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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 *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 *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	data was collected using Real Time Analysis 3 (RTA3, https://support.illumina.com/downloads.html),bcl2fastq2 v2.20 (Illumina, Inc., https://support.illumina.com/downloads.html),human genome assembly (GRCh38.p13; https://www.ensembl.org/Homo_sapiens/Info/Index),HISAT2 v2.2.1,CellRanger software (version 5.0.0),GraphPad Prism 9,Python Package,pySCENIC (version 0.10.3),velocyto (version 0.17.0),scVelo (version 0.2.2),CellPhoneDB (version 2.0) and R (version 3.6.1)
Data analysis	data was analyzed by R package including Seurat package (version 3.2.0),inferCNV (version 1.2.1),featureCounts v2.0.3,DESeq2 (version3.15 https://bioconductor.org/packages/DESeq2/),FeatureCounts v1.5.0-p3,NMF (version 0.25),ggplot2 (version 3.4.0),ComplexHeatmap (version 2.18.0),limma (version 3.58.0),Monocle2 (version 2.14.0),Slingshot (Version 1.5.1),clusterProfiler (version 4.10.0),GSVA (version 1.34.0),Survival (version 3.4-0) ,Survminer (version 0.4.9)

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

The data reported in this study have been deposited in the website of China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (https://www.cncb.ac.cn/services). Expression matrix of single cell sequence was deposited and can be downloaded in OMIX database (https://ngdc.cncb.ac.cn/omix: accession no. OMIX004730). Raw data of single cell sequence can be retrieved in GSA-Human database (https://ngdc.cncb.ac.cn/gsa-human/) under the accession number HRA007322 with controlled access (following the Guidance for Making Data Access Request of the GSA Human database, contact: hongmengyush@163.com, Professor Yu Hongmeng/ENT institute and Department of Otorhinolaryngology, Eye & ENT Hospital of Fudan University, Shanghai, China. The detailed guidance for data requesting process can be found at [https://ngdc.cncb.ac.cn/gsa-human/document/GSA-Human_Request_Guide_for_Users_us.pdf]). The published data used for validation or comparation in this study were retrieved from the NCBI Gene Expression Omnibus database under accession code GSE118995 and GSE139522. The remaining data are available within the Article, Supplementary Information files and Extended Data files of this study. All other data supporting the finding of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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	The term "sex" was used in the present study to describe the biological attribute of participants involved. The findings in the present study apply to both sexes. Sex and gender were not considered in the study design. Sex of participants involved in this study was determined based on self-reporting. The information of sex- or gender-disaggregate data has not been collected in this study. Sex- or gender-based analysis was not performed, since no significant difference was observed in the incidence rate or clinical outcomes between the sexes regarding the disease studied in this research, and sex or gender attributes of the participants was not the main focus of this study.
Reporting on race, ethnicity, or other socially relevant groupings	No socially relevant categorization variables were included in this manuscript. We confirm that no variables were used as proxies for any other socially constructed/relevant variables. Confounding bias could be partially evaded since there were no subgroups of race or ethnicity to classify, because all the candidates enrolled in this study were locally-born Asian Chinese.
Population characteristics	The covariate-relevant population in the study composed of 10 patients with single cells information and 23 with only bulk RNA-seq data. Among the 33 patients,the age ranged from 19 to 76(median 47)years, and the diagnosis was olfactory neuroblastoma pathologically.No previous treatment was adopted,which means all the patients were treatment naive.
Recruitment	Fresh specimens of 10 ONB patients were collected for scRNA-seq during surgical resection, from August, 2020 to August, 2021, under the supervision of a qualified pathologist. Tumor specimens for bulk RNA-seq analysis were harvested from 23 cases of ONB patients from February, 2019 to January, 2022. Formalin-fixed and paraffin embedded (FFPE) specimens of tumor samples and cancer adjacent mucosa were collected from 30 ONB patients, including 10 patients for scRNA-seq, under the permission of Pathology Department from February, 2019 to March, 2022, for hematoxylin and eosin (HE) and IHC staining. All FFPE specimens were independently reviewed by two pathologists (Y.C.C and C.X.K) who confirmed the diagnosis of ONB and determined relevant Hyams grading of each slide.
Ethics oversight	The study was conducted under the approval of ethics committee of the Eye, Ear, Nose and Throat (EENT) Hospital of Fudan University, Shanghai, China (No.2019096 and No.2021165). The collection and utility of patient specimens strictly followed procedures in accordance with the ethical standards formulated in the Helsinki Declaration. We have obtained written informed consent from all participants.

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	ONB is a very rare malignancy discovered in the paranasal and skull base area, with an incident rate of about 4/10,000,000 per year. No

Sample size	sample size calculation was performed at the start of this research, since it was very hard to precisely predict the number of ONB patients who visit our medical center every year, due to the rarity of this disease. Considering the timeliness of scientific research, the duration of specimen collection for scRNA was set up to around one year in this study, and we successfully collected fresh specimens from 10 ONB patients for scRNA sequencing and analysis. We believe that the sample sizes were sufficient for this rare disease, since the main goal of the study was to discover the tumoral heterogeneity among tumor cells. scRNA profiles of 85452 cells collected from 10 ONB patients were enough to reveal the heterogeneity among tumor cells thus projecting to the subtypes of patients. Moreover, literature review of studies reporting high throughput sequencing results of ONB has showed that the sample sizes of our study has exceed the vast majority of similar researches, with no recently published scRNA study of ONB using a patient cohort of more that 10 candidates, and no recent bulk RNA study of ONB involving more than 20 candidates.
Data exclusions	No data was excluded from analysis in this study.
Replication	We used 4-5 replicates to assure the reliability of experiments.To validate drug vulnerability in vivo by establishing PDX model, we set four treatment groups,namely Radiotherapy (N=5), Radiotherapy + Endostatin (N=5), Radiotherapy + Doxorubicin (N=4) and a Vehicle control group (N=5). Additionally,the findings revealed by scRNA sequencing were validated using an internal and an external (GSE118995) bulk RNA-seq cohort independently. We confirm that these attempts at replication were successful.
Randomization	No randomization or allocation was designed.In this study,we recruited patients with initial diagnosis of ONB without any treatment,and no allocation was needed.This did not affect the results because no comparison between tumors groups were made,alternatively,cell subtypes inside tumor was the focus.In this perspective,one patient represented a unique entity.Of course,the rarity of the malignancy does not warrant an allocation.
Blinding	During data collection and analysis, investigators were blinded to Hyams grade, which was evaluated only by two pathologists. Pathologists were blinded to other clinical information or neural/basal/mesenchymal characteristics regarding each patient.

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	anti-Ki67 (1:1500, Abcam, #ab15580), anti-CTGF/CCN2 (1:200, Thermofisher, #PA5-109248), anti-Chromogranin (1:50, Gene Tech (Shanghai, China), #GT211429), anti-Tubulin β3/TUBB3 (1:300, BioLegend, #MMS-435P) and anti-CD34 (1: 2000, Abcam, #ab81289). The following antibodies were used for IF: anti-CD8 alpha (1:800, Abcam, #ab237710), anti-CD4 (1:400, Abcam, #ab133616), anti-CD3 (1:200, Abcam, #ab16669), anti-FOXP3 (1:100, Abcam, #ab215206), anti-LAG3 (1:100, Abcam, #ab209236), anti-CD68 (1:00, Abcam, #ab955), anti-VEGFA (1:100, Abcam, #ab52917) and anti-S100A4 (1:1000, Abcam, #ab197896). For anti-Chromogranin,anti-CD8 alpha, and anti-Tubb3 primary antibodies,an HRP-conjugated secondary (1:400,Abcam,ab205719) were used to detect the primary.For other primary antibodies,an HRP-conjugated secondary (1:400,Abcam,ab6721） were used.
Validation	Anti-Ki67: Rabbit polyclonal antibody reacting with Human. Ab15580 is batch tested in ICC/IHC. A variability in IHC-Fr performance can occur with this antibody but the manufacturer guaranteed its consistency in IHC-P. This antibody has been validated in over 3000 published reports. (https://www.abcam.cn/products/primary-antibodies/ki67-antibody-ab15580.html) Anti-CTGF/CCN2: Rabbit polyclonal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human appendix tissue, human thyroid gland cancer tissue, human uterus tissue according to the validation statements on the manufacturer's website. (https://www.thermofisher.cn/cn/zh/antibody/product/CTGF-Antibody-Polyclonal/PA5-109248) Anti-Chromogranin: Mouse monoclonal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human pancreas tissue according to the validation statements on the manufacturer's website. (https://www.genetech.com.cn/goods/goods_detail/293.html) Anti-Tubulin β3/TUBB3: Mouse monoclonal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human brain tissue according to the manufacturer's website. This antibody has been validated in over 750 published reports. (https://www.biolegend.com/en-us/products/purified-anti-tubulin-beta-3-tubb3-antibody-11580) Anti-CD34: Rabbit monoclonal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human kidney tissue according to the manufacturer's website. This antibody has been validated in

about 500 published reports. (https://www.abcam.cn/products/primary-antibodies/cd34-antibody-ep373y-ab81289.html)
Anti-CD8 alpha:
Mouse monoclonal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human tonsil tissue according to the manufacturer's website. This antibody has been validated in about 20 published reports. (https://www.abcam.cn/products/primary-antibodies/cd8-alpha-antibody-cal67-ab237710.html)
Anti-CD4: Rabbit monoclonal antibody reacting with Human. This antibody has been validated in IF (Formalin/PFA-fixed paraffin-embedded sections) analysis using human tonsil and spleen tissues according to the manufacturer's website. This antibody has been validated in over 200 published reports. (https://www.abcam.cn/products/primary-antibodies/cd4-antibody-epr6855-ab133616.html)
Anti-CD3: Rabbit monoclonal antibody reacting with Human. This antibody has been validated in fluorescence multiplex immunohistochemical (Formalin/PFA-fixed paraffin-embedded sections) analysis using human colon tissue according to the manufacturer's website. This antibody has been validated in over 600 published reports. (https://www.abcam.cn/products/primary-antibodies/cd3-antibody-sp7-ab16669.html)
Anti-FOXP3: Rabbit monoclonal antibody reacting with Human. This antibody has been validated in fluorescence multiplex immunohistochemical (Formalin/PFA-fixed paraffin-embedded sections) analysis using human lung cancer tissue according to the manufacturer's website. This antibody has been validated in about 40 published reports. (https://www.abcam.cn/products/primary-antibodies/foxp3-antibody-epr22102-37-ab215206.html)
Anti-LAG3: Rabbit monoclonal antibody reacting with Human. This antibody has been validated in IHC analysis of paraffin-embedded human tonsil tissue and IF analysis of HEK-293T (Human epithelial cell line from embryonic kidney) cells, according to the manufacturer's website. This antibody has been validated in more than 500 published reports. (https://www.abcam.cn/products/primary-antibodies/lag-3-antibody-epr20261-ab209236.html)
Anti-CD68: Mouse monoclonal antibody reacting with Human. This antibody has been validated in IF analysis of paraffin-embedded human liver tissue according to the manufacturer's website. This antibody has been validated in more than 500 published reports. (https://www.abcam.cn/products/primary-antibodies/cd68-antibody-kp1-ab955.html)
Anti-VEGFA: Rabbit monoclonal [EP1176Y] to VEGFA - C-terminal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human kidney tissue according to the manufacturer's website. This antibody has been validated in about 200 published reports. (https://www.abcam.cn/products/primary-antibodies/vegfa-antibody-ep1176y-c-terminal-ab52917.html)
Anti-S100A4: Rabbit monoclonal antibody reacting with Human. This antibody has been validated in IHC (Formalin/PFA-fixed paraffin-embedded sections) analysis using human lung carcinoma tissue according to the manufacturer's website. This antibody has been validated in over 50 published reports. (https://www.abcam.cn/products/primary-antibodies/s100a4-antibody-epr146392-ab197896.html)

HRP-conjugated secondary:Goat Anti-Mouse IgG.This antibody has been validated in Formalin/PFA-fixed paraffin-embedded normal human colon tissue according to the manufacturer's website.This antibody has been validated in over 1026 published reports. https://www.abcam.cn/products/secondary-antibodies/goat-mouse-igg-hl-hrp-ab205719.html

HRP-conjugated secondary:Goat Anti-Rabbit IgG.This antibody has been validated in Formalin/PFA-fixed paraffin-embedded rat cardiac tissue sections according to the manufacturer's website.This antibody has been validated in over 4974 published reports. https://www.abcam.cn/products/secondary-antibodies/goat-rabbit-igg-hl-hrp-ab6721.html

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	Immunodeficient NOD SCID gamma (NSG) and Balb/c(Nu/Nu) mice (male, 18g-20 g, 6-8 weeks at the time of receipt) were used in the study. Mice were housed in individually ventilated polysulfone cages in a temperature- (20℃℃ °C-26°C℃,humidity:40-70%)and light-controlled room (12 h light12 h dark cycle, lights on at 7:00 a.m.) with free access to food and water.
Wild animals	This study did not involve wild animals.
Reporting on sex	NSG and Balb/c-nu mice used in this study were all male. Findings of this study applied to both sexes. Sex was not considered in this study design, thus there was no method used for sex assignment. Data disaggregate for sex were not collected. No sex-based analysis were performed in this study, since there was no significant difference observed in the incidence rate or clinical outcomes between the sexes regarding the disease studied in this research, and sex or gender attributes of the participants was not the main focus of this study.
Field-collected samples	NSG and Balb/c-nu mice were housed in individually ventilated polysulfone cages in a temperature- (20℃℃ °C-26°C℃, humidity:40%-70%) and light-controlled room (12h light 12h dark cycle, lights on at 7:00 a.m.) Experiment protocol will end when the maximal size of established tumors was around 2.0 cm in any direction. The maximal tumor size was not exceeded in this study.
Ethics oversight	The procedures of animal handling and tissue harvesting were approved by the Experimental Animal Department of Fudan University (Permit Number: 202104036S). All animal studies were conducted in accordance with the Animal Research Reporting of In Vivo (ARRIVE) guidelines. The maximal tumor size/burden permitted by the ethics committee was no greater than 2cm in all directions.And all the maximal tumor size burden was not exceeded accordingly.

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