

## 1 **Supplementary Methods**

### 2 **Mice**

3 By crossing *Tmem98<sup>tm1a/+</sup>* mice with mice carrying Flpe the *tm1a* 'knockout-first' allele was  
4 converted to a conditional allele *Tmem98<sup>tm1c(EUCOMM)Wtsi</sup>* (hereafter *Tmem98<sup>tm1c</sup>*). By crossing  
5 *Tmem98<sup>tm1c/+</sup>* mice with mice carrying Cre the floxed critical exon 4 is deleted generating a  
6 deletion allele that has a frame shift *Tmem98<sup>tm1d(EUCOMM)Wtsi</sup>* (hereafter *Tmem98<sup>tm1d</sup>*) that  
7 would be subject to nonsense mediated decay<sup>37</sup>.

8

### 9 **Embryonic Protein Preparations**

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

16

### 17 **Western Blotting**

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

27 **Supplementary Tables**

28

29 **Supplementary Table S1. Genotyping primers**

Primer Name	Sequence (5'-3')	Product size/allele information
<b>ex5F</b>	CTTCCACCCCATTTCTCT	501 bp (sequenced for <i>Tmem98</i> <sup>l135T</sup> genotyping)
<b>ex5R</b>	AGGCTCTGTCAGCCCAGTTA	
<b>ex7F</b>	CTTGGTGCTAGTGACCAGGA	228 bp (sequenced for <i>Tmem98</i> <sup>A193P</sup> and <i>Tmem98</i> <sup>H196P</sup> genotyping)
<b>ex7R</b>	ACAGGAAGTAGAAGGCTCGC	
<b>1532</b>	CCAAAGGGGTGCATTTGAAG	465 bp (WT)
<b>1533</b>	TGCAAACCCAAGTCAAAAAGC	595 bp ( <i>tm1c</i> )
<b>1532</b>	CCAAAGGGGTGCATTTGAAG	196 bp ( <i>tm1a</i> , <i>tm1b</i> , <i>tm1c</i> , <i>tm1d</i> )
<b>1490</b>	TCGTGGTATCGTTATGCGCC	
<b>LacZF</b>	ATCACGACGCGCTGTATC	108 bp ( <i>tm1a</i> , <i>tm1b</i> )
<b>LacZR</b>	ACATCGGGCAAATAATATCG	
<b>1604</b>	CCCCCTGAACCTGAAACATA	310 bp ( <i>tm1b</i> )
<b>838</b>	CTCAGACACCCAGCCTTCTC	
<b>1605</b>	ACCCTTCTCTCCCTAAGTAGTCT	867 bp (WT)
<b>1606</b>	CCCCAAGCCGTCCTTTCC	1030 bp ( <i>tm1c</i> ) 238 bp ( <i>tm1d</i> )
<b>FLPeF</b>	AGGGTGAAAGCATCTGGGAGA	~400 bp (Flpe)
<b>FLPeR</b>	TCAACTCCGTTAGGCCCTTCA	
<b>747</b>	CCTGAAAATGCTTCTGTCCG	4 primer reaction
<b>748</b>	CAGGGTGTTATAAGCAATCCC	290 bp (control product)
<b>749</b>	AACACACACTGGAGGACTGGCTA	450 bp (Cre)
<b>750</b>	CAATGGTAGGCTCACTCTGGGAG	

30

31

32 **Supplementary Table S2. *Tmem98*<sup>tm1a/+</sup> intercross genotyping results**

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

33 \*Test for significance using chi-square test

34

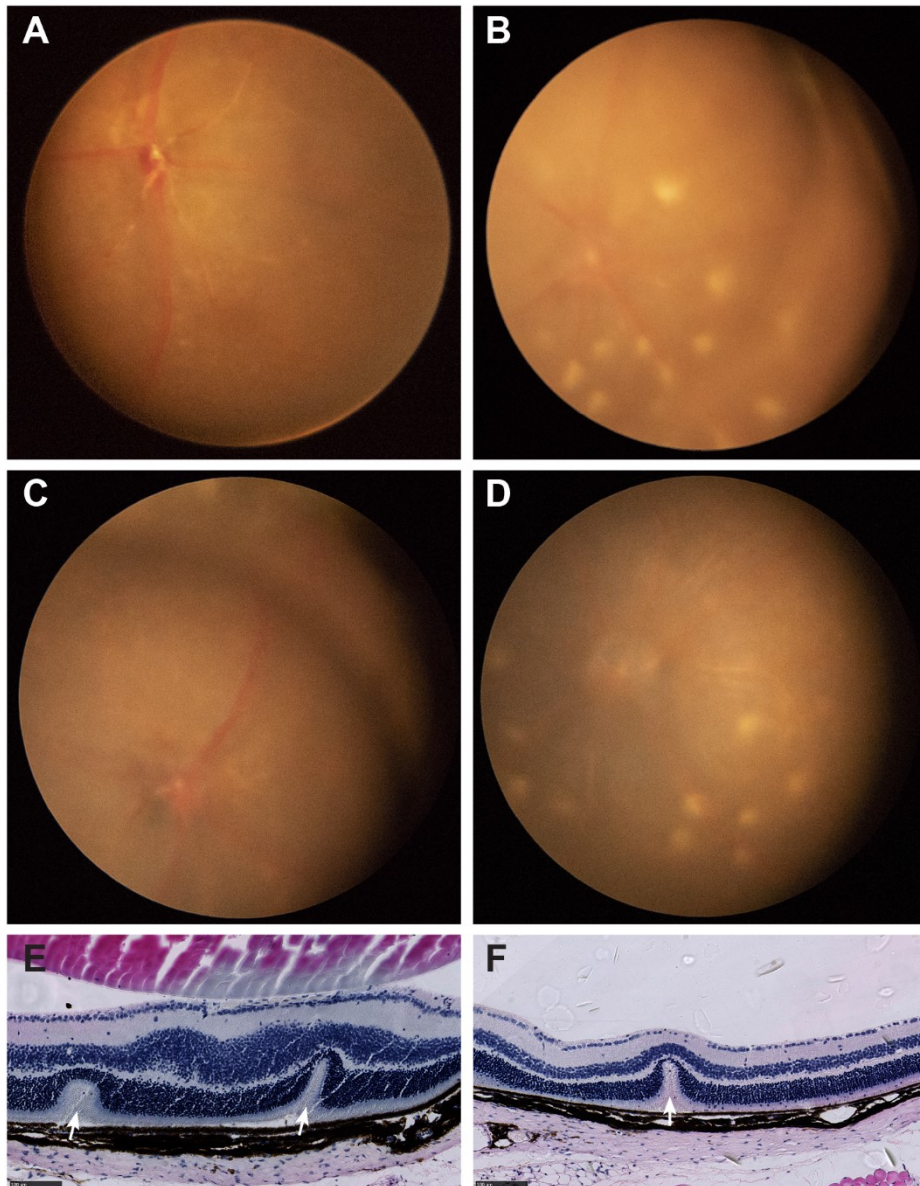
35 **Supplementary Table S3. Mouse homologues of the human nanophthalmos *TMEM98* mutations intercross genotyping results at**  
 36 **weaning**

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

37 \*Test for significance using chi-square test

38 N/A=not applicable

39 **Supplementary Figures**



40

41 **Supplementary Figure S1.** Effect of genetic background on the *Rwhs* dominant retinal  
42 phenotype. (A-D) Retinal images of *Tmem98*<sup>1357/+</sup> mice on the C57BL/6J genetic  
43 background. A and B are littermates and C and D are littermates. The mice shown in A and  
44 C have normal retinas whereas the mice shown in B and D have white spots on their retinas.  
45 (E-F) Haematoxylin and eosin stained sections of *Tmem98*<sup>1357/+</sup> retinas on the CAST strain  
46 background displaying invaginations of the outer nuclear layer indicated by arrows. Scale  
47 bars: 100  $\mu$ m.

48

CLUSTAL O(1.2.4) multiple sequence alignment

```

|Q9Y2Y6|TMM98_HUMAN      METVVIVAIGVLATIFLASFAALVLCRQRYC--RPRDLLQRYDSK-----PIVDLIGAM 53
|Q91X86|TMM98_MOUSE     METVVIVAIGVLATIFLASFAALVVVCRQRYC--RPRDLLQRYDSK-----PIVDLIGAM 53
|Q6AYS5|TMM98_RAT       METVVIVAIGVLATIFLASFAALVVVCRQRYC--RPRDLLQRYDSK-----PIVDLIGAM 53
|Q6INX1|TMM98_XENLA     METVVIVAIGVLATIFLASFAALVVVCRQRYC--RTKNLLTNYNNK-----PTVDLIGAM 53
|Q2HJB9|TMM98_BOVIN     METVVIVAIGVLATIFLASFAALVVVCRQRYC--RPRDLLQCYDSK-----PIVDLIGAM 53
|A1L279|A1L279_DANRE    METVVIVAIGVLATIFLASFVALVVVCRHRYC--HPPDFLHQFDSK-----PTVDLIGAM 53
|A0A1D5PUC5|A0A1D5PUC5_CHICK METVVIVAIGVLATIFLASFVALVVVCRQRYC--RPKDLLHPYDTK-----PIVDLIGAM 53
|H2UZU1|H2UZU1_TAKRU    METVVIVAIGVLATIFLASFVALVVVCRHRYC--HPHLLHHFDSK-----PTVDLIGAM 53
|H2XZ57|H2XZ57_CIOIN    METVVIVAVSILGVVFAVSLVTLIIICRQKYRLCRRHHSILSDNDDDEGSSETVVRVGN 60
*****:.*.:*:*:*:*:*:*:*:* : . : : . * :*

|Q9Y2Y6|TMM98_HUMAN      ETQSEPSELELDDVVIITNPHIEAILENEDWIEDASGLMSHCIAILKICHTLTEKLVAMTM 113
|Q91X86|TMM98_MOUSE     ETQSEPSELELDDVVIITNPHIEAILENEDWIEDASGLMSHCIAILKICHTLTEKLVAMTM 113
|Q6AYS5|TMM98_RAT       ETQSEPSELELDDVVIITNPHIEAILENEDWIEDASGLMSHCIAILKICHTLTEKLVAMTM 113
|Q6INX1|TMM98_XENLA     ETQSEPSDLELDDVVIITNPHIEAILEDEDWIEDASGLVSHCIAILKICHTLTEKLVAMTM 113
|Q2HJB9|TMM98_BOVIN     ETQSEPSELELDDVVIITNPHIEAILENEDWIEDASGLMSHCIAILKICHTLTEKLVAMTM 113
|A1L279|A1L279_DANRE    ETQSEPSELELDDVVIITNPHIEAMLENEDWIEDASGLVSHCIAILKICHTLTEKLVAMTM 113
|A0A1D5PUC5|A0A1D5PUC5_CHICK ETQSEPSELELDDVVIITNPHIEAILENEDWIEDCVPTLSPL---PSGGRGGGEKLVAMTM 110
|H2UZU1|H2UZU1_TAKRU    ETQSEPSELELDDVVIITNPHIEAILENEDWIEDASGLVSHCISILKICHTLTEKLVAMTM 113
|H2XZ57|H2XZ57_CIOIN    AN---ENHIQSTITFDGDIRHLLLETEDWANDIHGLVPHCIAILKMKREVTEKLVALLT 116
. . . : . : : * . : ** * * : * : . : * * * * : *

|Q9Y2Y6|TMM98_HUMAN      GSGAKMKT SASVSDIIVVAKRISPRVDDVVKSMYPPLDPKLLDARTTALLSVSHLVLT 173
|Q91X86|TMM98_MOUSE     GSGAKMKT SASVSDIIVVAKRISPRVDDVVKSMYPPLDPKLLDARTTALLSVSHLVLT 173
|Q6AYS5|TMM98_RAT       GSGAKMKT SASVSDIIVVAKRISPRVDDVVKSMYPPLDPKLLDARTTALLSVSHLVLT 173
|Q6INX1|TMM98_XENLA     GSGAKMKS PSSLSDIIVVAKRISPRVDDVVRSMYPPLDPKLLDARTTALLSVSHLVLT 173
|Q2HJB9|TMM98_BOVIN     GSGAKMKT SASLSDIIVVAKRISPRVDDVVKSMYPPLDPKLLDARTTALLSVSHLVLT 173
|A1L279|A1L279_DANRE    GSGAKVKAPASLNDIITVAKRISPRVDDVVRSMYPPLDPIILLDARATALLSVSHLVLT 173
|A0A1D5PUC5|A0A1D5PUC5_CHICK GSGARAKS PSSLGDIIVVAKRISPRVDDVVRSMYPPLDPKLLDARAAAALLSVSHLVLA 170
|H2UZU1|H2UZU1_TAKRU    GSGAKVKAPASLSDIITVAKRISPRVDDVVRSMYPPLDPIILLDARATALLSVSHLVLT 173
|H2XZ57|H2XZ57_CIOIN    DRKQENVQSSDMAIIVGAKRITPRVDDVISSIAAPLNPI SLESKCSALIYSVQHAILV 176
. . . : : * : * * * * : * * : * * * * : * * : : * * : * * * * : .

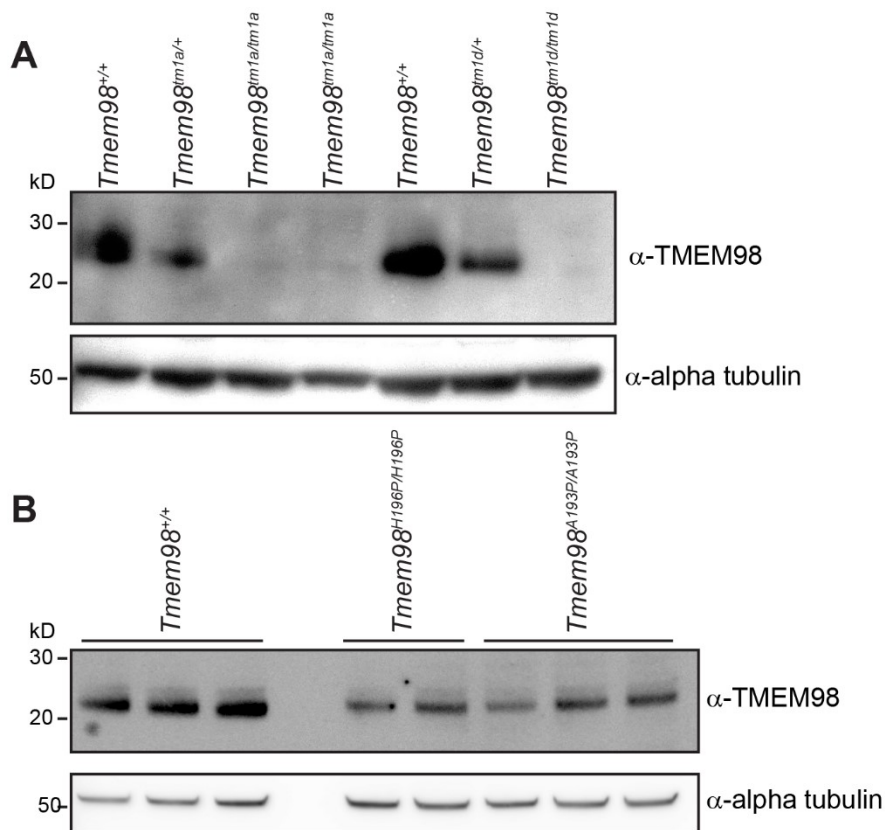
|Q9Y2Y6|TMM98_HUMAN      RNACHLTGGLDWIDQSLSAEEEHLEVLREAALASEPDKGLPGPEGFLQEQSAI 226
|Q91X86|TMM98_MOUSE     RNACHLTGGLDWIDQSLSAEEEHLEVLREAALASEPDKSLPNPEGFLQEQSAI 226
|Q6AYS5|TMM98_RAT       RNACHLTGGLDWIDQSLSAEEEHLEVLREAALASEPDKSLPNPEGFLQEQSAI 226
|Q6INX1|TMM98_XENLA     KNACHLTGGMWDIDQSLSAEEDH LAVLREAALATEPERPMTGADNFLQEQSAI 226
|Q2HJB9|TMM98_BOVIN     RNACHLTGGLDWIDQSLTAAEEHLEVLREAALASEPDKGLPGPEGFLQEQSAI 226
|A1L279|A1L279_DANRE    RNACHMSGSLDWIDQSLHAAEDH MVVLR EAALASEPERCFPDREQSI----- 220
|A0A1D5PUC5|A0A1D5PUC5_CHICK RSACPQPGDRDWDRSLAAEQHMAALRHAAMATEPERSAA-AEPFRQEQSAI 222
|H2UZU1|H2UZU1_TAKRU    RNACHMSGSLDWIDQSLHAAEDH MVVLR EAALASEPERSLPGADAQREQAI-- 224
|H2XZ57|H2XZ57_CIOIN    RKAWQSTGSLEWIDIAFDTMADHMRIISSARYYPQVSQHNNKPDVDANAESSQ 229
.: * * . : * * : : : : * : : * : : : :

```

49

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

59



60

61 **Supplementary Figure S3.** TMEM98 protein is expressed in the missense mutants. **(A)**

62 Western blot analysis of E12.5 embryonic protein lysates of the indicated genotypes.

63 TMEM98 protein can be detected in the wild-type and heterozygous samples but not the

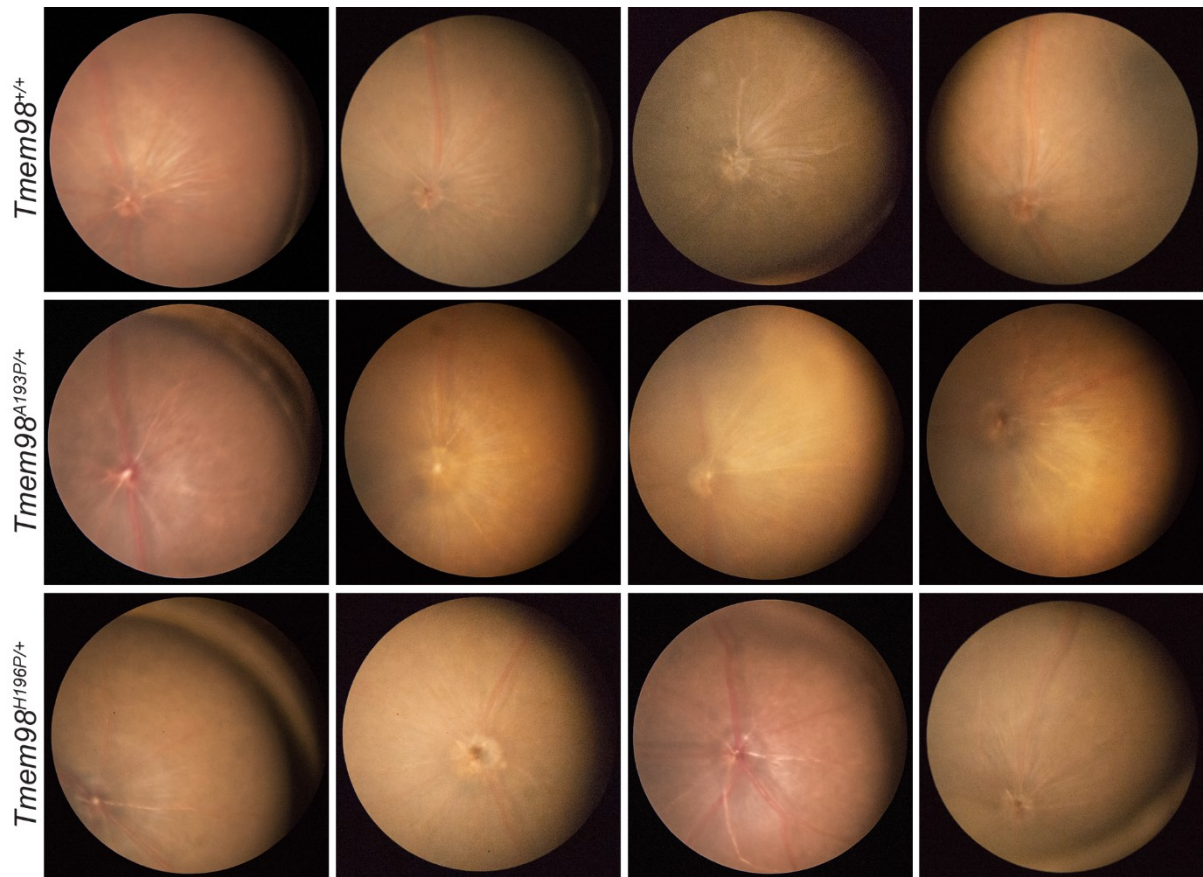
64 homozygous knock-out samples validating the antibody. **(B)** Western blot analysis of E12.5

65 embryonic protein lysates of the indicated genotypes. TMEM98 is present in the

66 homozygous mutants carrying the missense mutations found in human nanophthalmos

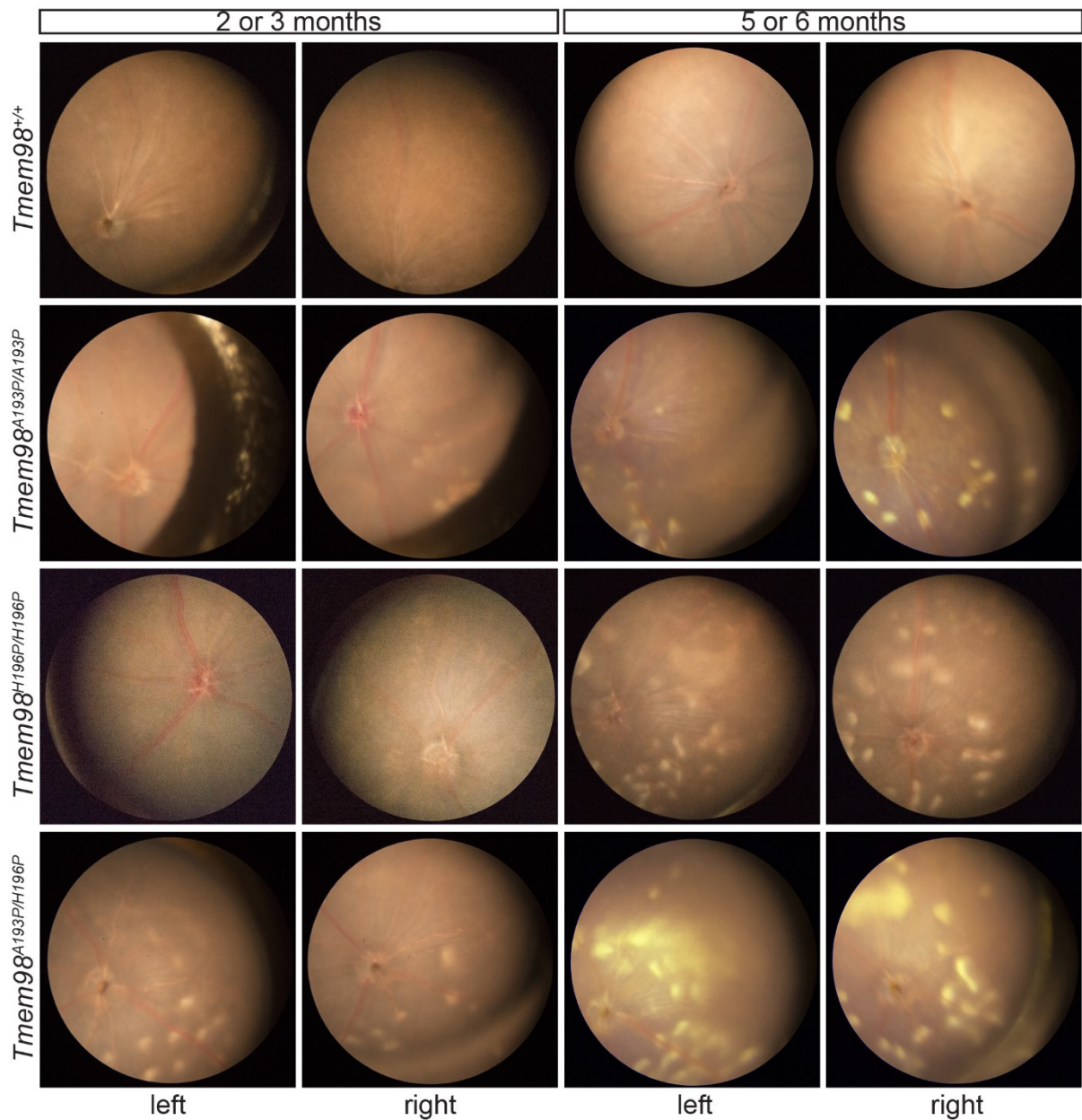
67 patients. Alpha tubulin was used as a loading control.





68

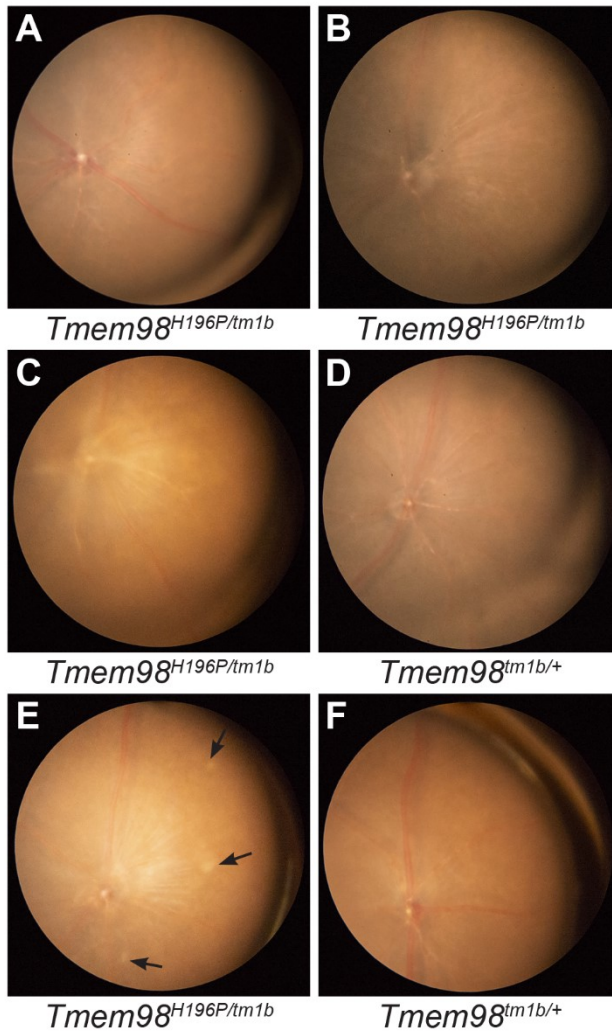
69 **Supplementary Figure S4.** Retinas of mice heterozygous for the human nanophthalmos  
70 missense mutations are normal. Retinal pictures from mice of between 5-10 months are  
71 shown. Top row wild-type mice, middle row  $Tmem98^{A193P/+}$  mice and bottom row  
72  $Tmem98^{H196P/+}$  mice.



73

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

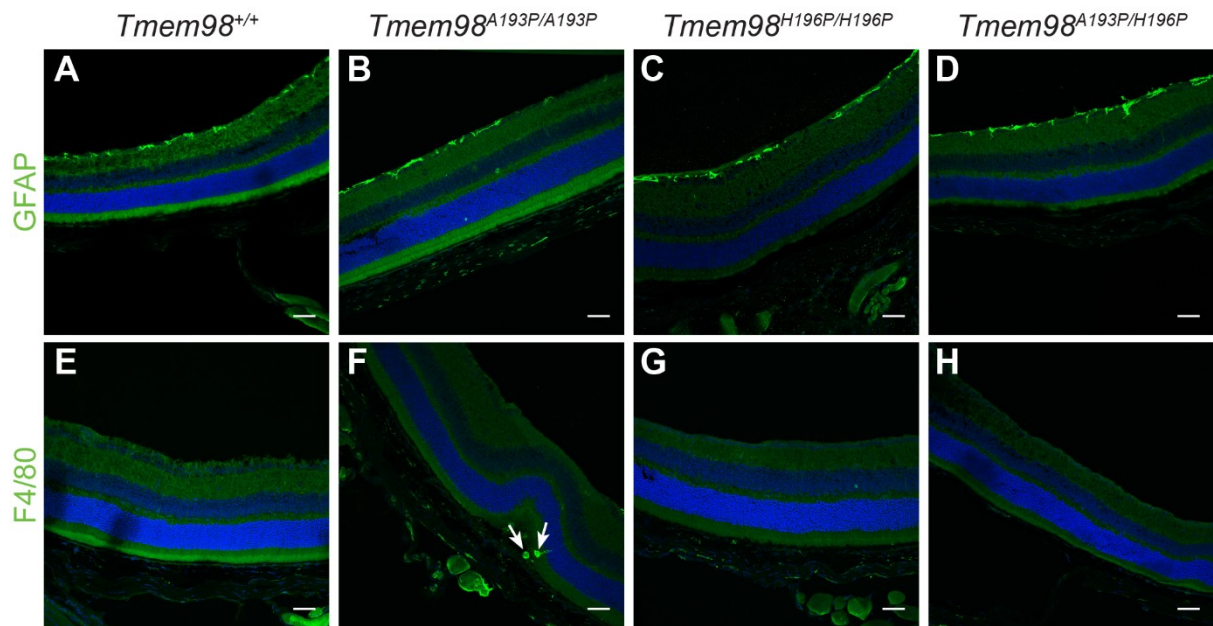




83

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

89



90

91 **Supplementary Figure S7.** GFAP and F4/80 is normal outside the areas with retinal folds in  
 92 the mutants. Immunostaining of retinal sections from wild-type mice (**A** and **E**),  
 93 *Tmem98<sup>A193P/A193P</sup>* mice (**B**, and **F**), *Tmem98<sup>H196P/H196P</sup>* mice (**C** and **G**), *Tmem98<sup>A193P/H196P</sup>*  
 94 mice (**D** and **H**). (**A-D**) GFAP staining (green) is normal for all genotypes. GFAP is  
 95 expressed in the ganglion cell layer where it is principally found in astrocytes. (**E-H**) F4/80  
 96 staining (green) was not observed outside the folds in the mutant retinas. The white arrows  
 97 indicate macrophages within a fold in the outer segments for *Tmem98<sup>A193P/H196P</sup>*. (**F**) DAPI  
 98 staining is shown in blue. Scale bars: 50  $\mu$ m.