Warsaw, 30.12.2025

Review of PhD thesis of Mr. Eryk Duński entitled
"Evolution of regulome in human astrocytes based on iPSC-derived astrocyte models"

The PhD project of Mr. Eryk Duński was carried out under the supervision of Dr. hab. Aleksandra Pękowska at the Nencki Institute of Experimental Biology, Polish Academy of Sciences. This doctoral dissertation addresses the role of astrocytes in the development and evolution of the human brain, with a particular focus on how evolutionary changes in cis-regulatory elements (CREs) have shaped human-specific gene expression programs. Using an in vitro astrocyte model derived from induced pluripotent stem cells of humans, chimpanzees, and rhesus macaques, the study combines transcriptomic, epigenomic, and chromatin conformation analyses with high-throughput expression assay to dissect the regulatory logic underlying astrocyte specialization in humans, comparing it to that of the chimpanzee and rhesus macaque. The work provides compelling evidence that rapidly evolving enhancers, including those harboring human-specific sequence variants, are enriched near genes linked to brain morphology and cognitive traits, and identifies transcription factor binding sites that are critical for human-specific gain in enhancer activity.

General comments:

The dissertation is generally well-written and follows a typical layout, consisting of abstract, a broad introduction to the topic, methodology, followed by a detailed description of the results, conclusions, discussions, and the limitations of the study.

In the introduction, Mr. Duński provides a comprehensive overview of the topic, providing the background information necessary for understanding the function of astrocytes, specifically in supporting brain cognitive function, and their evolutionary context. The introduction also describes the IPS system as well as the general application of the massively parallel reporter assay (MPRA) and the CRISPRi. However, this section is relatively brief and much of the details describing the principles and technicalities of these approaches were included under the results section instead. While this is not a major issue, providing more methodological context in the Introduction and reserving the results section primarily for experimental findings would improve readability.

The methods section is well organized, providing a comprehensive list of the materials used and sufficient details on the experimental procedures. The results are presented in 52 figures which are generally of high quality. The results section is logically structured, clearly written, and is followed by a concise conclusion that summarizes the key outcomes of the study. The discussion section is comprehensive, addressing the critical findings in the context of existing literature. The thesis also included a dedicated chapter outlining the limitations of the study, in which various aspects of design considerations and potential caveats are explicitly acknowledged and appropriately discussed.

Overall, the thesis is well written and scientifically sound. However, there are several spelling and grammatical mistakes, as well as editing errors noticeable throughout the text. For instance, p. 13 first line "Neurons, which are generally regarded as the brain's principal functional units."; p.13 last line "Inversely, he removal of astrocytes…"; p. 107 "This suggests that the evolutionary change HARs which are active enhancers in astrocytes occurs at locations which can potentially influence expression genes crucial for brain development"; p. 111 "… showing that a gain for USF binding sites in enhancers linked to genes that are differentially expression between human and NHP iAstrocytes."; p. 163 information in the first paragraph is duplicated in the subsequent one, etc. While these issues do not detract from the quality of the work, addressing them through careful proofreading would further improve the clarity and professionalism of the already strong dissertation.

Specific comments:

1. Results section 4.4

   The identification of TF binding sites as well as disease-associated variants among the enhancers identified through ATAC-seq provided valuable insights into potential upstream regulators as well as downstream processes. An additional means to strengthen the biological interpretation would be to perform GO enrichment analyses on genes associated with these enhancers. Several gene-enhancer association rules could be considered, e.g. nearest neighbor or inclusion of genes within a defined genomic range. While enhancers can act over long distances, the nearest-neighbor rule is generally applicable in the majority of cases. Such an analysis could reveal whether poised and active enhancers are enriched for distinct biological/molecular functions, potentially offering further insights into different active and repressed astrocytic programs.

2. Results section 4.6

   The comparison between human and ancient hominids is particularly interesting and could be an opportunity to identify highly specific factors that contribute to shaping the human mind. What are the genes that are associated with these SNC-containing enhancers, and could additional insight be gained in terms of how they provide selective evolutionary advantage of *Homo sapiens*?

3. Results section 4.7

   - LiftOver was mainly used with different stringency to identify common enhancers between human and NHPs. It is worth considering that enhancers are resistant to a high degree of sequence divergence while still retaining its function across different species. Hence, relying on sequence alone risks missing out on enhancers whose sequences had diverged substantially during evolution. Were alternative strategies considered to determine homology of enhancer elements, such as conserved syntenic relationship (e.g. Clement et al., 2020, Nucleic Acids Res. 48(5):2357-2371)?

- The term ‘linked’ and ‘unlinked’ used to denote proximal and distal enhancers is somewhat redundant and potentially confusing. In principle, distal enhancers are still functionally connected to specific target genes (i.e. linked to a particular gene). Could the candidate please clarify the intended distinction or rationale for this terminology?

4. Results section 4.8

   Which minimal promoter was used in the MPRA experiment? Could the candidate discuss the rationale for selecting this promoter, and whether it has been validated for driving the expression of neural genes?

5. Results section 4.8.4

   In p. 134 the candidate stated “…I did not detect an obvious correlation between the degree of sequence divergence and MPRA activity change. This indicates that even minor changes between human and chimpanzee variants can result in a change in MPRA enhancer activity, and the changes must be caused by specific sequence alterations.”

   An alternative interpretation is that the lack of correlation may indicate that the differences in MPRA activity is not ascribed only to sequence alterations, but could also reflect the influence of *trans*-acting factors such as TFs and epigenetic regulation. Could the candidate clarify this statement, and how these possibilities were considered and distinguished?

6. Results section 4.9.2

   The candidate convincingly demonstrated that substituting human enhancer sequence at specific nucleotide positions with the chimpanzee counterpart affected the resulting gene expression. Do these specific nucleotides identified as critical in the human vs. chimpanzee analysis also coincide with disease- associated polymorphism in humans?

7. Results section 4.10.3

   Bulk RNA-seq analysis following enhancer perturbation revealed its effects on biological processes related to extracellular matrix organization and structure. It would be valuable to complement these findings with cell morphology analyses, which could provide critical insights into the enhancer's role in astrocyte function and its interactions with the extracellular environment. Could the candidate comment on whether such analyses were performed or are feasible within the scope of this study?

8. In general, the analysis focuses on CREs which gained or increased in activity in humans compared to NHPs. Would you also expect that CREs that are lost or decreased in activity may equally play a role in the evolution of human intelligence? Do you also observe human CREs which behave in such a way (i.e. decreased in activity in MPRA assay compared to NHPs)? Similarly, the analysis prioritizes CREs associated with genes upregulated in human astrocytes, while downregulated genes were largely ignored. Could the candidate clarify whether there is any particular reason for this focus, and are there examples in the literature where the loss or silencing of specific gene's expression has contributed to the evolution of human-specific traits?

Overall, my evaluation of Mr. Duński's thesis is very positive. The topic of the dissertation is novel, addressing critical knowledge gaps on the regulatory mechanisms contributing to the emergence of astrocytic functions that contribute to human-specific brain function during evolution. The study reflects careful planning and potential limitations of each analysis are well justified and sufficiently discussed. Importantly, the work integrates data across multiple omics approaches, enabling a robust and comprehensive analysis that yields unique and biologically meaningful insights. The study is ambitious in scope, conceptually sound, and applies state-of-the-art methodology. Furthermore, the candidate's ability to perform well in both wet-lab and computational analyses highlights his comprehensive skill set and capacity to undertake complex research.

The dissertation complies with requirements listed in Art. 187 Sections 1 and 2 of the Act of July 20, 2018 - Law on Higher Education and Science (Journal of Laws 2018 item 1668 as amended). I am pleased to recommend that the candidate be admitted to the subsequent stages of the doctoral defense. Given the significant contribution to the current state of knowledge and the extensive scope of the work undertaken, I recommend that the doctoral dissertation be awarded a distinction.

Sincerely,

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UNIVERSITY
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Anna Malik, PhD DSc

Warszawa, 15/01/2026

Evaluation of the doctoral thesis

„Evolution of regulome in human astrocytes based on iPSC-derived astrocyte models”
by Eryk Duński, M.Sc.

prepared under the supervision of Dr. Aleksandra Pękowska, Ph.D., D.Sc., in the Dioscuri Centre for Chromatin Biology and Epigenomics of the Nencki Institute of Experimental Biology

The doctoral thesis of Eryk Duński, M.Sc., focuses on investigating evolutionary changes in enhancers that may have contributed to uniquely human patterns of gene expression in astrocytes. This research aims to deepen our understanding of the role of astrocytes in shaping the distinctive features of the human brain during evolution.

The thesis has been written in English as a standard monograph. The title reflects the scientific content of the thesis. The structure of the dissertation follows a standard format, including Polish and English Abstracts and seven chapters (Introduction, Aims of the work, Materials and methods, Results, Summary, Discussion, and Conclusion). The total length of the thesis is 224 pages. On the last page, three publications and two patents co-authored by the candidate have been listed, as well as one publication in preparation that will contain the main findings described in the dissertation.

In this review, I evaluate the key criteria to be fulfilled by the doctoral candidate, which are defined as follows:

1) The thesis presents the candidate's general theoretical knowledge in the scientific discipline
2) The thesis demonstrates the ability of the candidate to independently conduct scientific work
3) The dissertation constitutes an original solution to a scientific problem.

1) Candidate's general theoretical knowledge in the scientific discipline presented in the dissertation

The candidate's theoretical knowledge is presented primarily in the Introduction section and, to some extent, also in the Discussion. Introduction covers the following topics: processes regulated by astrocytes (formation and elimination of synapses; gliotransmission; blood-brain barrier transport and maintenance; brain metabolism); role of astrocytes in disease; role of astrocytes in cognition; evolution of astrocytes; astrocyte regulome and its role in brain evolution; modelling astrocytes and their regulome (IPS technology; Massively Parallel Reporter Assay (MPRA); CRISPRi technology).

The candidate presents a very good synthesis of major concepts in the field of astrocyte biology and evolution. He demonstrates an excellent understanding of modern tools in molecular biology, bioinformatics, and cellular models. It is worth pointing out that although the amount of cited literature is massive (576 publications), the knowledge is presented in a synthetic and non-overwhelming way, demonstrating the ability of Mr Duński to summarize and integrate concepts from different sources.

Among the weaker points of the thesis, I need to mention that in some instances the wording is misleading. I trust that this reflects inaccuracies rather than a lack of knowledge, although this is somewhat disturbing. Some of the examples are: (1) "In mice, knockout (KO) of Gpc4 impairs synapse formation, and was shown to induce the formation of excitatory synapses *in vitro*." I believe that it was Gpc4, and not the knockout of Gpc4, which was not shown to induce the formation of synapses. (2) "Reduction of astrocytic glycogen levels has been shown to cause seizures, as observed in Lafora disease." Lafora disease is caused by abnormal accumulation of glycogen. (3) "mice lacking S100β expression have shown increased LTP (220), whereas optogenetic inactivation of S100β decreased cognitive flexibility in more recent studies (221)". I have doubts whether optogenetic approach was used in the cited work. It is also a pity that there are no figures or schemes in the Introduction, which would help to comprehend the presented concepts.

These are only minor concerns and **I am fully convinced that the candidate demonstrates a good level of biological knowledge**, particularly in the areas of molecular biology, sequencing-based approaches, cellular models, and astrocyte biology.

2) The ability of the candidate to independently conduct scientific work

Conducting scientific work involves proposing hypotheses and designing the study with relevant methodology, followed by performing experiments, analyzing data, presenting results, drawing conclusions, and confronting them with existing knowledge.

1. Hypotheses, study design and methodology.

Hypotheses proposed by Mr Duński are novel and interesting as will be discussed in point #3 below.

Overall, the methods and materials used in the study are described in detail, although at times the presentation is somewhat disorganized. I found it difficult to link the sequences in the tables to their specific applications; for example, it is unclear to me which sequences correspond to sgRNAs. There is some confusion regarding the glioma cell line used: U87 and LN229 cell lines are mentioned in the methods, but only LN229 is mentioned in other sections. At times, editorial and spelling mistakes make the information less clear.

Nevertheless, the methods used are state of the art and have been very well chosen for the proposed hypotheses. I find the MPRA assay, which allows functional verification of enhancer activity, particularly interesting. Moreover, cellular model used in the study – IPSCs-derived human and primate astrocytes, is highly advanced, innovative and unique. Furthermore, Mr. Duński employed a range of bioinformatic analyses to test his assumptions on several complex datasets, including ATAC-seq, Hi-C, and ChIP-seq data. The combined analysis of these datasets required very good planning as well as advanced analytical skills.

2. Analysis and presentation of the results.

The analysis of the obtained results has been conducted correctly and rigorously, although I have one reservation. The use of the term "correlation" for some of the results is not accurate in my view. In its precise meaning, correlation is a statistical measure that expresses the extent to which two variables are linearly related. It should be tested using specific statistical methods and by computing a correlation coefficient. Throughout the dissertation, this term is misused to describe apparent associations between two measures (e.g. Fig. 25, 26, 27, 30, 32, 34). Moreover, an important weakness in data presentation is the lack of key information in the figure legends. It is unclear which measures (e.g., mean or median, error bars) are presented in the graphs. In some cases, panel descriptions are missing (e.g., Fig. 21, panel D; Fig. 24, panels C and D). Additionally, there are references to non-existent figures, such as Fig. R29 (page 125), R3 (page 129), and R5 (page 163). I would like to stress that while this aspect of the presented work could be improved, it does not significantly diminish the overall value of the study.

3. Conclusions and discussion.

Overall, Mr. Duński presents conclusions that are well supported by the results he obtained. In the Discussion section, he places his findings in the context of existing knowledge in a concise and mature manner. Importantly, he also addresses the limitations of the study, including those arising from the technical constraints of the methods used. Moreover, the candidate identifies several directions for future research, such as investigating the role of FOS, JUN, and CRE-binding transcription factors in astrocytes; the potential involvement of the uncovered regulatory mechanisms in shaping brain morphology; and the specificity of the obtained results to fetal astrocytes compared to mature cells.

In conclusion, I am convinced that the dissertation documents the ability of the candidate to independently conduct scientific work.

3) The dissertation constitutes an original solution to a scientific problem.

This research is based on the concept that a large share of the interspecies differences stems from evolutionary changes in the sequence of DNA regulatory elements. In this context, Mr Duński postulated the particular importance of the evolution of astrocyte regulome in driving the morphological and functional differences between human and non-human primate brains. Thus, he aimed at identifying the genetic changes that underlay the evolutionary expansion of astrocyte size, form, and function. To achieve this, he combined sequencing-based approaches, bioinformatics tools and advanced molecular tools, and IPSC-based models.

Overall, this research is very timely and ambitious. In my opinion, the crucial aspect of this work is combining bioinformatics analysis of sequencing data with an experimental validation of the regulatory activity of selected enhancer regions. For this purpose, Mr Duński applied an original approach based on massively parallel reporter assay (MPRA). He used it to validate the results of the previous work suggesting that the gain of binding sites for Universal Stripe Factors (USFs) in enhancers near genes more highly expressed in human then non-human primate astrocytes underlie the emergence of human-specific regulatory activity. He showed that the number of predicted USF binding sites is associated most strongly with enhancer activity compared with other epigenetic and sequence features. Furthermore, he used MPRA to test whether SNPs within active human enhancers modulate their activity. He obtained evidence for selective evolutionary pressure favoring the gain of USF sites and postulated that the emergence of new enhancers through the gain of USF binding sites might be a mechanism of enhancer evolution. I find these and other conclusion from this work extremely interesting and I am convinced they bring novel knowledge on astrocyte regulome and the evolution of human brain.

In summary, Mr Duński investigates a very timely and innovative topic, focusing on the role of astrocyte regulome in the evolution of this cell type from non-human primates to human. **There is no doubt that this dissertation constitutes an original solution to a scientific problem.**

I would be happy to further discuss the following topics with the candidate:

- The vast majority of our knowledge on astrocytes and brain development comes from murine studies. How relevant are these findings to human astrocyte function, and how useful are they for interpreting the data presented in this work? Do human astrocytes perform the same functions more efficiently, or have they acquired new ones?

- The concept that changes in fetal astrocytes can underlie neurodegeneration (e.g. in AD) is unclear to me.

- Some pathways active in astrocytes are also highly relevant to neuronal function, e.g. AP-1 and CREB. In MPRA, glioma cell line was used (which is completely justified given the technical limitations). To what extent the importance of these regulatory mechanisms for brain physiology/pathology solely depends on their activities in astrocytes? Could the same mechanisms also shape neuronal development and function?

Final conclusions

Based on the above assessment, I conclude that the doctoral dissertation by Eryk Duński, MSc, meets the requirements set forth by the Polish law (Art. 187 of The Act on Higher Education and Science of 20 July, 2018). In view of the above, I request the Scientific Council of the Nencki Institute of Experimental Biology, Polish Academy of Sciences, to take further steps toward defending the thesis.

Rozprawa doktorska spełnia warunki określone w art. 187 Ustawy z dnia 20 lipca 2018 r Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2023 r. poz. 742 z późn. zm). W związku z powyższym wnioskuję do Rady Naukowej Instytutu Biologii Doświadczalnej o dopuszczenie mgr. Eryka Duńskiego do dalszych etapów postępowania o nadanie stopnia doktora.

AMalik

Dr hab. Anna Malik

GHENT UNIVERSITY

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CENTER FOR MEDICAL GENETICS GHENT

Evaluation report of the doctoral dissertation

Date: February 15, 2026

PhD candidate: Eryk Duński, M.Sc.
Title: Evolution of regulome in human astrocytes based on iPSC-derived astrocyte models
Institution: Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Poland.
Supervisor: Dr. Aleksandra Pękowska, Ph.D., D.Sc.

Summary

The PhD thesis is based on the hypothesis that unique features of human astrocytes are driven by alterations in regulatory regions, e.g. enhancers, which finetune gene expression.
The overall goal of the doctoral thesis is to provide insight into the role of astrocytes in the human brain and into the role of evolutionary changes in enhancers in human-specific gene expression in astrocytes and in human brain traits during evolution. Specifically, it aims to uncover how genetic and regulatory changes in astrocytes contribute to the evolution of the human brain, focusing especially on enhancer activity and transcription factor (TF) function. It investigates which TFs, particularly Universal Stripe Factors (USFs), drive enhancer activity in fetal astrocytes, how astrocyte regulation relates to brain traits and disorders, and whether disease-associated genetic changes alter enhancer function. Finally, it investigates whether gain of USF-binding sites is responsible for increased activity of human-specific enhancers.
The thesis represents experimental and computational work focused on the evolutionary divergence of regulatory elements (enhancers) in astrocytes, leveraging iPSC-derived astrocyte models from humans and non-human primates (chimpanzee and macaque).
(1) The study uses human, chimpanzee, and macaque iPSC-derived astrocytes to map their transcriptomes, regulatory landscapes, and chromatin architecture, revealing that human astrocyte enhancers show rapid evolution and are enriched for variants linked to brain morphology.
(2) The work identifies human-specific enhancers containing modern-human DNA variants, supporting insights into mechanisms of brain evolution and cognition.
(3) Using MPRAs interrogating enhancers in primate iAstrocytes, it is shown that USF TFBSs are essential for the activity of human enhancers, have recently evolved, and are associated with disease-linked genetic variants.
The topic lies at the intersection of regulatory genomics/epigenomics, evolutionary biology, and cellular neurobiology, and is aligned with biological sciences.

Specific comments

The thesis is structured in an Abstract, Introduction, Aims, Materials & Methods, Results, Discussion, Conclusions, References, and Publication list.

Introduction

This provides an overview of astrocyte biology (physiology, metabolism, gliotransmission, BBB roles), astrocyte involvement in disease and cognition, astrocyte evolution, the astrocyte regulome and its role in brain evolution, modeling of astrocytes and its regulome.

-Although the text is interesting and accessible to non-experts, the addition of figures may enhance the reading and understanding.

Materials and Methods

The work integrates iPSC differentiation into astrocytes (human, chimpanzee, macaque), multiomics profiling (RNA-seq, ATAC-seq, ChIP-seq for H3K27ac/H3K4me3), chromatin conformation (Intact Hi-C), Massively Parallel Reporter Assays (MPRA), luciferase assays, and CRISPRi enhancer perturbations. Cross-species comparative analyses and bioinformatic pipelines (e.g., STAR, MACS2, DESeq2, HOCOMOCO/FIMO, Juicer, multiz, clusterProfiler) are used.

-The protocols are provided in detail in this section. The contributors' credits are listed in the beginning of this section. It would be useful however if the contributions of the PhD candidate could be specified and added.

-The Materials section mainly consists of lengthy tables that could be moved to Supplementary material.

-An Ethics section is missing, which is quite important given the research work on NHP (some from the Warsaw ZOO) and human material.

-On p.68 the subtitle "3.2.18. Genomic characterisations" is followed by "3.2.18.1. RNA-Seq", which seems a bit odd, as the latter concerns the transcriptional level.

-p.78: SNP is used for variant: do all tested variants qualify as SNPs or is SNV rather meant? The term 'single-nucleotide variants' is used on p.92, and single-nucleotide changes (SNCs) on p.103. Harmonizing the terms throughout thesis may improve clarity. SNVs are a frequently used term.

-GWAS variant: is this used as a proxy of GWAS hits? The variants themselves are not necessarily the 'causative' variants but can be a proxy of a neighbouring region. Same comment for p.94: "disease-associated variants."

Results

-p.84: (including disease-related single-nucleotide polymorphisms (SNPs) -> (SNPs))

-p.92: water circulation: is this commonly used? Rather liquid circulation?

-p.92: 'Thus, the association of astrocyte regulome with water circulation in the brain suggests broader implications for understanding the astrocytic contribution to brain homeostasis and neurodegenerative disease.' -> without validation, it may be better to soften this claim.

-p.93: the Impairment of cortical folding -> impairment

Conclusions

Based on the research findings presented, it can be concluded that: USFs are important for astrocytic enhancer activity, and their gain correlates with greater enhancer activity; the iAstrocyte regulome is enriched in sites of accelerated evolutionary change specific to humans, relative to both NHP are other hominids; the iAstrocyte regulome is enriched in human alterations associated with brain morphology, prioritizing astrocytes for the regulation of brain size in evolution; gain of USF binding sites in human-specific enhancers is essential for bolstering enhancer activity in evolution. The effects of human 283 alternations on enhancer activity were tested and 108 alterations were found that modulate enhancer activity in astrocytes.

Discussion and conclusions

The discussion integrates evolutionary genomics with functional data to propose a compelling mechanism by which astrocyte regulatory evolution may have supported human brain specialisation.

Although some limitations are acknowledged (e.g., mainly focusing on the MPRA-based approaches used in the study), another limitation that should be acknowledged is that the iAstrocytes rather represent a fetal profile than a profile of (postnatal) astrocyte cells, hence limiting potential conclusions about late-onset brain neuropsychiatric diseases where the GWAS hits used come from.

Future perspectives could be discussed, either in the thesis or during the public defense. Examples: are (1) Potential alternative models for regulatory astrocyte studies (e.g., primary native adult tissue); (2) Astrocyte datasets that are missing and that might be relevant to more sophisticated regulation of gene expression such as full-length transcriptional isoforms across time and species (humans, NHPs); (3) Criteria that could be used to select enhancer variants for therapeutic modulation (ASOs/CRISPRa) in neuropsychiatric disease.

Originality and innovation

The dissertation constitutes a very original solution to a significant scientific problem. Particular innovations include the study of evolutionary divergence of regulatory elements (enhancers) in astrocytes, leveraging iPSC-derived astrocyte models from humans and non-human primates (chimpanzee and macaque), which is very unique.

Demonstration of theoretical knowledge and ability to conduct independent research

The candidate demonstrates advanced knowledge of regulatory genomics, proficiency in both wet-lab and computational methods, and clear independence in experimental design, data analysis, and interpretation. The scope and technical complexity of the work meet doctoral expectations.

Depending on the institutional requirements, it is recommended to add an applicant's CV to give an idea of the PhD portfolio, in terms of participation in meetings and courses (research and soft skills), abstract and poster presentations, dissemination and outreach.

It would be useful to be informed about the status of the paper (first authorship) in preparation.

Compliance with legal requirements

The dissertation complies with requirements listed in Art. 187 Sections 1 and 2 of the Act of July 20, 2018 - Law on Higher Education and Science (Journal of Laws 2018 item 1668 as amended)"

1. The doctoral dissertation demonstrates the candidate's general theoretical knowledge in a discipline (or disciplines) and the ability to conduct research or artistic work independently.
2. The subject matter of the doctoral dissertation is an original solution to a scientific problem.

Reviewer's recommendation
Overall evaluation: POSITIVE.
The Doctoral student be admitted to the subsequent stages of the doctoral defense.
Optional (Distinction): Given the originality and conceptual novelty regarding USF-mediated enhancer evolution, and scale of functional validation, I support considering a distinction for this dissertation.

Yours sincerely,

Prof. Elfride De Baere, M.D., Ph.D.
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Belgium