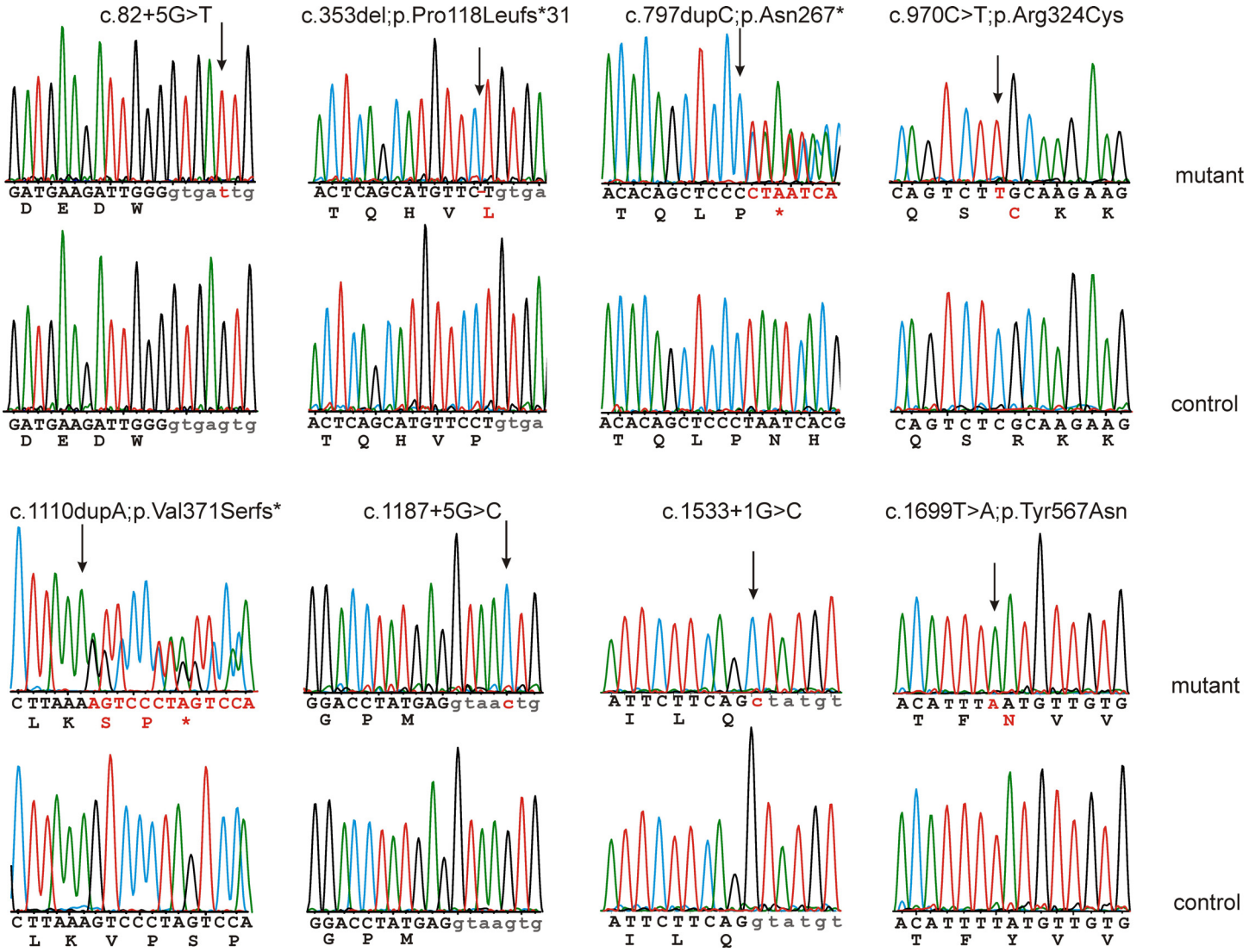
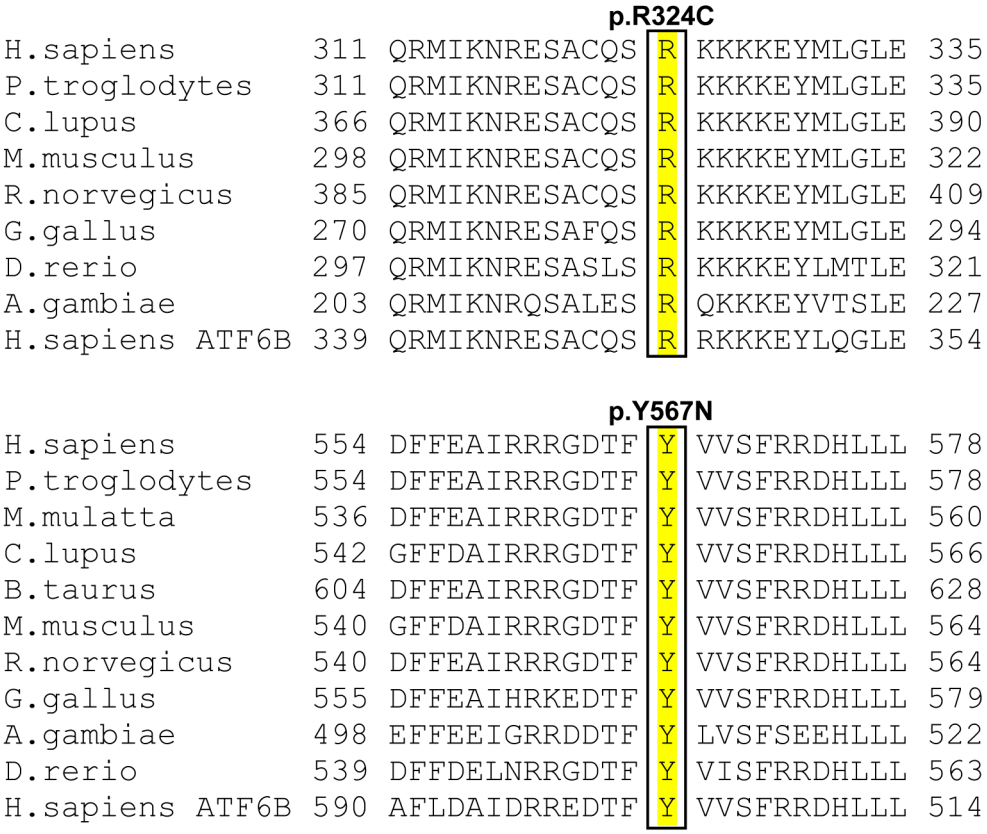


Supplementary Figure 1

Electropherograms of all identified mutations in *ATF6*.

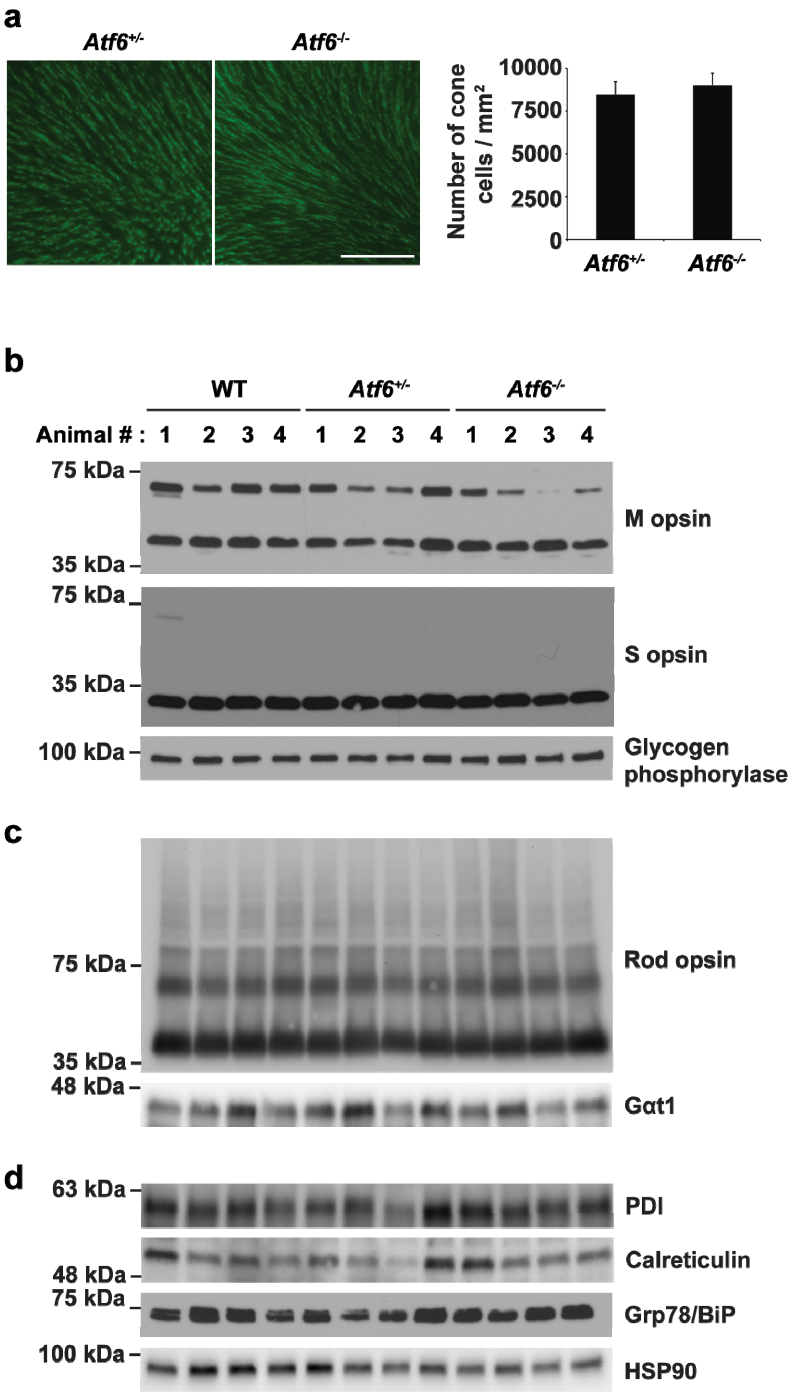
Mutant sequence (top) compared to wild-type sequence (bottom). Nucleotide and protein sequence (one-letter code) are presented beneath the electropherogram. Exonic sequence is given in uppercase letters, and intronic sequence is given in lowercase letters. Arrows indicate the mutation.



Supplementary Figure 2

Comparative sequence analysis showing conservation of ATF6A at the protein level.

Comparative sequence analysis showing conservation of ATF6A at the protein level according to HomoloGene. In addition, alignment with human ATF6B is shown on the last line for comparison. Top, protein alignment for the p.Arg324Cys missense mutation. Bottom, protein alignment for the p.Tyr567Asn mutation.



Supplementary Figure 3

***Atf6*$^{-/-}$ mouse whole-mount and biochemistry data**.

(**a**) Whole-mount retina preparations from 1-year-old *Atf6*$^{+/-}$ and *Atf6*$^{-/-}$ mice were stained with FITC-PNA (left immunofluorescent images), and the numbers of FITC-PNA–positive cone cells were quantified with Keyence BZ image analysis software for eight different eyes. Scale bar, 500 μm. (**b**–**d**) Whole retinas were collected and lysed from 90-d-old wild-type, *Atf6*$^{+/-}$ and *Atf6*$^{-/-}$ mice (n = 4 per genotype). (**b**) Cone-specific proteins (M opsin, S opsin and glycogen phosphorylase), (**c**) rod-specific proteins (rhodopsin and Gαt1 / rod transducin) and (**d**) ER stress-induced proteins (PDI, calreticulin and BiP (*Grp78*)) were detected by immunoblotting. HSP90 served as a protein loading control. WT, wild type; FITC, fluorescein isothiocyanate; PNA, peanut agglutinine.

Supplementary Tables:

Supplementary Table 1: Summary of patients' demographics, clinical data and *ATF6* mutations and genotypes

Patient ID	*ATF6* mutation Nucleotide level	ATF6 mutation Protein level	Ethnic descent / Age / Gender	VA (Refraction) OD, OS	Fundus	OCT	Color vision	Visual field	Scotopic / photopic ERG	Glare	Nystagmus	Progression
CHRO282-II:1	c.82+5G>T homozygous	Splice defect	South Tyrolean / 42 / M	20/100 (+0.5 +2.0 90°), 20/2000 (+1.0 +2.0 90°)	Macular changes	n.a.	Achromat	n.a.	n.a.	Yes	Nystagmus at age 5 months	No
CHRO628-II:2	c.970C>T homozygous	p.Arg324Cys	Irish / 19 / M	20/63 (+2.0 -1.5 162°), 20/100 (+2.25 -0.75 5°)	Small RPE-defect in the fovea, pathological reflexes	Foveal hypoplasia, missing foveal pit, IS/OS and RPE disruption	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Nystagmus at birth, currently no nystagmus	No
CHRO628-II:4	c.970C>T homozygous	p.Arg324Cys	Irish / 16 / F	20/100 (0.0 -1.0 180°), 20/63 (-1.0)	Small RPE-defect in the fovea, pathological reflexes	Foveal hypoplasia, missing foveal pit, IS/OS and RPE disruption	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Nystagmus at birth, currently no nystagmus	No
CHRO628-II:6	c.970C>T homozygous	p.Arg324Cys	Irish /9 / F	20/200 (+3.0), 20/200 (+3.0)	Small RPE-defect in the fovea, pathological reflexes	Foveal hypoplasia, missing foveal pit, IS/OS and RPE disruption	n.a.	n.a.	n.a.	Yes	Nystagmus at birth, currently no nystagmus	No
CHRO91-II:1	c.970C>T homozygous	p.Arg324Cys	British / 47 / F	20/448 (-4.0 -4.25 10°), 20/252 (-4.5 -4.00 2°)	Mild peripapillary atrophy, small amount of foveal atrophy	Foveal hypoplasia,, IS/OS absence	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Pendular nystagmus at birth, now mild nystagmus	No
CHRO91-II:2	c.970C>T homozygous	p.Arg324Cys	British / 45 / F	20/209 (+6.5 -2.0 5°), 20/115 (+6.0 -1.5 15°)	Mild peripapillary atrophy, small amount of foveal atrophy	Foveal hypoplasia, IS/OS absence	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Pendular nystagmus at birth, now mild nystagmus	No
CHRO91-II:3	c.970C>T homozygous	p.Arg324Cys	British / 43 / M	20/110 (-6.5), 20/152 (-9.0)	Marked peripapillary atrophy, marked foveal atrophy	Foveal hypoplasia, marked loss of outer retina and RPE	Achromat	Relative central scotoma	n.a.	Yes	Pendular nystagmus at birth, now mild nystagmus	No
CHRO709-II:1	c.1187+5G>C homozygous	Splice defect	Asian-Indian / 27 / F	20/132 (0.0 -2.5 15°), 20/145 (-1.5 -2.25 10°)	Mild pigmentary changes at macula	Foveal hypoplasia, IS/OS absence	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Pendular nystagmus at birth, now mild nystagmus	No
CHRO709-II:2	c.1187+5G>C homozygous	Splice defect	Asian-Indian / 23 / F	20/110 (+ 2.5 -2.0 180°), 20/166 (+2.0 -1.0 50°)	Mild pigmentary changes at macula	Foveal hypoplasia, IS/OS absence	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Pendular nystagmus at birth, now mild nystagmus	No
CHRO593-IV:1	c.1533+1G>C homozygous	Splice defect	French-Canadian / 17 / M	20/160 (+4.25 +0.75 92°), 20/160 (+2.0 +1.25 92°)	Bull's eye maculopathy	n.a.	Incomplete achromat	n.a.	Normal / Reduced	Yes	Currently convergence controlled nystagmus	n.a.
CHRO593-II:3	c.1533+1G>C homozygous	Splice defect	French-Canadian / 94 / M	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MOGL411-MOGL467-III:4	c.1533+1G>C homozygous	Splice defect	French-Canadian / 59 / F	20/100 (+6.0 + 1.0 90°), 20/100 (+6.0 +1.0 90°)	Foveal atrophy	Foveal hypoplasia with no umbo formation, cone loss in the central fovea	Achromat	Normal	Normal / Reduced	Yes	Yes	n.a.

Patient ID	*ATF6* mutation Nucleotide level	ATF6 mutation Protein level	Ethnic descent / Age / Gender	VA (Refraction) OD, OS	Fundus	OCT	Color vision	Visual field	Scotopic / photopic ERG	Glare	Nystagmus	Progression
MOGL411-MOGL467-IV:1	c.1533+1G>C homozygous	Splice defect	French-Canadian / 25 / M	20/100 (-6.5 + 3.5 90°), 20/100 (-9.5 + 5.0 90°)	Foveal atrophy	Foveal hypoplasia with no umbo formation, cone loss in the central fovea	Achromat	Normal	Reduced / Reduced	Yes	No	n.a.
MOGL5414-II:1	c.1533+1G>C homozygous	Splice defect	French-Canadian / 32 / M	20/70 (-5.5 +1.75 90°), 20/80 (-5.75 + 1.25 90°)	Foveal atrophy	Foveal hypoplasia with no umbo formation, cone loss in the central fovea	Achromat	Normal	Normal / Non-detectable	Yes	No	n.a.
CHRO649-II:1	c.1699T>A homozygous	p.Tyr567Asn	Iranian / 26 / F	20/200 (-1.5+3.5 85°), 20/100 (-1.5+2.75 105°)	Dull foveal reflex with RPE changes at the fovea OU, fine RPE mottling left eye	Partial foveal hypoplasia; presence of optical gap (hyporeflectivity) at the fovea in both eyes	Incomplete achromat	Relative central scotoma	Normal / Non-detectable	Yes	Nystagmus since infancy; now milder	Yes
ZD179-II:1	c.353delC homozygous	p.Pro118Leufs*31	Turkish / 41 / F	20/200 (0.0 -4.0 180°), 20/200 (0.0 -4.5 170°)	Dull foveal reflex with RPE changes at the fovea	No foveal impression, no subfoveal inner outer segment border	Achromat	n.a.	Normal / Non-detectable	Yes	Yes	n.a.
CHRO436-II:1	c.797dupC / c.1110dupA compound-heterozygous	p.Asn267* / p.Val371Serfs*3	German / 22 / M	20/100 (+7.25 -2.25 5°), 20/200 (+7.25 -2.25 5°)	Small RPE-defect in the fovea, pathological reflexes, small drusen	Foveal hypoplasia, missing foveal pit, IS/OS and RPE disruption	Achromat	Relative central scotoma	Normal / Non-detectable	Yes	Nystagmus at birth, currently no nystagmus	No
CHRO436-II:2	c.797dupC / c.1110dupA compound-heterozygous	p.Asn267* / p.Val371Serfs*3	German / 17 / F	20/200 (+7.25 -1.25 100°), 20/200 (+8.25 -1.25 20°)	Small RPE-defects in the fovea	Foveal hypoplasia, missing foveal pit, IS/OS and RPE disruption	Achromat	Slightly narrowed outer boundaries due to glare	Normal / Non-detectable	Yes	Yes	No

Abbreviations: OD: right eye; OS: left eye; OU: both eyes; n.a.: not available; M: male; F: female; RPE: retinal pigment epithelium; IS: inner segment; OS: outer segment.

Supplementary Table 2: PCR and Sequencing Primers for *ATF6* used in this study.

Exon	Forward Primer (5'-3')	Reverse Primer (5'-3')
ATF6-Exon 1	CTGAAAACTCCAAAAGGGAAA	GCGGAAGTAGGGAGGAGGAA
ATF6-Exon 2	GGTGACATAGGGACACAGTGC	AGCCTGAACCTGTTGTCCTG
ATF6-Exon 3	TGCCAAATTGTGTCTCACAG	GCCCAATAACCCCACCTAAT
ATF6-Exon 4	TTTTGGTGACCTTAGCTTCCA	CCCACAAGGCTCTTTCTTGA
ATF6-Exon 5	TCCTTTGAAGTTACCCTGAAGTG	AAAACAGCAAGCCAGCCTAA
ATF6-Exon 6	TGTGTAGAGAAAGGTGTGTGATAGAA	GCACGTGCTCAGAAATTATAACC
ATF6-Exon 7	TCCAATCCCTTGGTGTTGAT	TGTGCAATTCAGCACACTCTT
ATF6-Exon 8	AGTTTGAGCAGTGTGTTTTCAA	ACCCTCCTTTTCCTAGCACT
ATF6-Exon 9	AAGCGTTGCTTTTTCTGAAATC	TGACAAAAGCTAAAGAATAATGAGAA
ATF6-Exon 10	AGCTGCATGTAGCAGGCATA	CACTTAGGCCAAAAGATAGATGC
ATF6-Exon 11	TTTCCATGAAAAGTTAACACTAATGA	CCAACTCAGATGTTCTGCAA
ATF6-Exon 12	GTTTGAATGCATGGTTGACAGG	CAGGAGACAGTGGGAAGAGG
ATF6-Exon 13	TGTCAGAAATCTTAGCAATGGAA	TTTAAGGTGAAAGGGAAGTGTGA
ATF6-Exon 14	GAATGGGAAGTATTTTGGGAAA	GTGTACGGCAAGCACTCAAA
ATF6-Exon 15	TTTTTCATCATCCCAACGAA	TCCTGGTTAAACAAAGAAATCTCA
ATF6-Exon 16	CCTCTACACCCCTTGAAAGT	TTCTCTGCCTGCCACCAAG

PCR was performed with standard chemistry on ABI Veriti Cycler applying the following PCR program: 95°C 3min – 95°C 15sec, 55°C 15sec, 72°C 30sec, 35 cycles– 72°C 7min, cool down to 8°C (except for exons 2 and 13 where annealing temperatures of 65°C and 60°C, respectively, were used).

Supplementary Table 3: Primer sequences used for qPCR in this study.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH (human)	GTCGGAGTCAACGGATT	AAGCTTCCCGTTCTCAG
RPL19 (human)	ATGTATCACAGCCTGTACCTG	TTCTTGGTCTCTTCCTCCTTG
HSPA5 (GRP78/BiP) (human)	GCCTGTATTTCTAGACCTGCC	TTCATCTTGCCAGCCAGTTG
HERPUD1 (human)	AACGGCATGTTTTGCATCTG	GGGGAAGAAAGGTTCCGAAG
SEL1L (human)	ATCTCCAAAAGGCAGCAAGC	TGGGAGAGCCTTCCTCAGTC
EDEM1 (human)	TTCCCTCCTGGTGGAATTTG	AGGCCACTCTGCTTTCCAAC

For quantitative PCR analysis, cDNA was used as template in SYBR green qPCR supermix (Bio-Rad). *GAPDH* or *Rpl19* mRNA levels served as internal normalization standards. qPCR condition was 95°C for 5 min; 95°C for 10 sec; 60°C for 10 sec; 72°C for 10 sec, with 40 cycles of amplification.