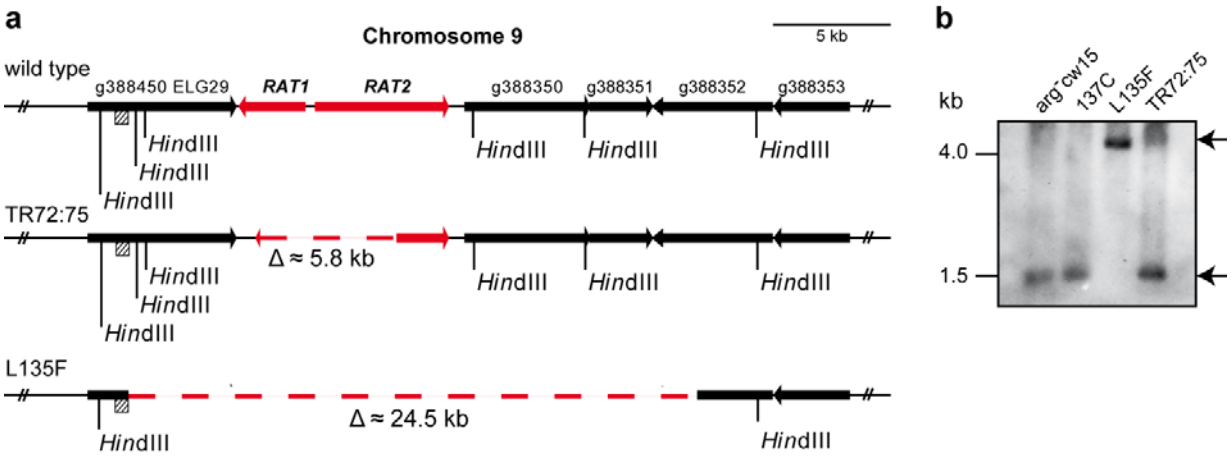
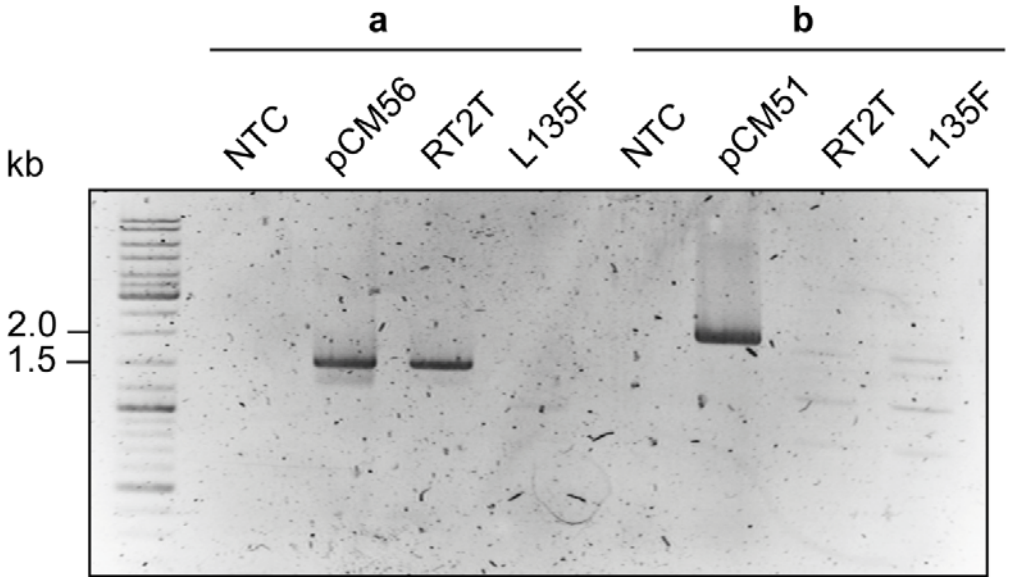
Supplementary Information

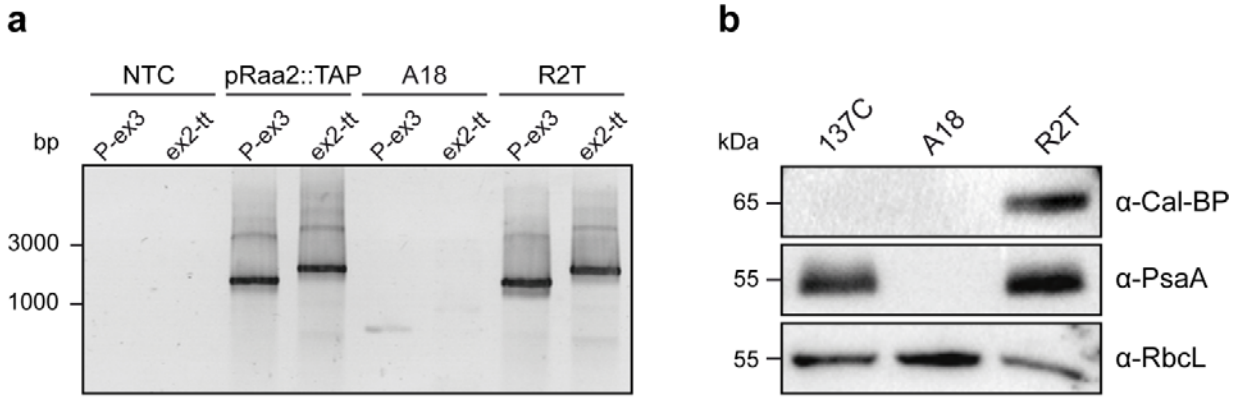
Supplementary Figures 1-9, Supplementary Tables 1-5



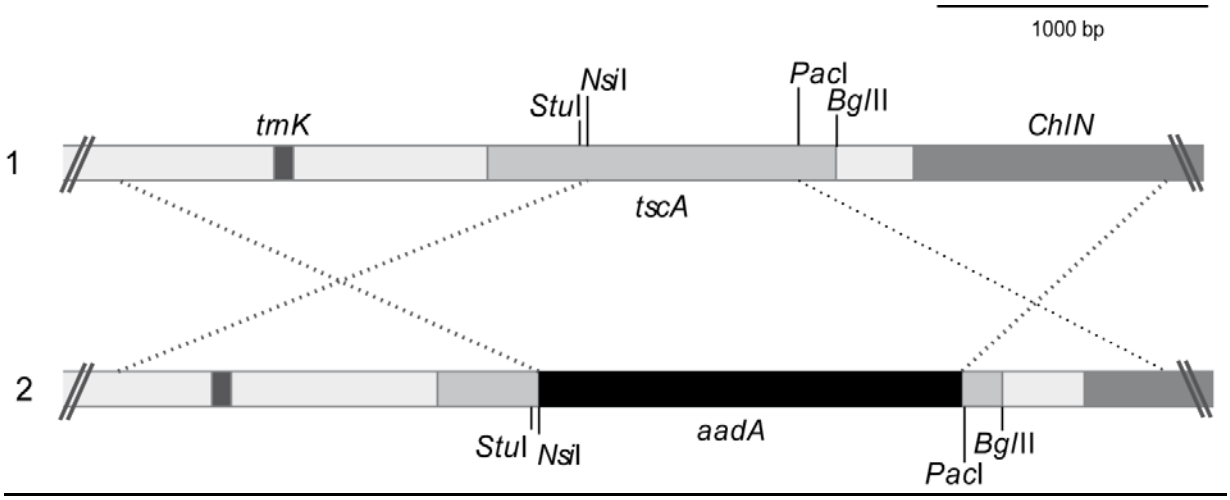
Supplementary Figure 1: Southern analysis of two wild type strains (arg-cw15, 137C) and two allelic mutants (TR72:75, L135F). (**a**) Comparative physical map of the *RAT2* locus and flanking regions in wild type (arg-cw15 and 137C) and the two allelic mutant strains TR72:75 and L135F. The probe used for hybridization is shown as dashed box. (**b**) Southern blot of gDNA digested with *Hind*III from arg-cw15, 137C, L135F, and TR72:75. Hybridization with a radioactively labeled PCR fragment revealed that several *Hind*III restriction sites are deleted in mutant L135F, leading to a 4 kb signal in Southern analysis compared to an 1.5 kb signal that represents the wild type situation.



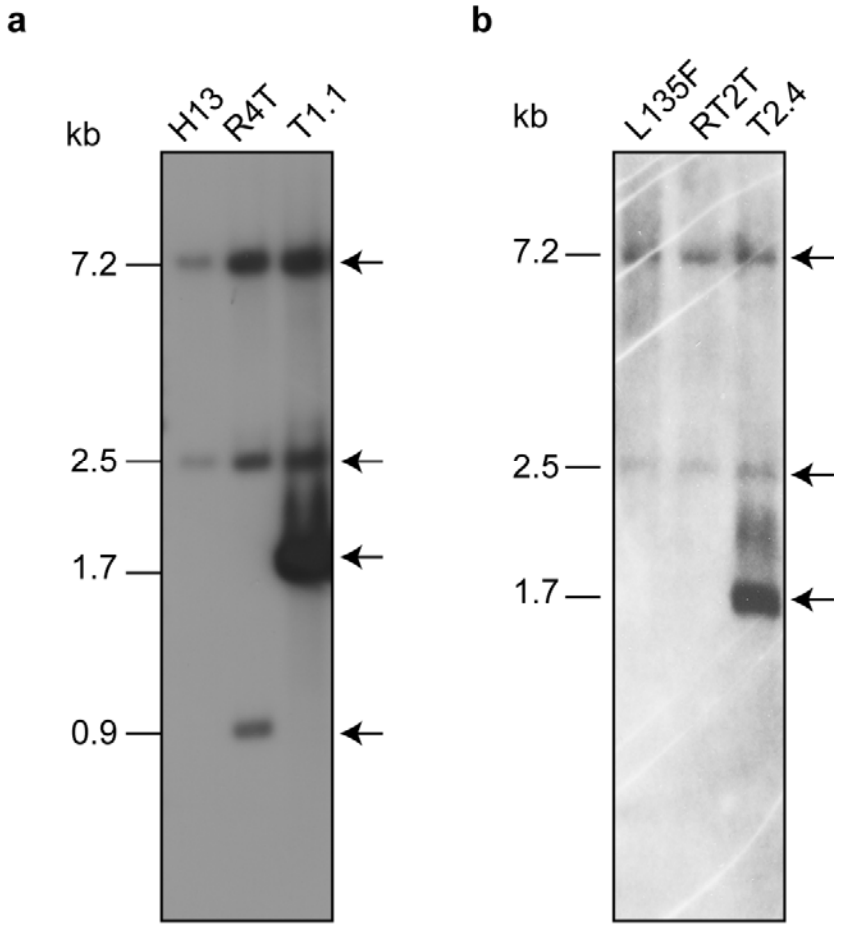
Supplementary Figure 2: PCR analysis of a *RAT2::TAP* transformant RT2T by PCR. (**a**) Genomic integration of the *RAT2::TAP* fusion gene of transformant RT2T was verified by PCR with gDNA as template using *TAP-tag*- and *RAT2* exon 9-specific primers. Isolated gDNA of mutant strain L135F served as negative control, whereas plasmid pCM56 was used as positive control. (**b**) Deletion of *RAT1* gene in mutant strain L135F and transformant RT2T was verified using *RAT1* specific primers. Plasmid pCM51 served as positive control. NTC = no template control.



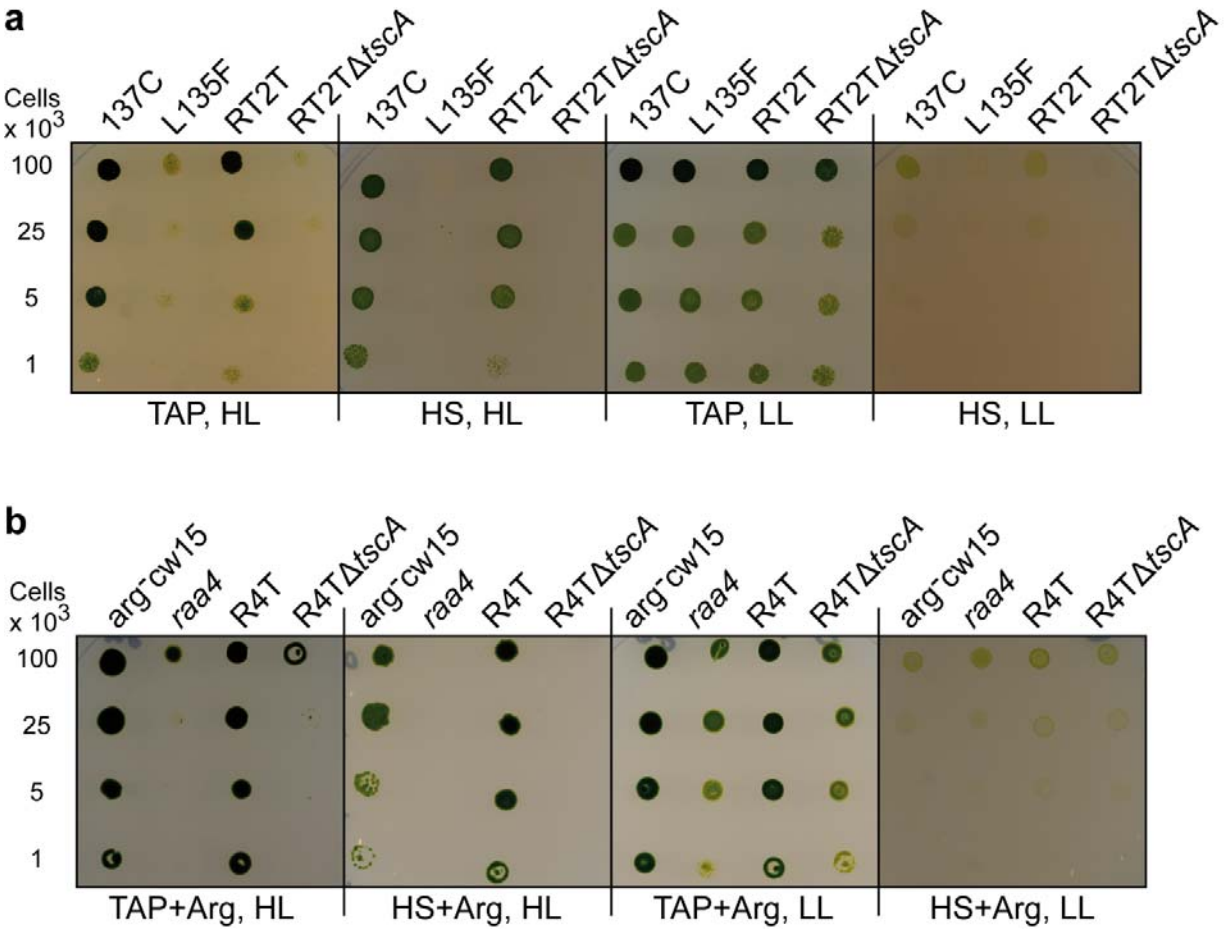
Supplementary Figure 3: Molecular characterization of strain R2T. For TAP experiments, *RAA2* was fused upstream of the *TAP* sequence and mutant A18 was transformed with pRaa2::TAP. (**a**) Genomic integration of the *RAA2::TAP* construct in transformant R2T was verified by amplification of promotor-gene fragments (P-ex3, primers: for_Chl1 and rev_Raa2_Ex3) and gene-TAP fragments (ex2-tt, primers: for_Raa2_Ex2 and taptag2). (**b**) Immunodetection of Raa2::TAP fusion protein, PsaA, and RbcL in wild type, mutant *Δraa2*, and R2T strain proved the expression of Raa2::TAP in R2T and photosynthetic restoration. Abbreviation: ctrl, control; P, promoter; ex, exon; tt, TAP-tag.



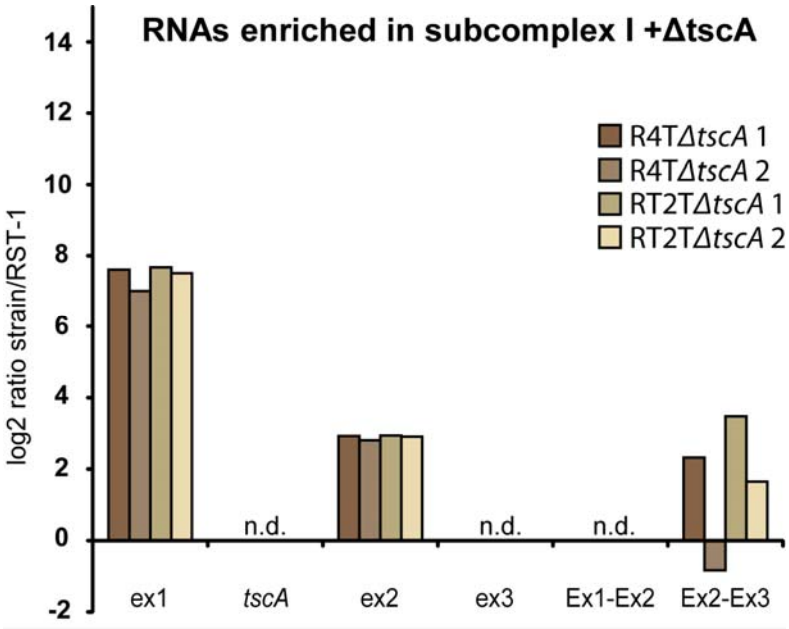
Supplementary Figure 4: Deletion of chloroplast *tscA* locus by homologous recombination. Physical map of the *tscA* locus and flanking regions (1). After transformation, the main part of the *tscA* gene is replaced by an *aadA* resistance cassette (2). For gene disruption the *aadA* resistance cassette was integrated via *Stu*I and *Pac*I restriction sites into plasmid pCM57.



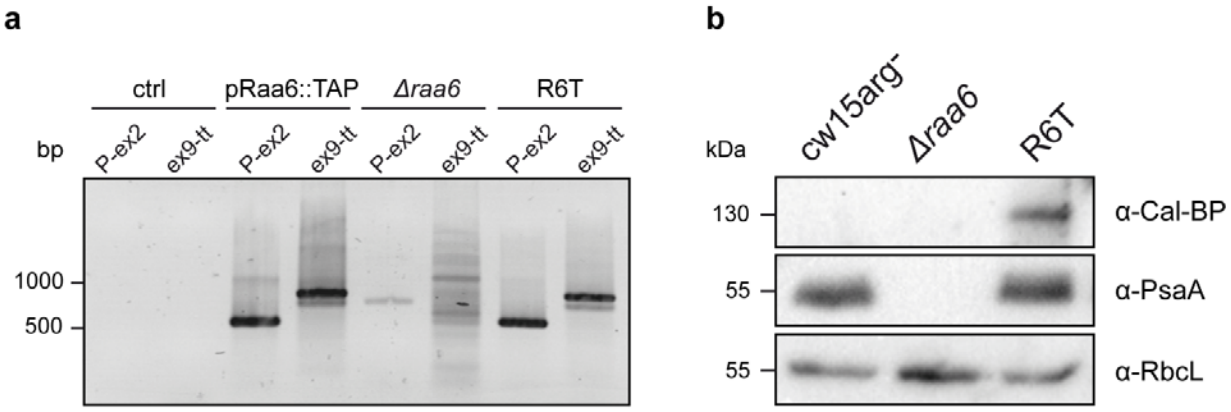
Supplementary Figure 5: Southern hybridization confirms deletion of the *tscA* gene in R4T and RT2T. (**a**) Total DNA from mutant strain H13, parental strain R4T, and several transformants was digested with *Nsi*I and *Bgl*II. A *Bgl*II and *Nsi*I restriction fragment of pCM59 was used as a *5' psbD-aadA-3' rbcL-``tscA* probe and revealed a 0.9 kb signal for *tscA* and an 1.7 kb signal for the *aadA* cassette (arrows). The 2.5 kb signal corresponds to the *psbD,* and the 7.2 kb signal to the *rbcL* locus as indicated with arrows. (**b**) Total DNA from mutant strain L135F, parental strain RT2T, and transformant T2.4 was digested with NsiI and BglII. Southern hybridization was performed as in (a).



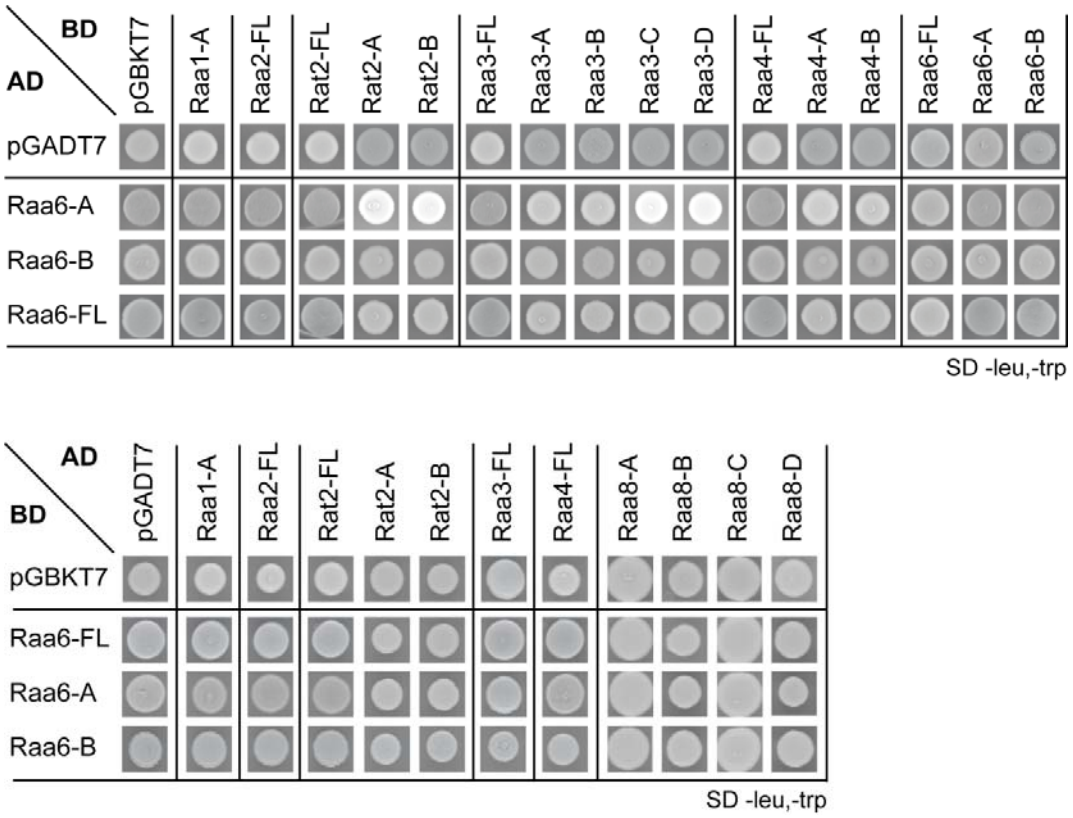
Supplementary Figure 6: Photosynthetic defect of tscA deletion strains RT2T*ΔtscA* and R4T*ΔtscA*. Cells were spread onto HS or TAP agar plates (for arg$^-$cw15 supplemented with arginine) with concentrations as indicated and incubated under high light (HL) or low light conditions (LL). (**a**) Wild type 137C and transformant RT2T are able to grow without acetate on HS plates under HL. Mutants L135F and RT2TΔtscA are photosynthetic deficient and can only grow on TAP plates under LL conditions. (**b**) Wild type arg-cw15 and transformant R4T are able to grow without acetate on HS plates under HL, whereas mutants raa4 and R4TΔtscA can only grow on TAP plates under LL conditions due to their photosynthetic defect.



Supplementary Figure 7: RNAs enriched in TAP eluates of Raa4::TAP*ΔtscA* and Rat2::TAP*ΔtscA*. After TAP purification, RNA was precipitated and RNA levels were analyzed with qRT-PCRs. Enrichment of RNAs was determined by calculating ratios of Raa4::TAP∆*tscA* and Rat2::TAP∆*tscA* results and data obtained with the control strain RST-1. The *rrnL* gene was used for normalization. Primer pair specific for exon 1 precursor (ex1), exon 2 precursor (ex2), exon 3 precursor (ex3), *tscA* RNA, and partially spliced *psaA* exons (Ex1-Ex2, Ex2-Ex3) were used in these qRT-PCRs.



Supplementary Figure 8: Molecular characterization of strain R6T. For TAP experiments, gDNA of *RAA6* was fused upstream of the *TAP* sequence under control of the *HSP70::RBCS2.* Mutant *Δraa6* was transformed with pRaa6::TAP. (**a**) Genomic integration of *RAA6::TAP* construct in transformant R6T was verified by amplification of promotor-gene fragments (P-ex2, primers: for_Chl1 and 29701_RT1_rev) and gene-TAP fragments (ex9-tt, primers: OOR_107 and tapatag2). (**b**) Immunodetection of Raa6::TAP fusion protein, PsaA, and RbcL in wild type, mutant *Δraa6*, and R6T strain proved the expression of Raa6::TAP in R6T and photosynthetic restoration. Abbreviation: ctrl, control; P, promoter; ex, exon; tt, TAP-tag.



Supplementary Figure 9: Growth control of the yeast-two hybrid analyses for detecting interaction between trans-splicng factors. Yeast strains were co-transformed with plasmids carrying either the empty GAL4 DNA-binding domain (pGADT7) or the GAL4 activation domain plasmid pGBKT7. Transformats were grown on SD medium lacking leucine and tryptophane for the selection of both plasmids.

Supplementary Table 1: Proteins co-purified with bait Rat2 in TAP experiments.

		Rat2		
		PSMs[a]		
Protein	Description	P1[b]	P2	P3
Trans-splicing factors				
Raa1	*trans*-splicing factor B, OPR domains	55	47	52
Raa3	*trans*-splicing factor C	28	11	41
Raa4	*trans*-splicing factor C	42	34	32
Raa8	*trans*-splicing factor C, OPR domains	90	43	106
Rat2	*trans*-splicing factor C, OPR domains	194	34	66
Not annotated				
Cre10.g426450	no functional annotations	4	5	5
Cre11.g467652	no functional annotations	32	7	26
Cre15.g643750	no functional annotations	7	10	8
Cre16.g690655	no functional annotations	5	4	2
Cre17.g724450	no functional annotations	22	13	27
Gene expression				
Cre01.g001501	OPR domains	35	16	22
Cre06.g262650	OPR domains, RAP domain	3	14	4
Cre17.g698750	OPR domains	30	38	38
Cre07.g347400	G-patch domain	55	7	10
Cre08.g373878	U1 snRNP-specific protein C	37	15	26
Cre13.g574200	Poly(A) polymerase, PAP2	7	8	6
Others				
Cre09.g387134	DUF 525	4	5	3
Cre12.g500550	calcium activated chloride channel regulator	19	5	3
Cre13.g567100	D-arabinono-1,4-lactone oxidase, FAD binding domain	9	4	5
Cre14.g610501	SDR34, predicted dehydrogenase	28	7	11

[a] PSMs, peptide spectral matches
[b] P, TAP purifications (P1-P3) performed with bait Rat2

Supplementary Table 2: Proteins co-purified with bait Raa2 in TAP experiments.

		Raa2			
		PSMs[a]			
Protein	Description	P4[b]	P5	P6	P7
Trans-splicing factors					
Raa1	*trans*-splicing factor B, OPR domains	13	22	22	158
Raa2	*trans*-splicing factor A, pseudouridine synthase	14	12	22	43
Raa7	*trans*-splicing factor A	10	12	15	74
Not annotated					
Cre17.g728850	no functional annotations	7	11	16	51
Cre09.g400960	no functional annotations	3	4	9	5
Cre06.g252100	no functional annotations	2	9	9	2
Cre03.g179000	no functional annotations	2	9	8	33
Cre02.g095900	no functional annotations	5	4	3	11
Gene expression					
Cre03.g158950	RNA recognition motif (RRM)	3	5	5	5
Cre12.g494750	Plastid ribosomal protein S20	10	3	7	2
Cre10.g466250	U3 small nucleolar RNA-associated protein 21	30	33	28	57
Cre10.g428678	tRNA uridine modification enzyme	6	7	5	6
Cre01.g022350	DEAD/DEAH box helicase	4	15	10	9
Cre07.g349300	DEAD/DEAH box helicase	3	11	21	4
Others					
Cre12.g507650	Chloroplast DnaJ-like protein	6	6	5	10
Cre02.g147302	Aminotransferase class I and II	2	3	6	5
Cre06.g260850	Nop14-like family	10	17	18	18
Cre09.g391652	Rad4 related protein	5	8	12	9
Cre02.g073200	Threonine dehydratase	6	17	6	27

[a] PSMs, peptide spectral matches
[b] P, TAP purifications (P4-P7) performed with bait Rat2

Supplementary Table 3: *C. reinhardtii* strains and growth conditions.

Strain	Characteristics	Growth conditions	References
arg$^-$cw15	*arg7*, *cw15*, mt$^-$; nuclear mutant; reduced cell wall, arginine auxotroph	TAP medium or HS (Sueoka 1960, Gillham 1965, Gorman and Levine 1965, Harris 1989) under HL conditions, supplemented with 500 mg/ l arginine	strain collection Prof. J.-D. Rochaix, Department de Biologie Moléculaire, Quai Ernest-Ansermet, Genève, Swiss; (Gillham 1965)
137C	mt$^+$, wild type strain	TAP medium or HS under HL conditions	(Goldschmidt-Clermont et al. 1990)
H13	chloroplast mutant; deletion of *tscA* gene	TAP medium under LL conditions	(Goldschmidt-Clermont et al. 1990)
L135F	mt$^+$; nuclear class C *trans*-splicing mutant; from UV mutagenesis of 137C	TAP medium under LL conditions	(Goldschmidt-Clermont et al. 1990, Hahn et al. 1998)
A18	mt$^+$; nuclear class A *trans*-splicing mutant; from transformation of mutant arg7 with plasmid pARG7.8	TAP medium under LL conditions	(Debuchy et al. 1989, Perron et al. 1999)
Δraa6	mt-, nuclear class A *trans*-splicing mutant; reduced cell wall, derived from transformation of CC-406 *cw15* with plasmid pHyg3	TAP medium under LL conditions	Jacobs, Glanz, Kück personal communication
R4T	mutant *raa4* expressing *RAA4::TAP* fusion gene	TAP medium or HS under HL conditions	(Jacobs et al. 2013)
TR72:75	*cw15*, mt$^-$; nuclear mutant; reduced cell wall, defect in PSI, class C *trans*-splicing mutant; from transformation of arg$^-$cw15 with plasmid pARG7.8□3	TAP medium under LL conditions	(Hahn et al. 1998, Balczun et al. 2005)
RT2T	mutant L135F expressing *RAT2::TAP* fusion gene	TAP medium or HS under HL conditions	this work
R2T	mutant A18 expressing *RAA2::TAP* fusion gene	TAP medium or HS under HL conditions	this work
R6T	mutant *raa6* expressing *RAA6::TAP* fusion gene	TAP medium or HS under HL conditions	this work
R4TΔ*tscA*	Integrated *RAA4::TAP*; chloroplast mutant; deletion of *tscA* gene	TAP medium with spectinomycin under LL conditions	this work
RT2TΔ*tscA*	Integrated *RAT2::TAP*; chloroplast mutant; deletion of *tscA* gene	TAP medium with spectinomycin under LL conditions	this work

Supplementary Table 4: Plasmids used in this study.

Plasmid	Characteristics	Reference
P78H3	Cosmid from *C. reinhardtii* wild type library	(Zhang et al. 1994)
pBatTL	spec^R, amp^R	(Nagai et al. 2002)
pCM6	2.3 kb *RAA2* gDNA in pDrive	this work
pCM10	codon optimized *TAP-tag* gene in pCrg1	(Jacobs et al. 2013)
pCM43	5'*psbD-aadA*-3'*rbcL* cassette in pRS426	this work
pCM45	full length *tscA* gene in pDrive	this work
pCM49	5'*psbD-aadA*-3*'rbcL* cassette in pCM45: *Pac*I, *Stu*I	this work
pCM51	gDNA of *Rat1::3xHA* in pCrg1	this work
pOR11	gDNA of RAA6 in pDrive	
pRAT2::TAP	gDNA of *RAT2*::*TAP* under control of *HSP70A/ 5'RBCS2* and *3'LHCB1*	this work
pRAA2::TAP	gDNA of *RAA2*::*TAP* under control of *HSP70A/ 5'RBCS2* and *3'LHCB1*	this work
pRAA6::TAP	gDNA of *RAA6*::*TAP* under control of *HSP70A/ 5'RBCS2* and *3'LHCB1*	this work
pCM57	R12 from pIG637.1 : *Lgu*I, *Ehe*I in pRS426; 0.9 kb; *Bgl*II, *Nsi*I-fragment was used as a *tscA* probe	this work
pCM59	*5'psbD-aadA-3'rbcL* cassette in pCM57 : *Pac*I, and deletion of 0.8 kb internal fragment of *tscA;* 1.7 kb *Bgl*II, *Nsi*I-fragment was used as a *tscA::aadA* probe	this work
pDrive	amp^R, kan^R, *lacZ*	Qiagen, Hilden, Germany
pGAD_RbcS1	*RBCS1*-cDNA in pGAD424/ *Eco*RI (BD Biosciences Clontech)	(Glanz et al. 2012)
pIG637.1	*C. reinhardtii* chloroplast DNA *Eco*RI restriction fragment R12 in pT3T7, contains the *tscA* gene	(Herdenberger et al. 1994)
pIG3197-3	*3xHA* in pCrg1	this work
pRS426	*URA3*, *lacZ_a*, Amp^R	(Christianson et al. 1992)
pGADT7	*ADH1(p)::gal4-AD::LEU2*	Clontech
pGBKT7	ADH1(p)::gal4-BD::TRP1	Clontech
pGADT7_Raa1-A	1.7 kb fragment of *RAA1* cDNA in pGADT7	(Jacobs et al. 2013)
pGBKT7_Raa1-A	1.7 kb fragment of *RAA1* cDNA in pGBKT7	(Jacobs et al. 2013)
pGADT7_Raa2-FL	1.1 kb fragment of *RAA2* cDNA in pGADT7	this work
pGBKT7_Raa2-FL		this work
pGADT7_Rat2-FL	2 kb fragment of *RAT2* cDNA in pGADT7	(Jacobs et al. 2013)
pGADT7_Rat2-A	0.7 kb fragment of *RAT2* cDNA in pGADT7	(Jacobs et al. 2013)
pGADT7_Rat2-B	0.8 kb fragment of *RAT2* cDNA in pGADT7	(Jacobs et al. 2013)
pGBKT7_Rat2-FL	2 kb fragment of *RAT2* cDNA in pGBKT7	(Jacobs et al. 2013)
pGBKT7_Rat2-A	0.7 kb fragment of *RAT2* cDNA in pGBKT7	(Jacobs et al. 2013)
pGBKT7_Rat2-B	0.8 kb fragment of *RAT2* cDNA in pGBKT7	(Jacobs et al. 2013)
pGADT7_Raa3-FL	5.1 kb fragment of *RAA3* cDNA in pGADT7	this work
pGBKT7_Raa3-FL	5.1 kb fragment of *RAA3* cDNA in pGBKT7	(Jacobs et al. 2013)

pGBKT7_Raa3-A	3.3 kb fragment of *RAA3* cDNA in pGBKT7	(Jacobs et al. 2013)
pGBKT7_Raa3-B	1.8 kb fragment of *RAA3* cDNA in pGBKT7	(Jacobs et al. 2013)
pGBKT7_Raa3-C	1.8 kb fragment of *RAA3* cDNA in pGBKT7	(Jacobs et al. 2013)
pGBKT7_Raa3-D	1.4 kb fragment of *RAA3* cDNA in pGBKT7	(Jacobs et al. 2013)
pGADT7_Raa4-FL	3.2 kb fragment of *RAA4* cDNA in pGADT7	(Jacobs et al. 2013)
pGBKT7_Raa4-A	1.6 kb fragment of *RAA4* cDNA in pGADT7	(Jacobs et al. 2013)
pGBKT7_Raa4-B	1.6 kb fragment of *RAA4* cDNA in pGADT7	(Jacobs et al. 2013)
pGBKT7_Raa4-FL	3.2 kb fragment of *RAA4* cDNA in pGBKT7	(Jacobs et al. 2013)
pGADT7_Raa6-A	1.6 kb fragment of *RAA6* cDNA in pGADT7	this work
pGBKT7_Raa6-A	1.6 kb fragment of *RAA6* cDNA in pGBKT7	this work
pGADT7_Raa6-B	1.8 kb fragment of *RAA6* cDNA in pGADT7	this work
pGBKT7_Raa6-B	1.8 kb fragment of *RAA6* cDNA in pGBKT7	this work
pGADT7_Raa6-FL	3.4 kb fragment of *RAA6* cDNA in pGADT7	this work
pGBKT7_Raa6-FL	3.4 kb fragment of *RAA6* cDNA in pGBKT7	this work
pGADT7_Raa8-A	0.5 kb fragment of *RAA8* cDNA in pGADT7	this work
pGADT7_Raa8-B	1.4 kb fragment of *RAA8* cDNA in pGADT7	this work
pGADT7_Raa8-C	0.5 kb fragment of *RAA8* cDNA in pGADT7	this work
pGADT7_Raa8-D	0.9 kb fragment of *RAA8* cDNA in pGADT7	this work
pGBKT7_Raa8-B	1.4 kb fragment of *RAA8* cDNA in pGADT7	this work
pGBKT7_Raa8-D	0.9 kb fragment of *RAA8* cDNA in pGADT7	this work

Supplementary Table 5: Oligonucleotides used in this study.

Oligonucleotide	Sequence (5' → 3')	Reference
for_Rat2_IF	TTACAAGAGAGCTAGCATGCAGTCTCCCGCGCTAG	this work
815.3D	CATCGAAGTCGGGCATGA	Balczun, pers. communication
for_Rat2-F2	TACTGAGCGCCAGCTTC	(Jacobs et al. 2013)
rev_Rat2-F2	TGCCGCCGCTGTTGCTGCTTTG	(Jacobs et al. 2013)
for_Rat2-F3	AGGTCCAGCAAAACCCAAAG	(Jacobs et al. 2013)
rev_Rat2-F4	TGTTGCCCAACGGGGATGAG	(Jacobs et al. 2013)
for_Rat2-F4	GTTTGTGACGGCCGAGGAG	(Jacobs et al. 2013)
rev_Rat2-IF	CTTAGATCTGCTAGCCCCGGCTGCGGACTGTTCC	this work
for_hsp70A_HR	CCCTCGAGGTCGACGGTATCGATAAAGCTCGCTGAGGC TTGACAT	this work
rev_5'rbcS2_HR	ACGGCGCTAGCGCGGGAGACTGCATGCTAGCTCTCTT GTAAAAAA	this work
for_TAP_3'lhcb_HR	AGCCCTGGAACAGTCCGCAGCCGGGGCTAGCAGATCT AAGCGCCG	this work
rev_3'lhcb_HR	CACCGCGGTGGCGGCCGCTCTAGAAGATATCATCGATG GCCTACC	this work
for_5'UTR_psbD	GACGGTATCGATAAGCTTGATATCGAGGCCTATGAAATT AAATGGATATTTGGTAC	this work
for_aadA	ATGCCTCGGGCATCCAAGCAGCAAG	this work
for_rbcL	GATCACCAAGGTAGTCGGCAAATAAAGCTTGTACTCAA GCTCGTAACGAA	this work
rev_3'UTR_rbcL	GGTGGCGGCCGCTCTAGAACTAGTGTTAATTAATACAT CCGCTTTAGTATGTTACT	this work
rev_aadA	TTATTTGCCGACTACCTTGGTGATC	this work
rev_psbD	CTTGCTGCTTGGATGCCCGAGGCATTGCGTGTATCTCC AAAATAAAAAAA	this work
for_tscA	TACTCCGAAGCAGGCAGTTG	this work
rev_tscA	GTATAGCAGCGAGATGCTAC	this work
OOR_59	TCTAGAATGGCGGAGGAGCTGCGG	this work
OOR_60	TCTAGACAGCCGCATTTCCGCCGC	this work
for_Raa2_TAP	TCTAGATAAAACAATTGGGTCCAAGAGCCGC	this work
rev_Raa2_TAP	TCTAGAATGCGCGTGCCGTTGCAGCAC	this work
for_Rat1-IF	TACAAGAGAGCTAGCATGTCACGCTTGAAATCTC	this work
rev_Rat2-IF	GTAAGATCTGCTAGCCCCGCCGGCGCCCATCTG	this work
for_3xHA	AGAGCTAGCAGATCTTACCCCTACGACGTGCCC	this work
pGAD_Raa6_Infu1	CAGATTACGCTCATATGCAGCAGCTGGGGACCAGT	this work
rev_pGAD_Raa6_Infu2	CACCCGGGTGGAATTCTCACAGCCGCATTTCCGCCGC	this work
pGAD_Raa6_Infu3	CAGATTACGCTCATATGATGGCGGAGGAGCTGCGGCG	this work
pGAD_Raa6_Infu4	CACCCGGGTGGAATTCTCACGCGGCATTCCTAGCTTCC TG	this work
for_29701_Fragment_2	GAGCAGCAGGAAACACAAGG	this work
for-IF-Raa8-F1	CAGATTACGCTCATATGGCAGGCGGTTATGTACAG	this work
rev-IF-Raa8-F1	CACCCGGGTGGAATTCCTATAGCCACTCCATGCTA	this work
for-IF-Raa8-F2	CAGATTACGCTCATATGCAGTTGGTGCGGGTGTTG	this work
rev-IF-Raa8-F2	CACCCGGGTGGAATTCCTACAGGTCTCGCTGCCA	this work
IF-42B-for	CAGATTACGCTCATATGTGTAGCATGGAGTGGCTAC	this work
IF-42B-rev	CACCCGGGTGGAATTCTTACGAGGACATGGCGCT	this work

IF-46A-for	CAGATTACGCTCATATGTGCGGCTGCTTACAAACC	this work
IF-46A-rev	CACCCGGGTGGAATTCTTACCCTTCCTGTACATAAC	this work
OOR_48	GCAGATGCTCATGACTTTGAC	this work
OOR_98	AAAGAATTTGAGCCGTGTGCAG	this work
OOR_99	TGAGTAGGGTCACTTAACCAAG	this work
OOR_113	AGGTCGCACGCCCATTTTTC	this work
OOR_114	TAGTACTCCAGAGCGCGAAG	this work
tscA_RT_for	CCAACTTCAGCTAGGTTTGATG	this work
tscA_RT_rev	TAAGCCCTTCTCTTTCTGCAAG	this work
rev_psaA_Ex2Int2	TCGTGTCGCACCCACTTAAC	this work
for_Chl1	CTAGGCGCCAATGCAAGCAG	this work
for_raa2_Ex2	CGGCCTTATGCTGTTGTGCT	this work
rev_Raa2_Ex3	CTCTTCGCTAAGCGGTGACG	this work
taptag2	AGATCTCTTGGGGGCCTGTGCGCC	this work
29701_RT1_rev	GTATGGTCCGCTGCATTCCC	this work
OOR_107	GCTGGCTGGCCGACACATGC	this work

Supplementary Table 6: LC-MS data of each TAP experiment

List of proteins purified by tandem affinity purification and identified by LC-MS. The results for each purification are presented in a separate worksheet. All proteins are reported with the accession number, the percentage of the protein sequence coverage (ΣCoverage), the number of identified unique peptides (Σ# Unique Peptides), the number of identified proteins in a protein group (Σ# Peptides), the number of peptide spectrum matches (Σ# PSMs), the number of amino acids (# AAs), the molecular weight (MW [kDa]), and the calculated isoelectric point (calc. pI).