Supplementary Material

Systematic benchmarking of tools for CpG methylation detection from Nanopore sequencing

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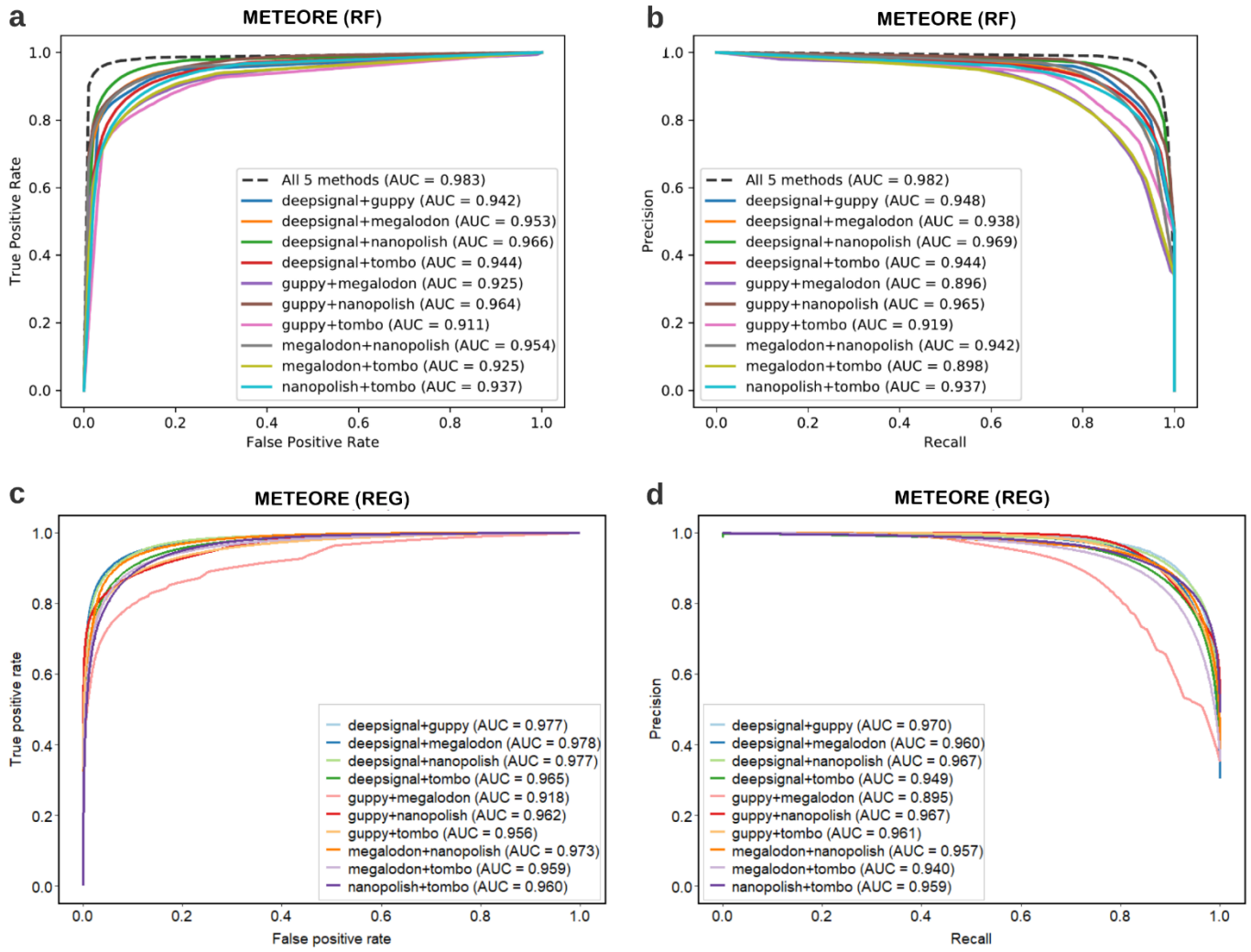
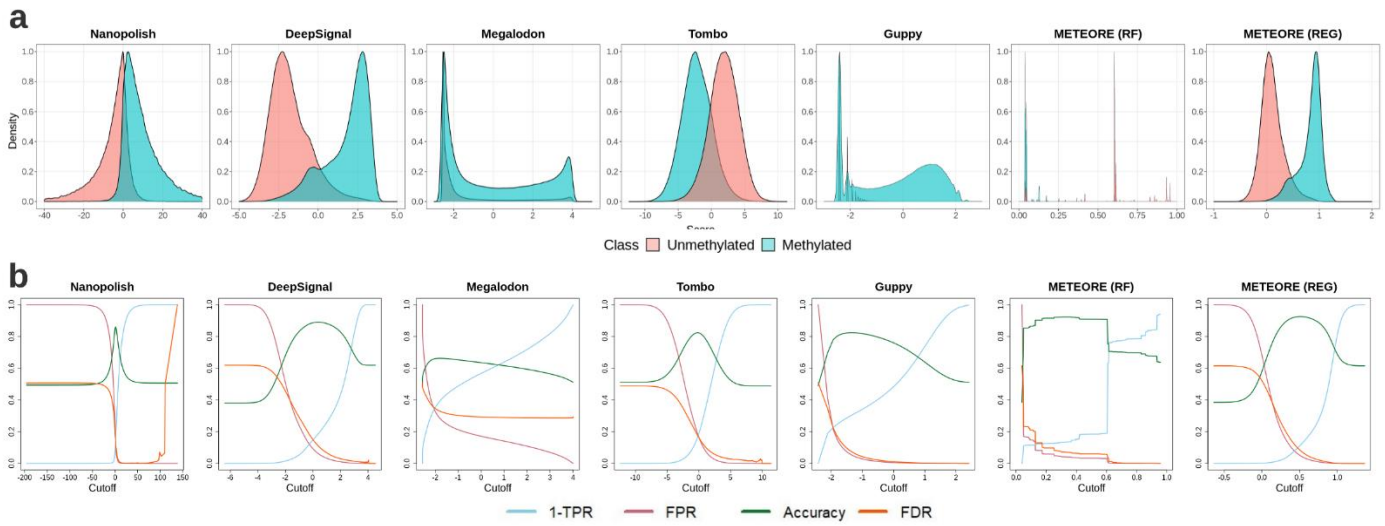
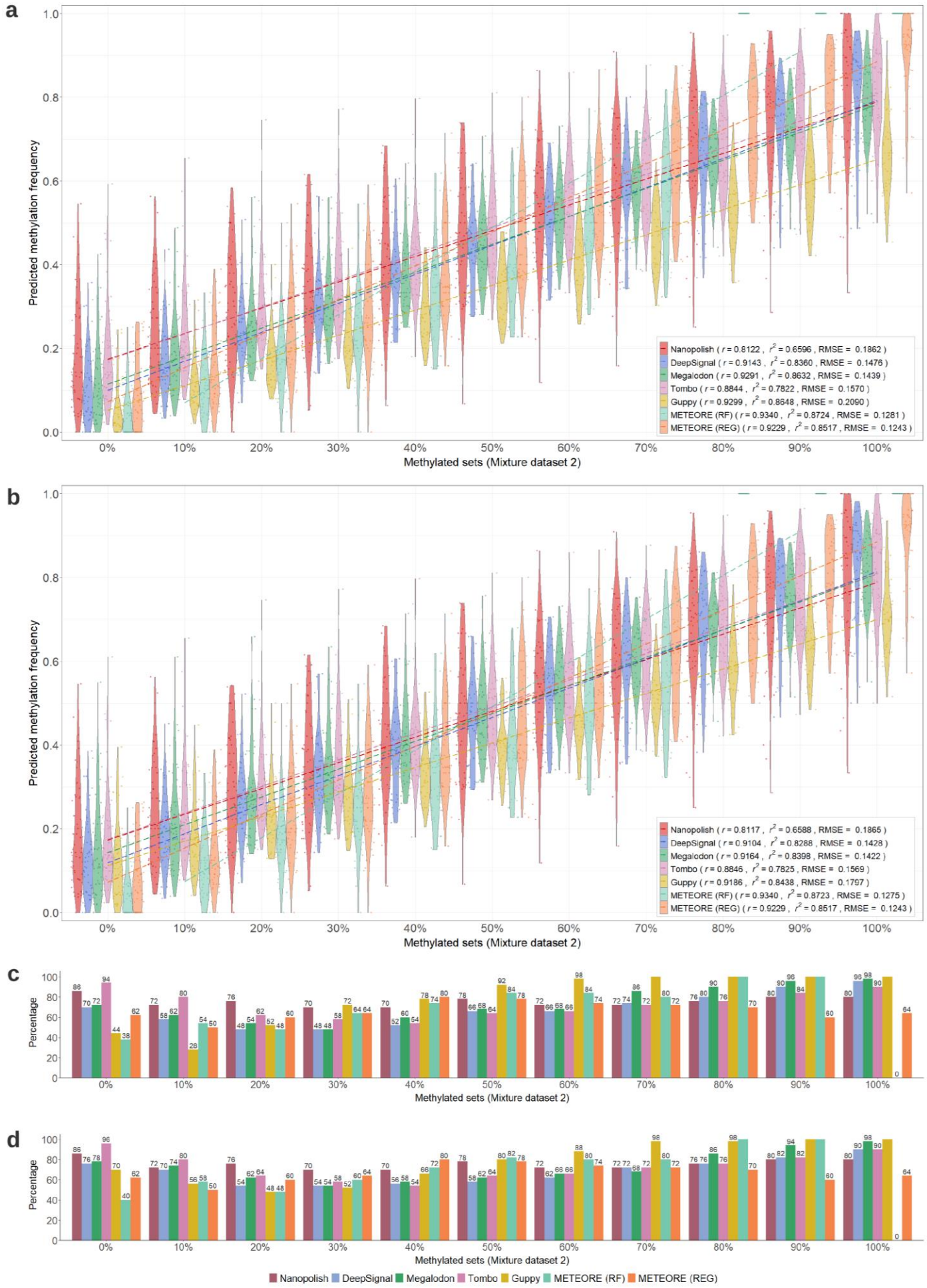


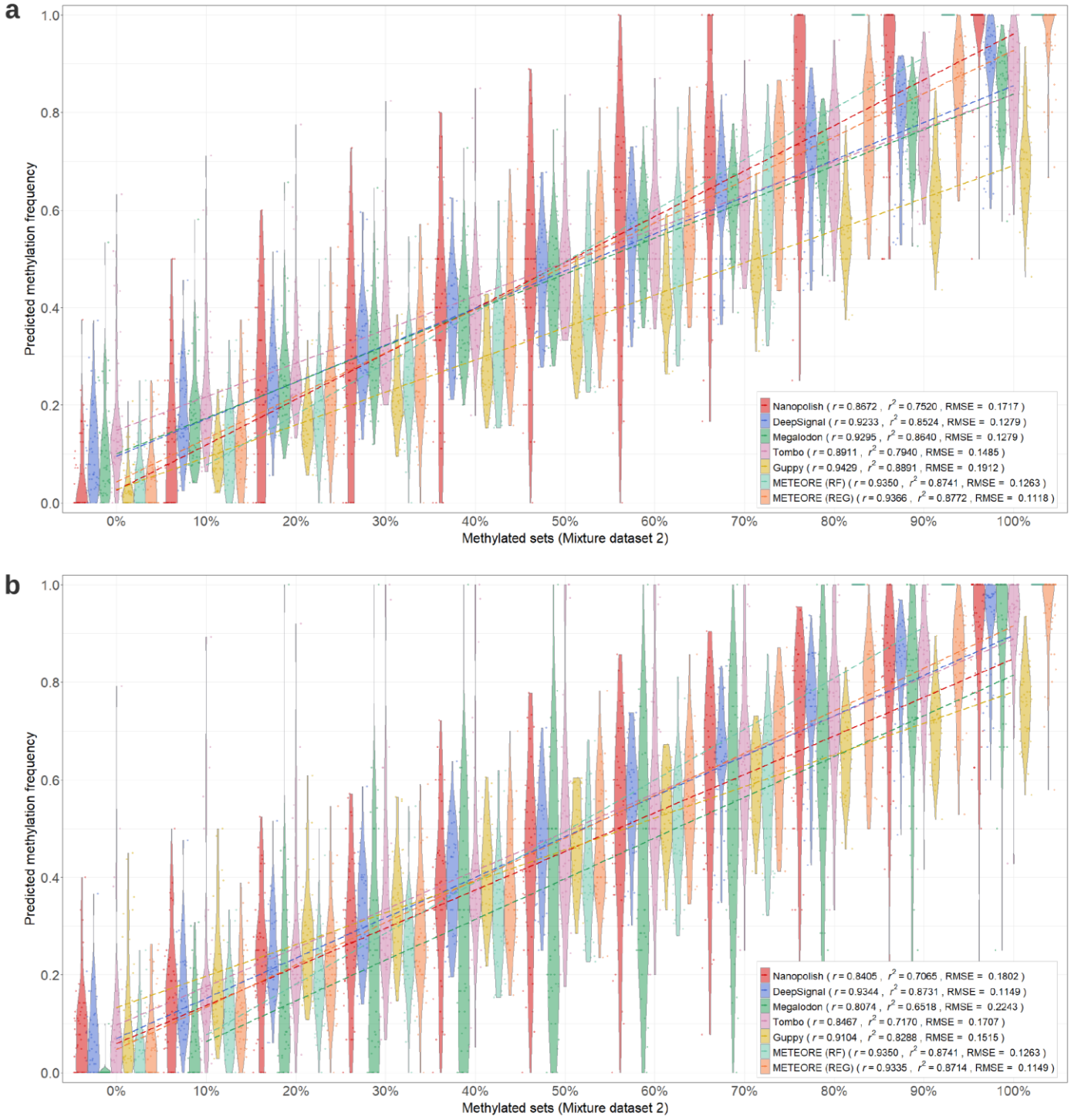
Supplementary Figure 1. Model accuracy for METEORE at the individual read level. (a) Receiver operating characteristic (ROC) curves showing the false positive rate (x axis) and true positive rate (y axis) for the predictions of the METEORE random forest (RF) model combining two methods (with default parameters: n_estimator = 10 and max_depth = None) using the sites of the mixture dataset 1 at individual read level. The curves were built from the average of a 10-fold cross validation. **(b)** Precision-recall (PR) curves showing the recall (x axis) and precision (y axis) for the RF model using the sites of the mixture dataset 1 at individual read level, also built from 10-fold cross validation. **(c)** ROC curves for the METEORE regression (REG) model combining two methods at individual read level. Curves were built from a 5-fold cross validation using the sites of mixture dataset 1. **(d)** PR curves for the METEORE REG model using the sites of the mixture dataset 1 at individual read level, built from a 5-fold cross validation.

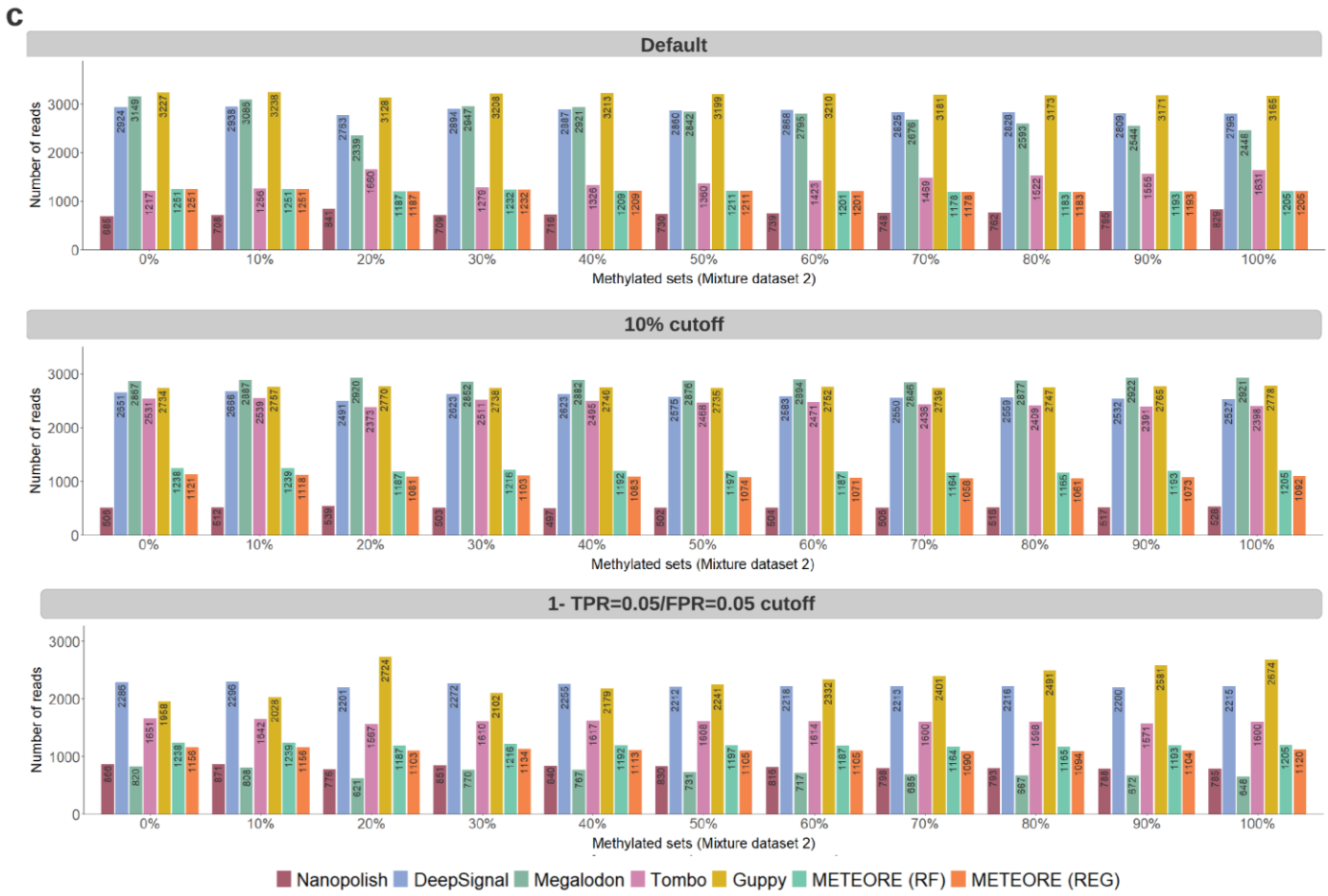


Supplementary Figure 2. Score distributions and accuracy metrics. (a) Score (x axis) distribution for methylated and unmethylated sites in individual reads for each tested tool on the mixture dataset 1, including METEORE. We used METEORE combining the predictions of Nanopolish and DeepSignal with a random forest (RF) (parameters: max_dep=3 and n_estimator=10) and with a regression model (REG). **(b)** Distribution of various accuracy metrics (y axis) according to the score (x axis) for each method shown in (a). We show the false positive rate (FPR), false discovery rate (FDR), 1 - true positive rate (TPR), and Accuracy curves as a function of the single score (x axis) cutoff for each tool, where FPR = FP/(FP+TN), TPR = TP/(TP+FN), FDR = FP/(TP+FP), accuracy = (TP + TN)/(TP + TN + FP + FN); and TP = true positives, FP = false positives, TN = true negatives, and FN = false negatives.

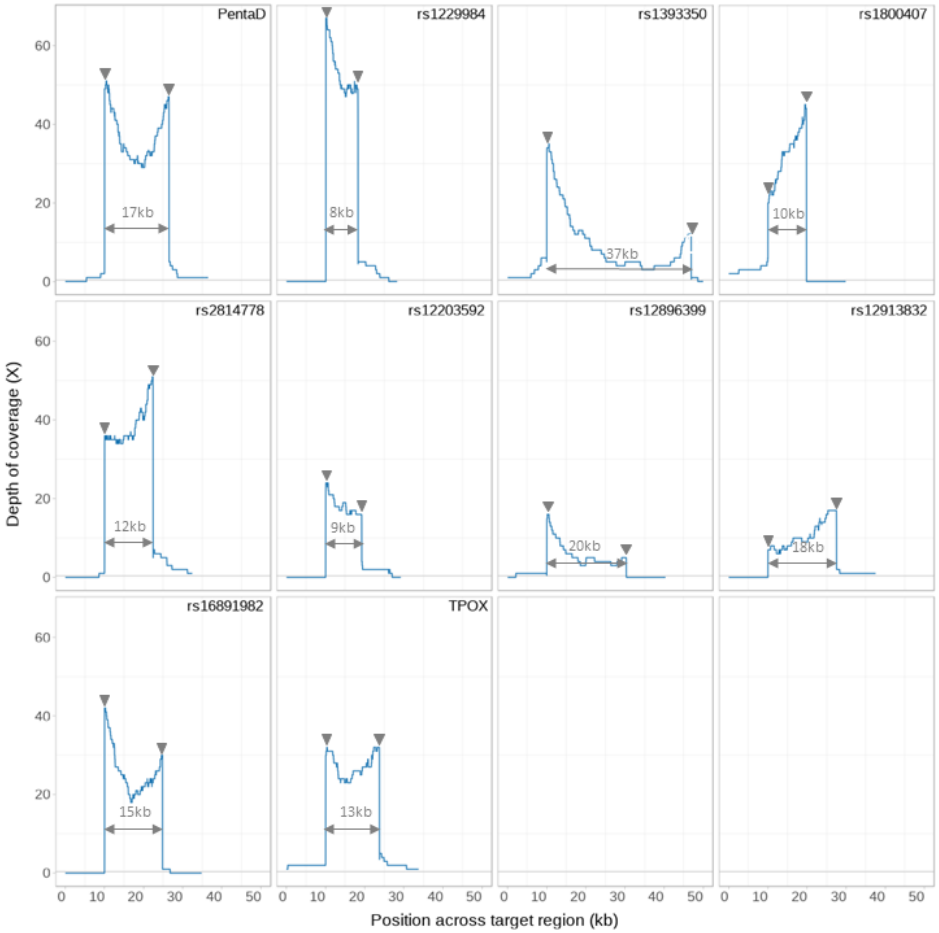

a
Predicted methylation frequency
Methylated sets (Mixture dataset 2)
Nanopolish (r = 0.8122 , r² = 0.6596 , RMSE = 0.1862)
DeepSignal (r = 0.9143 , r² = 0.8360 , RMSE = 0.1476)
Megalodon (r = 0.9291 , r² = 0.8632 , RMSE = 0.1439)
Tombo (r = 0.8844 , r² = 0.7822 , RMSE = 0.1570)
Guppy (r = 0.9299 , r² = 0.8648 , RMSE = 0.2090)
METEORE (RF) (r = 0.9340 , r² = 0.8724 , RMSE = 0.1281)
METEORE (REG) (r = 0.9229 , r² = 0.8517 , RMSE = 0.1243)
b
Predicted methylation frequency
Methylated sets (Mixture dataset 2)
Nanopolish (r = 0.8117 , r² = 0.6588 , RMSE = 0.1865)
DeepSignal (r = 0.9104 , r² = 0.8288 , RMSE = 0.1428)
Megalodon (r = 0.9164 , r² = 0.8398 , RMSE = 0.1422)
Tombo (r = 0.8846 , r² = 0.7825 , RMSE = 0.1569)
Guppy (r = 0.9186 , r² = 0.8438 , RMSE = 0.1797)
METEORE (RF) (r = 0.9340 , r² = 0.8723 , RMSE = 0.1275)
METEORE (REG) (r = 0.9229 , r² = 0.8517 , RMSE = 0.1243)
c
Percentage
Methylated sets (Mixture dataset 2)
d
Percentage
Methylated sets (Mixture dataset 2)
Nanopolish DeepSignal Megalodon Tombo Guppy METEORE (RF) METEORE (REG)

Supplementary Figure 3. Accuracy analysis of five individual tested tools using a single optimal cutoff. Violin plots showing the predicted methylation frequencies (y axis) for each control mixture set with a given proportion of methylated reads (x axis) from the mixture dataset 2 for the five tested tools plus METEORE combining Nanopolish and DeepSignal using random forest (RF) and regression (REG) models with the optimal threshold obtained by **(a)** the maximum value of **(**TPR-FPR) or **(b)** the minimum value of $FPR^2 + (1-TPR)^2$. Score thresholds are given in Supp. Table 3. The Pearson's correlation (r), coefficient of determination (r^2) and the root mean square error (RMSE) are given for each tool. **(c)** Barplot showing the proportion of sites predicted outside a 10% window around the expected methylation proportion for each method with the optimal threshold obtained by the maximum value of **(**TPR-FPR). Each predicted site in the m% dataset was classified as "outside" if its predicted percentage methylation was outside the interval [(m-5)%, (m+5)%] for intermediate methylation values, or outside the intervals [0,5%] or [95%,100%] for the fully unmethylated or fully methylated sets, respectively. **(d)** Barplot showing the proportion of sites predicted outside a 10% window around the expected methylation proportion for each method with the optimal threshold obtained by the minimum value of $FPR^2 + (1-TPR)^2$. TPR = true positive rate, FPR = false positive rate.

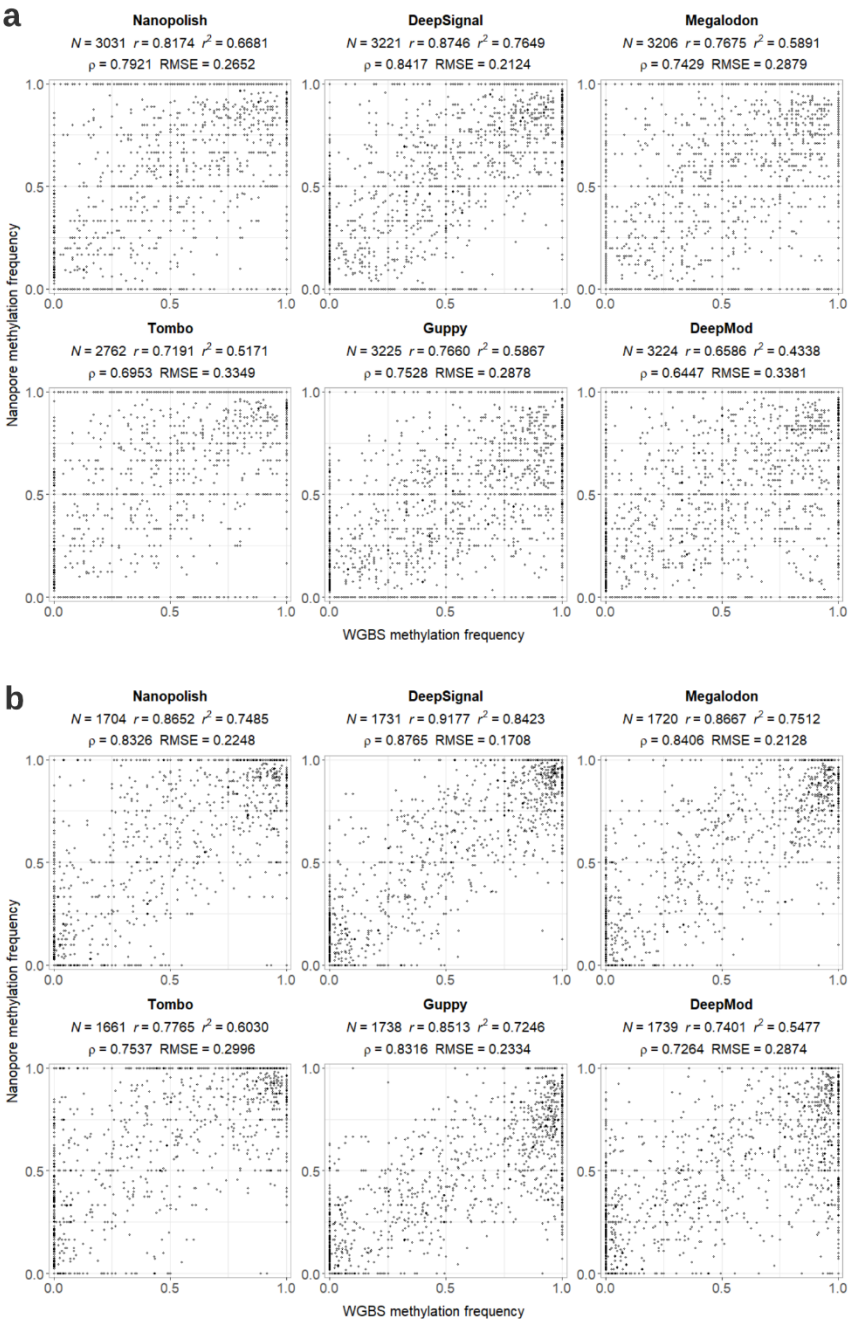

a
Predicted methylation frequency
1.0
0.8
0.6
0.4
0.2
0.0
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Methylated sets (Mixture dataset 2)
Nanopolish (r = 0.8672 , r² = 0.7520 , RMSE = 0.1717)
DeepSignal (r = 0.9233 , r² = 0.8524 , RMSE = 0.1279)
Megalodon (r = 0.9295 , r² = 0.8640 , RMSE = 0.1279)
Tombo (r = 0.8911 , r² = 0.7940 , RMSE = 0.1485)
Guppy (r = 0.9429 , r² = 0.8891 , RMSE = 0.1912)
METEORE (RF) (r = 0.9350 , r² = 0.8741 , RMSE = 0.1263)
METEORE (REG) (r = 0.9366 , r² = 0.8772 , RMSE = 0.1118)
b
Predicted methylation frequency
1.0
0.8
0.6
0.4
0.2
0.0
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Methylated sets (Mixture dataset 2)
Nanopolish (r = 0.8405 , r² = 0.7065 , RMSE = 0.1802)
DeepSignal (r = 0.9344 , r² = 0.8731 , RMSE = 0.1149)
Megalodon (r = 0.8074 , r² = 0.6518 , RMSE = 0.2243)
Tombo (r = 0.8467 , r² = 0.7170 , RMSE = 0.1707)
Guppy (r = 0.9104 , r² = 0.8288 , RMSE = 0.1515)
METEORE (RF) (r = 0.9350 , r² = 0.8741 , RMSE = 0.1263)
METEORE (REG) (r = 0.9335 , r² = 0.8714 , RMSE = 0.1149)



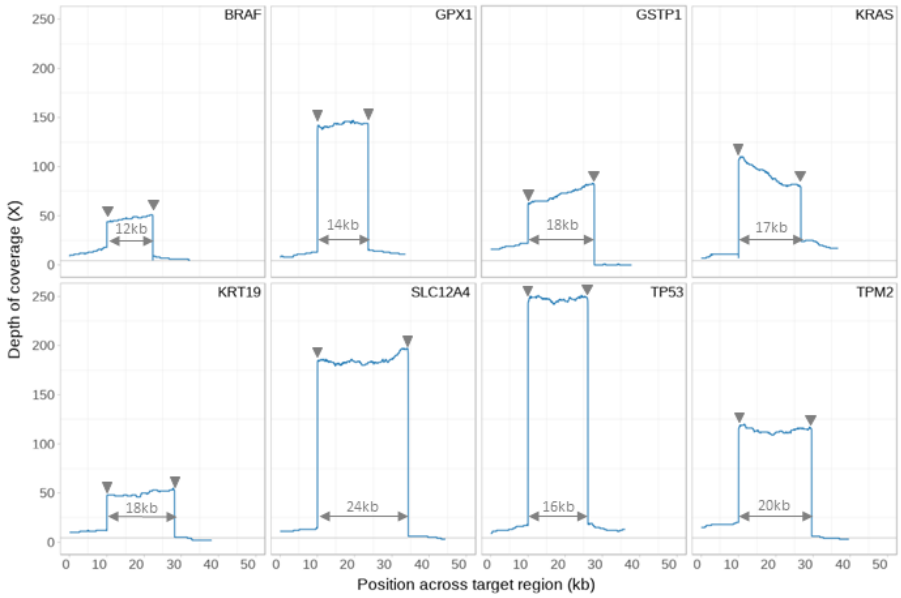
Supplementary Figure 4. Accuracy analysis using a double cutoff and discarding reads. (a) Violin plots showing the predicted methylation frequencies (y axis) for each control mixture set with a given proportion of methylated reads (x axis) from the mixture dataset 2 for the five tested tools plus METEORE combining Nanopolish and DeepSignal using random forest (RF) and regression (REG) models, after discarding 10% of the reads with a score closest to the value corresponding to the intersection between FPR and 1-TPR. See Supp. Table 4 for the cutoff values. The Pearson's correlation (r), coefficient of determination (r^2) and the root mean square error (RMSE) are given for each tool. **(b)** Similar plot as (a) but considering the scores at which FPR=0.05 and 1-TPR=0.05 and removing all sites in reads with a score between these two values. See Supp. Table 4 for the cutoff values. **(c)** Number of reads used by each method in different approach: default setting of each method (top), the use of a double cutoff to remove 20% of the reads (middle) and the use of a double cutoff to remove reads with the scores between the cutoff values at FPR=0.05 and 1-TPR=0.05 (bottom).



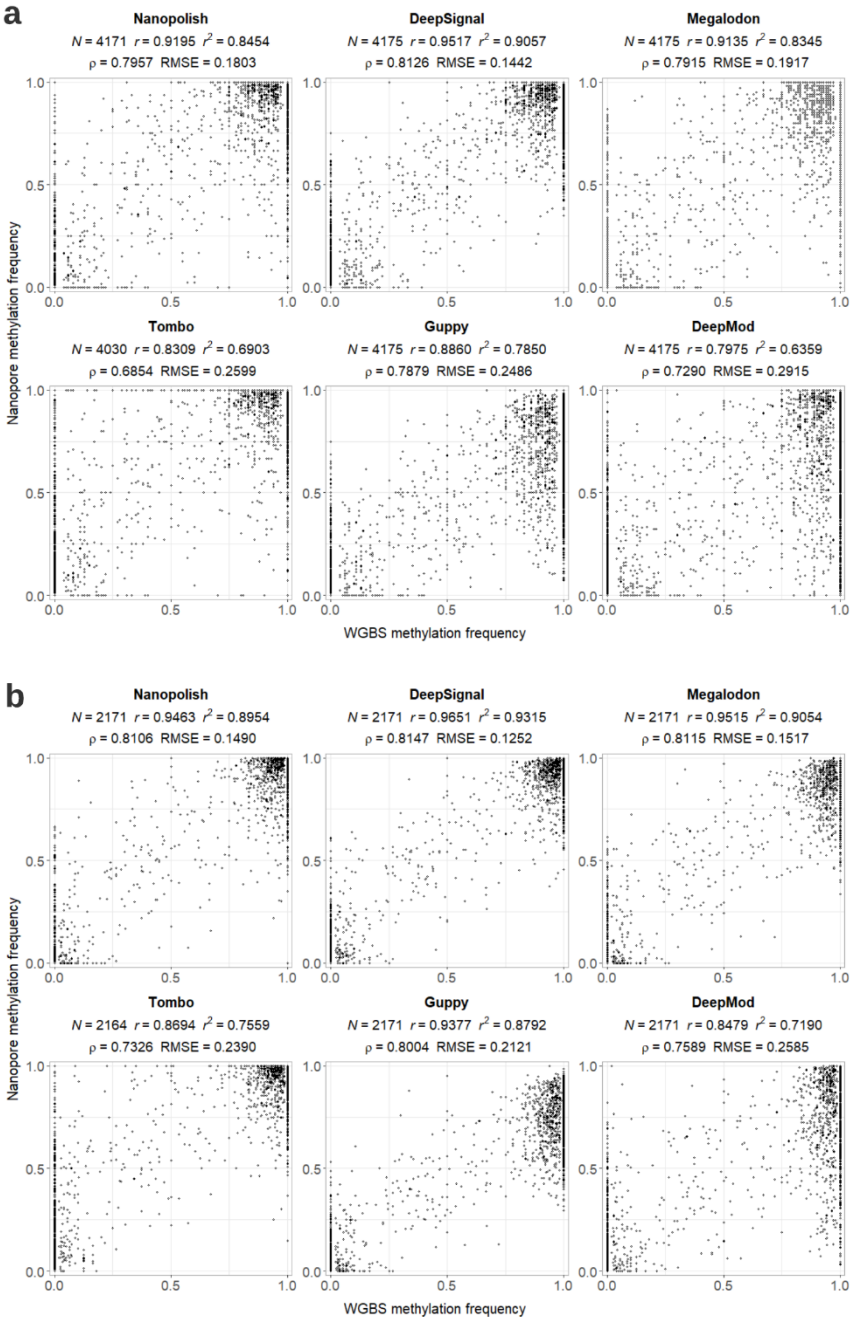
Supplementary Figure 5. Coverage plots for the 10 regions targeted with the nCATS protocol. (a) For each of our 10 sequenced regions (Supp. Table 4) we show the number of reads (y axis) aligning at each position along the region (x axis). The boundaries and length of each region are also indicated. For the coverage, reads mapped in forward and reverse were considered.



Supplementary Figure 6. Comparison of the nCATS data with whole genome bisulfite sequencing (WGBS). For each tool, we show the methylation fraction predicted by each tool (y axis) and the fraction calculated from WGBS (x axis), using either **(a)** individual predictions on both strands or **(b)** combined predictions from both strands. The number of sites (N), the Pearson's correlation (r), coefficient of determination (r2), the Spearman's rank correlation (ρ), and the root mean square error (RMSE) are provided for each tested tool.

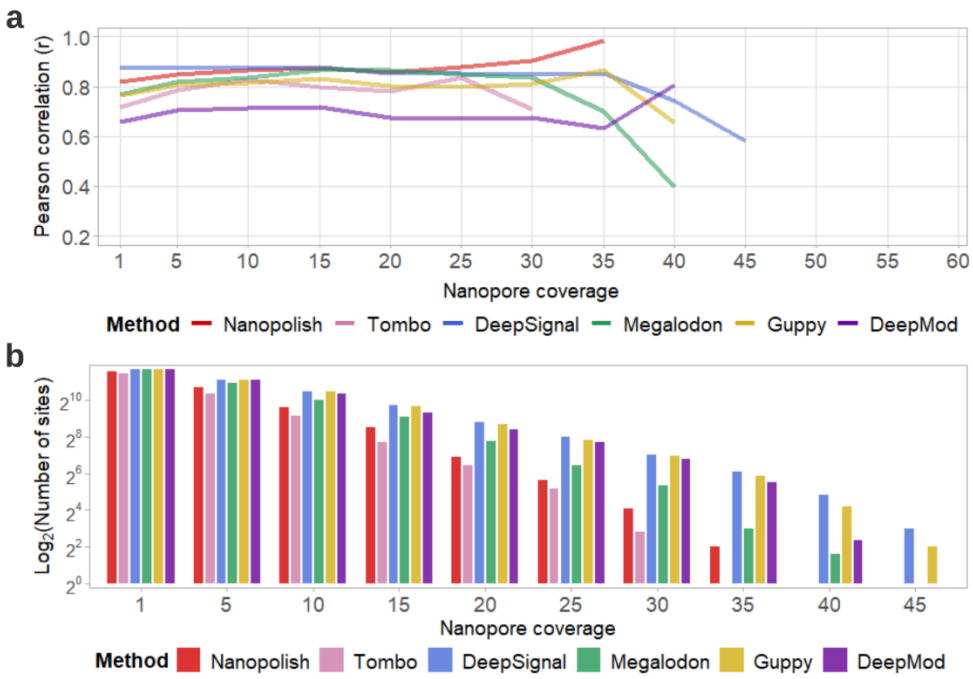


Supplementary Figure 7. Coverage plots for the regions targeted with the nCATS protocol from Gilpatrick et al. 2020. For each of the 8 regions tested in Gilpatrick et al. (2020), we show the number of reads (y axis) aligning at each position along the region (x axis). The boundaries and length of each region are also indicated. For the coverage, reads mapped in forward and reverse were considered.

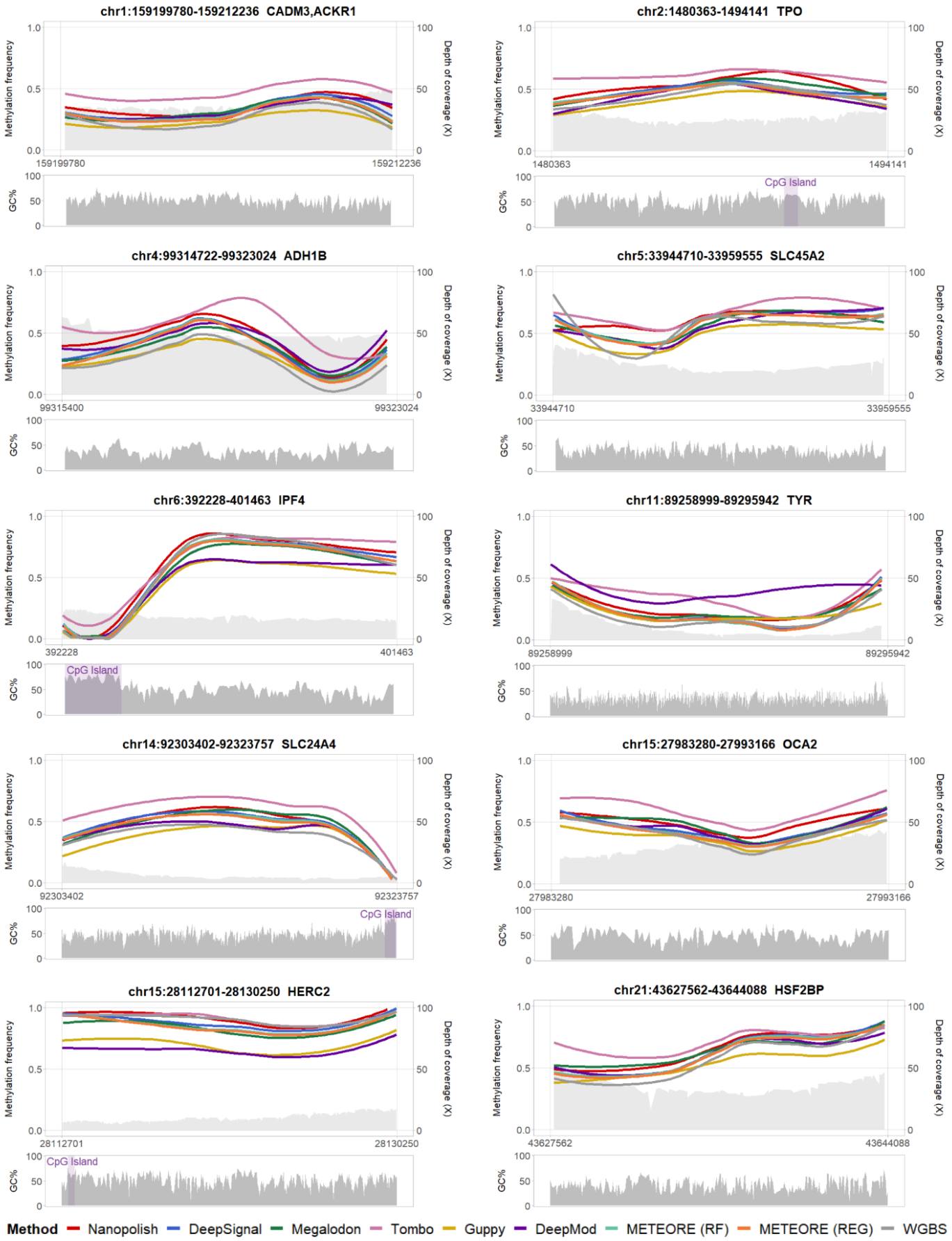


Supplementary Figure 8. Comparison of the nCATS data from Gilpatrick et al. with whole genome bisulfite sequencing (WGBS) data. For each tool, we show the methylation fraction predicted by each tool (y axis) and the fraction calculated from WGBS (x axis), using either **(a)** individual predictions on both strands or **(b)** combined predictions from both strands. The number of sites (N), the Pearson's correlation (r), coefficient of determination (r2), the Spearman's rank correlation (ρ), and the root mean square error (RMSE) are provided for each tested tool.

Supplementary Figure 9. Comparison of CpG methylation frequencies from whole genome bisulfite sequencing (WGBS) Illumina data with Cas9-targeted Nanopore data from Gilpatrick et al. (a) Distribution of nanopore methylation calls across three WGBS methylation bins unmethylated or lowly methylated (0.0-0.3), intermediate methylation (0.3-0.7), and highly or fully methylated (0.7-1.0). We show the seven tested tools: Nanopolish, DeepSignal, Megalodon, Tombo, Guppy, DeepMod, and METEORE. For METEORE, we used the combination of Nanopolish and DeepSignal using either a random forest model (RF) or a regression model (REG). **(b)** Pearson's correlation (r) (y axis) between nanopore methylation frequencies calculated from Nanopore by each of the tested tools and WGBS at sites with predictions from both strands combined at each level of minimal coverage, i.e. minimum number of Nanopore reads considered (x axis). **(c)** Number of sites on a logarithmic scale (y axis) considered at each value of minimum coverage in (b). METEORE (RF) is the combination of DeepSignal and Nanopolish using a random forest (parameters: max_depth=3 and n_estimator=10). METEORE (REG) is the combination of DeepSignal and Nanopolish using a regression model.

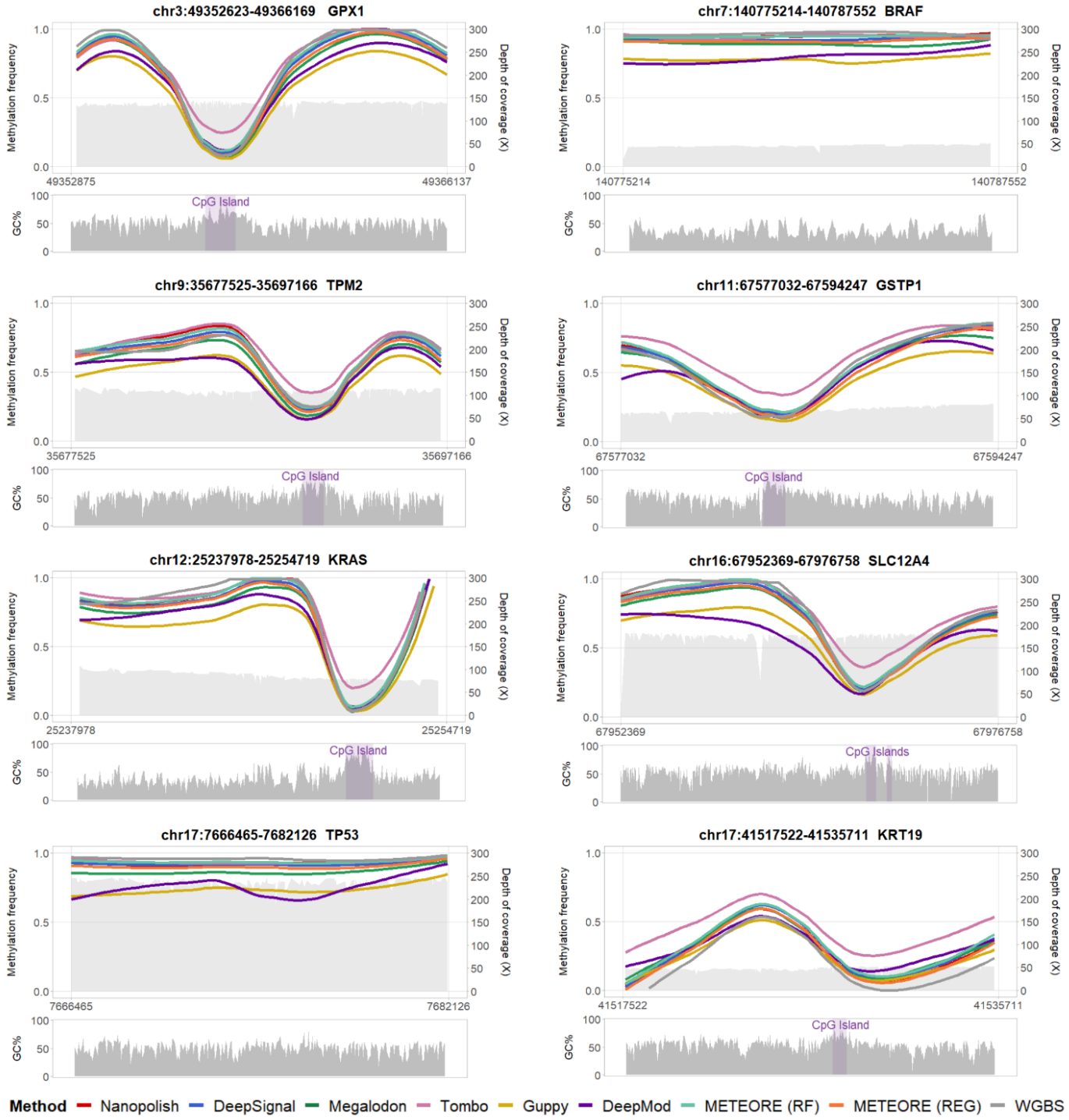


Supplementary Figure 10. (a) Pearson's correlation (r) (y axis) between nanopore methylation frequencies calculated from Nanopore by each of the tested tools and WGBS at individual sites at each level of minimal coverage, i.e. minimum number of Nanopore reads considered (x axis). **(b)** Number of sites on a logarithmic scale (y axis) considered at each value of minimum coverage in (b). METEORE is not included since it performs prediction only combining both strands.



Supplementary Figure 11. Comparison of CpG methylation frequencies from WGBS and Nanopore across our 10 targeted regions. LOESS smoothing line plots of methylation calls from WGBS Illumina and Nanopore data detected by the seven tested tools: Nanopolish, DeepSignal, Megalodon, Tombo, Guppy, DeepMod, and METEORE. We show METEORE with the combination of Nanopolish and DeepSignal using

either a random forest model (RF) or a regression model (REG). The plots include the Nanopore coverage, shown as a light grey area. Below, we include the GC content of the region.



Supplementary Figure 12. Comparison of CpG methylation frequencies from WGBS and Nanopore across the 8 regions tested in Gilpatrick et al. LOESS smoothing line plots of methylation calls from WGBS Illumina and Nanopore data detected by the seven tested tools: Nanopolish, DeepSignal, Megalodon, Tombo, Guppy, DeepMod, and METEORE. We show METEORE with the combination of Nanopolish and DeepSignal using either a random forest model (RF) or a regression model (REG). The plots include the Nanopore coverage, shown as a light grey area. Below, we include the GC content of the region.

a

Set name	% methylated	Total reads	Unmethylated reads (PCR)	Methylated reads (PCR+M.Sssl)
m0	0	2390	2390	0
m10	10	2437	2182	255
m20	20	2431	1946	485
m30	30	2434	1714	720
m40	40	2410	1458	952
m50	50	2432	1231	1201
m60	60	2414	981	1433
m70	70	2420	739	1681
m80	80	2410	498	1912
m90	90	2399	256	2143
m100	100	2225	0	2225

b

Set name	% methylated	Total reads	Unmethylated reads (PCR)	Methylated reads (PCR+M.Sssl)
m0	0	3420	3420	0
m10	10	3426	3081	345
m20	20	3413	2745	668
m30	30	3423	2410	1013
m40	40	3415	2068	1347
m50	50	3434	1736	1698
m60	60	3403	1377	2026
m70	70	3406	1039	2367
m80	80	3398	690	2708
m90	90	3396	354	3042
m100	100	3383	0	3383

Supplementary Table 1. Methylation control mixtures. We describe the mixture dataset 1 **(a)** and 2 **(b)** used for the benchmarking of different methylation proportions built from fully unmethylated and fully methylated reads.

Unmethylated if freq < 0.1, methylated if freq > 0.9					
	Accuracy	Specificity	Precision	Recall	Error rate
Nanopolish	0.675	0.490	0.628	0.860	0.325
DeepSignal	0.410	0.590	0.359	0.230	0.590
Megalodon	0.500	1.000	NA	0.000	0.500
Tombo	0.588	0.384	0.564	0.790	0.412
Guppy	0.500	1.000	NA	0.000	0.500
DeepMod	0.495	0.680	0.492	0.310	0.505

Unmethylated if freq < 0.2, methylated if freq > 0.8					
	Accuracy	Specificity	Precision	Recall	Error rate
Nanopolish	0.860	0.800	0.821	0.920	0.140
DeepSignal	0.805	0.910	0.886	0.700	0.195
Megalodon	0.510	1.000	1.000	0.020	0.490
Tombo	0.794	0.616	0.719	0.970	0.206
Guppy	0.500	1.000	NA	0.000	0.500
DeepMod	0.790	0.970	0.953	0.610	0.210

Unmethylated if freq < 0.3, methylated if freq > 0.7					
	Accuracy	Specificity	Precision	Recall	Error rate
Nanopolish	0.935	0.900	0.907	0.970	0.065
DeepSignal	0.930	1.000	1.000	0.860	0.070
Megalodon	0.600	1.000	1.000	0.200	0.400
Tombo	0.839	0.697	0.766	0.980	0.161
Guppy	0.505	1.000	1.000	0.010	0.495
DeepMod	0.935	1.000	1.000	0.870	0.065

Unmethylated if freq < 0.4, methylated if freq > 0.6					
	Accuracy	Specificity	Precision	Recall	Error rate
Nanopolish	0.955	0.930	0.933	0.980	0.045
DeepSignal	0.975	1.000	1.000	0.950	0.025
Megalodon	0.755	1.000	1.000	0.510	0.245
Tombo	0.884	0.778	0.818	0.990	0.116
Guppy	0.600	1.000	1.000	0.200	0.400
DeepMod	0.975	1.000	1.000	0.950	0.025

Unmethylated if freq < 0.5, methylated if freq > 0.5					
	Accuracy	Specificity	Precision	Recall	Error rate
Nanopolish	0.975	0.970	0.970	0.980	0.025
DeepSignal	0.990	1.000	1.000	0.980	0.010
Megalodon	0.880	1.000	1.000	0.760	0.120
Tombo	0.915	0.838	0.861	0.990	0.085
Guppy	0.720	1.000	1.000	0.440	0.280
DeepMod	0.980	1.000	1.000	0.960	0.020

Supplementary Table 2. Per-site performance. The table shows the accuracies in fully methylated or fully unmethylated CpG sites for the six tested tools using two different methylation frequency thresholds to classify methylated and unmethylated sites. For each pair of thresholds (a,b), we defined a site to be unmethylated if the predicted methylation frequency was <a, and methylated if the predicted methylation frequency was >b, for (a,b) = (0.1,0.9), (0.2,0.8), (0.3,0.7), (0.4,0.6), and (0.5,0.5).

	Nanopolish	DeepSignal	Megalodon	Tombo	Guppy	METEORE (RF)	METEORE (REG)
Maximum of (TPR –FPR)	Cutoff= 1.03 TPR =0.87 FPR=0.15	Cutoff= -0.05 TPR =0.86 FPR=0.10	Cutoff= -1.86 TPR =0.61 FPR=0.30	Cutoff= -0.13 TPR =0.83 FPR=0.18	Cutoff= -1.35 TPR =0.69 FPR=0.05	Cutoff= 0.32 TPR =0.87 FPR=0.04	Cutoff= 0.43 TPR =0.92 FPR=0.08
Minimum of $(FPR-0)^2 + (TPR-1)^2$	Cutoff= 1.04 TPR=0.87 FPR=0.15	Cutoff= -0.19 TPR =0.87 FPR=0.11	Cutoff= -1.97 TPR =0.64 FPR=0.32	Cutoff= -0.11 TPR =0.82 FPR=0.18	Cutoff= -1.70 TPR =0.73 FPR=0.12	Cutoff= 0.25 TPR =0.87 FPR=0.05	Cutoff= 0.43 TPR =0.92 FPR=0.08

Supp. Table 3. Optimal single score cutoffs obtained by maximising the value of TPR-FPR (first row) or by minimizing the value of $(FPR-0)^2 + (TPR-1)^2$ (second row). In both optimization we used all reads from the mixture dataset 1. These optimal cutoffs were applied to the per-read data generated by each tool. For all these tools except for Tombo, if a read with a score above the optimal cutoff, we consider it as methylated, and unmethylated for the scores below the cutoff. For Tombo, a read is considered methylated if its score is below the optimal cutoff, and unmethylated for a score above the cutoff. For METEORE REG, both strategies led to exactly the same cutoffs.

	Nanopolish	DeepSignal	Megalodon	Tombo	Guppy	METEORE (RF)	METEORE (REG)
Remove **10%** of reads around the cross point of FPR and 1-TPR curves	(-3.58 ,5.80)	(-0.60, 0.09)	(-2.14, -1.66)	(-0.54,0.34)	(-1.93, -0.91)	(0.12,0.20)	(0.33,0.53)
Remove the reads that fall between the score at **1-TPR=0.05** and **FPR=0.05**	(-0.65, 3.53)	(-1.07, 0.60)	(-2.56, 3.39)	(-1.82, 1.66)	(-2.41, -1.31)	(0.05,0.25)	(0.36,0.51)

Supp. Table 4. Double score cutoffs obtained by removing 10% of reads around the intersection point of the FPR curve and 1-TPR curve (first row) or removing the cases that fall between the score at 1- TPR = 0.05 and FPR = 0.05 (second row). In the first optimization we used all reads from the mixture dataset 1. In the second optimization we used the fully methylated and fully unmethylated sets from mixture dataset 1. For each double cutoff (a,b), all sites in reads with score < a are considered unmethylated, with score > b are considered methylated, and all cases between these values are discarded. For Tombo the score scale has the opposite orientation, i.e. a read is considered methylated if its score is < a, and unmethylated for a score > b. METEORE (RF) is the combination of DeepSignal and Nanopolish using a random forest (parameters: max_depth=3 and n_estimator=10). METEORE (REG) is the combination of DeepSignal and Nanopolish using a regression model. In the REG model, filtering is symmetric by the ranking of scores about the tipping point, i.e. 5% of reads with scores lower than the tipping point and 5% of reads with scores higher than the tipping point.

Chromosome	Start position	End position	Size (nt)	Target locus	Type of variants	Associated gene(s)
chr1	159199780	159212236	12456	rs2814778	aiSNP	CADM3, ACKR1
chr2	1480363	1494141	13778	TPOX	STR	TPO
chr4	99314722	99323024	8302	rs1229984	aiSNP	ADH1B
chr5	33944710	33959555	14845	rs16891982	piSNP	SLC45A2
chr6	392228	401463	9235	rs12203592	piSNP	IPF4
chr11	89258999	89295942	36943	rs1393350	piSNP	TYR
chr14	92303402	92323757	20355	rs12896399	piSNP	SLC24A4
chr15	27983280	27993166	9886	rs1800407	piSNP	OCA2
chr15	28112701	28130250	17549	rs12913832	piSNP	HERC2
chr21	43627562	43644088	16526	PentaD	STR	HSF2BP

Supp. Table 5. Ten forensically relevant regions used for the nCATS protocol. The table provides the coordinates (GRCh38) of the ten regions used to sequence native DNA with the nCATS protocol. The table also indicates whether the region contains an ancestry-informative SNP (aiSNP), a phenotypic-informative SNP (piSNP), or a short tandem repeat (STR).

Target	Guide RNA sequence	PAM	Cleaved site
rs12913832	CTTGTTCTCAATCCAACGAG	CGG	chr15:28112701(+)
	GATCAGATGACCATGTTCGA	AGG	chr15:28130250(-)
rs1800407	GTAGAGCTCTAACTAAGTGG	AGG	chr15:27983280(+)
	TATCCAATCCTGCTGACCAG	TGG	chr15:27993166(-)
rs12896399	GCTGGAACGCCCCATCAACA	CGG	chr14:92303402(+)
	GAGTGCAATCAGTGGCCGAG	CGG	chr14:92323757(-)
rs16891982	TGTGATCACCACGACGACAA	CGG	chr5:33944710(+)
	GAGTGCAACGAGGAACTAAG	AGG	chr5:33959555(-)
rs1393350	TCCTTGCTGCACGAATCAGT	GGG	ch11:89258999(+)
	GCTGGATGTGTTATAGACGC	TGG	chr11:89295942(-)
rs12203592	TAAGGGGCCCAAGCTCACGG	CGG	chr6:392228(+)
	ACGTGGTCAGCTCCTTCACG	AGG	chr6:401463(-)
TPOX	CGTATTTGAAAGATCCACGG	TGG	chr2:1480363(+)
	CTTACGTAAGAGTTGAATGG	TGG	chr2:1494141(-)
Penta D	CGGTACCTATCCCAGAACTA	TGG	chr21:43627562(+)
	TAACACGTAGATCATTCACT	TGG	chr21:43644088(-)
rs2814778	CCTACCACGCCATCATCGGT	GGG	chr1:159199780(+)
	GCAATTGTCTTTCAGTGCGT	TGG	chr1:159212236(-)
rs1229984	ACCATCTGCTAACACGTATG	AGG	chr4:99314772(+)
	GCGTTAACATATCTCCACAA	GGG	chr4:99323024(-)

Supplementary Table 6. Guide RNA (gRNA) panel used for the nCATs protocol. The table describe the ten pairs of gRNAs used to target the ten regions from Supp. Table 3. To enrich for each target region, two gRNAs were used to make a cut on each side, one upstream of the region of interest targeting the positive strand, and the other one downstream targeting the negative strand.

	N	*r*	r^2	*ρ*	*RMSE*
Nanopolish	2171	0.9463	0.8954	0.8106	0.1490
DeepSignal	2171	0.9651	0.9315	0.8147	0.1252
Megalodon	2171	0.9515	0.9054	0.8115	0.1517
Tombo	2164	0.8694	0.7559	0.7326	0.2390
Guppy	2171	0.9377	0.8792	0.8004	0.2121
DeepMod	2171	0.8479	0.7190	0.7589	0.2585
METEORE (RF)	2171	0.9657	0.9326	0.8161	0.1241
METEORE (REG)	2171	0.9637	0.9288	0.8132	0.1297

Supp. Table 7. Comparison of CpG methylation frequencies from whole genome bisulfite sequencing (WGBS) Illumina data with Cas9-targeted Nanopore data from Gilpatrick et al. 2020. For each tool we provide the number of sites (N), the Pearson's correlation (r), coefficient of determination (r^2), the Spearman's rank correlation (ρ), and the root mean square error (RMSE) for the comparison of the percentage methylation predicted from Nanopore with the percentage methylation calculated from whole genome bisulfite sequencing (WGBS) data. We show the results for five tested tools and METEORE combining DeepSignal and Nanopolish using a random forest (RF) (parameters: max_depth=3 and n_estimator=10) or a regression (REG) model.

	N	*r*	r^2	*ρ*	*RMSE*
Nanopolish	1724	0.8648	0.7478	0.8362	0.2171
DeepSignal	1731	0.9196	0.8456	0.8785	0.1693
Megalodon	1724	0.7294	0.5320	0.7704	0.3642
Tombo	1734	0.8037	0.6460	0.7871	0.2551
Guppy	1738	0.7706	0.5938	0.7741	0.2978
METEORE (RF)	1724	0.8998	0.8096	0.8568	0.1881
METEORE (REG)	1724	0.9183	0.8433	0.8771	0.1699

Supp. Table 8. Comparison of CpG methylation frequencies from whole genome bisulfite sequencing (WGBS) Illumina data with Cas9-targeted Nanopore data for each method using optimal thresholds obtained by the maximum value of (TPR-FPR). We used the score cutoffs that maximized TPR-FPR in the mixture dataset 1 (Supp. Table 3). For each method we provide the number of sites (N), the Pearson's correlation (r), coefficient of determination (r^2), the Spearman's rank correlation (ρ), and the root mean square error (RMSE) for the comparison of the percentage methylation predicted from Nanopore with the percentage methylation calculated from whole genome bisulfite sequencing (WGBS) data. We show the results for five tested tools and METEORE combining DeepSignal and Nanopolish using a random forest (RF) (parameters: max_depth=3 and n_estimator=10) or a regression (REG) model.

	N	*r*	r^2	*ρ*	*RMSE*
Nanopolish	1621	0.8726	0.7614	0.8327	0.2165
DeepSignal	1731	0.9220	0.8500	0.8805	0.1698
Megalodon	1721	0.6855	0.4699	0.7344	0.4021
Tombo	1733	0.8150	0.6642	0.7924	0.2468
Guppy	1733	0.7345	0.5394	0.7275	0.3243
METEORE (RF)	1575	0.0721	0.0052	0.0536	0.6496
METEORE (REG)	1724	0.9240	0.8537	0.8832	0.1661

Supp. Table 9. Comparison of CpG methylation frequencies from whole genome bisulfite sequencing (WGBS) Illumina data with Cas9-targeted Nanopore data for each method using the double cutoff obtained by discarding 10% of reads. For each site, we removed the 10% of reads with scores closest to the cross point of the FPR and 1-TPR curves in the mixture dataset 1 (Supp. Table 4). For each method we provide the number of sites (N), the Pearson's correlation (r), coefficient of determination (r^2), the Spearman's rank correlation (ρ), and the root mean square error (RMSE) for the comparison of the percentage methylation predicted from Nanopore with the percentage methylation calculated from whole genome bisulfite sequencing (WGBS) data. METEORE is the combination model with the adjusted parameters of a random forest (max_depth=3 and n_estimator=10) combining DeepSignal and Nanopolish.

	No. of CPUs used	Real time per CPU (min)	Peak memory (GB)	Bases per second
Nanopolish	2	10.2	0.2	25433
DeepSignal	9	334.8	23.7	775
Tombo	9	30.4	23.6	8533
Megalodon	11	3258.7	1.4	80
Megalodon (GPU)	1 GPU	4.0 per GPU	1.7	66516
Guppy	11	1494.6	3.3	174
Guppy (GPU)	1 GPU	7.0 per GPU	0.6	37596
DeepMod	8	176.0	2.5	1474

Supp. Table 10. Runtime and memory usage for each tested tool. We tested each pipeline on the m50 set (2,432 reads and a total of 15,564,827 bases) from the mixture dataset 1. We recorded the real time (wall clock time) from start to finish of the pipeline/command(s), which took a fast5 directory as an input and output a prediction at genome level (i.e. methylation frequency for each site). We ran Nanopolish, DeepSignal, Tombo, Megalodon, Guppy and DeepMod on a computer with 12 CPU processors (Intel Core i7 (8th Gen) 8700 @ 3.2 GHz). Guppy can be run on CPUs or GPUs, but on a GPU the basecalling speed increase significantly. Megalodon requires Guppy for the basecalling step and uses GPU-enabled Guppy by default. If Megalodon is used with Guppy (CPU), the --guppy-timeout argument should be specified (here we used 200 seconds) to allow sufficient time for calling a read during CPU basecalling. Additionally, we ran Megalodon and Guppy on another computer with a GPU (GeForce RTX 2080 Ti) and 32 CPU processors (AMD Ryzen threadripper

2950x). Real time per CPU = real (wall-clock) time taken x no. of CPUs. Bases per second = total no. of bases/(real time per CPU x 60).