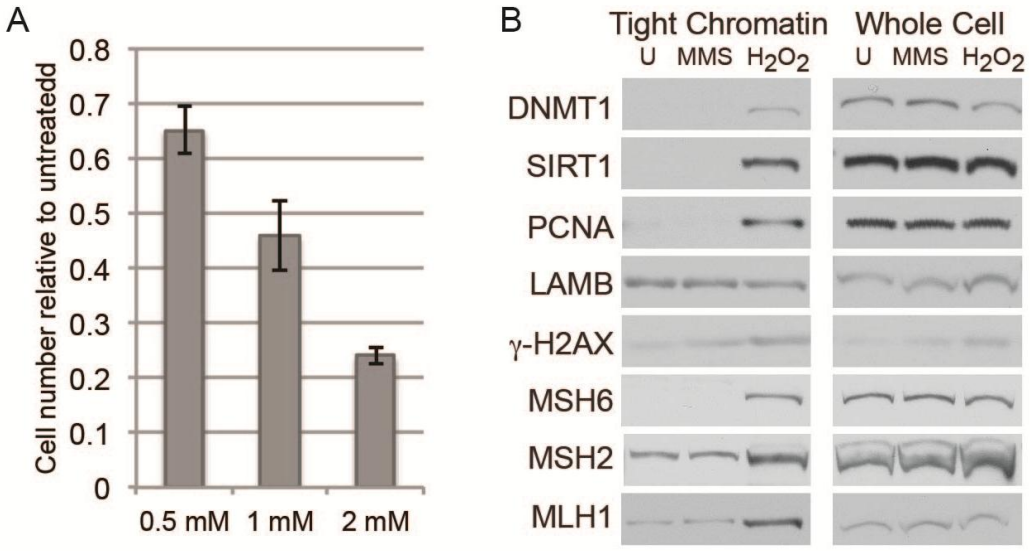
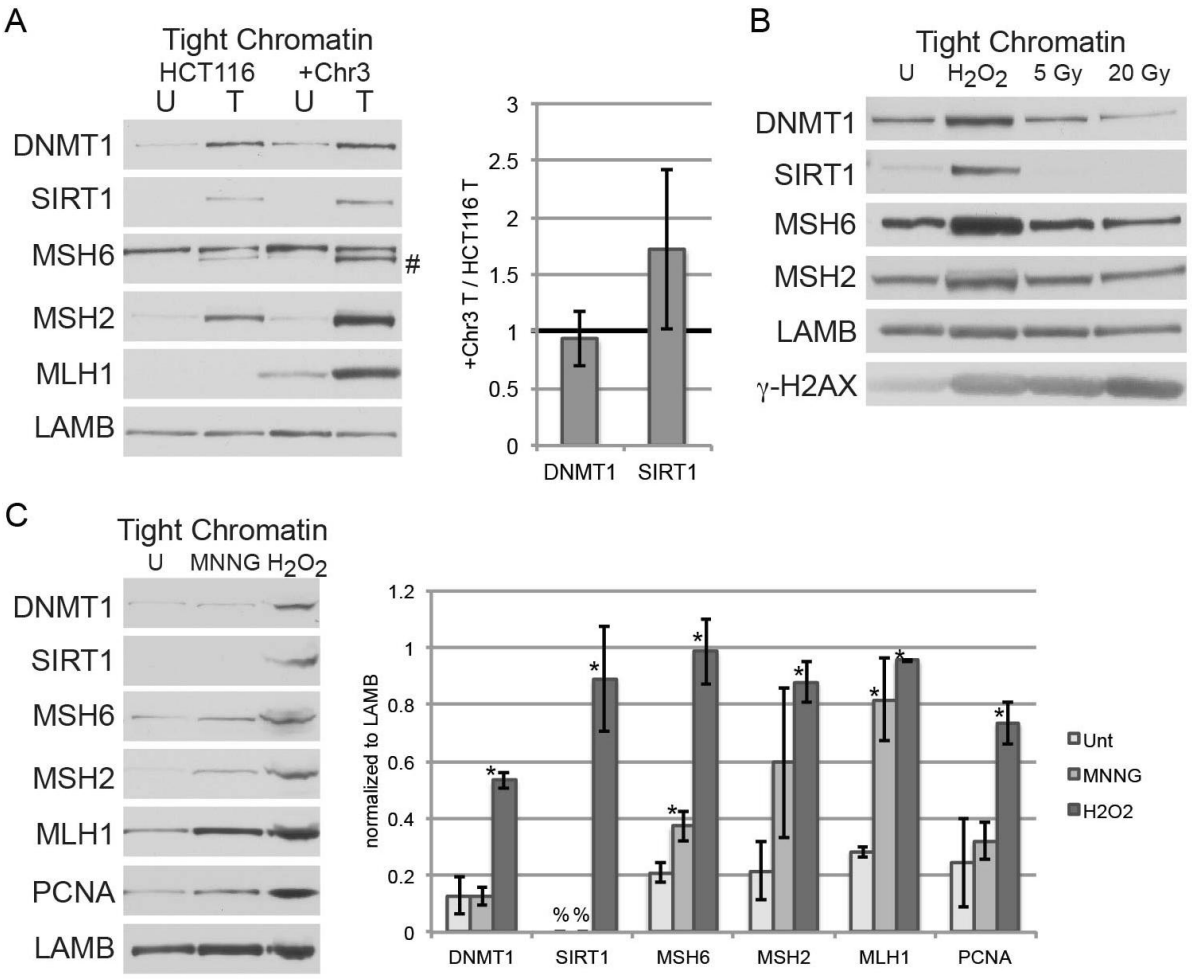
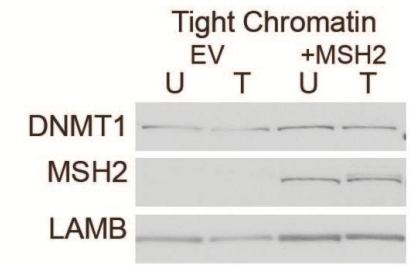
## Supplementary Figures



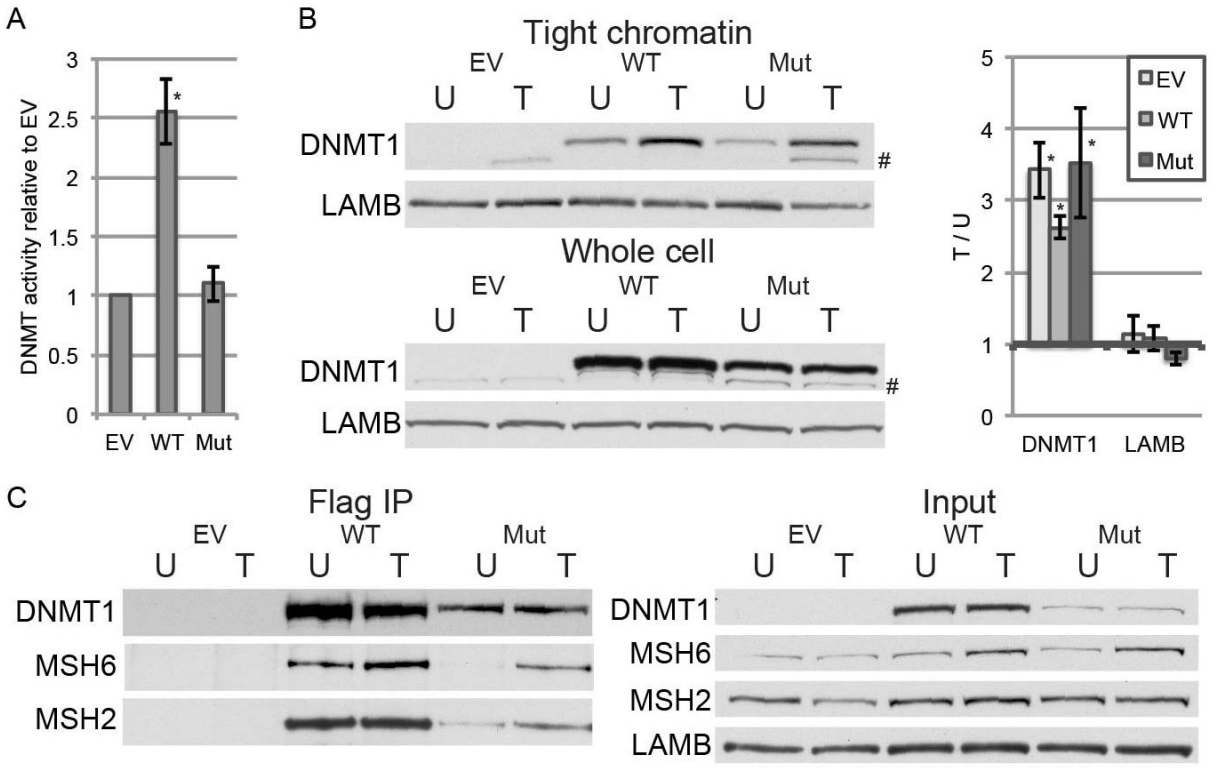

**Supplementary Figure S1. MMS treatment does not induce an increase in affinity of MSH2, MSH6 or DNMT1 for chromatin.** A) Cell survival 24 hours after exposure to $H_2O_2$ for 30 minutes. NCCIT cells were untreated or treated with the indicated concentration of $H_2O_2$ for 30 minutes. Media was then aspirated, cells were washed with PBS, and new media was added. 24 hours later viable cells were counted using trypan blue exclusion. B) NCCIT cells were either untreated, treated with 1.5 mM MMS for 1 hour, or 1 mM $H_2O_2$ for 30 minutes. Proteins tightly bound to chromatin were isolated (tight chromatin). The data presented is from one representative experiment of two biologic replicates.




**Supplementary Figure S2. MLH1 status does not affect the oxidative damage-induced increase in affinity of DNMT1 for chromatin.** A) Parental HCT116 cells or HCT116 expressing an additional chromosome 3 (+Chr3) were either untreated (U) or treated with 4 mM $H_2O_2$ for 30 minutes. Proteins tightly bound to chromatin were isolated (tight chromatin). # indicates MSH6 band. Upper band is non-specific. Data was first normalized to LAMB and then displayed as the mean of the ratio of the indicated protein levels in +Chr3 $H_2O_2$ treated cells over parental $H_2O_2$ treated cells +/- SEM (n=3). B) Ionizing radiation does not induce an increase in affinity of MSH2 or MSH6 for chromatin. NCCIT cells were untreated or treated with 1 mM $H_2O_2$ for 30 minutes or 5 or 20 Gy IR and collected 30 minutes later. The data presented is from one representative experiment of two biologic replicates. C) NCCIT cells were pretreated in media containing 25 μM *O*6-Benzylguanine for 2 hours to inhibit *O*6-methylguanine transferase activity. The cells were then untreated, treated by 20 μM MNNG for 1 hour, or treated with 1 mM $H_2O_2$ for 30 minutes. Data is displayed as mean of the levels of the indicated protein normalized to LAMB +/- SEM (n=3). *p<0.05.




**Supplementary Figure S3. MSH2 in the absence of MSH6 does not restore the oxidative damage-induced increase in affinity of DNMT1 for chromatin**. A) LOVO cells were transduced with lentiviral particles for expressing empty vector (EV) or MSH2 (+MSH2). Cells were either untreated (U) or treated with 4 mM $H_2O_2$ for 30 minutes. The data presented is from one representative experiment of two biologic replicates.




**Supplementary Figure S4. Catalytically inactive DNMT1 also has an increased affinity for chromatin and binds to MSH2/6 after oxidative damage.** A) HCT116 DNMT1 hypomorphic cells stably expressing flag-tagged wildtype DNMT1 (WT) have higher DNMT activity than HCT116 DNMT1 hypomorphic cells stably expressing an empty vector (EV) or flag-tagged catalytically inactive DNMT1 (Mut). A fluorescence-based DNMT activity assay was performed on nuclear lysates prepared from the indicated cell lines. Data is displayed as mean of the DNMT activity normalized to EV cells +/- SEM (n=3). *p<0.05. B) Indicated cells were untreated or treated with 4 mM $H_2O_2$ for 30 minutes. Proteins tightly bound to chromatin were isolated (tight chromatin). # indicates hypomorphic DNMT1.Data is displayed as the mean of the ratio of the indicated protein levels in $H_2O_2$ treated cells over untreated cells for the given cell line +/- SEM (n=3). *p<0.05. C) Cells were treated as in B. Nuclear extracts were prepared and co-immunoprecipitations were performed with anti-Flag antibody. Expression of flag-tagged DNMT1 is lower in the mutant cell line and therefore less protein was co-IP'd.

**Supplementary Table S1**. **ChIP and RTPCR primers.**

| | | Forward | Reverse |
|---|---|---|---|
| ChIP | *MYC* | TCCTACGTTGCGGTCACAC | GTTCACCATGTCTCCTCCCAG |
| | *MLH1* | TTCAGGAGTGAAGGAGGCCA | CGAGGCTGAGCACGAATACT |
| | *IL8* | ATCGTGGAATTTCCTCTGACAT | AGTTTGTGCCTTATGGAGTGCT |
| RTPCR | *ACTB* | GAAGCCGGCCTTGCACAT | AGCACAGAGCCTCGCCTTT |
| | *MYC* | GTCAAGAGGCGAACACACAA | GGCCTTTTCATTGTTTTCCA |
| | *SFRP2* | GTTTCCCCCAGGACAACGAC | CCTTTGGAGCTTCCTCGGTG |
| | *CDH1* | ATCCCACCACGTACAAGGGT | GTGTATACAGCCTCCCACGC |
| | *MLH1* | GAATGCGCTATGTTCTATTCCATCC | ATAGATCAGGCAGGTTAGCAAGCTG |
| | *MGMT* | CCCGCGCCCCGGATA | CAAGTACCAAGTCGCAAACGG |
| | *IL8* | ACCACCGGAAGGAACCATCTCA | AGCACTCCTTGGCAAAACTGCAC |

**Supplementary Materials and methods**

*Cell culture and treatments*

HCT116 parental and +chr3 cells were a generous gift from Drs. AB Clark and TA Kunkel and were maintained as described previously (Koi et al., 1994). HCT116 DNMT1 hypomorph cells stably expressing an empty vector (EV), flag-tagged DNMT1 (WT), or catalytically inactive DNMT1 (Mut) were maintained as previously described (Clements et al., 2012). For MMS exposure, cells were treated in media containing 1.5 mM MMS (Sigma) for 1 hour at 37°C. For MNNG exposure, cells were pretreated in media containing 25 μM *O*6-Benzylguanine (Sigma) for 2 hours at 37°C to inhibit *O*6-methylguanine transferase activity (MGMT) (Yanamadala and Ljungman, 2003). The cells were then treated by 20 μM MNNG for 1 hour at 37°C (Schroering and Williams, 2008). For $H_2O_2$ exposure, Hct116, Hct116+Chr3, DLD-1 and LOVO cells were treated in media containing 4 mM $H_2O_2$ for 30 min at 37°C. NCCIT cells were treated in media containing 2 mM $H_2O_2$ for 30 min at 37°C. For ionizing radiation exposure, cells were irradiated at 5 Gy or 20 Gy using a Cs-137 source at a dose rate of 9.58 Gy/min.

*DNMT activity assay*

DNMT activity assays were performed using the Fluorometric EpiQuik DNMT Activity Assay Kit (Epigentek) following the manufacturer's protocol using 10 ug of nuclear extract from indicated cells.

*Antibodies*

For western blot:

DNMT1 (Sigma – D4692), SIRT1 (Delta Biolabs – DB083), PCNA (Cell Signaling - 2586), MSH6 (AbD Serotec – AHP1529 and Cell Signaling – 5425), MSH2 (Cell Signaling - 2017), MSH3 (BD Biosciences - 611390), MLH1 (Cell Signaling - 3515), LAMB (Santa Cruz – sc-6216), ACTB (Sigma – 5441), GAPDH (Cell Signaling – 5174)

For immunofluorescence:

DNMT1 (SantaCruz – H-300), MSH6 (Cell signaling - 5425), g-H2AX (Millipore – 05-636)

For CoIP and ChIP:

DNMT1 (Sigma – D4692), SIRT1 (DeltaBiolabs – DB083), MSH6 (BD Biosciences - 610918), MSH3 (BD Biosciences - 611390), MLH1 (Cell Signaling - 3515), MSH2 (Cell Signaling - 2017), EZH2 (Cell Signaling - 5246), Flag (Sigma – F1804), Rabbit and mouse IgG (Millipore)

**Supplementary References**

Clements, E.G., Mohammad, H.P., Leadem, B.R., et al. (2012). Dnmt1 modulates gene expression without its catalytic activity partially through its interactions with histone-modifying enzymes. Nucleic Acids Res. 40, 4334-4346.

Koi, M., Umar, A., Chauhan, D.P., et al. (1994). Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces n-methyl-n'-nitro-n-nitrosoguanidine tolerance in colon tumor cells with homozygous hmlh1 mutation. Cancer Res. 54, 4308-4312.

Schroering, A.G. and Williams, K.J. (2008). Rapid induction of chromatin-associated DNA mismatch repair proteins after mnng treatment. DNA Repair. 7, 951-969.

Yanamadala, S. and Ljungman, M. (2003). Potential role of mlh1 in the induction of p53 and apoptosis by blocking transcription on damaged DNA templates. Mol. Cancer Res. 1, 747-754.