SUPPLEMENTAL MATERIAL

Supplemental Materials and Methods

Vector construction, protein purification and analysis. Oligonucleotides and primers used to generate recombinant GST-tagged BAL1 and FLAG-tagged PARP1 proteins are included in Tables S1 and S3. Using pFLAG-CMV2-BAL1 as template (2), BAL1 was PCR-amplified with primers containing Sal I and Not I restriction sites (Table S3). The Sal I- and Not I-digested BAL1 PCR product was then ligated into the vector, pGEX-4T-2 (GE Healthcare Bio-Sciences Corp., Piscataway, NJ), to generate pGEX-BAL1. Using pGEX-BAL1 as template, the double mutation BAL1 construct (DM) was generated by introducing the IE326,327AA (IE-AA) and D126A mutations into the pGEX-BAL1 vector with the indicated oligonucleotides (Table S1) and the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies). The pGEX-BAL1 recombinant proteins were generated and purified as previously described (1). Purified BAL1 and PARP1 recombinant proteins were subjected to NuPAGE and analyzed by Coomassie Blue staining.

Human PARP1 cDNA was purchased from OriGene Technologies (#SC119157). Using the PARP1 cDNA as a template, FLAG-tagged PARP1 was PCR-amplified with the indicated primers in Table S3. The resulting PCR product was ligated into pET101 following the manufacturer's instructions (Invitrogen). FLAG-PARP1 was purified using the FLAG immunoprecipitation kit according to the manufacturer's instructions (Sigma). Purified PARP1 recombinant proteins were size-fractionated and analyzed by Coomassie Blue staining.

Subcellular localization of GFP-proteins. Following transient transfection with GFP-BAL1 constructs (Fig. S1), 293T cells were subjected to subcellular fractionation (Nuclear Extraction kit; Active Motif). Nuclear and cytoplasmic fractions were size-fractionated on NuPage gels (Invitrogen) and immunoblotted with anti-GFP (#G10362, Invitrogen), and antibodies directed against nuclear (lamin B) and cytoplasmic (β-tubulin) proteins,

anti-Lamin B (#20682, Santa Cruz Biotechnology) and anti-β-tubulin (#5274, Santa Cruz Biotechnology).

BAL2 and BAL3 analyses. U2OS cells were grown on coverslips, subjected to laser microirradition and analyzed for BAL2 and BAL3 recruitment by indirect immunofluorescence with anti-BAL2 (#ab101010, Abcam) and anti-BAL3 (#H00165631-B01, Abnova) antibodies. U2OS whole-cell lysates were also size-fractionated and immunoblotted for BAL2 and BAL3 using these antibodies.

***In vitro* PARP activity assays.** *In vitro* poly(ADP-ribose) activity assays were carried out as previously described with minor modifications (1). Reactions including ≈ 200 ng of FLAG-tagged PARP1 and 0, 0.5 or 1mM of NAD+ (Roche Applied Science, Indianapolis, IN) substrate were incubated at 25 °C for 30 min in assay buffer (0.1 ml) containing 50 mM Tris-HCl, pH 8.0, 4mM MgCl2, 0.2mM dithiothreitol, 200 ng of activated DNA (Sigma). In certain assays, the PARP inhibitor, PJ-34 (Sigma), was included at 1 mM final concentration. Reactions were stopped by the addition of 20% trichloroacetic acid. Precipitated proteins were rinsed once in 5% trichloroacetic acid, suspended in SDS loading buffer, and fractionated by NuPAGE (Invitrogen). After size fractionation, the proteins were subjected to immunoblotting with anti-PARP1 antibody (mouse monoclonal antibody, BD Biosciences, #51-6639); and anti-PAR (Trevigen, #4335).

GST-pulldown. For GST-pulldown in Figure S3F and G, GST-BAL1 or –BAL1 mutant protein (1 µg) was immobilized on glutathione-Sepharose 4B beads and incubated with PARP assay samples (above) with or without NAD+ in 1 ml of TBST buffer (20 mM Tris [pH 7.0], 200 mM NaCl, 1% NP-40, 1 mM dithiothreitol [DTT], and 0.5 mM EDTA) for 5 min at 4°C on a rotating wheel. After 5 washes with TBST buffer, the samples were size-fractionated and immunoblotted with anti-PARP1 (BD Biosciences) and anti-PAR (Trevigen) antibodies.

***In vitro* Ubiquitylation assay.** For vector construction, the previously described human BBAP cDNA was utilized (3). Using the BBAP cDNA as a template, BBAP was PCR-amplified with the indicated primers in Table S3. The resulting PCR product was ligated into pET101 following the manufacturer's instructions (Invitrogen). His6-tagged BBAP was purified using the Ni-NTA Spin

Kit (Qiagen) according to the manufacturer's instructions. Purified BBAP-his6 recombinant protein was size-fractionated, analyzed by Coomassie Blue staining and utilized in the *in vitro* ubiquitylation assay which was performed as previously described (3). Using recombinant pET-BBAP-his6 wild type with minor modifications to the method, wild type ubiquitin, lysine 48 (K48)-only ubiquitin and lysine 63 (K63)-only ubiquitin were purchased from Boston Biochem (Cambridge, MA 02139). For immunoblotting of specific ubiquitin-chain, anti-ubiquitin-K48 rabbit monoclonal antibody (05-1307) and anti-ubiquitin-K63 rabbit monoclonal antibody (05-1308) were purchased from Millipore.

SUPPLEMENTAL REFERENCES

1. **Aguiar, R. C. T., K. Takeyama, C. He, K. Kreinbrink, and M. Shipp.** 2005. B-aggressive lymphoma (BAL) family proteins have unique domains which modulate transcription and exhibit PARP activity. J Biol Chem **280:**33756-33765.
2. **Takeyama, K., R. C. T. Aguiar, L. Gu, C. He, G. J. Freeman, J. L. Kutok, J. C. Aster, and M. A. Shipp.** 2003. The BAL-binding protein, BBAP, and related Deltex family members exhibit E3 ubiquitin ligase activity. J Biol Chem **278:**21930-21937.
3. **Yan, Q., S. Dutt, R. Xu, K. Graves, P. Juszczynski, J. P. Manis, and M. A. Shipp.** 2009. BBAP monoubiquitylates histone H4 at lysine 91 and selectively modulates the DNA damage response. Mol Cell **36:**110-120.

SUPPLEMENTAL FIGURES

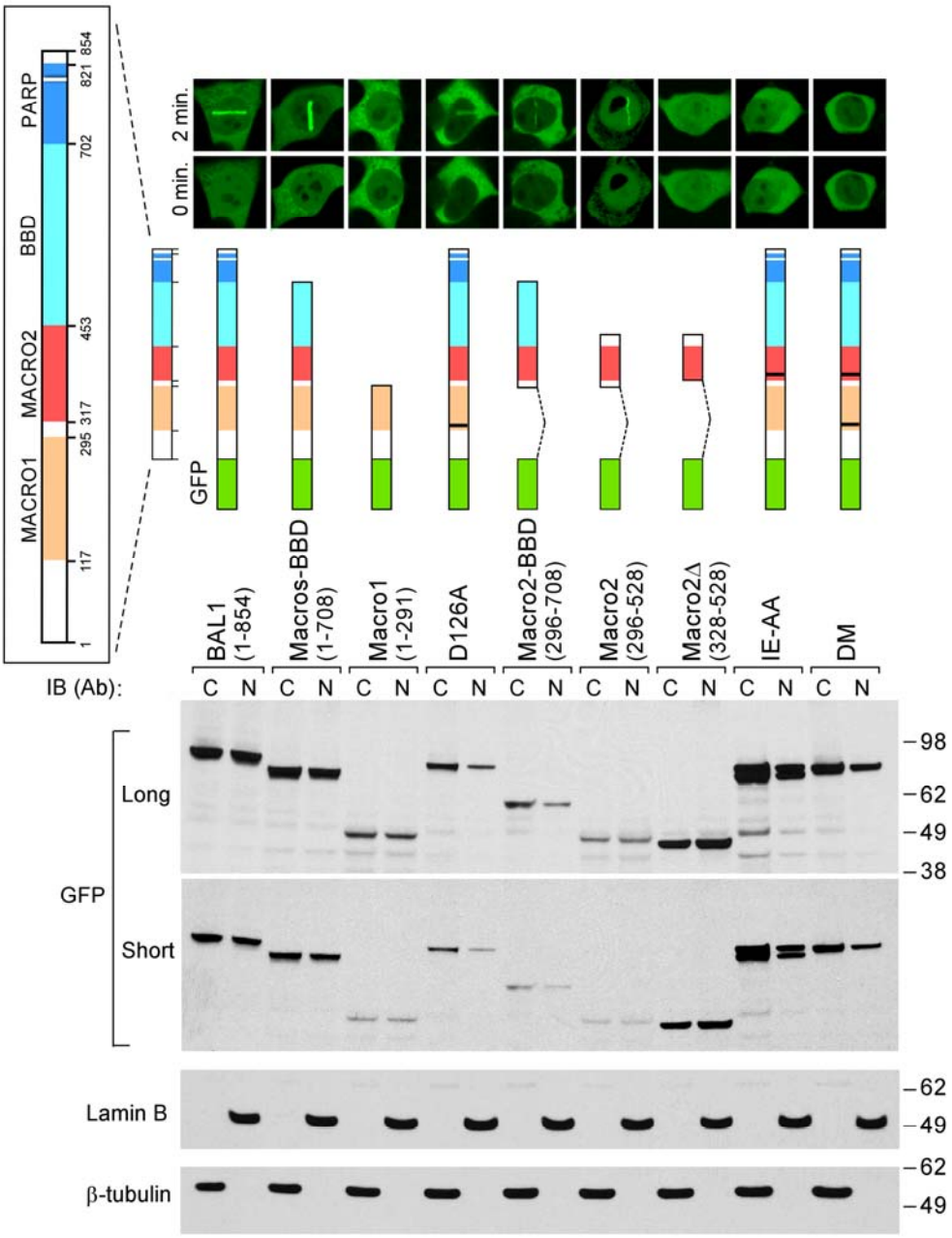


FIG. S1 Subcellular localization of GFP BAL1 domain-specific proteins. Top panel) Recruitment of BAL1 domain-specific proteins to DNA damage sites. BAL1 protein functional domains, including macro domains 1 and 2) (orange and red), BBAP binding domain (BBD, light blue in BAL1 [2]) and region with partial sequence homology to PARP catalytic domain (dark blue) (domain sizes and aa below). GFP-BAL1 constructs are labeled and represented below, left. Mutations in macro domain 1 (D126A) and macro domain 2 (IE 326, 327 AA [IE-AA]) in black. Representative images of GFP-BAL1 (293T cell) transfectants at baseline and 2 min following laser microirradiation, right**.** Assays were performed as in Figure 2, main manuscript. 293T cells were transfected with GFP-tagged BAL1 constructs for 24 hours. Bottom panel) Subcellular localization of GFP-tagged BAL1 domain-specific proteins. Cytosolic (C) and nuclear (N) fractions were obtained, size fractionated, and analyzed by immunoblotting with anti-GFP, anti-lamin B (nuclear protein control), and anti-β-tubulin (cytoplasmic protein control).

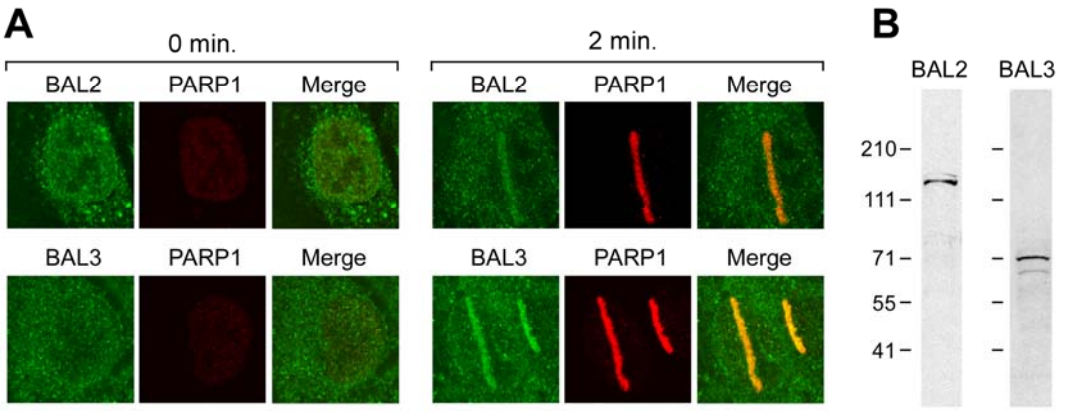


FIG. S2 BAL2 and BAL3 are recruited to DNA damage sites. A) Co-localization of PARP1 and BAL2 (top panel) or PARP1 and BAL3 (bottom panel) at laser-induced DNA breaks in U2OS cells. Images obtained at baseline and 2 min following laser-microirradiation. Endogenous BAL2 or BAL3 (FITC); PARP1 (cy5); and merged images. B) Confirmation of the specificity of the anti-BAL2 and BAL3 antibodies. U2OS whole-cell lysates were obtained, size-fractionated and analyzed by immunoblotting with anti- BAL2 (#ab101010, Abcam) and anti-BAL3 (#H00165631-B01, Abnova) antibodies to confirm specificity. MWM, right.

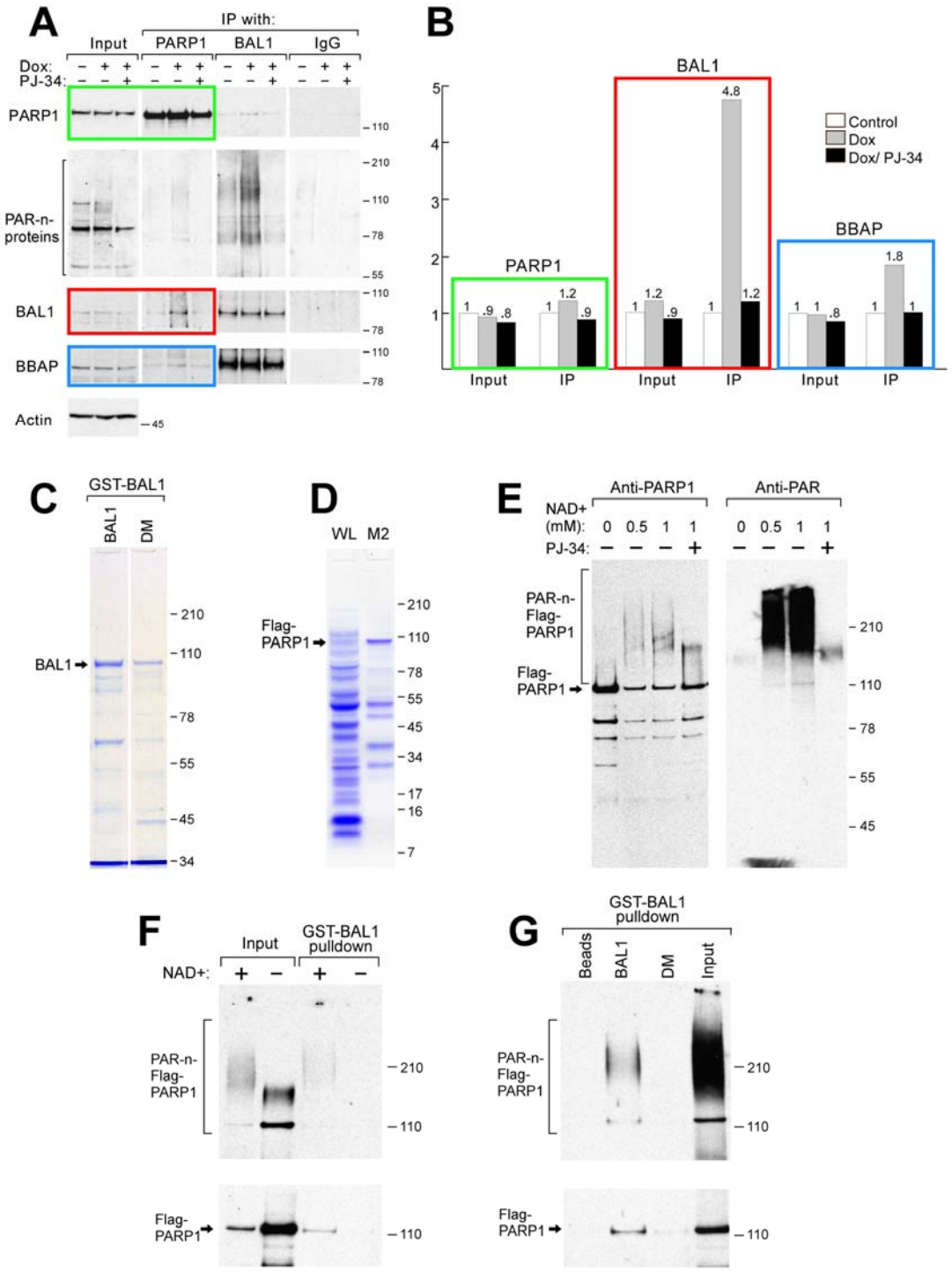
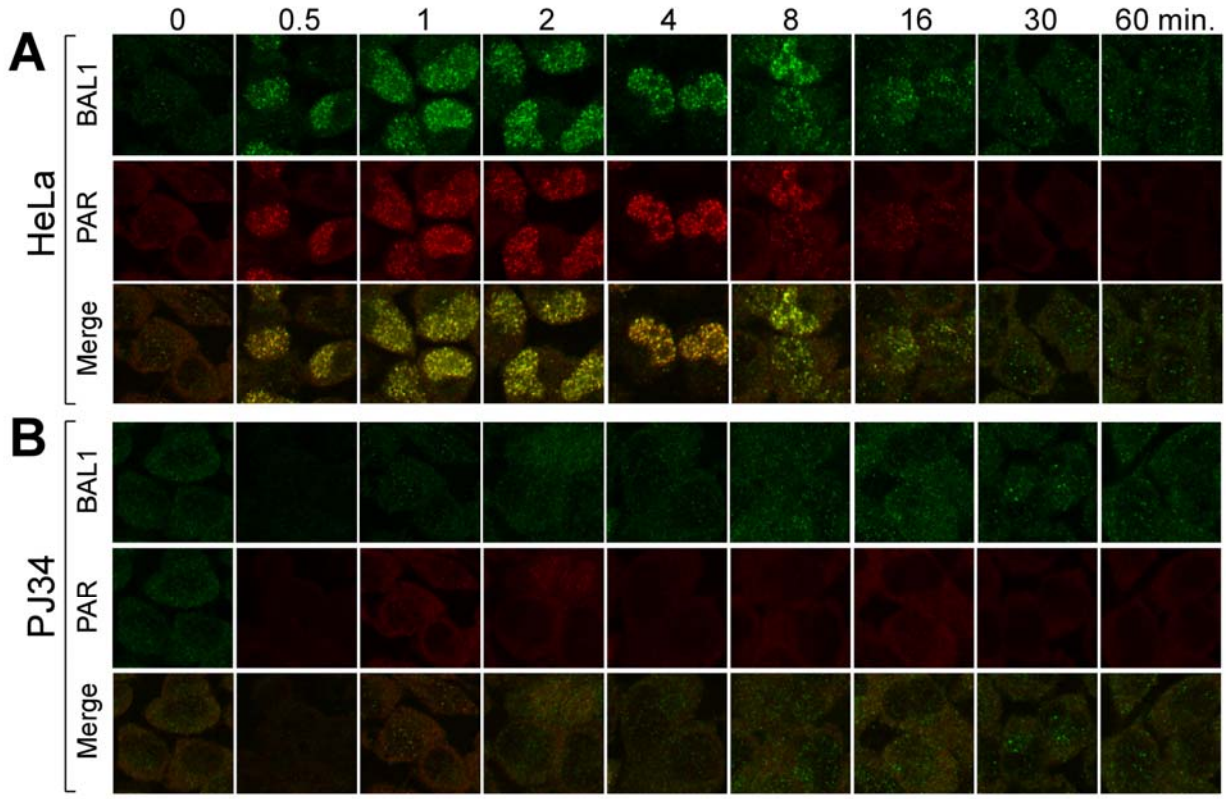


FIG. S3 Analyses of co-immunoprecipitated PARP1, BAL1 and BBAP and the interaction between GST-BAL1 and PAR. A) Co-immunoprecipitation of PAR-n- protein and BAL1 (Fig. 3D from main manuscript). Hela cells were untreated or treated with low-dose Dox (50 ng/ml) for 10 min with or without PJ-34 pretreatment. Cell lysates were immunoprecipitated (IP) with anti-PARP1, anti-BAL1 or control IgG and immunoblotted with anti-PARP1, -PAR, -BAL1 or -BBAP antibodies. Input whole cell lysates (left panel) were similarly analyzed and immunoblotted for actin as a loading control. Molecular weight markers, right. B) Scanning densitometric analysis of PARP1 immunoprecipitates in untreated cells and cells treated with Dox alone or Dox following PJ-34 pretreatment. PARP1, green box. BAL1 co-immunoprecipitated with PARP1, red box.

BBAP, blue box. There was only a modest increase in PARP1 immunoprecipitated following Dox treatment (1X control, 1.2X Dox-treated cells). In contrast, there was a 4.8 fold increase in BAL1 and a 1.8 X increase in BBAP co-immunoprecipitated with PARP1 in Dox-treated cells. Of importance, chemical PARP inhibition (PJ-34 pretreatment) decreased the co-immunoprecipitation of BAL1 and BBAP with PARP1 in Dox-treated cells. C) Recombinant GST-BAL1 proteins (BAL1 full length and DM [IE-AA and D126A] mutants [see schema, Fig. 1F, main manuscript]). Proteins were synthesized, size-fractionated by NuPAGE and analyzed by Coomassie Blue staining. D) FLAG-PARP1. FLAG-PARP1 (M2) was purified from whole cell lysate (WL), size-fractionated by NuPAGE and analyzed by Coomassie Blue staining. E) Immunoblotting of PAR-n-proteins following PARP1 activation *in vitro*. *In vitro* assays included ≈ 200ng purified FLAG- PARP1 and 0, 0.5 or 1mM NAD+. Assays were performed in the presence (+) or absence (-) of the PARP inhibitor, PJ-34. Thereafter, samples were size-fractionated and immunoblotted with anti-PARP1 and anti-PAR antibodies. FLAG-PARP1 was ADP-ribosylated in a dose-dependent manner by NAD+; this enzymatic activity was inhibited by PJ-34, confirming its specificity. F) Pulldown of PAR-n-FLAG-PARP1 by GST-BAL1. GST-BAL1 (1ug) was immobilized on glutathione-sepharose 4B beads and incubated with products of *in vitro* PARP1 activation (+ or – NAD+, as in E). After multiple washes, GST-BAL1 bound proteins were size-fractionated and immunoblotted with anti-PAR and anti-PARP1 antibodies (upper and lower panels, respectively). The products of the *in vitro* PARP1 assay (Input, + or - NAD+) were similarly analyzed. GST-BAL1 selectively pulled down PAR-modified PARP1 but did not bind unmodified PARP1 protein. G) Pulldown of PAR-n-FLAG-PARP1 by GST-BAL1 mutants. Assays including GST-BAL1, or -BAL1 DM were performed as in (F). GST-BAL1 pulled down PAR-modified PARP1 in a macro domain-dependent manner.



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145 FIG. S4 Co-localization of BAL1 and PAR foci in γ-irradiated control Hela cells (A), and Hela cells
146 pretreated with PJ-34 (B). Hela cells were treated with low-dose irradiation (100cGy) and
147 analyzed for BAL1 and PAR foci at baseline and at serial timepoints (0.5 – 60 min) thereafter.
148 BAL1, top; PAR, middle; merged images, bottom.

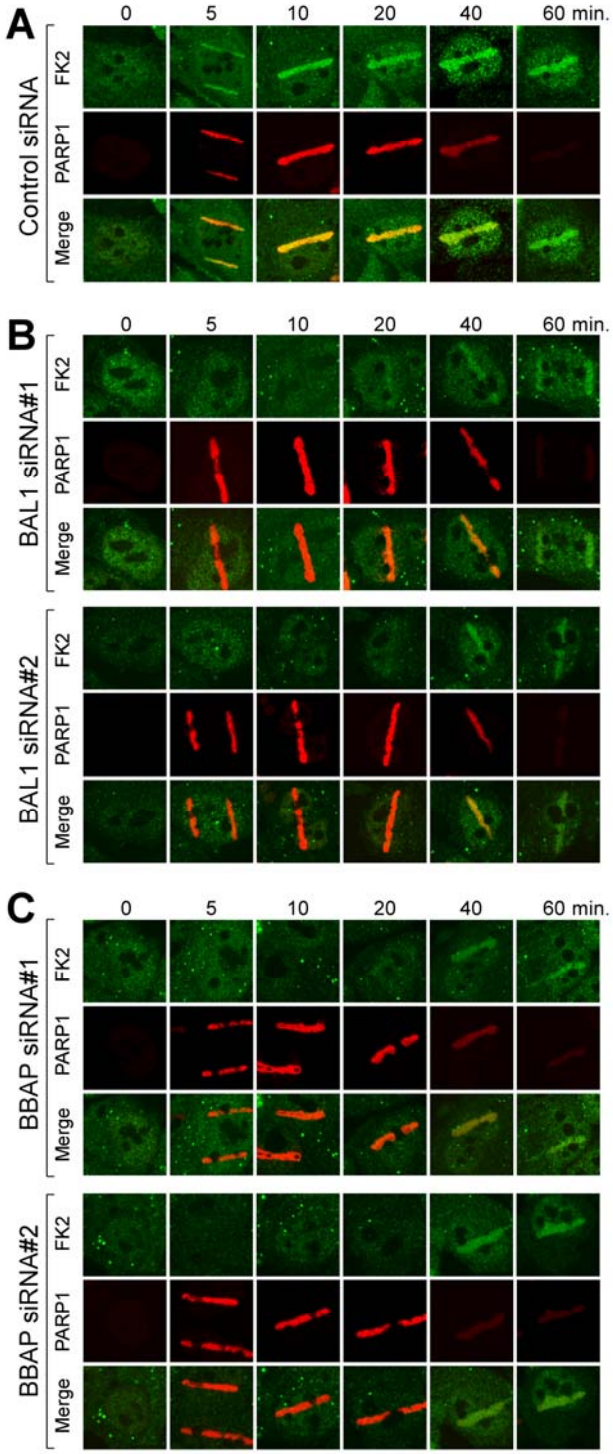


FIG. S5 PARP-dependent recruitment of BAL1 and BBAP to DNA damage sites is required for early ubiquitin chain formation. Ubiquitylation (conjugated ubiquitin, FK2 immunostaining) and PARP1 recruitment in laser-microirradiated control cells (A) or cells depleted of BAL with 2 independent siRNAs (B) or cells depleted of BBAP with 2 independent siRNAs (C) at serial timepoints (0 – 60 min) following DNA damage.

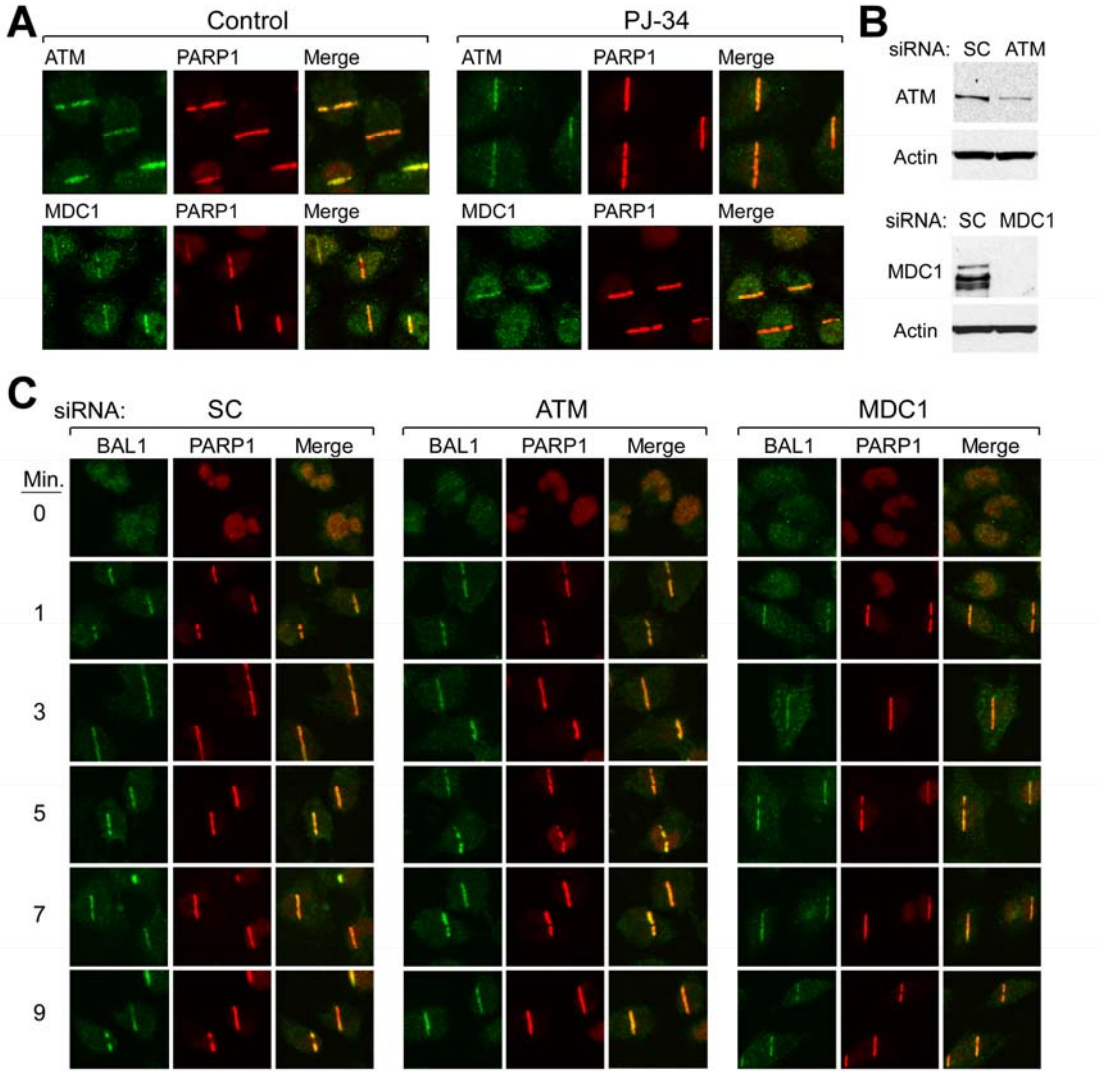


FIG. S6 PARP1 activation and BAL1/BBAP recruitment to DNA damage sites are independent of ATM and MDC1. A) Recruitment of PARP1, ATM and MDC1 to laser-induced breaks in control or PJ-34 pretreated cells (5 min following laser microirradiation). B) Depletion of ATM or MDC1 following siRNA. Hela cells treated with a scrambled control (SC) or ATM or MDC1 siRNA were lysed, size-fractionated and immunoblotted with the respective antibodies and actin (as a loading control). C) Recruitment of BAL1 and PARP1 to laser-induced breaks in control cells or cells depleted of ATM or MDC1. Assays were performed and analyzed at serial timepoints following laser microirradiation (baseline [0] and 1-9 min).

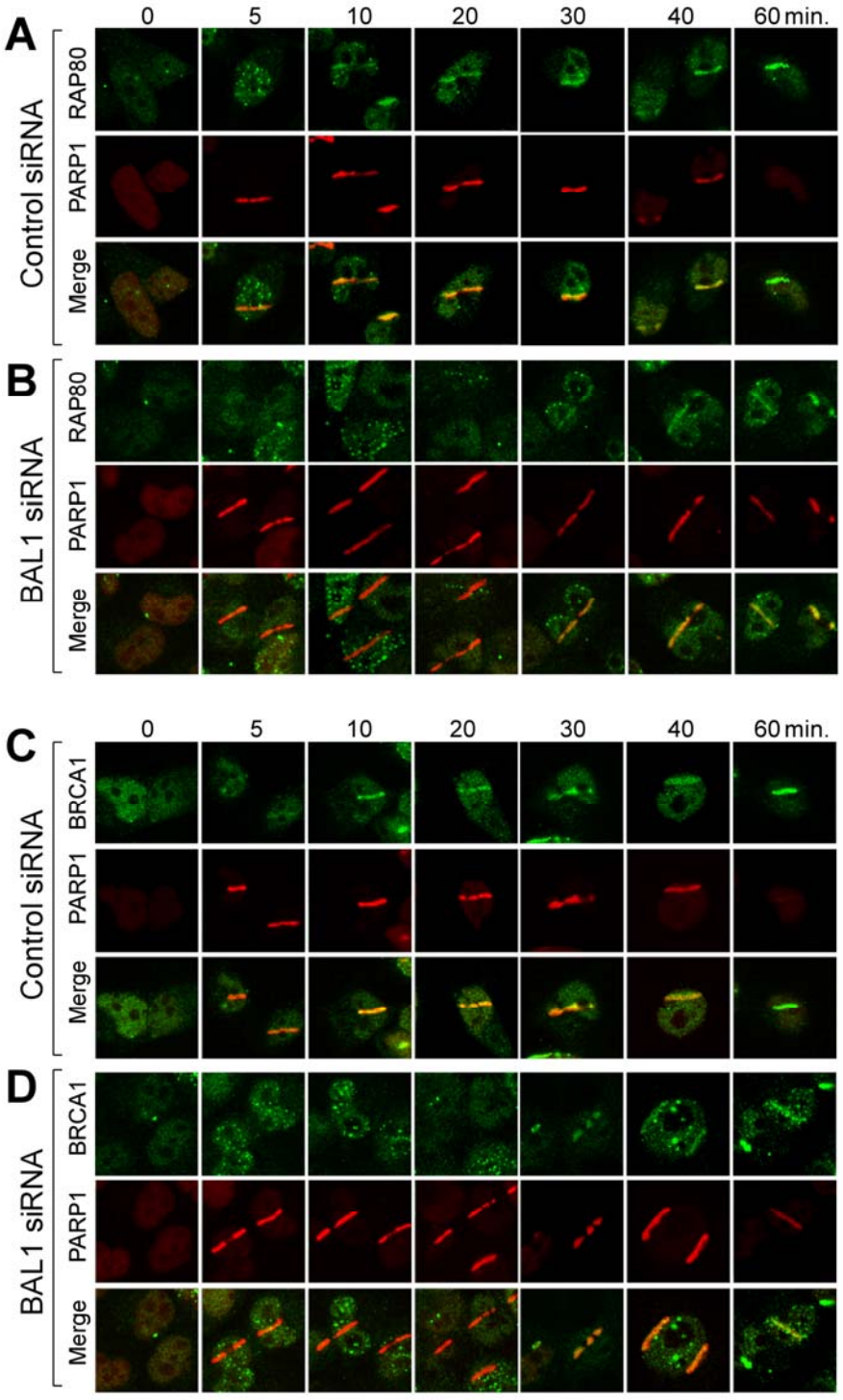
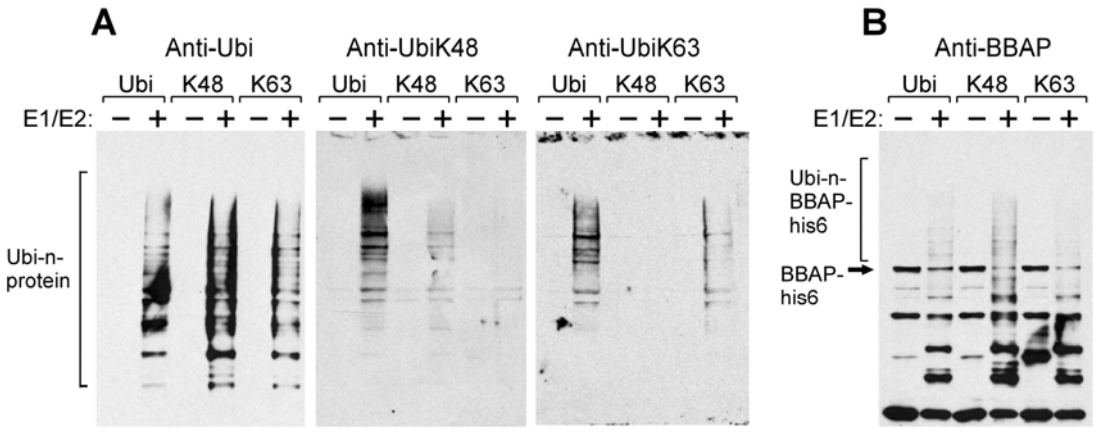


FIG. S7 Kinetics of recruitment of RAP80/BRCA1 and PARP1 to laser-induced breaks. (A, B) RAP80 or (C, D) BRCA1 and PARP1 localization in laser-induced breaks in control cells (A and C) or cells depleted of BAL1 (via siRNA) (B and D). Assays were performed as in Figure 8, main manuscript, and analyzed at serial timepoints following laser microirradiation (baseline [0] and 5-60 min).



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172 FIG. S8 *In vitro* analysis of BBAP ubiquitylation using lysine- specific ubiquitin. BBAP-his6 was
173 purified from E. coli. and was incubated with or without E1/E2 ligase and wild-type ubiquitin, K48-
174 only ubiquitin, or K63-only ubiquitin. Thereafter, samples were size-fractionated and
175 immunoblotted with the following antibodies: anti-ubiquitin, anti-ubiK48, anti-ubiK63 (A) and anti-
176 BBAP (B).
177

Table S1: Oligonucleotides and primers used to generate GFP-BAL1, GFP-BBAP and GFP-PARP1 constructs.

Vector name	Pair of Oligos or primers	Location* (bp)	Wild type (bp)
GFP-BAL1	5'_atggacttttccatggtggccggag_3'	104-128	
	5'_ttaatcaacagggctgccacttg_3'	2563-2541	
Macros-BBD	5'_gtttcagcaagtcccataAcagttctgcaatgtggt_3'	2336-2471	C nt2454
	5'_accacattgcagaactgTtatgggacttgctgaaac_3'	2471-2336	G
Macro1	5'_gggaagagtgagctgggacaaTaaaccacccttctttc_3'	1216-1255	C nt1202
	5'_gaaagaaggggtggtttattgtcccagctcactcttccc_3'	1255-1216	G
Marco2-BBD	5'_ctagggaagagtgagctgggacaagaaacc_3'	1213-1242	
	5'_accacattgcagaactgttatgggacttgctgaaac_3'	2471-2336	G nt2454
Marco2	5'_ctagggaagagtgagctgggacaagaaacc_3'	1213-1242	
	5'_ggatccatgcgtgggcctAttacatctcttccacgttg_3'	1930-1893	C nt1913
Macro2Δ	5'_tggcagacggcagatgtaattgtt_3'	1309-1332	
	5'_ggatccatgcgtgggcctAttacatctcttccacgttg_3'	1930-1893	C nt1913
D126A	5'_cagtctggaaagatgCcctcaccacacatgctg_3'	689-721	A nt704
	5'_cagcatgtgtggtgaggGcatctttccagactg_3'	721-689	T
IE326,327AA	5'_gtccagggccacGCtgCatggcagacggcagatg_3'	1291-1324	AT nt1303,nt1304, A nt1307
	5'_catctgccgtctgccatGcaGCgtggccctggac_3'	1324-1291	T, TA
GFP-BBAP	5'_atggcctcccacctgcgcccgccgtc_3'	90-115	
	5'_ttactcaattcctttggctttcagctc_3'	2312-2285	
GFP-PARP1	5'_atggcggagtcttcggataagctc_3'	172-295	
	5'_ttaccacagggaggtcttaaaattg_3'	3216-3192	

Mutated bases in the oligonucleotides are capitalized and their positions indicated (right column).

* NCBI reference access number: BAL1, NM_031458.2; BBAP, NM_138287.3; PARP1, NM_001618.3.

Table S2: **BAL1, PARP1, ATM, MDC1 and RNF8 siRNAs**

Gene*	RNA sequences	Location (bp)	3' DNA overhanging
BAL1 siRNA#1	5'_GCCCACGCAUGGAUCCAAAGAAU_3'	1915-1937	CC
	5'_GGAUUCUUUGGAUCCAUGCGUGGGCCU_3'	1939-1913	
BAL1 siRNA#2	5'_CCCAUACCAGUUCUGCAAUGUGG_3'	2448-2470	TA
	5'_UACCACAUUGCAGAACUGGUAUGGGAC_3'	2472-2446	
BAL1 siRNA#3**	5'_ GGAAGUAGCUCUCCAGCUUCCUU _3'	160-184	CG
	5'_CGAAGGAAGCUGGAGAGCUACUUCCAG _3'	186-158	
PARP1 siRNA	5'_CCAAAGGAAGGAACGCUAACAAU_3'	3758-3780	TT
	5'_AAAUUGUUAGCGUUCCUUCCUUUGGUC_3'	3782-3756	
ATM siRNA	5'_AGCUAUCAGAGAAGCUAAUAAAU_3'	12710-12732	TA
	5'_UAAUUUAUUAGCUUCUCUGAUAGCUUC_3'	12734-12708	
MDC1 siRNA	5'_CCACUAGGAGAAAGACAAAUAGG_3'	5350-5372	TC
	5'_GACCUAUUUGUCUUUCUCCUAGUGGCC_3'	5374-5348	
RNF8 siRNA	5'_CCCUUGUACAUAUAUCUUUAGAG_3'	3662-3684	AG
	5'_CUCUCUAAAGAUAUAUGUACAAGGGUG_3'	3686-3660	

* NCBI reference access number: BAL1, NM_031458.2; PARP1, NM_001618.3; ATM, N000051.3; MDC1, NM_014641.2; RNF8, NM_003958

** 5'-UTR specific BAL1 siRNA

Table S3: Oligonucleotides and primers used to generate recombinant BAL1 and PARP1 proteins in E. coli.

Vector name	Pair of Oligos or primers	Location (bp)
BBAP-his6	5'_caccatggcctcccacctgcgcccgccgtc_3'	90-115
	5'_ctcaattcctttggctttcagctc_3'	2312-2285
GST-BAL1*	5'_acccgGTCGACatggacttttccatggtggccggag_3'	104-128
	5'_caccGCGGCCGCttaatcaacagggctgccacttg_3'	2563-2541
FLAG-PARP1**	5'_caccATGGACTACAAGGATGACGATGACAAGatggcggagtcttcggataagctc_3'	172-295
	5'_ttaccacagggaggtcttaaaattg_3'	3216-3192

* The RE sites are capitalized.

** The DNA sequence encoding FLAG tag is capitalized.