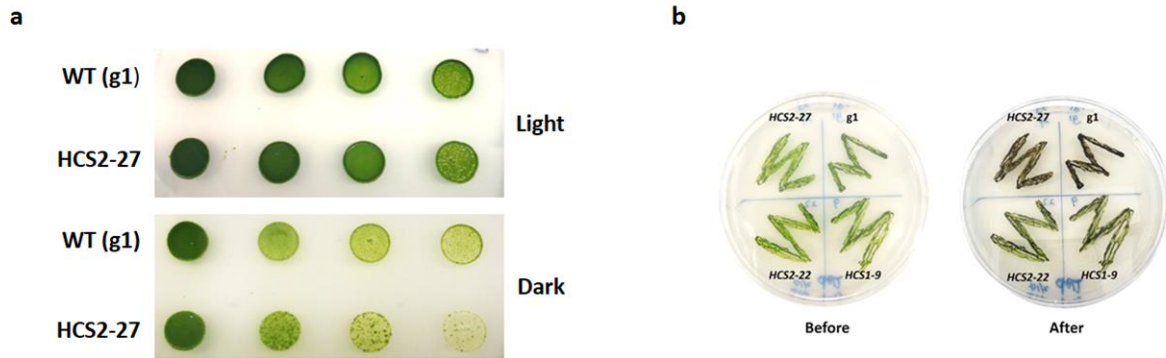
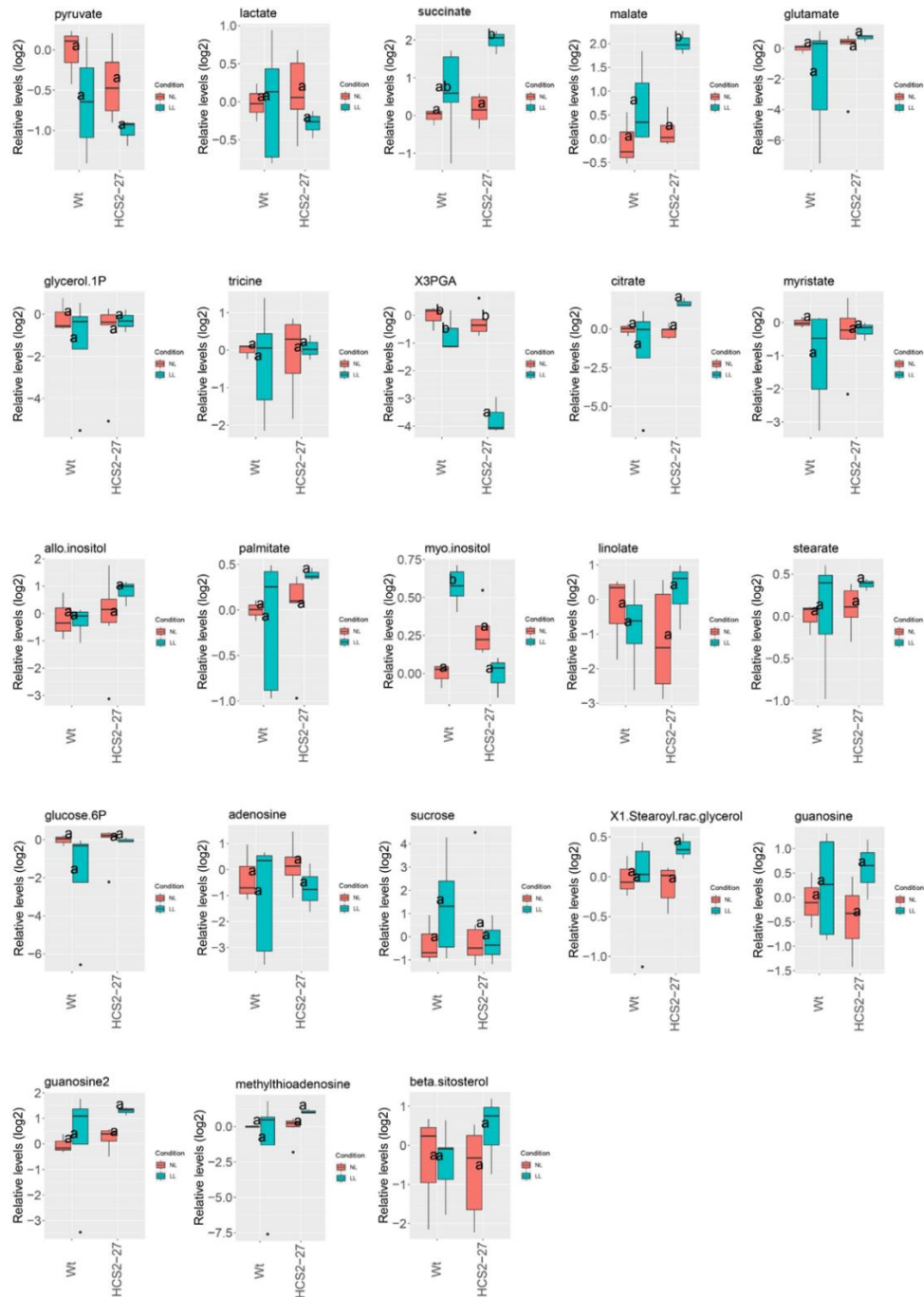


**Recurrent evolutionary switches of mitochondrial cytochrome *c* maturation systems in Archaeplastida**

Li *et al.*



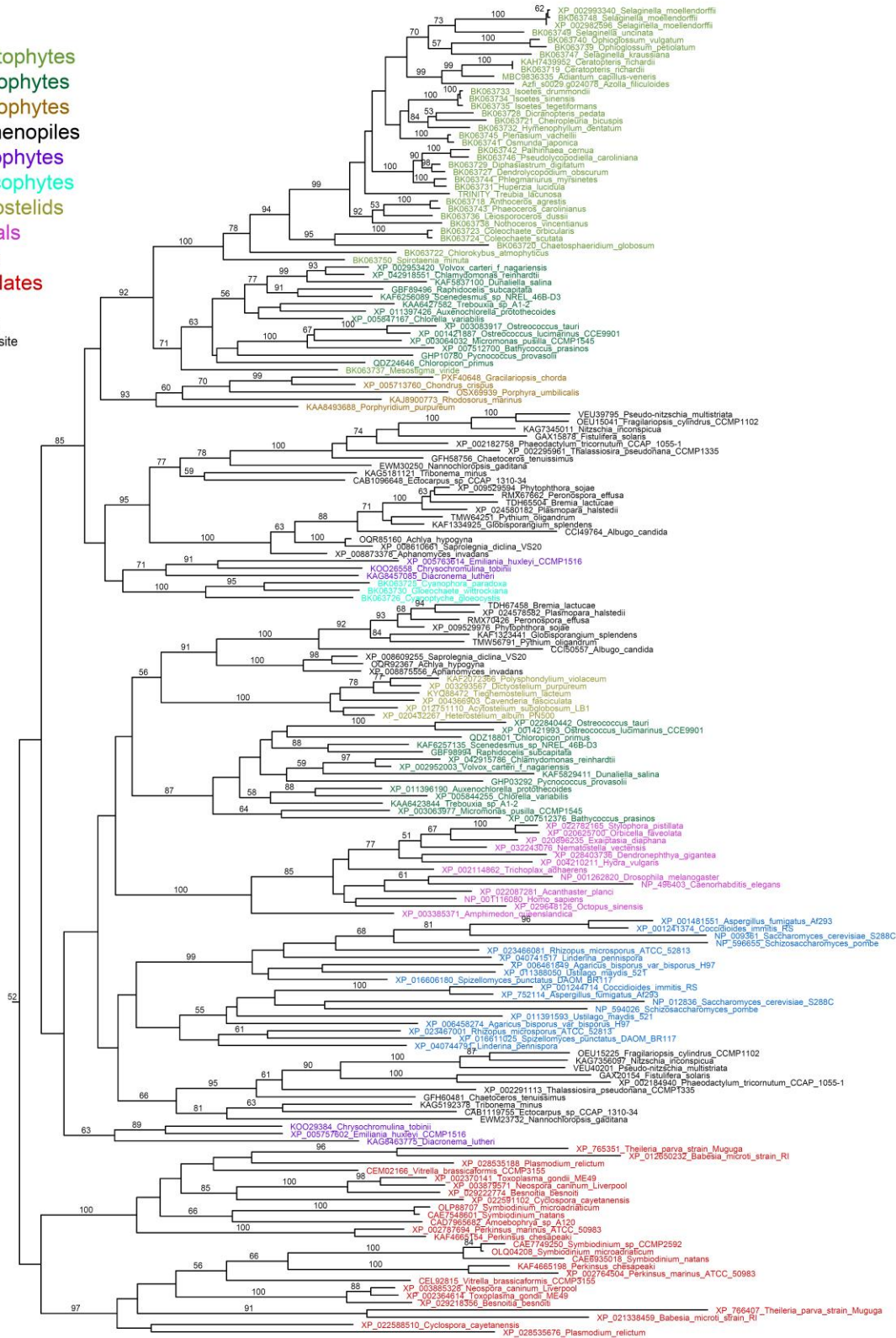
**Supplementary Fig. 1. Spot test assay and TTC staining of *Chlamydomonas* HCS mutants. a,** The in-frame HCS2-27 mutant exhibits growth deficiency in the dark. **b,** Compared to the WT g1 strain and HCS2-27, knock-out mutants HCS1-9 and HCS2-22 remain green after TTC staining, indicating a disruption of their mitochondrial ETC functionality.



**Supplementary Fig. 2. Metabolic analysis of *Chlamydomonas* HCS knock-down mutant (HCS2-27) and the wild type (Wt) grown under normal light (NL) and low light (LL) conditions.** The relative metabolite levels were normalized by the mean of those in Wt/NL conditions and log<sub>2</sub> transformed. The boxplots show the minimum, first quartile, median, third quartile, and maximum relative levels (n = 3, 6, 4, 3 for each sample). Letters indicate significant differences between mean values (one-way ANOVA: Tukey's multiple comparisons test, P < 0.05), and groups with the same letters are not significantly different. Source data are provided as a Source Data file.

Streptophytes  
 Chlorophytes  
 Rhodophytes  
 Stramenopiles  
 Haptophytes  
 Glaucophytes  
 Dictyostelids  
 Animals  
 Fungi  
 Alveolates

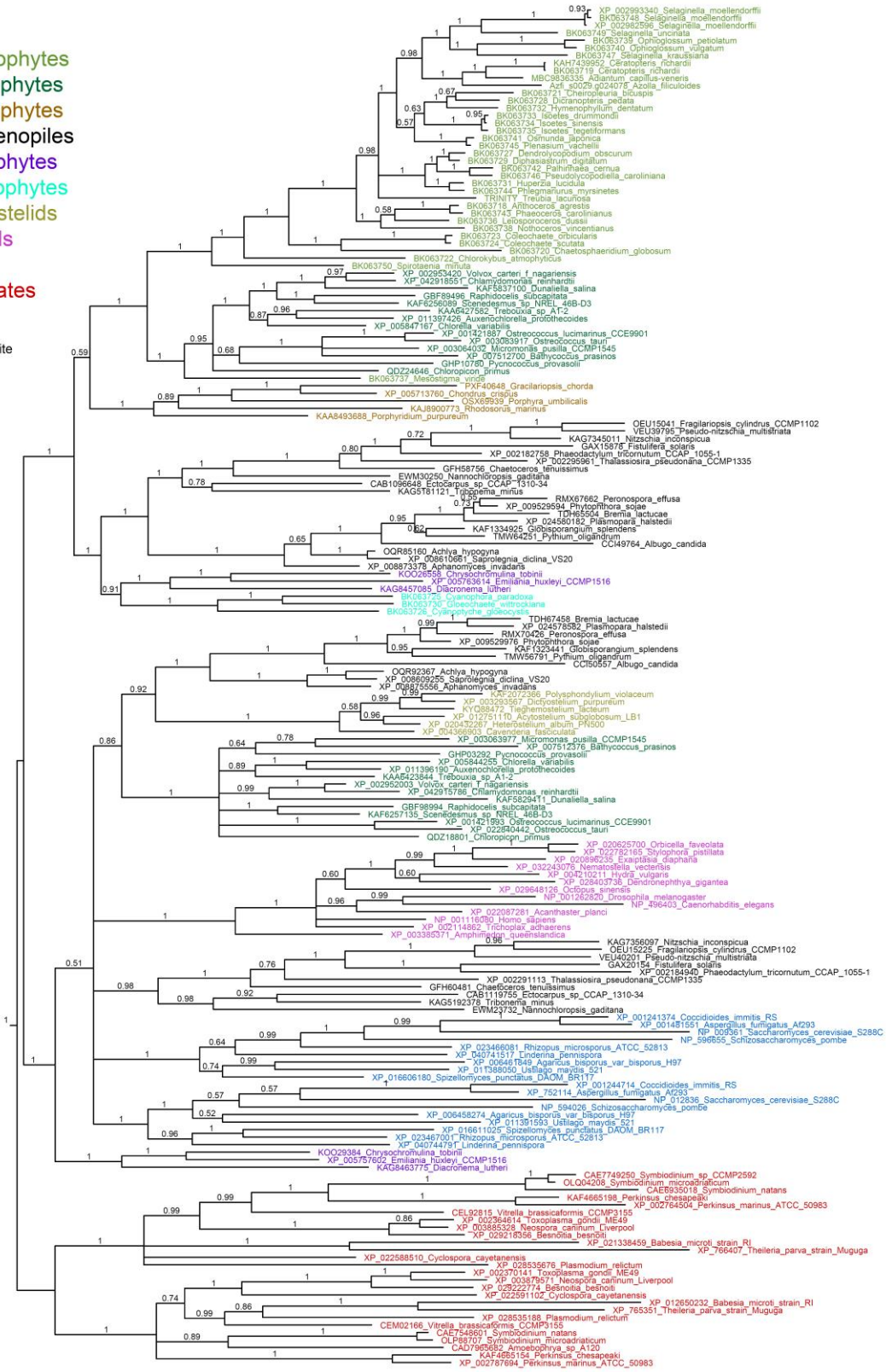
0.1 subs/site



**Supplementary Fig. 3. Fully labeled ML tree corresponding to Figure 7A.** Bootstrap values <50% not shown. Tree was rooted by midpoint rooting.

Streptophytes  
 Chlorophytes  
 Rhodophytes  
 Stramenopiles  
 Haptophytes  
 Glaucophytes  
 Dictyostelids  
 Animals  
 Fungi  
 Alveolates

0.1 subs/site



Supplementary Fig. 4. Fully labeled Bayesian tree corresponding to Figure 7A. Posterior probabilities <0.5 not shown. Tree was rooted by midpoint rooting.

**Supplementary Table 1. Sequence features and subcellular localization prediction of *C. reinhardtii* HCS.**

Gene name	Phytozome ID	Genbank ID	CDS (bp)	5'UTR (bp)	Exon	Intron	Subcellular localization	
							PredAlgo	WoLF PSORT
<i>CreHCS1</i>	Cre12.g525700	5722497	879	359	5	4	M	M
<i>CreHCS2</i>	Cre16.g690050	5724796	960	0	6	5	M	M

CDS, coding sequencing; UTR, untranslated region; M, mitochondrion; PredAlgo (<http://lobosphaera.ibpc.fr/predalgo>) and WoLF PSORT (<https://wolfsort.hgc.jp/>).

**Supplementary Table 2. Accession numbers for query sequences used for blast searches.**

Species	System I proteins		System III proteins	
Land plants				
<i>Arabidopsis thaliana</i>	CCMA	NP_176516	-	-
	CCMB	YP_009472099	-	-
	CCMC	YP_009472125	-	-
	CCME	NP_190747	-	-
	CCMF <sub>C</sub>	YP_009472113	-	-
	CCMF <sub>N1</sub>	YP_009472126	-	-
	CCMF <sub>N2</sub>	YP_009472124	-	-
	CCMH	NP_563966	-	-
<i>Selaginella moellendorffii</i>	-	-	HCCS	XP_002982596
Green algae				
<i>Chlamydomonas reinhardtii</i>	-	-	HCS1	XP_042918551
	-	-	HCS2	XP_042915786
<i>Klebsormidium nitens</i>	CCMA	GAQ83552	-	-
	CCMB	GAQ93830	-	-
	CCMC	GAQ93831	-	-
	CCME	GAQ86245	-	-
	CCMF	GAQ93806	-	-
	CCMH	GAQ81871	-	-
	-	-	-	-
Red algae				
<i>Cyanidioschyzon merolae</i>	CCMA	BAA36528	-	-
	CCMB	BAA36529	-	-
	CCMC	BAA36544	-	-
	CCME	XP_005536347	-	-
	CCMF	BAA36527	-	-
	CCMH	XP_005534965	-	-
	-	-	-	-
<i>Chondrus crispus</i>	-	-	HCCS	XP_005713760