Ubiquitin-Binding Domains Array 2.0

CUE A1) AMFR A2) ASCC2 A3) AUP1 A4) CUEDC1 A5) CUEDC2 A6) SMARCAD1(1-2) A7) TAB2 A8) TAB3 A9) TOLLIP	JAB/MPN B1) MPND B2) MYSM1 B3) PRPF8 B4) PSMD14 B5) PSMD7 B6) STAMBPL1 B7) AMSHLP B8) AMSH	UBA-like C1) C6orf106 C2) Cezanne/OTUD7B C3) DCUN1D1 C4) DCUN1D2 C5) FAM100A/UBALD1 C6) FAB100B/UBALD2 C7) NSFL1C C8) OTUD7A C9) TTRAP/TDP2 C10) USP25		A B C D E F G H I J K L M N 2 6 12 2 3 1 10 4 11 12 9 6 M 7 10 7 8 11 3 5 1 5 9 4 8
UBA D1) LATS1 D2) LATS2 D3) MARK1 D4) blank D5) MARK3 D6) MARK4 D7) NACA D8) NACA2 D9) NBR1 D10) NICE4/UBAP2L D10) NICE4/UBAP2L D11) NYREN18/NUB1(1-3) D12) p62/SOSTM1	UBA E1) RAD23A(1-2) E2) RAD23B(1-2) E3) RHBDD3 E4) STS2/UBASH3A E5) TNRC6C E6) UBAC(1-2) E7) UBAC2 E8) UBE2K E9) UBL7 UBA-like+UBA E10)TNK1 UBA-LIM E11)HUWE1	UBA F1) UBAP2 F2) UBQLN1 F3) UBQLN2 F4) UBQLN3 F5) UBQLN4 F6) UBXN1 F7) UBXN7 F8) USP13(1-2) F9) USP24 F10)USP5(1-2) F11)VPS13D UBA-like+UIM F12)USP25	UBA G1) CBL G2) CBLB • • G3) EFTS/TSFM G4) ETTEA/FAF2 G5) FAF1 G6) TDRD3 • UBA+SH3 G7) STS-1/UBASH3B UBA+Tudor G8) TDRD3 •	
UIM H 1) ANKIB1 H 2) ANKRD13/ANKRD13A(1-4) H 3) ANKRD13D(1-4) H 4) Ataxin-3/ATXN3L(1-2) H 5) Ataxin-3/ATXN3(1-3) H 6) DNAJB2(1-2) H 7) EPS15(1-2) H 7) EPS15(1-2) H 8) Epsin-1/EPN1(1-3) H 9) Epsin-2/EPN2(1-2) H 10) Epsin-3/EPN3(1-2) H 11) PSMD4(1-2) H 12) RAP80/UIMC2(1-2)	UIM 11) RNF166 12) UBXN7 13) USP25(1-2) • • 14) USP37(1-3) • • 15) ZFAND2B(1-2) • • 16) Hrs/HGS • • 17) DNMT1 18) STAM2 • • UIM+SH3 19) STAM1/STAM • 110)STAM2 • • UIM+UBX 111) UBXN7	GAT J1) GGA1 J2) GGA2 J3) GGA3 J4) Srcasm/TOM1L1 J5) TOM1 J6) TOM1L2 <u>VHS+GAT</u> J7) GGA1 J8) GGA2 J9) GGA3 J10) Srcasm/TOM1L1 J11) TOM1 J12) TOM1L2	VHS K1) GGA2 K2) GGA3 K3) Hrs/HGS K4) Srcasm/TOM1L1 K5) STAM1/STAM K6) STAM2 K7) TOM1 K8) TOM1L2 VHS+UIM+SH3 K9) STAM1/STAM K10)STAM2	
UMI+MIU L1) RNF168(UMI+MIU1) • • MIU L2) RNF168(2) • L3) RNF169(2) • PEU L4) PLAA UBX L5) ETEA/FAF2 L6) FAF1 L7) NSFL1C L8) UBXN1 L9) UEVLD MIU/UBD L10) UEVLD MIU/UBD L11) MINDY1 • L12) MINDY2 •	ZnF-UBZ M1) TAX1BP1(1-2) ZnF-UBP M2) USP13 M3) USP20 M4) USP3 M5) USP33 M6) USP39 M7) USP44 M8) USP49 M9) BRAP2 M10) USP5 USP M11) USP30 Znf M12) TRABID	UBM N1) hPol iota (1-2) N2) REV1L UBZ N3) hPol eta N4) hPol kappa (1-2) N5) PAD18 N6) SPARTAN Other N7) hRPN13/ADRM1 FL N8) SNF5 UBZ N9) WRNIP1	Robust Binders K63-Ub4 K48-Ub4	

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-ubiquitin 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 HAtagged 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 Δ UBA or Δ ZF, 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) K63antibody 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_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 48 h 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 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.