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MATR3-antisense LINE1 RNA meshwork scaffolds higher-order chromatin organization

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Dear Dr. Wen,

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are shown below.

Unfortunately, two of the referees remain unconvinced that the data is sufficient to support the conclusions here. Given these opinions and the fact that the EMBO Journal can only afford to accept papers which receive enthusiastic support from a majority of referees, I am afraid we cannot offer to publish it here.

That said, given the general interest in this topic, we still found this work potentially suitable for our sister journal EMBO reports, in light of their focus on interesting key observations that do not necessarily need to be fully mechanistically followed up. I therefore briefly discussed the work with my EMBO reports colleague, Dr. Esther Schnapp, who considered the study interesting and would be happy to publish this work. However, they would require you to change or remove your model and tone down the claims made, specifically removing claims of any local effect of MATR3 and L1 on chromatin/H3K27me3. They would require you to address all final referee comments in the text, and address the last comment by referee 1 experimentally, if possible (to investigate the interaction s between MATR3 mutants and AS L1 RNAs, at least through RNA IP). Should you be interested in this option, please simply follow the transfer link; no reformatting is required.

Thank you in any case for the opportunity to consider this manuscript. I am sorry we cannot be more positive on this occasion, but we hope nevertheless that you will find our referees' comments helpful.

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Referee #1:

Having carefully read the revised manuscript and considered the authors' rebuttal, this reviewer acknowledges that the authors have provided detailed answers to most of the questions and performed additional experiments. However, while the paper reports interesting functional correlations between MATR3 and AS L1 RNA, the presented data is insufficient to support the final model. Additionally, the authors' explanations of the mechanism rely heavily on

assumptions and lack critical evidence. Overall, it appears that the authors' conclusion is based on biased wishful thinking. This reviewer suggests that the authors revise the manuscript and modify their model by considering and discussing alternative explanations for their experimental results. For instance, the disruption of MATR3 or AS-L1 may inhibit transcription, indirectly altering the organization of H3K27me3 marked chromatin. Two specific comments are outlined below.

The revised manuscript introduces the use of SAMMY-seq as an attempt to support the notion that the MATR3/ASL1 meshwork may act as an organizing platform in the cell nucleus. However, there are several issues regarding SAMMY-seq that require clarification. Firstly, the authors should explain the S1-S4 fractions' representation in terms of nuclear fractions or structures. Secondly, in the large-scale analysis presented in Figure 6B, the regions that are enriched in S4 versus S2 seem to be depleted of H3K27me3. This raises questions about what kind of heterochromatin is primarily retained in S4 fractions. It would be helpful if the authors could include a western blot of H3K27me3 and H3K9me3 in the S1-S4 fractions in Figure 6A to address this concern. Thirdly, the observed 7% genome loss and 4% genome gain in S4 cannot fully explain the changes in overall genome organization. Fourthly, as depicted in Fig 2H, H3K9me2 and H3K9me3 ChIP-seq signals are also enriched in MATR3-associated AS-L1 regions. Therefore, the proximity of H3K27me3 and AS L1 in linear genomic distance alone may not be sufficient to explain the specific changes in H3K27me3 upon MATR3 KD. These issues raise doubts about the proposed model of MATR3/ASL1 meshwork acting as a platform to organize nearby H3K27me3 regions.

The authors stated in their manuscript that AS L1 RNAs act locally (i.e., cis-acting) and can form R-loop or RNA:DNA triplex structures in various genomic L1 loci. However, since AS L1 RNA has a similar half-life to Neat1 RNA, which has been shown to have trans-acting effects (as demonstrated in Figure EV2G), there is a possibility of trans-acting effects for AS L1 RNA as well. To support this possibility, the authors presented two cases where MATR3 interacts with independent AS L1 RNA fragments located within introns. This suggests that MATR3 binds to spliced intronic AS L1 RNAs that are likely to exit the transcription sites after splicing. The question then arises as to how the MATR3/AS L1 RNA network functions locally to affect nearby H3K27me3-marked chromatin.

Additionally, it would be worthwhile to investigate the interactions between MATR3 mutants and AS L1 RNAs, at least through RNA immunoprecipitation (RIP).

Referee #2:

Overall, I think that this paper has improved with revision. Specifically, the wording of this paper relative to their correlation of their MATR3 findings and the H3K27me3 domains is significantly improved, as this association still has tenuous data demonstrating a causative link as opposed to a mere correlative link.

The authors added Figure EV2E in response to my concern that the AS L1 RNAs are located in

gene introns and as such it is unclear if they function independently of their originating transcripts. However, the experiment outlined in Figure EV2E is not appropriate to address whether these AS L1 RNAs are actually independent of their parent genes encoding them. Specifically, per their methods, the RIP-seq protocol uses sonication prior to immunoprecipitation. As such, you would expect that the RIP-seq pelleted RNA to only be small fragments that correspond to sites of MATR3 occupancy. Consequently, this experiment does not show that the MATR3 transcripts are independent of the gene transcripts that they are imbedded in. Similarly, it is not expected that RIP-seq levels would correlate to gene expression level, as these are two completely orthogonal measures.

Page 5, line 106, missing a word between "which" and "adjacent"
Page 7, line 171, missing word between "29%" and "cells"
Pages 31-2, lines 838, 839 and 841, misspelling of "pallets" (should be pellets)
Page 14, line 335. Should be "on" average

Referee #3:

Authors performed an impressive amount of additional work. Indeed, the new version of the manuscript contains compelling data as weel as a clearer conceptual framework, definitely supporting the main conclusions of the work.

We thank all the referees for their highly insightful comments and constructive suggestions. Based on these comments and suggestions, we have conducted a large number of additional experiments and analyses, and carefully addressed the referees' concerns in our revised manuscript. We belive that our revised manuscript is much improved. Please see below for our point-to-point responses. *Referees' comments* in black and *italic* font; responses in blue font; revisions in the manuscript are highlighted in red.

Referee #1:

In this manuscript, Zhang et al reported that depletion of MATR3 altered the nuclear distribution of the H3K27me3 mark without affecting its protein level and genome-wide binding. Intriguingly, prolonged inhibition of transcription by DRB or amanitin for 12-24 hours or treatments with RNase all abolished MATR3-chromatin association, and drastically altered the nuclear distribution of MATR3 from dispersed nuclear signals into a few spheroidal condensates. Depletion of MATR3 by RNAi in human AML12 cells or acute degradation of MATR3 in mouse ESCs both appeared to affect the nuclear distribution of H3K27me3. Aberrant distribution of H3K27me3 was rescued by the wild-type MATR3, but not by disease-associated MATR3 mutants (F115 or S85C) which form fibre-like condensates in vivo and in vitro. In addition, Hi-C revealed minor defects on compartments (2.3% A-B switches) and altered distal and local interactions (inter- and intra-compartmental, respectively) upon MATR3 depletion.

Interestingly, RIP analysis revealed that MATR3 associates with antisense L1 RNAs (asL1), which is consistent with the previously reported eCLIP analysis. RNA FISH of asL1 showed dispersed distribution that partially overlaps with MATR3 signals throughout the nucleus. GFP-tagged MATR3 formed mesh-like assemblies in the present of AS L1 RNA in vitro. Strikingly, depletion of asL1 RNA by ASO also led to the formation of spheroidal MATR3 condensates, reminiscent of inhibition of transcription or RNase treatment. In addition, depletion of asL1 RNA also appeared to affect the nuclear distribution of H3K27me3. Moreover, TADs that transcribe MATR3-associated asL1s exhibited a decrease on local chromatin contacts, in contrast to an increase for TADs with no expression of asL1s. Together, these results suggest a role for MATR3 and asL1 RNA in chromatin organization.

This paper reported a number of interesting observations; however, it is unclear how MATR3 and asL1 RNA play a role in regulating H3K27m3 distribution and chromatin organization. The authors proposed that MATR3-asL1 RNA forms a gel-like meshwork as a platform for spatial organization of the chromatin. The mechanistic link underlying MATR3-asL1 RNA meshwork and the reported phenotypes remains unexplored in this work.

Response: We thank the referee for these critical and constructive comments. In our revised manuscript, we have added additional data and built up a more clearer action model of MATR3-ASL1 RNA meshwork on 3D chromatin organization (Fig 8). Briefly, MATR3 and *cis*-acting AS L1 RNAs form dynamic meshwork via phase separation, and the chromatin regions with high-AS L1 density could be gathered by the meshwork, including H3K27me3-modified chromatin regions, which enrich for MATR3 associated LINE1 elements nearby. Please see the detailed discuss in our point-to-point response below.

For example, MATR3 and asL1 show partially overlapped, punctuate staining throughout the nucleus and co-phase separated in vitro; however, the direct evidence to show a "gel-like" "meshwork" still lacks.

Response: Descriptions about “gel” or “hydrogel” states in literatures are variable (Bergeron-Sandoval, Safaee et al., 2016) (Kaganovich, 2017), and it is hard to find a well-acknowledged physical parameter to evaluate the “gel-like” property of condensates (Villegas, Heidenreich et al., 2022). However, the common observation is that, the liquid-to-gel phase transition is accompanied by reduced fluidity and protein movement, and *vice versa* (Aguzzi & Altmeyer, 2016). Our new FRAP data showed that, in cell nuclei, the meshwork of full-length MATR3 had a longer half-time (7.57 s vs. 6.09 s) and lower mobile fraction ratio (49.11% vs 87.33%) than the condensate of △RRM2-truncated MATR3, which lost the capability to bind ASL1 RNAs (Fig 4A and B), indicating that the former is closer to a “gel-like” phase than the latter. The new results have been added to the text (Line 296-299).

Partial co-localization is not sufficient to propose MATR3/asL1 as the "platform" for organizing the H3K27me3 mark. In addition, why only H3K27me3 was affected, but not other histone marks? The logic behind this phenotype? I don't find the explanation satisfactory as the authors suggested a higher correlation of MATR3 with the K27me3 mark than all other marks analyzed (Fig S1). Moreover, the redistribution of H3K27me3 and altered Hi-C signals were relatively moderate, compared to drastic changes in MATR3 distribution upon treatments with asL1 ASO or prolonged inhibition of transcription. Thus, despite interesting, I believe the overall conclusion overstated. The authors should seriously consider to tone down their conclusions before this work can be published. The claims, such that this meshwork provides "a partially dynamic platform for chromatin spatial organization", either depletion of MATR3 or asL1 leads to "chromatin reorganization in the nucleus", and ALS-associated MATR3 mutants cause "chromatin reorganization", should be modified to be more specific and accurately represent the experimental results. The last sentence in the abstract is also overstated and should be deleted, particularly considering the controversy of the "nuclear matrix".

Response: We agree that partial co-localization is not sufficient to support a platform role of MATR3/ASL1 meshwork. Our conclusion is now based on multiple pieces of data including: 1) H3K27me3 staining upon MATR3 and ASL1 depletion, as well phenotype rescued by re-expressing WT and △RRM2 MATR3 (Fig 3); 2) Hi-C upon MATR3 RNAi in AML12 and acute degradation in ES cells (Fig 5 and Fig EV4); 3) SAMMY-seq data upon MATR3 knockdown (Fig 6).

As mentioned in our manuscript, H3K27me3 staining displayed the most evident alteration after MATR3 depletion compared to other tested histone marks, and that’s why we took H3K27me3 as a representative mark to probe chromatin changes (Line 116-123). Our new analyses of Hi-C indicated that intra-TAD interactions are more correlated to MATR3 associated ASL1 rather than H3K27me3 (Fig 5H). Therefore, we believe that MATR3 globally regulates chromatin structure by interaction with AS L1 RNAs *in-cis*, not merely H3K27me3 domains. But why H3K27me3 showed greater changes than other histone marks? Integrated analyses of RIP-seq, ChIP-seq and SAMMY-seq data indicated that MATR3 associated ASL1 elements are overlapped or adjacent to H3K27me3 regions, and MATR3/ASL1 could affect the higher-order organization of H3K27me3-modified chromatin *in-cis*. These data have been presented in Figures (Fig 2H and 6H) and text of the revised manuscript (Line 218-222 and Line 445-454).

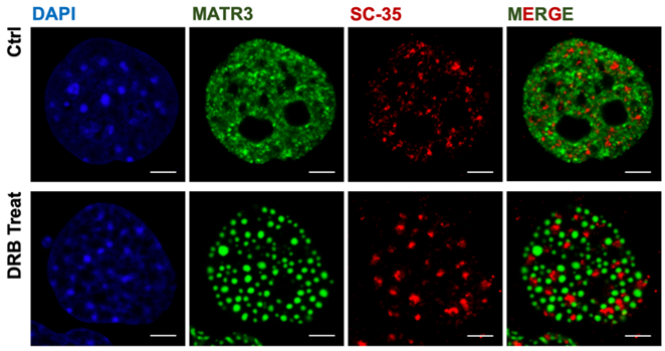
In the light of the referee's comments, we have toned down our conclusions in Abstract and the Text.

Overall, this work highlights a general role of transcription and nuclear RNAs and their binding proteins in chromatin organization. This paper is of an immediate interest and deserves to be published should the above and following concerns be properly addressed.

1. Prolonged treatments with DRB or amanitin for 12-24 hours led to the formation of spheroidal puncta of MATR3. What about the immediate effect of short-term (1-2 hr) treatment? Inhibition of transcription is reported to enlarge nuclear speckles. Could these spheroidal large condensates concentrate the markers of nuclear speckles? On page 7 lines158, the authors should indicate clearly the treatment time (12-24 hours), and discuss an indirect consequence of long-term inhibition of transcription.

Response: We have examined the distribution of MATR3 after DRB treatment at different time points (1h, 2h, 6h, 12h, 24h) (Fig EV2A and B).We observed that about 29% cells showed the spheroidal MATR3 puncta after 1 hours treating with 75 μ M DRB. And with increasing treating time, the percentage of cells containing MATR3 puncta increased (Fig EV2B). The overall RNA decay and the secondary consequences caused by protein synthesis inhibition can both contribute to this phenomenon (Tamm, 1984). We have added on the results and discussion in Line 171-176.

We co-stained the MATR3 and the nuclear speckle marker SC35 before and after DRB treatment, and the data is presented below. We did observe the enlargement of SC35 foci after DRB treatment, but they were not corelated with the MATR3 foci.



2. AS L1 RNAs are mainly from the introns. Possibly, asL1 ASO directly affects nascent transcription through unspliced introns, consequently altering MATR3 localization. Please validate and discuss this possibility. Would other RBPs such as FUS, and other nuclear matrix proteins, like SAF-A/B, also form spheroidal structures after AS L1 ASO? Thus, based on current evidence, the authors cannot conclude that AS L1 functions as 'RNA Glue' for MATR3 proteins in maintaining the meshwork structure.

Response: To test this possibility, we conducted rRNA-off and strand-specific RNA-seq to examine whether transcripts of host genes containing AS L1 in introns were degraded after AS L1 ASO treatment. The expression changes of these genes were subtle and showed no correlation with changes of the AS L1 RNA level (PCC=0.04) (Fig EV3H). Therefore, the phenotypes after AS L1 ASO treatment could be mainly caused by the disruption of AS L1 RNAs, rather than the disturbance of host genes. The data and related discussion have been added on Line 251-256.

Unlike MATR3, SAF-A and SAFB did not form the spherical foci after treating with AS L1 ASO (Fig EV3E and F). Interestingly, FUS proteins kept the dispersed distribution in most nuclear

regions while some of them assembled around the MATR3 foci (Fig EV3G). The protein-protein interaction between FUS and MATR3 (Kamelgarn, Chen et al., 2016) may contribute to this phenomenon.

3. Colocalization of MATR3 and asL1 is partial in both AML12 and ESCs, implying each of them have functions beyond the proposed role in this paper. It seems that sense L1 and antisense B1 also facilitated MATR3 forming insoluble assemblies (Fig 4G). If so, how to explain the specificity of AS L1 in mediating MATR3 meshwork?

Response: Fig 4G showed *in-vitro* droplet formation data, and only the addition of AS L1 RNAs led to meshwork-like structure, similar to the morphology seen in cell nucleus; while sense L1 and other repeat RNAs (sense/antisense B1 and MajSAT) led to the morphology similar to the phenomenon after addition of total RNAs (Appendix Fig S3A). The electrostatic forces drive the random interaction between MATR3 and RNAs, which interfere the interaction between MATR3-IDRs and led to the slight deformation of MATR3 phase. However, AS L1 RNAs contain much higher density of MATR3-binding motifs than sense L1 or sense/antisense B1 (Appendix Fig. S3B). The multivalent interactions between MATR3 and AS L1 RNAs could increase the avidity (the overall affinity between molecules) (Bhat, Honson et al., 2021) and the saturation concentration of system (Maharana, Wang et al., 2018, Wang, Choi et al., 2018), thereby promoting the meshwork-like assembly formation. We have elaborated on this point on Line 320-332.

And if most of AS L1 RNAs are located in gene introns, they function as independent transcripts or parts of gene transcripts? Please discuss.

Response: This is a great point. We conducted RT-PCR to test the independency of the MATR3-associated AS L1 RNAs. For one AS L1 locus, two pairs of primers were used. One targeted the RIP-seq peak (primer 1 or 3), and the other targeted flanking regions at both sides (primer 2 or 4). The former can be detected in MATR3 RIP samples, while the latter were hardly detected (Fig EV2E). Furthermore, we tested the correlation between the enrichment degree of MATR3-asscoiated AS L1 in RIP-seq and the expression level of their host genes. The PCC value is closed to zero (Fig EV2F). Therefore, MATR3-associated AS L1 RNAs are mainly independent RNA fragments, rather than parts of the un-spliced transcripts. We have added the new data in our revised manuscript (Line 194-203).

4. In the abstract, the authors claimed this meshwork-like structure is "gel-like". However, they didn't provide evidence to support their gel-like physicochemical property.

Response: Same question that has been responded above**.**

5. RRM2 deletion mutant was supposed to lack the ability to bind asL1 RNA. What about H3K27me3 distribution in cells expressing RRM2 mutant? In addition, Lines 220-228, the authors performed MATR3 domain deletion and analyzed the colocalization between these deletion mutants and AS L1 to infer the AS L1 binding by MATR3 mutants. I don't think colocalization equalizes association or binding. Without CLIP and EMSA, the author should tone down their conclusions.

Response: We appreciate this constructive suggestion. We conducted immuno-staining of H3K27me3 in AML12 cells expressing full-length and truncated MATR3. The full-length MATR3 can completely rescue changes of H3K27me3 that caused by MATR3 knock-down; the △ZF1, △ZF2 and △RRM1 truncations can partially rescue the phenotypes. However, the H3K27me3 in △RRM2 rescued cells showed no significant difference compared with the

MATR3-depleted cells (Fig 3G and H). We have changed the word “binding” to “interact” in Line 293.

6. Please indicate the half time and mobile fractions for all FRAP experiments.

Response: We have added on the values of half time and mobile fractions in Fig 4B and Fig 7E.

7. The concentration of AS L1 in cells (line 273-283) should be validated by q-PCR.

Response: It is technically challenged to evaluate AS L1 RNA concentration using q-PCR, due to the great heterogeneity of AS L1 elements, especially for those belong to different subfamilies. As an alternative, we evaluated the nuclear concentration of AS L1 RNAs based on the ribosome-off and strand-specific RNA-seq data. The estimated nuclear concentration of AS L1 RNAs is 469 nM. The calculation details have been described in Line 338-340 and Line 807-819.

The majority of AS L1 may come from truncated L1 which locates in intron regions. These AS L1 could be the by-products of their host genes.

Response: Same question that has been responded above**.**

8. The authors should provide the probe sequences for AS L1 RNA FISH.

Response: Probes for AS L1 RNA were designed by ACD company (Advanced Cell Diagnostics, Hayward, CA, USA) based on the consensus sequence on antisense L1_Mus1 RNAs provided by us. We have negotiated with the ACD company, but they refused to provide the probe sequence due to commercial confidentiality. The product code is 1084381-C1, and we have present this in **Methods**.

The consensus sequence of AS L1_Mus1 RNAs is:

UUUAUUAGAUAUUUUCUUUAUUUACAUUUCAAAUGCUAUCCCGAAAGUUCCCUAU
ACCUUCUCCCUGCCCUGCUCCCCUACCCACCCACUCCCACUUCUUGGCCCUGGGGU
UCCCCUGUACUGGGGCAUAUAAAGUUUGCAAGACCUAGGGGCCUCUCUUCCCAAU
GAUGGCCGACUAGGCCAUCUUCUGCUACAUAUGCAGCUAGAGAUAUGAGCUCUGG
UGGUACUGGUCAGUUCAUAUUGUUGUUUCACCUAUAGGGUUGCAGAUCCCUUUAG
CUCCUUGGGUACUUUCUCUAGCUCCUCUAUUGGAGACCCUGUGUUUCAUCUUAUA
GAUGACUGUGAGCGUCCACUUCUGUAUUU

9. The authors used standard deviation (SD) to quantify the distribution pattern for different histone marks. But what does SD mean? Please clarify.

Response: The standard deviation (SD) measures the dispersion degree of fluorescence signal in the nucleus. If the chromatin is equally distributed in nuclei, the SD value will be lower; and if the chromatin preferentially distributes in specific regions, the SD value will be higher. We have added the explanation in Line 118.

10. Line 207-209. "AS L1 RNAs may function as 'RNA Glue' for MATR3 proteins in maintaining the meshwork structure and their association with nuclear chromatin." Intronic regions transcribes most antisense L1 RNA, while the fate of introns is spliced and their half-life is supposed to be very short. What's the half-life of the MATR3-associated AS L1 RNAs and how could they participate nuclear organization since they need to be spliced and have short life-time?

Response: We designed two pairs of primers targeting different MATR3-AS L1 RNAs and generated the decay curves. In AML12 cells, the half-life of the AS L1 RNAs is 2-3 hours (Fig. EV2H), similar to that of Neat1 (2.54h), the architectural lncRNA for paraspeckles. The half-life of Neat1 RNA is close to the results obtained in N2A and 3T3 cells (Clark, Johnston et al., 2012).

Therefore, shorter half-time may not be the barrier for RNAs' function as the scaffold. We have added these results in our revised manuscript (Line 204-207).

11. The author claimed that 88% of AS L1 RNAs are located in intronic regions. In the MATR3 RIP-seq analysis (Fig. C, D and E), the 17.2% RIP-seq peaks in repeats are intergenic or including intronic peaks? Since L1 repeats occupy large proportion of the whole genome compared to other repeats, how about calculate peak density instead of peak counts?

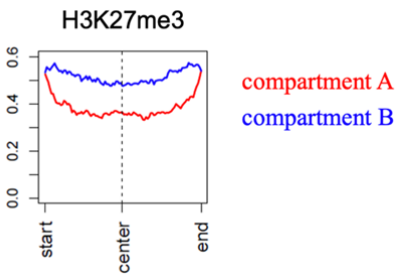
Response: To obtain the Pie chart in Fig 2C, the MATR3 RIP-seq peaks were overlapped with the genomic features. The priority of features are TSS, UTR, exonic, intronic, repeats, intergenic and others. Therefore, the 17.2% RIP-seq peaks in repeats (Fig.2C) are from intergenic regions. The MATR3 RIP-seq analysis in Fig.2D and 2E contained all RIP-Seq peaks. We calculated the peak density and the mean counts of MATR3-associated AS L1 RNAs in AML12 cells, separately. By two means of calculation, AS L1 RNAs ranked first (Fig.2E; Appendix Figure S2D). So did in ES cells (Fig.EV2C; Appendix Figure S2E).

12. The global changes in Hi-C results are not dramatic and not necessarily direct. And the conclusion that "TADs with the higher AS L1 density were more enriched in compartment A regions" is inconsistent with the results that show MATR3-AS L1 meshwork specifically regulates H3K27me3 distribution (Fig 1D, E), please explain.

Response: H3K27me3-modified regions are widely-distributed in genome including A type and B type compartments, especially enriched at the regions close to the border of compartment A and B (Johnstone, Reyes et al., 2020, Rao, Huntley et al., 2014, Zhang, Liu et al., 2021). Our data in AML12 also showed that there are more than half of the H3K27me3 regions locating in compartment A, particually at the boundaies (data showed as below). Furthermore, we clustered TADs into 6 groups according to the density of ASL1 and H3K27me3, and the intra-TAD interactions are more related to ASL1 density rather than H3K27me3(Fig 5H), suggested that MATR3-AS L1 meshwork regulates chromatin architecture globally, not merely for H3K27me3.

The number of H3K27me3 region located in compartment A/B

Number	Type
929	Boundary
1290	Compartment A
792	Compartment B
45	None



13. As gene intron region contains most of antisense L1 repeats, it doesn't make sense that AS L1 RNA FISH colocalized with H3K27me3 instead of active histone marks (Fig 2G).

Response: Besides RNA FISH, our integrated genomic analysis including RIP-seq, ChIP-seq and ATAC-seq indicated that the MATR3-associated AS L1 elements are enriched for H3K27me3 modification, rather than active marks such as H3K27ac and ATAC-seq peaks (Fig.2H). Of note, H3K27me3 is not fully overlapped but mainly enriched on the boundaries of ASL1 elements (Fig.2H, top panel).

To prove that MATR3-AS L1 meshwork regulates H3K27me3 distribution, why not using truncations with or without AS L1 RNAs binding ability to do the rescue experiments.

Response: Please see our response to point 5.

14. Why not use AID cells to capture direct effects of MATR3 degradation on the 3D genome structure? The quality of the figure data on H3K27me3 in MATR3-AID ESCs is poor. Please show high quality data figure.

Response: We thank the referee for this inspiring suggestion. We have conducted Hi-C with MATR3-AID and control ES cells. Although the cell types and experimental systems are different, 3D genome changes are similar in MATR3-AID ES cells (6 hours treatment) and MATR3 RNAi AML12 cells (72 hours treatment) at some important aspects. Particularly, TADs with higher AS L1 density presented more reduction of intra-TAD interactions (Fig EV4B and C), highly consistent with the results from AML12 cells. This data indicated that the decrease of local contacts within TADs is a direct effect upon MATR3 disruption. Furthermore, we have conducted the IF experiments again with MATR3-AID ES cells and replaced it with images of higher quality (Fig 1K).

15. Line 131. Misspell "MATR3".

Response: We have corrected this mistake. Thanks.

Referee #2:

The manuscript by Zhang et al uses a combination of techniques to evaluate the association between MART3 and antisense LINE RNA. Specifically, they demonstrate that LINE antisense RNA contains four MART3-binding motifs, and that MATR3 and antisense LINE RNA co-localize within the nucleus to regions that also preferentially colocalize with H3K27me3. In addition, they demonstrate that in vitro MATR3 undergoes phase-separation, and when incubated with antisense LINE RNA forms a gel-like meshwork. Finally, they demonstrate that the (ALS)-associated MATR3 mutant S85C alters the in vivo dynamics of MATR3. Overall, this manuscript raises an interesting hypothesis regarding the role of MATR3 binding to antisense LINEs in chromatin organization. I have indicated below some specific comments I have about the manuscript.

The authors identify that 88% of MATR3-associated AS L1 RNAs are intronic. As such, these AS L1 RNAs should be contained within spliced transcripts of mRNAs, as opposed to lncRNAs or other species of ncRNAs. Is the model that these spliced introns are being retained at the genomic loci that they were transcribed from via MATR3? This indicates that MATR3-AS L1 RNA interactions are occurring in a fundamentally different manner than traditionally thought of with nrRNAs like TERRA, XIST, etc. The authors need to elaborate on this in more detail.

Response: We thank the referee for these highly constructive comments. We totally agree that MATR3-associated AS L1 RNAs are different from the traditional lncRNAs. We showed that these RNAs are fragments from antisense transcribed L1 elements, rather than parts of the un-spliced transcripts (Fig EV2E and F). The interaction with MATR3 prevent the random spread of AS L1 RNAs (Fig 3C). Genome-wide data indicated that MATR3 associated AS L1 RNAs function as “landmarks” to sustain chromatin interactions *in cis*, including the H3K27me3-modified chromatin (Fig 5H and Fig 6). We have clearly elaborated our model in Fig 8 in our revised manuscript (Line 524-533).

Regarding the ASO results from knocking down L1 antisense transcripts. This experimental design is likely to result in significant off target effects, as this ASO should interfere with any mRNA that contains an intronic L1 element in the antisense orientation. Did the authors perform RNA-seq after treatment to determine potential off target impacts? Also, was this experiment performed using an ASO targeting sense L1 transcripts as a control?

Response: We appreciate these constructive comments. We conducted RNA-seq with ASL1 and control ASO treated cells. The expression changes of ASL1 contained host genes were subtle and had no correlation with changes of the AS L1 RNAs (PCC=0.04) (Fig EV3H). Therefore, the potential off target effects of ASL1 ASO may be not the major factor that causes the observed phenotypes. The data and related discussion have been added on Line 251-256.

We also presented the results after sense L1-targeted ASO treatment in Fig EV3A and B. The treatment with sense L1_Mus1 ASOs (24 hours) caused 10% of cells showing spheroidal MATR3 foci, substantially lower than the ratio caused by antisense L1_Mus1 ASOs treatment (69%). As shown by our RIP-seq data (Fig 2F), although much weaker, MATR3 also interact with sense L1_Mus1 to some extent. As additional controls, ASOs that targeting to MATR3 non-associated AS L1 RNAs (L1_MA7), no matter sense or antisense, showed no effects on MATR3 distribution (Fig EV3A).

In figure 6, the authors highlight 15 mutations in MATR3 that are associated with ALS/FTD/neurodegenerative diseases. They then focus on the S85C and F115C mutations to evaluate PONOR scores and chromatin organization to conclude that ALS-associated mutations in MATR3 are associated with chromatin redistribution. Why were these two variants chosen? What is the PONOR score for all of the other disease-associated MATR3 variants? Do the authors believe that all disease-associated MATR3 variants are acting through a similar mechanism as these two variants?

Response: The ASL related S85C and F115C mutations were reported to have more supportive pedigree data (Chen, Cai et al., 2018) and had been studied the pathogenicity in knock-in mice models (Kao, van Bruggen et al., 2020, van Bruggen, Maksimovic et al., 2021), we thus chose these two mutations for further studies. According to the data in knock-in mice models that developed to mimic ALS-associated MATR3 mutants, S85C mice recapitulated pathological features of ALS, while F115C could not (Kao et al., 2020, van Bruggen et al., 2021). In this study, we revealed that S85C shows greater PONDR score change, more dynamic loss and more effects on the chromatin mis-localization than F115C does, consistent with the results from mice models. We have added the explanation in our revised manuscript (Line 490-493).

The PONDR score changes of other disease-associated MATR3 variants were listed in Appendix Figure S8A. Besides S85C and F115C, other mutations also caused great changes in PONDR score, suggesting that these MATR3 mutations could follow the similar mechanism of action, which needs further studies. We have added the discussion about MATR3's pathogenicity in revised manuscript (Line 589-608).

Regarding their findings that dox-inducible shRNA MATR3 knockdown results in redistribution of H3K27me3 signal, did the authors perform a scrambled shRNA control to ensure that this finding is not merely a result of the dox or a cellular response to the shRNA as opposed to MART3.

Response: We confirmed that the changes of H3K27me3 staining were not resulted from the Dox or the cellular response to the shRNA, as no H3K27me3 alteration was observed in Dox-treated control cells that transfected with the scrambled shRNA (Appendix Fig S1A).

The H3K27me3 results in ES cells after AID degradation of MATR3 need further quantification. Specifically, the authors claim that "After rapid degradation of MATR3, H3K27me3 in ES Cells showed larger and brighter staining near the nuclear periphery". However,

the data to support this is merely an image of a single cell. Please provide further quantification of this finding.

Response: To improve the quality of this image, we performed the experiments again, and obtained same results. We showed representative image of an ES cell clone (consist of several cells) in Ctrl and MATR3-AID cells, separately. And we added on the quantification data in Fig.1K and L.

More generally, it is unclear how the results linking MATR3 with H3K27me3 are pertinent for the larger story of this manuscript. Overall, the imaging studies in Figures 1 and 2 linking MATR3 with H3K27me3 are subject to multiple possible confounders, and do not demonstrate a direct link between these two features at this resolution.

Response: We agree that the resolution of imaging data was not sufficient to link MATR3 with H3K27me3 directly. Besides imaging data, we also conducted genomic assays to investigate the association between these two features. Our integrated analyses with MATR3 RIP-seq and ChIP-seq data indicated that H3K27me3 was enriched at the boundaries of MATR3-associated ASL1 elements (Fig.2H). In addition, we conducted SAMMY-seq, which was reported to be more sensitive to detect the structure changes of heterochromatin including H3K27me3-modified regions (Sebestyen, Marullo et al., 2020). Interestingly, the loss of SAMMY-domains after MATR3 knockdown, accounted for 7% of the genome, are strongly associated with H3K27me3 regions, which are adjacent to MATR3-associated AS L1 elements (Fig 6). These data linked MATR3 and H3K27me3 directly, and also indicated that MATR3 regulates higher-order structure of H3K27me3-modified chromatin by interacting with the *cis*-acting AS L1 RNAs that transcribed nearby.

Page 3, line 50, missing a "has been" between "it suggested"

Page 8, line 212, there is a word missing in the following clause "AS L1 RNAs appeared a more..."

Page 14, line 374, this wording is awkward "we remained to examine"

Page 15, line 401, this wording is awkward "How do the antisense transcripts be regulated?"

Response: We have modified these sentences. Thanks!

Referee #3:

EMBOJ-2022-112823s

- general summary and opinion about the principle significance of the study, its questions and findings

The work by Wen and co-workers addresses the functional role of L1 retrotransposons noncoding transcriptome in nuclear architecture and cell identity homeostasis. Focusing on MATR3, a nuclear matrix protein component, authors identify antisense L1 transcript as a direct interactor. By loss of function experiments authors provide evidence for an association between AS L1 and MATR3 mediated stability of higher order structures and epigenome regulation, in particular on the contribution of PRC2 specific mark H3K27m3. On the mechanistic side, authors propose a role in controlling the biophysical properties of nuclear architecture via phase-separation.

While the findings represent an interesting contribution to the field, the work still lacks a clear explanation of H3K27m3 impairment and its functional implications. Indeed, authors do not investigate the role of PRC2, leaving the story incomplete. Moreover, phase separation may not provide a convincing mechanistic insight, at least not solving the evident cause-consequence issue.

Response: We appreciate these critical and constructive comments. As suggested, we have investigated the regulatory relationship between MATR3 and PcG proteins. As for the mechanism of action, phase separation is just a framework to explain molecule-molecule interactions, and our model was based on multidisciplinary data, which will be discussed in detail below.

- specific major concerns essential to be addressed to support the conclusions.

Previous work (Cesarini et al JCB 2015, Bianchi et al J. Clin Invest. 2018) showed a tight functional relationship between Lamin-A, a major NM component, and Polycomb proteins. Authors should take inspiration from those papers to further investigate the effects on PcG as result of MATR3/ASL1 interaction. Indeed, the effects on TADs and changes in cell specific gene expression should be evaluated looking at PRC2 (and PRC1) stability on chromatin (e.g. ChIPseq, chromatin fractionation, IF). In turn, MATR3-ASL1 interaction should be evaluated also in PRC2 loss of function as well as rescue experiments.

Response: The works accomplished by Cesarini et al and Bianchi et al provided excellent paradigms to investigate the interplay between nuclear matrix and PcG proteins. In these studies, Lamin A/C affect the epigenetic silencing of specific genes by altering chromatin occupancy of PcG proteins. As suggested by the referee, we investigated regulatory relationship between MATR3 and PcG proteins, including the PRC1 component BMI-1 and PRC2 component EZH2. As expected, MATR3 interacts with BMI-1 and PCR2 as indicated by the co-staining and the co-immunoprecipitation data (Fig EV5A and B). After depletion of MATR3, both BMI-1 and EZH2 were redistributed, seen as larger foci in the nucleus (Fig EV5C-E); However, MATR3 depletion did not change the protein level of BMI-1 and EZH2 or their association with chromatin fraction (Fig EV5F); BMI-1 and EZH2 maintained their colocalization with H3K27me3 (Fig EV5G and H). On the other hand, knockdown of BMI-1 or EZH2 had no effects on MATR3 expression and MATR3-AS L1 RNA interaction (Fig EV5I-L). These data suggested that MATR3 regulates the nuclear localization of PcG proteins with H3K27me3, but PcG proteins do not modulate MATR3.

Furthermore, we show that MATR3 knockdown resulted in H3K27me3 redistribution in the nucleus without change the modification level across the genome (Fig 1, Fig EV1G), and depletion of ASL1 abolished the association between MATR3 and H3K7me3(Fig 3A). In addition, LINE1 elements transcribed MATR3 associated ASL1 RNAs locate near H3K27me3 domains (Fig 2H and 6H). Therefore, the MATR3-AS L1 RNA meshwork may function as the physical scaffold gathering H3K3me3-modified chromatin and PRC complexes, rather than participating in the *de novo* epigenetic establishment.

Based on these data, we proposed a model on how does MATR3/ASL1 meshwork regulate 3D chromatin structure. MATR3 organize the higher-order chromatin structure via phase separation with AS L1 RNAs, which function as the landmarks recruiting MATR3 and mediate homogenous clustering of nearby chromatin regions (Fig 8, Line 524-533). As LINE1 elements transcribing AS L1 RNAs intersperse across the genome, this mechanism accounts for the overall chromatin organization, including the H3K27me3-modified region. The interaction between

MATR3 and PcG proteins may further enhanced the association of H3K27me3-modified chromatin with MATR3/ASL1 meshwork.

Association with NM could be analysed by chromatin fractionation experiments. In line with the same approach, higher order structures may be analysed by the recently reported SammySeq technology (Sebestyen et al Nat Comm 2020). This could also be used to follow the compartment association of ASL1 RNA and associated genes.

Response: We highly appreciate this constructive suggestion. We have conducted SAMMY-seq in control and MATR3-depleted AML12 cells. The results were presented in Fig 6 and Line 423-461. Interestingly, upon MATR3 depletion, SAMMY domains loss in 7% of the genome, and these regions are strongly associated with H3K27me3 (Fig 6C-E). Furthermore, H3K27me3 regions overlapped with SAMMY-domains altered the accessibility and the changed region are enriched of MATR3-associated AS L1 RNAs (Fig 6G and H). Differential gene expression tended to occur on the boundaries of changed SAMMY domains (Fig 6J). These new data greatly enhanced our conclusion regarding the regulatory roles of MATR3/ASL1 on higher-order organization of H3K27me3-modified chromatin.

- minor concerns that should be addressed

PRC2 function on chromatin is known to be directly influenced by RNA (Long et al Nat Genet 2020). Authors should address this point by checking PRC2-RNA interactions in MATR3/ASL1 contexts.

Response: We conducted the RIP-qPCR to examine whether MATR3 affect the RNA-binding ability of EZH2. Apart from the well-known EZH2-binding lncRNAs Malat1 and Neat1, we also examined the liver-specific EZH2-binding RNA Hnf1aos1 (Chen et al., 2018, Wang, Xie et al., 2018). The RNAs were enriched in anti-EZH2 RIP samples compared with IgG, while MATR3 depletion did not affect the enrichment (Fig EV5I).

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1st Editorial Decision **1st Jun 2023**

Dear Dr. Wen,

Thank you for the transfer of your manuscript to EMBO reports.

As discussed, we can offer to publish your manuscript provided that you change or remove your model and tone down the claims made, specifically removing claims of any local effect of MATR3 and L1 on chromatin/H3K27me3. Please address all final referee comments in the text, and address the last comment by referee 1 experimentally, if possible (to investigate the interactions between MATR3 mutants and AS L1 RNAs, at least through RNA IP). Please also co-submit a point-by-point response to the last referee comments.

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Referee #1:

Having carefully read the revised manuscript and considered the authors' rebuttal, this reviewer acknowledges that the authors have provided detailed answers to most of the questions and performed additional experiments. However, while the paper reports interesting functional correlations between MATR3 and AS L1 RNA, the presented data is insufficient to support the final model. Additionally, the authors' explanations of the mechanism rely heavily on assumptions and lack critical evidence. Overall, it appears that the authors' conclusion is based on biased wishful thinking. This reviewer suggests that the authors revise the manuscript and modify their model by considering and discussing alternative explanations for their experimental results. For instance, the disruption of MATR3 or AS-L1 may inhibit transcription, indirectly altering the organization of H3K27me3 marked chromatin. Two specific comments are outlined below.

Response: As suggested, we have modified our model and discussed the possibility of indirect effect by MATR3 or AS-L1 disruption (line 525-535).

The revised manuscript introduces the use of SAMMY-seq as an attempt to support the notion that the MATR3/ASL1 meshwork may act as an organizing platform in the cell nucleus. However, there are several issues regarding SAMMY-seq that require clarification. Firstly, the authors should explain the S1-S4 fractions' representation in terms of nuclear fractions or structures. Secondly, in the large-scale analysis presented in Figure 6B, the regions that are enriched in S4 versus S2 seem to be depleted of H3K27me3. This raises questions about what kind of heterochromatin is primarily retained in S4 fractions. It would be helpful if the authors could include a western blot of H3K27me3 and H3K9me3 in the S1-S4 fractions in Figure 6A to address this concern.

Response: We have explained these in our manuscript: the CSK buffer-soluble fraction (S1), DNase I-sensitive fraction (S2), DNase I-resistant fraction (S3), and the high-salt resistant fraction (S4) (line 428-430).

As shown in Figure 6E, H3K27me3 is depleted in the center but enriched at the boundaries of SAMMY domains (S4/S2). According to the literature, SAMMY domains (S4/S2) detect heterochromatin structure including H3K9me3 and H3K27me3 (Sebestyen, Marullo et al., 2020). We have clarified this in our manuscript (line 430-432).

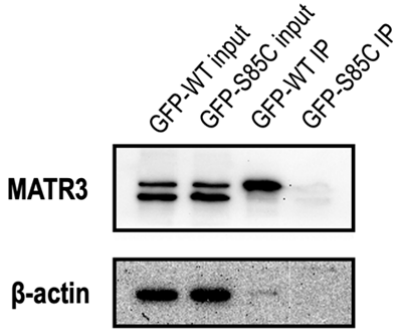
Thirdly, the observed 7% genome loss and 4% genome gain in S4 cannot fully explain the changes in overall genome organization. Fourthly, as depicted in Fig 2H, H3K9me2 and H3K9me3 ChIP-seq signals are also enriched in MATR3-associated AS-L1 regions. Therefore, the proximity of H3K27me3 and AS L1 in linear genomic distance alone may not be sufficient to explain the specific changes in H3K27me3 upon MATR3 KD. These issues raise doubts about the proposed model of MATR3/ASL1 meshwork acting as a platform to organize nearby H3K27me3 regions.

Response: As indicated by literatures (Jerkovic & Cavalli, 2021, Kempfer & Pombo, 2020, Quinodoz, Ollikainen et al., 2018) , imaging-based and genomic approaches may be more sensitive to detect different aspects of the nuclear architecture. In our study, the H3K27me3 localization changed upon MATR3 depletion as indicated by IF (Figure 1), and H3K27me3 domains showed changes in structure as detected by SAMMY-seq (signal changed not only in lost or gain domains, but also in common domains) (Figure 6G). Therefore, both imaging and genomic data demonstrate an alteration of H3K27me3. We agree that linear genomic distance alone may be insufficient to explain the changes in H3K27me3, and we have toned down our conclusion and improved our model by removing statements such as *in-cis* and local interactions.

The authors stated in their manuscript that AS L1 RNAs act locally (i.e., cis-acting) and can form R-loop or RNA:DNA triplex structures in various genomic L1 loci. However, since AS L1 RNA has a similar half-life to Neat1 RNA, which has been shown to have trans-acting effects (as demonstrated in Figure EV2G), there is a possibility of trans-acting effects for AS L1 RNA as well. To support this possibility, the authors presented two cases where MATR3 interacts

with independent AS L1 RNA fragments located within introns. This suggests that MATR3 binds to spliced intronic AS L1 RNAs that are likely to exit the transcription sites after splicing. The question then arises as to how the MATR3/AS L1 RNA network functions locally to affect nearby H3K27me3-marked chromatin.
Response: Yes, it is not easy to fully exclude the possibility of trans-acting function of ASL1 RNAs, we have added this in the discussion of the manuscript (line 540).

Additionally, it would be worthwhile to investigate the interactions between MATR3 mutants and AS L1 RNAs, at least through RNA immunoprecipitation (RIP).
Response: This is a good suggestion. However, our previous experiment showed that the solubility of MATR3 mutant decreased and cannot be successfully pulled-down by IP (figure below). This is consistent with the observation that the mobility of MATR3 mutants decreased (Fig 7D-F).



Referee #2:
Overall, I think that this paper has improved with revision. Specifically, the wording of this paper relative to their correlation of their MATR3 findings and the H3K27me3 domains is significantly improved, as this association still has tenuous data demonstrating a causative link as opposed to a mere correlative link.

The authors added Figure EV2E in response to my concern that the AS L1 RNAs are located in gene introns and as such it is unclear if they function independently of their originating transcripts. However, the experiment outlined in Figure EV2E is not appropriate to address whether these AS L1 RNAs are actually independent of their parent genes encoding them. Specifically, per their methods, the RIP-seq protocol uses sonication prior to immunoprecipitation. As such, you would expect that the RIP-seq pelleted RNA to only be small fragments that correspond to sites of MATR3 occupancy. Consequently, this experiment does not show that the MATR3 transcripts are independent of the gene transcripts that they are imbedded in. Similarly, it is not expected that RIP-seq levels would correlate to gene expression level, as these are two completely orthogonal measures.
Response: Our RIP protocol includes a step of mild sonication. The input, the starting material of RIP experiments, underwent the same sonication process; however, as shown in Fig EV2E, the larger bands can be detected by RT-PCR with input but not those with the RIP samples, indicating that larger RNA fragments remain in the input after sonication. However, as our data cannot fully exclude the possibility that MATR3 interacts with ASL1-containing long RNAs, we have toned down our conclusion in the revised manuscript (line 206-208).

Page 5, line 106, missing a word between "which" and "adjacent"
Page 7, line 171, missing word between "29%" and "cells"
Pages 31-2, lines 838, 839 and 841, misspelling of "pallets" (should be pellets)
Page 14, line 335. Should be "on" average
Response: We have corrected these errors.

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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.	Yes	Materials and Methods
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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.	Not Applicable	

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?	Not Applicable	

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	Results, Materials and methods
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
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.	Yes	Figure legends, Materials and methods
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	Figure legends

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
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure legends

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	Data Availability Section
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?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	