SUPPLEMENTAL FIGURE 1. FPLC chromatogram at A_{280} and A_{420} for the crude soluble extract from WT *Synechocystis* sp. PCC 6803. The crude soluble extract from WT *Synechocystis* sp. PCC 6803 (100 µl of a soluble extract, OD_{650}=10) was separated by FPLC and the absorption recorded at 280 and 420 nm (blue and red graphs, respectively). Twenty-two 0.5-ml fractions were collected for subsequent SDS-PAGE and Western blot analyses (see Figure 1). As a marker, the content of one vial of the high molecular weight marker kit (GE Healthcare, US) was also run over the column and the elution peaks for the respective proteins with their molecular weights indicated.

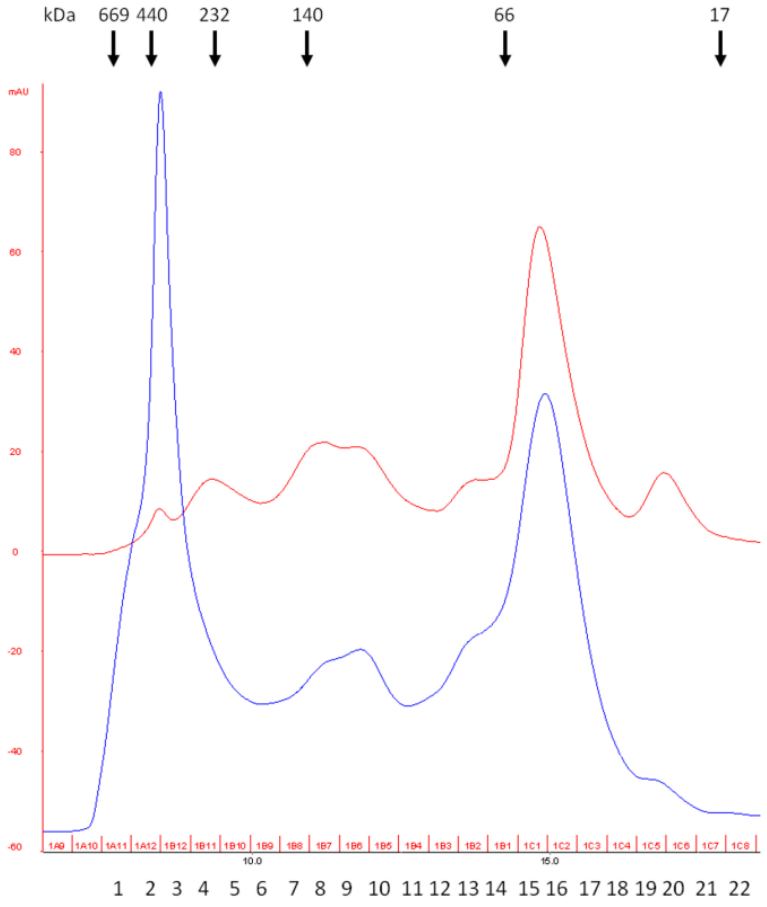
SUPPLEMENTAL FIGURE 2. Gene arrangement scheme for constructed *Synechocystis* sp. PCC 6803 *hox* mutants. The *hox* operon is depicted, illustrating the introduction of antibiotic resistance ORFs (cassette from pUC4K was used for *hoxH*-) for each gene. Not shown is the *hox*- strain, which disrupts the entire operon with a hygromycin resistance cassette (strain constructed and provided by T. Ogawa).

SUPPLEMENTAL FIGURE 3. *Synechocystis* sp. PCC 6803 *hox* operon overexpression in *hoxE*- strains. (A) Illustration of the *hox* operon in WT, our *hoxE*- strain in which the *hoxE* open reading frame (ORF) is replaced with the *aac3ia* ORF to confer gentamicin resistance (GmR), and the *hoxEF*- mutation constructed in (30) in which a 1.6 kb region between SmaI (190-bp into the *hoxE* ORF) and MscI (1181-bp into the *hoxF* ORF) is replaced with a chloramphenicol resistance cassette from pACYC184. The regions amplified for RT-PCR in *hox F* and *hoxH* are depicted. (B) Relative Hox protein levels in WT, *hoxE*-, and *hoxEF*- strains. Quantitation of Western blot Hox protein levels relative to WT are shown for *hoxEF*- (in our WT strain background) as in Figure 3, with a representative Western blot with Hox subunit-specific antibodies and PsaD as a loading control for WT, *hoxE*-, and *hoxEF*- whole cell lysates. (C) Fold-change versus WT of *hoxF* and *hoxH* transcripts (normalized to a *psbAII* transcript target (122-bp) levels as a control) in *hoxE*- or *hoxEF*- strains as determined by quantitative RT-PCR. Results are from two separate experiments using Agilent Brilliant II 1-Step SYBR Green qRT-PCR kit as per manufacturer's instructions. Reactions were performed and mean threshold values (C_T) were determined for each duplicate reaction using Applied Biosystems Step One Plus Real-Time PCR system and software (v. 2.2), and fold-change versus WT from both experiments were calculated and presented. (D) RNAfold plots generated using Vienna Websuite (57) depicting minimum free energy (mfe, red), the partition function (pf, green), and centroid folding (centroid, blue) of the mRNA from the +1 site through *hoxE* for WT and the +1 site through *aac3ia* for the *hoxE*- strain. Purple lines denote positions for the +1 site, the start site of either *hoxE* or *aac3ia* (+168), the SmaI site in *hoxE* through which remains intact in *hoxEF*- (+358), and the stop site for *hoxE* (+690) or *aac3ia* (+702). Minimum free energies are listed for the most probable structure for both mRNA sequences.

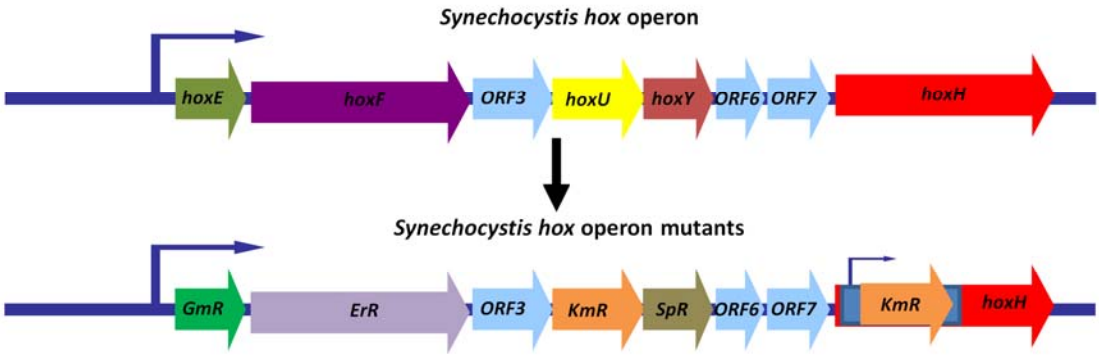
SUPPLEMENTAL FIGURE 4. *Synechocystis* sp. PCC 6803 HoxH processing in *hox* mutants. Percentages of processed versus unprocessed HoxH were determined for WT and *hox* mutants containing HoxH by quantitative analysis of multiple Western blots (representative blot in Figure 2).

SUPPLEMENTAL FIGURE 5. *Synechocystis* sp. PCC 6803 *hoxEF*- mutation in the Ogawa parental background does not exhibit light sensitivity. Serial dilution plating of Ogawa WT, Ogawa *hox*-, and the *hoxEF*- mutation from the original *hoxEF*- strain constructed by Howitt and Vermaas (30) and analyzed in Antal et al. (41) transferred (by PCR amplification of mutation plus 500-kb flanking sequence and transformation) to the Ogawa WT background. 10-fold serial dilutions were made in a 48-well holder and cells were transferred using a 48-prong replica plating device onto two identical BG11 plates. One plate was grown under 50 µE m^{-2} s^{-2} light while the other was grown under 200 µE $m^{-2}s^{-1}$ light. Plates were imaged after 5 days of growth at 30°C.

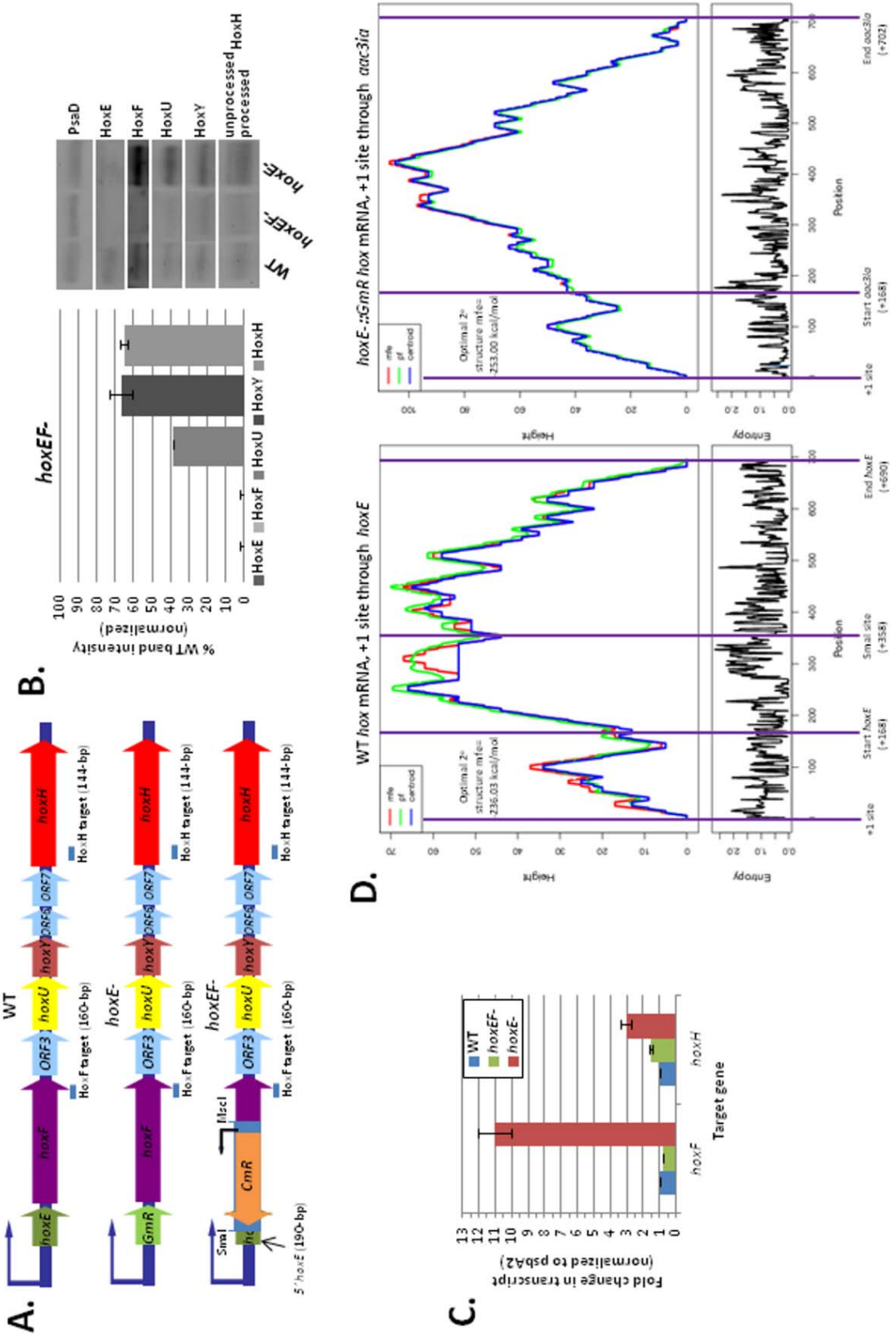
Supplemental Figure 1



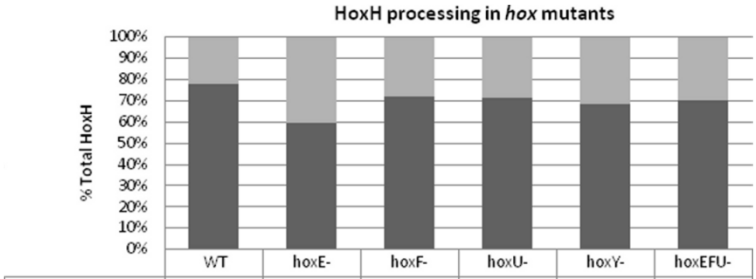
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



	WT	hoxE-	hoxF-	hoxU-	hoxY-	hoxEFU-
Unprocessed HoxH	22.1	40.2	27.9	28.3	31.7	29.5
Processed HoxH	77.9	59.8	72.1	71.7	68.3	70.5

Supplemental Table 1

Hydrogenase activity of *hox* mutants with different electron donors (nmol H_2/ml culture/min)									
	WT	***hox-***	***hoxE-***	***hoxF-***	***hoxU-***	***hoxY-***	***hoxH-***	***hoxEFU-***	***hoxYH-***
1mM NADH	0.20 ± 0.04	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected
in vivo	0.08 ± 0.01	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected
1mM MV	31 ± 6	None detected	22 ± 4	7 ± 2	5 ± 1	None detected	None detected	5 ± 2	None detected

Supplemental Figure 5