Supporting Information

Peng and Chen 10.1073/pnas.1109209108

SI Materials and Methods

BAC Clones and Mammalian Expression Plasmids. BAC clones used in chromosomal conformation capture (3C) assays are listed in Table S3. All were purchased from BACPAC Resource Center (http://bacpac.chori.org/). The human cone–rod homeobox (*CRX*) expression vector *hCRX-pcDNA3.1/HisC* and its parent vector were described previously (1).

Mouse Strains. C57BL/6J (WT) and nuclear receptor subfamily 2, group E, member 3 (NR2E3)-deficient *rd7* mice ($Nr2e3^{rd7/rd7}$) were purchased from The Jackson Laboratory. Neural retina leucine zipper (*Nrl*) knockout ($Nrl^{-/-}$) mice were provided by Anand Swaroop (University of Michigan, Ann Arbor, MI). *Crx* knockout ($Crx^{-/-}$) mice were a gift from Constance Cepko (Harvard University, Boston, MA). All mice were used in accordance with a protocol approved by the Washington University Animal Care and Use Committee and conforming with the guidelines for animal use of the Association for Research in Vision and Ophthalmology (*Policies*, www.arvo.org).

Human Retinas. Normal human retina samples from 31- and 34-y-old male cadaver donors were kindly provided by David Beebe (Washington University). Each retina was dissected into three subregions enriched for rods [far periphery (FP)], cones [macula lutea (ML)], or mixed rods/cones [peripheral macula lutea (PML)], respectively.

3C assays. 3C assays on mouse or human retinas were performed based on a protocol described by Hagège et al. (2). Briefly, dissected retinas were digested with 2% collagenase (C0130; Sigma) to make single-cell suspensions. Aliquots containing 1×10^7 cells were treated with 2% formaldehyde in 10% (vol/vol) FBS/PBS for 10 min to crosslink interacting chromatin segments. The samples were lysed in cell lysis buffer [10 mM Tris·HCl (pH 7.5), 10 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EGTA, and 1× complete protease inhibitor (11836153001; Roche)] for 10 min on ice. The nuclei were collected by centrifugation at 400 × *g* for 5 min and digested with 300–400 U of Bpm1 (R0565L; New England Biolabs) at 37 °C overnight. For positive controls, 10 μg of BAC clone containing each gene of interest were also digested by Bpm1. The resulting nuclei preparation and BAC-positive control DNA were treated with 100 U of T4 ligase (M0202S; New England Biolabs) at 16 °C for 4 h. After decrosslinking and DNA purification, 3C samples and controls were diluted 10-fold and analyzed by PCR. 3C PCR primers and sequence primers used for validating human *L/M* 3C products are listed in Tables S1 and S2. Predicted 3C PCR product sizes in base pairs are listed in Table S4. Quantitative PCR (qPCR) was performed with SYBR Green Jumpstart Taq ReadyMix qPCR Kit (S4438; Sigma) on a CFX96 Real-Time PCR System (Bio-Rad) according to the manufacturers' protocols. Looping frequencies were calculated as ratios (×100) relative to a constant DNA control using Fc/Rc primers located within the same Bpm1 restriction fragment (percentage Fc/Rc). Error bars represent the SEM from three independent experiments ($n = 3$).

Quantitative RT-PCR (qRT-PCR) assays. qRT-PCR assays were performed essentially as described by Peng and Chen (1) with β-*actin* as a reference. Relative mRNA levels normalized to β-*actin* were determined and presented as fold changes relative to the WT values. Error bars represent the SEM from three independent experiments ($n = 3$), and statistical significance was determined by using the paired Student's *t* test. All of the RT-PCR primers were described previously (1, 3).

ChIP assays. ChIP assays were performed with six pooled retinas of each mouse strain at age postnatal day 14 (P14) following the procedure described by Peng and Chen (3). Immunoprecipitation (IP) was carried out with 1 μg of a rabbit antibody to CRX (p261) (4), NR2E3 (p183) (5), or NRL (AB 5693; Chemicon) and normal rabbit IgG (Santa Cruz Biotechnology) as a baseline control. The immunoprecipitated DNA, input (without IP), and mock (without chromatin DNA) controls were analyzed by qPCR using primers spanning various regions of each murine *opsin* gene (Figs. 1*A*, 2*A*, and 3*A*). The results are presented as ratios of IP vs. input × 100 (percentage input) after normalization with the IgG control values. Error bars represent the SEM from three independent experiments ($n = 3$). Primer sets for genes are listed in Table S5.

Y79 cell cultures and transient transfection. Y79 cell cultures and transient transfection were performed as described previously by Peng and Chen (1). Four micrograms of *hCRX-pcDNA 3.1/HisC* (+CRX) or its empty vector *pcDNA 3.1/HisC* (−CRX) were transfected into Y79 cells cultured to 50–60% confluence on 100-mm plates. Over 50% transfection efficiency was achieved with DreamFect transfection reagent (Boca Scientific). 3C assays were performed at 48 h after transfection.

1. Peng GH, Chen S (2007) Crx activates *opsin* transcription by recruiting HAT-containing co-activators and promoting histone acetylation. *Hum Mol Genet* 16:2433–2452.
2. Hagège H, et al. (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nat Protoc* 2:1722–1733.
3. Peng GH, Chen S (2005) Chromatin immunoprecipitation identifies photoreceptor transcription factor targets in mouse models of retinal degeneration: New findings and challenges. *Vis Neurosci* 22:575–586.
4. La Spada AR, et al. (2001) Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. *Neuron* 31:913–927.
5. Peng GH, Ahmad O, Ahmad F, Liu J, Chen S (2005) The photoreceptor-specific nuclear receptor Nr2e3 interacts with Crx and exerts opposing effects on the transcription of rod versus cone genes. *Hum Mol Genet* 14:747–764.

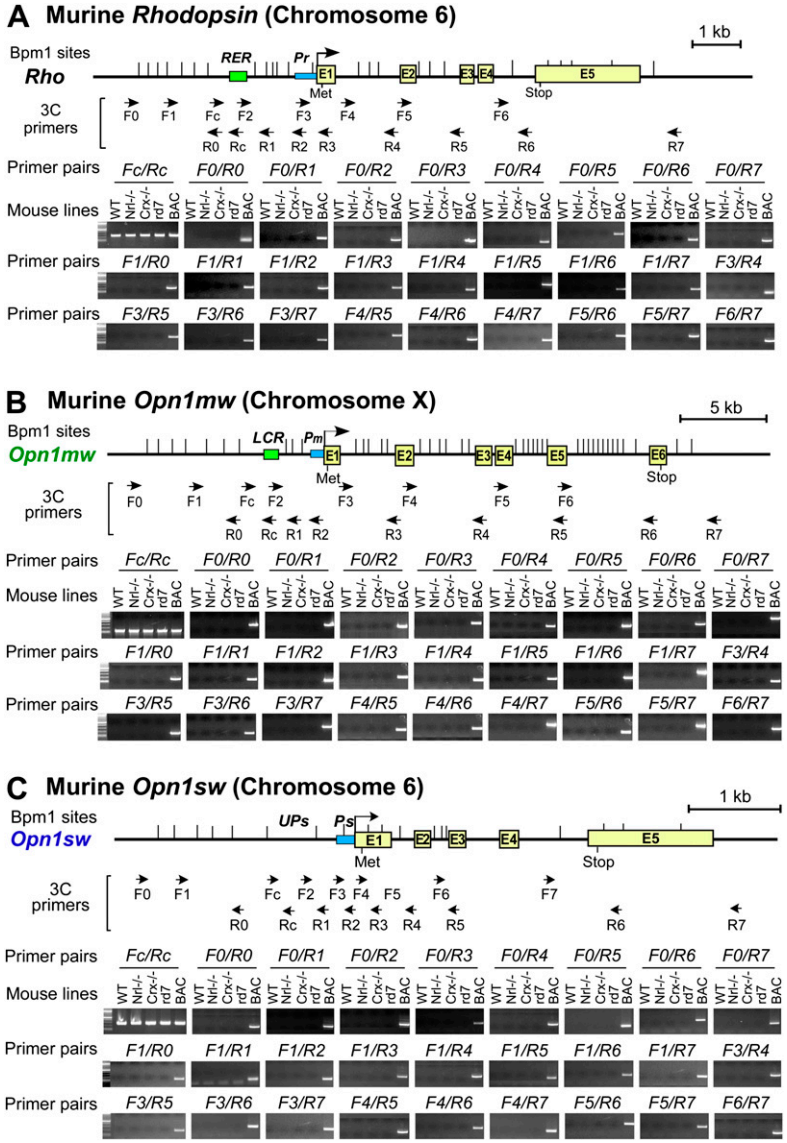

Fig. S1. Gel images of negative 3C results using retinas of the indicated mouse lines. (*A*) *Rhodopsin* (*Rho*). (*B*) *Opn1mw* (*M*). (C) *Opn1sw* (*S*). No chromosomal loops were detected with the indicated primer sets. The BAC sample in each set served as a positive control for PCR. A constant DNA control (Fc/Rc) amplified in parallel from each locus (see description in *3C assays* in *SI Materials and Methods*) served as a genomic DNA loading control for each sample.

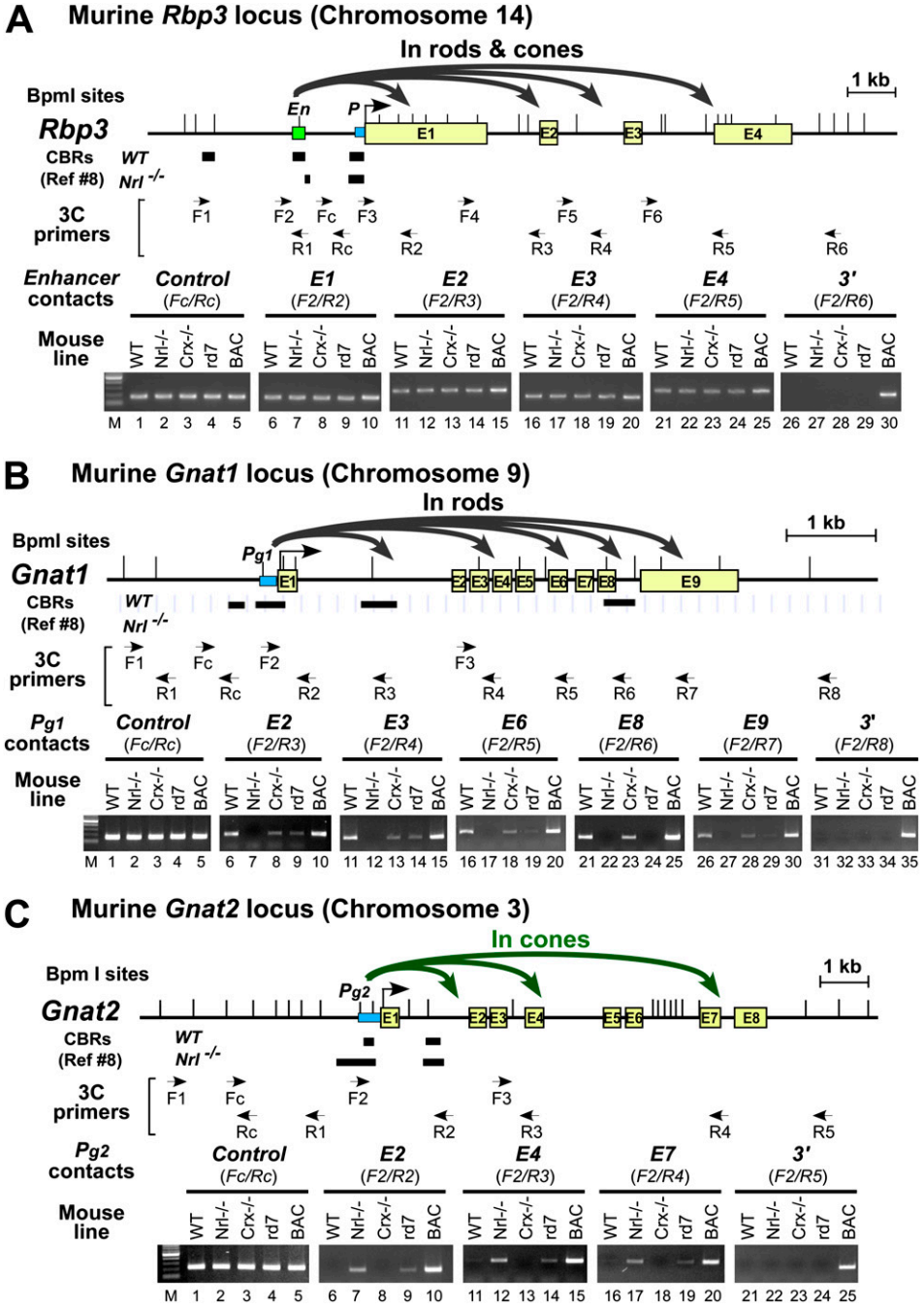

Fig. S2. Mouse *Rbp3*, *Gnat1*, and *Gnat2* loci exhibit intrachromosomal loops in the respective photoreceptor subtype(s) expressing them. (*A*) Diagram of *Rbp3* locus, showing gene structure (1, 2) and positions of CRX binding regions (CBRs) reported by Corbo et al. (3), Bpm1 restriction sites (|), and 3C primers. Gel images show PCR products from 3C assays on P14 retinas of the indicated mouse lines and BAC controls. *Rbp3* transcription occurs in both rods and cones and is unaffected by $Crx^{-/-}$, $Nrl^{-/-}$, and *rd7* mutations (4). As indicated by curved black arrows, *Rpb3* enhancer (*En*) loops with various *Rbp3* coding regions, but not 3′, in WT rods, $Nrl^{-/-}$ cones, defective rods ($Crx^{-/-}$), and rod/S-cone hybrid cells (*rd7*), consistent with *Rbp3* expression pattern in these mouse lines. (*B*) Diagram of *Gnat1* locus, showing gene structure and positions of CBRs reported by Corbo et al. (3), Bpm1 restriction sites (|), and 3C primers. Gel images show PCR products from 3C assays on P14 retinas of the indicated mouse lines and BAC controls. *Gnat1* promoter (*Pg1*) makes looping contacts with *Gnat1* coding regions in WT rods, as indicated by curved gray arrows. Weak looping signals were also detected in $Crx^{-/-}$-defective rods and *rd7* rod/S-cone hybrid cells, where *Gnat1* expression is reduced. (*C*) Diagram of *Gnat2* locus, showing gene structure and positions of CBRs, Bpm1 restriction sites (|), and 3C primers. Gel images show PCR products from 3C assays on P14 retinas of the indicated mouse lines. *Gnat2* promoter (*Pg2*) makes looping interactions with *Gnat2* coding regions in $Nrl^{-/-}$ cones but not in rods, as illustrated by curved green arrows. Weak looping signals were also detected in *rd7* hybrid cells, where *Gnat2* is partially derepressed (5).

1. Fong SL, Fong WB (1999) Elements regulating the transcription of human interstitial retinoid-binding protein (IRBP) gene in cultured retinoblastoma cells. *Curr Eye Res* 18:283–291.
2. Ying S, et al. (1998) A CAT reporter construct containing 277bp GNAT2 promoter and 214bp IRBP enhancer is specifically expressed by cone photoreceptor cells in transgenic mice. *Curr Eye Res* 17:777–782.
3. Corbo JC, et al. (2010) CRX ChIP-seq reveals the *cis*-regulatory architecture of mouse photoreceptors. *Genome Res* 20:1512–1525.
4. Hennig AK, Peng GH, Chen S (2008) Regulation of photoreceptor gene expression by Crx-associated transcription factor network. *Brain Res* 1192:114–133.
5. Chen J, Rattner A, Nathans J (2005) The rod photoreceptor-specific nuclear receptor Nr2e3 represses transcription of multiple cone-specific genes. *J Neurosci* 25:118–129.

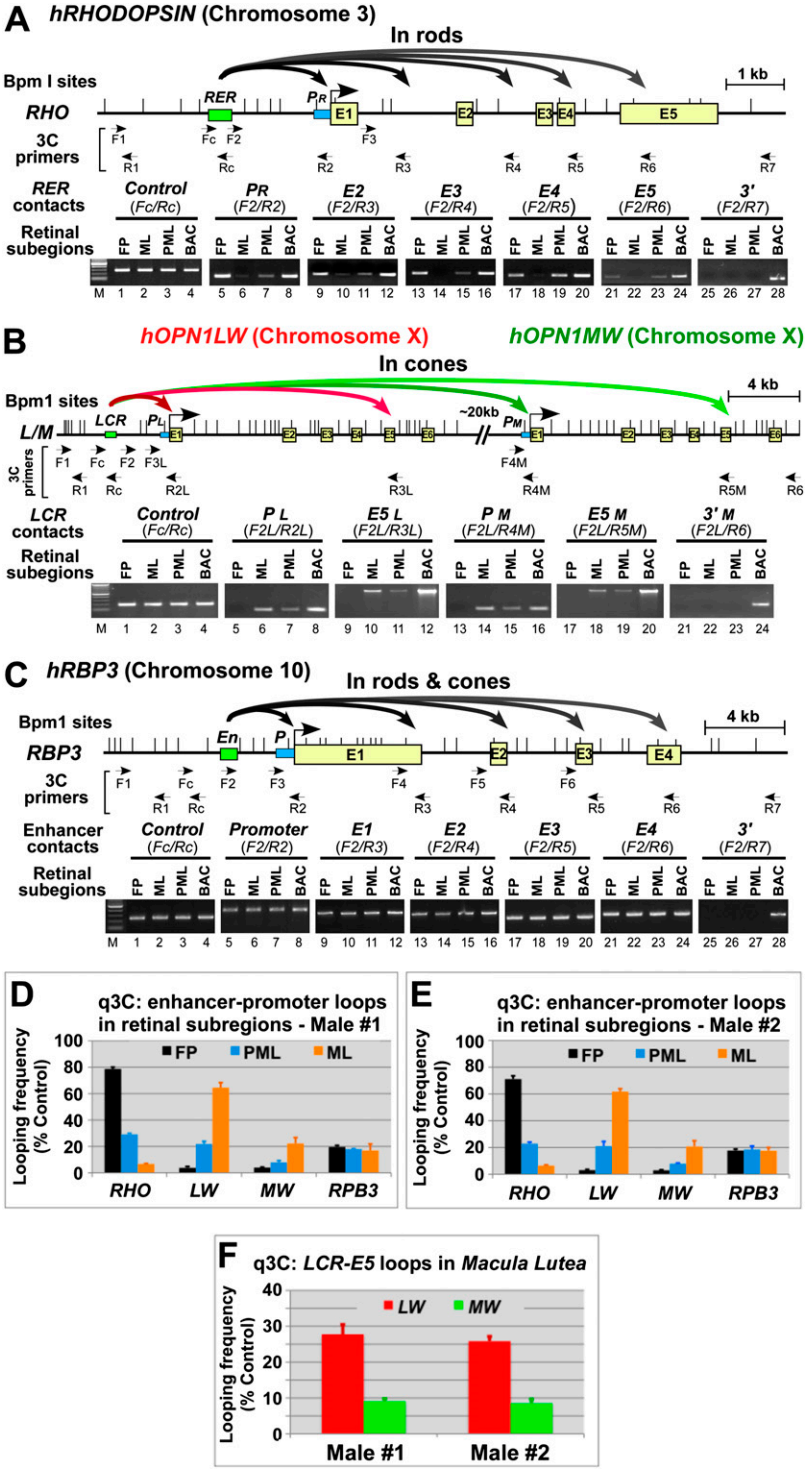

Fig. S3. Human *OPSIN* loci—*RHO*, *OPN1LW*, and *OPN1MW*—adopt looping organizations in the respective photoreceptor subtypes actively expressing them. (*A*) Human *RHO* locus, showing gene structure, Bpm1 restriction sites (|), 3C primers, and *Rho enhancer region* (*RER*)-organized chromosomal loops (black/gray arrows) in rods detected by 3C assays. Gel images show 3C results for the *RHO* locus in the indicated subregions of male no. 1 retinas: FP (rod-rich), ML (cone-rich), and PML (mixed rods and cones) with BAC-positive controls. The results show that *RHO RER* loops with the promoter (P_R) and coding regions in rod-rich FP and, to a lesser degree, in PML samples with mixed rods and cones, but no looping was detected in cone-rich ML samples, indicating the rod origin of these loops. (*B*) Human *OPN1LW* (*L*) and *OPN1MW* (*M*) loci, showing gene structure, Bpm1 restriction sites (|), 3C primers, and *LCR*-organized chromosomal loops in cones (red and green arrows represent the loops contacting *L* and *M* regions, respectively) as detected by 3C. Gel images show 3C results from the *L/M* loci in the indicated subregions of male no. 1 retinas and BAC controls. *L/M* loops are detected in cone-rich ML samples and, to a lesser degree, in PML samples but not in rod-rich FP samples, suggesting a cone origin of these loops. (*C*) Human *RBP3* locus, showing gene structure (1), Bpm1 restriction sites (|), 3C primers, and *RBP3* enhancer-organized chromosomal loops in both rods and cones detected by 3C. Gel images show 3C results from the indicated subregions, showing *RBP3* loops are present in all subregions, consistent with the *RBP3* rod/cone expression pattern. Similar results were obtained for male no. 2. (*D* and *E*) qPCR quantification of enhancer–promoter loop frequencies (relative to controls) of four genes in the indicated subregions dissected from male donors no. 1 and no. 2,

Legend continued on following page

respectively. Note that the difference in looping frequencies between rod-rich FP and cone-rich ML samples is reversed when *Rho* is compared with *L* or *M*, whereas *RBP3* looping frequency is constant in all subregions. (*F*) Comparison of *LCR* looping frequencies between *L* (red) and *M* (green) *E5* fragments for both male no. 1 and male no. 2. Only ML samples are used for the comparison because this subregion contains the highest cone density. The 3C products from *L*/*M* loci of each donor were confirmed by sequencing. Both donors show a near 3:1 *L*/*M* looping ratio for the two fragments examined, supporting distance-dependent competition between *L* and *M* for *LCR*, which gives *L* advantages over *M*.

1. Fong SL, Fong WB (1999) Elements regulating the transcription of human interstitial retinoid-binding protein (IRBP) gene in cultured retinoblastoma cells. *Curr Eye Res* 18:283–291.

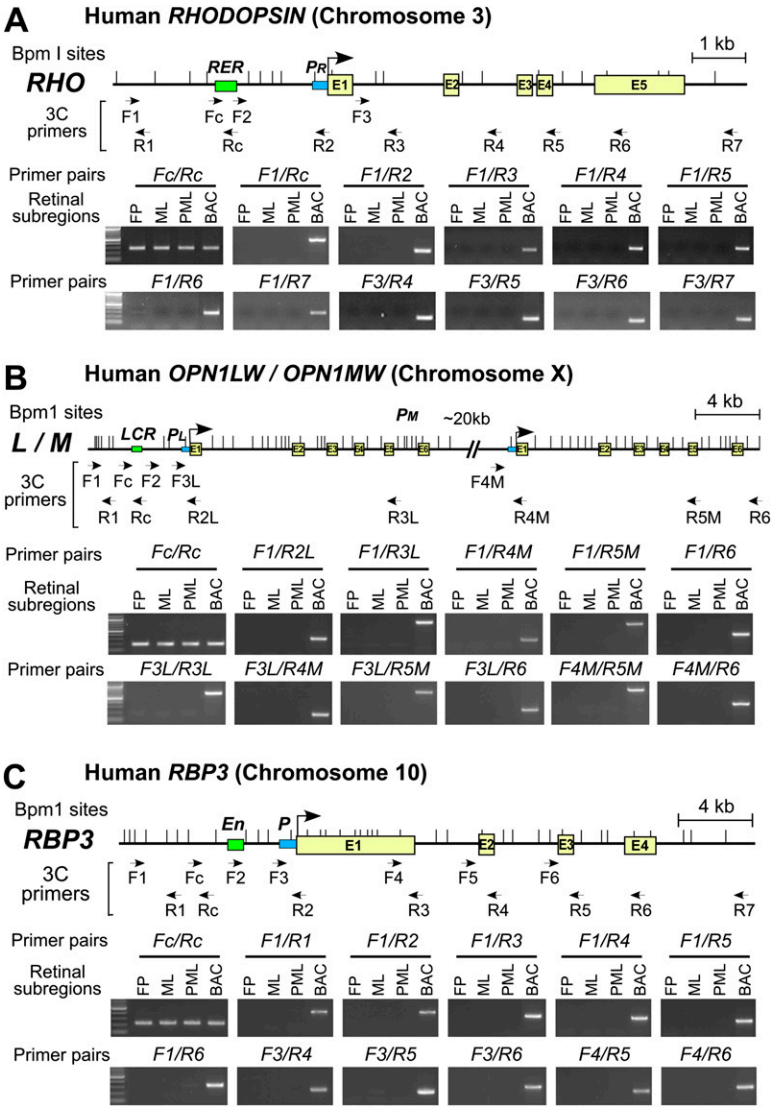

Fig. S4. Gel images of negative 3C results using subregions of human retinas. (*A*) *RHO*. (*B*) *OPN1LW* (*L*) and *OPN1MW* (*M*). (*C*) *RBP3*. FP (rod-rich), ML (cone-rich), and PML (mixed rods and cones). No chromosomal loops were detected with the indicated primer sets. The BAC sample in each set served as a positive control for PCR. A DNA segment (Fc/Rc) served as a constant DNA control for each 3C sample, as described in *SI Materials and Methods*.

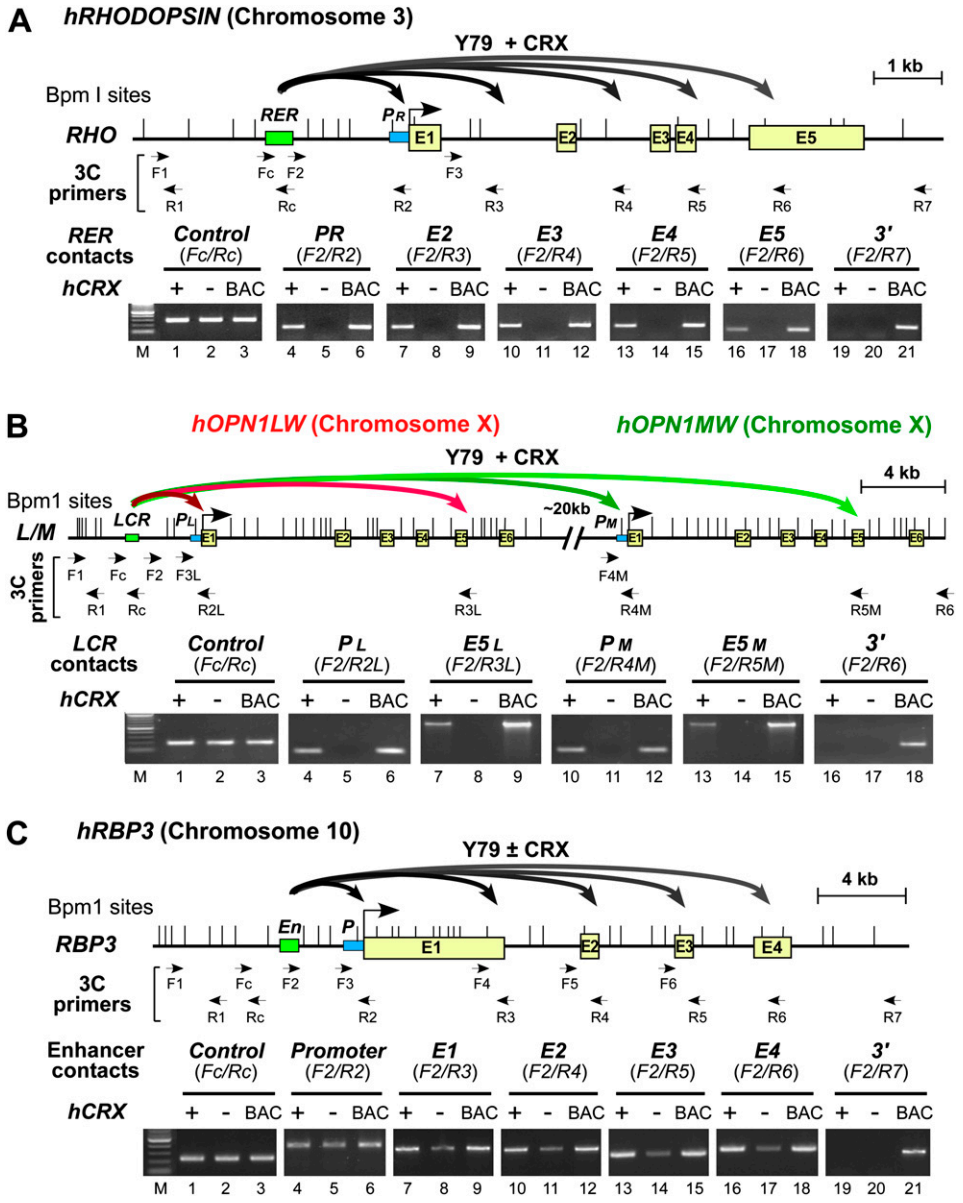

Fig. S5. Recombinant CRX capable of activating *OPSIN* transcription induces intrachromosomal loops in Y79 retinoblastoma cells. Diagrams of human *RHO*, *OPN1LW/OPN1MW*, and *RBP3* loci, respectively, showing gene structure, Bpm1 restriction sites (|), 3C primers, and intrachromosomal loops detected by 3C in Y79 cells with the indicated treatments. Gel images of PCR products below each gene diagram show 3C results from cultured Y79 cells transiently transfected with a recombinant CRX expression vector (+CRX) or an empty vector (−CRX) and the respective BAC-positive controls (BAC). *OPSIN* intrachromosomal loops were detected in *CRX*-transfected Y79 cells (+CRX) but not in the absence of *CRX*, consistent with the induction of *RHO* and *L/M* transcription reported previously (1). In contrast, *RBP3* loops were detected in the absence and presence of recombinant CRX, consistent with *RBP3* expression in these conditions (1).

1. Peng GH, Chen S (2007) Crx activates *opsin* transcription by recruiting HAT-containing co-activators and promoting histone acetylation. *Hum Mol Genet* 16:2433−2452.

Table S1. 3C primers

Gene	Forward primer	Sequence (5′ → 3′)	Reverse primer	Sequence (5′ → 3′)
Mouse				
Rho	Fc	GCGGAGACCACGATGAAG	Rc	GGGAACACAGGGCAGGTATG
	F0	CCATCTTGACAGTCTTGTGCTTG	R0	AGATGCTTGGATGCTGGGG
	F1	GTGACTGCTGAGCCAAAGTTG	R1	GCTGATGGTGGGGGAAATG
	F2	ATGGAAGGAAGGGGGTTGC	R2	TGCGACTGGAACCTTTGG
	F3	TCTGGCTTAGGGAGAGAAGGTC	R3	TTCGTAGACAGAGACCAAGGC
	F4	GATTCTTGTTGGTAGCACTCCTG	R4	CAGTTAGTAGCCGTCAGTCCTCC
	F5	GGAGAAGAGAGGGACTGAATGG	R5	CAGGAAGAGAACACACGCC
	F6	CCAAATGGTTCTGGAAGTCTGC	R6	TTGGGGAGAGGGGGACACAC
			R7	GGCTGAGTGTGTTGTATGTTCC
Opn1mw (*M*)	Fc	GCTATCTCTCAGTTTTGCCCTG	Rc	CCTTATTCCACCTCCTCTTTCC
	F0	CCACATACAGTAGCCAAACCG	R0	TTGTGAGTGCTGGTCAATGAAC
	F1	CACCAGAGCCTCAACATCTTG	R1	TTCTCTCCTCCTTCACCCTTTAC
	F2	GGGAAAGAGGAGGTGGAATAAG	R2	CCCCTCTCTCTACCCATCAAATAC
	F3	TTCCAACCCCTTTCTTCCTG	R3	CCACAAGAATCATCCAGGTGC
	F4	GCTGCCAAACCTGATGAGC	R4	CCATAGTTGCTTGTGCTCTGGAG
	F5	GGGGCTTGTTTAGAGTTTCAGAG	R5	AATGACCTGATTTGGGGGC
	F6	GGAGCAGGGGATTCCTTTC	R6	CGACATTTTGTTGCCTGATTC
			R7	AACAAGTTTTGGCTCACCTGG
Opn1sw (*S*)	Fc	TGGATGGGACACACACTTCC	Rc	CAGCAGGCAGCAGTAAGAGAAC
	F0	GCCTTCGGTTTAGTGCCAGAC	R0	CAAAGCACACATTCTCACCAAC
	F1	AAGTCCCCCAGAGAGGCATTC	R1	TGTAAGAGAAATCAGGCTGGAAC
	F2	AAACCCAAGCAGGAAGTTGC	R2	GGTCAGTATTGGTTTCTGTGGC
	F3	GGTCTCTGGATAGGGACACTCAG	R3	GGGCTGTCGCAACTTTTTG
	F4	AGCGGGAGATGTCAGGAGAG	R4	AGGAAAGCCAATGACCATCC
	F5	CTCAATGCCATAGTGCTGGTG	R5	GGGATGAACCTGAAAAGGACC
	F6	TGTCTTCTGGCATTCTTCCAAC	R6	ACCACTCCCCATCTTTCCC
	F7	TCCTATCCCGAGTGCTGGAGTC	R7	CAAGAAGAGGCTGAAGAGATGG
Rbp3	Fc	CCTGACAGTAAGTCTGCCTCATTTC	Rc	TGGAAGCCATTATCTGACGATG
	F1	GGATTATGGAGTTGAGGAGTTGATG	R1	CTCATCACCCCACTGTTTGC
	F2	TCCTGGTAGTCTTCACCCTCTCC	R2	TGTGAGGAAGAAGTTGGACTGG
	F3	TGAGAACCTGATGGGAATGC	R3	CTAAGGAAATGGGAAGGAGTGAG
	F4	CCAGCACCTCTACTCTGTTTTTGAC	R4	AGAGAATGGCTGTCCTGGGAG
	F5	CCAATGTGTGTTTGAGGTGAAG	R5	CCACTTGTCACTTCACCGATG
	F6	CCCCTCACCTTGCTAATCAAATAG	R6	TGTGCCCAGTCTCAGAAGAGC
Gnat1	Fc	CCTGAGTGGTAGGCAAATAGTCAC	Rc	AACTTGGAGGAAGGTGGTGC
	F1	AACACACACACACACAACATCTG	R1	CTGTCACTTCAGCCCTTGG
	F2	TGTGGAGAGCCAGTTGATTG	R2	CCCAGAAGCAGCAGTTTCAC
	F3	CAGAACCTGTCCTTCCCTACTTG	R3	GTCTGAGGGCAAAATGTGC
			R4	CATACTGAATGTTGAGCGTGG
			R5	CGAACATTCTGTCGGGTAGAG
			R6	GAGGTTCTCCTTGATGATAATGTC
			R7	TCAAGTCCTTCGTGGAAACTG
			R8	CAGCCAGCCCTGAGTATTG
Gnat2	Fc	TCCAGGGTATGTGGTCCTTG	Rc	TGACTCCTATTCTGCTACTGCTCTG
	F1	GCAGACTTTTCCAACTTGCCAG	R1	AACCAGAGGGAGAGGGATACAG
	F2	CAGGCACAAGCAAAGTGGG	R2	AGAGGTTGAGCCAGGGTTTC
	F3	GCAGCAAAGGACCTGTATCTTC	R3	CAACCTGTCTCAATGACGGTG
			R4	TTACTCACTCTAATGCTCCTGCTG
			R5	CCTGGCTCTTCATCAGACTGG
Human				
RHO	Fc	TTGTGAGCAAGACAGACCCAG	Rc	TGGTGATTATGCCCAACGC
	F1	CCTGTAACCCCAGCACTTTG	R1	CAAGCGATTCTCCTGCCTTAG
	F2	GCGTTGGGCATAATCACCAG	R2	CACAAGTTACCTCTGGAGTCATCC
	F3	GCCTCTGTTTCCCTTGGAGC	R3	CACCAGGAGACTTGGAACGC
			R4	TAGCAAGACTGAGGAGGAAAGG
			R5	TCTGTGTGGTGGCTGACTCC
			R6	ATGAGTTGGGAGGAGGAGGG
			R7	GCAACAAAACCCACCACCG
OPN1LW/MW (*L/M*)	Fc	CGTAATCCTGGACAAGGGCAG	Rc	GGGAGATAAGCAACTCTGAGGC
	F1	TCTTACCACCCTACTGTCGCC	R1	CACTCTGAATGGAATGAGGAGC
	F2	TTTTGTGTCTGGCTTCGCTC	R2L*	GTGGTTCTCTTGAAAGCCCAG
	F3L*	GGAAGCCAACAGCAGGATG	R3L*	CAGACGCAGTACGCAAAGAT

Table S1. Cont.

Gene	Forward primer	Sequence (5′ → 3′)	Reverse primer	Sequence (5′ → 3′)
	F4M*	GAATGTGGCAGAAGGTGATGTC	R4M*	GCTGGGCTTAGTGGCAAATAC
			R5M*	CAGAAGCAGAATGCCAGGAC
			R6	GGGTTAGAGTCGCTCATTCACC
RBP3	Fc	CTCAAGCAATGGCAGACAAG	Rc	GGTCACATCACTCCACCGTATG
	F1	TTGTCAGGGTCTGGATGGG	R1	CAGCCAATGGAAGTGAGCC
	F2	GCATACGGTGGAGTGATGTG	R2	GGAAGCAGTAGTTATCCAAGAGG
	F3	CACAACACAGCAAGATAAGATGC	R3	CAGGTCTCTCTTGGGTCTCAC
	F4	AGGACATAGTGGCTCTGCG	R4	TTTGACTGTCCCCTGGCAG
	F5	TCAGAAATGTTAGTTCCTGTCCC	R5	TACAACCTGGGCGTGTGTC
	F6	ATACTCTGGAAAATGCCAACC	R6	TTTGCTGCTCCTGGGAAC
			R7	ATGACTCTGGCTTGGCTGC

*Specific to either *L* or *M* as indicated (≥ 6-bp difference).

Table S2. Primers for sequencing human L/M LCR loops

Primer	Sequence (5′ → 3′)
L/M-Prom	TCACCGCAACCTCGACCTCC
L/M-*E5*	TGTCTCCCTTAGGTGGCAAAG

Table S3. Mouse and human BAC clones

Gene	Gene ID	BAC clone name
Mouse		
Rho	212541	RP24-288F10
Opn1mw (*M*)	14539	RP23-378I14
Opn1sw (*S*)	12057	RP24-285P4
Rbp3	19661	RP23-377H21
Gnat1	14685	RP24-211B23
Gnat2	14686	RP24-252F10
Human		
RHO	6010	RP11-263I1
OPN1LW/MW (*L/M*)	5956/2652	RP11-330B2
RBP3	5949	RP11-635L5

Table S4. Predicted sizes (in base pairs) of 3C PCR products

		Reverse primer								
Gene	Forward primer	Rc	R0	R1	R2	R3	R4	R5	R6	R7
Mouse										
Rho	Fc	234								
	F0		139	165	111	129	107	292	148	134
	F1		258	284	230	248	206	411	267	253
	F2				153	170	106	333	188	174
	F3						116	281	137	123
	F4							320	177	162
	F5								297	284
	F6									131
Opn1mw (*M*)	Fc	125								
	F0		288	287	275	331	270	282	310	508
	F1		154	153	141	198	137	149	176	374
	R2				218	275	214	226	253	451
	R3						104	115	142	340
	F4							242	280	478
	F5								195	394
	F6									293
Opn1sw (*S*)	Fc	245								
	F0		174	179	192	263	245	206	334	242
	F1		154	148	168	239	221	182	310	218
	F2				240	311	293	254	384	290
	F3						211	172	301	208
	F4							150	278	186
	F5								381	288
	F6									135
Rbp3	Fc	173								
	F1		228	173	284	184	266	234		
	F2			168	280	180	262	230		
	F3				296	190	272	240		
	F4					288	372	340		
	F5						310	277		
	F6							162		
Gnat1	Fc	163								
	F1		152	101	163	136	186	159	171	168
	F2				220	193	243	216	228	224
	F3						234	207	219	215
Gnat2	Fc	156								
	F1		138	160	217	235	210			
	F2			198	255	273	248			
	F3					324	299			
Human										
RHO	Fc	289								
	F1	566	181	245	263	275	270	250	265	
	F2			181	199	211	206	186	201	
	F3					173	168	148	163	
OPN1LW/MW (*L/M*)	Fc	197								
	F1		160	303	835	296	829	390		
	F2			125	656	116	656	211		
	F3L				702	162	702	257		
	F4M						796	351		
RBP3	Fc	224								
	F1		464	433	342	312	259	343	323	
	F2			449	358	328	275	359	339	
	F3					276	223	307	287	
	F4						210	294	274	

Table S5. ChIP primers (mouse)

Gene	Region	Forward (5′ → 3′)	Reverse (5′ → 3′)
Rho	5′a	TCGTTTTCATCAGTGTCTGCG	TCCTGGGCTCCAAGCACAAG
	5′b	GTGACTGCTGAGCCAAAGTTG	GCTGAGGACGCTGTATTCAAGC
	RER	CCAGGGTCAGAATCAGAACC	GCAGGTGTCTTGTTTGGGAAC
	Pr	GGGGCAGACAAGATGAGACAC	TTCGTAGACAGAGACCAAGGC
	CR1	TCTGTTTTGGCACACGGG	AGCGGAAGTTGCTCATCGG
	CR2	TGGGCGTGTGTTCTCTTCC	CCTGCTCATACCTCCTTGACTG
	CR3	AATGCCACTTGACAATACCCC	ATTCTTGCCGCAGCACAGC
	3′ UTR	CCTTAGGACTGAGAAAGCATCG	CCTGGAACTGTTGCCACTTG
	3′	CTGTGGCTTGAAAGTAGGAGTC	GGTCTTGGTGGATGGATGTC
Opn1mw (*M*)	5′a	TGGACATCAATGGGAAGAGC	TTTCTGGGTCTGTGTTACCTCAC
	5′b	TTGTGTGTTCTTTGCTGGGG	TAGGGAGTTTGCTGTGAGAGTATG
	LCR	CTCAGTTTTGCCCTGCCTG	TCTCTCCTCCTTCACCCTTTAC
	Pm	TGAGCACCCCTGTGGATTG	GGAACCTGTCAGACTTGGCAC
	CR1	AGGTGAACAGACACTGGACCAC	CACAGGTGCCAAAACCATCC
	CR2	CAGAGACCATTATTGCCAGCAC	TGAACACCATTTGTGAACCCC
	CR3	CATTTCCTGGGAGAGATGGC	CAGTCTGACCTTCCACACTTGG
	CR4	TGTAGGCAACTCTGAGGCACAC	CGGAGGCAAGTGGATTTCTG
	CR5	CTGAGTTTTCCCCATCCCAC	TTCTGTCTTGGAGGTGCTGG
	3′	TTCGGGTGAATGGCACTTC	ATTGGGCTTGGGACTTTGG
Opn1sw (*S*)	5′a	ACGACACGACCACCAGTCCTTC	GCTCCTGCTGCCCTGATTAG
	5′b	GTTTGCTGCTCTGATAAAGGACTG	CACTCTCGTTCTTGGGAATGC
	UPs	CCAGCCTGATTTCTCTTACACC	TGCTTCTCCCAGACTATGTGAG
	Ps	CACTCATCCTCTTCCTGTTTCC	GGTCAGTATTGGTTTCTGTGGC
	CR1	GGACCCCACTCAATGCCATAG	TAGCCCAGGTTGCCTTCAG
	CR2	GGTCCTGGCTACTTGGATTATTG	GAGAACTGGAAGAATGGGCTTAG
	CR3	CATTCCTCTTTCCCTCATCTGC	ACAGCGTTTTCACTCCCCC
	CR4	CTCCTGTGTCTACAACCCCATC	TGTGCCTACCAAGAGCATCC
	3′ UTR	TAGCAGCAAACACAACGCC	CCCTTCTCTCACAGTTCTCCAG
	3′	TTACACCTGCTGCTCCTGG	CCCTCACACTTAGTCCTTTGC

CR, coding region.