For research article

Response to Reviewer four Comments

1. Summary		
Thank you very much for taking the time to thoroughly review our manuscript and for your valuable suggestions. We also sincerely appreciate your recognition of our efforts in precisely identifying key genomic regions in yaks and detecting candidate genes associated with the investigated traits, which demonstrates significant theoretical value and promising applications for breeding improvement. The paper is well-structured overall and employs relatively advanced methodologies. Your comments have been highly instructive in improving the quality of this work. We have carefully examined and addressed each point raised, with all corresponding revisions highlighted in purple in the text. Below are our detailed responses.		
2. Questions for General Evaluation	**Reviewer's Evaluation**	**Response and Revisions**
Does the introduction provide sufficient background and include all relevant references?	Must be improved	We sincerely appreciate the reviewer's insightful feedback. Upon re-evaluation, we acknowledge that the introduction could indeed be strengthened by providing more comprehensive background information and incorporating additional relevant references. We have expanded the introduction to include a more detailed overview of the research field, key concepts, and previous studies. We have also added several new references to support the background information and contextualize our research within the existing literature.
Is the research design appropriate?	Yes	We are pleased that the reviewer finds our research design appropriate. We made concerted efforts to ensure that the design aligns with the research objectives and questions. No major revisions are needed

		for the research design. However, we have refined certain aspects based on the reviewer's comments to further enhance clarity and precision.
Are the methods adequately described?	Can be improved	We thank the reviewer for pointing out areas where the methods section can be improved. We agree that a more detailed and transparent description of the methods would facilitate better understanding and replication of our study. We have revised the methods section to include more specific details about the experimental procedures, sample collection protocols, and data analysis techniques. We have also added information about the statistical software used and the criteria for data inclusion/exclusion.
Are the results clearly presented?	Must be improved	We are grateful to the reviewer for highlighting the need for clearer presentation of the results. We recognize that the current presentation may hinder readers' ability to quickly grasp the key findings. We have reorganized the results section to present the data in a more logical and coherent manner. We have also created new figures and tables to better visualize the results and added concise explanations to accompany each figure/table.
Are the conclusions supported by the results?	Must be improved	We sincerely appreciate the reviewer's emphasis on ensuring that conclusions are well-supported by the results. We agree that this is crucial for the credibility and impact of our research. We have carefully reviewed the conclusions section and revised it to ensure that each conclusion is directly supported by the results presented in the paper. We have also added more discussion to contextualize the findings within the broader research landscape.
Are all figures and tables clear	Must be	We thank the reviewer for their valuable

and well-presented?	improved	feedback on the figures and tables. We acknowledge that there is room for improvement in terms of clarity and presentation. We have revised all figures and tables to enhance their clarity and readability. This includes adjusting font sizes, improving color contrasts, adding labels and legends, and ensuring that all elements are properly aligned. We have also added brief descriptions to each figure/table to explain their content and significance.

3. Point-by-point response to Comments and Suggestions for Authors

Comments 1: Data Presentation and Figure Quality:
Figure 2 (Fig2): The resolution of the currently provided image is too low, resulting in blurry critical details (e.g., branch structures, scale bars). Please provide a high-resolution version (recommended resolution ≥ 300 dpi) of the image file.

Response 1: Dear Reviewer,
Thank you for your valuable feedback regarding the readability of the figures and tables in the manuscript. In response to your suggestions, we have systematically optimized all figures and tables throughout the manuscript: each chart has been enlarged in size, and the image resolution has been uniformly upgraded to the publication standard (≥300 DPI). These adjustments have significantly improved the clarity and detail presentation of the figures and tables, further enhancing the readability and scientific quality of the manuscript. We sincerely appreciate your rigorous guidance, which has helped us improve the quality of our submission.

Comments 2: Figure 3 (Manhattan Plot): Currently, only the significant region is displayed, which provides insufficient information. We suggest explicitly annotating the key candidate gene names located within or adjacent to the most significant peak regions. This will more intuitively link the statistical signals to the biological candidate targets.

Response 2: Dear Reviewer,
Thank you sincerely for your valuable feedback and meticulous reminders regarding the figures and tables in our manuscript. In accordance with your suggestions, we have systematically optimized and adjusted all charts throughout the paper. Specifically, the following improvements have been made:
The Manhattan plot and QQ plot are now presented separately. Key regions in the Manhattan plot have been annotated to enhance the clarity of information presentation.
All figures have been enlarged in size, and the image resolution has been increased to the publication-standard level (≥300 DPI), ensuring clear display of graphical details in both print and digital formats.
Your guidance has helped us further improve the readability and scientific presentation of the figures and tables. We once again express our gratitude for the rigorous assistance you provided during the review process, which has been essential for refining our manuscript.

Comments 3: Phylogenetic Tree: The reviewer notes that samples representing different phenotypes (horned/polled) or populations do not form clear clusters in the phylogenetic tree. Please provide a necessary explanation for this observation.
Response 3: Dear Reviewer, We have attached great importance to this issue. Based on the suggestions from all reviewers, we have revised and restructured this part of the content into three sections to present the results more clearly. Moreover, we have given greater emphasis to this issue in the Discussion section, providing a reasonable interpretation of the findings. We preliminarily infer that this phenomenon may be related to genetic differences between polled and horned Xueduo yaks, and have discussed it prudently in the manuscript in light of existing evidence. Thank you once again for your rigorous review—your comments have significantly enhanced the clarity and logical flow of this study.
Comments 4: Table 2: The column header If_exonic is unclear and appears to be erroneous or an improper abbreviation. Please verify and correct all column headers to ensure they accurately and completely describe the column contents.
Response 4: Based on the reviewers' suggestions, we have carefully examined and revised all column headers. The original column "If_exonic" was intended to indicate whether a SNP is located in an exon. To improve readability and avoid ambiguity, it has been updated to "Genomic Location of SNP".
Comments 5: The term "nucleus herd" in the section title "3.3. Population genetic structure of the yak nucleus herd" is not sufficiently professional.
Response 5: Thank you for your professional correction. In accordance with your suggestion, we have revised the section heading "3.3. Population genetic structure of the yak nucleus herd" to a more precise academic expression: "3.3. Population Genetic Structure Analysis of the Experimental Yak Population." This modification not only clarifies the experimental nature of the study subjects but also aligns with the standard terminology used in population genetics research, making the title clearer and more rigorous.
Comments 6: Enhancing Reliability: In the haplotype analysis section, it is strongly recommended to present a comparative display of the genotype frequencies for the most significantly associated dominant haplotypes or core SNP loci in both the Xueduo yak (discovery population) and Ashdan yak (validation population). Furthermore, if possible, supplement the results of "expanded population validation" in a third independent yak population. This would substantially enhance the reliability and generalizability of the identified region and loci.
Response 6: We have comprehensively revised and optimized this section. The relevant changes have been marked in purple (corresponding to lines 350–386 in the original manuscript). A new independent subsection, "3.5.2 Identification, Quality Control, and Genetic Characteristic Analysis of Cross-Population Shared Core SNPs/INDELs," has been added to systematically present the validation results. Additionally, a new Table 3, "Genotype Distribution and Quality Parameters of Core SNPs and INDELs Related to the Polled Trait on Yak Chromosome 1," has been included. This table displays the genotype frequencies—wild-type homozygote (WT), heterozygote (Het), and variant homozygote (Hom) proportions—of the 6 core SNPs and 6 key INDELs in the "horned Xueduo yaks

(discovery population)," "polled Xueduo yaks (discovery population)," and "Ashdan yaks (validation population)." This allows a direct visual comparison of genetic consistency at the target loci between the validation and discovery populations.

Furthermore, targeted analysis has been added in the text, explicitly stating: "Horned Xueduo yaks were homozygous for the wild-type allele at all core loci, whereas polled Xueduo yaks and Ashdan yaks were predominantly heterozygous. Notably, Ashdan yaks, which have undergone long-term directional breeding, exhibited a higher degree of homozygosity at these variant sites." This further validates the stability and reliability of the association between the core loci and the polled phenotype. These points are emphasized in the Discussion section (lines 437–448).

Taking into account the current limitations in sample resources, we have also added the following as a follow-up research plan in the Discussion: "In future studies, we will collect samples from additional independent polled yak populations, such as Gannan yaks, Huanhu yaks, and Tianzhu white yaks, to conduct multi-breed joint GWAS analyses. This will help verify the specificity and stability of the target genetic loci across different genetic backgrounds and improve the generalizability of the findings." This response not only addresses your suggestion but also reflects the continuity and rigor of our research.

Thank you once again for your insightful comments, which have greatly helped us improve the clarity and robustness of the manuscript.

Comments 7：Depth of Discussion and Literature Support:

Insufficient Exploration of Regulatory Mechanisms: While the discussion correctly notes that the associated region resides within a lincRNA and hypothesizes that *PAXBP1* is a key gene, the biological logic connecting the two is weak. Please deepen the discussion: Integrate the known cis-regulatory functions of non-coding RNAs (especially lincRNAs) to specifically hypothesize potential molecular mechanisms through which this lincRNA region might influence *PAXBP1* expression, thereby participating in neural crest development and horn bud formation. This step is key to transforming genetic mapping into biological understanding.

Literature Update: The reference list is generally outdated and does not adequately reflect recent advancements in the field. Please systematically search for and cite important literature from the past 5 years (especially post-2020) on the genetic regulation of horn/polled trait development in Bovidae (including studies in cattle, sheep, and goats). This will not only provide a more solid foundation for your discussion but also demonstrate your grasp of the field's cutting edge.

Response 7: Dear Reviewer,

We have supplemented the discussion on the connection between lincRNA and the *PAXBP1* gene in lines 466–498 of the manuscript. It is noted that the finding regarding the role of lincRNA in regulating horn formation in this study is not the first of its kind. In cattle polledness research, lincRNAs have been shown to co-localize with horn development-related genes such as *OLIG2* within the same topologically associating domain (TAD) or in adjacent genomic regions. This suggests that lincRNAs may regulate the expression of neighboring genes through local chromatin interactions, thereby participating in horn development. Furthermore, studies on goat horns have identified two key structural

variants (SVs) located in intergenic regions on chromosome 1 as major loci influencing the polled phenotype, further supporting the central role of non-coding variants in regulating horn traits in ruminants.
In addition, research on the antisense lincRNA of *PAXBP1* (*PAXBP1-AS1*) indicates that it may influence *PAXBP1* expression through antisense mechanisms. This provides a direct theoretical reference for our hypothesis that the lincRNA region identified in this study may affect *PAXBP1* expression through similar molecular mechanisms, thereby leading to the polled phenotype.
Moreover, we have introduced six relevant references published within the past five years in both the Introduction and Discussion sections. These additions strengthen the context and support the updated discussion.
Thank you once again for your constructive suggestions, which have helped us enhance the depth and clarity of the manuscript.

Comments 8: Formatting and Referencing Throughout the Manuscript:
There are numerous formatting issues. You must thoroughly eliminate all placeholder errors such as Error! Reference source not found. appearing in the text. Please carefully check and correct all cross-references for figures, tables, and citations.

Response 8: Thank you for your careful and attentive feedback. In response to the formatting issues you identified in the manuscript, we have conducted a comprehensive review and made thorough corrections. All placeholder errors such as "Error! Reference source not found" have been completely removed. We have also meticulously verified and corrected the cross-referencing formats for all figures, tables, and literature citations to ensure accurate and standardized correspondence between figure labels, table references, and citation indexing. These adjustments eliminate any potential confusion caused by formatting inconsistencies and enhance the overall readability of the manuscript.

Comments 9: Keywords (Keywords): Remove any duplicates and ensure proper formatting. For example, genome-wide association Study should be corrected to genome-wide association study ("study" in lowercase).

Response 9: Dear Reviewer,
Thank you sincerely for your valuable suggestions. As per your recommendation, we have optimized and refined the keywords to: yak; horned; polled; GWAS. This revision makes the keywords more concise and representative, and fully aligns with the key focus of our study.

Comments 10: Language and Presentation:
Perform meticulous language polishing of the entire text, checking for and correcting potential grammatical errors and unclear sentences.
Ensure accurate and consistent use of professional terminology.

Response 10: Thank you for your professional suggestions. We have now conducted a comprehensive language polish and standardization review of the entire manuscript. This included systematically checking sentence by sentence to correct potential grammatical errors, ambiguous phrasing, and unclear expressions. We have optimized sentence structure and logical flow, ensured the accurate and consistent use of key terminology such as "lincRNA," "*PAXBP1*," "topologically associating domain (TAD)," and "structural variant (SV)," and verified details like the full-term annotation upon first use of abbreviations and

proper punctuation throughout the text. These revisions have further enhanced the linguistic quality, formality, and overall readability of the manuscript.
4. Response to Comments on the Quality of English Language
Point 1:
Response 1: We sincerely thank the reviewer for the positive feedback on the English expression. In accordance with the overall review comments, we have further polished and refined the language and presentation throughout the manuscript.
5. Additional clarifications
We would like to once again express our sincere gratitude for your rigorous review. Should there be any further findings during your continued evaluation, we will promptly address and refine them accordingly. We deeply appreciate the careful guidance and valuable suggestions provided. All requested revisions have been completed, and we have strived to enhance the manuscript in terms of academic standards, content accuracy, and readability. We kindly invite you to further review the revised manuscript. Wishing you a successful and productive year 2026, with all the best in your work and endeavors.