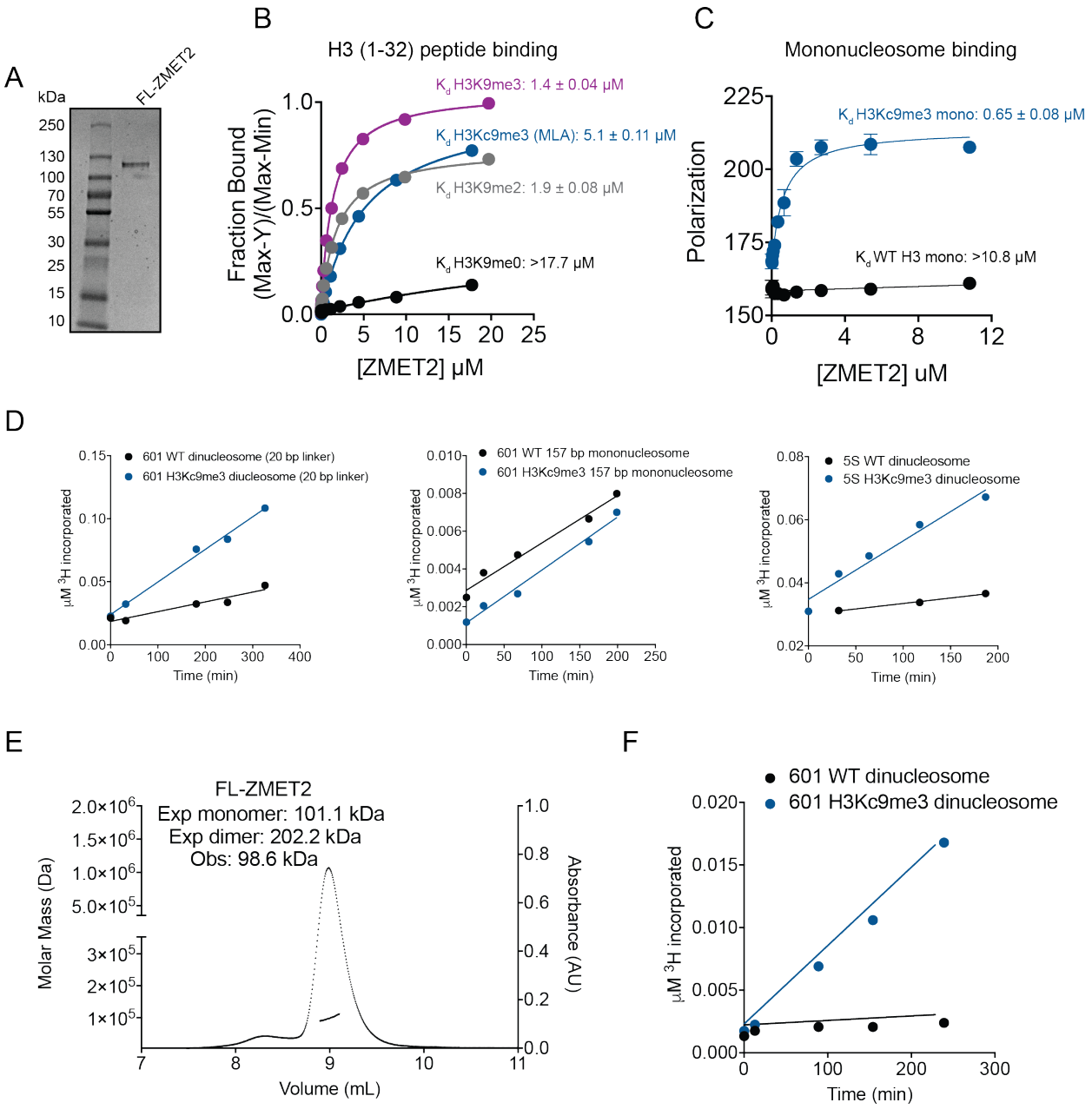


Figure S1. Binding and activation properties of full-length recombinant ZMET2. Related to Figure 1.

(A) Denaturing gel stained with Simply Blue showing purified recombinant full length ZMET2.

(B) Fluorescence polarization binding curves for ZMET2 binding to 32mer H3 tail peptides with differential methylation states. K_d values for H3K9me0, H3K9me2,

H3K9me3 and H3Kc9me3 (MLA) peptides are >17.7 μM, 1.9 μM, 1.4 μM, and 5.1 μM, respectively.

(C) Affinity of ZMET2 for mononucleosomes with and without methyl lysine analog (MLA) modification of lysine 9 measured by fluorescence polarization. Measurements were done in duplicate (n=2).

(D) Raw DNA methyltransferase time courses for ZMET2 and the substrates depicted in Figure 1B.

(E) Molar mass for full-length ZMET2 as measured by size exclusion chromatography coupled with multi angle light scattering. ZMET2 was injected onto the SEC-MALS system at a concentration of 19 μM.

(F) Raw DNA methyltransferase time course associated with Figure 1E for ZMET2 DNA methylation on WT and H3Kc9me3 dinucleosomes under multiple-turnover conditions.

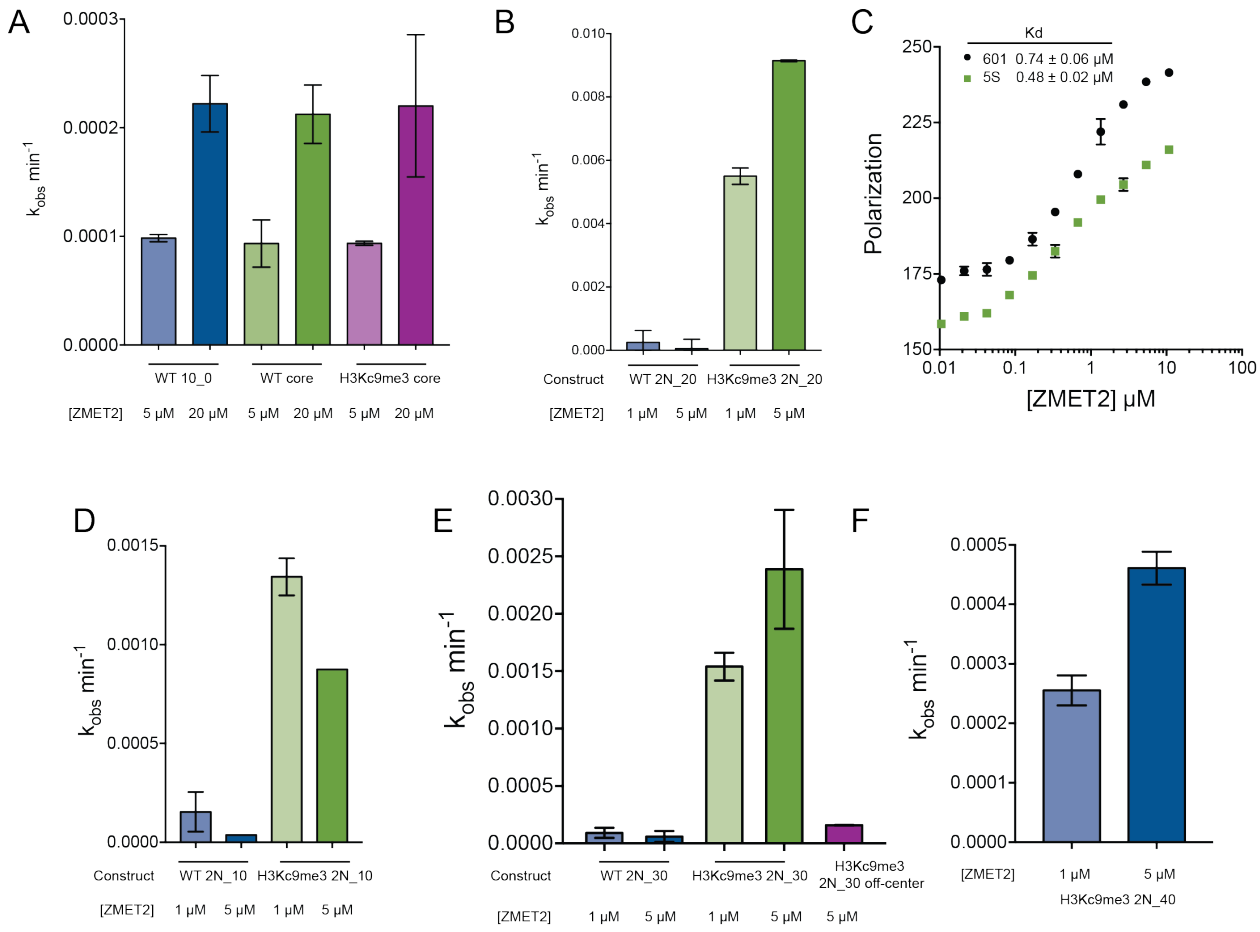


Figure S2. ZMET2 activity is dependent on nucleosome spacing. Related to Figures 1 and S1.

(A) Rate constants calculated for ZMET2 MTase activity on mononucleosome substrates at varying [ZMET2].
(B) Rate constants calculated for ZMET2 DNA MTase activity on dinucleosomes with a 20 bp linker at varying concentrations of ZMET2.
(C) Fluorescence polarization curve for ZMET2 bindng to mononucleosomes assembled with either 601 or 5S DNA.
(D) Rate constants calculated for ZMET2 DNA MTase activity on dinucleosomes with a 10 bp linker at varying concentrations of ZMET2.
(E) Rate constants calculated for ZMET2 DNA MTase activity on dinucleosomes with one CHG site (CTG) positioned in the center of the 30 bp linker (WT 2N_30 and H3Kc9me3 2N_30, varying concentrations of ZMET2) or two CHG sites (both CTG) positioned at either end of the linker (H3Kc9me3 2N_30_off-center).
(F) Rate constants calculated for ZMET2 DNA MTase activity on dinucleosomes with a 40 bp linker at varying concentrations of ZMET2.

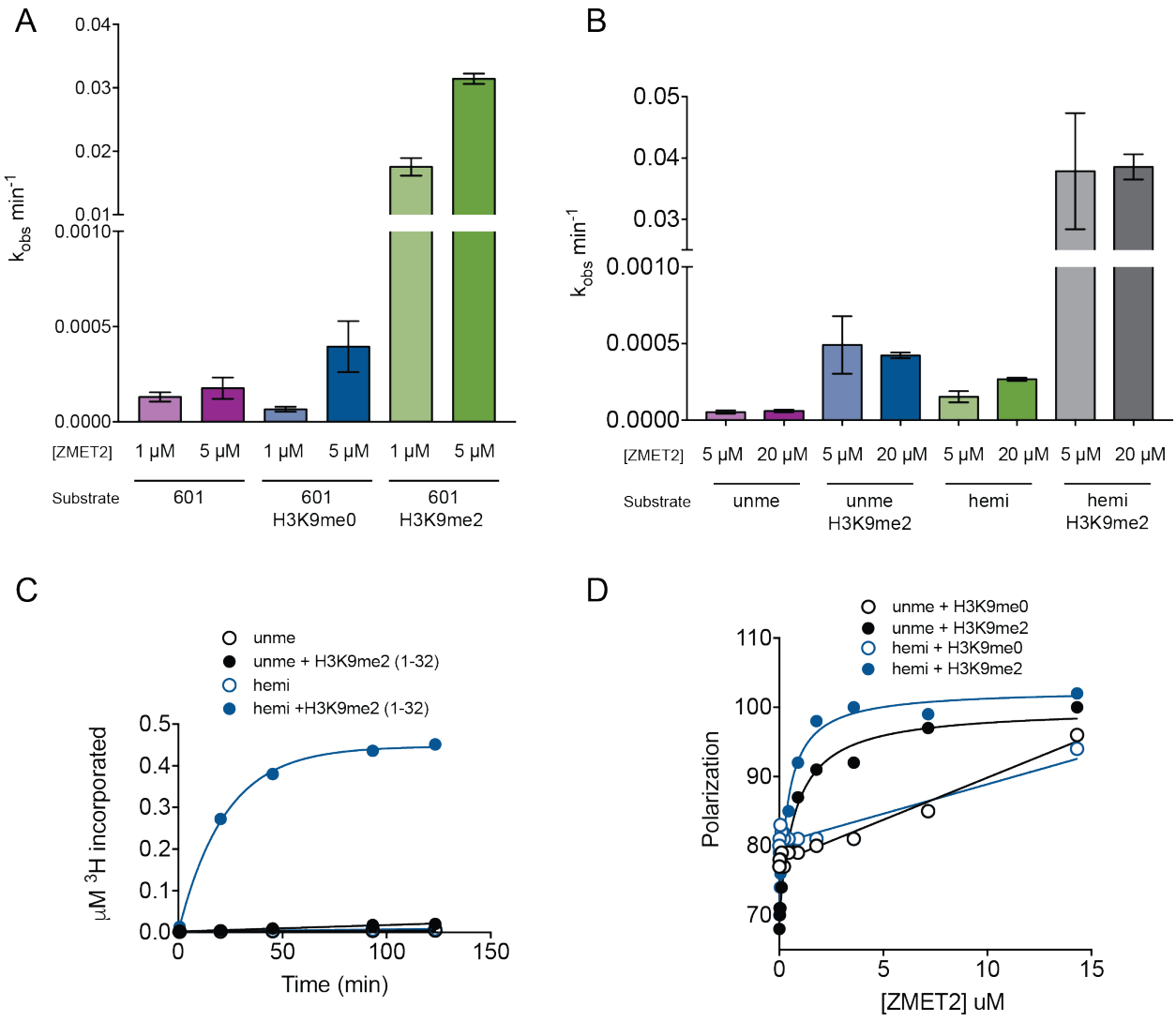


Figure S3. ZMET2 uses the H3K9me mark and hemimethylation for binding as well as activity. Related to Figure 2.

(A) Rate constants for ZMET2 DNA MTase activity on naked 157 bp 601 DNA in the presence and absence of H3 tail peptides at varying ZMET2 concentrations.
(B) Rate constants for ZMET2 DNA MTase activity on 38 bp DNA substrates in the presence and absence of H3 tail peptides at varying ZMETs2 concentrations.
(C) DNA methyltransferase time course for ZMET2 DNA MTase activity on 38 bp unmethylated or hemimethylated DNA substrates in the presence and absence of H3 tail peptides at varying ZMET2 concentrations.
(D) Fluorescence polarization binding curves for ZMET2 binding to 38 bp duplexed DNA in the presence or absence of methylated or unmethylated H3 tail peptides.

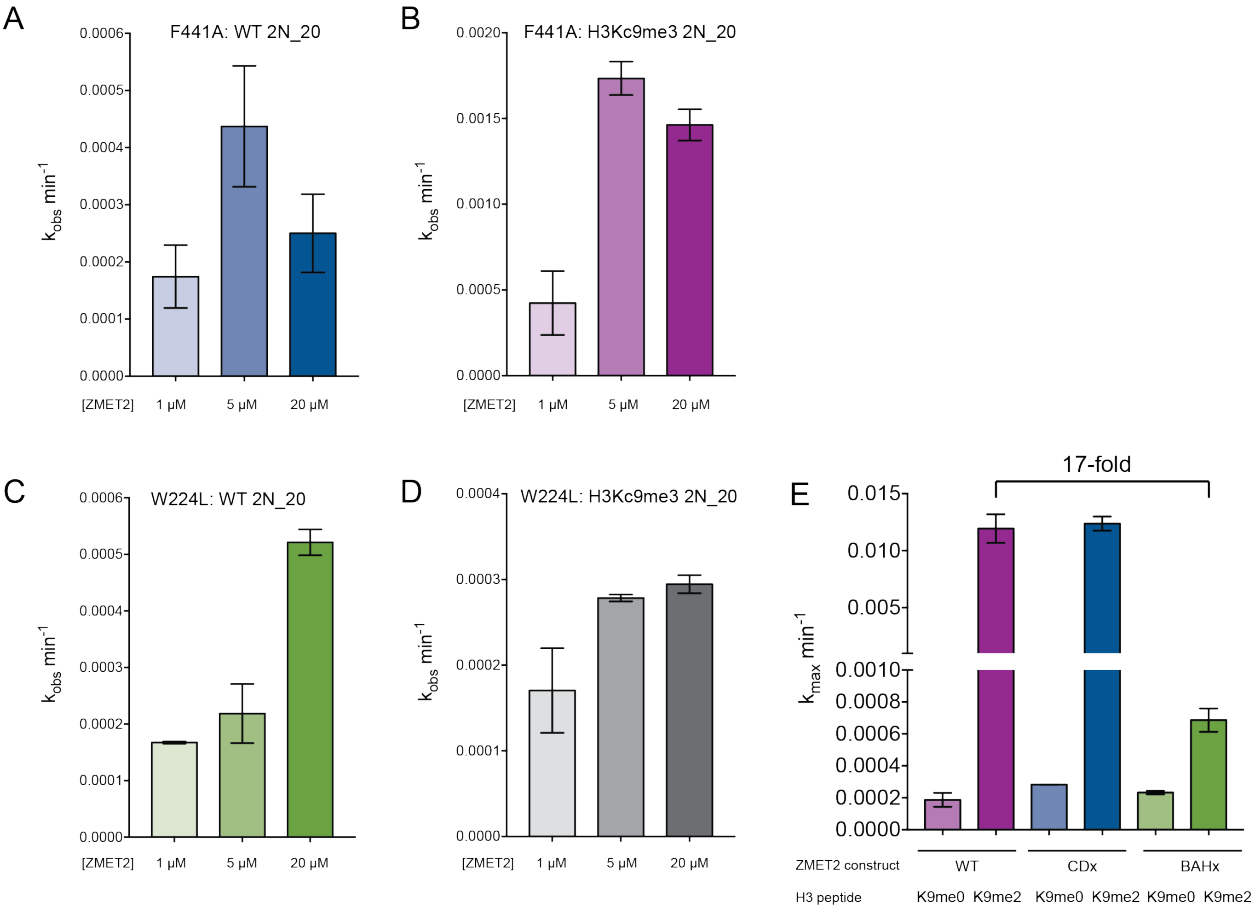
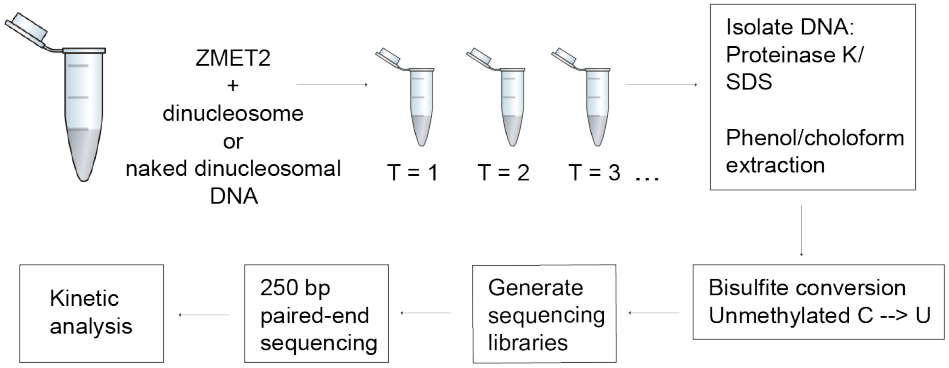


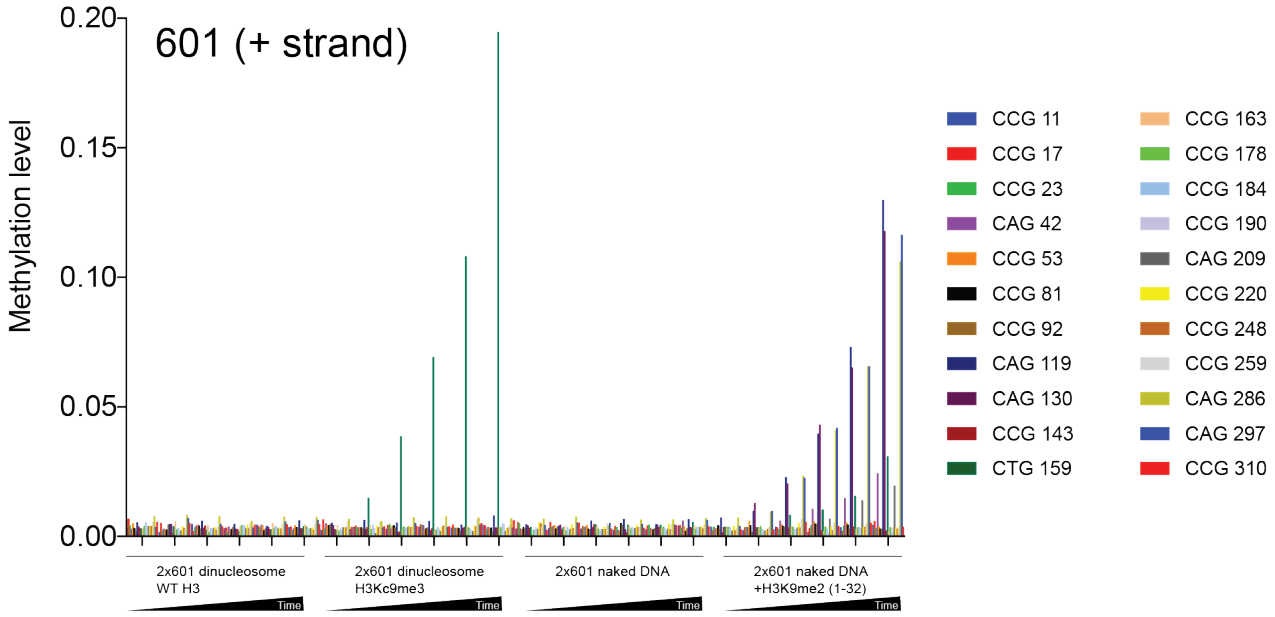
Figure S4. The ZMET2 CD recognizes H3K9me in the binding step and the BAH domain recognizes H3K9me in the catalytic step. Related to Figure 3.

(A) Rate constants for DNA methylation of WT dinucleosomes with a 20 bp linker (2N_20) under varying concentrations of F441A ZMET2.
(B) Rate constants for DNA methylation of H3Kc9me3 dinucleosomes with a 20 bp linker (2N_20) under varying concentrations of F441A ZMET2.
(C) Rate constants for DNA methylation of WT dinucleosomes with a 20 bp linker (2N_20) under varying concentrations of W224L ZMET2.
(D) Rate constants for DNA methylation of H3Kc9me3 dinucleosomes with a 20 bp linker (2N_20) under varying concentrations of W224L ZMET2.
(E) DNA methyltransferase activity for WT, CDx (F441A) and BAHx (W224L) ZMET2 on 157 bp DNA with 601 sequence in the presence of H3K9me0 (1-32) or H3K9me2 (1-32) *in trans*.

A


ZMET2
+
dinucleosome
or
naked dinucleosomal
DNA
T = 1
T = 2
T = 3 ...
Isolate DNA:
Proteinase K/
SDS
Phenol/choloform
extraction
Bisulfite conversion
Unmethylated C --> U
Generate
sequencing
libraries
250 bp
paired-end
sequencing
Kinetic
analysis

B


601 (+ strand)
Methylation level
0.20
0.15
0.10
0.05
0.00
2x601 dinucleosome
WT H3
2x601 dinucleosome
H3Kc9me3
2x601 naked DNA
2x601 naked DNA
+H3K9me2 (1-32)
Time
CCG 11
CCG 17
CCG 23
CAG 42
CCG 53
CCG 81
CCG 92
CAG 119
CAG 130
CCG 143
CTG 159
CCG 163
CCG 178
CCG 184
CCG 190
CAG 209
CCG 220
CCG 248
CCG 259
CAG 286
CAG 297
CCG 310

C

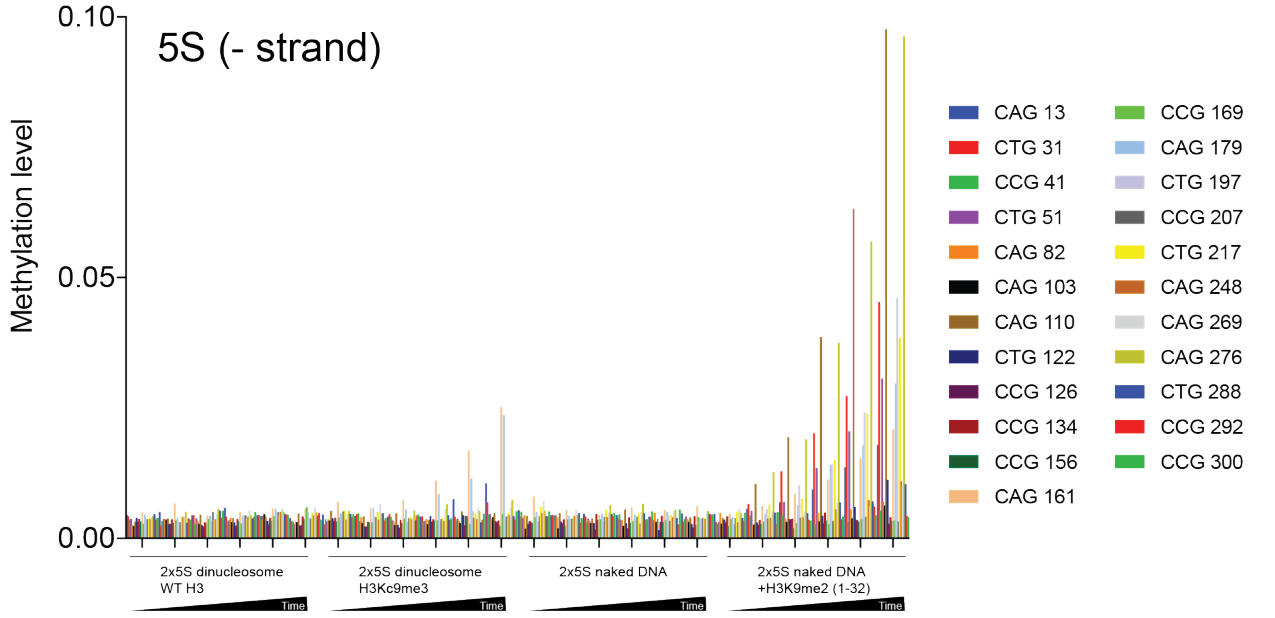

5S (- strand)
Methylation level
0.10
0.05
0.00
2x5S dinucleosome
WT H3
2x5S dinucleosome
H3Kc9me3
2x5S naked DNA
2x5S naked DNA
+H3K9me2 (1-32)
Time
CAG 13
CTG 31
CCG 41
CTG 51
CAG 82
CAG 103
CAG 110
CTG 122
CCG 126
CCG 134
CCG 156
CAG 161
CCG 169
CAG 179
CTG 197
CCG 207
CTG 217
CAG 248
CAG 269
CAG 276
CTG 288
CCG 292
CCG 300

Figure S5. ZMET2 preferentially methylates the linker DNA in H3Kc9me3 dinucleosomes. Related to Figure 4.

(A) Experimental scheme for DNA methyltransferase assay followed by bisulfite conversion and sequencing.
(B) Time courses for DNA methylation by ZMET2 on 601 dinucleosomes at all CHG sites on the plus strand.
(C) Time courses for DNA methylation by ZMET2 on 5S dinucleosomes at all CHG sites on the minus strand.

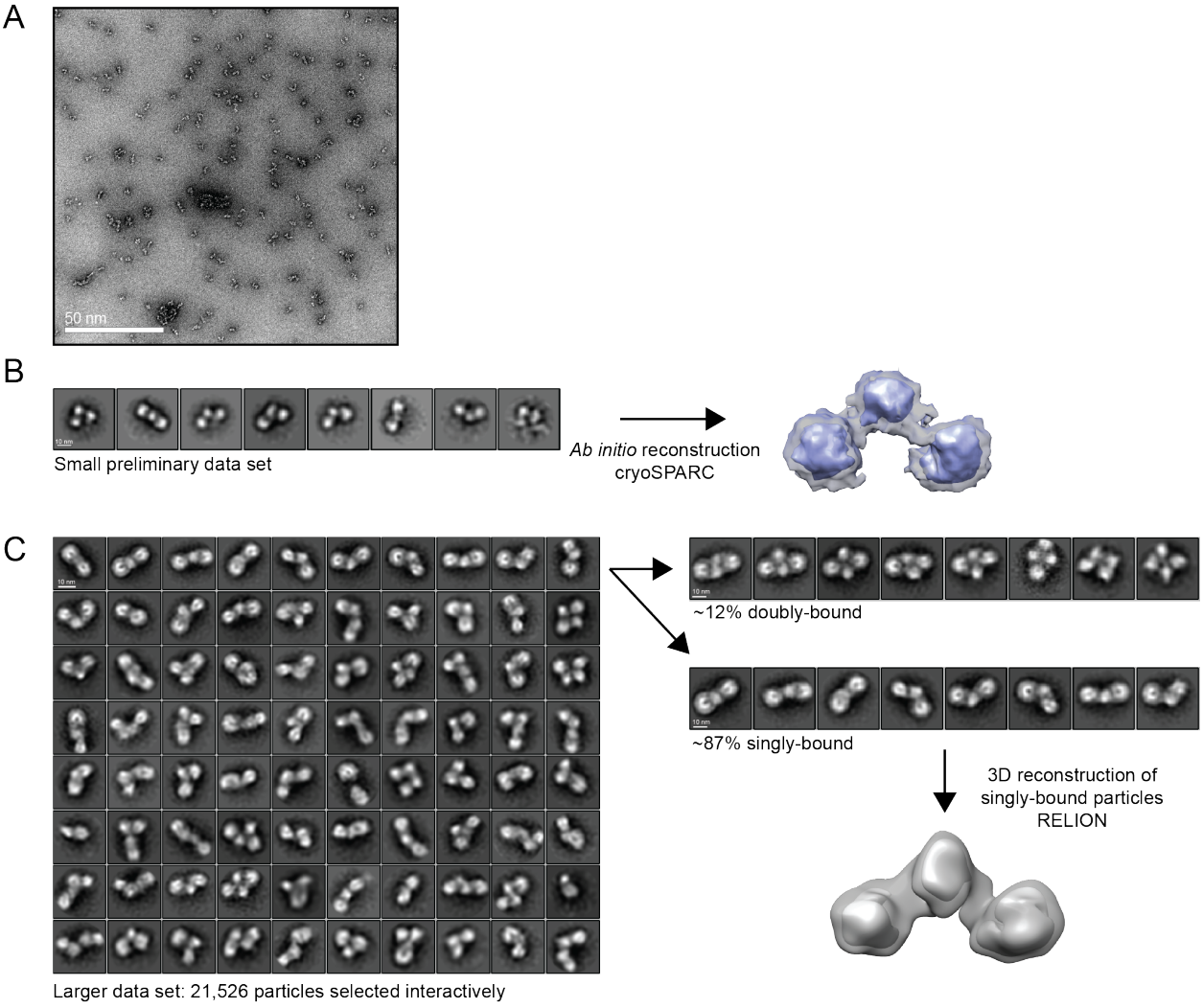


Figure S6. Visualization of ZMET2 bridging the dinucleosome by negative stain electron microscopy. Related to Figure 5.

(A) Representative raw micrograph of GraFix-treated ZMET2/H3Kc9me3 dinucleosome complexes.
(B) Two-dimensional classification of a small preliminary dataset shows dinucleosomes bound with a single ZMET2 molecule (left). Map resulting from *ab initio* 3D reconstruction in cryoSPARC (right).
(C) Representative 2D classes from RELION 2D classification of a larger dataset (left). Later iteration of 2D classification with only selected doubly-bound classes (right, upper); later iteration of 2D classification with only selected singly-bound classes (right, middle); final 3D reconstruction generated by 3D classification with singly-bound particles (right, lower)

Site	Naked (min^{-1})	Naked + H3K9me2 (min^{-1})	WT di (min^{-1})	H3Kc9me3 di (min^{-1})
601/159	3.8e-005	0.00058	1.4e-005	0.0055
601/297	2.7e-005	0.0028	3.2e-005	8.5e-007
601/209	1.6e-005	0.0004	ND	ND
601/11	ND	ND	ND	ND
601/130	1.509e-014	0.002829	ND	7.199e-006
5S/161	2e-015	0.00034	6.6e-006	0.00042
5S/13	ND	0.00029	ND	0.00014
5S/179	1.2e-015	0.00057	1.9e-005	0.0004
5S/110	ND	0.0023	2.2e-005	5.5e-006

Table S1. Rate constants for ZMET2 DNA methylation on naked and nucleosomal substrates at selected CHG sites. Related to Figure 4.

Table containing rate constants for selected sites mentioned in the text calculated from DNA methyltransferase/bisulfite sequencing time courses associated with Figures 4 and S5. ND: not detectable.