EMBO *reports*

Dual localized kinesin-12 POK2 plays multiple roles during cell division and interacts with MAP65-3

Arvid Herrmann, Pantelis Livanos, Elisabeth Lipka, Astrid Gadeyne, Marie-Theres Hauser, Daniël Van Damme, Sabine Müller

Review timeline:

Submission date:	8 March 2018
Editorial Decision:	20 April 2018
Revision received:	25 May 2018
Editorial Decision:	19 June 2018
Revision received:	21 June 2018
Accepted:	22 June 2018

Editor: Martina Rembold

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.)

1st Editorial Decision 20 April 2018

Thank you for the submission of your research manuscript to our journal. I apologize again for the delay in handling your manuscript. It has been sent to three referees and we have meanwhile received the two enclosed reports on it. Since both reports are very positive and both referees support publication in EMBO reports after minor revision, I would like to ask you to begin revising your study along the lines suggested by the referees. Please note that this is a preliminary decision made in the interest of time, and that it is subject to change should the third referee offer very strong and convincing reasons for this. As soon as we will receive the final report on your manuscript, we will forward it to you as well.

As you will see, referee 1 requests to provide a rationale for the decision not to analyse the central POK2 segment on localization and indicates that further data are required to substantiate the claim that MAP65-3/PLE retains POK2 at the phragmoplast midzone.
Please address these concerns in the manuscript and please also provide a point-by-point reponse. Depending on the outcome of this experiments your manuscript might be sent back to referee 1.

It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript. Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional

Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

- Please submit all movies as a ZIP file including the movie file and a separate text file with the movie title and its legend.

- Please provide the accession numbers in a separate section at the end of Materials and Methods that is called 'Data availabiltiy'

- Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied. Please also include scale bars in all microscopy images.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where the requested information can be found.
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution
(In order to avoid delays later in the publication process please check our figure guidelines before preparing the figures for your manuscript:
http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf)
- a separate PDF file for the Appendix (in its final format)
- all corresponding authors are required to provide an ORCID ID for their name. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (http://embor.embopress.org/authorguide).

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

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I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

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Referee #1:

The regulation of division planes is one of the most exciting areas of plant cell biology that has recently receiving much attention. Previous work has established the POK1&2 kinesins as one of the few known players in the process. POK kinesins were identified by virtue of their association with TANGLED (TAN), and loss of both POK1 and its close homolog POK2 results in slower expansion of the phragmoplast/cell plate and a frequent failure of the cell plate to fuse at the site predicted by the preprophase band (a similar phenotype as caused by the loss of TAN). POK1 protein is localized to the cell cortex with the preprophase band (PPB), but persists after the PPB disappears to mark the future site of cell plate fusion (similar to TAN protein).

In this manuscript, Herrmann et al. report in detail in the localization of POK2 protein, making use of a GFP-fusion that complements the defects of pok1/2 double mutants. Consistent with the idea that both POK proteins provide similar function at the site of cell plate fusion, POK2 localization at the cell cortex follows a very similar pattern and dynamic as POK1 localization. Different from POK1, POK2 protein also accumulates at the phragmoplast; this is in line with the observation that lateral expansion of the phragmoplast in pok2 single mutants is slow compared to wild type and similar in speed to pok1/2 doubles, suggesting that POK2 alone functions at the phragmoplast.

To begin describing the localization mechanisms, GFP-fusions of an N-terminal segment of POK2 (residues 1-589) and a C-terminal segment (2083-2771) were created and analyzed. As with POK1, the C-terminal segment is sufficient for localization to the cortex. The N-terminal motor domain mediates targeting to the phragmoplast midzone, and motor activity is required. In addition, targeting is dependent on the microtubule bundling protein MAP65-3/PLEIADE, and two binding domains are identified (by co-expression in tobacco cells and yeast two-hybrid interaction).

None of the presented results are surprising, but they constitute an important and significant advance. The technical standard of the analysis is high - in particular, the micrographs/movies included in the figures are of excellent quality. I would argue that this is a valuable contribution toward resolving a fascinating and important question.

To be addressed:

Why was the effect of the central POK2 segment on localization not analyzed (the segment was only tested for binding to MAP65-3/PLE)? No rationale is provided in the manuscript, although it constitutes almost half of the protein.

The localization of full length GFP-POK2 in map25-3/pleiade mutants would need to be shown to substantiate the claim that "MAP65-3/PLE retains POK2 at the phragmoplast midzone".

Referee #3:

The manuscript describes experiments designed to uncover the role of the POK2 kinesin in plant cell division. The core of the work is a structure function study looking at different POK2 domains for localization and interactions that could explain a complex pok1 pok2 double mutant phenotype. The manuscript presents data for three separate discoveries that lead to an important model for regulating the placement of the new cell wall during plant cytokinesis. The work provides evidence that there are both microtubule dependent and independent aspects to POK2 localization that place the protein at an appropriate position for division even in cells devoid of preprophase band microtubules. interesting work in a different cellular context presents in vivo evidence that the POK2 kinesin is a plus-end directed motor where the action of the motor is required for localization and function at the phragmoplast midline. And perhaps most broadly interesting, the authors show that a microtubule bundling protein, known to be central to phragmoplast organization, has a required interaction with the POK2 kinesin. Together, these data provide significant insight into the molecular mechanisms driving the eventual association of the circumferentially expanding phragmosome with the predetermined site of attachment at the plasma membrane.

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With the exception of the figure legend label 3C, misspelling Anaphase as Aanaphase, the entirety of the manuscript is very well written and provides as much quantitative detail as could be expected for the difficulty inherent in collecting these data. As a blanket approach to working through how these kinesin molecules potentially function in cytokinesis, the manuscript provides significant new insight and a possible view into why the early phragmosome positioning and later attachment as phragmoplast have been so refractory to genetic analyses. While the biochemically inclined might want more biochemistry, I have no major issues with this manuscript and commend the authors for executing what looks like a very difficult set of experiments to both perform and interpret. The discussion and conclusions stay within the bounds of the data and do not extrapolate too far forward.

1st Revision - authors' response 25 May 2018

EMBOR-2018-46085-T
POINT-BY-POINT Response

Referee #1:

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To be addressed:

Why was the effect of the central POK2 segment on localization not analyzed (the segment was only tested for binding to MAP65-3/PLE)? No rationale is provided in the manuscript, although it constitutes almost half of the protein.
The central domain of POK2 was not tested at all. Cloning and amplification of POK2 DNA fragments in *E. coli* and Agrobacterium have been proven most challenging. Our attempts to clone the central region or fuse it to the motor domain or the C-terminal domain have been hampered due to DNA recombinations in bacteria. Therefore, we could not explicitly address the function of the central domain. However, instead, we have created a construct *p35S:GFP-POK2(Δ590-2082)*, excluding the central domain. This fusion protein localizes like the full-length POK2, indicating that the motor domain and C-terminal domain are sufficient for the dual localization pattern of POK2.

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We have added a panel to Figure EV2 (EV2I-K, page 10, second to last paragraph; materials page 21, pENTR:POK2(Δ590-2082) showing the distribution of this fusion protein, lacking the central domain in dividing cells. Furthermore, we have added the frequencies of GFP-POK2(Δ590-2082) to Table 1 (page 34).

The localization of full length GFP-POK2 in map25-3/pleiade mutants would need to be shown to substantiate the claim that "MAP65-3/PLE retains POK2 at the phragmoplast midzone".
We have included the localization of GFP-POK2 in the *pleiade* mutant in Figure 5 (new panels A and B). There, we compare the localization pattern and the signal intensity of GFP-POK2 between a wild type and a *pleiade* cytokinetic cell, imaged successively, using exactly the same settings. GFP-POK2 localizes to the division site and the phragmoplast midzone in *pleiade* mutants but its signal at the phragmoplast midzone is diminished, indicating that POK2 may bind to other MAP65 proteins at the midzone, in the absence of MAP65-3/PLEIADE (see also page 11, starting with the second sentence). This is consistent with our co-expression analysis of different MAP65 and POK fragments in tobacco and with our interpretation in the discussion.

Referee #2

This is an interesting and well-executed study that reveals that the POK2 kinesin12 has a distinct distribution pattern to its closely related POK1 homologue. Moreover the study identifies two distinct domains of POK2 that can interact with the MAP65-3 microtubule cross linker. The results help to explain why pok1 pok2 double mutants have previously been shown to have delays in cell plate formation, which is dependent on phragmoplast reconstruction.

Overall the work has been carried out in a logical manner, with appropriate controls and quantification. I have only one major concern that should be addressed:

There is no mention which promoter is used to drive the expression of GFP-POK2 and the truncated variants of this construct. This is important because the reported cellular distribution patterns could be altered by the expression levels at different stages of the cell cycle. While the authors do show that the full length GFP-POK2 reporter does complement the pok1pok2 mutant phenotype, this alone does not guarantee that the distribution that is reported is identical to what would be occurring in the wild type.
Ideally the native promoter would be used to drive expression of the reporter fusion. In this study, if the endogenous promoter was not used, the authors should provide a caveat that the distribution patterns and expression levels could be different from wild-type cells.
In the previous version this information was included in the plasmid names in material list. We realize that it is not feasible to look up promoters of plasmids. Following the reviewer's advice, we have now noted the promoter used in the main text (page 5, last paragraph). We have used the p35S promoter, which was the only promoter among those tested (others were pUBQ10, pPOK1, pPOK2) that allowed visualization of GFP-POK2 in our hands. We added a note mentioning possible differences in expression/distribution between endogenous and transgenic POK2 (page 6, towards the end of the first paragraph). However, the restriction of the p35S- driven GFP-POK2 localization to mitotic cells suggest cell cycle dependency and post-translational regulation of the transgene. In addition, in terms of intracellular localization the presence of GFP-POK2 at the phragmoplast midzone is consistent with the phragmoplast expansion phenotype of *pok1 pok2* mutants. Since POK1 localizes exclusively to the division site and the GFP-POK2 rescues the mutant phenotype the phragmoplast expansion phenotype could not be explained if POK2 was not localized at the phragmoplast

Other comments:

1) It should be stated explicitly in the main text that the coding regions used for the reporter fusions were from dDNA.
In the previous version we have described the generation of the POK2 reporter fusion in detail in the Material and Methods section. Following the reviewer's recommendation, the current version contains a note in the results section addressing the generation of the reporter in the main text (page 5, last paragraph). In addition, a panel showing PCR-amplified cDNA from wild type, as well as transgene plants expressing GFP-POK2 in wild type and double mutant background was

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incorporated in Figure EV1 (Fig. EV1B). The different fragments cover the entire POK2 coding region and evidently, look identical in all cases indicating that the splicing of the transgene is correct.

2) Please comment on whether or not the POK2 N-terminal (motor) domain was able to complement the pok1pok2 phenotype.
Figure 4 C shows POK2-motor in the *pok1pok2* double mutant does not complement the phenotype. We have added a comment in the text. (Fig. 4C, page 8 last sentence)

3) For the GFP-POK2 introgression into the pok1pok2 double mutant, the genotyping that confirmed the pok1-1 pok-3 homozygosity should be shown in the supplemental data section.
We have added representative gel electrophoresis images (Figure S1E, F) to proof presence of the T-DNA in *pok1-1* in *pok1 pok2* homozygous plants that rescue the mutant phenotype. Since *POK1* and *POK2* are tightly linked (Müller et al., 2006), populations homozygous for *pok1-1* are also homozygous for *pok2-3* (Lipka et al., 2014). The parent plants of H420 and H419, which carry the GFP-POK2 transgene were sequenced to confirm the presence of the point mutation in *pok2-3* allele. We added the corresponding histogram to Figure S1 (Figure S1G). There, the double peaks indicate the presence of the point mutation and the simultaneous presence of the transgene.

4) The discussion could be much more concise.
We made some changes to shorten the discussion.

5) Summary line 2: I don't think that homolog is the correct term here. Ortholog might be better.
We have changed homolog to ortholog.

6) Introduction p. 5, line 6: I think it would be more accurate to say that expansion is slower. Is there evidence that it decelerates?
We have altered the text to "slowing"

7) For your expansion rate measurements and comparisons, did you monitor the temperature? It would be helpful to report this so that the rates can be compared to other studies. It is also critical that the temperatures were the same for both genotypes.
Genotypes were alternated during individual microscope sessions and the sessions were scheduled at the same time of day to be as consistent as possible. Moreover, the room temperature is set to constant 22 degrees in the microscopy room. We added this information in materials and methods – Imaging paragraph. (page 19)

8) The tobacco leaf cell analysis of MAP65-3 and GFP-POK2 is interesting but it relies on overexpression of MAP65-3 in a heterologous system. You might mention that there is no expression of a MAP65-3 homologue in tobacco leaf epidermal cells
This is clearly mentioned in the current manuscript and a relevant reference was added (page 12, first sentence).

9) page 15 lines 5-7: Sentence needs restructuring
The sentence was revised

Referee #3:

The manuscript describes experiments designed to uncover the role of the POK2 kinesin in plant cell division. The core of the work is a structure function study looking at different POK2 domains for localization and interactions that could explain a complex pok1 pok2 double mutant phenotype. The manuscript presents data for three separate discoveries that lead to an important model for regulating the placement of the new cell wall during plant cytokinesis. The work provides evidence that there are both microtubule dependent and independent aspects to POK2 localization that place the protein at an appropriate position for division even in cells devoid of preprophase band microtubules. interesting work in a different cellular context presents in vivo evidence that the POK2 kinesin is a plus-end directed motor where the action of the motor is required for localization and function at the phragmoplast midline. And perhaps most broadly interesting, the authors show that a microtubule bundling protein, known to be central to phragmoplast organization, has a required interaction with

the POK2 kinesin. Together, these data provide significant insight into the molecular mechanisms driving the eventual association of the circumferentially expanding phragmosome with the predetermined site of attachment at the plasma membrane.

With the exception of the figure legend label 3C, misspelling Anaphase as Aanaphase, the entirety of the manuscript is very well written and provides as much quantitative detail as could be expected for the difficulty inherent in collecting these data. As a blanket approach to working through how these kinesin molecules potentially function in cytokinesis, the manuscript provides significant new insight and a possible view into why the early phragmosome positioning and later attachment as phragmoplast have been so refractory to genetic analyses. While the biochemically inclined might want more biochemistry, I have no major issues with this manuscript and commend the authors for executing what looks like a very difficult set of experiments to both perform and interpret. The discussion and conclusions stay within the bounds of the data and do not extrapolate too far forward.

We would like to thank the reviewer for his/her kind comments. We have corrected the misspelling.

2nd Editorial Decision 19 June 2018

Thank you for the submission of your revised manuscript to EMBO reports. It has been sent back to former referee 1 and 2 and we have now received the full set of referee reports that is copied below.

As you will see, both referees are very positive about the study and support publication without further revision.

Browsing through the manuscript myself, I noticed a few things that we need before we can proceed with the official acceptance of your study.

- In the Authors Contributions paragraph it appears that the initials of Astrid Gadeyne are AD. Could you please double-check and correct this?

- Please note that et al should be used in the Reference list if there are more than 10 authors, i.e., the first 10 authors are listed followed by et al. Currently, your reference list does not conform to this formatting. You can also download the EMBO reports EndNote template from our Guide to Authors https://drive.google.com/file/d/0BxFM9n2lEE5oOHM4d2xEbmpxN2c/view

- When going through the manuscript we noticed that the following figures are either never mentioned in the text or the order of the figures is reverted.
- Fig 3G & 3H are not called out.
- Fig 6E is called out before 6D.
- Fig 6H is called out before 6D,E or F.
- Appendix Table S2 - callout missing
Could you please add the missing callouts and review Figure 6, if it can be arranged in a way that the order of the panels follows the description in the text?

- Fig EV1D - There is a magnification box in the GFP-POK2, which is not magnified. Only the overlay image shows a magnification. Do you want to highlight this area also in the single channel image?

- Our data editors have already inspected the figure legends for completeness and accuracy. Could you please have a look at the attached file and the editor's suggestions?

- Appendix Figure S1C, D: please specify the number of samples analysed in the Figure legend.

- Please fill section F in the Author Checklist (Data availability).

- Please shorten the title (max. 100 characters incl. spaces)

- Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of

the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (width x height). You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

We look forward to seeing a final version of your manuscript as soon as possible.

REFEREE REPORTS
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Referee #1:

The revised manuscript adds several helpful elements; most notably
- the design of T-DNA constructs at the basis of the functional analysis is clarified in the text and caveats (due to the use of 35S promoter and/or cDNA segments) are stated explicitly; since the authors can make a strong point that all necessary function is retained in their baseline POK2 construct, I don't see a problem there.
- images documenting the localization of a POK2 protein lacking the central domain as well as the localization of POC2 in plejade mutants have been added as additional controls.

The changes adequately address all the comments raised by the reviewers. Nice work, congratulations!

Referee #2:

The authors have addressed my major concerns by revising the manuscript and clarifying any important issues.

2nd Revision - authors' response 21 June 2018

We are very pleased that you consider our manuscript for publication. We have made the requested changes and added the missing information.

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Corresponding Author Name: Sabine Müller
Journal Submitted to: EMBO Reports
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Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Figs 1F, Fig. 4H and 4I: Experience and statistical tests (One Way Anova with Tukey HSD) were performed on trial experiments
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Analysis of the samples in parallel experiments performed by different investigators Fig.1F, Fig4H and I, Fig. S4I
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Figs 1F, Fig. 4H and 4I: Samples were collected by different investigators. Different investigators were involved in imaging to obtain raw data.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	YES (Material and Methods, Figs 1F Fig. 4H and I);
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	One way Anova and Tukey-HSD were performed, Whiskers are indicated in Figure Legends Fig. 1F, Fig 4H,I, Box blots are shown for these experiments indicating the signal distribution
Is there an estimate of variation within each group of data?	Standard deviation Fig. 1F, Fig.4H and I (Material and Methods)
Is the variance similar between the groups that are being statistically compared?	One way Anova with Tukey HSD test p 0.001 Fig. 1F, Fig.4H

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Materials and Methods, Figure legend 7; anti-HA Roche, Cat. 12013819001; anti-VP16 GeneTex, Cat. GTX30776; anti-rabbit-POD Merck-Millipore, Cat. AP307P,
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA