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Supplemental Material to:

Hongchang Zhao, Min Zhu, Gelin Dou, Hongli Zhao, Bingtao Zhu, Jing Li, Ji Liao, and Xingzhi Xu

BCL10 regulates RNF8/RNF168-mediated ubiquitination in the DNA damage response

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Zhao et al., Supplemental Figures

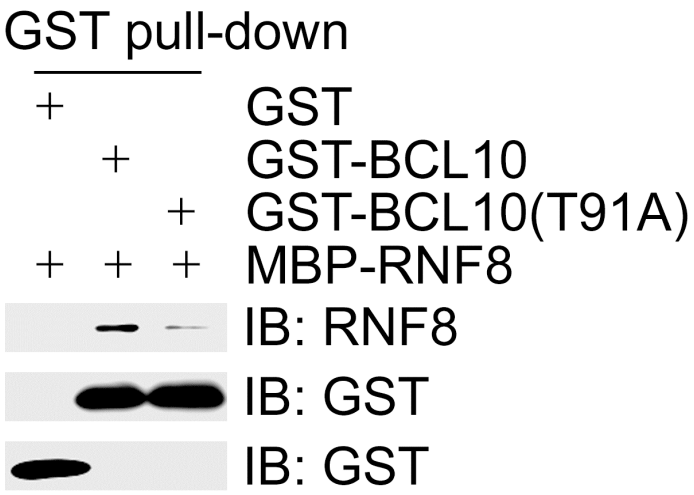

Figure S1. BCL10 directly binds to RNF8 and this binding is compromised with the point mutant BCL10(T91A). In a GST pull-down assay, bacterially produced MBP-RNF8 was incubated with recombinant protein GST, GST-BCL10, or GST-BCL10(T91A) at 4°C overnight, glutathione sepharose 4B was then added and incubated for an additional hour. GST pull-downs were extensively washed, resolved in SDS-PAGE, and immunoblotted with antibodies as indicated.

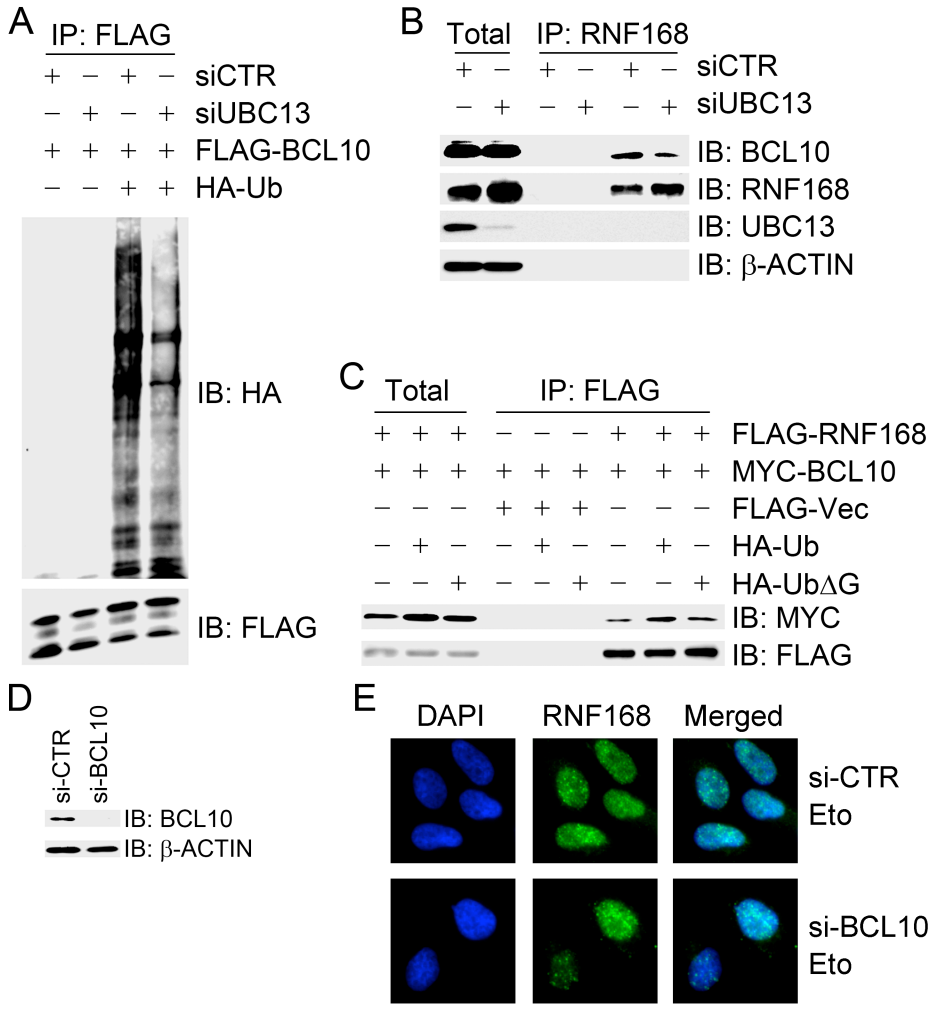

Figure S2. UBC13 mediated-ubiquitination of BCL10 is important for its interaction with RNF168. (A) Depletion of UBC13 compromised BCL10 ubiquitination. UBC13-depleted 293T cells were co-transfected with HA-Ub and FLAG-BCL10, total cell lysates were extracted for immunoprecipitation with an anti-FLAG antibody and immunoblotting with antibodies as indicated. (B) Depletion of UBC13 reduced the interaction between RNF168 and BCL10. Total cell lysates were extracted from UBC13-depleted 293T cells and subjected for immunoprecipitaion with an anti-RNF168 antibody and subsequently immunoblotting with antibodies as indicated. (C) Polyubiquitination of BCL10 promoted its interaction with RNF168. 293T cells were co-transfected with different combinations

of expression constructs for FLAG-RNF168, MYC-BCL10, and HA-Ub or chain elongation-defective HA-UbΔG. Total cell lysates were extracted 48 hours after transfection and subjected to immunoprecipitation with an anti-FLAG antibody followed by immunoblotting with antibodies as indicated. (D and E) DNA damage-induced RNF168 nuclear focus formation is BCL10-independent. HeLa cells were transfected with si-CTR or si-BCL10, total cell lysates were extracted 48 hours after transfection for immunoblotting with antibodies as indicated in (D). Mock or BCL10-depleted cells were treated with etoposide for 1 hour and subsequently immunostained with an anti-RNF168 antibody (E).

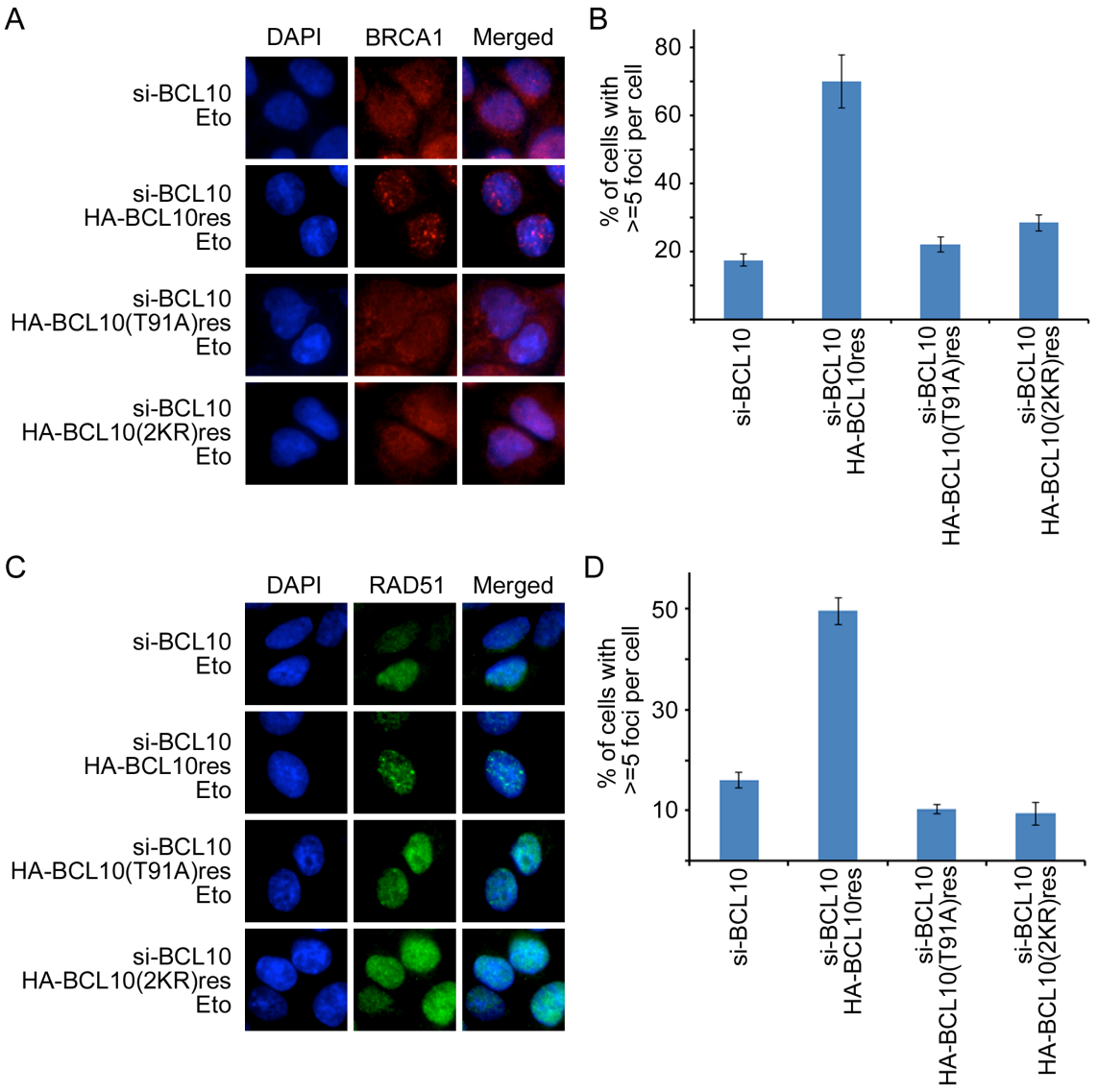

Figure S3. Both ATM-mediated phosphorylation and RNF8-dependnent ubiquitination are required for BCL10 to recruit BRCA1 and RAD51 to the DNA damage site. U2OS cells were stably transfected with HA-Vec, HA-BCL10res, HA-BCL10(T91A)res, or HA-BCL10(2KR)res. Transfectants were further transfected with si-BCL10. (A, B) Depletion of BCL10 expression compromised focus formation of BRCA1. Transfectants were treated with etoposide 1 hour before fixed with 4% paraformaldehyde and subsequently immunostained with an anti-BRCA1 antibody (A). Percentage of cells with

>=5 foci per cell was shown in (B). (C, D) Depletion of BCL10 expression compromised focus formation of RAD51. Experiments were performed as described in (A, B) except that antibody used for immunostaining was specific for RAD51. Percentage of cells with >=5 foci per cell was shown in (D).

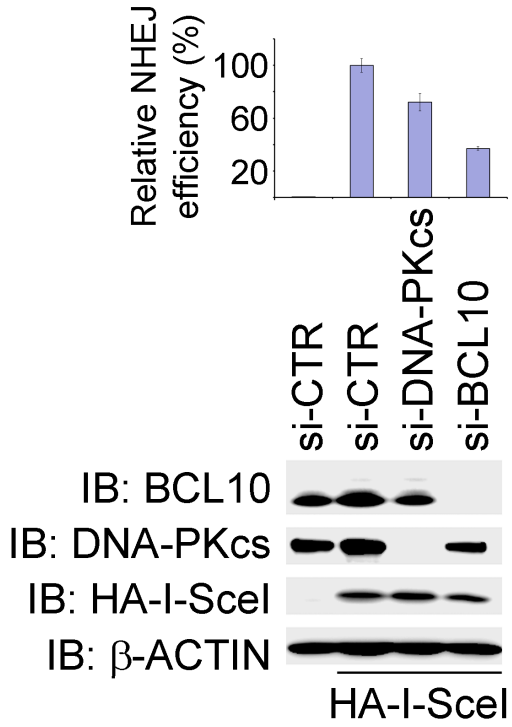

Figure S4. BCL10 promotes NHEJ-mediated DSB repair. Mock, DNA-PKcs, or BCL10-depleted NHEJ-GFP U2OS cells were transfected with HA-I-SceI. Total cell lysates were extracted 48 hours after transfection from a small aliquot of transfectants were subjected to immunoblotting with antibodies as indicated. The majority of transfectants were harvested for flow cytometry analysis for the percentage of GFP-positive cells that reflected NHEJ-mediated DSB repair efficiency. Relative NHEJ efficiency from a triplicate experiment was shown. The expression levels of HA-I-SceI served as a transfection efficiency control.

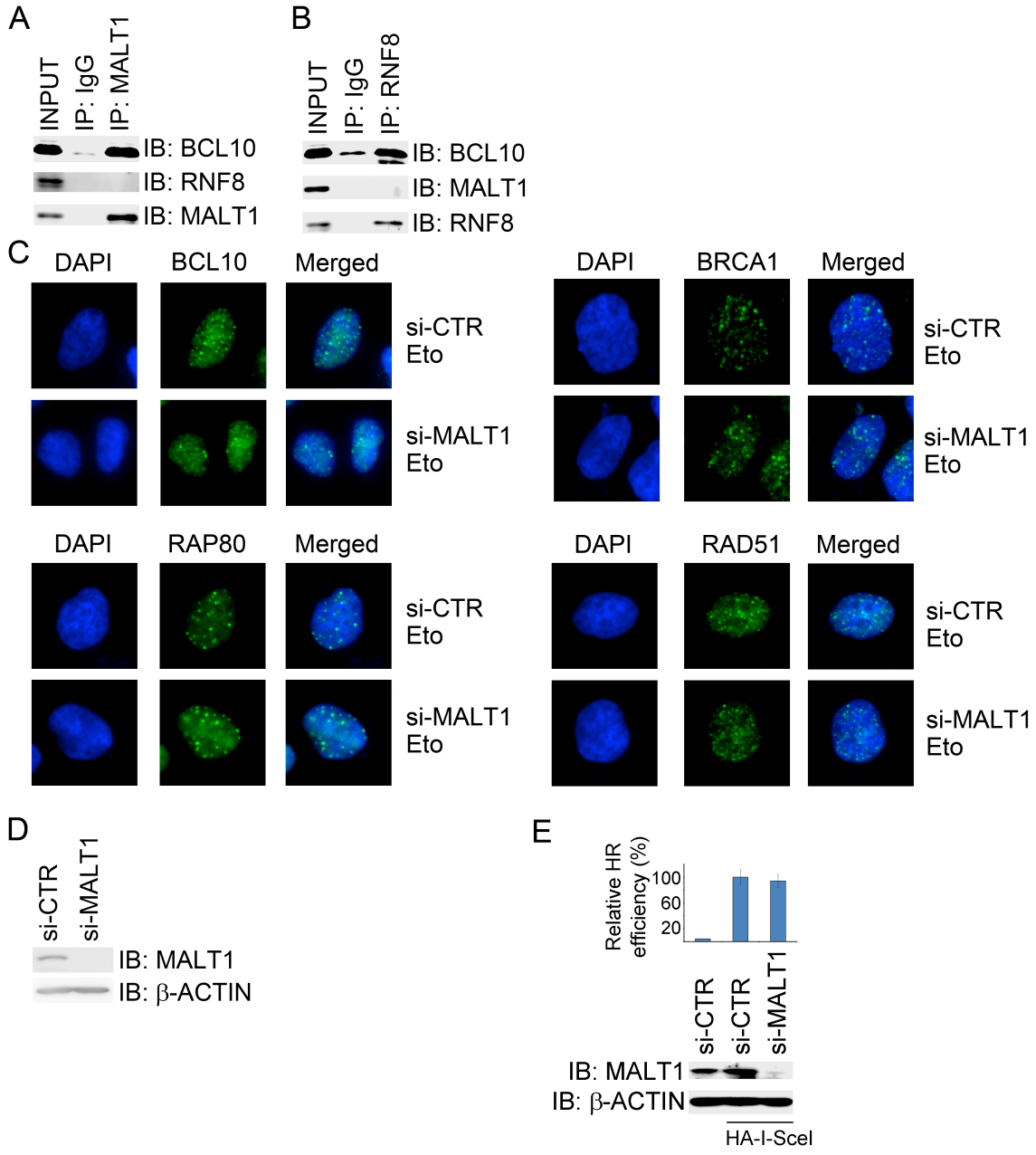

Figure S5. BCL10-mediated function in DDR is independent of MALT1. (A) RNF8 was not present in the BCL10-MALT1 complex. Total cell lysates were extracted from 293T cells and subjected to immunoprecipitaion with an anti-MALT1 antibody and immunoblotting with antibodies as indicated. (B) MALT1 was not present in the RNF8-BCL10 complex. Total cell lysates were extracted from 293T cells and subjected to

immunoprecipitaion with an anti-RNF8 antibody and immunoblotting with antibodies as indicated. (C, D) Depletion of MALT1 did not compromise the bleomycin-induced focus formation of BCL10, RAP80, BRCA1, and RAD51.Mock or MALT1-depleted HeLa were treated with or without etoposide for 1hours before fixed and subsequently immunostained with antibodies specific for BCL10, BRCA1, RAP80 and RAD51. MALT1 knockdown efficiency was determined by immunoblotting with antibodies as indicated (D). (E) Depletion of MALT1 did not compromise HR-mediated DSB repair. Mock or MALT1-depleted DRGFP U2OS cells were transfected with HA-I-SceI. Total cell lysates were extracted 48 hours after transfection for immunoblotting with antibodies as indicated, cells prepared in parallel were harvested for flow cytometry analysis and percentage of GFP-positive cells representing HR-mediated DSB repair efficiency was determined. Histogram showed the relative HR efficiency. Experiments were performed in triplicate, and error bars indicate standard deviation.