Supporting Information

Engineered green alga *Chlamydomonas reinhardtii* as a whole-cell photosynthetic biocatalyst for stepwise photoproduction of H_2 and ε-caprolactone

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CDS CHMO
1st Intron RBCS2
2nd Intron RBCS2
3rd Intron RBCS2
Plasmid backbone pAGM1287

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Figure S1. Sequence of pMBS596 containing the CHMO coding sequence in B3–B4 for level 0. The coding sequence of *Acinetobacter* NCIB 9871 CHMO (purple) was codon adapted and *Chlamydomonas* RBCS introns one (blue), two (green) and three (orange) were incorporated to ensure high-level transgene expression. Sequences of recipient vector pAGM1287 are in grey.

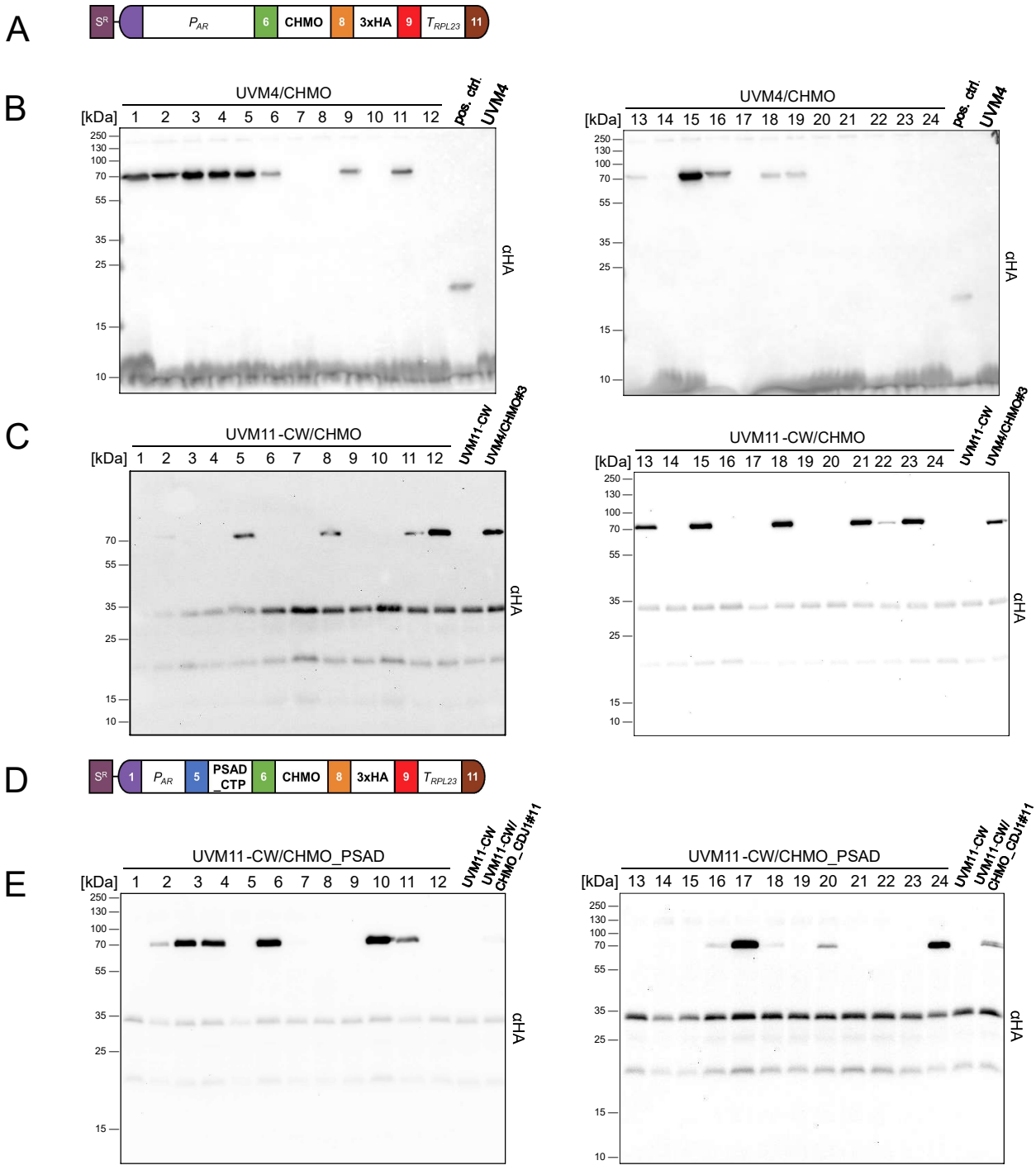

Figure S2: Generation of *Chlamydomonas* lines expression CHMO-3xHA in the cytosol and the chloroplast stroma. A) Level 2 device consisting of the *aadA* cassette (S^R) and the transcription unit for the expression of CHMO-3xHA in the cytosol (CHMO). The coding sequence of CHMO (B3–B4) was fused to the PAR (HSP70A-RBCS2 promotor, A1–B2), a 3xHA-tag (B5) and TRPL23 (RPL23 terminator, B6–C1). B) Screening for transformants expressing CHMO-3xHA in the cytosol. The level 2 construct shown in A) was transformed into *Chlamydomonas* UVM4. Whole-cell protein corresponding to 2 µg chlorophyll of 24 spectinomycin-resistant transformants was separated by SDS-PAGE and immunodetected with an antibody against HA. C) Screening for transformants overexpressing CHMO-3xHA in the cytosol. The level 2 construct shown in A) was transformed into *Chlamydomonas* UVM11-CW. Whole-cell protein corresponding to 2 µg chlorophyll of 24 spectinomycin-transformants was separated by SDS-PAGE and immunodetected with an antibody against HA. Additionally detected bands at ~35 kDa and ~20 kDa are cross reactions of the HA antibody with proteins in this strain background. D) Level 2 device consisting of the *aadA* cassette (S^R) and the transcription unit for the expression of CHMO-3xHA in the stroma (CHMO_PSAD). The coding sequence of CHMO (B3–B4) was fused to PAR (HSP70A-RBCS2 promotor, A1–B1), the chloroplast transit peptide of PSAD (B2), a 3xHA-tag (B5) and TRPL23 (RPL23 terminator, B6–C1). E) Screening for transformants overexpressing CHMO-3xHA in the chloroplast stroma. The level 2 construct shown in A) was transformed into *Chlamydomonas* UVM11-CW. Whole-cell protein corresponding to 2 µg chlorophyll of 24 spectinomycin-transformants was separated by SDS-PAGE and immunodetected with an antibody against HA. The additionally detected bands at ~35 kDa and ~20 kDa are known cross reactions of the antibody in this strain background.

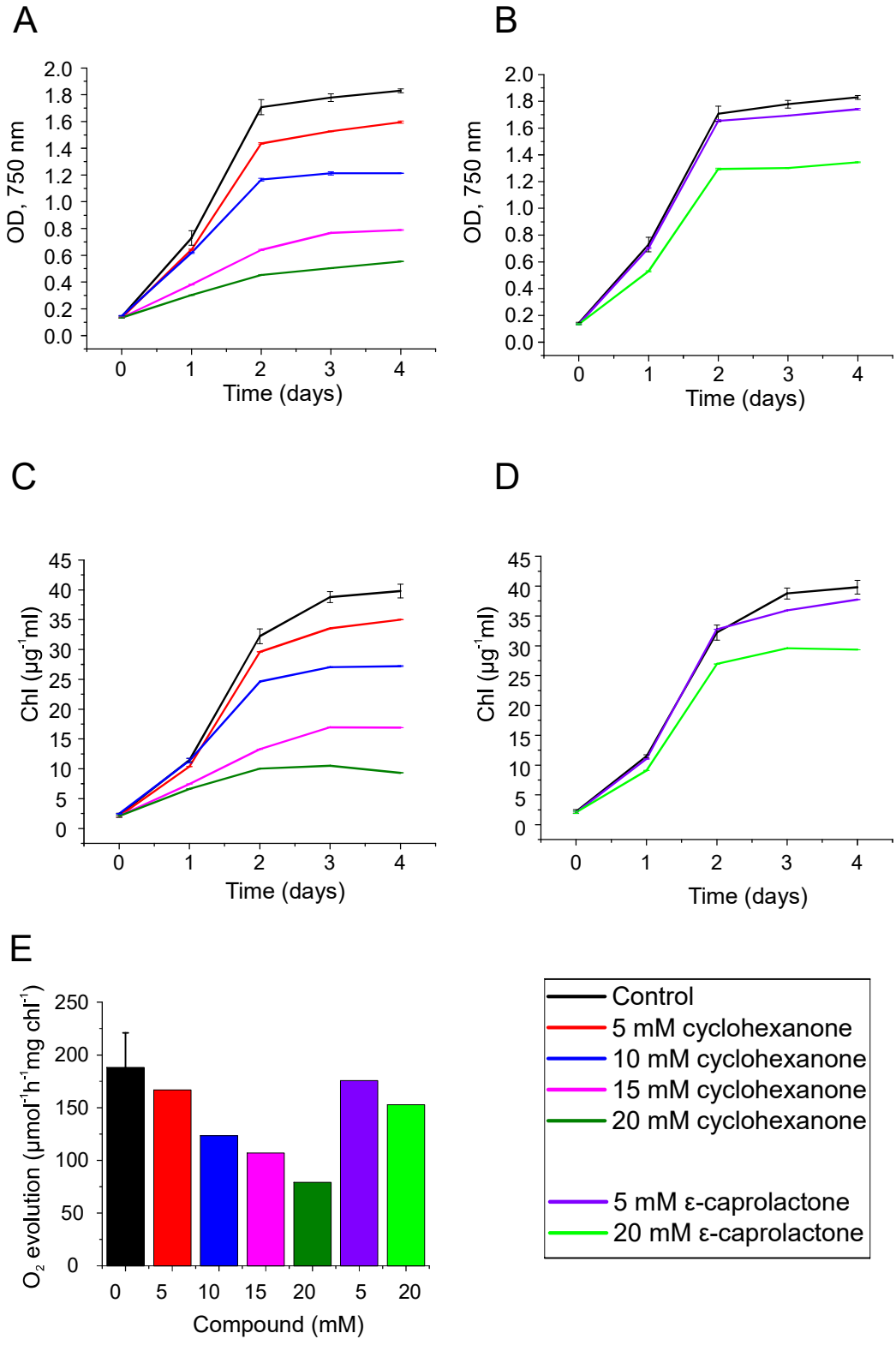

Figure S3. Evaluating toxicity of substrate and product towards the *Chlamydomonas* UVM4 strain. A) Following growth by $OD_{750\ nm}$ with no additives (black), 5 mM cyclohexanone (red), 10 mM cyclohexanone (blue), 15 mM cyclohexanone (pink), and 20 mM cyclohexanone (green). B) Following growth by $OD_{750\ nm}$ without additives (black), with 5 mM ε-caprolactone (purple) and with 20 mM ε-caprolactone (light green). C) Following growth by total chlorophyll concentration, same samples as in A. D) Following growth by total chlorophyll concentration, same samples as in B. E) Oxygen evolution on the 2nd day of cultivation (0mM, 5mM, 15mM, and 20mM cyclohexanone; 5 mM and 20 mM ε-caprolactone) and on the 3rd day of cultivation (0 mM and 10 mM cyclohexanone). The presented standard deviations in the curves are from two biological replicates for the control and from three technical replicates for all others.

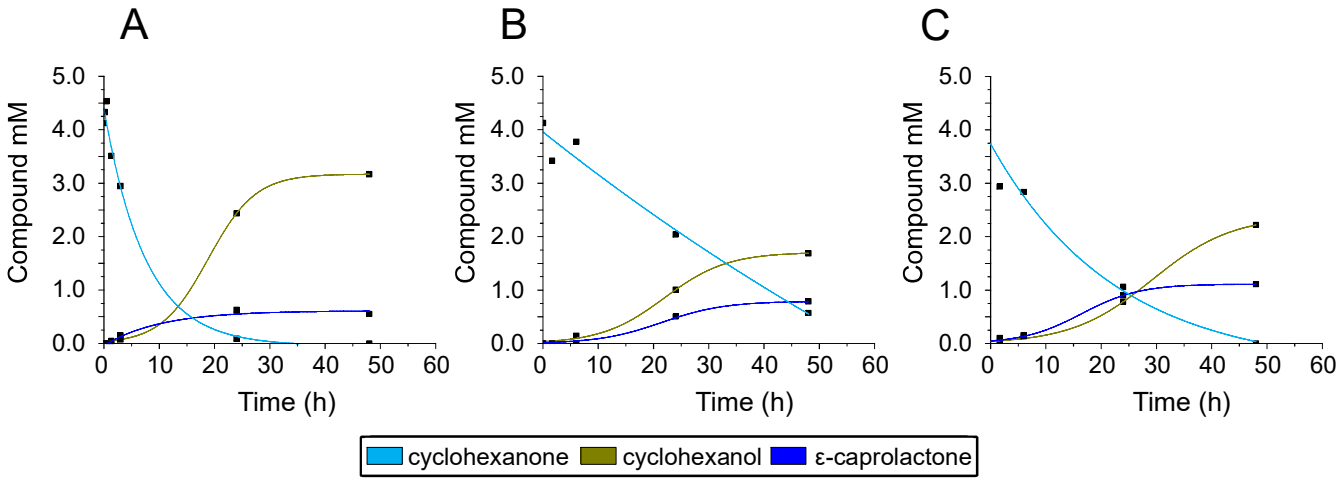

Figure S4. Effects of additives to the reaction with UVM4/CHMO clone 3. Illumination was set to 165 µmol photons m^{-2} s^{-1}. A) No additives. B) 30 mM fomepizole. C) 1.7 % (v/v) Ethanol.

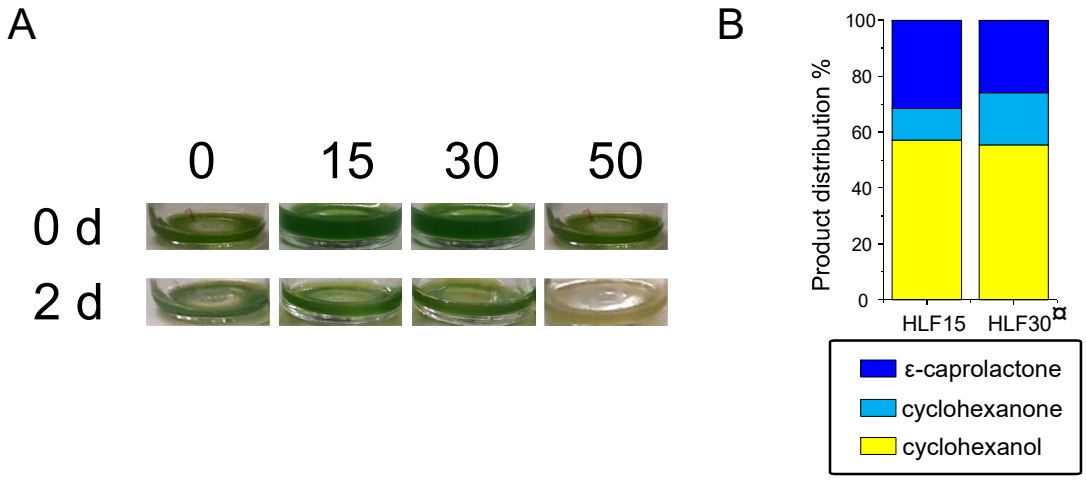

Figure S5. Effect of fomepizole on UVM4/CHMO clone 3. A) Effect of fomepizole on *Chlamydomonas* cells after 2 days of biotransformation in ML with 0, 15, 30, and 50 mM of fomepizole. B) Comparison of the effect of 15 mM (HLF15) and 30 mM (HLF30) fomepizole on product distribution after 48 h in HL. ¤ Same data as in Fig. 2B HLF to facilitate comparison.

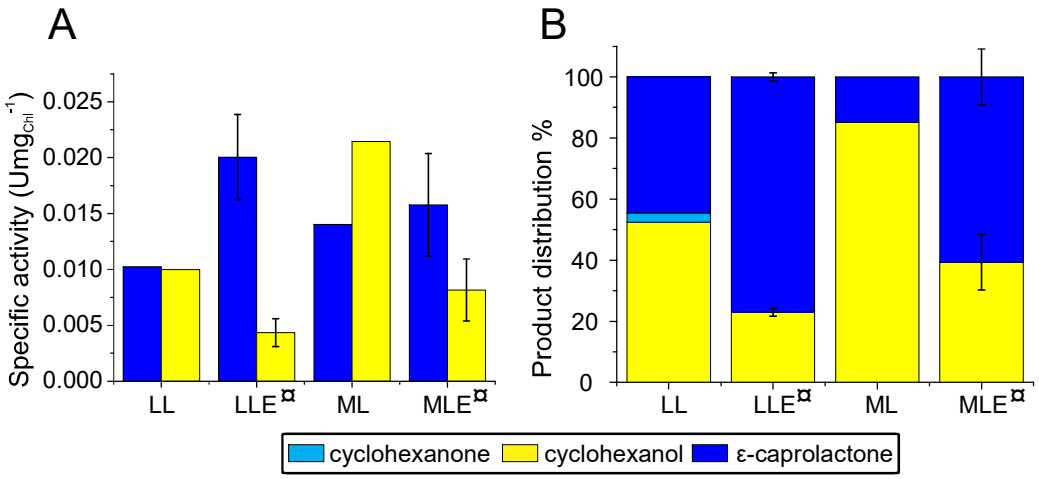

Figure S6. Comparing the effect of ethanol addition under different light conditions using UVM4/CHMO clone 3. A) Specific activity (Umg$_{Chl}^{-1}$) under the following conditions: LL) LL without additives. LLE) LL with ethanol [1.7%, (v/v)]. ML) ML without additives. MLE) ML with ethanol [1.7%, (v/v)]. B) Product distribution after 48 h of cultivation under the conditions described in A). Presented standard deviations are from three biological replicates. ¤ Data of the samples with ethanol are the same as in Fig. 2 to facilitate comparison.

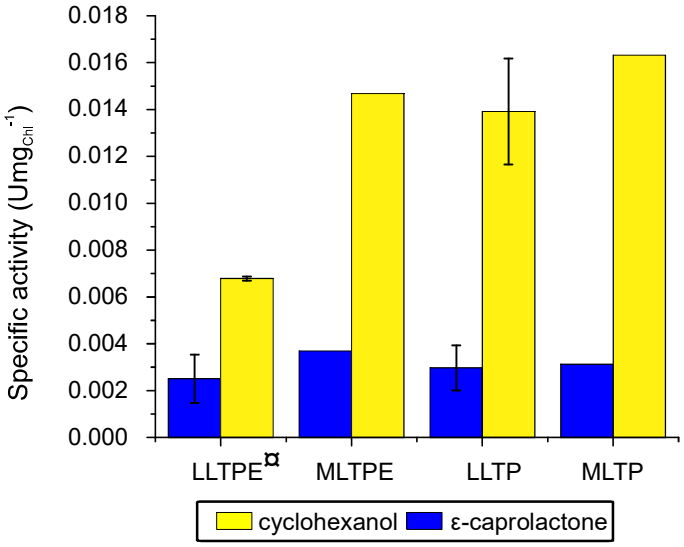

Figure S7. Comparing different conditions for phototrophic biotransformation using UVM4/CHMO clone 3. Specific activity (Umg$_{Chl}^{-1}$) under the following conditions: LLTPE) LL with ethanol [1.7%, (v/v)]. MLTPE) ML with ethanol [1.7%, (v/v)]. LLTP) LL without ethanol. MLTP) ML without ethanol. ¤ Data for LLTPE are the same as in Fig. 2 to facilitate comparison. Presented standard deviation are from 2–3 technical replicates.

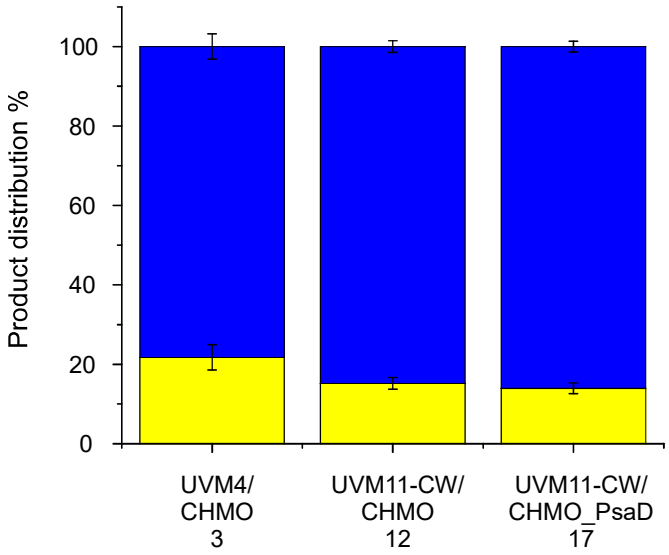

Figure S8. The ratios of the compounds present in different strains after 48h of biotransformation. Standard deviations are from three biological replicates.

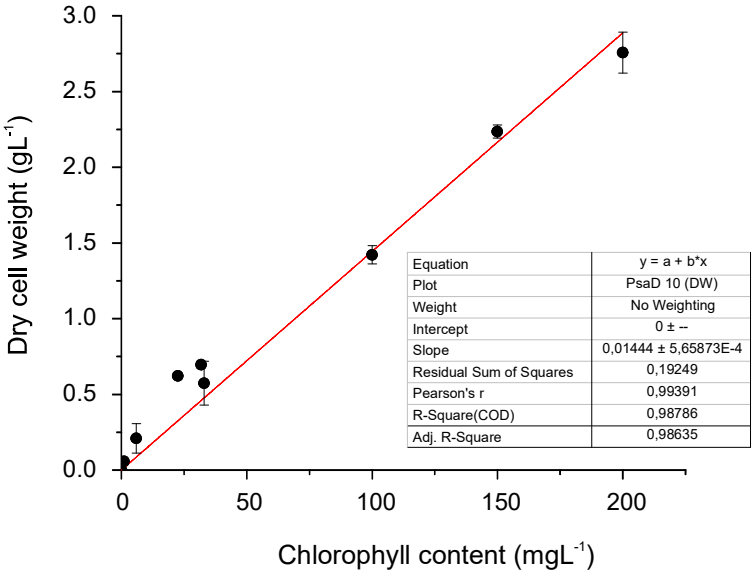

Figure S9. Chlorophyll and cell dry weight (DCW) correlation curve for UVM11-CW/CHMO_PSAD clone 10. Standard deviations are from three biological replicates.