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LETTER TO THE EDITOR

Improving growth performance across multiple sheep breeds by targeting the *MSTN* gene using an enhanced Cas12i system

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Dear editor,

Over the past decade, CRISPR-Cas technologies have transformed gene editing in eukaryotes across biomedical research, gene therapy, and agriculture. Recently, several functionally diverse type V systems (subtypes V-G, V-H, and V-I) have been identified, expanding the CRISPR toolbox. The Cas12i subfamily of type V-I nucleases has attracted attention for its gene-editing activities and smaller size (1033–1093 aa) compared with type II Cas9 and type V Cas12a. Cas12i autonomously processes pre-crRNA into mature crRNA and cleaves dsDNA via a single RuvC domain by sequentially nicking the non-target and target strands, enabling high-fidelity multiplex genome editing. However, the low editing efficiency of native nucleases in this system limits their broader application. Several studies have generated engineered variants—including hfCas12Max, ABR-001, Cas-SF01, EOCas12i, and Cas12i^{Max}—through protein engineering or crRNA structural modification (Chen et al., 2022; Duan et al., 2024; McGaw et al., 2022; Wang et al., 2025; Zhang et al., 2023). It has been reported that Cas9 nuclease activity is positively associated with increased chromatin accessibility (Ding et al., 2019). Some studies have shown that Cas9-dependent deletions or insertions are enhanced by fusion with chromatin-remodeling proteins or peptides, chromatin-modulating peptides, or synthetic transcription activation domains (Ding et al., 2019; Yin et al., 2023). Cas12 family nucleases have been characterized as generating DNA cleavage products with 5′ single-stranded overhangs, which preferentially undergo error-free rather than indel-prone NHEJ repair. By resecting 5′ single-stranded overhangs at double strand break (DSB) ends through exonuclease fusion, Cas12-mediated repair may be shifted toward error-prone NHEJ, enhancing knockout efficiency. Here, we developed optimized Cas12i systems by fusing non-sequence-specific double-strand DNA-binding domains (dsDBDs) or exonucleases. These modifications significantly enhanced genome-editing efficiency across multiple cell types and cell lines. Additionally, we demonstrated that the engineered Cas12i system, hfCas12Max-T5, can efficiently generate *MSTN*-edited sheep with improved growth performance across three breeds. Notably, family trio-based whole-genome sequencing (WGS) was used to trace variant origins and revealed no increase in off-target events in gene-edited Merino sheep trios. These findings demonstrate that the hfCas12Max-T5 system is both effective and safe for advancing genetic improvement in domestic animals.

Previous studies have shown that fusion of dsDBDs or exonuclease modules to RNA-guided nucleases enhances genome-editing efficiency (Ding et al., 2019; Wu et al., 2020; Yin et al., 2022; Yin et al., 2023; Zhang et al., 2020). To further enhance hfCas12Max genome-editing activity, we systematically evaluated N- or C-terminal fusions of five dsDBDs (HMG-D, HMGN1, HMGB1, H1G, and Sso7d) and three exonucleases (TREX1, TREX2, and T5 exonuclease) at seven endogenous loci using targeted deep sequencing across multiple cell lines (Figure 1A). Interestingly, all C-terminal fusions, except TREX1, showed improved or comparable editing efficiency relative to hfCas12Max across three human cell lines (Figure 1B–D; Figures S1 and S2). Among these variants, hfCas12Max-T5 achieved the highest indel efficiencies,

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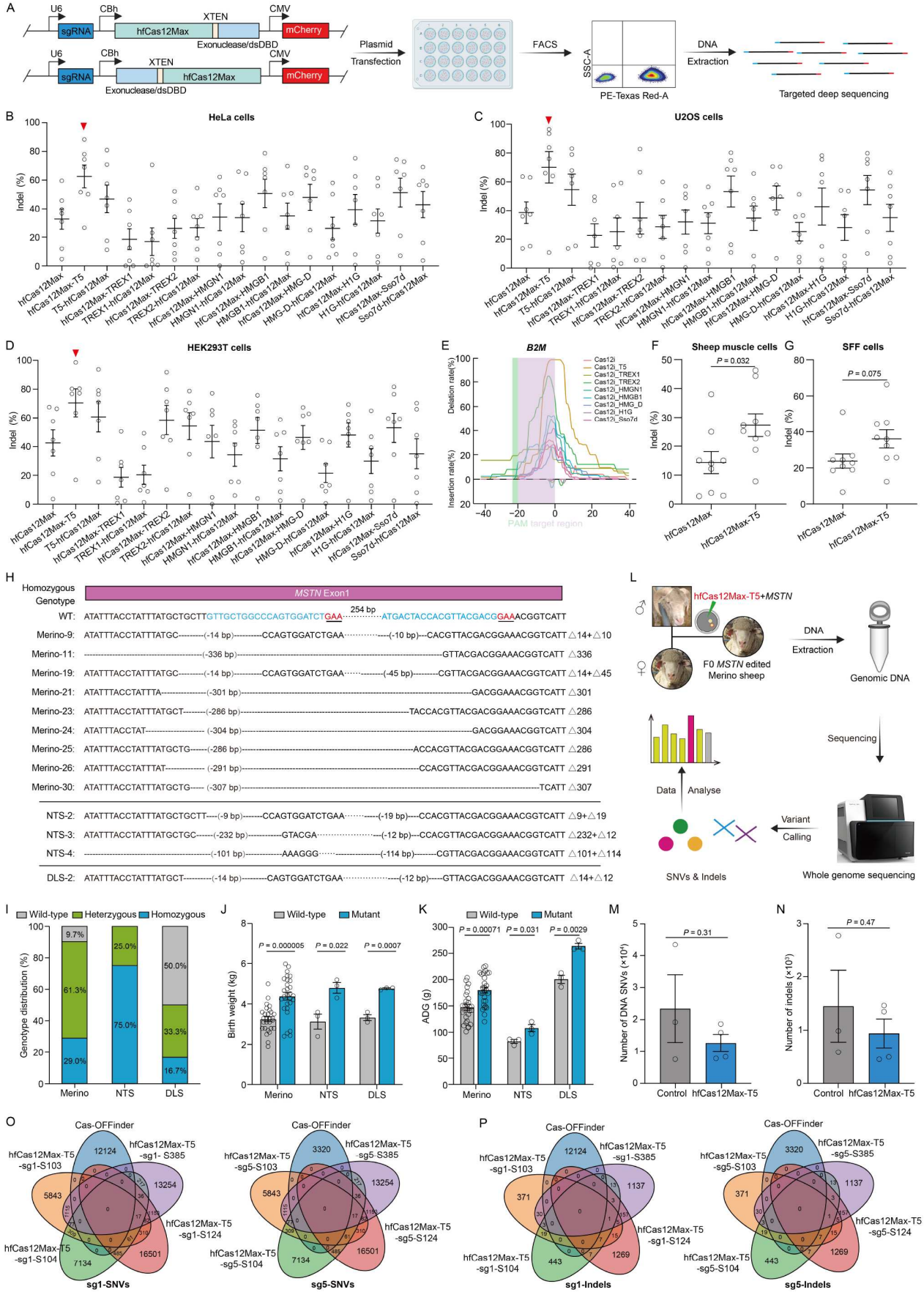

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Figure 1 Optimization of the hfCas12Max system via dsDBD or exonuclease fusions, and evaluation of genome-editing efficiency, specificity, and genetic improvement across multiple sheep breeds. A, Experimental workflow for detecting genome-editing activity of hfCas12Max variants at endogenous loci across multiple cell types. B–D, Genome-editing efficiencies of hfCas12Max with N- or C-terminal fusions of five dsDBDs and three exonucleases at seven genomic loci in HeLa (B), U2OS (C), and HEK293T (D) cells. E, Metaplot showing broader indel patterns generated by hfCas12Max variants at the endogenous *B2M* locus in HEK293T cells. F and G, Comparison of average genome-editing efficiencies between hfCas12Max-T5 and hfCas12Max across nine *MSTN* target sites in sheep muscle (F) and SFF (G) cells. Each data point represents the average genome-editing efficiency at each target site. H, Sequences of modified *MSTN* alleles in 13 homozygous mutant lambs across three sheep breeds. Note that (·) in the reference sequence (WT) and homozygous genotype sequence indicates omitted sequence due to space constraints, while (−) in the homozygous genotype sequence indicates deletions. Abbreviations: NTS, Ningxia Tan Sheep; DLS, Duolang sheep. I, Genotype distribution of all gene-edited lambs across three sheep breeds. J and K, Comparison of birth weight (J) and ADG (K) between *MSTN* mutant and wild-type lambs (Merino, n=28; NTS, n=3; DLS, n=3). L, Outline of identifying *de novo* variants potentially induced by hfCas12Max-T5 using WGS of four Merino sheep family trios. M and N, Comparison of total SNVs (M) and indels (N) detected by WGS in control versus hfCas12Max-T5-edited Merino lambs (Control, n=3; hfCas12Max-T5, n=4). O and P, Overlap among SNVs (O) and indels (P) detected by WGS with predicted off-target sites from Cas-OFFinder between control and hfCas12Max-T5-edited lambs. Data are shown as mean±SEM. The P value was determined using an unpaired two-tailed Student's t-test.

averaging 61.8% in HeLa cells, and 73.07% in U2OS cells, and 70.5% in HEK293T cells, and significantly outperformed hfCas12Max and other engineered variants (Figure 1B–D; Figure S1). Additionally, among all tested dsDBDs, the 63-amino-acid Sso7d from *Sulfolobus solfataricus* outperformed the others, yielding an average 1.25–1.57-fold improvement at seven endogenous loci across three cell lines (Figure 1B–D; Figure S2). We further compared the genome-editing activity and editing patterns of hfCas12Max and its C-terminal fusion variants and found that these variants exhibited higher genome-editing activity and a broader deletion size range at the endogenous *B2M* locus in HEK293T cells (Figure 1E). The hfCas12Max-T5 system exhibited superior editing performance compared with hfCas12Max at nine sites within the *MSTN* gene in sheep fetal fibroblasts (SFFs) and muscle cells (Figure 1F and G; Figure S3).

MSTN encodes myostatin, a negative regulator of skeletal muscle growth that inhibits myoblast proliferation and differentiation. Disruption of MSTN function results in increased muscle mass, commonly referred to as the double-muscling phenotype. Based on test results from sheep cells, we selected two highly efficient sgRNAs (sg1 and sg5) flanking the first exon and used them with the hfCas12Max-T5 system to disrupt the *MSTN* gene in three sheep breeds: Merino, Ningxia Tan (NTS), and Duolang (DLS). In total, we generated gene-edited sheep across three breeds: 31 Merino (90.3% editing frequency: nine homozygous, 19 heterozygous, and three wild-type); four NTS (100.0%: three homozygous and one heterozygous); six DLS (50.0%: one homozygous, two heterozygous, and three wild-type) (Figure 1H and I). Comprehensive phenotypic evaluations of gene-edited sheep from three breeds revealed significant increases in both birth weight and average daily gain (ADG) compared with controls (Figure 1J and K). Additionally, we longitudinally tracked and statistically analyzed phenotypic data from gene-edited Merino and Tan sheep over a six-month period (0–190 d), revealing significant improvements in growth traits, including body weight, body height, body length, hip width, chest width, chest circumference, and cannon circumference (Figures S4 and S5). It is noted that we minimized confounding effects in both the control and gene-edited groups by including only singletons, matching parental backgrounds across groups, balancing sex where possible, and managing all animals under identical feeding and housing protocols.

Unintended off-target mutations induced by CRISPR/Cas nucleases may lead to adverse consequences, limiting the broader application of this technology in livestock genetic improvement. Here, we leveraged hfCas12Max-T5-mediated gene-edited Merino sheep and performed family trio-based WGS to distinguish inherited variants from naturally occurring and gene-editing—induced mutations in the edited progeny (Figure 1L). In total, seven family trios-four hfCas12-Max-T5-edited and three wild-type—were sequenced to a high average genomic coverage depth (~30.2×). Specifically, 7,814, 8,336, 18,645, and 15,951 single-nucleotide variants (SNVs) were identified in the four gene-edited trios following SNV calling, quality control, and exclusion of variants present in the published sheep SNP database (Figure 1M). Additionally, following stringent indel filtering based on read depth, PL values, and manual inspection, 451, 496, 1,464, and 1,349 indels were identified as candidate *de novo* indels in the four gene-edited trios (Figure 1N). The data indicated that hfCas12Max-T5 treatment did not result in a significant increase in SNV or indel counts in gene-edited trios compared with wild-type controls (Figure 1M and N). Furthermore, these *de novo* SNVs were dispersed across all chromosomes, showing no enrichment near the target sites (Figure S6). We then compared the predicted off-target sites generated by Cas-OFFinder (mismatch threshold=6) with the off-target sites empirically identified in the four hfCas12-Max-edited family trios. Interestingly, none of the SNVs or indels identified in the hfCas12Max-treated four trios overlapped with the predicted off-target mutations (Figure 1O and P). However, varying numbers of shared variants were observed among the four edited trios, indicating that these mutations are likely naturally occurring single nucleotide polymorphism (SNP) across different breeds rather than editing-induced off-target events (Figure 1O and P). Overall, fusion of the T5 exonuclease to the C-terminus of hfCas12Max enhanced editing efficiency while preserving specificity, underscoring its considerable potential for applications in animal genetic improvement.

In summary, we tested a series of dsDBDs and exonuclease modules, and found that C-terminal fusion of the Sso7d domain and T5 exonuclease to hfCas12-Max yielded the greatest improvement in editing efficiency in HeLa, U2OS, and HEK293T cells. We also demonstrated that the hfCas12Max-T5 variant enhanced the genome-editing efficiency compared with hfCas12Max in sheep primary cells. *MSTN*-edited Merino, Tan, and Duolang sheep were efficiently generated using the hfCas12Max-T5 system and exhibited improved growth performance. Furthermore, family trio-based WGS was performed to better discriminate variant origins and revealed no obvious off-target effects in four family trios compared with wild-type Merino sheep. These results support hfCas12Max-T5 as a robust gene-editing platform with high efficiency and specificity for livestock genetic improvement.

Compliance and ethics

The authors declare that they have no conflict of interest. Experiments involving different sheep breeds were approved by the Executive Committee on Laboratory Animal Management and Ethical Review at Northwest A&F University. All animal studies were conducted in accordance with relevant ethical guidelines for animal research (NWAFU-DK2024096).

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Supporting information

The supporting information is available online at https://doi.org/10.1007/s11427-026-3369-1. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

References

Chen, Y., Hu, Y., Wang, X., Luo, S., Yang, N., Chen, Y., Li, Z., Zhou, Q., and Li, W. (2022). Synergistic engineering of CRISPR-Cas nucleases enables robust mammalian genome editing. Innovation 3, 100264.

Ding, X., Seebeck, T., Feng, Y., Jiang, Y., Davis, G.D., and Chen, F. (2019). Improving CRISPR-Cas9 genome editing efficiency by fusion with chromatin-modulating peptides. CRISPR J 2, 51–63.

Duan, Z., Liang, Y., Sun, J., Zheng, H., Lin, T., Luo, P., Wang, M., Liu, R., Chen, Y., Guo, S., et al. (2024). An engineered Cas12i nuclease that is an efficient genome editing tool in animals and plants. Innovation 5, 100564.

McGaw, C., Garrity, A.J., Munoz, G.Z., Haswell, J.R., Sengupta, S., Keston-Smith, E., Hunnewell, P., Ornstein, A., Bose, M., Wessells, Q., et al. (2022). Engineered Cas12i2 is a versatile high-efficiency platform for therapeutic genome editing. Nat Commun 13, 2833.

Wang, L., Wang, Y., Chen, J., Zhu, Y., Qin, H., Liu, J., Ai, Y., Lai, J., Lian, Z., and Han, H. (2025). An engineered CRISPR-Cas12i tool for efficient multiplexed genome editing. Nucleic Acids Res 53, gkaf806.

Wu, Y., Yuan, Q., Zhu, Y., Gao, X., Song, J., and Yin, Z. (2020). Improving FnCas12a genome editing by exonuclease fusion. CRISPR J 3, 503–511.

Yin, J., Lu, R., Xin, C., Wang, Y., Ling, X., Li, D., Zhang, W., Liu, M., Xie, W., Kong, L., et al. (2022). Cas9 exo-endonuclease eliminates chromosomal translocations during genome editing. Nat Commun 13, 1204.

Yin, S., Zhang, M., Liu, Y., Sun, X., Guan, Y., Chen, X., Yang, L., Huo, Y., Yang, J., Zhang, X., et al. (2023). Engineering of efficiency-enhanced Cas9 and base editors with improved gene therapy efficacies. Mol Ther 31, 744–759.

Zhang, H., Kong, X., Xue, M., Hu, J., Wang, Z., Wei, Y., Wang, H., Zhou, J., Zhang, W., Xu, M., et al. (2023). An engineered xCas12i with high activity, high specificity and broad PAM range. Protein Cell 14, 538–543.

Zhang, X., Chen, L., Zhu, B., Wang, L., Chen, C., Hong, M., Huang, Y., Li, H., Han, H., Cai, B., et al. (2020). Increasing the efficiency and targeting range of cytidine base editors through fusion of a single-stranded DNA-binding protein domain. Nat Cell Biol 22, 740–750.