Supplementary data

Nuclear pore components affect distinct stages of intron-containing gene expression

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- **Supplementary Table 1:** Strains used in this study
- **Supplementary Table 2:** Plasmids used in this study
- **Supplementary Table 3:** Primers used in this study

- ***Supplementary Table 4:*** *Raw data for β-galactosidase activities provided as a separate excel file.*

- **Supplementary References**

- **Supplementary Figure 1:** LacZ reporters allow to discriminate between distinct mRNA biogenesis/export defects in nuclear pore mutants.
- **Supplementary Figure 2:** The differential effect of THO mutants on intronless and intron-containing LacZ reporters is not a mere consequence of transcriptional inhibition or heterogeneity in plasmid maintenance.
- **Supplementary Figure 3:** The differential effect of THO mutants on intronless and intron-containing LacZ reporters is also observed with centromeric vectors.
- **Supplementary Figure 4:** Nuclear pore mutants triggering *bona fide* pre-mRNA leakage do not modulate cellular sumoylation patterns.

Supplementary Table 1: Strains used in this study

Heterozygous and homozygous deletion strains were obtained from the *EUROSCARF* deletion collection (http://web.uni-frankfurt.de/fb15/mikro/euroscarf). Except *MEX67+/mex67-5* strains, all strains are isogenic to S288C and were obtained by homologous recombination and/or successive crosses.

Strain code	Name	Relevant génotype	Source
BY4742	*wt*		Euroscarf
RS453	*MEX67+*		(34)
YV287	*mex67-5*	*mex67::HIS3 pUN100-mex67-5*	(34)
Y24217	*yra1Δ/+*	*yra1::kanMX/YRA1*	Euroscarf
YV1480	*YRA1 shuffle*	*yra1::kanMX YCplac33-YRA1*	This study [a]
YV1486	*YRA1+*	*yra1::kanMX YCpHIS-HA-YRA1*	This study [b]
YV1496	*yra1-KR*	*yra1::kanMX YCpHIS-HA-yra1-KR*	This study [b]
Y24489	*nab2Δ/+*	*nab2::kanMX/NAB2*	Euroscarf
YV1403	*nab2-F73D*	*nab2::kanMX pRS315-nab2-F73D*	This study [c]
Y14268	*npl3Δ*	*npl3::kanMX*	Euroscarf
Y14072	*hpr1Δ*	*hpr1::kanMX*	Euroscarf
Y10508	*mft1Δ*	*mft1::kanMX*	Euroscarf
Y12861	*thp2Δ*	*thp2::kanMX*	Euroscarf
YV1542	*sus1Δ*	*sus1::kanMX*	This study [d]
Y15828	*sem1Δ*	*sem1::KanMX*	Euroscarf
YV1685	*hpr1-K60/65R*	*hpr1-K60-65R::hphMX*	(19)
YV1684	*hpr1-K60/65R ulp1*	*hpr1-K60-65R::hphMX ulp1::kanMX YCpLac111-ulp1-333*	(19)
YV1478	*siz1 siz2*	*siz1::kanMX siz2::kanMX*	(19)
Y14906	*nup120Δ*	*nup120::kanMX*	Euroscarf
Y15998	*nup133Δ*	*nup133::kanMX*	Euroscarf
Y16503	*nup188Δ*	*nup188::kanMX*	Euroscarf
YV929	*ULP1-GFP*	*ULP1-GFP::HIS3*	Invitrogen
Y17104	*mlp1Δ*	*mlp1::kanMX*	Euroscarf
YV1413	*ULP1-GFP mlp1Δ*	*ULP1-GFP::HIS3 mlp1::kanMX*	This study *
Y16507	*pml39Δ*	*pml39::kanMX*	Euroscarf
YV829	*ULP1-GFP pml39Δ*	*ULP1-GFP::HIS3 pml39::kanMX*	This study *
YV1262	*ulp1 mat alpha*	*ulp1::kanMX YCplac111-ulp1-333*	(19)
YV1263	*ulp1 mat a*	*ulp1::kanMX YCplac111-ulp1-333*	(19)
YV1328	*ulp1 mlp1Δ*	*mlp1::hphMX ulp1::KanMX YCplac111-ulp1-333*	This study [e]
YV1290	*ulp1-ΔN*	*6HA-Δ172-340-ulp1*	This study [f]
YV1339	*mlp1Δ ulp1-ΔN*	*6HA-Δ172-340-ulp1 mlp1::HIS3*	This study *

a. Segregant of a heterozygous diploid *yra1::kanMX/YRA1+* transformed with the YCplac33-YRA1 plasmid.
b. Obtained by shuffling the YCpLac22-HA-YRA1 or YCpLac22-HA-yra1-KR plasmids into the YV1480 strain.
c. Segregant of a heterozygous diploid *nab2::kanMX/NAB2+* transformed with the pRS315-nab2-F73D plasmid.
d. *SUS1* complete CDS was deleted by a KanMX cassette amplified from pFA6a-KanMX6.
e. *MLP1* complete CDS was deleted in YV1263 (*ulp1*) by a hphMX cassette amplified from pFA6a-hphMX6
f. The *ulp1-ΔN* strain (which encodes a mutant Ulp1 protein with an internal replacement of aminoacids 172 to 340 with a 6 HA tag) was obtained by homologous recombination at the *ULP1* locus as described (52) except that the pOM12 template (*lox-URA3-lox-6HA*) was used.

* Obtained by crosses.

<u>Supplementary Table 2:</u> Plasmids used in this study

Name	Description	Source
pFA6a-KanMX6	*for deletion*	(59)
pFA6a-hphMX6	*for deletion*	(60)
pTL7	*trp1::LEU2 disruption fragment*	(61)
pTH4	*trp1::HIS3 disruption fragment*	(61)
YCplac111-ulp1-333	*CEN/LEU2/ulp1-333 (ulp1)*	(25) [a]
pLGS-D5 (intronless reporter)	*2µ/URA3/GAL1-LacZ*	(42)
pJCR51 ("*splicing*" reporter)	*2µ/URA3/GAL1-intron-out-of-frame-LacZ*	(62)
pJCR1 ("*leakage*" reporter)	*2µ/URA3/GAL1-intron-in-frame-LacZ*	(62)
YCpLac33-YRA1	*CEN/URA3/YRA1*	(63)
YCpLac111-YRA1gen	*CEN/LEU2/YRA1*	(64)
YCpLac22-Yra1-5'-HA-3'	*CEN/TRP1/YRA1 promoter-ATG-HA-SalI-stop-YRA1 terminator*	Provided by F. Stutz
YCpHIS-HA-YRA1	*CEN/HIS3/HA-YRA1*	This study [b]
pUC57-yra1-K1-22R	*yra1-K1-22R CDS*	ATG Biosynthetics [c]
YCpHIS-HA-yra1-KR	*CEN/HIS3/HA-yra1-K1-22R*	This study [d]
pRS315-nab2-F73D	*CEN/LEU2/NAB2 gene F73D with point mutation*	(35)
pRS426-GAL1-LacZ	*2µ/URA3/GAL1-LacZ*	This study [e]
pRS426-GAL1-RP51A*i-LacZ	*2µ/URA3/GAL1-RP51A* intron-out-of-frame-LacZ*	This study [e]
pRS316-GAL1-LacZ	*CEN/URA3/GAL1-LacZ*	This study [e]
pRS316-GAL1-RP51A*i-LacZ	*CEN/URA3/GAL1-RP51A* intron-out-of-frame-LacZ*	This study [e]
pRS426-GAL1-PRE3i-LacZ	*2µ/URA3/GAL1-PRE3 intron-out-of-frame-LacZ*	This study [f]
pRS426-GAL1-ACT1i-LacZ	*2µ/URA3/GAL1-ACT1 intron-out-of-frame-LacZ*	This study [f]
pRS426-GAL1-RPL35Ai-LacZ	*2µ/URA3/GAL1-RPL35A intron-out-of-frame-LacZ*	This study [f]
pSCh247	*CEN/URA3/GAL1-YAT1*	(40)
pRS316-GAL1-YAT1	*CEN/URA3/GAL1-YAT1*	This study [g]
pRS316-GAL1-RP51A*i-YAT1	*CEN/URA3/GAL1-RP51A* intron-out-of-frame-YAT1*	This study [g]
pRS316-NUP49-mCherry	*CEN/URA3/NUP49-mCherry*	(65)
pRS315-NOP1-GFP-ULP1	*CEN/LEU2/NOP1-GFP-ULP1*	(49)
pOM12	*lox-URA3-lox-6HA, for homologous recombination*	(66)
pET15-HisScSMT3	for bacterial protein production	This study[h]

a. Original TRP1 marker was swapped by homologous recombination with a disruption fragment from pTL7.
b. A XhoI-SalI fragment encompassing *YRA1* CDS (including its intron) was amplified from YCpLac111-YRA1gen and subcloned in YCpLac22-Yra1-5'-HA-3'. TRP1 marker was swapped into HIS3 by homologous recombination in yeast with a disruption fragment from pTH4.
c. An artificial fragment encompassing *YRA1* CDS (including its intron) with all Lys codons mutated to Arg codons was synthesized by ATG-Biosynthetics.
d. A XhoI-SalI fragment from pUC57-yra1-K1-22R carrying *yra1 K1-22R* mutations was subcloned in YCpLac22-Yra1-5'-HA-3'. TRP1 marker was swapped into HIS3 by homologous recombination in yeast with a disruption fragment from pTH4.
e. The GAL1promoter-LacZ or GAL1promoter-*RP51A**intron-LacZ cassettes were amplified by PCR from pLGS-D5 or pJCR51, respectively, and subcloned at the HindIII site of pRS426 or pRS316 by In-Fusion (Clontech).
f. The GAL1 promoter (+ATG) and the LacZ coding sequence were independently amplified by PCR from pJCR51. Intronic sequences were amplified by PCR from BY4742 genomic DNA. The three fragments were fused and subcloned at the HindIII site of pRS426 by In-Fusion (Clontech).
g. GAL1 promoter (+ATG) or GAL1 promoter (+ATG)- *RP51A**intron encompassing fragments were amplified by PCR from pLGS-D5 or pJCR51, respectively. The *YAT1* coding sequence (-ATG) was amplified by PCR from pSCh247. The two fragments were fused and subcloned at the HindIII site of pRS316 by In-Fusion (Clontech).
h. The coding sequence for mature Smt3 was amplified from yeast genomic DNA and further cloned in pET15b (Novagen). The obtained construct allowed to produce in bacteria a His-tagged version of Smt3 that was further purified and used for rabbit immunization.

All plasmids were checked by sequencing.

<u>Supplementary Table 3:</u> Primers used in this study

Name	Sequence
LacZ–5'-F	TTCCTGAGGCCGATACTGTC
LacZ-5'-R	TGGGATAGGTTACGTTGGTG
LacZ–3'-F	ATTAGGGCCGCAAGAAAACT
LacZ-3'-R	GTGGGCCATAATTCAATTCG
25S-F	AACGTCTATGCGAGTGTTTGG
25S-R	TTCCTCTGGCTTCACCCTATT
YAT1–5'-F *	ACTGCAGGACACGCTCAAC
YAT1-5'-R *	GTTTTCTGCGGAGAGCACAG
YAT1–3'-F *	TCTGTGGTGGTGTCCTCAAG
YAT1-3'-R *	CTTGCTGCCGTTTGAAGATG
ACT1-F	ACGTTACCCAATTGAACACG
ACT1-R	AGAACAGGGTGTTCTTCTGG

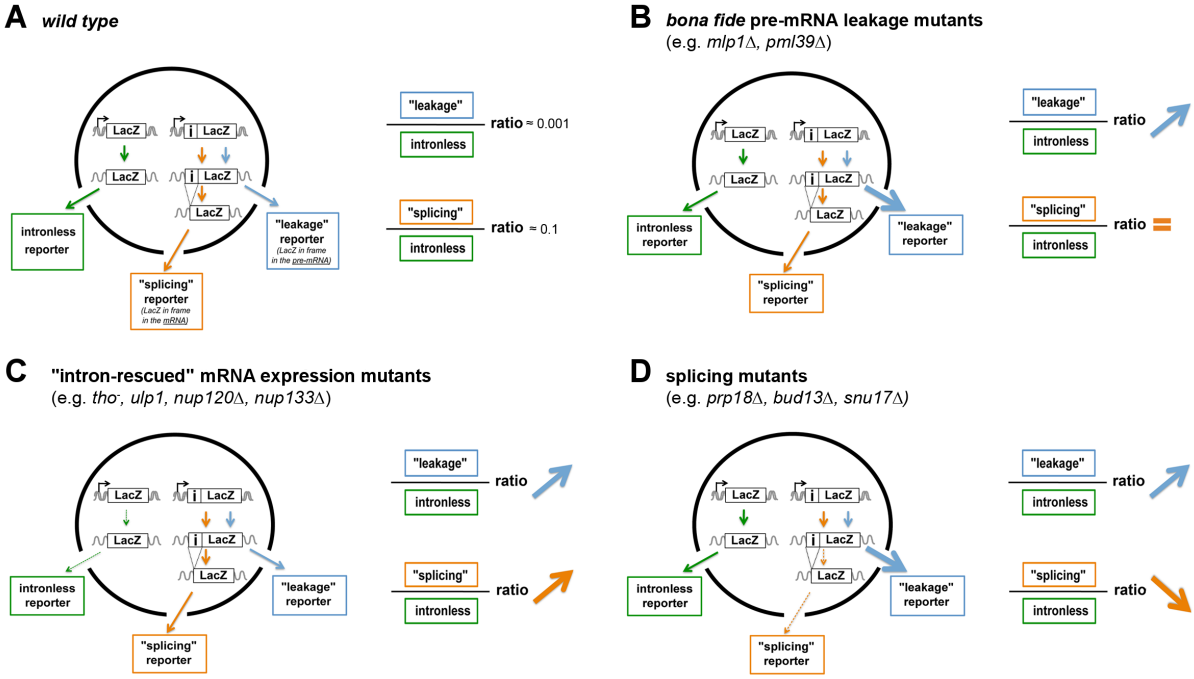
* Note that these *YAT1*-specific primers also amplify the genomic copy of *YAT1* ; however, this version of *YAT1* is ~500 times less expressed that its plasmid-borne counterpart in our growth conditions and therefore, does not account for the changes in expression observed in mutant situations.

Supplementary References

19. Bretes, H., Rouviere, J.O., Leger, T., Oeffinger, M., Devaux, F., Doye, V. and Palancade, B. (2014) Sumoylation of the THO complex regulates the biogenesis of a subset of mRNPs. *Nucleic Acids Res*, 42, 5043-5058.
20. Galy, V., Gadal, O., Fromont-Racine, M., Romano, A., Jacquier, A. and Nehrbass, U. (2004) Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. *Cell*, 116, 63-73.
25. Lewis, A., Felberbaum, R. and Hochstrasser, M. (2007) A nuclear envelope protein linking nuclear pore basket assembly, SUMO protease regulation, and mRNA surveillance. *J Cell Biol*, 178, 813-827.
26. Dziembowski, A., Ventura, A.P., Rutz, B., Caspary, F., Faux, C., Halgand, F., Laprevote, O. and Seraphin, B. (2004) Proteomic analysis identifies a new complex required for nuclear pre-mRNA retention and splicing. *EMBO J*, 23, 4847-4856.
34. Segref, A., Sharma, K., Doye, V., Hellwig, A., Huber, J., Luhrmann, R. and Hurt, E. (1997) Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)+ RNA and nuclear pores. *EMBO J*, 16, 3256-3271.
35. Fasken, M.B., Stewart, M. and Corbett, A.H. (2008) Functional significance of the interaction between the mRNA-binding protein, Nab2, and the nuclear pore-associated protein, Mlp1, in mRNA export. *J Biol Chem*, 283, 27130-27143.
40. Chavez, S., Garcia-Rubio, M., Prado, F. and Aguilera, A. (2001) Hpr1 is preferentially required for transcription of either long or G+C-rich DNA sequences in Saccharomyces cerevisiae. *Mol Cell Biol*, 21, 7054-7064.
41. Chavez, S. and Aguilera, A. (1997) The yeast HPR1 gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability. *Genes Dev*, 11, 3459-3470.
42. Legrain, P. and Rosbash, M. (1989) Some cis- and trans-acting mutants for splicing target pre-mRNA to the cytoplasm. *Cell*, 57, 573-583.
49. Zhao, X., Wu, C.Y. and Blobel, G. (2004) Mlp-dependent anchorage and stabilization of a desumoylating enzyme is required to prevent clonal lethality. *J Cell Biol*, 167, 605-611.

52. Texari, L., Dieppois, G., Vinciguerra, P., Contreras, M.P., Groner, A., Letourneau, A. and Stutz, F. (2013) The nuclear pore regulates GAL1 gene transcription by controlling the localization of the SUMO protease Ulp1. *Mol Cell*, 51, 807-818.
59. Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P. and Pringle, J.R. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast*, 14, 953-961.
60. Hentges, P., Van Driessche, B., Tafforeau, L., Vandenhaute, J. and Carr, A.M. (2005) Three novel antibiotic marker cassettes for gene disruption and marker switching in Schizosaccharomyces pombe. *Yeast*, 22, 1013-1019.
61. Cross, F.R. (1997) 'Marker swap' plasmids: convenient tools for budding yeast molecular genetics. *Yeast*, 13, 647-653.
62. Rain, J.C. and Legrain, P. (1997) In vivo commitment to splicing in yeast involves the nucleotide upstream from the branch site conserved sequence and the Mud2 protein. *EMBO J*, 16, 1759-1771.
63. Zenklusen, D., Vinciguerra, P., Strahm, Y. and Stutz, F. (2001) The yeast hnRNP-Like proteins Yra1p and Yra2p participate in mRNA export through interaction with Mex67p. *Mol Cell Biol*, 21, 4219-4232.
64. Zenklusen, D., Vinciguerra, P., Wyss, J.C. and Stutz, F. (2002) Stable mRNP formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p. *Mol Cell Biol*, 22, 8241-8253.
65. Chadrin, A., Hess, B., San Roman, M., Gatti, X., Lombard, B., Loew, D., Barral, Y., Palancade, B. and Doye, V. (2010) Pom33, a novel transmembrane nucleoporin required for proper nuclear pore complex distribution. *J Cell Biol*, 189, 795-811.
66. Gauss, R., Trautwein, M., Sommer, T. and Spang, A. (2005) New modules for the repeated internal and N-terminal epitope tagging of genes in Saccharomyces cerevisiae. . *Yeast* 22, 1-12.

Bonnet, Bretes et al - Supplementary Figure 1



Supplementary Figure 1 - LacZ reporters allow to discriminate between distinct mRNA biogenesis/export defects in nuclear pore mutants.

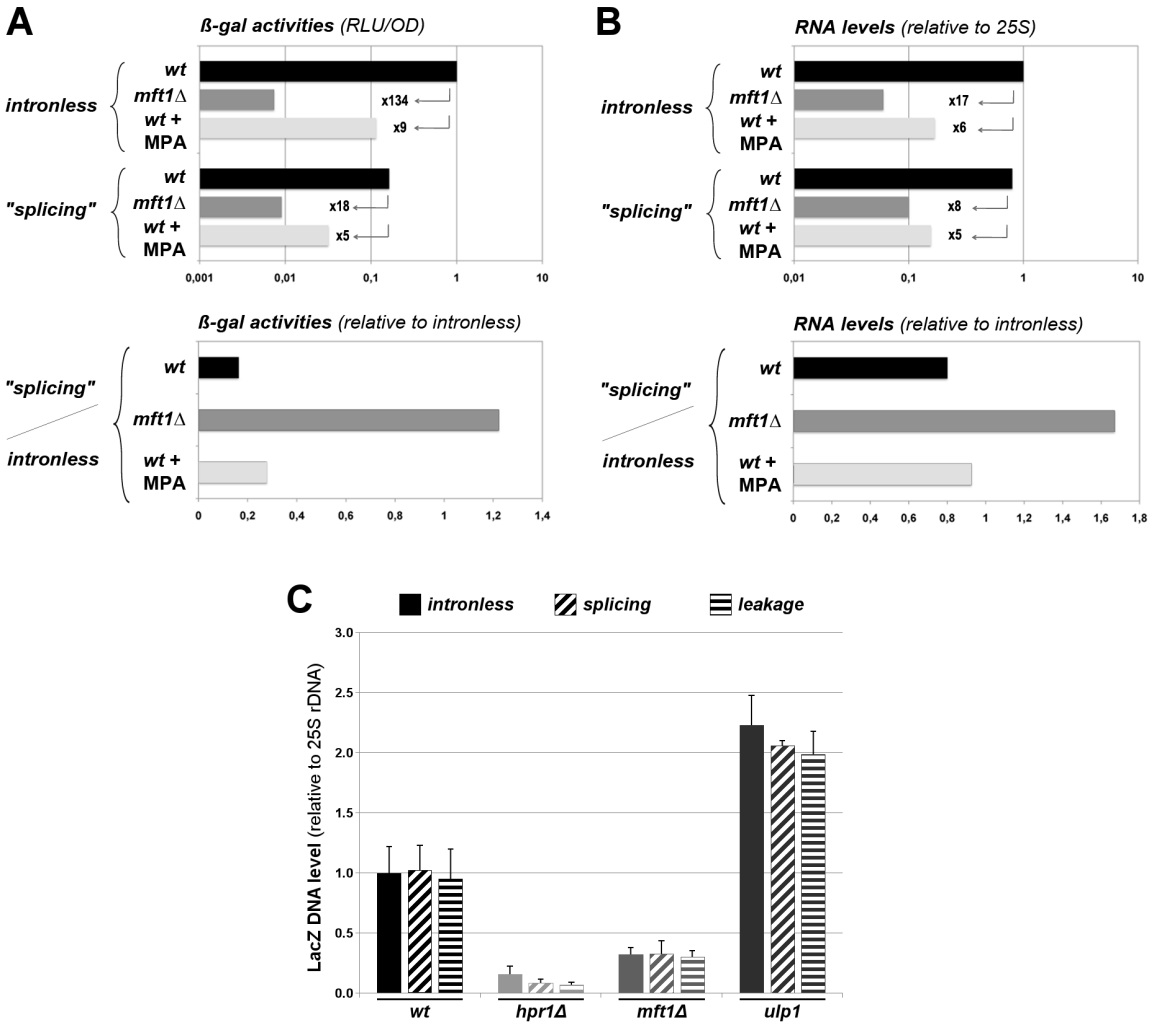
A, The ratios of β-gal activities obtained from the "*leakage*" or "*splicing*" reporters normalized to the ones obtained from their intronless counterpart are well characterized in *wt* cells.

B, An increase in the "*leakage*" ratio without change of the "*splicing*" ratio in the *mlp1Δ* and *pml39Δ* mutants reveals a function for the corresponding proteins in pre-mRNA retention.

C, The increase of both "*leakage*" and "*splicing*" ratios is a signature of THO, *ulp1* and Nup84 complex mutants which have a lower impact on the expression of intron-containing reporters, regardless of the frame of the intron, than on their intronless counterpart.

D, An increased "*leakage*" ratio in association with a decreased "*splicing*" ratio is typical of mutants affected at the splicing stage, as previously reported (20,26).

Bonnet, Bretes et al - Supplementary Figure 2

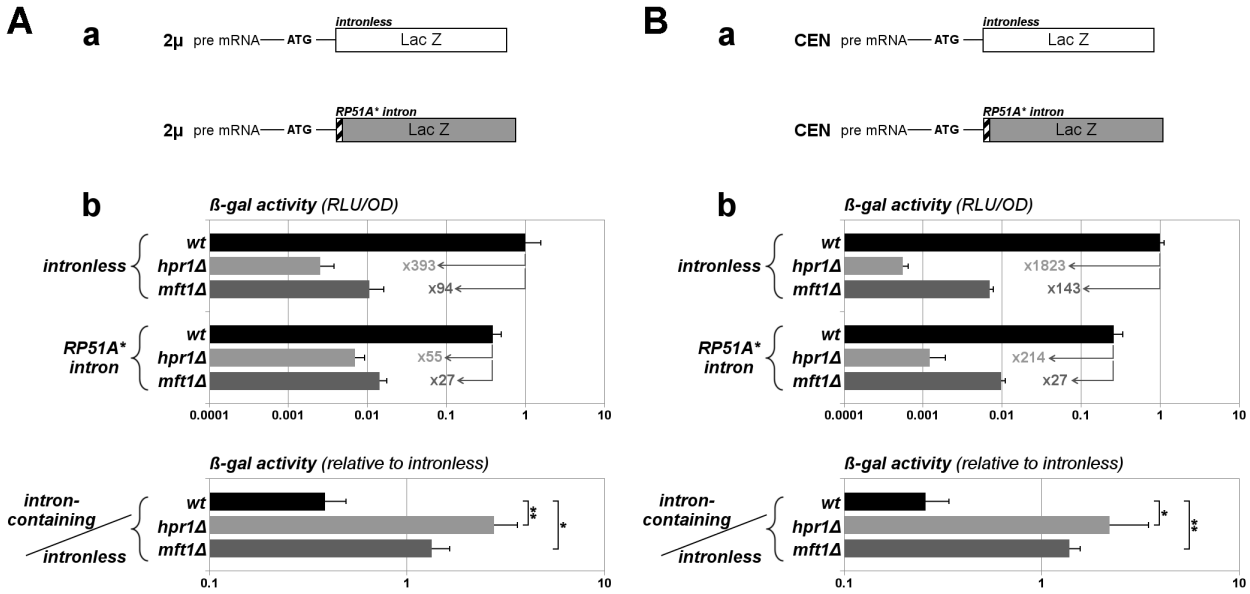


Supplementary Figure 2 – The differential effect of THO mutants on intronless and intron-containing LacZ reporters is not a mere consequence of transcriptional inhibition or heterogeneity in plasmid maintenance.

A, B, β-gal activities (**A**) and LacZ mRNA levels (**B**) from intronless and "*splicing*" reporters were measured in *wt* cells treated or not with mycophenolic acid (MPA, 100 µg/mL for 5 h) or in *mft1Δ* cells. Raw data (top panels) and data normalized to intronless (bottom panels) are indicated. Fold decreases relative to *wt* are indicated by numbers.

C, The amount of intronless, "*splicing*" or "*leakage*" LacZ reporter 2μ-plasmids were quantified in *wt* and mutant cells by qPCR (normalized to 25S rDNA ; mean ± SD; *n*=3) using LacZ-5'primers (see **Supplementary Table 3**); similar results were obtained with LacZ-3' primers (our unpublished data). Values were set to 1 for *wt* cells with the intronless reporter. Note that THO and *ulp1* mutants exhibit decreased and increased levels of 2μ-plasmids, respectively, in agreement with published reports (41,49) ; however, the three reporters plasmids are similarly affected for each mutant.

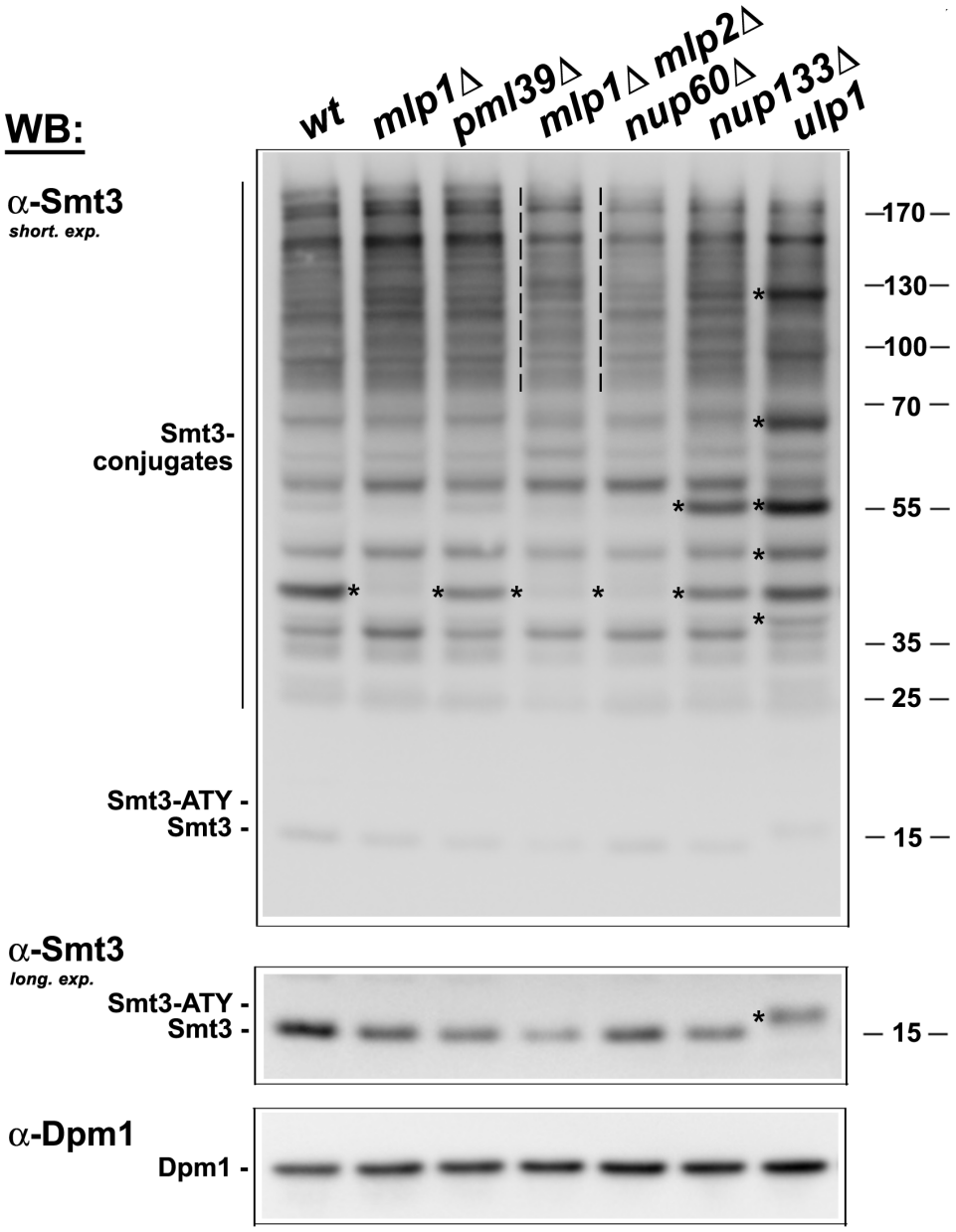
Bonnet, Bretes et al - Supplementary Figure 3



Supplementary Figure 3 – The differential effect of THO mutants on intronless and intron-containing LacZ reporters is also observed with centromeric vectors.

A, B, a, Schematic representation of the LacZ reporters used in this figure. **b**, β-gal activities from intronless and intron-containing reporters expressed from 2µ (*pRS426 series,* **A**) or centromeric (*pRS316 series,* **B**) plasmids were measured in *wt* and mutant cells. Raw data (top panels) and data normalized to intronless (bottom panels) are presented. Fold decreases relative to *wt* are indicated by numbers. Note that the more pronounced reduction of intronless LacZ expression (as compared to its intron-containing counterpart) is observed for both types of vectors in *tho* mutants.

Bonnet, Bretes et al - Supplementary Figure 4



Supplementary Figure 4 – Nuclear pore mutants triggering *bona fide* pre-mRNA leakage do not modulate cellular sumoylation patterns.

Whole cell extracts of the indicated strains were analyzed by western blotting using anti-SUMO (Smt3) antibodies. Unconjugated, mature ("Smt3") and unprocessed ("Smt3-ATY") SUMO molecules are visible upon longer exposition times (lower panel). Note that *mlp1*Δ and *pml39*Δ mutants only affect the level of a 40-kDa SUMO-conjugate, as opposed to mutants strongly impairing Ulp1 activity (e.g., *ulp1*) or its NPC localization (e.g., *mlp1*Δ*mlp2*Δ*, nup60*Δ*, nup133*Δ). Stars and lines (on the left sideof the lanes) point to the SUMO-conjugates reproducibly affected in the different mutants. Dpm1 is used as a loading control. Molecular weights are indicated (kDa).