

Spatial polarization of endothelial ICAM 1 governs T cell exclusion in melanoma

Minah Kim, Ha-Ram Park, Shawn Kim, Nashat Zaman, Nika Kozlov, Somnath Tagore, Lawrence Wu, and Benjamin Izar

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3rd Nov 2025

Decision on your manuscript EMM-2025-22887

Dear Dr. Kim,

Thank you for submitting your manuscript to EMBO Molecular Medicine. I have now read your article carefully and discussed it with the other members of our editorial team. I am sorry to inform you that we find that the manuscript is not well suited for publication in EMM, and that we therefore have decided not to proceed with peer review. However, our colleagues from our sister journal Life Science Alliance would be happy to offer peer review if you were to transfer your manuscript there. Life Science Alliance is an open access journal launched in partnership between EMBO Press, Rockefeller Press, and Cold Spring Harbor Laboratory Press, and publishes work that is of high value to the respective communities across all areas in the life sciences.

Your study uses transcriptomic analysis of endothelial cells isolated from tumor cores versus peripheries in a mouse melanoma model to investigate the vascular mechanisms that govern intratumoral T-cell positioning. Similar to human melanoma tumors, these murine tumors exhibited a progressive emergence of T-cell exclusion with tumor growth, accompanied by the accumulation of CD8+ T cells at the tumor periphery. This process paralleled ICAM-1 upregulation in endothelial cells. Targeting ICAM-1 with an antibody slowed tumor growth and improved the balance of CD8+ T cells between the tumor core and periphery. While we appreciate your finding that endothelial ICAM-1 regulates T cell infiltration during melanoma progression, unfortunately, the manuscript does not further develop the translational applications, which detracts from its consideration for publication in EMBO Molecular Medicine. Consequently, we cannot consider your manuscript for publication in our journal.

That being said - and as mentioned above - my colleagues would be pleased to send your manuscript out for formal peer-review at Life Science Alliance in its current form. You can take advantage of this offer by transferring your study to Life Science Alliance using the link below; no re-formatting is required.

I am very sorry to disappoint you on this occasion and would like to reassure you that this is not a judgment of the quality or interest of your work, but a decision based on the scope requirement of our journal.

With kind regards,

Lise Roth

Lise Roth, PhD
Senior Editor
EMBO Molecular Medicine

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Dear Lise,

Thank you for your thoughtful consideration and clear summary of our manuscript. As a vascular biologist, I have regarded EMM as a trusted journal since my PhD training. Before we decide on next steps, could you please clarify what specific "translational applications" EMM expects for studies of this type? Guidance on the threshold for translational advance would help us determine how best to strengthen the current manuscript and future submissions to EMM for other ongoing projects.

Thank you again for your time and advice.

Best,
Minah

Dear Minah,

Thank you for your kind email and for requesting clarification on the translational advance required for consideration in EMBO Molecular Medicine. As mentioned in my decision letter, we value your research into the role of ICAM-1 in CD8+ T cells tumor distribution and its potential as a therapeutic target. To demonstrate this therapeutic potential fully, however, we would expect to see validation in additional tumour models, a longer timeframe for measuring the impact of targeting ICAM-1 and/or combination therapy.

I hope this helps you understand the requirements for the journal.

With kind regards,

Lise

Lise Roth, PhD
Senior Editor
EMBO Molecular Medicine

Dear Lise,

Thank you for your clear guidance on the level of translational advance expected for *EMBO Molecular Medicine*. We truly appreciate your thoughtful assessment of our work on ICAM-1 and CD8⁺ T-cell tumor distribution.

Building on our prior work demonstrating ANGPT2/TIE2 regulation of T-cell exclusion (<https://pubmed.ncbi.nlm.nih.gov/37093870/>), this study expands our investigation to identify a novel vascular mediator of T-cell positioning. Beyond the well-established role of ICAM-1 in T-cell adhesion and migration, our data reveal a tumor stage-dependent redistribution of ICAM-1 and show that its spatial expression, coinciding with vascular destabilization, promotes CD8⁺ T-cell exclusion from the tumor core.

We plan to include additional data from other tumor models and evaluate combination therapy to strengthen the therapeutic relevance of our findings. If these data substantively enhance the translational potential, would *EMBO Molecular Medicine* be willing to reconsider our manuscript?

Thank you again for your time and valuable feedback.

Best,
Minah

Dear Minah,

We would indeed be ready to reconsider our decision should you strengthen the translational aspect of your study.

With kind regards,

Lise

Lise Roth, PhD
Senior Editor
EMBO Molecular Medicine

Dear Lise,

Thank you for your encouraging response. We will revise the manuscript to more clearly emphasize the translational significance of our findings and look forward to resubmitting it for your consideration.

Best,
Minah

We are grateful for the guidance provided during our previous submission and have substantially strengthened the manuscript to address the requested translational advances. Specifically, we have:

1. Added a complementary tumor model. We incorporated the immune-refractory melanoma model YUMM1.7 to determine whether tumor immunogenicity influences CD8⁺ T-cell spatial positioning and responsiveness to ICAM-1–targeted intervention (Fig. 1J–K; Fig. 5).
2. Combination therapy and therapeutic validation. We show that ICAM-1 inhibition sensitizes the immune cold tumor to immune checkpoint blockade in vivo. These results are supported by tumor growth data together with analyses of T-cell infiltration and function (Fig. 5A–G), demonstrating therapeutic benefit when ICAM-1 targeting is combined with checkpoint inhibition.
3. Longitudinal and spatial analyses. We expanded our spatial transcriptomic comparisons to include multiple immune subsets and assessed their localization between tumor periphery and core across time points (Fig. 1D–F). This longitudinal, spatial profile emphasizes immune exclusion in melanoma, which is most pronounced in T cells of immunogenic tumors.

13th Apr 2026

Dear Dr. Kim,

Thank you for submitting your manuscript to EMBO Molecular Medicine, and please accept my apologies for the delay in getting back to you as I was waiting for a referee report. Unfortunately, referee #1 has not responded yet, but since referees #2 and #3 made similar recommendations, we would like to make a decision now to avoid further delay in the process. If referee #1 provides a report, we will send it to you on the understanding that we will not ask you to carry out extensive additional experiments beyond those requested in the attached reports from referees #2 and #3. As you will see from the reports below, the reviewers recognize the importance of your study but also express major concerns about the lack of mechanistic understanding and the need for additional melanoma models that are not BRAF-mutated.

If you feel you can satisfactorily address the points listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will once again be subject to review, and we cannot guarantee at this stage that the eventual outcome will be favorable. We expect your revised manuscript within three months. If you anticipate any delay, please contact us.

If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

When preparing your revised manuscript, please refer to our guidelines: <https://link.springer.com/journal/44321/submission-guidelines#cms-Revised-submissions>. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

1) A .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF': <https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>.

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

4) A complete author checklist, which you can download from our author guidelines. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public.

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

8) At EMBO Press we ask authors to provide source data for the main manuscript figures. You will receive a separate email with instructions for providing source data with your revised manuscript, including how to upload and organize the files.

9) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as

follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference.

10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2' etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc.

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

11) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting

- the medical issue you are addressing,
- the results obtained and
- their clinical impact.

This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

12) Author contributions: You will be asked to provide CRediT (Contributor Role Taxonomy) terms in the submission system. These replace a narrative author contribution section in the manuscript.

13) A Conflict of Interest statement should be provided in the main text.

14) Every published paper includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one-sentences bullet points that summarizes the paper. Please write the bullet points to summarize the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300 px high.

15) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines.

16) As part of the EMBO Publications transparent editorial process initiative (see our Editorial at <http://embomolmed.embopress.org/content/2/9/329>), EMBO Molecular Medicine will publish online a Review Process File (RPF) to accompany accepted manuscripts.

In the event of acceptance, this file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. Let us know whether you agree with the publication of the RPF and as here, if you want to remove or not any figures from it prior to publication. Please note that the Authors checklist will be published at the end of the RPF.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

I look forward to receiving your revised manuscript.

Yours sincerely,

Lise Roth

Lise Roth, PhD
Senior Editor

***** Reviewer's comments *****

Referee #2 (Comments on Novelty/Model System for Author):

I've suggested a III melanoma model carrying genetic mutations different from BRAFV600E

Referee #2 (Remarks for Author):

In my opinion, the manuscript clearly demonstrates the role of endothelial ICAM in promoting the spatial distribution of recruited cytotoxic lymphocytes in melanoma. Furthermore, the results presented open interesting translational perspectives for combining immune checkpoint (ICP) therapies with ICAM blockade. However, the current version of the manuscript requires further effort to better explain the zonal modulation of vascular ICAM and to extend these observations to additional melanoma models, particularly those that are not BRAF-mutated.

The authors use two cell lines (YUMMER1.7 and YUMM1.7), both carrying the BRAFV600E mutation. Since ICP therapies are indicated for advanced melanomas regardless of BRAF mutational status, I suggest including a third syngeneic cell line harboring different genetic alterations.

Figure 1E. The results shown here raise an intriguing question. It appears that the ratio of lymphocyte subset accumulation changes in relation to tumor size. Is this phenomenon simply correlated with the duration of the experiment? If so, do different lymphocyte subsets exhibit distinct accumulation kinetics within melanoma tissue? I suggest an experiment based on the intravenous injection of labeled subsets of murine lymphocytes into mice bearing melanomas of different sizes, followed by evaluation of their accumulation kinetics. Alternatively, the authors could inject bulk labeled lymphocytes and subsequently phenotype them within the tumor at different time points. Reliable near-infrared fluorescent cell labeling dyes are available. Obviously, alternative approaches to address this question are also welcome.

Figure 1F-K. It is important to obtain information on both the localization and the abundance of leukocytes with immunosuppressive activity (e.g., Tregs, MDSCs). Is there any correlation with CD8+ T cell and NK cell recruitment?

Figures 2 and 3. These data clearly demonstrate the spatial distribution of endothelial ICAM and its correlation with CD8+ infiltrates. However, they do not fully explain the molecular mechanisms underlying this spatial pattern. A plausible explanation is that different melanoma secretomes influence endothelial cell behavior. By exploiting spatial transcriptomic datasets, I suggest applying computational tools capable of analyzing intercellular ligand-receptor interactions while accounting for spatial relationships (e.g., 10.1038/s41588-024-01890-9; <https://github.com/Caochris/SCOTIA>; 10.1101/2025.11.24.690257). These predictions could then be validated in biological samples and/or co-culture systems.

Alternatively, or in a complementary manner, the authors could isolate cancer cells from tumors with distinct characteristics and analyze in vitro the ability of their secretome to induce ICAM expression in dermal microvascular endothelial cells.

On page 12 (last three lines), vascular leakiness is indirectly assessed through fibrin deposition. While suggestive, this approach does not strongly support the authors' conclusions. Given the importance of vascular permeability in this context, I recommend the use of more appropriate and dynamic techniques, such as tail vein injection of fluorescent dextrans followed by immunofluorescence analysis in tumor sections. This point is particularly relevant because ICAM expression may be regulated by HIF; therefore, assessing vascular permeability in conjunction with markers of hypoxia (e.g., pimonidazole, carbonic anhydrase IX) would strengthen the study.

Referee #3 (Comments on Novelty/Model System for Author):

The model systems are adequate, also in terms of technical quality. What is missing is a mechanistic layer.

Referee #3 (Remarks for Author):

In this manuscript, Park and coauthors examine CD8+ T-cell localization in both patient-derived and murine melanoma samples, linking their peripheral localization to ICAM1 expression on endothelial cells. Blocking ICAM1 promotes deeper CD8+ T-cell infiltration into tumors and leads to partial control of tumor growth. When ICAM1 inhibition is combined with anti-PD-1 therapy in

immunologically "cold" tumors, a similar tumor control effect is observed. These findings are interesting; however, the study remains largely correlative and lacks mechanistic insight into how ICAM1 blockade enhances immune cell infiltration. Moreover, the therapeutic rationale for combining ICAM1 blockade with PD-1 inhibition is insufficiently justified.

Major points:

1. The authors do not assess tumor necrosis in larger lesions, which could independently account for the lack of T cells in the tumor core and the reduced ICAM1 expression on core-associated vessels.
2. In Figure 1, the most pronounced difference between tumor periphery and core occurs in the CD4⁺ compartment, yet Treg abundance and localization are not evaluated. The analysis of the Treg-to-effector T-cell ratio is also missing.
3. In Figure 2, it would be important to present at least some gene ontology (GO) terms enriched in core endothelial cells (ECs) and to discuss further the biological meaning of the genes shown as upregulated in panels 2C and 2D, especially since other gene modules in the figure are clearly annotated.
4. It is surprising that the "adhesion molecule" gene set in Figure 2 does not include VCAM1, ICAM2, or selectins (E-selectin, P-selectin), particularly since these are analyzed later in Figure 3. Given the claim that ICAM1 is the most significantly induced transcript in peripheral ECs, expression data for these other adhesion molecules should be shown. The omission of P-selectin, especially since it is differentially expressed at the protein level, appears inconsistent.
5. In Figure 3, CD11a is analyzed on immune cells, but PSGL-1 is not, despite P-selectin following a similar pattern to ICAM1. Including PSGL-1 analysis would strengthen the interpretation.
6. In Figures 4 and 5, the authors do not examine vessel normalization, permeability, or leakage in anti-ICAM1-treated tumors, nor do they evaluate the expression of other adhesion molecules. These assessments are critical to elucidate the mechanism involved. If ICAM1 blockade leads to vessel normalization, it would be informative to compare its effects with those of established vessel normalization therapies to determine whether ICAM1 inhibition provides superior enhancement of immune cell infiltration.
7. It is unclear whether ICAM1 blockade improves survival when combined with dual immune checkpoint blockade (anti-CTLA-4/PD-1) in the immunologically cold YUMM1.7 tumors. This seems essential to argue for the therapeutic relevance of this approach in melanoma. Recent studies (Hua et al., Cancer Cell 2022; Asir et al., Cancer Cell 2022) have shown that combined checkpoint blockade induces high endothelial venule formation at the tumor periphery. These vessels express high levels of ICAM1 and other adhesion molecules and facilitate CD8⁺ T-cell infiltration into the tumor core despite peripheral localization. Since combined ICB can already normalize vessels and enhance T-cell infiltration, the added benefit of ICAM1 blockade should be clearly demonstrated.
8. Please report any possible gender-specific effects of your preclinical studies.

Responses to Reviewers' comments

Referee #2 (Comments on Novelty/Model System for Author):

I've suggested a III melanoma model carrying genetic mutations different from BRAFV600E

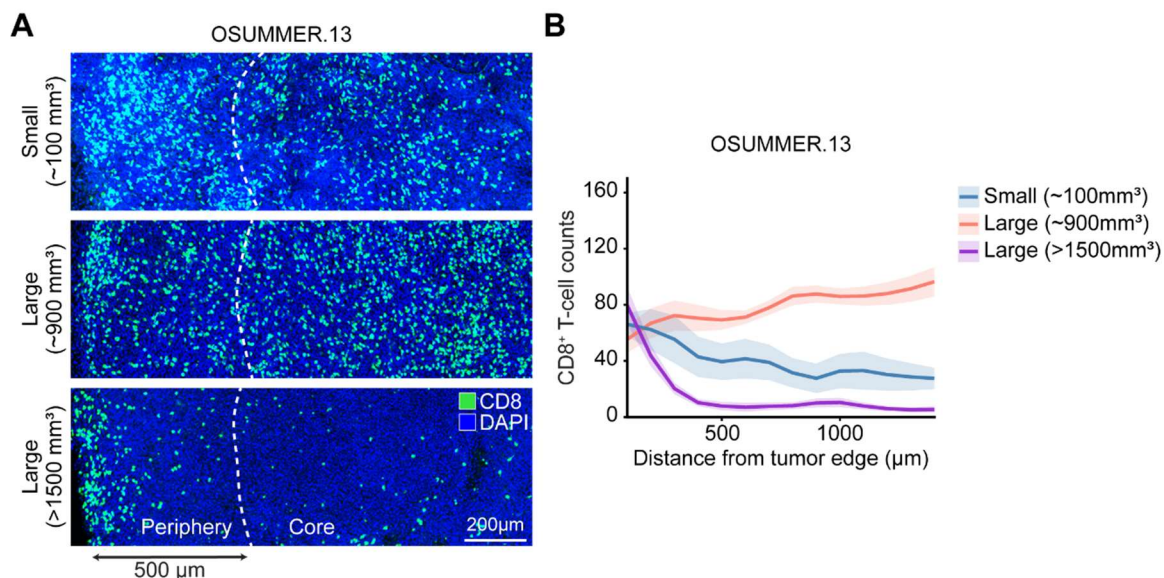
Referee #2 (Remarks for Author):

In my opinion, the manuscript clearly demonstrates the role of endothelial ICAM in promoting the spatial distribution of recruited cytotoxic lymphocytes in melanoma. Furthermore, the results presented open interesting translational perspectives for combining immune checkpoint (ICP) therapies with ICAM blockade. However, the current version of the manuscript requires further effort to better explain the zonal modulation of vascular ICAM and to extend these observations to additional melanoma models, particularly those that are not BRAF-mutated.

Response to general comment: We sincerely thank the reviewer for the positive assessment of our work and for the constructive comments. To address the concerns raised, we performed additional experiments, expanded our analyses to include an additional non-BRAF-mutated melanoma model, and revised the manuscript accordingly. We are confident that these additions have substantively strengthened the study.

1. The authors use two cell lines (YUMMER1.7 and YUMM1.7), both carrying the BRAFV600E mutation. Since ICP therapies are indicated for advanced melanomas regardless of BRAF mutational status, I suggest including a third syngeneic cell line harboring different genetic alterations.

Response: We agree that validating our findings in a melanoma model with a distinct mutational background is important. To address this, we employed the OSUMMER.13 melanoma model, which harbors an NRAS Q61R mutation, to assess CD8⁺ T-cell spatial distribution. OSUMMER.13 tumors developed a T-cell exclusion pattern consistent with that observed in YUMMER1.7—characterized by peripheral CD8⁺ T-cell accumulation with relative core exclusion—although this pattern emerged at a larger tumor size (~2000 mm³) than in YUMMER1.7, suggesting that the kinetics of immune exclusion may differ by mutational context. These data extend our findings beyond BRAF-mutated melanoma and have been incorporated into the Results section of the revised manuscript (**Appendix Figure S2; Page 6, paragraph 1**).



Appendix Figure S2 in the revised manuscript. Spatial distribution of CD8⁺ T cells in OSUMMER.13 tumors. 1×10^6 OSUMMER.13 cells were subcutaneously inoculated in C57BL/6NHsd mice. Tumors were harvested at ~100 mm³ (small), ~900 mm³ (large), and > 1500 mm³ (advanced

large). **(A)** Representative CD8 immunofluorescence staining of small, large, and advanced large tumors. Dashed lines indicate the periphery–core boundary. Scale bar, 200 μm . **(B)** Corresponding quantification of CD8⁺ T cells (cells per 0.05 mm²) as a function of distance from the tumor margin is shown for tumors (n = 4 – 5 mice per group).

2. Figure 1E. The results shown here raise an intriguing question. It appears that the ratio of lymphocyte subset accumulation changes in relation to tumor size. Is this phenomenon simply correlated with the duration of the experiment? If so, do different lymphocyte subsets exhibit distinct accumulation kinetics within melanoma tissue? I suggest an experiment based on the intravenous injection of labeled subsets of murine lymphocytes into mice bearing melanomas of different sizes, followed by evaluation of their accumulation kinetics. Alternatively, the authors could inject bulk labeled lymphocytes and subsequently phenotype them within the tumor at different time points. Reliable near-infrared fluorescent cell labeling dyes are available. Obviously, alternative approaches to address this question are also welcome.

Response: We thank the reviewer for this thoughtful suggestion. We agree that different immune subsets may exhibit distinct recruitment or retention kinetics during tumor progression, and this is an important question for future investigation. However, the primary goal of our study was to define the spatial distribution patterns of endogenous immune populations during melanoma progression—specifically, how their regional localization changes between early and advanced tumor stages—rather than to directly measure trafficking kinetics. The two experimental timepoints (~100 mm³ and ~900 mm³) were chosen to represent physiologically distinct tumor states rather than to isolate the effect of time per se, and the spatial redistribution we observe is consistent across multiple independent approaches. While adoptive transfer of labeled lymphocyte subsets could provide complementary information on recruitment kinetics, we note that such acute transfer assays present interpretive challenges: they primarily capture initial vascular arrest and short-term homing rather than the longer-term intratumoral retention and spatial positioning that govern the excluded phenotype we describe. The endogenous approaches we employed — spatial transcriptomics, regional flow cytometry, immunofluorescence distance mapping, and treatment-induced redistribution — are better suited to capturing steady-state physiologic T-cell localization during tumor progression. We have acknowledged the question of subset-specific trafficking kinetics as an important direction for future work in the revised Discussion (**Page 12, paragraph 2**).

3. Figure 1F-K. It is important to obtain information on both the localization and the abundance of leukocytes with immunosuppressive activity (e.g., Tregs, MDSCs). Is there any correlation with CD8⁺ T cell and NK cell recruitment?

Response: We thank the reviewer for this important suggestion. To address the localization and abundance of immunosuppressive immune subsets and their relationship to CD8⁺ T-cell and NK-cell distributions, we performed additional flow cytometry and gene-set scoring analysis of immunosuppressive programs using our Xenium spatial transcriptomic data.

We first examined Tregs (Foxp3⁺CD4⁺), assessing their spatial distribution by both Xenium and flow cytometry. By Xenium, Tregs showed a periphery-enriched pattern in large tumors (**Figure 1E**), but their overall abundance was low (0.18% in small tumors and 0.13% in large tumors). Flow cytometry confirmed low Treg frequencies in both the periphery and core of large tumors (0.4% and 0.24% of live cells, respectively), with no statistically significant regional difference (**Figure 1G**). The Treg-to-effector T-cell ratio also did not differ significantly between compartments (**Response Figure 2; see response to Reviewer 3, Comment 2**), suggesting that regional Treg variation does not substantially alter the immunosuppressive balance across the tumor. For MDSCs, monocyte and neutrophil populations in our flow cytometry panel overlap phenotypically with M-MDSC (CD11b⁺Ly6C⁺Ly6G⁻) and PMN-MDSC (CD11b⁺Ly6G⁺Ly6C^{low/-}) subsets; we therefore did not formally designate these populations as MDSCs based on the available markers alone, characterizing them instead by their canonical surface phenotype.

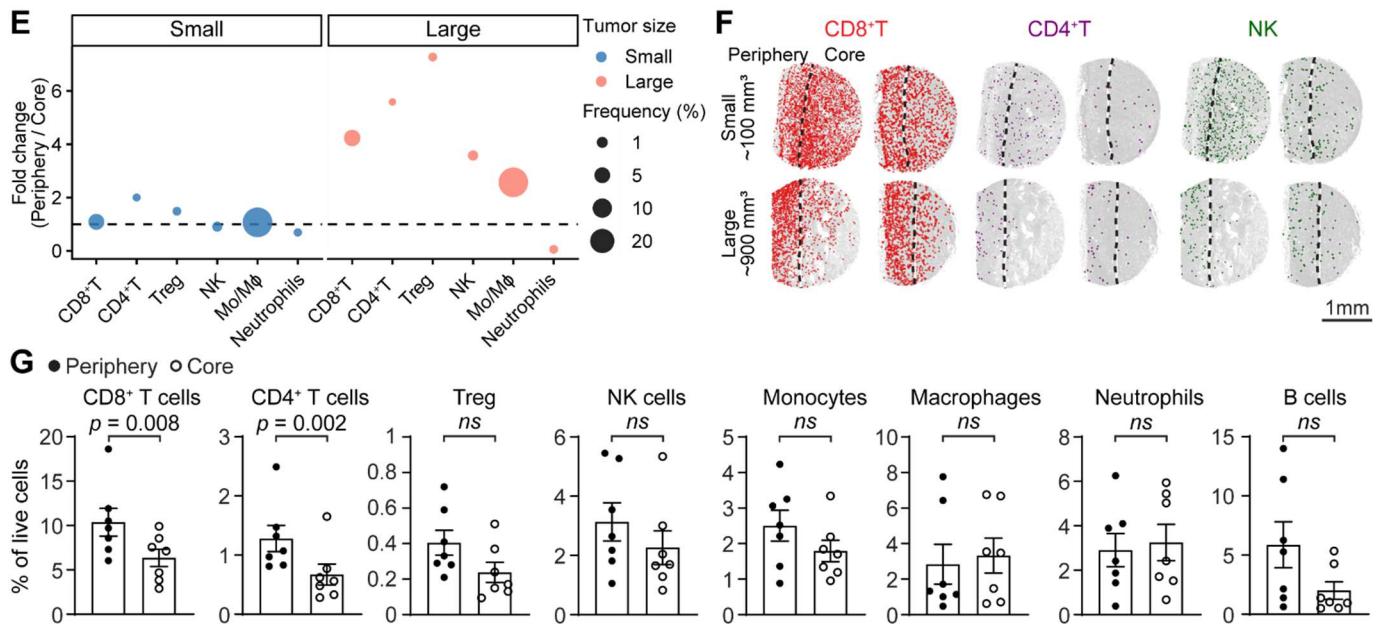


Figure 1E-G in the revised manuscript. Spatial distribution of immune subsets in YUMMER1.7 tumors during tumor progression. (E) Fold change of immune populations (CD8⁺ T, CD4⁺ T, Treg, NK, Mo/Mφ, and neutrophils) in the periphery relative to the core, comparing small (blue) and large (red) tumors. Fold change was calculated as the ratio of the frequency of each cell population in the periphery to that in the core. A value of 1 (dashed line) indicates equal distribution. Dot size represents the overall frequency of each population across all tumors. **(F)** Representative spatial maps of CD8⁺ T, CD4⁺ T, and NK cells in the periphery and core of small and large tumors. Dashed lines mark the periphery–core boundary (500 μm from the tumor margin). Scale bar, 1 mm. **(G)** Flow cytometric comparison of immune-cell frequencies in the periphery vs. core of large YUMMER1.7 tumors (n = 7), including CD8⁺ T cells (TCRβ⁺CD8⁺), CD4⁺ T cells (TCRβ⁺CD4⁺), Treg (TCRβ⁺CD4⁺Foxp3⁺), NK cells (TCRβ⁺NK1.1⁺), monocytes (CD11b⁺Ly6G⁺Ly6C^{high}), macrophages (CD11b⁺Ly6G⁺Ly6C⁺F4/80⁺), neutrophils (CD11b⁺Ly6G⁺Ly6C^{low}), and B cells (TCRβ⁺CD19⁺) among live cells. Each point represents one mouse; bars show mean ± SEM; *p*-values are shown; ns, not significant. Statistical analysis was performed using a paired two-tailed Student's *t*-test. Mo/Mφ, monocytes/macrophages; Treg, regulatory T cells.

To assess immunosuppressive programs more broadly, we performed gene-set scoring of regulatory and immunosuppressive signatures within each immune subset using the Xenium data. Although the 407-gene panel was not designed for deep immunosuppressive subclustering, the included regulatory genes allowed us to score relevant programs within annotated cell populations. This analysis revealed increased immunoregulatory programs in large tumors, including CD8⁺ T-cell exhaustion, Treg-like regulatory, and suppressive neutrophil signatures (**Table EV2** and **Figure EV2C**). Regional differences were minimal in small tumors but more pronounced in large tumors (**Figure EV2D**). This spatial divergence was most evident in myeloid populations: M2-like macrophage and immunosuppressive neutrophil programs were enriched in the tumor core, whereas the M1-like macrophage program was relatively higher at the periphery, showing that the core is dominated by suppressive myeloid activity while T cells and NK cells are retained at the margin. These spatially distinct immunoregulatory programs in large tumors align with the progressive peripheral exclusion of CD8⁺ T cells and NK cells observed in our study. These analyses have been added to **Figure EV2**, and the Results and Discussion sections have been updated accordingly in the revised manuscript (**Page 5, paragraph 1; Page 10, paragraph 2**).

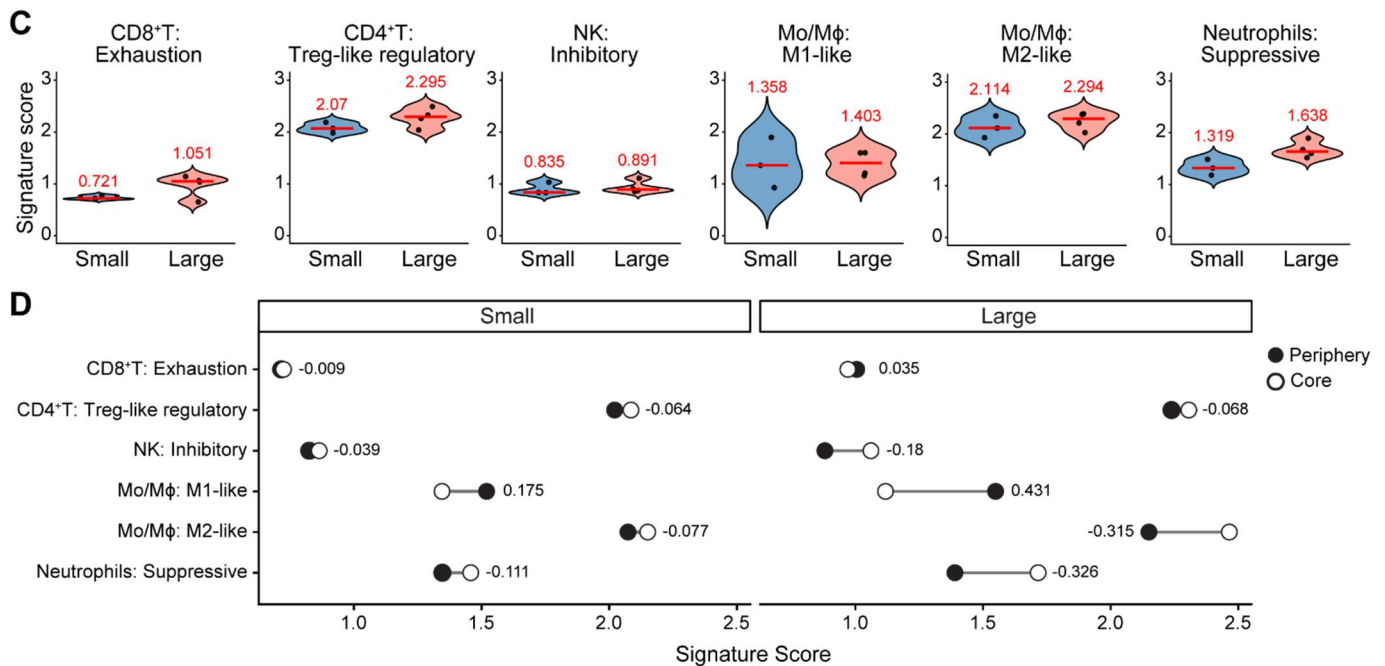
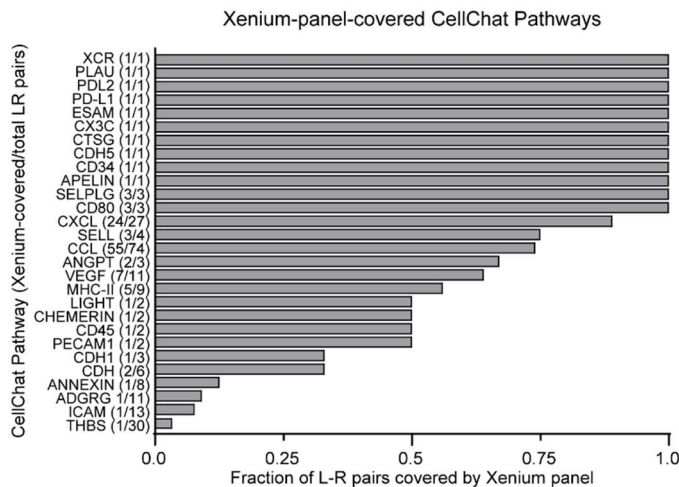


Figure EV2C-D in the revised manuscript. Immunosuppressive signatures in small and large YUMMER1.7 tumors. (C) Immunoregulatory signature scores by annotated immune cell subset in small and large tumors. **(D)** Differences in immunoregulatory signature score between tumor periphery and core in small and large tumors. Signature scores were calculated within the corresponding immune cell subsets using selected phenotype-associated genes detected in the Xenium panel: a T cell exhaustion score in CD8⁺ T cells, a Treg-like regulatory score in CD4⁺ T cells, an NK inhibitory score in NK cells, an M1-like or M2-like macrophage score in Mo/Mφ, and a neutrophil-associated suppressive score in neutrophils.

4. Figures 2 and 3. These data clearly demonstrate the spatial distribution of endothelial ICAM and its correlation with CD8⁺ infiltrates. However, they do not fully explain the molecular mechanisms underlying this spatial pattern. A plausible explanation is that different melanoma secretomes influence endothelial cell behavior. By exploiting spatial transcriptomic datasets, I suggest applying computational tools capable of analyzing intercellular ligand-receptor interactions while accounting for spatial relationships (e.g., 10.1038/s41588-024-01890-9; <https://github.com/Caochris/SCOTIA>; 10.1101/2025.11.24.690257). These predictions could then be validated in biological samples and/or co-culture systems. Alternatively, or in a complementary manner, the authors could isolate cancer cells from tumors with distinct characteristics and analyze in vitro the ability of their secretome to induce ICAM expression in dermal microvascular endothelial cells.

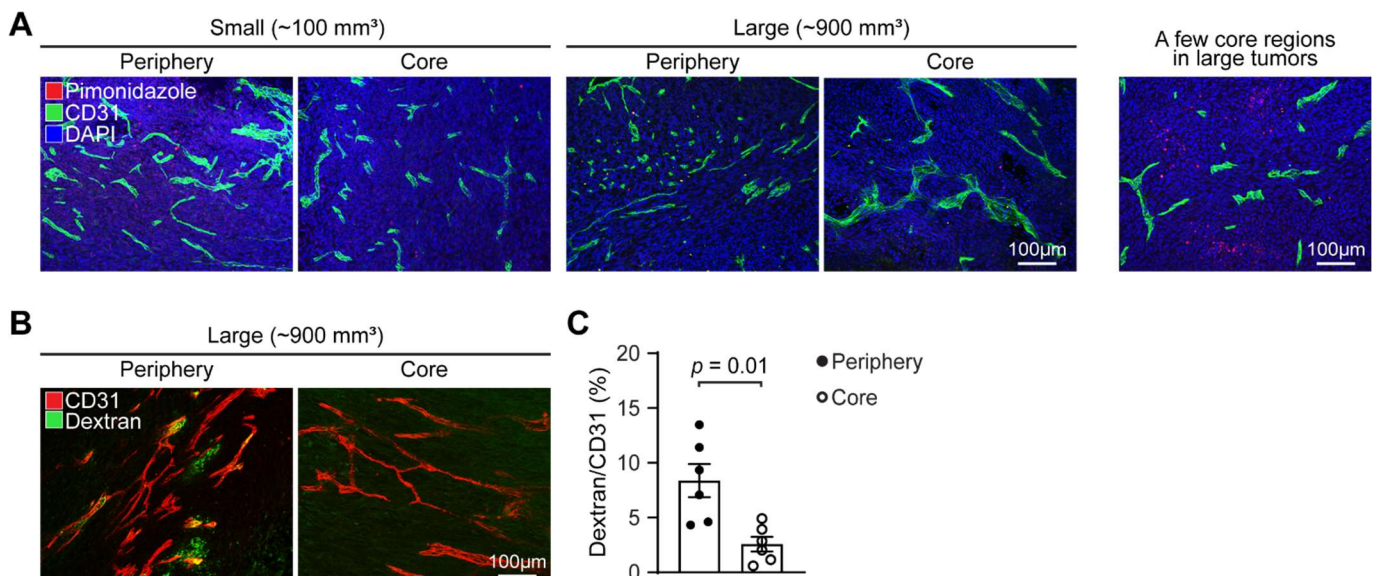
Response: We thank the reviewer for this constructive suggestion and agree that identifying the upstream signals driving spatial ICAM-1 polarization is an important next step. We attempted to apply ligand-receptor interaction analysis to our Xenium dataset; however, the majority of known upstream regulators of ICAM-1 were not included in our custom 407-gene panel, which was designed to resolve immune states rather than to capture the broader receptor-ligand interactome. As a result, the CellChatDB-based cell-cell interaction analysis returned insufficient gene coverage to draw interpretable conclusions (**Response Figure 1**). We have therefore addressed this point by expanding the Discussion (see revised Discussion, **page 11, paragraph 3**) to explicitly acknowledge the mechanistic gap, propose biologically plausible upstream regulators grounded in the existing literature, and identify spatial ligand-receptor analysis with a broader gene panel as a priority for future work. We believe this framing accurately represents the current state of the data while offering a scientifically grounded mechanistic framework for the reader.



Response Figure 1. Limited coverage of CellChatDB ligand-receptor pathways by the custom Xenium 407-gene panel. Coverage was assessed for all ligand-receptor interaction pathways in CellChatDB by determining the fraction of complete ligand-receptor pairs for which all component genes—including all subunits of multimeric ligand or receptor complexes—were present in the custom 407-gene Xenium panel. Numbers adjacent to bars indicate the number of complete ligand-receptor pairs detected over the total annotated pairs for each pathway. Only pathways with coverage greater than zero are shown (28 of 293 total pathways).

5. On page 12 (last three lines), vascular leakiness is indirectly assessed through fibrin deposition. While suggestive, this approach does not strongly support the authors' conclusions. Given the importance of vascular permeability in this context, I recommend the use of more appropriate and dynamic techniques, such as tail vein injection of fluorescent dextrans followed by immunofluorescence analysis in tumor sections. This point is particularly relevant because ICAM expression may be regulated by HIF; therefore, assessing vascular permeability in conjunction with markers of hypoxia (e.g., pimonidazole, carbonic anhydrase IX) would strengthen the study.

Response: We thank the reviewer for this suggestion. We agree that dextran extravasation provides a real-time assessment of active vascular permeability, complementing the cumulative readout of leakage that fibrin(ogen) deposition reflects. To address the concern about indirectly assessing vascular permeability, we performed fluorescent dextran injections in YUMMER1.7 tumor-bearing mice and evaluated leakage across the periphery–core axis by immunofluorescence. Dextran extravasation revealed a periphery-biased permeability pattern in large tumors, consistent with the spatial distribution of fibrin(ogen) deposition (**Appendix Figure S3B and C**). This confirms that vascular destabilization is preferentially localized to the tumor periphery, accompanied by elevated ICAM-1 expression and T-cell exclusion. As suggested, we also assessed the potential contribution of hypoxia to regional ICAM-1 expression by performing pimonidazole (Hypoxyprobe) labeling in the same model. Robust pimonidazole staining was absent from viable tumor regions in both small and large tumors, aside from focal signal in the core of some large tumors, suggesting that the peripheral ICAM-1 pattern is unlikely to be explained by hypoxia (**Appendix Figure S3A**). These findings have been incorporated into the revised manuscript (**Page 7, paragraph 3; Page 8, paragraphs 1-2**).



Appendix Figure S3 in the revised manuscript. Hypoxia and vascular permeability in YUMMER1.7 tumors of different sizes. (A) Representative immunofluorescence image showing pimonidazole (red), CD31⁺ vessels (green), and DAPI-stained nuclei (blue) in small (~100 mm³) and large (~900 mm³) tumors at the periphery and core. Robust pimonidazole signal was not observed in viable tumor regions, although focal pimonidazole-positive areas were detected in a subset of core regions in large tumors (right). **(B-C)** Representative immunofluorescence showing 40-kDa fluorescein isothiocyanate-dextran (green) and CD31⁺ vessels (red) in large tumors (B) and corresponding quantification of the extravasated dextran⁺/CD31⁺ area (%) (C, n = 5). Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using a paired two-tailed Student's *t*-test. *P*-value is shown. Scale bar in A and B, 100 μ m.

Referee #3 (Comments on Novelty/Model System for Author):

The model systems are adequate, also in terms of technical quality. What is missing is a mechanistic layer.

Referee #3 (Remarks for Author):

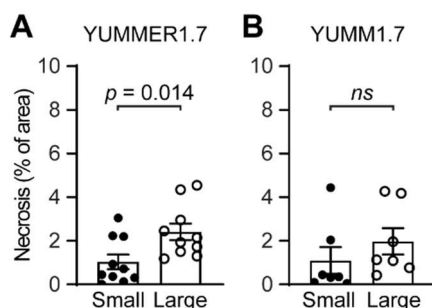
In this manuscript, Park and coauthors examine CD8⁺ T-cell localization in both patient-derived and murine melanoma samples, linking their peripheral localization to ICAM1 expression on endothelial cells. Blocking ICAM1 promotes deeper CD8⁺ T-cell infiltration into tumors and leads to partial control of tumor growth. When ICAM1 inhibition is combined with anti-PD-1 therapy in immunologically "cold" tumors, a similar tumor control effect is observed. These findings are interesting; however, the study remains largely correlative and lacks mechanistic insight into how ICAM1 blockade enhances immune cell infiltration. Moreover, the therapeutic rationale for combining ICAM1 blockade with PD-1 inhibition is insufficiently justified.

Response to general comment: We sincerely thank the reviewer for the insightful and constructive comments. We have made a sincere effort to address the reviewer's concerns and to more clearly articulate both the mechanistic interpretation of our findings and the rationale for combining ICAM-1 blockade with immune checkpoint inhibition.

Major points:

1. The authors do not assess tumor necrosis in larger lesions, which could independently account for the lack of T cells in the tumor core and the reduced ICAM1 expression on core-associated vessels.

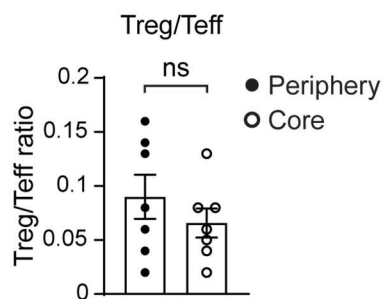
Response: We thank the reviewer for raising this important point. To address whether tumor necrosis could independently account for the absence of T cells in the core or the reduction in endothelial ICAM-1 expression, we quantified necrotic regions in both small and large tumors by measuring DAPI-low areas across entire tumor sections. Necrotic regions accounted for only 1.03% and 2.41% of tumor area in small and large YUMMER1.7 tumors, respectively, and 1.10% and 1.97% in small and large YUMM1.7 tumors, respectively (**Appendix Figure S1**). Although the difference between small and large YUMMER1.7 tumors reached statistical significance, necrotic area remained low in absolute terms across all conditions, indicating that necrosis is unlikely to account for the observed T-cell exclusion or reduced endothelial ICAM-1 expression in the tumor core. These data have been incorporated into the revised manuscript (**Page 5, paragraph 3**).



Appendix Figure S1 in the revised manuscript. Necrotic area in mouse melanomas of different sizes. (A-B) Quantification of necrotic regions, defined by low DAPI signal intensity, across entire tumor sections in small (~100 mm³) and large (~900 mm³) YUMMER1.7 (A, n = 10 per group) and YUMM1.7 (B, n = 7 per group) tumors. Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using an unpaired two-tailed Student's *t*-test. *P*-values are shown; ns, not significant.

2. In Figure 1, the most pronounced difference between tumor periphery and core occurs in the CD4⁺ compartment, yet Treg abundance and localization are not evaluated. The analysis of the Treg-to-effector T-cell ratio is also missing.

Response: We agree that Treg abundance and localization, as well as the Treg-to-effector balance, represent important dimensions of the spatial immune analysis. To address Treg localization, we revisited our Xenium spatial transcriptomic data and analyzed the periphery-to-core fold change of the Treg subset (Foxp3⁺CD4⁺), which showed a periphery-enriched pattern in large tumors, consistent with the broader CD4⁺ T-cell exclusion phenotype (**Figure 1E; see response to Reviewer 2, Comment 3 above**). To further assess Treg abundance and the Treg-to-effector T-cell balance using an independent approach, we performed flow cytometry on dissected periphery and core regions of large YUMMER1.7 tumors. Tregs (TCRβ⁺CD4⁺Foxp3⁺) were detected at low frequencies (below 1% of live cells) in both regions, with no statistically significant difference between the periphery and core (**Figure 1G; see response to Reviewer 2, Comment 3 above**). These data have been incorporated into the revised manuscript (**Page 5, paragraphs 1-2**). The Treg-to-Teff ratio—calculated as the frequency of Tregs divided by the frequency of CD8⁺ effector T cells (TCRβ⁺CD8⁺) in each region—also did not differ significantly between compartments (**Response Figure 2**). Together, these data indicate that regional Treg variation does not substantially alter the Treg-to-effector balance across the tumor, and that the spatial exclusion of CD8⁺ T cells from the core occurs independently of Treg enrichment at the periphery.



Response Figure 2. Treg-to-effector T-cell ratio in the tumor periphery and core. The Treg/Teff ratio was calculated as the frequency of Tregs (TCRβ⁺CD4⁺Foxp3⁺) divided by the frequency of effector T cells (TCRβ⁺CD8⁺) within each dissected tumor region. Each point represents one mouse; bars show mean ± SEM. Statistical analysis was performed using a paired two-tailed Student's *t*-test; ns, not significant.

3. In Figure 2, it would be important to present at least some gene ontology (GO) terms enriched in core endothelial cells (ECs) and to discuss further the biological meaning of the genes shown as upregulated in panels 2C and 2D, especially since other gene modules in the figure are clearly annotated.

Response: We performed GO analysis on genes downregulated in peripheral relative to core endothelial cells and present these results in **Figure EV3A**. The enriched terms include extracellular matrix organization, apoptotic signaling, T cell receptor signaling, and immune response; however, each pathway is supported by only two to three contributing genes, with *Lgals3* present in all four terms and *IL33* contributing to two, indicating that these pathways reflect the enrichment of a small number of transcripts rather than a broad core-specific endothelial program. We have also expanded the Results section to address the biological significance of core-enriched genes in the context of spatially distinct endothelial states (**Page 6, paragraph 2**).

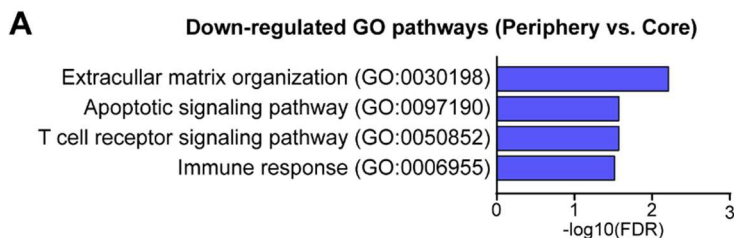
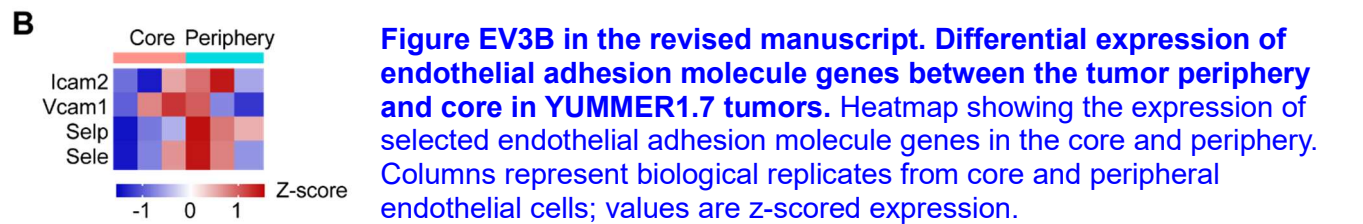


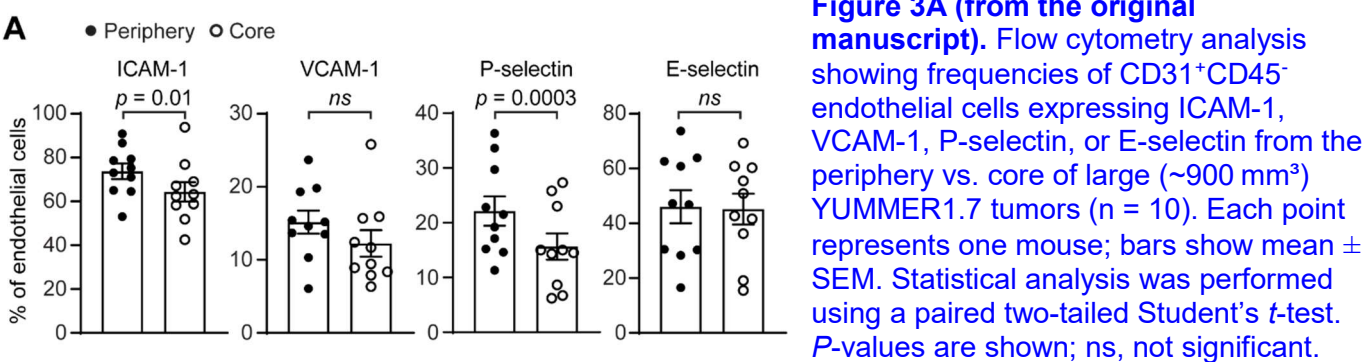
Figure EV3A in the revised manuscript. GO pathways downregulated in peripheral endothelial cells in YUMMER1.7 tumors. Gene Ontology enrichment for Biological Process analysis of genes downregulated in peripheral endothelial cells relative to core endothelial cells. Bars show the top terms ranked by -log₁₀ false discovery rate (FDR).

4. It is surprising that the "adhesion molecule" gene set in Figure 2 does not include VCAM1, ICAM2, or selectins (E-selectin, P-selectin), particularly since these are analyzed later in Figure 3. Given the claim that ICAM1 is the most significantly induced transcript in peripheral ECs, expression data for these other adhesion molecules should be shown. The omission of P-selectin, especially since it is differentially expressed at the protein level, appears inconsistent.

Response: We thank the reviewer for this observation. We examined the transcript-level expression of additional adhesion molecules — *Vcam1*, *Icam2*, *Selp*, and *Sele* — in peripheral and core endothelial cells and present these data in **Figure EV3B**. Among these, *Icam1* showed the most pronounced and statistically significant peripheral enrichment, whereas *Vcam1*, *Icam2*, and *Sele* showed no consistent regional difference. *Selp* showed a trend toward higher peripheral expression that did not reach statistical significance. We have noted this point in the revised manuscript (**Page 6, paragraph 2**).

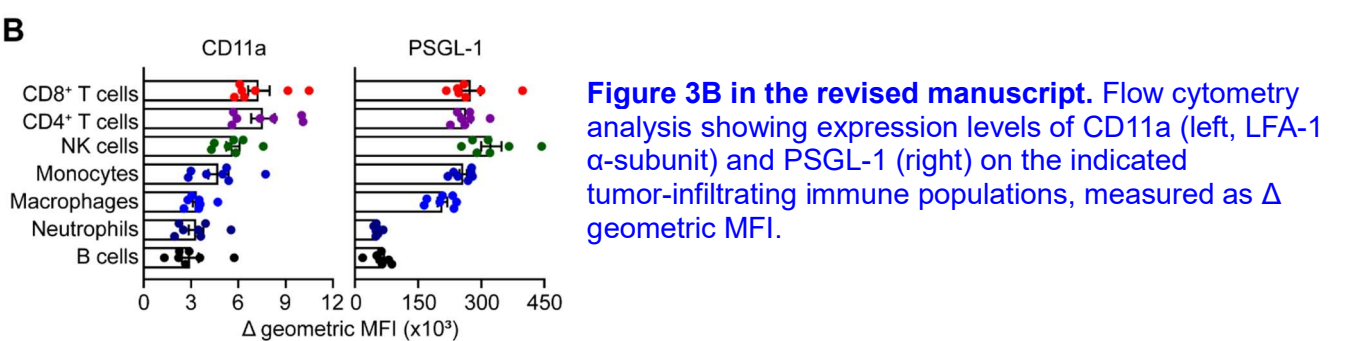


The discordance between *Selp* transcript and P-selectin protein levels—the latter being significantly enriched at the periphery by flow cytometry (**Figure 3A**)—likely reflects post-transcriptional regulation: adhesion molecules are tightly regulated at the levels of translation, membrane trafficking, and surface localization, so mRNA levels do not reliably predict surface protein abundance (PMID: 41038299, 12096112, 37290530, 19346756).



5. In Figure 3, CD11a is analyzed on immune cells, but PSGL-1 is not, despite P-selectin following a similar pattern to ICAM1. Including PSGL-1 analysis would strengthen the interpretation.

Response: We appreciate the reviewer's suggestion to examine PSGL-1 expression, given the peripheral enrichment of P-selectin observed in our analysis. PSGL-1 is a major ligand for P-selectin and mediates the initial tethering and rolling step of leukocyte adhesion, complementing the LFA-1/ICAM-1 axis that governs firm arrest and transmigration. We therefore incorporated PSGL-1 expression into our flow cytometric analysis of tumor-infiltrating immune cells (**Figure 3B; Page 7, paragraph 2**). Among immune populations, the highest PSGL-1 expression was observed on NK cells, followed by CD8⁺ and CD4⁺ T cells. The notable PSGL-1 expression on CD8⁺ T cells—which also express high levels of LFA-1 (CD11a)—suggests that these cells are equipped to engage both P-selectin and ICAM-1 at the peripheral vasculature, consistent with a multi-step adhesion mechanism contributing to their retention at the tumor margin.



6. In Figures 4 and 5, the authors do not examine vessel normalization, permeability, or leakage in anti-ICAM1-treated tumors, nor do they evaluate the expression of other adhesion molecules. These assessments are critical to elucidate the mechanism involved. If ICAM1 blockade leads to vessel normalization, it would be informative to compare its effects with those of established vessel normalization therapies to determine whether ICAM1 inhibition provides superior enhancement of immune cell infiltration.

Response: We thank the reviewer for this important suggestion. To assess whether ICAM-1 blockade affects vascular integrity, we examined fibrin deposition as a marker of vascular leakage and desmin staining as a marker of pericyte coverage in anti-ICAM-1-treated and control YUMMER1.7 tumors. ICAM-1 blockade reduced vascular leakage and increased pericyte coverage in both the tumor periphery and core relative to IgG-treated controls (**Figure 4G–J; Page 9, paragraph 1**), indicating that ICAM-1 inhibition is accompanied by improved vascular integrity across the tumor. These findings suggest that ICAM-1 blockade not only relieves peripheral T-cell retention by disrupting the ICAM-1/LFA-1 adhesive interaction but may also promote vascular stabilization that facilitates broader immune cell access to the tumor parenchyma.

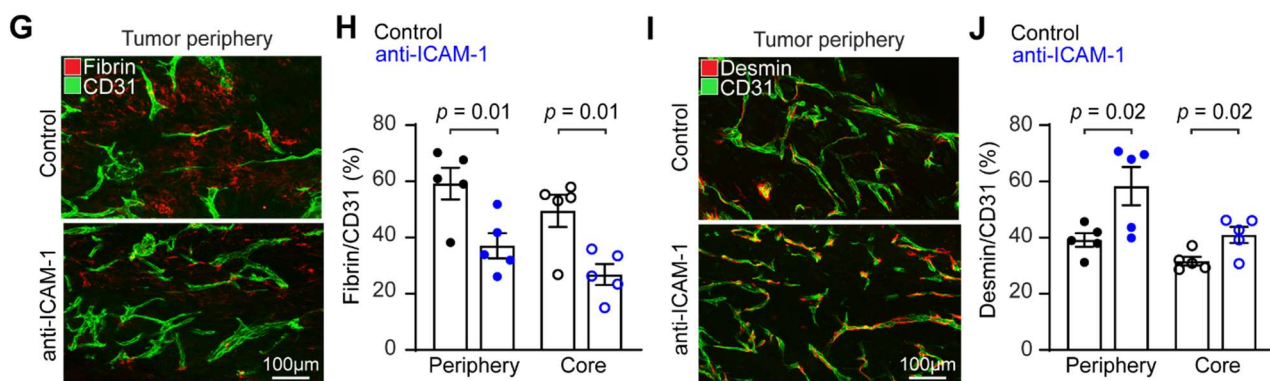
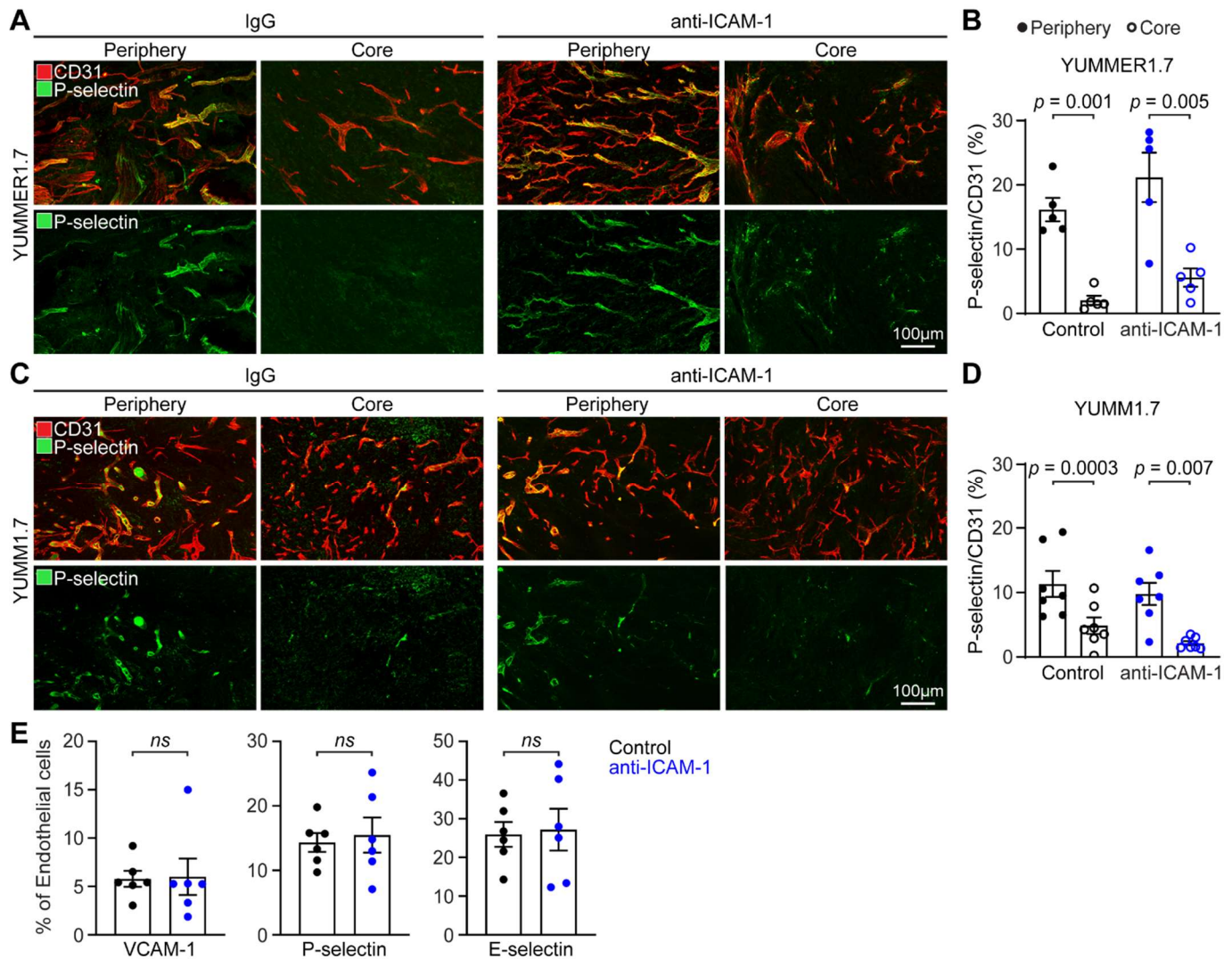


Figure 4G-J in the revised manuscript. Improved vascular integrity following ICAM-1 blockade in YUMMER1.7 tumors. (G-J) Representative images of extravasated fibrin(ogen) (red) (G) and pericytes (desmin, red) (I) relative to CD31⁺ vessels (green) at the tumor periphery in IgG-treated control and anti-ICAM-1-treated YUMMER1.7 tumors. Corresponding quantification of extravasated fibrin(ogen)⁺/CD31⁺ area (%) (H) and desmin⁺/CD31⁺ area (%) (J) at the tumor periphery and core (n = 5 per group). Each point represents one mouse; bars show mean ± SEM. Statistical analysis was performed using an unpaired two-tailed Student's *t*-test. *P*-values are shown. Scale bars for G and I, 100 μm.

Additionally, we evaluated whether ICAM-1 blockade altered the expression of other adhesion molecules. Because P-selectin showed peripheral enrichment in YUMMER1.7, we assessed whether this spatial pattern was altered by ICAM-1 blockade in YUMMER1.7 and YUMM1.7 tumors. P-selectin remained higher at the tumor periphery than at the core in both control and anti-ICAM-1-treated tumors across both models. In YUMM1.7 tumors, we further assessed the expression of additional adhesion molecules, including VCAM-1 and E-selectin, by flow cytometry; their expression did not change significantly with anti-ICAM-1 treatment. Together, these results suggest that ICAM-1 blockade improves vascular integrity while preserving endothelial adhesion molecule expression (**Appendix Figure S4**). The results section was updated accordingly in the revised manuscript (**Page 9, paragraphs 1 and 3**).

Regarding the comparison with established vessel normalization therapies, while this is an informative direction, a head-to-head comparison with anti-angiogenic agents such as anti-VEGF falls outside the scope of the current study. We have noted the potential complementarity of ICAM-1 blockade with vascular normalization approaches in the revised Discussion (**Page 11, paragraph 2**).



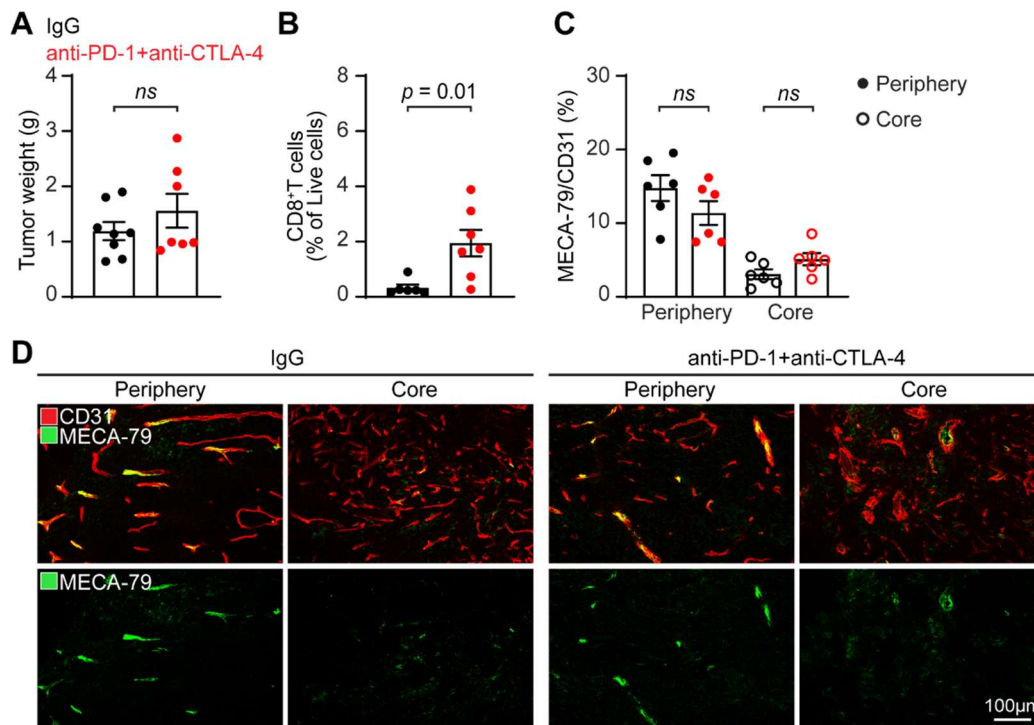
Appendix Figure S4 in the revised manuscript. Expression of adhesion molecules in mouse melanomas following ICAM-1 blockade. (A-D) Representative immunofluorescence showing P-selectin (green) on CD31⁺ vessels (red) at the tumor periphery and core in IgG-treated (left) and anti-ICAM-1-treated (right) YUMMER1.7 (A) and YUMM1.7 (C) tumors. Corresponding quantification of P-selectin⁺/CD31⁺ area (%) for YUMMER1.7 (B, n = 5 per group) and YUMM1.7 (D, n = 7 per group) tumors. No significant treatment effect was detected between control and anti-ICAM-1-treated tumors. **(E)** Flow cytometry analysis showing frequencies of CD31⁺CD45⁻ endothelial cells expressing VCAM-1, P-selectin, and E-selectin in IgG-treated and anti-ICAM-1-treated YUMM1.7 tumors (n = 6 per group). Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using a paired or unpaired two-tailed Student's *t*-test; *P*-values are shown; ns, not significant. *P*-values are shown; ns, not significant. Scale bars in A and C, 100 μ m.

7. It is unclear whether ICAM1 blockade improves survival when combined with dual immune checkpoint blockade (anti-CTLA-4/PD-1) in the immunologically cold YUMM1.7 tumors. This seems essential to argue for the therapeutic relevance of this approach in melanoma. Recent studies (Hua et al., Cancer Cell 2022; Asir et al., Cancer Cell 2022) have shown that combined checkpoint blockade induces high endothelial venule formation at the tumor periphery. These vessels express high levels of ICAM1 and other adhesion molecules and facilitate CD8⁺ T-cell infiltration into the tumor core despite peripheral localization. Since combined ICB can already normalize vessels and enhance T-cell infiltration, the added benefit of ICAM1 blockade should be clearly demonstrated.

Response: We thank the reviewer for raising this important point. The studies cited demonstrate that combined ICB (anti-PD-1 plus anti-CTLA-4) induces HEV formation in certain tumor models, facilitating

CD8⁺ T-cell infiltration into the tumor. To directly test whether this mechanism operates in our melanoma model, we performed a new experiment in which YUMM1.7 tumor-bearing mice were treated with dual ICB (anti-PD-1 plus anti-CTLA-4), and we assessed both CD8⁺ T-cell infiltration and MECA-79⁺ HEV-like structures.

We found that dual ICB increased CD8⁺ T-cell infiltration in YUMM1.7 tumors, but this was not accompanied by measurable tumor growth inhibition, suggesting that the increased T-cell infiltration was insufficient to overcome therapeutic resistance in this immunologically cold model (**Response Figure 3A and B**). Notably, MECA-79⁺ HEV-like vascular structures were rarely detected in YUMM1.7 tumors at baseline and did not increase appreciably with dual ICB treatment (**Response Figure 3C and D**). This is consistent with a recent study (Blanchard et al, *Cell Reports Medicine*, 2025) showing that tumor-associated HEV induction by anti-CTLA-4 depends critically on Fc effector function, and that Fc-optimized anti-CTLA-4 antibodies effectively increase HEVs in the immunologically cold B16-F10 melanoma model, supporting the notion that HEV induction by dual ICB is not a universal effect. We acknowledge, however, that our therapeutic experiments tested ICAM-1 blockade only in combination with anti-PD-1 monotherapy, and whether ICAM-1 inhibition provides additional benefit in the setting of dual ICB remains an important open question. We have noted this limitation in the revised Discussion (**Page 12, paragraph 3**) and agree that future studies testing ICAM-1 blockade combined with dual ICB will be needed to establish the therapeutic relevance of this approach in immunologically cold melanoma.



Response Figure 3. Therapeutic effect of dual immune checkpoint blockade in YUMM1.7 tumors. 2.5×10^5 cells were inoculated subcutaneously in C57BL/6J mice. Mice were treated intraperitoneally with anti-PD-1 (5 mg/kg) plus anti-CTLA-4 (5 mg/kg) or a rat IgG isotype control every other day for seven doses once tumors reached $\sim 70 \text{ mm}^3$; tumors were collected on days 22-25. **(A)** Tumor weight at perfusion showing group means $\pm$ SEM for IgG (black) and anti-PD-1 plus anti-CTLA-4 (red) ($n = 10$ per group). **(B)** Flow cytometry analysis of whole tumors comparing the frequency of CD8⁺ T cells among live cells ($n = 7$ per group). **(C, D)** Quantification of MECA-79⁺/CD31⁺ area (%) in the tumor periphery and core of IgG- or anti-PD-1 plus anti-CTLA-4-treated tumors (C, $n = 6$ per group), with corresponding representative immunofluorescence images showing MECA-79 (green) on CD31⁺ vessels (red) (D). Scale bar in D, 100 μm . Each point represents one mouse; bars show the mean $\pm$ SEM. Statistical analysis was performed using an unpaired two-tailed Student's *t*-test. *P*-values are shown; ns, not significant.

8. Please report any possible gender-specific effects of your preclinical studies.

Response: All experiments with YUMMER1.7 and YUMM1.7 melanoma models were performed in male mice, and OSUMMER.13 experiments in female mice, matching the sex of origin of each model, as specified in the revised Materials and Methods (**Page 17, paragraph 2**). Because each model was studied in a single sex, we could not formally evaluate sex-specific effects on tumor growth or treatment response. We note this limitation in the revised manuscript (**Page 12, paragraph 2**). Future studies incorporating both sexes will be needed to determine whether the observed vascular and immune phenotypes are sex-dependent.

26th Aug 2026

Dear Dr. Kim,

Thank you for submitting your revised manuscript, and please accept my apologies for the delay in getting back to you, due to annual leave of both the referees and members of the editorial team. Referees #2 and #3 have now evaluated the revised manuscript and, as you will see from their comments below, are satisfied with the revisions. I am therefore pleased to let you know that I will be able to accept the manuscript once the following editorial requests have been addressed:

1/ Manuscript text

- Please accept all previous tracked changes and use Track Changes only for any new modifications to the text.
 - Please update the order and headings of the manuscript sections as follows: Abstract / The Paper Explained / Introduction / Results / Discussion / Methods / Data Availability / Acknowledgements / Disclosure and Competing Interests Statement / References / Figure Legends / Tables / Expanded View Figure Legends.
 - Methods:
 - o Remove the Reagents and Tools table from the manuscript and upload it as a separate file.
 - o Human samples: Please provide the IRB approval/protocol number, clarify whether informed consent was obtained or waived, and confirm that the study was conducted in accordance with the Declaration of Helsinki.
 - o Animal experiments: Please provide the IACUC protocol/approval number and clarify the method of euthanasia.
 - o Cell lines: Please indicate whether and how the identity of the cell lines was authenticated/verified, or state if no additional authentication was performed.
 - o Antibodies: Please provide the antibody dilutions.
 - o Statistics: Please provide statements on sample-size determination and inclusion/exclusion criteria.
 - Data Availability: Please list here only deposited datasets generated as part of this study and remove the remaining text. The datasets must be made publicly available upon publication. Please provide a specific URL for each dataset.
 - Author contributions: Please remove this section from the manuscript and provide the CRediT (Contributor Role Taxonomy) terms in the submission system instead.
 - Disclosure and Competing Interests: Please rename the current "Conflict of Interest" section "Disclosure and Competing Interests Statement."
 - Acknowledgements: The information provided in the manuscript should match that entered in our submission system. Currently, "R21" is missing for CA293766 in the submission system but is included in the Acknowledgements.
 - Use of a human scRNA-seq dataset: If the dataset is publicly available, please include a data citation at the relevant point in the text and a corresponding [DATASET] entry in the reference list, following the journal's data citation format outlined below. If the associated study becomes available before publication of this manuscript, please also update the corresponding bibliographic reference accordingly.
- Our journal encourages inclusion of data citations in the reference list to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifier, and a resolvable link to the landing page from which the data can be accessed.

2/ Figures

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- Please remove Tables EV1-EV3 from the manuscript and upload them as separate files, one file per table.
- Appendix: You are welcome to convert some of the Appendix figures into EV figures. If you do so, please make sure to update the corresponding figure callouts.
- Please address the query from our data editors in the figure legends: information regarding n is missing from the legends of Figures 2D, EV2C, EV4C, and EV5A-C.
- During our standard figure checks, similarities were identified between two images. Please carefully review the composition of Figure 3G and correct it if necessary.

3/ Author checklist

Please complete the Ethics section, subsections on informed consent and Declaration of Helsinki.

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Source Data for Figure 1B (microscopy images) are missing. Please provide these.

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Please note that the Author Checklist will be published at the end of the RPF.

I look forward to receiving your revised manuscript.

Yours sincerely,

Lise Roth

Lise Roth, PhD
Senior Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #2 (Comments on Novelty/Model System for Author):

The syngenetic mouse models exploited represent the state of the art in the field

Referee #2 (Remarks for Author):

All criticisms have been properly addressed

Referee #3 (Remarks for Author):

The authors have put a substantial amount of effort to improve the manuscript, and addressed the most important issues raised by the Reviewers.

The authors addressed the remaining editorial issues.

1st Oct 2026

Dear Dr. Kim, Dear Minah,

Thank you for sending your revised files. I am pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

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If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to EMBO Molecular Medicine.

Yours sincerely,

Lise

Lise Roth, Ph.D
Senior Editor
EMBO Molecular Medicine

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Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if $n < 5$, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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/changed/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.

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

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	
Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For antibodies provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and/or clone number - Non-commercial: RRID or citation	Yes	Reagents and Tools Table, Materials and Methods, and Table EV3
DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	
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	Reagents and Tools Table, Materials and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods
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.	Yes	Reagents and Tools Table, Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail housing and husbandry conditions .	Yes	Materials and Methods
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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.	Yes	Table EV1
Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

Design

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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)
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Include a statement about sample size estimate even if no statistical methods were used.	Yes	Results and 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	Materials and Methods
Include a statement about blinding even if no blinding was done.	Yes	Materials and Methods
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Yes	Materials and Methods
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.		
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	Materials and Methods, 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.	Yes	Materials and Methods
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.	Yes	Materials and Methods
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.	Yes	Materials and Methods
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	
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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	
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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.

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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	Materials and Methods
Were human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Yes	Materials and Methods