Supporting Information

Cross-Link Structure Affects Replication-Independent DNA Interstrand Cross-Link Repair in Mammalian Cells[†]

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Supplementary Methods

siRNA knockdowns of Rev1. To ensure efficient siRNA delivery, reverse tranfections were performed with 20pmol Rev1 siRNA with Lipofectamine 2000 (Invitrogen) in 13 x 10^4 Hela cells in a 24-well plate. Serum-free media containing the DNA/liposome complex was replaced after 6 h with serum-containing medium. Cells were lysed 48 h after transfection and probed for Rev1 using an anti-human Rev1 antibody (Santa Cruz). Tubulin was used as a loading control. Host-cell reactivation repair assays were performed as previously noted in the Methods section between 24 and 48 h after transfection with Rev1 siRNA.

Figure S1

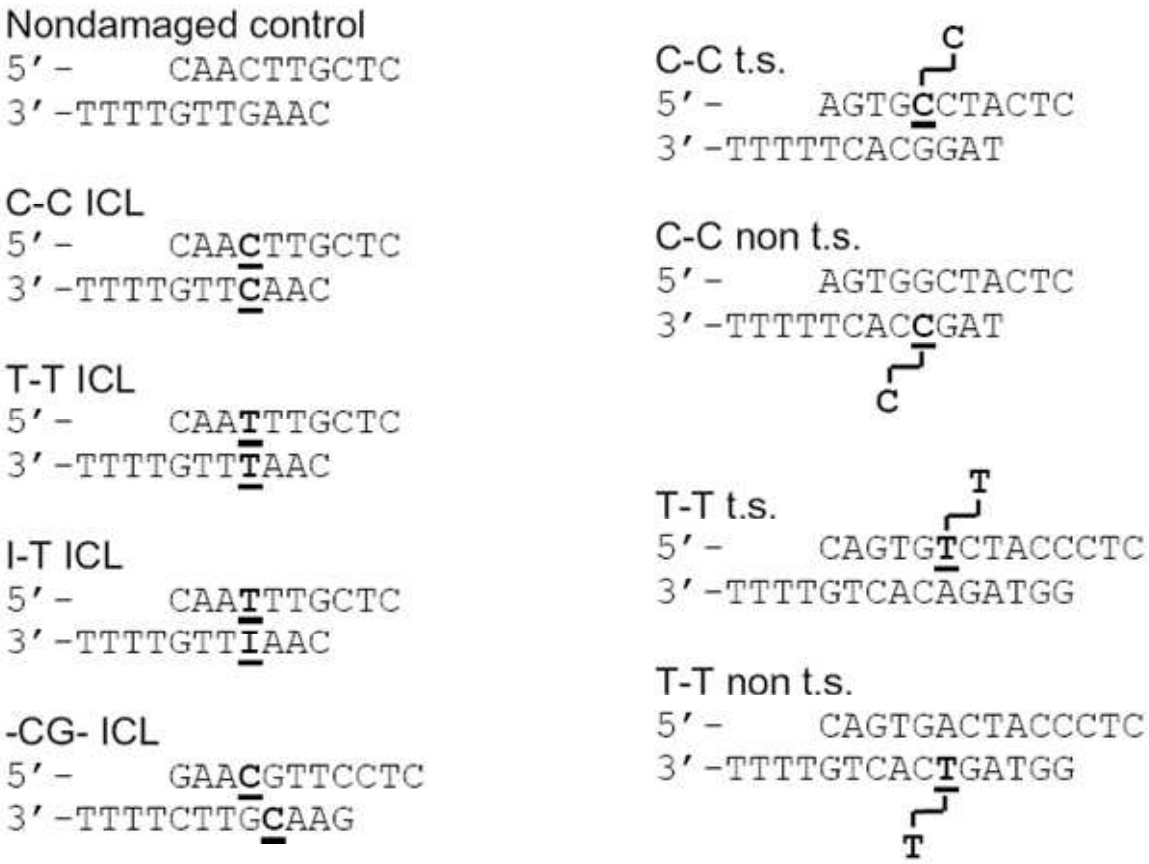

Figure S1: Sequences of non-damaged, cross-linked, and cross-link remnant containing duplexes used to construct the reporter plasmids. Cross-linked nucleotides are indicated by the bold underline. Transcribed strand and non-transcribed strands are indicated by “t.s.” and “non t.s.,” respectively.

Figure S2

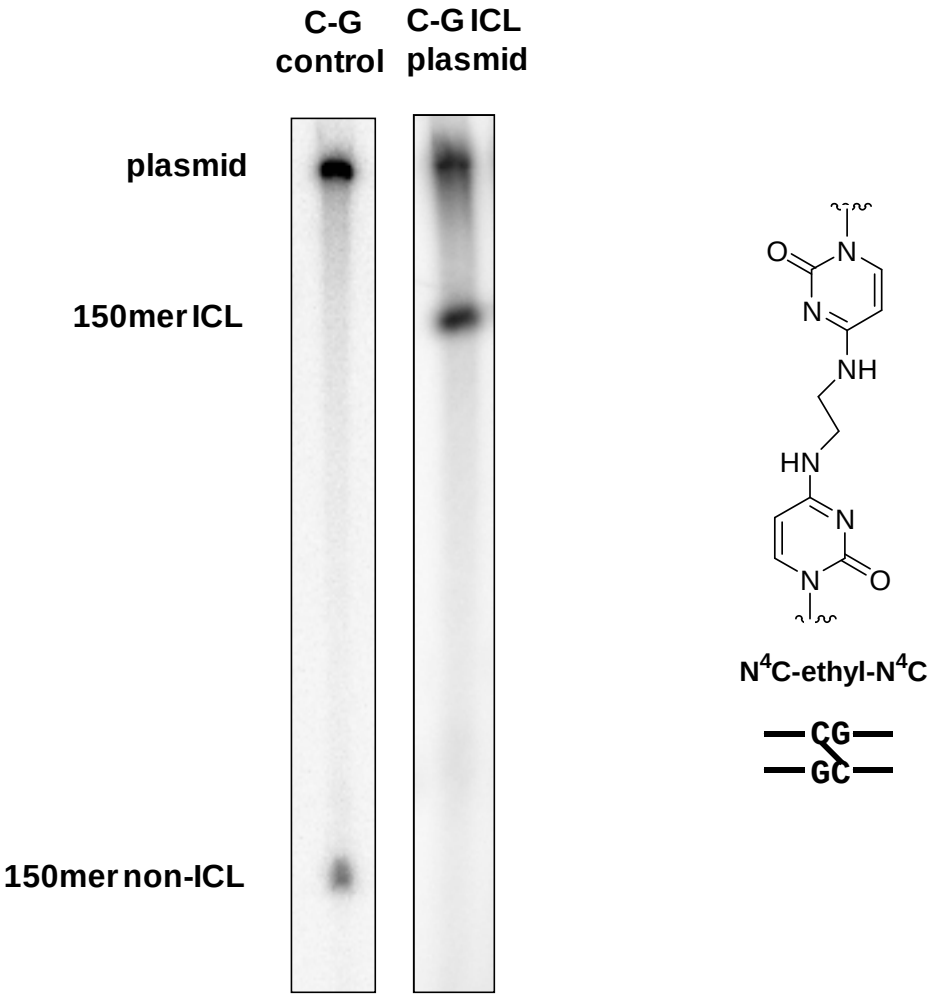

Figure S2: Structure of a N^4C-ethyl-N^4C interstrand cross-link placed in a -CG- sequence and characterization of a -CG- cross-linked plasmid. The non-damaged control plasmid or the -CG- cross-linked plasmid was digested with a restriction enzyme to release a 150bp fragment; the fragment was radiolabeled using the Klenow fragment of *E. Coli* DNA polymerase I, and the products were analyzed on a 6% denaturing gel.

Figure S3

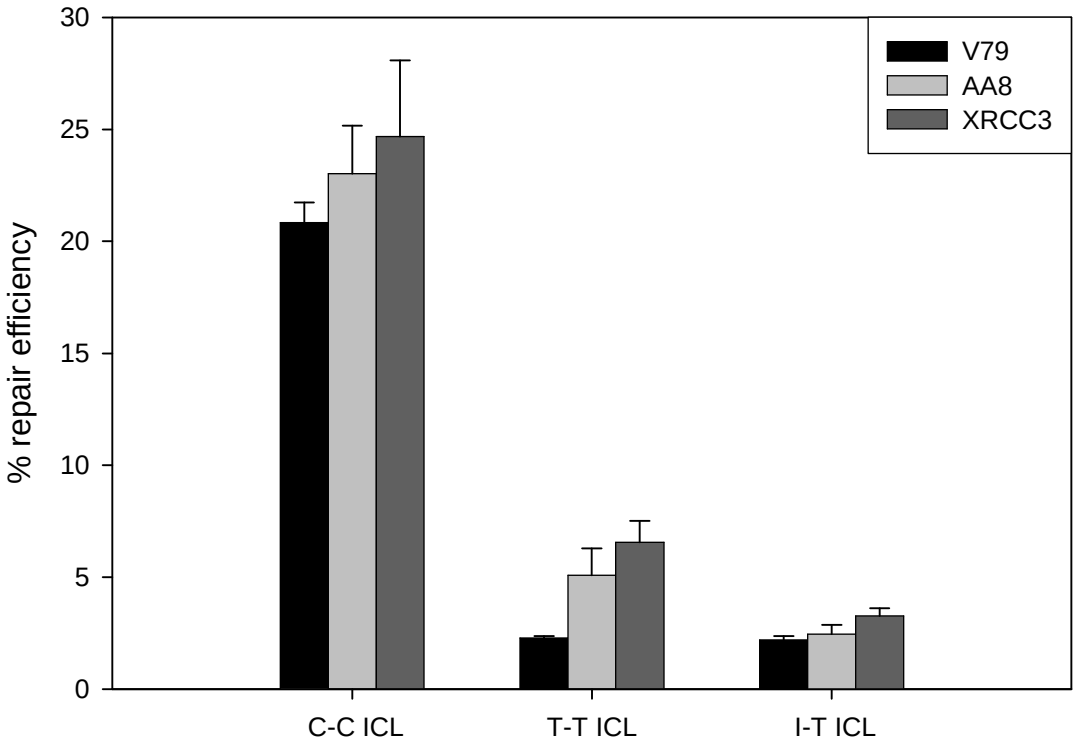

Figure S3: Repair efficiencies of C-C, T-T and I-T interstrand cross-linked plasmids transfected into wild-type CHO (AA8 or V79) and XRCC3-deficient cells derived from the V79 cell line. Percent repair efficiency is the relative level of luciferase expression from a damaged plasmid compared to that from a non-damaged control plasmid. Six replicates were performed and the error bars represent the standard error for each data point.

Figure S4

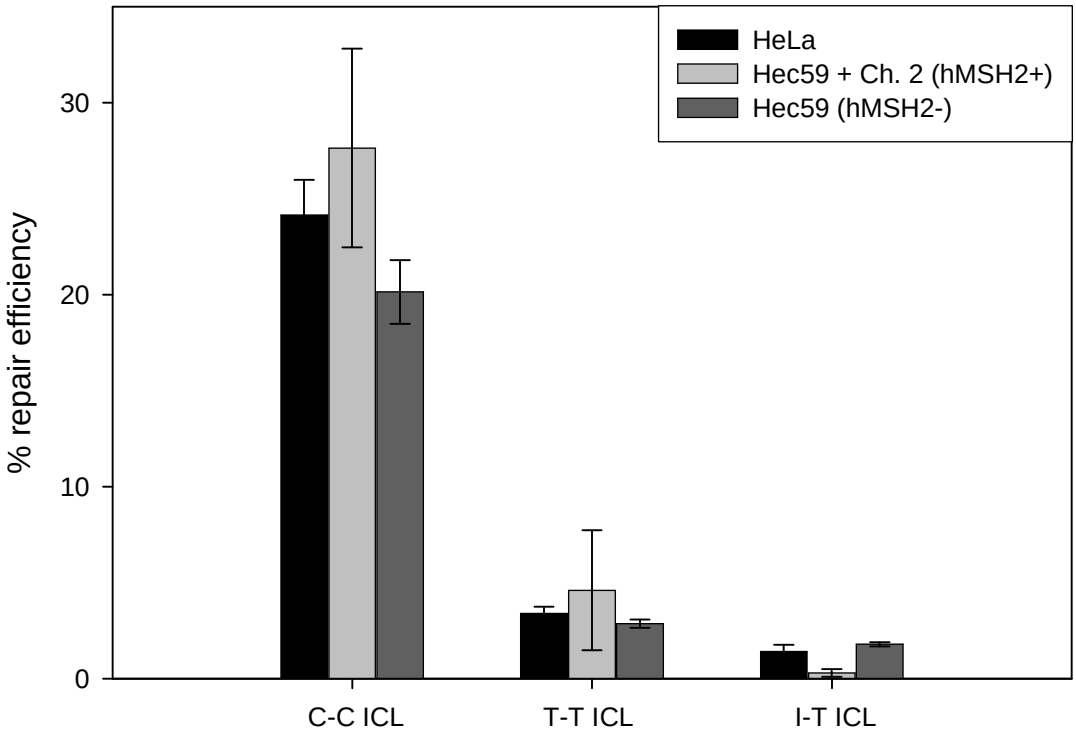

Figure S4: Repair efficiencies of C-C, T-T and I-T interstrand cross-linked plasmids transfected into wild-type HeLa cells, hMSH2-deficient (Hec59) cells, and Hec59 cells complemented with chromosome 2 (hMSH2+). Percent repair efficiency is the relative level of luciferase expression from a damaged plasmid compared to that from a non-damaged control plasmid. Six replicates were performed and the error bars represent the standard error for each data point.

Figure S5

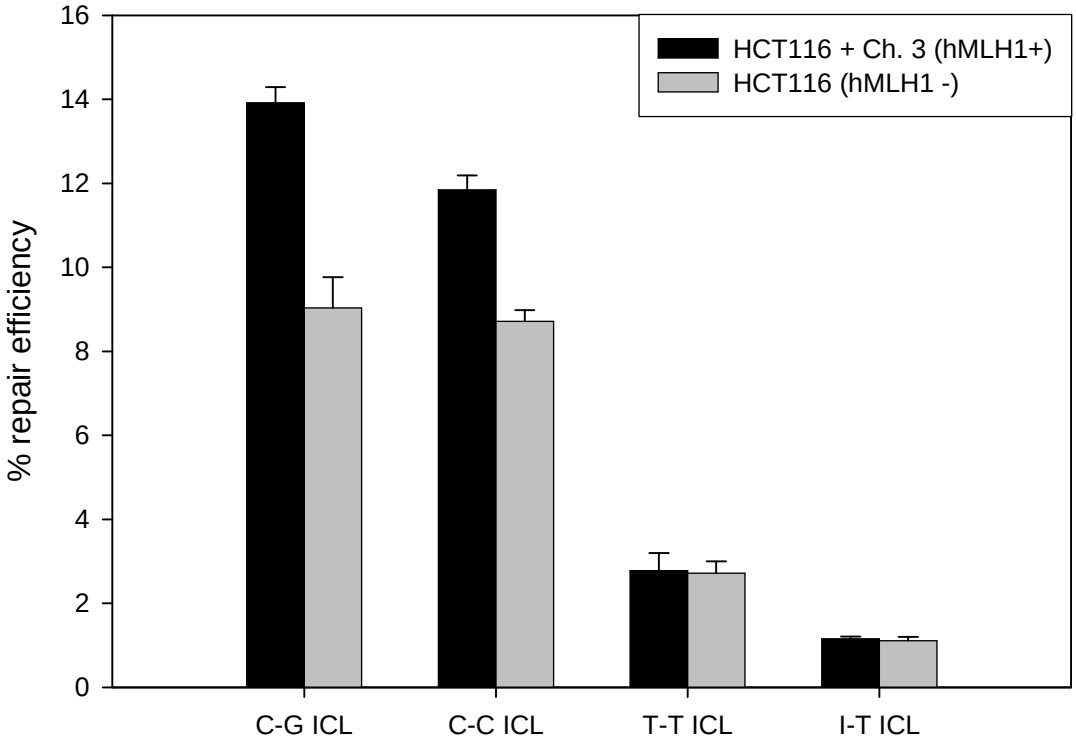

Figure S5: Repair efficiencies of -CG-, C-C, T-T and I-T interstrand cross-linked plasmids transfected into the hMLH1-deficient cell line HCT116 (hMLH-) and the parental cell line, HCT116 complemented with chromosome 3 (hMLH1+). Percent repair efficiency is the relative level of luciferase expression from a damaged plasmid compared to that from a non-damaged control plasmid. Six replicates were performed and the error bars represent the standard error for each data point.

Figure S6

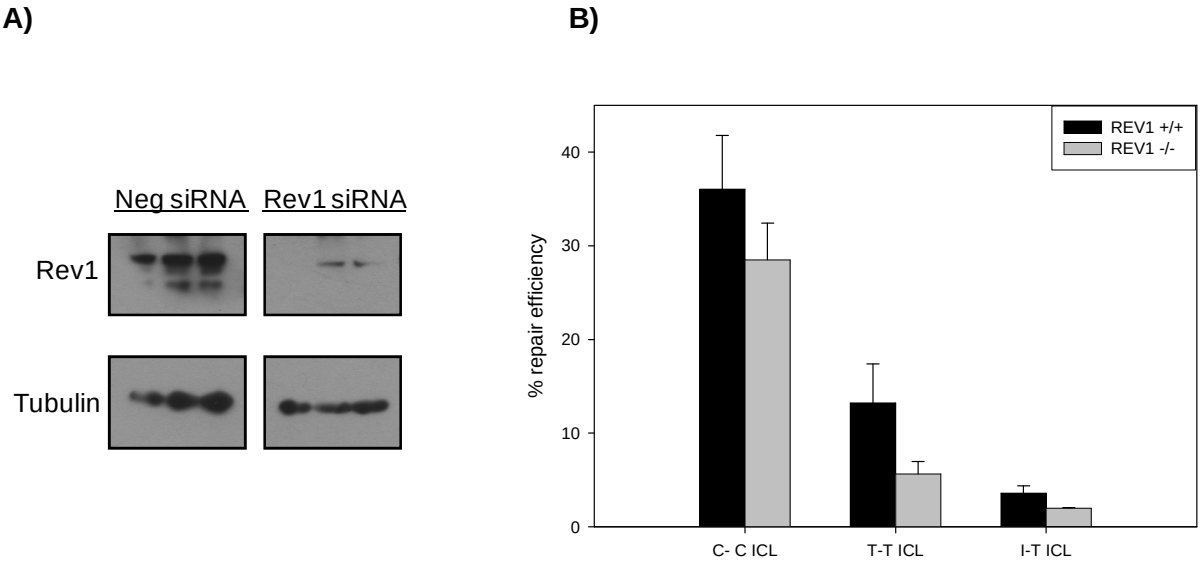

Figure S6: A) Rev1 siRNA knockdown in HeLa cells. Three independent experiments are shown simultaneously for each blot. A human anti-Rev1 antibody was used to determine protein expression of Rev1 and an anti-Tubulin antibody was used for the loading control. B) Repair efficiencies of C-C, T-T, and I-T interstrand cross-linked plasmids transfected into wild-type DT40 (REV1+/+) and REV1-/- DT40 cells. Percent repair efficiency is the relative level of luciferase expression from a damaged plasmid compared to that from a non-damaged control plasmid. Six replicates were performed and the error bars represent the standard error for each data point.