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 *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

Imaging devices:
- Device for confocal microscopy image acquisition: Confocal microscope Olympus, FV1000, X81
- Software for confocal microscopy image acquisition: FV10-ASW, v4.0, Olympus
- Device for Light sheet microscopy image acquisition: Light sheet Ultramicroscope, Miltenyi Biotec
- Software for Light sheet microscopy image acquisition: ImSpector Pro (v7.1.15) LaVision BioTec GmbH

RNASeq device:
- cellenONE technology (Cellenion®)
- Bioanalyzer 2100, Agilent
- NextSeq500, Illumina
- NovaSeq600, Illumina

Data analysis

Image analysis:
- Image J (1.53aa)
- Arivis Vision4D (Zeiss, v3.1.3)

Analysis was performed with R (v3.6 and v4.0.) and Python v3.8.15

Bioinformatic tools and packages:
- Bcl2fastq v2.17.1.14

- CutAdapt v1.9.1
- FastQ Screen v0.5.2
- featureCounts v1.5.2
- GENCODE v26
- ENSEMBL v93
- STAR v2.6.1 and v2.7
- HTSeq-count v0.12.4
- GATK v4.1.4
- Picard-tools v2.1.0
- Seurat v4.0.1
- SCTransform v2
- Clustree v0.4.3
- DoubletFinder v2.0
- SingleR v1.0.5
- Scenic v1.1.2
- Genie3 v3.16
- scVelo v0.2.5
- Velocyto v0.17
- Cellrank v1.5.1
- Scanpy v1.9.1
- scFates v1.0
- Gprofiler2 v0.2.1
- fgsea v1.16.0
- limma v3.28.14
- MuSiC v1.0
- Survival v3.5.0
- Maftool v2.17
- DENDRO v0.1.1
- DepMap (https://depmap.org/portal/)
- InferCNV v1.10.0

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

Source data are provided with this paper. Information and data provided in the present manuscript, figures, supplementary information and source data is sufficient to assess whether the study claims are supported by the data. Raw lightsheet and confocal microscopy files generated in the study are available upon simple request. NB raw sequencing data from pairs of primary tumor and invaded bone marrow have been deposited in GEO database under the accession number GSE245175 (patients #1 to #7 data were analyzed in the present study, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE245175]). Raw sequencing data from NB implanted in the avian chick embryo have been deposited in GEO database under the accession number GSE237881 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237881]). ScRNA-seq data of NB patient samples from Dong et al. and from Kildisiute et al. used in this study were respectively obtained from the GEO (GSE137804, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137804]) and from the European Genome-phenome Archive (EGAD00001008345, [https://ega-archive.org/datasets/EGAD00001008345]). scRNA-seq data of human embryonic adrenal glands and NB patient samples from Jansky et al. 15 were obtained from the European Genome-phenome Archive (EGAS00001004388, [https://ega-archive.org/studies/EGAS00001004388]; and EGAD00001006624, [https://ega-archive.org/datasets/EGAD00001006624]). scRNA-seq data of human embryonic adrenal glands from Kameneva et al. were obtained from the GEO (GSE147821, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147821]). Bulk RNA-seq data of NB from SEQC cohort were obtained from the GEO (GSE49711, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49711]). Bulk RNA-seq data of NB from Kocak cohort were obtained from the GEO (GSE45547, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45547]).

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	Findings apply to any sex/gender. For the 7 patients for which data were generated in this study, sex information was collected based on self-report. Informed consent for biological material use and associated clinical data was obtained from patients / parents and the study was approved by the biobank committee at the Princess Máxima Center. The sex ratio in neuroblastoma was analyzed in multiple previous studies, with a slightly more frequent occurence in males (M/F= 1.2-1.3/1), but no significant difference in disease features between sexes.
Reporting on race, ethnicity, or other socially relevant groupings	N/A

Population characteristics	Primary tumor- and bone marrow samples of 7 high-risk patients between the age of 11 and 151 months were included in this cohort.
Recruitment	Primary tumor biopsies and bone marrow aspirates were taken at the timepoint of diagnosis prior to chemotherapeutic treatment.
Ethics oversight	Samples were used if written informed consent from patients and/or parents was obtained. The use of these samples was reviewed and approved by the biobank committee at the Princess Máxima Center.

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	No statistical tests or analyses were used to predetermine sample size. A total of 7 paired PT/BM neuroblastoma samples were included in this study to analyze neuroblastoma evolution during dissemination. A sufficient number of neuroblastoma cells were harvested and sequenced from chick embryo to perform statical analysis of the dynamics of neuroblastoma cell plasticity across early steps of metastatic progression.
Data exclusions	No exclusion was applied to the raw data. Sequenced cells from count matrix that did not meet quality control criteria explained in the methods section were removed from the downstream analysis.
Replication	Patient samples were sequenced using same sequencer. NB cells from chick embryo were sequenced from 3 different sets of experiments, with partially overlapping experimental conditions and with two different sequencing approaches (SMART-Seq2 and seqWell). Imaging analyses were performed on at least 3 chicken embryos from 2 independent experiments per experimental conditions, with the occurence of phenotypes given in each figure legend. The development of chicken embryos used for graft experiments (correct morphology and number of somites, indicative of a correct developmental stage) was systematically controlled in all experiments. Experiments on chicken embryos were performed by 5 collaborators, with a systematic replication of the graft outcome and phenotypes observed.
Randomization	No randomization was performed for this study.
Blinding	scRNA-Seq data generated here or from previous studies were not collected nor analyzed in blind as the objective was to define objectively the differences between experimental groups.

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	- Mouse anti-NF160 IgG Monoclonal Antibody Thermo Fisher Scientific, Life Technologies, Cat# 130700; RRID: AB_2532998 (dilution 1/200) - Mouse anti-SMA mouse IgG Monoclonal Antibody Millipore – Sigma-Aldrich, Cat# A2547 (dilution 1/200)

- Rabbit anti-Green Fluorescent Protein (GFP) Polyclonal Antibody Thermo Fisher Scientific Cat# A-11122; RRID: AB_221569 (dilution 1/200)
- Goat anti-Green Fluorescent Protein (GFP) IgG Polyclonal Antibody Abcam, Cat# ab5450 (dilution 1/500)
- Mouse anti-TOP2A IgG Monoclonal Antibody Novus Biologicals, Cat# 53281 (dilution 1/200)
- Mouse anti-LMNB1 IgG Monoclonal Antibody Abcam, Cat# ab8982 (dilution 1/200)
- Rabbit anti-ATAD2 rabbit IgG Polyclonal Antibody Thermo Fisher Scientific - Invitrogen, Cat# 720138; RRID: AB_2607225 (dilution 1/200)
- Rabbit anti-CHGB IgG Polyclonal Antibody Millipore – Sigma-Aldrich, Cat# HPA008759; RRID: AB_1846704 (dilution 1/200)
- Mouse anti-CD13 Antibody conjugated to BD Horizon BV421 BD, Cat# 562596 (dilution 1/100)
- Mouse anti-CD45 Antibody conjugated to BD Horizon BV510 BD, Cat# 563204 (dilution 1/800)
- Mouse anti-CD34 Antibody conjugated to BD Horizon BV605 BD, Cat# 745247 (dilution 1/200)
- Mouse anti-CD81 Antibody conjugated to BD Horizon BV650 BD, Cat# 740590 (dilution 1/100)
- Mouse anti-CD90 Antibody conjugated to BD Horizon BV711 BD, Cat# 740786 (dilution 1/200)
- Mouse anti-GD2 Antibody conjugated to BD Horizon BV786 BD, Cat# 744073 (dilution 1/400)
- Mouse anti-CD146 Antibody conjugated to BD Horizon PerCP Cy5.5 BD, Cat# 746081 (dilution 1/200)
- Mouse anti-CD271 Antibody conjugated to BD Horizon PE-CF594 BD, Cat# 563452 (dilution 1/200)
- Mouse anti-CD73 Antibody conjugated to BD Horizon PE-Cy7 BD, Cat# 561258 (dilution 1/400)
- Mouse anti-CD3 Antibody conjugated to BD Horizon APC BD, Cat# 561804 (dilution 1/50)
- Mouse anti-105 Antibody Biorad, Cat# MCA 1557F (dilution 1/25)
- Mouse anti-CD56 Antibody Biolegend, Cat# 355504 (dilution 1/200)
- Goat anti-L1CAM Antibody R&D, Cat# AF277 (dilution 1/50)
- Rabbit anti-ERBB4 Antibody Millipore – Sigma-Aldrich, Cat# HPA012016 (dilution 1/50)
- Rabbit anti-PLXNB2 Antibody Proteintech, Cat#10602-1-AP (dilution 1/100)
- Rabbit anti-TUBB3 Antibody Biolegend Cat# 802001 (dilution 1/200)

Validation

- Mouse anti-NF160 IgG Monoclonal Antibody: The antibody was validated in more than 49 publications. The manufacturer provides data showing that this Antibody was verified by Knockout to ensure that the antibody binds to the antigen stated, in IHC and immunofluorescence: https://www.thermofisher.com/antibody/product/NEFM-Antibody-clone-RMO-270-Monoclonal/13-0700
- Mouse anti-SMA mouse IgG Monoclonal Antibody: The antibody was validated in more than 2380 publications. The manufacturer provides validation data for immunohistochemistry, immunofluorescence, western blot: https://www.sigmaaldrich.com/FR/fr/product/sigma/a2547
- Rabbit anti-Green Fluorescent Protein (GFP) Polyclonal Antibody: the antibody was validated in more than 1303 publications and was validated by the manufacturer specifically for Immunofluorescence: https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-11122
- Goat anti-Green Fluorescent Protein (GFP) IgG Polyclonal Antibody: the antibody was validated in more than 210 publications and was validated by the manufacturer specifically for Immunofluorescence: https://www.abcam.com/products/primary-antibodies/gfp-antibody-ab5450.html?productWallTab=ShowAll
- Mouse anti-TOP2A IgG Monoclonal Antibody: the antibody was validated by the manufacturer for immunohistochemistry, immunofluorescence, western blot, Protein Array: https://www.novusbio.com/products/top2a-antibody-top2a-1361_nbp2-53281?utm_source=antibodypedia&utm_medium=referral&utm_campaign=product#datasheet
- Mouse anti-LMNB1 IgG Monoclonal Antibody: The antibody was validated in more than 40 publications. The manufacturer provides data showing that this Antibody was verified by Knockout to ensure that the antibody binds to the antigen stated, in flow cytometry, immunohistochemistry and immunofluorescence: https://www.abcam.com/products/primary-antibodies/lamin-b1-antibody-119d5-f1-nuclear-envelope-marker-ab8982.html?productWallTab=ShowAll
- Rabbit anti-ATAD2 rabbit IgG Polyclonal Antibody: the antibody was validated by the manufacturer for immunohistochemistry, immunofluorescence and western blot: https://www.thermofisher.com/antibody/product/ATAD2-Antibody-Polyclonal/720138
- Rabbit anti-CHGB IgG Polyclonal Antibody: The antibody was validated in more than 4 publications. The manufacturer provides validation data for immunohistochemistry, immunofluorescence, western blot: https://www.sigmaaldrich.com/FR/fr/product/sigma/hpa008759
- Mouse anti-CD13 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-mouse-anti-human-cd13.562596
- Mouse anti-CD45 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv510-mouse-anti-human-cd45.563204
- Mouse anti-CD34 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-mouse-anti-human-cd34.745247
- Mouse anti-CD81 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv650-mouse-anti-human-cd81.740590
- Mouse anti-CD90 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-mouse-anti-human-cd90.740786
- Mouse anti-GD2 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-mouse-anti-human-disialoganglioside-gd2.744073
- Mouse anti-CD146 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bb700-mouse-anti-human-cd146.746081
- Mouse anti-CD271 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cf594-mouse-anti-human-cd271.563452
- Mouse anti-CD73 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-mouse-anti-human-cd73.561258

- Mouse anti-CD3 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bdbiosciences.com/en-fr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-mouse-anti-human-cd3.561804
- Mouse anti-CD105 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.bio-rad-antibodies.com/monoclonal/human-cd105-antibody-sn6-mca1557.html?f=purified&JSESSIONID_STERLING=A289C7C0E48BF8635085BB0321232287.ecommerce1&evCntryLang=FR-fr&EU_COOKIE_PREFS=111&cntry=FR&thirdPartyCookieEnabled=true
- Mouse anti-CD56 Antibody: the antibody was validated by the manufacturer for flow cytometry: https://www.biolegend.com/nl-nl/products/pe-anti-human-cd56-subset-msc-marker-antibody-8191?GroupID=BLG11118
- Goat anti-L1CAM IgG Monoclonal Antibody : the antibody was validated by the manufacturer for immunohistochemistry, immunofluorescence, western blot, Protein Array: https://www.rndsystems.com/products/human-l1cam-antibody_af277?gad_source=1&gclid=Cj0KCQjwq86wBhDiARIsAJhuphmqfdJJUc0_WOlgFFaLcb6khisgTWANRvVl-vtj0xcXkxLKZktvzx8aApufEALw_wcB&gclsrc=aw.ds#product-details
- Rabbit anti-ERBB4 IgG Monoclonal Antibody : the antibody was validated by the manufacturer for immunohistochemistry, immunofluorescence, western blot, Protein Array: https://www.sigmaaldrich.com/FR/fr/product/sigma/hpa012016?utm_source=google&utm_medium=cpc&utm_campaign=20855489261&utm_content=155355395734&gclid=Cj0KCQjwq86wBhDiARIsAJhuphkdyrmNjg3H20xyz2thC5Tofm0embkBrrvoPDk32r8KL2HGdf4pMZ8aAoQFEALw_wcB
- Rabbit anti-PLXNB2 IgG Monoclonal Antibody : the antibody was validated by the manufacturer for immunohistochemistry, immunofluorescence, western blot, Protein Array: https://www.ptglab.com/products/PLXNB2-Antibody-10602-1-AP.htm
- Rabbit anti-TUBB3 IgG Monoclonal Antibody : the antibody was validated by the manufacturer for immunohistochemistry, immunofluorescence, western blot, Protein Array: https://www.biolegend.com/fr-ch/explore-new-products/purified-anti-tubulin-beta-3-tubb3-antibody-11579

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	IGR-N91 cell line (RRID:CVCL_8883) was given by the team of J. Bénard (Gustave Roussy Institute) and described in Ferrandis and Bénard, 1993. SH-SY5Y cell line was obtained from ATCC (ATCC® CRL-2266™). SHEP cell line was provided by the team of V. Combaret (Centre Léon Bérard): (RRID:CVCL_0524) IGR-N91::GFP, SHEP::GFP and SH-SY5Y::GFP cell lines were previously described in Delloye-Bourgeois et al., Cancer Cell; 2017 and were generated upon lentiviral infection of source cell lines.
Authentication	Cell lines were regularly checked for the expression of key markers by qRT-PCR (MYCN, SEMA3C). Their typical morphology in in vitro culture conditions was also verified at each step of the project.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination. Tests were carried out monthly for the duration of the study.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	Naked Neck strain embryonated eggs were obtained from a local supplier (Elevage avicole du Grand Buisson, Saint Maurice sur Dargoire, France). Laying hen's sanitary status was regularly checked by the supplier according to French laws.
Wild animals	N/A
Reporting on sex	N/A
Field-collected samples	The study did not involved samples collected from the field.
Ethics oversight	Chick embryos were used within the 14 first days of gestation, stages that do not require approved protocol by ethics committee, in accordance with the revised EU directive 2013 86/609/EEC.

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