Supplementary Methods

Analysis of MSI

Genomic DNA was isolated by standard methods including proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation. MSI status was determined with 2 mononucleotide markers (BAT25, BAT26) according to the methods described by Miyakura et al. (1). As a microsatellite-stable control, we used normal blood DNA.

Analysis of frameshift mutations of MRE11 and RAD50

PCR was carried out using genomic DNA extracted from the cell lines. Amplification was performed in 25 μl reaction mixtures, with approximately 100 ng of genomic DNA, 10x PCR buffer, 1.25 mM dNTP, 0.5 μM of each primer, and 0.125 units of AmpliTaq Gold DNA polymerase (Life Technologies Japan). The PCR was carried out as follows: 95°C for 10 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, and then a final extension at 72°C for 10 minutes. The sequences of the PCR primers of MRE11 and RAD50 were as follows: MRE11, 5'-aatattttggaggagaatcttaggg-3' (forward) and 5'-aattgaaatgttgaggttgcc-3' (reverse); RAD50, 5'-aactgcgacttgctccagat-3' (forward) and 5'-caagtcccagcatttcatca-3' (reverse). The amplified DNA fragments were analyzed by direct sequencing using a 3100 Genetic Analyzer (Life Technologies Japan) with a Big-Dye Terminator v1.1 Sequencing Standard Kit (Life Technologies Japan).

^{3}H-thymidine incorporation assay

A total of 2-4 x 10^{3} cells in 100 μl of culture medium were plated in 96-well culture plates. After 24 hours, the cells were exposed to various concentrations of SN-38 and/or olaparib for 48 hours. In the case of combination treatment, olaparib was added to the culture plates 1 hour prior to the addition of SN-38. ^{3}H-thymidine (1 μCi/well) was

added to each well, and the plates were incubated at 37°C for 2 hours. Then, the ^{3}H-thymidine-containing medium was discarded, and each well was washed twice with 100 µl of PBS. For cell fixation, 100 µl of 5% trichloroacetic acid (TCA) was added to each well, and then the plates were incubated on ice for 10 min. After discarding the 5% TCA, 50 µl of 1 N NaOH was added to each well, and the plates were incubated at 37°C for 10 min. Next, 30 microliters of the cell lysate was transferred to 96-well LumaPlates (Perkin-Elmer, CA, USA) and air-dried. Finally, the radioactivity of ^{3}H-thymidine was assessed using the TopCount NXT microplate scintillation counter (Perkin-Elmer). Data are expressed as mean ± SD values for replicated wells (n = 6). The half-maximal inhibitory concentration (IC_{50}) values were calculated by regression analysis of the cell growth inhibition curves using the GraphPad Prism V5.0 software (GraphPad Software, Inc., San Diego, CA). Data of IC_{50} are expressed as mean ± SD values of three independent experiments.

Cell counting assay using the Scepter™ 2.0 cell counter

A total of 1-3 x 10^{4} cells were plated in 24-well culture plates (500 µl/well). After 24 hours, the cells were exposed to various concentrations of SN-38 and/or 10 nM olaparib for 48 hours. Cell count assay using the Scepter™ 2.0 cell counter (Merck Millipore, Billerica, MA) was carried out according to the manufacturer's recommendations (2). Cell counts are expressed as the mean ± standard deviation (SD) values per well of the triplicate experiments, and representative diagrams of the cell diameter measured in triplicate using the Scepter™ 2.0 cell counter were plotted using the Scepter™ Software Pro 2.1 computer software (Merck Millipore).

Clonogenic assay

A total of 1-2 x 10^{2} cells were plated in triplicates into 6-well culture plates (2 ml/well). After 24 hours, the cells were exposed to SN-38 (1 nM for HCT116 and 2 nM for HT29)

and/or 10 nM olaparib for different periods ranging from 6, 12, 24 and 48 hours. After that, treated cultures were incubated for additional 7-10 days in drug-free medium to allow colony formation. The colonies were stained with 0.25% crystal violet in ethanol and containing more than 50 cells were counted. The survival fraction was calculated relative to non-treated cells. Data were shown as mean ± SDs.

Western blot analysis

Cells were plated in 100 mm dishes and treated with SN-38 and/or olaparib, then washed with PBS and lysed in RIPA buffer (Sigma-Aldrich). Protein concentrations were quantified using DC Protein Assay Kit II (Bio-Rad, CA) and a DU7500 spectrophotometer (Beckman Coulter, Inc., CA). A total of 10-20 μg of protein was separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 1% casein for 1 hour, the membrane was incubated with primary antibody diluted with Tris-buffered saline (TBS, pH 7.4) containing 0.1% Tween-20 overnight. After washing three times, membrane was incubated with secondary antibody as described previously (3). The bound secondary antibody was detected using ECL Prime Western Blotting Detection System (GE Healthcare Japan, Tokyo, Japan).

Immunofluorescence microscopy

A total of 0.5-1 x 10^5 cells in 1 ml of culture medium were grown on polylysine-coated chamber slides for 24 hours, followed by exposure to SN-38 (1 nM for HCT116 and 2 nM for HT29) and/or 10 nM olaparib for indicated hours (6-48 hours). Then, the medium was aspirated and cells were fixed with 4% paraformaldehyde for 20 minutes. After incubation, the cells were washed twice with PBS and permeabilized in 0.5% TritonX-100 for 15 minutes. The cells were then washed twice with PBS and blocked for 1 h in 2% goat serum (Nichirei Biosciences Inc., Tokyo, Japan) and incubated with primary antibody for 1 hour at room temperature. For examination of Rad51 staining,

cells were permeabilized with cytoskeleton buffer (10 mM PIPES (pH: 6.8), 100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 1 mM EGTA and 0.5% Triton-X 100) for 5 minutes on ice before fixation. Cells were washed twice with PBS and incubated with goat anti-mouse IgG antibody labeled with Alexa Fluor 647 (1:250, Life Technologies Japan), goat anti-rabbit IgG antibody labeled with Alexa Fluor 488 (1:250, Life Technologies Japan) and 4',6-diamidino-2-phenylindole (DAPI) for 1 hour in the dark at room temperature. Slides were mounted with Fluoromount/Plus (Cosmo Bio Co., Tokyo, Japan) until imaging.

For examination of 5-bromo-2'-deoxyuridine (BrdU) staining, treated cells were incubated with 10 μM BrdU for one hour before fixation. After fixation and permeabilization, the cells were incubated with 1N HCl on ice for 10 minutes, then incubated 2N HCl for 10 minutes at room temperature following incubation 2N HCl for 20 minutes at 37°C to denaturalize the double DNA helix. HCl was aspirated and the cells were then neutralized with 0.1 M borate buffer before blocking. The following procedure is described above.

References

1. Miyakura Y, Sugano K, Konishi F, Ichikawa A, Maekawa M, Shitoh K, et al. Extensive methylation of hMLH1 promoter region predominates in proximal colon cancer with microsatellite instability. Gastroenterology 2001;121:1300-9.
2. Tahara M, Inoue T, Miyakura Y, Horie H, Yasuda Y, Fujii H, et al. Cell diameter measurements obtained with a handheld cell counter could be used as a surrogate marker of G2/M arrest and apoptosis in colon cancer cell lines exposed to SN-38. Biochem Biophys Res Commun 2013;434:753-9.
3. Miyake M, Ishii M, Kawashima K, Kodama T, Sugano K, Fujimoto K, et al. siRNA-mediated knockdown of the heme synthesis and degradation pathways: modulation of treatment effect of 5-aminolevulinic acid-based photodynamic

therapy in urothelial cancer cell lines. Photochem Photobiol 2009;85:1020-7.

Supplementary Figure and Table legends

Supplementary Fig. S1. Cell counts with cell diameters in colon cancer cells exposed to SN-38 and/or olaparib. **A**, cell counts and diameters were measured using the Scepter 2.0™ cell counter in HCT116, HT29, RKO and SW1116 exposed to SN-38 (1 nM for HCT116, RKO and SW1116 and 4 nM for HT29) and/or 10 nM olaparib. The X-axis of each graph represents the cell diameter (μm) and the Y-axis represents the cell count (demonstrated in the inset of Supplementary Fig. S5). Experiments were performed in triplicate in each condition and each graph represents the result of a single run in triplicate experiments. **B**, data of cell counts (large diameter cells shown as L in Supplementary Fig. S1A) and the proportion of apoptotic cells (small diameter cells shown as S in Supplementary Fig. S1A) are presented as the mean ± SD of triplicate experiments. The significance of differences between the four groups was analyzed using one-way ANOVA with Tukey's test, and the data between control cells and SN-38 and/or olaparib treated cells are shown as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The mean ± SD of cell count, mean cell diameter and the proportion of small diameter cells in each condition were also shown in Supplementary Tables S3.

Supplementary Fig. S2. Clonogenic assay of HCT116 and HT29 cells exposed to SN-38 and/or olaparib. Clonogenic assay was performed in HCT116 and HT29 cells exposed to SN-38 (1 nM for HCT116 and 2 nM for HT29) and/or 10 nM olaparib for 6, 12, 24 and 48 hours. Experiments were performed in triplicate in each condition. Representative photographs of HCT116 (**A**) and HT29 (**B**) in 6-well plates treated for 48 hours are shown. The survival fraction was calculated relative to non-treated cells in HCT116 (**C**) and HT29 (**D**) exposed to SN-38 and/or olaparib for 6, 12, 24 and 48 hours. Error bars indicate mean ± SD. The significance of differences between the four groups was

analyzed using one-way ANOVA with Tukey's test, and the data between control cells and SN-38 and/or olaparib treated cells are shown as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplementary Fig. S3. The proportion of cells in S-phase and 53BP1 foci formation in SN-38 and/or olaparib treated HCT116 cells. **A**, the proportion of BrdU stained cells in HCT116 cells treated with 1 nM SN-38 and/or 10 nM olaparib for 6, 12, 24 and 48 hours. Error bars indicate mean ± SD. The significance of differences between the four groups was analyzed using one-way ANOVA with Tukey's test, and the data between control cells and SN-38 and/or olaparib treated cells are shown as follows: *, $P < 0.05$; **, $P < 0.01$; NS, not significant). **B**, immnofluorescence double staining for BrdU and 53BP1 was examined in HCT116 cells treated with SN-38 and olaparib for 6 and 24 hours.

Supplementary Fig. S4. Western blot analysis of proteins associated with DNA repair in colon cancer cell lines. ß-actin was used as an internal control.

Supplementary Fig. S5. Cell diameter of SW1116 exposed to 1 nM SN-38 and/or 10 nM olaparib after transfection with control siRNA, MRE11 siRNA, RAD50 siRNA or RAD51 siRNA. Cell counting and measuring the cell diameter were performed using the Scepter 2.0™ cell counter. The X-axis represents the cell diameter (μm) and the Y-axis represents the cell count as shown in enlarged view. Experiments were performed in triplicate in each condition and each graph represents the result of a single run in triplicate experiments. The mean ± SD of the cell diameters and the proportion of the cells with small diameter in each condition were shown in Supplementary Tables S4.

Supplementary Fig. S6. The siRNA-mediated knockdown targeting Rad51 potentiates the sensitivity to SN-38 and/or olaparib in HCT116. **A**, cell counting assay of HCT116

exposed to 1 nM SN-38 and/or 10 nM olaparib for 48 hours after transfection with control siRNA or RAD51 siRNA. Data are expressed as mean ± SD of triplicate experiments. Control means non-treated cells. Student's t-test was performed between cells transfected with control siRNA and cells transfected with RAD51 siRNA (*, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$). **B**, cell diameter of HCT116 exposed to 1 nM SN-38 and/or 10 nM olaparib after transfection with control siRNA or RAD51 siRNA. The X-axis of each graph represents the cell diameter (μm) and the Y-axis represents the cell count (demonstrated in the inset of Supplementary Fig. S5). Experiments were performed in triplicate in each condition and each graph represents the result of a single run in triplicate experiments. The mean ± SD of the cell diameters and the proportion of the cells with small diameter in each condition were shown in Supplementary Tables S5. **C**, effect of knockdown was confirmed by the Western blot analysis 24 hours after siRNA-mediated transfection targeting Rad51 in HCT116.

Supplementary Table S1. Mismatch repair, *TP53*, *MRE11* and *RAD50* gene status of colon cancer cell lines.

Supplementary Table S2. SN-38 IC_{50} in the presence or absence of 10 nM olaparib in colon cancer cell lines measured by ^{3}H-thymidine incorporation assay.

Supplementary Table S3. Cell count, mean cell diameter and the proportion of small-diameter cells in SN-38- and/or olaparib-treated cells measured using the Scepter™ 2.0 cell counter.

Supplementary Table S4. Mean cell diameter and the proportion of small-diameter cells in SN-38- and/or olaparib-treated cells of SW1116 transfected with control siRNA, MRE11 siRNA, RAD50 siRNA or RAD51 siRNA measured using the Scepter™ 2.0 cell

counter.

Supplementary Table S5. Mean cell diameter and the proportion of small-diameter cells in SN-38- and/or olaparib-treated cells of HCT116 transfected with control siRNA or RAD51 siRNA measured using the Scepter™ 2.0 cell counter.

Supplementary Table S6. Data of blood samples of SW1116 xenografted SHO mice on day 30.