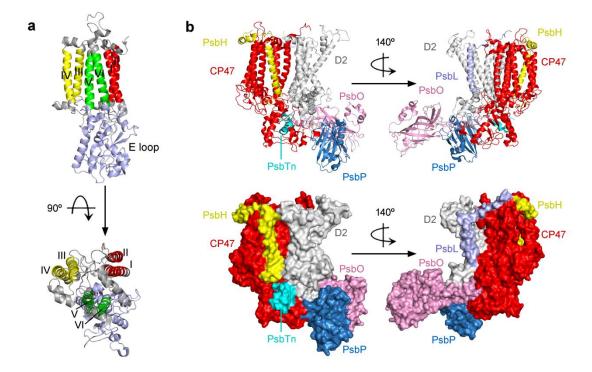
Supplementary Material for

Thylakoid Protein FPB1 Synergistically Cooperates with PAM68 to Promote CP47 Biogenesis and Photosystem II Assembly

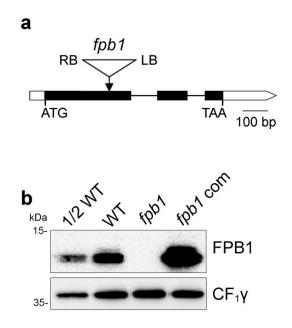
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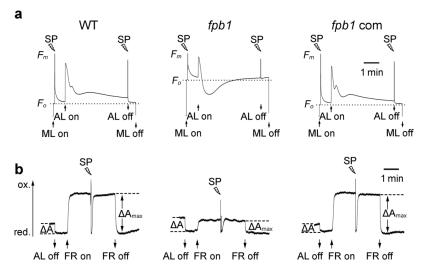
This PDF file includes: Supplementary Figures 1 to 11 Supplementary Table 1



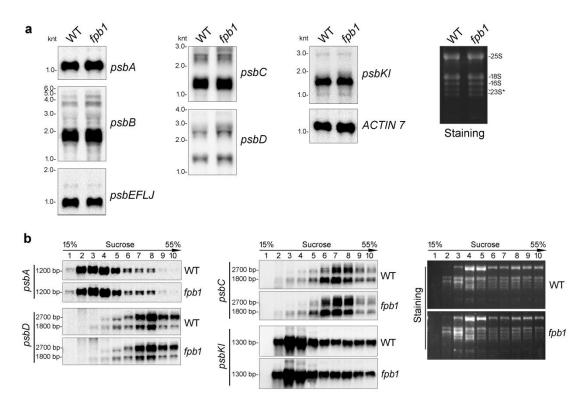
Supplementary Fig. 1 Overall structure of CP47 and its associated PSII subunits in spinach. a Structure of CP47 viewed along the membrane plane (upper) and along the membrane normal from the stromal side (bottom). The TMDs of I/II, III/IV, V/VI, and the E loop are coloured as indicated. **b** Structure of CP47 and its associated subunits viewed along the membrane plane. The proteins are shown with ribbon models (upper) and surface models (bottom) and are coloured as indicated. Protein structures are visualized using PyMOL based on PDB ID 3JCU¹.



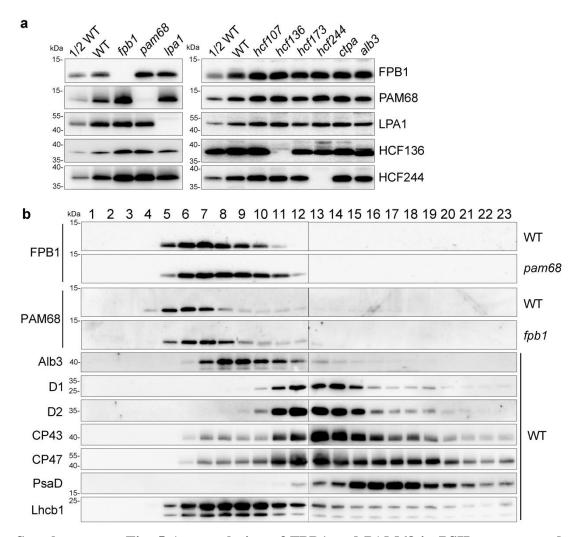
Supplementary Fig. 2 Genetic characterization of the *fpb1* **mutant. a** Structure of the *FPB1* gene and T-DNA insertion site in the *fpb1* mutant. White boxes, black boxes, and lines represent UTRs, exons, and introns, respectively. T-DNA insertion occurred in the first exon of *fpb1* (SALK_048033). **b** Immunoblot of FPB1 protein. Equal amount of total protein was separated by SDS-urea-PAGE and transferred onto nitrocellulose membranes for immunobloting using antibodies against FPB1 and CF₁ γ (loading control). Blots are representative of two independent experiments. Source data are provided as a Source Data file.



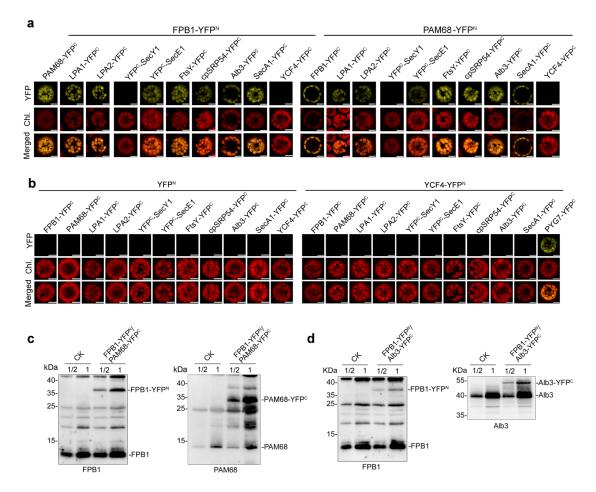
Supplementary Fig. 3 Photosynthetic characterization of *fpb1*. a Chlorophyll *a* fluorescence transients. After dark adaption for 20 min, leaves from four-week-old plants were illuminated with measuring light (ML) to determine the minimum chlorophyll *a* fluorescence (F_o). Subsequently the maximum chlorophyll *a* fluorescence (Fm) was measured by firing a saturating pulse (SP). Then the leaves were exposed to the actinic light (AL, 64 µmol photons m⁻² s⁻¹) for 4 min. **b** P700 absorbance kinetics. After exposure to AL (209 µmol photons m⁻² s⁻¹) for 3 min, leaves were illuminated with FR (far-red) light for 4 min to induce maximal oxidation of P700 (Δ Amax). During illumination, re-reduction of P700⁺ was induced with a saturating pulse (SP). Source data are provided as a Source Data file.



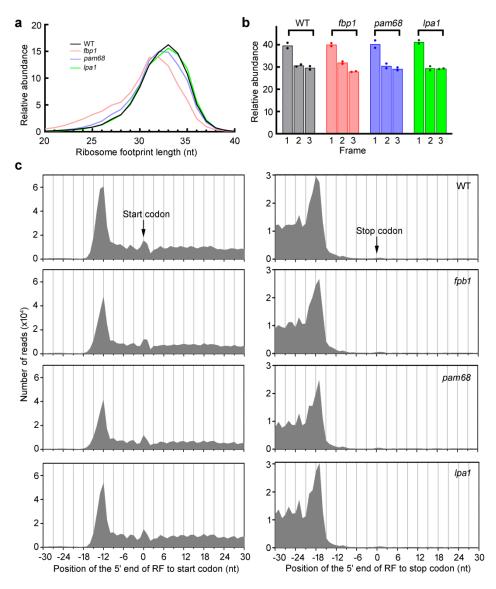
Supplementary Fig. 4 Abundance of the *psb* **transcripts and their association with polysomes. a** RNA gel blot analyses. Total RNA isolated from WT and *fpb1* leaves of four-week-old plants was probed with DIG-labelled specific probes as indicated. An *ACTIN 7* probe and RNA staining were used as loading controls. The sizes for the RNA lad der are indicated. **b** Polysome association studies of WT and *fpb1* plants. Whole leaf extracts from three-week-old plants were separated by centrifugation in 15-55% sucrose density gradients. The gradients were then divided into 10 fractions and RNA was isolated for gel-blot analyses with DIG-labelled DNA probes corresponding to the *psbA*, *psbD*, *psbC*, and *psbKI* mRNAs. The rRNAs were stained and used as loading controls. Approximate sizes of the main bands are shown and the sizes of the corresponding bands can found in (**a**). Blots are representative of at least two independent experiments. Source data are provided as a Source Data file.



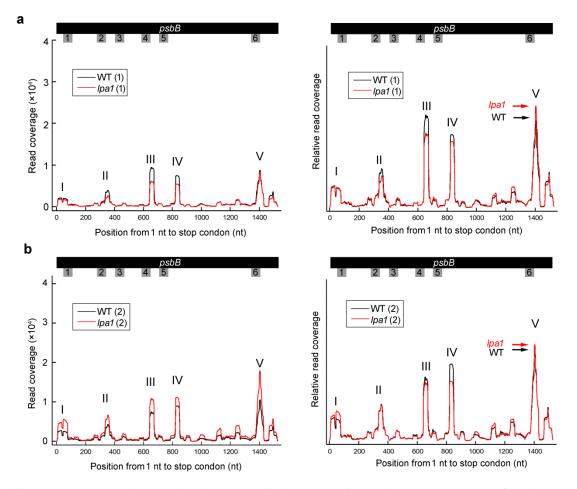
Supplementary Fig. 5 Accumulation of FPB1 and PAM68 in PSII mutants and their complex formation. a Immunoblot analysis of FPB1, PAM68, LPA1, HCF136, and HCF244 in various mutants. Thylakoid membranes isolated from four-week-old *fpb1*, *pam68*, and wild-type plants grown on soil as well as from the other mutants grown on MS medium containing 3% sucrose were subjected to SDS-urea-PAGE with equal protein loading. b Sucrose density gradient analyses of FPB1 and PAM68. Freshly isolated thylakoids from four-week-old plants were solubilized with 1% β -DM and protein complexes were separated on a linear sucrose gradient (0.1 M to 1.0 M). Twenty-three fractions of equal volume were collected from top to bottom of the gradients and the proteins were subjected to SDS-PAGE (for Alb3) or SDS-urea-PAGE (for others) and then probed with antibodies as indicated. Blots are representative of at least two independent experiments. Source data are provided as a Source Data file.



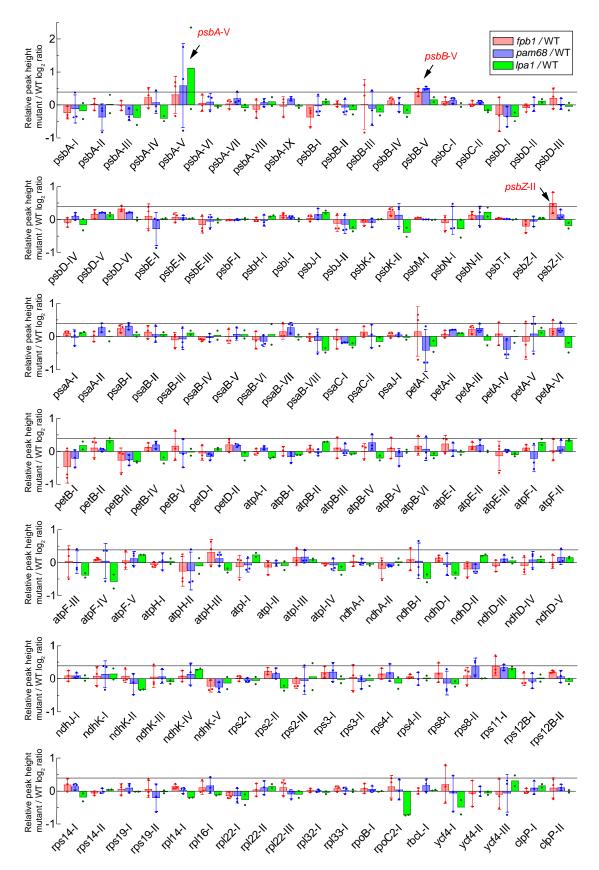
Supplementary Fig. 6 BiFC assay for the interaction of FPB1 and PAM68 with chloroplast proteins in Arabidopsis protoplasts. a Full-length FPB1 and PAM68 were fused with the N-terminal part of YFP (YFP^N) at their C-terminus. Other proteins indicated at the top were fused with the C-terminal part of YFP (YFP^C) at their N- or C-terminus to ensure that the fused YFP^C is present on the stromal side of thylakoids (See Methods). The fusions were transiently co-expressed in Arabidopsis mesophyll protoplasts and the signal was observed using confocal microscopy. Bars =10 µm. b Chloroplast targeting sequence of RbcS and the PSI assembly factor YCF4 were fused with the N-terminal part of YFP (YFP^N) at their C-terminus. The fusions and the proteins fused with the C-terminal part of YFP (YFP^C) were transiently co-expressed in protoplasts. Bars =10 μ m. c, d Immunoblot analysis of the fusion proteins in protoplasts expressing FPB1-YFP^N/PAM68-YFP^C (c) and FPB1-YFP^N/Alb3-YFP^C (**d**). Thylakoids were isolated from protoplasts and the proteins were separated by SDS-urea-PAGE (FPB1 and PAM68) or SDS-PAGE (Alb3) and immunoblotted with antibodies. Protoplasts without plasmid transformations were used as a negative control (CK). Protein dilutions were loaded as reference in the blots. Data are representative of two independent experiments. Source data are provided as a Source Data file.



Supplementary Fig. 7 Assessments of Ribo-seq data. a Read length distribution of ribosome footprints. Values are the means from two independent replicate samples in the first experiment. **b** Three-nucleotide (nt) periodicity of the Ribo-Seq data. Fractions of 31-33 nt at the first (1), second (2), and third (3) nucleotide positions of the start codon are shown. Values are the means with single dots for two independent replicate samples in the first experiment. **c** Meta-analysis of ribosome footprints mapping near the start and stop codon. Values show the number of total footprints with 5' end at each position from the first replicate sample. All genes (22895 detected genes) with unique mapped reads of ribosome footprints were analysed. Source data are provided as a Source Data file.

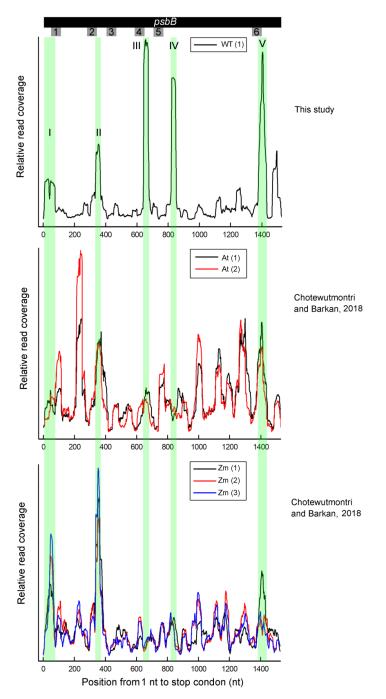


Supplementary Fig. 8 Distribution of ribosome footprints along the *psbB* **ORF in WT and** *lpa1* **mutant plants.** The Y-axis shows the total reads at each position (left) or normalized based on the total reads (right). Transmembrane domain (TMD)-encoding positions on the *psbB* mRNA are indicated from 1 to 6. The data from the first (**a**) and second (**b**) replicates of WT are the same as in Figs. 6c and d, since these genotypes were analysed in a same experiment. Source data are provided as a Source Data file.



Supplementary Fig. 9 Chloroplast genome-wide analysis of ribosome pausing between the different mutants and WT. A threshold was set in this analysis, in which the ribosome footprint reads at each position along the ORF less than 500 (38

chloroplast genes) were excluded. For the remaining 50 chloroplast genes, ribosome footprints of each gene from three replicates of WT, *fpb1*, *pam68* and two replicates of *lpa1* were normalized based on the total reads (Supplementary Data 3). The relative heights of the main peaks of each gene of the mutants compared with WT were calculated and plotted in a log_2 scale on the Y-axis. The numbers of major peaks of each gene are indicated as Roman numerals. Values are the means \pm SD from three independent replicates of *fpb1* and *pam68* samples and the means from two replicates of *lpa1*. Lines with the value corresponding to the relative height of *psbB*-V in *fpb1* are shown. Source data are provided as a Source Data file.



Supplementary Fig. 10 Comparison of distribution of ribosome footprints along the *psbB* **ORF in Arabidopsis and maize.** Distribution of ribosome footprints along the *psbB* ORF of WT (1) in this study is compared with that of Arabidopsis and maize reported by Chotewutmontri and Barkan². Ribosome footprints of *psbB* from two Arabidopsis and three maize samples were normalized based on the total reads, respectively, and the relative read coverage is shown. Transmembrane domain (TMD)-encoding positions on the *psbB* mRNA are indicated from 1 to 6. Five peaks from I to V are indicated with light green shadows. Accession number for At(1), At(2), Zm(1), Zm(2) and Zm(3) are SRX3744028, SRX3744024, SRX3744002, SRX3744022 and SRX3744035, respectively. Source data are provided as a Source Data file.

a 1 10 20 30 40 1 50 60 70 80 1	90 100
AtpsbB ATCCCTTCCCTCCCTCCCTCCTCCTCCTCCTCCTCCTCCT	GTTICCTCGCTTGGG IGTTICCTCGCTTGGG 190 200
AtpsbB CITCENTCEATCECH (TATA GAATTACO CITTETTICATCCCTC (CA CC CITTETI (ATCCAMTETGGAACAAGGTATCHTCCT) (A ZmpsbB CITCENTCAATCCCI IIAAA GAATTACO CITTETTICATCCCTC ICA ICC CITTETI (ATCCAMTETGGAACAAAGGTATCHTCCI A 20 220 220 220 220 220 220 220 220 220	ATACC T <u>TTCATGAC</u> ATCCC (TTCATGAC
AtpsbB TCCHTHACGANTANG (ANTIC TIGGGC (GCHTCGA) TATTI ACAGG (GC ACH) TAACGANTCCGGGT (TTIGGACTA) (GAAGG (G ZmpsbB TCCHTHAGGANTANG (ANTIC TIGGGC (GCHTGGA) TATTI ACAGG (GC ACH) TAACGANTCCGGGT (TTIGGACTA) (GAAGG (GT	I G GC A GG I GO GCA T
AtpsbB All CIGNUTCICCC INCIDENCECCACCIAN INCCCATICACCIAN AND BASE	390 400 MAAACCTTCTTTGG MAAACCTTCTTTGG
410 430 440 3 450 490 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 480 470 470 470 470 470 470 470 470 470 47	490 500 FC TCCTGCAATATC FC TCCTGC ATATC
AtosbB (CCI) Incode (CCI) And (CCI)	590 600 CACCAATACC OTO T CACCAATACC TITO G
AtosbB CATCATANTICG ICC ICC ACC HATATCACCCCCCCCATCIL ACTACCCCCCCCAACC LATAAAAACC HATATCACCCCCCCCAACC LATAAAAACC HATATCACCCCCCCCCAACC LATAAAAAACC HATATCACCACCCCCCCAACC LATAAAAAAACC HATATCACCACCCCCCCAACC LATAAAAAAACC HATATCACCACCCCCCAACC LATAAAAAAACC HATATCACCACCCCCCAACC LATAAAAAAACC HATATCACCACCCCCCAACCACCCCCAACCACCACCACCAC	690 700 ACGTATGGGCAATA
AtosbB Internate Children (Children and Children and Chil	790 800 TCC (ATCGAATT
810 820 820 10 820 850 850 850 870 870 870 870 870 870 870 870 870 87	890 900
	990 1,000
	1,090 1,100 TTTGAAAO TTTTCC
ZIIIpsbB MINE AIMABELDI GOARDON I, 120 (1300 MINE) 1400 (1400 MINE) 1400 (140 MINE) 1400 (140 MINE) 1400 (1400 MINE) 1400 (14	1,190 1,200
1,210 1,230 1,230 1,240 1,250 1,260 1,260 1,270 1,280 Atosbb Thi Thytochtoc (Iganobi (Matterna) (Activity) (Actornal (Activity) (Actornal (1,290 1,300 TTTTGAATTAGATC
Zmpsbb III (INNIGGIEGE GAAGEI) IANIGEAGEI ACHINIT (IGUEGE IG INDICH) AMAMANANGE (G. CC IGUEGAANI) (G. ANA 1300 - 1300 - 1300 - 1300 Alpsbb II (INNIGGIEGE GAAGEI) CANGERENNIG (GAAGE CAAGE CAAGE CAAGE) (GENTGEN) (M. HINGE CAACEI) (G. INNIGGIE I INGER CAACEINIGEANIG (GANGE) (I. CE) (GANGE) (I. CE) (INNIGGIE II. CE) (INNIGEI II. CE) (INNIGGIE II. CE) (INNIGGIE II. CE) (INNIGGIE II. CE) (INNIGEI III. CE) (INNIGEI II. CE) (INNIGEI II. CE) (INNIGEI II. CE) (INNIGEI III. CE) (INNIGEI IIII. CE) (INNIGEI III. CE) (INNIGEI IIII. CE) (INNIGEI IIIIIIII	1,390 1,400
	1,490 1,500
Zmpsbb Hitggenige (CG) (GANCO II) INCO (ENGANGEDINYIGCIGGIANICATICEAGA) (Incentigoteangigeanni) (CC AA (ANTICEA 1.500 Atpsbb (COANCETACAM) A (CACAN (CCC) (CICA)	AAAA]GTTGGAGAT
ZmpsbB CCANCINACAM CGACATCA CCONCIUTA	
b 100 100 100 100 100 100 100 100 100 10	
ZmCP47 MCL2WWWHWWWNDCCRUISWHWHWHWAAXSCWACSMADYELAW DDRSDEVEDDAWROCKIEV PENTREGENSUSCCUVIN	190 200

ZmCP47	MGLPWYRVHTVVLN	DPGRLLSVHIM	HTALVSGWAC	SMALYELAVI	FDPS DPV LDPM	WRQGMFVIPF	MTRLGITNSW	GGWSISGGTV	TNPGIWS YE	GVAGAH
	2 110	120	130	140	3 150	160	170	180	190	200
AtCP47	IVFSGLCFLAAIWH	WVYWDLEIFCD	ERTGKPSLDL	PKIFGIHLFI	SGVACFGFGA	FHV TGL YGPG	IWVSDPYGLT		GVEGFDPFVI	
ZmCP47	IVFSGLCFLAAIWH	WVYWDLEIFCD	ERTGKPSLDL	.PKIFGIHLFI	AGVACFGFGA	FHVTGLYGPG	HWSDPYGLT	GKVQAVNPAW	GAEGFDPFVI	PGGIAS
	4 210	220	230	240	5 250	260	270	280	290	300
AtCP47	HHIAAGTLGILAGL	FHLSVRPPQRL	YKGLRMGNIE	TVLSSS IAAV	FFAAFVVAGT	MWYGSATTPI	ELFGPTRYQW	DQGYFQQEIY	RRVSAGLAE	
ZmCP47	HHIAAGTLGILAGL	FHLSVRPPQRL	YKGLRMGNIE	ETVLSSS IAAV	FFAAFVVAGT	MWYGSATTPI	ELFGPTRYQW	DQGYFQQEIY	RRVSDGLAE	NISLSE
	310	320	330	340	350	360	370	380	390	400
AtCP47		IGNNPAKGGLF	RAGSMDNGDC	JAV GWLGHPV	FRNKEGRELF	VRRMPTFFET	FPVVLVDGDG	IVRADVPFRR	AESKYSVEQ	VGVTVE
ZmCP47	AW <mark>S</mark> KIPEKLAFYDY	IGNNPAKGGLF	RAGSMDNGDC	JAVGWLGHP\	FRDKEGRELF	VRRMPTFFET	FPVVLVDEEG	IVRADV PFRR	AESKYSVEQ	VGVTVE
	410	420	430	440	450	460 6	470	480	490	500
AtCP47		ATVKKYARRAQ	LGEIFELDRA	TLKSDGVFRS	SPRGWFTFGH	ASFALLFFFG	HIWHGARTLF		LDAQVEFGAI	FQKLGD
ZmCP47	FYGGELNGVSYSDP	ATVKKYARRAQ	LGEIFELDRA	TLKSDGVFRS	SPRGWFTFGH	ATFALLFFFG	HIWHGARTLF	RDVFAGIDPD	LDAQVEFGI	FQKNGD
	508									
	PTTKRQAV									
ZmCP47	PTTRRQAA									

Supplementary Fig. 11 Sequence alignment of CP47 and *psbB* **of Arabidopsis and maize.** a Sequence alignment of *psbB* CDS from Arabidopsis and maize. The sequence of *AtpsbB* (*ATCG00680*) and *ZmpsbB* (*GRMZM5G808939_P01*) were aligned on Geneious 6.1.8 using parameters 65% similarity and global alignment with free end gaps. The sequences of *AtpsbB* and *ZmpsbB* share 87.8% sequence identity. **b** Alignment of CP47 from Arabidopsis and maize. The sequence of AtCP47 (ATCG00680) and ZmCP47 (GRMZM5G808939_P01) were aligned on Geneious 6.1.8 using Blosum62 and global alignment with free end gaps. AtCP47 shares 96.1% amino acid sequence identity with ZmCP47. Regions of the ribosome footprint peaks are indicated with red lines from I to V. The transmembrane domains (TMDs) are indicated with blue lines from 1 to 6.

Supplementary Table	e 1 Primers	used in th	is work
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Primer names	Sequences 5' to 3'		
	ection of T-DNA insertion sites		
FPB1-TDNA-F	CAAAACTATAACGTGTGGCTGC		
FPB1-TDNA-R	CAGAAGTGGGGATTCCAGTAAG		
PAM68-TDNA-F	CGAACTGGATTAAAGCGGTATT		
PAM68-TDNA-R	GGTTTGTTTTTTGCGTGTGTG		
LPA1-TDNA-F	AGATATTGGCGATAATTTCCG		
LPA1-TDNA-R	CTCTACTCAATTCTAAGGTTTTACG		
CtpA-TDNA-F	CCGAGATTGATGAACTAATGAG		
CtpA-TDNA-R	ATGGTGGTGAATCAGTGGTAAC		
SAIL-LB2	GCTTCCTATTATATCTTCCCAAATTACCAATACA		
SALK-LBa1	TGGTTCACGTAGTGGGCCATCG		
TLB2	ATAATAACGCTGCGGACATCTACATTTT		
	nplementation vector construction		
FPB1-com-F	CAATCTAGAGAAGATTCCTACTCCAAAACAACC		
FPB1-com-R	CAACTCGAGTTAAAGAAAGCCATCTCTTTTATC		
	pare hybridization probes for RNA gel blot analyses		
psbA-F	TTATCCATTTGTAGATGGAGCCTCA		
psbA-R	ATGACTGCAATTTTAGAGAGACGCG		
psbB-F	GCAAGGATCCATGGGTTTGCCTTGG		
psbB-R	GCAACTCGAGATCAGACTGCTTGTCG		
psbC-F	GCGGGATCCATGAAAACCTTATATTCC		
psbC-R	GGCCTCGAGTTAGTTAAGAGGAGTCATG		
psbD-F	TGTTCGGAAATGGTTGAAGTAGATG		
psbD-R	GGTAGAACCTCCTCAGGGAATATAA		
psbEFLJ-F	CGCCTCGAGAATTTCTACAGGGATG		
psbEFLJ-R	GCGGGATCCATGTCTGGAAGCACAGGAG		
psbKI-F	CCGCTCGAGTATCCACAAGAACACC		
psbKI-R	CTTGGATCCTTATTCTTCACGTCCCGG		
psbH-F	ATGGCTACAAAACTGTTGAAG		
psbH-R	GAAATTCCATCCAGTAGAACAG		
ACT7-F	GGTGTCATGGTTGGTATGGGTC		
ACT7-R	CCTCTGTGAGTAGAACTGGGTGC		
Primers used for protein expression vectors construction			
FPB1-GFP-F	TAA <u>TCTAGA</u> ATGGCCGCGTCTCTAACATCTC		
FPB1-GFP-R	AAG <u>GGATCC</u> AAAGAAAGCCATCTCTTTTATCTTG		
FPB1-pET28a-ab1-F	CAT <u>GAATTC</u> GCTGGGAGGAGGAAGGGTC		
FPB1-pET28a-ab1-R	AAA <u>CTCGAG</u> AACCTCAAAGCTCTCTTGGG		
FPB1-pET28a-ab2-F	CAT <u>GGATCC</u> GCTGGGAGGAGGAGGAGGGTC		
FPB1-pET28a-ab2-R	AAA <u>GAATTC</u> AACCTCAAAGCTCTCTTGGG		
Alb3-pET28a-F	TGG <u>GGATCC</u> AATAATGTACTTAGTACCGCCG		
Alb3-pET28a-R	TTA <u>CTCGAG</u> TACAGTGCGTTTCCGCTTCGATC		
Primers used for vec	tor construction of split-ubiquitin assay		

51510-NCW-F	TTA <u>CTGCAG</u> GGCTGGGAGGAGGAAG
51510-NCW-R	GCA <u>CCATGG</u> TTAAAGAAAGCCATCTC
PAM68-NCW-F	TTACTGCAGAGATAAAACGAAGAGTC
PAM68-NCW-R	GCACCATGGCTATCTCTTGTCTGAG
51510-Nx-F	AGTGGATCCGCTGGGAGGAGGAAG
51510-Nx-R	GCTGAATTCTTAAAGAAAGCCATCTC
PAM68-Nx-F	AGT <u>GGCCATTACGGCC</u> GATAAAACGAAGAGTC
PAM68-Nx-R	GCT <u>GGCCGAGGCGGCC</u> CTATCTCTTGTCTGAG
PsbB-Nx-F	ATGGATCCGGTTTGCCTTGGTATCGTGTTC
PsbB-Nx-R	AT <u>CTCGAG</u> TCAGACTGCTTGTCGTTTTGTA
PsbC-Nx-F	AT <u>GGATCC</u> AAAACCTTATATTCCCTGAGGAG
PsbC-Nx-R	GC <u>GAATTC</u> GCTTAGTTAAGAGGAGTCATGGAA
LPA1-Nx-F	AT <u>GGATCC</u> GATGCTCTTGTTCAGTTTGA
LPA1-Nx-R	GC <u>GAATTC</u> GCTCATCTTTCTAACTTGCTGAGA
LPA2-Nx-F	AT <u>GGATCC</u> TCAAAGAATTCAAGCTCTTCCG
LPA2-Nx-R	AT <u>CTCGAG</u> TCACTCTTGACCCTTCATTTTC
PsaA-Nx-sfiI-F	CGCAGAGT <u>GGCCATTACGGCC</u> ATGATTATTCGTTCGCCGG
PsaA-Nx-sfiI-R	CTCGAGAGGCCGCCACGGCCTTATCCTACTGCAATAATTCTTG
YCF4-2Nx-sfiI-F	GGCCATTACGGCCGTTGGAACTTCCAGTTATC
YCF4-2Nx-sfiI-R	<u>GGCCGAGGCGGCC</u> TTTCAAAATACTTCAATTGG
Alb3-CCW-F	AT <u>GGCCATTACGGCC</u> TTCAGCTTAAACGAGATTCCTC
Alb3-CCW-R	CG <u>GGCCGAGGCGGCC</u> AATACAGTGCGTTTCCGCTTCGATC
SCY1-CCW-F	TAT <u>GGCCATTACGGCC</u> AGCTCTGAGGCTTCGGTTTTTG
SCY1-CCW-R	ATA <u>GGCCGAGGCGGCC</u> CATGGATCATACTTGTCAAGC
Primers used for Bi	iFC assay vector construction
FPB1-BiFC-F	TAA <u>ACTAGT</u> ATGGCCGCGTCTCTAAC
	TAA <u>ACTAGT</u> ATGGCCGCGTCTCTAAC AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT
FPB1-BiFC-R PAM68-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT
FPB1-BiFC-F FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F LPA2-BiFC-R	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F LPA2-BiFC-R Alb3-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGTTCTAGTCTC
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA2-BiFC-R LPA2-BiFC-R Alb3-BiFC-R	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGTTCTAGTCTC TTT <u>GTCGAC</u> TACAGTGCGTTTCCG
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F LPA2-BiFC-R Alb3-BiFC-F Alb3-BiFC-R SecY1-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGTTCTAGTCTC TTT <u>GTCGAC</u> TACAGTGCGTTTCCG AT <u>CTCGAG</u> AGCTCTGAGGCTTCGGTTTTTG
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA2-BiFC-R LPA2-BiFC-F Alb3-BiFC-F Alb3-BiFC-R SecY1-BiFC-R	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGTTCTAGTCTC TTT <u>GTCGAC</u> TACAGTGCGTTTCCG AT <u>CTCGAG</u> AGCTCTGAGGCTTCGGTTTTTG AT <u>GGATCC</u> TCATGGATCATACTTGTCAAGC
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F LPA2-BiFC-R Alb3-BiFC-F Alb3-BiFC-R SecY1-BiFC-F SecY1-BiFC-R SecE1-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGTTCTAGTCTC TTT <u>GTCGAC</u> TACAGTGCGTTTCCG AT <u>CTCGAG</u> AGCTCTGAGGCTTCGGTTTTTG AT <u>GGATCC</u> TCATGGATCATACTTGTCAAGC GAG <u>CTCGAG</u> ACTATGACGACGAGTAATC
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F LPA2-BiFC-R Alb3-BiFC-F Alb3-BiFC-R SecY1-BiFC-F SecE1-BiFC-R SecE1-BiFC-R FtsY-BiFC-F	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGATTCTAGTCTC TTT <u>GTCGAC</u> TACAGTGCGTTTCCG AT <u>CTCGAG</u> AGCTCTGAGGCTTCGGTTTTTG AT <u>GGATCC</u> TCATGGATCATACTTGTCAAGC GAG <u>CTCGAG</u> ACTATGACGACGAGTAATC TTC <u>GGATCC</u> TCAGCTGAAGAAGTCTTGAAC
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA2-BiFC-R LPA2-BiFC-F Alb3-BiFC-R SecY1-BiFC-R SecY1-BiFC-R SecE1-BiFC-R	AGT <u>GTCGAC</u> AAGAAAGCCATCTCTTTT GAC <u>ACTAGT</u> ATGGCTTCTGTACCATG TAT <u>GTCGAC</u> TCTCTTGTCTGAGGA TAA <u>ACTAGT</u> ATGGCTGTGGCTACAG ACA <u>GTCGAC</u> TCTTTCTAACTTGCTG CAA <u>ACTAGT</u> ATGGCGCTACAAATCCAC ATA <u>GTCGAC</u> CTCTTGACCCTTCATTTTC AGA <u>ACTAGT</u> ATGGCGAGAGTTCTAGTCTC TTT <u>GTCGAC</u> TACAGTGCGTTTCCG AT <u>CTCGAG</u> AGCTCTGAGGCTTCGGTTTTTG AT <u>GGATCC</u> TCATGGATCATACTTGTCAAGC GAG <u>CTCGAG</u> ACTATGACGACGAGAGTCTTGAAC ACA <u>ACTAGT</u> ATGGCAACTTCTTCTG
FPB1-BiFC-R PAM68-BiFC-F PAM68-BiFC-R LPA1-BiFC-F LPA1-BiFC-R LPA2-BiFC-F Alb3-BiFC-F Alb3-BiFC-R SecY1-BiFC-R SecE1-BiFC-R SecE1-BiFC-R FtsY-BiFC-R	AGTGTCGACAGTGTCGACGACACTAGTATGTCGACTATGTCGACTCTCTTGTCTGAGGATAAACTAGTACAGTCGACACAGTCGACACAGTCGACCAAACTAGTATAGTCGACATAGTCGACAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGTAGAACTAGAACTAGAACTAGAACTAGAACTATCCGAGACTATGGATCCTCAGCTGAAGAAGTCTTGAACAGAACTAGTAGACTAGTATGGCAACTTCTTCGCTCGCTGTCGACAAGAAGAATATAGCATTCAC

SecA1-BiFC-R	ATA <u>GGATCC</u> GGCGCTTGCAATTGAG
YCF4-BiFC-F	TAG <u>GGATCC</u> ATGAGTTGGCGATCAGAATC
YCF4-BiFC-R	CCG <u>CTCGAG</u> AAATACTTCAATTGGTACACG
PYG7-BiFC-F	GCC <u>GGATCC</u> ATGTTCGAATCGAACATGGTTC
PYG7-BiFC-R	GCC <u>CTCGAG</u> CCGTTTCTTAGACTTAAC
RbcS-TP-BiFC-F	TGC <u>TCTAGA</u> ATGGCTTCCTCTATGCTCTCTCC
RbcS-TP-BiFC-R	CGG <u>GGTACC</u> AGGCCACACCTGCATGCAG

Supplementary References

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