Supplementary Data

REGULATION OF HISTONE H2A AND H2B DEUBIQUITINATION AND XENOPUS DEVELOPMENT BY USP12 AND USP46

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This file contains Supplementary Figure S1-S4 with Figure legends and Supplemental Table S1.

Figure S1. Characterization of USP12 and USP46 antibodies. Different amounts of purified USP12 and USP46 (as indicated on the top of the panel; purified from Sf9 cells, Figure S3) were analyzed by Western blot assay with antibodies as indicated on the left side of the panels.

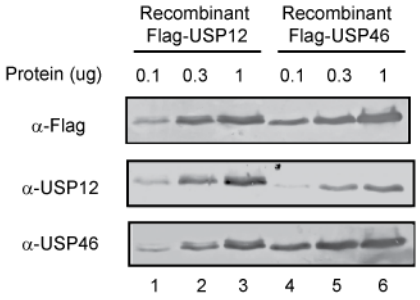
Figure S2. H2A deubiquitination assay (first and third panels) and Western blot analysis (second and fourth panels) of fractions derived from the Hydroxyapatite (top two panels) and Mono S (bottom two panels) columns. Antibodies used are indicated on the left side of the panels.

Figure S3. USP12 and USP46 single subunits could not deubiquitinate histone H2A. *A,* Schematic representation of the steps used to purify USP12 and USP46 from Sf9 cells. *B* and *C,* Silver staining of an SDS-PAGE (top panels) and Western blot analysis (bottom panels) of fractions derived from the Superose 6 column. The elution profile of the protein markers is indicated on the top of the panels. Antibodies used are indicated on the left side of the panels. *D,* Comparison of the H2A deubiquitination activity of USP12 and USP46 single subunits with native fractions. Top panel shows the Western blot analysis of USP12, USP46, and native fractions. Bottom two panels show the histone H2A deubiquitination assay of USP12, USP46, and native fractions.

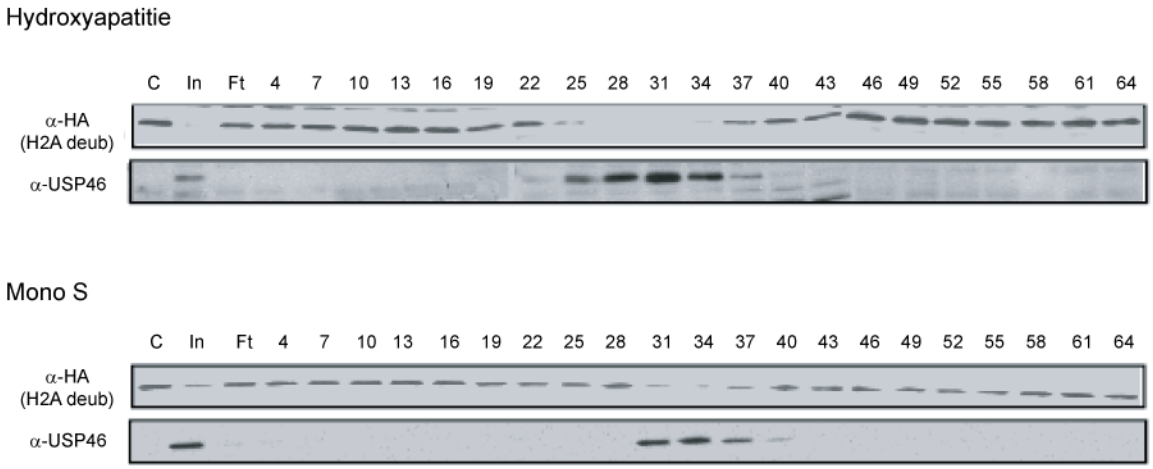
Figure S4. Semi-quantitative RT-PCR of the expression of *USP12* and *USP46* (top two panels) and selective *Hox* genes (third to sixth panels) in control and cells transfected with siRNA against USP12 and USP46 as indicated on the top of the panels. *GAPDH* was used as a control. Cells were harvested 96 hrs after initial transfection. siRNA transfection was carried out three times with two days interval.

Table S1. Identified peptides in the epitope-tagged purified USP12 complex. Affinity-purified samples were resolved by SDS-PAGE and individual bands were excised for mass spectrometry analysis as described in the materials and methods section. MASCOT and SEQUEST results were refined and combined using Peptide and Protein Prophet. Proteins listed had a Protein probability of 1.00 and a NSP adjusted peptide probability greater than 0.90 in order to be included.

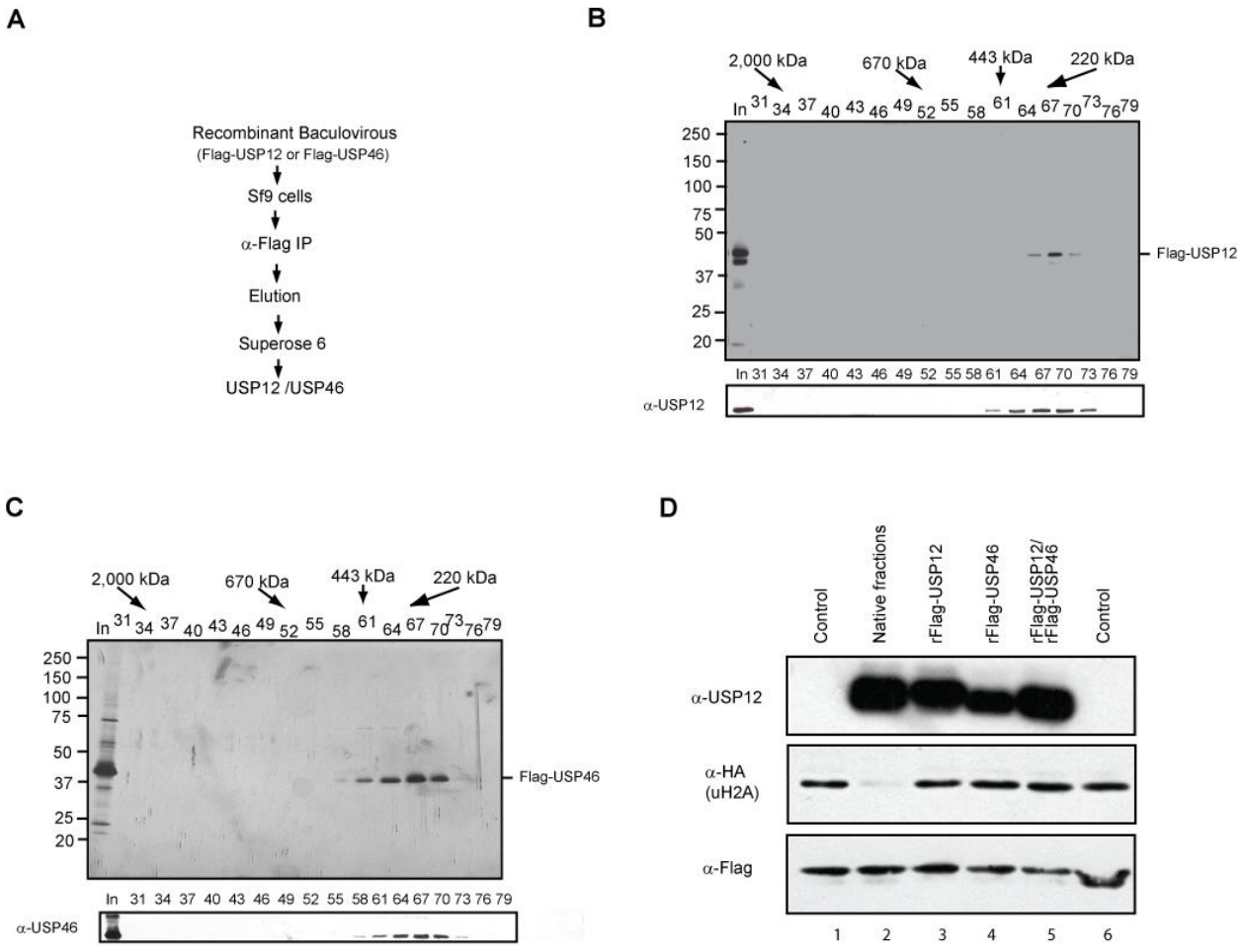
Supplemental Figure 1 (Joo et al.)



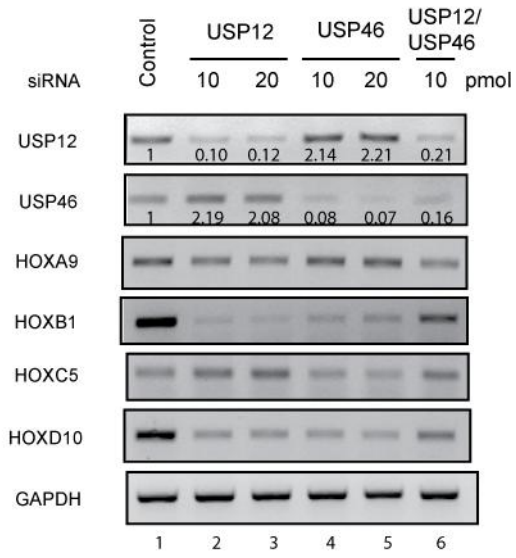
Supplemental Figure 2 (Joo et al.)



Supplemental Figure 3 (Joo et al.)



Supplemental Figure 4 (Joo et al.)



Supplemental Table S1 (Joo et al.)

Protein	Acession number	MW	Unique peptides
WDR48	Q8TAF3	76.1kDa	46
DMWD	Q09019	70.4kDa	9
WDR26	Q9H7D7	72.1kDa	9
MEP50 (WDR77)	Q9BQA1	36.7kDa	12
USP12	O75317	42.8kDa	22
WDR20	Q8TBZ3	62.8kDa	13