SUPPLEMENTAL DATA

Figure legends to supplementary data

Figure S1. Intracellular localization and degradation of ΔssPrA-3A (A) ΔssCPY* is degraded by the proteasome. Cycloheximide decay experiments were performed in Δ*pdr5* cells in the absence and presence of MG132 (20 μM) as described in Figure 1E. (B) Wild type cells expressing ΔssPrA and ΔssPrA-3A were fixed and stained with anti-HA antibodies to visualize substrates. Nuclei were stained with DAPI and anti-Kar2p antiserum was used to stain the ER and nuclear envelope. Arrowheads indicate the position of nuclei. Scale bar 2 μm. (C) Cycloheximide-chase experiments were performed in wild type and Δ*san1* cells expressing ΔssPrA and ΔssPrA-3A.

Figure S2. Effect of substrate expression level on degradation. (A) Relative substrate expression levels. Wild type cells expressing ΔssPrA or Δ2GFP controlled by the *GAS1* (LE, *Low Expression*) or *TDH3* (HE, *High Expression*) promoters were pulsed labeled for 10 min. Substrate proteins were immunoprecipitated from detergent extracts and quantified as described in materials and methods. (B) Degradation of ΔssPrA (LE) and Δ2GFP (LE) was measured in *SSA1* and *ssa1-45*^{ts} by pulse chase analysis as described in Figure 1D. (C) ΔssPrA (LE) and Δ2GFP (LE) degradation in wild type and Δ*ydj1* cells. (D) ΔssPrA (LE) and Δ2GFP (LE) degradation in wild type and Δ*san1* cells. (E) ΔssPrA (LE) and Δ2GFP (LE) degradation in wild type and Δ*san1*Δ*ubr1*. Error bars reflect the standard deviation of three independent experiments (panels A-E). (F) Wild type and Δ*san1*Δ*ubr1* cells expressing ΔssPrA (LE) and Δ2GFP (LE) were processed and stained as described in Figure 2. Arrowheads indicate the position of nuclei. Scale bar, 2 μm.

Figure S3. Effect of San1p-V5 overexpression on its localization and substrate degradation (A) Intracellular localization of overexpressed San1p-V5. Wild type cells expressing ΔssPrA/San1p-V5 and Δ2GFP/San1p-V5 were grown fixed and stained with anti-V5 antibodies to visualize San1p-V5. Nuclei were stained with DAPI and anti-Kar2p antiserum was used to mark ER membranes. Arrowhead indicates the position of the nucleus. Scale bar, 2 μm. (B) Turnover rates of ΔssPrA and Δ2GFP in wild type and San1p-V5 (OE) cells were determined by pulse chase analysis as in Figure 1C. Error bars reflect the standard deviation of three independent experiments. San1p-V5 expression was induced in 2 % galactose for 4 hours prior to experiments in panels A and B.

Figure S4. Substrate degradation in nuclear export defective *xpo1-1* cells. (A) Stability of ΔssPrA and Δ2GFP were examined in *XPO1* and *xpo1-1* cells by pulse chase analysis at 23˚C (RT) and 30°C as described in Figure 1C. Error bars reflect the standard deviation of three independent experiments. (B) Localization of NLS-GFP-NES and NLS-GFP-P12 in *xpo1-1* cells were performed by confocal microscopy at temperatures indicated. NLS-GFP-NES is a reporter protein for nuclear export (Stade *et al.*, 1997). Nuclei were stained with Hoechst. Scale bar, 2 μm.

Figure S5. Analysis of *SSA1-4* single and double mutants in CytoQC. (A) Pulse chase analysis of ΔssPrA and Δ2GFP were performed on wild type and single mutant strains indicated as described in Figure 1C. (B) Degradation of ΔssPrA and Δ2GFP was measured in *ssa* double mutants by pulse-chase analysis as described in Figure 1C. Error bars reflect the standard deviation of three independent experiments (panels A and B). Data obtained for Δ*ssa1*Δ*ssa2* are shown in Figure 7.

Figure S6. Temperature effect on substrate localization. Wild type cells expressing ΔssPrA were grown to log phase at 30°C and incubated at 30˚C or 37°C for 60 min. Cells were fixed and stained for ΔssPrA (green channel), ER/nuclear envelope (anti-Kar2p, red channel), and DNA (DAPI) as described in Figure 2. Arrowheads indicate the positions of ΔssPrA cytosolic forms. Scale bar, 2 µm.

Figure S7. Doa10p is not required for ΔssPrA and Δ2GFP degradation. Turnover rates of ΔssPrA and Δ2GFP in wild type and *Δdoa10* cells were determined by pulse chase analysis as in Figure 1C. Error bars reflect the standard deviation of three independent experiments.

TABLES

Table S1. Strains used in this study

Strain	Genotype	Source
W303	*MAT***a**, *leu2-3,112, his3-11, trp1-1, ura3-1, can1-100, ade2-1*	P. Walter (UCSF)
SMY676	*MAT***a**, *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa2*::*LEU2, ssa3*::*TRP1, ssa4*::*LYS2*	J.Brodsky (U. Pittsburgh)
SMY677	*MAT***a** *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa1-45::URA3, ssa2::LEU, ssa3::TRP1, ssa4::LYS2*	J. Brodsky (U. Pittsburgh)
RPY147	*MAT***a**, pRP21, W303 background	This study
RPY338	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pRP21, W303 background	This study
RPY340	*MAT***a**, pRP61, W303 background	This study
RPY341	*MAT***a**, pRP58, W303 background	This study
RPY205	*MAT***a**, pRP42, W303 background	This study
RPY206	*MAT***a**, pRP44, W303 background	This study
RPY235	*MAT***a**, pRP51, W303 background	This study
RPY236	*MAT***a**, pRP52, W303 background	This study
RPY252	*MAT***a**, *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa2*::*LEU2, ssa3*::*TRP1, ssa4*::*LYS2*, pRP51	This study
RPY239	*MAT***a**, leu2-*3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa1-45::URA3,*	This study

	ssa2::LEU2, ssa3::TRP1, ssa4::LYS2, pRP51	
RPY253	*MAT***a**, *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa2*::*LEU2, ssa3*::*TRP1, ssa4*::*LYS2*, pRP52	This study
RPY240	*MAT***a**, *leu2-3, 112 his3-11, ura3-52, trp1Δ1, lys2, ssa1-45::URA3, ssa2::LEU2,ssa3::TRP1, ssa4::LYS2*, pRP52	This study
RPY250	*MAT***a**, *ydj1::KANMX*, pRP52, W303 background	This study
RPY251	*MAT***a**, *ydj1::KANMX*, pRP51, W303 background	This study
RPY267	*MAT***a**, *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa2::LEU2, ssa3::TRP1, ssa4*::*LYS2*, pRP42	This study
RPY266	*MAT***a**, *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa1-45::URA3, ssa2::LEU2, ssa3::TRP1, ssa4::LYS2*, pRP42	This study
RPY269	*MAT***a**, *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa2::LEU2, ssa3::TRP1*, *ssa4*::*LYS2*, pRP44	This study
RPY268	*MAT***a** *leu2-3,112, his3-11, ura3-52, trp1Δ1, lys2, ssa1-45::URA3, ssa2::LEU2, ssa3::TRP1, ssa4::LYS2*, pRP44	This study
RPY275	*MAT***a**, *san1::KANMX*, pRP42, W303 background	This study
RPY276	*MAT***a**, *san1::KANMX*, pRP44, W303 background	This study
RPY277	*MAT***a**, *san1::KANMX*, pRP51, W303 background	This study
RPY278	*MAT***a**, *san1::KANMX*, pRP52, W303 background	This study
RPY299	*MAT***a**, *ubr1::KANMX*, pRP42, W303 background	This study
RPY300	*MAT***a**, *ubr1::KANMX*, pRP44, W303 background	This study
RPY301	*MAT***a**, *ydj1::KANMX*, pRP42, W303 background	This study
RPY302	*MAT***a**, *ydj1::KANMX*, pRP44, W303 background	This study
RPY342	*MAT***a**, *pdr5::KANMX*, pRP42, W303 background	This study
RPY343	*MAT***a**, *pdr5::KANMX*, pRP44, W303 background	This study
RPY403	*MAT***a**, *san1::KANMX, ubr1::KANMX*, pRP42, W303 background	This study
RPY404	*MAT***a**, *san1::KANMX, ubr1::KANMX*, pRP44, W303 background	This study
RPY344	*MAT***a**, *ssa1::KANMX*, pRP42, W303 background	This study
RPY345	*MAT***a**, *ssa2::KANMX*, pRP42, W303 background	This study
RPY346	*MAT***a**, *ssa3::KANMX*, pRP42, W303 background	This study
RPY347	*MAT***a**, *ssa4::KANMX*, pRP42, W303 background	This study
RPY348	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pRP42, W303 background	This study
RPY349	*MAT***a**, *ssa1::KANMX, ssa3::KANMX*, pRP42, W303 background	This study
RPY350	*MAT***a**, *ssa1::KANMX, ssa4::KANMX*, pRP42, W303 background	This study
RPY351	*MAT***a**, *ssa2::KANMX, ssa3::KANMX*, pRP42, W303 background	This study
RPY352	*MAT***a**, *ssa2::KANMX, ssa4::KANMX*, pRP42, W303 background	This study
RPY353	*MAT***a**, *ssa3::KANMX, ssa4::KANMX*, pRP42, W303 background	This study
RPY354	*MAT***a**, *ssa1::KANMX*, pRP44, W303 background	This study
RPY355	*MAT***a**, *ssa2::KANMX*, pRP44, W303 background	This study
RPY356	*MAT***a**, *ssa3::KANMX*, pRP44, W303 background	This study
RPY357	*MAT***a**, *ssa4::KANMX*, pRP44, W303 background	This study
RPY358	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pRP44, W303 background	This study
RPY359	*MAT***a**, *ssa1::KANMX, ssa3::KANMX*, pRP44, W303 background	This study
RPY360	*MAT***a**, *ssa1::KANMX, ssa4::KANMX*, pRP44, W303 background	This study
RPY361	*MAT***a**, *ssa2::KANMX, ssa3::KANMX*, pRP44, W303 background	This study
RPY362	*MAT***a**, *ssa2::KANMX, ssa4::KANMX*, pRP44, W303 background	This study
RPY363	*MAT***a**, *ssa3::KANMX, ssa4::KANMX*, pRP44, W303 background	This study
RPY401	*MAT***a**, *san1::KANMX, ubr1::KANMX*, pRP51, W303 background	This study
RPY402	*MAT***a**, *san1::KANMX, ubr1::KANMX*, pRP52, W303 background	This study
RPY403	*MAT***a**, *san1::KANMX, ubr1::KANMX*, pRP42, W303 background	This study
RPY404	*MAT***a**, *san1::KANMX, ubr1::KANMX*, pRP44, W303 background	This study
RPY430	*MAT***a**, *doa10::KANMX*, pRP42, W303 background	This study
RPY431	*MAT***a**, *doa10::KANMX*, pRP44, W303 background	This study
RPY437	*MAT***a**, *pdr5::KANMX,* pSK146 and pRS313, W303 background	This study
RPY438	*MAT***a**, *pdr5::KANMX,* pSK146 and pRP42, W303 background	This study
RPY439	*MAT***a**, *pdr5::KANMX,* pSK146 and pRP42, W303 background	This study

KWY120	*MATα, xpo1::Leu2*, expressing Xpo1p, W303 background	K. Weis (UC Berkeley)
KWY121	*MATα, xpo1::Leu2*, expressing Xpo1-1p, W303 background	K. Weis (UC Berkeley)
SKY328	*MATα, xpo1::Leu2*, expressing Xpo1p, pRPB42, W303 background	This study
SKY329	*MATα, xpo1::Leu2*, expressing Xpo1p, pRPB44, W303 background	This study
SKY332	*MATα, xpo1::Leu2*, expressing xpo1-1p, pRPB42, W303 background	This study
SKY333	*MATα, xpo1::Leu2*, expressing xpo1-1p, pRPB44, W303 background	This study
SKY599	*MATα, xpo1::Leu2*, expressing xpo1-1p, pKW430, W303 background	This study
SKY600	*MATα, xpo1::Leu2*, expressing xpo1-1p, pKW431, W303 background	This study
SKY557	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pSK112 and pRP42, W303 background	This study
SKY558	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pSK112 and pRP44, W303 background	This study
SKY560	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pRS316 and pRP42, W303 background	This study
SKY561	*MAT***a**, *ssa1::KANMX, ssa2::KANMX*, pRS316 and pRP44, W303 background	This study
SKY563	*MAT***a**, pSK145 and pRP42, W303 background	This study
SKY566	*MAT***a**, pSK145 and pRP44, W303 background	This study
SKY565	*MAT***a**, pTS210 and pRP42, W303 background	This study
SKY568	*MAT***a**, pTS210 and pRP44, W303 background	This study
SKY564	*MAT***a**, pSK146 and pRP42, W303 background	This study
SKY567	*MAT***a**, pSK146 and pRP44, W303 background	This study

Table S2. Plasmids used in this study

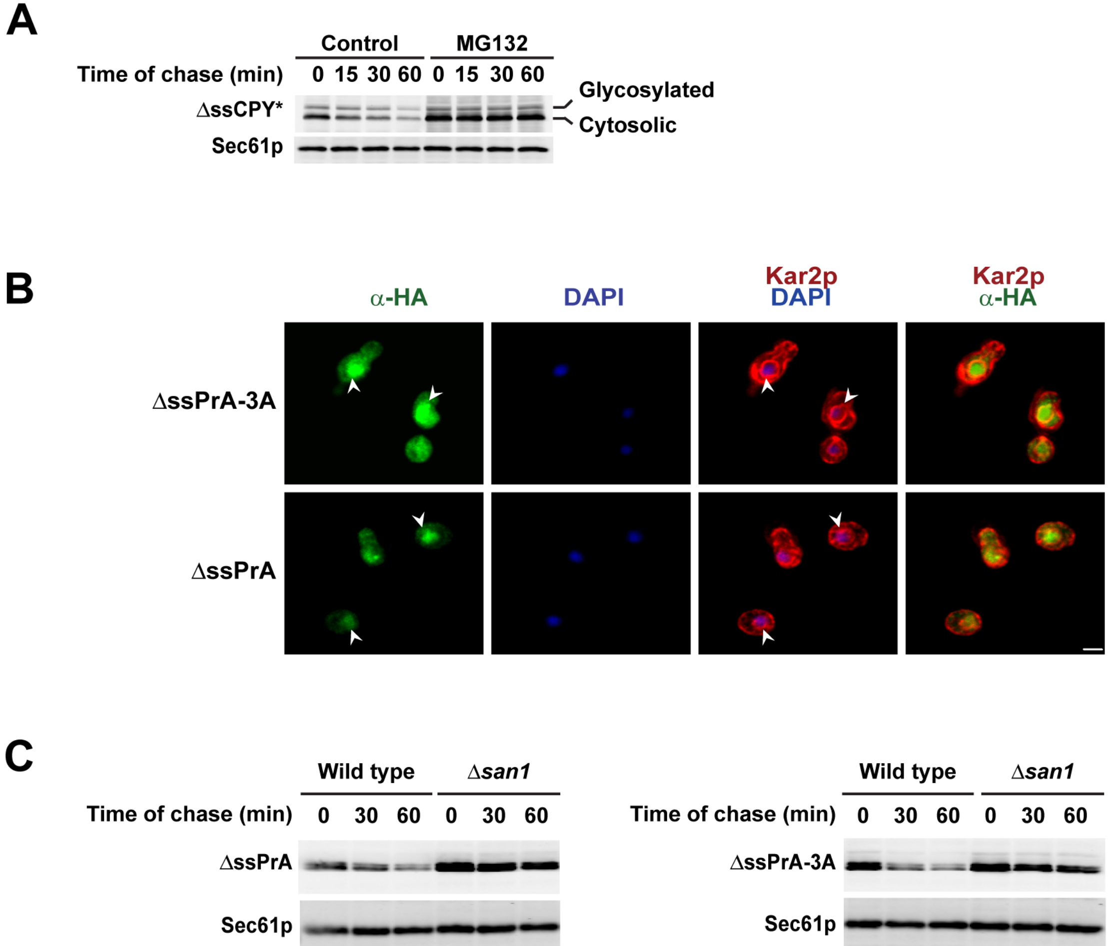
Plasmid	Protein	Promoter	Vector	Source
pRP21	ΔssCPY*	P_{TDH3}	pRS315*	This study
pRP42	ΔssPrA	P_{TDH3}	pRS313*	This study
pRP44	Δ2GFP	P_{TDH3}	pRS313*	This study
pRP51	ΔssPrA	P_{GAS1}	pRS313*	This study
pRP52	Δ2GFP	P_{GAS1}	pRS313*	This study
pRP58	PrA	P_{TDH3}	pRS313*	This study
pRP61	GFP	P_{TDH3}	pRS313*	This study
pKW430	NLS-GFP-NES	P_{ADH}	pRS426	K. Weis (UC Berkeley)
pKW431	NLS-GFP-P12	P_{ADH}	pRS426	K. Weis (UC Berkeley)
pSK112	FLAG-Ssa1	P_{TDH3}	pRS316	This study

pSK145	San1	P_{GAL}	pTS210	This study
pSK146	San1-V5H6	P_{GAL}	pTS210	This study

*(Sikorski and Hieter, 1989)

Table S3. Oligonucleotide primers used in this study

Primer	Plasmid	Sequence (5'→ 3')
RP06	pRP21	AGTTTCGACGGATCATACATACGCATGATCTCATTGCAAAGACCG TTGGGTAC
RP57	pRP58	CGGGATCCCGATGTTCAGCTTGAAAGCATTATTGCC
RP61	pRP58	TCCCCCGGGGGATCAAGCGTAATCTGGAACATCATAT
RP63	pRP61	CGGGATCCCGATGAGTAAAGGAGAAGAACTTTTCACTGG
RP64	pRP61	GCTCTAGAGCTTAAGCGTAATCTGGAACATCATATGGGTATTTGTAT AGTTCATCCATGCCATGT
RP59	pRP42	AACTTGTTTCGACGGATCCCGATGAAAGTCCACAAGGCTAAAATT TATA
RP65	pRP44	GAATTAGATGGTGATGTTAATGGGGCAACATACGGAAAACTTACC CTTA
SK165	pSK112	ATGGATTACAAGGACGATGACGATAAAATGTCAAAAGCTGT CGGTATTGATTTAG
SK166	pSK112	TTAATCAACTTCTTCAACGGTTGG
SK232	pSK112	CTGAGGATCCCGATGGATTACAAGGACGATGACGATAA
SK233	pSK112	ACTGTCTAGAGCTTAATCAACTTCTTCAACGGTTGGA
SK246	pSK145 and pSK146	GTCAGGATCCCGATGAGTGAAAGTGGTCAAGAAC
SK247	pSK145	GTCATCTAGACGTTATTGTGATGATCGTTGCTCATTG
SK176	pSK146	GCGTGACATAACTAATTACATGATGCG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Control
MG132
Time of chase (min)
0 15 30 60 0 15 30 60
ΔssCPY*
Glycosylated
Cytosolic
Sec61p
B
α-HA
DAPI
Kar2p
DAPI
Kar2p
α-HA
ΔssPrA-3A
ΔssPrA
C
Wild type
Δsan1
Time of chase (min)
0 30 60 0 30 60
ΔssPrA
Sec61p
Wild type
Δsan1
Time of chase (min)
0 30 60 0 30 60
ΔssPrA-3A
Sec61p

Figure S1

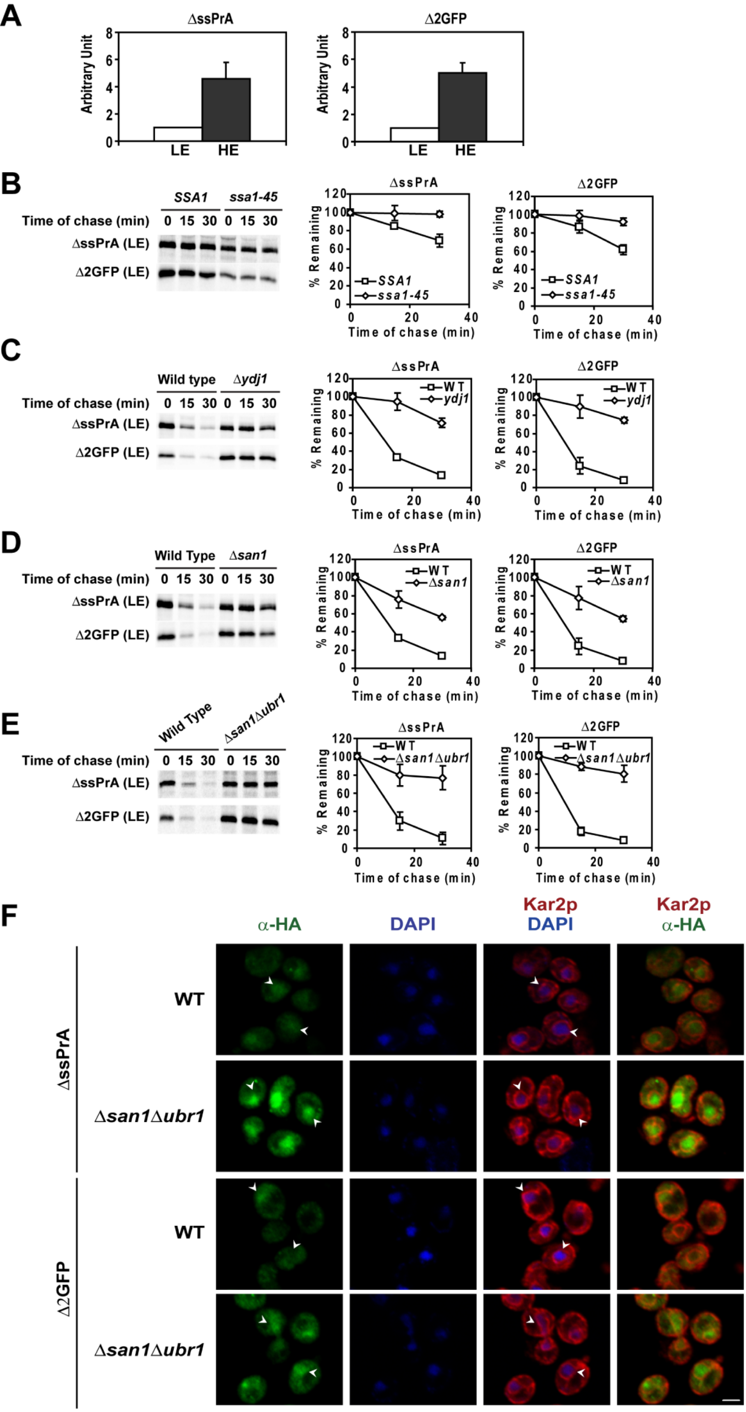

A
ΔssPrA
Δ2GFP
Arbitrary Unit
LE
HE
B
SSA1
ssa1-45
Time of chase (min)
0 15 30 0 15 30
ΔssPrA (LE)
Δ2GFP (LE)
% Remaining
Time of chase (min)
C
Wild type
Δydj1
WT
ydj1
D
Wild Type
Δsan1
E
Δsan1Δubr1
F
α-HA
DAPI
Kar2p
DAPI
Kar2p
α-HA
WT
Δsan1Δubr1

Figure S2

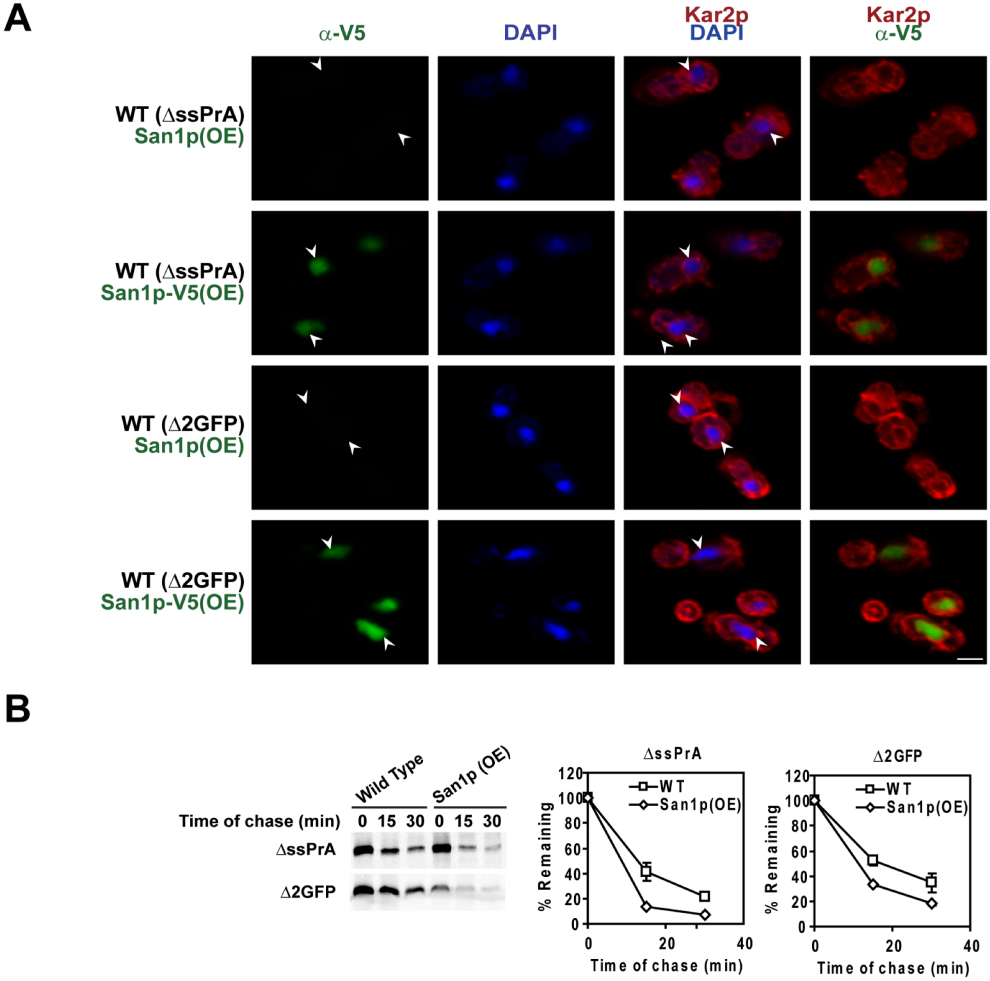
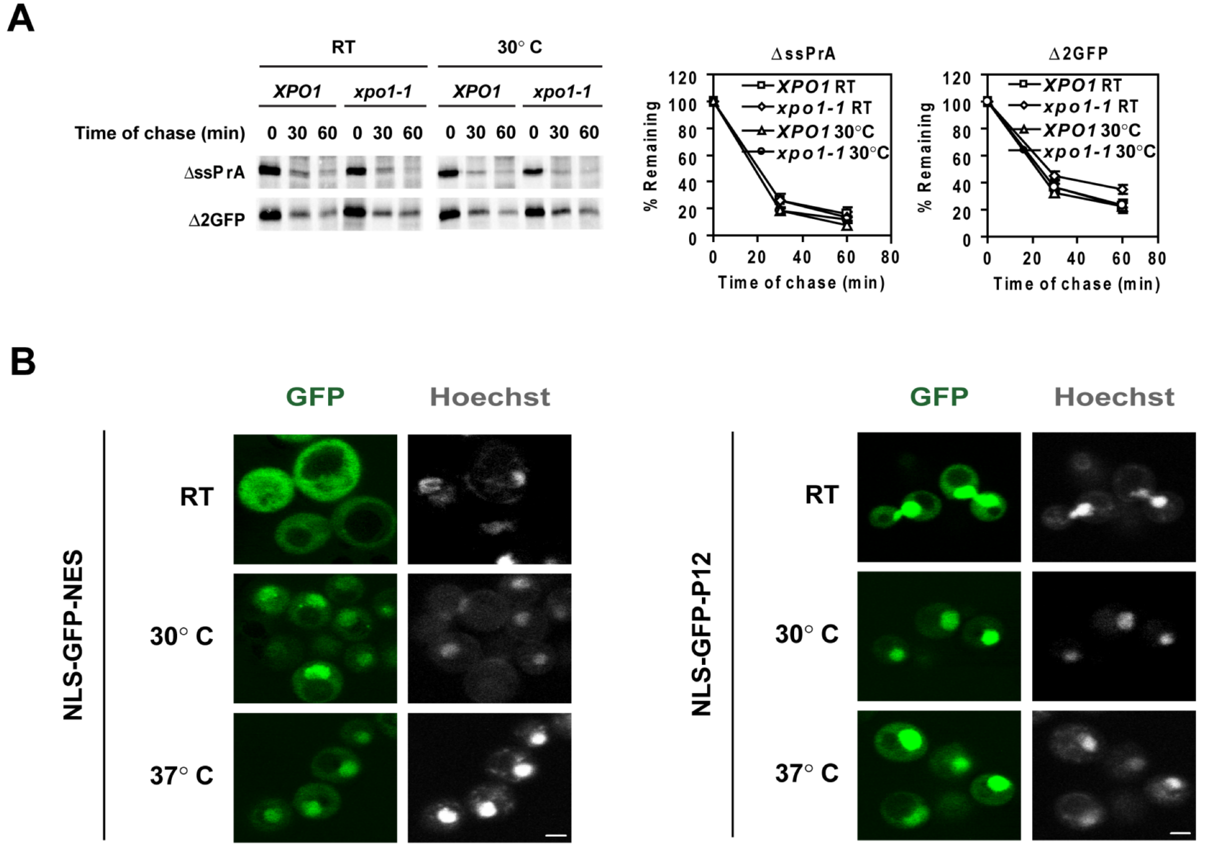

A
α-V5
DAPI
Kar2p DAPI
Kar2p α-V5
WT (ΔssPrA) San1p(OE)
WT (ΔssPrA) San1p-V5(OE)
WT (Δ2GFP) San1p(OE)
WT (Δ2GFP) San1p-V5(OE)
B
Wild Type
San1p (OE)
Time of chase (min)
0 15 30 0 15 30
ΔssPrA
Δ2GFP
ΔssPrA
% Remaining
Time of chase (min)
WT
San1p(OE)
Δ2GFP
% Remaining
Time of chase (min)
WT
San1p(OE)

Figure S3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30° C
XPO1
xpo1-1
XPO1
xpo1-1
Time of chase (min)
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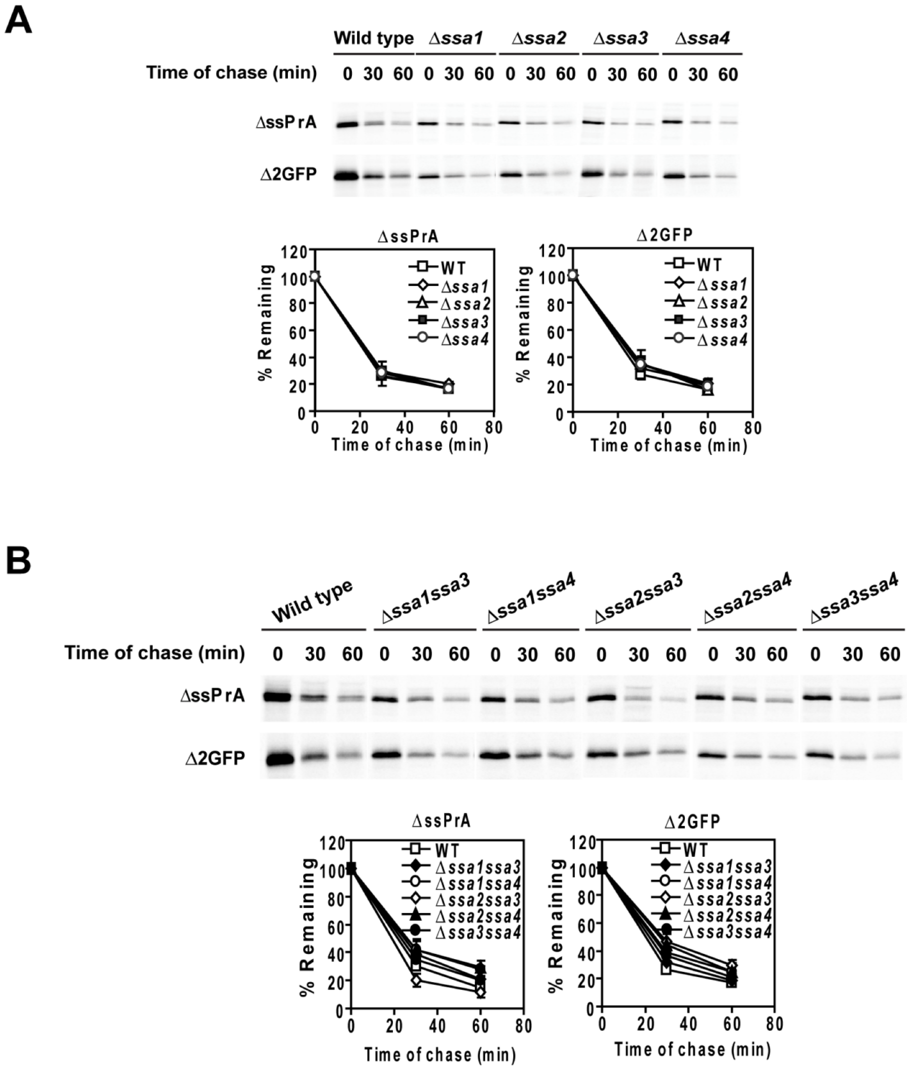
ΔssPrA
Δ2GFP
ΔssPrA
Δ2GFP
XPO1 RT
xpo1-1 RT
XPO1 30°C
xpo1-1 30°C
% Remaining
Time of chase (min)
B
GFP
Hoechst
NLS-GFP-NES
RT
30° C
37° C
GFP
Hoechst
NLS-GFP-P12
RT
30° C
37° C

Figure S4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Wild type
Δssa1
Δssa2
Δssa3
Δssa4
Time of chase (min)
0 30 60 0 30 60 0 30 60 0 30 60 0 30 60
ΔssPrA
Δ2GFP
ΔssPrA
Δ2GFP
% Remaining
Time of chase (min)
WT
Δssa1
Δssa2
Δssa3
Δssa4
B
Wild type
Δssa1ssa3
Δssa1ssa4
Δssa2ssa3
Δssa2ssa4
Δssa3ssa4
Time of chase (min)
0 30 60 0 30 60 0 30 60 0 30 60 0 30 60 0 30 60
ΔssPrA
Δ2GFP
ΔssPrA
Δ2GFP
% Remaining
Time of chase (min)
WT
Δssa1ssa3
Δssa1ssa4
Δssa2ssa3
Δssa2ssa4
Δssa3ssa4

Figure S5

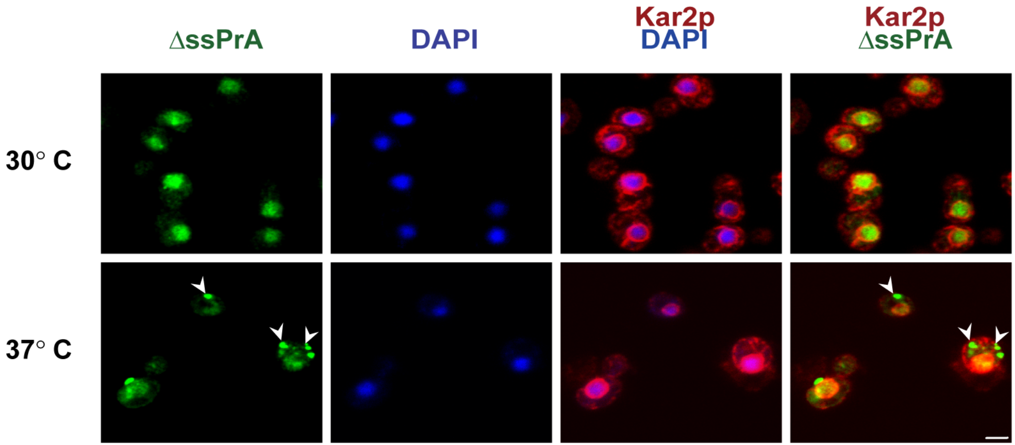

Figure S6

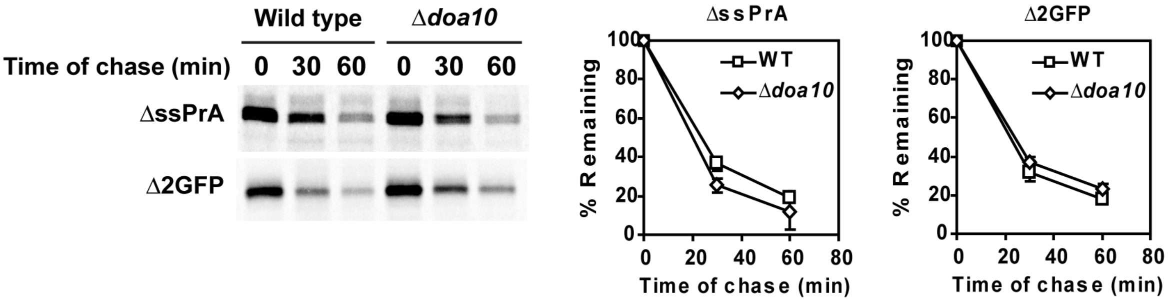
Wild type
Δdoa10
Time of chase (min)
0
30
60
0
30
60
ΔssPrA
Δ2GFP
ΔssPrA
% Remaining
Time of chase (min)
WT
Δdoa10
Δ2GFP
% Remaining
Time of chase (min)
WT
Δdoa10

Figure S7