SUPPLEMENTAL DATA

The *Synechocystis* MANGANESE EXPORTER Mnx is essential for manganese homeostasis in cyanobacteria

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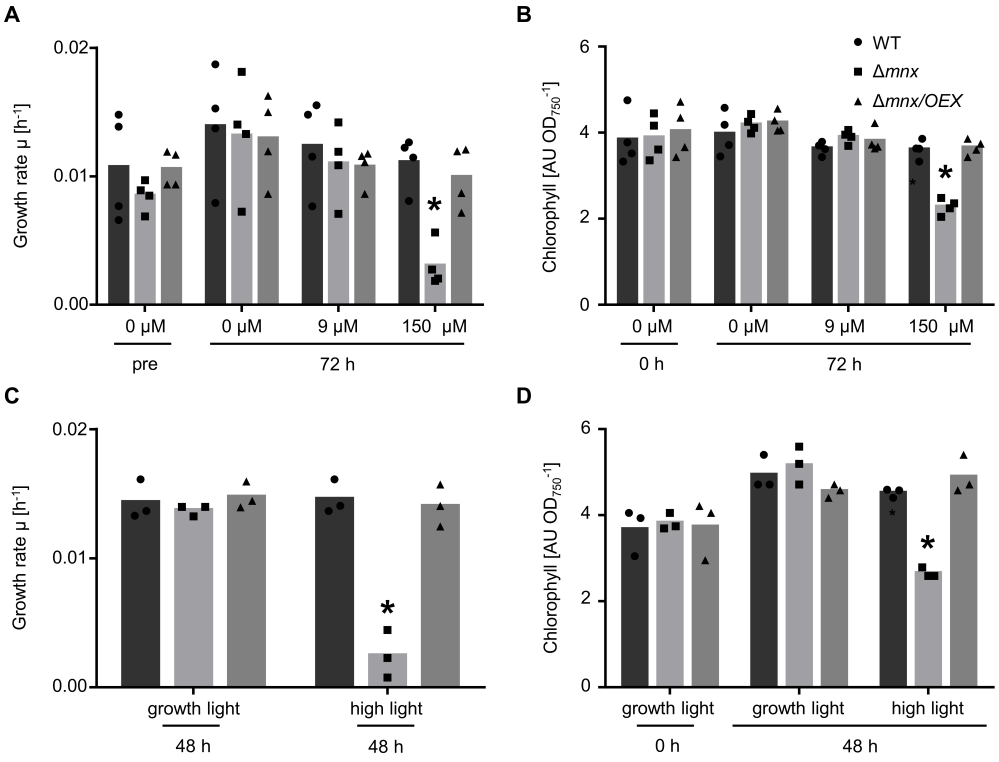

Fig. S1: Statistical analysis of growth rates and chlorophyll content. (A) Growth rates of WT, Δ*mnx*, and Δ*mnx/OEX* during 5 d of Mn limited pre-cultivation (pre) and during 72 h treatment with Mn concentrations as indicated. (B) Chlorophyll content after 5 d of Mn limited pre-cultivation (0 h) and after 72 h treatment with Mn concentrations as indicated. (C) Growth rates after 48 h cultivation under standard growth light (100 µmol photons m^{-2} s^{-1}) or high light (1,000 µmol photons m^{-2} s^{-1}) conditions. (D) Chlorophyll content at the beginning of the experiment (0 h) and after 48 h cultivation under standard growth light (100 µmol photons m^{-2} s^{-1}) or high light (1,000 µmol photons m^{-2} s^{-1}) conditions. Asterisks (*) indicate significant changes to the respective WT value according to a two-way ANOVA ($P \leq 0.05$).

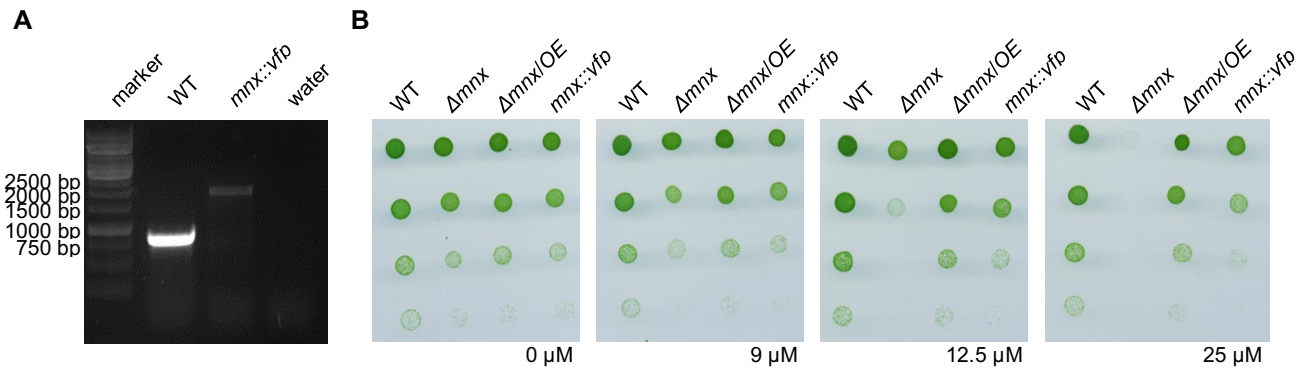

Fig. S2: Verification of genotype of *mnx::yfp* and functionality of Mnx::YFP proteins. (A) The genotype of the mutant *mnx::yfp* was verified by PCR using genomic DNA and primers binding to the *mnx* coding sequence and to the 3' region of *mnx*. The amplified fragment for *mnx::yfp* includes *mnx*, the inserted *yfp* and *KmR* genes, and 255 bp of the 3' region downstream of *mnx*. Water was used as a negative control. (B) The cells in mid log phase were diluted to an OD_{750} of 0.25. 2 µL of these cultures and subsequent 1:10, 1:100 and 1:1,000 dilutions were spotted onto BG11 plates with increasing concentrations of $MnCl_2$. Pictures were taken after incubation for 4 d. The concentration of $MnCl_2$ in standard BG11 medium is 9 µM.

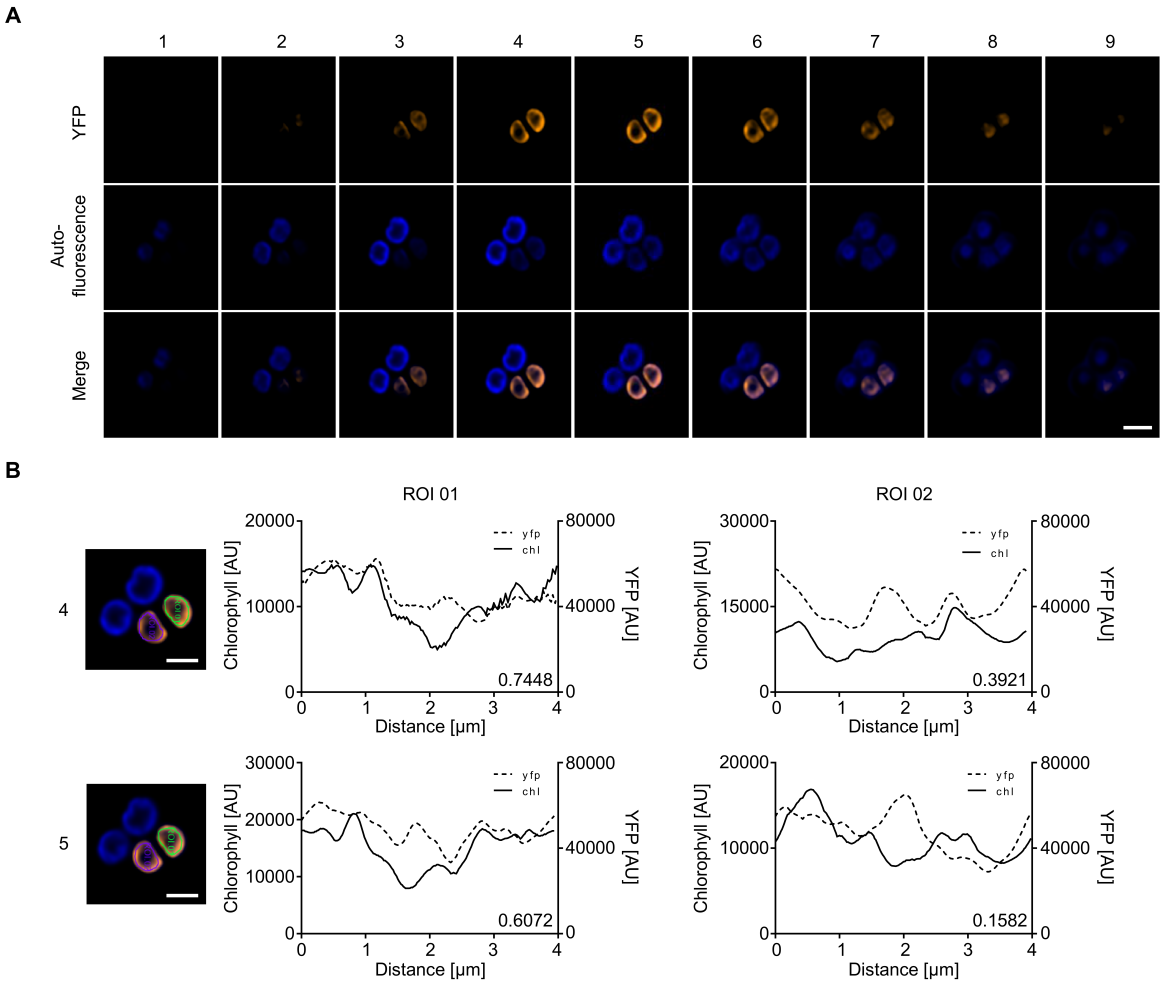

Fig. S3: Overview of fluorescence microscopy pictures of WT and *mnx::yfp* cells. (A) Full series of z-stacks used for localization of Mnx. Fig. 7 shows stack 5. The distance between two images is approximately 0.1 µm. (B) Quantification of YFP and chlorophyll fluorescence along the cellular circumference for images 4 and 5. Region of interest (ROI) 01 is indicated in green and ROI 02 in purple. Spearman's rank correlation coefficients are given for each graph. The length of the scale bars is 1 µm.

Table S1: List of GreenCut proteins strictly conserved in cyanobacterial genomes with predicted but so far unknown function in chloroplast transport processes. Proteins were extracted from the GreenCut2 inventory (Karpowicz et al., 2011). *Cre* protein ID according to JGI v3.0 of the Cre genome, *Syn* protein ID according to Cyanobase

C: Chloroplast; *Cre*: *Chlamydomonas reinhardtii;* iV: inner envelope; *Syn: Synechocystis* sp. PCC 6803;

Cre* protein name	***Cre* protein ID***	***Syn* protein ID**	**Proposed function***	**Domain***	**Predicted location***	**Encoded in cyanobacterial genomes (number)***
CGL98	114780	Sll0355		Integral membrane protein DUF6	C	37
PCD4	205606	Slr0941	possible lipid transport protein	Polyketide_cyc(1)	C	37
TIC21	183668	Sll1737	inner envelope membrane transporter protein, regulated by signaling pathway	Similar to AtTic20-like proteins	iV	37
CPLD63	105873	Sll0615	possible solute transporter	Uncharacterized protein family UPF0016	iV	36
SNR2	146768	Slr0305	SNARE associated Golgi protein, green tissues	SNARE associated Golgi protein	C	35

* according to Karpovicz et al., 2011

Table S2: Oligonucleotides used in this study. Restriction sites are underlined.

Name	**Sequence (5`-> 3`)**	**Experiment**
ME29	TTAACGGTCAGGCGATCA	Generation of knockout mutant in *mnx*
ME30	TAGATCTGATCGCCTTGC	Generation of knockout mutant in *mnx*
ME57	CATATGATGCTGACCGCTTTTACT	Generation of *mnx* overexpression line
ME58	GTTAACCTATGCAATCTTGGTCCA	Generation of *mnx* overexpression line
FB24	TCTTGGCCATACCATCTGTGC	RT-qPCR analysis, expression of *mnx*
FB25	CCACCAGGAAACCACAGCAA	RT-qPCR analysis, expression of *mnx*
FB26	AAGGTTGGTCTTTTTCCTGCC	RT-qPCR analysis, expression of *rnpB*
FB27	AGAGAGTTAGTCGTAAGCCGGG	RT-qPCR analysis, expression of *rnpB*
SK102	AGGAGTCGACATGCTGACCGCTTTTAC	Expression of *mnx* in yeast
SK103	AGGAACTAGTCTATGCAATCTTGGTCCACC	Expression of *mnx* in yeast
FB16	cgagtttttcagcaagatcaTTTGCCCTCACCTTTGTG	Generation of *mnx::yfp* line
FB17	tgctcactccTGCAATCTTGGTCCACCA	Generation of *mnx::yfp* line
FB18	caagattgcaGGAGTGAGCAAGGGCGAG	Generation of *mnx::yfp* line
FB19	gttgcaaaatTTATAACTTGTACAGCTCGTCCATG	Generation of *mnx::yfp* line
FB20	caagttataaATTTTGCAACAAATAACCAC	Generation of *mnx::yfp* line
FB21	aatccgagtcGAGAGTGAGGACCTGCAG	Generation of *mnx::yfp* line
FB22	cctcactctcGACTCGGATTTGCAGATC	Generation of *mnx::yfp* line
FB23	agaatattgtaggagatcttCACGTAGGGTTGAAACTTTTTC	Generation of *mnx::yfp* line
FB31	CACGTAGGGTTGAAACTTTTTCA	Together with ME57 verification of *mnx::yfp*