Supplementary:

S1.
(A) In vitro analysis of PAR vs. poly(ADP-ribosyl)ated PARP1 binding by the wild-type ALC1 and C1 fragment. Proteins were dot-blotted onto a nitrocellulose

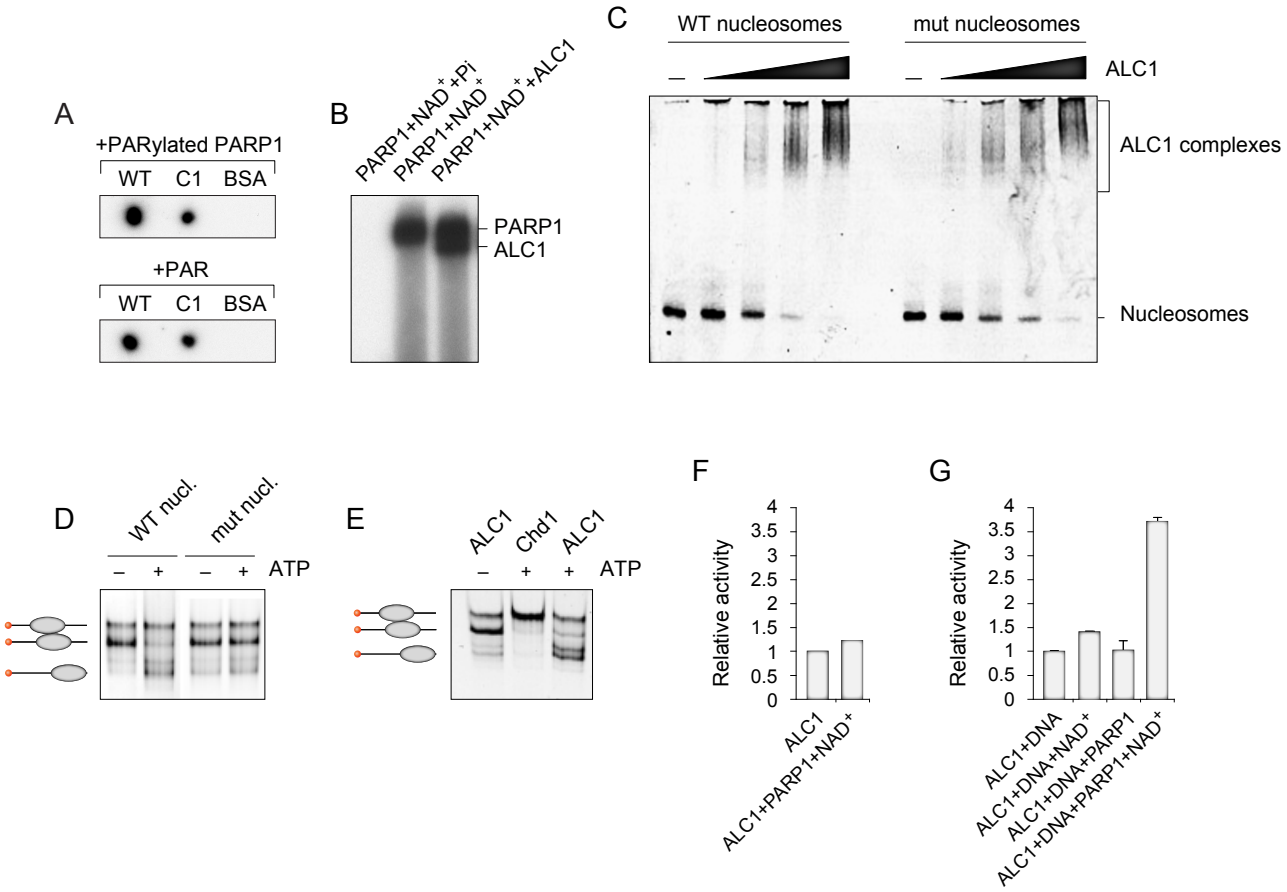
membrane and incubated with ^{32}P-labelled PAR or PARylated PARP1. No significant difference in binding was observed.
(B) In vitro poly(ADP-ribosyl)ation of ALC1 by PARP1. Poly(ADP-ribosyl)ation was detected using [32P]-labelled NAD^+ followed by SDS-PAGE and autoradiography.
(C) WT or H4(16-19)A nucleosomes, assembled on Cy3 labelled 0w0 DNA, were incubated for 5 min at 30° C with increasing amounts of ALC1 (87 nM, 175 nM, 350 nM, 700 nM). Reactions were loaded onto 5% native gels and scanned using Phosphoimager. At higher concentrations supershifted ALC1 complexes were observed, indicating that ALC1 can interact with both wild type and mutant nucleosomes.
(D) Mutant H4 tail inhibits nucleosome sliding by ALC1. Xenopus wild-type and H4 mutant (H4(16-19)A) octamers assembled on Cy5-labelled linear 236 bp DNA fragment were incubated with 2.7 pmol of ALC1 in the absence or presence of 1 mM ATP.
(E) Comparison of ALC1 and Chd1 nucleosome-sliding ability. Xenopus wild-type octamer assembled on Cy5-labelled linear 236 bp DNA fragment was incubated with 2.7 pmol of ALC1 (1 and 3) and yeast Chd1 (lane 2) protein in the absence or presence of 1 mM ATP. ALC1 repositions nucleosomes from the centre of a DNA fragment towards its end, whereas Chd1 has the opposite directionality.
(F) ALC1 ATPase activity is not stimulated by purified PAR, or by PARP1 and NAD^+ in the absence of effector DNA. ATP hydrolysis by recombinant ALC1 (2.3 pmol) was monitored in the presence of NAD^+ (0.5mM) and PARP1 (0.16 pmol) as indicated. Rates of ATP hydrolysis are expressed relative to the activity of ALC1 alone.
(G) Stimulation of ALC1 ATPase activity by PARP1 and NAD^+ in the presence of DNA. ATP hydrolysis was measured as in C. Rates of ATP hydrolysis are expressed relative to the activity of ALC1 in the presence of DNA.

S2.
(A) ALC1 shifts from soluble to chromatin-bound fraction after H_2O_2 treatment. Cells were either untreated, treated with 0.5 mM H_2O_2 for 10 min and immediately processed (H_2O_2), treated with 0.5 mM H_2O_2 for 10 min and allowed to recover in media for 30 min (H_2O_2 recov.), or incubated with PARP inhibitor for 1 h prior to H_2O_2 treatment (H_2O_2+PARPi). Cells were fractionated into extracts by successive solubilisation in 150 and 400 mM salt. Pellet contains chromatin-bound fraction as shown by the presence of HP1α (Heterochromatin Protein 1α) and histones.
(B) Endogenous ALC1 co-localizes with PAR in mouse 3T3 cells.
(C) Expression levels of YFP-tagged ALC1 WT, K77R, D723A, N2 and C1 in transiently transfected U2OS cells are not notably different.
(D) Comparative kinetics of the wild-type ALC1 recruitment to sites of laser micro-irradiation with and without PARP inhibitor treatment. Cells treated with PARP inhibitor were followed over time up to 15 min after laser damage and local accumulation of ALC1 could not be observed.
(E) Over-expression of the Macro domain fragment C1 leads to persistence of XRCC1 at sites of DNA breaks. U2OS cells were transfected with YFP-ALC1 or YFP-C1 construct, exposed to laser damage, fixed and stained against XRCC1 and γH2AX.

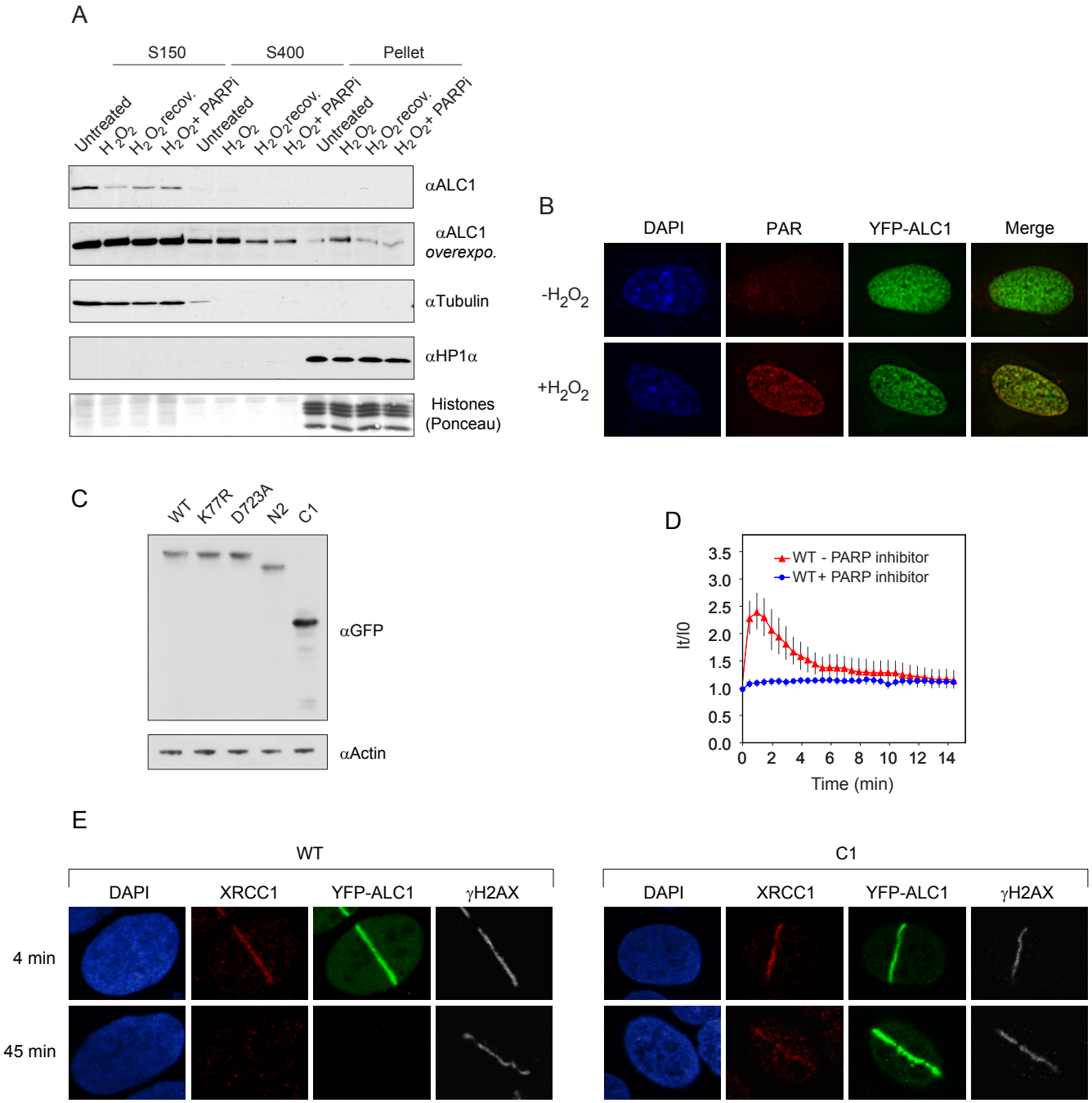
S3.
(A) ALC1 co-localizes with PARP1, XRCC1 and APLF at sites of laser micro-irradiation. 5 min after laser damage, cells were subject to detergent pre-extraction prior to fixation and immunostaining.
(B) Knockdown efficiency of ALC1 in U2OS stable sh cell lines examined by immunofluorescence.

(C) PARP-1 and XRCC1 interaction with damaged chromatin (sites of laser-induced damage) is not dependent on the presence of ALC1. Cells were processed as in A.
(D) Comparison of acetylated H3K9 levels in U2OS stable sh cell lines following DNA damage. Small reduction in H3K9ac after H_2O_2 damage (500 μM, 30 min) (*35*) is comparable between shControl and shAlc1 cells. Anti-phospho-SMC1 was used as a control for DNA damage.
(E) Ubiquitinated H2A is detected at damage sites in shALC1 as in shControl cells. Cells were allowed to recover for 20 min after laser micro-irradiation. Pre-extraction was performed prior to immunostaining.

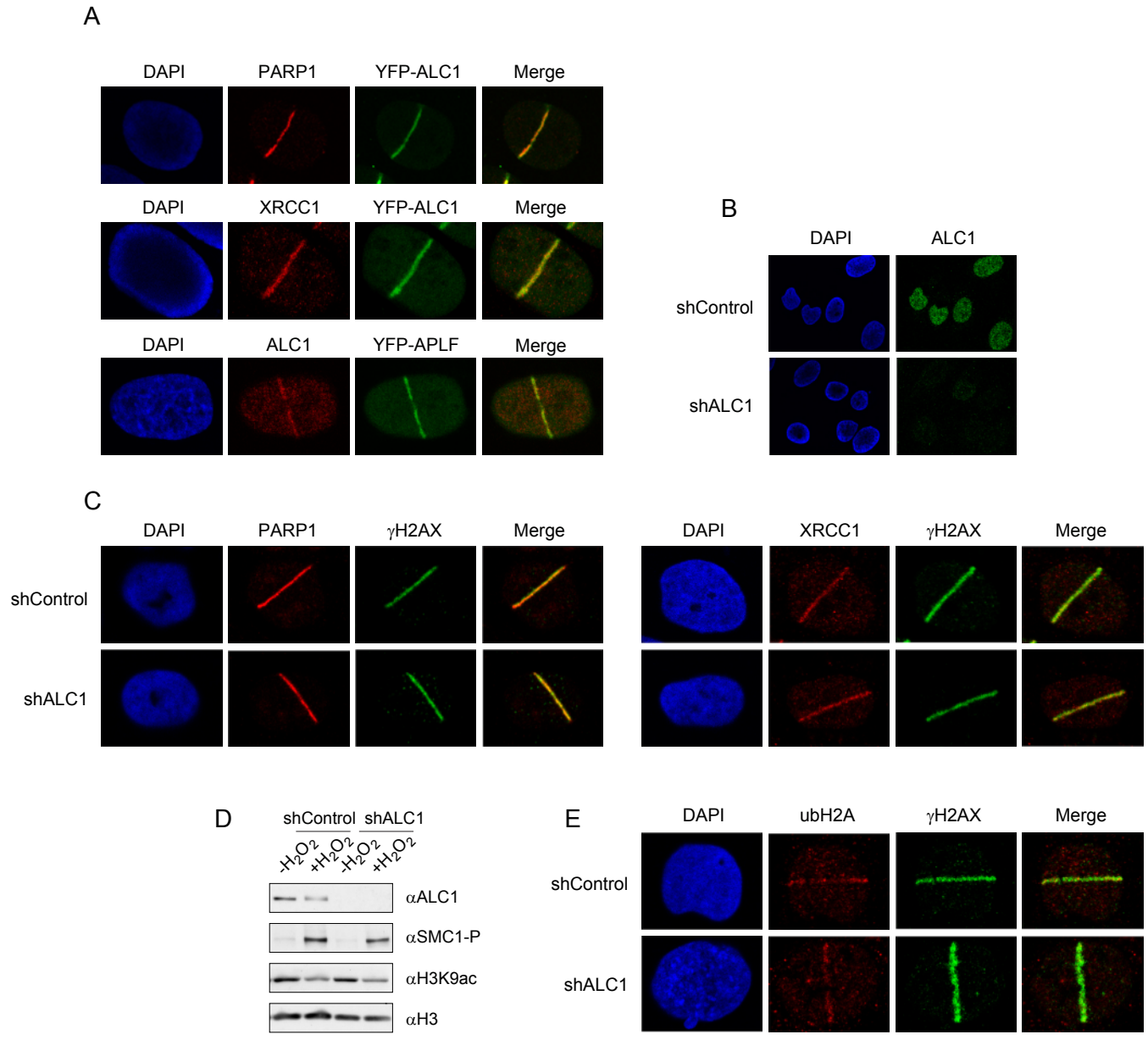
S4.
(A) Expression levels of FLAG-tagged ALC1 proteins in stable Flp-In cell lines.
(B) Increased H2AX phosphorylation is compromised in the Macro domain expressing cell line. Quantitative γH2AX FACS analysis of stable control (Flp-In-FLAG), wild-type ALC1 expressing (Flp-In-ALC1) and the Macro domain expressing (Flp-In-C1) cell lines. Cells were untreated or exposed to 300 μM phleomycin for 1 h, fixed, stained with FITC-γH2AX antibody and analyzed by FACS.
(C) H2AX phosphorylation is not affected by the treatment of ALC1 overexpressing cells with PARP inhibitor. Cells were exposed to PARP inhibitor overnight and to 300 μM phleomycin for 1 h where indicated. They were processed as in (B).



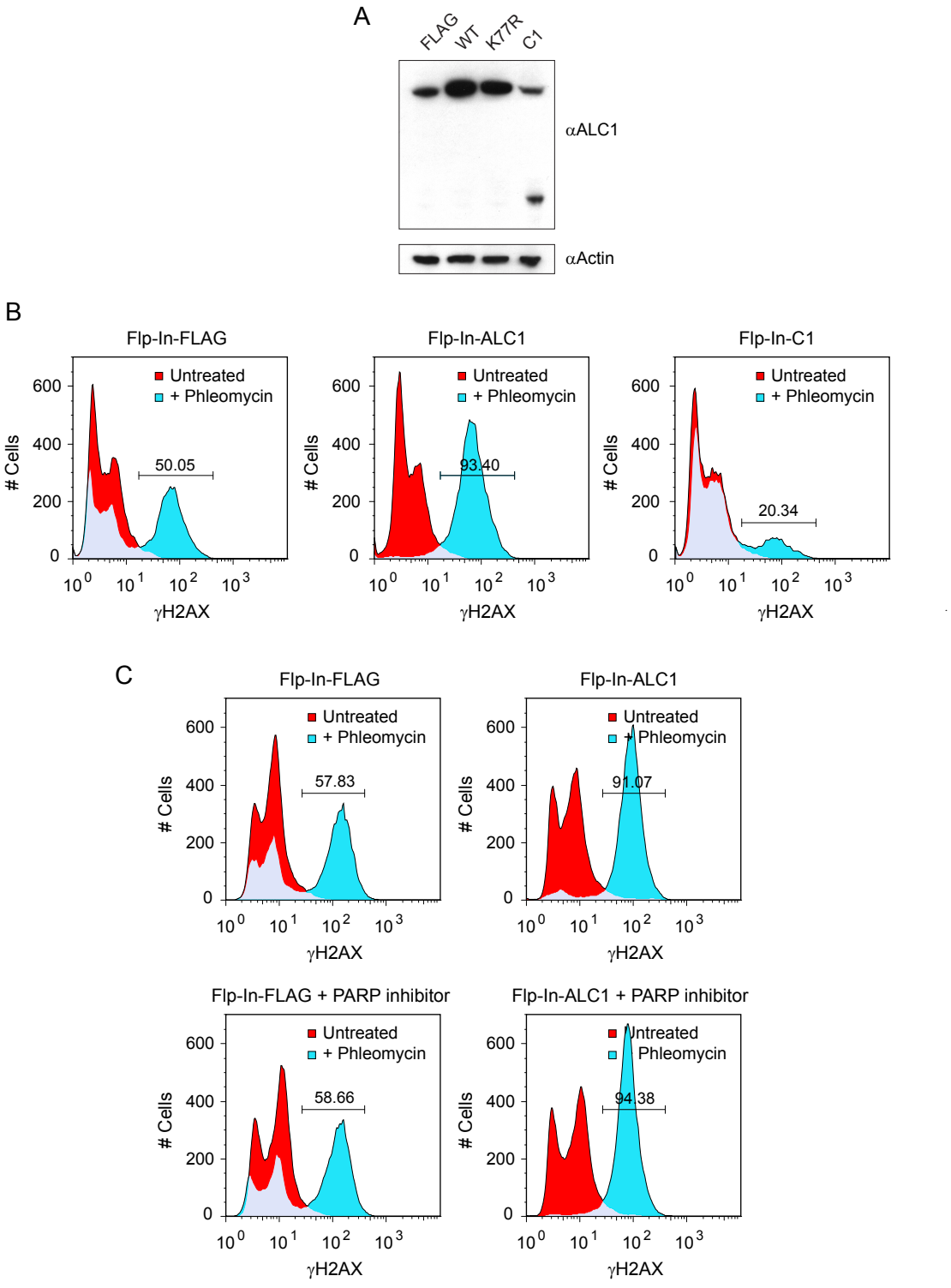
Ahel Supplementary fig. 1.



Ahel Supplementary fig. 2.



Ahel Supplementary fig. 3.



Ahel Supplementary fig. 4.

Supplementary references

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