Supporting Information

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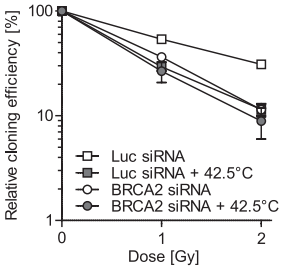

Fig. S1. Hyperthermia radiosensitizes control cells but not cells transfected with BRCA2 siRNA. Cloning efficiency of HeLa cells transfected with siRNA directed against luciferase (squares) or BRCA2 (circles), incubated for 75 min at 37 °C (open symbols) or 42.5 °C (filled symbols), and exposed to the indicated dose of γ-rays.

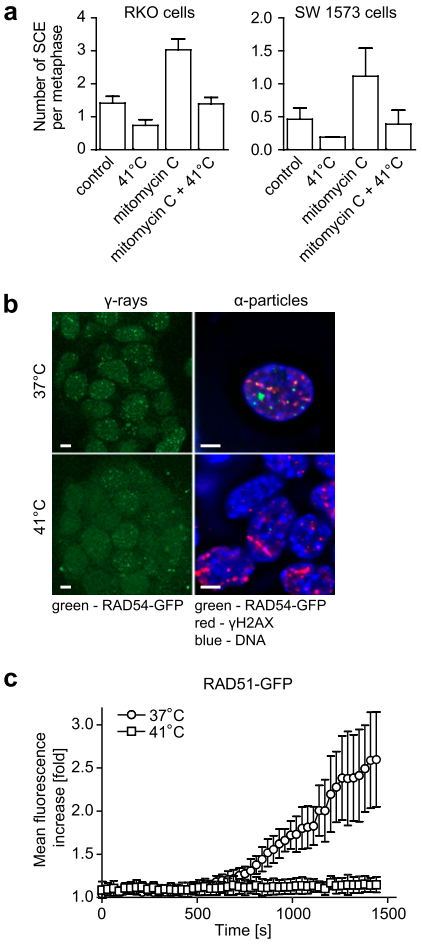

Fig. S2. A mild temperature increase interferes with HR. (*A*) Influence of temperature on the frequencies of spontaneous and mitomycin C-induced sister-chromatid exchanges. RKO (*Left*) or SW-1573 (*Right*) cells were incubated for two cell cycles in the presence of BrdU, then for 60 min in the presence or absence of mitomycin C at 37 °C or 41 °C and processed to obtain metaphase spreads. Graph presents average number of sister-chromatid exchanges (SCE) per scored metaphase. Error bars indicate SEM obtained from three independent experiments. (*B*) Influence of temperature on the accumulation of RAD54-GFP at DSB sites. Mouse knock-in ES cells expressing RAD54-GFP were incubated at 37 °C or 41 °C for 75 min, then irradiated with γ-rays (8 Gy, left column) or α-particles (right column) and fixed 30 min after irradiation. Cells were then either directly imaged using a confocal microscope (left column) or stained for γH2AX and DNA and imaged using a wide-field fluorescence microscope (right column). (Scale bar: 5 μm.) (*C*) Quantification of accumulation of GFP-RAD51 at sites of DNA damage induced by UVA laser microirradiation in living cells preincubated at 37 °C or 41 °C. V79 cells expressing GFP-RAD51 were incubated at 37 °C (○) or 41 °C (□) for 60 min, then exposed to UVA light in predefined areas of the nuclei and imaged for indicated period. Graphs represent mean increase of fluorescence in the exposed areas as a function of time. Error bars represent SD around the mean from at least 10 measurements.

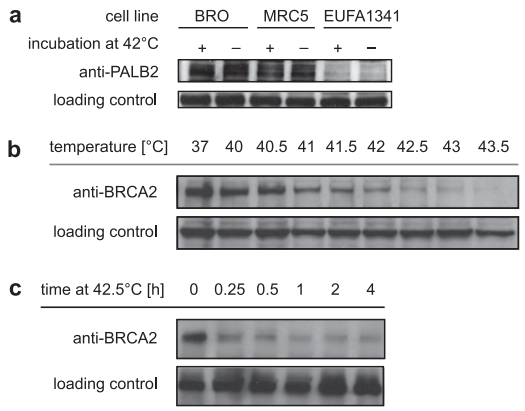

Fig. S3. BRCA2 degradation is time and temperature dependent. (*A*) Hyperthermia does not influence PALB2 levels. Immunoblotting of BRO cells and human fibroblasts expressing (MRC5) or lacking PALB2 (EUFA1341). Cells were subjected to 42.5 °C for 75 min and lysed. Lysates were analyzed by immunoblotting with antibodies against PALB2 (*Upper*). Loading of samples was controlled by a nonspecific band detected by the anti-PALB2 antibody (*Lower*). (*B*) Immunoblotting of cells subjected to increasing temperature. BRO cells have been incubated for 75 min at indicated temperatures. Next, cells were lysed and lysates were analyzed by immunoblotting with antibodies against BRCA2 (*Upper*). Equal sample loading was verified by probing for ORC2 (*Lower*). (*C*) Immunoblotting of cells subjected to 42.5 °C for increasing time spans. BRO cells were incubated for indicated time spans at 42.5 °C and subsequently lysed. Lysates were analyzed by immunoblotting with antibodies against BRCA2 (*Upper*). Equal sample loading is indicated by a nonspecific band (*Lower*).

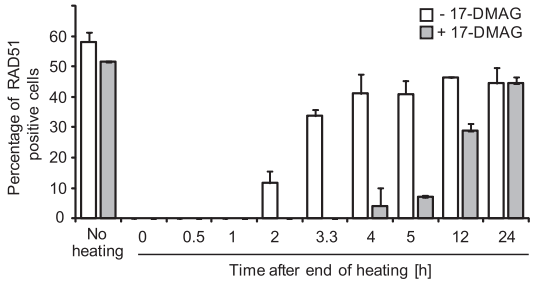

Fig. S4. 17-DMAG prolongs the temperature-mediated inhibition of RAD51 IRIF formation. Quantification of accumulation of RAD51 at DSB sites in cells preincubated at 37 °C or 42 °C in the presence or absence of 17-DMAG. U2OS cells were incubated for 60 min in the presence or absence of 17-DMAG at 37 °C, then incubated for 90 min at 37 °C (no heating) or 42 °C. Next, cells were washed and medium was refreshed to remove the 17-DMAG. Cells were then incubated at 37 °C for the indicated period, irradiated with α-particles, incubated for 15 min at 37 °C, and fixed. Cells were stained for γH2AX and RAD51 and scored as positive if they contained at least three IRIF of RAD51 colocalizing with γH2AX IRIF. Graphs represent average percentages of positive cells. Error bars represent the range of percentages obtained from two independent experiments. At least 100 cells containing damage induced by α-particles were scored per experiment.