Supplementary Methods

KillerRed System

The system is described in previous paper (23). To activate KR, KR was exposed to 559-nm laser light for 50 scans (over a total of 10 s) at a power rate of 1 mW/scan (equal to 50 mW). At "the KR-TRE array" induced localized damage, we have detected γ-H2AX at the site of TA-KR but not TA-monomer cherry (TA-mcherry) after laser light exposure (unpublished data). For bleaching KR, a 559-nm laser (1 mW/scan) in a selected area was used (FV1000 SIM Scanner set with 405-nm laser diode, Cat. F10OSIM405, Olympus). The dose that was delivered to the KillerRed spot was calculated on the basis of the pixel size, where the pixel size for irradiation was 0.138 μm/pixel and the dwell time per pixel was 8 μs/pixel. The irradiation was at 1.0 mW (1.0 mJ/s). With the dwell time of 8 μs/pixel, each pixel was irradiated with 8.0 nJ/pixel/scan. Multiplying by the number of scans gives the total energy per pixel. For statistical analysis, 10~20 cells from each experiment were counted to calculate the positive percentage, in total, over 50 cells were examined from three independent experiments.

Flow Cytometric Analyses

Cell cycle profiles were measured by flow cytometry using propidium iodide (PI). Briefly, HCT116 or U2OS cells were plated at 3×10^5 cells/plate in 60-mm plates 24 h before treatment. Cells were treated with IR at various dosages. Paclitaxel (2 μM) was added into the medium 1 h after irradiation and left in the medium. At the end of the treatment, cells were trypsinized and collected by centrifugation (1000 RPM for 5 min at room temperature). Cell pellets were resuspended in PBS and fixed in 70% ethanol for 1 h at 4°C. After being washed twice with PBS, cells were incubated at room temperature for 30 min in the dark with PI staining solution

containing 50 μg/mL PI (Calbiochem), 20 μg/mL RNase A (Novagen), and 0.05% Triton X-100. For p-H3 staining, the cell pellets were incubated with buffer (PBST + 0.1% NP40 + 0.1% Triton X-100) containing anti-p-H3-Alex673 antibody at 4°C overnight. The cells were washed twice with PBS and stained with PI staining solution. Stained cells were analyzed in a Beckman Coulter Gallios flow cytometer using Kaluza Flow Analysis software.

Comet Assay

Comet assay was performed as previously described (21). Briefly, the presence of DNA damage was analyzed by alkaline buffer using Trevigen's comet assay kit according to the manufacturer's instructions. Cells were exposed to 7 Gy of IR and subjected to comet analysis at indicated time points. After staining with SYBR green, comet images were captured by fluorescence microscopy. Tail moments (percentage of DNA in tail x tail length) were quantitated for 100 cells/slide by using CometScore software.

Colony-forming Assay

Cells were seeded at low density (500 cells/well for 6-well plate) and the drug treatment was added the next day. The cells were left for further two weeks to allow colonies to form. Colonies were fixed with 4% buffered paraformaldehyde and stained with 0.01% Crystal violet. Colonies containing 50 or more cells were scored as positive for statistical analysis.

Tet-ON expression of ARID1A

HCT116-ARID1A (-/-) cells were infected by the lentivirus pLenti-3-TR and pLenti6-ARID1A, then briefly selected by puromycin (1 μg/ml). The resulting cells were treated by doxycycline (1 μg/ml) for 48 h to induce the expression of ARID1A.