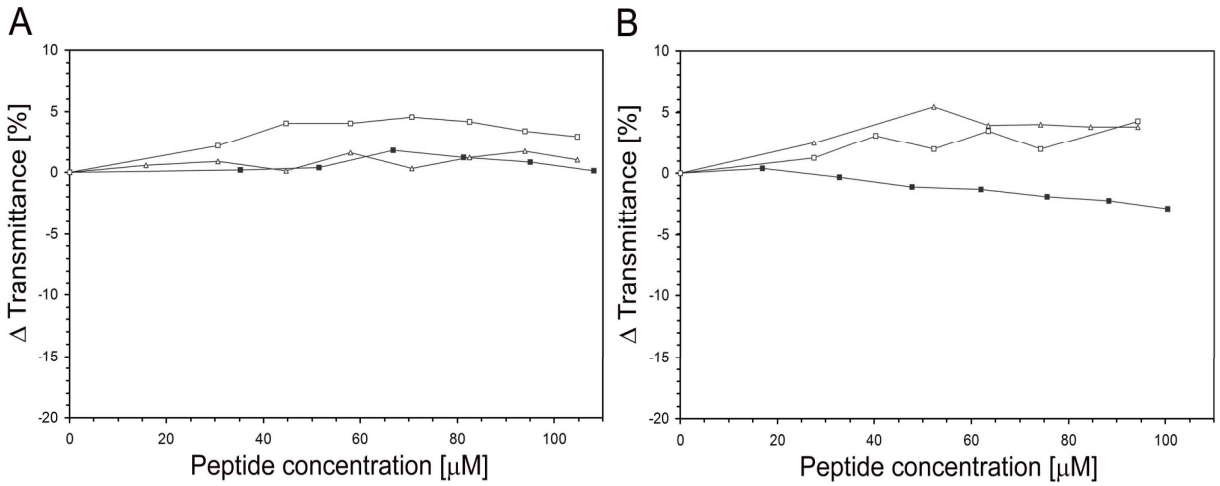
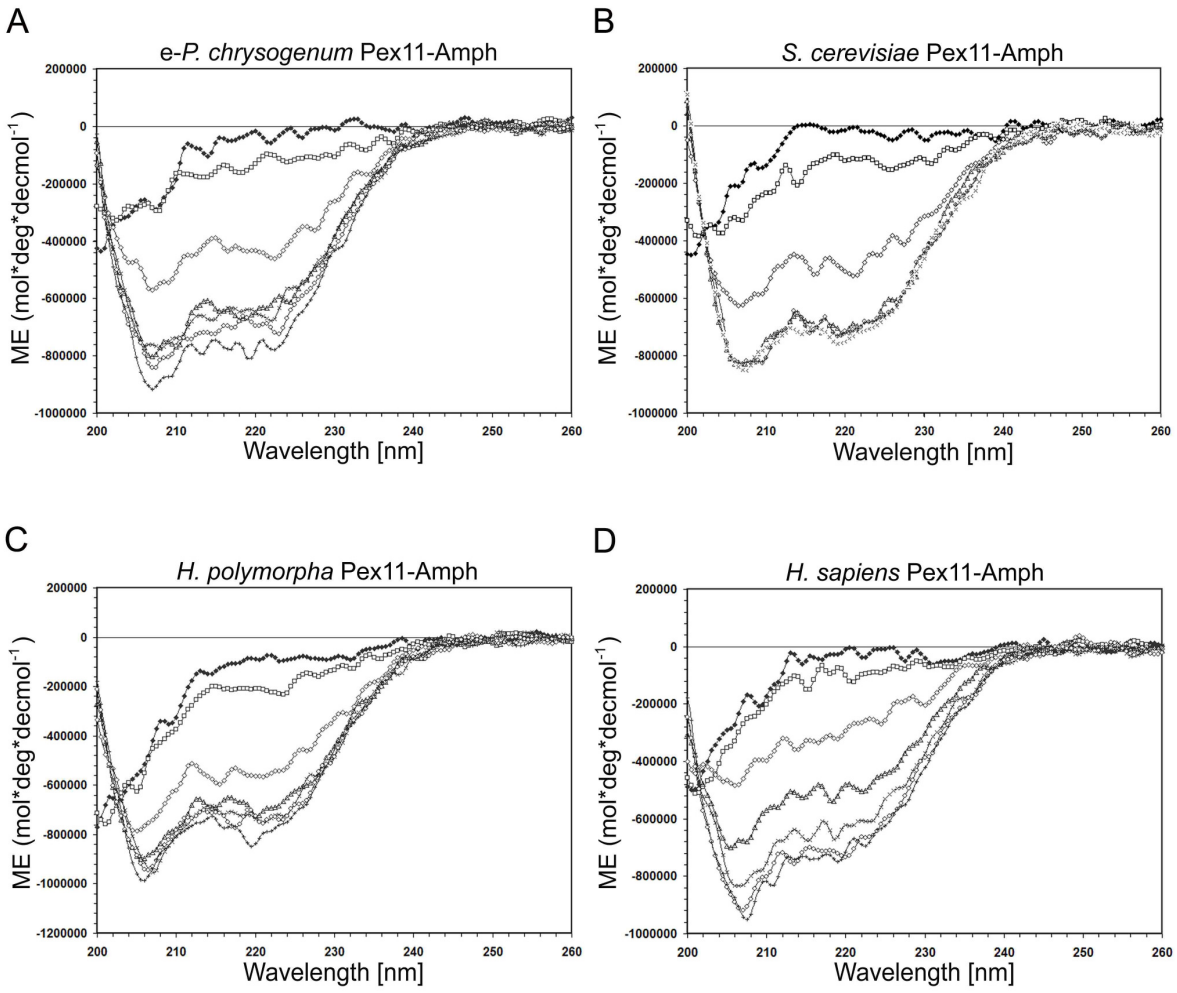
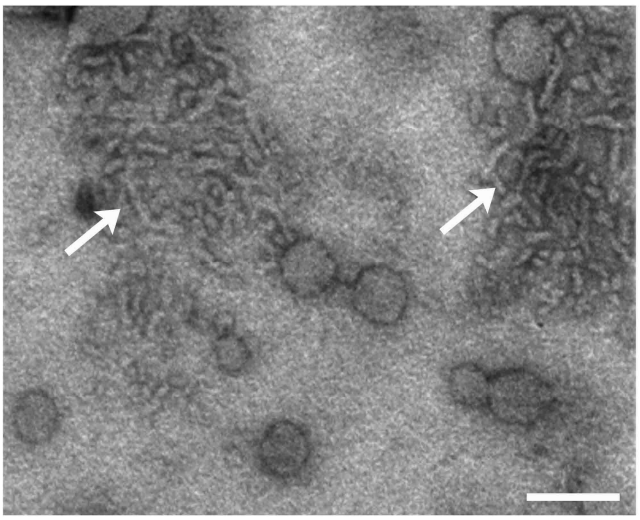


Supplementary Fig. S1. The effect of Pex11-Amph mutation on liposome turbidity. Increasing amounts of mutant peptides Pex11-Amph-Φ (A) and Pex11-Amph-P (B) were added to suspension of SUVs of different phospholipid composition and the effect on liposome shape/size was monitored at 400 nm. In contrast to wild type Pex11-Amph (see Fig.2B), mutant peptides are not able to significantly change the turbidity of a SUV solution (□- PC SUVs; Δ - PC/PE SUVs; ■ - PC/PE/PS/PI/CL SUVs).



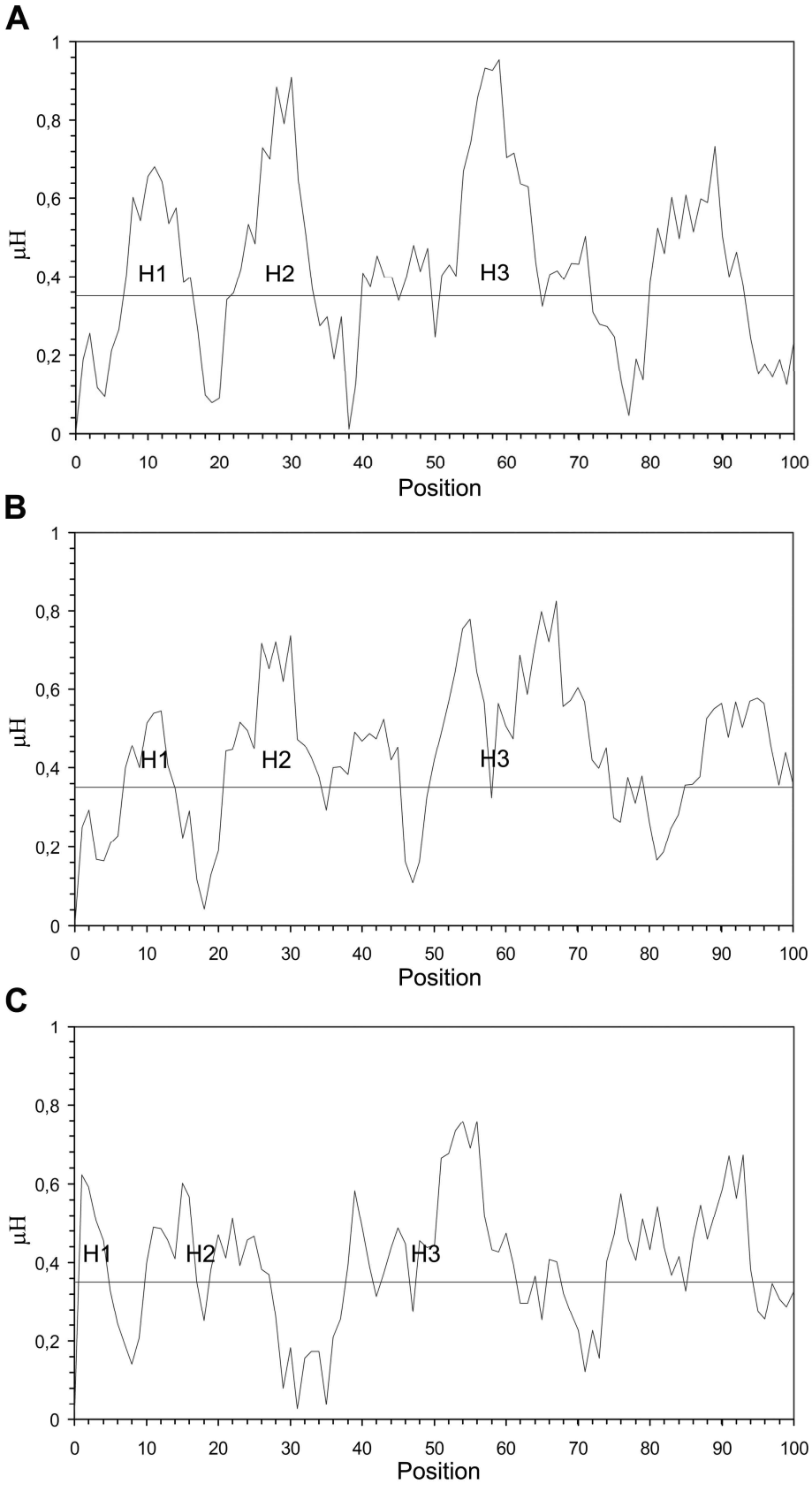
Supplementary Fig. S2. Secondary structure analysis of *P. chrysogenum* e-Pex11-Amph and Pex11-Amph's from different species.

The secondary structures of the *e*-Pex11-Amph mutant (**A**) and Pex11-Amph peptides from *S. cerevisiae* Pex11 (residues 49-78) (**B**), *H. polymorpha* Pex11 (residues 54-86) (**C**) and *H. sapiens* Pex11α (**D**) were analysed using CD spectroscopy. The spectra show that all 4 studied peptides are unstructured in phosphate buffer (♦). Addition of increasing amounts of 2,2,2-trifluoroethanol (TFE) induces changes in the spectrum, resulting in minima at 208 nm and 220 nm, typical for α-helical structure; 10 % TFE (□), 20 % TFE (◊), 30 % TFE (Δ), 40 % TFE (x), 50 % TFE (○), 60 % TFE (+).



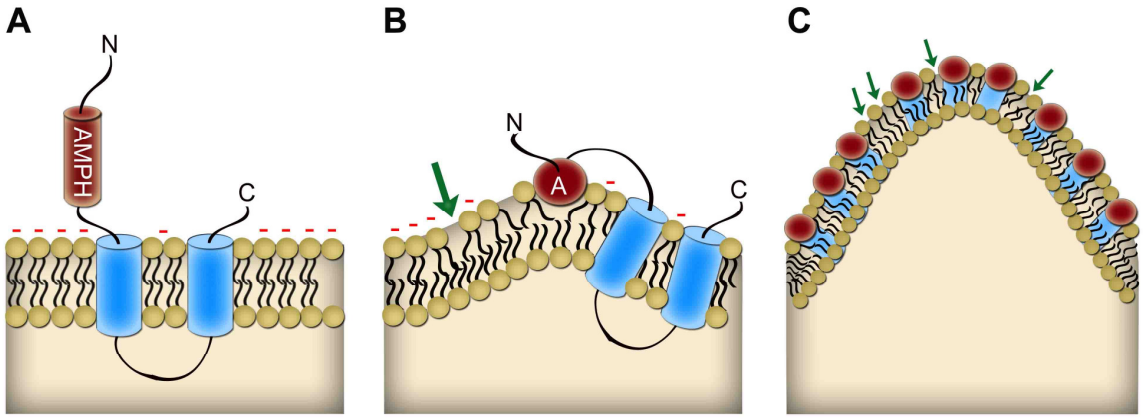
Supplementary Fig. S3. The mutant peptide *e*-Pex11-Amph efficiently induces formation of large and low diameter tubules and vesiculation of liposomes.

The *e*-Pex11-Amph mutant peptide was incubated with SUVs with phospholipid content resembling the peroxisomal membrane and analysed by electron microscopy. Besides typical tubules with 40-50 nm diameter, observed previously for Pex11-Amph, low diameter tubules (10-15 nm) that seem to connect in network-like structures (white arrows) could be often observed. Bar represents 100 nm.


A
B
C
μH
H1
H2
H3
Position
0
0,2
0,4
0,6
0,8
1
0
10
20
30
40
50
60
70
80
90
100

Supplementary Fig. S4. The hydrophobic moments of N-terminal regions of Pex11 proteins from *S. cerevisiae, H. polymorpha* and *H. sapiens*.

Plot of hydrophobic moments (μH) for the N-termini of Pex11 proteins from *S. cerevisiae* (**A**)*, H. polymorpha* (**B**) and *H. sapiens* (**C**). Similarly to *P. chrysogenum* Pex11 the highest values of the μH were obtained for the long putative helix H3, suggesting strong amphipathic properties for this motif in all three Pex11 proteins.



Supplementary Fig. S5. Hypothetical model of Pex11-Amph-induced tubulation of the peroxisomal membrane.

A) In the inactive state, the amphipathic helices of Pex11 are exposed to cytosol.

B) To allow membrane fission, the amphipathic helix of Pex11 inserts into one leaflet of the peroxisome membrane bilayer. This leads to a membrane curvature that is characterized by the presence of mismatches in phospholipid packaging (marked by the green arrow). An increased amount of mismatches forms potential insertion sites for additional amphipathic helices and an initial grouping of Pex11 can be observed.

C) The increased recruitment of Pex11 to curved regions of the membrane via the Pex11-Amph region results in the formation of Pex11-enriched domains. The accumulated amphipathic helices of Pex11 further remodel the membrane of the peroxisome, resulting in tubulation.

Supplementary Table I. Plasmids used in this study

Plasmid	Description	Reference
pDONR221	Gateway vector, Kan^R	Invitrogen
pDONR-P4-P1r-P_{AMO}	pDONR4 P4-1R containing P_{AMO}, Kan^R	(Nagotu et al., 2008)
pBBK-002	pDONR221 containing *P.chrysogenum PEX11* without stop codon, Kan^R	This study
pHIPZ4-DsRed-T1-SKL	Plasmid containing P_{AOX}-*DsRed•SKL*, Amp^R, Zeo^R	(Monastyrska et al., 2005)
pDONR-P2r-P3-eGFP-T_{AMO}	pDONR P2-P3 containing eGFP and T_{AMO}, Kan^R	(Nagotu et al., 2008)
pDEST-R4-R3-NAT	pDESTR4-R3 containing a nourseothricin resistance marker, Amp^R Nat^R	(Nagotu et al., 2008)
LMOPPcPex11	Plasmid containing a P_{AMO}-*PcPEX11•GFP*-T_{AMO} expression cassette, Amp^R Nat^R	This study
LMOPPcPex11-Φ	Plasmid containing a P_{AMO}-*PcPEX11-Φ•GFP*-T_{AMO} expression cassette, Amp^R Nat^R	This study
LMOPPcPex11-P	Plasmid containing a P_{AMO}-*PcPEX11-P.GFP*-T_{AMO} expression cassette, Amp^R Nat^R	This study
pQE60	Expression vector for C-terminal His-tag fusions, Amp^R	Qiagen
pQE60-Pex11N	Expression vector encoding the 97 N-terminal residues of *P. chrysogenum* Pex11 fused C-terminally to the His-Tag, Amp^R	This study

Supplementary Table II. Oligonucleotides used in this study

Oligonucleotide	Sequence (5' – 3')
BB-JK001	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTAAGATGGTCGCTAACACTCTCGTC
BB-JK002	GGGGACCACTTTGTACAAGAAAGCTGGGTCGGCAGGTCCGGCGGTCTTGC
LMOp062	GGCACGACGCGCAAGGAGATGCGTGAGGGCAAAGAGCTCGAGCATCTCAAGG
LMOp063	CCTTGAGATGCTCGAGCTCTTTGCCCTCACGCATCTCCTTGCGCGTCGTGCC
LMOp064	CGACGCGCAAGATCCCACGTATCGGCAAATTCCTCCCACATCTCAAGGCCG
LMOp065	CGGCCTTGAGATGTGGGAGGAATTTGCCGATACGTGGGATCTTGCGCGTCG
LMOp067	AAACCATGGTCGCTAACACTCTCGTCTACC
LMOp068	TATAGATCTCAGGACGGGGTCAACGGG

Supplementary Table III. Composition of the small unilamellar vesicles used in this study

	PC	**PE**	**PS**	**CL**	**PI**
PC	100 mole %	-	-	-	-
PC/PE	70 mole %	30 mole %	-	-	-
PC/PE/PS/CL/PI	55 mole %	30 mole %	5 mole %	5 mole %	5 mole %

PC-phosphatidylcholine, PE- phosphatidylethanolamine, PS – phosphatidylserine, PI – phosphatidylinositol, CL - cardiolipin