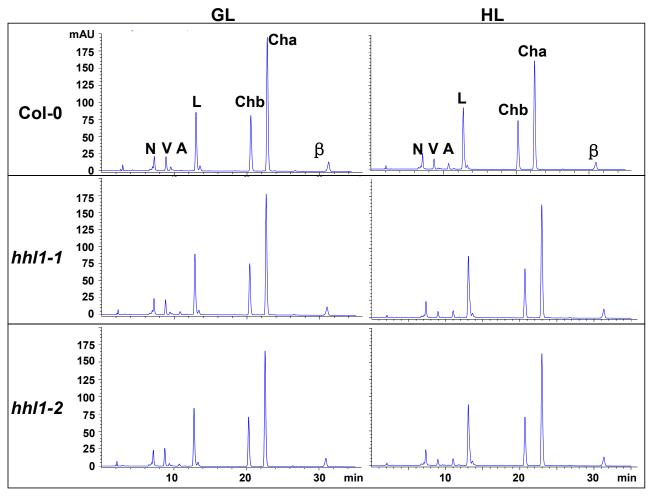


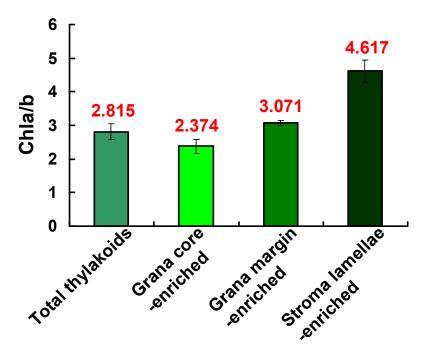
## Supplemental Figure 1. Isolation of the hhl1-0 Mutant.

(A) The  $F_v/F_m$  image after high-light treatment. Arrows indicate the *hhl1-0* mutant with abnormal  $F_v/F_m$  after a 3 h high light ( $\sim$ 1200  $\mu$  mol photons m<sup>-2</sup>s<sup>-1</sup>). The false color ranged from black (0) via red, orange, yellow, green, blue and violet to purple (1) as indicated at the bottom. (B) The  $F_v/F_m$  image after 2-d recovery ( $\sim$  100  $\mu$  mol photons m<sup>-2</sup>s<sup>-1</sup>) following 3 h high-light treatment. Arrows indicate the *hhl1-0* mutant with abnormal  $F_v/F_m$  following high-light treatment recovered to normal levels following recovery under growth light for 2 days. (C) Identification of insert site of T-DNA in *hhl1-0* mutant by TAIL-PCR. M, DL2000 marker; 1, the primary PCR product; 2, the secondary PCR product. (D) Model of insertion site of T-DNA in the *hhl1-0* mutant based on BLAST analysis after sequencing the secondary TAIL-PCR product.

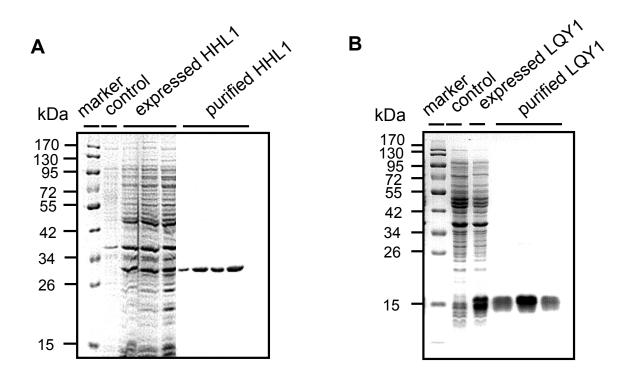


**Supplemental Figure 2.** HPLC Analysis of Pigments in the Wild Type (Col-0), *hhl1-1*, and *hhl1-2* Plants.

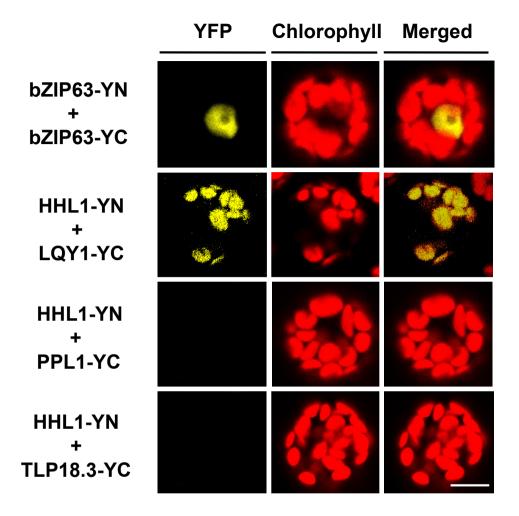
Comparison of pigment profile of the wild type (Col-0), *hhl1-1*, and *hhl1-2* before and after exposure to high light ( $\sim$ 1200  $\,\mu$  mol photons m<sup>-2</sup> s<sup>-1</sup>) for 3h. Each profile represent pigment extracted from 100 mg fresh weight of grinded leaves. The retention time and N, neoxanthin; V, violaxanthin; A, antheraxanthin; L, lutein;  $\beta$ ,  $\beta$ -carotene; Chl a, Chlorophylls a; Chl b, Chlorophylls b were indicated.



**Supplemental Figure 3.** The Chla/b Ratios of Grana Core-, Grana Margin-, Stroma Lamellae-Enriched, and Total Thylakoids. The Chla/b ratios from grana core-, grana margin-, stroma lamellae-enriched and total thylakoids in Figure 4D were measured by spectrofluorometry to confirm the successful fraction of thylakoids. The values are means  $\pm$  SE from three independent samples.

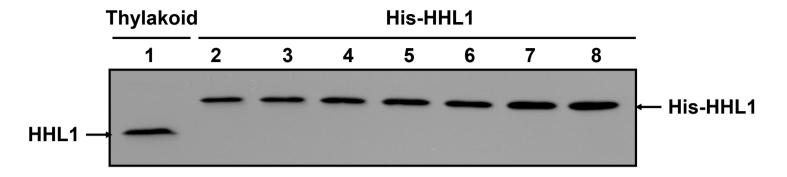


**Supplemental Figure 4.** Expression and Purification of HHL1 and LQY1. The BL21 cells were harvested after the addition of isopropylthio-  $\beta$  -D-gala ctoside for overnight, and the overexpressed His-HHL1 (A) and His-LQY1 (B) proteins were purified on a Ni-NTA agarose resin matrix. Samples were resolved by SDS-PAGE and stained with Coomassie blue.



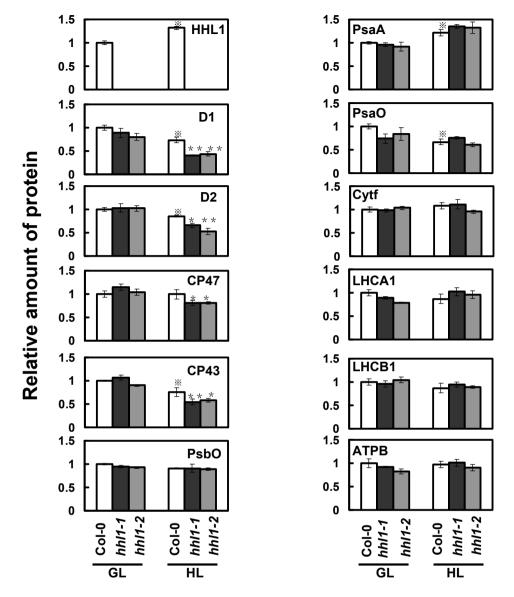
**Supplemental Figure 5.** BiFC Analyses Show HHL1 Interacts with LQY1, but not with PPL1 or TLP18.3.

HHL1 fused with the N terminus of YFP (YN), LQY1,PPL1 and TLP18.3 fused with the C terminus of YFP (YC) were cotransfected into protoplasts and visualized using confocal microscopy. As a positive control, both bZIP663 fused with YN and bZIP663 fused with YC were cotransfected into protoplasts. Bars =  $10 \,\mu$  m. All experiments were repeated at least two times with similar results.

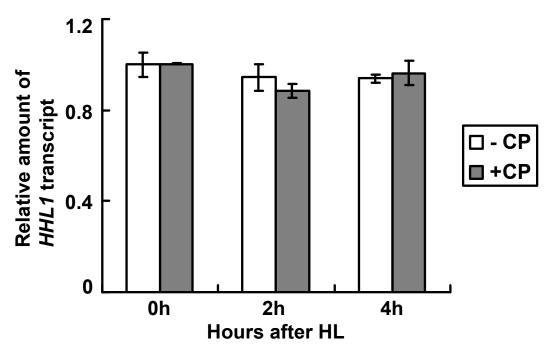


**Supplemental Figure 6.** Example of Titration Used for Stoichiometry Determination.

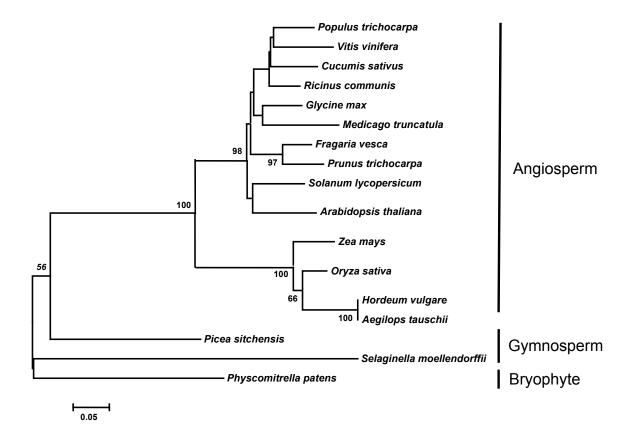
Various dilutions of thylakoid membranes were loaded on an SDS-PAGE gel, together with a dilution series of the recombinant HHL1 protein. Following transfer to nitrocellulose and immunodetection with anti-HHL1-specific antibody, the intensity of the immunological reaction was estimated by densitometry and related to the amount of chlorophyll (Chl) loaded. The first lane is loaded with thylakoid based on the amounts of chlorophyll are shown. 1,11.394  $\mu$  g Chlorophyll. Six lanes (3-8) loaded with different amounts of His tagged HHL1 purified from *E.coli* are shown. 2, 0.0096  $\mu$  g; 3, 0.0108  $\mu$  g; 4, 0.0120  $\mu$  g; 5, 0.0132  $\mu$  g; 6, 0.0144  $\mu$  g; 7, 0.0156  $\mu$  g; 8, 0.0168  $\mu$  g. Four independent titration were done with similar results, and the result of a representative experiment is shown.



**Supplemental Figure 7.** Relative Abundance of HHL1 and PSII proteins. Proteins immunodetected as in Figure 7 were analyzed with Phoretix 1D Software (Phoretix International, UK). The values (mean  $\pm$  SE, n = 3 independent biological replicates) are given as the ratio to protein amount of the wild type (Col-0) before 2-d high-light treatment. ' $\times$ ' indicates that the value is significantly different from the wild-type (Col-0) value under growth light conditions (Student's *t*-test; ' $\times$ ', *p* < 0.05). Asterisks indicate that the value is significantly different from the wild-type (Col-0) value after 2-d high-light treatment (Student's *t*-test; \*, *p* < 0.05; \*\*\*, *p* < 0.01).GL, growth light; HL, after 2-d high-light treatment.



**Supplemental Figure 8.** Transcript Analysis of *HHL1* Gene under High-light Treatment. Total RNA were isolated from wild type (Col-0) plants during exposure to high light in the absence of chloramphenicol (-CP) and in the presence of 200  $\,\mu$  g mL<sup>-1</sup> chloramphenicol (+CP), and the relative amount of *HHL1* transcript were determined through quantitative real-time PCR analysis. Relative mRNA levels of *HHL1* gene under different conditions were normalized with respect to *ACTIN* (*At3g18780*). *UBQ4* (*At5g20620*) was also used as reference gene with similar results (data not shown). The data represent means  $\pm$  SE. Three biological replicates for each sample were used, and at least two technical replicates were analyzed for each biological replicate.



**Supplemental Figure 9.** Phylogenetic Analysis of HHL1 Protein and Its Homologs in Other Land Plants.

Phylogenetic analyses of plant HHL1 were performed using the MEGA program (www.megasoftware.net). The phylogenetic tree was generated using MEGA5. Full-length homologous amino acid sequences of Arabidopsis HHL1 proteins in Physcomitrella patens, Selaginella moellendorffii, Picea stichensis, Populus trichocarpa, Vitis vinifera, Cucumis sativus, Ricinus communis, Glycine max Medicago truncatula, Fragaria ananassa, Prunus trichocarpa, Solanum lycopersicum, Oryza sativa, Zea mays, Hordeum vulgare and Aegilops tauschii were selected to generate a bootstrap neighbor-joining phylogenetic unrooted tree. Percentages over 50% from 1000 bootstrap replicates are shown. Bar = 0.05 amino acid substitutions.

## Supplemental Data. Jin et al. (2014). Plant Cell 10.1105/tpc.113.122424 -----altrlplkntg-rfeevg----larhslfssrtacreta---voorrmvfvvbak<mark>c</mark>kk -----alvrlplsns--kll<mark>edg----vv</mark>rhslfsartmhkphh---tyrr--p<mark>llve</mark>ak<mark>c</mark>kk Arabidopsis thaliana Cucumis sativus Ricinus communis Glycine max Populus trichocarpa Fragaria vesca Vitis vinifera Solanum lycopersicum Prunus persica Medicago truncatula Zea mays Hordeum vulgare Oryza sativa Aegilops tauschii Picea sitchensis Selaginella moellendorffii 1 ----GSAALKSC-----SKAGLEEHANRRDVS-----Physcomitrella patens Arabidopsis thaliana Cucumis sativus Ricinus communis 144 Glycine max Populus trichocarpa 145 137 Fragaria vesca 135 Vitis vinifera Solanum lycopersicum 139 Prunus persica 136 Medicago truncatula Zea mays 136 136 Hordeum vulgare Oryza sativa Aegilops tauschii Picea sitchensis Selaginella moellendorffii 43 109 Physcomitrella patens 52 119 139 EKETOKTATKOHRVLRTATEFRYGYKLVENI 137 EKETOKTAFKOFRVLRTATEFRYGYKLVENI 140 EKETOKTAFKOFRVLRSATEFRYGYKLVENI 145 EKEYOKSAFKOYRVLRSATEFRYGYKLVENI 146 EKETOKTAFKOHRVLRSATEFRYGYKLVENI 138 Arabidopsis thaliana -DAKESFEKLSSLNPGSDEKTE-- 222 NLRSALSTT<mark>DVIELPTQDQLKTVL</mark>DKVK<mark>DFF</mark> ---D<mark>akd</mark>sf<mark>gkltalgspegee</mark>sd-- 220 Cucumis sativus ---D<mark>akesfgkltaldstt-de</mark>pe-- 222 Ricinus communis ---D<mark>ake</mark>sf<mark>ckit</mark>slcttete<mark>e</mark>se--Glycine max Populus trichocarpa TS<mark>DVIELP#PD</mark>QLRT<mark>#FD</mark>KVKDFF( -DAKESFCKLTAIGSSESEESVKE 223 Fragaria vesca <mark>EKE</mark>IQKTAFKQYRVLRSATEFRY<mark>G</mark>YKIVE 136 EKELOKTAFROYRVLRSAKE FRYGYKLVENN 135 EKELKKLAMKOHRVLKSATE FRYGYKLVENN 140 EKELOKTAFKOYRVLRSATD FRYGYKLVENG 137 EKELOKSAFKOHRVLKSATE FRYGYKLVENG TT<mark>DVIE</mark>LP<mark>EQGELKTVL</mark>DKVRDFF --DAKESFCKLTALNSTT-EESE-- 218 Vitis vinifera ---D<mark>akesfg</mark>klt<mark>alpesseed</mark>sp-- 218 Solanum lycopersicum TS<mark>DVIE</mark>LP<mark>TP</mark>DKLKTV<mark>V</mark>DKVK<mark>DFF</mark> TSDVI<mark>E</mark>LP<mark>TKD</mark>KLKT<mark>TF</mark>DKVKI Prunus persica KLTTLNLSESEESE--140 EKETOKTA KOYRVLRSATDERYGYKIVE NENMRAJI STSDVIELPEKDKLKT TEDKVKDFFG----DIKESF 137 EKETOKSA EKOHRVLKSATEFRYGYKIVENGNVKAAL STSDVIQLPTEDKLKTVADKVKDFFG----DVKESF 137 EDDITDTAKEQYRVLKTENOFRYGYKVVEKGNIRS JITTSNVIELPEKDELKTVVDKVKDFFGDVTTGCKESF 148 EDDITDTAKEQFRVLKGETEFRYGYKIVEKGNIRS JITTSNVIELPEKEELKSVVDKVRDFFGDVTSGAKESF 157 EDDITDTAKEQFRVLKGETEFRYGYKIVEKGNIRS JITTSNVIELPEKEELKSVVDKVRDFFGDVTSGAKESF 158 EDDITDTAKEQFRVLKGETEFRYGYKIVEKGNIRS JITTSNVIELPEKEELKSVVDKVRDFFGDVTSGAKESF 159 EDDITDTAKEQFRVLKGETEFRYGYKIVEKGNIRS JITTSNVIELPEKEELKSVVDKVRDFFGDVTSGAKESF 160 EKKITAKUTUR ASSTORY STORY S Medicago truncatula ---DVKESFGEITSL-VTATDESE-- 219 OIT<mark>gsavskee</mark>eaes 226 Zea mays Hordeum vulgare QIT<mark>GSASAAEAE</mark>AEGK 226 AOITGSVS-AEABAPVE 236 AOITGSASAAEABAEAK 246 SISNLDTGAEEDEK-- 228 Orvza sativa Aegilops tauschii Picea sitchensis Selaginella moellendorffii 110 Physcomitrella patens Arabidopsis thaliana 220 --- ETSDEKEN 230 220 --- ENSTEKEKVKT 231 222 --- EVSKEKEKVKG 233 228 --- EDTKEKEKVKG 239 Cucumis sativus Ricinus communis Glycine max Populus trichocarpa ---- Possible signal peptide Fragaria vesca - - - Possible transmembrane domain Vitis vinifera Solanum lycopersicum Predicted VWA domain Prunus persica Medicago truncatula Zea mays Hordeum vulgare Oryza sativa Aegilops tauschii

Supplemental Figure 10. Alignment Analysis of HHL1 Protein in Other Land Plants.

Picea sitchensis

Physcomitrella patens

Selaginella moellendorffii

The amino acid sequence of the At1g67700 protein was compared with homologous sequences from other land plants, including *Physcomitrella patens*, *Selaginella moellendorffii*, *Picea stichensis*, *Populus trichocarpa*, *Vitis vinifera*, *Cucumis sativus*, *Ricinus communis*, *Glycine max*, *Medicago truncatula*, *Fragaria ananassa*, *Prunus trichocarpa*, *Solanum lycopersicum*, *Oryza sativa*, *Zea mays*, *Hordeum vulgare and Aegilops tauschii*. Same colors indicate conserved amino acids. Possible chloroplast signal peptide, transmembrane domain, and predicted VWA domain are shown above. The sequences similar to Thimet Oligopeptidase (TOP) in *Arabidopsis thaliana* (28-222 amino acids ) are not shown. The sequences were aligned with ClustalW.

203 -----QSKDNK-----

Supplemental Table 1. Chlorophyll Contents and Chlorophyll Fluorescence Parameters in Wild-Type (Col-0) and hhl1 Mutants

	Growth Light			After 3h High Light		
_	Col-0	hhl1-1	hhl1-2	Col -0	hhl1-1	hhl1-2
Chl a (mg/g FW)	1.423±0.031	1.424±0.011	1.454±0.047	1.276±0.030	1.238±0.038	1.336±0.031
Chl b (mg/g FW)	0.484±0.001	0.483±0.009	0.486±0.018	0.464±0.003	0.451±0.006	0.483±0.008
Chl a/b	2.940±0.056	2.946±0.079	2.998±0.205	2.753±0.048	2.768±0.037	2.768±0.037
Total Car (mg/g FW)	0.242±0.015	0.249±0.003	0.241±0.033	0.248±0.026	0.251±0.009	0.244±0.021
$F_0$	0.108±0.001	0.115±0.007	0.113±0.001	0.121±0.001	0.139±0.007*	0.144±0.007*
F <sub>m</sub>	0.584±0.011	0.585±0.007	0.612±0.044*	0.399±0.003	0.247±0.010**	0.214±0.015**
$F_{v}/F_{m}$	0.823±0.004	0.821±0.005	0.822±0.004	0.696±0.002	0.514±0.010**	0.518±0.011**
1-qP	0.184±0.001	0.186±0.007	0.187±0.001	0.125±0.036	0.021±0.008**	0.025±0.013**
1-qL	0.421±0.012	0.425±0.006	0.426±0.009	0.279±0.001	0.068±0.009**	0.074±0.006**

Measurements of chlorophyll fluorescence parameters were done on plants after 30 min of dark adaption. For 1-qP and 1-qL measurements, Actinic light intensity was 100 µmol photons m<sup>-2</sup> s<sup>-1</sup>. Chl a, chlorophyll a; Chl b, chlorophyll b; FW, fresh weight; Total Car, total carotenoids. The concentration of total carotenoids (mgL<sup>-1</sup>) was calculated as (OD<sub>480nm</sub>–0.6383OD<sub>645nm</sub>+0.1143OD<sub>663nm</sub>)/0.218. F<sub>0</sub>, minimal fluorescence; F<sub>m</sub>, maximal fluorescence; F<sub>v</sub>/F<sub>m</sub>, maximum quantum yield of PSII; 1-qP and 1-qL, parameters estimating the fraction of PSII in close states or excitation pressure of PSII based on a lake mode; Data are presented as means  $\pm$  SE of five biological repeats. The asterisk indicates a significant difference between the mutant and wild type in corresponding light conditions (Col-0) (Student's t test; \*, P < 0.05; \*\*\*, P < 0.01).

hhl1-2/Col-0

General Light

hhl1-1/Col-0

were analyzed for each biological replicate.

Genes

## Supplemental Table 2. Transcript Analysis of Wild-Type (Col-0) and hhl1 Mutants

After 3h-High Light

hhl1-2/Col-0

hhl1-1/Col-0

PsbA	1.544±0.106	1.688±0.074	0.671±0.122	0.517±0.005	
PsbD	1.214±0.018	1.035±0.112	0.871±0.029	0.652±0.010	
PsbO	1.956±0.172	1.395±0.013	1.132±0.007	1.404±0.029	
PsbS	1.373±0.029	1.459±0.035	0.886±0.039	0.824±0.012	
PsaA	1.079±0.037	0.902±0.035	0.908±0.002	0.836±0.004	
PsaE	1.227±0.001	1.157±0.023	0.438±0.001	0.578±0.007	
LHCB1	1.081±0.085	1.069±0.148	1.110±0.013	1.041±0.001	
ATPB	1.212±0.107	1.324±0.019	1.083±0.043	0.941±0.006	
YCF2	1.031±0.020	0.972±0.010	0.454±0.067	0.463±0.033	
ELIP2	1.231±0.042	1.433±0.001	1.032±0.206	1.190±0.125	
ZAT12	1.347±0.033	1.866±0.001	6.302±0.233*	6.573±0.033*	
ZAT10	1.697±0.420	1.711±0.309	7.304±0.025*	7.769±0.263*	
The transcript levels of representative genes encoding chloroplast proteins and					
stress related proteins were analyzed by quantitative real-time RT-PCR. For					
individual genes, relative mRNA levels were normalized with respect to ACTIN					
(At3g18780). The relative mRNA levels of hhl1 mutants relative to wild type (Col-0)					
in corresponding conditions were given. UBQ4 (At5g20620) was also used as					
reference gene with similar results (data not shown). The data represent means ±					
SE. '*', stress related genes of which transcription are significantly induced. Three					

biological replicates for each sample were used, and at least two technical replicates

## Supplemental Table 3. A List of Primers Used in This Study

Drimor	Coguence				
Primer Sequence					
Mutant identification					
` '	5'-TAGCCATATCGAAACTCGGTG-3'				
` '	5'-CGGTTCTGGTTCGGTTTAAAC-3'				
	5'-GCAAAAGATAACCTCTTGGGG-3'				
	5'-CTCACCTTCACGAAGCTTTTG-3'				
` ,	5'-TTGGTGAACAAAACAGCATTG-3'				
	5'-TACGTTTAAGCGGGTGTATCG-3'				
RT PCR					
HHL1-F	5'-TTGGATCCGAGACGCCGT-3'				
HHL1-R	5'-CTCGAGGGCCTTGGCTTTCTC-3'				
LQY1-F	5'-GGATCCATGCCAGTTTCAGCTC-3'				
LQY1-R	5'-CTCGAGGTCATCGTCCTTGAACTC-3'				
UBQ10-F	5'-GATCTTTGCCGGAAAACAATT GGAGGATGGT-3'				
UBQ10-R	5'-CGACTTGTCATTAGAAAGAAAGAGATAACAGG-3'				
His-tag Constru	uct				
HHL1-F	5'-TTGGATCCGAGACGGCGGT-3'				
HHL1-R	5'-CTCGAGGGCCTTGGCTTTCTC-3'				
LQY1-F	5'-ACACAGGATCCGAGACTCAAATCGACAATGC-3'				
LQY1-R	5'-ACACACTCGAGTTAGTCATCGTCCTTGAACT-3'				
GFP assay					
HHL1-F	5'-GGGGATCCATGGAAGTGAGTATGTCT-3'				
HHL1-R	5'-AACTCGAGGGCCTTGGCTTTCTC-3'				
BiFC assay					
HHL1-F	5'-GGGGATCCATGGAAGTGAGTATGTCT-3'				
HHL1-R	5'-AACTCGAGGGCCTTGGCTTTCTC-3'				
LQY1-F	5'-GGATCCATGCCAGTTTCAGCTC-3'				
LQY1-R	5'-CTCGAGGTCATCGTCCTTGAACTC-3'				
Quantitive real					
PsbS-F	5'-CTCTTCAAACCCAAAACCAAAGCT-3'				
PsbS-R	5'-GCCTTTGTGAAACCAATCCCA-3'				
PsbO-F	5'-CAGCCTCTCCCAATCCAC-3'				
PsbO-R	5'-GAGGTGGCAAGAGCGAATC-3'				
PsbA-F	5'-GCATAGCACTGAATAGGGAGCCG-3'				
PsbA-R	5'-GCGACCTTGGATTGCTGTTGC-3'				
PsbD-F	5'-CAGCGAAGATCCAGAATT-3'				
	5'-TGGTAGAACCTCCTCAGGGA-3'				
PsbD-R					
PsaA-F	5'-CTACTTTGCCACCCACTGC-3'				
PsaA-R	5'-TGAGTGCTTTAGGGCGTCC-3'				
LHCB1-F	5'-CGTGACCATGCGTCGTACCGTC-3'				
LHCB1-F	5'-CCT CAG GGAATGTGCATCCG-3'				
ATPB-F	5'-TCTCAGAAACCCTAGTTGAAGTT-3'				
ATPB-R	5'-GTTGAATCCACCACATAATCC-3'				
ZAT10-F	5'-ATCACACGTTTGCACCATCT-3'				
ZAT10-R	5'-TGCTAACGTGGCTAGTGGAC-3'				
ZAT12-F	5'-TGGGAAGAGTGGCTTGTTT-3'				
ZAT12-R	5'-TAAACTGTTCTTCCAAGCTCCA-3'				
YCF2-F	5'-TCTTTATTGGTTCTACCTCCTA-3'				
YCR2-R	5'-TGCCTCCATTATGTTGTTG-3'				
HHL1-F	5'-AGCAGCGGCGGATGGTTTT-3'				
HHL1-R	5'-GCATAGGAGGAGGTGTACGTTGATAC-3'				
ACTIN-F	5'-GGTAACATTGTGCTCAGT GGTG-3'				
ACTIN-R	5'-CTCGGCCTTGGAGATCCACATC-3'				
-					