Supplemental Figure Legend

**Supplementary Figure 1.** RNF8-deficient MEFs were transduced with retroviral vector encoding flag-WT, -ΔFHA, -ΔRING and -I405A mutants of RNF8. Ectopic expressions of flag-tagged RNF8 and its mutants were analysed by Western blotting.

**Supplementary Figure 2.** Immunoflorescence staining of (A) FK2 and (B) 53BP1 foci of WT RNF8 and I405A reconstituted RNF8-deficient MEFs were shown at indicated time points after 10Gy IR. Numbers of foci per cell (average of 100 cells) were examined at each time points. Bars represent standard error of the mean from three independent experiments.

**Supplementary Figure 3.** RNF8-deficient MEFs reconstituted with WT, ΔFHA, ΔRING and I405A of RNF8 were irradiated with indicated dose of IR and were allowed to grow for additional 10 days. Cells were fixed and stained with Coomassie blue solution, and colonies with more than 50 cells were counted. Error bars represent standard deviations of the mean from three independent experiments.

**Supplementary Figure 4.** (A) Efficiency of siRNA targeting UBCH8 (GenePharma) was tested by co-transfecting myc-UBCH8 into 293T cells. 48-hour after transfection, cell lysates were analyzed by Western blotting to examine UBCH8 protein expression. (B) HeLa cells were transfected with pooled UBCH8 siRNAs (siUBCH8) or control siRNAs (siCTR) twice at 24 hr intervals. Cells were seeded, and fresh medium supplemented with cycloheximide was added. At indicated time cells were lysed and processed for Western blotting analyses using antibodies against RNF8 and β-actin (loading control). (C) HeLa cells were transfected with pooled UBCH8-specific siRNAs (siUBCH8), control siRNAs (siCONTROL) or oligofectamine (mock). 48-hour after transfection, cells were irradiated with 10 Gy, and were recovered for 4 hr before immunofluorescence experiments were performed to assess 53BP1 and FK2 IRIF formation.
Supplementary Figure 2

A

FLJ foci per cell

WT
H405A

Untreated 2 4 8
Time after IR (h)

B

53BP1 foci per cell

WT
H405A

Untreated 2 4 8
Time after IR (h)