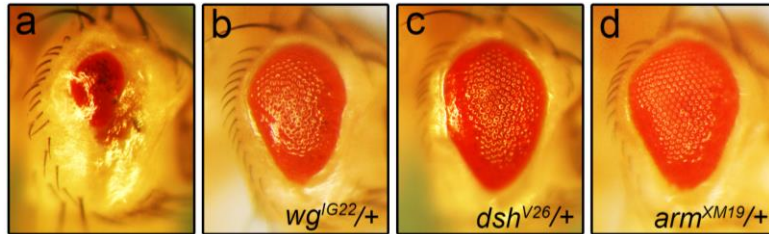


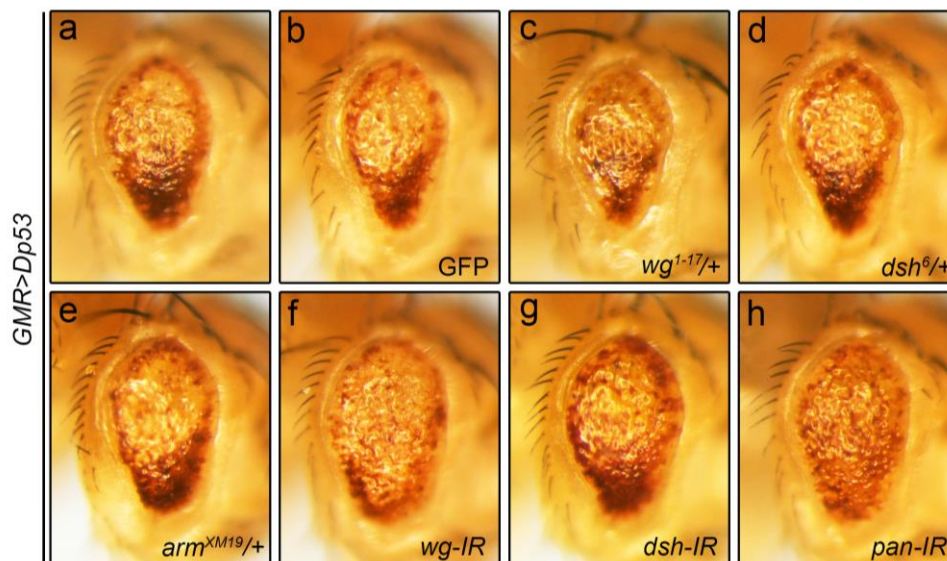
## Supplementary Information

Zhang et al., Figure S1



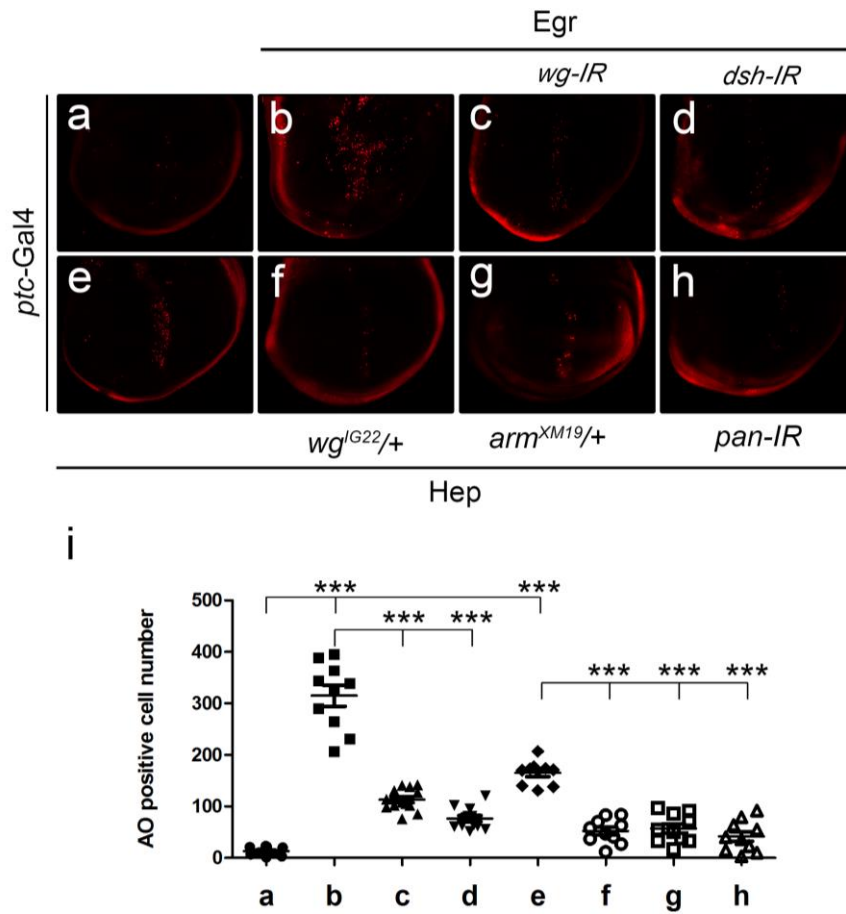
### Figure S1. Loss of Wg signaling suppresses Egr-induced cell death in eyes

Light micrographs of *Drosophila* adult eyes are shown. *GMR>Egr* induced small eye phenotype (a) is strongly suppressed by mutations in *wg* (b), *dsh* (c) or *arm* (d).



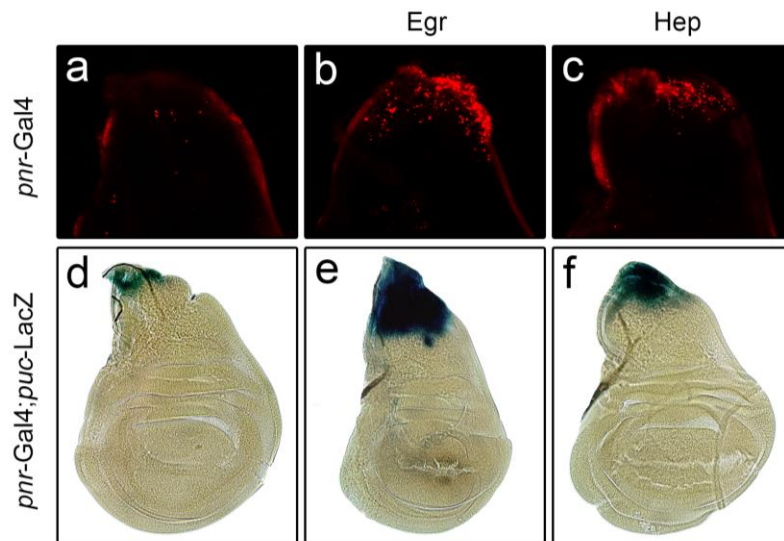
**Figure S2. Loss of Wg signaling does not suppress Dp53-induced cell death phenotype**

Expression of Dp53 under *GMR*-Gal4 generates a small and rough eye phenotype (a), which remains unaffected by expression of GFP (b), mutations in *wg* (c), *dsh* (d) or *arm* (e), and RNAi-mediated knocking-down of *wg* (f), *dsh* (g) or *pan* (h).



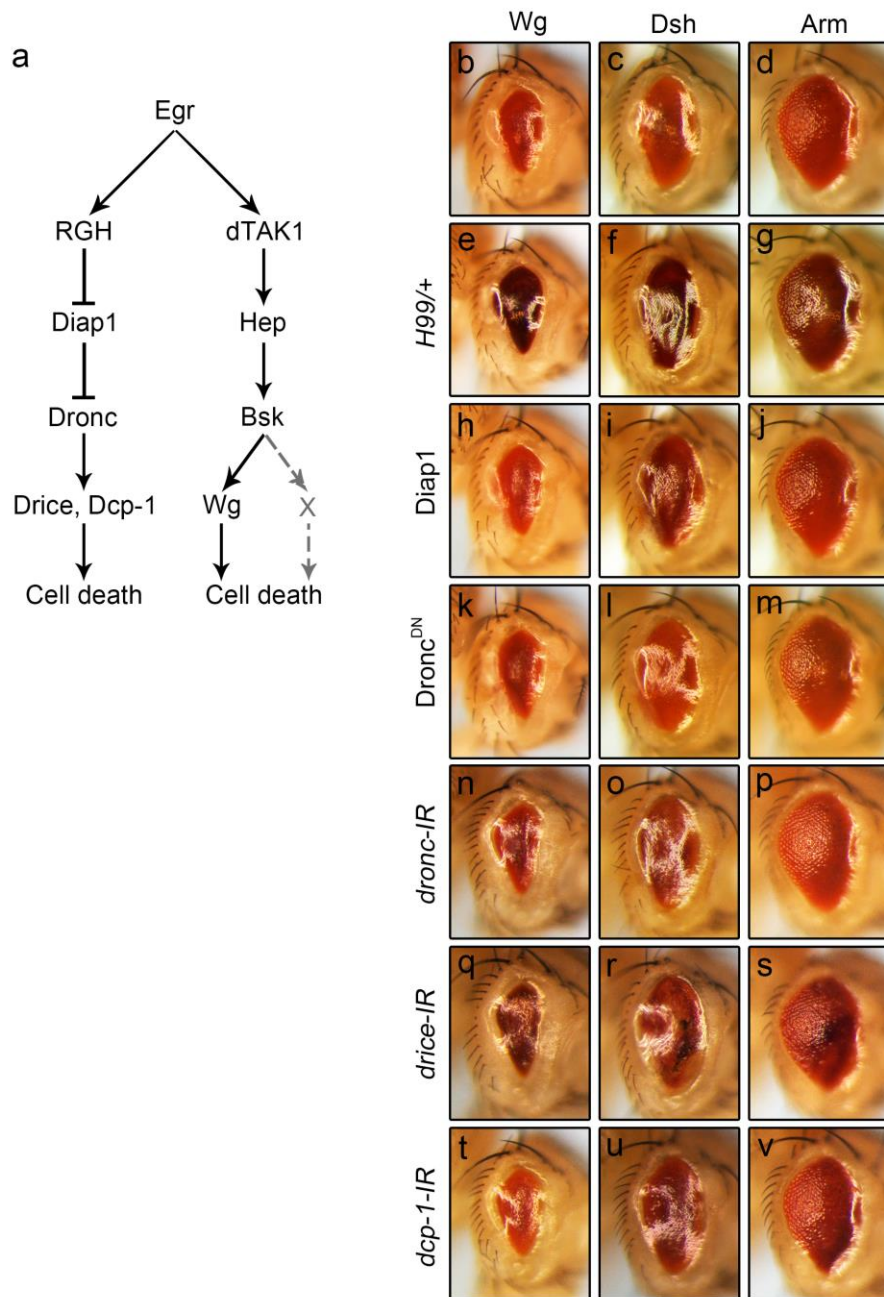
**Figure S3. Loss of Wg signaling suppress Bsk-mediated cell death in the wing discs**

Fluorescent micrographs of 3<sup>rd</sup> instar wing discs (**a-h**) are shown. Compared with the *ptc-Gal4* control (**a**), expression of Egr induces extensive cell death (**b**), which is suppressed significantly by RNAi knocking-down of *wg* (**c**) or *dsh* (**d**). Expression of Hep induces weaker cell death (**e**), which is also suppressed significantly by mutation in *wg* (**f**) or *arm* (**g**) and RNAi knocking-down of *pan* (**h**). (**i**) Statistics of the AO positive cell number in a-h. For each genotype, at least 10 discs were analyzed. Three asterisks,  $p < 0.001$ .



**Figure S4. Egr induces more Bsk-activation and cell death than Hep**

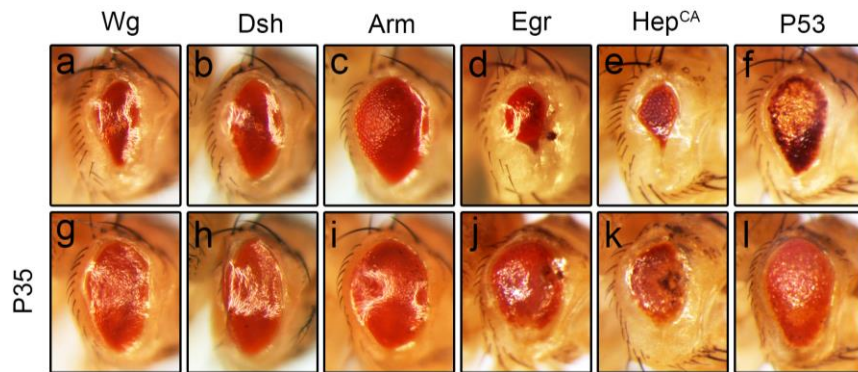
Fluorescent (a-c) and light (d-f) micrographs of 3<sup>rd</sup> instar wing discs are shown. Compared with the *pnr*-Gal4 control (a, d), expression of Egr induces much stronger cell death (b, AO staining) and *puc*-LacZ expression (e, X-Gal staining) than that of Hep (c, f).



**Figure S5. Wg signaling promotes caspase-independent cell death**

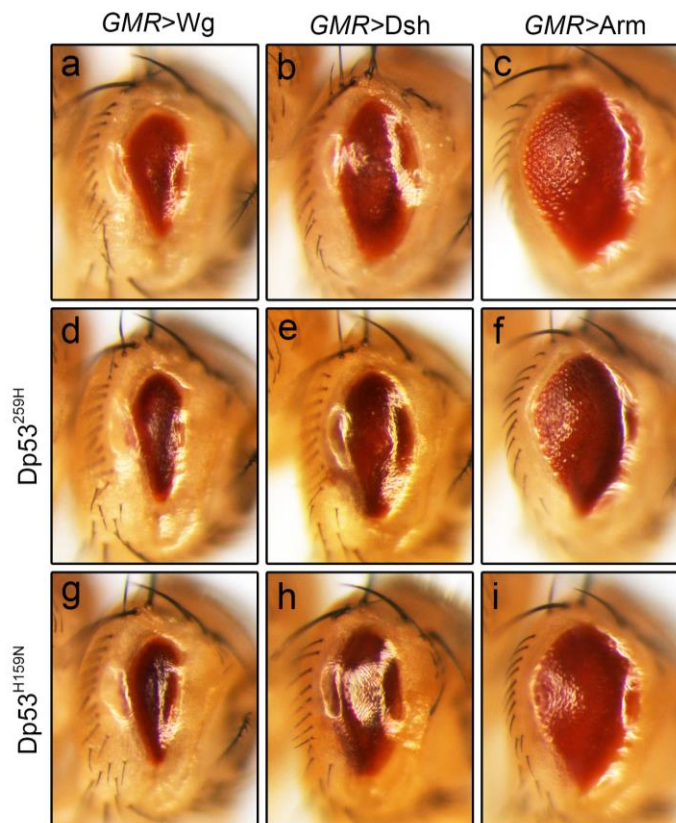
**(a)** A model showing Egr-induced cell death is mediated by two distinct pathways.

**(b-v)** Light micrographs of *Drosophila* adult eyes are shown. The small eye phenotype triggered by ectopic expression of Wg **(b)**, Dsh **(c)** or Arm **(d)** cannot be suppressed by deficiency *Df(3L)H99* **(e-g)**, expression of Diap1 **(h-j)**, a dominant negative form of DRONC **(k-m)**, and RNAi knocking-down of *dronc* **(n-p)**, *drice* **(q-s)** or *dcp-1* **(t-v)**.



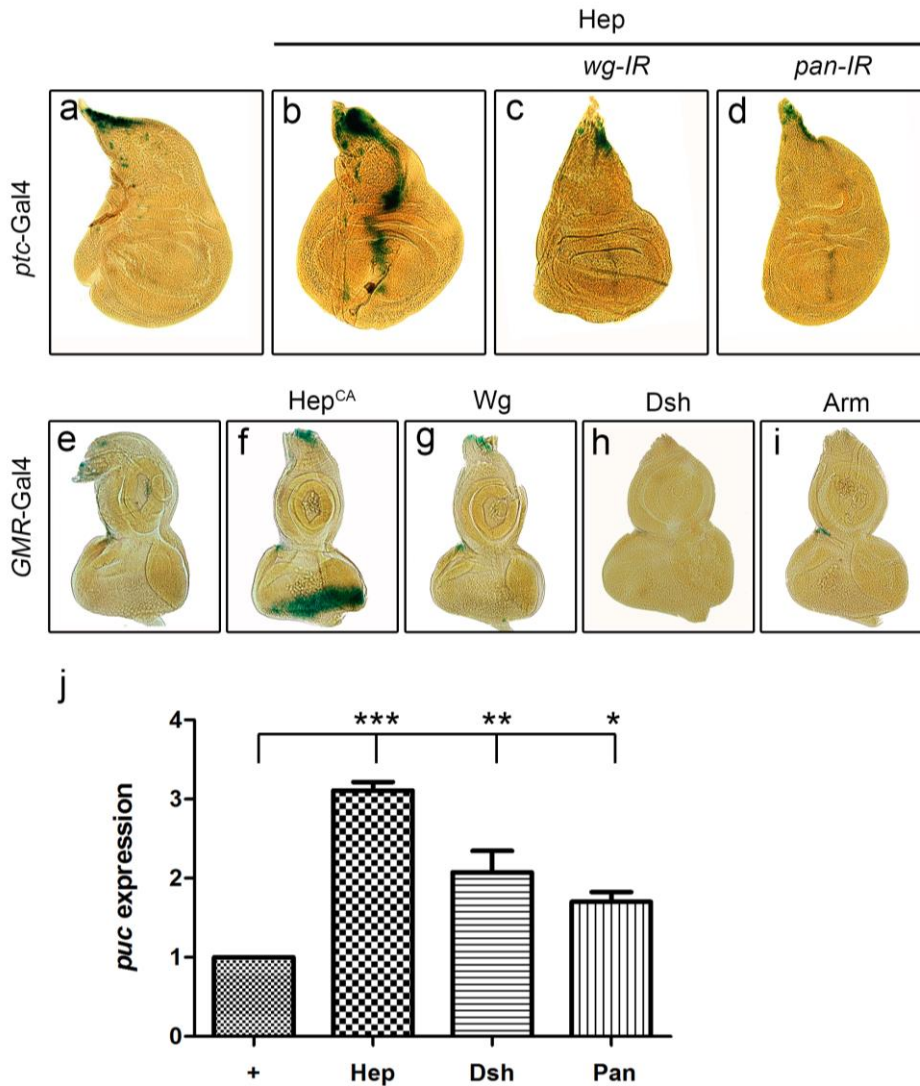
**Figure S6. P35 is a general suppresser of cell death**

Light micrographs of *Drosophila* adult eyes are shown. The small eye phenotype induced by ectopic Wg, Dsh, Arm, Egr, Hep<sup>CA</sup> or P53 (a-f) is suppressed by P35 (g-l).



**Figure S7. Wg signaling-induced cell death is independent of Dp53**

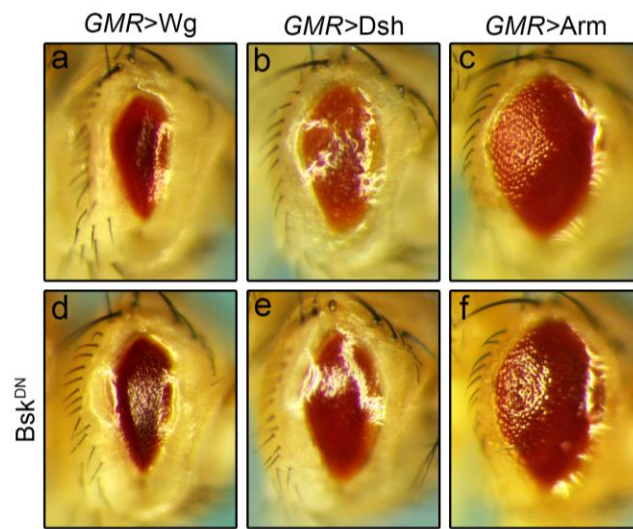
Light micrographs of *Drosophila* adult eyes are shown. Ectopic Wg, Dsh or Arm induced small eye phenotype (a-c) cannot be suppressed by the expression of two independent dominant negative forms of Dp53 (d-i).



**Figure S8. Wg signaling modulates Bsk activation in a tissue specific manner**

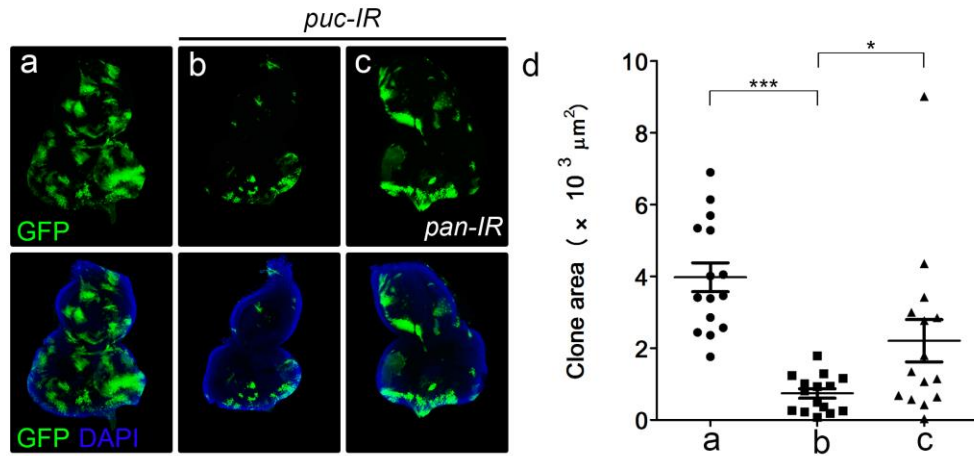
Light micrographs of *Drosophila* 3<sup>rd</sup> instar wing (a-d) and eye discs (e-i) with X-Gal staining are shown. Compared with the *ptc-Gal4* control (a), ectopic expression of Hep along the A/P compartment boundary in the wing disc activates *puc-LacZ* expression (b), which is suppressed by knocking-down *wg* (c) or *pan* (d). Compared with the *GMR-Gal4* control (e), ectopic expression of *Hep<sup>CA</sup>* (f), but not *Wg* (g), *Dsh* (h) or *Arm* (i), activates *puc-LacZ* expression in the eye discs. (j) Quantification of *puc* expression level in 3<sup>rd</sup> instar wing discs by qRT-PCR. Compared with the *sd-Gal4* control (+), expression of Hep, Dsh, or Pan significantly activates *puc* expression. Three asterisks,  $p < 0.001$ . Two asterisks,  $p < 0.01$ . One asterisks,  $p < 0.05$ .





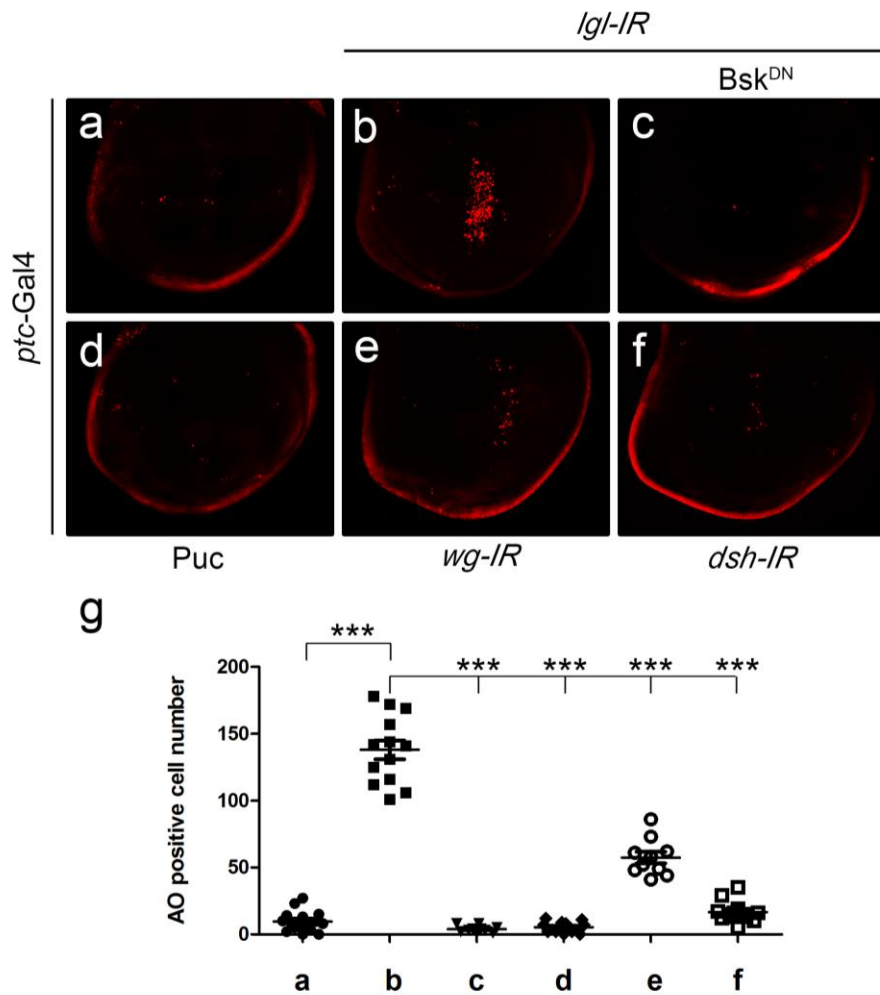
**Figure S9. Wg signaling acts downstream of Bsk**

Light micrographs of *Drosophila* adult eyes are shown. Ectopic Wg (a), Dsh (b) or Arm (c) induced small eye phenotype cannot be suppressed by expression of Bsk<sup>DN</sup> (d-f).



**Figure S10. Wg signaling contributes to the physiological function of Bsk**

(a-c) Fluorescent micrographs of 3<sup>rd</sup> instar eye discs are shown. Compared with wild type clones induced in the eye disc (a), *puc* loss-of-function clones show dramatically reduced area (b), which is partially rescued by RNAi knocking-down of *pan* (c). (d) Statistics of the total clone areas in a-c. For each genotype, at least 15 clones were analyzed. Three asterisks,  $p < 0.001$ ; one asterisks,  $p < 0.05$ .



**Figure S11. Wg signaling modulates loss of cell polarity-induced Bsk-mediated cell death**

(a-f) Fluorescent micrographs of 3<sup>rd</sup> instar wing discs are shown. Compared with the *ptc*-Gal4 control (a), depletion of *lgl* induces extensive cell death (b), which is suppressed by expression of Bsk<sup>DN</sup> (c) or Puc (d), or RNAi-mediated knocking-down of *wg* (e) or *dsh* (f). For each genotype, at least 10 discs were analyzed. Three asterisks,  $p < 0.001$ .

## Detailed Genotypes

### Figure 1

- (a) *GMR-Gal4/+*
- (b) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/+*
- (c) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-GFP*
- (d) *UAS-Egr<sup>Regg1</sup>/Sp; GMR-Gal4/+*
- (e) *UAS-Egr<sup>Regg1</sup>/wg<sup>1-17</sup>; GMR-Gal4/+*
- (f) *dsh<sup>6</sup>/+; UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/+*
- (g) *arm<sup>1</sup>/+; UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/+*
- (h) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/+; pan<sup>13a</sup>/+*
- (i) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-wg-IR*
- (j) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-dsh-IR*
- (k) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-pan-IR*
- (l) *Sgg<sup>EP1576</sup>/+; UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/+*
- (m) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-Apc2*
- (n) *UAS-Egr<sup>Regg1</sup>/bsk<sup>1</sup>; GMR-Gal4/+*
- (o) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-Bsk<sup>DN</sup>*

### Figure 2

- (a) *GMR-Gal4 UAS-Hep<sup>CA</sup>/+*
- (b) *GMR-Gal4 UAS-Hep<sup>CA</sup>/UAS-GFP*
- (c) *wg<sup>IG22</sup>/+; GMR-Gal4 UAS-Hep<sup>CA</sup>/+*
- (d) *arm<sup>XM19</sup>/+; GMR-Gal4 UAS-Hep<sup>CA</sup>/+*
- (e) *GMR-Gal4 UAS-Hep<sup>CA</sup> /UAS-wg-IR*
- (f) *GMR-Gal4 UAS-Hep<sup>CA</sup>/UAS-arm-IR*
- (g) *GMR-Gal4 UAS-Hep<sup>CA</sup>/UAS-pan-IR*
- (h) *Sgg<sup>EP1576</sup>/+; GMR-Gal4 UAS-Hep<sup>CA</sup>/+*
- (i) *GMR-Gal4 UAS-Hep<sup>CA</sup>/UAS-Axn*
- (j) *bsk<sup>1</sup>/+; GMR-Gal4 UAS-Hep<sup>CA</sup>/+*

### Figure 3

- (a) *pnr-Gal4/+*
- (b) *UAS-Hep/+; pnr-Gal4/+*
- (c) *UAS-Hep/wg<sup>IG22</sup>; pnr-Gal4/+*
- (d) *dsh<sup>V26</sup>/+; UAS-Hep/+; pnr-Gal4/+*
- (e) *arm<sup>XM19</sup>/+; UAS-Hep/+; pnr-Gal4/+*
- (f) *ptc-Gal4/+; puc<sup>E69</sup>/+*
- (g) *ptc-Gal4 UAS-Hep/+; puc<sup>E69</sup>/+*
- (h) *ptc-Gal4 UAS-Hep/+; puc<sup>E69</sup>/UAS-LacZ*
- (i) *dsh<sup>V26</sup>/+; ptc-Gal4 UAS-Hep/+; puc<sup>E69</sup>/+*
- (j) *ptc-Gal4 UAS-Hep/+; puc<sup>E69</sup>/UAS-dsh-IR*
- (k) *ptc-Gal4/+*
- (l) *ptc-Gal4 UAS-Hep/+*
- (m) *ptc-Gal4 UAS-Hep/+; UAS-LacZ/+*
- (n) *dsh<sup>V26</sup>/+; ptc-Gal4 UAS-Hep/+*
- (o) *ptc-Gal4 UAS-Hep/+; UAS-dsh-IR/+*

### Figure 4

- (a, g, m and s) *GMR-Gal4/+*
- (b, h, n and t) *GMR-Gal4 UAS-Hep<sup>CA</sup>/+*
- (c, i, o and u) *GMR-Gal4 UAS-Wg/+*
- (d, j, p and v) *GMR-Gal4 UAS-Dsh/+*
- (e, k, q and w) *UAS-Arm/+; GMR-Gal4/+*
- (f, l, r and x) *UAS-Rpr/+; GMR-Gal4/+*

### Figure 5

- (a, e and m) *ptc-Gal4/+; puc<sup>E69</sup>/+*
- (b, f) *ptc-Gal4 UAS-Egr/+; puc<sup>E69</sup>/+*
- (c) *ptc-Gal4 UAS-Egr/+; puc<sup>E69</sup>/UAS-wg-IR*

- (d) *ptc-Gal4 UAS-Egr/+; puc<sup>E69</sup>/UAS-arm-IR*
- (g) *ptc-Gal4 UAS-Egr/+; puc<sup>E69</sup>/UAS-pan-IR*
- (h) *Sgg<sup>EP1576</sup>/+; ptc-Gal4 UAS-Egr/+; puc<sup>E69</sup>/+*
- (i) *sd-Gal4/+; puc<sup>E69</sup>/+*
- (j) *sd-Gal4/+; UAS-Hep/+; puc<sup>E69</sup>/+*
- (k) *sd-Gal4/+; puc<sup>E69</sup>/UAS-Dsh*
- (l) *sd-Gal4/+; UAS-Pan/+; puc<sup>E69</sup>/+*
- (n) *ptc-Gal4/UAS-Hep; puc<sup>E69</sup>/+*
- (o) *ptc-Gal4/UAS-Arm; puc<sup>E69</sup>/+*
- (p) *ptc-Gal4/UAS-Pan; puc<sup>E69</sup>/+*

### Figure 6

- (a) *en-Gal4 UAS-GFP/+*
- (b) *en-Gal4 UAS-GFP/UAS-Egr<sup>Regg1</sup>*
- (c) *en-Gal4 UAS-GFP/+; tub-Gal80<sup>ts</sup>/UAS-Hep<sup>CA</sup>*
- (d) *en-Gal4 UAS-GFP/wg-LacZ*
- (e) *en-Gal4 UAS-GFP/ wg-LacZ UAS-Egr<sup>Regg1</sup>*
- (f) *en-Gal4 UAS-GFP/wg-LacZ; tub-Gal80<sup>ts</sup>/UAS-Hep<sup>CA</sup>*

### Figure 7

- (a) *hs-Flp; act>CD2>Gal4 UAS-GFP/+*
- (b) *hs-Flp; act>CD2>Gal4 UAS-GFP/UAS-puc-IR*
- (c) *hs-Flp; act>CD2>Gal4 UAS-GFP/UAS-puc-IR; UAS-pan-IR/+*
- (e) *ptc-Gal4/+*
- (f) *ptc-Gal4 UAS-puc-IR/+*
- (g) *ptc-Gal4 UAS-puc-IR/+; UAS-wg-IR/+*
- (h) *Sgg<sup>EP1576</sup>/+; ptc-Gal4 UAS-puc-IR/+*

### Figure S1

- (a) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/+*

- (b) *UAS-Egr*<sup>Regg1/wg<sup>IG22</sup></sup>; *GMR-Gal4*/+
- (c) *dsh*<sup>V26</sup>/+; *UAS-Egr*<sup>Regg1/+</sup>; *GMR-Gal4*/+
- (d) *arm*<sup>XM19/+</sup>; *UAS-Egr*<sup>Regg1/+</sup>; *GMR-Gal4*/+

### Figure S2

- (a) *GMR-Gal4 UAS-Dp53*/+
- (b) *GMR-Gal4 UAS-Dp53/+; UAS-GFP*/+
- (c) *GMR-Gal4 UAS-Dp53/wg<sup>1-17</sup>*
- (d) *dsh*<sup>6</sup>/+; *GMR-Gal4 UAS-Dp53*/+
- (e) *arm*<sup>XM19/+</sup>; *GMR-Gal4 UAS-Dp53*/+
- (f) *GMR-Gal4 UAS-Dp53/+; UAS-wg-IR*/+
- (g) *GMR-Gal4 UAS-Dp53/+; UAS-dsh-IR*/+
- (h) *GMR-Gal4 UAS-Dp53/+; UAS-pan-IR*/+

### Figure S3

- (a) *ptc-Gal4*/+
- (b) *ptc-Gal4 UAS-Egr*/+
- (c) *ptc-Gal4 UAS-Egr/+; UAS-wg-IR*/+
- (d) *ptc-Gal4 UAS-Egr/+; UAS-dsh-IR*/+
- (e) *ptc-Gal4 UAS-Hep*/+
- (f) *ptc-Gal4 UAS-Hep/wg<sup>IG22</sup>*
- (g) *arm*<sup>XM19/+</sup>; *ptc-Gal4 UAS-Hep*/+
- (h) *ptc-Gal4 UAS-Hep/+; UAS-pan-IR*/+

### Figure S4

- (a) *pnr-Gal4*/+
- (b) *UAS-Egr*/+; *pnr-Gal4*/+
- (c) *UAS-Hep*/+; *pnr-Gal4*/+
- (d) *pnr-Gal4/puc<sup>E69</sup>*
- (e) *UAS-Egr*/+; *pnr-Gal4/puc<sup>E69</sup>*

(f) *UAS-Hep/+; pnr-Gal4/puc<sup>E69</sup>*

### Figure S5

(b) *GMR-Gal4 UAS-Wg/+*

(c) *GMR-Gal4 UAS-Dsh/+*

(d) *UAS-Arm/+; GMR-Gal4/+*

(e) *GMR-Gal4 UAS-Wg/Df(3L)H99*

(f) *GMR-Gal4 UAS-Dsh/Df(3L)H99*

(g) *UAS-Arm/+; GMR-Gal4/Df(3L)H99*

(h) *GMR-Gal4 UAS-Wg/UAS-Diap1*

(i) *GMR-Gal4 UAS-Dsh/UAS-Diap1*

(j) *UAS-Arm/+; GMR-Gal4/UAS-Diap1*

(k) *UAS-Dronc<sup>DN</sup>/+; GMR-Gal4 UAS-Wg/+*

(l) *UAS-Dronc<sup>DN</sup>/+; GMR-Gal4 UAS-Dsh/+*

(m) *UAS-Arm/UAS-Dronc<sup>DN</sup>; GMR-Gal4/+*

(n) *UAS-dronc-IR/+; GMR-Gal4 UAS-Wg/+*

(o) *UAS-dronc-IR/+; GMR-Gal4 UAS-Dsh/+*

(p) *UAS-Arm/UAS-dronc-IR; GMR-Gal4/+*

(q) *UAS-drice-IR/+; GMR-Gal4 UAS-Wg/+*

(r) *UAS-drice-IR/+; GMR-Gal4 UAS-Dsh/+*

(s) *UAS-Arm/UAS-drice-IR; GMR-Gal4/+*

(t) *GMR-Gal4 UAS-Wg/UAS-dcp-1-IR*

(u) *GMR-Gal4 UAS-Dsh/UAS-dcp-1-IR*

(v) *UAS-Arm/+; GMR-Gal4/UAS-dcp-1-IR*

### Figure S6

(a) *GMR-Gal4 UAS-Wg/+*

(b) *GMR-Gal4 UAS-Dsh /+*

(c) *UAS-Arm/+; GMR-Gal4/+*

(d) *UAS-Egt<sup>Regg1</sup>/+; GMR-Gal4/+*



- (e) *GMR-Gal4 UAS-Hep<sup>CA</sup>/+*
- (f) *GMR-Gal4 UAS-Dp53/+*
- (g) *GMR-Gal4 UAS-Wg/UAS-P35*
- (h) *GMR-Gal4 UAS-Dsh /UAS-P35*
- (i) *UAS-Arm/+; GMR-Gal4/UAS-P35*
- (j) *UAS-Egr<sup>Regg1</sup>/+; GMR-Gal4/UAS-P35*
- (k) *GMR-Gal4 UAS-Hep<sup>CA</sup>/ UAS-P35*
- (l) *GMR-Gal4 UAS-Dp53/+; UAS-P35/+*

### Figure S7

- (a) *GMR-Gal4 UAS-Wg/+*
- (b) *GMR-Gal4 UAS-Dsh /+*
- (c) *UAS-Arm/+; GMR-Gal4/+*
- (d) *GMR-Gal4 UAS-Wg/GUS-Dp53<sup>259H</sup>*
- (e) *GMR-Gal4 UAS-Dsh/GUS-Dp53<sup>259H</sup>*
- (f) *UAS-Arm/+; GMR-Gal4/GUS-Dp53<sup>259H</sup>*
- (g) *UAS-Dp53<sup>H159N</sup>/+; GMR-Gal4 UAS-Wg/+*
- (h) *UAS-Dp53<sup>H159N</sup>/+; GMR-Gal4 UAS-Dsh/+*
- (i) *UAS-Arm/UAS-Dp53<sup>H159N</sup>; GMR-Gal4/+*

### Figure S8

- (a) *ptc-Gal4/+; puc<sup>E69</sup>/+*
- (b) *ptc-Gal4 UAS-Hep/+; puc<sup>E69</sup>/+*
- (c) *ptc-Gal4 UAS-Hep/UAS-wg-IR; puc<sup>E69</sup>/+*
- (d) *ptc-Gal4 UAS-Hep/+; puc<sup>E69</sup>/UAS-pan-IR*
- (e) *GMR-Gal4/+; puc<sup>E69</sup>/+*
- (f) *GMR-Gal4/+; puc<sup>E69</sup>/UAS-Hep<sup>CA</sup>*
- (g) *GMR-Gal4/+; puc<sup>E69</sup>/UAS-Wg*
- (h) *GMR-Gal4/+; puc<sup>E69</sup>/UAS-Dsh*

(i) *GMR-Gal4/UAS-Arm; puc<sup>E69</sup>/+*

### Figure S9

(a) *GMR-Gal4 UAS-Wg/+*

(b) *GMR-Gal4 UAS-Dsh /+*

(c) *UAS-Arm/+; GMR-Gal4/+*

(d) *GMR-Gal4 UAS-Wg/UAS-Bsk<sup>DN</sup>*

(e) *GMR-Gal4 UAS-Dsh/UAS-Bsk<sup>DN</sup>*

(f) *UAS-Arm/+; GMR-Gal4/UAS-Bsk<sup>DN</sup>*

### Figure S10

(a) *hs-Flp; act>CD2>Gal4 UAS-GFP/+*

(b) *hs-Flp; act>CD2>Gal4 UAS-GFP/UAS-puc-IR*

(c) *hs-Flp; act>CD2>Gal4 UAS-GFP/UAS-puc-IR; UAS-pan-IR/+*

### Figure S11

(a) *ptc-Gal4/+*

(b) *ptc-Gal4/+; UAS-lgl-IR/+*

(c) *ptc-Gal4/+; UAS-lgl-IR/UAS-Bsk<sup>DN</sup>*

(d) *ptc-Gal4/+; UAS-lgl-IR/UAS-Puc*

(e) *ptc-Gal4/+; UAS-lgl-IR/UAS-wg-IR*

(f) *ptc-Gal4/+; UAS-lgl-IR/UAS-dsh-IR*