Supplementary Table S2. List of mutants in this study, Related to STAR Methods

Mutants	Mution type	Notes
ddm1-2	EMS mutant (nucleotide: G to A)	Hypomorphic; the splicing defect leads to a deletion, a frameshift and premature translation termination; DNA hypomethylation
ddm1-10	T-DNA (SALK_093009)	T-DNA insertion into exon region; used for crossing with MGH3-GFP
met1-1	EMS mutant (amino acid: P 1300 S)	Hypomorphic; amino acid substitution in catalytic domain; DNA hypomethylation; delayed flowering
met1-7	T-DNA (SALK_076522)	T-DNA insertion into exon region; used for crossing with MGH3-GFP
cmt3-11	EMS mutant	Single nucleotide substitution leading to a nonsense mutation; used for crossing with MGH3-GFP
fas2-4	T-DNA (SALK_033228)	Null; no transcript detectable; fasciation
hira-1	WiscDsLox362H05	Reduced fertility
atrx-2	SAIL_861_B04	Reduced fertility

Supplementary Table S3. Primer sequences, Related to STAR Methods

Cloning primers

Name	Primer sequence (5' to 3')	Locus	Note
DDM1_attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCT ATTACTAATTGTGTCGACAAATCC	AT5G66750	for cloning into pDONR221
DDM1_attB2	GGGGACCACTTTGTACAAGAAAGCTGGGT AATCCCAAATCCAAAACATAAGATC	AT5G66750	for cloning into pDONR221
MET1_attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCT TCATGGTAAAATGTTAGTTCTCG	AT5G49160	for cloning into pDONR221
MET1_attB2	GGGGACCACTTTGTACAAGAAAGCTGGGT CTGGACAAACTTTATTTCGAC	AT5G49160	for cloning into pDONR221

Genotyping

Name	Primer sequence (5' to 3')	Locus	Note
ddm1-2 CAPS F	GTTGGACAGTGTGGTAAATTCCGCT	AT5G66750	RsaI digestion
ddm1-2 CAPS R	GAGCTACGAGCCATGGGTTTGTGAAACGTA	AT5G66750	RsaI digestion
ddm1-10 F	GCAAGCCATGGACAGATGCCACAG	AT5G66750	
ddm1-10 R	CAGAGGGCCAATTGTTTTCATCAC	AT5G66750	
met1-1 F	CTCTTTAGTAGAAGTTGGCATG	AT5G49160	HaeIII digestion
met1-1 R	ATATGTATGTATAGATATTTTCTCC	AT5G49160	HaeIII digestion
hira-1 LB	CTACTAAAATTTGAGGCCGGG	AT3G44530	
hira-1 RB	GAGAGTCACTGTTTTGGCTGG	AT3G44530	
atrx-2 LB	AGGAACCCTCACAGCTTCTTC	AT1G08600	
atrx-2 RB	TCACATGGATGGCTTCTTTTC	AT1G08600	
HTR3-GFP-F	TAGTGCAGTCGCAGCTCTTC	AT3G27360	
HTR3-GFP-R	TTTGTACAAGAAAGCTGGGTCAG	AT3G27360	

McrBC-qPCR

Name	Primer sequence (5' to 3')	Locus	Note
ATGP1 F	CGAATGAATCCCTTACCCAAC	AT2G01022	
ATGP1 R	AGCGACATTCGGGAGGAT	AT2G01022	
ATHILA2_1 F	ACCAAGCCGAGTACAACCATAT	AT5G33257	
ATHILA2_1 R	CATTGTGCTCGAGTGTCTGG	AT5G33257	
ATHILA2_2 F	TGCTAGATCGAGTGAGTGTCGT	AT5G35057	
ATHILA2_2 R	CCGAGCCTAGAGAGCAGAAG	AT5G35057	

ChIP-qPCR and RT-qPCR

Name	Primer sequence (5' to 3')	Locus	Note
TSI F	ATCCAGTCCGAAGAACGCGAACTA		
TSI R	TCACTTGTGAGTGTTCGTGAGGTC		
Ta3 F	AAGAGAGCTGGCAGAAGCAGTTGA	AT1G37110	
Ta3 R	ACGCCCTTTACCTTGACCTCCTTT	AT1G37110	
ATHILA6A F	ACAGGAAGTGGGCGCACACC	AT5G32511	
ATHILA6A R	CTCACAACGACGCAAGTGATCT	AT5G32511	
ATHILA2_1 F	ACCAAGCCGAGTACAACCATAT	AT5G33257	
ATHILA2_1 R	CATTGTGCTCGAGTGTCTGG	AT5G33257	
ATHILA2_2 F	TGCTAGATCGAGTGAGTGTCGT	AT5G35057	
ATHILA2_2 R	CCGAGCCTAGAGAGCAGAAG	AT5G35057	

ATPase assay substrates

Name	Primer sequence (5' to 3')	Locus	Note
Widom601 0N60 F	AGAGTGGGAGCTCGGAACACTATCCGAC		
Widom601 0N60 R	CTGGAGAATCCCGGTGCC		

Supplementary Table S4. ChIP and Bisulfite-sequencing libraries metrics, related to STAR Methods.

Genotype	Sample	Group	Replicate	Total reads	All mapped reads	(% total)	Uniquely mapped reads	(% total)	Cytosines covered (WGBS only)	Average coverage (WGBS only)	Non conversion rate (% mC/C in Pt) (WGBS only)
WT	WGBS	A	Rep1	32,230,036	30,942,651	96.01%	24,745,052	76.78%	90.41%	8.17	0.269861
WT	WGBS	A	Rep2	53,613,049	52,389,181	97.72%	42,053,916	78.44%	88.18%	6.78	0.51281
hira	WGBS	A	Rep1	17,362,101	16,566,981	95.42%	12,957,483	74.63%	77.90%	3.93	0.270725
hira	WGBS	A	Rep2	44,991,888	42,569,624	94.62%	33,798,990	75.12%	78.21%	3.22	0.706732
ddm1hira	WGBS	A	Rep1	21,758,809	17,461,284	80.25%	13,837,031	63.59%	77.38%	3.94	0.287453
ddm1hira	WGBS	A	Rep2	48,425,900	36,805,003	76.00%	28,725,572	59.32%	84.11%	6.16	0.548739
ddm1	WGBS	A	Rep1	27,502,435	26,294,284	95.61%	20,404,561	74.19%	83.48%	5.30	0.266461
ddm1	WGBS	A	Rep2	37,878,589	37,470,061	98.92%	29,204,434	77.10%	83.05%	6.49	0.537523
WT	WGBS	B	Rep1	30,561,004	24,364,737	79.72%	18,151,083	59.39%	77.57%	7.07	0.589116
WT	WGBS	B	Rep2	34,550,917	30,417,035	88.04%	22,452,116	64.98%	79.20%	9.83	0.587723
ddm1atrx	WGBS	B	Rep1	23,525,857	19,197,760	81.60%	14,136,528	60.09%	71.88%	6.43	0.595414
ddm1atrx	WGBS	B	Rep2	37,170,647	35,040,723	94.27%	24,906,077	67.00%	79.15%	10.33	0.667431
ddm1	WGBS	B	Rep1	36,520,861	35,544,986	97.33%	26,379,181	72.23%	78.73%	7.54	0.621665
ddm1	WGBS	B	Rep2	20,358,084	19,257,875	94.60%	14,528,944	71.37%	73.49%	4.70	0.923219
atrx	WGBS	B	Rep1	39,661,456	39,246,987	98.95%	29,154,984	73.51%	79.63%	5.21	0.845968
atrx	WGBS	B	Rep2	38,694,008	38,125,949	98.53%	28,077,230	72.56%	79.89%	6.57	0.661121
WT	DDM1	IP	Rep1	40,515,724	37,212,763	91.85%	21,468,053	52.99%	-	-	-
WT	DDM1	Input	Rep1	47,290,771	45,973,121	97.21%	32,105,406	67.89%	-	-	-
WT	DDM1	IP	Rep2	34,645,829	32,569,880	94.01%	19,276,714	55.64%	-	-	-
WT	DDM1	Input	Rep2	51,279,688	49,817,225	97.15%	34,561,985	67.40%	-	-	-
ddm1	DDM1	IP	Rep1	50,102,086	47,183,497	94.17%	27,125,034	54.14%	-	-	-
ddm1	DDM1	Input	Rep1	39,982,483	39,028,473	97.61%	26,984,304	67.49%	-	-	-
ddm1	DDM1	IP	Rep2	49,577,213	43,872,422	88.49%	24,804,809	50.03%	-	-	-
ddm1	DDM1	Input	Rep2	33,380,544	32,612,138	97.70%	22,752,619	68.16%	-	-	-
WT	H3K27me1	IP	Rep1	30,078,663	29,656,796	98.60%	11,522,922	38.31%	-	-	-
WT	H3K27me1	H3	Rep1	22,622,734	22,483,854	99.39%	16,142,362	71.35%	-	-	-
WT	H3K27me1	IP	Rep2	50,994,633	40,503,003	79.43%	13,950,016	27.36%	-	-	-
WT	H3K27me1	H3	Rep2	60,442,954	59,862,967	99.04%	45,680,564	75.58%	-	-	-
ddm1	H3K27me1	IP	Rep1	26,031,998	25,885,357	99.44%	18,435,013	70.81%	-	-	-
ddm1	H3K27me1	H3	Rep1	28,806,975	28,258,079	98.09%	10,926,824	37.93%	-	-	-
ddm1	H3K27me1	IP	Rep2	37,494,713	28,836,811	76.91%	9,554,115	25.48%	-	-	-
ddm1	H3K27me1	H3	Rep2	52,356,409	51,800,730	98.94%	38,115,613	72.80%	-	-	-
WT	H4K16ac	IP	Rep1	29,867,512	29,113,254	97.47%	19,477,351	65.21%	-	-	-
WT	H4K16ac	H4	Rep1	32,025,461	31,151,067	97.27%	17,149,656	53.55%	-	-	-
WT	H4K16ac	IP	Rep2	49,958,674	48,846,091	97.77%	33,687,425	67.43%	-	-	-
WT	H4K16ac	H4	Rep2	49,482,528	48,239,575	97.49%	26,621,503	53.80%	-	-	-
ddm1	H4K16ac	IP	Rep1	44,578,514	43,566,178	97.73%	28,553,612	64.05%	-	-	-
ddm1	H4K16ac	H4	Rep1	59,698,668	20,431,979	34.23%	12,114,825	20.29%	-	-	-
ddm1	H4K16ac	IP	Rep2	40,697,481	39,804,154	97.81%	26,494,041	65.10%	-	-	-
ddm1	H4K16ac	H4	Rep2	33,698,770	32,821,242	97.40%	17,607,160	52.25%	-	-	-
WT	HTR5	IP	Rep1	89,816,986	89,262,349	99.38%	74,274,600	82.70%	-	-	-
WT	HTR5	H3	Rep1	43,362,049	42,608,947	98.26%	33,866,720	78.10%	-	-	-
WT	HTR5	IP	Rep2	47,569,522	47,259,571	99.35%	40,618,380	85.39%	-	-	-
WT	HTR5	H3	Rep2	62,187,338	61,578,996	99.02%	46,846,643	75.33%	-	-	-
ddm1	HTR5	IP	Rep1	91,478,171	90,971,481	99.45%	68,533,360	74.92%	-	-	-
ddm1	HTR5	H3	Rep1	43,901,988	43,551,892	99.20%	31,424,017	71.58%	-	-	-
ddm1	HTR5	IP	Rep2	88,251,305	87,728,045	99.41%	67,051,725	75.98%	-	-	-
ddm1	HTR5	H3	Rep2	77,052,248	76,533,910	99.33%	51,185,737	66.43%	-	-	-
WT	MGH3	IP	Rep1	114,691,469	110,674,811	96.50%	97,134,182	84.69%	-	-	-
WT	MGH3	Input	Rep1	42,239,090	39,899,471	94.46%	25,189,691	59.64%	-	-	-
ddm1/+	MGH3	IP	Rep1	28,958,163	23,043,589	79.58%	17,882,624	61.75%	-	-	-
ddm1/+	MGH3	Input	Rep1	25,645,477	24,623,469	96.01%	14,422,150	56.24%	-	-	-

Supplementary Table S5. Cryo-EM data collection and reconstruction statistics for the DDM1-nucleosome complex, Related to STAR Methods

Data collection	
Microscope	Titan Krios
Voltage (keV)	300
Magnification	81,000×
Defocus range (μm)	−1.0 to −2.2
Detector	K3
Pixel Size (Å)	1.1
Total exposure ($e^-/Å^2$)	71.2
Exposure rate ($e^-/Å^2$/sec)	14.8
Exposure per frame ($e^-/Å^2$)	2.37
Micrographs collected	8,165

Initial processing	
Micrographs used	7,811
Initial particles	3,788,872

Reconstruction	
Final particles	215,066
Symmetry	C1
Map sharpening B-factor ($Å^2$)	57.8
Half maps resolution (unmasked / masked)	
FSC 0.143	3.4 / 3.2

Supplementary Table S6. Model refinement and validation statistics for the DDM1-nucleosome complex, Related to STAR Methods

Statistics are provided for the full model as well as the individual octamer, DNA and DDM1 components.

Refinement	Full model	Octamer	DNA	DDM1
Protein residues	1217	749	——	468
Nucleic acid residues	282	——	282	——
Model resolution (unmasked / masked)				
FSC 0.5	3.2 / 3.2			
FSC 0.143	2.8 / 2.8			
Map correlation coefficients				
CC mask	0.74	0.78	0.73	0.6
CC box	0.65	——	——	——
CC peaks	0.65	——	——	——
CC volume	0.72	0.73	0.72	0.58

Model geometry	Full model	Octamer	DNA	DDM1
Ramachandran plot				
Outliers (%)	0	0	——	0
Allowed (%)	0.67	0	——	1.72
Favored (%)	99.33	100	——	98.28
Ramachandran plot Z-score				
Whole	−0.32 ± 0.23 (N = 1197)	0.18 ± 0.30 (N = 733)	——	−1.11 ± 0.36 (N = 464)
Helix	0.25 ± 0.19 (N = 681)	0.72 ± 0.23 (N = 492)	——	-0.96 ± 0.34 (N = 189)
Sheet	1.72 ± 0.73 (N = 55)	——	——	1.72 ± 0.73 (N = 55)
Loop	−1.05 ± 0.26 (N = 461)	−1.00 ± 0.36 (N = 241)	——	−1.10 ± 0.38 (N = 220)
CaBLAM outliers (%)	0.4	0.3	——	0.7
Rotamers				
Poor (%)	0.1	0	——	0.24
Favored (%)	98.28	98.72	——	97.62
R.M.S. deviations				
Bond lengths (Å)	0.004	0.004	0.004	0.004
Bond angles (°)	0.759	0.757	0.716	0.837
Geometry outliers				
C_α deviations (%)	0.08	0	——	0.22
C_β deviations	0	0	——	0
Bad bonds	0 / 9,894	0 / 5,991	0 / 6,484	0 / 3,903
Bad angles	0 / 13,296	0 / 8,040	0 / 10,003	0 / 5,256
Cis prolines	0 / 39	0 / 24	——	0 / 15
Chiral volume outliers	0 / 2633	0 / 931	0 / 1,128	0 / 574
Clashscore (all atoms)	3.22	1.97	2.79	5.57
MolProbity score	1.11	0.96	——	1.3
B-factors (Å^2) (min / max / mean)				
Protein	12.6 / 98.4 / 45.8	12.6 / 93.9 / 30.7	——	36.1 / 98.4 / 69.2
Nucleotide	28.84 / 201.26 / 87.65	——	28.8 / 201.3 / 87.7	——