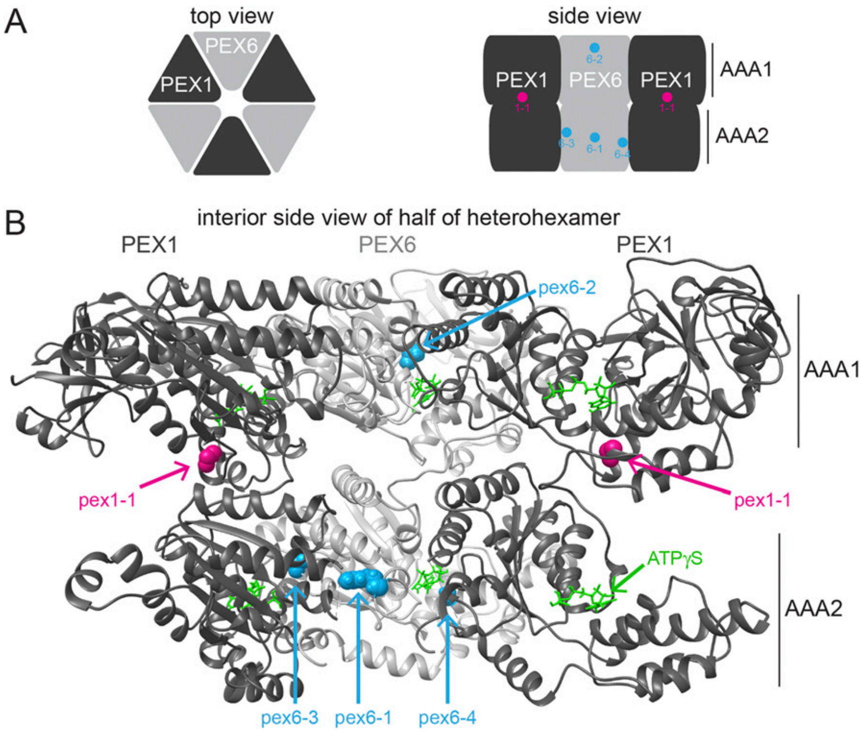
Supporting Information

Gonzalez et al. 10.1073/pnas.1721279115



C

Analogous residues

Arabidopsis PEX1-PEX6	human p97
pex1-1 (Glu748Lys)	Gly376
pex6-1 (Arg766Gln)	Arg586
pex6-2 (Leu328Phe)	Val201
pex6-3 (Gly817Asp)	Gly637
pex6-4 (Ala867Val)	Ala685

Fig. S1. Predicted positions of residues altered in pex1 and pex6 proteins based on the crystal structure of the related human ATPase p97. (*A*) Cartoon depictions of PEX1–PEX6 heterohexamer, viewed from the top to highlight the central pore (*Left*) and from the side to visualize the stacked AAA1 and AAA2 domains, with dots marking the relative positions of pex1-1 and pex6 alterations from *B* (*Right*). (*B*) Side view of three adjacent subunits of the human p97 homohexamer viewed from the interior and colored to illustrate the possible ramifications of *pex1* and *pex6* mutations. The dark gray backbone ribbons represent PEX1 subunits, and the light gray backbone ribbon represents a PEX6 subunit. p97 residues at the positions of pex1 or pex6 alterations (*C*) are shown with side-chain and backbone atoms in spherical representations. Cocrystallized ATPγS is depicted in green. The structure in *B* was generated from the p97 structure deposited in the Protein Data Bank under ID code 5C18 (62) using University of California, San Francisco, Chimera software (63). (*C*) Human p97 residues at the positions of *Arabidopsis* pex1 or pex6 alterations.

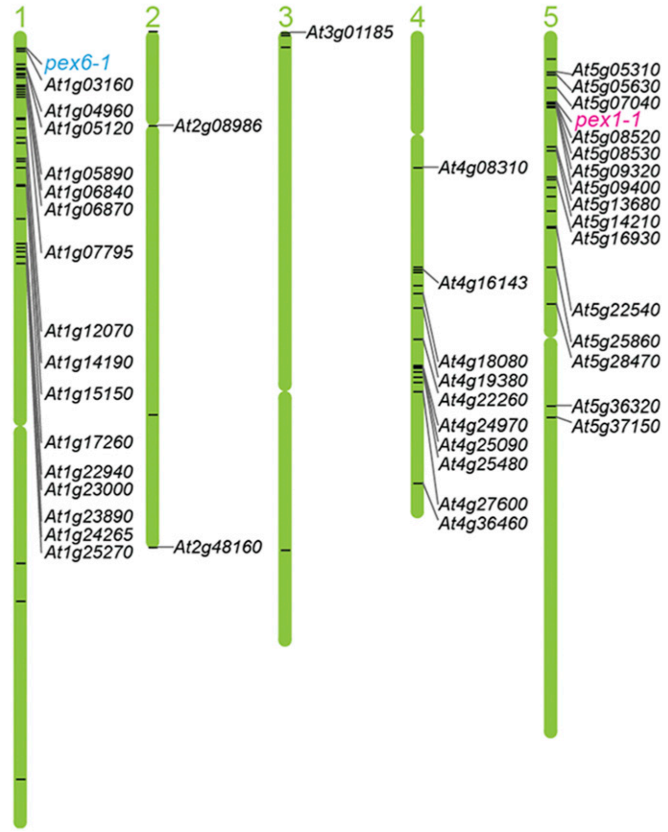


Fig. S2. Whole-genome sequencing results of once-backcrossed *pex1-1 pex6-1*. DNA prepared from pooled backcrossed suppressor seedlings was sequenced and inspected for EMS-consistent SNPs (G/C to A/T transitions) in exons, introns, and predicted UTRs that were absent in our laboratory WT strain. Homozygous mutations in exons are highlighted by locus identifiers or the *pex1* or *pex6* allele name to the right of the five *Arabidopsis* chromosomes; homozygous intron and UTR mutations are identified with tick marks lacking gene identifiers. The *Arabidopsis* Information Resource Chromosome Map Tool was used to generate the map.

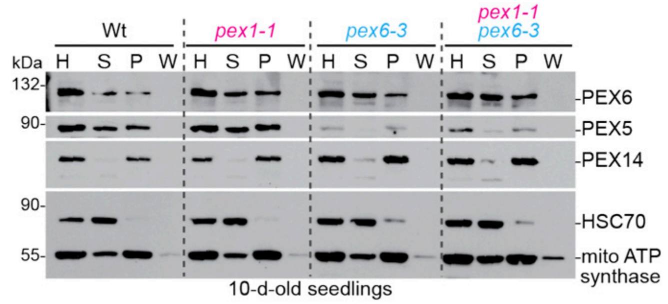


Fig. S3. PEX5 remains excessively membrane-associated in *pex1-1 pex6-3*. Homogenates (H) prepared from 10-d-old light-grown WT, *pex1-1*, *pex6-3*, and *pex1-1 pex6-3* seedlings were separated by centrifugation to isolate cytosolic supernatant (S) and an organellar pellet, which was resuspended and recentrifuged to provide a final organellar pellet (P) and a wash (W) fraction. Fractions were subjected to immunoblotting with the indicated antibodies. HSC70 is cytosolic, and mitochondrial (mito) ATP synthase subunit α and PEX14 localize in the organelle fraction. The positions of molecular mass markers (in kilodaltons) are indicated on the left.

Table S1. Summary of mutant phenotypes

Genotype	Growth without sucrose	IBA response	PEX5 Levels	PTS2 processing (PMDH)	GFP-PTS1 localization	Oil bodies present at 5 d
WT	Robust	Sensitive	Moderate	Complete	Puncta	Few
pex1-1	Robust	Sensitive	Moderate	Complete	Puncta	Few
pex6-1	Poor	Resistant	Low	Defect	Cytosolic	Abundant
pex1-1 pex6-1	Robust	Sensitive	Low	Intermediate defect	Mixed	Moderate
pex6-2	Robust	Sensitive	High	Complete	Puncta	Few
pex1-1 pex6-2	Robust	Sensitive	High	Complete	Puncta	Few
pex6-3	Robust	Resistant	Low	Defect	Cytosolic	Moderate
pex1-1 pex6-3	Robust	Sensitive	Low	Intermediate defect	Mixed	Few
pex6-4	Poor	Resistant	Low	Defect	Cytosolic	Abundant
pex1-1 pex6-4	Moderate	Resistant	Low	Defect	Mixed	Moderate
pex26-1	Moderate	Resistant	Low	Intermediate defect	Mixed	Abundant
pex1-1 pex26-1	Poor	Resistant	Low	Defect	Mixed	Abundant
WT *35S:PEX5*	Robust	Sensitive	High	Complete	ND	ND
pex1-1 35S:PEX5	Robust	Sensitive	High	Complete	ND	ND
pex6-1 35S:PEX5	Robust	Resistant	High	Intermediate defect	ND	ND
pex1-1 pex6-1 35S:PEX5	Robust	Sensitive	High	Intermediate defect	ND	ND
atg7-3	Robust	Sensitive	Moderate	Complete	Puncta	Few
pex1-1 atg7-3	Robust	Sensitive	Moderate	Complete	ND	ND
pex6-1 atg7-3	Moderate	Resistant	Moderate	Defect	Mixed	Few
pex1-1 pex6-1 atg7-3	Robust	Sensitive	Low	Intermediate defect	Puncta	Few
pex13-1	Robust	Sensitive	Moderate	Complete	ND	ND
pex1-1 pex13-1	Robust	Sensitive	Moderate	Complete	ND	ND
pex6-1 pex13-1	Moderate	Resistant	Low	Defect	ND	ND
pex1-1 pex6-1 pex13-1	Robust	Sensitive	Low	Intermediate defect	ND	ND

ND, not determined. Gray fill indicates phenotypes similar to WT, blue indicates phenotypes similar to *pex6-1*, lavender indicates intermediate phenotypes, purple indicates phenotypes different from WT or *pex6-1*.

Table S2. PCR-based genotyping markers used to track mutations and transgenes

			Product size, bp	
Mutation or transgene	PCR primers (5′ to 3′)	Restriction enzyme	WT	Mutant
atg7-3	LB1-SAIL (GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC) ATG7-24 (GTCGATTTAAACTTAAAGTTAATGAGATG)	—	—	~350
ATG7	ATG7-23 (AGACGGTAACCACCTGCTTTCTC) ATG7-24 (GTCGATTTAAACTTAAAGTTAATGAGATG)	—	539	—
pex1-1	PEX1-F5 (CTTGCATTCGTTGCTTCTGTCCAG) PEX1-F6 (GCATCATATCCTTCACATTTAGC)	TaqαI	300	197, 103
pex6-1	F10O3-7 (CAGACTTTACTGGCAAAAGCTGTGGCG) F10O3-T (GCTTGCACCTATAATAAACAGATCCTGGG)	XhoI	270, 115	385
pex6-2	PEX6-19 (AGGAACCTTTGATCTATACACCAGT) PEX6-AvaII (AGTGAATCACTCCCAAACCGCCCTGGTC)	AvaII	62, 29	91
pex6-3	PEX6-3F1 (AACAGACCTGACTTGATTGAT) PEX6-3R2 (GTCAAAAACAAGAATCAGGAAG)	BstNI	134, 38	172
pex6-4	PEX6-4F (TTACAGGGAAAGGTTAGG) PEX6-4R (GCCTGAAACCAAGCATC)	HhaI	180, 40	220
pex13-1	PEX13-1 (AGAATTCAATAAATCGAGACCCTAAAAT) LB1-SALK (CAAACCAGCGTGGACCGCTTGCTGCAACTC)	—	—	300
PEX13	PEX13-1 (AGAATTCAATAAATCGAGACCCTAAAAT) PEX13-2 (TATAGGGGCTGATACATAATAACCTAAAA)	—	285	—
pex26-1	PEX26-1 DdeF1 (GATGCTACACTAAACTGTCTATCTCA) PEX26-9 (CATCATTCTTTTCATTACCCAACGACTTCTT)	DdeI	131, 23	154
pxa1-1	T5J17-24 (ATGGGAGTCACTTTCATAACCTCATCTCAA) T5J17-25 (CCATCAATCAGCCTTAGCTCCAAGGAATGG)	SmlI	142, 30	172
35S:HA-PEX1	PEX1-F1 (CATTCGTTGCTTCTGTCCA) PEX1-E2 (GCCCCACGTTCCGAGGTAG)	—	206	206, 112
35S:HA-PEX6	PEX6-3F1 (AACAGACCTGACTTGATTGAT) PEX6-3R1 (GAGGGACACTTCTTTGCTAC)	—	252	252, 176
35S:PEX5	PEX5-38 (TGAAGACCAACAGATAAGG) PEX5-39 (CCCATTGGAGGCATAGG)	—	264	264, 168
GFP-PTS1	35S-F (GGATGACGCACAATCCCACTATCCTTCG) GFP-1 (TTGAAAAGCATTGAACACCATAAGAGAAAGT)	—	—	321