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Supplemental Information

Corneal fibrosis abrogation by a localized

AAV-mediated inhibitor of differentiation

3 (Id3) gene therapy in rabbit eyes in vivo

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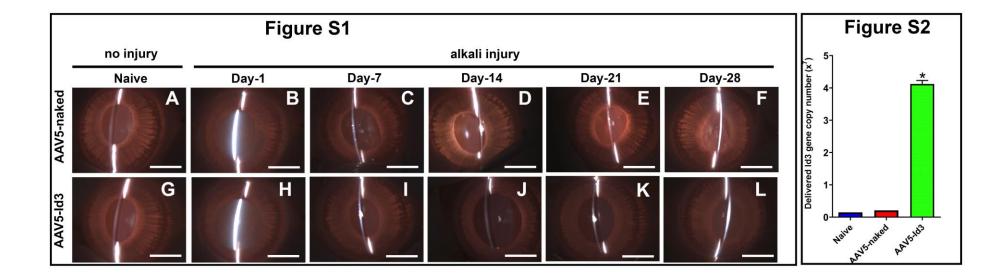


Figure S1. AAV5-Id3 gene therapy regulates corneal fibrosis and maintains corneal transparency. (A-F) The slit lamp microscopic images show the progress of alkali trauma in the AAV5-naked vector group in a time-dependent manner. (G-L) The slit-lamp microscopic images show the progress and efficacy of AAV5-Id3 gene therapy after alkali trauma in the AAV5-Id3 group in a time-dependent manner. The slit beam pattern in AAV5-Id3 group images shows that Inflammation and corneal scaring were reduced notably from day 7 onwards and much appreciable on day 28 to naïve corneal tissue. Scale bar = 3.0 mm.

Figure S2. AAV5-Id3 gene copies are retained in corneal tissue. The qRT-PCR analysis showed that efficient delivery of Id3 gene copies carried through AAV5 vectors was retained in corneal tissue via topical customized method after day -28. There were six samples in each group and error bars represent \pm SEM. *p<0.001, and tissues were collected on day 28.

Table S1. The sequence of primers used in the study

| Gene Name | Gene Abbreviation | Forward Primer (5' to 3') | Reverse Primer (5' to 3') |
|-------------------------|-------------------|---------------------------|---------------------------|
| β-actin | β-actin | CGGCTACAGCTTCACCACCA | CAGGCAGCTCGTAGCTCTTC |
| α-Smooth muscle actin | α-SMA | TGGGTGACGAAGCACAGAGC | CTTCAGGGGCAACACGAAGC |
| Fibronectin | FN | CGCAGCTTCGAGATCAGTGC | TCGACGGGATCACACTTCCA |
| Collagen I | Col-I | TGTGGCCCAGAAGAACTGGTACAT | ACTGGAATCCATCGGTCATGCTCT |
| Collagen III | Col-III | TATCGAACACGCAAGGCTGTGAGA | GGCCAACGTCCACACCAAATTCTT |
| Transcription Factor-3 | E2A | GCAGGGTCCTCATGAGAGTG | GGTAGGGCAGCAGTTTGT |
| Transcription Factor-4 | E2-2 | CCATCCAGGAACTATGGAGATG | GAAGAAGGAGCTAGGGAAAGTG |
| Transcription factor-12 | HEB | GACCATACCAGCAGTAGTTTCC | GCCTTTCCTTCTCCCTTTCTATC |
| ld3-mCherry | Id3-MCH | CGCGTCATCGACTACATTCTC | CCCATGGTCTTCTTCTGCATT |

The sequence of the forward and reverse primers was used in the study to confirm the mRNA expression of different proteins using PCR amplification.