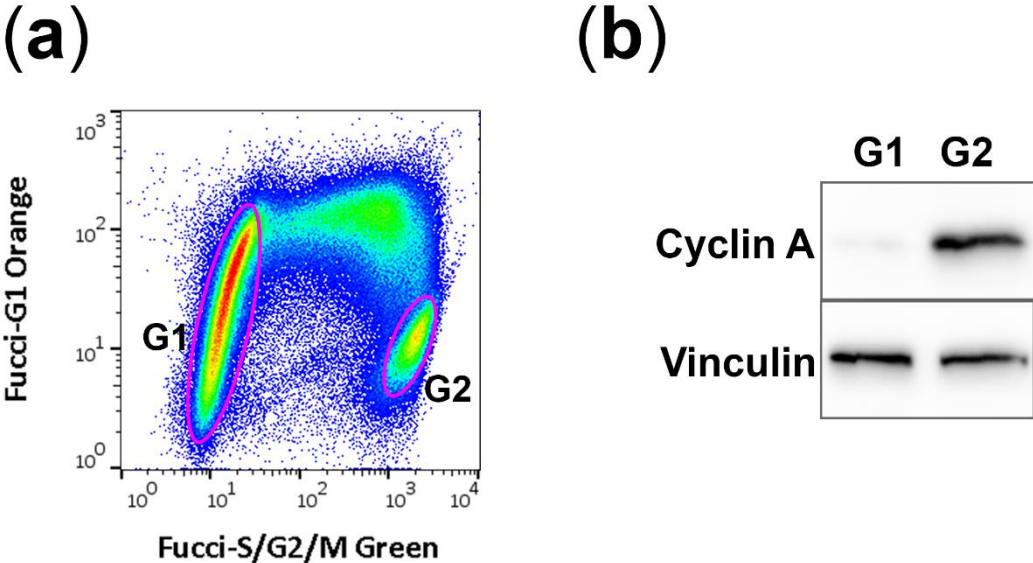


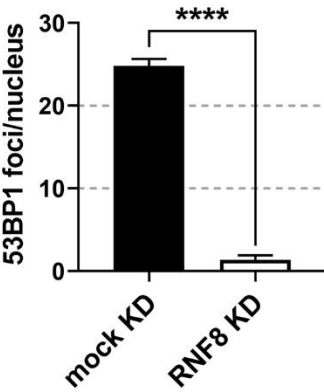
# Supplemental Figure S1



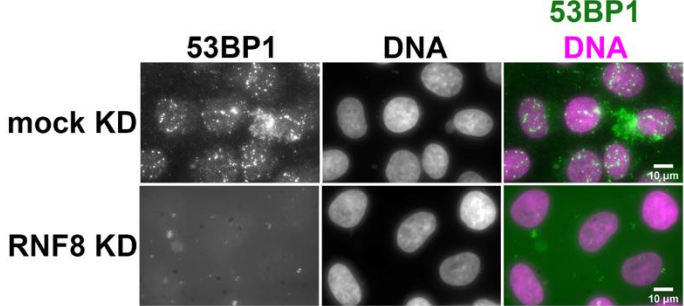
**Supplemental Figure S1: Cell-cycle specific sorting of HeLa.S-Fucci.** (a) Scatter plot of the cell-cycle specific fluorescence signals of Fucci-G1 Orange (G1 cells) and Fucci-S/G2/M Green (S, G2, and M cells). The gates to sort for G1 and G2 cells are highlighted. (b) Western blot analysis of cells sorted by flow cytometry for G1 and G2 cells. Whole cell extracts of sorted cells were immunoblotted for Cyclin A (S/G2 marker) and Vinculin (loading control).

# Supplemental Figure S2

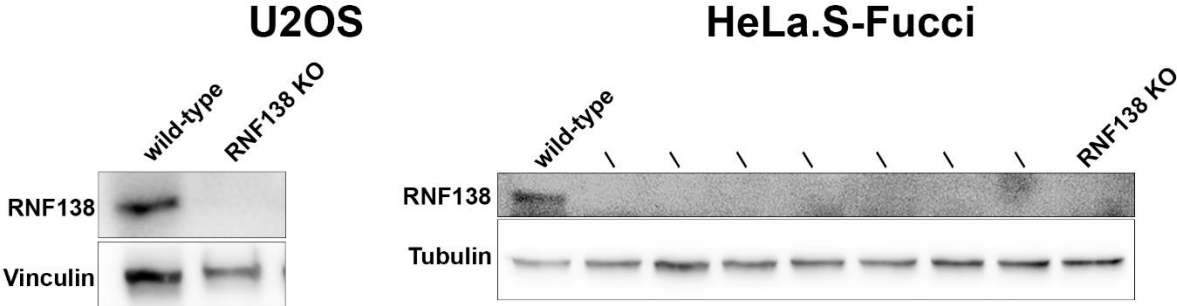
(a)



(b)

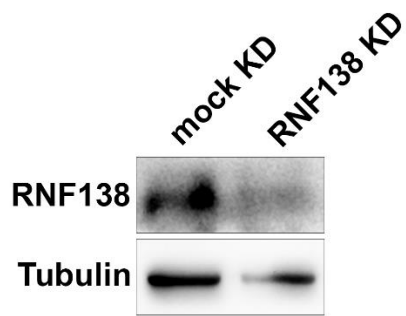


(c)



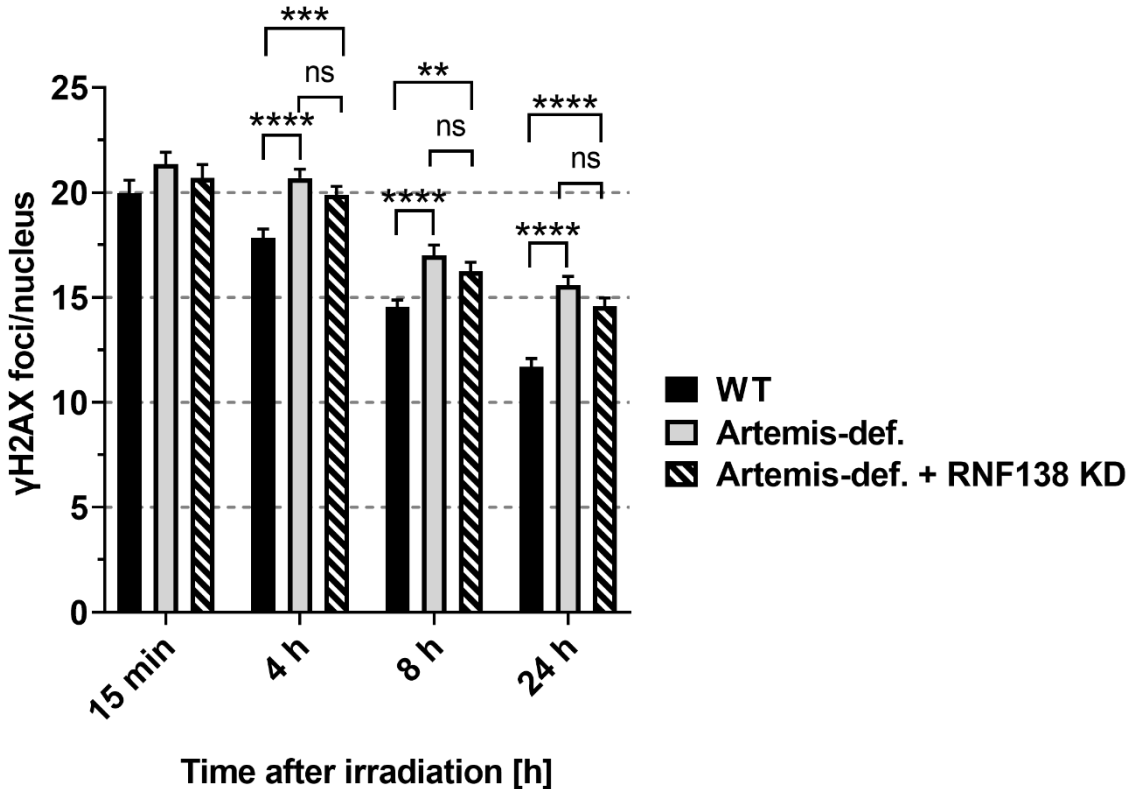
**Supplemental Figure S2: Verification of RNF8 knockdown and RNF138 knockout.** (a) Representative image of 53BP1 immunofluorescence stained cells irradiated with  $\alpha$ -particles. DSB recruitment of 53BP1 requires histone ubiquitination by RNF8 and thus, quantification of radiation-induced 53BP1 foci can serve as a measure for an RNF8 knockdown (KD). U2OS cells were irradiated with 2 Gy  $\alpha$ -particles and fixed 1 h after irradiation. 53BP1 was immunofluorescence stained and DNA was counterstained with DAPI. (b) 53BP1 foci were quantified and the foci number of non-irradiated cells was subtracted. Per condition, 100 cells were analyzed; n=1, error=SEM. Two-tailed Mann-Whitney-test, p values: \*\*\*\* p < 0.0001. (c) To verify the RNF138 knockout (KO) in U2OS and HeLa.S-Fucci cells, a western-blot analysis was performed, in which RNF138 and the loading control tubulin or vinculin was immuno-stained.

## Supplemental Figure S3



**Supplemental Figure S3: Verification of RNF138 KD in NFFhTERT cells.** Knockdown (KD) of RNF138 was verified by a western blot analysis. Tubulin served as loading controls.

# Supplemental Figure S4



**Supplemental Figure S4: At complex DSBs, RNF138 deficiency does not allow bypassing Artemis.** Artemis proficient cells (82-6 hTERT) and deficient cells (CJ179hTERT) as well as Artemis deficient cells that were depleted for RNF138 by RNAi (CJ179hTERT + RNF138 KD) were irradiated with 2 Gy  $\alpha$ -particles and fixed at different time points after irradiation. The samples were immunofluorescence stained for  $\gamma$ H2AX and DNA was counterstained with DAPI.  $\gamma$ H2AX foci/nucleus were quantified in at least 50 cells and averaged; n=1, error=SEM. Brackets indicate comparisons between samples. Two-tailed t-test, p values: ns, not significant; \*\* p < 0.01; \*\*\* p < 0.001; \*\*\*\* p < 0.0001.