

RASSF1A disrupts the NOTCH signaling axis via SNURF/RNF4-mediated ubiquitination of HES1

Angelos Papaspyropoulos, Andriani Angelopoulou, Ioanna Mourkioti, Aikaterini Polyzou, Daniela Pankova, Konstantinos Toskas, Simone Lanfredini, Anastasia Pantazaki, Nefeli Lagopati, Athanassios Kotsinas, Konstantinos Evangelou, Efstathios Chronopoulos, Eric O'Neill, and Vassilis Gorgoulis

DOI: 10.15252/embr.202051287

Corresponding author(s): Vassilis Gorgoulis (vgorg@med.uoa.gr), Eric O'Neill (eric.oneill@oncology.ox.ac.uk), Angelos Papaspyropoulos (a.papaspyropoulos@med.uoa.gr)

Review Timeline:	Submission Date:	9th Jul 20
	Editorial Decision:	4th Aug 20
	Revision Received:	29th Jun 21
	Editorial Decision:	28th Jul 21
	Revision Received:	22nd Oct 21
	Editorial Decision:	16th Nov 21
	Revision Received:	23rd Nov 21
	Accepted:	26th Nov 21

Editor: Achim Breiling

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND lacksquare

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Vassilis Gorgoulis, Eric O'Neill, Angelos Papaspyropoulos Journal Submitted to: EMBO Reports

Manuscript Number: EMBOR-2020-51287V2-Q

porting 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 x2 tests, Wilcoxon and Mann-Whitney

- tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods
- · 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;

 deficition of (authors plant a profile to the pr
- 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.

n the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itsel ncourage you to include a specific subsection in the methods section for statistics, reagents, animal models and hi

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com

http://1degreebio.org

http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm

http://ClinicalTrials.gov

http://www.consort-statement.org

http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tume

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/

http://ijj.biochem.sun.ac.za https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/ http://www.selectagents.gov/

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	All experiments performed are representative of at least 3 biological replicates
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.	NA .
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.	NA .
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
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Based on the sample size, all the requirements were met in order to safely conduct the indicated statistical tests. Statistical analyses were validated using the GraphPad Prism 6 software.
Is there an estimate of variation within each group of data?	Yes

To travel the antibulate ware yer filted for our mice of patient, color than the property of the control of the color of t		
The contract entropic contract printed to some or the systems control to the contract printed by the c	Is the variance similar between the groups that are being statistically compared?	Yes
The contract entropic contract printed to some or the systems control to the contract printed by the c		
The contract entropic contract printed to some or the systems control to the contract printed by the c		
The contract entropic contract printed to some or the systems control to the contract printed by the c	eagents	
Insulation analote code number, organized preference to the organized preference to an extraction, which preference to the company of the preference to the company of the code of the cod		_
The soft his source of cell lines and report if they were recently authenticated (e.g., by 378 profiling) and tested for procedures accommodate citature for ro large than 25 mores. This life most for commodate and the form to be provided as a commodate citature for ro large than 25 mores. This life most for commodate citature for ro large than 25 mores. This life most for commodate citature for ro large than 25 mores. This life most for commodate citature for ro large than 25 mores. This life most for commodate citature for ro large than 25 mores. This life most for commodate citature for ro large than 25 mores. This life most for commodate citature for row large than 25 mores. This life most for commodate citature for row large than 25 more in the foreign and the course of commodate citature for row large than 25 mores. This life most foreign and the course of commodate citature for row large than 25 more in the foreign and the course of commodate citature for row large than 25 more in the foreign and the course of commodate citature for commodate citature for foreign and the course of commodate citature for commodate citature for foreign and commodate citature for commodate	number and/or clone number, supplementary information or reference to an antibody validation profile. e.g.,	15404 and sc-101199), HES1 (AB5702), GAPDH (Abcam;2251-1), TAZ (sc-48805 and Ab118373), FLAG (M2; Agilent; 200472-21), RASSF1A (sc-58470), NANOG (Cell Signaling; 4893S), OCT4 (ab19857), SOX2 (MAB4423), LAMIN B1 (Ab16048), RNF4 (Novus Biologicals), TEAD1 (BD Transduction Laboratories; 610922), p73 (EP4367), Ub (Cell Signaling; 3936), HA (Cell Signaling; 3724S), ICN1 (Cell Signaling; 4971), IgG (Cell Signaling; 49725), HRP conjugated anti-mouse (Jackson Immunoresearch; 315-035-048) and anti-rabbit (Jackson Immunoresearch; 711-035-152) secondary antibodies and Alexa secondary antibodies (Invitrogen; A-21422; A-11005; A-11012; A-11001; A-31576; A-11008). This information can be found in the Materials and Methods, Co-immunoprecipitation, immunoblotting, immunofluorescence and
Claricity and wave maintained in culture for on brought that 2 months. This information can be pound in the Materians and Mechadic, call fore and Response section. **To all hyperidak, prises see the stable at the top rigid of the document **Animal Models** For appartments invalving the control of the document For appartments invalving the control of the document of the document of the Materials and Mechadic control of the document of the Materials and Mechadic control of the Mechadic control o	7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for	
To all hyporholis, please we the table at the tilty right of the document **Unitarial Models **Be Roort species, strong gender, age of animals and genetic modification ablas where applicable. Please detail housing **In Planta Species** **Be Roort species, strong gender, age of animals.** **Be Roort species, strong gent species, strong gender, age of animals.** **Be Roort species, strong gender, age of animals.** **Be Roort species, strong gent spe	mycoplasma contamination.	Clontech, and were maintained in culture for no longer than 2 months. This information can be
B. Roport species, strain, geoder, age of animals and genetic modification status where applicable. Please detail housing conditions and the source of animals. 5. For experiments involving the ventebraces, include a statement of compliance with ethical regulations and identify the Committeetic approving the optimients. 15. Van accommod channeling and ARPER guidelines, ion in with a training and the committeetic approving the optimients. 16. Van accommod channeling and ARPER guidelines ion in with a training and the committeetic approving the part of the committeetic approving the study produces. 16. Van accommod channeling and ARPER guidelines ion in with a training and the committeetic approving the study produces. 17. Include a statement confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained and the Department of Health and Funna Provincial Selection of guident photos, include a statement confirming that consent to publish was obtained. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration to the line of the new of human data or samples. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where containing the line of the line o	* for all hyperlinks, please see the table at the top right of the document	
B. Roport species, strain, geoder, age of animals and genetic modification status where applicable. Please detail housing conditions and the source of animals. 5. For experiments involving the ventebraces, include a statement of compliance with ethical regulations and identify the Committeetic approving the optimients. 15. Van accommod channeling and ARPER guidelines, ion in with a training and the committeetic approving the optimients. 16. Van accommod channeling and ARPER guidelines ion in with a training and the committeetic approving the part of the committeetic approving the study produces. 16. Van accommod channeling and ARPER guidelines ion in with a training and the committeetic approving the study produces. 17. Include a statement confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained and the Department of Health and Funna Provincial Selection of guident photos, include a statement confirming that consent to publish was obtained. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where applicable. 18. Report the clinical train registration to the line of the new of human data or samples. 18. Report the clinical train registration number (all ClinicalTrinity) or or equivalently, where containing the line of the line o	unimal Models	
and budshardy concloses and the source of animals. 3. For experiments involving the experiments. 10. We recommend consulting the experiments. 10. We recommend consulting the ARRIVE guidelines (see link list at too right) (PLoS Blok Bligk, e200012, 2003) to essuare MA. 10. We recommend consulting the ARRIVE guidelines (see link list at too right) (PLoS Blok Bligk, e200012, 2003) to essuare MA. 10. When recommend consulting the ARRIVE guidelines (see link list at too right) (PLoS Blok Bligk, e200012, 2003) to essuare MA. 10. When recommend consulting the ARRIVE guidelines (see link list at too right) (PLoS Blok Bligk, e200012, 2003) to essuare MA. 11. Services as the link list in the too to pright and MAC (see link list at too pright) (PLoS Blok Bligk, e200012, 2003) to essuare MA. 12. Includes, statement confirming that informed consent was disalsed from all subjects and that the experiments of recommendations of the commendations of		
10. We recommend consulting the ARRIVE guideline; (see Intel list at too rigin) (Plud Silot, IRE), e1000112, 2010) to ensure IN to this review aspects of animal studes are adequately reported. See author guidelines, under "Apporting Guidelines," See Soit Will cere list list at too rigit) or anomaly guidelines. See Soit Will cere list list at too rigit) or anomaly guidelines, under "Apporting Guidelines," See Soit Will cere list list at too rigit) or anomaly guidelines. We are adequated to the state of the s		NA .
10. We recommend consulting the ARRW guidelines (or link list as too right) Profit dist, 46(6, at 200612, 2010) to ensure that other relevant appects of arms and studies are extensively reported, See author guidelines, under Paporting Guidelines, see also, 1914 (see link list at too right) and MRC (see link list at too right) are commendations. Rease confirm compliance. **Luman Subjects*** **Luman Subjects*** **Luman Subjects*** **Luman Subjects*** **Luman Subjects*** **Link at attement confirming that informed consent was obtained from all subjects and that the experiments confirming that informed consent was obtained and the Department of Iterath and Luman Services Belmont Report. **Link at attement confirming that informed consent was obtained and the Department of Iterath and Luman Services Belmont Report. **Link at attement confirming that informed consent was obtained and the Department of Iterath and Luman Services Belmont Report.** **Link at a section of patient photos, include a statement confirming that consent to publish was obtained.** **Link at a section of patient photos, include a statement confirming that consent to publish was obtained.** **Link at a section of the availability (and/or on the use) of human data or samples.** **Link at a confirming that information number (at Clinical Trials, gov or equivalent, where applicable.** **Link at a confirming that information number (at Clinical Trials, gov or equivalent, where applicable.** **Link at a confirming that information number (at Clinical Trials, gov or equivalent, where applicable.** **Link at a confirming that information number (at Clinical Trials, gov or equivalent, where applicable.** **Link at a confirming that information number (at Clinical Trials, gov or equivalent, where applicable.** **Link at a confirming that and its analysis to the pu		NA .
that other relevant aspects of animal studies are adequately reported. See author guidelines, under "Reporting Guidelines". See but NIII (bee link list at top right) and MRC (bee link list at top right) recommendations. Please confirm compliance. 11. Identify the committee(s) approximg the study protocol. 12. Include a statement confirming that informed consent was addulated from all subjects and that the apparent conformation of the principles set out in the WMA Declaration of Heisina's and the Department of Health and Human Services Belmont Report. 13. Tor publication of patient photos, include a statement confirming that consent to publish was obtained. 14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at ClinicalTrials gov or equivalent), where applicable. 16. For phase I and Ill stationated controlled trials, greate refer to the CONSORT flew diagram (one link bit at laying). 17. For humor marker progressis studies, we recommend that you follow the REMARK reporting guidelines, under "Reporting Guidelines". Please confirm you have followed these guidelines. 18. Provise or "Ose a Availability" section at the end of the Materials & Methods has been added generated in this study and deposited in a public clashase (e.g. RNA-deplate followed these guidelines. 18. Provise or "Ose a Availability" section at the end of the Materials & Methods has been added generated in this study and deposited in a public clashase (e.g. RNA-deplate) reporting Guidelines. Provision of datases for the public pub	commutee(s) approving the experiments.	
that other relevant aspects of animal studies are adequately reported. See author guidelines, under "Reporting Guidelines". See but NIII (bee link list at top right) and MRC (bee link list at top right) recommendations. Please confirm compliance. 11. Identify the committee(s) approximg the study protocol. 12. Include a statement confirming that informed consent was addulated from all subjects and that the apparent conformation of the principles set out in the WMA Declaration of Heisina's and the Department of Health and Human Services Belmont Report. 13. Tor publication of patient photos, include a statement confirming that consent to publish was obtained. 14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at ClinicalTrials gov or equivalent), where applicable. 16. For phase I and Ill stationated controlled trials, greate refer to the CONSORT flew diagram (one link bit at laying). 17. For humor marker progressis studies, we recommend that you follow the REMARK reporting guidelines, under "Reporting Guidelines". Please confirm you have followed these guidelines. 18. Provise or "Ose a Availability" section at the end of the Materials & Methods has been added generated in this study and deposited in a public clashase (e.g. RNA-deplate followed these guidelines. 18. Provise or "Ose a Availability" section at the end of the Materials & Methods has been added generated in this study and deposited in a public clashase (e.g. RNA-deplate) reporting Guidelines. Provision of datases for the public pub		
Goodeline'. See also thit (pee link list at top right) and MRC (pee link list at top right) recommendations. Please confirm (compliance.) **Ituman Subjects** 11. Identify the committee(s) approving the study protocol. 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 Isuman Services Bellions the Sport. 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. 14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at ClinicalTrials gor or equivalent), where applicable. 16. For phase ill and ill randomized controlled trials, glease refer to the CDNSORT flow diagram (see link list at top right) and submit the CDNSORT flow diagram (see link list at top right) and submit the CDNSORT flow diagram (see link list at top right) and submit the CDNSORT flow diagram (see link list at top right) and submit the CDNSORT flow diagram (see link list at top right). Sees confirm you have flowed the REMARK reporting guidelines, under Reporting Guidelines. Please confirm you have followed these guidelines. Judicelines. Please confirmed to Please Please confirmed to Please Please Please confirmed to Please		NA NA
turnan Subjects 11. Identify the committee(s) approving the study protocol. 12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles are out in the WMA Declaration of Helsinki and the Department of Health and Numan Services Belmont Report. 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. 14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at Clinical Trials goor or equivalent), where applicable. 16. For phase III and III randomized controlled trials, please refer to the CONSORT flow diagram (see link III at top right) and submit the CONSORT flow is given like III at top right). NA 16. For phase III and III randomized controlled trials, please refer to the CONSORT flow diagram (see link III at top right). NA 17. For turn or marker prognotics clinic, like III it at top right). See author guidelines. Please confirm you have submitted this III. 17. For turn or marker prognotics clinic, where referent that you follow the REMARK reporting guidelines (see IIIR III at a top right). See author guidelines, under Reporting Guidelines. Please confirm you have followed these guidelines. If the author guidelines, under Reporting Guidelines. Please confirm you have followed these guidelines. 18. Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in his subyl and disposited in a public database (e.g., RNA-See datas. Gene Expression Omnibus GSS39462, Proteomics data. PRIDE PRODUZOSE etc.) Please refer to our author guidelines for 'Data Deposition'. 18. Provide a "Data Availability" section at the end of the Materials & Methods has been added. Provides a "Data Availability section at the end of the Materials & Methods has been added. Provides a "Data Deposition" in a public database (e.g., RNA-See datas. Gene Expre	Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm	
11. Identify the committee(s) approxing the study protocol. 12. Nelvolute a statement confirming that informed consent was obtained from all subjects and that the opportments of conformed to the privileges set out in the WMAD Declaration of Heldinal and the Department of Health and Human Services Belmont Report. 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. 14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. 16. For phase II and III randomized controlled trials, please refer to the CONORT flow diagram (see link list at too right). 17. For tumor marker prognosis Leidel, we recommend that you follow the REMARK reporting guidelines, under "Reporting Guidelines," Please confirm you have submitted this list. 17. For tumor marker prognosis Leidel, we recommend that you follow the REMARK reporting guidelines (see link list at too right). See author guidelines, under "Reporting Guidelines," Please confirm you have submitted this list. 18. Provide a "Quals Availability section at the end of the Materials & Methods, listing the accession codes for data generated in his study and deposition in a public repolation, in a public repolation in the public repolation in the public repolation in the	compliance.	
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 Helains and the Department of Health and Human Services Belmont Report. 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. 14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at ClinicalTrials gov or equivalent), where applicable. 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 flowding (see link list at top right) with your submission. See author guidelines, under Reporting Guidelines, See author guidelines, under Reporting Guidelines,	uman Subjects	
conformed to the principles set out in the WMAN Declaration of Helsinki and the Department of Health and Human Services Belmont Report. 33. For publication of patient photos, include a statement confirming that consent to publish was obtained. 34. Report any restrictions on the availability (and/or on the use) of human data or samples. 35. Report the clinical trial registration number (at ClinicalTrials gov or equivalent), where applicable. 36. For ghaze I and III randomized controlled trials, please refer to the CONSORT flow diagram (use limit list at top right) and submit in CONSORT flow diagram (use limit list at top right) and submit in CONSORT flow diagram (use limit list at top right). What is advantaged to the CONSORT flow diagram (use limit list at top right) and submit in CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the consensation of the CONSORT flow diagram (use limit list at top right). What is to pright is on a submit of the consensation of the CONSORT flow of the CONSORT	11. Identify the committee(s) approving the study protocol.	NA NA
conformed to the principles set out in the WMAN Declaration of Helsinki and the Department of Health and Human Services Belmont Report. 33. For publication of patient photos, include a statement confirming that consent to publish was obtained. 34. Report any restrictions on the availability (and/or on the use) of human data or samples. 35. Report the clinical trial registration number (at ClinicalTrials gov or equivalent), where applicable. 36. For ghaze I and III randomized controlled trials, please refer to the CONSORT flow diagram (use limit list at top right) and submit in CONSORT flow diagram (use limit list at top right) and submit in CONSORT flow diagram (use limit list at top right). What is advantaged to the CONSORT flow diagram (use limit list at top right) and submit in CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the CONSORT flow diagram (use limit list at top right). What is a diagram of the consensation of the CONSORT flow diagram (use limit list at top right). What is to pright is on a submit of the consensation of the CONSORT flow of the CONSORT		
14. Report any restrictions on the availability (and/or on the use) of human data or samples. 15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. 16. For phase if and ill candomized controlled talis, 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. 17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines, under "Reporting guidelines, under "Repor	conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human	NA .
25. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. 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 checkist (see link list at top right) with your submission. See author guidelines, under "Reporting Guidelines". Please confirm you have submitted this list. 17. For turnor 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. 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 PRODO2028 ect.) Please refer to our author guidelines for "Data Deposition". 19. 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 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 "Expression of the study; please consider the journal's data policy, if no structured large in the study of the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under "Expression of the study please consider the journal's data policy, if no structured large in submit and the provision of datasets in the manuscript as a supplementary Document (see author guidelines under "Expression of the major public access-controlled repositories such as dbsAP (see link list	13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	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. 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 (see link list at top right). See author guidelines, under Reporting Guidelines'. Please confirm you have followed these guidelines (see link list at top right). 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 and molecular interactions 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 Documents are provided. 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 Documents are provided. 19. Deposition is strongly recommended for any datasets that are central and integral to a strongly or in unstructured repositories such as Oryal Deposition or in unstructu	14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA 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. 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 (see link list at top right). See author guidelines, under Reporting Guidelines'. Please confirm you have followed these guidelines (see link list at top right). 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 and molecular interactions 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 Documents are provided. 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 Documents are provided. 19. Deposition is strongly recommended for any datasets that are central and integral to a strongly or in unstructured repositories such as Oryal Deposition or in unstructu		
and submit the CONSONT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines', Please confirm you have submitted this list. 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. 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 PXD0002028 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. Crystalligraphic data for small molecules d. Functional genomics data molecular interactions 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) and the position of the major public access-controlled repositories and should be deposited in one of the major public accession numbers or links should be provided with as few restrictions as possible while respective the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as Object (see link list at top right) or GEA (see link list at top right). 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	15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA NA
12. 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. 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 molecular interactions 19. Peposition 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 Dryal (see link list at top right) or Fighare (see link list at top right). 20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting etchal obligations to the patients and relevant medical and legal sisues. 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 dDAGA (see link list at top right). 21. Computational models that are central and integral to a study should be shared without restrictions and provided in an amachine-readable form. The relavant accession numbers or links should be provided. When possible in this at top right). 21. Computational models that are central and integral to a study should be shared without restrictions and provided in an amachine-re	and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting	
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 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) or Figshare (see link list at top right) or Eigshare (see link list at top right) and deposit derived or link should be deposited in one of the major public access- controlled repositories such as doEAP (see link list at top right) or Eigshare (see link list at top right) or Eigsha	,	NA NA
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 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). 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). 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, sandardized 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). If computer source code is provided with the paper, it should be deposited	top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	
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 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). 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). 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, sandardized 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). If computer source code is provided with the paper, it should be deposited	ata Accessibility	
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 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) or EGA (see link list at top right) or EGA (see link list at top right) or IVS Online (see link list at top right) or IVS Online (see link list at top right) for Ome of the major ded. With the propersion of the Michael of the provided of the study, such data 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) if computer source code is provided with the paper, it should be deposited in a public database such as Blomodels (see link list at top right) if computer source code is provided with the paper, it should be deposited in the provided with the paper, it should be deposited in the paper.		A Data Availability section at the end of the Materials C Mastered has been added
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 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 Dyrad (see link list at top right) or Figshare (see link list at top right). 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) or EGA (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or IWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in	generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462,	A Data Availability section at the end of the Materials & Methods has been added.
a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions 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 Dyrad (see link list at top right) or Figshare (see link list at top right) or INF and the 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). 21. Computational models that are central and integral to the study, ycle accession of datasets in the manuscript as a Supplementary Documents are provided.		
c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions 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). 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 FGA (see link list at top right) or FGA (see link list at top right) or SGA (see link list at top right) and amachine-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) in Computer source code is provided with the paper, it should be deposited	a. Protein, DNA and RNA sequences	
e. Proteomics and molecular interactions 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). 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) or EGA (see link list at top right) or Hongard to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. NA machine-readable form. The relevant accession numbers or links should be provided. NA machine-readable form. The relevant accession numbers or links should be growided. NA machine-readable form. The relevant accession numbers or links should be deposited in a machine-readable form. The relevant accession numbers or links should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) or IWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited		
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). 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) or EGA (see link list at top right) or IWS Online (see link list at top right), a thors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right). If computer source code is provided with the paper, it should be deposited		
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). 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) at top right). 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) at top right). If computer source code is provided with the paper, it should be deposited		Supplementary Documents are provided.
repositories such as Dryad (see link list at top right) or Figshare (see link list at top right). 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) or FGA (see link list at top right) or IWA of link list at controlled repositories such as dbGAP (see link list at top right) or IWA of link should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) or IWA Online (see link list at top right). If computer source code is provided with the paper, it should be deposited		
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) or EGA (see link list at top right). 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 MIRIAMM 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	repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	
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) and seposit their model in a public database such as Biomodels (see link list at top right). If computer source code is provided with the paper, it should be deposited	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-	NA .
(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	21. Computational models that are central and integral to a study should be shared without restrictions and provided in a	
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		
	guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top	
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.