

Coordinated demethylation of H3K9 and H3K27 is required for rapid inflammatory responses of endothelial cells

Yoshiki Higashijima, Yusuke Matsui, Teppei Shimamura, Ryo Nakaki, Nao Nagai, Shuichi Tsutsumi, Yohei Abe, Verena Link, Mizuko Osaka, Masayuki Yoshida, Ryo Watanabe, Toshihiro Tanaka, Akashi Taguchi, Mai Miura, Xiaolan Ruan, Guoliang Li, Tsuyoshi Inoue, Masaomi Nangaku, Hiroshi Kimura, Tetsushi Furukawa, Hiroyuki Aburatani, Youichiro Wada, Yijun Ruan, Christopher Glass and Yasuharu Kanki.

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision

4th Dec 2019

Thank you for submitting your manuscript reporting a role for KDM7A and UTX in NF- κ B dependent regulation of inflammatory genes for consideration by The EMBO Journal. We have now received two referee reports on your study, which are included below for your information.

As you will see, the reviewers are overall positive and acknowledge the interest in the findings and the extent of the analysis. However, both referees raise some concerns that would need to be addressed in a revised version of the manuscript. In addition to the specific points, the referees also find that the manuscript should be restructured to put a more clear focus on the key findings of the study, in particular that the discussion should be shortened. To address this issue, you should also consider moving experiments and data less crucial for the overall understanding of the conclusions to expanded view (EV) figures or an appendix. Thus we would now like to invite you to prepare and submit a revised version of the manuscript. Please note that it is our policy to allow only a single round of major revision and that it is therefore important to clarify all key concerns raised at this stage.

REFeree REPORTS

Referee #1:

In this study, the authors investigated the role of KDM7A and UTX and TNF- α -induced chromatin interactions and gene transcription regulation. They started by analyzing TNF- α -induced changes in

gene transcription and miRNAs after 4 and 24h of TNF- α -stimulation. These analyses confirmed many of previously reported TNF- α -regulated genes, and miRNAs, and identified several new ones. Of these, they tested the functional relevance of the three most downregulated miRNAs on TNF- α -induced adhesion-regulating genes. They showed that overexpression of miR-3679-5p caused downregulation of several key adhesion regulating genes, including VCAM1 and ICAM1, and reduced binding of monocytes to endothelial cells. To identify the putative target of miR-3679-5p, they performed unbiased RNA immunoprecipitation-Chip analyses, and identified the demethylases UTX and KDM7A as putative targets. Using various assays, they confirmed that overexpression of miR-3679-5p reduced the expression of UTX and KDM7A. Through knockdown and RNA-seq analyses they showed that knockdown of UTX and KDM7A impaired upregulation of many TNF- α -induced mRNAs. Consistent with this, knockdown of UTX and KDM7A impaired TNF- α -induced upregulation of adhesion-promoting genes and impaired monocyte adhesion to ECs. Chromatin binding mapping showed that UTX and KDM7A co-occupy p65 binding sites, and their binding at key TNF- α -regulated genes was increased in response to TNF- α stimulation. TNF- α stimulation decreases H3K9me3 and H3K27me3, and knockdown of KDM7A and UTX prevented TNF- α -induced demethylation of these marks at key target genes. Their results implicated the demethylases in the formation of TNF- α -induced super-enhancer (SE). ChIA-PET analysis of active RNA Pol2 showed that TNF- α -stimulation increased chromatin interactions at TNF- α specific SE that are bound by KDM7A and UTX, indicating their role in TNF- α -induced SE formation. Further analyses revealed that TNF- α stimulation increased chromatin interactions at sub-TAD level, at the regulated genes. Finally, their data suggest a catalytic role of demethylases in leukocyte adhesion in mouse model.

This is an interesting manuscript with massive amount of high-quality data. The work provides important mechanistic insights into the role of KDM7A and UTX-dependent demethylation in TNF- α -induced chromatin interactions and transcription regulation. In particular, it is interesting that rapid demethylation is important for early steps of TNF- α -induced transcription regulation. While one can always ask for more experiments, the manuscript already includes a large amount of data, and importantly, the main conclusions are adequately supported by the presented data. Thus, the manuscript can be considered for publication with minor changes.

Specific points:

- The manuscript is excessively long; for example, the discussion section is more than 5 pages long, and starts to read almost like a review article. It would be better if the authors primarily focus on discussing the novelty of the presented findings, and their implications in TNF- α -regulated transcription regulation. Shortening of the text will help to better focus on the key findings of the manuscript.
- It would be good if the authors could more discuss previous findings that suggested that the demethylase activity of UTX is dispensable for its function in enhancer-regulated gene expression in other experimental cell systems.
- Is demethylation of both H3K9me3 and H3K27me3 similarly important for TNF- α -induced gene expression, or one of them is important than the other?
- In the results section, it would be helpful to clarify the molecular target of BAY 11-7082.

Referee #2:

In this manuscript Higashijima et al describe the role of two demethylases in acute inflammatory responses in endothelial cells. The authors first scan for microRNAs that regulate the expression of adhesion genes in human endothelial cells. Indeed they identify miR-3679-5p as a regulator of KDM7A and UTX demethylases. Based on expression assays, they then show that KDM7A and UTX are essential for the induction of NF- κ B target genes after TNF α stimulation of endothelial cells. Next the authors demonstrate with CHIP-seq analysis that p65 co-localizes with KDM7A and UTX at multiple genomic regions and that binding of the demethylases precedes the binding of the NF- κ B transcription factor. The authors then characterize the functional role of KDM7A and UTX by Chip-seq for H3K9me2 and H3K27me3 as methylation marks. They see that KDM7A and UTX are essential for removing repressive methylation marks from adhesion genes in endothelial cells after inflammatory stimulation. They then call SEs and find that KDM7A and UTX are co-localized

in SEs and a number of SNPs can be found in or close to these regions. They also characterize chromosome conformation and active interactions in endothelial cells during inflammatory stimulation by HiC and ChIA-PET for active RNAP. Finally, they show that KDM7A and UTX are important for leukocyte adhesion by performing *in vivo* experiments.

In general this paper has an impressive amount of work and it is obvious that the authors have put a lot of effort and time on this manuscript. The findings are novel enough and technically this paper is impressive. Additionally the manuscript is very well written and easy to follow. Indeed till the middle of the manuscript the experiments are logical, thorough with all the right controls and the outcome of these experiments provides information helpful for the major message of this manuscript. However, towards the end the authors use many techniques, for example HiC and ChIA-PET that provide some descriptive information which is really not connected with the function of KDM7A and UTX. Although it would be helpful to connect these experiments with the KDM7A and UTX function by including experiments with KDM7A and UTX knockouts, the manuscript is already so data dense that this may not be absolutely fair.

Major points

-As mentioned above the manuscript includes many lines of investigation that are not followed up thoroughly.

1. Mapping of SNPs to SEs that KDM7A and UTX co-localize. This is an interesting idea with an important outcome. However, it is almost randomly placed in one paragraph within the manuscript. The authors do not continue to investigate this at all. If the authors want to include this information they may choose to perform some luciferase assays to see how the SNPs affect adjacent genes. They may also explore whether the function of the SNPs is affected by KDM7A and UTX knockouts. Since this information is probably not the most important for this manuscript they may keep this as a discussion point.

2. I have similar thoughts for the ChIA-PET and HiC parts of the manuscript. Here the authors provide only descriptive information about how the 3D interactions are affected by TNF α stimulation. However, they never connect these assays to KDM7A and UTX. Does knockout of KDM7A and UTX affect chromosome conformation and active interactions?

I will say again that I recognize that this paper has already a lot of data so maybe the authors will opt in shrinking these parts that are not entirely connected with the main message of the paper.

-The *in vivo* experiments are extremely interesting for this paper. Can the authors try their drugs on atherosclerosis or other models?

Minor comments

-The authors could include the information about the miR-3679-5p as regulator of KDM7A and UTX in the abstract.

-The authors should diminish the discussion.

-In the heatmap on Figure 3F, H3K27ac seems to be present before TNF stimulation. How can the authors explain this?

Author's responses to the reviewer's comments

Referee #1:

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adhesion regulating genes, including VCAM1 and ICAM1, and reduced binding of monocytes to endothelial cells. To identify the putative target of miR-3679-5p, they performed unbiased RNA immunoprecipitation-Chip analyses, and identified the demethylases UTX and KDM7A as putative targets. Using various assays, they confirmed that overexpression of miR-3679-5p reduced the expression of UTX and KDM7A. Through knockdown and RNA-seq analyses they showed that knockdown of UTX and KDM7A impaired upregulation of many TNF- α -induced mRNAs. Consistent with this, knockdown of UTX and KDM7A impaired TNF- α -induced upregulation of adhesion-promoting genes and impaired monocyte adhesion to ECs. Chromatin binding mapping showed that UTX and KDM7A co-occupy p65 binding sites, and their binding at key TNF- α -regulated genes was increased in response to TNF- α stimulation. TNF- α stimulation decreases H3K9me3 and H3K27me3, and knockdown of KDM7A and UTX prevented TNF- α -induced demethylation of these marks at key target genes. Their results implicated the demethylases in the formation of TNF- α -induced super-enhancer (SE). ChIA-PET analysis of active RNA Pol2 showed that TNF- α -stimulation increased chromatin interactions at TNF- α specific SE that are bound by KDM7A and UTX, indicating their role in TNF- α -induced SE formation. Further analyses revealed that TNF- α stimulation increased chromatin interactions at sub-TAD level, at the regulated genes. Finally, their data suggest a catalytic role of demethylases in leukocyte adhesion in mouse model.

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We are very grateful for the reviewer's positive comments and suggestions to help enhance the impact of our study. In response to the points and suggestions raised by the reviewer, we have substantially revised the paper. Our point-by-point responses to the reviewer's comments are as follows:

Specific points:

- The manuscript is excessively long; for example, the discussion section is more than 5 pages long, and starts to read almost like a review article. It would be better if the authors primarily focus on discussing the novelty of the presented findings, and their implications in TNF- α -regulated transcription regulation. Shortening of the text will help to better focus on the key findings of the manuscript.

We highly appreciate the reviewer's comment on this point. According to the advice, we have shortened the discussion and tried to focus on the key findings of our manuscript. As a consequence, total words of the discussion were reduced from 2,285 to 1,616 (p.17-20 in the revised MS).

- It would be good if the authors could more discuss previous findings that suggested that the demethylase activity of UTX is dispensable for its function in enhancer-regulated gene expression in other experimental cell systems.

- It demethylation of both H3K9me3 and H3K27me3 similarly important for TNF- α -induced gene expression, or one of them is important than the other?

We highly appreciate these two comments. As described in the discussion, KDM7A and UTX control large and small numbers of NF- κ B dependent genes, respectively, in TNF- α -treated human ECs, which is consistent with the results that KDM7A is recruited to many more TNF- α -induced gene loci than UTX. Thus, we are now considering that in comparison with UTX, KDM7A seems to play a dominant role for regulating inflammatory responses in human ECs (i.e. H3K9me2 could be more important role during inflammatory responses). In line with this, recent studies have demonstrated that genes involved in immune responses were more likely targeted by H3K9me2 than H3K27me3 during early mouse development (Zylicz *et al*, 2015) and UTX H3K27me3 demethylase activity might not be required for conversion of inactive enhancer in mouse ESCs to active enhancer during differentiation (Wang *et al*, 2017). We have put this sentence “In line with this, a recent study” into the discussion section (p.18, lines 19-23 in the revised MS).

- In the results section, it would be helpful to clarify the molecular target of BAY 11-7082.

In accordance with the reviewer’s comments, we have included the information about the molecular target of BAY 11-7082 in the result section (p.10, lines 10-11 in the revised MS).

Referee #2:

In this manuscript Higashijima et al describe the role of two demethylases in acute inflammatory responses in endothelial cells. The authors first scan for microRNAs that regulate the expression of adhesion genes in human endothelial cells. Indeed they identify miR-3679-5p as a regulator of KDM7A and UTX demethylases. Based on expression assays, they then show that KDM7a and UTX are essential for the induction of NF- κ B target genes after TNF α stimulation of endothelial cells. Next the authors demonstrate with CHIP-seq analysis that p65 co-localizes with KDM7A and UTX at multiple genomic regions and that binding of the demethylases precedes the binding of the NF- κ B transcription factor. The authors then characterize the functional role of KDM7A and UTX by Chip-seq for H3K9me2 and H3K27me3 as methylation marks. They see that KDM7A and UTX are essential for removing repressive methylation marks from adhesion genes in endothelial cells after inflammatory stimulation. They then call SEs and find that KDM7A and UTX are co-localized in SEs and a number of SNPs can be found in or close to these regions. They also characterize chromosome conformation and active interactions in endothelial cells during inflammatory stimulation by HiC and ChIA-PET for active RNAP. Finally, they show that KDM7A and UTX are important for leukocyte adhesion by performing in vivo experiments.

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descriptive information which is really not connected with the function of KDM7A and UTX. Although it would be helpful to connect these experiments with the KDM7A and UTX function by including experiments with KDM7A and UTX knockouts, the manuscript is already so data dense that this may not be absolutely fair.

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Major points

-As mentioned above the manuscript includes many lines of investigation that are not followed up thoroughly.

1. Mapping of SNPs to SEs that KDM7A and UTX co-localize. This is an interesting idea with an important outcome. However, it is almost randomly placed in one paragraph within the manuscript. The authors do not continue to investigate this at all. If the authors want to include this information they may choose to perform some luciferase assays to see how the SNPs affect adjacent genes. They may also explore whether the function of the SNPs is affected by KDM7A and UTX knockouts. Since this information is probably not the most important for this manuscript they may keep this as a discussion point.

We highly appreciate the reviewer's comment on this point. As the reviewer pointed out, it would be needed to perform additional experiments such as genome editing experiments to determine the target gene of the regulatory element at SNPs (Gupta *et al*, 2017). We think over again and the relationship between vascular diseases related SNPs and inflammatory SEs is not the key finding of this manuscript. Accordingly, we have followed the reviewer's suggestion to delete GWAS analysis from the result section. Because the discussion section was long as the reviewer pointed out (although we have shortened as described below), we have also excised the descriptions about GWAS and SNP completely from the discussion section.

2. I have similar thoughts for the ChIA-PET and HiC parts of the manuscript. Here the authors provide only descriptive information about how the 3D interactions are affected by TNF α stimulation. However, they never connect these assays to KDM7A and UTX. Does knockout of KDM7A and UTX affect chromosome conformation and active interactions? I will say again that I recognize that this paper has already a lot of data so maybe the authors will opt in shrinking these parts that are not entirely connected with the main message of the paper.

We have demonstrated the possibility of rapid chromatin conformation change under TNF- α -stimulation on HUVECs by 4C-seq (Papantonis *et al*, 2012). However, other group showed not only large TADs but also small TADs have not drastically changed under same signaling pathway (TNF- α) and same cell lines (HUVECs) by using Hi-C (Jin *et al*, 2013). In this manuscript, we investigated the sub-TADs level conformational changes around inflammatory response genes by two independent 3C-derived method, ChIA-PET and in situ Hi-C. As shown in Fig 5 and 6 (old MS), ChIA-PET and in situ Hi-C have demonstrated the same important result. It is meaningful that the results of two methods were identical, but we moved in situ Hi-C results to expanded figures (Fig EV5) in the revised manuscript because those data only supported ChIA-PET data. In addition, we have not tested whether knockdown of KDM7A and UTX affected these chromatin loop change. Although it may be of interest to show Hi-C data under KDM7A

and UTX knockdown, our main result is ‘SE-SE interactions have rapidly formed within sub-TADs level under inflammatory signal on vascular endothelial cells’. Given that our manuscript has a lot of data as you pointed out, the relationship between two histone modifying enzymes and chromatin looping would be resolved in the next work.

3. The *in vivo* experiments are extremely interesting for this paper. Can the authors try their drugs on atherosclerosis or other models?

We appreciate and agree the reviewer’s comment on this point. As the reviewer know, atherosclerosis models require long term experiments (i.e. 10 weeks) (Brown *et al*, 2014). In the preliminary experiment, we administered Daminozide and GSK-J4 to wild-type mice (not ApoE^{-/-} or LDL-R^{-/-} mice), but we observed chronic toxic effects, which would make it difficult to assess the drug effect on atherosclerosis accurately. Indeed, several mice died during the preliminary experiment (within 2-3 weeks). We think that these toxicities are due to the ubiquitous expression of KDM7A and UTX. It will be needed to make EC-specific knockout mice or develop the system specifically delivered drugs to endothelial cells. We think that this is out of scope of our current manuscript and warrants further investigation.

Minor comments

-The authors could include the information about the miR-3679-5p as regulator of KDM7A and UTX in the abstract.

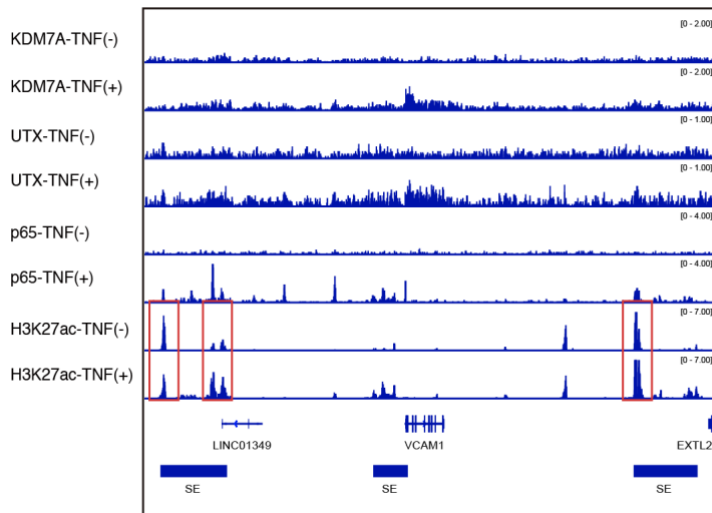
In accordance with the reviewer’s comments, we have included the information about the miR-3679-5p in the abstract section (p.3, lines 4-7 in the revised MS).

-The authors should diminish the discussion.

We thank the reviewer’s comment. According to the advice, we have shortened the discussion to help to better focus on the key findings of the manuscript. As a consequence, total words of the discussion were reduced from 2,285 to 1,616 (p.17-20 in the revised MS).

-In the heatmap on Figure 3F, H3K27ac seems to be present before TNF stimulation. How can the authors explain this?

We highly appreciate the reviewer’s comment on this point. As the reviewer pointed out, H3K27ac can be present at KDM7A- and UTX-binding sites before TNF- α -stimulation (Fig 3F) although Fig 3G demonstrated H3K27ac seemed to be barely enriched at *VCAMI* loci before TNF- α -treatment. However, when we looked at *VCAMI* loci with lower magnification, H3K27 mark was substantially enriched in KDM7A- and UTX-binding SEs (see below figure, red boxes) either TNF- α -stimulated or unstimulated ECs. That might be why H3K27ac could be present before TNF- α -stimuli when ChIP-seq data was analyzed genome-widely as Fig 3F. In consistent with this, a previous study (similar heatmap) has shown that H3K27ac marks was substantially present before TNF- α -stimuli at NF- κ B-binding site (Brown *et al*, 2014). Otherwise we could also speculate that a certain level of active histone mark (i.e. H3K27ac) before TNF- α -stimuli might be required for rapid formation of SEs during inflammatory responses although functional meanings of pre-deposited active histone marks merit further investigation.



Brown JD, Lin CY, Duan Q, Griffin G, Federation A, Paranal RM, Bair S, Newton G, Lichtman A, Kung A *et al* (2014) NF-kappaB directs dynamic super enhancer formation in inflammation and atherogenesis. *Mol Cell* 56: 219-231

Gupta RM, Hadaya J, Trehan A, Zekavat SM, Roselli C, Klarin D, Emdin CA, Hilvering CRE, Bianchi V, Mueller C *et al* (2017) A Genetic Variant Associated with Five Vascular Diseases Is a Distal Regulator of Endothelin-1 Gene Expression. *Cell* 170: 522-533 e15

Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, Yen CA, Schmitt AD, Espinoza CA, Ren B (2013) A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* 503: 290-4

Papantonis A, Kohro T, Baboo S, Larkin JD, Deng B, Short P, Tsutsumi S, Taylor S, Kanki Y, Kobayashi M *et al* (2012) TNFalpha signals through specialized factories where responsive coding and miRNA genes are transcribed. *EMBO J* 31: 4404-14

Wang SP, Tang Z, Chen CW, Shimada M, Koche RP, Wang LH, Nakadai T, Chramiec A, Krivtsov AV, Armstrong SA *et al* (2017) A UTX-MLL4-p300 Transcriptional Regulatory Network Coordinately Shapes Active Enhancer Landscapes for Eliciting Transcription. *Mol Cell* 67: 308-321 e6

Zylicz JJ, Dietmann S, Gunesdogan U, Hackett JA, Cougot D, Lee C, Surani MA (2015) Chromatin dynamics and the role of G9a in gene regulation and enhancer silencing during early mouse development. *eLife* 4

2nd Editorial Decision

22nd Jan 2020

Thank you for submitting your revised manuscript for our consideration, it has now been seen once more by the original referees (see comments below). I am pleased to say that the referees find that their concerns have been satisfactorily addressed and now support publication.

REFeree REPORTS

Referee #1:

The authors adequately addressed my concerns, and I am happy to recommend its publication.

Referee #2:

The authors have addressed all my comments. This is an exciting manuscript, ready for publication.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓
PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Christopher K. Glass and Yasuharu Kanki

Journal Submitted to: EMBO Journal

Manuscript Number: EMBOJ-2019-103949

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was determined based on the previous experience with specific experimental setup. Experiments were conducted with cell lines or laboratory animals with multiple available biological replicates.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Animal numbers were kept at a minimum to repeatedly observe a result. Appropriateness of the animal sizes were evaluated by IACUC at the University of Tokyo.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded from analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	Drugs were administered to mice in a blind manner (by a separate operator).
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The key experiments were performed by at least two independent investigator. In vitro adhesion assays, multiple fields were automatically photographed at random and quantification was also automatically performed.
4.b. For animal studies, include a statement about blinding even if no blinding was done	As described above, drugs were administered to mice in a blind manner. In addition, procedure and analysis of intravital microscopy (IVM) were also performed in a blinded fashion.
5. For every figure, are statistical tests justified as appropriate?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Statistical differences were analyzed by the Tukey-Kramer test for multiple comparisons. Differences between two groups were compared by the Student's t test. In all tests, differences with P values of <0.05 were considered statistically significant.
Is there an estimate of variation within each group of data?	Data were presented including standard deviation (SD) or standard error (SE).
Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

<http://www.antibodypedia.com>
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<http://www.mrc.ac.uk/Ourresearch/Ethic-researchguidance/Useofanimals/index.htm>
<http://ClinicalTrials.gov>
<http://www.consort-statement.org>
<http://www.consort-statement.org/checklists/view/32-consort/66-title>

<http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tum>
<http://datadrivad.org>

<http://figshare.com>

<http://www.ncbi.nlm.nih.gov/gap>
<http://www.ebi.ac.uk/ega>

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<http://biomodels.net/miriam/>
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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
<http://www.selectagents.gov/>

<p>6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).</p>	<p>Certified and company-validated antibodies were purchased and used in this study except for anti-active polymerase II antibody. Anti-active polymerase II antibody has been validated in these previous studies (PMID:24797675, 23103767). We provided the antibody information as follows. VCAM1 Abcam Cat#ab134047; RRID:AB_2721053 ICAM1 Cell Signaling Technology Cat#4915; RRID:AB_2280018 SELE Santa Cruz Biotechnology Inc. Cat#sc-14011; RRID:AB_2186684 β-actin Sigma-Aldrich Cat#A1978; RRID:AB_476692 Goat Anti-Mouse IgG (H+L)-HRP Conjugate Bio-Rad Cat#1721011; RRID:AB_11125936 Goat Anti-Rabbit IgG (H+L)-HRP Conjugate Bio-Rad Cat#1706515; RRID:AB_11125142 EIF2/AGO2 MBL Cat#RN003M; RRID:AB_10694871 Mouse IgG2a Isotype Control MBL Cat#MO76-3; RRID:AB_593055 p65 for CHIP qPCR Santa Cruz Biotechnology Inc. Cat#sc-8008; RRID:AB_628017 p65 for CHIP-seq Abcam Cat#ab7970; RRID:AB_306184 FLAG M2 Sigma-Aldrich Cat#F3615; RRID:AB_259529 H3K4me3 MAB Institute Cat#MAB10304; RRID:AB_11123891 H3K27ac MAB Institute Cat#MAB10309; RRID:AB_11126964 H3K9me2 MAB Institute Cat#MAB10307; RRID:AB_11124951 H3K27me3 MAB Institute Cat#MAB10323; RRID:AB_11123929 active RNA polymerase II Gift from Dr. Kimura Pd75C9</p>
<p>7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.</p>	<p>HUVECs and U937 were purchased from Lonza Japan Ltd. (Tokyo, Japan) and the JCRB Cell Bank (Osaka, Japan), respectively and not maintained more than 6 months prior to returning to low passage stocks. Cell lines were routinely tested for mycoplasma contamination. No commonly misidentified cell lines were used in this study.</p>

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

D- Animal Models

<p>8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.</p>	<p>Male C57BL/6N mice aged 10 weeks were purchased from Japan SLC (Shizuoka, Japan). The animals were housed in individual cages in a temperature- and light-controlled environment and had ad libitum access to chow and water.</p>
<p>9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.</p>	<p>All mouse experiments were approved by The University of Tokyo Animal Care and Use Committee (approval number; H29-1).</p>
<p>10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.</p>	<p>Yes, we confirmed.</p>

E- Human Subjects

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

F- Data Accessibility

<p>18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'.</p> <p>Data deposition in a public repository is mandatory for:</p> <ol style="list-style-type: none"> Protein, DNA and RNA sequences Macromolecular structures Crytalographic data for small molecules Functional genomics data Proteomics and molecular interactions 	<p>The array and sequence data can be accessed through the Gene Expression Omnibus (GEO) under the NCBI accession number GSE121522.</p>
<p>19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).</p>	<p>N/A</p>
<p>20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).</p>	<p>N/A</p>
<p>21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomedels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.</p>	<p>N/A</p>

G- Dual use research of concern

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