Elimination of Unfit Cells Maintains Tissue Health and Prolongs Lifespan

Graphical Abstract

Highlights
- Fitness-based cell culling maintains tissue health
- Azot ensures the elimination of less fit cells
- Lack of azot accelerates tissue degeneration
- Improving the efficiency of cell selection extends lifespan

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In Brief
Elimination of less fit cells ensured by the expression of a cell-fitness checkpoint, Azot, is important for maintaining tissue health and prolonging lifespan in Drosophila.
Elimination of Unfit Cells Maintains Tissue Health and Prolongs Lifespan

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SUMMARY

Viable yet damaged cells can accumulate during development and aging. Although eliminating those cells may benefit organ function, identification of this less fit cell population remains challenging. Previously, we identified a molecular mechanism, based on “fitness fingerprints” displayed on cell membranes, which allows direct fitness comparison among cells in Drosophila. Here, we study the physiological consequences of efficient cell selection for the whole organism. We find that fitness-based cell culling is naturally used to maintain tissue health, delay aging, and extend lifespan in Drosophila. We identify a gene, azot, which ensures the elimination of less fit cells. Lack of azot increases morphological malformations and susceptibility to random mutations and accelerates tissue degeneration. On the contrary, improving the efficiency of cell selection is beneficial for tissue health and extends lifespan.

INTRODUCTION

Individual cells can suffer insults that affect their normal functioning, a situation often aggravated by exposure to external damaging agents. A fraction of damaged cells will critically lose their ability to live, but a different subset of cells may be more difficult to identify and eliminate: viable but suboptimal cells that, if unnoticed, may adversely affect the whole organism (Moskalev et al., 2013).

What is the evidence that viable but damaged cells accumulate within tissues? The somatic mutation theory of aging (Kennedy et al., 2012; Szilard, 1959) proposes that over time cells suffer insults that affect their fitness, for example, diminishing their proliferation and growth rates, or forming deficient structures and connections. This creates increasingly heterogeneous and dysfunctional cell populations disturbing tissue and organ function (Moskalev et al., 2013). Once organ function falls below a critical threshold, the individual dies. The theory is supported by the experimental finding that clonal mosaicism occurs at unexpectedly high frequency in human tissues as a function of time, not only in adults due to aging (Jacobs et al., 2012; Laurie et al., 2012), but also in human embryos (Vanneste et al., 2009).

Does the high prevalence of mosaicism in our tissues mean that it is impossible to recognize and eliminate cells with subtle mutations and that suboptimal cells are bound to accumulate within organs? Or, on the contrary, can animal bodies identify and get rid of unfit viable cells?

One indirect mode through which suboptimal cells could be eliminated is proposed by the “trophic theory” (Levi-Montalcini, 1987; Moreno, 2014; Raff, 1992; Simi and Ibáñez, 2010), which suggested that Darwinian-like competition among cells for limiting amounts of survival-promoting factors will lead to removal of less fit cells. However, it is apparent from recent work that trophic theories are not sufficient to explain fitness-based cell selection, because there are direct mechanisms that allow cells to exchange “cell-fitness” information at the local multicellular level (Moreno and Rhiner, 2014).

In Drosophila, cells can compare their fitness using different isoforms of the transmembrane protein Flower. The “fitness fingerprints” are therefore defined as combinations of Flower isoforms present at the cell membrane that reveal optimal or reduced fitness (Merino et al., 2013; Rhiner et al., 2010). The isoforms that indicate reduced fitness have been called Flowerlose isoforms, because they are expressed in cells marked to be eliminated by apoptosis called “Loser cells” (Rhiner et al., 2010). However, the presence of Flowerlose isoforms at the cell membrane of a particular cell does not imply that the cell will be culled, because at least two other parameters are taken into account: (1) the levels of Flowerlose isoforms in neighboring cells: if neighboring cells have similar levels of Lose isoforms, no cell will be killed (Merino et al., 2013; Rhiner et al., 2010); (2) the levels of a secreted protein called Sparc, the homolog of the Sparc/Osteonectin protein family, which counteracts the action of the Lose isoforms (Portela et al., 2010).

Remarkably, the levels of Flower isoforms and Sparc can be altered by various insults in several cell types, including: (1) the appearance of slowly proliferating cells due to partial loss of ribosomal proteins, a phenomenon known as cell competition (Bailon and Basler, 2014; de Beco et al., 2012; Hogan et al., 2011; Morata and Ripoll, 1975; Moreno et al., 2002; Tamori and Deng, 2011); (2) the interaction between cells with slightly higher levels of d-Myc and normal cells, a process termed supercompetition (de la Cova et al., 2004; Moreno and Basler, 2004); (3) mutations in signal transduction pathways like Dpp signaling (Portela et al., 2010; Rhiner et al., 2010); or (4) viable neurons forming part of incomplete ommatidia (Merino et al., 2013). Intriguingly, the role of Flower isoforms is cell type specific, because certain isoforms acting as Lose marks in epithelial cells...
**A**

*azot::dsRed*

`azot` promoter ~3 Kb

**B**

*azot::dsRed*  
40µm

**C**

dsRed  
DAPI  
Merge

**D**

**E**

48hr ACI  
*Azot::dsRed*

**F**

48hr ACI  
wt  
tub>dmyc  
GFP  
Merge

**G**

48hr ACI  
*Azot::dsRed*

**H**

48hr ACI  
*Azot::dsRed*

**I**

Loser clones  
Wt  
Loser

**J**

24hr ACI  
*Azot::dsRed*

**K**

24hr ACI  
UASbrinker  
GFP  
Merge

**L**

24hr ACI  
*Azot::dsRed*

**M**

24hr ACI  
UASfwe-3  
GFP  
Merge

**N**

**O**

Homogeneous lose  
Wt  
Loser

**P**

Mouse flower  
*Azot::dsRed*

**Q**

24hr ACI  
*Azot::dsRed*

**R**

24hr ACI  
*Azot::dsRed*

**S**

*Azot::dsRed*

**T**

*Azot::dsRed*

**U**

40µm  
*Azot::dsRed*

**V**

40µm  
*Azot::dsRed*

**W**

Genomic context  
5’arm  
white  
3’arm  
Homologous recombination

**X**

44hr APF  
*Azot::dsRed*

**Y**

44hr APF  
*Azot::dsRed*

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Azot Is Required to Eliminate Loser Cells and Unwanted Neurons

To understand Azot function in cell elimination, we generated azot knockout (KO) flies, whereby the entire azot gene was deleted (Figure 1W). Next, we analyzed Azot function using

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Azot maintains tissue fitness during development

Next, we asked what could be the consequences of decreased cell selection at the tissue and organismal level. To this end, we took advantage of the viability of homozygous azot KO flies. We observed an increase of several developmental aberrations. We focused on the wings, where cell competition is best studied and, because aberrations were easy to define, which comprised melanosomal areas, blisters, and wing margin nicks (Figures 3A–3E). Wing defects of azot mutant flies could be rescued by introducing two copies of azot:dsRed, showing that the phenotypes are specifically caused by loss of Azot function (Figures 3A–3E).

Next, we reasoned that mild tissue stress should increase the need for fitness-based cell selection after damage. First, in order to generate multicellular tissues scattered with suboptimal cells, we exposed larvae to UV light (Figure 3F) and monitored Azot expression in wing discs of UV-irradiated WT larvae, which were stained for cleaved caspase-3, 24 hr after treatment (Figures 3G–3K). Under such conditions, Azot was found to be expressed in cleaved caspase-3-positive cells (Figures 3H–3K). All Azot-positive cells showed caspase activation and 17% of cleaved caspase-positive cells expressed Azot (Figure 3G). This suggested that Azot-expressing cells are culled from the tissue. To confirm this, we looked at later time points (3 days after dmyc-induced competition. In the absence of Azot function, loser cells were no longer eliminated (Figures 2A–2F), showing a dramatic 100-fold increase in the number of surviving clones (Figures 2B and 2E). Loser cells occupied more than 20% of the tissue 72 hr after clone induction (ACI) (Figures 2B and 2F). Moreover, using azot(KO; gfp) homozygous flies (that express GFP under the azot promoter but lack Azot protein), we found that loser cells survived and showed accumulation of GFP (Figures S2A and S2B). From these results, we conclude that azot is expressed by loser cells and is essential for their elimination.

In addition, clone removal was delayed in an azot heterozygous background (50-fold increase, 15%) (Figures 2E and 2F), compared to control flies with normal levels of Azot (1-fold, 1%) (Figures 2A, 2E, and 2F). Cell elimination capacity was fully restored by crossing two copies of Azot:dsRed into the azot KO background (0.5-fold, 0.2%) demonstrating the functionality of the fusion protein (Figures 2C, 2E, and 2F). Silencing azot with two different RNAis was similarly able to halt selection during dmyc-induced competition (Figures S2C–S2P). Next, in order to determine the role of Azot’s EF hands, we generated and overexpressed a mutated isoform of Azot (Pm40Q12) carrying, in each EF hand, a point mutation known to abolish Ca2+ binding (Maune et al., 1992). Although overexpression of wild-type azot in negatively selected cells did not rescue the elimination (Figures 2E, S2I, S2L, and S2P), overexpression of the mutant AzotPm40Q12 reduced cell selection (Figures S2H, S2I, S2O, and S2P), functioning as a dominant-negative mutant. This shows that Ca2+ binding is important for Azot function. Finally, staining for apoptotic cells corroborated that the lack of Azot prevents cell elimination, because cell death was reduced 8-fold in mosaic epithelia containing loser cells (Figure 2D).

Next, we analyzed the role of azot in elimination of peripheral photoreceptor neurons in the pupal retina using homozygous azot KO flies (Figures 2G–2L). Pupal retinas undergoing photoreceptor culling (44 hr APF) of azot+/+ and azot−/− flies were stained for the cell death marker TUNEL (Figures 2G and 2L) and the proapoptotic factor Hid (Figures 2H and 2J). Consistent with the expression pattern of Azot, the number of Hid and TUNEL-positive cells was dramatically decreased in azot−/− retinas (Figures 2I–2L) compared to azot+/+ retinas (Figures 2G, 2H, 2K, and 2L).

Those results showed that Azot was required to induce cell death and Hid expression during neuronal culling. Therefore, we tested if that was also the case in the wing epithelia during dmyc-induced competition. We found that Hid was expressed in loser cells and that the expression was strongly reduced in the absence of Azot function (Figures 2M–2Q).

Finally, forced overexpression of Flower+/+/+ isoforms from Drosophila (Figures S2Q, S2R, and S2T) and mice (Figures 2R–2T; Figures S2S and S2U) were unable to mediate WT cell elimination when Azot function was impaired by mutation or silenced by RNAi.

These results suggested that azot function was dose sensitive, because heterozygous azot mutant flies displayed delayed elimination of loser cells when compared with azot WT flies (Figure 2E). We therefore took advantage of our functional reporter Azot:dsRed (Figures 2C and 2E) to test whether cell elimination could be enhanced by increasing the number of genomic copies of azot. We found that tissues with three functional copies of azot were more efficient eliminating loser cells during dmyc-induced competition and most of the clones were culled 48 hr ACI (Figures 2U–2W).

From these results, we conclude that azot expression is required for the elimination of Loseer cells and unwanted neurons (Figure 2X).
Developmental aberrations in the wing/fly

Without irradiation:

With irradiation:

Suboptimal viable cells

Elimination of developmental aberrations

Appearance of developmental aberrations

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irradiation; Figure S3A) and found that the increase in Azot-positive cells was no longer detectable (Figures S3B–S3D). The elimination of azot-expressing cells after UV irradiation required azot function, because cells revealed by reporter azot[KO; gfp], that express GFP instead of Azot, persisted in wing imaginal discs from azot-null larvae (Figures S3E–S3G). We therefore tested if lack of azot leads to a faster accumulation of tissue defects during organ development upon external damage. We irradiated azot+/- pupae 0 stage (Figures 3L–3P) and compared the number of morphological defects in adult wings to those in nonirradiated azot KO flies (Figures 3A–3E). We found that aberrations increased more than 2-fold when compared to nonirradiated azot+/- flies (Figures 3L–3P).

In order to functionally discriminate whether azot belongs to genes regulating apoptosis in general or is dedicated to fitness-based cell selection, we examined if azot silencing prevented Eiger/TNF-induced cell death (GMR-Ga4, UASeiger) (Figures S3H–S3N). Inhibiting apoptosis (UASp35) or eiger (UASRNAi-eiger) rescued eye ablation, whereas azot silencing and overexpression of AzotPm4Q12 did not (Figures S3I–S3N). Furthermore, azot silencing did not impair apoptosis during genitalia rotation (Figures S3O–S3R) (Suzanne et al., 2010) or cell death of epithelial precursors in the retina (Figures S3S–S3V) (Wolff and Ready, 1991).

The results showed above highlight the consequences of nonfunctional cell-quality control within developing tissues (Figure 3Q).

azot Promoter Computes Relative FlowerLose and Sparc Levels

Next, we performed epistasis analyses to understand at which level azot is transcriptionally regulated. For this purpose, we again used the assay where WT cells are outcompeted by dMyc-overexpressing supercompetitors (Figure 1D). We have previously observed that azot induction is triggered upstream of caspase-3 activation and accumulated in outcompeted cells unable to die (Figures 1G and 1H). Then, we genetically modified upstream events of cell selection (Figures 4A–4G): silencing hveLose transcripts by RNAi or overexpressing Sparc, both blocked the induction of Azot::dsRed in WT loser cells (Figures 4A–4D and 4G). In contrast, when outcompeted WT cells were additionally “weakened” by Sparc downregulation using RNAi, Azot is detected in almost all loser cells (Figures 4E–4G) compared to its more limited induction in the presence of endogenous Sparc (Figures 1E and 1F and 4G). Inhibiting JNK signaling with UASpuc (Martin-Blanco et al., 1998; Moreno et al., 2002) did not suppress Azot expression (Figures S4A and S4B).

Next, we analyzed the activation of Azot upon irradiation. Strikingly, we found that all Azot expression after irradiation was eliminated when Flower Lose was silenced and also when relative differences of Flower Lose where diminished by overexpressing high levels of Lose isoforms ubiquitously (Figures 4H–4K; Figure S4C). On the contrary, Azot was not suppressed after irradiation by expressing the prosurvival factor Bcl-2 or a p53 dominant negative (Brodsky et al., 2000; Gaumer et al., 2000) (Figures S4C–S4G). Those results show that Azot expression during competition and upon irradiation requires differences in Flower Lose relative levels.

Finally, we analyzed the regulation of Azot expression in neurons. Silencing five transcripts by RNAi blocked the induction of Azot::dsRed in peripheral photoreceptors (Figures 4L and 4M; Figure S4H). Because Wingless signaling induces FlowerLose-B expression in peripheral photoreceptors (Merino et al., 2013), we tested if overexpression of Daxin, a negative regulator of the pathway (Willert et al., 1999), affected Azot levels and found that it completely inhibited Azot expression (Figures S4H–S4J). Similarly, overexpression of the cell competition inhibitor Sparc also fully blocked Azot endogenous expression in the retina (Figures S4H, S4K, and S4L). Finally, ectopic overexpression of FlowerLose-B in scattered cells of the retina was sufficient to trigger ectopic Azot activation (Figures S4M–S4O). Those results show that photoreceptor cells also can monitor the levels of Sparc and the relative levels of FlowerLose-B before triggering Azot expression (Figure S4P).

The results described above suggest that the azot promoter integrates fitness information from neighboring cells, acting as a relative “cell-fitness checkpoint” (Figures 4N–4Q).

Cell Selection Is Active during Adulthood

To test if fitness-based cell selection is a mechanism active not only during development, but also during adult stages, we exposed WT adult flies to UV light and monitored Azot and Flower expression in adult tissues (Figures 5A–5T). UV irradiation of adult flies triggered cytoplasmic Azot expression in several adult tissues including the gut (Figures 5B–5E; Figures S5A and S5B) (Lemaître and Miguel-Aliaga, 2013) and the adult brain (Figures 5F–5J) (Fernández-Hernández et al., 2013). Likewise, UV irradiation of adult flies triggered Flower Lose expression in the gut (Figures 5K–5N) and in the brain (Figures 5O–5T). Irradiation-induced Azot expression was unaffected by Bcl-2 but was eliminated when Flower Lose was silenced or when relative differences of Flower Lose where diminished in the gut (Figures S5C–S5E) and in the adult brain (Figures S5F–SSH). This suggests that the process of cell selection is active throughout the life history of the animal. Further confirming this conclusion,

Figure 3. Azot Mutants Show Developmental Aberrations

(A–E) Wings of 10- to 13-day-old flies and quantification of developmental aberrations in the wing of each genotype. **p < 0.01. (A and B) azot+/-; (A and C) azot+/+;azot+/-, (A and D) azot+/-; (A and E) azot+/-;azot+/-; (B–E) (A and D) azot+/- and (A and E) azot+/-;azot+/-.

(F–K) Azot and cleaved caspase-3 expression upon UV irradiation (2 x 10^-2 J irradiation dose during second instar larvae, treatment as shown in F). (G) Quantification of the percentage of Azot and cleaved caspase-3-expressing cells after UV irradiation. (H) Azot::dsRed expression after UV irradiation and irradiation (red), (I) cleaved caspase-3 (green) after UV irradiation, (J) merge, and (K) merge with DAPI (blue).

(Q) Scheme showing the requirement of azot function for preventing developmental aberrations. Data are represented as mean ± SEM.
Figure 4. The azot Promoter Computes Relative FlowerLose and Sparc Levels

(A–F) Epistasis analysis of the following genotypes during dmyc-induced supercompetition. (A and B) UASRNAifweLhp, (C and D) UASsparc, and (E and F) UASRNAisparc. Azot::dsRed is shown in red (A, C, and E) and merges with GFP in (B, D, and F).

(G) Graph showing the probability of finding Azot expression in a GFP marked clone in several genotypes.

(H–J) Azot::dsRed expression after UV irradiation (red) is suppressed when UASRNAifweLhp (H and I) or UASfweLose-B and UASfweLose-A (J and K) are expressed ubiquitously. Quantified in Figure S4C.

(L and M) Epistasis analysis of Azot expression in the Drosophila retina. Pupal retinas dissected 44 hr APF of GMR-Gal4; RNAifwe (GD). Azot expression shown in red (L) and merge with nuclear marker DAPI in blue (M). Quantified in Figure S4H.

(N) Azot is not expressed in cells without FlowerLose isoforms.

(O–Q) Cells expressing FlowerLose but that are either surrounded by cells with equal or higher levels of FlowerLose (O) or express high levels of Sparc (P) also do not activate azot expression. Cells with higher relative levels of Lose and not enough Sparc induce the expression of azot and are eliminated (Q).
Azot function was essential for survival after irradiation, because more than 99% of azot mutant adults died 6 days after irradiation, whereas only 62.4% of WT flies died after the same treatment (Figure S5I). The percentage of survival correlated with the dose of azot because adults with three functional copies of azot had higher median survival and maximum lifespan than WT flies, or null mutant flies rescued with two functional azot transgenes (Figure S5J).

Those results show that in adult tissues external damage can induce cell-fitness deficits.

**Role of Cell Selection during Aging**

Lack of cell selection could affect the whole organism by two nonexclusive mechanisms. First, the failure to detect precancerous cells, which could lead to cancer formation and death of the individual. Second, the time-dependent accumulation of unfit
**Figure Legend**

**A** Posterior

**B** - **V**

- **B** - **D**: azot+/+
- **E** - **G**: azot−/−; azot−/−
- **H** - **L**: azot−/−
- **M** - **Q**: azot+/+; azot−/+; azot−/−
- **R** - **V**: azot(KO; gfp)

**N** - **W**

- **N**: Number of neurodegenerative vacuoles/1 day
- **O**: Number of neurodegenerative vacuoles/1 day
- **P**: Number of neurodegenerative vacuoles/1 day
- **W**: Number of GFP-positive cells/1 day

**X**

- **X**: Lifespan

**Y**

- **Y**: Lifespan

**Supplementary Table**

| Genotype | Median survival (d) | % Median survival difference | Max. lifespan | % Max. lifespan difference | Log-Rank p |
|----------|---------------------|----------------------------|--------------|---------------------------|------------|
| azot+/+  | 16.3                | 0%                         | 24           | 0%                        | -          |
| azot−/+  | 18.2                | 12%                        | 24           | 0%                        | >0.05      |
| azot−/−  | 7.8                 | -52.14%                    | 18           | -25%                      | <0.001     |
| azot+/+; + | 25.1               | 54%                        | 28           | 17%                       | <0.001     |

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but viable cells could lead to accelerated tissue and organ decay. We therefore tested both hypotheses.

It has been previously shown that cells with reduced levels for cell polarity genes like scrib or dlg are eliminated but can give rise to tumors when surviving (Igaki et al., 2009; Parisi et al., 2014; Tamori et al., 2010). We therefore checked if azot functions as a tumor suppressing mechanism in those cells (Figures S6A–S6M). Elimination of dlg and scrib mutant cells was not affected by RNAi against azot (Figures S6D–S6M) or when Azot function was impaired by mutation (Figures S6N–S6R), in agreement with the absence of azot induction in these mutant cells (Figures S1O and S6A–S6C). However, azot RNAi or the same azot mutant background efficiently rescued the elimination of clones with reduced Wg signaling (Vincent et al., 2011) (Figures S6J–S6S). Interestingly, azot{KO; gfp} flies were crossed with reporter KO; Gal4 flies were crossed with WT flies as a control; and finally three genotypes (azot{KO; gfp} flies (azot{+/-})), flies with one extra genomic copy of the gene (azot{+/-}; azot{+/-}), and mutant flies rescued with two genomic copies of azot (azot{+/-};azot{+/-}). For all the genotypes analyzed, we observed a progressive increase in the number and size of vacuoles in the brain over time (Figures 6N–6P; Figure S6S). Interestingly, azot{+/-} brains showed higher number of vacuoles compared to control flies (azot{+/-} and azot{+/-};azot{+/-}) and a higher rate of vacuole accumulation developing over time (Figures 6N–6P). In the case of flies with three genomic copies of the gene (azot{+/-};azot{+/-}), vacuole number tended to be the lowest (Figures 6E, 6I, and 6M–6P).

Next, we analyzed the cumulative expression of azot during aging of the adult brain. We detected positive cells as revealed by reporter azot{KO; gfp}, in homozygosis, that express GFP instead of Azot. We observed a time-dependent accumulation of azot-positive cells (Figures 6Q–6W).

From this, we conclude that azot is required to prevent tissue degeneration in the adult brain and lack of azot showed signs of accelerated aging. This suggested that azot could affect the longevity of adult flies (Figures 6X and 6Y). We found that flies lacking azot (azot{+/-}) had a shortened lifespan with a median survival of 7.8 days, which represented a 52% decrease when compared to WT flies (azot{+/-}), and a maximum lifespan of 18 days, 25% less than WT flies (azot{+/-}). This effect on lifespan was azot dependent because it was completely rescued by introducing two functional copies of azot (Figures 6X and 6Y). On the contrary, flies with three functional copies of the gene (azot{+/-}; azot{+/-}) showed an increase in median survival and maximum lifespan of 54% and 17%, respectively.

In conclusion, azot is necessary and sufficient to slow down aging, and active selection of viable cells is critical for a long lifespan in multicellular animals.

**Death of Unfit Cells Is Sufficient and Required for Multicellular Fitness Maintenance**

Our results show the genetic mechanism through which cell selection mediates elimination of suboptimal but viable cells. However, using flip-out clones and MARCM (Lee and Luo, 2001), we found that Azot overexpression was not sufficient to induce cell death in wing imaginal discs (Figures S6T–S6Y). Because Hid is downstream of Azot, we wondered whether expressing Hid under the control of the azot regulatory regions could substitute for Azot function.

In order to test this hypothesis, we replaced the whole endogenous azot protein-coding sequence by the cDNA of the proapoptotic gene hid (azot{KO; hid}) flies; see Figure 7A). In a second strategy, the whole endogenous azot protein-coding sequence was replaced by the cDNA of transcription factor Gal4, so that the azot promoter can activate any UAS driven transgene (azot{KO; Gal4} flies (Figure 7B). We then compared the number of morphological aberrations in the adult wings of six genotypes: first, homozygous azot{KO; Gal4} flies that lacked Azot; second, azot{KO; hid} homozygous flies that express Hid with the azot pattern in complete absence of Azot; third, azot{+/-} WT flies as a control; and finally three genotypes where the azot{KO; Gal4}/UASHid, UASsickle, another proapoptotic gene (Srinivasula et al., 2002), or UASp35, an apoptosis inhibitor. In the case of UASsickle flies, we introduced a second azot mutation to eliminate azot function. Interestingly, the number of morphological aberrations was brought back to WT levels in all the situations where the azot promoter was driving proapoptotic genes (azot{KO; hid}), azot{KO; Gal4} × UASHid, azot{KO; Gal4} × UASsickle, see Figures 7A–7J) with or without irradiation. On the contrary, expressing p35 with the azot promoter was sufficient to produce morphological aberrations despite the presence of one
**A**

**azot**(KO; hid)

![Graph showing developmental aberrations in the wing/fly](image)

**Without irrad.**

**With irrad.**

**B**

**azot**(KO; Gal4)

![Graph showing lifespan analysis](image)

**C**

**azot+/-**

**azot(KO; Gal4)/azot(KO; Gal4)**

**azot(KO; hid)/azot(KO; hid)**

**azot(KO; Gal4)/azot+; UAS-hid**

**azot(KO; Gal4)/azot-; UAS-sickle**

**D**

**azot(KO; hid)/azot(KO; hid)**

**E**

**azot(KO; Gal4)/azot-; UAS-sickle**

**F**

**azot(KO; Gal4)/azot+; UAS-hid**

**G**

**Lifespan analysis 29°C**

| Genotype | Median survival (d) | % Median survival difference | Max. lifespan | % Max. lifespan difference | Log-Rank p |
|----------|---------------------|----------------------------|--------------|---------------------------|------------|
| azot -/- | 12.1                | 0%                         | 24           | 0%                        | -          |
| azot(KO; hid) | 27.2            | 124.74%                    | 34           | 41.6%                     | <0.001     |

**H**

**azot(KO; hid)/azot(KO; hid)**

**I**

**azot(KO; Gal4)/azot-; UAS-sickle**

**J**

**azot(KO; Gal4)/azot+; UAS-hid**

**K**

**Lifespan analysis 25°C**

| Genotype | Median survival (d) | % Median survival difference | Max. lifespan | % Max. lifespan difference | Log-Rank p |
|----------|---------------------|----------------------------|--------------|---------------------------|------------|
| azot +/- | 34.2                | 0%                         | 58           | 0%                        | -          |
| azot -/- | 25.9                | -24.27%                    | 40           | -31.04%                   | <0.001     |
| azot +/- | 53                  | 54.97%                     | 65           | 10.06%                    | <0.001     |
| azot(KO; hid) | 56              | 63.74%                     | 72           | 24.13%                    | <0.001     |

**T**

**Suboptimal cells**

**Targeted Culling**

**Elimination of suboptimal cells**

**Legend on next page**
functional copy of azot (Figures S7A–S7H). Likewise, p35-expressing flies (azot[KO; Gal4]/azot+; UASp35) did not survive UV treatments (Figure S7I), whereas a percentage of the flies expressing hid (26%) or sickle (28%) in azot-positive cells were able to survive (Figure S7I).

From this, we conclude that specifically killing those cells selected by the azot promoter is sufficient and required to prevent morphological malformations and provide resistance to UV irradiation.

Death of Unfit Cells Extends Lifespan
Next, we checked if the shortened longevity observed in azot−− flies could be also rescued by killing azot-expressing cells with hid in the absence of Azot protein. We found that azot (KO; hid) homozygous flies had dramatically improved lifespan with a median survival of 27 days at 29 °C, which represented a 125% increase when compared to azot−− flies, and a maximum lifespan of 34 days, 41% more than mutant flies (Figures 7K and 7L).

Similar results were obtained at 25 °C (Figures 7M and 7N). We found that flies lacking azot (azot−−) had a shortened lifespan with a median survival of 25 days, which represented a 24% decrease when compared to WT flies (azot+/+), and a maximum lifespan of 40 days, 31% less than WT flies (azot++). On the contrary, flies with three functional copies of the gene (azot+/+; azot−) or flies where azot is replaced by hid (azot[KO; hid] homozygous flies) showed an increase in median survival of 54% and 63% and maximum lifespan of 12% and 24%, respectively.

Finally, we tested the effects of dietary restriction on longevity of those flies (Partridge et al., 2005) (Figures S7J and S7K). We found that dietary restriction could extend both the median survival and the maximum lifespan of all genotypes (Figures S7J and S7K). Interestingly, dietary restricted flies with three copies of the gene azot showed a further increase in maximum lifespan of 35% (Figure S7K). This shows that dietary restriction and elimination of unfit cells can be combined to maximize lifespan.

In conclusion, eliminating unfit cells is sufficient to increase longevity, showing that cell selection is critical for a long lifespan in Drosophila.

**DISCUSSION**

Here, we show that active elimination of unfit cells is required to maintain tissue health during development and adulthood. We identify a gene (azot), whose expression is confined to suboptimal or misspecified but morphologically normal and viable cells. When tissues become scattered with suboptimal cells, lack of azot increases morphological malformations and susceptibility to random mutations and accelerates age-dependent tissue degeneration. On the contrary, experimental stimulation of azot function is beneficial for tissue health and extends lifespan. Therefore, elimination of less fit cells fulfills the criteria for a hallmark of aging (López-Otín et al., 2013).

Although cancer and aging can both be considered consequences of cellular damage (Greaves and Maley, 2012; López-Otín et al., 2013), we did not find evidence for fitness-based cell selection having a role as a tumor suppressor in Drosophila. Our results rather support that accumulation of unfit cells affect organ integrity and that, once organ function falls below a critical threshold, the individual dies.

We find Azot expression in a wide range of “less fit” cells, such as WT cells challenged by the presence of “supercompetitors,” slow proliferating cells confronted with normal proliferating cells, cells with mutations in several signaling pathways (i.e., Wingless, JAK/STAT, Dpp), or photoreceptor neurons forming incomplete ommatidia. In order to be expressed specifically in “less fit” cells, the transcriptional regulation of azot integrates fitness information from at least three levels: (1) the cell’s own levels of Flower isoforms, (2) the levels of Sparc, and (3) the levels of Lose isoforms in neighboring cells. Therefore, Azot ON/OFF regulation acts as a cell-fitness checkpoint deciding which viable cells are eliminated. We propose that by implementing a cell-fitness checkpoint, multicellular communities became more robust and less sensitive to several mutations that create viable but potentially harmful cells. Moreover, azot is not involved in other types of apoptosis, suggesting a dedicated function, and—given the evolutionary conservation of Azot—pointing to the existence of central cell selection pathways in multicellular animals.

**EXPERIMENTAL PROCEDURES**

**In Situ Hybridization**

We followed the protocol described in Rhiner et al. (2010). Probe sequences are available upon request.

**Drosophila Genetics**

Stocks and crosses were kept at 25 °C in standard media. The following stocks were used: yw; tub > dmyc > Gal4;Cyo;UAsgfp; azot;dsRed/TM6B; GMR-Gal4; azot::dsRed/TM6B; yw; tub > dmyc > Gal4,azot+/Cyo;UAsgfp; yw; tub

**Figure 7. Culling Azot-Expressing Cells Is Sufficient and Required for Multicellular Fitness Maintenance**

(A and B) Knockin (Kl) schemes (A) azot[KO; Gal4] and (B) azot[KO;hid].

(C–F) Wings from 10- to 13-day-old flies and quantification of developmental aberrations of the following five genotypes: (C) azot+/+, (C and D) azot[KO; Gal4]/azot (KO; Gal4), (C and E) azot[KO;hid]/azot[KO;hid], (C and F) azot[KO; Gal4]/azot−, UASsickle, and (C) azot[KO; Gal4]/azot−/UAShid.

(G–J) Wings from 10- to 13-day-old flies and quantification of developmental aberrations after UV irradiation of the same five genotypes. Irradiation dose of 2 × 10^2 J administered during pupal stage 0.

(K and L) Comparative lifespan studies of genotypes azot[KO;hid]/azot[KO;hid] and azot−− at 29 °C.

(KL) Median and maximum survival of genotypes azot[KO;hid]/azot[KO;hid] and azot−− at 29 °C.

(M and N) Lifespan studies at 25 °C of the following four genotypes: (1) azot+/+, (2) azot−−, (3) azot+/−, azot+, and (4) azot[KO;hid]/azot[KO;hid]. (N) Median and maximum survival of the four genotypes.

(O) Scheme showing that specifically killing Azot-expressing cells with the general proapoptotic factor Hid is sufficient to prevent morphological malformations and rescue azot mutant phenotypes.

Data are represented as mean ± SEM.
Treatments were performed using a UV Stratalinker 2400 machine (UV-B). Confocal images acquired with Leica SP2 and SP5 cam, anti-cytochrome (Rhiner et al., 2010). For the generation of specific antibodies against Azot, Primer sequences are available upon request.

Clone Induction
Flip-out clones were generated after heat shock at 37°C between 5 and 15 min. For ubiquitous expression experiments larvae were subjected to 45 min heat shock for all cells to perform flip-out and activate Gal4 under the control of the actin promoter (act-Gal4).

Azot Reporter: azot::dsRed
The genomic region 3 kb upstream plus the full exon was cloned in pFet sterile vector using Xbal and Kpn1 restriction sites. Primer sequences are available upon request.

Overexpressing Constructs
cDNA of azot was fully sequenced and subcloned into the pUASattB vector using Xbal and Kpn1 restriction sites. In order to generate N- and C-terminal HA-tagged forms, the respective cDNAs were amplified with primers containing the HA sequence and subcloned into Kpn1 and Xba1 sites of pUASattB. Primer sequences are available upon request.

Azotpm4Q12
Site-directed mutagenesis was used to create point mutations that changed glutamic acid (E) to glutamine (Q) as shown in Figure S1A. Primer sequences are available upon request.

Azot Knockout Generation
We followed the genomic engineering strategy described in Huang et al. (2009); homologous regions are shown in (Figure 1A). Primer sequences are available upon request.

Knockin Generation
Knockout founder line (Figure 2A) was used for the generation of knockin flies as described in Huang et al. (2009). cDNA of gfp, hid, and Gal4 was used for the generation of azot(KO; gfp), azot(KO; hid), and azot(KO; Gal4) knockin lines. Primer sequences are available upon request.

Immunohistochemistry
Standard immunohistochemistry protocol was used for antibody detection (Rhiner et al., 2010). For the generation of specific antibodies against Azot, N-terminal peptide MEDISHEEVILDFTF was used to immunize rabbits. Anti-Wingless (ms, 1:50) was from DSHB, anti-caspase-3 (rabbit, 1:100) was from Cell Signaling Technology, anti-KDEL (rabbit, 1:100) was from Abcam, anti-cytochrome c (mouse, 1:800) was from BD PharMingen, anti-Hid (rabbit, 1:50) and anti-HA (rat, 1:250) were from Roche, and anti-jI-Gal (mouse, 1:200) was from Promega. TUNEL staining performed as described (Lolo et al., 2012). Confocal images acquired with Leica SP2 and SP5s microscopes.

UV Treatments
Treatments were performed using a UV Stratallinker 2400 machine (UV-B 254 nm). Adult flies were subjected to 2 × 10⁻² J dose of UV irradiation when they were 1–3 days old and analyzed for Azot and Flower isoform expression 24 hr later. For lifespan experiments after irradiation, a dose of 5 × 10⁻² J was used. Larvae and pupae were subjected to 2 × 10⁻² J dose of UV irradiation, and Azot expression or developmental aberrations were analyzed.

Longevity Assays
cohorts of 100 female flies (1–3 days old) of the same genetic background were collected and kept at 29°C or 25°C on standard food (3.4 l water, 280 g maize, 36 g agar, 120 g yeast, 300 g sugar syrup, 32 g potassium, 6 g methyl, 20 ml propionic acid). Surviving flies were counted every 2 days (He and Jasper, 2014).

Dietary Restriction Assays
cohorts of 100 female flies (1–3 days old) were collected and kept at 29°C on water-diluted standard food (one to one). Surviving flies were counted every 2 days.

Brain Studies
Brain Integrity
Adult flies kept at 29°C of the selected time points and genotypes were analyzed for the appearance of neurodegenerative vacuoles over time in the central brain as previously described (Kretzschmar et al., 1997).

Azot Expression
Adult flies azot(KO; gfp)/azot(KO; gfp) were kept at 29°C. The selected time points were analyzed for the number of GFP-positive cells in the central brain.

Statistical Analysis
For the rescue assay using azot KO in supercompetition (Figure 2E), rescue assay in supercompetition with azot RNAi and overexpression of the protein (Figures S2J–S2P), the rescue assay of clones with apicobasal defects and the clones with deficient Wg signaling (Figures S6N–S6R), and brain integrity studies over time (Figures 6A–6P), the data were analyzed with the K independent samples test. The post hoc DMS test was then used to detect significant differences.

For the caspase-positive cells in azot⁻¹⁻ and azot⁻¹⁻ background (Figure 2D), the rescue assay in overexpression of Flowerisoforms (Figures 2R–2T, Figure S2T), and azot overexpression in clones (Figures S6T–S6Y), all data were analyzed with two independent samples test (Mann-Whitney U test). Levene test was used to analyze number of cleaved caspase-3-positive cells, rescue assay of Flowerisoforms, and number of azot-overexpressing clones.

For the quantification of the number of developmental aberrations before and after irradiation treatment in azot⁻¹⁻, azot⁻¹⁻, and azot⁻¹⁻; azot⁻¹⁻ background (Figures 3A–3E, 3L–3P, 7C–7J, and S7A–S7H), data were analyzed with the K independent samples test (Levene), and Levy-Tukey was used for post hoc analyses.

In the rescue assay in supercompetition using RNAi (24 hr ACI) (Figures 2G–2I, S2G–S2I), the data were analyzed with ANOVA test.

In the quantification of eye size in apoptosis assay (Figures S3H–S3N), the data were analyzed with ANOVA. Bonferroni post hoc test was used to detect significant differences among genotypes.

For the functional assays of azot in retinas (Figures 2G–2L), azot dose sensitive (Figures 2U–2W), rescue assay in overexpression of mouse flower isoform (Figure S2U), and rescue assay of clones with apicobasal defects, and clones with deficient Wg signaling by azot RNAi (Figures S6D–S6M), all data were analyzed with Student’s t test.

In the lifespan analysis (Figures 6X, 7K, 7M, and S7J), the log-rank test was used to study significant differences among the genotypes.

SUPPLEMENTAL INFORMATION
Supplemental Information includes seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.12.017.

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REFERENCES

Baillon, L., and Basler, K. (2014). Reflections on cell competition. Semin. Cell Dev. Biol. 32, 137–144.

Brodsky, M.H., Nordstrom, W., Tsang, G., Kwan, E., Rubin, G.M., and Abrams, J.M. (2000). Drosophila p53 binds a damage response element at the reaper locus. Cell 101, 103–113.

de Beco, S., Ziosi, M., and Johnston, L.A. (2012). New frontiers in cell competition. Developmental dynamics 247, 831–841.

de la Cova, C., Abril, M., Bellosta, P., Gallant, P., and Johnston, L.A. (2004). Drosophila myc regulates organ size by inducing cell competition. Cell 117, 107–116.

Fernández-Hernández, I., Rhiner, C., and Moreno, E. (2013). Adult neurogenesis in Drosophila. Cell Rep. 3, 1857–1865.

Fuchs, Y., and Steller, H. (2011). Programmed cell death in animal development and disease. Cell 147, 742–758.

Gaumer, S., Guénel, I., Brun, S., Théodore, L., and Mignotte, B. (2000). Bcl-2 and Bax mammalian regulators of apoptosis are functional in Drosophila. Cell Death Differ. 7, 804–814.

Greaves, M., and Maley, C.C. (2012). Clonal evolution in cancer. Nature 481, 306–313.

He, Y., and Jhaver, H. (2014). Studying aging in Drosophila. Methods 68, 129–133.

Hogan, C., Kajita, M., Lawrenson, K., and Fujita, Y. (2011). Interactions between normal and transformed epithelial cells: their contributions to tumourigenesis. Int. J. Biochem. Cell Biol. 43, 496–503.

Huang, J., Zhou, W., Dong, W., Watson, A.M., and Hong, Y. (2009). From the cover: directed, efficient, and versatile modifications of the Drosophila genome by genomic engineering. Proc. Natl. Acad. Sci. USA 106, 8284–8289.

Igaki, T., Pastor-Pareja, J.C., Aonuma, H., Miura, M., and Xu, T. (2009). Intrinsinc tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev. Cell 16, 458–466.

Jacobs, K.B., Yeager, M., Zhou, W., Wacholder, S., Wang, Z., Rodríguez-Santiago, B., Hutchinson, A., Deng, X., Liu, C., Horner, M.J., et al. (2012). Detectable clonal mosaiicism and its relationship to aging and cancer. Nat. Genet. 44, 651–658.

Kennedy, S.R., Loeb, L.A., and Herr, A.J. (2012). Somatic mutations in aging, cancer and neurodegeneration. Mech. Ageing Dev. 133, 118–126.

Kretzschmar, D., Hasan, G., Sharma, S., Heisenberg, M., and Benzer, S. (1997). The Swiss cheese mutant causes gial hyperwrapping and brain degeneration in Drosophila. The Journal of Neuroscience 17, 7425–7432.

Laurie, C.C., Laurie, C.A., Rice, K., Doheny, K.F., Zeinick, L.R., McHugh, C.P., Ling, H., Hetrick, K.N., Pugh, E.W., Amos, C., et al. (2012). Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat. Genet. 44, 642–650.

Lee, T., and Luo, L. (2001). Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci. 24, 251–254.

Lemaitre, B., and Miguel-Aliaia, I. (2013). The digestive tract of Drosophila melanogaster. Annu. Rev. Genet. 47, 377–404.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. Science 237, 1154–1162.

Liu, N., Landreth, M., Cao, K., Abe, M., Hendriks, G.J., Kennedell, J.R., Zhu, Y., Wang, L.S., and Bonini, N.M. (2012). The microRNA miR-34 modulates ageing and neurodegeneration in Drosophila. Nature 482, 519–523.

Lolo, F.N., Casas-Tintó, S., and Moreno, E. (2012). Cell competition time line: winners kill losers, which are extruded and engulfed by hemocytes. Cell Rep. 2, 526–539.

López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell 153, 1194–1217.

Martin-Blanco, E., Gampel, A., Ring, J., Virdee, K., Kirov, N., Tolkovsky, A.M., and Martinez-Arias, A. (1998). Puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. Genes Dev. 12, 557–570.

Mauve, J.F., Klee, C.B., and Beckingham, K. (1992). Ca2+ binding and conformational change in two series of point mutations to the individual Ca(2+)-binding sites of calmodulin. J. Biol. Chem. 267, 5286–5295.

Merino, M.M., Rhiner, C., Portela, M., and Moreno, E. (2013). “Fitness fingerprints” mediate physiological culling of unwanted neurons in Drosophila. Curr. Biol. 23, 1300–1309.

Morata, G., and Ripoll, P. (1975). Minutes: mutants of Drosophila autonomously affecting cell division rate. Dev. Biol. 42, 211–221.

Moreno, E. (2014). Cancer: Darwinian tumour suppression. Nature 509, 435–436.

Moreno, E., and Basler, K. (2004). dMyc transforms cells into super-competitors. Cell 117, 117–129.

Moreno, E., and Rhiner, C. (2014). Darwin’s multicellularity: from neurotrophic theories and cell competition to fitness fingerprints. Curr. Opin. Cell Biol. 31C, 16–22.

Moreno, E., Basler, K., and Morata, G. (2002). Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. Nature 416, 755–759.

Moskailev, A.A., Shaposhnikov, M.V., Pyusnina, E.N., Zhavornokov, A., Budovsky, A., Yanai, H., and Fraifeld, V.E. (2013). The role of DNA damage and repair in aging through the prism of Koch-like criteria. Ageing Res. Rev. 12, 661–684.

Parisi, F., Stefanatos, R.K., Strathdee, K., Yu, Y., and Vidal, M. (2014). Transformed epithelia trigger non-tissue-autonomous tumor suppressor response by adipocytes via activation of Toll and Eiger/TNF signaling. Cell Rep. 6, 855–867.

Partridge, L., Piper, M.D., and Mair, W. (2005). Dietary restriction in Drosophila. Mech. Ageing Dev. 126, 938–950.

Petrova, E., López-Gay, J.M., Rhiner, C., and Moreno, E. (2012). Flower-deficient mice have reduced susceptibility to skin papilloma formation. Dis. Model. Mechan. 5, 553–561.

Portela, M., Casas-Tinto, S., Rhiner, C., López-Gay, J.M., Domínguez, O., Soldini, D., and Moreno, E. (2010). Drosophila SPARC is a self-protective signal expressed by loser cells during cell competition. Dev. Cell 19, 562–573.

Raff, M.C. (1992). Social controls on cell survival and cell death. Nature 356, 397–400.

Reeves, H.M. (2006). Sahagún’s “Florentine codex,” a little known Aztecan natural history of the Valley of Mexico. Arch. Nat. Hist. 33, 302–321.

Rhiner, C., López-Gay, J.M., Soldini, D., Casas-Tinto, S., Martín, F.A., Lombardía, L., and Moreno, E. (2010). Flower forms an extracellular code that reveals the fitness of a cell to its neighbors in Drosophila. Dev. Cell 18, 985–998.

Rodrigues, A.B., Zoránovic, T., Ayala-Camargo, A., Grewal, S., Reyes-Robles, T., Krasny, M., Wu, D.C., Johnston, L.A., and Bach, E.A. (2012). Activated STAT regulates growth and induces competitive interactions independently of Myc. Yorkie, Wingless and ribosome biogenesis. Development 139, 4051–4061.

Simi, A., and Ibañez, C.F. (2010). Assembly and activation of neurotrophic factor receptor complexes. Dev. Neurobiol. 70, 323–331.

Simpson, P. (1979). Parameters of cell competition in the compartments of the wing disc of Drosophila. Dev. Biol. 69, 182–193.

Sririvasula, S.M., Datta, P., Kobayashi, M., Wu, J.W., Fujikoa, M., Hegde, R., Zhang, Z., Mukattash, R., Fernandes-Alemanni, T., Shi, Y., et al. (2002). ). sickle, srtc, dme, tct, and yorkie form a feedback loop regulating JNK activity during dorsal closure in Drosophila. Genes Dev. 12, 557–570.

Swiss Cancer League.

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a novel Drosophila death gene in the reaper/hid/grim region, encodes an IAP-inhibitory protein. Curr. Biol. 12, 125–130.

Suzanne, M., Petzoldt, A.G., Speder, P., Coutelis, J.B., Steller, H., and Noselli, S. (2010). Coupling of apoptosis and L/R patterning controls stepwise organ looping. Curr. Biol. 20, 1773–1778.

Szilard, L. (1959). On the Nature of the Aging Process. Proc. Natl. Acad. Sci. USA 45, 30–45.

Tamori, Y., and Deng, W.M. (2011). Cell competition and its implications for development and cancer. Journal of genetics and genomics 38, 483–495.

Tamori, Y., Bialucha, C.U., Tian, A.G., Kajita, M., Huang, Y.C., Norman, M., Harrison, N., Poulton, J., Ivanovitch, K., Disch, L., et al. (2010). Involvement of Lgl and Mahjong/VprBP in cell competition. PLoS Biol. 8, e1000422.

Vanneste, E., Voet, T., Le Caignec, C., Ampe, M., Konings, P., Melotte, C., Debrock, S., Amyere, M., Vikkula, M., Schuit, F., et al. (2009). Chromosome instability is common in human cleavage-stage embryos. Nat. Med. 15, 577–583.

Vidal, M., Larson, D.E., and Cagan, R.L. (2006). Csk-deficient boundary cells are eliminated from normal Drosophila epithelia by exclusion, migration, and apoptosis. Dev. Cell 10, 33–44.

Vincent, J.P., Kolahgar, G., Gagliardi, M., and Piddini, E. (2011). Steep differences in wingless signaling trigger Myc-independent competitive cell interactions. Dev. Cell 21, 366–374.

Willert, K., Logan, C.Y., Arora, A., Fish, M., and Nusse, R. (1999). A Drosophila Axin homolog, Daxin, inhibits Wnt signaling. Development 172, 4165–4173.

Wolff, T., and Ready, D.F. (1991). Cell death in normal and rough eye mutants of Drosophila. Development 113, 825–839.