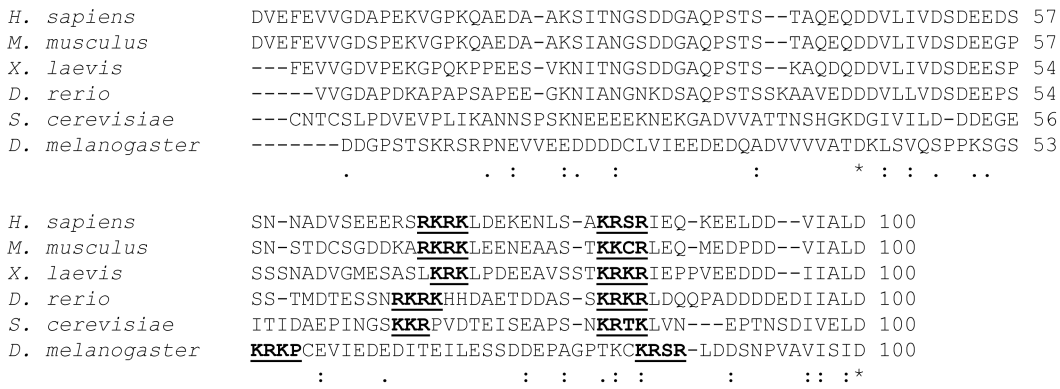
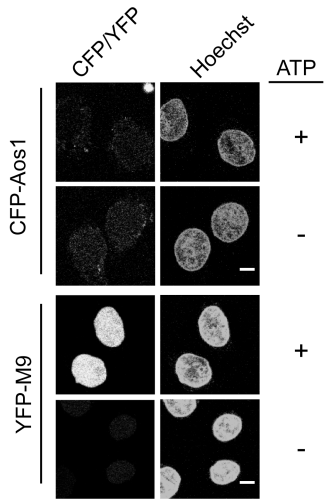
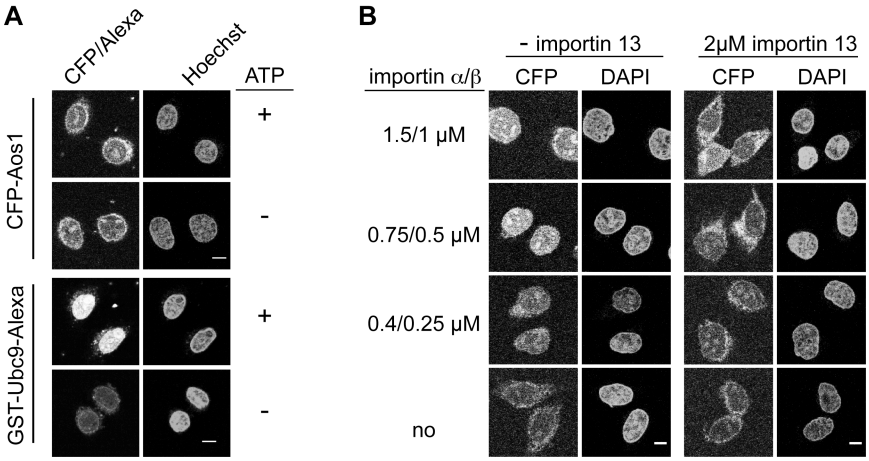


Supplemental Figure 1. Sequence alignment of the C-terminus of Uba2. The C-terminal 100 amino acids of Uba2 from *H. sapiens, M. musculus, D. rerio, X. laevis, D. melanogaster and S. cerevisiae* were aligned using ClustalW2. Conserved clusters of basic amino acids potentially participating in nuclear import are underlined and highlighted in bold.

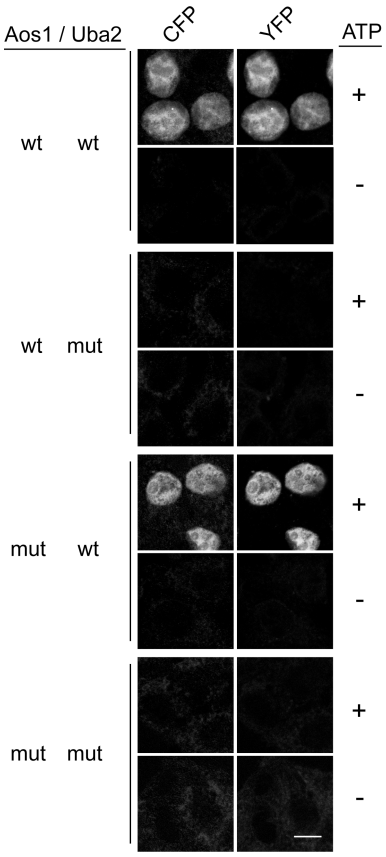
Supplemental Figure 2. Sequence alignment of Aos1. Amino acid sequences of Aos1 from *H. sapiens, M. musculus, D. rerio, X. laevis, D. melanogaster and S. cerevisiae* were aligned using ClustalW2. Conserved clusters of basic amino acids potentially participating in nuclear import are underlined and highlighted in bold.



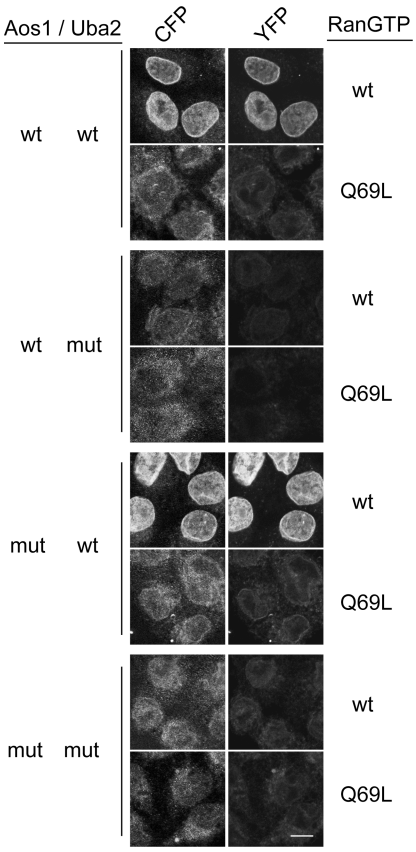
Supplemental Figure 3. Transportin does not mediate import of CFP-Aos1 *in vitro*. *In vitro* import of CFP-Aos1 (1μM) in semipermeable HeLa cells was tested in the presence of Transportin (1μM) and 12 μM Ran with or without ATP. The transportin dependent cargo YFP-M9 (1μM) (Siomi and Dreyfuss, 1995; Nakielny et al., 1996) was included as a positive control. Nuclear accumulation of fluorescently labelled cargo proteins was analyzed by confocal microscopy. Bar, 10 μm.



Supplemental Figure 4. Importin 13 does not support import of CFP-Aos1 *in vitro*. (A) CFP-Aos1 (5 μM) import was tested in semi-permeabilized HeLa cells in the presence of importin 13 (1 μM) and Ran (12 μM) with or without ATP. Alexa488 labelled Gst-Ubc9 (Mingot et al., 2001) served as a positive control. Nuclear accumulation of CFP-Aos1 and Gst-Ubc9-Alexa488 was analyzed by fluorescence microscopy. Bar, 10 μm. (B) Importin 13 does not synergize with importin α/β for the import of CFP-Aos1. *In vitro* import of CFP-Aos1 (1μM) using semipermeable HeLa cells was performed with the indicated concentrations of Importin α and importin β in the presence of Ran (12μM), with or without addition of 2 μM importin13. Nuclear accumulation of CFP-Aos1 was analyzed by confocal microscopy. Bar, 10μm.



Supplemental Figure 5. The Uba2 NLS is required and sufficient for import of the SUMO E1 holo-enzyme. *In vitro* import of E1 complexes reconstituted from wild type proteins (CFP-Aos1-wt, Uba2-wt-YFP) and/or NLS mutants (CFP-Aos1-KR195,196A_2, Uba2-KR623,624A_2-YFP). The assays were performed in semipermeabilized HeLa cells in the presence of cytosol and ATP-regenerating system (+). Control experiments contained ATP-depleting system (-) instead. Nuclear accumulation of CFP-Aos1 and Uba2-YFP was analyzed by fluorescence microscopy. Bar, 10 μm.



Supplemental Figure 6. Importin α/β mediates *in vitro* import of the SUMO E1 holo-enzyme via interaction with the Uba2 NLS. *In vitro* import of E1 complexes reconstituted from wild type proteins (CFP-Aos1-wt, Uba2-wt-YFP) and/or NLS mutants (CFP-Aos1-KR195,196A_2, Uba2-KR623,624A_2-YFP). The assays were performed in semi-permeabilized HeLa cells in the presence of importin α/β in the presence of wt Ran or RanQ69L. Nuclear accumulation of CFP-Aos1 and Uba2-YFP was analyzed by fluorescence microscopy. Bar, 10 μm.