

## SUPPORTING INFORMATION

The antisense RNA As1\_flv4 in the cyanobacterium *Synechocystis* sp. PCC 6803 prevents premature expression of the *flv4-2* operon upon shift in inorganic carbon supply\*

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\*Running title: *Effects of an asRNA on expression in Synechocystis*

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SUPPLEMENTAL TABLE

**TABLE S1:** Primer used in this work. Restriction sites are underlined.

| Name                   | Sequence (5' -> 3')   | Experiment   |
|------------------------|---|--|
| T7_as1_flv4_fw         | TAA TAC GAC TCA CTA TAG GGA<br>GAA AGC GGG GGA GGA CAG<br>GAT GAG | Northern Hybridization,<br>Probe to detect As1_flv4                                      |
| as1_flv4_rev           | GTA CCC GCT TCA CGG CAA AGC<br>TGA                                | Northern Hybridization,<br>Probe to detect As1_flv4                                      |
| T7_as2_flv4_fw         | TAA TAC GAC TCA CTA TAG GGA<br>ACC GAC CTT TTG AGT CAC AGA<br>TTG | Northern Hybridization,<br>Probe to detect As2_flv4                                      |
| as2_flv4_rev           | GTC GTT CTG GCG TTT CAT CGA<br>AAA                                | Northern Hybridization,<br>Probe to detect As2_flv4                                      |
| T7_as_flv2_fw          | TAA TAC GAC TCA CTA TAG GGA<br>CAC TGG ATC AGT TTG ATC CGT<br>TAA | Northern Hybridization,<br>Probe to detect As_flv2                                       |
| as_flv2_rev            | CTG GCT AGG TTG TTG TTG ACA<br>CCA                                | Northern Hybridization,<br>Probe to detect As_flv2                                       |
| T7_ncr0080_fw          | TAA TAC GAC TCA CTA TAG GGA<br>AGT TCG TGG GCA AGA TGG AGC<br>CAC | Northern Hybridization,<br>Probe to detect Ncr0080                                       |
| ncr0080_rev            | CAG TCA ACT TAA TCT ATC GAG<br>AGG GCT CTA TGG C                  | Northern Hybridization,<br>Probe to detect Ncr0080                                       |
| flv4_fw                | CCA GTA CCT CAC CCA GAA ACA                                       | Northern Hybridization,<br>Probe to detect <i>flv4-2</i><br>(Zhang <i>et al.</i> , 2009) |
| flv4_rev               | AAG CTA GGG TTT CCA ACA GGA                                       | Northern Hybridization,<br>Probe to detect <i>flv4-2</i><br>(Zhang <i>et al.</i> , 2009) |
| 5'-RNA Adaptor-sense_F | GAA TTC CTG TAG AAC GAA CAC<br>TAG                                | 5'RACE   |
| flv4_5'RACE_1          | GGA TTA AAA ATG AGT TGT AGG<br>TTG                                | 5'RACE (1. PCR)  |
| flv4_5'RACE_2          | ATG GCG TAA CTC AAA CTC AAT                                       | 5'RACE (2. PCR)  |
| as1_flv4_prom_fw       | <u>GGT ACC</u> AGA TAA GCT AGG GTT<br>TCC                         | Promoter- <i>luxAB</i> fusion  |

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|                       |  |                               |
|-----------------------|--|-------------------------------|
| as1_flv4_prom_rev     | <u>GGT ACC GAC TGT ATA TTT TAA</u><br>CCG  | Promoter- <i>luxAB</i> fusion |
| flv4-2_prom_fw        | <u>GGT ACC ACA ACC AGT TGG AAT</u><br>GGT TAA AGT                                    | Promoter- <i>luxAB</i> fusion |
| flv4-2_prom_rev       | <u>GGT ACC AGA AAA CTT TGT GGT</u><br>TTT  | Promoter- <i>luxAB</i> fusion |
| 5' ApaI_petJ          | <u>GAAGGGCCCGGGAATTGCTCTGG</u><br><u>CAACTGATTAATC</u>                               | Mutagenesis                   |
| 3'petJ_AsuII_oop_sall | <u>GAAGTCGACAATAAAAAACGCC</u><br><u>GGCGGCAACCGAGCGTTCGAAGG</u><br>TATTATGGGAGGCGGTC | Mutagenesis                   |
| as_flv4_asuII_for     | GAA TTCGAA<br>CCAGTGCTGAGGCGACCGATC  | Mutagenesis                   |
| as_flv4_asuII_rev     | GAATTCGAATTTCCCATTTGGCTTG<br>GACAGTACGATG  | Mutagenesis                   |
| spK_seg_for           | GATCGATGCCATTGAAGCGGAAA<br>TC  | Mutagenesis<br>(Segregation)  |
| spK_seg_rev           | CAATTTTCCTTCAGCGGCGTTGAG   | Mutagenesis<br>(Segregation)  |

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## SUPPLEMENTAL FIGURE LEGENDS

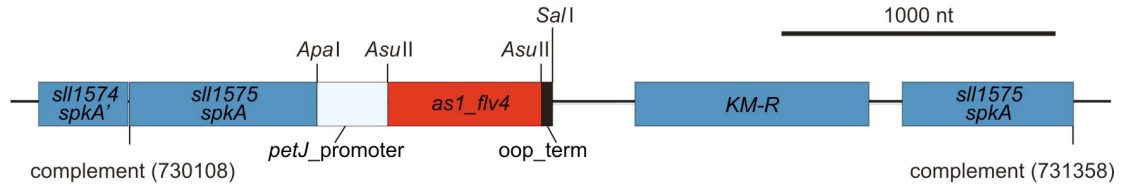
**FIGURE S1:** Artificial modulation of asRNA *As1\_flv4* levels. A. Cloning strategy for fusion of *as1\_flv4* gene with *petJ* promoter and integration into the *spkA* gene. For further explanation see Experimental procedures. B) Verification of overexpression of *As1\_flv4* in the overexpression strains *As1\_flv4*[+]/2 and *As1\_flv4*[+]/3. Accumulation of *As1\_flv4* transcript was measured 24 and 46 h after induction of heterologous expression by diminishing  $\text{Cu}^{2+}$  from the medium. *As* loading control *rnpB* was used.

**FIGURE S2:** Effect of changing  $\text{C}_i$  levels on accumulation of asRNA *As1\_flv4* transcript and proteins encoded by the *flv4-2* operon. *Synechocystis* WT cells were pre-cultivated under HC conditions, shifted to LC for 24 h, and then shifted back to HC conditions. Samples were collected 0, 1, 3, 4.5, 6, 12, and 24 h after each shift. A. Results of blotting experiments. 5S rRNA and AtpB were used as loading controls for RNA and protein, respectively. B. Quantification of signal intensities. The strongest signal intensity for each probe was set to 100% and the other signal intensities were related accordingly. Shown are the results of one representative experiment.

**FIGURE S3:** Impact of loss of the transcriptional regulator NdhR on the LC-induced expression of the asRNA *As1\_flv4* and the *flv4-2* operon. A. Accumulation of transcripts and proteins in WT and  $\Delta\text{ndhR}$  after LC shift. B. Activity of the *flv4-2* promoter in WT (WT- $P_{flv4-2}$ ) and  $\Delta\text{ndhR}$  ( $\Delta\text{ndhR}$ - $P_{flv4-2}$ ) after LC shift. Strains were generated, verified and treated as described in Experimental procedures. Each sample was measured in triplicates. Given are the means and SD of triplicates of a representative time course.

Figure S1

**A**



**B**

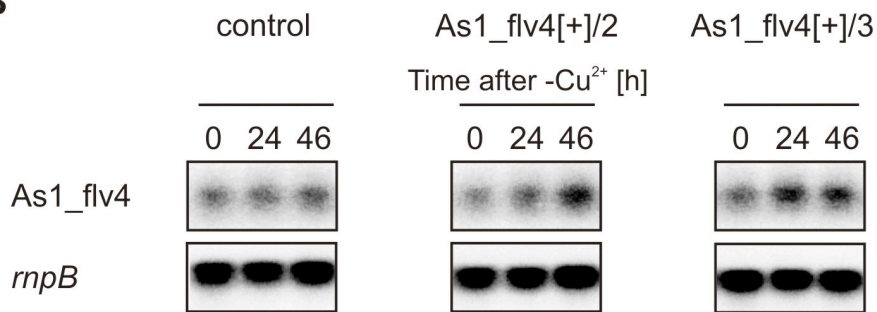
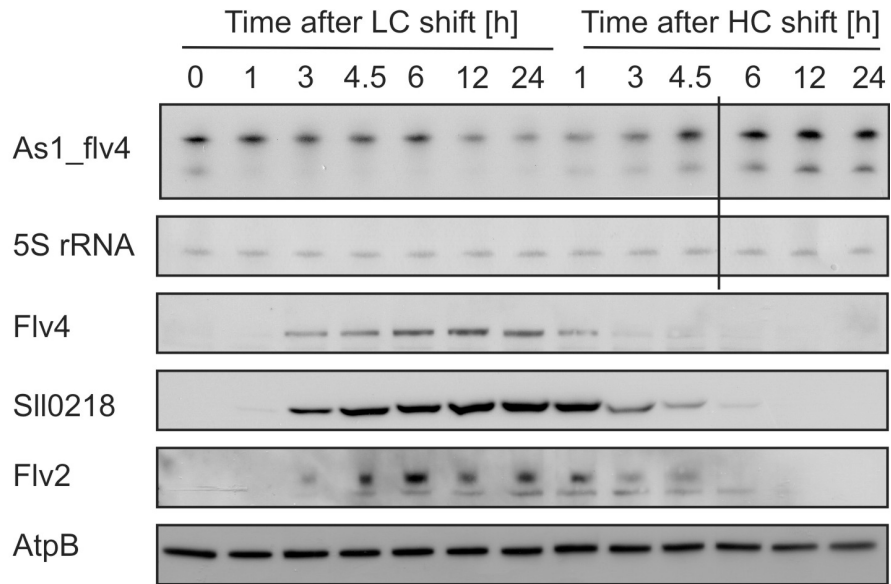


Figure S2

**A**



**B**

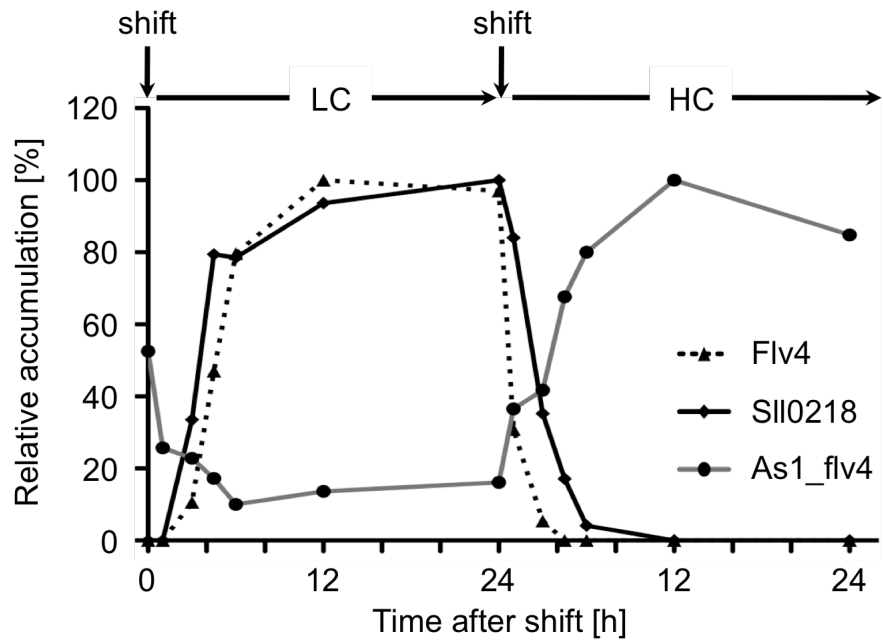
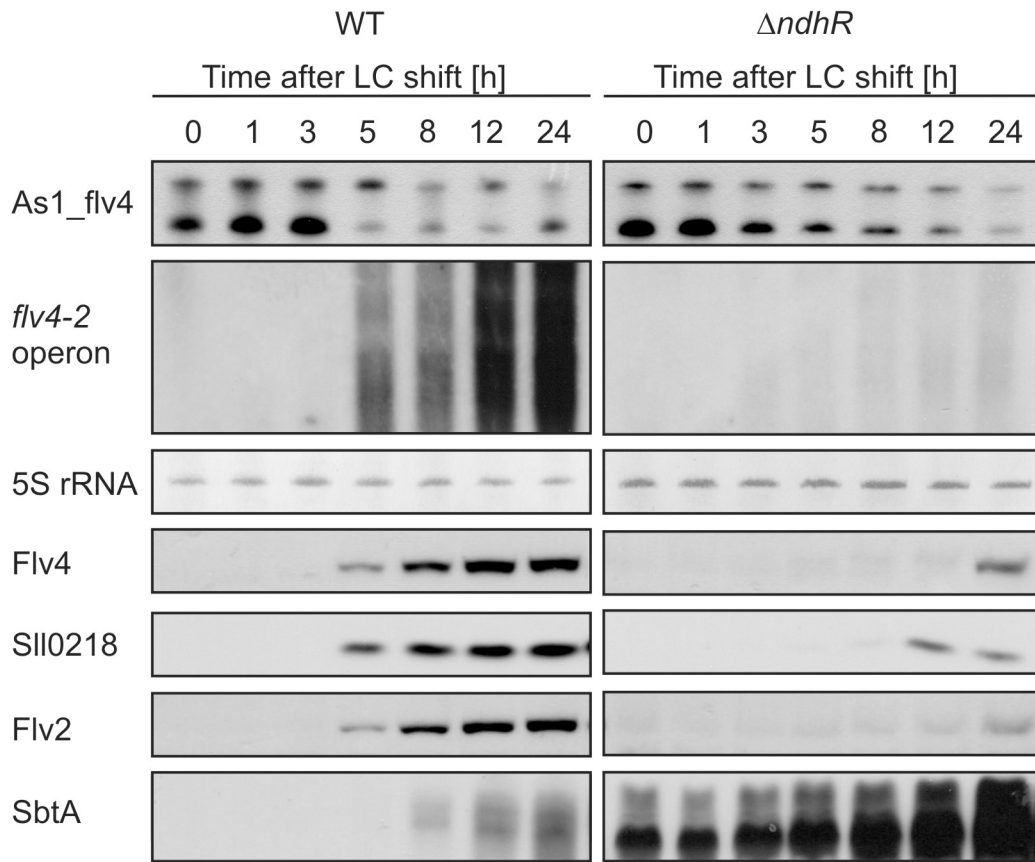


Figure S3

**A**



**B**

