Supporting Information

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SI Methods

Size Exclusion Chromatography. Flag-immunopurified F-Alc1 (≈35 μg in 100 μL) from HEK293/FRT cells was fractionated on a Superose6 sizing column using a SMART FPLC microSeparation system. The column was equilibrated in 40 mM HEPES-NaOH, pH 7.9, 0.1 M NaCl, 0.1 mM EDTA, 10% glycerol, and was eluted using the same buffer at a flow rate of 50 μL/min. Fifty-microliter fractions were collected and analyzed on silver-stained polyacrylamide gels and western blotting before use in assays.

Purification of F-Alc1 from HEK293T Cell Whole Cell Extracts. To purify F-Alc1 and associated proteins from whole cell extracts, cells were washed with PBS and then lysed by resuspension in 1 mL/dish 40 mM HEPES-NaOH, pH 7.9, 0.2 M NaCl, 1.5 mM $MgCl_2$, 10% glycerol, 1 mM DTT, 0.2% Triton X-100. The resulting suspension was incubated with rotation at 4 °C for 30 min and spun at 40,000 rpm for 60 min at 4 °C in a 70.1 Ti rotor (Beckman-Coulter). Supernatants were subjected to anti-FLAG agarose chromatography as described in the *Materials and Methods*, except that beads were washed with buffer containing 0.2 M NaCl.

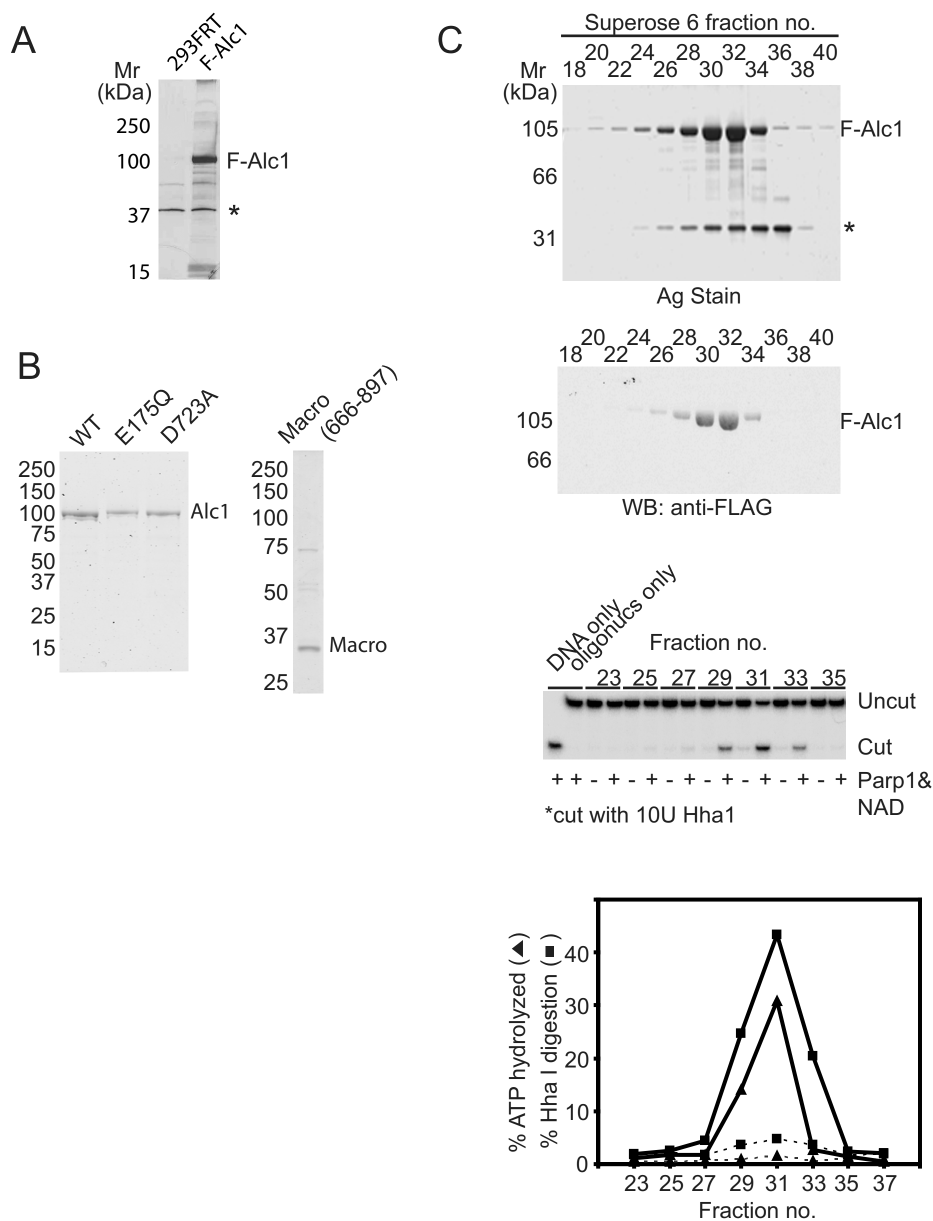


Fig. S1. (*A*) SDS/PAGE analysis of F-Alc1 from HEK293/FRT cells. Aliquots of Flag-immunoprecipitates from equivalent amounts of nuclear extract from HEK293/FRT cells or HEK293/FRT cells stably expressing F-Alc1 were analyzed by SDS/PAGE and silver staining. In this panel and in Fig. S2C, the asterisk denotes a protein that nonspecifically binds and is eluted from Flag agarose beads. (*B*) SDS/PAGE analysis of recombinant wild-type and mutant versions of Alc1, expressed in insect cells using baculovirus expression system and purified by Flag-immunopurification. (*C*) Co-purification of ATPase and nucleosome remodeling activities with Alc1. F-Alc1 purified from nuclear extracts of HEK 293/FRT cells was subjected to Superose 6 chromatography. The indicated fractions were analyzed by SDS/PAGE and silver staining or anti-FLAG western blotting (2 upper panels) or were assayed for ATPase or nucleosome remodeling (lower panels). Assays were performed in the presence (solid lines) or absence (dotted lines) of Parp1 and NAD.

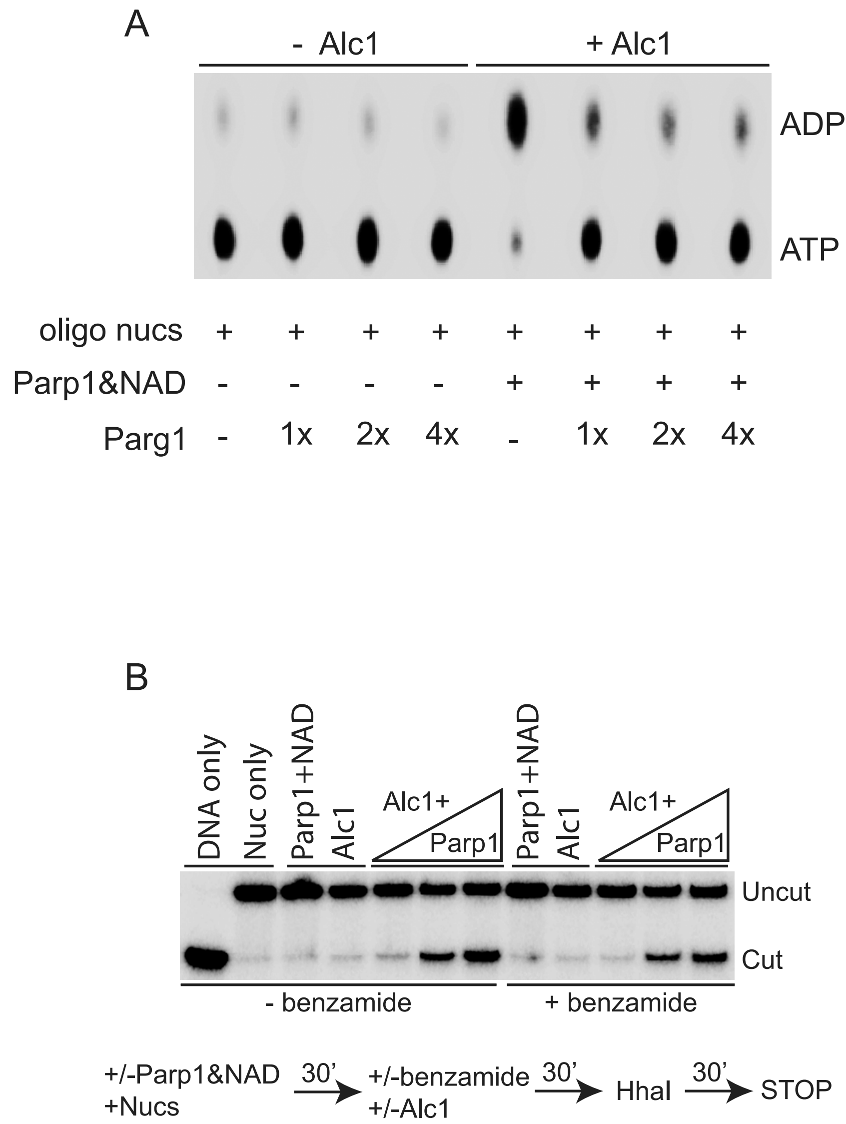


Fig. S2. (*A*) Parp1- and NAD-dependent Alc1 ATPase is inhibited by Parg1. Reactions were performed as described in the *Materials and Methods* with or without 1 ng (1×), 2 ng (2×), or 4 ng (4×) Parg. (*B*) Alc1 PARylation is not required for nucleosome remodeling. Nucleosome remodeling assays were performed as described with nucleosomes containing HeLa cell histones except that nucleosomes were preincubated for 30 min with Parp1 and NAD before addition of Alc1, with or without 2 mM benzamide.

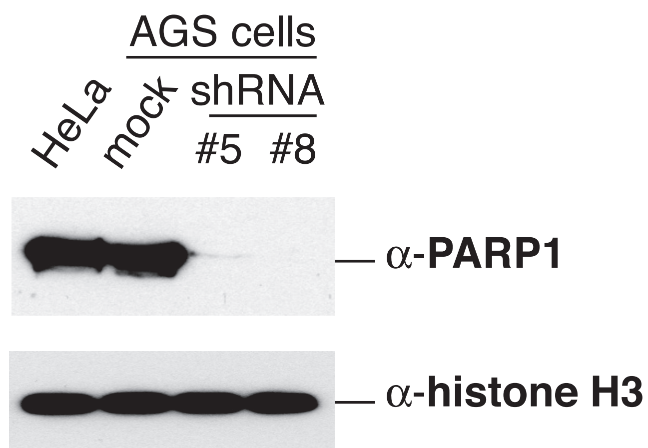


Fig. S3. Anti-Parp1 and histone H3 western blots of lysates from HeLa cells or from AGS cells expressing 2 different shRNAs targeting PARP1 or a nontargeting shRNA (mock).

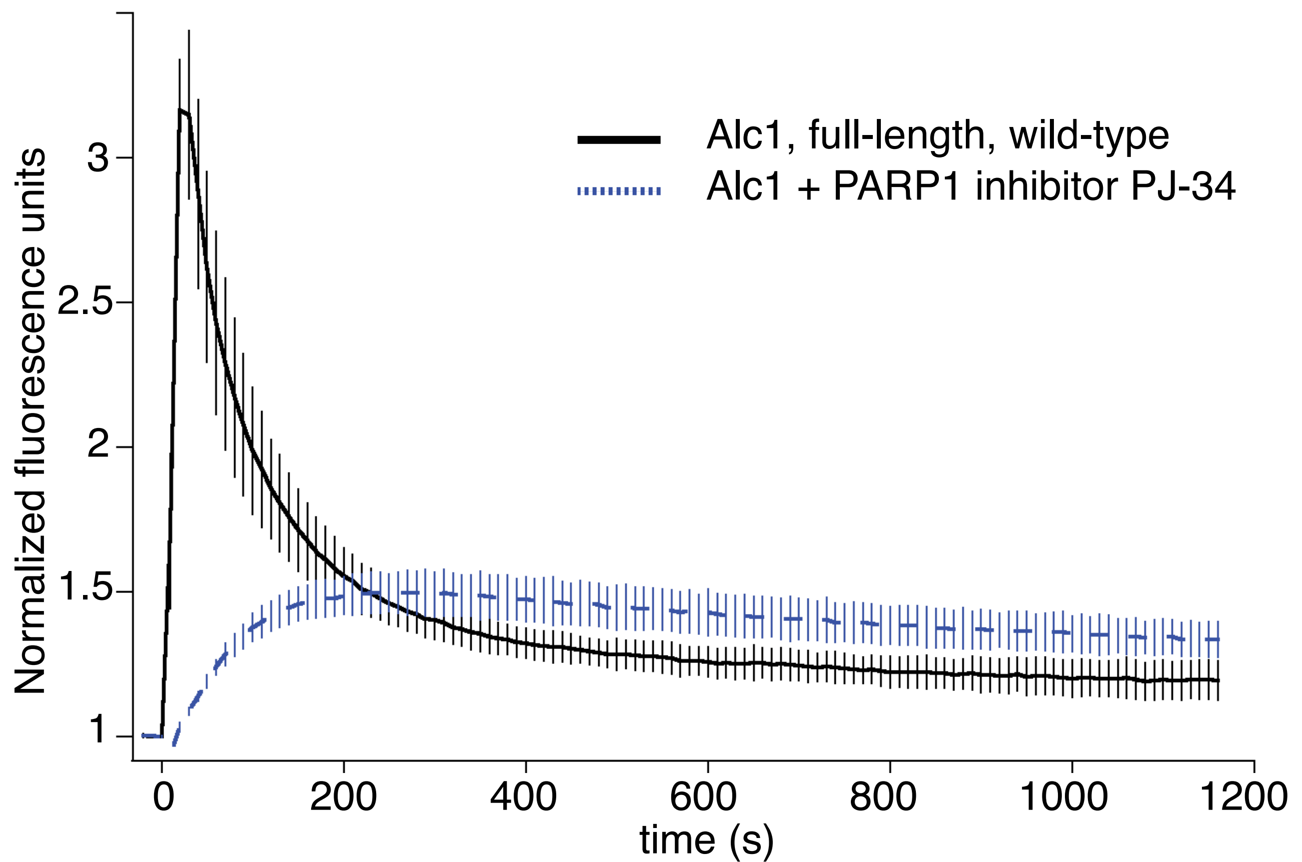


Fig. S4. The effect of PARP inhibitor PJ-34 on Alc1 recruitment kinetics. (*A*) Real-time recruitment kinetics ($n \geq 6$) of wild-type Alc1 at the site of laser microirradiation in HeLa cells in the presence (blue dashed line) and absence (black line) of the PARP inhibitor PJ-34.

Table S1. Whole cell lysates from HEK 293/FRT cells expressing wild type or mutant F-Alc1 were immunoprecipitated with anti-FLAG (M2) agarose

Locus	Description	NSAF × 10^4		
		F-Alc1 (wt)	F-Alc1 (E175Q)	F-Alc1 (D723A)
gi|148612870|ref|NP_004275.3|	Alc1	2574	1537	1382
gi|4503841|ref|NP_001460.1|	Ku70	137	21	ND
gi|156523968|ref|NP_001609.2|	Parp1	111	12	ND
gi|106775678|ref|NP_001035807.1|	Histone H2A	105	33	ND
gi|15617199|ref|NP_254280.1|	Histone H2A	105	33	ND
gi|10863945|ref|NP_066964.1|	Ku80	102	14	ND
gi|4504253|ref|NP_002096.1|	Histone H2AX	83	38	ND
gi|4506587|ref|NP_002938.1|	RPA3	57	21	ND
gi|6005757|ref|NP_009123.1|	Spt16	51	ND	ND
gi|169167131|ref|XP_001720197.1|	Histone H3	46	15	ND
gi|4504255|ref|NP_002097.1|	Histone H2AZ	44	20	ND
gi|4506585|ref|NP_002937.1|	RPA2	39	ND	ND
gi|13654237|ref|NP_008835.5|	DNAPK	38	14	1
gi|110825961|ref|NP_005475.2|	Parp2	29	19	ND
gi|18105048|ref|NP_542160.1|	Histone H2B	20	12	ND
gi|10800140|ref|NP_066406.1|	Histone H2B	20	12	ND

Immunopurified proteins were identified using a modification of the multidimensional protein identification (MudPIT) procedure (1, 2). Shown are the most abundant proteins that were detected by MudPIT mass spectrometry in Flag immunopurified material from cells expressing wild type F-Alc1 but not the macrodomain mutant F-Alc1 (D723A). The normalized spectral abundance factor (NSAF) is proportional to the amount of protein present in the sample (3, 4) and is calculated using the formula:

$$(NSAF)_k = \frac{(SpC/L)_k}{\sum_{i=1}^{N}(SpC/L)_i}$$

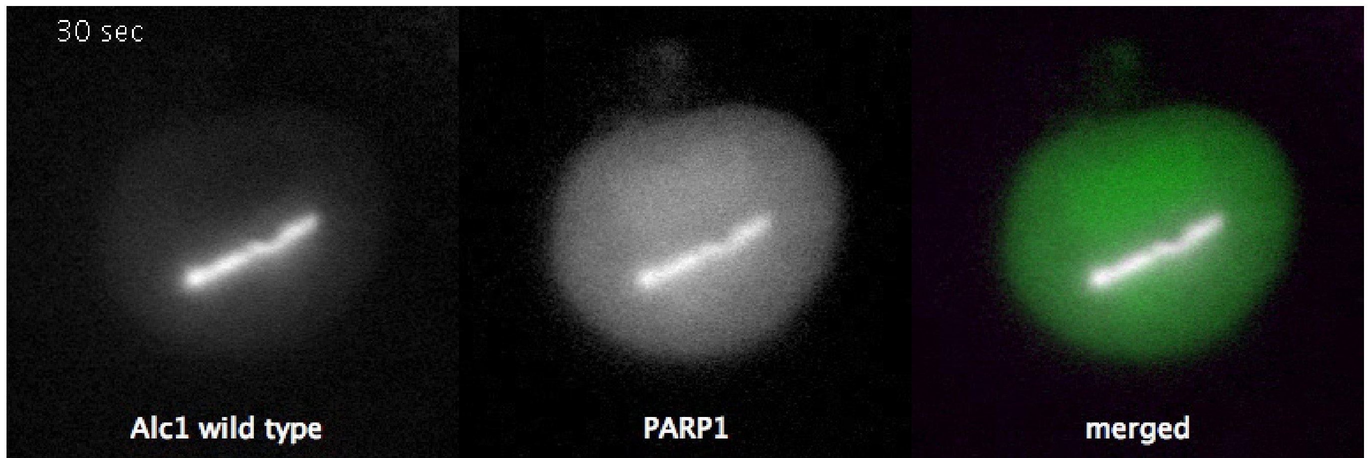
where SpC = spectal count, L = protein length in amino acids, and i = all proteins detected in the MudPIT runs. ND, not detected.

1. Washburn MP, Wolters D, Yates JR, III (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotechnol* 19:242–247.
2. Wolters D, Washburn MP, Yates JR (2001) An automated multidimensional protein identification technology for shotgun proteomics. *Anal Chem* 73:5683–5690.
3. Paoletti AC, et al. (2006) Quantitative proteomic analysis of distinct mammalian Mediator complexes using normalized spectral abundance factors. *Proc Natl Acad Sci USA* 103:18928–18933.
4. Zybailov B, et al. (2006) Statistical analysis of membrane proteome expression changes in *Saccharomyces cerevisiae*. *J Proteome Res* 5:2339–2347.

Table S2. Neither poly(ADP-ribose) nor ADP-ribose activate Alc1 ATPase activity

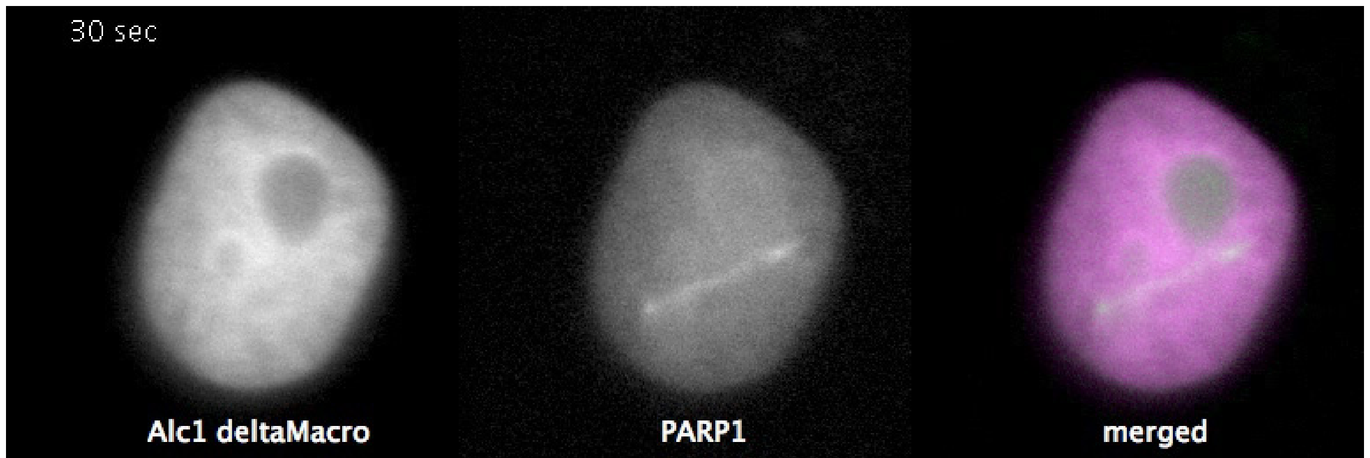
Reaction components	ATP hydrolysis, pmol/min
Alc1, Parp-1, NAD (34 μM), nucleosomes	4.6
Parp-1, NAD (34 μM), nucleosomes	0.9
Alc1, PAR (1.5 μM), nucleosomes	0.8
Alc1, PAR (15 μM), nucleosomes	1.0
Alc1, PAR (150 μM), nucleosomes	0.9
PAR (150 μM), nucleosomes	0.8
Alc1, ADPr (1.5 μM), nucleosomes	1.2
Alc1, ADPr (15 μM), nucleosomes	1.0
Alc1, ADPr (150 μM), nucleosomes	1.0
ADPr (150 μM), nucleosomes	1.1
nucleosomes	0.9

Thirty-minute ATPase reactions were performed as described in *Methods* with or without 1 pmol Alc1, 1 pmol Parp-1, and the indicated concentrations of NAD, free ADP(ribose) (ADPr), or free PAR. All reactions contained 1 pmol HeLa cell long oligonucleosomes, 40 μM ATP. Concentrations of PAR and ADPr are expressed as mole equivalents of adenosine determined using the extinction coefficient of adenosine [A_{260nm} = 15 O.D. ($cm^2/\mu mol$) at pH 7]. PAR was prepared as described [Karras GI, et al. (2005) The macro domain is an ADP-ribose binding module. *EMBO J* 24:1911–1920].



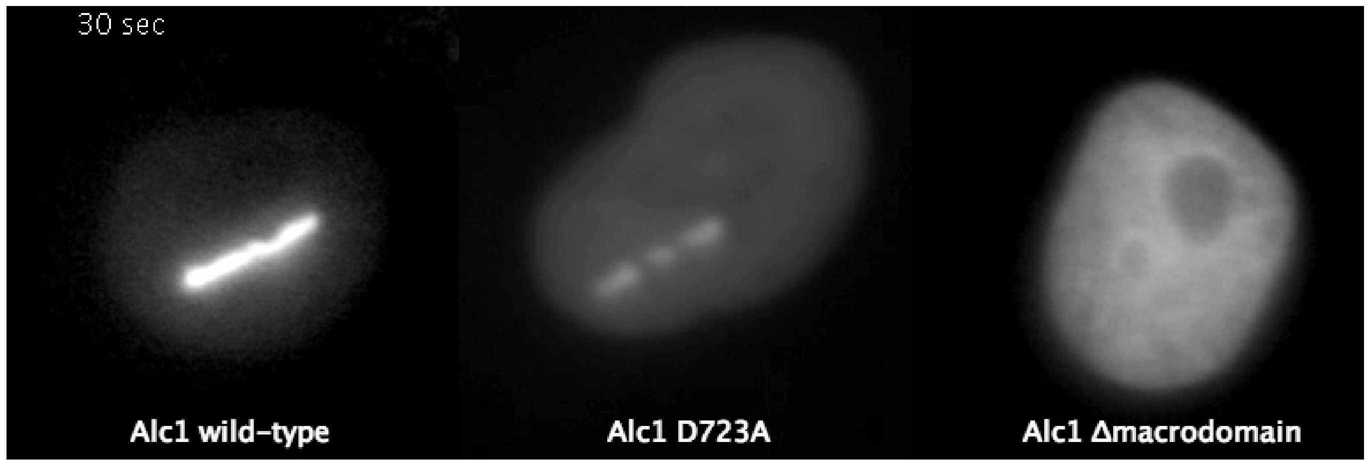
Movie S1. Recruitment kinetics of wild-type Alc1 and PARP1 to DNA damage sites. Representative movie of a HeLa cell transiently co-expressing EYFP-Alc1 (cyan) and PARP1-mCherry (green) following laser microirradiation. Right panel shows the merge.

Movie S1 (MOV)



Movie S2. Recruitment kinetics of a Alc1 fragment lacking its macrodomain and of PARP1 to microirradiated sites. Representative movie of a HeLa cell transiently co-expressing EYFP-Alc1-Δmacrodomain (cyan) and PARP1-mCherry (green) following laser microirradiation. Right panel shows the merge.

Movie S2 (MOV)



Movie S3. Recruitment of wild-type, D723A mutant and macrodomain deletion Alc1 following laser microirradiation. Representative movie of HeLa cells transiently expressing either wild-type, D723A mutant or macrodomain deletion Alc1 tagged with EYFP following laser microirradiation.

Movie S3 (MOV)