Supplementary Table S1. Oligonucleotide primers used in this study

Primers for PCR	
WDHD1-F	GCA GCA GGA ACT CTT AAT GA
WDHD1-R	ACT TGA CTT CTG ACC CGT GG
MajF1[a]	GAC GAC TTG AAA AAT GAC GAA ATC
MajR1[a]	CAT ATT CCA GGT CCT TCA GTG TGC
MinF1[a]	CAT GGA AAA TGA TAA AAA CC
MinR1[a]	CAT CTA ATA TGT TCT ACA GTG TGG
GAPDH-F	AAC AAC CCC TTC ATT GAC CTC
GAPDH-R	TTC TGA GTG GCA GTG ATG GC
U5 snRNA	GGT TTC TCT TCA GAT CGT ATA AAT C
U5 snRNA	CTC AAA AAA TTG GTT TAA GAC TCA GA
Primers for amplifying mitochondrial DNA probe	
mitochondria DNA-F	CCT CCT TAG TCC TTT AGT TTC
mitochondria DNA-R	GTC ATA CGA TTA ACC CAA AC
Primers for generating probes for RNA pull-down assay[b]	
T7-MajF1	GCG TAA TAC GAC TCA CTA TAG GGA GAG ACG ACT TGA AAA ATG ACG AAA TC
T7-MajR1	GCG TAA TAC GAC TCA CTA TAG GGA GAC ATA TTC CAG GTC CTT CAG TGT GC
T7-MinF1	GCG TAA TAC GAC TCA CTA TAG GGA GAC ATG GAA AAT GAT AAA AAC C
T7-MinR1	GCG TAA TAC GAC TCA CTA TAG GGA GAC ATC TAA TAT GTT CTA CAG TGT GG
T7-18S rRNA-F	GCG TAA TAC GAC TCA CTA TAG GGA GAG ACT CTT TCG AGG CCC TGT A
T7-18S rRNA-R 300	GCG TAA TAC GAC TCA CTA TAG GGA GAG GCC TGC TTT GAA CAC TCT A
T7-18S rRNA-R 162	GCG TAA TAC GAC TCA CTA TAG GGA GAC GCT CCC AAG ATC CAA CTA C
Oligonucleotide probes for Northern blot analysis	
Maj23	AAT GTC CAC TGT AGG ACG TGG AAT ATG GCA
Maj12	CAC GGA AAA TGA GAA ATA CAC ACT TTA GGA TGT G
Min1	CAT GGA AAA TGA TAA AAA CCA CAC TGT AGA ACA TAT TAG
Min2	TGA GTG AGT TAC ACT GAA AAA CAC ATT CGT TGG AAA CCG
Min3	CAT TGT AGA ACA GTG TAT ATC AAT GAG TTA CAA TTA GAA
U6 snRNA-AS	GAA TTT GCG TGT CAT CCT TGC GCA GGG GCC ATG CTA A
Gene-targeting siRNAs	
WDHD1-1	TCT GTT GGG ATT ATT CGC TGC TAT A
WDHD1-2	CCA CGA CCT GCT GTA GCT ATA TTA T
WDHD1-3	GGA CAG AGA GGA CAC TGT TTC TGA A
Dicer-1	TCT GGG ACC CTG GTG TGC AGA TAA A
Dicer-2	CCA GGT TCT CAT TAT GAC TTG CTA T
Dicer-3	CAT CAA CAG ATA TTG TGC TCG ATT A
Drosha-1	CCG AAG TGA TCG CTT TCC CGA TTA T
Drosha-2	GAG GAG TGC AAA GGC ATG ATC GTT A
Drosha-3	CAA AGA AAC TTG AAC TGG ATC GAT T
ADAR1-1	GGG CCA CAG ATG ACA TCC CAG ATA A
ADAR1-2	TCT CAT GGC TTT GCT GCT GAG TTC A
ADAR1-3	CAG GAC CAA TGA GAC TGC GGC CTC A

[a]These primers were also used in ChIP assays

[b]Sequence for the T7 promoter is underlined.

Supplementary Table S2. WDHD1 expression profiles in normal tissue in Human Protein Atlas (HPA)

		IHC staining[a]				
Tissue	No. of cell types	+3	+2	+1	−	Positive rate (%)
Central nervous system (Brain)	9				9	0
Blood and immune system (Hematopoietic)	7	3	3	1		100
Liver and pancreas	5				5	0
Digestive tract (GI-tract)	11	5	4	1	1	90
Respiratory system (Lung)	4	1			3	25
Cardio vascular system (Heart and blood vessels)	1				1	0
Breast and reproductive system (Female tissues)	10	3	2	4	1	90
Placenta	2		2			100
Male reproductive system (Male tissues)	5	1		1	3	40
Urinary tract (Kidney and bladder)	3	1			2	33
Skin and soft tissues	4	1	1		2	50
Endocrine glands	3			1	2	33

[a]IHC staining scores in the HPA: 3+, strong staining; 2+, moderate staining; 1+, weak staining; −, negative staining.

Supplementary Table S3. WDHD1 expression profile in different cancer types in the Human Protein Atlas (HPA)

		IHC staining[a]				
cancer type	Detected no.	+3	+2	+1	−	Positive rate (%)
Colorectal cancer	8	2	6			100
Breast cancer	8	1	5	2		100
Prostate cancer	8		1	4	3	62
Ovarian cancer	12	3	5	3	1	92
Cervical cancer	11	7	3		1	91
Endometrial cancer	11	1	4	1	5	55
Malignant carcinoid	4		2	1	1	75
Head and neck cancer	4	4				100
Thyroid cancer	4		1		3	25
Malignant glioma	11	5	2		4	64
Malignant lymphoma	12	10	2			100
Lung cancer	12	6	3	2	1	92
Malignant melanoma	12	8	2	2		100
Skin cancer	11	6	1	4		100
Testis cancer	12	11	1			100
Urothelial cancer	12	9	2	1		100
Renal cancer	12				12	0
Stomach cancer	12	3	3	3	3	75
Pancreatic cancer	11	2	1	4	4	64
Liver cancer	12	1	1		10	17

[a]IHC staining scores in the HPA: 3+, strong staining; 2+, moderate staining; 1+, weak staining; −, negative staining.

FIGURE LEGENDS

Figure S1

Expression of the PCT homologous small RNA fragments is Dicer dependent

Total RNA from control (Ctrl) or Dicer knockdown NIH-3T3 cells was size fractionated, and equivalents amounts of small RNA fraction (<200nt) were resolved in a denaturing 12% acrylamide gel (EtBr staining shown in the middle). The gel was subjected to Northern blot analysis using probe specific for major satellite repeats. Blot was re-hybridized with a U6 snRNA-complementary probe to demonstrate equal loading (bottom).

Figure S2

Loss of WDHD1 does not alter the nuclear distribution of centromeric RNA transcripts

Representative RNA-FISH analysis of the major satellite transcripts in NIH-3T3 cells transfected with control (Ctrl) or WDHD1 siRNA. RNA-FISH was done as described in the MATERIALS AND METHODS section. Nuclear DNA was stained by DAPI (blue). Individual and merged images were captured by laser scanning confocal microscope and single sections are shown (scale bar is 10 μm).

Figure S3

Characteristics of the ectopically expressed FLAG-WDHD1 in 293T cells

293T cells were transfected with constructs encoding FLAG or FLAG-tagged WDHD1 (Fig. 3E). Immunoblotting analysis of the cell lysates (top panel) was done using antibodies specific to WDHD1 and β-actin, as indicated. Subcellular localization of the FLAG-WDHD1 protein (green) is shown in the bottom panel. Nuclear DNA was stained by DAPI (blue). Individual and merged images were captured by laser scanning confocal microscope and single sections are shown (scale bar is 10 μm).

Figure S4

Nuclear distribution of the Dicer protein

Indirect immunofluorescence analysis was performed to observe localization of endogenous Dicer in the NIH-3T3 cells. Cells were grown on coverslips before being fixed and subjected to immunostaining. Nuclear DNA was stained by DAPI. Both individual and merged (Dicer, green; DAPI, red) images are shown. Images were captured by laser scanning confocal microscope and single sections are shown (scale bar is 10 μm).

Figure S5

Abundance of various histone modifications in the control vs. knockdown cells

(A) Representative indirect immunofluorescence analysis of H3K9me3 distribution in NIH-3T3 cells transfected with control (Ctrl) or WDHD1 siRNA. Nuclear DNA was stained by

DAPI (blue). Individual and merged images were captured by laser scanning confocal microscope and single sections are shown.
(B) Nuclear extracts were prepared from control (Ctrl) or WDHD1 knockdown cells (as in Fig. 3B & 3C). Abundance of the indicated histone marks and H3 (as loading control) was monitored by Western blot analysis. The anti-WDHD1 blot shows the degree of knockdown.

Figure S6
(A) A shorter-exposed version of the blots shown in Figure 5D.
(B) Comparison of the proliferation status between the control and knockdown cultures used in the methylation-sensitive restriction analysis (Figure 5D). Proliferation status was determined as described in the MATERIALS AND METHODS based on the relative proportion of replicating cells.

Figure S1

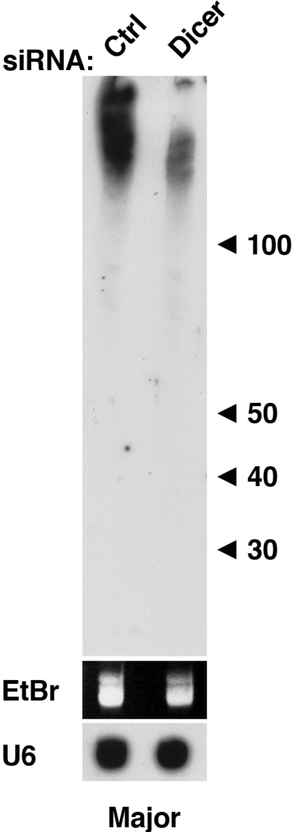


Figure S2

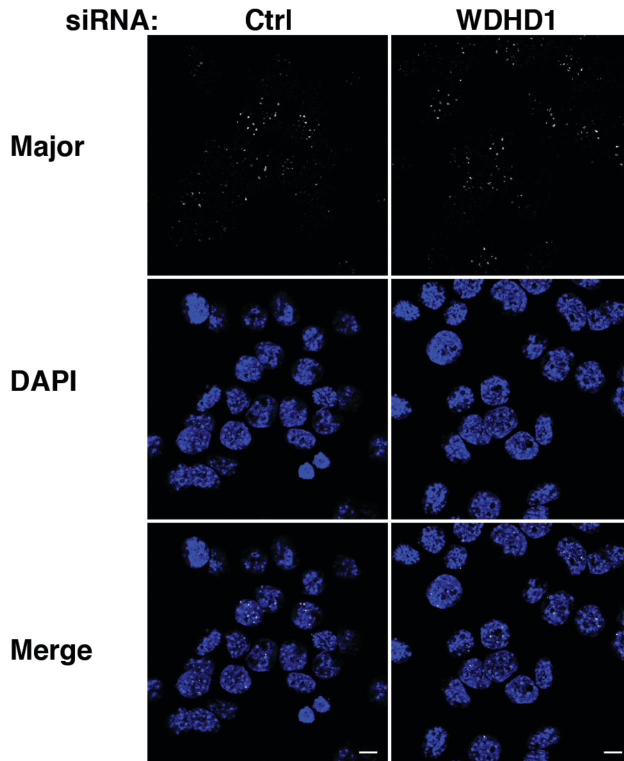


Figure S3

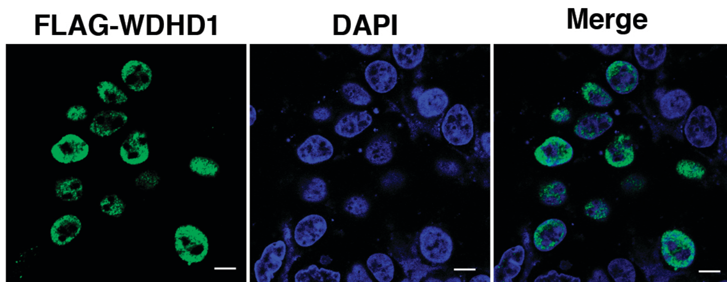


Figure S4

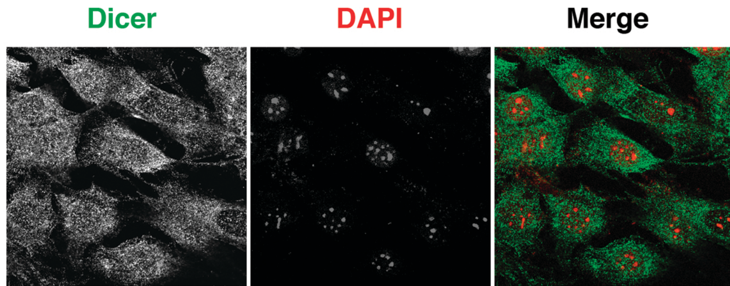
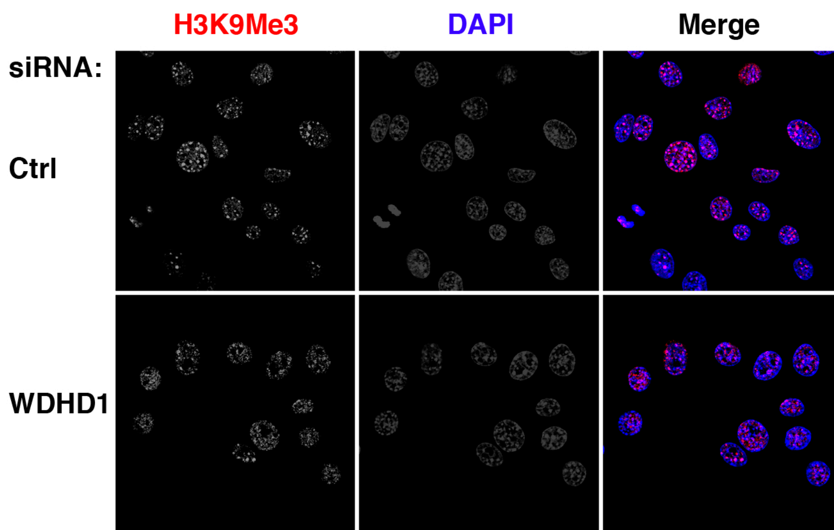


Figure S5

A



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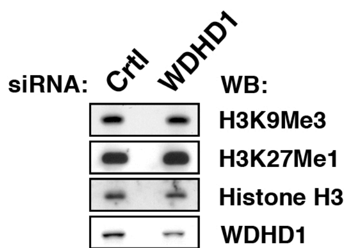
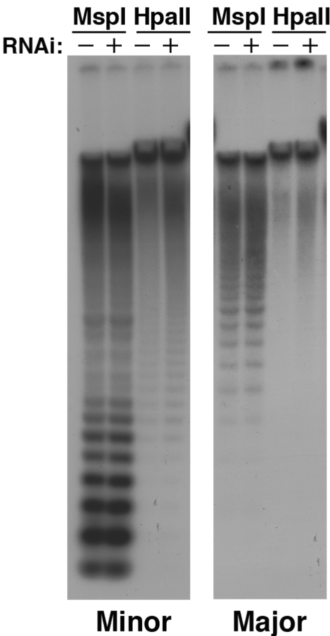


Figure S6

A



B