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Poly(ADP-ribose)–Dependent Regulation of DNA Repair by the Chromatin Remodeling Enzyme ALC1

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Posttranslational modifications play key roles in regulating chromatin plasticity. Although various chromatin-remodeling enzymes have been described that respond to specific histone modifications, little is known about the role of poly[adenosine 5′-diphosphate (ADP)–ribose] in chromatin remodeling. Here, we identify a chromatin-remodeling enzyme, ALC1 (Amplified in Liver Cancer 1, also known as CHD1L), that interacts with poly(ADP-ribose) and catalyzes PARP1-stimulated nucleosome sliding. Our results define ALC1 as a DNA damage–response protein whose role in this process is sustained by its association with known DNA repair factors and its rapid poly(ADP-ribose)–dependent recruitment to DNA damage sites. Furthermore, we show that depletion or overexpression of ALC1 results in sensitivity to DNA-damaging agents. Collectively, these results provide new insights into the mechanisms by which poly(ADP-ribose) regulates DNA repair.

Processes such as transcription, repair, and replication that require efficient DNA recognition are dependent on modulation of chromatin structure (1–3). Chromatin relaxation is a critical event that occurs during DNA repair and is associated with the negatively charged polymer of adenosine 5′-diphosphate (ADP)–ribose (PAR) (4). PAR is synthesized from nicotinamide adenine dinucleotide (NAD⁺) by the poly(ADP-ribose) polymerase protein family (PARPs), of which PARP1 (and to a lesser extent PARP2) respond to DNA strand breaks (5–7). As a consequence of poly(ADP-ribosylation), chromatin adopts a more relaxed structure (8–11), and this is thought to facilitate DNA repair (4, 12–14). However, the molecular mechanism by which PAR modulates chromatin during DNA repair is largely unknown.

One potential mechanism for PAR function is to bind and recruit chromatin modifiers. In a search for DNA repair–associated chromatin factors, we investigated the function of human ALC1 (Amplified in Liver Cancer 1, also known as CHD1L), which contains a helicase domain (Fig. 1A) that is also found in the Snf2 family of adenosine 5′-triphosphate (ATP)–dependent chromatin-remodelers (such as Snf2, ISWI, and CHD1) (15). ATPases of

this family are modular in nature and often combine a helicase domain with motifs that mediate selective recognition of protein modifications. Unlike CHD1, ALC1 does not contain a chromo domain, which can recognize methylated histone tails. Instead, ALC1 contains a macro domain, which is an ADP-ribose/PAR-binding element (16). Thus, ALC1 might possess a PAR-dependent chromatin-remodeling activity and facilitate DNA repair reactions within a chromatin context.

To assess this, we determined whether ALC1 binds PAR *in vitro*. We constructed a number of ALC1 domain deletions, as well as the putative ATPase dead mutant K77R, and the macro-domain mutant D732A, which is predicted to affect PAR binding (Fig. 1A) (16–19). Purified recombinant FLAG-tagged wild-type (WT) ALC1 and the various mutated ALC1 derivatives (fig. S1A) were dotted onto a nitrocellulose membrane, and their ability to bind ³²P-radiolabeled PAR was measured. Aprataxin PNK-like factor (APLF), which binds PAR via an unrelated PAR-binding Zn-finger, was used as a positive control (20). PAR binding was detected with the macro domain–containing ALC1 proteins (WT, K77R, and C1) but not with the N-terminal helicase domain fragments N1 and N2 or with the macro-domain mutant D732A (Fig. 1B). We also demonstrated PAR binding in cells by means of immunoprecipitation of the FLAG-tagged ALC1 proteins from transiently transfected 293T cells. Endogenous PAR was immunoprecipitated with the macro domain–containing proteins but not with the D723A mutant or the helicase domain fragments N1 and N2 (Fig. 1C). Most of the immunoprecipitated PAR was associated with PARP1 and histones; we observed signals on the blot of antibody to PAR whose molecular weights corresponded to these proteins (Fig. 1C). We also detected increased levels of endogenous PAR in the extracts of cells that expressed the macro-domain proteins (WT, K77R, and C1) (Fig. 1C, inputs).

Possibly, overexpression of these PAR-binding proteins restricted accessibility of PAR to be degraded by the catabolic enzyme poly(ADP-ribose) glycohydrolase (PARG), giving rise to the observed effect. The ALC1 immunoprecipitates also revealed interactions of the macro-domain proteins with PARP1 and core nucleosome components (Fig. 1C). The interaction with PARP1 was severely reduced with the D723A mutant.

We next tested ALC1 for nucleosome-stimulated ATPase activity. ALC1 displayed only weak ATPase activity on its own, but the activity was stimulated modestly by the addition of DNA and more markedly by the addition of nucleosomes (Fig. 1D). This enhanced activity was, however, less pronounced with nucleosomes containing histone H4 mutated at residues 16 to 19 [H4(16-19)A], demonstrating that the ALC1 ATPase activity required the histone H4 N-terminal tail (fig. S1D). A single amino acid change in the ATP-binding Walker A motif (K77R) abolished the ATPase activity of ALC1 (Fig. 1D).

To establish ALC1 as a bona fide chromatin-remodeling enzyme, we tested its ability to reposition nucleosomes. Native-gel analyses showed that WT ALC1 and the macro-domain mutant D732A, but not the ALC1 ATPase dead mutant K77R, catalyzed nucleosome sliding in an ATP-dependent manner (Fig. 1E). The ability of ALC1 to reposition nucleosomes was histone H4 tail-dependent; it was not observed with the mutant nucleosome H4(16-19)A (fig. S1F). The dependence on this epitope within the H4 tail has been observed with other remodeling enzymes (21, 22) and provides strong evidence that nucleosomes are the relevant substrate for ALC1.

Given that ALC1 interacts with PAR and PARP1, we investigated the effect of PARP1 action on the activities of ALC1. PARP1 stimulated the ATPase activity of ALC1 approximately fourfold, depending on the assay conditions (Fig. 1F and fig. S1H). Stimulation was not observed in the absence of NAD⁺ or DNA (Fig. 1F and fig. S1, D and H) and was abolished by PARP inhibitor treatment (Fig. 1F), indicating that poly(ADP-ribosylation) was required for ALC1 stimulation. Preincubation of PARP1 with nucleosomes and NAD⁺, followed by the addition of PARP inhibitor, was sufficient for ALC1 stimulation (Fig. 1F), suggesting that the stimulation is not dependent on the PARylation of ALC1 itself (fig. S1C). Moreover, addition of purified PAR did not stimulate ALC1 (Fig. 1F), indicating that PAR binding alone is insufficient to stimulate ATPase activity. Thus, we propose that stimulation of ALC1 requires PARylated PARP1. PARP1 and NAD⁺ also stimulated the nucleosome-repositioning activity of ALC1 (Fig. 1G), thereby defining ALC1 as a PARP1-stimulated chromatin-remodeling enzyme.

To investigate the cellular functions of ALC1, we analyzed ALC1-associated immunocomplexes by means of mass spectrometry (MS). Chromatin extracts prepared from stable Flp-In-FLAG and Flp-In-ALC1 cell lines were subjected to immu-

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noprecipitation with antibody to FLAG. Comparative MS analysis revealed the presence of proteins implicated in DNA repair but also in other cellular processes (Fig. 2A and fig. S2A) (23). Interactions with DNA-PKcs, Ku, and PARP1, as well as those with the DNA damage responsive XRCC1 and APLF proteins, were confirmed through immunoblot analyses (Fig. 2B). These interactions were largely abrogated by PARP inhibitor treatment, indicating that the association of ALC1 with these DNA repair proteins is dependent on PAR modifications.

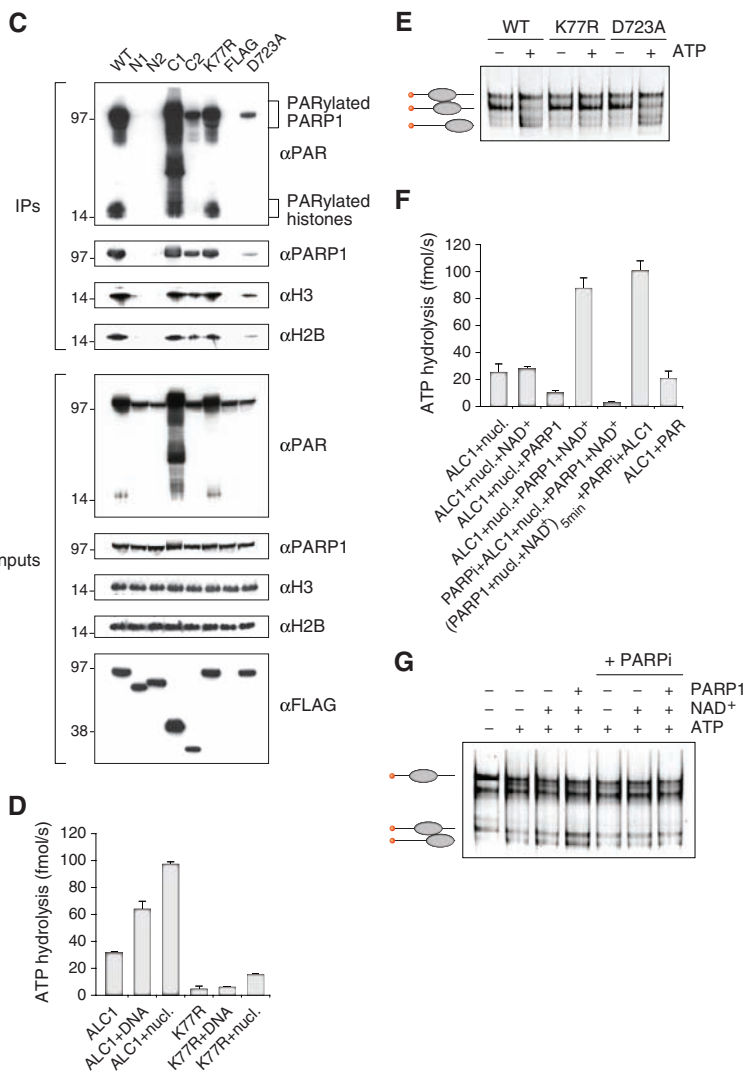
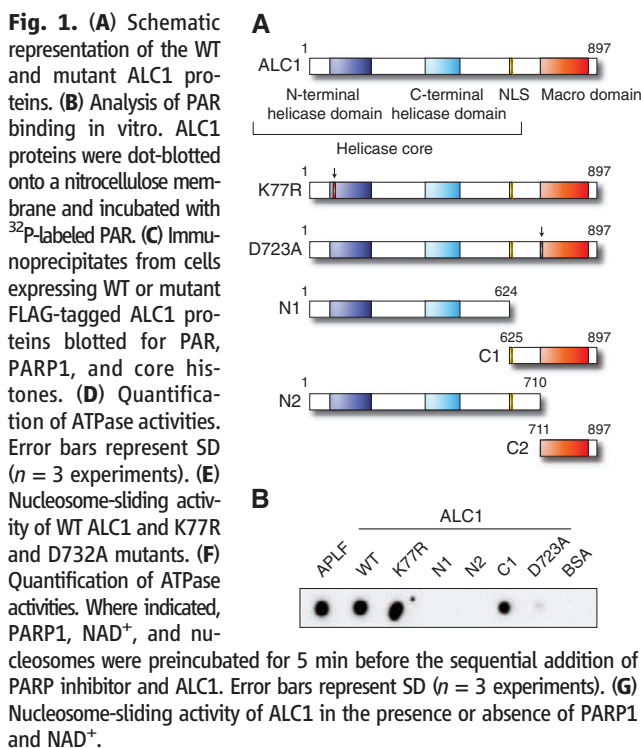
The stimulation of ALC1 nucleosome-repositioning activity by PARP1 and the PAR-dependent interactions between ALC1 and several known DNA repair factors suggested a possible role for ALC1 in the DNA damage response. Indeed, we observed reversible mobilization of ALC1 from the soluble to chromatin-bound fraction in response to DNA damage induced by H₂O₂ treatment, which was largely dependent on active PAR synthesis (fig. S2B). Furthermore, we observed the colocalization of ALC1 with sites of active PAR synthesis in mouse 3T3 cells after

exposure to H₂O₂ (fig. S2C). To further investigate the role of ALC1 in the DNA damage response, we analyzed the recruitment of ALC1 to sites of DNA damage induced by laser micro-irradiation. Endogenous ALC1 efficiently localized to sites of laser-induced DNA breaks, as marked by γ H2AX staining (Fig. 3A). Recruitment of fluorescently tagged yellow fluorescent protein (YFP)-ALC1 to laser-induced DNA breaks was also observed in transiently transfected U2OS cells (Fig. 3B), which was abolished in cells treated with the PARP inhibitor (Fig. 3B and fig. S2E).

The kinetics of ALC1 recruitment to DNA damage sites closely mirrored the dynamics of PAR, which is known to be a short-lived modification. Live cell imaging revealed an almost instant mobilization of ALC1 to DNA damage sites, and a relatively transient retention of ALC1 at such regions (Fig. 3C and fig. S2F). Moreover, the ALC1 K77R mutant, which is defective for nucleosome sliding *in vitro*, exhibited prolonged retention at DNA damage sites as compared with WT ALC1 (Fig. 3, D and E). This was even more pronounced with the macro domain fragment of

ALC1 (C1 mutant), which persisted at DNA damage sites beyond 40 min (Fig. 3D). In contrast, the helicase core fragment (N2 mutant) was not mobilized to DNA damage sites, and the recruitment of the D723A mutant was severely impaired (Fig. 3D). Thus, ALC1 recruitment required macro domain-mediated recognition of PAR at sites of DNA damage. Conversely, the timely disengagement of ALC1 from DNA damage sites required the ATPase activity of its helicase core. Expression of the macro domain fragment (C1) also led to prolonged accumulation of the single-strand-break repair factor XRCC1 at sites of DNA damage (fig. S3A), potentially indicating delayed repair kinetics in the absence of ALC1-mediated chromatin remodeling.

We also assessed the sensitivity of ALC1-deficient cells to various DNA-damaging agents. U2OS cells that were stably reduced for ALC1 expression by an integrated ALC1 short hairpin RNA (shRNA) construct (Fig. 4A) were more sensitive toward H₂O₂ and the radiomimetic drug phleomycin but not to camptothecin (Fig. 4B). We conclude that ALC1 is a nucleosome-repositioning



enzyme that is specifically targeted to sites of DNA damage through interaction with PAR and functions to regulate chromatin during DNA repair.

ALC1 was recently identified as a target oncogene within the 1q21 amplicon, which is the most frequent genetic alteration in human hepato-

cellular carcinoma (HCC) and occurs in 58 to 78% of primary HCC cases (24). Although the precise molecular impact of ALC1 overexpression is unclear, ALC1-overexpressing cells display increased colony formation in soft agar and tumorigenicity in nude mice (25). To assess the possible

consequences of ALC1 overexpression, we analyzed induction of H2AX phosphorylation (a DNA damage marker) in ALC1-overexpressing and control cells after phleomycin treatment (fig. S4A). No measurable differences in γ H2AX profiles were observed between untreated control and ALC1-overexpressing cells. In contrast, phleomycin induced H2AX phosphorylation in 44 to 50% of control cells, whereas 80 to 93% of ALC1-overexpressing cells exhibited H2AX phosphorylation (Fig. 4C and fig. S4B). Phleomycin treatment also consistently produced longer tails (more damage) in alkaline Comet assay in ALC1-overexpressing cells as compared with control cells (Fig. 4, D and E). This phenotype was abrogated by the K77R ALC1 mutation (Fig. 4C) and was unaffected by PARP inhibitor treatment (fig. S4C), suggesting that the phenotype required ALC1 chromatin-remodeling activity but not its recruitment to the sites of DNA damage. Furthermore, the phenotype was specific to phleomycin and could not be observed with ionizing radiation or H₂O₂ (fig. S4D and E), possibly reflecting a difference in the accessibility of chromatin-embedded DNA to these DNA-damaging agents.

Fig. 2. (A) Identification of ALC1-interacting partners by means of MS. **(B)** Western blotting of ALC1 immunoprecipitates from transiently transfected 293T cells for known DNA repair factors.

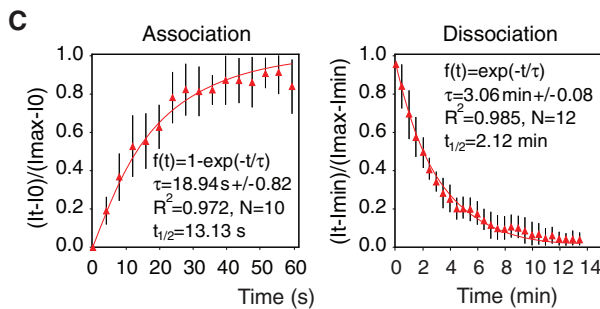
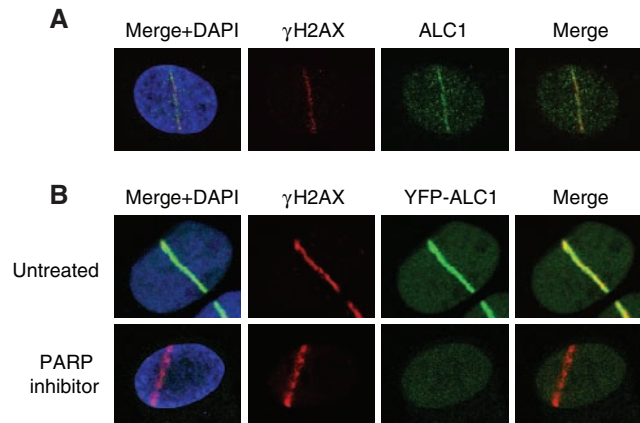
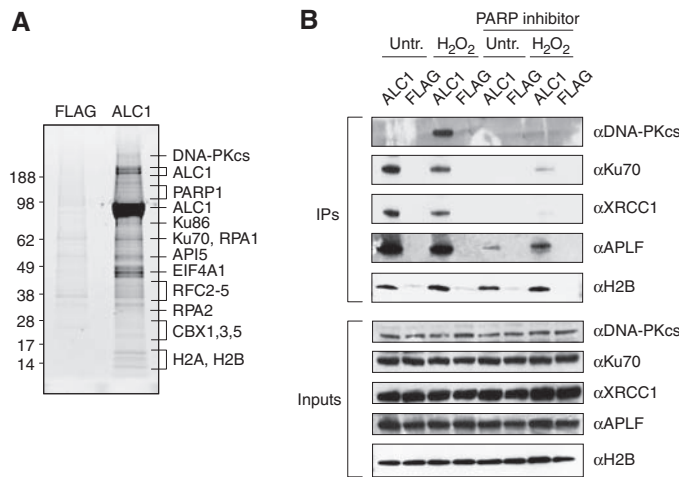
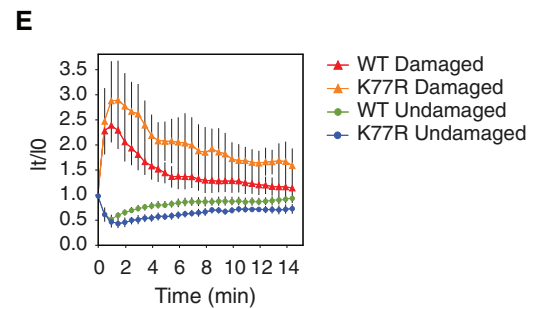
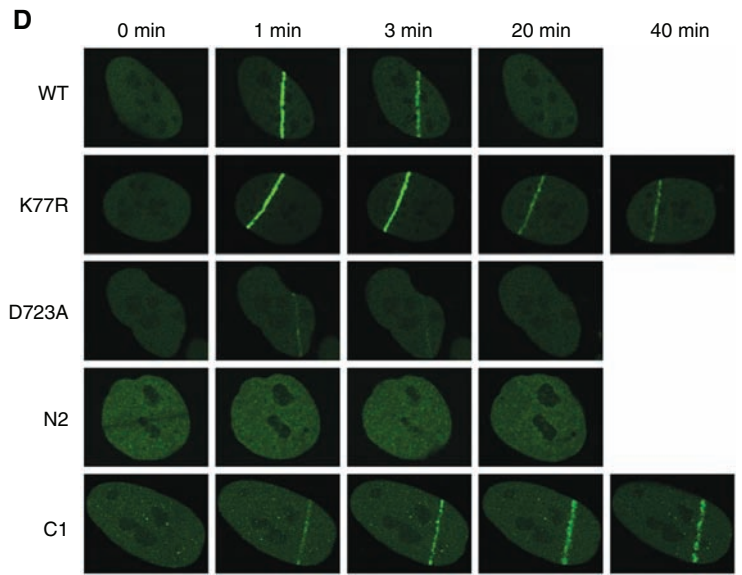
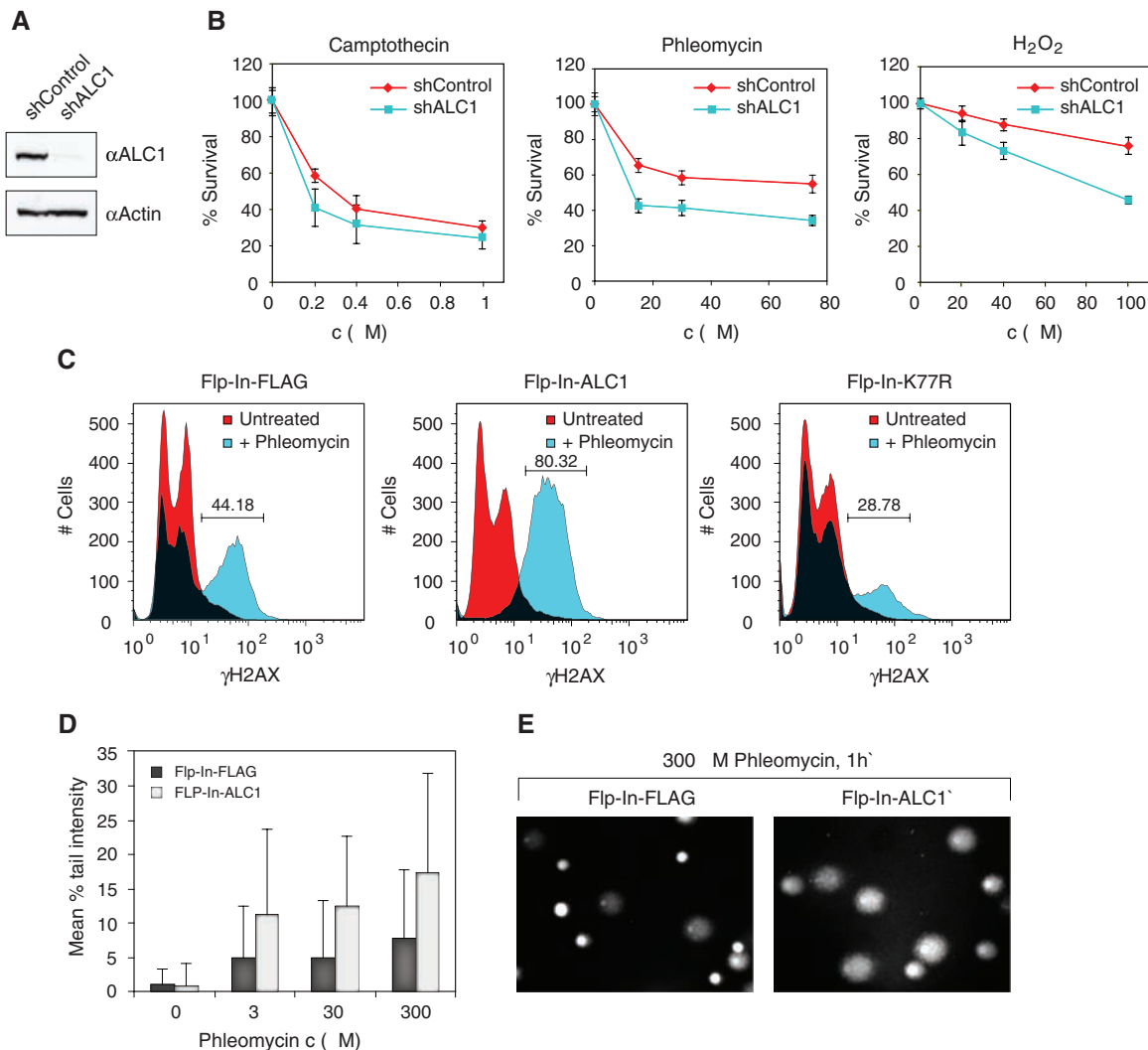


Fig. 3. (A) Recruitment of endogenous ALC1 to sites of laser-induced DNA damage, 1 min after laser damage. **(B)** Recruitment of the transiently expressed YFP-ALC1 to laser-induced DNA breaks, with and without PARP inhibitor. **(C)** Kinetics of ALC1 association and dissociation from DNA breaks.



τ , time constant; N, number of cells. Error bars represent SD. **(D)** Recruitment of the ALC1 derivatives and mutants to laser-induced DNA breaks. **(E)** Comparison of the WT and K77R mutant ALC1 kinetics at laser-induced DNA breaks.

Fig. 4. (A) Knockdown efficiency of ALC1 in U2OS-stable shRNA cell lines. **(B)** Sensitivity of ALC1-deficient cells to DNA-damaging agents. Data are averaged values from 3 experiments. **(C)** γ H2AX levels assessed by fluorescence-activated cell sorting analysis in cell lines of the indicated genotype, 1 hour after 300 μ M phleomycin treatment. **(D)** DNA damage levels in cells ($n > 200$ cells) of the indicated genotype assessed by means of Comet assay. Error bars represent SD. **(E)** Representative images of control and ALC1-overexpressing cells analyzed by means of Comet assay.



Indeed, these results are in agreement with the observation that the structurally related DNA-damaging agent bleomycin induces DNA breaks in the linker region but not in core DNA and therefore acts preferentially on relaxed chromatin (25). We thus conclude that ALC1 overexpression leads to chromatin relaxation and an increased susceptibility to phleomycin-induced DNA damage. These findings provide a key insight into the molecular consequence of ALC1 deregulation, emphasizing the importance of chromatin reorganization as a critical element in genome stability and cancer.

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18. In the mutants, other amino acids were substituted at certain locations; for example, R182Q indicates that arginine at position 182 was replaced by glutamine. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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