Supplementary Material legends

Supplementary Figure S1. List of the *CRX* human mutations leading to the production of a premature stop codon at amino acid 185 compared to the human wild-type and feline mutant sequence.

Supplementary Figure S2. Retinal regions imaged by SD-OCT. Cross sectional retinal images were captured for measurement of retinal layer thicknesses at the following regions (as indicated on the fundus image by an asterisk): the *area centralis*; 4 optic nerve diameters from the optic nerve edge superiorly, inferiorly, temporally and nasally.

Supplementary Figure S3. Sites of retinal sampling for qRT-PCR. A. For the 2-week-old kittens, due to the small globe size, retina was collected from two regions as indicated (central and peripheral retinal regions). **B.** In the 6, 12 and 20 weeks of age kittens retinal biopsies were collected from the indicated five regions (*area centralis* – *AC*; inferior mid-peripheral – Inf MP; inferior far-peripheral – Inf FP; superior mid-peripheral – Sup MP; and superior far-periphery – Sup FP).

Supplementary Figure S4. TR, REC+, ONL and IR layer thicknesses on SD-OCT images, four optic nerve distance from the optic nerve rim itself **A. Superiorly, B. Inferiorly, C. Temporally and D. Nasally** of $Crx^{Rdy/+}$ kittens normalized to control WT kittens at 4, 6, 8, 10, 12, 15, 20 weeks-old and 6 months of age.

The ONL and REC+ thicknesses showed thinning with age in *Crx*$^{Rdy/+}$ kittens compared to WT. TR was not significant thinned until 6 months of age inferiorly. Conversely, the IR became thicker in *Crx*$^{Rdy/+}$ kittens compared to the wild-type kittens in all regions from 10 weeks of age.

Supplementary Figure S5. Immunolabeling of the retina for S cones and inner retinal cells.

Frozen sections of central retinal labeled with PNA combined with S-opsin, and with GFAP, and PKCalpha.

The *Crx*$^{Rdy/+}$ retina showed a lack of S-opsin staining cells. Cone nuclei (PNA positive) became mislocalized to the subretinal space but did not stain for S-opsin (indicated by white arrowheads in the bottom panel – high magnification view). There was marked Müller cell activation as indicated by GFAP upregulation at 12 and 20 weeks of age. Rod bipolar cells were labeled by PKCalpha and showed dendrite retraction in the *Crx*$^{Rdy/+}$ retina.

Key: OS= Photoreceptor Outer segment, IS= Photoreceptor Inner segment, ONL= Outer Nuclear Layer, OPL= Outer Plexiform Layer, INL= Inner Nuclear Layer, IPL= Inner Plexiform Layer, GCL/NFL= Ganglion Cell Layer/Nerve Fiber Layer; White arrow head= Mislocalized photoreceptor nuclei.

Supplementary Figure S6. Western blot for Crx protein in retinal nuclear and cytoplasmic extracts from 2 week old kittens.

Note that the truncated mutant Crx protein was exclusively detected in the nuclear extract from the *Crx*$^{Rdy/+}$ kitten and was at a higher level than the wild-type protein (immunolabeled with anti-Crx antibody 119b1).

Beta-actin was used as protein loading control.

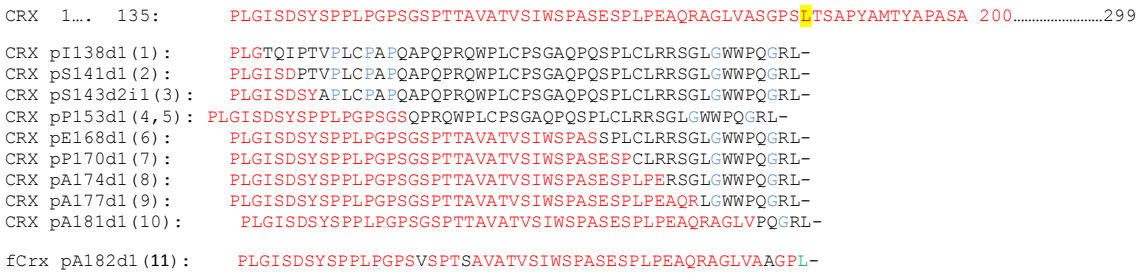
Supplementary Figure S7. Dual-Luciferase assays for CRX transactivation activity on mouse *Crx-Luc* reporter.

Crx auto-activation ability of WT or mutant Crx protein on its own promoter *Crx* (containing 2 binding sites within 500-bp upstream region of the mouse *Crx* gene) was tested using HEK293 cells transfected by plasmids containing the 500bp mouse *Crx* promoter-luciferase reporter (*mCrx-Luc*) and the indicated Crx protein expression vector. Comparing to *pcDNA3.1hisc control,* only *pCAGIG-feline Crx WT* significantly activated the *mCrx-Luc* reporter. *pCAGIG-feline* Crx^{Rdy} *mutant* did not show any transactivation compared to the control vector.

P-values indicate as followed: $^{**}P<0.01$ and $^{***}P<0.001$.

Supplementary Table S1. List of antibodies used for IHC – their origins and dilutions.

Supplementary Table S2. Primer sequences for qRT-PCR assays.



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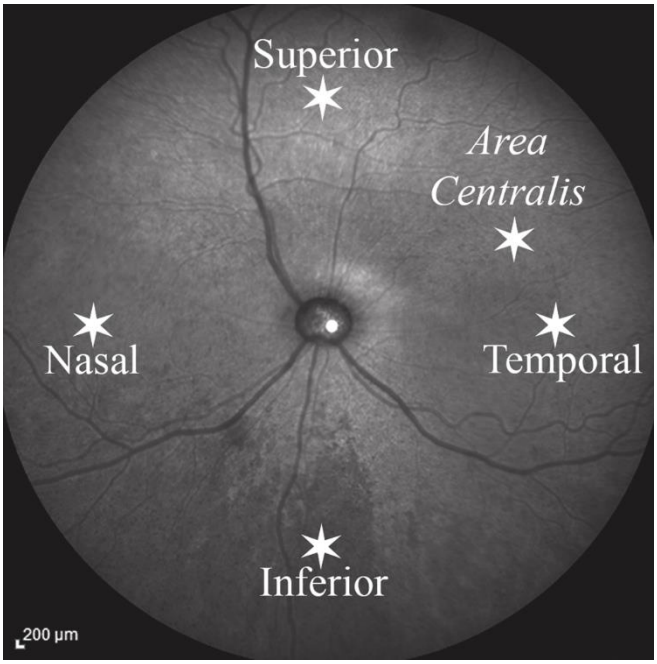
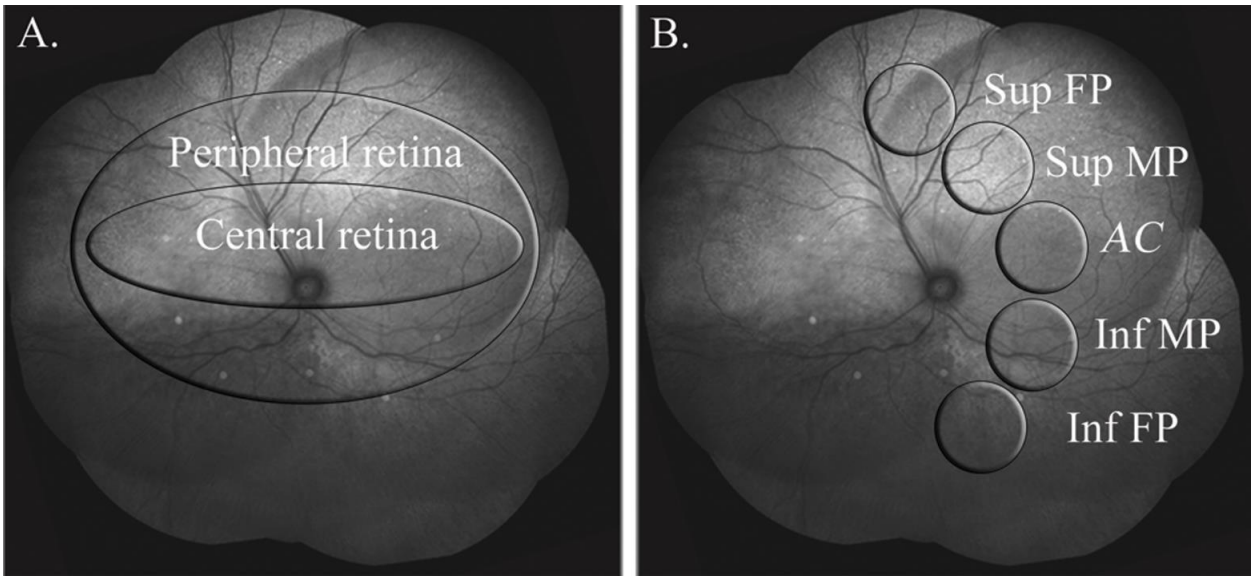
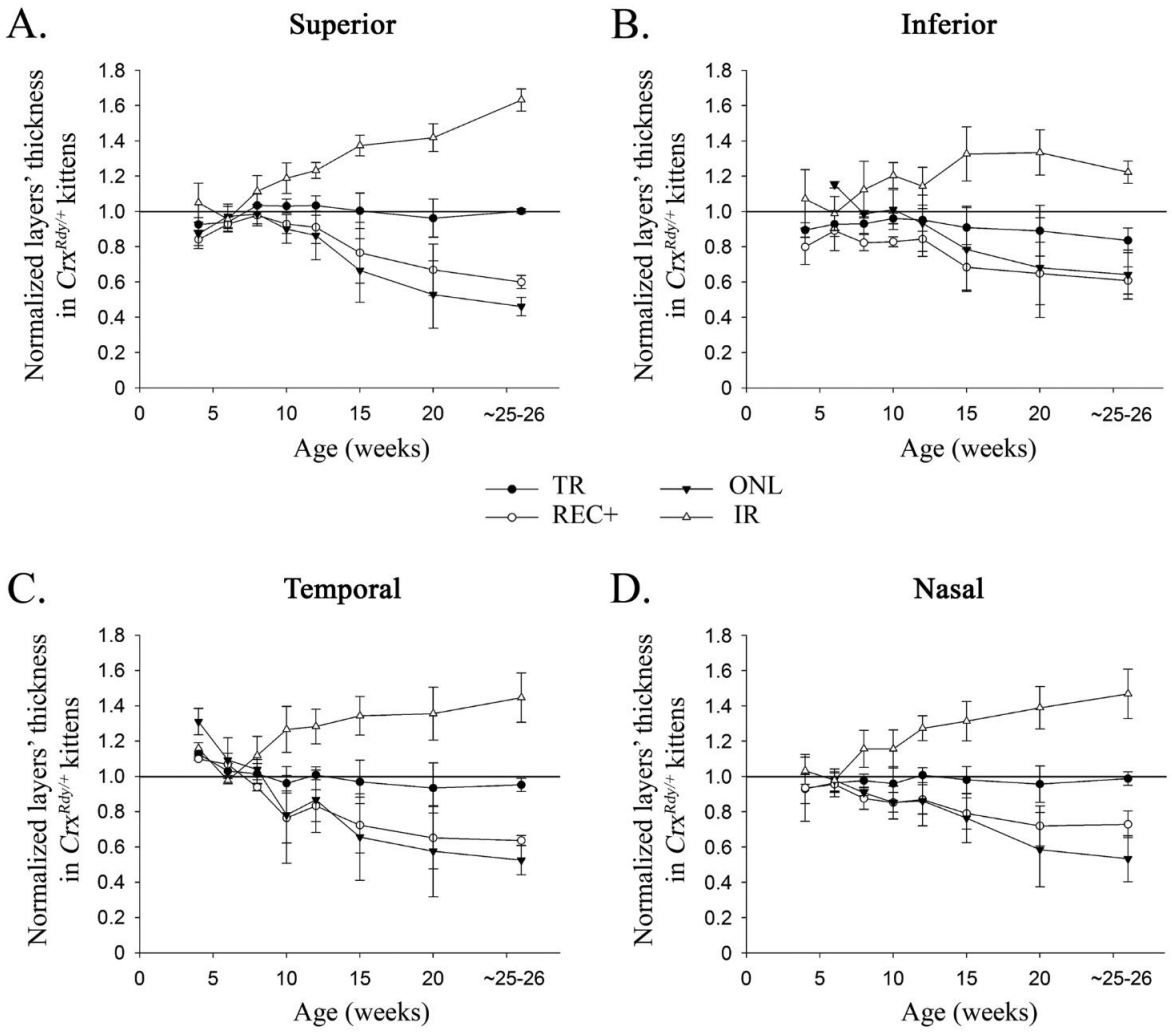


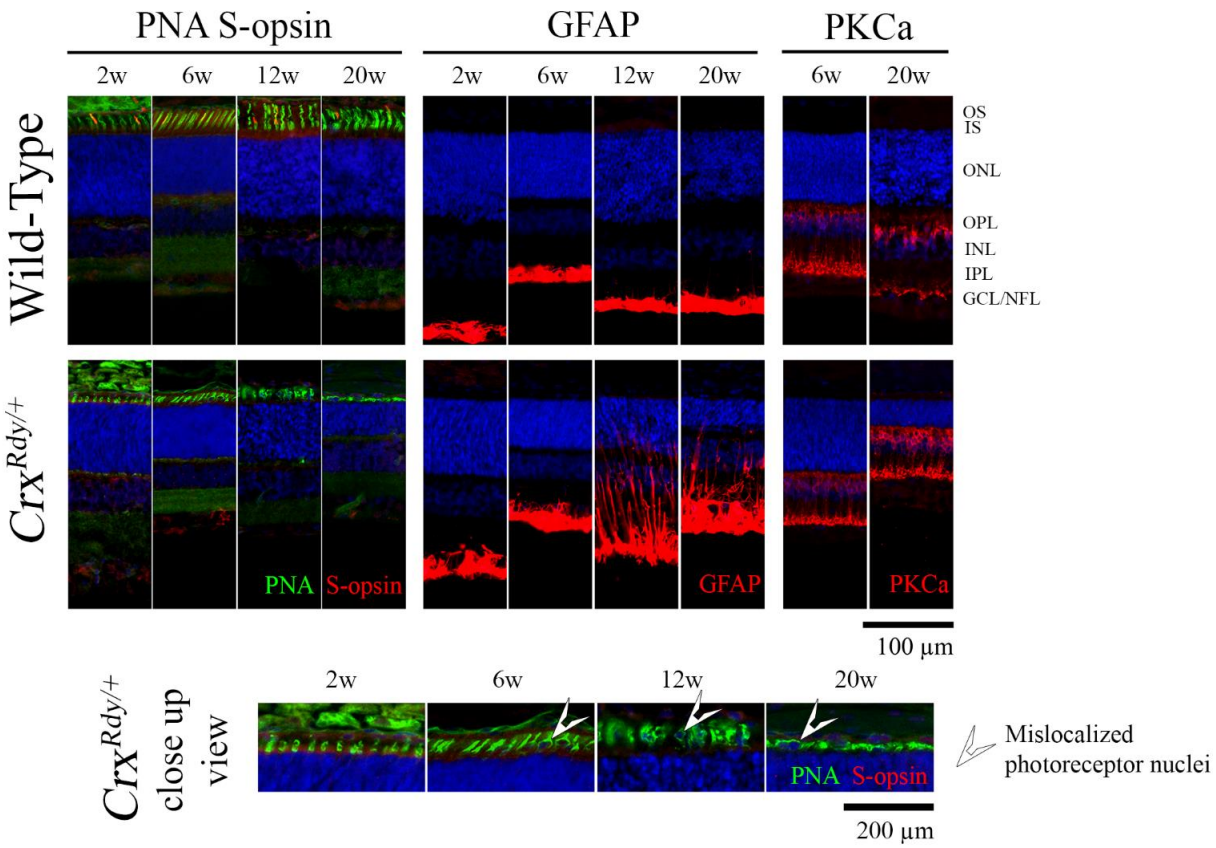
Supplementary Figure S2



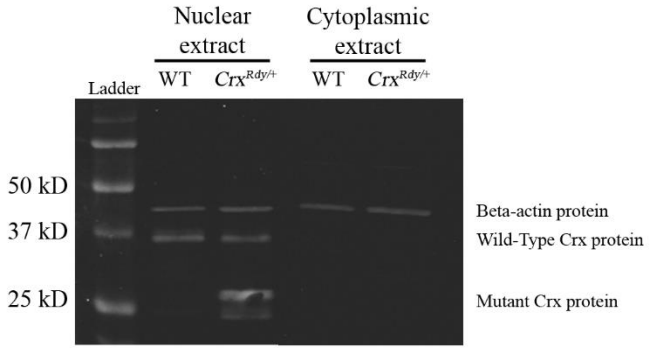
Supplementary Figure S3



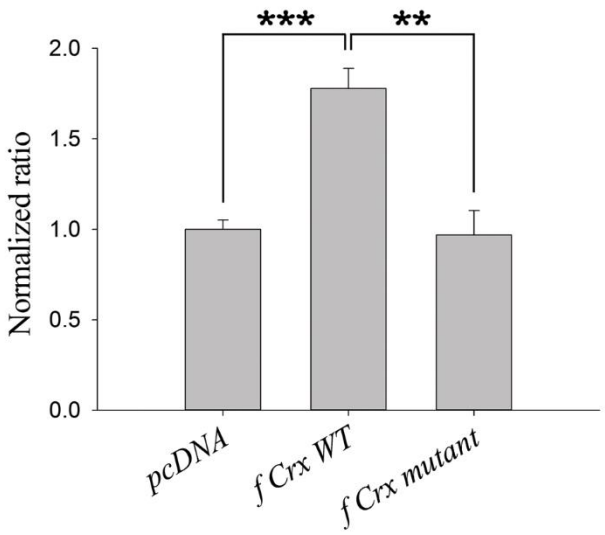
Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6



Supplementary Figure S7

Supplementary Table S1. List of antibodies used for IHC – their origins and dilutions.

Antibody – Source	Type	Primary Dilution	Secondary Antibody – Source	Secondary Dilution
hCAR (Human cone arrestin) Dr. Cheryl Craft; LUMIJ, University of Southern California, Los Angeles, CA, USA	Polyclonal rabbit	1:10,000	Alexa Fluor 488 Goat anti-rabbit IgG Life technologies, Carlsbad, CA, USA	1:500
PNA (Biotinylated Peanut Agglutinin) Vector Labs Inc., Burlin-game, CA, USA	Biotinylated Lectin	1:500	Alexa Fluor 488 Streptavadin Life technologies, Carlsbad, CA, USA	1:500
ML-opsin (Anti-Opsin, Red/Green; Medium/ Long wavelength cone opsin) Millipore Corp., Billerica, MA, USA	Polyclonal rabbit	1:1,000	Alexa Fluor 568 or 594 Goat anti-rabbit IgG Life technologies, Carlsbad, CA, USA	1:500
S-opsin (Anti-Opsin, Blue; Short wavelength cone opsin) Millipore Corp., Billerica, MA, USA	Polyclonal rabbit	1:1,000	Alexa Fluor 568 or 594 Goat anti-rabbit IgG Life technologies, Carlsbad, CA, USA	1:500
RetP1 (Rhodopsin Ab-1) Thermo Scientific, Rockford, IL, USA	Monoclonal mouse	1:2	Alexa Fluor 594 Rabbit anti-mouse IgG Life technologies, Carlsbad, CA, USA	1:500
GFAP (Anti-Glial Fibrillary Acidic Protein) Cell Signaling Technology Inc., Danvers, MA, USA	Monoclonal mouse	1:300	Alexa Fluor 594 Rabbit anti-mouse IgG Life technologies, Carlsbad, CA, USA	1:500
PKCa (Protein Kinase C-alpha) BD Biosciences, San Jose, CA, USA	Monoclonal mouse	1:500	Alexa Fluor 594 Rabbit anti-mouse IgG Life technologies, Carlsbad, CA, USA	1:500

Supplementary Table S2. Primer sequences for qRT-PCR assays.

Primer name	Forward primer	Reverse primer	Amplicon size (bp)	Annealing temperature (°C)
Crx Total	5' AAGACTCAGTACCCGGATGTGTA 3'	5' GGGGCTGTAGGAGTCTGAGAT 3'	223	60
Arr3	5' CGTTGTCCTGTATTCCCTAGAC 3'	5' GCTAGAGGCCAGATTAGTATCAC 3'	190	60
Rho	5' GGTGCCCTACGCCAGCGTG 3'	5' CAGTGGGTTCTTGCCACAG 3'	190	60
Tuba1b	5' GCTCTATTGCCTGGAACACG 3'	5' CATCTTCCTTGCCCGTGATG 3'	230	60
GAPDH	5' GGTCTTCACCACCATGGAGA 3'	5' TGGACTGTGGTCATGAGTCC 3'	237	60