SUPPLEMENTARY FIGURES

Non-cell autonomous control of apoptosis by ligand-independent Hedgehog signaling

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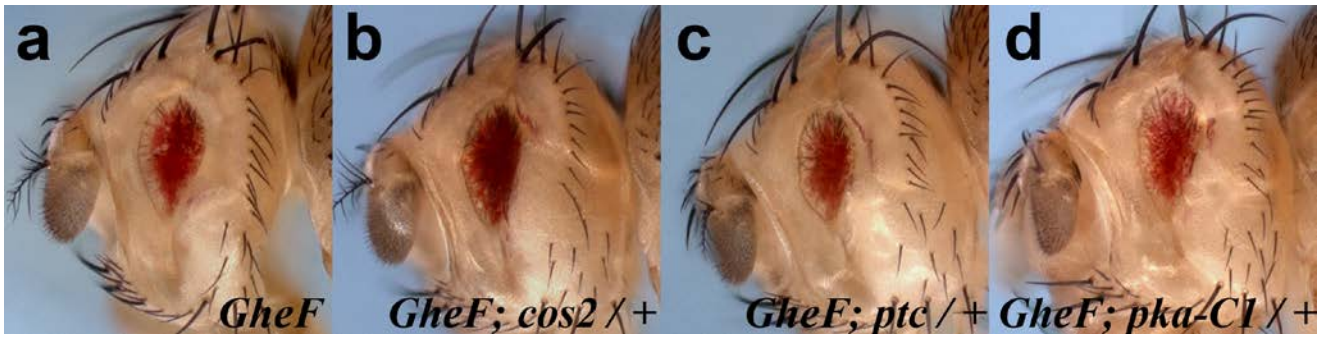


Supplementary Figure S1. Heterozygosity of *cos2, ptc* and *pka-C1* does not suppress *GMR-hid*.

The suppression of *GMR-hid* by *cos2*, *ptc* and *pka-C1* is not dominant, suggesting that the suppression observed in Figure 1 is due to recessive mutant clones. *GheF = GMR-hid ey-FLP*.

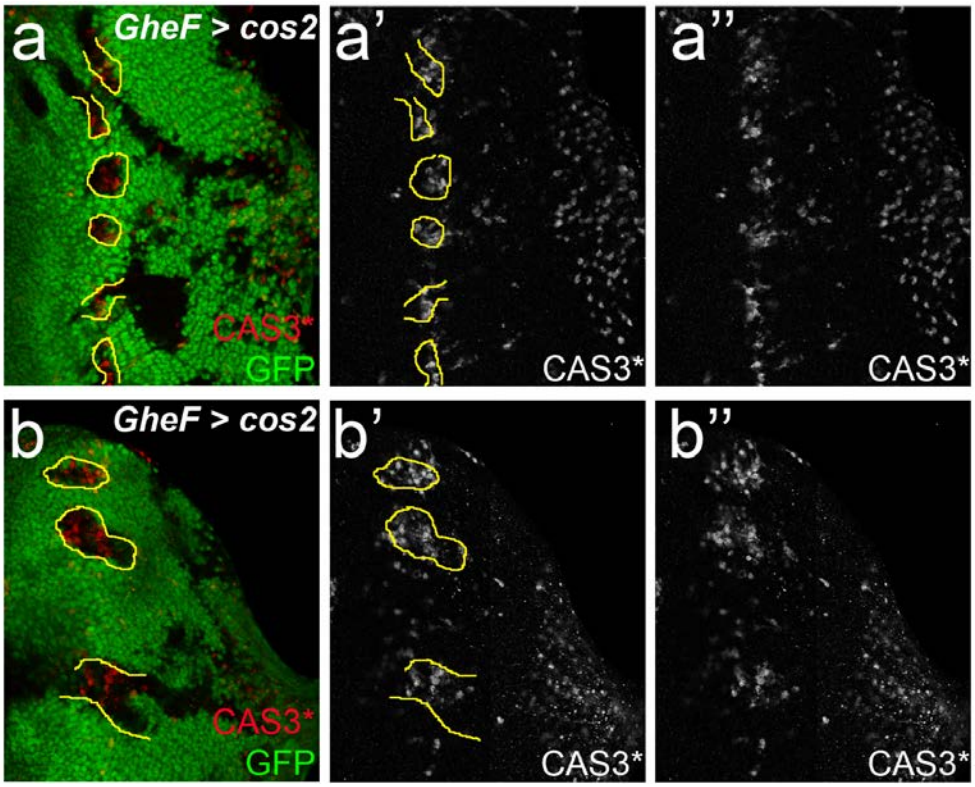
Genotypes:

(a) *GheF /+*.

(b) *GheF /+; cos2^{H29}/+*.

(c) *GheF /+; ptc^{C}/+*.

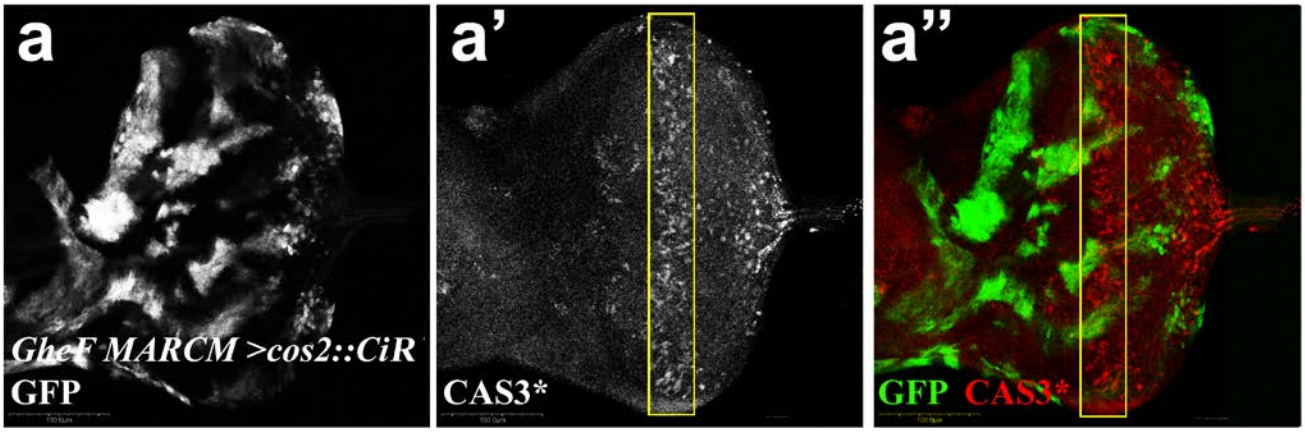
(d) *GheF /+; pka-C1^{B3}/+*.



Supplementary Figure S2. Non-cell autonomous suppression of *GMR-hid* in *cos2* mosaics.

Shown are two additional *GMR-hid* eye discs mosaic for *cos2* to support Figure 1j,j'. *cos2* clones are marked by the absence of GFP and those clones that cross the first apoptotic wave of *GMR-hid* are outlined by yellow lines in (a), (a'), (b) and (b'). CAS3* labeling is high in *cos2* clones (outlined in yellow in (a') and (b')), but low in adjacent non-mutant tissue in the first apoptotic wave (a'') and (b''). The second apoptotic wave does not appear to be affected by *cos2* clones. Panels (a) and (b) are the merge of CAS3* and GFP. *cos2* clones crossing the first apoptotic wave of *GMR-hid* are outlined with yellow lines or circles. Panels (a') and (a'') as well as (b') and (b'') are identical, but highlight different things. Panels (a') and (b') highlight the location of *cos2* mutant clones (yellow lines and circles) which are unprotected from apoptosis; panels (a'') and (b'') are unedited to highlight the lack of apoptosis in non-mutant tissue.

Genotype in a and b**:** *y w GMR-hid ey-FLP; FRT42 cos2*^{H29} */ FRT42 P[ubi-GFP]*.

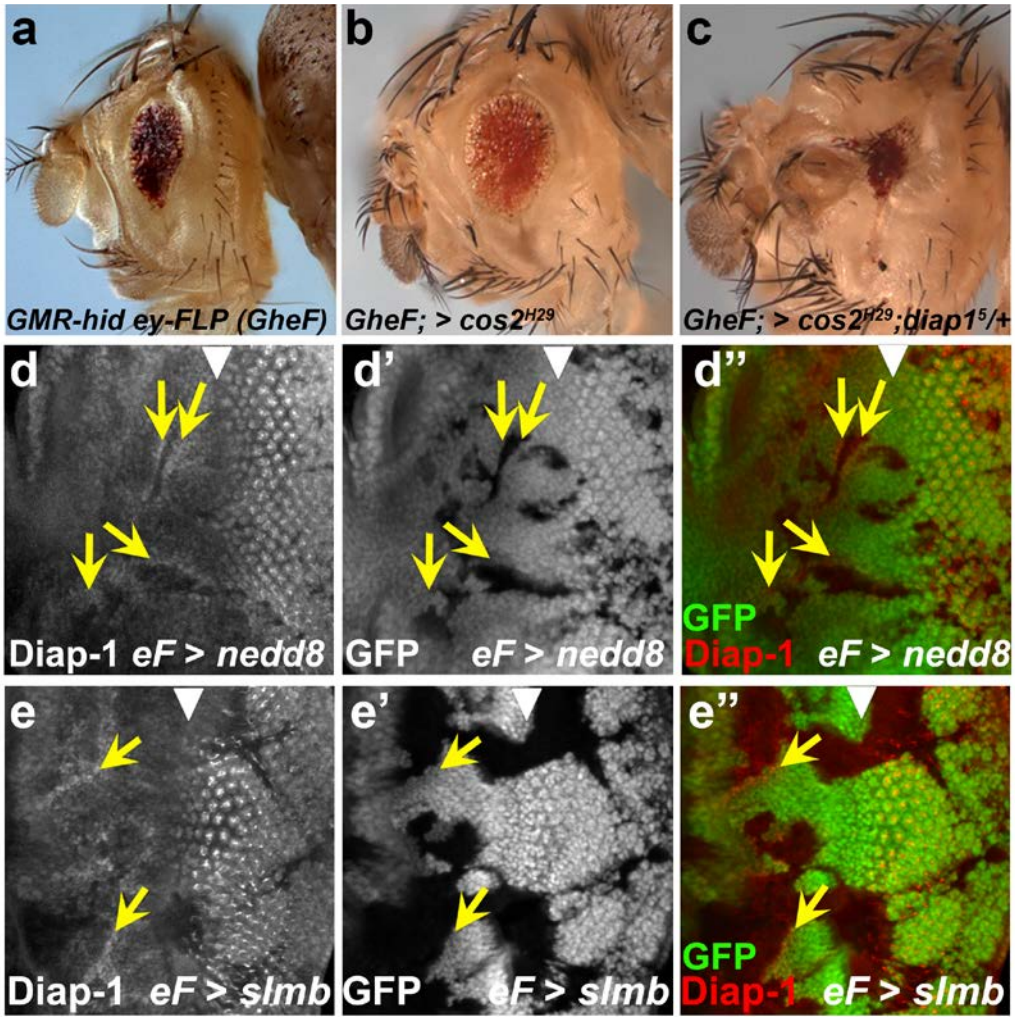


Supplementary Figure S3. Overexpression of *CiR* in *cos2* clones normalizes the caspase pattern in *cos2* mosaic *GMR-hid* eye imaginal discs.

Overexpression of *CiR* in *cos2* mutant clones using MARCM suppressed the non-autonomous suppression of caspase activity (CAS3*) in the first apoptotic wave of *GMR-hid* discs (boxed in yellow in (a') and (a")). Note, *cos2* clones expressing *CiR* are GFP^{+} due to MARCM.

Genotype:

hs-FLP UAS-mCD8-GFP/GMR-hid ey-FLP; FRT42 cos2^{H29} */ FRT42 GAL80; tubP-GAL4/UAS-ci*^{CE}.



Supplementary Figure S4. Supporting information for Diap-1 being the critical target for *cos2*-mediated suppression of *GMR-hid*.

(a-c) Removing 50% of *diap-1* gene function (c) is sufficient to reverse the suppression of *GMR-hid* by *cos2* mosaics (a, b). This observation suggests that accumulation of Diap-1 is essential for the non-autonomous control of apoptosis by signal-independent, deregulated Hh signaling. Please note that other aspects of the *cos2* mutant phenotype such as overgrowth and patterning defects are not affected by removing *diap-1* function.

(d, e) *nedd8* (**d,d',d''**) and *slmb* (**e,e',e''**) mosaic eye discs were labeled with anti-Diap-1 antibody. Mutant clones are marked by the absence of GFP. Clones located in or anterior to the MF (arrowhead) promote non-autonomous increase of DIAP-1 levels (arrows). *slmb* clones posterior to the MF lose Diap-1 labeling, indicating a different requirement of Slmb protein posterior to the MF.

Genotypes:

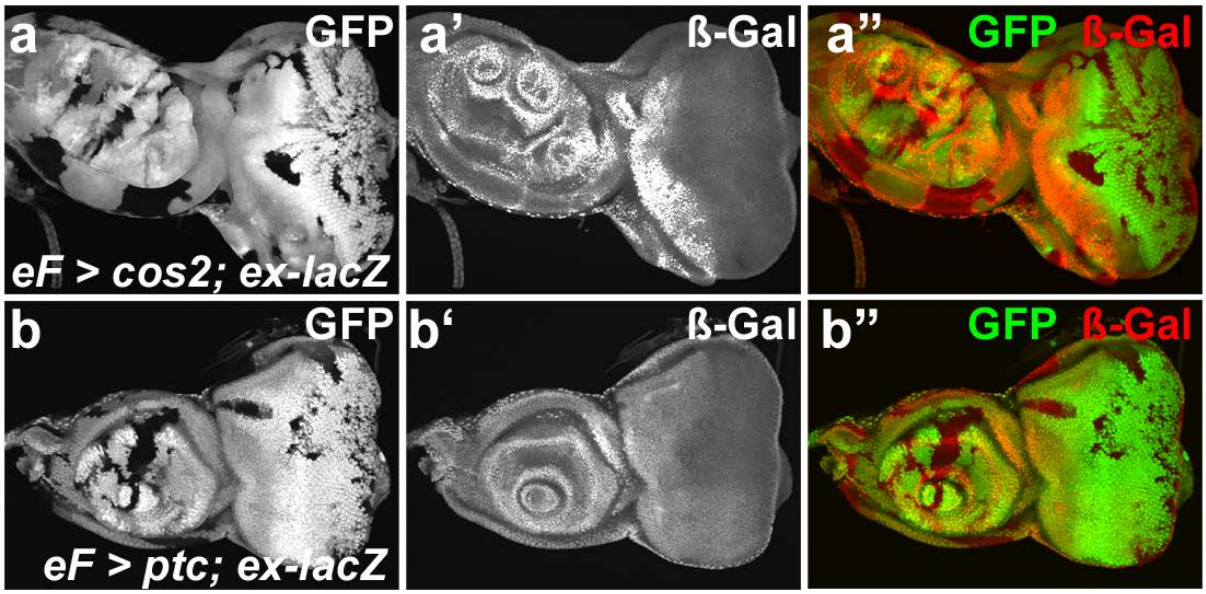
(a) *GMR-hid ey-FLP (GheF).*

(b) *y w GheF ;; FRT42D cos2^{H29} / FRT42D P[ubi-GFP].*

(c) *y w GheF ;; FRT42D cos2^{H29} / FRT42D P[ubi-GFP]; th^{5} /+ .*

(d,d',d'') *y w ey-FLP; nedd8^{AN015} FRT40 / P[ubi-GFP] FRT40.*

(e,e',e'') *y w ey-FLP; FRT82B slmb^{1} / FRT82B P[ubi-GFP].*



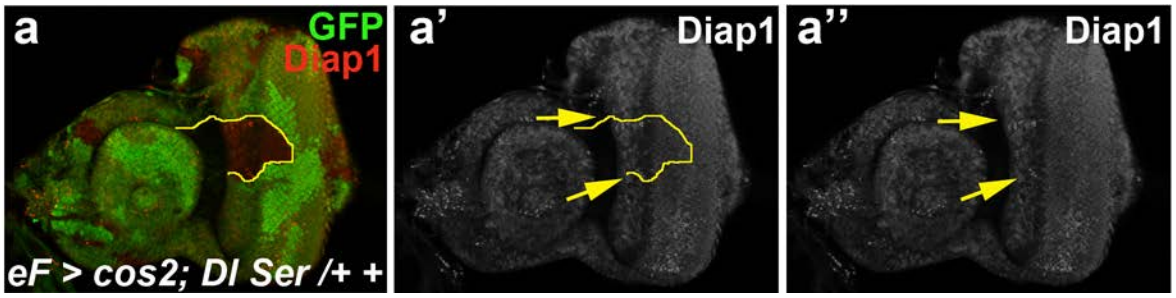
Supplementary Figure S5. *ex-lacZ* is unchanged in (a) *cos2* and (b) *ptc* mosaic eye antennal imaginal discs.

cos2 and *ptc* mutant clones throughout the eye disc do not display increased β-Gal immunoreactivity (*ex-lacZ*) either autonomously or non-cell autonomously. *cos2* and *ptc* mosaics frequently have pattern duplications (note the three organized regions in the antennal disc in (a')).

Genotypes:

(a) *y w ey-FLP; ex^{697} FRT42 ubi-GFP / FRT42 $cos2^{H29}$*.

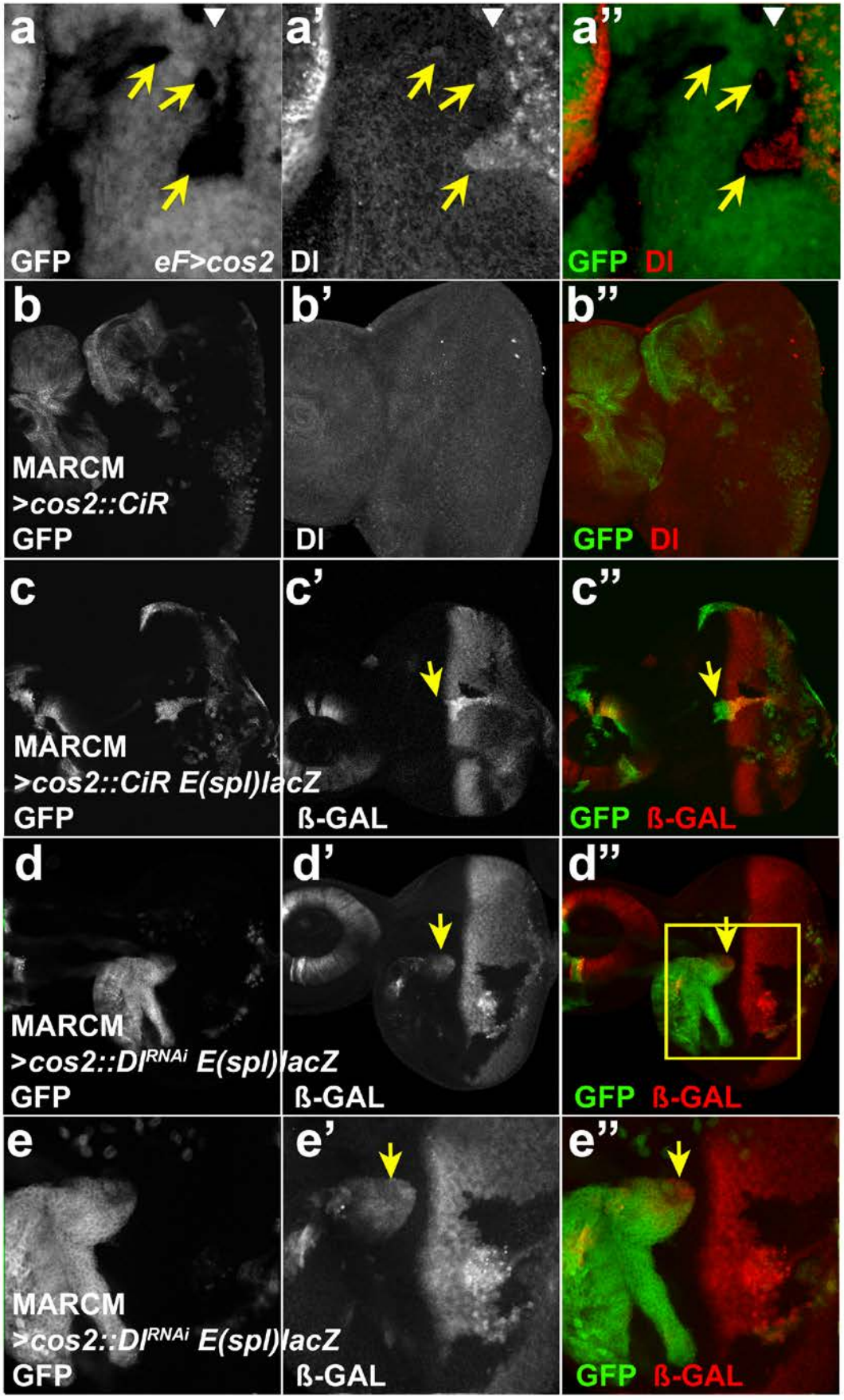
(b) *y w ey-FLP; ex^{697} FRT42 ubi-GFP / FRT42 ptc^{X115}*.



Supplementary Figure S6. Non-cell autonomous accumulation of Diap1 depends on Dl and Ser.

Heterozygosity of *Dl* and *Ser* suppresses the non-autonomous accumulation of Diap1 in *cos2* mosaics. A *cos2* mutant clone is outlined in yellow in (a) and (a'). There is no non-cell autonomous upregulation of Diap1 at the clonal boundary (see arrows in (a''')). Panels (a') and (a'') are the same, but highlight different things. Panel (a') highlights the location of *cos2* mutant clones (yellow lines); panel (a'') highlights the lack of non-autonomous upregulation of Diap1 (yellow arrows).

Genotype: *y w ey-FLP ; FRT42D cos2^{H29} / FRT42D P[ubi-GFP]; Dl^{RevF10} Ser^{RX82}/+ +*.


a
GFP
eF>cos2
a'
Dl
a''
GFP Dl
b
MARCM
>cos2::CiR
GFP
b'
Dl
b''
GFP Dl
c
MARCM
>cos2::CiR E(spl)lacZ
GFP
c'
ß-GAL
c''
GFP ß-GAL
d
MARCM
>cos2::Dl^RNAi E(spl)lacZ
GFP
d'
ß-GAL
d''
GFP ß-GAL
e
MARCM
>cos2::Dl^RNAi E(spl)lacZ
GFP
e'
ß-GAL
e''
GFP ß-GAL

Supplementary Figure S7.

(**a-a’’**) **Accumulation of Dl proteins in *cos2* clones anterior to the MF.** *cos2* mosaic eye discs were labeled with anti-Dl antibodies. Mutant clones are marked by the absence of GFP. Accumulation of Dl occurs only in *cos2* clones located in or anterior to the MF (arrows).

(b-e) Test for dependence of Dl levels and N activity on deregulated Hh signaling. Using MARCM technology, we generated *cos2* clones (marked with GFP) which expressed *CiR* (b,c) and *Dl* RNAi (d,e).

(b) Expression of the Ci repressor, *CiR*, in *cos2* mutant tissue blocked increased Dl levels (b’, b”) as compared to *cos2* mutant tissue (a’, a’’) suggesting that Dl accumulation in *cos2* clones is dependent on Ci ($CiR \equiv Ci^{CE}$).

(c) Expression of *CiR* in *cos2* mutant tissue blocks ectopic N activity as detected by the *E(spl)lacZ* reporter (c’, c”) anterior to the MF (arrow) ($CiR \equiv Ci^{CE}$).

(d,e) *Dl* RNAi in the *cos2* mutant tissue blocked non-autonomous N activity in *cos2* clones anterior to the MF (arrows) as detected by the *E(spl)lacZ* reporter, but has no effect on autonomous N activity, suggesting that the non-autonomous induction of N activity depends on Dl. (e,e’,e’’) is an enlargement of (d,d’,d’’) (yellow box).

Genotypes:

(**a-a’’**) *y w ey-FLP; FRT42D cos2^{H29} / FRT42D P[ubi-GFP]*.

(b,c): *hs-FLP UAS-mCD8-GFP/GMR-hid ey-FLP; FRT42 cos2^{H29} E(spl)-M8-2.61-lacZ/FRT42 tubP-GAL80; tubP-GAL4/UAS-ci^{CE}*,

(d,e): *hs-FLP UAS-mCD8-GFP; FRT42 cos2^{H29} E(spl)-M8-2.61-lacZ/FRT42 tubP-GAL80; tubP-GAL4/UAS-Dl RNAi*.