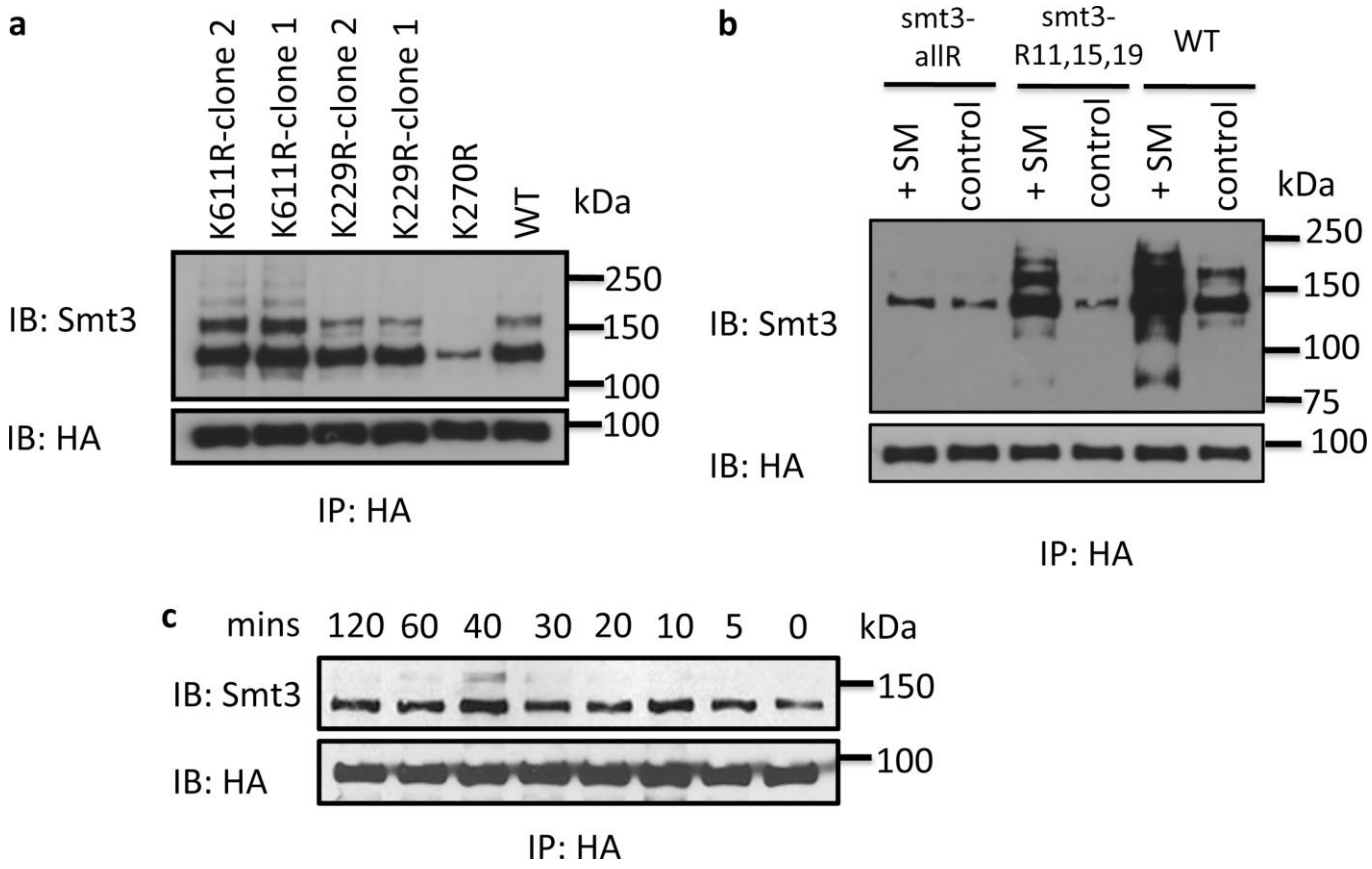
Supplementary Figure 1



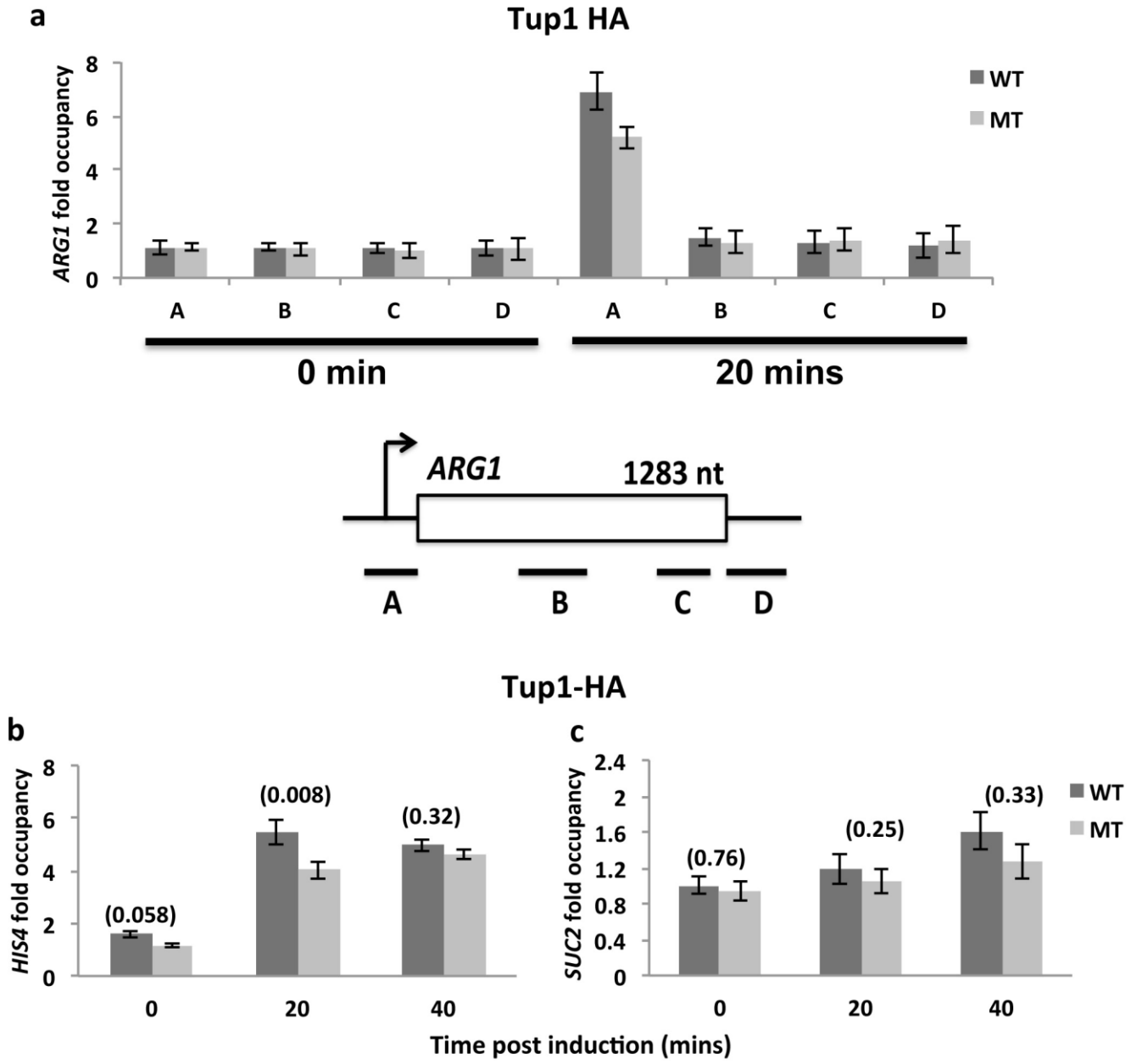
Characterization of sumoylated Tup1 protein profile

(a) Tup1 K611R mutation results in Tup1 hypersumoylation. HA immunoprecipitations of SM-treated WT, K229R, K270R and K611R mutants were analyzed by HA and yeast SUMO (Smt3) immunoblots.

(b) Tup1 is polysumoylated. HA immunoprecipitations of untreated (control), or SM-treated WT, smt3-R11,15,19 or smt3 allR mutants were analyzed by HA and yeast SUMO (Smt3) immunoblots.

(c) Time-course analysis of Tup1 immunoprecipitation after SM treatment. HA immunoprecipitations of SM-treated WT at the indicated time points were analyzed by HA and yeast SUMO (Smt3) immunoblots.

Supplementary Figure 2

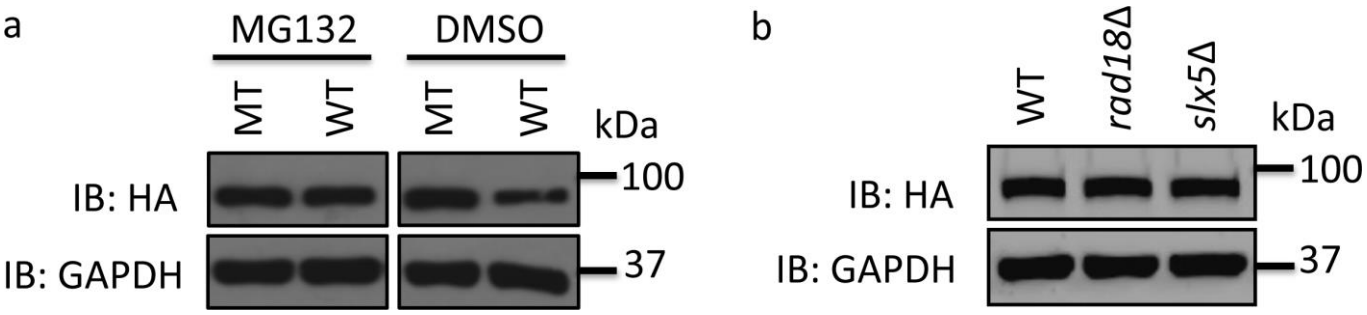


Characterization of sumoylated Tup1 ChIP profile

(a) Tup1-WT and Tup1-MT(K229,270R) are found mostly on *ARG1* promoter after SM treatment. Tup1-HA ChIP analysis of Tup1-WT or Tup1-MT (K229,270R) on indicated *ARG1* gene positions at 0 and 20 mins after SM treatment and gene diagrams for *ARG1* indicating gene length, approximate position of transcriptional start site (bent arrow), and regions amplified by indicated ChIP primers.

(b-c) Tup1 sumoylation enhances its own association with *HIS4* promoter, but not with *SUC2* promoter after SM treatment. Tup1-HA ChIP analysis of Tup1-WT or Tup1-MT (K229,270R) on (b) *HIS4* and (c) *SUC2* promoters at indicated times after SM induction. Data are represented as mean +/- SD of three independent experiments. *P*-values (refer to Materials and Methods for calculation) are indicated in parentheses above paired bars where relevant.

Supplementary Figure 3

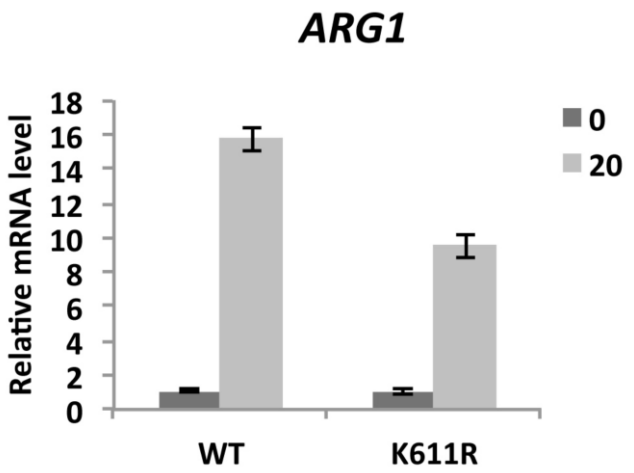


Tup1 protein stability is affected by its sumoylation, but SUMO-targeted ubiquitin ligases Slx5 and Rad18 do not regulate Tup1 protein level

(a) Tup1-HA immunoblot analysis of extract from yeast cells transformed with Tup1-HA WT or Tup1-HA MT (K229,270R). In order to make cells permeable to MG132, yeast strains were grown at 30°C in a synthetic medium (0.17% yeast nitrogenous base without ammonium sulfate) supplemented with 0.1% proline, appropriate amino acids, and 2% glucose. The overnight culture was reinoculated into 50 ml fresh media with 0.003% SDS at OD of 0.5. The cells were grown for an additional 3 h at 30°C. The transformed cells were either treated with DMSO or MG132. GAPDH was used as a loading control.

(b) Tup1-HA immunoblot analysis of extract from yeast cells (wild-type, *slx5Δ* or *rad18Δ* strains) transformed with Tup1-WT HA protein level. The cells were grown in at 30°C in a synthetic medium (0.17% yeast nitrogenous base without ammonium sulfate) supplemented with 5 g l^{-1}, appropriate amino acids, and 2% glucose. GAPDH was used as a loading control.

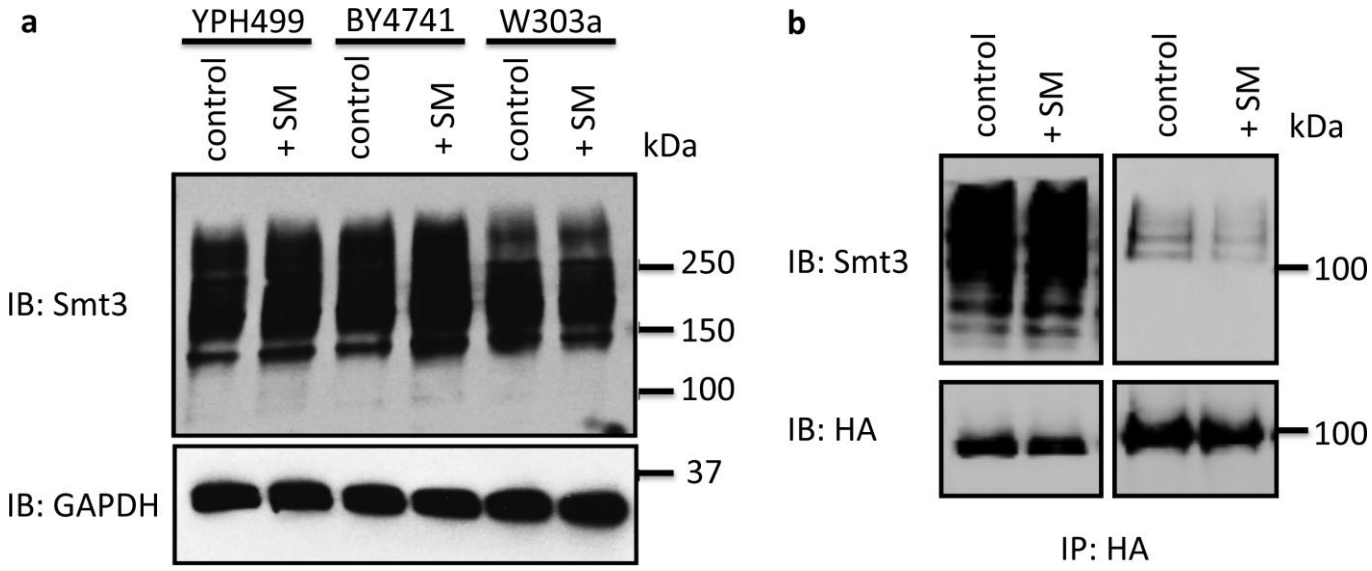
Supplementary Figure 4



Hypersumoylated Tup1 results in repression of *ARG1* mRNA gene expression

Steady-state RNA levels were determined by RT-PCR for Tup1 target gene, *ARG1* and control housekeeping gene, *ACT1* at the indicated time after exposure to SM. Analysis was performed in strains expressing Tup1-WT, or Tup1-K611R as indicated. Values were normalized to *ARG1* RNA level in strain expressing Gcn4-WT and Tup1-WT at 0 min. Data are represented as mean +/- SD of three independent experiments.

Supplementary Figure 5

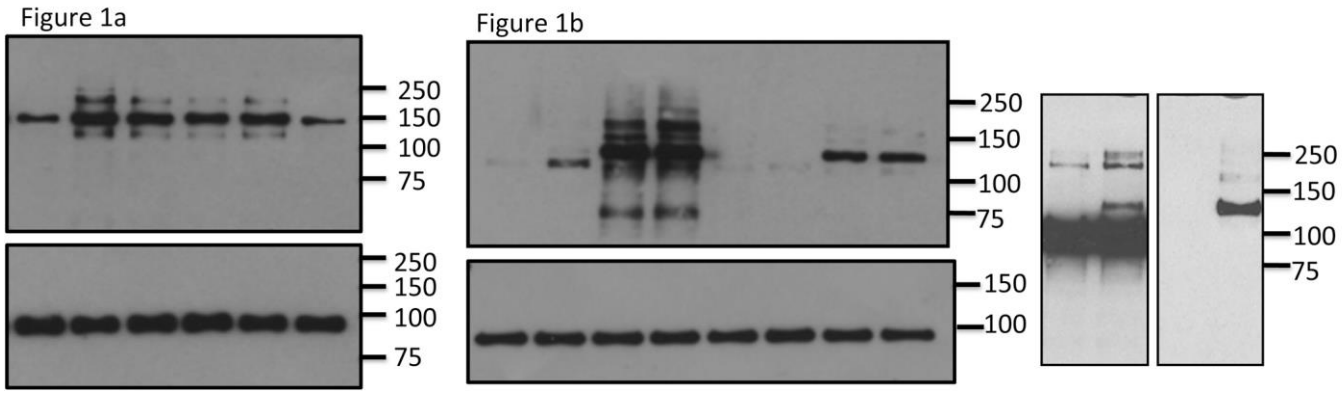


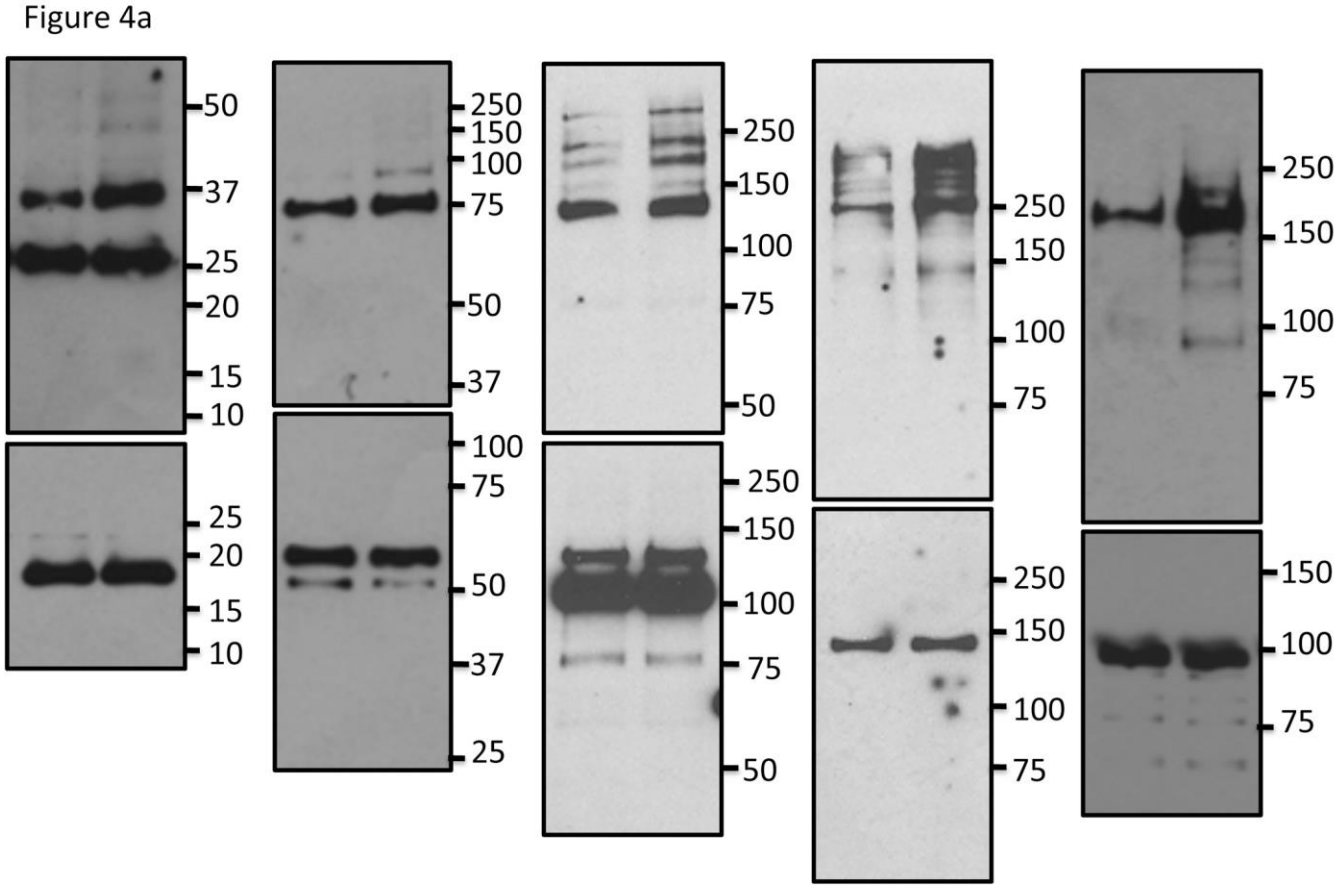
Treatment of SM does not result in a global increase in sumoylation

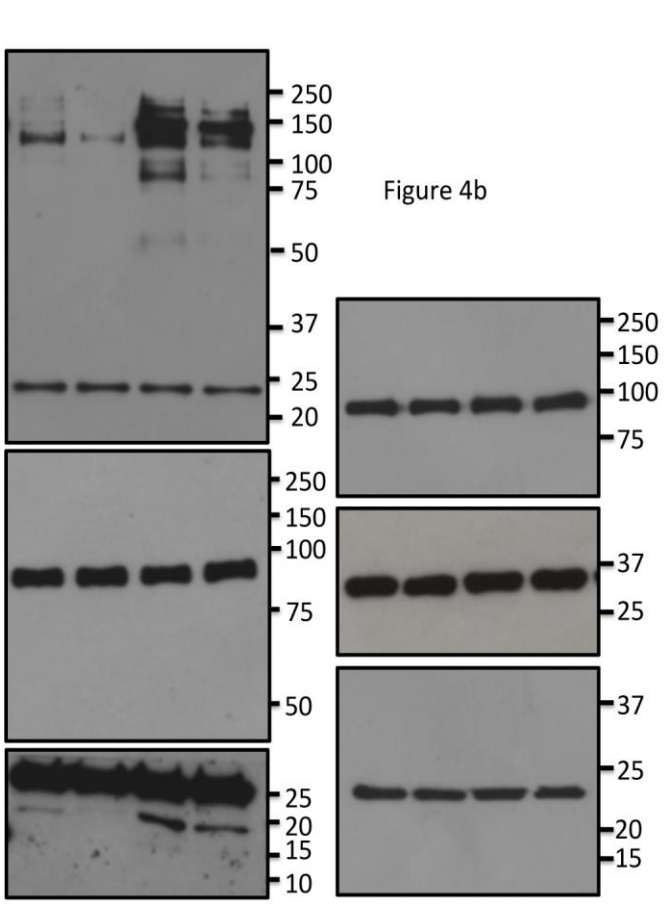
(a) Total sumoylated proteins in three different WT (YPH499, BY4741 and W303a strains) before and after SM treatment were determined by Western blot analysis. GAPDH was used a loading control.

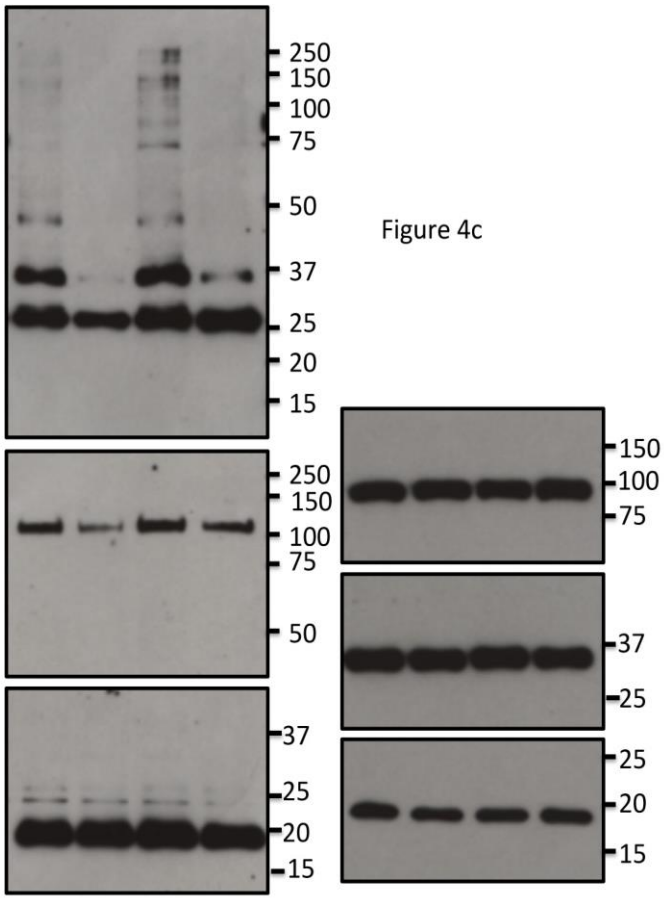
(b) SM treatment does not result in an increase in Tfg1 sumoylation level. HA immunoprecipitations of control (untreated) and SM-treated Tfg1-HA tagged wild-type strains were analyzed by HA and yeast SUMO (Smt3) immunoblots.

Supplementary Figure 6









Uncropped Western blot scans of Figures 1a, 1b, 4a, 4b and 4c

Supplementary Table 1 Yeast strains used in this study

Strain	Genotype	Source
The following strains are derived from the YPH499 strain (*MAT*a *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 his3-Δ200 leu2-Δ1)*		
CHNM1	*TUP1::TUP1-6HA–K. lactis TRP1*	This study
CHNM2	*CYC8::CYC8-6HA–K. lactis TRP1*	This study
CHNM3	*GAL11::GAL11-6HA–K. lactis TRP1*	This study
CHNM4	*GCN5::GCN5-6HA–K. lactis TRP1*	This study
CHNM5	*HHT1::HHT1-6HA–K. lactis TRP1*	This study
CHNM6	*CTI6::CTI6-6HA–K. lactis TRP1*	This study
CHNM7	*TUP1::TUP1-6HA-K270R–K. lactis TRP1*	This study
CHNM8	*TUP1::TUP1-6HA-K611R–K. lactis TRP1*	This study
CHNM9	*TUP1::TUP1-6HA–K. lactis TRP1 gcn4Δ::kanMX [pGCN4-6HA/CEN URA3*]	This study
CHNM10	*TUP1::TUP1-6HA–K. lactis TRP1 gcn4Δ::kanMX [pGCN4-6HA-K50,58R/CEN URA3]*	This study
CHNM11	*TUP1::TUP1-6HA-K270R–K. lactis TRP1 gcn4Δ::kanMX [pGCN4-6HA/CEN URA3]*	This study
CHNM12	*TUP1::TUP1-6HA-K270R–K. lactis TRP1 gcn4Δ::kanMX [pGCN4-6HA-K50,58R/CEN URA3]*	This study
CHNM13	*tup1Δ::kanMX [pTUP1-6HA/CEN URA3]*	This study
CHNM14	*tup1Δ::kanMX [pTUP1-6HA-K229R/CEN URA3]*	This study
CHNM15	*tup1Δ::kanMX [pTUP1-6HA-K270R/CEN URA3]*	This study
CHNM16	*tup1Δ::kanMX [pTUP1-6HA-K229,270R/CEN URA3]*	This study
CHNM17	*TUP1::TUP1-6HA–K. lactis TRP1 GAL11::GAL11-FLAG kanMX*	This study
CHNM18	*TUP1::TUP1-6HA-K270R–K. lactis TRP1 GAL11::GAL11-FLAG kanMX*	This study
CHNM19	*TUP1::TUP1-6HA–K. lactis TRP1 GCN5::GCN5-FLAG kanMX*	This study
CHNM20	*TUP1::TUP1-6HA–K270R-K. lactis TRP1 GCN5::GCN5-FLAG kanMX*	This study
CHNM21	*TUP1::TUP1-6HA–K. lactis TRP1 HHT1::HHT1-FLAG kanMX*	This study

CHM22	*TUP1::TUP1-6HA–K270R-K. lactis TRP1 HHT1::HHT1-FLAG kanMX*	This study
The following strains are derived from the strains used in Bylebyl et al. 2003.		
CHNM23	*MAT***a** *trp1-Δ1 ura3-52 his3-Δ200 leu2-3,112 lys2-801[pTUP1-HA/CEN URA3]*	This study
CHNM24	*MAT***a** *smt3-R11,15,19*::*TRP1[pTUP1-HA/CEN URA3]*	This study
CHNM25	*MAT***a** *smt3-R38,40,41*::*TRP1[pTUP1-HA/CEN URA3]*	This study
The following strains are derived from W303a (*MAT*a *leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*)		
CHNM26	*TFG1::TFG1-6HA–K. lactis TRP1*	This study
The following strains are derived from BY4741 (*MAT*a *his3*Δ0 *leu2*Δ0 *met15*Δ0 *ura3*Δ0)		
YAA018	*TUP1:TUP1-3HA-HIS3MX6*	This study
YAA019	*slx5Δ::kanMX TUP1:TUP1-3HA-HIS3MX6*	This study
YAA020	*rad18Δ::kanMX TUP1:TUP1-3HA-HIS3MX6*	This study

Supplementary Table 2 Primers sequences used in this study

Primer name	Primer sequence	Note
Tup1F-HA-Trp	GATTGTAAAGCAAGGATTTGGAAGTATAAAAAAATAGC GCCAAAT**CGTACGCTGCAGGTCGAC**	HA-tagging of Tup1
Tup1R-HA-Trp	GTTTAGTTAGTTACATTTGTAAAGTGTTCCTTTTGTGTTC TGTTC**ATCGATGAATTCGAGCTCG**	
Cyc8F-HA-Trp	GAAAATGTAGTAAGGCAAGTGGAAGAAGATGAAAACT ACGACGAC**CGTACGCTGCAGGTCGAC**	HA-tagging of Cyc8
Cyc8R-HA-Trp	TCGTTGATTATAAATTAGTAGATTAATTTTTTGAATGCA AACTTT**ATCGATGAATTCGAGCTCG**	
Gal11F-HA-Trp	GAACAATTCAATGTATGGGATTGGAATAATTGGACAAG TGCTACT**CGTACGCTGCAGGTCGAC**	HA-tagging of Gal11
Gal11R-HA-Trp	ACGAAGTAACTTCAAAAGTATCAAAAGTATGGAAACTT CAAATGT**ATCGATGAATTCGAGCTCG**	
Gcn5F-HA-Trp	AATAATAAAGTAAAAGAAATACCTGAATATTCTCACCT TATTGAT**CGTACGCTGCAGGTCGAC**	HA-tagging of Gcn5
Gcn5R-HA-Trp	TTTCTTCTTCGAAAGGAATAGTAGCGGAAAAGCTTCTTC TACGCA**ATCGATGAATTCGAGCTCG**	
Hht1F-HA-Trp	CAAAAGAAGGATATCAAGTTGGCTAGAAGATTAAGAG GTGAAAGATCA**CGTACGCTGCAGGTCGAC**	HA-tagging of Hht1
Hht1R-HA-Trp	TATTGTGTTTTTGTTCGTTTTTTACTAAAACTGATGACA ATCAACAAA**ATCGATGAATTCGAGCTCG**	
Cti6F-HA-Trp	CTACTATGGGAGAAAAAATATTCAAATAACACTAATGC CATTCAA**CGTACGCTGCAGGTCGAC**	HA-tagging of Cti6
Cti6R-HA-Trp	ATTACAGTTATACTTTGGTTGAGAATAATATTGCAGTGT TTTGCG**ATCGATGAATTCGAGCTCG**	
Tup1-K229R-F-Int	GAGACCACTACTTTACCCTCTGTCA**G**GGCACCTGAATC TACGTTGAAAGAA	Creation of integrative Tup1 mutations
Tup1-K270R-F-Int	GCAACTGAAACTGAAATCAAACCTA**G**GGAGGAAGACG CCACCCCGGCTAGT	
Tup1-K611R-F-Int	TGGAATTTGCAGAATGCAAACAACA**G**GAGCGATTCGA AAACTCCAAATTCC	
Tup1-R-Int	GAATAGTTTAGTTAGTTACATTTGTAAAGTGTTCCTTTT GTGTTCTGTTC	
Tup1-K229-LF	CCCTCTGTCA**G**GGCACCTGAA**TCTACGTTGAAAGAAA CTGAACCG**	Creation of Tup1-K229R mutation using SLIM
Tup1-K229-SF	**TCTACGTTGAAAGAAACTGAACCG**	
Tup1-K229-LR	TTCAGGTGCC**C**TGACAGAGGG**TAAAGTAGTGGTCTCT TTAGAAGTAGGAG**	
Tup1-K229-SR	**TAAAGTAGTGGTCTCTTTAGAAGTAGGAG**	
Tup1-K270-LF	ATCAAACCTA**G**GGAGGAAGAC**GCCACCCCGGCTAGT TTG**	Creation of Tup1-K270R mutation using SLIM
Tup1-K270-SF	**GCCACCCCGGCTAGTTTG**	
Tup1-K270-LR	GTCTTCCTCC**C**TAGGTTTGAT**TTCAGTTTCAGTTGCGG TGGTAGT**	
Tup1-K270-SR	**TTCAGTTTCAGTTGCGGTGGTAGT**	
Tup1-K611-LF	AACA**G**GAGCGATTCGAAAACT**CCAAATTCCGGCACTT GTGAAG**	Creation of Tup1-K611R mutation using SLIM
Tup1-K611-SF	**CCAAATTCCGGCACTTGTGAAG**	
Tup1-K611-LR	AGTTTTCGAATCGCTC**C**TGTT**GTTTGCATTCTGCAAAT TCCAGA**	
Tup1-K611-SR	**GTTTGCATTCTGCAAATTCCAGA**	
pFA6a-Gal11F	GAACAATTCAATGTATGGGATTGGAATAATTGGACAAG	Flag-tagging

	TGCTACT**GGGGGAGGCGGGGGTGGA**	of Gal11
pFA6a-Gal11R	ACGAAGTAACTTCAAAAGTATCAAAAGTATGGAAACTT CAAATGT**GAATTCGAGCTCGTTTAAAC**	
pFA6a-Gcn5F	AATAATAAAGTAAAAGAAATACCTGAATATTCTCACCT TATTGAT**GGGGGAGGCGGGGGTGGA**	Flag-tagging of Gcn5
pFA6a-Gcn5R	TTTCTTCTTCGAAAGGAATAGTAGCGGAAAAGCTTCTTC TACGC**GAATTCGAGCTCGTTTAAAC**	
pFA6a-Hht1F	AAGAAGGATATCAAGTTGGCTAGAAGATTAAGAGGTG AAAGATCA**GGGGGAGGCGGGGGTGGA**	Flag-tagging of Hht1
pFA6a-Hht1R	TGTGTTTTTGTTCGTTTTTTACTAAAACTGATGACAATC AACAAA**GAATTCGAGCTCGTTTAAAC**	
Cpa2F	CCACTGTCTTCTTTCCTGCGGC	ChIP analysis
Cpa2R	GATGCTTAAACTTAAATAGCGC	
Arg1A-F	GATAGTGTAATCTGAGCAGTTG	
Arg1A-R	GTATCTAAACCACCAGAATAAGC	
Arg1B-F	GTAATGATCAAATCAGATTCG	
Arg1B-R	GGTCAAATCTTGTGGTTGGTCCG	
Arg1C-F	GACTTTAGACAAAGAAGTCCG	
Arg1C-R	GTATCGGTAGGTAAGAAACCG	
Arg1D-F	CATCGCCTCAAGATAGATAACG	
Arg1D-R	GAGGACTTAATGGTGCAGTGC	
Tel-VI-F	GCTGAGTTTAACGGTGATTATTAGG	
Tel-VI-R	CCAGTCCTCATTTCCATCAATAG	
Arg1F+454	AGATTTGCTGGCAGAAAGGA	Quantitative RT-PCR
Arg1R+609	CTTTGGTGGGGTGGTATCTG	
Cpa2F+102	TGGATCAGGAGGGCTCTCTA	
Cpa2R+280	ATTCTGGTGTAACGGGCAAG	
25s-F	CAAGTGCACCGTTGCTAGC	
25s-R	GCCTCTAAGTCAGAATCCATGC	