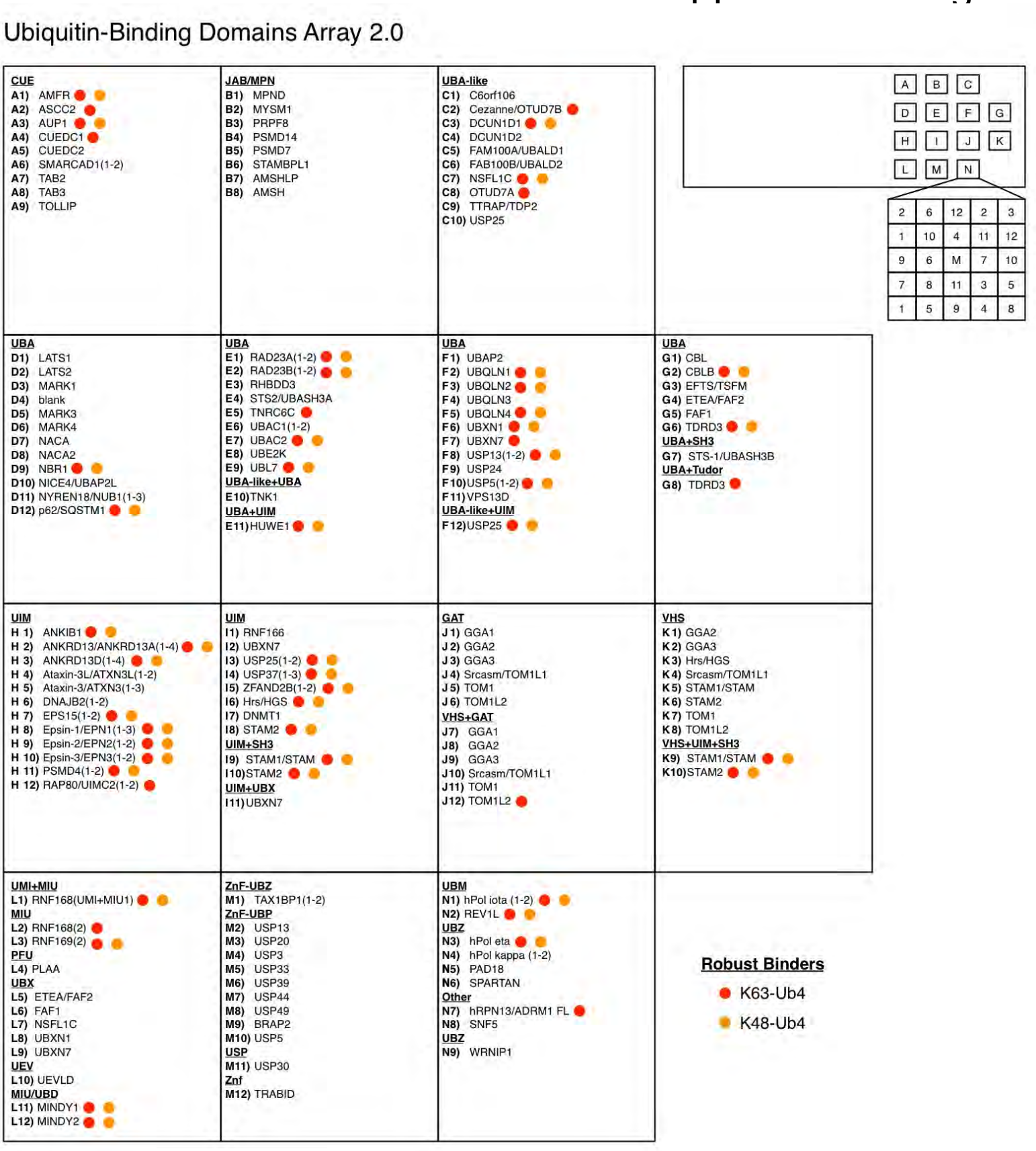
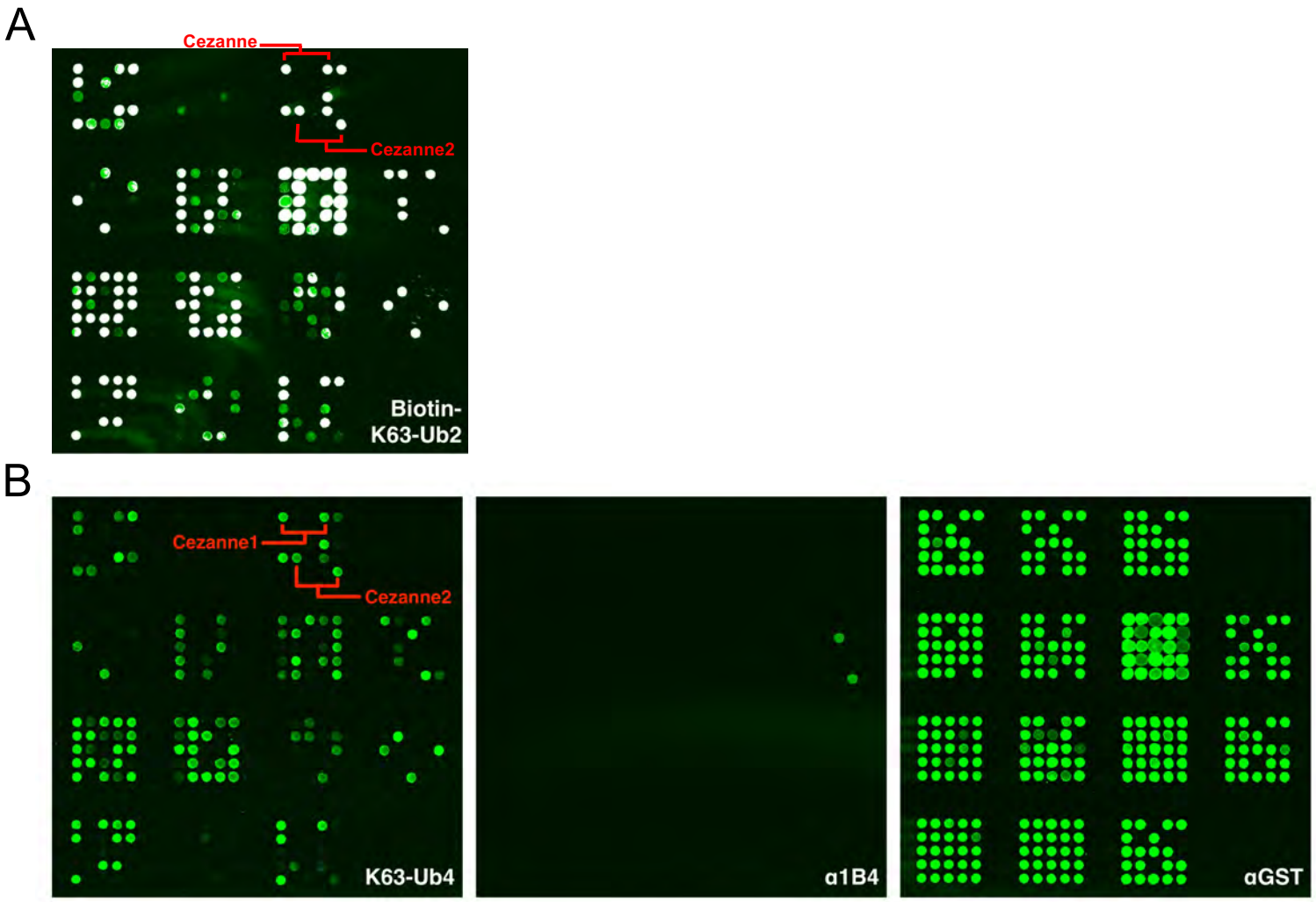
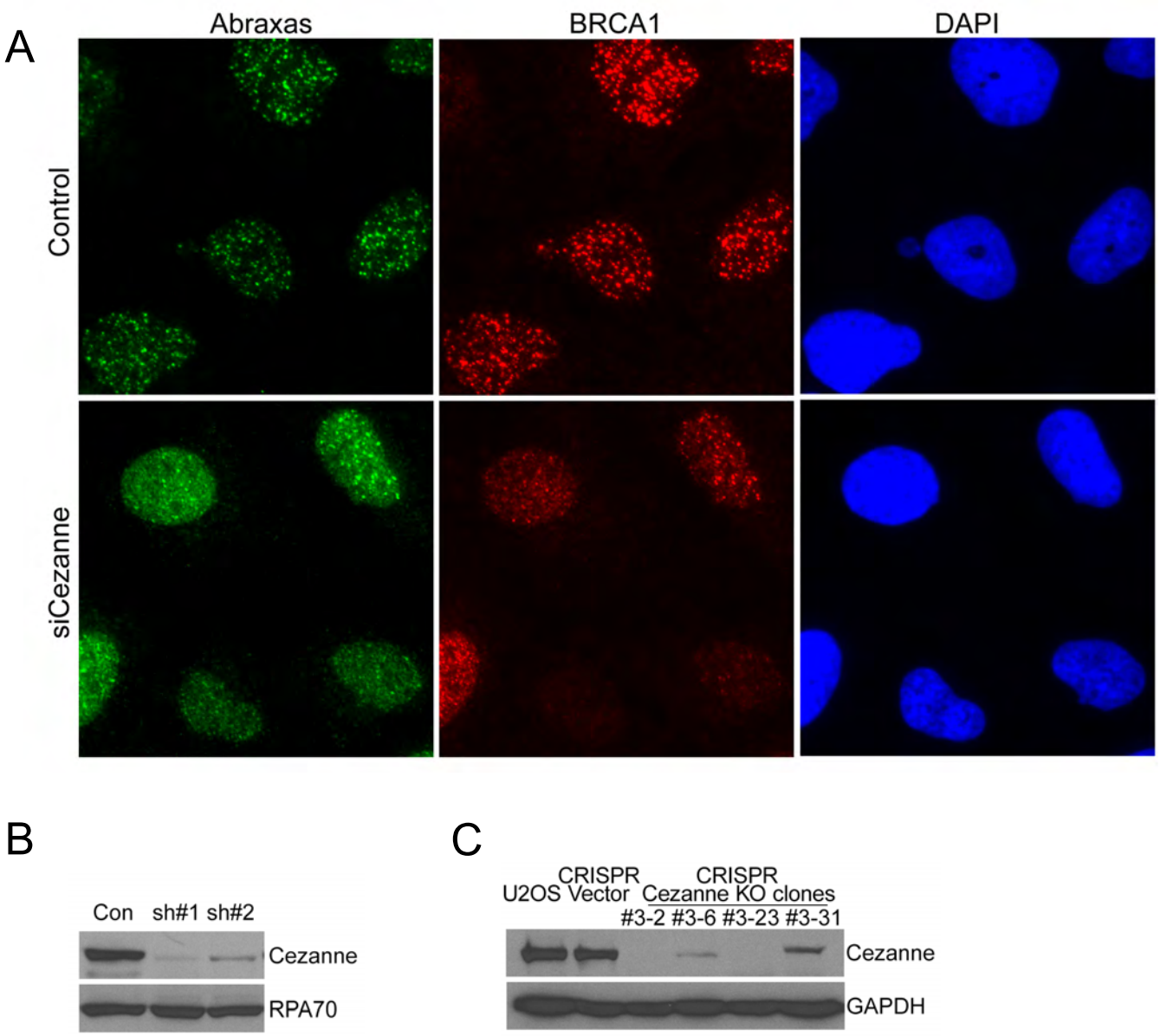


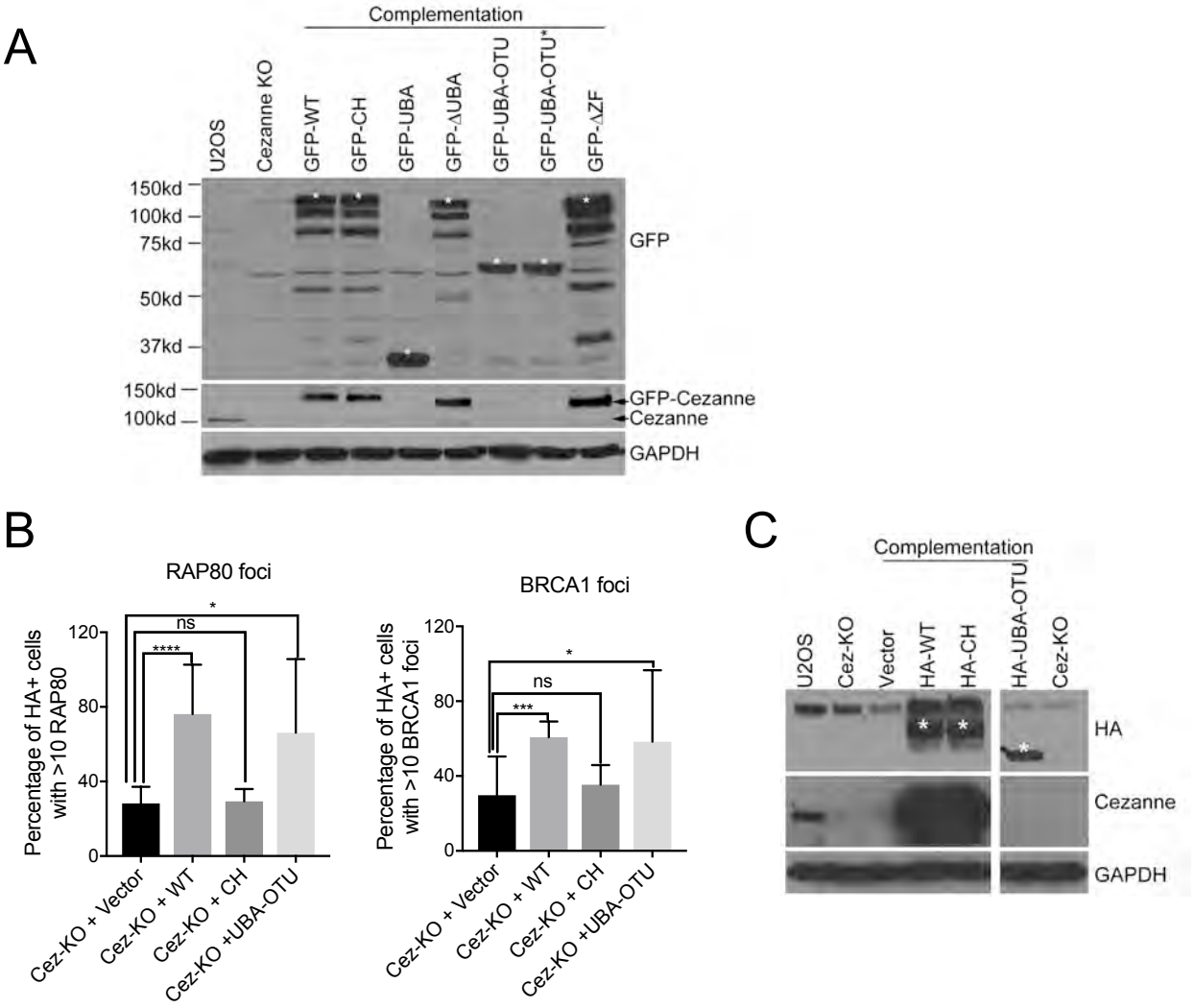
Fig_S1. A list of the 148 recombinant ubiquitin-binding domains, separated into block by domain type. A key to the domain position on the array is shown in the top right. "M" represents a GST control spot. All GST-UBD fusions are arrayed in duplicate.



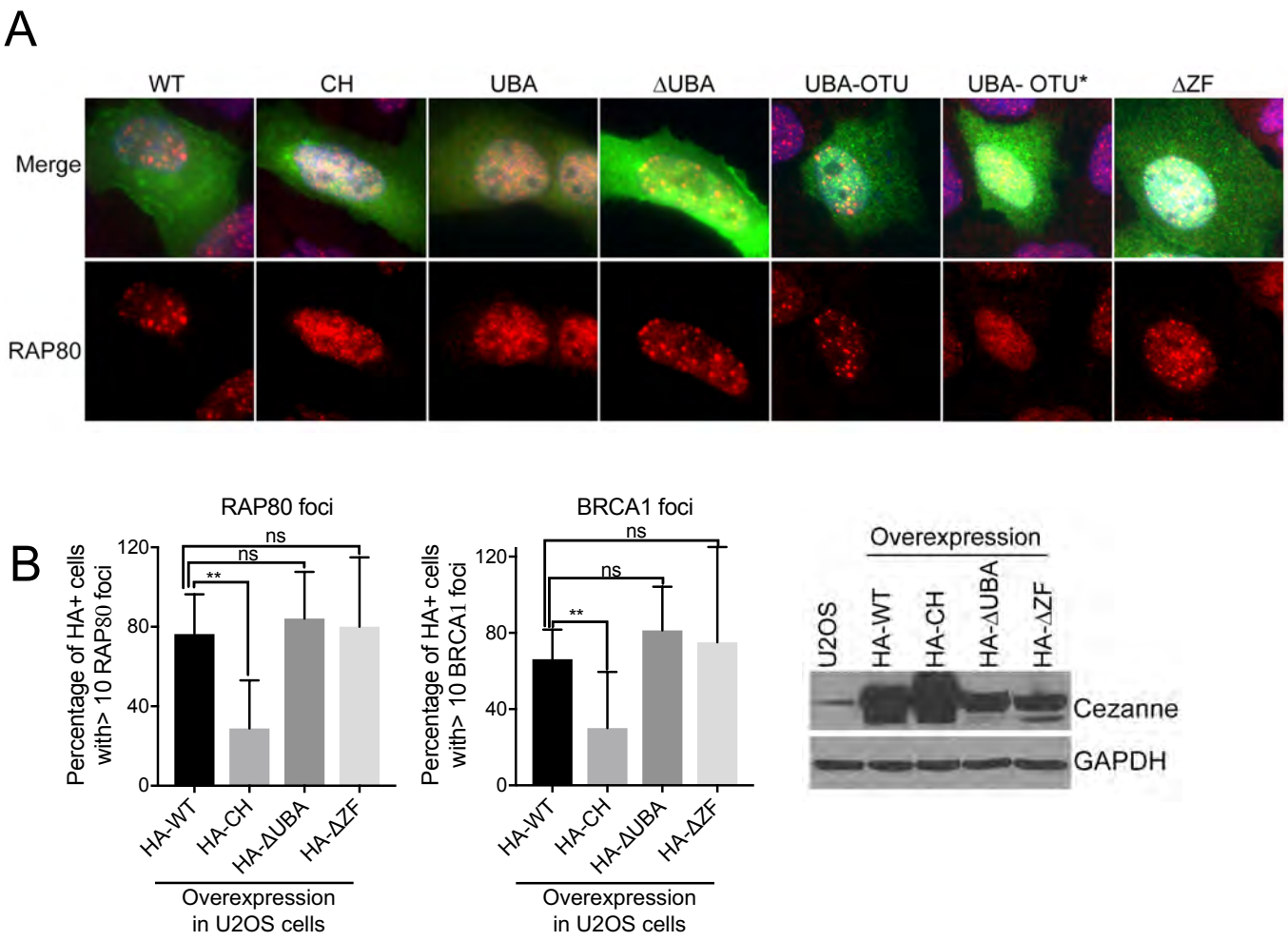
Fig_S2. Cezanne and Cezanne2 UBA bind to K63-linked ubiquitin. (A) biotin-K63-diUb is used as a probe for a screen of the UBD microarray. **(B)** Screen using hybridization with untagged K63-tetra-Ub followed by anti-ubiquitin antibody and FITC-labeled secondary antibody. Left panel: hybridization with K63 tetra-ubiquitin chains followed by anti-ubiqitin antibody (a1B4) then FITC-labeled secondary antibody; Middle panel: hybridization with anti-ubiquitin antibody (a1B4) followed by FITC-labeled secondary antibody; Right panel; hybridization with anti-GST antibody followed by incubation with a FITC-labeled secondary antibody.



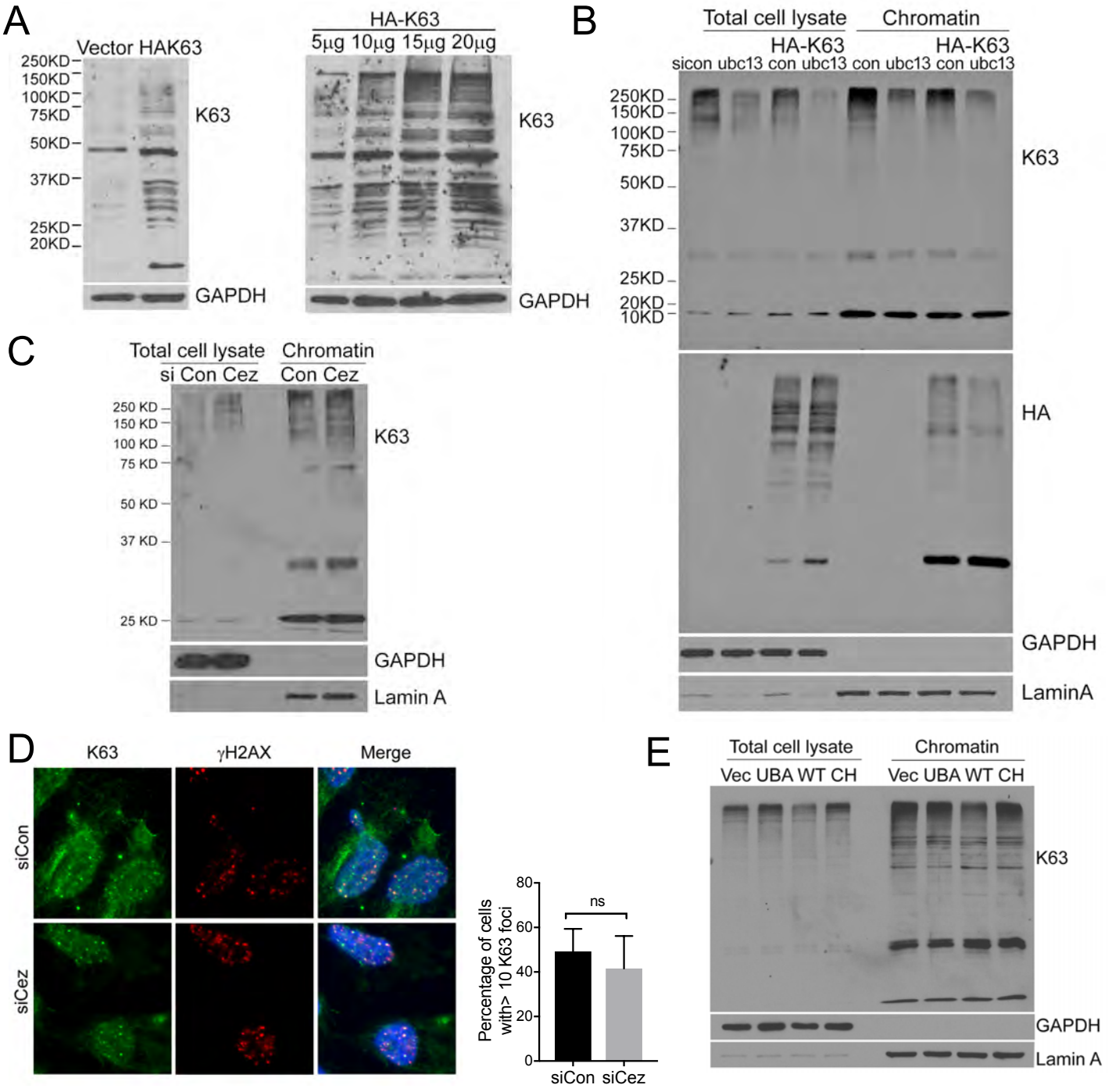
Fig_S3. Cezanne regulates Rap80/Abraxas/BRCA1 recruitment to DSBs. (**A)** Representative images of Abraxas and BRCA1 IRIF in Cezanne siRNA treated cells treated with 10 Gy IR followed by 2 h incubation before fixation and staining. (**B**) Depletion of Cezanne by shRNAs (**C**) Generation of Cezanne knockout (KO) cells using CRISPR-Cas9. Individual clones were screened by western blot using anti-Cezanne antibody. Clone #3-2 and #3-23 were picked and named as KO#1 and KO#2.



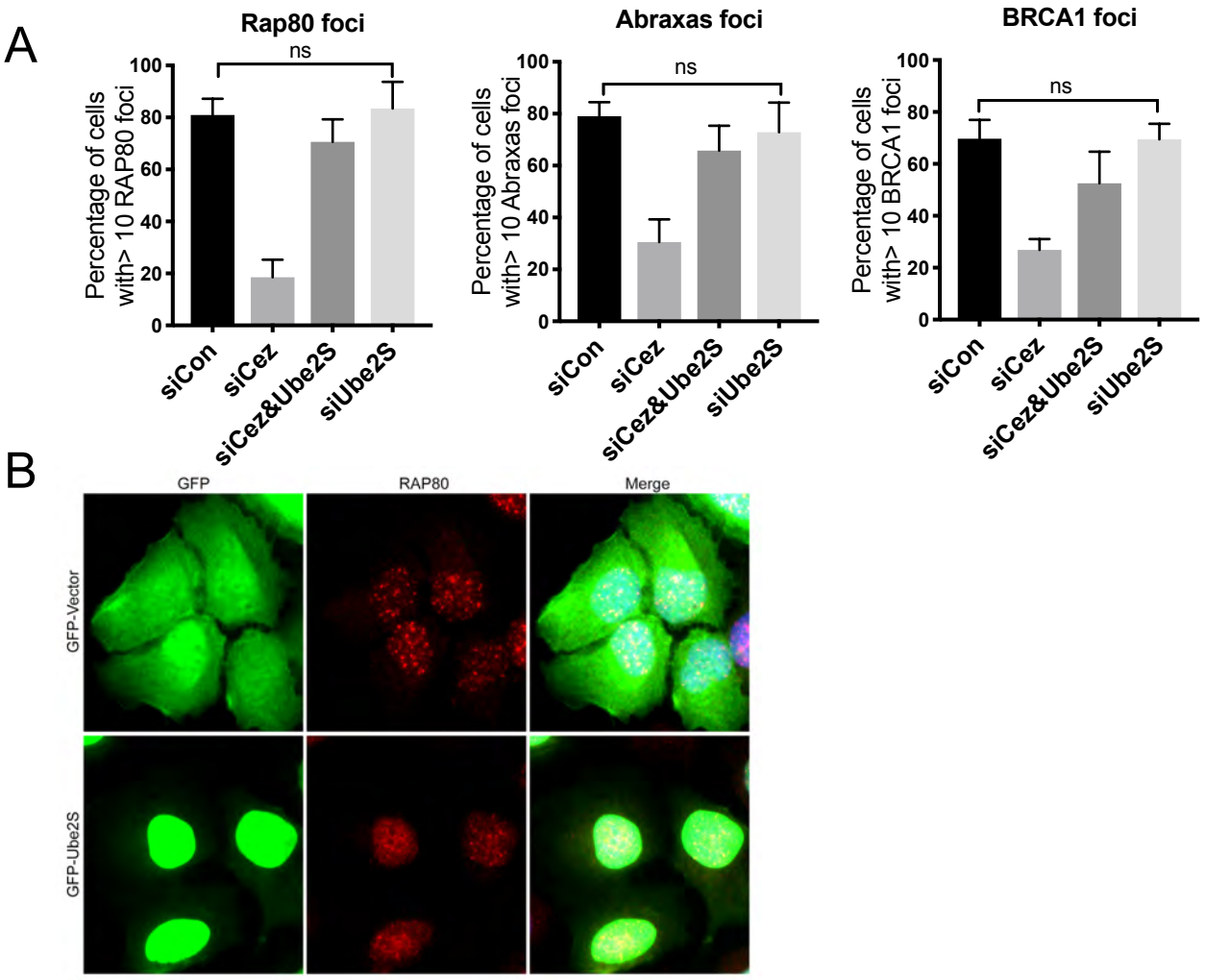
Fig_S4. Cezanne UBA domain and DUB activity are required for IRIF of Rap80/Abraxas/BRCA1. (**A**) Western blot showing complementation of Cezanne KO cells with expression of GFP-tagged Cezanne WT and mutants. * indicates bands with correct size. (**B**) Quantification of Cezanne KO cells complemented with expression of HA-tagged Cezanne WT, CH or UBA-OUT mutant. Non-parametric Kruskal-Wallis ANOVA was used for statistical analysis. (**C**) Western blot showing the expression of the indicated HA-tagged proteins in Cezanne KO cells using indicated antibodies.



Fig_S5. Overexpression of Cezanne mutants lacking DUB activity plays a dominant negative effect on Rap80 recruitment. (A) Representative images of cells overexpressing GFP-tagged Cezanne WT and mutants. Cells treated with 10 Gy IR followed by incubation at 37°C for 2h were fixed and stained with antibodies to GFP and Rap80 followed by appropriate secondary antibodies and DAPI staining. **(B)** Overexpression of HA-tagged Cezanne DUB mutant (CH), but not ΔUBA or ΔZF, showed dominant negative effect on IRIF of Rap80 and BRCA1. Non-parametric Kruskal-Wallis ANOVA was used for statistical analysis. Protein levels of overexpression were shown in western blot with antibodies to Cezanne and GAPDH (right panel).

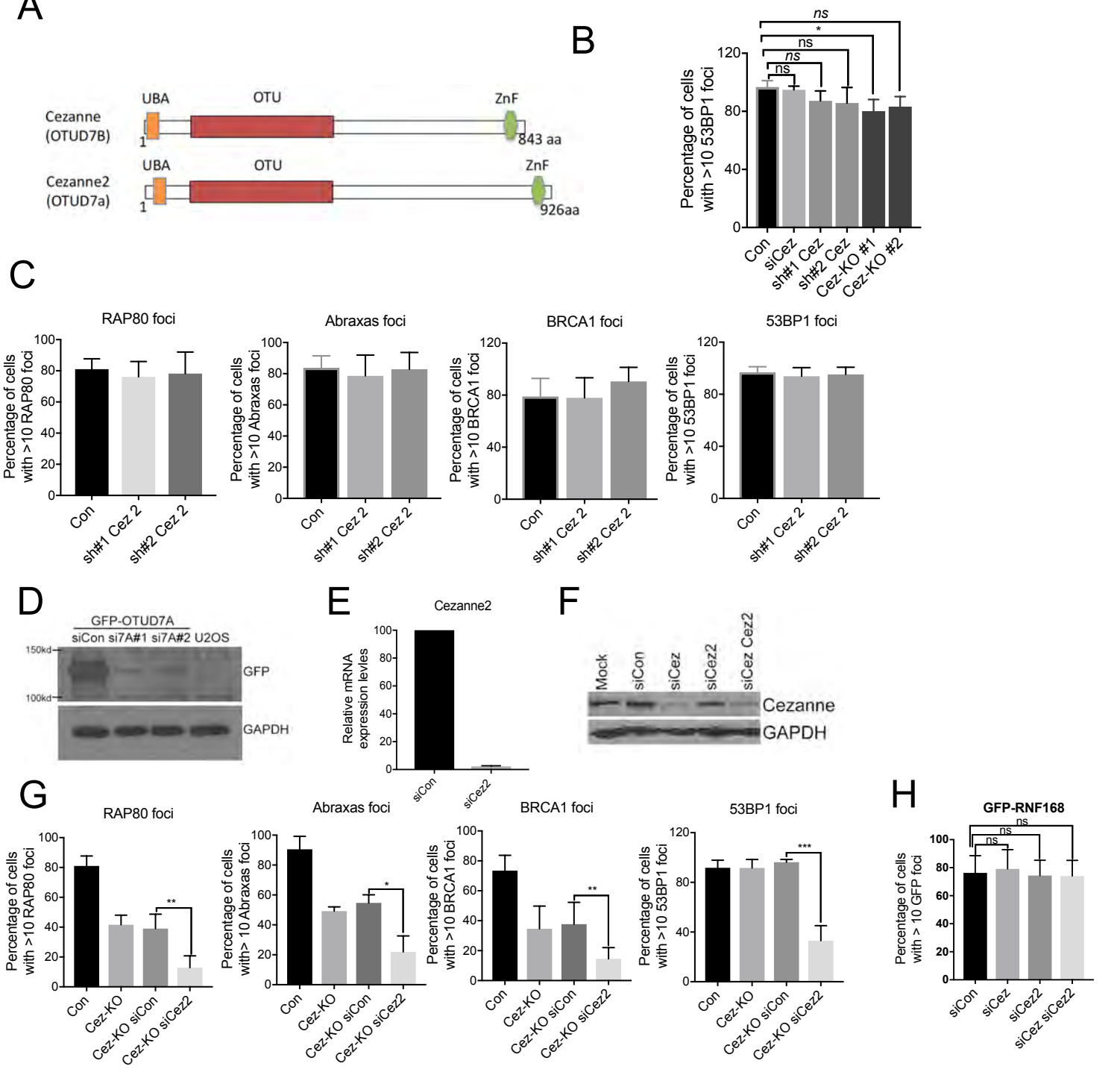


Fig_S6. Cezanne deficiency does not lead to decreased K63 conjugation on damaged chromatin. (A) K63-antibody specificity. Cells were transfected with either vector or HA-tagged ubiquitin K63 mutant which all of the lysine residue except lysine 63 are mutated to arginine (HA-K63). Western blot was carried out with the antibody to K63. **(B)** K63-linkage ubiquitin levels are decreased in Ubc13 siRNA treated cells. Cells not or transiently expressing HA-K63 were treated with control or siRNAs to Ubc13. Both total cell lysate and chromatin fraction were analyzed by western blots with indicated antibodies. **(C)** K63- ubiquitin levels on damaged chromatin are not affected in Cezanne siRNA . Cells were treated with 10 Gy IR, collected at 2h after incubation. **(D)** IF staining with indicated antibodies of control and Cezanne siRNA treated cells treated with 10 Gy IR and incubated for 2h. Student's *t*-test was used for statistical analysis. **(E)** K63- ubiquitin levels on damaged chromatin in cells overexpressing GFP-tagged Cezanne UBA-only, WT or CH mutant. Total lysates or chromatin fraction from cells treated with 10 Gy IR followed by 2h incubation were examined in western blot with indicated antibodies.

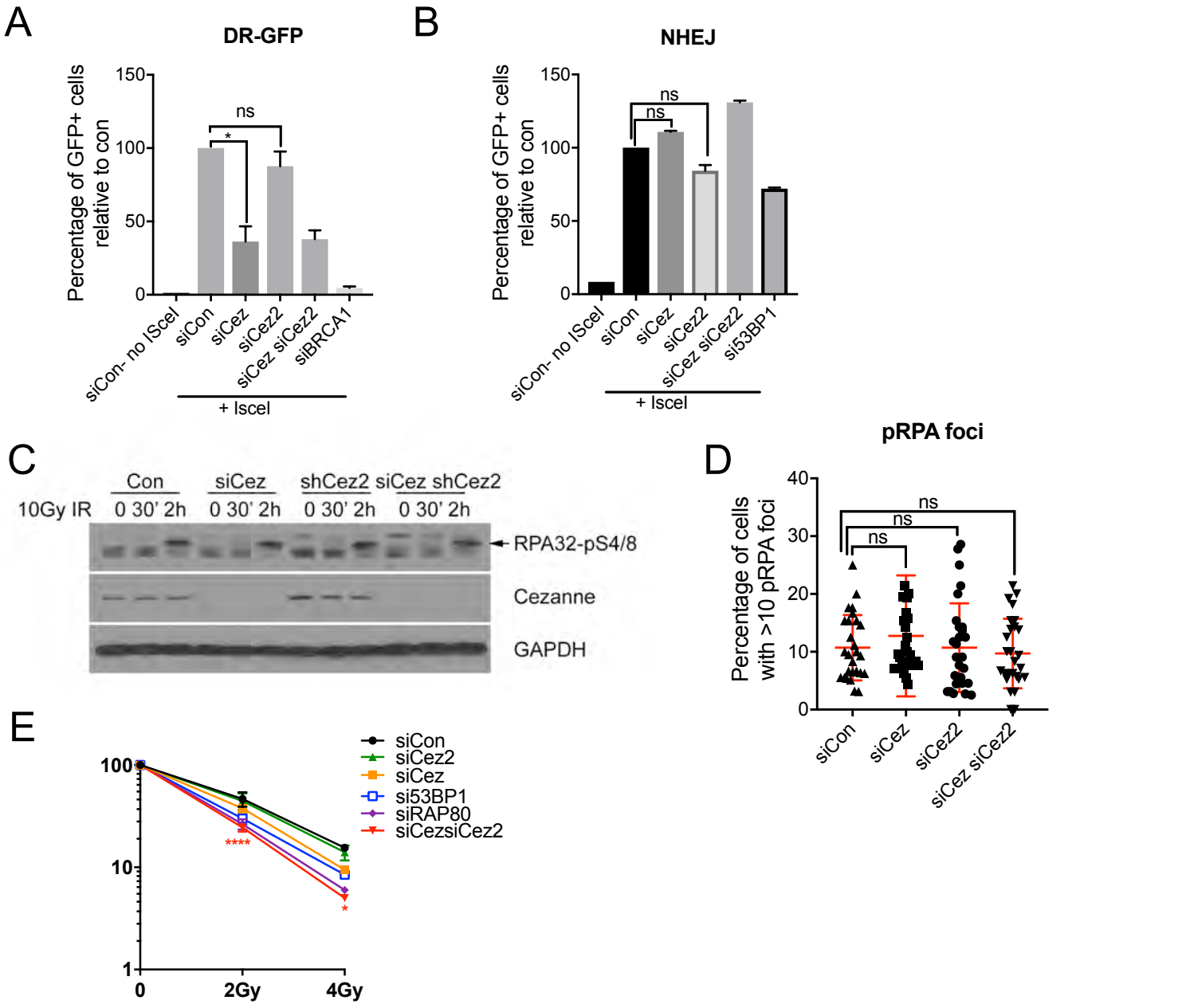


Fig_S7. Cezanne promotes Rap80/Abraxas/BRCA1 recruitment through its K11-linkage DUB activity. (A) Knockdown of Ube2S alone does not have an effect on Rap80/Abraxas/BRCA1 IRIF. U2OS cells were treated with 10 Gy IR followed by 2h incubation before fixation and staining with antibodies to indicated antibodies. Non-parametric Kruskal-Wallis ANOVA was used for statistical analysis. **(B)** Representative images of IF staining of cells overexpressing Ube2S. Cells expressing GFP vector or GFP-Ube2S were treated with 10 Gy IR followed by 2 h incubation before fixation and IF staining

Wu_Supplemental_FigS8



Fig_S8. Cezanne and Cezanne 2 promote recruitment of Rap80 and 53BP1 to DNA damage sites. (A) A diagram of Cezanne and Cezanne2. **(B)** Depletion of Cezanne does not affect 53BP1 IRIF. **(C)** knockdown of Cezanne2 had minimal effect on Rap80, Abraxas and BRCA1 and 53BP1 IRIF. **(D)** Cezanne2 siRNAs depleted GFP-Cezanne2 in U2OS cells expressing GFP-Cezanne2 (OTUD7A). **(E)** RT-PCR of Cezanne2 gene in control and cells depleted with Cezanne2 by siRNAs. (**F**) Western blot of Cezanne in cells depleted of Cez, Cez2 and both. **(G)** Quantification of IRIF of Rap80, Abraxas and BRCA1 in Cezanne KO cells depleted of Cezanne2 by siRNAs. **(H)** RNF168 IRIF is not affected by depletion of Cezanne and Cezanne2. Non-parametric Kruskal-Wallis ANOVA was used for statistical analysis.



Fig_S9. Cezanne promotes HR repair. (A) HR repair efficiency was examined by DR-GFP reporter assay. U2OS/DR-GFP cells were treated with indicated siRNAs. 24 h later, cells were then transiently transfected with plasmid expressing I-SceI. Percentage of GFP+ cells were quantified by flow cytometry 48 h after plasmid transfection. (**B**) NHEJ repair efficiency was examined by EJ-5 reporter assay. U2OS/EJ-5 cells were transfected with indicated siRNAs, then transfected with I-SceI expression plasmid. Percentage of GFP+ cells were quantified using flow cytometry 48h after plasmid transfection. Non-parametric Kruskal-Wallis ANOVA was used for statistical analysis. **(C)**. Phosphorylated RPA32 levels in U2S cells treated with indicated siRNA and shRNAs. Cells were treated with 10 Gy IR followed by incubation at 37°C. Total cell lysate were prepared from cells collected at 30 min or 2h after IR. RPA32pS4/8 levels were detected by western blot with indicated antibodies. **(D)** Phosphorylated RPA32pS4/8 foci were quantified from U2OS cells treated with indicated siRNAs. Cells were treated with 10 Gy IR and fixed at 2h after IR. IF staining was carried out with pRPA32-S4/8 antibody and appropriate secondary antibodies. Non-parametric Kruskal-Wallis ANOVA was used for statistical analysis. (**E**) Colony formation assay of cells treated with indicated siRNAs. Two-way ANOVA statistical analysis was performed to compare the deficiency between siCez (orange) with siCezsiCez2 (red)at indicated doses.