Supplementary information

Systematic comparison of tools used for m6A mapping from nanopore direct RNA sequencing

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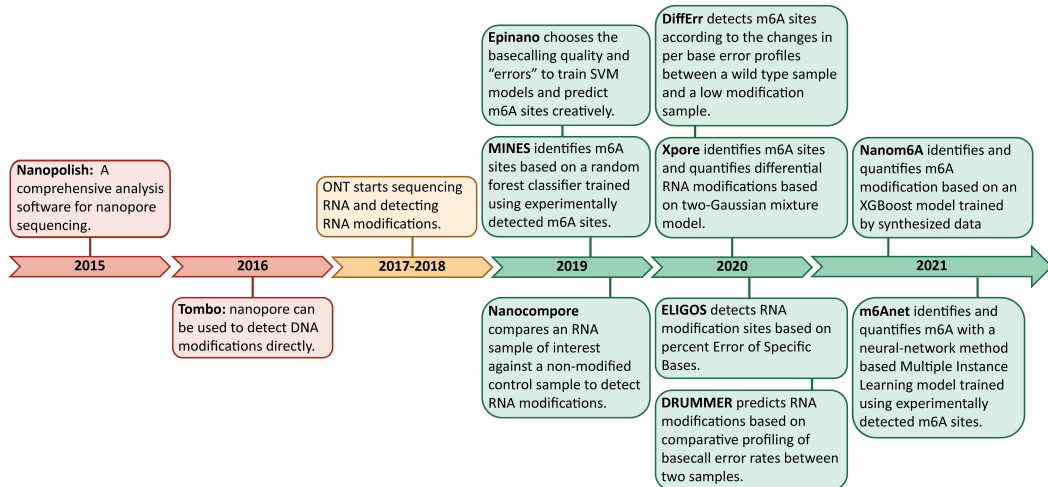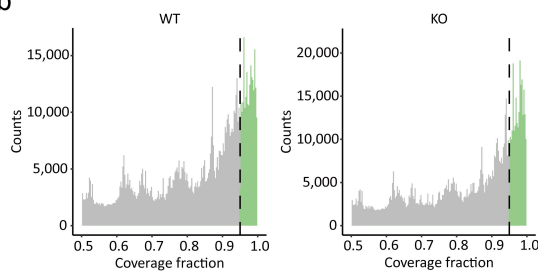4 These authors contributed equally
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a

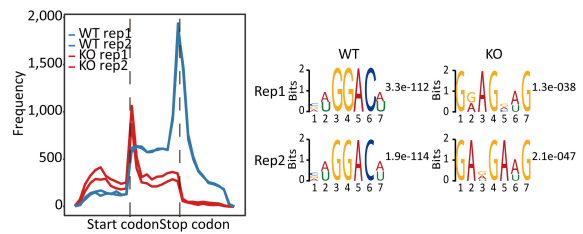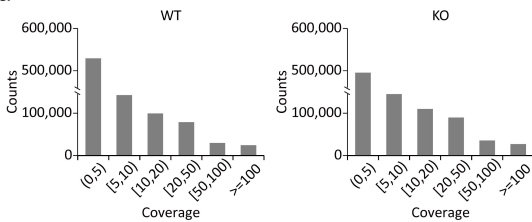**Nanopolish:** A comprehensive analysis software for nanopore sequencing.

Tombo: nanopore can be used to detect DNA modifications directly.

ONT starts sequencing RNA and detecting RNA modifications.

Epinano chooses the basecalling quality and "errors" to train SVM models and predict m6A sites creatively.

MINES identifies m6A sites based on a random forest classifier trained using experimentally detected m6A sites.

Nanocompore compares an RNA sample of interest against a non-modified control sample to detect RNA modifications.

DiffErr detects m6A sites according to the changes in per base error profiles between a wild type sample and a low modification sample.

Xpore identifies m6A sites and quantifies differential RNA modifications based on two-Gaussian mixture model.

ELIGOS detects RNA modification sites based on percent Error of Specific Bases.

DRUMMER predicts RNA modifications based on comparative profiling of basecall error rates between two samples.

Nanom6A identifies and quantifies m6A modification based on an XGBoost model trained by synthesized data

m6Anet identifies and quantifies m6A with a neural-network method based Multiple Instance Learning model trained using experimentally detected m6A sites.
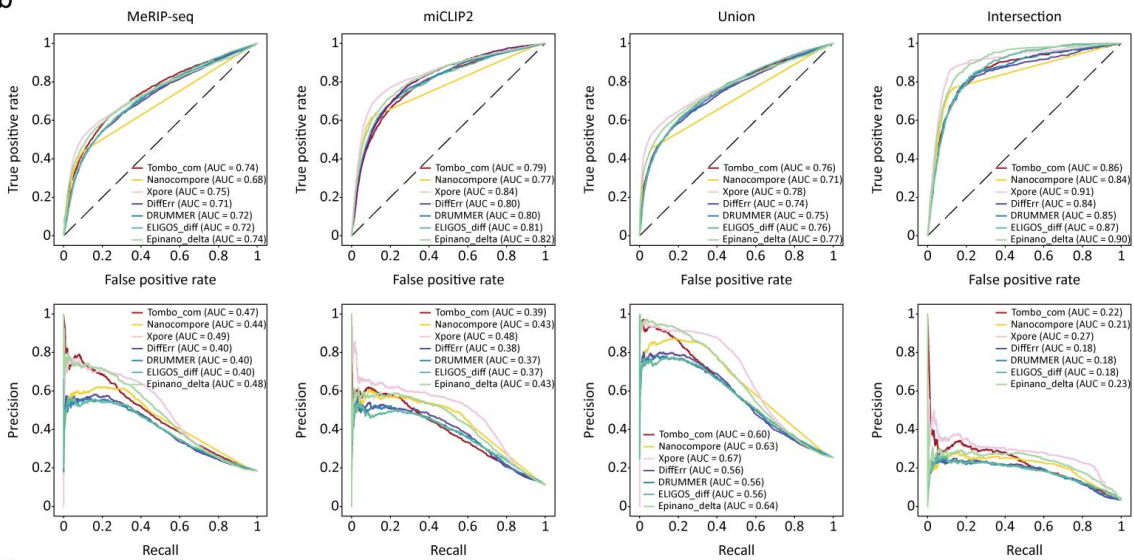
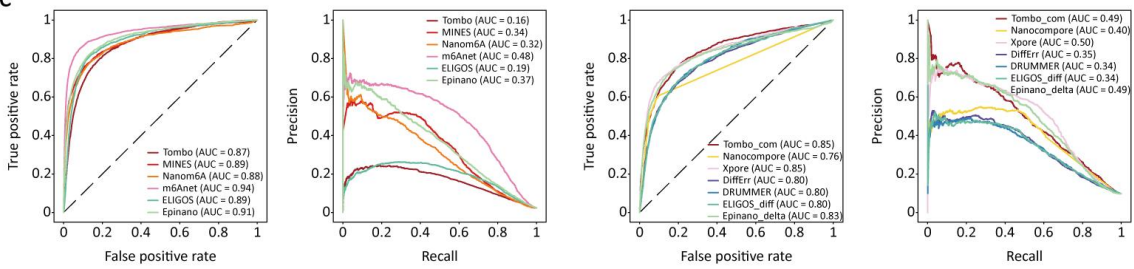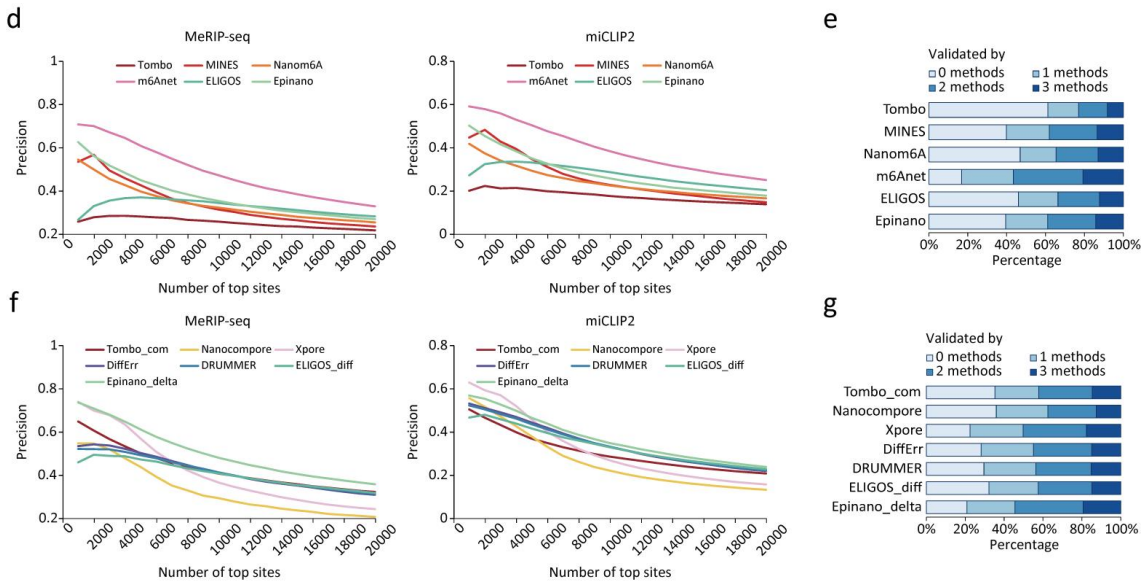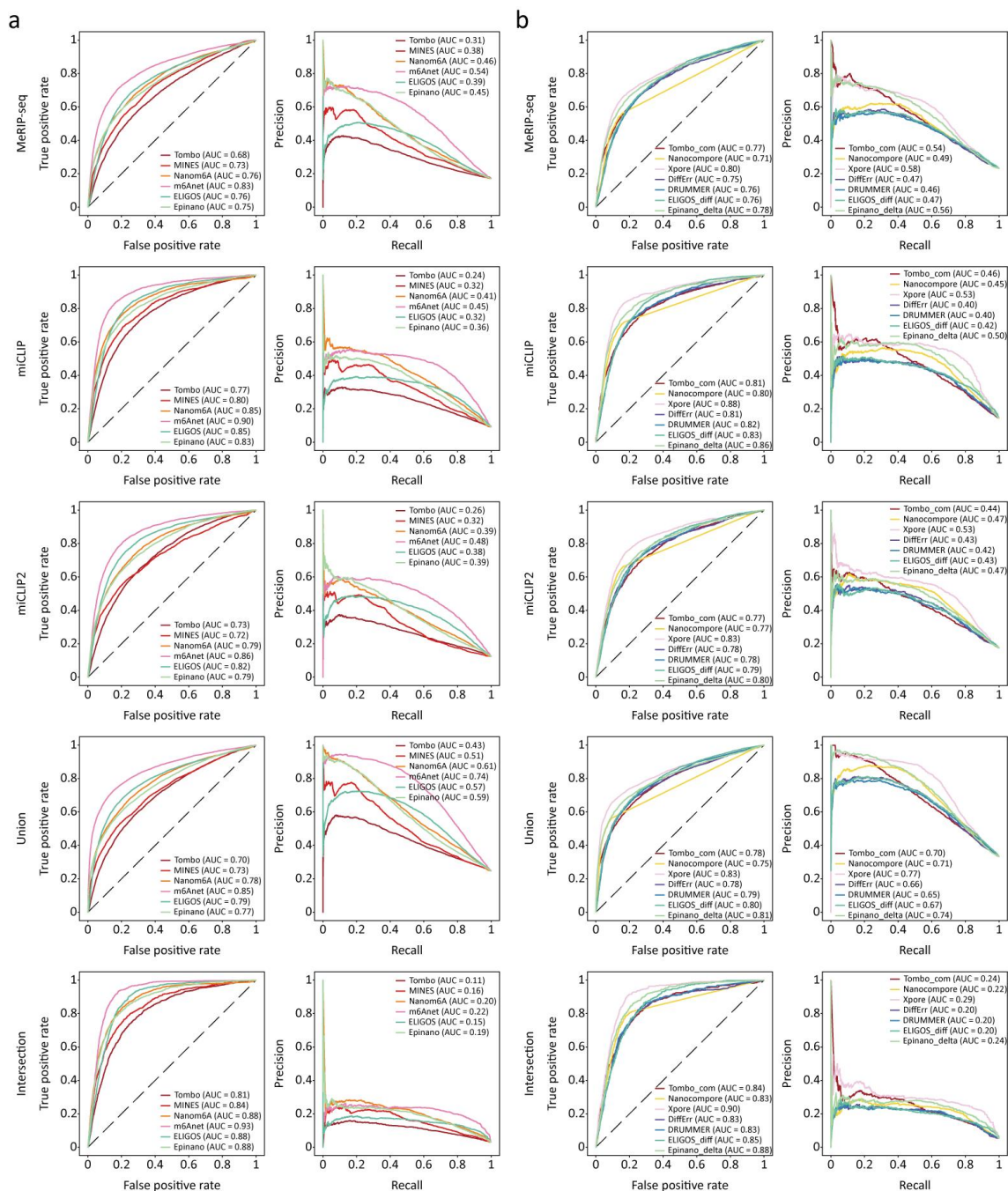| 2015 | 2016 | 2017-2018 | 2019 | 2020 | 2021 |

b

c

d

Supplementary Fig. 1 Development of m6A detection using nanopore sequencing and datasets used for benchmarking. a Timestamp of the advent of ONT tools for m6A detection. **b** Frequency of reads with different coverage fraction. Coverage fraction, fraction of annotated transcripts from GENCODE.VM18 covered by reads. Reads in the right side (green-shaded) of dotted line (coverage fraction=0.95) could be considered as full-length reads. **c** Metagene plots and motif preferences of m6A peaks from MeRIP-seq results. **d** Counts of sites in RRACH motifs with different coverage.
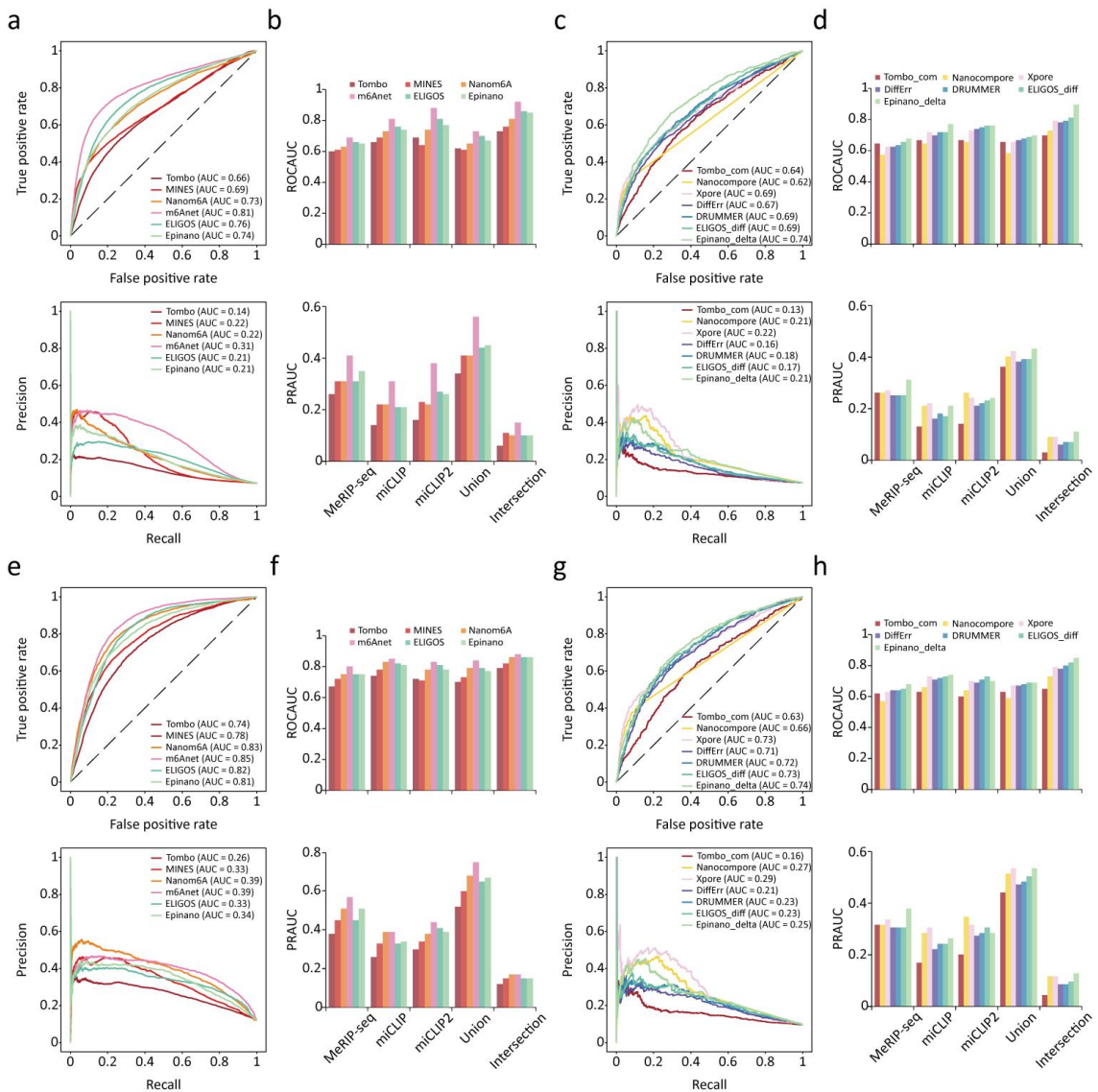
a

MeRIP-seq

Tombo (AUC = 0.59)
MINES (AUC = 0.62)
Nanom6A (AUC = 0.64)
m6Anet (AUC = 0.68)
ELIGOS (AUC = 0.65)
Epinano (AUC = 0.64)

miCLIP2

Tombo (AUC = 0.69)
MINES (AUC = 0.64)
Nanom6A (AUC = 0.75)
m6Anet (AUC = 0.89)
ELIGOS (AUC = 0.80)
Epinano (AUC = 0.77)

Union

Tombo (AUC = 0.61)
MINES (AUC = 0.62)
Nanom6A (AUC = 0.66)
m6Anet (AUC = 0.72)
ELIGOS (AUC = 0.68)
Epinano (AUC = 0.67)

Intersection

Tombo (AUC = 0.74)
MINES (AUC = 0.77)
Nanom6A (AUC = 0.83)
m6Anet (AUC = 0.94)
ELIGOS (AUC = 0.85)
Epinano (AUC = 0.85)

Tombo (AUC = 0.20)
MINES (AUC = 0.26)
Nanom6A (AUC = 0.26)
m6Anet (AUC = 0.37)
ELIGOS (AUC = 0.25)
Epinano (AUC = 0.29)

Tombo (AUC = 0.14)
MINES (AUC = 0.21)
Nanom6A (AUC = 0.21)
m6Anet (AUC = 0.41)
ELIGOS (AUC = 0.24)
Epinano (AUC = 0.26)

Tombo (AUC = 0.27)
MINES (AUC = 0.34)
Nanom6A (AUC = 0.35)
m6Anet (AUC = 0.52)
ELIGOS (AUC = 0.36)
Epinano (AUC = 0.38)

Tombo (AUC = 0.05)
MINES (AUC = 0.11)
Nanom6A (AUC = 0.11)
m6Anet (AUC = 0.20)
ELIGOS (AUC = 0.09)
Epinano (AUC = 0.12)

b

MeRIP-seq

Tombo_com (AUC = 0.74)
Nanocompore (AUC = 0.68)
Xpore (AUC = 0.75)
DiffErr (AUC = 0.71)
DRUMMER (AUC = 0.72)
ELIGOS_diff (AUC = 0.72)
Epinano_delta (AUC = 0.74)

miCLIP2

Tombo_com (AUC = 0.79)
Nanocompore (AUC = 0.77)
Xpore (AUC = 0.84)
DiffErr (AUC = 0.80)
DRUMMER (AUC = 0.80)
ELIGOS_diff (AUC = 0.81)
Epinano_delta (AUC = 0.82)

Union

Tombo_com (AUC = 0.76)
Nanocompore (AUC = 0.71)
Xpore (AUC = 0.78)
DiffErr (AUC = 0.74)
DRUMMER (AUC = 0.75)
ELIGOS_diff (AUC = 0.76)
Epinano_delta (AUC = 0.77)

Intersection

Tombo_com (AUC = 0.86)
Nanocompore (AUC = 0.84)
Xpore (AUC = 0.91)
DiffErr (AUC = 0.84)
DRUMMER (AUC = 0.85)
ELIGOS_diff (AUC = 0.87)
Epinano_delta (AUC = 0.90)

Tombo_com (AUC = 0.47)
Nanocompore (AUC = 0.44)
Xpore (AUC = 0.49)
DiffErr (AUC = 0.40)
DRUMMER (AUC = 0.40)
ELIGOS_diff (AUC = 0.40)
Epinano_delta (AUC = 0.48)

Tombo_com (AUC = 0.39)
Nanocompore (AUC = 0.43)
Xpore (AUC = 0.48)
DiffErr (AUC = 0.38)
DRUMMER (AUC = 0.37)
ELIGOS_diff (AUC = 0.37)
Epinano_delta (AUC = 0.43)

Tombo_com (AUC = 0.60)
Nanocompore (AUC = 0.63)
Xpore (AUC = 0.67)
DiffErr (AUC = 0.56)
DRUMMER (AUC = 0.56)
ELIGOS_diff (AUC = 0.56)
Epinano_delta (AUC = 0.64)

Tombo_com (AUC = 0.22)
Nanocompore (AUC = 0.21)
Xpore (AUC = 0.27)
DiffErr (AUC = 0.18)
DRUMMER (AUC = 0.18)
ELIGOS_diff (AUC = 0.18)
Epinano_delta (AUC = 0.23)

c

Tombo (AUC = 0.87)
MINES (AUC = 0.89)
Nanom6A (AUC = 0.88)
m6Anet (AUC = 0.94)
ELIGOS (AUC = 0.89)
Epinano (AUC = 0.91)

Tombo (AUC = 0.16)
MINES (AUC = 0.34)
Nanom6A (AUC = 0.32)
m6Anet (AUC = 0.48)
ELIGOS (AUC = 0.19)
Epinano (AUC = 0.37)

Tombo_com (AUC = 0.85)
Nanocompore (AUC = 0.76)
Xpore (AUC = 0.85)
DiffErr (AUC = 0.80)
DRUMMER (AUC = 0.80)
ELIGOS_diff (AUC = 0.80)
Epinano_delta (AUC = 0.83)

Tombo_com (AUC = 0.49)
Nanocompore (AUC = 0.40)
Xpore (AUC = 0.50)
DiffErr (AUC = 0.35)
DRUMMER (AUC = 0.34)
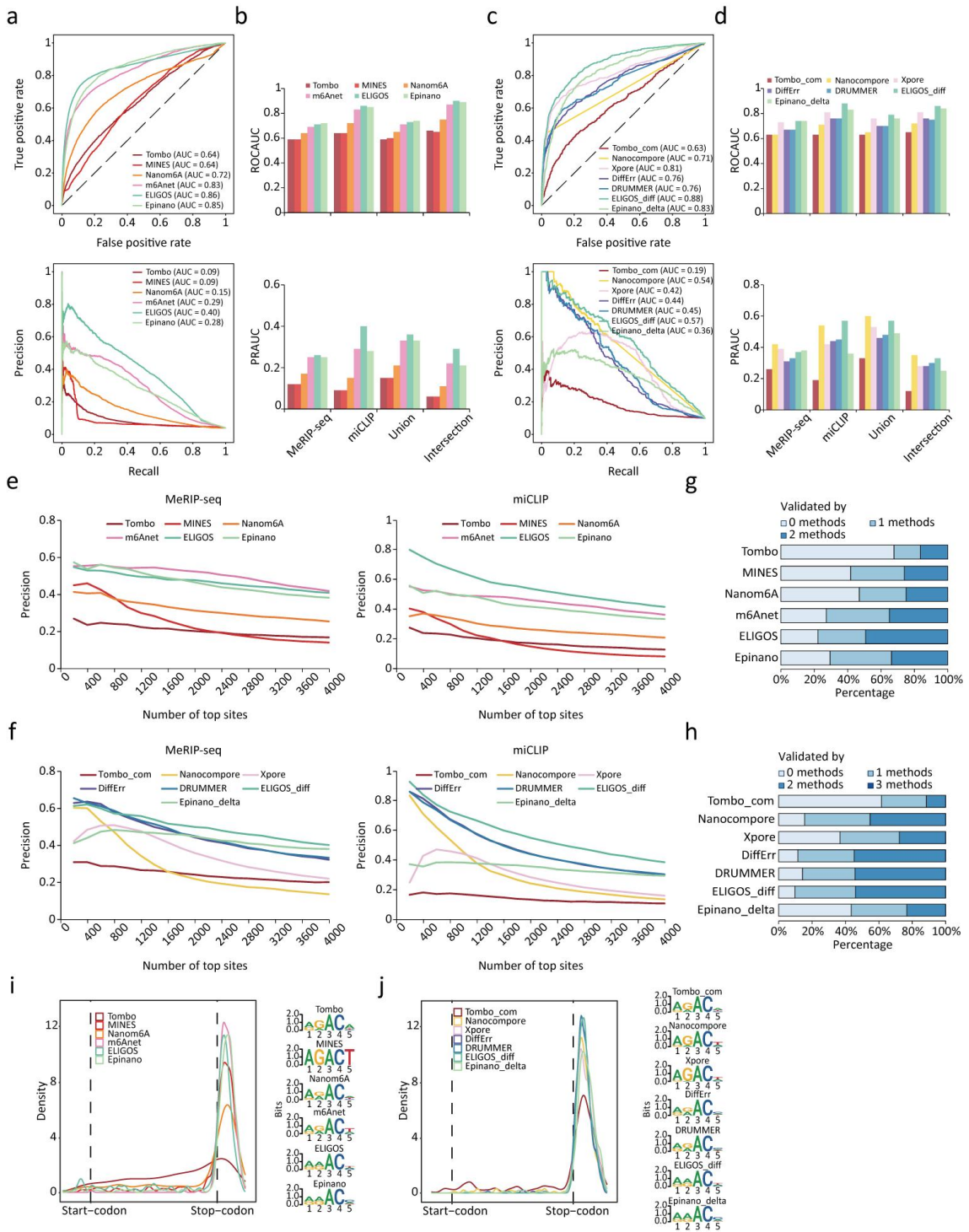ELIGOS_diff (AUC = 0.34)
Epinano_delta (AUC = 0.49)

Supplementary Fig. 2 Performance for the ten ONT tools evaluated for m6A detection capability in mouse. a-b Receiver Operating Characteristic (ROC) curve (top) and Precision Recall (PR) curve (bottom) for candidate sites detected by single-mode tools (**a**) and compare-mode tools (**b**) using different data as ground truth. Area under the curve (AUC) indicated in parentheses. **c** ROC curve and PR curve for candidate sites detected by tools using MeRIP-seq data as ground truth, but sites with maximum of the predicted probability within each peak are selected to represent the results. Precision for top 20,000 m6A sites detected by single-mode tools (**d**) and compare tools (**f**) using the MeRIP-seq and miCLIP2 data as ground truth. Percentage for top 5,000 m6A sites detected by single-mode tools (**e**) and compare-mode tools (**g**) validated by different number of NGS-based methods.

Supplementary Fig. 3 Continuous evaluation metrics for the ten ONT tools for m6A detection of sites in AGACT/GGACH motifs in mouse. a-b ROC curve (left) and PR curve (right) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**a**) and compare-mode tools (**b**) using different data as ground truth. AUC indicated in parentheses.
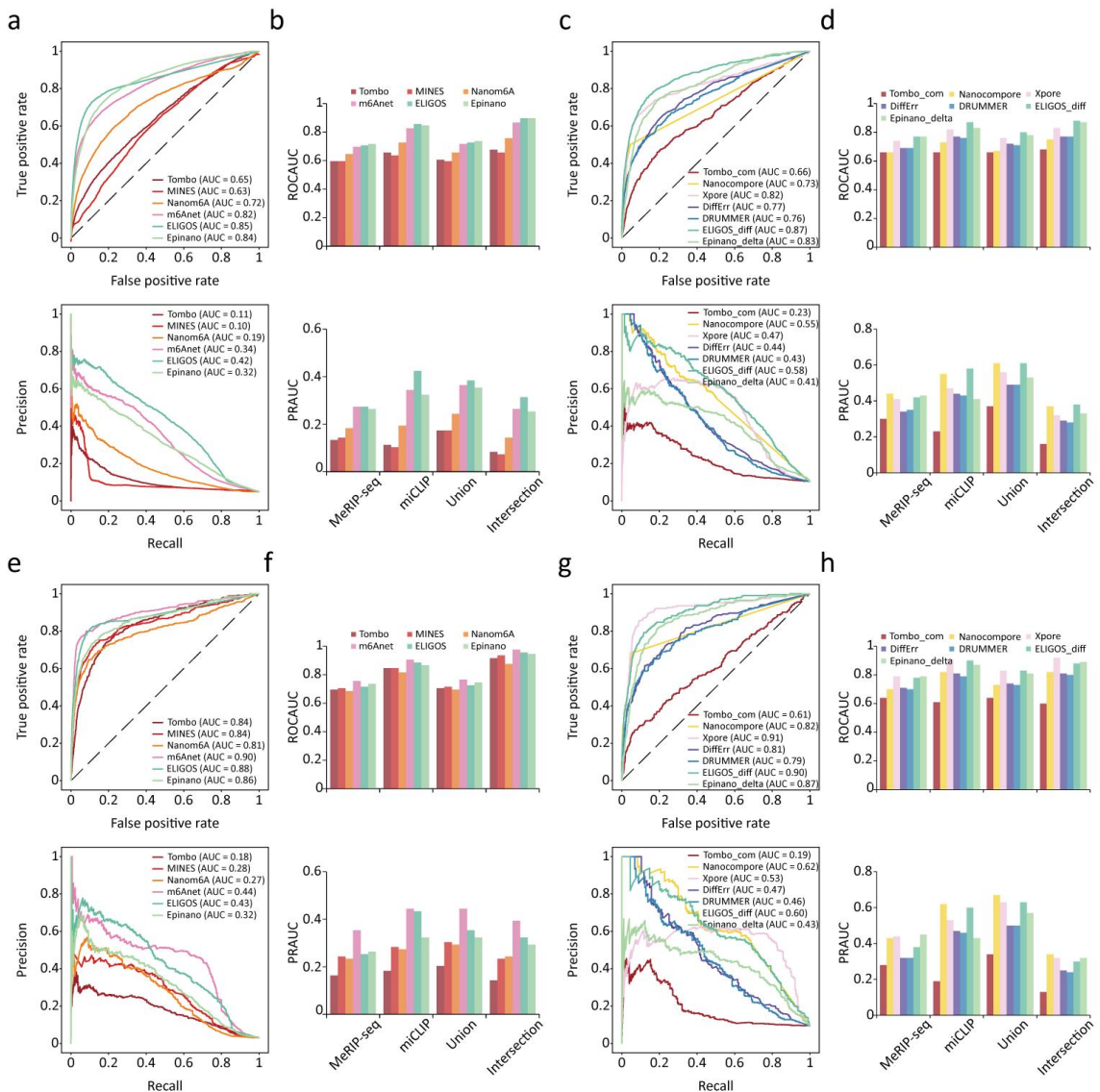
Supplementary Fig. 4 Continuous evaluation metrics for the ten ONT tools for m6A detection of sites in mouse (replicate). ROC curve (top) and PR curve (bottom) for candidate sites detected by single-mode tools (**a**) and compare-mode tools (**c**) using the miCLIP data as ground truth. AUC indicated in parentheses. ROC AUC (top) and PR AUC (bottom) for candidate sites detected by single-mode tools (**b**) and compare tools (**d**) using different data as ground truth. ROC curve (top) and PR curve (bottom) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**e**) and compare-mode tools (**g**) using the miCLIP data as ground truth. AUC indicated in parentheses. ROC AUC (top) and PR AUC (bottom) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**f**) and compare tools (**h**) using different data as ground truth.

a

b

c

d

e

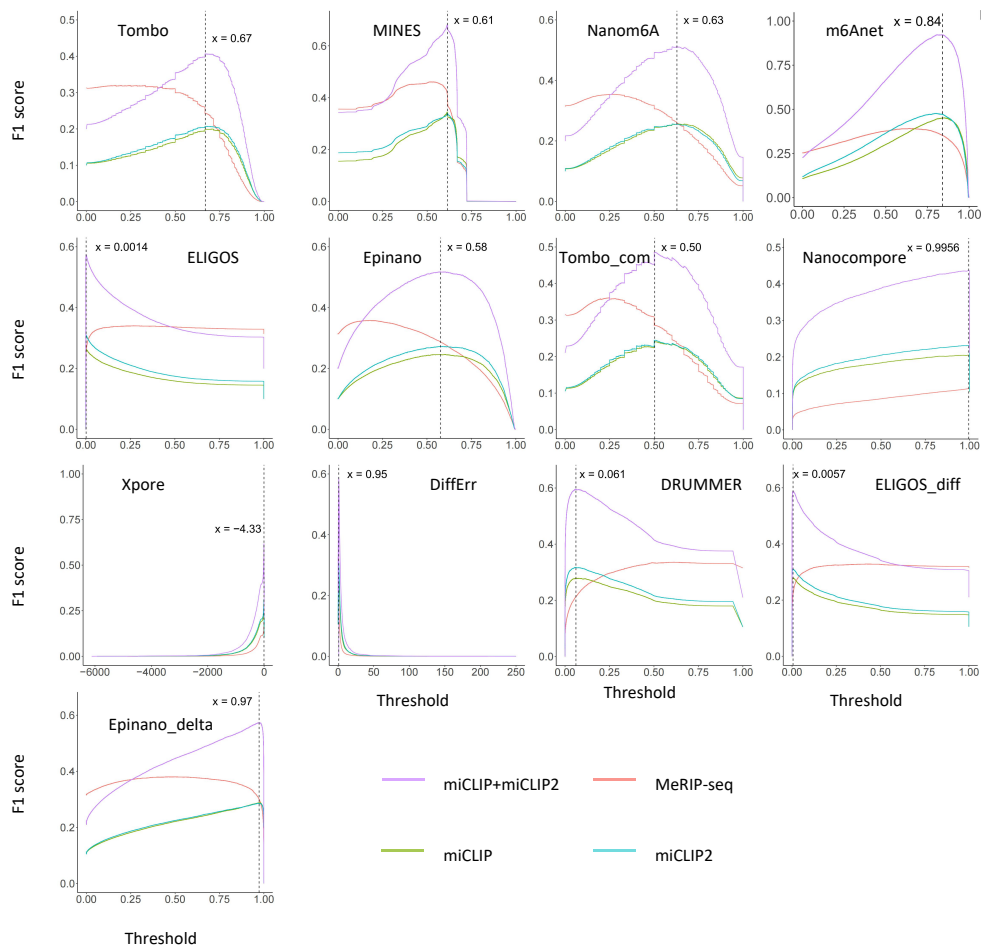MeRIP-seq

miCLIP

g

f

MeRIP-seq

miCLIP

h

i

j

Supplementary Fig. 5 Performance for the ten ONT tools evaluated for m6A detection capability in Arabidopsis. ROC curve (top) and PR curve (bottom) for candidate sites detected by single-mode tools (**a**) and compare-mode tools (**c**) using the miCLIP data as ground truth. AUC indicated in parentheses. ROC AUC (top) and PR AUC (bottom) for candidate sites detected by single-mode tools (**b**) and compare tools (**d**) using different data as ground truth. **e-f** Precision for top 4,000 m6A sites detected by single-mode tools (**e**) and compare tools (**f**) using the MeRIP-seq and miCLIP data as ground truth. **g-h** Percentage for top 400 m6A sites detected by single-mode tools (**g**) and compare-mode tools (**h**) validated by different number of NGS-based methods. **i-j** Metagene plots (left) of the transcriptome-wide m6A distribution for top 400 m6A sites detected by single-mode tools (**i**) and compore-mode tools (**j**). Seqlogo plots (right) show the frequency of the RRACH motif for top 400 m6A sites detected by single-mode tools (**i**) and compore-mode tools (**j**).

Supplementary Fig. 6 Continuous evaluation metrics for the ten ONT tools for m6A detection of sites in AGACT/GGACH motifs in Arabidopsis. ROC curve (top) and PR curve (bottom) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**a**) and compare-mode tools (**c**) using the miCLIP data as ground truth. AUC indicated in parentheses. ROC AUC (top) and PR AUC (bottom) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**b**) and compare tools (**d**) using different data as ground truth.
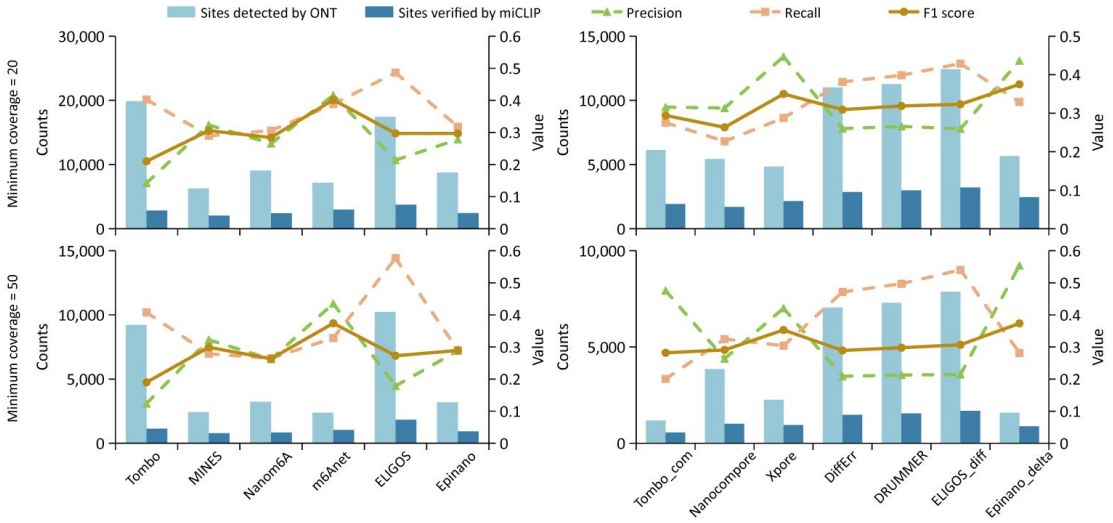
Supplementary Fig. 7 Continuous evaluation metrics for the ten ONT tools for m6A detection of sites in Arabidopsis (replicate). ROC curve (top) and PR curve (bottom) for candidate sites detected by single-mode tools (**a**) and compare-mode tools (**c**) using the miCLIP data as ground truth. AUC indicated in parentheses. ROC AUC (top) and PR AUC (bottom) for candidate sites detected by single-mode tools (**b**) and compare tools (**d**) using different data as ground truth. ROC curve (top) and PR curve (bottom) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**e**) and compare-mode tools (**g**) using the miCLIP data as ground truth. AUC indicated in parentheses. ROC AUC (top) and PR AUC (bottom) for candidate sites in AGACT/GGACH motifs detected by single-mode tools (**f**) and compare tools (**h**) using different data as ground truth.
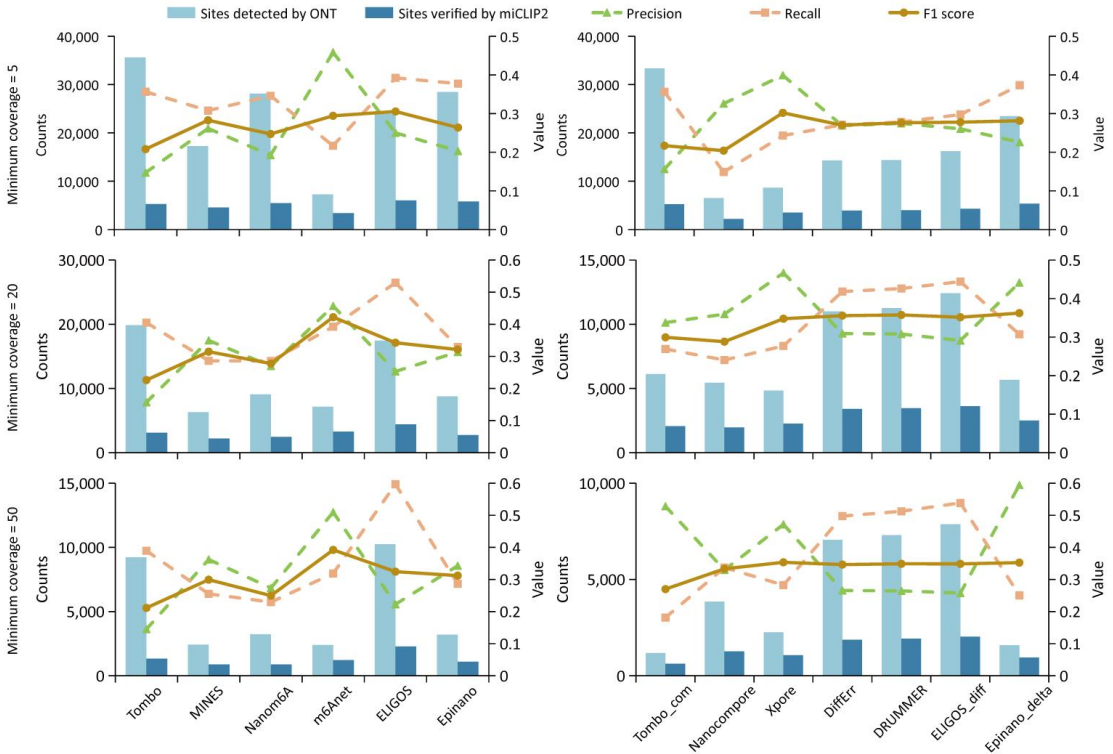
Supplementary Fig. 8 Selection of the best cut-offs for ONT tools. Plots show the distribution of F1 scores with varing cut-offs of all tools using MeRIP-seq, miCLIP and miCLIP2 results as validation sets. "miCLIP and miCLIP2" represents the sum of F1 scores from miCLIP and miCLIP2.
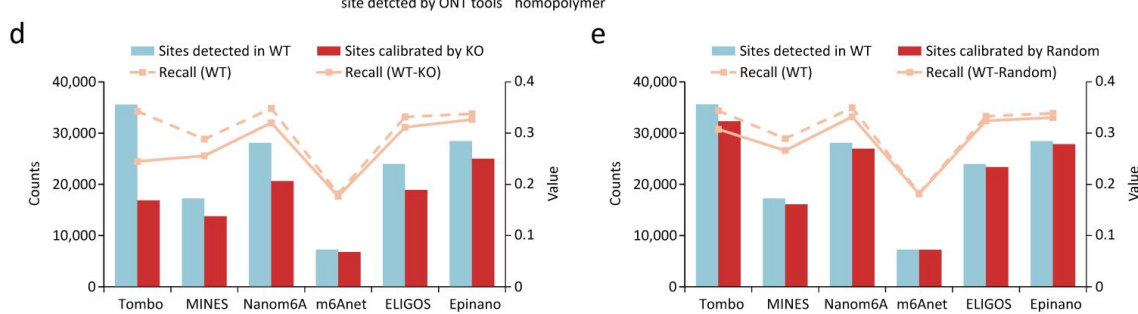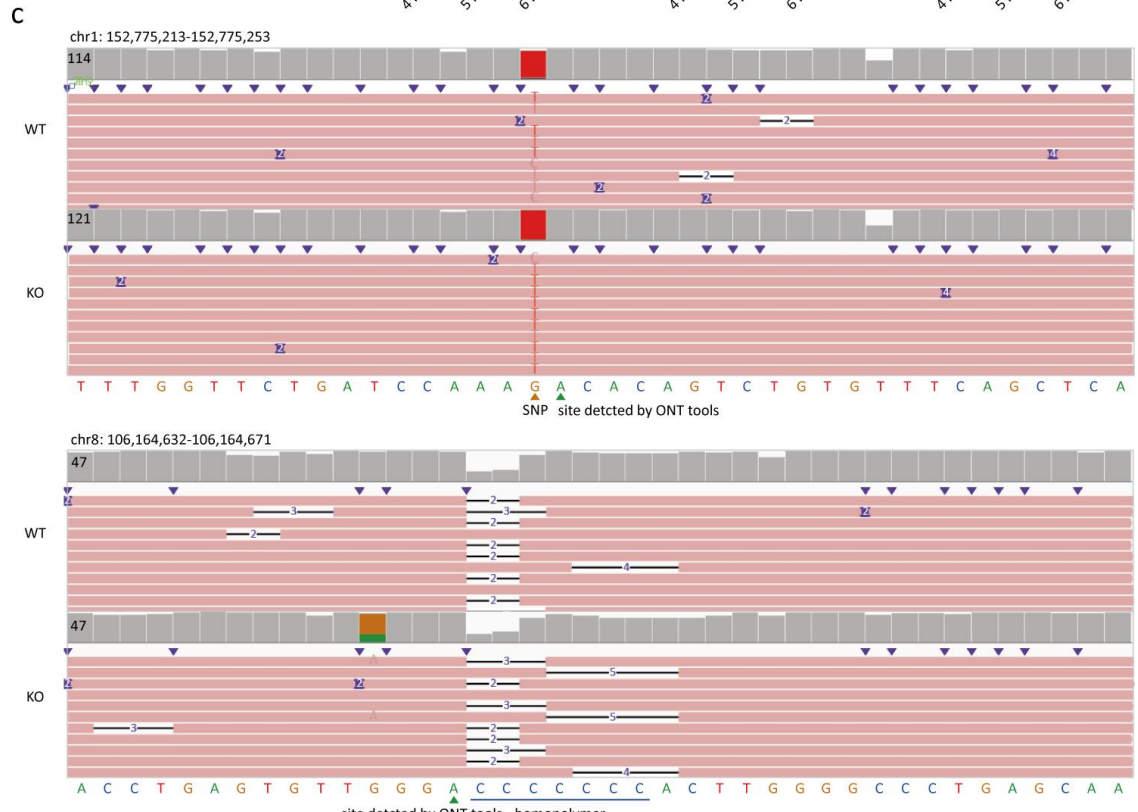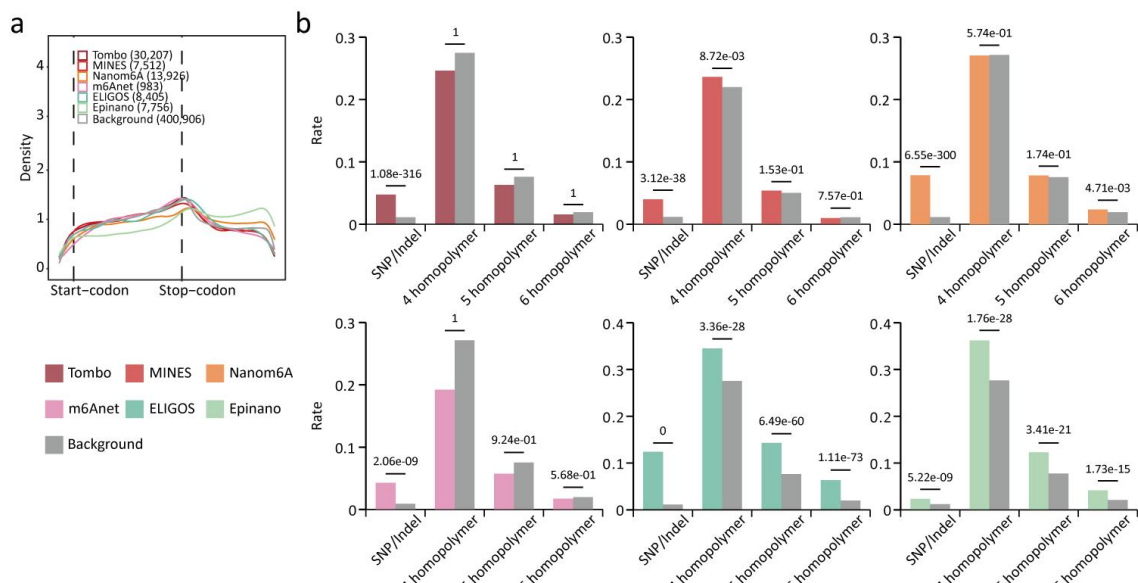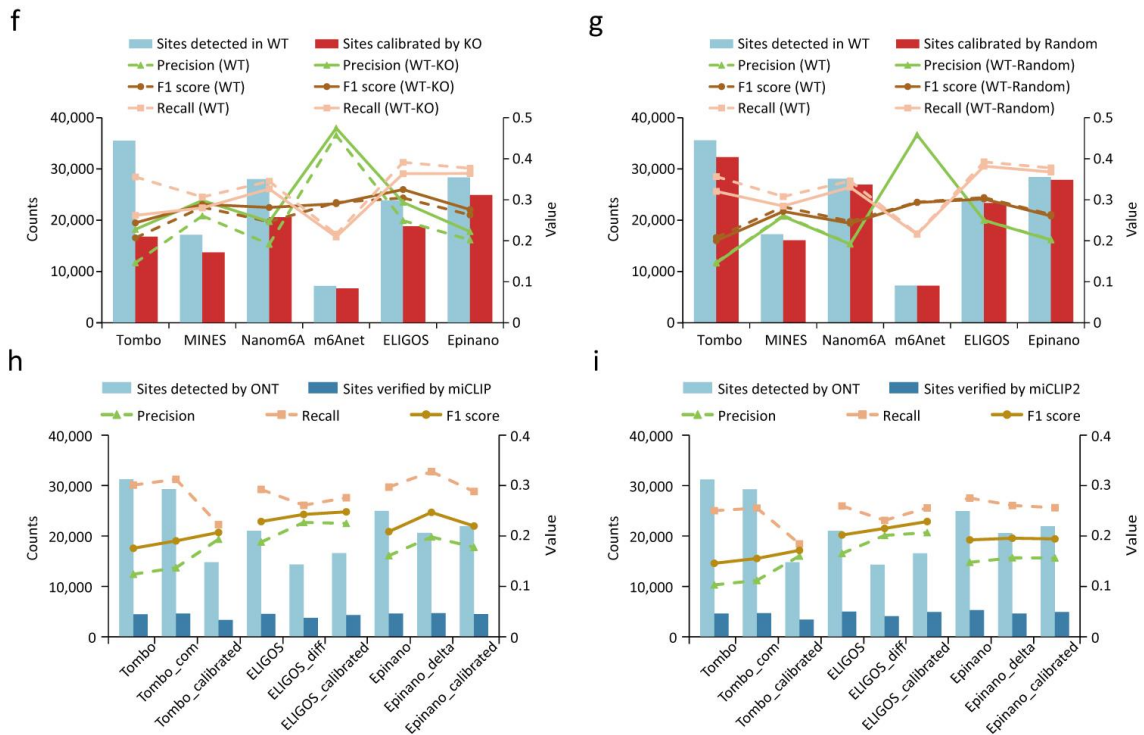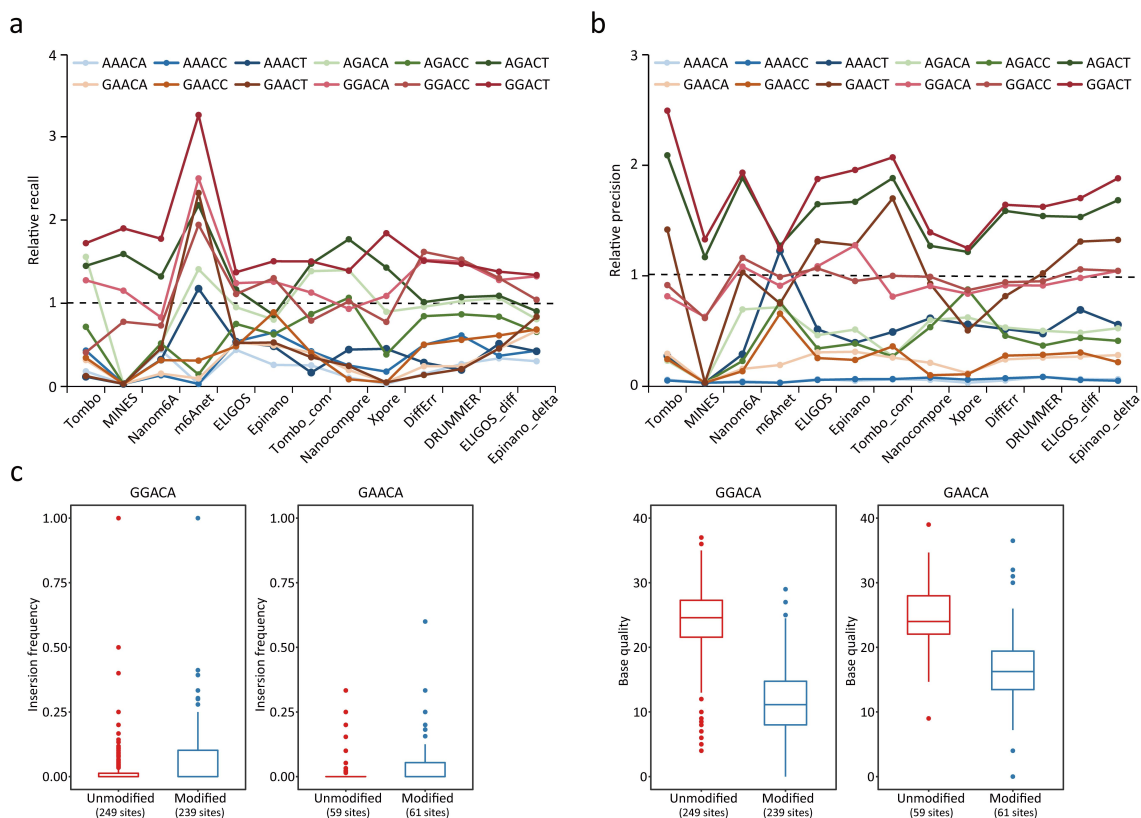
Supplementary Fig. 9 Performance of m6A detection for ONT tools under the optimal cut-off. a-b Precision, recall and F1 scores for ONT tools using miCLIP results (**a**) and miCLIP2 results (**b**) as validation set. Minmum coverage requirements of sites indicated in the left side.
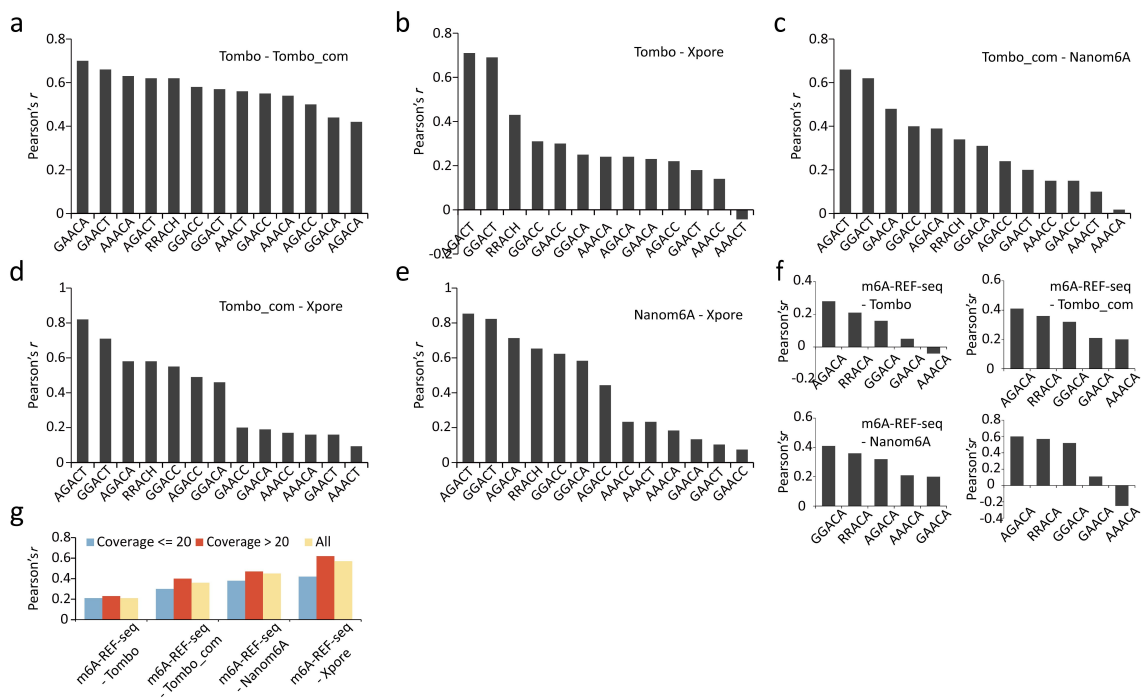
Supplementary Fig. 10 Assessment of intrinsic bias and the effects of using a negative control on m6A detection. a Metagene plots of m6A sites detected in KO samples under the best cut-off. "Background" means all RRACH sites with coverage >= 5 nanopore reads in KO samples. Number of sites indicated in parentheses. **b** Rates of different types intrinsic bias in the sites detected both in WT and KO samples. "Background" means all RRACH sites with coverage >= 5 nanopore reads. One-side Binomial tests were applied to calculate significance. **c** IGV snapshots of two highly probable false positives around SNP/homopolymer in WT and KO samples. Coverage for each site is indicated on the left of each short sequence. **d-e** Comparison of recall before and after calibration with the KO sample results (**d**) and a random dataset (**e**). WT shows the recall for m6A sites detected in the WT samples, WT-KO shows the recall for sites calibrated using KO results, and WT-random shows the recall for sites calibrated with a randomly selected dataset. miCLIP results were used as a validation set. **f-g** Comparison of precision, recall and F1 score before and after calibration with the KO sample results (**f**) and a random dataset (**g**). miCLIP2 results were used as a validation set. **h-i** Comparison of number of m6A sites, precision, recall and F1 scores using original mode, compare mode and calibration with the KO sample results for Tombo, ELIGOS and Epinano. The validation set is derived from the miCLIP results in **h**, the miCLIP2 results in **i**.

Supplementary Fig. 11 Detection capacity for m6A among sequence motifs. a-b
Relative recall rates (**a**) and precision (**b**) for all tools for 12 motifs bearing the
consensus sequence RRACH. Relative recall rates/precision were calculated as the ratio
of the recall/precision for the sites in a specific motif to the overall mean (across all
sites). A m6A profile created from miCLIP2 results was used for validation. **c** A
comparison of insertion frequency and base quality for modified and unmodified sites in
GGACA and GAACA motifs. In the Boxplot, the upper and lower limits represent the
75th and 25th percentiles, respectively, while the center line represents the median;
upper and lower whiskers indicate ±1.5 × the interquartile range (IQR). The number of
sites is indicated in parentheses.

Supplementary Fig. 12 Performance of m6A quantification in different motifs and coverage. a-f Correlation coefficients for methylation rates observed between each paired comparison for m6A sites in different motifs. **g** Correlation coefficients for methylation rates observed between each tool and m6A-REF-seq for m6A sites with different sequencing coverage.

Supplementary Fig. 13 Detection sensitivity for m6A sites varying in coverage. a-b
Relative recall rates (**a**) and precision (**b**) for m6A sites versus coverage. Relative recall
rates/precision were calculated as the ratio of the recall rate/precision for m6A sites with
a given coverage to the overall mean (across all sites). The validation set used came
from the miCLIP2 results.

Supplementary Fig. 14 Performance of m6A detection by integrating results from all tools. a-c Precision (**a**), recall (**b**) and F1 scores (**c**) of m6A sites for integrated predictions and each individual tool versus the number of supported tools. For each category, the number of top m6A sites is identical to integrated predictions. The validation sets used came from the miCLIP2 results. **d** Overlap ratio between m6A sites not detected by NGS-based methods but supported by different number of ONT tools and m6A sites detected in mESCs KO samples.

	Published time	Classification strategy	Classification method	Specific algorithms	Model source	Control sample required	Reference	Motif
Tombo	2016.12.15	current	statistical test	Fisher's test	integral k-mers from nanopore	alternative	transcriptome	NNNNN
Epinano	2019.09.09	base-calling "errors"	machine learning	Support Vector Machine(SVM)	"curlcakes"	alternative	transcriptome/ genome	RRACH
MINES	2019.10.17	current	machine learning	Random Forest(RF)	miCLIP data from HEK293T cells	no	transcriptome	AGACT, GGACH
Nanocompore	2019.11.15	current	clustering	Gaussian mixture model(GMM)	\	yes	transcriptome	NNNNN
DiffErr	2020.01.14	base-calling "errors"	statistical test	G-test	\	yes	transcriptome/ genome	NNNNN
Xpore	2020.06.20	current	clustering	Gaussian mixture model(GMM)	\	no	transcriptome	NNNNN
ELIGOS	2020.07.25	base-calling "errors"	statistical test	Fisher's exact test	In vitro transcription	alternative	transcriptome/ genome	NNANN
DRUMMER	2020.11.26	base-calling "errors"	statistical test	G-test	\	yes	transcriptome/ genome	NNANN
Nanom6A	2021.01.07	current	machine learning	XGBoost	"curlcakes"	no	genome	RRACH
m6Anet	2021.09.22	current	machine learning	Multiple Instance Learning based Neural Network	miCLIP data from HCT116 cell line	no	transcriptome	DRACH

Supplementary Table 1 Detailed information of all ONT tools. "curlcakes" are sequences that that they include all possible 5-mers by in-vitro synthesized.

Supplementary Table 2 Reads quality and alignment statistics for all samples.

		Basecalled reads	Mean read length	Mean read quality	Aligned reads
mESCs	**wide type**	1,309,027	1,058.5	10.2	1,282,198
	Mettl3 **knockout**	1,316,561	1,072.2	10.8	1,289,348
mESCs (replicate)	**wide type**	3,079,421	528.0	9.9	2,979,700
	Mettl3 **knockout**	1,486,950	438.4	10.0	1,431,246
Arabidopsis	**wide type**	1,059,560	1,034.1	10.6	972,804
	mutants defective in m6A	1,093,477	1,061.9	10.3	966,780
Arabidopsis (replicate)	**wide type**	790,015	989.9	10.6	741,436
	mutants defective in m6A	1,843,192	1,058.8	10.6	1,680,748

Supplementary Table 3 Final datasets used for evaluating ONT tools of all samples.

	mESCs		mESCs (replicate)		Arabidopsis		Arabidopsis (replicate)	
	single-mode	compare-mode	single-mode	compare-mode	single-mode	compare-mode	single-mode	compare-mode
Evaluated sites (coverage)	105,241 (147)	124,676 (127)	73,509 (261)	73,816 (261)	86,531 (121)	104,552 (104)	63,736 (114)	77,995 (97)
Validated sites by miCLIP (coverage)	6,026 (83)	7,414 (72)	5,200 (187)	5,255 (183)	3,422 (620)	3,842 (555)	3,102 (523)	3,596 (455)
Validated sites by miCLIP2 (coverage)	6,652 (121)	8,060 (104)	5,611 (252)	5,692 (251)	-	-	-	-

Supplementary Table 4 Counts of validated sites in different motifs of all samples.

Validated sites by miCLIP

Motif	mESCs		mESCs (replicate)		Arabidopsis		Arabidopsis (replicate)	
	single-mode	compare-mode	single-mode	compare-mode	single-mode	compare-mode	single-mode	compare-mode
AAACA	278	328	216	200	659	747	599	689
AAACC	157	186	120	119	356	400	325	377
AAACT	440	524	365	363	775	865	689	793
AGACA	417	520	329	341	267	297	243	282
AGACC	219	272	165	170	83	99	76	94
AGACT	807	972	724	735	273	308	250	293
GAACA	342	402	264	261	265	303	240	285
GAACC	167	199	140	136	204	220	186	209
GAACT	830	971	720	726	327	360	298	344
GGACA	818	1,079	700	728	79	94	73	89
GGACC	393	509	343	344	63	71	58	69
GGACT	1,158	1,452	1,114	1,132	71	78	65	72

Validated sites by miCLIP2

Motif	mESCs		mESCs (replicate)	
	single-mode	compare-mode	single-mode	compare-mode
AAACA	39	47	35	34
AAACC	11	13	9	9
AAACT	479	550	364	358
AGACA	191	233	156	165
AGACC	114	133	94	103
AGACT	1,091	1,306	938	950
GAACA	211	254	177	170
GAACC	99	114	89	88
GAACT	1,169	1,361	1,003	1,005
GGACA	822	1,069	688	732
GGACC	554	702	461	474
GGACT	1,872	2,278	1,597	1,604

Supplementary Table 5 The Best F1 score for each tool and the corresponding threshold using miCLIP and miCLIP2 results as validation sets. "Sum" represents the sum of F1 scores from miCLIP and miCLIP2.

	Threshold	F1 score using different validation sets		Sum
		miCLIP	miCLIP2	
Tombo	0.67	0.20	0.21	0.41
MINES	0.61	0.34	0.34	0.68
Nanom6A	0.63	0.26	0.26	0.51
m6Anet	0.84	0.45	0.47	0.92
ELIGOS	0.0014	0.26	0.31	0.57
Epinano	0.58	0.25	0.27	0.52
Tombo_com	0.50	0.24	0.24	0.49
Nanocompore	0.9956	0.21	0.23	0.44
Xpore	-4.33	0.30	0.31	0.62
DiffErr	0.95	0.27	0.32	0.59
DRUMMER	0.061	0.28	0.32	0.60
ELIGOS_diff	0.0057	0.28	0.31	0.59
Epinano_delta	0.97	0.29	0.29	0.57

Supplementary Table 6 Recall and precision for all tools for 12 motifs bearing the consensus sequence RRACH. m6A profiles created from miCLIP and miCLIP2 results were used for validation separately.

Recall (miCLIP)

Motif	AAACA	AAACC	AAACT	AGACA	AGACC	AGACT	GAACA	GAACC	GAACT	GGACA	GGACC	GGACT
Tombo	0.03	0.06	0.03	0.42	0.12	0.56	0.07	0.03	0.04	0.47	0.14	0.73
MINES	-	-	-	-	-	0.54	-	-	-	0.42	0.31	0.71
Nanom6A	0.11	0.06	0.12	0.11	0.11	0.52	0.04	0.07	0.17	0.36	0.28	0.76
m6Anet	0.00	0.00	0.24	0.18	0.01	0.58	0.02	0.02	0.67	0.63	0.50	0.89
ELIGOS	0.08	0.04	0.16	0.21	0.10	0.43	0.11	0.09	0.20	0.48	0.39	0.57
Epinano	0.06	0.07	0.16	0.18	0.08	0.33	0.11	0.16	0.18	0.49	0.49	0.65
Tombo_com	0.03	0.02	0.06	0.36	0.15	0.60	0.10	0.06	0.16	0.44	0.31	0.64
Nanocompore	0.00	0.00	0.05	0.14	0.08	0.31	0.03	0.00	0.04	0.15	0.15	0.26
Xpore	0.00	0.00	0.09	0.16	0.02	0.40	0.00	0.00	0.00	0.30	0.22	0.58
DiffErr	0.02	0.02	0.06	0.17	0.10	0.29	0.03	0.09	0.03	0.50	0.48	0.51
DRUMMER	0.02	0.03	0.05	0.20	0.12	0.32	0.04	0.08	0.06	0.50	0.46	0.51
ELIGOS_diff	0.07	0.02	0.13	0.21	0.12	0.35	0.10	0.10	0.15	0.44	0.42	0.50
Epinano_delta	0.06	0.06	0.16	0.18	0.09	0.39	0.20	0.17	0.37	0.55	0.42	0.61

Recall (miCLIP2)

Motif	AAACA	AAACC	AAACT	AGACA	AGACC	AGACT	GAACA	GAACC	GAACT	GGACA	GGACC	GGACT
Tombo	0.05	0.14	0.03	0.54	0.24	0.51	0.10	0.11	0.03	0.44	0.13	0.60
MINES	-	-	-	-	-	0.48	-	-	-	0.34	0.23	0.57
Nanom6A	0.14	0.04	0.10	0.17	0.17	0.45	0.04	0.10	0.15	0.28	0.24	0.60
m6Anet	0.00	0.00	0.25	0.30	0.02	0.46	0.01	0.06	0.50	0.53	0.41	0.70
ELIGOS	0.16	0.20	0.20	0.36	0.28	0.45	0.19	0.18	0.19	0.48	0.42	0.53
Epinano	0.09	0.23	0.17	0.29	0.22	0.31	0.17	0.32	0.19	0.46	0.48	0.56
Tombo_com	0.08	0.14	0.05	0.49	0.31	0.53	0.14	0.14	0.12	0.40	0.28	0.54
Nanocompore	0.01	0.04	0.07	0.23	0.17	0.29	0.03	0.01	0.04	0.15	0.17	0.23
Xpore	0.00	0.04	0.11	0.22	0.09	0.35	0.00	0.00	0.00	0.26	0.19	0.45
DiffErr	0.04	0.15	0.08	0.29	0.26	0.31	0.07	0.15	0.03	0.47	0.50	0.47
DRUMMER	0.08	0.19	0.05	0.32	0.27	0.33	0.07	0.17	0.06	0.47	0.48	0.46
ELIGOS_diff	0.10	0.11	0.16	0.34	0.27	0.35	0.14	0.19	0.14	0.41	0.42	0.44
Epinano_delta	0.10	0.15	0.15	0.29	0.23	0.32	0.24	0.24	0.30	0.48	0.38	0.49

Precision (miCLIP)

Motif	AAACA	AAACC	AAACT	AGACA	AGACC	AGACT	GAACA	GAACC	GAACT	GGACA	GGACC	GGACT
Tombo	0.02	0.02	0.04	0.05	0.03	0.28	0.05	0.01	0.17	0.13	0.10	0.30
MINES	-	-	-	-	-	0.28	-	-	-	0.20	0.16	0.29
Nanom6A	0.02	0.02	0.06	0.20	0.05	0.36	0.04	0.02	0.17	0.28	0.20	0.32
m6Anet	0.00	0.00	0.48	0.40	0.33	0.53	0.20	0.14	0.31	0.47	0.38	0.44
ELIGOS	0.03	0.02	0.11	0.15	0.06	0.33	0.07	0.04	0.25	0.28	0.18	0.34
Epinano	0.02	0.02	0.08	0.14	0.06	0.30	0.07	0.03	0.18	0.29	0.14	0.31
Tombo_com	0.01	0.01	0.10	0.07	0.04	0.28	0.05	0.04	0.27	0.15	0.13	0.27
Nanocompore	0.01	0.02	0.18	0.29	0.19	0.40	0.11	0.00	0.29	0.35	0.25	0.39
Xpore	0.01	0.00	0.20	0.42	0.18	0.47	0.00	0.00	0.16	0.40	0.31	0.44
DiffErr	0.03	0.02	0.13	0.21	0.11	0.38	0.05	0.07	0.17	0.32	0.21	0.37
DRUMMER	0.03	0.04	0.14	0.22	0.10	0.38	0.08	0.06	0.26	0.31	0.21	0.38
ELIGOS_diff	0.05	0.02	0.17	0.19	0.11	0.37	0.09	0.06	0.30	0.31	0.23	0.37
Epinano_delta	0.04	0.02	0.14	0.16	0.07	0.37	0.09	0.05	0.27	0.28	0.19	0.36

Precision (miCLIP2)

Motif	AAACA	AAACC	AAACT	AGACA	AGACC	AGACT	GAACA	GAACC	GAACT	GGACA	GGACC	GGACT
Tombo	0.00	0.00	0.04	0.03	0.03	0.30	0.04	0.03	0.20	0.11	0.13	0.36
MINES	-	-	-	-	-	0.30	-	-	-	0.16	0.15	0.34
Nanom6A	0.00	0.00	0.05	0.13	0.04	0.36	0.02	0.02	0.19	0.20	0.22	0.37
m6Anet	0.00	0.00	0.54	0.31	0.33	0.57	0.07	0.29	0.33	0.40	0.44	0.55
ELIGOS	0.01	0.01	0.12	0.11	0.08	0.40	0.07	0.06	0.32	0.26	0.26	0.46
Epinano	0.00	0.01	0.07	0.10	0.07	0.33	0.06	0.04	0.25	0.25	0.19	0.39
Tombo_com	0.00	0.01	0.07	0.04	0.04	0.29	0.04	0.05	0.26	0.12	0.15	0.32
Nanocompore	0.01	0.02	0.21	0.21	0.18	0.45	0.07	0.02	0.33	0.32	0.35	0.50
Xpore	0.00	0.01	0.22	0.24	0.34	0.48	0.04	0.03	0.19	0.33	0.34	0.50
DiffErr	0.01	0.01	0.15	0.16	0.13	0.48	0.07	0.08	0.24	0.27	0.28	0.50
DRUMMER	0.02	0.02	0.14	0.15	0.10	0.47	0.07	0.08	0.31	0.27	0.29	0.49
ELIGOS_diff	0.01	0.01	0.19	0.13	0.12	0.43	0.07	0.08	0.36	0.27	0.29	0.48
Epinano_delta	0.01	0.00	0.12	0.11	0.08	0.37	0.06	0.04	0.29	0.22	0.22	0.41

Supplementary Table 7 Recall and precision for all tools for m6A sites versus coverage. m6A profiles created from miCLIP and miCLIP2 results were used for validation separately.

Recall (miCLIP)

Coverage	5-10	10-20	20-40	40-60	60-80	80-100	>=100
Tombo	0.23	0.34	0.39	0.42	0.40	0.40	0.41
MINES	0.28	0.30	0.30	0.30	0.27	0.30	0.27
Nanom6A	0.41	0.37	0.33	0.31	0.28	0.28	0.24
m6Anet	-	-	0.44	0.38	0.33	0.32	0.30
ELIGOS	0.08	0.26	0.41	0.49	0.55	0.60	0.63
Epinano	0.37	0.34	0.34	0.31	0.32	0.28	0.28
Tombo_com	0.44	0.41	0.34	0.27	0.24	0.20	0.14
Nanocompore	0.04	0.07	0.14	0.23	0.26	0.31	0.43
Xpore	0.15	0.22	0.27	0.29	0.32	0.29	0.32
DiffErr	0.09	0.18	0.30	0.38	0.43	0.52	0.53
DRUMMER	0.08	0.18	0.32	0.39	0.46	0.55	0.56
ELIGOS_diff	0.07	0.20	0.33	0.43	0.48	0.58	0.61
Epinano_delta	0.44	0.41	0.37	0.31	0.30	0.27	0.27

Recall (miCLIP2)

Coverage	5-10	10-20	20-40	40-60	60-80	80-100	>=100
Tombo	0.24	0.35	0.41	0.42	0.42	0.41	0.36
MINES	0.33	0.32	0.31	0.29	0.29	0.27	0.22
Nanom6A	0.45	0.39	0.34	0.29	0.29	0.25	0.18
m6Anet	0.00	0.00	0.47	0.40	0.35	0.35	0.26
ELIGOS	0.10	0.32	0.46	0.54	0.59	0.59	0.61
Epinano	0.45	0.42	0.37	0.34	0.35	0.27	0.24
Tombo_com	0.50	0.44	0.35	0.28	0.26	0.18	0.10
Nanocompore	0.04	0.08	0.15	0.21	0.27	0.29	0.43
Xpore	0.18	0.23	0.28	0.27	0.32	0.27	0.26
DiffErr	0.11	0.22	0.33	0.43	0.46	0.47	0.55
DRUMMER	0.09	0.21	0.34	0.42	0.48	0.47	0.57
ELIGOS_diff	0.08	0.23	0.35	0.44	0.51	0.54	0.58
Epinano_delta	0.48	0.44	0.37	0.30	0.32	0.25	0.19

Precision (miCLIP)

Coverage	5-10	10-20	20-40	40-60	60-80	80-100	>=100
Tombo	0.14	0.14	0.16	0.15	0.15	0.13	0.10
MINES	0.18	0.24	0.32	0.36	0.33	0.34	0.29
Nanom6A	0.13	0.19	0.26	0.29	0.29	0.29	0.22
m6Anet	-	-	0.40	0.45	0.44	0.43	0.42
ELIGOS	0.16	0.23	0.27	0.25	0.25	0.23	0.13
Epinano	0.11	0.19	0.27	0.29	0.35	0.30	0.26
Tombo_com	0.10	0.15	0.26	0.38	0.49	0.51	0.53
Nanocompore	0.37	0.39	0.43	0.42	0.38	0.37	0.20
Xpore	0.28	0.37	0.46	0.48	0.47	0.45	0.38
DiffErr	0.26	0.31	0.35	0.33	0.31	0.31	0.14
DRUMMER	0.26	0.32	0.37	0.33	0.34	0.33	0.14
ELIGOS_diff	0.21	0.28	0.34	0.32	0.32	0.32	0.15
Epinano_delta	0.12	0.21	0.37	0.49	0.56	0.58	0.59

Precision (miCLIP2)

Coverage	5-10	10-20	20-40	40-60	60-80	80-100	>=100
Tombo	0.12	0.14	0.17	0.16	0.16	0.16	0.13
MINES	0.18	0.25	0.34	0.37	0.39	0.36	0.34
Nanom6A	0.12	0.19	0.26	0.28	0.32	0.30	0.24
m6Anet	-	-	0.42	0.49	0.50	0.54	0.50
ELIGOS	0.17	0.26	0.30	0.29	0.28	0.26	0.18
Epinano	0.11	0.21	0.29	0.33	0.40	0.35	0.30
Tombo_com	0.09	0.16	0.28	0.42	0.56	0.57	0.57
Nanocompore	0.34	0.41	0.45	0.41	0.41	0.41	0.28
Xpore	0.27	0.38	0.47	0.47	0.49	0.49	0.44
DiffErr	0.25	0.35	0.39	0.38	0.35	0.33	0.21
DRUMMER	0.25	0.35	0.39	0.37	0.37	0.34	0.21
ELIGOS_diff	0.18	0.30	0.35	0.35	0.36	0.35	0.20
Epinano_delta	0.11	0.21	0.37	0.50	0.63	0.62	0.60

Supplementary Table 8 Recall for all tools for m6A sites versus versus methylation rate/site enrichment. m6A profiles created from m6A-REF-seq, miCLIP and miCLIP2 results were used for validation separately.

m6A-REF-seq

Methylation rate	0-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1
Tombo	0.16	0.24	0.28	0.30	0.41
MINES	0.04	0.11	0.18	0.26	0.38
Nanom6A	0.07	0.17	0.27	0.36	0.57
m6Anet	0.05	0.11	0.17	0.31	0.34
ELIGOS	0.22	0.34	0.38	0.54	0.66
Epinano	0.09	0.23	0.34	0.50	0.65
Tombo_com	0.07	0.25	0.31	0.34	0.44
Nanocompore	0.11	0.13	0.16	0.23	0.22
Xpore	0.08	0.15	0.19	0.29	0.46
DiffErr	0.22	0.30	0.39	0.54	0.67
DRUMMER	0.24	0.31	0.41	0.52	0.65
ELIGOS_diff	0.22	0.30	0.38	0.49	0.65
Epinano_delta	0.11	0.28	0.34	0.54	0.71

miCLIP

Site enrichment	0-1	1-2	2-3	3-4	4-5	5-6	>6
Tombo	0.31	0.32	0.31	0.32	0.36	0.35	0.36
MINES	0.25	0.24	0.26	0.27	0.30	0.31	0.32
Nanom6A	0.25	0.29	0.30	0.32	0.36	0.36	0.40
m6Anet	0.24	0.21	0.20	0.18	0.19	0.20	0.18
ELIGOS	0.34	0.33	0.31	0.32	0.32	0.35	0.34
Epinano	0.24	0.25	0.28	0.30	0.32	0.34	0.40
Tombo_com	0.24	0.28	0.29	0.33	0.36	0.37	0.41
Nanocompore	0.19	0.16	0.14	0.13	0.15	0.15	0.15
Xpore	0.17	0.20	0.21	0.22	0.23	0.25	0.28
DiffErr	0.28	0.26	0.26	0.27	0.27	0.29	0.27
DRUMMER	0.28	0.27	0.27	0.28	0.27	0.29	0.28
ELIGOS_diff	0.30	0.29	0.28	0.29	0.31	0.30	0.30
Epinano_delta	0.24	0.28	0.31	0.33	0.37	0.41	0.44

miCLIP2

Site enrichment	0-1	1-2	2-3	3-4	4-5	5-6	>6
Tombo	0.21	0.31	0.33	0.36	0.39	0.42	0.42
MINES	0.11	0.22	0.28	0.32	0.36	0.40	0.38
Nanom6A	0.09	0.22	0.29	0.34	0.41	0.43	0.48
m6Anet	0.12	0.23	0.25	0.23	0.25	0.25	0.22
ELIGOS	0.27	0.35	0.41	0.42	0.42	0.44	0.43
Epinano	0.11	0.24	0.33	0.40	0.41	0.44	0.54
Tombo_com	0.11	0.25	0.31	0.39	0.43	0.43	0.50
Nanocompore	0.13	0.16	0.18	0.16	0.19	0.19	0.17
Xpore	0.08	0.17	0.23	0.26	0.28	0.33	0.34
DiffErr	0.21	0.29	0.31	0.33	0.33	0.37	0.36
DRUMMER	0.22	0.30	0.32	0.34	0.33	0.37	0.35
ELIGOS_diff	0.21	0.31	0.33	0.34	0.36	0.38	0.37
Epinano_delta	0.09	0.22	0.32	0.39	0.42	0.47	0.55