EMBO reports

Manuscript EMBO-2012-36261

Caspase Signaling in the Absence of Apoptosis Drives Jnk-Dependent Invasion

Vivek A.Rudrapatna, Erdem Bangi and Ross L. Cagan

Corresponding author: Ross L. Cagan, Mount Sinai School of Medicine

Review timeline:

Submission date:	11 June 2012
Editorial Decision:	05 July 2012
Revision received:	11 November 2012
Editorial Decision:	28 November 2012
Revision received:	04 December 2012
Accepted:	12 December 2012

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Barbara Pauly

1st Editorial Decision 05 July 2012

Thank you for your submission to EMBO reports. We have now received reports from the three referees that were asked to evaluate your study, which can be found at the end of this email. As you will see, although referee 1 is more positive, referees 2 and 3 consider the study would need to be strengthened for publication here. Referees 1 and 2 work on apoptosis, whereas referee 3 is an expert on signal transduction in cell migration and invasion.

Given that all referees provide suggestions on how to strengthen the work, we would like to give you the opportunity of revising your manuscript. All concerns of referees 1 and 2 would have to be addressed in full -including providing proof of the causal link of MMP1 to the cell migration phenotype- as would referee 3's concerns regarding figure 2 and incorporating the immunofluorescence images of pJNK in the main text. Regarding referee 3's concern about the lack of clarity of the role of JNK, referee 1 suggested after further discussion that the use of msn-lacZ would be a better readout of JNK activity than puc-lacZ, as it doesn't activate the JNK pathway. In addition, as is clear from referee 3's report and also acknowledged by the other referees upon further discussion, the current manuscript is very hard to understand for people outside the Drosophila apoptosis field. As EMBO reports caters to a general readership, this issue would have to be addressed by making the text more accessible to a non-expert and clarifying the different tools used, which would address several of referee 3's concerns.

If the referee concerns can be adequately addressed, we would be happy to consider your manuscript for publication. Please note that it is EMBO reports policy to undergo one round of revision only

and thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

Please note that your current manuscript is longer than we can accept for publication. Given that you need to include additional explanations as indicated above, some rewriting will be needed. Shortening may be made easier by combining the Results and Discussion into a single section, which we require, and which will help eliminate the redundancy that is inevitable when discussing the same experiments twice. In addition, although basic Materials and Methods required for understanding the experiments performed must remain in the main text, additional detailed information may be included as Supplementary Material.

Revised manuscripts must be submitted within three months of a request for revision unless previously discussed with the editor; they will otherwise be treated as new submissions. Revised manuscript length must be a maximum of 28,500 characters (including spaces). When submitting your revised manuscript, please also include editable TIFF or EPS-formatted figure files, a separate PDF file of any Supplementary information (in its final format) and a letter detailing your responses to the referees.

We also welcome the submission of cover suggestions or motifs that might be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, do not hesitate to get in touch with me if I can be of any assistance.

Referee #1:

In this report, Rudrapatna et al describe the importance of caspase activation in the absence of apoptosis in JNK-mediated cell invasion in Drosophila. This study is novel and timely, since it was an unresolved issue that upon Src-driven delamination and cell migration associated with active-caspase staining as to whether these cells actually died (Vidal & Cagan publications). In this study they nicely examine this issue by inducting cell death with the hid cell death induced and blocking effector caspases with the caspase inhibtor p35 using the wing anterior-posterior boundary driver, patched-GAL4. This study has important implications for the Drosophila and mammalian cell death and tumorigenesis fields, since many investigators use active-caspase 3 staining as an indicator of cell death, when it may not necessarily be so. This point should be highlighted more in the conclusions of the paper.
This paper also makes some important mechanistic findings showing that activation of the initiator caspase Dronc as well as the effector caspase Drice contribute to the induction of JNK and the expression of the MMP1 metalloproteinase to facilitate invasion. The paper was generally well written and the quality of the data was excellent and well quantified. With minor corrections detailed below, I recommend acceptance of this important paper.

(1) Results - 4rd paragraph - it should be more clearly stated that from the study of Lammer et all p35 acts downstream of Drice activation to block the action of Drice, but that p35 does not ibhibit the initiator caspase Dronc.

(2) Results - "p35-hid cells activate caspases but not apoptosis" - 2nd to last sentence - Do the authors mean that is was "likely" (rather than "unlikely") to have been undead cells that lost ptc or GFP expression? Otherwise the following sentence does not make sense. Also how were the lineage tracing experiments done?

(3) Quantification of the invasion phenotype - Fig 2, 3, Supp Fig 1, 3 and experimental procedures - more detail is needed for how the bins (strong, medium and weak) were defined?

(4) Fig 3D - arrows pointing to lamellopodia-like processes would help.

(5) Fig 1 and supp Fig 2 - it is curious why p35 expression alone induced some activation of Dronc, JNK (pJNK), the JNK target puc-Z and migratory behaviour - Do the authors have any thoughts how this may occur - this could be mentioned in the Discussion?

(6) It is also curious that since p35 has been shown to inhibit the function of Drice (Lammer et al) that knocking down Drice resulted in some suppression of the invasive phenotype (Fig 3B). Can the authors comment on possible reasons for this in the discussion?

(7) Discussion - It would be helpful to expand upon the discepancy between the Fan & Bergmann vs Kondo studies - is it just due to different contexts?

(8) Can the authors comment on whether in Drosophila Mst (Hpo) activation might activate JNK? Certainly the recent study of Verghese S, Bedi S, & Kango-Singh M (Cell Death Diff 2012) would suggest that Hpo can activate Dronc. This should be cited.

Referee #2:

While it has been known for some time that caspase activity regulates cell migration in Drosophila, this manuscript presents novel findings that implicate caspase activity in invasion and metastasis. This is an important observation with potential implications for cancer.

A few points should be addressed before the manuscript is accepted for publication:

• The results show that Hid expression in combination with the effector caspase inhibitor p35, but not DIAP1, promotes cell invasion. Based on knockdown eperiments, the authors conclude that caspase-mediated cell migration of 'undead cells' is the result of drICE activity. Although their knockdown result is convincing, this conclusion is rather provocative as p35 should smother all but drICE's catalytic activity. P35 acts as s suicide substrate that blocks the catalytic activity of drICE. Hence, the simplest explanation would be that the migration phenotype is mediated directly by Dronc. To address the contribution of drICE in regulating migration of undead cells, it the authors should confirm their knockdown experiment using drICE mutants.

• Under conditions where drICE mediates compensatory proliferation, p35 completely blocks this effect. This questions whether the expression level of p35 is high enough in the Hid expressing clones in this study. The authors should introduce two copies of p35, or express it throughout the disc using an additional driver.

• In Fig 2, p35 expression alone leads to MMP1 expression. The authors suggest that this is due to stochastic caspase activation, but this theory should be tested by expressing a Dronc dominant-negative mutant to see if this suppresses the MMP1 expression.

• Although the authors demonstrate that undead cells express MMP1, they haven't demonstrated that expression of this metalloprotease is actually important for the cell migration phenotype.

• The authors should provide some statistics on actual cell numbers. It is important to know how penetrant this phenotype actually is: how many cells move away, is it a small number of cells, or is it a general feature of 'undead cells'? Presenting the data after binning into arbitrary migration categories is not informative enough.

Referee #3:

This paper investigates the relationship between apoptosis and invasion using a model of Drosophila larva expressing positive and negative regulators of apoptosis under the control of the ptc promoter in a strip of epithelial cells. The invasion phenotype, as detected by the detachment of cells from the ptc domain, was observed following the expression of Hid to initiate the activation of the caspase cascade together with p35, an inhibitor of effector caspase. Cells expressing both Hid and p35 display low level active caspase 3 and low level TUNEL staining. Further analysis indicated that

cells invading locally within the ptc domain expressed high level MMP1. JNK signaling appears to be implicated in this process.

The study addresses an important question related to the understating on cell migration and invasion in the context of tumor. However, the work is preliminary. For example there is no evidence that MMP1 is important in this process, if anything MMP1 expression is elevated in p35 discs that do not display the invasion phenotype; there is no information on how p35 controls MMP1 expression and JNK phosphoryation, in fact p35 does not even appear on the diagram at the end of paper (Fig. 3F). Furthermore, the data investigating the role of JNK are very confusing. For example, why staining of pJNK in hid discs is weak, while hid puc-lacZ discs exhibit high level of reporter expression. Overall, the paper is very confusing and the data do not support the conclusion.

Other comments:

Fig. 1E-F: based on the labeling of the figure and the information provided in the figure legend, these panels appear to present immunofluorescence images of active caspase 3. However, the description in the text refers to Dronc activity. Therefore, I assume that Dronc is caspase 3, although this is surprising considering that in mammalian cells caspase 3 is an effector/executioner, and not an initiator, caspase. This is very confusing.

Fig 2 D (p5): In contrast to the authors' statement, there is little evidence that migrating cells (detected in green indicating the absence of red stain) retained low MMP1 level. Furthermore, there is no strong evidence that MMP1 expression correlates with caspase activation. To be more convincing the authors will have to demonstrate the presence of activate caspase 3/DronC? in areas of p35 discs displaying significant increased MMP1 expression in particular regions associated with attached cells possessing elongated processes.

There is no reason why immunofluorescence images showing pJNK should be placed in supplementary data.

How does p35 block apoptosis in Hid expressing discs? Is it by inhibiting caspase 3? It is not clear what is caspase 3 in Drosophila.

It is not clear what the message of supp Fig 3 is. Considering that hid-expressing cells are not observed outside of the ptc domain (Fig. 1 and supp Fig. 1), how can the effect of down-regulating Dronc expression on cell migration be tested in hid discs? hid discs expressing drice RNAi display a migratory phenotype that does not seem to be consistent with the pictures provided in Fig. 1C or 2C.

1st Revision - authors' response 11 November 2012

Response to Reviewers

Referee #1:

The paper was generally well written and the quality of the data was excellent and well quantified. With minor corrections detailed below, I recommend acceptance of this important paper.

• *...This study has important implications for the Drosophila and mammalian cell death and tumorigenesis fields, since many investigators use active-caspase 3 staining as an indicator of cell death, when it may not necessarily be so. This point should be highlighted more in the conclusions of the paper.*

This point is now strengthened (page 4 paragraph 4).

(1) Results - 4rd paragraph - it should be more clearly stated that from the study of Lammer et all p35 acts downstream of Drice activation to block the action of Drice, but that p35 does not inhibit the initiator caspase Dronc.

This is clarified in the first paragraph of the Results section (page 4)

(2) Results - "p35-hid cells activate caspases but not apoptosis" - 2nd to last sentence - Do the authors mean that is was "likely" (rather than "unlikely") to have been undead cells that lost ptc or GFP expression? Otherwise the following sentence does not make sense. Also how were the lineage tracing experiments done?

Lineage tracing experiments were done using the G-TRACE system established by Utpal Banerjee's laboratory (Evans et al., 2009). Briefly, this method combines the UAS-Gal4 and FLP-FRT systems to permanently mark all cells that express (in our case) *ptc-gal4*. In other words, lineally-related cells remain GFP-positive even if they have lost *ptc-gal4* expression. In our experiments we found that this was not the case. We therefore concluded that the cells that were caspase and TUNEL positive but GFP negative were not undead cells that have lost *ptc-gal4* (and therefore GFP) expression. But as the reviewer pointed out, the sentence following that statement was wrong and is now corrected (page 5 paragraph 2).

(3) Quantification of the invasion phenotype - Fig 2, 3, Supp Fig 1, 3 and experimental procedures - more detail is needed for how the bins (strong, medium and weak) were defined?

The phenotypic categories were defined based on numbers of invading cells observed in the posterior compartment. None: no invasion; weak: 1-5 cells; Moderate: 6-15 cells; Strong: >15 cells. To facilitate the detection of suppressor genotypes, category thresholds and cutoffs were defined such that the distribution of migration strength in *p35 hid* was centered around the "Moderate" class. This is now stated in Methods and in Supplementary Figure 1.

(*4) Fig 3D - arrows pointing to lamellopodia-like processes would help.*

Arrows have been added (this panel is now figure 3F).

(5) Fig 1 and supp Fig 2 - it is curious why p35 expression alone induced some activation of Dronc, JNK (pJNK), the JNK target puc-Z and migratory behaviour - Do the authors have any thoughts how this may occur - this could be mentioned in the Discussion?

We agree this is surprising and expect these represent cells that activated apoptosis as part of the normal developmental program but have become "undead" due to p35 expression. This is now discussed on page 5 paragraph 3.

(6) It is also curious that since p35 has been shown to inhibit the function of Drice (Lammer et al) that knocking down Drice resulted in some suppression of the invasive phenotype (Fig 3B). Can the authors comment on possible reasons for this in the discussion?

We found that active caspase levels induced by Hid expression can be reduced to a level that suppresses apoptosis but is still sufficient to direct migration by either blocking Drice activity (*hid p35*) or by reducing total Drice levels (*hid drice*^{RNAi}). On the other hand, reducing Drice levels and simultaneously inhibiting Drice function (*hid p35 drice*^{RNAi}) brings active Drice levels to a level too low to induce invasion. Overall, these experiments indicated that caspase activation must be precisely controlled to promote invasion. We have elaborated on this point in the revised manuscript (page 6, last paragraph)

(7) Discussion - It would be helpful to expand upon the discepancy between the Fan & Bergmann vs Kondo studies - is it just due to different contexts?

The difference between these studies is likely due to different developmental contexts (e.g. differentiation status of the cells) as discussed in a recent review by Bergmann and Steller (2010).

As these studies mostly focused on compensatory proliferation rather than invasion, we were not able to include a discussion of this point in our revised manuscript given space constraints. We can add a discussion of this point if the reviewer feels it is key.

(8) Can the authors comment on whether in Drosophila Mst (Hpo) activation might activate JNK? Certainly the recent study of Verghese S, Bedi S, & Kango-Singh M (Cell Death Diff 2012) would suggest that Hpo can activate Dronc. This should be cited.

This is an interesting point, which we now include in our discussion along with the reference the reviewer suggested (page 7, paragraph 3)

Referee #2:

While it has been known for some time that caspase activity regulates cell migration in Drosophila, this manuscript presents novel findings that implicate caspase activity in invasion and metastasis. This is an important observation with potential implications for cancer…A few points should be addressed before the manuscript is accepted for publication:

• The results show that Hid expression in combination with the effector caspase inhibitor p35, but not DIAP1, promotes cell invasion. Based on knockdown experiments, the authors conclude that caspase-mediated cell migration of 'undead cells' is the result of drICE activity. Although their knockdown result is convincing, this conclusion is rather provocative as p35 should smother all but drICE's catalytic activity. P35 acts as s suicide substrate that blocks the catalytic activity of drICE. Hence, the simplest explanation would be that the migration phenotype is mediated directly by Dronc. To address the contribution of drICE in regulating migration of undead cells, it the authors should confirm their knockdown experiment using drICE mutants.

We have now confirmed these findings in *drice* mutants. Specifically, we show that a *drice*$^{17/D1}$ allelic combination suppresses *p35 hid* mediated invasion to a similar extent as a *drice* knock-down (figure 3C,G)

• Under conditions where drICE mediates compensatory proliferation, p35 completely blocks this effect. This questions whether the expression level of p35 is high enough in the Hid expressing clones in this study. The authors should introduce two copies of p35, or express it throughout the disc using an additional driver.

This is an interesting point. Introducing two copies of *p35* modestly enhanced migration in our hands, suggesting that a single copy of *p35* is not sufficient to block Drice activity in *hid p35*(1x) discs. More broadly, we do not know how the level of Drice activity required to direct compensatory proliferation relates to levels required to direct invasion. The most likely model is that compensatory proliferation requires higher caspase activity to ensure that it is only activated in the presence of apoptotic cells, making it more sensitive to a reduction of Drice activity. Consistent with this argument and also previous work mentioned by the reviewer, we did not observe compensatory proliferation in *p35 hid* discs. We propose that the level of Drice activity required to direct invasion is significantly lower; in this view, modest p35 levels are insufficient to bring it to this 'invasion threshold' in the presence of *hid*.

• In Fig 2, p35 expression alone leads to MMP1 expression. The authors suggest that this is due to stochastic caspase activation, but this theory should be tested by expressing a Dronc dominant-negative mutant to see if this suppresses the MMP1 expression.

We found that MMP1 expression is indeed significantly reduced in *p35 drice*$^{17/D1}$ mutants (supplementary figure 2B). We also show that MMP1 staining in *p35* discs is observed in cleaved caspase-3 positive cells within *p35* discs (supplementary figure 2A). This data is consistent with our conclusion that MMP1 positive cells in *p35* discs are indeed undead cells generated when *p35* has blocked normal developmental apoptosis.

• *Although the authors demonstrate that undead cells express MMP1, they haven't demonstrated that expression of this metalloprotease is actually important for the cell migration phenotype.*

We now demonstrate that co-expression of tissue inhibitor or metalloproteinase (TIMP) partially suppresses p35-hid mediated invasion (fig 2J), indicating a functional requirement for MMPs in this process

• *The authors should provide some statistics on actual cell numbers. It is important to know how penetrant this phenotype actually is: how many cells move away, is it a small number of cells, or is it a general feature of 'undead cells'? Presenting the data after binning into arbitrary migration categories is not informative enough.*

As described above, the phenotypic categories were defined based on numbers of invading cells observed in the posterior compartment. Our categories were as follows: None: no invasion; weak: 1-5 cells; Moderate: 6-15 cells; Strong: >15 cells. To facilitate the detection of suppressor and enhancer genotypes, category thresholds and cutoffs were defined such that the distribution of migration strength in p35 hid would be centered around the "Moderate" class. This is now stated in Methods and in supplementary figure 1.

Referee #3:

The study addresses an important question related to the understating on cell migration and invasion in the context of tumor. However, the work is preliminary.

• *...there is no evidence that MMP1 is important in this process, if anything MMP1 expression is elevated in p35 discs that do not display the invasion phenotype;*

We found that co-expression of tissue inhibitor or metalloproteinase (TIMP) partially suppresses *p35-hid*-mediated invasion (fig 2J), indicating a functional requirement for MMPs in this process.

• *...there is no information on how p35 controls MMP1 expression and JNK phosphorylation, in fact p35 does not even appear on the diagram at the end of paper (Fig. 3F).*

P35 is a baculovirus derived protein that inhibits the activity of the effector caspase Drice (Drosophila caspase 3) by acting as a suicide substrate (page 4 paragraph 1). In our first submission we proposed that p35 expression blocks apoptosis that happens as part of the normal developmental program in these discs, thereby creating undead cells that show JNK pathway activation and MMP1 expression. We now strengthen this point by showing that MMP1 expression in p35 discs is observed in cleaved caspase-3 positive cells (supplementary figure 2A), consistent with the view that they have activated the apoptosis program but failed to execute it because of p35 expression. Furthermore, MMP1 expression is strongly reduced in *p35* discs that are also mutant for *drice* (*p35 drice*$^{17/D1}$; supplementary figure 2B). We now include p35 in our model (now Figure 3H)

• *...the data investigating the role of JNK are very confusing. For example, why staining of pJNK in hid discs is weak, while hid puc-lacZ discs exhibit high level of reporter expression.*

As we stated in the original manuscript, the JNK reporter pucZ disrupts the *puckered* locus and is therefore a *puc* loss of function allele. Since *puc* is a negative regulator of the pathway—it is a JNK phosphatase—JNK pathway activity in *pucZ/+* animals is enhanced. This is why *hid pucZ* discs exhibit high level of reporter activity and also why invasion is enhanced in *pucZ/+* animals.

To get around this problem, we used another commonly used reporter for JNK pathway activity as suggested by the other reviewers: *msn-LacZ*. *hid msn-LacZ* animals did not show elevated reporter activity, consistent with the very weak pJNK staining in *hid* discs. Importantly, *msn-lacZ* is also a loss of function allele for the JNK pathway component msn (*JNKKKK*); in this case we

anticipate *p35 hid* mediated invasion will be suppressed in the *msn-LacZ* background. Despite this caveat, *msn-lacZ* more faithfully recapitulated the pJNK results. Therefore responding to the reviewer's point, we removed the *pucZ* experiments from the manuscript, moved the pJNK panels to figure 2, and added the *msn-lacZ* data to supplementary figure 2.

• Fig. 1E-F: based on the labeling of the figure and the information provided in the figure legend, these panels appear to present immunofluorescence images of active caspase 3. However, the description in the text refers to Dronc activity. Therefore, I assume that Dronc is caspase 3, although this is surprising considering that in mammalian cells caspase 3 is an effector/executioner, and not an initiator, caspase. This is very confusing.

We now clarify these points in the text. We more clearly state the mammalian ortholog for each gene: Drice is the Drosophila caspase 3 and Dronc is the Drosophila caspase 9. Furthermore, previous work has shown that the human cleaved caspase 3 antibody is a reporter for Dronc activity. This is now more clearly stated and referenced in the revised manuscript.

• Fig 2 D (p5): In contrast to the authors' statement, there is little evidence that migrating cells (detected in green indicating the absence of red stain) retained low MMP1 level.

We now present this data in supplementary figure 1D,E.

Furthermore, there is no strong evidence that MMP1 expression correlates with caspase activation. To be more convincing the authors will have to demonstrate the presence of activate caspase 3/DronC? in areas of p35 discs displaying significant increased MMP1 expression in particular regions associated with attached cells possessing elongated processes.

We now show that MMP expression is observed in regions with cleaved caspase 3 staining in p35 discs (supplementary figure 2A). We also show that MMP1 expression is lost from most cleaved caspase-3 positive cells in *drice* mutants (supplementary figure 2B), indicating that caspase activation is required for MMP expression. Note that as previously published, cleaved caspase 3 antibody still labels cells in *drice* mutants since Dronc is still active in these cells.

• There is no reason why immunofluorescence images showing pJNK should be placed in supplementary data.

We have moved the pJNK panels to Figure 2.

• How does p35 block apoptosis in Hid expressing discs? Is it by inhibiting caspase 3? It is not clear what is caspase 3 in Drosophila.

As discussed above, we now clarify the point that Drice is the Drosophila caspase 3 and p35 inhibits apoptosis by blocking Drice activity by acting as a suicide substrate (page 4 paragraph 1).

• It is not clear what the message of supp Fig 3 is. Considering that hid-expressing cells are not observed outside of the ptc domain (Fig. 1 and supp Fig. 1), how can the effect of down-regulating Dronc expression on cell migration be tested in hid discs? hid discs expressing drice RNAi display a migratory phenotype that does not seem to be consistent with the pictures provided in Fig. 1C or 2C.

This is a central point to our model: that achieving a precise level of caspase activity is required to achieve invasion. Our data indicate that we do not see invasion in *hid* discs because caspase activity is too high, leading to apoptosis instead of invasion. The data in supplemental figure 3 is consistent with the view that when *hid* activity is reduced to a level that is too low to induce apoptosis, a level of caspase activity is achieved that directs invasion. Invasion was observed in *hid*

drice^{RNAi} discs but not in *hid dronc*^{RNAi} discs, suggesting that active caspase levels are too low to induce invasion in the latter case.

In summary, we show that active caspase levels induced by Hid expression can be reduced to a level that suppresses apoptosis but is still sufficient to direct migration by two different ways: 1) by blocking Drice activity (*hid p35*), or 2) by reducing total Drice levels (*hid drice*^{RNAi}). On the other hand, 1) reducing Drice levels and simultaneously inhibiting Drice function (*hid p35 drice*^{RNAi}), or 2) reducing Dronc levels (*hid dronc*^{RNAi}) bring Drice activity levels to a level too low to induce invasion. Overall, these experiments indicate that caspase activation must be precisely controlled to promote invasion. We now elaborate on this point (page 6, last paragraph).

2nd Editorial Decision 28 November 2012

Thank you for your patience while we have reviewed your revised manuscript. As you will see from the reports below, the referees are now all positive about its publication in EMBO reports. I am therefore writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once the minor issues raised by referee 1 have been addressed (please see his/her comments below).

I also thought you would be happy to hear that I have commissioned a highlight on your article (a News&Views-type of piece) to accompany the publication of your paper, as I thought the findings that you presented here were very cool and I wanted them to be more exposed.

Once you have made these minor revisions, please use the following link to submit your corrected manuscript:

<http://mts-embor.nature.com/cgi-bin/main.plex?el=A2BA3hw4A4Tn6J3A91W4SncJsoF1gLRV56awZ>

If all remaining corrections have been attended to, you will then receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Thank you for your contribution to EMBO reports.

Referee #1 (Remarks to the Author):

In this revised version of their manuscript, Rudrapatna et al have addressed all of the reviewers comments and added new data substantially improving the manuscript and its signficance.
I now recommend publication of this important paper, however the following minor corrections would improve the manuscript:

(1) p4 para 2 - ref 17 is incorrect - it has nothing to do with p35 - it should be replaced by ref 23.

(2) p4 para 4 - It would be more correct to say "no significant effect on invasion" rather than "no evidence of invasion" since compared with hid alone the hid diap1 samples had more samples that showed strong invasion (Fig S1A).

(3) p5 para 2- It would be informative to the reader if the reference for the lineage tracing expt was added here.

(4) p6 para 2 - it should be made clear that this is halving the dosage of msn.

(5) p7 para 2 - should add ref 28 along with 39.

(6) The discussion here that JNK also lies downstream of Dronc to promote migration is not bourne out in the model - Fig 3H - the model needs to incorporate JNK acting downstream of Dronc in promoting actin remodelling and MMP1 expression.

(7) Fig leg 1 - "by included ptc>GFP" does not make sense, and needs to be altered.

(8) Fig leg 3 - the arrow in "F" needs to be referred to in the legend.

(9) Fig S3 - the labelling of the genotypes and stainings on the panels A', B' need to be moved more to the left.

Referee #2 (Remarks to the Author):

I am happy with the response and the additional experiments, and fully support publication of this article.

2nd Revision - authors' response 04 December 2012

Thank you for 'accepting in principle' our manuscript. We have made the requested changes and uploaded the files.

3rd Editorial Decision 12 December 2012

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

