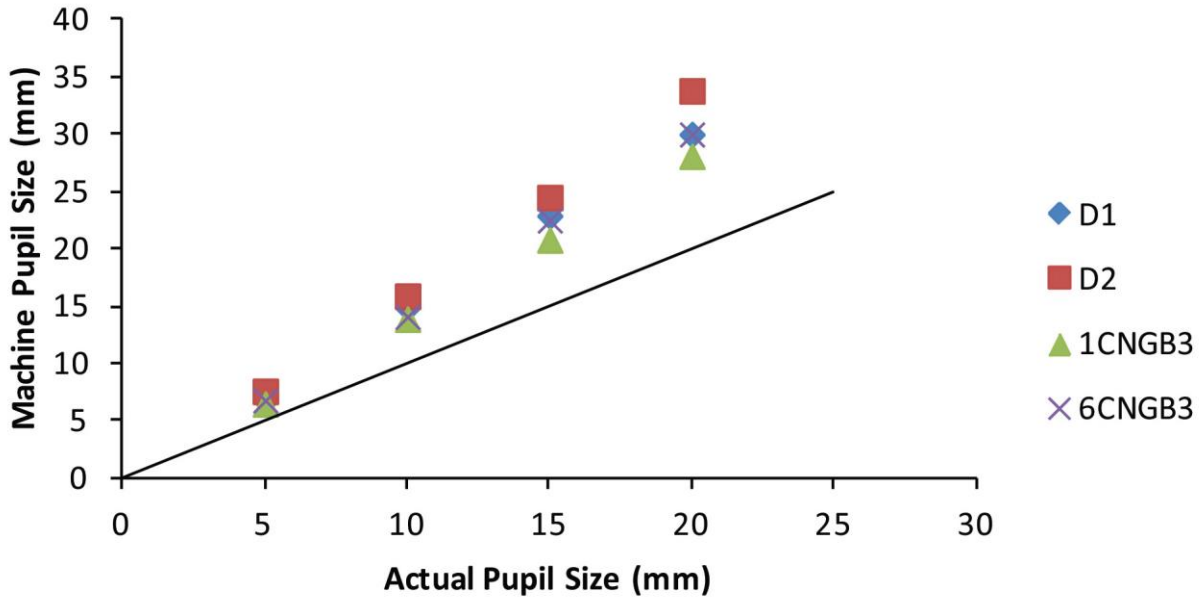
Supplementary Table S1. Summary of Dogs Used for Molecular Analyses

Disease	Dog	Gender	Age (years)	Studies
WT	1984	F	0.8	Sequence/cloning
	1985	F	0.9	Sequence/cloning/qRT-PCR
	V11614	-	0.06	qRT-PCR
	M656	M	0.5	qRT-PCR
	GS170	M	0.8	qRT-PCR
	Mateo	M	0.5	IHC
CNGB3-ACHM	GS86	M	0.6	qRT-PCR
	M550	M	0.08	qRT-PCR
	GS53	F	2.2	qRT-PCR
	M676	M	0.4	qRT-PCR
	M681	F	0.4	qRT-PCR
	M614	F	2.5	qRT-PCR
	GS171	M	0.8	qRT-PCR
	M501	F	7.3	IHC
PRCD-prcd	X168	F	4.8	qRT-PCR
	X225	M	3.5	qRT-PCR
	P774	F	3	qRT-PCR
	P1450*	F	8.2	IHC
RPGR-XLPRA2	Z234	M	0.06	qRT-PCR
PDE6B-rcd1	2055	F	0.06	qRT-PCR
	1888	M	0.3	IHC
	2016	F	1.5	IHC
STK38L-erd	E1044*	M	8.4	IHC

* Chromatic pupillometry performed



Supplementary Figure S1. Machine-measured pupil size *vs*. actual pupil size in 4 dogs. D1 and D2 are WT dogs that were not included in the main study. *Solid black line* shows $x = y$.

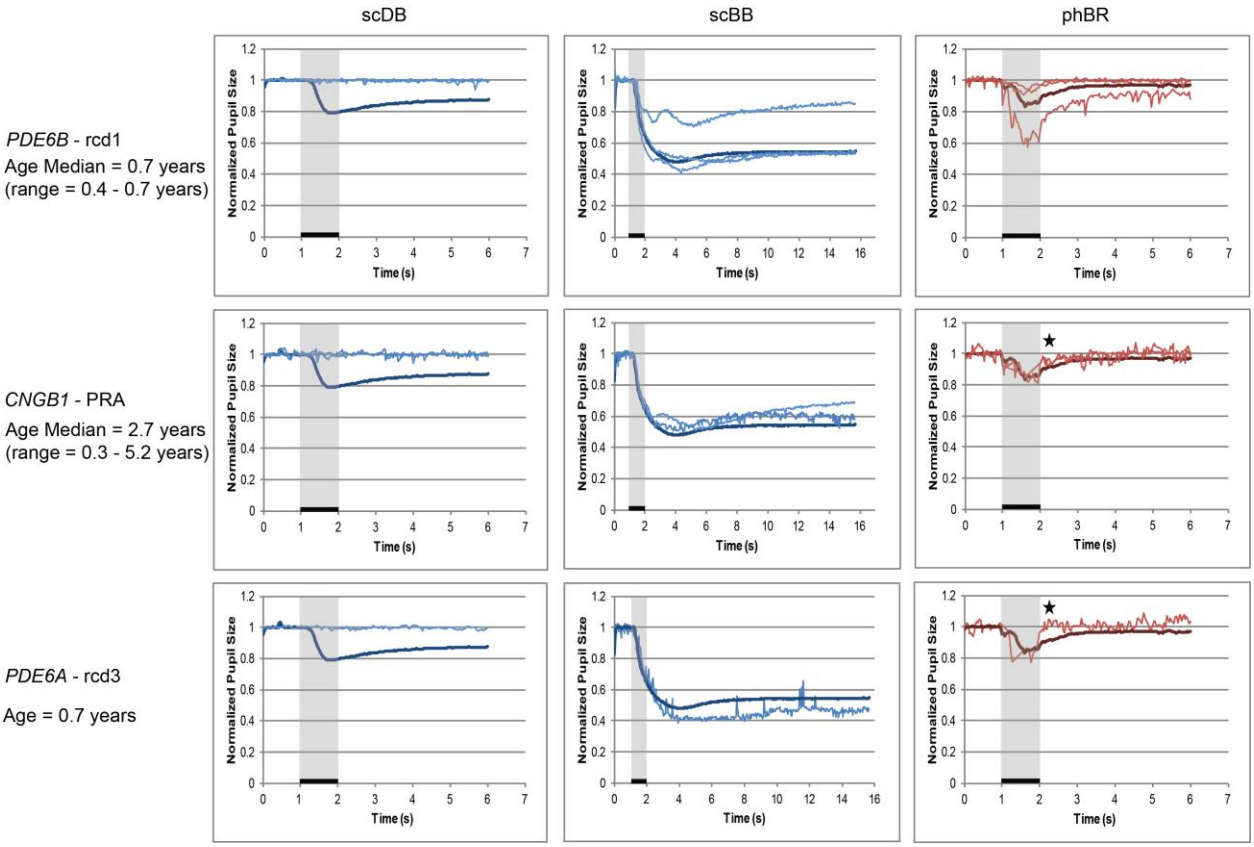
Supplementary Table S2. Light Intensities Used for Chromatic Pupillometry and Corresponding Corneal Irradiances.

Light Intensities (cd/m^2)	Irradiance - Blue Stimulus (470 nm) ($\mu W/cm^2$)	Irradiance – Red Stimulus (640 nm) ($\mu W/cm^2$)
1	2.8	0.2
10	22.6	9.9
32	73.4	31.0
100	237.0	98.8
400	926.7	402.1

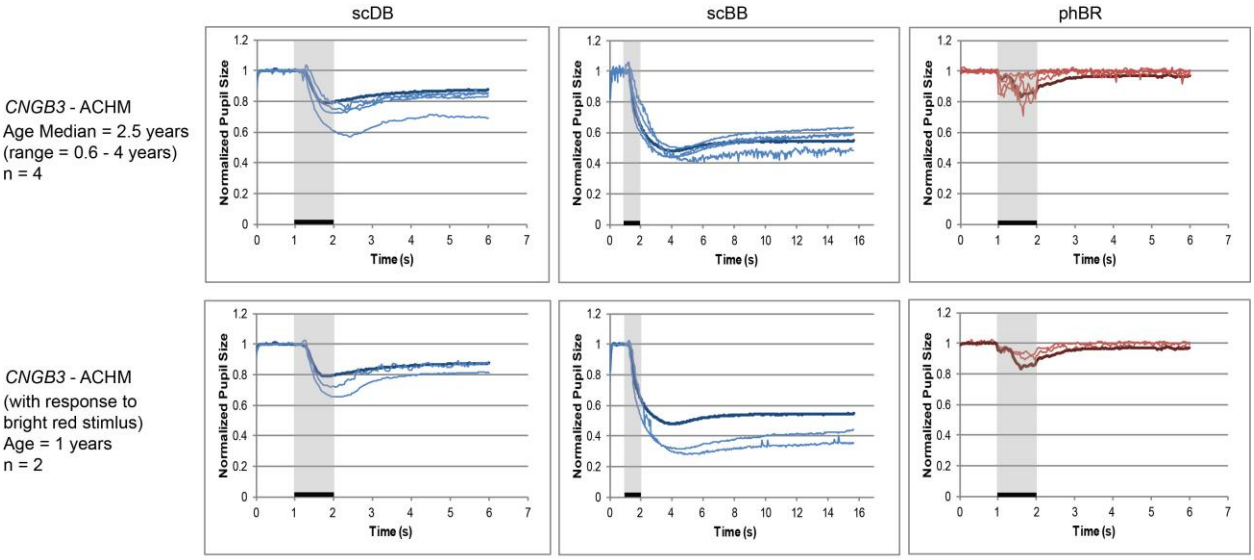
Supplementary Table S3. Antibodies Used

Antigen	Host	Catalogue No./Source	Working Dilution	Normal Retina Localization
Cone *alpha* transducin (GNAT2)	Rabbit polyclonal	Santa Cruz sc-390	1:5000	Cone outer segments
Human cone arrestin (hCAR)	Rabbit polyclonal	Cheryl Craft (University of Southern California)	1:5000	Cone photoreceptors
Neural Nuclei (NeuN) cloneA60	Mouse polyclonal	Millipore MAB377	1:2000	Ganglion cells
Neurofilament 200 (NF200)	Rabbit polyclonal	Sigma N4142	1:1000	Nerve fiber, outer and inner plexiform layer
L/M opsin	Rabbit polyclonal	Millipore AB5405	1:500	Outer segments of long- and medium-wavelength-absorbing (L/M)-cones
Melanopsin 1	Rabbit	21st Century	1:1000	ipRGCs
Rhodopsin	Mouse monoclonal	Millipore MAB5316	1:1000	Rod outer segments/axons and pedicles

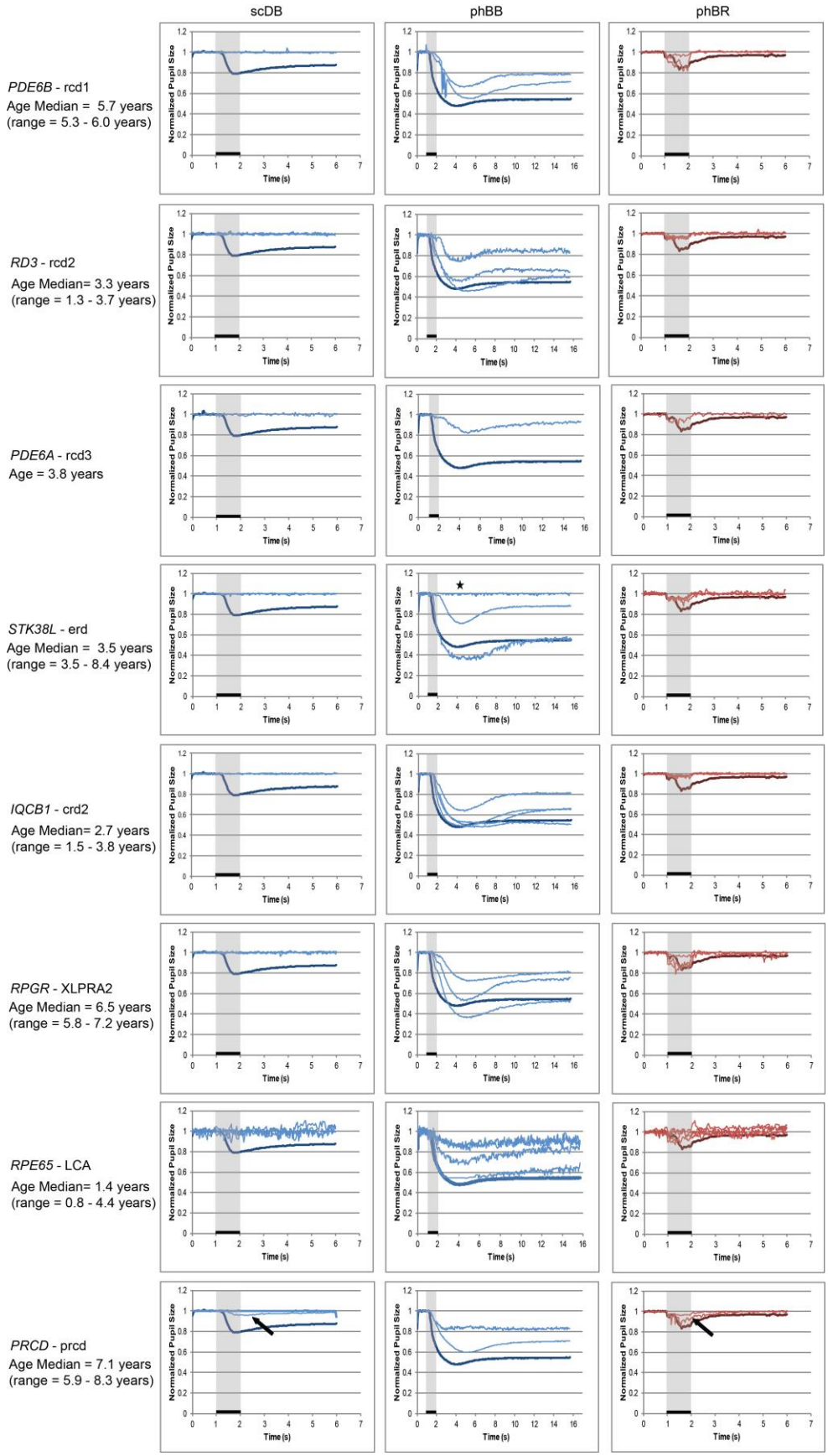
Santa Cruz Biotechnology Inc., Santa Cruz, CA; Millipore Corporation, Temecula, CA; Sigma-Aldrich Co. LLC., St. Louis, MO; 21st Century Biochemicals, Marlboro, MA



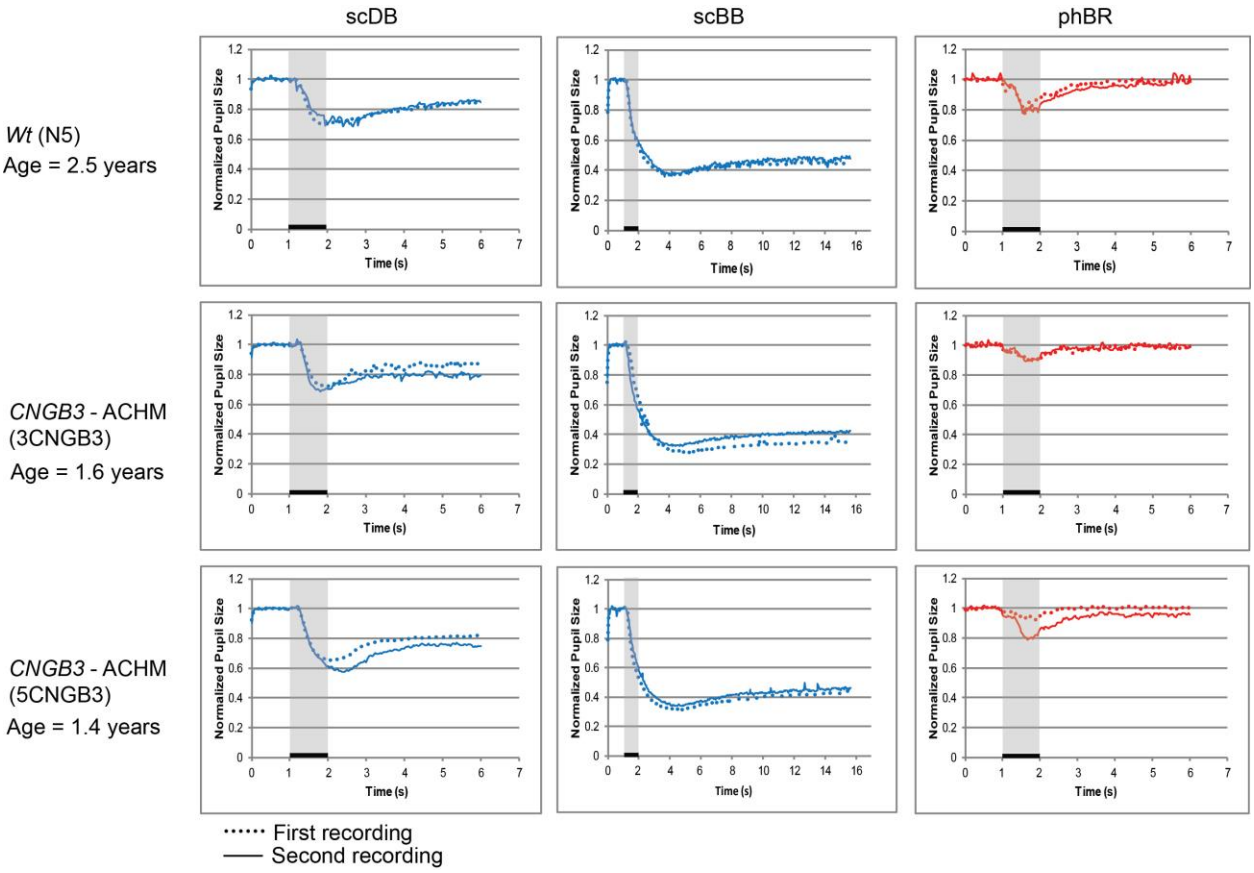
Supplementary Figure S2. Effects of mutations in *PDE6B, CNGB1*, and *PDE6A* on PLRs compared to average *wt* PLRs. *Bold blue* and *red lines* represent mean PLRs of *wt* dogs. *Lighter blue* and *red lines* represent individual PLRs from affected dogs. ★ indicates undetectable PLRs from *PDE6A*- and *CNGB1*-mutant dogs overshadowed by a light artifact. S*haded* area/*black bar* represents 1-s stimulus presentation.



Supplementary Figure S3. PLRs from *CNGB3*-mutant dogs with ACHM with the expected abrogation of phBR responses. In contrast, a subset of *CNGB3*-mutant dogs with incomplete ACHM showed persistent but reduced phBR responses. *Bold blue* and *bold red lines* represent averaged PLRs from *wt* dogs. *Lighter blue* and *red lines* represent individual PLRs from affected dogs. *Shaded area/black bar* represents the 1-s light stimulus presentation.


scDB
phBB
phBR
PDE6B - rcd1
Age Median = 5.7 years
(range = 5.3 - 6.0 years)
RD3 - rcd2
Age Median= 3.3 years
(range = 1.3 - 3.7 years)
PDE6A - rcd3
Age = 3.8 years
STK38L - erd
Age Median = 3.5 years
(range = 3.5 - 8.4 years)
IQCB1 - crd2
Age Median= 2.7 years
(range = 1.5 - 3.8 years)
RPGR - XLPRA2
Age Median = 6.5 years
(range = 5.8 - 7.2 years)
RPE65 - LCA
Age Median= 1.4 years
(range = 0.8 - 4.4 years)
PRCD - prcd
Age Median = 7.1 years
(range = 5.9 - 8.3 years)
Normalized Pupil Size
Time (s)

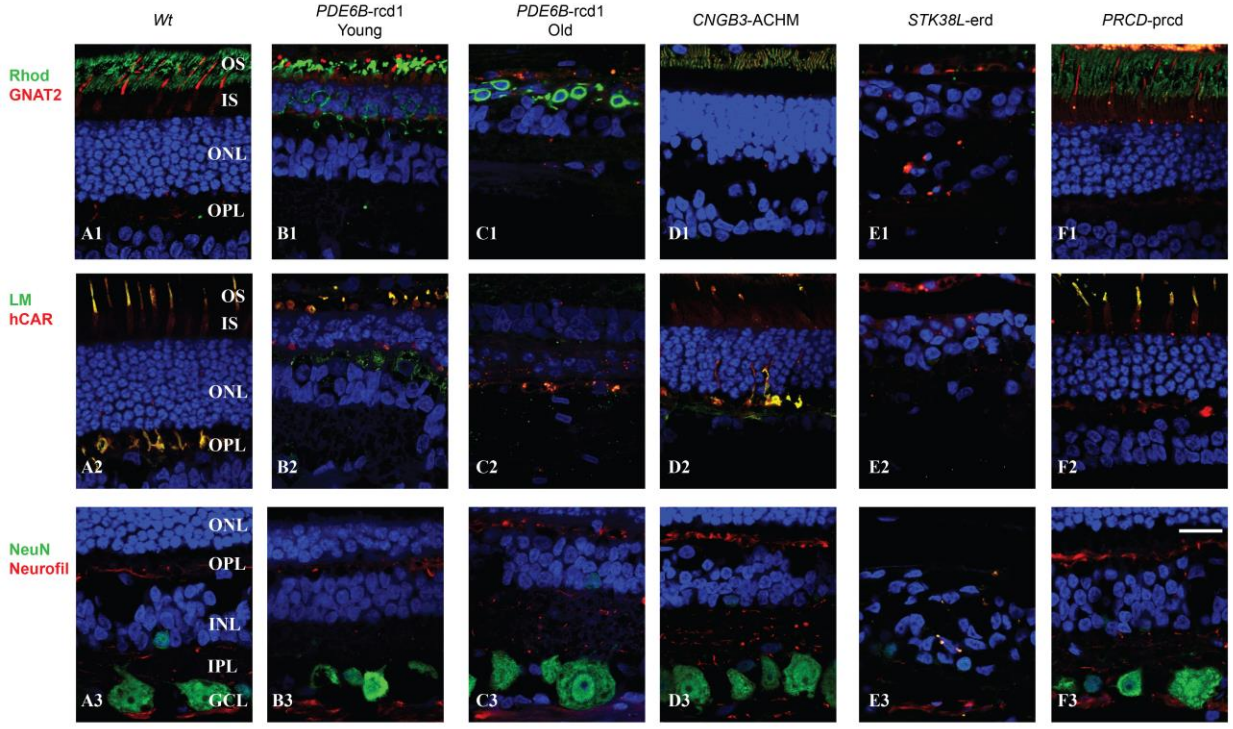
Supplementary Figure S4. PLRs of dogs bearing mutations in *STK38L*, *IQCB1*, *RPGR*, *RPE65*, and *PRCD*, and older dogs with *PDE6B*, *RD3*, and *PDE6A* mutations. *Bold blue* and *red lines* represent averaged PLRs of *wt* dogs. *Lighter blue* and *red lines* represent individual PLRs of affected dogs. *Shaded area/black bar* represents the 1-s light stimulus presentation. ★ indicates absent PLR to scBB in one older *STK38L*-mutant. *Arrows* indicate small residual rod function and near-normal preserved cone function in an older *PRCD*-mutant.



Supplementary Figure S5. Comparison between initial chromatic pupillometry testing results in 1 *wt* and 2 *CNGB3*-mutant dogs with those seen 4-5 months after the first recording. No obvious differences were seen between the first and second recordings for any PLR parameters. The *CNGB3*-mutant dogs were affected by incomplete ACHM with recordable response to phBR.

```
Podarcis siculus     ----------------------------------------------------MGTQH     5
Mus musculus         MDSPSGPRVLSSLTQDPSFTTSPA-LQGIWNGTQN-VSVRAQLLSVSPTTSAHQAAAWVP     58
Rattus norvegicus    MNSPSESRVPSSLTQDPSFTASPALLQGIWNSTQN-ISVRVQLLSVSPTTPGLQAAAWVP     59
Homo sapien          MNPPSGPRVPPSPTQEPSCMATPAP-PSWWDSSQSSISSLGRLPSISPTAPGTWAAAWVP     59
Felis catus          MNPPSGPR-----TQEPSCVATPAS-PSRWDGYRSSTSSLDQPLPISPTAARAQAAAWIP     54
Canis familiaris     MNPPSGPG-----AQEPGCVATAAS-PGRWHGSPRSTVGLDQALPTGPTAAGARAAAWAP     54
                                                                      .

Podarcis siculus     RIKVDVPDRVLYTVGSCVLVIGSIGITGNLLVLYAFYSNKRLRTPANYFTMNLAASDFLM     65
Mus musculus         FPTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCRNRGLRTPANMFIINLAVSDFLM     118
Rattus norvegicus    FPTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCRNRGLRTPANMLIINLAVSDFLM     119
Homo sapien          LPTVDVPDHAHYTLGTVILLVGLTGMLGNLTVIYTFCRSRSLRTPANMFIINLAVSDFLM     119
Felis catus          FPTVDVPDHAHYTLGTVILLVGLTGILGNLMVIYTFCRSRGLRTPANMFIINLAVSDFFM     114
Canis familiaris     FPTVDVPDHAHYILGTVILLVGLTGMLGNLMVIYTFCRTRGLRTPSNMFIINLAVSDFFM     114
                       .*****:. * :*: :*::*  *: *** *:*:*  .: ****:* :*:***.***:*

Podarcis siculus     SATQAPICFLNSMHTEWILGDIGCNFYVFCGALFGITSMMTLLAISVDRYCVITKPLQSI     125
Mus musculus         SVTQAPVFFASSLYKKWLFGETGCEFYAFCGAVFGITSMITLTAIAMDRYLVITRPLATI     178
Rattus norvegicus    SFTQAPVFFASSLYKKWLFGETGCKFYAFCGAVFGIVSMITLTAIAMDRYLVITRPLATI     179
Homo sapien          SFTQAPVFFTSSLYKQWLFGETGCEFYAFCGALFGISSMITLTAIALDRYLVITRPLATF     179
Felis catus          SFTQAPVFFASSLHKRWLFGEAGCEFYAFCGALFGITSMITLMAIALDRYLVITHPLATI     174
Canis familiaris     SFTQAPVFFASSLHKRWLFGEAGCEFYAFCGALFGITSMITLTAIALDRYLVITHPLAAV     174
                     * ****: * .*::..*::*: **:**.****:*** **:** **::*** ***:** :.

Podarcis siculus     KRSSKKRSCIIIAFVWLYSLGWSVCPLFGWSSYIPEGLMISCTWDYVSYSPANRSYTMML     185
Mus musculus         GRGSKRRTALVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYMTFTPQVRAYTMLL     238
Rattus norvegicus    GMRSKRRTALVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYVTFTPLVRAYTMLL     239
Homo sapien          GVASKRRAAFVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYMSFTPAVRAYTMLL     239
Felis catus          GVVSKRRAALVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYMSFTPSVRAYTMLL     234
Canis familiaris     GVVSKRRAALVLLGVWLYALAWSLPPFFGWSAYVPEGLLTSCSWDYMSFTPSVRAYTMLL     234
                        **:*:.:::  ****:*.**: *:****:*:****: **:***:::*  *:***:*

Podarcis siculus     CCFVFFIPLIIIFHCYLFMFLAIRSTGRNVQKLGSTY------NRKSNVSQSVKSEWKLA     239
Mus musculus         FCFVFFLPLLIIIFCYIFIFRAIRETGRA------CEGCGESPLRQRRQWQRLQSEWKMA     292
Rattus norvegicus    FCFVFFLPLLIIIFCYIFIFRAIRETGRA------CEGCGESPLR-RRQWQRLQSEWKMA     292
Homo sapien          CCFVFFLPLLIIIYCYIFIFRAIRETGRALQTFGACKGNGESL----WQRQRLQSECKMA     295
Felis catus          FCFVFFLPLLVIVYCYIFIFRAIRETGQALQTFRACEGGGRSP----RQRQRLQREWKMA     290
Canis familiaris     FCFVFFLPLLVIVYCYVFIFRAIRETGQALQTFRACEGGARSP----RQRQRLQREWKMA     290
                      *****:**::*..**:*:* ***.**:                    * :: * *:*

Podarcis siculus     KIAFVAIVVFVLSWSPYACVTLIAWAGYAKTLNPYSKSVPAVIAKASAIYNPIIYAIIHP     299
Mus musculus         KVALIVILLFVLSWAPYSTVALVAFAGYSHILTPYMSSVPAVIAKASAIHNPIIYAITHP     352
Rattus norvegicus    KVALIVILLFVLSWAPYSTVALVGFAGYSHILTPYMSSVPAVIAKASAIHNPIIYAITHP     352
Homo sapien          KIMLLVILLFVLSWAPYSAVALVAFAGYAHVLTPYMSSVPAVIAKASAIHNPIIYAITHP     355
Felis catus          KIELLVILLFVLSWAPYSIVALMAFAGYAHVLTPYMNSVPAVIAKASAIHNPIIYAITHP     350
Canis familiaris     KMELLVILLFVLSWAPYSAVALTAFAGYSHVLTPYMNSVPAVIAKASAIHNPIIYAITHP     350
                     *: ::.*::*****:**: *:* .:***:: *.** .************:******* **

Podarcis siculus     RYRRTIRSAVPCLRFLIRISPSDLSTSSVNESSFRASMSSRHS---F-AARNKSSCVSSI     355
Mus musculus         KYRVAIAQHLPCLGVLLGVSGQRSHPSLSYRSTHRSTLSSQSSDLSWISGRKRQ--ESLG     410
Rattus norvegicus    KYRAAIAQHLPCLGVLLGVSGQRSHPSLSYRSTHRSTLSSQSSDLSWISGQKRQ--ESLG     410
Homo sapien          KYRVAIAQHLPCLGVLLGVSRRHSRPYPSYRSTHRSTLTSHTSNLSWISIRRRQ--ESLG     413
Felis catus          KYRMAIAQHLPCLGVLLGVSGQHTGPYASYRSTHRSTLSSQASDLSWISGRRRQ--ASLG     408
Canis familiaris     KYRMAIAQHLPCLGVLLGVSGQRTGPYASYRSTHRSTLSSQASDLSWISGRRRQ--ASLG     408
                     :** :* . :*** .*: :*          .*:.*::::*: *   : : :.:.   *

Podarcis siculus     SAAETTWSDMELEPVEAARKKQQPHRSRSFSKQAEEETGLLL--KTQSCNVLTGEKVAVS     413
Mus musculus         SESEVGWTDTETTAAWGAA---QQASGQSFCSQNLEDGELKASSSPQVQRSKTPKV-PGP     466
Rattus norvegicus    SESEVGWTDTETTAAWGAA---QQASGQSFCSHDLEDGEVKAPSSPQEQKSKTPKT-KRH     466
Homo sapien          SESEVGWTHMEAAAVWGAA---QQANGRSLYGQGLEDLEAKAPPRPQGHEAETPGK-TKG     469
Felis catus          SESEVGWMDTEAAAVWGAA---QQVSGRFPCSQGLEDREAKAPVRPQGREAETPGQ-AMT     464
Canis familiaris     SESEVGWMDTEAAAVWGAA---QPAGGRFLCTQGLEDAEAKAPLRPRGQAVETPGK-VVT     464
                     * :*. * . *   . .*    *   .:    :  *:         :     *

Podarcis siculus     SISLHDPFERSFGENAPELLLRP-------SCLRTSSLPFGLNS----SSTEENADTSDM     462
Mus musculus         S--TCRPM---KGQGARPSSLRGDQKGRLAVCTGLSECPHPHTSQFPLAFLEDDVTL---     518
Rattus norvegicus    LPSLDRRM-----------------------------------------------------     474
Homo sapien          LIPSQ-----------------D-----------PRM-----------------------     478
Felis catus          MAMAPW--------------DT-----------PANCELPLHPGWAFH-----------     487
Canis familiaris     TATAAW--------------DP----------------PLHPGWAFQ-----------     481


Podarcis siculus     SISLHDPFERSFGENAPELLLRP-------SCLRTSSLPFGLNS----SSTEENADTSDM     462
Mus musculus         S--TCRPM---KGQGARPSSLRGDQKGRLAVCTGLSECPHPHTSQFPLAFLEDDVTL---     518
Rattus norvegicus    LPSLDRRM-----------------------------------------------------     474
Homo sapien          LIPSQ-----------------D-----------PRM-----------------------     478
Felis catus          MAMAPW--------------DT-----------PANCELPLHPGWAFH-----------     487
Canis familiaris     TATAAW--------------DP----------------PLHPGWAFQ-----------     481

                                                        Identity
Podarcis siculus     EVQEQHQMEASSH     475                46.63%
Mus musculus         ----RHL------     521                75.37%
Rattus norvegicus    -------------     474                75.38%
Homo sapien          -------------     478                78.94%
Felis catus          -------------     487                89.60%
Canis familiaris     -------------     481                ------
```

Supplemental Figure S6. Alignment of the predicted amino acid sequence of canine melanopsin with those of other species: Italian wall lizard (*Podarcis siculus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), human (*Homo sapiens*), and cat (*Felis catus*). Residues that are identical to those of the canine sequence are *shaded*. Percentage identity of canine melanopsin to those of other species is reported at the end of the alignment.



Supplementary Figure S7. Immunolabeling of *wt* and mutant retinas. In young and old *PDE6B*-mutant dogs rhodopsin (Rho) is mislocalized from OS to IS and cell bodies; rod OS are stunted (*young, B1*) and lost (*old, C1*). Cone OS (*GNAT2*, *hCAR*, and *LM*) are short (*young, B1 and B2*) and eventually lost (*old, C1 and C2*), and the ONL shows progressive thinning. In *CNGB3*-mutant dogs, rods (Rhod) are present with normal OS (*D1*). Fewer and abnormally-shaped cone OS are seen compared to those in *wt* (*hCAR*, *LM* in D2), and they lack detectable GNAT2 (D1). *STK38L*-affected dog with advanced disease shows severe degeneration of retina, with loss of characteristic layering: No rod and cone photoreceptors are present (*E1, E2*). In *PRCD*-mutant dogs, both rods and

cones are still present with normal localization of their specific markers Rho, GNAT2, LM, and hCAR despite reduced ONL thickness (*F1, F2*). In all mutants except the *STK38L*-mutant dog (*E3*), dendrites/axons of secondary neurons (*Neurofil*) as well as RGC (*NeuN*) are well-preserved (A2, B3, C3, D3, F3). Cell nuclei are shown in *blue* with DAPI. Calibration *bar* = 20 µm. OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; Rhod, rhodopsin; LM, long- and medium-wavelength-absorbing cone opsin; hCAR, human cone arrestin; NeuN, Neuronal Nuclei; Neurofil, neurofilament.

Photopigment	Canine	Human
Rhodopsin	506-510 nm	495 nm
L-opsin		560 nm
M-opsin		530 nm
L/M-opsin	555 nm	
S-opsin	429-435 nm	430 nm
Melanopsin	480 nm	480 nm

Supplementary Table S4. Maximum spectral sensitivity of canine *vs*. human photoreceptors. Dogs are functional dichromats, having combined red/green (long- and medium-wavelength-absorbing, L/M) and blue (short-wavelength-absorbing, S) cone pigments with a maximal sensitivity of 555 nm and 429-435 nm, respectively.[1,2] Humans are trichromats having red (L), green (M), and blue (S) cone pigments with a maximal sensitivity of 560 nm, 530 nm, and 430 nm, respectively.[3,4] Canine rhodopsin has peak sensitivity of 506-510 nm (human rhodopsin maximal sensitivity: ~495 nm).[1,5-7] The spectral sensitivity of canine melanopsin has not been validated but it is assumed to be 480 nm, similar to other species, including humans.[8-13]

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