# Supporting Information

## Kohzuma et al. 10.1073/pnas.1115728109

### 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′-GGGGACAAGTTTGTAC-AAAAAAGCAGGCTYYAAGACGGCGGCGATTTTGAGA-GC-3′, 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTYT-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 introduced 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 $\gamma_2$ 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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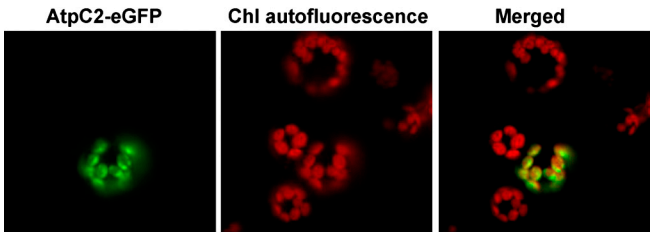



**Fig. S1.** *ATPC2*-eGFP localization in transiently transformed *Arabidopsis* protoplasts. Images were recorded 24 h after protoplast transformation using Axiovert 200 M inverted microscope and 20xW NA1.3 lenses (Carl Zeiss).

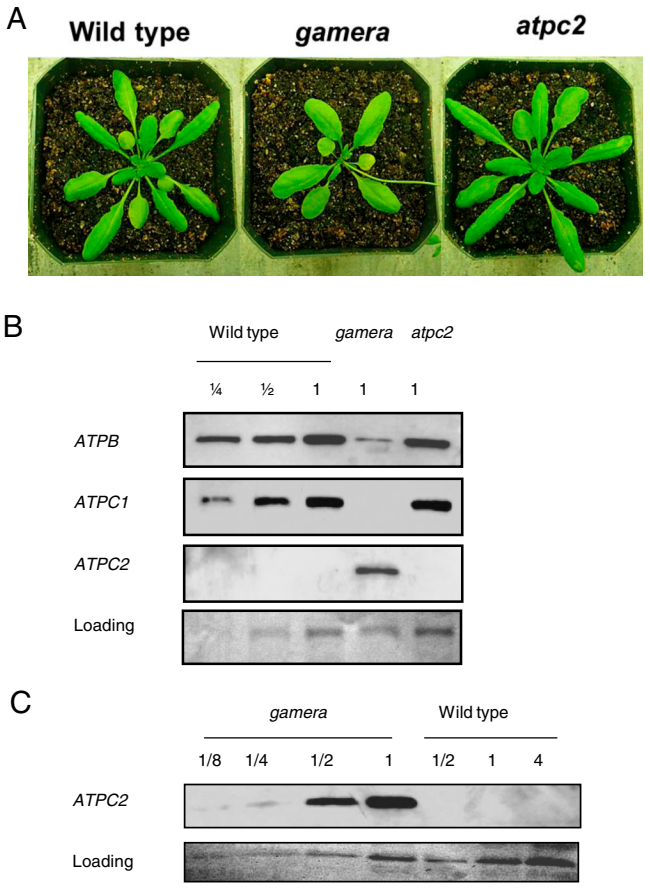



**Fig. S2.** *A*. Images of wild type and mutant plants. Plants were grown under continuous light at 20–30 $\mu$mol photons $m^{-2}$ $s^{-1}$ at 22 °C for 4 wks. All plants, wild type, *ATPC2* over expressing plants (*gamera*), and *ATPC2* knockout plant (*atpc2*) are *Arabidopsis* cv. Columbia. *B* and *C*. Immunodetection of ATP synthase subunits ($\beta$, $\gamma_1$, and $\gamma_2$) in thylakoid membrane proteins isolated from wild type, *gamera*, and *atpc2*. (*B*) shows wild-type proteins loaded at 1, ½, and ¼ 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 $\gamma_2$ subunit (*ATPC2*). Equal protein loading was confirmed by Ponceau staining (1) as shown in the bottom panels.

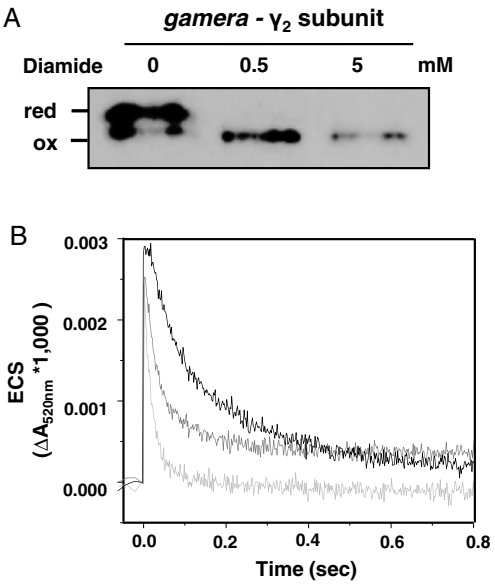
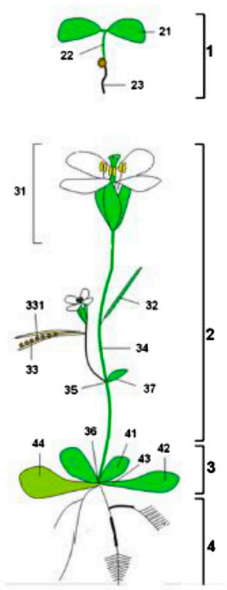



**Fig. S3.** Effects of infiltration of the strong thiol oxidant diamide on the activity and redox state of the $\gamma_2$-ATP synthase. *A*. The redox state of the ATP synthase was probed by measuring the shift in apparent molecular weight after modification by 4-acetamido-4′-maleimidylstilbene-2,2′-disulfonate, 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 |
| Cotyledons (21) | 36488 | 284 |
| Hypocotyls (22) | 6725 | 433 |
| Radicle (23) | 460 | 514 |
| **Inflorescence (2)** | 12230 | 550 |
| Flower (31) | 12230 | 449 |
| Silique (32) | 19353 | 425 |
| Seed (33) | 4095 | 707 |
| Stem (34) | 20574 | 364 |
| Node (35) | 18176 | 254 |
| shoot apex ( 36) | 8863 | 661 |
| cauline leaf (37) | 32003 | 164 |
| **Rosette (3)** | 31163 | 734 |
| juvenile leaf (41) | 24516 | 344 |
| adult leaf (42) | 27770 | 263 |
| 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.

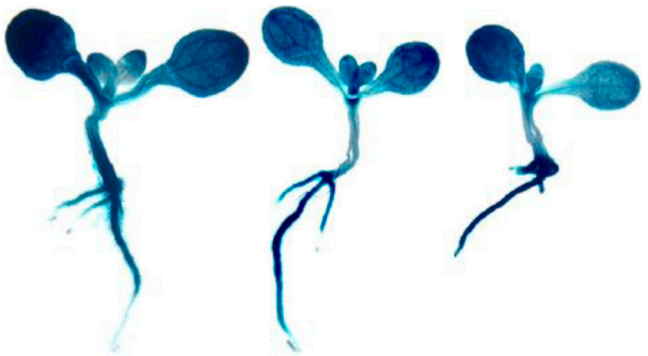


**Fig. S5.** *ATPC1*::GUS staining in 10 d old seedlings.

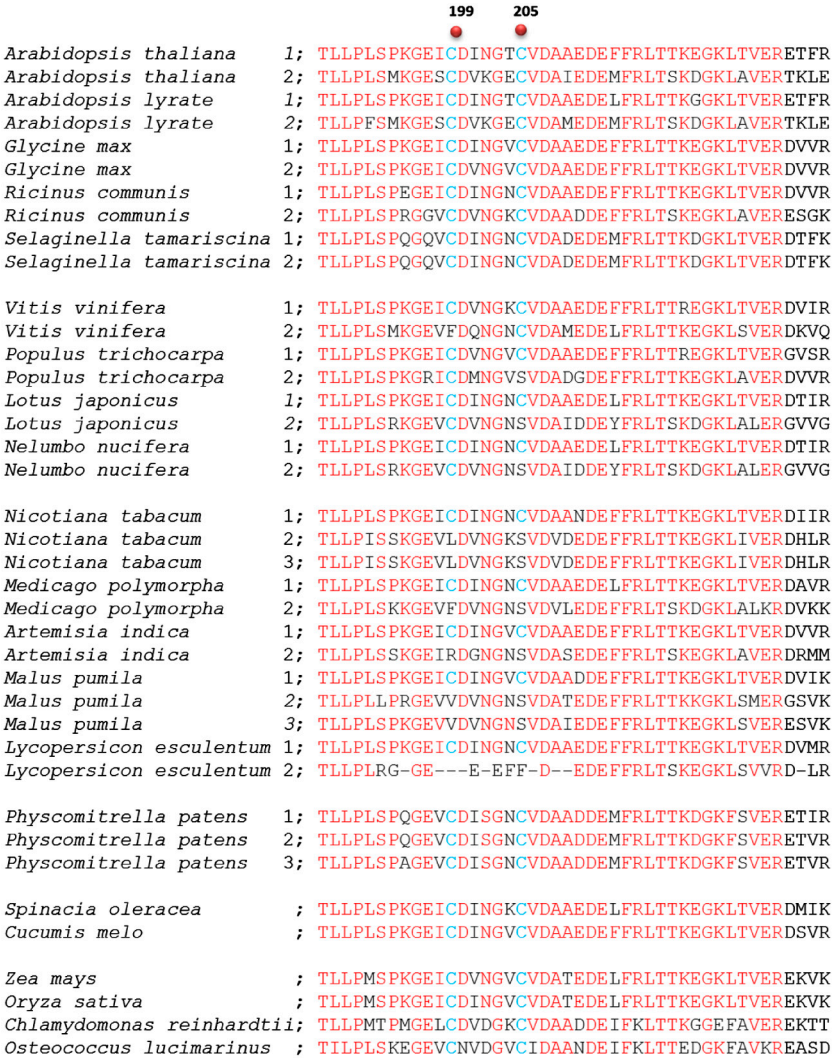



**Fig. S6.** Alignment of amino acid sequences of chloroplast γ subunits using *Arabidopsis* numbering. Red amino acid residues are conserved. Blue amino acids are cysteine residues involved in redox regulation. Amino acids 199 and 205 are key regulators.

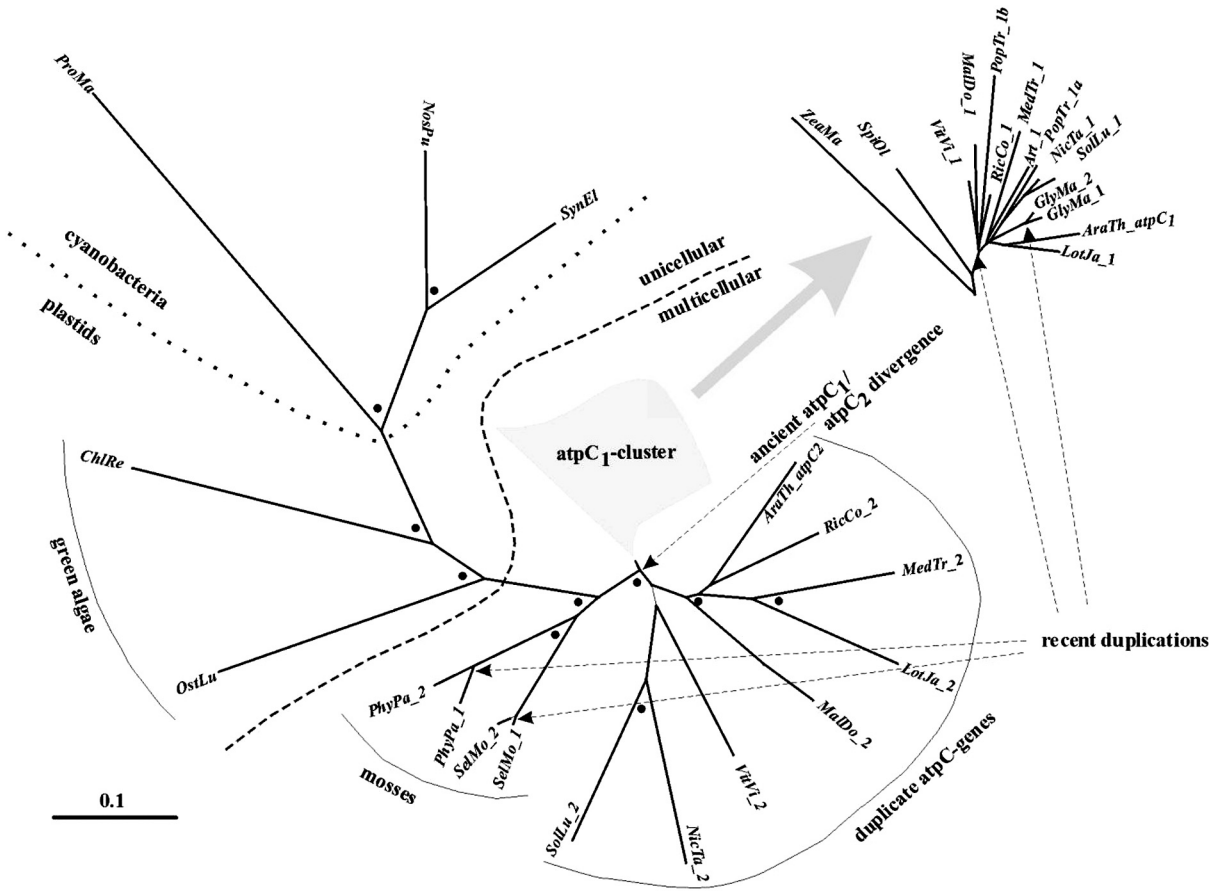



**Fig. S7.** Schematic phylogenetic tree of the γ subunit of chloroplast ATP synthase (see SI Materials and Methods).