Supplementary Material

Supplementary Table S1: Strains and plasmids constructed and used in the present study.

Designation	Size (kb)	Description
pGT1803	4.2	pGEMT containing a 1.2-kb fragment, the coding sequence of *slr1803* amplified by PCR
pGT1803Km-	5.3	pGT1803 containing a partial deleted *slr1803* (*aph*II gene inserted after deletion of the internal *Hin*cII fragment opposite to the transcription direction of *slr1803*)
p1803pBADHISB	5.0	pBADHISB containing a 0.9-kb fragment, the coding sequence of *slr1803* amplified by PCR
pGT0729	4.8	pGEMT containing a 1.8-kb fragment, the coding sequence of *sll0729* amplified by PCR
pGT0729ΔKm	5.6	pGT0729 containing a partial deleted *sll0729* (*aph*II gene inserted after deletion of the internal *Eco*RI/*Bcl*I fragment opposite to the transcription direction of *sll0729*)
p0729pBADHISC	5.0	pBADHISC containing a 0.9-kb fragment, the coding sequence of *sll0729* amplified by PCR
pVZ322-P*ziaA ::sll0729*	8.6	Self-replicating pVZ322 plasmid carrying the *sll0729* gene in fusion with the promoter of the *ziaA* gene of *Synechocystis*, the used promoter sequence covers the range between genome pos. 3060796 and 3061359 (in Genbank file BA000022) and also includes the *ziaR* gene encoding the transcriptional regulator of *ziaA*
pVZ322-P*ziaA ::ssl1378*	8.0	Self-replicating pVZ322 plasmid carrying the *ssl1378* gene in fusion with the promoter of the *ziaA* gene of *Synechocystis*
pGT6095	4.4	pGEMT containing a 1.4-kb fragment, the coding sequence of *slr6095* amplified by PCR
pGT6095Km-	5.7	pGT6095 containing an *aph*II gene inserted opposite to the transcription direction of *slr6095*
pGT8009	5.8	pGEMT containing a 2.6-kb fragment, the coding sequence of *sll8009* amplified by PCR

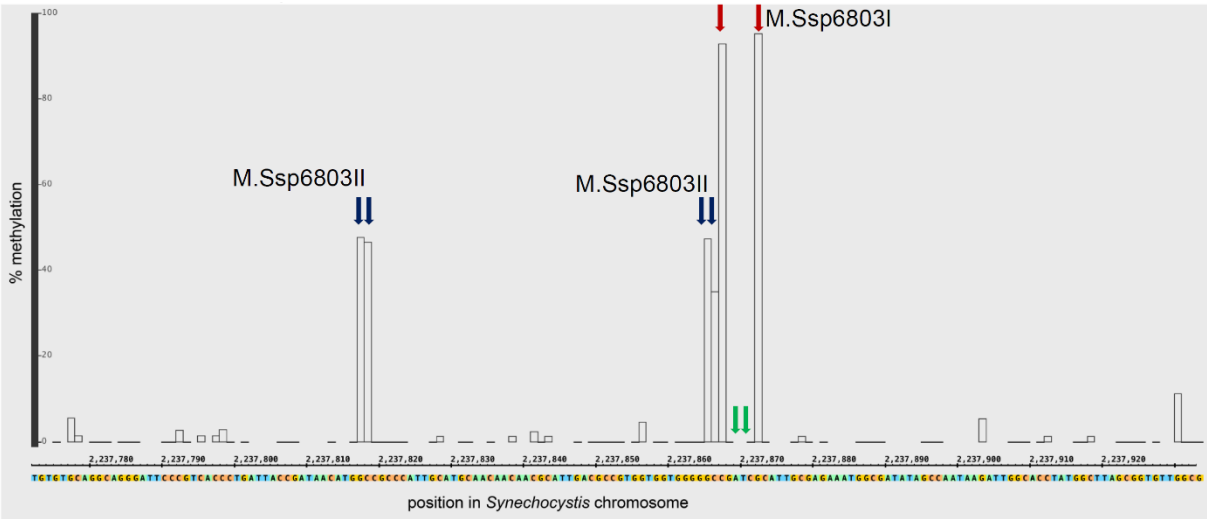
pGT8009ΔKm	6.0	pGT8009 containing a partial deleted *sll8009* (*aph*II gene inserted after deletion of the internal *Nhe*I/*Nhe*I fragment opposite to the transcription direction of *sll8009*)
pGT6050	7.3	pGEMT containing a 3.3-kb fragment, the coding sequence of *slr6050* amplified by PCR
pGT6050ΔKm	7.6	pGT6050 containing a partial deleted *slr6050* (*aph*II gene inserted after deletion of the internal *Cla*I/*Cla*I fragment opposite to the transcription direction of *slr6050*)
Δ*slr1803*		*Synechocystis* mutant obtained after transformation of the wild type with pGT1803Km-
Δ*sll0729*		*Synechocystis* mutant obtained after transformation of the wild type with pGT0729Km-
Δ*slr0214*		*Synechocystis* mutant with interrupted gene *slr0214* for MSsp6803I [18]
Δ*slr6095*		*Synechocystis* mutant obtained after transformation of the wild type with pGT6095Km-
Δ*sll8009*		*Synechocystis* mutant obtained after transformation of the wild type with pGT8009ΔKm
Δ*slr6050*		*Synechocystis* mutant obtained after transformation of the wild type with pGT6050ΔKm
Δ*sll0729+sll0729*		*Synechocystis* Δ*sll0729* strain harbouring the pVZ322-P*ziaA ::sll0729*
Δ*ssl1378+ssl1378*		*Synechocystis* Δ*sll0729* strain harbouring the pVZ322-P*ziaA ::ssl1378*

Supplementary Table S2: Primers used in the present study.

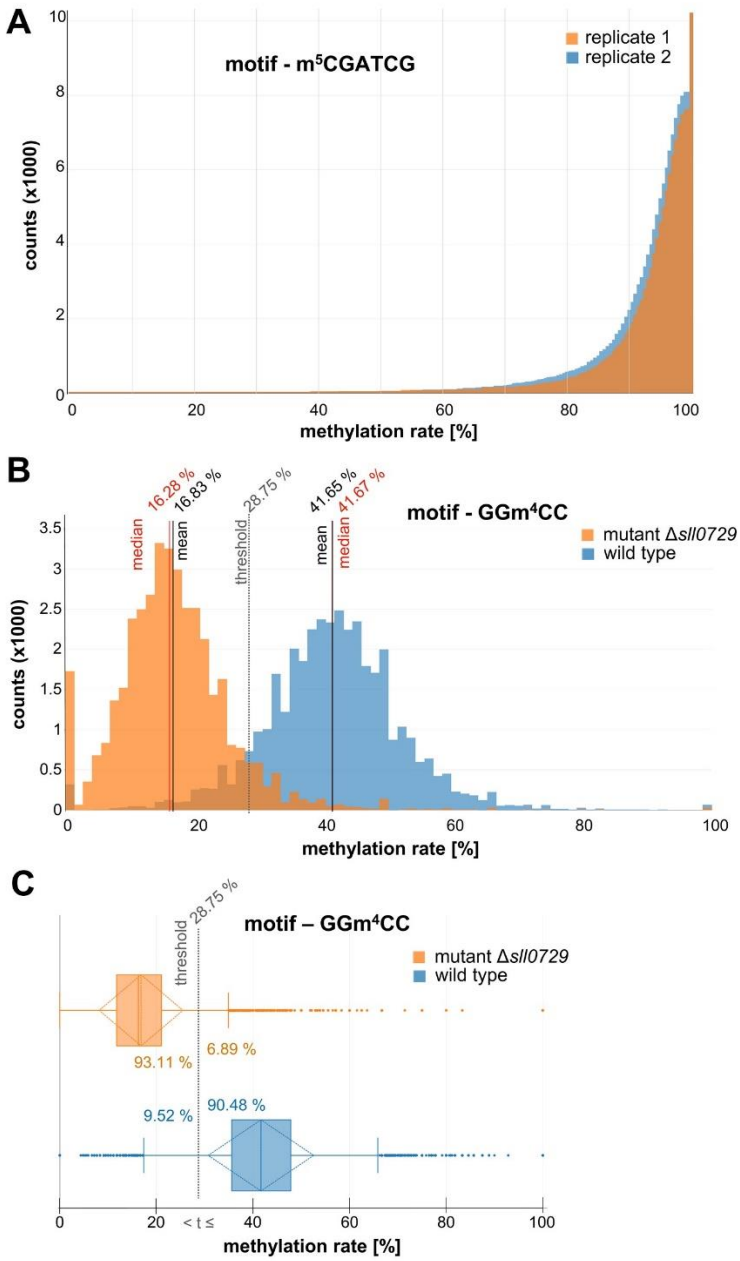
Description	**Sequence (5'→3')**	**amplified ORF or sequence**
1803fw	TCCTGAAGGCATGGT	*slr1803*
1803re	TTGCCATGCTCCATC	*slr1803*
1803pBADfw	CTCGAGAGTGAAAATTAAACA	*slr1803*
1803pBADre	CTGCAGTCAGCGATGGTAATT	*slr1803*
0729DELfw	ATGATCTAGCTCCATGGCGA	*sll0729*
0729DELre	AGGTTATGACGATCCGGCTT	*sll0729*
0729pBADfw	CTGCAGTTAATAATTTTT	*sll0729*
0729pBADre	CTG CAG TTA ATA ATT TTT	*sll0729*
PziaA_XbaI_fw	TAACGATCTAGACGTCCATCTCCTTAATCCGATTCCTG	*ziaR+PziaA+5'UTR*
PziaA_ClaI_rev	TATCGAATCGATCATGGCGGCCAACGTGATTTAAAGAAAAAC	*ziaR+PziaA+5'UTR*
sll0729_ClaI_fw	TATCGAATCGATGAGGAAACCGGGGAAATAAATAAATCAGC	*sll0729*
sll0729_XhoI_rev	AATCGACTCGAGTTAATAATTTTTGATCATTAACTCTTTACCTTTGGAAGC	*sll0729*
ssl1378_ClaI_fw	TATCGAATCGATAGTAATCCTGAAGGTTATAACGTTTGGTCAG	*ssl1378*
ssl1378_XhoI_rev	AATCGACTCGAGTTAATTGGACTTTGCCTGGGAATTGGG	*ssl1378*
slr6095_EcoRI_fw	GAATTCATGTTATTCCTTAA	*slr6095*
slr6095_XhoI_rev	CTCGAGCTAAGCCTTTTCCA	*slr6095*
sll8009_fw	GTTTCATCGCTCCACCTCTC	*sll8009*
sll8009_rev	TAGGACGGGATACGCTCTTG	*sll8009*

slr6050_fw	ATATTGAGCAAGTTCGGGGA	*slr6050*
slr6050_rev	AAACCCCATGCCTACGCCTT	*slr6050*
slr6050_i_fw	TTAAGTGAGCCTTTGCCTCA	*slr6050*
slr6050_i_rev	GGTTCTAAAAGGTGTTGATG	*slr6050*
aphII_fw	ATGAGCCATATTCAACGGGAAACGT	*aminoglycoside phosphotransferase II*
aphII_rev	TTAGAAAAACTCATCGAGCATCAAA	*aminoglycoside phosphotransferase II*

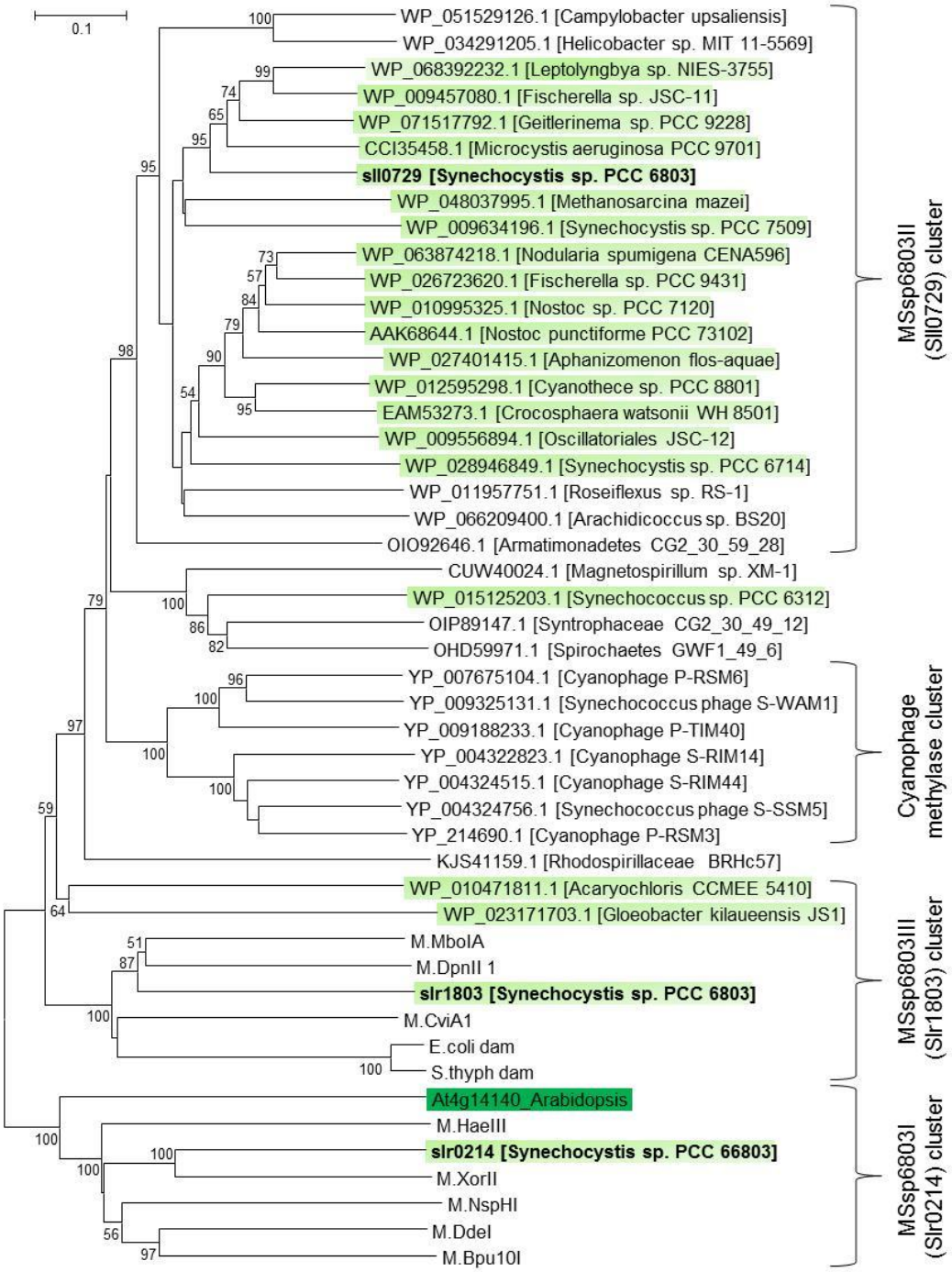
Supplementary Figures



Supplementary Figure S1. Analysis of the *Synechocystis* methylome by bisulfite sequencing. The snapshot shows the chromosomal section from nt position 2,237,773 to position 2,237,934, which is within gene *slr1938* encoding methylthioribose-1-phosphate isomerase. The bars indicate cytosine methylation on both strands (the bars are displayed on a G for the C at the reverse strand). ^{m5}CGATCG and GG^{m4}CC methylations are clearly detectable and highlighted by the red and blue arrows, respectively. The complexity of DNA methylation in *Synechocystis* is highlighted by the region from nt 2,237,865 to 2,237,873, in which six out of nine base pairs are methylated (GGCCGATCG). The 2nd and 3rd positions in this sequence stretch are methylated by M.Ssp6803II, the 4th and 9th by M.Ssp6803I, interspersed by methylation on the 6th and 7th by M.Ssp6803III, which targets G^{m6}ATC and was detected only by SMRT sequencing (green arrows). Please note that ^{m5}CGATCG methylation (red arrows) is quantitatively (nearly 100%) displayed by the bisulfite sequencing method, whereas the GG^{m4}CC methylations (blue arrows) are less sensitive to the bisulfite modifications (around 50%) as shown previously by reference [37].

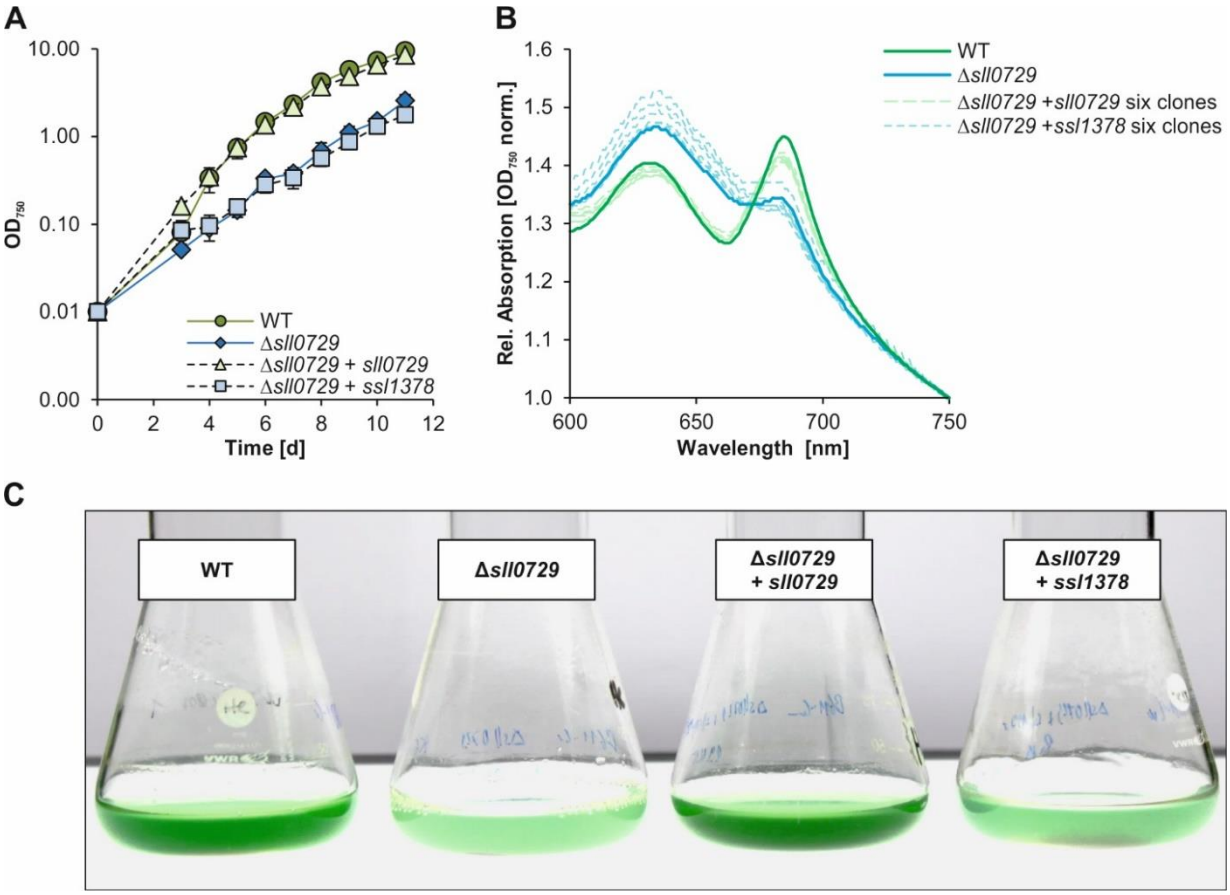


Supplementary Figure S2. Global analysis of the *Synechocystis* methylome by bisulfite sequencing.
A. Bisulfite analysis of ^{m5}CGATCG methylation. There is a total of 38,512 analyzed sites on the chromosome. The cumulative plot shows the percentage of methylation for two replicates. 84.3% (replicate 1, DNA from Δ*sll0729* mutant, orange) and 79.6% (replicate 2 wild-type DNA, blue) of all sites were found to be methylated ≥90%.
B. Bisulfite analysis of GG^{m4}CC methylation. There is a total of 29,852 analyzed sites on the chromosome. The detected percentages of methylation are shown for wild-type DNA (blue) and for the Δ*sll0729* mutant (orange) lacking M.Ssp6803II methylase activity. Because ^{m4}C is only partially resistant to bisulfite-mediated deamination, an average detected methylation of only 41.65% was detected for the wild type. The 16.83% (mean) detected apparent methylation in the Δ*sll0729* mutant is indicative for the background of the bisulfite conversion rate.
C. Box plot of the bisufite analysis data of GG^{m4}CC methylation from panel B. The local minimum at 28.75% apparent methylation was taken as threshold to separate the two datasets. This classification revealed 90.48% of all sites in the wild type to show methylation and 9.52% to be unmethylated. In contrast, 6.89% of all sites were falsely qualified as methylated in the mutant (false-positives).

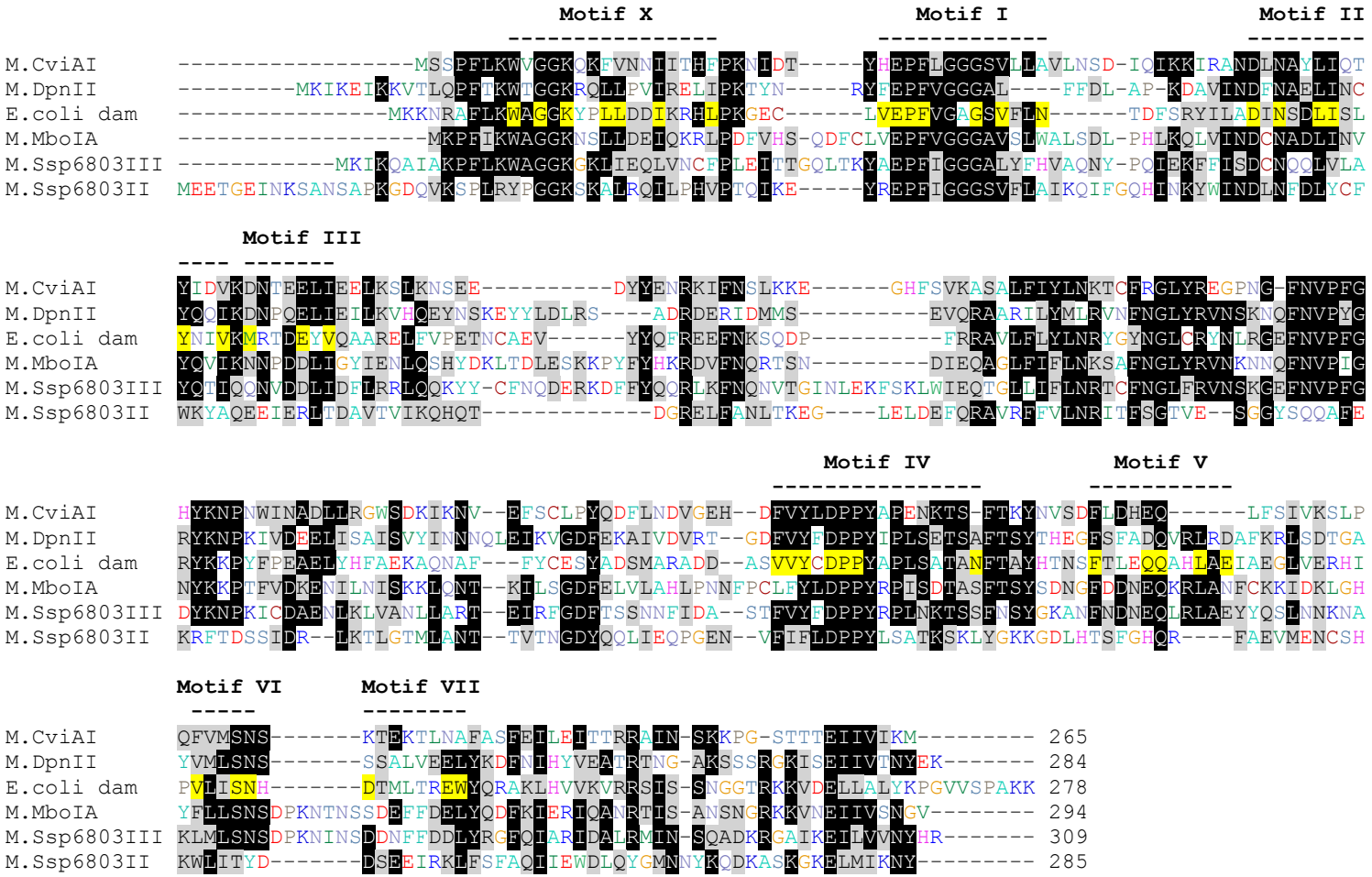


Supplementary Figure S3: Phylogenetic relation of methyltransferases from *Synechocystis* sp. PCC 6803 to related proteins.

Unrooted neighbor joining tree showing the relation of the characterized DNA-specific methyltransferases (MSsp6803I - Slr0214, MSsp6803II – Sll0729, MSsp6803III – Slr1803) to related enzymes found in databases. For the phylogenetic comparison up to 10 of the most similar proteins found in BlastP searches [ref. 32,33] among cyanobacteria (light-green shading) or other bacteria. In addition, functionally characterized methyltransferases were included. Bootstrap values after 1000 replications are indicated when ≥50. The evolutionary history was inferred using the Neighbor-Joining algorithm. Evolutionary analyses were conducted in MEGA5 [ref. 35]. Accession numbers of the compared sequences are mentioned in the tree or are as follows: M.DpnII – Acc.No. P04043; M.MboIA – Acc.No. P34720; M.CviA1 – Acc.No. NP_048937, M.EcoRV – Acc.No. P04393; Dam E.coli – Acc.No. P0AEE9; Dam S.Thyph – Acc.No. P0DMP4; M.XorII – Acc.No. P52311; M.HaeIII – Acc.No. P20589; M.NspHI – Acc.No. AAC97192; M.DdeI – Acc.No. P05302; M.Bpu10I – Acc.No. CAA74996.



Supplementary Figure S4: Complementation of the Δ*sll0729* phenotype.
The gene *sll0729* or the downstream located gene *ssl1378* were ectopically expressed in the Δ*sll0729* background driven by the Zn^{2+}-dependent promoter of the *Synechocystis ziaA* gene.
A: Growth curves for the WT, Δ*sll0729* and the strains harboring a pVZ321-derivative with P*ziaA*-fused *sll0729* or *ssl1378* genes. Cells were cultivated in presence of 0.77 µM Zn^{2+}. Data are the mean of 3 biological replicates.
B: Whole cell absorption spectra including six biological replicates (= independent conjugants) for the complementation strains.
C: Photograph of representative cultures after 7 days of growth.



Supplementary Figure S5: Multiple sequence alignment of characterized adenine-specific methyltransferases as well as the Dam-like protein M.Ssp6803III and the cytosine-specific M.Ssp6803II from *Synechocystis* (second lowest and lowermost rows). Motifs I-VII and X were assigned according to Fig.1a in Malone et al. [ref. 52] and are indicated. The conserved amino acid residues in the Dam protein of *E. coli* (acc.no. P0AEE9) are highlighted in yellow. Identical amino acid residues are shaded in black and biochemically synonymous amino acid residues in grey. For the corresponding accession numbers see Supplementary Fig. S2.