nature portfolio

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | 1. Labeled cells were visualized and images were captured using a Nikon Eclipse Ti or Ti2 laser scanning confocal microscope. Measurements were performed with Nikon NIS-Elements AR 3.10 software.<br>2. Western blots data were collected and analyzed using ODYSSEY Infrared Imaging System (LI-COR).<br>3. Flow cytometry data were collected on a Gallios flow cytometer and Kaluza analysis software (Beckman Coulter).<br>4. Single-cell sequencing data were collected using the HiSeq3000 platform (Illumina). |
| Data analysis | 1. Quantitation used Image J (Fuji, version 1.54g). Retinal blood vessels were analyzed by Angiotool (Version 0.6a).<br>2. Kaplan–Meier survival curves and statistical analysis was performed using the GraphPad Prism software.<br>3. Western blots data were analyzed using ODYSSEY Infrared Imaging System (LI-COR).<br>4. Flow cytometry data were analyzed by Kaluza analysis software (Beckman Coulter).<br>5. Single-cell sequencing data were analyzed by CellRanger pipelines (10x Genomics, V2.2.0), 10x Genomics Loupe Browser, Scanpy python toolkit, Seurat R toolkit, biomaRt package, the python package scrublet |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data files for transcriptomic scRNAseq analyses are available at the Gene Expression Omnibus (GEO) under accession numbers GSE245137. Mouse reference index provided by 10x Genomics (refdata-cellranger-mm10) can be downloaded from: https://www.10xgenomics.com/support/software/cell-ranger-arc/downloads.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | *N/A* |
| Reporting on race, ethnicity, or other socially relevant groupings | *N/A* |
| Population characteristics | *N/A* |
| Recruitment | *N/A* |
| Ethics oversight | *N/A.* |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | Sample size was determined by power analysis (Cohen's d). Different genotypes were compared within the same litter and across four to six litters. |
| Data exclusions | No data were excluded. Both sex of animals were used in the study. |
| Replication | Findings were reliably reproduced across four to six litters |
| Randomization | The experimental approaches did not require samples to be randomized. Samples, and the subsequent data collection and analysis, were handled the same way in all experiments. |
| Blinding | The investigators were not blinded to group allocation during data collection or subsequent analysis for two reasons: (1) they were performing the experiments themselves and were aware of experimental conditions, groups and outcomes. All mice in each group were analyzed in the same way. (2) Non-blinding also facilitates veterinary and staff monitoring tumor growth. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

Antibodies used

The following antibodies were obtained from commercial sources: Active caspase 3 (Cell Signaling Technology 9661, WB: 1:200, IF:1:500), Ap2a (Santa Cruz SC-8975, IF: 1:500), aPKC zeta (BD Transduction lab 610176, IF: 1:500), BrdU (Abcam ab1893, IF: 1:500;DSHB, G3G4, 1:1000), Brn3 (Santa Cruz SC-6062, IF: 1:500), Calretinin (Santa Cruz SC-11644, IF: 1:500), CCSP (Seven Hills Bioreagent,WRAB-3950, 1:1000), CGRP (Sigma, C8198, 1:1000), Cone arrestin (Millipore AB15282, IF: 1:500), Cyclin A2 (Abcam, Ab181591,1:500),Cyclin B1 (Cell Signaling Technology, 4138S, IF: 1:500), Galectin 3 (Santa Cruz, SC-19283,IF: 1:500), Ki67 (BD science Pharmingen 550609, IF: 1:500; Thermo Fisher Scientific, 14-5698-82,IF:1:500), MSHa (Fisher Scientific,AB508MI, IF:1:200), Mycn(Santa Cruz, SC-791, IF:1:500), N-Cadherin (Santa Cruz SC7939, IF: 1:500), OC2(R&D systems, AB6294, IF: 1:500), P21 cip1 (Abcam,ab188224, IF:1:500), P27 kip1 (BD Biosciences 554069, IF: 1:500, WB: 1:200), Phospho-aPKCzeta/lamda(ThermoFisher 44-968G, IF: 1:500), Phospho-histone H3 (Santa Cruz SC-8656, IF: 1:500),Protein kinase C alpha(Sigma, P5704, IF:1:500), Rhodopsin (Santa Cruz SC-57433, IF: 1:500), Skp2 (SC-74477; Santa Cruz, WB:1:1000), Sox9 (EMD Millipore, MAB5535, IF:1:500), SPC (Abcam, ab40879, 1:1000), beta-actin (A5441, Sigma, WB: 1:2000 ).Flow cytometry used the following antibodies: anti-B220 (0.5 g, ThermoFisher Scientific Cat# 12-0452-81), anti-CD3 (0.75 g,ThermoFisher Scientific Cat# 17-0032-80), anti-CD335/NKp46 (0.75 g, ThermoFisher Scientific Cat# 12-3351-80), anti-CD45 (0.1 g,ThermoFisher Scientific Cat# 12-0451-82), and anti-CD11b (0.25 g, ThermoFisher Scientific Cat# 17-0112-81). F-actin was labelled by Alexa Fluor® 488 Phalloidin (ThermoFisher Scientific, A12379, IF: 1:500) or Alexa Fluor® 568 Phalloidin(ThermoFisher Scientific, A12380, IF: 1:500). Blood vessel endothelium cells were labelled by FITC-IB4 (Sigma, L2895, 1:200). Crx antibody was from Dr. CY Gregory-Evans at Imperial College School of Medicine. Ptf1a antibody was from Dr. Pierre Cordelier,INSERM, France.

Validation

The vendors validated the commercially available antibodies, Phalloidin and IB4.
Crx antibody had been validated by Dr. CY Gregory-Evans: Bibb LC et al., Temporal and spatial expression patterns of the CRX transcription factor and its downstream targets. Critical differences during human and mouse eye development. Human Molecular Genetics, 2001, 10 (15): 1571-1579; in our previous studies, see Chen D, Livne-bar I, Vanderluit JL, Slack RS, Agochiya M, Bremner R. Cell-specific effects of RB or RB/p107 loss on retinal development implicate an intrinsically death-resistant cell-of-origin in retinoblastoma. Cancer Cell. 2004 Jun;5(6):539-51.
Ptf1a antibody had been validated by Dr. Pierre Cordelier: Hanoun et al., The E3 ubiquitin ligase thyroid hormone receptor-interacting protein 12 targets pancreas transcription factor 1a for proteasomal degradation J Biol Chem. 2014 Dec 19;289(51):35593-604.

## Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

α-Cre mice (P. Gruss, age E14-P400), B6.129P2(Cg)-Braftm1Mmcm/J (Jackson Laboratory, strain# 017837, Common Name: BRafCA, age P0-P42), B6.Cg-Gt (ROSA)26Sortm14(CAG-tdTomato)Hze/J, also known as Ai14(tdTomato) (Jackson Laboratory, stock#007914, age P0-P91), B6.129-Gt (ROSA)26Sortm1(cre/ERT2) Tyj/J (Jackson Laboratory, Stock No: 008463, age P0-P91), B6.129S4-Krastm4Tyj/J (Jackson Laboratory, Stock No: 008179, age P0-P91), Cdk1f/f (D.Santamaria and M. Barbacid, age P0-P400), Cdk2f/f (D. Santamaria and M. Barbacid, age P0-P400) , p107-/- mice (M.Rudnicki, age E14-P400), p27Ck-/Ck- mice (A. Besson and Roberts, age P0-P400), p27T187A (A. Besson and J. Roberts, age P0-P400), P53f/f mice (A. Berns, age E18-P56), Rbf/f mice (A.Berns, age E14-P400), Skp2-/- mice (K. Nakayama, age P0-P400), Spc-rtTA(Whitsett, age E18-P60), TetO-Cre (Whitsett, age E18-P60) and Z/Red (Jackson Laboratory, stock#005438, age E16) mice

Wild animals

Study did not involve wild animals

Reporting on sex

No studies have reported sex-dependent differences in the tumor types examined, thus sex was not assessed.

Field-collected samples

The study did not involved field samples.

| | |
|---|---|
| Ethics oversight | Mice were treated according to institutional and national guidelines approved by the Toronto Centre for Phenogenomics and Cincinnati Children's Hospital Medical Center. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Flow Cytometry

## Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

| | |
|---|---|
| Sample preparation | 1. P8 eyeballs of p107-/-, α-Cre; Rbf/f;p107-/- and α-Cre; Rbf/f;p107-/-; Skp2+/- mice were enucleated, and peripheral retinas were dissected in fresh cold 1X HBSS.<br>2. Dissected peripheral retinas were transferred to 200µl of cold HBSS per retina.<br>3. An equivalent amount of Papain solution was added and incubated at 37°C for 10 min, and the tube inverted gently every 2 min.<br>4. Next, the digestion solution was discarded by pipetting without disturbing the retina.<br>5. Mechanical trituration of the retina was performed in 600µl of Neurobasal Media supplemented with 10% FBS by pipetting slowly 10 to 15 times with a P1000 pipette tip.<br>6. Samples were then DNAse treated for 5 min at 37°C.<br>7. Cell suspensions were centrifuged using a swing-bucket rotor at 200×g for 5 min.<br>8. The supernatant was carefully aspirated off the cell pellet, and the pellet was suspended in 1-5ml Neurobasal media with 1% FBS.<br>9. Cellular aggregates were removed by straining cells through a 50 µm cell strainer (pluriSelect).<br>10. Splenocytes were collected by grinding a p107-/- mouse spleen through a 40µm mesh and then red blood cells (RBC) were removed by incubating cells for 10 min in RBC lysis buffer (Sigma, Cat# 11814389001). |
| Instrument | Gallios flow cytometer |
| Software | Kaluza analysis software (Beckman Coulter) |
| Cell population abundance | FACS analysis of dissociated tumor-prone DKO and tumor-resistant DKO-Skp2+/- retina revealed very low levels of B, T, or NK cells, macrophage/microglia, or leukocytes in both genotypes |
| Gating strategy | Cells were first gated on Forward Scatter by Side Scatter Density Plots to exclude debris. The resulting cells (~90% of total) then underwent doublet exclusion by comparing Forward Scatter Peak Height vs. Forward Scatter Time-of-flight (or peak width) and then again for Side Scatter Peak Height vs. Side Scatter Time-of-flight. Approximately 0.5% of the cells were excluded as doublets at this stage. Finally, the triple-gated cells (FS x SS, 2 rounds of doublet exclusion) were analyzed using Single Parameter Histograms to identifying cells positive for each marker. Isotype control antibodies were used to define negative cells and splenocytes were used as a positive control to define positive cells. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.