SUPPLEMENTAL MATERIAL

Fig. S1. Time-course experiment showing developmental upregulation of *pknE*. Wild-type *Anabaena* filaments containing reporter plasmid pAM3853 ($P_{pknE\text{-}P}$-*gfp*) showed increased GFP fluorescence in all cells by 3 h after nitrogen step-down and patterned expression by 12 h. Size bar, 10 μm.

Fig. S2. Analysis of mutagenized *pknE* promoter activity. Wild-type *Anabaena* filaments containing reporter plasmid pAM3919 ($P_{pknE\text{-}PmutntcA}$-*gfp*) or pAM3920 ($P_{pknE\text{-}P2mutntcA}$-*gfp*) showed patterned increases in GFP reporter expression 24 h after nitrogen step-down. The *gfp* gene was driven by *pknE* promoter containing mutagenized NtcA binding site. Top panels show GFP and autofluorescence (AF) and lower panels show only GFP fluorescence. Size bar, 10 μm.

Fig. S3. Analysis of *pknE* promoter activity in Δ*hetR* and extracopy *hetR* strains. Fluorescence micrographs show GFP reporter expression from plasmid pAM4012 ($P_{pknE\text{-}P}$-*gfp*) in Δ*hetR* (strain UHM103) and extracopy *hetR* (strain AMC1761) backgrounds 24 h after nitrogen step-down. Top panels show GFP fluorescence and autofluorescence (AF) and lower panels show only GFP fluorescence. Size bar, 10 μm.

Fig. S4. Affinity purification of GST-HetR and GST. *E. coli* BL21(DE3) containing expression plasmid pGEX-4T1-*hetR* or pGEX-4T1 was used to purify GST-HetR and GST. Coomassie stained gels of purification fractions of GST-HetR (A) and GST (B).

Lane M, PageRuler Pre-stained Protein Ladder (Fermentas); lane FT, column flow through; lane W, column wash; lanes 1-7, collected fractions.

Materials and Methods for Purification of GST-HetR from *E. coli.* *E. coli* BL21(DE3) cells harboring pGEX-4T1-*hetR* (a kind gift of Prof. Zhao, Peking University, Beijing, China) or pGEX-4T1 were grown at 37°C in Luria-Bertani (Lennox L) liquid medium supplemented with ampicillin (100 μg ml^{-1}) to an optical density of 0.6-0.7 at 600 nm. The cultures were induced overnight with 1 mM IPTG at 28ºC for overproduction of GST-HetR or GST. The washed cells were re-suspended in PBS buffer (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, pH 7.3) and sonicated for 15 min (15 sec ON, 20 sec OFF) on ice with 50% amplitude of medium probe (Branson Sonic Dismembrator). The crude lysate was centrifuged at 32,500 × g for 1 h and the supernatant was passed through a 0.45 μm Millipore filter prior to binding to a GSTrap HP column (GE Healthcare). After washing the column with 20 column volumes of wash buffer (50 mM Tris-Cl, 150 mM NaCl, pH 7.5), the proteins were collected as fractions with elution buffer (50 mM Tris-Cl, 150 mM NaCl, 7 mM glutathione, pH 8). After SDS-PAGE analysis (Fig. S4), appropriate fractions were concentrated and dialyzed at 4ºC by centrifugation using 10 kDa cutoff filter devices (Amicon Ultra-15 10K MWCO, Millipore) in elution buffer without glutathione for further use.