Supporting Information

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SI Materials and Methods.

Construction of Gamma Exchange-Revised ATP Synthase (Gamera). The null mutant for *ATPC1 (dpa1)* was as described in (1). The full-length coding sequence of the intronless *ATPC2* gene was amplified by *Pyrococcus furiosus* (Pfu) polymerase from wild-type genomic DNA using the forward primer 5' -AACAAAAAAATG-ACAGGTTCGATCTCG-3' and the reverse primer 5' -CACAG-CTGTAGTTTCTACCTAAGACTCTCG-3' that inserts an XbaI restriction site at the 3' end of the gene. The PCR fragment was cloned in the binary vector pSEX001-VS under control of the cauliflower mosaic virus 35S promoter. Successful cloning was verified by sequencing. The construct was introduced into plants heterozygous for *dpa1* mutation via *Agrobacterium*-mediated transformation (2) and the segregating transformant progenies were selected on rock wool (Grodan, Hobro) immersed in 10 mg/L sulfadiazine (3).

Construction of ATPC1 and ATPC2-β-Glucuronidase (GUS) Lines. Promoter regions of ATPC1 and ATPC2 genes were amplified from Arabidopsis cv. Columbia genomic DNA with the primer pairs: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTYYTTT-CGTTCTAATTTCTAACCAAATATT-3', 5'-GGGGACCACT-TTGTACAAGAAAGCTGGGTYTGTTGTTTGTTTCAGAC-AGTGTTT-3'for ATPC1 and 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTYYAAGACGGCGGCGATTTTGAGA-GC-3', 5'-GGGGACCACTTTGTACAAGAAAGCAGGCTYYAAGACGGCGGCGATTTTGAGA-GC-3', 5'-GGGGACCACTTGTACAAGAAAGCTGGGTYT-TTGGGAAAGAAGACCCTGA-3'for ATPC2. The corresponding PCR products were first cloned in pDONOR207 vector (Invitrogen) and then transferred to the binary vector pKGWFS7,0 (4) to drive the expression of GUS gene. The constructs were in-

- 1. Bosco CD, et al. (2004) Inactivation of the chloroplast ATP synthase gamma subunit results in high non-photochemical fluorescence quenching and altered nuclear gene expression in *Arabidopsis thaliana*. J Biol Chem 279:1060–1069.
- Clough SJ, Bent AF (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of adult Arabidopsis thaliana plants by vacum infiltration. *Plant* J16:735–743.
- Hadi MZ, Kemper E, Wendeler E, Reiss B (2002) Simple and versatile selection of Arabidopsis transformants. Plant Cell Rep 21:130–135.
- Karimi M, Inzé D, Depicker A (2002) Gateway vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci* 7:193–195.
- Dovzhenko A, Dal Bosco C, Meurer J, Koop HU (2003) Efficient regeneration from cotyledon protoplasts in Arabidopsis thaliana. Protoplasma 222:107–111.

troduced into *Arabidopsis* cv. Columbia plants via *Agrobacterium*mediated transformation (2) and the transformant plants were selected on rock wool (Grodan, Hobro) immersed in 25 mg/L kanamycin (3).

Localization of the γ_2 **Protein.** The first 297 nucleotides of *ATPC2* encoding a putative transit peptide were fused to the sequence of enhanced green fluorescence protein (eGFP) by overlap extension PCR using the primers:

5'-CACCATGACAGGTTCGATCTCGACC-3', 5'-TGCTCA-CCATTATGACTGCATCTTGAG-3', 5'-TGCAGTCATAAGG-TGAGCAAGGGCGAGGAGC-3' 5'-AGTACACAGTCCTTG-TACAGCTCGTCCA-3'. The PCR fragment was first cloned into pENTR/TOPO vector (Invitrogen) and then transferred to p2GW7,0 containing the 35S promoter and terminator (4). *Arabidopsis* protoplasts were isolated from 7 d old seedlings according to (5) and polyethylene glycol mediated transient transformation was performed according to (6)

Phylogenetic Tree Reconstruction. Neighbor-joining trees were reconstructed using ClustalX (7) and Molecular Evolutionary Genetics Analysis 4 (8) These two methods yielded tree topologies differing only in a few very late branching orders. The tree shown in Fig. S5 has been obtained using Clustal X. Gap positions were taken into account. Multiple substitutions were allowed for using Kimura's correction algorithm (7, 9). Calculated bootstrap values correspond to the frequency of occurrence of nodes in 1,000 bootstrap replicates. All sequences were retrieved from the plant genome database (PlantGDB) http://www.plantgdb.org.

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- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.
- Galtier N, Gouy M, Gautier C (1996) SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* 12:543–548.
- Ariizumi T, Lawrence PK, and Steber CM (2011) The role of two F-box proteins, SLEE-PY1 and SNEEZY, in *Arabidopsis* gibberellin signaling. *Plant Physiol* 155: 765–775.



Fig. S1. ATPC2-eGFP localization in transiently transformed Arabidopsis protoplasts. Images were recorded 24 h after protoplast transformation using Axiovert 200 M inverted microscope and 20xW NA1.3 lenses (Carl Zeiss).



Fig. 52. *A*. Images of wild type and mutant plants. Plants were grown under continuous light at 20–30 μ mol photons m⁻² s⁻¹ at 22 °C for 4 wks. All plants, wild type, *ATPC2* over expressing plants (*gamera*), and *ATPC2* knockout plant (*atpc2*) are *Arabidopsis* cv. Columbia. *B* and *C*. Immunodetection of ATP synthase subunits (β , γ_1 , and γ_2) in thylakoid membrane proteins isolated from wild type, *gamera*, and *atpc2*. (*B*) shows wild-type proteins loaded at 1, $\frac{1}{2}$, and $\frac{1}{4}$ loading levels, compared to *gamera*, and *atpc2*. (*C*) shows assays at fourfold higher protein loading levels in an attempt to detect small low levels of the γ_2 subunit (*ATPC2*). Equal protein loading was confirmed by Ponceau staining (1) as shown in the bottom panels.



Fig. S3. Effects of infiltration of the strong thiol oxidant diamide on the activity and redox state of the γ_2 -ATP synthase. *A*. The redox state of the ATP synthase was probed by measuring the shift in apparent molecular weight after modification by 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonate, as described in the main text. *B*. The activity of the ATP synthase was probed by the decay of flash-induced electrochromic shift (ECS) signal in *gamera* after infiltration with water (light gray line) or solutions of 0.5 mM (dark gray line) or 5 mM (black line) diamide.

		atpC1	atpC2
	Seedling (1)	19551	362
22 21 1	Cotyledons (21)	36488	284
23	Hypocotyls (22)	6725	433
	Radicle (23)	<mark>460</mark>	<mark>514</mark>
	Inflorescence (2)	12230	550
	Flower (31)	12230	449
31	Silique (32)	19353	425
	Seed (33)	4095	707
11	Stem (34)	20574	364
331 2 32	Node (35)	18176	254
34 2	shoot apex (36)	8863	661
33	cauline leaf (37)	32003	164
35 01	Rosette (3)	31163	734
44 36 41 42	juvenile leaf (41)	24516	344
	adult leaf (42)	27770	263
The	Petiole (43)	28984	257
	senescent leaf (44)	13226	222
/ 🐨	Roots (4)	1329	526

Fig. S4. Expression profiles of ATPC1 and ATPC2 genes using the Genevestigator gene atlas.



Fig. S5. ATPC1::GUS staining in 10 d old seedlings.

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Arabidopsis thaliana	1; TLLPLSPKGEICDINGTCVDAAEDEFFRLTTKEGKLTVERETFR
Arabidopsis thaliana	2; TLLPLSMKGESCDVKGECVDAIEDEMFRLTSKDGKLAVERTKLE
Arabidopsis lyrate	1; TLLPLSPKGEICDINGTCVDAAEDELFRLTTKGGKLTVERETFR
Arabidopsis lyrate	2; TLLPFSMKGESCDVKGECVDAMEDEMFRLTSKDGKLAVERTKLE
Glycine max	1; TLLPLSPKGEICDINGVCVDAAEDEFFRLTTKEGKLTVERDVVR
Glycine max	2; TLLPLSPKGEICDVNGVCVDAAEDEFFRLTTKEGKLTVERDVVR
Ricinus communis	1; TLLPLSPEGEICDINGNCVDAAEDEFFRLTTKEGKLTVERDVVR
Ricinus communis	2; TLLPLSPRGGVCDVNGKCVDAADDEFFRLTSKEGKLAVERESGK
Selaginella tamariscina	1; TLLPLSPQGQVCDINGNCVDADEDEMFRLTTKDGKLTVERDTFK
Selaginella tamariscina :	2; TLLPLSPQGQVCDINGNCVDADEDEMFRLTTKDGKLTVERDTFK
Vitis vinifera	1; TLLPLSPKGEICDVNGKCVDAAEDEFFRLTTREGKLTVERDVIR
Vitis vinifera	2; TLLPLSMKGEVFDQNGNCVDAMEDELFRLTTKEGKLSVERDKVQ
Populus trichocarpa	1; TLLPLSPKGEICDVNGVCVDAAEDEFFRLTTREGKLTVERGVSR
Populus trichocarpa	2; TLLPLSPKGRICDMNGVSVDADGDEFFRLTTKEGKLAVERDVVR
Lotus japonicus	1; TLLPLSPKGEICDINGNCVDAAEDELFRLTTKEGKLTVERDTIR
Lotus japonicus .	2; TLLPLSRKGEVCDVNGNSVDAIDDEYFRLTSKDGKLALERGVVG
Nelumbo nucifera	1; TLLPLSPKGEICDINGNCVDAAEDELFRLTTKEGKLTVERDTIR
Nelumbo nucifera	2; TLLPLSRKGEVCDVNGNSVDAIDDEYFRLTSKDGKLALERGVVG
Nicotiana tabacum	1; TLLPLSPKGEICDINGNCVDAANDEFFRLTTKEGKLTVERDIIR
Nicotiana tabacum	2; TLLPISSKGEVLDVNGKSVDVDEDEFFRLTTKEGKLIVERDHLR
Nicotiana tabacum	3; TLLPISSKGEVLDVNGKSVDVDEDEFFRLTTKEGKLIVERDHLR
Medicago polymorpha	1; TLLPLSPKGEICDINGNCVDAAEDELFRLTTKEGKLTVERDAVR
Medicago polymorpha	2; TLLPLSKKGEVFDVNGNSVDVLEDEFFRLTSKDGKLALKRDVKK
Artemisia indica	1; TLLPLSPKGEICDINGVCVDAAEDEFFRLTTKEGKLTVERDVVR
Artemisia indica	2; TLLPLSSKGEIRDGNGNSVDASEDEFFRLTSKEGKLAVERDRMM
Malus pumila	1; TLLPLSPKGEICDINGVCVDAADDEFFRLTTKEGKLTVERDVIK
Malus pumila .	2; TLLPLLPRGEVVDVNGNSVDATEDEFFRLTTKKGKLSMERGSVK
Malus pumila	3; TLLPLSPKGEVVDVNGNSVDAIEDEFFRLTTKEGKLSVERESVK
Lycopersicon esculentum	1; TLLPLSPKGEICDINGNCVDAAEDEFFRLTTKEGKLTVERDVMR
Lycopersicon esculentum 2	2; TLLPLRG-GEE-EFF-DEDEFFRLTSKEGKLSVVRD-LR
Physcomitrella patens	1; TLLPLSPQGEVCDISGNCVDAADDEMFRLTTKDGKFSVERETIR
Physcomitrella patens	2; TLLPLSPQGEVCDISGNCVDAADDEMFRLTTKDGKFSVERETVR
Physcomitrella patens	3; TLLPLSPAGEVCDISGNCVDAADDEMFRLTTKDGKFSVERETVR
Spinacia oleracea	; TLLPLSPKGEICDINGKCVDAAEDELFRLTTKEGKLTVERDMIK
Cucumis melo	; TLLPLSPKGEICDINGVCVDAAEDEFFRLTTKEGKLTVERDSVR
Zea mays	; TLLPMSPKGEICDVNGVCVDATEDELFRLTTKEGKLTVEREKVK
Oryza sativa	; TLLPMSPKGEICDINGVCVDATEDELFRLTTKEGKLTVEREKVK
Chlamydomonas reinhardti.	<i>i;</i> TLLPMTPMGELCDVDGKCVDAADDEIFKLTTKGGEFAVEREKTT
Osteococcus lucimarinus	* TILPLSKEGEVCNVDGVCTDAANDETEKLTTEDGKEAVKREASD

Fig. S6. Alignment of amino acid sequences of chloroplast γ subunits using *Arabidopsis* numbering. Red amino acid residues are conserved. Blue amino acids are cysteine residues involved in redox regulation. Amino acids 199 and 205 are key regulators.

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Fig. S7. Schematic phylogenetic tree of the γ subunit of chloroplast ATP synthase (see SI Materials and Methods).

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