

Supplementary material – Hedgehog morphogen gradient is robust towards variations in tissue morphology in *Drosophila*

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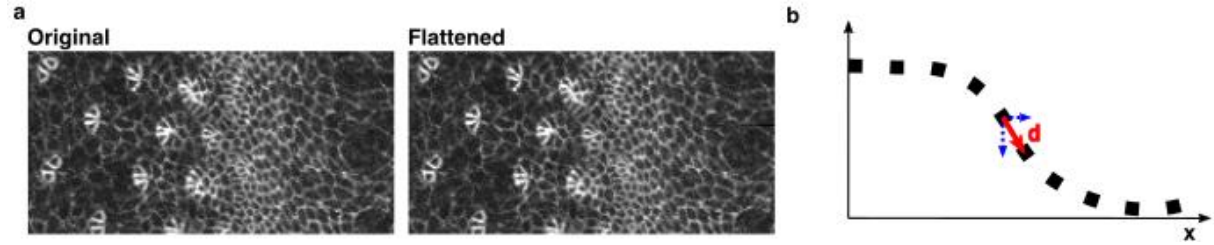


Figure S1: Validation of the flattening procedure and schematics of the contour length computation.

(a) z-projection of a section of an eye disc immunostained for E-cadherin before (left) and after (right) flattening. (b) Schematics of the Euclidean distance (d , red arrow) over the apical edge. The blue arrows indicate the x and z components of d ($d_x = \Delta x = 0.21 \mu\text{m}$, $d_z = (a(x) - a(x-1))\Delta z$ with $\Delta z = 0.45 \mu\text{m}$).

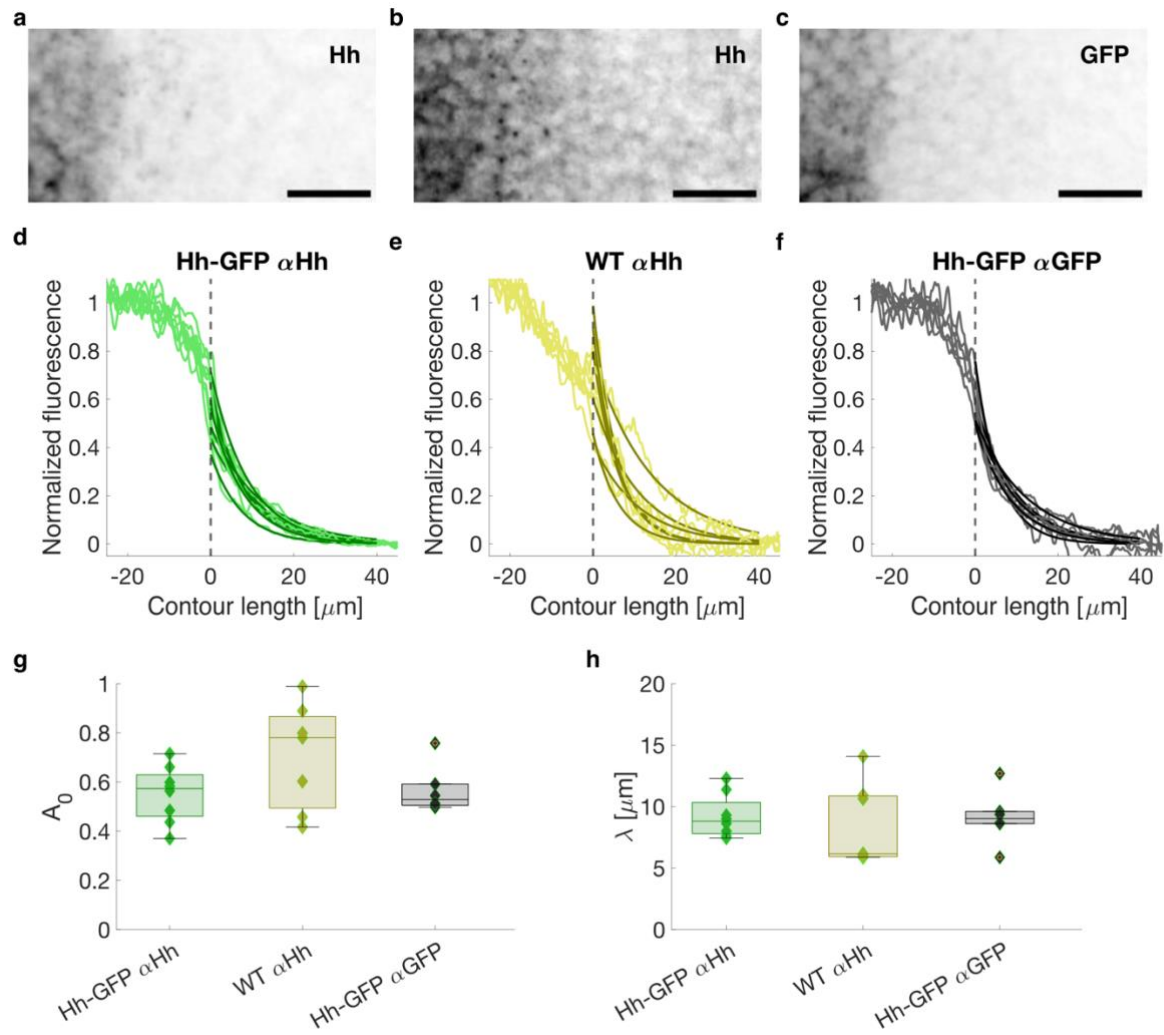


Figure S2: Quantification of the characteristic length of the Hedgehog gradient in the wing disc of wild-type (WT) and Hh-GFP larvae stained for Hh and GFP.

(a-c) z-projection (sum) of a region of interest of wing discs expressing Hh-GFP (a) or wild type wing discs (b) stained for Hh, or wing discs expressing Hh-GFP stained for GFP (c). Scale bars are 10 μ m. (d-f) Normalized fluorescence intensities relative to the contour length resulting from staining for Hh of wing discs expressing Hh-GFP(d) or wild type wing discs (e), or for staining for GFP of wing discs expressing Hh-GFP (f). In each panel, the number of replicates was: N=8 (d), N=7 (e), N=6 (f) wing discs. The x-axis was repositioned setting the PA boundary (estimate) as 0. Darker lines show the result of single exponential fitting in the anterior compartment. (g-h) Parameters estimated by single exponential fitting. A_0 is the amplitude of the exponential and λ is the characteristic length of the exponential.

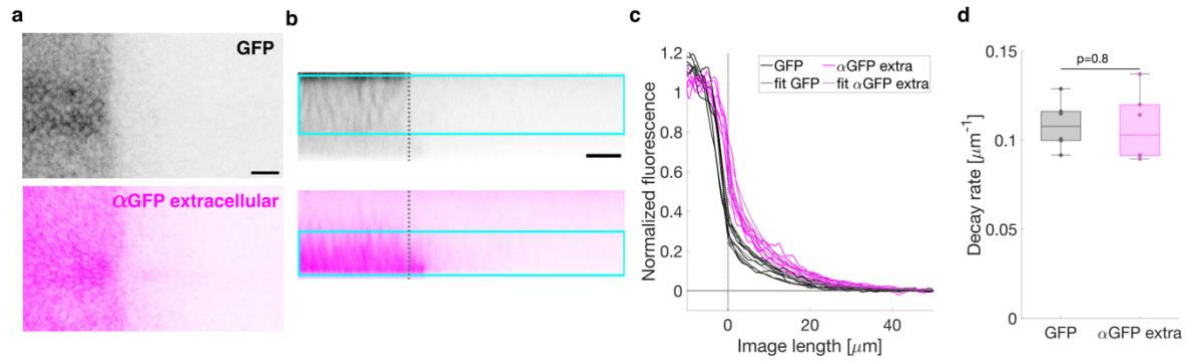


Figure S3: The decay rate of the intracellular Hh-GFP gradient resembles the decay rate of the extracellular Hh-GFP gradient.

(a) z-projection (sum) of a region of interest of wing discs expressing Hh-GFP (*hh-Gal4/UAS-hh-GFP*). GFP fluorescence (GFP, grey) and extracellular GFP antibody staining (magenta) are shown. **(b)** y-projection (sum) of a cross-section of the same region of interest shown in the previous panel **(a)**. Regions of interest used for quantification are highlighted in cyan (apico-lateral (20 μm height) for GFP and baso-lateral (15 μm height) for extracellular GFP). The dotted lines represent the estimated PA boundary. Scale bars are 10 μm. **(c)** Normalized fluorescence intensities for N=6 wing discs. The x-axis was repositioned setting the PA boundary (estimate) as 0. Lighter lines show the result of single exponential fitting in the anterior compartment. **(d)** Decay rate estimated by single exponential fitting. P-value resulting from a two-sample t-test.

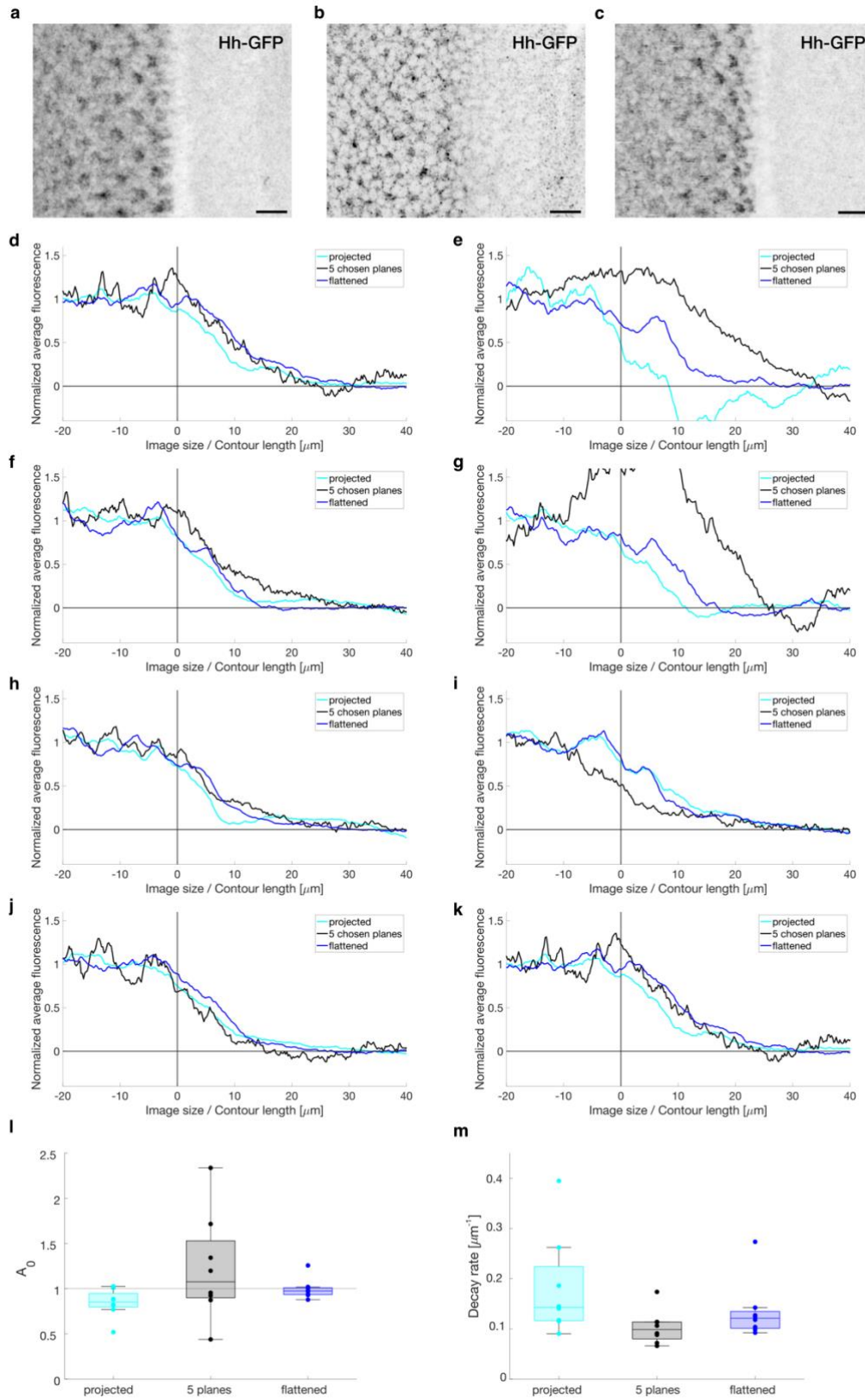


Figure S4: Comparison between different analysis methods: z-projection of the whole image, choice of 5 quantification planes (chosen according to the strongest fluorescence intensity in

the region of the gradient) and our analysis pipeline (flattening and correction for contour length).

(a-c) Top views of a region of interest of an eye disc expressing Hh-GFP and stained for GFP. z-projections (sum) **(a)** over the whole image stack, **(b)** over 5 chosen z-planes or **(c)** over z-planes located within 15 μm from the apical side after flattening **(c)**. Scale bars are 10 μm . **(d-k)** Normalized fluorescence intensities as a function of contour length relative to the position of the PA boundary for different eye discs obtained from standard z-projection, choice of 5 planes and according to our methodology (flattening and correcting for contour length). The intensities are averaged over the whole region of interest. **(l-m)** Amplitude and decay rate estimated by single exponential fitting of the curves in anterior compartment for N=8 eye discs (same as Fig. 3 main text) are shown.

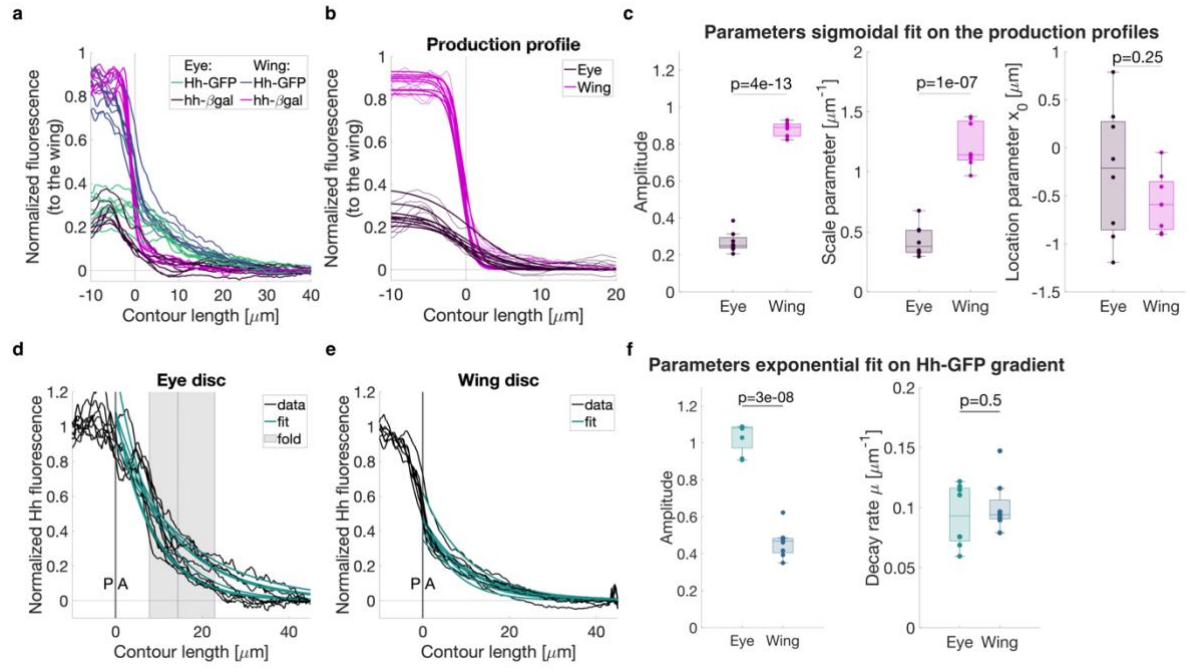


Figure S5: Quantification of the expression level, expression profile and gradient slope for the morphogen Hedgehog in the eye and wing disc.

(a) Hh-GFP and hh- βgal fluorescence intensities normalized to the maximum intensity value of the wing and shown as a function of contour length relative to the position of the PA boundary for N=8 eye discs and N=8 wing discs. Pairs of eye and wing discs were extracted from the same larvae. (b) Hh expression profile (hh- βgal in a): single curves (thin) and sigmoidal fit (thick) are shown. (c) Amplitude, scale parameter and location parameter resulting from the sigmoidal fits in panel b. (d-e) Normalized Hh-GFP fluorescence intensities as a function of contour length for N=8 eye discs (d) and N=8 wing discs (e). Single curves (black) and exponential fit results (dark cyan) are shown. (f) Amplitude and decay rate resulting from the exponential fits shown in panels d and e. Statistical significance testes by a two-sample t-test.

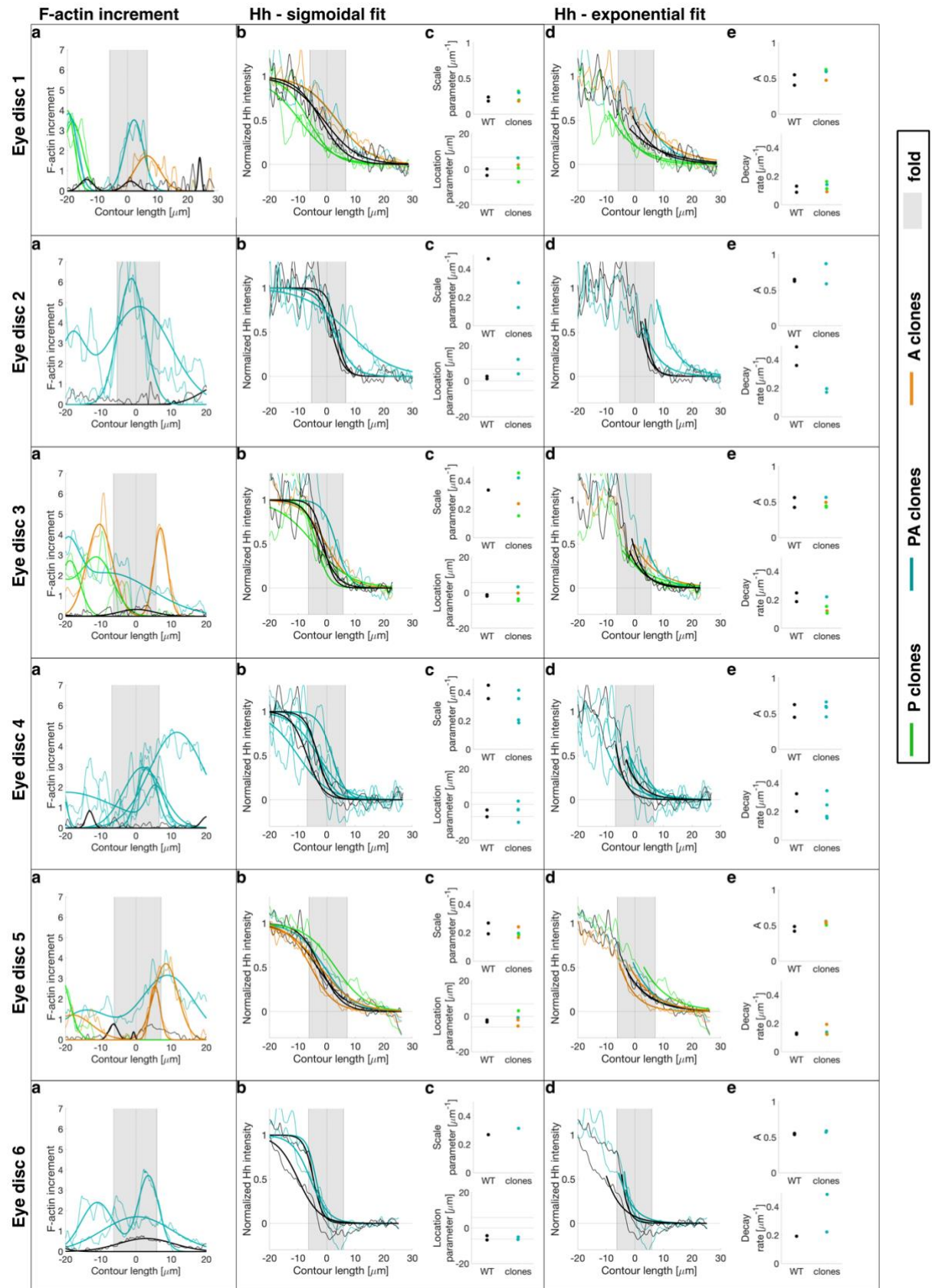


Figure S6: Analysis of N=6 eye discs presenting *capt* mutant clones analogous to (and containing) the examples presented in Fig. 4e-l and resulting in the data shown in Fig. 4m-o.

(a) Increment of F-actin as a function of contour length relative to wild-type areas of the tissue, defined as $\frac{A_{\text{clone}} - A_{\text{WT}}}{\sqrt{\Delta A_{\text{clone}}^2 + \Delta A_{\text{WT}}^2}}$ where A is the F-actin signal. Thick lines represent the Gaussian fits

(single or double) used to identify the clone location relative to the fold and to categorize it as P, PA, or A. **(b)** Normalized Hh fluorescence intensity as a function of contour length (thin lines). Thick lines represent the sigmoidal fits. **(c)** Parameters resulting from the sigmoidal fits in panel **b**: scale parameter (top) and location parameter, i.e. inflection point (bottom). **(d)** Normalized Hh fluorescence as a function of contour length (thin lines). Thick line represents the exponential fit. **(e)** Parameters resulting from the exponential fit in panel **d**: amplitude (top), decay rate (bottom).

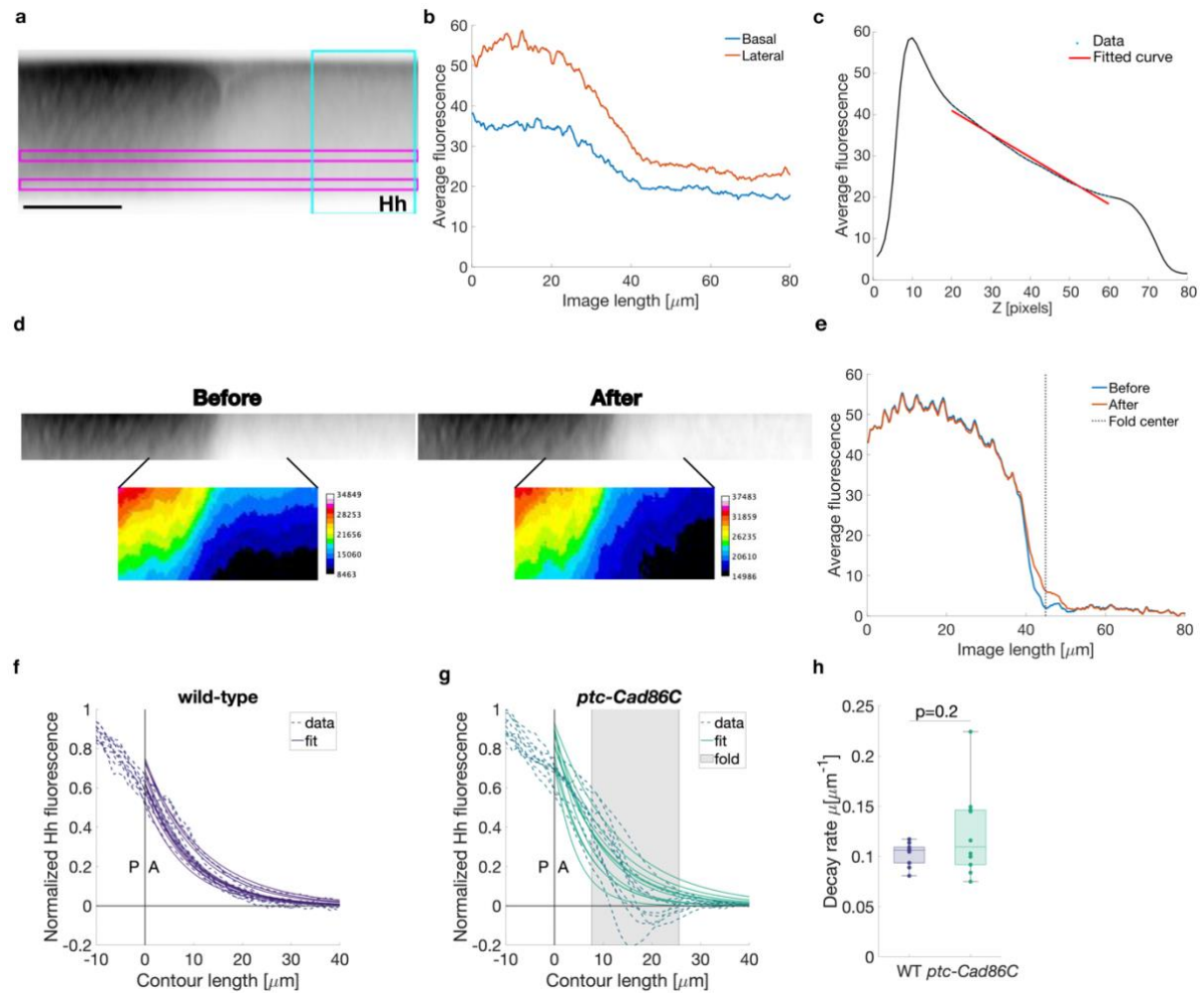


Figure S7: Correction for the distance from the objective in the experiments where we induced a fold in the wing disc (Fig. 5 in the main text).

(a) y-projection (sum) of the Hh channel in a region of interest in a *ptc-Cad86C* wing disc. (b) Plot of the averaged fluorescence intensity of the two regions of interest shown in magenta in (a) (bottom=basal, top=lateral). (c) Variation in background fluorescence relative to the z location in the image. Intensities extracted from the cyan region of interest shown in (a) (far anterior) and averaged over x. To estimate the scaling factor, we performed a linear fit (red line). (d) Comparison between regions of interest used for quantification before or after correcting for the distance from the objective. Zoom around the PA boundary is shown and color-coded with the lookup table of 16 colors in Fiji. (e) Comparison between the average fluorescence intensity obtained before or after correction for the distance from the objective. The baseline value (far anterior) has been set to 0. (f-h) Analogous to Fig. 5d-f in the main text prior to correction for the distance from the objective.

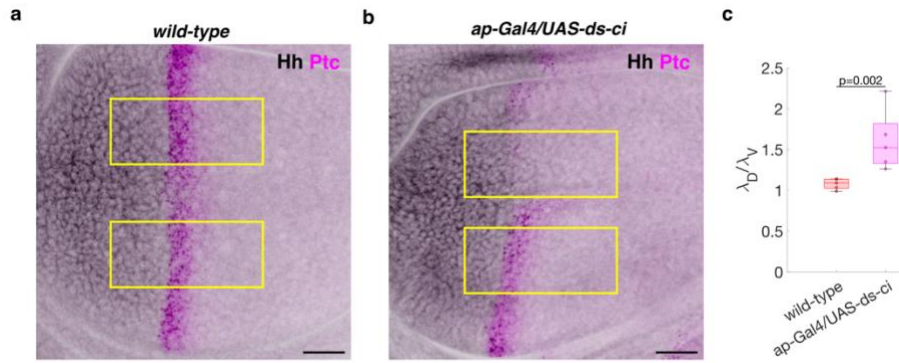


Figure S8: Knockdown of *ci* results in the downregulation of the Hedgehog receptor Ptc and in a significantly extended Hedgehog gradient.

(a-b) z-projection (sum) of a region of interest of wild type **(a)** and *ap-Gal4/UAS-ds-ci* **(b)** wing discs stained for Hh and Ptc. The knockdown of *ci* is limited to the dorsal region (to the top), where *ap-Gal4* is active. Regions of interest used for quantification are highlighted in yellow. Scale bars are 20 μm . **(c)** The characteristic length of the dorsal Hedgehog gradient normalized to the ventral one for wild-type (N=5) and *ap-Gal4/UAS-ds-ci* (N=5) wing discs.