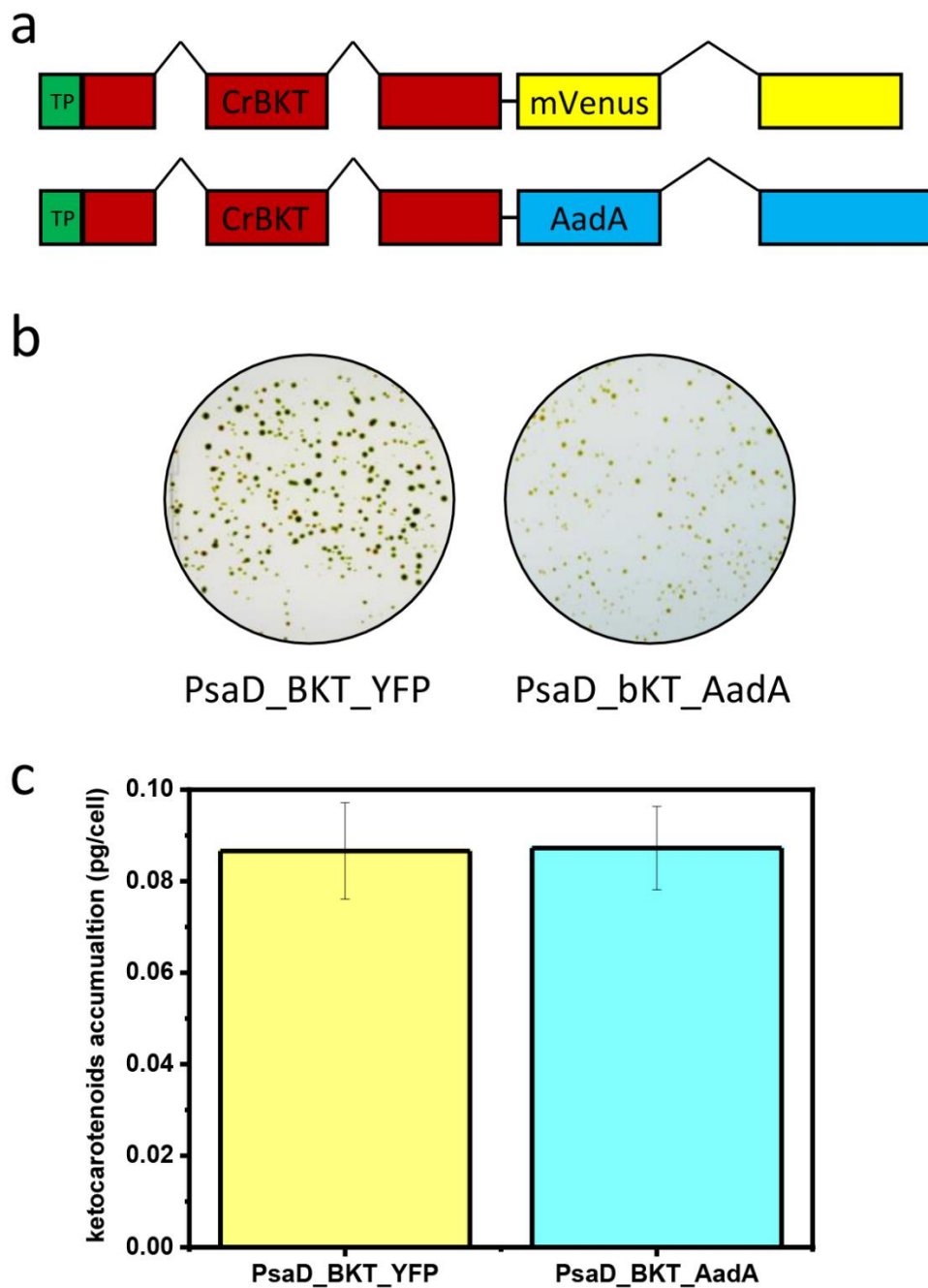


## SUPPLEMENTARY INFORMATION

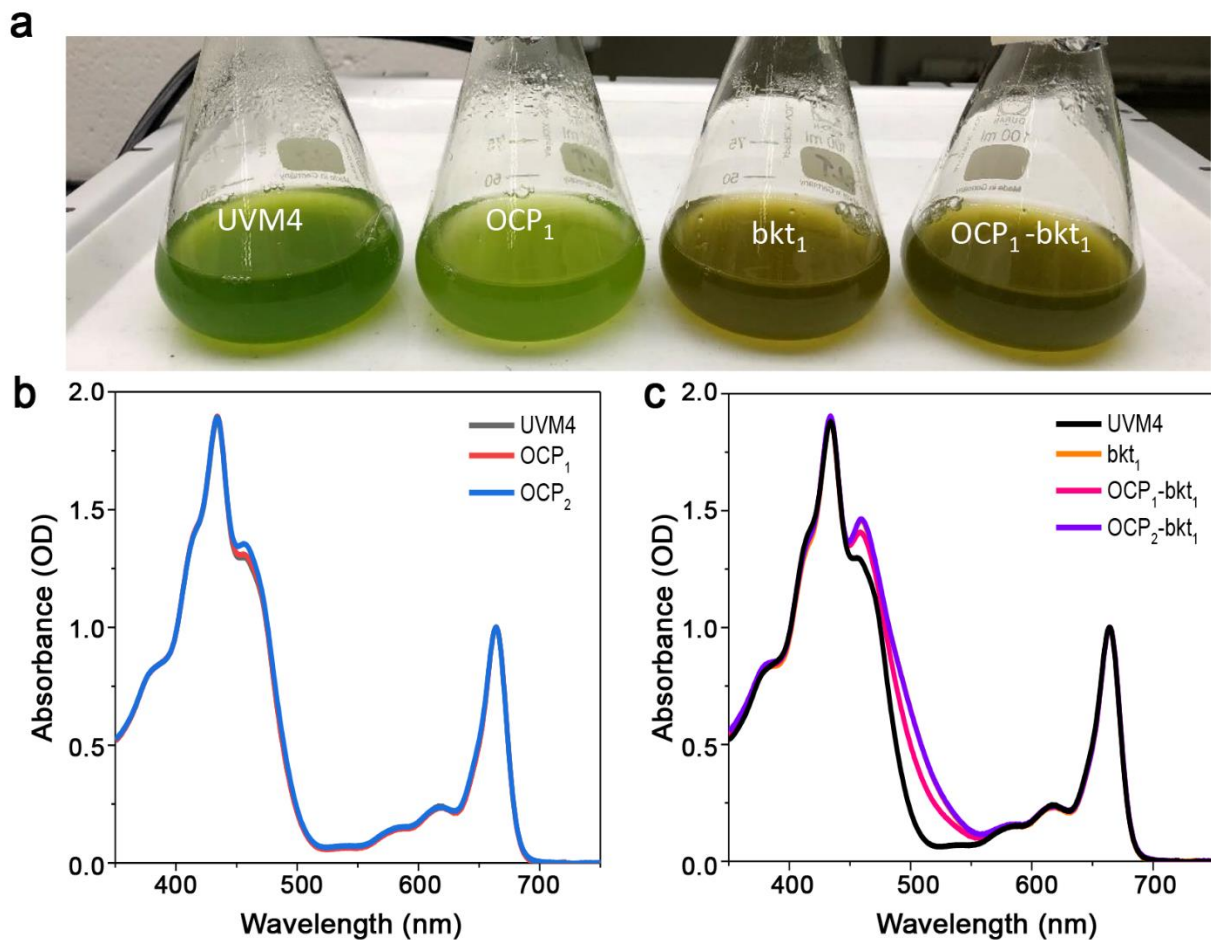
**Figure S1 *FtOCP* sequence.** Aminoacidic sequence of *Fischerella termalis* OCP (WP\_009459388.1)

MSFTIESARSIFPETEVANAI PAIVESFNQLSAEDQLALLWFAYTEMGVTITPAAMGAA  
NMVFAEKTTLTQIKQMSAREQTQVMCDLINHADTPICRTYSSFGTNVKLGFWYQLGEWMK  
QGIVAPIPEGYKLSAQASDVLQTI RQLEGGQQLTVLQNI VVNMGYAPT VSAQKVKEPVV  
PPKDIAPRAKVSIEGIDNLTVLSY MENMNAFDFQGAVALFAENGALQPPFQDP I VGQEN  
ILAYMREECYGLKLIPERGI SEPAEGGFTQVKVTGKVQTPWFGDSVGINLAWRFLLN PQ  
GKIFFVAIDVLATPQELLMGFIQ

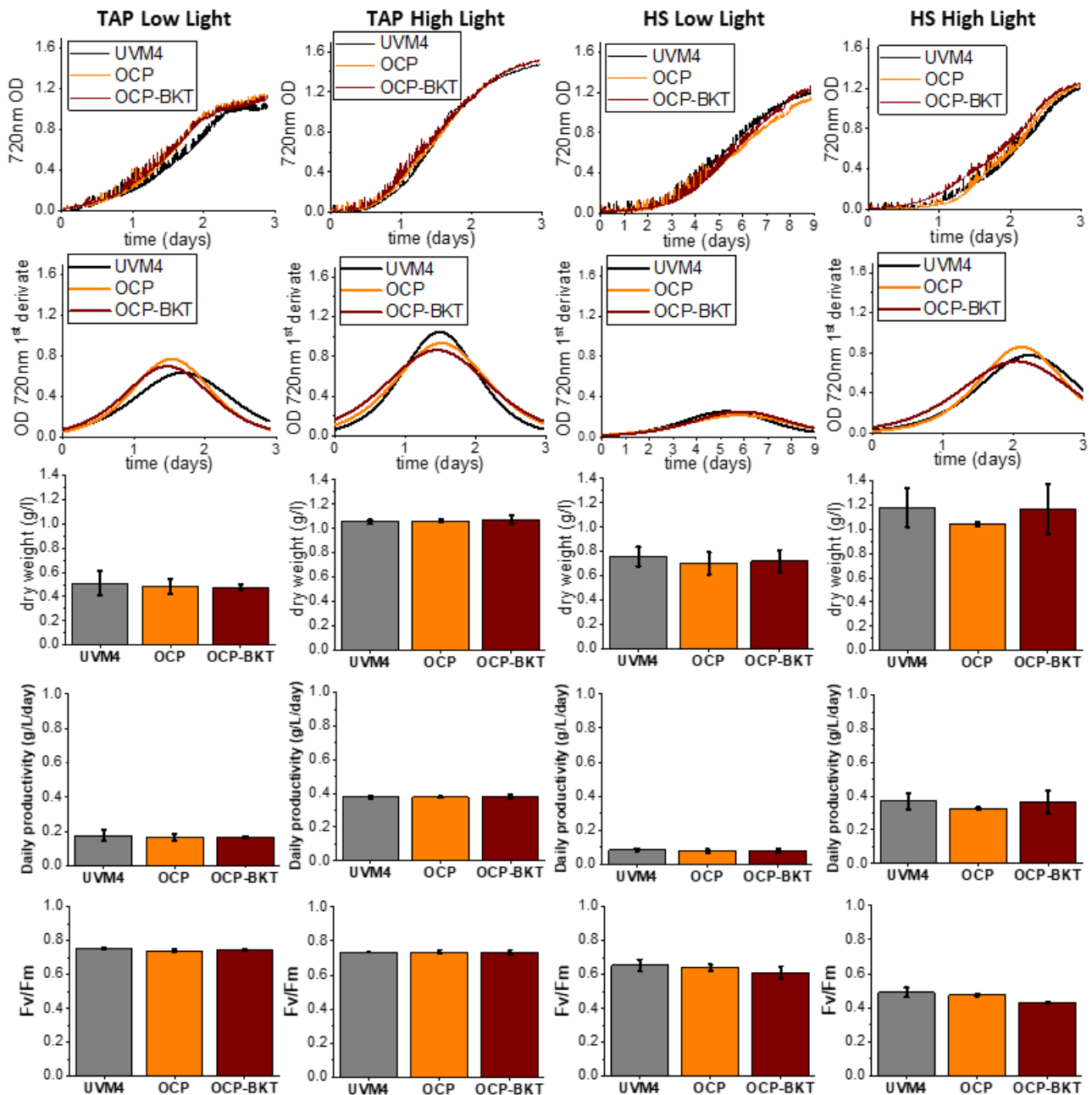
**Figure S2. *bkt* expression and ketocarotenoids accumulation.** a) schematic overview of construct tested for CrBKT (*Chlamydomonas reinhardtii*  $\beta$ -carotene ketolase) expression. Both vectors use Heat shock protein 70/ Ribulose biphosphate carboxylase small chain 2 (Hsp70A/Rbcs2) hybrid promoter and PsaD transit peptide (TP) for chloroplast localization. In the case of PsaD\_BKT\_YFP vector (upper) CrBKT is fused to YFP (Yellow Fluorescent Protein). PsaD\_BKT\_AadA vector contains the spectinomycin resistance gene (AadA, aminoglycoside-3''-adenylyltransferase gene). b) Green/orange/red phenotype obtained upon transformation of *C. reinhardtii* with PsaD\_BKT\_YFP or PsaD\_BKT\_AadA vectors. c) Ketocarotenoids accumulation in PsaD\_BKT\_YFP or PsaD\_BKT\_AadA transformed lines. 50 colonies pre-screened by eyed for orange/red phenotype were tested for each transformation. Error bars are reported as standard deviations (n=3).



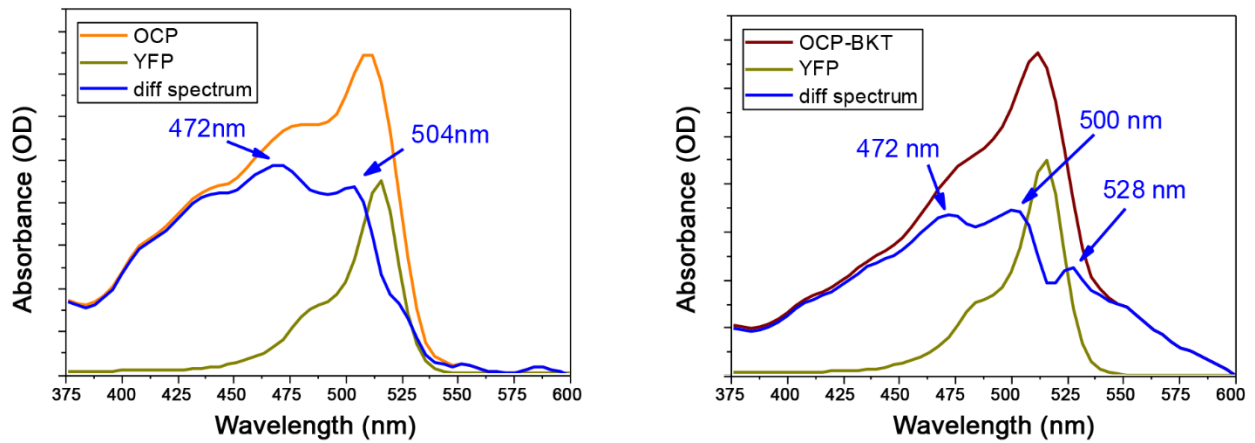
**Figure S3. Influence of *ocp* and *bkt* expression on pigments composition.** a) Cultures of *Chlamydomonas reinhardtii* strains UVM4 (UV-mediated mutant 4, herein used as background strain), *ocp<sub>1</sub>* (transformed line expressing Orange Carotenoid-binding Protein), *bkt<sub>1</sub>* (transformed line expressing  $\beta$ -carotene ketolase) and *ocp<sub>1</sub>-bkt<sub>1</sub>* (transformed line expressing both Orange Carotenoid-binding Protein and  $\beta$ -carotene ketolase ). b) absorption spectra (reported as optical density, O.D.) of pigments extracted from UVM4, *ocp<sub>1</sub>* and *ocp<sub>2</sub>* lines; c) absorption spectra (reported as optical density, O.D.) of pigments extracted from UVM4, *bkt<sub>1</sub>*, *ocp<sub>1</sub>-bkt<sub>1</sub>* and *ocp<sub>2</sub>-bkt<sub>1</sub>*. The data reported are representative of three independent experiments.



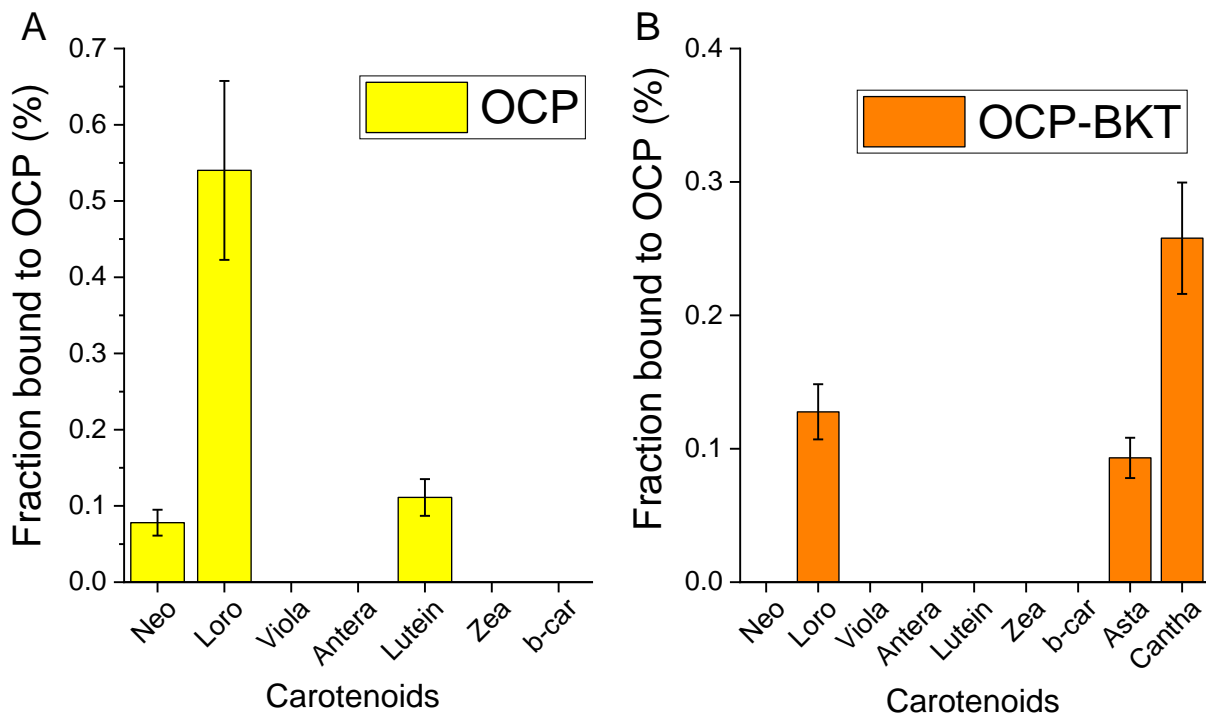
**Figure S4. Effect of OCP and BKT expression on algal growth.** UVM4 (UV-mediated mutant 4, herein used as background strain), *ocp1* (transformed line expressing Orange Carotenoid-binding Protein), *bkt1* (transformed line expressing  $\beta$ -carotene ketolase) and *ocp1-bkt1* (transformed line expressing both Orange Carotenoid-binding Protein and  $\beta$ -carotene ketolase) growth curve, dry weight, productivity and Photosystem II maximum quantum yield (Fv/Fm) in mixotrophy (growth in Tris-Acetate-Phosphate, TAP, medium) or autotrophy (growth in High Salt, HS). Two different light intensities were used: 100  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  for low light (LL) and 500  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  for high light (HL). Error bars are reported as standard deviations (n=3).



**Figure S5. Difference absorption spectra.** Difference absorption spectra of purified OCP (Orange Carotenoid-binding Protein) proteins OCP and OCP-BKT deprived by YFP (Yellow Fluorescent Protein) signal. OCP and OCP-BKT were isolated respectively from *Chlamydomonas reinhardtii* strains expressing recombinant OCP protein (OCP) or both BKT ( $\beta$ -carotene ketolase) and OCP (OCP-BKT). Main peaks are indicated. The data reported are representative of three independent experiments.



**Figure S6. Percentage of the different carotenoids in *ocp* and *ocp-bkt* strains bound to FtOCP.** FtOCP (Orange Carotenoid-binding Protein from *Fischerella thermalis*) content in *ocp<sub>1</sub>* (*Chlamydomonas reinhardtii* transformed line expressing OCP), and *ocp<sub>1</sub>-bkt<sub>1</sub>* (*Chlamydomonas reinhardtii* transformed line expressing both OCP and  $\beta$ -carotene ketolase, BKT) strains were determined by western blot reported in Figure 3b where isolated OCP (recombinant OCP purified from *ocp<sub>1</sub>*) and OCP-BKT (recombinant OCP purified from *ocp<sub>1</sub>-bkt<sub>1</sub>*) were used a standard, which protein concentration was determined before loading. On the base of the FtOCP concentration in *ocp<sub>1</sub>* and *ocp<sub>1</sub>-bkt<sub>1</sub>* strains, the carotenoid content in isolated FtOCP proteins (Figure 3e) and in whole cells (Table S3), the percentage of each carotenoid bound to FtOCP compared to the amount of the same carotenoid in *ocp<sub>1</sub>* or *ocp<sub>1</sub>-bkt<sub>1</sub>* strains was calculated. Neo: neoxanthin; Loro: loroxanthin; Antera: anteraxanthin; Viola: violaxanthin; Lutein: lutein, Zea: zeaxanthin; b-car:  $\beta$ -carotene, Asta: astaxantin; Cantha: canthaxanthin. Error bars are reported as standard deviations (n=3).



**Table S1 strains nomenclature.** Nomenclature and characteristics of *Chlamydomonas reinhardtii* (Cr) strains generated in this work. UVM4: UV-mediated mutant 4. *ocp<sub>x</sub>*: transformed lines expressing Orange Carotenoid-binding protein from *Fischerella Thermalis* (Ft). PsaD: Photosystem I Subunit D. BKT:  $\beta$ -carotene ketolase. YFP: Yellow Fluorescent Protein. AadA: aminoglycoside-3"-adenylyltransferase gene conferring resistance to spectinomycin. *ocp<sub>x</sub>-bkt<sub>y</sub>*: transformed lines expressing both OCP and BKT.

background	vector used	gene inserted	nomenclature
UVM4	-	-	UVM4
UVM4	pOpt2_PsaD_BKT_YFP	CrBKT-YFP	PsaD_BKT_YFP
UVM4	pOpt2_PsaD_BKT_AadA	CrBKT-AadA	PsaD_BKT_AadA
UVM4	pOpt2_PsaD_FtOCP_YFP	FtOCP-YFP	<i>ocp<sub>x</sub></i>
<i>ocp<sub>x</sub></i>	pOpt2_PsaD_BKT_AadA	BKT-AadA	<i>ocp<sub>x</sub>-bkt<sub>y</sub></i>

**Table S2 protein nomenclature.** Nomenclature of protein isolated in this work. *ocp<sub>1</sub>*: *Chlamydomonas reinhardtii* transformed line expressing Orange Carotenoid-binding Protein, OCP, fused to Yellow Fluorescent protein (YFP). *ocp<sub>1</sub>-bkt<sub>1</sub>*: *Chlamydomonas reinhardtii* transformed line expressing both OCP-YFP and  $\beta$ -carotene ketolase, BKT.

host strain	Protein	nomenclature
<i>ocp<sub>1</sub></i>	OCP-YFP	OCP
<i>ocp<sub>1</sub>-bkt<sub>1</sub></i>	OCP-YFP	OCP-BKT

**Table S3 Pigment profile** HPLC analysis of pigments extracted from *C. reinhardtii* strains UVM4 (UV-mediated mutant 4), *ocp<sub>1</sub>* (transformed lines expressing OCP, Orange Carotenoid-binding protein) and *ocp<sub>1</sub>-bkt<sub>1</sub>* (*Chlamydomonas reinhardtii* transformed line expressing both OCP and  $\beta$ -carotene ketolase, BKT). Neo: neoxanthin; Loro: lorenzoanthin; Viola: violaxanthin; Antera: anteraxanthin; Lutein: lutein; Zea: zeaxanthin;  $\beta$ -car:  $\beta$ -carotene, Asta: astaxanthin; Cantha: canthaxanthin. Carotenoids are normalized to 100 chlorophylls (Chls). Chlorophylls (in pg) per cell content (Chl/cell) and chlorophylls to carotenoids ratio (Chl/Car) are also reported. Errors are reported as standard deviation (s.d., n=3).

	Neo	Loro	Viola	Antera	Lutein	Zea	$\beta$ -car	Asta	Cantha	Chls	Chl/cell	Chl/Car
<b>UVM4</b>	4.6	2.9	6.4	0.9	12.2	1.3	5.8	-	-	100.0	1.1	3.0
<i>s.d.</i>	0.5	0.2	0.4	0.4	1.0	0.7	0.6	-	-	-	0.2	0.1
<b>ocp1</b>	4.4	2.4	3.5	2.1	10.2	3.5	8.1	-	-	100.0	1.1	2.8
<i>s.d.</i>	0.1	0.3	0.6	1.0	0.1	0.4	1.1	-	-	-	0.2	0.1
<b>ocp1-bkt1</b>	3.2	2.2	4.4	1.6	7.9	1.5	5.3	10.8	3.8	100.0	0.6	2.6
<i>s.d.</i>	0.1	0.4	1.3	0.1	1.0	0.4	0.4	1.2	0.1	-	0.1	0.1

