

A Nucleus-Encoded Chloroplast Protein YL1 Is Involved in Chloroplast Development and Efficient Biogenesis of Chloroplast ATP Synthase in Rice

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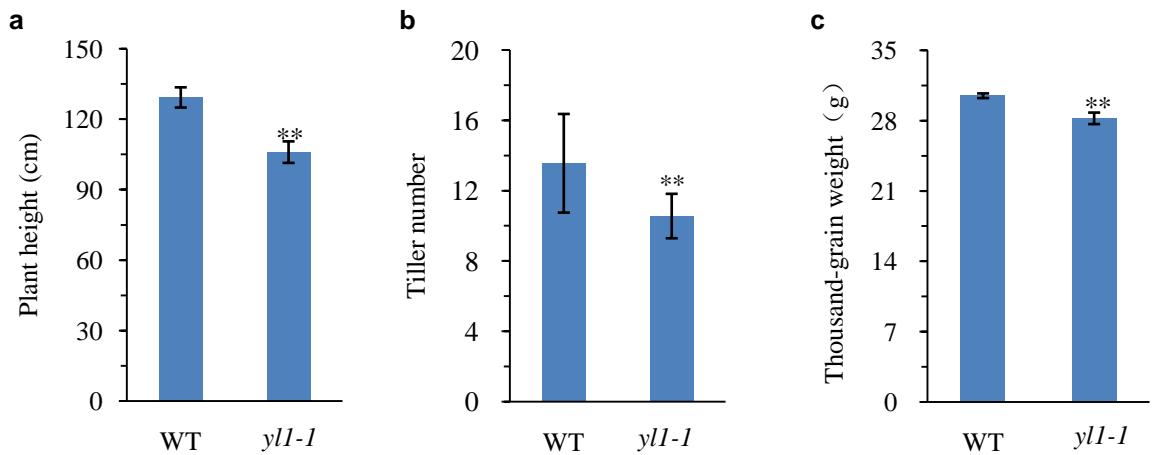
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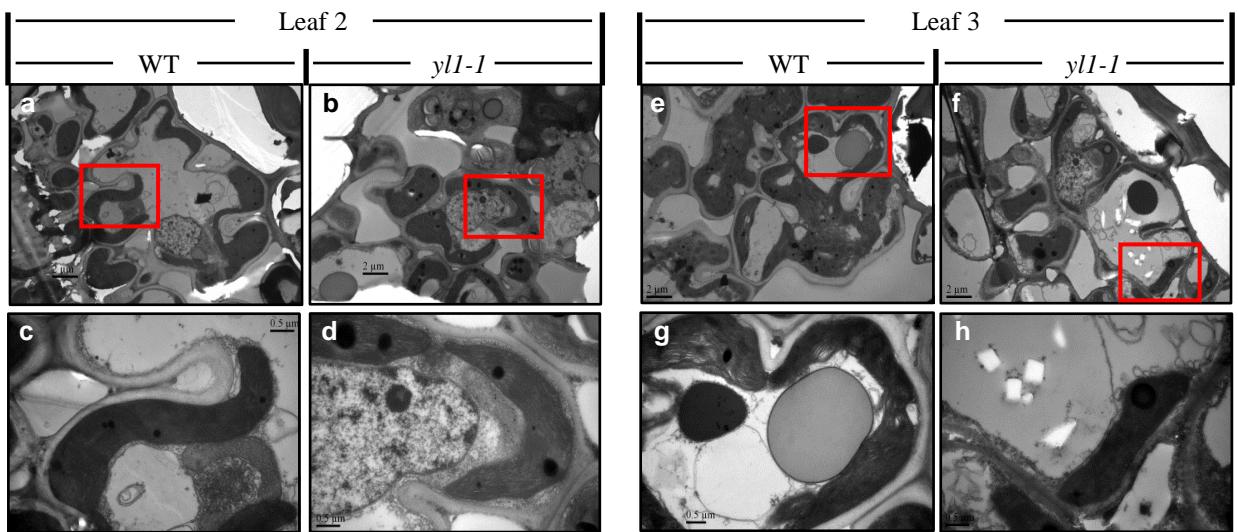
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Supplemental Figure S1



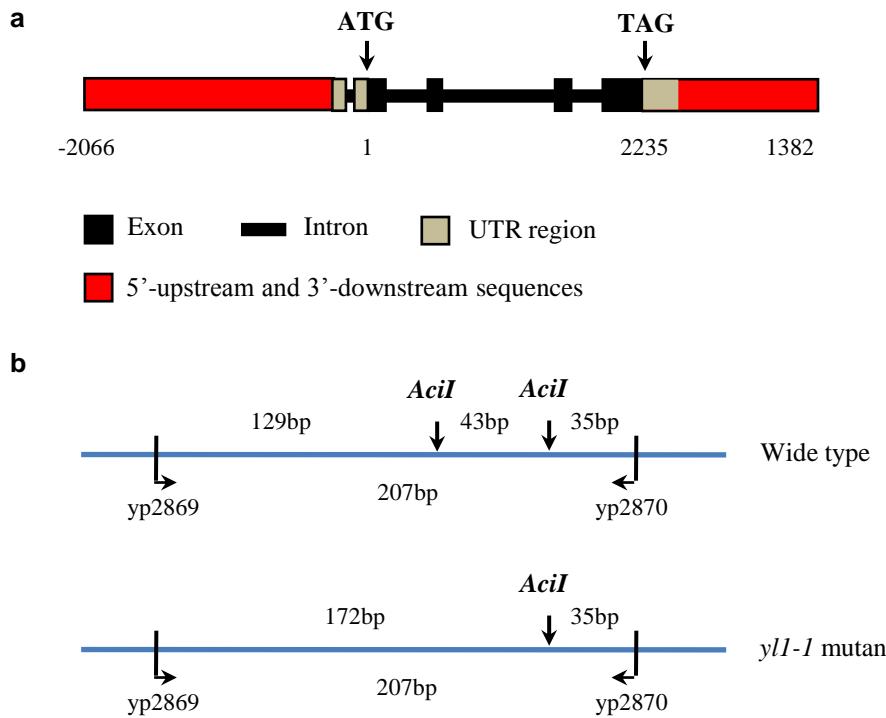
Supplemental Figure S1. Comparison of tiller number per plant (a), seed setting ratio (b), and 1,000-grain weight (c) between wild-type and *ylI-1* mutant plants. Data in (a)-(c) were measured from plants grown within 60 × 60 cm spacing in field. All the data are means ± SD (n = 10). **, P<0.05.

Supplemental Figure S2



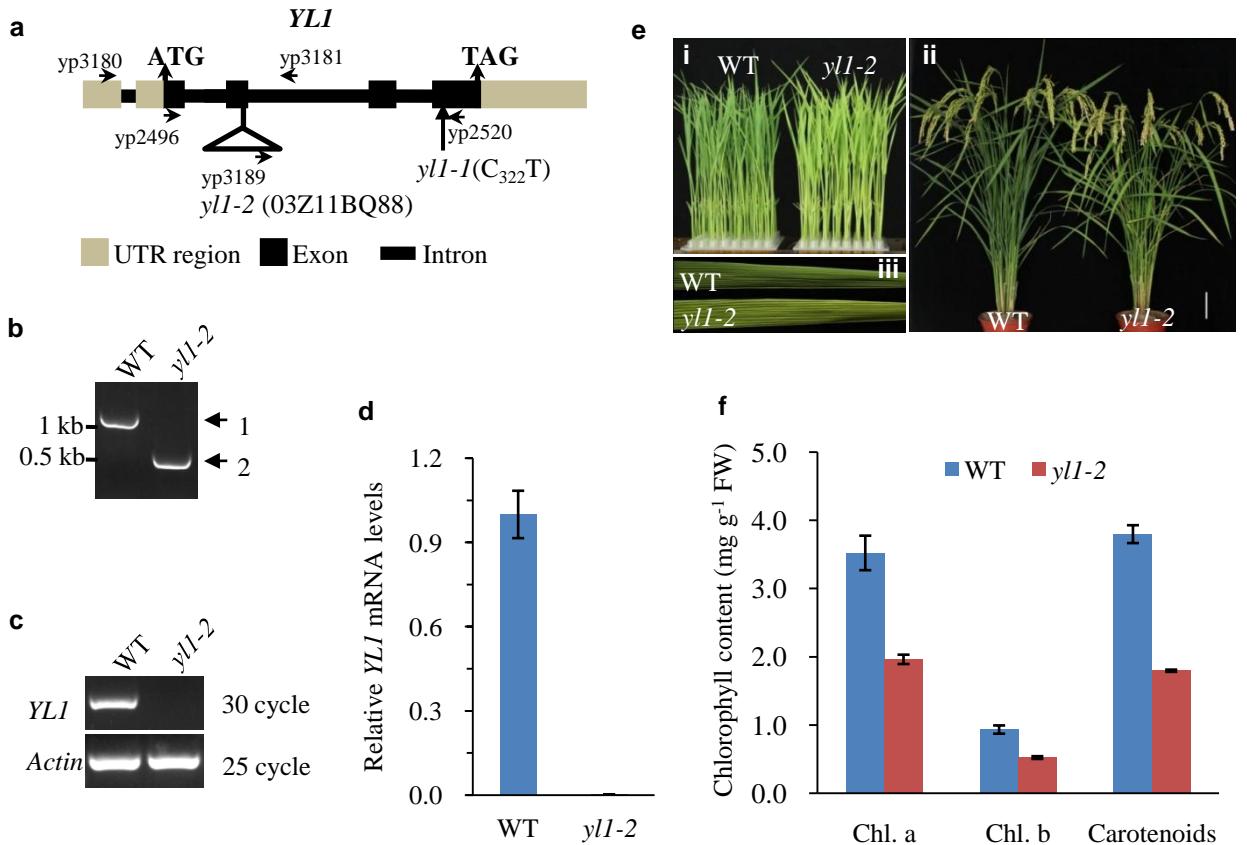
Supplemental Figure S2. Transmission electron microscopy (TEM) of chloroplasts from leaf 2 (a, b, c, d) and leaf 3 (e, f, g, h) of 40-day-old wild-type (WT, left) and *yll-1* (right) seedlings. Leaf 1 to Leaf 4 represent leaves from the youngest to the oldest ones. (c), (d), (g), and (h) Enlarged images of chloroplasts shown in (a), (b), (e), and (f), respectively. Bars = 2.0 μm in (a), (b), (e) and (f); and 0.5 μm in (c), (d), (g) and (h).

Supplemental Figure S3



Supplemental Figure S3. Complementation test of YL1. (a) Schematic diagram of the complementation plasmid containing the entire *YL1* (genomic DNA). (b) Restriction enzyme map of *AciI*. An *AciI* restriction site was abolished by the C-to-T transition in mutation sequence. Primers (YP2869 and YP2870) were used for amplify a 207-bp DNA fragment around the mutation position.

Supplemental Figure S4



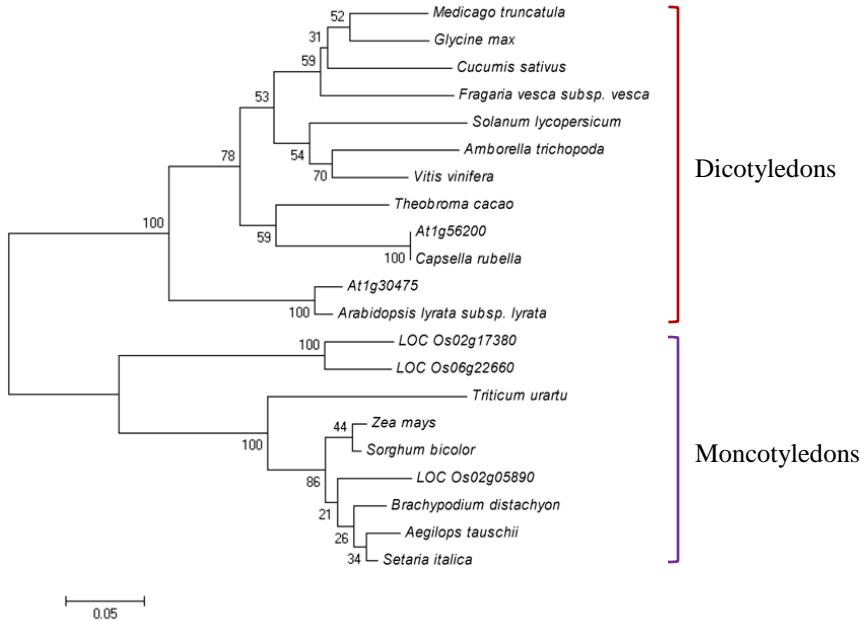
Supplemental Figure S4. Knockout mutant *yll-2*, by T-DNA insertion, presents similar phenotype of *yll-1*. a, Localization of T-DNA insertion site in the *YL1* gene. Exons, introns and UTR regions are indicated in black boxes, black lines and brown boxes, respectively. The triangle indicates the T-DNA insertion in *yll-2* (RMD_03Z11BQ88). Positions of primers used in (b), (c) and (d) are indicated by arrowheads. b, PCR analysis of genomic DNA from the wild type (WT) and *yll-2* mutants confirms the homozygosity of the mutants. Band 1, amplification with primers YP3180 and YP3181; Band 2, amplification with primers YP3189 and YP3181; c and d, RT-PCR (c) and qRT-PCR (d) analysis of *YL1* expression in the wild type and *yll-2* plants. Data for WT and *yll-2* plants are presented as mean ± SD (n = 3). e, Phenotypes of WT and *yll-2* mutant plants. (i) Phenotypes of two-week-old wild type and *yll-2* seedlings cultured in nutrition solution. (ii) Phenotypes of wild type and *yll-2* plants at grain filling stage. (iii) Enlarged views of flag leaves from (ii). f, Pigment contents in two-week-old leaves in wild type and *yll-2* mutant. Chl.a, Chlorophyll a; Chl.b, chlorophyll b; Carotenoids. Data are means ± SD (n = 5).

Supplemental Figure S5

a



b



c

0.05

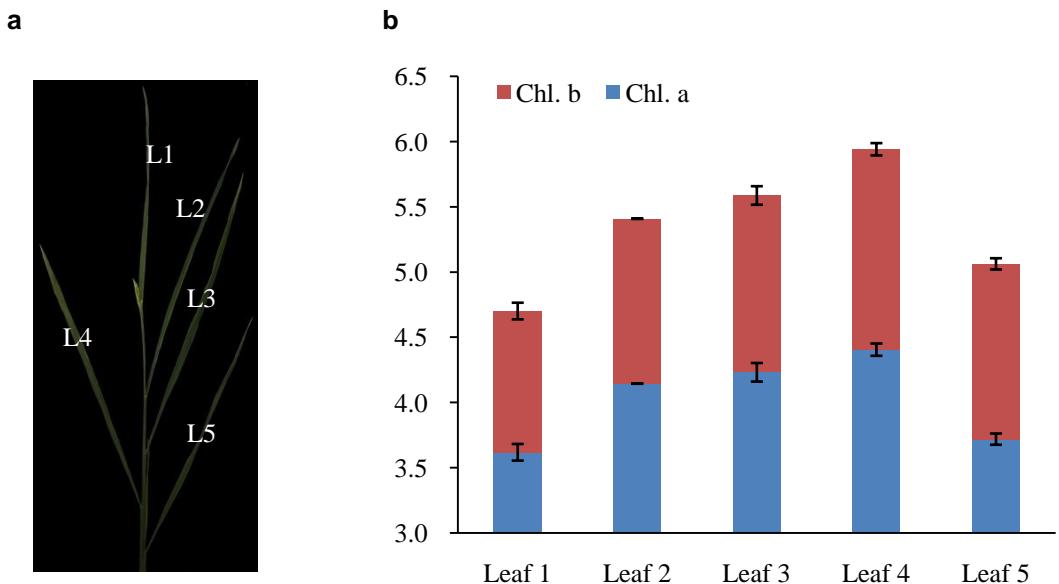
At1g30475	MIEKLRGATKIPVNFS-----STKN-----LPAIQLISMVMPVMNPNSLCLGTYQTSV-----YLAF-----RE-KSPTNSNGNSQRRTYPTVSVMQR-----QFWS-----QSKEPPSL	83
<i>A. lyrata</i> subsp. <i>Lyrata</i>	-TSRSNQTPDFS-----RQKT-----YILLIQIPMVMPVMNPNSLCLGTPSV-----YLAF-----RGHNFITNGNSHRSPTSVSMQR-----QFSS-----QSKEPPSL	80
<i>A. trichopoda</i>	-----MASIT-MS-PLNT-IPAAVPSVFSPE-IPLGKVNVPSAGSINLLKSGLAMPKISTRMMS-----QCKTG-----61	
<i>F. vesca</i> subsp. <i>F. vesca</i>	-----MVMILTAASIAASS-PL-----LPPVQHNGMMKAANNLRLWYISSQHPTN-----SRR-LS-----MVAKAGPVFLKF	60
<i>C. sativus</i>	-----MEML-TTNSGAML-PL-----LPPVQHNGMMKAANNLRLWYISSQHPTN-----SRR-LS-----C-----TQ	50
<i>M. truncatula</i>	-----ML-ATSAVSL-PK-----LPPVQHNGMMKAANNLRLWYISSQHPTN-----SIS-LS-----C-----TQ	50
<i>G. max</i>	-----ML-AISAIALS-PV-----LPPVQHNGMMKAANNLRLWYISSQHPTN-----SIS-LS-----S-----TQ	50
<i>V. vinifera</i>	-----ML-AISAIALS-IA-----IPAKRPLESEC-----RQGIKIC-LGFVFVGLYIQLSLSFGGTQAIISPWLTR	55
<i>T. cacao</i>	-----ML-AISAIALS-PP-----L-LSPG-----SPL-----EAOIHK-S-----GFCGACRISTQVR-P	41
At1g56200	-----MMAAIIIIQSS-PL-----S-FNSHNAKPRI-----HS-S-----GSLGKISQNRRVS-P	41
<i>C. rubella</i>	-----MMAAIIIIQSS-PL-----S-LNSRNEKPRI-----EVKVPFS-S-----GSLGKISQNRRVS-P	45
LOC_Os02g05890	-----MPLPLAT-MSS-PGSLLLTAVQYQIGRN-----RGGQSQ-----EQQSISS-SS	41
<i>Z. mays</i>	-----MAA-AAA-ASAFSRLRVSNQGTG-----HV-----LY	28
<i>S. italica</i>	-----MFIPIAA-ASA-PSLRLPLTRARQGIR-----GGQ-GW-----GERSLY-GS	37
<i>S. bicolor</i>	-----MFSSMAA-PA-VSAPSRLRTSQQMIGGG-----VGG-GW-----GERCRY-GS	39
<i>T. urartu</i>		0
<i>A. tauschii</i>		
<i>B. distachyon</i>	-----MSRCPVRLPKPHCLRALYLSSGSSGGNYGIAPPRLACTYTAMPLPLAA-----VSA-PSSL-LLRFAAYHRIGRD-----LQQ-SW-----QIFPSVS-GS	82
LOC_Os02g17380	-----MSTLV-T-CSL-PFGATVTHASTK-----PQ-----GS	25
LOC_Os06g22660	-----MFASPLT-CSL-PQTIVVASAATAQ-----FTE-----RC-----NDLFMPF-CS	34

At1g30475	QD-----AVQPVWSIKMGPVPAFCN-----SALNSKCS-QQQTQTVTRECPPTTITQAP-----THGKVSPLKIDDGNGKTFP-----IDGGGG-G-C-GGGGGGSSGGFFLP-----GFLLFWGFLYLDLEGEHENHH-----	191
<i>A. lyrata</i> subsp. <i>Lyrata</i>	QALGTRPVGSWVSKIDESVFACh-----SALNSKCS-QQQTQTVTRECPPTTITQV-----THGKVSPLKIDDGNGKTFP-----IDGGGG-G-C-GGGGGGSSGGFFLP-----GFLLFWGFLYLDLEGEHENHH-----	192
<i>A. trichopoda</i>	-----VSLAHKMASTGACMCERGASAIICA-----LAVINARCT-----EQQTIQHQSATVITNAP-----AQNEKSPFLDIDGTFP-----IDDGNGKTFP-----IDGGGG-G-C-GGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YEDR	173
<i>F. vesca</i> subsp. <i>F. vesca</i>	-----TGSBRMGSAPVICA-----SALNARCG-AEQTQTVTREAPTITMLP-----GKESKPLDIDGDSGTFP-----IDDGNGKTFP-----IDGGGG-G-C-GGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YERENKERS-Y	173
<i>C. sativus</i>	-----TPSTR-TKGVLCLSSKMKNAFICF-----AALNARCA-----AEQTQTVTREAPTITVLP-----GKESKPLDIDGDSGTFP-----IDDGNGKTFP-----IDGGGG-G-C-GGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YENDRER	174
<i>M. truncatula</i>	-----PPL-----GRGTRNSTQAAITIICA-----AALNARCS-----AEQTQTVTREAPTITMLP-----GKESKPLDIDGDSGTFP-----IDDGNGKTFP-----IDDGNGG-G-C-GGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YENDRER	163
<i>G. max</i>	-----GPF-----GRKA-BSTQPATITICA-----AALNARCG-----AEQTQTVTREAPTITVLP-----GKESKPLDIDGDSGTFP-----IDDGNGKTFP-----IDGGGG-G-C-GGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YDRDERR	159
<i>V. vinifera</i>	-----QSLFGRHTQAHTRKQASLTC-----SALNARCG-AEQTQTVTREAPTITVLP-----VQKEKSPLDIDGDTGTFP-----IDDGNGG-G-CGGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YDRER	170
<i>S. hyperosideum</i>	-----SQLNRRRRLPAQQRKRSATICA-----SALNARCA-----EQQQTQTVTREAPTITVLP-----VQKEKSPLDIDGDTGTFP-----IDDGNGG-G-CGGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YDRQRR	160
At1g56200	-----IQLGLQLOPIVGSSRQSSVICA-----AAALNATNCASQQTQTVTREAPTITVLP-----VQKEKSPLDIDGDTGTFP-----IDDGNGG-G-CGGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YDRQRR	161
<i>C. rubella</i>	-----LSA-VGLS-----SGLGKRSLSLICH-----SALNACKS-----EQQQTQTVTREAPTITVLP-----VQKEKSPLDIDGDTGTFP-----IDDGNGG-G-CGGGGGGWNCGKCFPTGFLAFLGLDQESEGS-----YDRQRR	154
LOC_Os02g05890	-----KTL-----SVSARAVSS-CESAMRITCC-----ANQQTQAAKRSFSGTSPSGSVEKVSPLDIDGDTGTFP-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	185
<i>Z. mays</i>	-----S-----ISDR-A-----ATVS-ARASAMNITCC-----ANQQTQAAKRSFSGTSPSGSVEKVSPLDIDGDTGTFP-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	146
<i>S. italica</i>	-----QSR-----RATR-G-----ATIS-ARASAMNITCC-----ANQQTQAAKRSFSGTSPSGSVEKVSPLDIDGDTGTFP-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	156
<i>S. bicolor</i>	-----RSQ-----RATR-G-----ATVS-ARASAMNITCC-----ANQQTQAAKRSFSGTSPSGSVEKVSPLDIDGDTGTFP-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	158
<i>T. urartu</i>	-----MA-----LSHDL-LTSLLWVHLIAEK-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	91
<i>A. tauschii</i>	-----RNNL-S-----NSIC-VK-----ANITCC-----ANQQTQAAKRSFSGTSPSGSVEKVSPLDIDGDTGTFP-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	156
<i>B. distachyon</i>	-----ADKL-S-----NTIS-AKASMNITCC-----ANQQTQAAKRSFSGTSPSGSVEKVSPLDIDGDTGTFP-----PFGGGGG-----GGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----NGPR-GSDYDINGLAPQ	201
LOC_Os02g17380	-----TSQASC1SPFKEVSAKAWLBSVRKC-----ATQTQSAQKRSSTTATV-----KED5KGKTCQKPLDIDGSGTFP-----PFGGGGGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----ARBBRAGYDANNDMFPQ	152
LOC_Os06g22660	-----QIL-----BARKNSLSSRNVKPTKPLBLRCH-----ATQTQSTQKRSSTATI-----QSDTKGKLGKPLDIDGGGGTFP-----PFGGGGGGGGGNSNSAGGVILPVIVLILLYLDEPERFLNQ-----LQHNRIGEATLGLAQ	160

* * * * * Transmembrane region

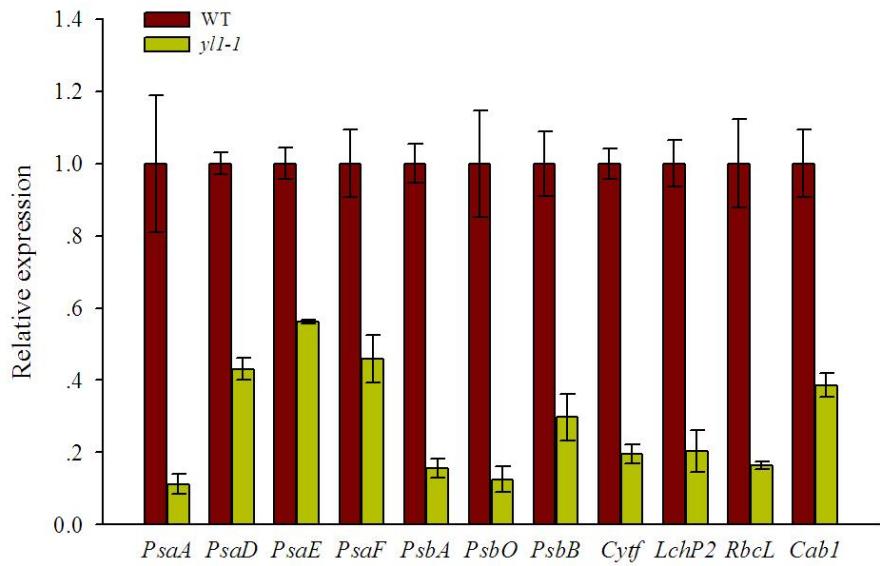
Supplemental Figure S5. Sequence analysis and phylogenetic tree of YL1 homologs. a, Schematic representation of the predicted protein structure of YL1. cTP, chloroplast transit peptide; tm, transmembrane domain. b, Phylogenetic analysis of MEGA5 software (version 5.05) with neighbor-joining method. The numbers at the nodes represent percentage bootstrap values based on 1,000 replications. YL1 (LOC_Os02g05890) is highlighted by the red box. c, Sequence alignment of YL1 and its homologous proteins. The alignment was performed using ClustalW (<http://www.ebi.ac.uk/Tools/msa/>). High and low consensus residues are shown in red and blue, respectively. Transmembrane region is predicted by TMHMM (<http://www.cbs.dtu.dk/>).

Supplemental Figure S6



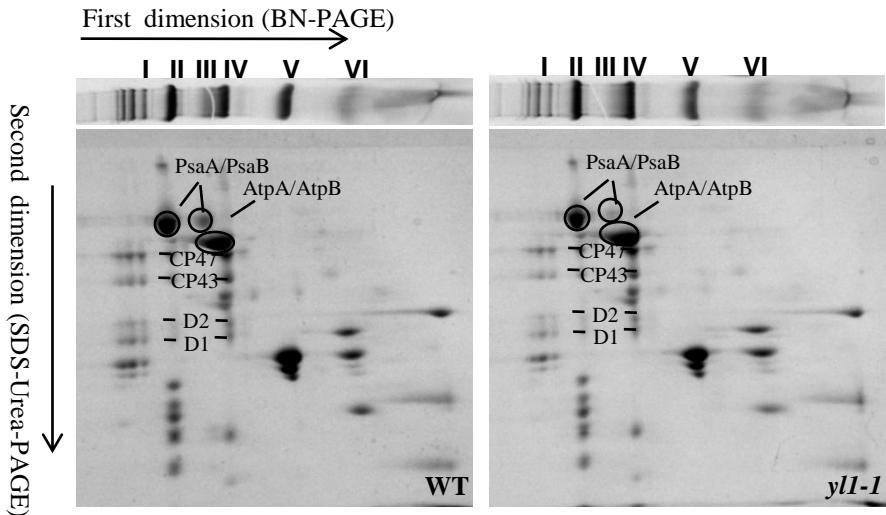
Supplemental Figure S6. Diagram (a) and chlorophyll contents (b) of different leaves from wild-type plants. L1 to L5 (Leaf1 to Leaf5) represent leaves from the youngest to the oldest ones in 80-day-old wild-type plants grown under field condition. Data are means \pm SD ($n = 5$).

Supplemental Figure S7



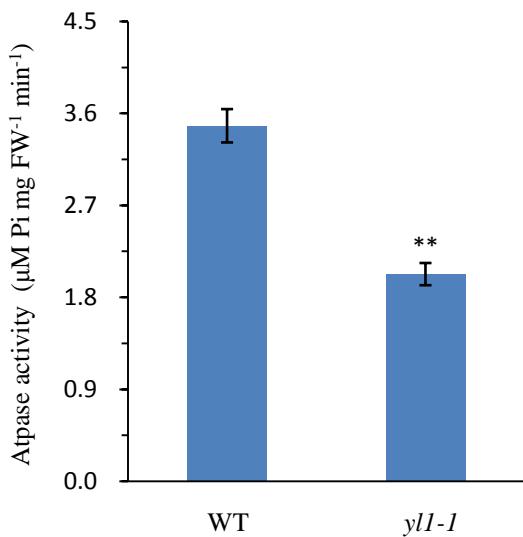
Supplemental Figure S7. Expression analysis of genes involved in photosynthesis in wild type (WT) and *yll-1* leaves. The relative expression level of each gene were analyzed by qRT-PCR and normalized using the Actin gene (LOC_Os03g50885) as an internal control (mean \pm SD, n=3).

Supplemental Figure S8



Supplemental Figure S8. 2D BN/SDS-PAGE fractionation of thylakoid membrane protein complexes. Thylakoid membrane proteins were extracted from leaves of 4-week-old wild type and *yll-1* mutant plants. Proteins were loaded on an equal chlorophyll content basis. I, PSI-PSII supercomplex; II, PSI-PSII dimer; III, PSI monomer; IV, CP43-less PSII core monomer; V, LHCII dimer; VI, LHCII monomer.

Supplemental Figure S9



Supplemental Figure S9. ATPase activity in isolated chloroplasts of wild type and *yll-1* mutant plants. Intact chloroplasts were isolated from the leaves of wild type and *yll-1* mutant, and ATPase activity (equal fresh weight basis) was determined as described in Materials and Methods. Data are means \pm SD (n = 4). Asterisks indicate a statistically significant difference from the wild type (**, P<0.01).

Supplemental table S1. Photosynthetic parameters in wild type and *yII-2* mutant plants.

	Pn (μmol CO ₂ m ⁻² s ⁻¹)	Gs (mol CO ₂ m ⁻² s ⁻¹)	Tr (mol CO ₂ m ⁻² s ⁻¹)
Wide type	20.6±0.28	0.65±0.06	7.44±0.23
<i>yII-2</i>	15.0±0.26**	0.31±0.03**	4.92±0.30**

Data are presented as means ± SD (n = 6). The asterisk indicates significant difference between the wild type and *yII-1* mutant (Student's t-test, **, p < 0.01). Chl, chlorophyll; Pn, net photosynthetic rate; Gs, stomatal conductance; Tr, transpiration rate.

Supplemental Table S2. A list of primers used in this study.

Primer name	Forward primer
Map-based cloning	
RM7562-F	AGACATGCCAATGTGATGGC
RM7562-R	TCGGTAGTATGGGGCTTGTGTC
RM3703-F	GAGAGAGAGGGAAAGGGAAAGG
RM3703-R	GCTCCCCGACATTAAACTG
YP1957-F	ATTCCTCTACATGGTTGATTT
YP1957-R	TTGTCCTCGTCCGAATCGCT
YP1959-F	ATGCTTGATTGCCATGCTAC
YP1959-R	CTAGGAGATATTGTAGCTA
YP2033-F	AGGTGCGTCTCTCGCTCGAT
YP2033-R	GCCTCTGATTCAAGAACACT
YP1963-F	ACAATAGCGTAGTACTACCA
YP1963-R	GAGGCAGAGATGAAGCTGCA
YP2039-F	AGCGTAGTACTACCAGTACT
YP2039-R	CATTGCATTGCAACACAGAT
RM3495-F	ACTCTCTAAACTGGAGCAAT
RM3495-R	CTTGATGCCATTCTAACATCC
YP1755-F	AATCCGATCGTACGAGCAAC
YP1755-R	GACAAGGGAAAGGAAACCCCTC
RM279-F	GCGGGAGAGGGATCTCCT
RM279-R	GGCTAGGAGTTAACCTCGCG
YP2344-F	TCTCACTCACGTGGACTCT
YP2344-R	CTCACCCCTAGGCTTGATAT
YP2392-F	GACGTTGAAGACAAGCCTC
YP2392-R	AGGGAGAAGGCATGGCTTAC
YP2869-F	CACTGCATTGCTCACAGT
YP2870-R	ACGAACAGGATGAATCCACC
<i>yII-2</i> mutant genotyping	
YP3180-F	CATCTTCTCTCCTGCGG
YP3181-R	GAATATGCAAGGAGCGCTTC
YP3189-F	ATAGGGTTCGCTCATGTGTTGAGCAT
Quantitive real time PCR	
YL1-F (YP2496)	ATGCCTCCACTGCCACAAT
YL1-R (YP2520)	CACGAACAGGATGAATCCAC
V1-F	AGAATCAGCGCGAGAAGAGAACCT
V1-R	TACACCAGCTTGGAGGAGCTGAA
V2-F	AGCAGATCCGTGATTACATGGCGA
V2-R	TGCCTCTTCACTCTGCAACCAA
V3-F	AACGAGAGATCTGGCTGAATGCT
V3-R	CTCTCATTAACATGTGTTGC
RpoA-F	TAGATGCTGTATCCATGCCT

RpoA-R	CTCTTCCTCCGTGTGAAGAA
OsSig2A-F	AGTCTTATGGCATCTTGAGTG
OsSig2A-R	GACCGCTTCTTCTTGAGG
Rps15-F	AGATAACGGAGACTTGCTCA
Rps15-R	GCTCCCTAATATCCAAC TGACT
RpoTP-F	AAGCAGACAGTGATGACATC
RpoTP-R	ATCTTGACACAATCACCAAG
Cab1-F	CCGGAGACGTTGCCAAGA
Cab1-R	ATGAGCACACCTGCACCG
RbcL-F	CTTGGCAGCATTCCGAGTAA
RbcL-R	TGTTAGTAACAGAACCTCT
PsaA-F	TAGCCTGGTCCAAGACGTA
PsaA-R	TTTGGACCAATTCAAGGTGA
PsbA-F	AGAGACGCGAAAGTACAAGC
PsbA-R	AAGITGCGGTCAATAAGGTA
LchP2-F	GAAGgAGATCAAGAACGGCC
LchP2-R	TTGCCGGGACGAAGTTGGT
PsbB-F	ATGGGTTGCCCTGGTATCGT
PsbB-R	CTCCACATTGGATCCAGAACAGG
PsbO-F	CGAGTTCCAGAACGACCAAGC
PsbO-R	GTGGCGACCAGATTCTGAT
Cytf-F	GGTCTATAATGCAACGTCACAGGT
Cytf-R	CCTTGAACCGTAATGGATCCTG
Actin-F	CATCTTGGCATCTCTCAGCAC
Actin-R	AACTTGTCCACGCTAATGAA
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Complementation Construct	
pCYL1-F	tctagaAGTCCCAGTAAATAGGGTC
pCYL1-R	gtcgacGCTTCTCGGATAACGACTCT
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Gus Staining	
pGUS-F	ggtaccAGTCCCAGTAAATAGGGTC
pGUS-R	ccatggGATATGCAGCAGCTGTGTAG
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GFP Assay	
YL1-GFP-F	gagtcATGCCTCCACTGCCACAAT
YL1-GFP-R	gtcgacTTGGGGAGCGAGCCCATTGT
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Yeast Two-Hybrid Assay	
YL1-F	catatgATGCCTCCACTGCCACAAT
YL1-R	ggatcccTTGGGGAGCGAGCCCATTGT
PsaA-F	gaattcTTGGCGGGTCTCTTGATGTC
PsaA-R	gagtcCTATCCTACTGCAATAATTCTCGCTAAG
AtpA-F	catatgTTGAACCTACTTCTTCTGAGGAAATTAGGC
AtpA-R	ctgcggTTATGTTGTTCTGAAGGGAAAACC
AtpB-F	catatgATGAGAACCAATCCTACTACTTCTCG
AtpB-R	ggatccTCATTCTCAATTGTTCTCCTCTTCT
Cytf-F	catatgTTGGACATGGAAAATAGAAACTT

Cytf-R	ctgcagCTAGAAATTCAATTGTACAATTGAAC
BiFC assay	
YL1-F	ggatccATGCCTCCACTTGCCACAAT
YL1-R	gtcgacTTGGGGAGCGAGCCCATTGT
YL1 _{CDS285} -R	gtcgacCCTCACTTCTCTTAAGTGAC
YL1 _{CDS286} -F	ggatccAGTCAAAGCTTGACGATGG
AtpB-F	ggatccATGAGAACCAATCCTACTACTTCT
AtpB-R	ggtaccTTTCTTCAATTGTTCTCCTCTTCT