Tumor suppression by cell competition through regulation of the Hippo pathway

Chiao-Lin Chen1,b,1, Molly C. Schroeder1,c,1, Madhuri Kango-Singhd, Chunyao Taoa, and Georg Haldera,b,c,e,2

*Department of Biochemistry and Molecular Biology and 1Program in Genetics and Development, University of Texas MD Anderson Cancer Center, Houston, TX 77030; 1Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030; 2Department of Biology, Center for Tissue Regeneration and Engineering at Dayton, University of Dayton, Dayton, OH 45469; and 4Vlaams Instituut voor Biotechnologie (VIB) Center for the Biology of Disease, and Center for Human Genetics, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

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Homeostatic mechanisms can eliminate abnormal cells to prevent diseases such as cancer. However, the underlying mechanisms of this surveillance are poorly understood. Here we investigated how clones of cells mutant for the neoplastic tumor suppressor gene scribble (scrib) are eliminated from Drosophila imaginal discs. When all cells in imaginal discs are mutant for scrib, they hyperactivate the Hippo pathway effector Yorkie (Yki), which drives growth of the discs into large neoplastic masses. Strikingly, when discs also contain normal cells, the scrib cells do not overproliferate and eventually undergo apoptosis through JNK-dependent mechanisms. However, induction of apoptosis does not explain how scrib cells are prevented from overproliferation. We report that cell competition between scrib and wild-type cells prevents hyperproliferation by suppressing Yki activity in scrib cells. Suppressing Yki activation is critical for scrib clone elimination by cell competition, and experimental elevation of Yki activity in scrib cells is sufficient to fuel their neoplastic growth. Thus, cell competition acts as a tumor-suppressing mechanism by regulating the Hippo pathway in scrib cells.

Animals have evolved homeostatic mechanisms to eliminate abnormal and cancerous cells, protecting the animal from harm (1). A prominent example of an organism removing abnormal cells that have the potential to form tumors is the elimination of scribble mutant (scrib) cells from Drosophila imaginal discs (2–8). scrib is a conserved tumor-suppressor gene that is essential for the establishment of apical–basal cell polarity (8–10). scrib is a scaffold protein that localizes to basal lateral cell junctions and functions together with the Discs large (Dlg) and Lethal giant larvae (Lgl) adaptor proteins to govern apical–basal junctions and functions. However, induction of apoptosis does not explain how scrib cells are prevented from overproliferation. We report that cell competition between scrib and wild-type cells prevents hyperproliferation by suppressing Yki activity in scrib cells. Suppressing Yki activation is critical for scrib clone elimination by cell competition, and experimental elevation of Yki activity in scrib cells is sufficient to fuel their neoplastic growth. Thus, cell competition acts as a tumor-suppressing mechanism by regulating the Hippo pathway in scrib cells.

The presence of normal cells is required for the elimination of tumorigenic scrib clones because genetically ablating the normal tissue surrounding scrib cells results in hyperproliferation of the scrib cells (2, 3). It has been suggested that scrib cells compete with other cells, a process by which viable cells of lower fitness are removed from a tissue and replaced through extra proliferation of fitter neighbors (17), is responsible for the elimination of scrib and lgl cell clones (2, 14). However, the hypothesis that scrib and lgl clones are eliminated by cell competition is in conflict with other reports and thus is controversial.

It has been reported that cells with compromised Scrib or Lgl function exhibit elevated activity of Yorkie (Yki), a transcriptional coactivator and downstream effector of the Hippo growth-control pathway (13, 14, 18–20). The Hippo pathway is a conserved tumor-suppressor pathway that suppresses growth by antagonizing the activity of Yki (21). Thus, loss of Hippo pathway activity or elevated levels of Yki activity result in hyperproliferation of imaginal disc cells and resistance to apoptosis that normally would eliminate extra cells (21). Notably, an increase in Yki activity can rescue weak cells, such as cells heterozygous for Minute (M) mutations, from being eliminated by cell competition (22). M mutations occur in ribosomal protein-encoding genes and were the first class of genes identified as having cell-competition phenotypes (23). Homozygous M mutations are lethal, but heterozygous M animals are viable, although their cells have reduced growth rates (23). In genetic mosaics, however, interaction between wild-type and M+ cells leads to the elimination of the M+/− cells and expansion of the wild-type population, a phenomenon termed “cell competition” (17). Thus, M+/− cells are less competitive than wild-type cells. Importantly, elevated levels of Yki can rescue M+ cells from being eliminated by cell competition and also can transform normal cells into supercompetitors that induce apoptosis in their neighbors and proliferate at their neighbors’ expense (24, 25). Yki may increase the competitiveness of cells by inducing the expression of Myc, a known regulator of cell competition (24–27). However, the reports that scrib cells have high levels of Yki activity and the hypothesis that scrib cell competition present a paradox. If scrib cells indeed have elevated levels of Yki activity, why does that elevated Yki activity not protect scrib cells from cell competition?

Here we investigated this paradox further. We show that scrib cells are indeed eliminated by cell competition. We found that for this elimination to occur, scrib cells undergo a JNK-dependent suppression of Yki activity; this suppression of Yki activity


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1C.-L.C. and M.C.S. contributed equally to this work.
2To whom correspondence should be addressed. E-mail: ghalder@mdanderson.org.

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prevents \(scrib^-\) cells from hyperproliferating and enables their removal. The modulation of Yki activity in \(scrib^-\) cells thus is a critical effect of the JNK-dependent cell-competition process that removes such tumorigenic cells from imaginal discs. Finally, we show that the Myc and Ras onco-genes, which can rescue \(scrib^-\) cells from elimination (2, 4, 15), do so by conferring competitive fitness to \(scrib^-\) cells and thereby prevent the down-regulation of Yki activity in \(scrib^-\) cells. Our results thus further characterize the effects of cell-competition pathways in removing tumorigenic \(scrib^-\) cells from imaginal discs.

**Results**

**Normal Cells Inhibit the Proliferation of \(scrib^-\) Clones.** \(scrib^-\) clones activate JNK signaling and induce JNK-dependent apoptosis (2–4, 6). However, the induction of apoptosis is not sufficient to explain how \(scrib^-\) clones are eliminated, because blocking apoptosis by coexpression of the caspase inhibitor p35 does not rescue the small clone size of \(scrib^-\) clones to the size observed when JNK activity is inhibited (2, 3). To confirm that apoptosis is not sufficient for the removal of \(scrib^-\) clones, we generated large and consistent numbers of GFP-marked \(scrib^-\) cell clones by combining an eye-specific source of Flippase (ey-Flp) using the mosaic analysis with a repressible cell marker (MARCsystem) (28) and examined the contribution of these mutant cells to third-instar eye discs as a measure of their proliferation and survival. Corroborating previous observations, \(scrib^-\) clones comprised only a small fraction of eye discs compared with wild-type control clones (Fig. 1 A and B) (2–5, 7), as did \(scrib^-\) clones that coexpressed p35 or the antiapoptotic gene Drosophila inhibitor of apoptosis 1 (Diap1) (Fig. 1 C and Fig. S1 A–C) (3). Blocking apoptosis thus is not sufficient to rescue the growth defects of \(scrib^-\) clones. In contrast, \(scrib^+\) cells in which JNK signaling was blocked by coexpressing a dominant-negative form of the Drosophila JNK Basket (BskDN), overexpression is indicated as \(+Bsk^DN\) or because they were generated in animals that were homozygous mutants for \(eiger^{egr^-}\), an extracellular ligand that activates JNK signaling (16), no longer eliminated and grew into large clones (Fig. 1 D and G and Fig. S1D) (2–4, 12). In addition to surviving, these clones hyperproliferated, as revealed by an excess of BrdU-incorporating cells in mutant clones, in contrast to \(scrib^-\) clones with normal JNK activity, which did not grow and remained small (Fig. 1 E–G and Fig. S2 A–F) (12). Therefore, in addition to triggering apoptosis, JNK signaling counteracts the potential of \(scrib^-\) cells to hyperproliferate (2, 5). Notably, \(scrib^-\) cells that cannot activate JNK still showed defects in photoreceptor differentiation, observed through ELAV expression, and in cell polarity, observed through anti-Patj staining, forming multilayered structures of tumorigenic cells (Fig. 1 H and I) (12). These data show that \(scrib^-\) cells have the potential to hyperproliferate and in genetic mosaics this potential is counteracted by JNK activity (2, 3).

**Cell Competition Regulates Hippo Pathway Activity in \(scrib^-\) Cells.** The observation that the proliferation of \(scrib^-\) cells is restricted in the presence of wild-type neighbors raised questions about the role of neighboring cells in maintaining homeostasis and eliminating \(scrib^-\) cells. Removal of \(scrib^-\) clones may depend on cell competition (2), on the presence of neighboring cells with normal apical–basal polarity (3), or on circulating hemocytes that attach to \(scrib^-\) cells and secrete Egr (6). To determine the contribution of cell competition to the elimination of \(scrib^-\) cells, we decreased the fitness of the surrounding \(scrib^-\) cells by making them heterozygous for \(M^{+/}\) cells with \(M^{++}\) neighbors formed large clones of proliferating cells, revealed by high levels of BrdU incorporation, that often resulted in deformed and overgrown imaginal discs (Fig. 1 J and Fig. S2 B and G). This result demonstrates that the suppression of the tumorigenic potential of \(scrib^-\) cell clones depends on the fitness of their neighboring cells rather than on the mere presence of cells with normal polarity. Thus, cell competition between \(scrib^-\) cells and neighboring wild-type cells is essential for the elimination of \(scrib^-\) cells.

To gain insight into the effects of cell competition on \(scrib^-\) cells and to explore how \(scrib^-\) cells are prevented from hyperproliferating, we analyzed the activity of pathways known to regulate imaginal disc growth in \(scrib^-\) cells that were protected from cell competition and then compared that activity with that of \(scrib^-\) cells facing cell competition. Readouts for the Decapentaplegic (Dpp) and Hedgehog (Hh) pathways (29) were not affected significantly in \(scrib^-\) clones in \(egr^-\) discs, demonstrating that \(scrib^-\) cells protected from cell competition do not misregulate these signaling pathways (Fig. S3). In contrast, expanded-lacZ (ex-lacZ), a reporter for the Hippo tumor-suppressor pathway and Yki activity (30), was dramatically up-regulated in \(scrib^-\) cells in \(egr^-\) discs as well as in \(scrib^-\) cells with \(Bsk^DN\) clones (Fig. 2 A–C and E–H and Figs. S2F and S4 A and B) (13). In addition, Yki was more concentrated in the nuclei of \(Egr^-\) cells, a finding that is consistent with elevated Yki activity (Fig. 2 D). Similarly, \(scrib^-\) clones surrounded by \(M^{++}\) cells also displayed high levels of ex-lacZ expression (Fig. 3 A–D). In addition, \(scrib^-\) homozygous discs, in which all cells are \(scrib^-\) and therefore do not face cell
competition, displayed high levels of the Yki activity reporters ex-lacZ and Diap1-GFP (Fig. 3 E and F and Fig. S4 C and D) (31). Thus, scrib−/− cells not facing cell competition have abnormally high levels of Yki activity.

To test whether these elevated levels of Yki activity are required for the hyperproliferation phenotype of “noncompeted” scrib−/− cells, we decreased Yki activity in scrib−/−+BskDN cells by coexpressing Warts (Wts), a Hippo pathway serine threonine kinase that phosphorylates Yki and inactivates it (Fig. 3 H and I and Fig. S5A) (21). We found that such cells made only small contributions to third-instar eye discs, indicating that Yki is important for the proliferation of noncompeted scrib−/− clones. Thus, scrib−/− cells not facing cell competition have high levels of Yki activity, which is required for their hyperproliferation.

The finding that Yki activity is elevated in noncompeted scrib−/− cells raised the question of what happens to Yki in scrib−/− cells that do face cell competition. Elevation of Yki levels is sufficient to protect M−/− cells from cell competition and can even transform normal cells into supercompetitors (22, 24, 25). Remarkably, ex-lacZ, which was up-regulated in noncompeted scrib−/− clones, was not induced in scrib−/− clones surrounded by wild-type cells in most regions of eye and wing discs (Figs. 2 C and 3G). Thus, ex-lacZ generally was not elevated in scrib−/− cells that faced cell competition, whereas scrib−/− clones rescued from cell competition (scrib−/−+BskDN clones) had elevated ex-lacZ levels in all regions of eye and wing discs (Fig. 2, quantified in Fig. S6). The failure of competed scrib−/− clones to up-regulate Yki activity may be caused by the perdurance of Scrib, because competed scrib−/− clones generally were much smaller than rescued clones. However, ex-lacZ also was up-regulated in noncompeted scrib−/− clones that were small (Figs. S7 and S8B). Such small clones also had the polarity and differentiation defects seen in big clones, indicating that it is not Scrib perdurance that prevents the up-regulation of Yki activity in scrib−/− clones subject to cell competition. Thus, these data show that cell competition prevents the up-regulation of Yki activity in scrib−/− cells.

We noted that a minority of scrib−/− clones in the hinge region of wing discs and in the posterior region of eye discs displayed some increase in ex-lacZ expression, which has been observed by other groups (13, 20). Thirty-one percent of clones in the hinge and 16% of clones in the posterior eye had at least one cell in which ex-lacZ was up-regulated (Fig. S6). Notably, the hinge region has been proposed to be a less competitive environment than the wing pouch, and the posterior region of eye discs may similarly be a less competitive environment, since cells in that region start to differentiate earlier than those located more anteriorly (15, 26, 27). Therefore, some scrib−/− clones may face less cell competition in these regions, allowing them to elevate ex-
lacZ levels. However, even in the wing hinge region and posterior eye disc region there was a significant difference in ex-lacZ expression profiles between scrib−/− clones that were subjected to or protected from cell competition (Fig. S6).

To test whether the suppression of Yki activity by cell competition is required for the elimination of scrib−/− clones, we experimentally increased Yki activity in scrib−/− cells by overexpression of Yki (+Yki) or loss of wts. Both these manipulations were sufficient to rescue scrib−/− clones from being outcompeted (Fig. 3 J and K and Fig. S5 B and C). Therefore, the prevention of Yki up-regulation is key to the elimination of scrib−/− clones. We conclude that cell competition acts as a tumor-suppression mechanism by preventing Yki activation in scrib−/− cells.

scrib−/− Cells Not Subjected to Cell Competition Have Enhanced Non–Cell-Autonomous Effects on the Hippo Pathway. scrib−/− clones can cause non–cell-autonomous up-regulation of ex-lacZ in neighboring wild-type cells (Fig. 3G) (20). This non–cell-autonomous effect on Hippo signaling also was observed around scrib−/− clones rescued from elimination: scrib−/− clones in M−/− tissues showed non–cell-autonomous effects on ex-lacZ (Fig. 3B–D). Such non–cell-autonomous induction of ex-lacZ was observed most dramatically around scrib−/− clones that coexpressed oncogenic RasV12, which compete with scrib−/− cells from being out-competed and acts synergistically with loss of scrib to form tumors (Figs. 3A–D and S8A) (2, 4, 7). Clones of scrib−/− cells overexpressing RasV12 (scrib−/−+RasV12) expressed high levels of ex-lacZ and also showed strong non–cell-autonomous up-regulation of ex-lacZ expression (Figs. 3A–D and S8A) (13, 14). Such rescued scrib−/− clones grew into multilayered masses that expanded beyond the epithelial monolayer. This effect, combined with extra growth caused by non–cell-autonomous Hippo pathway regulation, caused non-competed scrib−/− clones to distort the morphology of the discs (Fig. 5A–D). Non–cell-autonomous regulation of Hippo signaling by abnormal or damaged cells has been observed previously and has been suggested as a mechanism for ensuring that compensatory growth restores the tissue (19, 20).

This regenerative signal has been proposed to depend upon JNK signaling (19, 20). In contrast to these reports, however, we observe non–cell-autonomous effects on ex-lacZ in scrib−/− clones in scrib−/− clones in egr−/− animals (Fig. 2E–H and Fig. S4B). Therefore, scrib−/− cells that are not cleared efficiently from imaginal discs are competent to elevate Yki activity in their normal neighbors via a JNK-independent signal.

Increased Relative Myc Levels Protect scrib−/− Cells from Cell Competition. To test further the importance of cell competition in the elimination of scrib−/− cells, we increased their fitness by overexpressing Myc (+Myc), which turns cells into supercompetitors (26, 27). We found that overexpression of Myc in scrib−/− cells rescued their poor growth and resulted in strong up-regulation of ex-lacZ expression (Fig. 4 A and C and Fig. S8B). This result is striking because overexpression of Myc in wild-type cells did not cause up-regulation of ex-lacZ expression; rather, it slightly suppressed ex-lacZ expression levels (Fig. 4 B and D) (25). This indicates that the increase of Yki activity in scrib−/+Myc clones is an indirect consequence of these cells being able to evade cell competition due to the increased fitness conferred by Myc overexpression, rather than Myc directly inducing Yki activity. Thus, Myc has different effects on Hippo signaling in scrib−/− and wild-type cells and the oncogenic potential of Myc is more dramatically realized in scrib−/− cells than in wild-type cells. This suggests that elevated Myc may most potently increase the proliferation of tumorigenic cells by counteracting the growth suppressing effects of cell competition that they may face.

This result could be explained by two different kinds of effects. One possibility is that the absolute level of Myc in scrib−/− cells determines whether scrib−/− cells can survive in the presence of normal neighbors. Alternatively, it could be that high levels of Myc in scrib−/− cells transform them into supercompetitors. In the latter case, the relative levels of Myc between scrib−/− cells and their neighbors would determine whether the scrib−/− cells will be eliminated. To distinguish between these two possibilities, we overexpressed Myc throughout the posterior wing compartment and produced scrib−/− clones in this uniformly high-Myc environment. If Myc contributes to the absolute growth ability of scrib−/− clones rather than to relative growth ability, we would expect that scrib−/− clones would not be eliminated and would be able to grow when Myc is overexpressed in the entire tissue. We observed that scrib−/− clones generated in compartments in which Myc is overexpressed are not rescued from elimination (Fig. 4E). This result indicates that high levels of Myc are insufficient to rescue scrib−/− clones from being eliminated by cell competition if surrounding normal cells also have high levels of Myc. Thus, the effects of Myc on the survival of scrib−/− clones are not a simple result of a cell-autonomous increase in proliferation rate. Rather, the relative level of Myc in scrib−/− cells compared with their normal neighbors is important. When scrib−/− cells have more Myc

Fig. 4. Myc overexpression promotes tumorigenesis of scrib−/− clones. Shown are confocal images of mosaic eye and wing imaginal discs. (A–D) Clones of cells are positively marked by GFP expression (green), and discs are stained for β-Gal to reveal ex-lacZ expression (red in A–D and grey in A–D). (A) scrib−/− clones overexpressing Myc (+Myc) in an eye disc are not eliminated by cell competition and induce ex-lacZ. (B) Myc clones in an eye disc show no notable defects. (C) scrib−/−+Myc clones in a wing disc elevate ex-lacZ expression. (D) +Myc clones in a wing disc suppress ex-lacZ. (E and F) scrib−/− clones marked by the absence of GFP expression were generated in discs in which engrailed-Gal4 drove overexpression of transgenes in the posterior compartment. (E) scrib−/− clones were generated in a wing disc in which Myc, tagged with c-Myc (grey in E ′), was overexpressed in the posterior compartment. No scrib−/− clones are observed, but a large GFP+ twinspot in the posterior compartment shows that scrib−/− tissue was eliminated. (F) scrib−/− clones overproliferate and induce ex-Z (grey in F ″) in a wing disc in which BskDN was expressed. The posterior compartment is marked by the absence of Cubitus interruptus (grey in F ′′). Genotypes are listed in SI Methods.
than their neighbors, they are protected from elimination; when both populations have high Myc levels, the
scrib− cells are eliminated. This result confirms that scrib+ cells are eliminated by cell
competition. In contrast to these results with Myc overexpression, scrib+ clones were rescued from elimination when BskDN was
overexpressed in entire posterior compartments, showing that the overexpression in this system is early enough to rescue scrib−
clones (Fig. 4F). Altogether, we conclude that Myc acts as an oncogene in scrib− cells by increasing their relative fitness.

Discussion
In this study we show that tumorigenic scrib− cells are removed from Drosophila imaginal discs by a cell–cell signaling event that
suppresses elevated Yki activity in scrib+ cells. Previous reports implicated JNK as a mediator of cell competition of
scrib− clones, where it induces apoptosis and suppresses proliferation (2–5, 7). However, it was not known how JNK prevents scrib−
clones from hyperproliferating. We now provide evidence that JNK prevents scrib− clones from hyperproliferating by regulating
the activity of the Hippo pathway effector Yki. First, scrib+ clones that do not face cell competition up-regulate Yki activity,
which drives their hyperproliferation. Second, when scrib+ clones do face cell competition, then JNK signaling prevents the up-
regulation of Yki activity. Third, experimental up-regulation of Yki activity is sufficient to rescue scrib− clones from being
eliminated by cell competition. Fourth, experimental suppression of Yki activity in scrib− clones not subjected to cell competition
is sufficient to suppress their hyperproliferation. Therefore, cell competition suppresses up-regulation of Yki activity in scrib−
cells, and this suppression is important for the elimination of scrib− clones by cell competition. Previous reports showed that
Hippo pathway reporters can be up-regulated in scrib− and lgl− mutant discs and clones (13, 14, 18, 20) and that Yki is required for
the overgrowth of scrib+ + BskDN cells not subjected to cell competition (13). However, these studies did not analyze the
effects of cell competition on Yki activity in scrib+ cells. Our analysis now shows that scrib− cells facing cell competition do not
up-regulate Yki activity and thereby identifies a mechanism that is critical for the elimination of scrib− cells.

Although it was reported that scrib+ and lgl+ can up-
regulate ex-lacZ expression and Yki activity (13, 14, 18, 20).
However, upon quantification we found that the majority of
scrib− clones have normal or reduced levels of ex-lacZ expression,
and only a small percentage of scrib− clones have elevated
levels of ex-lacZ expression. Clones with elevated ex-lacZ
expression were observed mainly in the hinge region of wing discs,
which may provide an environment of reduced cell competition
(15, 26, 27). Thus, outcompeted scrib+ clones do not have ele-
vated levels of Yki activity. In contrast, when scrib− clones are rescued from cell competition, they show highly elevated levels of
ex-lacZ expression (this study and refs. 13 and 14). Similarly,
discs that are entirely mutant for scrib, thereby creating an en-
vironment that does not have competing normal cells, show
hyperactivation of Yki (this study and ref. 13). Cell competition
thus prevents the hyperactivation of Yki in scrib− clones and
turns a potential high-Yki “supercompeting” scrib+ cell into a
cell of lower fitness and less resistance to apoptosis. Importantly,
scrib+ cells and scrib+ + Yki clones show greatly increased growth
and survival compared with scrib− clones. These results show that
elevated levels of Yki are sufficient to protect scrib− cells from
being outcompeted. Thus, if Yki activity already was high in
scrib− cells facing cell competition, those cells would not be
outcompeted, and overexpression of Yki or loss of wts would not
cause such dramatic effects on the survival and growth of scrib−
clones. Apparently, Yki levels in scrib− cells facing cell competition
are not high enough for these cells to evade cell competition.
Thus, the amount of Yki activity in scrib− cells is a critical
determinant of whether scrib− clones are eliminated or form
malignant cells, and the suppression of Yki activity in scrib−
cells is important for the elimination of scrib− clones by cell
competition.

Our studies show that JNK activity is required in scrib− cells for
the suppression of Yki activity by cell competition. In contrast,
JNK signaling can induce Yki activity during regeneration and
compensatory proliferation in imaginal discs (19, 20). Therefore,
the effects of JNK signaling on Yki activity in scrib+ cells are different from those in normal cells: JNK signaling activates Yki
in normal cells promoted to regenerate but suppresses Yki in
scrib− cells induced to be eliminated. Interestingly, both these
effects are observed in discs with scrib+ clones. In scrib− cells, JNK
activity suppresses the hyperactivation of Yki, but in neighboring
cells that are stimulated to proliferate and compensate for the loss
of scrib+ cells, the activities of both JNK and Yki are elevated (11,
19, 20). However, we still observed non–cell–autonomous effects
on Yki reporters in engrailed animals and in discs that ubiquitously
inhibited JNK signaling by BskDN Therefore, JNK-independent
signals contribute to the non–cell–autonomous induction of Yki
activity around scrib− clones. The regulation of Yki by JNK sig-
naling thus is complex and context dependent and may involve
several mechanisms.

The observation that wts− scrib− clones overgrow indicates that
JNK and Wts function in parallel to regulate Yki or that JNK
regulates the Hippo pathway upstream of Wts. JNK can phos-
phorylate and activate Yap1 to regulate apoptosis in mammalian
cells (32, 33). Notably, the JNK phosphorylation sites of Yap1
are different from the Lats phosphorylation sites (21), supporting

![Fig. 5.](#) Model for cell competition acting as a tumor-suppressor mechanism. (A) Wild-type cells have normal apical
basal polarity (Upper). In such cells, Scrib limits the amount of
Yki activity (Lower). (B) When scrib+ cells arise in a disc, they
face cell competition, which leads to their elimination (Upper).
In such tissues, cell competition leads to activation of
JNK in scrib− cells; JNK activation antagonizes Yki activity,
leading to the elimination of the clone. The presence of
scrib+ cells leads to a non–cell–autonomous activation of Yki
activity in neighboring cells, which promotes compensatory
proliferation (Lower). (C) scrib− cells surrounded by poorly
competing M+ cells (Upper) do not suppress the high levels of
active Yki caused by loss of Scrib (Lower). These scrib−
cells are not eliminated, hyperproliferate, and produce a sus-
tained signal that activates Yki in neighboring cells, stimu-
ating overproliferation.
a model in which JNK functions in parallel with Wts to regulate Yki activity. However, it is not known whether the same sites also act to suppress the activity of Yki in other contexts.

Although several models have been proposed to explain how cell–cell interactions between scrib−/− normal cells lead to the elimination of scrib−/− clones from epithelia, it was not clear what properties normal cells must possess to perform this tumor-suppressive role (16, 17). Our data demonstrate that for scrib−/− cells to be eliminated they must be juxtaposed with cells that have higher levels of competitive fitness, not just proper cellular architecture. Overexpression of the Myc or Ras−/− oncogenes in scrib−/− clones increases their fitness. As a result, in scrib−/− clones cell competition does not suppress Yki activity, which protects these clones from being eliminated. Interestingly, Myc expression also synergizes with loss of scrib to form tumors in mammals (9), and our data offer a model to explain this phenomenon.

In addition to the cell-autonomous hyperproliferation, scrib−/− cells that are not removed from imaginal discs have profound non–cell-autonomous effects on the Hippo pathway. This non–cell-autonomous Hippo pathway-regulating signal may serve normally as a regenerative growth signal that facilitates the replacement of eliminated or dying cells, such as outcompeted scrib−/− cells (19, 20). If scrib−/− clones are not eliminated efficiently, however, this signal may persist longer than required to restore the tissue, thereby causing overgrowth and deformation of neighboring tissue. Thus, continued residence of tumorigenic cells can stimulate growth beyond that needed for compensation, essentially hijacking the proliferation and regeneration programs of their normal neighbors. Therefore, the non–cell-autonomous activation of Yki by scrib−/− cells may have important implications for tumor–stromal interactions in human cancers.

In summary, we conclude that cell competition is crucial in suppressing the tumorigenic capacity of scrib−/− cells and does so by regulating their Yki activity (Fig. 5 A and B). Loss of this regulation results in overproliferation of both tumorigenic cells and neighboring wild-type cells (Fig. 5C). Efficient elimination of scrib−/− clones by cell competition prevents Yki-fueled overgrowth of mutant cells and prevents them from disrupting proliferation control of their normal neighbors. Thus, we identified a tumor-suppression mechanism that depends on signaling between normal and tumorigenic cells. These data identify evasion of cell competition as a critical step toward malignancy and illustrate a role for wild-type tissue in preventing the formation of cancers.

**Methods**

**Drosophila Stocks and Culture.** All crosses were maintained at 25 °C. Mutant clones were induced by mitotic recombination using the Flippase/Flippase recognition target (Flip/FRT) system. Flip recombinase was expressed in a tissue-specific manner using ey-Flip and ubx-Flip or was induced conditionally using hs-Flip. The Upstream Activation System (UAS)-Gal4 system was used to overexpress genes of interest. The scrib−/− null allele was flipped against corresponding ubi-GFP-marked FRT chromosomes to generate scrib−/− clones. To express GFP and other genes of interest in mutant clones, the MARCM system was used (28). Heat shocks were performed at 37 °C for 30 min during the first or second larval stage. Information regarding immunostaining procedures and Drosophila strains used is given in SI Methods.

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Supporting Information

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SI Methods

Strains. The following strains were used in this study: (i) y w, hs-Flp; FRT82Bubi-gfp; (ii) y w, ey-flp; FRT82Bubi-gfp; (iii) y w, ubx-Flp; FRT82Bubi-gfp; (iv) y w, hs-Flp, FRT82B Minute(3), ubi-GFP/TM6B; (v) y w, ey-flp; act > y+>GAL4, UAS-GFP, FRT82B tub-GAL80; (vi) y w, hs-Flp, tub-GAL4, UAS-GFP, FRT82B tub-GAL80/TM6B; (vii) act, FRT82B Minute(3), ubi-GFP/TM6B; (viii) y w; UAS-Diap1; (ix) y w, UAS-Flp; (xii) y w, UAS-Ras112 (II); (xiii) y w, UAS-Ras112 (II); (xv) y w; UAS-dMyc with c-Myc tag (III); (xvi) act, act, FRT82B Minute(3), ubi-GFP, FRT82Bubi-gfp; (xvii) y w, hs-Flp; eng-railed-GAL4/CyO; (xviii) y w; Diap1-GFP (II); (xix) y w; scrib1/TM6B.

Immunostaining. Antibody staining of imaginal discs and BrdU incorporations were performed as previously described (1). The following antibodies were used: mouse anti-BrdU (1:50; Becton-Dickinson); mouse anti-β-galactosidase (anti-β-Gal) (1:2,000; Promega); rabbit anti-β-Gal (1:600; Cappel); rabbit anti-phospho-Mothers against decapentaplegic (anti-p-Mad) (1:600; E. Lauffer, Columbia University, New York); rat anti-ELAV (1:60; Developmental Studies Hybridoma Bank); rat anti-Cubitus interruptus (anti-Ci) (1:150; R. Holmgren, Northwestern University, Evanston, IL); mouse anti-Patj (1:50; H. Bellen, Baylor College of Medicine, Houston, TX); rabbit anti-Yorkie (anti-Yki) (1:500; D. Pan, Johns Hopkins University, Baltimore, MD), and rabbit anti-cMyc (1:200; Cell Signaling).

Detailed Genotypes. Genotypes in Fig. 1:

A: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/++; FRT82B tub-GAL80/FRT82B
B: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/++; FRT82B tub-GAL80/FRT82B scrib2
C: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/++; FRT82B tub-GAL80/FRT82B scrib2
D: y w, ey-flp/+; UAS-bskDN (II); act > y+>GAL4, UAS-GFP/++; FRT82B tub-GAL80/FRT82B scrib2
E: y w, hs-Flp; FRT82Bubi-gfp/FRT82B
F: y w, hs-Flp; FRT82Bubi-gfp/FRT82B scrib2
G: y w, hs-Flp; act, FRT82Bubi-gfp/FRT82B scrib2
H: y w, hs-Flp; FRT82Bubi-GFP/TUB82B scrib2
J: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/UAS-Yki; FRT82B tub-GAL80/FRT82B scrib2
K: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/++; FRT82B tub-GAL80/FRT82B scrib2

Genotypes in Fig. 2:

A: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B
B and D: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
C: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
E–H: y w, hs-Flp, tub-GAL4, UAS-GFP/+; UAS-bskDN; ey-flp+/+; FRT82B tub-GAL80/FRT82B scrib2

Genotypes in Fig. 3:

A: y w; ey-flp+/+; FRT82B Minute(3), ubi-gfp/FRT82B
B–D: y w, hs-Flp, ey-flp+/+; FRT82B Minute(3), ubi-gfp/FRT82B scrib2
E: y w; ey-flp+/+; scrib1/FRT82B scrib2
F: y w; ey-flp+/+; scrib1/ FRT82B scrib2
G: y w, ubx-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
H: y w, ey-flp/+; UAS-bskDN; act > y+>GAL4, UAS-GFP/UAS-Wts; FRT82B tub-GAL80/FRT82B
I: y w, ey-flp/+; UAS-bskDN; act > y+>GAL4, UAS-GFP/UAS-Wts; FRT82B tub-GAL80/FRT82B scrib2
J: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/UAS-Yki; FRT82B tub-GAL80/FRT82B scrib2
K: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/++; FRT82B tub-GAL80/FRT82B scrib2

Genotypes in Fig. 4:

A and C: y w, hs-Flp, tub-GAL4, UAS-GFP/++; ex697/+; FRT82B tub-GAL80/UAS-Myc, FRT82B scrib2
B and D: y w, hs-Flp, tub-GAL4, UAS-GFP/++; ex697/+; FRT82B tub-GAL80/UAS-Myc, FRT82B
E: y w, hs-Flp, en-GAL4 ex697/++; UAS-Myc, FRT82B scrib2
F: y w, hs-Flp, UAS-bskDN, en-GAL4 ex697/++; FRT82B tub-GAL80/FRT82B

Genotypes in Fig. S1:

A: y w, hs-Flp; ey-flp+/+; act > y+>GAL4, UAS-GFP/UAS-p35; FRT82B tub-GAL80/FRT82B
B: y w, hs-Flp; FRT82B Minute(3), ubi-gfp/FRT82B
C: y w, hs-Flp; FRT82Bubi-gfp/FRT82B
D: y w, hs-Flp; FRT82Bubi-gfp/FRT82B scrib2
E: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
F: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
G: y w, hs-Flp; FRT82B Minute(3), ubi-gfp/FRT82B scrib2
H: y w, hs-Flp, tub-GAL4, UAS-GFP/+; UAS-bskDN; ex697/++; FRT82B tub-GAL80/FRT82B

Genotypes in Fig. S2:

A: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B
B: y w, hs-Flp; FRT82B Minute(3), ubi-gfp/FRT82B
C: y w, hs-Flp; FRT82Bubi-gfp/FRT82B
D: y w, hs-Flp; FRT82Bubi-gfp/FRT82B scrib2
E: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
F: y w, hs-Flp; ey-flp+/+; FRT82Bubi-gfp/FRT82B scrib2
G: y w, hs-Flp; FRT82B Minute(3), ubi-gfp/FRT82B scrib2
H: y w, hs-Flp, tub-GAL4, UAS-GFP/+; UAS-bskDN; ex697/++; FRT82Bubi-gfp/FRT82B

Genotypes in Fig. S3:

A and B: y w, hs-Flp; ey-flp+/+; ex697; FRT82Bubi-gfp/FRT82B

Genotypes in Fig. S4:

A: y w, hs-Flp; ey-flp+/+; ex697; FRT82Bubi-gfp/FRT82B
B: y w, hs-Flp; ey-flp+/+; ex697; FRT82Bubi-gfp/FRT82B scrib2
C: y w; Diap1-GFP/+; scrib1/FRT82B scrib2
D: y w; Diap1-GFP/+; FRT82Bscrib2/TM6B

Genotypes in Fig. S5:

A: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/UAS-Wts; FRT82B tub-GAL80/FRT82B
B: y w, ey-flp+/+; act > y+>GAL4, UAS-GFP/UAS-Yki; FRT82B tub-GAL80/FRT82B
C: y w; ey-flp+/+; act > y+>GAL4, UAS-GFP, FRT82B tub-GAL80/FRT82B wts1

Genotypes in Fig. S7:

A–D: y w, hs-Flp, tub-GAL4, UAS-GFP/++; ex697/UAS-Ras112; FRT82B tub-GAL80/FRT82B scrib2

Genotypes in Fig. S8:
A: y w, hs-FLP, tub-GAL4, UAS-GFP/++; ex^{697}/UAS-Ras^{V12}; FRT82B tub-GAL80/FRT82B

B: y w, hs-FLP, tub-GAL4, UAS-GFP/++; ex^{697}/+; FRT82B tub-GAL80/UAS-Myc, FRT82B scrib^{2}


**Fig. S1.** Preventing apoptosis in scribble- (scrib^{-}) cells does not rescue clone size. Shown are confocal images of eye imaginal discs containing clones of cells with different genotypes as indicated. Clones were made using the mosaic analysis with a repressible cell marker (MARCM) system to label positively mutant clones with GFP expression (green) and eye-specific source of Flippase (ey-FLP) to induce recombination in eye discs. Cell nuclei are labeled with DAPI (blue). (A) Clones overexpressing p35 (+p35). (B) Clones overexpressing Drosophila inhibitor of apoptosis 1 (+Diap1). (C) scrib^{-}+Diap1 clones do not hyperproliferate. (D) Clones overexpressing a dominant-negative form of the Drosophila JNK Basket (+Bsk^{DN}) are normal size. Genotypes are listed in SI Methods.
Fig. S2. *scrib*− clones rescued from cell competition hyperproliferate in wing discs. Shown are confocal images of wing imaginal discs containing clones of cells with different genotypes as indicated. Clones of cells are marked by the absence of GFP expression (green), and discs are stained for BrdU incorporation to reveal cells in S-phase (red in A–G, grey in A′–G′). (A) Wild-type clones in eiger− (egr−/−) animals have no proliferation defects. (B) Wild-type clones in a Minute heterozygous (M+/−) background also display no proliferation defects. (C) Wild-type clones show a normal BrdU incorporation pattern. (D) *scrib*− cells with normal neighbors have normal proliferation patterns. (E) Wild-type clones in an egr−/− animal show no proliferation defects. (F) *scrib*− clones in an egr−/− animal show high levels of BrdU incorporation. (G) *scrib*− clones surrounded by M+/− cells show high levels of BrdU incorporation. (H) Clones overexpressing BskDN, positively marked by expression of GFP, show no change in expression of the Yki activity reporter expanded-lacZ (ex-Z) (red in H, grey in H′). Genotypes are listed in SI Methods.
**Fig. S3.** *scrib*− clones protected from cell competition do not display noticeable defects in Hedghog (Hh) and Decapentaplegic (Dpp) signaling. Shown are confocal images of wing imaginal discs containing *scrib*− clones in *egr−/−* animals. Clones of cells are marked by the absence of GFP expression. (A) Discs are stained for Ci, a transcription factor that undergoes proteolytic cleavage in the absence of Hh signaling and whose levels reveal the amount of Hh signaling (red in A, grey in A’). (B) Discs stained for pMad to reveal the activity of Dpp signaling, which causes Mad phosphorylation (red in B, grey in B’). Genotypes are listed in SI Methods.

**Fig. S4.** Hippo signaling is suppressed in *scrib*− cells not facing cell competition. Shown are confocal images of eye and wing imaginal discs containing clones of cells with different genotypes as indicated. (A and B) Clones of cells are marked by absence of GFP expression (green), and discs are stained for β-Gal to reveal the levels of ex-lacZ (ex-Z) expression (red in A and B, grey in A’ and B’). (A) Wild-type clones in an *egr−/−* animal show no changes in ex-lacZ expression. (B) High-magnification view of a *scrib*− clone in an *egr−/−* animal. Cells within the clone as well as those bordering it show elevation of ex-lacZ. (C and D) Single-slice confocal images of wing imaginal discs taken at the same settings with Hippo pathway reporter Diap1-GFP expression shown in grey. (C) *scrib*− discs show uniform elevation of Diap-GFP expression. (D) Discs heterozygous for *scrib* show normal size and Diap1-GFP expression pattern. Genotypes are listed in SI Methods.
Fig. S5. Hippo signaling levels regulate clone growth. Shown are confocal images of wing imaginal discs containing clones positively marked by expression of GFP. Clones were made using the MARCM system to label mutant clones positively with GFP expression (green) and ey-Flp to induce recombination in eye discs. Cell nuclei are labeled with DAPI (blue). (A) Clones overexpressing Warts (+Wts) are small. (B) Clones overexpressing Yki (+Yki) overgrow. (C) wts⁻ clones also grow large. Genotypes are listed in SI Methods.

Fig. S6. Quantification of ex-lacZ expression in scrib⁻ clones with and without overexpression of BskDN. Bars represent populations of clones in different regions of the discs, labeled below the graph. The level of ex-lacZ expression within each clone was compared with ex-lacZ expression levels in that region and assigned to one of three categories based on the relative ex-lacZ expression. The fraction of clones in which no cells exhibited elevation of ex-lacZ expression is indicated in blue; the fraction of clones in which at least one but not all cells displayed elevated ex-lacZ expression is shown in green; and the fraction of clones in which all cells showed elevated ex-lacZ expression is indicated in yellow. Numbers at the base of each bar indicate the sample size.

Fig. S7. scrib⁻ cells that are rescued by RasV12 overexpression show non–cell-autonomous effects on Hippo signaling. Shown are confocal images of wing imaginal discs containing clones of scrib⁻ clones overexpressing RasV12 (+RasV12). Discs are stained for ex-lacZ, shown in red (A–D) or grey (A’–D’), and DAPI, shown in blue (C and D). (A) scrib⁻+RasV12 marked by GFP expression (green in A–D, grey in B’). (B) Optical cross-section through a scrib⁻ +RasV12 clone showing that ex-lacZ expression is induced both inside and outside the clone. (C and D) Apical and basal sections of the disc in A at higher magnification. RasV12 expression increases the fitness of scrib⁻ cells, thus relieving the suppression of Yki. Genotypes are listed in SI Methods.
Fig. S8. Small scrib− clones protected from cell competition elevate ex-lacZ expression. Shown are confocal images of wing imaginal discs containing clones positively marked by expression of GFP. Discs are stained for β-Gal to reveal the levels of ex-lacZ expression (red in A–B, grey in A′–B′). (A) +RasV12 clones show variable changes in ex-lacZ expression levels. (B) Small scrib− clone overexpressing Myc has increased levels of ex-lacZ expression. Genotypes are listed in SI Methods.