



Supplemental Figure 1. The glyoxylate enzyme isocitrate lyase is degraded with near wild-type kinetics in identified putative peroxisomal protease mutants.

- (A) Immunoblots of protein extracts from wild-type (Col-0), *deg15-1*, *lon2-1*, and *lon2-2* cotyledons (12 per lane) cut from 3- to 8-day-old light-grown seedlings.
- (B) Immunoblot of protein extracts from *deg15-1*, *pxm16-1*, *pxm16-2*, and *lon2-2* cotyledons (12 per lane) from 3- and 6-day-old light-grown seedlings.
- (C) Immunoblot of protein extracts from cotyledons of mutants disrupted in putative peroxisomal proteases *At2g18080* and *At2g35615* from 3- and 6-day-old light-grown seedlings. Blots were probed with antibodies raised against isocitrate lyase (ICL), hydroxypyruvate reductase (HPR), PED1 (thiolase), and HSC70. Positions of molecular weight markers (in kDa) are indicated on the left. Processed and unprocessed thiolase are marked by one or two asterisks, respectively, and a filled circle marks the position of a protein that cross-reacts with the ICL antibody but is not found in the *icl* null mutant (Zolman et al., 2005).

Supplemental Table I. Primers used to determine mutant genotypes.

Genotype	Primers ¹	Amplicon size (bp)
<i>deg15-1</i>	R005: CCTCCCTGTGGTGATGATTATGAAGCC LB1-SALK: CAAACCAGCGTGGACCGCTGCTGCAACTC	246
<i>DEG15</i>	R005: CCTCCCTGTGGTGATGATTATGAAGCC R006: TGGCCATGAAGTTAGCGTTGTGTG	487
<i>lon2-1</i>	R008: TTACCATATGGGCCAACACACAGTCC LB1-SALK	278
<i>lon2-2</i>	R008: TTACCATATGGGCCAACACACAGTCC LB1-SALK	248
<i>LON2</i>	R007: ATTGGCTGCACACCGGTATGGTAT R008: TTACCATATGGGCCAACACACAGTCC	466
<i>pxm16-1</i>	R002: ACTTTACGAATCAGTAAGCGATAATCCA LB1-SALK	378
<i>PXM16-1</i>	R001: GGGATATATT CATTATACAAATGACGTGGC R002: ACTTTACGAATCAGTAAGCGATAATCCA	403
<i>pxm16-2</i>	R004: GGGTAGAGCCATCTGGTGGTAGAA LB1-SALK	273
<i>PXM16-2</i>	R003: GGCAGCCATATCACCAAGCAATGT R004: GGGTAGAGCCATCTGGTGGTAGAA	458
<i>ped1-96</i>	PED1-16: ATCTGGGCTTCAGGCTGTTGCTGAT PED1-17: TGCCTTCTGTGCGAGTCAACCTA	² 502
<i>PEDI</i>	PED1-16: ATCTGGGCTTCAGGCTGTTGCTGAT PED1-17: TGCCTTCTGTGCGAGTCAACCTA	³ 305, 197

¹Primers are given in the 5' to 3' orientation.

²The *ped1-96* mutation destroys a *Hin*P1I site.

³The 502-bp *PEDI* amplicon is cleaved once by *Hin*P1I.

Supplemental Table II. Accession numbers for LON homologs used in phylogenetic analysis.

Organism	Protein name	Protein accession number
<i>Anaeromyxobacter</i> sp. Fw109-5	La / Anae109_2098	YP_001379285
<i>Arabidopsis thaliana</i>	LON1 / At5g26860	NP_568490
<i>Arabidopsis thaliana</i>	LON2 / At5g47040	NP_568675
<i>Arabidopsis thaliana</i>	LON3 / At3g05780	NP_566258
<i>Arabidopsis thaliana</i>	LON4 / At3g05790	NP_566259
<i>Caenorhabditis elegans</i>	C34B2.6	NP_492796
<i>Danio rerio</i>	LONP1	XP_691712
<i>Danio rerio</i>	LONP2	NP_001008573
<i>Debaryomyces hansenii</i>	DEHA0G02310g	XP_461623
<i>Drosophila melanogaster</i>	CG8798	NP_649133
<i>Escherichia coli</i>	La	YP_003053061
<i>Gallus gallus</i>	LONP2	XP_414106
<i>Homo sapiens</i>	LONP1	NP_004784
<i>Homo sapiens</i>	LONP2	NP_113678
<i>Kluyveromyces lactis</i>	KLLA0B01892g	XP_451615
<i>Micromonas</i> sp. RCC299	MICPUN_113675	ACO63129
<i>Myxococcus xanthus</i>	La	YP_632173
<i>Oryza sativa</i>	Os09g0533400	NP_001063769
<i>Physcomitrella patens</i>	PHYPADRAFT_161045	XP_001757793
<i>Pichia angusta</i>	PLN	ABB88892
<i>Pichia pastoris</i>	PAS_chr1-1_0174	XP_002489820
<i>Populus trichocarpa</i>	POPTRDRAFT_827676	XP_002329591
<i>Saccharomyces cerevisiae</i>	Pim1p	NP_009531
<i>Schizosaccharomyces pombe</i>	LON1	NP_593035
<i>Yarrowia lipolytica</i>	YALI0F23595g	XP_505795