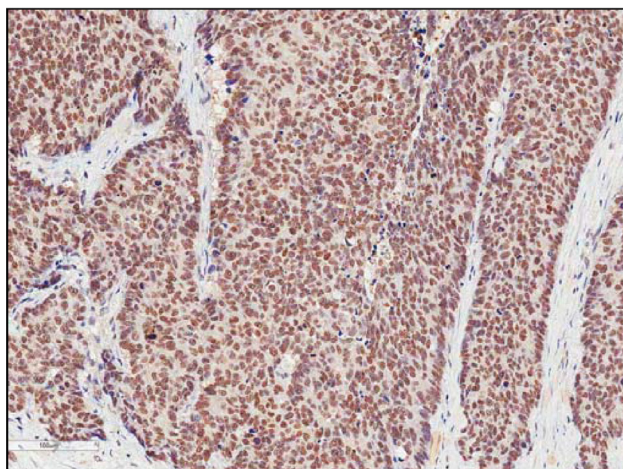


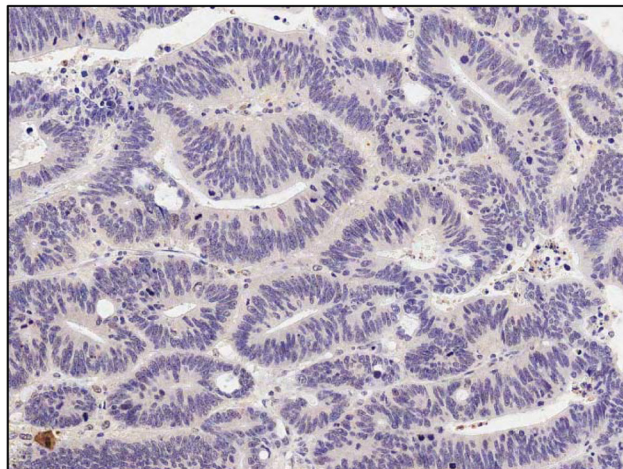
## The prognostic significance of protein arginine methyltransferase 6 expression in colon cancer

### SUPPLEMENTARY MATERIALS

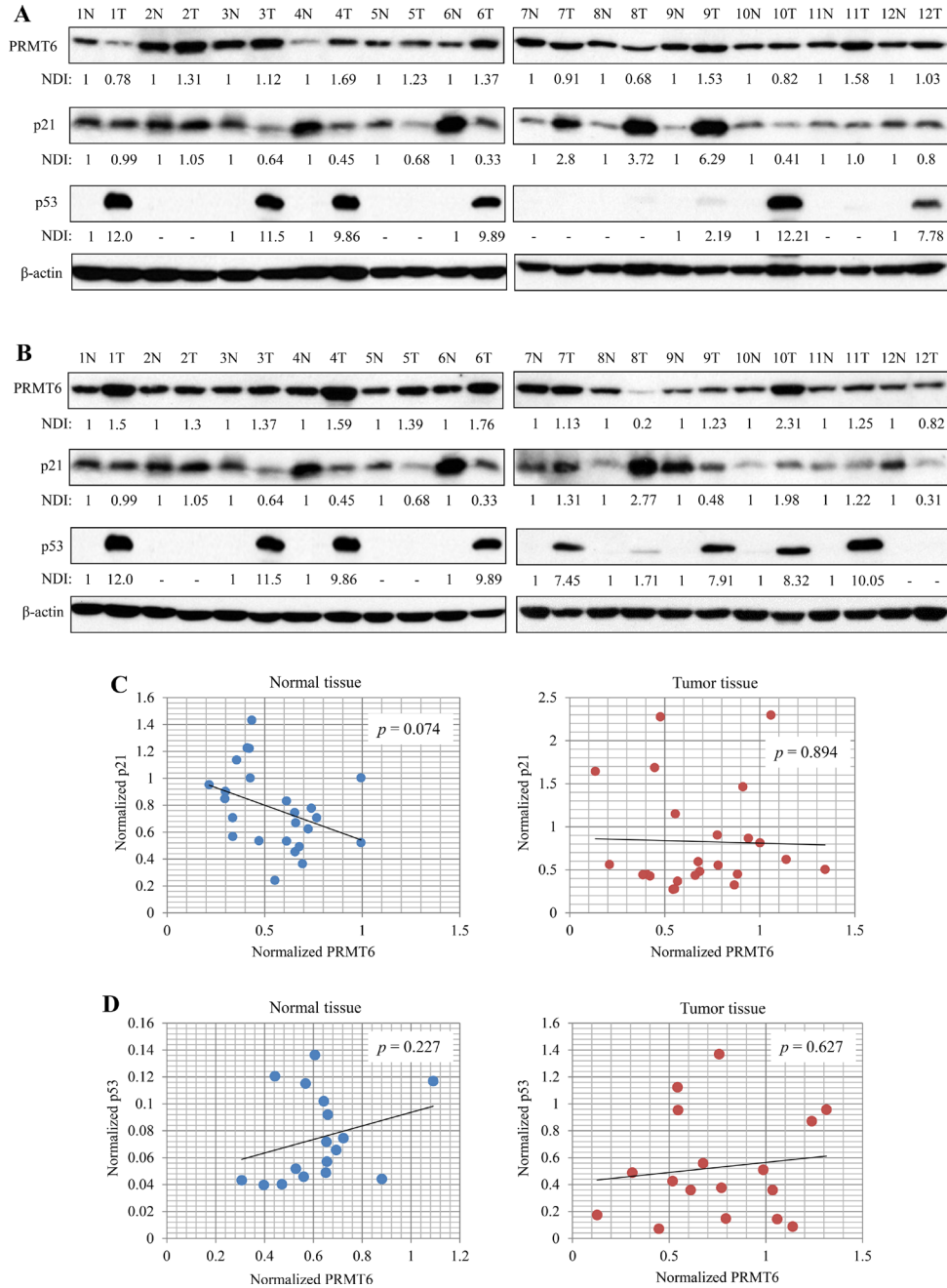
**A**



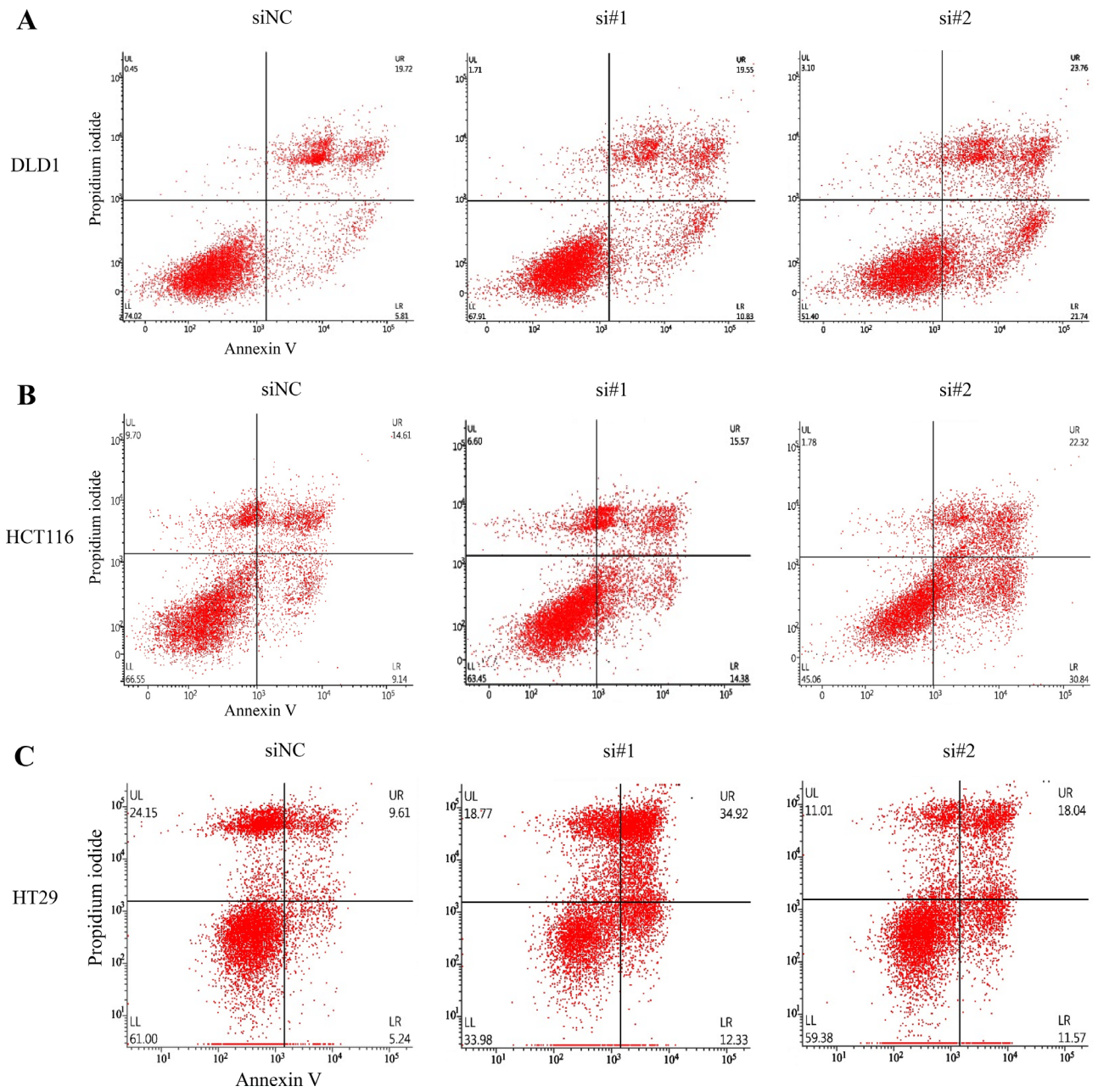
**B**



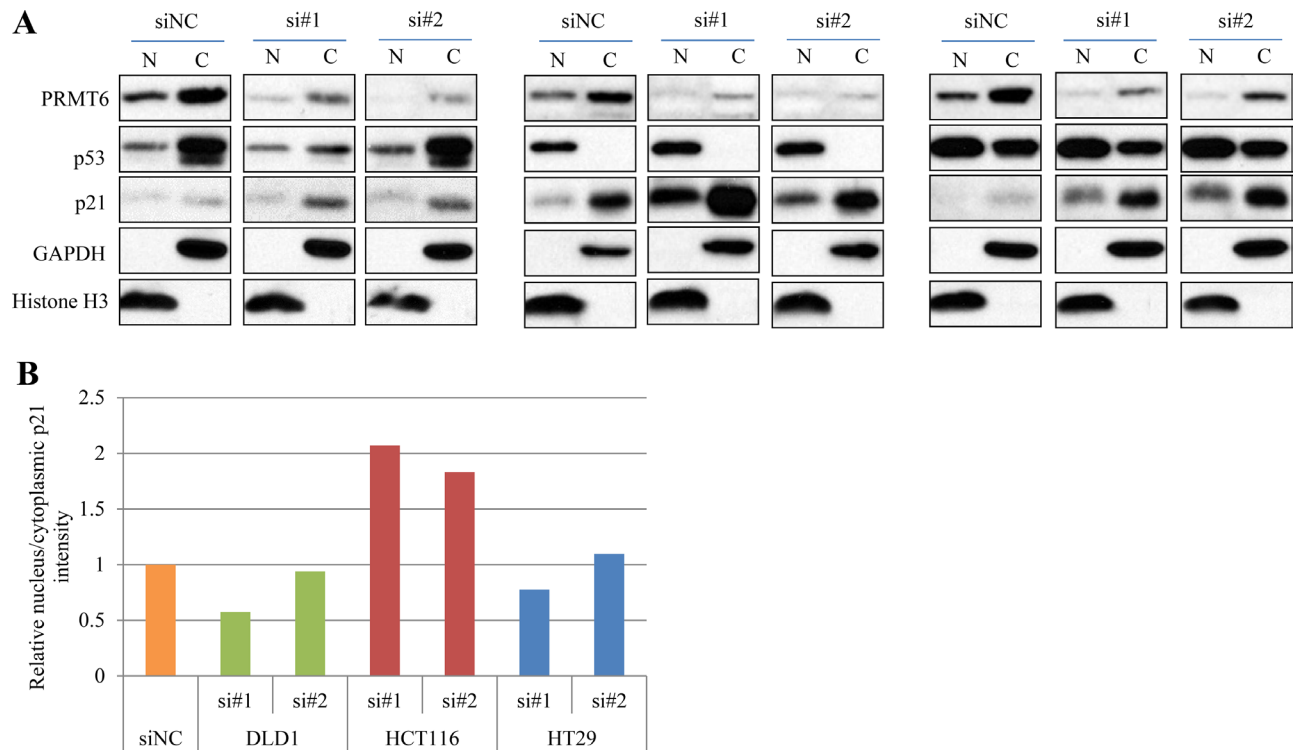
**Supplementary Figure 1: Immunohistochemical PRMT6 staining CRC images.** Images ( $\times 100$ ) of PRMT6 positive (A) and PRMT6-negative tumors (B) from stage III CRC patients.



**Supplementary Figure 2: Interrelationship between PRMT6 and tumor suppressors in 24 matched samples from the primary CRC and adjacent normal tissues paired from the same patient.** Expressions of PRMT6, p21 and p53 in patients with stage II (A) and stage III (B) were examined by western blotting. Densitometric intensity of each protein was normalized to  $\beta$ -actin. NDI indicates normalized densitometric intensity. Densitometric values corresponding to each protein were plotted and interrelationships between PRMT6 and p21 (C) and p53 (D) analyzed.



Supplementary Figure 3: Representative flow cytometry plots of siNC- and siPRMT6s-transfected CRC cells.



**Supplementary Figure 4: Nuclear and cytoplasmic fractions of DLD1, HCT116 and DLD1 cells were prepared and analyzed by western blot.** (A) The signal intensity of nuclear and cytoplasmic p21 was normalized by histone H3 signal and  $\beta$ -actin, respectively (B).

**Supplementary Table 1: Sequences of primers used in the real-time PCR**

Gene Name	Primer sequence	
PRMT6	Forward	5'-TGCTGCCACCTCGCCTTTTC-3'
	Reverse	5'-TTGGTCTTCTCCTCCTGGTCTCC-3'
p21	Forward	5'-GGCAGACCAGCATGACAGATT-3'
	Reverse	5'-GCGGATTAGGGCTTCCTCTT-3'
p53	Forward	5'-AGACTGGGTCTCGCTTTGTTG-3'
	Reverse	5'-AGGCAAAGGCTGCAGTAAGC-3'
GAPDH	Forward	5'-ATCATCCCTGCCTCTACTGG-3'
	Reverse	5'-CTGCTTCACCACCTTCTTGA-3'

**Supplementary Table 2: Patients' demographics**

	<b>Number (%)</b> <b>(Total N = 586)</b>
<b>Gender <i>n</i> (%)</b>	
Female	221 (37.7)
Male	365 (62.3)
<b>Median age (range, years)</b>	59 (28–90)
<b>Location</b>	
Colon	436 (74.4)
Rectum	150 (25.6)
<b>Preoperative CEA (ng/ml)</b>	
Median (range)	1.93 (0.14-148.32)
<b>AJCC stage</b>	
IIA	269 (45.9)
IIB	29 (4.9)
IIC	10 (1.7)
IIIA	38 (6.5)
IIIB	240 (41.0)
<b>Tumor stage</b>	
T1	14 (2.4)
T2	26 (4.4)
T3	476 (81.2)
T4	70 (11.9)
<b>Nodal stage</b>	
N0	308 (52.6)
N1	216 (36.9)
N2	62 (10.6)
<b>Cell type</b>	
Well-differentiated	44 (7.5)
Moderately-differentiated	488 (83.3)
Poorly-differentiated	27 (4.6)
Mucinous	25 (4.3)
Signet ring cell	2 (0.3)
<b>Lymphatic invasion</b>	
Negative	430 (73.4)
Positive	156 (26.6)
<b>Vascular invasion</b>	
Negative	394 (67.2)
Positive	192 (32.8)
<b>Perineural invasion</b>	
Negative	411 (70.1)
Positive	175 (29.9)
<b>Adjuvant chemotherapy</b>	
No	45 (7.7)
Yes	458 (78.2)
Undescribed	83 (14.2)
<b>PRMT6 expression</b>	
Negative	447 (76.3)
Positive	139 (23.7)

**Supplementary Table 3: Univariate and multivariate analyses for prognostic variables of disease-free and overall survival using Cox-proportional-hazards regression**

**(1) Disease-free survival**

Predictors	Univariate	<i>p</i> -value	Multivariate	<i>p</i> -value
	Ratio of risk (95% CI)		Ratio of risk (95% CI)	
<b>Gender</b>		0.862		
Female	1.000			
Male	1.039 (0.672–1.607)			
<b>Age (years)</b>		0.107		
< 60	1.000			
≥ 60	0.697 (0.449–1.081)			
<b>Location</b>		< 0.001		0.006
Colon	1.000		1.000	
Rectum	2.175 (1.420–3.333)		1.851 (1.196–2.865)	
<b>CEA level</b>		0.001		0.076
≤ 5 ng/ml	1.000		1.000	
> 5 ng/ml	2.105 (1.330–3.332)		1.548 (0.956–2.505)	
<b>T stage</b>		0.174		
T1–2	1.000			
T3–4	2.223 (0.703–7.033)			
<b>N stage</b>		0.003		0.155
N0	1.000		1.000	
N+	1.944 (1.257–3.005)		1.448 (0.870–2.410)	
<b>Cell type</b>		0.689		
WD/MD	1.000			
PD/MUC/SRC	0.854 (0.394–1.849)			
<b>Lymphatic invasion</b>		< 0.001		0.094
Negative	1.000		1.000	
Positive	2.448 (1.604–3.735)		1.526 (0.930–2.503)	
<b>Vascular invasion</b>		< 0.001		0.003
Negative	1.000		1.000	
Positive	3.334 (2.126–5.229)		2.216 (1.318–3.725)	
<b>Perineural invasion</b>		0.010		0.195
Negative	1.000		1.000	
Positive	2.223 (1.208–4.088)		1.527 (0.805–2.897)	
<b>Adjuvant chemotherapy</b>		0.001		< 0.001
Yes	1.000		1.000	
No/undescribed	2.133 (1.359–3.349)		2.557 (1.602–4.080)	
<b>PRMT6 expression</b>		0.018		0.035
Negative	1.000		1.000	
Positive	1.711 (1.096–2.673)		1.630 (1.034–2.569)	

**(2) Overall survival**

Predictors	Univariate	<i>p</i> -value	Multivariate	<i>p</i> -value
	Ratio of risk (95% CI)		Ratio of risk (95% CI)	
<b>Gender</b>		0.441		
Female	1.000			
Male	1.295 (0.671–2.500)			
<b>Age (years)</b>		<u>0.014</u>		0.219
≤ 60	1.000		1.000	
> 60	2.191 (1.169–4.105)		1.509 (0.783–2.910)	
<b>Location</b>		0.124		
Colon	1.000			
Rectum	1.646 (0.872–3.107)			
<b>CEA level</b>		<u>≤ 0.001</u>		<u>0.003</u>
≤ 5 ng/ml	1.000		1.000	
> 5 ng/ml	3.482 (1.870–6.483)		2.654 (1.384–5.089)	
<b>T stage</b>		0.241		
T1–2	1.000			
T3–4	3.280 (0.451–23.863)			
<b>N stage</b>		<u>0.014</u>		0.282
N0	1.000		1.000	
N+	2.273 (1.177–4.388)		1.519 (0.710–3.254)	
<b>Cell type</b>		0.925		
WD/MD	1.000			
PD/Muc/SRC	1.051 (0.374–2.947)			
<b>Lymphatic invasion</b>		<u>≤ 0.001</u>		<u>0.013</u>
Negative	1.000		1.000	
Positive	3.732 (2.014–6.915)		2.389 (1.200–4.759)	
<b>Vascular invasion</b>		<u>≤ 0.001</u>		0.079
Negative	1.000		1.000	
Positive	3.230 (1.694–6.160)		1.895 (0.928–3.870)	
<b>Perineural invasion</b>		<u>0.001</u>		<u>0.012</u>
Negative	1.000		1.000	
Positive	3.676 (1.755–7.703)		2.694 (1.247–5.819)	
<b>Adjuvant chemotherapy</b>		<u>≤ 0.001</u>		<u>≤ 0.001</u>
Yes	1.000		1.000	
No/undescribed	5.662 (3.061–10.476)		5.615 (2.914–10.819)	
<b>PRMT6 expression</b>		0.353		
Negative	1.000			
Positive	1.376 (0.702–2.696)			