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Supplemental Information

CHD4 Has Oncogenic Functions in Initiating and Maintaining Epigenetic Suppression of Multiple Tumor Suppressor Genes

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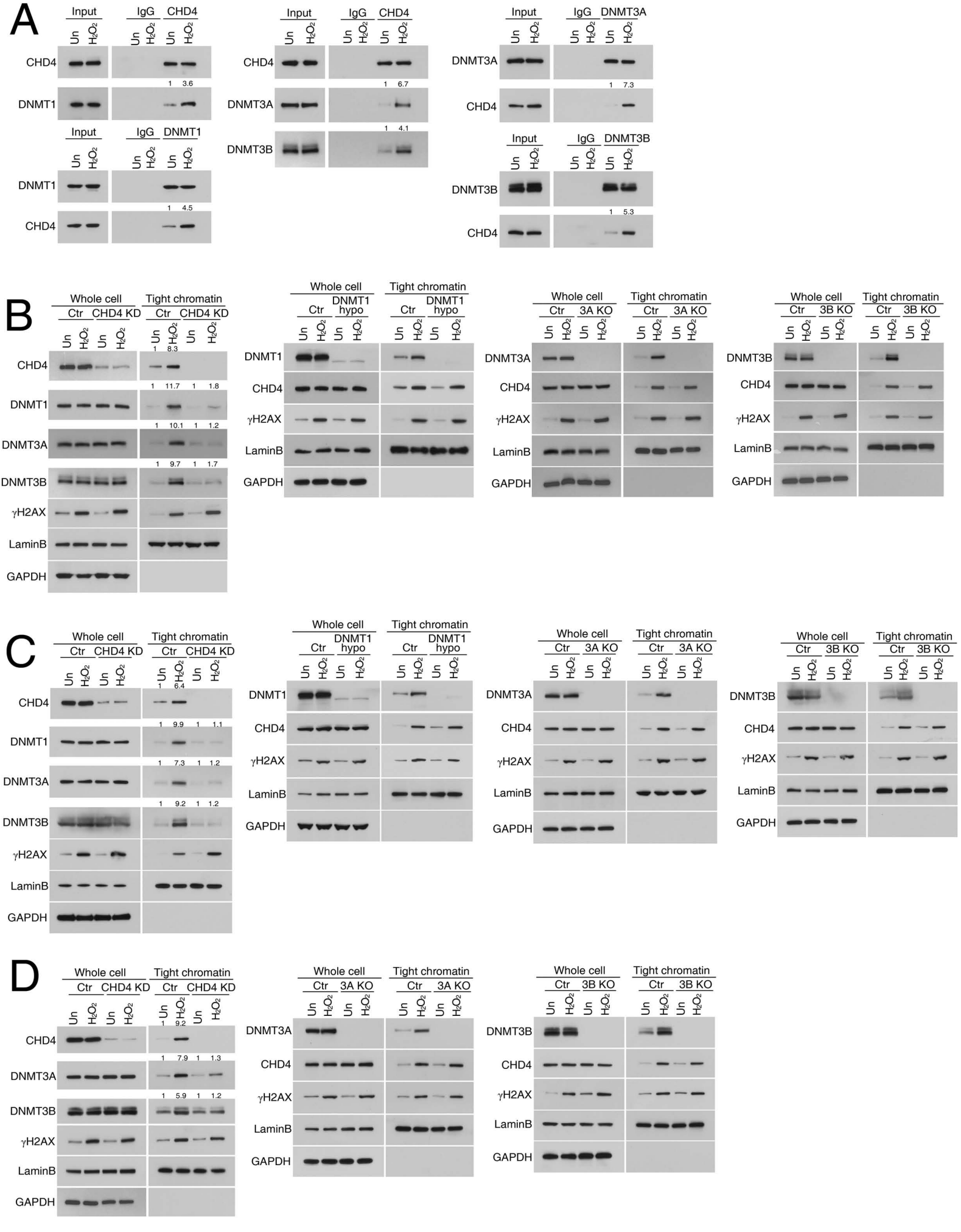

A
Input
IgG
CHD4
DNMT1
DNMT3A
DNMT3B
Un
H2O2
B
Whole cell
Tight chromatin
Ctr
CHD4 KD
DNMT1 hypo
3A KO
3B KO
γH2AX
LaminB
GAPDH
C
D

Figure S1. The effect of CHD4 on the binding of DNMT1, DNMT3A, and DNMT3B to chromatin after oxidative damage, Related to Figure 1

(A) SW480 or NCCIT cells, were treated with 2 mM H_2O_2 for 30 min and compared to untreated cells for all analyses. CoIPs were performed with antibodies consisting of control IgG, anti-CHD4, anti-DNMT1, anti-DNMT3A, or anti-DNMT3B, respectively.

(B) SW480 Ctr, SW480 CHD4 knockdown cells (CHD4 KD), SW480 genetically deleted for DNMT1 generating severe hypomorphic cells (SW480 DNMT1 hypomorph), SW480 DNMT3A KO, and SW480 DNMT3B KO cells were not treated (Un) or treated with 2 mM H_2O_2 for 30 min. The tight chromatin analyzed represents the remaining protein in the nuclear, chromatin pellet after extraction with 0.45 M NaCl buffer. Whole cell extracts and the tight chromatin fractions were analyzed by immunoblotting with the antibodies designated in panel A.

(C) HCT116 Ctr, HCT116 CHD4 KD, HCT116 DNMT1 hypomorph, HCT116 DNMT3A KO, and HCT116 DNMT3B KO cells were untreated (Un) or treated with 2 mM H_2O_2 for 30 min. Whole cell extracts and the tight chromatin fractions were analyzed by immunoblotting as in (B).

(D) NCCIT Ctr, NCCIT CHD4 KD, NCCIT DNMT3A KO, and NCCIT DNMT3B KO cells were not treated (Un) or treated with 2 mM H_2O_2 for 30 min. Whole cell extracts and the tight chromatin fractions were analyzed by immunoblotting as in (B).

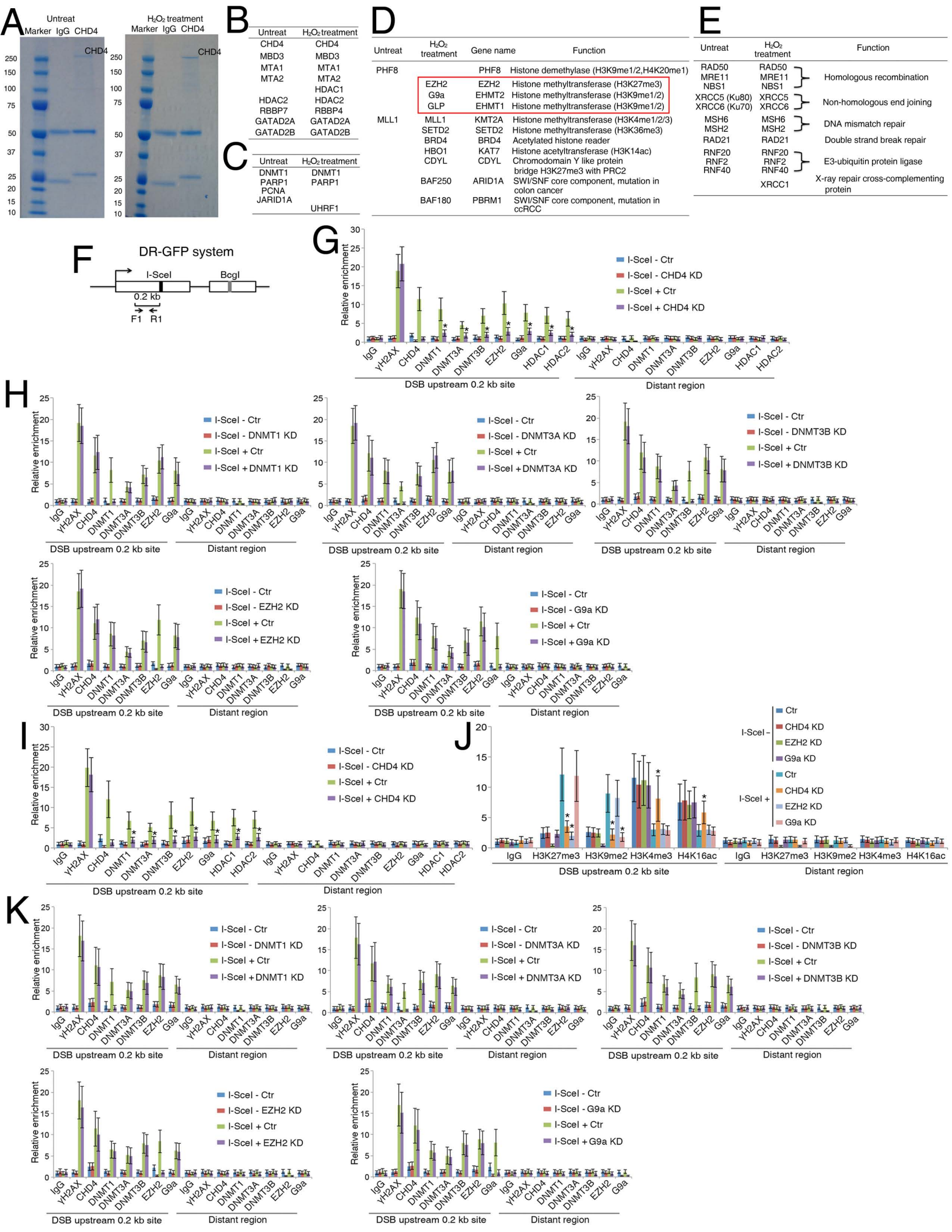

A
Untreat
Marker IgG CHD4
CHD4
H2O2 treatment
Marker IgG CHD4
CHD4
250
150
100
75
50
37
25
20
15
10
B
Untreat
H2O2 treatment
CHD4
CHD4
MBD3
MBD3
MTA1
MTA1
MTA2
MTA2
HDAC1
HDAC2
HDAC2
RBBP7
RBBP4
GATAD2A
GATAD2A
GATAD2B
GATAD2B
C
Untreat
H2O2 treatment
DNMT1
DNMT1
PARP1
PARP1
PCNA
JARID1A
UHRF1
D
Untreat
H2O2 treatment
Gene name
Function
PHF8
PHF8
Histone demethylase (H3K9me1/2,H4K20me1)
EZH2
EZH2
Histone methyltransferase (H3K27me3)
G9a
EHMT2
Histone methyltransferase (H3K9me1/2)
GLP
EHMT1
Histone methyltransferase (H3K9me1/2)
MLL1
MLL1
KMT2A
Histone methyltransferase (H3K4me1/2/3)
SETD2
SETD2
Histone methyltransferase (H3K36me3)
BRD4
BRD4
Acetylated histone reader
HBO1
KAT7
Histone acetyltransferase (H3K14ac)
CDYL
CDYL
Chromodomain Y like protein
bridge H3K27me3 with PRC2
BAF250
ARID1A
SWI/SNF core component, mutation in
colon cancer
BAF180
PBRM1
SWI/SNF core component, mutation in
ccRCC
E
Untreat
H2O2 treatment
Function
RAD50
RAD50
MRE11
MRE11
NBS1
NBS1
Homologous recombination
XRCC5 (Ku80)
XRCC5
XRCC6 (Ku70)
XRCC6
Non-homologous end joining
MSH6
MSH6
MSH2
MSH2
DNA mismatch repair
RAD21
RAD21
Double strand break repair
RNF20
RNF20
RNF2
RNF2
RNF40
RNF40
E3-ubiquitin protein ligase
XRCC1
X-ray repair cross-complementing
protein
F
DR-GFP system
I-SceI
BcgI
0.2 kb
F1
R1
G
Relative enrichment
I-SceI - Ctr
I-SceI - CHD4 KD
I-SceI + Ctr
I-SceI + CHD4 KD
IgG
γH2AX
CHD4
DNMT1
DNMT3A
DNMT3B
EZH2
G9a
HDAC1
HDAC2
DSB upstream 0.2 kb site
Distant region
H
I-SceI - DNMT1 KD
I-SceI + DNMT1 KD
I-SceI - DNMT3A KD
I-SceI + DNMT3A KD
I-SceI - DNMT3B KD
I-SceI + DNMT3B KD
I-SceI - EZH2 KD
I-SceI + EZH2 KD
I-SceI - G9a KD
I-SceI + G9a KD
I
J
I-SceI -
I-SceI +
Ctr
CHD4 KD
EZH2 KD
G9a KD
H3K27me3
H3K9me2
H3K4me3
H4K16ac
K

Figure S2, Related to Figure 2

(A) Endogenous CHD4 was immunoprecipitated from nuclear extracts of SW480 cells that was untreated or treated with 2 mM H_2O_2 for 30 min, resolved by SDS-PAGE and stained. IgG served as a negative control. Protein bands were retrieved and analyzed by mass spectrometry.

(B) Summary of several NuRD components that was identified by mass spectrometry in SW480 untreated or H_2O_2 treated cells.

(C) Summary of previously known CHD4-interacting proteins that was identified by mass spectrometry in SW480 untreated or H_2O_2 treated cells.

(D) Summary of CHD4-interacting-histone/chromatin modifiers that was identified by mass spectrometry in SW480 untreated or H_2O_2 treated cells.

(E) Summary of CHD4-interacting-DNA damage/repair proteins that was identified by mass spectrometry in SW480 untreated or H_2O_2 treated cells.

(F) Schematic representation of the DR-GFP/I-SceI system. The DR-GFP reporter contains an upstream GFP gene with insertion of an I-SceI recognition site and a downstream internal GFP repeat. Expression of the I-SceI endonuclease leads to DSB induction in the upstream GFP.

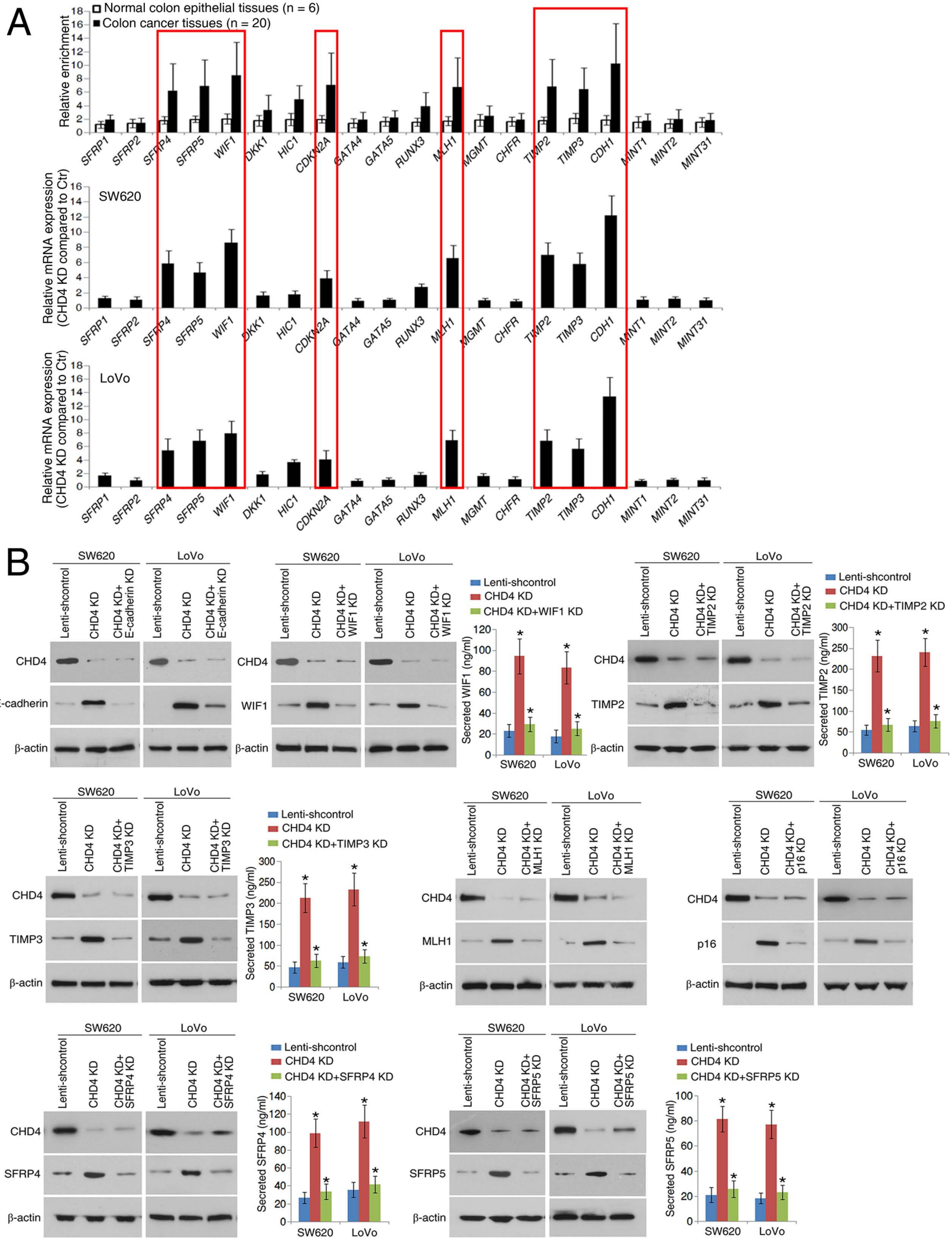
(G) SW480 DR-GFP cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, cells were infected with lentivirus expressing I-SceI. After 24 hr, ChIP assay was used to analyze the enrichment of epigenetic silencing proteins near the DSB site.

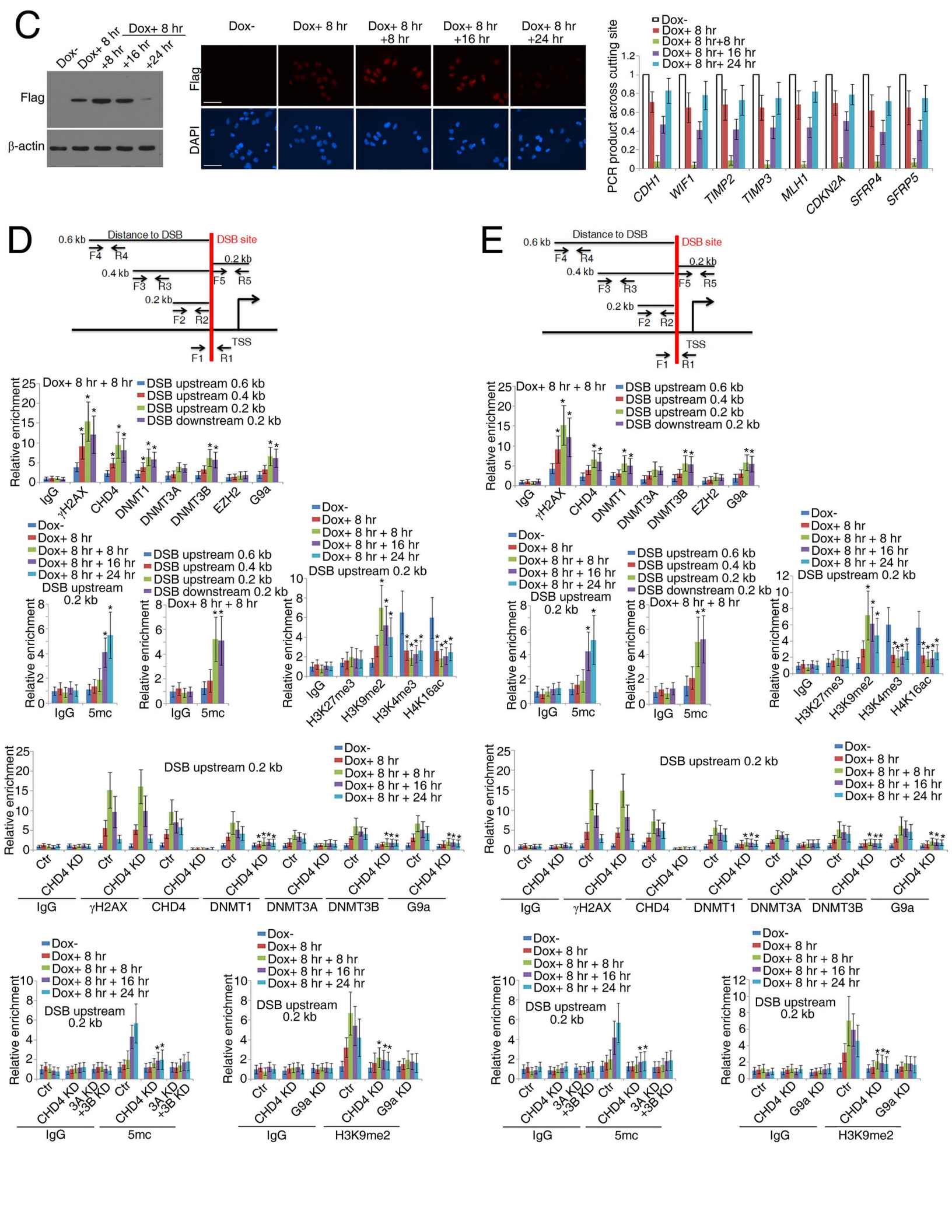
(H) The recruitment of DNMTs and EZH2 or G9a to DNA damage sites are not dependent on each other. SW480 DR-GFP cells were infected with the indicated lentivirus. After 72 hr, cells were infected with lentivirus expressing I-SceI. After 24 hr, ChIP assay was used to analyze the enrichment of the indicated proteins near the DSB site. The y-axis represents the relative enrichment of the indicated proteins compared to the IgG control.

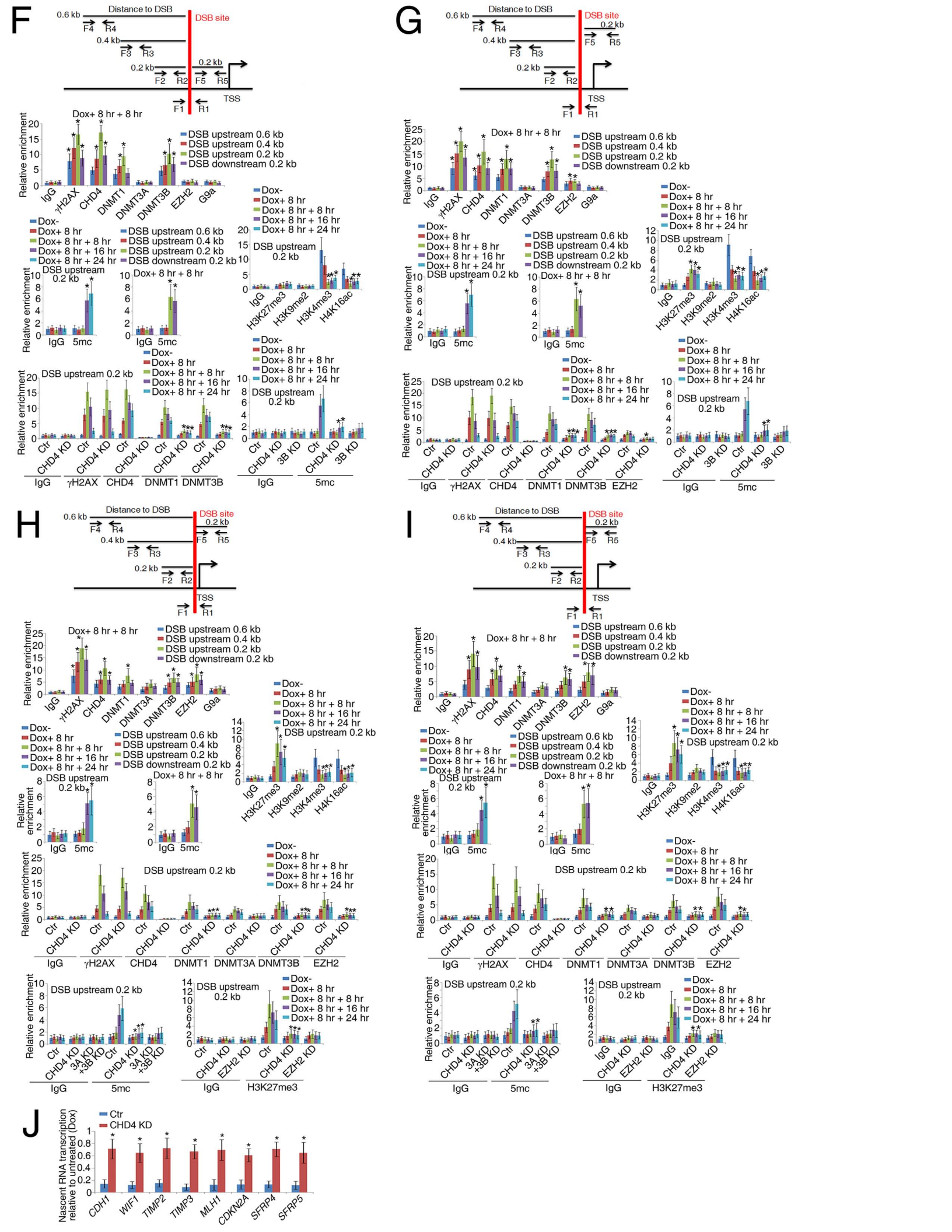
(I, J) ChIP analyses of the enrichment of epigenetic silencing proteins and histone modification changes at I-SceI-induced DSBs in U2OS DR-GFP cells infected with the indicated lentivirus.

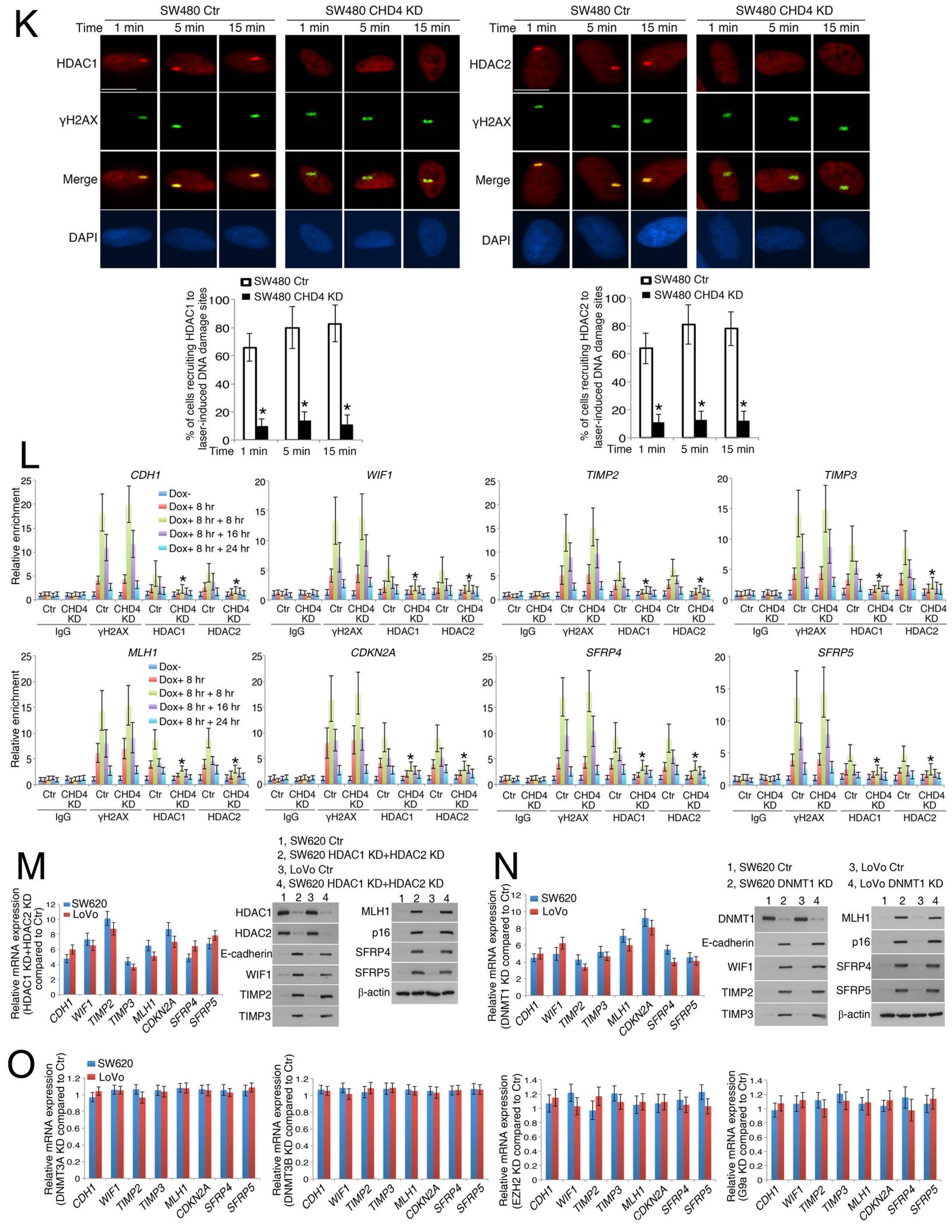
(K) ChIP analyses of the enrichment of the indicated proteins at I-SceI-induced DSBs in U2OS DR-GFP cells infected with the indicated lentivirus. The y-axis represents the relative enrichment of the indicated proteins compared to the IgG control.

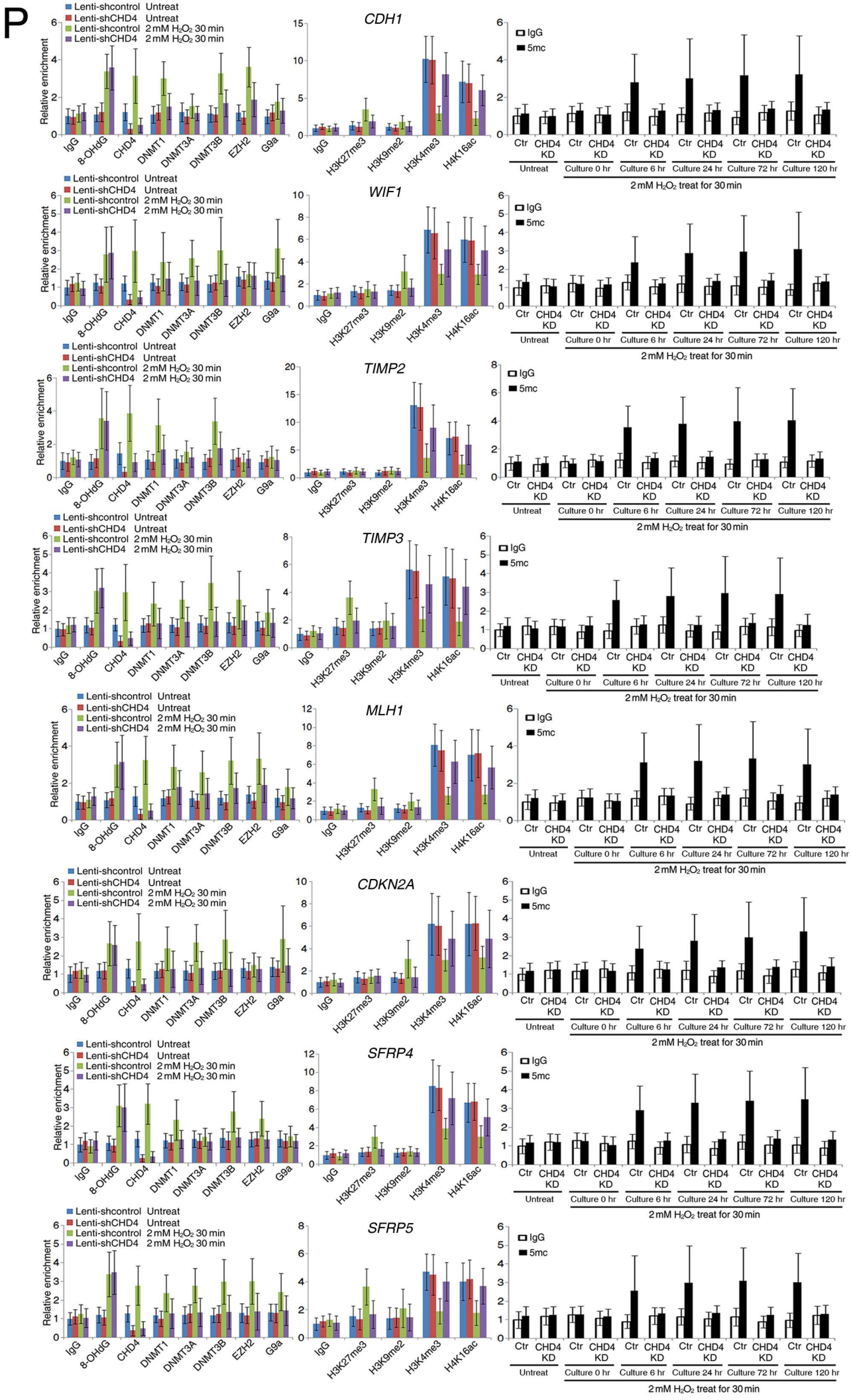
Data are represented as mean ± SEM for triplicate experiments. *p value < 0.05


A
Normal colon epithelial tissues (n = 6)
Colon cancer tissues (n = 20)
Relative enrichment
Relative mRNA expression (CHD4 KD compared to Ctr)
SW620
LoVo
SFRP1
SFRP2
SFRP4
SFRP5
WIF1
DKK1
HIC1
CDKN2A
GATA4
GATA5
RUNX3
MLH1
MGMT
CHFR
TIMP2
TIMP3
CDH1
MINT1
MINT2
MINT31
B
SW620
LoVo
Lenti-shcontrol
CHD4 KD
CHD4 KD+ E-cadherin KD
CHD4 KD+ WIF1 KD
CHD4 KD+ TIMP2 KD
CHD4 KD+ TIMP3 KD
CHD4 KD+ MLH1 KD
CHD4 KD+ p16 KD
CHD4 KD+SFRP4 KD
CHD4 KD+SFRP5 KD
CHD4
E-cadherin
WIF1
TIMP2
TIMP3
MLH1
p16
SFRP4
SFRP5
β-actin
Secreted WIF1 (ng/ml)
Secreted TIMP2 (ng/ml)
Secreted TIMP3 (ng/ml)
Secreted SFRP4 (ng/ml)
Secreted SFRP5 (ng/ml)


C
Dox-
Dox+ 8 hr
Dox+ 8 hr +8 hr
Dox+ 8 hr +16 hr
Dox+ 8 hr +24 hr
Flag
β-actin
DAPI
PCR product across cutting site
CDH1
WIF1
TIMP2
TIMP3
MLH1
CDKN2A
SFRP4
SFRP5
D
E
0.6 kb
Distance to DSB
DSB site
0.4 kb
0.2 kb
TSS
F1
F2
F3
F4
F5
R1
R2
R3
R4
R5
Relative enrichment
DSB upstream 0.6 kb
DSB upstream 0.4 kb
DSB upstream 0.2 kb
DSB downstream 0.2 kb
IgG
γH2AX
CHD4
DNMT1
DNMT3A
DNMT3B
EZH2
G9a
5mc
H3K27me3
H3K9me2
H3K4me3
H4K16ac
DSB upstream 0.2 kb
Ctr
CHD4 KD
3A KD
+3B KD
G9a KD


F
G
H
I
J
Distance to DSB
DSB site
TSS
0.6 kb
0.4 kb
0.2 kb
F1
F2
F3
F4
F5
R1
R2
R3
R4
R5
Dox+ 8 hr + 8 hr
DSB upstream 0.6 kb
DSB upstream 0.4 kb
DSB upstream 0.2 kb
DSB downstream 0.2 kb
Relative enrichment
IgG
γH2AX
CHD4
DNMT1
DNMT3A
DNMT3B
EZH2
G9a
Dox-
Dox+ 8 hr
Dox+ 8 hr + 8 hr
Dox+ 8 hr + 16 hr
Dox+ 8 hr + 24 hr
DSB upstream 0.2 kb
5mc
H3K27me3
H3K9me2
H3K4me3
H4K16ac
Ctr
CHD4 KD
3B KD
3A KD +3B KD
EZH2 KD
Nascent RNA transcription relative to untreated (Dox)
CDH1
WIF1
TIMP2
TIMP3
MLH1
CDKN2A
SFRP4
SFRP5

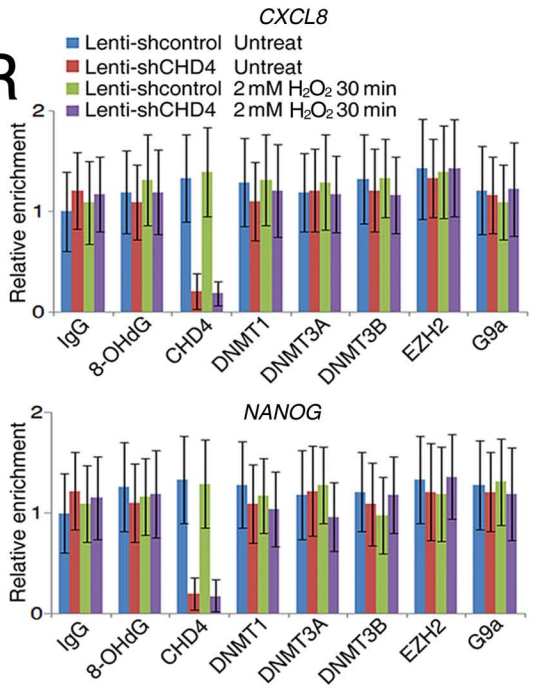

K
SW480 Ctr
SW480 CHD4 KD
Time
1 min
5 min
15 min
HDAC1
HDAC2
γH2AX
Merge
DAPI
SW480 Ctr
SW480 CHD4 KD
% of cells recruiting HDAC1 to laser-induced DNA damage sites
% of cells recruiting HDAC2 to laser-induced DNA damage sites
L
CDH1
WIF1
TIMP2
TIMP3
MLH1
CDKN2A
SFRP4
SFRP5
Relative enrichment
Dox-
Dox+ 8 hr
Dox+ 8 hr + 8 hr
Dox+ 8 hr + 16 hr
Dox+ 8 hr + 24 hr
Ctr
CHD4 KD
IgG
γH2AX
HDAC1
HDAC2
M
1, SW620 Ctr
2, SW620 HDAC1 KD+HDAC2 KD
3, LoVo Ctr
4, SW620 HDAC1 KD+HDAC2 KD
SW620
LoVo
Relative mRNA expression (HDAC1 KD+HDAC2 KD compared to Ctr)
HDAC1
HDAC2
E-cadherin
WIF1
TIMP2
TIMP3
MLH1
p16
SFRP4
SFRP5
β-actin
N
1, SW620 Ctr
2, SW620 DNMT1 KD
3, LoVo Ctr
4, LoVo DNMT1 KD
Relative mRNA expression (DNMT1 KD compared to Ctr)
DNMT1
O
Relative mRNA expression (DNMT3A KD compared to Ctr)
Relative mRNA expression (DNMT3B KD compared to Ctr)
Relative mRNA expression (EZH2 KD compared to Ctr)
Relative mRNA expression (G9a KD compared to Ctr)


P
Lenti-shcontrol Untreat
Lenti-shCHD4 Untreat
Lenti-shcontrol 2 mM H2O2 30 min
Lenti-shCHD4 2 mM H2O2 30 min
Relative enrichment
IgG
8-OHdG
CHD4
DNMT1
DNMT3A
DNMT3B
EZH2
G9a
CDH1
WIF1
TIMP2
TIMP3
MLH1
CDKN2A
SFRP4
SFRP5
H3K27me3
H3K9me2
H3K4me3
H4K16ac
5mc
Ctr
CHD4 KD
Untreat
Culture 0 hr
Culture 6 hr
Culture 24 hr
Culture 72 hr
Culture 120 hr
2 mM H2O2 treat for 30 min

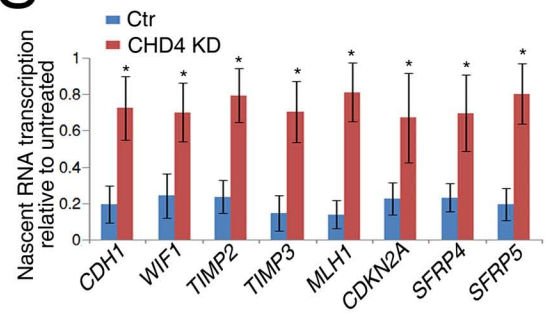
Q

Genes	Recruitment of proteins by CHD4 in the vicinity of DSBs	Epigenetic changes in the vicinity of DSBs
CDH1	DNMT1, DNMT3B, EZH2	5mc ↑ , H3K27me3 ↑ , H3K4me3 ↓ , H4K16ac ↓
WIF1	DNMT1, DNMT3A, DNMT3B, EZH2	5mc ↑ , H3K27me3 ↑ , H3K4me3 ↓ , H4K16ac ↓
TIMP2	DNMT1, DNMT3A, DNMT3B, G9a	5mc ↑ , H3K9me2 ↑ , H3K4me3 ↓ , H4K16ac ↓
TIMP3	DNMT1, DNMT3A, DNMT3B, G9a	5mc ↑ , H3K9me2 ↑ , H3K4me3 ↓ , H4K16ac ↓
MLH1	DNMT1, DNMT3B	5mc ↑ , H3K4me3 ↓ , H4K16ac ↓
CDKN2A	DNMT1, DNMT3B, EZH2	5mc ↑ , H3K27me3 ↑ , H3K4me3 ↓ , H4K16ac ↓
SFRP4	DNMT1, DNMT3A, DNMT3B, EZH2	5mc ↑ , H3K27me3 ↑ , H3K4me3 ↓ , H4K16ac ↓
SFRP5	DNMT1, DNMT3A, DNMT3B, EZH2	5mc ↑ , H3K27me3 ↑ , H3K4me3 ↓ , H4K16ac ↓

R



S



T

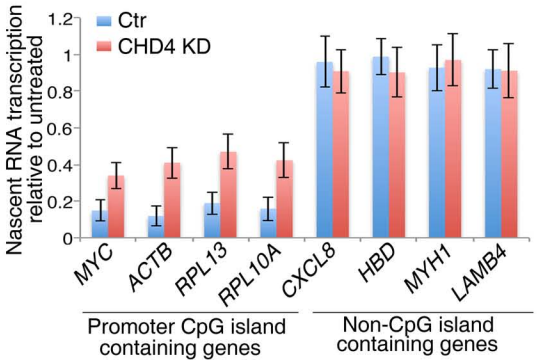


Figure S3, Related to Figure 4

(A) (Upper panel) ChIP analyses of CHD4 enrichment at the promoter CpG islands of twenty genes in fresh frozen human colon cancer tissues (n = 20) and normal colon epithelial tissues (n = 6). (Lower panel) SW620 and LoVo cells were infected with Lenti-shcontrol or Lenti-shCHD4 for 96 hr. Real-time RT-PCR was performed to detect the mRNA levels of twenty genes.

(B) SW620 and LoVo cells were infected with the indicated lentivirus. Seventy-two hours after infection, cells were selected for 7 days using 2 μg/ml puromycin. Western blot and ELISA analyses were performed to detect the protein levels and secreted protein levels of the indicated proteins.

(C) (Left panel) Treatment of cells with 1 μg/ml doxycycline (Dox) induces expression of the flag-tagged dCas9-FokI fusion protein by western blot. (Middle panel) Immunofluorescence staining of flag-tagged dCase9/FokI fusion protein in cells treated with 1 μg/ml doxycycline at the indicated time point. Localization of dCase9/FokI fusion protein was determined using anti-Flag primary antibody followed by an anti-rabbit Red secondary antibody (Red light). Blue, nuclear DAPI staining. The scale bar represents 50 μm. (Right panel) DNA was collected from cells treated with doxycycline at the indicated time points. PCR was performed using primers (F1, R1) on either side of the cut site.

(D-I) (Upper panel) Map of the doxycycline-inducible DSB site at the endogenous promoter CpG islands of *TIMP2* (D), *TIMP3* (E), *MLH1* (F), *CDKN2A* (G), *SFRP4* (H), and *SFRP5* (I), and PCR strategy for detection of protein enrichment and histone modification near the DSB site. Doxycycline-induced DSB was monitored by a PCR assay with F1, R1 primers spanning the cut site. Four pairs of primers (F2, R2---F5, R5) were used for ChIP at indicated distances from the DSB site. (Lower panel) ChIP analyses of the indicated protein enrichment, 5mc enrichment, and histone modifications in the vicinity of DSB sites at the indicated time points.

(J) SW480 cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, doxycycline was added to medium for 8 hr followed by doxycycline washput. Cells were collected at 8 hr and nascent RNA was labeled concurrently.

(K) SW480 Ctr and SW480 CHD4 KD cells were micro-irradiated with a 455 nm laser and fixed at the indicated time points. The recruitment of endogenous HDAC1 and HDAC2 to DNA damage sites and their co-localization with γH2AX was examined by immunofluorescence staining in the indicated cells. Representative images at the indicated time points are shown. The graphs represent the percentages of cells with γH2AX micro-irradiation tracks observed that have visible accumulation of HDAC1 or HDAC2 co-localizing with γH2AX. The scale bar represents 10 μm.

(L) Real-time PCR analyses of ChIP samples from the indicated cells infected with Lenti-shcontrol (Ctr) or Lenti-shCHD4 (CHD4 KD). The y-axis represents the relative enrichment of the indicated proteins compared to the IgG control.

(M) SW620 and LoVo cells were infected with Lenti-shcontrol or Lenti-shHDAC1 + Lenti-shHDAC2. After 96 hr, Real-time RT-PCR and Western blot analyses were used to detect the mRNA and protein levels of eight TSGs.

(N) SW620 and LoVo cells were infected with Lenti-shcontrol or Lenti-shDNMT1. After 10 days, Real-time RT-PCR and Western blot analyses were used to detect the mRNA and protein levels of eight TSGs.

(O) SW620 and LoVo cells were infected with the indicated lentivirus. After 96 hr, Real-time RT-PCR analyses were used to detect the mRNA levels of eight TSGs.

(P) SW480 cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, cells were untreated or treated with 2 mM H_2O_2 for 30 min followed by ChIP for control IgG, 8-OHdG, CHD4, DNMT1,

DNMT3A, DNMT3B, EZH2, G9a, H3K27me3, H3K9me2, H3K4me3, and H4K16ac at the promoter CpG islands of eight representative genes and analyzed by real-time PCR. 8-OHdG was used as DNA oxidation marker. For 5mc ChIP at the promoter CpG islands of eight representative genes, SW480 cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, cells were untreated or treated with 2 mM H_2O_2 for 30 min followed by washing out H_2O_2 and cultured free from H_2O_2 for additional time, and collected cells at indicated time points. The y-axis represents the relative enrichment of the indicated proteins compared to the IgG control.

(Q) Summary of the CHD4-mediated recruitment of epigenetic silencing proteins and epigenetic changes at the promoter CpG islands of eight TSGs.

(R) SW480 cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, cells were untreated or treated with 2 mM H_2O_2 for 30 min followed by ChIP for control IgG, 8-OHdG, CHD4, DNMT1, DNMT3A, DNMT3B, EZH2, and G9a at the promoter of non CpG island genes *CXCL8* and *NANOG*.

(S) SW480 cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, cells were untreated or treated with 2 mM H_2O_2 for 30 min, and nascent RNA was labeled concurrently. Real-time RT-PCR was used to detect the nascent RNA transcription of eight TSGs.

(**T)** SW480 cells were infected with Lenti-shcontrol or Lenti-shCHD4. After 96 hr, cells were untreated or treated with 2 mM H_2O_2 for 30 min, and nascent RNA of the indicated genes was labeled concurrently. Real-time RT-PCR was used to detect the nascent RNA transcription of the indicated genes.

Data are represented as mean ± SEM for triplicate experiments. *p value < 0.05

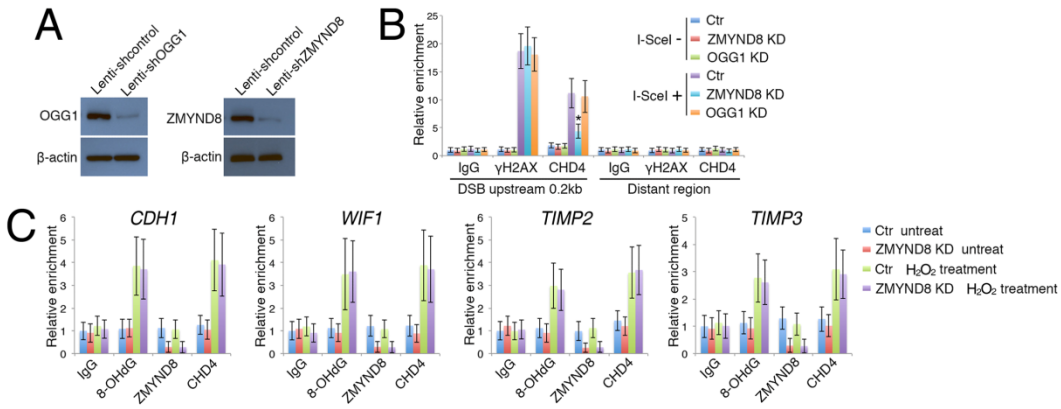


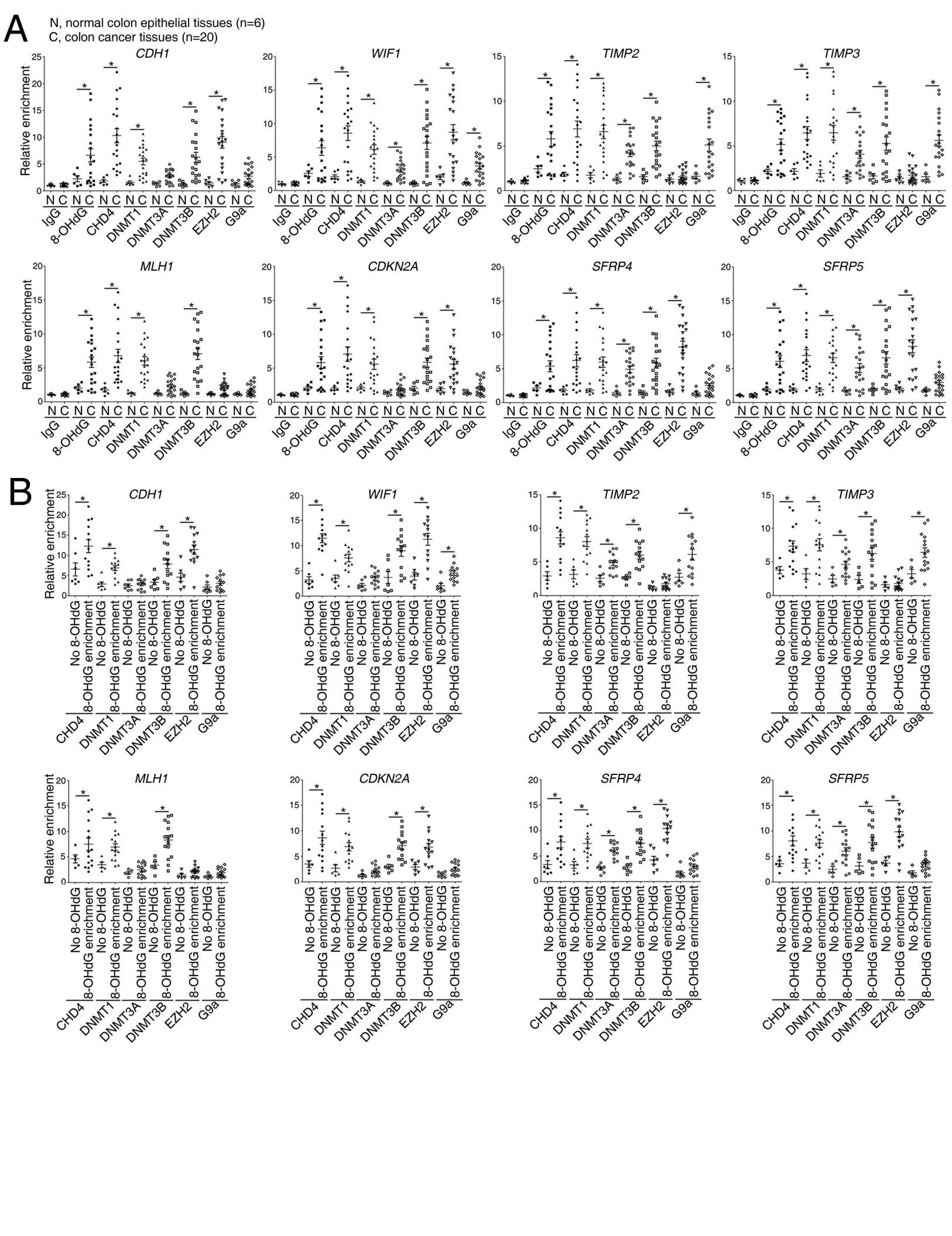
Figure S4, Related to Figure 5

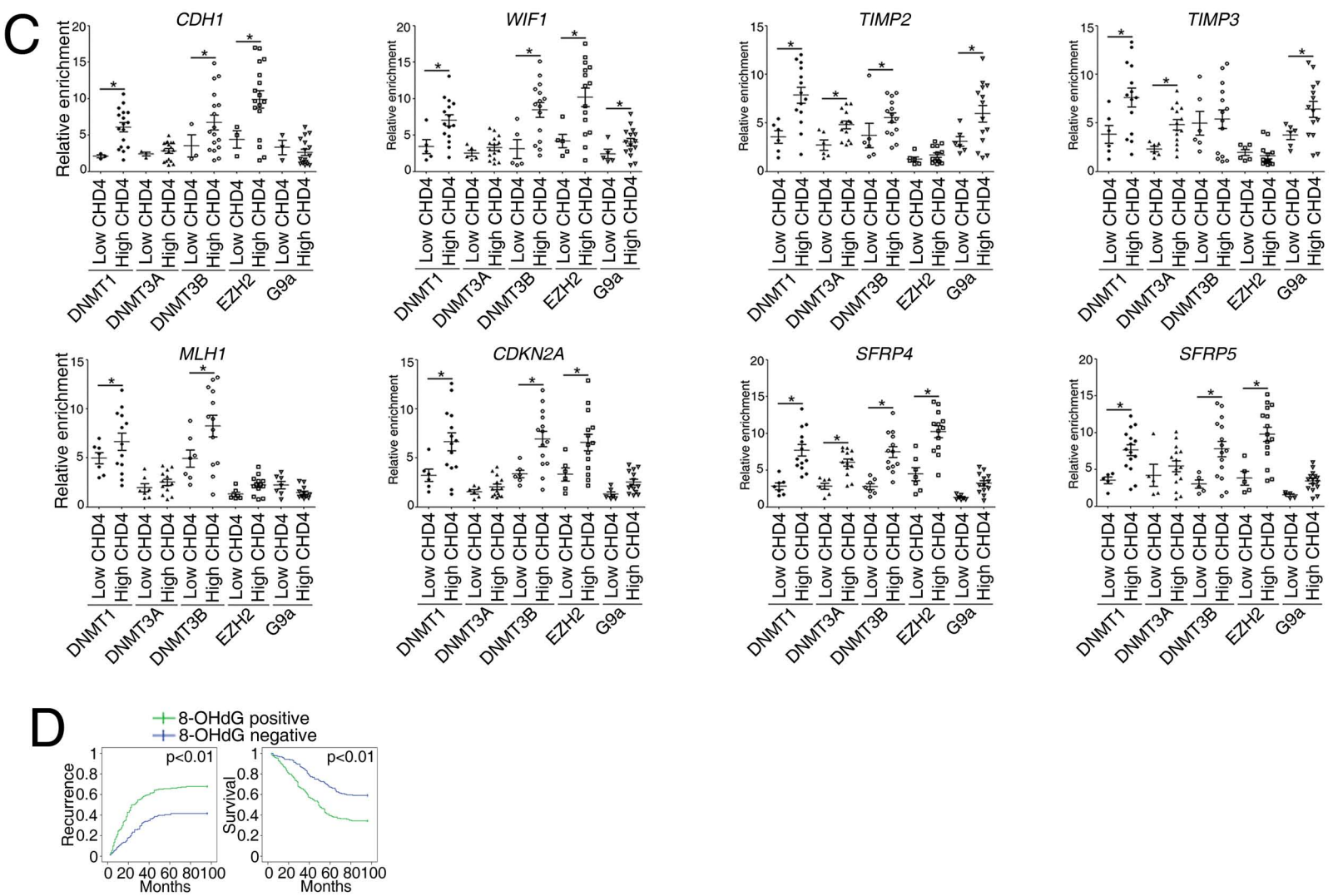
(A) SW480 DR-GFP cells were infected with Lenti-shOGG1 or Lenti-shZMYND8. After 72 hr, Western blot analyses were used to detect the protein levels of OGG1 and ZMYND8.

(B) SW480 DR-GFP cells were infected with Lenti-shcontrol, Lenti-shZMYND8, or Lenti-shOGG1. After 72 hr, cells were infected with lentivirus expressing I-SceI. After 24 hr, ChIP assay was used to analyze the enrichment of indicated proteins near the DSB site.

(C) Cells were untreated or treated with 2 mM H_2O_2 for 30 min followed by ChIP for control IgG, 8-OHdG, ZMYND8 and CHD4 at the promoter CpG islands of four representative genes. The y-axis represents the relative enrichment of the indicated proteins compared to the IgG control.

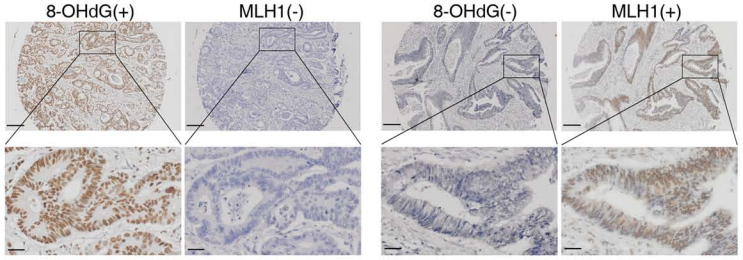
Data are represented as mean ± SEM for triplicate experiments. *p value < 0.05


A
N, normal colon epithelial tissues (n=6)
C, colon cancer tissues (n=20)
CDH1
WIF1
TIMP2
TIMP3
MLH1
CDKN2A
SFRP4
SFRP5
Relative enrichment
N C
IgG
8-OHdG
CHD4
DNMT1
DNMT3A
DNMT3B
EZH2
G9a
B
No 8-OHdG
8-OHdG enrichment

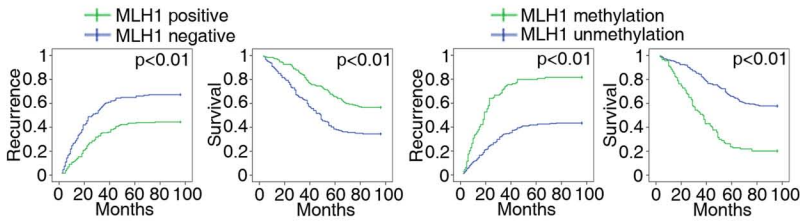

C
CDH1
WIF1
TIMP2
TIMP3
MLH1
CDKN2A
SFRP4
SFRP5
Relative enrichment
Low CHD4
High CHD4
DNMT1
DNMT3A
DNMT3B
EZH2
G9a
*

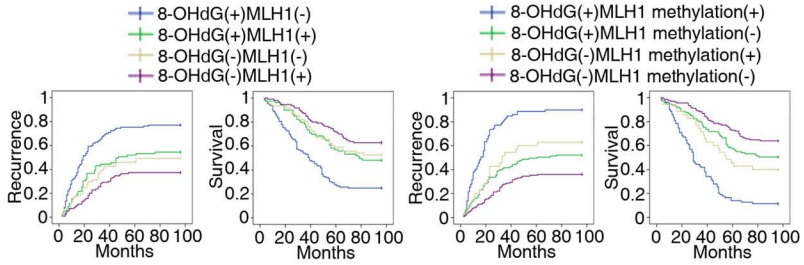
D
8-OHdG positive
8-OHdG negative
p<0.01
Recurrence
Survival
Months
0 20 40 60 80100

E

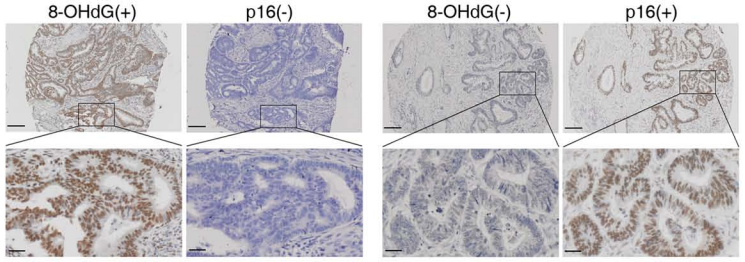


		8-OHdG positive(n=192)	8-OHdG negative(n=171)	p value
MLH1	positive (n=189)	79	110	p<0.001
	negative (n=174)	113	61	
MLH1 methylation(n=114)		79	35	p<0.001
MLH1 unmethylation(n=249)		113	136	

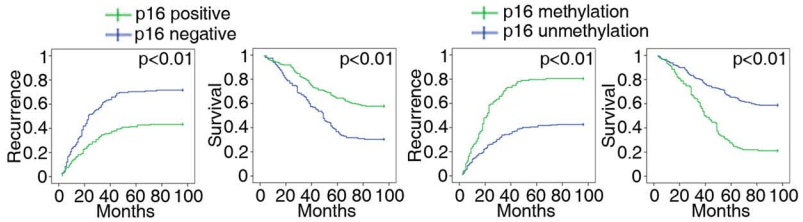


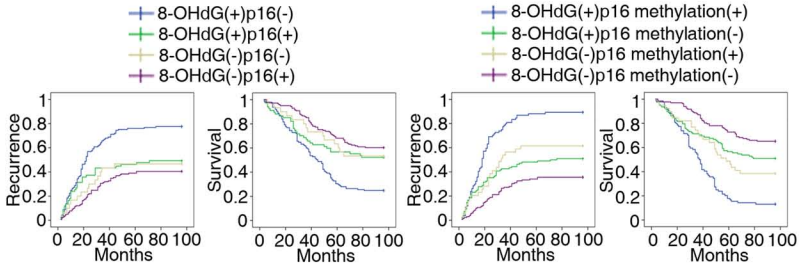


F

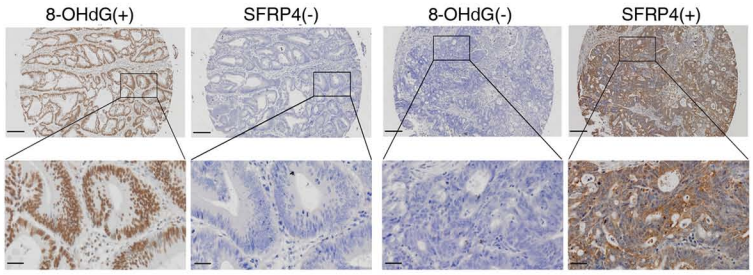


		8-OHdG positive(n=192)	8-OHdG negative(n=171)	p value
p16	positive (n=208)	67	141	p<0.001
	negative (n=155)	125	30	
p16 methylation(n=123)		84	39	p<0.001
p16 unmethylation(n=240)		108	132	

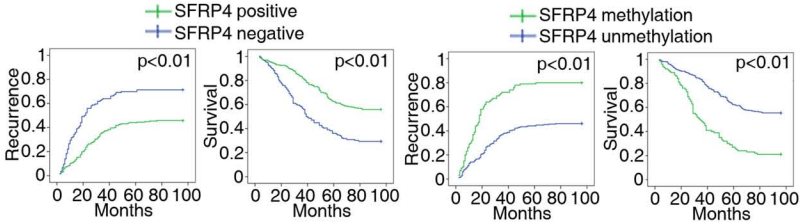


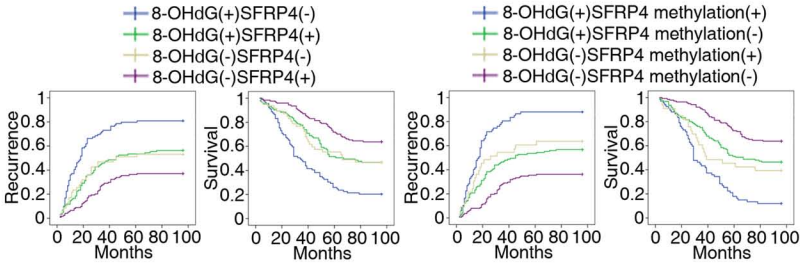


G

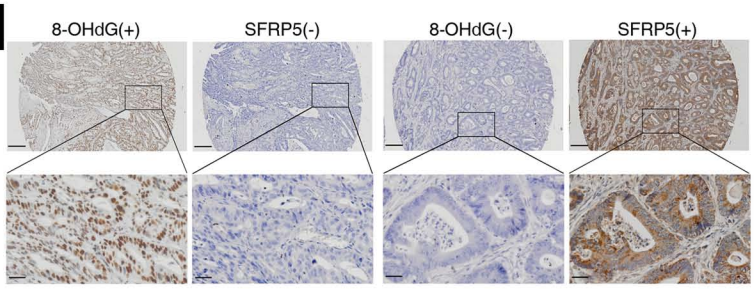


		8-OHdG positive(n=192)	8-OHdG negative(n=171)	p value
SFRP4	positive (n=227)	103	124	p<0.001
	negative (n=136)	89	47	
SFRP4 methylation (n=100)		67	33	p=0.001
SFRP4 unmethylation (n=263)		125	138	

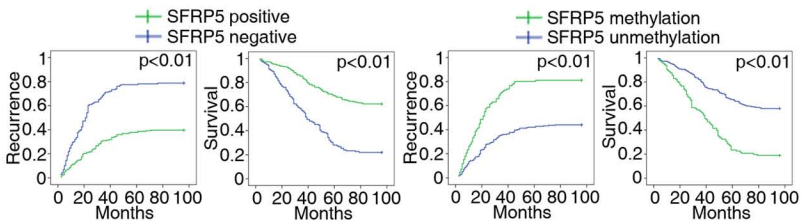


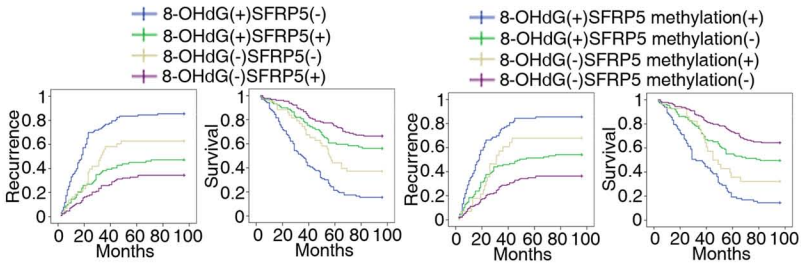


H



		8-OHdG positive(n=192)	8-OHdG negative(n=171)	p value
SFRP5	positive (n=217)	89	128	p<0.001
	negative (n=146)	103	43	
SFRP5 methylation (n=111)		83	28	p<0.001
SFRP5 unmethylation (n=252)		109	143	





	E-cadherin expression		p value
	negative (n=201)	positive (n=162)	
E-cadherin methylation (n=93)	66	27	p<0.001
E-cadherin unmethylation (n=270)	135	135	

	WIF1 expression		p value
	negative (n=189)	positive (n=174)	
WIF1 methylation (n=95)	67	28	p<0.001
WIF1 unmethylation (n=268)	122	146	

	TIMP2 expression		p value
	negative (n=162)	positive (n=201)	
TIMP2 methylation (n=115)	77	38	p<0.001
TIMP2 unmethylation (n=248)	85	163	

	TIMP3 expression		p value
	negative (n=156)	positive (n=207)	
TIMP3 methylation (n=109)	81	28	p<0.001
TIMP3 unmethylation (n=254)	75	179	

	MLH1 expression		p value
	negative (n=174)	positive (n=189)	
MLH1 methylation(n=114)	87	27	p<0.001
MLH1 unmethylation(n=249)	87	162	

	p16 expression		p value
	negative (n=155)	positive (n=208)	
p16 methylation(n=123)	97	26	p<0.001
p16 unmethylation(n=240)	58	182	

	SFRP4 expression		p value
	negative (n=136)	positive (n=227)	
SFRP4 methylation (n=100)	68	32	p<0.001
SFRP4 unmethylation (n=263)	68	195	

	SFRP5 expression		p value
	negative (n=146)	positive (n=217)	
SFRP5 methylation (n=111)	80	31	p<0.001
SFRP5 unmethylation (n=252)	66	186	

Figure S5, Related to Figure 6

(A) ChIP analyses of the enrichment of oxidative DNA damage marker 8-OHdG and epigenetic silencing proteins (CHD4, DNMT1, DNMT3A, DNMT3B, EZH2, and G9a) at the promoter CpG islands of eight representative TSGs in fresh frozen human CRC tissues (n = 20) and normal colon epithelial tissues (n = 6). N, normal colon epithelial tissues; C, colon cancer tissues

(B) The association of 8-OHdG enrichment and enrichment of epigenetic silencing proteins at the promoter CpG islands of eight representative TSGs in fresh frozen human CRC tissues (n = 20). Colon cancer tissues were divided into two groups based on the value of 8-OHdG enrichment. The value of 8-OHdG enrichment < 2 was considered as "No 8-OHdG enrichment", whereas the value ≥ 2 was considered as "8-OHdG enrichment".

(C) The association of CHD4 enrichment and enrichment of other epigenetic silencing proteins at the promoter CpG islands of eight representative TSGs in fresh frozen human CRC tissues (n = 20). Colon cancer tissues were divided into two groups based on the value of CHD4 enrichment. The value of CHD4 enrichment < 4 was considered as "Low CHD4 enrichment", whereas the value ≥ 4 was considered as "High CHD4 enrichment". The y-axis represents the relative enrichment of the indicated proteins compared to the IgG control.

(D) A Kaplan-Meier analyses of the correlation of 8-OHdG with recurrence and overall survival in human CRC patients.

(E-H) (Upper panels) Representative images of immunohistochemistry of 8-OHdG, MLH1 (E), p16 (F), SFRP4 (G), and SFRP5 (H) in human CRC tissues. Scale bars represent 200 μm (low magnification) and 50 μm (high magnification). (Middle panels) The association between 8-OHdG expression and the methylation of representative TSGs or expression of TSGs in human CRC tissues. (Lower panels) A Kaplan-Meier analyses of the correlation of TSGs expression, TSGs methylation, 8-OHdG/TSGs co-expression or 8-OHdG/TSGs methylation status with recurrence and overall survival in patients with CRC.

(I) The association between eight representative TSGs expression and their methylation in 363 human CRC tissues.

Data are represented as mean ± SEM. *p value < 0.05

Table S1, related to Figure 6 Correlation between 8-OHdG staining and clinicopathological characteristics of human colorectal cancer tissues

Clinicopathological variables		Tumor 8-OHdG expression		p value
		Negative (n=171)	Positive (n=192)	
Age		66.37(10.96)	66.23(11.69)	0.911
Sex	female (n=161)	76	85	1
	male (n=202)	95	107	
Tumor location	right colon	71	102	0.05
	left colon	64	64	
	rectum	36	26	
Tumor size	＜5cm (n=154)	74	80	0.832
	≥5cm (n=209)	97	112	
Tumor differentiation	well or moderate (n=250)	141	109	<0.001
	poor (n=113)	30	83	
Tumor invasion	T1 (n=7)	6	1	<0.001
	T2 (n=30)	26	4	
	T3 (n=242)	106	136	
	T4 (n=84)	33	51	
Lymph node metastasis	absent (n=199)	123	76	<0.001
	present (n=164)	48	116	
Distant metastasis	absent (n=298)	158	140	<0.001
	present (n=65)	13	52	
AJCC stage	Stage I (n=35)	32	3	<0.001
	Stage II (n=159)	91	68	
	Stage III (n=104)	35	69	
	Stage IV (n=65)	13	52	

Table S2, related to Figure 6 Correlation between eight TSGs expression or their methylation and clinicopathological characteristics of human colorectal cancer tissues. Provided as an Excel file.

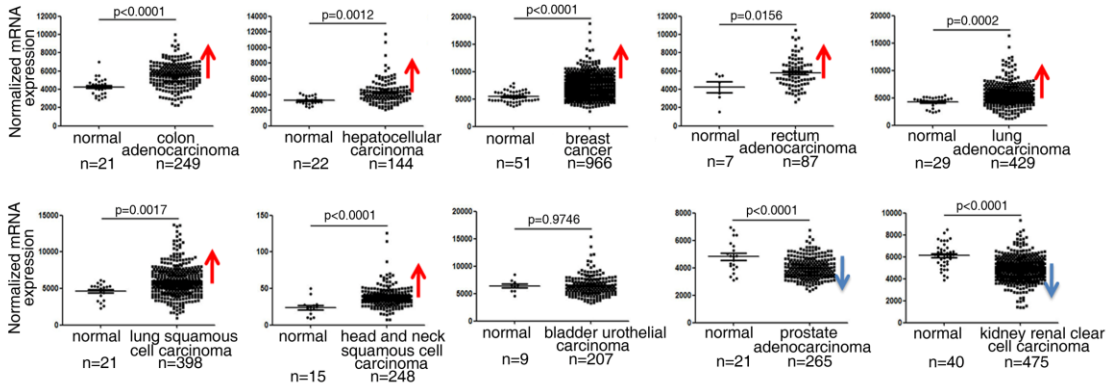


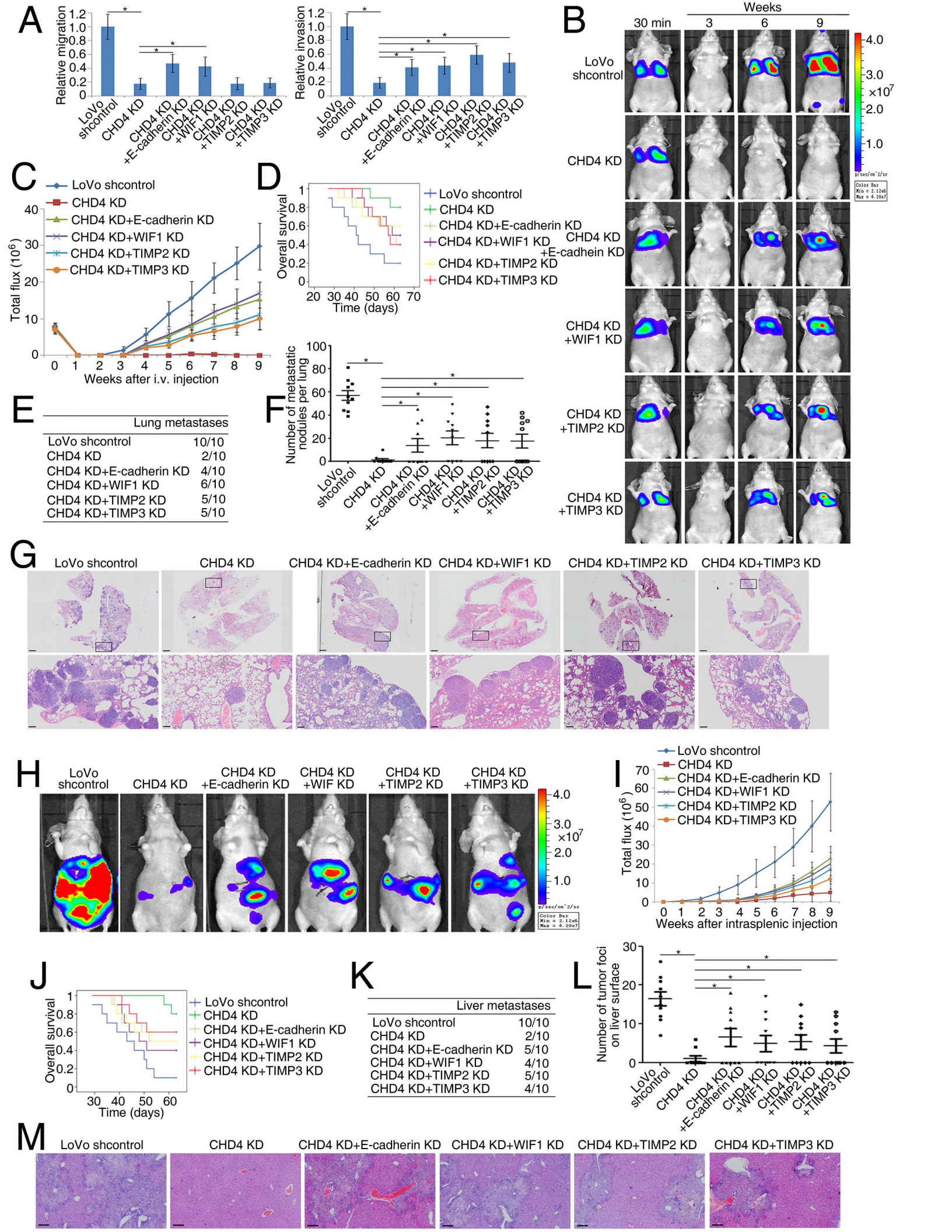
Figure S6, Related to Figure 7 Analyses of CHD4 expression in several human cancers using TCGA database. Data are represented as mean ± SEM.

Table S3, related to Figure 7 Correlation between CHD4 staining and clinicopathological characteristics of human colorectal cancer tissues

Clinicopathological variables		Tumor CHD4 expression		
		Negative (n=161)	Positive (n=202)	p value
Age		66.75(11.53)	65.94(11.20)	0.56
Sex	female	69	92	0.671
	male	92	110	
Tumor location	right colon	68	105	0.011
	left colon	55	73	
	rectum	38	24	
Tumor size	＜5cm	71	83	0.594
	≥5cm	90	119	
Tumor differentiation	well or moderate	140	110	<0.001
	poor	21	92	
Tumor invasion	T1	6	1	<0.001
	T2	23	7	
	T3	100	142	
	T4	32	52	
Lymph node metastasis	absent	125	74	<0.001
	present	36	128	
Distant metastasis	absent	155	143	<0.001
	present	6	59	
AJCC stage	Stage I	29	6	<0.001
	Stage II	98	61	
	Stage III	28	76	
	Stage IV	6	59	

Table S4, related to Figure 7 Multivariate analysis of factors associated with survival and recurrence of 363 human CRCs

Variables	Recurrence			Survival		
	Multivariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Tumor differentiation(well/moderate versus poor)	0.756	0.518-1.102	0.146	0.798	0.543-1.171	0.249
Tumor invasion(T1-T3 versus T4)	0.592	0.425-0.825	0.002	0.610	0.434-0.858	0.005
Lymph node metastasis (absent versus present)	0.340	0.120-0.964	0.043	0.318	0.110-0.923	0.035
Distant metastasis (absent versus present)	0.385	0.256-0.578	<0.001	0.373	0.246-0.564	<0.001
AJCC stage(I-III versus IV)	0.443	0.217-0.679	<0.001	0.378	0.223-0.569	<0.001
CHD4 expression (negative versus positive)	0.678	0.484-0.950	**0.024**	0.678	0.481-0.955	**0.026**


A
Relative migration
Relative invasion
LoVo shcontrol
CHD4 KD
CHD4 KD +E-cadherin KD
CHD4 KD +WIF1 KD
CHD4 KD +TIMP2 KD
CHD4 KD +TIMP3 KD
B
Weeks
30 min
3
6
9
LoVo shcontrol
CHD4 KD
CHD4 KD +E-cadhein KD
CHD4 KD +WIF1 KD
CHD4 KD +TIMP2 KD
CHD4 KD +TIMP3 KD
Color Bar
C
Total flux (10^6)
Weeks after i.v. injection
D
Overall survival
Time (days)
E
Lung metastases
LoVo shcontrol 10/10
CHD4 KD 2/10
CHD4 KD+E-cadherin KD 4/10
CHD4 KD+WIF1 KD 6/10
CHD4 KD+TIMP2 KD 5/10
CHD4 KD+TIMP3 KD 5/10
F
Number of metastatic nodules per lung
G
H
I
Weeks after intrasplenic injection
J
K
Liver metastases
LoVo shcontrol 10/10
CHD4 KD 2/10
CHD4 KD+E-cadherin KD 5/10
CHD4 KD+WIF1 KD 4/10
CHD4 KD+TIMP2 KD 5/10
CHD4 KD+TIMP3 KD 4/10
L
Number of tumor foci on liver surface
M

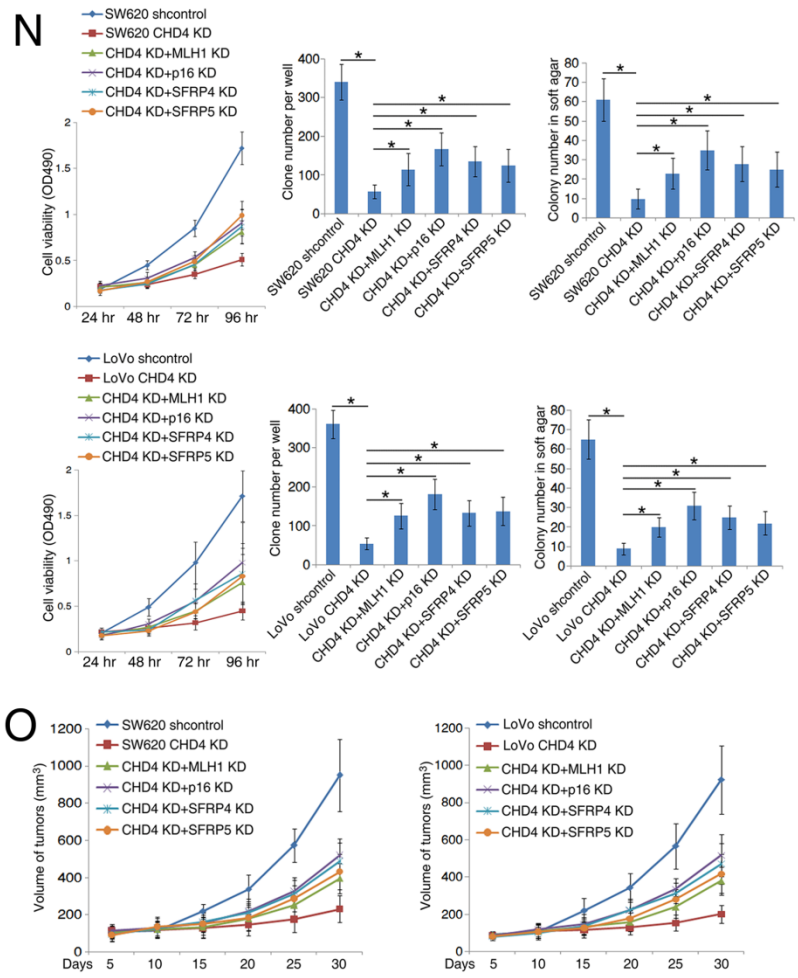


Figure S7, Related to Figure 8

(A) Transwell assay analyses of the migration and invasion abilities of the indicated CRC cells.

(B-G) LoVo cells infected with the indicated shRNA lentiviruses for the indicated genes were injected into the tail vein of immune-incompetent mice, followed by noninvasive bioluminescence imaging for weeks. Representative bioluminescent imaging (B)**,** bioluminescence signals (C)**,** overall survival (D)**,** incidence of lung colonization (E)**,** the number of lung colonization foci (F), and representative H&E staining of lung tissues (G) from the different groups is shown. Scale bars represent 1 mm (low magnification) and 100 μm (high magnification).

(H-M) A mouse model of liver metastases was established by intrasplenic injection of the indicated colon cancer cells. Representative bioluminescent imaging 9 weeks after injection (H), signals recorded at the indicated time points(I), overall survival (J), incidence of liver metastases (K)**,** number of tumor foci on the liver surface (L), and representative HE staining of liver tissues (M) from different groups is shown. The scale bar represents 200 μm.

(N) Indicated cells were subjected to MTT assays, colony formation assays, and soft agar colony formation assays.

(O) Tumor growth of the indicated colon cancer cells was investigated by subcutaneous xenograft tumor models. Each experimental group contained 10 nude mice.

Data are represented as mean ± SEM. *p value < 0.05

Table S5. Sequence for shRNA and CRISPR guide RNA

Gene name	Sequence
shRNA	
CHD4	CCGGGCGGGAGTTCAGTACCAATAACTCGAGTTATTGGTACTGAACTCCCGCTTTTT
DNMT1	CCGGCGATGAGGAAGTCGATGATAACTCGAGTTATCATCGACTTCCTCATCGTTTTTG
DNMT3A	CCGGCCACCAGAAGAAGAGAAGAATCTCGAGATTCTTCTCTTCTTCTGGTGGTTTTTG
DNMT3B	CCGGGACGATGGCTATCAGTCTTACCTCGAGGTAAGACTGATAGCCATCGTCTTTTTTG
EZH2	CCGGTATGATGGTTAACGGTGATCACTCGAGTGATCACCGTTAACCATCATATTTTTG
G9a	CCGGCGAGAGAGTTCATGGCTCTTTCTCGAGAAAGAGCCATGAACTCTCTCGTTTTTG
E-cadherin	CCGGGAACGAGGCTAACGTCGTAATCTCGAGATTACGACGTTAGCCTCGTTCTTTTTG
WIF1	CCGGGCAAGAGTACTCATAGGATTTCTCGAGAAATCCTATGAGTACTCTTGCTTTTTG
TIMP2	CCGGCAAGTTCTTCGCCTGCATCAACTCGAGTTGATGCAGGCGAAGAACTTGTTTTTG
TIMP3	CCGGCGAGAGTCTCTGTGGCCTTAACTCGAGTTAAGGCCACAGAGACTCTCGTTTTTG
MLH1	CCGGCCAAGTGAAGAATATGGGAAACTCGAGTTTCCCATATTCTTCACTTGGTTTTTG
p16	CCGGGCTCTGAGAAACCTCGGGAAACTCGAGTTTCCCGAGGTTTCTCAGAGCTTTTTG
SFRP4	CCGGGAACTCAAGTCCCGCTCATTACTCGAGTAATGAGCGGGACTTGAGTTCTTTTTG
SFRP5	CCGGCCACTCGGATACGCAGGTCTTCTCGAGAAGACCTGCGTATCCGAGTGGTTTTT
OGG1	CCGGCGGATCAAGTATGGACACTGACTCGAGTCAGTGTCCATACTTGATCCGTTTTT
ZMYND8	CCGGCCCTGACTATGCGGAATACATCTCGAGATGTATTCCGCATAGTCAGGGTTTTTG
CRISPR guide RNA	
DNMT3A	sense: 5'-caccgCGATGACGAGCCAGAGTACG-3'
	antisense: 5'-aaacCGTACTCTGGCTCGTCATCGc-3'
DNMT3B	sense: 5'-caccgATCCGCACCCCGGAGATCAG-3'
	antisense: 5'-aaacCTGATCTCCGGGGTGCGGATc-3'
OGG1	sense: 5’-caccgTAGCCTCCACTCCTGCCCTG-3’
	antisense: 5’-aaacCAGGGCAGGAGTGGAGGCTAc-3’

Table S6. Primer sequences used in the study.

Primer name	Primer sequences	
Primers for Real-time PCR:		
E-cadherin sense:	5'-GAACGCATTGCCACATAC-3'	
E-cadherin antisense:	5'-ACCTTCCATGACAGACCC-3'	
WIF1 sense:	5'-ATGCCAATGTCAAGAAGG-3'	
WIF1 antisense:	5'-ATGTCGGAGTTCACCAGA-3'	
TIMP2 sense:	5'-GCACCACCCAGAAGAAGAG-3'	
TIMP2 antisense:	5'-ACCCAGTCCATCCAGAGGC-3'	
TIMP3 sense:	5'-GCTGACAGGTCGCGTCTA-3'	
TIMP3 antisense:	5'-CACAAAGCAAGGCAGGTAG-3'	
MLH1 sense:	5'-AAGTTGTTGGCAGGTATT-3'	
MLH1 antisense:	5'-GGTTGAGGCATTGGGTAG-3'	
p16 sense:	5'-TTCCTGGACACGCTGGTGGT-3'	
p16 antisense:	5'-CTATGCGGGCATGGTTACTGC-3'	
SFRP4 sense:	5'-GATGTTGACTGTAAACGCCTAA-3'	
SFRP4 antisense:	5'-AGGGATGGGTGATGAGGA-3'	
SFRP5 sense:	5'-TTGACATCCCTGCCGACCTG-3'	
SFRP5 antisense:	5'-GAAGACCTGCGTATCCGAGTG-3'	
β-actin sense:	5'-CGGGAAATCGTGCGTGAC-3'	
β-actin antisense:	5'-CAGGAAGCAAGGCTGGAA-3'	
MYC sense:	5'-CTGCGACGAGGAGGAGAA-3'	
MYC anti-sense:	5'-CCGAAGGGAGAAGGGTGT-3'	
ACTB sense:	5'-CGGGAAATCGTGCGTGAC-3'	
ACTB antisense:	5'-CAGGAAGGAAGGCTGGAAG-3'	
RPL13 sense:	5'-ACCGCTCCAAACTCATCCTC-3'	
RPL13 antisense:	5'-TTTGCCCGTATGCCGAAG-3'	
RPL10A sense:	5'-TTCCCTTCCCTGCTCACA-3'	
RPL10A antisense:	5'-CAGCCAGACATAACACCTTC-3'	
IL-8 sense:	5'-AGCCTTCCTGATTTCTGC-3'	
IL-8 antisense:	5'-AACCCTCTGCACCCAGTT-3'	
HBD sense:	5'-GATGCAGTTGGTGGTGAG-3'	
HBD antisense:	5'-AAGTGCCCTTGAGGTTGT-3'	
MYH1 sense:	5'-CACCACCAACCCATACGA-3'	
MYH1 antisense:	5'-CTGCCTTGTCAGCAACTTCA-3'	
LAMB4 sense:	5'-CCAACCACTACGGACTAAG-3'	
LAMB4 antisense:	5'-AGCACCTCCAATATCACA-3'	
Primers for Methylation specific PCR:		Annealing temperature
E-cadherin methylated (M) allele-specific primers (F) :	5'-TGTAGTTACGTATTTATTTTTAGTGGCGTC-3'	60°C
E-cadherin M-R:	5'-CGAATACGATCGAATCGAACCG-3'	
E-cadherin unmethylated (U) allele-specific primers (F):	5'-TGGTTGTAGTTATGTATTTATTTTTAGTGGTGTT-3'	60°C

E-cadherin U-R:	5'-ACACCAAATACAATCAAATCAAACCAAA-3'	
WIF1 M-F:	5'-ATTTAGGTCGGGAGGCGACGC-3'	65℃
WIF1 M-R:	5'-GACCTCCGCCCGCAATACCAA-3'	
WIF1 U-F:	5'-TGGTATTTAGGTTGGGAGGTGATGT-3'	56℃
WIF1 U-R:	5'-AACCTCCACCCACAATACCAA-3'	
TIMP2 M-F:	5'-TTTGGTGTTTTGGAAGAACGGGCG-3'	60℃
TIMP2 M-R:	5'-CGACCCCGATCCCCGCTACG-3'	
TIMP2 U-F:	5'-TTTGGTGTTTTGGAAGAATGGGTG-3'	60℃
TIMP2 U-R:	5'-CCAACCCCAATCCCCACTACA-3'	
TIMP3 M-F:	5'-CGTTTCGTTATTTTTTGTTTTCGGTTTC-3'	59℃
TIMP3 M-R:	5'-CCGAAAACCCCGCCTCG-3'	
TIMP3 U-F:	5'-TTTTGTTTTGTTATTTTTTGTTTTTGGTTTT-3'	59℃
TIMP3 U-R:	5'-CCCCCAAAAACCCCACCTCA-3'	
MLH1 M-F:	5'-ACGTAGACGTTTTATTAGGGTCGC-3'	60℃
MLH1 M-R:	5'-CCTCATCGTAACTACCCGCG-3'	
MLH1 U-F:	5'-TTTTGATGTAGATGTTTTATTAGGGTTGT-3'	60℃
MLH1 U-R:	5'-ACCACCTCATCATAACTACCCACA-3'	
p16 M-F:	5'-TTATTAGAGGGTGGGGCGGATCGC-3'	65℃
p16 M-R:	5'-GACCCCGAACCGCGACCGTAA-3'	
p16 U-F:	5'-TTATTAGAGGGTGGGGTGGATTGT-3'	60℃
p16 U-R:	5'-CAACCCCAAACCACAACCATAA-3'	
SFRP4 M-F:	5'-GGGTGATGTTATCGTTTTTGTATCGAC-3'	60℃
SFRP4 M-R:	5'-CCTCCCCTAACGTAAACTCGAAACG-3'	
SFRP4 U-F:	5'-GGGGGTGATGTTATTGTTTTTGTATTGAT-3'	60℃
SFRP4 U-R:	5'-CACCTCCCCTAACATAAACTCAAAACA-3'	
SFRP5 M-F:	5'-AAGATTTGGCGTTGGGCGGGACGTTC-3'	60℃
SFRP5 M-R:	5'-ACTCCAACCCGAACCTCGCCGTACG-3'	
SFRP5 U-F:	5'-GTAAGATTTGGTGTTGGGTGGGATGTTT-3'	60℃
SFRP5 U-R:	5'-AAAACTCCAACCCAAACCTCACCATACA-3'	

Primers for ChIP and PCR of cutting site in Dox-inducible DSB system:

CDH1 F1:	5'-GCAACTCCAGGCTAGAGG-3'
CDH1 R1:	5'-GCAAGCTCACAGGTGCTT-3’
CDH1 F2:	5'-AGAACTCAGCCAAGTGTA-3'
CDH1 R2:	5'-TCTAGCCTGGAGTTGCTA-3’
CDH1 F3:	5'-CGACTTGTCTCTCTACAA -3'
CDH1 R3:	5'-TGCGATCACAGCTCACTG-3’
CDH1 F4:	5'- GCAAGACAGAGCGAGACT-3'
CDH1 R4:	5'-CTGAAGCGATCCTCCTGC-3’
CDH1 F5:	5'-GAAGTCAGTTCAGACTCC-3'
CDH1 R5:	5'-TGCAGCAGCAGCAGCAGC-3’
WIF1 F1:	5’-CTCGAGCCAAGGCCAGCG-3’
WIF1 R1:	5’-ATGGTGATGGTGATGGTG-3’
WIF1 F2:	5’-TCCACGATGTTAGAGGAG-3’
WIF1 R2:	5’-GCGTCCAGCTGGTACTTG-3’

WIF1 F3:	5’-GGTAGCTAGGACTACAGG-3’
WIF1 R3:	5’-ATGGCTCACGCCTGTAAT-3’
WIF1 F4:	5’-CACGCACCTGTGAATTCG-3’
WIF1 R4:	5’-CAGGAGAATCACTTGAAC-3’
WIF1 F5:	5’-GAGAGAGGAATCCTACTG-3’
WIF1 R5:	5’-GCTTGACTGAGTGCTGAT-3’
TIMP2 F1:	5’-CGCGCTGAGTCAGGGACC-3’
TIMP2 R1:	5’-GTGCCCGGCCTGGCACGT-3’
TIMP2 F2:	5’-CAGTTTCTCAATAGGCCA-3’
TIMP2 R2:	5’-CTTCAGCTCGACTCTGGA-3’
TIMP2 F3:	5’-TCTCTTGTTGGCTGGTCA-3’
TIMP2 R3:	5’-TGAGAAACTGACAGGATC-3’
TIMP2 F4:	5’-CAGGTTCACAGCCAGGAA-3’
TIMP2 R4:	5’-AGTAGGATCGCAGCAGAG-3’
TIMP2 F5:	5’-GCCAGGCCGGGCACAACA-3’
TIMP2 R5:	5’-TCGGAGGATTTTCTGCTC-3’
TIMP3 F1:	5’-GGAGCTCTGTCAGCCATG-3’
TIMP3 R1:	5’-GCTCTCTCTGCGATAGTA-3’
TIMP3 F2:	5’-TATCTGCAATCATCTATT-3’
TIMP3 R2:	5’-GAGGAAGTGGTTCCCTGC-3’
TIMP3 F3:	5’-GTGACGAGTTCCTGGCTG-3’
TIMP3 R3:	5’-AGCAGGATTCTGTATCTC-3’
TIMP3 F4:	5’-AGAGGAGTTCTGAGAAAG-3’
TIMP3 R4:	5’-CTGTTCCATCTAACTGCA-3’
TIMP3 F5:	5’-TGTCCACTTCTCAGCGAG-3’
TIMP3 R5:	5’-GACATCCATGAATGCACA-3’
MLH1 F1:	5'-ACTAGAGCCTCGTCGACT-3'
MLH1 R1:	5'-CCAATAGGAGCAGAGATG-3'
MLH1 F2:	5'-TCGTAGTATTCGTGCTCA-3'
MLH1 R2:	5'-AGCAAGATGGAAGTCGAC-3'
MLH1 F3:	5'-CAGGACGCTTACATGCTC-3'
MLH1 R3:	5'-GAGAGAGCTGCTCGTGCA-3’
MLH1 F4:	5'-ATTATGGTCAGAAGATCT-3'
MLH1 R4:	5'-TAAAGCCTATTCTCTTGC-3’
MLH1 F5:	5'-CTCCTATTGGCTGGATAT-3'
MLH1 R5:	5'-CCTGAAGCGGCTACTGCC-3’
CDKN2A F1:	5’-AAGGACTCGGTGCTTGTC-3’
CDKN2A R1:	5’-TCCGCTCCTCTTCTAGAT-3’
CDKN2A F2:	5’-CATTCTACGCGAGGACGC-3’
CDKN2A R2:	5’-CGAGTCCTTTGTGTCTAG-3’
CDKN2A F3:	5’-TTCCTGTCTCCCAGCTGG-3’
CDKN2A R3:	5’-GCGTACGTTCTCTCTCCG-3’
CDKN2A F4:	5’-GGATCCAGGCAGACCGCA-3’
CDKN2A R4:	5’-CTCTCGTCCAGGAACTCG-3’

CDKN2A F5:	5’-CTAGAAGAGGAGCGGAGC-3’
CDKN2A R5:	5’-CTCGTCGAAAGTCTTCCA -3’
SFRP4 F1:	5’-AAAGACTGGCCAGACTAA-3’
SFRP4 R1:	5’-GCTCGGAACGCTCCCTTG-3’
SFRP4 F2:	5’-CTCTAAGGCAGAGGGAGC-3’
SFRP4 R2:	5’-TTTAGTCTGGCCAGTCTT-3’
SFRP4 F3:	5’-TCCTCAGCGCTCCTCATG-3’
SFRP4 R3:	5’-AGAATTTCAAGAGAGAGA-3’
SFRP4 F4:	5’-TCCACTAATGCTGCAGAG-3’
SFRP4 R4:	5’-TGCTAGTAGACAGTTTGG-3’
SFRP4 F5:	5’-GCGGCTGCAGCTGCCAAG-3’
SFRP4 R5:	5’-GAGCAGCGCCAGCTCTCA-3’
SFRP5 F1:	5’-GCAGGGGAGCCGAGCCGG-3’
SFRP5 R1:	5’-GACTGATCCTGGCGCCTC-3’
SFRP5 F2:	5’-AAGGCGAGGGTCGAGGAA-3’
SFRP5 R2:	5’-TGGCCCCAGCTCCAAGGC-3’
SFRP5 F3:	4’-GAGGACCAGAAGATCCAG-3’
SFRP5 R3:	5’-TCACTCACTTCACTTGCT-3’
SFRP5 F4:	5’-CTTGCACCGCACCTTGCA-3’
SFRP5 R4:	5’-TCAAAGATGGATATGACC-3’
SFRP5 F5:	5’-AGGCGCCAGGATCAGTCG-3’
SFRP5 R5:	5’-CCTCTCCAGGTGCGCGCC-3’
Primers for ChIP in H2O2 treatment-induced oxidative DNA damage:	
CDH1 sense:	5’-GTCTATGCGAGGCCGGGT-3’
CDH1 antisense:	5’-AGTTCCGACGCCACTGAG-3’
WIF1 sense:	5’-CTGCCATCGGCACCATCG-3’
WIF1 antisense:	5’-GATGATGATGATGAGGTG-3’
TIMP2 sense:	5’-TCGCCTGGTGTCCTGGAA-3’
TIMP2 antisense:	5’-TTGTGCCCGGCCTGGCAC-3’
TIMP3 sense:	5’-GAGCTCTGTCAGCCATGG-3’
TIMP3 antisense:	5’-ATCCTCGCTGAGAAGTGG-3’
MLH1 sense:	5’ CTCGTCGACTTCCATCTT-3’
MLH1 antisense:	5’-CTCTGCTGAGGTGATCTG-3’
CDKN2A sense:	5’-AGGACTCGGTGCTTGTCC-3’
CDKN2A antisense:	5’-CCGCTCCTCTTCTAGATT-3’
SFRP4 sense:	5’-ACAACTGCCAGAGGTTCT-3’
SFRP4 antisense:	5’-CCTTGGCAGCTGCAGCCG-3’
SFRP5 sense:	5’-TGCGGCAGGGGAGCCGAG-3’
SFRP5 antisense:	5’-GACTGATCCTGGCGCCTC-3’

Table S7. Sequence for CRISPR guide RNA in the Dox-inducible DSB system

Gene name	Guide RNA sequence 1	Guide RNA sequence 2	Spacer
CDH1	TAGTCGTCGCGCCTGGGGAG**GGG**	TCAGCCAATCAGCGGTACGG**GGG**	24bp
WIF1	GAGCTCCGAGCTGACAGGCT**ACC**	AGTGCCAGCCTATCGCAGAA**CGG**	24bp
TIMP2	TGGGGCCAGGGGCGACGCAC**CGG**	CGGGGACCCCCGCGCGGGTG**CGG**	18bp
TIMP3	TGTCGTTCACGTTTCTGGGA**CCC**	GTGGCTTGCCCCAGAGCTGA**TCC**	25bp
MLH1	GGAACGCCGGAAAGATTGCA**ACC**	GAGAAGCGCCAAGCACCTCC**TCC**	15bp
CDKN2A	GGCCTCGACAGCTGGGCCGG**ACC**	GTGTCCAGATGTCGCGTCAG**AGG**	18bp
SFRP4	CGCCACTGTAGTGGGGGAGA**CCC**	CCTGGGGGTGGGGCGGCCGA**GGG**	15bp
SFRP5	TCGGTTAGAGGCCGGCGGAC**CGG**	CCGGGCTGGAGCCCCGAGGT**GGG**	17bp

Table S8. Sequences of DNA oligonucleotides

Control oligo	GGAACTAGTGGCTCCCCCGGGCTGC
8-OHdG oligo	GGAACTAGTGG (8-OHd) CTCCCCCGGGCTGC
8-OHdG oligo	GGAACTAGTGG (8-OHd) CTCCCCCGGGCTGC
(double strand)	GCAGCCCGGGGGAGCCACTAGTTCC
Biotin-8-OHdG oligo	GGAACTAGTGG (8-OHd) CTCCCCCGGGCTGC
(double strand)	GCAGCCCGGGGGAGCCACTAGTTCC(Biotin)