Src42A modulates tumor invasion and cell death via Ben/dUev1a-mediated JNK activation in Drosophila

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Loss of the cell polarity gene could cooperate with oncogenic Ras to drive tumor growth and invasion, which critically depends on the c-Jun N-terminal Kinase (JNK) signaling pathway in Drosophila. By performing a genetic screen, we have identified Src42A, the ortholog of mammalian Src, as a key modulator of both RasV12/lgl−/−-triggered tumor invasion and loss of cell polarity gene-induced cell migration. Our genetic study further demonstrated that the Bendless (Ben)/dUev1a ubiquitin E2 complex is an essential regulator of Src42A-induced, JNK-mediated cell migration. Furthermore, we showed that ectopic expression of sorcin in wing disc epithelia of the developing wing disc, loss of the cell polarity gene-induced cell migration. Our genetic study further demonstrated that the Bendless (Ben)/dUev1a ubiquitin E2 complex is an essential regulator of Src42A-induced, JNK-mediated cell migration. Furthermore, we showed that ectopic expression of sorcin in wing disc epithelia, with increased MMP1 production in wing disc epithelia. Moreover, Ben/dUev1a could cooperate with RasV12 to promote tumor overgrowth and invasion. In addition, we found that the Ben/dUev1a complex is required for ectopic Src42A-triggered cell death and endogenous Src42A-dependent thorax closure. Our data not only provide a mechanistic insight into the role of Src in development and disease but also propose a potential oncogenic function for Ubc13 and Uev1a, the mammalian homologs of Ben and dUev1a.

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Cancer is the leading cause of death worldwide, causing an estimated total of 577,190 deaths in United States alone in 2012. Cancer-related mortalities are mainly caused by metastasis, rather than the primary tumor that arises from a malignant lesion. Although significant progress has been made towards the understanding of cancer development, the molecular and genetic mechanisms of tumor metastasis have remained poorly understood. With reduced genome redundancy and amenable genetic tools, Drosophila melanogaster has become an excellent model system to investigate the genetic mechanism of cancer biology over the past decades. Several invasion and metastasis models have been established in both adult flies and developing larvae. In the eye–antennal discs, expression of oncogenic mutants, such as RasV12, can cooperate with oncogenes that disrupt cell polarity, such as scrib or lethal giant larvae (ig), to induce invasive tumors into the ventral nerve cord (VNC). In the epithelia of the developing wing disc, loss of the cell polarity gene (scrib) or C-terminal SRC kinase (Csk) along the anterior/posterior (A/P) boundary using patched-Gal4 driver produces an invasive migration phenotype, which has been used to model cell migration in vivo.

The c-Jun N-terminal Kinase (JNK) pathway is evolutionarily conserved from fly to human and has an essential role in regulating a wide range of cellular activities including proliferation, differentiation, migration and apoptosis. Recently, JNK signaling has been shown to have an important role in modulating RasV12/scrib−/−-triggered tumor growth and metastasis, as well as RafG12V/RhoGEF2-induced tumorgenesis. Src was the first discovered oncogene encoding a non-receptor membrane-associated tyrosine kinase. Nine Src family members have been identified in mammals, whereas the Drosophila genome encodes only two Src homologs, Src42A and Src64B. Src42A and Src64B have a redundant role in regulating dorsal closure and are both required for tracheal cell morphogenesis. Recent studies found that Src64B could induce JNK-dependent overgrowth and expression of Yorkie’s (Yki) target genes when cell death was blocked by expressing p35. When Src64B was overexpressed in a clone context, it induced non-autonomous tumor overgrowth through JNK-dependent propagation of Yki activity. Consistent with the well-documented role of Src family kinases in promoting mammalian tumor invasion, Cagan and colleagues found that inhibition of Csk, a negative regulator of Src family kinases, triggered JNK signaling-mediated cell invasion in Drosophila wing discs. However, a direct involvement of Src in tumor metastasis and cell invasion, and its underlying mechanisms, remain largely elusive.

We have performed a genetic screen and identified Src42A as a crucial regulator of RasV12/lgl−/−-induced tumor

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Abbreviations: VNC, ventral nerve cord; JNK, c-Jun N-terminal Kinase; MMP1, matrix metalloproteinase 1; AEL, after egg laying; A/P, anterior/posterior; AO, acridine orange; MARCM, mosaic analysis with a represensible cell marker; Ey, eyeless

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invaded, as well as the loss of scrib-triggered cell migration in development. Our genetic epistasis analysis demonstrated that Src42A modulates JNK signaling-mediated cell invasion, cell death and thorax closure upstream of Bendless (Ben) and dUev1a. Furthermore, we showed that ectopic expression of Ben/dUev1a not only induces cell migration and matrix metalloproteinase 1 (MMP1) upregulation but also cooperates with RasV12 to promote tumor growth and invasive behavior. Together, these data highlight the importance of Src42A-Ben/dUev1a-JNK signaling in regulating cell invasion and extend our knowledge towards the underlying mechanism of Src42A in tumor progression.

Results and discussion

Src42A is required for tumor invasion and cell migration. As previously described, co-expression of oncogenic Ras (RasV12) in gll-mutant cells in eye–antenna discs using the ey-FLP/MARCM (mosaic analysis with a repressible cell marker) system induces strong tumor-like growths (Figure 1a), with invasive migration into the VNC 8 days after egg laying (AEL) (Figure 1a).10,16,29 Such animals kept growing as oversized larvae carrying huge tumors in their head and died before pupation (Supplementary Figure S1A).

Blocking JNK signaling by expressing the JNK phosphatase Puc dramatically suppressed the growth and invasion behaviors of RasV12/gll−/− tumors (Figures 1b and b).12,16 To identify additional genes required for tumor growth and invasion, we performed a genetic screen for dominant suppressors of the RasV12/gll−/− tumor progression phenotype and identified dUev1a as a crucial regulator.12 From this screen, we also found that the loss of src42A dramatically suppressed tumor cell invasion into the VNC (Figure 1c) and enabled the animals to survive to the pupa stage (Supplementary Figure S1B), whereas the tumor size remained largely unaffected (compare Figures 1a and c). Loss of src64B produced a similar suppression effect (Supplementary Figure S2), consistent with previous finding that the two Drosophila Src proteins share redundant functions in development.24

To examine whether Src42A modulates cell invasion in other contexts, we turned to another well-characterized model in the larval wing imaginal disc.3,12,13,30–32 Compared with the controls (Figures 1d–d″), RNAi downregulation of the cell polarity gene scrib driven by ptc-Gal4 triggered strong cell migration phenotype towards the posterior part of the discs (Figure 1e′), along with JNK-dependent upregulation of matrix metalloprotease 1 (MMP1, Figure 1e′),12,14 a protein that is essential for basement membrane degradation.29,33 We found that the loss of src42A dramatically suppressed the epithelial migration phenotype as well as the upregulation of MMP1 (Figures 1f–f″). Taken together, these data indicate that Src42A is required for RasV12/gll−/−-triggered tumor invasion in the eye discs as well as the loss of cell-polarity-induced cell migration and MMP1 expression in the developing wing.

Src42A induce JNK-mediated cell migration through Ben/dUev1a. Ectopic expression of Src42A along the A/P boundary of wing imaginal discs driven by ptc-Gal4 (ptc→Src42A) resulted in strong cell migration phenotype along with the upregulation of MMP1 (Figures 2b–b″). To examine whether cell invasion is a primary consequence of Src42A activation, or a secondary effect of cell death triggered by elevated Src activity,34–36 we expressed the caspase inhibitor p35 to block cell death. Co-expression of
p35 with Src42A resulted in a widening of the ptc domain and enhanced the production of MMP1 (Supplementary Figure S3). Importantly, these "undead cells" can still migrate to the posterior part (Supplementary Figure S3), indicating that cell migration is a primary event induced by Src42A expression.

JNK signaling is misregulated in many human cancers and contributes to invasion in different Drosophila models. We found that ptc-Src42A-induced cell migration and MMP1 induction (Figure 2b) were significantly suppressed by the expression of a dominant-negative form of Basket (Bsk) (Figure 2c), indicating that JNK signaling is required for Src42A-induced cell migration. We performed genetic epistasis analysis between Src42A and components of JNK signaling and found that ptc-Src42A-induced migration phenotype was significantly blocked by the loss of JNK kinase Hep, JNK Kinase Kinase dTAK1 and the E3 ubiquitin ligase dTRAF2 (Figure 3c), suggesting that Src42A induces cell migration upstream of dTRAF2.

Our recent work identified the E2 ubiquitin-conjugating enzyme complex consisting of Bendless (Ben) and dUev1a as the upstream regulators of dTRAF2 in JNK signaling. We explored the genetic interactions between the Src42A and Ben/dUev1a complex and found that ptc-Src42A-triggered migration phenotype and MMP1 expression (Figures 3a–a”) were suppressed strongly by RNAi downregulation of either Ben or dUev1a alone (Figure 3c), and completely by both (Figures 3b–b”), suggesting that the Ben/dUev1a complex is necessary for Src42A-induced cell migration. We previously showed that co-expression of Ben and dUev1a resulted in JNK activation; consistently, Ben and dUev1a expression along the A/P boundary induced a mild cell migration phenotype and upregulation of MMP1 (Figures 4a,b and d), which was significantly enhanced by removing one copy of endogenous puc (Figure 4c), indicating that Ben/dUev1a expression could induce JNK-dependent cell migration.

Together, the above data suggested that the Ben/dUev1a complex is necessary and sufficient for Src42A-induced JNK-dependent cell migration.

Ben/dUev1a cooperates with RasV12 to induce tumor growth and invasion. Gain of function of cell morphology regulators or loss of cell polarity could promote JNK-dependent cell migration and cooperate with RasV12 to drive tumor growth and invasion. As Ben/dUev1a expression could also induce JNK-dependent cell migration, we wonder whether Ben/dUev1a could cooperate with RasV12 to promote tumor progression. As reported previously, clonal expression of RasV12 in eye disc induced benign overgrowth (Figure 4e), with no cells invading into the VNC.
On the other hand, expression of Ben/dUev1a resulted in no obvious growth or invasion phenotype (Figures 4f and f'). When Ben/dUev1a was simultaneously expressed with RasV12, tumor overgrowth (Figure 4g) and invasion into the VNC region (Figure 4g') were observed in the eye discs 8 days AEL, suggesting that Ben/dUev1a could cooperate with RasV12 to promote tumor growth and invasion behavior.

Src42A activates JNK signaling 

Src42A triggers JNK-dependent cell death in the developing eye. Apart from the role of Src42A in regulating tumor invasion and cell migration showed above, we and others also found that ectopic Src42A expression in developing eyes could induce extensive cell death, as shown with acridine orange (AO) staining (Supplementary Figure S4B) that detects dying cells and produce a small eye phenotype (Figure 6b). Consistent with the genetic epistasis analysis in cell invasion, the GMR>Src42A-triggered cell death and small eye phenotype were dramatically suppressed by inactivation of Ben, dUev1a or downstream components of the JNK signaling (Supplementary Figure S4 and Figures 6c–g). Besides, the ectopic Src42A-caused small eye phenotype was also reverted by co-expressing two
independent RNAi lines against src42A (Figure 6h and Supplementary Figure S5), which further confirmed the efficiency of the two RNAi lines.

**Ben/dUev1a is physiologically required for Src42A.** Apart from its role in modulating cell migration and cell death, src42A also regulates JNK-dependent dorsal closure. Consistently, JNK signaling, as revealed by puc expression, is activated in the dorsal patch of the third instar larval wing disc (Figure 7a). Downregulation of src42A under the pannier (pnr) promoter resulted in reduced puc expression in the wing disc (Figure 7b) and produced a cleft phenotype in the adult thorax (Figure 7d). This cleft phenotype resembled that of JNK inactivation and could be partially rescued by deleting one copy of endogenous puc (Figure 7f), or expression of a wild-type Hep (Hep WT) (Figure 7h), or co-expression of Ben and dUev1a (Figure 7j). Together, these results suggested that the Ben/dUev1a complex is required for the physiological function of Src42A in thorax development.

**Conclusion**

We have identified Src42A as a key modulator of both RasV12/\(lgl^{-/}\) -triggered tumor invasion and loss of cell-polarity-induced cell migration in Drosophila. Our genetic evidence established the Ben/dUev1a complex as an essential positive regulator that mediates Src42A-induced, JNK-dependent cell migration and death (Figure 7k). We showed that ectopic Ben/ dUev1a expression not only induced MMP1 upregulation and cell migration but also cooperated with RasV12 to promote tumor growth and invasion, suggesting that Ben and dUev1a
Src42A modulates endogenous JNK activity upstream of Ben/dUev1a. (a–b) X-Gal staining of the puc-LacZ reporter gene in the developing wing are shown. The endogenous puc expression pattern in the notum region of wild-type wing disc (a) was reduced by expressing a src42A RNAi (b) indicated by the arrow head. (c–j) Light micrographs of Drosophila adult thoraxes are shown. Compared with the controls (c), loss of src42A-produced strong thorax cleft phenotype (d) was restored by deleting on copy of puc (f), overexpression of Hep (h) or overexpression of Ben/dUev1a (j), whereas loss of puc, expression of Hep or Ben/dUev1a alone gave no obvious phenotype (e, g, i respectively). (k) A schematic diagram of Ben/dUev1a-JNK signaling in regulating Src42A-induced cell migration and death are candidate proto-oncogenes. Consistent with our findings, Uev1A, the mammalian homolog of dUev1a, is upregulated in many human cancer cell lines. Our data suggested a link between Src activation and Ben/dUev1a-JNK signaling in regulating cell migration and death in development and disease, which provided beneficial information for mammalian studies and target therapies for cancer.

Materials and Methods
Drosophila strains and generation of clones. All stocks were raised on standard Drosophila media, and crosses were performed at 25°C unless otherwise indicated. Fluorescently labeled invasive tumors were produced in the eye discs as previously indicated. Using the following strains: y, w, ey-Flp; tub-Ga4A, FRT40A, Act>γ>Gal4, UAS-GFP (40A tester), ify; FRT40A UAS-RasV12 (40A tester) and ey-Flp, Act>γ>Gal4, UAS-GFP. Additional strains, including GMR-Ga4A, ptc-Ga4A, pnr-Gal4, UAS-p35, UAS-GFP and UAS-Src42A[l], were obtained from Bloomington stock center; UAS-ben-IR (no. 9413) and UAS-src42A-IR (no. 26019) were obtained from the VDRC center; UAS-dTAK1-IR, UAS-Bak[l], UAS-hep-IR, UAS-dTraf2-IR, UAS-Puc, puc[2,6,46] UAS-src42A-IR[l], UAS-src64B-IR[l], UAS-Src42A[l], UAS-Ben[l], UAS-dUev1a, UAS-dUev1a-IR and UAS-scrib-IR[l] were previously described. Genotypes of flies used in each figure can be found in online Supplementary Information.

Immunohistochemistry. Antibody staining of imaginal discs was performed as previously described. The following antibodies were used: rabbit anti-phospho-JNK (1:200, Cell Signaling, Danvers, MA, USA), mouse anti-MMP1 (1:200, Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Secondary antibodies were anti-rabbit-Alexa (1:1000, Cell Signaling Technology, Danvers, MA, USA) and anti-mouse-Cy3 (1:1000, Jackson Immuno Research, West Grove, PA, USA).

X-gal staining. Eye and wing discs were dissected from the third instar larvae in PBT (1× PBS pH 7.0, 0.1% Triton X 100) and stained for β-galactosidase activity as described.

AO staining. Eye and wing discs were dissected from the third instar larvae in PBT and incubated in 1×10^{-5} M AO for 5 min at room temperature prior to imaging.

Conflict of Interest
The authors declare no conflict of interest.

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1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10–29.
2. Valsalvani S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011; 147: 275–292.
3. Miles WD, Dyson NJ, Walker JA. Modeling tumor invasion and metastasis in Drosophila. Dis Model Mech 2011; 4: 753–761.
4. Gonzalez C. Drosophila melanogaster: a model and a tool to investigate malignancy and identify new therapeutics. Nat Rev Cancer 2013; 13: 172–183.
5. Willoughby LF, Schlisser T, Manning SA, Parisot JP, Street IP, Richardson HE et al. An in vivo large-scale screening platform using Drosophila for anti-cancer drug discovery. Dis Model Mech 2013; 6: 521–529.

6. Elsum I, Yates L, Humbert PO, Richardson HE. The Scribble-Dlg-Lgl polarity module in development and cancer: from flies to man. Essays Biochem 2012; 53: 141–168.

7. Rudrapatna VA, Cagan RL. Das TK. Drosophila cancer models. Dev Dyn 2012; 241: 107–118.

8. Brumby AM, Richardson HE. Using Drosophila melanogaster to map human cancer pathways. Nat Rev Cancer 2005; 5: 268–269.

9. Simon MA, Drees B, Kornberg T, Bishop JM. The nucleotide sequence and the tissue-specific expression of Drosophila c-sis. Cell 1985; 42: 831–840.

10. Tadokoro F, Endo S, Kojima T, Saigo K. Regulation of cell-cell contacts in developing Drosophila. EMBO J 2003; 22: 5769–5779.

11. Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. Science 2003; 302: 1227–1231.

12. Ma X, Yeatman TJ. A renaissance for SRC. Annu Rev Cell Dev Biol 2010; 26: 33–44.

13. Cordero JB, Macagno JP, Stefanatos RK, Strathdee KE, Cagan RL. Cdk-deficient boundary cells are eliminated from normal Drosophila epithelia by exclusion, migration, and apoptosis. Dev Cell 2006; 10: 37–44.

14. Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Cell Biol 2007; 19: 142–149.

15. Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. Curr Biol 2006; 16: 1139–1146.

16. Chen X, Wu M, Pastor-Pareja JC, Xu T. Interaction between Ras(V12) and scribbled clones induces tumour growth and invasion. Nature 2010; 463: 545–548.

17. Kho P, Alkan K, Willoughby L, Brumby AM, Richardson HE. In Drosophila, RhoGEF2 cooperates with activated Ras in tumorigenesis through a pathway involving Rho1-Rok-Mycin-II and JNK signalling. Dia Model Mech 2013; 6: 661–678.

18. Yeatman TJ. A renaissance for SRC. Nat Rev Cancer 2004; 4: 470–480.

19. Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 1997; 13: 513–609.

20. Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. Curr Opin Cell Biol 2004; 26: 7906–7909.

21. Simon MA, Drees B, Kornberg T, Bishop JM. The nucleotide sequence and the tissue-specific expression of Drosophila c-sis. Cell 1985; 42: 831–840.

22. Tadokoro F, Endo S, Kojima T, Saigo K. Regulation of cell-cell contacts in developing Drosophila eyes by Drosophila. Nature 1996; 10: 1645–1656.

23. Dodson GS, Guarnieri DJ, Simon MA. Src64 is required for ovarian ring canal morphogenesis during Drosophila oogenesis. Development 1998; 125: 2883–2892.

24. Tateno M, Ishida Y, Adachi-Yamada T. Regulation of JNK by Src during Drosophila development. Science 2000; 2047–2050.

25. Shindo M, Wada H, Kaido M, Tateno M, Agaki T, Tsuda L et al. Dual function of Src in the maintenance of adherens junctions during tracheal epithelial morphogenesis. Development 2000; 172: 1395–1396.

26. Fernandez BG, Jezowska B, Janody F. Drosophila actin-Capping Protein limits JNK activation by the Src proto-oncogene. Oncogene 2013; 13: e-pub ahead of print 3 May 2013; doi:10.1038/onc.2013.155.

27. Enomoto M, Igaki T, Src controls tumorigenesis via JNK-dependent regulation of the Hippo pathway in Drosophila. EMBO Rep 2013; 14: 65–72.

28. Guarino M. Src signaling in cancer invasion. J Cell Physiol 2010; 223: 14–26.

29. Uhlirwa M, Bohmman D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. EMBO J 2006; 25: 5294–5304.

30. Rudrapatna VA, Bangi E, Cagan RL. Caspase signalling in the absence of apoptosis drives JNK-dependent invasion. EMBO Rep 2013; 14: 172–177.

31. Rudrapatna VA, Bangi E, Cagan RL. A Jnk-Rho-Actin remodeling positive feedback network directs Src-driven invasion. Oncogene 2013; e-pub ahead of print 8 July 2013; doi:10.1038/onc.2013.312.

32. Portela M, Richardson HE. Death takes a holiday:non-apoptotic role for caspases in cell migration and invasion. EMBO Rep 2013; 14: 107–108.

33. Srivastava A, Pastor-Pareja JC, Igaki T, Pagliarini R, Xu T. Basement membrane remodeling is essential for Drosophila disc evisceration and tumor invasion. Proc Natl Acad Sci USA 2007; 104: 2721–2726.

34. Singh J, Aaronson SA, Midzuki M. Drosophila Abelson kinase mediates cell invasion and proliferation through two distinct MAPK pathways. Oncogene 2010; 29: 4033–4045.

35. Pedraza LG, Stewart RA, Li DM, Xu T. Drosophila Src-family kinases function with Csk to regulate cell proliferation and apoptosis. Oncogene 2004; 23: 4754–4762.

36. Vidal M, Warner S, Read R, Cagan RL. Differing Src signaling levels have distinct outcomes in Drosophila. Cancer Res 2007; 67: 10278–10285.

37. Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the Drosophila wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. Curr Biol 2004; 14: 1382–1386.

38. Ryoo HD, Gorec T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Dev Cell 2004; 7: 491–501.

39. Ma X, Huang J, Yang L, Yang Y, Li W, Xue L. NOPO modulates Egr-induced JNK-independent cell death in Drosophila. Cell Res 2012; 22: 425–431.

40. Brumby AM, Goulding KR, Schlisser T, Loi S, Galea R, Kho P et al. Identification of novel Ras-cooperating oncoproteins in Drosophila melanogaster: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. Genetics 2011; 188: 105–125.

41. Agnes F, Suzanne M, Noselli S. The Drosophila JNK pathway controls the morphogenesis of imaginal discs during metamorphosis. Development 1999; 126: 5453–5462.

42. Abrams JM, White K, Fessler LJ, Steller H. Programmed cell death during Drosophila embryogenesis. Development 1993; 117: 29–43.

43. Campuzano S, Modolell J. Patterning of the Drosophila nervous system: the achaete-scute gene complex. Trends Genet 1992; 8: 202–208.

44. Xiao W, Lin SL, Broomefield S, Chow BL, Wei YF. The products of the yeast MMS2 and two human homologs (hMMS2 and CROC1-1) define a structurally and functionally conserved Ub-like protein family. Nucleic Acids Res 1998; 26: 3908–3914.

45. Syed NA, Andersen PL, Warrington RC, Xiao W. Uev1a a ubiquitin conjugating enzyme variant, inhibits stress-induced apoptosis through NF-kappaB activation. Apoptosis 2008; 11: 2147–2157.

46. Xue L, Igaki T, Kuranaga E, Kanda H, Miura M, Xu T. Tumor suppressor CYLD regulates JNK-induced cell death in Drosophila. Dev Cell 2007; 13: 446–454.

47. Pechkovsky A, Lahav M, Bitman E, Salzberg A, Kleinberger T, E4orf4 induces PP2A- and Src-family kinase-mediated cell death. Cell Death and Disease 2013; 4: 1043–1048.

48. Ma X, Huang J, Yang L, Yang Y, Li W, Xue L. NOPO modulates Egr-induced JNK-independent cell death in Drosophila. Cell Res 2012; 22: 425–431.

49. Brumby AM, Goulding KR, Schlisser T, Loi S, Galea R, Kho P et al. Identification of novel Ras-cooperating oncoproteins in Drosophila melanogaster: a RhoGEF/Rho-family/ JNK pathway is a central driver of tumorigenesis. Genetics 2011; 188: 105–125.

50. Agnes F, Suzanne M, Noselli S. The Drosophila JNK pathway controls the morphogenesis of imaginal discs during metamorphosis. Development 1999; 126: 5453–5462.

51. Abrams JM, White K, Fessler LJ, Steller H. Programmed cell death during Drosophila embryogenesis. Development 1993; 117: 29–43.