Dear Editor,

We report a gene therapy strategy using CRISPR/Cas9-mediated cellular reprogramming by switching a mutation-vulnerable/sensitive cell type to a mutation-insensitive/resistant cell type, therefore restoring tissue architecture and function. We applied this strategy to retinitis pigmentosa (RP), a major cause of blindness characterized by retinal rod photoreceptor degeneration caused by numerous mutations in many genes. By reprogramming rod to cone-like photoreceptors \textit{in situ} by inactivating \textit{Nrl} or \textit{Nr2e3}, we show an increase in cone-like cells with remarkable concomitant preservation of both cone and rod photoreceptors and retinal tissue, with restoration of visual function. Our approach demonstrates the feasibility of cellular reprogramming in preventing degeneration and preserving tissue and function, and points to a novel approach in treating human diseases in a gene and mutation independent manner.

Gene therapy shows great promise in treating many human diseases, yet a major drawback of the current technology is that it can only be directed to a particular mutation or a single gene at best. This makes it difficult to apply gene therapy to a spectrum of diseases and a broad patient population. Similarly, repair and regeneration of tissues using endogenous stem cells represents an important goal in regenerative medicine and promising efforts have been demonstrated in mouse liver [1], zebra fish heart [2], and human lens [3]. However, the starting cells used to regenerate tissues must possess normal genetic makeup and function, which represents a challenge in many cases of human disease. One approach to overcome the above drawbacks in tissue repair and regeneration is therapeutic cellular reprogramming, which switches a cell type sensitive to a mutation to a functionally related cell type that is resistant to the same mutation. Therefore, this strategy would render the underlying mutation irrelevant, with consequent preservation of tissue architecture and function.

We tested this hypothesis on RP, a retinal rod photoreceptor-specific disease. RP is one of the most common degenerative diseases of the eye, affecting over one million patients worldwide. RP is characterized by primary rod photoreceptor death and degeneration, followed by secondary cone death [4]. It can be caused by numerous mutations in over 200 genes, which limits the therapeutic impact of conventional gene therapy strategies. Acute gene knockout of rod determinant \textit{Nrl} was shown to reprogram adult rods into cone-like cells, rendering them resistant to effects of mutations in RP-specific genes and consequently preventing secondary cone loss [5]. \textit{Nrl} acts as a master switch gene between rods and cones and activates a key downstream transcription factor \textit{Nr2e3}. \textit{Nrl} and \textit{Nr2e3} function in concert to activate a rod-specific gene transcription network and control rod differentiation and fate [6]. Loss of function in either \textit{Nrl} or \textit{Nr2e3} reprograms rods to a cone cell fate.

To test a CRISPR/Cas9-based cellular reprogramming strategy to treat RP, we employed two AAV vectors, one expressing Cas9, and another carrying gRNAs targeting the \textit{Nrl} or \textit{Nr2e3} gene (Figure 1A a, b). To assess if simultaneously targeting two sites by two gRNAs in the same gene has a higher targeting and inactivation efficiency than that by a single gRNA, we designed constructs that have either one or two gRNAs targeting \textit{Nrl} or \textit{Nr2e3}. We tested gene mutagenesis rates in mouse fibroblasts using a T7E1 nuclease assay, which cuts a mismatched double stranded DNA template. The results showed that the two-gRNA system had a much higher editing efficiency than that by a single gRNA, we designed constructs that have either one or two gRNAs targeting \textit{Nrl} or \textit{Nr2e3}. We tested gene mutagenesis rates in mouse fibroblasts using a T7E1 nuclease assay, which cuts a mismatched double stranded DNA template. The results showed that the two-gRNA system had a much higher editing efficiency than that by a single gRNA, we designed constructs that have either one or two gRNAs targeting \textit{Nrl} or \textit{Nr2e3}. We tested gene mutagenesis rates in mouse fibroblasts using a T7E1 nuclease assay, which cuts a mismatched double stranded DNA template. The results showed that the two-gRNA system had a much higher editing efficiency than that by a single gRNA, we designed constructs that have either one or two gRNAs targeting \textit{Nrl} or \textit{Nr2e3}. We therefore adopted a double gRNA targeting strategy in all subsequent \textit{in vivo} experiments.

We next delivered the two gRNAs/Cas9 constructs using AAV vectors to normal mice via subretinal injection at postnatal day 7 (P7) and sacrificed mice for histology at P30. Retinas were frozen-sectioned and stained for cone markers, including cone arrestin (mCAR) and short wavelength opsin (S-opsin) [7]. We observed a reprogrammed photoreceptor phenotype with two gRNAs \textit{in vivo}. In normal retinas, cone nuclei reside at the top layer of outer nuclear layer (ONL), while rod nuclei fill
CRISPR-Cas9 mediated rod to cone reprogramming

The FVB/N mouse strain provides another model of rapid photoreceptor degeneration [9], which is caused by another mutation in PDE. We injected AAV-gRNAs/Cas9 into the subretinal space at P7, and tested ERG responses at P50, followed by histology analysis. Consistent with results from rd10 mice, histology analysis of the AAV-gRNAs/Cas9-treated FVB/N retinas showed greater preservation of mCAR+ cells and significantly improved preservation of ONL thickness (Figure 1D a-c). The eyes treated with AAV-gRNAs/Cas9 also exhibited significantly improved photopic b-wave values, suggesting improved cone function (Figure 1D d).

In this study, we showed that targeted inactivation of Nrl or Nr2e3 via CRISPR/Cas9-mediated genome editing can reprogram rod to cone-like photoreceptors, rescue retinal rod and cone degeneration and restore visual function in two different mouse RP models. In addition, our double gRNA strategy towards a single gene significantly increased targeting efficiency. In RP, cone photoreceptor death with consequent loss of day light...
and color vision function has the most severe impact on patient quality of life, yet it appears to be secondary to rod death. Our cellular reprogramming approach significantly rescues and restores both rod and cone function. This reprogramming strategy is especially attractive as the natural course of the disease inevitably results in loss of both rods and cones at an advanced stage, which eventually leads to legal blindness.

Our approach shows promise of cellular reprogramming in preventing degeneration and restoring tissue function, and points to a novel approach in treating human diseases in a gene and mutation independent manner.

Note added in proof: an independent study with similar results has been published recently in *Nat Commun* [10].

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(Supplementary information is linked to the online version of the paper on the *Cell Research* website.)