

Expanded View Figures

Figure EV1. *rpk* knockdown decreases DiBAC staining and Rpk expression at the A-P compartment boundary.

- A Expression of *rpk*-RNAi in the wing pouch decreases patterned DiBAC staining (white arrowhead).
- B Expression of *rpk*-RNAi in the wing pouch decreases anti-Rpk staining anterior to the A-P compartment boundary (white arrowhead).
- C, D Control discs. Increased DiBAC staining and anti-Rpk staining are visible in the centre of the wing pouch (white arrowheads).

Data information: Scale bars are 100 μ m.

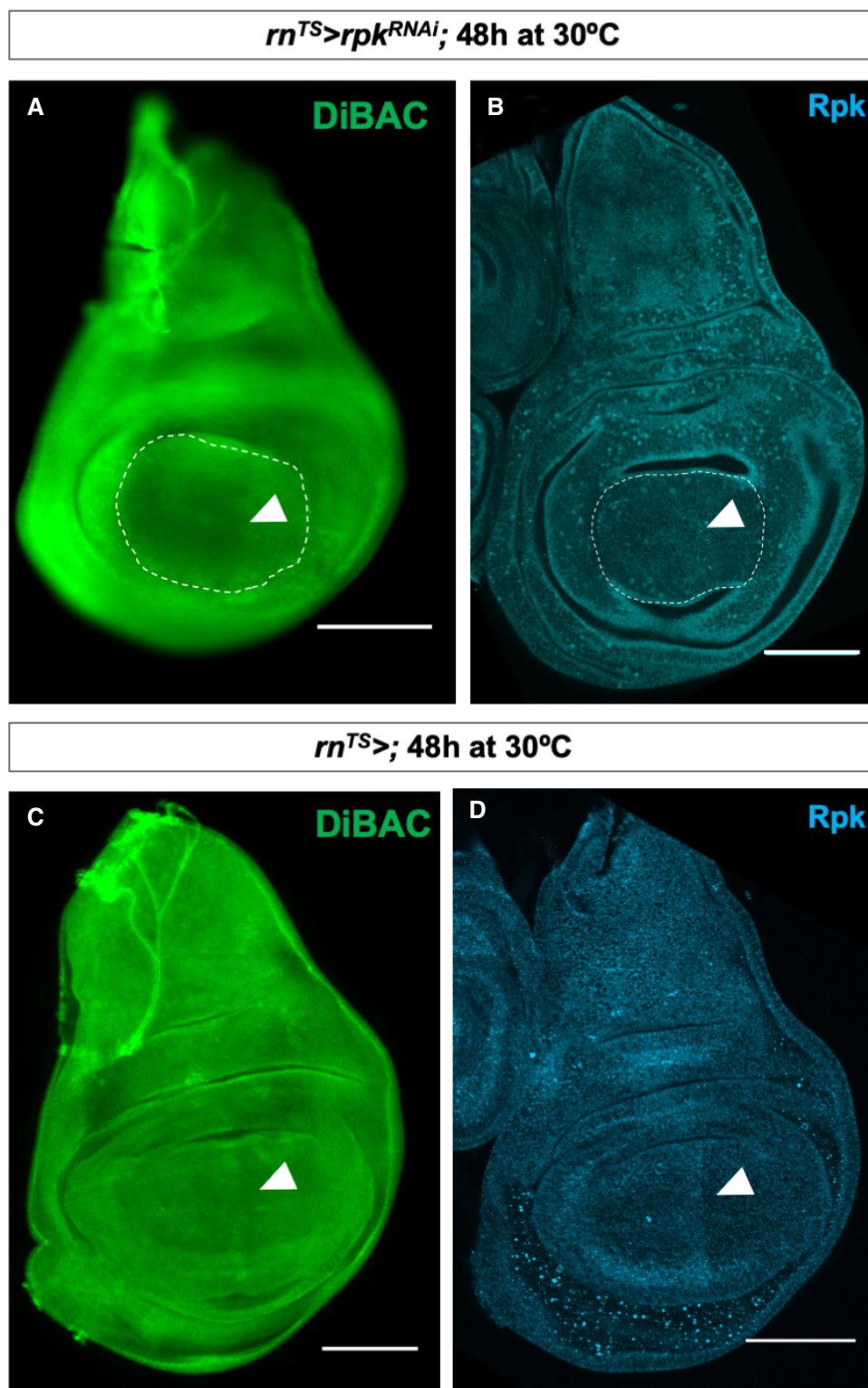


Figure EV1.

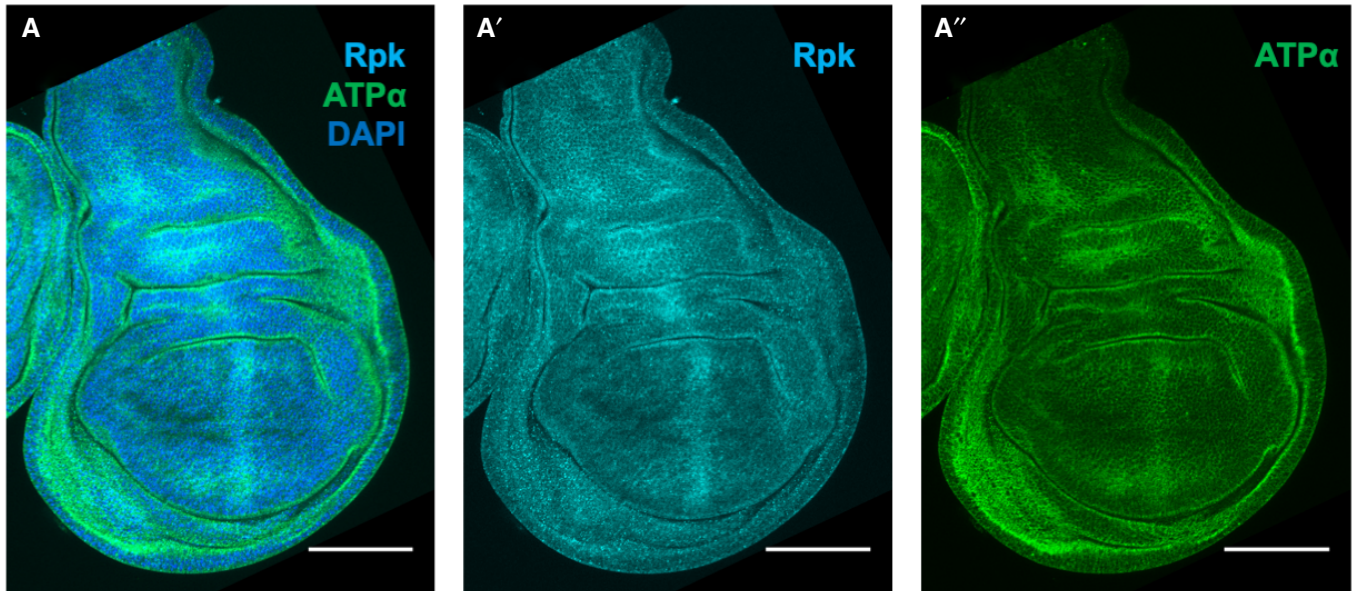
***hh^{AC}/+*, restrictive temperature for 12h**

Figure EV2. Patterned Rpk and ATPα expression and membrane depolarization require Hh signalling.

A–A'' Immunostaining of discs heterozygous for the *hh^{AC}* allele and upshifted to 30°C for 12 h with antibodies to Rpk and ATPα showing elevated levels of both proteins anterior to the A-P compartment boundary. All scale bars are 100 μm.

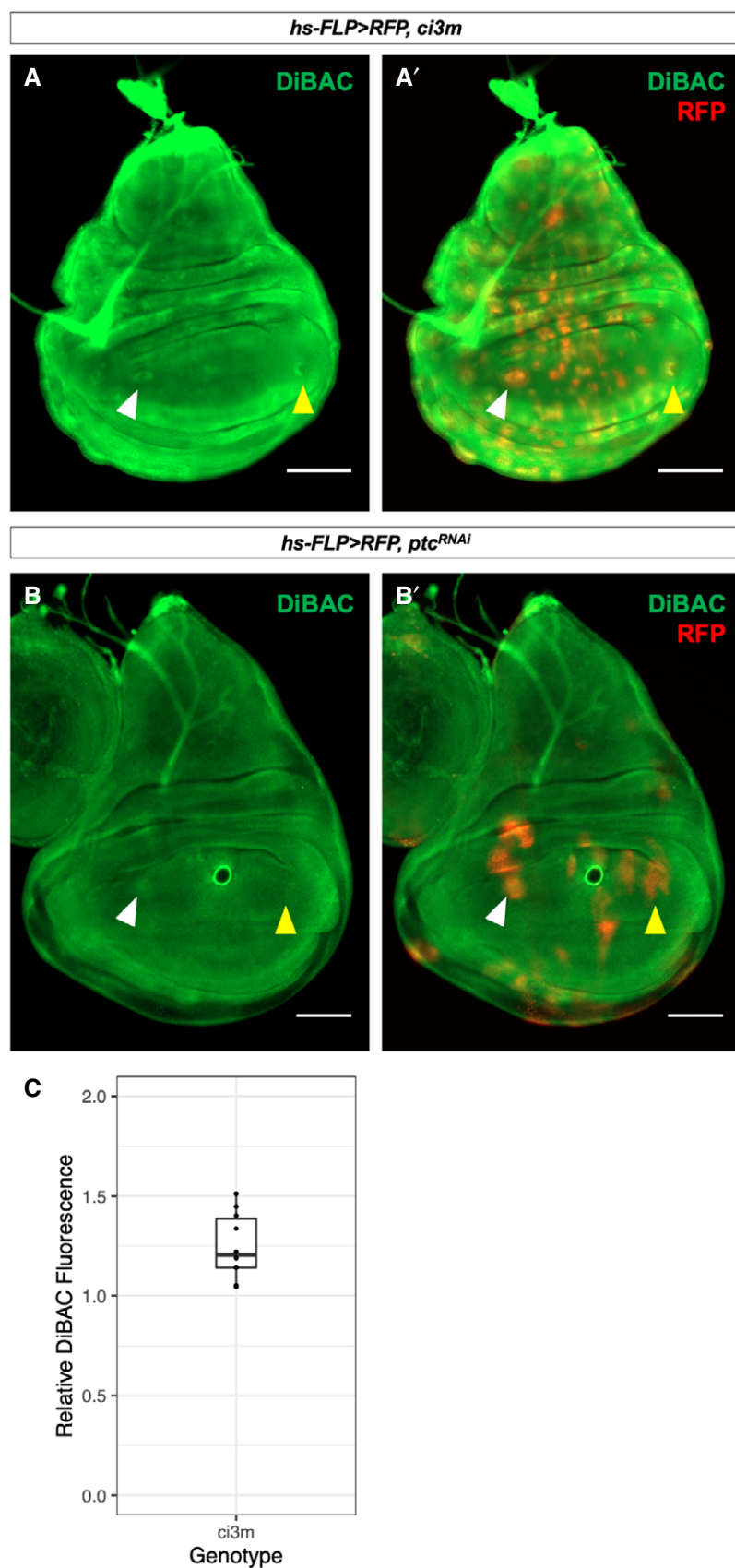


Figure EV3. Hh pathway activation results in depolarization.

- A–A' Clones of cells expressing *ci3m* accumulate more DiBAC relative to surrounding tissue in both the anterior (white arrow) and posterior (yellow arrow) compartments.
- B–B' Clones of cells expressing *ptc*-RNAi accumulate more DiBAC relative to surrounding tissue in the anterior compartment (white arrow), but not the posterior compartment (yellow arrow).
- C The ratio of DiBAC fluorescence in *ci3m*-expressing clones to equally-sized regions of tissue not expressing *ci3m*. $N = 10$ clones from three imaginal discs. Individual data points are shown, as well as a box plot showing descriptive statistics. Bounds of the box show lower and upper quartiles, bar within the box shows the median of the data, and whiskers show minimum and maximum bounds of data.

Data information: All scale bars are 100 μm .

Figure EV4. Manipulating levels of the ion channel Rpk impacts both Hh and Wg signal transduction.

- A, B Expression patterns of *ap-Gal4* (A) and *mn-Gal4* (B).
- C, D Immunostaining of Smo protein (red), full-length Ci (light blue) (C–C') and Ptc protein (D) in discs expressing *ATPα^{RNAi}* in the dorsal compartment. Brackets indicate tissue expressing RNAi and control tissue.
- E–F'' Immunostaining of Smo protein (red) and full-length Ci (light blue) in (E–E'') control discs and (F–F'') discs expressing *rpk^{RNAi}* for 48 h before dissection.
- G–H' Immunostaining of Ptc protein in (G, G') control discs and (H, H') discs expressing *rpk^{RNAi}* for 48 h before dissection.
- I–J'' Immunostaining for Wg protein in (I–I'') control discs and (J–J'') discs expressing *rpk^{RNAi}* for 48 h before dissection.
- K–L' Immunostaining for Cut protein in (K, K') control discs and (L, L') discs expressing *rpk^{RNAi}* for 48 h before dissection.

Data information: All scale bars are 100 μm except (D), where scale bar is 50 μm.

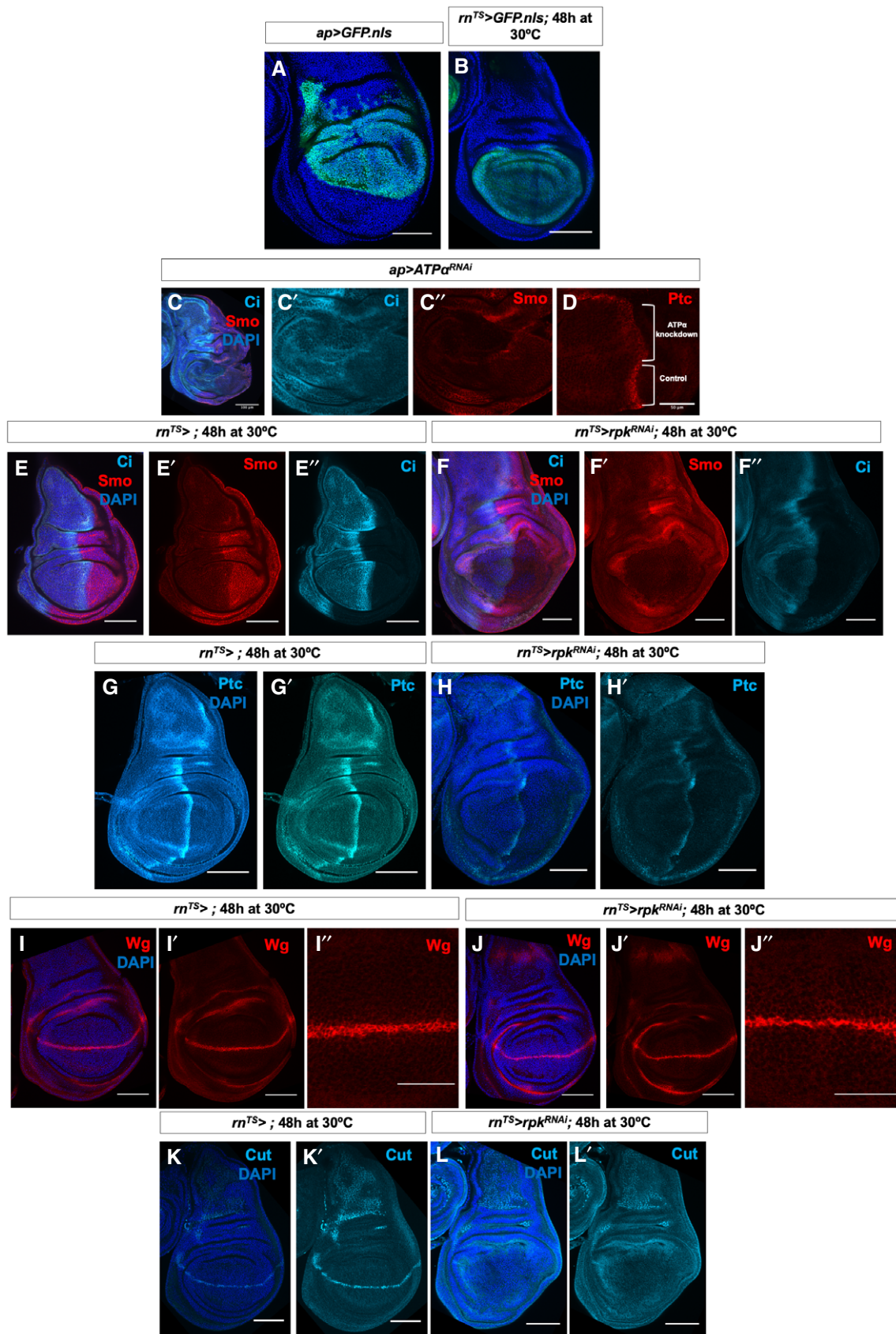


Figure EV4.

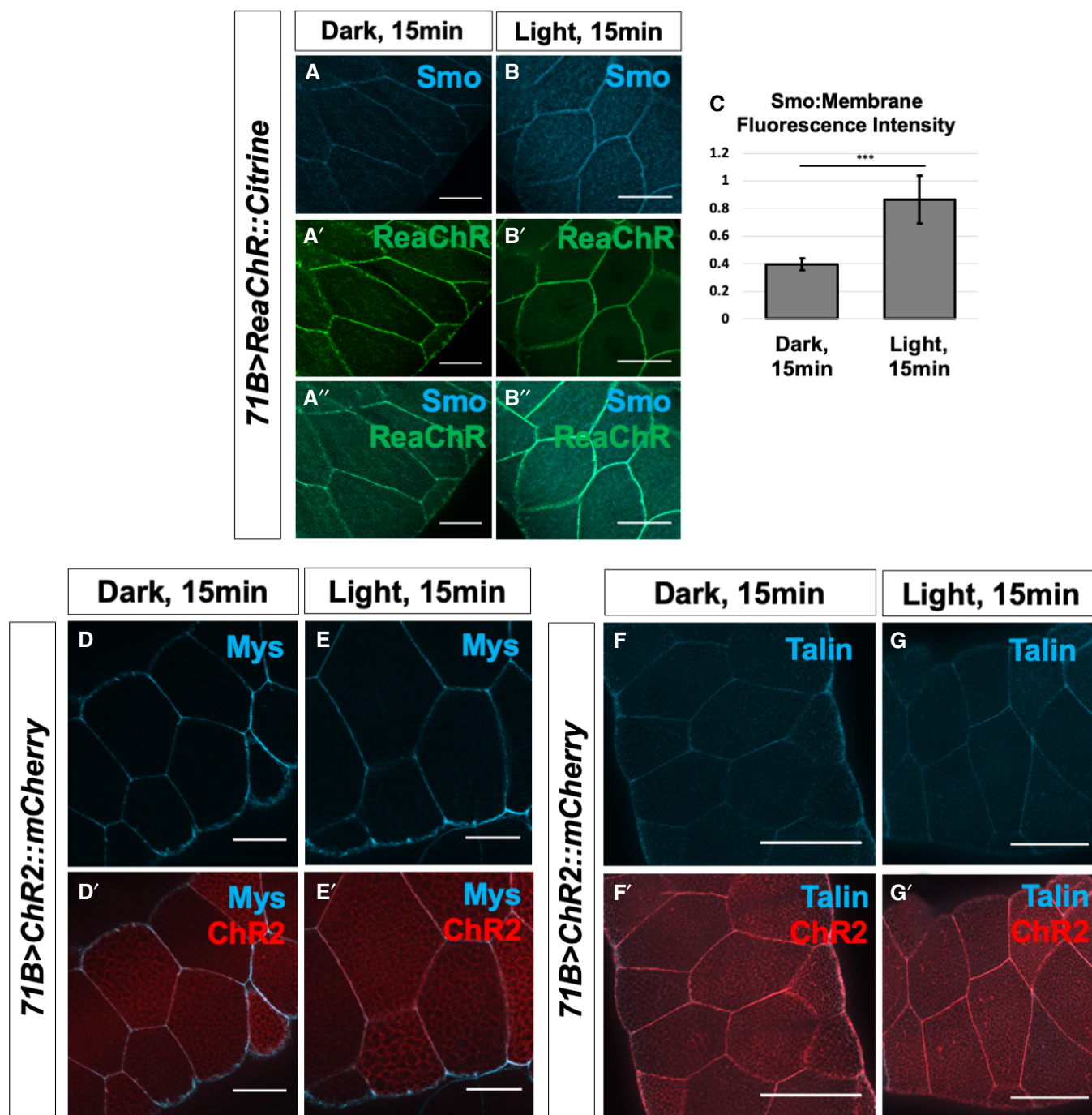


Figure EV5. Optogenetic manipulation of V_{mem} in *Drosophila* salivary glands.

A–G' Salivary glands expressing the channelrhodopsin ReaChR were subjected to 15 min in activating light, then fixed and stained for Smo protein (A–C). $N = 9$ glands, data were compared using an unpaired t -test ($***P < 0.001$), error bars are standard deviations. Membrane abundance of the beta integrin subunit Mys and the integrin-associated protein Talin are not altered upon ChR2 activation (D–G'). Scale bars are 50 μ m in all panels.