Supporting Information

Thazar-Poulot et al. 10.1073/pnas.1403322112

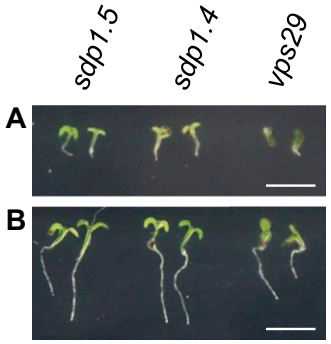
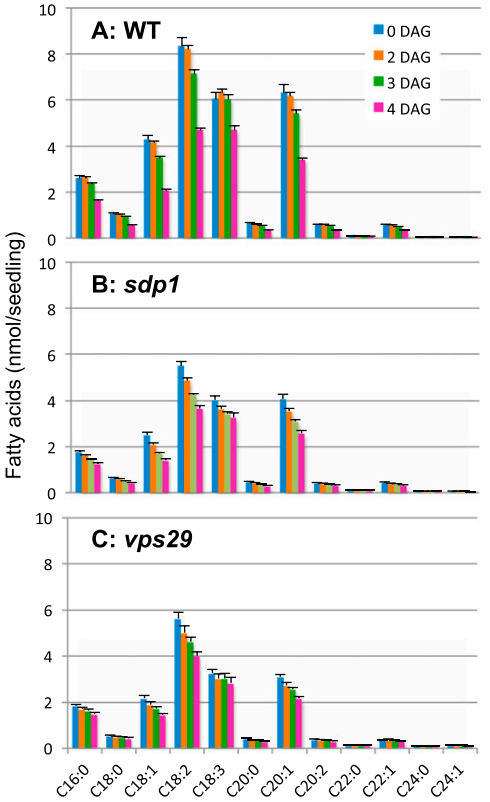


Fig. S1. Sugar-dependent phenotype of *sdp1* and *vps29* mutants. (*A*) Mutant seedlings were grown on MS medium without sugar for 12 d. (*B*) After 8 d of sugar starvation, mutant seedlings were transferred on MS medium supplemented with 1% (wt/vol) sucrose for 4 d. Three independent experiments were performed, and a total of at least 11 seedlings were analyzed. (Scale bars: 5 mm.) This figure relates to Fig. 1.



D

	% VLCFA			
	0 DAG	2 DAG	3 DAG	4 DAG
WT	27,4	26,9	26,6	25,6
sdp1	27,7	27,7	27,7	26,7
vps29	24,9	25	24,8	24,2

Fig. S2. (*A–C*) FA composition of germinating WT (*A*), *sdp1.4* (*B*), and *vps29* (*C*) seeds. The values are the means ± SEM. (*D*) VLCFAs expressed as percentage of TFAs in germinating WT, *sdp1.4*, and *vps29* seeds. Experiments were repeated twice on four to six batches of 20 dry seeds (0 DAG) or 20 seedlings (2, 3, and 4 DAG). Along with Fig. S3, this figure relates to Fig. 2.

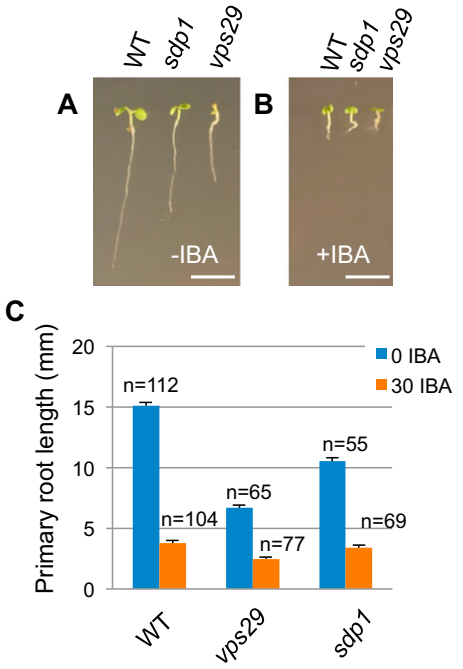


Fig. S3. β-oxidation is functional in *vps29* and *sdp1* mutants. (*A* and *B*) To test the functionality of the β-oxidation process, the primary root elongation of WT, *sdp1.4*, and *vps29* mutant seedlings were compared after 7 d of growth on MS medium with 1% (wt/vol) sucrose (*A*) or MS medium with 1% (wt/vol) sucrose containing 30 μM IBA (*B*). (Scale bars: 5 mm.) (*C*) Primary roots were measured by using ImageJ software (NIH). Values are mean ± SEM. n, number of measured roots. Along with Fig. S2, this figure relates to Fig. 2.

Fig. S4. The GFP–SDP1 fusion protein is functional. *sdp1.5* seedlings, WT, and *sdp1.5* seedlings expressing the GFP–SDP1 protein under the control of the 35S promoter were grown on MS medium without sucrose for 8 DAG. This experiment was performed three times independently on 100 mutant and complemented seedlings. Mutant, complemented mutant, and WT seedlings were grown simultaneously and two representative seedlings of each were selected for photography. (Scale bar: 5 mm.) Along with Fig. S5, this figure relates to Fig. 3.

	WT	*vps29*
2 DAG	0%	0%
3 DAG	0%	0%
5 DAG	81%	12%
8 DAG		72%

Fig. S5. Kinetics of SDP1 translocation toward the OB surface. WT and *vps29* seedlings expressing GFP–SDP1 under the control of the 35S promoter were grown on MS medium with 1% (wt/vol) sucrose. At different growth points, seedlings were incubated in Nile Red for OB staining, and hypocotyls were imaged by confocal microscopy. The percentage of seedlings that showed GFP–SDP1 labeling surrounding OBs was reported. At least 15 seedlings were observed for each time point. Along with Fig. S4, this figure relates to Fig. 3.

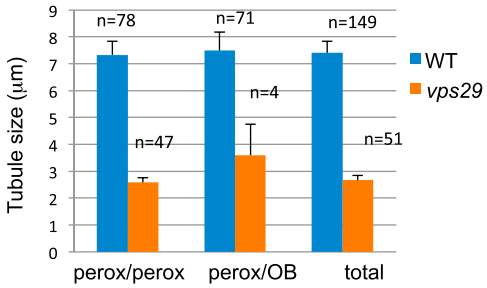
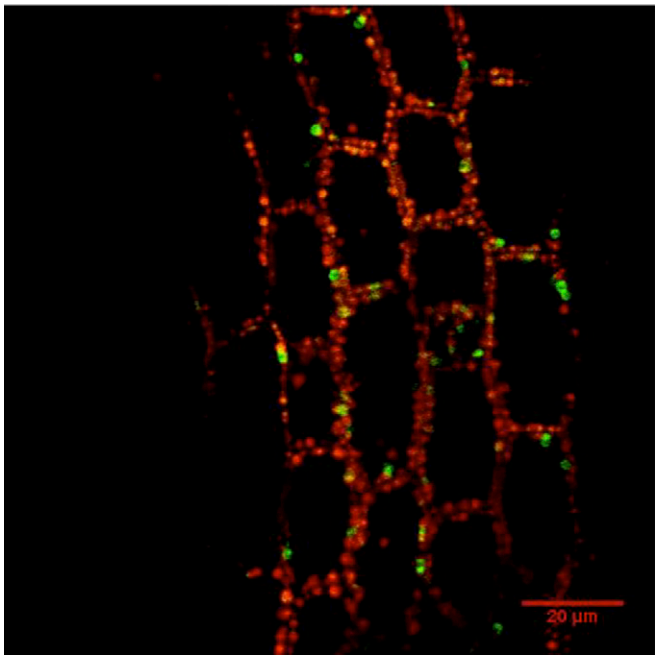


Fig. S6. Using ImageJ software (NIH), time series of 4 DAG SDP1-expressing lines stained with Nile Red were used to quantify and measure tubules connecting peroxisomes or connecting peroxisomes to OBs. A total of 149 tubules (78 perox/perox + 71 perox/OB) were counted for 10 WT hypocotyls. A total of 51 tubules were counted for 9 *vps29* hypocotyls; among these tubules, only 4 connected peroxisomes to OBs. Values are means ± SEM. This figure relates to Fig. 4.

Table S1. Average values of primary root length of retromer and *sdp1* mutants

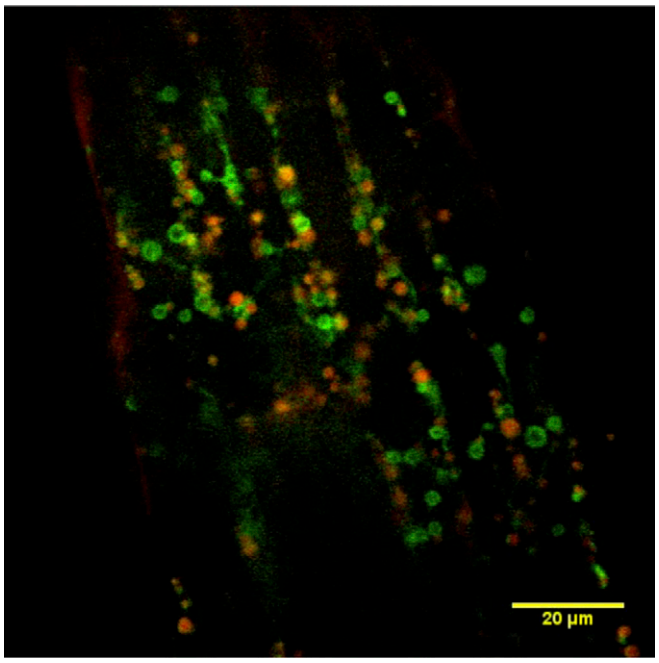
		WT		*snx triple*		*vps29*		*vps35ac*		*sdp1.4*		*sdp1.5*	
Exp.		+ Suc	− Suc	+ Suc	− Suc	+ Suc	− Suc	+ Suc	− Suc	+ Suc	− Suc	+ Suc	− Suc
1	Mean	1.7806	0.9474	1.4606	0.6007	1.0819	0.1821	0.5881	0.1267	0.8948	0.1684	0.9635	0.0919
	±SEM	0.0809	0.0817	0.0624	0.0691	0.0593	0.0111	0.0758	0.0164	0.0829	0.0210	0.0653	0.0058
	n	36	34	60	34	20	10	28	10	30	13	42	21
2	Mean	1.9104	1.1926	1.4697	0.4272	1.2446	0.0855	0.8285	0.0956	0.7667	0.1012	1.1504	0.1079
	±SEM	0.0866	0.0746	0.089	0.0302	0.1039	0.0063	0.1200	0.013	0.0996	0.0081	0.0895	0.0114
	n	42	35	18	18	23	26	11	14	4	27	18	29
3	Mean	1.7561	1.1060	1.0735	0.3987	1.1600	0.0870	0.638	0.1527	0.9464	0.1531	1.0784	0.0842
	±SEM	0.2211	0.2499	0.1648	0.0441	0.1609	0.0121	0.0913	0.0453	0.1252	0.0158	0.0664	0.0063
	n	12	7	14	17	11	6	24	18	11	18	8	9

This table relates to Fig. 1. WT, retromer (*snx* triple, *vps29*, *vps35a vps35c*), *sdp1.4*, and *sdp1.5* mutant seedlings grown on MS medium with or without 1% (wt/vol) sucrose for 8 DAG. Three independent experiments (Exp. 1, 2, and 3) were performed. Values are mean ± SEM; *n*, number of measured roots.



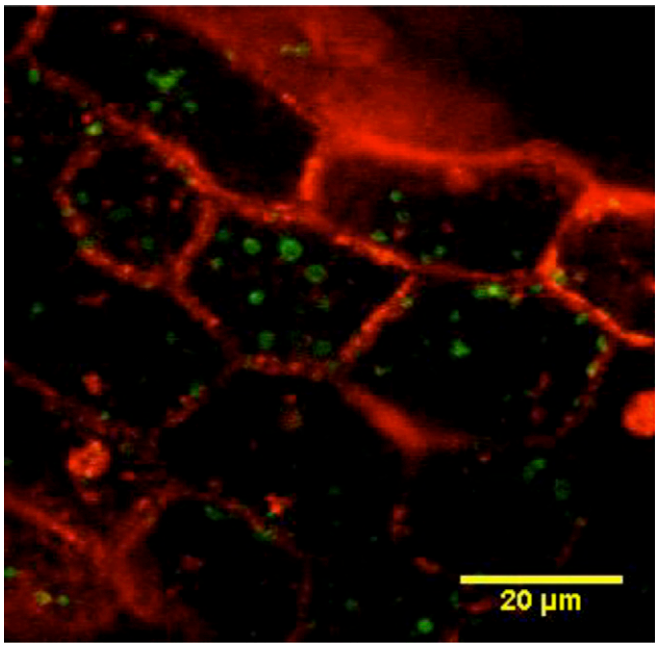
Movie S1. Time-lapse imaging of SDP1 in hypocotyl of WT seedlings at 3 DAG. WT seedlings expressing GFP–SDP1 were grown on MS medium with 1% (wt/vol) sucrose. At 3 DAG, seedlings were treated with Nile Red and imaged with a spinning disk confocal microscope (Leica) during 15 min. Green labeling corresponds to GFP–SDP1 and red labeling to OBs. (Scale bars: 20 µm.) Along with Movies S2–S4, this movie relates to Fig. 4.

Movie S1



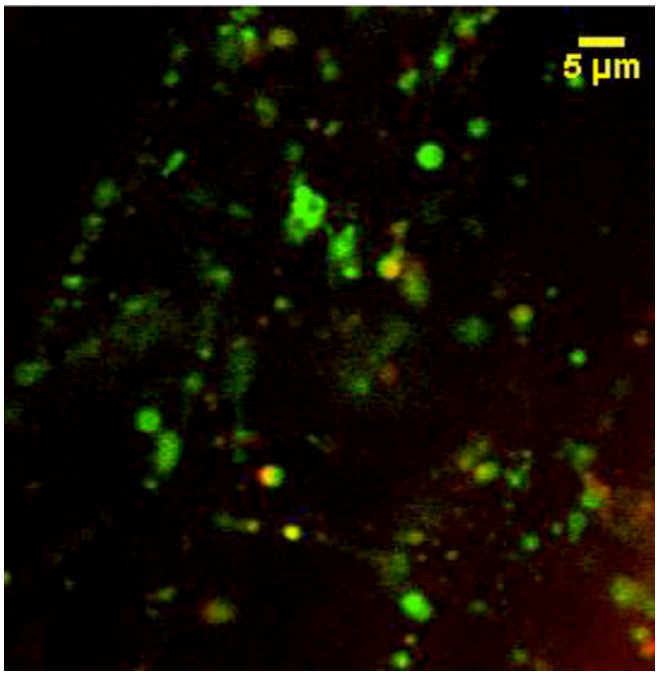
Movie S2. Time-lapse imaging of SDP1 in hypocotyl of WT seedlings at 4 DAG. WT seedlings expressing GFP–SDP1 were grown on MS medium with 1% (wt/vol) sucrose. At 4 DAG, seedlings were treated with Nile Red and imaged with a spinning disk confocal microscope (Leica) during 15 min. Green labeling corresponds to GFP–SDP1 and red labeling to OBs. (Scale bars: 20 µm.) Along with Movies S1, S3, and S4, this movie relates to Fig. 4.

Movie S2



Movie S3. Time-lapse imaging of SDP1 in hypocotyl of *vps29* seedlings at 4 DAG. *vps29* mutant seedlings expressing GFP–SDP1 were grown on MS medium with 1% (wt/vol) sucrose. At 4 DAG, seedlings were treated with Nile Red and imaged with a spinning disk confocal microscope (Leica) during 15 min. Green labeling corresponds to GFP–SDP1 and red labeling to OBs. (Scale bars: 20 µm.) Along with Movies S1, S2, and S4, this movie relates to Fig. 4.

Movie S3



Movie S4. Time-lapse imaging of SDP1 in hypocotyl of *vps29* seedlings at 8 DAG. *vps29* mutant seedlings expressing GFP–SDP1 were grown on MS medium with 1% (wt/vol) sucrose. At 8 DAG, seedlings were treated with Nile Red and imaged with a spinning disk confocal microscope (Leica) during 15 min. Green labeling corresponds to GFP–SDP1 and red labeling to OBs. (Scale bars: 5 µm.) Along with Movies S1–S3, this movie relates to Fig. 4.

Movie S4