

## Supplemental Data

### **Integrative Omics reveals changes in the cellular landscape of peroxisome-deficient pex3 yeast cells**

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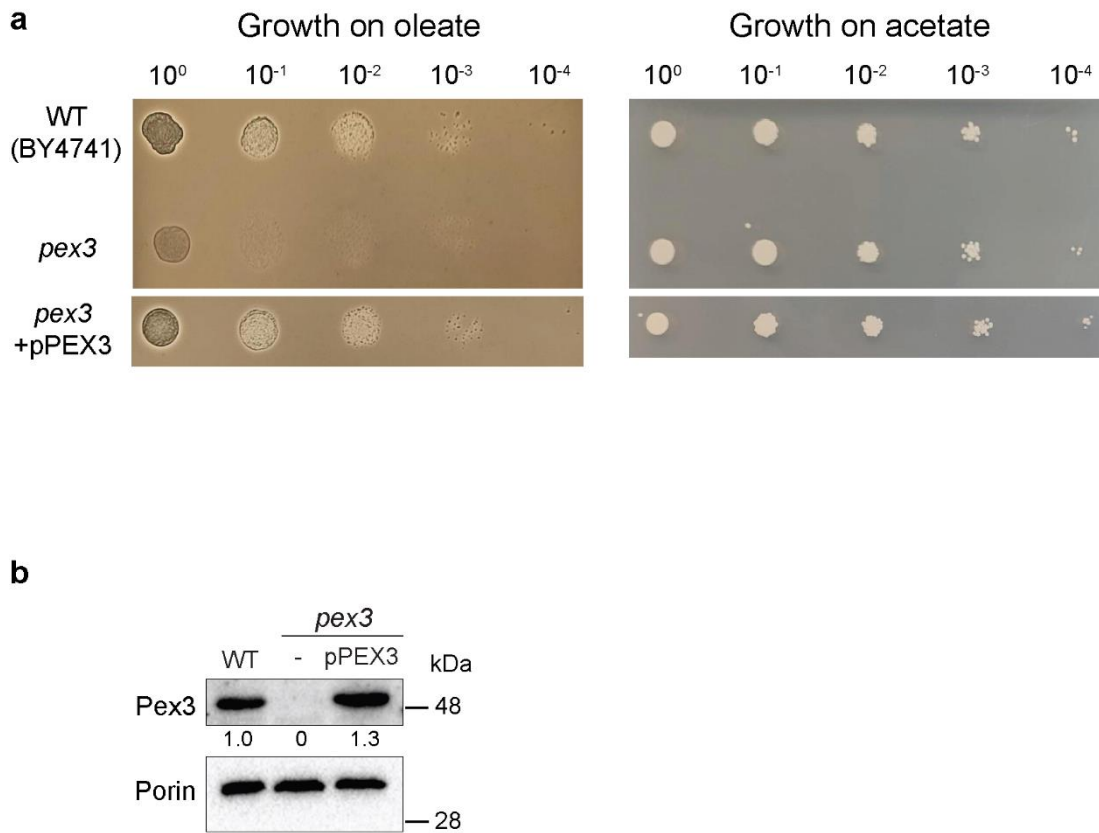
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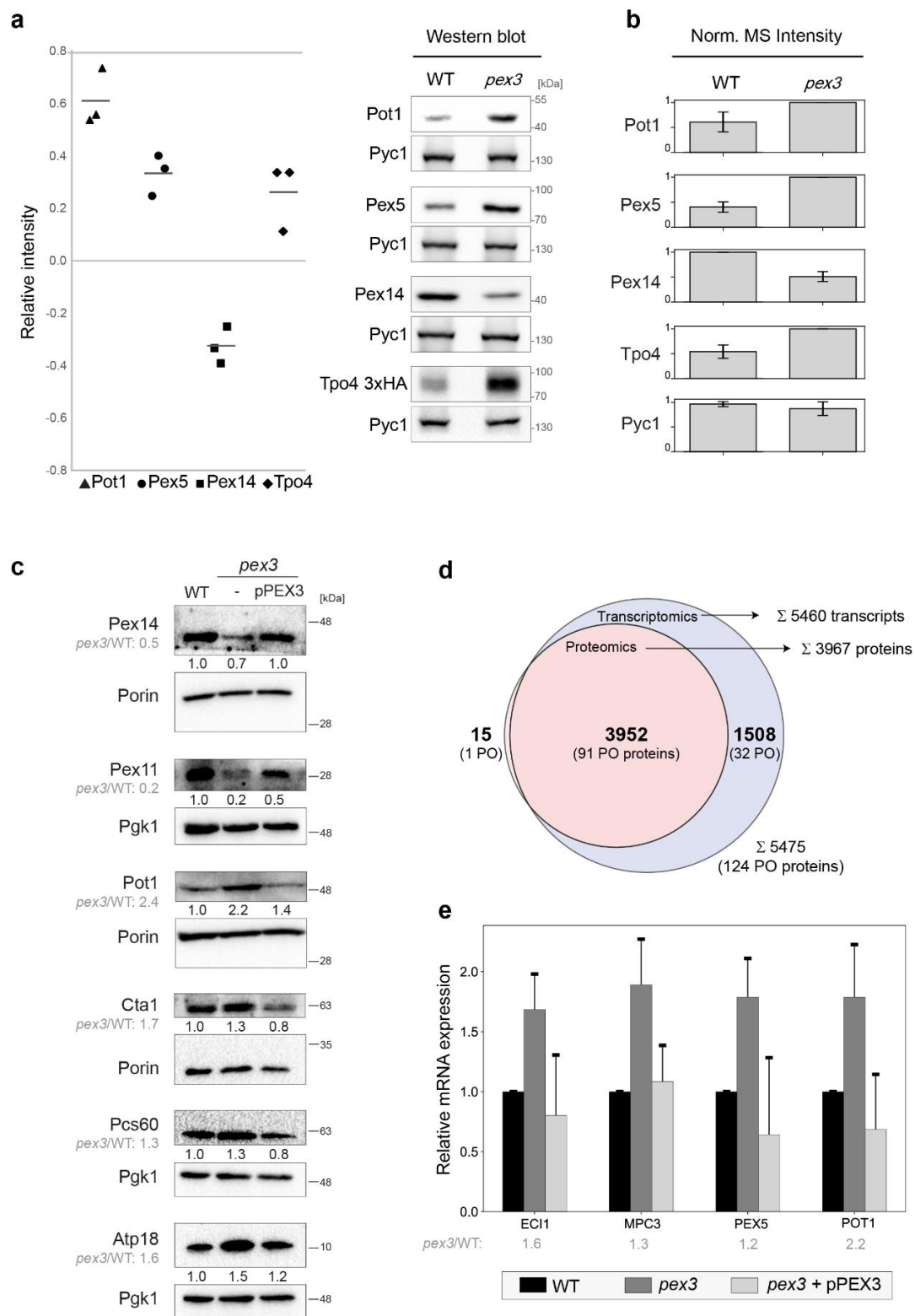
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## Supplemental Figures

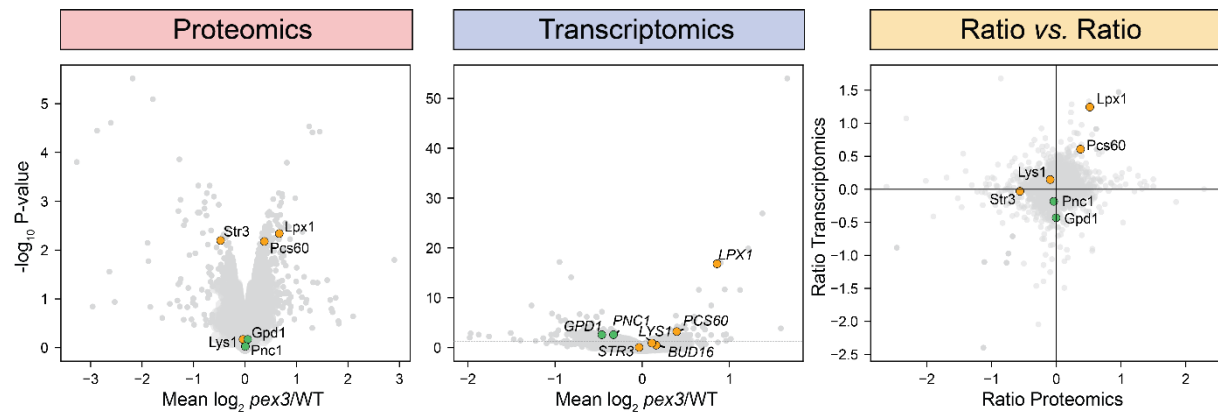


**Figure S1. Validation of the *PEX3* deletion strain used for the proteomics and transcriptomics experiment.** (a) Wild-type (WT) cells, *pex3* mutant cells, and *pex3* cells transformed with a plasmid for expression of *PEX3* (pPEX3) were spotted at 10-fold dilutions as indicated on agar containing oleate or acetate as carbon source. The inability of *pex3* cells to grow on oleate and the WT-like growth observed after reintroduction of *PEX3* into *pex3* cells confirms the lack of the *PEX3* gene in these cells. (b) Immunoblot analysis of Pex3 levels in whole cell lysates of acetate-grown WT, *pex3* and *pex3*+pPEX3 cells. Immunoblot signals were quantified by densitometry. Numbers below Pex3 immunoblot signals indicate relative signal intensities. Pex3 signals were normalized to the corresponding signal of the loading control Porin, and the value for WT cells was set to 1. Results confirm the absence of Pex3 at protein level in *pex3* cells and reconstitution of Pex3 levels following transformation with pPEX3.

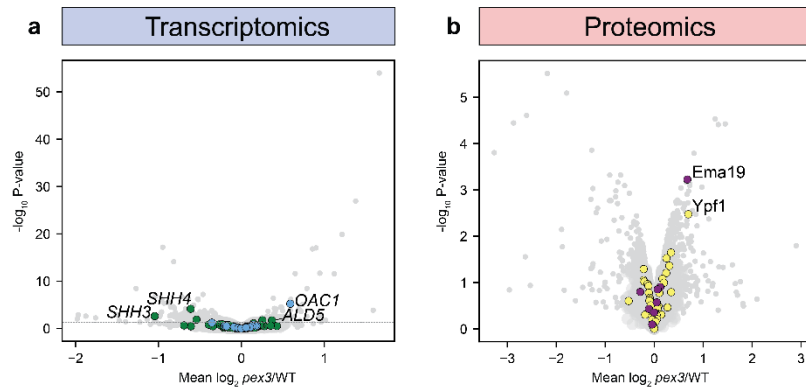


**Figure S2. Validation of selected regulated proteins and comparison of proteomics versus transcriptomics data.** (a) Validation of differences in protein abundance in whole cell lysates of wild-type (WT) and *pex3* cells by Western blotting using antibodies specifically recognizing the indicated

proteins. HA-tagged Tpo4 was recognized using an anti-HA antibody. Pyruvate carboxylase 1 (Pyc1) served as loading control. Signal intensities of three independent replicates were quantified by densitometry. Relative protein abundances for WT and *pex3* cells were determined by normalizing the signal intensities of Pot1, Pex5, Pex14, and Tpo4 to the signal of the corresponding Pyc1 control. The scatter plot shows the log-transformed ratios between the relative protein abundances determined for *pex3* and WT cells (*i.e.*, relative intensity) for the different proteins. **(b)** Normalized MS intensities for the proteins selected for Western blot validation in (a). Data are extracted from the proteomics analysis of WT *versus* *pex3* cells (Table S1a). **(c)** Immunoblot analysis of selected proteins showing changed abundance in *pex3* *versus* WT cells in the proteomics analysis. Whole cell extracts of WT, *pex3* and *pex3*+pPEX3 cells were analyzed using antibodies recognizing the indicated proteins. Numbers below immunoblot signals of the different proteins indicate relative signal intensities. Signals were normalized to the signal of the corresponding loading control (Porin or Pgk1), and the value for WT cells was set to 1. Protein abundance ratios (*pex3*/WT) determined in the proteomics analysis (Table S1a) are shown. Immunoblot data (n=1) for the selected proteins are in good accordance with the proteomics data, and WT-like protein levels are generally restored after transformation of *pex3* cells with pPEX3. **(d)** Overlap of proteins and transcripts identified in this study in the proteomics and transcriptomics analysis. Please note that the number of individual proteins reported in this figure (*i.e.*, 3967 in total) is higher than the number of protein groups (3921) that were identified in the proteomics analysis. This is due to the matching of the transcriptomics data to the proteomics data. Several of the protein groups reported by the analysis software MaxQuant contain more than one protein (see Table S1a) because the identified peptides do not allow to discriminate between the proteins within the protein group. However, we obtained data for several transcripts of distinct proteins that were combined in a protein group. Hence, these proteins with matching transcripts are considered and counted here as individual proteins (see also Tables S1c and S1d). PO, peroxisomal. **(e)** Levels of selected transcripts with altered abundance in the transcriptome analysis of *pex3* *versus* WT cells were analyzed by qPCR in WT, *pex3* and *pex3*+pPEX3 cells (three biological replicates each). *pex3*/WT ratios determined in the transcriptomics analysis (Table S1b) are indicated. Results of the qPCR analysis show good correlation with the transcriptomics data. Following reintroduction of the *PEX3* gene in *pex3* cells, WT-like transcript levels are observed. Error bars indicate standard error of the mean.



**Figure S3. Effect of Pex3 deficiency on peroxisomal enzymes and their transcripts associated with diverse metabolic processes.** Same plots as shown in Figure 3. Stress-inducible peroxisomal enzymes are marked in green.



**Figure S4. Abundance of mitochondrial transcripts (a), ERAD and ER-SURF components (b) in *pex3* versus WT cells.** (a) Same plot as shown in Figure 2c highlighting transcripts of mitochondrial respiratory chain components (green), mitochondrial carrier, and mitochondrial import proteins (both in blue). (b) Same plot as shown in Figure 2b highlighting proteins associated with the endoplasmic reticulum unfolded protein response (ERAD) (yellow) and ER-SURF (purple) [163]. A list of ERAD components was retrieved from the *Saccharomyces* Genome Database (<https://www.yeastgenome.org/>) using the Gene Ontology identifier GO:0036503. Transcripts/proteins annotated in the subplots are discussed in the manuscript.

## Supplemental Tables

**Table S1. Results of quantitative proteomics and transcriptomics analyses of *Saccharomyces cerevisiae* wild-type versus *pex3* cells** (xlsx file). **(a)** Whole cell extracts of *Saccharomyces cerevisiae* wild-type (WT) and *pex3* cells were analyzed by quantitative mass spectrometry using peptide stable isotope dimethyl labeling (n=3). Raw MS data and complete MaxQuant results files are available via Proteome-Xchange with the identifier PXD047234. **(b)** Transcriptomics data obtained from mRNA extracted from the same samples that were used for the proteomics analysis. **(c)** Combined omics data. **(d)** Heat map of proteomics and transcriptomics data visualizing *pex3*/WT ratios ( $\log_2$  fold-changes) for all proteins/transcripts identified in this study.

**Table S2. Results of GO term enrichment analysis** (xlsx file). GO term enrichment analysis for the domains "Biological Process" and "Cellular Component" was performed for proteins/transcripts with a minimum fold-change of 1.5 (reduced and increased; p-value < 0.05) between wild-type and *pex3* cells using shinyGO (version 0.77; <http://bioinformatics.sdstate.edu/go/>). GO terms with Benjamini-Hochberg corrected p-values of < 0.05 were considered enriched.

**Table S3. Yeast strains used in this study.**

Strain	Description and genotype	Reference
<i>Sc</i> BY4741 (WT)	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Euroscarf #Y00000
<i>Sc</i> BY4741 <i>pex3</i>	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 <i>pex3</i> (YDR329c)::kanMX4	Euroscarf #Y03688
<i>Sc</i> BY4741 <i>pex3</i> + pPEX3	<i>Sc</i> BY4741 <i>pex3</i> with pRSP3/2.4	This study
<i>Sc</i> WT P <sub>NOP1</sub> sfGFP- Mdh3	MATa can1Δ::GAL1pr-SceI::STE2pr-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hphΔn::URA3::SpNOP1pr- sfGFP-Mdh3	[107, 128]
<i>Sc</i> WT P <sub>NOP1</sub> sfGFP- Mdh3 P <sub>PEX14</sub> Pex14- mCherry	<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Mdh3 with integration of Pex14- mCherry fragment.	This study
<i>Sc pex3</i> P <sub>NOP1</sub> sfGFP- Mdh3	<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Mdh3 with integration of <i>pex3</i> deletion cassette.	This study
<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cat2	MATa can1Δ::GAL1pr-SceI::STE2pr-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hphΔn::URA3::SpNOP1pr- sfGFP-Cat2	[107, 128]
<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cat2 P <sub>PEX14</sub> Pex14-mCherry	<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cat2 with integration of Pex14- mCherry fragment.	This study
<i>Sc pex3</i> P <sub>NOP1</sub> sfGFP-Cat2	<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cat2 with integration of <i>pex3</i> deletion cassette.	This study
<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cit2	MATa can1Δ::GAL1pr-SceI::STE2pr-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hphΔn::URA3::SpNOP1pr- sfGFP-Cit2	[107, 128]
<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cit2 P <sub>PEX14</sub> Pex14-mCherry	<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cit2 with integration of Pex14- mCherry fragment.	This study
<i>Sc pex3</i> P <sub>NOP1</sub> sfGFP-Cit2	<i>Sc</i> WT P <sub>NOP1</sub> sfGFP-Cit2 with integration of <i>pex3</i> deletion cassette.	This study
<i>Sc</i> WT P <sub>MDH3</sub> Mdh3-mNG	MATa can1Δ::GAL1pr-SceI::STE2pr-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2+/lys+ lyp1Δ::STE3pr-LEU2 Mdh3-mNeonGreen-ADH1term:Hygro	[129]
<i>Sc</i> WT P <sub>CAT2</sub> Cat2-mNG	MATa can1Δ::GAL1pr-SceI::STE2pr-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2+/lys+ lyp1Δ::STE3pr-LEU2 Mdh3-mNeonGreen-ADH1term:Hygro	[129]



<i>Sc</i> WT P <sub>CIT2</sub> Cit2-GFP	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; Cit2-GFP HIS3MX6	[130]
<i>Sc</i> WT P <sub>MPC1</sub> Mpc1-GFP	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; Mpc1-GFP HIS3MX6	[130]
<i>Sc</i> WT P <sub>MPC1</sub> Mpc1-GFP:: P <sub>PEX14</sub> Pex14-mCherry	<i>Sc</i> P <sub>MPC1</sub> Mpc1-GFP with integration of Pex14-mCherry fragment	This study
<i>Sc</i> WT P <sub>MPC3</sub> Mpc3-GFP	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; Mpc3-GFP HIS3MX6	[130]
<i>Sc</i> WT P <sub>MPC3</sub> Mpc3-GFP:: P <sub>PEX14</sub> Pex14-mCherry	<i>Sc</i> P <sub>MPC3</sub> Mpc3-GFP with integration of Pex14-mCherry fragment.	This study
<i>Sc</i> WT P <sub>TPO4</sub> Tpo4-GFP	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; Tpo4-GFP HIS3MX6	[130]
<i>Sc</i> WT P <sub>TPO4</sub> Tpo4-GFP:: P <sub>PEX14</sub> Pex14-mCherry	<i>Sc</i> P <sub>TPO4</sub> TPO4-GFP with integration of Pex14-mCherry fragment	This study
<i>Sc</i> WT P <sub>TPO4</sub> Tpo4 3xHA	<i>Sc</i> BY4741 (WT) with integration of plasmid pHIPZ Tpo4 3xHA.	This study
<i>Sc pex3</i> P <sub>TPO4</sub> Tpo4 3xHA	<i>Sc</i> BY4741 <i>pex3</i> with integration of plasmid pHIPZ Tpo4 3xHA.	This study
<i>Sc tpo4</i>	<i>Sc</i> BY4741 (WT) with integration of <i>tpo4</i> deletion cassette.	This study
<i>Sc pex3 tpo4</i>	<i>Sc</i> BY4741 <i>pex3</i> with integration of <i>tpo4</i> deletion cassette.	This study
<i>Hp</i> NCYC495 (WT)	<i>yku80::URA3; leu1.1</i>	[164]
<i>Hp</i> WT P <sub>PEX3</sub> Pex3- mKate2	<i>Hp</i> WT with integration of plasmid pHIPN Pex3-mKate2.	[165]
<i>Hp</i> WT P <sub>PEX3</sub> Pex3- mKate2:: P <sub>ADHI</sub> Mpc1- meGFP	<i>Hp</i> WT P <sub>PEX3</sub> Pex3-mKate2 with integration of plasmid pHIPZ18 Mpc1-meGFP.	This study
<i>Hp</i> WT P <sub>PEX3</sub> Pex3- mKate2:: P <sub>ADHI</sub> Mpc3- meGFP	<i>Hp</i> WT P <sub>PEX3</sub> Pex3-mKate2 with integration of plasmid pHIPZ18 Mpc3-meGFP.	This study

**Table S4. Plasmids used in this study.**

Plasmid	Description	Reference
pHIPH Pex14-mCherry	Plasmid containing C-terminal region of <i>Sc PEX14</i> fused to mCherry (Hph <sup>R</sup> , Amp <sup>R</sup> ).	[166]
pHIPN Pex3-mKate2	Plasmid containing C-terminal region of <i>Hp PEX3</i> fused to mKate2 (Nat <sup>R</sup> , Amp <sup>R</sup> ).	[165]
pHIPZ18 Inp1-meGFP	Plasmid for integration of genes the <i>ADH1</i> promoter (Zeo <sup>R</sup> , Amp <sup>R</sup> ).	[165]
pHIPZ18 Mpc1-meGFP	Plasmid containing <i>Hp MPC1</i> fused to mEGFP under the <i>ADH1</i> promoter (Zeo <sup>R</sup> , Amp <sup>R</sup> ).	This study
pHIPZ18 Mpc3-meGFP	Plasmid containing <i>Hp MPC3</i> fused to mEGFP under the <i>ADH1</i> promoter (Zeo <sup>R</sup> , Amp <sup>R</sup> ).	This study
pUG6	Plasmid used to obtain the <i>PEX3</i> deletion cassette (Kan <sup>R</sup> , Amp <sup>R</sup> ).	Euroscarf #P30114
pHIPZ mGFP-fusinator	Plasmid used for the introduction of Tpo4 3xHA fragment (Zeo <sup>R</sup> , Amp <sup>R</sup> ).	[167]
pHIPH4	Plasmid used to obtain the <i>TPO4</i> deletion cassette (Hph <sup>R</sup> , Amp <sup>R</sup> ).	[168]
pHIPZ Tpo4 3xHA	Plasmid containing C-terminal region of <i>Sc TPO4</i> fused to 3xHA (Zeo <sup>R</sup> , Amp <sup>R</sup> ).	This study
pRSP3/2.4	Plasmid for expression of <i>Sc PEX3</i> (Amp <sup>R</sup> , URA3)	[44]

**Table S5. Primers used in this study.**

Name	Sequence
R-MDH3-CP	CAACAGCGGTTACCAGGC
R-CAT2-CP	CCTTACATAACAGCTGCTGTCT
R-CIT2-CP	GGTTGTGAGCTTCCTTTTGC
F-sfGFP-CP	TCAGGCACAACGTCGAAGAT
F-Pex3-del	AGTTAATACTAGTCATCGTAAAAGCAGAAGCACGAAA CAAGGAGGCCAAACCACTAAAAGGGGCCGCCAGCTGAA GCTTCGTA
R-Pex3-del	ATTAAAGATTACGCTATATATATATATATTCTGGTGTG AGTGTCAGTACTTATTCAGAGAGGCATAGGCCACTAGT GGATCTG
F-Pex3pr-del-CP	CGTCGTAGTCTATGCGTTTG
R-KanMX-CP	CATGCATCATCAGGAGTACGG
R-Pex3-del-CP	CTCCAAGACGCCCCGTAAATC
F-MDH3-CP	GGCCAAGTTTGCTGAAGAAG
F-CAT2-CP	GGTAATGGTGTGTCGATCGTCA
R-mNG-CP	CCGATATGAGGGACCAGTATCC
F-CIT2-CP	TGCCATGGACCATTTTCCAG
R-GFP(S65T)-CP	CAACAAGAATTGGGACAACCTCC
F_Prom-Pex3_Sc	GGTAGTTAATACTAGTCATCGT
R_Pex3_Sc	TGGTGTGAGTGTCAGTAC
R_KanMX4	ATCATCAGGAGTACGGATA
F-Mpc1-CP	CAACCGGTTCAACGCG
F-Mpc3-CP	GGTCTTCGCAGGGCTAAATG
TER214	AGATCAGTGTCCCTGACTGGCAAAATGGACAGGTCGA AGACTCCATCCCAATGGTGAGCAAGGGCGAGGAGGAT
TER215	GTTACAATTACAATTTCCGTTAAAAAACTAATTACTTA CATAGAATTGCGCGTTTTTCGACACTGGATGGCGGCGTT
TER216	TAACCGTATGGAATCCGGTA
R-mCherry-CP	GGCCTTGAGCCGTACATGA
MPC1-C-fw	CCCAAGCTTATGAGCTCGTCCTCGGCGTG
MPC1-C-rev	CGCGGATCCTTTCTGCACCTTGTTGCCGT
cPCR MPC1-Cfw	CACGGCAGCAGAATTGGAATTG

MPC3-C-fw	CCCAAGCTTATGGCCACGGCATTTCAGAAG
MPC3-C-rev	GGAAGATCTTGCCTCTGTTTTCGCTGGTGTAGTGC
cPCR MPC3-Cfw	CACGGCAGCAGAATTGGAATTG
mGFP rev check	AAGTCGTGCTGCTTCATGTG
F_Tpo4 3xHA	CGTAAATGCTGTTCGATGGTGAATTG
pHIPZ-pHIPZ5 rew seq.	CGAGGTTTTGTCCTTGGTCT
F-Tpo4-del	TCGACAGGGTCAATAACAGAGGACGAAAAAAGATCAG AACCGAATGCAGATAAGATCCCCCACACACCATAGC
R-Tpo4-del	CCTTGATAGCTGAGTATAAACTATTGGAAATGGGTTCA TTACGATTTTGCCATCATCGATGAATTCGAG
F-Tpo4-del-CP	GACACATCGATTTTCTAGTCATCAC
R-Tpo4-del-CP	GAATTTTATCCAGCTATCCCCC
F-ECI1-RT	TTTGCTATACCCCTTTGCTAAC
R-ECI1-RT	GCCCTTTCACTTTCTCCCTC
F-MPC3-RT	TTATCTTTATTAGCGACGGCAC
R-MPC3-RT	TGCAACCCAGGAAAAAATTGAC
F-PEX5-RT	TCAACAACCGCATCAAGC
R-PEX5-RT	TTCACTCACATTTTCCTCCTTC
F-POT1-RT	CTTCATCCTGGTCCACAGTAATAG
R-POT1-RT	GAGGAGATTATCCCATCCAAGTAG
F-ALG9-RT	CACGGATAGTGGCTTTGGTGAACAATTAC
R-ALG9-RT	TATGATTATCTGGCAGCAGGAAAGAACTTGGG

**Table S6. DNA fragment used in this study.**

<b>Name</b>	<b>Sequence</b>
Tpo4 3xHA	GGTGAATTGAATCTAACAAGGATGACCACGTTAAGGACCATGGAAA CAGACCCTTCGACTAGAGAAAAACCAGGTGAAAGGCTATCTCTGCG CAGGACCCATACGCAGCCCGTTCCTGCCTCGTTTGATCGCGAGGACG GGCAACATGCGCAAAATCGTAATGAACCCATTTCGAATAGTTTATAC TCAGCTATCAAGGATAATGAAGACGGTTATTCGTATACGGAAATGG CCACCGATGCTTCCGCCAGAATGGTTTACCCATACGACGTTCCAGAT TACGCTTACCCATACGACGTTCCAGATTACGCTTACCCATACGACGT TCCAGATTACGCTTGA