

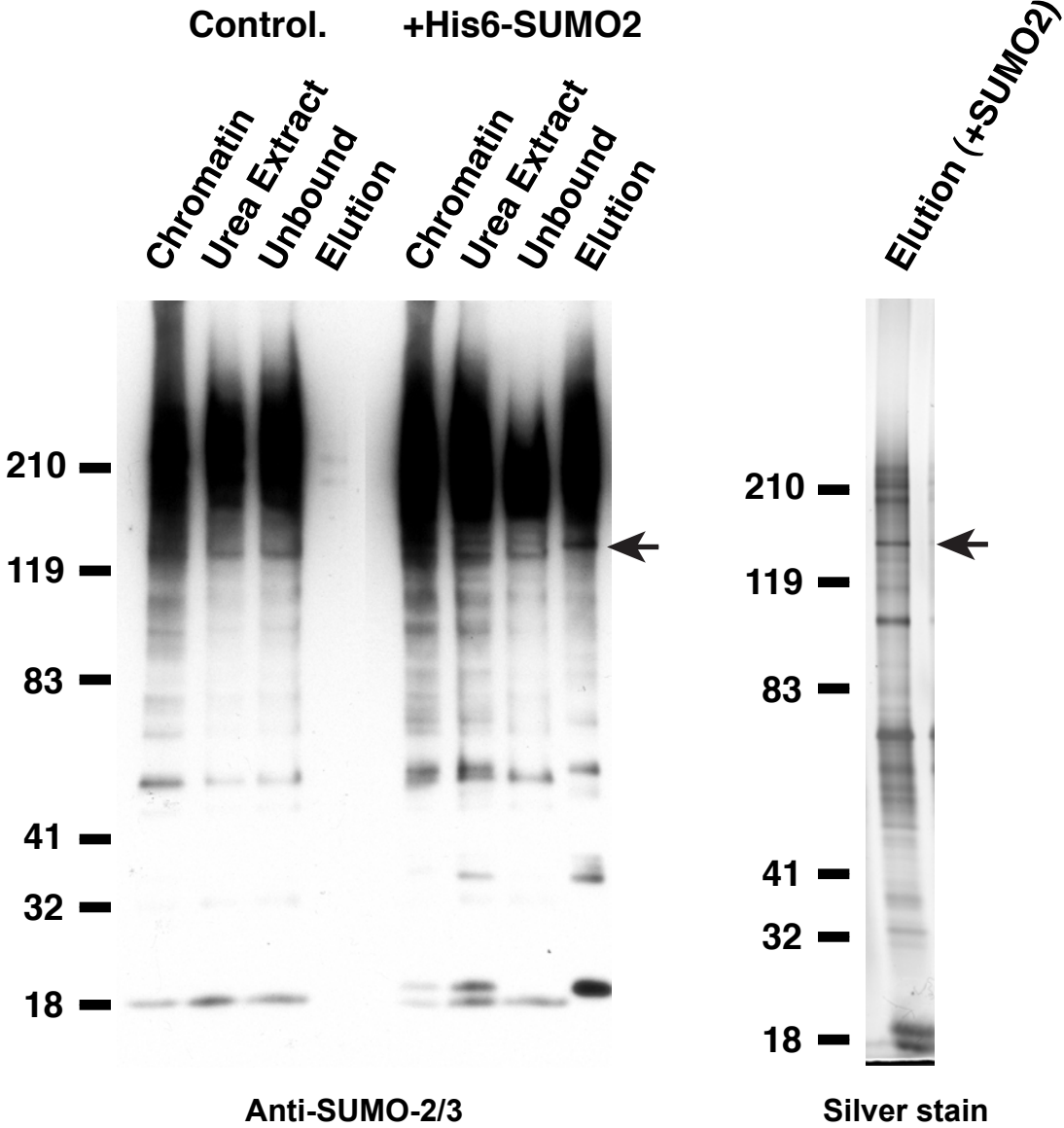
Supplemental Figure 1: Purification of novel SUMO-2 modified proteins from mitotic chromosomes.

Mitotic chromosomes were prepared with the CSF extract with or without His6-SUMO-2. After sixty minutes of incubation, the mitotic chromosomes were separated by centrifugation (Chromatin). Chromosomal proteins were extracted in a buffer containing 8.5M urea (Urea Extract), and the recovered fractions were subjected to further purification using metal affinity chromatography. Aliquots from unbound fractions and eluted fractions were analyzed by immunoblotting with an anti-SUMO-2/3 antibody together with Chromatin and Urea extract fractions (Left panel). Proteins in the elution from the His6-SUMO-2 containing sample were analyzed by silver staining (Right panel). Arrows indicate the isolated His6-SUMO-2 modified protein, which was subjected to identification by LS-MS/MS analysis.

Supplemental Figure 2. Mapping of SUMOylation site of PARP1 by MS/MS.

Chromosomal proteins were isolated by denaturing buffer containing SDS from mitotic chromosomes that were assembled in the indicated condition of CSF extracts. After renaturation with buffer containing thesitol, extracted fractions were subjected to immunoprecipitation with affinity purified anti PARP1 antibody. Precipitated fractions were separated by SDS-PAGE. Both SUMOylated (asterisk) and non-SUMOylated PARP1 (arrow head) were subjected to MS/MS analysis for mapping SUMOylation site. The SUMOylation site was identified by mass spectrometry following a double digestion with trypsin and chymotrypsin. The Chymotrypsin is responsible for generating the QQQTGG signature tag on the modified lysine which was observed for PARP1. MS/MS analysis indicates the Lysine at 482 as a candidate site of SUMOylation with over 70% of sequence coverage of PARP1. B) The diagram shows the comparison of primary sequences of *Xenopus laevis* PARP1 (PARP1 xl) and *Homo sapiens* PARP1 (PARP1 hs) around the identified SUMOylation sites. The bold and underlined letter indicates lysine 482, the mapped candidates site of SUMOylation.

Supplemental Figure 1



Supplemental Figure 2

