Reviewing process: Short Note sn20261014-17r

Limited contribution of CNS1-dependent peripheral Tregs to tumor immune suppression across multiple mouse tumor models

Peer-review Report
Short Notes applies an anonymous, free-of-charge peer-review process conducted by experts in the relevant field. After acceptance, reviewers' reports, authors' responses and revisions, and the editorial decision are published, and reviewers may choose to disclose their identity.

Peer-reviewers:

- Reviewer 1 : choose not to disclose their identity
- Reviewer 2 : choose not to disclose their identity

Executive Editor: Marc Le Bert, FC3R

Course of the Short Note sn20261014-17r

Short Note submitted on: June 17, 2026

1st editorial response: August 24, 2026

Subject: Short Note 017 - Accepted with minor revisions

Dear Authors,

Thank you very much for submitting your Short Note "Limited contribution of CNS1-dependent peripheral Tregs to tumor immune suppression across multiple mouse tumor models"

First of all, congratulations on the very positive feedback received from both reviewers.

Both reviewers support publication of the Short Note. Reviewer 1 considers the manuscript suitable for publication with only minor comments, while Reviewer 2 found the manuscript "overall very well written and highly enjoyable to read", with clearly presented experimental systems and data, and conclusions appropriately supported by the results.

Following peer review and our editorial assessment, I am therefore pleased to inform you that your Short Note is accepted subject to minor revisions.

The remaining points mainly concern clarification, reporting, figures and minor editorial adjustments. Once these points have been addressed, the revised version will be

checked directly by the Editorial Team and will not require a further round of external peer review.

Revision procedure and deadline

The Short Note will now be returned to Draft status in your author space. This will allow you to upload the revised manuscript and any modified supplementary documents.

Please submit your revised files and point-by-point responses within one month, i.e. by 24 September 2026 at the latest.

For each editorial or reviewer comment, please indicate how you have addressed the point and, where appropriate, where the corresponding modification can be found in the revised manuscript.

If you decide not to implement a suggestion, please simply explain the reason in your response.

Please upload:

- a clean revised version of the Short Note, leaving sufficient space at the top and bottom of the first page for the Short Notes and FC3R logos and publication information;
- the revised supplementary files, where applicable.

For each editorial or reviewer comment, please enter your response directly in the dedicated "Authors' response" field available in your author space, indicating how the point has been addressed and, when applicable, where the corresponding change can be found in the revised manuscript. If you experience any difficulty accessing or using your author space, you may simply reply to this email and enter your answers directly under the corresponding "Authors' response" sections provided.

Once these minor revisions and clarifications have been satisfactorily addressed, the Short Note can proceed directly to final editorial validation and publication.

Thank you again for submitting these results to the Short Notes platform, and congratulations again on this very positive peer-review outcome.

We look forward to receiving your revised version.

Best regards,

Marc Le Bert
The Short Notes Editorial Team

NB: If you wish, this Short Note can also participate in the OPAL Short Notes Challenge, which rewards the publication and sharing of previously unpublished research results. Participation is entirely optional.

Author's first response: September 02, 2026

A- Editorial check

In addition to the reviewers' comments, please check the revised manuscript carefully for compliance with the **Short Notes Author Guidelines**, particularly the following points.

Keywords
Please provide **three keywords that do not already appear in the title or Abstract**. Choosing complementary terms is important to improve the discoverability and indexing of the Short Note. You may consider using keywords like **"Tumor microenvironment", "Cancer immunotherapy" and "TGF-beta signaling", etc** , as these terms are absent from the title and abstract and may provide additional entry points that broaden the scientific reach of the Short Note.
=> Authors' response: We thank the reviewer for this suggestion. We have added a Keywords section including the following three terms, which are complementary to the title and abstract: Regulatory T cell heterogeneity, TGF-beta signaling, Tumor microenvironment. (p1)

Authors' contributions

Please add an **Authors' contributions** section, preferably using the CRediT terminology.
=> Authors' response: We thank the reviewer for this suggestion. We have included an Authors' contributions section, following the CRediT taxonomy. (p10)

Funding information

If applicable, please indicate the **relevant funding agency/agencies, project name(s) and grant number(s)**. Funding agencies are increasingly attentive to the **publication and valorisation of all research results**, so linking the Short Note to the corresponding funded project(s) can be particularly valuable, especially for **ANR-funded projects**. If the results derive from experiments supported by several grants, please acknowledge all relevant funding sources where possible.
If no specific funding supported this work, or if the results cannot be clearly attributed to a particular project or grant, this can simply be stated explicitly.

=> Authors' response: We have included a complete Funding section. (p10)

Supplementary material

Please clearly identify and list all supplementary files at tne end of the SN (before mat&Meth) and ensure that their naming is consistent between the manuscript and the uploaded documents.
=> Authors' response: We have included Supplementary material section, listing all 3 supplementary files. (p10)

Animal and experimental reporting

Where this information is available, please consider adding or clarifying relevant details that may improve the transparency and reproducibility of the study, such as sample sizes, the number of independent experiments, allocation/randomisation or blinding procedures, and any inclusion or exclusion criteria.Please also check whether the rationale for using only male animals should be stated more explicitly.

=> Authors' response: We thank the reviewer for this important point regarding transparency and reproducibility.

Randomization: Mice were not randomized in the traditional sense, as genotype (CNS1KO vs. CNS1WT) is a fixed genetic trait determined at birth. To control for genetic background and litter-related confounders, we systematically used littermate controls generated from CNS1WT/KO heterozygous females crossed with CNS1KO males.

Blinding: The operator measuring tumor volume with the calliper was blinded to the genotype of the mice, which was of particular importance given that tumor growth was the primary readout of the study.

Inclusion/exclusion criteria: No animals were excluded from the analyses presented.

The number of animals is now indicated on each figure.

Rationale for using only male mice: As *Foxp3* is located on the X chromosome, our breeding strategy (CNS1WT/KO heterozygous females × CNS1KO males) generates male offspring that are either fully CNS1WT or fully CNS1KO as littermates.

We have clarified this rationale in the Methods section (p10-11).

Consistency of animal ages

Please check and harmonise the information concerning animal age throughout the manuscript. Different sections currently refer to animals as being **3 months**, **3-4 months**, or **6-14 weeks** old.
=> Authors' response: We thank the reviewer for pointing out this inconsistency. We have carefully reviewed the manuscript and harmonised all references to animal age to "3- to 4-month-old" throughout the text (p3, p6, p11of the manuscript and in Fig. S1 and S2 legends).

Statistical analysis and software

Please verify the description of the statistical analyses, particularly those applied to longitudinal tumour-growth data, and ensure consistency regarding the version of **GraphPad Prism**, which appears to be reported as both Prism 6 and Prism 8.

=> Authors' response: We thank the reviewer for catching this discrepancy. All statistical analyses reported in this study were performed using GraphPad Prism 6, and we have corrected the Key Resources Table accordingly to consistently refer to this version throughout the manuscript.

Final editorial check

Please carefully proofread the revised version for any remaining typographical, grammatical or formatting inconsistencies, including references, figure citations and terminology. In particular, please check and harmonise the following points that I noted during the editorial review, some may simply reflect my understanding of the text outside its specific scientific context, so please check them and amend only if appropriate: **"selected by determined by elbow plot inspection"**, **"We greatly acknowledge"**, **MOUSET'IC/MOUSTIC**, **Biolegend/BioLegend**, **"Tumors growth"**, **"during 20 min"**, the use of **anti-tumor/antitumor**, **WT/wild-type**, **CNS1WT/CNS1KO** , units and spacing. Please also check the terminology used for animal killing: **"euthanised"** may be retained for animals removed at a humane endpoint, whereas **"killed"** is preferable to **"sacrificed"** for planned killing at an experimental endpoint. Also verify whether **"pLNs" in Supplementary Figure S2** is intended or should read **"mLNs"**, and check consistency of reference formatting throughout.

=> Authors' response: We thank the reviewer for this thorough editorial review and for flagging these inconsistencies. We have carefully proofread the revised manuscript and addressed each point as follows:

"selected by determined by elbow plot inspection" has been corrected to "selected by elbow plot inspection" (p12).

"MOUSET'IC" has been harmonised throughout (p8 and 10).

"Biolegend" has been harmonised to "BioLegend" throughout (p11 and Key Resources

Table).

"anti-tumor" has been used consistently throughout the manuscript, with the exception of reference n°3, where the original title could not be altered.

"Sacrificed" has been replaced by "killed" to describe planned euthanasia at experimental endpoints, in line with the reviewer's suggestion (p3 and p12).

"pLNs" in Supplementary Figure S2 has been corrected to "mLNs".

We have additionally re-checked the manuscript for remaining inconsistencies in terminology (WT/wild-type, CNS1WT/CNS1KO), units and spacing, and reference formatting, and made corrections where needed.

Authors' contributions

Although not mandatory, you may consider adding an **Authors' contributions** section, just before or after "Acknowledgments" section, preferably using the **CRediT taxonomy**, as this can help clarify and recognise each author's contribution to the work.

=> Authors' response: We thank the reviewer for this suggestion. An Authors' contributions section, following the CRediT taxonomy, was already included in the initial submission and can be found before the Acknowledgements section (p13).

Striking image / graphical abstract:

The label **"CD8 Tconvs"** is currently difficult to read; please either use a lighter background colour or display the text in white to improve contrast. As the striking image will also serve as a graphical abstract and may be used to promote the Short Note, you may also consider adding a **short take-home title** rather than the full manuscript title (for example, *"Tumor growth is largely unaffected by loss of CNS1-dependent peripheral Tregs"*), together with a **brief accompanying legend** describing the main message of the graphical abstract.
=> Authors' response: We thank the reviewer for these helpful suggestions. We have improved the contrast of the "CD8 Tconvs" label by displaying it in grey. We have also replaced the full manuscript title with a short take-home title, "Tumor growth is largely unaffected by loss of CNS1-dependent peripheral Tregs", and added a brief legend summarizing the main message of the graphical abstract.

B- Peer-reviewing

QUALITY OF WRITING

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Reviewer 1 ticked : yes

Reviewer 1 comment :

This Short Note is understandable by non-specialists, the instructions are respected and the approach is easy to follow.

in the abstract the sentence beginning with "This role in preventing autoimmune diseases..." is not clear : were they talking about autoimmune diseases? did they mean "their role in..."?

=> Authors' response: We thank the reviewer for this comment. This sentence has been changed for "Their crucial role in preventing autoimmune diseases limits"

Finally, results are correctly interpreted, except one overinterpretated data in the discussion section:
"Together, these findings indicate that CNS1-dependent pTreg differentiation is not a dominant driver of tumor progression in subcutaneous transplantation models, and allow us to exclude this pathway..." which is exaggerated to my opinion since subcutaneous tumor models are not perfectly representative of in situ sponatneously developed tumors

=> Authors' response: We thank the reviewer for this important observation. We agree that the original phrasing overstated our conclusions. Subcutaneous transplantation tumor models, while allowing standardized comparison across five immunologically distinct tumor types, do not fully recapitulate the kinetics, tissue context, and chronic antigen exposure of spontaneously arising, *in situ* tumors. We have revised the corresponding sentence in the Discussion to soften this claim and to explicitly acknowledge this limitation of our experimental system.

Revised manuscript sentence (p4):

"Together, these findings indicate that CNS1-dependent pTreg differentiation is not a dominant driver of tumor progression in the subcutaneous transplantation models tested here."

Reviewer 2 ticked : no

Reviewer 2 comment :

The manuscript is overall very well written and highly enjoyable to read. The experimental systems are clearly presented, and the data are described accurately and objectively, without overstating conclusions. Only the abstract would benefit from some minor revision, as in its current form it may not be sufficiently explicit for a non-specialist audience. For example, the statement that regulatory T cells (Tregs) "differentiate in the periphery" could be clarified by specifying what is meant by "the periphery" and from which precursor population these cells arise. Similarly, the term "CNS1" is introduced without explanation and would benefit from a brief definition.

=> Authors' response: We thank the reviewer for this constructive suggestion. We agree that the original abstract lacked sufficient detail for a non-specialist audience regarding the precursor population and the definition of CNS1. We have revised the abstract as follows:

Regulatory CD4â•º T cells, characterized by the expression of transcription factor Foxp3, promote tumor development and progression through their inhibitory effects on anti-tumor immune responses. Their crucial role in preventing autoimmune diseases limits their therapeutic targeting in cancer settings. However, the regulatory CD4â•º T cell compartment is heterogeneous in ontogeny, comprising cells that arise naturally in the thymus and others that differentiate from naïve CD4â•º T cells in Secondary Lymphoid Organs during immune responses. This peripheral differentiation is driven, at least in part, by CNS1 (conserved non-coding sequence 1), an enhancer region of the *Foxp3* gene. Here, we investigated the contribution of these CNS1-dependent regulatory CD4â•º T cells to anti-tumor immunity by comparing tumor growth and tumor-infiltrating immune cells in CNS1-deficient mice and control littermates. Our results suggest that CNS1-dependent regulatory CD4â•º T cells play only a minor role in the inhibition of anti-tumor responses in C57BL/6J mice housed under specific pathogen-free conditions.

In addition, the beginning of the second sentence ("This role in preventing autoimmune diseases") appears to refer back to the role of Tregs described in the first sentence. However, the first sentence focuses on their suppression of anti-tumor immune responses and consequent promotion of tumor growth, rather than on their function in maintaining self-tolerance and preventing autoimmunity. Rephrasing this transition would improve clarity and logical flow.

=> Authors' response: We thank the reviewer for this comment. This sentence has been changed for "Their crucial role in preventing autoimmune diseases limits"

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS

Reviewer 1 ticked : no

Reviewer 1 comment :

The figures are well presented but would benefit from integrating, when missing, the number of mice used in each experiment (sometimes the dots are too near to count them), also the colour dot to indicate CNS1 KO or Wt is not so clear on fig 1D (writting it on the top of the graphs would be easier to follow). Finally the resolution is too low (pixellisation) but it may result from the version I have??
To my opinion, it is no use to have the orange line around the figure S1A as well as the arrows, in contract precise the ladder used.

=> Authors' response: We thank the reviewer for these constructive suggestions to improve figure clarity.

The number of mice has been indicated in Figure S1C-F for the comparison of WT and CNS1KO mice at steady state, and in Figure 1 for each tumor model. The corresponding figure legends have been modified accordingly.

To improve readability, "WT" and "KO" labels have been added directly above the graphs for each panel, except in Figure 2, where the addition of these labels to every individual panel would have made the figure overly cluttered given the number of tumor models already displayed; genotype is indicated in the corresponding pie chart and heatmap labels in this case.

The resolution of all figures has been improved.

Regarding Figure S1A, the ladder used has been specified in the legend (100 bp DNA Ladder) and removed the orange frame and arrows.

Reviewer 2 ticked : yes

QUALITY OF THE EXPERIMENTAL DESIGN

Reviewer 1 ticked : yes

Reviewer 1 comment :

we can wonder why a n of 12 was included since the data are homogeneous. How was that number defined? does it come from a will to evidence a light or inexistent difference ? then it would not be that "3Rs-designed"... Also why testing so manby tumor cell lines? but I agree that now that the experiments were done, it's good to publish them to avoid unnecessary duplication of experiments. Unsing non parametric test is correct.

=> Authors' response: We thank the reviewer for raising this important point regarding experimental design and the 3Rs framework.

The sample size of n=12 per group was determined a priori using a power calculation, based on the expected variance and effect size for tumor growth in these models, to ensure adequate statistical power while avoiding the use of unnecessarily large cohorts. This *a priori* approach is itself consistent with the 3Rs principle of Reduction, as it aims to define the minimal number of animals required to detect a biologically meaningful difference, rather than over- or under-powering the study.

Regarding the use of five distinct tumor models, the reviewer is correct to suspect that this design choice was indeed motivated by a wish to maximize our chances of detecting a genuine effect of CNS1 deletion, however light, were one to exist. Rather than relying on a single tumor model -- which carries an inherent risk of being non-representative -- we tested tumors with distinct immunogenic and histological profiles (melanoma, lymphoma, colon carcinoma, sarcoma, and pancreatic adenocarcinoma) to give this hypothesis its best chance of being supported by the data. We do not view this as inconsistent with the 3Rs framework: testing across independent models rather than increasing the sample size within a single model remains a Reduction-compatible strategy, and the completion of this multi-model design is precisely why we consider it important to report the negative results obtained across all five models, to prevent unnecessary duplication of this work by other groups.

Reviewer 2 ticked : yes

QUALITY OF THE REPORTING _____

Reviewer 1 ticked : yes

Reviewer 1 comment :

in the MM section, the ethics statement should appear first before animal description. also, in the MM page 11: " previously coated with anti-CD3 (145-2C11; produced in-house) and anti-CD28 antibodies (Biolegend) at a final concentration of 4 mug/mL" each?

=> Authors' response: We thank the reviewer for these points. The ethics statement has been moved to appear before the animal description in the Materials and Methods section. We have also clarified that the 4 µg/mL concentration applies to each antibody (anti-CD3 and anti-CD28) individually (p10-11).

Also please align the bibliography on the right side of the document.
=> Authors' response: We thank the reviewer for this formatting note. The bibliography has been aligned on the right side of the document as suggested (p8-9).

Revised version of the Short Note submitted on: September 02, 2026

2nd editorial response: September 10, 2026

Final editorial check - remaining minor corrections before publication

Dear Dr. Auffray and co-authors,

Thank you very much for taking the time to address all the reviewers' and editorial comments. I have carefully reviewed the revised version of the Short Note. This is a very nice Short Note and, importantly, a useful piece of work to share.

The Short Note has already been accepted with minor revisions. The points below are therefore part of the final editorial check and verification of the reviewers' requests and will be handled editorially, without returning the manuscript to the reviewers.

For workflow tracking in the submission interface, the Short Note will remain under accepted with minor editorial revisions, which will return it to Draft status in your author space and allow you to upload the final corrected files.

These are only very minor final points and should be straightforward to address. No additional experiments are requested and the Short Note will not be returned to the reviewers.

Once these corrections have been made, please:

- upload the clean final Short Note without highlights and the corrected supplementary files;

- update the abstract and keywords in the submission interface.

I will then perform the final conformity check.

The Short Note will then enter the **post-editorial process**, including final formatting, addition of the Short Notes logos and identifier/number, deposit in HAL and moderation of the HAL record. Once the deposit has been validated, the DOI will be registered and the Short Note will be made available online. The authors will then receive the DOI and publication links by email.

Many thanks again for these final corrections.

Best regards,
Marc Le Bert
The Short Notes editorial board

Author's 2nd response: September 28, 2026

1. Abstract, keywords and final clean version

Please replace the abstract and keywords currently entered in the submission interface with the revised versions included in the manuscript. The title has not changed, as far as I can see.

Please also upload the **final clean version of the Short Note without highlights or tracked changes**.

During the very final post-editorial preparation, I may still make extremely minor copy-editing corrections where necessary, for example removing the page number from the first page or correcting a last-minute typographical error.
=> Authors' response: Done

2. Sample size and power calculation

In your response to the reviewer, you indicate that: "**n = 12/group was determined a priori using a power calculation.**"

This important information does not currently appear in the Materials & Methods or Statistical Analysis section and should therefore be added there.

In addition, the actual numbers shown in Figure 1 appear to vary substantially between tumor models -- approximately **3/6, 10/11, 7/6, 6/9 and 11/12** animals for the two genotypes -- whereas the manuscript also states that no animals were excluded from the analyses.

Please therefore reconcile these different pieces of information by briefly indicating:
- how the initial sample size was determined;
- the planned number of animals per group;
- why the actual numbers analysed were lower than 12 in several tumor models.

If applicable, this could for example reflect animals that were unavailable or could not be included at the planned experimental date, preliminary/pilot experiments, or any other practical reason. If there were losses or non-inclusions, these should also be stated.
No additional experiment is requested; this is only to ensure transparent reporting of the experiments already performed.

=> Authors' response:
We thank the editor for pointing out this inconsistency, which we have now clarified (see p15 - Tumoral transplantation / *Sample size*)

The target group size (n = 12/genotype) was determined *a priori* by power calculation for an unpaired Mann-Whitney test, to detect a significant difference of 10% between groups with an estimated variance of 15%, at a 5% alpha risk and 80% power. This information has been added to the Statistical Analysis section.

The lower numbers observed in some tumor models reflect the fact that, for these models, we restricted the analysis to experiments in which CNS1WT and CNS1KO littermates were run in parallel, so that genotype comparisons remained matched within the same experimental batch. This is representative of the full set of experiments performed in each model, but results in group sizes below the planned n = 12 where fewer littermate-matched cohorts were available. We have clarified this point in the Statistical Analysis section. No animal within an analysed cohort was excluded from the results shown; the group-size variation reflects this *a priori* matching criterion rather than post-hoc data exclusion.

3. Longitudinal analysis of tumor growth

Please clarify how the longitudinal tumor-growth data were statistically analysed.

In particular, were statistical comparisons performed at specific time points or at the experimental endpoint, or was the **overall tumor-growth kinetics analysed**?

Again, the aim here is simply to make the analysis actually performed explicit in the manuscript.

=> Authors' response: Statistical comparisons were performed at the 2 last experimental endpoints, on tumor volume and tumor weight, using an unpaired Mann-Whitney test. Tumor growth kinetics (i.e., the shape of the growth curves over time) were compared qualitatively/visually rather than through formal statistical testing at each time point. We have clarified this in the *Statistical Analysis* part of the Tumoral transplantation section of the Materials and Methods and in the relevant figure legends.

4. Randomisation, blinding and potential sources of bias

Please indicate briefly in the Materials & Methods that formal randomisation was not performed and explain why this was not feasible given the experimental design and/or the available numbers of animals.

Please also indicate, where relevant, whether **any potential sources of bias** (ie cage effect) related to animal allocation, housing or experimental handling were considered, and how they were minimised.

=> Authors' response: Formal randomisation was not performed, as animal allocation to the WT or CNS1KO group was determined by genotype at birth rather than by experimenter assignment. Littermates of both genotypes were co-housed in the same cages to limit cage-related environmental variability. Tumor measurements were performed by the same operator throughout to limit inter-operator measurement bias. Tumor measurements were performed blind to genotype. We have clarified this in the *Randomisation, blinding* part of the Tumoral transplantation section of the Materials and Methods

5. Terminology for animal killing

Please correct the following minor points:The manuscript currently states that *"Mice were euthanised when tumor volume approached 1 cm³"* and later that *"Mice were killed by cervical dislocation."*

Please clarify whether **1 cm³ corresponds to a humane endpoint**, and whether animals reaching this limit before the planned experimental endpoint were included in the analyses.

Please then harmonise the terminology accordingly and remove the remaining use of **"sacrifice"**.

=> Authors' response: We thank the reviewer for this clarification. We have revised the terminology throughout the manuscript: "euthanised" is retained specifically for animals removed at the humane endpoint (tumor volume approaching 1 cm³), while "killed" is now used for planned killing at the experimental endpoint, replacing all instances of "sacrificed".

A tumor volume of 1 cm^3 constituted a predefined humane endpoint under our animal protocol (APAFIS #18068). No animals reached this endpoint before the planned experimental endpoint in the experiments reported here; all animals were killed at the planned endpoint for their respective tumor model.

6. Remaining minor typographical and formatting corrections

Please correct the following minor points:
- page 12: "under agitation during 20min" -> "**under agitation for 20 min**"; **Done**

- please harmonise spacing between numbers and units throughout the manuscript, for example: **100 µL, 1 cm^3, 0.1 mg/mL, 1 mg/mL, 100 µm, etc.; Done**

- Figure 1 legend: "Each dot represents one one mouse" -> "**Each dot represents one mouse.**" **Done**

=> Authors' response: Changes have been done

7. Key Resources Table / Supplementary Table S1

The list of supplementary material on page 10 refers to the **Key Resources Table**, whereas page 12 refers to "**Supplementary Table S1**" for the antibodies.

As there is currently no file named Supplementary Table S1, I assume that "**Key Resources Table**" should be used throughout. Please harmonise the terminology, unless you actually intended to rename this document as Table S1.

=> Authors' response: Change has been done

8. Minor corrections in the Key Resources Table

A few typographical points remain in the Key Resources Table. Please check, in particular:
- "Catalogue Noner" Done
- "form hybridoma supernatant" Done
- "recombiant proteins" Done
- probably "INFg" -> "**IFN-gamma**"
- The **anti-PD1 BV421** entry also appears to be duplicated. Please check whether both entries are required.

=> Authors' response: Changes have been done

9. Figure S1

Please make the following final minor corrections to Figure S1 and its legend:
- in the title, please italicise **in vitro**
- replace "n are indicated" with "**n values are indicated**"
- in the legend, replace "(E." and "(F." with "**(E)**" and "**(F)**"
- In panel F, the circles/gates identifying the cell populations extend partly outside the plot boundaries. Usually, these graphical gates remain within the plotting area. Please check whether this presentation can be adjusted if appropriate. This is only a graphical observation on my part, as I am not a flow-cytometry gating specialist, so please retain the current representation if it is scientifically appropriate.

=> Authors' response: Changes have been done. Concerning the panel F, we thank the reviewer for this observation. This reflects standard FlowJo gating behaviour: gate shapes (e.g., elliptical/polygon gates) can extend graphically beyond the plot boundaries even when no additional events are captured beyond what is visible within the plot -- the gate outline itself is not clipped to the axis limits. This is a minor graphical feature of the gating software rather than an indication of cells being included or excluded from the population shown. We consider the current representation scientifically appropriate and have therefore retained it as is.

10. Figure S2

Please indicate in the Figure S2 legend the number of animals (n) in the CNS1WT and CNS1KO groups, and for each tissue if the sample sizes differ.

=> Authors' response: The following sentence has been added in the Figure legend "Data are from n = 5 CNS1WT and n = 6 CNS1KO mice for mesenteric lymph nodes (mLNs) and spleen, and n = 3 CNS1WT and n = 3 CNS1KO mice for Peyer's patches"

Revised version of the Short Note submitted on: September 28, 2026

Final editorial response: October 14, 2026

Subject: Short Note 017 - Accepted for publication

Dear Dr. Auffrey, dear Cédric and co-authors,

We are pleased to inform you that your Short Note 017, entitled "Limited contribution of

CNS1-dependent peripheral Tregs to tumor immune suppression across multiple mouse tumor models", has been formally accepted for publication.

The peer-review and final editorial checks are complete, and the publication files have been validated. Thank you for carefully addressing the reviewers' and editorial requests, and for your commitment to sharing previously unpublished results.

Your Short Note will be deposited in HAL and assigned a DOI. It will also be made available in the FC3R Short Notes Library, together with the peer-review report annexed to the publication.

We will notify you once publication is complete and provide the corresponding links. No further revision is requested at this stage; we will contact you if a technical clarification is needed during publication.

With best regards,
Marc Le Bert
Short Notes Editorial Team

Final validation and publication: October 14, 2026