Efficient base editing in spermatogonial stem cells enables the establishment of a PSEN1^{M146V} mutant tree shrew model

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Dear Editor,

The tree shrew (*Tupaia belangeri*), a squirrel-like mammal widely distributed in south and southeast Asia, possesses several advantages for biomedical research, including a high brain-to-body mass ratio, diurnal activity, and a well-developed visual system (Yao et al., 2024). Phylogenetically, tree shrews share a close evolutionary relationship with primates, exhibiting greater similarity to non-human primates (NHPs) than to rodents in nervous system organization (Liu et al., 2025) and immune function (Yu et al., 2025). Their practical advantages, such as a lifespan of 6–8 years in captivity, a short gestation period (~6 weeks), and relatively low maintenance costs, further enhance the utility of tree shrews relative to NHPs (Yao et al., 2024). These combined anatomical, phylogenetic, and practical characteristics establish the tree shrews as a valuable translational model, bridging the gap between rodent and NHP studies (Yao et al., 2024).

Establishing genetically modified animal models is fundamental to elucidating gene function and modeling human diseases. Among current strategies, spermatogonial stem cell (SSC)-mediated genome editing provides an efficient route for generating genetically engineered animals with stable germline transmission (Li et al., 2017; Wu et al., 2015). While well-established in rodents (Wu et al., 2015), adapting this approach to species more closely related to humans, such as NHPs, remains technically challenging.

We have previously established the first SSC-based genetically modified tree shrews overexpressing green fluorescent protein (GFP), which remains the only

method for achieving heritable genetic modification in this species (Li et al., 2017). Compared with traditional transgenesis, recently developed precision gene-editing technologies, such as CRISPR-Cas9 mediated knock-in, base editing, and prime editing (Anzalone et al., 2020), provide distinct advantages for modeling human diseases. Consequently, establishing efficient and precise genome-editing strategies in tree shrew SSCs is essential for developing more accurate disease models in this translational species.

In this study, we aimed to optimize the base editing system in tree shrew SSCs and apply it to generate a tree shrew model harboring a pathogenic Alzheimer's disease (AD) mutation (Figure 1A). We targeted the *PSEN1* c.436A>G (p.M146V) mutation, which causes extremely early-onset familial AD (average onset age < 40 years old) (Jia et al., 2020). This mutation is inherited in an autosomal dominant manner, such that heterozygosity alone is sufficient to drive AD phenotypes, establishing it as a well-validated target for AD modeling (Oddo et al., 2003). The targeted nucleotide and the corresponding residue are fully conserved between tree shrews and humans (Figure 1B), indicating that tree shrews represent an ideal model for studying the pathogenic mechanism of this mutation.

To establish an efficient base editing system for tree shrew SSCs, we optimized three factors: (1) electroporation conditions, (2) base editor variant, and (3) the ratio of ABE mRNA to guide RNA (gRNA). Using the Neon NxT system (Thermo Fisher), we first tested electroporation settings. Under the optimized parameters of 1150 V, 10 ms pulse width, and 3 pulses, tree shrew SSCs maintained relatively high cell viability

(>80%) and transfection efficiency (>66%) (Supplementary Table S1, Supplementary Figure S1A).

Because editing efficiency varies with sequence context and cell type, we tested two ABE8 variants in tree shrew SSCs. The target site (*PSEN1* c.436A>G) is located at protospacer position A5, with additional adenines at positions A8 and A11 within the same gRNA-targeted region (Figure 1B). ABE8.8m has been reported to achieve comparable editing efficiency at position A5 while exhibiting lower efficiency at positions A8-A11 compared to other ABE8 variants (e.g., ABE8.20m) (Gaudelli et al., 2020). To minimize potential bystander editing, we selected ABE8.8m for evaluation in tree shrew SSCs. We additionally assessed ABE8e (Richter et al., 2020), a variant reported to have substantially increased editing efficiency compared with ABE7.10, in tree shrew cells. Co-electroporation of 1 μg gRNA with 1 μg ABE mRNA showed that ABE8.8m yielded higher editing efficiency at the *PSEN1* c.436A>G site (Figure 1C). We further performed parallel experiments in tree shrew Sertoli cells using both ABE variants. In contrast to the observations in SSCs, ABE8e exhibited high editing efficiency at positions A5, A8, and A11 in Sertoli cells, whereas ABE8.8m primarily edited positions A5 and A8 (Supplementary Figure S1B). These results suggest that the editing efficiency of ABE variants may vary across cell types and that ABE8.8m may be more suitable for precisely introducing the PSEN1^{M146V} mutation (c.436A>G) in tree shrew SSCs.

Using ABE8.8m, we next optimized the gRNA-to-mRNA ratio. Increasing the gRNA amount to 3.2 μg significantly enhanced editing efficiency (Figure 1D). We

95 further quantified the editing efficiency using the next-generation sequencing
96 technology. Under the final optimized conditions, namely co-electroporation of 3.2 μg
97 synthetic gRNA (GenScript) and 1 μg ABE8.8m mRNA into 5×10^5 SSCs using the
98 Neon NxT system (Thermo Fisher) with parameters of 1150 V, 10 ms pulse width, and
99 3 pulses in GE buffer, we achieved a base editing efficiency exceeding 70% for the
100 *PSEN1* c.436A>G mutation in tree shrew SSCs (mutant SSCs) (Figure 1E). Bystander
101 editing was observed at positions A8 (~34.8%) and A11 (~6.5%).
102 The presenilin 1 (PSEN1) is a core component of γ-secretase complex essential
103 for the proteolytic activation of Notch signaling, a pathway implicated in stem cell
104 maintenance and differentiation (Mitra et al., 2025). We therefore investigated
105 whether the $PSEN1^{M146V}$ mutation affects self-renewal or differentiation in tree shrew
106 SSCs. Mutant SSCs harboring the heterozygous *PSEN1* c.436A>G (p.M146V) allele
107 exhibited a proliferation rate comparable to that of wild-type (WT) SSCs (Figure 1F).
108 This result was corroborated by the comparable percentage of EdU-positive cells
109 between the mutant and WT SSC populations (Figure 1G-H). Moreover, mRNA
110 expression levels of key undifferentiated spermatogonial markers, including *GFRA1*,
111 *ZBTB43*, *MSL3*, *LIN28A*, and *FMR1*, were similar between genotypes (Figure 1I). In
112 contrast, expression levels of the early differentiation markers *STRA8* and *ID3* were
113 significantly downregulated in mutant cells (Figure 1I). These results suggest that
114 although the $PSEN1^{M146V}$ mutation does not alter the molecular signature of
115 undifferentiated SSCs in culture, it may be associated with suppression of early
116 differentiation markers. Whether this suppression reflects preferential maintenance of

the undifferentiated state or a blockade of differentiation initiation warrants further investigation. Apoptosis rates also showed no significant difference between mutant and WT SSC populations (Figure 1J). Collectively, these results indicate that the PSEN1^{M146V} mutation does not impair the stem cell properties of tree shrew SSCs *in vitro*.

To obtain a homozygous PSEN1^{M146V} SSC line, we isolated and expanded single-cell clones from the edited SSC pool. Following one month of culture, six single-cell clones were successfully propagated (Supplementary Figure S2). Two of these clones (clones 4 and 6) were homozygous for the PSEN1^{M146V} mutation. However, both clones also carried bystander edits at positions A11 and A8, respectively (Supplementary Figure S2). Furthermore, generating sufficient amount of SSCs for transplantation would have required prolonged *ex vivo* expansion, a process we previously found can compromise spermatogenic capacity in this species (Li et al., 2023). To circumvent potential confounds from bystander edits and extended culture, we instead proceeded with transplantation using the initial heterogeneous edited cell pool, which exhibited >70% editing efficiency for the *PSEN1* c.436A>G (p.M146V) allele based on the next-generation sequencing result (Figure 1E). Following our established transplantation protocol (Li et al., 2017), approximately 1×10^6 cells harboring the heterozygous PSEN1^{M146V} mutation were transplanted into each testis of two busulfan-pretreated male recipients (Figure 1K). After recovery, the recipients were naturally mated, yielding 40 offspring. Sanger sequencing identified two offspring from recipient R1 (Supplementary Table S2) that carried the precisely edited

PSEN1^{M146V} allele (Figure 1L, Supplementary Table S2), whereas all offspring from recipient R2 were wild-type. The proportion of edited offspring (5%) was comparable to that reported in our previous study (Li et al., 2017).

We performed whole-genome sequencing on two mutant pups (#4 and #37) and one WT littermate to screen for potential off-target sites containing canonical (NGG) or non-canonical (NAG) protospacer adjacent motifs (PAMs). Compared with the WT control, insertions or deletions (indels) were identified at 27 potential off-target sites in pup #4 and at 22 sites in pup #37 (Supplementary Table S3). Notably, none of these sites were located within coding regions of the tree shrew genome. Owing to the limited genomic data currently available for tree shrews, further investigation is required to determine whether these identified indels represent genuine off-target events caused by *PSEN1* gRNA or merely reflect polymorphisms within the tree shrew population. Nonetheless, the mutant tree shrews exhibited normal growth and development during early life, with body length and weight gain comparable to those of WT littermates (Figure 1M-P). These results suggest that the AD-causing mutation PSEN1^{M146V}, together with any potential off-target edits, does not impair baseline physiological development in juvenile tree shrews.

The PSEN1^{M146V} mutation causes early-onset familial AD in an autosomal dominant pattern (Jia et al., 2020). However, in mouse models, introduction of the PSEN1^{M146V} mutation alone is insufficient to recapitulate key AD-like pathologies or cognitive deficits (Guo et al., 1999). Owing to their high sequence identity with humans in AD-related genes (including an identical Aβ42 peptide sequence (Fan et al.,

2018)) and the similarity of their expression patterns, tree shrews may naturally produce pathogenic Aβ42 and develop AD-like phenotypes with aging (Li et al., 2024). These findings collectively support the premise that introducing *PSEN1* mutations into tree shrews offers a promising strategy for modeling PSEN1-driven AD pathogenesis. Although a full phenotypic characterization of the mutant tree shrews remains beyond the scope of this study, the precisely edited PSEN1^{M146V} tree shrew line established here provides a valuable new resource for studying AD mechanisms and therapeutic development. Future work will include longitudinal behavioral assessments, biochemical analyses (e.g., changes in the Aβ42/Aβ40 ratio and pTau217 levels in blood, cerebrospinal fluid, and brain tissue; calcium homeostasis), and neuropathological profiling (e.g., glial cell morphology, neuronal loss, synaptic dysfunction) to validate the relevance of this model to AD. We plan to expand the breeding colony of this genetically edited PSEN1^{M146V} line to make it available to the broader research community.

The base editing efficiency achieved at the *PSEN1* locus in tree shrew SSCs (~70%) is moderate compared with the >90% efficiency reported at the *SGCA* locus in human muscle stem cells (Escobar et al., 2021). This discrepancy may arise from several factors, including differences in chromatin accessibility at the target locus, the quiescent versus activated state of the stem cells, and species- or cell type-specific variations in DNA repair pathways. Further optimization of culture conditions to promote a more open chromatin state (e.g., through HDAC inhibitors) or transient cell cycle activation may elevate editing efficiencies in SSCs to levels observed in more

permissive stem cell types.

The SSC-based strategy yielded a successful rate of approximately 5% in generating gene-edited pups, which is primarily attributed to the proportion of gene-edited SSCs available for transplantation and to individual variability in recipient response to busulfan, which can result in incomplete depletion of endogenous WT germ cells. Future efforts focusing on optimizing recipient preparation, refining the timing of cell transplantation, and employing immunosuppression to mitigate immune rejection are likely to improve the efficiency of generating gene-edited animals.

In summary, we have established an optimized base editing platform in tree shrew SSCs and used it to generate the first gene-edited tree shrew carrying a canonical pathogenic AD mutation. This work provides a critical technological foundation for developing more physiologically relevant, genetically defined disease models in this primate-like species.

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Conflict of interest

The authors declare no competing interests.

Supplementary materials

Supplementary Figure S1-S2, Table S1-S3, and detailed materials and methods are provided in online supplementary materials.

References

Anzalone, A.V., Koblan, L.W., and Liu, D.R. (2020). Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. Nat Biotechnol *38*, 824-844.

Escobar, H., Krause, A., Keiper, S., Kieshauer, J., Muthel, S., de Paredes, M.G., Metzler, E., Kuhn, R., Heyd, F., and Spuler, S. (2021). Base editing repairs an SGCA mutation in human primary muscle stem cells. JCI Insight *6*.

Fan, Y., Luo, R.C., Su, L.Y., Xiang, Q., Yu, D.D., Xu, L., Chen, J.Q., Bi, R., Wu, D.D., Zheng, P., *et al.* (2018). Does the genetic feature of the Chinese tree shrew (*Tupaia belangeri chinensis*) support its potential as a viable model for Alzheimer's disease research? J Alzheimers Dis *61*, 1015-1028.

Gaudelli, N.M., Lam, D.K., Rees, H.A., Sola-Esteves, N.M., Barrera, L.A., Born,

D.A., Edwards, A., Gehrke, J.M., Lee, S.J., Liquori, A.J., *et al.* (2020). Directed evolution of adenine base editors with increased activity and therapeutic application. Nat Biotechnol *38*, 892-900.

Guo, Q., Fu, W., Sopher, B.L., Miller, M.W., Ware, C.B., Martin, G.M., and Mattson, M.P. (1999). Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. Nat Med *5*, 101-106.

Jia, L., Fu, Y., Shen, L., Zhang, H., Zhu, M., Qiu, Q., Wang, Q., Yan, X., Kong, C., Hao, J., *et al.* (2020). PSEN1, PSEN2, and APP mutations in 404 Chinese pedigrees with familial Alzheimer's disease. Alzheimers Dement *16*, 178-191.

Li, C., Bi, R., Wang, L., Ma, Y.H., Yao, Y.G., and Zheng, P. (2023). Characterization of long-term ex vivo expansion of tree shrew spermatogonial stem cells. Zool Res *44*, 1080-1094.

Li, C.H., Yan, L.Z., Ban, W.Z., Tu, Q., Wu, Y., Wang, L., Bi, R., Ji, S., Ma, Y.H., Nie, W.H., *et al.* (2017). Long-term propagation of tree shrew spermatogonial stem cells in culture and successful generation of transgenic offspring. Cell Res *27*, 241-252.

Li, H., Xiang, B.L., Li, X., Li, C., Li, Y., Miao, Y., Ma, G.L., Ma, Y.H., Chen, J.Q., Zhang, Q.Y., *et al.* (2024). Cognitive deficits and Alzheimer's disease-like pathologies in the aged Chinese tree shrew. Mol Neurobiol *61*, 1892-1906.

Liu, Y., McDaniel, J.A., Chen, C., Yang, L., Kipcak, A., Savier, E.L., Erisir, A., Cang, J., and Campbell, J.N. (2025). Co-Conservation of synaptic gene expression and circuitry in collicular neurons. Nat Commun *16*, 9146.

Mitra, M., Batista, S.L., and Coller, H.A. (2025). Transcription factor networks in cellular quiescence. Nat Cell Biol *27*, 14-27.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., and LaFerla, F.M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron *39*, 409-421.

Richter, M.F., Zhao, K.T., Eton, E., Lapinaite, A., Newby, G.A., Thuronyi, B.W., Wilson, C., Koblan, L.W., Zeng, J., Bauer, D.E., *et al.* (2020). Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. Nat Biotechnol *38*, 883-891.

Wu, Y., Zhou, H., Fan, X., Zhang, Y., Zhang, M., Wang, Y., Xie, Z., Bai, M., Yin, Q., Liang, D., *et al.* (2015). Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells. Cell Res *25*, 67-79.

Yao, Y.G., Lu, L., Ni, R.J., Bi, R., Chen, C., Chen, J.Q., Fuchs, E., Gorbatyuk, M., Lei, H., Li, H., *et al.* (2024). Study of tree shrew biology and models: A booming and prosperous field for biomedical research. Zool Res *45*, 877-909.

Yu, D., Kang, W.B., Ma, Y.H., Lv, L.B., Zhang, S., Tang, L., Liu, S., Zhong, J., and Yao, Y.G. (2025). Single-cell atlas of hepatitis C virus inoculated tree shrew liver reveals immune activation, metabolic reprogramming, and persistent inflammation. EBioMedicine *123*, 106080.

Figure Legend

Figure 1. Generation of a PSEN1^{M146V} tree shrew model using efficient base editing in spermatogonial stem cells.

(A) Experimental workflow and timeline for generating precisely gene-edited tree shrews.

(B) Sequence alignment of the region containing the PSEN1^{M146V} mutation between tree shrew and human. The guide RNA (gRNA) target site is indicated by red line; PAM, protospacer adjacent motif.

(C) Sequencing chromatograms showing successful base editing in tree shrew SSCs mediated by ABE8.8m. The targeted nucleotide for the *PSEN1* c.436A>G (p.M146V) mutation is marked by a red arrow.

(D) Editing efficiency for *PSEN1* c.436A>G at different gRNA/ABE mRNA ratios.

(E) Editing efficiency of sites A5, A8 and A11 in the gRNA targeting region identified by the next-generation sequencing.

(F) Growth curves of WT and PSEN1^{M146V} mutant SSCs.

(G) EdU incorporation assay in WT and PSEN1^{M146V} mutant SSCs. Scale bars, 50 μm.

(H) Quantification of EdU positive cells in (G).

(I) Relative mRNA levels of spermatogonial marker genes in wild-type (WT) and PSEN1^{M146V} mutant SSCs. *GAPDH* was used for normalization in quantitative real-time PCR.

(J) Proportions of early and late apoptotic SSCs.

(K) Transplantation of PSEN1^{M146V}-edited SSCs into recipient testes, visualized with trypan blue. Scale bar, 5 mm.

(L) Sequencing-based genotyping of offspring. Offspring #4 and #37 carry the *PSEN1* c.436A>G (p.M146V) mutation.

(M-P) Photograph and body weight changes of offspring #4 and littermates **(M-N)** and of offspring #37 and littermates **(O-P)**.

Data in (**H**, **I** and **J**) were presented as mean ±SD from three independent replicates. ns, not significant; **, $P < 0.01$; two-tailed Student's *t*-test.