

## Supplementary Materials

### Supplementary tables

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

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
<i>ENSA</i>	AGGACACGCAGGAGAAAAGAA	CACACACCCAAATGCTCCAA	365
<i>DACT2</i>	TTTACGAGATGAGCGACGGT	CCTTCCAGCGTCCCTTAGAG	410
<i>EXTL2</i>	GGTGCTTTGACTGCCTTACT	CCAGACGGTTAAGCAGAAGG	428
<i>DDR1</i>	TTCCTGATGCCTCGTCCTGT	CCTCCTTACCTCCCCAAACT	297
<i>CCDC188</i>	CTGCTGTGCTTACCTCCTCT	CTGCAGCCACTTTTCCGATG	448

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

Name	Species	Forward primer (5'-3')	Reverse primer (5'-3')
<i>ENSA</i> (F1+R1)	Human	TGCCTGAGAGAGCTGAAGAG	TGGCCATGTTGTAGTCTCCT
<i>ENSA</i> (F2+R2)	Human	AAGAAGAGAACCTGCGGAG	GAGACTATGCCCCATACCCC
<i>ACTB</i>	Human	TGGCACACACCTTCTACAA	CCAGAGGCGTACAGGGATAG
<i>Ensa</i> (f1+r1)	Mouse	TGAGAAAGCTGAGGAGGCAA	TCACCAGGTTCTGTCTGCT
<i>Ensa</i> (f2+r2)	Mouse	GAAGAAGAAAACCTGCGGA	CTTCATCCAAGGTGCCACAG
<i>Actb</i>	Mouse	CCTCTATGCCAACACAGTGC	CCTGCTTGCTGATCCACATC
<i>DACT2</i>	Human	CGGTTGATGAGACTACTGTGC	GGGCTCTGTCAAGATCACCT
<i>EXTL2</i>	Human	ACAACAGACAGCAAACAGGA	AAAACAAGGTCTGGGGTGCT
<i>GAPDH</i>	Human	GGACTCATGACCACAGTCCATGCC	TCAGGGATGACCTTGCCCACAG
<i>dact2</i>	zebrafish	CTGAACAGAGACCAGCCAGA	TAGGTGTTCCGGTGGATGCTT
<i>extl2</i>	zebrafish	GGAACAACCCAGGAGAAACG	TGTCATCGTCCAGCATCAA
<i>β actin</i>	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')
<i>dact2</i> MO TB	TCAGACGTGCCGTTTAGACATATCC
<i>dact2</i> MO SP	AATCTCTTTTGATTCTTACCAGCT
<i>extl2</i> MO TB	CAGCACAAGTGAACCCTCATGGTAT
<i>extl2</i> MO SP	CCAATCGTCCCGTACCTACCATCAG
std MO	CCTCTTACCTCAGTTACAATTTATA

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

<b>Proband ID</b>	<b>Chromosome position</b>	<b>Gene</b>	<b>Nucleotide and amino acid change</b>	<b>Reference transcript</b>
ZOCR0009	chr02: 27695182	<i>IFT172</i>	c.1459C>T (p.Arg487Cys)	NM_015662
ZOCR0009	chr04: 187115722	<i>CYP4V2</i>	c.283G>A (p.Gly95Arg)	NM_207352
ZOCR0009	chr08: 10480459	<i>RP1L1</i>	c.253C>T (p.Arg85Trp)	NM_178857
ZOCR0156	chr02: 234217866	<i>SAG</i>	c.31G>T (p.Glu11*)	NM_000541
ZOCR0156	chr05: 149264115	<i>PDE6A</i>	c.1954C>T (p.Arg652Cys)	NM_000440
ZOCR0559	chr11: 46724722	<i>ZNF408</i>	c.581_592del (p.Val194_Val197del)	NM_024741
ZOCR0830	chr04: 47945255	<i>CNGA1</i>	c.392A>T (p.Lys131Met)	NM_000087

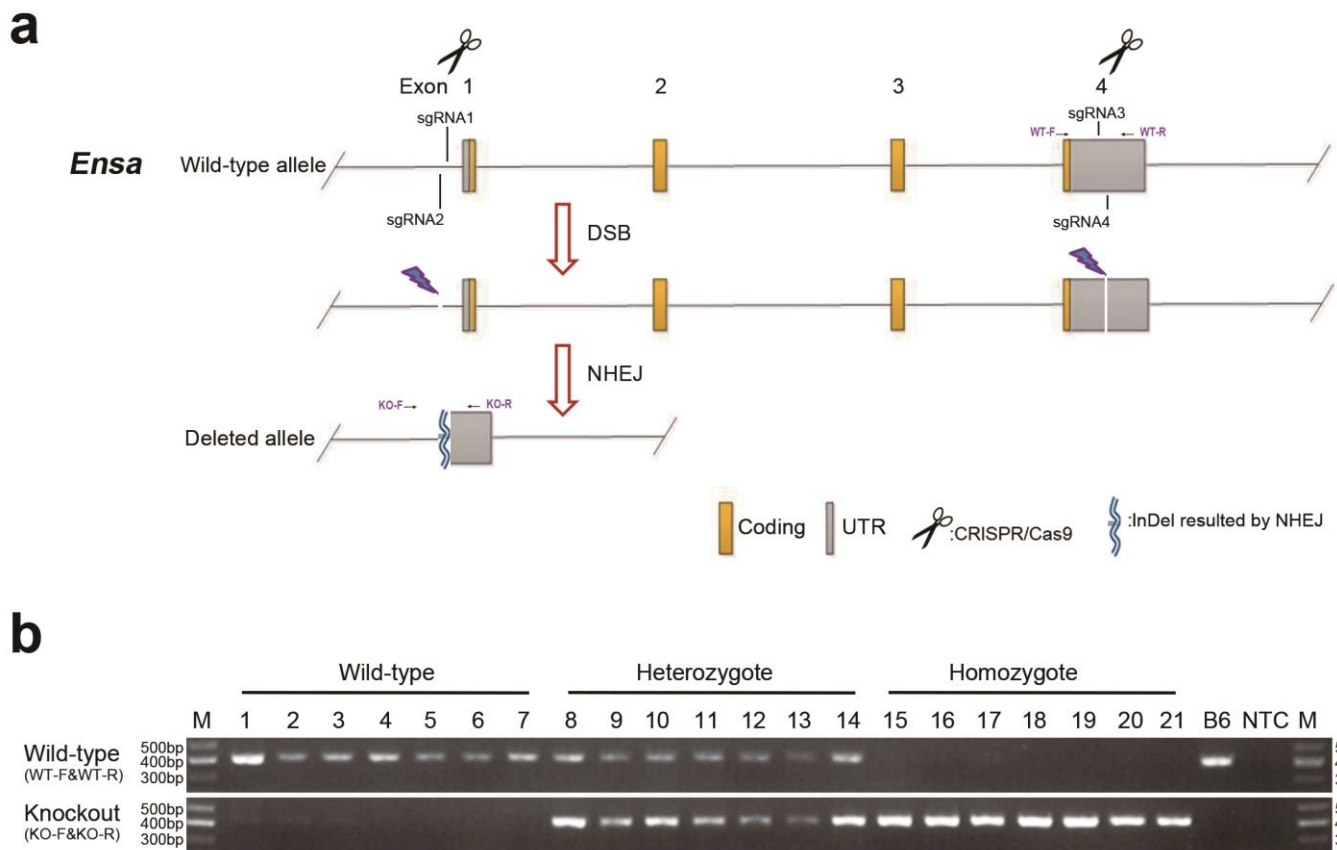
**Table S7. Homozygous or compound heterozygous rare variants for the four probands with RP**

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
<b>ZOCP0009</b>	<b>chr01</b>	<b>150599938</b>	<b>ENSA</b>	<b>NM_207168</b>	<b>c.188delG</b>	<b>p.Gly64Alafs*2</b>	<b>Homo</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>1/251460</b>	<b>1/18394</b>	<b>0</b>
ZOCP0156	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
ZOCP0156	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
<b>ZOCP0156</b>	<b>chr01</b>	<b>101343065</b>	<b>EXTL2</b>	<b>NM_001439</b>	<b>c.400C&gt;T</b>	<b>p.Arg134*</b>	<b>Homo</b>	<b>36</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>3/247450</b>	<b>3/18342</b>	<b>0</b>
ZOCP0156	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
<b>ZOCP0156</b>	<b>chr06</b>	<b>168710973</b>	<b>DACT2</b>	<b>NM_214462</b>	<b>c.533C&gt;A</b>	<b>p.Ser178*</b>	<b>Homo</b>	<b>40</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>
ZOCP0156	chr06	170181532	ERMARD	NM_018341	c.1960A>G	p.Lys654Glu	Homo	22.3	0.171	D (0.006)	B (0.442)	NA	NA	NA
ZOCP0156	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
ZOCP0156	chr07	150711145	NOS3	NM_000603	c.3500A>G	p.Gln1167Arg	Homo	24.6	0.125	T (0.387)	B (0.064)	NA	NA	NA
ZOCP0156	chr08	22438199	PDLIM2	NM_021630	c.832A>C	p.Met278Leu	Het	17.16	0.071	T (0.3)	B (0.002)	NA	NA	NA
ZOCP0156	chr08	22451269	PDLIM2	NM_021630	c.1655A>C	p.Glu552Ala	Het	27.6	0.660	D (0.004)	P (0.616)	NA	NA	NA
ZOCP0156	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
ZOCP0559	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
ZOCP0559	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
ZOCP0559	chr05	122728995	CEP120	NM_153223	c.809A>C	p.Gln270Pro	Het	33	0.365	D (0.02)	D (0.996)	NA	NA	NA
ZOCP0559	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
ZOCP0559	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
ZOCP0559	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
ZOCP0559	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
<b>ZOCP0559</b>	<b>chr22</b>	<b>20136745</b>	<b>CCDC188</b>	<b>NM_001243537</b>	<b>c.937C&gt;T</b>	<b>p.Arg313*</b>	<b>Homo</b>	<b>40</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>16/118792</b>	<b>0/10622</b>	<b>NA</b>
ZOCP0830	chr06	17628900	NUP153	NM_001278209	c.3623C>G	p.Thr1208Ser	Homo	22.4	0.112	T (0.083)	B (0.384)	NA	NA	NA
<b>ZOCP0830</b>	<b>chr06</b>	<b>30864445</b>	<b>DDR1</b>	<b>NM_001202523</b>	<b>c.1616dupT</b>	<b>p.Pro540Alafs*15</b>	<b>Homo</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>
ZOCP0830	chr06	35747143	CLPSL2	NM_001286550	c.298T>C	p.Tyr100His	Homo	5.961	0.056	D (0)	B (0)	3/202712	2/15228	0
ZOCP0830	chr10	104210698	C10orf95	NM_024886	c.290G>C	p.Ser97Thr	Homo	13.86	0.018	D (0)	B (0.069)	NA	NA	NA
ZOCP0830	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
ZOCP0830	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, Homozygosity; Het, Heterozygosity; 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**

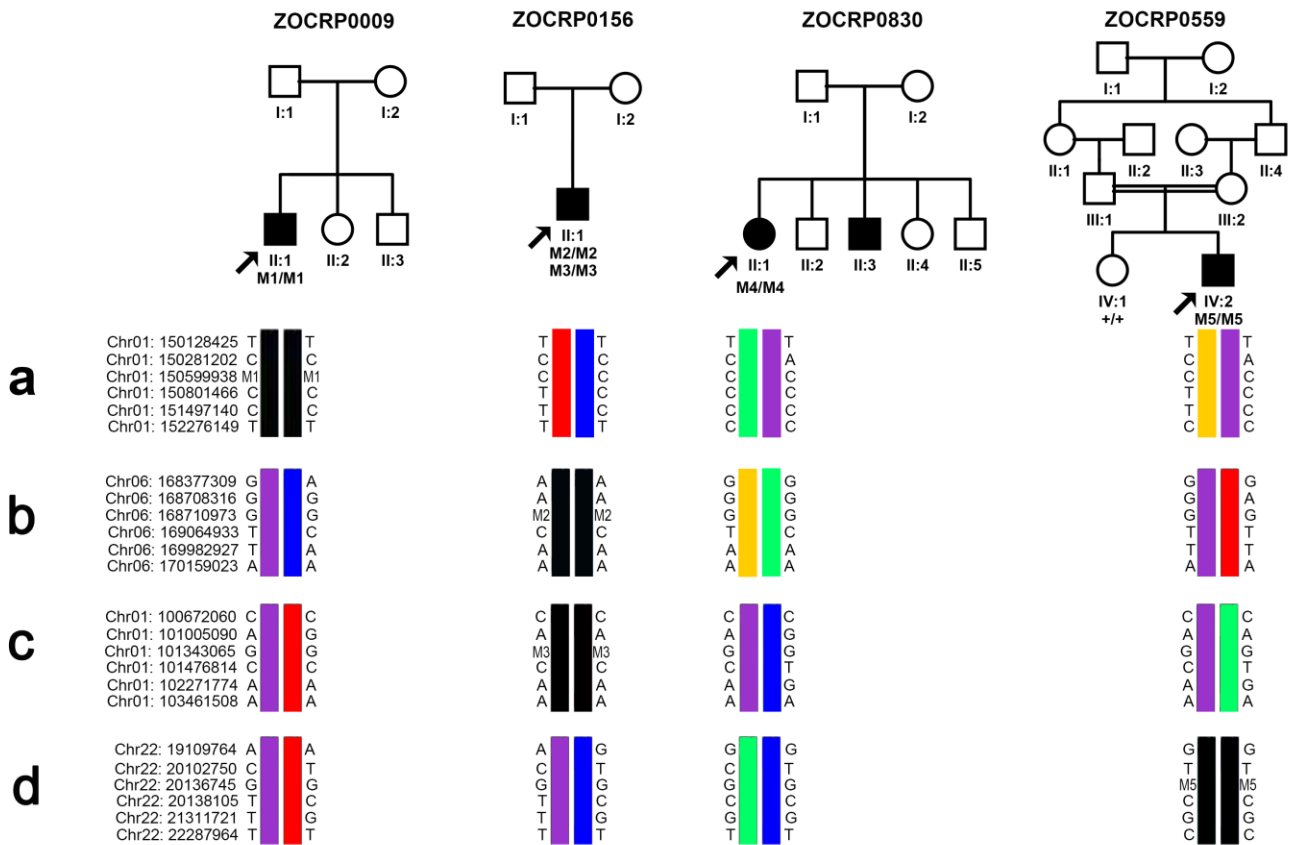


**Fig. S1. Graphical display of the mouse knockout strategy and gel pictures of genotyping**

(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 ZOCRPO009, Family ZOCRPO156, Family ZOCRPO830, and Family ZOCRPO559 according to the whole exome sequencing data**

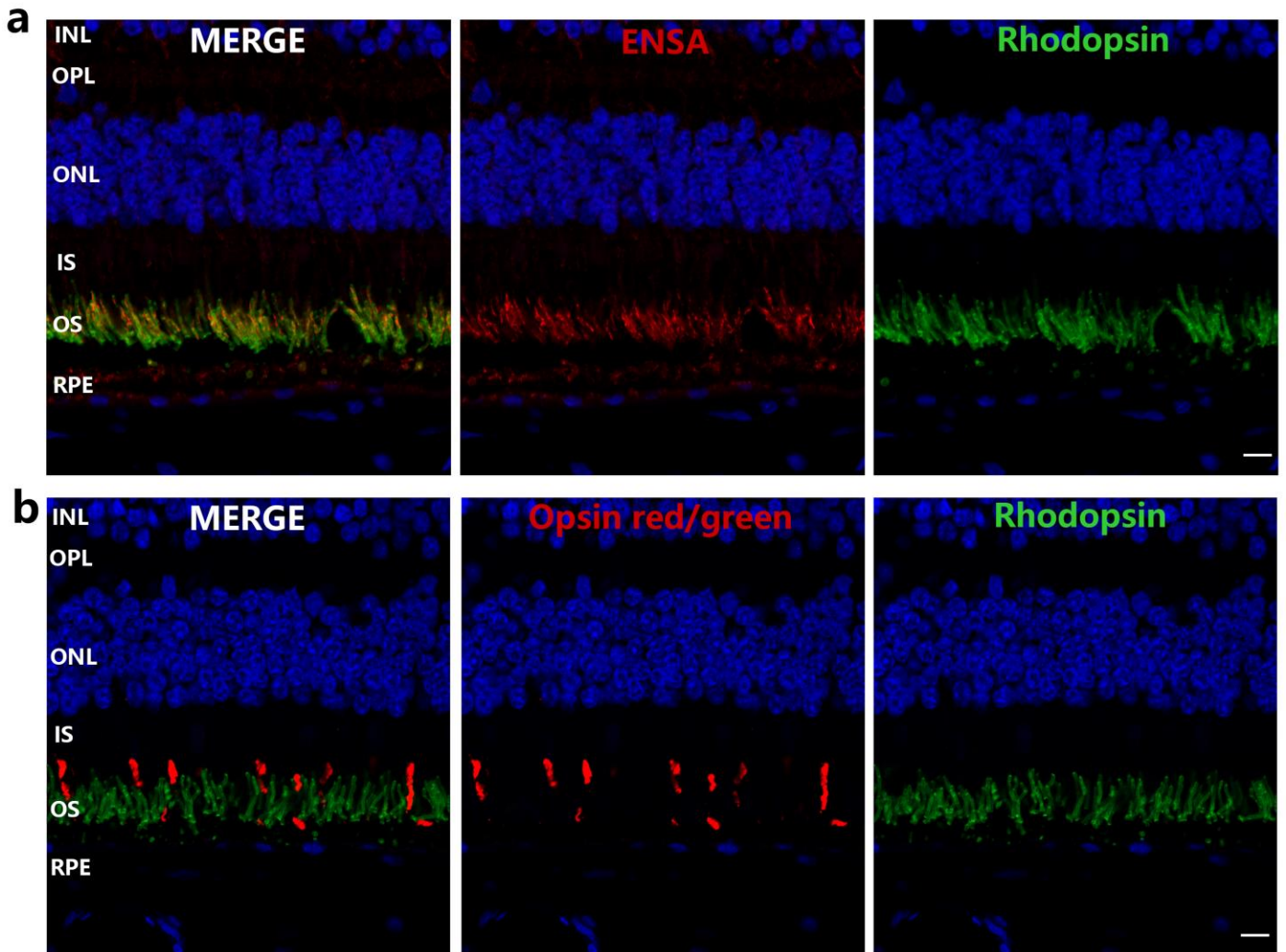
(a) Local SNP Haplotypes in area around M1 indicate a homozygous region spans approximately 2148kb in the proband from Family ZOCRPO009. M1, the c.191delG (p.Gly64Alafs\*2) mutation in *ENSA*.

(b) Local SNP Haplotypes in area around M2 indicate a homozygous region spans approximately 1782kb in the proband from Family ZOCRPO156. 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 ZOCRPO156. 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 ZOCRPO559. M5, the c.937C>T (p.Arg313\*) mutation in *CCDC188*.

Figure S3



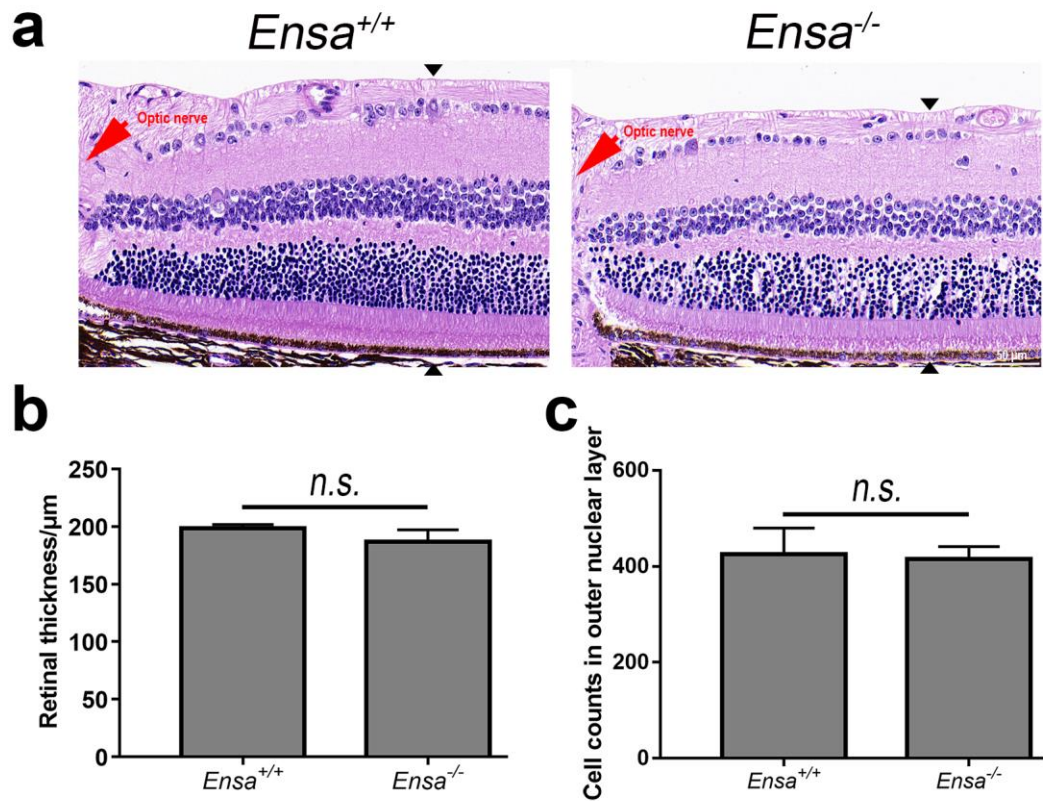
**Fig. S3. Immunostaining for ENSA as well as rod- and cone-specific markers in the human retina**

(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  $\mu\text{m}$ .

Figure S4



**Fig. S4. Haematoxylin & eosin staining in the retinas from 10-month-old *Ensa*<sup>+/+</sup> and *Ensa*<sup>-/-</sup> mice**

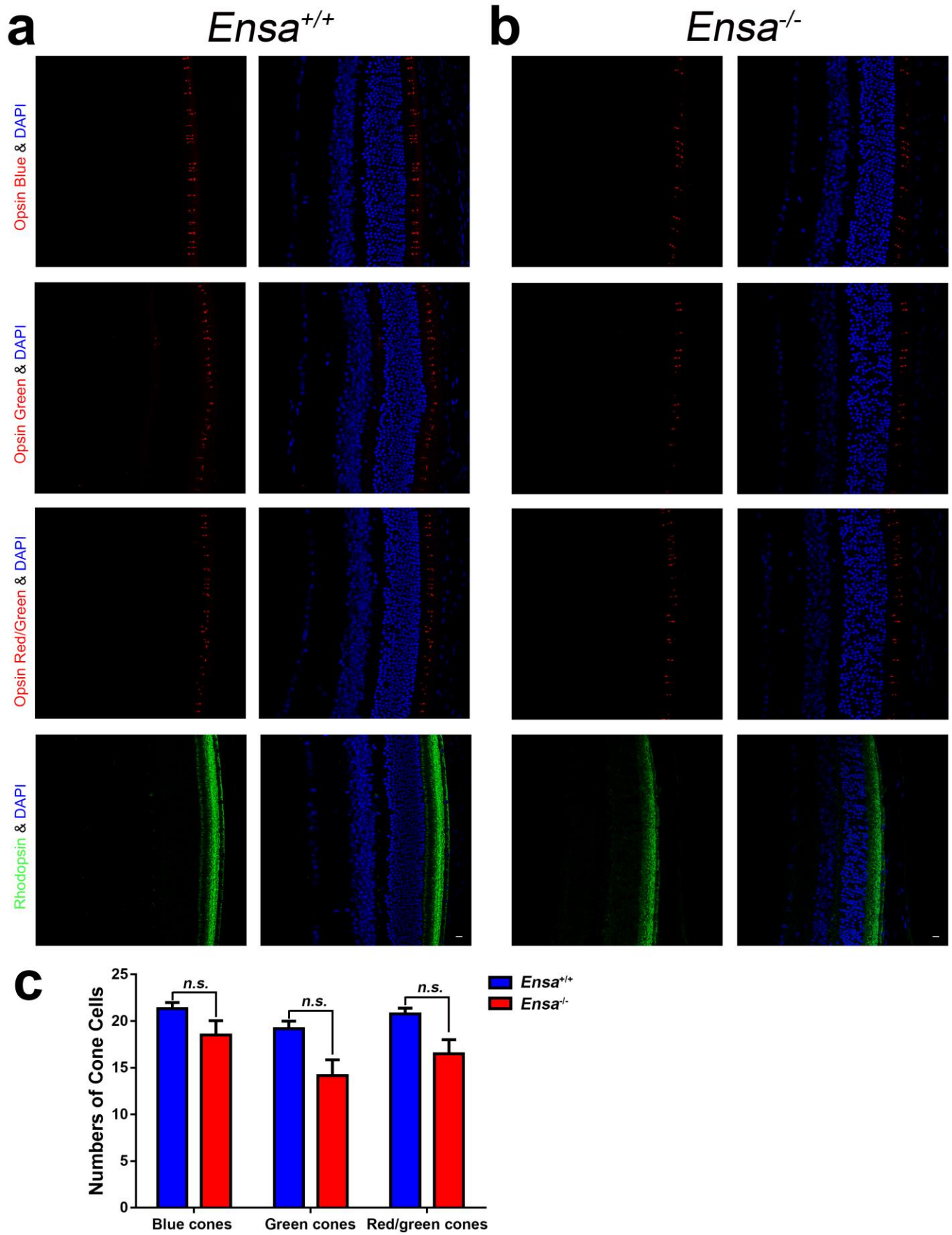
(a) Although the overall structure of the retina is preserved, histological sections show a thinner retina in *Ensa*<sup>-/-</sup> mice compared with *Ensa*<sup>+/+</sup> mice. In addition, staining revealed a decreased cell density in the outer and inner nuclear layers, and the photoreceptor layer in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

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



Figure S5



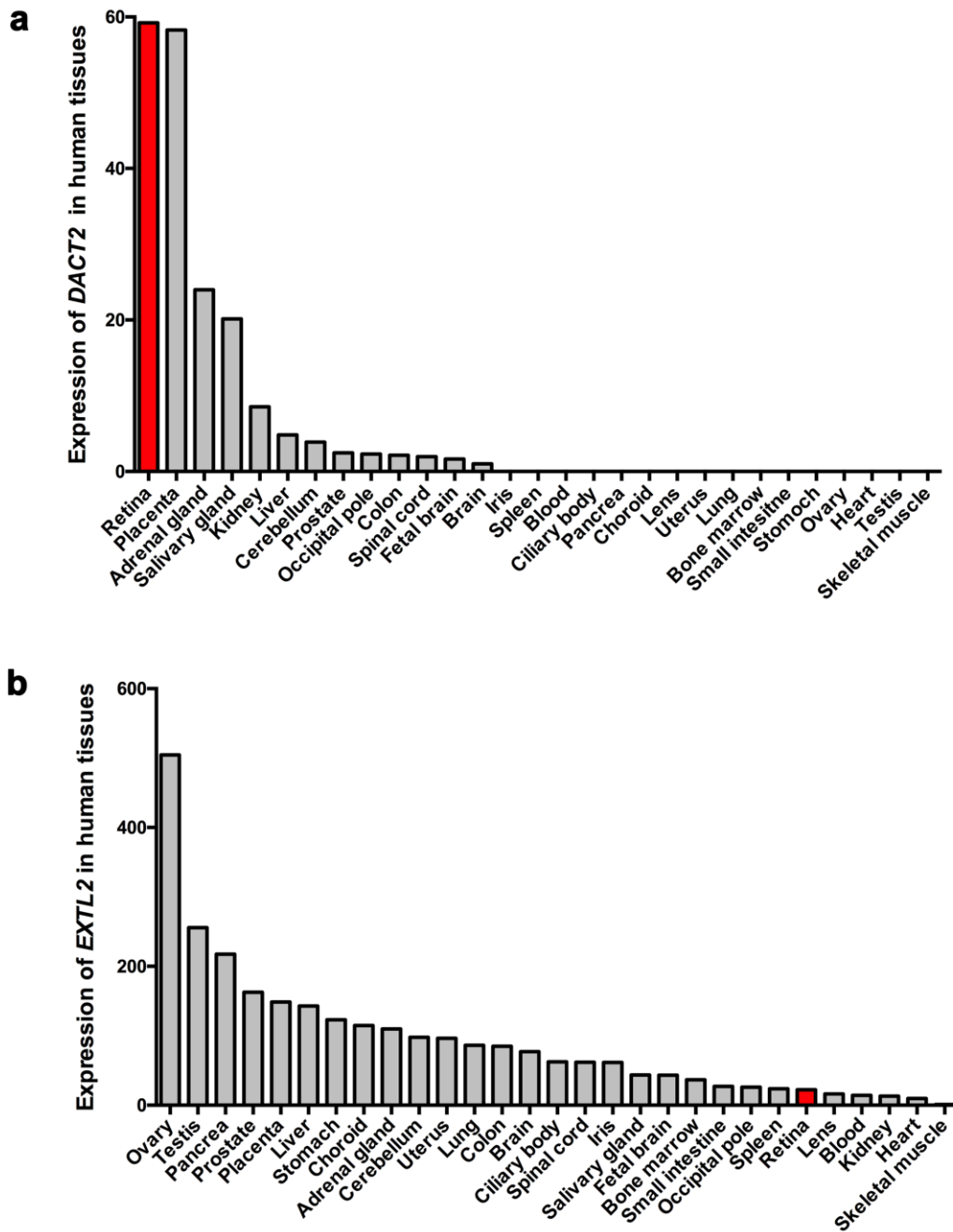
**Fig. S5. Expression of photoreceptor-specific proteins in the retinas of 10-month-old *Ensa*<sup>+/+</sup> and *Ensa*<sup>-/-</sup> mice**

(a) Images of stained sections of *Ensa*<sup>+/+</sup> mice, showing the normal distribution of cones and rods. Scale bar represents 10  $\mu$ m.

(b) Images of stained sections of *Ensa*<sup>-/-</sup> mice, showing reduced cone numbers compared with those found in sections of *Ensa*<sup>+/+</sup> mice. Scale bar represents 10  $\mu\text{m}$ .

(c) Numbers of blue, green and red/green cones in *Ensa*<sup>+/+</sup> and *Ensa*<sup>-/-</sup> mice. The area size used for quantification is 320  $\mu\text{m} \times 320 \mu\text{m}$ , which corresponds to the coverage of each picture in a and b. The values are shown as the means  $\pm$  SEMs (n = 4). Two-tailed Student's t-test, *n.s.*, no significance. SEMs, standard errors of the means.

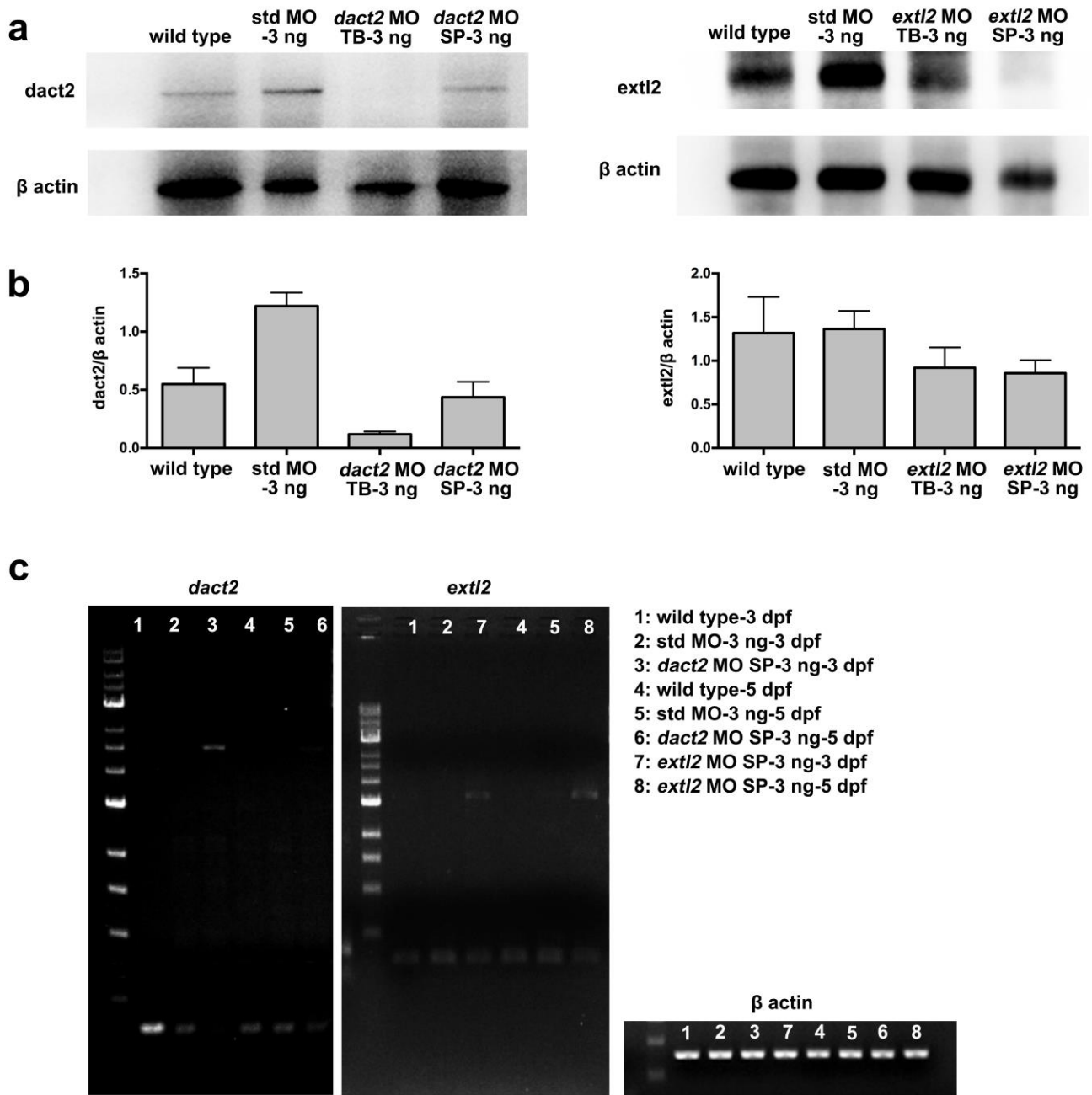
Figure S6



**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



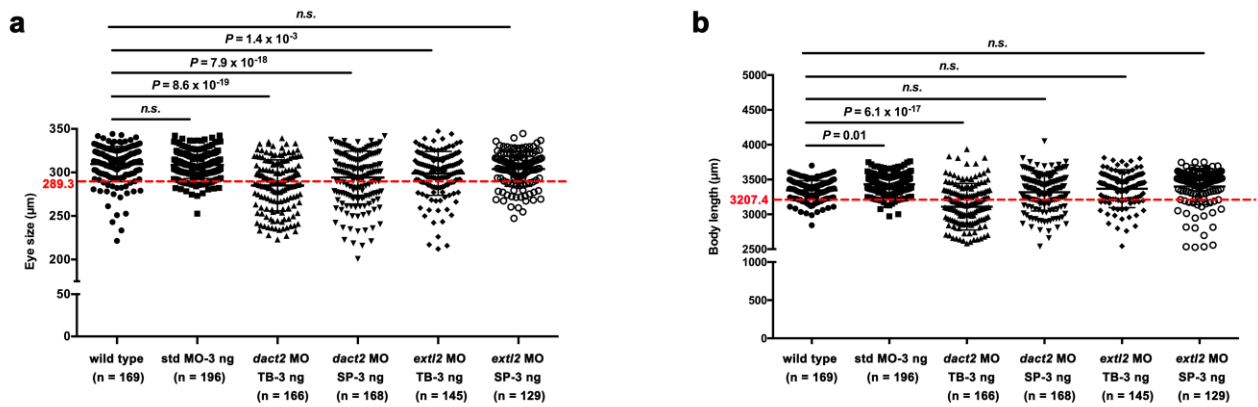
**Fig S7. Efficacy test in *dact2* MO TB, *dact2* MO SP, *extl2* MO TB and *extl2* MO SP**

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

(b) The graph of the ratio of *dact2* or *extl2* to  $\beta$  actin. Left: the graph of the ratio of *dact2* to  $\beta$  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  $\beta$  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

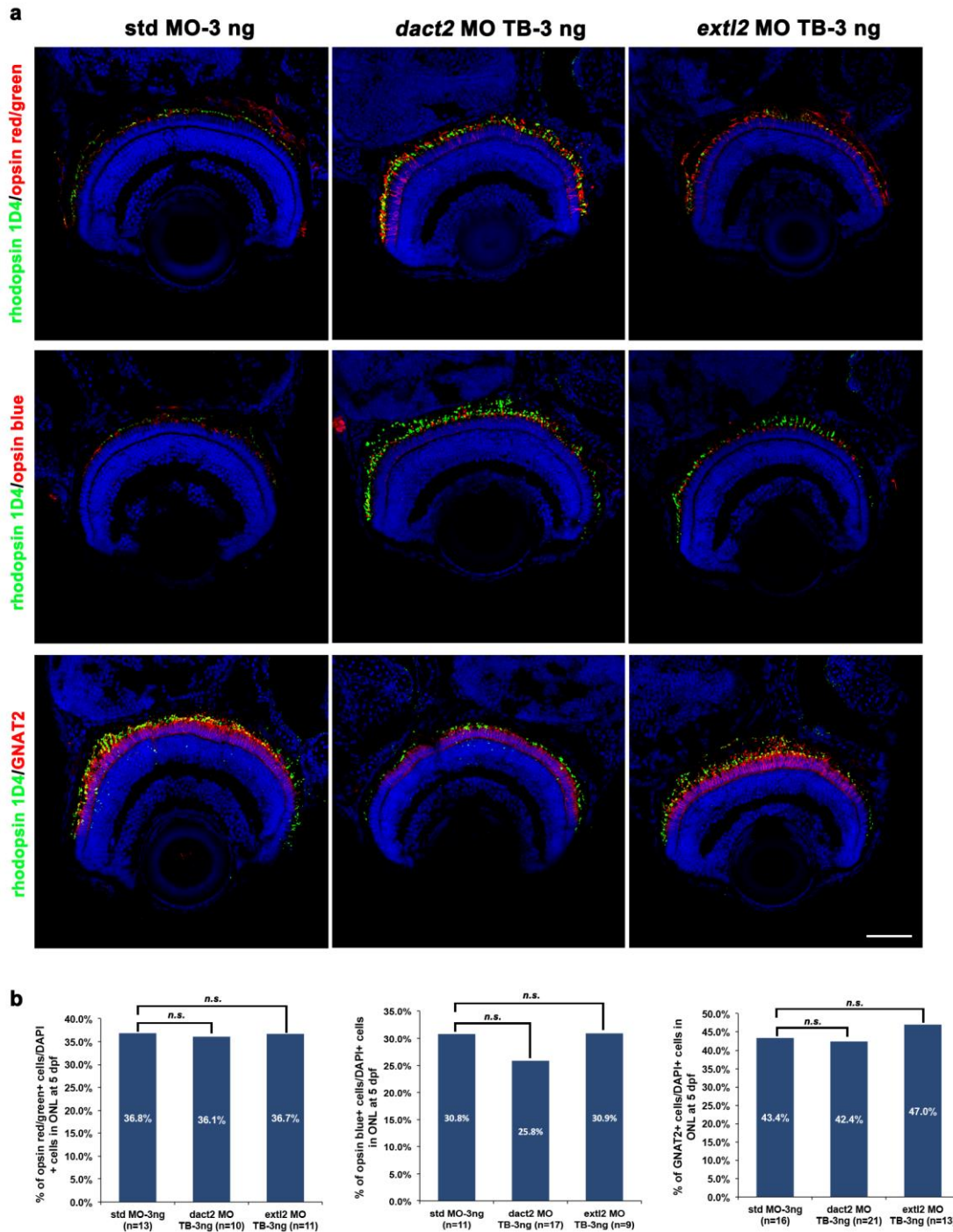


**Fig. S8. Eye size and body length in each larva at 3 dpf**

(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  $\mu\text{m}$ , the mean minus SD for wild type, with diameters greater than 289.3  $\mu\text{m}$  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  $\mu\text{m}$ , the mean minus SD for wildtype larvae, were classed as short, while the body length greater than 3207.4  $\mu\text{m}$  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.