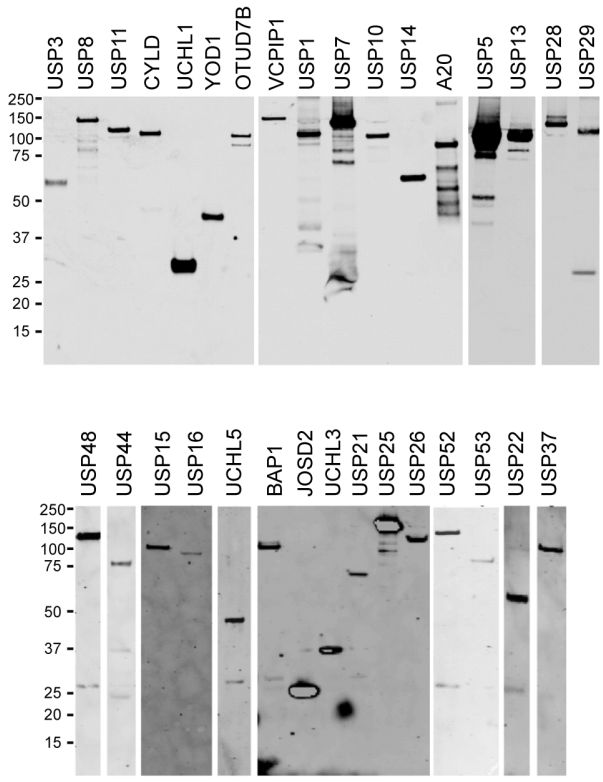
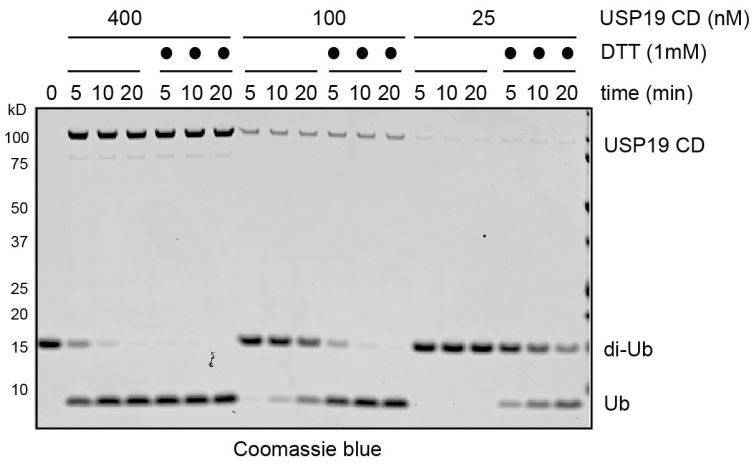


Supplementary Figure S1 Purification of deubiquitinases

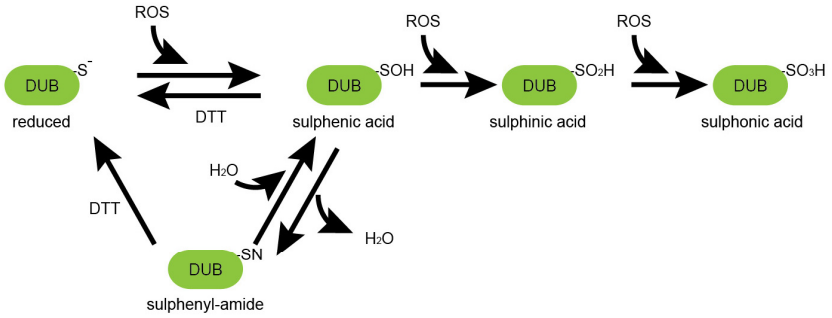
HEK293 cells were transfected with the indicated DUB-expressing plasmids. The cells were harvested 72 h after transfection. FLAG-tagged deubiquitinases were affinity-purified using FLAG-agarose beads. Purified proteins were analyzed by immunoblotting with the FLAG antibody.



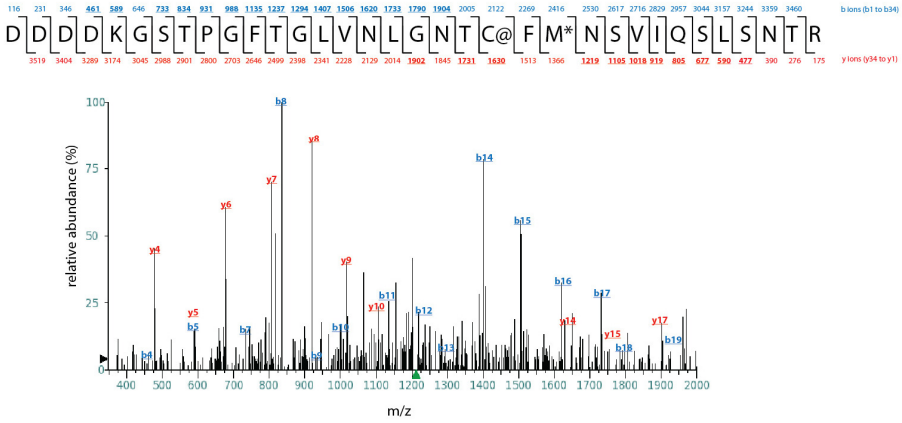
Supplementary Figure S2 Activation of USP19 CD by DTT

Purified USP19 CD was treated with or without 1mM DTT and incubated with di-ubiquitin for the indicated time periods at 37 °C. The cleavage of di-ubiquitin was detected by SDS-PAGE electrophoresis followed by Coomassie blue staining.

a

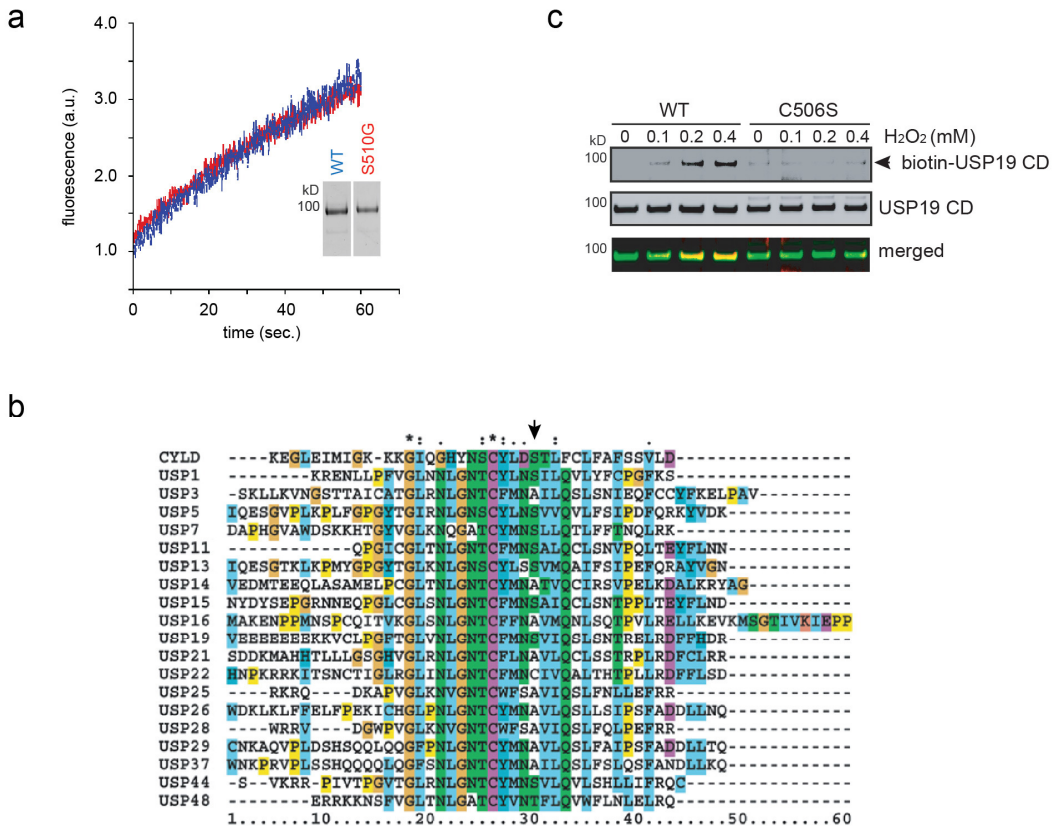


b



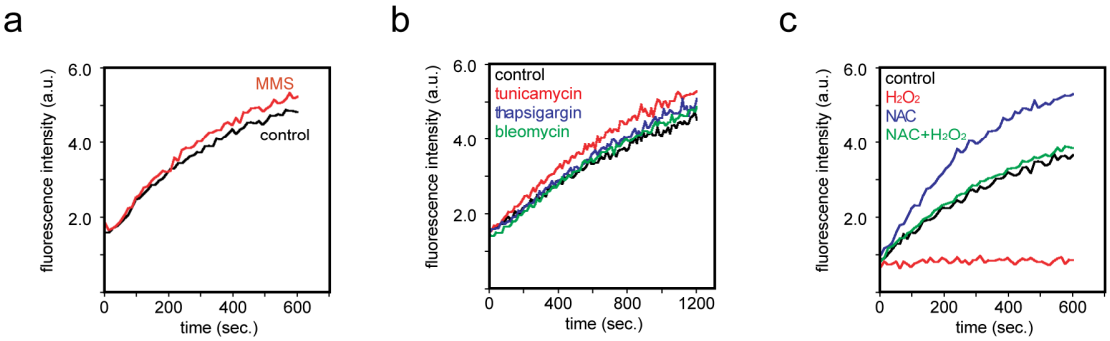
Supplementary Figure S3 Oxidation of the catalytic cysteine of USP19 in the cell

(a) The DUB redox species and their interrelationship. (b) The band indicated by the asterisk in Figure 3a was subject to trypsin digestion and mass spectrometry analysis. @ indicates the sulphenyl-amide modification, * indicates oxidized methionine.



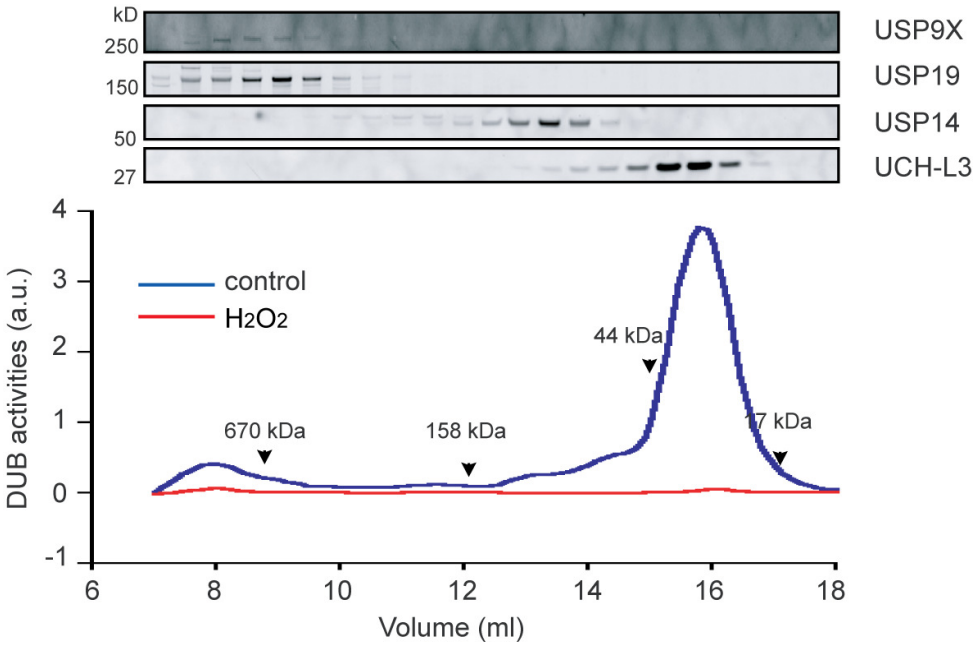
Supplementary Figure S4 Reversible inactivation of USP19 requires serine510

(a) The activities of USP19CD S510G and USP19CD WT as determined by the Ub-AFC assay. The gel shows the purified proteins. (b) Alignment of DUB sequences around the active site cysteine. The arrow indicates the Serine residue conserved among many DUBs. (c) The catalytic cysteine of USP19 can be oxidized by H_2O_2 *in vivo*. HEK293 cells transfected with the indicated DUB-expressing plasmids were treated with the indicated concentrations of H_2O_2 for 5min followed by *in vitro* biotin labeling.



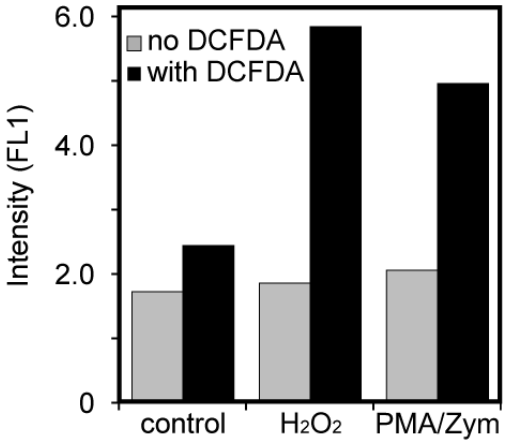
Supplementary Figure S5 Oxidative stress reduces DUB activities in cells

(a) HEK293 cells were treated with or without 1mM MMS for 1 hour. Same number of cells (~3,000) was directly added to a deubiquitinating assay buffer containing 0.5% NP40 and Ub-AFC. DUB activities were assayed by monitoring the fluorescence. (b) HEK293 cells were treated with 5µg/ml tunicamycin, 1 µM thapsigargin, or 200 µg/ml bleomycin, or as a control with DMSO for 1 hour. The DUB activities were analyzed as in (a). (c) The anti-oxidant NAC prevents DUB inhibition by H_2O_2. HEK293 cells were pretreated with or without 20mM NAC for 30min before addition of 1mM H_2O_2. Where indicated, cells were incubated with H_2O_2-containing medium for 2min prior to the DUB activity assay. 2,000 cells were assayed for each sample.



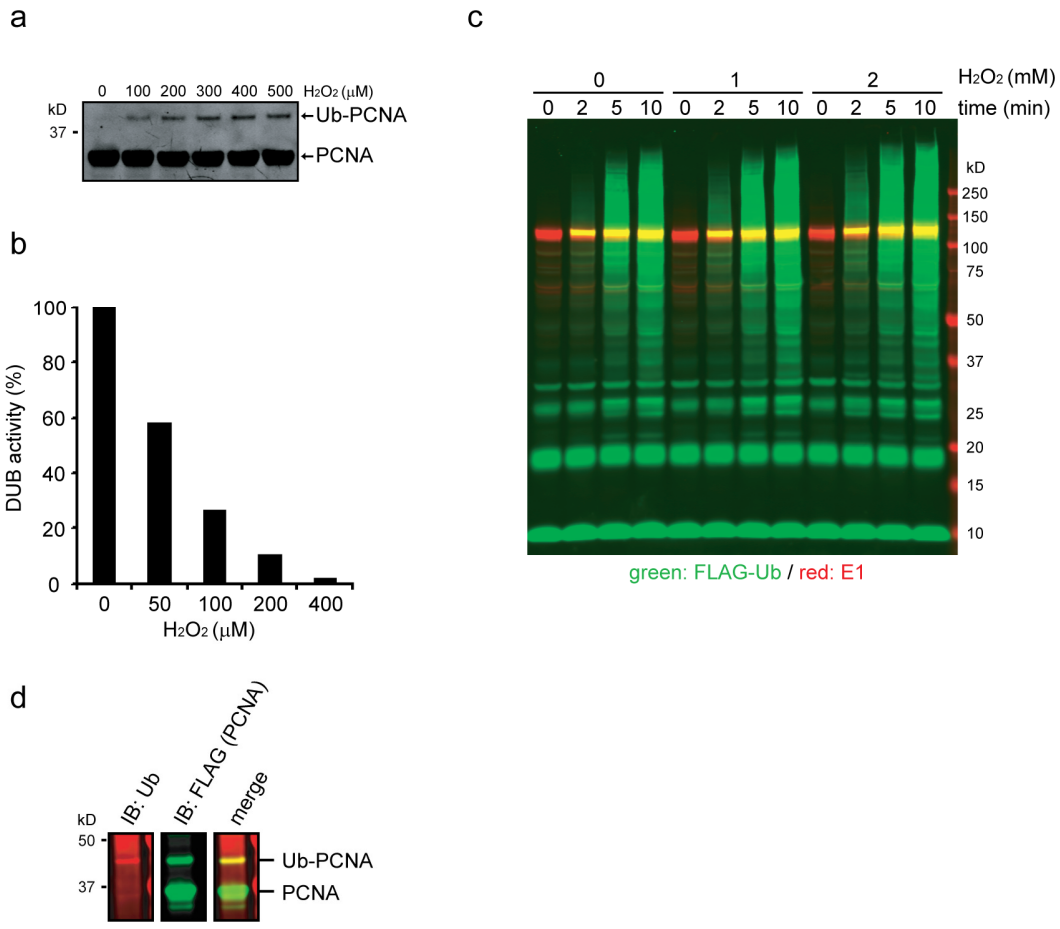
Supplementary Figure S6 DUB inhibition by ROS occurs in a global manner

Whole cell extract from HEK293 cells were subject to gel filtration analysis by a Superdex200 HR 10/30 column in a buffer containing 2mM DTT. The DUB activities in the resulting fractions were determined by incubating 2µl of each fraction in 100µl DUB assay buffer containing Ub-AFC. A portion of the samples (2µl) were treated with H_2O_2 (0.5mM, 1min) before the addition of Ub-AFC. Another fraction of the samples were also analyzed by immunoblotting with antibodies against the indicated DUBs.



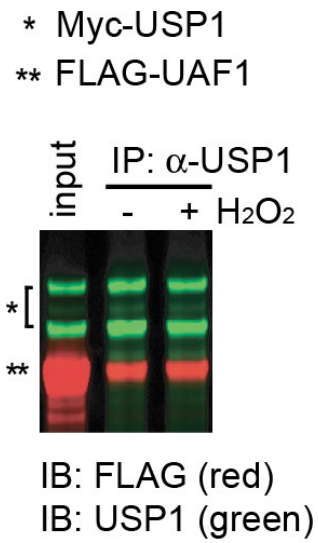
Supplementary Figure S7 ROS generation in macrophage cells

ROS generation was stimulated by an inflammatory response. RAW264.7 cells treated with 1mM H_2O_2 or 500nM PMA plus 0.5mg/ml Zymosan for 1hr were stained with 10μM DCFDA. Unstained cells were used as a negative control. Shown are the mean values of fluorescence intensity.



Supplementary Figure S8 Oxidative stress inhibits DUBs to modulate PCNA ubiquitination

(a) PCNA ubiquitination is induced by H_2O_2 in a dose dependent manner. HEK293 cells were treated with H_2O_2 as indicated for 10min. Cells were lysed in the Laemmli buffer for immunoblotting analyses. (b) Dose dependent inhibition of DUBs by H_2O_2. (c) H_2O_2 does not affect the ubiquitin conjugating system in HEK293 cells. Cell extracts from control or H_2O_2-treated cells were incubated with FLAG-tagged ubiquitin together with an energy regenerating system. *In vitro*-synthesized ubiquitin chains by endogenous enzymes were detected by immunoblotting. (d) Ubiquitination of PCNA *in vitro*. The purified PCNA-FLAG protein was incubated with UBE1 (E1), RAD6B (E2), RAD18 (E3), and bovine ubiquitin in the presence of ATP before immunoblotting analysis.



Supplementary Figure S9 H_2O_2 does not affect the interaction of USP1 with UAF1.

The purified USP1-UAF1 complex was incubated in a buffer in the absence (-) or presence (+) of 1mM H_2O_2 for 5min. The samples were then subject to immunoprecipitation using the USP1 antibody. The precipitated proteins were analyzed by immunoblotting (IB). A fraction of the untreated sample was included as the input.