dGLYAT modulates Gadd45-mediated JNK activation and cell invasion

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Abstract

Background: Cell invasion is a crucial step of tumor metastasis, finding new regulators of which offers potential drug targets for cancer therapy. Aberrant *GLYAT* expression is associated with human cancers, yet its role in cancer remains unknown. This study aims to understand the function and mechanism of *Drosophila GLYAT* in cell invasion.

Results: We found that dGLYAT regulates Gadd45-mediated JNK pathway activation and cell invasion. Firstly, loss of *dGLYAT* suppressed *scrib* depletion- or Egr overexpression-induced JNK pathway activation and invasive cell migration. Secondary, mRNA-seq analysis identified *Gadd45* as a potential transcriptional target of dGLYAT, as depletion of *dGLYAT* decreased *Gadd45* mRNA level. Finally, *Gadd45* knockdown suppressed *scrib* depletion-induced JNK pathway activation and cell invasion.

Conclusions: These evidences reveal the role of dGLYAT and Gadd45 in cell polarity disruption-induced JNK-dependent cell invasion, and provide insight for the roles of their human homologs in cancers.

Background

Tumor metastasis, rather than primary tumor formation, is the main cause of fatality in cancer patients [1]. Therefore, identifying additional factors involved in tumor cell invasion and metastasis is of great significance to develop novel strategies for cancer prevention and therapies [2]. Various approaches and model systems have been employed to comprehend the mechanisms underlying cancer metastasis, among which the fruit fly, *Drosophila melanogaster*, has emerged as an excellent model organism to dissect different cancer processes [3]. For example, depletion of cell polarity genes or C-terminal SRC kinase (*Csk*) in *Drosophila* larval wing disc epithelia induces epithelia-mesenchymal transition (EMT)-like cell migration [4].

The Jun N-terminal Kinase (JNK) signaling is evolutionarily conserved from *Drosophila* to human, and plays critical roles in cancer initiation and progression [5]. In *Drosophila*, the JNK pathway consists of a kinase cascade including the JNK kinase kinase such as dTAK1 [6], the JNK kinase hemipterous (Hep) [7], and the sole fly JNK Basket (Bsk) [8]. Upon activation of the kinase cascade by sequential phosphorylation, Bsk phosphorylates and activates downstream transcription factors including Jun and Fos, which form the AP-1 heterodimers [9]. *puckered (puc)*, a transcriptional target of JNK signaling, encodes a JNK phosphatase that inhibits Bsk activity in a negative feed-back manner [10]. Previous studies in *Drosophila* have found that JNK signaling plays pivotal roles in cell proliferation, migration and apoptosis in normal development, and promotes tumorigenic cell death and invasion in a context dependent manner [11].

Human glycine N-acyltransferases (GLYAT, GLYATL1, GLYATL2, and GLYATL3) promote conjugation of carboxylic acids to glycine and glutamine, which play crucial roles in the detoxification of endogenous and exogenous acyl-CoA. Previous studies suggest potential roles of GLYAT family members in various cancers, for instance, aberrant GLYAT expression has been associated with hepatocellular carcinomas

and breast cancer [12], and reduced GLYATL1 expression is correlated with short overall survival in hepatocellular carcinoma patients [13]. On the other hand, data from the Oncomine Platform (<https://www.oncomine.org>) show that *GLYATL1* mRNA expression is up-regulated in colorectal and prostate cancers, but is down-regulated in kidney and liver cancers. Despite these association, the exact functions of GLYAT family proteins in cancers remain elusive. Our previous work found a *Drosophila* homolog of GLYAT (*dGLYAT*) is required for JNK-mediated cell death [14], yet two critical questions remain unanswered. Whether *dGLYAT* is required for other *in vivo* functions of JNK pathway, and by which mechanism does *dGLYAT* regulate JNK signaling?

To address the above questions, we took advantage of the well-established *Drosophila* cell invasion model. In this model, knockdown of a cell polarity gene, e.g. *scrib*, *lgl* or *dlg*, along the anterior/posterior (A/P) compartment boundary in 3rd instar larval wing imaginal discs by *ptc*-Gal4, induces JNK-dependent invasive cell migration [15]. To assess whether *dGLYAT* contributes to JNK-mediated cell invasion, we depleted *dGLYAT* by mutation or RNAi-mediated knockdown, and found *dGLYAT* is required for loss-of-cell polarity-induced JNK-dependent cell invasion. In particular, loss of *dGLYAT* suppresses *scrib* depletion- or Egr overexpression-induced JNK-dependent cell invasion, and impedes *scrib* knockdown-triggered JNK pathway activation. To investigate the mechanism by which dGLYAT regulates JNK signaling, we performed mRNA-seq analysis, and found a significant reduction of *Gadd45* (Growth Arrest and DNA Damage-inducible 45) mRNA level upon *dGLYAT* depletion. Finally, knockdown of *Gadd45* suppresses loss-of-*scrib*-induced JNK activation and cell invasion. Thus, these data provide the first *in vivo* evidence that dGLYAT modulates JNK-dependent cell invasion through Gadd45.

Results

***dGLYAT* is required for cell polarity disruption-induced cell invasion**

It is well known that disrupting cell polarity by depleting *scrib* along the anterior/posterior (A/P) compartment boundary of the wing discs leads to JNK-dependent invasive cell migration [16], in which cells migrate away from the A/P boundary with up-regulated expression of matrix metalloprotease 1 (MMP1) that is able to degrade the basement membrane [17]. Using this well-established *in vivo* cell invasion model, we examined the role of *dGLYAT* in cell polarity disruption-induced cell invasion. Compared with *ptc*>GFP control (Figure 1a-a"), knockdown of *scrib* induced extensive cell migration and MMP1 up-regulation (Figure 1b-b" and quantified in Figure 1f-g). Both phenotypes were significantly suppressed in heterozygous *dGLYAT*^{C02982} mutants (Figure 1c-c"), which has a piggyBac insertion into the second exon that deletes the critical Gcn5-related N-acetyltransferases (GNAT) domain [14]. To corroborate this result, we depleted *dGLYAT* by RNAi-mediated knockdown, and confirmed that expression of a *dGLYAT-IR* dramatically inhibited *scrib* depletion-induced cell migration and MMP1 elevation, compared with LacZ expression as a negative control (Figure 1d-d", e-e").

To further confirm the effect of *dGLYAT* on cell invasion, we checked other markers of epithelial-mesenchymal transition (EMT), a critical process in malignant transformation [18]. During the process of EMT, expressions of cell-cell junction proteins, such as E-cadherin, are switched off in epithelial cells [19]. Consistently, loss-of-*scrib* resulted in reduced expression of E-cadherin (Figure 2a-a' and 2b-b'), which was suppressed by expression of *dGLYAT-IR*, but not LacZ (Figure 2c-c' and 2d-d'). Taken together, these data suggest that *dGLYAT* is required for cell polarity disruption-induced EMT-like cell migration.

***dGLYAT* is necessary for Egr-JNK pathway-triggered invasive cell migration**

Scrib depletion triggers Egr-JNK pathway-mediated invasive cell migration [15]. To investigate whether *dGLYAT* is involved in Egr-JNK pathway-activated cell migration, we used *ptc*-Gal4 to drive ectopic Egr expression. Compared with the control (Figure 3a-a''), ectopic expression of Egr driven by *ptc*-Gal4 resulted in invasive cell migration, accompanied by up-regulated MMP1 expression (Figure 3b-b''). Both phenotypes were considerably suppressed in heterozygous *dGLYAT* mutants, or by expressing *dGLYAT-IR*, but not LacZ (Figure 3c-e'' and quantified in Figure 3f-g). As MMP1 also serves as a reporter of JNK signaling, these results suggest that *dGLYAT* is necessary for Egr-JNK pathway-triggered invasive cell migration.

***dGLYAT* is required for cell polarity disruption-induced JNK pathway activation**

As loss-of-cell polarity-induced cell invasion depends on JNK signaling, and depletion of *dGLYAT* impedes *ptc>scrib-IR*-induced cell invasion and MMP1 expression, a transcriptional target of JNK signaling, we proposed that *dGLYAT* might be required for cell polarity disruption-triggered JNK pathway activation. To test this possibility, we examined the expression of two well-recognized JNK pathway reporters, *puc*-LacZ and *TRE*-RFP [20]. In agreement with previous reports, knockdown of *scrib* along the A/P compartment boundary triggered JNK pathway activation, as indicated by elevated expression of *puc*-LacZ (Figure 4a-b) and *TRE*-RFP (Figure 4d-e), both of which were significantly suppressed by knockdown of *dGLYAT* (Figure 4c, f). Collectively, these results suggest that *dGLYAT* is required for loss-of-cell polarity-induced JNK pathway activation. *dGLYAT* regulates *Gadd45* transcription

According to the Flybase (<http://flybase.org/reports/FBgn0054010>), *dGLYAT* encodes a Gcn5-related N-acetyltransferases (GNAT) family member that catalyzes the transfer of an acetyl moiety from Coenzyme A (Ac-CoA) to diverse substrates [21]. GNATs are evolutionarily conserved regulators that acetylate key amine of small molecules and proteins involved in numerous cellular processes. Some members of GNAT superfamily are involved in histone acetylation [22], which affects gene transcription [23]. Based on these information, we proposed that *dGLYAT* might be required for the transcriptional activation of JNK pathway positive regulator(s). In this scenario, loss of *dGLYAT* would compromise JNK pathway

activation via reduced expression of the positive regulator(s). To find the potential regulator(s), we conducted the whole genome mRNA-seq assays, and analyzed differentially expressed genes (DEGs) between control and *dGLYAT*-depleted groups. We found that 13 genes were up-regulated and 29 genes down-regulated upon *dGLYAT* knockdown (Figure 5a, $\log_2FC \geq 1.0$ or $\log_2FC \leq -1.0$, adjusted P value < 0.001 and Additional file 1: Table S1). We paid specific attention to the 29 down-regulated genes, whose expression profiles are presented (Figure 5b). Intriguingly, among the 29 down-regulated genes, *Gadd45* has been previously reported as a positive regulator of JNK pathway in apoptosis and egg asymmetric development [24, 25]. We performed reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay to validate the mRNA-seq results, and confirmed that *Gadd45* mRNA level was significantly reduced 2 hours and 6 hours after heat shock-induced *dGLYAT* knockdown (Figure 5c). The relative short time interval (2 hours) between *dGLYAT* knockdown and *Gadd45* mRNA reduction implies *dGLYAT* may directly affect *Gadd45* transcription.

Loss of *Gadd45* suppresses *scrib* depletion-induced JNK activation and cell invasion

Given that loss of *dGLYAT* decreased *Gadd45* mRNA expression and suppressed JNK-dependent cell invasion, we hypothesized that *dGLYAT* regulates JNK-dependent cell invasion through *Gadd45*. In agreement with this assumption, *ptc>scrib-IR*-induced invasive cell migration was significantly suppressed by knockdown of *Gadd45* with two independent *RNAi* lines (Figure 6a-d). Furthermore, *scrib* depletion-triggered JNK pathway activation, revealed by up-regulated MMP1 and *puc-LacZ* expression, were significantly impeded by loss of *Gadd45* (Figure 6e-l, quantified in Figure 6m-n). Collectively, these results suggest that *Gadd45* is required for loss-of-cell polarity-induced JNK-dependent cell invasion.

Discussion

In this study we found that loss of *Drosophila GLYAT* suppresses cell polarity disruption-induced JNK-dependent cell invasion. Furthermore, loss of *dGLYAT* impedes Egr-JNK pathway-triggered invasive cell migration. Moreover, *dGLYAT* regulates the transcription of *Gadd45*, a positive regulator of JNK signaling in apoptosis and egg development. Finally, depletion of *Gadd45* blocks loss-of-cell polarity-triggered JNK activation and cell invasion. Thus, *dGLYAT* regulates *Gadd45*-mediated JNK activation and EMT-like cell migration in *Drosophila*.

Consistent with our findings, data from the Cancer Genome Atlas (TCGA) project imply a possible role of human GLYAT family members in breast cancer invasion. Firstly, *GLYATL1* mRNA expression is significantly increased in breast invasive carcinoma. Secondly, high *GLYATL1* expression is associated with poor overall survival in breast cancer patients. Finally, a recent study shows that *GLYAT* knockdown in MDA-MB-231 cells (triple negative breast cancer cell) results in decreased E-cadherin expression and

reduced EMT progression [26]. Collectively, these data suggest that GLYAT family members might execute an evolutionarily conserved role in regulating EMT-like cell migration from fly to human.

The mammalian GADD45 family consists of three members, GADD45 α , GADD45 β and GADD45 γ , which interacts with cellular proteins in response to physiological or environmental stressors, and participates in cell cycle arrest, DNA repair, apoptosis, cell survival and senescence [27]. The role of GADD45 in P38 and JNK signaling have been extensively studied, yet divergent mechanisms were reported. GADD45 proteins activate MTK1 by promoting its dimerization and autophosphorylation [28], or bind and activate MTK1 MAPKKK to regulate P38 and JNK signaling upon environmental stress [29]. In addition, GADD45 α could interact directly with P38 and regulates oncogene-induced growth [30], while GADD45 β interacts with ASK1 and MKK7 to regulate both JNK and P38 [31-33]. GADD45 proteins could execute either tumor suppressor or tumor promoter function in tumor initiation [34], yet their roles in tumor invasion have not been explored previously. The *Drosophila* genome encodes only one GADD45 member, *Gadd45*, which is involved in inflammatory response, egg development, wing disc regeneration and lifespan [24, 25, 35]. *Gadd45* has been shown as a positive regulator of JNK signaling in germline development and cell death [24, 25], yet its role in JNK-mediated cell invasion has not been known previously. In this study, we found that *Gadd45* is required for cell polarity disruption-induced JNK-mediated cell invasion in *Drosophila*, suggesting a potential role of GADD45 in tumor invasion and cancer progression, which deserves further investigation.

Although we found that dGLYAT regulates *Gadd45* mRNA expression, the underlying mechanism remains unknown. We hypothesize that dGLYAT, which contains a GNAT domain, may promote histone acetylation at the promoter of *Gadd45*. In support of this assumption, the yeast GNAT domain-containing protein Gcn5 (general control nonderepressible-5) is reported to regulate *PHO5* transcription by promoting histone acetylation at its promoter [36], and this histone acetylation activity has been conserved by Gcn5 homologs in *Tetrahymena* and human [37]. Future studies are needed to address whether GLYAT family proteins promote histone acetylation, and whether this activity is required for their roles in cancer initiation and progression.

Conclusions

In summary, we found dGLYAT is required for loss of cell polarity and Egr overexpression induced cell invasion and JNK activation. Through analysing mRNA-seq results, we identified *dGLYAT* RNAi reduced *Gadd45* mRNA expression. Downregulation of *Gadd45* also suppressed cell polarity disruption induced cell invasion and JNK activation.

Methods

Drosophila Genetics and Stocks

Fly stocks were raised on standard cornmeal and agar medium at 25 °C. For cell migration assay, larvae were reared at 29 °C unless indicated. Fly strains used in this study are as follow: *w¹¹¹⁸*, *ptc-Gal4*, *UAS-GFP*, *UAS-scrib-RNAi*, *puc^{E69}*, *TRERFP*, *UAS-Egr^{Regg1}*, *dGLYAT^{c02982}* and *UAS-dGLYAT-IR* have been described previously [14, 17]. *UAS-Gadd45-RNAi* (V100413 and BL35023) were gifts from Dr. Erjun Ling (Chinese Academy of Sciences, China).

Immunohistochemistry

Antibody staining was performed according to standard procedures. The following primary antibodies were used: mouse anti-MMP1 (3A6B4, 1:200, Developmental Studies Hybridoma Bank, DSHB), Rat anti-E-cad (DCAD2-c, 1:100, Developmental Studies Hybridoma Bank, DSHB) and mouse anti β -gal (40-1a, 1:500, Developmental Studies Hybridoma Bank, DSHB). The following secondary antibodies were used: anti-mouse CY3 (A11032, 1:1000, Cell Signaling Technology) and anti-Rat CY3 (104086, 1:500, Jackson Immuno research).

X-gal Staining

Wing discs were dissected from third instar larvae in PBST (1× PBS pH 7.0, 0.1% Triton X-100) and stained for β -galactosidase activity as described [38].

RNA Library Preparation and Data Analysis

Third instar larvae were subjected to heat shock at 37 °C for 30 minutes and recovered at 29 °C for 6 hours prior to tissues dissection. RNA extraction, library construction and sequencing were performed by Hua Gen Biotechnology (Shanghai, China). The sequencing platform is Illumina. The quality of sequenced raw reads was controlled by the FastQ Screen program. The reference genome sequence is *Drosophila melanogaster* (BDGP6.28) downloaded from Ensembl.

(<http://www.ensembl.org/info/data/ftp/index.html>). Differentially expressed genes (DEGs) were identified by comparing the expression levels of genes between *hs>dGLYAT* RNAi and *hs-Gal4* control groups. The threshold for DEGs was set at *P*-adjusted <0.001 and $\log_2$ fold change ($\log_2FC$) ≥ 1.0 or ≤ -1.0 .

RT-qPCR

Total RNAs were extracted from fifteen third instar larval tissues of indicated genotypes with Trizol (Ambion, Life Technologies, Carlsbad, CA, USA) following the protocol of RNA preparation kit, and quantitative polymerase chain reaction (qPCR) was performed using SYBR Green PCR Premix Kit (TaKaRa). The primers used are as follow:

Gene	Forward primer	Reverse primer
<i>rp49</i>	TCTCCTTGCGCTTCTTGGA	TACAGGCCCAAGATCGTGAA
<i>Gadd45</i>	GGTACTGCTGGAGGCCTTTT	CGCAGTAGTCGACTAGCTGG

Abbreviations

Csk: C-terminal SRC kinase; JNK: c-Jun N-terminal kinase; GLYAT: glycine N-acyltransferases; Gadd45: Growth Arrest and DNA Damage-inducible 45; MMP1: matrix metalloprotease 1; EMT: epithelial-mesenchymal transition; GNAT: Gcn5-related N-acetyltransferases; Ac-CoA: acetyl moiety from Coenzyme A; DEGs: differentially expressed genes; RT-qPCR: reverse transcription-quantitative polymerase chain reaction; TCGA: the Cancer Genome Atlas.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and agreed to the final version of the manuscript.

Availability of data and materials

The data that support the finding of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

M.X., P.R., W.L. and L.X. conceived and designed the experiments. M.X., P.R., J.T., L.X., P.H. and W.L. conducted experiments. P. C., X.X. and L.X. supervised/advised on the study, M.X., P.R., W.L. and L.X. analyzed the data, M.X. and L.X. wrote the manuscript. All authors approved the final manuscript.

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Figures

Figure 1

***dGLYAT* is required for cell polarity disruption-induced cell invasion.**

Fluorescent micrographs of *Drosophila* third instar larval wing discs are shown (a-e). Compared with the *ptc*-Gal4 control (a-a"), *ptc>scrib-IR* induced cell migration from A/P boundary toward posterior and elevated MMP1 expression (b-b"). Both phenotypes were significantly suppressed by heterozygous mutation (c-c") or *RNAi* expression of *dGLYAT* (e-e"). Expression of LacZ served as a negative control (d-d"). Statistics of migrating cells number (f) and MMP1 positive dots (g) are shown. **P<0.01,

****P<0.0001. Detailed genotypes: (a-a'') *ptc*-Gal4 UAS-GFP/+, (b-b'') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+, (c-c'') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/ *dGLYAT*^{c02982}, (d-d'') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+; *UAS-LacZ*/+, (e-e'') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+; *UAS-dGLYAT-IR*/+.

Figure 2

***dGLYAT* is necessary for cell polarity disruption-induced EMT-like phenotype.**

Fluorescent micrographs of third instar larval wing discs are shown (a-d). Compared with the *ptc*-Gal4 control (a-a'), *ptc*>*scrib-IR* induced cell migration and reduced E-cadherin expression (b-b'), which were suppressed by expressing *dGLYAT* RNAi (d-d'), but not LacZ (c-c'). Detailed genotypes: (a-a') *ptc*-Gal4 UAS-GFP/+, (b-b') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+, (c-c') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+; *UAS-LacZ*/+, (d-d') *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+; *UAS-dGLYAT-IR*/+.

Figure 3

***dGLYAT* is necessary for Eiger-JNK pathway-induced invasive cell migration.**

Fluorescent micrographs of third instar larval wing discs are shown (a-e). Compared with the *ptc*-Gal4 control (a-a''), *ptc*>*Egr*-induced cell migration and up-regulated MMP1 expression (b-b'') were suppressed by *dGLYAT* mutation (c-c'') or RNAi (e-e''), but not by LacZ expression (d-d''). Statistics of migrating cells number (f) and MMP1 positive dots (g) are shown. **P<0.01, ****P<0.0001. Detailed genotypes: (a-a'') *ptc*-Gal4 UAS-GFP/+, (b-b'') *ptc*-Gal4 UAS-GFP *UAS-Egr^{Regg1}*/+, (c-c'') *ptc*-Gal4 UAS-GFP *UAS-Egr^{Regg1}*/ *dGLYAT*^{c02982}, (d-d'') *ptc*-Gal4 UAS-GFP *UAS-Egr^{Regg1}*/+; *UAS-LacZ*/+, (e-e'') *ptc*-Gal4 UAS-GFP *UAS-Egr^{Regg1}*/+; *UAS-dGLYAT-IR*/+.

Figure 4

***dGLYAT* is required for cell polarity disruption-induced JNK pathway activation.**

Light micrographs of third instar wing discs with X-gal staining (a-c) and fluorescent micrographs of third instar wing discs (d-f) are shown. Compared with the *ptc*-Gal4 control (a, d), *ptc*>*scrib-IR* activated *puc*-LacZ (b) and *TRE-RFP* (e) expression, which were suppressed by *dGLYAT* RNAi (c, f). Detailed genotypes: (a) *ptc*-Gal4 UAS-GFP/+, *puc*-LacZ/+, (b) *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+; *puc*-LacZ/+, (c) *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/+; *puc*-LacZ/ *UAS-dGLYAT-IR*, (d) *ptc*-Gal4 UAS-GFP/ *TRE-RFP*, (e) *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/*TRE-RFP*, (f) *ptc*-Gal4 UAS-GFP *UAS-scrib-IR*/*TRE-RFP*; *UAS-dGLYAT-IR*/+

Figure 5

***Gadd45* mRNA level is reduced upon *dGLYAT* depletion.**

(a) Volcano plot between *dGLYAT* RNAi and control groups. Compared with the *hs-Gal4* control groups, there are 29 down-regulated genes (indicated by blue points) and 13 up-regulated genes (showed by red points) in the *hs>dGLYAT*-IR groups, when $\log_2FC > 1.0$ or < -1.0 and adjusted P value < 0.001 . Grey dotted lines show thresholds of $\log_2FC$ (1.0 and -1.0) and adjusted P value (0.001). (b) Heat map of 29 down-regulated genes between *dGLYAT* RNAi (R1-R6) and control (W1-W6) groups when $\log_2FC < -1.0$ and adjusted P value < 0.001 . Genes showing significant changes are listed in Supplemental Table S1. (c) RT-qPCR data showed that *GADD45* mRNA level was decreased upon *dGLYAT* depletion. * $P < 0.05$, ** $P < 0.01$. Third instar larvae were heat shocked at 37 °C for 30 minutes and recovered at 29 °C for 2 hours (c) or 6 hours (a-c) prior to tissues dissection and RNA extraction.

Figure 6

***Gadd45* knockdown suppresses *scrib* depletion-induced JNK activation and cell invasion.**

Compared with the *ptc-Gal4* control (a, e, i), *scrib* RNAi-induced cell migration (b), elevated expression of MMP1 (f) and *puc-LacZ* (j) were significantly suppressed by expressing two independent *Gadd45* RNAi (c-d, g-h and k-l). (m, n) Statistics of numbers of migrated cells and MMP1 positive dots are shown. **** $P < 0.0001$, *** $P < 0.001$. Larvae were reared at 25°C. Detailed genotypes: (a, e, i) *ptc-Gal4* UAS-GFP/+; *puc-LacZ*/+, (b, f) *ptc-Gal4* UAS-GFP UAS-*scrib*-IR; *puc-LacZ* / UAS-LacZ, (c, g, k) *ptc-Gal4* UAS-GFP UAS-*scrib*-IR/ UAS-*Gadd45*-IR^{V100413}; *puc-LacZ*/+, (d, h, l) *ptc-Gal4* UAS-GFP UAS-*scrib*-IR/ UAS-*Gadd45*-IR^{BL35023}; *puc-LacZ*/+ (j) *ptc>GFP* UAS-*scrib*-IR; *puc-LacZ*/ UAS-GFP.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AdditionalfileTableS1.xlsx](#)