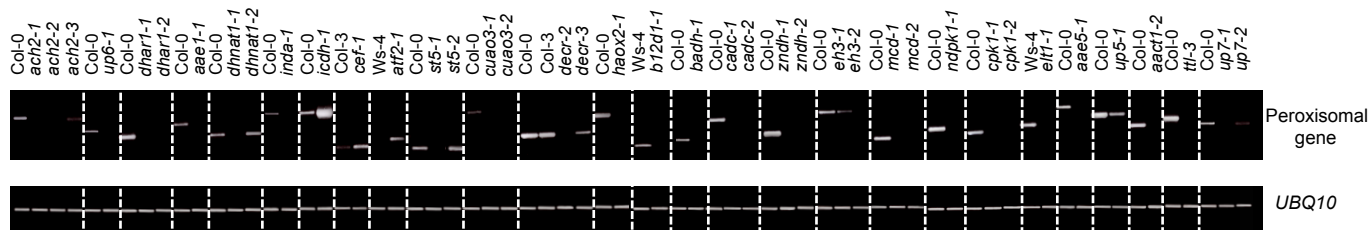


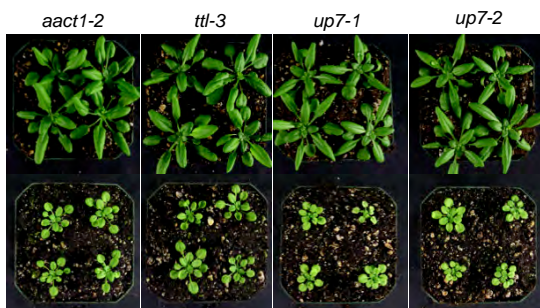
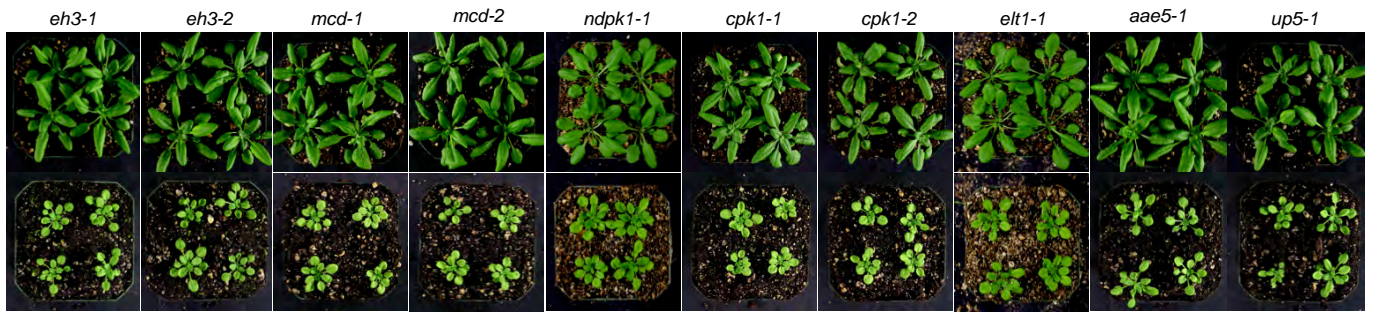
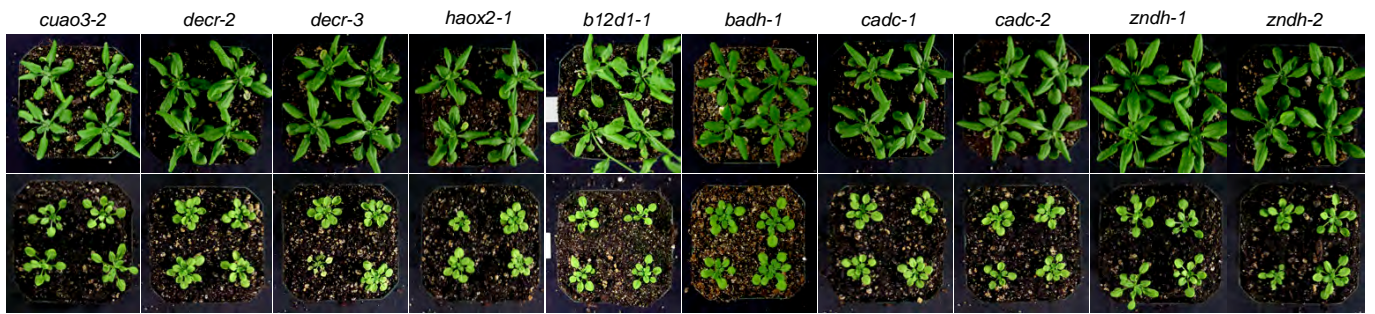
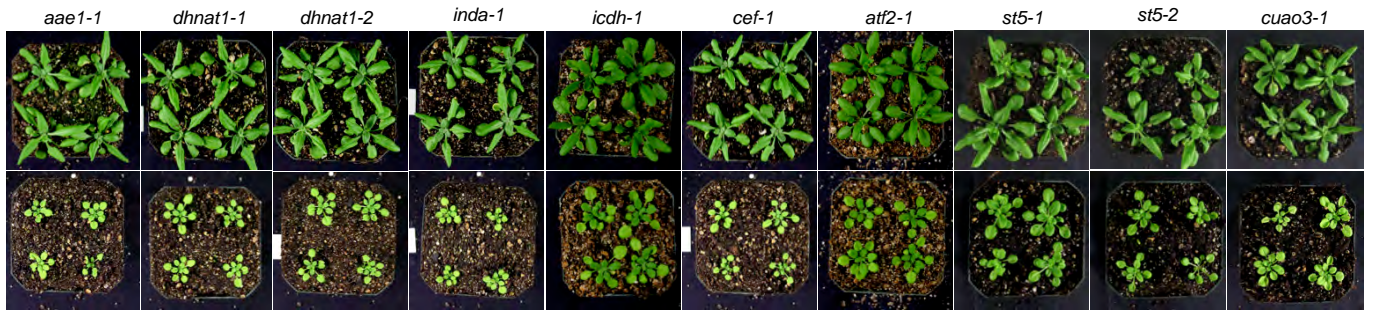
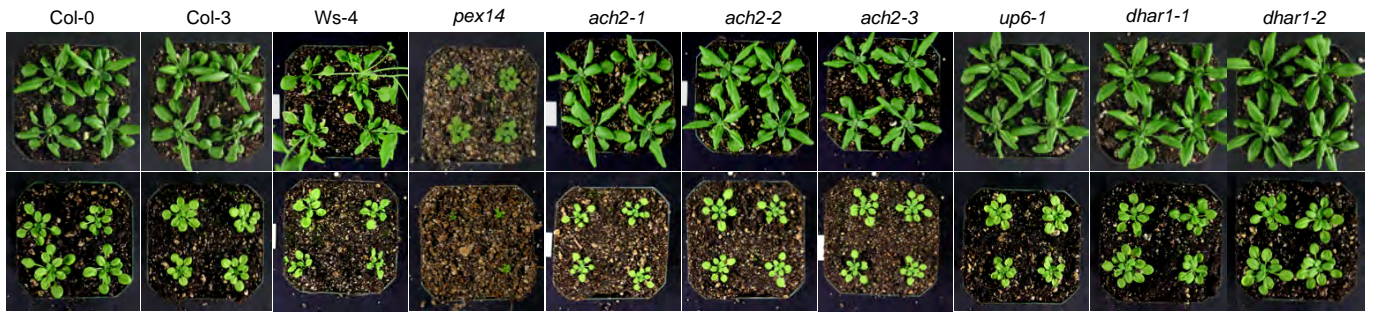
Supplemental Figure S1. Schematics of peroxisomal genes analyzed in this study.

Black boxes, coding regions; open boxes, untranslated regions; solid lines, introns; dashed lines, intergenic regions. Positions of the T-DNA insertions are indicated. Primers used for genotyping (P3 and P4) and RT-PCR (P1 and P2) are also indicated.



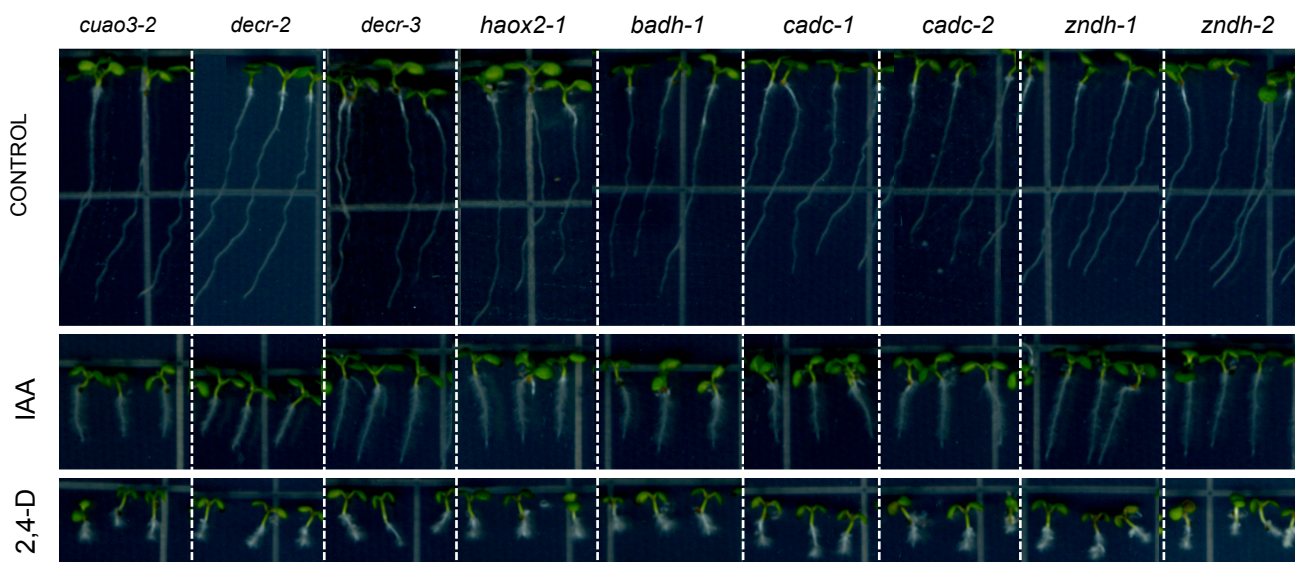
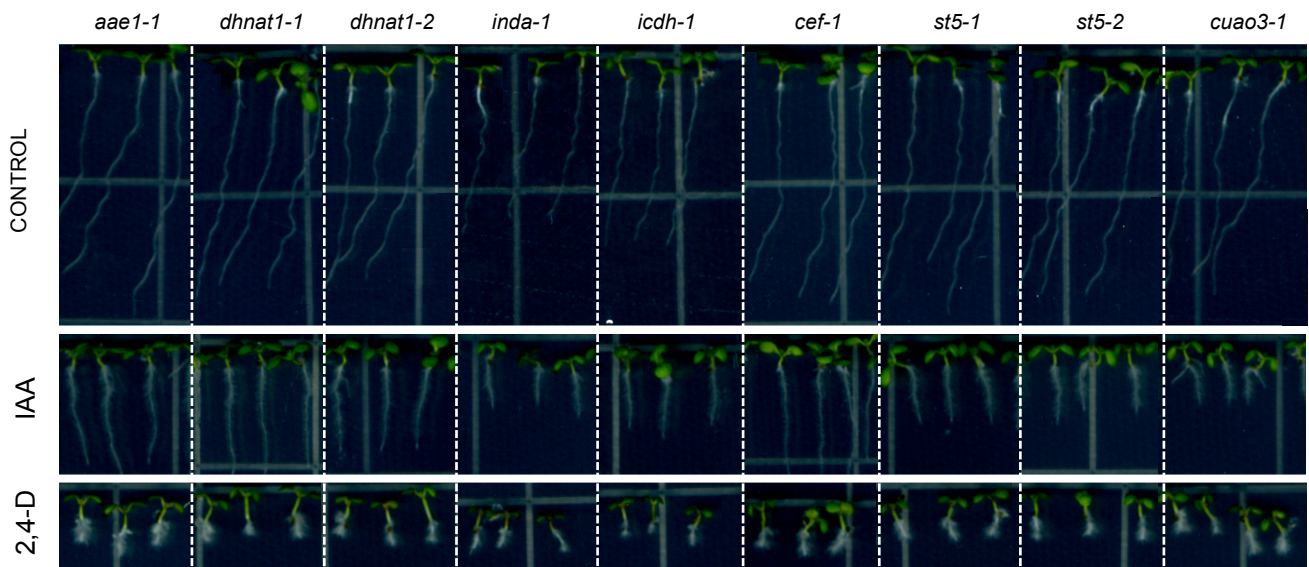
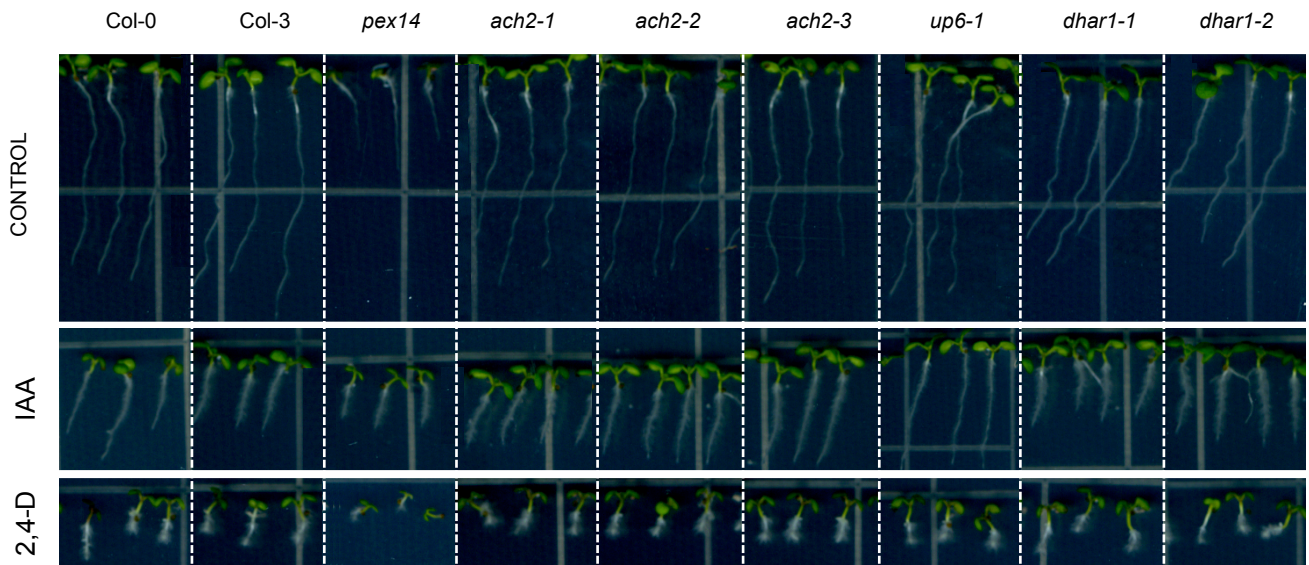
Supplemental Figure S2. RT-PCR analysis of 7d mutant seedlings.

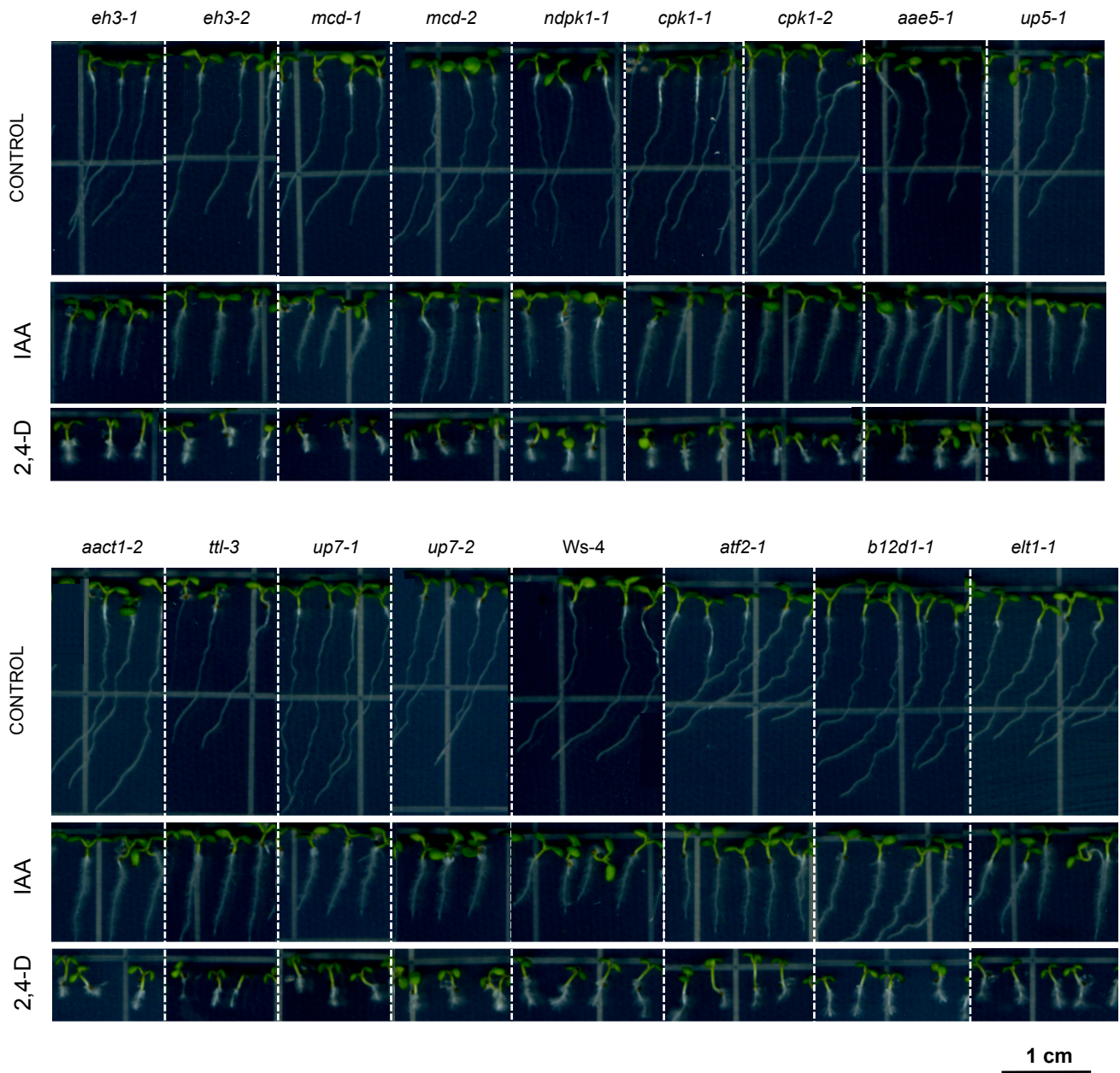
Gene-specific primers used for each pair of PCR reaction (35 cycles) are listed in the Supplemental Table S5. *UBQ10* was used to normalize the expression level (25 cycles).



1 cm

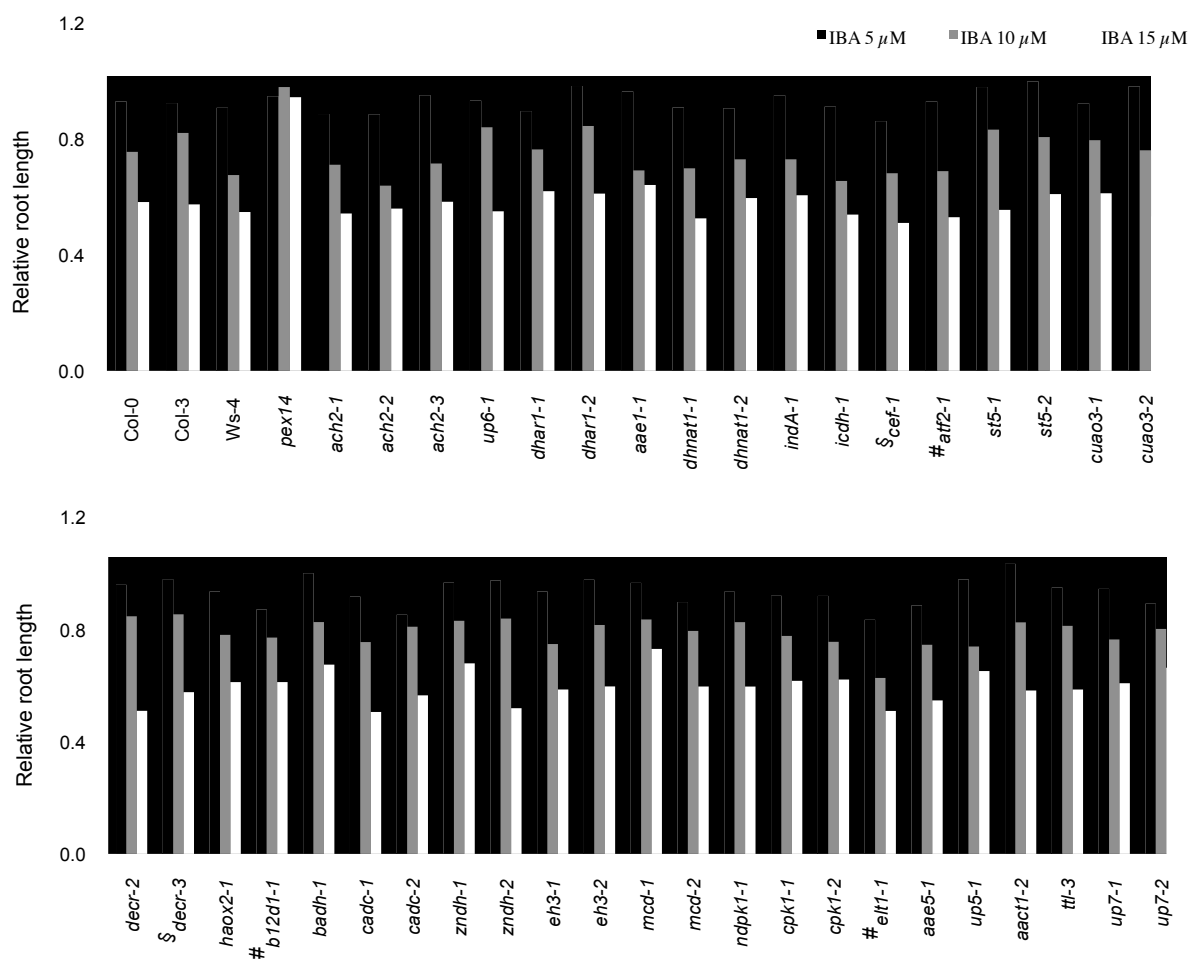
Supplemental Figure S3. Images of four-week-old plants grown in ambient air (400 ppm CO₂, top row) or under low CO₂ (80 ppm CO₂, bottom row).





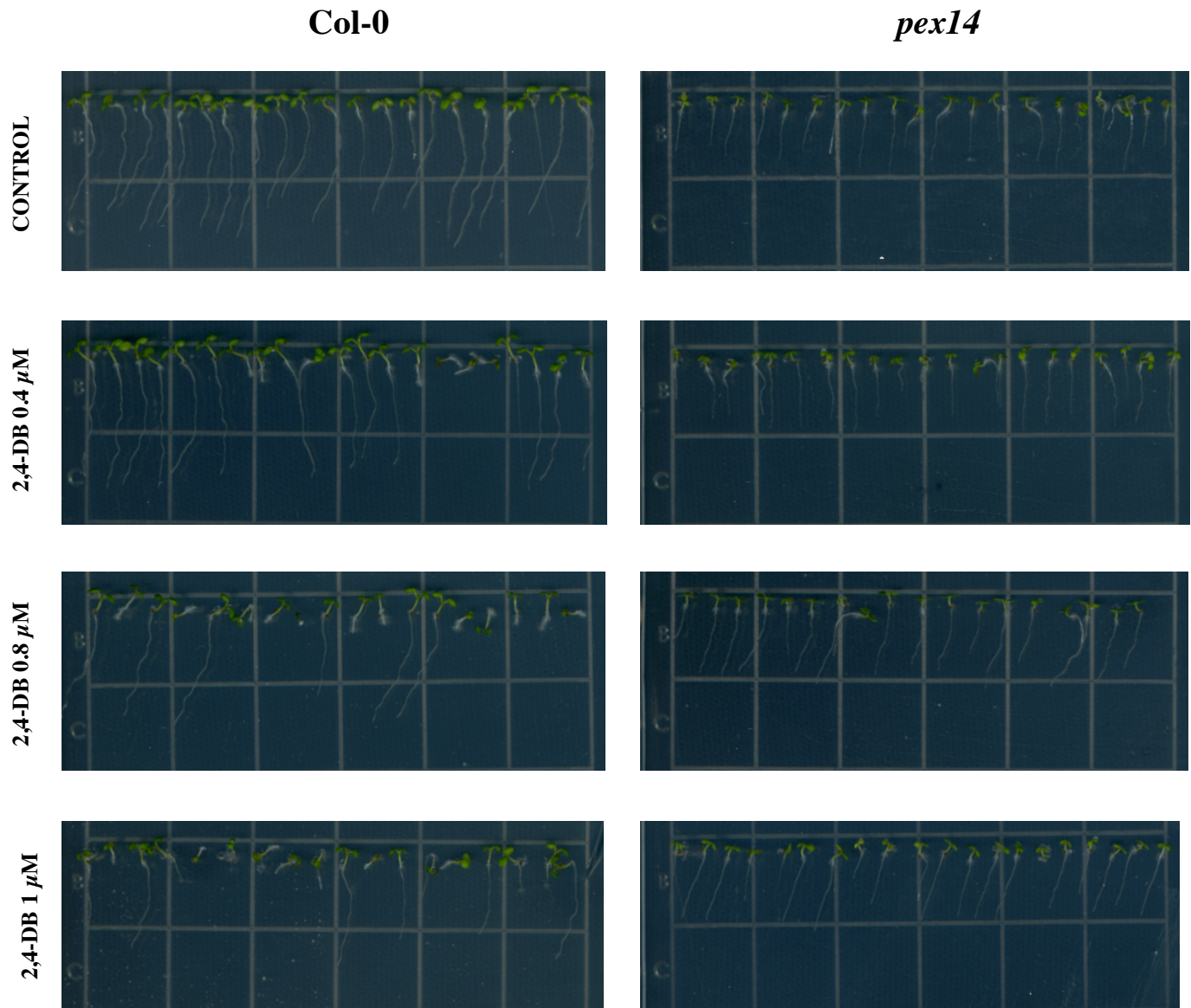
Supplemental Figure S4. IAA and 2,4-D responses.

Seven-day-old seedlings grown on 1/2 LS medium supplemented with 0.5% Suc and 100 nM IAA or 2,4-D.



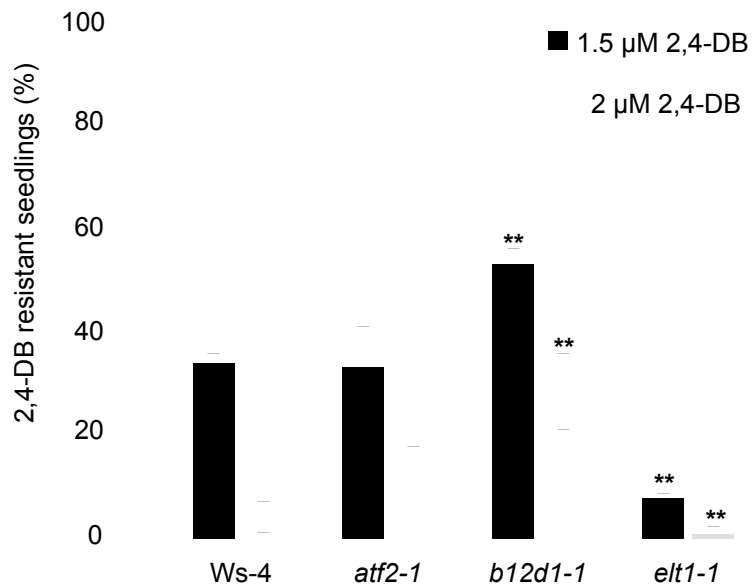
Supplemental Figure S5. IBA response of peroxisomal mutants.

Relative root lengths (ratio between root length on IBA vs. without IBA) of 7d seedlings grown on 1/2 LS medium supplemented with 0.5% Suc and 5 μ M (black bars), 10 μ M (grey bars) or 15 μ M (white bars) of IBA are shown. Data represent means \pm se of three independent experiments. For each experiment, $n \geq 30$. Section and hashtag signs indicate mutants in Col-3 and Ws-4 backgrounds respectively. Except for the control mutant *pex14*, none of the mutants display statistically significant reduction in the response to IBA.



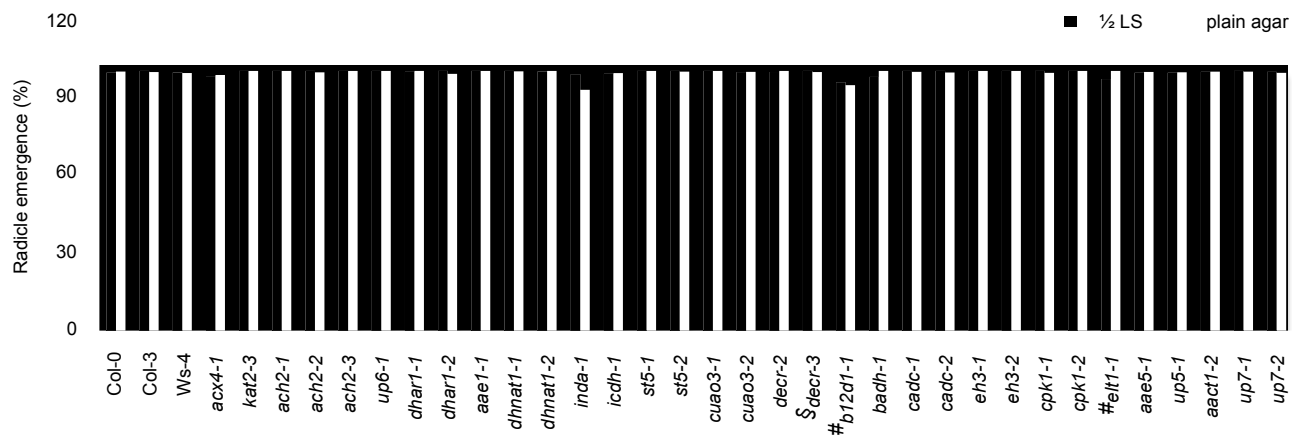
Supplemental Figure S6. Responsiveness of Col-0 and *pex14* to 2,4-DB.

Images show 7d seedlings grown on 1/2 LS medium supplemented with 0.5% Suc and 0.4 μM, 0.8 μM or 1 μM 2,4-DB. Scale bar = 1 cm.



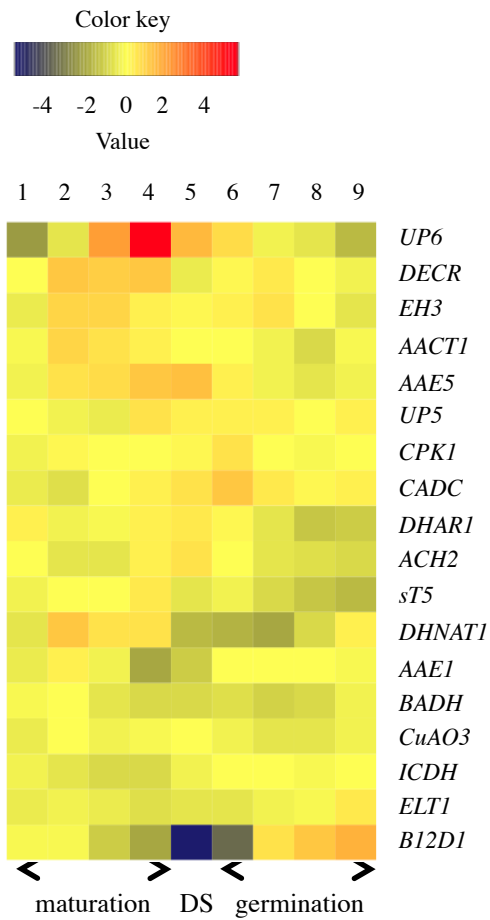
Supplemental Figure S7. Responsiveness of mutants in Ws-4 background to 2,4-DB.

Percentage of 7d seedlings with long (resistant) root after growing on 1/2 LS medium supplemented with 0.5% Suc and 1.5 or 2.0 μM of 2,4-DB. Data represent means \pm se of three independent experiments. For each experiment, $n \geq 30$. Asterisks indicate changes significantly different from that in the wild type Ws-4. Student's *t* test, * $p < 0.01$, ** $p < 0.001$.



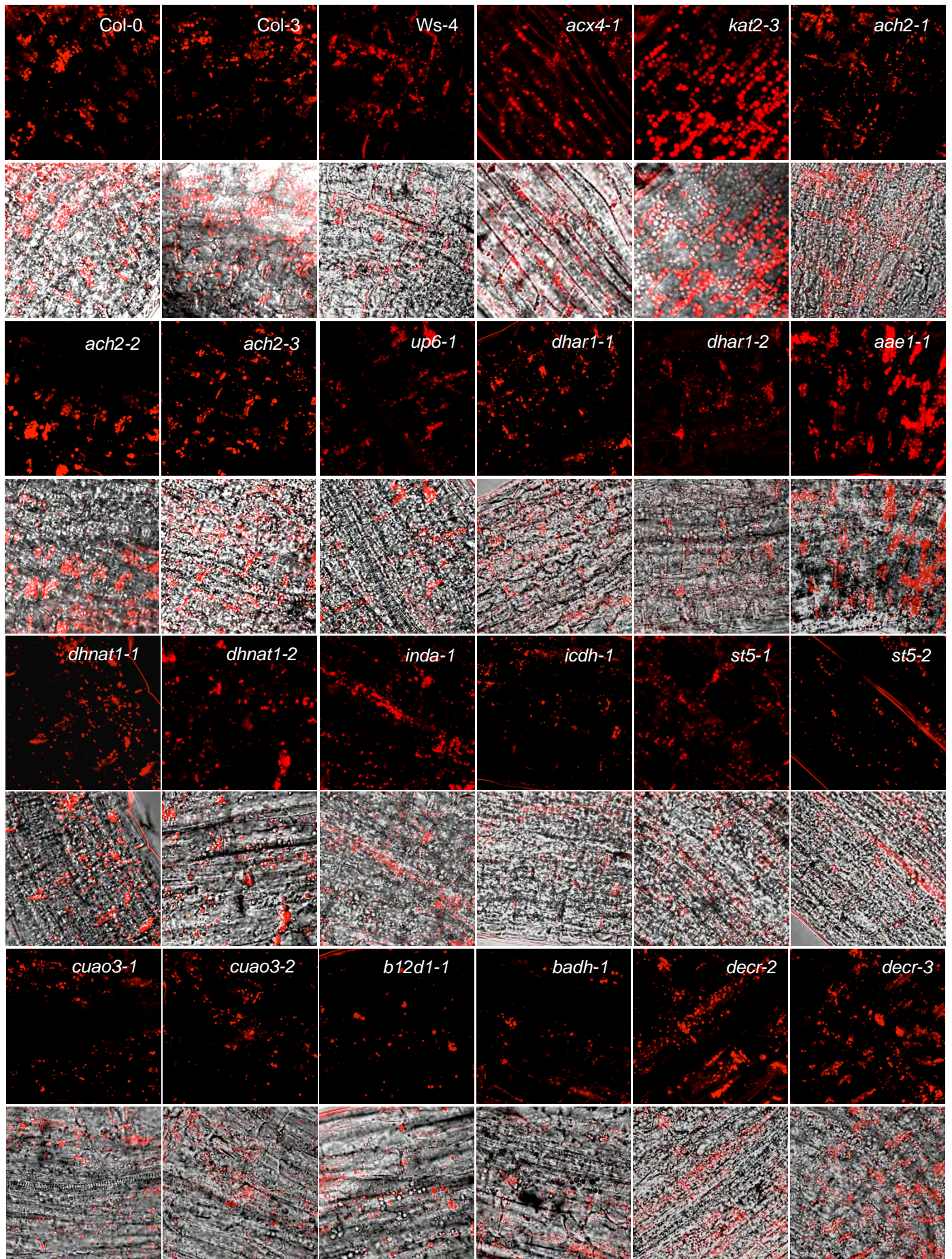
Supplemental Figure S8. Seed germination potential of peroxisomal mutants.

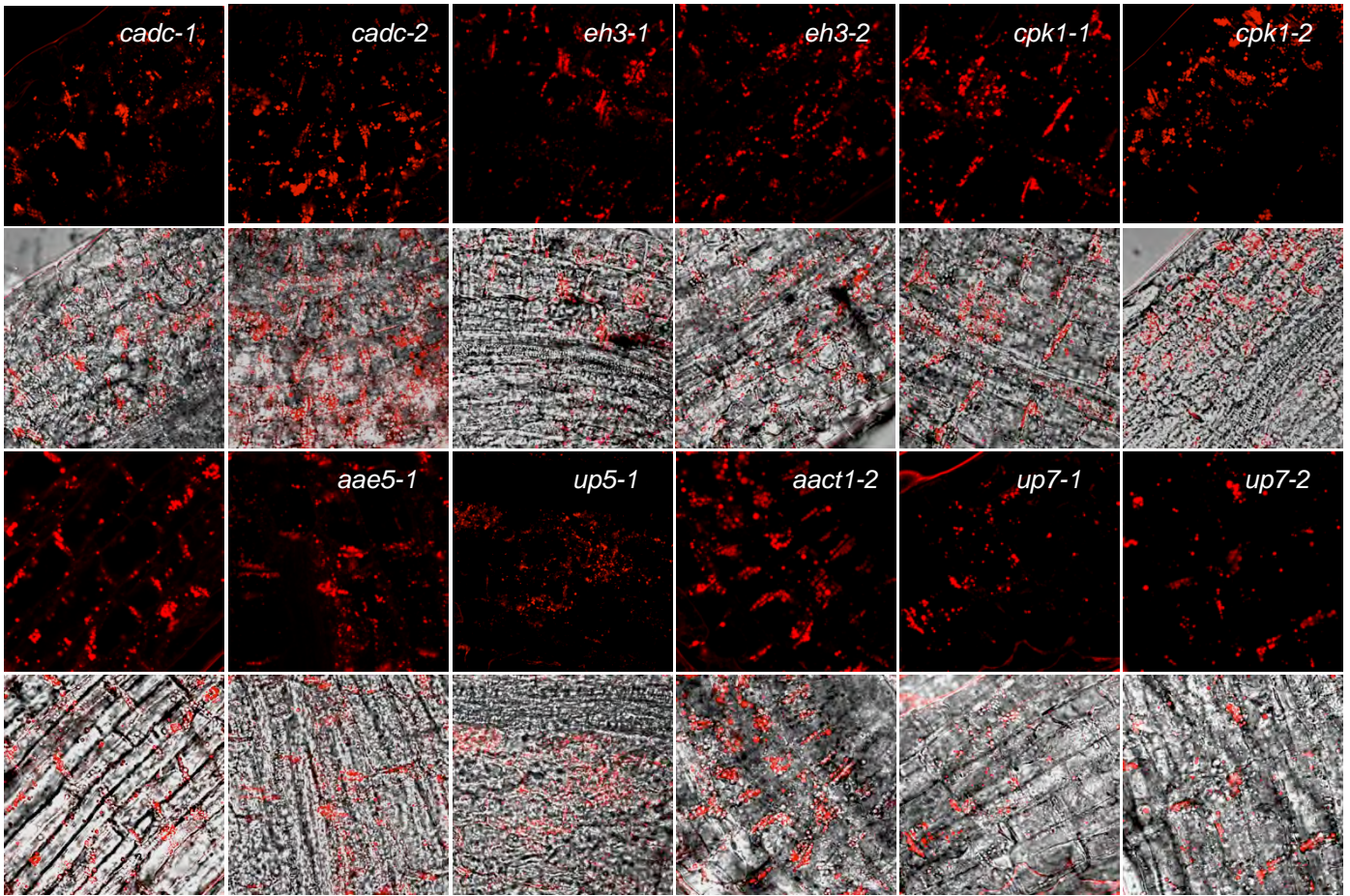
Percentage of radicle emergence from seeds grown on plain agar (0.8% agar) or 1/2 LS medium is presented. Data represent means \pm SE of three independent experiments. For each experiment, n = 50. Section and hashtag signs indicate mutants in Col-3 and Ws-4 backgrounds respectively.



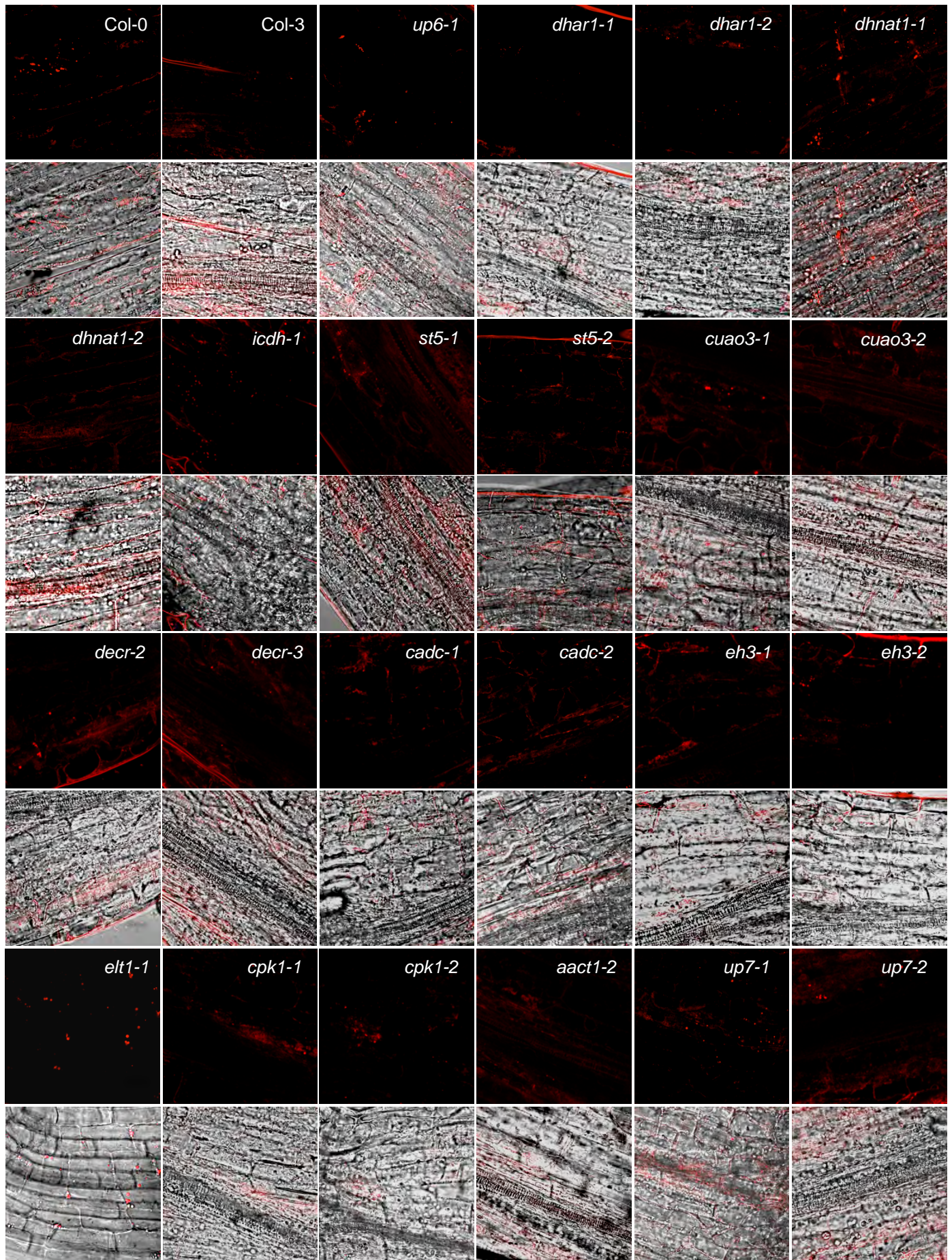
Supplemental Figure S9. Heatmap showing relative expression levels of candidate genes in seed maturation and germination.

Log2-normalized expression values during seed maturation and germination were downloaded from BAR expression browser. 1, Seed stage 3 with siliques; 2, Seed stage 5 with siliques; 3, Seed stage 7 without silique; 4, Seed stage 9 without silique; 5, Dry seed (DS); 6, 3-h imbibed seed ; 7, 6-h imbibed seed; 8, 12-h imbibed seed ; 9, 24-h imbibed seed.





Supplemental Figure S10. Visualization of hypocotyl oil bodies in 5d etiolated seedlings. Confocal images are from representative 5d mutant or wild-type (Col-0, Col-3, or Ws-4) seedlings grown on 0.8% agar plates. Top row, Nile red fluorescence; bottom row, bright-field images. Scale bar = 10 μ m.



Supplemental Figure S11. Visualization of hypocotyl oil bodies in 7d etiolated seedlings. Confocal images of representative seedlings grown on 0.8% agar plates are shown. Top row, Nile red fluorescence; bottom row, merge of Nile red images with bright-field images. Scale bar, 10 μ M.

Supplemental Table S1. Lines without T-DNA insertion and lines for which homozygotes could not be identified.

Mutants without T-DNA insertion:

Gene Locus	Acronym	Annotation	PTS	Line	Ecotype
At1g20560	AAE1	Acyl-activating enzyme isoform 1	SKL	SALK_082448	Col-0
				SALK_082430	Col-0
				SALK_082437	Col-0
At2g26230	Uri	Uricase	SKL	CS812424	Col-3
				SALK_131436	Col-0
At5g58220	TTL1/ ALNS	Transthyretin-like protein 1/ Allantoin synthase	RLx ₅ HL	CS875458	Col-0

List of heterozygous mutants:

Gene Locus	Acronym	Annotation	PTS	Line	Ecotype
At1g54340	ICDH / IDHP1	NADP-dependent isocitrate dehydrogenase	SRL	SALK_039193C	Col-0
At2g26230	Uri	Uricase	SKL	SALK_131438	Col-0
At3g02360	6PGDH	Phosphogluconate dehydrogenase	SKI	SALK_071687	Col-0
				GK_692E09	Col-0
At3g48140	B12D1	Senescence-associated protein/ B12D-related protein		SALK_006284C	Col-0
At4g39660	AGT2	Alanine: glyoxylate aminotransferase 2	SRL	SALK_003381	Col-0
				GK_325A04	Col-0

Supplemental Table S2. Identification of peroxisomal proteins analyzed in this study from previous proteomic studies.

Gene Locus	Acronym	Annotation	Etiolated seedlings		Greenings cotyledons	Cell culture	Green Leaves
			Fukao et al, 2003	Quan et al, 2013	Fukao et al., 2002	Eubel et al, 2008	Reumann et al, 2009
At1g01710	ACH2	Acyl-CoA thioesterase 2		X		X	X
At1g16730	UP6	Unknown protein 6		X			X
At1g19570	DHAR1	Dehydroascorbate reductase					X
At1g20560	AAE1	Acyl-activating enzyme isoform 1			X	X	X
At1g48320	DHNAT1/sT1	DHNA-CoA thioesterase 1/ Small thioesterase 1		X			X
At1g50510	INDA	Indigoidine synthase A		X		X	X
At1g54340	ICDH	NADP-dependent isocitrate deshydrogenase	X	X		X	X
At1g64850	CEF	Calcium binding EF-hand					X
At1g77540	ATF2	Acetyltransferase 2		X			X
At2g29590	sT5	Small thioesterase 5					X
At2g42490	CuAO3	Copper amine oxidase 3		X		X	X
At3g12800	SDRb / DECR	Short-chain dehydrogenase/ reductase isoform b/ 2,4-dienoyl-CoA reductase		X		X	X
At3g14150	HAOX2	Hydroxy-acid oxidase isoform 2					X
At3g48140	B12D1	B12D-related protein					X
At3g48170	BADH	Aldehyde deshydrogenase		X		X	X
At3g55640	CADC	Ca ²⁺ -dependent carrier		X		X	X
At3g56460	ZnDH	Zinc-binding dehydrogenase		X		X	X
At4g02340	EH3	Epoxide hydrolase isoform 3				X	X
At4g04320	MCD	Malonyl-CoA decarboxylase				X	X
At4g09320	NDPK1	Nucleoside diphosphate kinase type 1		X			X
At5g04870	CPK1	Calcium dependent protein kinase 1					
At5g11910	ELT1	Esterase/ lipase/ thioesterase family isoform 1					X
At5g16370	AAE5	Acyl-activating enzyme 5		X	X	X	X
At5g44250	UP5	Unknown protein 5					X
At5g47720	AACT1	Acetoacetyl-CoA thiolase 1.3		X			
At5g58220	TTL	Transthyretin-like protein				X	X
At5g65400	UP7	Serine Hydrolase 1					X

Supplemental Table S3. Measurements for the sucrose dependence assays.

Relative hypocotyl (dark) and root (light) lengths (-Suc versus +Suc) of 7d seedlings grown on 1/2 LS medium supplemented with or without Suc. Data represent means \pm SE of three independent experiments. For each experiment, n = 50.

		DARK			LIGHT		
		mean	SE	Student test	mean	SE	Student test
	Col0	0.85	0.027	-	0.84	0.056	-
	Col3	0.90	0.045	-	0.88	0.038	-
	Ws4	0.80	0.024	-	0.94	0.039	-
	<i>pex14</i>	0.22	0.030	2.70113E-21	0.14	0.019	5.06518E-28
At1g01710	<i>ach2-1</i>	0.81	0.046	0.099780022	0.85	0.054	0.781488227
	<i>ach2-2</i>	0.88	0.066	0.14394992	0.95	0.036	0.000733983
	<i>ach2-3</i>	0.91	0.034	0.000904445	0.83	0.042	0.628189991
At1g16730	<i>up6-1</i>	0.94	0.078	0.004495419	0.89	0.039	0.163499548
At1g19570	<i>dhar1-1</i>	0.87	0.014	0.088216133	0.88	0.021	0.232105387
	<i>dhar1-2</i>	0.88	0.017	0.06028705	0.80	0.049	0.267459055
At1g20560	<i>aae1-1</i>	0.97	0.056	6.87515E-05	0.85	0.052	0.791591679
At1g48320	<i>dhnat1-1</i>	0.81	0.060	0.121543694	0.82	0.060	0.538924628
	<i>dhnat1-2</i>	0.81	0.039	0.327294242	0.82	0.029	0.438858416
At1g50510	<i>inda-1</i>	0.83	0.027	0.184775749	0.76	0.010	0.0139893
At1g54340	<i>icdh-1</i>	0.74	0.037	0.000153897	0.85	0.045	0.941075033
At1g64850	<i>cef-1</i>	0.83	0.028	0.152712563	0.83	0.077	0.33154256
At1g77540	<i>atf2-1</i>	0.84	0.013	0.223973757	0.78	0.069	0.010691507
At2g29590	<i>st5-1</i>	0.98	0.063	5.76434E-05	0.80	0.096	0.260361059
	<i>st5-2</i>	0.94	0.025	3.03002E-05	0.96	0.086	0.003391833
At2g42490	<i>cuao-1</i>	0.86	0.037	0.415983857	0.90	0.041	0.146159872
	<i>cuao-2</i>	0.92	0.034	0.009217858	0.82	0.017	0.595812737
At3g12800	<i>decr-2</i>	1.02	0.017	1.65719E-07	0.86	0.117	0.672608646
	<i>decr-3</i>	1.04	0.022	0.013135616	0.90	0.065	0.672542951
At3g14150	<i>haox1-1</i>	0.95	0.030	2.211E-05	0.91	0.057	0.03432197
At3g48140	<i>b12d1-1</i>	0.79	0.014	0.737902312	0.89	0.065	0.252919227
At3g48170	<i>badh-1</i>	0.86	0.116	0.692022112	0.79	0.058	0.086638398
At3g55640	<i>cadc-1</i>	1.00	0.021	1.6556E-07	0.90	0.034	0.064334466
	<i>cadc-2</i>	0.80	0.066	0.0520033	1.05	0.033	3.16612E-07
At3g56460	<i>zndh-1</i>	0.97	0.027	3.21522E-06	0.90	0.066	0.09468511
	<i>zndh-2</i>	0.99	0.050	6.47175E-06	0.89	0.012	0.309884302
At4g02340	<i>eh3-1</i>	0.92	0.037	0.00082387	0.89	0.107	0.175933598
	<i>eh3-2</i>	0.96	0.054	0.000134123	0.89	0.087	0.198442622
At4g04320	<i>mcd-1</i>	1.01	0.168	0.009532992	0.90	0.160	0.236621439
	<i>mcd-2</i>	0.86	0.111	0.797343451	0.90	0.091	0.10510667
At4g09320	<i>ndpk-1</i>	0.81	0.057	0.150957756	0.89	0.028	0.287200891
At5g04870	<i>cpk1-1</i>	0.94	0.076	0.005890754	0.85	0.073	0.784468002
	<i>cpk1-2</i>	0.98	0.063	8.16885E-05	0.83	0.071	0.693940174
At5g11910	<i>elt1-1</i>	0.89	0.053	0.067961956	0.91	0.039	0.324510445
At5g16370	<i>aae5-1</i>	0.95	0.067	0.003761092	0.88	0.024	0.290013306
At5g44250	<i>up5-1</i>	0.79	0.047	0.00818022	0.86	0.054	0.49299674
At5g47720	<i>aact1-2</i>	0.81	0.099	0.355669223	0.89	0.052	0.233434107
At5g58220	<i>tll-3</i>	0.99	0.028	5.63043E-07	0.94	0.038	0.009693737
At5g65400	<i>up7-1</i>	0.89	0.103	0.266844759	0.76	0.024	0.013005102
	<i>up7-2</i>	0.86	0.092	0.671106101	0.86	0.040	0.713764227

Supplemental Table S4. Log2 expression values downloaded from BAR expression browser to generate heatmap shown in Supplemental Figure S8

Locus	Annotation	Acronym	1	2	3	4	5	6	7	8	9
At1g16730	Unknown protein 6	UP6	-2.3	-0.4	2.3	5.9	1.7	0.9	-0.2	-0.5	-1.6
At3g12800	Short-chain dehydrogenase/ reductase isoform b	DECR	0.1	1.4	1.2	1.3	-0.3	0.3	0.6	0.2	-0.1
At4g02340	Epoxide hydrolase isoform 3	EH3	-0.3	1	1.1	0.5	0.3	0.5	0.7	0.1	-0.4
At5g47720	Acetoacetyl-CoA thiolase 1.3	AACT1	0	1.1	0.8	0.4	0.2	0.1	-0.1	-0.7	0
At5g16370	Acyl-activating enzyme 5	AAE5	-0.1	0.8	0.9	1.4	1.6	0.5	-0.1	-0.4	-0.2
At5g44250	Unknown protein 5	UP5	0.2	-0.2	-0.3	0.7	0.5	0.5	0.5	0.2	0.5
At5g04870	Calcium dependent protein kinase 1	CPK1	-0.1	0.3	0.1	0.1	0.3	0.7	0.1	0	0.2
At3g55640	Ca ²⁺ -dependent carrier	CADC	-0.3	-0.6	0.2	0.5	0.7	1.4	0.6	0.3	0.4
At1g19570	Dehydroascorbate reductase	DHAR1	0.4	-0.1	0	0.4	0.6	0.3	-0.5	-1.2	-1.1
At1g01710	Acyl-CoA thioesterase 2	ACH2	0.1	-0.4	-0.5	0.4	0.7	0.2	-0.4	-0.6	-0.8
At2g29590	Small thioesterase 5	sT5	-0.1	0.1	0.2	0.6	-0.4	-0.1	-0.7	-1.2	-1.6
At1g48320	DHNA-CoA thioesterase 1/ Small thioesterase 1	DHNAT1	-0.5	1.3	0.8	0.7	-1.5	-1.7	-2	-0.8	0.4
At1g20560	Acyl-activating enzyme isoform 1	AAE1	-0.3	0.5	-0.1	-2	-1	0.1	0.2	0.1	0
At3g48170	Aldehyde deshydrogenase	BADH	0	0.2	-0.4	-0.7	-0.7	-0.6	-0.9	-0.7	-0.1
At2g42490	Copper amine oxidase 3	CuAO3	-0.3	0.1	-0.1	0	0.2	-0.2	-0.4	-0.4	-0.1
At1g54340	NADP-dependent isocitrate deshydrogenase	ICDH	-0.1	-0.4	-0.7	-0.7	-0.1	0.2	0.1	0	0.1
At5g11910	Esterase/ lipase/ thioesterase family isoform 1	ELT1	-0.3	-0.2	-0.3	-0.6	-0.5	-0.5	-0.1	0	0.6
At3g48140	B12D-related protein	B12D1	0	0	-1	-2	-5.6	-3.6	0.7	1.4	1.8

Number as	Developmental stage
1	Seed stage 3 with siliques
2	Seed stage 5 with siliques
3	Seed stage 7 without silique
4	Seed stage 9 without silique
5	Dry seed
6	3-h imbibed seed
7	6-h imbibed seed
8	12-h imbibed seed
9	24-h imbibed seed

Supplemental Table S5. Primers used for genotyping and RT-PCR analysis. P1 and P2 were used for RT-PCR; P3 and P4 were used for genotyping.

Name		Sequence	
LB_SALK		ATTTTGCCGATTTTCGGAAC	
LB_SAIL		TAGCATCTGAATTTTCATAACCAATCTCGATACAC	
LB_GABI		ATATTGACCATCATACTCATTGC	
LB_Ws4		CGTGTGCCAGGTGCCACGGAATAGT	
At1g01710	ACH2	P1	CGAGTTCCTTGGAAACGTCC
		P2/ P4.2	TCAGAGCTTCGACGTGCCGG
		P3.1	GATGTGGTTTTACCGACCTC
		P3.2	CACCATTATCTGCTACAGCTTC
		P4.1	GATCCAAAATGGGTCTGACG
At1g16730	UP6	P1/ P3	CATCACCGAATATCCGCCAC
		P2	AGGTTTGGCGAGAGAGCTGA
		P4	ACACATCTCAAGCTCTGATAC
At1g19570	DHAR1	P1	TCCTGATCATCTCGGCGACT
		P2	TCAAGGGTTAACCTTGGGAG
		P3	CTCTCTGACAAACCCAGTG
		P4	GTCCTTGCCGAAGAGCGACT
At1g20560	AAE1	P1/ P3	GCTCCGTCAAGTACACGTG
		P2/ P4	GCACTGGCCTTACACCCGTC
At1g48320	DHNAT1	P1/ P3.1	GCATCGTCCAACACCAAAGC
		P2/ P4.1/ P4.2	CTTTGCGACCATTTTGAGCATG
		P3.2	CTTTGGTCCGAAAGACTCTC
At1g50510	INDA	P1	ATGGCTTCTTCTTTAGCTCAAT
		P2/ P4	CGTGCAAGTGATGTGCCTCC
		P3	CGGTGCAACTACAGTCTCTG
At1g54340	ICDH	P1	ATGGAATTCGAGAAGATCAA
		P2	TGGAGTTGTTGCCTAACAAATC
		P3	TCACTGCGGGGAATTCAAAG
		P4	CGGACTAATCGTCGCACAC
At1g64850	CEF	P1	GAGTGGAGACATAAGCAGGT
		P2	CATTACGTATTGACTAGATCC
		P3	GCTCCATGTTTCTGTGGTATTC
		P4	GATCGAAATGAGGACCAGGC
At1g77540	ATF2	P1	ATGACGAACACGGCGGCGAC
		P2/ P4	CGTAGACTTGAATACCTCTG
		P3	GCTCAATTGGCAGAGAGAAG
At2g29590	sT5	P1	GCAATTGAGCGATGAGGTGG
		P2/ P4	TCAGAGCTTACTAGCTTGTCT
		P3	GTGGAGGAATCTGAGGAACA
At2g42490	CUAO3	P1	GCTGTCACTCGGGGTGGACA
		P2/ P4.1	ACCAGTCTGAAGAGCTTTCG
		P3.1	GACGAGCGAGGTGAGAACAA
		P3.2	GATTGGCCGTTATGCCCGT
		P4.2	CTGGTTCGTCCGAGTTGGAT
At3g12800	DECR	P1/ P3.1	CTCTCCGTTCAAACCTGATG
		P2	ACAGCTTGCTGGTTGGGAGAC
		P3.2	GCATTGGAGTGGGGAAGT
		P4.1	GGACCTGGAGCAATCCCGT
		P4.2	ATGAGAAAACAGAGAGGTGAC
At3g14150	HAOX2	P1	GGCTAAACAAGCCCTCCCTA
		P2	GAAGTCTCTCATTCTCTGTCC
		P3	GATTGCTTCCAGTTGTAACGC
		P4	CTCAGGACAAAGGGGAGAGC
At3g48140	B12D1	P1/ P3	ATGGCGAGCCGTTGGTTGAG
		P2/ P4	CAGTATGTAGGATCAGTGAAG
At3g48170	BADH	P1/ P3	ATGGCGATTACGGTGCCGAGAC
		P2	CAGAGCTTGAAGGAGGTTTG
		P4	ACGCCAATGGGTTCCCTTCAG
At3g55640	CADC	P1/ P3.1	GCGTCGCAGCTCTTAGCC
		P2/ P4.2	GCAAATCCCTACACCAGGTAC
		P3.2	GGTGTGGGCCTAGTATTGC
		P4.1	CTGAGCAGCAAGACGAGTTC
At3g56460	ZNDH	P1	ATGGAGGCTTTGGTTGCGG

		P2	CAGTCTTGTGGTCGAGAGCA
		P3	TAAACGGCGTCGTATCAAATC
		P4	TAAAGACCATGAACCGTCCAG
At4g02340	<i>EH3</i>	P1	ATGGAGAAGATAGAGCACAC
		P2	TGTTCCGGCGATGATTAACA
		P3	CTACAAGGATGCGGTTTCGC
		P4	TGTTATGTCTAGATCACCAC
At4g04320	<i>MCD</i>	P1	GCAAGAAAAATCTAGCGATT
		P2/ P4.1	TGCCTCCACTTTGACGAATAC
		P3.1	ACCAGAGAACCAAGCAACATG
		P3.2	TGAAGGAGAACTCGGTATGT
		P4.2	GAGGGCATGTTCCCTTCTG
At4g09320	<i>NDPK1</i>	P1/ P3	TTGCCTCCTACGAATCACAAG
		P2/ P4	ATAGACCCAAGGGTGAACGC
At5g04870	<i>CPK1</i>	P1	CAAGCAGAAATGGCTTCTTGC
		P2	CAGGTCTCCTGTGATGCTTC
		P3	AGTTCCTCCATTCTGGGCTG
		P4	GTGATATAGCCGCTCCCATC
At5g11910	<i>ELT1</i>	P1/ P3	ATGTCAGAGACTCGCCGAA
		P2	CGCGATGTAATAGGAACAAC
		P4	AAGTAGTCCTTACCAAGCCG
At5g16370	<i>AAE5</i>	P1/ P3	GCGCCGCAAACCTCGCCGCCG
		P2	CGACTCAACCTCGTCGTACC
		P4	CTGAAATTCGTTAGTCGCCG
At5g44250	<i>UP5</i>	P1	CAACGGAGGAGAAAGCGAAGC
		P2	CTCTTCCCCGGTCTCCGCCT
		P3	AAAACATCATCAAATAGTTACGGATG
		P4	AGGAACTGGCTTTGCTTTGAG
At5g47720	<i>AACT1</i>	P1	ATGGCTCCGCCTGTCTCTGA
		P2	TCAGAGTGCCGAATATCCGA
		P3	CTACAGGTGGTAGCTCTAGC
		P4	GCTGTGTCCGCTCCCTAGCC
At5g58220	<i>TTL</i>	P1	GAATGGAAGGTTTGCTGTGG
		P2/ P4	CTAGCTCCCACGGTATGTGG
		P3	ACAGCTCTAGCTCTCCT
At5g65400	<i>UP7</i>	P1	ATGTTAGGATACTCCTATCC
		P2/ P4.1	AGAAAGCATCTGCCTAATGCT
		P3.1	CTGCAGCTATTCTGGAATG
		P3.2	CAGTCAAAATTGCCGAAAGAC
		P4.2	CGCCAAAGAATCAAACAAC