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Supplemental Material to:

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Two distinct sites in Nup153 mediate interaction with the SUMO proteases SENP1 and SENP2

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Supplementary Methods

SENP1 and Nup153 indirect immunofluorescence analysis. Hela cells treated with control or Nup153 siRNA were seeded on cover slips. After 48 hours, cells were rinsed with PBS followed by fixation with -20°C methanol for 4 min. Following fixation, cells were rinsed and blocked with PBS containing 3% FBS, 0.02% Tx100 for 30 min. Subsequent antibody treatments were performed as described in Mackay *et al.* (19). Images were acquired with a confocal microscope (FV1000 Olympus IX81) on a 60x objective.

Nup153 SUMO modification analysis with $SENP2_{CD}$. Hela cells were co-transfected with Flag-tagged $SENP2_{CD}$ along with T7-tagged SUMO1, 2 or 3 and lysates were generated using lysis buffer (1xPBS, 0.25% Tx100, 60mM β-Glycerophosphate, 10mM Sodium Orthovanadate, 2x Roche COMPLETE protease inhibitor, 40mM N-Ethylmaleimide). Cell lysates were later incubated with protein-G beads, with Nup153 antibody (SA1) pre-immobilized, for 4 hr at 4°C. Beads were subsequently washed 3 times with the above lysis buffer (without N-Ethylmaleimide). Proteins were eluted with SDS loading buffer and subjected to western analysis.

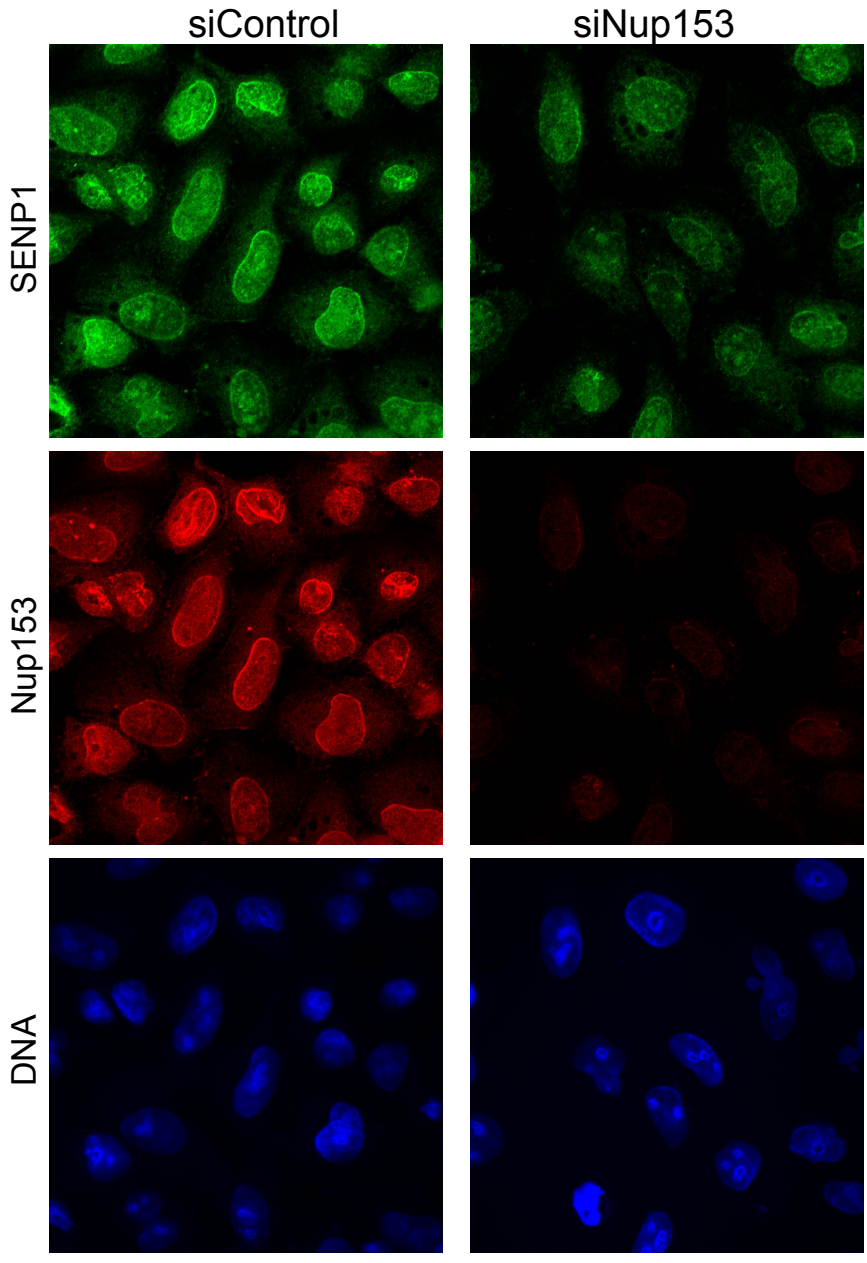
Indirect immunofluorescence analysis of GFP-tagged Nup153 C-terminal domains. Hela cells seeded on cover slips were transfected with constructs of GFP or GFP-tagged Nup153 C-terminal domain truncation mutants: C_{Δ} (875-1457), C-distal (1263-1475), C-distal$_{\Delta}$ (1263-1457), and C-proximal (875-1262). After 24 hours, cells were fixed with 2% PFA for 10 minutes followed by -20°C methanol for 2 min. Cells were later processed for indirect immunofluorescence analysis as described above. Images were acquired with a Zeiss Axioskop 2 microscope (Carl Zeiss, Inc.) on 63x objective.

Supplementary Figure 1. SENP1 localizes to the nuclear periphery. Hela cells treated with control or Nup153 siRNA were subjected to indirect immunofluorescence analysis using antibodies against Nup153 and SENP1.

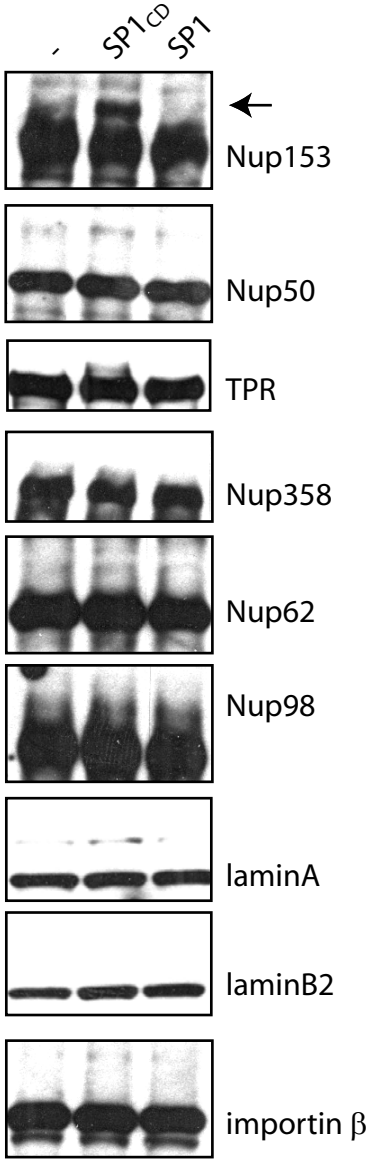
Supplementary Figure 2. Nup153 is a unique target of SENP1. Flag fusion proteins of SENP1 (SP1) or catalytically dead SENP1 (SP1 CD) were expressed in Hela cells for 24hr. Cell lysates were then harvested for western analysis using antibodies against indicated proteins.

Supplementary Figure 3. Expression of $SENP2_{CD}$ results in sumoylation of Nup153. A flag-tagged fusion protein of catalytic dead SENP2 was coexpressed with either T7-tagged SUMO1 (S1), SUMO2 (S2), or SUMO3 (S3) in Hela cells. After 24hr, Nup153 was immunoprecipitated from cell lysates and subjected to western analysis using antibodies against T7 and Nup153. Arrow indicates sumoylated Nup153.

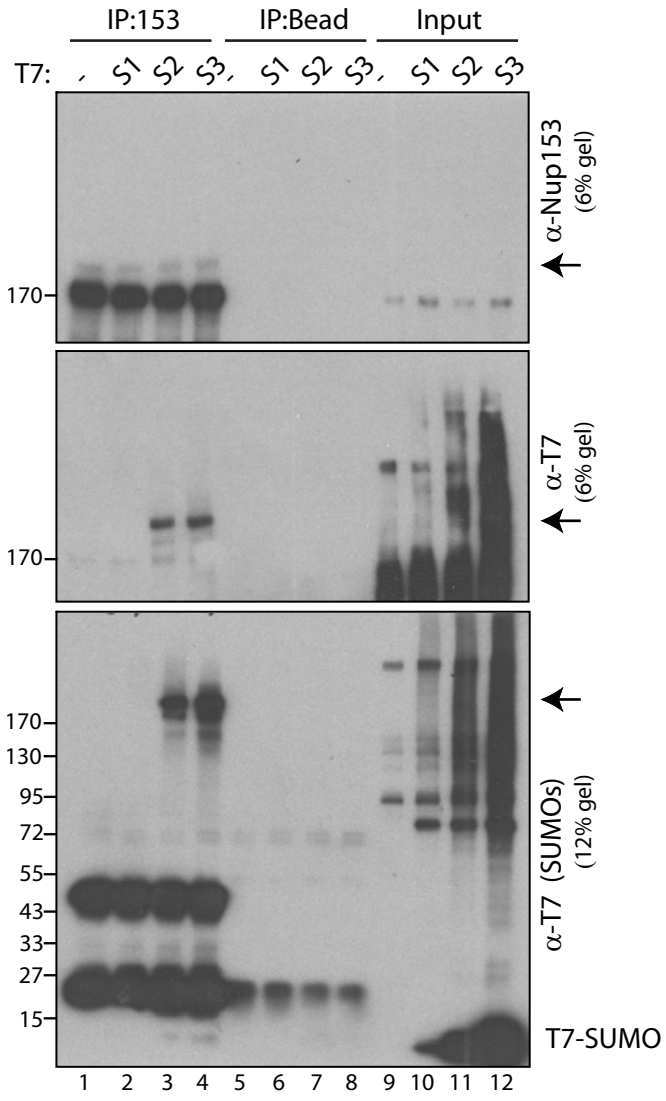
Supplementary Figure 4. Localization of Nup153 C-terminal domains. GFP or GFP-tagged Nup153 C-terminal domain truncation contructs were transfected into Hela cells and subjected to indirect immunofluorescence using GFP antibody. DNA is stained with Hoechst.

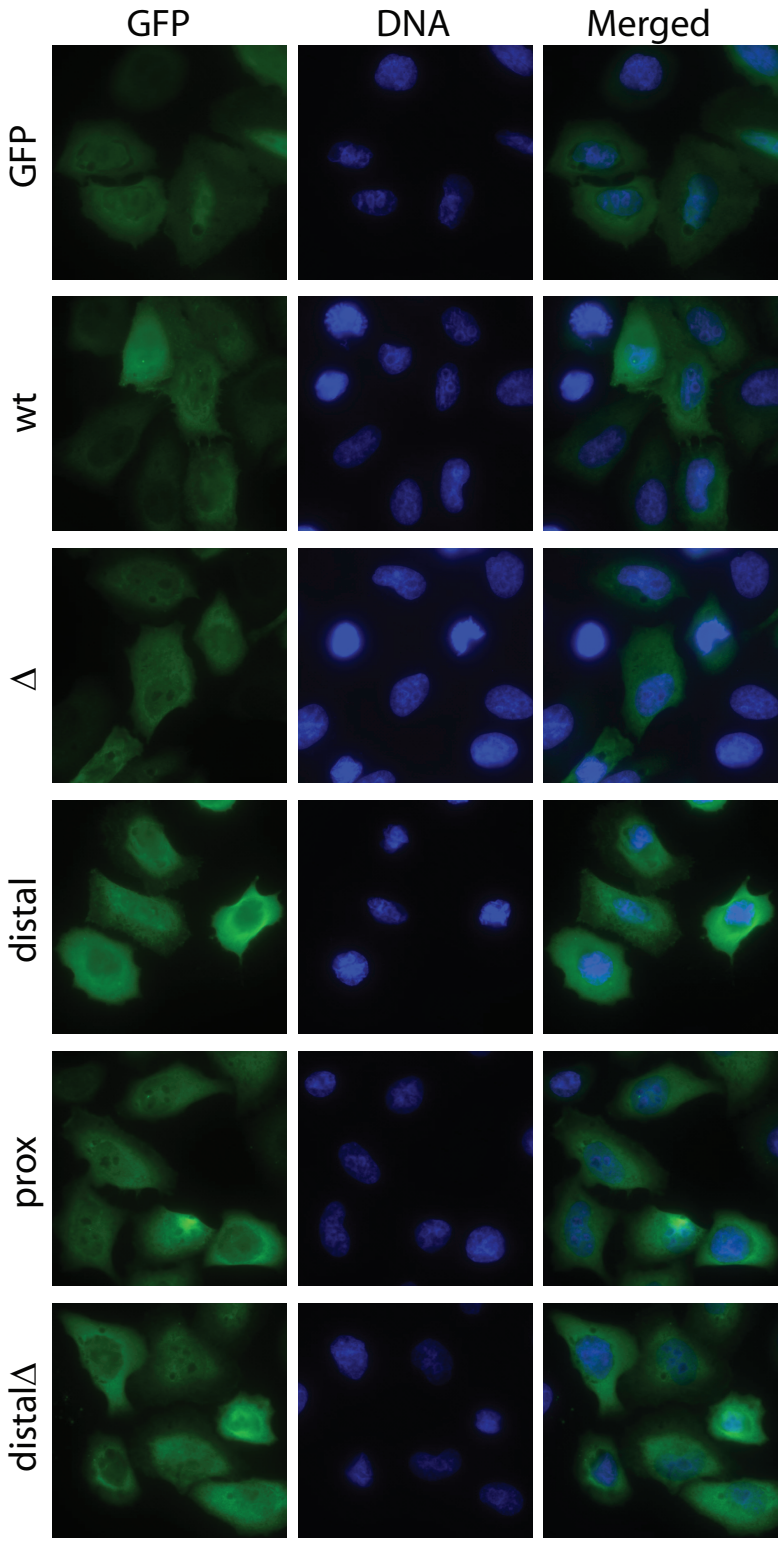

siControl
siNup153
SENP1
Nup153
DNA

Supplementary Figure 2
Chow et al.



Supplementary Figure 3
Chow et al.




GFP
DNA
Merged
GFP
wt
Δ
distal
prox
distalΔ