SUPPLEMENT

Protein misfolding and the pathogenesis of *ABCA4*-associated retinal degenerations

Ning Zhang[1,§], Yaroslav Tsybovsky[1,§], Alexander V. Kolesnikov[2], Malgorzata Rozanowska[3], Malgorzata Swider[4], Sharon B. Schwartz[4], Edwin M. Stone[5,6], Grazyna Palczewska[7], Akiko Maeda[3], Vladimir J. Kefalov[2], Samuel G. Jacobson[4], Artur V. Cideciyan[4], and Krzysztof Palczewski[1,*]

From the Departments of [1]Pharmacology and Cleveland Center for Membrane and Structural Biology; [2]Ophthalmology & Visual Sciences, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8096, Saint Louis, MO 63110, USA; [3]Ophthalmology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, USA; [4]Scheie Eye Institute, Department of Ophthalmology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [5]Department of Ophthalmology, University of Iowa Carver College of Medicine, Iowa City, IA, USA; [6]Howard Hughes Medical Institute, Iowa City, IA, USA; and [7]Polgenix, Inc., 11000 Cedar Ave, Suite 260, Cleveland, OH 44106, USA

[8]**Correspondence to:** Krzysztof Palczewski, Ph.D., or Yaroslav Tsybovsky, Ph.D. Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44160, USA. Tel: +1−216−368−4631; Fax: +1−216−368−1300. Email: kxp65@case.edu or yxt76@case.edu.

Figure S1

RNA-Seq identified 474 significantly differentially expressed genes between *Abca4*$^{-/-}$ and WT, 290 between *Abca4*$^{PV/PV}$ and WT, and 323 between *Abca4*$^{-/-}$ and *Abca4*$^{PV/PV}$ mouse retina samples. Also, RNA-Seq identified 430 significantly differentially expressed genes between *Abca4*$^{-/-}$ and WT, 354 between *Abca4*$^{PV/PV}$ and WT, and 347 between *Abca4*$^{-/-}$ and *Abca4*$^{PV/PV}$ RPE samples in total. A considerable number of genes were found significantly differentially expressed in one genotype *versus* the other two genotypes in both retinal and RPE samples. Fifty-eight genes in the retina samples and fifty-three genes in the RPE samples were significantly differentially expressed among all three genotypes. These statistics are based on all significant genes that RNA-Seq detected including those sparsely expressed (low FPKM genes).

Figure S2

Genes with a two-fold or greater up- or down-regulation (Log 2 <-1 or >1) that also exhibited considerable expression levels (FPKM>10) in retina transcriptomes. RNA-Seq identified 31 significantly differentially expressed genes in *Abca4*$^{-/-}$ *versus* WT, 21 in *Abca4*$^{PV/PV}$ *versus* WT and 25 in *Abca4*$^{-/-}$ *versus Abca4*$^{PV/PV}$ RPE samples. Individual genes are listed on the *right*.

Figure S3

Two-fold or greater up- or down-regulated genes (Log2 <-1 or >1) which also had considerable expression levels (FPKM>10) in mouse RPE transcriptomes. RNA-Seq identified 28 significantly differentially expressed genes in knockout versus wild-type, 18 in dual knock-in versus wild-type and 6 in knockout versus dual knock-in RPE samples. Individual genes are listed on the *right*.

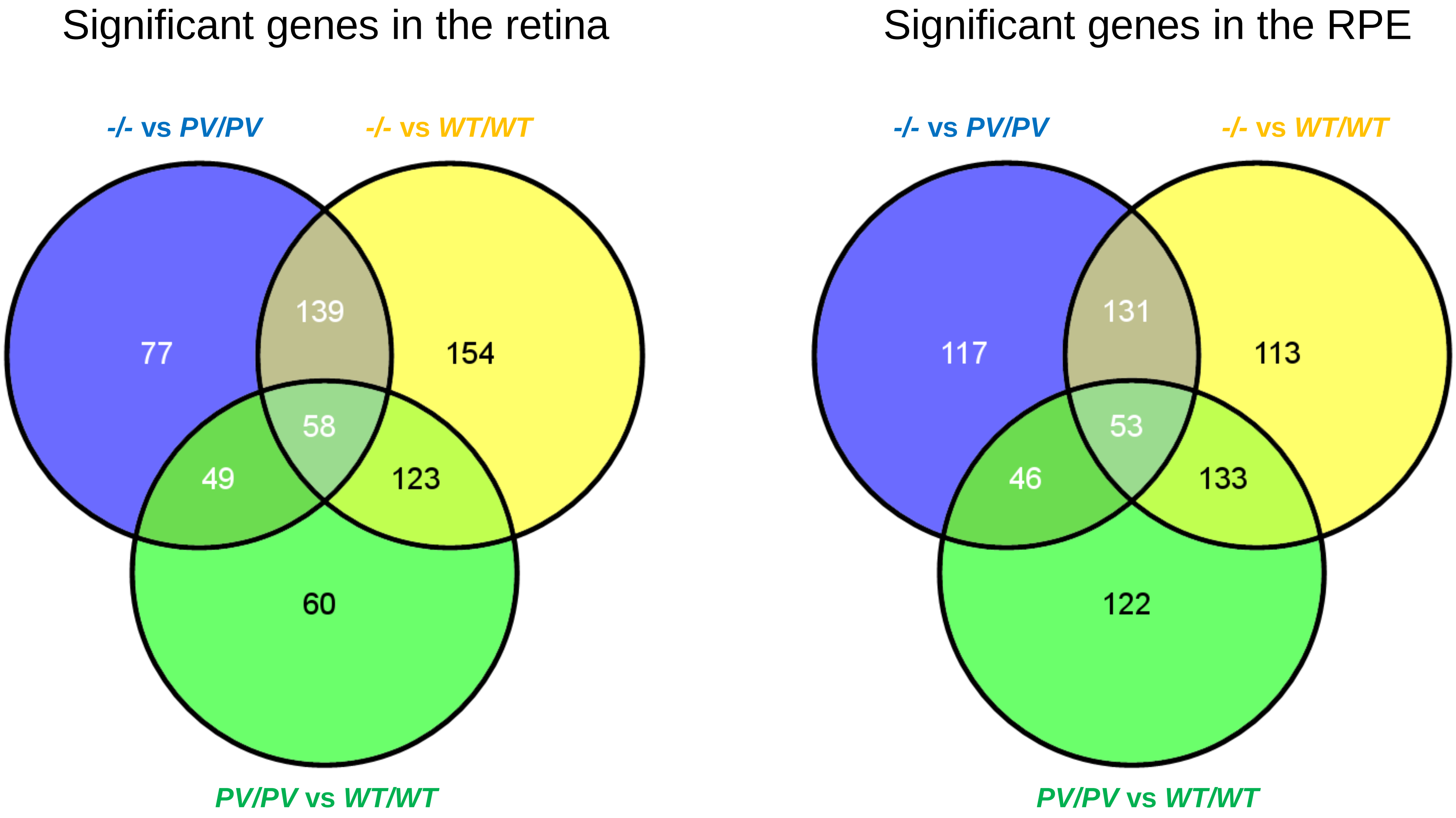
Figure S4

Immunohistochemistry with GFAP labeling (*green*) reveals a similar pattern in 1-month-old (**A**) and 6-month-old (**B**) *Abca4*$^{PV/PV}$, *Abca4*$^{-/-}$ and WT mouse retinas indicating that Müller cells are not activated in the absence or upon mutation of ABCA4 in the retina. This observation contrasts with the robust GFAP signals evident in the retina of a 3-month-old rhodopsin E150K mouse which served as a model for retinitis pigmentosa. DAPI was used to counter-stain nuclei. Scale bars: 40 μm. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Figure S5

Rod photo-response recovery kinetics after photo-bleaching determined from ERGs of anesthetized 1-month-old and 8-month-old *Abca4*$^{PV/PV}$, *Abca4*$^{-/-}$ or WT mice. (**A**) Representative full-field scotopic ERG responses from 1-month-old mice at the light intensity of -0.286 (log cd × s / m^2) before and at various times after photo-bleaching. Scotopic ERG a-wave amplitude recovery after >90% bleaching of rod visual pigment was slightly but not statistically significantly delayed in *Abca4* mutant mice both at 1 (**B**) and 8 (**C**) months of age. The results are presented with standard errors.

Figure S1

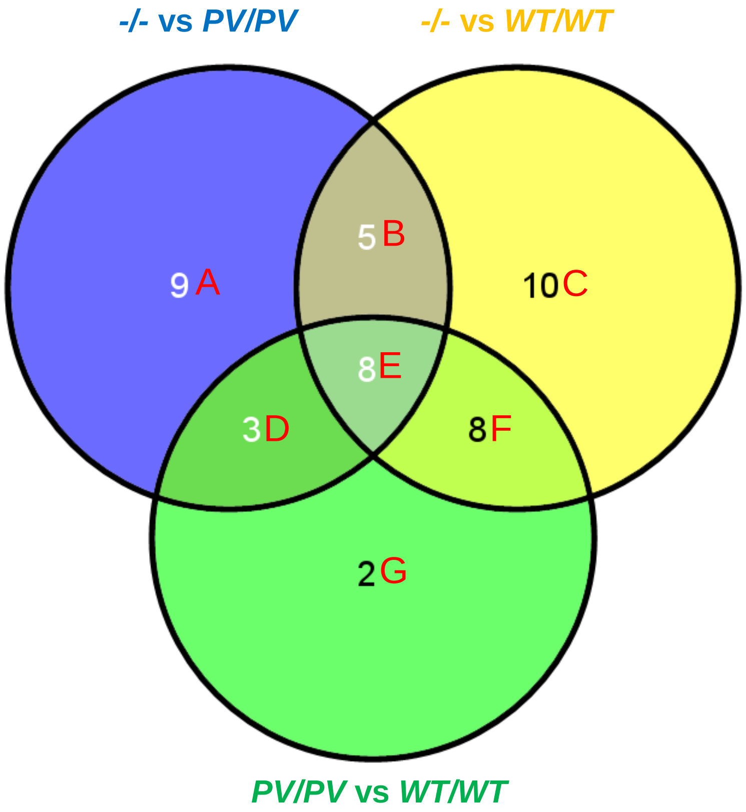


Significant genes in the retina

Significant genes in the RPE

Figure S2

Significant genes in the retina
(FPKM>10, Log2<-1 or >1)



A: *Nupr1, Grifin, Crygn, Cd24a, Lim2, Ndufs5, Pop4, Mip, Glo1*

B: *Trf, Rnu12, Dusp1, Abca4, BC002163*

C: *Cryge, Fabp5, Gstm1, Dapl1, Rwdd4a, S100a6, Tuba1c, Beta-s, Gm16119, Fxyd6*

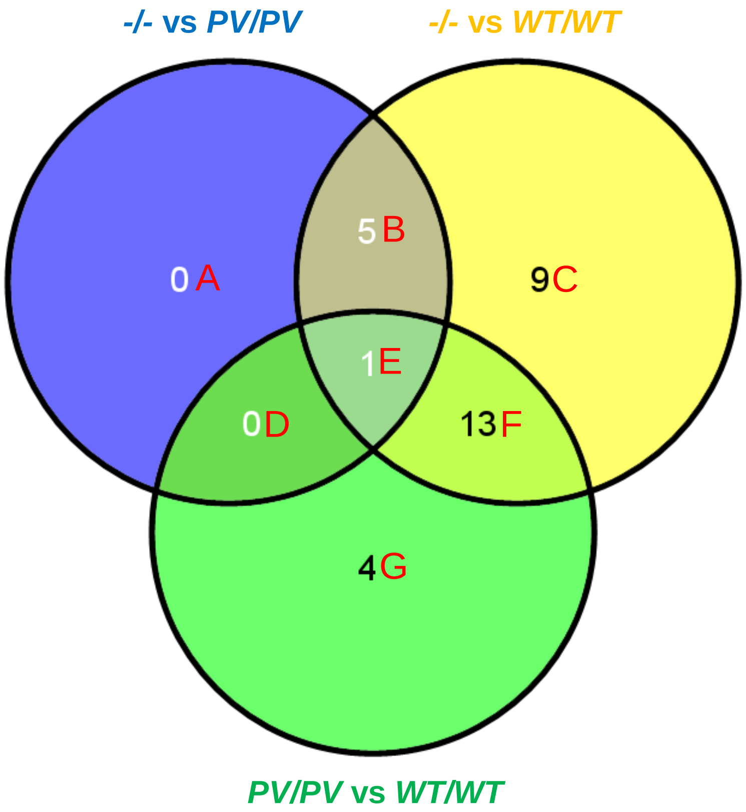
D: *Gpsm3, Gm1821, Ssbp4*

E: *Cryba1, Crygs, Crybb2, Cryba2, Cryab, Cryba4, Crybb1, Crybb3*

F: *Cryaa, Crygc, Ptgds, Crygb, Crygd, Cryga, Cd59a, Spc25*

G: *Xist, Gm5506*

Significant genes in the RPE
(FPKM>10, Log2<-1 or >1)



B: *Gpx3, Mrps12, Gpr180, Krt12, Retnla*

C: *Fkbp5, A130040M12Rik, Hbb-b2, 4930583H14Rik, Crygc, Pde6a, Rpph1, Hist4h4, Pde6b*

E: *Gm1821*

F: *Sag, Cryba2, Cryba4, Crybb1, Gnat1, Crybb3, Pde6g, Rcvrn, Gngt1, Pdc, Nrl, Prph2, Rho*

G: *Mbp, Cryga, Crygs, Crygf*

Figure S4

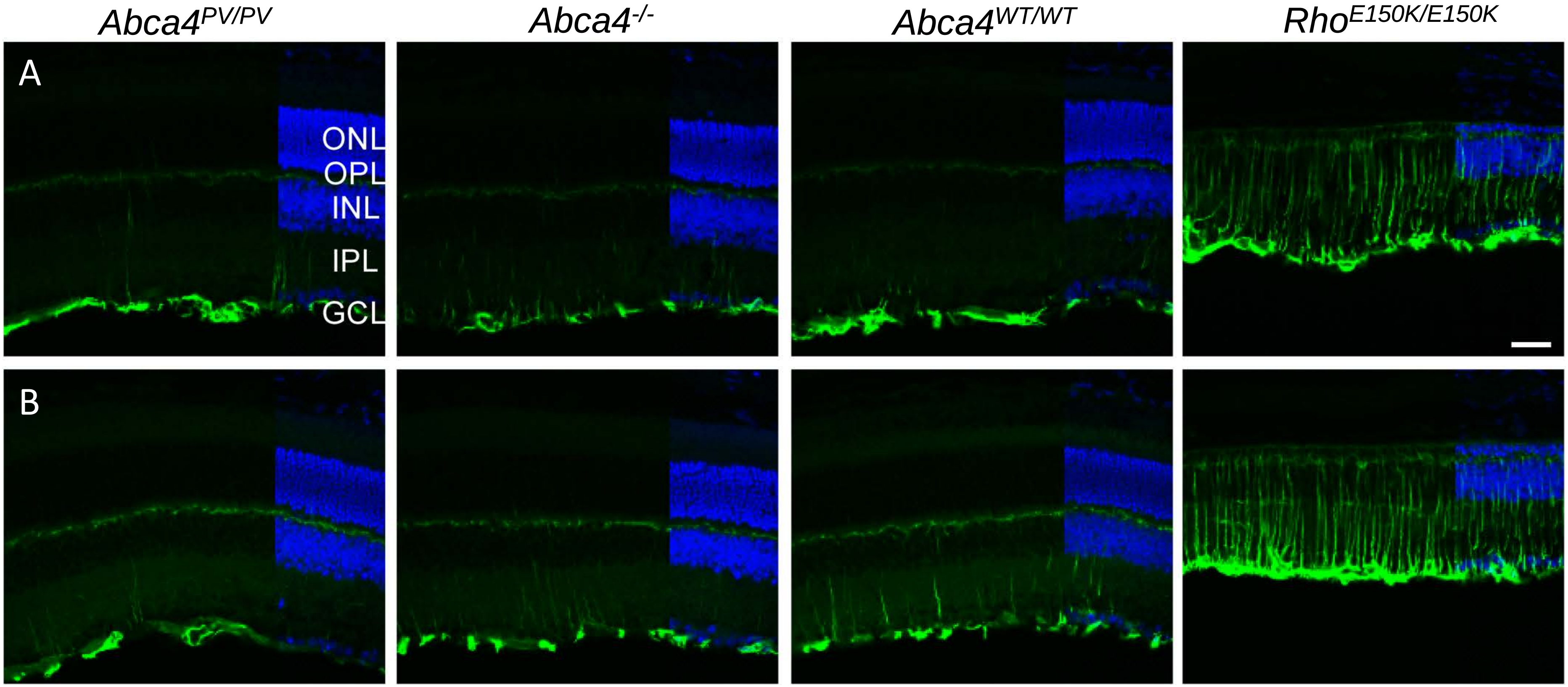

Abca4$^{PV/PV}$
Abca4$^{-/-}$
Abca4$^{WT/WT}$
Rho$^{E150K/E150K}$
A
ONL
OPL
INL
IPL
GCL
B

Figure S5

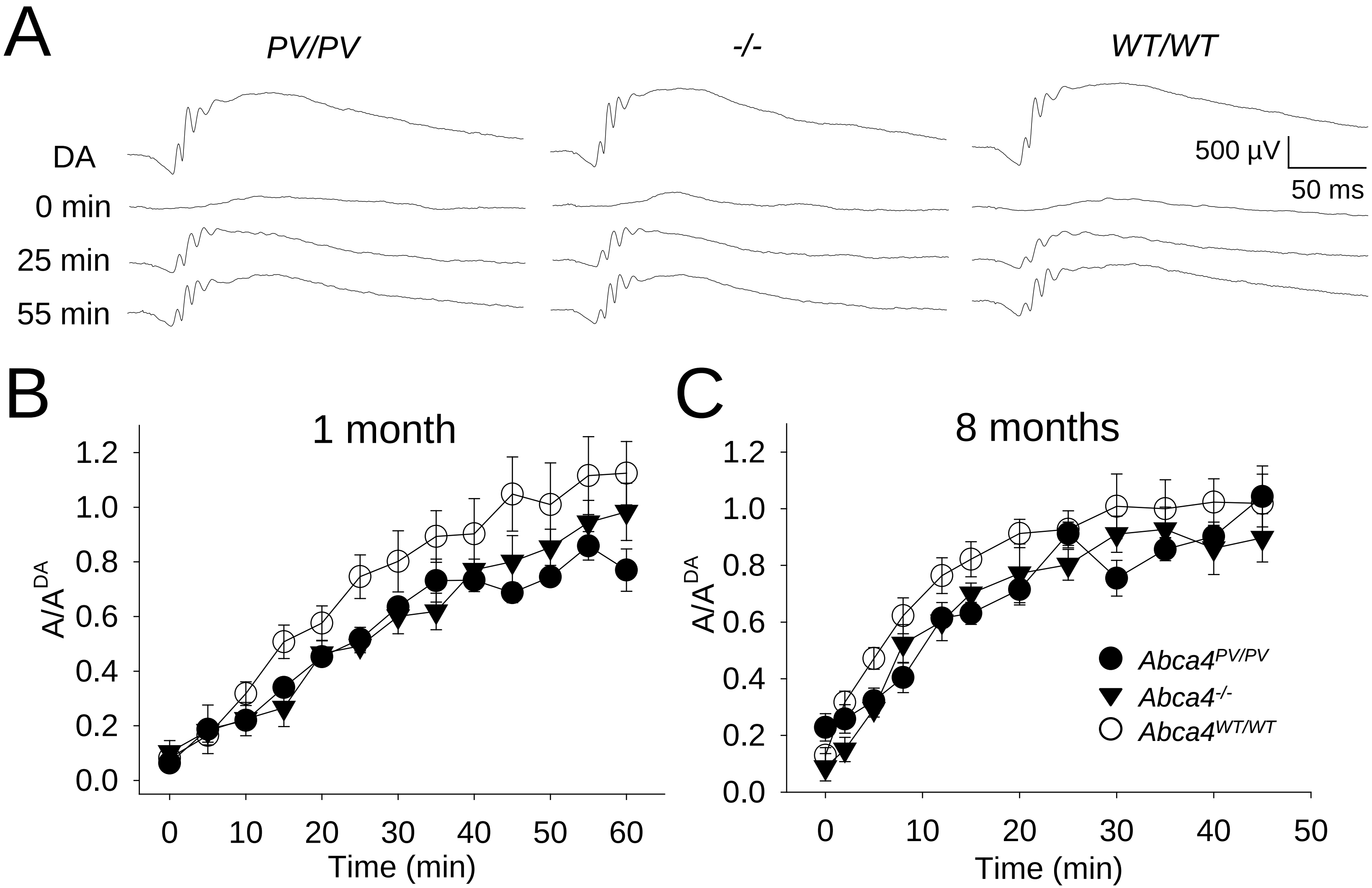

A
PV/PV
-/-
WT/WT
DA
0 min
25 min
55 min
500 µV
50 ms
B
1 month
A/A^{DA}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Time (min)
C
8 months
A/A^{DA}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Time (min)
● Abca4$^{PV/PV}$
▼ Abca4$^{-/-}$
○ Abca4$^{WT/WT}$

TABLE S1. Genes with altered expression in mouse retinas.

Gene[1]	**Name**	**FPKM**			**Log2 fold change (q value)**		
		WT	*Abca4*$^{-/-}$	*Abca4*$^{PV/PV}$	*Abca4*$^{-/-}$ vs. WT	*Abca4*$^{PV/PV}$ vs. WT	*Abca4*$^{PV/PV}$ vs. *Abca4*$^{-/-}$
Nupr1	nuclear protein transcription regulator 1	5.46	66.91	24.31 24.309	3.61 (0.0029)	2.15 (0.0029)	-1.46 (0.0029)
Grifin	galectin-related inter-fiber protein	1.16	29.40	10.98	4.66 (0.0029)	3.24 (0.0029)	-1.42 (0.0029)
Crygn	crystallin, gamma N	1.78	48.04	17.96	4.75 (0.0029)	3.33 (0.0029)	-1.42 (0.0029)
Cd24a	CD24a antigen	9.41	66.16	25.75	2.81 (0.0029)	1.45 (0.0029)	-1.36 (0.0029)
Lim2	lens intrinsic membrane protein 2	1.96	36.88	15.45	4.23 (0.0029)	2.98 (0.0029)	-1.26 (0.0029)
Ndufs5	NADH dehydrogenase (ubiquinone) Fe-S protein 5	49.30	95.75	43.90	0.96 (0.0029)	-0.17 (0.9993)	-1.13 (0.0029)
Pop4	processing of precursor 4, ribonuclease P/MRP family, (S. cerevisiae)	8.03	26.02	12.05	1.70 (0.0029)	0.58 (0.0055)	-1.11 (0.0029)
Mip	major intrinsic protein of eye lens fiber	1.15	74.06	35.05	6.01 (0.0029)	4.93 (0.0029)	-1.08 (0.0029)
Glo1	glyoxalase 1	258.24	342.00	168.53	0.41 (0.0762)	-0.61 (0.0029)	-1.02 (0.0029)
Trf	transferrin	19.55	45.49	19.46	1.22 (0.0029)	-1.22(0.0029)	-0.006 (0.9993
Rnu12	RNA U12, small nuclear	191.62	70.86	142.86	-1.44 (0.0029)	-0.42 (0.9310)	1.01 (0.0478)
Dusp1	dual specificity phosphatase 1	30.47	14.07	29.90	-1.11 (0.0029)	-0.027 (0.9310)	1.088 (0.0029)
Abca4	ATP-binding cassette, sub-family A (ABC1), member 4	253.68	78.66	203.91	-1.69 (0.0029)	-0.32 (0.8862)	1.374 (0.0029)
BC002163	cDNA sequence BC002163	90.59	15.49	88.62	-2.55 (0.0029)	-0.03 (0.9993)	2.52 (0.0029)
Cryge	crystallin, gamma E	36.58	101.53	62.36	1.47 (0.0029)	0.77 (0.0029)	-0.70 (0.0029)
Fabp5	fatty acid binding protein 5, epidermal	35.62	86.35	54.30	1.28 (0.0029)	0.61 (0.0029)	-0.67 (0.0029)
Gstm1	glutathione S-transferase, mu 1	10.94	25.09	15.39	1.20 (0.0029)	0.49 (0.0401)	-0.70 (0.0029)
Dapl1	death associated protein-like 1	31.96	69.47	37.62	1.12 (0.0029)	0.24 (0.9425)	-0.88 (0.0029)
Rwdd4a	RWD domain containing 4A	24.32	52.74	41.51	1.12 (0.0029)	0.77 (0.0029)	-0.34 (0.0699)
S100a6	S100 calcium binding protein A6	11.32	22.80	16.82	1.01 (0.0029)	0.57 (0.0852)	-0.44 (0.2426)
Tuba1c	tubulin, alpha 1C	44.47	21.85	43.23	-1.03 (0.0029)	-0.04 (0.9993)	0.98 (0.0029)
Beta-s	Hbb-bs, hemoglobin, beta adult s chain	81.52	37.89	44.21	-1.11 (0.0029)	-0.88 (0.0029)	0.22 (0.9717)
Gm16119	predicted gene 16119	53.63	21.87	30.55	-1.29 (0.0029)	-0.81 (0.6681)	0.48 (0.9993)
Fxyd6	FXYD domain-containing ion transport regulator 6	34.71	12.97	22.34	-1.42 (0.0029)	-0.64 (0.0029)	0.78 (0.0029)
Gpsm3	G-protein signalling modulator 3 (AGS3-like, C. elegans)	11.27	11.78	23.90	0.064 (0.9993)	1.08 (0.0029)	1.02 (0.0029)
Gm1821	**predicted gene 1821 (pseudo)**	**29.23**	**46.72**	**100.11**	**0.68 (0.0029)**	**1.78 (0.0029)**	**1.10 (0.0029)**
Ssbp4	single stranded DNA binding protein 4	39.96	40.73	89.08	0.027 (0.9993)	1.16 (0.0029)	1.13 (0.0029)

Cryba1	crystallin, beta A1	53.50	1547.41	494.22	4.85 (0.0029)	3.21 (0.0029)	-1.65 (0.0029)
Crygs	**crystallin, gamma S**	**35.71**	**706.20**	**251.70**	**4.31 (0.0029)**	**2.82 (0.0029)**	**-1.49 (0.0029)**
Crybb2	crystallin, beta B2	57.60	1372.33	492.96	12.33 (0.0029)	9.76 (0.0029)	-3.54 (0.0029)
Cryba2	**crystallin, beta A2**	**24.04**	**614.04**	**229.78**	**4.67 (0.0029)**	**3.26 (0.0029)**	**-1.42 (0.0029)**
Cryab	crystallin, alpha B	39.98	644.73	254.07	4.01 (0.0029)	2.67 (0.0029)	-1.34 (0.0029)
Cryba4	**crystallin, beta A4**	**18.90**	**347.71**	**140.16**	**4.20 (0.0029)**	**2.89 (0.0029)**	**-1.31 (0.0029)**
Crybb1	**crystallin, beta B1**	**20.17**	**286.54**	**127.77**	**3.83 (0.0029)**	**2.66 (0.0029)**	**-1.17 (0.0029)**
Crybb3	**crystallin, beta B3**	**18.60**	**258.71**	**123.47**	**3.80 (0.0029)**	**2.73 (0.0029)**	**-1.07 (0.0029)**
Cryaa	crystallin, alpha A	82.14	2070.94	800.27	4.66 (0.0029)	3.28 (0.0029)	-1.37 (0.0510)
Crygc	**crystallin, gamma C**	**52.93**	**221.52**	**126.98**	**2.07 (0.0029)**	**1.26 (0.0029)**	**-0.80 (0.0029)**
Ptgds	prostaglandin D2 synthase (brain)	44.01	166.76	114.73	1.92 (0.0029)	1.38 (0.0029)	-0.54 (0.0029)
Crygb	crystallin, gamma B	50.16	185.44	109.44	1.89 (0.0029)	1.13 (0.0029)	-0.76 (0.0029)
Crygd	crystallin, gamma D	31.51	88.03	73.51	1.48 (0.0029)	1.22 (0.0029)	-0.26 (0.6297)
Cryga	crystallin, gamma A	10.61	25.71	23.02	1.28 (0.0029)	1.12 (0.0029)	-0.16 (0.9993)
Cd59a	CD59a antigen	35.25	12.52	15.64	-1.49 (0.0029)	-1.17 (0.0029)	0.32 (0.3230)
Spc25	SPC25, NDC80 kinetochore complex component, homolog (S. cerevisiae)	156.92	36.90	63.40	-2.09 (0.0029)	-1.31 (0.0029)	0.78 (0.0029)
Xist	inactive X specific transcripts	23.77	37.92	63.74	0.67 (0.1631)	1.42 (0.0029)	0.75 (0.0055)
Gm5506	Eno1b, enolase 1B, retrotransposed	274.73	184.79	133.48	-0.57 (0.0029)	-1.04 (0.0029)	-0.47 (0.0029)

[1] Genes shared by RPEs are shown in bold type.

TABLE S2. Genes with altered expression in mouse RPEs

Gene[1]	**Name**	**FPKM**			**Log2 fold change (q value)**		
		WT	*Abca4*$^{-/-}$	*Abca4*$^{PV/PV}$	*Abca4*$^{-/-}$ vs. WT	*Abca4*$^{PV/PV}$ vs. WT	*Abca4*$^{PV/PV}$ vs. *Abca4*$^{-/-}$
Gpx3	glutathione peroxidase 3	128.76	279.12	69.74	1.12 (0.0035)	-0.88 (0.0035)	-2.00 (0.0035)
Mrps12	mitochondrial ribosomal protein S12	16.36	34.64	13.99	1.08 (0.0035)	-0.23 (0.9992)	-1.31 (0.0035)
Gpr180	G protein-coupled receptor 180	75.00	35.84	72.78	-1.07 (0.0035)	-0.043 (0.9992)	1.02 (0.0035)
Krt12	keratin 12	181.18	68.02	140.75	-1.41 (0.0035)	-0.36 (0.1499)	1.05 (0.0035)
Retnla	resistin like alpha	46.93	20.10	53.23	-1.22 (0.0035)	0.18 (0.9992)	-1.40 (0.0035)
Fkbp5	FK506 binding protein 5	10.50	23.12	7.57	1.14 (0.0035)	-0.47 (0.0460)	-1.61 (0.0035)
A130040M12Rik	RIKEN cDNA A130040M12 gene	32.30	64.96	33.11	1.01 (0.0035)	0.04 (0.9992)	-0.97 (0.0035)
Hbb-b2	hemoglobin, beta adult minor chain	77.00	38.30	4.33	-1.01 (0.0035)	-4.15 (0.0159)	-3.14 (0.0621)
4930583H14Rik	Mgarp, mitochondria localized glutamic acid rich protein	108.79	53.32	59.82	-1.03 (0.0035)	-0.86 (0.0035)	0.17 (0.9992)
Crygc	**crystallin, gamma C**	**241.78**	**117.10**	**126.52**	**-1.05 (0.0035)**	**-0.93 (0.0035)**	**0.11 (0.9992)**
Pde6a	phosphodiesterase 6A, cGMP-specific, rod, alpha	21.43	10.22	6.92	-1.07 (0.0035)	-1.63 (0.0035)	-0.56 (0.0035)
Rpph1	ribonuclease P RNA component H1	22103.7	10353.2	15143.6	-1.09 (0.0035)	-0.55 (0.3913)	0.55 (0.2282)
Hist4h4	histone cluster 4, H4	31.73	12.39	17.25	-1.36 (0.0035)	-0.88 (0.0035)	0.48 (0.0686)
Pde6b	phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide	28.17	10.76	7.56	-1.39 (0.0035)	-1.90 (0.0035)	-0.51 (0.0311)
Gm1821	**predicted gene 1821**	**28.26**	**13.29**	**59.44**	**-1.09 (0.0035)**	**1.07 (0.0035)**	**2.16 (0.0035)**
Sag	S-antigen, retina and pineal gland (arrestin)	156.82	76.50	39.66	-1.04 (0.0035)	-1.98 (0.0035)	-0.95 (0.0035)
Cryba2	**crystallin, beta A2**	**90.18**	**41.96**	**44.99**	**-1.10 (0.0035)**	**-1.00 (0.0035)**	**0.10 (0.9992)**
Cryba4	**crystallin, beta A4**	**95.24**	**43.23**	**45.13**	**-1.14 (0.0035)**	**-1.08 (0.0035)**	**0.06 (0.9992)**
Crybb1	**crystallin, beta B1**	**91.94**	**39.83**	**42.99**	**-1.21 (0.0035)**	**-1.10 (0.0035)**	**0.11 (0.9992)**
Gnat1	**guanine nucleotide binding protein, alpha**	**160.68**	**68.52**	**47.73**	**-1.23 (0.0035)**	**-1.75 (0.0035)**	**-0.52 (0.0035)**
Crybb3	**crystallin, beta B3**	**68.62**	**28.40**	**32.69**	**-1.27 (0.0035)**	**-1.07 (0.0035)**	**0.20 (0.9992)**
Pde6g	phosphodiesterase 6G, cGMP-specific, rod, gamma	75.52	28.68	23.10	-1.40 (0.0035)	-1.71 (0.0035)	-0.31 (0.6138)
Rcvrn	recoverin	36.48	13.75	10.71	-1.41 (0.0035)	-1.77 (0.0035)	-0.36 (0.6465)
Gngt1	guanine nucleotide binding protein (G protein), gamma transducing activity	101.30	36.93	33.83	-1.46 (0.0035)	-1.58 (0.0035)	-0.13 (0.9992)

	polypeptide 1						
Pdc	phosducin	94.75	34.42	20.81	-1.46 (0.0035)	-2.19 (0.0035)	-0.73 (0.0035)
Nrl	neural retina leucine zipper gene	42.36	15.22	12.02	-1.48 (0.0035)	-1.82 (0.0035)	-0.34 (0.4938)
Prph2	peripherin 2	66.08	22.12	13.17	-1.58 (0.0035)	-2.33 (0.0035)	-0.75 (0.0035)
Rho	rhodopsin	287.12	84.59	53.10	-1.76 (0.0035)	-2.43 (0.0035)	-0.67 (0.0035)
Mbp	myelin basic protein	23.78	24.69	49.00	0.05 (0.9992)	1.04 (0.0035)	0.99 (0.0035)
Cryga	crystallin, gamma A	49.38	40.55	24.02	-0.28 (0.9683)	-1.04 (0.0035)	-0.76 (0.0257)
Crygs	**crystallin, gamma S**	**168.61**	**102.88**	**79.44**	**-0.71 (0.0035)**	**-1.09 (0.0035)**	**-0.37 (0.4025)**
Crygf	crystallin, gamma F	37.28	19.30	15.59	-0.95 (0.0035)	-1.26 (0.0035)	-0.31 (0.9992)

[1] Genes shared by retinas are shown in bold type.