EMBO reports

Prostate cancer cell-intrinsic interferon signaling regulates dormancy and metastatic outgrowth in bone

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Editor: Achim Breiling

Transaction Report: Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at EMBO Reports. Since the original reviews are not subject to EMBO Press' transparent review process policy, the reports and author response cannot be published.

(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 6 March 2020

Thank you for the submission of your research manuscript to EMBO reports. We have now received the reports from the two referees that were asked to re-evaluate your study, which can be found at the end of this email. Both have assessed your manuscript before at a journal not belonging to EMBO press.

As you will see, both referees now support the publication of your revised study in EMBO reports. Nevertheless, referee #1 has remaining concerns and suggestions to improve the manuscript, I ask you to address in a final revised version of the manuscript. Please also provide a detailed point-by-point response that addresses these remaining points of the referee.

When submitting your revised manuscript, please also carefully review the instructions that follow below.

PLEASE NOTE THAT upon resubmission revised manuscripts are subjected to an initial quality control prior to exposition to re-review. Upon failure in the initial quality control, the manuscripts are sent back to the authors, which may lead to delays. Frequent reasons for such a failure are the lack of the data availability section (please see below) and the presence of statistics based on n=2 (the authors are then asked to present scatter plots or provide more data points).

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that the changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

Please order the manuscript sections like this:
Title page - Abstract - Introduction - Results - Discussion - Materials and Methods - DAS - Acknowledgements - Author contributions - Conflict of interest - References - Figure legends - Expanded View Figure legends

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

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 file labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

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3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq and array data) are deposited in an appropriate public database.

See also: http://embor.embopress.org/authorguide#datadeposition

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (DAS - placed after Materials & Methods) that follows the model below. Please note that the Data Availability Section is restricted to new primary data that are part of this study. This is now mandatory (like the COI statement). If no primary datasets have been deposited in any database, please state this in this section (e.g. 'No primary datasets have been generated and deposited').

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843
(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

6) Please restrict the number of keywords to 5.

7) Please provide the abstract written in present tense.

8) We 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. If you want to provide source data, please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

9) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at:
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10) Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable, and also add a paragraph detailing this to the methods section. See:
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11) Please check that all microscopic images have scale bars. Please refrain from writing the size on or near the scale bars. Please add the size information to the respective figure legend.

12) It seems Stefano Mangiola, John Mariadason, Tri Gian Phan, Alexander Swarbrick, Niall Corcoran and Paul Herzog are presently missing from the author contributions. Please add their contributions. Is DJZ and DZ the same person?

13) - Please format the references according to our journal style. We need the 10 first authors, and if there are more 'et al.'.
See: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

14) Please enter the complete funding information also into our submission system (including grant numbers). Please check that in the online form and the manuscript the funding information is the same and complete.

15) It seems several panels are duplicated (show up again in the supplemental figures). Some bone images in Fig. 4E are also displayed in Fig. S4F; cell images in 4K are also shown in Fig. S4J; mouse and bone images in Fig. 6C are also displayed in Fig. S5E. Please mentions this in the respective figure legends, or replace the duplicated images with replicate images.

16) As I understand Fig. 7E shows enlargements of the images shown in S7C. Please clearly state this in the legends of these figures, and also indicate (using boxes) the areas in S7C that are shown enlarged in 7E.

Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see the modifications done.

In addition I would need from you:
- a short, two-sentence summary of the manuscript
- two to three bullet points highlighting the key findings of your study
- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

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

REFEREE REPORTS

Referee #1:

The study reported the loss of tumor-intrinsic IFN signaling compromised immune recognition, thereby increasing bone metastatic outgrowth; HDACi restored IFN signaling and inhibited bone metastasis. This work is exciting and may inform the role of IFN in bone (or other) metastases of other cancers in addition to the prostate. The reduction in IFN signaling in dormant prostate cancer cells are evaluated in both animal models and in clinical cohorts. The manuscript has improved since last submission, I would support publication when the following concerns are addressed.

Major concern:
Regarding the clinical translational relevance, it remains difficult to conclude that IRGs is downregulated specifically in bone metastasis or in metastasis in general (i.e. soft tissue metastases), by simply comparing primary vs. bone metastases. While it may be valid that the low IRGs are specific to bone metastasis and this bone metastasis may seed other soft tissue metastases, it is important to assess soft vs. bone, especially in larger cohorts of metastases, to determine whether the IRG effect is specific to bone or potentially shared across all metastases (which may have a higher impact).

Minor concerns:
1. Line 57-58: '... dormancy, a state in which cancer cells persist undetected prior to overt lesion formation.' Dormancy refers to a state that cells remain inactive in proliferation, however prior reports have shown that the cells were readily detected e.g. using surface marker EpCAM staining. Suggest to remove the word 'undetected'.
2. The identification of two sub-populations (C1 and C2) of 'dormant' tumor cells in the revised manuscript is biologically important, suggesting niche-specific or disease-state specific cell populations that are susceptible to dormancy escape than the others. Similar findings have been reported in prostate cancer (PMID: 25301725) which warrants discussion.
3. Fig 1f: while it is informative to present key DE IRGs between dormant and proliferating cells, the enrichment of these genes in dormant cells are not evident in the current presentation. May consider ranking the genes from the largest to lowest DE between dormant vs proliferating cells. Statistical analysis e.g. p-value will help readers to determine the actual possibility of difference between dormant vs proliferating cells.
4. Fig. 2e: It is unclear how statistical significance can be achieved by comparing 1 bone lesion to 1 lung lesion.
5. It will be helpful to address (i) limitations of this study e.g. technical limitation precluding direct assessment on bone TME for immune population and using one prostate cancer line for the study, and (ii) potentially important work related to the clinical translational relevance e.g. HDACi effect on the bone stroma that produces a high level of IFN and systemic immune response detected in

blood by HDACi.

Referee #2:

The authors have satisfactorily addressed all my comments. I have no additional comments.

1st Revision - authors' response 15 March 2020

Reviewers comments

Major concern:

Regarding the clinical translational relevance, it remains difficult to conclude that IRGs is downregulated specifically in bone metastasis or in metastasis in general (i.e. soft tissue metastases), by simply comparing primary vs. bone metastases. While it may be valid that the low IRGs are specific to bone metastasis and this bone metastasis may seed other soft tissue metastases, it is important to assess soft vs. bone, especially in larger cohorts of metastases, to determine whether the IRG effect is specific to bone or potentially shared across all metastases (which may have a higher impact).

The specific analysis of soft tissue metastases derived from patients with prostate cancer is difficult due to sample/database availability. Visceral or soft tissue metastases in prostate cancer are described as 'atypical' and the metastatic patterns of prostate adenocarcinoma (rather than those that arise from neuroendocrine origin, associated with more frequent visceral metastases https://www.targetedonc.com/case-based-peer-perspectives/prostate-cancer/george-metastatic-crpc/bone-versus-visceral-metastases-in-mcrpc) have been evidenced by several large studies to arise from bone[1–4], as we have previously pointed out, with the most robust findings generated through the rapid autopsy investigation conducted by Bubendorf et al.[1] on 1,589 patients. Data availability of visceral metastatic tissue samples in castrate-resistant prostate cancer beyond lymph node origin (which occur with concurrent overt bone lesions in over 50% diagnosed cases) is scarce, with the exception of the small yet widely cited, Varambally (array)[4,5] dataset. **We have now probed the Varambally dataset (which includes metastatic sites from bone, liver, lung, undefined soft tissues) with our 8-gene signature through the Oncomine database, which we have added to the current manuscript as EV4B, including related text (lines 660-669, 842-843). Findings revealed that our signature is downregulated in all metastatic samples, compared to primary tumors, suggesting that the impact of IRG loss may extend to other metastatic sites.** It has been noted, however, in a previous Varambally study [4] which described the metastatic tissues, that only 16% of patients had visceral involvement without the presence of overt bone metastases, and, as such, it cannot be ruled out that gene alterations in sites other bone may result from bone seeding secondary metastases—as has also been recently reported as a confounding factor in prostate cancer datasets by Chen et al. [6]. Interestingly, while the pan-cancer TCGA-PRAD dataset (which we have probed in the current study, see Fig 7H) included in the pan-cancer MET500 report[7] is largely restricted to prostate primary tumor analysis (with some primaries inaccurately inferred as 'metastatic' by Robinson et al. (2017), when in actuality tissues sampled were primary tumors from patients *bearing* metastases rather than sampling the actual met site, including the Taylor dataset: GSE21034, and as such, any met data is from the GSE6469 contribution of lymph node metastases) it was reported that in comparison to other cancers, metastases from prostate cancer, were associated with low immunogenicity scores, as has previously reported [3] and which we also reference in the current manuscript.

Minor concerns:

1. Line 57-58: '... dormancy, a state in which cancer cells persist undetected prior to overt lesion formation.' Dormancy refers to a state that cells remain inactive in proliferation, however prior reports have shown that the cells were readily detected e.g. using surface marker EpCAM staining. Suggest to remove the word 'undetected'.

Thank you for your advice. The word undetected has been removed.

2. The identification of two sub-populations (C1 and C2) of 'dormant' tumor cells in the revised manuscript is biologically important, suggesting niche-specific or disease-state specific cell populations that are susceptible to dormancy escape than the others. Similar findings have been reported in prostate cancer (PMID: 25301725) which warrants discussion.

Such evidence has now been incorporated (lines 204-205) along with inclusion of the suggested reference.

3. Fig 1f: while it is informative to present key DE IRGs between dormant and proliferating cells, the enrichment of these genes in dormant cells are not evident in the current presentation. May consider ranking the genes from the largest to lowest DE between dormant vs proliferating cells. Statistical analysis e.g. p-value will help readers to determine the actual possibility of difference between dormant vs proliferating cells.

Genes in Fig 1f were included based solely on ranked criteria. Clarification of this ranking (by ExpLogFC—a test of significance in the BASiCS package) has been made in the figure legend. The figure legend and methods section for single cell analysis also clearly states that all analysis associated with Figure 1 was only performed on genes that were deemed significant statistically different through BASiCS computation.

4. Fig. 2e: It is unclear how statistical significance can be achieved by comparing 1 bone lesion to 1 lung lesion.

Significance values associated with Fig 2E, which were specifically comparing bone to the parental line (as noted in the figure legend) have now been removed. The heatmap now only shows the mean normalized voom of each sample (lung and bone met from a single host), with the *n* outlined for full transparency as a *preliminary* finding, as reported in all versions of the manuscript.

5. It will be helpful to address (i) limitations of this study e.g. technical limitation precluding direct assessment on bone TME for immune population and using one prostate cancer line for the study, and (ii) potentially important work related to the clinical translational relevance e.g. HDACi effect on the bone stroma that produces a high level of IFN and systemic immune response detected in blood by HDACi.

Several limitations have been addressed in the current study, which have now been highlighted throughout the discussion, including the use of a single model, which we have now expanded upon at the reviewer's request (see line 697). In regard to the impact of HDACi on stromal cells, we have indicated that that further studies need to be conducted and have now included an additional passage on what further studies might entail (lines 816-820). However, as reported in the manuscript, our HDACi alone did not actually impact systemic immune activation status (Fig 6A and B; as also discussed in manuscript lines 812-814 and also reported by others, as also included), with the exception of promoting a low-moderate specific T cell memory response as single agent (Fig 6D). It is in the combination setting with a systemic IFN inducer that we see the robust benefit of using a HDACi. As such, the benefit of using a HDACi alone appears to rest largely on releasing the brakes of tumour cell IFN signaling suppression in order to boost tumour cell immunogenicity, rather than inducing global increases in IFN signaling at the concentrations we have used.

1. Bubendorf L, Schöpfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC, Mihatsch MJ (2000) Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. *Hum Pathol* **31**: 578–583.
2. Thysell E, Vidman L, Ylitalo EB, Jernberg E, Crnalic S, Iglesias-Gato D, Flores-Morales A, Stattin P, Egevad L, Widmark A, et al. (2019) Gene expression profiles define molecular subtypes of prostate cancer bone metastases with different outcomes and morphology traceable back to the primary tumor. *Mol Oncol* **13**: 1763–1777.
3. Ylitalo EB, Thysell E, Jernberg E, Lundholm M, Crnalic S, Egevad L, Stattin P, Widmark A, Bergh A, Wikstrom P (2017) Subgroups of Castration-resistant Prostate Cancer Bone Metastases

Defined Through an Inverse Relationship Between Androgen Receptor Activity and Immune Response. *Eur Urol* **71**: 776–787.
4. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, MacVicar GR, Varambally S, Harwood J, Bismar TA, et al. (2004) Androgen-independent prostate cancer is a heterogeneous group of diseases: Lessons from a rapid autopsy program. *Cancer Res* **64**: 9209–9216.
5. Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, Shah RB, Chandran U, Monzon FA, Becich MJ, et al. (2005) Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* **8**: 393–406.
6. Chen F, Zhang Y, Varambally S, Creighton CJ (2019) Molecular correlates of metastasis by systematic pan-cancer analysis across the cancer genome atlas. *Mol Cancer Res* **17**: 476–487.
7. Robinson DR, Wu Y-M, Lonigro RJ, Vats P, Cobain E, Everett J, Cao X, Rabban E, Kumar-Sinha C, Raymond V, et al. (2017) Integrative clinical genomics of metastatic cancer. *Nature* **548**: 297–303.

Accepted 20 March 2020

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

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

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Corresponding Author Name: Belinda S Parker
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2020-50162V1

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 $\chi 2$ 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)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Based on previous data in the laboratory utilising the RM model of prostate cancer for metastasis assays, at least 5 mice per group are required to order to detect a minimum difference of 30% metastatic burden (via bioluminescence , PCR and survival time) between 2 groups. For qRT-PCR analysis of gene expression between cell lines and Flow Cytometry measurement of immune markers, 3 biological replicates are sufficient due to the consistency of relative tumor abundance and Mean Fluorescence Intensity between replicates. The single cell RNAseq analysis was conducted on the maximum number of cells yielded and numbers were sufficient due to the low heterogeneity within groups.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	As above, based on the intracardiac route of injection in the RM model, we have calculated that a minimum of 5 mice per group is required. This is due to the parental/control intracardiac RM1 model of metastasis reproducibly yielding bone metastasis within a predicted time frame, reducing variability in the control group .
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For survival assays, all animals that developed full or partial paralysis directly associated with bone metastases (as confirmed via bioluminescent imaging) were included as a "death". All animals that developed tumors in the heart (where tumour burden was merely the byproduct of intracardiac injection) with no metastasis evident were excluded from analysis. Animals will illness or injury independent of experimental procedures were excluded from analysis. All survival inclusion/exclusion criteria were established prior to experiment commencement.
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.	Treatment groups for tumor-bearing animal experiments were assigned randomly by a blind, independent investigator who selected cages arbitrarily in order to prevent any bias that may be associated with prior inoculation (such as order of animal injection/length of time from cell culture to inoculation).
For animal studies, include a statement about randomization even if no randomization was used.	Where possible, randomisation was used for all animal inoculation and treatment assignments to minimize the effect of subjective bias.
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.	For animals receiving different tumor cell lines, mice were inoculated alternately to prevent bias associated with one cell line sitting in PBS longer than the other, which may impact tumor cell take in vivo. For endpoint imaging, termination and/or animal group inclusion decisions, more than one investigator (and, additionally, animal facility technicians who were blinded to groups and with no vested interest) was involved to prevent selective bias. For ex vivo two-photon experiments, imaging and analysis of tumour-bearing bones was performed by a blind investigator. All groups were age matched.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Where possible, treatment was assigned blindly by an independent investigator to minimize the effect of subjective bias. This was also the case for metastatic-endpoint survival studies where animal facility technicians, who were blinded to treatment groups, alerted the lead researcher of signs of metastasis, which were then confirmed by bioluminescence.
5. For every figure, are statistical tests justified as appropriate?	All statistical tests are described for every figure and are deemed appropriate for the experiments in question.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	All data meets the assumptions of the tests employed, as determined through appropriate analysis programs (ie. Prism, edgeR).
Is there an estimate of variation within each group of data?	All statistical tests include a measure of variance, as described for each relevent figure (mostly SEM or residual dispersion tests for single cell data).

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Is the variance similar between the groups that are being statistically compared?	All data points are shown individually for >95% of experiments for transparency in regard to data spread, and largely illustrate that variance comparable between groups.

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).	CFP/GFP (ab6556; abcam), donkey anti-rabbit HRP (AP182P; Chemicon), IRF9 (ab51639; abcam), RMMA-1 (22100-1; PBL), rabbit polyclonal mouse IFN-α (32100-1; PBL). CD8a-PE-Cy7 (53-6.7), CD4-APC-Cy7 (GK1.5), CD69-APC (H1.2F3), CD44-FITC (IM7), CD62L-BV421 (MEL-14), PD-1-PE (J43), NK1.1-BV421 (PK136), FOXP3-FITC (MF23), TNF-a-FITC (MP6-XT22), IFN-g PE (XMG 1.1) (all from BD Biosciences) and NKg2D-PE-Cy7 (CX5) (eBioscience). CD11b-BV421/BV605 (M1/70), CD3-PE (17A2), TCR-b FITC (H57-597), Ly6G-PE (1A8; all BD Biosciences), CD11c-PercP (N418; Biolegend), Ly6C-APC (HK1.4; eBioscience), F4/80-PeCy7 (BM8; Invitrogen). H2-Kb-PE (AF6-88.5; BD
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Parental RM1 cells were developed and sourced by Timothy Thompson (The Urology Research Laboratory, Houston and Baylor College of Medicine, Houston, TX, USA). All cell lines used in the current study were reported as mycoplasma negative by VIDRL (The Peter Doherty Institute for Infection and Immunity, Melbourne, 3000, Australia).

* 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.	Male C57BL/6 mice (~8 weeks) were obtained from the Walter and Eliza Hall Institute of Medical Research (Melbourne, VIC, Australia). C57BL/6 Ifnar-/- mice were bred inhouse as described in Bidwell et al., (2012) and Rautela et al., CIR, 2015. All experimental mice were age matched. Experimental mice were housed under PC2 conditions at the La Trobe Animal Research and Teaching Facility, with 3-4 mice per cage.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All mouse experiments were approved by the La Trobe Animal Experimentation Ethics Committee and conducted in accordance.
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.	All aspects of animal studies meet the compliance criteria of the ARRIVE guidelines, including study design, the recording of procedures, outcomes and analysis.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Epworth Clinical Cohort patient samples were obtained and processed with consent and institutional ethics approval from the Royal Melbourne Hospital and Epworth Hospital Human Ethics Committees. Primary and bone metastases prostate cancer specimes were obtained with written and informed consent through (a) the Australian Prostate Cancer BioResource with approvals from the St Andrew's Hospital and University of Adelaide Human Research Ethics Committees and (b) the CASCADE project (11/102) from the Peter MacCallum Cancer Centre Human Research Ethics Committees. St Vincent's-Garvan Clinical Cohort biospecimens were obtained in consent and approval by the St Vincent's Human Research Ethics Committee (SVH
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.	We declare that informed consent was obtained from all subjects involved in the current study and all experiments conformed to the principles set out in the WMA Declaration of Helsinki 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.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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.	N/A
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.	N/A

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	RNAseq datasets pertaining to Figure 1 and 2 have been deposited on GEO with the relevant codes provided in the Data Availability section.
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).	Key large datasets have been provided as source matieral to the journal.
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).	Human datasets will be deposited to GEO upon subsequent publication of additional manuscript (within approximately 18 months of current manuscript acceptance).
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.	N/A

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.	N/A