Peer-Review

Chae, Nathaniel. 2026. “Cell-Type-Specific Chromatin Accessibility Patterns in Aging Hippocampal Neural Stem Cells Reveal MEF2 Family Motif Enrichment.” *Journal of High School Science* 10 (1): 140–58. https://doi.org/10.64336/001c.155781.

The manuscript contains interesting, plausible findings, and is a valuable contribution; but as currently written, it overstates what the data can support. The claims exceed the evidence. This is true even though in some instances you have extrapolated and inferred from published literature (which may not have had the same experimental design, datasets, analysis software…..). The current approach provides suggestive, not definitive evidence. It identifies candidate regions, but cannot establish differential accessibility or causality. Please address my specific comments below.

1.You cannot prove differential chromatin accessibility: You cannot claim that a region is more open in old vs young, only that it is only called as a peak in one group. A peak could exist in both groups but with different strength (read depth). Your binary (yes/no) method discards any shared peaks, even if they show large quantitative differences.

2.You cannot quantify the magnitude of change: You cannot determine whether accessibility increased slightly or dramatically, or whether differences are biologically meaningful. This is because no read count data is used. It only mentions BED files with coordinates, which are just regions, not quantitative data. No statistical modeling of effect size is performed.

3.You cannot separate biological change from technical noise. Peak calling can vary due to sequencing depth, batch effects, different sample quality, peak-calling thresholds. Peak presence or absence can be driven by technical variation, not biology. Without replicate-based statistics, you cannot distinguish noise from real effects.

4.You cannot show reproducible age-related patterns. You cannot say the identified peaks are consistently different across replicates because the method does not account for replicate variability.

5.you cannot attribute TF binding or activity. Even if MEF2 motifs are enriched in “old-only” peaks, you cannot claim that MEF2C binds those regions, MEF2C activity is increased, MEF2C is the driver of chromatin changes because motif presence ≠ TF binding and chromatin accessibility ≠ transcription factor occupancy.

6.You cannot prove age-specific regulatory programs: You cannot claim the identified TFs are functionally responsible for aging changes because no functional validation is linked to specific peaks or genes. There is no direct TF binding data (ChIP/CUT&Tag).

7.You cannot show that MEF2C is the only or main factor. The presence of motifs does not imply MEF2C is the dominant TF because motifs are shared among MEF2 family members. Other factors may bind similar motifs.

8.ATAC-seq data acquisition and preprocessing: Inconsistent age definitions (5 days, 10 weeks, 24 weeks, 80 weeks, 18 months) are not justified or harmonized across datasets. It is unclear whether: scATAC-seq peaks were aggregated across cells or whether any cell-type filtering or quality control was performed.The Methods state that data were “aligned and mapped,” yet only preprocessed .bed or .bedgraph files were used. No description is provided of peak-calling algorithms, alignment parameters or quality control thresholds. It is difficult to assess whether observed differences reflect biology or preprocessing artifacts.

9.Motif analysis using HOMER: No background set is described (e.g., matched GC content or genomic regions). Motifs with $p < 0.05$ are reported, but: No multiple-testing correction is mentioned, q-values are not consistently reported. Motif enrichment is treated as evidence of transcription factor “activation,” which is not methodologically justified. Motif analysis supports permissive binding environments but NOT transcription factor occupancy or activity.

10.Gene expression analysis: Student’s t-test is used for scRNA-seq data, which may violate normality. No tests for normality or variance equality are described. No correction for multiple comparisons is reported. Sample size justification and power analysis are absent. Ignores zero inflation and heterogeneity. There is no description of cell filtering criteria, normalization methods,

differential expression pipelines. This means that statistical conclusions about MEF2C expression changes may not be reliable.
11.Cell culture and transfection methods: No justification is given for use of an immortalized human progenitor line to model mouse aging, overexpression levels relative to physiological MEF2C expression. ReN VM cells are human, immortalized, and not aged and may not serve as a proper proxy for this experiment. Morphological changes may reflect altered adhesion, motility, or stress responses rather than synaptic regulation. The inducible nature of the pInducer vector is mentioned, but the inducing agent is not specified, induction timing and concentration are not reported. This means that overexpression may introduce non-physiological effects, limiting biological relevance.
12.Cytoskeleton staining and image analysis: Cell-to-cell contact" is not operationally defined. Quantification is manual and subjective. Only three fields per condition were analyzed. No blinding or automated image analysis is reported. This means that morphological conclusions are vulnerable to observer bias and limited sampling.
13.Your conclusion or inference that MEF2C functions through inhibiting synapse formation" is not directly supported because you did not measure synapses, synaptic proteins or functional connectivity. The invitro assay shows altered cell morphology and contact, not synaptogenesis. The cells used do not form functional synapses. Your data can only support that MEF2C overexpression alters cell morphology and reduces cell–cell contact in vitro.
14.You have not demonstrated a causal role in neural stem cell functional decline because you have no loss-of-function experiments, no rescue experiments, no invivo manipulation. Your data therefore cannot distinguish whether MEF2C drives aging-related decline, responds to aging-related stress or acts as a compensatory mechanism.
15.Cognitive decline is not supported because you have no behavioral data, no manipulation of MEF2C in animals, no link between MEF2C levels and cognition. You over-reach with your claims well beyond what your data can support.
16.You have Overused the analogy to cancer therapeutics. While the examples of transcription factor degraders are interesting, Cancer biology ≠ aging neurobiology. Success in cancer does not imply feasibility or safety in the brain. This analogy weakens the conclusion by overpromising translational relevance.
17.The Limitations section omits discussion of the reliance on motif enrichment as a proxy for transcription factor binding, the lack of quantitative differential accessibility analysis, the heterogeneity of public datasets, and the constraints of the in vitro overexpression assay. Explicitly acknowledging these limitations would strengthen the rigor and credibility of the conclusions.
18.The statement that "MEF2C is responsible for neural stem cell aging" is too strong given the evidence. The data show association, not necessity or sufficiency. Motif similarity explains ambiguity in motif enrichment but does not rule out MEF2D involvement at the protein or activity level. The conclusion should be reframed to state that MEF2C is a candidate contributor or marker of aging-associated transcriptional changes, rather than the causal driver.
19.The explanation that MEF2C promotes commitment but restricts synapse integration remains speculative. No direct evidence is provided showing that aged hippocampal NSCs commit but fail to integrate. The Discussion implicitly assumes that adult neurogenesis failure is due to synaptic integration defects, which is only one of several possibilities (e.g., reduced proliferation, survival, or fate switching). This section would benefit from explicitly framing this model as a hypothesis generated by the current study, rather than a demonstrated mechanism.
20.Cross-dataset variability: Replicating AP-1 enrichment does not directly validate the MEF2C-specific findings. Differences in age definitions, tissue handling, and peak-calling pipelines may disproportionately affect subtler signals like MEF2 motifs. Therefore, claims of robustness should be limited to general aging signatures, not the central MEF2C conclusion.
21.Age definition and unidirectionality: The conclusion that aging-associated transcriptional changes are "unidirectional" is not sufficiently justified. This would require longitudinal or

trajectory-based analysis. Similar motif enrichment does not imply monotonic or linear biological change.
22.The Discussion currently implies generality to hippocampal aging broadly, which is premature given: Mouse-only data, Cell-type–restricted analysis, Artificial in vitro manipulation.
The paper is a valuable contribution. You will however need to tone down your claims to what your data supports. For example, you have overinterpreted correlative data, particularly in attributing a causal role to MEF2C in neural stem cell aging and synaptic integration failure. You will need to replace causal language with associative language, framing MEF2C as a candidate target for future investigation. You will need more cautious phrasing and clearer distinction between experimentally supported conclusions and hypotheses generated by the study. You will also need to re-analyze data for proper statistical treatment and re-perform some experiments to eliminate observer bias (or put this down in the limitations section). Please expand the limitations section to explicitly include all the concerns that I have listed.

Point-by-Point Response to Reviewer

The manuscript contains interesting, plausible findings, and is a valuable contribution; but as currently written, it overstates what the data can support. The claims exceed the evidence. This is true even though in some instances you have extrapolated and inferred from published literature (which may not have had the same experimental design, datasets, analysis software…..). The current approach provides suggestive, not definitive evidence. It identifies candidate regions, but cannot establish differential accessibility or causality. Please address my specific comments below.

: I thank the reviewers for their careful and rigorous evaluation of the manuscript. I agree that the original version overstated what the data could support. In the revised manuscript, all causal language has been removed, and the findings are consistently framed as associative and hypothesis-generating. The analyses are now presented as identifying candidate regulatory features rather than definitive mechanisms. These revisions have improved both the clarity and rigor of the manuscript. Brackets indicate the sections in which changes were made.

1.You cannot prove differential chromatin accessibility: You cannot claim that a region is more open in old vs young, only that it is only called as a peak in one group. A peak could exist in both groups but with different strength (read depth). Your binary (yes/no) method discards any shared peaks, even if they show large quantitative differences.

: I agree that the current analysis cannot establish quantitative differential chromatin accessibility between age groups. The revised manuscript no longer claims that regions are “more open” or exhibit increased accessibility with age. Instead, I consistently describe the results in terms of peak presence or absence under identical preprocessing and peak-calling thresholds, which reflects how the data were analyzed. I also explicitly acknowledge that this binary approach necessarily excludes shared peaks that may differ quantitatively in read depth or signal intensity and therefore cannot capture subtle or continuous accessibility changes. This limitation is now clearly stated to prevent overinterpretation of the peak sets as evidence of differential accessibility (p 1, 3,10-11). [Abstract, Methods, Limitations]

2. You cannot quantify the magnitude of change: You cannot determine whether accessibility increased slightly or dramatically, or whether differences are biologically meaningful. This is because no read count data is used. It only mentions BED files with coordinates, which are just regions, not quantitative data. No statistical modeling of effect size is performed.

: I agree that the magnitude of accessibility changes cannot be inferred from BED-based analyses. All statements implying effect size or biological magnitude have been removed. The revised manuscript now states that the analysis identifies age-associated candidate regions without quantifying effect size (p 1, 10-11). [Abstract, Discussion]

3. You cannot separate biological change from technical noise. Peak calling can vary due to sequencing depth, batch effects, different sample quality, peak-calling thresholds. Peak presence or absence can be driven by technical variation, not biology. Without replicate-based statistics, you cannot distinguish noise from real effects.

: I acknowledge that peak presence/absence can reflect technical variation. I have added explicit language noting that without replicate-aware statistical modeling, biological signal cannot be definitively separated from technical noise (p 10-11). [Limitations]

4. You cannot show reproducible age-related patterns. You cannot say the identified peaks are consistently different across replicates because the method does not account for replicate variability.

: I agree that reproducibility across biological replicates cannot be assessed with the current approach. Claims of consistency across replicates have been removed, and this limitation is now clearly stated (p 10-11). [Limitations]

5. you cannot attribute TF binding or activity. Even if MEF2 motifs are enriched in "old-only" peaks, you cannot claim that MEF2C binds those regions, MEF2C activity is increased, MEF2C is the driver of chromatin changes because motif presence ≠ TF binding and chromatin accessibility ≠ transcription factor occupancy.

: I fully agree that motif enrichment does not demonstrate transcription factor binding or activity. All claims of MEF2C binding, activation, or occupancy have been removed. The manuscript now consistently states that motif enrichment reflects permissive sequence environments only (p 1, 6, 11). [Abstract, Results, Limitations]

6. You cannot prove age-specific regulatory programs: You cannot claim the identified TFs are functionally responsible for aging changes because no functional validation is linked to specific peaks or genes. There is no direct TF binding data (ChIP/CUT&Tag).

: I explicitly state that motif enrichment identifies permissive sequence environments rather than transcription factor binding, regulatory programs, or causal mechanisms, and that conclusions are associative and hypothesis-generating only (p 1, 9, 11). [Abstract, Discussion, Limitations]

7. You cannot show that MEF2C is the only or main factor. The presence of motifs does not imply MEF2C is the dominant TF because motifs are shared among MEF2 family members. Other factors may bind similar motifs.

: I agree that motif similarity among MEF2 family members prevents attribution to MEF2C alone. The revised manuscript acknowledges this ambiguity and refrains from claiming MEF2C exclusivity (p 1, 6, 9, 11). [Title, Abstract, Results, Discussion, Limitations]

8. ATAC-seq data acquisition and preprocessing: Inconsistent age definitions (5 days, 10 weeks, 24 weeks, 80 weeks, 18 months) are not justified or harmonized across datasets. It is unclear whether: scATAC-seq peaks were aggregated across cells or whether any cell-type filtering or quality control was performed.The Methods state that data were "aligned and mapped," yet only preprocessed .bed or .bedgraph files were used. No description is provided of peak-calling algorithms, alignment parameters or quality control thresholds. It is difficult to assess whether observed differences reflect biology or preprocessing artifacts.

: These are important points. I have clarified age definitions and included the description of data preprocessing steps provided by the original data contributors under the Methods section (p 3-4), and also acknowledge heterogeneity across datasets. First, mouse ages were classified according to commonly used murine aging categories (neonatal/early postnatal <1 week; young adult 2–3 months; old adult 3–6 months; aged ≥18 months) (p 4). The publicly available datasets analyzed here span multiple studies with distinct experimental designs and age sampling strategies; therefore, ages were not synchronized across datasets. Instead, analyses were performed within each dataset using the age group defined by the original studies, and results were interpreted as reflecting broad age-associated patterns rather than precisely matched chronological stages.

For preprocessing, details are now included according to the original contributors' description (p 3). [Methods]

9. Motif analysis using HOMER: No background set is described (e.g., matched GC content or genomic regions). Motifs with $p < 0.05$ are reported, but: No multiple-testing correction is mentioned, q-values are not consistently reported. Motif enrichment is treated as evidence of transcription factor "activation," which is not methodologically justified. Motif analysis supports permissive binding environments but NOT transcription factor occupancy or activity.

: I apologize for missing details of the HOMER analysis and added it (p 4). Motif enrichment was performed using HOMER with the default background model implemented by the software, which samples genomic regions matched for size and GC content from the mm10 genome. Reported p-values reflect nominal enrichment significance as computed by HOMER using a hypergeometric test. Although HOMER outputs both p-values and estimated false discovery rates (q-values), motif rankings and interpretation in this study were based on nominal p-values, and no additional multiple-testing correction was applied. Accordingly, motif enrichment results are treated as exploratory and descriptive rather than confirmatory. The revised manuscript avoids interpreting motif enrichment as TF activation and explicitly frames it as exploratory (p 4, 9, 11). [Methods, Discussion, Limitations]

10. Gene expression analysis: Student's t-test is used for scRNA-seq data, which may violate normality. No tests for normality or variance equality are described. No correction for multiple comparisons is reported. Sample size justification and power analysis are absent. Ignores zero inflation and heterogeneity. There is no description of cell filtering criteria, normalization methods, differential expression pipelines. This means that statistical conclusions about MEF2C expression changes may not be reliable.

: I agree that Student's *t*-tests are not appropriate for single-cell RNA-seq data and that the analysis does not account for zero inflation, cellular heterogeneity, multiple testing, or replicate-aware statistical modeling. In the revised manuscript, I have removed all claims that rely on statistical inference from the scRNA-seq analysis. Gene expression results are now explicitly described as descriptive and supportive only, rather than definitive evidence of differential expression (p 4, 11) [Methods, Limitations]. Figure 8 was reformatted as violin plot to show data distribution pattern and removing p-values. Conclusions regarding MEF2C do not depend on this analysis and are framed as associative and hypothesis-generating (p 1, 11). [Abstract, Limitations]

11. Cell culture and transfection methods: No justification is given for use of an immortalized human progenitor line to model mouse aging, overexpression levels relative to physiological MEF2C expression. ReN VM cells are human, immortalized, and not aged and may not serve as a proper proxy for this experiment. Morphological changes may reflect altered adhesion, motility, or stress responses rather than synaptic regulation. The inducible nature of the pInducer vector is mentioned, but the inducing agent is not specified, induction timing and concentration are not reported. This means that overexpression may introduce non-physiological effects, limiting biological relevance.

: I fully agree that immortalized human progenitor cells do not model mouse neural stem cell aging. The revised manuscript explicitly states that the in vitro assay tests the effect of MEF2C overexpression on cell-to-cell contact only (p 9). I also added missing details and justifications (p 5, 8-9, 10-11). [Methods, Results, Discussion, Limitations]

12. Quantification is manual and subjective. Only three fields per condition were analyzed. No blinding or automated image analysis is reported. This means that morphological conclusions are vulnerable to observer bias and limited sampling.

: I acknowledge limited sampling and subjective quantification. Morphological conclusions are now described cautiously (p 9)[Results], and observer bias is explicitly noted (p 5). [Methods]

13.Your conclusion or inference that MEF2C functions through inhibiting synapse formation" is not directly supported because you did not measure synapses, synaptic proteins or functional connectivity. The invitro assay shows altered cell morphology and contact, not synaptogenesis. The cells used do not form functional synapses. Your data can only support that MEF2C overexpression alters cell morphology and reduces cell–cell contact in vitro.

: I agree that synapse formation was not measured. All claims regarding synaptogenesis or synaptic integration have been removed. The data are now described as changes in cell morphology and contact only (p 1, 9). [Abstract, Results]

14. You have not demonstrated a causal role in neural stem cell functional decline because you have no loss-of-function experiments, no rescue experiments, no in vivo manipulation. Your data

therefore cannot distinguish whether MEF2C drives aging-related decline, responds to aging-related stress or acts as a compensatory mechanism.
: I agree that no causal role or cognitive impact can be claimed. All statements causally linking MEF2C to cognitive decline or neural stem cell functional decline have been removed (p 1, 9-11) [Abstract, Discussion, Limitations]
15. Cognitive decline is not supported because you have no behavioral data, no manipulation of MEF2C in animals, no link between MEF2C levels and cognition. You over-reach with your claims well beyond what your data can support.
: I agree that cognitive outcomes cannot be inferred from the present data. All statements causally linking MEF2C to cognitive decline or behavioral effects have been removed, and references to cognition are now limited to background context only (p 2)[Introduction]. The revised manuscript restricts conclusions to molecular and cellular associations and explicitly avoids claims regarding cognitive or functional outcomes (p 9-11). [Discussion]
16. You have Overused the analogy to cancer therapeutics. While the examples of transcription factor degraders are interesting, Cancer biology ≠ aging neurobiology. Success in cancer does not imply feasibility or safety in the brain. This analogy weakens the conclusion by overpromising translational relevance.
: I agree that the cancer therapeutic analogy overreached. This section has been substantially toned down and reframed as speculative future context (p 11). [Conclusion]
17. The Limitations section omits discussion of the reliance on motif enrichment as a proxy for transcription factor binding, the lack of quantitative differential accessibility analysis, the heterogeneity of public datasets, and the constraints of the in vitro overexpression assay. Explicitly acknowledging these limitations would strengthen the rigor and credibility of the conclusions.
: I have substantially expanded the [Limitations] section to address motif-based inference, lack of quantitative accessibility analysis, dataset heterogeneity, in vitro overexpression constraints, and absence of causal validation (p 11). [Limitations]
18. The statement that “MEF2C is responsible for neural stem cell aging” is too strong given the evidence. The data show association, not necessity or sufficiency. Motif similarity explains ambiguity in motif enrichment but does not rule out MEF2D involvement at the protein or activity level. The conclusion should be reframed to state that MEF2C is a candidate contributor or marker of aging-associated transcriptional changes, rather than the causal driver.
: I agree and have revised the manuscript to remove all causal language. As suggested by the reviewer, MEF2C is now described as a candidate marker or contributor associated with aging-related chromatin features (p 1, 10). I acknowledge MEF2 family motif similarity and state that “motif enrichment alone cannot distinguish among MEF2 paralogs or infer transcription factor binding or protein-level activity. Accordingly, these findings are interpreted as identifying permissive sequence environments within age-associated peak sets rather than evidence of MEF2C- or MEF2D-specific regulatory activity” (p 6, 11). [Results, Limitations]
19. The explanation that MEF2C promotes commitment but restricts synapse integration remains speculative. No direct evidence is provided showing that aged hippocampal NSCs commit but fail to integrate. The Discussion implicitly assumes that adult neurogenesis failure is due to synaptic integration defects, which is only one of several possibilities (e.g., reduced proliferation, survival, or fate switching). This section would benefit from explicitly framing this model as a hypothesis generated by the current study, rather than a demonstrated mechanism.
: I appreciate the suggestion and have revised the discussion to remove mechanistic assumptions. The model linking MEF2C to impaired synaptic integration is now framed as a hypothesis generated by the data, not a demonstrated mechanism (p 9). Alternative explanations for reduced neurogenesis (e.g., proliferation, survival, fate changes) are acknowledged, and in vitro findings are limited to morphology and cell–cell contact (p 10). [Discussion]
20. Cross-dataset variability: Replicating AP-1 enrichment does not directly validate the MEF2C-specific findings. Differences in age definitions, tissue handling, and peak-calling pipelines may

disproportionately affect subtler signals like MEF2 motifs. Therefore, claims of robustness should be limited to general aging signatures, not the central MEF2C conclusion.
: I agree and have narrowed claims of robustness. Replication of AP-1 motif enrichment is presented only as validation of general aging-associated signatures, not of MEF2C-specific findings (p7).[Results] I also added a statement that dataset heterogeneity may disproportionately affect detection of subtler motifs such as MEF2 (p7). [Results]
21. Age definition and unidirectionality: The conclusion that aging-associated transcriptional changes are "unidirectional" is not sufficiently justified. This would require longitudinal or trajectory-based analysis. Similar motif enrichment does not imply monotonic or linear biological change.
: I agree and have removed all claims of unidirectional or monotonic aging-associated change. The revised manuscript states that cross-sectional comparisons across heterogeneous age groups cannot establish directionality or trajectories, which would require longitudinal or trajectory-based analyses. All interpretations are now limited to age-associated differences observed within the analyzed datasets (p10). [Discussion]
22. The Discussion currently implies generality to hippocampal aging broadly, which is premature given: Mouse-only data, Cell-type–restricted analysis, Artificial in vitro manipulation.
: I have revised the discussion to limit the scope of interpretation. The discussion section states that the findings are restricted to mouse hippocampal neural stem cell datasets and an exploratory in vitro overexpression assay, and should not be generalized to hippocampal aging broadly, other cell types, or functional outcomes (p 10). [Discussion]
The paper is a valuable contribution. You will however need to tone down your claims to what your data supports. For example, you have overinterpreted correlative data, particularly in attributing a causal role to MEF2C in neural stem cell aging and synaptic integration failure. You will need to replace causal language with associative language, framing MEF2C as a candidate target for future investigation. You will need more cautious phrasing and clearer distinction between experimentally supported conclusions and hypotheses generated by the study. You will also need to re-analyze data for proper statistical treatment and re-perform some experiments to eliminate observer bias (or put this down in the limitations section). Please expand the limitations section to explicitly include all the concerns that I have listed. Please also change the title and abstract accordingly.
: Thank you for the opportunity to submit our revised manuscript. We have systematically updated the Title, Abstract, and main text to remove causal language, instead framing MEF2C as a candidate marker associated with age-related chromatin and transcriptional changes. We have also expanded the Limitations section to address all reviewer concerns comprehensively. Regarding the data, Figure 8 has been replotted to show overall distribution omitting the previously used Student's t-test. Additionally, we have updated the Methods and Figure 10 legend to transparently disclose that quantitation was performed without blinding, acknowledging potential observer bias.
Thank you.
Sincerely,
Nathaniel Chae

Thank you for addressing my comments. The paper now becomes descriptive and hypothesis testing; but its novelty is obviously reduced. However, there is still latent novelty that can be extracted from the revised manuscript if the framing is shifted.
To strengthen its contribution without expanding claims, I recommend explicitly reframing the novelty around cell-type specificity rather than transcription factor causality. In particular, you should emphasize that MEF2-family motif enrichment emerges selectively in hippocampal neural stem cell peak sets and is not observed in whole hippocampus or subventricular zone neural stem cells, underscoring the context-dependent nature of aging-associated regulatory signatures. Clarifying this point in the Discussion (and, if possible, the title or abstract) would help readers understand what is genuinely new in this work while remaining fully consistent with the acknowledged limitations and descriptive scope of the analyses.

Below is a novelty and significance paragraph (courtesy of chatgpt), which I agree with, that you can expand on, rewrite, and spread the relevant content across pertinent sections in the manuscript.
Novelty and Significance
This study provides a cell-type–restricted view of aging-associated regulatory features in the brain by comparing ATAC-seq peak-set motif enrichment across hippocampal neural stem cells, whole hippocampal tissue, and subventricular zone neural stem cells. Rather than identifying universal aging signatures, the analysis reveals that certain motif enrichments—exemplified by MEF2-family motifs—are selectively associated with hippocampal neural stem cell peak landscapes and are not detected in bulk hippocampus or in other neurogenic niches. This specificity highlights that aging-associated regulatory signatures can be strongly context-dependent and may be obscured in tissue-level analyses. Importantly, the work explicitly frames motif enrichment as reflecting permissive regulatory sequence environments rather than transcription factor binding or activity, thereby clarifying the interpretive limits of peak presence–based ATAC-seq analyses. Together, these findings contribute a cautious, cell-type–resolved perspective on chromatin-associated aging signatures and provide a transparent framework for generating testable hypotheses in future mechanistic studies.
Please also write the manuscript in third person, past perfect tense where-ever possible.

Response to Reviewers
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Clarifying this point in the Discussion (and, if possible, the title or abstract) would help readers understand what is genuinely new in this work while remaining fully consistent with the acknowledged limitations and descriptive scope of the analyses.
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: I sincerely thank the reviewer for these careful and constructive suggestions. In response, I have made the following revisions:
(1) The title has been revised to “Cell-Type-Specific Chromatin Accessibility Patterns in Aging Hippocampal Neural Stem Cells Reveal MEF2 Family Motif Enrichment.”

(2) The discussion of novelty and significance has been integrated into the Discussion section. As suggested, this revision reframes the primary contribution of the study around the cell-type specificity of aging-associated regulatory signatures (hippocampal neural stem cells versus whole hippocampus and subventricular zone neural stem cells), rather than implying MEF2 causality. This revised framing has also been incorporated into the Abstract, Introduction, and Limitations sections.
Please also write the manuscript in third person, past perfect tense where-ever possible.
: As suggested, the manuscript was revised to use third person narration throughout. Past tense was used to describe analyses and observations performed in this study, while past perfect tense was applied selectively to prior preprocessing steps and previously published datasets. Present tense was retained only for established background information.

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Thank you for addressing my comments. Accepted.