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Drosophila endocytic neoplastic tumor suppressor genes regulate Sav/Wts/Hpo signaling and the c-Jun N-terminal kinase pathway

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Genetic screens in the fruit fly *Drosophila melanogaster* have identified a class of neoplastic tumor suppressor genes (endocytic nTSGs) that encode proteins that localize to endosomes and facilitate the trafficking of membrane-bound receptors and adhesion molecules into the degradative lysosome. Loss of endocytic nTSGs transforms imaginal disc epithelia into highly proliferative, invasive tissues that fail to differentiate and display defects in cellular apicobasal polarity, adhesion and tissue architecture. As vertebrate homologs of some Drosophila nTSGs are linked to tumor formation, identifying molecular changes in signaling associated with nTSG loss could inform understanding of neoplastic transformation in vertebrates. Here, we show that mutations in genes that act at multiple steps of the endolysosomal pathway lead to autonomous activation of the Sav/Wts/Hpo (SWH) transcriptional effector Yki (YAP/TAZ in vertebrates) and the Jun N-terminal kinase (JNK), which is known to promote Yki activity in cells with disrupted polarity. Yki and JNK activity are elevated by mutations at multiple steps in the endolysosomal pathway, including mutations in the *AP-2σ* gene, which encodes a component of the AP-2 adaptor complex that recruits cargoes into clathrin-coated pits for subsequent internalization. Moreover, reduction of JNK activity can decrease elevated Yki signaling caused by altered endocytosis. These studies reveal a broad requirement for components of the endocytic pathway in regulating SWH and JNK outputs and place Drosophila endocytic nTSGs into a network that involves two major signaling pathways implicated in oncogenesis.

Introduction

Genetic screens have identified an assortment of genes that are required to restrict growth of developing epithelia in the fruit fly *Drosophila melanogaster*. Among these are a relatively small subset of genes, termed neoplastic tumor suppressor genes (nTSGs), whose inactivation transforms imaginal disc epithelia into highly proliferative, invasive tissues that fail to differentiate and display defects in cellular apicobasal polarity, adhesion and tissue architecture.[1-3] These phenotypes indicate that nTSGs affect many cellular processes, including proliferation, differentiation, polarity control and adhesion. Understanding these nTSG phenotypes will allow for a greater understanding of how these processes are coupled in cells. Moreover, as a number of studies have linked the vertebrate homologs of these Drosophila nTSGs to tumor formation (reviewed in refs. 4 and 5), identifying molecular changes in signaling associated with nTSG loss could inform understanding of neoplastic transformation in vertebrates.

The *erupted* (*ept*) nTSG is a member of a subgroup of nTSGs that encode factors required for targeting of internalized transmembrane and membrane-associated proteins to the lysosome.[4-7] The Ept protein is a homolog of the *S. cerevisiae* vacuolar protein sorting 23 (Vps23) and vertebrate tumor susceptibility gene 101 (TSG101) proteins, which are components of the endosomal-sorting complex required for transport-I (ESCRT-I) complex. ESCRT-1 functions sequentially with the ESCRT-2 and -3 complexes to promote multivesicular body (MVB) biogenesis of late endosomes, a step required for complete exposure of cargo proteins to the proteolytic environment of the lysosome (reviewed in ref. 8). The mechanisms by which defects in endolysosomal trafficking elicit such strong growth phenotypes are only partially understood. Interestingly, mutations in genes that act at multiple steps of the endolysosomal pathway, including for example *syntaxin-7/avalanche*,[9] *ept/tsg101*,[4] *vps25* [5,7] and *vps22*,[10] produce overtly similar neoplastic disc phenotypes, indicating that they may share a regulatory target(s) or pathway. Genetic and molecular studies of *ept* mutant cells show central roles of the Notch and JAK-STAT signaling pathways in *ept* phenotypes.[4,11,12] However, because the phenotypes of these animals cannot be fully rescued by reducing Notch and JAK/STAT signaling, and because neoplasia ensues in endocytic mutants which do not activate both of these pathways (e.g., *syx7/avl* [9]), it is likely that other signaling pathways are altered in these genetic backgrounds.

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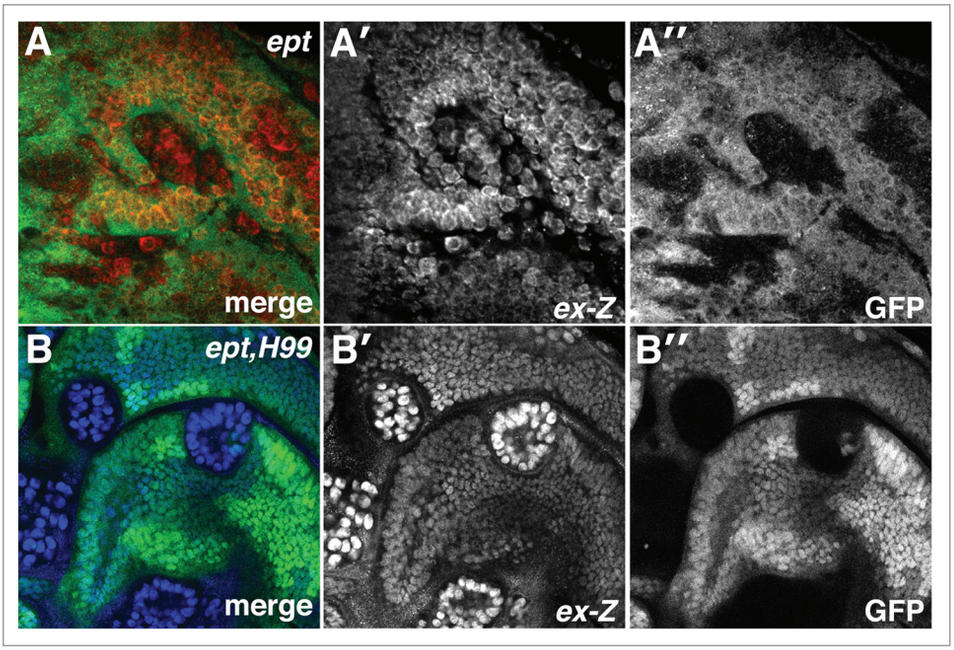

Figure 1. *ept* loss elevates Yki activity. α-β-gal staining to detect the *ex-lacZ* reporter (red in A; blue in B) in (A) *ept* or (B) *ept,H99* mosaic imaginal eye discs (clones are marked by the absence of GFP).

The Salvador-Warts-Hippo (SWH) pathway is emerging as a central integrator of signals from membrane proteins that control cell proliferation and survival in metazoans (reviewed in ref. 13). The pathway consists of a core cassette composed of two kinases Hippo (Hpo) and Warts (Wts) and the scaffolding protein Salvador (Sav). Hpo activation by upstream signals promotes its association with Sav, allowing for Hpo-dependent phosphorylation and activation of Wts. Active Wts phosphorylates the pro-growth transcription factor Yorkie (Yki), rendering it inactive via 14-3-3-dependent cytosolic sequestration. SWH signal strength is modulated in response to a variety of upstream inputs,[14] including those involving transmembrane proteins such as the cadherins Fat and Dachsous,[15-18] the apicobasal polarity factor Crumbs[19-23] and the Dpp receptor Thickveins.[24] As transmembrane proteins are likely to be trafficked through the endolysosomal system, defects in internalization and/or trafficking of these proteins in cells lacking endocytic nTSGs may deregulate SWH signaling and contribute to nTSG overgrowth phenotypes.

In order to better understand how mutations in endocytic nTSGs promote neoplastic growth of Drosophila imaginal discs, we explored the state of Yki activity in the background of *ept*-deficient cells. We find that Yki activity is autonomously elevated in *ept* mutant cells, and that this phenotype is strongly enhanced when *ept* loss is combined with a block in cell death. Unlike Notch and JAK-STAT signaling, which are only activated by later blocks in the endolysosomal pathway (e.g., *ept* and *vps25*), we find that Yki activity is elevated by manipulating genes at each step in the endolysosomal pathway including *AP-2σ*, which encodes a component of the AP-2 adaptor complex that recruits cargoes into clathrin-coated pits for subsequent internalization (reviewed in ref. 25). Moreover, we show that Yki activation in endocytosis-defective cells is accompanied by activation of the JNK signaling pathway, a MAP kinase pathway that has been linked to Drosophila neoplasia and control of Yki activity.[26-29] Additionally, we find that elevated JNK activity is required for the altered Yki signaling observed in endocytic mutants, as reduction of JNK activity can suppress the elevated Yki signaling observed in the background of a block in endocytosis. These studies highlight a requirement for multiple endocytic factors in regulating SWH outputs and place Drosophila endocytic nTSGs into a network that involving two major signaling pathways implicated in oncogenesis.

Results

***ept* mutant cells display altered *yki* activity.** Drosophila imaginal discs composed of *ept* mutant cells overgrow as neoplastic masses.[4,11,12] Because SWH signaling is linked with two key features of neoplasia, altered contact inhibition and excessive proliferation (reviewed in ref. 13), we examined whether activity of the SWH transcriptional effector Yki was modified in *ept*-deficient cells. To test this model, we generated *ept* mutant clones in the Drosophila larval eye disc and analyzed the expression of the Yki transcriptional reporter *expanded-lacZ* (*ex-lacZ*).

The pattern of *ex-lacZ* expression is variable in *ept* mosaic eye discs, but a portion *ept* mutant clones display non-cell autonomous activation of *ex-lacZ* in a ring of normal cells immediately surrounding mutant clones (**Fig. 1A–A''**). This non-autonomous effect has been previously associated with Yki-dependent regenerative growth of normal cells elicited by high rates of apoptosis within *ept* mutant clones.[27,30] To assess whether blocking apoptotic signals in *ept* cells might reveal an autonomous effect on Yki activity, we tested *ex-lacZ* activity in clones of cells doubly mutant for *ept* and the small deletion *Df(3L)H99* (or *H99*), which removes genes (*reaper*, *grim* and *hid*) required for developmental apoptosis in Drosophila.[31]

We have previously shown that *ept,H99* clones overgrow aggressively and produce large neoplastic eye discs.[11] *ept,H99* cells show strong cell-autonomous activation of *ex-lacZ* (**Fig. 1B–B''**). The *H99* genes are not themselves required to restrict *ex-lacZ* expression,[32] indicating that blocking apoptosis of *ept* mutant cells leads to strong cell-autonomous inactivation of Yki.

Loss of both early and late endocytic nTSGs elevates Yki activity in disc cells. As a component of the ESCRT-I complex, *ept* acts at a relatively late step in the endolysosomal pathway. To test whether earlier blocks within the endolysosomal pathway

also autonomously elevate Yki activity, specific endolysosomal pathway members were depleted in the posterior domain of the larval wing by *engrailed-Gal4* (*en>*) expression of *UAS-inverted repeat* (IR) RNA-interference constructs and tested for effect on expression of *ex-lacZ*.

Reduction of the clathrin-adaptor protein AP-2σ, which is required for cargo recruitment and internalization (reviewed in ref. 25), activates *ex-lacZ* and causes the posterior compartment of the wing disc to overgrow (**Fig. 2B**). Similarly, depletion of Syntaxin-7 (*syx7/avl*) or Rab-5, which promote early endosome formation in Drosophila,[9] results in strong activation of the *ex-lacZ* Yki-reporter and tissue overgrowth (**Fig. 2C and D**). Overgrowth caused by depletion of the ESCRT-II complex member Vps25, which acts after Ept/ESCRT-I, is also associated with activation of *ex-lacZ* (**Fig. 2E**). Interestingly, AP-2σ-depleted cells showed very mild activation of a second Yki reporter, *thread-lacZ* (*th-lacZ*) (**Fig. S1**), suggesting that endocytic blocks affect some Yki targets more so than others. This result parallels the finding that mutations in the endocytic adaptor and Yki-binding protein Myopic elevate *ex-lacZ* but not *th-lacZ*.[32] These data indicate that reduction of several members of the endolysosomal pathway can elevate expression of a SWH reporter gene in the nucleus. Moreover, this link between defective endocytosis and SWH signaling appears to map to multiple steps along the pathway including cell-surface internalization, early endosome formation and MVB biogenesis.

Endocytic neoplastic tumor suppressor genes require *yki* to overgrow. Given the effect of altered endocytosis on *ex-lacZ*, we next tested whether Yki, the pro-growth effector of the SWH-pathway, was required for phenotypes observed in endolysosomal mutants. One hallmark of mutations that produce disc neoplasia is a strong reduction and/or absence in pupariation.[6] Therefore, we utilized the *eyeless-FLP* system to generate eye-antennal discs composed of mostly *syx7/avl* mutant cells and analyzed whether overexpression of the SWH scaffolding protein Sav, which antagonizes Yki activity,[33,34] could affect pupariation rates among animals with *syx7/avl* mutant eye discs. While animals bearing *syx7/avl* mutant eye discs normally die during an extended larval stage and rarely form pupae (reviewed in ref. 9), those that also express Sav in *syx7/avl* mutant eye discs pupariate more frequently and a fraction can eclose as adult animals with small, roughened eyes (**Fig. 3A and B**). Importantly, Sav overexpression itself has only a mild effect on control eyes [*FRT/M(3)*], suggesting that *syx7/avl*-deficient cells are especially sensitive to the dosage of *sav* activity.

In parallel experiments, we co-depleted AP-2σ and Yki from cells by *en-GAL4*-driven expression of *UAS-AP-2σ-IR* and *UAS-yki-IR* transgenes. We were unable to assess whether Yki depletion could rescue *AP-2σ-IR*-driven lethality as *en-GAL4* expression of the *UAS-yki-IR* transgene led to significant lethality alone (data not shown). However, as with the *syx/avl*-deficient animals noted above, co-depletion of Yki led to a significant reduction in pupariation delay in the *en > AP-2σ-IR* background (**Fig. 3C**). Moreover, depletion of Yki blocks the effect of $AP\text{-}2\sigma^{IR}$ on *ex-lacZ* expression and prevents overgrowth of the posterior domain of the wing disc (**Fig. 3D–D''**). Thus *yki* is required for the effect of $AP\text{-}2\sigma^{IR}$ loss on disc growth and the SWH transcriptional program.

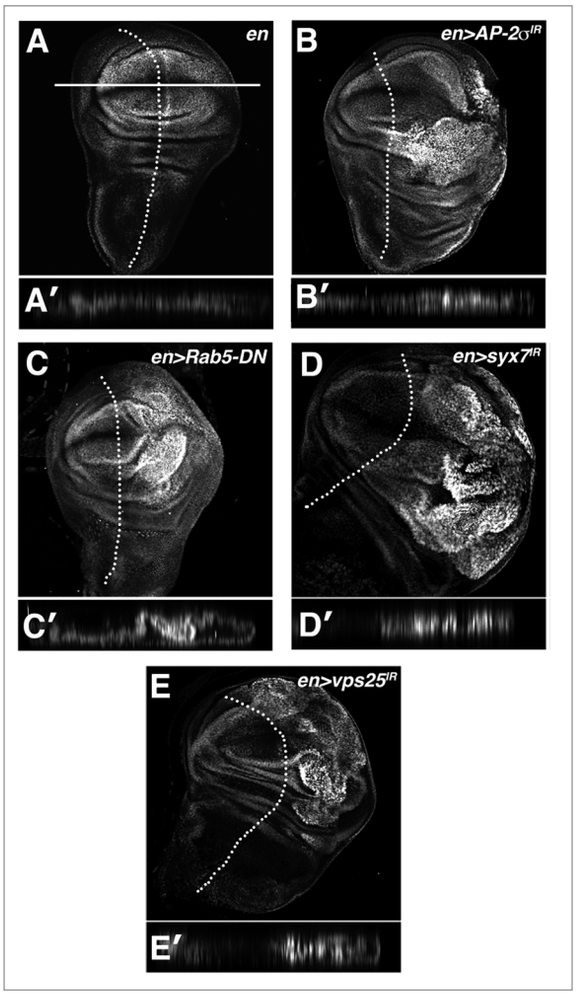

Figure 2. Multiple blocks in the endolysosomal pathway elevate Yki activity. α-β-gal staining of larval imaginal wing discs in which the *ex-lacZ* SWH-reporter has been placed in the background of (A) *en,* (B) *en > AP-2σ-IR* (C) *en > Rab5*^{DN} (D) *en > syx7-IR* and (E) *en > vps25-IR* animals. Tangential and lateral sections are shown for each genotype. Lateral sections were obtained from the dorsal-ventral margin of the wing epithelium as indicated by the line in (A). Posterior compartment is to the right of the dotted line.

Endocytic neoplastic tumor suppressor genes elevate JNK activity in vivo. The transmembrane polarity protein Crumbs (Crb) is a target of ESCRT-dependent endolysosomal turnover.[4,5,7] Crb also controls the levels and localization apical SWH-pathway component Expanded (Ex) by recruiting it to the apical membrane and stimulating its phosphorylation and proteasomal turnover.[19-22] As Ex inhibits Yki by direct binding to it and by promoting its phosphorylation by Wts (reviewed in ref. 13), this Crb-Ex link could contribute to Yki activation in endolysosomal mutants that block Crb turnover (e.g., *ept*). Depletion of *syx7/avl*, *rab7* or *vps25* caused Crb to accumulate, as has been reported previously in references 5, 7 and 9, although depletion of *AP-2σ* had a more minor effect on Crb

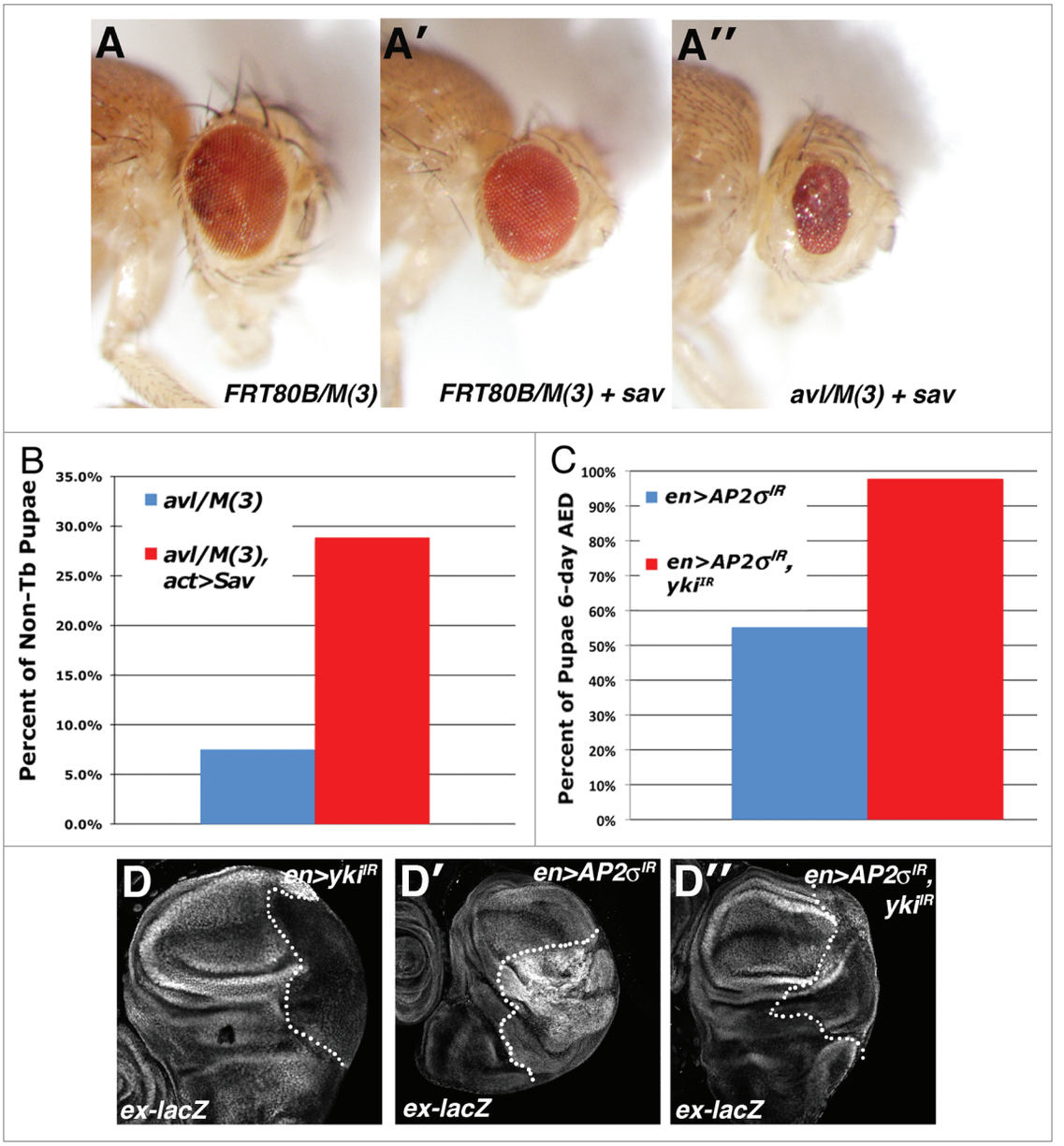

Figure 3. Endolysosomal growth phenotypes are sensitive to the genetic dose of Yki. Light microscopic images of (A) *eyFLP;FRT80B/M(3)*, (A′) *eyFLP;Act > CD2 > Gal4, UAS-sav;FRT80B/M(3)* and (A″) *eyFLP,Act > CD2 > Gal4,UAS-sav;avl*^{1}*/M(3)* adult eyes, in which *sav* is overexpressed using the *Act > CD2 > Gal4* 'Flp-out technique'. (B and C) Quantitative analysis showing the percent of pupae at 6 d AED (after egg deposition) in the indicated genotypes. A minimum of 50 animals was counted per genotype. (D) α-β-gal staining to detect *ex-lacZ* reporter in the background of the indicated genotypes: (D) *en > yki-IR* (D′) *en > AP-2σ-IR* and (D″) *en > AP-2σ-IR,yki-IR*. Posterior compartment is to the right of the dotted line.

(**Fig. S2**). Depletion of *AP-2σ* also led to mild reduction in the polarity factor aPKC (**Fig. S3**), indicating a subtle but reproducible effect of AP-2σ loss on polarity proteins. Consistent with a previous report showing that *vps25* mutant cells express reduced Ex,[35] we found that *ept*-deficient cells also contain reduced levels of Ex (**Fig. S4A**). Ex levels are elevated in *AP-2σ* mutant cells (**Fig. S4B**), which is consistent the well-established role of *ex* as a Yki target gene (reviewed in ref. 34). Thus *ept* loss and *AP-2σ* loss have opposite effects on Ex levels, but nonetheless elevate Yki activity to a similar degree. Accordingly, if a common mechanism links *AP-2σ* and *ept* to Yki, then it is unlikely to be exclusively via control of the Crb/Ex arm of the SWH pathway.

Cells lacking either of the apical-basal polarity factors *lethal (2) giant larvae* (*lgl*) or *discs-large* (*dlg*) elevate Yki activity in part via the c-Jun N-terminal kinase (JNK) signaling pathway.[27,30] JNK activity also promotes Yki-activation in Drosophila intestinal stem cells,[28,29] and in vertebrates Yap1 (e.g., Yki) is a target of JNK1/2 activity in cultured cells.[36] We therefore analyzed JNK signaling in cells lacking early, middle and late endocytic proteins. JNK-pathway signaling involves upstream activation of a MAPKKK, which leads to the eventual phosphorylation and

activation of Basket (Bsk), the terminal JNK kinase in Drosophila.[37] Active p-Bsk then phosphorylates the transcription factor Jun-related antigen (Jra),[38] which heterodimerizes with the transcription factor Fos to form an active AP-1 transcriptional complex in the nucleus. Several transcriptional targets of active AP-1 have been identified in Drosophila, including *matrix-metalloprotease-1* (*MMP-1*) and the *puckered* (*puc*) dual-specificity phosphatase.[39,40]

MMP-1 protein levels are strongly elevated in *ept* and *ept,H99* clones mutant clones compared with surrounding wild type tissue (**Fig. 4A–A'' and C–C''**). MMP-1 expression is also elevated in the posterior compartment of *en* > *AP-2σ-IR*, *en* > *syx7/avl-IR* and *en* > *vps25-IR* wing discs (**Fig. 5A–C**). Similar results were observed with the JNK-reporter *puc-lacZ*, which is elevated in *en* > *AP-2σ-IR* and *en* > *syx/avl7-IR* wing discs relative to an *en* > control (**Fig. 5D–F**). We also analyzed levels of phospho-JNK in endocytic mutants. As with MMP-1, increased p-JNK staining was evident in *ept* clones, in *ept,H99* clones (**Fig. 4B–B'' and D-D''**) and in the posterior compartment of *en* > *syx7/avl-IR* and *en* > *vps25-IR* animals (**Fig. 5H–I**). Although *en* > *AP-2σ-IR* cells showed robust elevation of the JNK reporters *puc-lacZ* and MMP-1, these cells showed only mild elevation of p-JNK levels (**Fig. 5G**). In summary, these data suggest that altered endocytosis is accompanied by elevations in Yki activity and JNK signaling.

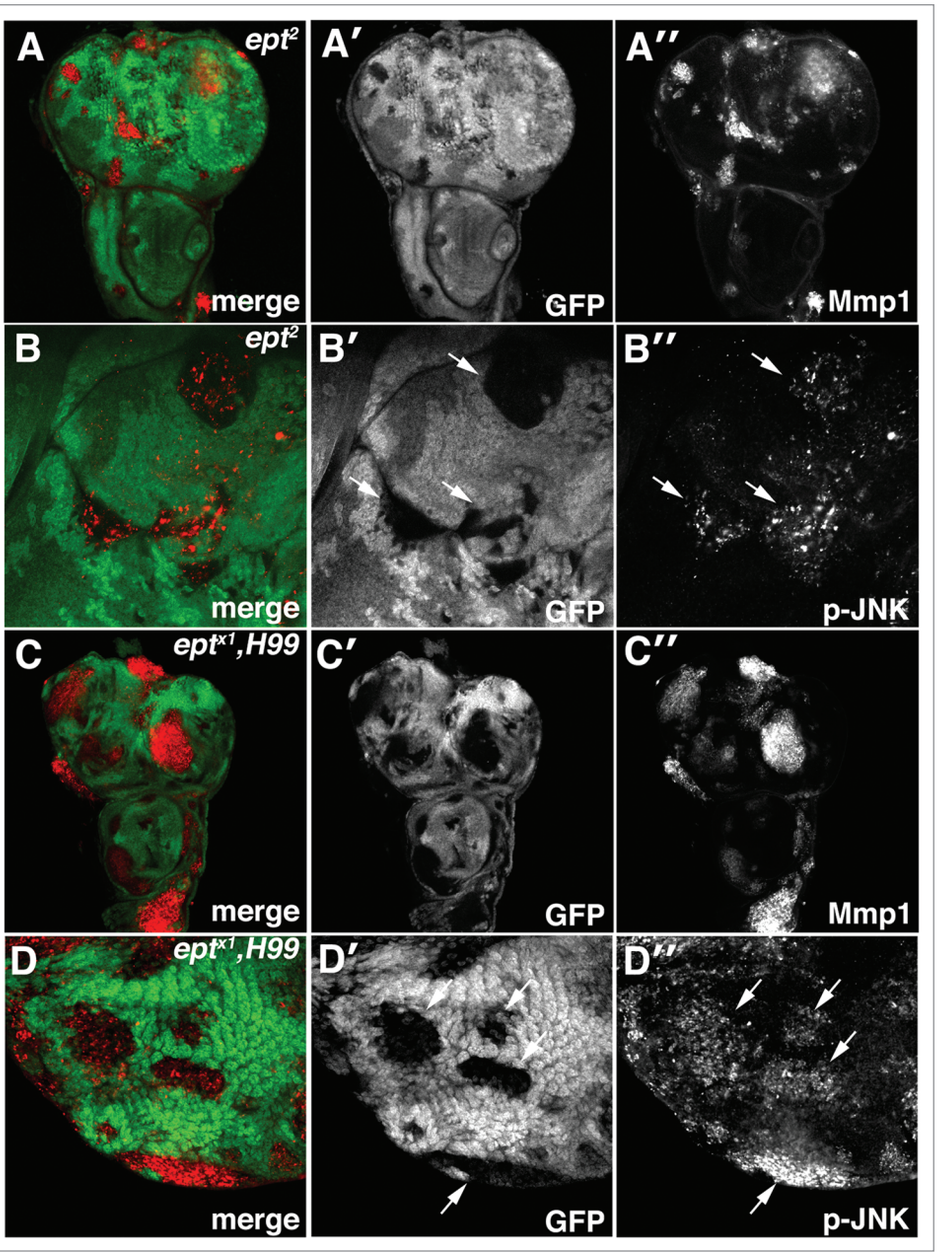

Figure 4. Loss of *ept* elevates JNK activity. Confocal images of (A–B) *ept*^{2} and (C–D) *ept*^{x1}*,H99* clones marked by the absence of GFP stained for (A and C) MMP1 and (B and D) phosphorylated-JNK (p-JNK). Arrows denote p-JNK in mutant clones.

Endocytic neoplastic tumor suppressor genes require *bsk* to activate Yki. The established role of JNK upstream of Yki suggests that the activation of these two pathways could be linked in cells with defective endocytic nTSGs. To test this, we analyzed the effect of expression of a dominant-negative allele of *bsk* (*DN-Bsk*) on Yki activity in the background of *en* > *syx7/avl-IR* animals. Co-expression of *DN-Bsk* with *avl-IR* reduces both the elevated *ex-lacZ* and MMP-1 expression observed in *en* > *avl-IR* discs (**Fig. 6A and B**). Additionally, we found that expression of the *DN-bsk* transgene was sufficient to rescue the disorganized morphology associated with a block in endocytosis (**Fig. 6C**), paralleling similar results from studies that found a reduction of JNK activity could restore defective tissue architecture and polarity in *lgl* mutant animals.[41]

Discussion

The link between neoplastic transformation and mutations in genes required for the endolysosomal trafficking in Drosophila is well established (reviewed in refs. 42 and 43). Here we provide insight into this link further by highlighting a connection between endocytic neoplastic tumor suppressor genes (nTSGs) and two major proliferation control pathways, the JNK pathway

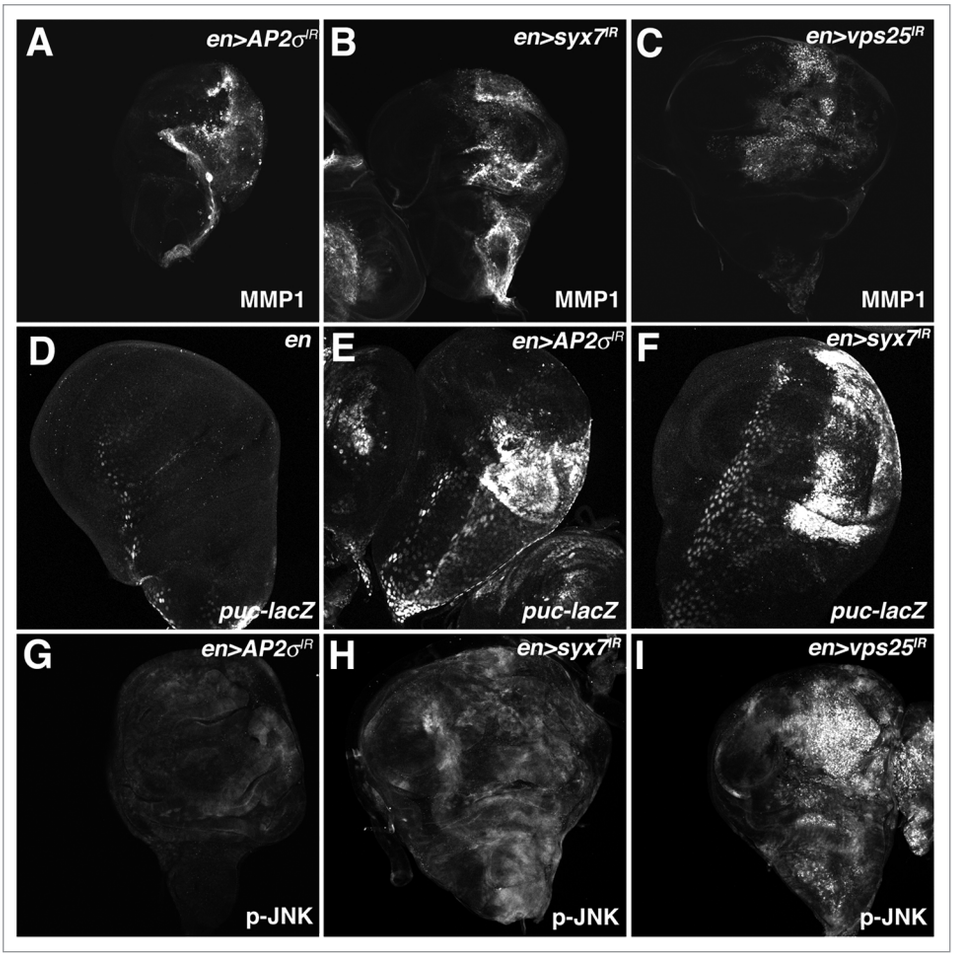

Figure 5. Depletion of AP-2σ, Syx7 or Vps25 elevates JNK activity. Confocal images of larval imaginal wing discs from (A and G) *en > AP-2σ-IR*, (B and H) *en > syx7-IR* and (I) *en > vps25-IR* flies stained for (A–C) MMP1 and (G–I) p-JNK. (D–F) α-β-Gal staining of larval imaginal wing discs in which the *puc-lacZ* JNK-pathway reporter has been placed into the background of (D) control *en >*, (E) *en > AP-2σ-IR* or (F) *en > syx7-IR* flies. Wing discs are oriented with the posterior compartment to the right.

and the SWH pathway. We show that loss of genes encoding factors that act at early (*AP-2*σ), middle (*syx7/avl*, *rab5*), or late (*ept*, *vps25*) steps in the endolysosomal pathway is associated with elevated nuclear activity of the SWH component Yki, which controls expression of genes involved in cell division, cell growth and cell death (reviewed in ref. 13). Moreover, we show that *yki* is required for overgrowth of discs lacking the clathrin adaptor gene *AP-2*σ and for the elevated expression of the Yki transcriptional target *ex*. In parallel to these effects on Yki, we find that loss of endocytic nTSGs is accompanied with significant upregulation of JNK-pathway activity, which is linked to disc neoplasia and SWH-regulation, and that reduction of JNK activity in this background can suppress ectopic Yki activation in cells.

The data presented here reinforce an emerging role for JNK signaling in driving Yki-mediated proliferative phenotypes in cells with disrupted polarity and/or endocytosis. JNK signaling is required for disc neoplasia phenotypes produced by loss of the basolateral polarity components *lgl* and *scrib*,[30,44-46] and this has been proposed to occur through Yki. JNK1/2 can phosphorylate Yap1 in cultured cells,[36] and JNK-activation also promotes Yki activity in Drosophila gut intestinal stem cells,[28,29] indicating that Yki can be a target of JNK signaling in normal physiologic contexts as well. The mechanism by which JNK is activated by defective polarity/endocytosis is not clear. The data presented here do not indicate whether JNK activation elicited by loss of endocytic nTSGs occurs independent of altered apicobasal polarity. Studies of *dlg* and *lgl* alleles demonstrate a clear role for polarity factors in control of JNK signaling in imaginal discs cells.[27,47] Moreover, recent evidence of a pathway involving the polarity factor Cdc42, the Rho1 GTPase, and JNK suggests that altered cell polarity triggers JNK fairly directly,[48] and that polarity defects in endocytic nTSG mutants such as *ept* and *vps25* are the primary driver of JNK hyperactivity. However, the polarity kinase aPKC, which is a component of the Cdc42/Par6/aPKC complex,[49] is a regulator of endocytic trafficking from the apical membrane.[50] Defective polarity could disrupt endocytic internalization and trafficking of apical regulators of the JNK-pathway, such as the TNF receptor Wengen.[51] Indeed, the finding that loss of the clathrin-adaptor protein AP-2σ is sufficient to activate the Jun reporter *puc-lacZ* and the Yki reporter *ex-lacZ* with relatively subtle effects on the polarity markers Crb and aPKC (**Figs. S2 and S3**) seems to suggest that altered clathrin-mediated endocytosis (CME) of membrane receptors could contribute to Yki activation by endocytic blocks. Some signaling receptors such as EGF and TGFβ are internalized via alternate CME and non-CME pathways that produce distinct downstream signaling outputs.[43,52] This type of mechanism could conceivably play an important role in controlling receptor signaling in normal Drosophila epithelial cells and in those lacking proteins required for various steps in the CME endolysosomal degradation pathway.

Our data also provide insight into phenotypes of ept and perhaps TSG101 mutant cells. First, the proliferation of *ept* mutant cells has been linked to JAK-STAT and Notch signaling,[4,11,12] but our findings suggest that JNK-stimulated activation of Yki may also act as a pro-proliferative factor in these cells. This pro-proliferative role of JNK only becomes apparent when apoptosis is blocked. Uhlirova and Bohmann (2006) noted a similar conditional relationship between a block in apoptosis at the appearance

of JNK-dependent invasive phenotypes.[40] Second, a pro-death role for JNK in ept mutant cells very much mirrors recent work identifying a pro-apoptotic role for JNK in cells mutant for the ESCRT-III component *vps4*,[53] and raises the possibility that JNK plays an equivalent role in apoptosis of mouse cells lacking the *ept* vertebrate ortholog *TSG101*,[54-56] which is a candidate tumor suppressor (reviewed in ref. 57). If so, then blockade of JNK-driven death may be necessary for the survival and overproliferation of TSG101 mutant cells in vertebrate tumors.

Materials and Methods

Genetics. Crosses were performed at 25°C unless otherwise noted. For analysis of larval wing discs, crosses were maintained at at 28°C to enhance GAL4 activity. For analysis of adult wings, crosses were maintained at 20°C. Alleles used: *ept*;[2] *ept*^{x1}*,Df(3L)H99* (M. Gilbert); *UAS-sav* (G. Halder); *ex*^{697};[58,59] *th*^{ic58} (B. Hay); *UAS-AP-2σ-IR* (Bloomington Stock Center, stock 27322); *UAS-syx7/avl-IR* (Bloomington Stock Center, stock 29546); *UAS-rab7-IR* (Bloomington Stock Center, stock 27501); *UAS-vps25-IR* (Bloomington Stock Center); *avl*^{1} (D. Bilder); *UAS-Rab5-DN* (M. Scott); *UAS-Yorkie-IR* (Vienna Drosophila RNAi Center); *UAS-Bsk-DN* (Bloomington Stock Center, stock 6409). Clonal analysis was performed using: *eyFLP;ubi-GFP,FRT80B.* 'Flp-out' analysis was performed using: *eyFLP;Act* > *CD2* > *Gal4;*Rps17,[4] FRT80B.

Immunohistochemistry and immunoblotting. Immuno-staining and confocal microscopy were performed as described previously in reference 60. Antibodies used: mouse α-β-Gal 1/1,000 (Promega); mouse α-MMP-1, 1/100 of 1:1:1 aliquot of clones 5H7B11, 3B8D12, 3A6B4 (Developmental Studies Hybridoma Bank); rabbit α-aPKC 1/1,000 (Santa Cruz, C-20); guinea pig α-Ex 1/5,000 (R. Fehon); rabbit α-phospho-JNK 1/1,000 (Promega); rat α-Crb-Extra 1/500 (U. Tepass and E. Knust).

Pupariation analysis. Briefly, adults were allowed to deposit embryos for a span of 24 h, after which their progeny was tracked and the number of pupae and larvae were compared at 144 h AED. A minimum of 50 flies was analyzed per genotype.

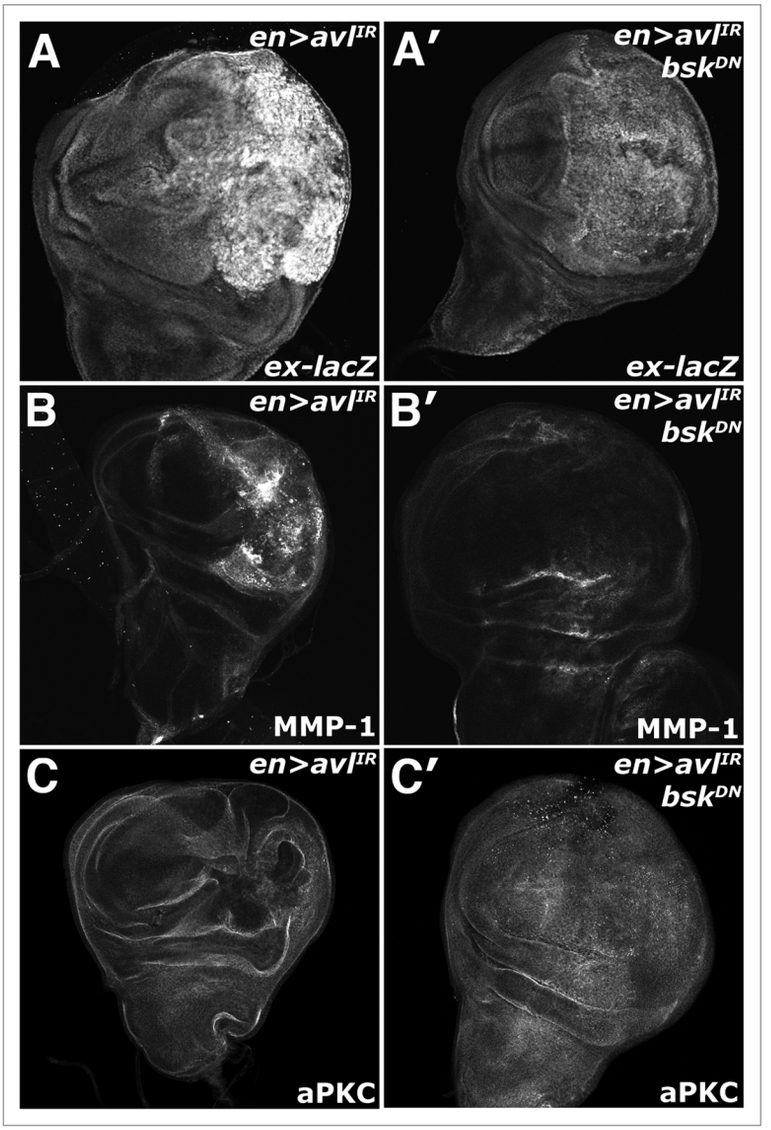

Figure 6. Endolysosomal activation of Yki requires Bsk activity. Confocal images of larval imaginal wing discs in which the *ex-lacZ* reporter has been placed in the background and of (A–C) *en* > *syx7-IR* and (A′–C′) *en* > *syx7-IR*, DN-Bsk animals and stained for (A) α-β-gal, (B) MMP1 and (C) aPKC to show disc morphology. Wing discs are oriented with the posterior compartment to the right.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Note

Supplemental material can be found at:
www.landesbioscience.com/journals/cc/article/18243

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