

# Topical application of the HSV-1-based gene therapy vector KB801 results in sustained nerve growth factor expression in wounded murine corneas

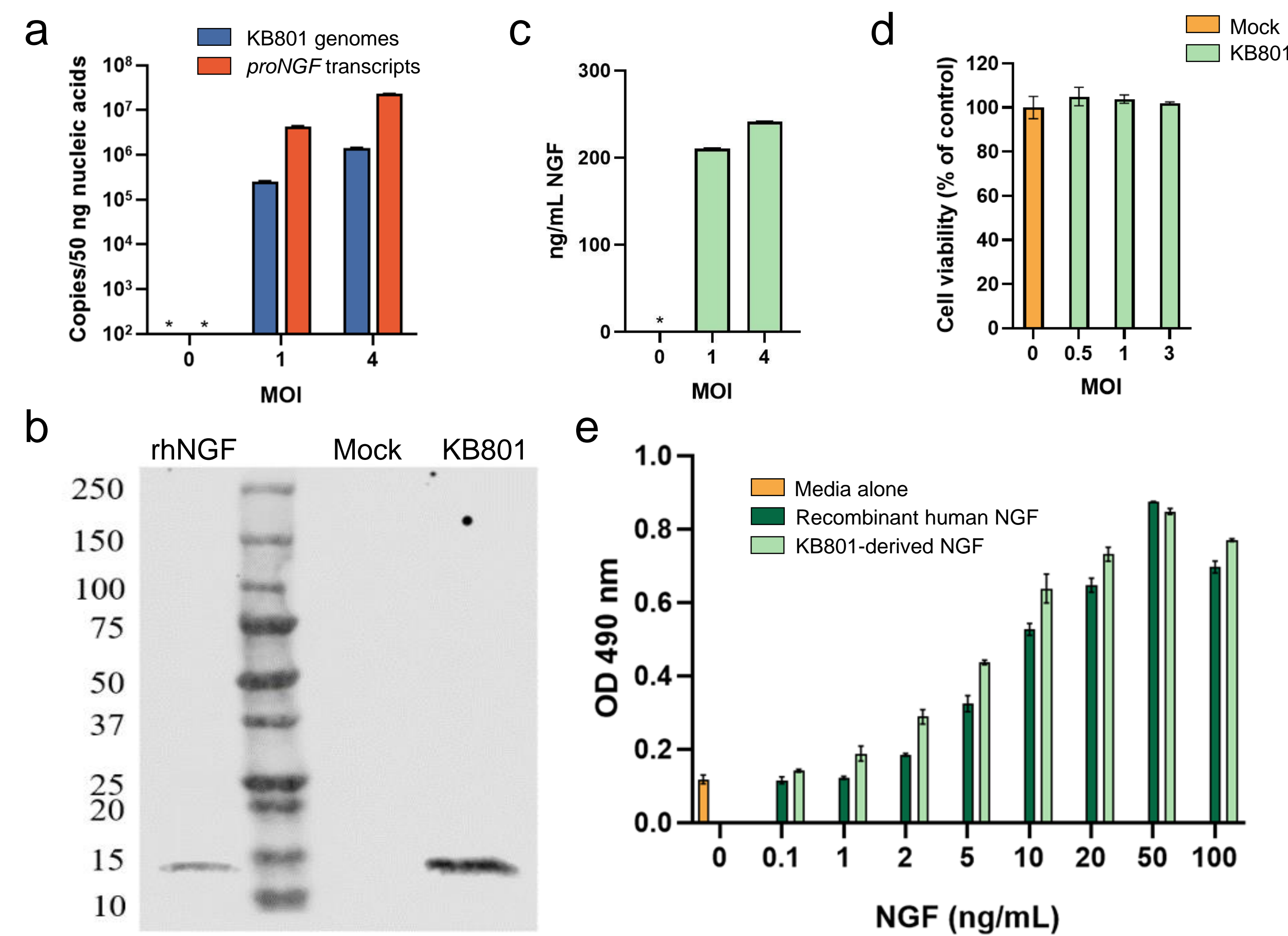
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## Purpose & Methods

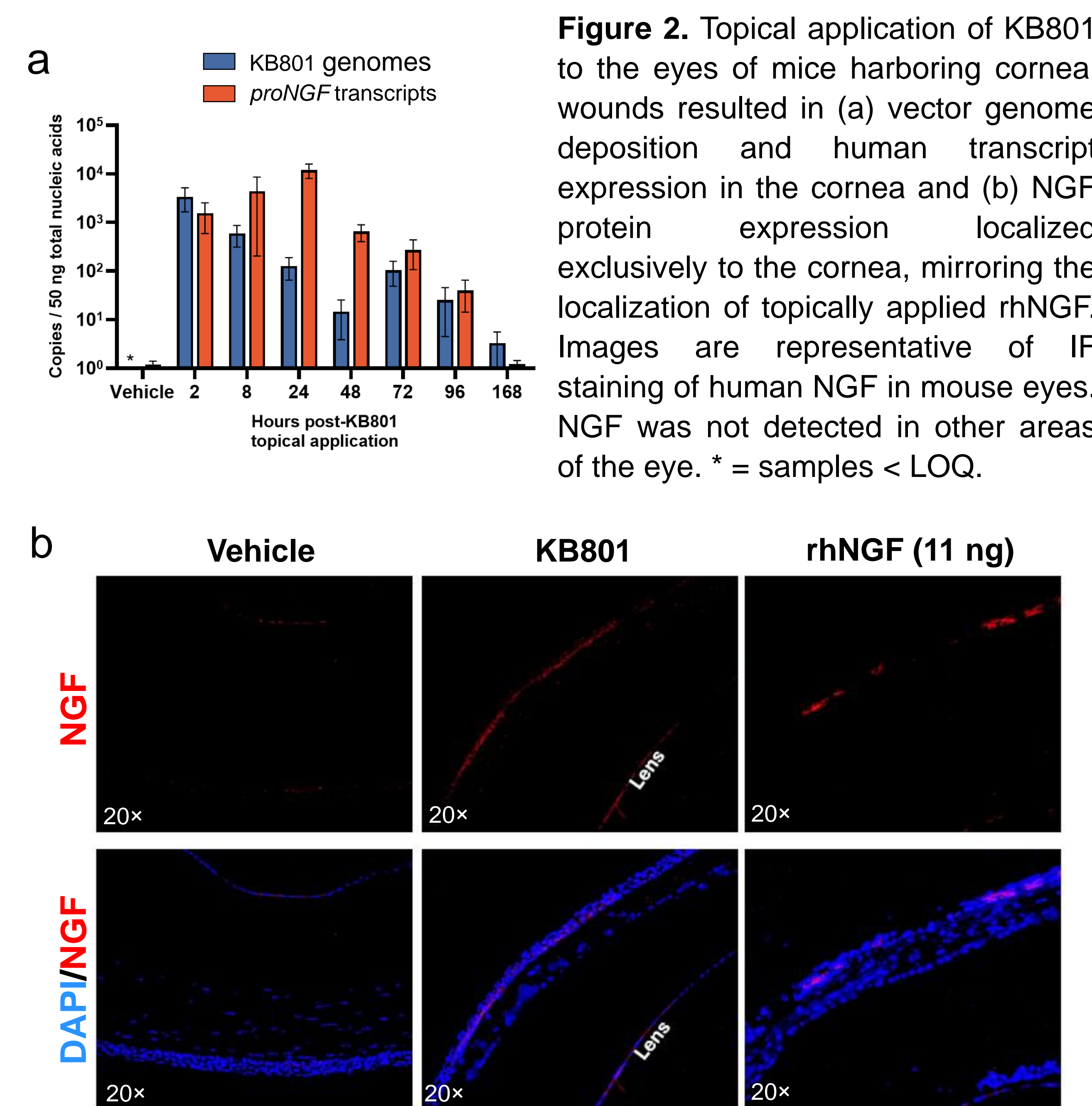
Beta-nerve growth factor (NGF) has emerged as an effective treatment for the rare degenerative disease neurotrophic keratitis (NK) due to its ability to promote corneal wound healing. The only approved therapeutic option for NK is cenegermin-bkjb, an ophthalmic solution of recombinant human NGF (rhNGF) administered six times daily for eight weeks; however, the high dosing frequency that is required due to rapid rhNGF clearance is burdensome and may lead to suboptimal treatment outcomes. Krystal Biotech, Inc. is currently developing KB801, a replication-defective, non-integrating herpes simplex virus type 1 (HSV-1)-based vector encoding human NGF as a topical treatment for NK. Here, KB801 was evaluated for its ability to durably express mature, fully functional NGF in both cell culture and murine corneal wound models. Primary human corneal epithelial cells were transduced with KB801 and assessed for transcript and protein expression. A cell-based proliferation assay was used to investigate the bioactivity of KB801-derived human NGF protein compared to commercially available rhNGF. NGF pharmacokinetics and protein localization were evaluated in vivo after a single topical dose of KB801 vs. one or six applications of rhNGF to wounded murine corneas via enzyme-linked immunosorbent assay (ELISA) and immunofluorescence (IF). Hematoxylin and eosin (H&E) staining was used to assess inflammation and histological changes.

## KB801 delivers its transgene to primary corneal epithelial cells, resulting in mature, functional human NGF protein secretion



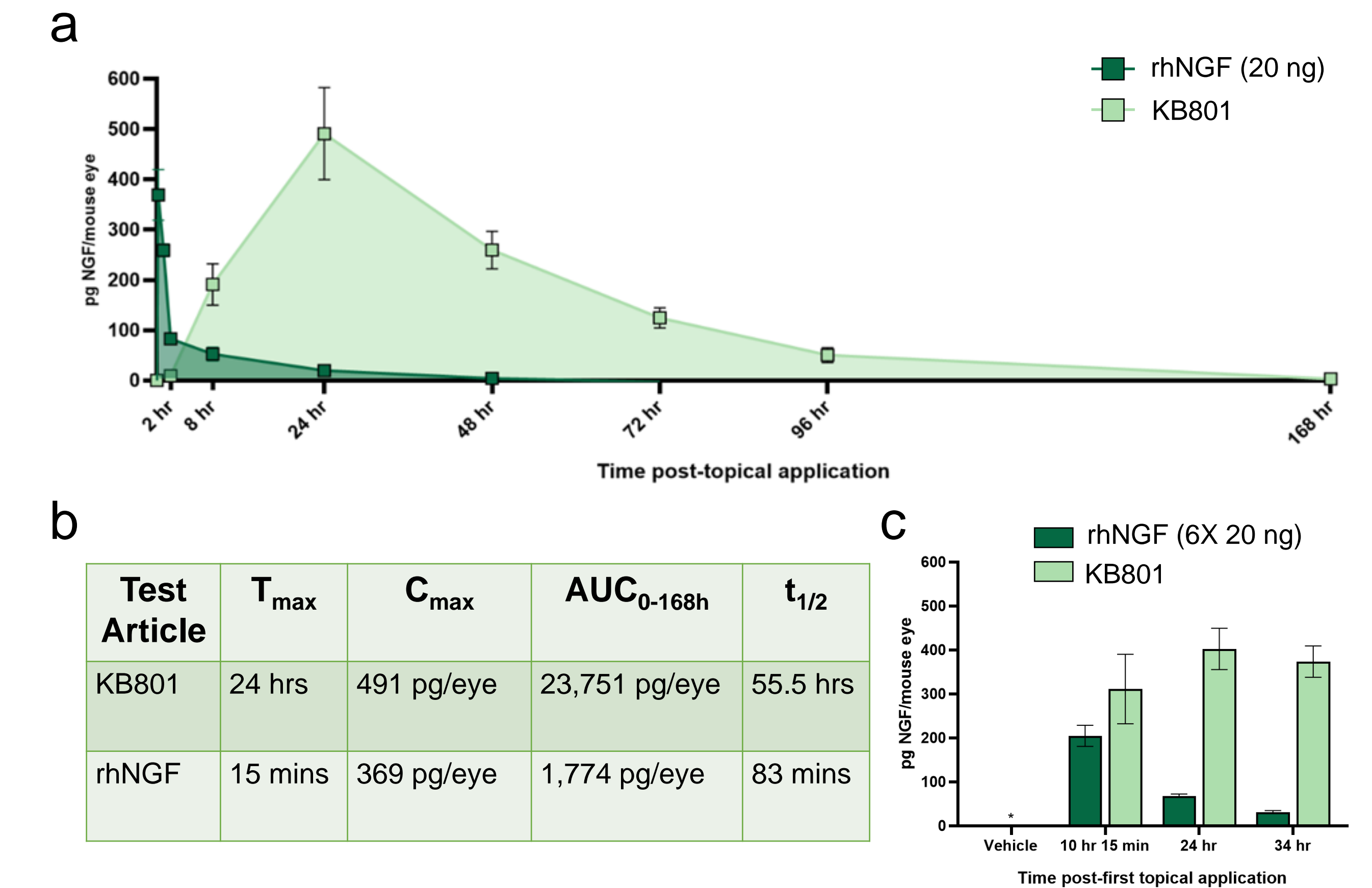
**Figure 1.** (a) KB801 transduction of primary human corneal epithelial cells resulted in dose-dependent vector deposition (via quantitative PCR) and transcript expression (via reverse transcription qPCR). Data presented as the average of duplicates  $\pm$  standard error of the mean (SEM). \* = samples < limit of quantification (LOQ). (b) Mature NGF protein secretion following KB801 transduction (MOI 1) of human corneal epithelial cells via western blotting. (c) Dose-dependent secretion of human NGF in the culture medium of primary corneal epithelial cells 48 hours post-transduction via ELISA. Data presented as the average of duplicates  $\pm$  SEM. \* = samples < limit of detection (LOD) (d) KB801 transduction of primary corneal epithelial cells resulted in no significant dose-dependent effects on cell metabolism or toxicity, as assessed by Mosmann's Tetrazolium Toxicity assay, at 24 hours. Data presented as the average of triplicates  $\pm$  SEM. (e) KB801 transduction of human cells results in the secretion of NGF protein with analogous bioactivity to commercially available rhNGF as assessed in a TF-1 cell-based proliferation assay. Data presented as the average of triplicates  $\pm$  SEM. MOI, multiplicity of infection; OD, optical density.

## KB801-delivered human NGF protein localizes to the cornea



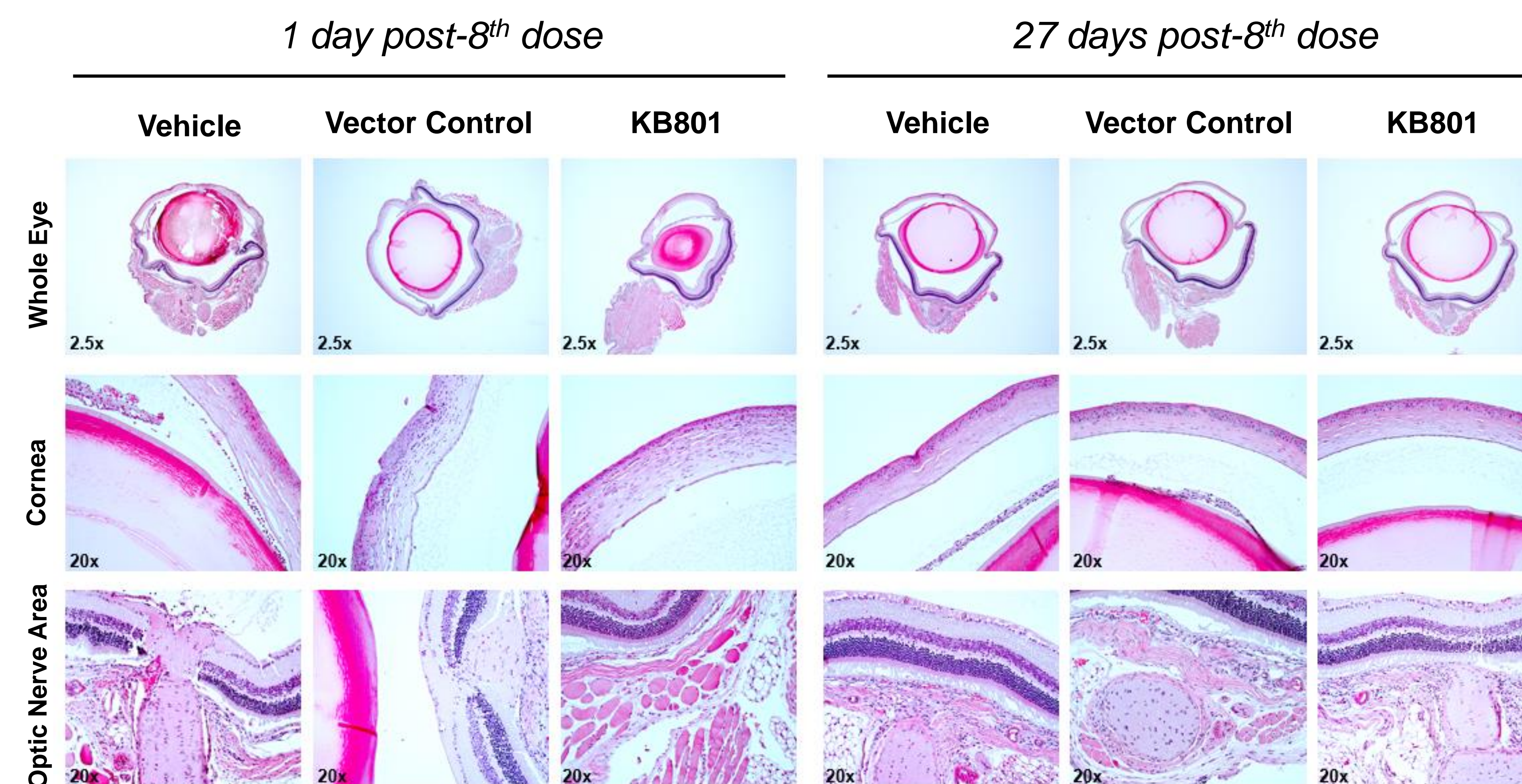
**Figure 2.** Topical application of KB801 to the eyes of mice harboring corneal wounds resulted in (a) vector genome deposition and human transcript expression in the cornea and (b) NGF protein expression localized exclusively to the cornea, mirroring the localization of topically applied rhNGF. Images are representative of IF staining of human NGF in mouse eyes. NGF was not detected in other areas of the eye. \* = samples < LOQ.

## Topical ocular KB801 application results in lasting NGF protein expression in the cornea



**Figure 3.** (a) Topical application of KB801 to the eyes of mice harboring corneal wounds resulted in both higher peak NGF levels in the eye and more prolonged total NGF exposure time as compared to mouse eyes treated with 20 ng rhNGF, an equivalently scaled concentration of the approved dose of cenegermin-bkjb. (b) Calculated pharmacokinetic parameters from (a). (c) Single KB801 topical ocular treatment resulted in higher NGF protein levels in wounded mouse eyes at every timepoint assessed as compared to six doses of 20 ng rhNGF given at two-hour intervals, mimicking the cenegermin-bkjb daily clinical dosing regimen. Data is presented at average  $\pm$  SEM of n=6 eyes per group; \* = samples < LOD.

## KB801 topical ocular dosing is well-tolerated in mice with a favorable safety profile after repeat dosing



**Figure 4.** Weekly topical application of KB801 to the eyes of mice harboring acute corneal wounds resulted in no signs of ocular necrosis and only mild inflammatory infiltrate in vehicle-, vector control- (HSV-mCherry), and KB801-treated eyes, as assessed by H&E staining. By 27 days after treatment cessation, all eyes were clear of inflammatory infiltrate. Images are representative of the whole eye (upper row), cornea (middle row), and optic nerve area (bottom row).

## Conclusions

KB801 is a potentially safe and effective redosable topical ocular therapy for sustained NGF expression in the cornea of NK patients.

## Acknowledgements

Krystal Biotech, Inc. would like to thank Hilltop Lab Animals, Inc. for their contributions to the work presented here. All animal studies were performed in an AAALAC accredited facility, and protocols were IACUC approved prior to initiation. We thank Dr. Meghan Conner (Krystal Biotech) for drafting this poster.

## Disclosures

These studies were funded by Krystal Biotech, Inc. All authors are current employees of Krystal Biotech, Inc.