Supporting Information

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SI Materials and Methods

Isolation of Mitochondria. For enzyme assays, mitochondria were isolated as described previously (1). Briefly, ~20 g of whole rosette leaves was disrupted with a tissue grinder in 150 mL of cold extraction buffer [0.3 M sucrose, 5 mM tetrasodiumpyrophosphate (10 H_2O), 2 mM EDTA, 10 mM KH_2PO_4, 1% polyvinylpyrrolidone (PVP-40), 1% BSA, 20 mM ascorbic acid, 5 mM Cys (pH 7.5)]. The homogenate was filtered twice through four layers of Miracloth (CalBioChem). The material that was retained was recovered and ground in a cold stone mortar with extraction buffer. This step was repeated three times. The preparation was centrifuged first at 1,100 × *g* for 10 min to separate chloroplast (pellet) and mitochondrial (supernatant) fractions. The supernatant containing the mitochondria-enriched fraction was further centrifuged for 10 min at 18,000 × *g*. The pellet was resuspended in a small volume of washing buffer [0.3 M sucrose, 10 mM 3-(N-morpholino)propansulfonic acid (Mops), 1 mM EGTA (pH 7.2)] and homogenized with a Potter–Elvehjem homogenizer. An additional 15 mL of washing buffer was added, and the mixture was centrifuged again at 1,100 × *g* for 10 min. The supernatant was transferred to new tubes (40 mL), making sure that no pellet was transferred. The suspension was then centrifuged at 18,000 × *g* for 10 min. The obtained pellet was resuspended in 1 mL of washing buffer and loaded on top of a Percoll (GE Healthcare) step gradient. Each gradient consisted of mitochondria gradient buffer [1.5 M sucrose, 50 mM Mops (pH 7.2)] and a 0–4.4% (vol/vol) PVP gradient in 28% (vol/vol) Percoll (1). The gradient loaded with the sample was centrifuged at 40,000 × *g* for 45 min. The bottom part of the gradient containing the mitochondria was collected, washed three times, divided into aliquots, frozen, and stored at −80 °C. The purity of the mitochondria was assessed by determining the activity of marker enzymes phosphoenolpyruvate carboxylase (PEPc, cytosolic), catalase (peroxisome), and cytochrome *c* oxidase (COX, mitochondrial), as well as by measuring the chlorophyll content (2). The protocol for mitochondria isolation leads to a highly concentrated mitochondrial extract, as evidenced by the higher COX activity compared with whole leaves with low contamination of cytosol, peroxisome, and chloroplast, as evidenced by the activity of the marker enzymes PEPc (cytosolic marker) and catalase (peroxisome marker) and by the chlorophyll content (chloroplastidic marker) (Table S5).

Enzyme Assays. Enzymes were extracted as described previously (3), except that Triton X-100 was used at a concentration of 1% and glycerol at 20% (vol/vol). The enzyme assays were performed as follows: ACL (4), NADP-MDH (5), CS and ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco) (6), PEPc, IDH, FUM, AGPase and NADP-GAPDH (3), NAD-MDH (7), oxoglutarate dehydrogenase (8), ACO (9), SDH (10), SCoAL (11), COX (12), and catalase (13). Activity was expressed by FW or by protein content determined using the method of Bradford (14). For immunoblotting, total leaf protein was extracted as described previously (3). Proteins were separated on a 10% (vol/vol) SDS/PAGE gel and electrotransferred onto a PVDF membrane. Equal loading was validated using Ponceau red staining of the membrane.

Metabolite Assays. Leaf samples were taken at end of the day, immediately frozen in liquid nitrogen, and stored at −80 °C until further analysis. Sugars, starch, and nitrate contents were determined as previously described (15). Metabolite extraction for GC-MS was also performed as reported previously (16). Briefly, *Arabidopsis* leaf tissue (~100 mg) was homogenized using a ball-mill precooled with liquid nitrogen and extracted in 1,400 μL of methanol. Sixty microliters of internal standard (0.2 mg of ribitol per milliliter of water) was subsequently added as a quantification standard. The extraction, derivatization, standard addition, and sample injection were exactly as described previously (16). Metabolites were identified by comparison with database entries of authentic standards (17, 18) and are presented in Datasets S2 and S3 following current reporting standards (19). Profiling of secondary metabolites was performed as described previously (20). Obtained data were normalized with the peak area of internal standard (isovitexin). All data were processed using Xcalibur 2.1 software (Thermo Fisher Scientific).

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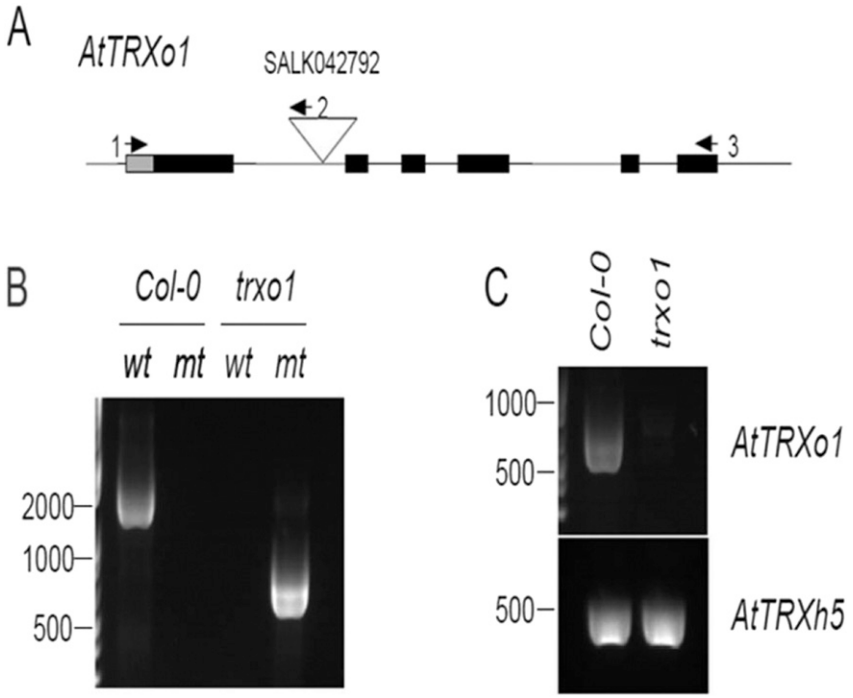

Fig. S1. Genetic mapping of the *trxo1* insertion line. (*A*) T-DNA insertion within the *trxo1* (At2g35010, SALK042792) line was mapped by PCR. The oligonucleotides used are represented with arrows and numbers. Intergenic regions and introns are shown with black lines, and exons are shown with boxes. A gray box represents the putative mitochondrial transit peptide. (*B*) Genotyping of the *trxo1* homozygous plants. WT copy (wt) was amplified using primers 1 (CTCGAGTGATGAAGGGAAATTGGTCG) and 3 (CAACACGTTCTTTACTAGAACGG). The mutant copy (mt) was amplified using primers 1 and 2 (TGGTTCACGTAGTGGGCCATCG). (*C*) *AtTRXo1* mRNAs in the WT (Col-0) and *trxo1* homozygote mutant line. RT-PCR assays (40 cycles) were performed using AtTRXo1 gene-specific oligonucleotides. The AtTRXh5 gene was used as a positive PCR control.

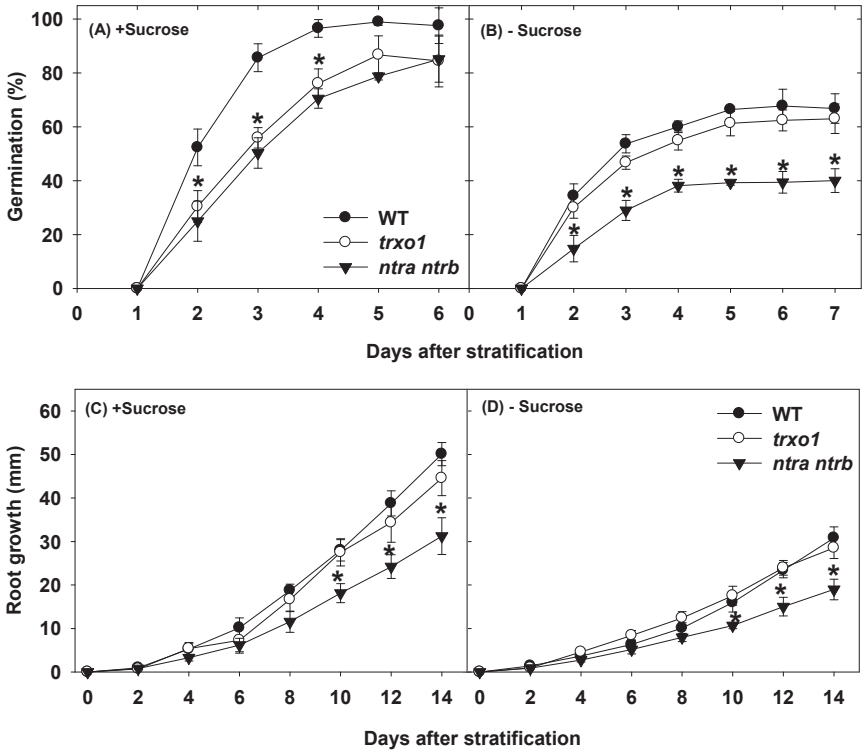

Fig. S2. Effect of TRX inactivation on seed germination of *Arabidopsis thaliana* plants. Radicle emergence was used as the criterion of seed germination. Germination tests were performed in a minimum of triplicate experiments using at least 100 seeds for an individual assay. The average germination percentage ± SEM of triplicate experiments is shown. Asterisks indicate values significantly different from WT by the Student's *t* test (*$P < 0.05$).

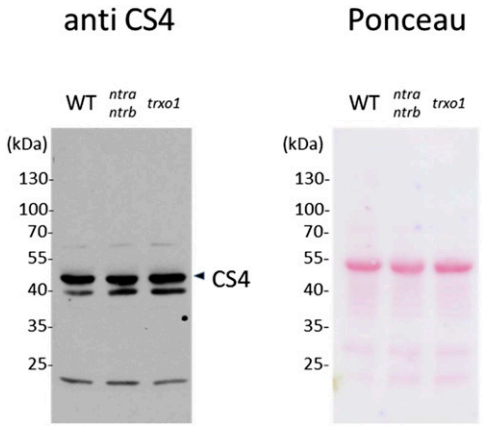

Fig. S3. Abundance of CS isoform 4 (CS4) protein. Total proteins were extracted from leaves of *A. thaliana* WT, *ntra ntrb* double mutant, and *trxo1* mutant; separated by 1D SDS/PAGE; and analyzed by Western blot using an anti-CS4 antibody (~50 kDa).

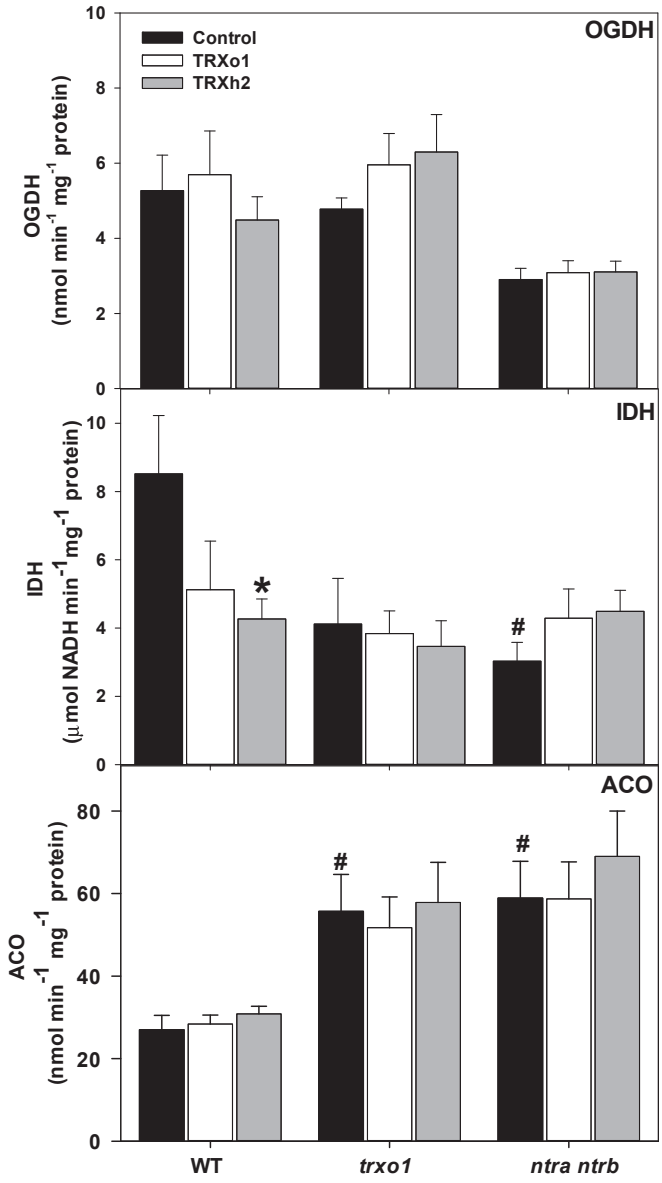

Fig. S4. Enzyme activities and protein-based complementation in WT, *trxo1*, and *ntra ntrb* mutants. Oxoglutarate dehydrogenase (OGDH), NAD-IDH, and ACO activities were measured in mitochondrial extracts of WT, *trxo1*, and *ntra ntrb* mutants. These extracts were also used to perform protein-based complementation assays. The extracts were untreated (Control) or treated with TRXo1 (3 μg; 180 nM) or TRXh2 (3 μg; 210 nM), both reduced by NTRB (7.5 μg; 100 nM) and NADPH (100 μM). Data presented are mean ± SEM ($n = 5$). A number symbol indicates values significantly different from WT by Student's *t* test at 5% ($^{\#}P < 0.05$) and an asterisk indicates values significantly different from the control in the same genotype by the Student's *t* test at 5% ($^{*}P < 0.05$).

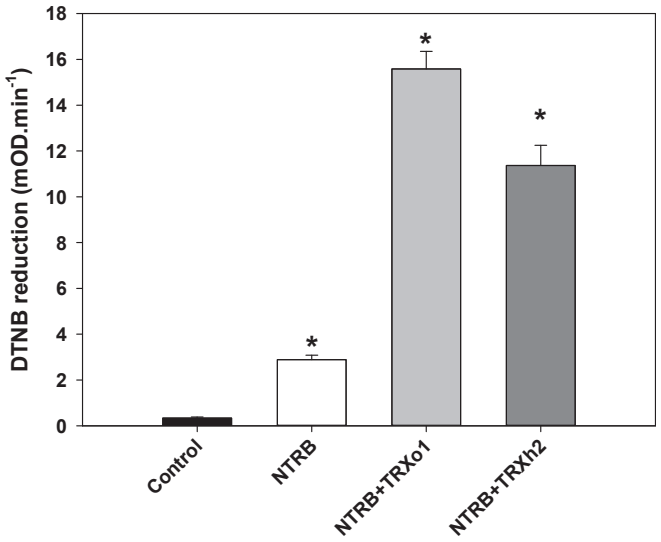

Fig. S5. Activity of the TRX system determined with the DTNB reduction assay. TRXo1 (3 μg; 180 nM) and TRXh2 (3 μg; 210 nM) were reduced with NTRB (7.5 μg; 100 nM) and NADPH (100 μM) at room temperature and then incubated with DTNB (130 μM). The rate of DTNB reduction was measured at 412 nm. Control values indicate the assay performed without NTRB (Control) or without TRX (NTRB). mOD, mili optical density. Asterisks indicate values significantly different from control by the Student's *t* test ($P < 0.05$).

Table S1. Growth phenotype of WT, *trxo1*, and *ntra ntrb* mutants

Age	WT	*trxo1*	*ntra ntrb*
2-wk-old	120 ± 14	**44 ± 2**	**28 ± 0.1**
4-wk-old	729 ± 41	**604 ± 48**	**484 ± 60**
6-wk-old	2,553 ± 328	**3,384 ± 284**	2,746 ± 29
8-wk-old	3,638 ± 264	3,743 ± 570	3,564 ± 63

The shoot FW (mg) was determined in plants grown in a growth chamber as described in *Material and Methods*. Similar results were observed in at least three independent experiments. Data presented are mean ± SEM ($n = 5$). Values set in bold and underlined type were determined by the Student's *t* test to be significantly different ($P < 0.05$) from the WT.

Table S2. Enzyme activities in whole-leaf extract of WT, *trxo1* mutant, and *ntra ntrb* double mutant

Enzymes	WT	*trxo1*	*ntra ntrb*
RUBISCO	0.45 ± 0.0	0.45 ± 0.0	0.46 ± 0.0
NADP-MDH total	1.85 ± 0.0	1.91 ± 0.1	2.00 ± 0.1
AGPase	1.26 ± 0.0	**1.15 ± 0.0**	1.25 ± 0.0
NADP-GAPDH	0.17 ± 0.0	**0.11 ± 0.0**	0.16 ± 0.0

Activities ($nmol \cdot min^{-1} \cdot g^{-1} \cdot FW^{-1}$) were determined in leaf material harvested at the end of the day from 4-wk-old plants before bolting. Rubisco, ribulose-1,5-biphosphate carboxylase oxygenase. Data presented are mean ± SE ($n = 6$). Values set in bold and underlined type were determined by the Student's *t* test to be significantly different ($P < 0.05$) from the WT.

Table S3. Content of metabolites in WT, *trxo1* mutant, and *ntra ntrb* double mutant

Metabolite	WT	*trxo1*	*ntra ntrb*
GC-MS			
Val	1.00 ± 0.1	0.86 ± 0.1	1.26 ± 0.3
Ile	1.00 ± 0.1	1.15 ± 0.2	1.23 ± 0.1
Fumarate	1.00 ± 0.1	0.99 ± 0.1	1.03 ± 0.1
Urea	1.00 ± 0.2	0.77 ± 0.1	1.14 ± 0.3
Spermidine	1.00 ± 0.1	1.01 ± 0.1	1.15 ± 0.2
Glycolic acid	1.00 ± 0.1	0.83 ± 0.0	1.00 ± 0.1
Glyceric acid	1.00 ± 0.1	0.83 ± 0.1	0.98 ± 0.0
Raffinose	1.00 ± 0.2	0.65 ± 0.1	1.12 ± 0.2
Sucrose	1.00 ± 0.1	0.89 ± 0.0	1.05 ± 0.0
Gal	1.00 ± 0.2	0.96 ± 0.2	0.96 ± 0.1
Talose	1.00 ± 0.1	0.89 ± 0.2	0.88 ± 0.1
Trehalose	1.00 ± 0.2	1.06 ± 0.1	1.80 ± 0.2
Myoinositol	1.00 ± 0.2	0.76 ± 0.1	1.43 ± 0.1
LC-MS			
4 MTBG	1.00 ± 0.3	0.79 ± 0.1	0.32 ± 0.1
3 MSPG	1.00 ± 0.2	0.63 ± 0.0	0.64 ± 0.3
4 MSBG	1.00 ± 0.1	0.53 ± 0.2	0.63 ± 0.3
5 MSPG	1.00 ± 0.1	0.51 ± 0.2	0.78 ± 0.2
7 MSHG	1.00 ± 0.2	0.61 ± 0.2	1.26 ± 0.3
8 MSOG	1.00 ± 0.2	0.66 ± 0.1	1.60 ± 0.5
IndoleGLS 1	1.00 ± 0.3	0.36 ± 0.2	0.75 ± 0.3
IndoleGLS 2	1.00 ± 0.8	0.34 ± 0.3	0.43 ± 0.8
Sinapoyl Glu	1.00 ± 0.1	0.21 ± 0.2	0.72 ± 0.2
Sinapoyl Mal	1.00 ± 0.0	0.68 ± 0.2	0.68 ± 0.1
Kaempferol 1	1.00 ± 0.1	0.90 ± 0.0	1.17 ± 0.0
Kaempferol 2	1.00 ± 0.1	1.13 ± 0.1	1.36 ± 0.0
Kaempferol 3	1.00 ± 0.0	0.91 ± 0.1	0.92 ± 0.1
Anthocyanin	1.00 ± 0.7	0.35 ± 0.6	2.19 ± 0.3

Metabolites were determined at end of the day as described in *Materials and Methods*. The GC-MS data were normalized with the values obtained for ribitol (internal standard) and the FW used for the extraction (~100 mg). The liquid chromatography (LC)-MS data were normalized with the peak area of internal standard (isovitexin) and FW used for the analysis. The data presented are normalized with respect to the mean response calculated for WT plants. Data presented are mean ± SEM ($n = 6$). Values in underlined type indicate values significantly different from WT by the Student's *t* test at 5% ($P < 0.05$). Glucosinolates: 3 MSPG, 3-methylsulfinylpropyl glucosinolate; 4 MSBG, 4-methylsulfinylbutyl glucosinolate; 5 MSPG, 5-methylsulfinylpentyl glucosinolate; 7 MSHG, 7-methylsulfinylheptyl glucosinolate; 8 MSOG, 8-methylsulfinyloctyl glucosinolate; 4 MTBG, 4-methylthiobutyl glucosinolate. Indole glucosinolates: IndoleGLS 1, 1-methoxy-3-indolylmethyl glucosinolate, 1-methoxyindol-3-ylmethylglucosinolate; IndoleGLS 2, indole-3-methylglucosinolate. Phenylpropanoids: Sinapoyl Mal, Sinapoyl (S)-malate; Sinapoyl Glu, Sinapoyl glucoside. Flavonoids: Kaempferol 1, kaempferol 3-O-rhamnoside 7-O-rhamnoside; Kaempferol 2, kaempferol 3-O-glucoside 7-O-rhamnoside; Kaempferol 3, kaempferol 3-O-[2′′-O-(rhamnosyl) glucoside] 7-O-rhamnoside; Anthocyanin, cyanidin 3-O-[2′′-O-(6′′′-O-(sinapoyl) xylosyl) 6′′-O-(p-O-(glucosyl)-p-coumaroyl) glucoside] 5-O-(6′′′′-O-malonyl) glucoside.

Table S4. Redistribution of the total ^{13}C-enrichment in selected metabolites after 4 h of ^{13}C- labeling in leaves of WT, *trxo1* mutant, and *ntra ntrb* double-mutant *A. thaliana* plants

Metabolite	WT	*trxo1*	*ntra ntrb*
^{13}C-Glc			
Pyruvate	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.0
Pro	0.05 ± 0.0	**0.02 ± 0.0**	**0.01 ± 0.0**
Gly	0.19 ± 0.1	0.15 ± 0.0	0.10 ± 0.0
Fumarate	0.49 ± 0.1	0.37 ± 0.1	0.48 ± 0.1
Ser	0.05 ± 0.0	0.03 ± 0.0	0.04 ± 0.0
Ala	0.007 ± 0.0	0.007 ± 0.0	**0.014 ± 0.0**
Malate	0.001 ± 0.0	**0.002 ± 0.0**	**0.004 ± 0.0**
Asp	0.01 ± 0.0	0.01 ± 0.0	**0.001 ± 0.0**
Glu	0.10 ± 0.0	0.12 ± 0.0	0.11 ± 0.0
Succinate	0.002 ± 0.0	0.003 ± 0.0	**0.003 ± 0.0**
Fructose	0.36 ± 0.2	0.36 ± 0.1	0.53 ± 0.1
Sucrose	5.75 ± 0.7	4.88 ± 0.4	5.20 ± 0.6
Raffinose	0.45 ± 0.1	0.18 ± 0.1	**0.05 ± 0.0**
^{13}C-malate			
Pyruvate	0.004 ± 0.0	0.004 ± 0.0	0.01 ± 0.0
Pro	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.0
Fumarate	0.62 ± 0.1	0.58 ± 0.1	0.57 ± 0.1
Ala	0.002 ± 0.0	0.001 ± 0.0	**0.01 ± 0.0**
Malate	0.06 ± 0.0	0.05 ± 0.0	0.07 ± 0.0
Asp	0.05 ± 0.0	0.04 ± 0.0	**0.001 ± 0.0**
Glu	0.12 ± 0.0	0.08 ± 0.0	0.09 ± 0.0
Succinate	0.002 ± 0.0	0.002 ± 0.0	**0.004 ± 0.0**
^{13}C-pyruvate			
Pyruvate	0.19 ± 0.1	0.20 ± 0.1	0.08 ± 0.0
Val	0.92 ± 0.1	1.19 ± 0.1	**1.55 ± 0.1**
Pro	0.08 ± 0.0	0.12 ± 0.0	0.11 ± 0.0
Gly	0.18 ± 0.1	0.22 ± 0.1	0.06 ± 0.0
Fumarate	0.44 ± 0.2	0.60 ± 0.1	0.59 ± 0.1
Ser	0.06 ± 0.0	**0.01 ± 0.0**	**0.02 ± 0.0**
Ala	0.04 ± 0.0	0.11 ± 0.0	**0.15 ± 0.0**
Malate	0.003 ± 0.0	0.004 ± 0.0	0.003 ± 0.0
Asp	0.01 ± 0.0	0.02 ± 0.0	**0.001 ± 0.0**
Glu	0.48 ± 0.1	0.74 ± 0.2	0.57 ± 0.1
Succinate	0.02 ± 0.0	0.03 ± 0.0	0.02 ± 0.0
Sucrose	1.29 ± 0.2	1.57 ± 0.2	1.20 ± 0.3

Fully expanded leaves of 6-wk-old plants were harvested in the middle of the light period and fed via the petiole with [U-^{13}C]Glc, [U-^{13}C]pyruvate, or [U-^{13}C]malate solution. Data presented are mean ± SEM ($n = 6$). Values (μmol of fractional ^{13}C enrichment g^{-1}·FW^{-1}·h^{-1}) in bold and underline type were determined by the Student's *t* test to be significantly different from the WT ($P < 0.05$).

Table S5. Enzyme activities and chlorophyll content in leaf extract and mitochondria isolated

Markers	Leaf	Mitochondria
PEPc	1,238 ± 79	**196 ± 25**
Catalase	32 ± 2.0	**1.5 ± 0.7**
COX	120 ± 12	**1,011 ± 80**
Chlorophyll	717 ± 8.3	**82 ± 2.0**

Catalase (μmol of H_2O_2 per min^{-1}·mg^{-1} of protein), COX (nmol·min^{-1}·mg^{-1} of protein), phosphoenolpyruvate carboxylase (PEPc; nmol·min^{-1}·mg^{-1} of protein) and total chlorophyll content (mg·mg^{-1} of protein) were used as marker for peroxisome, mitochondria, cytosol, and chloroplast, respectively. These subcellular markers were measured in WT, *trxo1* mutant, and *ntra ntrb* double mutant ($n = 5$). The results are the average of the genotypes. Values in bold and underlined type were determined by the Student's *t* test to be significantly different from leaf ($P < 0.05$).

Other Supporting Information Files

Dataset S1 (PPT)
Dataset S2 (XLS)
Dataset S3 (XLS)