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Peer Review File

ROCK1 mechano-signaling dependency of human malignancies driven by TEAD/YAP activation

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this manuscript the authors identify a crosstalk between p53 DNA contact mutants and TEAD/YAP that act indirectly through deregulation of homeostatic RhoA mechano-signaling. Most of the experiments aimed to demonstrate the mechanistic details through which the reported cross-talk takes place, are conducted in MCF10A cells, which have a wt-p53 intracellular environment, with overexpression of the diverse p53 mutants. The authors should repeat key experiments in cells endogenously expressing mutant p53 proteins and intervening by silencing of p53. Clinical relevance of the reported findings is also lacking. These lacks significantly flaw the strength of the reported findings and make the manuscript in its present form unsuitable for publication in Nature Communications.

Major points:

1. In the “materials and methods”, the authors did not describe the immunoprecipitation methods and the antibodies used. In the Supplementary Figure 2, it appears that the anti-YAP antibody is not efficient in recognizing the protein either in the total extract or in the IP-YAP sample where the band is expected to be enriched. The run of the proteins appears to be slowed down by chromatin still present in the sample which then creates waves and streaks in the bands. I suggest the authors should repeat IPs, perhaps from nuclear extracts, making sure the DNA has been perfectly sonicated, even making a step in DNAase.

2. WB in the Fig. 2B and Suppl. Fig 3B lacks staining for non-phosphorylated cofilin, PAK1/2 and PAK4/5.

3. The p-value is missing in all the graphs and in the legends of the figures. For example, in the ChIP in Fig. 1I (MCF10A-p53R175H), does the p53 enrichment on the sequences -2100 and -150 reach significance (0.05)? Authors should perform a ChIP with endogenous mutp53 levels from MDA-MB-468 and HCC1395. It could be interesting to test also the YAP recruitment.

4. Figure 2K, L, M: to reinforce the molecular mechanism, the authors should perform the same experiments in endogenous p53 interference condition and H-1152 treatment.

5. Fig. 3 A, B, E: what about performing the same experiments in MDA-MB-468 cells depleted for mutant p53 proteins?

6. Does the double depletion ROCK1/mutp53 and ROCK1/YAP produce an attenuation of the effect shown in Figures 4A-E?

7. This study needs to further substantiate the data produced in vitro in a cohort of patients. Taking as example the Suppl. Figures 2D, the authors should analyze either breast cancer datasets from Metabric or from TCGA in which TP53 mutations are known and verify a broader signature of YAP (Wang Y, Xu X, Maglic D, et al. Comprehensive Molecular Characterization of the Hippo Signaling Pathway in Cancer. Cell Rep. 2018; 25 (5): 1304-1317.e5. Doi: 10.1016 / j.celrep.2018.10.001) and how it behaves in a survival curve. The authors should show the expression of ROCK1 and 2 in the two groups, conformational and DNA contact mutant p53.

8. The images of the colony assays have no high definition.

9. In figure 2F 2G, 2H and S4, the authors should show that ROK1/2 inhibition, as well as lantruculin B and blebbastatin treatment, do not affect TEAD4-mediatd transcription in cell lines expressing p53 conformational mutants.

10. Since ROCK inhibition does not relocate YAP in the cytoplasm, differently from serum deprivation, the authors should dissect the mechanism through which ROCK inhibition may impair YAP/TEAD

transcriptional oncogenic function. The authors may, for example, analyze whether the interaction between YAP and TEAD, or their binding on target genes analyzed by ChIP, may be reduced upon ROCK inhibition, or analyze the post-translational modifications of YAP beyond ser127 phosphorylation.
11. Based on the model proposed by the authors, one could speculate that YAP pharmacological inhibition with statins would have a stronger effect in patients bearing p53 DNA contact mutants tumors. If possible, the authors should analyze the effect of statins in vitro, in cells bearing p53 DNA contact mutations or conformational mutations.
12. Moreover, in figure 5B, the authors need to analyze the in vivo effect of the combined treatment H-1125 with statin in cells that are less responsive to H-1125 alone.

Reviewer #2 (Remarks to the Author):

In this MS, Esposito et al. show compelling data demonstrating a new interesting connection between common cancer-associated genetic lesions (i.e. missense p53 mutations) and the actin cytoskeleton cell machinery, ultimately impacting on canonical mechano-responsive pathways. The authors provide convincing evidence that a subclass of p53 missense mutants (DNA contact) indirectly foster the activity of the well-known RhoA/ROCK1 signaling axis and, as a direct effect, promote actin polymerization. Interestingly, they found that the mechano-sensitive transcriptional complex YAP/TEAD is a key downstream effector of ROCK1 in the context of both mutant p53 tumors and Hippo pathway lesions. Finally, the authors demonstrate ROCK1 inhibition as a previously unidentified vulnerability in the context of missense p53 mutations and designed an innovative therapeutic approach to target mutant p53 tumors, a still unmet clinical need.

This paper is straightforward, easy to read and conclusions mostly follow the data, with very few exceptions. The results are of broad interest and provide significant new insights in the understanding of the molecular crosstalk between mutant p53, cell mechano-transduction and the Hippo pathway. The experiments are in general well planned and the figures are of good quality, although an important point should be addressed in order to make this work suitable for publication in Nature Communications. In particular, the link between mutant p53 and YAP/TEAD activation has been explained as an effect of increased mevalonate and isoprenoids (i.e. geranylgeranylation) production. However, no data have been shown in this regard. This hypothesis should be experimentally proved in order to confirm the authors' working model. To this aim, the authors should demonstrate that geraylgerany-transferase inhibitor (e.g. GGTI-298) phenocopy the effect of ROCK1 inhibitor on key phenotypes shown in the manuscript, in particular in the context of mutant p53-expressing cells. Moreover, the authors should comment, within the discussion section, how their results relate to, or diverge from, a relevant paper on this topic showing that mutant p53 function is impaired upon RhoA inhibition (Ingallina et al. Nat Cell Biol 2018)

Reviewer#1:

General Comments: *In this manuscript the authors identify a crosstalk between p53 DNA contact mutants and TEAD/YAP that act indirectly through deregulation of homeostatic RhoA mechano-signaling. Most of the experiments aimed to demonstrate the mechanistic details through which the reported cross-talk takes place, are conducted in MCF10A cells, which have a wt-p53 intracellular environment, with overexpression of the diverse p53 mutants. The authors should repeat key experiments in cells endogenously expressing mutant p53 proteins and intervening by silencing of p53. Clinical relevance of the reported findings is also lacking. These lacks significantly flaw the strength of the reported findings and make the manuscript in its present form unsuitable for publication in Nature Communications.*

In response to Reviewer#1's general comments, we did analyze multiple human tumor lines for responses to p53 silencing but may not have made sufficiently clear that they contained endogenous p53 missense mutations. We showed that p53 knockdown or exogenous expression of dnTEAD4 effectively antagonized the high levels of TEAD/YAP transcription and cell proliferation/motility of tumors with endogenous p53 DNA contact mutations. While p53 knockdown, antagonized the proliferation of tumor lines with endogenous p53 conformational mutations, which lacked upregulated TEAD/YAP transcription, exogenous expression of dnTEAD4 had no effect. In the revised manuscript, we have also included results with another NSCLC line, PC-9, harboring an endogenous p53 DNA contact mutation. Like other tumor lines analyzed with endogenous p53 DNA contact mutations, PC-9 cells exhibited TEAD/YAP transcriptional upregulation, and p53 knockdown, dnTEAD4, or ROCK inhibition antagonized this transcription as well as their proliferation (see revised Figs. 1D-F and 2K, L).
These mechanistic studies involved analysis of 18 human tumor lines with endogenous p53 DNA contact or conformational mutations as well as tumors with representative Hippo pathway mutations (see Figs 1D-F, 2K-M, 3G-I, 4A-H, 5A-D). We also utilized MCF10A cells, an immortalized human breast epithelial line as a cell model, because it has neither endogenous p53 missense mutations nor Hippo pathway genetic aberrations. Thus, it was possible to compare in the same wild type cell background, the effects on signaling and proliferation induced by exogenously expressed p53 missense mutants or by aberrations afflicting Hippo pathway components. Our results with this cell model were completely consistent with those involving human tumor lines, independently confirming the conclusions drawn.

In the MCF10A model, we were also able to establish that p53 DNA contact mutants hyperactivated RhoA/ROCK mechano-signaling, while Hippo pathway mutations did not detectably perturb homeostatic RhoA/ROCK mechano-signaling. We showed further that the physiological levels of RhoA/ROCK signaling observed were required for upregulation of TEAD/YAP transcription and induction of the transformed phenotype by Hippo pathway aberrations and that ROCK inhibitors selectively antagonized proliferation/motility of human tumors with upregulated TEAD/YAP transcription induced by endogenous p53 DNA contact mutations or Hippo pathway lesions. These findings have potential clinical relevance for application of ROCK inhibitors to human tumors driven by p53 DNA contact mutations or Hippo pathway lesions, which as yet lack biologically targeted therapies.

In response to Reviewer#1's suggestions, we have also mined RNA-seq data for human triple negative breast tumors and for mesotheliomas with or without Hippo pathway lesions. Our new results utilizing GSEA with a well-established YAP conserved signature indicate that this gene signature is preferentially enriched in TNBCs with p53 DNA contact mutations as compared to those with p53 conformational mutations and in Hippo pathway mutant mesotheliomas as compared to Hippo pathway wild type mesotheliomas (see new Supplemental Fig. 15). In response to Reviewer#1's general comments, we have also revised the text in an effort to clarify these points.

Major Points:
1. In the ???materials and methods???, the authors did not describe the immunoprecipitation methods and the antibodies used. In the Supplementary Figure 2, it appears that the anti-YAP antibody is not efficient in recognizing the protein either in the total extract or in the IP-YAP sample where the band is expected to be enriched. The run of the proteins appears to be slowed down by chromatin still present in the sample which then creates waves and streaks in the bands. I suggest the authors should repeat IPs, perhaps from nuclear extracts, making sure the DNA has been perfectly sonicated, even making a step in DNAase.

Agree. We have included a section in the revised Materials and Methods describing the protocol used for immunoprecipitation. We appreciate Reviewer #1's constructive suggestion, and we have repeated these experiments as suggested. Revised Supplemental Fig. 2A includes these results, which more clearly show the lack of detectable interaction of YAP and p53 by coimmunoprecipitation.

2. WB in the Fig. 2B and Suppl. Fig 3B lacks staining for non-phosphorylated cofilin, PAK1/2 and PAK4/5.

Agree. We thank Reviewer#1 and in response we have included results showing immunodetection of the non-phosphorylated forms of cofilin, PAK1/2 and PAK4/5 in revised Fig. 2B and Supplemental Fig. 4B.

3. The p-value is missing in all the graphs and in the legends of the figures. For example, in the ChIP in Fig. 1I (MCF10A-p53R175H), does the p53 enrichment on the

sequences -2100 and -150 reach significance (0.05)? Authors should perform a ChIP with endogenous mutp53 levels from MDA-MB-468 and HCC1395. It could be interesting to test also the YAP recruitment.

We thank Reviewer#1 for their constructive suggestions. In response, we have included p-values in the legends of the figures. The ChIP data in Fig.1l for MCF10A-p53 R175H did not reach significance as is now indicated in the revised legend. At the reviewer's suggestion, we also performed ChIP with endogenous P53 in MDA-MB-468 and HCC1395, and these results are included in new Supplemental Fig. 2B. They show that endogenous P53 R273H shows statistically significant binding to the promoter of the Mevalonate pathway gene HMGCR in MDA-MB-468 cells and that endogenous p53 R175H does not do so in HCC1395 cells. These findings are consistent with our evidence that p53 DNA contact and conformational mutants possess different GOFs.

4. Figure 2K, L, M: to reinforce the molecular mechanism, the authors should perform the same experiments in endogenous p53 interference condition and H-1152 treatment.

The experiments in Figs 2K, L, and M were performed with the ROCK1/2 dual inhibitor, H-1152, which antagonized upregulated TEAD/YAP transcription (Fig. 2K), proliferation (Fig. 2L), and motility (Fig. 2M) of tumor cells with endogenous p53 DNA contact mutations but had no effect on proliferation of tumor cells with p53 conformational mutants or parental MCF10A breast epithelial cells at 5-10-fold higher concentrations (Fig. 2M). The initial manuscript also included evidence that p53 interference antagonizes both upregulated TEAD/YAP transcription (Fig. 1E) and proliferation (Fig. 1F) of multiple tumors including MDA-MB-468 with endogenous p53 DNA contact mutations. Of note, whereas p53 knockdown also inhibited proliferation of tumor cells with endogenous p53 conformational mutations, dnTEAD4 inhibited proliferation of only those human tumors with endogenous p53 DNA contact mutations (Fig. 1F).These findings along with complementary results in the MCF10A model establish that the mechanism by which p53 DNA contact mutations drive cell proliferation/motility is through upregulation of TEAD/YAP transcription and that the gain of function by p53 conformational mutations involves a different mechanism.

5. Fig. 3 A, B, E: what about performing the same experiments in MDA-MB-468 cells depleted for mutant p53 proteins?

Agree. We performed experiments that address this comment. Fig. 3A, B and E show that ROCK inhibition antagonized TEAD reporter activity, ROCK signaling, cell motility and agar growth of MCF10A cells with exogenous overexpression of p53 R273H or YAP or knock down of Hippo core components NF2 or LATS1/2. We also showed that depletion of an endogenous p53 DNA contact mutant in MDA-MB-468 cells inhibited TEAD reporter activity (see Fig. 1E), ROCK signaling (see Supplemental Fig. 4C) and cell proliferation (see Fig. 1F).

6. Does the double depletion ROCK1/mutp53 and ROCK1/YAP produce an attenuation of the effect shown in Figures 4A-E?

ROCK1 depletion (see Fig. 4B), or depletion of an endogenous p53 DNA contact mutant (see Fig. 1E), each markedly antagonized upregulated TEAD/YAP transcription. These findings argue strongly against the likelihood that these effects would be attenuated by double ROCK1/ p53 knockdown. Many tumors express YAP and TAZ, both of which serve as co-activators of TEAD dependent transcription. Thus, knockdown of YAP alone is only effective if TAZ is not present[1]. For these reasons, we utilized exogenous dnTEAD4 expression to antagonize TEAD dependent transcription (see Fig.1E) and our findings that it or ROCK1 knockdown (see Fig. 4A) similarly markedly inhibited TEAD dependent transcription strongly argue against the likelihood that these effects would be attenuated by expressing both dnTEAD4 and shROCK1.

7. This study needs to further substantiate the data produced in vitro in a cohort of patients. Taking as example the Suppl. Figures 2D, the authors should analyze either breast cancer datasets from Metabric or from TCGA in which TP53 mutations are known and verify a broader signature of YAP (Wang Y, Xu X, Maglic D, et al. Comprehensive Molecular Characterization of the Hippo Signaling Pathway in Cancer. Cell Rep. 2018; 25 (5): 1304-1317.e5. Doi: 10.1016 / j.celrep.2018.10.001) and how it behaves in a survival curve. The authors should show the expression of ROCK1 and 2 in the two groups, conformational and DNA contact mutant p53.

Agree. In response, we have revised the manuscript to include both a comparison of RNA-seq data sets of triple negative breast tumors with p53 DNA contact vs p53 conformational mutations and mesotheliomas with or without Hippo pathway mutations. When we applied a well-known YAP conserved signature[2] to our GSEA in both tumor sets, we observed enrichment of this gene set for Hippo signaling both in breast tumors with p53 DNA contact mutations and mesotheliomas with Hippo pathway aberrations (see new Supplemental Fig. 15).

In response to Reviewer#1's other comments, we did not observe significant differences in the expression of ROCK1 or ROCK2 at the RNA level between breast tumors with p53 DNA contact or conformational mutations or mesotheliomas with or without lesions in Hippo pathway components (see new Supplemental Fig 12b). This is not unexpected since the mechanism of RhoA/ROCK signaling activation by p53 DNA contact mutations is through hyperactivation of ROCK function at the protein rather than RNA level. Data mining revealed no statistically significant differences in survival of patients whose breast tumors had p53 DNA contact vs conformational mutations. These findings are consistent with the fact that both p53 DNA contact and conformational mutations confer a gain of function, which though different, provides in both cases a growth advantage to tumor cells.

8.The images of the colony assays have no high definition.

Agree, and in response, we have revised the manuscript to include high-definition images for all figures with colony assays.

9. In figure 2F 2G, 2H and S4, the authors should show that ROK1/2 inhibition, as well as lantruculin B and blebbastatin treatment, do not affect TEAD4-mediatd transcription in cell lines expressing p53 conformational mutants.

TEAD/YAP transcription in tumors with p53 conformational mutations was very low or undetectable (see revised Fig. 1E), so it was not possible to meaningfully assess inhibition of this transcription by H-1152, Latrunculin B or Blebbistatin. To address Reviewer#1's comment, we have added results showing that in human breast cancers, HCC1395 and MDA-MB-468 cells, with endogenous p53 DNA conformational and DNA contact mutations, respectively, the ROCK inhibitor, H-1152, antagonized downstream signaling similarly in a dose dependent manner (see new suppl. Fig. 6). However, HCC1395 tumor cells were not growth inhibited by H-1152 at 10-fold higher levels than were growth inhibitory for MDA-MB-468 cells (see revised Fig. 2L). Of note, HCC1395 tumor cells exhibited an undetectable TEAD/YAP transcription level (see revised Fig.1E) and were not growth inhibited by exogenous dnTEAD4 expression (see revised Fig. 1F) or by knockdown of ROCK1 or ROCK1/2 (see Fig. 4G). These results argue strongly that endogenous p53 DNA contact mutations activate oncogenic TEAD/YAP transcription in the tumors analyzed and that these tumors are selectively sensitive to inhibition of ROCK signaling. Complementary findings in the MCF10A cell model indicated that exogenously expressed p53 DNA contact mutants activated TEAD/YAP transcription, while p53 conformational mutants did not (see Fig. 1A). Further, in this cell model, H-1152, Latrunculin B or Blebbistatin each antagonized hyperactivated RhoA/ROCK/actomyosin signaling by p53 DNA contact mutants (see Fig. 2I) as well as upregulated levels of TEAD/YAP transcription (see Fig. 2F-H). Moreover, H-1152 antagonized the transformed phenotype induced by p53 DNA contact but not by conformational mutants (see Fig. 2J). We have revised the text in an effort to clarify these points.

10. Since ROCK inhibition does not relocate YAP in the cytoplasm, differently from serum deprivation, the authors should dissect the mechanism through which ROCK inhibition may impair YAP/TEAD transcriptional oncogenic function. The authors may, for example, analyze whether the interaction between YAP and TEAD, or their binding on target genes analyzed by ChIP, may be reduced upon ROCK inhibition, or analyze the post-translational modifications of YAP beyond ser127 phosphorylation.

Agree. In response to Reviewer#1's suggestions, we have included new results showing by co-ip that H-1152 markedly reduced interactions between TEAD and YAP as detected by co-ip similarly to the effects of Hippo pathway activation (see new Suppl. Fig. 9H). As shown in the original manuscript, Hippo pathway activation more rapidly antagonized the TEAD reporter (Suppl. Fig. 9F) and induced YAP phosphorylation, while H-1152 did so with slower kinetics and did not affect YAP phosphorylation at a

major LATS phosphorylation site(Suppl. Fig. 9E). Hippo activation also led to rapid YAP translocation to the cytosol while H-1152 treatment resulted in retention of YAP in the nucleus (Suppl. Fig. 9G). We have added further discussion of these differences in mechanism to the revised text.

11. Based on the model proposed by the authors, one could speculate that YAP pharmacological inhibition with statins would have a stronger effect in patients bearing p53 DNA contact mutants tumors. If possible, the authors should analyze the effect of statins in vitro, in cells bearing p53 DNA contact mutations or conformational mutations.

In response to the Reviewer#1's suggestion, we have included results in new Supplemental Fig. 3 showing that Simvastatin exhibited variable growth inhibitory effects on tumor cells with p53 DNA contact mutations or p53 conformational mutants. This lack of specificity compared to ROCK inhibition is not unexpected due to the fact that statins act upstream of RhoA/ROCK signaling and, thus, likely have more widespread biochemical effects.

12. Moreover, in figure 5B, the authors need to analyze the in vivo effect of the combined treatment H-1125 with statin in cells that are less responsive to H-1125 alone.

Agree. In response to Reviewer#1's suggestion, we have included new Supplemental Fig. 7 in the revised manuscript showing that H-1152 and Simvastatin cooperate for growth inhibition of tumor cells with endogenous p53 DNA contact mutations without having any detectable growth effects on tumors with p53 conformational mutations under the same conditions.

Reviewer #2:
General Comments: *In this MS, Esposito et al. show compelling data demonstrating a new interesting connection between common cancer-associated genetic lesions (i.e. missense p53 mutations) and the actin cytoskeleton cell machinery, ultimately impacting on canonical mechano-responsive pathways. The authors provide convincing evidence that a subclass of p53 missense mutants (DNA contact) indirectly foster the activity of the well-known RhoA/ROCK1 signaling axis and, as a direct effect, promote actin polymerization. Interestingly, they found that the mechano-sensitive transcriptional complex YAP/TEAD is a key downstream effector of ROCK1 in the context of both mutant p53 tumors and Hippo pathway lesions. Finally, the authors demonstrate ROCK1 inhibition as a previously unidentified vulnerability in the context of missense p53 mutations and designed an innovative therapeutic approach to target mutant p53 tumors, a still unmet clinical need.*
This paper is straightforward, easy to read and conclusions mostly follow the data, with very few exceptions. The results are of broad interest and provide significant new insights in the understanding of the molecular crosstalk between mutant p53, cell mechano-transduction and the Hippo pathway.

We appreciate Reviewer #2's positive comments concerning our manuscript.

Specific points:

1. *An important point should be addressed in order to make this work suitable for publication in Nature Communications. In particular, the link between mutant p53 and YAP/TEAD activation has been explained as an effect of increased mevalonate and isoprenoids (i.e. geranylgeranylation) production. However, no data have been shown in this regard. This hypothesis should be experimentally proved in order to confirm the authors??? working model. To this aim, the authors should demonstrate that geraylgerany-transferase inhibitor (e.g. GGTI-298) phenocopy the effect of ROCK1 inhibitor on key phenotypes shown in the manuscript, in particular in the context of mutant p53-expressing cells. Moreover, the authors should comment, within the discussion section, how their results relate to, or diverge from, a relevant paper on this topic showing that mutant p53 function is impaired upon RhoA inhibition (Ingallina et al. Nat Cell Biol 2018).*

Agree. In response to Reviewer#2's constructive suggestion, we tested the ability of the geranylgeranyl-transferase I inhibitor (e.g. GGTI-298) to phenocopy the effects of the ROCK inhibitor or ROCK1 knockdown on TEAD/YAP driven transcription and proliferation in tumor lines expressing endogenous p53 DNA contact mutants. The results show that in tumor cells with endogenous p53 DNA contact mutants or conformational mutations as well as immortalized MCF10A cells, growth inhibition varied (see new Supplemental Fig. 3). These findings are consistent with previous studies indicating that this inhibitor acts upstream of RhoA, has more biochemical targets and exerts pleiotropic effects on various tumor types[3-5].

2. Moreover, the authors should comment, within the discussion section, how their results relate to, or diverge from, a relevant paper on this topic showing that mutant p53 function is impaired upon RhoA inhibition (Ingallina et al. Nat Cell Biol 2018).

Agree. This paper indicated that Simvastatin and GGTI inhibitors decreased mutant p53 protein levels. These authors did not distinguish between effects on protein levels of p53 DNA contact and conformational mutants, while we showed that ROCK inhibition selectively impaired growth of cells expressing p53 DNA contact mutants. Based on Reviewer#2's constructive comment, we have also included results in new Supplementary Fig. 6 showing that ROCK inhibition did not alter the levels of p53 mutant protein expression. We appreciate this comment, which has helped us to exclude a possible mechanism that further strengthens our conclusions. We have also commented on the paper by Ingallina et al in Nat Cell Biol in the revised discussion.

We greatly appreciate the reviewers' constructive comments, which have helped us to strengthen the manuscript. We hope that the revised manuscript is now considered acceptable for publication in Nature Communications.

Sincerely,

Stuart Aaronson, M.D.

1 Plouffe, S. W. *et al.* The Hippo pathway effector proteins YAP and TAZ have both distinct and overlapping functions in the cell. *J Biol Chem* **293**, 11230-11240, doi:10.1074/jbc.RA118.002715 (2018).

2 Cordenonsi, M. *et al.* The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147**, 759-772, doi:10.1016/j.cell.2011.09.048 (2011).

3 Dan, H. C. *et al.* Phosphatidylinositol-3-OH kinase/AKT and survivin pathways as critical targets for geranylgeranyltransferase I inhibitor-induced apoptosis. *Oncogene* **23**, 706-715, doi:10.1038/sj.onc.1207171 (2004).

4 Sonnemann, J., Bumbul, B. & Beck, J. F. Synergistic activity of the histone deacetylase inhibitor suberoylanilide hydroxamic acid and the bisphosphonate zoledronic acid against prostate cancer cells in vitro. *Mol Cancer Ther* **6**, 2976-2984, doi:10.1158/1535-7163.MCT-07-0221 (2007).

5 Berndt, N., Hamilton, A. D. & Sebti, S. M. Targeting protein prenylation for cancer therapy. *Nat Rev Cancer* **11**, 775-791, doi:10.1038/nrc3151 (2011).

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Reviewer #1 (Remarks to the Author):

The authors have adequately addressed to the concerns raised previously by this reviewer. The manuscript is suitable for publication in Nat Comm.

Reviewer #2 (Remarks to the Author):

The authors have addressed my concerns and I have no further requests.