

Supplementary Material

1 Supplementary Tables

Supplementary Table 1. *Hansenula polymorpha* strains used in this study

Strains	Description	Reference
WT	NCYC495, <i>leu1.1</i>	(Sudbery et al, 1988)
<i>yku80</i>	NCYC495 <i>YKU80::URA3</i>	(Saraya et al, 2012)
WT <i>PMP47</i> -mGFP	<i>pMCE7::sh ble</i>	(Manivannan et al, 2013)
<i>pex11</i> <i>PMP47</i> -mGFP	<i>PEX11::URA3 pMCE07::sh ble</i>	(Thomas et al, 2015)
<i>pex23</i> <i>PMP47</i> -mGFP	<i>PEX23::sh ble YKU80::URA3 pHIPN-PMP47-GFP::NAT</i>	(Wu et al, 2020)
<i>pex24</i> <i>PMP47</i> -mGFP	<i>PEX24::sh ble YKU80::URA3 pHIPN-PMP47-GFP::NAT</i>	(Wu et al, 2020)
<i>pex11</i>	<i>PEX11::URA3</i>	(Krikken et al, 2009)
<i>vps13</i>	<i>VPS13::sh ble YKU80::URA3</i>	This study
<i>pex11 vps13</i>	<i>PEX11::URA3 VPS13::HPH</i>	This study
HF246 GFP-SKL	<i>pHI-GFP-SKL::LEU2</i>	(van Dijk et al, 2001)
<i>vps13</i> GFP-SKL	<i>VPS13::sh ble pHIPX7-GFP-SKL::LEU2 YKU80::URA3</i>	This study

<i>pex11</i> GFP-SKL	<i>PEX11::URA3 pHIPZ4-GFP-SKL::sh ble</i>	(Nagotu et al, 2008)
<i>pex11 vps13</i> GFP-SKL	<i>PEX11::URA3 VPS13::HPH pHIPZ7-GFP-SKL::sh ble</i>	This study
<i>pex11 vps13 PEX14-mCherry PEX3-mGFP</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PEX14-mCherry::NAT pHIPZ-PEX3-GFP::sh ble</i>	This study
<i>pex11 vps13 PEX14-mCherry PEX8-mGFP</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PEX14-mCherry::NAT pMCE4::sh ble</i>	This study
<i>pex11 vps13 PEX14-mCherry PEX10-mGFP</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PEX14-mCherry::NAT pMCE5::sh ble</i>	This study
<i>pex11 vps13 PEX14-mCherry PEX13-mGFP</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PEX14-mCherry::NAT pSEM03::sh ble</i>	This study
<i>pex11 vps13 PEX14-mCherry PMP47-mGFP</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PEX14-mCherry::NAT pMCE7::sh ble</i>	This study
<i>dnm1</i> DsRed-SKL	<i>DNM1::LEU2 pHI-DsRed-SKL::URA3</i>	(Cepinska et al, 2011)
<i>dnm1 vps13</i> DsRed-SKL	<i>VPS13::sh ble DNM1::HPH pHIPN4-DsRed-SKL::NAT YKU80::URA3</i>	This study
<i>pex23</i> DsRed-SKL	<i>PEX23::sh ble pHIPN4-DsRed-SKL::NAT YKU80::URA3</i>	This study
<i>pex23 vps13</i> .DsRed-SKL	<i>PEX23::sh ble VPS13::HPH pHIPN4-DsRed-SKL::NAT YKU80::URA3</i>	This study
<i>pex24</i> GFP-SKL	<i>PEX24::sh ble YKU80::URA3 pHIPX4-GFP-SKL::LEU2</i>	(Wu et al, 2020)

<i>pex24 vps13</i> GFP-SKL	<i>PEX24::sh ble VPS13::HPH</i> <i>YKU80::URA3 pHIPX4-GFP-SKL::LEU2</i>	This study
<i>pex23</i>	<i>PEX23::sh ble YKU80::URA3</i>	(Wu et al, 2020)
<i>pex24</i>	<i>PEX24::sh ble YKU80::URA3</i>	(Wu et al, 2020)
<i>pex23 vps13</i>	<i>PEX23::sh ble VPS13::HPH</i> <i>YKU80::URA3</i>	This study
<i>pex24 vps13</i>	<i>PEX23::sh ble VPS13::HPH</i> <i>YKU80::URA3</i>	This study
<i>pex11 vps13 PMP47-GFP P_{ADH1}PEX14</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PMP47-GFP::NAT pARM059::sh ble</i>	This study
<i>pex11 vps13 PMP47-GFP P_{ADH1} PEX14-2xHA-UBC6</i>	<i>PEX11::URA3 VPS13::HPH pHIPN-PMP47-GFP::NAT pARM053::sh ble</i>	This study
<i>pex23 vps13 PMP47-GFP P_{ADH1}PEX14</i>	<i>PEX23::sh ble VPS13::HPH pHIPN-PMP47-GFP::NAT pARM069::LEU2</i> <i>YKU80::URA3</i>	This study
<i>pex23 vps13 PMP47-GFP P_{ADH1}PEX14-2xHA-UBC6</i>	<i>PEX23::sh ble VPS13::HPH pHIPN-PMP47-GFP::NAT pARM072::LEU2</i> <i>YKU80::URA3</i>	This study
<i>pex24 vps13 PMP47-GFP P_{ADH1}PEX14</i>	<i>PEX24::sh ble VPS13::HPH pHIPN-PMP47-GFP::NAT pARM069::LEU2</i> <i>YKU80::URA3</i>	This study
<i>pex24 vps13 PMP47-GFP P_{ADH1}PEX14-2xHA-UBC6</i>	<i>PEX24::sh ble VPS13::HPH pHIPN-PMP47-GFP::NAT pARM072::LEU2</i> <i>YKU80::URA3</i>	This study

Supplementary Table 2. *S. cerevisiae* strains used in this study

WT	BY4742	Euroscarf collection
WT <i>GFP-SKL</i>	BY4742, pSL34:: <i>sh ble</i>	This study
<i>pex11 GFP-SKL</i>	BY4742, <i>PEX11::KanMX</i> , pSL34:: <i>sh ble</i>	This study
<i>vps13 GFP-SKL</i>	BY4742, <i>VPS13::KanMX</i> , pSL34:: <i>sh ble</i>	This study
<i>pex11 vps13 GFP-SKL</i>	BY4742, <i>VPS13::KanMX</i> <i>PEX11::NAT</i> , pSL34:: <i>sh ble</i>	This study

Supplementary Table 3. Plasmids used in this study

Plasmids	Description	References
pREMI-Z	REMI plasmid for transposon mutagenesis, Zeo ^R , Amp ^R	(van Dijk et al, 2001)
pMCE7	pHIPZ plasmid containing gene encoding C-terminal of Pmp47 fused to mGFP; Zeo ^R , Amp ^R	(Saraya et al, 2012)
pHIPN-PMP47-GFP	pHIPN plasmid containing C-terminal of Pmp47 fused to mGFP; Nat ^R , Amp ^R	This study
pHI-GFP-SKL	pHI plasmid containing GFP-SKL under the control of P _{AOX} ; URA3, Amp ^R	(Cepinska et al, 2011)
pHIPX7-GFP-SKL	pHIPX plasmid containing GFP-SKL under the control of P _{TEF} ; LEU2, Kan ^R	(Baerends et al, 1997)
pHIPZ4-GFP-SKL	pHIPZ plasmid containing GFP-SKL under the control of P _{AOX} ; Zeo ^R , Amp ^R	(Leao-Helder et al, 2003)
pHIPZ7-GFP-SKL	pHIPZ plasmid containing GFP-SKL under the control of P _{TEF} ; Zeo ^R , Amp ^R	(Knoops et al, 2014)
pSL34	Plasmid containing GFP-SKL under the control of P _{MET25} ; Zeo ^R , Amp ^R	(Lefevre et al, 2013)
pSEM01	pHIPN plasmid containing gene encoding C-terminal part of Pex14 fused to mCherry; Nat ^R , Amp ^R	(Knoops et al, 2014)
pHIPZ-PEX3-mGFP	pHIPZ plasmid containing gene encoding C-terminal of Pex3 fused to mGFP; Zeo ^R , Amp ^R	This study

pMCE4	pHIPZ plasmid containing gene encoding C-terminal of Pex8 fused to mGFP; Zeo ^R , Amp ^R	(Cepinska et al, 2011)
pMCE5	pHIPZ plasmid containing gene encoding C-terminal of Pex10 fused to mGFP; Zeo ^R , Amp ^R	(Cepinska et al, 2011)
pSEM03	pHIPZ plasmid containing gene encoding C-terminal of Pex13 fused to mGFP; Zeo ^R , Amp ^R	(Knoops et al, 2014)
pMCE7	pHIPZ plasmid containing gene encoding C-terminal of Pmp47 fused to mGFP; Zeo ^R , Amp ^R	(Cepinska et al, 2011)
pHI-DsRed-SKL	pHI plasmid containing DsRed-SKL under the control of P _{AOX} ; URA3, Amp ^R	(Nagotu et al, 2008)
pHIPN4-DsRed-SKL	pHIPN plasmid containing DsRed-SKL under the control of P _{AOX} ; Nat ^R , Amp ^R	(Cepinska et al, 2011)
pHIPX4-GFP-SKL	pHIPX plasmid containing GFP-SKL under the control of P _{AOX} ; LEU2, Kan ^R	(Faber et al, 2002)
pARM059	pHIPZ plasmid containing <i>PEX14</i> under the control of P _{ADH1} ; Zeo ^R , Amp ^R	(Wu et al, 2020)
pARM053	pHIPZ plasmid containing <i>PEX14</i> -2xHA- <i>UBC6</i> under the control of P _{ADH1} ; Zeo ^R , Amp ^R	(Wu et al, 2020)
pARM069	pHIPX plasmid containing <i>PEX14</i> under the control of P _{ADH1} ; LEU2, Kan ^R	This study
pARM072	pHIPX plasmid containing <i>PEX14</i> -2xHA- <i>UBC6</i> under the control of P _{ADH1} ; LEU2, Kan ^R	This study

Supplementary Table 4. Genes identified by transposon mutagenesis of *H. polymorpha* *pex11* cells

Genes identified	Times found	Function
<i>PEX1</i>	1	Matrix protein import
<i>PEX2</i>	2	Matrix protein import
<i>PEX4</i>	1	Matrix protein import
<i>PEX5</i>	3	Matrix protein import
<i>PEX6</i>	3	Matrix protein import
<i>PEX8</i>	4	Matrix protein import
<i>PEX10</i>	2	Matrix protein import
<i>PEX12</i>	3	Matrix protein import
<i>PEX25</i>	2	PMP with unknown function
<i>PEX26</i>	2	Matrix protein import
<i>AMO</i>	3	Amine oxidase
<i>IRA1</i>	1	GTPase activating protein
<i>MUT3</i>	1	Alcohol oxidase activation
<i>HPODL_04236</i>	1	Hypothetical protein
<i>HPODL_05268</i>	1	Putative transcription factor
<i>MPP1</i>	3	Transcription factor
<i>VPS13</i>	9	Involved in prospore membrane formation, localizes to several membrane contact sites;

References

- Baerends RJ, Salomons FA, Faber KN, Kiel JA, Van der Klei IJ, Veenhuis M (1997) Deviant Pex3p levels affect normal peroxisome formation in *Hansenula polymorpha*: high steady-state levels of the protein fully abolish matrix protein import. *Yeast* **13**: 1437-1448
- Cepinska MN, Veenhuis M, van der Klei IJ, Nagotu S (2011) Peroxisome fission is associated with reorganization of specific membrane proteins. *Traffic* **12**: 925-937
- Faber KN, Haan GJ, Baerends RJ, Kram AM, Veenhuis M (2002) Normal peroxisome development from vesicles induced by truncated *Hansenula polymorpha* Pex3p. *The Journal of biological chemistry* **277**: 11026-11033
- Knoops K, Manivannan S, Cepinska MN, Krikken AM, Kram AM, Veenhuis M, van der Klei IJ (2014) Preperoxisomal vesicles can form in the absence of Pex3. *The Journal of cell biology* **204**: 659-668
- Krikken AM, Veenhuis M, van der Klei IJ (2009) *Hansenula polymorpha* pex11 cells are affected in peroxisome retention. *The FEBS journal* **276**: 1429-1439
- Leao-Helder AN, Krikken AM, van der Klei IJ, Kiel JA, Veenhuis M (2003) Transcriptional down-regulation of peroxisome numbers affects selective peroxisome degradation in *Hansenula polymorpha*. *The Journal of biological chemistry* **278**: 40749-40756
- Lefevre SD, van Roermund CW, Wanders RJ, Veenhuis M, van der Klei IJ (2013) The significance of peroxisome function in chronological aging of *Saccharomyces cerevisiae*. *Aging cell* **12**: 784-793
- Manivannan S, de Boer R, Veenhuis M, van der Klei IJ (2013) Lumenal peroxisomal protein aggregates are removed by concerted fission and autophagy events. *Autophagy* **9**: 1044-1056
- Nagotu S, Krikken AM, Otzen M, Kiel JA, Veenhuis M, van der Klei IJ (2008) Peroxisome fission in *Hansenula polymorpha* requires Mdv1 and Fis1, two proteins also involved in mitochondrial fission. *Traffic* **9**: 1471-1484

Saraya R, Krikken AM, Kiel JA, Baerends RJ, Veenhuis M, van der Klei IJ (2012) Novel genetic tools for *Hansenula polymorpha*. *FEMS yeast research* **12**: 271-278

Sudbery PE, Gleeson MA, Veale RA, Ledeboer AM, Zoetmulder MC (1988) *Hansenula polymorpha* as a novel yeast system for the expression of heterologous genes. *Biochemical Society transactions* **16**: 1081-1083

Thomas AS, Krikken AM, van der Klei IJ, Williams CP (2015) Phosphorylation of Pex11p does not regulate peroxisomal fission in the yeast *Hansenula polymorpha*. *Scientific reports* **5**: 11493

van Dijk R, Faber KN, Hammond AT, Glick BS, Veenhuis M, Kiel JA (2001) Tagging *Hansenula polymorpha* genes by random integration of linear DNA fragments (RALF). *Molecular genetics and genomics* : *MGG* **266**: 646-656

Wu F, de Boer R, Krikken AM, Aksit A, Bordin N, Devos DP, van der Klei IJ (2020) Pex24 and Pex32 are required to tether peroxisomes to the ER for organelle biogenesis, positioning and segregation in yeast. *Journal of cell science* **133**