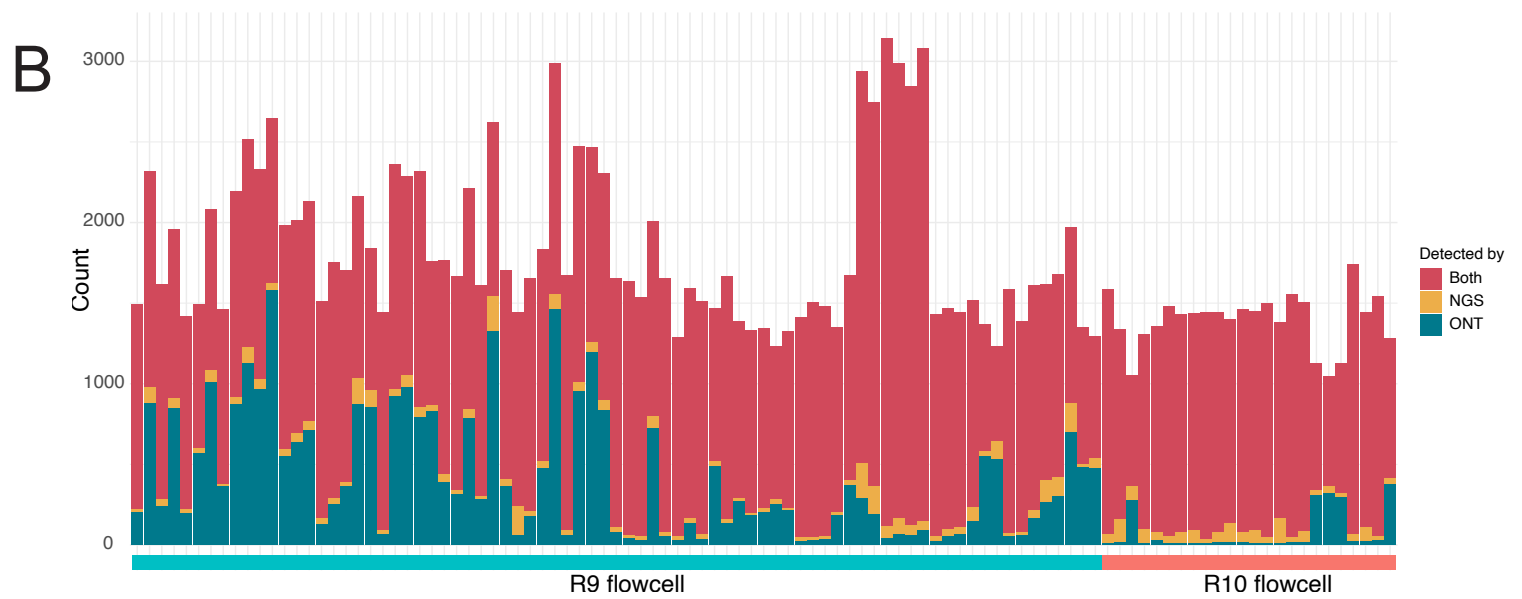
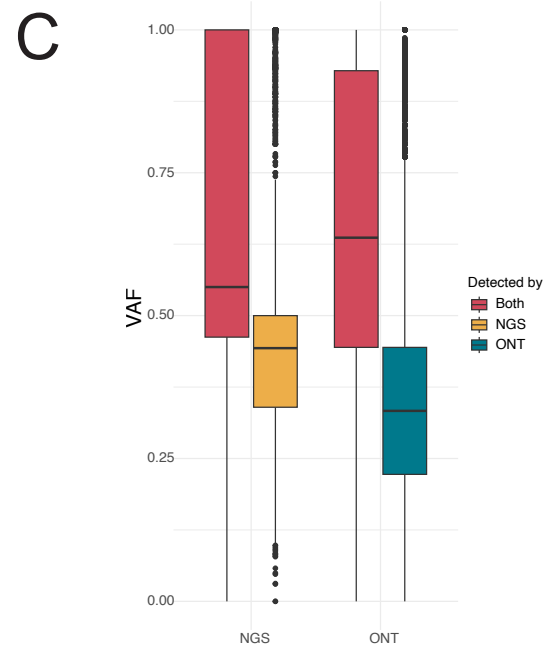
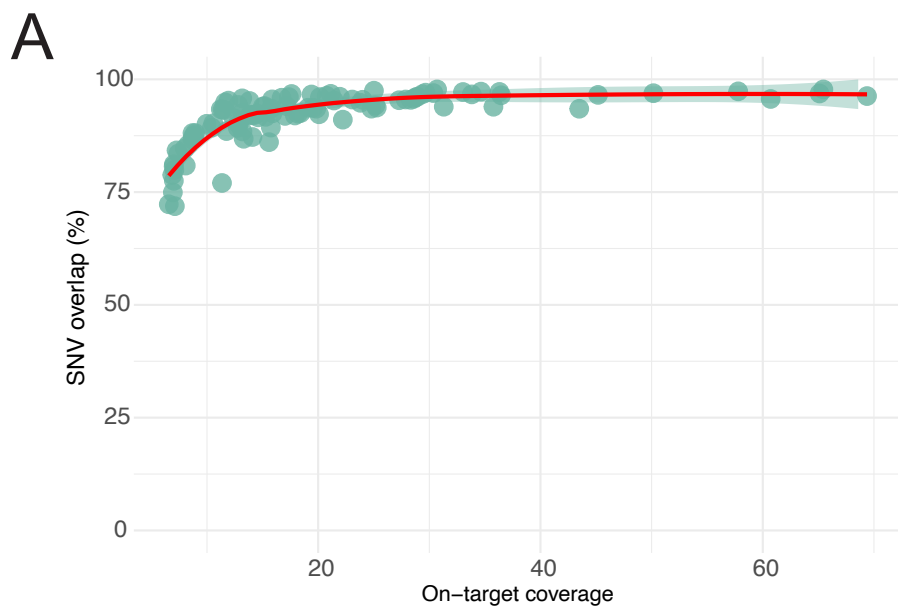


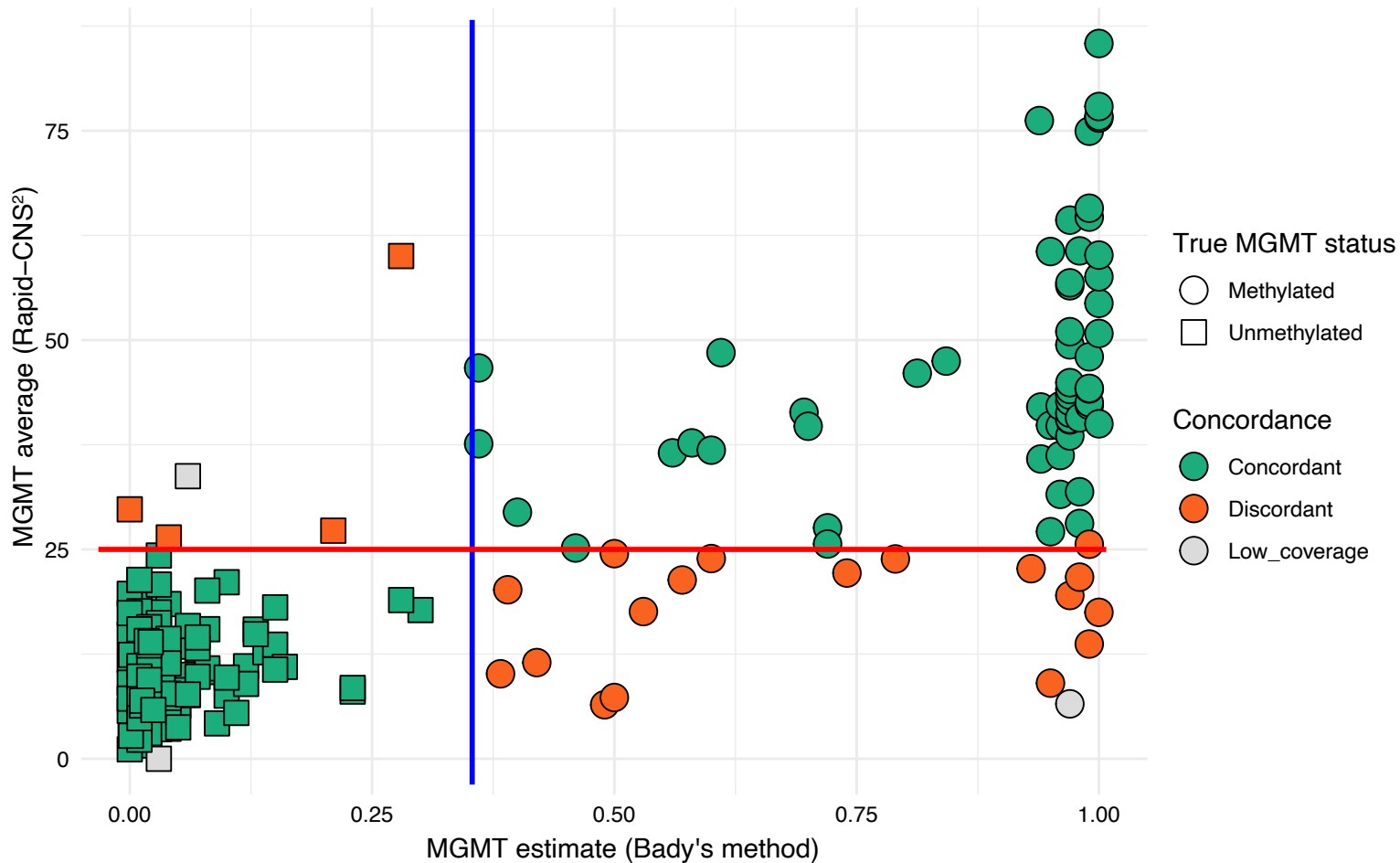
Prospective, multicenter validation of a platform for rapid molecular profiling of central nervous system tumors

In the format provided by the
authors and unedited



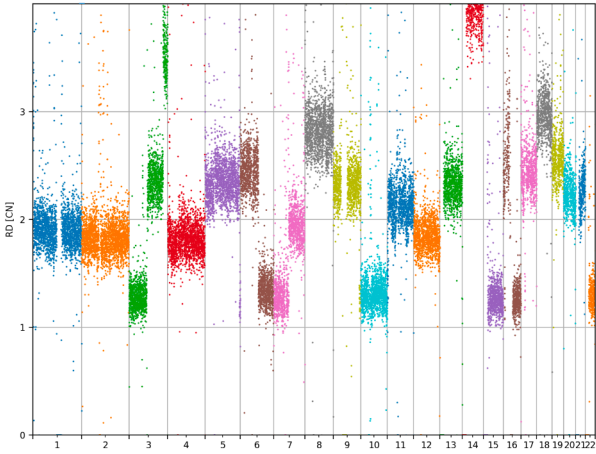
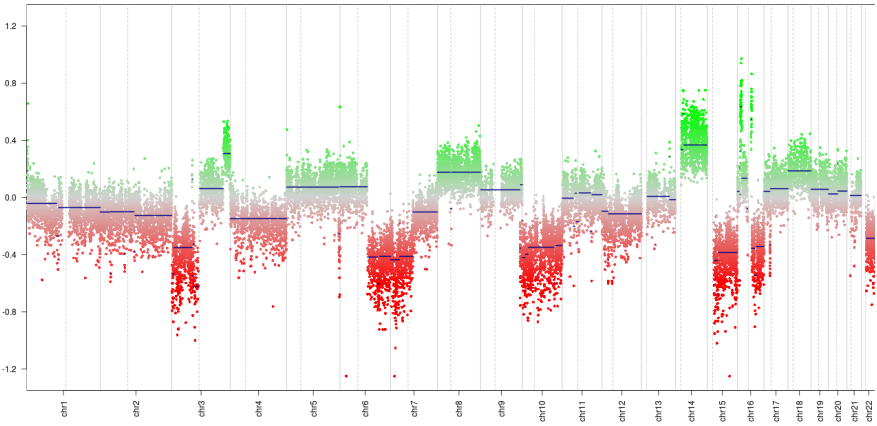
Supplementary Figure 1: Concordance for SNVs. A) Percentage of SNVs reported by NGS recovered in their respective Rapid-CNS² libraries. X axis indicates on-target coverage for the library and Y-axis indicates % overlap. B) Barplot showing number of shared and unique mutations across ONT and NGS. Samples are grouped by flowcell they were run on. R9 flowcells displayed higher false positive rates than R10.

C) Boxplots indicate variant allele frequency (VAF) for shared and unique mutations across ONT and NGS. Box plots illustrate the median (horizontal line), with the box boundaries indicating the 25th and 75th percentiles (interquartile range). Whiskers extend up to 1.5 times the interquartile range beyond the box edges. Data points falling outside these limits are plotted individually as outliers. Despite high false positive rate, ONT-specific variants can easily be excluded by VAF filtering.

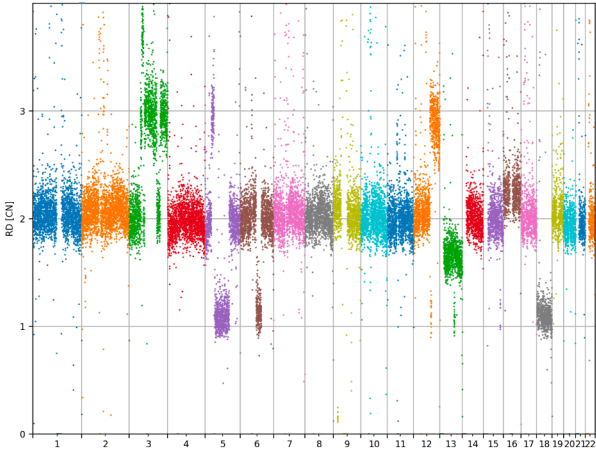
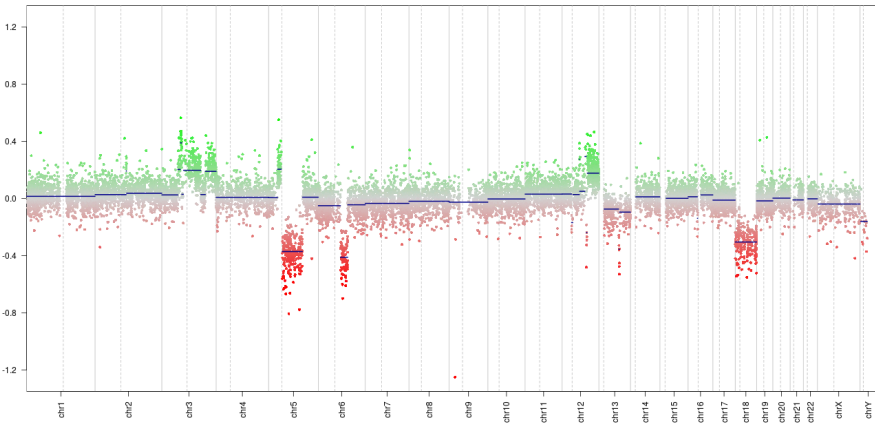


Supplementary Figure 2: MGMT concordance. X axis shows MGMT estimate by Bady's method, while Y-axis shows the average CpG methylation over the best predictive 137 CpGs. Colour indicates concordance. Shape indicates ground truth MGMT status. Red and blue lines indicate cutoffs for Rapid-CNS2 and Bady's method respectively.

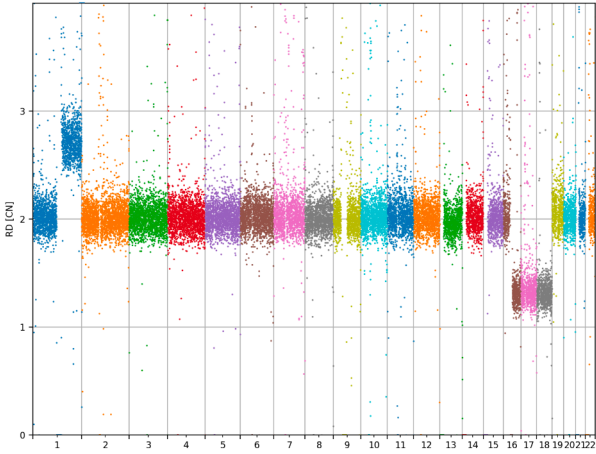
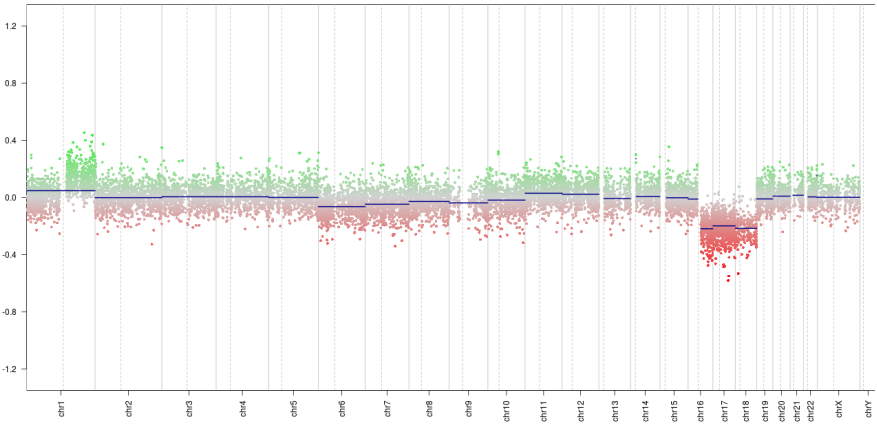
Study ID 109 (melanoma metastasis)



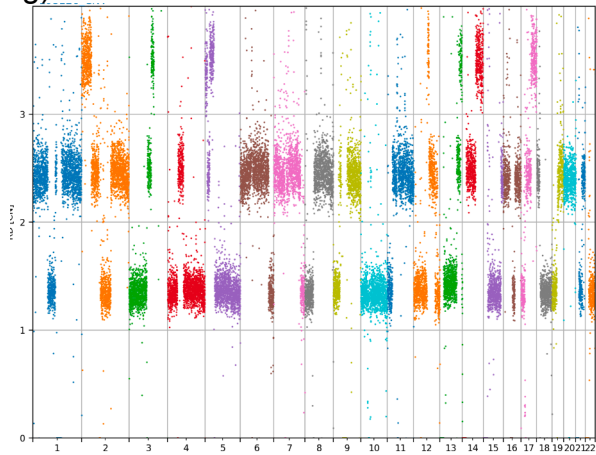
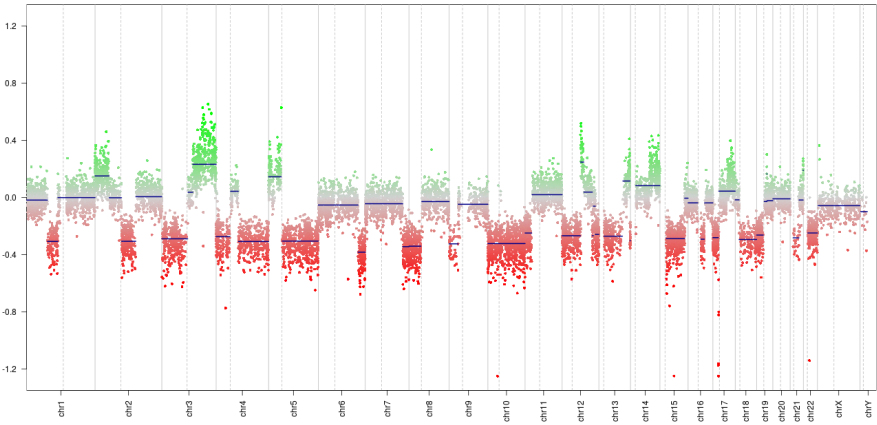
Study ID 110 (metastasis cancer of unknown primary)



Study ID 111 (metastasis mammary carcinoma)



Study ID 112 (metastasis adenosquamous carcinoma of the lung)



Supplementary Figure 3: Copy number profiles of metastases samples. EPIC array generated profiles (left) and corresponding Rapid-CNS2 profiles (right)

UCSC Genome Browser on Human (GRCh37/hg19)

move << < > >> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

multi-region chr7:138,054,596-140,978,703 2,924,108 bp. gene, chromosome range, search terms, help pages, see € go [examples](#)

chr7 (q33-q34) 21.3 14.3 14.1 21.11 31.1 q33 34 35

Scale
chr7: 138,500,000 | 139,000,000 | 139,500,000 | 140,000,000 | 140,500,000 | hg19

Reference Assembly Fix Patch Sequence Alignments

UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)

JB175279 | TMEM213 |
DQ573766 | TMEM213 |
DQ587956 | KIAA1549 |

TRIM24
TRIM24
TRIM24

SVOPL
SVOPL
ATP6V0A4
ATP6V0A4
ATP6V0A4
KIAA1549
KIAA1549
KIAA1549
RNU6-1272P |

ZC3HAV1L | UBN2 |
ZC3HAV1 | TRNA |
ZC3HAV1 | LUC7L2 |
ZC3HAV1 | FMC1 |

TTC26 |
TTC26 |
TTC26 |
TTC26 |
TTC26 |
TTC26 |
TTC26 |
TTC26 |

HIPK2 |
HIPK2 |
HIPK2 |
HIPK2 |
HIPK2 |
HIPK2 |
HIPK2 |
HIPK2 |

TBXAS1 |
TBXAS1 |
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TBXAS1 |
TBXAS1 |

KDM7A |
KDM7A |
KDM7A |
KDM7A |
KDM7A |
KDM7A |
KDM7A |
KDM7A |

SLC37A3 |
SLC37A3 |
SLC37A3 |
SLC37A3 |
SLC37A3 |
SLC37A3 |
SLC37A3 |
SLC37A3 |

MRPS33 |
ADCK2 |
NDUFB2 |
BRAF |
NDUFB2 |
BC065735 |
AK098095 |
RAB19 |
RAB19 |
MKRN1 |
MKRN1 |
MKRN1 |
MKRN1 |
DENND2A |
DENND2A |
DENND2A |
DENND2A |
DENND2A |
NDUFB2-AS1 |

MRPS33 |
MRPS33 |
TMEM178B |

RefSeq Curated

Sequences

Publications: Sequences in Scientific Articles

140,485 kb 140,490 kb 140,495 kb 138,541,500 bp 138,542,500 bp 138,543,500 bp 138,544,500 bp

Study ID 78_sorted_merged.bam

Study ID 78_sniffles_non_germline.vcf

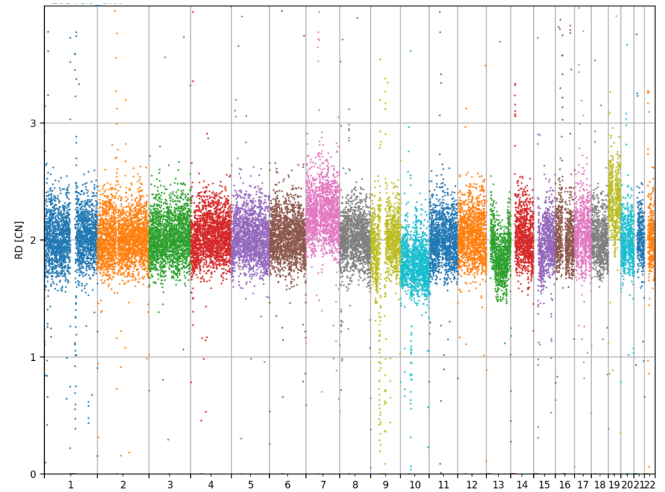
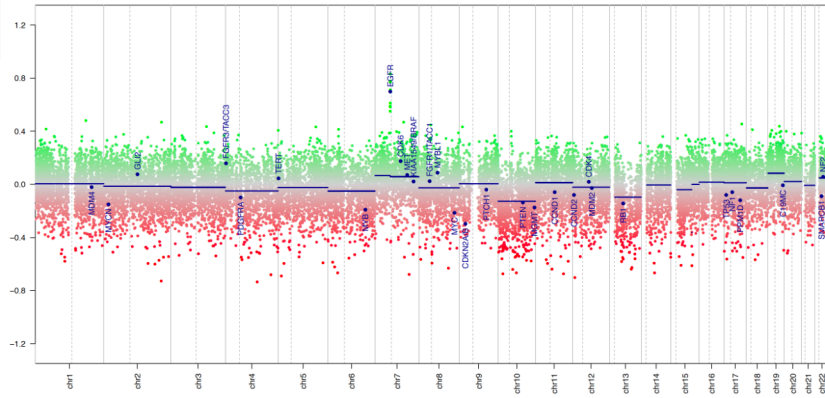
Refseq Genes

BRAF KIAA1549

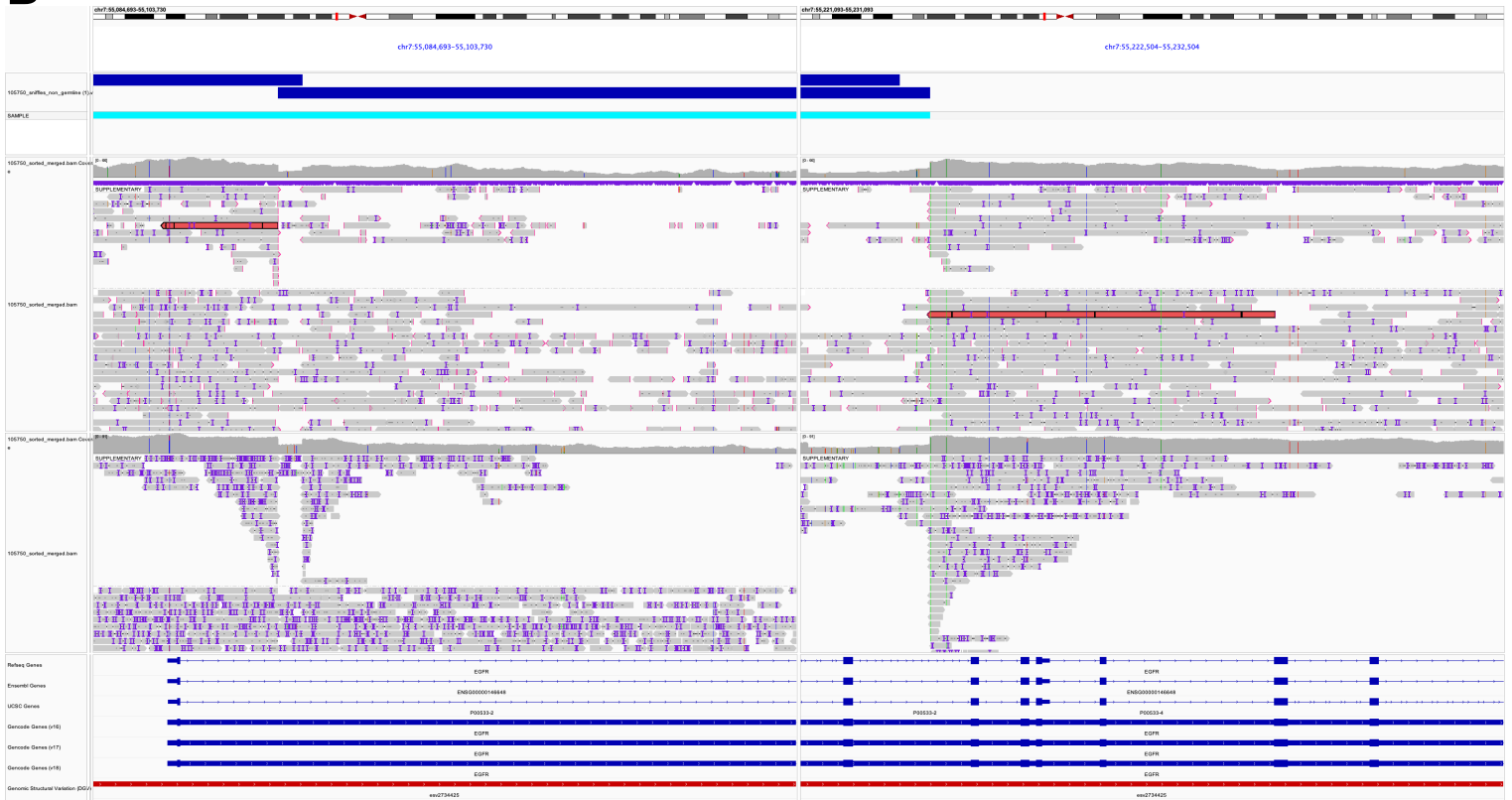
BRAF KIAA1549

Supplementary Figure 4: A) 19Mb duplication region on chr7.q34 detected by Rapid-CNS2 (highlighted in blue) in Study ID 78. B) IGV screenshot of the breakpoints in *BRAF* and *KIAA1549*, blue and green bars below indicate duplicated region.

A

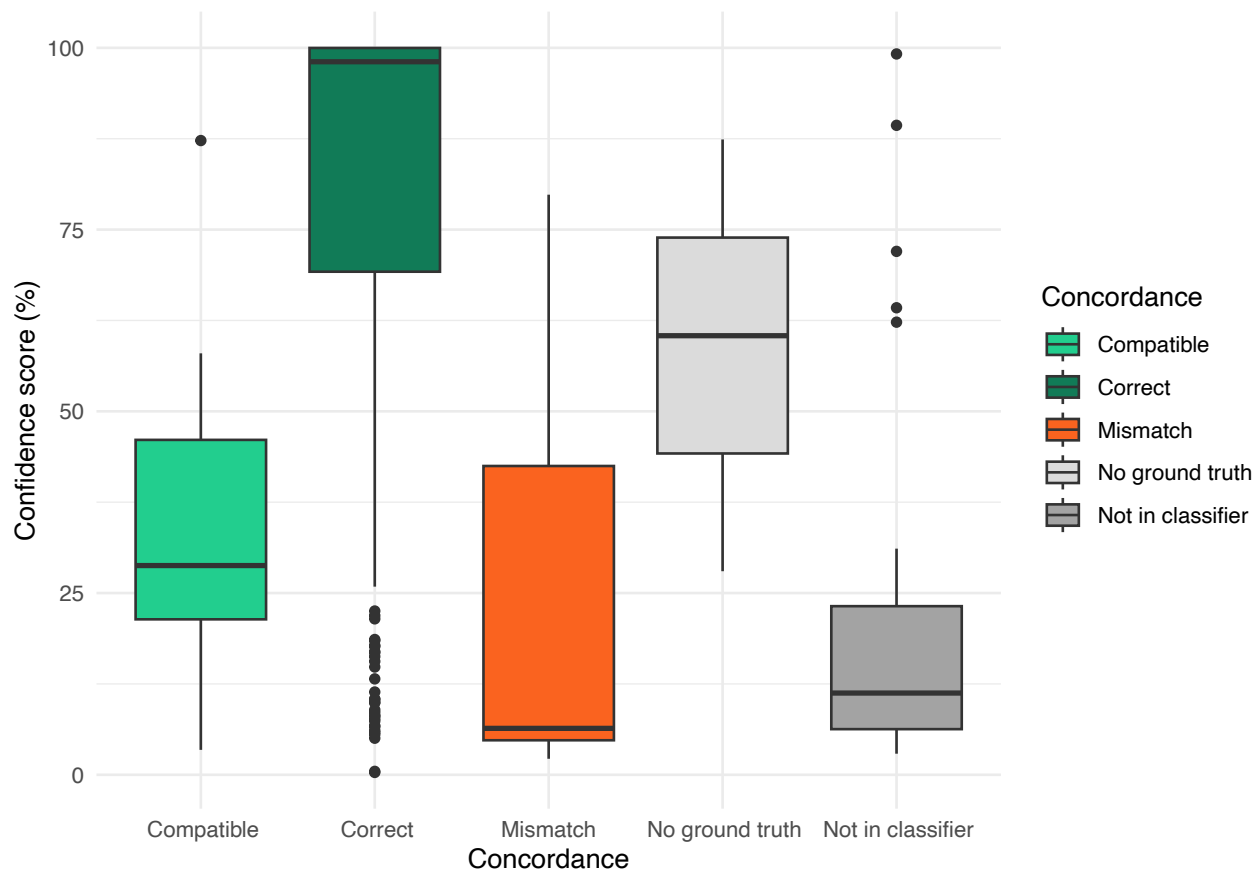


B

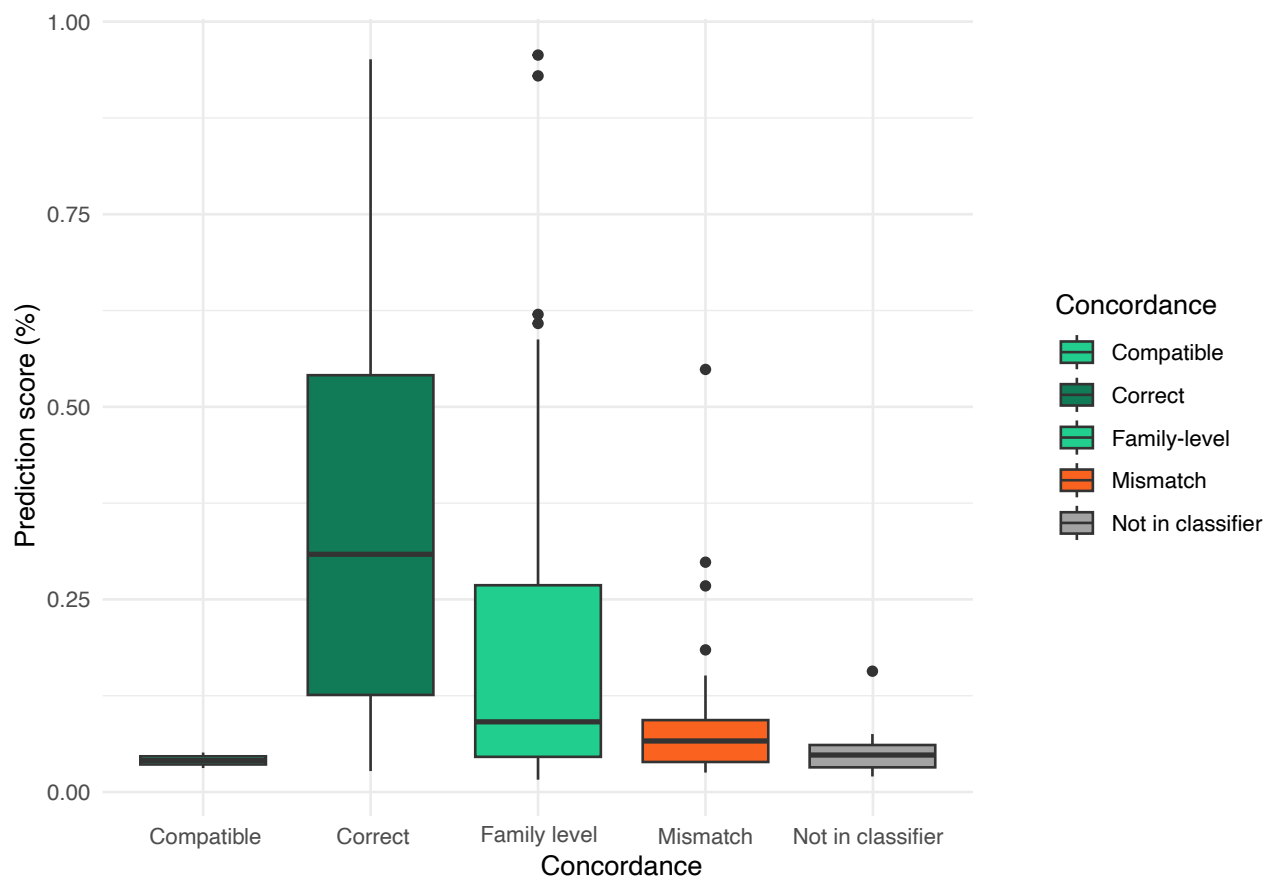


Supplementary Figure 5: EGFR vIII detection- A) Copy number profiles showing *EGFR* amplification- (left) EPIC array and (right) Rapid-CNS2. B) IGV screenshot of regions spanning exon 2 (left) and exon 7 (right) in two sequencing runs of the same sample. Read highlighted in orange indicates a single read spanning both breakpoints. ~20 reads out of the average 100 reads covering the region display the deletion.

A

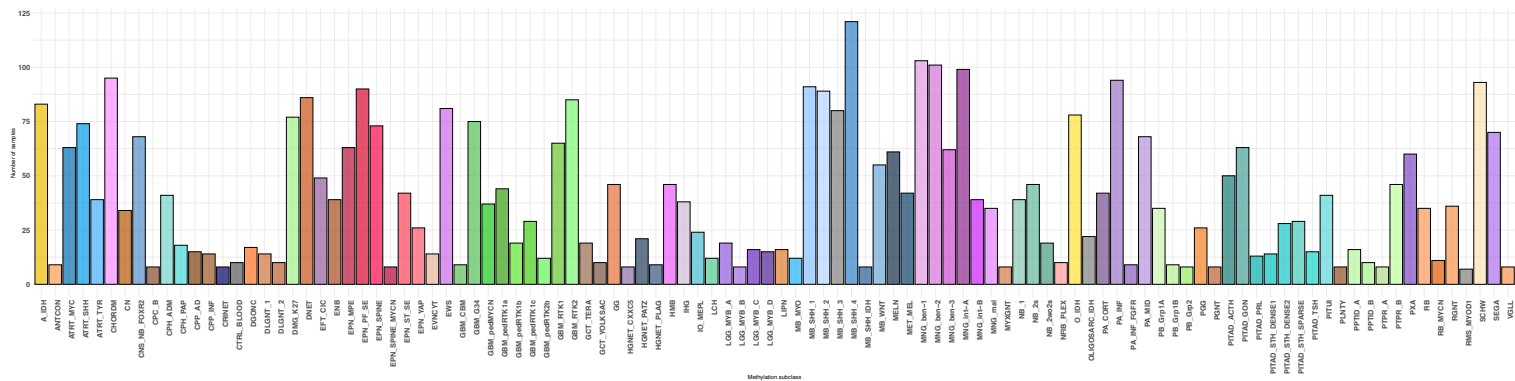


B

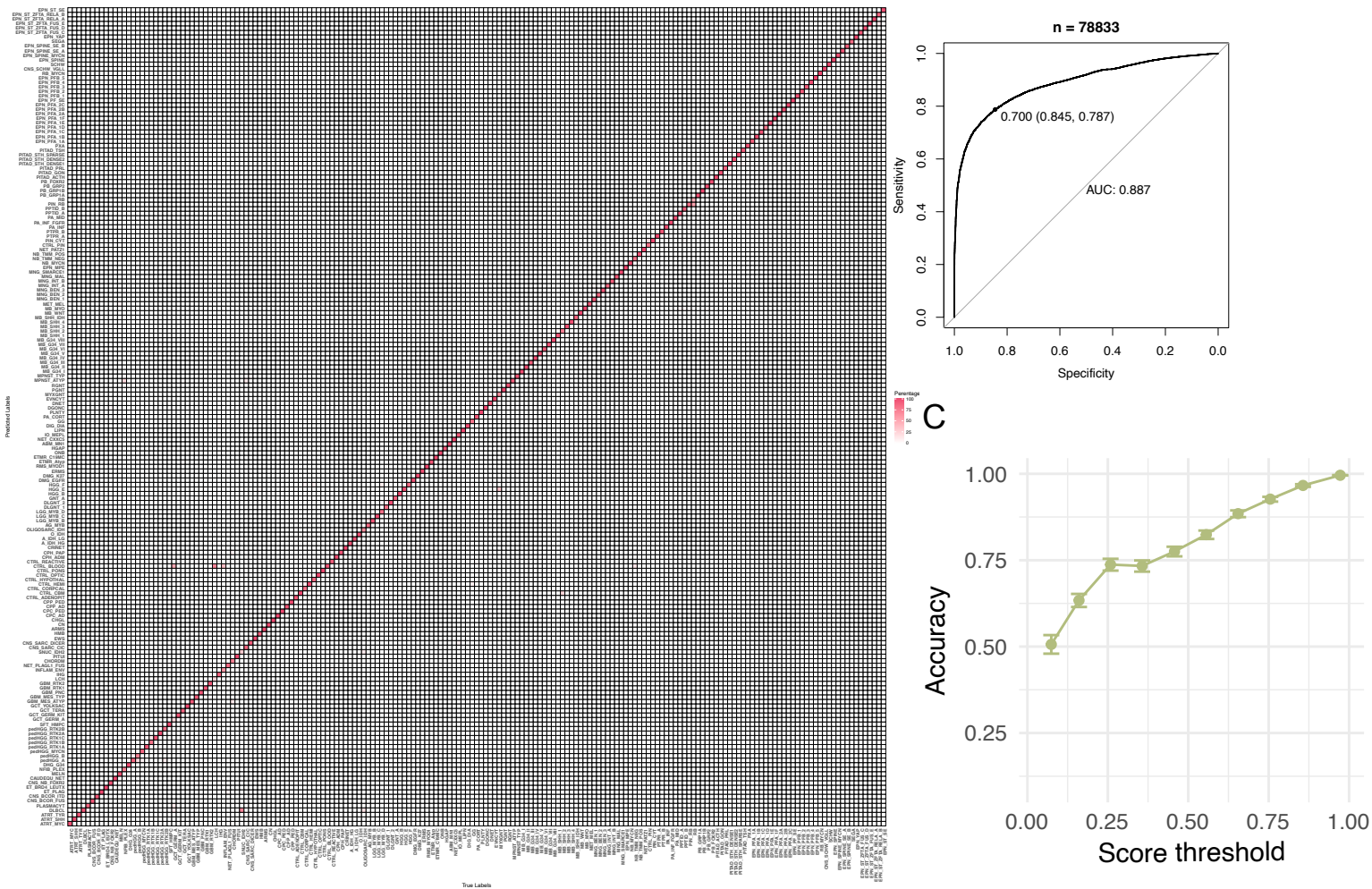


Supplementary Figure 6: Boxplots show confidence scores for the A) Rapid-CNS2 and B) MNP-Flex classifier across different concordance levels. "Compatible" includes cases predicted as tumour microenvironment or similar, which indicate presence of tumour but are not an exact match. "Not in classifier" includes cases belonging to classes outside the scope of the reference set. Box plots illustrate the median (horizontal line), with the box boundaries indicating the 25th and 75th percentiles (interquartile range). Whiskers extend up to 1.5 times the interquartile range beyond the box edges. Data points falling outside these limits are plotted individually as outliers.

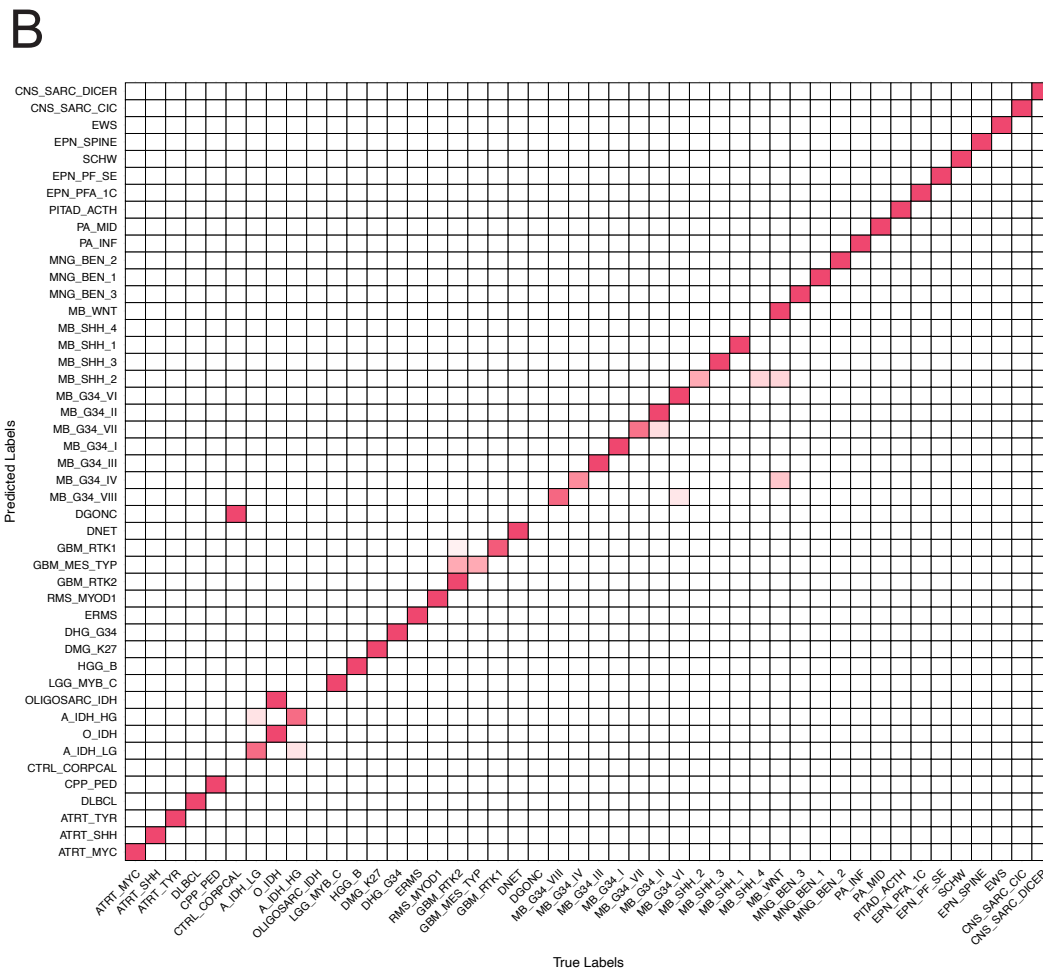
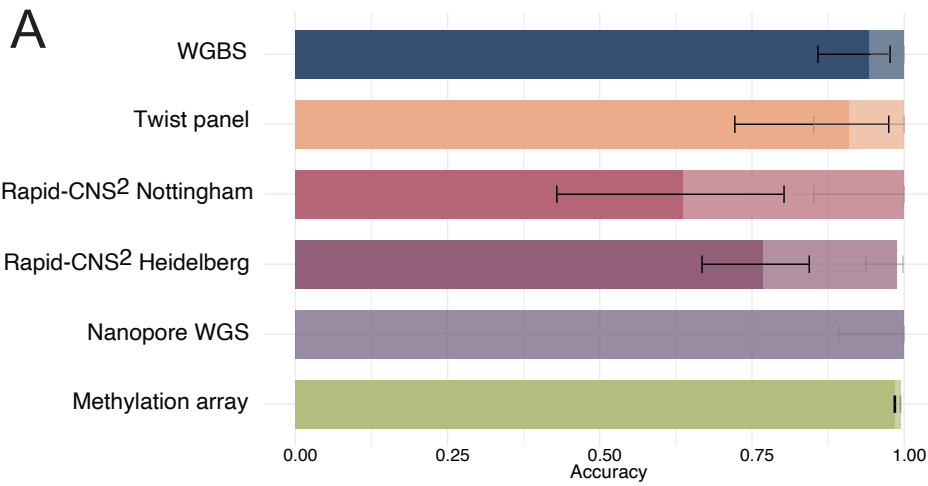
A



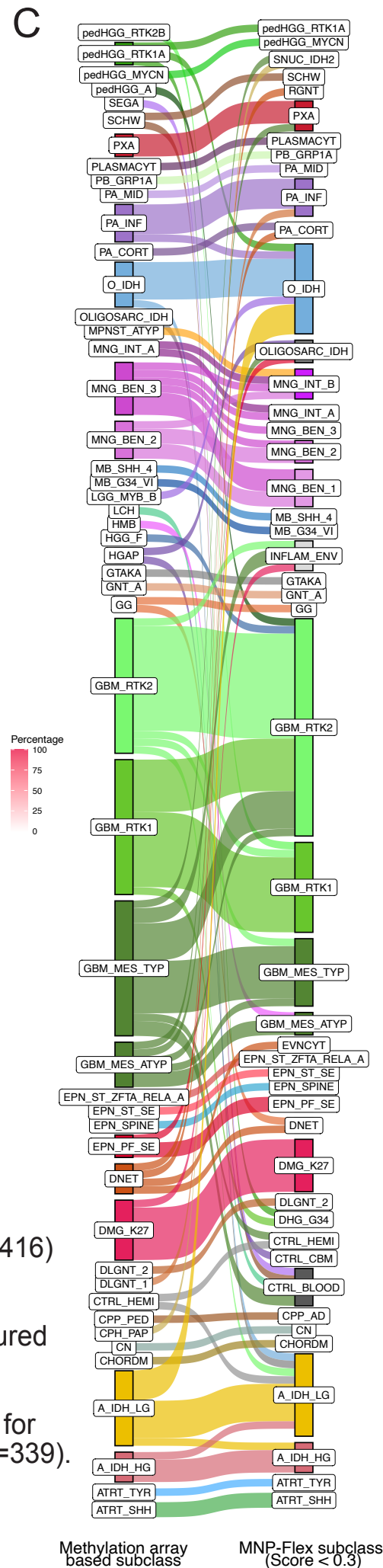
B

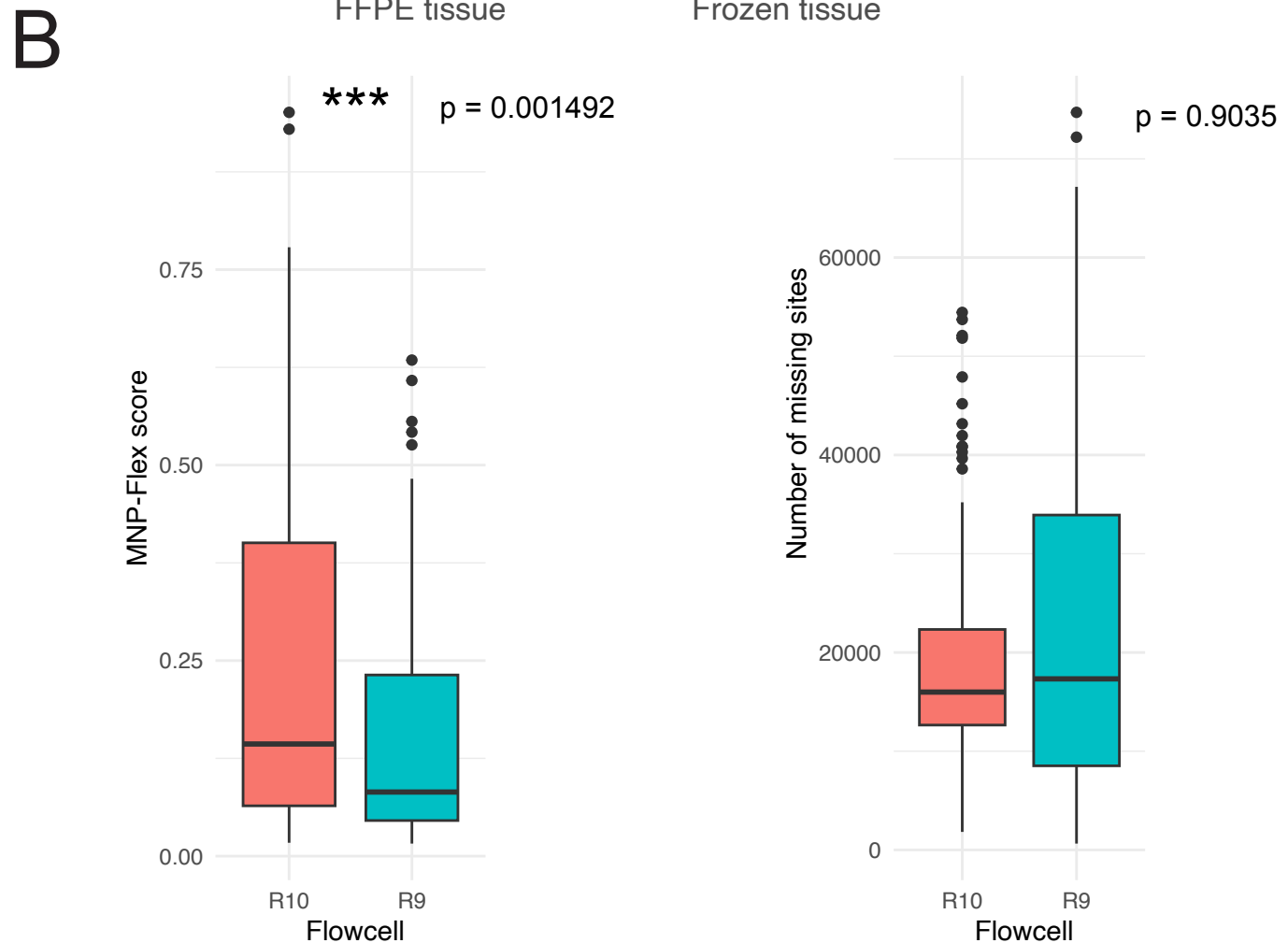
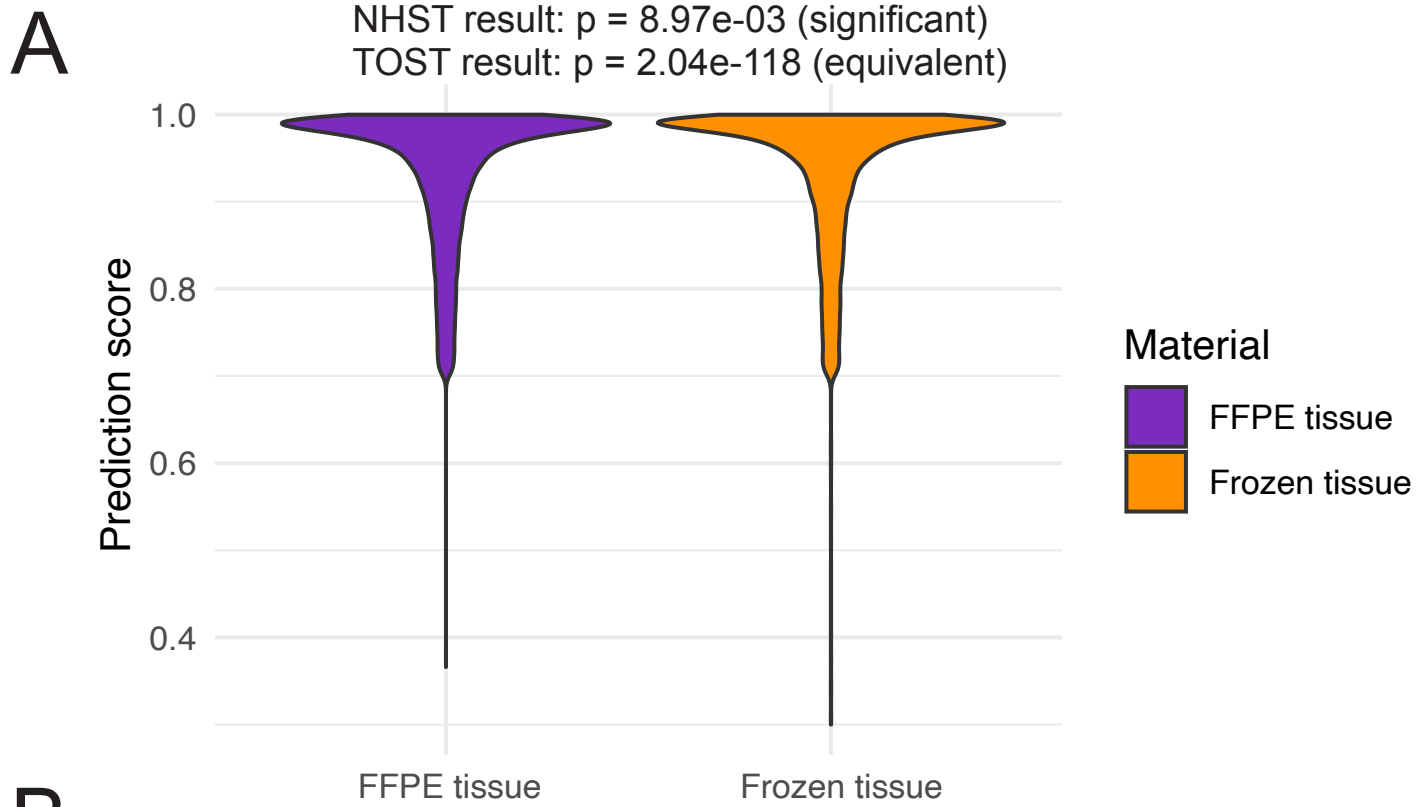


Supplementary Figure 7: A) Training set for MNP-Flex. B) Confusion matrix and precision-recall curve for 78,883 methylation array samples with MNP-RF subclass scores >0.7 C) Accuracy vs score threshold for array validation set (n = 78,883). Error bars represent the 95% confidence intervals calculated using a binomial proportion confidence interval via the binom.confint function in R.

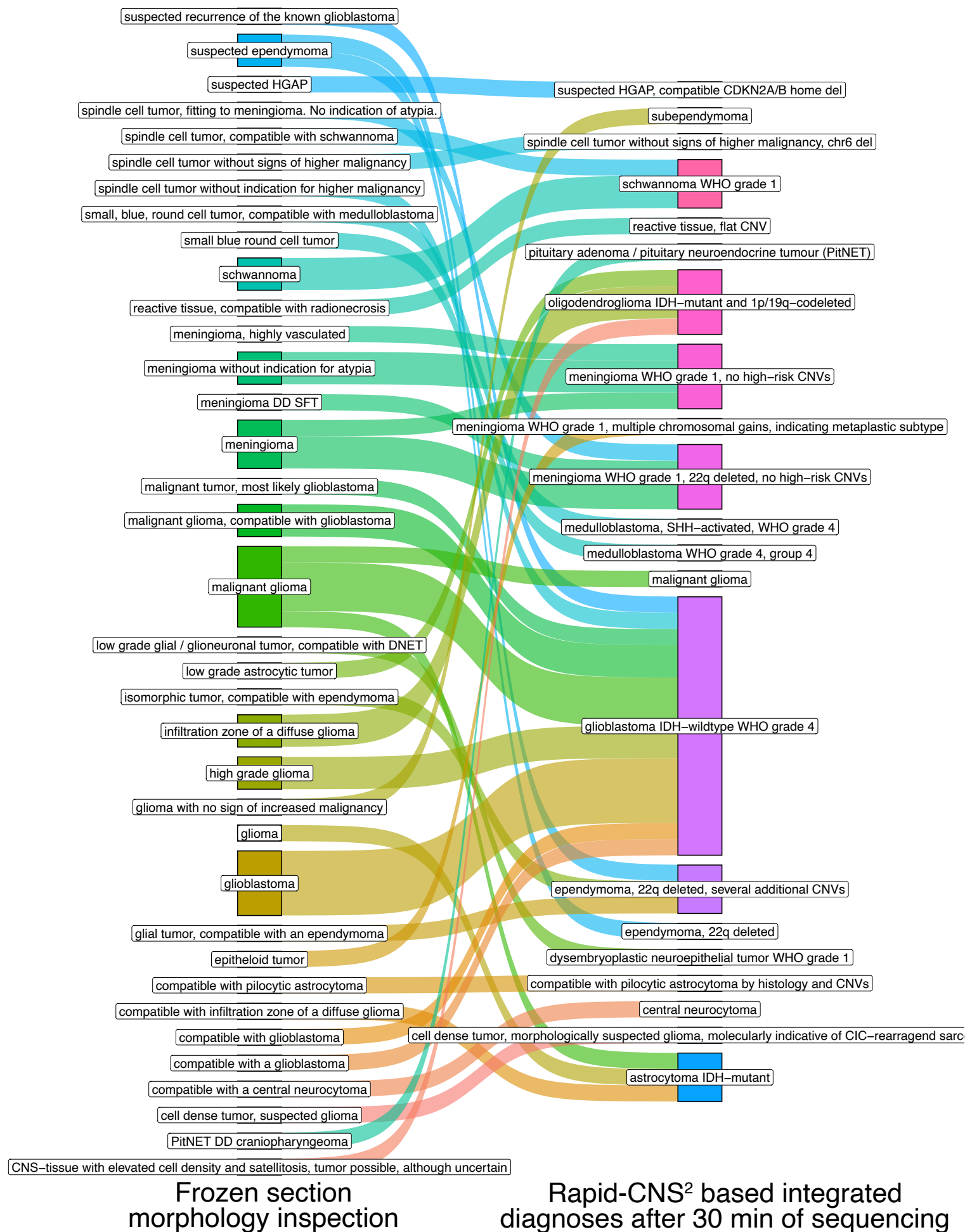


Supplementary Figure 8: A) MNP-Flex accuracy for different technologies for array samples with scores >0.7 ($n = 58,416$) and non-array samples >0.3 ($n = 339$). Error bars represent the 95% confidence intervals calculated using a binomial proportion confidence interval via the `binom.confint` function in R. Solid coloured bars indicate subclass level accuracy and bars with increased alpha indicate family level accuracy. B) Confusion matrix for non-methylation array dataset with matched array data for MNP-Flex, only samples with prediction score >0.3 are shown ($n=339$). Colour indicates percentage. C) Sankey plot for low-confidence samples i.e. with MNP-Flex score <0.3 ($n=159$)





Supplementary Figure 9: A) MNP-Flex prediction scores over tissue preparation types were compared using a Wilcoxon TOST (two one-sided test) test with equivalence bounds ± 0.01 . The prediction scores for FFPE ($n=48,621$) vs frozen ($n=30,378$) tissue had a detectable difference (NHST: $p = 8.97e-03$), but it was small enough to be considered negligible (TOST: $p = 2.04e-118$). B) Comparison between R9 ($n=95$) and R10 ($n=116$) flowcells on a GridION. (left) Boxplots indicate a statistically significant increase in prediction scores for MNP-Flex with R10 flowcells (unpaired two-sided Wilcoxon rank sum test) despite (right) number of missing sites not being significantly different (unpaired two-sided Wilcoxon rank sum test). Box plots illustrate the median (horizontal line), with the box boundaries indicating the 25th and 75th percentiles (interquartile range). Whiskers extend up to 1.5 times the interquartile range beyond the box edges. Data points falling outside these limits are plotted individually as outliers.



Supplementary Figure 10: We simulated an intraoperative setting for 51 prospective samples sequenced from February to May 2024 at University Hospital Heidelberg. Sankey plot compares frozen section morphology inspection to integrated diagnoses issued using methylation class and CNV profiles obtained from Rapid-CNS² within 30 minutes of sequencing.