# CvkR is a MerR-type transcriptional repressor of class 2 type V-K CRISPR-associated transposase systems

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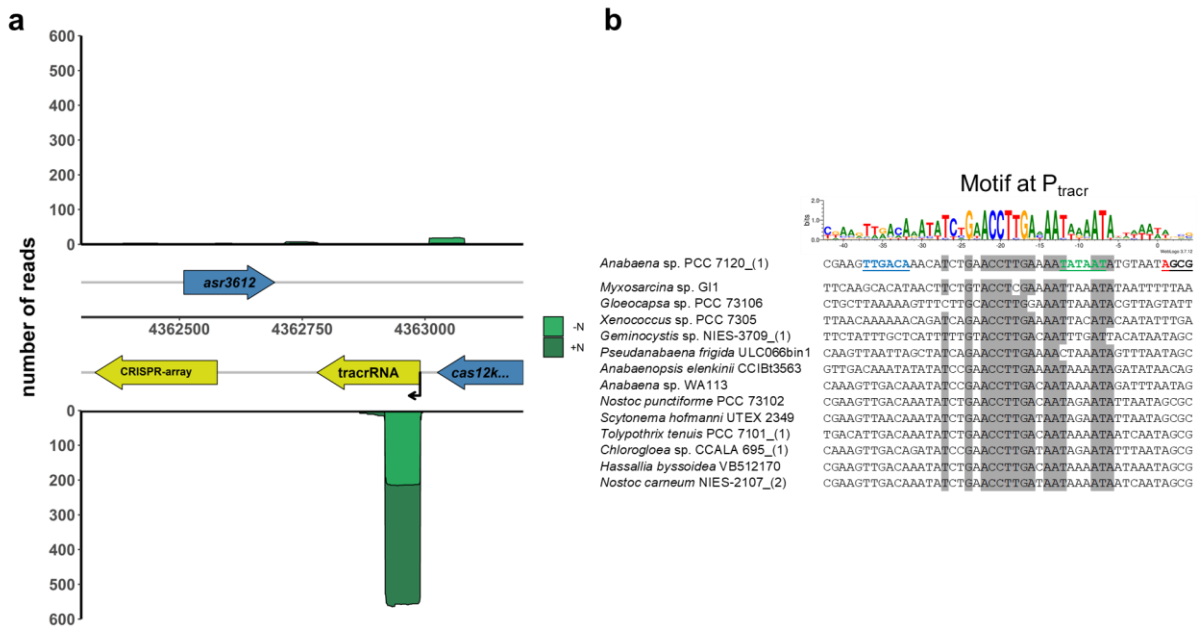
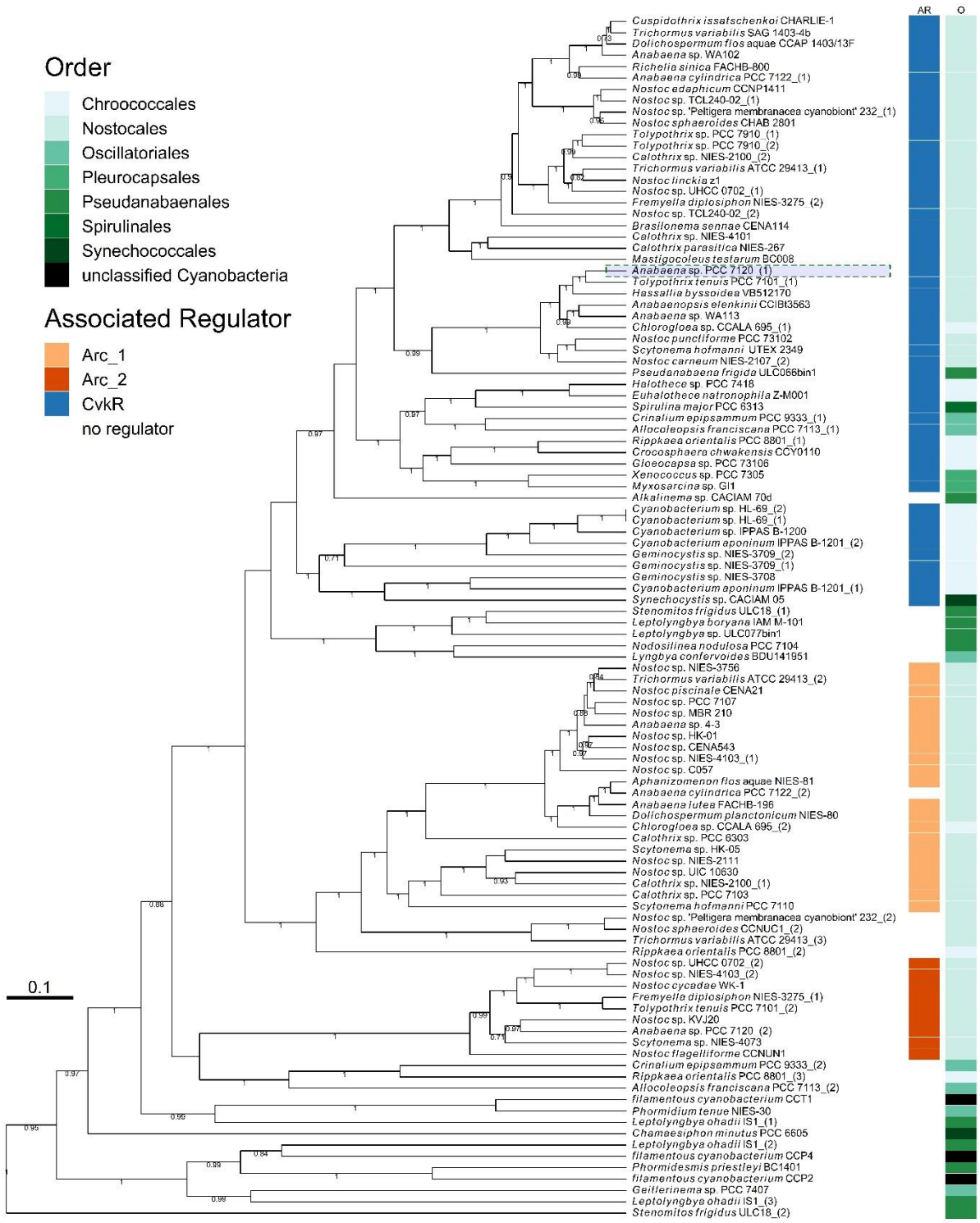
**Supplementary Material**

## Supplementary Figures

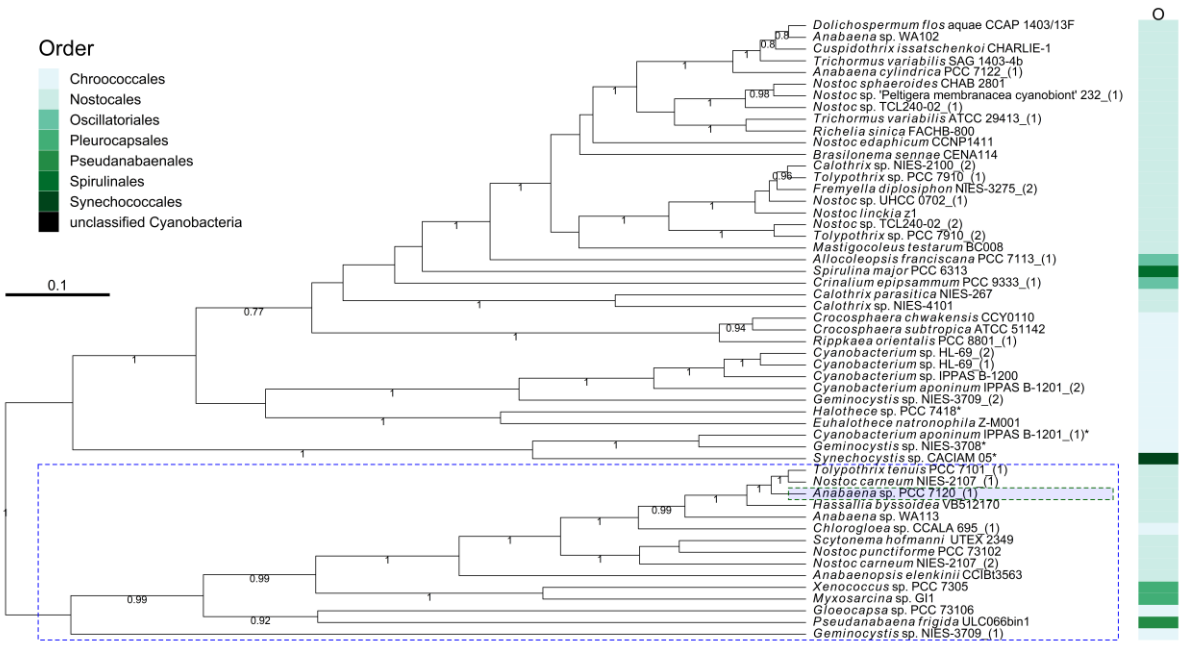



**Supplementary Figure 1. The tracrRNA promoter ($P_{tracr}$). a** Location of the tracrRNA TSS 35 nt downstream of *cas12k* in *Anabaena* 7120 according to the previous genome-wide mapping of TSS[1]. **b** Sequence conservation in the tracrRNA promoters of different CAST systems belonging to the same subgroup as the *Anabaena* 7120 CvkR according to the phylogenetic analysis (**Supplementary Fig. 3**). Positions with ≥92% conservation are shaded. Putative -10 and -35 elements of the promoter in the AnCAST system are colored green and blue, respectively, and the first tracrRNA nucleotides are underlined and in boldface letters (TSS in *Anabaena* 7120 is highlighted in red). One of the more closely related 15 systems according to **Supplementary Fig. 3** lacked a recognizable tracrRNA, therefore only 14 sequences were compared.

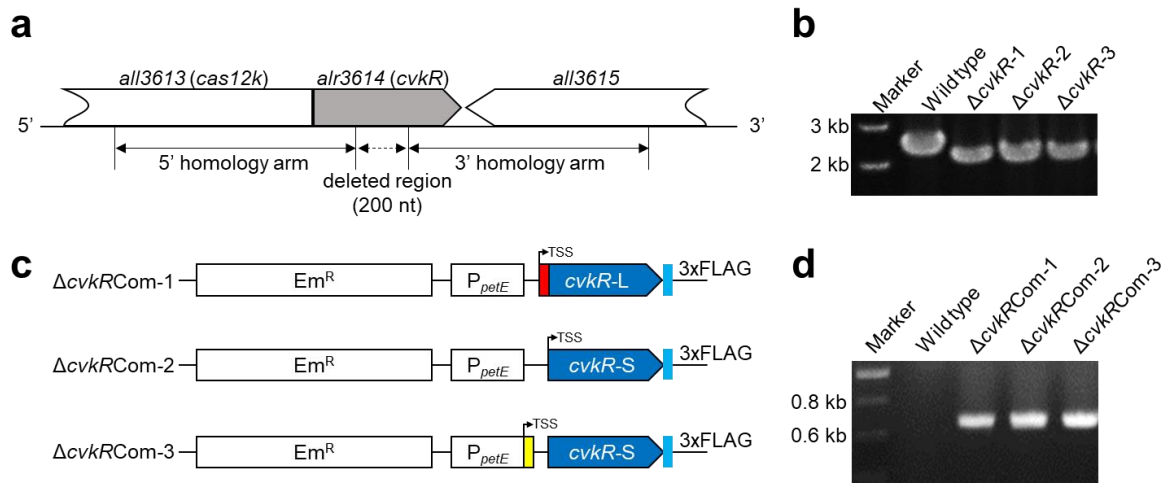



**Supplementary Figure 2. Phylogenetic tree of Cas12k.** The identified 106 complete Cas12k proteins were aligned using M-coffee[2,3] and analyzed by BEAST[4]. Eleven instances of degenerated Cas12k proteins were intentionally let out to avoid misinterpretation. The resulting tree is depicted with branches labeled with their respective posterior probability until a threshold of 0.7. For better recognition, the proteins were

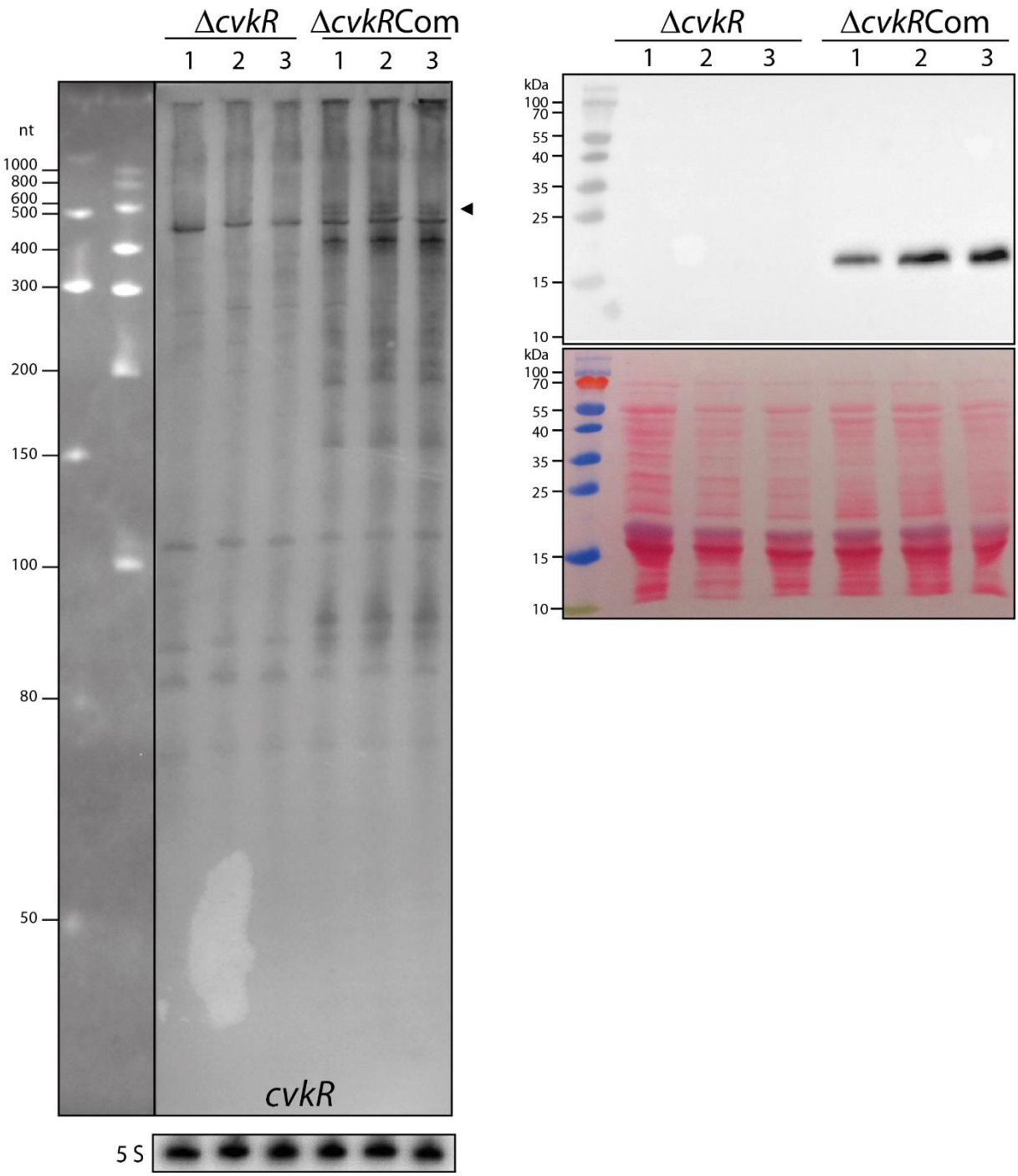
labeled with their respective host organism (see **Supplementary Data 1**). The associated regulator type (AR) and taxonomic order (O) of the respective host organism are also depicted. The *Anabaena* 7120 Cas12k (All3613) (NCBI: BAB75312.1) is marked with a green dashed box. The multiple sequence alignments are available in **Supplementary Data 4**.




**Supplementary Figure 3. Phylogenetic tree of CvkR.** Phylogenetic tree of CvkR homologs. The proteins were aligned using M-coffee[2,3] and analyzed by BEAST[4]. The resulting tree is depicted with branches labeled with their respective posterior probability until a threshold of 0.7. For better recognition, the proteins were labeled with their respective host organism (see **Supplementary Data 1**). The taxonomic order (O) of the respective host organism are also depicted. *Anabaena* 7120 CvkR (Alr3614) (NCBI: BAB75313.1) is highlighted by a green dashed box and its most similar homologs by a blue-dashed box. Asterisks label four instances of CvkRs fused to an *hsdR* restriction enzyme domain. The multiple sequence alignments are available in **Supplementary Data 5**.

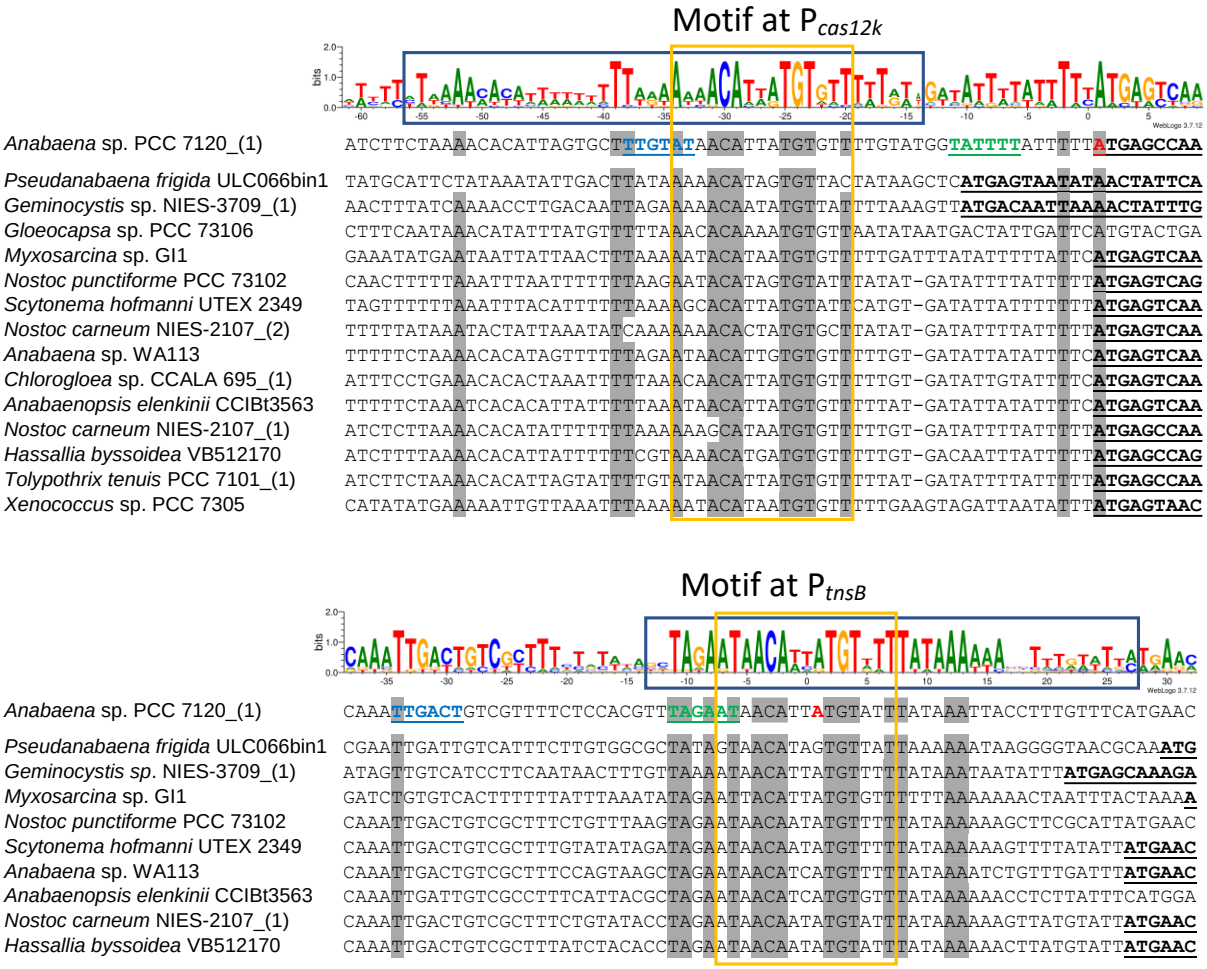



**Supplementary Figure 4. Generation of *cvkR* deletion mutants and complementation of *ΔcvkR*. a** Schematic drawing of the deleted regions and flanking regions of *cvkR*. **b** PCR assays showing successful segregation of deletion mutants in three independent replicates each. **c** Schematic drawing of the three cassettes for analyzing the leaderless expression of *cvkR*. The red bar indicates the extra 54-bp fragment at the 5'-end of *cvkR-L* compared to *cvkR-S*. The yellow bar indicates the 31 nt long 5'UTR of *petE*. **d** PCR assays showing successful construction of reporter strains for monitoring leaderless expression of *cvkR*. Please note that the result of one positive colony out of five (n=5) is shown for each reporter strain.

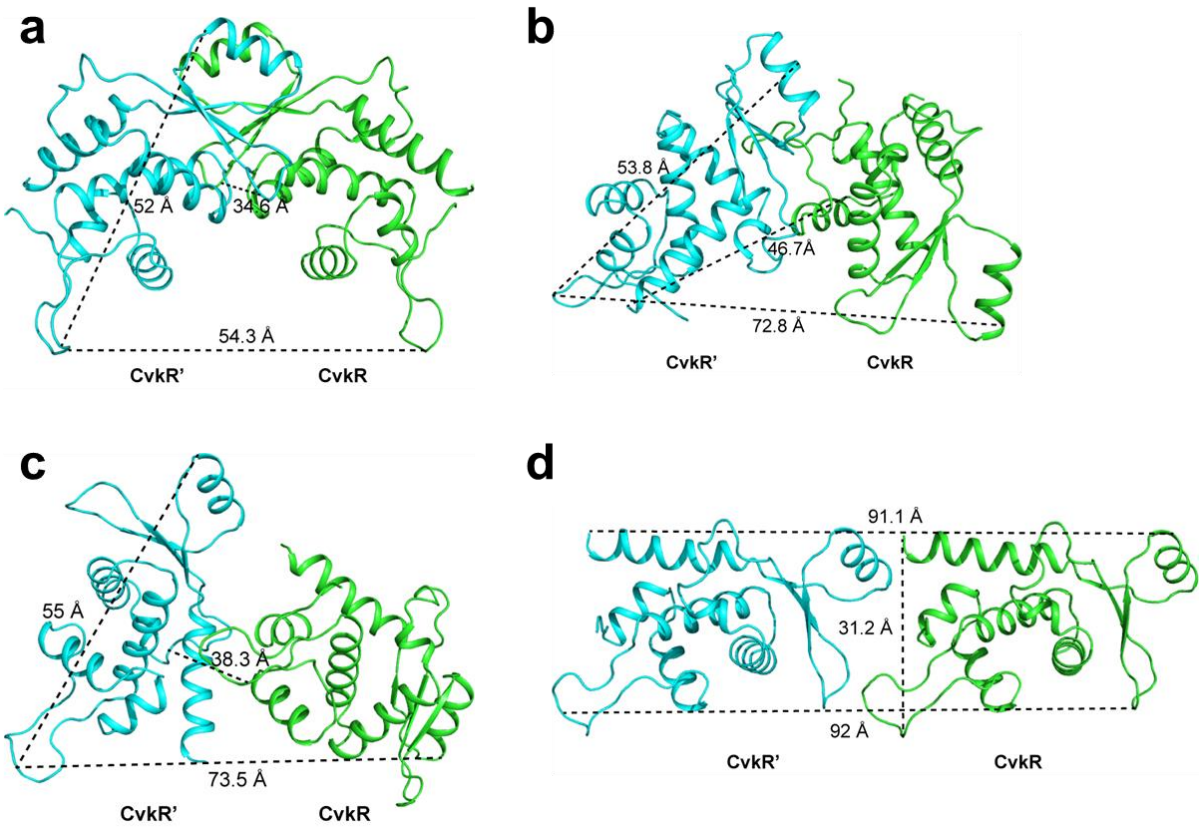



**Supplementary Figure 5. Verification of absence or overexpression of CvkR in Δ*cvkR* or Δ*cvkR*Com, respectively.** Left: Northern blot hybridization against *cvkR* in three independent replicates of Δ*cvkR* and Δ*cvkR*Com. The precise length of the *cvkR* mRNA is unknown because the transcript level in *Anabaena* 7120 WT is below the detection limit. The *cvkR* gene length is 453 bp. Taking the short-added sequence and

3’UTR into account, a correspondingly-sized additional signal in Δ*cvkR*Com represents the target *cvkR* band (labeled by triangle). Total RNA (20 µg) was loaded on a 10% PAA 8.3 M urea gel. The Low Range ssRNA Ladder (NEB) and the RiboRuler Low Range RNA Ladder (Thermo Fisher Scientific) were used as size markers. Right: Western blot against CvkR with an N-terminal 3xFLAG tag (upper panel) in three independent replicates of Δ*cvkR* and Δ*cvkR*Com. The stained membrane is shown in the lower panel. The calculated molecular mass for CvkR is 20.16 kDa. The prestained PageRuler (Thermo Fisher Scientific) was used as a size marker. Ten micrograms of total protein were loaded on a 15% SDS–PAA gel. Two separate experiments were performed, which showed consistent results.




**Supplementary Figure 6. Comparison of *cas12k* (top panel) and *tnsB* (lower panel) promoter homologs.** The promoters of these genes belonging to the same CvkR subgroup as the AnCAST CvkR according to phylogenetic analysis (**Fig. 2b**) were compared to each other (≥92% conservation is shaded). Putative -10 and -35 elements of the genes in the AnCAST system are colored green and blue, respectively and the first codons of respective reading frames are underlined. The TSSs of the AnCAST genes are highlighted in red. Four of the observed CAST systems lack a *tnsB* gene and one *tnsB* was degraded, so the remaining 10 *tnsB* promoter sequences were aligned. The protected areas in the DNase I footprinting assays are blue boxed and the potential CvkR binding motif AnnACATnATGTnnT is yellow boxed.




**Supplementary Figure 7. Analysis of CvkR homodimer in structure**. **a-d** four potential dimeric partners (CvkR', cyan) of CvkR (green) are found through different crystallographic symmetry operations. The resulting four homodimers have interface areas of 997.6 Å$^2$, 843.3 Å$^2$, 372.2 Å$^2$, and 276.6 Å$^2$, respectively, as calculated by webtool PDBePISA[5]. The sizes of CvkR-CvkR' homodimers are respectively further measured and labeled from several structural angles. The dimeric architecture, assembled by two vertically symmetric CvkR monomers shown in panel a, is similar to other reported MerR family members and is further approved by PISA assembly analysis and DLS assay.

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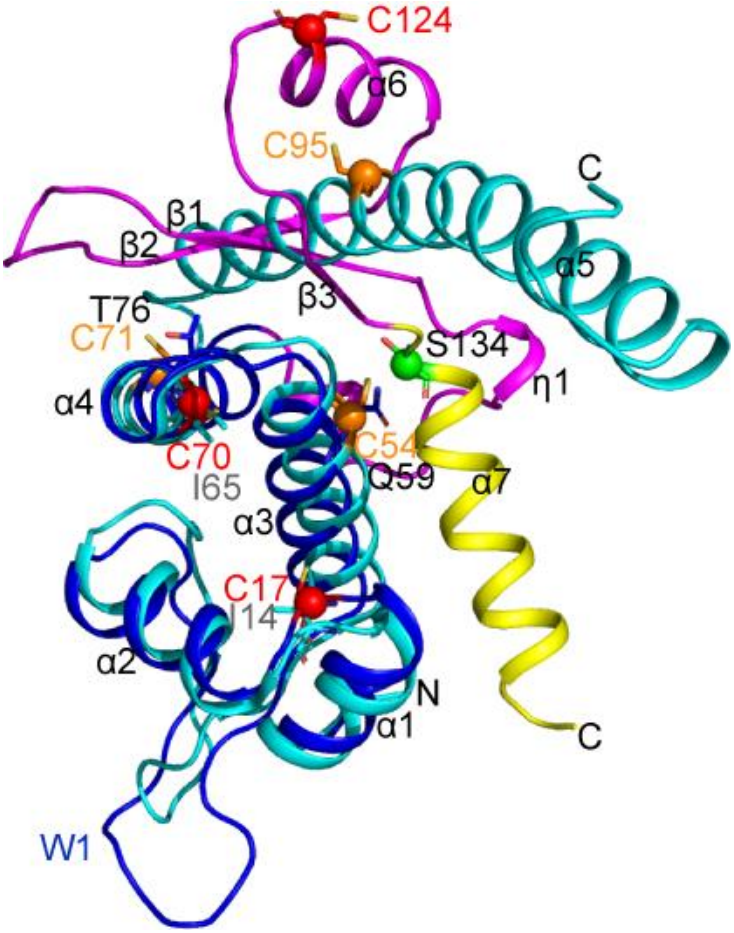
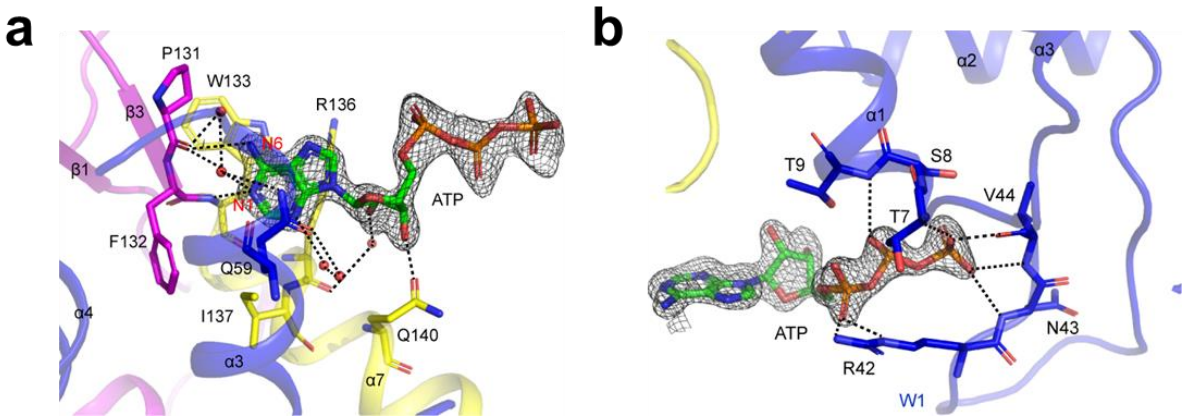
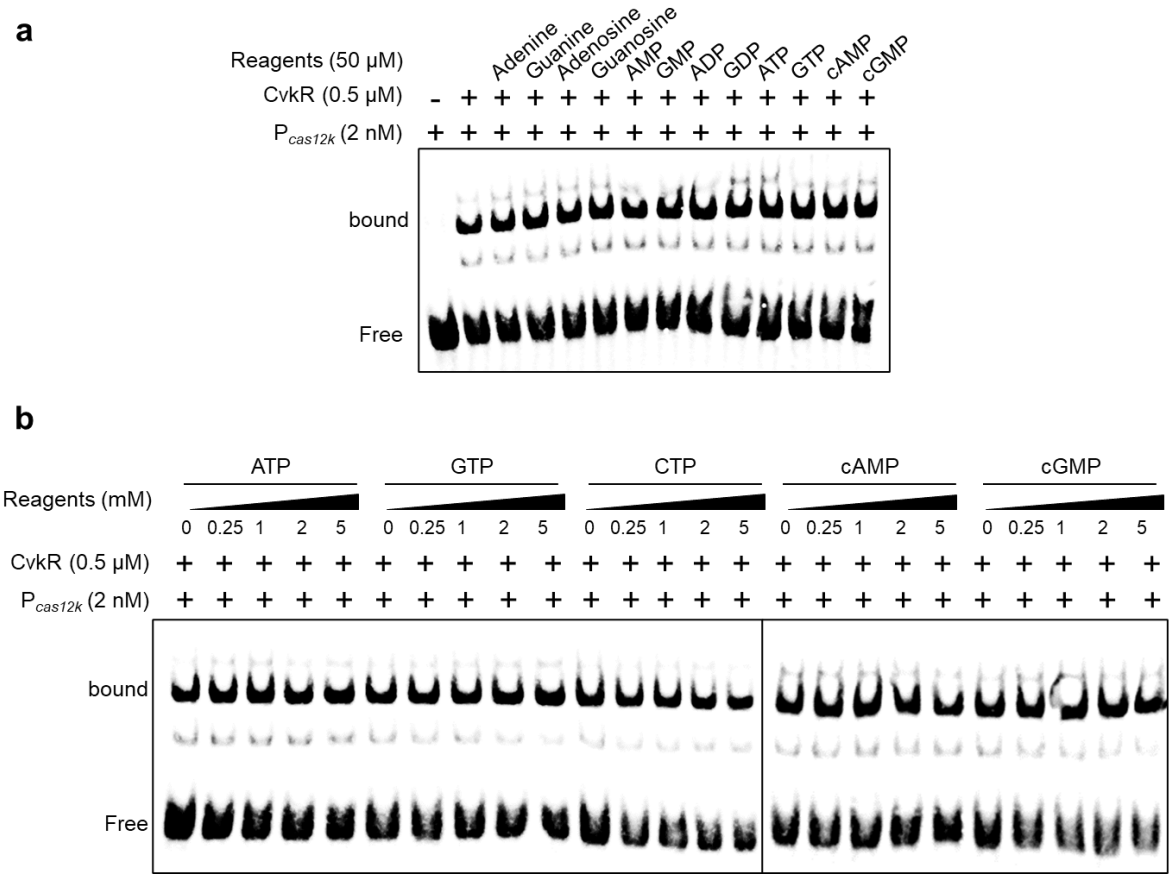


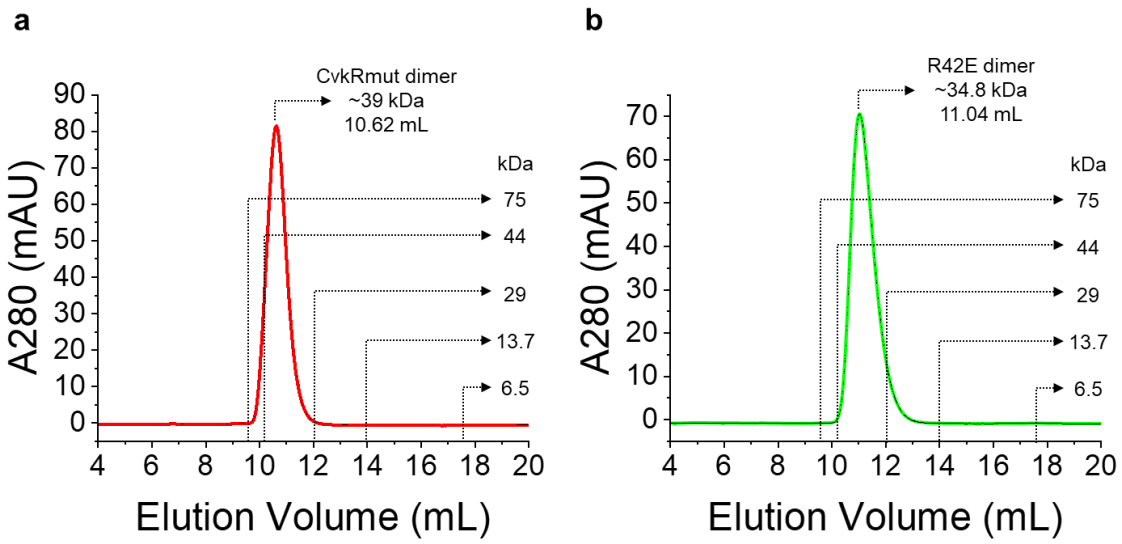

**Supplementary Figure 8. Representation of cysteine sites in CvkR.** The cysteine residues are represented in spheres and sticks and labeled with red in CvkR and with orange in HiNmlR (PDB code 5D8C, cyan). The Ser134 residue in CvkR is shown in green sphere and stick. Residues matching the positions of cysteines are shown in sticks and marked with black (CvkR) and gray (HiNmlR).



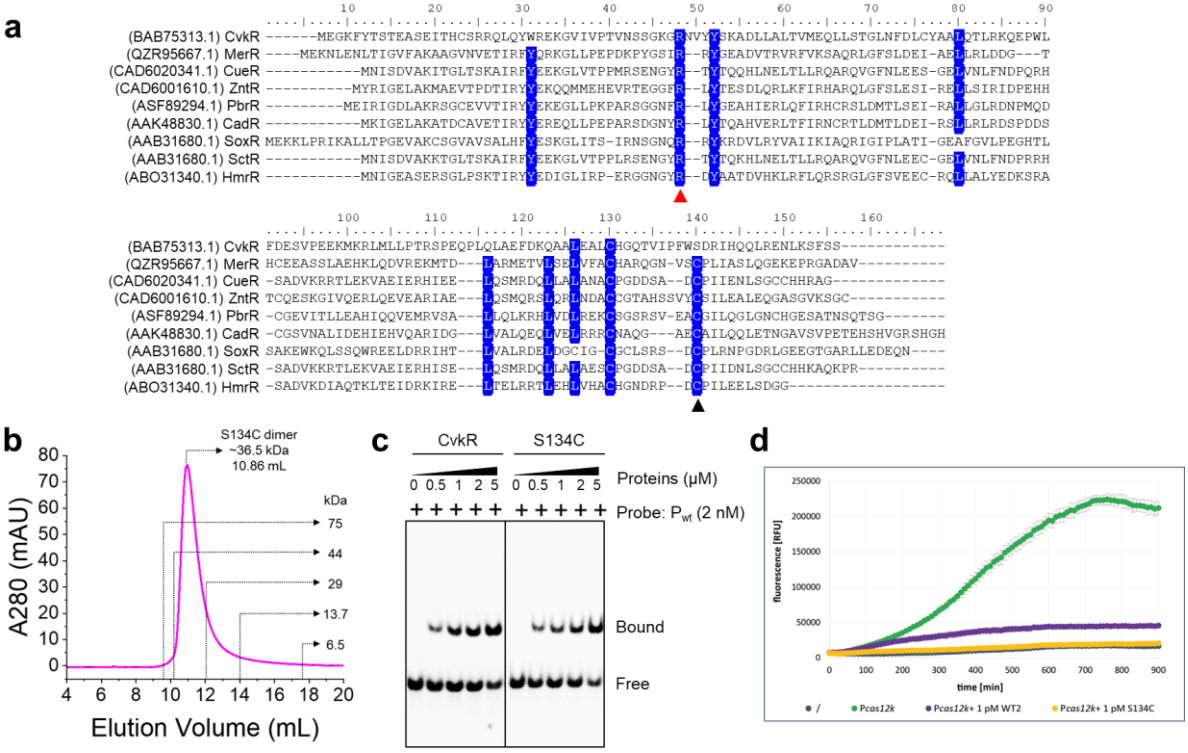

**Supplementary Figure 9. ATP binding site of CvkR. a** Close view of the ATP binding site in the CvkR monomer. **b** The ATP triphosphate group interacts with the wHTH domain of the adjacent CvkR monomer. The ATP ligand is represented in green stick and colored by the atom type. The N1 and N6 sites of ATP are labeled in red. The 2Fo-Fc density for the ATP is contoured in gray at 1.5 σ. The key residues involving in ATP binding are shown in sticks and labeled in black. Hydrogen bonds are indicated by dashed lines. The water molecules involved in hydrogen bonds are presented as red spheres.




**Supplementary Figure 10. EMSA assays showing the binding of CvkR to P*cas12k* in the presence of multiple molecules with similar chemical structures to ATP. a** EMSA showing the DNA binding ability of CvkR with the addition of adenine, adenosine, AMP, ADP, ATP, cAMP, guanine, guanosine, GMP, GDP, GTP and cGMP. **b** EMSA showing the DNA binding ability of CvkR with concentration gradients of ATP, GTP, CTP, cAMP, and cGMP. EMSA assays showing the binding of S134C to the *cas12k* promoter. The P*cas12k* probe was used for the assay. The data are presentative from three independent experiments. The free probe and complexes of P*cas12k* with CvkR are marked as “Free” and “Bound”, respectively.

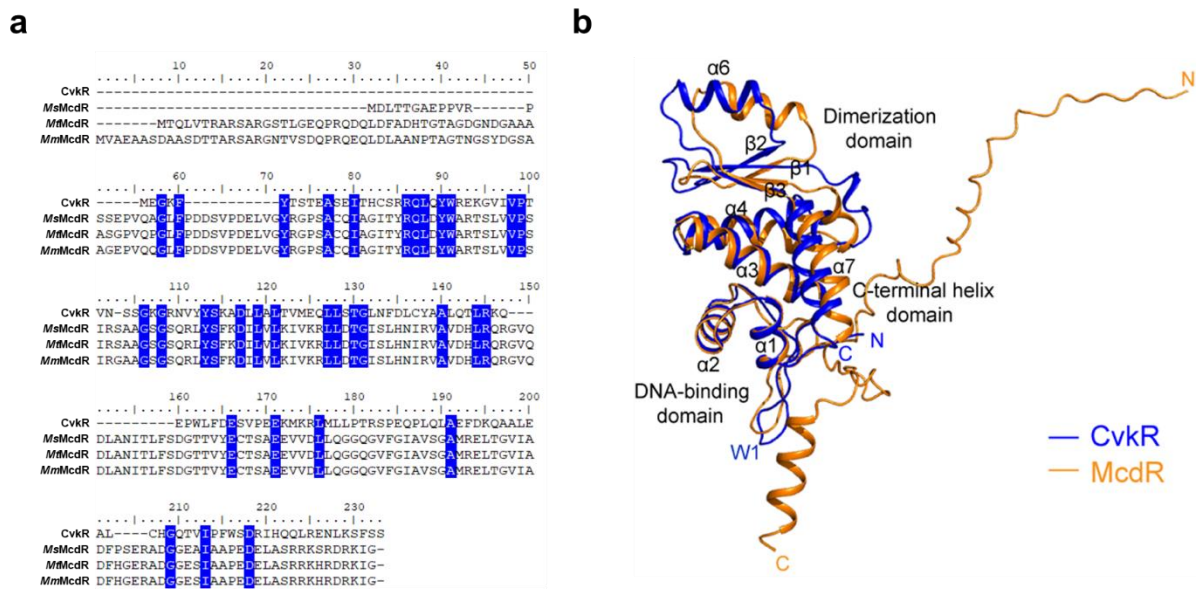



**Supplementary Figure 11. Molecular weight estimates of CvkR mutant proteins by size exclusion chromatography (SEC) with Superdex 75 10/300 GL column (GE Healthcare)**. Calibration standards are indicated. **a** The elution volume of CvkRmut was 10.62 mL, corresponding to a mass of ~39 kDa. **b** The elution volume of R42E was 11.04 mL, corresponding to a mass of ~34.8 kDa. The estimated masses of both mutant proteins correspond to twice their calculated theoretical molecular weights.




**Supplementary Figure 12. Characterization of S134C mutant of CvkR. a** Multiple sequence alignment of CvkR with selected MerR-type regulators. The NCBI accession numbers of the MerR-type regulators are given in brackets. The sequence alignment was performed using MAFFT[6], and visualized with BioEdit[7]. Threshold for shading is 80% identity. The black triangle indicates CvkR lacks a cysteine residue in the C-terminal sensor domain which is conserved in the selected members of the MerR-type regulators. The red triangle indicates one of the conserved arginine reported to interact with the phosphate backbone of promoter DNA[8,9]. **b** Molecular weight estimates of S134C mutant protein on SEC. Calibration standards are indicated. The elution volume of S134C was 10.86 mL, corresponding to a mass of ~36.5 kDa (twice its calculated molecular weight). **c** EMSA assays showing the binding of S134C to the *cas12k* promoter. The 33-nt $P_{wt}$ probe was used for the assay. The data are presentative from three independent experiments. The free probe and complexes of $P_{wt}$ with S134C are

marked as “Free” and “Bound”, respectively. **d** TXTL assay to test the regulatory capacity of S134C compared to CvkR WT2 for repressing deGFP fluorescence expressed from p70a under the control of $P_{cas12k}$ (5 nM). Error bars show the standard deviation and are derived from 2 technical replicates. The experiments were repeated twice independently. The TXTL reactions were performed at 29°C overnight, and fluorescence was measured every 10 min in a Wallac 1420 Victor2 microplate reader. Error bars show the standard deviation derived from two technical replicates.




**Supplementary Figure 13. Comparison of CvkR and McdR**. **a** Multiple sequence alignment of CvkR and McdR. CvkR: Alr3614 of *Anabaena* sp. PCC 7120 (NCBI: BAB75313.1). MsMcdR: McdR of *Mycolicibacterium smegmatis* MC2 155 (NCBI: ABK74795.1). MtMcdR: McdR of *Mycobacterium tuberculosis* H37Ra (NCBI: ABQ73597.1). MmMcdR: McdR of *Mycobacterium marinum* M (NCBI: ACC41150.1). The sequences were aligned using MAFFT[2] and visualized with BioEdit[7]. **b** Superimposition of CvkR (blue, NCBI: BAB75313.1) onto AlphaFold2[10] predicted McdR (orange, NCBI: ABK74795.1) [https://alphafold.ebi.ac.uk/entry/A0QYF9]. The secondary structure elements and the DNA-binding, dimerization, and C-terminal helix domains of CvkR are labeled.

## Supplementary Tables

**Supplementary Table 1.** Number of identified CAST associated regulator homologs and of CAST typical components in close vicinity.

| | Total hits | near *cas12k* | near Left end | near CRISPR-array | near tracrRNA | near tRNA | no CAST-components |
|---|---|---|---|---|---|---|---|
| CvkR | 157 | 130 | 93 | 61 | 85 | 100 | 11 |
| Arc_1 | 32 | 25 | 22 | 19 | 24 | 25 | 5 |
| Arc_2 | 21 | 14 | 15 | 10 | 10 | 14 | 2 |
| total | 210 | 169 | 130 | 90 | 119 | 139 | 18 |

**Supplementary Table 2. Microarray analysis of Δ*cvkR* or Δ*cvkR*Com strains.** Transcripts with significantly different accumulation levels in Δ*cvkR* compared to the complementation strain Δ*cvkR*Com. The microarray data was normalized using the R-implementation in package limma[11] and resulted p-values from t-tests were corrected using the Benjamini and Hochberg method to control the False Discovery Rate (FDR) of differential expression[12]. Threshold: $|\log_2| \geq 1$, p-value ≥0.01. Two replicates were used for the microarray experiment.

| Probe_Label | Gene/region_Function | location | $\log_2$FC | adj. pvalue |
|---|---|---|---|---|
| alr0738_igFwd | intergenic | chr | -2.413 | 1.41E-03 |
| *rtcB* | RNA-splicing ligase | chr | -2.382 | 4.19E-03 |
| *cvkR* | CvkR | chr | -2.203 | 4.77E-04 |
| nTSS_52243_52243 | tRNA L-array | delta | -1.987 | 4.77E-04 |
| *all8564* | restriction endonuclease | delta | -1.743 | 1.41E-03 |
| *alr0739* | uncharacterized conserved protein YdeI | chr | -1.616 | 5.37E-04 |
| *alr0740* | slipin family protein | chr | -1.500 | 1.06E-04 |
| *asr0855* | unknown | chr | -1.328 | 4.19E-03 |
| *all7121* | cytochrome c domain-containing protein | alpha | 1.075 | 1.41E-03 |
| all0328_igFwd | intergenic | chr | 1.109 | 3.81E-03 |
| alr0786_igRev | intergenic | chr | 1.268 | 3.28E-03 |
| *pecB* | phycoerythrocyanin beta-chain | chr | 1.300 | 4.19E-03 |
| all0736_igRev | intergenic | chr | 1.344 | 8.26E-03 |
| alr1198_igFwd | intergenic | chr | 1.387 | 1.12E-03 |
| *pecA* | phycoerythrocyanin alpha-chain | chr | 1.416 | 1.68E-03 |
| *pecC* | phycoerythrocyanin-associated rod linker protein | chr | 1.435 | 1.69E-04 |
| tracrRNA | CAST tracrRNA | chr | 1.493 | 4.33E-03 |
| *cas12k* probe 1 | CAST effector gene | chr | 1.550 | 1.41E-03 |
| all3391_igFwd | intergenic | chr | 1.567 | 4.77E-04 |
| *tnsB* | CAST transposase | chr | 1.594 | 4.67E-04 |
| *cas12k* probe 2 | CAST effector gene | chr | 1.870 | 9.16E-05 |

**Supplementary Table 3. Oligonucleotide primers used in this work.** Sequences belonging to the T7 promoter are underlined. Primers were purchased from IDT or Tsingke.

| | | |
|---|---|---|
| *alr3614* deletion mutant (Δ*cvkR*) construction | | |
| alr3614gRNA-1 | AGATCATTAACAGTAATGGAGCAG | gRNA for *cvkR* |
| alr3614gRNA-2 | AGACCTGCTCCATTACTGTTAATG | |
| alr3614KO-1 | GGTCATTTTTTTGTCTAGCTTTAATGCGGTAGTTG GTACCGGTTAAGAGAATATCCTGCC | 5' homology arm for *cvkR* knock out |
| alr3614KO-2 | CAGGCGATCGCGTGGGTAATTACTATAACTCCTT TCTCTC | |
| alr3614KO-3 | GAGAGAAAGGAGTTATAGTAATTACCCACGCGAT CGCCTG | 3' homology arm for *cvkR* knock out |
| alr3614KO-4 | GCGCTGCCCGGATTACAGATCCTCTAGAGTCGA CGGTACCAAAATCTTCGATGCAGATGA | |
| alr3614-3 | GACGACTATCGCAGTAAACTTC | Genotype confirmation |
| alr3614-4 | TTAGCTGCTTTACTACGACG | |
| *alr3614* complementation strain (Δ*cvkR*Com) construction | | |
| 59M-F | GATTATAAAGATCATGATGGTGATTATAAAGATCAT GATATTGATTATAAAGATGATGATGATAAATGAAAG GGTGGGCGCGCCGACCCAG | Amplification of pRL59EH-Cm/Em backbone with overlap to 3xFlag tag |
| 59M-R1 | GTTAATTTCACAGGCTTTAGGTCGACCTGCATCC CTTAAC | Amplification of pRL59EH-Cm/Em backbone with overlap to $P_{petE}$ |
| $P_{petE}$-F | GTTAAGGGATGCAGGTCGACCTAAAGCCTGTGA AATTAAC | With overlap to pRL59EH-Cm/Em backbone |
| $P_{petE}$-R4 | CACATTAGTGCTTTGTATAACATTTATTTCATTTTA AATAAAATCGACACC | $P_{petE\_no5'UTR}$-*cvkR-L*-3xFlag |
| $P_{petE}$-R5 | GCTTGTGTAGAACTTTCCTTCCATTTATTTCATTT TAAATAAAATCGACACC | $P_{petE\_no5'UTR}$-*cvkR-S*-3xFlag |
| $P_{petE}$-R6 | GTGTAGAACTTTCCTTCCATGGCGTTCTCCTAAC CTGTAG | $P_{petE}$-*cvkR-S*-3xFlag |
| 3614L-F | GGTGTCGATTTTATTTAAAATGAAATAAATGTTATA CAAAGCACTAATGTG | $P_{petE\_no5'UTR}$-*cvkR-L*-3xFlag |
| 3614S-F1 | CGATTTTATTTAAAATGAAATAAATGGAAGGAAAG TTCTACACAAGC | $P_{petE\_no5'UTR}$-*cvkR-S*-3xFlag |
| 3614S-F2 | CTACAGGTTAGGAGAACGCCATGGAAGGAAAGT TCTACAC | $P_{petE}$-*cvkR-S*-3xFlag |
| 3614-R | TCATTTATCATCATCATCTTTATAATCAATATCATGA TCTTTATAATCACCATCATGATCTTTATAATCGCTA CTAAAGCTTTTAAGATTC | Amplification of *cvkR*-3xFlag with overlap to pRL59EH-Cm/Em backbone |
| 3614TesT-F | GCCGCCAGTTGCAGTATT | Genotype confirmation |
| 3614TesT-R | CGGGCAAGTACGACATCA | |
| qRT-PCR | | |
| alr3614 RT-F | GTGGCAAAGGTCGTAATGTT | qRT-PCR of *cvkR* |
| alr3614 RT-R | CGCTTCATCTTTTCTTCTGGG | |
| rnpB RT-F | CGTGAGGATAGTGCCACAGA | Internal standard |
| rnpB RT-R | CCAACCATAGTTCCTTCGGC | |
| Northern hybridizations | | |
| CR_9_fwd | TTTGAATATTCAGAACTTTATATTGTGCGCGAT | as published[13], for mutant analysis |

| | | |
|---|---|---|
| CR_9_rev | TAATACGACTCACTATAGGGGCAAGCTGATTTG GTAGAAGCTGTTAAT | for mutant analysis |
| all3613-Nblot1 | GGAGTAAGCTTGGGGCTAGAA | for mutant analysis |
| all3613-Nblot2 | TAATACGACTCACTATAGGGGCCTGCTTTATAGG TCTGTGC | |
| tracrRNA_Fw_T7_1 | TAATACGACTCACTATAGGGCTCTTTGGTGCGTC AAATCAAG | for mutant analysis |
| tracrRNA_Rev | CAGTTCATGCTGCTTGCAGC | |
| Alr3614_Nblot_f | GCACAGAAGCATCAGAAATTAC | for mutant analysis |
| Alr3614_Nblot_r | TAATACGACTCACTATAGGGAGACAGCAACTGC TCCATTAC | |
| 5S_7120 | TAGCAGCGTTTCACCTCTGAGTTCGG | as published[13] |
| TXTL assay | | |
| pet28a_HisTEV_fwd | CTCGAGCACCACCACCAC | Amplification of pET-28a(+) with 6xHis tag and TEV site |
| pet28a_HisTEV_fwd | GGATCCCATATGCTGAAAATACAGG | |
| 3614mut_fwd | ATTTTCAGCATATGGGATCCATGGAAGGAAAGTT CTACAC | Amplification of *cvkR*mut with overlaps to pET-28a(+) |
| 3614mut_rev | TGGTGGTGGTGGTGCTCGAGTTAGCTACTAAAG CTTTTAAGATTC | |
| p70a-deGFP_fwd | GCTAGCAATAATTTTGTTTAACTTTAAGAAGGAG ATATACC | Amplification of p70a with deGFP and 5´UTR but without promoter |
| p70a-deGFP_rev | GCATGCCCAGCGGAACAG | |
| P3613_fwd | TGCTGTTCCGCTGGGCATGCAAGAAACATCCTA TAGAAGC | Amplification of the *all3613* promoter with overlaps to p70a |
| P3613_rev | TAAACAAAATTATTGCTAGCAAAAATAAAATACCA TACAAAACAC | |
| P3614_fwd | TGCTGTTCCGCTGGGCATGCAAAAATAAAATAC CATACAAAACAC | Amplification of the *alr3614* promoter with overlaps to p70a |
| P3614_rev | TAAACAAAATTATTGCTAGCAAGAAACATCCTAT AGAAGC | |
| P43_fwd | TGCTTTGTATAACATTATGTGTTTTGTATGGTATT TTATTTTTGCTAGCAATAATTTTGTTTAACTTTAA GAAGGAGATATACC | Amplification of p70a with deGFP under control of a shorter version of the *all3613* promoter |
| P43_rev | AAAAATAAAATACCATACAAAACACATAATGTTAT ACAAAGCAGCATGCCCAGCGGAACAG | |
| Ptracr_fwd | TGCTGTTCCGCTGGGCATGCCGCAGGATAAAGC AAAAG | Amplification of p70a with deGFP under control of the tracrRNA promoter |
| Ptracr_rev | TAAACAAAATTATTGCTAGCTATTACATATTATAT TTTCAAGGTTCAG | |
| PpsbAI_TXTL_fwd | TGCTGTTCCGCTGGGCATGCAAGGATTCCCAAA GATAGG | Amplification of p70a with deGFP under control of the *psbAI* promoter |
| PpsbAI_TXTL_rev | TAAACAAAATTATTGCTAGCCTCATAAAATTTACA TGA | |
| P3630_TXTL_fwd | TGCTGTTCCGCTGGGCATGCGTCGCTTCAGTAA TTACAAAAAAG | Amplification of p70a with deGFP under control of the *all3630* promoter |
| P3630_TXTL_rev | TAAACAAAATTATTGCTAGCAATTTATAAATACAT AATGTTATTCTAAACG | |
| Alr3614S protein heterologous expression | | |
| 3614S-BamHI-F | CGGGATCCATGTTATACAAAGCACTAATGTG | Producing 3614S-pET-28a(+)-smt3 |
| 3614S-taa-XhoI-R | CCGCTCGAGTTAGCTACTAAAGCTTTTAAGATTC TC | |
| Promoter fragments for TXTL assay | | |

| | | |
|---|---|---|
| $P_{cas12k}$ | AAGAAACATCCTATAGAAGCATCTTCTAAAACACA TTAGTGCTTTGTATAACATTATGTGTTTTGTATGGT ATTTTATTTTT | TXTL |
| $P_{tracr}$ | CGCAGGATAAAGCAAAAGAATTAGCCCTCTATGC TTATAGTCTCCGCCTAGCTAGGCGAAGTTGACAA ACATCTGAACCTTGAAAATATAATATGTAATA | TXTL |
| $P_{cvkR}$ | AAAAATAAAATACCATACAAAACACATAATGTTATA CAAAGCACTAATGTGTTTTAGAAGATGCTTCTATA GGATGTTTCTT | TXTL |
| P43 | TGCTTTGTATAACATTATGTGTTTTGTATGGTATTT TATTTTT | TXTL |
| $P_{psbAI}$ | CAAGGATTCCCAAAGATAGGGGGAATAATTAACA TTAAGAATTATTAATTCATGGGTTTTTAGTCTAGTA AATTTGCGTGAATTCATGTAAATTTTATGAG | TXTL |
| $P_{tnsB}$ | GTCGCTTCAGTAATTACAAAAAAGGTTTTGTATAT TTTCATAATGACAAATTGACTGTCGTTTTCTCCAC GTTTAGAATAACATTATGTATTTATAAATT | TXTL |
| DNase I footprinting | | |
| P3613-F(FAM) | AACTCCTTTCTCTCGCCAATAC | Producing FAM-labeled probes |
| P3613-R | GCTGCTGAAGCAGTTCGTT | |
| M13F(FAM) | CCCAGTCACGACGTTGTAAAACG | Producing FAM-labeled probes |
| M13R | AGCGGATAACAATTTCACACAGG | |
| CvkR protein mutant construction | | |
| CvkRMut-F | GGTGGTCATATGGGATCCATGGAAGGAAAGTTCT ACAC | CvkRmut mutant |
| CvkRMut-R | CGTTTAGAGGCCCCAAGGGGTTATGCTAGTTATT GCTCAG | |
| pET28a-sumo-F | GAGCAATAACTAGCATAACCCCTTGGGGCCTCTA AACGGG | |
| pET28a-sumo-R | GTAGAACTTTCCTTCCATGGATCCCATATGACCA CCAA | |
| R42E-F | TTAACAGTAGTGGCAAAGGTGAAAATGTTTACT | R42E mutant |
| R42E-R | AGTAAACATTTTCACCTTTGCCACTACTGTTAA | |
| S134C-F | AAACTGTGATTCCTTTTTGGTGCGATCGCATTCA TCAACAA | S134C mutant |
| S134C-R | GCACCAAAAAGGAATCACAGTTTGTCCATGAC | |

**Supplementary Table 4. Data collection and refinement statistics.**

| | SeMet CvkR | Native CvkR |
|---|---|---|
| **Data collection** | | |
| Space group | C121 | C121 |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 78.813, 49.778, 41.017 | 78.579, 49.703, 40.52 |
| α, β, γ (°) | 90, 116.38, 90 | 90, 116.044, 90 |
| Resolution (Å) | 24.90-1.93 (2.03-1.93) | 50.00-1.60 (1.66-1.60) |
| $R_{sym}$ or $R_{merge}$ | 0.054(0.25) | 0.054(0.441) |
| $I / \sigma I$ | 14.6 (3.8) | 32.1 (2.93) |
| Completeness (%) | 94 (84.4) | 99.8 (99.2) |
| Redundancy | 3.2 (3.1) | 2.5 (2.6) |
| | | |
| **Refinement** | | |
| Resolution (Å) | | 1.6 |
| No. reflections | | 18599 |
| $R_{work}$ / $R_{free}$ | | 0.194/0.217 |
| No. atoms | | |
| Protein | | 1199 |
| Ligand/ion | | 38 |
| Water | | 92 |
| *B*-factors | | |
| Protein | | 45.39 |
| Ligand/ion | | 45.50 |
| Water | | 51.62 |
| R.m.s. deviations | | |
| Bond lengths (Å) | | 0.01 |
| Bond angles (°) | | 1.29 |

Values in parentheses are for highest-resolution shell.

**Supplementary References**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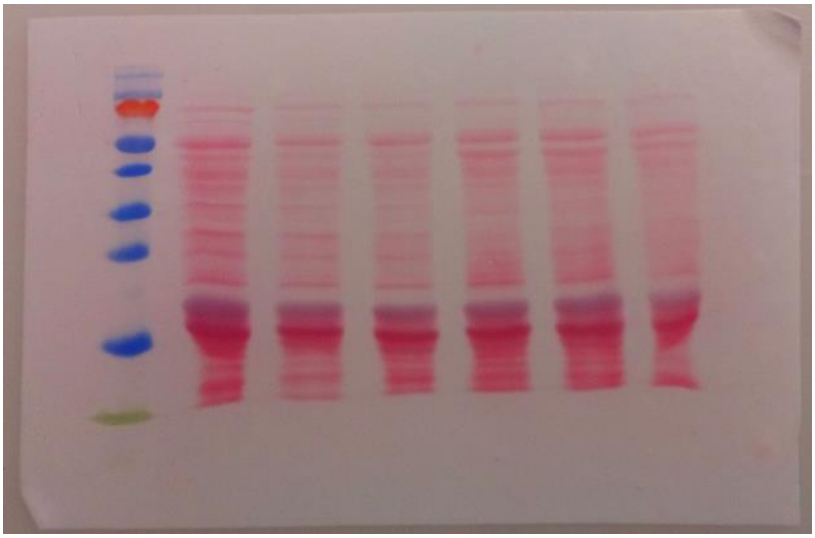
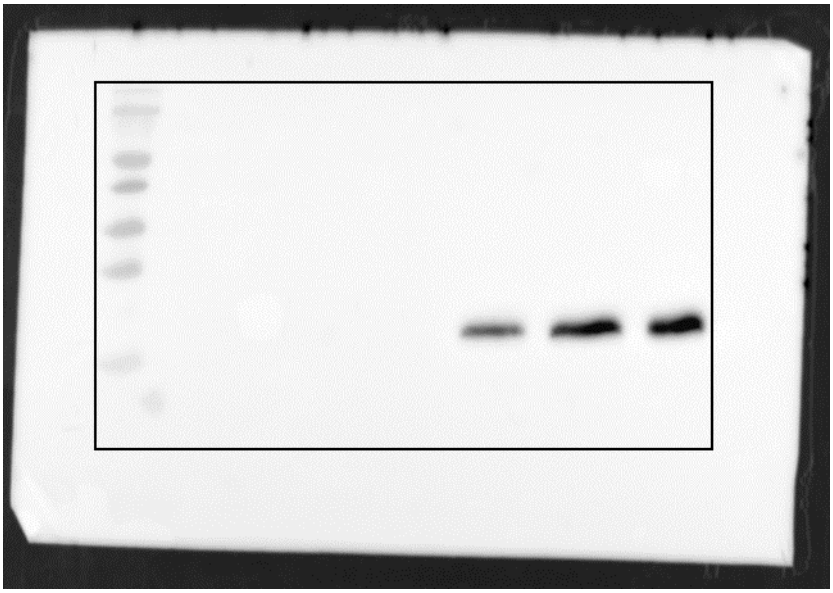
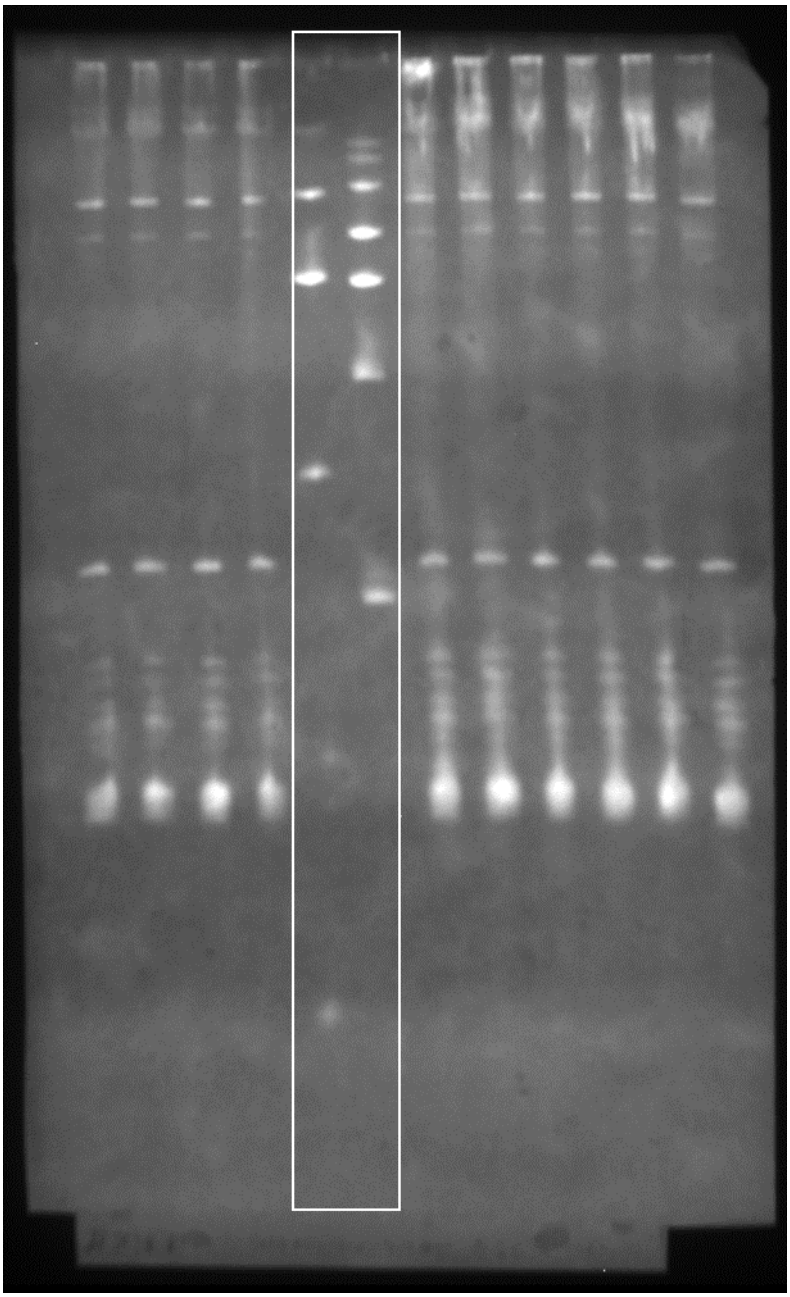
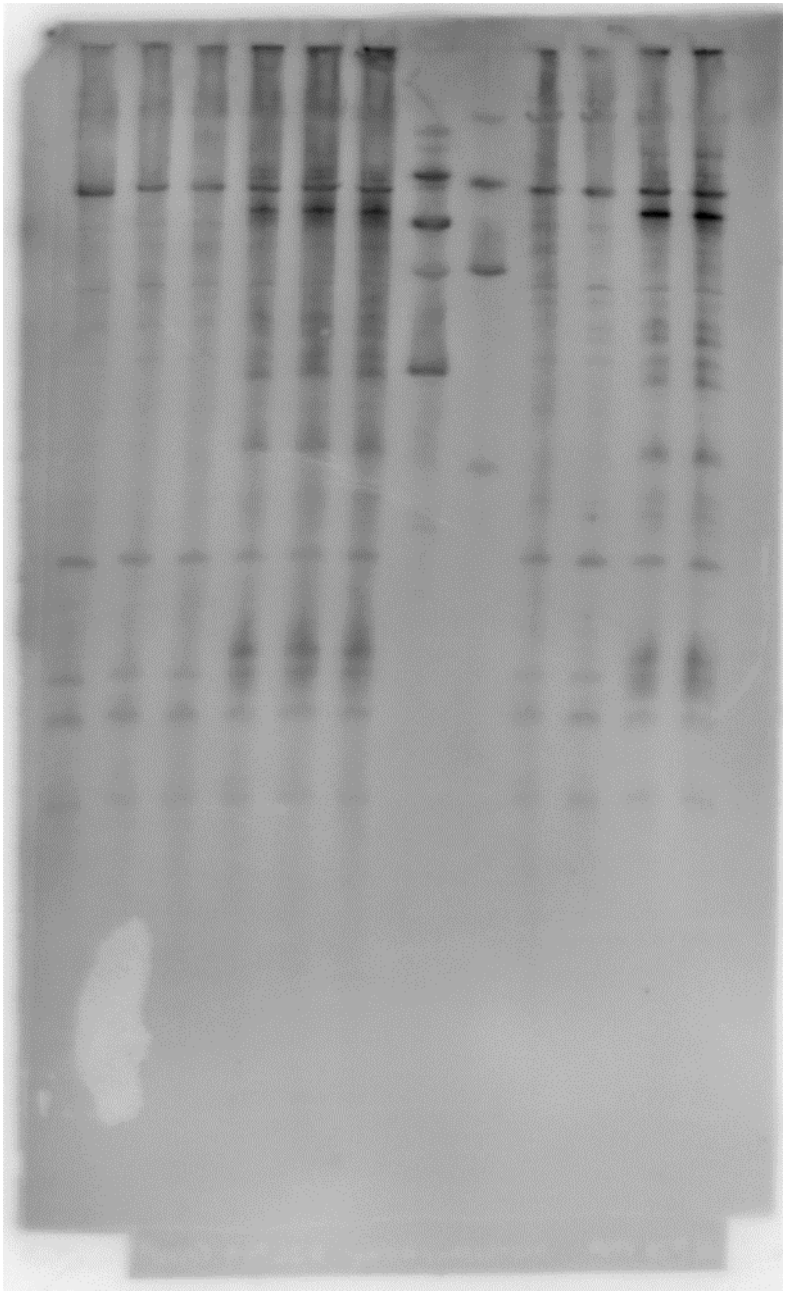
**Uncropped scans of blots and gels in Supplementary Figures**

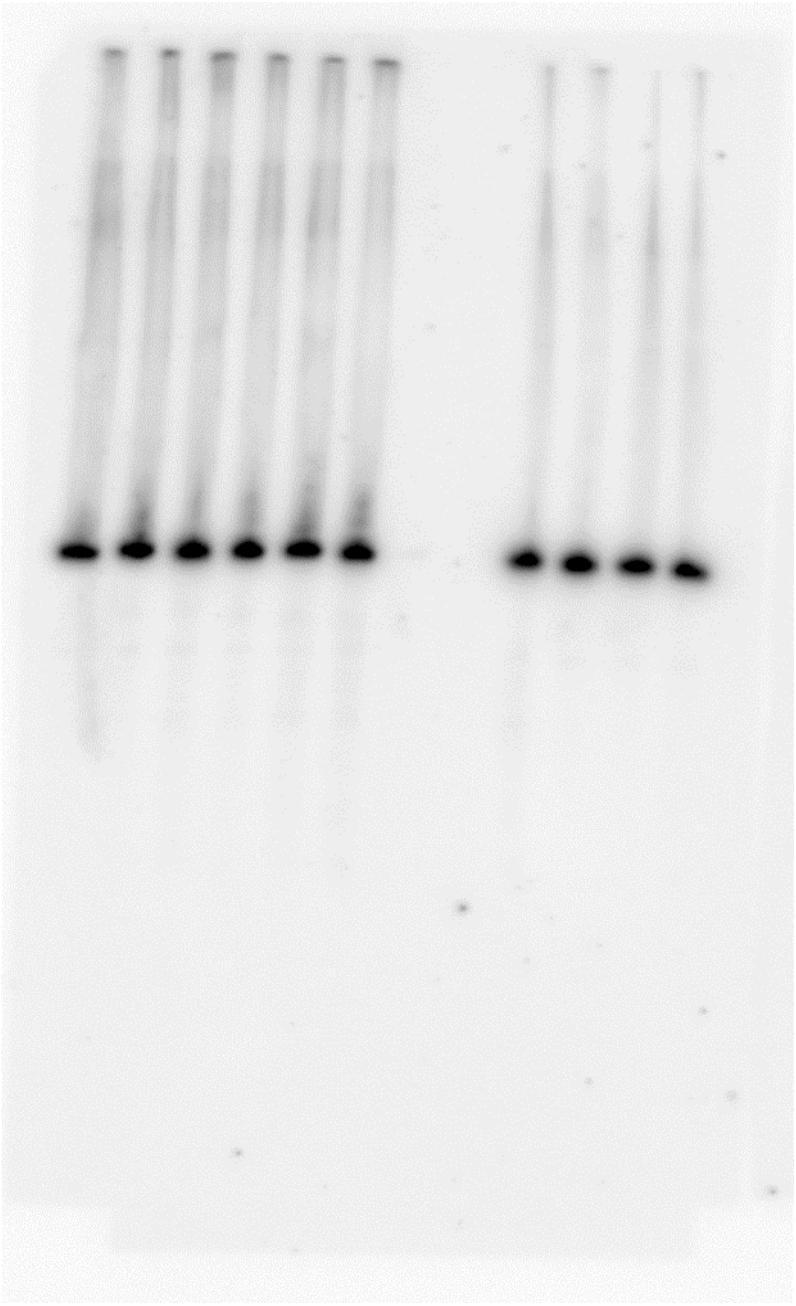
Supplementary Figure 5.



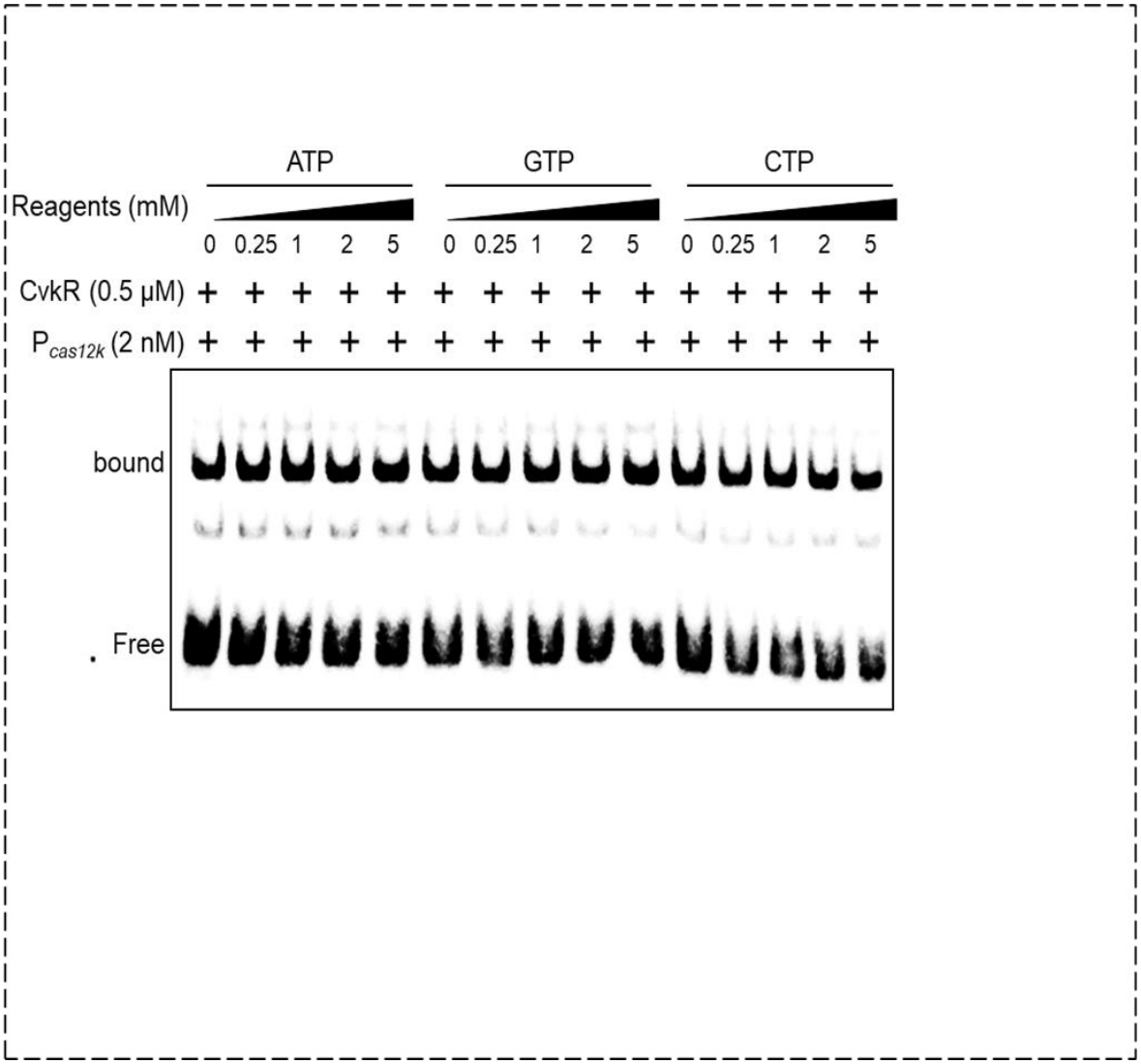


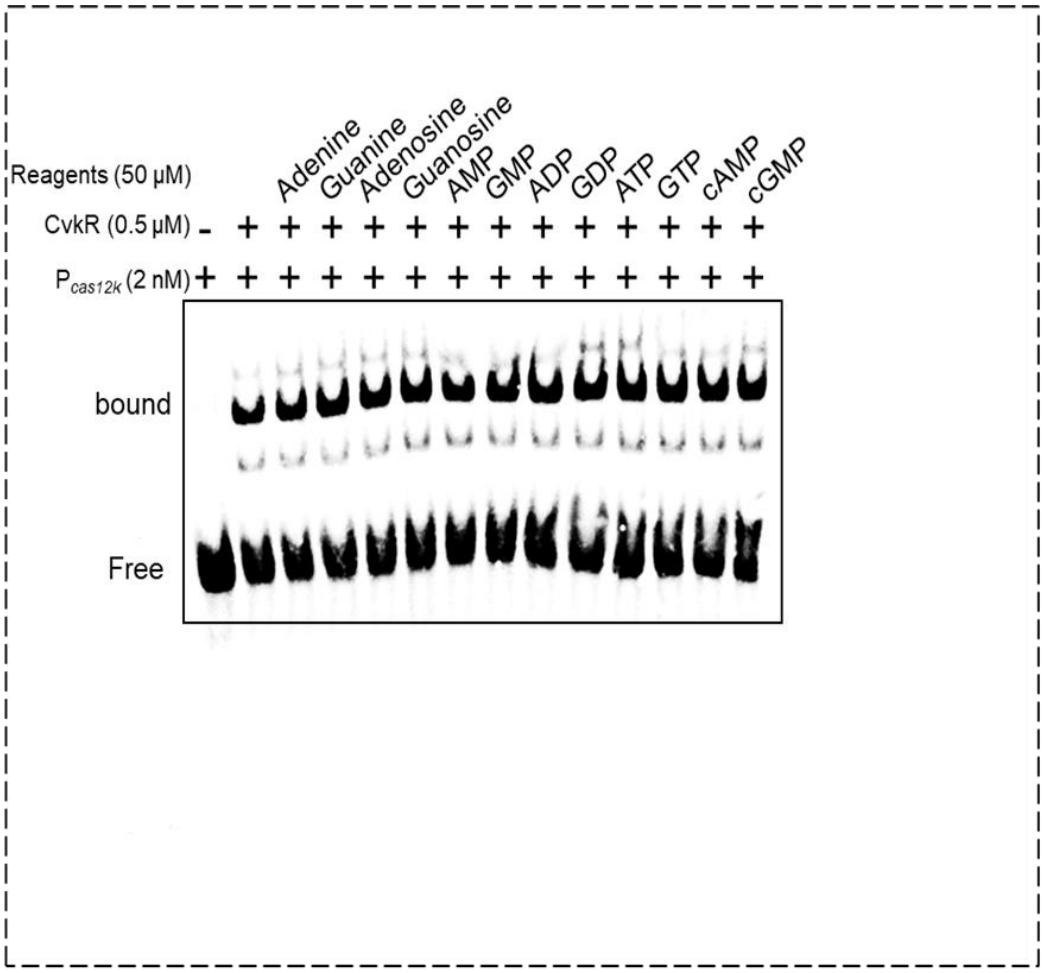


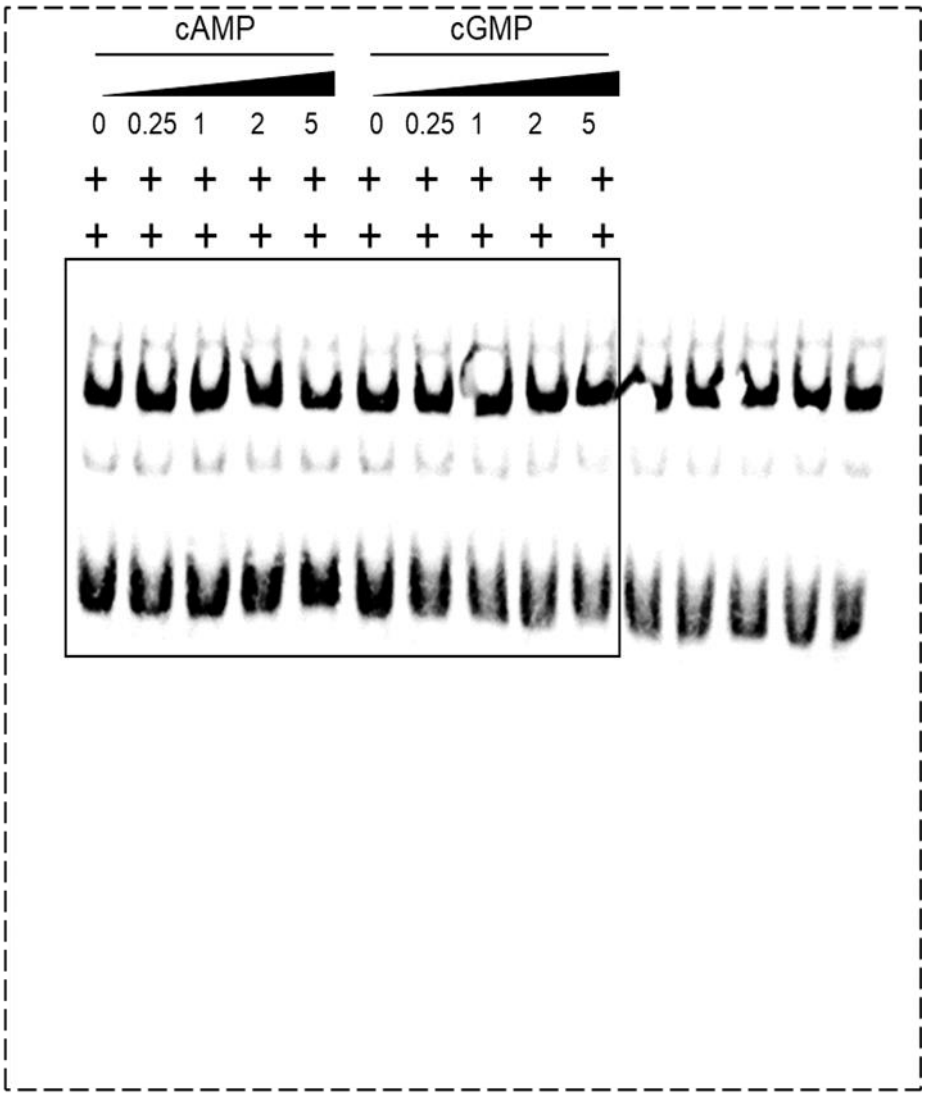




Supplementary Figure 10.




Reagents (50 μM)
Adenine
Guanine
Adenosine
Guanosine
AMP
GMP
ADP
GDP
ATP
GTP
cAMP
cGMP
CvkR (0.5 μM) - + + + + + + + + + + + + +
$P_{cas12k}$ (2 nM) + + + + + + + + + + + + + +
bound
Free


cAMP
cGMP
0 0.25 1 2 5 0 0.25 1 2 5
+ + + + + + + + + +
+ + + + + + + + + +

Supplementary Figure 12.