

# NEAT1 Deficiency Promotes Corneal Epithelial Wound Healing by Activating cAMP Signaling Pathway

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**PURPOSE.** This study aimed to investigate the role of the long non-coding RNA (lncRNA) *NEAT1* in corneal epithelial wound healing in mice.

**METHODS.** The central corneal epithelium of wild-type (WT), *MALAT1* knockout (M-KO), *NEAT1* knockout (N-KO), and *NEAT1* knockdown (N-KD) mice was scraped to evaluate corneal epithelial and nerve regeneration rates. RNA sequencing of the corneal epithelium from WT and N-KO mice was performed 24 hours after debridement to determine the role of *NEAT1*. Quantitative PCR (qPCR) and ELISA were used to confirm the bioinformatic analysis. The effects of the cAMP signaling pathway were evaluated in N-KO and N-KD mice using SQ22536, an adenylate cyclase inhibitor.

**RESULTS.** Central corneal epithelial debridement in N-KO mice significantly promoted epithelial and nerve regeneration rates while suppressing inflammatory cell infiltration. Furthermore, the expression of *Atp1a2*, *Ppp1r1b*, *Calm4*, and *Cngb1*, which are key components of the cAMP signaling pathway, was upregulated in N-KO mice, indicative of its activation. Furthermore, the cAMP pathway inhibitor SQ22536 reversed the accelerated corneal epithelial wound healing in both N-KO and N-KD mice.

**CONCLUSIONS.** *NEAT1* deficiency contributes to epithelial repair during corneal wound healing by activating the cAMP signaling pathway, thereby highlighting a potential therapeutic strategy for corneal epithelial diseases.

**Keywords:** *NEAT1*, corneal epithelium, wound healing, inflammation, nerve regeneration, cAMP signaling

The cornea is a transparent avascular tissue that serves as the primary structural barrier to the remaining ocular tissue. The cornea consists of three main layers: corneal epithelium, stroma, and endothelium.<sup>1</sup> The corneal epithelium, which is the outer surface of the eye, is susceptible to physical, chemical, and infectious insults that often result in corneal epithelial damage. Corneal epithelial wounds typically heal rapidly and effectively within a few days, restoring structural and functional integrity.<sup>2-4</sup> Corneal epithelial regeneration is a complex and dynamic process that involves the repair of the epithelial layer, cell migration, and proliferation during wound healing.<sup>5-7</sup> However, untreated corneal epithelial injury can lead to complications, such as inflammation, neovascularization, ulceration, scarring, and other complications, significantly impairing vision.<sup>8</sup>

Long non-coding RNAs (lncRNAs) are transcripts that are at least 200 nucleotides in length and possess limited protein-encoding ability.<sup>9</sup> Due to a higher total quantity than coding genes,<sup>10</sup> the diversity and size of lncRNAs are strongly correlated with organismal complexity, surpass-

ing the correlation observed for protein-coding genes.<sup>11,12</sup> Aberrant lncRNA expression has been implicated in various human diseases, including tumorigenesis, neurological diseases, and cardiovascular diseases.<sup>13</sup> lncRNAs have been extensively studied in the field of ophthalmology, particularly in relation to Fuchs' endothelial corneal dystrophy (FECD), glaucoma, cataracts, retinal disease, and ocular tumors.<sup>14-18</sup> Among these lncRNAs, nuclear paraspeckle assembly transcript 1 (*NEAT1*) has shown promise in its involvement in various ocular diseases and its ability to exert functional effects.<sup>19,20</sup> Previous studies have indicated that the downregulation of *NEAT1* expression inhibits neovascularization of the cornea,<sup>21</sup> whereas *NEAT1* deficiency impairs the oxidant-antioxidant balance in the corneal endothelium.<sup>14</sup> In the corneal epithelium, *NEAT1* accelerating diabetes-related dry eye disease development and *NEAT1* knockdown will alleviate corneal damage.<sup>22</sup> Moreover, *NEAT1* plays a role in abnormal immune activation and is related to the disease progression of primary Sjögren's syndrome, affecting the health of the corneal epithelium.<sup>23</sup> These studies suggest that the expression of *NEAT1* in

the cornea maintains a balance, and excessive or insufficient expression affects the corneal homeostasis. Such studies also indicate diverse functions of *NEAT1* across different cell types. However, the role of *NEAT1* in protecting corneal epithelium during wound healing remains unclear.

This study aimed to delineate the role of *NEAT1* in corneal epithelial wound healing in mice. The findings show that the regeneration of both epithelial and nerve tissues was significantly enhanced in *NEAT1* knockout (N-KO) mice, whereas inflammatory cell infiltration was reduced. The cAMP signaling pathway was significantly activated in N-KO mice, which was confirmed by the upregulated expression of *Atp1a2*, *Ppp1r1b*, *Calm4*, *Cngb1*, and cAMP production in the corneal epithelium. Additionally, administration of the cAMP pathway inhibitor SQ22536 reversed the accelerated healing of corneal epithelial wounds in N-KO and *NEAT1* knockdown (N-KD) mice. In conclusion, our data substantiate the role of *NEAT1* in corneal epithelial wound healing and suggest its potential as a therapeutic target for corneal epithelial diseases.

## METHODS

### Animals

Male C57BL/6 mice (8 weeks old) were procured from Beijing Vital River Laboratory Animal Technology (Beijing, China). N-KO mice (strain number T011757) were purchased from GemPharmatech (Nanjing, China) and bred as heterozygotes in a specific pathogen-free environment. Homozygous mice derived from heterozygote breeding were used in the present study.

Recombinant adeno-associated virus (AAV) was constructed using inverted terminal repeats from serotype 2 AAV and capsid protein from serotype 9. AAV containing *NEAT1* short hairpin RNA (shRNA) and control shRNA (NC) target sequences were obtained from GeneChem (Shanghai, China). All of the sequences are shown in Table 1. Following intraperitoneal injection of 0.6% pentobarbital sodium (50 mg/kg) and the application of topical anesthesia (2% xylocaine), the male C57BL/6 mice were administered the AAV virus by subconjunctival injection (at  $\sim 5 \times 10^{12}$  vector genomes per milliliter, 5  $\mu$ L per eye) to generate N-KD mice. RNA was extracted from mouse corneal epithelium after at least 4 weeks to detect infection efficiency.

The 200- $\mu$ M cAMP inhibitor SQ22536 (Selleck Chemicals, Houston, TX, USA) was administered by subconjunctival injection. Mice were systemically anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) and topically anesthetized with 2% xylocaine. SQ22536 (5  $\mu$ L per eye) was injected 24 hours before, at 0 hour, and at 24 hours after wounding. cAMP production of epithelium after SQ22536 treatment was determined to detect an inhibitory effect.

The mice were housed at the animal center of the Shandong Eye Institute, where they had unrestricted access

TABLE 1. Target Sequences

	shRNA Target Sequences
<i>NEAT1</i>	5'-ATGGGTAGGGTTGTGGTTTA-3'
Control	5'-CGCTGAGTACTTCGAAATTC-3'

to food and water. All protocols involving animals were approved by the Ethics Committee of Shandong Eye Institute and conducted in accordance with the guidelines outlined in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Data Processing and Downstream Analysis of Single-Cell RNA Sequencing

We employed the Cell Ranger 3.1.0 pipeline to map the cleaned RNA sequencing (RNA-seq) reads to the human reference genome (GRCh38-3.0.0) based on the early sequencing results (databases hra00781 and hra00728). The count data were subsequently imported into the Seurat 3.2.0 R package (R Foundation for Statistical Computing, Vienna, Austria).<sup>24</sup> The raw reads underwent processing, which involved the removal of adaptor sequences, short sequences, low-quality bases, and ambiguous sequences (reads with more than two unknown bases *N*). Subsequently, the canonical correlation analysis algorithm was used to exclude batch effects during data integration,<sup>25</sup> and the data were normalized using the Seurat LogNormalize function. To complete cell clustering, principal component analysis was performed for highly variable genes. Genes selected for this analysis underwent visualized cell clustering using Uniform Manifold Approximation and Projection (UMAP) stochastic neighbor embedding to generate four cluster types.

### Corneal Epithelial Wound Healing

To establish the corneal epithelial debridement model, mice were anesthetized with intraperitoneal injections of 0.6% pentobarbital sodium (50 mg/kg), followed by topical administration of 2% xylocaine. The central corneal epithelium (2.5 mm in diameter) was excised using AlgerBrush II Corneal Rust Ring Remover (Alger, Lago Vista, TX, USA).<sup>26</sup> The procedure was performed to prevent infection, and ofloxacin eye drops were administered after debridement. Corneal epithelial defects were evaluated at 0, 12, 24, and 36 hours after debridement using 0.25% fluorescein sodium and were visualized using a slit-lamp microscope. The defect area was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).<sup>27</sup>

### Hematoxylin and Eosin Staining

Eyeballs were collected after a 24-hour interval following the induction of corneal epithelial injury. The collected eyeballs were fixed overnight with tissue fixative. Subsequently, the samples were dehydrated, paraffin embedded, and sectioned at a 5- $\mu$ m thickness. The sections subsequently underwent hematoxylin and eosin (H&E) staining with a diaminobenzidine chromogenic solution.

### Quantitative Real-Time PCR

Mouse eyeballs were placed in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12; Wuhan Pricella Biotechnology, Wuhan, China) containing 15 mg/mL Dispase II (Roche, Mannheim, Germany) and incubated overnight at 4°C. Subsequently, the epithelium was delicately detached using a dissecting microscope.<sup>28</sup> Total RNA was extracted from corneal epithelial tissue using the TransZol Up Plus

TABLE 2. Primer Pairs Used for qRT-PCR Analyses

Gene	Forward Primer	Reverse Primer
m- $\beta$ -actin	ACGGCCAGGTCATCACTATTG	AGAGGTCTTTACGGATGTCAACGT
m-MALAT1	TTTTCCCCACATTTCCAAATA	CAGAGGCAAGCGTTATATGC
m-NEAT1	AGGCTATCCCAGCGTCCTATTAA	AGCCGTGTAAGCACAGGTCACT
m-CD45	GGTTGTTCTGTGCCCTTGTTCAA	TGGCGATGATGCATAGAGGAA
m-IL-6	ACCACTCCCAACAGACCTGTCT	CAGATTGTTTCTGCAAGTGCAT
m-IL-1 $\beta$	CTTTCCCGTGGACCTTCCA	CTCGGAGCCTGTAGTGCAGTT
m-Atp1a2	TGCAGCCAGGCTCAACATT	GAGGTCCGGGCAAAAGACAAT
m-Calm4	CGAGGAACCTGGAGATGTAATGAA	TGGCCTTCTTGTACTTCTATGG
m-Cngb1	CCCAGCTCCTGGATGTTGAG	TCCCCAGTCCACCAAGGTT
m-Ppp1r1b	GCACCACCCAAAGTGAAGA	TACCCAAGCTCCCGAAGCT

RNA Kit (TransGen Biotech, Beijing, China), and cDNA was synthesized using a reverse transcription kit (Vazyme Biotech, Nanjing, China). Subsequently, quantitative PCR (qPCR) was conducted employing the ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech) and a quantitative real-time PCR (qRT-PCR) instrument (Applied Biosystems, Carlsbad, CA, USA). Each experiment was performed in triplicate. The cycling conditions for qPCR consisted of an initial step at 95°C for 30 seconds followed by 40 two-step cycles of 10 seconds at 95°C and 30 seconds at 60°C.  $\beta$ -Actin served as the internal control for messenger RNA (mRNA) and lncRNA. The primer pairs (Delohaida, Qingdao, China) used for the qRT-PCR analyses are listed in Table 2.

### Corneal Whole-Mount Staining for Nerve Fibers

Mouse eyeballs were immersed in Zamboni fixative (Beijing Solarbio Science & Technology, Beijing, China) for 1 hour 5 days following corneal epithelial injury. Subsequently, the cornea was completely separated and immersed in PBS containing 0.3% Triton X-100 and 3% BSA. The samples were sealed airtight and left overnight at 4°C. Next, the cornea was tagged with an Alexa Fluor 647 anti-Tubulin- $\beta$  III Antibody (1:400; BioLegend, San Diego, CA, USA) and incubated overnight at 4°C. After each cornea was thoroughly rinsed six times with PBS containing 0.3% Triton X-100 and 0.05% Tween 20, the cornea was divided into six segments or “petals.” These segments were observed under a confocal microscope.<sup>29</sup> Corneal nerve fiber density was quantified using ImageJ.

### RNA Sequencing and Data Processing

Thirty-two regenerative corneal epithelium samples were collected from equal numbers of wild-type (WT) and N-KO mice. The samples were collected 24 hours after debridement and were subsequently combined to form eight distinct groups, with four groups consisting of normal mice with corneal injury and four groups comprised of N-KO mice with corneal injury. Each group consisted of corneal epithelial samples obtained from four mice. The collected epithelial samples were stored at -80°C for the subsequent experimental procedures.

Total RNA was extracted using Invitrogen TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the libraries were constructed utilizing the VAHTS Universal V6 RNA-seq Library Prep Kit for Illumina (Vazyme Biotech). Transcriptome sequencing and analysis were conducted by Shanghai OE Biotech (Shanghai, China). The libraries were

sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). The obtained raw reads in fastq format were first processed using fastp,<sup>30</sup> and the low quality reads were then removed to obtain the clean reads. These clean reads were mapped to the reference genome using HISAT2.<sup>31</sup> The fragments per kilobase per million mapped fragments (FPKM)<sup>32</sup> of each gene were calculated, and the read counts of each gene were determined with HTSeq-count.<sup>33</sup> Principal component analysis was conducted using R 3.2.0 to evaluate the biological duplication of samples. DESeq2<sup>34</sup> was employed for differential expression analysis.  $Q < 0.05$  and fold change  $> 2$  or fold change  $< 0.5$  were set as the thresholds for significantly differential expression genes (DEGs). Based on the hypergeometric distribution, Kyoto Encyclopedia of Genes and Genomes (KEGG)<sup>35</sup> pathway enrichment analyses of DEGs were performed to screen the significant enriched term using R 3.2.0.

### ELISA Analysis

Mouse corneal epithelium was collected 48 hours post-injury to assess cAMP expression levels. The corneal epithelium was treated with 1 $\times$  cell lysis buffer and centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant. The cAMP concentration was determined using a Cyclic AMP XP Assay Kit (Cell Signaling Technology, Danvers, MA, USA) following the manufacturer's instructions, and absorbance was measured using a microplate reader.

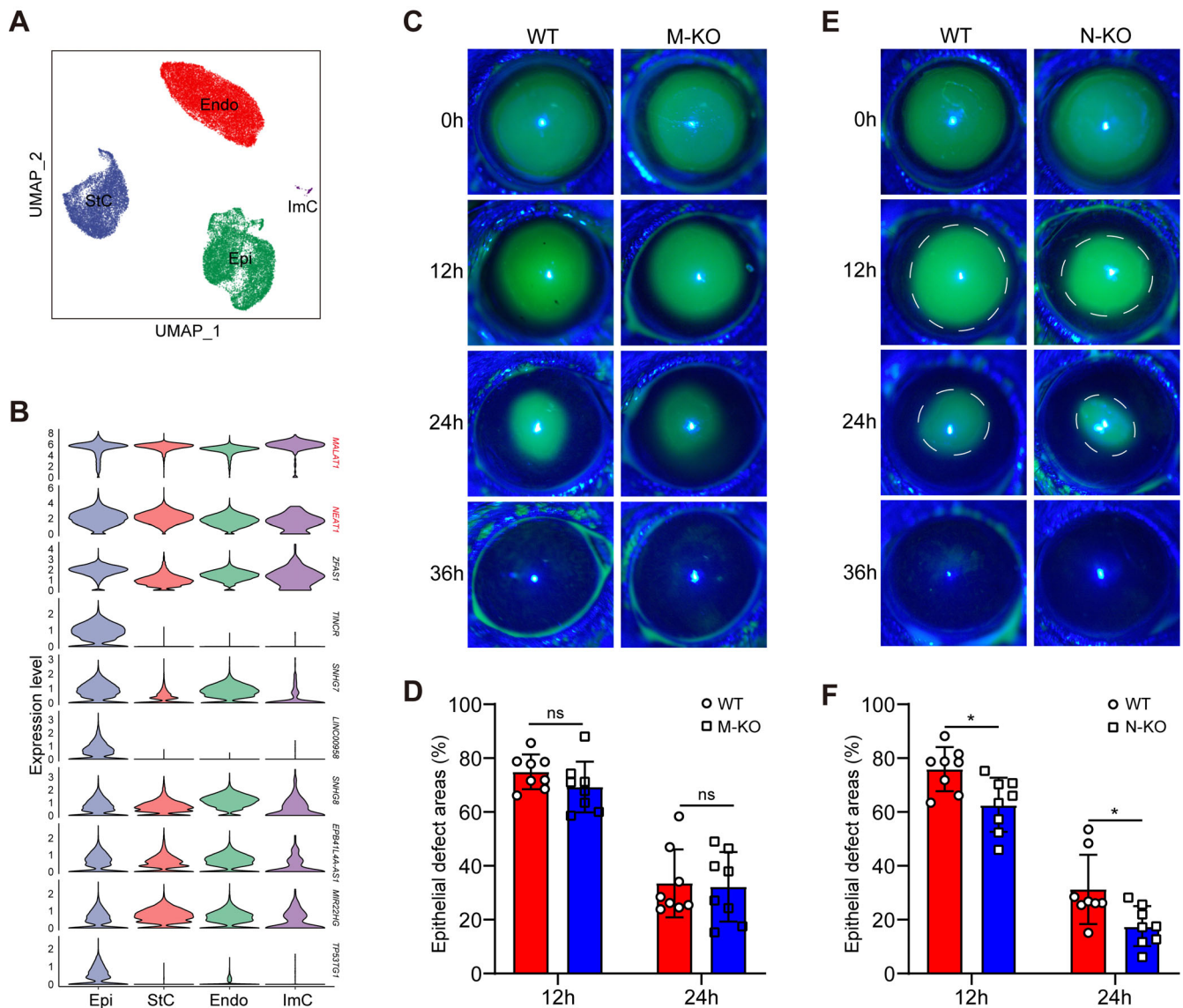
### Statistical Analyses

Statistical analyses were conducted using Prism 8 (GraphPad, Boston, MA, USA). Data are presented as the mean  $\pm$  standard error. For normally distributed data, differences between or among groups were compared using two-tailed unpaired Student's *t*-test or one-way ANOVA. For non-normally distributed data, a non-parametric test (Wilcoxon rank-sum test) was used. The levels of significance are indicated as follows: ns, no significance; \* $P < 0.05$ ; \*\* $P < 0.01$ ; and \*\*\* $P < 0.001$  (statistical significance). All experiments were validated using at least three replicates.

## RESULTS

### NEAT1 Deficiency Contributes to Corneal Epithelial Wound Healing in Mice

We conducted a systematic analysis of lncRNA profiles in four human corneas derived from two healthy individuals

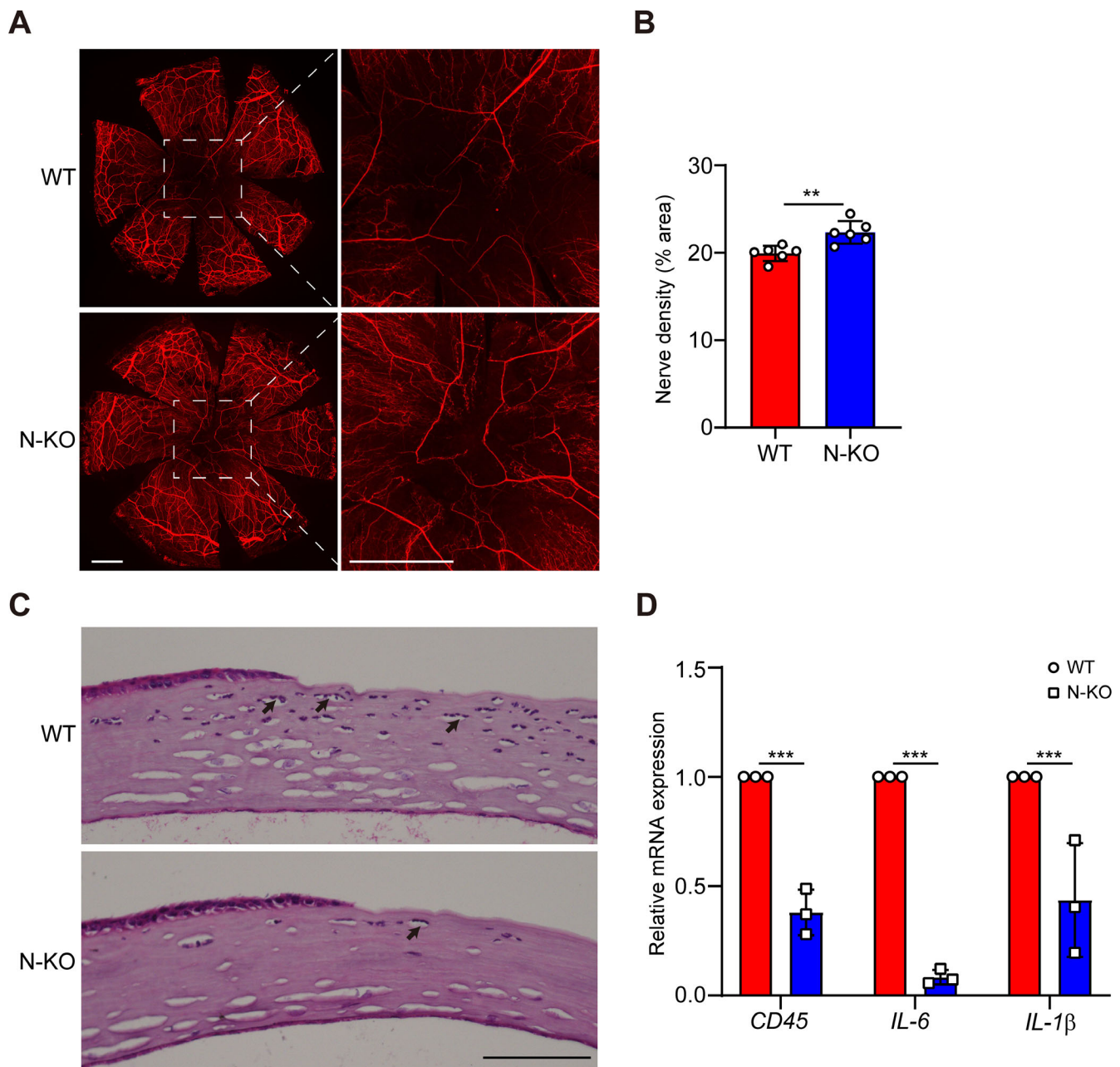


**FIGURE 1.** *NEAT1* knockout promotes corneal epithelial wound healing in mice. (A) UMAP clustering depicting the spatial distribution of human corneal cells with distinct subtypes represented by different colors. (B) Violin plot showing the expression levels of the top 10 lncRNAs for each subtype. (C, E) Corneal fluorescein staining of M-KO and N-KO mice at 0, 12, 24, and 36 hours after epithelial debridement. (D, F) Analysis of the epithelial defect area using ImageJ ( $n = 8$ ).

using our previously published single-cell RNA-seq data to explore their biological functions in the cornea.<sup>14</sup> Unsupervised clustering was used to annotate four primary cell types, including corneal epithelial cells, corneal stromal cells, corneal endothelial cells, and immune cells, based on classical specific markers using uniform manifold approximation and projection (UMAP) (Fig. 1A). *MALAT1* and *NEAT1* were the most highly expressed lncRNAs across the different subtypes and were among the most abundant genes (Fig. 1B). To further investigate the role of *MALAT1* and *NEAT1* in corneal epithelial wound healing, which is crucial for maintaining corneal function, we scraped the central corneal epithelium from the eyes of age-matched WT, *MALAT1* knockout (M-KO),<sup>36</sup> and *NEAT1* N-KO mice (Supplementary Fig. S1). Interestingly, N-KO mice, but not M-KO mice, displayed accelerated epithelial wound closure at 12 and 24 hours post-wounding (Figs. 1C–1F).

### ***NEAT1* Knockout Promotes Corneal Nerve Regeneration and Suppresses Inflammatory Cell Infiltration**

Corneal re-epithelialization is a complex, multistep process involving nerve regeneration and an inflammatory response.<sup>37,38</sup> We investigated the role of *NEAT1* in corneal nerve function and inflammation by analyzing corneal subbasal nerve fiber density and inflammatory cytokine levels. N-KO mice exhibited increased regeneration of subbasal nerve fibers compared with WT mice (Figs. 2A, 2B). Moreover, N-KO mice showed alleviated pathology after epithelial debridement, including decreased inflammatory cell infiltration (Fig. 2C) and reduced mRNA expression of *CD45*, *IL-6*, and *IL-1 $\beta$*  (Fig. 2D). These findings suggest that *NEAT1* negatively regulates corneal epithelial and nerve regeneration.

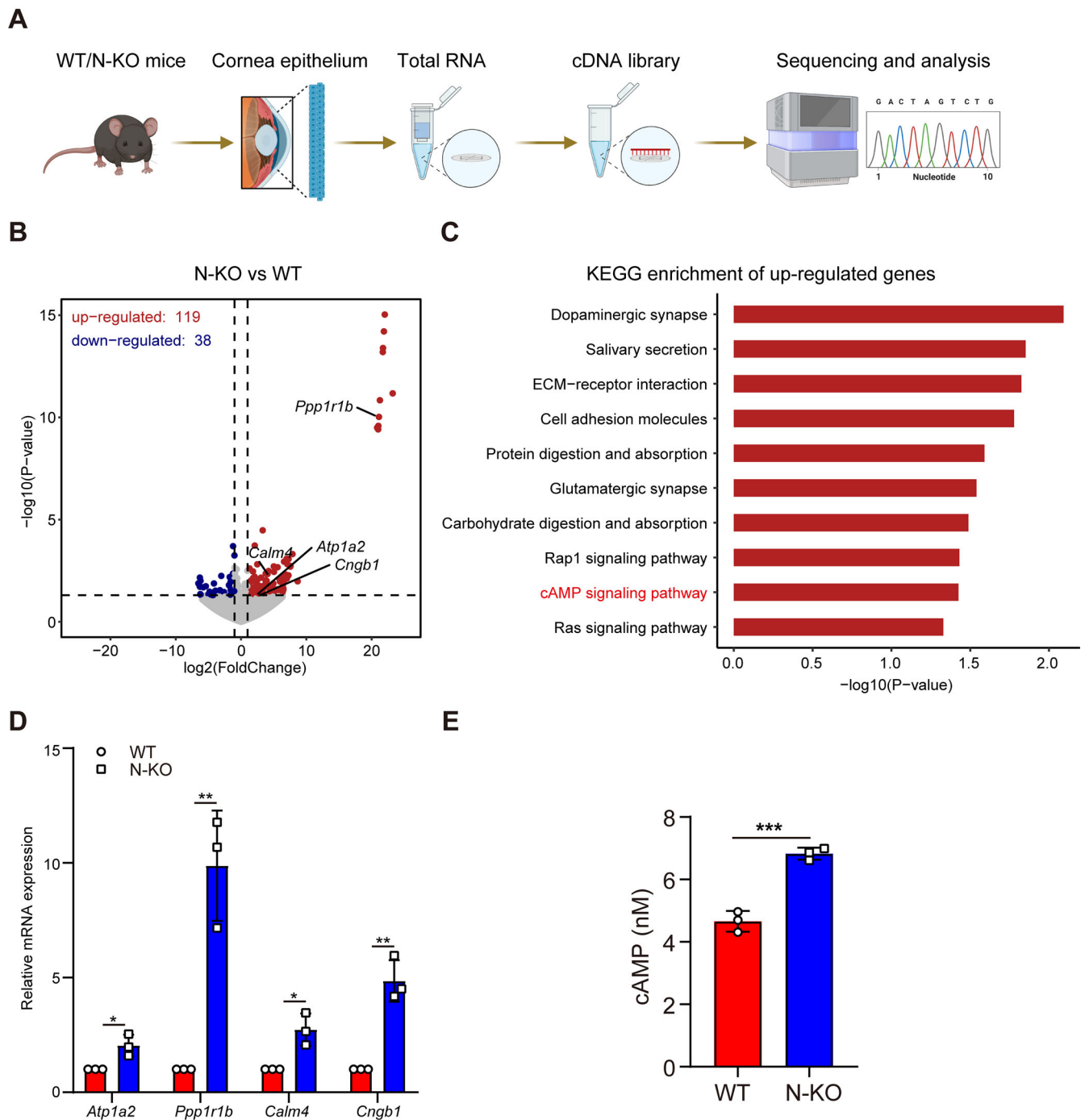


**FIGURE 2.** *NEAT1* deficiency improves corneal nerve regeneration and alleviates inflammation. (A) Representative images of neuronal  $\beta$ -tubulin III-stained subbasal nerve fibers in corneas from WT and *NEAT1* knockout mice 5 days after epithelium removal. Scale bar: 500  $\mu$ m. (B) Quantification of nerve densities in the entire cornea by analyzing the areas that stained positive for  $\beta$ -tubulin III ( $n = 6$ ). (C, D) H&E staining of corneal sections and mRNA expression levels of *CD45*, *IL-6*, and *IL-1 $\beta$*  in WT and *NEAT1* knockout mice 24 hours after epithelial debridement ( $n = 3$ ). Scale bar: 100  $\mu$ m.

### ***NEAT1* Knockout Activates the cAMP Signaling Pathway During Corneal Re-Epithelialization**

We investigated the cellular mechanism underlying the accelerated corneal epithelial regeneration observed in *NEAT1* deficiency by conducting bulk RNA-seq of the corneal epithelium from WT and N-KO mice 24 hours after epithelial debridement (Fig. 3A). RNA-seq identified 119 upregulated and 38 downregulated genes (Fig. 3B). KEGG enrichment analysis revealed significant upregula-

tion changes in cell growth-related categories, including the RAP1, cAMP, and RAS pathways, in N-KO corneal epithelium (Fig. 3C). Subsequently, we studied the cAMP signaling pathway, which regulates corneal epithelial proliferation, migration, and apoptosis.<sup>39–41</sup> To validate this comparative analysis, we assessed the mRNA expression of cAMP pathway-related genes (*Atp1a2*, *Ppp1r1b*, *Calml4*, and *Cngb1*) and cAMP production in the corneal epithelium. As shown in Figures 3D and 3E, *NEAT1* deficiency resulted in significant activation of the cAMP signaling pathway.

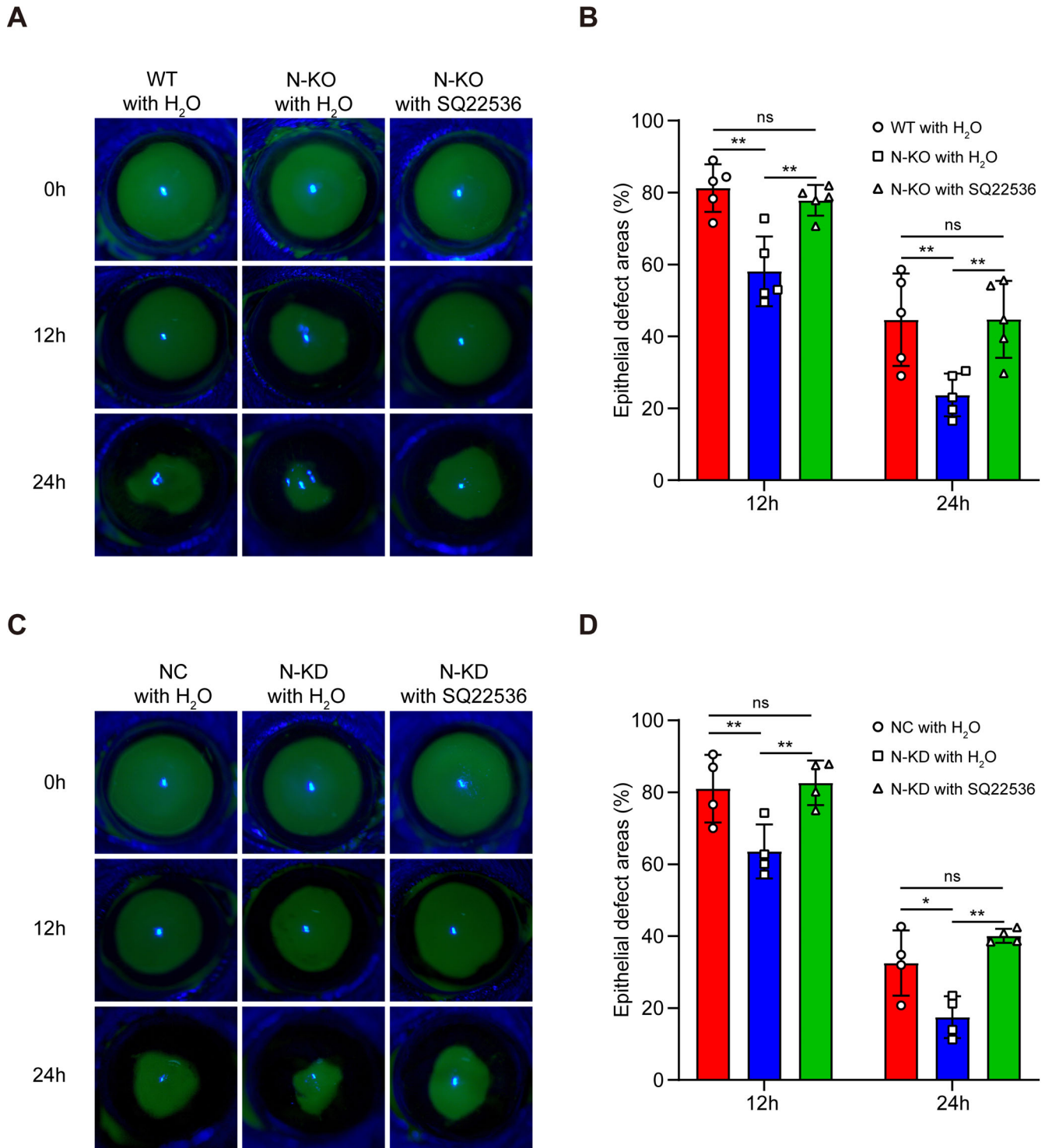


**FIGURE 3.** The cAMP signaling pathway is activated in *NEAT1* knockout mice. **(A)** Flowchart illustrating the experiments conducted in this study. **(B)** Volcano plot depicting differentially expressed genes in the corneal epithelium of WT and *NEAT1* knockout mice 24 hours after wounding ( $n = 4$ ). Identification of cAMP pathway-associated mRNAs. **(C)** KEGG analysis depicting upregulated mRNAs. **(D)** qPCR validation of the upregulation of *Atp1a2*, *Ppp1r1b*, *Calm4*, and *Cngb1* ( $n = 3$ ). **(E)** cAMP production in the corneal epithelium ( $n = 3$ ).

### Blockade of cAMP Signaling Inhibits Accelerated Corneal Epithelial Repair in *NEAT1* Knockout Mice

To further confirm the role of cAMP signaling in promoting corneal epithelial wound healing, we used SQ22536, an effective adenylate cyclase inhibitor, to specifically disrupt cAMP signaling activation. Subcon-

junctival injection of 200  $\mu$ m SQ22536 in N-KO mice resulted in a significant reduction in cAMP production (Supplementary Fig. S2A). Moreover, the administration of SQ22536 in N-KO mice significantly reduced the rate of epithelial repair compared with that in sham-operated animals at 12 and 24 hours post-wounding (Figs. 4A, 4B). Next, *NEAT1*-targeting shRNAs were injected into WT mice to specifically knockdown *NEAT1*

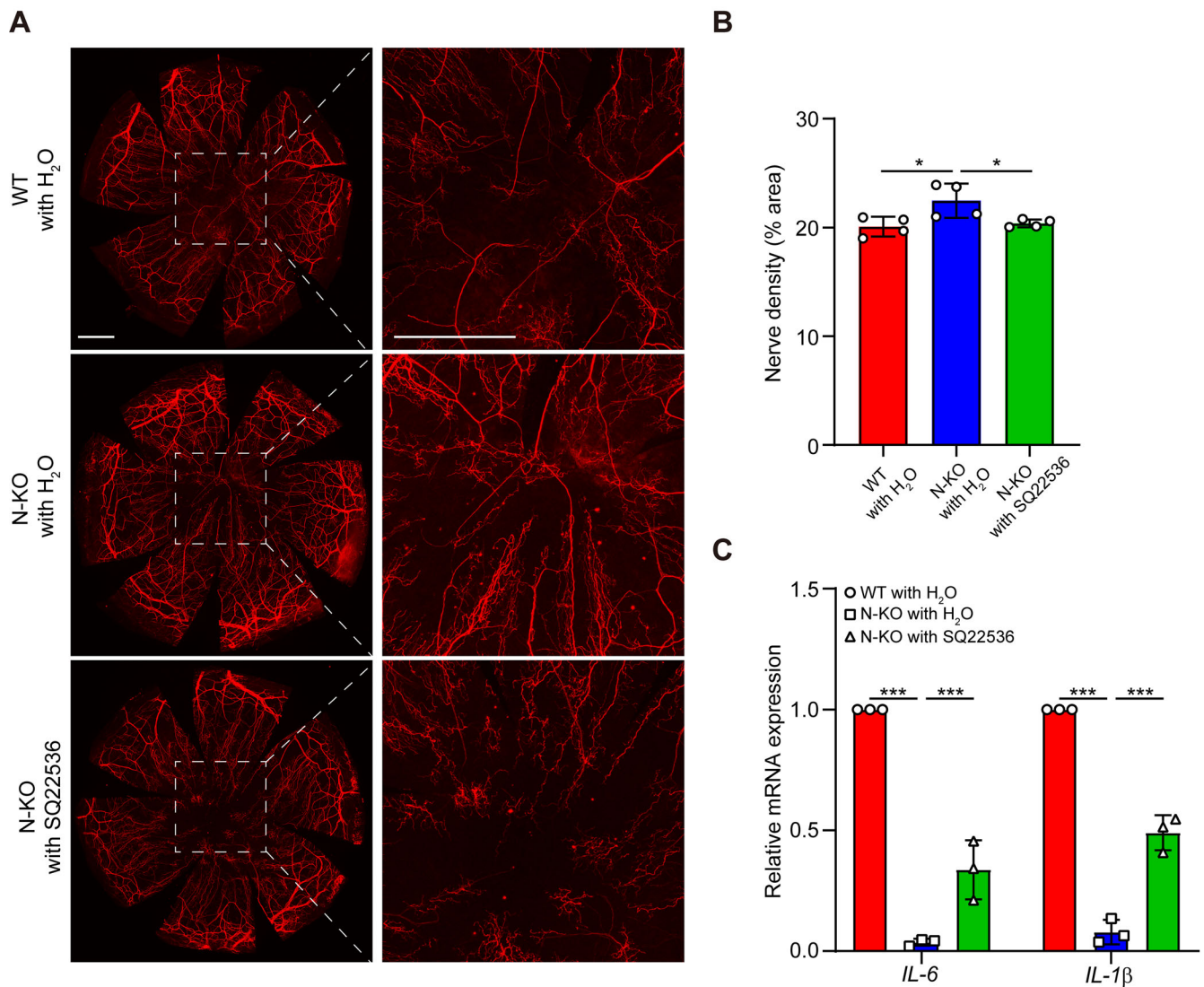


**FIGURE 4.** The cAMP pathway inhibitor SQ22536 delays corneal epithelial wound healing in *NEAT1* knockout or knockdown mice. (A, C) Corneal fluorescein staining of *NEAT1* knockout or knockdown mice at 0, 12, and 24 hours after epithelial debridement, following subconjunctival injection of SQ22536. (B, D) Analysis of the epithelial defect area using ImageJ (N-KO, *n* = 5; N-KD, *n* = 4). NC, negative control shRNA; N-KD, *NEAT1* knockdown shRNA.

expression in the eyes before SQ22536 administration (Supplementary Figs. S2B, S3A–S3C). Consistent with the results obtained in the N-KO mice, SQ22536 delayed corneal epithelial wound healing in the N-KD mice (Figs. 4C, 4D).

**Activation of the cAMP Pathway Regulates Nerve Regeneration and Inflammation**

Given the profound effects of *NEAT1* knockout on nerve regeneration and inflammation (Fig. 2), we investigated the



**FIGURE 5.** SQ22536 reverses the improvement in corneal nerve regeneration and inflammation in N-KO mice. (A) Representative images depicting wholemount-stained corneal subbasal nerve fibers in WT and *NEAT1* knockout mice with or without SQ22536 5 days after epithelium removal. Scale bar: 500  $\mu$ m. (B) Corneal nerve density was quantified by analyzing the areas that stained positive for  $\beta$ -tubulin III ( $n = 4$ ). (C) Validation of *IL-6* and *IL-1 $\beta$*  expression levels using qPCR ( $n = 3$ ).

influence of cAMP activity on these mechanisms. Consistent with the findings in N-KO mice, N-KD mice also exhibited significantly increased subbasal nerve fiber density and weakened inflammatory responses. Compared with N-KO or N-KD mice, SQ22536-treated mice showed nerve density and gene expression similar to those of the WT or control mice (Figs. 5, 6), suggesting that *NEAT1* deficiency exerts tissue-protective effects by modulating cAMP pathway activity.

## DISCUSSION

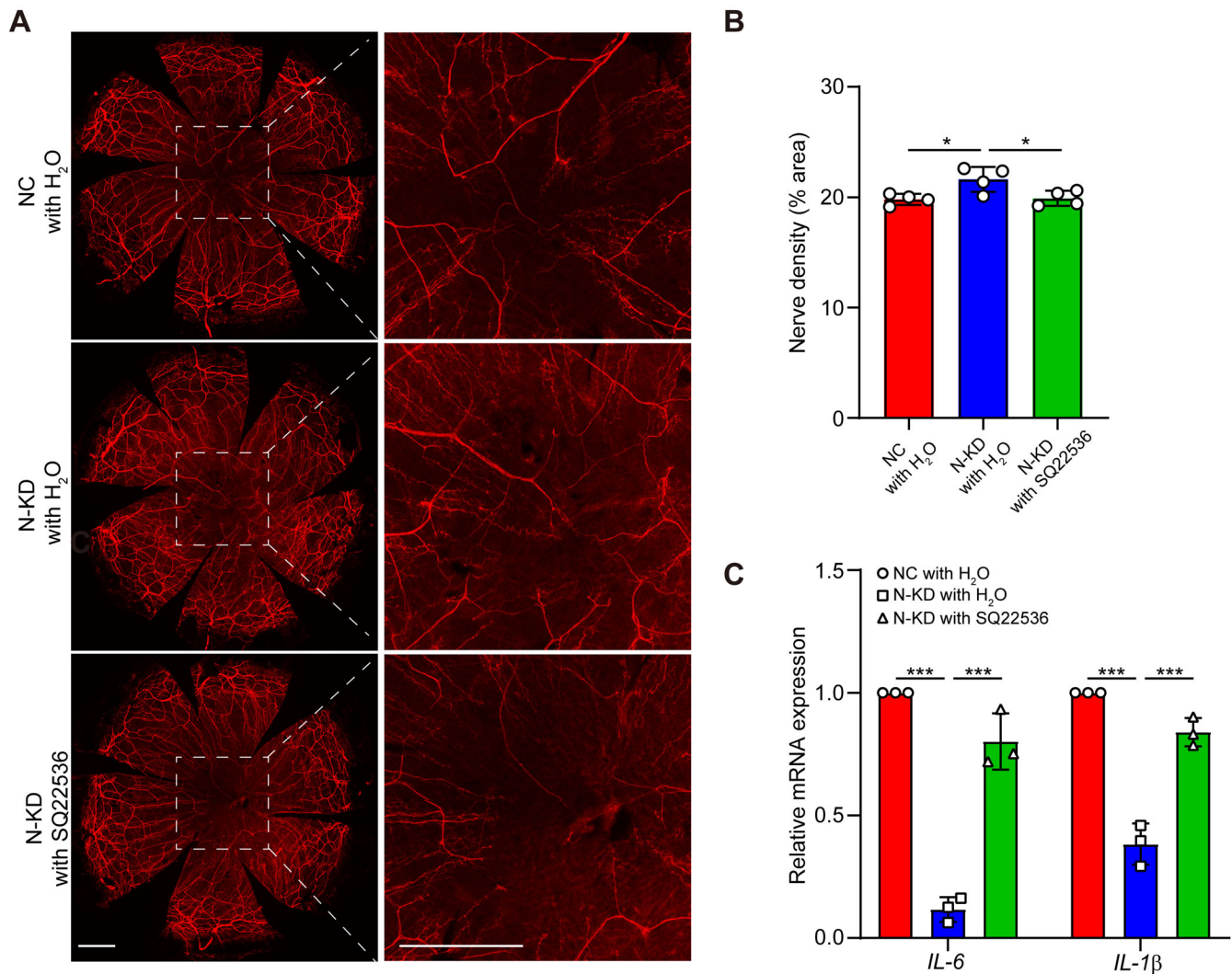
This study investigated the function of *NEAT1* in the regulation of corneal re-epithelialization and nerve regeneration. Our findings indicate that *NEAT1* deficiency accelerates corneal epithelial wound healing via activation of the cAMP signaling pathway.

lncRNAs have diverse biological functions and are known to play a significant role in tissue repair and injury. *MALAT1*

upregulation can stimulate the inflammatory response by targeting the miR-146a/nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling pathway in lipopolysaccharide (LPS)-induced kidney injury both in vitro and in vivo.<sup>42</sup> Furthermore, *PRINS* may inhibit *RANTES* and improve acute tubular necrosis and inflammation in renal ischemia-reperfusion injury.<sup>43</sup> *NEAT1* promotes the release of inflammatory factors and corneal neovascularization progression during corneal tissue repair.<sup>21</sup> Moreover, *NEAT1* protects the corneal endothelium from ultraviolet A (UVA)-induced FECD.<sup>14</sup> This study provides novel evidence to support the hypothesis that *NEAT1* plays a crucial role in corneal epithelial integrity and repair.

Recent studies have highlighted the significant role of lncRNAs in neuronal regeneration. Silencing BC089918 and uc.217 using small interfering RNA (siRNA) significantly promoted the outgrowth of dorsal root ganglion neurites.<sup>44</sup> *Silc1*, a conserved lncRNA, regulates nerve regeneration by *cis*-activating *SOX11*.<sup>45</sup> In contrast, lncRNA *Arr11* inhibits axon regeneration via an lncRNA-mRNA-microRNA





**FIGURE 6.** SQ22536 reverses the improvement in corneal nerve regeneration and inflammation in N-KD mice. (A) Representative images depicting wholemount-stained corneal sub-basal nerve fibers in control and *NEAT1* knockdown mice with or without SQ22536, five days after epithelium removal. Scale bar: 500  $\mu$ m. (B) Corneal nerve densities were quantified by analyzing the areas staining positive for  $\beta$ -tubulin III ( $n = 4$ ). (C) Validation of *IL-6* and *IL-1 $\beta$*  expression levels using qPCR ( $n = 3$ ).

co-expression network.<sup>46</sup> The deficiency of *NEAT1* contributes to corneal nerve regeneration during corneal wound healing, which is mediated by activation of the cAMP signaling pathway in the corneal epithelium. However, corneal nerve fibers are primarily sensory in nature and originate from the nasociliary branch of the ophthalmic division of the trigeminal ganglion (TG). The precise role of *NEAT1* in nerve regeneration and the TG remains unclear and requires further investigation.

The cAMP pathway is a ubiquitous and versatile signaling pathway in eukaryotic cells that regulates diverse cellular functions in almost all tissues. cAMP-dependent protein kinase A (PKA) activity has been observed in corneal epithelial and endothelial cells.<sup>47</sup> The proliferation of the corneal epithelium is endogenously regulated by the balance between adrenergic cAMP-dependent and cholinergic cGMP-dependent pathways.<sup>48</sup> In bovine corneal endothelial cells, depolarization of plasma membrane potential activates the cAMP/PKA pathway, leading to cytoskeletal reorganization.<sup>49</sup> The cAMP pathway regulates various cellu-

lar processes, such as the cell cycle, proliferation, differentiation, microtubule dynamics, intracellular transport, and ion fluxes.<sup>50–54</sup> This study revealed the contribution of cAMP signaling in the promotion of corneal epithelial wound healing. For the possible molecular mechanisms of cAMP overstimulation, previous studies have shown the activation of serotonin (5-HT7),<sup>55</sup> endothelin-1,<sup>56</sup> epidermal growth factor,<sup>57</sup> and  $\beta$ -adrenergic receptors<sup>47</sup> could activate the cAMP pathway in the cornea epithelium. Even so, the regulation mechanism upon *NEAT1* deficiency requires further exploration in follow-up studies.

Collectively, the knockout or specific knockdown of *NEAT1* in the corneal epithelium of mice enhances the regeneration rates of both epithelial and nerve tissues while reducing inflammatory cell infiltration. Moreover, RNA-seq has revealed a crucial role for increased cAMP signaling in the wound repair process. These findings are potentially valuable for understanding the function of *NEAT1* in corneal epithelial wound healing and the development of therapeutic strategies for corneal epithelial diseases.

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