Yun et al., http://www.jcb.org/cgi/content/full/jcb.200807185/DC1

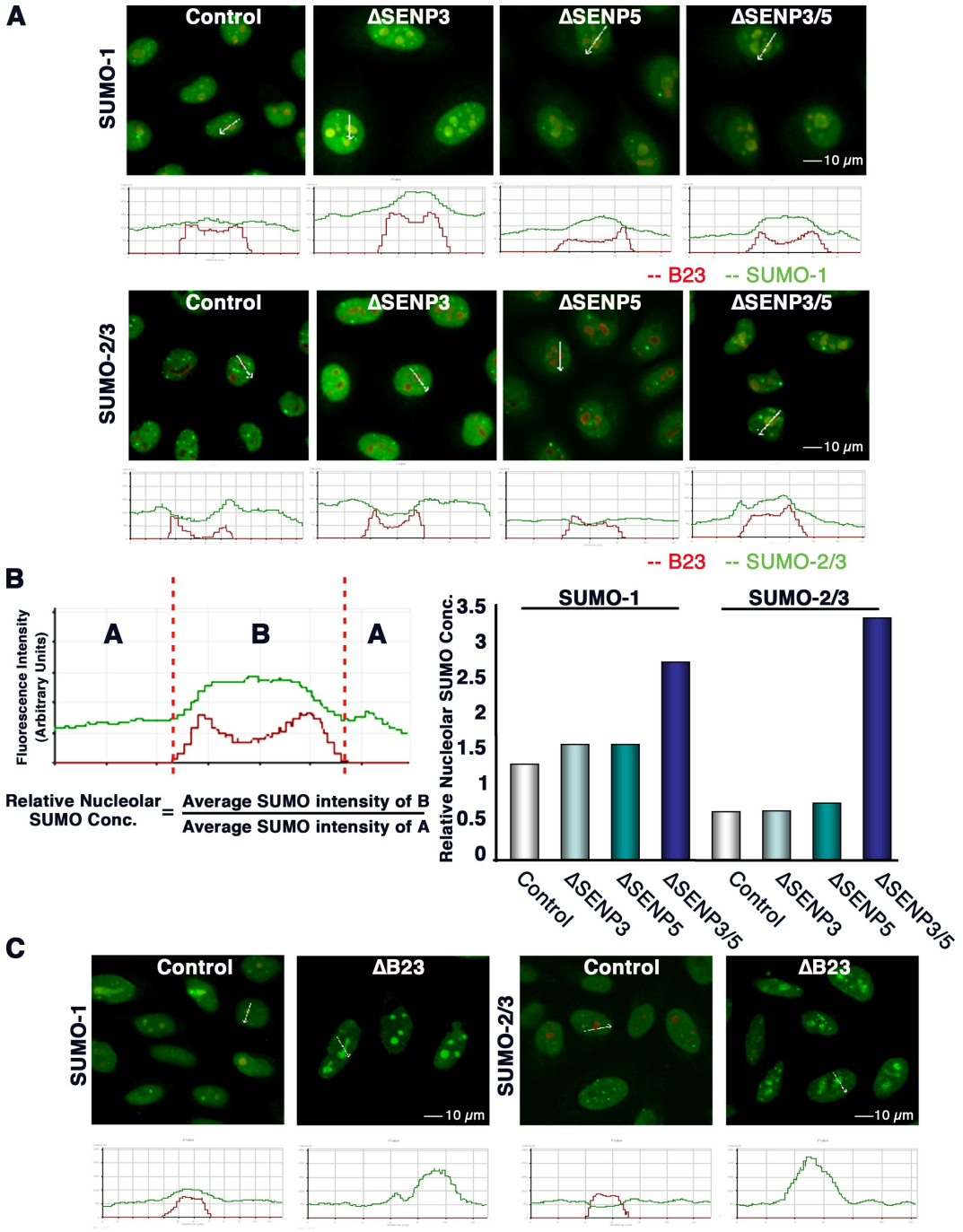

Figure S1. **Quantitation of SUMO redistribution after SENP3 and SENP5 depletion.** Cells prepared as in Fig. 2 B were imaged using a confocal microscope (LSM510; Carl Zeiss, Inc.) . (A) Line-scans were made for B23/nucleophosmin (red) and SUMO-1 (top, green) or SUMO-2 (bottom, green) along the indicated paths. The intensities for both signals are shown graphically below each image. (B) The localization of B23/nucleophosmin was used to define the boundary of individual nucleoli (B region). The mean intensity within this region was divided by the mean intensity of line-scan regions outside of it (A regions), to obtain a rough measure of the relative enrichment of SUMO paralogues within nucleoli. These measurements represent averages obtained from analysis of 20 nuclei within each sample. Consistent with earlier findings (Ayaydin, F., and M. Dasso. 2004. *Mol. Biol. Cell.* 15:5208–5218), SUMO-1 levels were slightly higher within nucleoli than in nucleoplasm. Codepletion of SENP3 and SENP5 caused a further twofold enhancement of SUMO-1 concentration. Also consistent with earlier findings (Ayaydin and Dasso, 2004), SUMO-2/3 levels were lower within nucleoli than in nucleoplasm. However, codepletion of SENP3 and SENP5 caused a substantial concentration of SUMO-2/3 within nucleoli, roughly threefold higher than nucleoplasmic levels. (C) Similar analysis was performed using B23/nucleophosmin-depleted cells. Although quantitation of nucleolar SUMO levels was not possible because nucleolar boundaries were not defined in the absence of B23/nucleophosmin, we observed clear accumulation of all paralogues in structures that appear to be nucleoli in a pattern that appears very similar to the SENP3/5-depleted cells.

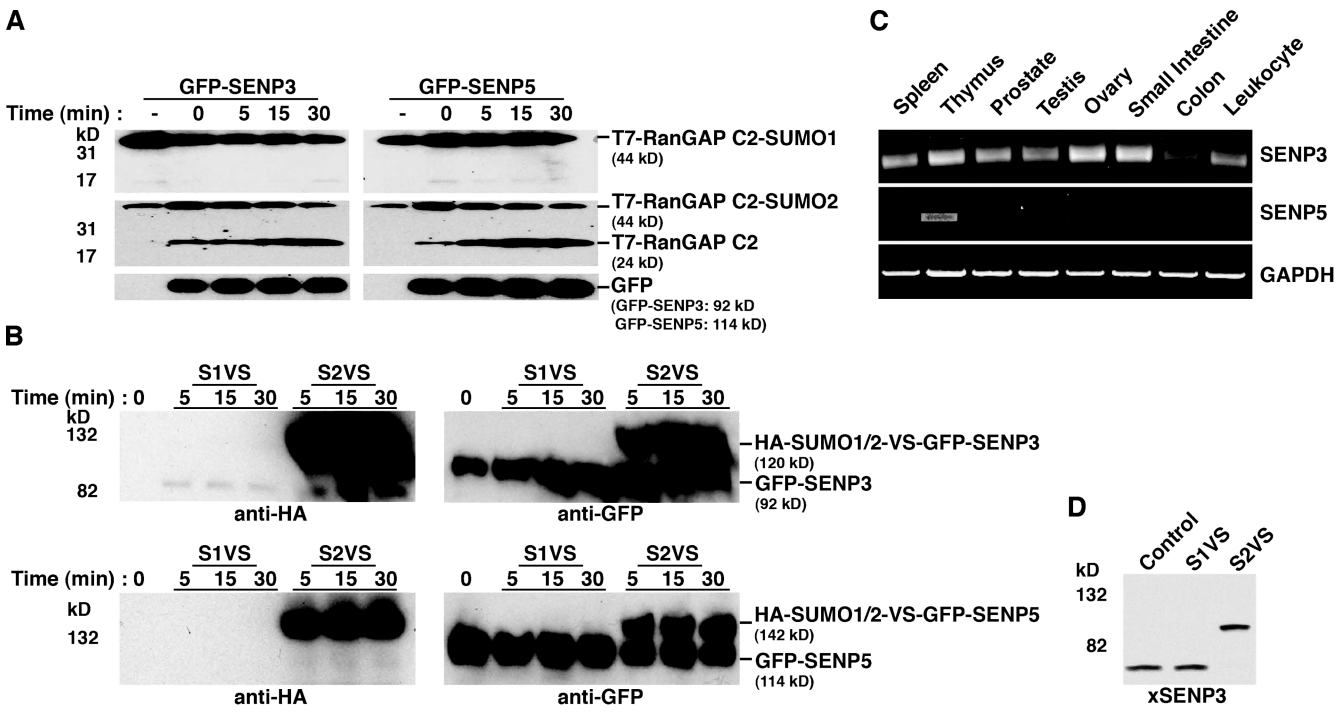

Figure S2. **SENP3 and SENP5 paralogue preferences and expression patterns.** (A) To examine the activity of GFP-SENP3 and GFP-SENP5 for deconjugation, we prepared a purified, recombinant C-terminal fragment of Ran GTPase-activating protein 1 (RanGAP1; His_6-T7-RanGAP1-C2), which contains its primary acceptor site. We incubated this fragment with purified E1 and E2 enzymes plus SUMO-1 or -2 in vitro to obtain monoconjugated species containing each paralogue (Mukhopadhyay, D., F. Ayaydin, N. Kolli, S.H. Tan, T. Anan, A. Kametaka, Y. Azuma, K.D. Wilkinson, and M. Dasso. *J. Cell Biol.* 2006. 174:939–949). Lysates of U2OS cells stably expressing GFP-SENP3 and GFP-SENP5 were subjected to immunoprecipitation with anti-GFP antibodies. After extensive washing, proteins bound to the antibody beads were incubated with the model substrates for the indicated times at room temperature (in minutes). The reactions containing His_6-T7-RanGAP1 C2-SUMO-1 model substrates (top) and for His_6-T7-RanGAP1 C2-SUMO-2 (middle) were immunoblotted with anti-T7 antibodies. The cleavage product (His_6-T7-RanGAP1 C2) should appear as a lower band with the indicated mobility. We also blotted each sample with anti-GFP antibodies to confirm equivalent concentrations of the fusion proteins (bottom). In each set of reactions minus (−) indicates a reaction were the model substrate was incubated for 30 min without the addition of beads. (B) Lysates of U2OS cells stably expressing GFP-SENP3 and GFP-SENP5 were subjected to immunoprecipitation with anti-GFP antibodies. After extensive washing, proteins bound to the antibody beads were incubated with HA-tagged vinyl sulfone derivatives of SUMO-1 and -2 (HA-SU1-VS and HA-SU2-VS) for the indicated times at room temperature. VS reagents derived from ubiquitin-like proteins covalently react with the nucleophilic active site residues of their deconjugation enzymes (Mukhopadhyay et al., 2006). The reactions were terminated with SDS sample buffer and analyzed by Western blotting with the indicated antibodies. Long exposures are shown to demonstrate that both fusion proteins showed little reactivity with HA-SU1-VS, if any. (C) SENP3 and SENP5 were amplified by quantitiative PCR from a human multiple tissue cDNA library (Clontech Laboratories, Inc.). (D) 5 µl of interphase XEE was incubated with buffer (control) with SUMO-1-vinyl sulfone (S1VS) or SUMO-2-vinyl sulfone (S2VS) for 30 min at 23°C (top). Reactions were terminated with SDS sample buffer and Western blotted with anti-*Xenopus* SENP3 (xSENP3) antibodies.

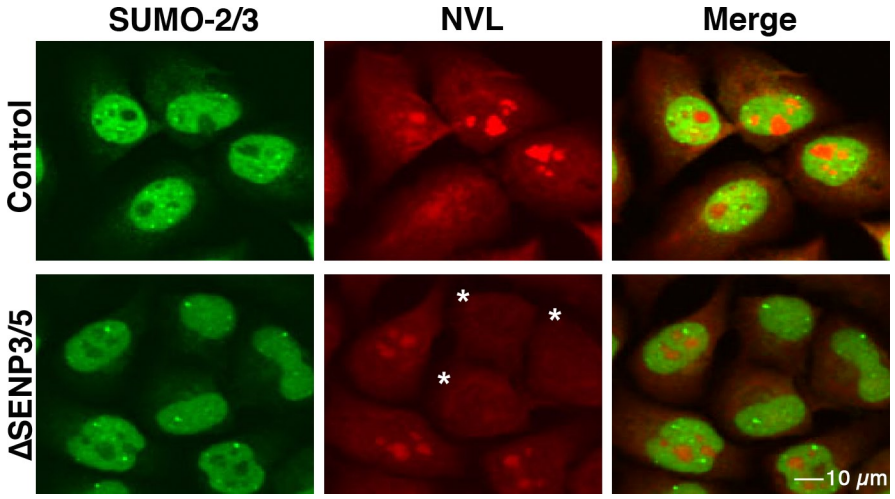

Figure S3. **Mislocalization of ribosomal processing factor NVL (nuclear valosin-containing protein-like protein 2) in the absence of SENP3 and SENP5.** NVL2 is a nucleolar member of the AAA ATPase family that mediates release of DOB1, a DExD/H-box RNA helicase, from 60S preribosomes at a late step in the maturation pathway (Nagahama, M., T. Yamazoe, Y. Hara, K. Tani, A. Tsuji, and M. Tagaya. 2006. *Biochem. Biophys. Res. Commun.* 346:1075–1082). HeLa cells were transfected with control oligonucleotides (top) or siRNAs directed against SENP3 and SENP5 (bottom). 72 h after transfection, the cells were stained with antibodies against NVL (middle, red) and against SUMO-2/3 (left, green). Cells that showed extensive relocalization of SUMO-2/3 into nucleoli (asterisks) were assumed to be effectively depleted of SENP3 and SENP5, and they are intentionally imaged in juxtaposition to cells within the same culture that were not effectively transfected. In this case, the accumulation of SUMO-2/3 within the nucleoli of SENP3- and SENP5-depleted cells coincides with loss of NVL2 nucleolar accumulation.