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Supplementary Information for

Low-oxygen response is triggered by an ATP-dependent shift in oleoyl-CoA in *Arabidopsis*

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Datasets S1 to S2 as supplemental tables in Excel format.

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Supplementary Information Text

Material and Methods

RNA-SEQ analysis. RNA quality and integrity was assessed on a QIAxcel platform (Qiagen, Hilden, Germany). The TruSeq Stranded mRNA sample preparation 96 rcx kit (Illumina) following the low sample protocol according to Illumina™ guidelines was used to construct the library using 2.5 µg of total RNA. Adaptors were ligated in 12-plex formations and the libraries were quantified using PicoGreen (Life Technologies). Groups of 12 samples were pooled at equal concentrations to create the eight pools. The Kapa SYBR FAST universal qPCR kit (Kapa Biosystems) for Illumina sequencing was used to quantify the number of the amplifiable molecules in the pools and the Bioanalyzer (Agilent Technologies) to determine the average fragment size of our pools and optimization of the flow cell clustering. An illumina HiSeq 1500 sequencer was used in the high throughput mode using the TruSeq SBS Kit in a 50-cycle pair-end run.

Transcriptome analysis was performed by means of CLC Genomics Workbench v.6 using the *Arabidopsis thaliana* reference sequence (Tair10). The expression values were normalized using quantile normalisation and pair-wise statistical analyses comparing the treatments performed using FDR-corrected p-values based on Baggerly's test [1].

***In vitro* binding assay.** To determine whether acyl-CoAs affect the interaction between ACBP1 and RAP2.12, both proteins were synthesized using the TNT SP6 high-yield wheat germ extract system (Promega) as described previously [2] with several modifications. The full CDS of RAP2.12, fused C-terminally with the CDS of CFP, and the CDS of ACBP1, N-terminally fused with a FLAG-tag, were cloned into pF3A WG (BYDV) Flexi vector (Promega) using the PmeI and SgfI restriction sites. For *in vitro* protein synthesis, 3 µg of plasmid was added to 30 µl wheat germ extract and the synthesis reaction was run for 2.5 hours at 25°C.

After successful synthesis, the ACBP1 and RAP2.12 proteins were mixed and incubated in binding buffer (10 mM Tris pH 7.5; 50 mM NaCl; 0.5 mM EDTA) for 3 - 4 h on a tube rotator at 10 rpm. To equilibrate the GFP-Trap-A beads (Chromotek), 20 µl of beads slurry was resuspended in 500 µl ice-cold binding buffer and centrifuged at 2,500g for 2 min at 4°C. The supernatant was discarded, and the washing step was repeated twice. The equilibrated beads were resuspended in the ACBP1-RAP2.12 binding-reaction mix and incubated overnight at 4°C on a tube rotator at 10 rpm. The next day, the samples were centrifuged at 2,500g for 2 min at 4°C. The beads were washed twice with wash buffer (10 mm Tris-HCl, pH 7.6, 150 mm NaCl, 0.5 mm EDTA). For the third wash step, the beads were resuspended in wash buffer containing different acyl-CoAs at a final concentration of 0.1% in 0.01% Pluronic F68, or as a mock control only 0.01% F68. Subsequently, the samples were incubated for 10 min on a tube rotator at 10 rpm at 4°C. After this wash, the samples were centrifuged at 2500g for 2 min at 4°C. The beads were resuspended in SDS-page sample buffer, and boiled for 10 min at 94°C. To elute the ACBP1-RAP2.12 protein complexes, the samples were centrifuged at 2,500g for 2 min at 4°C. Eluted protein complexes were subjected to SDS-PAGE and immunoblot analysis. For the detection of RAP2.12-CFP and FLAG-ACBP1, mouse anti-GFP (10,000-fold diluted; Roche) and mouse anti-FLAG (10,000-fold diluted; Sigma) antibodies were used, respectively. As secondary antibody an anti-mouse HRP antibody (Sigma) was used (10,000-fold dilution) and Luminata Crescendo Western HRP substrate (Millipore) was added to detect bands. Blots were imaged using a BioRad Chemidoc system, and the provided ImageLab software was used to quantify band intensities.

The procedure is further illustrated in SI Appendix Fig. S1.

Analysis of acyl-CoA esters. Acyl-CoAs were extracted, derivatized, and analysed by HPLC as described earlier [3]. Briefly, 100 mg of plant tissue was ground under liquid nitrogen before 400 µl extraction buffer (2 ml isopropanol, 2 ml KH_2PO_4, pH 7.2, 50 µl glacial acetic acid, 80 µl bovine serum albumin (50 mg ml^{-1} H_2O, defatted) was added together with 37 pmol heptadecanoyl-CoA as internal standard. To remove contaminants, the samples were washed three times with 600 µl washing buffer (petroleum ether (40-60°C) saturated with isopropanol:water (1:1, v/v). The samples were mixed with the washing buffer, the phases

were separated by centrifugation at 100 g for 1 min and the upper phase was discarded. After the washing steps, 5 µl saturated $(NH_4)_2SO_4$ and 600 µl methanol:chloroform (2:1, v/v) was added to the extracts. The samples were mixed and incubated for 20 min at 22°C. The samples were centrifuged at 21,000 g for 2 min, the supernatant was transferred to a new Eppendorf tube and then evaporated under a stream of nitrogen. To the dried samples 200 µl derivatization reagent (0.5 M chloroacetaldehyde, 0.15 M citrate buffer, pH 4.0, 0.5% (w/v) sodium dodecyl sulphate) was added. The samples were kept at 80°C for 20 min. Prior to HPLC analysis, the samples were centrifuged at 16,000 g for 2 min to remove any precipitates and the supernatant was transferred into HPLC vials. The derivatized acyl-CoAs were analysed on a Agilent 1100 HPLC system (Agilent, Waldbronn) equipped with a LUNA 150 x 2.0 mm column together with a 4 x 2 mm phenyl-propyl guard column (Phenomenex; Aschaffenburg). The derivatized acyl-CoAs were eluted by a quaternary gradient system as described earlier [3] and detected using a UV detector (Agilent, Waldbronn). Acyl-CoA profiles were analysed by using ChemStation Software (Agilent, Waldbronn) and quantified by known concentrations of internal standards.

LACS4 *in vitro* enzyme assay. For expression in *E.coli* the LACS4 cDNA was amplified by a standard PCR protocol using Phusion DNA polymerase (New England Biolabs) and LACS4f (5' CATACATATGTCGCAGCAGAAGAAATAC-3') and LACS4r (5'-CATCGGATCCTACCCTCTGGAAGCA-3') as primers. The resulting fragment was digested by NdeI / BamHI and inserted into the vector pET22b (Merck, Darmstadt) digested correspondingly. The resulting plasmid pET-AtLACS4 was transformed into competent K27 *E.coli* cells [4] to generate the strain K27/pET-AtLACS4. As control the empty vector pET22b was transformed to establish strain K27 /pET22b. K27 is a fadD mutant strain deficient in endogenous LACS activity. The strain was made compatible to the pET protein expression system by integrating the T7 RNA polymerase gene into the E. coli chromosome using the λDE3 lysogenization kit (Novagen, Madison, WI). Enzyme overproduction was achieved as described earlier [5]. Briefly, 200 ml cultures were grown in presence of carbenicillin (100 µg ml^{-1}) at 16 °C and induced with 1 mM isopropylthio-β-galactoside at mid-log phase. The cells were cultured for another 24 h before they were harvested by centrifugation. All following procedures were carried out at 4 °C. The cell pellet was resuspended in 5 ml buffer A (KH_2PO_4, pH 6.8, 5 mM dithiothreitol, 200 µM Pefabloc SC (Serva, Heidelberg)) and subjected to sonication for three times 45 seconds each. Cell debris was removed by centrifugation at 6,000 g for 15 min, the supernatant was collected and subjected to ultracentrifugation at 100,000 g for 1 h. The supernatant was discarded and the pellet representing the membrane fraction was washed twice with buffer B (Bis-Tris-propane, pH 7.6, 5 mM dithiothreitol, 200 µM Pefabloc SC). Finally, the membrane pellet was resuspended in 300 µl buffer B containing 20 % (v/v) glycerol.

The *in vitro* LACS enzyme assay was carried out in a volume of 100 µl as described previously [5]. The assay mixture contained 100 mM Bis-Tris-propane (pH 7.6), 10 mM $MgCl_2$, variable concentrations of ATP as indicated, 2.5 mM dithiothreitol, 0.5 mM CoA, 30 µM 1-[^{14}C] oleic acid (specific activity 55 mCi $mmol^{-1}$), and 4.5 ng E. coli membrane fraction as enzyme source. As negative control a membrane fraction of cells containing the empty vector was used. The assay was initiated by addition of oleic acid and incubated at room temperature for 10 min. The reaction was terminated by addition of 100 µl of 10% (v/v) acetic acid in isopropanol and extracted twice with 900 µl of hexane (previously saturated with 50% [v/v] isopropanol). To estimate the enzymatic activity 150 µl aliquots of the aqueous phase were analysed by liquid scintillation counting.

ATP and ADP quantification. To measure ATP and ADP levels, plant material was shock-frozen directly into liquid nitrogen, then homogenized to a fine powder in a liquid-nitrogen cooled ball mill (MM400; Retsch, Haan, Germany) and subsequently extracted using 300 µl 16 % trichloroacetic acid containing 5 mM EGTA for 50 mg of frozen plant powder as described previously [6]. Prior to analysis of ATP and ADP by high-performance liquid chromatography (HPLC), adenosine nucleotides were derivatised according to literature [7]. 20 µl 50 % chloracetaldehyde and 20 µl citrate buffer (pH 5.2) consisting of 62 mM citric acid and 76 mM potassium dihydrogen phosphate were mixed with 85 µl of plant extract and the samples were then heated at 80 °C for 15 min, cooled on ice for 1 min and centrifuged at 18,000 g for 4 min. 20 µL of supernatant were subsequently injected into the HPLC system consisting of a Rheos 2200 pump (Flux

Instruments, Basel, Switzerland), a HTC PAL autosampler (PAL System, Zwingen, Switzerland), an EC 250/4 Nucleodur 100-5 C18ec column with an EC 4/3 UNIVERSAL RP guard column (Macherey-Nagel, Düren, Germany), and a LaChrom Elite L-2485 fluorescence detector (Hitachi, Berkshire, England). For chromatographic separation mobile phase A contained 5.7 mM tetrabutylammonium hydrogen sulfate and 10 mM potassium dihydrogen phosphate (pH 5.4), and mobile phase B consisted of 90 % acetonitrile. With a flow rate of 800 µl min^{-1} there was a linear gradient from 100 % A / 0 % B to 86 % A / 14 % B in 18 min, continuing to 56 % A / 44 % B in 18 min, to 17.5 % A / 82.5 % B in 2.4 min, and finally to 0 % A / 100 % B in 1.2 min, staying at this concentration for 2.4 min. The wavelength for excitation was 280 nm and for emission 410 nm. For peak analysis, the software Xcalibur (Thermo Fisher Scientific, Waltham, USA) was used and amounts of ATP and ADP were quantified by comparison with standards.

References

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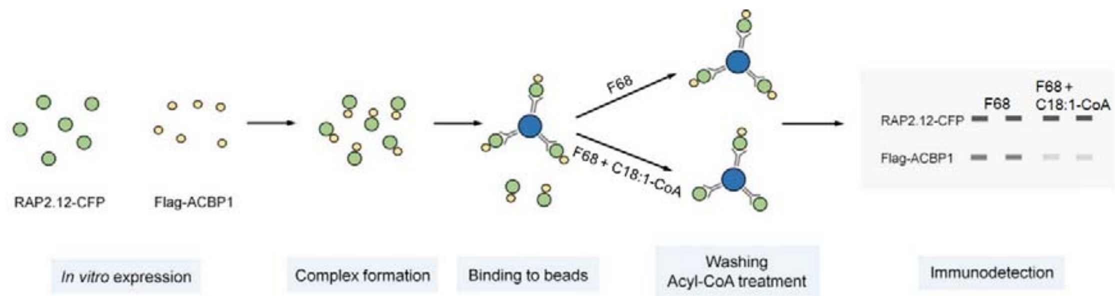

Fig. S1. C18:1-CoA causes dissociation of the RAP2.12-ACBP1 complex *in vitro*.
Schematic illustration of co-immunopurification of the ACBP1:RAP2.12 protein complex. Both proteins were produced using wheat germ extract. Subsequently, RAP2.12-CFP and Flag-ACBP1 were mixed and incubated to form a complex. Thereafter, complexes were immobilized on GFP-TRAP beads (represented here by the blue balls) via the CFP-tag of RAP2.12. Next, the beads were washed with different acyl-CoAs (0.1% acyl-CoA solved in 0.01% F68). Treatment with Pluronic F68 only served as control. After washing, the solution was removed from the vial and the proteins that remained being attached to the GFP-TRAP beads were subsequently eluted from the beads and loaded onto a SDS-page gel, followed by immunoblotting.

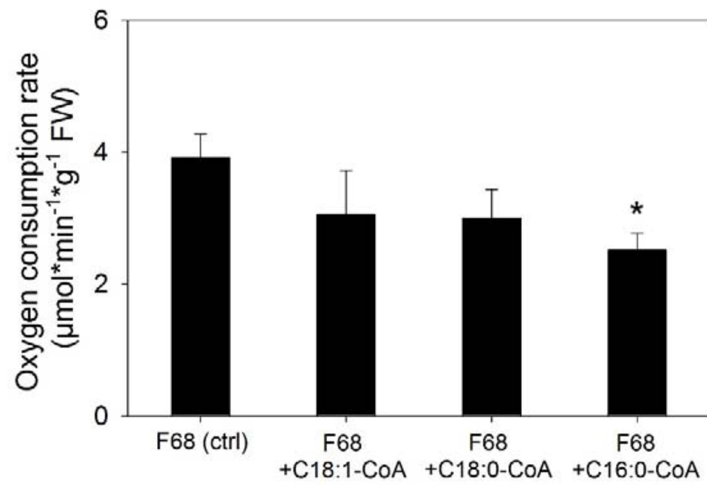

Fig. S2. No increase in oxygen consumption rates is observed after acyl-CoA treatment.
For the measurements, leaf discs pre-incubated with either 0.1% C18:1-CoA, C18:0-CoA or C16:0-CoA in air-saturated buffer (0.01% Pluronic F68) were kept in closed vials in which the oxygen concentration was measured through time. Pluronic F68 served as control. Oxygen consumption rates were calculated from the raw data. Data are mean ± s.d.; *$p < 0.05$, $n = 5$.

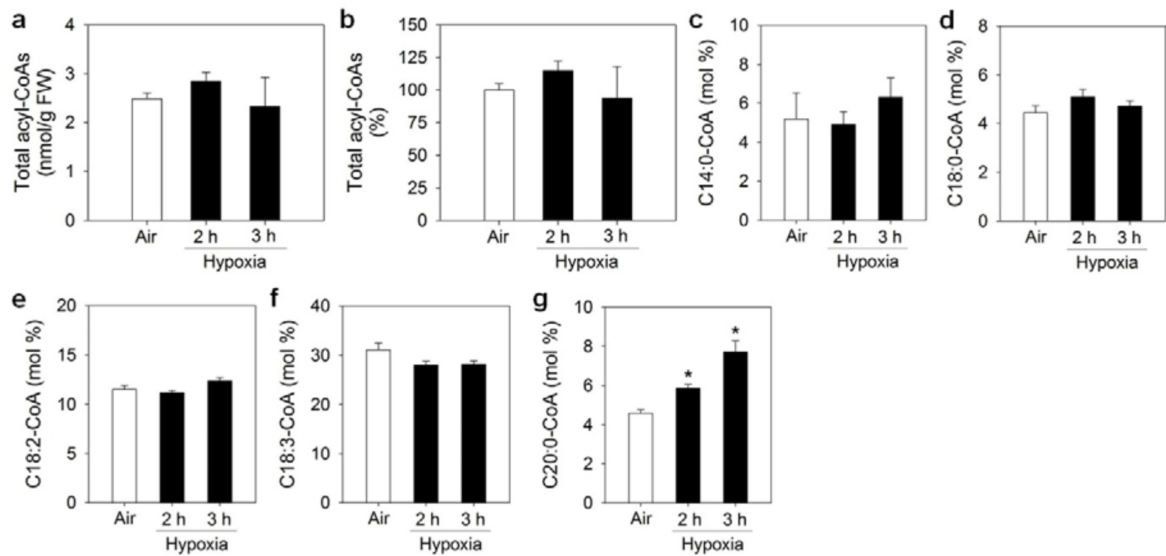

Fig. S3. The effect of hypoxia on acyl-CoA levels in wild-type plants.
a, Total concentration of acyl-CoAs in wild-type seedlings grown under long-day conditions and exposed to hypoxia. Data shown are given in nmol per gram fresh weight and represent the mean ± s.e. of independent replicates (n = 3). **b,** Relative amount of total acyl-CoAs in wild-type seedlings exposed to hypoxia as compared to control treatment in air. **c-g,** Relative levels of individual acyl-CoAs in wild-type seedlings exposed to hypoxia: **c,** C14:0-CoA, **d**, C18:0-CoA, **e**, C18:2-CoA, **f**, C18:3-CoA, and **g**, C20:0-CoA. Data represent the mean ± s.e. (n = 3). *$p < 0.05$. Relative levels of C18:1-CoA and C16:0-CoA are shown in Figure 3d,e.

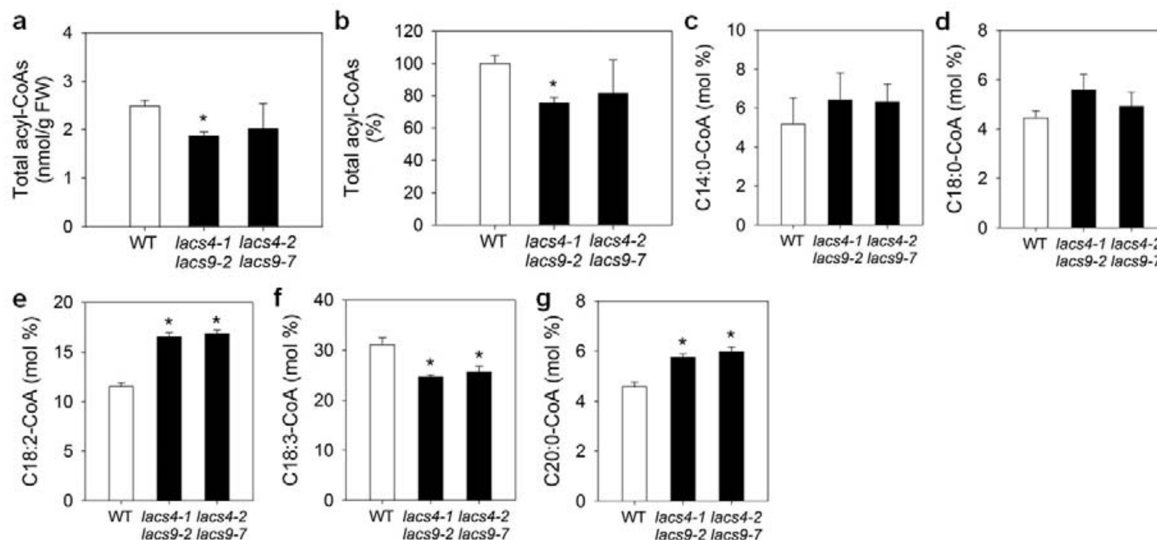

Fig. S4. Acyl-CoA levels in *lacs4 lacs9* knock-out lines under normoxic conditions.
a, Total concentration of acyl-CoAs in *lacs4 lacs9* seedlings grown in air under long-day conditions. Data shown are given in nmol per gram fresh weight and represent the mean ± s.e. of independent replicates (n = 3). **b,** Relative amount of total acyl-CoAs of *lacs4 lacs9* seedlings as compared to wildtype. **c-g,** Relative levels of individual acyl-CoAs in *lacs4 lacs9* seedlings as compared to wild-type seedlings, including **c**, C14:0-CoA, **d**, C18:0-CoA, **e**, C18:2-CoA, **f**, C18:3-CoA, and **g**, C20:0-CoA. Data represent the mean ± s.e. (n = 3). *$p < 0.05$. Relative levels of C18:1-CoA and C16:0-CoA can be found in Figure 3f,g.

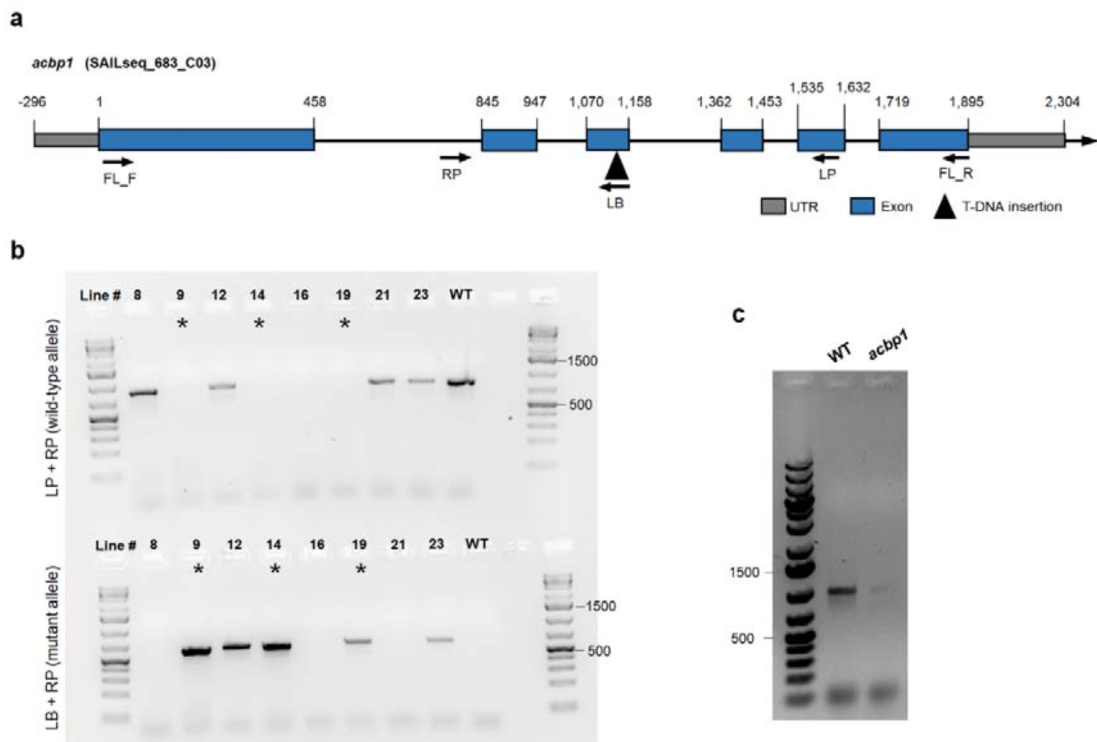

Figure S5. Identification of homozygous *acbp1* knock-out mutant. (a) Gene-structure of *ACBP1* highlighting location of the T-DNA insertion site within *acbp1* (SAILseq_683_C03). Positions of primers for detection of wild-type (LP + RP) and mutant allele (LB + RP) as well as those for the amplification of *ACBP1* from cDNA (FL_F and FL_R) are shown. (b) Identification of homozygous T-DNA insertion lines for *ACBP1* by PCR. Absence of wild-type allele (upper part) but presence of mutant allele (lower part) is shown for lines 9, 14 and 19 (marked with *). Genomic DNA of wild-type (WT) served as control. (c) Semi-quantitative PCR was used to determine the transcript level of *ACBP1* in wildtype and the identified *acbp1* mutant. Full-length *ACBP1* transcripts (1,017 bp) were only detected in wildtype (WT) but not in the *acbp1* mutant.

Table S1. Comparison of the mRNA levels of hypoxia-responsive genes in wild-type seedlings upon acyl-CoA treatment under aerobic conditions. Data (log_2FC) are means relative to the reference (WT, air) ± SEM for n=5 (Student´s t-test, bold numbers indicate $p < 0.05$).

Gene name	F68	SEM	F68 + C18:1-CoA	SEM	p value	F68 + C18:0-CoA	SEM	p value	F68 + C16:0-CoA	SEM	p value
PDC1	0	0.22	**1.53**	**0.32**	**0.00**	-0.55	0.67	0.39	1.15	0.70	0.10
HUP7	0	0.65	0.96	0.60	0.27	-0.34	0.71	0.70	0.86	0.82	0.31
HUP9	0	0.53	**1.96**	**0.58**	**0.03**	0.47	0.89	0.55	1.39	1.25	0.21
ADH1	0	1.00	1.00	0.82	0.34	0.76	1.07	0.48	0.73	1.47	0.60
HB1	0	0.61	**2.35**	**0.62**	**0.03**	**1.48**	**0.55**	**0.05**	**2.53**	**0.91**	**0.05**
HRA1	0	0.91	1.15	0.74	0.22	0.25	0.91	0.78	1.66	1.00	0.11
LBD41	0	0.71	**1.81**	**0.71**	**0.05**	0.08	0.76	0.92	1.61	0.86	0.09
SUS1	0	0.71	0.65	0.63	0.39	1.05	0.69	0.20	1.09	1.03	0.26
SUS4	0	0.44	0.45	0.58	0.44	0.33	0.83	0.65	0.79	0.92	0.33
PCO1	0	0.59	1.07	0.47	0.11	0.42	0.55	0.47	0.76	0.79	0.37

Table S2. Comparison of the mRNA levels of hypoxia-responsive genes in wild-type and *lacs4-1 lacs9-2* seedlings under aerobic conditions and after 2 h hypoxic treatment. Data (log_2FC) are means relative to the reference (WT, air) ± SEM for n=4 (Student´s t-test, bold numbers indicate $p < 0.05$).

Aerobic conditions

Gene name	WT	SEM	*lacs4-1 lacs9-2*	SEM	p value
PDC1	0	0.35	**1.27**	**0.71**	**0.04**
HUP7	0	0.51	**2.50**	**0.46**	**0.01**
HUP9	0	0.12	**1.80**	**0.19**	**0.00**
ADH1	0	0.35	**1.49**	**0.31**	**0.01**
HB1	0	0.13	**2.28**	**0.41**	**0.01**
HRA1	0	0.25	0.60	0.39	0.23
LBD41	0	0.27	-0.86	0.56	0.25
SUS1	0	0.08	-1.02	0.43	0.11
SUS4	0	0.14	-0.65	0.64	0.43
PCO1	0	0.33	0.32	0.24	0.31

Hypoxic conditions

Gene name	WT	SEM	*lacs4-1 lacs9-2*	SEM	p value
PDC1	7.78	0.45	8.53	1.38	0.20
HUP7	4.15	0.38	4.51	0.69	0.48
HUP9	12.16	0.29	12.87	0.61	0.32
ADH1	6.34	0.47	6.22	0.35	0.82
HB1	6.96	0.33	6.45	1.25	0.68
HRA1	7.33	0.36	6.84	0.75	0.36
LBD41	6.12	1.69	5.18	1.56	0.76
SUS1	2.19	0.44	1.39	1.05	0.33
SUS4	3.85	0.59	2.90	1.62	0.45
PCO1	3.84	0.59	4.38	0.19	0.45

Table S3. Comparison of the mRNA levels of hypoxia-responsive genes in wild-type seedlings upon antimycin-A (50 µM) and/or 1 mM dimethylthiourea (DMTU) treatment (3 hours) under aerobic conditions. Data (log_2FC) are means relative to the reference (WT, air) ± SEM for n=5 (Student´s t-test, bold numbers indicate $p < 0.05$).

	Aerobic conditions										
Gene name	**Control**	**SEM**	**DMTU**	**SEM**	**p value**	**Antimycin-A**	**SEM**	**p value**	**Antimycin-A + DMTU**	**SEM**	**p value**
PDC1	0	0.82	-0.26	0.60	0.68	**3.83**	**0.68**	**0.00**	**5.06**	**0.70**	**0.00**
HUP7	0	0.13	0.36	0.14	0.16	**1.15**	**0.18**	**0.01**	-0.21	0.33	0.65
HUP9	0	0.89	-0.06	0.76	0.13	0.15	0.89	0.88	-0.31	0.85	0.73
ADH1	0	0.60	-0.19	0.51	0.72	**1.73**	**0.45**	**0.01**	**1.85**	**0.78**	**0.05**
HB1	0	0.70	**1.37**	**0.53**	**0.04**	**1.74**	**0.67**	**0.05**	**1.35**	**0.57**	**0.05**
HRA1	0	0.70	0.75	0.51	0.18	-0.66	0.55	0.64	-0.28	0.57	0.64
LBD41	0	0.22	-0.21	1.00	0.33	**3.73**	**0.34**	**0.00**	**3.45**	**0.29**	**0.00**
SUS1	0	0.33	**-1.04**	**0.27**	**0.03**	0.05	0.26	0.88	0.14	0.28	0.63
SUS4	0	0.64	0.51	0.63	0.64	**1.45**	**0.49**	**0.05**	**1.83**	**0.52**	**0.01**
PCO1	0	0.71	-0.46	0.54	0.42	-0.40	0.57	0.51	-0.51	0.54	0.37

Table S4. Full list of primers used in this study. (F = forward primer, R = reverse primer)

Primer name	Sequence 5' → 3'	Task
d30ACBP1_flag_Flex_F	TTTGCGATCGCATGGATTACAAGGATGACGATGACAAGGCAGCCGGTATGTTTAAAGAC	cloning
d30ACBP1_Flex_R	AAAGTTTAAACCTAATTGGAATCCTTCTTC	cloning
RAPCFP_Flex_F	TTTGCGATCGCATGTGTGGAGGAGCTATAATA	cloning
RAPCFP_Flex_R	AAAGTTTAAACTTACTTGTACAGCTCGTCCATGC	cloning
ADH1_qF	TATTCGATGCAAAGCTGCTGTG	qPCR
ADH1_qR	CGAACTTCGTGTTTCTGCGGT	qPCR
HB1_qF	TTTGAGGTGGCAAGTATGCA	qPCR
HB1_qR	TGATCATAAGCCTGACCCCAA	qPCR
HRA1_qF	ACAACCACCGCAACAGAATCC	qPCR
HRA1_qR	TCTCCGCAATTCTCGCCAT	qPCR
HUP7_qF	ACCAATGTTGGCAACCCGCTTC	qPCR
HUP7_qR	TTTCCCTCAGCTCACGAACCTG	qPCR
HUP9_qF	TCATCGGCGGACATAGCAA	qPCR
HUP9_qR	ATCATCAACCACCCAACCTCC	qPCR
LBD41_qF	TGAAGCGCAAGCTAACGCA	qPCR
LBD41_qR	ATCCCAGGACGAAGGTGATTG	qPCR
PCO1_qF	TGCGGCGGTTGTTTAATACTTGC	qPCR
PCO1_qR	ATGGTCGGAGTTAAACCGACATCC	qPCR
PDC1_ qF	CGATTATGGCACTAACCGGATT	qPCR
PDC1_qR	TGTTCACCACCGCCTGATAAC	qPCR
SUS1_qF	ACGCTGAACGTATGATAACGCG	qPCR
SUS1_qR	AACCCGGAAAGCAAGGCAAG	qPCR
SUS4_qF	CGCAGAACGTGTAATAACGCG	qPCR
SUS4_qR	CAACCCTTGAGAGCAAAGCAAA	qPCR
Lacs1_qF	GCTTGTGCAGCTCACACTCTAATC	qPCR
Lacs1_qR	TGTAATCGACTGCTCCTGAACCC	qPCR
Lacs2_qF	CGCGTAACGACTGATTCTAAGGTC	qPCR
Lacs2_qR	CAATCCGAATTGCAGCGTCGTG	qPCR
Lacs3_qF	TGGGACCAATTCTTGAAGCTAGGC	qPCR
Lacs3_qR	TGGTGCAAACATCGCTTCTCCTC	qPCR
Lacs4_qF	AGTACAAGCTGTTGATTCCGTGTG	qPCR
Lacs4_qR	AGGATATGCTGGTTTGGGTTGGC	qPCR
Lacs6_qF	GGCAGCTTCAGAAGGCATTAAGGG	qPCR
Lacs6_qR	CAGCCTTTGCAAACTCGAAGCC	qPCR
Lacs7_qF	TGGAACCACAGGAACACCAAAGGG	qPCR
Lacs7_qR	ACCAGCGACATTCGCGATCAAG	qPCR
Lacs8_qF	TCTTGGGAAGAAGGTGGCTACAG	qPCR
Lacs8_qR	TGTGCCCTTCTCATCGACCTTG	qPCR
Lacs9_qF	AACTGTGCACGAAAGAGCAAGC	qPCR
Lacs9_qR	TCGTGATTGTTTAGCCGCCTTC	qPCR
UBI10_qF	AAGCAGTTGGAGGATGGCAGAAC	qPCR
UBI10_qR	CGGAGCCTGAGAACAAGATGAAGG	qPCR
Acbp1 LP	CTTCAGCAACGTTAAGATGGC	genotyping
Acbp1 RP	TAGGGATGCATTTTGATCGAC	genotyping
Acbp1 FL_F	ATGGCTGATTGGTATCAGCTTGC	Semi-quantitative PCR
Acbp1 FL_R	CGAGAGAAGAAGGATTCCAATTAG	Semi-quantitative PCR

Captions to Supplementary Datasets

Supplementary Dataset S1: Identification of differentially expressed genes after different acyl-Coa treatments (18:1-CoA; 18:0-CoA; 16:0-CoA) of seedlings. At day 14, seedlings were treated with different Acyl-CoAs (C18:0, C18:1 or C16:0) at a final concentration of 0.1% in 0.01% Pluronic F68. For control treatments plants were exposed to 0.01% Pluronic F68. After 90 minutes, whole seedlings were collected and immediately frozen in liquid nitrogen. Shown are the mean expression values (FC) based on 3 biological replicates between control and different acyl-CoA treatments. Differentially expressed genes ($FC \geq 2$ or ≤ -2 and FDR adj $p. \leq 0.05$) are presented, which are summarized in Figure 1f.

This table is provided as an Excel data file with the supplementary materials.

Supplementary Dataset S2: GO enrichment of genes responsive to acyl-CoA treatment. At day 14, seedlings were treated with different Acyl-CoAs (C18:0, C18:1 or C16:0) at a final concentration of 0.1% in 0.01% Pluronic F68. For control treatments plants were exposed to 0.01% Pluronic F68. After 90 minutes whole seedlings were collected and immediately frozen in liquid nitrogen. GO enrichment analysis was performed using the workbench in PLAZA3.0 (Proost et al., 2009). Shown are enriched biological processes (BP; $p \leq 0.05$) and their GO terms for each acyl-CoA treatment.

This table is provided as an Excel data file with the supplementary materials.