Supplementary information for

Pias3-dependent SUMOylation controls mammalian cone photoreceptor differentiation.

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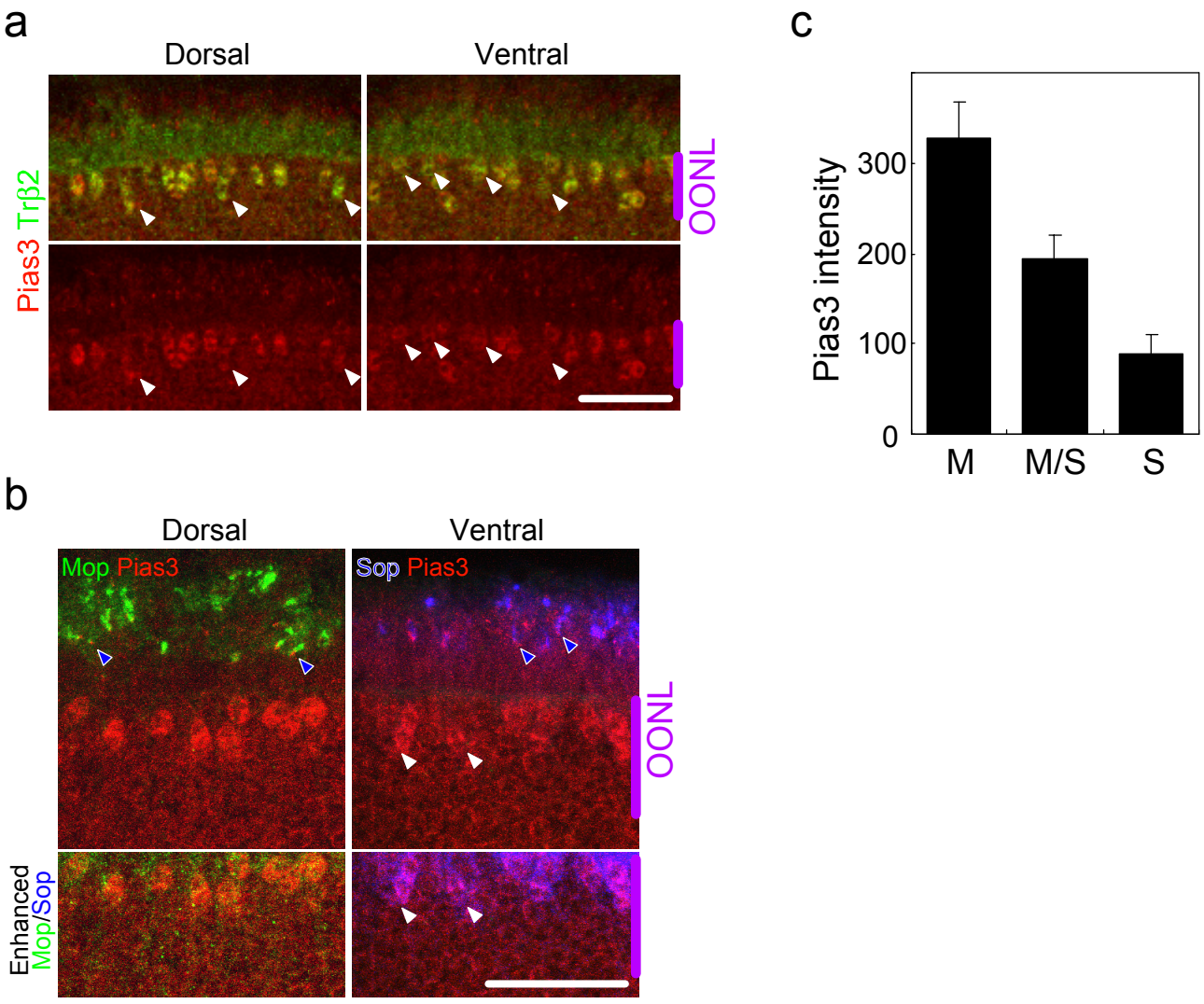
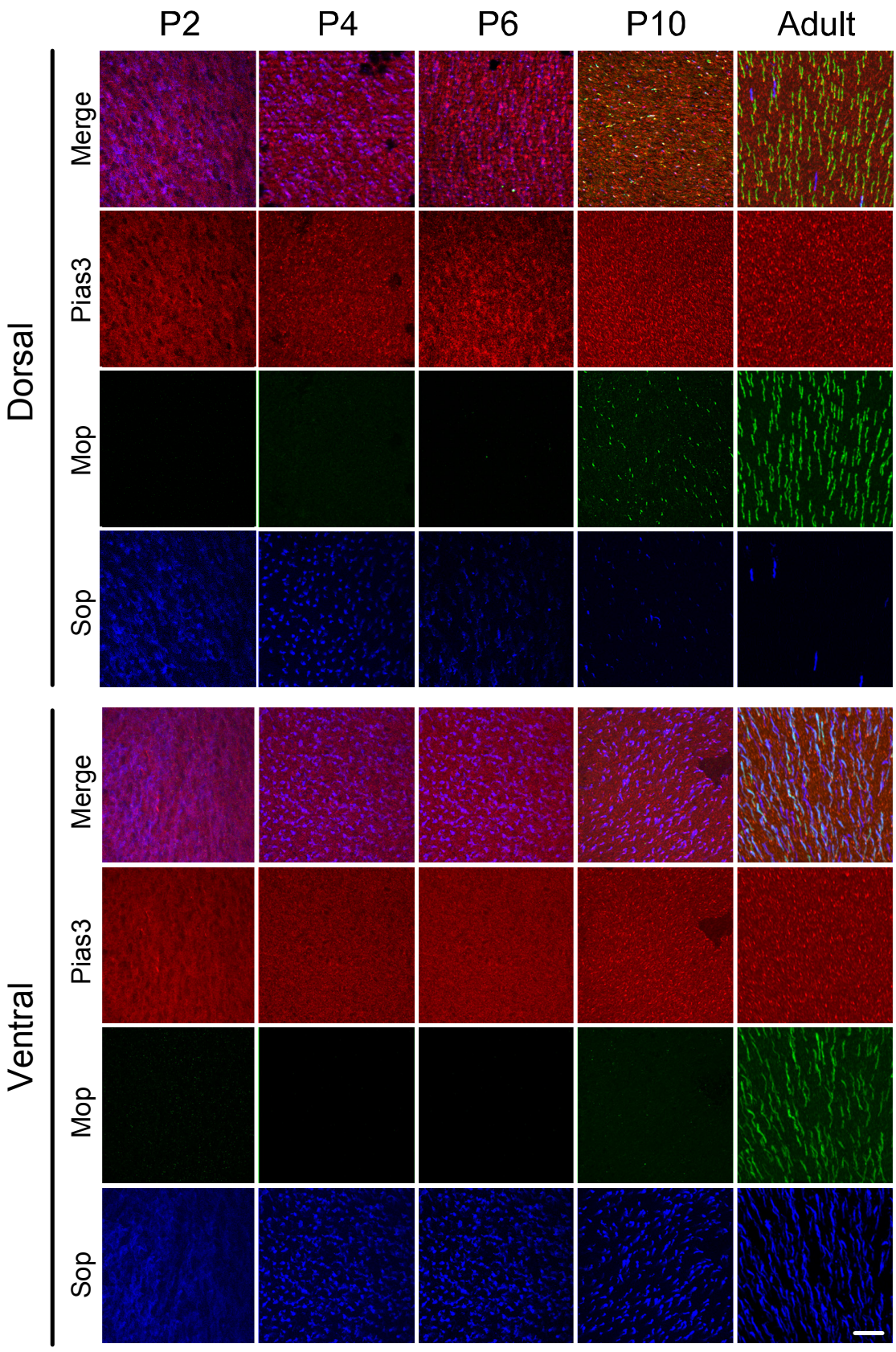
Supplementary Figures:

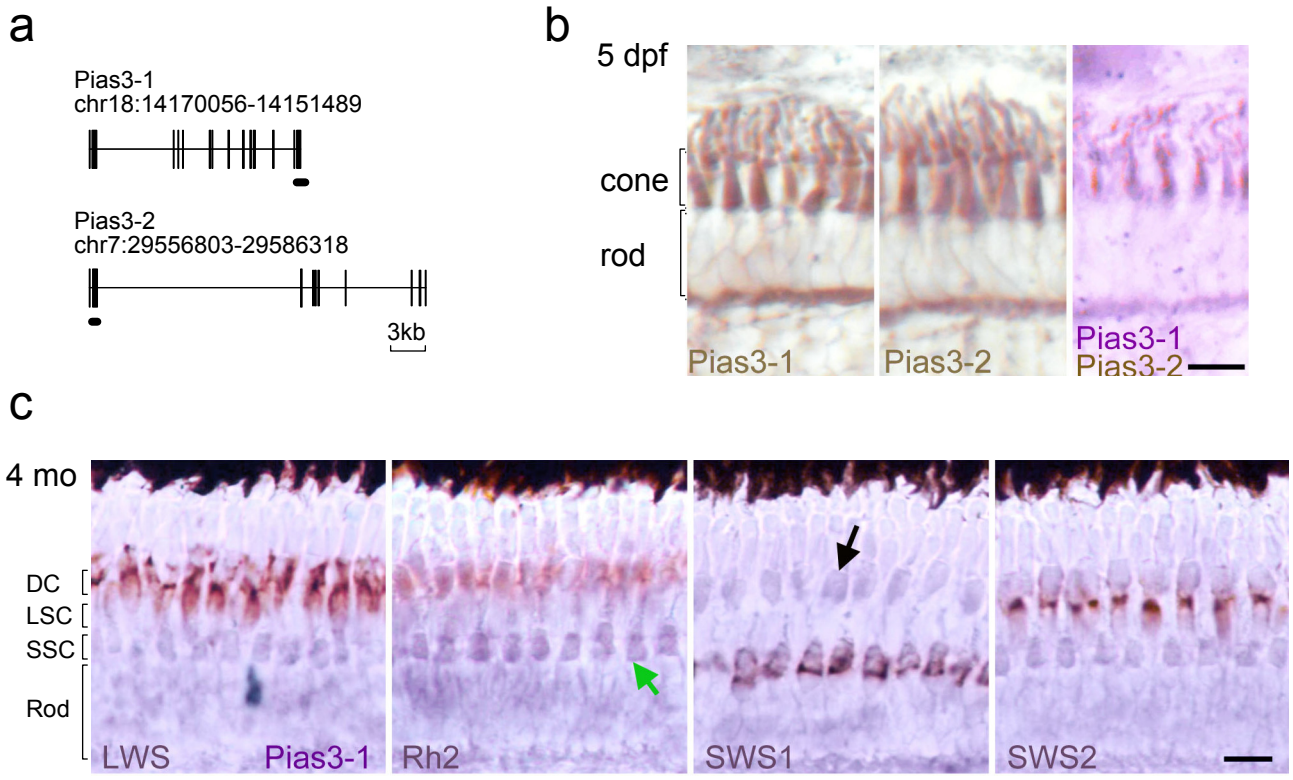


Supplementary Figure 1. Section and flat mount immunohistochemistry (IHC) analysis of Pias3 in 8wk C57BL/6 mouse retinas. (a and b) Section IHC of dorsal and ventral retinas immunostained with Pias3 (red) and Trβ2 (green) (a) and with Sop (blue)/Mop (green) and Pias3 (red) (b). Pias3 signals in cone photoreceptor nuclei were double-labeled with Trβ2, Mop or Sop. A subset of cone photoreceptor

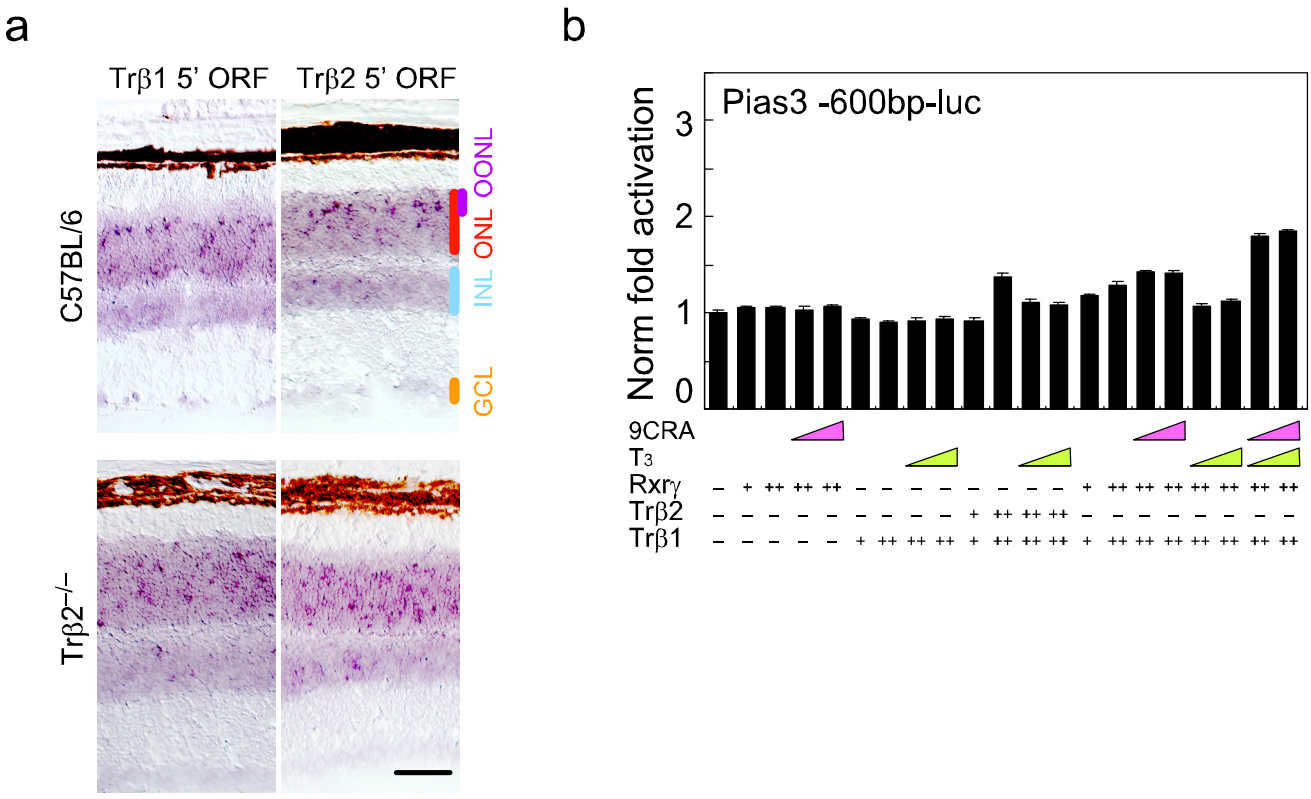
nuclei label relatively weakly with the anti-Pias3 antibody (white arrowheads). These nuclei are preferentially labeled with Sop (b, white arrowheads), indicating that S-dominant cones express less Pias3 than M-dominant cones. Pias3 is also expressed in the inner segment of cone photoreceptors, in a position corresponding to the connecting cilium (blue arrowheads). In the lower panels of Figure S1b, the exposure time is increased to enable S- and M-cone opsin immunostaining to be detected in cone photoreceptor cell bodies. Scale bar: 10 μm. (c) Quantification of nuclear Pias3 immunofluorescence of each cone subtype (M, M/S and S) from confocal Z projections obtained from flat mount immunohistochemistry data shown in Figure 1a. All data are represented as mean ± s.d. (n = 6 cells of each type). The immunofluorescence intensity of nuclear Pias3 levels shows good concordance with cone photoreceptor subtype, matching the data for Pias3 levels in cone photoreceptor inner segments that are shown in Figure 1b.


P2
P4
P6
P10
Adult
Dorsal
Ventral
Merge
Pias3
Mop
Sop
Merge
Pias3
Mop
Sop

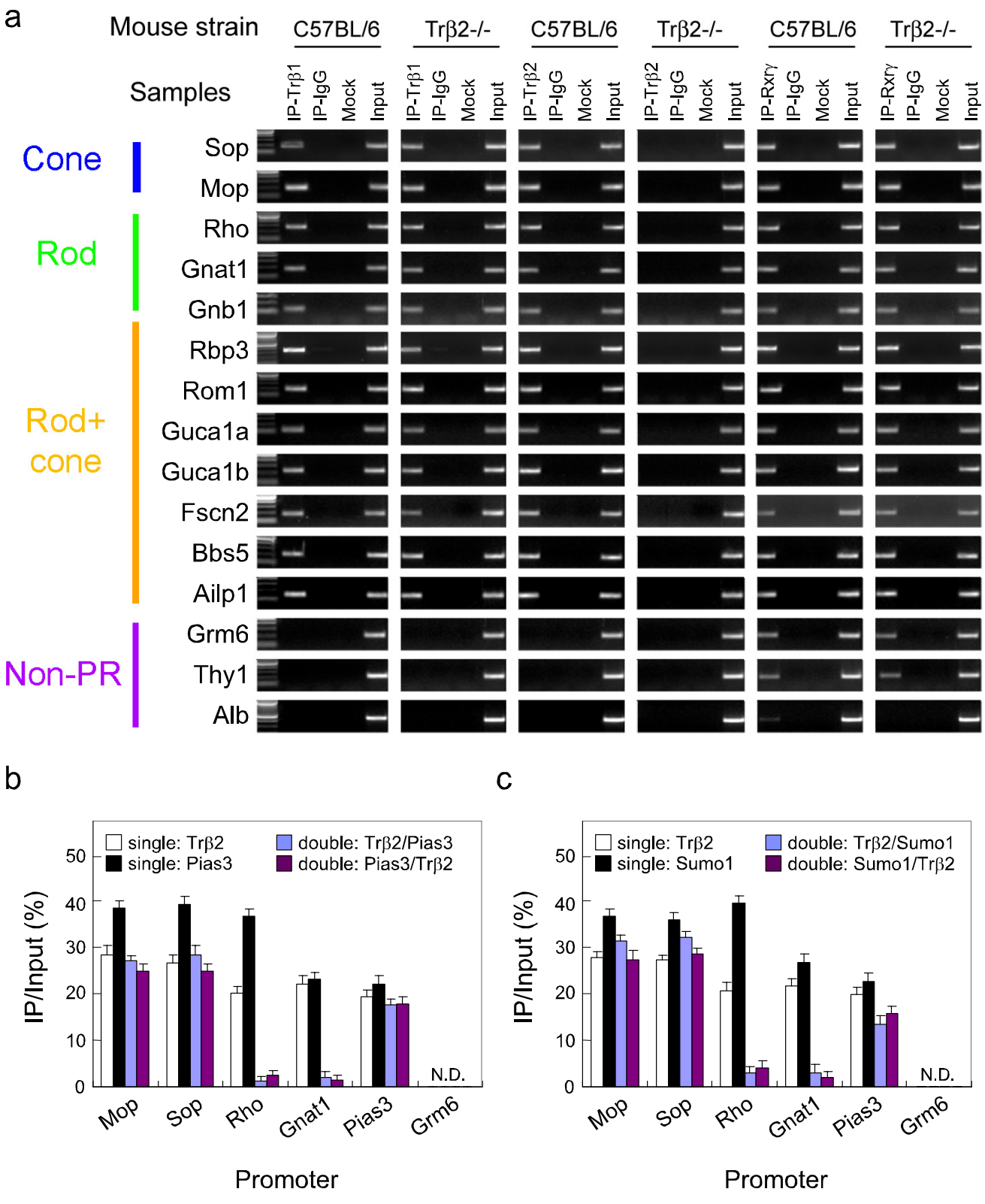
Supplementary Figure 2. Developmental expression of Pias3 (red) in dorsal and ventral region of the mouse retina, and co-immunostained with Sop (blue) and Mop (green) antibodies. S-opsin is expressed both in dorsal and ventral region at P2. The selective expression of Pias3 in cone photoreceptors precedes both the downregulation of S-opsin expression and induction of M-opsin expression (P6~P10). In the adult, Pias3 is preferentially expressed in M-opsin expressing cones (see Figure 1). Scale bar: 20 μm.



Supplementary Figure 3. *Pias3* orthologues are expressed in *LWS*, *Rh2* and *SWS1* cones of zebrafish (a) Genomic structure and location of the two Pias3 homologues of Zebrafish. (b) Single or two-color chromogenic *in situ* hybridization analysis of *Pias3-1* (brown) and *Pias3-2* (brown) in 5 days post fertilization (5 dpf) zebrafish retina. *Pias3-1* (purple) and *Pias3-2* (brown) are colocalized in cone photoreceptors. (c) Two-color chromogenic *in situ* hybridization of *Pias3-1* (purple) and each cone subtype (*LWS, SWS1, SWS2, Rh2*, shown in brown) in four months post-fertilization zebrafish retinas. The result suggests that *SWS2*-positive long single (LCS) cones do not express this *Pias3* homologue. Black arrow indicates *Pias3-1* expression in *LWS/Rh2*-positive double cones (DC), while green arrow indicates *Pias3-1* expression in *SWS1*-positive short-single cones (SSC) Scale bars: Figures B and C, 5 μm.

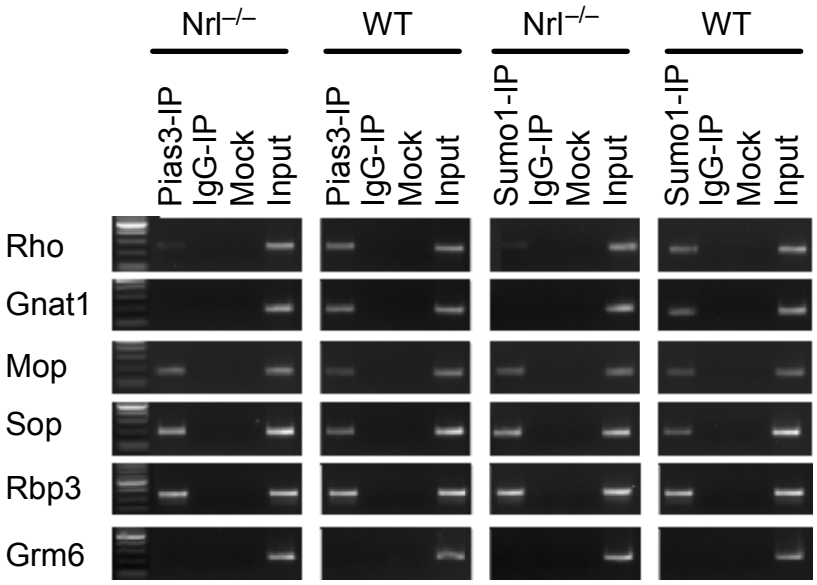


Supplementary Figure 4. (a) *In situ* hybridization analysis of *Trβ1* and *Trβ2* mRNA in adult mouse retina. Probes used are generated from divergent sequences at the 5'end of the transcripts, including untranslated and ORF-derived sequence. In C57BL/6, *Trβ1* and *Trβ2* transcripts are primarily observed in outer region of outer nuclear layer (OONL), where cone photoreceptors are located. In the *Trβ2*$^{-/-}$ mouse retina, both mRNAs are expressed in a subset of cells scattered throughout the ONL. Since the *in situ* probes hybridization probes used here are complimentary to the first untranslated exon of *Trβ1* and *Trβ2*, were are able to detect expression of this portion of the *Trβ2* transcript in *Trβ2*$^{-/-}$ mice, as the first exon is not disrupted by the targeted mutation of *Trβ2*. Scale bar: 50 μm. (b) Trβ1 and Rxrγ can weakly activate expression of a Pias3 promoter luciferase reporter construct in the presence of 9-*cis* retinoic acid (9CRA), but do so much less efficiently than Trβ2 and Rxrγ. Pink and green right triangles represent increasing concentrations of 9CRA (5 μM and 10 μM) and 3,5,3′-triiodothyronine (T_3, 1 nM and 5 nM), respectively. '+' and '++' represent 15 ng and 30 ng of transfected plasmid, respectively. All data are represented as mean ± s.d. (n = 3).

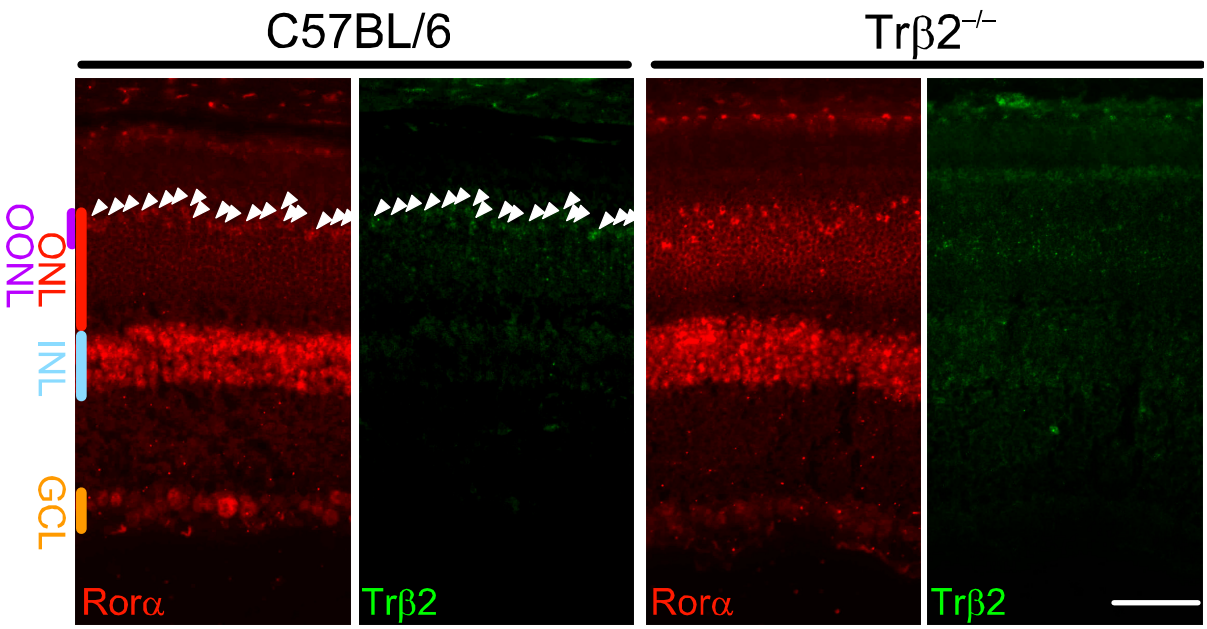


Supplementary Figure 5. ChIP assays performed using P14 retinas from C57BL/6 and *Trβ2*$^{-/-}$ mice. (a) Trβ1, Trβ2, and Rxrγ preferentially occupy cone photoreceptor-specific gene

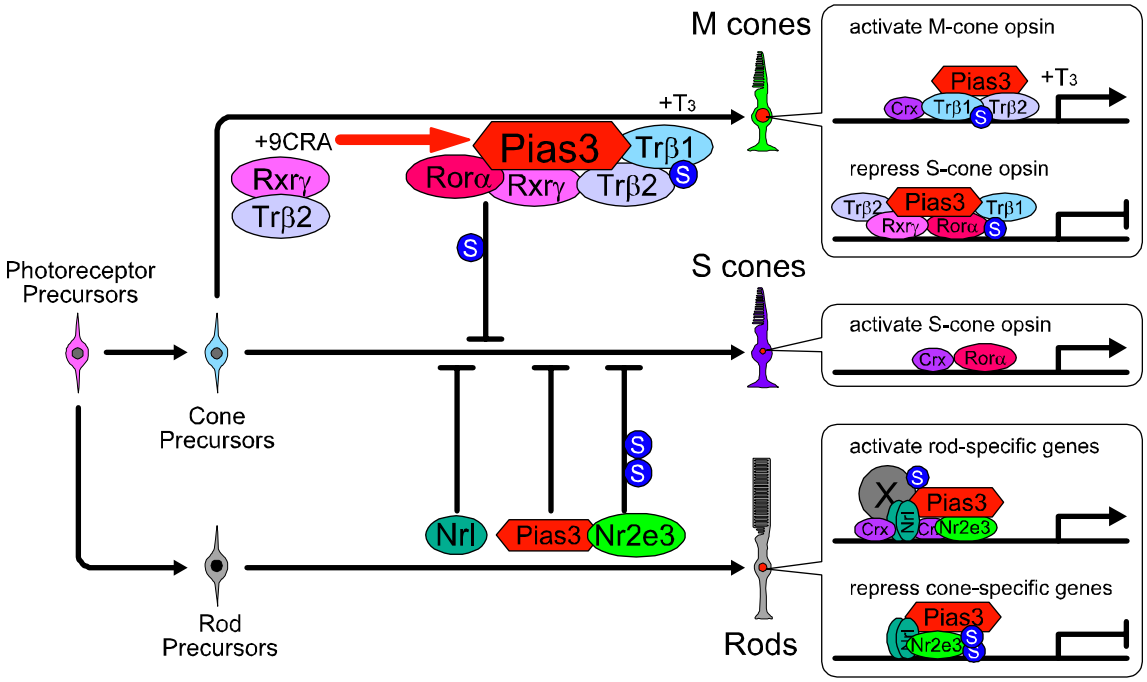
promoters. (b) and (c) Quantitative PCR for the promoters for Mop, Sop, Rhodopsin (Rho), rod transducin (Gnat1), Pias3 and metabotropic glutamate receptor 6 (Grm6) using chromosomal DNA immunoprecipitated with antibodies for Trβ2 and Pias3 (b) and Trβ2 and Sumo1 (c). Input ratio is calculated as in Figure S4A. Data are presented as mean ± s.d. (n = 3).



Supplementary Figure 6. ChIP assays performed using P14 retinas from C57BL/6 and *Nrl*$^{-/-}$ mice. PCR analysis for the promoters for Mop, Sop, Rhodopsin (Rho), rod transducin (Gnat1), Pias3 and metabotropic glutamate receptor 6 (Grm6) using chromosomal DNA immunoprecipitated with antibodies for Pias3 and Sumo1 is shown.



Supplementary Figure 7. Section immunohistochemistry of adult C57BL/6 and *Trβ2*$^{-/-}$ mouse retinas immunostained with Rorα (red) and Trβ2 (green) antibodies. White arrowheads represent cone photoreceptors expressing Trβ2, which are immunonegative in *Trβ2*$^{-/-}$ animals. Scale bar: 50 μm.



Supplementary Figure 8. Model of the mechanism of action of Pias3 in mouse photoreceptor development. "S" indicates SUMOylated proteins, while "X" indicates an unidentified SUMOylated factor present on the promoters of rod-specific genes as suggested in our recent study[22]. In cone precursors, Trβ2 and Rxrγ activated by 9CRA enriched in dorsal retina activate expression of Pias3. Pias3 expression in cones causes downregulation of S-opsin expression and induction of M-opsin expression by SUMOylation of cone-enriched transcription factors (Rorα, Rxrγ, Trβ1 and Trβ2). T_3-bound Trβ1 and/or Trβ2 enhance Pias3-dependent M-opsin expression.

Suipplementary Table 1. DNA constructs used in this study

construct		cloning method
description in the text	cloned DNA/gene	
constructs cloned into pCAGIG(1)		
CAG-GFP	ires-GFP (pCAGIG)	gift from C. Cepko (Proc Natl Acad Sci U S A. 101:16-22)
CAG-Pias3	mouse Pias3 (accession no. NM_018812.1)	RT-PCR
Pias3 ΔSUMO	Pias3 mutant in RING domain (C299S, H301A)	PCR from myc-tagged Pias3 RING mutant (gift from F. Liu)
CAG-Gam1	Gam1 (accession no. AC_000014.1:37391-38239)	PCR from myc-tagged Gam1 (gift from S. Chiocca)
constructs cloned into pcDNA3.1/HisC or pcDNA3.1/nV5-DEST(2)		
Pias3	mouse Pias3 (accession no. NM_018812.1)	RT-PCR
Pias3 ΔSUMO	Pias3 mutant in RING domain (C299S, H301A)	PCR from myc-tagged Pias3 RING mutant (gift from F. Liu)
Rxrγ	human RXRG (accession no. NP_001009598.1)	Gateway cloning from Ultimate Human ORF Collection (Invitrogen)
Trβ2	mouse Trβ2 (accession no. NP_033406.1)	PCR from Trβ2 cDNA (gift from D. Forrest)
Crx	bovine Crx	Hum Mol Genet. 14:747-764
Trβ1	human THRB (accession no. BC106929.1)	Gateway cloning from Ultimate Human ORF Collection (Invitrogen)
Rorα	human RORA (accession no. NM_134262.1)	Gateway cloning from Ultimate Human ORF Collection (Invitrogen)
Rorα K185R	RORA K185R mutant	QuikChange XL site-directed mutagenesis Kit (Stratagene)
constructs cloned into pSilencer2.1/Hygro(3)		
U6-Pias3	Pias3 shRNA (516-532)	Synthetic oligo
U6-control	Negative control shRNA	Silencer® Negative Control #1 siRNA (Ambion)
constructs cloned into pGL2/3(4)		
Pias3 -600bp-luc	mouse Pias3 promoter region (chr3:96,500,554-96,501,097)	PCR
Mop -250bp-luc	human M opsin promoter (chrX:153,447,873-153,448,163)	Hum Mol Genet. 14:747-764
Sop -600bp-luc	human S opsin promoter (chr7:128,415,845-128,416,406)	gift from D. Forrest (Mol Endocrinol. 20:1728-1741)
constructs cloned into pBluescript KS-(5)		
Pias3-1	Zebrafish Pias3-1 (chr18:14,151,257-14,151,760)	RT-PCR
Pias3-2	Zebrafish Pias3-2 (chr7:29,557,068-29,557,473)	RT-PCR
LWS	Zebrafish LWS opsin (accession no. NM_131175: 111-1184)	RT-PCR
SWS1	Zebrafish SWS1 opsin (accession no. NM_131319: 62-1072)	RT-PCR
SWS2	Zebrafish SWS2 opsin (accession no. NM_131192: 128-1192)	RT-PCR
Rh2	Zebrafish Rh2 opsin (accession no. NM_131253: 68-1117)	RT-PCR
Trb1	mouse Trb1 5' region (accession no. NM_001113417.1: 0 bp-469 bp)	PCR
Trb2	mouse Trb2 5' region (accession no. NM_009380.3: 0 bp-554 bp)	PCR

(1) gift from C. Cepko, (2) Invitrogen (3) Ambion (4) Promega (5) Stratagene

Suipplementary Table 2. PCR primers used for ChIP analysis.

Gene	Forward primer	Reverse primer
Aipl1	GGAGCAGGGGAAAGTGGAG	GGAAGGAGAGAAGAAAGCAGGC
Bbs5	AGCCCCATCAAACCAGTAATG	CTTCCACCAGGTAGTGTGCG
Fscn2	AAGAAGAGAGACTAAGAACCGTGG	AGAAGCAGTAGCCAACTTGCC
Gnat1	TTCAGCACTCTCCTGCCAGC	GCCCCAATCCTTCAACTGC
Gnb1	TCTTTCCTGTCCCACCTGG	TGTGTCCTGCCTGAATGACC
Guca1a	CACACAAGATGCCCACTCAAG	GAACCCACAATGCTCTGCC
Guca1b	CCCAGGCTCTTACCCTCATAG	CCTTAGGCTCCTCTCTCCTTGG
Pias3	TGGCGGGACTCTGGGATTTC	TAACGACGAGAAGGCGGACC
Rom1	AGGTAACCCTTCCCTGTCCG	GCTTCTTAGCCTTGCCCTTTAG