Supplementary Materials for

HOLMESv2: a CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation

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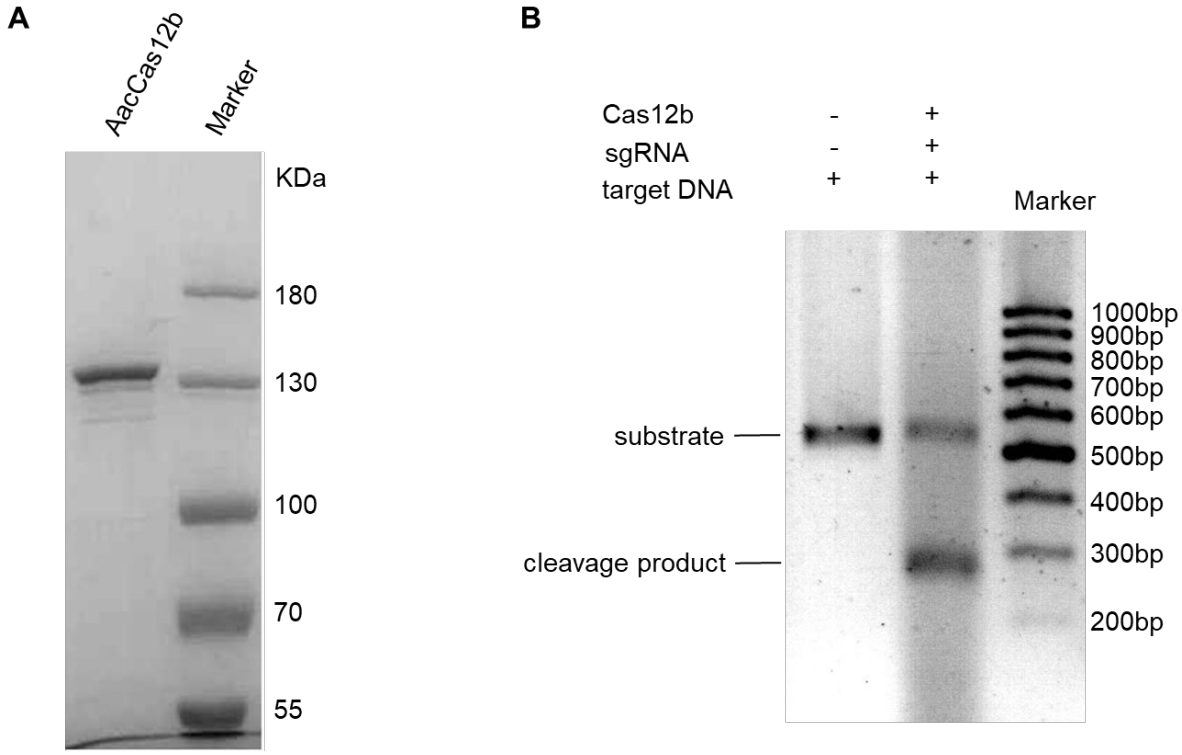

Figure S1. Cleavage of target dsDNA by purified recombinant AacCas12b. (A) SDS-PAGE analysis of purified recombinant AacCas12b. (B) Verification of the *cis*-cleavage activity of Cas12b against target dsDNA. The 536-bp PCR fragment (T1-536bp) was cleaved by purified AacCas12b with the guide sgRNA of sgRNA-T1 at 48 °C, obtaining two expected fragments of about 270 bps.

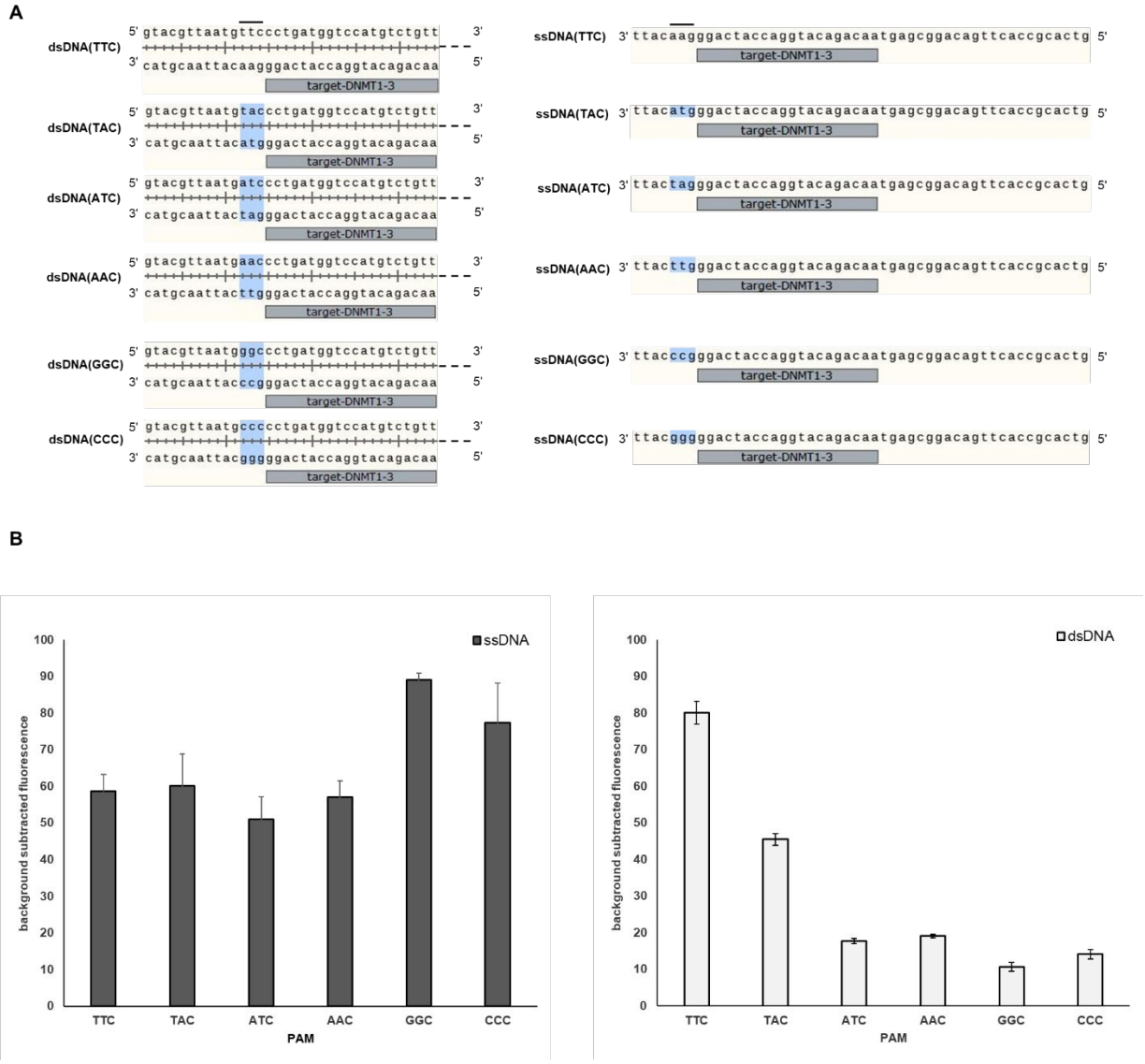

Figure S2. Mutational analysis of the PAM sequences in AacCas12b target DNAs. (A) Representation of target sequences (DNMT1-3) analyzed in Figure 2b. The PAM sequence in the wild type ("TTC") was overlined, while mutated sequences were indicated by blue background (e.g. 5′-TTC-3′, 5′-TAC-3′, 5′-ATC-3′, 5′-AAC-3′, 5′-GGC-3′ and 5′-CCC-3′). (B) Analysis of the *trans*-cleavage activity of AacCas12b with different PAM sequences indicated in Figure 2a. The fluorescence signal intensity of reaction systems with no target was taken as the background, which was subtracted from tested systems to obtain the real fluorescence intensity.

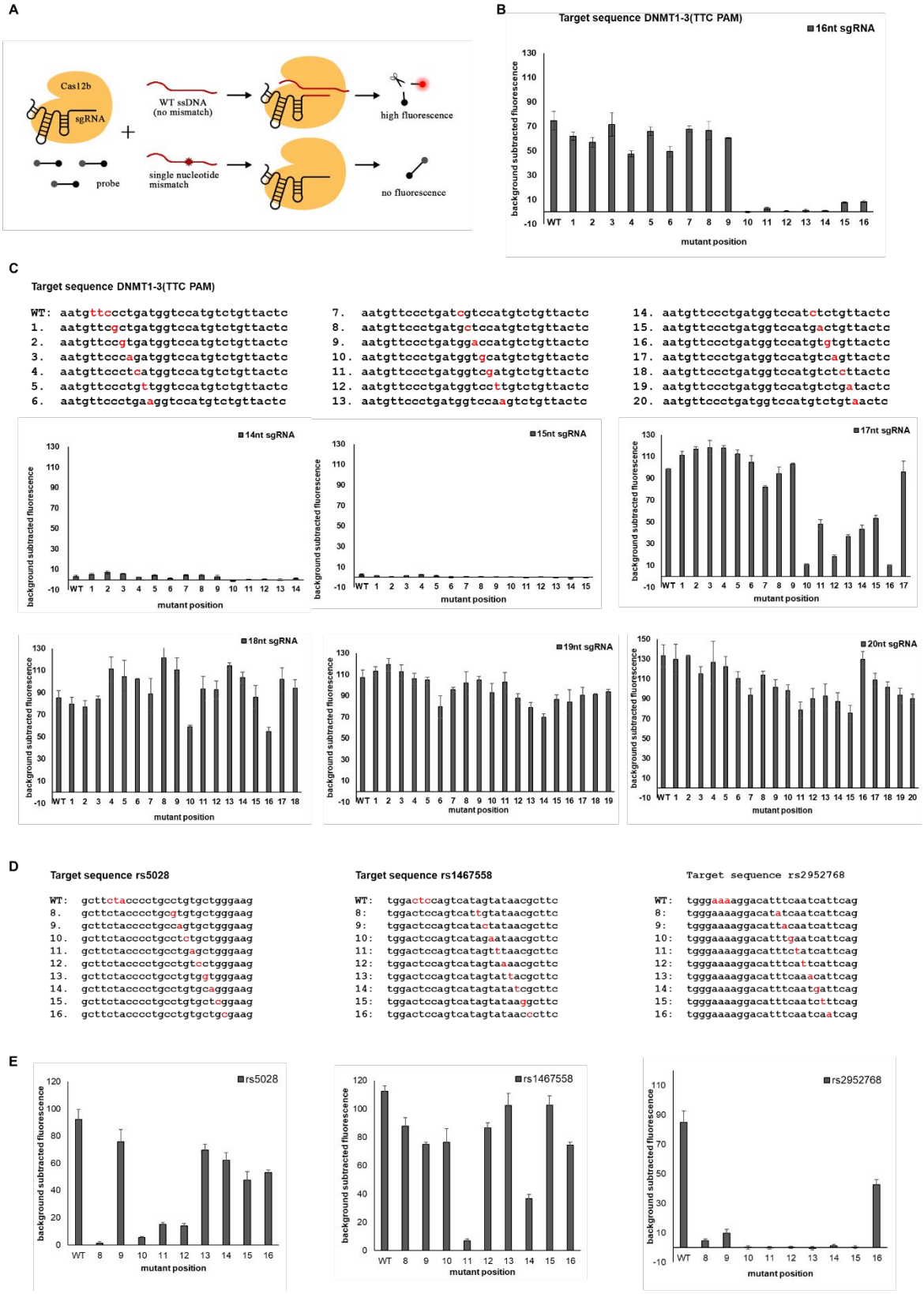

Figure S3. Analysis of the AacCas12b *trans*-cleavage activity with ssDNA targets containing single-nucleotide mismatch (SNM). (A, B) The schematic was illustrated (A), and the results (B) were obtained with the use of an sgRNA with 16-nt wild-type guide sequence. Mutated target sequences were listed in Figure 3C, and mutated sequences of 1 to

16 were analyzed with the 16-nt sgRNA. (C) Comparative analysis of the fluorescence signals with ssDNA targets containing SNM and sgRNAs with different lengths of guide sequences. (D) Presentation of ssDNA target sequences (i.e. rs5028, rs1467558, rs2952768) with SNM. (E) Comparative analysis of the fluorescence signals for target ssDNAs containing SNM, using16-nt sgRNAs. The fluorescence intensity was measured with a Varioskan Flash (ThermoFisher) with the background intensity subtracted.

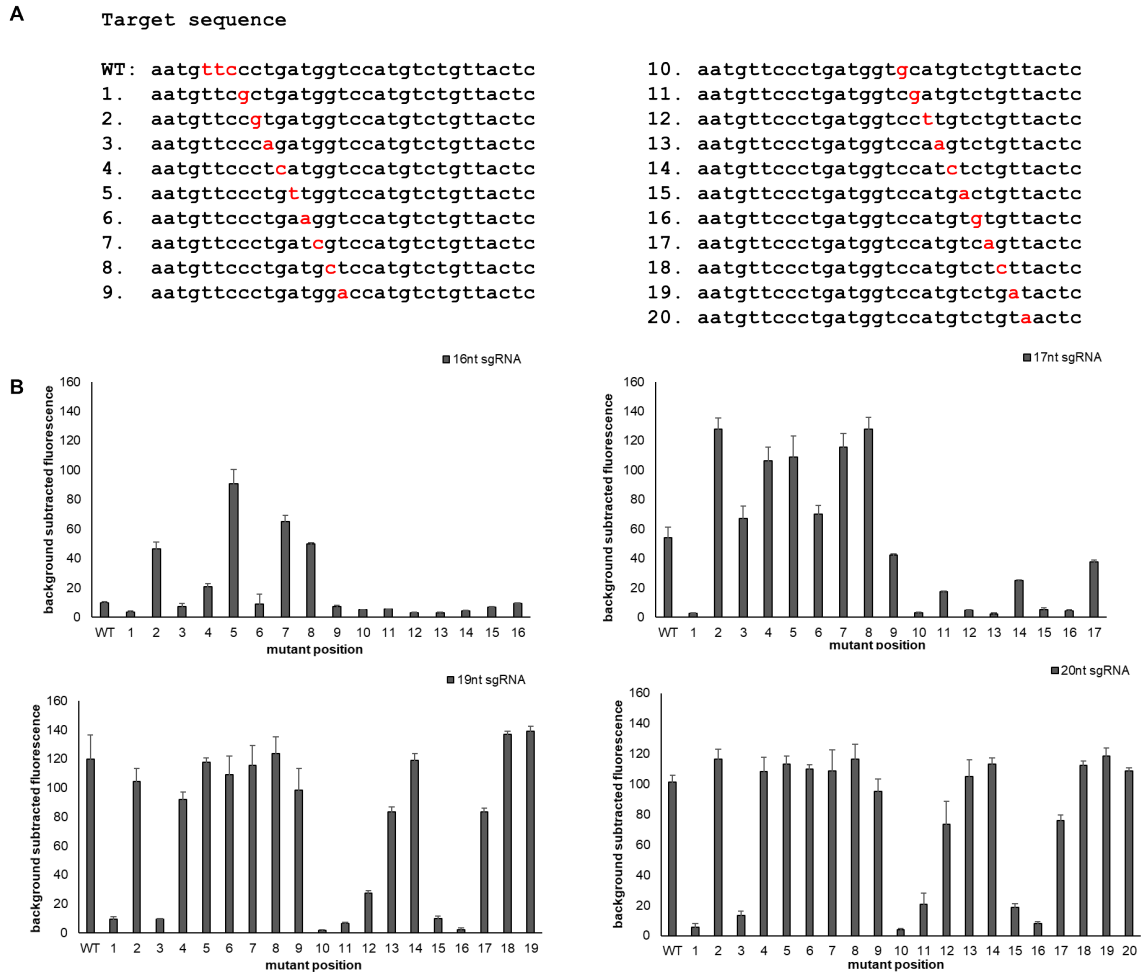

Figure S4. Analysis of the AacCas12b *trans*-cleavage activity with dsDNA targets containing SNM. (A) Presentation of dsDNA target sequences (DNMT1-3) with SNM. (B) Comparative analysis of the fluorescence intensity with target dsDNAs containing SNM. Target sequences were listed in Figure 4A, and sgRNAs with different lengths of guide sequences were analyzed. The fluorescence intensity was measured with a Varioskan Flash (ThermoFisher) with the background intensity subtracted.

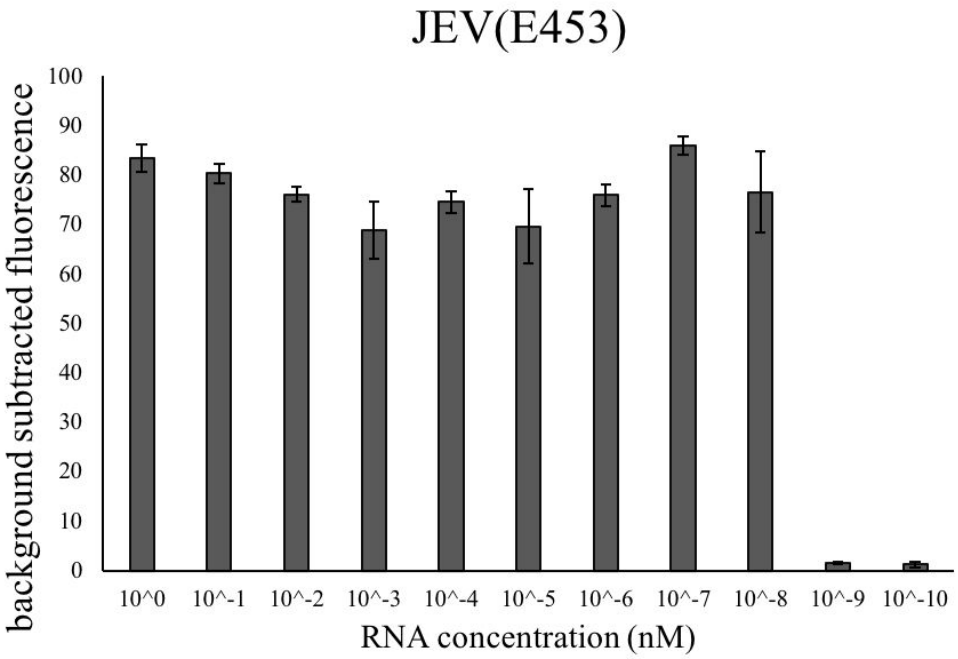

Figure S5. Determination of HOLMESv2 sensitivity for target RNA. Target RNA was prepared by *in vitro* transcription using a template containing the JEV E453 site, which was then purified, quantitated, serially diluted, amplified by LAMP. The LAMP product was then *trans*-cleaved by Cas12b, followed by fluorescence determination with a fluorescence reader. Error bars represented s.d. from n = 3 replicates.

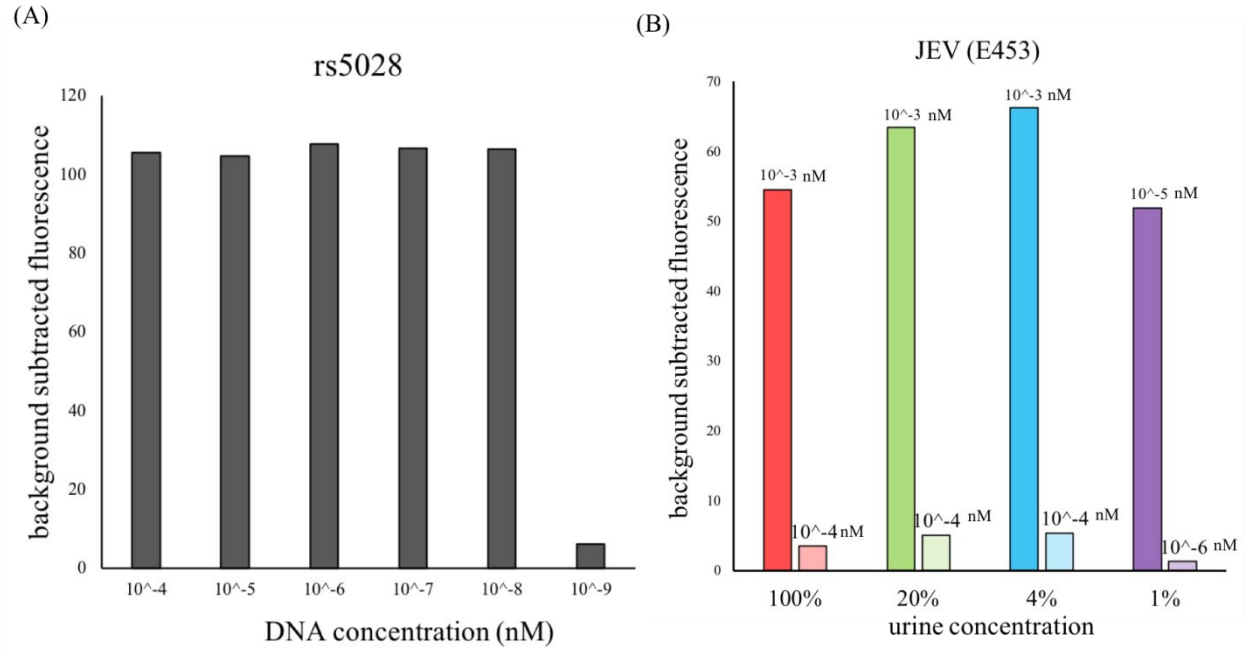

Figure S6. Determination of HOLMESv2 sensitivity for urine spike-ins of target DNA and target RNA. (A) Target DNA of pUC18-rs5028 was serially diluted by fresh urine, which was used as the DNA template for further HOLMESv2 detection. (B) Target RNA containing the JEV E453 site was diluted by fresh pure or diluted urine, which was then used as the RNA template for HOLMESv2 detection. The concentration of target RNA was labelled on each column. The above experiments were repeated twice with consistent results obtained.

COL1A2:

GGAGGCACCCTAGGGCCAGGGAAACTTTTGCCGTATAAATAGGGCAGATCCGGGCTTTATTATTTTAGCAC
CACGGCAGCAGGAGGTTTCGGCTAAGTTGGAGGTACTGGCCACGACTGCATGCCCGCGCCCGCCAGGTGAT
ACCTCCGCCGGTGACCCAGGGGCTCTGCGACACAAGGAGTCTGCATGTCTAAGTGCTAGACATGCTCAGCT
TTGTGGATACGCGGACTTTGTTGCTGCTTGCAGTAAC

COL1A2(BSP)-C:

GGAGGTATTTTAGGGTTAGGGAAATTTTTGTCGTATAAATAGGGTAGATTCGGGTTTTATTATTTTAGTAT
TACGGTAGTAGGAGGTTTCGGTTAAGTTGGAGGTATTGGTTACGATTGTATGTTCGCGTTCGTTAGGTGAT
ATTTTCGTCGGTGATTTAGGGGTTTTGCGATATAAGGAGTTTGTATGTTTAAGTGTTAGATATGTTTAGTT
TTGTGGATACGCGGATTTTGTTGTTGTTTGTAGTAAT

COL1A2(BSP)-T:

GGAGGTATTTTAGGGTTAGGGAAATTTTTGTTGTATAAATAGGGTAGATTTGGGTTTTATTATTTTAGTAT
TATGGTAGTAGGAGGTTTTGGTTAAGTTGGAGGTATTGGTTATGATTGTATGTTTGTGTTTGTCAGGTGAT
ATTTTTGTTGGTGATTTAGGGGTTTTGTGATATAAGGAGTTTGTATGTTTAAGTGTTAGATATGTTTAGTT
TTGTGGATATGTGGATTTTGTTGTTGTTTGTAGTAAT

Figure S7. Sequences of the promoter region of *COL1A2* gene in human cell line. The 13 CpG methylation islands were shown both in the wild type fragment and two fragments of COL1A2 (BSP)-C and COL1A2 (BSP)-T, representing bisulphite treated methylated and unmethylated *COL1A2* promoter region, respectively, were shown. The target sequence for quantitation of M3 site by HOLMESv2 was highlighted, and the sgRNA guide sequence (*ref to* Figure 4B) perfectly matched the target sequences in wild-type COL1A2 and COL1A2 (BSP)-C, but had SNM with that in COL1A2 (BSP)-T.

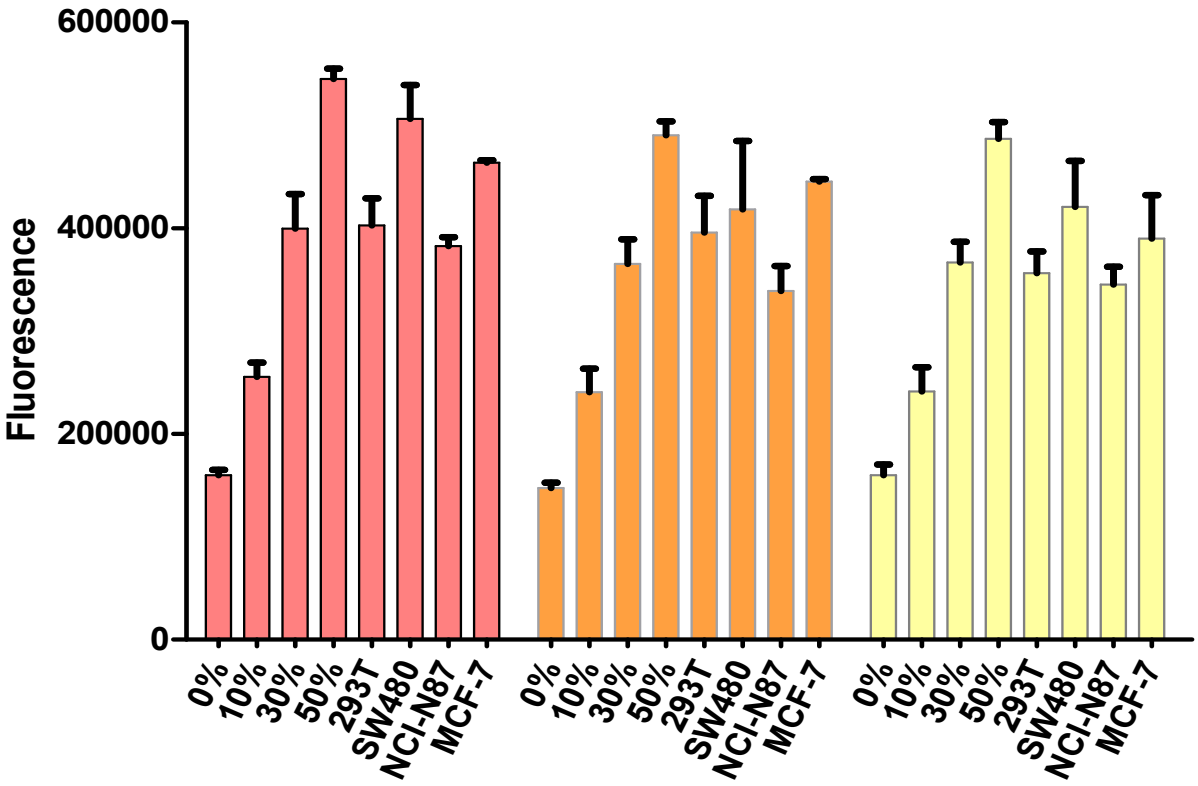

Figure S8. Quantitation of the methylation degree of M3 CpG site in *COL1A2* promoter region by HOLMESv2. The methylation degree of M3 in four different human cell lines (i.e. 293T, SW480, NCI-N87 and MCF-7) was determined as described in Supplementary Materials and Methods. The experiments were independently repeated three times, and error bars in each experiment represented s.d. from n = 3 replicates. The results obtained were compared with other methods in Figure 4D.

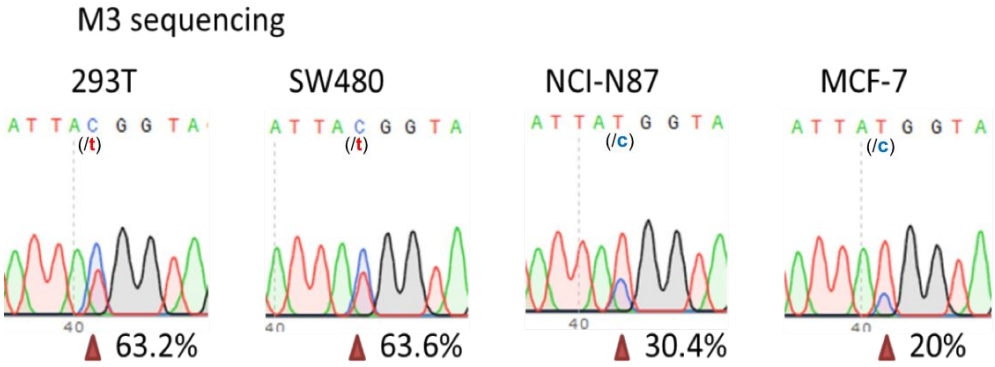

Figure S9. Determination of the methylation degree of M3 site in *COL1A2* promoter region by BSP-direct sequencing. The degree of M3 site methylation was calculated by the ratio of C peak area to the peak areas of both C and T of the M3 site, which was indicated by solid brown triangles. The results obtained were compared with other methods in Figure 4D.

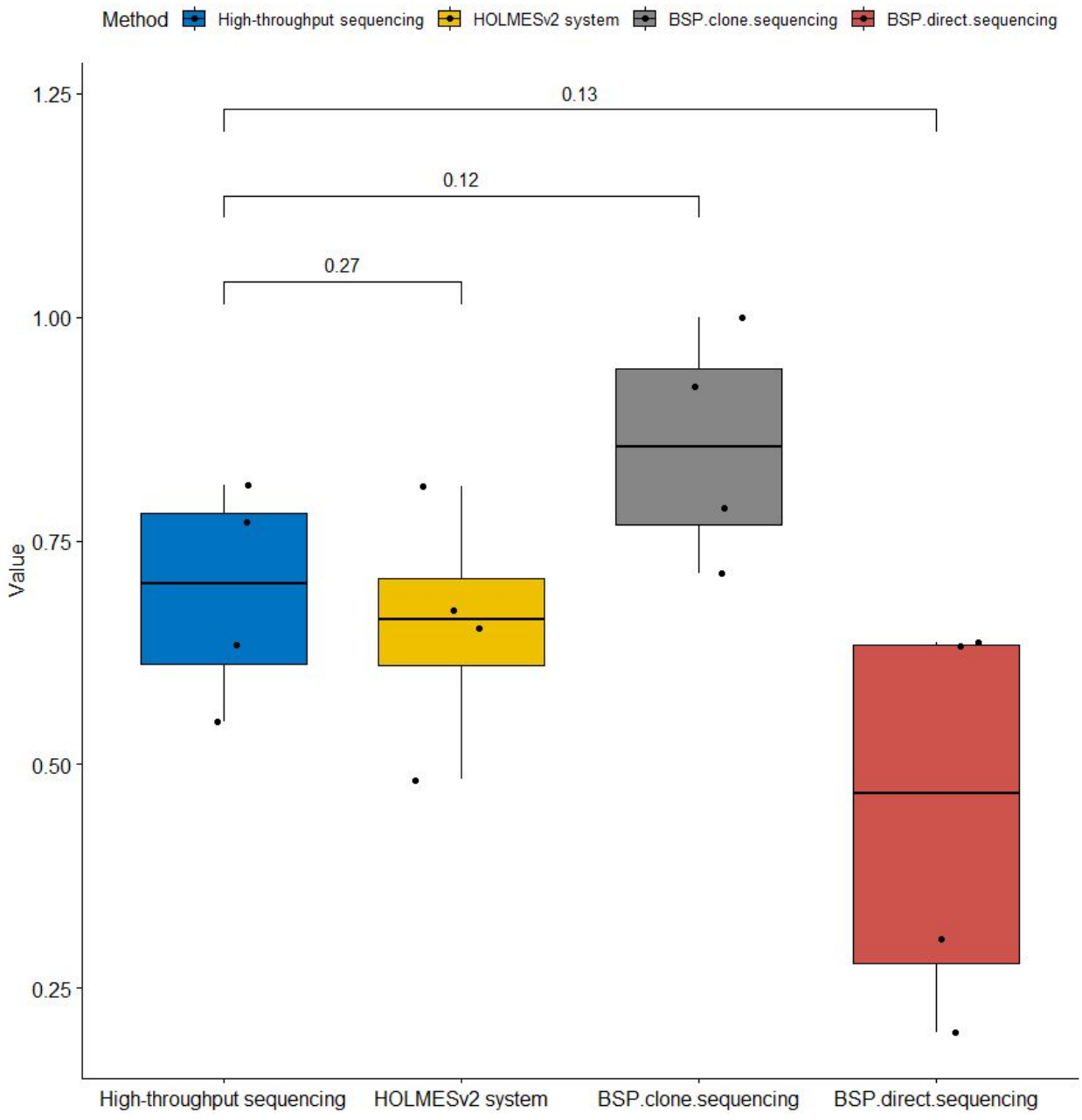

Figure S10. Paired t-test analysis of the results of DNA methylation degree measured by different methods. The p-values of the four groups of data were 0.615, 0.8098, 0.7583 and 0.1652, respectively, which indicated these data obeyed normal distribution. Statistical analysis using the paired t-test was performed between the high-throughput sequencing result and others, and the p-value was labelled.

Table S1. Oligonucleotides used for preparation of cleavage templates in this study

Oligo names	Sequences (5′-3′)
target-T1-F	tttctgtttgttatcgcaactttctactgaattcaagctttactctagaaagaggagaaaggatcc
target-T1-R	ggatcctttctcctctttctagagtaaagcttgaattcagtagaaagttgcgataacaaacagaaa
rs5082-F-198bp	aggaaatataggctggaaaggtcaag
rs5082-R-198bp	acattaggggtttgttgcacagtcc
pUC18-5082-F	tgcaacaaaccccctaatgtcgagctcgaattcgtaatcatgg
pUC18-5082-R	ctttccagcctatatttccttgcaggcatgcaagcttggcactg
DNMT1-3(TTC PAM)-F	aatgttccctgatggtccatgtctgttactcgcctgtcaagtggcgtgac
DNMT1-3(TTC PAM)-R	gtcacgccacttgacaggcgagtaacagacatggaccatcagggaacatt
DNMT1-3(AAC PAM)-R	gtcacgccacttgacaggcgagtaacagacatggaccatcagggttcatt
DNMT1-3(ATC PAM)-R	gtcacgccacttgacaggcgagtaacagacatggaccatcagggatcatt
DNMT1-3(TAC PAM)-R	gtcacgccacttgacaggcgagtaacagacatggaccatcagggtacatt
DNMT1-3(GGC PAM)-R	gtcacgccacttgacaggcgagtaacagacatggaccatcagggcccatt
DNMT1-3(CCC PAM)-R	gtcacgccacttgacaggcgagtaacagacatggaccatcaggggcatt
DNMT1-3-C1G-F	aatgttcgctgatggtccatgtctgttactcgcctgtcaagtggcgtgac
DNMT1-3-C1G-R	gtcacgccacttgacaggcgagtaacagacatggaccatcagcgaacatt
DNMT1-3-C2G-F	aatgttccgtgatggtccatgtctgttactcgcctgtcaagtggcgtgac
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DNMT1-3-T17A-R	gtcacgccacttgacaggcgagtaactgacatggaccatcagggaacatt
target-rs5028-R	agacttagatctgagccctccctcttcccagcacaggcaggggtagaagc

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target-rs2952768-T13A-R	aaaatagtgctttttacttttatctgaatgtttgaaatgtccttttccca
target-rs2952768-C14G-R	aaaatagtgctttttacttttatctgaatcattgaaatgtccttttccca
target-rs2952768-A15T-R	aaaatagtgctttttacttttatctgaaagattgaaatgtccttttccca
target-rs2952768-T16A-R	aaaatagtgctttttacttttatctgattgattgaaatgtccttttccca
FAM-N12-Eclipse	FAM-nnnnnnnnnnn-Eclipse
HEX-N12-BHQ1	HEX-nnnnnnnnnnn-BHQ1

Table S2. Oligonucleotides used for preparation of transcription templates in this study

Oligo names	Sequences (5′-3′)
T7-sgRNA-F	gaaattaatacgactcactataggg
ZL-sgRNA-T1-R	tcagtagaaagttgcgataagtgc
ZLsgRNA-DNMT1-3-R	aacagacatggaccatcagggtg
ZLsgRNA-DNMT1-3-14	catggaccatcagggtgccac
ZLsgRNA-DNMT1-3-15	acatggaccatcagggtgccac
ZLsgRNA-DNMT1-3-16	gacatggaccatcagggtgcc
ZLsgRNA-DNMT1-3-17	agacatggaccatcagggtgc
ZLsgRNA-DNMT1-3-18	cagacatggaccatcagggtg
ZLsgRNA-DNMT1-3-19	acagacatggaccatcagggtg
ZL-rs5028-16-R	ccagcacaggcagggggtgccacttctcagatttgagaag
ZL-rs1467558-16-R	cgttatactatgactggtgccacttctcagatttgagaag
ZL-rs2952768-16-R	atgattgaaatgtcctgtgccacttctcagatttgagaag
ZL-rs5028-16-G12T-R	ccagaacaggcagggggtgccacttctcagatttgagaag
ZL-rs5028-10SNP-18-R	cttcccagcacaggcagggtgccacttctcagatttgagaag
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ZL-rs5028-10SNP-20	ctcttcccagcacaggcagggtgccacttctcagatttgagaag
ZL-sgRNA-JEV-E453-R	tgtgatccaagacattccccgtgccacttctcagatttgagaag
ZL-sgRNA-JEV-NS170-R	ctcgtcagtgctctcctctcgtgccacttctcagatttgagaag
sgRNA-GAPDH-target3-7	ggcgtcttcaccaccatggagtgccacttctcagatttgagaag
sgRNA-CDR1as-target1-3	ctccaagtcttccagtaaatgtgccacttctcagatttgagaag
sgRNA-COL1A2m3-C12-17	ctaccgtaatactaaaagtgccacttctcagatttgagaag
JEV-E-T7-F	gaaattaatacgactcactatagggctggaagcacgctgggcaaagc
JEV-E-R	gtgtcagcatgcacattggtcgc

Table S3. Oligonucleotides used for amplification or other usage in this study

Oligo names	Sequences (5′-3′)
LAMP-DNM-F3	gtgaacgttcccttagcact
LAMP-DNM-B3	gggagggcagaactagtcc
LAMP-DNM-FIP	cgccacttgacaggcgagtaactgccacttattgggtcagc
LAMP-DNM-BIP	gcgtgttccccagagtgacttagcagcttcctcctcctt
LAMP-DNM-LoopF	aggaaacattaacgtactgatg
LAMP-DNM-LoopB	ttccttttatttcccttcagc
ASP-primer	ggtttcggatgttacagcgt
ASP-rs5028-F	caagcaccccacccgctcacccacctcctcctttg
ASP-rs5028-R	ggtttcggatgttacagcgtgtgctggaagacttagatctgag
LAMP-rs5028-F3	gctggaaaggtcaagggac
LAMP-rs5028-B3	ggggtttgttgcacagtcc
LAMP-rs5028-FIP-10PAM	gaaagaagcaaaggcaggaggtttgcccaaggtcacacag
LAMP-rs5028-BIP	ctgggaagagggagggctcagtgttgccacactttcactgg
LAMP-rs5028-LoopF	gtgagcgggtggggtgct
LAMP-rs5028-LoopB	tctaagtcttccagcacgggatc
LAMP-E453-F3	tgacacagcctgggactt
LAMP-E453-B3	cacacctcctgtggctaag
LAMP-E453-FIP	gagtgttctgaaggcaccaccagctccattggaggggtct
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LAMP-E453-LoopB	tgctctggatgggcgtcaacg
LAMP-NS170-F3	gagacaaaggaatgccctga
LAMP-NS170-B3	gccctctcaagtttccatgt
LAMP-NS170-FIP	cgggttgatgtgatgccaaagcgagcacagagcttggaaca
LAMP-NS170-BIP	ggagcgatcataggtacggctggcgactctcaatccagtacg
LAMP-NS170-LoopF	gaagtcttcgatttgcatg
LAMP-NS170-LoopB	acatgtggcagtccatagtgac
GAPDH-LAMP-target3-FIP	agggatctcgctcctggaagatcaccgtcaaggctgagaac
GAPDH-LAMP-target3-BIP	cgatgctggcgctgagtacgaatgagccccagccttct
GAPDH-LAMP-target3-F3	tccacccatggcaaattcc
GAPDH-LAMP-target3-B3	agggggcagagatgatgac
GAPDH-LAMP-target3-LoopF	tccattgatgacaagcttccc
GAPDH-LAMP-target3-LoopB	tcgtggagtccactggcgtc
CDR1as-LAMP-FIP	ccagatcttccaggaaaatccacatctgtatttgatggaagacctt
CDR1as-LAMP-BIP	agaccagtaattgctggaagacttgtcttccaagaagctcc
CDR1as-LAMP-F3	agatttttctggaagacatgg
CDR1as-LAMP-B3	atgtcttccggacaatcc
CDR1as-LAMP-LoopB	tgctggaagacttgatttactgg
pUC18-1-F	atctgagaagtggcacttatcgcaactttctactgaggtcatagctgtttcctgtgtga
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sgRNA-DNMT1-3-F	cctgatggtccatgtctgttggtcatagctgtttcctgtgtg
sgRNA-DNMT1-3-R	tggaccatcagggtgccacttctcagatttgagaag
T1-(TTC)PAM-F	cttatcgcaactttctactgaattcg
T1-(TTC)PAM-R	aaaactggccgtcgttttacaacg

T1-300-F	ttaactatgcggcatcagagcag
T1-300-R	gcgcagcgagtcagtgagcgagg
DNMT1-3-800-F	agccccacgtgtctttgtctcaag
DNMT1-3-1200-R	tgggcaacaagaacgaaactgtctc
PAM-DNMT1-3-TTC(PAM)-F	gtacgttaatgttccctgatggtccatg
PAM-DNMT1-3-AAC(PAM)-F	gtacgttaatgaaccctgatggtccatg
PAM-DNMT1-3-ATC(PAM)-F	gtacgttaatgatccctgatggtccatg
PAM-DNMT1-3-TAC(PAM)-F	gtacgttaatgtaccctgatggtccatg
PAM-DNMT1-3-GGC(PAM)-F	gtacgttaatgggccctgatggtccatg
PAM-DNMT1-3-CCC(PAM)-F	gtacgttaatgcccccctgatggtccatg
PAM-DNMT1-3-(PAM)-R	acaacagcttcatgtcagccaag
COL1A2-F	ggaggcaccctagggccagggaaa
COL1A2-R	gttactgcaagcagcaacaaagtcc
COL1A2(BSP)-F	ggaggtattttagggttagggaaa
COL1A2(BSP)-R	attactacaaacaacaacaaaatcc

Table S4. DNA methylation detection by NGS-based high-throughput sequencing

293T_CpG						
chrBase	chr	base	strand	coverage	freqC	freqT
COL1A2.32	COL1A2	32	F	2378988	64.5	35.5
COL1A2.51	COL1A2	51	F	2338459	64.57	35.43
COL1A2.74	COL1A2	74	F	2358444	65.26	34.74
COL1A2.90	COL1A2	90	F	2443064	65.85	34.15
COL1A2.114	COL1A2	114	F	4655434	57.76	42.24
COL1A2.126	COL1A2	126	F	4684781	60.55	39.45
COL1A2.128	COL1A2	128	F	4675700	60.51	39.49
COL1A2.132	COL1A2	132	F	4673665	60.99	39.01
COL1A2.148	COL1A2	148	F	4668904	62.14	37.86
COL1A2.151	COL1A2	151	F	2436989	64.52	35.48
COL1A2.170	COL1A2	170	F	2362032	64.82	35.18
COL1A2.223	COL1A2	223	F	2380493	64.61	35.39
COL1A2.225	COL1A2	225	F	2376481	64.4	35.6
COL1A2.10	COL1A2	10	F	17	0	100
COL1A2.17	COL1A2	17	F	458	0	100
COL1A2.45	COL1A2	45	F	3	0	100
COL1A2.77	COL1A2	77	F	3	0	100
COL1A2.80	COL1A2	80	F	111	0	100
COL1A2.135	COL1A2	135	F	277	19.49	80.51
COL1A2.159	COL1A2	159	F	165	26.67	73.33
COL1A2.175	COL1A2	175	F	1	0	100
COL1A2.209	COL1A2	209	F	15	0	100
SW480_CpG						
chrBase	chr	base	strand	coverage	freqC	freqT
COL1A2.32	COL1A2	32	F	2076441	81.02	18.98
COL1A2.51	COL1A2	51	F	2043831	80.57	19.43
COL1A2.74	COL1A2	74	F	2059679	81.06	18.94
COL1A2.90	COL1A2	90	F	2090001	81.04	18.96

COL1A2.114	COL1A2	114	F	4044019	70.87	29.13
COL1A2.126	COL1A2	126	F	4073003	77.83	22.17
COL1A2.128	COL1A2	128	F	4063524	78.57	21.43
COL1A2.132	COL1A2	132	F	4064823	76.54	23.46
COL1A2.148	COL1A2	148	F	4059230	76.93	23.07
COL1A2.151	COL1A2	151	F	2089376	79.9	20.1
COL1A2.170	COL1A2	170	F	2053498	80.02	19.98
COL1A2.223	COL1A2	223	F	2072221	80.77	19.23
COL1A2.225	COL1A2	225	F	2068422	80.17	19.83
COL1A2.10	COL1A2	10	F	1	0	100
COL1A2.17	COL1A2	17	F	101	0	100
COL1A2.45	COL1A2	45	F	1	0	100
COL1A2.77	COL1A2	77	F	2	0	100
COL1A2.80	COL1A2	80	F	81	0	100
COL1A2.135	COL1A2	135	F	88	6.82	93.18
COL1A2.159	COL1A2	159	F	66	3.03	96.97
COL1A2.175	COL1A2	175	F	1	0	100
COL1A2.209	COL1A2	209	F	25	0	100
NCI-N87_CpG						
chrBase	chr	base	strand	coverage	freqC	freqT
COL1A2.32	COL1A2	32	F	2029759	46.55	53.45
COL1A2.51	COL1A2	51	F	1992909	46.92	53.08
COL1A2.74	COL1A2	74	F	2013393	48.27	51.73
COL1A2.90	COL1A2	90	F	2042482	47.87	52.13
COL1A2.114	COL1A2	114	F	3989985	38.85	61.15
COL1A2.126	COL1A2	126	F	4010707	39.68	60.32
COL1A2.128	COL1A2	128	F	4005485	39.46	60.54
COL1A2.132	COL1A2	132	F	4005468	41.21	58.79
COL1A2.148	COL1A2	148	F	4010833	43.23	56.77
COL1A2.151	COL1A2	151	F	2058953	47.01	52.99
COL1A2.170	COL1A2	170	F	2020069	48.06	51.94
COL1A2.223	COL1A2	223	F	2029231	47.47	52.53
COL1A2.225	COL1A2	225	F	2027590	46.99	53.01
COL1A2.16	COL1A2	16	F	1	0	100
COL1A2.17	COL1A2	17	F	55	0	100
COL1A2.45	COL1A2	45	F	3	0	100
COL1A2.77	COL1A2	77	F	7	0	100
COL1A2.80	COL1A2	80	F	98	0	100
COL1A2.107	COL1A2	107	F	1	0	100
COL1A2.135	COL1A2	135	F	133	30.83	69.17
COL1A2.159	COL1A2	159	F	116	50	50
COL1A2.209	COL1A2	209	F	9	0	100
MCF-7_CpG						
chrBase	chr	base	strand	coverage	freqC	freqT
COL1A2.32	COL1A2	32	F	2441346	65.81	34.19
COL1A2.51	COL1A2	51	F	2398016	66.29	33.71

COL1A2.74	COL1A2	74	F	2421164	67.3	32.7
COL1A2.90	COL1A2	90	F	2485535	67.85	32.15
COL1A2.114	COL1A2	114	F	4773382	59.78	40.22
COL1A2.126	COL1A2	126	F	4803428	62.63	37.37
COL1A2.128	COL1A2	128	F	4794842	62.6	37.4
COL1A2.132	COL1A2	132	F	4792864	63.09	36.91
COL1A2.148	COL1A2	148	F	4785523	64.28	35.72
COL1A2.151	COL1A2	151	F	2484995	66.55	33.45
COL1A2.170	COL1A2	170	F	2423406	66.95	33.05
COL1A2.223	COL1A2	223	F	2441330	66.57	33.43
COL1A2.225	COL1A2	225	F	2437003	66.25	33.75
COL1A2.10	COL1A2	10	F	3	0	100
COL1A2.17	COL1A2	17	F	257	0	100
COL1A2.45	COL1A2	45	F	8	0	100
COL1A2.77	COL1A2	77	F	12	8.33	91.67
COL1A2.80	COL1A2	80	F	90	1.11	98.89
COL1A2.107	COL1A2	107	F	1	0	100
COL1A2.135	COL1A2	135	F	226	11.5	88.5
COL1A2.159	COL1A2	159	F	181	28.73	71.27
COL1A2.209	COL1A2	209	F	18	0	100

Note: COL1A2.74 was the M3 site in the promoter region of *COL1A2* gene, and the results of COL1A2.74 highlighted in bold were compared with other methods in Figure 4D.

Table S5. Comparison of HOLMESv2 and HOLMES

Methods	Sensitivity (nM)	Speed (min)	Steps	Reference
HOLMES	10^{-8}	< 60	2	[1]
HOLMESv2	10^{-8}	< 60	2	This study
one-step HOLMESv2	10^{-5}	70 - 120	1	This study

REFERENCES

[1] Li, S. Y., Cheng, Q. X., Wang, J. M., Li, X. Y., Zhang, Z. L., Gao, S., Cao, R. B., Zhao, G. P., and Wang, J. (2018) CRISPR-Cas12a-assisted nucleic acid detection, *Cell discovery 4*, 20.