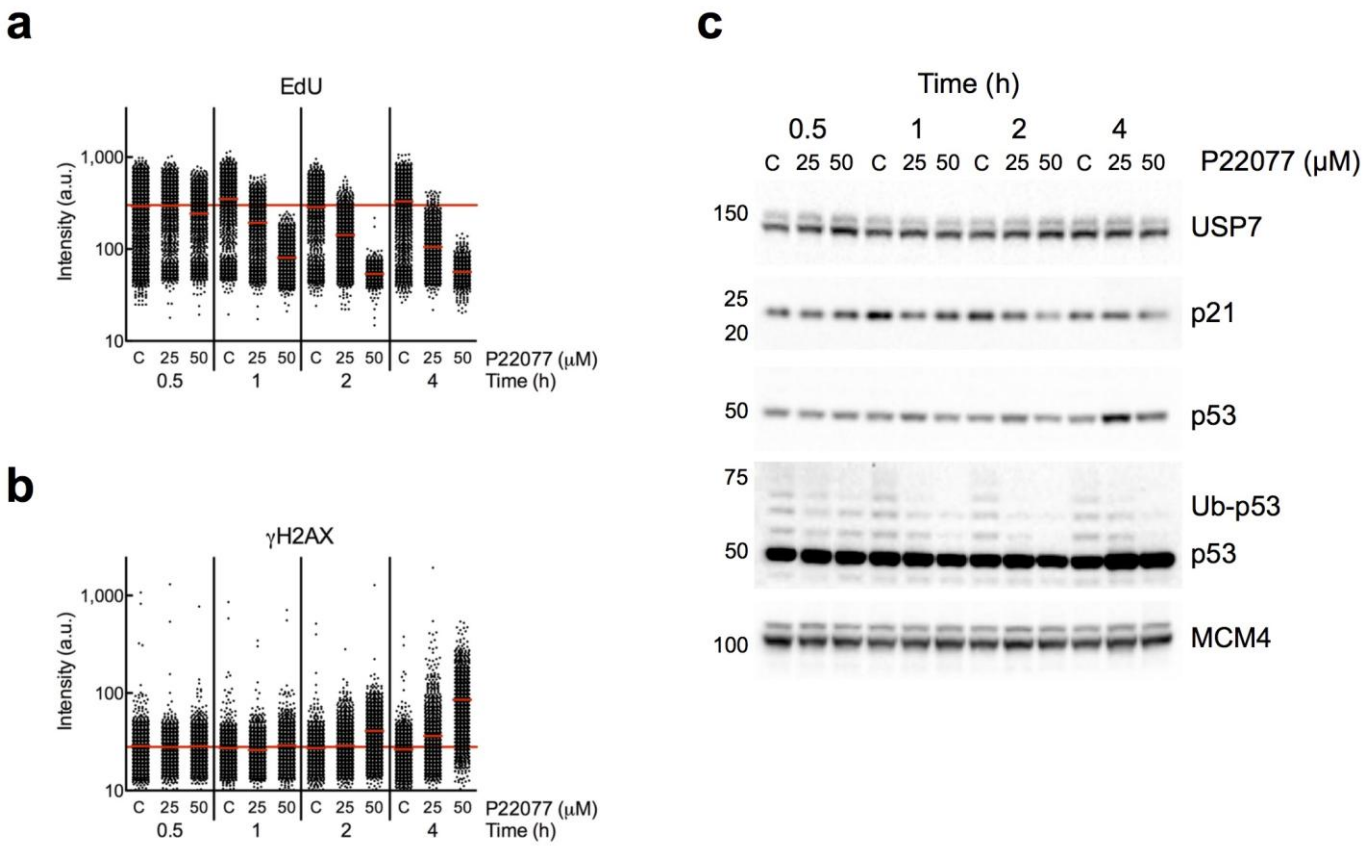
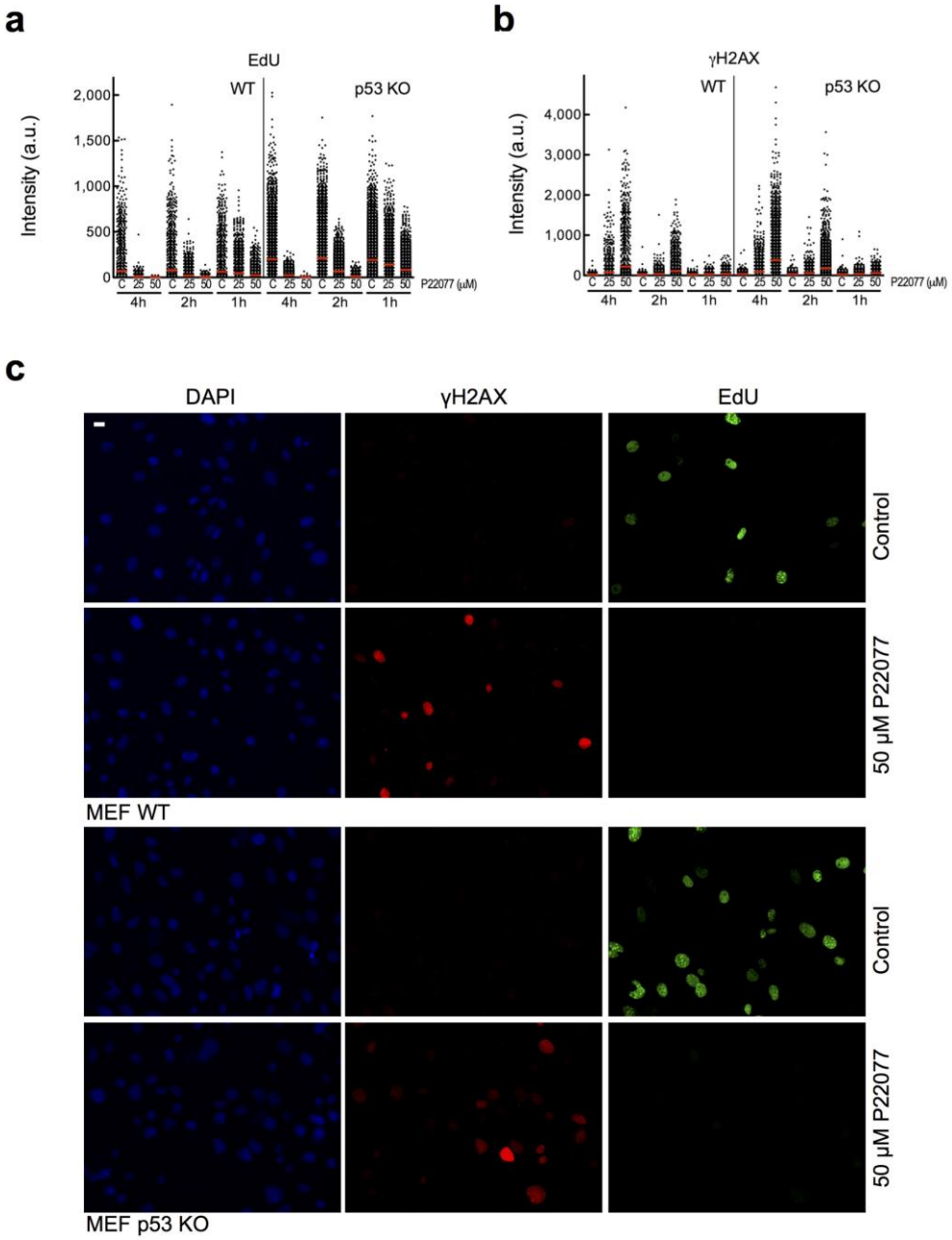


Supplementary Figure 1

USP7 inhibition stops DNA replication in a time- and dose-dependent manner.

(**a-b**) HCT116 cells were treated with increasing concentrations of P22077 or DMSO as a control for the indicated times. After incubation with 20 μM EdU for 30 min, cells were fixed and analyzed by HTM that quantified EdU (**a**) and γH2AX (**b**) levels per individual nucleus. DAPI was used to identify nuclei.

(**c**) Whole cell extracts of HCT116 cells treated with increasing concentrations of P22077 or DMSO (C) for the indicated times were analyzed by WB with antibodies against USP7, p21, p53 and MCM4.

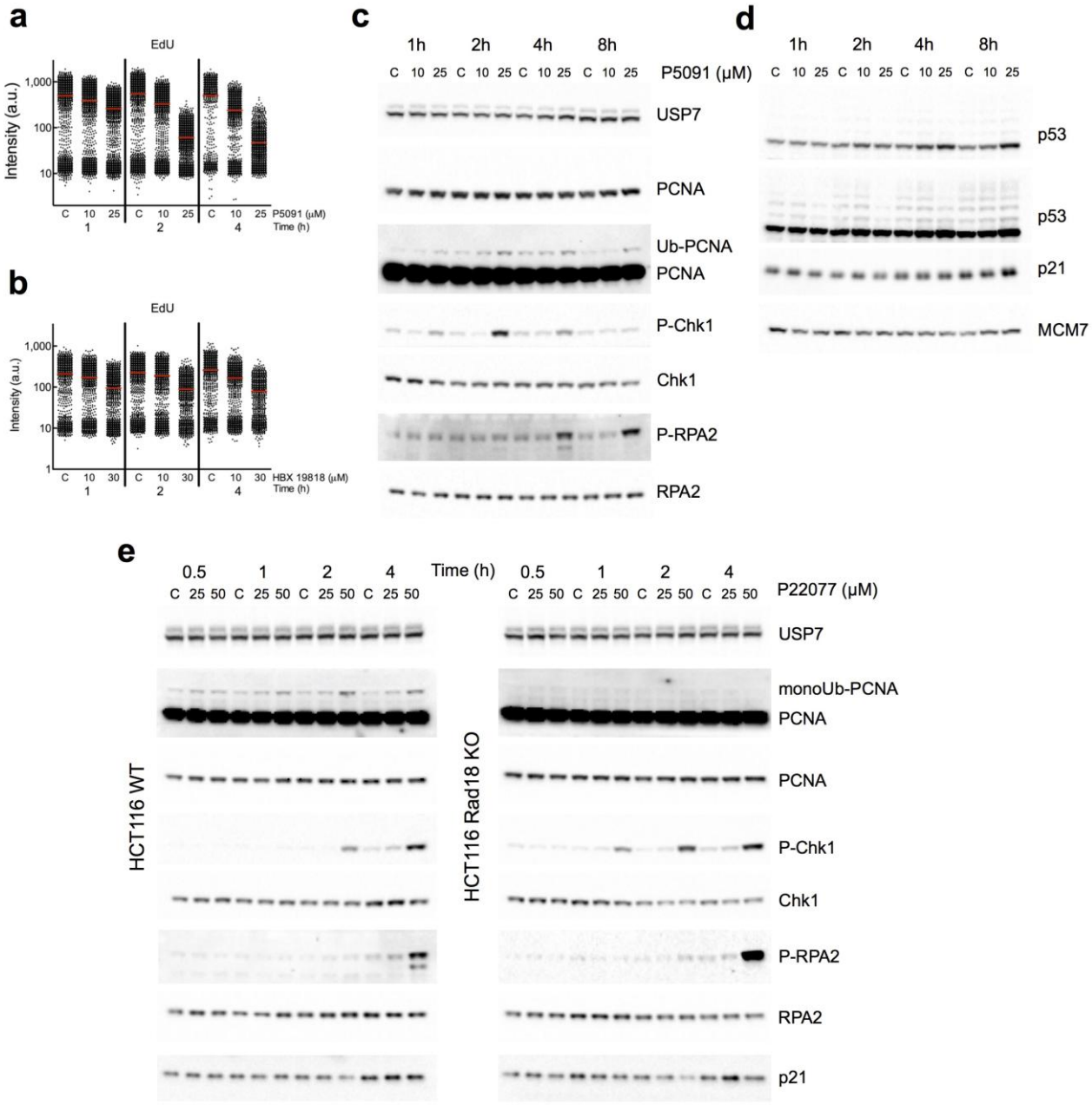


Supplementary Figure 2

The effects of USP7 inhibition on DNA replication and replicative stress are independent of p53.

(**a-b**) Wild type (WT) and p53-deficient (p53 KO) MEF were treated with increasing concentrations of P22077 or DMSO (C) for the indicated times. After incubation with 20 μM EdU for 30 min, cells were fixed and analyzed by HTM that quantified EdU (**a**) and γH2AX (**b**) levels per individual nucleus.

(**c**) Representative IF examples illustrating the levels of EdU (green) and γH2AX (red) observed in WT and p53 KO MEF after 4 h of treatment. DAPI (blue) was used to identify nuclei. Scale bar, 20 μm.



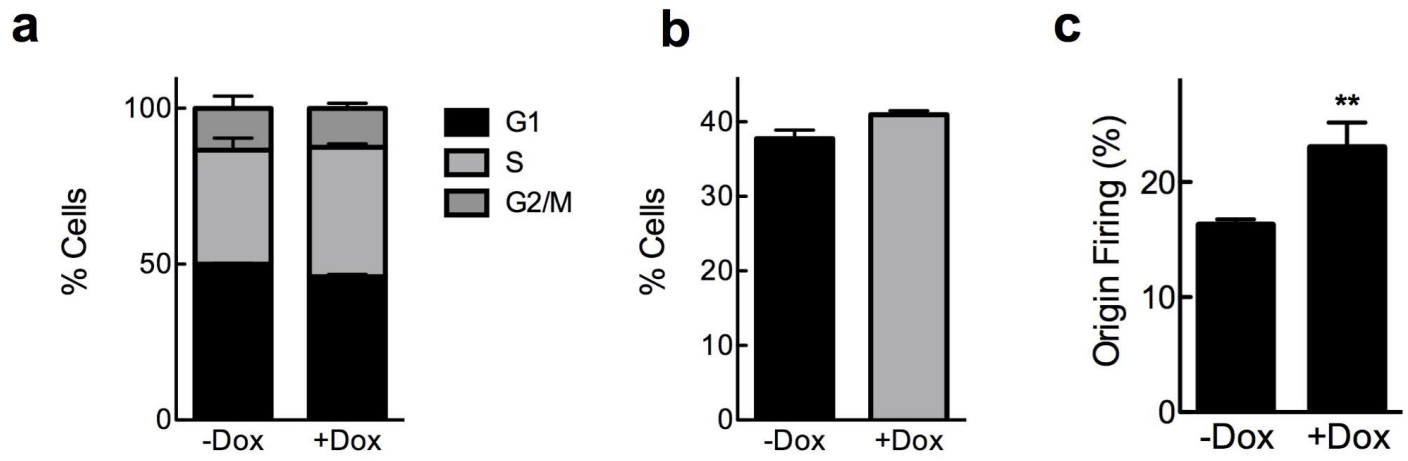
Supplementary Figure 3

Two independent USP7 inhibitors recapitulate the effects of P22077, independently of PCNA ubiquitination.

(**a-b**) HCT116 cells were treated with increasing concentrations of P5091 (**a**) or HBX19818 (**b**), using DMSO as a control for the indicated times. After incubation with 20 μM EdU for 30 min, cells were fixed and analyzed by HTM that quantified EdU levels per individual nucleus. Equivalent results were obtained in two independent experiments.

(**c-d**) Whole cell extracts of HCT116 cells treated with increasing concentrations of P5091 or DMSO as a control for the indicated times were analyzed by WB with antibodies against USP7, PCNA, total and phosphorylated Chk1 (S345), and total and phosphorylated RPA2 (S4/S8) (**c**), or with antibodies against p53, p21 and MCM7 (**d**). Equivalent results were obtained in two independent experiments.

(**e**) Whole cell extracts of HCT116 wild-type (WT) and Rad18 KO cells treated with increasing concentrations of P22077 or DMSO (C) for the indicated times were analyzed by WB with antibodies against USP7, PCNA, total and phosphorylated Chk1 (S345), and total and phosphorylated RPA2 (S4/S8), and p21. Equivalent results were obtained in two independent experiments.

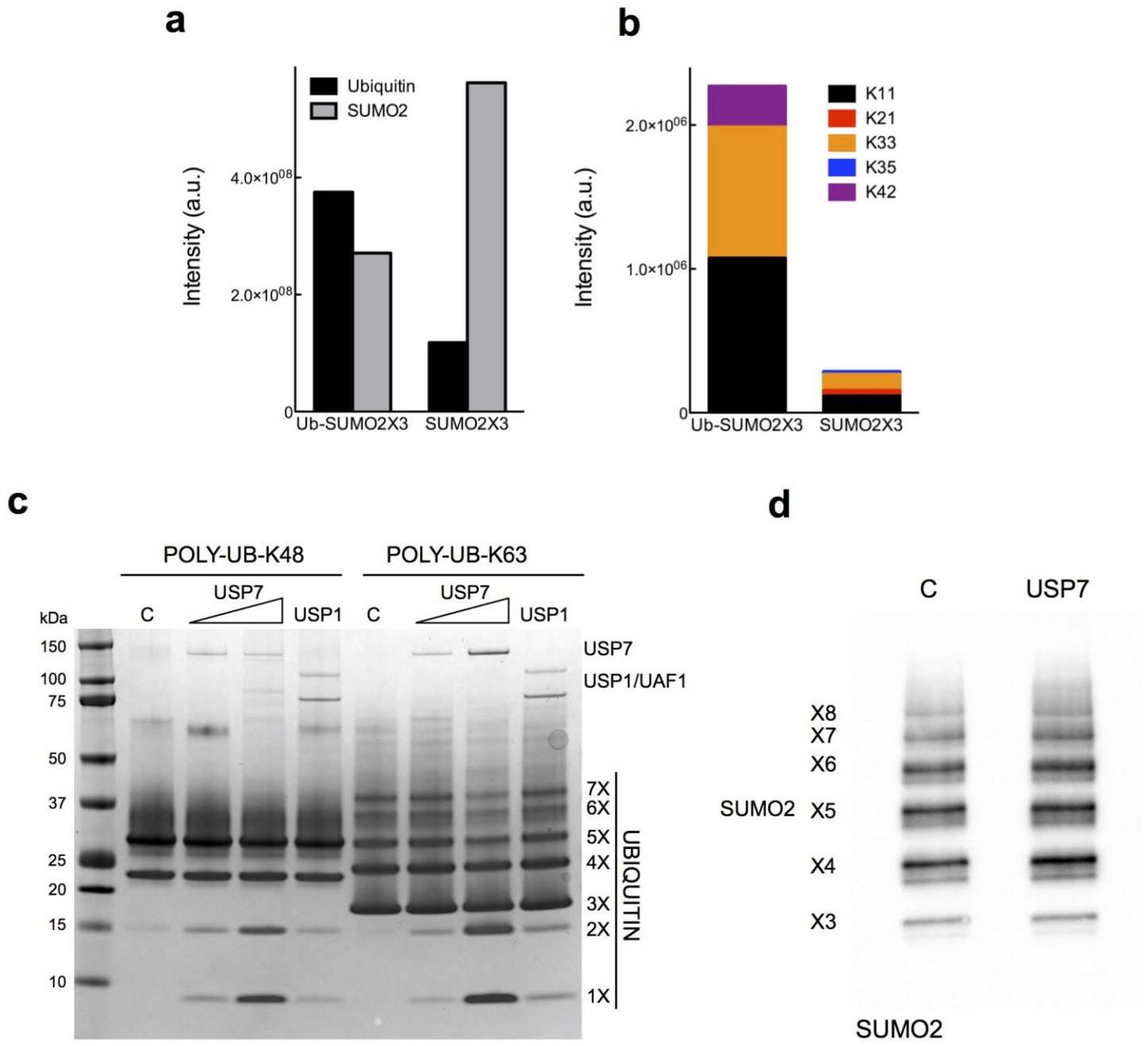


Supplementary Figure 4

Effect of USP7 overexpression on cell-cycle progression and origin firing.

(**a**) Cell cycle distribution of 293T-REx cells OneStrep-FLAG-HA-USP7 after being incubated for 1 day with (+Dox) or without (-Dox) 1 μg/ml doxycycline, measured by FACS staining with propidium iodide (PI) and BrdU. The percentage of BrdU cells is shown in (**b**).

(**c**) DNA fibers were extracted from 293T-REx OneStrep-FLAG-HA-USP7 cells incubated for 1 day with (+Dox) or without (-Dox) 1 μg/ml doxycycline and then sequentially treated with CldU and IdU. The firing of new origins was quantified as in **Fig. 2e**. ** $p<0.01$, t-test, two tails.



Supplementary Figure 5

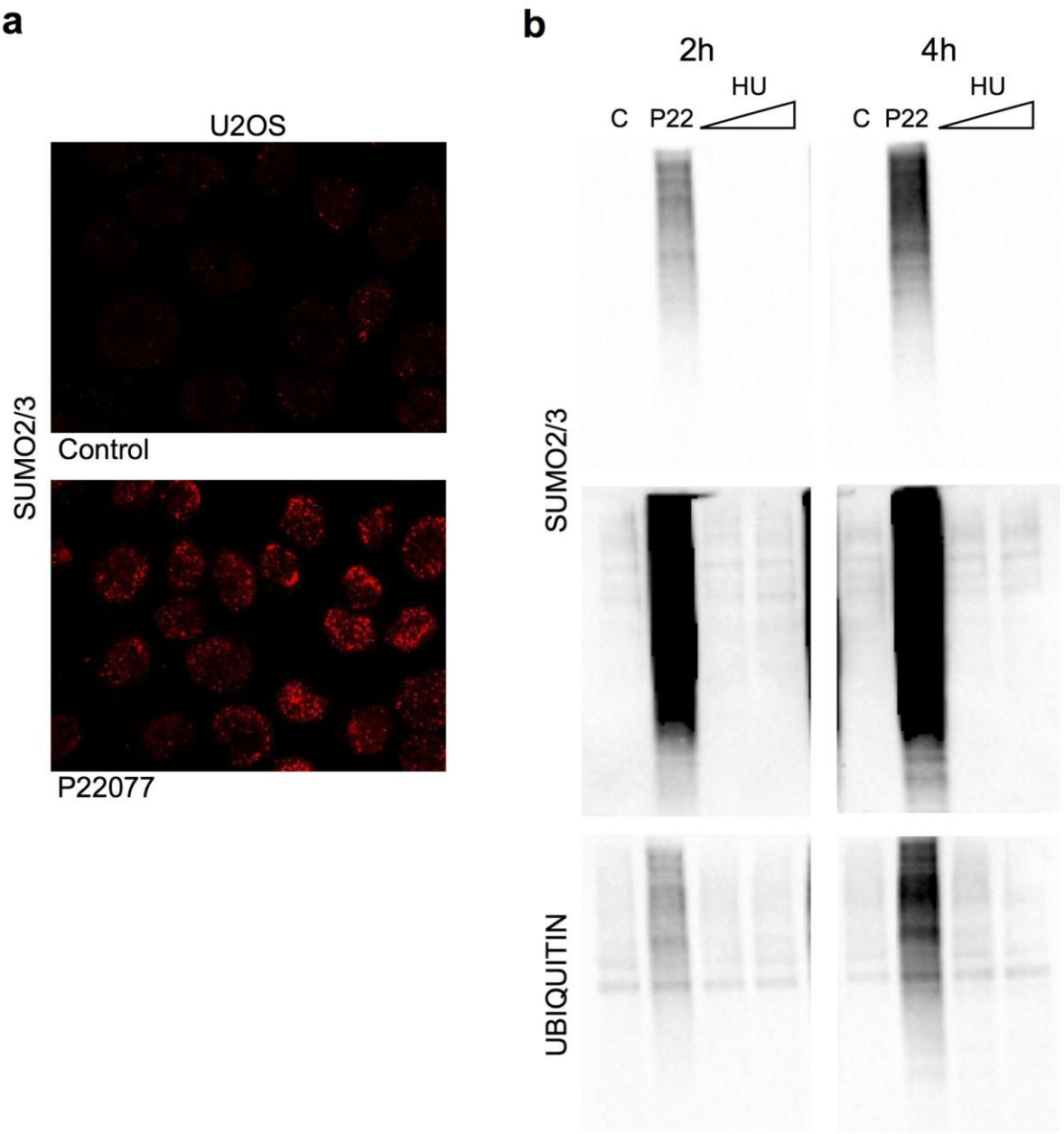
Mass spectrometry analysis of the effect of USP7 on poly-SUMO2 and polyubiquitin chains.

(**a**) The intensity of the peptides for ubiquitin (black) and SUMO2 (grey) in the Ub-SUMO2X3 and the SUMO2X3 bands is shown.

(**b**) Di-Gly containing peptides for SUMO2 were identified and the cumulative intensity of all these peptides is shown for both bands.

(**c**) Poly-Ubiquitin (3-7) K48 or K63 chains were incubated with 25-100 nM USP7 or 100 nM USP1/UAF1 or in the absence of enzyme (C) for 2h at 37ºC. The products of the reaction were detected by Coomassie staining. The position of the different chains of Ubiquitin is indicated, together with the position of USP7 and the USP1/UAF1 dimmer.

(**d**) Poly-SUMO2 (3-8) chains were incubated with 20 nM USP7 or in the absence of enzyme (C) and the levels of SUMO2 were measured by WB.

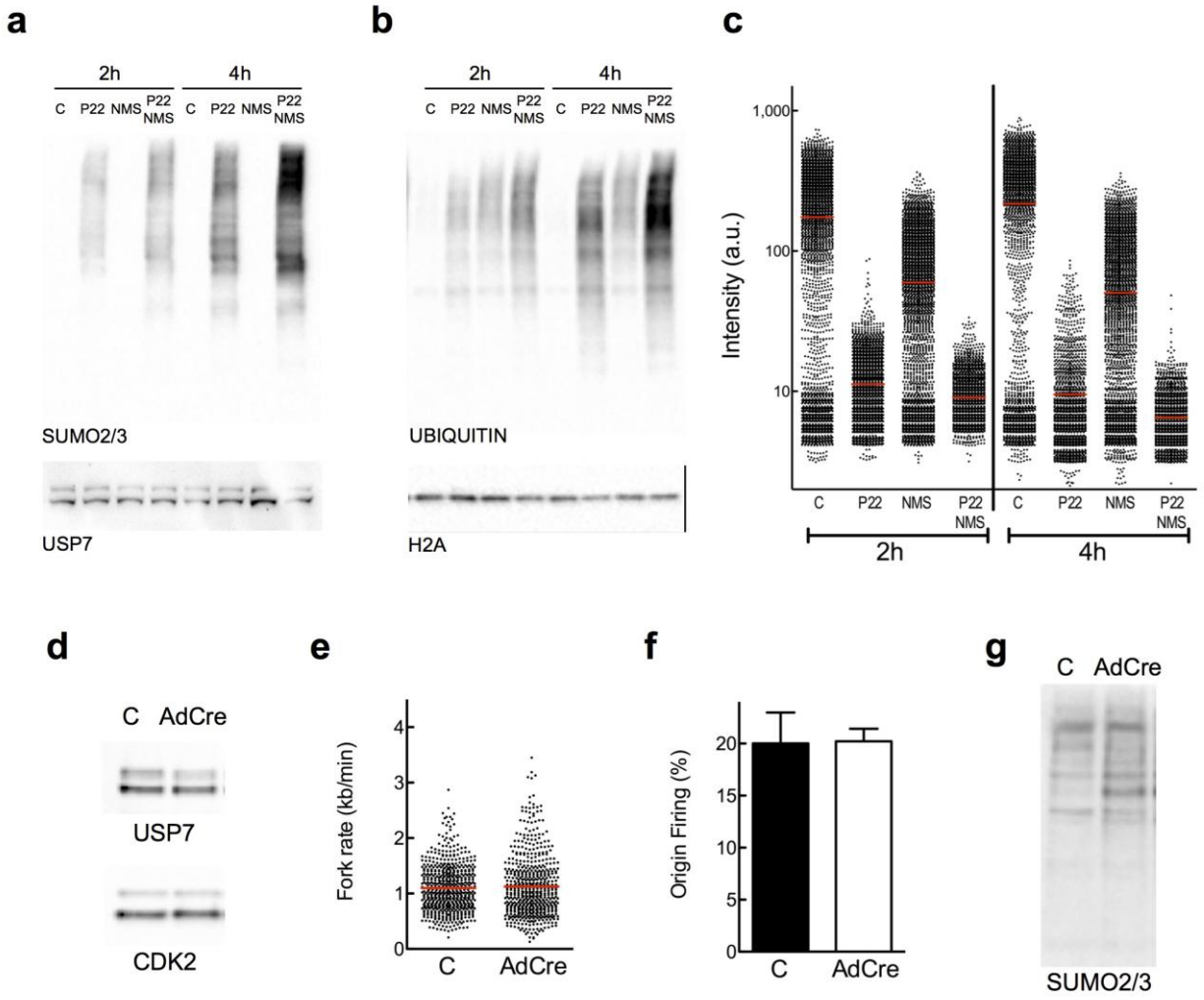


Supplementary Figure 6

Replication stress does not induce accumulation of SUMOylated proteins on chromatin.

(**a**) Representative IF images of U2OS cells treated with 50 μM P22077 or DMSO (C) for 4 h. Chromatin-bound levels of SUMO2 (red) were analyzed by a IF protocol that previously extracts the nuclear-soluble fraction of proteins. Besides this isolated example, the overall levels of chromatin-bound SUMO2/3 per individual nucleus as quantified by HTM are shown in **Fig. 4e**.

(**b**) HCT116 cells were treated with DMSO (C), 50 μM P22077 (P22) or 2-5 mM hydroxyurea (HU) for 2 h (left) or 4 h (right). The chromatin fraction was extracted and analyzed by Western blot with antibodies against SUMO2/3 or ubiquitin.



Supplementary Figure 7

Effects of inhibition of USP7 and/or p97 on DNA replication and effects of AdCre infection on WT MEFs.

(**a-b**) HCT116 cells were treated with 50 μM P22077 (P22), 5 μM NMS873 (NMS), both (P22-NMS) or DMSO as a control (C), for the indicated times. Chromatin fractions were analyzed by WB with antibodies against SUMO2/3 and USP7 (**a**), or Ub and histone H2A (**b**). Equivalent results were obtained in two independent experiments.

(**c**) HCT116 cells treated as in (**a**) were incubated with 20 μM EdU for 30 min. After incubation with 20 μM EdU for 30 min, cells were fixed and analyzed by HTM that quantified EdU levels per individual nucleus. The experiment was repeated three times and one representative experiment is shown.

(**d**) Western blot analysis of whole cell extracts from WT MEF mock infected (C) or infected with AdCre for 4 days. The levels of USP7 and CDK2 were measured using specific antibodies.

(**e-f**) DNA fibers were extracted 4 days after mock or AdCre infection of WT MEF and the fork rate (**e**) and percentage of origin firing (**f**) were measured. The experiments were repeated three times; the pool of the three experiments (fork rate) or the average (origin firing) is shown.

(**g**) Chromatin fraction of mock or AdCre infected WT MEF were assayed by Western blot with antibodies against SUMO2/3. Equivalent results were obtained in two independent experiments