

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	No commercial or open source software was used for data collection in this study
Data analysis	Analysis code for different use cases of Rapid-CNS2 and preprocessing code to convert bedmethyl files to MNP-Flex compatible input is available on Github (https://github.com/areebapatel/Rapid-CNS2_sh; https://github.com/areebapatel/Rapid-CNS2_nf). Latest versions of MinKNOW were used as applicable on the devices. Readfish v2024.2.0 was used for intraoperative analysis. Tools used included: Rapid-CNS2 Pipeline: Initial version: Guppy v4.4.0, Megalodon Updated version: Guppy v5+ (basecalling, methylation calling via Remora, alignment). Dorado for basecalling/alignment; modbam2bed v0.5.3 for methylation values; liftover tool, SNV detection: PEPPER-Margin-DeepVariant (r0.4 for R9, r0.8 for R10 flowcells). CNV calling: cnvpytor (v1.2.1 for R9, v1.3.1 for R10 flowcells). Structural variants: Sniffles2 (v2.2), annotated using AnnotSV (v3.0.7). Annotation: ANNOVAR (downloaded May 7, 2021). Subset BAM creation: Bedtools v2.30.0. Methylartist (v1.2.8) for methylation visualization. Alignment: wf-human-variation (ONT pipeline) and Nextflow. Intraoperative Protocols: Real-time analysis: Dorado (dna_r10.4.1_e8.2_400bps_sup), modbam2bed (Heidelberg), and modkit (Nottingham). Nanopore WGS: Basecalling and mapping: MinKNOW (v23.07), Dorado (v7.1.14), wf-human-variation (v1.8.1), modbam2bed (v0.10.0), modkit (v0.2.0). Methylation Array Analysis: Beta-value processing: minfi (v1.21.4), limma (v3.30.11). WGBS Analysis: Methylation analysis: MethylCtools (ODCF Bisulfite core workflow).

MNP-Flex Model:

Machine learning: XGBoost (v2.01) with Caret (v6.0-94).

Visualization: ggplot2 (v3.5.1), ggscankey (v0.0.99999), ggridges (v0.5.4), patchwork (v1.1.3).

Confidence intervals: Binom R-package.

Equivalence testing: TOSTER (v0.8.3).

Twist Panel Sequencing:

Methylation values: nf-core/methylseq (v1.6.1).

Time to Classification:

Basecalling: Guppy v6.4.6 (super accuracy configuration).

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 generated in this study has been uploaded to Zenodo and is accessible at <https://doi.org/10.5281/zenodo.13351527>. Raw sequencing data will be made available upon reasonable request to Felix Sahm (felix.sahm@med.uni-heidelberg.de). Requests will be reviewed and responded to within 14 working days

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	Patient sex and/or gender were not considered in the design of the study, and were also not considered in the inclusion criteria. Patient sex was self-reported and available for 239 patients in the Rapid-CNS2 cohort. Age, sex, and/or gender were not included in the study design, as the focus was on validating a platform for molecular profiling. Based on available literature, such profiling accuracy is not known to be influenced by age, sex, or gender.
Reporting on race, ethnicity, or other socially relevant groupings	Patient race, ethnicity and/or other socially relevant groupings were not considered in the design of the study, and were also not considered in the inclusion criteria
Population characteristics	Patients were recruited from each of the local sites, the only inclusion criteria was suspected CNS tumour with a neurosurgical specimen. No age, sex, gender or ethnicity factors were taken into account.
Recruitment	Archival samples with sufficient cryopreserved tissue were selected for Rapid-CNS2. Diagnostic samples were also similarly recruited if at least 1.5mm diameter tissue was available.
Ethics oversight	This study complied with ethics regulations as approved by the Ethics Committee Heidelberg (S-318/2022), Ethics Committee Nottingham (11/EM/0076), and Ethical Committee, Ghent University Hospital (Clinical Trial Number/IRB B6702021000850). Patients were not compensated for participation. Patient samples were included if they presented with a suspected CNS tumour and had undergone surgery to ensure tissue availability.

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 sample size calculation was performed. Sample sizes were determined based on the availability of patients presenting with suspected CNS tumors who had undergone surgery, ensuring sufficient tissue for analysis. The sample size is considered adequate as it encompasses a diverse range of CNS tumour subtypes, allowing for robust validation of the Rapid-CNS2 pipeline and the MNP-Flex model across a broad spectrum of real-world cases.
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Data exclusions	No data exclusion criteria was set for Rapid-CNS2. For non-IDAT validation samples for MNP-Flex, all samples submitted by collaborating groups were included
Replication	Technical robustness of the Rapid-CNS2 protocol was established by running the protocol in two laboratories- University Hospital Heidelberg and University of Nottingham
Randomization	There were no experimental groups or randomization in the study design
Blinding	As there were no groups, blinding of participants was not necessary in this study. Neuropathologists and scientists performing molecular analyses and evaluation were blinded to the respective results until final concordance analyses

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

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>