Condensation of Rubisco into a proto-pyrenoid in higher plant chloroplasts

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Supplementary Table 1. Photosynthetic parameters from gas exchange and fluorescence measurements for $S2_{Cr}$ transgenic lines of Arabidopsis.

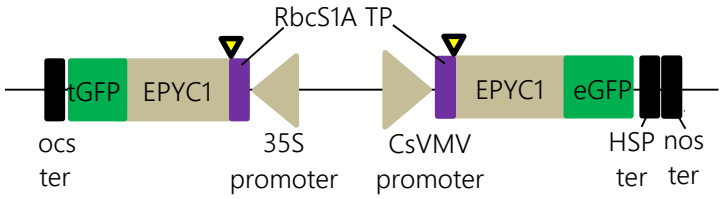
	Ep1	Az1	Ep2	Az2	Ep3	Az3	EpWt	AzWt
V_{cmax} (µmol CO_2 m^{-2} s^{-1})	35.6±1.5[a]	36.4±2.0[a]	32.2±1.9[a]	33.6±1.6[a]	33.1±1.9[a]	33.8±2.2[a]	44.9±1.6[b]	43.3±1.7[b]
J_{max} (µmol e^- m^{-2} s^{-1})	59.2±2.3[a]	61.9±6.3[a]	57.2±2.6[a]	56.1±3.5[a]	52.9±4.4[a]	58.6±5.2[a]	76.4±2.4[b]	74.9±7.5[b]
Γ (µmol CO_2 m^{-2} s^{-1})	63±8[a]	53±5[a]	52±6[a]	54±7[a]	53±7[a]	56±8[a]	51±7[a]	64±12[a]
g_s (mol H_2O m^{-2} s^{-1})	0.249±0.031[a]	0.279±0.051[a]	0.233±0.017[a]	0.251±0.015[a]	0.233±0.021[a]	0.236±0.016[a]	0.287±0.018[a]	0.306±0.011[a]
g_m (mol CO_2 m^{-2} s^{-1})	0.034±0.001[b]	0.035±0.003[b]	0.032±0.002[b]	0.033±0.002[b]	0.034±0.003[b]	0.032±0.002[b]	0.045±0.002[a]	0.046±0.003[a]
F_v/F_m (ML)	0.848±0.002[a]	0.849±0.002[a]	0.848±0.001[a]	0.847±0.001[a]	0.847±0.002[a]	0.845±0.002[a]	0.851±0.002[a]	0.850±0.001[a]
F_v/F_m (HL)	0.852±0.002[a]	0.845±0.002[a]	0.850±0.001[a]	0.855±0.004[a]	0.846±0.002[a]	0.849±0.001[a]	0.850±0.003[a]	0.852±0.002[a]

Note: The mean and SEM are shown for 35- to 45-d-old rosettes for gas exchange variables (n = 5-8), and 32-d-old rosettes for F_v/F_m. F_v/F_m is shown for attached leaves dark-adapted for 45 min prior to fluorescence measurements (n = 6-23). Letters above the SEM indicate significant difference ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's HSD tests. Values followed by the same letter are not statistically significantly different. Abbreviations: Γ, CO_2 compensation point (C_i–A); A, CO_2 assimilation rate, C_i, inorganic carbon concentration; F_v/F_m, maximum potential quantum efficiency of photosystem II; g_s, stomatal conductance to water vapour; g_m, mesophyll conductance to CO_2 (i.e. conductance of CO_2 across the pathway from intercellular airspace to chloroplast stroma) using the Ethier and Livingston method (see Methods section); J_{max}, maximum electron transport rate; V_{cmax}, maximum rate of Rubisco carboxylation. Source data are provided as a Source Data file.

Supplementary Table 2. Transcript abundances of tGFP and eGFP in EPYC1-dGFP plant lines.

	tGFP expression			eGFP expression		
	Rep 1	Rep 2	Average	Rep 1	Rep 2	Average
Ep1	0.222	0.294	0.258	0.299	0.315	0.307
Ep2	0.857	0.482	0.669	1.093	0.524	0.808
Ep3	1.430	0.569	1	1.7E-05	4.98E-05	3.34E-05

Note: Quantitative reverse transcription PCR (RT-qPCR) was carried out on three T2 $S2_{Cr}$ transgenic plants expressing EPYC1-dGFP (Ep1 - Ep3, as in Fig. 1B) using gene-specific primers for tGFP and eGFP. Abundances of tGFP and eGFP transcripts are shown relative to the highest tGFP expression level and normalised to reference genes PP2A (At1g13320) and RHIP1 (At4g26410). Each biological replicate shown (e.g. Rep 1) is an average of two technical replicates.



ocs terminator
turbo GFP (Pontellina pluma)
mature EPYC1
Rbcs1A transit peptide
35S promoter
linking region
CSVMV promoter
eGFP (with N-terminal HA-tag)
HSP terminator
nos terminator

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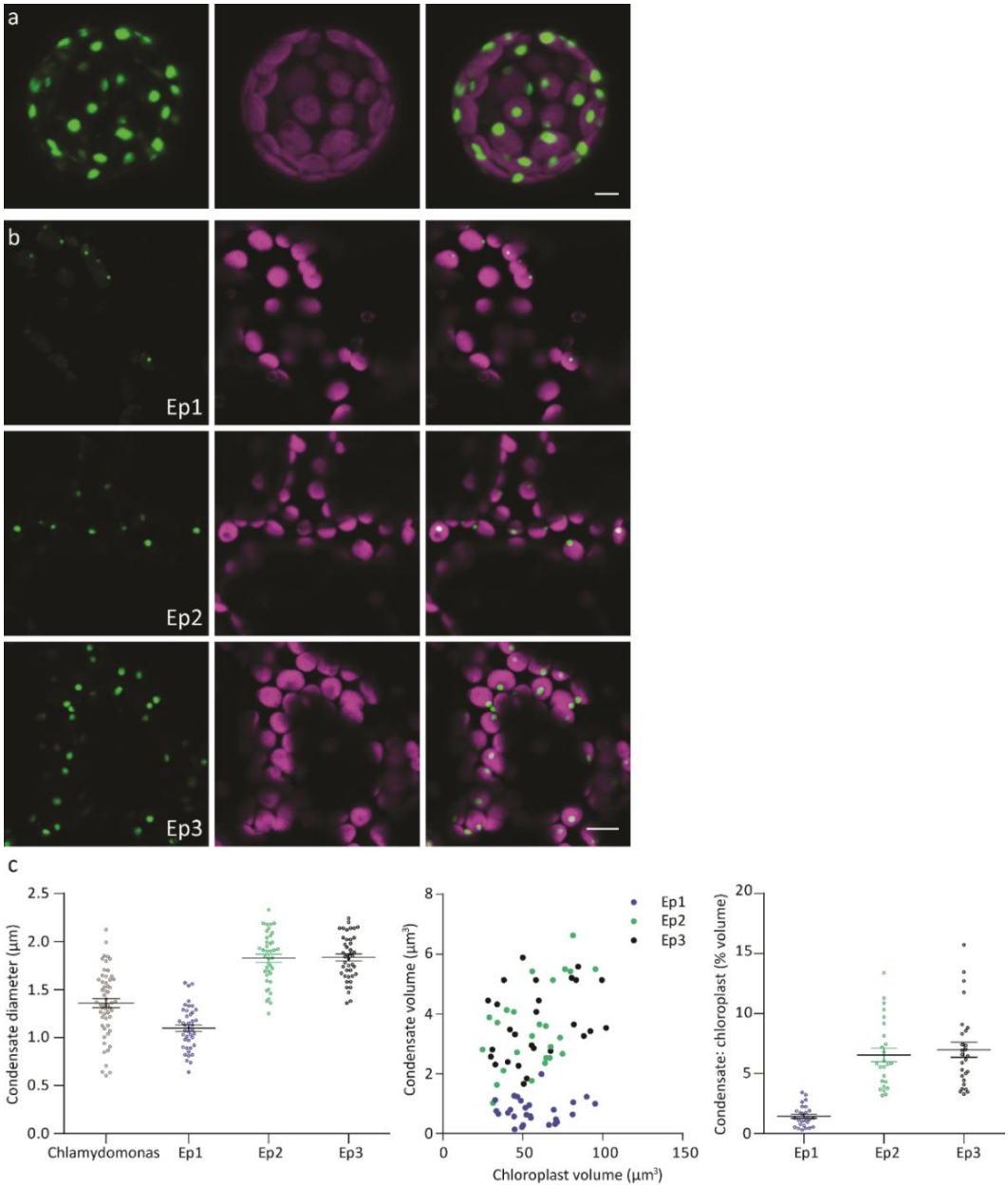
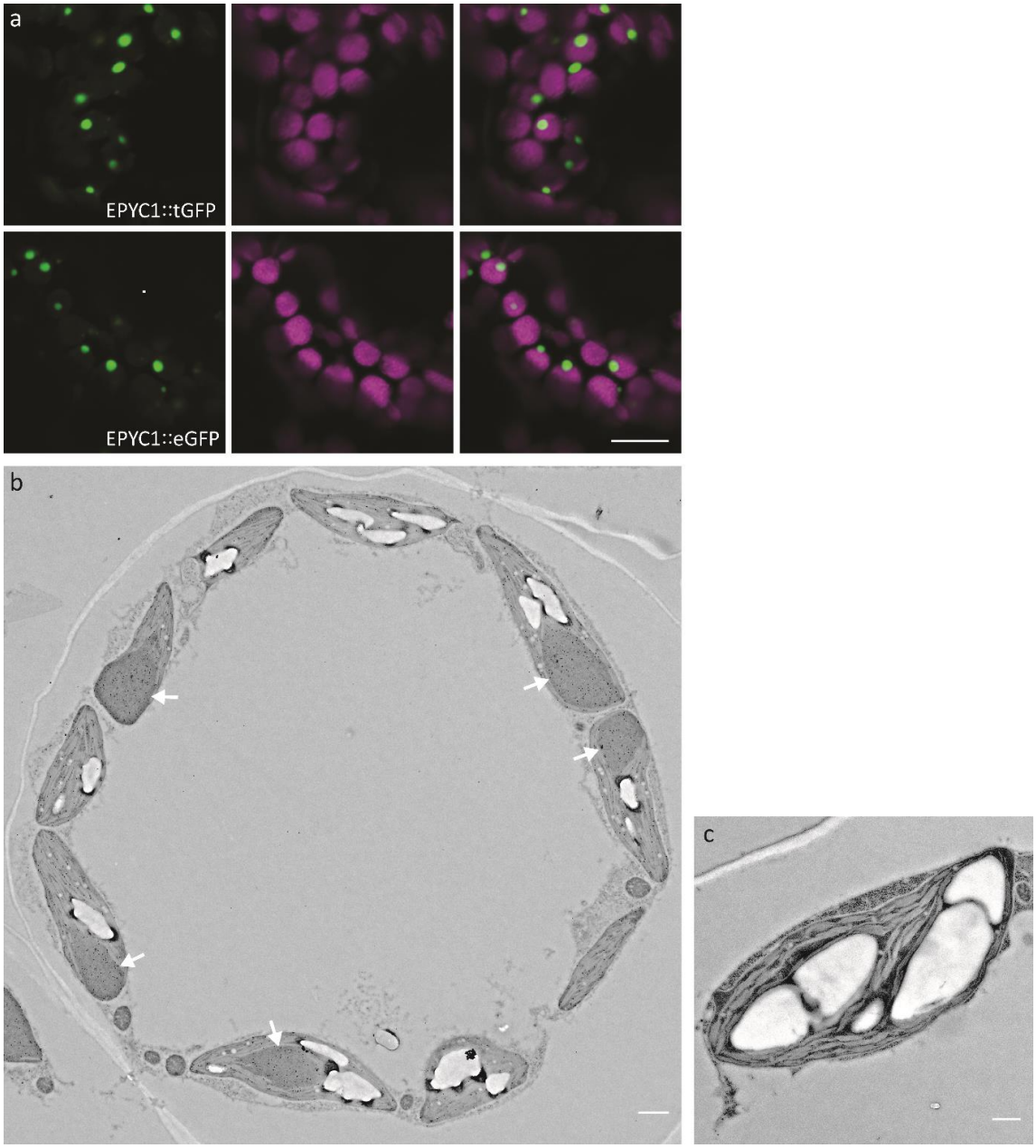
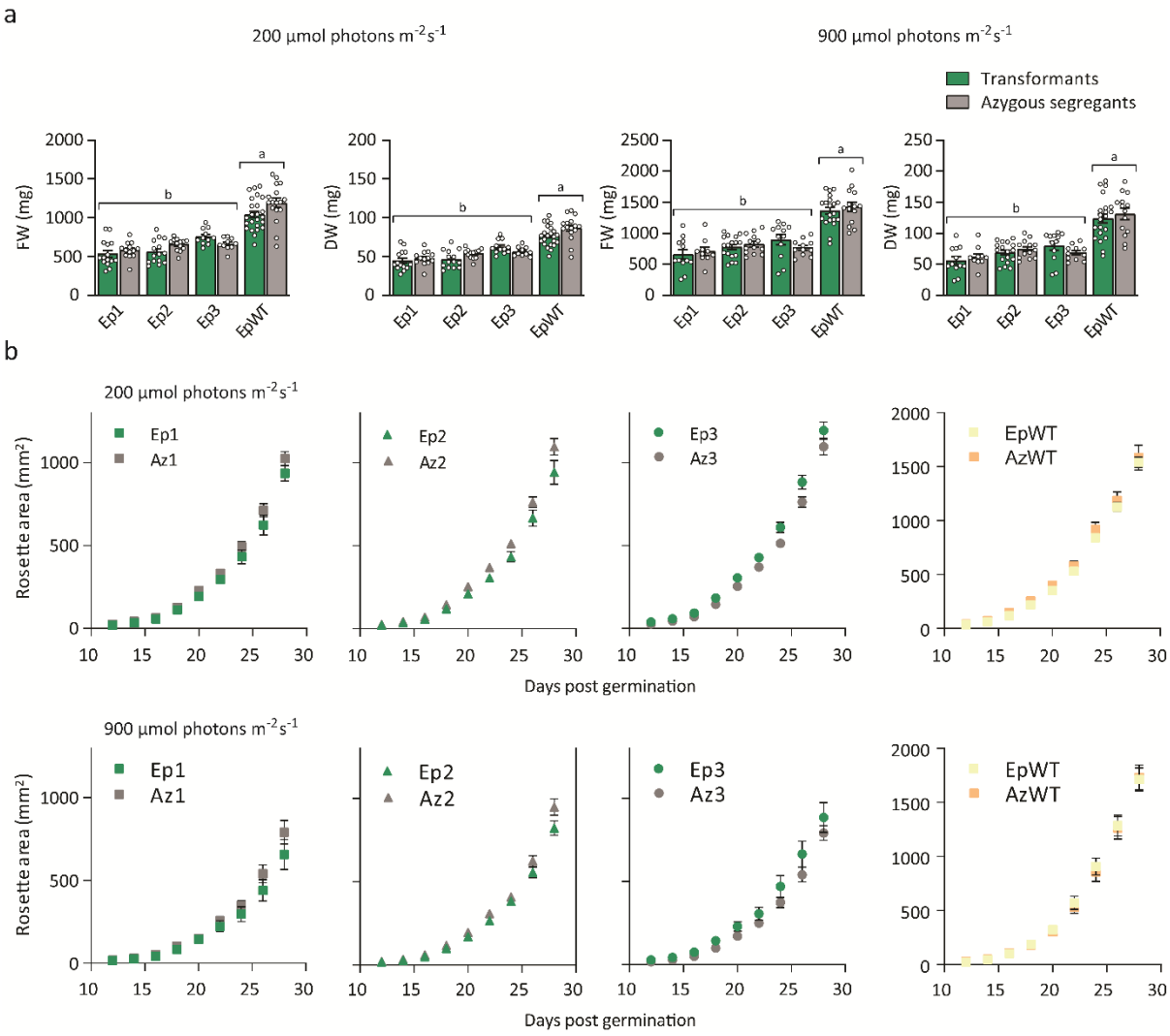
Supplementary Figure 1. Full sequence of the EPYC1 expression cassettes. Sequence parts are colour coded, and MoClo overhangs are shown in lower case. Source data are provided as a Source Data file.



Supplementary Figure 2. Characterisation of condensates in S2$_{Cr}$ plants. a, Maximum projection of a z stack showing condensates in every chloroplast. Scale bar = 5 µm for all images. Similar stacked images were reproduced for six individual protoplasts. **b**, The size of condensates is dependent on the expression level of EPYC1. Arabidopsis lines Ep1-3 with different expression levels of EPYC1-dGFP (see Figure. 1b) have different sizes of condensates. Scale bar = 10 µm for all images. Confocal images are representative of images captured from Ep1 plants (42), Ep2 plants (21) and Ep3 plants (>100). **c**, Data derived from confocal images of Chlamydomonas pyrenoids (n = 55) and chloroplasts (n = 40-42) from each of the three Ep transgenic lines (Ep1-3) showing the mean diameter and SEM of pyrenoids and condensates (left). The volume of the condensate is shown plotted against estimated chloroplast volume (centre) and the estimated proportion of chloroplast volume occupied by the condensate (right) (n = 25-27 chloroplasts for each line). Source data underlying Supplementary Figure 2c are provided as a Source Data file. Raw image data corresponding to Supplementary Figure 2c are available online at the open access Edinburgh DataShare repository (https://doi.org/10.7488/ds/2945).



Supplementary Figure 3. Condensate formation in $S2_{Cr}$ plants. a, Condensation occurs in $S2_{Cr}$ plants expressing a single EPYC1 expression cassette. Representative images are shown for EPYC1 fused at the C-terminus to either tGFP (top) or eGFP (bottom). Scale bar = 10 µm. Confocal images are representative of images captured from EPYC1:tGFP plants (14) and EPYC1:tGFP plants (17). **b**, TEM image of a mesophyll cell cross-section showing chloroplasts with EPYC1-dGFP condensates. Visible condensates are marked by a white arrowhead. The section has been probed by immunogold labelling with anti-Rubisco antibodies. Scale bar = 1 µm. The TEM image is representative of 5 images. c, TEM image of a chloroplast from a wild-type plant expressing EPYC1-dGFP. No condensates were observed in this background. Scale bar = 0.5 µm. The TEM image is representative of 5 images.



Supplementary Figure 4. Growth of plant lines expressing EPYC1 under different light levels. a, Fresh (FW) and dry weight (DW) of three T2 EPYC1-dGFP $S2_{Cr}$ transgenic lines (Ep1-3, green) and an EPYC1-dGFP WT transformant (EpWT, yellow) with their respective azygous segregants (Az1-3, grey and AzWT, orange) measured after 32 days of growth at 200 or 900 µmol photons m^{-2} s^{-1} light. The mean ± SEM are shown for n=10-26 individual plants for each line. Letters indicate significant difference ($p < 0.05$) of EpWT lines compared to Ep lines as determined by one-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc tests. **b,** Rosette area expansion rates for individual transgenic lines and azygous segregants at two different light levels in (a). Source data are provided as a Source Data file.