SUPPLEMENTAL DATA

MISMATCH REPAIR-DEPENDENT ITERATIVE EXCISION AT IRREPARABLE O^6-METHYLGUANINE LESIONS IN HUMAN NUCLEAR EXTRACTS*

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Supplemental Fig. 1. Repair synthesis is specific for heteroduplex DNA and requires MutSα. **(A)** Homoduplex and heteroduplex 5' substrates were incubated with *MSH6*$^{-/-}$ HCT15BBR nuclear extracts under repair conditions (**Materials and Methods**) except that reactions (10 μl) contained 5 μM dCTP, 50 μM each dATP, dGTP, and dTTP, and 5 μCi [α-^{32}P]dCTP (3000 Ci/mmol). MutSα (500 fmol) was present as indicated. Samples were removed at 5, 15 or 30 minutes as indicated and reactions quenched. After recovery, DNA was digested with AflII, nicked with N.BbvCIB, and subjected to denaturing electrophoresis through alkaline agarose followed by phosphorimager analysis. Substrates were 50 ng 5' A•T (lanes 1-4), 50 ng 5' G-T (lanes 5-8), a mixture of 25 ng A•T and 25 ng G-T (lanes 9-12), or 50 ng 5' MeG-T (lanes 13-16) for a total of 37 fmol DNA/reaction. **(B)** Counts in fragments A, B, and C after 15 min of incubation were estimated using IQMac software and specific activities estimated as the quotient of counts in each fragment divided by length in nucleotides. Note that fragment C corresponds to the full-length continuous strand of the original heteroduplex. Black bars - G-T heteroduplex in presence of MutSα (lane 8, 15 min incubation); Gray bars - MeG-T heteroduplex in presence of MutSα (lane 16, 15 min incubation); White bars - MeG-T heteroduplex in absence of MutSα (lane 14, 30 min incubation).

Supplemental Fig. 2. Rate of repair synthesis is reduced on a MeG-T heteroduplex. Kinetics of repair DNA synthesis was determined by calculation of the specific activity of fragment A from the gel of **Fig. 3** (lanes 1-3, 7-9 and 13-15). DNAs were 5' G-T (❑), AGT-pretreated MeG-T (○) and MeG-T (●).

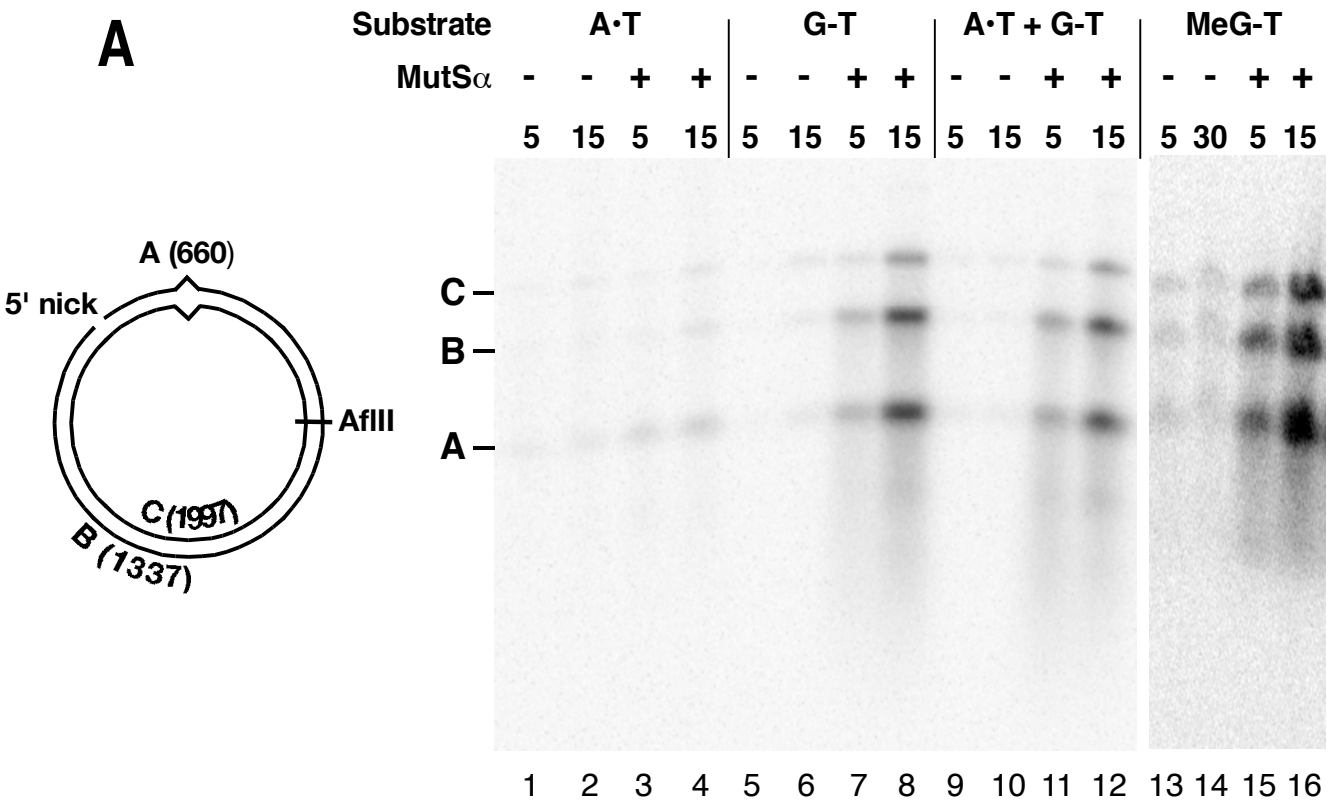
Supplemental Fig. 3. Iterative excision does not occur on a closed circular heteroduplex. (**A**) Reactions in O^6BG-treated HeLa extract contained covalently closed circular (ccc) MeG-T heteroduplex, 5' MeG-T heteroduplex, or AGT-treated ccc MeG-T heteroduplex, as indicated, and were performed the presence of [α-^{32}P]dATP under label-chase conditions as in **Figure 3** and **Materials and Methods**. Reactions were sampled at 5, 15 and 30 min and quenched. Immediately after removal of the 5 min sample, the remainder of the reaction was supplemented with either buffer (lanes 2, 3, 11 and 12) or excess cold dATP (lanes 5, 6, 8, 9, 14 and 15) and incubation continued prior to removal of 15 and 30 minute samples. Recovered DNA was digested with N.BbvCIB and AflII as indicated in schematic to the left. Digests were analyzed by electrophoresis through alkaline agarose and radiolabel visualized by phosphorimager. (**B**) Qnatitative phosphorimager results are presented for lanes 4 and 6 (**left panel**) and lanes 7 and 9 (**right panel**).

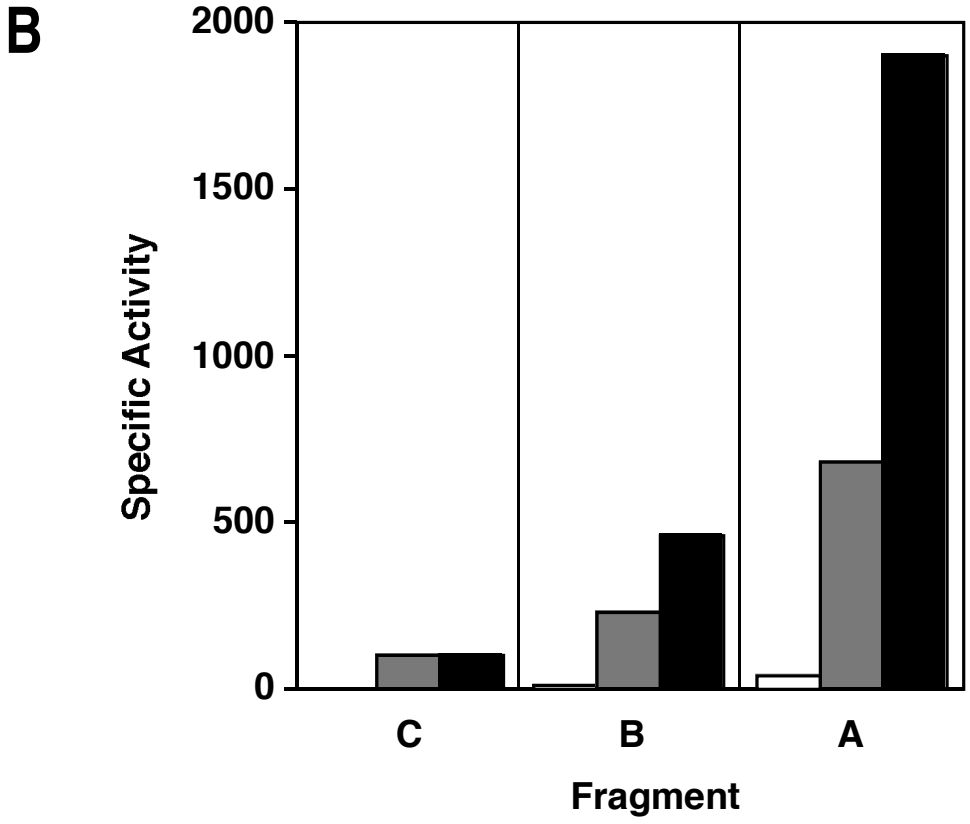
Supplemental Fig. 4. Ligation of irreparable substrates is delayed. **(A)** Kinetics of ligation of 5'-heteroduplexes was determined in O^6BG-treated HeLa extract under repair conditions after 5 min of labeling in the presence of dNTPs and [α-^{32}P]dATP followed by 10 or 25 min chase with excess cold dATP. DNAs were digested with BanI, products resolved by electrophoresis through alkaline agarose, gels analyzed by phosphorimager, and the fraction of label in the full

length linear strand determined. Results shown are the mean (± one standard deviation) for at least three experiments. Heteroduplexes were: G-T (❑), MeG-T (●), AGT-pretreated MeG-T (❍), and T-MeG (■). **(B)** Ligation of 3' substrates was determined as in **panel A** using HCT116BBR extract in the absence or presence of MutLα, or O^6BG-treated HeLa extract. Black bars correspond to ligation after 15 min labeling in the presence of [α-^{32}P]dATP, and cross-hatched bars to values determined after a subsequent 15 min chase with cold dATP.

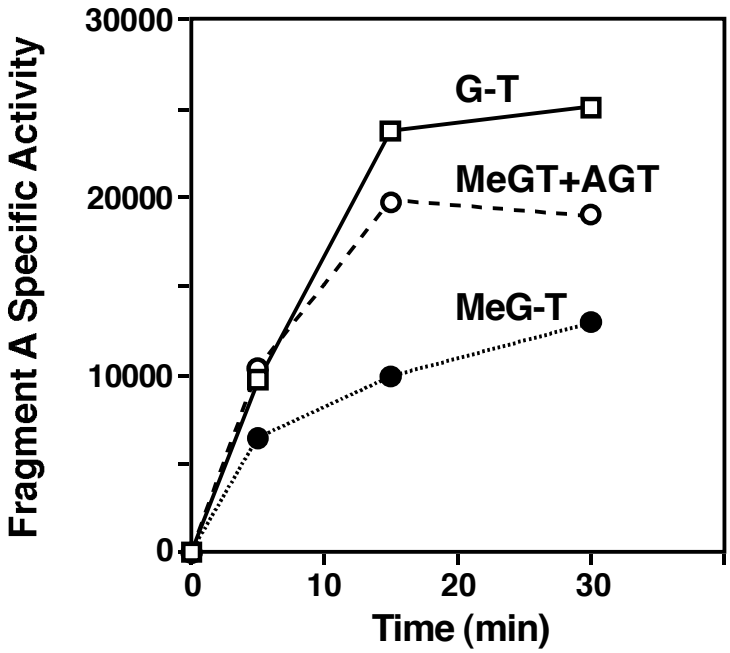
Supplemental Fig. 5. Relative labeling intensity of 3' substrates is altered by MutLα. Phosphorimager results for 15 min incubation times shown in **Fig. 4A** (lanes 1, 3, 7, 9, 13, and 15) were quantitated using IQMac software. The specific activity of each species was estimated as the quotient of the label present in that species divided by the number of deoxyadenylate residues contained therein. Fragment E was derived from the continuous heteroduplex strand (see **Fig. 4A**), and label incorporated into this fragment was used to estimate the specific activity of the continuous strand. Labeling of fragments B and C includes that incorporated into both DNA strands. The specific activities shown for these two fragments were corrected for incorporation into the continuous heteroduplex strand by subtraction of the specific activity of fragment E.

Supplemental Figure 1

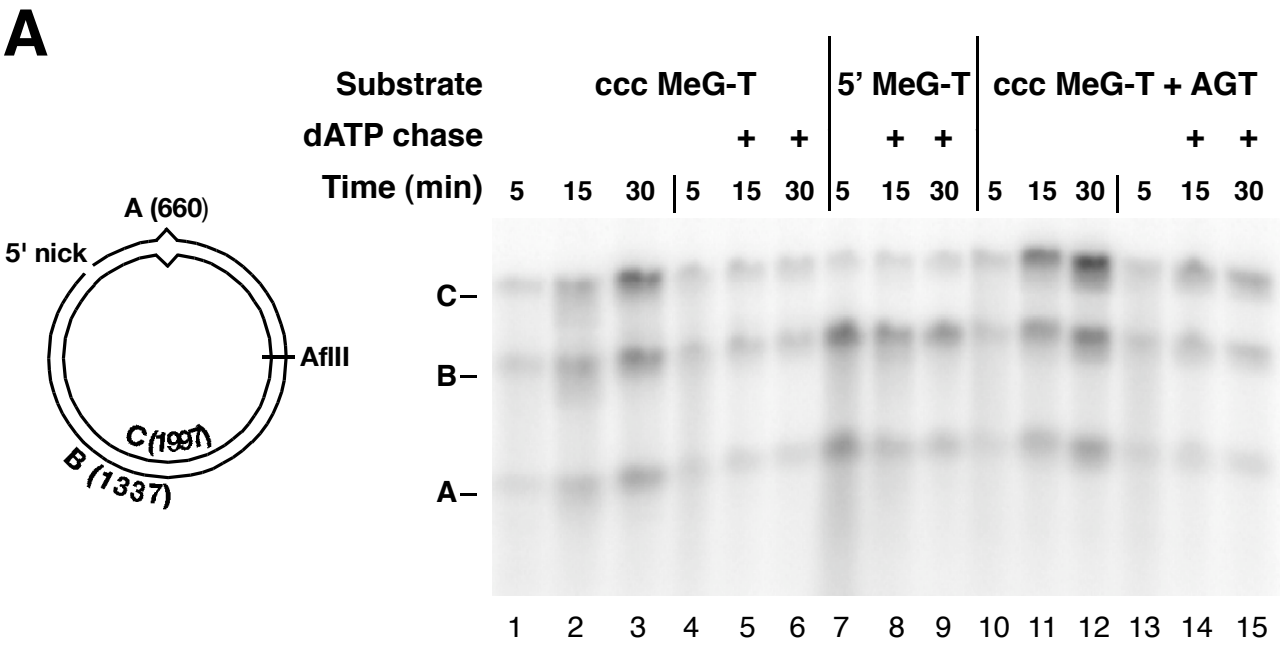


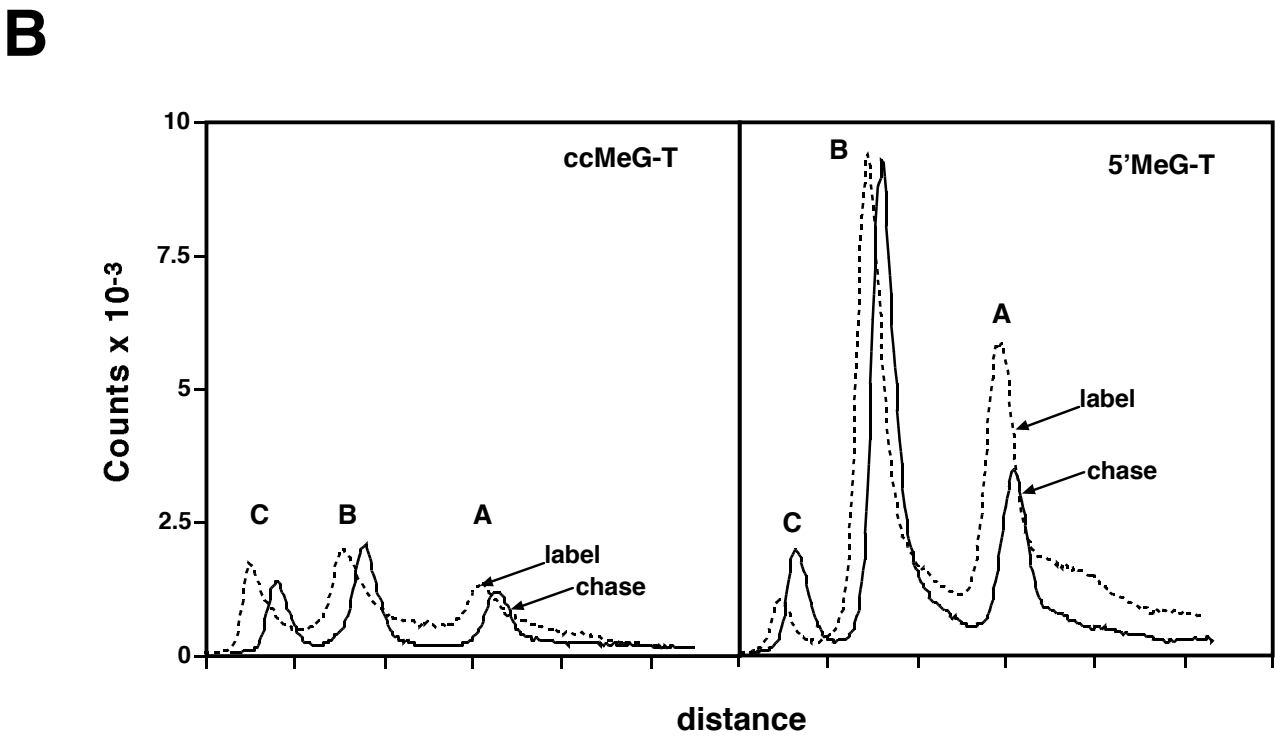


Supplemental Figure 2

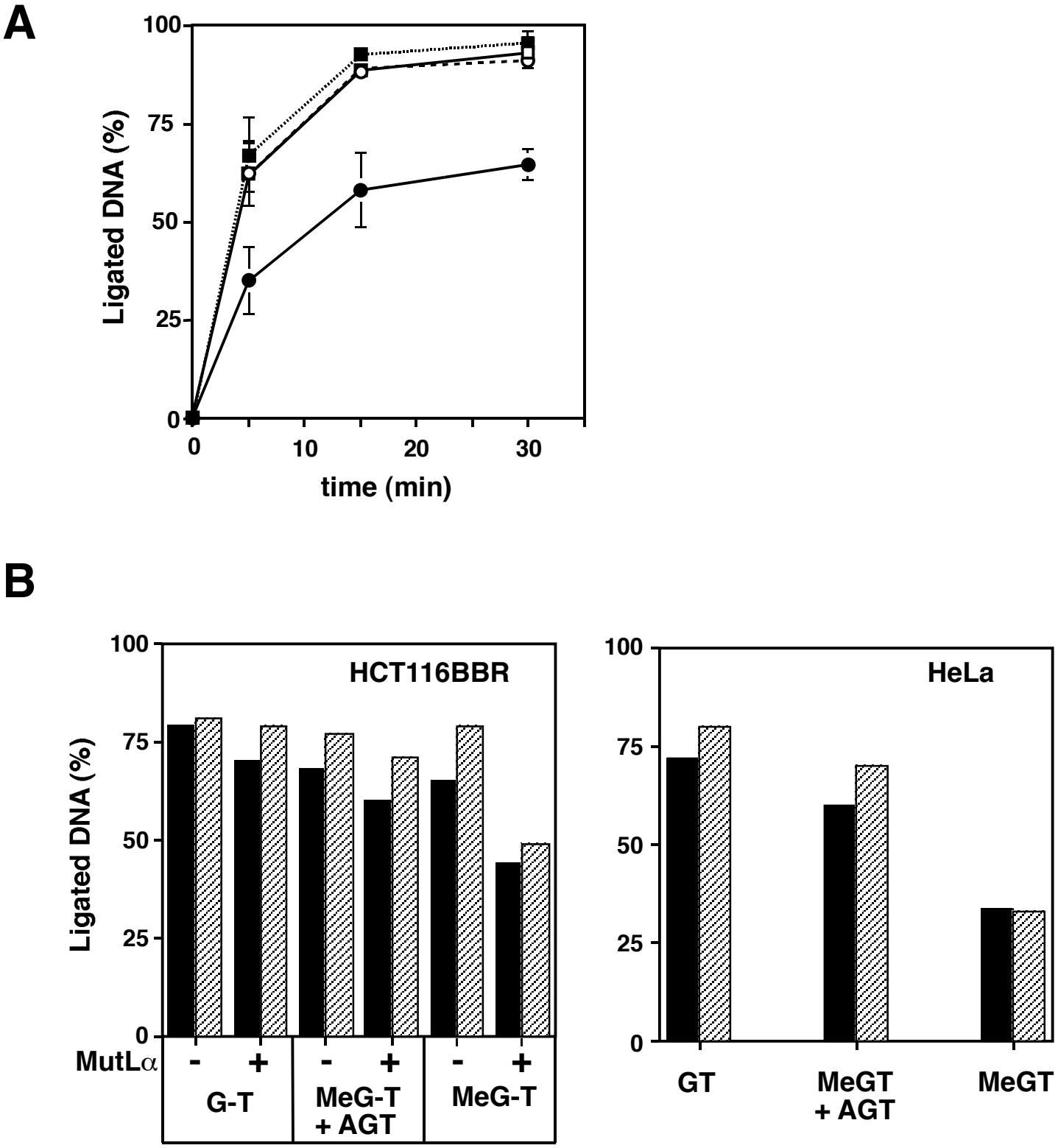


Supplemental Figure 3





Supplemental Figure 4



Supplemental Figure 5