Supporting Information.

Figure. S1

Figure. S2

Figure. S3

A

WT   Δ*hsp31*   Δ*hsp34*   Δ*31Δ34*
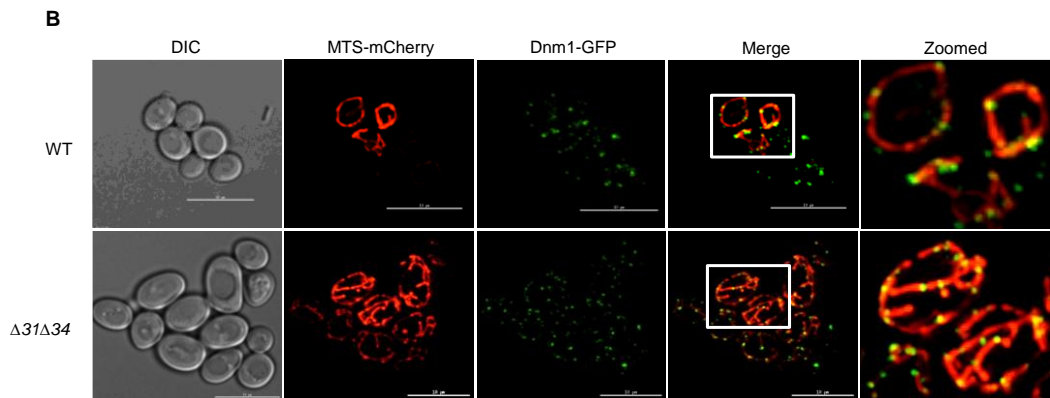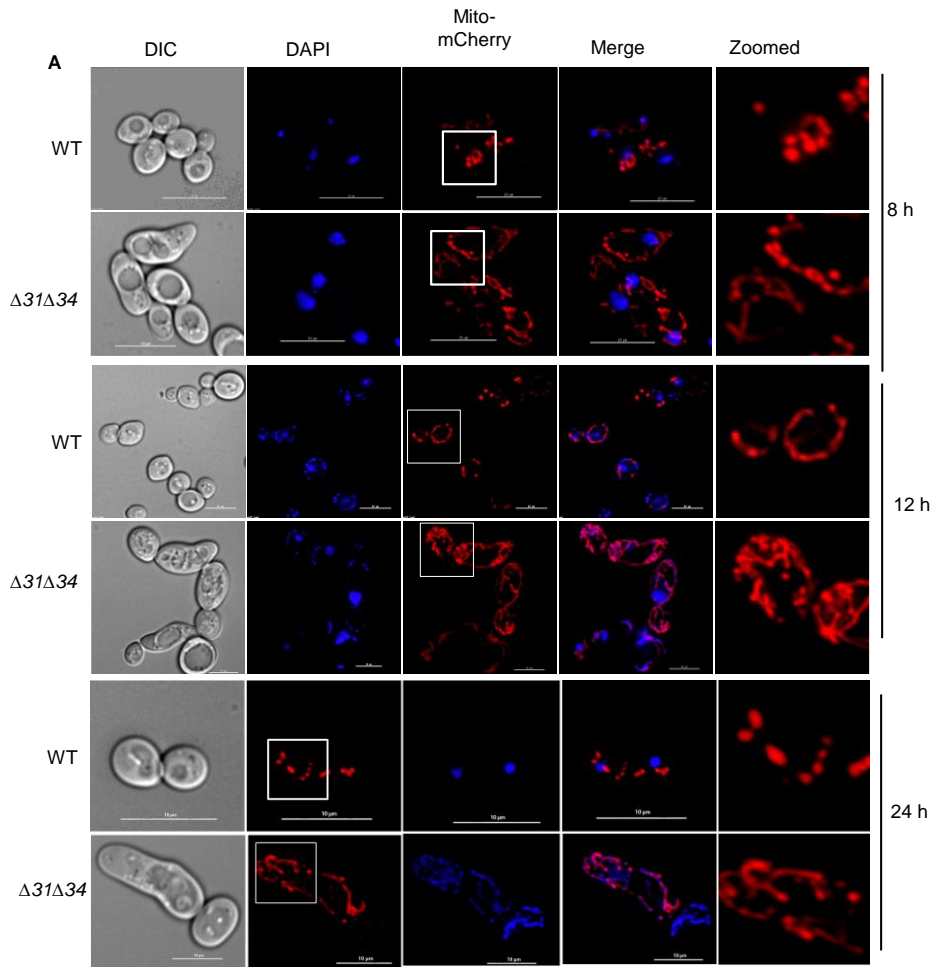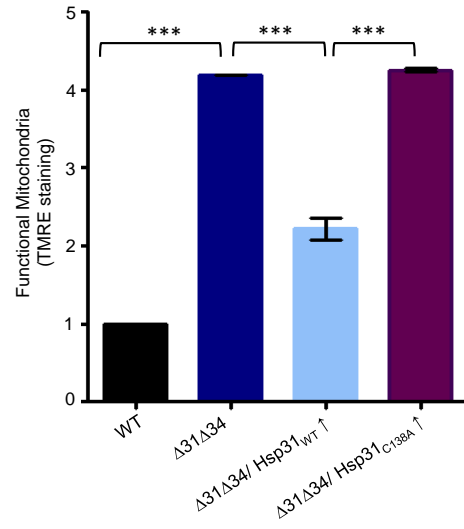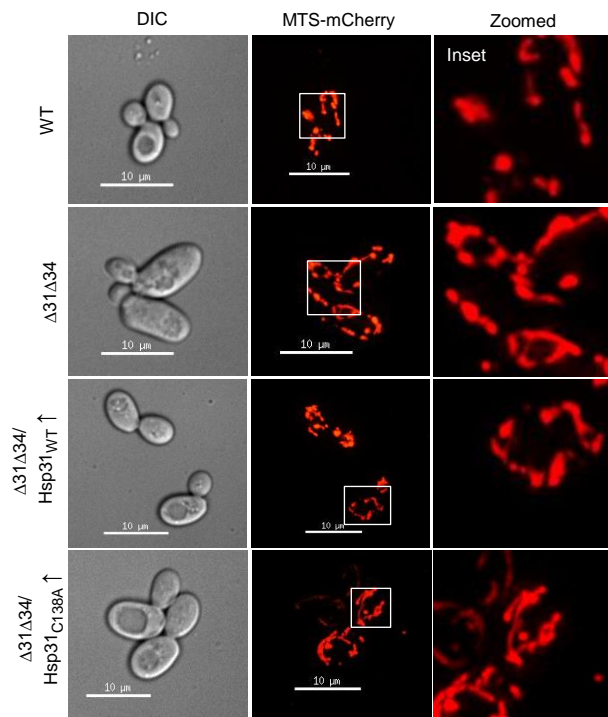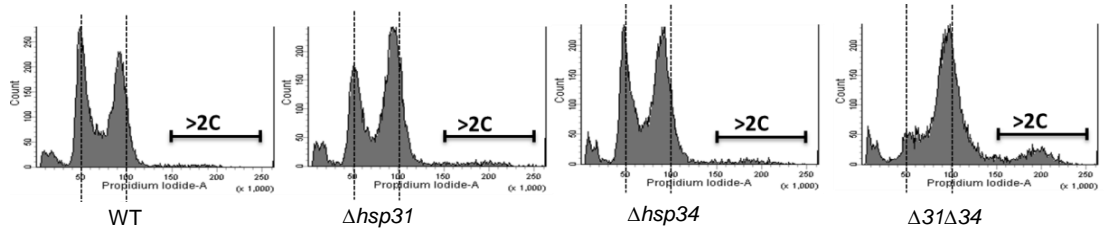
B
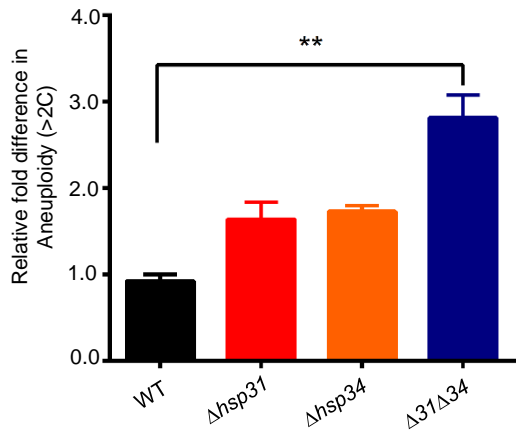
C
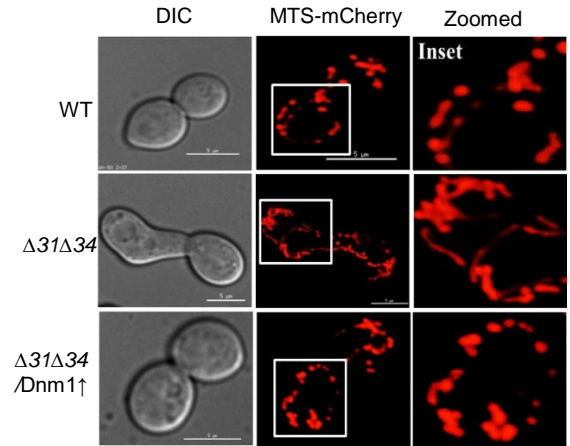
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Figure. S4

A

| | 0 min | 20 min | 40 min | 60 min | 80 min |

WT

Δ31 Δ34

Δ31 Δ34/Dnm1↑

B

Untreated   0.05 % MMS   200 mM HU

WT

Δ31Δ34   *

C

Rad9-HA

WT   Δ31Δ34   WT+MMS

*
* Non-specific band

Rad53- HA

WT   Δ31Δ34   WT+MMS

Figure. S5

A

WT Δ*hsp31* Δ*hsp34* Δ*31Δ34*

B

WT Δ*31Δ34* Δ*31Δ34/Dnm1↑*

DIC

C

DIC DAPI

WT

Δ*31Δ34*
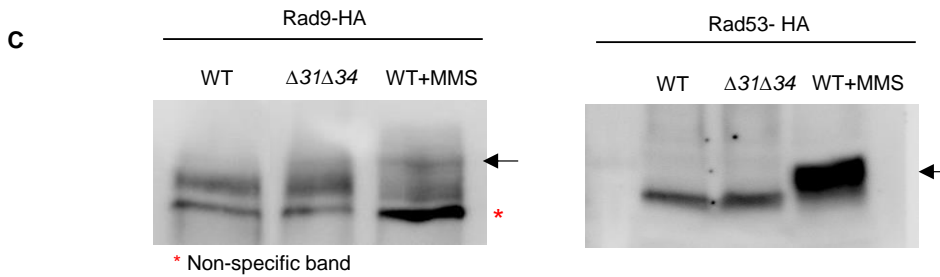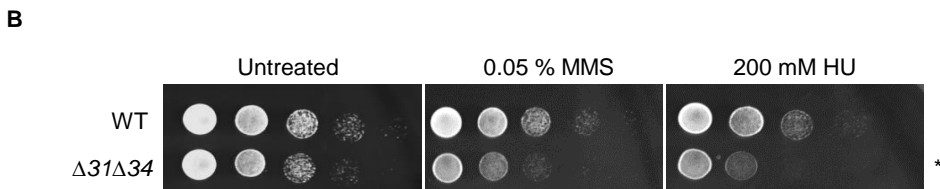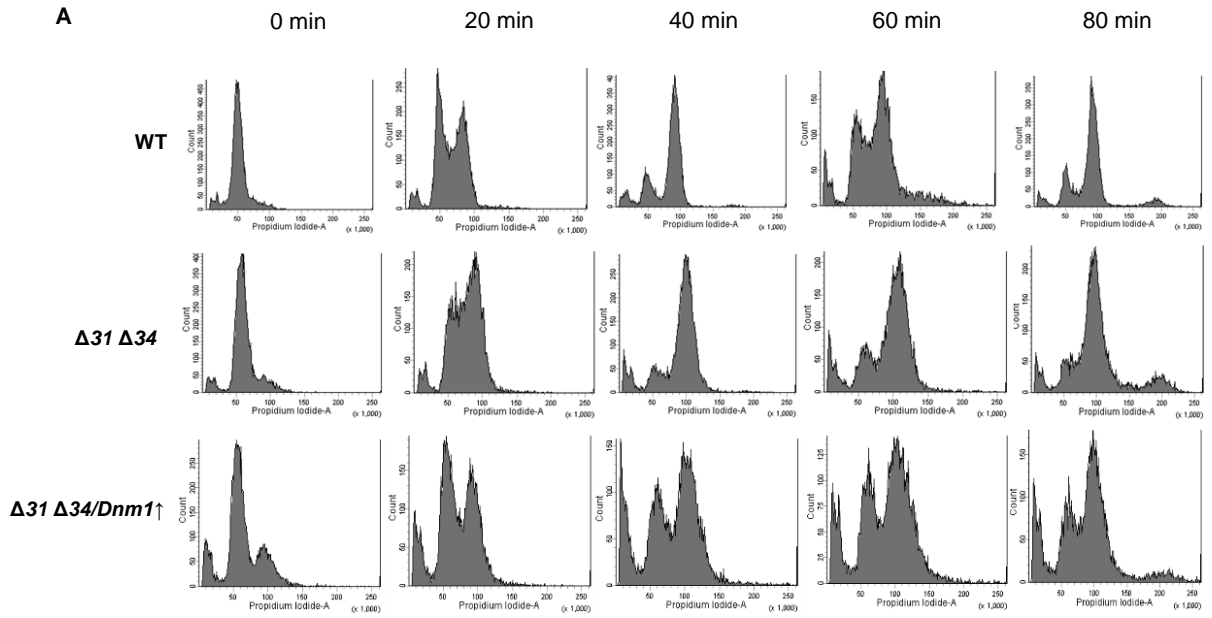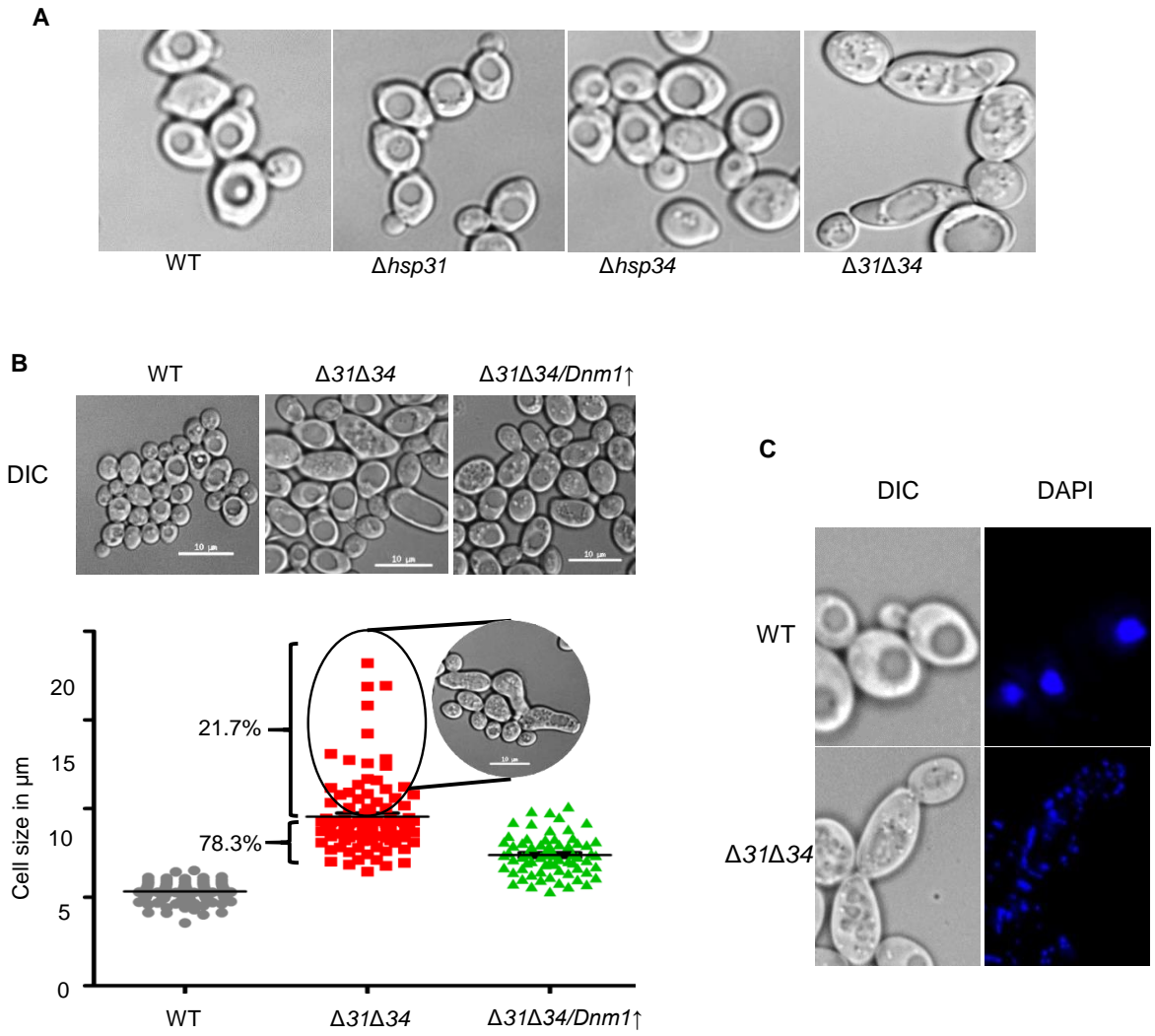
66 **Table.S1** List of yeast strains used in this study.

Strain	Genotype	Source
BY4741 WT	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
Δhsp31	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4*	Bankapalli et al. 2015 [1]
Δhsp32	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp32::hphNT1*	Bankapalli et al. 2015
Δhsp33	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp33::hphNT1*	Bankapalli et al. 2015
Δhsp34	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp34::URA3*	Bankapalli et al. 2015
Δ31Δ32	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp32::hphNT1*	This study
Δ31Δ33	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp33::hphNT1*	This study
Δ31Δ34	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3*	Bankapalli et al. 2015
Δ31Δ32Δ34	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp32::hphNT1; Δhsp34::URA3*	This study
Δ31Δ33Δ34	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp33::hphNT1; Δhsp34::URA3*	This study
Δatg32	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δatg32:: HIS3*	This study
WT-Om45-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 OM45-GFP::hphNT1*	This study
Δhsp31-Om45-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; OM45-GFP::hphNT1*	This study
Δhsp34-Om45-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp34::URA3; OM45-GFP::hphNT1*	This study
Δ31Δ34-Om45-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; OM45-GFP::hphNT1*	This study
Δatg32-Om45-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δatg32:: HIS3; OM45-GFP::hphNT1*	This study
Δbar1	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δbar1::LEU2*	This study
Δhsp31Δbar1	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δbar1::LEU2*	This study
Δhsp34Δbar1	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp34::URA3; Δbar1::LEU2*	This study
Δ31Δ34Δbar1	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; Δbar1::LEU2*	This study
WT/Dnm1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 DNM1-HA::KanMX4*	This study
WT/Fzo1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 FZO1-HA::KanMX4*	This study
WT/Mgm1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MGM1-HA::KanMX4*	This study

Δ31Δ34/Dnm1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; DNM1::hphNT1*	This study
Δ31Δ34/Fzo1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; FZO1::hphNT1*	This study
Δ31Δ34/Mgm1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; MGM1::hphNT1*	This study
WT/Dnm1-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 DNM1-GFP::hphNT1*	This study
Δ31Δ34/Dnm1-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; DNM1-GFP::hphNT1*	This study
WT/Nup49-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 NUP49-GFP::hphNT1*	This study
Δ31Δ34/Nup49-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δhsp31::KanMX4; Δhsp34::URA3; Nup49-GFP::hphNT1*	This study

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70 **Table S2.** List of primers used in this study.
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Primer name	5' → 3'	Comments
P1	CTAGAACACTTTTCTCCTTCATTCAAAAGAAAACTGGCCTTGCA	Forward, Excision of Hsp32 or Hsp33
P2	CAAGCCAAAAAAAGAAAAAAAAAGGAAAAAAAAGAAAACACAGC	Reverse, Excision of Hsp32 or Hsp33
P3	ATGATAAGGGTGATGGTAAATTCTGGAGCTCGAAAAAGGAC	Forward, Om45 GFP tagging
P4	TTATGCGGGAACCAACCCTTTACAATTAGCTATCTAACTA	Reverse, Om45 GFP tagging
P5	ATGGCCCCAAAAAAAGTTTTAC	Forward, Hsp31 cloning
P6	TTAGTTTTTTAAAGCGTCGATGGATCT	Reverse, Hsp31 cloning
P7	ATGACTCCAAAAAGAGCGCTAATA	Forward, Hsp34 cloning
P8	TCAGCTATATAATGCGTTTATAGC	Reverse, Hsp34 cloning
P9	CGGAGTTTATAAAAAGGCTGCAACCCTTATTAGTAATATTCTG	Forward, Dnm1-HA and GFP tagging
P10	CGCAATGTTGAAGTAAGATCAAAAATGAGATGAATTATGCAATTA	Reverse, Dnm1-HA and GFP tagging
P11	GTGGCTCAAAAATTGATGGTGGAAGAAATAAATTTAGACATCGAT	Forward, Fzo1-HA tagging

P12	GTATATTGATTTGAAAAGACCTCATATATTTACAAGAATATCTA	Reverse, Fzo1-HA tagging
P13	CAATCTTGGTTTTCAAGAAAAGCTACAAGGGCGTCTCCAAAAATTTA	Forward, Mgm1-HA tagging
P14	GTAAAAAATGCTATTTACAAATTCTCTAATGACACTATTTATTTTACATCA	Reverse, Mgm1-HA tagging
P15	GTTACATCAAAAAACGAAACACTGGCATCATTGAGCATA	Forward, Nup49-GFP tagging
P16	CTTGTTATACGCACTATATAAACTTTCAGGGCGATTTACTCA	Reverse, Nup49-GFP tagging
P17	TGAATCGCTCGCAACAGGAT	Forward, *DNM1* q-PCR
P18	CTACGCCACCGTATCTAGCG	Reverse, *DNM1* q-PCR
P19	CGAGCCCAGTACGGCTTTTA	Forward, *MGM1* q-PCR
P20	TCCATGACCACCGTCTTGTG	Reverse, *MGM1* q-PCR
P21	AGAGGCTCGGGAAAATGACG	Forward, *FZO1* q-PCR
P23	TGTCGGGACATTACTTCGGC	Reverse, *FZO1* q-PCR
P24	AATAAGGATTGACAGATTGAGAG	Forward, q-PCR control
P25	TTATCGCAATTAAGCAGACAA	Reverse, q-PCR control
P26	AGTTGATGCTACTCCTGGTAGA	Forward, *COX2*
P27	TTTGCATGACCTGTCCCACA	Reverse, *COX2*

Table S3. List of plasmids used in this study.

Plasmid Name	Comments
pRS 415 TEF- Hsp31	For complementation analysis.
pRS 415 TEF- Hsp31(C138A)	For complementation analysis.
pRS 415 TEF- Hsp34	For complementation analysis.
pRS 415 TEF- MTS-mCherry	For the visualization of mitochondrial morphology.
pRS 413 TEF- Dnm1	For expression in yeast strains.

References

1.   Bankapalli K, Saladi S, Awadia SS, Goswami AV, Samaddar M, D'Silva P. Robust glyoxalase activity of Hsp31, a ThiJ/DJ-1/PfpI family member protein, is critical for oxidative stress resistance in Saccharomyces cerevisiae. J Biol Chem. 2015;290(44):26491-507. Epub 2015/09/16. doi: M115.673624 [pii] 10.1074/jbc.M115.673624. PubMed PMID: 26370081.

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