

Supplemental Materials

Molecular Biology of the Cell

Jurado *et al.*

The recruitment of ACF1 and SMARCA5 to DNA lesions relies on ADP-ribosylation dependent chromatin unfolding

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*equal contributions

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SUPPLEMENTARY INFORMATION

Table S1. List of primers used in this work.

Primer	Sequence (5' - 3')
ACF1ΔWAC Forward	AATTCTGCAGTCGACGGTACCATGCCGCTGCCTCCATCA
ACF1ΔWAC Reverse	CACCATGGTGGCGACCGGTGGATCCCAGATTCGTGACTTTT TCGCAG
ACF1ΔDBD2 Forward	AATTCTGCAGTCGACGGTACCATGCCGGAAGATATGGAATG
ACF1ΔDBD2 Reverse	CACCATGGTGGCGACCGGTGGATCCCAGATTCGTGACTTTT TCGC
ACF1ΔWACΔDB D2 Forward	GGAAGTCCTCGATATGGAATGTGATGACCTTAAG
ACF1ΔWACΔDB D2 Reverse	CACCATGGTGGCGACCGGTGGATCCCAGATTCGTGACTTTT TCG
ACF1 WAC Forward	ATATATGGTACCATGCCGCTGCTACACCG
ACF1 WAC Reverse	ATATATGGATCCAAGAGGACTTCCAAAATCC
ACF1 DBD2 Forward	ATATATGGTACCATGCCTCCATCACATCAAATG

ACF1 DBD2 Reverse	ATATATGGATCCGATTCTCTAGGTTTACTCC
ACF1 WAC-DBD2 Forward	ATATATGGTACCATGCCGCTGCTACACCG
ACF1 WAC-DBD2 Reverse	ATATATGGATCCGATTCTCTAGGTTTACTCC
ACF1ΔC Forward	TCGACGGTACCCatgccgctgctacaccgaaagc
ACF1ΔC Reverse	GGTGGATCCCacagtatagatttagaccatatacagc

SUPPLEMENTARY FIGURE LEGENDS

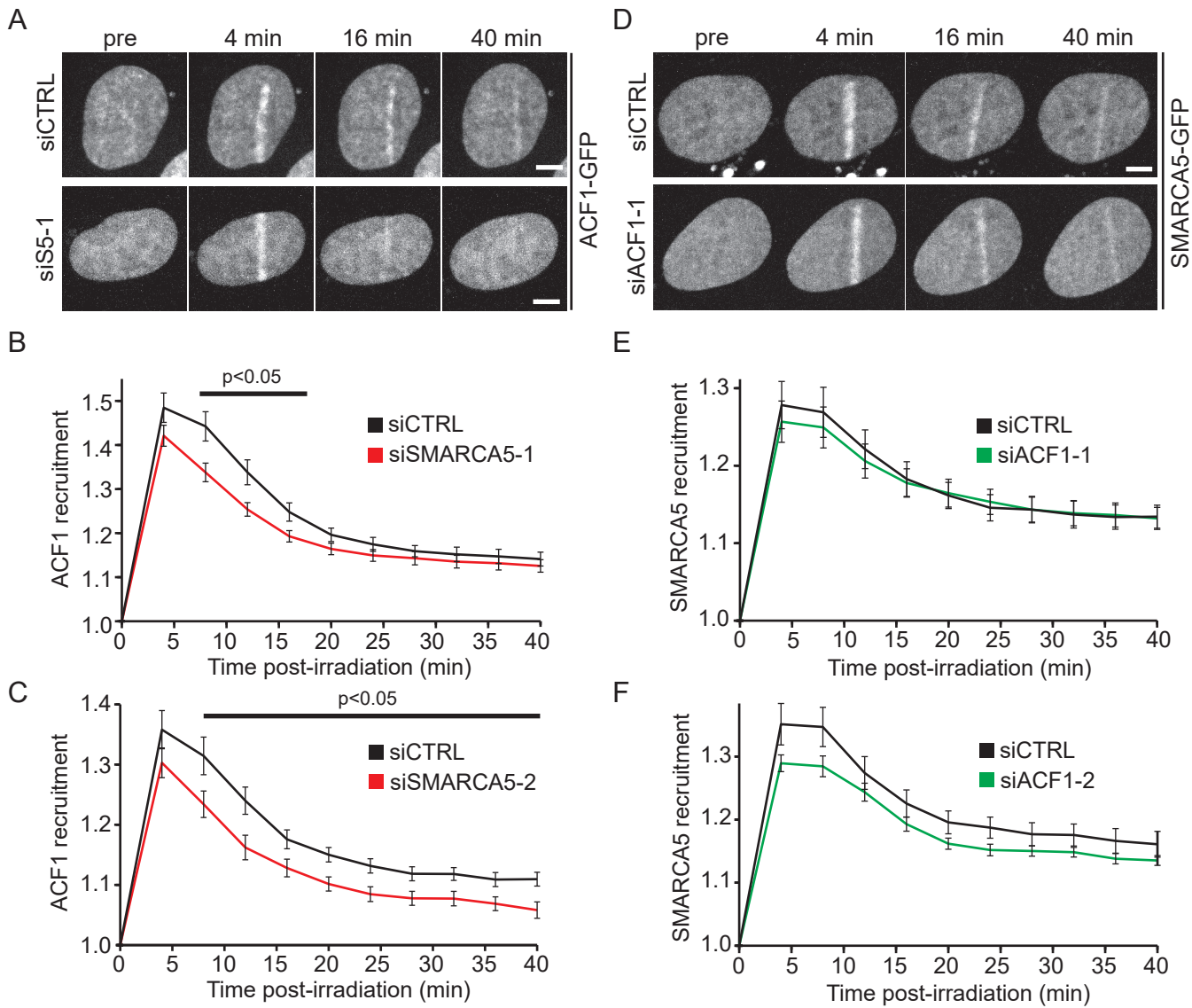
Supplementary figure 1. SMARCA5 promotes ACF1 stabilization at DNA lesions. (A-B) Images (A) and kinetics (B) of ACF1-GFP recruitment to sites of DNA damage induced by 405 nm laser irradiation in U2OS cells knocked-down for SMARCA5 (siS5-1) or treated with scrambled siRNA (siCTRL). Curves are mean±SEM of 14-18 cells per condition. (C) Kinetics of ACF1-GFP recruitment to sites of DNA damage induced by 405 nm laser irradiation in U2OS cells knocked-down for SMARCA5 (siS5-2) or treated with scrambled siRNA (siCTRL). Curves are mean±SEM of 14-18 cells per condition. (D-E) Images (D) and kinetics (E) of SMARCA5-GFP recruitment to sites of DNA damage induced by 405 nm laser irradiation in U2OS cells knocked-down for ACF1 (siACF1-1) or treated with scrambled siRNA (siCTRL). Curves are mean±SEM of 19 cells per condition. (F) Kinetics of SMARCA5-GFP recruitment to sites of DNA damage induced by 405 nm laser irradiation in U2OS cells knocked-down for ACF1 (siACF1-2) or treated with scrambled siRNA (siCTRL). Curves are mean±SEM of 16-18 cells per condition. For B and E, scale bars, 5 μm.

Supplementary figure 2. Mechanisms underlying the recruitment of ACF1 and SMARCA5 at DNA lesions. (A-B) Representative images (A) and quantification (B) of the recruitment of endogenous SMARCA5 to sites of DNA damage in U2OS cells treated or not with 30 μM of the PARP inhibitor Olaparib (PARPi). Cells were fixed 5 min after irradiation and stained for Hoechst, the DNA damage marker γH2AX and SMARCA5. Quantification was performed on 32-37 cells. (C) Immunoprecipitation of YFP-tagged PARP1 in U2OS cells treated or not with 2 mM H₂O₂ for 15 min and with 30 μM of the PARP inhibitor Olaparib (PARPi). Blots were probed for YFP and SMARCA5. (D) The GFP tagged macrodomain of macroH2A1.1 (macro-GFP) tethered to the LacO array is able to recruit ADP-ribosylated mCherry-PARP1 after DNA damage induction. Insets, pseudocolored according to the look-up table on the right, show magnification of the LacO array. (E) Boxplots show mCherry-PARP1 intensity at the LacO array tethering macro-GFP. The average intensity at the spot is quantified pre and 60 s post damage, corrected for background and

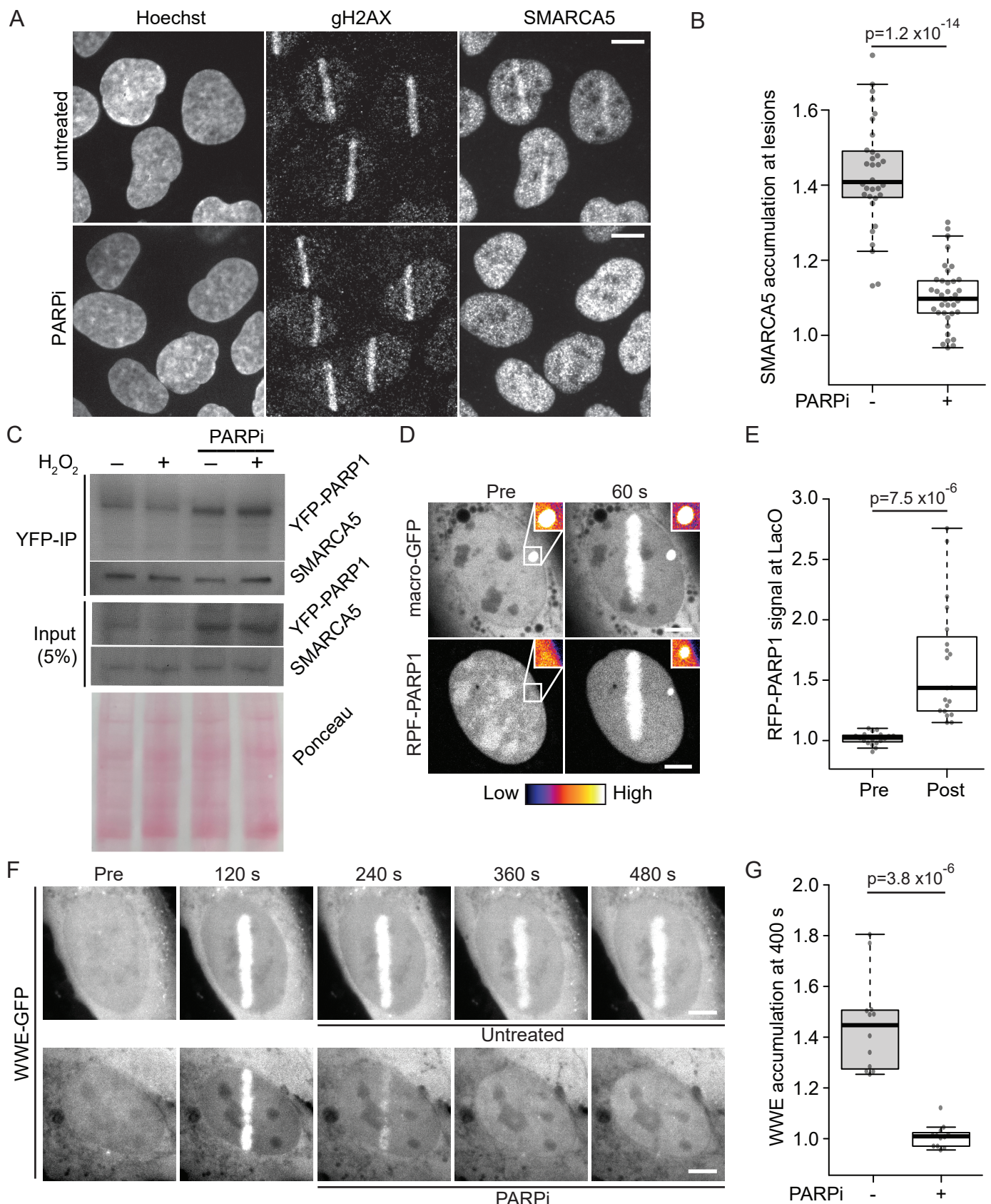
normalised to average intensity of the nucleus. 18-20 cells per condition. (F-G) Images (F) and quantification (G) of the accumulation of the GFP tagged WWE domain of RNF146 (WWE-GFP) at sites of DNA damage in cells were left untreated or treated with 30 μ M Olaparib (PARPi) 180 s after DNA damage induction. Quantification of protein accumulation was done 400 s after irradiation for 12 cells per condition. For A, scale bar, 10 μ m. For D and F, scale bars, 5 μ m.

Supplementary figure 3. The increased recruitment of SMARCA5 upon ALC1 overexpression relies on active ADP-ribose signaling. Images (A) and kinetics (B) of the recruitment to sites of damage of GFP tagged SMARCA5 in cells co-expressing mCherry tagged wild-type ALC1 or the ATPase-dead ALC1-K77R mutant, and treated or not with 30 μ M Olaparib (PARPi). Curves are mean \pm SEM of 16-19 cells per condition. For A, scale bars, 5 μ m.

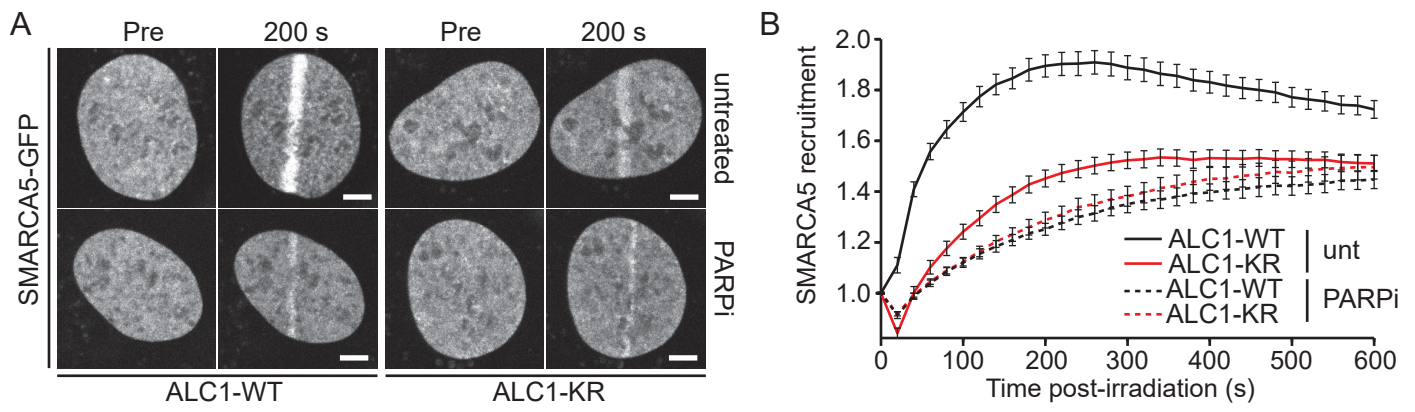
Supplementary figure 4. The recruitment of ACF1 to sites of damage is not controlled by histone binding. Images (A) and kinetics (B) of the recruitment to sites of damage of GFP tagged full-length ACF1 or a mutant lacking the PHD and BRD domains (ACF1- Δ C). Curves are mean \pm SEM of 18-19 cells per condition. For A, scale bars, 5 μ m.



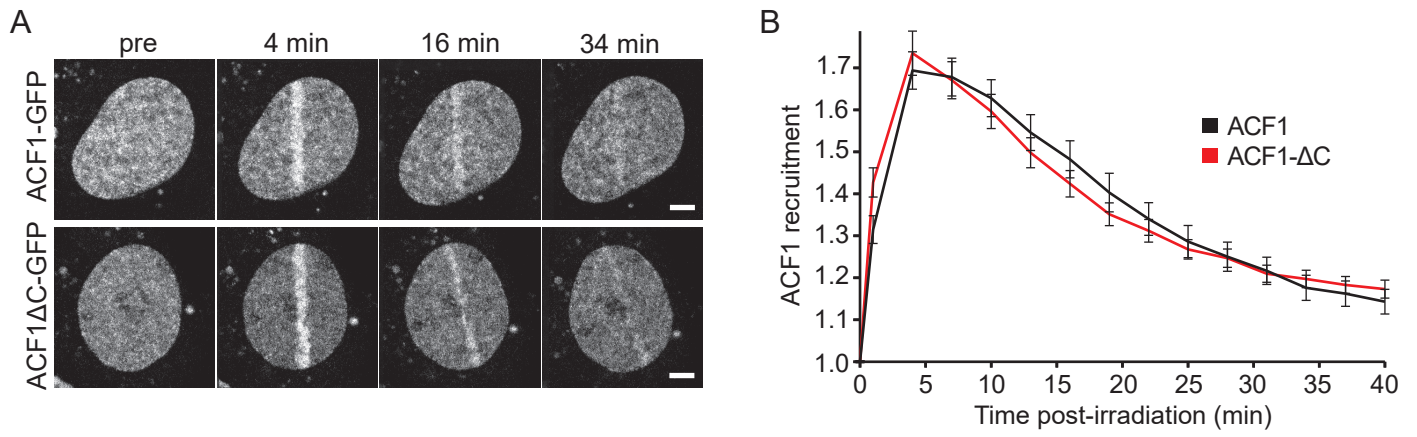
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