



HLA3



(b) GFP





chlorophyll+GFP

Figure S1. Expression of fluorescent-tagged CCM components in Chlamydomonas and tobacco (from Fig. 1). Expression of Venus-fused CCM-components in *Chlamydomonas reinhardtii* (a). Expression in tobacco of GFP-fused CCM components from Chlamydomonas (b). Green and purple signals are Venus or GFP fluorescence and chlorophyll autofluorescence, respectively. Overlaid images of these signals are shown: overlaps are white. Arrowhead shows the label-free area is the algal pyrenoid, a micro-compartment composed primarily of RuBisCO. A non-transformed control is included. Scale bar = 5 μ m (all 5 μ m for Chlamydomonas images).



Figure S2. Co-expression of Venus-fused CCM components with Mitotracker[®] Red CMXRos in Chlamydomonas. Purple, green and cyan signals are chlorophyll autofluorescence, Venus and Mitotracker fluorescence, respectively. Overlaid images of these signals are shown: overlaps of Venus and Mitotracker are pale green. Scale bar = $5 \mu m$.



Figure S3. Expression of GFP-fused CCM components carrying native Arabidopsis chloroplast transit peptides in tobacco (from Fig. 3). Green and purple signals are GFP fluorescence and chlorophyll autofluorescence, respectively. Overlaid images of these signals are shown: overlaps are white. A non-transformed control is included. Scale bar = $10 \mu m$.



Figure S4. Stable expression of LCIA:GFP and HLA3:GFP in Arabidopsis (from Fig. 5a). Green and purple signals are GFP fluorescence and chlorophyll autofluorescence, respectively. Overlaid images of these signals are shown: overlaps are white. A non-transformed control is included. Scale bar = $10 \mu m$.



Figure S5. Chlorophyll content of transgenic Arabidopsis plants expressing LCIA or HLA3. Chlorophyll content measurements are shown for 8 separate plants, bars are means \pm SE. ANOVA revealed that there were no statistically significant differences between samples (P< 0.05).



Figure S6. Specific leaf area (area/DW) of transgenic LCIA or HLA3 Arabidopsis plants. Values are the means ± SE of measurements made on 24 rosettes. Growth conditions refer to those outlined in Fig. 6.

Codon optimised LCIA (1011 bp)

Codon optimised HLA3 (3978 bp)

ATGGGAGAGGGGAAGGTGGCCGAGAGAGGTCATGATCCTACTGCAGGCTTCTTCAACAAGTTTGCTTTCGGG TGGATGTACAAATGGATTGGTGCTGCTAGGCGAGGTGAAGAACTTAATGCCGAAGAAATGGGTATGCCACCA GAAAACATGGCACACGAGGCTTATGATAAGTTTGCGGCCCATTGGGCTGCGGAGCAGAAACTTAAAGACCAG GATGGGCAGAAGCCGTCATTGGTAAGGGCTCTTAGAAAATCATTTGGTCTATTCTACATGTTGGGAGGTCTTT GCAAATGCGGATGGTCAACATTCGTGATTCTTGGTGCTTTTTACTTTGTGCGTTCACTCTTGTCGCATGTCAACT ATGTAGATGCTCCTACGAAAAAGTGGACAACGGCTGAAACTTTGTATGGCCAAACAACCGCTGGATGGCTCTT AATGATGGGTTTCACAATAGATGCTTGGCTTTTGGGACTTTGTCTCCAAAGAATGGGTTACATATGCATGACG GTTGGTATCCGTGCGAGAGCTGCTCTTGTCCAAGCGGTTACTCATAAAGCTTTCCGACTTAATACGGTTAGAG CTGATCAATCAGCCGCGATTGTCAACTTCGTGGCTTCCGATATCCAGAAAATCTACGATGGGGCGTTAGAGTT TCATTATCTTTGGACCGCCCCTTTTGAAGCCGCAGCAATCCTTGCCCTCTTGGGATACTTGACACAAGACTCCAT GCTCCCAGGATTGGGGGGTCATTTGCTAGTGTTGCCTATGCAGTATTACTTCGGTTATAAGATTGTACAAATCA AGCTTCAAAACGCTAAGCATGTTGCTCCCGGTCAGCAATTATGCAGGAAGTGCTTCCTGCTATCAAGCTTGTT AAGTACTACGCATGGGAACAGTTCTTCGAGAACCAAATCTCAAAAGTACGTAGGGAGGAAATAAGGTTGAAC TTCTGGAATTGCGTCATGAAGGTCATTAACGTTGCATGTGTATTTTGTGTTCCGCCTATGACAGCCTTTGTGAT CTTTACCACTTATGAGTTTCAACGCGCTAGATTAGTATCTTCTGTGGCGTTTACTACACTCAGCTTATTCAATAT CCTGCGCTTTCCGTTGGTTGTTTTGCCAAAGGCTCTGAGAGCTGTTTCTGAAGCTAACGCTAGCTTGCAAAGAT TGGAAGCGTATTTGTTGGAAGAGGTTCCATCTGGAACAGCAGCAGTAAAGACTCCTAAGAATGCGCCACCGG GAGCGGTCATTGAAAACGGGGTGTTTCATCATCCCTCTAACCCAAATTGGCATCTCCACGTACCAAAGTTTGA GGTAAAGCCAGGTCAGGTGGTCGCTGTTGTTGGTCGGATTGCCGCTGGTAAGAGCTCGTTGGTACAAGCTAT ATTGGGTAATATGGTTAAGGAACACGGTTCCTTCAATGTTGGTGGCCGGATCAGCTACGTACCTCAGAATCCT TGGCTCCAAAACCTTTCTTTGCGAGACAACGTGCTTTTTGGTGAACAATTTGACGAGAATAAGTACACGGATG TCATTGAATCTTGTGCATTGACTCTGGACCTTCAGATACTTTCCAACGGGGATCAGAGTAAAGCTGGTATTAGA GGAGTGAATTTCTCCGGTGGGCAAAGACAAAGGGTCAACCTTGCTCGTTGTGCCTACGCAGACGCCGACCTT GTCCTGTTGGACAACGCGCTGTCCGCGGTGGATCATCATACGGCGCATCACATTTTTGATAAATGTATCAAAG GATTATTCAGTGATAAAGCCGTAGTGCTCGTTACTCACCAGATTGAGTTCATGCCTAGGTGTGATAACGTTGCT ATCATGGATGAGGGCCGATGTCTCTACTTCGGTAAGTGGAACGAAGAGGCTCAACATCTTCTGGGAAAATTG CTCCCTATCACACATCTCCTTCACGCCGCAGGCTCGCAGGAAGCTCCTCCAGCACCGAAGAAAAAGGCAGAAG ATAAGGCAGGGCCACAAAAGTCACAGAGTTTACAGTTAACACTTGCCCCAACCTCTATAGGAAAGCCCACCGA AAAACCAAAAGATGTTCAAAAGCTTACGGCCTACCAGGCTGCACTTATCTATACCTGGTATGGAAATTTGTTCT

TGGTGGGAGTTTGTTCTTCTTCTTCCTGGCTGCTCAATGTTCCCGCCAGATCTCAGACTTTTGGGTGAGGTGG TGGGTTAATGATGAATACAAGAAGTTTCCTGTCAAGGGAGAACAAGACTCGGCTGCAACTACCTTTTACTGCT TGATATATCTTCTACTAGTTGGTCTGTTTTACATTTTCATGATCTTTAGGGGTGCGACCTTTCTCTGGTGGGTAT TGAAAAGCTCCGAAACAATTCGTCGTAAGGCACTGCATAACGTCCTAAATGCTCCAATGGGGTTCTTCCTTGTC ACACCAGTTGGAGATCTGTTGCTTAACTTTACAAAAGATCAAGACATAATGGATGAAAATCTTCCGGACGCCG TGCACTTTATGGGAATCTATGGCTTGATATTGTTAGCCACCACTATAACTGTAAGTGTGACTATTAACTTCTTCG AGAAAGCAAGAGCAGTGTCAGGTGGGATGCTAGTGGGACTTGTTGCTGAAGTCTTGGAGGGGGCTCGGTGTG GTGCAAGCATTCAATAAGCAAGAGTATTTCATTGAAGAGGCTGCTAGACGTACAAACATCACTAATAGTGCTG GTTTCTGCGTTTGCAGTTGGGATGGCAAAGGATTTAGGTGGCGCTACTGTTGGTCTGGCATTTTCTAATATCAT GAATGGCTTATTTGGCAGATTATGTGCCACACGATGGAGTGTTTTATGACCAGCGTCAAAAAGATGGAGTCGC TAAGCAAATAGTTTTACCGGATGGTAATATCGTCCCAGCGGCTTCCAAGGTTCAAGTTGTTGTGGATGACGCA GCGTTGGCTCGATGGCCTGCTACAGGAAATATCAGATTTGAAGATGTGTGGATGCAATATCGTTTAGATGCCC TCTGGTAAAAGTACAACGCTTTTAGCTCTGTACAGGATGTTCGAACTCGGAAAAGGGAGAATCTTAGTCGACG GCGTGGATATAGCTACATTGTCTTTGAAGAGACTCCGTACCGGGCTCAGTATTATCCCCCCAAGAGCCAGTTAT GTTCACAGGAACTGTTCGGTCTAATCTGGATCCCTTTGGAGAGTTCAAGGATGACGCCATACTTTGGGAAGTG TGGCAAAGCTTGGTCGCTCGGACAAATGCAATTGGTATGCCTTGCTAGAGCAGCACTCAGAGCCGTGCCCATT TATTCGATGACCGGACCACTATCACTATCGCACATCGGTTAGATACGATCATAGAAAGCGACAAGATTATCGT TATGGAGCAAGGCTCTCTTATGGAATATGAATCGCCTAGTAAGCTGCTGGCTAACCGAGATTCGATGTTCTCC AAACTCGTTGATAAAACTGGACCTGCAGCTGCGGCAGCTCTACGTAAGATGGCTGAAGATTTTTGGAGTACGC GTTCAGCGCAAGGCCGTAATCAATGA

Figure S7. DNA sequences of codon-optimised LCIA and HLA3.

Table S1. Sequences of synthetic oligonucleotides used in this study.

Gene	Phytozome 10.2 Cr v5.5	Gene length				
name	(Augustus u11.6)	(bp)	Forward primer	Reverse primer	Internal forward primer	Internal reverse primer
Full-length ORFs						
LCIA	Cre06.g309000	2001		GAGCCACCCAGATCTCCGTTCGCGGTGGCG		
LCIB	Cre10.g452800	2172	GCTACTCACAACAAGCCCAGTTATGTTCGCTCTG	GAGCCACCCAGATCTCCGTTGTTCTTGGGG		
LCIC	Cre06.g307500	2161	GCTACTCACAACAAGCCCAGTTATGGCCCTTGC TCAGAAGATGAACGTG	GAGCCACCCAGATCTCCGTTGTTCATGTTGG CGAGCTCGGGC		
LCI1	Cre03.g162800	1616	GCTACTCACAACAAGCCCAGTTATGTCGGGCTT GAACAAGTTCATC	GAGCCACCCAGATCTCCGTTCACCTTGGTG GCGCCGTC		
HLA3*	Cre02.g097800	6068	GCTACTACAACAAGCCCAGTTATGGGCGAGGG	GAGCCACCCAGATCTCCGTTCTGGTTGCGG	ACTTCTCCGGTGGC	CAGCACCAGGTCGG
CCP1	Cre04.g223300	1891	GCTACTCACAACAAGCCCAGTTATGTCATCCGA	GAGCCACCAGATCTCCGTTCTGCGCGGCG	CAGOGO	001000
CCP2	Cre04.g222750	1801	GCTACTACAACAACAACGAGTTATGGCATCCGA	GAGCCACCAGATCTCCGTTCTGAGCGGTG		
CAH2	Cre04.g223050	3309	GCTACTACAACAACAAGCCCAGTTATGGCGCGTAC	GAGCCACCCAGATCTCCGTTCTCGTAGCGG		
CAH3	Cre09.g415700	1594	GCTACTCACAACAAGCCCAGTTATGCGCTCAGC	GAGCCACCAGATCTCCGTTCAGCTCGTATT		
CAH6	Cre12.g485050	1226	GCTACTCACAACAAGCCCAGTTATGGGATGCGG	GAGCCACCAGATCTCCGTTCTCGGCGCTG		
cDNA			1000,00010			
LCIA	Cre06.g309000	1008	ATGCAGACCACTATGACTCGCC	CGCGGTGGCGGGCTTGGC		
LCIB	Cre10.g452800	1344	ATGTTCGCTCTGTCTTCGCG	GTTCTTGGGGGCCTCGAAG		
LCIC	Cre06.g307500	1329	ATGGCCCTTGCTCAGAAGATG	GTTCATGTTGGCGAGCTCG		
LCI1	Cre03.g162800	576	ATGTCGGGCTTGAACAAGTTCATCTATGTGG	CACCTTGGTGGCGCCGTCAG		
HLA3	Cre02.g097800	3975	ATGGGCGAGGGCAAGGTG	CTGGTTGCGGCCCTGCGC		
CCP2	Cre04.g222750	1065	ATGGCATCCGACGCTATGACTATCAACGAGG	CTGAGCGGTGGCGGGGCCGAT		
CAH1	Cre04.g223100	1131	ATGGCGCGTACTGGCGCTC	GTGATAGCGGGCCAGCTTGATG		
CAH3	Cre09.g415700	930	ATGCGCTCAGCCGTTCTAC	CAGCTCGTATTCGACCAGG		
CAH6	Cre12.g485050	792	ATGGGATGCGGTGCCAGCGTGC	CTCGGCGCTGCTGCCGCCCTT		
LCIA	synthetic codon optimised	1008	ATGCAGACAACTATGACAAGACCT	TGCTGTAGCAGGTTTAGCAGAT		
HLA3	(Fig. S7) (Fig. S7)	3975	ATGGGAGAGGGGAAGGTG	TTGATTACGGCCTTGCG		