are not conducive to expression (such as hairpin structures), changes in GC content, CpG dinucleotide content, mRNA secondary structure, cryptic splice sites, early polyadenylation sites, internal ribosome entry and binding sites, negative CpG islands, RNA instability regions, repeats (direct repeats, inverted repeats, etc.) and restriction sites that may affect cloning. The optimized sequence (SEQ ID NO: 1) was added with two restriction sites of Cla I and Xba I, or the product produced by PCR amplification with the primers designed with the new gene and the pAAV plasmid vector were subjected to Cla I and Xba I double-enzyme digestion, respectively, and then the digestion products were recovered and ligated with T4 DNA Ligase overnight, and then the ligation products were transformed into competent cells to obtain recombinant pAAV-Nrf2. A single clone was picked out for bacterial liquid sequencing, and the sequence obtained by sequencing was subjected to sequence alignment analysis to successfully obtain a recombinant adeno-associated virus plasmid pAAV-Nrf2 with the correct sequence, as shown in Figure 7, including enhancer/promoter, gene sequence, polyA, and viral packaging ITR sequence, wherein Figures 7a, 7b, 7c, and 7d are the profiles of pAAV-CMV-Nrf2_native, pAAV-CMV-Nrf2_opt, pAAV-CAG-Nrf2_opt, and pAAV-SYN-Nrf2_opt plasmids, respectively.

1.2 Coating of pAAV-Nrf2 recombinant adeno-associated virus

The pAdHelper, pAAV-r2c~~5~~2, pAAV-Nrf2 plasmids were transfected into 293T cells (seeded in a 225cm^2 cell culture flask), and the cells were harvested after 48 hours. Cells were resuspended in PBS and freezed-thawed 3 times.

1.3 Purification and concentration of rAAV2/2-Nrf2 virus

The rAAV2/2-Nrf2 virus was isolated, concentrated and purified using three steps of chloroform treatment-PEG/NaCl precipitation-chloroform extraction. Total recovery = number of virus particles in final product/number of virus particles in starting material.

1.4 Fluorescence quantitative PCR method to detect the physical titer of rAAV2/2-

7-month-old DBA/2J mice (intraocular pressure>18mmHg) were divided into three groups, wherein 6 mice were injected with PBS as the model group, and the other two groups were intravitreally injected with rAAV2/2-CAG-Nrf2_opt virus (n=7) and rAAV2/2-SYN-Nrf2_opt virus (n=7), respectively in single eyes. Five C57/BL6 mice of the same age were used as the normal group.

4.2 Intraocular pressure and fundus examinations for mice

Fundus and intraocular pressure examinations were performed every 3-5 days after surgery. There were no obvious abnormalities in the eyes of all mice, with no conjunctival hyperemia, secretion, endophthalmitis, or obvious changes in intraocular pressure.

4.3 Staining and counting of retinal ganglion cells

5 months after dosing, the mice were sacrificed by breaking spines, and the retinas were harvested for immunofluorescence staining and counting of ganglion cells. The results shown in Figure 3 shows that the density of surviving ganglion cells in mice of the model group (971±146 RGCs/mm^2, Figure 3b) was significantly lower than that of the normal group (~~1506±152~~ 971±146 RGCs/mm^2, Figure 3a), with $P<0.05$. The densities of ganglion cells in the rAAV2/2-CAG-Nrf2_opt group (1426±179 RGCs/mm^2, Figure 3c) and the rAAV2/2-SYN-Nrf2_opt group (1384±88 RGCs/mm^2, Figure 3d) were significantly increased as compared with that in the model group (1506±152 RGCs/mm^2, Figure 3b) ($P<0.05$), indicating a protective effect on retinal ganglion cells in modeling mice of drug groups. However, there was no significant difference between the rAAV2/2-CAG-Nrf2_opt group and the rAAV2/2-SYN-Nrf2_opt group. Figure 3e shows the statistical results of the RGC cell counts per unit retinal area of mice in each group.

4.4 Retinal expression level

The eyeballs were taken for performing cryosection and immunofluorescence staining with Nrf2 antibody. The expression levels of rAAV2/2-CAG-Nrf2_opt and rAAV2/2-SYN-