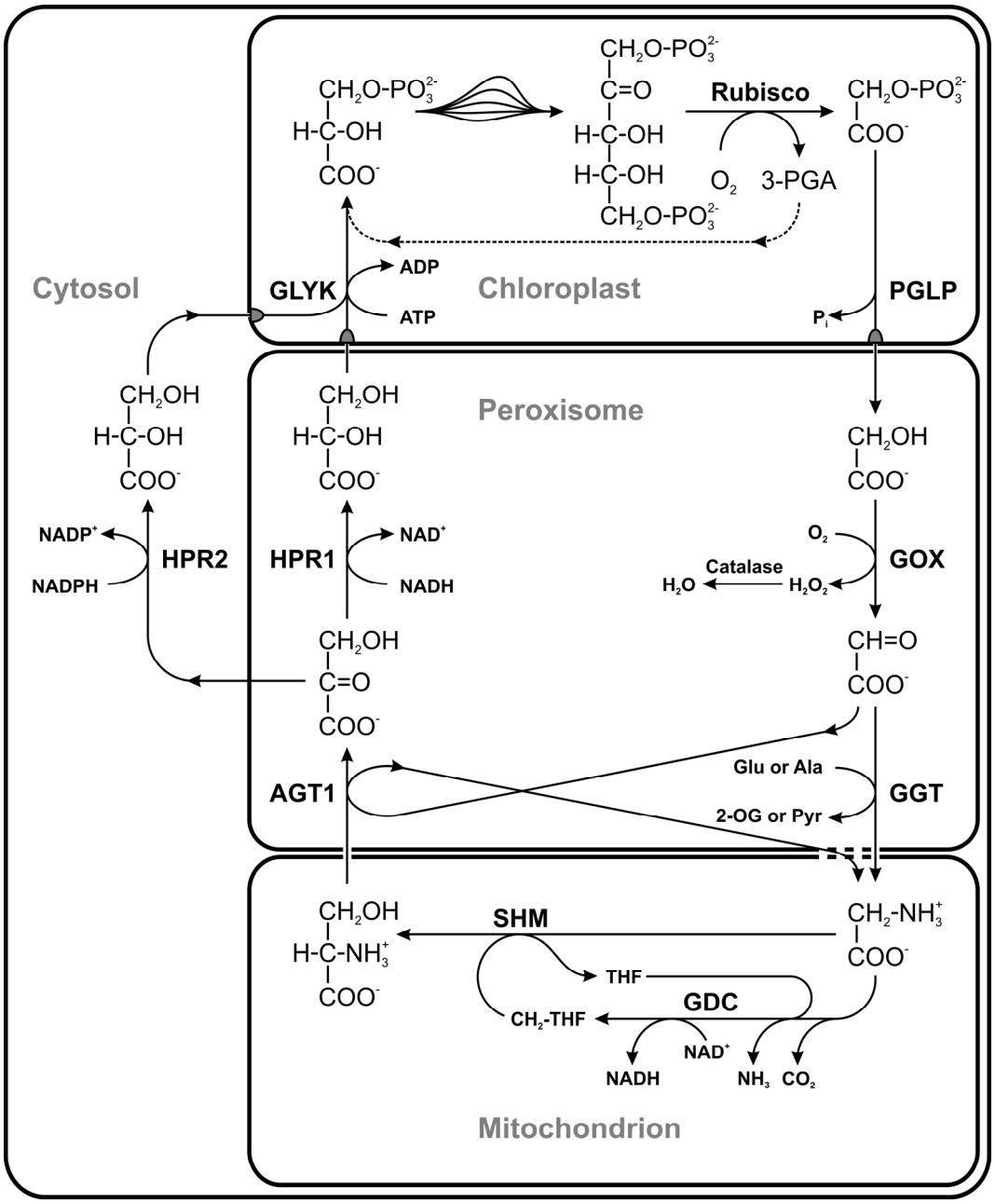

Supplemental Figure 1. The Photorespiratory Cycle Spans Four Cellular Compartments Including the Cytosol.

Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; PGLP, 2-phosphoglycolate phosphatase; GOX, glycolate oxidase; GGT, glutamate:glyoxylate aminotransferase; GDC, glycine decarboxylase; SHM, serine hydroxymethyltransferase; AGT1, serine(alanine):glyoxylate aminotransferase; HPR1, peroxisomal NADH-dependent hydroxypyruvate reductase; HPR2, cytosolic NAD(P)H-dependent hydroxypyruvate reductase; GLYK, D-glycerate kinase. Further contributing enzymes, such as malate dehydrogenase or those involved in ammonia reassimilation, are not shown.

Supplemental Table 1. True Photosynthesis and Decarboxylation Rates of Metabolite Pools With Different Turnover Half-Times Measured at 21% Oxygen.

The data represent mean values ± SD from three independent experiments with different plants grown for 10 weeks with an 8-h photoperiod.

	Col-0	*hpr1-1*	Col-0	*hpr1-1*
	µmol CO_2 m^{-2} s^{-1}		% of true photosynthesis	
True photosynthesis	6.84 ± 0.26	6.53 ± 0.23		
Decarboxylation of primary and stored photosynthates in the light				
total	1.66 ± 0.06	1.71 ± 0.14	24.2 ± 0.9	26.1 ± 1.4
photorespiratory	1.30 ± 0.08	1.16 ± 0.04	18.9 ± 1.2	17.8 ± 0.4
respiratory	0.36 ± 0.04	0.55 ± 0.11	5.3 ± 0.6	8.3 ± 1.4
Decarboxylation of primary photosynthates				
total	1.17 ± 0.06	1.05 ± 0.04	17.1 ± 0.9	16.1 ± 0.3
photorespiratory	0.91 ± 0.08	0.73 ± 0.06	13.3 ± 1.1	11.2 ± 1.3
respiratory	0.26 ± 0.02	0.32 ± 0.08	3.8 ± 0.3	4.9 ± 1.1
Decarboxylation of stored photosynthates				
total	0.49 ± 0.05	0.66 ± 0.12	7.1 ± 0.7	10.0 ± 1.6
photorespiratory	0.39 ± 0.03	0.43 ± 0.10	5.6 ± 0.4	6.6 ± 1.5
respiratory	0.10 ± 0.04	0.23 ± 0.03	1.5 ± 0.5	3.4 ± 0.3
Respiration in the dark	0.99 ± 0.05	1.43 ± 0.05	14.5 ± 0.7	22.0 ± 0.9

Supplemental Table 2. Purification Scheme for the Isolation of Arabidopsis HPR2 from *hpr1* Knockout Plants.

	Total activity µmol min^{-1}	Specific activity µmol min^{-1} mg^{-1}	Purification -fold
Crude extract	1524	0.1	1
Ammonium sulphate (45-75%)	27.72	0.7	7
DEAE-cellulose	8.51	1.15	11
Q-Sepharose blue	4.27	1.94	19
2',5'-ADP Sepharose	0.44	not determined	463

Supplemental Table 3. Alteration of the Leaf Content of Selected Metabolites in the Individual *hpr1-1* and *hpr2-1* Knockout Plants and the *hpr1-1 hpr2-1* Double Mutant.

Plants were grown in normal air and in air with 1% CO_2, respectively. Mutant-to-wild type ratios of mean relative metabolite contents ± SD (n = 6) are shown. Rosette leaves of six individual plants were analyzed per line. Rows in grey contain data used for Figure 8.

Leaf Metabolite Content Relative To the Wild-Type Plants								
Metabolite	*Ambient Air*				*1% CO_2*			
	Col-0	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*	*Col-0*	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*
Aconitate	1.00 ± 0.20	1.64 ± 0.24	1.46 ± 0.14	2.24 ± 0.15	1.00 ± 0.46	0.34 ± 0.07	1.05 ± 0.21	0.42 ± 0.11
Alanine	1.00 ± 0.11	0.49 ± 0.05	0.78 ± 0.26	0.64 ± 0.10	1.00 ± 0.09	0.57 ± 0.08	0.80 ± 0.08	0.39 ± 0.02
Arginine	1.00 ± 0.23	5.55 ± 0.86	1.34 ± 0.32	16.30 ± 8.05	1.00 ± 0.39	1.46 ± 0.68	0.45 ± 0.18	1.26 ± 0.52
Asparagine	1.00 ± 0.18	2.96 ± 0.54	0.93 ± 0.04	14.07 ± 7.47	1.00 ± 0.28	0.77 ± 0.13	0.69 ± 0.08	0.56 ± 0.12
Aspartate	1.00 ± 0.23	1.82 ± 0.29	2.23 ± 0.64	2.34 ± 0.35	1.00 ± 0.18	0.80 ± 0.10	0.54 ± 0.11	0.92 ± 0.18
Citramalic acid	1.00 ± 0.23	1.35 ± 0.25	1.68 ± 0.65	2.48 ± 0.33	1.00 ± 0.16	0.74 ± 0.16	0.85 ± 0.15	0.88 ± 0.09
Citrate	1.00 ± 0.22	0.63 ± 0.15	0.87 ± 0.10	1.48 ± 0.15	1.00 ± 0.17	0.84 ± 0.32	0.74 ± 0.18	1.38 ± 0.78
Cysteine	1.00 ± 0.11	0.58 ± 0.07	1.30 ± 0.16	0.81 ± 0.12	1.00 ± 0.21	1.05 ± 0.15	0.87 ± 0.10	0.90 ± 0.22
Ethanolamine	1.00 ± 0.08	1.66 ± 0.17	1.11 ± 0.11	3.01 ± 0.72	1.00 ± 0.10	1.92 ± 0.17	0.78 ± 0.08	1.65 ± 0.33

	Col-0	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*	*Col-0*	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*
Fructose	1.00 ± 0.11	0.48 ± 0.03	0.85 ± 0.16	0.51 ± 0.04	1.00 ± 0.15	4.89 ± 4.22	1.06 ± 0.25	0.84 ± 0.09
Fumarate	1.00 ± 0.21	0.98 ± 0.16	1.16 ± 0.13	1.19 ± 0.12	1.00 ± 0.11	0.79 ± 0.17	0.76 ± 0.21	0.34 ± 0.11
Galactose	1.00 ± 0.30	3.14 ± 1.62	9.97 ± 5.27	3.91 ± 2.67	1.00 ± 0.57	0.53 ± 0.32	0.14 ± 0.07	1.51 ± 0.83
GABA	1.00 ± 0.26	1.17 ± 0.15	1.13 ± 0.30	3.15 ± 1.11	1.00 ± 0.19	1.26 ± 0.48	1.22 ± 0.11	1.10 ± 0.22
Glucose	1.00 ± 0.39	0.25 ± 0.06	0.92 ± 0.18	0.18 ± 0.07	1.00 ± 0.45	1.70 ± 1.33	0.28 ± 0.11	2.49 ± 0.85
Gluconic acid	1.00 ± 0.14	0.78 ± 0.08	1.05 ± 0.07	0.83 ± 0.12	1.00 ± 0.12	0.99 ± 0.09	0.86 ± 0.06	0.95 ± 0.13
Glutamine	1.00 ± 0.24	2.17 ± 0.60	2.07 ± 0.41	10.67 ± 4.90	1.00 ± 0.34	1.03 ± 0.34	0.97 ± 0.19	0.53 ± 0.24
Glycerate	1.00 ± 0.14	2.77 ± 0.34	0.88 ± 0.14	3.30 ± 0.65	1.00 ± 0.13	0.69 ± 0.11	0.51 ± 0.06	0.79 ± 0.15
Glycerate-3-P	1.00 ± 0.18	1.51 ± 0.20	1.21 ± 0.11	2.01 ± 0.50	1.00 ± 0.10	1.02 ± 0.13	0.82 ± 0.18	1.04 ± 0.16
Glycerol	1.00 ± 0.22	0.89 ± 0.08	0.94 ± 0.08	1.00 ± 0.08	1.00 ± 0.11	0.79 ± 0.05	1.01 ± 0.12	0.74 ± 0.09
Glycine	1.00 ± 0.38	6.93 ± 1.29	0.97 ± 0.37	4.31 ± 0.93	1.00 ± 0.12	1.35 ± 0.81	0.32 ± 0.06	1.02 ± 0.26
Glycolate	1.00 ± 0.12	3.25 ± 0.39	1.23 ± 0.18	3.59 ± 0.42	1.00 ± 0.14	0.92 ± 0.10	1.34 ± 0.43	1.44 ± 0.17
Homocysteine	1.00 ± 0.09	0.64 ± 0.06	1.54 ± 0.33	0.88 ± 0.29	1.00 ± 0.19	1.09 ± 0.12	0.81 ± 0.20	0.96 ± 0.13
Homoserine	1.00 ± 0.43	0.80 ± 0.17	0.14 ± 0.03	0.76 ± 0.19	1.00 ± 0.18	0.88 ± 0.14	0.45 ± 0.05	0.96 ± 0.18
Hydroxylamine	1.00 ± 0.12	1.17 ± 0.06	0.84 ± 0.04	1.14 ± 0.07	1.00 ± 0.10	0.98 ± 0.12	0.88 ± 0.09	0.81 ± 0.07
Hydroxybenzoic acid	1.00 ± 0.19	1.22 ± 0.12	1.69 ± 0.16	1.72 ± 0.14	1.00 ± 0.11	0.83 ± 0.10	0.85 ± 0.11	0.93 ± 0.32

	Col-0	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*	*Col-0*	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*
Hydroxypyruvate	1.00 ± 0.19	5.72 ± 1.02	1.48 ± 0.23	9.15 ± 2.85	1.00 ± 0.35	1.15 ± 0.24	0.51 ± 0.09	0.88 ± 0.27
Isoleucine	1.00 ± 0.31	1.60 ± 0.12	1.50 ± 0.72	2.64 ± 0.51	1.00 ± 0.20	1.21 ± 0.35	1.60 ± 0.30	1.21 ± 0.23
α-Ketoglutarate	1.00 ± 0.14	6.13 ± 0.58	1.65 ± 0.54	9.93 ± 1.98	1.00 ± 0.12	0.89 ± 0.08	0.49 ± 0.15	1.85 ± 0.87
L-Ascorbate	1.00 ± 0.18	0.61 ± 0.15	0.94 ± 0.11	0.15 ± 0.05	1.00 ± 0.41	1.15 ± 0.37	0.42 ± 0.21	1.26 ± 0.32
Leucine	1.00 ± 0.37	1.52 ± 0.17	1.60 ± 0.78	3.14 ± 0.43	1.00 ± 0.14	1.12 ± 0.40	1.54 ± 0.31	1.25 ± 0.22
Malate	1.00 ± 0.15	1.36 ± 0.18	1.27 ± 0.41	1.81 ± 0.15	1.00 ± 0.11	1.15 ± 0.02	0.78 ± 0.15	1.25 ± 0.15
Maltose	1.00 ± 0.08	0.02 ± 0.04	1.19 ± 0.30	1.24 ± 0.09	1.00 ± 0.19	0.83 ± 0.16	1.08 ± 0.12	0.84 ± 0.13
Methionine	1.00 ± 0.35	3.80 ± 0.77	1.34 ± 0.30	3.56 ± 1.18	1.00 ± 0.17	1.12 ± 0.25	0.60 ± 0.13	1.23 ± 0.12
Inositol	1.00 ± 0.21	0.20 ± 0.02	1.04 ± 0.08	0.21 ± 0.03	1.00 ± 0.22	0.94 ± 0.13	0.66 ± 0.09	1.01 ± 0.16
Phenylalanine	1.00 ± 0.28	0.28 ± 0.26	1.55 ± 0.75	2.71 ± 0.47	1.00 ± 0.13	1.16 ± 0.19	1.05 ± 0.10	1.01 ± 0.09
Phosphoric acid	1.00 ± 0.12	1.12 ± 0.08	1.28 ± 0.10	1.38 ± 0.05	1.00 ± 0.09	0.94 ± 0.06	0.99 ± 0.10	0.97 ± 0.06
Pyroglutamate	1.00 ± 0.14	1.03 ± 0.09	1.18 ± 0.04	1.32 ± 0.16	1.00 ± 0.16	0.97 ± 0.12	0.12 ± 0.12	0.99 ± 0.10
Serine	1.00 ± 0.19	2.70 ± 0.18	1.28 ± 0.52	3.87 ± 0.20	1.00 ± 0.04	2.13 ± 0.20	0.94 ± 0.10	2.15 ± 0.21
Shikimate	1.00 ± 0.22	1.30 ± 0.16	1.12 ± 0.08	1.75 ± 0.39	1.00 ± 0.10	1.03 ± 0.13	0.83 ± 0.18	1.03 ± 0.14
Succinate	1.00 ± 0.11	1.99 ± 0.11	1.55 ± 0.28	2.12 ± 0.10	1.00 ± 0.11	1.02 ± 0.06	0.74 ± 0.09	1.20 ± 0.15
Sucrose	1.00 ± 0.07	0.56 ± 0.07	0.92 ± 0.20	0.37 ± 0.05	1.00 ± 0.08	0.65 ± 0.08	0.76 ± 0.05	0.84 ± 0.06

	Col-0	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*	*Col-0*	*hpr1-1*	*hpr2-1*	*hpr1 hpr2*
Proline	1.00 ± 0.16	1.05 ± 0.10	1.35 ± 0.08	1.55 ± 0.29	1.00 ± 0.21	1.03 ± 0.21	0.77 ± 0.12	0.97 ± 0.14
Threonic acid	1.00 ± 0.13	0.57 ± 0.07	1.24 ± 0.17	0.78 ± 0.13	1.00 ± 0.25	1.05 ± 0.17	0.82 ± 0.10	0.88 ± 0.25
Threonine	1.00 ± 0.20	3.76 ± 0.31	1.83 ± 1.00	7.51 ± 2.60	1.00 ± 0.11	1.30 ± 0.21	0.90 ± 0.10	1.14 ± 0.20
Trehalose	1.00 ± 0.26	0.93 ± 0.21	1.75 ± 0.57	1.44 ± 0.32	1.00 ± 0.29	1.15 ± 0.48	0.77 ± 0.31	0.88 ± 0.20
Valine	1.00 ± 0.23	1.01 ± 0.23	1.58 ± 0.63	1.52 ± 0.22	1.00 ± 0.08	0.83 ± 0.21	1.00 ± 0.14	0.83 ± 0.11
Xylose	1.00 ± 0.17	0.40 ± 0.06	1.37 ± 0.55	0.62 ± 0.13	1.00 ± 0.20	1.01 ± 0.18	0.89 ± 0.14	0.88 ± 0.26

Supplemental Table 4. Primers used for PCR amplification of genomic DNA and cDNA. Underlined sequences indicate the introduced *Xho*I, *Spe*I and *Kpn*I sites in the primers used to produce expression constructs. ATG in bold print highlight the start codon for methionine.

R175	(SALK LB)	5'-AATCAGCTGTTGCCCGTCTCACTGGTGAA-3'
R409	(SALK RB)	5'-ATTAAACTCCAGAAACCCGCGGCTGAG-3'
R576	(gene trap)	5'-CCGTTTTGTATATCCCGTTTCCGT-3'
R379	(HPR1-S)	5'-TAGCACAAAACCGATGCCTGGAAC-3'
R380	(HPR1-A)	5'-GTTTCCATGTCACAGGTTGTTCTC-3'
R666	(HPR2-S)	5'-CTTCTGGACTTCTCCGGAGAAATC-3'
R668	(HPR2-A)	5'-CCAAATCCCAAATGTGTCACATGAC-3'
R663	(HPR1-S3)	5'-CGAACATCTCAAAGAGAACCCGATG-3'
R664	(HPR1-A3)	5'-GAGAGTGGGTAATCGTCTAATAGACTC-3'
R176	(S16 sense)	5'-GGCGACACAACCAGCTACTGA-3'
R177	(S16 anti)	5'-CGGTAACTCTTCTGGTAACGA-3'
R659	(sense)	5'-ACTAGTGGAGAT**ATG**GAATCAATCGGAGTCC-3'
R660	(antisense)	5'-GGTACCTCAGACGACCGGAGTCAGAAGTGATTTC-3'
R686	(sense)	5'-AACTCGAG**ATG**GCGAAACCGGTGTCCATTGAA-3'
R687	(antisense)	5'-AACTCGAGTCATAGCTTCGAAACAGGCAATCC-3'