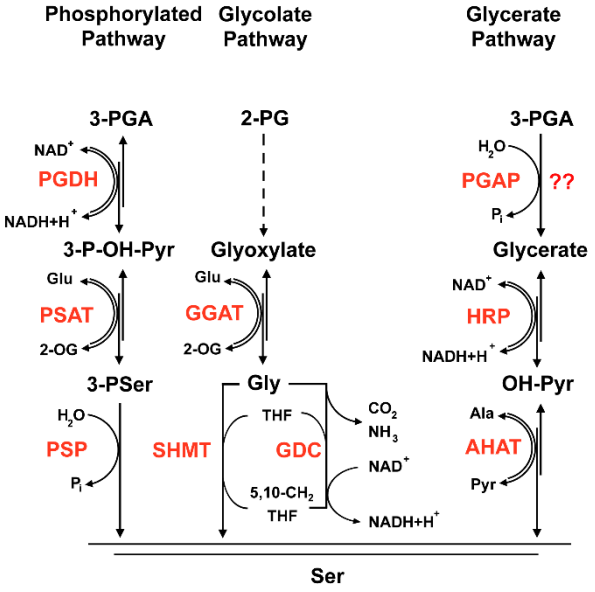
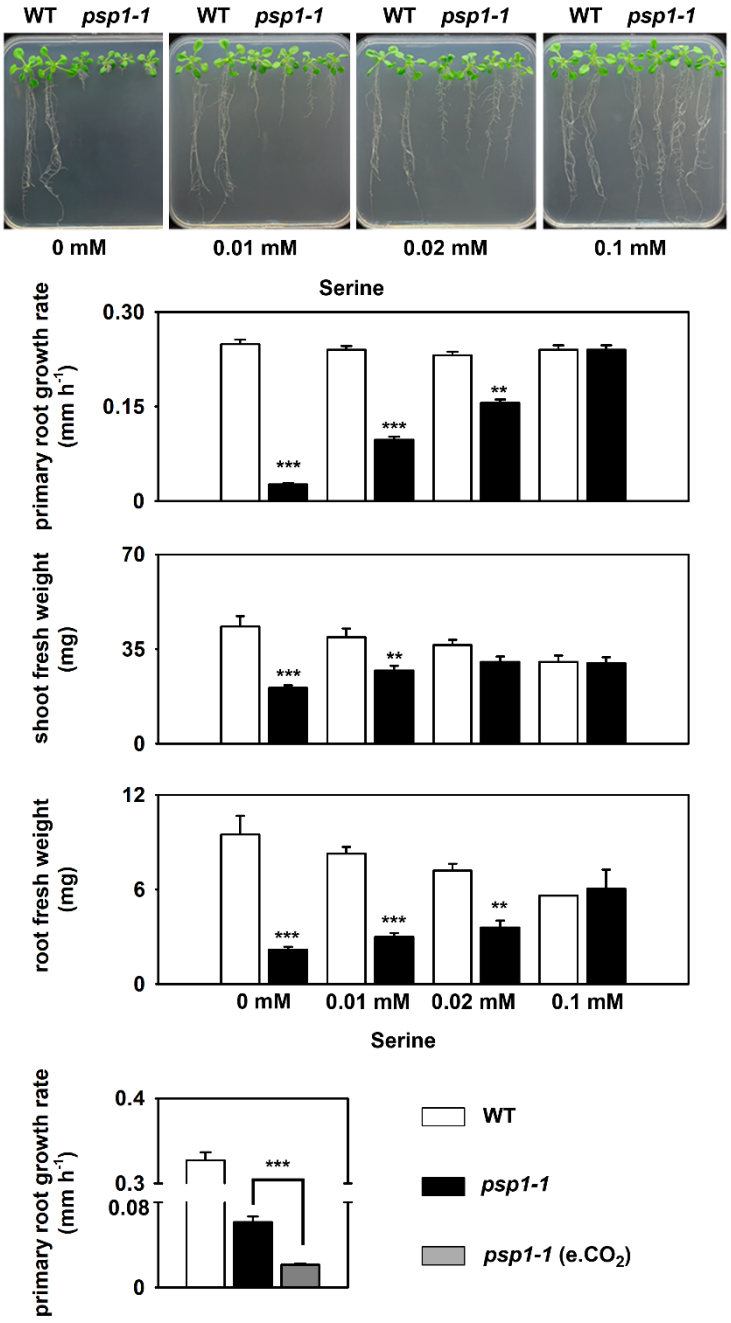
Supplemental Figures

Supplemental Figure S1



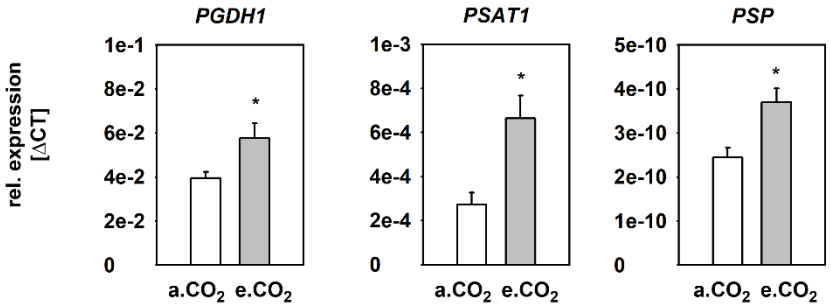
Supplemental Figure S1. Pathways for serine biosynthesis in plants. In theory, three serine biosynthesis pathways exist in plants: the phosphorylated pathway, the glycolate pathway and the glycerate pathway. The phosphorylated pathway is catalyzed by the three enzymes, 3-phosphoglycerate dehydrogenase (PGDH), 3-phosphoserine aminotransferase (PSAT), and 3-phosphoserine phosphatase (PSP), and starts from 3-phosphoglycerate (3-PGA) generated by plastidial glycolysis (Ros et al., 2014). The glycolate pathway is part of photorespiration and therefore starts from 2-phosphoglycolate (2-PG). The reactions directly involved in photorespiratory serine biosynthesis are catalyzed by glutamate-glyoxylate aminotransferase (GGAT), glycine decarboxylase (GDC) and serine hydroxymethyltransferase (SHMT). The glycerate pathway functions in reverse orientation to photorespiration and is carried out by 3-phosphoglycerate phosphatase (PGAP), hydroxypyruvate dehydrogenase (HRP) and alanine-hydroxypyruvate aminotransferase (AHAT). Abbreviations: pyruvate (Pyr), 3-phosphohydroxypyruvate (3-P-OH-Pyr), 3-phosphoserine (3-PSer), tetrahydrofolate (THF), 5,10-methylenetetrahydrofolate (5,10-CH2-THF), carbon dioxide (CO_2), ammonia (NH_3), inorganic phosphate (P_i,), nicotinamide dinucleotide (NAD(H)), 2-oxoglutarate (2-OG). Red font indicates enzymes. Solid lines indicate one reaction and dashed lines indicate multiple reactions.

Supplemental Figure S2



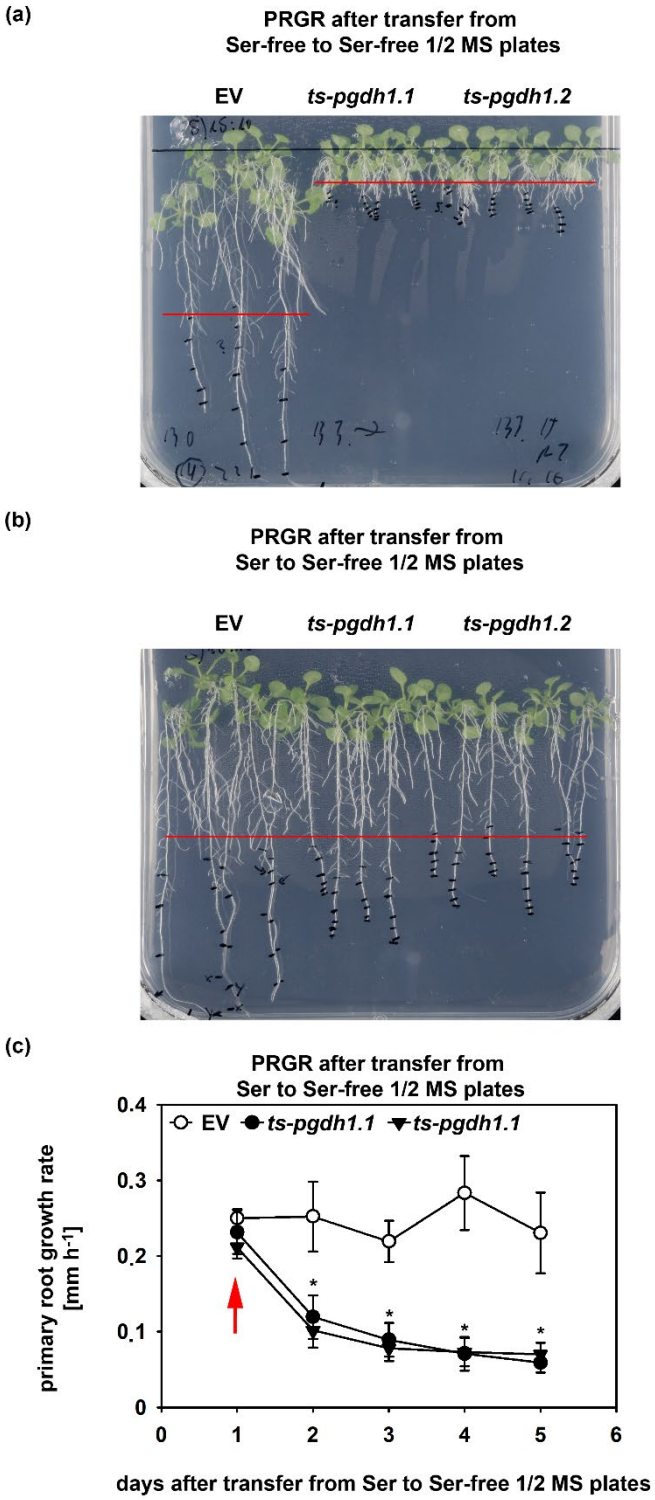
Supplemental Figure S2. Growth of *psp1-1* mutants. Growth phenotype of *psp1-1* mutants and wild type (WT) plants on growth medium supplemented with 0, 0.01, 0.02 and 0.1mM serine or at elevated (4000 µl l^{-1}) CO_2 (e.CO_2). Data presented are means ± SE of n = 20. Asterisks indicate significant differences based on Student's t test (** $P < 0.01$; *** $P < 0.001$).

Supplemental Figure S3



Supplemental Figure S3. Expression analysis of PPSB genes at ambient and elevated CO_2. Expression analysis of PPSB genes in empty vector (EV) control plants grown at ambient or elevated CO_2. Data presented are means ± SE of n = 5. Asterisks indicate significantly different values by the Student's t test (* $P < 0.05$).

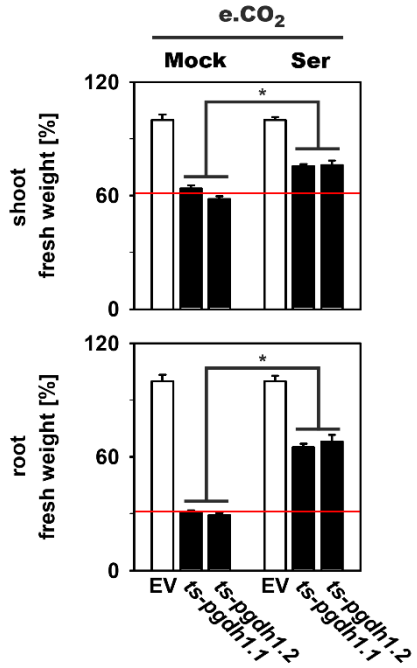
Supplemental Figure S4.



Supplemental Figure S4. *PGDH1*-silenced lines require continuous supply of external serine to maintain normal plant growth. *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) and empty vector (EV) control plants were germinated and pre-cultivated on half-strength (1/2) MS medium without *(a)* or with *(b)* supplementation of 0.1mM serine. After ten days plants were transferred to serine-free half-strength MS medium and the primary root growth rate (PRGR) was determined further. Red lines indicate the average root length of plants before transfer to serine-free half-strength MS medium. *(c)* Data presented are means ± SE of n > 10. Asterisks indicate significant differences based on Student's t test (* $P < 0.05$). Red arrow

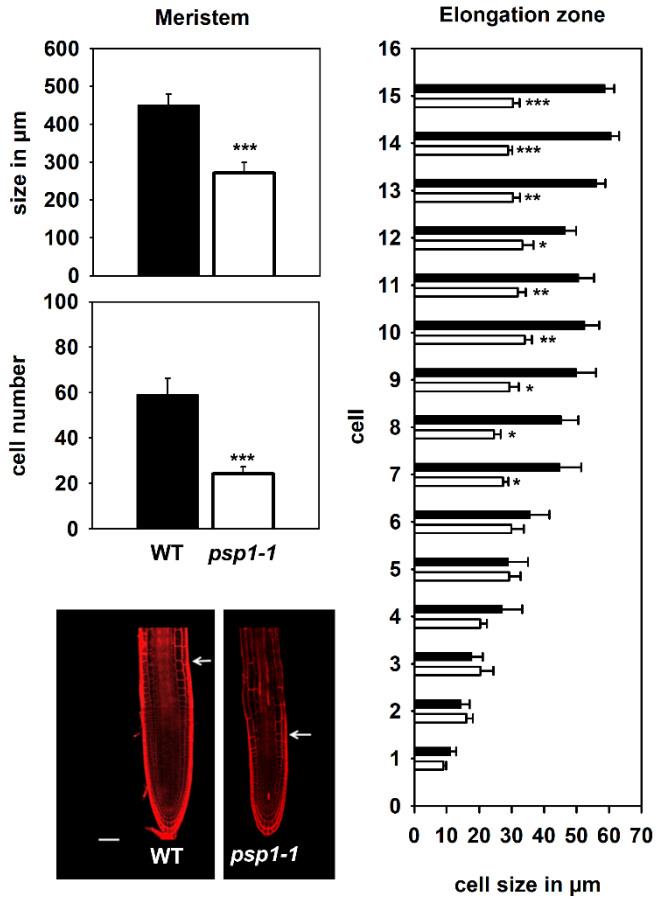
indicates the average root length of plants before transferred to serine-free half-strength MS medium.

Supplemental Figure S5



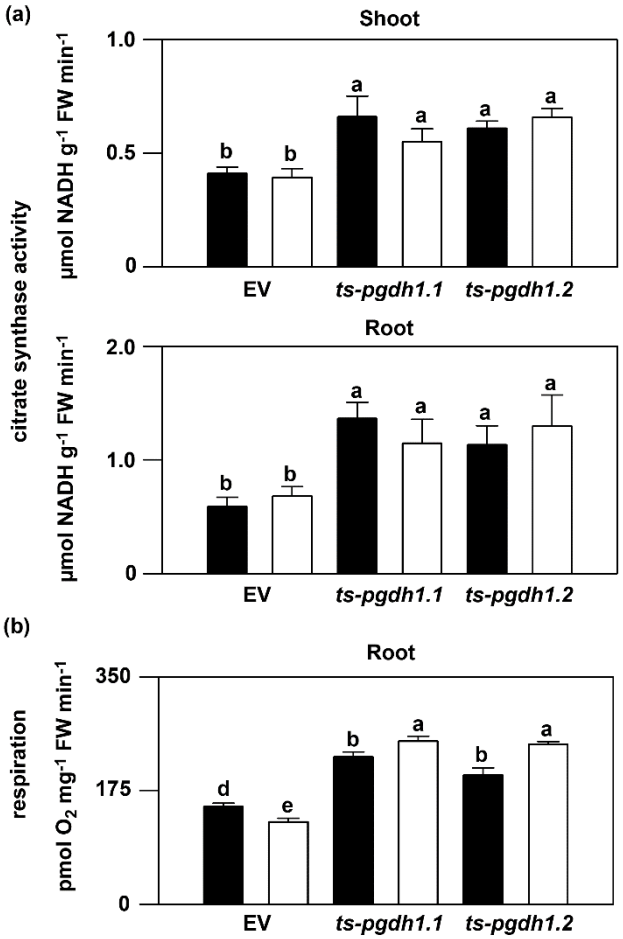
Supplemental Figure S5. Growth of *PGDH1*-silenced lines at elevated CO_2 is significantly improved by external serine. Shoot and root biomasses of empty vector (EV) control plants and *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) were determined at e.CO_2 (elevated CO_2, 4000 µl l^{-1}) and e.CO_2 supplemented with 0.1 mM serine conditions. Therefore, plants were germinated and pre-cultivated on half-strength MS medium at ambient CO_2 for eight days before transfer to vertical plates containing either half-strength MS medium (Mock) or half-strength MS medium supplemented with 0.1mM serine (Ser), and incubated for additional eight days at elevated CO_2. Relative fresh weight as percent of EV control plants was calculated. Data presented are means ± SE of n > 5. Asterisks indicate significant differences based on Student's t test (* $P < 0.05$). Red lines indicate the average shoot or root fresh weight of *PGDH1*-silenced lines grown on Mock.

Supplemental Figure S6



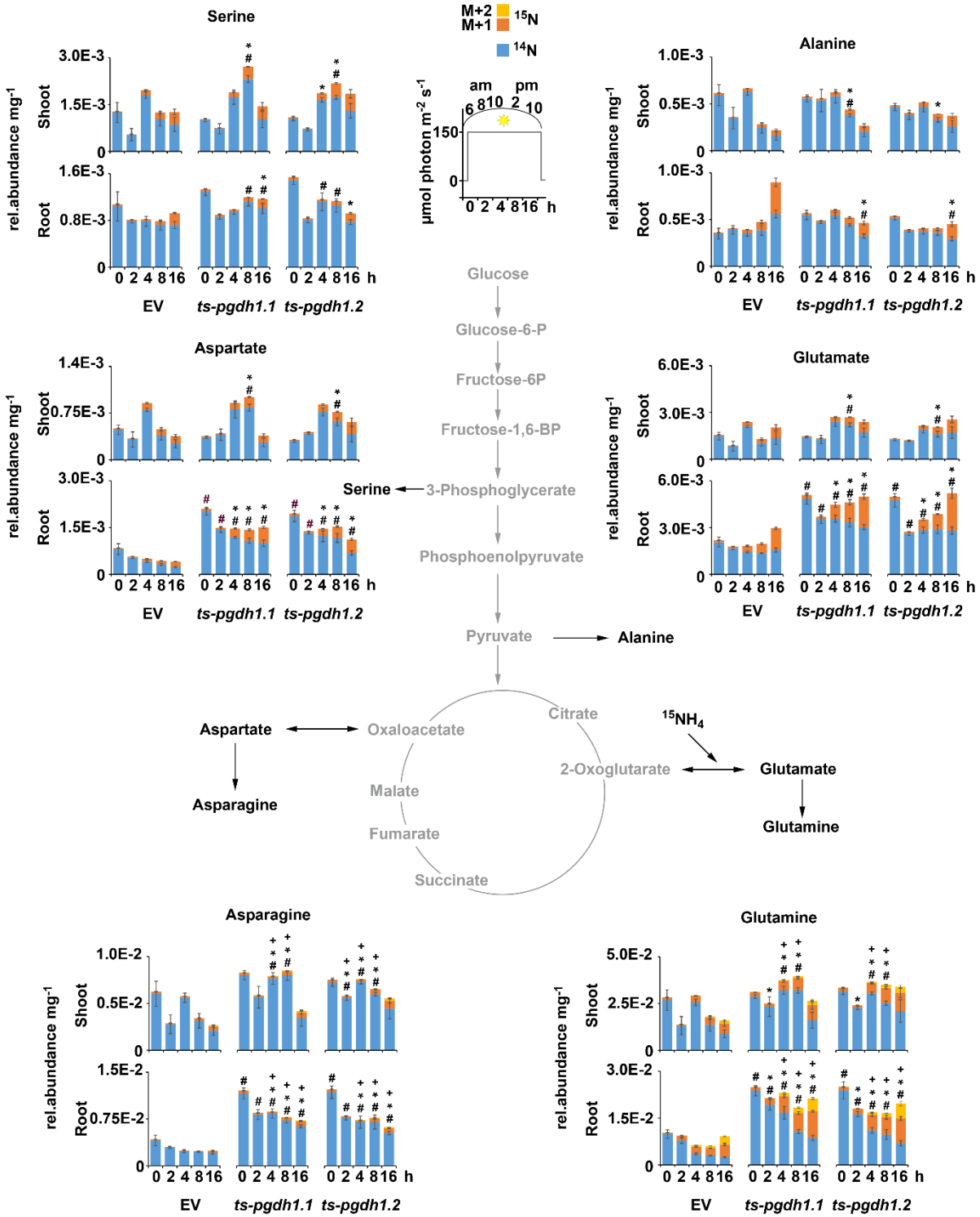
Supplemental Figure S6. Size of the meristem and elongation zone in wild type and *psp1-1* mutant plants. Quantification of size and cell number of the meristem and sizes of the first fifteen cells of the elongation zone of WT (black bars) and *psp1-1* mutant (white bars) plants and visualization of roots by propidium iodide staining. White arrows indicate the beginning of the elongation zone in epidermal cell layers. Data presented are means ± SE. Asterisks indicate significant differences based on Student's t test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Scale bar: 50 µm.

Supplemental Figure S7



Supplemental Figure S7. Citrate synthase activity in *PGDH1*-silenced lines. The activity of citrate synthase was analyzed in shoot and root tissue of empty vector (EV) control plants and *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) grown at ambient CO_2 (a.CO_2, 400 µl l^{-1}; black bars) or elevated CO_2 (e.CO_2, 4000 µl l^{-1}; white bars). *(b)* The oxygen consumption in pmol O_2 mg^{-1} min^{-1} is shown for roots of empty vector (EV) control plants and *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) grown at ambient CO_2 (a.CO_2, 400 µl l^{-1}; black bars) or elevated CO_2 (e.CO_2, 4000 µl l^{-1}; white bars). For *(a)* and *(b)*, data presented are means ± SD of n = 5; one-way ANOVA followed by Fisher's least significant difference test ($P < 0.05$) were performed and columns with the same letter are not significantly different.

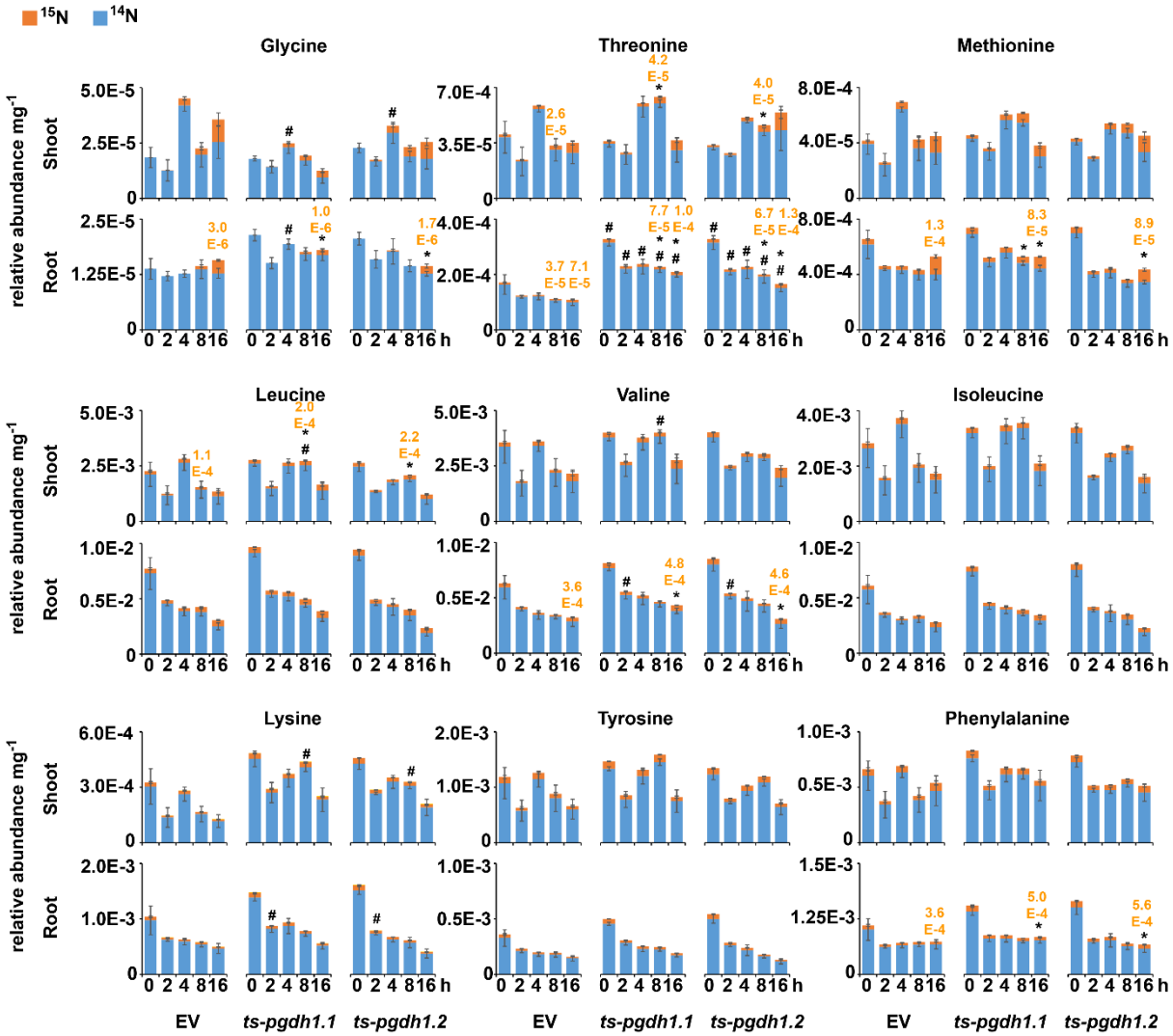
Supplemental Figure S8



Supplemental Figure S8. Quantification of ^{15}N-labeled amino acids in *PGDH1*-silenced lines. ^{14}N- (blue) and ^{15}N-isotopes (M+1 orange; M+2 yellow) of major amino acids are shown in shoot and root tissue of empty vector (EV) control plants and *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) in a 16 hour time course (see inset). Data presented as relative (rel.) abundance per mg fresh weight (FW) are means ± SE of n = 5. Sharp (M0), asterisk (M+1) and

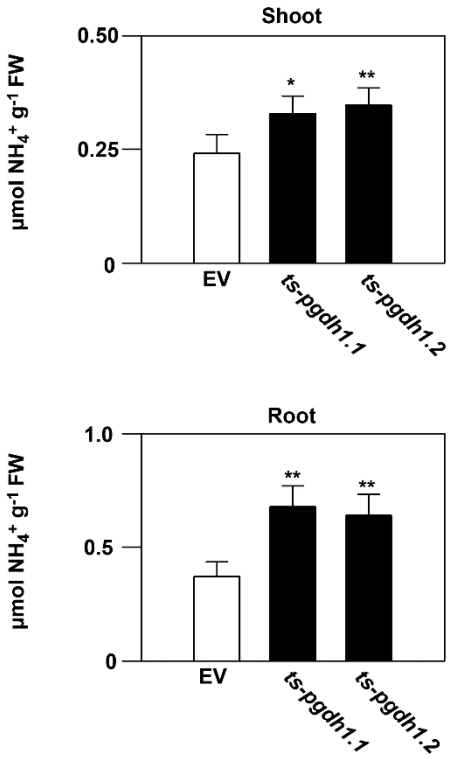
cross (M+2) indicate significantly different values between EV and *PGDH1*-silenced lines by the Student's t test (#, *, + $P < 0.05$).

Supplemental Figure S9



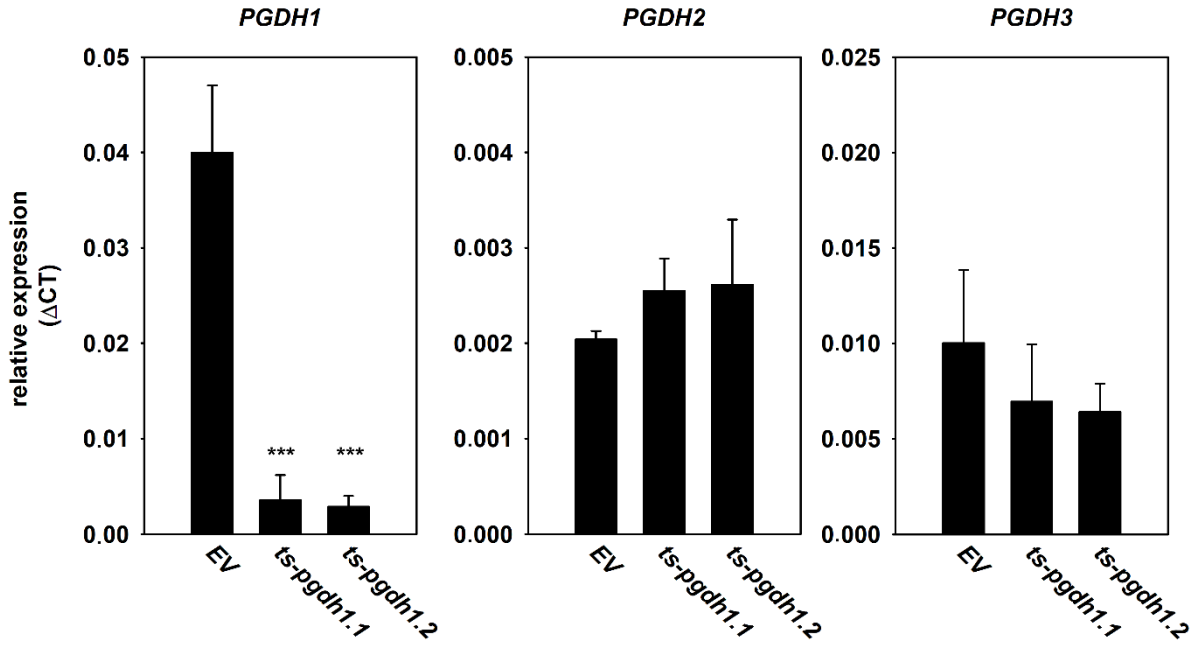
Supplemental Figure S9. Quantification of ^{15}N-labeled amino acids in *PGDH1*-silenced plants. ^{14}N- (blue) and ^{15}N- isotopes (M+1; orange) of minor abundant amino acids are shown for shoot and root tissues of empty vector (EV) control plants and *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) in a 16 hour time course. Data presented are means ± SE of n = 5. Orange Numbers are significantly different values for M+1 between EV and *PGDH1*-silenced lines. Sharp (M0) and asterisk (M+1) indicate significantly different values between EV and *PGDH1*-silenced lines by the Student's t test (#, * $P < 0.05$).

Supplemental Figure S10.



Supplemental Figure S10. Changes in the ammonium content in *PGDH1*-silenced lines. The ammonium content was analyzed in shoot and root tissues of empty vector (EV) control plants and *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*). Data presented are means ± SD of n = 5. Asterisks indicate significantly different values between EV and *PGDH1*-silenced lines by the Student's t test (* $P < 0.05$; ** $P < 0.01$).

Supplemental Figure S11



Supplemental Figure S11. Expression analysis of *PGDH* genes in *PGDH1*-silenced lines. Expression of the *PGDH* genes was quantified by RT-qPCR in *PGDH1*-silenced lines (*ts-pgdh1.1* and *ts-pgdh1.2*) and empty vector (EV) control plants. Data presented are means ± SE of n = 5. Asterisks indicate significant differences between *PGDH1*-silenced lines and EV plants based on Student's t test (***$P < 0.001$). For detailed characterization of *PGDH1*-silenced plants please see Benstein et al. (2013).

Supplemental Table S1

Lab.ID	AGI	Gene	Enzyme	Orientation	Sequence
859	At4g34200	*PGDH1*	3-phosphoglycerate dehydrogenase	FWD	gacactgaagaagattgggg
739	At4g34200	*PGDH1*	3-phosphoglycerate dehydrogenase	REV	ccggtgagatttaagattcaag
743	At4g35630	*PSAT1*	phosphoserine aminotransferase	FWD	ggagaagtctgaattggaagc
744	At4g35630	*PSAT1*	phosphoserine aminotransferase	REV	gcaaaaacccttaaaactaagcatgc
410	At1g18640	*PSP*	phosphoserine phosphatase	FWD	caagagacccccaaggctat
411	At1g18640	*PSP*	phosphoserine phosphatase	REV	tgccaagaatcgatgctaca
1562	AT5G04140	*GLU1*	glutamate synthase	FWD	gggaatctcattgctttcaagt
1563	AT5G04140	*GLU1*	glutamate synthase	REV	tgacacacttccagtgaatgc
1600	AT2G41220	*GLU2*	glutamate synthase	FWD	tccgtggtagtgcatctcaa
1601	AT2G41220	*GLU2*	glutamate synthase	REV	aacgtctccctagccagctc
1602	AT5G53460	*GLT1*	glutamate synthase	FWD	tgttggtctgcctcatgatt
1603	AT5G53460	*GLT1*	glutamate synthase	REV	ccagcagaaggaagtacaaagc
1729	AT3G03910	*GDH3*	glutamate dehydrogenase	FWD	acttcgagtgggttcagaacat
1730	AT3G03910	*GDH3*	glutamate dehydrogenase	REV	tcacatgagtgcgtctgaca
1731	AT5G07440	*GDH2*	glutamate dehydrogenase	FWD	ggacgcaactggaagtctca
1732	AT5G07440	*GDH2*	glutamate dehydrogenase	REV	accaagagcgcatggaatga
1733	AT5G18170	*GDH1*	glutamate dehydrogenase	FWD	atggatactcgcctgcagtt
1734	AT5G18170	*GDH1*	glutamate dehydrogenase	REV	tgccctgatatggtctttccg
1592	AT5G37600	*GLN1,1*	glutamine synthetase	FWD	ccactgacaaaatcattgctg
1593	AT5G37600	*GLN1,1*	glutamine synthetase	REV	tcactggtccaggtagagtcct
1594	AT3G17820	*GLN1,3*	glutamine synthetase	FWD	ggccctcagggaccttacta
1595	AT3G17820	*GLN1,3*	glutamine synthetase	REV	tccacaatgtcacgaccaat
1596	AT5G35630	*GLN2*	glutamine synthetase	FWD	gtggaggcaataacatcttgg
1597	AT5G35630	*GLN2*	glutamine synthetase	REV	tgtttgttggaattggctca
1723	AT1G48470	*GLN1;5*	glutamine synthetase	FWD	tagcaccgatcaagctgccg
1724	AT1G48470	*GLN1;5*	glutamine synthetase	REV	gatctccggccggtctgtaa
1725	AT5G16570	*GLN1;4*	glutamine synthetase	FWD	ggtctgggttgctcgttaca
1726	AT5G16570	*GLN1;4*	glutamine synthetase	REV	cccctgcaccattccaatct
2004	AT1G66200	*gln1.2*	glutamine synthetase	FWD	gtctcacgggacaccatgaa
2005	AT1G66200	*gln1.2*	glutamine synthetase	REV	caagggttccagaggagtgt
2006	AT1G64780	*AMT1;2*	ammonium transporter	FWD	tgggtgggtgacggtaacta
2007	AT1G64780	*AMT1;2*	ammonium transporter	REV	tcttccagcgaaatgacccc
1590	AT1G37130	*NIA2*	nitrate reductase	FWD	ctttggtagacgccgaactc
1591	AT1G37130	*NIA2*	nitrate reductase	REV	tttagctcgttgattatgtactctg
1560	AT1G77760	*NIA1*	nitrate reductase	FWD	aagccgtacacattaaaaggcta
1561	AT1G77760	*NIA1*	nitrate reductase	REV	tcacctcaaccctcgttacc
1566	AT1G08090	*NRT2.1*	nitrate transporter	FWD	tggaaatcgagctaccttgg
1567	AT1G08090	*NRT2.1*	nitrate transporter	REV	gtaacggcataccacagaatctt

Supplemental Table S1. Oligonucleotide sequences.