Supplementary Methods

Mice

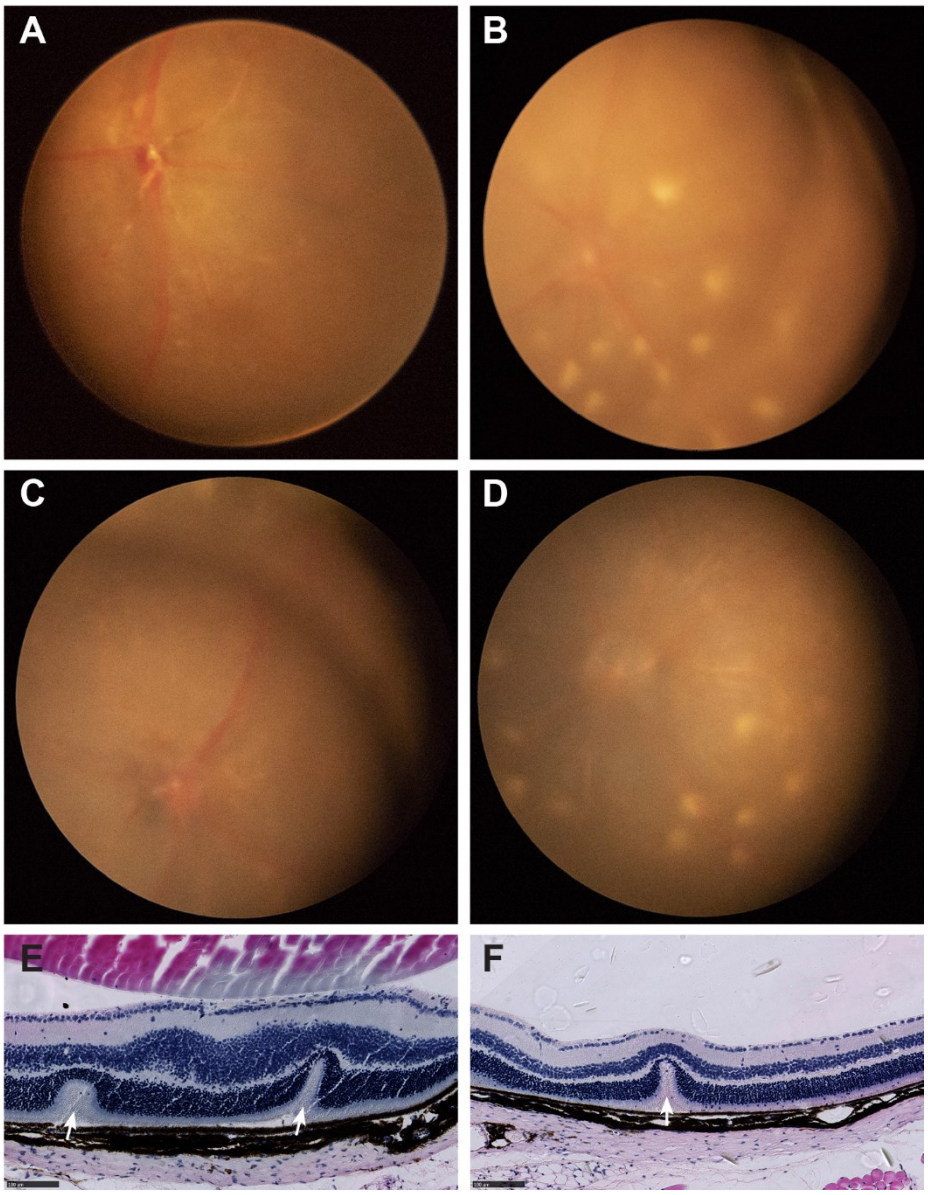
By crossing *Tmem98*$^{tm1a/+}$ mice with mice carrying Flpe the *tm1a* 'knockout-first' allele was converted to a conditional allele *Tmem98*$^{tm1c(EUCOMM)Wtsi}$ (hereafter *Tmem98*^{tm1c}). By crossing *Tmem98*$^{tm1c/+}$ mice with mice carrying Cre the floxed critical exon 4 is deleted generating a deletion allele that has a frame shift *Tmem98*$^{tm1d(EUCOMM)Wtsi}$ (hereafter *Tmem98*^{tm1d}) that would be subject to nonsense mediated decay[39].

Embryonic Protein Preparations

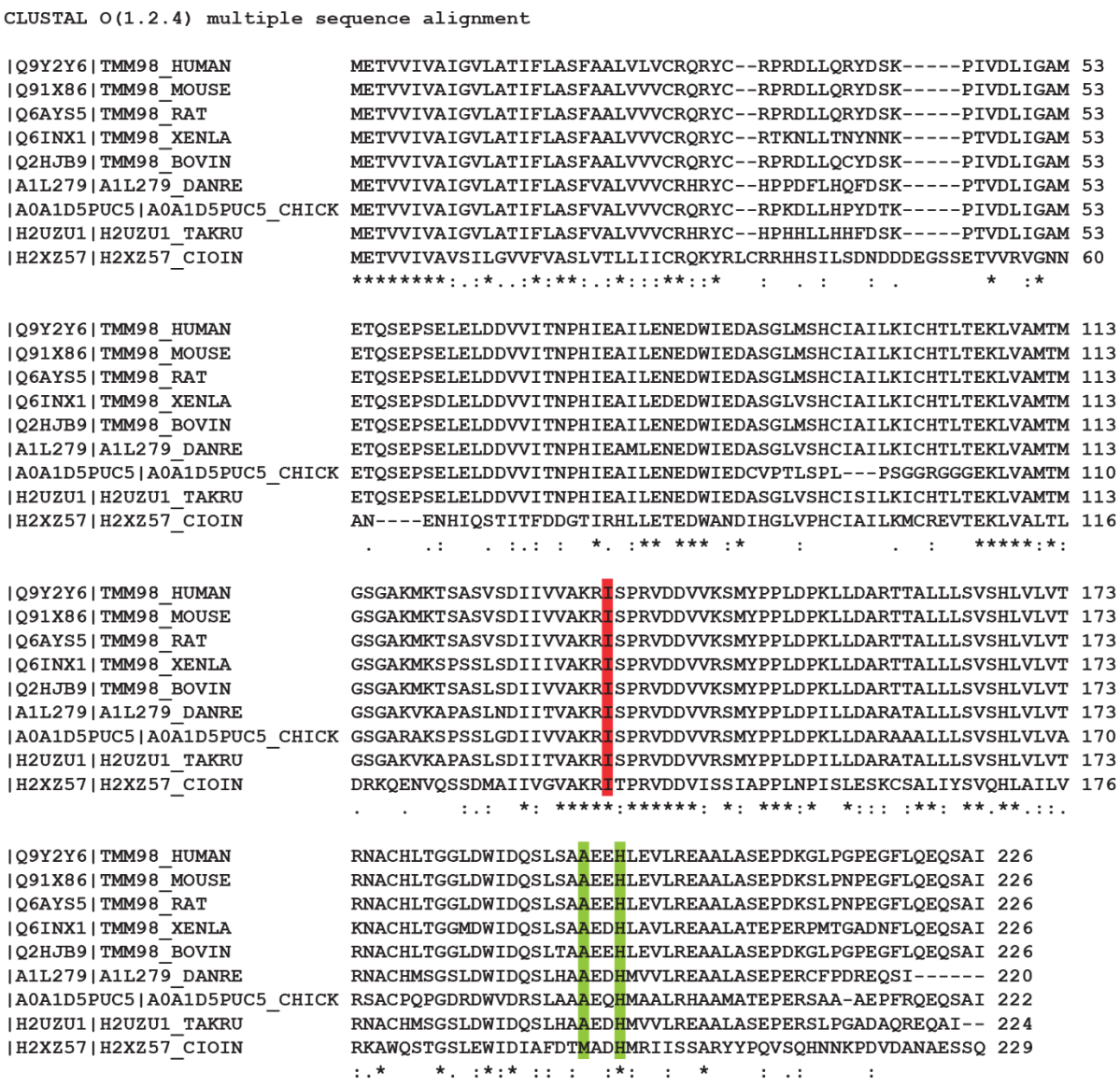
Embryonic day 12.5 (E12.5) embryos were collected and a small piece of tail used for genotyping. Tissue was homogenised in RIPA buffer (Cell Signaling Technology) plus 1 mM phenymethysulfonyl (ThermoFisher Scientific) and Complete Protease Inhibitor Cocktail (Roche) and sonicated for 30s 3 times. Crude lysates were cleared by centrifugation (20,000 g for 30 minutes at 4°C) and protein concentrations determined by Bradford assay (Bio-Rad). 20 µg samples were used for Western blotting.

Western Blotting

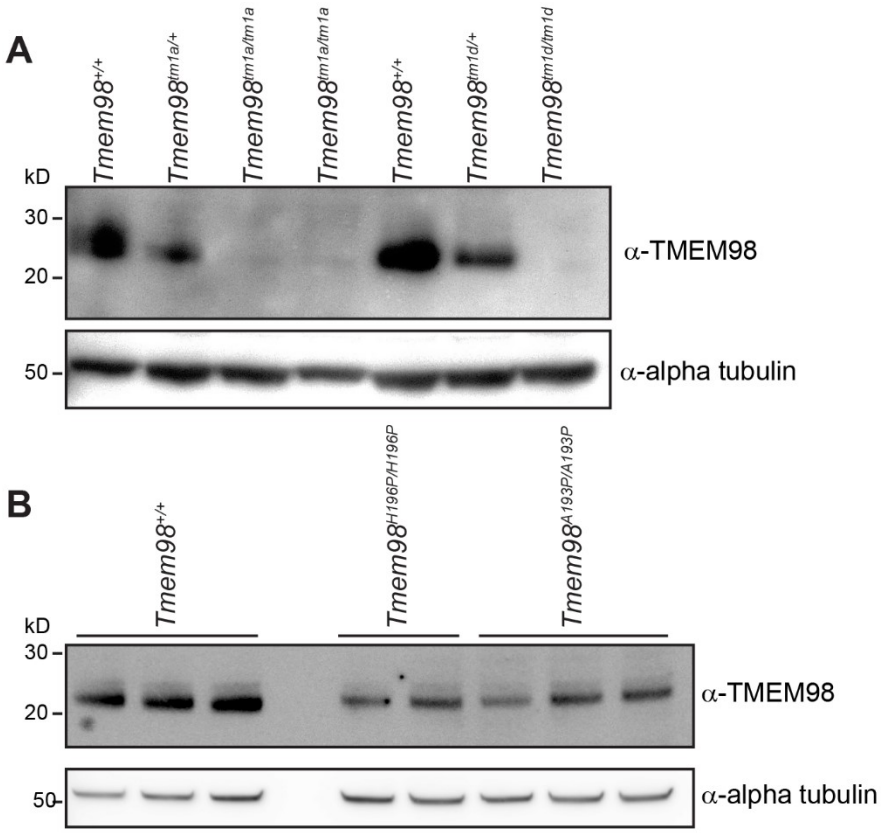
Equal amounts of protein lysates were separated on 4-12% Nupage Bis-Tris gels (ThermoFisher Scientific) and transferred to polyvinylidene difluoride or nitrocellulose membranes. Membranes were blocked for one hour at room temperature in SuperBlock T20 (TBS) Blocking Buffer (ThermoFisher Scientific) and incubated with primary antibodies for one hour at room temperature or overnight at 4°C in blocking buffer with shaking. Following washing with TBST membranes were incubated with ECL horse radish peroxidase (HRP)-conjugated secondary antibodies (GE Healthcare) diluted 1:5000 in blocking buffer for one hour at room temperature, washed with TBST and developed using SuperSignal™ West Pico PLUS (ThermoFisher Scientific).



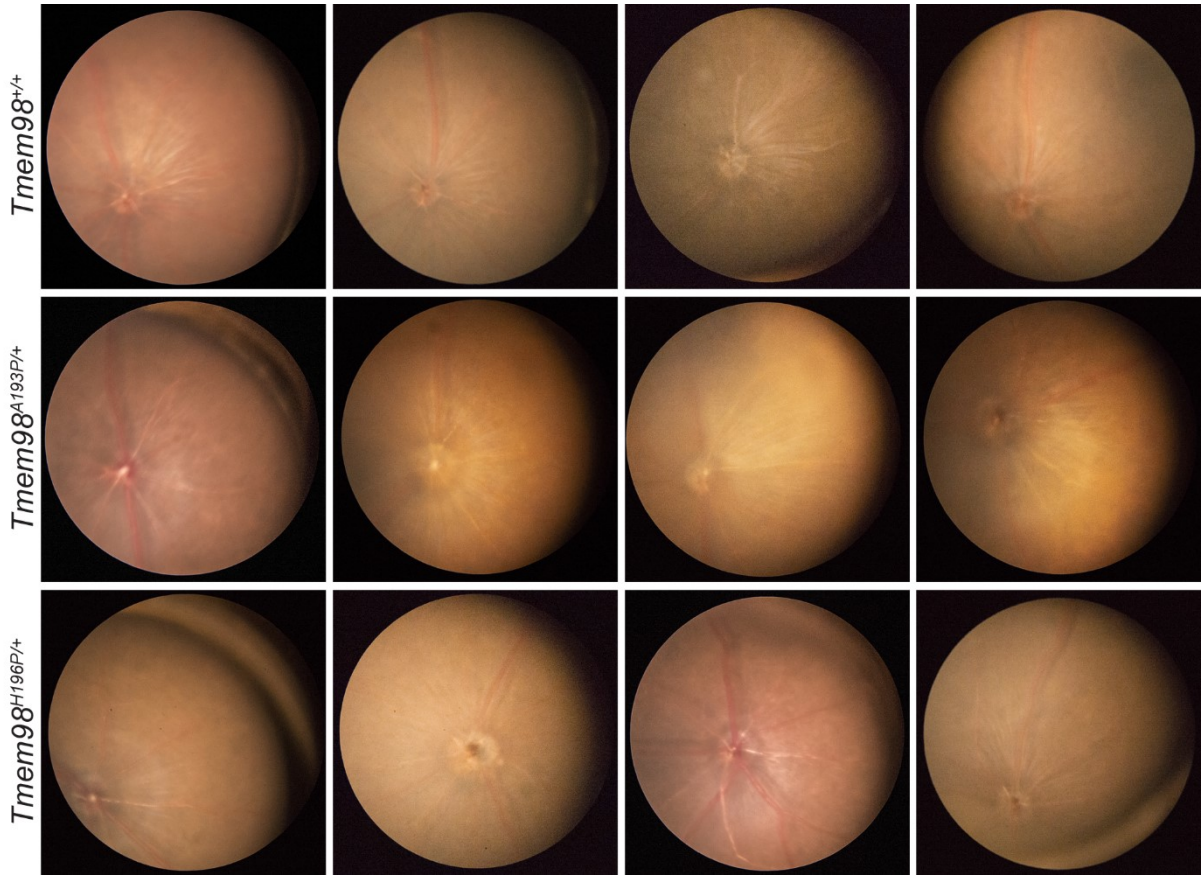
Supplementary Figure S1. Effect of genetic background on the *Rwhs* dominant retinal phenotype. (**A**-**D**) Retinal images of *Tmem98*$^{135T/+}$ mice on the C57BL/6J genetic background. **A** and **B** are littermates and **C** and **D** are littermates. The mice shown in **A** and **C** have normal retinas whereas the mice shown in **B** and **D** have white spots on their retinas. (**E**-**F**) Haematoxylin and eosin stained sections of *Tmem98*$^{135T/+}$ retinas on the CAST strain background displaying invaginations of the outer nuclear layer indicated by arrows. Scale bars: 100 μm.



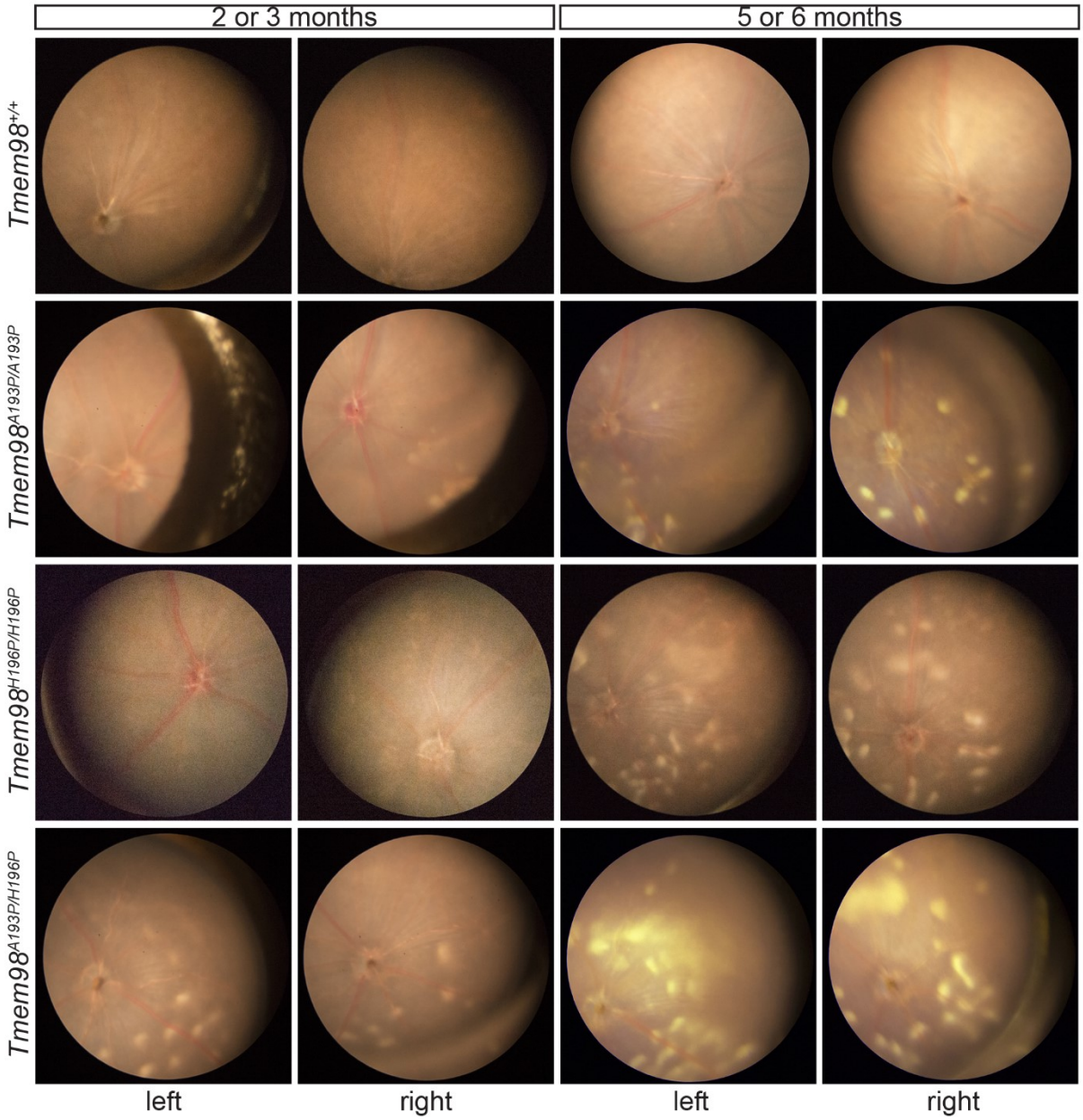
Supplementary Figure S2. TMEM98 protein sequences from different species aligned using the Clustal Omega program. The uniprot accession numbers and identifiers are shown on the left. An * indicates a completely conserved residue, a : indicates that residues have strongly similar properties (scoring > 0.5 in the Gonnet PAM 250 matrix), a . Indicates that residues have weakly similar properties (scoring =< 0.5 in the Gonnet PAM 250 matrix). I135, which is mutated in *Rwhs*, is completely conserved and highlighted in red. The two residues affected in the human nanophthalmos patients, A193 and H196, are highlighted in green. Both are completely conserved except that a methionine is substituted for A193 in *Ciona intestinalis*.



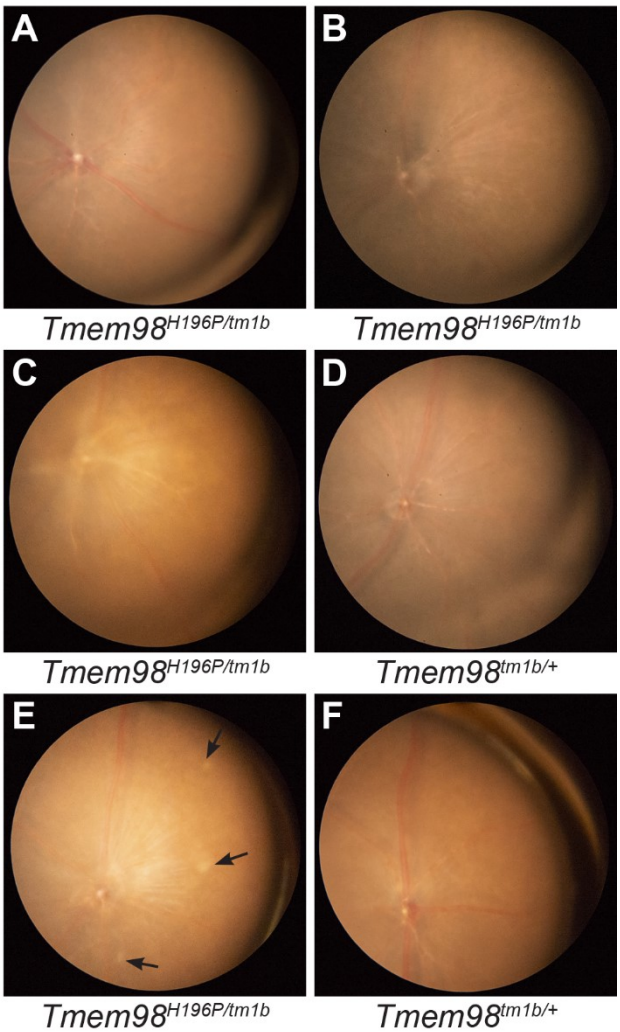
Supplementary Figure S3. TMEM98 protein is expressed in the missense mutants. (**A**) Western blot analysis of E12.5 embryonic protein lysates of the indicated genotypes. TMEM98 protein can be detected in the wild-type and heterozygous samples but not the homozygous knock-out samples validating the antibody. (**B**) Western blot analysis of E12.5 embryonic protein lysates of the indicated genotypes. TMEM98 is present in the homozygous mutants carrying the missense mutations found in human nanophthalmos patients. Alpha tubulin was used as a loading control.



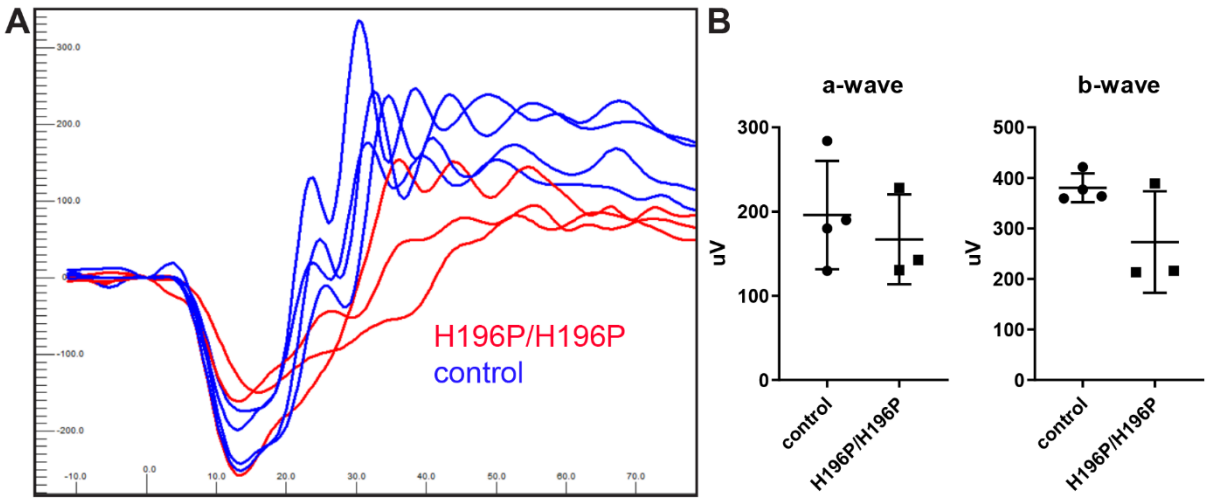
Supplementary Figure S4. Retinas of mice heterozygous for the human nanophthalmos missense mutations are normal. Retinal pictures from mice of between 5-10 months are shown. Top row wild-type mice, middle row *Tmem98*$^{A193P/+}$ mice and bottom row *Tmem98*$^{H196P/+}$ mice.



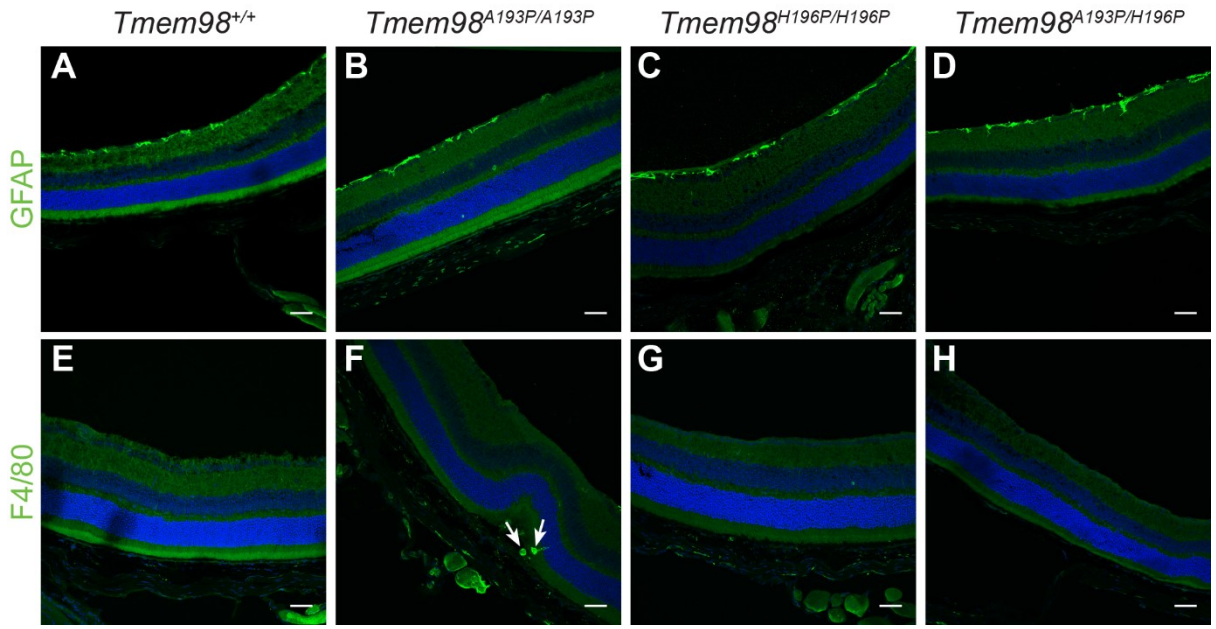
Supplementary Figure S5. The retinal white spotting recessive phenotype in mice carrying the human nanophthalmos missense mutations is progressive. Retinal pictures of the left and right eyes of mice taken at the indicated ages. First row wild-type, second row *Tmem98*$^{A193P/A193P}$, third row *Tmem98*$^{H196P/H196P}$ and fourth row *Tmem98*$^{A193P/H196P}$. The wild-type eyes have normal retinas at 5 months. On *Tmem98*$^{A193P/A193P}$ retinas only a few spots are present at 3 months but there is extensive spotting at six months. The *Tmem98*$^{H196P/H196P}$ retinas appear normal at 3 months but there is extensive spotting at six months. On *Tmem98*$^{A193P/H196P}$ retinas a few spots are present at 2 months but there is extensive spotting at 5 months.



Supplementary Figure S6. *Tmem98*$^{H196P/tm1b}$ retinas rarely have retinal white spots. Retinal pictures of *Tmem98*$^{H196P/tm1b}$ mice (**A**-**C** and **E**) and *Tmem98*$^{tm1b/+}$ mice (**D** and **F**). The retinas shown in **E** and **F** are from littermates. *Tmem98*$^{tm1b/+}$ retinas are normal. *Tmem98*$^{H196P/tm1b}$ retinas are normal except for the one shown in **E** at one year of age which has three faint white spots indicated by arrows.



Supplementary Figure S7. ERG response of *Tmem98*$^{H196P/H196P}$ mice at 9-11 months of age is normal. Three *Tmem98*$^{H196P/H196P}$ mice (two at 11 months of age and one at 9 months of age) and four 11 month old control mice (three *Tmem98*$^{H196P/+}$ and one wild-type) were tested. (**A**) ERG traces of *Tmem98*$^{H196P/H196P}$ mice (red lines), and control mice (blue lines). Shown are the responses at 3 cd.s/m^2 (average of 4 flashes) for the right eye. (**B**) Comparison of a-wave amplitudes (left) and b-wave amplitudes (right), average of left and right eye for each mouse. There is no significant difference between *Tmem98*$^{H196P/H196P}$ and control mice (a-wave, unpaired t test with Welch's correction, P = 0.55 and b-wave, unpaired t test with Welch's correction, P = 0.20).



Supplementary Figure S8. GFAP and F4/80 is normal outside the areas with retinal folds in the mutants. Immunostaining of retinal sections from wild-type mice (**A** and **E**), *Tmem98*$^{A193P/A193P}$ mice (**B** and **F**), *Tmem98*$^{H196P/H196P}$ mice (**C** and **G**), *Tmem98*$^{A193P/H196P}$ mice (**D** and **H**). (**A**-**D**) GFAP staining (green) is normal for all genotypes. GFAP is expressed in the ganglion cell layer where it is principally found in astrocytes. (**E**-**H**) F4/80 staining (green) was not observed outside the folds in the mutant retinas. The white arrows indicate macrophages within a fold in the outer segments for *Tmem98*$^{A193P/A196P}$ (**F**). DAPI staining is shown in blue. Scale bars: 50 μm.

Supplementary Table S1. Sequences of oligos

Oligo Name	Sequence (5'-3')*
ex7_Guide1	CACCGGCCAATCACTGTCTGCCGCTG
ex7_Guide2	AAACCAGCGGCAGACAGTGATTGGCC
A193P	AACCGCCCTGCTGCTGTCCGTTAGTCACTTGGTGCTAGTGACCAGGA ACGCCTGCCATCTAACCGGGGGCCTGGACTGGATTGACCAATCACT GTCTGCCCCTGAaGAGCACCTGGAAGTCCTTCGAGAGGCAGCCCTG GCTTCTGAGCCAGATAAAAGCCTCCCCAACCCTGAGGGCTTCCTGCA GGAACAGTCGGCCA
H196P	AACCGCCCTGCTGCTGTCCGTTAGTCACTTGGTGCTAGTGACCAGGA ACGCCTGCCATCTAACCGGGGGCCTGGACTGGATTGACCAATCACT GTCTGCCGCTGAaGAGCCCCTGGAAGTCCTTCGAGAGGCAGCCCTG GCTTCTGAGCCAGATAAAAGCCTCCCCAACCCTGAGGGCTTCCTGCA GGAACAGTCGGCCA

*For the repair oligos base changes introducing amino acid changes are underlined and silent base changes destroying the PAM site are in lower case. In the A193P line only the mutation causing the A193P change was found, in the H196P line both base changes were incorporated.

Supplementary Table S2. Genotyping primers

Primer Name	Sequence (5'-3')	Product size/allele information
ex5F	CTTTCCACCCCATTTCCTCT	501 bp (sequenced for *Tmem98*^{I135T}
ex5R	AGGCTCTGTCAGCCCAGTTA	genotyping)
ex7F	CTTGGTGCTAGTGACCAGGA	228 bp (sequenced for *Tmem98*^{A193P}
ex7R	ACAGGAAGTAGAAGGCTCGC	and *Tmem98*^{H196P} genotyping)
1532	CCAAAGGGGTGCATTTGAAG	465 bp (WT)
1533	TGCAAACCCAAGTCAAAAAGC	595 bp (*tm1c*)
1532	CCAAAGGGGTGCATTTGAAG	196 bp (*tm1a*, *tm1b*, *tm1c*, *tm1d*)
1490	TCGTGGTATCGTTATGCGCC	
LacZF	ATCACGACGCGCTGTATC	108 bp (*tm1a*, *tm1b*)
LacZR	ACATCGGGCAAATAATATCG	
1604	CCCCCTGAACCTGAAACATA	310 bp (*tm1b*)
838	CTCAGACACCCAGCCTTCTC	
1605	ACCCTTCTCTCCCTAAGTAGTCT	867 bp (WT)
1606	CCCCAAGCCGTCCTTTCC	1030 bp (*tm1c*)
		238 bp (*tm1d*)
FLPeF	AGGGTGAAAGCATCTGGGAGA	~400 bp (Flpe)
FLPeR	TCAACTCCGTTAGGCCCTTCA	
747	CCTGGAAAATGCTTCTGTCCG	4 primer reaction
748	CAGGGTGTTATAAGCAATCCC	290 bp (control product)
749	AACACACACTGGAGGACTGGCTA	450 bp (Cre)
750	CAATGGTAGGCTCACTCTGGGAG	

Supplementary Table S3. *Tmem98*$^{tm1a/+}$ intercross genotyping results

Age	WT	*tm1a*/+	*tm1a/tm1a*	Total	P*
at weaning	21	38	0	59	<0.0001
E16.5-E17.5	10	20	10	40	1.0000

*Test for significance using chi-square test

Supplementary Table S4. Mouse homologues of the human nanophthalmos *TMEM98* mutations intercross genotyping results at weaning

Cross	WT	*A193P/+*	*A193P/A193P*	*H196P/+*	*H196P/H196P*	*A193P/H196P*	Total	P*
A193P/+ x *A193P/+*	10	21	12	N/A	N/A	N/A	43	0.9006
H196P/+ x *H196P/+*	68	N/A	N/A	94	49	N/A	211	0.0516
A193P/+ x *H196P/+*	23	18	N/A	20	N/A	14	75	0.5164

*Test for significance using chi-square test
N/A=not applicable