

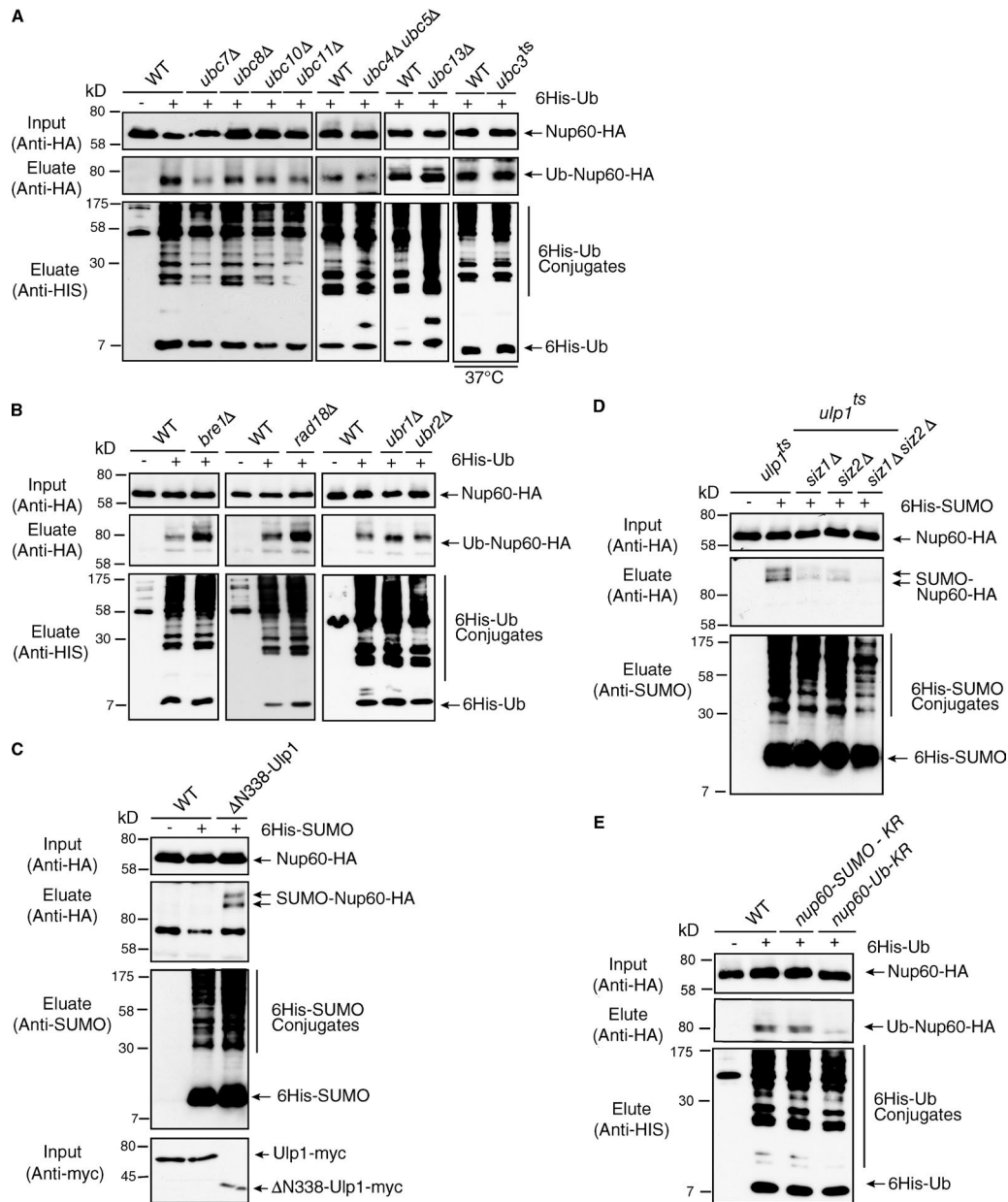
Niño et al., <http://www.jcb.org/cgi/content/full/jcb.201506130/DC1>

Figure S1. **Ubiquitin conjugation of genomically HA-tagged Nup60.** Ubiquitin conjugation of genomically HA-tagged Nup60 was analyzed as in Fig. 1 in WT and different *E2Δ* cells (A) and in WT and different *E3* mutant cells (B). SUMO conjugation of genomically HA-tagged Nup60 was analyzed as in Fig. 1 in WT and indicated N-terminal deletion mutants of Ulp1 (C) in WT and *ulp1* *ts* mutant cells deleted for different E3 ligases after a 2-h shift to 37°C (D). Myc-tagged Ulp1 expression in C was evaluated by Western blotting using an anti-myc antibody. (E) Ubiquitylation of Nup60-HA was determined in WT, *nup60-Ub-KR*, and *nup60-SUMO-KR* cells.

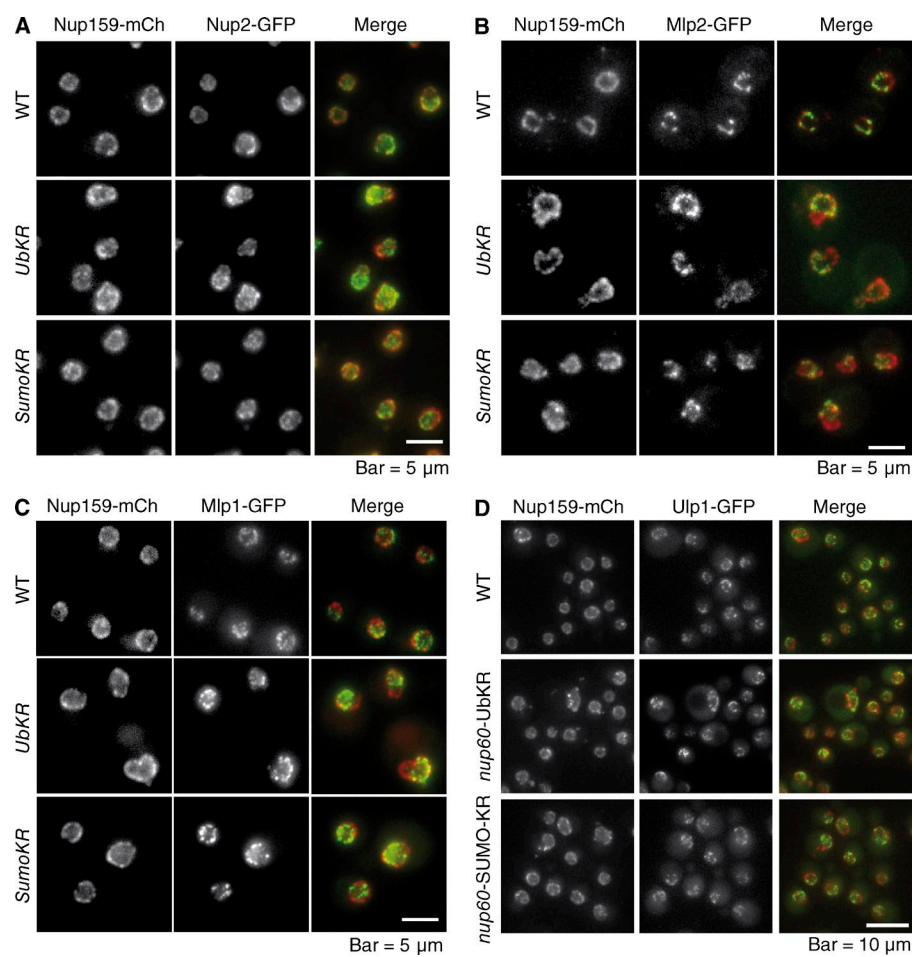


Figure S2. **Steady-state localization.** Steady-state localization of GFP-tagged Nup2 (A), Mlp2 (B), Mlp1 (B), and Ulp1 (C) in WT, *nup60-UbKR*, and *nup60-SUMO-KR* strains. The nuclear pore complex is shown by Nup159-mCherry.

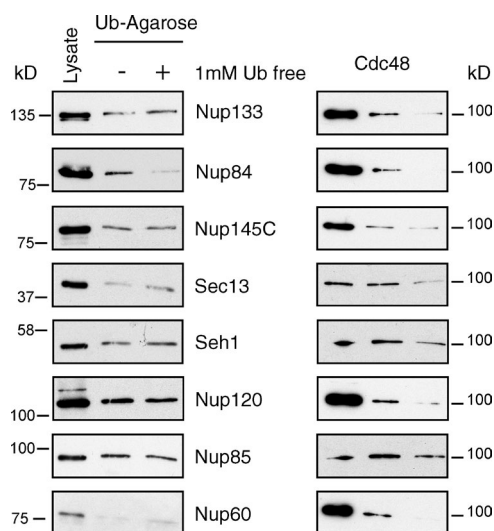


Figure S3. **Nup84 interacts with monoubiquitin.** Lysates from cells expressing HA-tagged indicated Nups were purified on monoubiquitin-coupled agarose beads in the absence (-) or presence (+) of 1 mM ubiquitin. Bound proteins were analyzed by Western blotting using anti-HA or anti-Cdc48 antibodies as an internal control for ubiquitin-binding protein. Results shown in Fig. 4 A correspond to a duplicate of results presented here for Nup60-HA, Nup84-HA, Nup133HA, and corresponding Cdc48 controls.

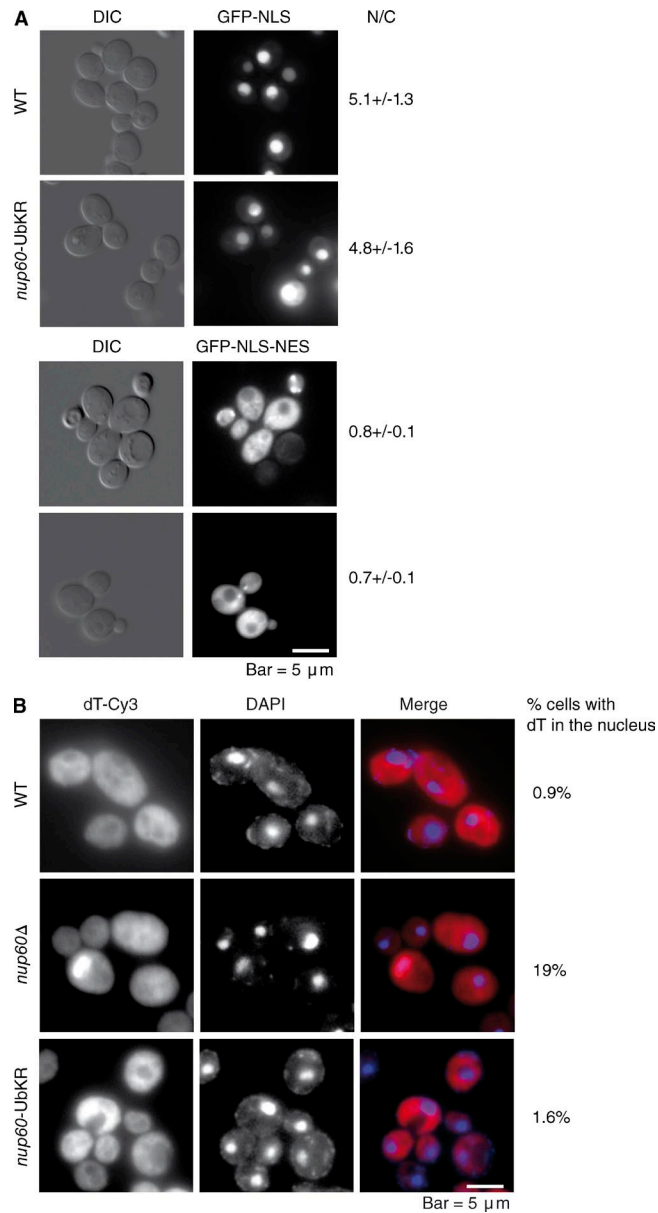


Figure S4. **The effect of Nup60 modification on the major nuclear transport pathways.** (A) WT or *nup60-UbKR* cells were transfected with a plasmid encoding GFP-NLS or GFP-NLS-NES. Cells grown at 30°C were examined by both fluorescence microscopy and DIC. Fluorescence intensity was quantified in the nucleus (N) and cytoplasm (C) using ImageJ, and N/C ratios are indicated. (B) Subcellular localization of poly(A)<sup>+</sup> RNA was analyzed by FISH using a Cy3-labeled oligo-dT probe in WT (*n* = 301), *nup60* $\Delta$  (*n* = 337), and *nup60-UbKR* (*n* = 378) strains. Percentage of cells accumulating poly(A)<sup>+</sup> RNA in the nucleus is shown on the right. Bar, 5  $\mu$ m.

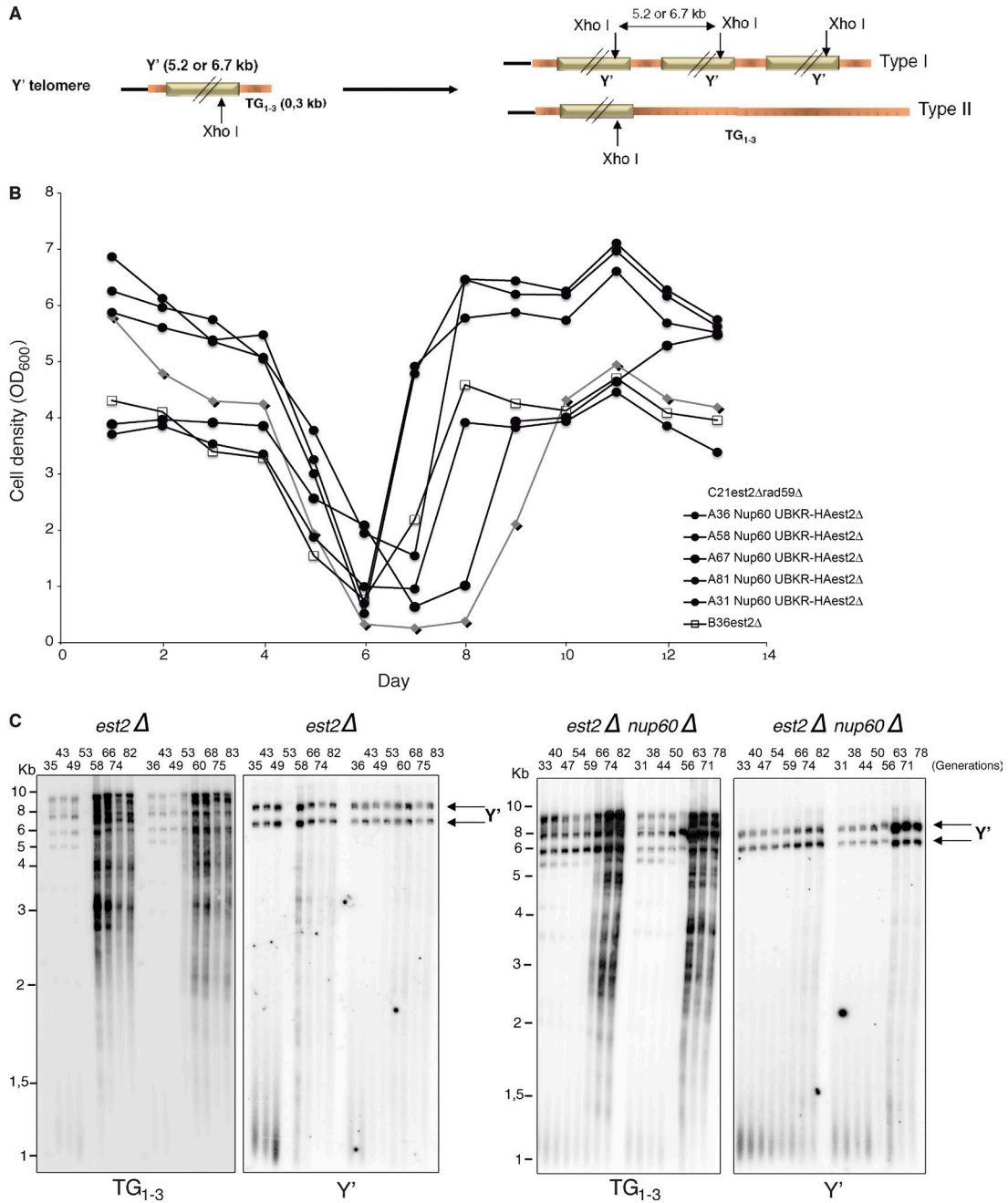


Figure S5. **Senescence curves for the individual clones analyzed in Fig. 7 B.** (A) Schematic representation of a Y' telomere and type I and type II survivors. Y' elements are of two size classes (6.7 and 5.2 kb long). (B) Senescence curves of the clones shown in Fig. 6 B. Senescence assays were started from individual spore colonies with the indicated genotypes and performed in liquid culture by propagating the cells via serial dilutions to  $10^5$  cells/ml every 24 h. (C) Survivor types of two representative clones of *est2Δ* and *est2Δ nup60Δ* were determined by Southern blot of telomeric DNA digested by XhoI and probed with either a TG<sub>1-3</sub> or a subtelomeric Y'.

Table S1. *S. cerevisiae* strains used in this study

Strain	Genotype	Reference
BY4741	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	EUROSCARF
<i>nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS</i>	This study
<i>ubcXΔ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, UBCX::kanMX6</i>	This study
<i>ubc3ts Nup60-HA</i>	<i>MATa his3Δ1 ura3-52 trp1D leu2-3_112 ade2-1 can1-100, cdc34-2, nup60-HA:HIS</i>	This study
<i>rad6Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, RAD6::kanMX6</i>	This study
<i>bre1Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, BRE1::kanMX6</i>	This study
<i>rad18Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, RAD18::kanMX6</i>	This study
<i>ubr1Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, UBR1::kanMX6</i>	This study
<i>ubr2Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, UBR2::kanMX6</i>	This study
<i>slx5Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, SLX5::kanMX6</i>	This study
<i>slx8Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, SLX8::HPH</i>	This study
<i>uls1Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, ULS1::kanMX6</i>	This study
<i>ubcpΔ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, UBXP::kanMX6</i>	This study
<i>ubp10Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:HIS, UBP10::kanMX6</i>	This study
<i>Ulp1ts</i>	<i>Mat a, ULP1::KanMX, YCplac111-ulp1-333</i>	Vitaliano-Prunier et al., 2008
<i>ulp1ts nup60-HA</i>	<i>Mat a, ULP1::KanMX, YCplac111-ulp1-333, nup60-HA:HIS</i>	This study
<i>ulp1ts siz1Δ nup60-HA</i>	<i>Mat a, ULP1::KanMX, YCplac111-ulp1-333, nup60-HA:HIS, SIZ1::HPH</i>	This study
<i>ulp1ts siz2Δ nup60-HA</i>	<i>Mat a, ULP1::KanMX, YCplac111-ulp1-333, nup60-HA:HIS, SIZ2::HPH</i>	This study
<i>ulp1ts siz1Δ siz2Δ nup60-HA</i>	<i>Mat a, ULP1::KanMX, YCplac111-ulp1-333, nup60-HA:HIS, SIZ1::HPH, SIZ2::NAT</i>	This study
<i>Ulp1-MYC nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ulp1-MYC:HPH nup60-HA:HIS</i>	This study
<i>ΔN388-ulp1</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ΔN388-ulp1:LEU</i>	This study
<i>ΔN388-ulp1-MYC</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ΔN388-ulp1-MYC:HPH</i>	This study
<i>ΔN388-ulp1-MYC nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ΔN388-ulp1-MYC:HPH, nup60-HA:HIS</i>	This study
<i>nup60Δ</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, NUP60::kanMX6</i>	This study
<i>nup60Δ ulp1ts</i>	<i>Mat a, ULP1::KanMX, YCplac111-ulp1-333, NUP60::HPH</i>	This study
<i>nup60-HA LEU</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU</i>	This study
<i>nup60-UbKR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU</i>	This study
<i>nup60-SUMO-KR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU</i>	This study
<i>nup60-SUMO-Site1KR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442)R-HA:LEU</i>	This study
<i>nup60-SUMO-Site2KR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(505)R-HA:LEU</i>	This study
<i>nup60-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-GFP:HIS</i>	This study
<i>nup60-GFP nup159-mCh nup84Δ</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-GFP:HIS, nup84:HPH, nup159-mCherry:KAN</i>	This study
<i>nup60-UbKR-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-GFP:HIS</i>	This study
<i>nup60-SUMO-KR-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-GFP:HIS</i>	This study
<i>nup159-mChe</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, nup159-mCherry:KAN</i>	This study
<i>nup60-UbKR nup159-mChe</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, nup159-mCherry:KAN</i>	This study
<i>nup60-SUMO-KR nup159-mChe</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, nup159-mCherry:KAN</i>	This study
<i>nup2-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, nup2-GFP:HIS</i>	This study
<i>nup60-UbKR nup2-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, nup2-GFP:HIS</i>	This study
<i>nup60-SUMO-KR nup2-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, nup2-GFP:HIS</i>	This study
<i>nup2-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, nup2-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>nup60-UbKR nup2-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, nup2-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>nup60-SUMO-KR nup2-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, nup2-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>mlp1-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, mlp1-GFP:HIS</i>	This study
<i>nup60-UbKR mlp1-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, mlp1-GFP:HIS</i>	This study
<i>nup60-SUMO-KR mlp1-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, mlp1-GFP:HIS</i>	This study
<i>mlp1-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, mlp1-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>nup60-UbKR mlp1-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, mlp1-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>nup60-SUMO-KR mlp1-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, mlp1-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>ulp1-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, ulp1-GFP:HIS</i>	This study
<i>nup60-UbKR ulp1-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, ulp1-GFP:HIS</i>	This study
<i>nup60-SUMO-KR ulp1-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, ulp1-GFP:HIS</i>	This study
<i>ulp1-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, ulp1-GFP:HIS, nup159-mCherry:KAN</i>	This study

Table S1. *S. cerevisiae* strains used in this study (Continued)

Strain	Genotype	Reference
<i>nup60-UbKR ulp1-GFP nup159-mCh</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, ulp1-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>nup60-SUMO-KR ulp1-GFP nup159-mCh</i>	<i>Mat a, Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(440,442,505)R-HA:LEU, ulp1-GFP:HIS, nup159-mCherry:KAN</i>	This study
<i>rad6Δ nup60-GFP</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-GFP:HIS, RAD6::KAN</i>	This study
<i>ulp1-GFP nup60Δ</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ulp1-GFP:HIS, NUP60:HPH</i>	This study
<i>NSP1-UPL1C</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ulp1::HPH, pRS315-promULP1-GFP-NSP1-UPL1C-KAN, nup60-HA:LEU</i>	This study
<i>NSP1-UPL1C nup60-UbKR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, ulp1::HPH, pRS315-promULP1-GFP-NSP1-UPL1C-KAN, nup60-K(105-175)R-HA:LEU</i>	This study
<i>mlp1Δ mlp2Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, MLP1::HPH, MLP2::KAN</i>	This study
<i>rad53k227A bar1Δ nup60-UbKR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, BAR1::HPH, rad53K227A::KAN</i>	This study
<i>rad53k227A bar1Δ nup60 HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, BAR1::HPH, rad53K227A::KAN</i>	This study
<i>mrc1Δ nup60-UbKR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, mrc1::HPH</i>	This study
<i>mrc1Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, mrc1::HPH</i>	This study
<i>rad9Δ nup60-UbKR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, rad9::KAN</i>	This study
<i>rad9Δ nup60-HA</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, rad9::KAN</i>	This study
<i>mlp1Δ mlp2Δ nup60-UbKR</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-K(105-175)R-HA:LEU, MLP1::HPH, MLP2::KAN</i>	This study
<i>ubp10Δnup60-HA nup84-myc</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-HA:LEU, ubp10::HPH, nup84-13myc:HIS</i>	This study
<i>nup60-UbKR-HA nup84-myc</i>	<i>Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, nup60-UbKR-HA:LEU, nup84-13myc:HIS</i>	This study
<i>nup60-ProtA</i>	<i>his3-D200; leu2-3,2-112; lys2-801; trp-1; ura3-52, nup60-ProtA:HIS</i>	Rout et al., 2000
<i>nup49-ProtA</i>	<i>his3-D200; leu2-3,2-112; lys2-801; trp-1; ura3-52, nup49-ProtA:HIS</i>	Rout et al., 2000
<i>nup60-GFP nup133Δ</i>	<i>MATα, leu2, his3, ura3, lys2, nup133::KanMX, NUP60-GFP::HIS3</i>	Palancade et al., 2007
<i>nup84-GFP nup133Δ</i>	<i>MATα, leu2, his3, ura3, lys2, nup133::KanMX, NUP84-GFP::HIS3</i>	Palancade et al., 2007

Table S2. Plasmids used in this study

Plasmid	Description	Reference
p415-nup60-HA	CEN6/LEU2/ADH promoter/nup60-HA	This study
p415-nup60-KRall-HA	CEN6/LEU2/ADH promoter/nup60-KRall-HA (all 52 Lys to Arg)	This study
YE <sub>p</sub> 352-6His-Ub	2μ/URA3/CUP promoter/6-His-Ub	Bretes et al., 2014
YE <sub>p</sub> 352-6His-SUMO	2μ/URA3/CUP promoter/6-His-SUMO	This study
pRS315-GFP-NSP1-ΔNULP1	CEN6/LEU2/UPL1 promoter/ GFP-NSP1-ΔNULP1	Texari et al., 2013
pRS315-GFP-NSP1-ΔNULP1 KAN	CEN6/KAN/UPL1 promoter/ GFP-NSP1-ΔNULP1	This study
pWJ11344	RAD52-YFP	Lisby et al., 2001

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