Supplementary Material

Supplementary Figure Legends

Figure S1: Histogram showing amplitudes of dark-adapted (scotopic) a- (**A**) and b- (**B**) wave (corresponding to rod function) and light-adapted (photopic) b-wave (**C**) (corresponding to cone function) from the retina of 1, 3, 6, 9 and 18-month old wild type (WT) (Blue), *Reep6*$^{+/-}$ (Red), and *Reep6*$^{-/-}$ mice.

Figure S2: RNA-seq analysis of *Reep6*$^{-/-}$ retina. (**A**) Principal component analysis (PCA) of the expressed genes from postnatal (PN) day 9 and 21 WT and *Reep6*$^{-/-}$ retina. (**B**) Heatmap showing hierarchical clustering of significantly differentially expressed (DE) genes at PN21. (**C**) Significantly DE genes related to positive regulation of immune response gene ontology (GO:0050778). (**D**) Significantly DE genes relating to the response to oxygen-containing compound gene ontology (GO:1901700).

Figure S3: Fundus images of the two Chinese RP patients with *REEP6* mutation.

Figure S4: Control images of adult mouse retina immunostained with anti-REEP6 (green) and anti-Clathrin (red) antibodies. Note that the green and red channels in the middle and right panels do not show any bleed-through. On the left is a merged image showing co-localization of REEP6 and Clathrin.

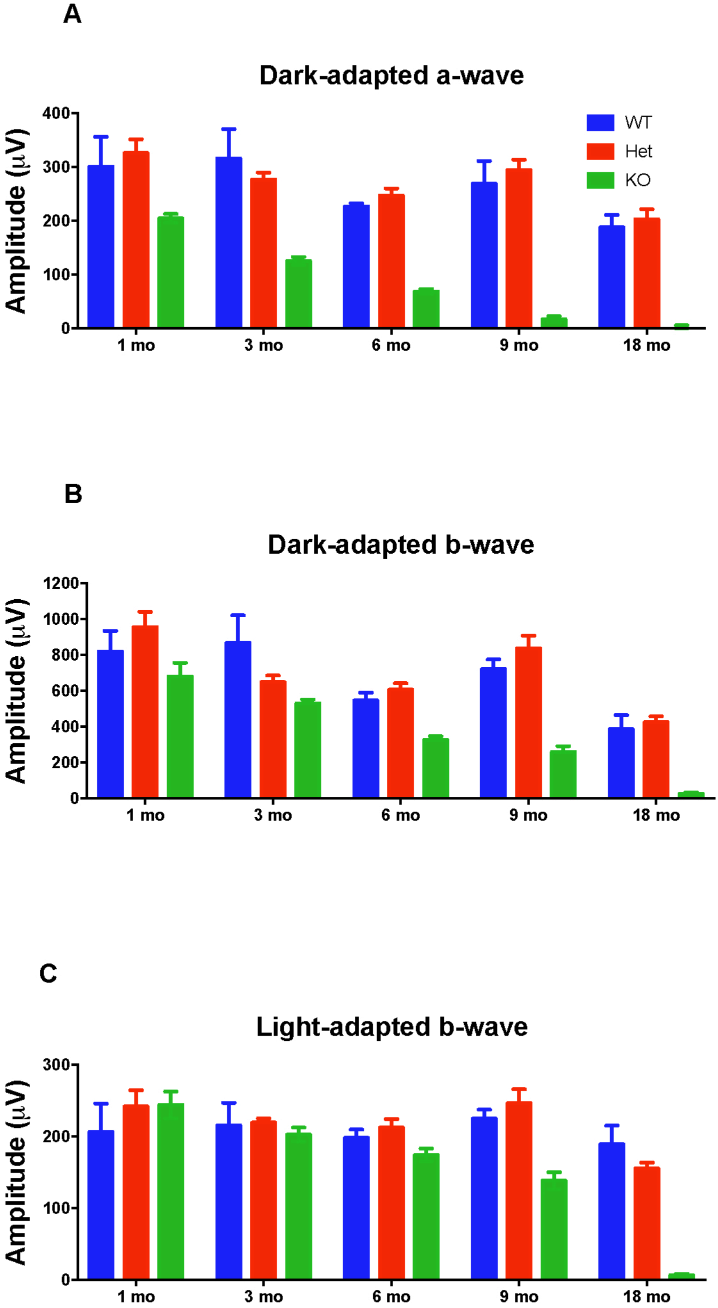
A
Dark-adapted a-wave
Amplitude (μV)
400
300
200
100
0
1 mo
3 mo
6 mo
9 mo
18 mo
WT
Het
KO
B
Dark-adapted b-wave
Amplitude (μV)
1200
1000
800
600
400
200
0
1 mo
3 mo
6 mo
9 mo
18 mo
C
Light-adapted b-wave
Amplitude (μV)
300
200
100
0
1 mo
3 mo
6 mo
9 mo
18 mo

Figure S1

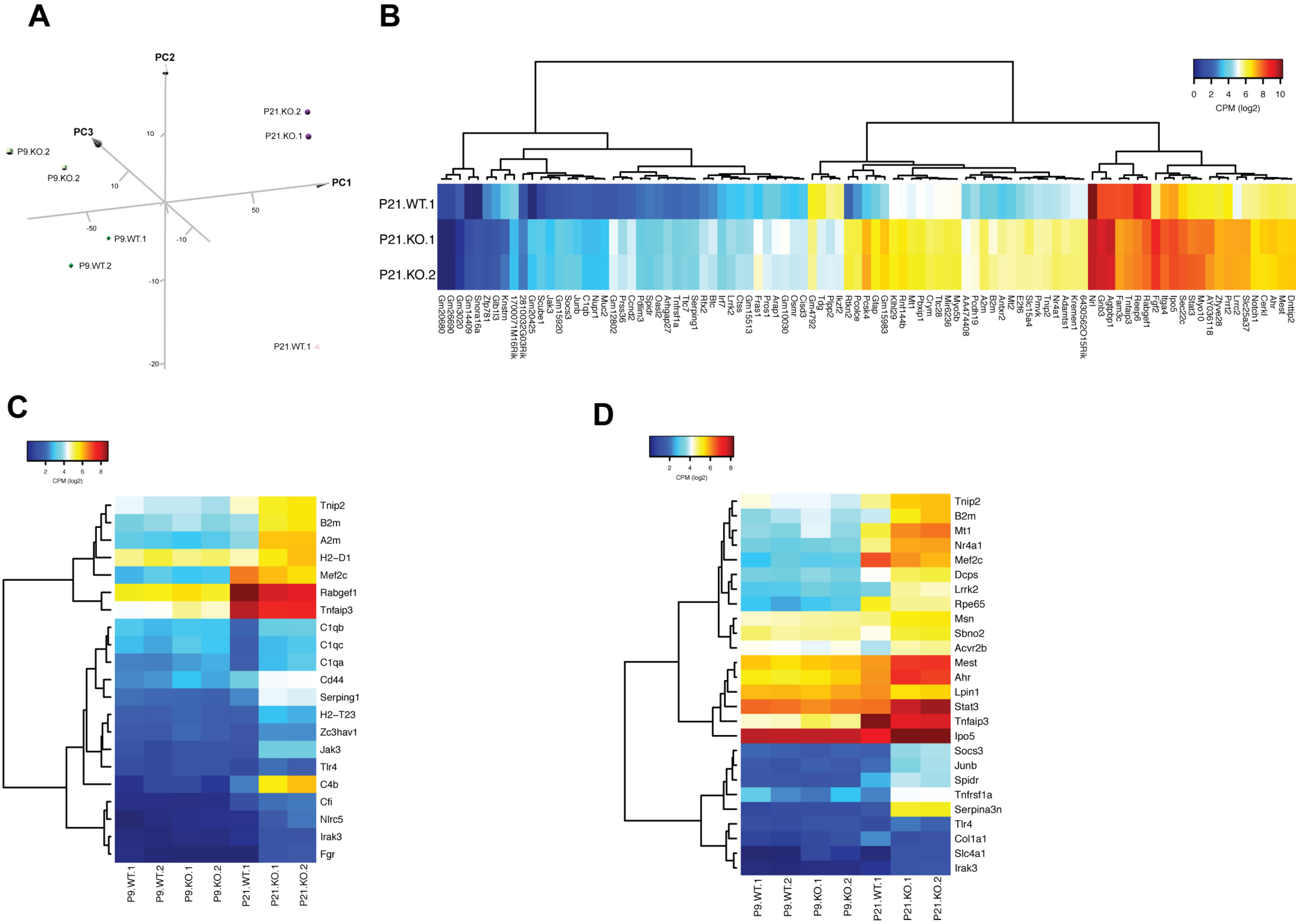
A
PC2
PC3
PC1
10
10
50
-50
-10
-10
-20
P21.KO.2
P21.KO.1
P9.KO.2
P9.KO.2
P9.WT.1
P9.WT.2
P21.WT.1
B
0 2 4 6 8 10
CPM (log2)
P21.WT.1
P21.KO.1
P21.KO.2
Gm20680
Gm26690
Gm3020
Gm14409
Snora16a
Zfp781
Glb1l3
Knstrn
1700071M16Rik
2810032G03Rik
Gm20425
Scube1
Jak3
Gm15920
Socs3
Junb
C1qb
Nupr1
Muc2
Gm12802
Prss36
Ccnd2
Pdlim3
Spidr
Oasl2
Arhgap27
Tnfrsf1a
Ttc7
Serping1
Rfx2
Btc
Irf7
Lrrk2
Ctss
Gm15513
Fras1
Pros1
Arap1
Gm10030
Osmr
Cisd3
Gm4792
Tdg
Plpp2
Ikzf2
Rtkn2
Pcolce
Pcsk4
Gfap
Gm15983
Klhl29
Rnf144b
Mt1
Pbxip1
Crym
Ttc28
Mir6236
Myo5b
AA474408
Pcdh19
A2m
B2m
Antxr2
Mt2
E2f6
Slc15a4
Pmvk
Tnip2
Nr4a1
Adamts1
Kremen1
6430562O15Rik
Nrl
Gnb3
Agtpbp1
Fam3c
Tnfaip3
Reep6
Rabgef1
Fgf2
Itga4
Ipo5
Sec22c
Stat3
Myo10
AY036118
Zfyve28
Prrt2
Lrrc2
Slc25a37
Notch1
Cerkl
Ahr
Mest
Dnttip2
C
2 4 6 8
CPM (log2)
Tnip2
B2m
A2m
H2−D1
Mef2c
Rabgef1
Tnfaip3
C1qb
C1qc
C1qa
Cd44
Serping1
H2−T23
Zc3hav1
Jak3
Tlr4
C4b
Cfi
Nlrc5
Irak3
Fgr
P9.WT.1
P9.WT.2
P9.KO.1
P9.KO.2
P21.WT.1
P21.KO.1
P21.KO.2
D
2 4 6 8
CPM (log2)
Tnip2
B2m
Mt1
Nr4a1
Mef2c
Dcps
Lrrk2
Rpe65
Msn
Sbno2
Acvr2b
Mest
Ahr
Lpin1
Stat3
Tnfaip3
Ipo5
Socs3
Junb
Spidr
Tnfrsf1a
Serpina3n
Tlr4
Col1a1
Slc4a1
Irak3
P9.WT.1
P9.WT.2
P9.KO.1
P9.KO.2
P21.WT.1
P21.KO.1
P21.KO.2

Figure S2

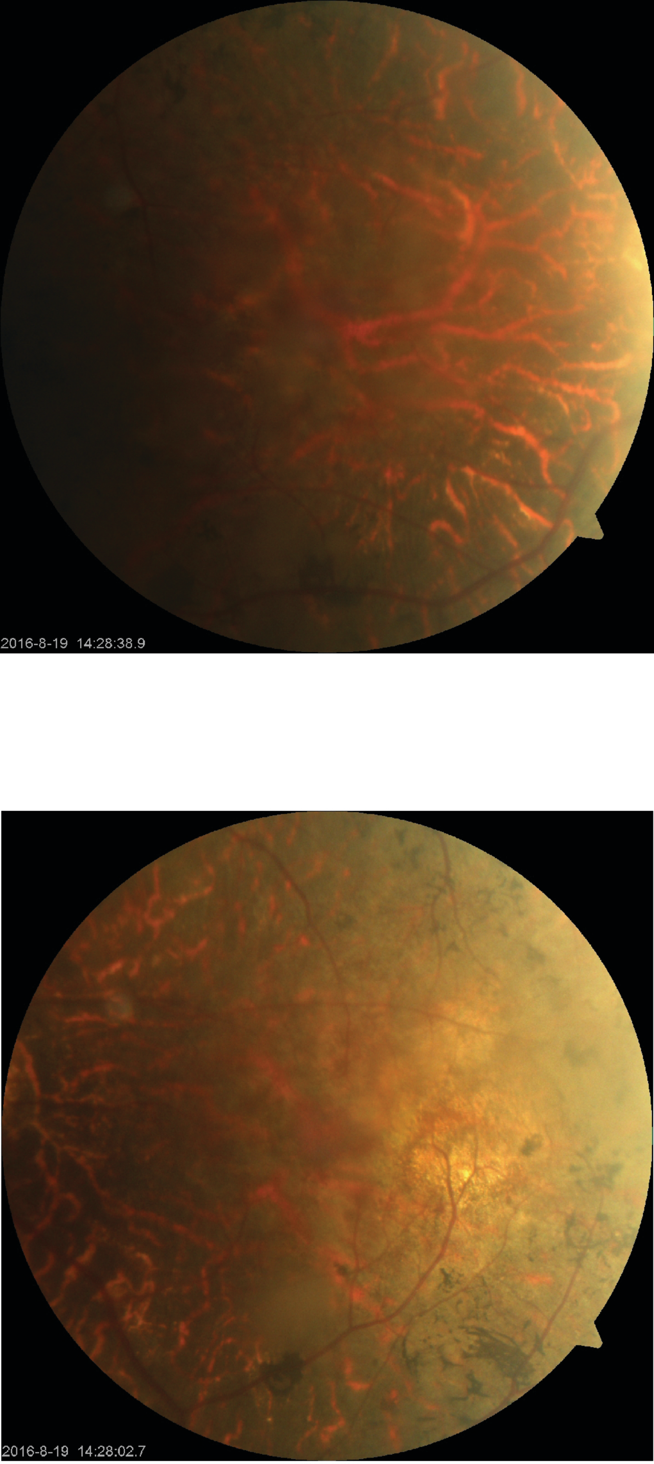

Figure S3

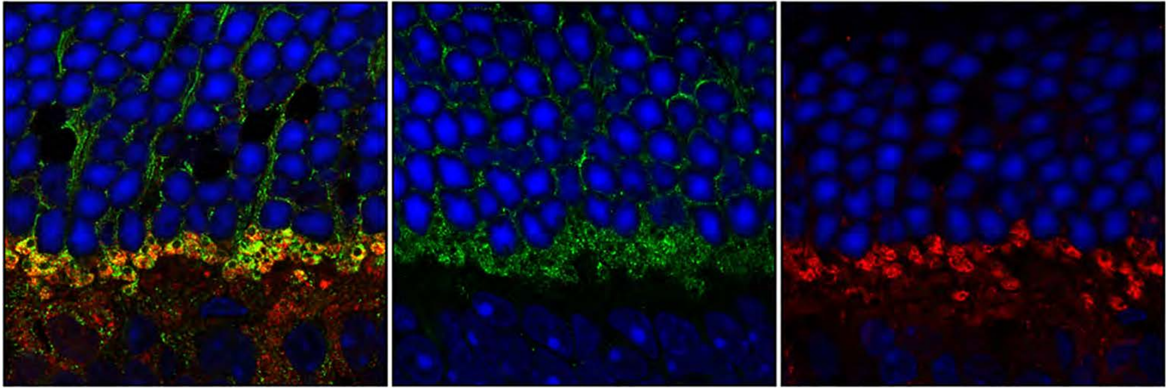

Figure S4

Table S1: Differentially expressed genes comparing PN9 *Reep6*$^{-/-}$ (KO) to WT retina. Listed are averages of count per million (CPM) values for each time point, the fold change, and associated adjusted P-value.

Gene Symbol	P9.WT	P9.KO	P21.WT	P21.KO	Fold Change	Adj P-value
Tdg	193.6	57.3	85.8	28.8	-3.39	2.00E-04
Tdg-ps	0.1	25.1	0.1	9.5	169.56	2.00E-04
Gm9855	0	41.2	0.1	14.9	1074.54	2.00E-04
Rpl30-ps9	0	1.3	0	0	76.27	2.00E-04
Rtkn2	2.6	26.1	3.4	68.4	9.82	2.00E-04
Gm15210	0	2.1	0.2	0	111.4	0.0012
Gm7993	0.3	5.2	4.6	3.7	14.89	0.0012
Gm9493	40.1	9.7	4	4.3	-4.12	0.0016
Gm13304	0	2.4	0	0	123.86	0.0024
Speer5-ps1	0	1.4	0.7	0.9	46.21	0.0024
Prima1	3	0.5	5.3	4.6	-5.5	0.0116
Reep6	113.7	50.3	589.5	200.4	-2.28	0.0219
Dffa	38.6	19.3	38.1	37.3	-2.01	0.022
Plk5	93.9	46.5	104.6	58.8	-2.03	0.022
A930033H14Rik	2	8	44.2	67.6	4.02	0.022
Gm6639	0.2	1.2	1.1	1.2	5.98	0.0223
Gm15920	1.7	18.7	1.6	12.1	13.44	0.0237
Amd2	2.6	13.8	13.4	18.6	5.47	0.0244
A730036I17Rik	0.7	3	3.5	3.9	4.09	0.0362
2610005L07Rik	293.4	177	125.9	120.8	-1.66	0.0362
Gm10575	0.1	2.9	0.8	0.8	29.67	0.0362
Zfp433	59.5	33.7	41.5	26.9	-1.77	0.0362
Ndufa12	50.2	28.2	29.9	16.1	-1.78	0.0375

Table S2A: Differentially expressed genes comparing PN21 *Reep6*$^{-/-}$ (KO) to WT retina. Listed are averages of count per million (CPM) values for each time point, the fold change, and associated adjusted P-value.

Gene Symbol	P9.WT	P9.KO	P21.WT	P21.KO	Fold Change	Adj P-value
Pcsk4	18.2	25.7	10.5	143.9	13.8	0.001
Gfap	21.2	14.7	13.3	93.9	7.12	0.001
Pcolce	4.2	3.8	6.2	65.4	10.64	0.001
Lrrc2	5.2	5.5	31.4	149.6	4.82	0.001
Rtkn2	2.6	26.1	3.4	68.4	20.52	0.001
Fgf2	9.9	14.1	53.2	411.5	7.78	0.0012
Gm15983	1.7	1.6	21.3	116.7	5.52	0.002
A2m	7.6	7	12.8	61.9	4.89	0.0021
Gm12802	22.6	27.7	2.1	30.2	14.63	0.0023
Reep6	113.7	50.3	589.5	200.4	-2.91	0.0023
Ccnd2	2.6	28.6	3.2	28.1	8.74	0.0027
Slc25a37	35.2	37.7	50.3	150.7	3.03	0.0032
Mt2	6.8	7.6	11.6	46.5	4.06	0.0033
Tdg	193.6	57.3	85.8	28.8	-2.94	0.0033
Rnf144b	28.2	31.7	32.9	100	3.08	0.0033
Klhl29	70.3	57.9	33.9	92.7	2.77	0.0052
Antxr2	8.4	7.8	10.6	47.9	4.55	0.0052
Scube1	4.7	4.2	0.8	11	14.09	0.0064
Myo10	121.7	123.3	85.6	230.5	2.72	0.0064
Rfx2	3.4	4.4	5.3	25.7	4.9	0.0064
Arhgap27	2	10.6	4	20.6	5.18	0.0064
Prss36	3.1	6.8	3.1	23.6	7.64	0.0064
Agtpbp1	147	176	304.5	693.9	2.3	0.009
Gnb3	299.7	299.2	307.7	668.7	2.2	0.009
Tnfrsf1a	5.4	4.5	3.7	16.7	4.56	0.009
Ttc7	5.5	6.2	4.2	18.2	4.4	0.009
E2f6	14.8	16.9	18.7	48.8	2.64	0.009
Sec22c	57	51.1	109	260.4	2.41	0.009
Gm15920	1.7	18.7	1.6	12.1	7.41	0.009
Btc	3.6	3.1	6	26.4	4.45	0.009
Gm20425	0.6	2	0	9.9	154.9	0.009
Serping1	3.2	2.8	4.3	18.1	4.28	0.0093
Fras1	12.5	12.2	8.7	37.5	4.32	0.0103
Nr4a1	8.2	9.6	24.7	59	2.42	0.0104
Pbxip1	27.3	29	33.6	76.5	2.3	0.0126
Osmr	4	4.6	9.3	26.9	2.91	0.0128
Zfyve28	15.1	18.3	70.1	141.3	2.04	0.0128

Dnttip2	119.5	113.3	58.3	119.7	2.08	0.0129
Stat3	82.1	72.6	79.5	229.4	2.9	0.0132
Ipo5	217	212.8	160.1	305	1.93	0.0132
Mt1	10.6	13.3	30.1	73.7	2.47	0.0132
Jak3	1.1	1.4	1.5	9.3	6.11	0.0132
Socs3	2.6	2.1	2.3	12	5.21	0.0132
Cisd3	20.2	16.3	10.1	27	2.71	0.0132
Gm26690	1.3	0.9	0.9	0	-52.56	0.0132
Notch1	92.9	66.4	52	102.1	1.99	0.0157
Gm20680	1.5	0.8	0.8	0	-46.46	0.0157
Snora16a	0.8	1.7	0	2.1	124.93	0.0163
Fam3c	96.6	95.4	293.6	157.2	-1.85	0.0169
Muc2	7.8	10.9	2.2	8.9	4.02	0.0259
Mest	45.7	50	62.3	115.5	1.88	0.0259
Mir6236	39	47.3	35.2	67.5	1.94	0.0259
Gm3020	2.1	1.7	2.8	0.4	-7.05	0.0268
Tnip2	17.4	15.2	23.8	48.9	2.07	0.0272
Oasl2	0.9	4.1	4.5	15.8	3.52	0.0274
Gm10030	1.2	0.8	9.8	31.3	3.2	0.0295
Nupr1	0.9	0.9	2.2	8.4	3.82	0.03
B2m	11.9	13.7	14	45	3.21	0.03
Crym	122.9	117.2	31.7	62.6	1.99	0.0306
Zfp781	2.1	2.8	5.5	1.4	-3.74	0.0306
Pdlim3	2.5	2.2	4.3	12.7	3.01	0.0315
Gm14409	0.7	0.4	0	1.2	72.75	0.0315
Kremen1	27	26.1	29.7	55.4	1.89	0.0327
6430562O15Rik	2.1	1.8	30.1	55.8	1.88	0.0327
Myo5b	15.8	15	35.9	68.4	1.93	0.0339
Pmvk	13.5	15	24.8	46.7	1.9	0.0363
Junb	1.6	2.1	2.9	11.3	3.96	0.0379
Ttc28	63.6	67.6	34.7	65.4	1.91	0.039
Slc15a4	50.4	40.8	22.3	54.2	2.44	0.0413
Glb1l3	2.5	2.6	7	2.2	-3.05	0.0413
Ikzf2	12.3	13.3	55.1	30.1	-1.81	0.0422
Itga4	85.7	106.1	143	256.2	1.81	0.0422
Cerkl	39.6	46.4	58.9	111.7	1.91	0.0446
Ahr	38.5	43.8	64.1	115.4	1.82	0.0467
Tnfaip3	20.1	26.2	299.8	179.7	-1.65	0.0467
Adamts1	7.4	7.9	27	53.2	1.99	0.0467
Pros1	8.8	10	12.9	27.8	2.18	0.0467
Rabgef1	45.2	49	420.8	244.2	-1.71	0.0467
Knstrn	1.2	1.4	12.1	3.9	-3.11	0.0467
Arap1	42.6	47.2	12.7	29.8	2.37	0.0467

Lrrk2	6.4	7.8	9.7	21.5	2.24	0.0467
C1qb	6.5	6.8	2.8	8.4	3.03	0.0467
Nrl	775.5	815.7	1194.9	744.5	-1.59	0.0467
Spidr	1.7	1.6	5	12.9	2.6	0.0467
Gm4792	17.3	9.5	91.7	52.2	-1.74	0.0467
AA474408	23.2	24.5	16.5	34.6	2.12	0.0467
Gm15513	2.6	2.8	10.7	22.8	2.14	0.0467
AY036118	116.7	130.5	91.3	193.2	2.13	0.0467
Irf7	1.5	3.2	7.8	17.2	2.24	0.049
2810032G03Rik	2.4	2.1	1.3	5.1	3.84	0.049
Ctss	11.6	12.5	9.3	22.4	2.43	0.049
Prrt2	93.1	90	94.9	152.9	1.63	0.049
Pcdh19	37.9	39	21.7	39.8	1.85	0.049
1700071M16Rik	4.9	5	17.4	8.4	-2.05	0.049

Table S2B: Enrichment of biological process gene ontologies for the PN21 differentially expressed genes. Listed are end-of-branch terms and their significance values.

GO Term	Description	Ontology	Genes in Ont	DE Genes	P-value	FDR
GO:0032102	negative regulation of response to external stimulus	BP	210	9	1.63E-06	0.0042
GO:1903035	negative regulation of response to wounding	BP	108	7	1.65E-06	0.0021
GO:0019221	cytokine-mediated signaling pathway	BP	199	8	1.03E-05	0.0053
GO:0031348	negative regulation of defense response	BP	109	6	2.43E-05	0.0090
GO:0050778	positive regulation of immune response	BP	297	9	2.69E-05	0.0087
GO:0001869	negative regulation of complement activation, lectin pathway	BP	2	2	2.79E-05	0.0072
GO:0060548	negative regulation of cell death	BP	740	14	3.03E-05	0.0071
GO:1901700	response to oxygen-containing compound	BP	857	15	3.73E-05	0.0080
GO:0050727	regulation of inflammatory response	BP	176	7	4.08E-05	0.0081
GO:0006959	humoral immune response	BP	73	5	4.22E-05	0.0078
GO:0002698	negative regulation of immune effector process	BP	78	5	5.81E-05	0.0094

Table S3: Expression, fold change, and adjusted P-value for the rod and cone signal transduction pathway genes that are differentially expressed at PN21.

Gene Symbol	P9.WT	P9.KO	P21.WT	P21.KO	Fold Change	Adj P-value
Rod Genes						
Rho	3045	4047.2	33264.1	22643.4	-1.45	0.127
Gnat1	173.2	278	8913.8	6670	-1.32	0.224
Gnb1	3321.5	3354.9	5279.3	3512.1	-1.49	0.07
Gngt1	147.3	178.2	717.8	530.4	-1.34	0.194
Pde6a	64.7	106.8	2345.9	1902.1	-1.22	0.321
Pde6b	913.2	1066	2304	1496.1	-1.52	0.06
Pde6g	280.1	336	1436	1045	-1.36	0.136
Cnga1	1849.8	2202.8	3396.3	2363	-1.42	0.136
Cngb1	536.2	579.6	757.3	585.5	-1.28	0.22
Slc24a1	59	102.6	1506.1	1101.4	-1.35	0.136
Guca1a	19.9	22.2	302	250.6	-1.19	0.435
Guca1b	9.2	18	875.9	653.4	-1.33	0.195
Gucy2f	96.3	112.2	401.7	264.3	-1.51	0.126
Cone Genes						
Opn1sw	159.2	192.2	439.5	343.9	-1.26	0.253
Opn1mw	1.9	5.1	167.7	183.3	1.11	0.684
Gnat2	37.6	46.7	216.9	186.8	-1.15	0.551
Gnb3	299.7	299.2	307.7	668.7	2.2	0.009
Gngt2	83.3	59.5	48.6	43.2	-1.11	0.717
Pde6c	47.1	51.8	166	161.9	-1.01	0.969
Pde6h	139.3	148.9	503.8	408	-1.22	0.351
Slc24a2	28	31.5	104.8	113.3	1.09	0.738
Cnga3	9.1	8.2	15.3	14.3	-1.05	0.912
Cngb3	20.5	19.8	35.5	30.6	-1.15	0.641
Arr3	12.8	11.5	311.8	287.5	-1.07	0.792
Photoreceptor Genes						
Gnb5	183	261.8	388.1	353.5	-1.09	0.771
Rgs9	476.8	544.6	762	672	-1.12	0.614
Rgs9bp	166.3	207.8	920.5	685.6	-1.33	0.164
Sag	1790.1	2093.4	4864.8	3823.7	-1.26	0.275
Grk1	175.9	254.9	2203.1	1952.3	-1.12	0.634
Rcvrn	75.8	115.1	645.7	592.1	-1.08	0.787

Table S4A. The frequency of variant E75K in various databases.

Database	Allele	Frequency
ExAC	8	6.654e-05 (8/120224)
ESP6500	2	0.0001538 (2/13004)
1000G	0	0

Table S4B. Population specific AMF of E75K rare variant in ExAC database

Population	Allele count	Allele number	Number of homozygotes	AMF
African	6	10296	0	0.0005828
East Asian	2	8638	0	0.0002315
European (Finnish)	0	6604	0	0
European (Non-Finnish)	0	65746	0	0
Latino	0	11544	0	0
Other	0	896	0	0
South Asian	0	16500	0	0
Total	**8**	**120224**	**0**	**6.65E-05**

Table S4C. Damage prediction of the variant E75K.

Software	Score	Prediction
Polyphen2_HDIV	1	Probably damaging
Polyphen2_HVAR	0.992	Probably damaging
MutationTaster	1	Disease causing
SIFT	0.2	Tolerable
LRT	-	-
MutationAssessor	2.27	Medium
FATHMM	-3.13	Damaging
RadialSVM	-0.14	Tolerable
LR	0.393	Tolerable
VEST3	0.799	Damaging
CADD	21.4	Damaging
GERP++	4.53	Conserved
phyloP100way	9.668	Conserved
SiPhy_29way	16.28	Conserved