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Editorial Note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I would like to express my appreciation to the authors for their hard work and dedication throughout the review process. The revisions made have significantly improved the quality and clarity of the manuscript, addressing the concerns raised in previous rounds of review. I have carefully reviewed the current version and have no further comments or suggestions. I am pleased to recommend this manuscript for acceptance in *Nature Communications*.

Reviewer #2 (Remarks to the Author):

Most of my comments have been sufficiently addressed, though a puzzle remains in comment #2. The reviewer fully acknowledges the authors' assertion that METTL14-mediated m6A accelerates the decay of apoptosis-related transcripts in a YTHDF2/3-dependent manner in WAT, while its deficiency enhances the stability of PTGES2 and CBR1 mRNAs, also in the same reader-dependent manner. Yet, this raises the question of how precision targeting could translate into therapeutic potential. Specifically, would the authors anticipate that inhibiting YTHDF2/3 could provide therapeutic benefits by targeting both BAT and WAT? Additionally, as a point of discussion, the reviewer suggests considering whether METTL14-mediated m6A might engage distinct readers in BAT compared to WAT. The reviewer also seeks clarification on whether YTHDF2/3 expression levels are comparable in BAT and WAT.

Reviewer #3 (Remarks to the Author):

Overall, several of the issues raised in the reviewer 3 report have been adequately addressed, and several additions and modifications have been made to the manuscript that have improved the content. However, some points of concern remain.

Regarding the response to point 1b (assessment of the BAT transcriptome), the BAT alterations in the total adipose tissue (Adipoq-driven) knockout model for comparison with the BAT-specific (Ucp1-driven) model are valuable. However, the considerably discrepant pattern of alterations does not contribute to clarifying the scenario, and the possible explanations provided are speculative and not particularly robust (rather slight differences in the ontogeny of Adipoq and Ucp1 expression or in the degree of METTL14 knockout in the two models). The proposal that the distinct effects of BAT could be triggered by the presence of the apoptotic process in WAT that occurs in the Adipoq-driven invalidation model is plausible but speculative.

Regarding point 3b (marked discrepancies in the results obtained with the Adipoq-driven METTL14 invalidation model in the current manuscript and in the Kang paper published in 2023) the authors provide a thorough discussion of the potential mechanisms underlying this, but again the explanations provided are plausible but remain speculative. One might question the actual potency of METTL14-driven m(6)A mRNA methylation if only mild changes in genetic background or diet can have such an impact as to give rise to metabolic responses opposite to invalidation.

Making these two unresolved aspects more explicit in the “Limitations section” would enhance a balanced provision of conclusions in the manuscript.

Finally, the authors should tone down the strength of the conclusions drawn from surgical removal of interscapular BAT. It should be noted that interscapular BAT depot represents a relevant amount of total BAT in rodents, but there are other BAT depot such as subscapular, axillary and even periaortic depots that, taken together, are not quantitatively insignificant at all. Interpretation of the data should take this limitation into account.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

I would like to express my appreciation to the authors for their hard work and dedication throughout the review process. The revisions made have significantly improved the quality and clarity of the manuscript, addressing the concerns raised in previous rounds of review. I have carefully reviewed the current version and have no further comments or suggestions. I am pleased to recommend this manuscript for acceptance in Nature Communications

Response to reviewer #1: We appreciate your recognition and thank you for the endorsement.

Reviewer #2 (Remarks to the Author):

Most of my comments have been sufficiently addressed, though a puzzle remains in comment #2. The reviewer fully acknowledges the authors' assertion that METTL14-mediated m6A accelerates the decay of apoptosis-related transcripts in a YTHDF2/3-dependent manner in WAT, while its deficiency enhances the stability of *PTGES2* and *CBR1* mRNAs, also in the same reader-dependent manner. Yet, this raises the question of how precision targeting could translate into therapeutic potential.

Response to reviewer #2: Thank you. We have addressed your remaining queries as follows:

(1) Specifically, would the authors anticipate that inhibiting YTHDF2/3 could provide therapeutic benefits by targeting both BAT and WAT?

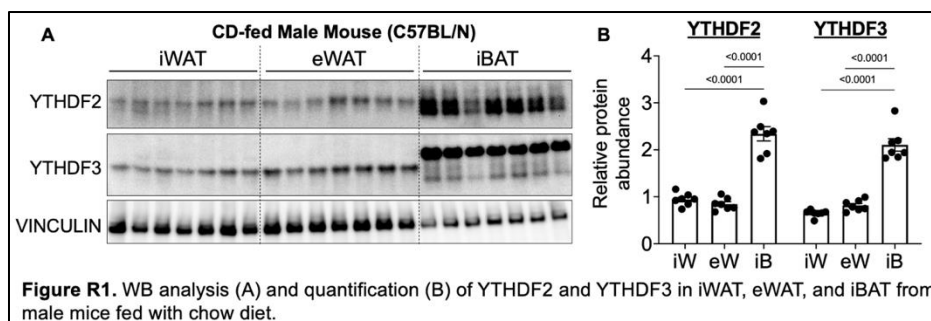
Response (1): We do not anticipate that inhibiting YTHDF2/3 would produce identical/similar metabolic benefits in both BAT- and WAT-mediated systemic metabolism. Specifically, in WAT, YTHDF2/3 inhibition may be detrimental, as it would prevent the decay of apoptosis-related genes, leading to their upregulation. However, in BAT, inhibiting YTHDF2/3 could indeed have beneficial effects on metabolic homeostasis. Specifically, a deficiency in YTHDF2/3 in BAT could decrease the decay of *PTGES2* and *CBR1* mRNAs, leading to their higher expression and subsequently elevating the production of PGE2/PGF2a, which is important for systemic insulin sensitivity. However, as you commented in #2 and #3, given the potential engagement of other m⁶A readers, as well as the differential expression of YTHDF2 and YTHDF3 in WAT and BAT, simply inhibiting YTHDF2/3 in BAT might not reproduce the metabolic benefits.

(2) Additionally, as a point of discussion, the reviewer suggests considering whether METTL14-mediated m6A might engage distinct readers in BAT compared to WAT.

Response (2): We are grateful for this insightful suggestion regarding the potential engagement of distinct m⁶A readers in BAT versus WAT. Indeed, we have examined specific readers in the current study and cannot rule out the engagement of reader proteins. We have incorporated this point into the discussion section (Lines 548-551) as suggested.

(3) The reviewer also seeks clarification on whether YTHDF2/3 expression levels are comparable in BAT and WAT.

Response (3): In response to your suggestion we analyzed YTHDF2/3 protein abundance in iWAT, eWAT, and iBAT tissues from the same mice under physiological conditions (chow diet). Interestingly, we observed significantly higher levels of YTHDF2/3 in iBAT compared to iWAT and eWAT (Figure R1), suggesting a potential tissue-specific role for the readers in BAT versus WAT. Additionally, the presence of multiple bands in iBAT may indicate posttranslational modifications, such as glycosylation or SUMOylation of YTHDF2/3, which we are currently investigating. Thank you again for this useful input.



Reviewer #3 (Remarks to the Author):

Overall, several of the issues raised in the reviewer 3 report have been adequately addressed, and several additions and modifications have been made to the manuscript that have improved the content.

Response to reviewer #3: Thank you. Please see our response to each of your remaining concerns below:

However, some points of concern remain. Regarding the response to point 1b (assessment of the BAT transcriptome), the BAT alterations in the total adipose tissue (Adipoq-driven) knockout model for comparison with the BAT-specific (Ucp1-driven) model are valuable. However, the considerably discrepant pattern of alterations does not contribute to clarifying the scenario, and the possible explanations provided are speculative and not particularly robust (rather slight differences in the ontogeny of Adipoq and Ucp1 expression or in the degree of METTL14 knockout in the two models). The proposal that the distinct effects of BAT could be triggered by the presence of the apoptotic process in WAT that occurs in the Adipoq-driven invalidation model is plausible but speculative.

Regarding point 3b (marked discrepancies in the results obtained with the Adipoq-driven METTL14 invalidation model in the current manuscript and in the Kang paper published in 2023) the authors provide a thorough discussion of the potential mechanisms underlying this, but again the explanations provided are plausible but remain speculative. One might question the actual potency of METTL14-driven m(6)A mRNA methylation if only mild changes in genetic background or diet can have such an impact as to give rise to metabolic responses opposite to invalidation.

(1) Making these two unresolved aspects more explicit in the “Limitations section” would enhance a balanced provision of conclusions in the manuscript.

Response (1): We agree and have revised the manuscript to explicitly discuss these two points in the Limitations section (Lines 551-555).

(2) Finally, the authors should tone down the strength of the conclusions drawn from surgical removal of interscapular BAT. It should be noted that interscapular BAT depot represents a relevant amount of total BAT in rodents, but there are other BAT depot such as subscapular, axillary and even periaortic depots that, taken together, are not quantitatively insignificant at all. Interpretation of the data should take this limitation into account.

Response (2): Per your suggestion we have revised our conclusion derived from the iBAT removal experiment, please see lines 239-243 in the revised manuscript.

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

My comments and concerns have been fully addressed.

Reviewer #3 (Remarks to the Author):

The additions in the text by the authors discussing the extent of the limitations of the study have appropriately addressed my previous comments in the Reviewer report.