Supplementary data

Experimental

Mammalian

Cell culture and fluorescence staining

Skin fibroblasts from a healthy volunteer were cultured in low glucose Eagle's minimal essential medium (EMEM) with 1% (w/v) glutamine, 10% (v/v) fetal calf serum (FCS), and 100 units/ml penicillin, 100 µg/ml streptomycin) in 5% CO2 at 37°C. Mitochondria were stained by 100 nM Mitotracker Red dye (Invitrogen) in the medium without FCS for 15 min at 37°C. Cells were washed once with PBS and fixation was performed with 10% formaldehyde. After blocking with 10% bovine serum albumin (BSA) in PBS (20 min at 37°C) the primary rabbit anti-PEX14 antibody (ProteinTech, 1:1000) and secondary antibodies anti-rabbit Alexa647 (Invitrogen, 1:1000) were applied in blocking solution (2% BSA) and incubated at 37°C for 1 hour each. The sample was mounted in Mowiol mounting medium and analyzed by fluorescence microscopy.

Microscopy, data processing, and analysis

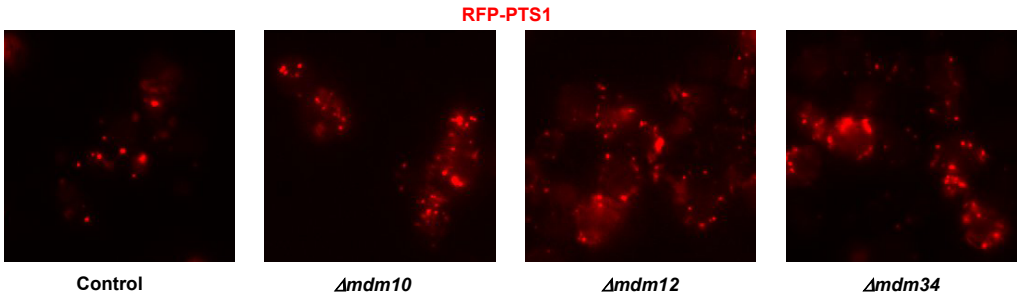
Fluorescence microscopy was done using the 100x oil objective (1.3 NA) with a Zeiss Imager M1 fluorescence wide field scope equipped with the Zeiss Axiocam HRm Camera and Zeiss Axiovision 4.8 acquisition software. z-Stacks with 30 images and 0.2 µm spacing were recorded and subjected deconvolution. Adobe Photoshop CS2 was used for linear contrast enhancement. ImageJ and the Volume Viewer plugin were used to build 3D model from z-stack deconvoluted images by applying the volume method with tricubic interpolation to create smooth volume contours.

Quantification of the percentage of peroxisomes that are localized adjacent to mitochondria sites

RFP-PTS1, Mdm34-GFP and RFP-PTS1, Pda1-GFP strains were grown to stationary and logarithmic growth phases. The cells were then transferred onto glass bottom 96-well microscope plates (Matrical Bioscience) coated with Concanavalin A (Sigma-Aldrich), and nine images from each well including hundreds of cells were acquired using a 60X air lens - GFP (excitation at 490/20 nm, emission at 535/50 nm) and mCherry (excitation at 572/35 nm, emission at 632/60 nm) channels. Images were then manually reviewed using the ScanR analysis program to identify RFP puncta that are in close proximity (3 pixels) to at least one GFP punctum.

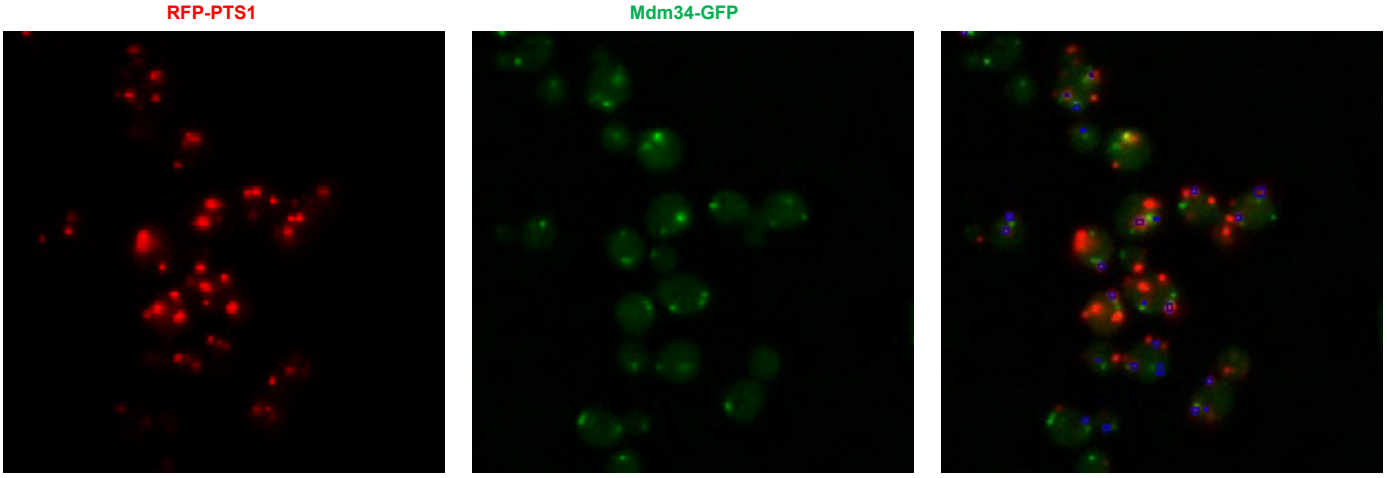
Supplemental figures and tables

Figure S1



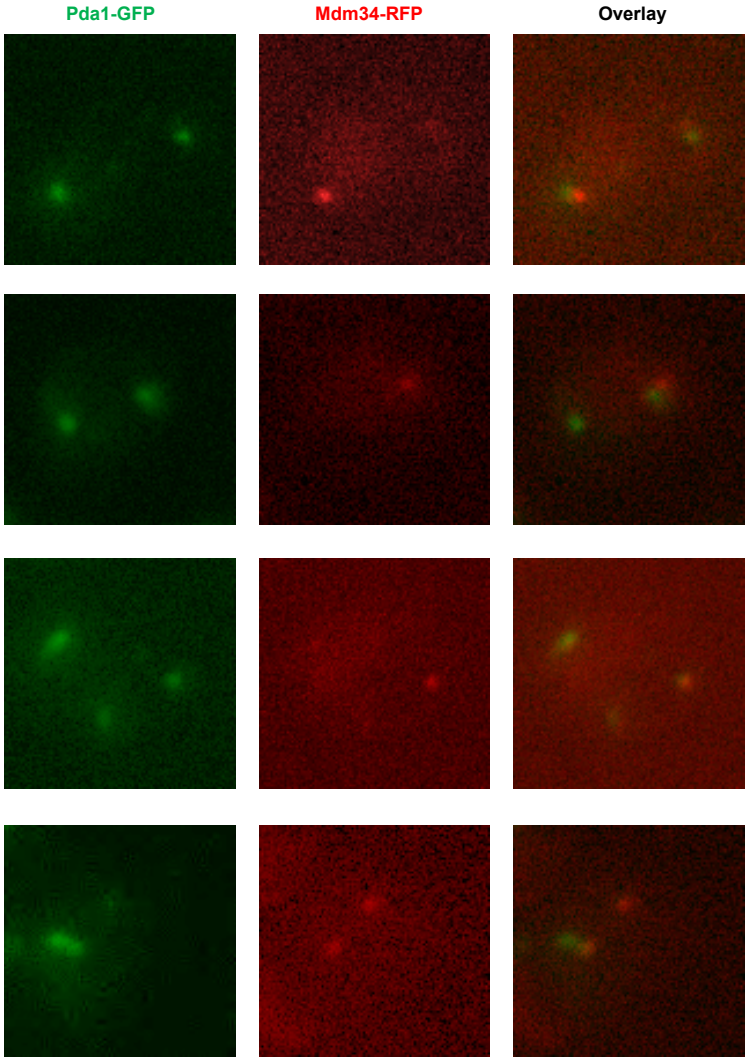
Cells with deletions of different components of the ERMES complex display several small peroxisomes. To uncover if loss of other components of the ERMES complex affect similarly on peroxisomes as the loss of Mdm10 we expressed RFP-PTS1 in the relevant, confirmed, yeast deletions. Representative images of the control and the deletion strains during logarithmic growth are shown, demonstrating a similar effect on peroxisomes when each of the three different ERMES components was deleted.

Figure S2



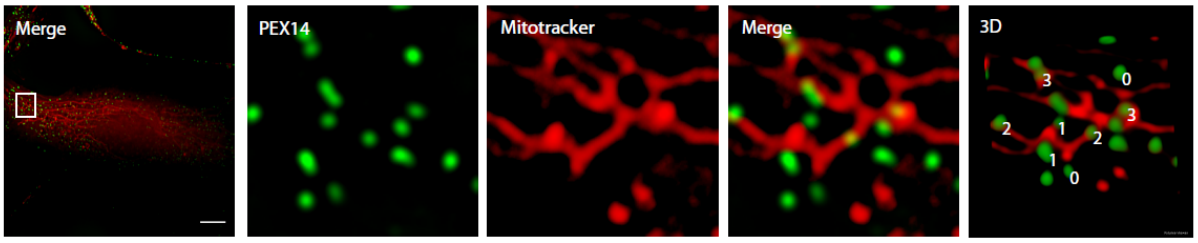
Quantification of the percentage of peroxisomes that are localized adjacent to mitochondria/ER junction (ERMES). To quantify what is the percentage of peroxisomes that are localized adjacent to ERMES foci a RFP-PTS1, Mdm34-GFP strain was grown to stationary phase and cells were then transferred onto glass bottom 96-well microscope plates coated with Concanavalin A. Nine images of fields including hundreds of cells were acquired using RFP (left picture) and GFP (middle picture) channels, and were automatically assigned, using the ScanR analysis program, as being colocalized if the program could identify RFP puncta that are in close proximity (3 pixels) to GFP puncta (right picture, Peroxisomes that were identified to have a close ERMES focus are marked by a blue square). Results were verified by manual inspection. Under the conditions used, 33% of the peroxisomes had adjacent ERMES foci.

Figure S3



PDH sites can be observed adjacent to mitochondria/ER junctions. Representative images of PDH and ERMES markers (Pda1-GFP and Mdm34-RFP respectively). The markers showed that PDH sites can be localized adjacent to ERMES foci.

Figure S4



Peroxisomes and mitochondria can be found in proximity in mammalian cells.

Wide field deconvolution microscopy and 3D reconstruction of mitochondria-peroxisome proximity in human fibroblasts. Mitotracker identified mitochondria and anti-PEX14 immunostain identified peroxisomes. In the 3D representation scores were assigned to peroxisomes with different degrees of apparent peroxisome-mitochondria proximity: (0) peroxisomes with no obvious proximity to mitochondria, (1) peroxisomes close to mitochondria, (2) juxtaposition with partial overlap, (3) peroxisomes partially or fully wrapped by mitochondria. Scale bar 2.5µm.

Table S1 summarizes a list of mutant strains that affected peroxisome structure or transport of peroxisomal proteins.

Table S2 summarizes a list of strains whose GFP tagged proteins were affected by *SPF1* loss.

Table S3 summarizes a list of strains whose GFP tagged proteins display a punctate phenotype and that were used to identify proteins that are localized to mitochondrial subdomains.