Supplemental Figures and Tables

Figure S1. Identification of HDR cassette incorporation in patient 1 iPSCs. Representative gel image of genomic PCR obtained from 25 individual clones following puromycin selection. To demonstrate HDR sequence incorporation the forward primer was placed inside HDR cassette and the reverse primer was placed outside the HDR cassette (i.e. beyond the homology arm).

A Uncorrected allele

B Clone 16- Corrected allele

Figure S2. Sequencing of patient 1 iPSCs. Sanger sequencing chromatograms of iPSC clone 16 depicting the uncorrected c.119-2A>C containing allele (A) and the HDR corrected allele (B). Arrows indicate the location of the disease causing c.119-2A>C mutation and the synonymous C>T PAM site variation, which was introduced in the HDR cassette to prevent re-cutting events.
Figure S3. Off-target analysis of sg4 in patient 1 iPSCs. Representative gel images of T7E1 assays for each off-target locus in non-CRISPR treated (-) and CRISPR corrected (+) iPSCs (clone 16).

Figure S4. Identification of HDR cassette incorporation in patient 2 iPSCs. Representative gel image of genomic PCR obtained from 24 individual clones following puromycin selection. To demonstrate HDR sequence incorporation the forward primer was placed inside HDR cassette and the reverse primer was placed outside the HDR cassette (i.e. beyond the homology arm).
**Figure S5. Sequencing of patient 2 iPSCs.** A-C: Chromatograms depicting the uncorrected paternal allele (i.e., non-targeted control) containing the p.(Arg73Ser) mutation (A), the CRISPR corrected paternal allele of clone 6 containing both the corrected p.(Arg73Ser) mutation and the synonymous C>T PAM site variation introduced to prevent re-cutting events (B), and the maternal allele from clone 6 displaying the untargeted p.(Arg311Gln) mutation (C).

**Figure S6. Off-target analysis of sg4 in Patient 2 iPSCs.** Representative gel images of T7E1 assays for each off-target locus in non-CRISPR treated (-) and CRISPR corrected (+) iPSCs (clone 6).
**Figure S7. Analysis of NR2E3 transcript expression.**

A-C: Chromatograms depicting NR2E3 transcript sequence of uncorrected cells with inclusion of 111 bases of intron 1 (A), uncorrected cells with inclusion of 143 bases of intron 1 (B) and corrected cells demonstrating CRISPR based restoration of normal splicing in iPSC derived photoreceptor precursor cells (clone 16). Note inclusion of the synonymous C>T PAM site variation in the HDR corrected allele, which was included to prevent re-cutting events.
### Table S1. List of sgRNAs and primers used.

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| T7E1 assay               | F2         | GCCGGGTTCTGTTCAATGT                  |
|                          | R2         | TCCAGCTTAGCACAGGGTTC               |

| HDR incorporation        | F1         | AACTTGTTTATTGAGCTTATAATGG          |
|                          | R1         | GATATAAGGCTGGCGCATAGGTG            |

| c.119-2/Arg73 Sequencing | F2         | GCCTGGGTTCTGTTCAATGT               |
|                          | R2         | TCCAGCTTAGACAGAGGTTTC           |

| Arg311 Sequencing        | F3         | GAACCAGTGCTCTCAGATGATAG          |
|                          | R3         | AGGATAGAGGCTCAGACAC               |

| NR2E3 RT-PCR             | F4         | GCACAGAGACAGAGAGGGTTCAT          |
|                          | R4         | GTCTGACAGGCGCTCCT                |

| 18s rRNA                 | 18s F      | CGGCTACCACACATCTCAAAGAAG         |
|                          | 18s R      | GCTGAAATACGCAGGGCTGCT           |

### Sequencing

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### Table S2. List of off-target sequences, PAM sites, off-target scores, chromosomal location of off-targets and primer sequences.

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