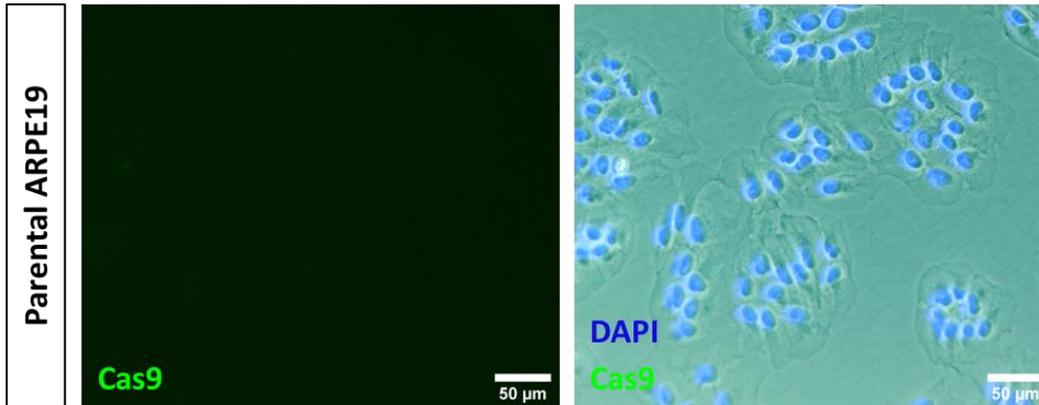
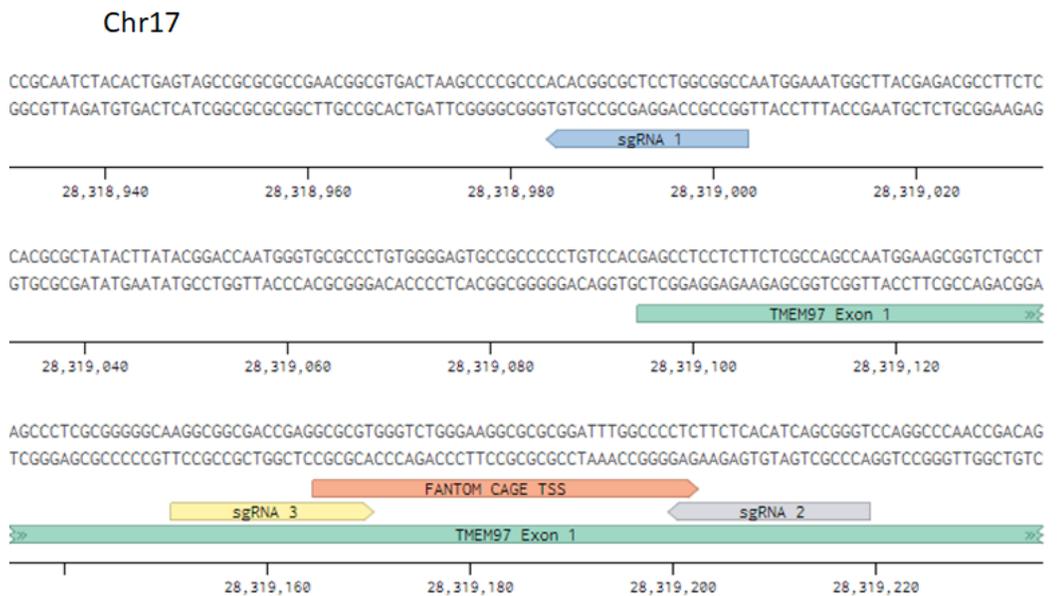


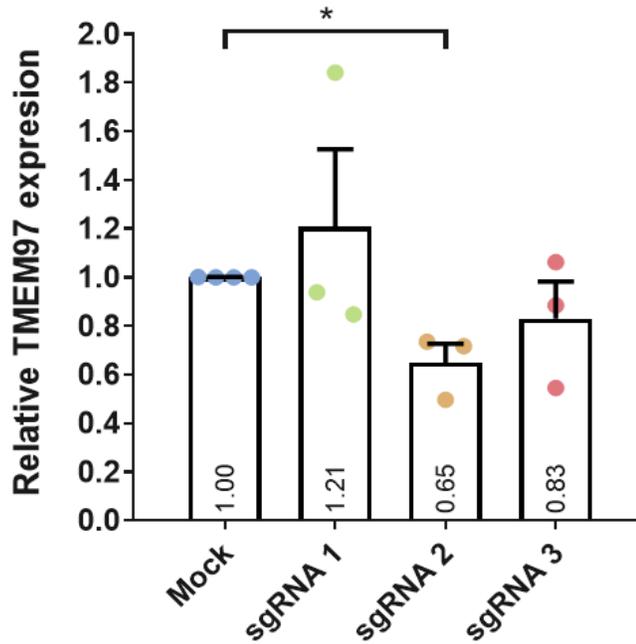
## Supplementary information



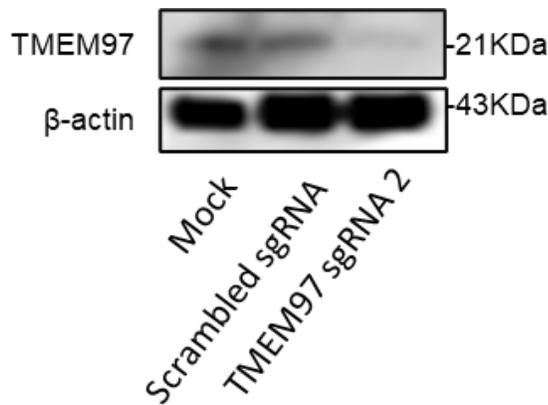
**Figure S1.** Immunostaining of SpCas9 in parental cell line ARPE19 confirmed absence of SpCas9 signal.



**Figure S2.** Schematic diagram of the 5' region of human *TMEM97* gene with sgRNA target areas near the transcription start site (TSS). The green region marks exon 1 of *TMEM97* gene as determined by Ensembl. The orange region marks the TSS as determined by FANTOM CAGE data. Target sites for sgRNA are marked in blue (sgRNA 1), grey (sgRNA 2) and yellow (sgRNA 3).



**Figure S3.** qPCR analysis of CRISPRi knockdown of *TMEM97* expression in HEK293A using 3 different sgRNA. Results represented as the mean  $\pm$  SEM of three biological repeats, each with three technical repeats. \* indicates  $p < 0.05$ .



**Figure S4.** Westernblot analysis of *TMEM97* expression in ARPE19-KRAB transfected with *TMEM97* sgRNA 2 or scrambled sgRNA as a negative control.

**Table S1.** Details for donor RPE samples used for RNAseq.

| Retina | Donor ID | Eye bulb | Age (yrs) | Sex | Retrieval time (hrs) | Ocular history                           |
|--------|----------|----------|-----------|-----|----------------------|--|
| 1      | 17-080   | Right    | 77        | F   | 4 hrs                | Healthy                                  |
| 2      | 17-172   | Left     | 69        | F   | 4 hrs                | Healthy                                  |
| 3      | 18-011   | Left     | 71        | M   | 6 hrs                | Bilateral intraocular lens, exophthalmos |

**Table S2.** Information of sgRNAs used in this study. TSS distance is based on the transcription start site defined by Ensembl. On-target score is based on [23].

| Name           | TSS distance | Strand | Sequence                 | PAM | Design         | On-target score |
|----------------|--------------|--------|--------------------------|-----|----------------|-----------------|
| TMEM97 sgRNA 1 | -91bp        | -      | GGCCGCCAGGAGCGCC<br>GTGT | GGG | Ensembl        | 50.6            |
| TMEM97 sgRNA 2 | +105bp       | -      | ACCCGCTGATGTGAGAA<br>GAG | GGG | FANTOM<br>CAGE | 65.9            |
| TMEM97 sgRNA 3 | +56bp        | +      | AGGCGGCGACCGAGGC<br>GCGT | GGG | FANTOM<br>CAGE | 56              |

**Table S3.** Off-targets alignment of *TMEM97* sgRNA2 using CasOFFinder with up to 3 mismatches. TSS: transcription start site.

| Target   | Chromosome | Position  | Direction | Mismatch | Genomic location                       | TSS within 1kbp upstream and 1kbp downstream |
|--|------------|-----------|-----------|----------|--|--|
| crRNA:<br>ACCCGCTGATGTGAGAAGAGNG<br>G<br>DNA:<br>ACCCGCTGATGTGAGAAGAGGG<br>G | chr17      | 28319196  | -         | 0        | TMEM97<br>exon 1                       | TMEM97                                       |
| crRNA:<br>ACCCGCTGATGTGAGAAGAGNG<br>G<br>DNA:<br>ACatGCTGATGTGAGAtGAGTGG     | chr8       | 17732170  | +         | 3        | N/A                                    | N/A  |
| crRNA:<br>ACCCGCTGATGTGAGAAGAGNG<br>G<br>DNA:<br>gCCCGCTtTGTTGAGAAGAGCGG     | chr5       | 157491565 | -         | 3        | Intronic<br>region of<br>ADAM19        | N/A  |
| crRNA:<br>ACCCGCTGATGTGAGAAGAGNG<br>G<br>DNA:<br>gCCCTcATGTGAGAAGAGAGG       | chr14      | 71953121  | +         | 3        | Intronic<br>region of<br>RGS6          | N/A  |
| crRNA:<br>ACCCGCTGATGTGAGAAGAGNG<br>G<br>DNA:<br>ACCCGCTGATGTtAGAAGctGGG     | chr10      | 70942247  | +         | 3        | Intronic<br>region of<br>LINC0262<br>2 | N/A  |

|                                       |       |          |   |   |                               |     |
|---------------------------------------|-------|----------|---|---|-------------------------------|-----|
| crRNA:<br>ACCCGCTGATGTGAGAAGAGNG<br>G | chr13 | 93671677 | - | 3 | Intronic<br>region of<br>GPC6 | N/A |
| DNA:<br>ACCCGCTGtgGTGAGAAGtGAGG       |       |          |   |   |                               |     |

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