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The DEAD box RNA helicase DDX42 is an intrinsic inhibitor of positive-strand RNA viruses

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1st Editorial Decision **18th Oct 2021**

Dear Dr. Goujon,

Thank you for the submission of your research manuscript to EMBO reports. We have now received the reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, the referees think that these findings are of interest. However, they have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and all their points need to be addressed, I will not detail them here. However, considering the timely topic, more emphasize should be put on SARS-CoC-2 (see the comments of referee #3). Moreover, we would need some molecular insight how DDX42 exerts the function described.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript or in the detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision. Please contact me to discuss the revision should you need additional time.

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 changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

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.

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Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines:
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5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

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 (placed after Materials & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

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) 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.

7) 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: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

9) Please also note our reference format:
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10) Please add up to five key words to the title page.

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

Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

The DEAD box RNA helicase DDX42 is an intrinsic inhibitor of positive-strand RNA viruses
Using a CRISPR-based genome wide screen, the authors identify DDX42 as an interferon-independent antiviral factor against HIV-1. The authors validate the antiviral phenotype of DDX42 against HIV-1 in different cell lines and primary cells. DDX42 also appears to be antiviral against a range of retroviruses. Their data suggests that DDX42 may act at the level of reverse transcription by direct interaction with viral components. In addition to retroviruses, DDX42 seems to be antiviral against a broad range of positive stranded-viruses, including CHIKV and SARS-CoV-2 but not negative viruses. The authors conclude, that DDX42 is a novel intrinsic antiviral factor against positive-stranded viruses.

One of the main strengths of the manuscript is that authors validate the phenotype of DDX42 in multiple cell lines, with both CRISPR KOs and siRNA KDs, and complement them with overexpression studies. The paper is also strengthened by RNA-immunoprecipitation (RNA-IP) experiments and proximity ligation assays (PLA) showing direct interactions between DDX42 and viral RNA and/or viral components only for viruses sensitive to DDX42. In addition, while there are many non-canonical antiviral factors, there are very few that seem to have such a broad antiviral phenotype.

However, the major weaknesses are described below.

Major issues:
The magnitude of the antiviral effect appears to be quite small as quantified by luciferase assays or flow cytometry. Given that the authors claim that DDX42 may act by directly interacting with viral RNA it would be useful to supplement such data with viral titers and qPCR data. In addition, given that there is a difference between the phenotype of DDX42 against ZIKV in U87-MG (nanoluciferase-expressing construct used) cells versus Huh-7 (wild type virus used) cells it would be revealing to use wild-type viruses as opposed to nanoluciferase-based constructs, whenever possible

The authors suggest an IFN-independent mechanism for DDX42 against HIV, but they only look at the infection when IFN is added or not. A better experiment would be to look at the antiviral phenotype of DDX42 when an IFN inhibitor is used. Moreover, additional studies should address whether the antiviral activity of DDX42 against plus sense viruses is IFN dependent.

Mechanistic insight into how DDX42 is antiviral is somewhat lacking, especially when it comes to the tested positive stranded RNA viruses. While the RNA-IP suggest direct binding between DDX42 and viral RNAs, additional studies are required.

RNA-seq data: There is poor concordance between differentially expressed genes for the different siRNAs and no explanation is provided. There is also no pathway analysis for the genes identified to be differentially expressed for each of the siRNA. Such an analysis should be done not only for the genes that were common between the different siRNAs.

Minor issues:
Literature references: The authors identify only DDX17 as the only other helicase with IFN-independent antiviral activities. However, other helicases such as DDX56 and DDX60 have been shown to have such an IFN-independent antiviral mechanism.

Taschuk, Frances, et al. "DDX56 Binds to Chikungunya Virus RNA To Control Infection." Mbio 11.5 (2020): e02623-20.

Oshiumi, Hiroyuki, et al. "DDX60 is involved in RIG-I-dependent and independent antiviral responses, and its function is attenuated by virus-induced EGFR activation." Cell reports 11.8 (2015): 1193-1207.
In figure 3A, the authors do a time course to assess which step of HIV-1 life cycle is affected by DDX42. However, the relative amounts of DNA are smaller at 24 hours compared to 6 hours or 2 hours and that is confusing.

KO levels of DDX42 do not seem to be complete, the authors suggesting that DDX42 might have a role in proliferation or survival. Analyses of RNA-seq data or proliferation assays could assess whether these claims are appropriate. The alternative is that different sgRNAs should have been used for more efficient KO.

Typos: the authors use either full stop "." comma "," to indicate decimals (Fig. 3G, Fig. 4A-F, S4B) and should use a full stop for all such figures, figure legends and materials and methods.

Missing statistics: none of the knockdown figures (Fig 2C, 3B, S4A) have any associated statistical tests. In addition, figures 3A, 3D, 3F, S2C, S2D do not have any statistics.

Data graphing: Fig. 3D should have the siCTRL plotted so the figure is easier to read. Figures showing knockdown should have the siRNAs specific to DDX42 colored in shades of gray to be consistent with the other graphs in the same figure (Fig 3B, 3E).

Figure legends: none of the figure legends indicate the p-value cutoff values for the various * of significance.

Referee #2:

Bonaventure et al. have identified that DDX42 can restrict positive-strand RNA viruses. They performed a genome-wide screen to identify DDX42 as a new HIV-1 inhibitor. The experiments were performed well but the manuscript is having problems, that need more work and explanations. They discussed the probable mode of action of DDX42 but did not provided any evidence in the manuscript. They say it's a direct mode of action on RNP complexes, but no evidence is provided. Please see below the major concerns.

Major concerns:
1. No attempts are made to understand the mechanism of action of DDX42. I am not looking for extensive evidence, but at least the some insight on how DDX42 might work. Just PLA for the presence of protein in the vicinity is not sufficent, given that the role of DDX42 in +ve RNA viruses is already worked out (please see below).
2. It is difficult to understand the CRISPR screen strategy used. The authors have treated T98G cells with IFNalpha followed VSV-G-pseudotyped HIV-1 lentivirus and selected for infection. There appear problems in this screen, which need explanation and work:
A. T98G is a glioblostoma cell line. What are the levels of ISG response in these cells? If they are highly permissive for HIV, chances are that they are severely compromised for IFN responses. If that's the case, how do you justify its use for such a positive screen? Please provide data and an explanation.
B. Did the author use as control a pseudotyped LV virus that is not HIV-1-based to understand specificity for HIV restriction?
3. Is DDX42 a positive regulator of the IFN response to positive-strand RNA viruses? This is not difficult to find out, given that they have all the required resources and reagents for DDX42.
4. I would like to point that a role of DDX42 in positive RNA viruses (JEV) was shown before (Lin et al 2008, Virus Research). They showed that the NS4A protein of JEV specifically interacts with DDX42 and the expression of N-terminal DDX42 can overcome JEV-induced antagonisms of IFN responses. This is interesting. Such an experiment will be useful also here. They also show that DDX42 can induce IFN responses. Please reference this paper, discuss it and also refer to Carlin et al 2018, PNAS. Thus, it is incorrect to claim in your manuscript that DDX42 functions have not been determined previously. Please check the whole manuscript and correct this claim.

Minor Comments:
1. In Fig. 1C, the authors have show that T98G cells are permissive for HIV 1 Infection. The authors have analyzed the infection efficiency after 30 hours. Did the authors also look at early time points such as 6 hrs or 12 hrs?
2. In Fig. 1C, the authors have shown that KO of two candidate genes WARS2 and DDX42 allowed the partial rescue of HIV 1 Infection from IFN induced inhibition. What was the reason for not choosing WARS2 for this study? Any previous studies done on WARS2?
3. The authors should find the possible reason behind DDX42 impacting positive-strand RNA virus and not having any effect on negative-strand RNA virus. What could be the reason for this specific effect?
4. The knockout and knockdown efficiency of sgRNA and siRNA cannot be correlated from the western blot data in Figure 2A and Figure 2C.
5. Although the authors have shown a possible interaction between DDX42 and virus capsid protein, they have not shown whether DDX42 affects the translation of viral proteins, which should be the ultimate read-out.
6. In Page 3, Line no. 62, Reference is missing.
7. In Fig. 3A, 3D, 3F, 4A, and 4B, the stats are missing.

Referee #3:

Bonaventure et al have done a whole genome CRISPR screen to try to identify IFN-induced genes that inhibit HIV-1 infection. While they did not find any novel ISGs that explain the IFN-effect in the cell line used for the screen, they did identify an interesting hit, DDX42 that has low activity against a wide variety of lentiviruses and positive-strand RNAs viruses with very high antiviral activity against SARS-CoV2. There is an impressive amount of work in this paper. While none of the studies are definitive for a role of DDX42 in controlling natural infections, this paper will likely be a starting point for future studies.

Major Comments:

1. The results of the whole genome screen were possibly disappointing to the authors since one of the top hits, DDX42, has a relatively modest 2-3 fold effect on HIV-1, and also is not an ISG. However, the big story here seems to be the 3-log effect of DDX42 knockout on SARS-CoV-2 infection in Figure 3. I am surprised the authors do not seem to give this more emphasis. Certainly, it would be easier to further investigate mechanism with a 3-log effect than a 3-fold effect.

2. Related to the huge effect seen with DD42 on SARS-CoV-2, can the authors speculate on why this gene has not come up as a bigger hit in other CRSIPR screens against SARS-CoV-2 including the authors' own preprint (https://www.biorxiv.org/content/10.1101/2021.05.19.444823v2). Did I miss it there?

3. I find it hard to interpret the PLA against HIV-1 CA and DDX42 given the lack of specificity for different viruses and the later results where they suggest that DD42 is found in viral RNP complexes. Are the authors suggesting that DDX42 is binding HIV-1 genomic RNA inside the capsid core?

4. One caveat of a 2-3 fold effect on most viruses is the authors' suggestion that knockout/down of DDX42 is detrimental to the cell in line 154. The RNA-seq data suggests that this is not a transcriptional response to DDX42 knockout. However, could it be a stress response that is inducing virus replication? I do not think additional experiments need to be done, but the authors might acknowledge some caution that the effect of DDX42 could be indirect (despite the lack of transcriptional difference and lack of effect on the negative-sense RNA viruses).

Minor comments

5. It is not obvious which infections were done with wtHIV versus which ones are done with a vector system. Please point that out in the figure legends. Was there a difference between entry with VSV-G versus HIV-1 env?

6. Line 89: A glioblastoma cell line is an odd choice for doing an IFN screen for HIV-relevant factors. ISGs show quite a bit of cell-specificity, so one would not necessarily expect that ISGs relevant to HIV would be expressed in this cell type. I am more concerned that readers will feel that this screen is comprehensive, whereas the authors might well have missed important ISGs because they are not expressed in the T98G glioblastoma cell line. Another caveat that could be mentioned is alluded to in line 127 where there is a statement that there is little over-lap between independent screen replicates. This seems to me to suggest that the screen is not comprehensive, and might have missed bonafide ISGs that contribute to the IFN effect against HIV-1.

We thank the reviewers for their time, their careful evaluation of our work and useful suggestions. Please find our answers to their reviews (in blue) below.

Referee #1:

The DEAD box RNA helicase DDX42 is an intrinsic inhibitor of positive-strand RNA viruses
Using a CRISPR-based genome wide screen, the authors identify DDX42 as an interferon-independent antiviral factor against HIV-1. The authors validate the antiviral phenotype of DDX42 against HIV-1 in different cell lines and primary cells. DDX42 also appears to be antiviral against a range of retroviruses. Their data suggests that DDX42 may act at the level of reverse transcription by direct interaction with viral components. In addition to retroviruses, DDX42 seems to be antiviral against a broad range of positive stranded-viruses, including CHIKV and SARS-CoV-2 but not negative viruses. The authors conclude, that DDX42 is a novel intrinsic antiviral factor against positive-stranded viruses.

One of the main strengths of the manuscript is that authors validate the phenotype of DDX42 in multiple cell lines, with both CRISPR KOs and siRNA KDs, and complement them with overexpression studies. The paper is also strengthened by RNA-immunoprecipitation (RNA-IP) experiments and proximity ligation assays (PLA) showing direct interactions between DDX42 and viral RNA and/or viral components only for viruses sensitive to DDX42. In addition, while there are many non-canonical antiviral factors, there are very few that seem to have such a broad antiviral phenotype.

However, the major weaknesses are described below.

Major issues:
The magnitude of the antiviral effect appears to be quite small as quantified by luciferase assays or flow cytometry. Given that the authors claim that DDX42 may act by directly interacting with viral RNA it would be useful to supplement such data with viral titers and qPCR data.

Answer to Reviewer#1 - 1. We have supplemented the data with experiments performed with WT viruses and assessed viral replication by RT-qPCR analysis and viral titers when they were lacking. More specifically:
- For HIV-1 infection: qPCR data performed on the different forms of viral DNA in cells infected with wild-type (WT) HIV-1 were included in the first version of the manuscript (Fig. 3A). We have now added RT-qPCR data to measure the accumulation of WT HIV-1 transcripts 2 days post-infection, and AlphaLisa data to measure viral production (i.e. CA p24 accumulation) in the supernatant **(Fig. EV2B** and **EV2C**, respectively**)**.
- For ZIKV infection: the first version of the manuscript contained experiments performed with reporter virus in U87-MG cells (**Fig. 4D**) and with WT ZIKV in Huh-7 cells (former Fig. S4B, now **EV4B**). We have added now experiments performed in U87-MG cells silenced for DDX42 and infected with WT ZIKV. Viral replication was assessed using RT-qPCR analysis **(Fig. EV4C)**.

Since the effects of DDX42 depletion were modest on ZIKV RNA levels (~2-fold; **Fig. EV4C**), we did not perform viral titrations in the supernatants from these experiments.
- For CHIKV infection: we added data from experiments performed in cells silenced for DDX42 and infected with WT CHIKV. We measured viral RNA abundance in cells with RT-qPCR and assessed viral production by plaque assays **(Fig. EV5A** and **Fig. 4F**, respectively**)**.
- For SARS-CoV-2 infection: the data in the first version of the manuscript showed RT-qPCR analysis of SARS-CoV-2 RNAs as well as viral titration using plaque assays **(Fig. 4G-H).**

In addition, given that there is a difference between the phenotype of DDX42 against ZIKV in U87-MG (nanoluciferase-expressing construct used) cells versus Huh-7 (wild type virus used) cells it would be revealing to use wild-type viruses as opposed to nanoluciferase-based constructs, whenever possible.

Answer to Reviewer#1 - 2. As mentioned above (point **1.**), we added experiments performed in U87-MG cells silenced for DDX42 and infected with WT ZIKV (in addition to the NLuc reporter virus experiments in U87-MG and WT ZIKV experiments with FACS staining in Huh-7 cells).

The authors suggest an IFN-independent mechanism for DDX42 against HIV, but they only look at the infection when IFN is added or not. A better experiment would be to look at the antiviral phenotype of DDX42 when an IFN inhibitor is used. Moreover, additional studies should address whether the antiviral activity of DDX42 against plus sense viruses is IFN dependent.

Answer to Reviewer#1 - 3. We have now added new experiments to address these concerns, please see the details below:

Rather than using an IFN inhibitor, we chose to generate IRF9/STAT1 double knockout U87-MG and A549-ACE2 cell populations, as well as control cells (which express Cas9 and 2 irrelevant sgRNAs). These IRF9/STAT1 KO cells were silenced for DDX42 and then pre-treated or not with IFN. Our western blot analysis confirmed that IRF9/STAT1 KO cells were, as expected, severely impacted for their responses to IFN since they were unable to efficiently induce prototype ISGs such as IFITM3, and MX1 or MX2 (**Fig. EV2E** and **EV5E**). These cell lines remained fully permissive to viral infection following IFN treatment, for the 3 selected viruses, namely HIV-1 (**Fig. EV2F**), CHIKV (**Fig. EV5C),** SARS-CoV-2 (**Fig. EV5F**). In these KO cells, DDX42 silencing had a similar positive impact than in control cells on the replication of HIV-1 (**Fig. EV2F**), CHIKV (**Fig. EV5C),** and SARS-CoV-2 (**Fig. EV5F**), both in the absence and in the presence of exogenous IFN. These novel results confirmed that DDX42 antiviral activity is IFN-independent in the context of infection with these three viruses. As specified by the reviewer, we agree that this was essential to check and our results here clearly argue against an IFN-dependent activity of DDX42, at least against these three viruses.

We also added experiments to address the impact of exogenous IFN on the replication of WT ZIKV in cells silenced for DDX2 (**Fig. EV4C**). Interestingly, these novel experiments showed that, whereas DDX42 impact was modest on ZIKV infection in untreated cells, it was increased upon IFN treatment (the impact of DDX42 silencing was between 4.5 to 7-fold in presence of IFN versus

~2-fold in its absence; **Fig. EV4C**). These results are now discussed **lines 411-413**. As IFN impedes ZIKV replication, DDX42's ability to inhibit ZIKV replication might be facilitated as the virus replicates less efficiently in the presence of IFN. One can envisage for instance that some flavivirus-specific ISGs may render the replication complexes more accessible to DDX42.

Mechanistic insight into how DDX42 is antiviral is somewhat lacking, especially when it comes to the tested positive stranded RNA viruses. While the RNA-IP suggest direct binding between DDX42 and viral RNAs, additional studies are required.

Answer to Reviewer#1 - 4. To investigate further the mode action of DDX42, we produced purified recombinant DDX42 to perform *in vitro* RNA binding assays (**Fig. EV3B**). Interestingly, DDX42 was known to bind G-quadruplexes (Zyner et al, eLife 2019; PMID: **31287417**), and while our manuscript was in revision, DDX42 had been shown to bind dsRNA, through the use of biotinylated poly(I:C) pull-downs and cell lysates (Pennemann et al, Nat comm, 2021; PMID: **34853303**). Using recombinant DDX42, we have confirmed that it binds synthetic dsRNA such as poly(I:C) and G4 RNAs, but not an RNA sequence unable to form a G4 (**Appendix Fig. S1**). Moreover, and of utmost interest, using an *in vitro* radioactive assay to measure HIV-1 reverse transcription, we showed that DDX42 significantly impeded reverse transcription *in vitro* (**Fig. 3C-D and EV3C-D**). These novel experiments provide novel and important mechanistic insights on DDX42's antiviral activity.

RNA-seq data: There is poor concordance between differentially expressed genes for the different siRNAs and no explanation is provided.

Answer to Reviewer#1 - 5. As now shown in **Appendix Fig. S2B-C**, there is a good correlation in the observed changes in transcript abundance when comparing the different siRNAs directed against DDX42. However, the magnitude of these changes is so mild that most transcripts fail to be significantly differentially expressed, therefore explaining the apparently poor concordance. This strengthens the hypothesis that the effect on viral replication that we observed is more likely due to DDX42 depletion itself than to the regulation of gene expression by DDX42 silencing.

There is also no pathway analysis for the genes identified to be differentially expressed for each of the siRNA. Such an analysis should be done not only for the genes that were common between the different siRNAs.

Answer to Reviewer#1 - 6. A pathway analysis has been added of both the DEG in common for the individual siRNA and the different DEG, please see **Appendix Fig. S2B-C.** This analysis showed that all siRNA tested induced similar effect within a cell type and that, interestingly, median log2 fold change of each pathway was lower than 0.6, suggesting a weak effect of DDX42 depletion on cell function.

Minor issues:

Literature references: The authors identify only DDX17 as the only other helicase with IFN-independent antiviral activities. However, other helicases such as DDX56 and DDX60 have been shown to have such an IFN-independent antiviral mechanism.

Taschuk, Frances, et al. "DDX56 Binds to Chikungunya Virus RNA To Control Infection." Mbio 11.5 (2020): e02623-20.

Oshiumi, Hiroyuki, et al. "DDX60 is involved in RIG-I-dependent and independent antiviral responses, and its function is attenuated by virus-induced EGFR activation." Cell reports 11.8 (2015): 1193-1207.

Answer to Reviewer#1 - 7. We have now added two sentences referencing these studies, please see lines 430-433.

In figure 3A, the authors do a time course to assess which step of HIV-1 life cycle is affected by DDX42. However, the relative amounts of DNA are smaller at 24 hours compared to 6 hours or 2 hours and that is confusing.

Answer to Reviewer#1 - 8. These observations may be surprising at first. However, this is not unusual to observe this type of kinetics for HIV-1 DNA accumulation, depending on the cell type and the experimental conditions. For instance, in primary CD4+ T cells, we had previously observed a slight decrease of viral DNA at 24h compared to 6h post-infection, followed by a drastic increase at 48h (please see PMID: **20610724,** Goujon and Malim, JVI 2010). Moreover, in our experiments the cells were siRNA-treated, which might have affected the kinetics of reverse transcription. Importantly this pattern was highly reproducible and didn't affect our main conclusions stating that DDX42 silencing favored viral DNA accumulation.

KO levels of DDX42 do not seem to be complete, the authors suggesting that DDX42 might have a role in proliferation or survival. Analyses of RNA-seq data or proliferation assays could assess whether these claims are appropriate. The alternative is that different sgRNAs should have been used for more efficient KO.

Answer to Reviewer#1 - 9. Indeed, we have made this hypothesis on the basis of the observation that our bulk DDX42 KO populations tended to derive quite rapidly when kept for longer than 2.5 weeks in culture, suggesting the cells that were not KO within the population had a growth advantage. With siRNA experiments, the duration of the experiment was a lot shorter and we did not see any effect on cell growth (as assessed by cell counting and toxicity assays). Of note, RNA-seq analyses were performed on these samples.
As for the usage of different sgRNAs, unfortunately, we don't believe this would really help (again we observed that KO cell populations derived, and KO efficiency in these populations decreased over time). The 3 sgRNAs routinely used in cell lines were the first 3 ones used in primary T cells (sgRNA-1 -2 -3, **Fig. 2F**) along with 2 new sgRNAs, designed on the IDT website (sgRNA-4 -5, **Fig. 2F**) and they are all similarly, highly efficient. We have also observed that primary T cells tended to grow slightly slower upon DDX42 depletion, but we systematically adjusted cell

numbers at the time of infection and performed short duration experiments (i.e. 4 days between Cas9/sgRNA RNP electroporation and infection, and analysis 24h later) to prevent any bias. Of note, this prevented us from testing the impact of DDX42 depletion on HIV-1 growth over long periods of time.

Typos: the authors use either full stop "." comma "," to indicate decimals (Fig. 3G, Fig. 4A-F, S4B) and should use a full stop for all such figures, figure legends and materials and methods.

Answer to Reviewer#1 - 10. This has been corrected.

Missing statistics: none of the knockdown figures (Fig 2C, 3B, S4A) have any associated statistical tests. In addition, figures 3A, 3D, 3F, S2C, S2D do not have any statistics.

Answer to Reviewer#1 - 11. The statistical analyses have now been added, when relevant (see *), i.e. in former Figures 3D, 3F, now named Fig. 3F and Fig. 3H, respectively, as well as in former Fig. S2C and Fig. S2D, now named Fig. EV2G and Fig. EV2H.

* Of note, the Figures 2C / 3B / EV4A (i.e. former S4A) show the relative quantification of DDX42 RNA levels in the knockdown experiments. We do not believe that statistical analyses are necessary here.

Data graphing: Fig. 3D should have the siCTRL plotted so the figure is easier to read. Figures showing knockdown should have the siRNAs specific to DDX42 colored in shades of gray to be consistent with the other graphs in the same figure (Fig 3B, 3E).

Answer to Reviewer#1 - 12. This has now been changed (of note former Fig. 3D is now Fig. 3F).

Figures showing knockdown should have the siRNAs specific to DDX42 colored in shades of gray to be consistent with the other graphs in the same figure (Fig 3B, 3E).

Answer to Reviewer#1 - 13. This has now been changed (for **Fig. 3B,** for **former Fig. 3E** now **Fig. 3G,** and also for **Fig. 2C,** former **Fig. S4A** now **Fig. EV4A**).

Figure legends: none of the figure legends indicate the p-value cutoff values for the various * of significance.

Answer to Reviewer#1 - 14. This was initially indicated in the methods, but has now been added in each of the main figure legend as requested**.**

Referee #2:

Bonaventure et al. have identified that DDX42 can restrict positive-strand RNA viruses. They performed a genome-wide screen to identify DDX42 as a new HIV-1 inhibitor. The experiments were performed well but the manuscript is having problems, that need more work and explanations. They discussed the probable mode of action of DDX42 but did not provided any evidence in the manuscript. They say it's a direct mode of action on RNP complexes, but no evidence is provided. Please see below the major concerns.

Major concerns:

1. No attempts are made to understand the mechanism of action of DDX42. I am not looking for extensive evidence, but at least the some insight on how DDX42 might work. Just PLA for the presence of protein in the vicinity is not sufficent, given that the role of DDX42 in +ve RNA viruses is already worked out (please see below).

Answer to Reviewer#2 - 1.

To better understand DDX42's mechanism of action, in addition to PLA experiments, in the original version of the manuscript, we had performed cross-linking RNA immunoprecipitations and shown a specific interaction between DDX42 and RNAs from targeted viruses (former **Fig. J-K** now **Fig. 4K-L**) or LINE-1 (former **Fig. 3J** now **Fig. 3L**). Moreover, there was no specific interaction with RNA from an untargeted virus (IAV; former **Fig. 4L** now **Fig. 4M**).

To gain further insights into DDX42's mechanism of action, we have now produced and purified recombinant DDX42 and confirmed that: 1) DDX42 was able to bind dsRNA *in vitro*, as recently shown by Andreas Pichlmair's group (Pennemann et al, Nat comm, 2021; PMID: 34853303); 2) DDX42 was able to specifically bind to G4 structures, using a predicted RNA G4 sequence from CHIKV as well as a well-characterized RNA G4 from a cellular gene (TRF2), as shown before by Balasubramanian's group (Zyner et al, eLife 2019; PMID: 31287417) (**Appendix Fig. S1**). Importantly, the use as a control of a mutated version of this TRF2 RNA, unable to form G4s, showed that this binding was not an unspecific binding to any type RNA (**Appendix Fig. S1**).

Finally, using recombinant DDX42 we performed *in vitro* assays and measured the accumulation of HIV-1 reverse transcription products (the "minus strand strong stop"). These assays showed that DDX42 alone was able to impede reverse transcription elongation (new **Fig. 3C and Fig. EV3C**). Of utmost interest, these novel data showed that DDX42 had the intrinsic capacity of directly inhibiting reverse transcription.

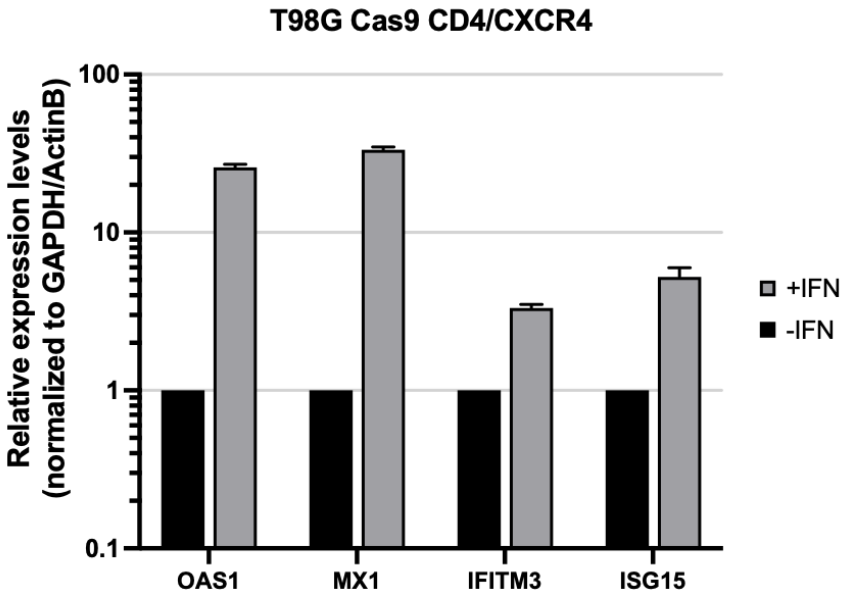
Regarding the comment "that the role of DDX42 in +ve RNA viruses is already worked out", we address this point below, please see **Answer to Reviewer#2 - 4.**

2. It is difficult to understand the CRISPR screen strategy used. The authors have treated T98G cells with IFNalpha followed VSV-G-pseudotyped HIV-1 lentivirus and selected for infection. There appear problems in this screen, which need explanation and work:

A. T98G is a glioblostoma cell line. What are the levels of ISG response in these cells? If they are highly permissive for HIV, chances are that they are severely compromised for IFN responses. If that's the case, how do you justify its use for such a positive screen? Please provide data and an explanation.

Answer to Reviewer#2 - 2A:

The screen was initially aimed at identifying novel ISGs inhibiting HIV-1 infection. For such a goal, the chosen model cell line must establish a potent antiviral state upon IFN treatment. In the first version of our manuscript, we showed in **Fig. EV1A** (formerly named **S1A**) that T98G became refractory to HIV-1 infection following IFN treatment (almost 2-log decrease in infection efficiency at the intermediary viral inputs). We had also confirmed in **Fig. EV1C** (formerly named **S2A**) that they respond to IFN through the induction of the prototype ISG ISG15. We have now extended this analysis to other prototypal ISGs in addition to ISG15: namely MX1, OAS1 and IFITM3 (**Rebuttal Fig. 1**; see below). These data further validate that these cells are IFN responsive.



Rebuttal Figure 1: T98G Cas9/CD4/CXCR4 cells are interferon-responsive. T98G Cas9/CD4/CXCR4 cells were treated with type 1 IFN (1000 U/mL) for 24 h or left untreated. RNA was subsequently extracted and prototype ISGs mRNA levels were quantified by RT-qPCR; Actin B and GAPDH were used as endogenous controls for normalization. The bar chart shows the relative levels of expression of OAS1, MX1, IFITM3 and ISG15 in the presence or absence of IFN treatment. Data represent the mean ± S.E.M of three independent experiments.

"If they are highly permissive for HIV, chances are that they are severely compromised for IFN responses. If that's the case, how do you justify its use for such a positive screen? Please provide data and an explanation."

We believe that this is highly unlikely. CD4+ T cells, which HIV-1's main primary target cells, respond really well to IFN stimulation and are highly permissive to infection (there are many examples in the literature, but please see for instance: Goujon and Malim, JVI 2010, PMID: **20610724**). HIV-1 is well-known to go "under the radar" and to not being well sensed by PRRs in its target cells. However, HIV-1 is highly sensitive to the IFN-induced antiviral state once it is established (see Doyle et al, Nature Reviews Microbiology 2015, PMID: **25915633** for instance). IFN induction by a virus and IFN sensibility are two different things and, here, only the latter (and the effectors of the antiviral state) was of interest to us.

We do show in **Fig. EV1A** (formerly **S1A**) both the permissiveness of T98G/CD4/CXCR4 cells to HIV-1 infection and their ability to potently inhibit replication following IFN treatment, as was previously shown for another glioblastoma cell line (namely U87-MG cells, see Goujon et al, Nature 2013, PMID: **24048477**; Goujon and Schaller et al, Retrovirology 2013, PMID: **23442224**) or many other cell types including primary CD4+ T cells or monocyte-derived macrophages (Goujon and Malim, JVI 2010, PMID: **20610724**).

For this screen, we selected a cell line that i) is easy to handle (e.g. growing fast) and easy to transduce with lentiviral vectors (in order to express Cas9 and the library of sgRNAs); ii) responds well to IFN through ISG induction and the establishment of a potent antiviral state (see **Fig. EV1C** (formerly named **S2A**), **Fig. EV1A** and **Rebuttal Figure 1**); iii) is permissive to HIV-1 infection (**Fig. EV1A**); and, iv) is able to grow well while being surrounded by large amounts of dying cells, as seen in the antibiotic selection phases of the screens. None of the T cell lines we tested fulfilled these criteria (the main point being that they don't block well HIV-1 infection following IFN treatment, see for instance Goujon and Malim 2010, PMID: **20610724**), and an alternative cell line of myeloid origin (THP-1 cells), responded well to IFN but grew too slowly in our hands for such a multi-round screen setup.
The idea here was not to get a comprehensive analysis of the full landscape of cellular inhibitors of HIV-1, but just to identify novel potential inhibitors. Importantly we validated our data the impact of DDX42 on HIV-1 replication in multiple cell lines, including primary T cells.

We have now added a sentence to highlight the fact that T98G are indeed an uncommon model to study HIV-1, please see Line 94.

B. Did the author use as control a pseudotyped LV virus that is not HIV-1-based to understand specificity for HIV restriction?

Answer to Reviewer#2 - 2B. We didn't do this at the time of the screen (i.e. we didn't do parallel screens with LVs from other origins). However, when focusing on DDX42, we studied DDX42's effect on a panel of VSV-G pseudotyped lentiviruses (HIV-1 IIIB and founder/transmitted CH106.C, CH077.t and REJO.C, HIV-2rod and SIVmac; **Former Fig. 3D**, now **Fig. 3F**) as well as lentiviral vectors which were not based on HIV-1 but on HIV-2, SIVmac or FIV (**Former Fig. 3F**, now **Fig. 3H**).
Of note, we were not particularly looking for HIV-1 specific inhibitors. At the time we initiated these screens, a few years ago, the ISGs potently inhibiting HIV-1 reverse transcription (Goujon and Malim, 2010, PMID: **20610724** and Cheney and McKnight, 2010, PMID: **20975956**), were still unknown but we knew that other retroviruses, such as MLV, were also affected (Goujon and Malim, 2010, PMID: **20610724**).

3. Is DDX42 a positive regulator of the IFN response to positive-strand RNA viruses? This is not difficult to find out, given that they have all the required resources and reagents for DDX42.

Answer to Reviewer#2 – 3.

To investigate whether DDX42 could be a positive regulator of the IFN response, first, we assessed ISG induction upon viral infection in the context of DDX42 presence or absence (**Fig. EV2D, Fig. EV4E** and **Fig. EV5B**). We didn't observe a significant difference in ISG induction upon viral infection, as you can see in **Fig. EV2D** in the context of HIV-1 infection, **Fig. EV4E** in the context of ZIKV and JEV infection, or in **Fig. EV5B** in the context of CHIKV infection. Importantly, the absence of DDX42 didn't substantially impact ISG induction following infection and/or interferon treatment (**Fig. EV2D, Fig. EV4E** and **Fig. EV5B**).

Moreover, we generated IRF9/STAT1 double knockout U87-MG and A549-ACE2 cell populations, as well as control cells (which express Cas9 and 2 irrelevant sgRNAs). These IRF9/STAT1 KO cells were silenced for DDX42 and then pre-treated or not with IFN. Our western blot analysis confirmed that IRF9/STAT1 KO cells were, as expected, severely impacted for their responses to IFN since they were unable to efficiently induce several ISGs such as IFITM3, and MX1 or MX2 (**Fig. EV2E** and **EV5E**). These cell lines remained fully permissive to viral infection following IFN treatment, for the 3 selected viruses, namely HIV-1 (**Fig. EV2F**), CHIKV (**Fig. EV5C),** SARS-CoV-2 (**Fig. EV5F**). In these KO cells, DDX42 silencing had a similar positive impact than in control cells on the replication of HIV-1 (**Fig. EV2F**), CHIKV (**Fig. EV5C),** and SARS-CoV-2 (**Fig. EV5F**), both in the absence and in the presence of exogenous IFN. These novel results confirmed that DDX42 antiviral activity is IFN-independent in the context of infection with these three viruses. As specified by the reviewer, we agree that this was essential to check and our results here clearly argue against an IFN-dependent activity of DDX42, at least against these three viruses.

4. I would like to point that a role of DDX42 in positive RNA viruses (JEV) was shown before (Lin et al 2008, Virus Research). They showed that the NS4A protein of JEV specifically interacts with DDX42 and the expression of N-terminal DDX42 can overcome JEV-induced antagonisms of IFN responses. This is interesting. Such an experiment will be useful also here. They also show that DDX42 can induce IFN responses. Please reference this paper, discuss it and also refer to Carlin et al 2018, PNAS. Thus, it is incorrect to claim in your manuscript that DDX42 functions have not been determined previously. Please check the whole manuscript and correct this claim.

Answer to Reviewer#2 - 4:

We were aware of this study on JEV NS4A and DDX42 (Lin et al, Virus Research 2008, PMID: **18588927**). This study didn't actually show a role of endogenously expressed DDX42 in the IFN responses during JEV infection. The authors first showed that JEV NS4A inhibited IFN signaling (using an ISRE reporter assay and ectopic expression, as well as immunoblots to assess phosphorylation of the JAK/STAT pathway members upon IFN treatment in the presence and absence of transfected NS4A). They identified the C-terminal domain of DDX42 as an interactor of NS4A using phage display. The domain of DDX42 they identified corresponded to residues 760-871, as specified in the article (of note DDX42 contains 938 amino-acids), although when looking at the sequence of the forward primer they used for PCR amplification, it seems that residue 750 would be at the N-terminus of their fragment, rather than residue 760. Confusingly enough, in the text and legend of the manuscript, the authors called this domain "N-terminal DDX42". The interaction between DDX42 fragment and NS4A was validated through the use of

recombinant proteins and pull-down and colocalizations studies. Finally, an impact of what the author called “N-terminal DDX42” on an ISRE reporter activity assay was only seen in very specific conditions, when cells were pre-infected with JEV and then treated with IFNb (there was no effect in the opposite settings: i.e. IFN pre-treatment followed by JEV infection) (PMID: **18588927** Fig. 8). The impact of both full-length DDX42 and of DDX42 silencing on IFN induction upon JEV replication should have been tested, at the very least, to be able to actually draw the conclusions proposed in this paper. However, we have acknowledged the fact that there was an initial study on DDX42 and an RNA-positive virus and have added this reference, please see Lines 285 and 405-406. Moreover, we have now investigated DDX42’s impact on JEV flavivirus in U87-MG cells (**Fig. EV4D**). DDX42 depletion had no significant impact on JEV infection efficiency as measured by RT-qPCR (i.e. ~1.8-fold increase in infection as compared to siCTRL-treated cells, for all 3 siRNAs). As seen for ZIKV, the impact of DDX42 depletion was slightly higher in the context of the IFN-induced antiviral state (4.3, 2.3, and 6.6-fold increase in JEV infection efficiency with siDDX42-1, -2 and -4, respectively, in IFN-treated U87-MG cells). When looking at ISG induction upon JEV replication in U87-MG cells, we didn’t really observe a differential induction of prototype ISGs (i.e. ISG15, MX1, OAS1) in the infected versus non-infected, IFN-treated or not, siCTRL- and siDDX42-treated cells (**Fig. EV4E**). Therefore, in the model cells and conditions used here, DDX42 doesn’t seem to favor JEV sensing and IFN production following JEV sensing.

In the Carlin et al. study (PNAS, 2018, PMID: **30206152**), transcriptomic and epigenetic analyses were performed in monocyte-derived macrophages infected with clinical isolates of ZIKV. The study aimed to investigate the host response of ZIKV infected cells versus non-infected bystander cells. DDX42 and other helicases were identified as negatively differentially expressed genes, in a ZIKV strain-specific manner. Indeed, there was a 2-fold decrease of DDX42 RNA upon infection with the wFSS13025 ZIKV strain, which was not further investigated. Therefore, we do not feel that the study from Carlin et al., could particularly be relevant to quote in our manuscript.

Minor Comments:

1. In Fig. 1C, the authors have show that T98G cells are permissive for HIV 1 Infection. The authors have analyzed the infection efficiency after 30 hours. Did the authors also look at early time points such as 6 hrs or 12 hrs?

Answer to Reviewer#2 - minor point 1:

No, we haven’t looked at very early time points, as these would be too short to analyze infection efficiency for HIV-1. At 6-12h post-infection, reverse transcription and integration are still ongoing.

2. In Fig. 1C, the authors have shown that KO of two candidate genes WARS2 and DDX42 allowed the partial rescue of HIV 1 Infection from IFN induced inhibition. What was the reason for not choosing WARS2 for this study? Any previous studies done on WARS2?

Answer to Reviewer#2 - minor point 2:

WARS2 is a mitochondrial aminoacyl-tRNA synthetase, and like WARS (a cytoplasmic aminoacyl-tRNA synthetase), have been found in other screens (see for instance: Jimenez-Guardeño et al,

Nature Microbiology 2019 or Yang et al., EMBO reports. PMID 32924251, which had identified WARS and WARS2, respectively). They are known to catalyze the aminoacylation of tRNA by their cognate amino acid, and we suspected here that an indirect effect on HIV-1 replication would be likely. We had, however, tried to validate WARS2 phenotype using siRNA-based depletion and ectopic expression. These experiments gave inconsistent phenotypes and WARS2 overexpression impacted cell viability, which led us to focus on DDX42.

3. The authors should find the possible reason behind DDX42 impacting positive-strand RNA virus and not having any effect on negative-strand RNA virus. What could be the reason for this specific effect?

Answer to Reviewer#2 - minor point 3:

There are several reasons as to why DDX42 could only affect the positive-strand RNA viruses and not the negative-strand RNA viruses we have tested. Notably, incoming genetic material from negative strand viruses are associated with their polymerases, which is suspected to decrease recombination events (Han et al., 2011 PMID: 21994784), whereas positive-strand RNA viruses generally require a first round of transcription/translation to initiate replication (with the notable exception of retroviruses which undergo reverse transcription). Interestingly, +sRNA virus replication complexes are less processive than the ones of nsRNA viruses (Simon-Loriere and Holmes, 2011 PMID: 21725337; Chare et al., 2003, PMID: 13679603). In particular, viral polymerases from alphaviruses (Filomatori et al., 2019 PMID: 30986247), retroviruses (Mansky et al., 1998 PMID: 9634073; Neher et al.,2010 PMID: 20126527) and coronaviruses (Makino et al., 1986 PMID: 2829575) are highly distributive, favoring template switching events and recombinations. Coronavirus discontinuous transcription fits particularly well with the idea that DDX42 might act preferentially on viruses encoding distributive polymerases. While a distributive replication complex may favor recombination and viral fitness (by generating diversity and evading RNA based restriction Aguado et al., 2018 PMID: 30209219), viral RNPs might be more sensitive to destabilization/inhibition. In addition, multiple secondary structures are involved in recombination (White KA, Morris TJ., 1995 PMID: 8595558, Galetto et al., 2006 PMID: 16291743) which could also be determinants for DDX42 antiviral activity.

Interestingly, while we were revising this manuscript, Andreas Pichlmair's team has identified DDX42 among poly(I:C)-binding proteins in a screen and has shown an impact of DDX42 in THP-1 cells (Pennemann et al Nature Communications, 2021, PMID: 34853303). Indeed, Pennemann et al found that, in undifferentiated THP-1 cells, DDX42 KO rescued influenza A virus infection, whereas it inhibited VSV infection in differentiated THP-1 cells. This means that DDX42 could potentially also impact negative-stranded viruses, and the differences observed between our studies could be cell-type dependent (and/or virus reporter type dependent) and would definitely require more exploration. We are now mentioning the novel findings (**Lines 374-375**). However, it is noteworthy that, for their IAV experiments, Pennemann et al used a mouse-adapted variant of a highly pathogenic avian H7N7 influenza A virus SC35, called SC35M, in which Gaussia CDS was inserted in the NS segment (influenza A SC35M NS1_2A_Gaussia_2A_NEP). Of note, the NS segment of IAV is normally spliced to allow NEP (also called NS2) expression, but that's not the case anymore for this genetically engineered virus (NEP expression is driven by a 2A peptide

from the same mRNA as NS1). However, there are likely remaining, splicing-related elements on this genetically modified NS mRNA (branch point site, cytosine and thymidine rich sequences) (Reuther et al, Sci Reports 2015, PMID: **26068081**) and it is possible that DDX42 impacts this (after all, DDX42, also named SF3b125, was initially identified as associated with splicing factors; please see Will et al, EMBO J 2022, PMID: **12234937**). It would be highly interesting to test DDX42 impact on IAV in THP-1 cells with our reporter virus (we have tried to address this, but, unfortunately, so far, we haven't managed to get sufficient depletion of DDX42 in THP-1 cells).

4. The knockout and knockdown efficiency of sgRNA and siRNA cannot be correlated from the western blot data in Figure 2A and Figure 2C.

Answer to Reviewer#2 - minor point 4:
We apologize but we're not sure to what exactly Reviewer #2 refers to here.
Fig. 2A shows the impact of DDX42 depletion on HIV-1 infection as well as DDX42 immunoblots on matching samples. These data revealed that DDX42 depletion was more efficient in cells transduced with sgRNA-3 and there was a slightly better effect on HIV-1 infection of this sgRNA in the absence of interferon. The fact that the impact was not stronger (or not observed in the presence of IFN) might indicate that this was the maximum effect that could be observed in these settings.
Fig. 2C shows that the silencing for all 3 siRNAs is around 90% as measured by RT-qPCR and this is well reflected on the immunoblot where DDX42 could barely be detected in cells in which it had been silenced.

5. Although the authors have shown a possible interaction between DDX42 and virus capsid protein, they have not shown whether DDX42 affects the translation of viral proteins, which should be the ultimate read-out.

Answer to Reviewer#2 - minor point 5. Indeed, we haven't studied DDX42's potential impact on viral translation. However, we showed that: i) there was more viral DNA accumulated and integrated in the absence of DDX42 (**Fig. 3A**); ii) there were more viral RNAs in the absence of DDX42 (**Fig EV2B**); iii) the percentage of cells expressing CA p24 was higher (as shown by p24 intracellular staining) in the absence of DDX42 (**Fig. 2D**); and there was also more CA p24 released in the supernatant in the absence of DDX42 (as shown by p24 AlphaLisa) (**Fig EV2C**). Moreover, in the context of CHIKV and SARS-CoV-2 infections, we showed that there were more infectious viral particles produced in the absence of DDX42 (**Fig. 4F** and **Fig. 4H**, respectively). We believe that measuring viral production is an appropriate read-out for assessing the role of an antiviral factor on the viral cycle.
Of note, we do not believe that there is an interaction between DDX42 and virus capsid (PLA only shows a proximity, not an interaction), but that there is an interaction between DDX42 and viral nucleic acids, as shown by the RIP experiments (**Fig. 4K-L**) and the novel *in vitro* experiments (**Fig. 3C-D, Fig. EV3C-D** and **Appendix Fig. S1**).

6. In Page 3, Line no. 62, Reference is missing.

Answer to Reviewer#2 - minor point 6. We have now added a reference (Tenthorey, Emerman and Malik, Annual Review of Immunology, 2022 PMID: **35080919**).

7. In Fig. 3A, 3D, 3F, 4A, and 4B, the stats are missing.

Answer to Reviewer#2 - minor point 7. The statistical analyses have now been added in these figures (of note Fig. 3D and Fig. 3F are now **Fig. 3F** and **Fig. 3H**, respectively).

Referee #3:

Bonaventure et al have done a whole genome CRISPR screen to try to identify IFN-induced genes that inhibit HIV-1 infection. While they did not find any novel ISGs that explain the IFN-effect in the cell line used for the screen, they did identify an interesting hit, DDX42 that has low activity against a wide variety of lentiviruses and positive-strand RNAs viruses with very high antiviral activity against SARS-CoV2. There is an impressive amount of work in this paper. While none of the studies are definitive for a role of DDX42 in controlling natural infections, this paper will likely be a starting point for future studies.

Major Comments:

1. The results of the whole genome screen were possibly disappointing to the authors since one of the top hits, DDX42, has a relatively modest 2-3 fold effect on HIV-1, and also is not an ISG. However, the big story here seems to be the 3-log effect of DDX42 knockout on SARS-CoV-2 infection in Figure 3. I am surprised the authors do not seem to give this more emphasis. Certainly, it would be easier to further investigate mechanism with a 3-log effect than a 3-fold effect.

Answer to Reviewer #3 - 1. Experiments performed in the context of SARS-CoV-2 infection will indeed be interesting and this has now been emphasized (lines 437-439). We obtained the SARS-CoV-2 data late during the preparation of our manuscript (towards the end of the first author PhD work), which is why less characterization work was done than for HIV-1 for instance. Moreover, the new experiments aiming at studying the impact of DDX42 on viral replication *in vitro* were done on HIV-1 reverse transcription, because the system was well in place in our collaborators' lab (Dr J.C. Paillard's lab) and we obviously didn't have an equivalent system for SARS-CoV-2 to measure in vitro RNA replication.

2. Related to the huge effect seen with DD42 on SARS-CoV-2, can the authors speculate on why this gene has not come up as a bigger hit in other CRSIPR screens against SARS-CoV-2 including the authors' own preprint (https://www.biorxiv.org/content/10.1101/2021.05.19.444823v2). Did I miss it there?

Answer to Reviewer #3 – 2. As mentioned in our manuscript, DDX42 was identified as a potential

SARS-CoV-2 inhibitor in Craig Wilen's screen performed in Vero E6 cells (Wei et al, Cell 2021), however, we didn't identify DDX42 in our own CRISPR/Cas9 KO screens when looking for regulators of SARS-CoV-2 replication (Rebendenne et al bioRxiv 2021, Nature Genetics, in press). DDX42 wasn't identified either in the other published screens performed in Huh7 (or derivatives) and in A549 cells (Wang et al, Cell 2021; Schneider et al, Cell 2021; Daniloski et al, Cell 2021; Baggen et al, Nature Genetics 2021; Zhu et al, Nature communication 2021). Our whole-genome screens performed in Calu-3 and Caco-2 cells were actually not powered to identify inhibitors (none were retrieved). In contrast, in our whole-genome screens performed in Vero E6 cells, we identified potential inhibitors, but DDX42 wasn't among them. Compared to Craig Wilen's screens and as explained in our bioRxiv manuscript, our screens had a much stronger selection pressure than theirs. This meant that we had massive cell death and therefore had to keep the cells in culture for a lot longer post-SARS-CoV-2 infection in order to retrieve enough genomic DNA material for the deep sequencing step. Therefore, it is likely that DDX42 KO cells were lost during this long period of culture (as we know that DDX42 KO populations do derive). In addition, Cas9 activity in our Vero E6-Cas9 cells was quite low for whole-genome screening, which could also explain that we've missed some interesting candidate genes. Of note, one can argue that we have kept our T98G cells for a long time in culture for our HIV screens, but it is important to mention here that T98G are pentaploid and this might have worked in our favor. Importantly, we can highlight that DDX42 is not an exception in that regard: for instance, LY6E is a well-known coronavirus inhibitor (Pfaender et al, Nature Microbiology 2020) and whereas we could identify it in our whole-genome activation screens (and in some of our secondary screens present in the revised version of our manuscript), it was not found in our whole-genome KO screens or in the other published whole-genome KO screens (i.e. Wang et al, Cell 2021; Schneider et al, Cell 2021; Daniloski et al, Cell 2021; Baggen et al, Nature Genetics 2021; Zhu et al, Nature communication 2021).

3. I find it hard to interpret the PLA against HIV-1 CA and DDX42 given the lack of specificity for different viruses and the later results where they suggest that DD42 is found in viral RNP complexes. Are the authors suggesting that DDX42 is binding HIV-1 genomic RNA inside the capsid core?

Answer to Reviewer #3 - 3. As mentioned above (Answer to Reviewer#2 - minor point 5.), we don't believe at all that there is a specific interaction occurring between HIV-1 Capsid and DDX42, considering the broad effect of DDX42 against several families of viruses. Actually, PLA data just suggest that there is proximity between Capsid and DDX42 during infection. HIV-1 Capsid was recently shown to remain associated with HIV-1 RNA/DNA up until proviral DNA integration (e.g. see the work from V. Pathak's lab, Burdick et al, PNAS 2020 PMID: **32094182**). Therefore, here we believe that the PLA data showed DDX42 proximity with the reverse transcription complexes. Interestingly, Wes Sunquist's lab has very elegantly shown that cores are likely to be sequentially disrupted during the reverse transcription process, using an *in vitro* approach (Christensen et al, Science 2020, PMID: **33033190**). This would explain how DDX42 could gain access to HIV-1 nucleic acids. Besides, even if Sunquist's model was not accurate in living cells, it seems highly likely that not all cores remain fully intact during infection and DDX42 could have access to the genetic material these partly disrupted cores contain.

4. One caveat of a 2-3 fold effect on most viruses is the authors' suggestion that knockout/down of DDX42 is detrimental to the cell in line 154. The RNA-seq data suggests that this is not a transcriptional response to DDX42 knockout. However, could it be a stress response that is inducing virus replication? I do not think additional experiments need to be done, but the authors might acknowledge some caution that the effect of DDX42 could be indirect (despite the lack of transcriptional difference and lack of effect on the negative-sense RNA viruses).

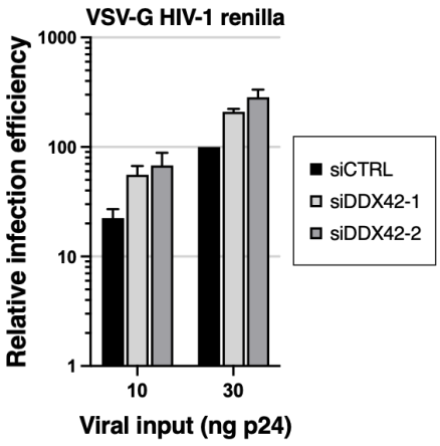
Answer to Reviewer #3 – 4. Concerning the first sentence, we only mentioned that DDX42 KO, not DDX42 KD, had a detrimental impact because we observed that DDX42 KO cell populations derived. With DDX42 KD, experiments were conducted over shorter periods of time and the cells were completely fine throughout the experiments (as notably shown by the absence of toxicity, **Fig. EV2A**). We have now clarified this in the text, see Lines 161-162.
We have now acknowledged that we can't formally exclude an indirect effect of DDX42 on viral replication both in the results and in the discussion, please see **Line 358** and **Lines 402-403**.

Minor comments

5. It is not obvious which infections were done with wtHIV versus which ones are done with a vector system. Please point that out in the figure legends. Was there a difference between entry with VSV-G versus HIV-1 env?

Answer to Reviewer #3 - 5. We have now clearly specified in all figure (and EV figure) legends which experiments were performed with replication-competent HIV-1 (or Renilla-expressing NL4-3 HIV-1 or replication-competent lentiviruses) versus lentiviral / retroviral reporter systems.

Concerning entry, VSV-G pseudotyped HIV-1 renilla (full length virus bearing a Nef-RES-Renilla cassette; NL4-3/Nef-IRES-Renilla) was modestly impacted by DDX42, as shown in Rebuttal **Figure 2** below (of note, VSV-G pseudotyped lentiviral vectors were also slightly impacted by DDX42, see **Fig. 3F**). Importantly, we showed in the manuscript that DDX42 did indeed not impact entry of WT- HIV-1, using a BlaM-VPR assay **(Fig. EV3A)**.



Rebuttal Figure 2. siRNA-treated U87-MG cells were infected with two doses of VSV-G pseudotyped, NL4-3 Renilla (VSV-G-NL4-3/Nef-IRES-Renilla) (indicated in ng $p24^{Gag}$). Renilla activity was normalized to Firefly activity and the relative infection efficiencies are shown. Data represent the average and standard deviation (s.d.) of 3 independent experiments.

6. Line 89: A glioblastoma cell line is an odd choice for doing an IFN screen for HIV-relevant factors. ISGs show quite a bit of cell-specificity, so one would not necessarily expect that ISGs relevant to HIV would be expressed in this cell type. I am more concerned that readers will feel that this screen is comprehensive, whereas the authors might well have missed important ISGs because they are not expressed in the T98G glioblastoma cell line. Another caveat that could be mentioned is alluded to in line 127 where there is a statement that there is little over-lap between independent screen replicates. This seems to me to suggest that the screen is not comprehensive, and might have missed bonafide ISGs that contribute to the IFN effect against HIV-1.

Answer to Reviewer #3 - 6. We have added a sentence in order to emphasize that the screen was by no means comprehensive and doesn't give an exhaustive view of HIV-1 (interferon-induced or not) restriction factor landscape, please see Lines 115-117.

T98G cells are indeed not physiologically relevant to study HIV-1 replication and we have now added a sentence to specify that they are an uncommon model, see Line 94. However, we've extensively used another glioblastoma cell line (U87-MG) in the past to characterize HIV-1 restriction (Goujon et al 2013 Nature, PMID: **24048477**, and Goujon et al 2013, Retrovirology, PMID: **23442224**) and the IFN-induced blocks in these cells of glioblastoma origin were similar to the ones observed in monocytic THP-1 cells or primary cells for instance. We characterized the IFN-induced restriction in T98G cells and showed that it was potent (**Fig. EV1A**).
For this screen, we selected this cell line because: i) it is easy to handle (e.g. growing fast) and easy to transduce with lentiviral vectors (in order to express Cas9 and the library of sgRNAs); iii) it is permissive to HIV-1 infection (**Fig. EV1A**); ii) it responds well to IFN through ISG induction and the establishment of a potent antiviral state (see **Fig. EV1C** (formerly named **S2A**), **Rebuttal Fig. 1** and **Fig. EV1A**; and, iv) it is able to grow well while being surrounded by large amounts of dying cells (not shown), as seen in the antibiotic selection phases of the screens. None of the T cell lines we tested fulfilled these criteria (the main point being that they don't block well HIV-1 infection following IFN treatment, see for instance Goujon and Malim 2010, PMID: **20610724**), and an alternative cell line of myeloid origin (THP-1 cells), responded well to IFN but grew too slowly in our hands for such a multi-round screen setup. The idea here was not to get a comprehensive analysis of the full landscape of cellular inhibitors of HIV-1, but just to identify novel potential inhibitors. Importantly we validated our data the impact of DDX42 on HIV-1 replication in multiple cell lines, including primary T cells. Of note, we had performed our CRISPR screens both in U87-MG cells and in T98G cells in parallel. However, our CRISPR screens with U87-MG cells were unsuccessful (likely because these cells do not tolerate well to remain in culture with lots of dying cells).
Finally, and as mentioned in our manuscript Lines 119-120, poor overlap between biological replicates from genome-wide screens has been observed before (e.g. Parnas et al, Cell 2015,

PMID: **26189680**, which was highlighted by John Doench, Nat Rev Gen, 2018, PMID: **29199283**) and didn't preclude the identification of important genes.

Dear Dr. Goujon,

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the reports from the three referees that was asked to re-evaluate your study, you will find below. As you will see, the referees now fully support the publication of your work. Referee #1 has some further comments and suggestions (further minor comments) to improve the manuscript I ask you to address in a final revised manuscript.

Moreover, I have these editorial requests I also ask you to address:

- Please provide the abstract written in present tense throughout.

- We plan to publish your manuscript in the Report format (as also selected by you upon submission). For a Scientific Report we require that results and discussion sections are combined in a single chapter called "Results & Discussion". Please do this for your manuscript. For more details please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#researcharticleguide

- Please ad subtitles to the results & discussion part. This will render the manuscript text more comprehensive.

- We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

- We now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please use the free text box to provide more detailed descriptions. Thus, please remove the author contributions section from the manuscript text file. See also guide to authors:
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- Please move all the methods information the main manuscript text and add the Appendix references to the main reference list. Then please remove this part from the Appendix.

- Please name the methods section "Materials and Methods".

- I wonder if the 2 Appendix figures could be included in the main or EV figures. There would be space for a fifth main figure. Please consider this, as then we would not need an Appendix.

- Please add the correct accession code for the GEO submission (GSE207288) to the data availability section and a direct link to access the dataset. Please make sure that the dataset is public latest upon publication of the study.

- There is one file uploaded named 'Supplementary File 1_RNA seq data'. Is this also part of GSE207288? I yes, please remove the file. If not, this should be a dataset. Please upload the file as dataset file, using the name Dataset EV1. Please use this name also for the callouts in the text and change these accordingly. Please make sure the item is called out in the text. Moreover, please put a legend for the item on the first TAB of the excel file.

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (main, EV and Appendix figures), and that statistical testing has been done where applicable. Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates. Please add complete statistical testing to all diagrams (main, EV and Appendix figures). Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant.

- For Fig. 1C and EV1B you indicate that the data from 2 replicates is shown. In this case, please show the two datasets separately (next to each other in the same diagram), without error bars. I think this is more transparent.

- Please make sure that all the funding information is also entered into the online submission system and that it is complete and similar to the one in the acknowledgement section of the manuscript text file.

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- 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 use this file for final revision and provide your final manuscript file with track changes, in order that we can see any modifications done.

In addition, I would need from you:
- a short, two-sentence summary of the manuscript (not more than 35 words).
- two to four short bullet points highlighting the key findings of your study (two lines each).
- 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.

Best,

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Senior Editor
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After revisions, the manuscript was strengthened by the addition of data performed with WT viruses, which confirmed the initial data. The IFN-independent mechanism of DDX42 was furthered confirmed by phenotypic analysis in IRF9/STAT1 KO cells. The authors also show that DDX42 specifically binds dsRNA in poly(I:C) and G4 RNAs, suggesting that binding viral RNA structures may be important for the antiviral phenotype of DDX42. The other concerns or suggestions were addressed by the authors in their point-by-point response. These revisions strengthened the authors' original hypotheses and added another layer of mechanistic insight. Therefore, I recommend the acceptance of the manuscript, after the minor editing issues below are addressed.

Previous issues:
1. The magnitude of the antiviral effect appears to be quite small as quantified by luciferase assays 28 or flow cytometry. Given that the authors claim that DDX42 may act by directly interacting with viral RNA it would be useful to supplement such data with viral titers and qPCR data.
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a. The authors added figures showing that broadly DEGs are similar and induce similar pathways.
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The authors have sufficiently managed to answer the concerns. I recommend accept at this stage.

Referee #3:

The authors have done extensive work to revise the manuscript and strengthen the conclusions. They have addressed all of my original comments and I have no further concerns. This is a nice study.

Dear Dr Breiling,

We warmly thank you and the reviewers for your time and the evaluation of this work.

Please find below the detailed point-by-point answer to your previous email.

Best regards,

Caroline

Dear Dr. Goujon,

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- Please provide the abstract written in present tense throughout.
This has been corrected.

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This has been done.

- Please ad subtitles to the results & discussion part. This will render the manuscript text more comprehensive.
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Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates.
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This has been done, using individual dots for the technical duplicates (Fig. 1C) and biological duplicates (Fig. EV1B).

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This has been done.

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- a short, two-sentence summary of the manuscript (not more than 35 words).

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- two to four short bullet points highlighting the key findings of your study (two lines each).
    - DDX42 is an intrinsic inhibitor of various positive-strand viruses including retroviruses, coronaviruses and alphaviruses as well as LINE-1 retroelements
    - DDX42 impedes HIV-1 reverse transcription in cells and in vitro
    - DDX42 is found in close proximity of viral components and interacts with viral RNAs

- 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.

This has been provided. Illustration credit: Zhanna Santybayeva (illustration4science.com)

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A sentence highlighting this possibility has now been added (see line 344).
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This has been done.

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Dr. Caroline Goujon
IRIM - UM - CNRS UMR9004
IRIM UMR9004
1919 route de Mende
Montpellier cedex 5, Occitanie 34293
France

Dear Dr. Goujon,

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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Editor
EMBO Reports

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This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

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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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Materials and Methods

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and Methods

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Materials and Methods

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Materials and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	For primary T cells, blood was obtained from French "Etablissement du sang" and the information on the sex of the donors was not provided.
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	All cell lines were routinely checked for potential mycoplasma contamination and were negative at all times. No cell line was recently authenticated but all cel lines were kept in culture for short periods of time and new ultures were regularly started from the original stocks.

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Yes	Materials and Methods

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgments

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Figure legends
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Material and Methods (PLA)
Include a statement about **blinding** even if no blinding was done.	Yes	Material and Methods (PLA)
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	No samples were excluded.
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	All the statistical analyses were performed by a bio-informatician, Dr Reiner Schulz, who is a co-author.

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figure Legends, EV Figure Legends.
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure Legends. All the data shown are from biological replicates (apart from Fig. 1C) and this has been stated.

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: 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.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
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.	Not Applicable	
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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Material and Methods
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Yes	Material and Methods
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	