Expanded View Figures

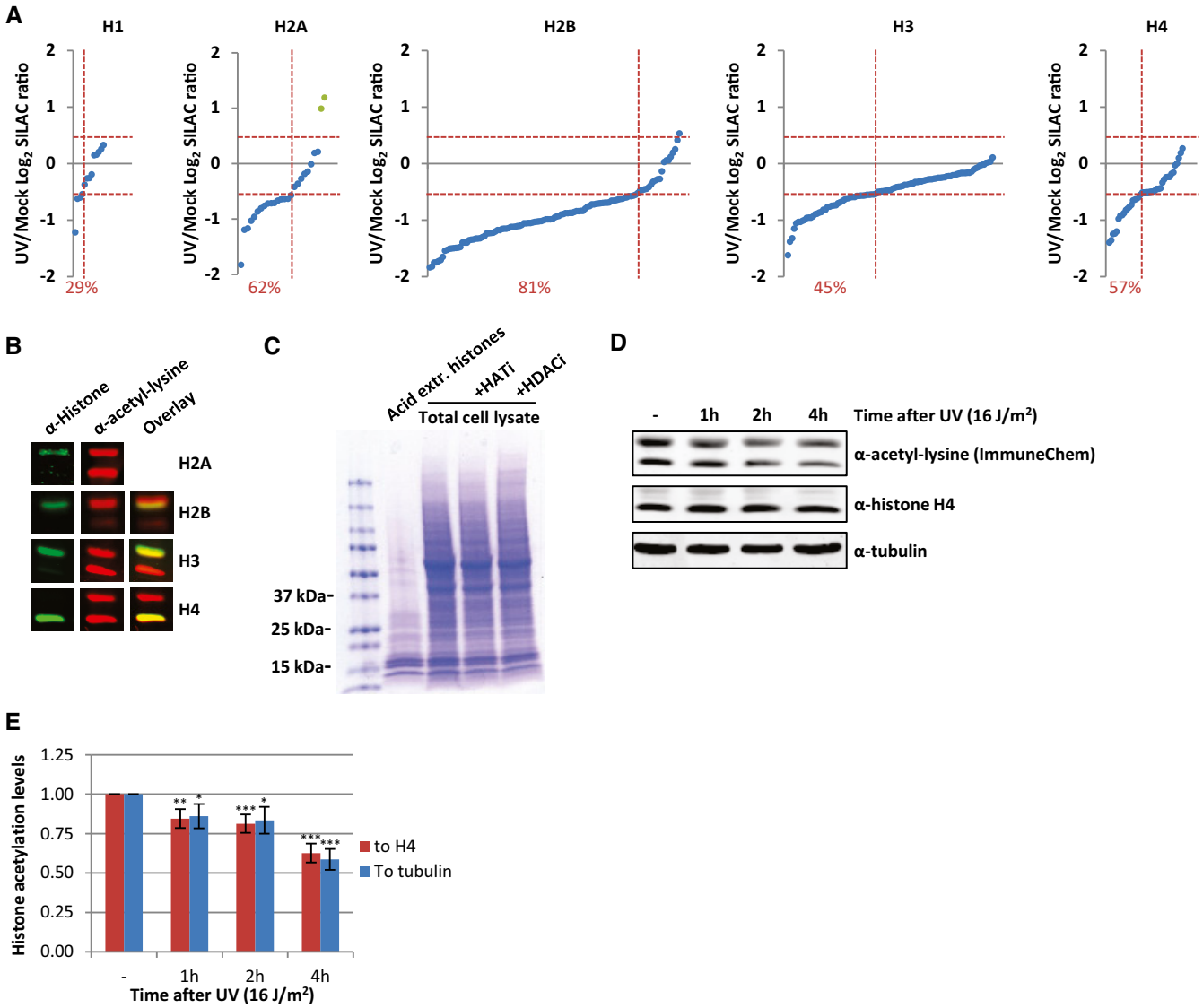

Figure EV1. UV-induced decrease in acetylated histones.

A Identified acetylated peptides per histone plotted against their $\log_2$ SILAC ratio (1 h after 16 J/m^2 UV/mock), ranked by SILAC ratio. Green dots represent the identified peptides from histone variant H2AZ. The percentage of peptides with a SILAC ratio < −0.5 are indicated in red.

B Western blots of WCE from HeLa cells stained with α-histone H2A, α-histone H2B, α-histone H3, and α-histone H4 (depicted in green) and α-acetyl-lysine (depicted in red) indicating that the strong acetyl-lysine signal of the low molecular weight bands originates from acetylated core histones. H2A and acetyl-lysine antibodies were raised in the same species and therefore did not allow an overlay.

C Coomassie staining of extracted histones and WCE from the same number of HeLa cells, treated with HATi (CTK7A, 100 μM and CPTH2, 50 μM) or HDACi (TSA, 1 μM) or mock-treated.

D Western blots of HeLa cells lysed at different time points after irradiation with UV (16 J/m^2). Blots were stained with α-acetyl-lysine (Immunechem, top panel), α-histone H4 (middle panel), and α-tubulin (bottom panel).

E Quantification of the α-acetyl-lysine signal normalized against either the histone H4 levels (red bars) or tubulin levels (blue bars). Error bars represent SEM. $N = 7$. Significant differences between UV-treated and mock-treated conditions are calculated with *t*-test and indicated with *$P < 0.1$, **$P < 0.05$, and ***$P < 0.01$.

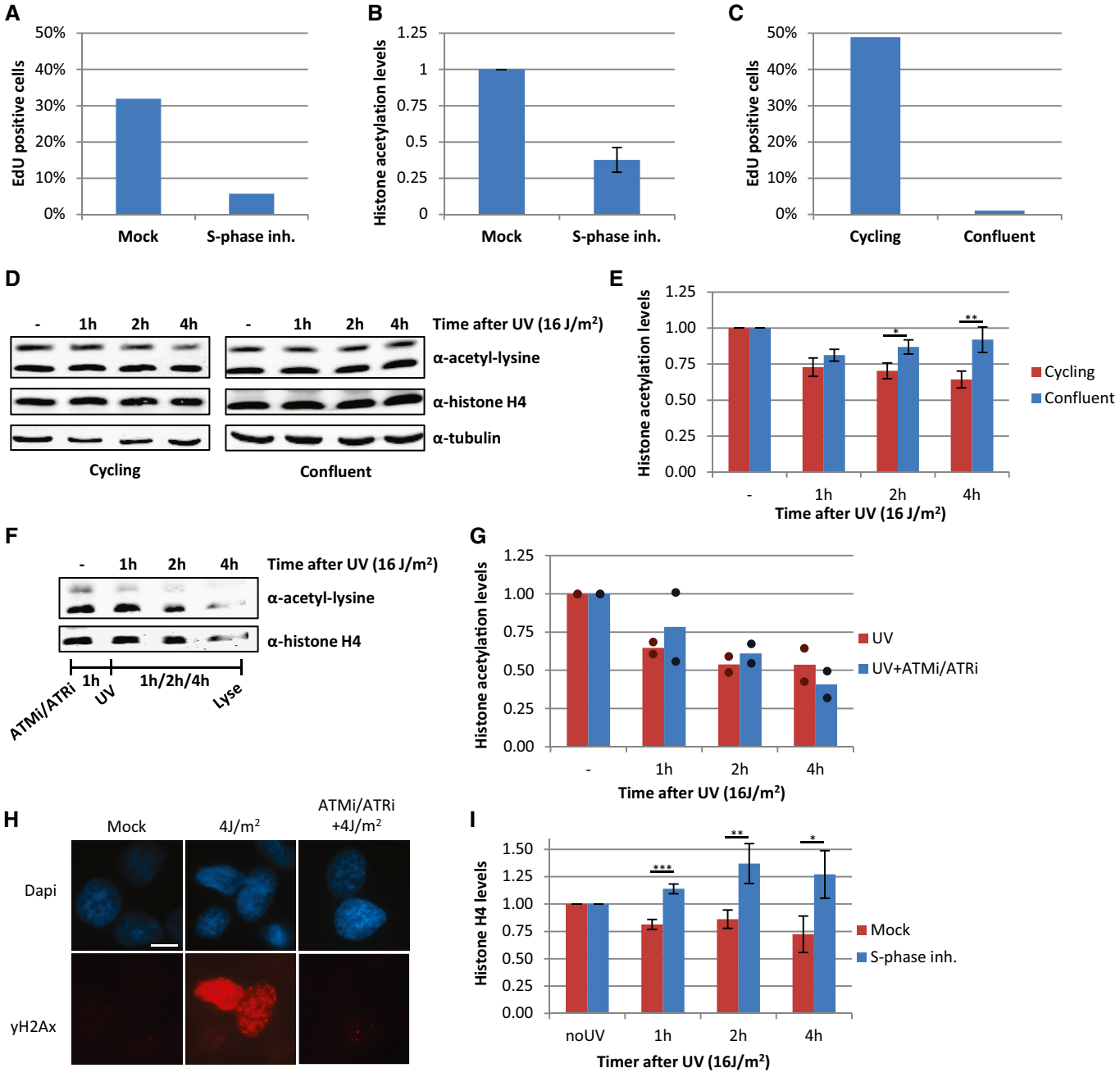
A
EdU positive cells
50%
40%
30%
20%
10%
0%
Mock
S-phase inh.
B
Histone acetylation levels
1.25
1
0.75
0.5
0.25
0
Mock
S-phase inh.
C
EdU positive cells
50%
40%
30%
20%
10%
0%
Cycling
Confluent
D
- 1h 2h 4h - 1h 2h 4h Time after UV (16 J/m²)
α-acetyl-lysine
α-histone H4
α-tubulin
Cycling
Confluent
E
Histone acetylation levels
1.25
1.00
0.75
0.50
0.25
0.00
- 1h 2h 4h
Time after UV (16 J/m²)
Cycling
Confluent
F
- 1h 2h 4h Time after UV (16 J/m²)
α-acetyl-lysine
α-histone H4
ATMi/ATRi
1h
UV
1h/2h/4h
Lyse
G
Histone acetylation levels
1.25
1.00
0.75
0.50
0.25
0.00
- 1h 2h 4h
Time after UV (16J/m²)
UV
UV+ATMi/ATRi
H
Mock
4J/m²
ATMi/ATRi +4J/m²
Dapi
γH2Ax
I
Histone H4 levels
1.50
1.25
1.00
0.75
0.50
0.25
0.00
noUV 1h 2h 4h
Timer after UV (16J/m²)
Mock
S-phase inh.

Figure EV2.

Figure EV2. Loss of acetylated histones is caused by replication stress.

A Relative amount of HeLa cells positively stained for EdU incorporation (2 h, 20 μM), quantified from immunofluorescence images (> 120 cells analyzed).
B Quantification of histone acetylation levels, normalized against histone H4 levels of HeLa cells that are cultured with S-phase inhibitor (10 μM, o/n) or mock-treated. Error bars represent SEM, $N = 5$.
C Relative amount of EdU-positive, cycling, or contact-inhibited (confluent) VH10 cells, quantified from immunofluorescence images (> 100 cells analyzed).
D Western blots of WCE from confluent (right panel) or cycling (left panel) VH10 cells lysed at different time points after UV (16 J/m^2). Blots were stained with α-acetyl-lysine (top panel), α-histone H4 (middle panel), and α-tubulin (bottom panel).
E Quantification of histone acetylation levels in confluent or cycling VH10 cells at different time points in which the histone acetylation levels are normalized against histone H4 levels after UV (16 J/m^2). $N = 3$, error bars represent SEM. Significance of differences between cycling and confluent cells is calculated by *t*-test and indicated with $*P < 0.1$ and $**P < 0.05$.
F Western blot of WCE, made at different time points after UV irradiation (16 J/m^2) from HeLa cells pre-treated for 1 h with a combination of ATM (Ku55933, 10 μM) and ATR (VE-821, 10 μM) inhibitors.
G Quantification of histone acetylation levels in cells treated with ATM and ATR inhibitors an hour before UV irradiation (16 J/m^2). Bar graph represents mean, and dots represent individual data points. $N = 2$
H Representative immunofluorescence images of γ-H2AX staining following, mock treatment, UV-induced DNA damage (4 J/m^2) with or without ATM and ATR inhibitors as indicated. Scale bar 10 μm.
I Quantification of histone H4 levels in HeLa cells pre-treated o/n with an S-phase inhibitor (PHA 767491 hydrochloride, 10 μM) and lysed at indicated time points after UV (16 J/m^2). $N = 5$, error bars represent SEM, and data are normalized against tubulin levels. Significant differences, calculated with *t*-test, between UV-irradiated and inhibitor-treated conditions are indicated with $*P < 0.1$, $**P < 0.05$, and $***P < 0.01$.

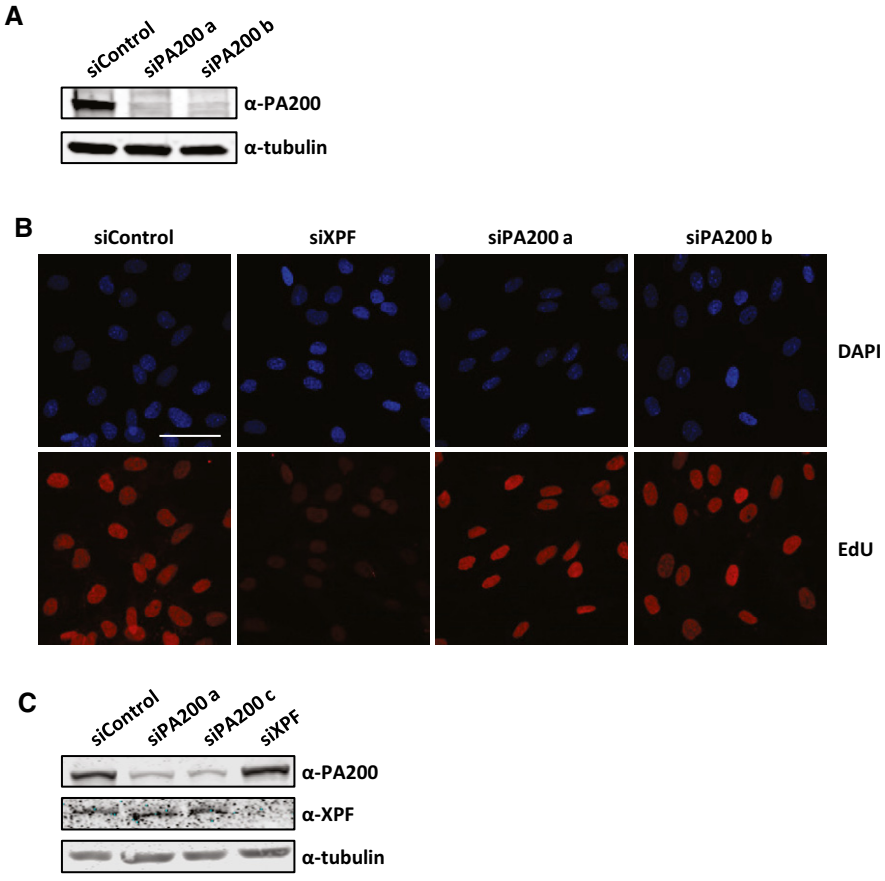

Figure EV3. Histone degradation does not affect repair of UV lesions.

A Western blot showing the knockdown efficiency of the siRNAs targeting PA200.
B Representative images of non-cycling C5RO cells treated with EdU (20 μM) for 3 h after UV irradiation (16 J/m^2) visualized by ATTO594 labeling using Click-chemistry. Scale bar 50 μm.
C Western blot showing the knockdown efficiency of the siRNAs targeting PA200 and XPF.

