SUPPLEMENTARY MATERIALS AND METHODS

Animal Housing and breeding

NOD.CB17-Prkdcscid/J (NOD SCID), CBA/J, BALB/cByJ (BALB/c) and transgenic C57/B6-GFP (C57BL/6-Tg (UBC-GFP) 30Scha/J) mice were obtained from the Jackson Laboratory, USA. The animals were housed in individually ventilated cages (IVC) with ad libitum access to acidified autoclaved water. The animal room was maintained at 21-23 °C on a 14-h light-10 h dark cycle, and all procedures were carried out in accordance with the guidelines for care and use of animals in scientific research (Indian National Science Academy, New Delhi, India) in a committee for the purpose of control and supervision of experiment on animals (CPCSEA) registered animal facility. 6-8 week-old female CBA/J mice having *Pde6b* mutation were crossed with 6-8 week old male NOD SCID mice. The progeny of F1 generation were intercrossed, and all the F2 generation pups were screened for *Pde6b* and *Prkdc* mutation. The homozygous double mutant phenotype was selected for further breeding program for over 15 generations to make a homozygous double mutant colony.

Isolation of crude DNA by high salt method

Tail biopsies (around 1 mm) were obtained from 2-3 week old mice and were incubated overnight at 55°C in 600ul TNES buffer supplemented with 35ul proteinase K (10mg/ml). 166.7 ul of 6M sodium chloride (NaCl) was further added and shaken vigorously for 20 seconds. The samples were then centrifuged at 12000 X g for 5 minutes at room temperature (RT). The supernatant was collected and the DNA was precipitated with 1 volume of chilled 95% ethyl alcoholfollowed by two washes with 70 % ethyl alcohol. The DNA thus obtained was dissolved and resuspended in 100ul Tris-EDTA (TE) buffer.

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Genotyping for screening of homozygous double mutant phenotype

1) SNP-RFLP (Single nucleotide polymorphism-restriction fragment length polymorphism) method for *Pde6b* mutation screening

The oligonucleotide primers were prepared using Beacon Designer for the amplification of *Pde6b* gene present in exon 7, codon 349 of chromosome 5 in mice which undergoes a single nucleotide polymorphism from cytosine (C) to adenosine (A) in the mutant phenotype. PCR amplification was performed to yield a 485 bp product in all the wild type, heterozygous or homozygous mutant animals (Initial denaturation: 95 ºC for 5 minutes, Denaturation: 95 degree for 30 seconds, Annealing: 52 C, 45 seconds for 30 cycles, Extension: 72 ºC for 30 seconds, Final extension: 7 minutes). The amplicons were then digested with HypCH4IV whose cutting site is A CG T as per manufacturer's protocol (NEB, UK). The digested product was run on 1% agarose gel and visualized in BioRad UV gel doc system. Since the sequence becomes AAGT on mutation, the restriction enzyme failed to digest the amplified product in *Pde6b* mutated animal, however the digested PCR product yielded two bands in wild type (316 bp, 169 bp) and three bands in heterozygous animals (485bp, 316bb, 169bp) owing to only one defective pde6b allele.

2) PCR-CTPP (Polymerase Chain Reaction-Confronting Two Primer Pairs)method for *Prkdc* mutation screening

The screening of *Prkdc* gene involves two primer pairs carrying out simultaneous amplification of three different sizes of DNA segments in a single tube reaction. Genomic DNA was subjected to PCR in a total volume of 20 ul containing 5X Firepolmastermix (Solis Biodyne) 1uM each of primers, DNA template and MilliQ. Thermal cycling conditions were as follows: Initial

denaturation: 94 ºC, 2 minutes; Denaturation: 94 ºC, 30 sec; Annealing: 60 ºC, 30 sec; Extension: 72 ºC, 20 sec; Final extension: 72 ºC, 1 min; Cycles: 40

Immune cell analysis in NOD.SCID- *rd1* mice

50 ul of anticoagulated whole blood was incubated with the respective antibody (1:1) at a dilution of 1:200 for 40 min at room temperature (RT). After incubation, the red blood cell (RBC) lysis was performed and the cells were fixed with RBC lysis and fixing solution (BD Biosciences, USA). The spleen was crushed between two frosted slides and sieved using 100um filter (BD Biosciences, USA) to obtain a single cell suspension. The antibodies used were FITC-conjugated anti-mouse CD3, allophycocyanin (APC)-conjugated anti-mouse CD4, APC Cy7-conjugated anti-mouse CD8, Phycoerethrin Cy5.5 (PE Cy 5.5) anti mouse B220 and Phycoerethrin (PE) conjugated anti mouse NKT (All from BD Biosciences, USA).Staining procedures were similar to that followed for peripheral blood. Thereafter, samples were run on BD FACSVerse and analyzed in FACSDiva. The analysis was performed in lymphocyte gated population.

Relative quantification of immunoglobulin secretion

Appropriate amount of each isotype specific rat anti-mouse purified monoclonal antibody (1:500) was coated overnight and the plate was further blocked with 5% BSA in PBS for 1 hour at RT. After 3 washes with PBST for 5 minutes each, samples at a dilution of 1:50 were added to the plate and incubated for 2 hours at RT. The plate was then washed thrice for 5 minutes each with PBST. Thereafter, detection antibody at a dilution of 1: 1000 was added to the plate and incubated for 1 hour at RT followed by three washes with PBST (5 minutes each). In the next step, streptavidin HRP was added at a dilution of 1:4000 and incubated for 30 minutes at RT

followed by 5 washes for 2 minutes each. Finally, 100ul TMB substrate was added per well and allowed to incubate till color developed and the reaction was stopped by adding stop solution.Absorption maxima were measured spectrophotometrically at 450 nm and 570 nm. Wavelength correction was performed by subtracting the readings obtained at 570 nm from 450 nm. The readings obtained were plotted to obtain a comparative result for total immunoglobulin secretion.

Quantitative RT-PCR analysis for retina specific genes and immune privilege markers

Total RNA was extracted from the whole eye tissue of mice by TRI Reagent (Sigma, USA). cDNA synthesis was performed by Verso cDNA Synthesis Kit (Thermo Fisher Scientific, USA) using 1µg total RNA and quantitative real-time PCR was performed usingDyNAmo Flash SYBRGreen (Thermo Fisher Scientific, USA) on the Master cycler Realplex platform (Eppendorf, Germany). The PCR conditions used were as follows: initial denaturation 95ºC for 5 minutes, followed by 40 cycles of 95ºC for 15 seconds (denaturation), 60ºC for 1 minute (annealing and extension).

Table 1: List of primers.

Gene	Forward primer	Reverse primer
Pde6b	5’ GGTATCATGAATGAAGAA 3’	5’ CACTAACCTACTTAACCT 3’
Rhodopsin	5’ GTCCACTTCACCATTCCTA 3’	5’ TTCCTTCTCTGCCTTCTG 3’
Recoverin	5’ TCGTCATGGCTATCTTCAA 3’	5’ TGGATCAGTCGCAGAATT 3’
IRBP	5’ GCTGCTGGTAGAACACAT 3’	5’ CACAAGGCTGAGATGGAG 3’
S-opsin	5’ TACTTGGATTATTGGTATCG 3’	5’ ACAGAAGATGAAGAGGAA 3’
Rom-1	5’ ATGGCTACAAGGATTGGT 3’	5’ GGATTACAACAGGAGAAGG 3’
Peripherin	5’ GAGAAGGTGGTGACAGAG 3’	5’ GCTTAGGAATAGGCTGAGA 3’
Gfap	5’ CAAGGAGATACTCTGAAC 3’	5’ GTGAAGCAATAGAACTCT 3’
S100	5’ GCTGTGGACAAGGTAATGAAGGAA 3’	5’ AGCCACCAGCACAACATACTC 3’

CD44	5’ ACAACTTCTGGTCCTATGA 3’	5’ CCACACCTTCTCCTACTAT 3’
IL-17	5’ CTCACACGAGGCACAAGT 3’	5’ GCAGCAACAGCATCAGAGA 3’
IL-7	5’ ATCCTTGTTCTGCTGCCTGTC 3’	5’ TTCGGGCAATTACTATCAGTTCCT
IL-10	5’ CTGGGTGAGGAAGCTGAAG 3’	5’ CCACTGCCTTGCTCTTAT 3’
IL-1b	5’ AAGGGCTGCTTCCAAACC 3’	5’ GATGTGCTGCTGCGAGAT 3’
IL-6	5’ CGCTATGAAGTTCCTCTC 3’	5’ TCTGTGAAGTCTCCTCTC 3’
IL-33	5’ TGCATGAGACTCCGTTCTGG 3’	5’ TCCCGTGGATAGGCAGAGAA 3’
Mac-1	5’ TTCACGGCTTCAGAGATG 3’	5’ CCATACGGTCACATTGTTG 3’
Ly6G	5’ CGTTGCTCTGGAGATAGAAGTTA 3’	5’ GTTGACAGCATTACCAGTGAT 3’
CD14	5’ GAAGCCAGAGAACACCAC 3’	5’ CAACAGCAACAAGCCAAG 3’
NK	5’ GGTTCTGGACAAGATGAAGT 3’	5’ CGATGCCGATGTTGATGA 3’
NKT	5’ CTCACGCTCAAGGCAATCA 3’	5’ CCGAGTCCAGACCTCCATT 3’
PEDF	5’ CTTACGATACGGCTTGGA 3’	5’ CTTGGATAGTCTTCAGTTCTC 3’
VEGF	5’ CGACAGAAGGAGAGCAGAAG	5’ CTCAATCGGACGGCAGTAG 3’
MMP-1	5’ TATTGTTGCTGCCCATGA 3’	5’ TTGCCAGTGTAGGTATAGATG 3’
MMP-2	5’ GACCACAACCAACTACGATGA 3’	5’ GCTGCCACGAGGAATAGG 3’
MCP-1	5’ CTACTCATTCACCAGCAAGAT 3’	5’ TCAGCACAGACCTCTCTC 3’
TNF-a	5’ CACCACCATCAAGGACTCA 3’	5’ GGCAACCTGACCACTCTC 3’
IFN-g	5’ CTGAGACAATGAACGCTACAC 3’	5’ TCTTCCACATCTATGCCACTT 3’
TGF-b	5’ AGCTTTGCAGGGTGGGTATC 3’	5’ GGGGCCATTCACTAGCAGTT 3’

Western immunoblotting analysis

After euthanasia by cervical dislocation, eyes were enucleated from mice and homogenized at 10 %(w/v) in ice cold RIPA buffer supplemented with a protease inhibitor cocktail (PIC). Protein content of the homogenate was estimated using bicinconinic acid (BCA) kit (Pierce,Rockford,IL). 50µg of protein was denatured and resolved on 12% SDS gel and transferred on a PVDF membrane. Immunochemical staining was performed using primary antibodies at a dilution of 1:1000 and the membrane was incubated overnight at 4 ºC. Further

incubation of the membrane with secondary antibodies (dilution 1: 5,000) was performed for 2 hours at RT followed by 3 washes for a total of 30 minutes with PBST. Bands were visualized with the enhanced chemiluminiscence kit according to manufacturer's protocol (Biorad, USA).

Immunocytochemistry for the analysis of photoreceptor cells

Whole eyewas enucleated after euthanizing the animals by cervical dislocation. The tissue was fixed overnight in 4% paraformaldehyde (4% PFA), processed in 30% sucrose and embedded in OCT (optimal cutting temperature) medium. Tissue sections of 5 micron thickness were obtained on poly-l-lysine (Sigma Aldrich, USA) coated slides using cryotomeand stained with rod and cone retinal cell specific markers *Rhodopsin and S-opsin* (Thermo fisher scientific, USA) at a dilution of 1:100 for 1 hour at RT. Secondary antibody was added for 40 minutes at a dilution of 1:200 followed by 3 washes with PBST, 5 minutes each. The slides were again washed thrice and nucleus staining was done using DAPI for 5 minutes at a dilution of 1:500. The representative confocal images were taken at 63 X magnification using a system incorporated in the microscope (Zeiss LSM Version 4.2.0.121).

Fundoscopic retinal imaging

The animals were dark adapted for 40 minutes before the imaging procedure. Thereafter, they the pupils were dilated using 1% tropicamide and anaesthetized using ketamine-xylazine (80mg/kg body weight). Upon dilation, the mice were laterally placed on the stand with their eye focused towards the camera. The retina was focused with the objective lens and posterior pole images of both eyes were captured using streampix software using MICRON III rodent imaging system (Phoenix Research Labs, USA).

Electroretinography (ERG)

Mice were dark-adapted for at least 12 hoursand anaesthetized. After pupil dilation (1 drop Atropine, 1%), individual mice were fixed on a movable sled and gold wires(as active electrodes) were placed on the cornea. Theground electrode was inserted in the tail and a reference electrode was placed subcutaneously between theeyes near cornea. The retina was stimulated using white light of 1cds/$m^2$and the mean of 25 averages was taken for obtaining single ERG response from the mouse retina. The ‘a’ and ‘b’ wave amplitude and latency were measured in all groups, using inbuilt algorithm of labscribe software.The ERG responses were obtained through ERG attachment of MICRON III rodent imaging system using labscribe software (Phoenix laboratory, USA).

Terminal deoxynucleotidyltransferasedUTP nick end labeling (TUNEL) assay for the analysis of apoptosis in RP

Paraffin sections of whole eye tissue were obtained, deparaffinized and rehydrated by successive serial washings with ethanol and treated with proteinase K for permeabilization of cells followed by equilibration for 10 minutes at RT. Fragmented DNA was labeled with terminal deoxynucleotidyltransferase (TdT) and biotin deoxynucleotides (dNTPs) and incubated for 1 hour at 37°C in a humidified chamber.The reaction was stopped by immersing the slides in 2X SSC solution for 15 minutes and was counterstained by DAPI to visualize nuclei. The image acquisition was performed under confocal microscope at 63X (Zeiss LSM Version 4.2.0.121)

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Behavioral analysis

1. Visual cliff test

The visual cliff apparatus was prepared in a wooden box (62 x 62 x62 cm) with the four edges emerging 19 cm above the top and a wooden platform running along the middle. The platform was 3.75 cm in height, 60 cm in length and 2.5 cm in width. The platform separated the box into two sections, one with checker paper placed on the top surface of the box, whereas the other had the same checker paper placed on the bottom surface of the box. The experiment was conducted by placing the mouse on the platform and observing the decision of mouse to step towards either safe zone or cliff zone in 5 minutes. Safe zone (shallow) results represented the events of the mouse stepped down to the reasonable side, which has the checker paper on the top surface of the box. Cliff zone (deep) represented the events of the mouse stepped down on the "fake cliff" side, which has the checker paper on the bottom surface of the box. 10 mice of each strain (BALB/c, CBA/J, and NOD.SCID- *rd1*) were used in this study and each mouse was tested for 5 trials. To reduce the effect of learning and memory, the apparatus was turned 180 degrees after two trials so that the "safe zone" and the "cliff zone" were at different sides for the next three trials. The glass surface was cleaned thoroughly between each trial in order to prevent mice from finding visual clues about depth. The percentage of stepping towards each zone (shallow /deep or safe/cliff) was calculated.

2. Light / dark latency test

Light / dark latency test apparatus consisted of a box sized 21 X 42 X 25cm divided in two equal halves by a partition with a 5 cm connecting door. One chamber was brightly illuminated (approx 400 lux) and the other was kept completely dark with black background. The animal was introduced in the light chamber (LC) and observed for five minutes. Time spent by the animal in each chamber was recorded. The number of transitions from light to dark chamber (DC) and vice

versa was also counted along with the initial time taken by the animal to enter the DC from LC where it was introduced.

3. Optokinetic response (OKR)

The optokinetic drum with stripes of varying grating frequencies (0.03, 0.13, 0.26, 0.52 and 1.25 cpd) contains a wooden platform 15 cm above the base and 10 cm farther to the periphery of the drum (diameter: 63 cm; height: 35 cm). Movement behavior and head tracking were analyzed in ambient room light (illuminance = 400 lux; measured in the center of the cylinder with a digital luxmeter (MASTECH®) with a rotation speed of 2 rpm and a spatial frequency of 0.1 cyc/deg. Prior to the analysis, the mice were allowed to adapt to the environment of the non-rotating drum for ten minutes. Thereafter the drum was rotated clockwise and anticlockwise, 1 minute each for every stripe used with a time interval of 30 seconds between two rotations. Occurrence of head-tracking reflexes was judged by the observer during each 1 minute period. Head tracking was defined as horizontal head movement at the same rate and the same direction as the drum for at least 15° (as described previously). If the spatial frequency of the black and white stripes was increased, a threshold was reached beyond which no tracking movements of the head were detected. The visual spatial resolution (visual acuity) of the animal was estimated to be greater than or equal to this threshold but below the next spatial frequency tested.

Cell transplantation in the retina and post transplantation engraftment analysis

Retinal cells from GFP transgenic mice were isolated by digesting retina with 0.05% trypsin at 37 C for 30 minutes with continuous agitation. These cells were passed through 70 micron sieve to eliminate debris and centrifuged at 200g, and resuspended in DMEM/F-12 to obtain single cell suspension. 1X10^6 cells were transplanted sub retinally into the eye of wild type CBA/J, immune suppressed CBA/J and NOD.SCID- *rd1* mice (n=4). Thereafter, the animals were

euthanized and eyes were isolated post 48 hours. Whole eye was sectioned and retina was stained with anti-GFP antibody (Santa Cruz, USA) at a dilution of 1:200 overnight at 4 C. Thereafter,the tissue sections were stained with secondary antibody (anti rabbit alexa fluor 488 at a dilution of 1:400) for 45 minutes at RT. The nuclei were stained with PI (Propidium Iodide) and imaged in confocal microscope (63X). The GFP +ve cells were individually counted for ONL, INL and GCL layers of retina in a minimum of 5 fields using Image-J software and was compared amongst the groups.

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