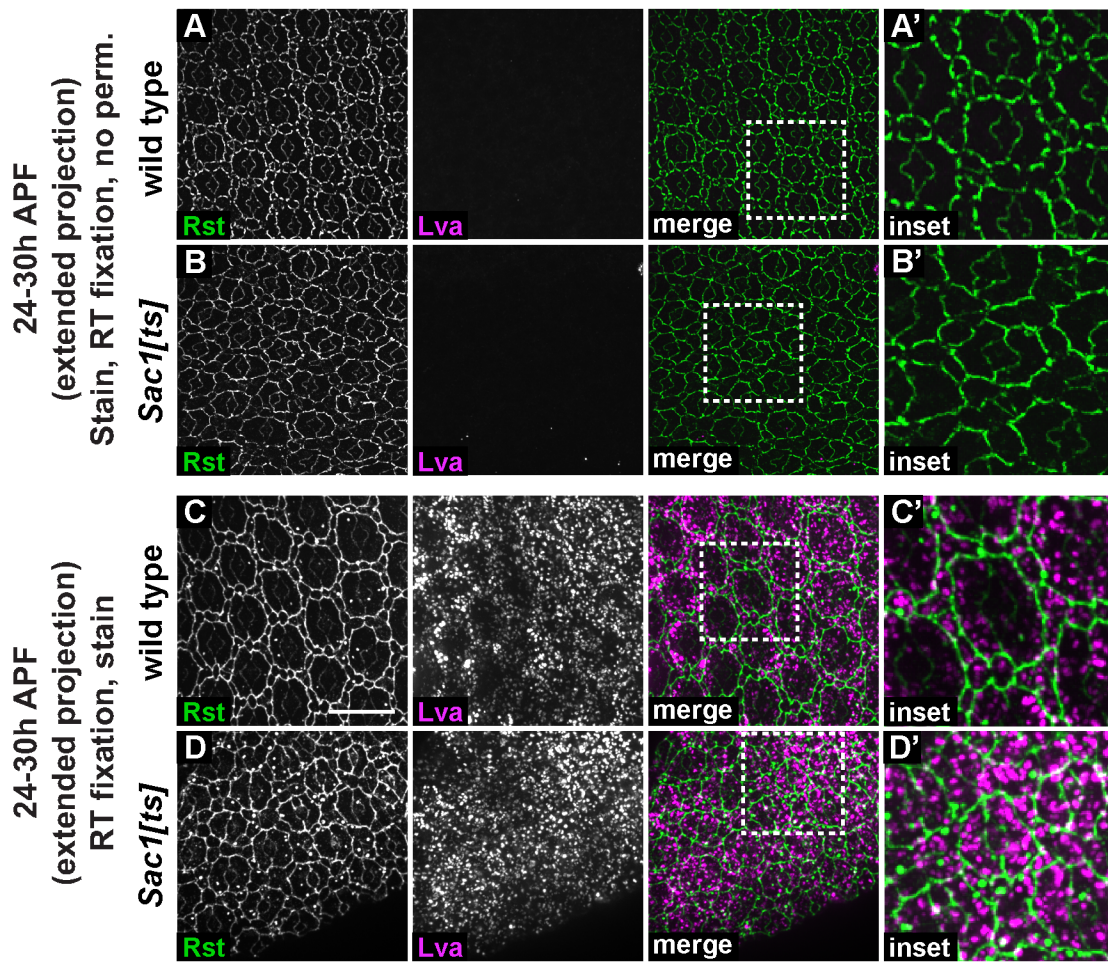
(A-J) Confocal images of pupal retinas at 30h APF fixed on ice and stained for Rst (A,B), Kirre (C,D), for Hibris (E,F), DE-cad (G,H) or Notch (I,J). Scale bar: 15  $\mu$ m. Flies were raised at 25°C.



**Figure S5. Rst accumulation is not affected by cold fixation in wild type retinas. (A-B')**

Apical confocal sections of 24h APF wild type retinas fixed at RT (A) or on ice (cold fixation, B) and stained for Rst (green) and F-actin (magenta). Boxed regions in A,B are magnified 2-fold in A',B'. White in merged images indicates colocalization. (C) Rst intensities at the 1°pc:IOC border, normalized to RT fixation. Error bars, SD. n=90 from a total of 29 different eyes from 3 independent experiments. Scale bar: 15  $\mu$ m. Flies were raised at 25°C.





**Figure S6. Rst is present at the plasma membrane in *sac1<sup>ts</sup>*.** (A-B') Extended projection of confocal images of WT and *Sac1<sup>ts</sup>* pupal retinas at 24-30h APF stained for Rst (green) and Lva (magenta). Retinas were fixed and imaged without permeabilization. (C-D') Extended projection of confocal images of WT and *Sac1<sup>ts</sup>* pupal retinas at 24-30h APF. Retinas were fixed and stained for Rst (green) and Lva (magenta). Boxed regions in A-D are magnified 2-fold in A'-D'. White in merged images indicates colocalization. Scale bar: 15µm. Flies were raised at 25°C.