# Supplementary Materials

## Supplementary tables

### Table S1. Primers used for PCR and sequencing

| Gene | Forward primer (5’-3’) | Reverse primer (5’-3’) | Size (bp) |
|---|---|---|---|
| *ENSA* | AGGACACGCAGGAGAAAGAA | CACACACCCAAATGCTCCAA | 365 |
| *DACT2* | TTTACGAGATGAGCGACGGT | CCTTCCAGCGTCCCTTAGAG | 410 |
| *EXTL2* | GGTGCTTTGACTGCCTTACT | CCAGACGGTTAAGCAGAAGG | 428 |
| *DDR1* | TTCCTGATGCCTCGTCCTGT | CCTCCTTACCTCCCCAAACT | 297 |
| *CCDC188* | CTGCTGTGCTTACCTCCTCT | CTGCAGCCACTTTTCCGATG | 448 |

### Table S2. Primers used for RT-PCR or qPCR

| Name | Species | Forward primer (5'-3') | Reverse primer (5'-3') |
|---|---|---|---|
| *ENSA* (F1+R1) | Human | TGCCTGAGAGAGCTGAAGAG | TGGCCATGTTGTAGTCTCCT |
| *ENSA* (F2+R2) | Human | AAGAAGAGAACCCTGCGGAG | GAGACTATGCCCCATACCCC |
| *ACTB* | Human | TGGCACCACACCTTCTACAA | CCAGAGGCGTACAGGGATAG |
| *Ensa* (f1+r1) | Mouse | TGAGAAAGCTGAGGAGGCAA | TCACCAGGTTCTTGTCTGCT |
| *Ensa* (f2+r2) | Mouse | GAAGAAGAAAACCCTGCGGA | CTTCATCCAAGGTGCCACAG |
| *Actb* | Mouse | CCTCTATGCCAACACAGTGC | CCTGCTTGCTGATCCACATC |
| *DACT2* | Human | CGGTTGATGAGACTACTGTGC | GGGCTCTGTCAAGATCACCT |
| *EXTL2* | Human | ACAACAGACAGCAAACAGGA | AAAACAAGGTCTGGGGTGCT |
| *GAPDH* | Human | GGACTCATGACCACAGTCCATGCC | TCAGGGATGACCTTGCCCACAG |
| *dact2* | zebrafish | CTGAACAGAGACCAGCCAGA | TAGGTGTTCGGTGGATGCTT |
| *extl2* | zebrafish | GGAACAACCCAGGAGAAACG | TGTCATCGTCCAGCATCAAA |
| *β actin* | zebrafish | ATGGATGATGAAATTGCCGCAC | ACCATCACCAGAGTCCATCACG |

### Table S3. Sequence of single-guide RNAs used for creating the *Ensa*-knockout mice

| Name | Sequence (5'-3') | PAM |
|---|---|---|
| sgRNA 1 | CACCTCCGGCCAACGCTTAT | TGG |
| sgRNA 2 | GGCGGATTCTTTAGAGCGAC | AGG |
| sgRNA 3 | TCAGGAGGTGGCCCGGGTGC | AGG |
| sgRNA 4 | CCATGTGTTGGGACACCAGT | GGG |

### Table S4. Primers used for mouse genotyping

| Name | Forward primer (5’-3’) | Reverse primer (5’-3’) | Size (bp) |
|---|---|---|---|
| Wild-type | GTTCGTCCACAGTGGCCAAGTTG | GGGTTGCTGTGGTTGACTGTAAGGT | 400 |
| Knockout | GCTCGGATGCAACATTCAGCG | GGGTTGCTGTGGTTGACTGTAAGGT | 433 |

### Table S5. Sequence of morpholinos used in this study

| Name | Oligo (5'-3') |
|---|---|
| *dact2* MO TB | TCAGACGTGCCGTTTAGACATATCC |
| *dact2* MO SP | AATCTCTTTTGATTTCTTACCAGCT |
| *extl2* MO TB | CAGCACAAGTGAACCCTCATGGTAT |
| *extl2* MO SP | CCAATCGTCCCGTACCTACCATCAG |
| std MO | CCTCTTACCTCAGTTACAATTTATA |

**Table S6. Single heterozygous variants in recessive known RP genes in the four probands**

| Proband ID | Chromosome position | Gene | Nucleotide and amino acid change | Reference transcript |
|---|---|---|---|---|
| ZOCRP0009 | chr02: 27695182 | *IFT172* | c.1459C>T (p.Arg487Cys) | NM_015662 |
| ZOCRP0009 | chr04: 187115722 | *CYP4V2* | c.283G>A (p.Gly95Arg) | NM_207352 |
| ZOCRP0009 | chr08: 10480459 | *RP1L1* | c.253C>T (p.Arg85Trp) | NM_178857 |
| ZOCRP0156 | chr02: 234217866 | *SAG* | c.31G>T (p.Glu11*) | NM_000541 |
| ZOCRP0156 | chr05: 149264115 | *PDE6A* | c.1954C>T (p.Arg652Cys) | NM_000440 |
| ZOCRP0559 | chr11: 46724722 | *ZNF408* | c.581_592del (p.Val194_Val197del) | NM_024741 |
| ZOCRP0830 | chr04: 47945255 | *CNGA1* | c.392A>T (p.Lys131Met) | NM_000087 |

**Table S7. Homozygous or compound heterozygous rare variants for the four probands with RP**

| Patient ID | Chr | Position | Gene | Reference Transcript | Nucleotide Change | Amino Acid Change | State | CADD | REVEL | SIFT Score | PolyPhen-2 Score | Allele Frequency in gnomAD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | All | EA | Homo |
| **ZOCRP0009** | **chr01** | **150599938** | ***ENSA*** | **NM_207168** | **c.188delG** | **p.Gly64Alafs*2** | **Homo** | **NA** | **NA** | **NA** | **NA** | **1/251460** | **1/18394** | **0** |
| ZOCRP0156 | chr01 | 39781318 | *MACF1* | NM_012090 | c.3419A>C | p.Glu1140Ala | Homo | 24.3 | 0.384 | D (0.012) | B (0.07) | 2/282722 | 2/19948 | 0 |
| ZOCRP0156 | chr01 | 55078220 | *FAM151A* | NM_176782 | c.739C>T | p.Arg247Trp | Homo | 24.7 | 0.269 | D (0.001) | D (0.938) | 2/251424 | 0/18394 | 0 |
| **ZOCRP0156** | **chr01** | **101343065** | ***EXTL2*** | **NM_001439** | **c.400C>T** | **p.Arg134*** | **Homo** | **36** | **NA** | **NA** | **NA** | **3/247450** | **3/18342** | **0** |
| ZOCRP0156 | chr06 | 159654785 | *FNDC1* | NM_032532 | c.3241G>C | p.Asp1081His | Homo | 13.23 | 0.062 | T (0.072) | B (0.008) | 2/142354 | 2/10646 | 0 |
| **ZOCRP0156** | **chr06** | **168710973** | ***DACT2*** | **NM_214462** | **c.533C>A** | **p.Ser178*** | **Homo** | **40** | **NA** | **NA** | **NA** | **NA** | **NA** | **NA** |
| ZOCRP0156 | chr06 | 170181532 | *ERMARD* | NM_018341 | c.1960A>G | p.Lys654Glu | Homo | 22.3 | 0.171 | D (0.006) | B (0.442) | NA | NA | NA |
| ZOCRP0156 | chr07 | 64168594 | *ZNF107* | NM_016220 | c.1912T>C | p.Cys638Arg | Homo | 24.1 | 0.343 | D (0.009) | D (1.00) | 19/281180 | 15/19924 | 0 |
| ZOCRP0156 | chr07 | 150711145 | *NOS3* | NM_000603 | c.3500A>G | p.Gln1167Arg | Homo | 24.6 | 0.125 | T (0.387) | B (0.064) | NA | NA | NA |
| ZOCRP0156 | chr08 | 22438199 | *PDLIM2* | NM_021630 | c.832A>C | p.Met278Leu | Het | 17.16 | 0.071 | T (0.3) | B (0.002) | NA | NA | NA |
| ZOCRP0156 | chr08 | 22451269 | *PDLIM2* | NM_021630 | c.1655A>C | p.Glu552Ala | Het | 27.6 | 0.660 | D (0.004) | P (0.616) | NA | NA | NA |
| ZOCRP0156 | chr21 | 46193528 | *UBE2G2* | NM_003343 | c.319G>A | p.Ala107Thr | Homo | 22.3 | 0.070 | T (0.048) | B (0.002) | 4/249640 | 0/18380 | 0 |
| ZOCRP0559 | chr04 | 126240633 | *FAT4* | NM_024582 | c.3067A>G | p.Lys1023Glu | Homo | 12.71 | 0.108 | T (0.97) | B (0.155) | 16/280774 | 16/19538 | 0 |
| ZOCRP0559 | chr05 | 122682368 | *CEP120* | NM_153223 | c.2806G>A | p.Val936Ile | Het | 9.204 | 0.051 | T (0.457) | B (0.006) | 14/250910 | 4/18386 | 0 |
| ZOCRP0559 | chr05 | 122728995 | *CEP120* | NM_153223 | c.809A>C | p.Gln270Pro | Het | 33 | 0.365 | D (0.02) | D (0.996) | NA | NA | NA |
| ZOCRP0559 | chr09 | 140006355 | *DPP7* | NM_013379 | c.1177G>A | p.Asp393Asn | Het | 15.77 | 0.077 | T (0.74) | B (0.003) | 68/279644 | 0/19900 | 0 |
| ZOCRP0559 | chr09 | 140007703 | *DPP7* | NM_013379 | c.658G>A | p.Val220Met | Het | 25.2 | 0.281 | D (0.002) | D (0.945) | 1/250990 | 0/18388 | 0 |
| ZOCRP0559 | chr12 | 4383270 | *CCND2* | NM_001759 | c.64C>G | p.Arg22Gly | Homo | 15.58 | 0.023 | T (0.609) | B (0) | 6/281848 | 6/19910 | 0 |
| ZOCRP0559 | chr15 | 75653510 | *MAN2C1* | NM_006715 | c.1337A>G | p.Asn446Ser | Homo | 24 | 0.517 | T (0.166) | D (0.912) | 6/278844 | 6/19818 | 0 |
| **ZOCRP0559** | **chr22** | **20136745** | ***CCDC188*** | **NM_001243537** | **c.163C>T** | **p.Arg55*** | **Homo** | **40** | **NA** | **NA** | **NA** | **NA** | **NA** | **NA** |
| ZOCRP0830 | chr06 | 17628900 | *NUP153* | NM_001278209 | c.3623C>G | p.Thr1208Ser | Homo | 22.4 | 0.112 | T (0.083) | B (0.384) | NA | NA | NA |
| **ZOCRP0830** | **chr06** | **30864445** | ***DDR1*** | **NM_001202523** | **c.1616dupT** | **p.Pro540Alafs*15** | **Homo** | **NA** | **NA** | **NA** | **NA** | **NA** | **NA** | **NA** |
| ZOCRP0830 | chr06 | 35747143 | *CLPSL2* | NM_001286550 | c.298T>C | p.Tyr100His | Homo | 5.961 | 0.056 | D (0) | B (0) | 3/202712 | 2/15228 | 0 |
| ZOCRP0830 | chr10 | 104210698 | *C10orf95* | NM_024886 | c.290G>C | p.Ser97Thr | Homo | 13.86 | 0.018 | D (0) | B (0.069) | NA | NA | NA |
| ZOCRP0830 | chr16 | 87742876 | *KLHDC4* | NM_017566 | c.1442A>C | p.Asp481Ala | Het | 26.7 | 0.412 | D (0.033) | P (0.835) | 2/247224 | 2/18316 | 0 |
| ZOCRP0830 | chr16 | 87743067 | *KLHDC4* | NM_017566 | c.1251C>A | p.Asp417Glu | Het | 0.795 | 0.096 | T (0.492) | B (0.01) | 2/248020 | 2/18268 | 0 |

Notes: Chr, Chromosome; EA, East Asian; gnomAD, genome aggregation database; Homo, Homozygousity; Het, Heterozygousity; T, Tolerant; B, Benign; D, Probably Damaging; PD,

Possibly Damaging; NA, not available. All these variants were not present in Exome Variant Server and 1000 Genomes.

**Figure S1**

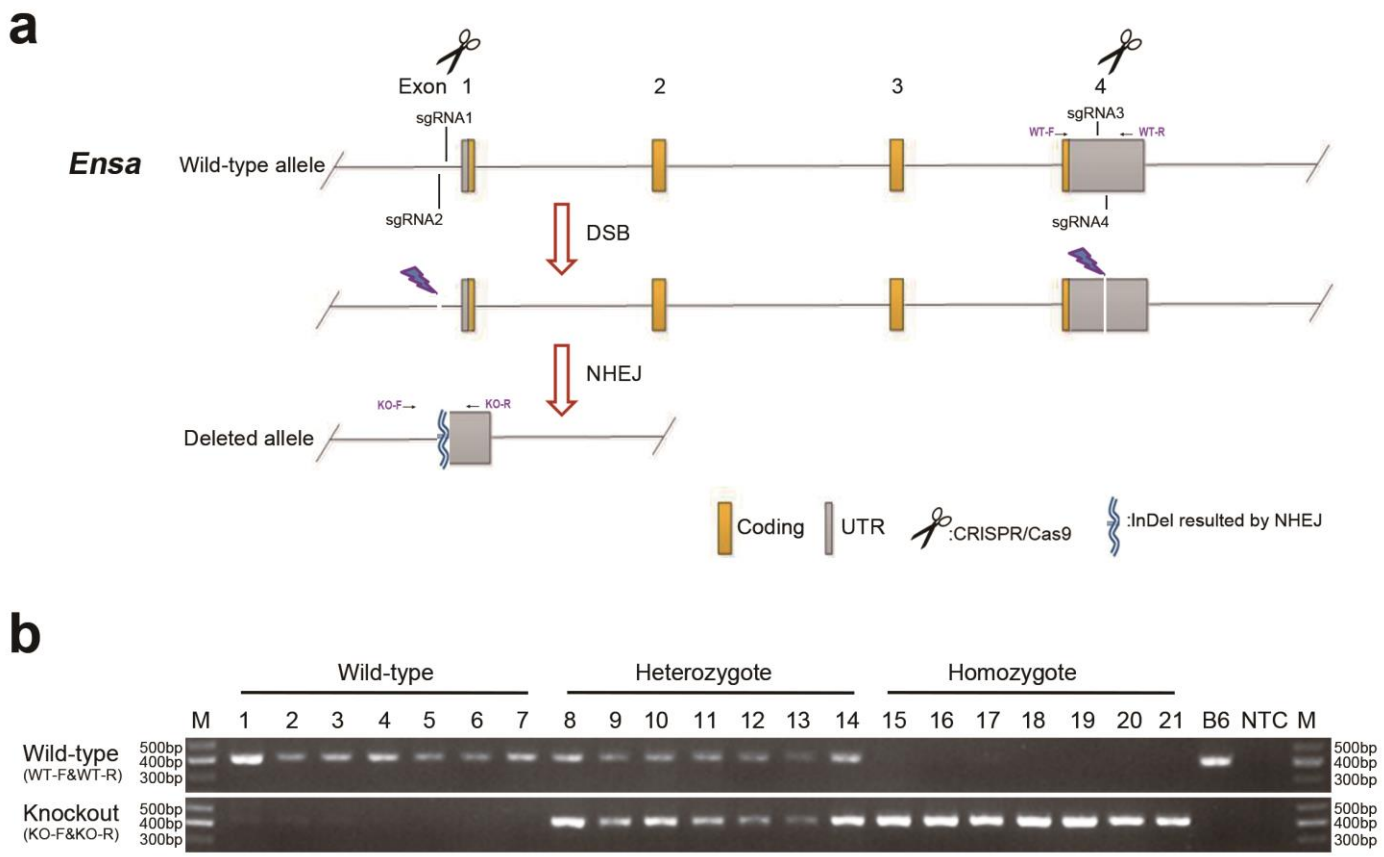


**Fig. S1. Graphical display of the mouse knockout strategy and gel pictures of genotyping**

(a) Schematic diagram of the strategy used for knockout of *Ensa* in mice. DSB, double-strand break; KO, knockout; NHEJ, non-homologous end joining; sgRNA, single-guide RNA; WT, wild-type; UTR, untranslated region.

(b) Gel pictures of the wild-type, heterozygous, and homozygous mice. B6, C57BL/6; M, marker; NTC, no template control. B6, negative control in which genomic DNA from C57BL/6J mice is used as the template. NTC, blank control without template.

**Figure S2**

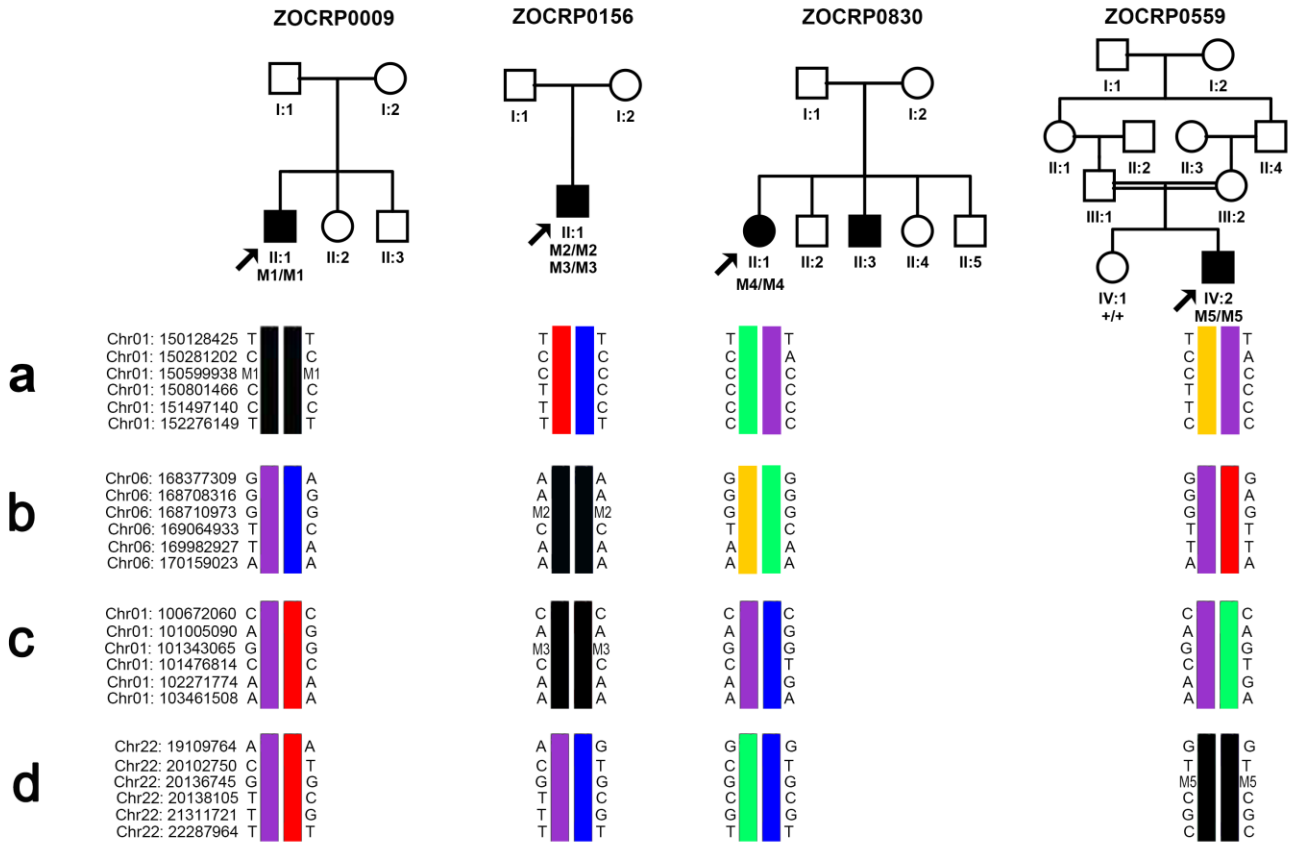


**Fig. S2. Local SNP Haplotypes of Family ZOCRP0009, Family ZOCRP0156, Family ZOCRP0830, and Family ZOCRP0559 according to the whole exome sequencing data**

(a) Local SNP Haplotypes in area around M1 indicate a homozygous region spans approximately 2148kb in the proband from Family ZOCRP0009. M1, the c.191delG (p.Gly64Alafs*2) mutation in *ENSA*.

(b) Local SNP Haplotypes in area around M2 indicate a homozygous region spans approximately 1782kb in the proband from Family ZOCRP0156. M2, the c.533C>A (p.Ser178*) mutation in *DACT2*.

(c) Local SNP Haplotypes in area around M3 indicate a homozygous region spans approximately 2789kb in the proband from Family ZOCRP0156. M3, the c.400C>T (p.Arg134*) mutation in *EXTL2*.

(d) Local SNP Haplotypes in area around M5 indicate a homozygous region spans approximately 3178kb in the proband from Family ZOCRP0559. M5, the c.163C>T (p.Arg55*) mutation in *CCDC188*.

**Figure S3**

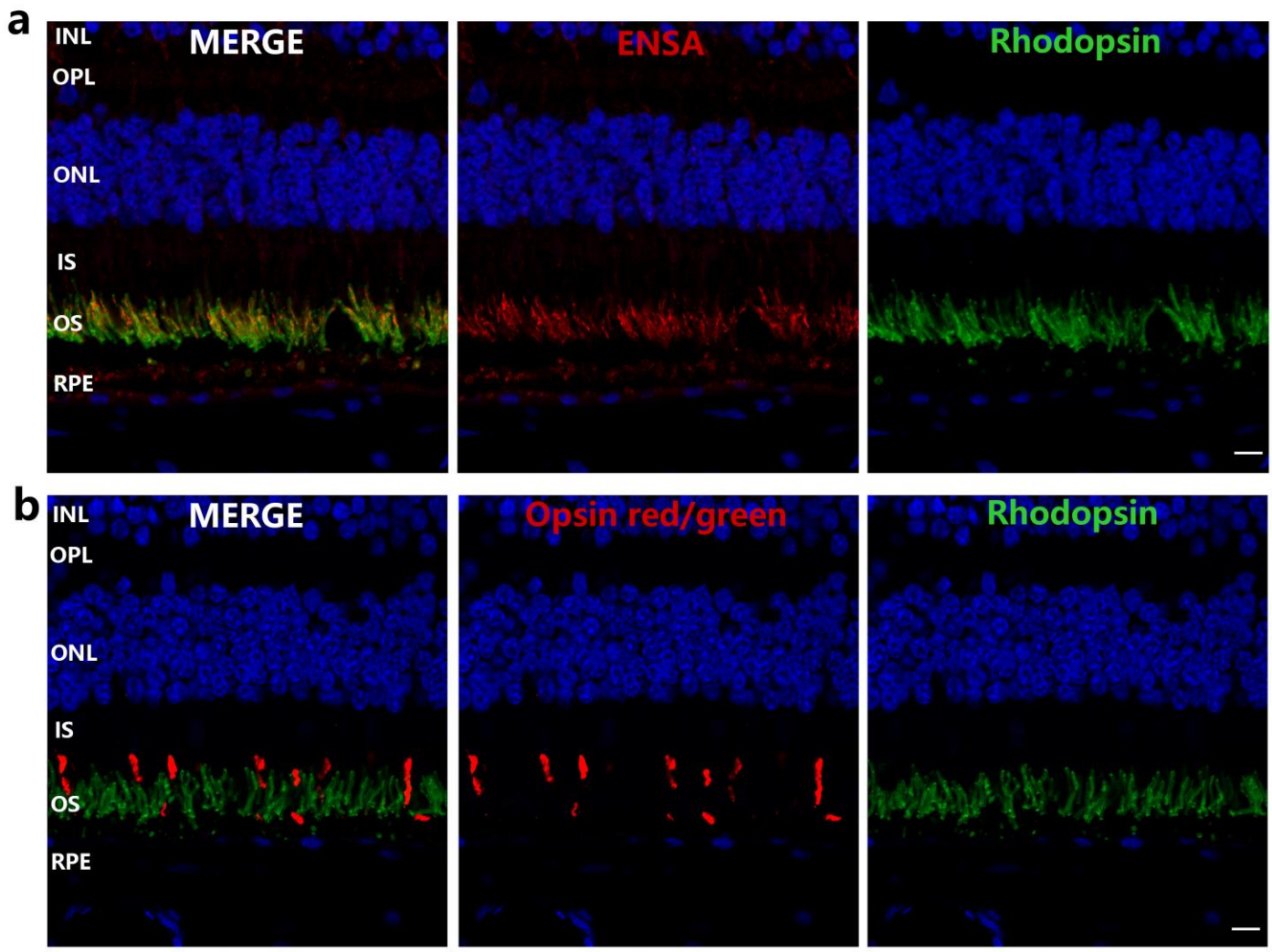


**Fig. S3. Immunostaining for ENSA as well as rod- and cone-specific markers in the human retina**

(a) Staining of ENSA with rhodopsin (rod-specific marker). All of the staining of ENSA was seen to be involved in the staining of rhodopsin in the outer segment of photoreceptors.

(b) Staining of rhodopsin with opsin red/green (cone-specific marker). No overlapping region was detected between the staining of the cone-specific red/green opsin and the rhodopsin.

The staining of rhodopsin indicates the location of rod outer segments. The staining of opsin red/green indicates the location of cone outer segments. INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segments of photoreceptors; OS, outer segments of photoreceptors; RPE, retinal pigment epithelium layer. All the scale bars represent 10 μm.

**Figure S4**

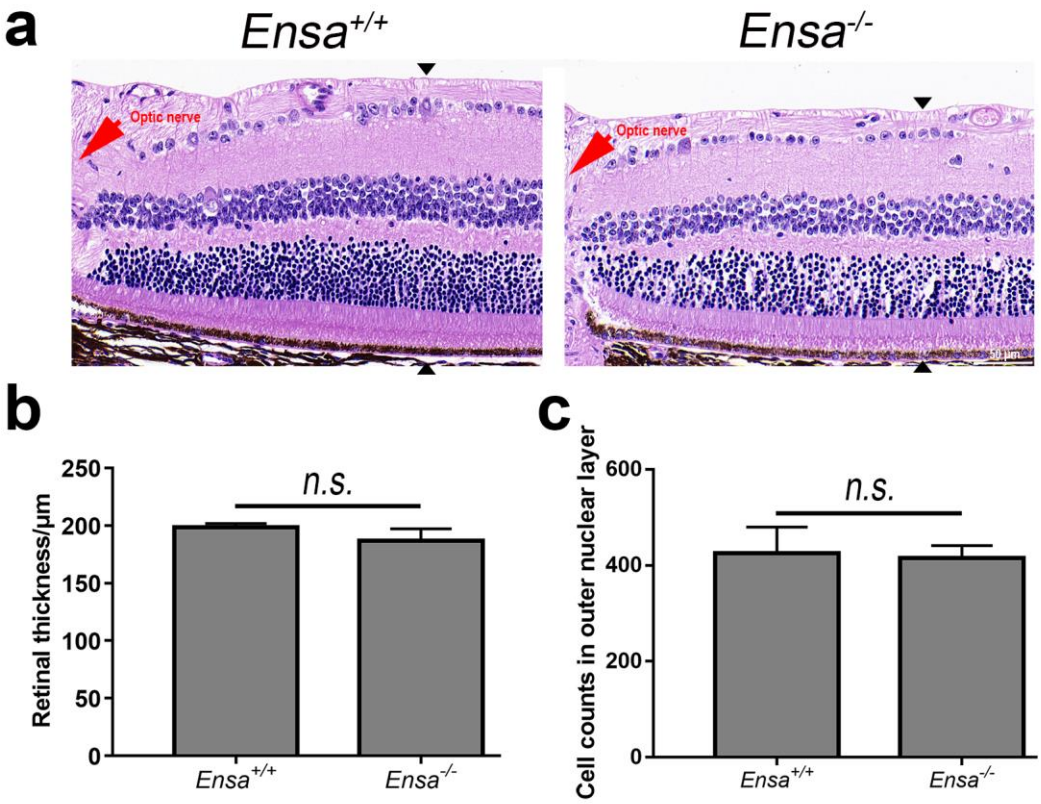


**Fig. S4. Haematoxylin & eosin staining in the retinas from 10-month-old *Ensa*$^{+/+}$ and *Ensa*$^{-/-}$ mice**

(a) Although the overall structure of the retina is preserved, histological sections show a thinner retina in *Ensa*$^{-/-}$ mice compared with *Ensa*$^{+/+}$ mice. In addition, staining revealed a decreased cell density in the outer and inner nuclear layers, and the photoreceptor layer in *Ensa*$^{-/-}$ mice compared with *Ensa*$^{+/+}$ mice. Each retinal section shows the optic nerve as an internal reference. Scale bar, 50 µm.

(b) Retinal thickness (black arrows in A) 280 µm superior to the optic disc (red arrows in A) of *Ensa*$^{+/+}$ and *Ensa*$^{-/-}$ mice. The values are shown as the means ± SEMs (n = 3). Two-tailed Student's t-test, *n.s.*, no significance. SEMs, standard errors of the means.

(c) Cell counts in the outer nuclear layer (from the optic disc [red arrows in A] to 280 µm superior to this disc) of the *Ensa*$^{+/+}$ and *Ensa*$^{-/-}$ mice. The values are shown as the means ± SEMs (n = 3). Two-tailed Student's t-test, *n.s.*, no significance. SEMs, standard errors of the means.

**Figure S5**

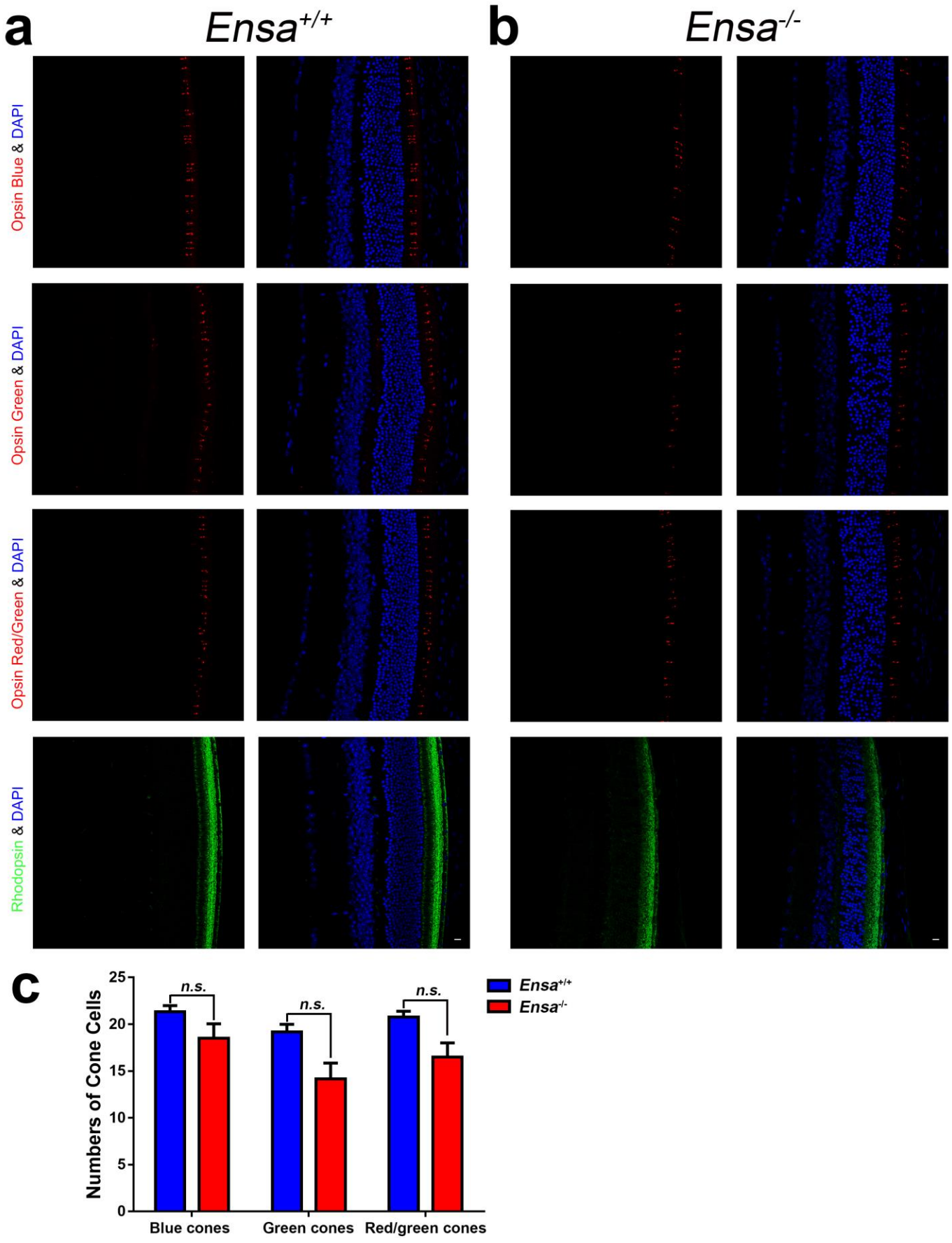


**Fig. S5. Expression of photoreceptor-specific proteins in the retinas of 10-month-old *Ensa*$^{+/+}$ and *Ensa*$^{-/-}$ mice**

(a) Images of stained sections of *Ensa*$^{+/+}$ mice, showing the normal distribution of cones and rods. Scale bar represents 10 μm.

(b) Images of stained sections of *Ensa*$^{-/-}$ mice, showing reduced cone numbers compared with those found in sections of *Ensa*$^{+/+}$ mice. Scale bar represents 10 μm.
(c) Numbers of blue, green and red/green cones in *Ensa*$^{+/+}$ and *Ensa*$^{-/-}$ mice. The area size used for quantification is 320 μm × 320 μm, which corresponds to the coverage of each picture in a and b. The values are shown as the means ± SEMs (n = 4). Two-tailed Student's t-test, *n.s.*, no significance. SEMs, standard errors of the means.

**Figure S6**

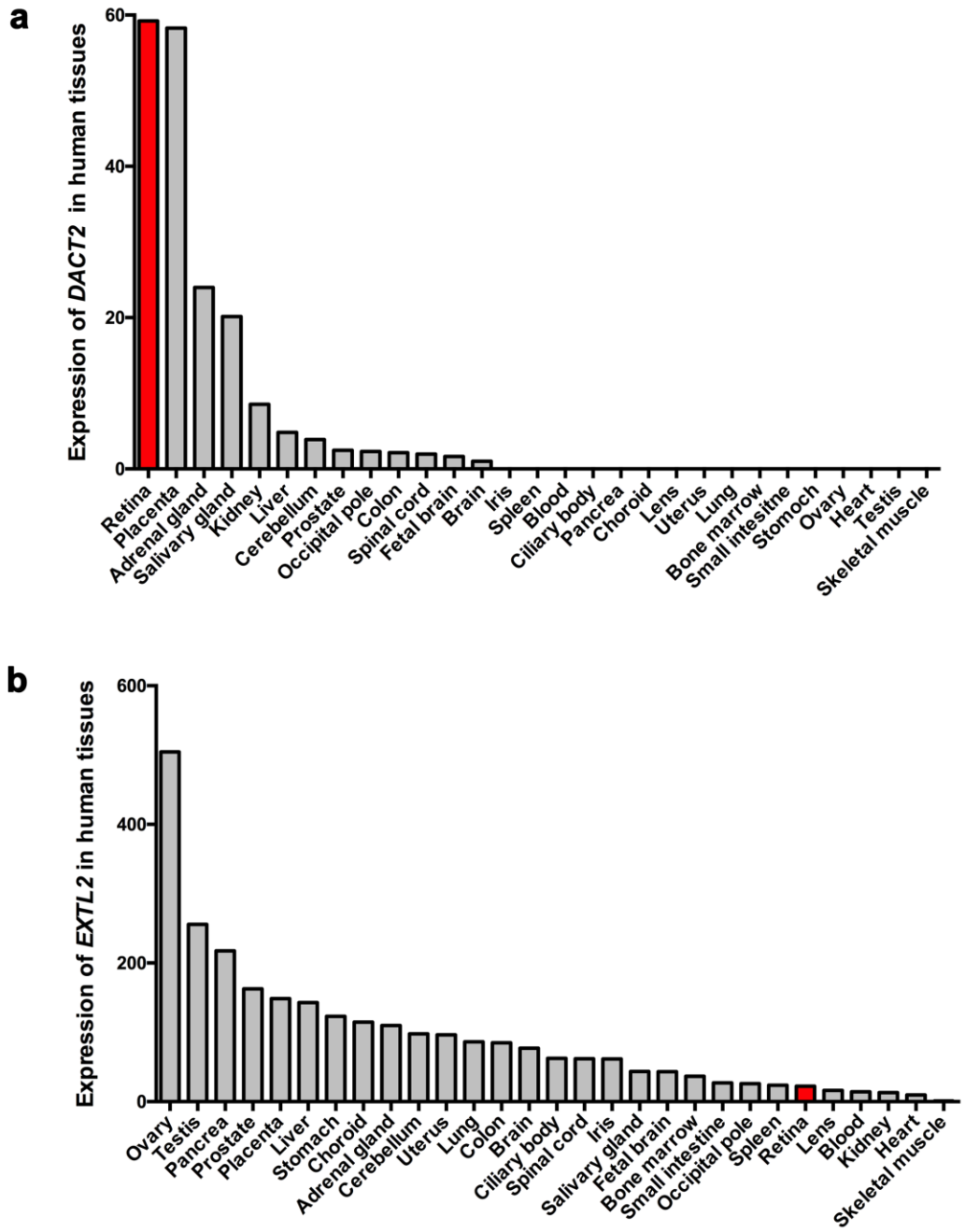


**Fig. S6. Analysis of the expression profile of *DACT2* and *EXTL2* in different human tissues by RT-qPCR**

A RT-qPCR analysis of 32 human tissues revealed that the highest expression of *DACT2* was found in the retina (a), whereas *EXTL2* was expressed at a relatively low level in the retina (b). *GAPDH* was used as the normalizing gene.

**Figure S7**

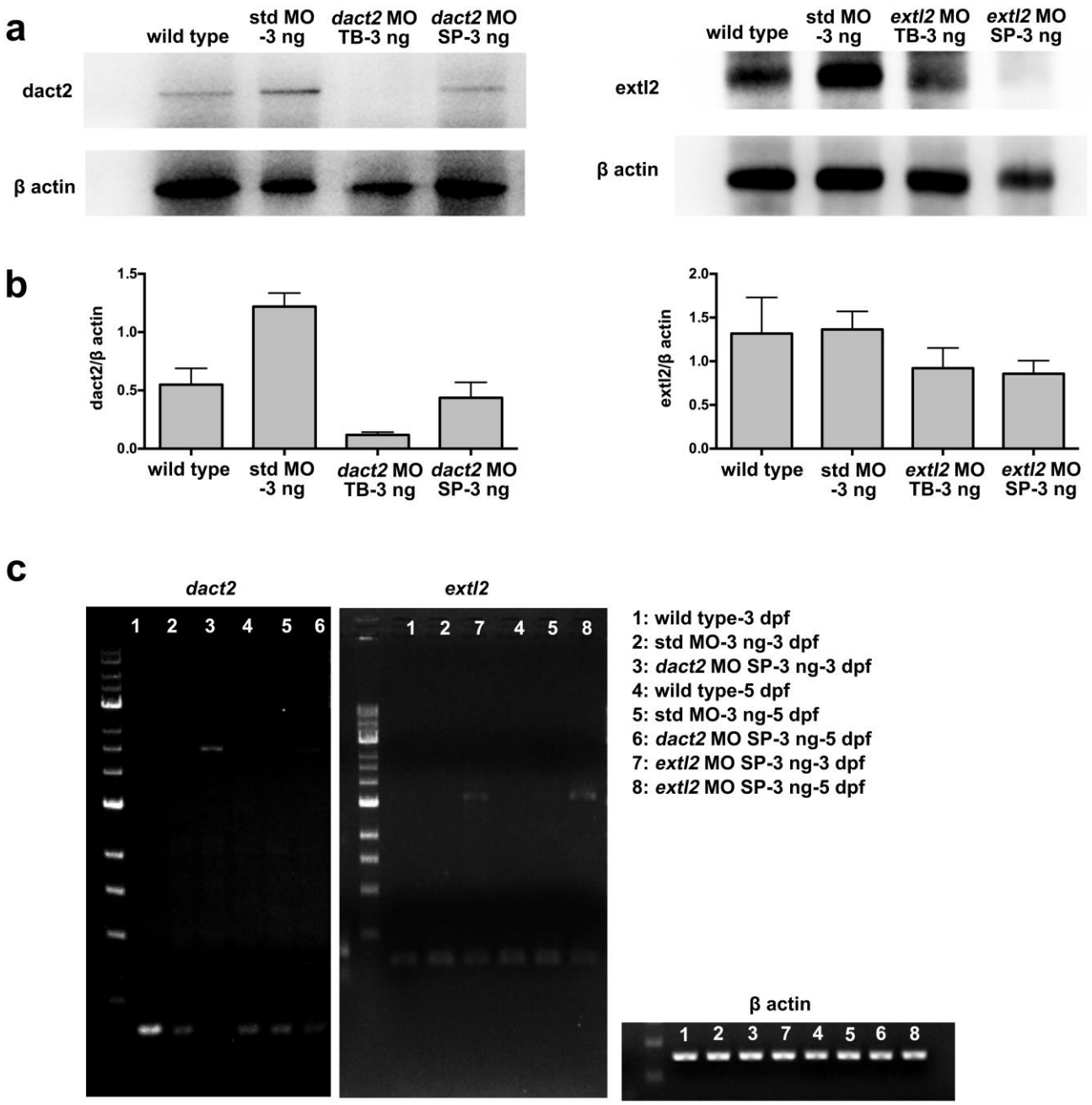


**Fig S7. Efficacy test in *dact2* MO TB, *dact2* MO SP, *extl2* MO TB and *extl2* MO SP**

(a) Protein level test of *dact2* and *extl2* at 24 hpf using western blot. Left: protein level of dact2 (upper) and β actin as control (below). Right: protein level of extl2 (upper) and β actin as control (below).

(b) The graph of the ratio of dact2 or extl2 to β actin. Left: the graph of the ratio of dact2 to β actin, showing a reduced dact2 protein level in the groups of *dact2* MO TB and *dact2* MO SP. Right: the graph of the ratio of extl2 to β actin, showing a reduced extl2 protein level in the groups of *extl2* MO TB and *extl2* MO SP.

(c) The efficacy test in *dact2* MO SP and *extl2* MO SP at 3 dpf and 5 dpf using RT-PCR. The *dact2* MO SP injection and *extl2* MO SP injection resulted in an intron insertion, respectively.

**Figure S8**

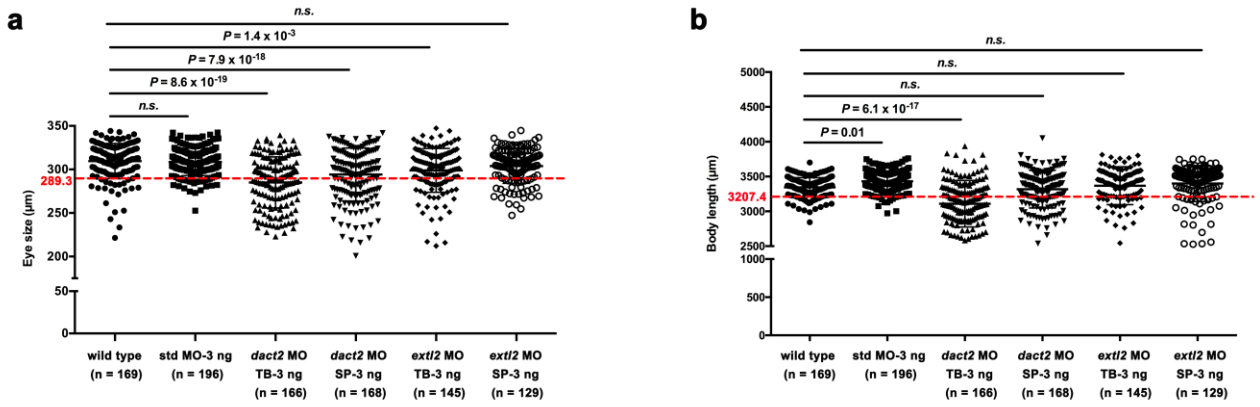


**Fig. S8. Eye size and body length in each larva at 3 dpf**

(a) Eye size in each larva at 3 dpf from six groups, i.e., wild type, std MO-3 ng, *dact2* MO TB-3 ng, *dact2* MO SP-3 ng, *extl2* MO TB-3 ng, and *extl2* MO SP-3 ng. The cut-off for small eye size classification was a diameter of less than 289.3 μm, the mean minus SD for wild type, with diameters greater than 289.3 μm defined as normal. One-Way ANOVA ($P < 0.001$, or 0.05/5, was considered statistically significant), *n.s.*, no significance.

(b) Body length in each larva at 3 dpf from six groups, i.e. wild type, std MO-3 ng, *dact2* MO TB-3 ng, *dact2* MO SP-3 ng, *extl2* MO TB-3 ng, and *extl2* MO SP-3 ng. Body lengths of less than 3207.4 μm, the mean minus SD for wildtype larvae, were classed as short, while the body length greater than 3207.4 μm was defined as normal body length. One-Way ANOVA ($P < 0.001$, or 0.05/5, was considered statistically significant), *n.s.*, no significance.

**Figure S9**

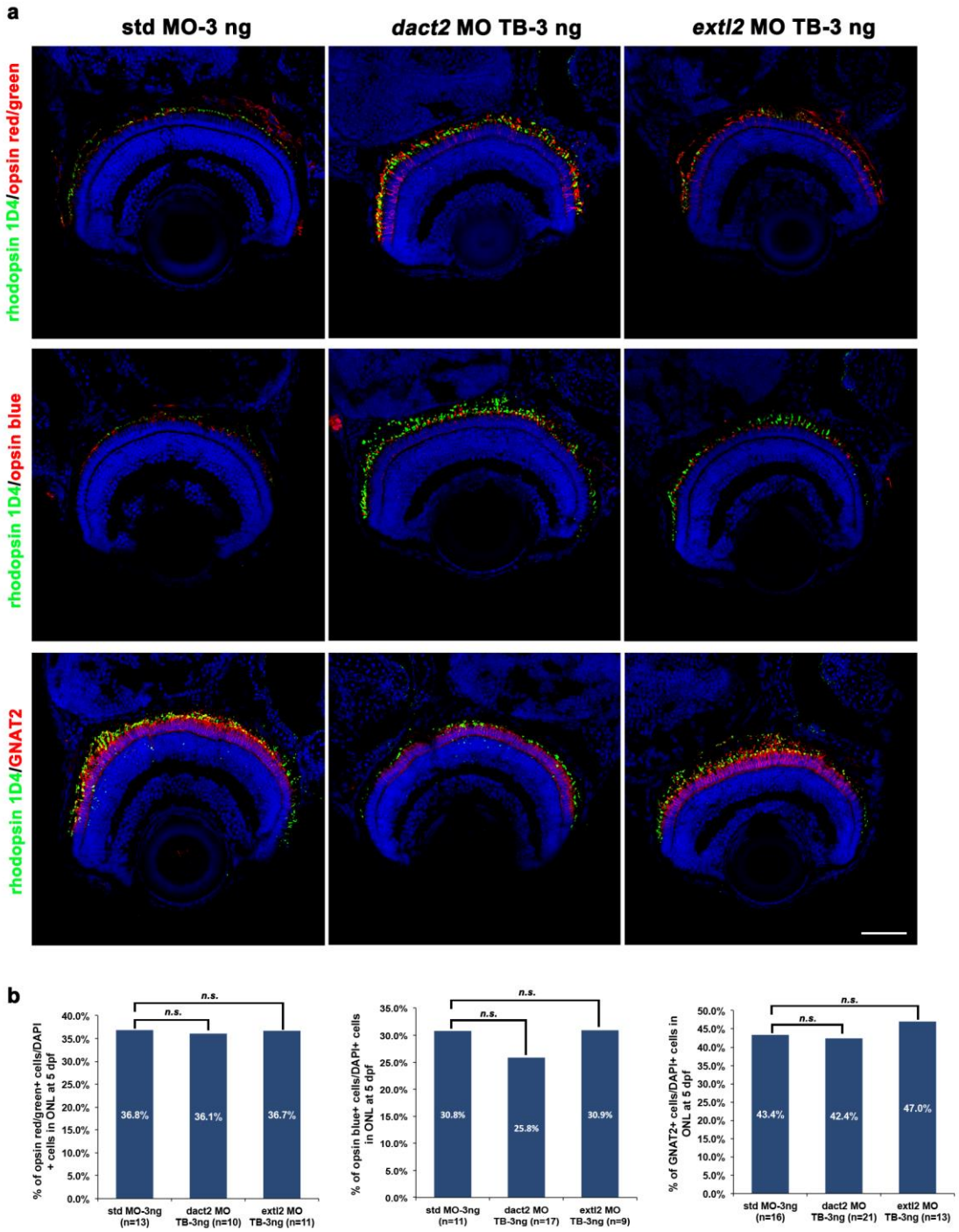


**Fig. S9. Expression of photoreceptor-specific proteins in *dact2* knockdown zebrafish and *extl2* knockdown zebrafish**

(a) Retinal cells developed normally in std MO larvae, *dact2* MO TB morphants, and *extl2* MO TB morphants: co-labelling with anti- rhodopsin 1D4 (for long double-cone outer segments, green) and anti-opsin red/green (for red and green cone cells, red) antibodies, anti- rhodopsin 1D4 (for long double-cone outer segments, green) and anti- opsin blue (for blue cone cells, red) antibodies, anti- rhodopsin 1D4 (for long double-cone outer segments, green) and anti- GNAT2 (for cone transducin, red) antibodies.

(b) The proportions of cone cells with opsin red/green staining, opsin blue staining, and GNAT2 staining in std MO injected larvae, *dact2* MO TB morphants and *extl2* MO TB morphants. The proportion of cone cells with opsin red/green staining, opsin blue staining, and GNAT2 staining did not show significant differences among std MO injected larvae, *dact2* morphant and *extl2* morphant by Chi-square test ($P < 0.025$, or 0.05/2, was considered statistically significant). ONL, outer nuclear layer.