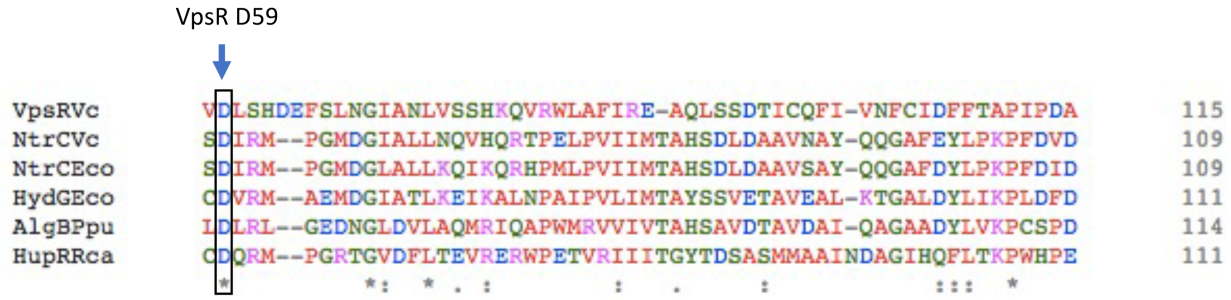

Figure S1. CLUSTAL Omega alignment of the N-terminal domain of VpsR with other Enhancer Binding Proteins. VpsR (VpsRVc) is aligned with the EBPs: nitrogen regulatory protein C (NtrC) from *V. cholerae* (NtrCVc), NtrC from *E. coli* (NtrCEco), the DNA-binding transcriptional activator HydG from *E. coli* (HydGEco), alginate biosynthesis transcriptional regulatory protein AlgB from *Pseudomonas putida* (AlgBPpu), and the RR HupR from *Rhodobacter capsulatus* (HupRRca). Asterisks indicate conserved, identical signature residues; double dots indicate conserved, highly similar residues; single dots indicate conservative replacements. The residue possibly involved in phosphorylation (D59) is boxed in black.

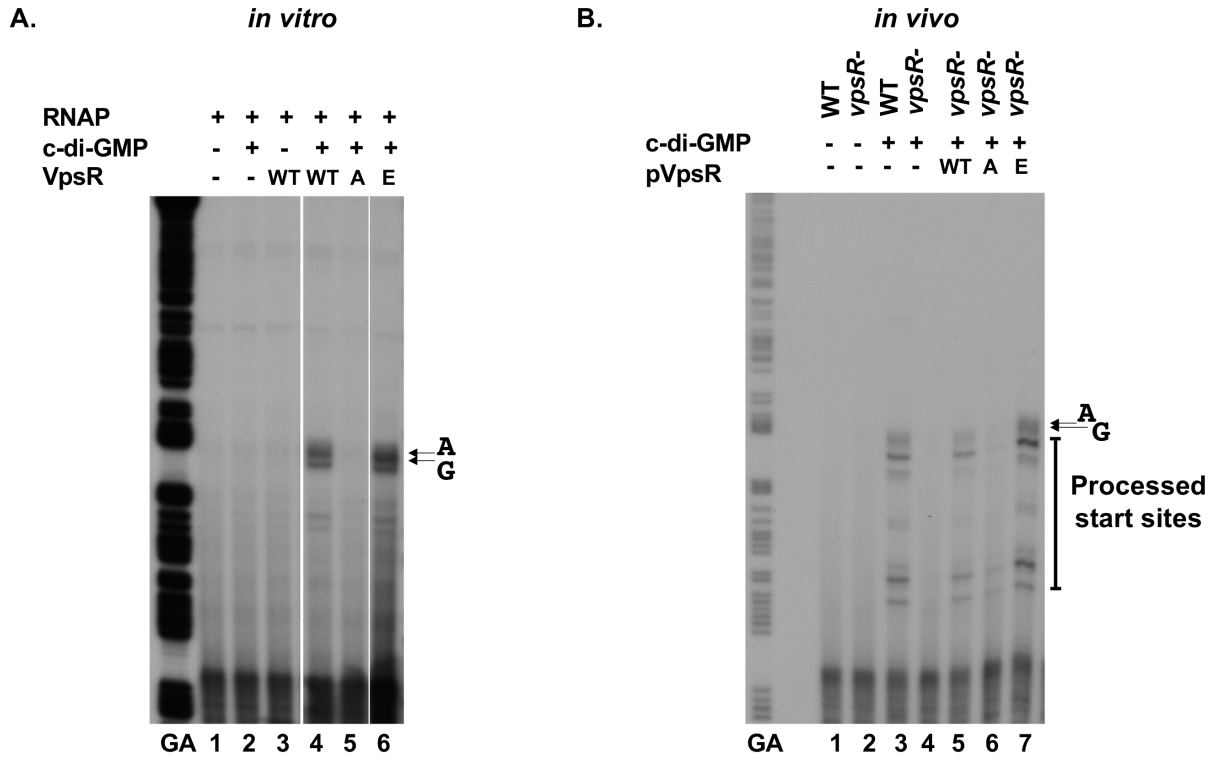

Figure S2. ***In vitro*** **and** ***in vivo*** **primer extensions at P_{vpsL} using RNA isolated from (A)** ***in vitro*** **transcriptions or (B)** ***V. cholerae*** **in the presence of WT VpsR, VpsR D59E, and VpsR D59A.** Two major primer extension products, at an A nucleotide and a G nucleotide, which are significantly increased in the presence of both c-di-GMP and either WT VpsR or the phospho-mimic D59E, are indicated with black arrows.

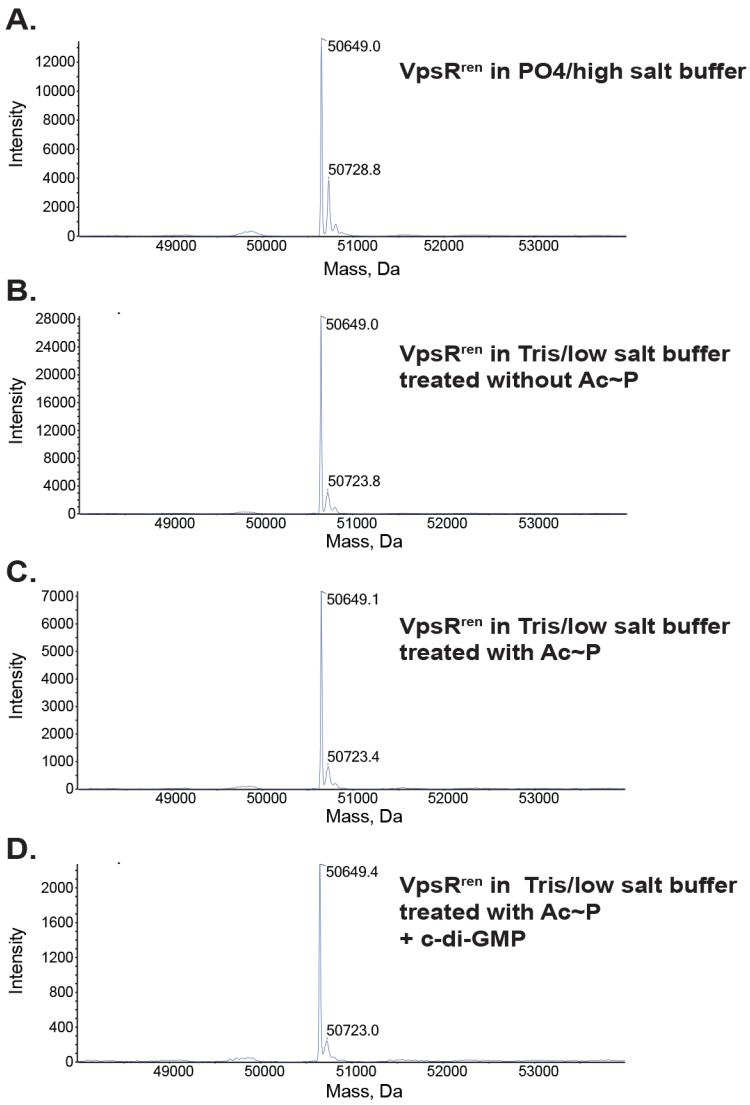

Figure S3: Mass spectrometry analysis of VpsR^{ren} indicates that it is not acetylated upon incubation with Ac~P. Reconstructed mass spectra of VpsR^{ren} following incubation in phosphate/high salt buffer (A); Tris/low salt buffer without Ac~P (B); Tris/low salt buffer with Ac~P (C); and Tris/low salt buffer with Ac~P and c-di-GMP (D). In all conditions, the expected mass was observed and was similar (See text for details.) The low intensity masses observed at 50,728 (A) and 50,723 (B-D) likely represent electrospray adducts.

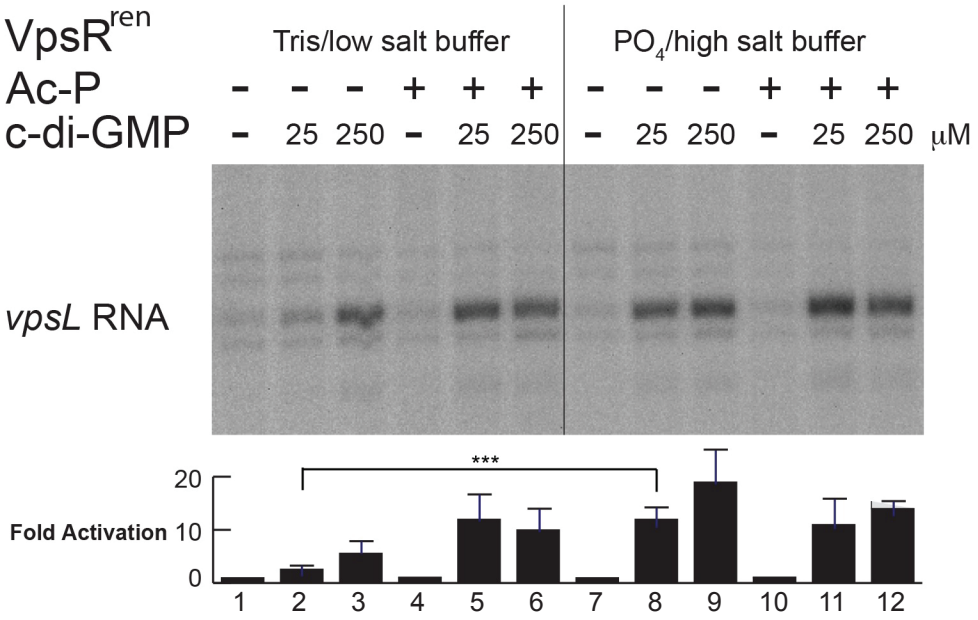

Figure S4. VpsR^{ren} stored in the Pi/high salt buffer is active without pretreatment with Ac~P. Gel (top) shows the products of *in vitro* transcription reactions obtained using plasmid template P_{vpsL}, and VpsR^{ren} in the indicated storage buffer pretreated with or without 20 mM Ac~P and the indicated concentration of c-di-GMP. For the histogram below, values represent averages relative to lane 1 for lanes 2-6 and relative to lane 7 for lanes 8-12, +/- standard deviation calculated from 3-5 experiments. Values are as follows. Lane 2: 2.43, 2.20, 3.70, 1.80, 2.70; lane 3: 9.82, 3.94, 6.14, 3.60, 4.50; lane 4: 1.04, .900, 1.18, 1.10, .920; lane 5: 10.1, 14.6, 20.1, 7.40, 9.00; lane 6: 8.65, 11.1, 17.1, 8.30, 5.50; lane 8: 9.21, 11.9, 14.7; lane 9: 12.1, 18.4, 27.0; lane 10: .87, 1.1, 1.1; lane11: 17.9, 10.1, 6.00; lane 12: 13.1, 15.6, 12.3. Horizontal line with *** indicates $P < 0.001$ from a one-tailed t-test for 2 independent means.

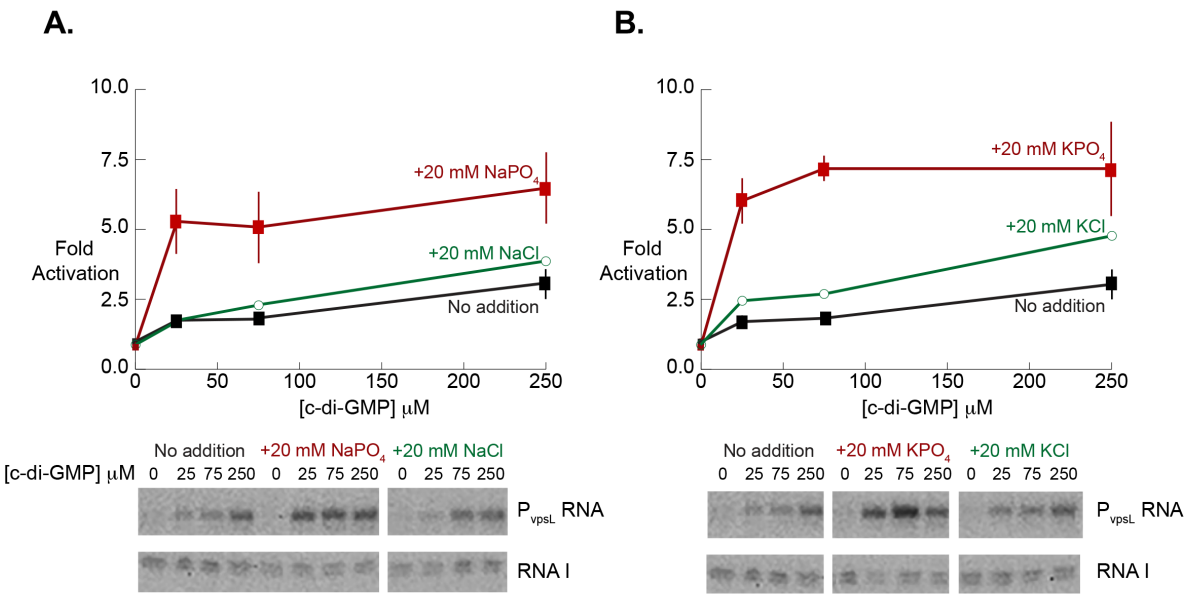

Figure S5. The presence of Pi rather than Na^+ or K^+ increases VpsR transcriptional activity. Top graphs (A, $NaPO_4$ vs. NaCl; B, KPO_4 vs. KCl) show the fold activation for transcription activation from P_{vpsL} vs. the c-di-GMP concentration present using $VpsR^{ren}$ in the Tris/low salt storage buffer. Values are averages from 4 experiments (no addition with 25 or 250 μM c-di-GMP, addition of 20 mM $NaPO_4$, pH 7.8 or 20 mM KPO_4, pH 7.8 with 0, 25, 75, or 250 μM c-di-GMP; bars indicate standard deviation] or 2 experiments (other values). Lines were drawn through the averages of the points. Underneath are sections of representative gels indicating the transcription products obtained using the plasmid template P_{vpsL} and $VpsR^{ren}$ in the Tris/low salt storage buffer pretreated in the presence or absence of 20 mM sodium phosphate or sodium chloride (A) or 20 mM potassium phosphate or potassium chloride (B). The products of P_{vspL} and the control promoter for RNAI present on the plasmid are indicated.