SUPPLEMENTARY MATERIALS AND METHODS

ERG recording and analysis

A typical ERG trace is composed of the a-wave, which is a negative component originating from the photoreceptors, and the b-wave, which is a positive component generated by the bipolar and Müller cells complex stimulation. Two major parameters can be derived from these waves, namely the amplitude (μV) and the implicit time (ms). By convention, the amplitude of the a-wave is measured from the baseline to trough, and the b-wave amplitude is measured from the trough of the a-wave to the peak of the b-wave. The implicit time of the waves represents the number of milliseconds at which the maximal amplitude is reached. For each of the four parameters, a photopic and scotopic luminance response function (LRF) was generated, where the values of the parameters were plotted against flash luminance. Repeated measures analyses of variance with Bonferroni correction were performed to assess the difference between the three genotypes for each one of the parameters. All analyses were conducted using SPSS for Windows, version 22.0.

Immunohistochemistry and immunofluorescence

For immunofluorescence labeling, sections were incubated overnight with primary antibody solutions at 4ºC in a humidified chamber. After three washes in PBS, sections were incubated with secondary antibodies for 1 h at room temperature. Slides were mounted on coverslips in DAPI-containing mounting medium (Vector Laboratories, CA). For immunohistochemistry labeling, slices were analyzed by using the Vectastain® ABC kit (Vector) according to the manufacturer instructions. Peroxidase substrates used are the Vector® VIP (Pink) (Vector), and DAB (brown) (Sigma). Observations were made under a fluorescence microscope (Leica DMRE, Leica Microsystems) and images were captured with a digital camera (Retiga EX; QIMAGING; with OpenLab, ver.3.1.1 software; Open-Lab, Canada). Confocal microscopy analyses were performed using 60x objectives with an IX81 confocal microscope (Olympus, Richmond Hill, Canada), and images were obtained with Fluoview software version 3.1 (Olympus). 3D reconstructions were obtained with Fluoview software version 3.1 from 18-25 z-stack image. Primary antibodies used in this study are: sheep anti-Chx10 (1:250, Exalpha Biologicals), rabbit anti-Pax6 (1:500, Chemicon), mouse anti-Bmi1 (1:200, US Biological), rabbit anti-Bmi1 (1:150, US Biological), mouse anti-Syntaxin (1:200, Sigma), mouse anti-4D2 (Rhodopsin) (1:100, R. Molday, UBC), mouse anti-Gad65 (1:300, BD Pharmingen), rabbit anti-CORD2 (CRX) (1:300, Abcam), rabbit anti-S-Opsin (1:200, Invitrogen), rabbit anti-M-Opsin (1:100, Chemicon), Rip3 (1:250, Santa Cruz), rabbit anti-H3K9me3 (1:500, Abcam), rabbit anti-H3K9ac (1:300, Cell Signaling), rabbit anti-H3K27me3 (1:300, Cell Signaling), mouse anti-glutamine synthetase (GS) (1:100, Chemicon), rabbit anti-Pkcα

(1:500, Sigma), rabbit anti-Calbidin (1:500, Chemicon), rabbit anti-Recoverin (1:1000, Millipore), mouse anti-γH2Ax (1:250, Millipore), rabbit anti-Cralbp (1:500, kindly given by Dr Saari's lab). Secondary antibodies are: donkey AlexaFluor488-conjugated anti-mouse (1:1000, Life Technologies), donkey AlexaFluor488-conjugated anti-rabbit (1:1000, Life Technologies), goat AlexaFluor594-conjugated anti-mouse IgM (1:1000, Invitrogen), donkey AlexaFluor633-conjugated anti-sheep (1:1000, Molecular Probes), goat AlexaFluor647-conjugated anti-mouse (1:1000, Life Technologies) goat AlexaFluor texas red-conjugated anti-rabbit (1:1000, Life Technologies), donkey FITC-conjugated anti-mouse (1:300, Chemicon), goat FITC-conjugated anti-rat (1:300, Caltag Laboratories), donkey Rhodamine-conjugated anti-rabbit (1:300, Chemicon). Fluorescein labeled Peanut Agglutinin (PNA) (1:200, Vector laboratories) was used to stain the outer segment of PRs. Superoxide production was measured by red fluorescence intensity as described by standard protocol indicated by manufacturer (MitoSoxRed mitochondrial superoxide indicator, Molecular Probes, U.S.).

Quantitative RT-PCR

Mouse primer sets used are:

Tnf (F) 5' AAAATTCGAGTGACAAGCCTGTAG 3'; Tnf (R) 5' CCCTTGAAGAGAACCTGGGAGTAG 3'; Tnfrsf1a (F) 5' GCCGGATATGGGCATGAAGC 3'; Tnfrsf1a (R) 5' TGTCTCAGCCCTCACTTGAC 3'; Ripk1 (F) 5' TGTCATCTAGCGGGAGGTTG 3'; Ripk1 (R) 5' TCACCACTCGACTGTGTCTCAG 3'; Ripk3 (F) 5' CTCCGTGCCTTGACCTACTG 3'; Ripk3 (R) 5' AACCATAGCCTTCACCTCCC3'; Bmi1 (F) 5'-GGAGACCAGCAAGTATTGTCCTATTTG-3', Bmi1 (R) 5'-CTTACGATGCCCAGCAGCAATG-3'; $p16^{Ink4a}$ (F) 5'-CAACGCCCCGAACTCTTTC-3', $p16^{Ink4a}$ (R) 5'-GCAGAAGAGCTGCTACGTGAAC-3'; $p19^{Arf}$ (F) 5'-GGCTAGAGAGGATCTTGAGAAGAGG-3', $p19^{Arf}$ (R) 5'-GCCCATCATCATCACCTGGTCCAGG-3'; Sox2 (F) 5'-TAAGGGTTCTTGCTGGGTTTT-3', Sox2 (R) 5'-AGACCACGAAAACGGTCTTG-3'; Lhx2 (F) 5'-GATCTCGCCTGGAAACAGAG -3', Lhx2 (R) 5'-TCGCTCAGTCCACAAAACTG -3'; Otx2 (F) 5'-AGAGGAGGTGGCACTGAAAA-3', Otx2 (R) 5'-TGACCTCCATTCTGCTGTTG-3'; Lpo (F) 5'-AGGTCTGTTGGCCAAGAATG-3', Lpo (R) 5'-ATGTTGATGGAAGCCAGGTC-3'; Apaf1 (F) 5'-TGCTCAGCGGATAAGAAGGT-3', Apaf1 (R) 5'TCCCAGAGCTTGAGGAAGAA-3'; Fas (F) 5'-AAACAAACTGCACCCTGACC-3', Fas (R) 5'CAACCATAGGCGATTTCTGG-3'; Nqo1 (F) 5'-TTCTCTGGCCGATTCAGAGT-3', Nqo1 (R) 5'GAGTGTGGCCAATGCTGTAA-3'; Gsta1 (F) 5'-CGCCACCAAATATGACCTCT-3', Gsta1 (R) 5'CCATGGCTCTTCAACACCTT-3';

Cyp24a1 (F) 5'-GGCGGAAGATGTGAGGAATA-3', Cyp24a1 (R) 5'GTTGTGAATGGCACACTTGG-3'; Duox2 (F) 5'-ACAAGGGGTGTATGCCTTTG-3', Duox2 (R) 5'-CACAGGTTGTGGTAGCGAAA-3'. Crx (F) 5'-CCTTCTGACAGCTCGGTGTT-3', Crx (R) 5'-CCACTTTCTGAAGCCTGGAG)-3'; Sesn2 (F) 5'-CCTCCTTTGTGTTGTGCTGT-3', Sesn2 (R) 5'-ACGGTTCTCCATTTCCTCCT-3'; Opn1sw (F) 5'-CAGCCTTCATGGGATTTGTCT-3', Opn1sw (R) 5'-CAAAGAGGAAGTATCCGTGAC-3'; Rax (F) 5′-TGGGCTTTACCAAGGAAGACG-3', Rax (R) 5′-GGTAGCAGGGCCTAGTAGCTT-3'; Six6 (F) 5′-GCAAGTAGCCGGGGTATGTG-3', Six6 (R) 5′-CGACTCATTCTTGTTAAGGGCTT-3'; Nrl (F) 5′-CCCAGTCCCTTGGCTATGGA-3', Nrl (R) 5′-ACCGAGCTGTATGGTGTGGA-3'; Notch1 (F) 5′-GATGGCCTCAATGGGTACAAG-3', Notch1 (R) 5′-TCGTTGTTGTTGATGTCACAGT-3'; Pax6 (F) 5′-TGGCAAACAACCTGCCTATG-3', Pax6 (R) 5′-TGCACGAGTATGAGGAGGTCT-3'; Gapdh (F) 5′-AGGTCGGTGTGAACGGATTTG-3′, Gapdh (R) 5′-TGTAGACCATGTAGTTGAGGTCA-3';

Human primer sets used are:

PAX6 (F) 5'-AGATTTCAGAGCCCCATATTCG-3', PAX6 (R) 5'-CCATTTGGCCCTTCGATTAG-3'; ARR3 (F) 5'-CCCAGAGCTTTGCAGTAACC-3', ARR3 (R) 5'-CACAGGACACCATCAGGTTG-3'; SOX1 (F) 5'-AAAGTCAAAACGAGGCGAGA-3', SOX1 (R) 5'-AAGTGCTTGGACCTGCCTTA-3'; RAX (F) 5'-GGCAAGGTCAACCTACCAGA-3', RAX (R) 5'-GCTTCATGGAGGACACTTCC-3'; SIX6 (F) 5′- ACAGACTCCAGCAGCAGGTT-3′, SIX6 (R) 5′-AGATGTCGCACTCACTGTCG-3′; OPN1SW (F) 5′-TGTGCCTCTCTCCCTCATCT-3′, OPN1SW (R) 5′-GGCACGTAGCAGACACAGAA-3′; $p16^{INK4A}$ (F) 5′-GATCCAGGTGGGTAGAAGGTC-3′, $p16^{INK4A}$ (R) 5′-CCCCTGCAAACTTCGTCCT-3′; CDKN1A (F) 5′-CCGAAGTCAGTTCCTTGTGG-3′, CDKN1A (R) 5′-GTCGAAGTTCCATCGCTCAC3′; SOX2 (F) 5′-CACAACTCGGAGATCAGCAA-3′, SOX2 (R) 5′-CGGGGCCGGTATTTATAATC-3′; LHX2 (F) 5′-CCAAGGACTTGAAGCAGCTC-3′, LHX2 (R) 5′-TAAGAGGTTGCGCCTGAACT-3′; BMI1 (F) 5-AATCCCCACCTGATGTGTGT-3, BMI1 (R) 5-GCTGGTCTCCAGGTA ACGAA-3; GAPDH (F) 5-TCACCAGGGCTGCTTTTAAC-3′, GAPDH (R) 5′-ATCCACAGTCTTCTGGGTGG -3′.

Western blot

Total protein extracts were prepared in the Complete Mini protease inhibitor cocktail solution (Roche Diagnostics). Proteins contents were quantified using the Bradford reagent. Proteins were

resolved in Laemmli buffer by SDS-PAGE and transferred to a 0.2µm Nitrocellulose Blotting Membrane (BioRad) that was exposed to the primary antibodies: mouse anti-Bmi1 (1:800, Millipore), mouse anti-H2Aub (1:1000, Millipore), mouse anti- βActin (1:1000, abcam), p63, mouse anti-p73 (1:500, abcam), mouse anti-p53 (1:500, Santa Cruz Biotecnology), goat anti-CRX (1:500, Santa Cruz Biotecnology), mouse anti-α-Tubulin (1:1000, Sigma), anti-Rip3 (1:1000, Santa Cruz Biotecnology), anti-Bmi1 (1:500, abgent), S-Opsin (1:400, Santa Cruz Biotechnology) and histone H3 (1:1000, upstate). Membranes were treated with corresponding horseradish peroxidase-conjugated secondary antibodies (Sigma) and developed using the Immobilon Western (Millipore).

Cell cultures

Cultures of retinal cells were obtained by dissecting the mice eyes at P5 in 1X oxygenated HBSS (Life Technologies) in order to extract only the neural retina. Retinal cells were then re-suspended and incubated 10 minutes at 37 °C in the enzyme solution composed of 10ml 1X HBSS, 9.3 mg of Papain (Worthington), 1.6 mg of N-acetyl L-cysteine (Sigma), 0.5 mg of DNaseI (Roche) and 10µl EDTA 500mM (Fisher Scientific). After centrifugation cells were dissociated into Neurobasal^{tm} medium (Life technologies) with 0.02 µg/µl NGF (Invitrogen), 0.02 µg/µl BDNF (Invitrogen), 1% B27 (Invitrogen), 70 µg/ml gentamycin (Invitrogen), 1% fetal bovine serum (Wisent), 0.5% glucose (Sigma) and 10 µM Forskolin (Sigma). The cells were then spread and cultivated on coverslips treated with Poly-L-Lysine hydrobromide (Sigma) and BD Matrigel Matrix (BD Biosciences).

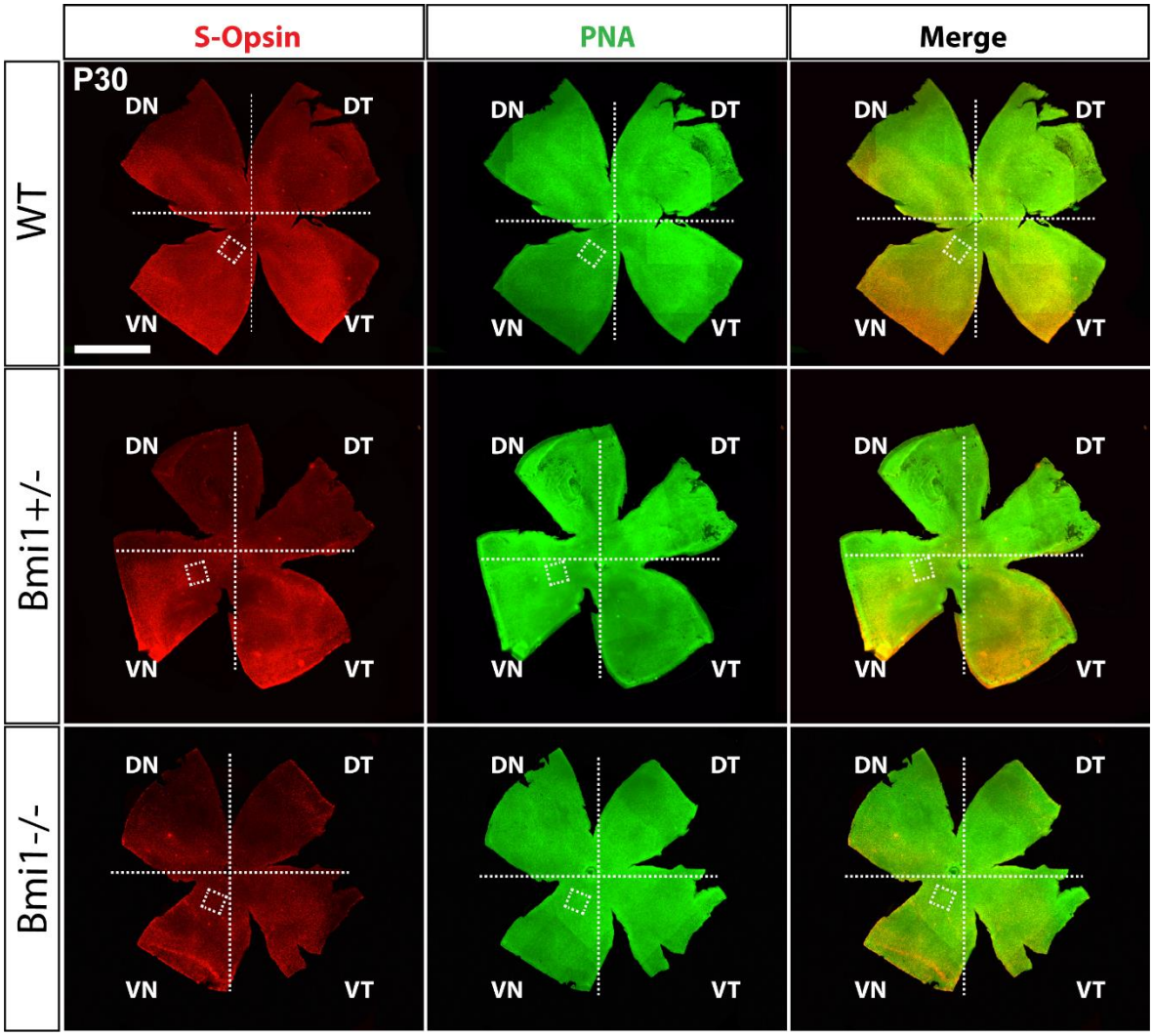

Figure S1. *Bmi1* is required for cone photoreceptor maintenance after birth

(A) Representative images from S-Opsin/PNA double-stained retinal flat mounts from WT, *Bmi1*$^{+/-}$ and *Bmi1*$^{-/-}$ mice at P30. Dorso-nasal (DN), dorso-temporal (DT), ventro-nasal (VN), ventro-temporal (VT). Scale bars: 1 mm.

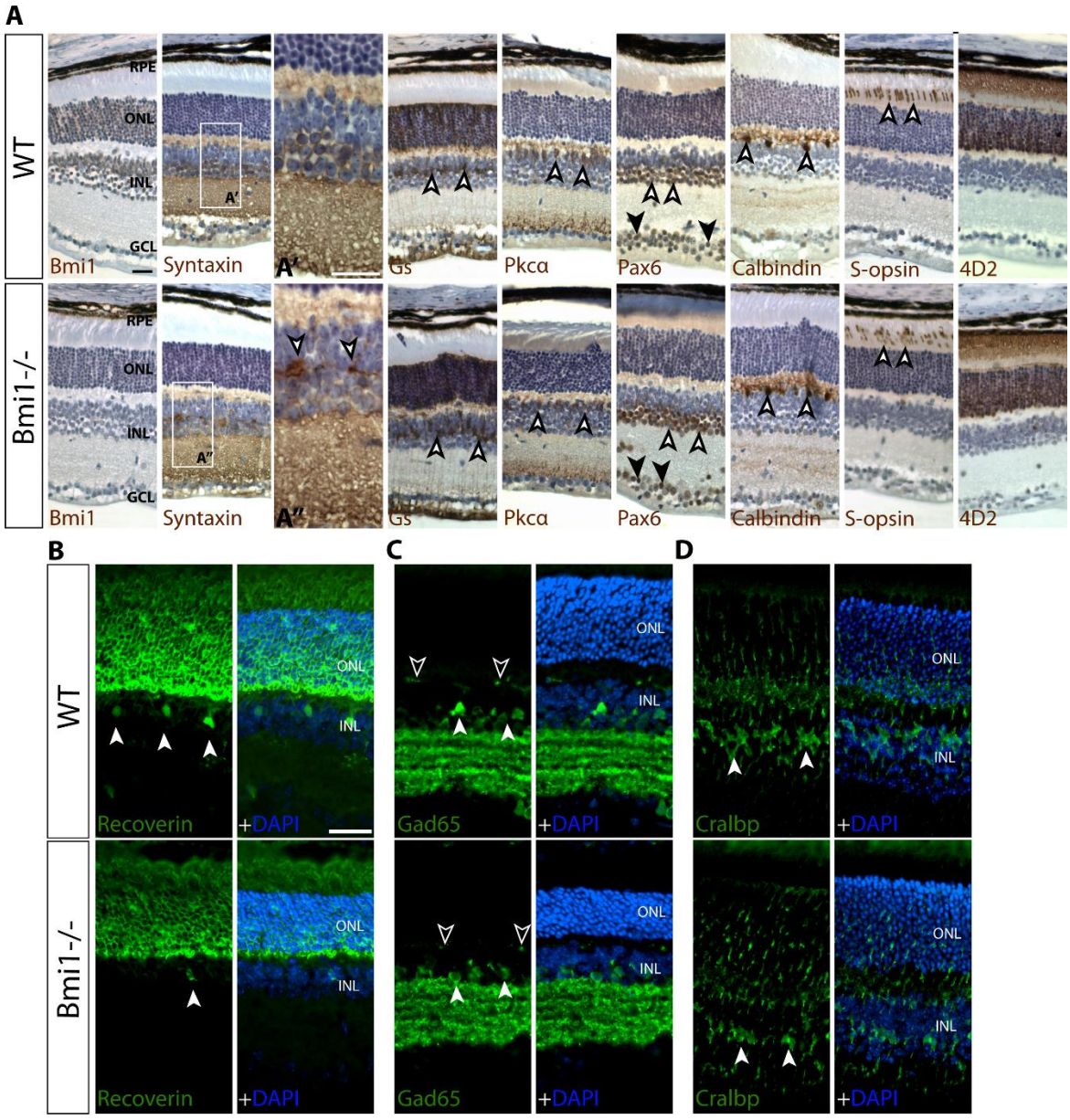

Figure S2. Histological anomalies in *Bmi1*$^{-/-}$ mouse retinas

(A, B, C, D) IHC and IF analyses of WT and *Bmi1*$^{-/-}$ retinas at P30 using specific markers for different retinal cell types. (A', A'') crop of the area indicated by the respective dashed rectangles. Amacrine cells' membrane (Syntaxin), müller glial cells (Gs and Cralbp), rods bipolar cells (Pkcα), amacrine (white-edged arrows) and ganglion cells (black arrows) (Pax6), horizontal cells (Calbindin), c-cones (S-Opsin), rod photoreceptors (4D2), T2 OFF and T8 ON cone bipolar cells (Recoverin), amacrine cells (white arrows) and horizontal cells (white-edged arrows) (Gad65). Retinal pigmented epithelium (RPE); outer nuclear layer (ONL); inner nuclear layer (INL); and ganglion cell layer (GCL). Scale bars: 40μm.

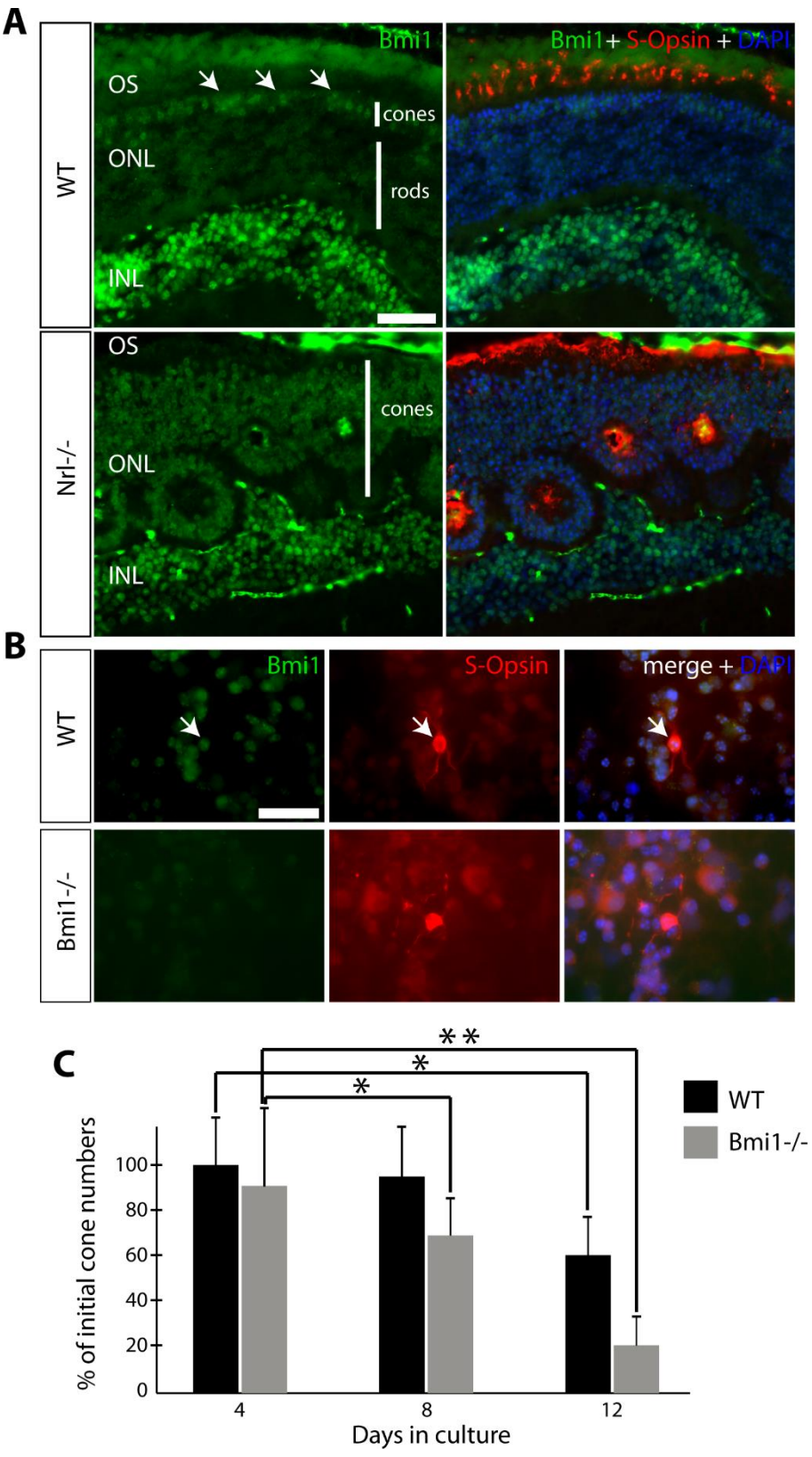

Figure S3. Bmi1 is preferentially expressed in mouse cone photoreceptors

(A) IF analysis of WT and *Nrl*$^{-/-}$ mouse retinal cryosections at P30. Arrows: Note the preferential expression of Bmi1 in neurons of the INL and in cone photoreceptors located in the ONL (white arrows). In contrast, Bmi1 is evenly distributed in the cone-only ONL of *Nrl*$^{-/-}$ mice. (B) IF analysis

of dissociated retinal cultures from WT and *Bmi1*$^{-/-}$ mice at P1 after 4 days *in vitro* (DIV). Note the expression of Bmi1 in WT cones labeled with S-Opsin, and the absence of Bmi1 in S-Opsin positive cells of *Bmi1*-mutants. (C) Quantification of S-Opsin positive cones in dissociated WT and *Bmi1*$^{-/-}$ retinal cultures, expressed as percentage of the initial cone number evaluated at 1 DIV. Outer nuclear layer (ONL); inner nuclear layer (INL); outer segment (OS). Scale bar: 50µm (A) and 20µm (B). WT n=4; *Bmi1*$^{-/-}$ n= 5. All values are mean ±SEM. (*) $P \leq 0.05$; (**) ≤ 0.01; Student t-test.

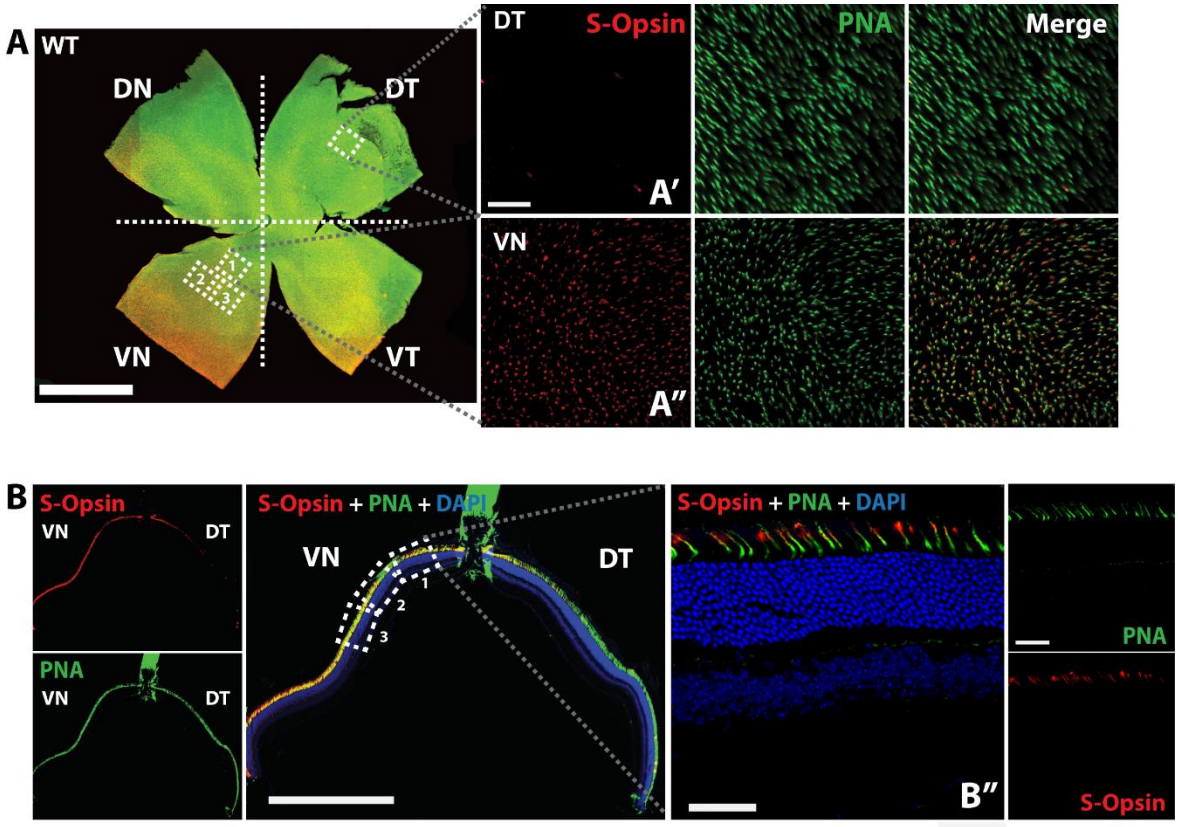

Figure S4. Methodology for the quantification of photoreceptors and other retinal cells types

(A) Representative mosaic reconstruction from 10x images from S-Opsin/PNA double-stained retinal flat mounts from P30 WT mice. (A', A'') 60x confocal microscopy images from retinal flat mount of the DT (A') and from the VN (A'') portion indicate with dashed rectangles in (A). (A') Note the almost complete absence of S-cone (S-Opsin+ cells) in the DT portion where almost only M-pure cones are present. (A") Note that in the VN portion approximately all cones express S-Opsin. The majority of these are dual-photoreceptors expressing both S and M Opsin and a minority of pure S-cone (data not shown). For PRs quantification, 3 images from the VN portion were quantified and averaged as shown by the dashed rectangles in (A). (B) 20x Mosaic Image of the whole retinal section at the level of the optic nerve of an orientated block. The eyes are oriented in the blocks in order to always have VN and DT in opposite positions with respect to the optic nerve on sections. For quantification on retinal sections, 3 consecutive images for sample (as shown by the boxes 1, 2, 3) were taken in the VN side, then quantified and averaged. (B') Representative 60x confocal microscopy image from retinal section used for quantification. Ventro-nasal (VN), dorso-temporal (TD). *n*= 3 to 6 retinas were used for genotype. Scale bars: (A) 1 mm, (B) 0,5mm.

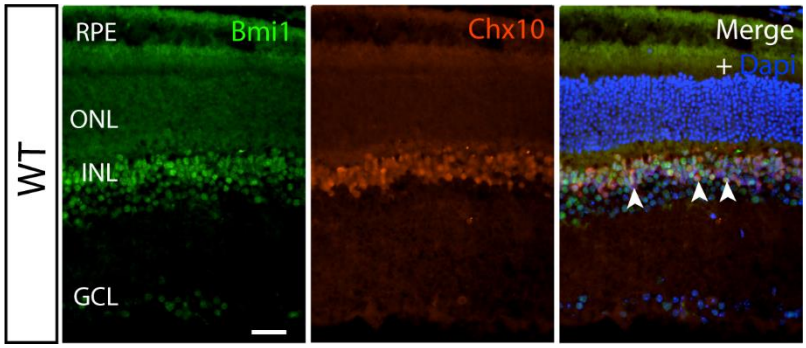

Figure S5. Bmi1 is highly expressed in bipolar neurons

IF analysis of a WT mouse retina (cryosections) using Chx10 and Bmi1 antibodies. Note Bmi1 expression in Chx10-positive cells of the INL. Retinal pigment epithelium (RPE); outer nuclear layer (ONL); inner nuclear layer (INL); ganglion cell layer (GCL). Scale bar: 20µm.

Figure S6. *Chk2* deletion partially improves the *Bmi1*$^{-/-}$ phenotype

Bmi1$^{-/-}$ */Chk2*$^{-/-}$ and *Chk2*$^{+/-}$ mice at P30. Note: *Bmi1*$^{-/-}$ */Chk2*$^{-/-}$ mice were healthier than *Bmi1*$^{-/-}$ mice but remained smaller than *Chk2*$^{+/-}$ control mice.

Table S1. Statistical analysis of ERG parameters

Global values from ERG experiment. Each parameter is analyzed by repeated measures ANOVA. The comparisons between the ERG parameters values at the Vmax are analyzed by one-way ANOVA. All values are mean ± SEM. N.S. = not significant; (*) P ≤ 0.05; (**) ≤ 0.01; (***) ≤ 0.001;

	Photopic system			Scotopic system		
	WT	+/-	-/-	WT	+/-	-/-
Overall a-wave amplitude	F(2,7) = 6.195, p = 0.028 *			F(2,7) = 1.297, p = 0.332 N.S.		
a-wave amplitude at Vmax	-215.4 ± 32.69 µV	-182.5 ± 15.29 µV	-88.35 ± 6.706 µV	-116.1 ± 12.17 µV	-117.4 ± 12.22 µV	-138.0 ± 10.05 µV
Comparison with the other groups (p-value)	+/- = 0.375 N.S.	WT = 0.375 N.S.	WT = 0.008 **	+/- = 0.939 N.S.	WT = 0.939 N.S.	WT = 0.226 N.S.
	-/- = 0.008 **	-/- = 0.039 *	+/- = 0.039 *	-/- = 0.226 N.S.	-/- = 0.281 N.S.	+/- = 0.281 N.S.
Overall a-wave implicit time	F(2,7) = 5.127, p = 0.043 *			F(2,7) = 0.596, p = 0.577 N.S.		
a-wave implicit time at Vmax	8.000 ± 0.4082 ms	8.667 ± 0.3333 ms	9.333 ± 0.3333 ms	21.75 ± 2.175 ms	19.67 ± 0.3333 ms	22.00 ± 2.517 ms
Comparison with the other groups (p-value)	+/- = 0.246 N.S.	WT = 0.246 N.S.	WT = 0.039 *	+/- = 0.484 N.S.	WT = 0.484 N.S.	WT = 0.932 N.S.
	-/- = 0.039 *	-/- = 0.275 N.S.	+/- = 0.275 N.S.	-/- = 0.932 N.S.	-/- = 0.464 N.S.	+/- = 0.464 N.S.
Overall b-wave amplitude	F(2,7) = 18.970, p = 0.001 ***			F(2,7) = 10.440, p = 0.008 **		
b-wave amplitude at Vmax	333.8 ± 34.78 µV	241.9 ± 17.32 µV	132.3 ± 9.479 µV	509.5 ± 24.04 µV	603.7 ± 51.01 µV	371.4 ± 40.33 µV
Comparison with the other groups (p-value)	+/- = 0.044 *	WT = 0.044 *	WT = 0.001 ***	+/- = 0.112 N.S.	WT = 0.112 N.S.	WT = 0.032 *
	-/- = 0.001 ***	-/- = 0.029 *	+/- = 0.029 *	-/- = 0.032 *	-/- = 0.004 **	+/- = 0.004 **
Overall b-wave implicit time	F(2,7) = 20.852, p = 0.001 ***			F(2,7) = 0.034, p = 0.966 N.S.		
b-wave implicit time at Vmax	27.25 ± 0.6292 ms	31.00 ± 1.000 ms	39.33 ± 2.848 ms	57.00 ± 1.472 ms	56.67 ± 1.453 ms	53.00 ± 2.517 ms
Comparison with the other groups (p-value)	+/- = 0.136 N.S.	WT = 0.136 N.S.	WT = 0.001 ***	+/- = 0.899 N.S.	WT = 0.899 N.S.	WT = 0.158 N.S.
	-/- = 0.001 ***	-/- = 0.010 **	+/- = 0.010 **	-/- = 0.158 N.S.	-/- = 0.217 N.S.	+/- = 0.217 N.S.