SUPPLEMENTAL INFORMATION

Engineering an osmosensor by pivotal histidine positioning within disordered helices

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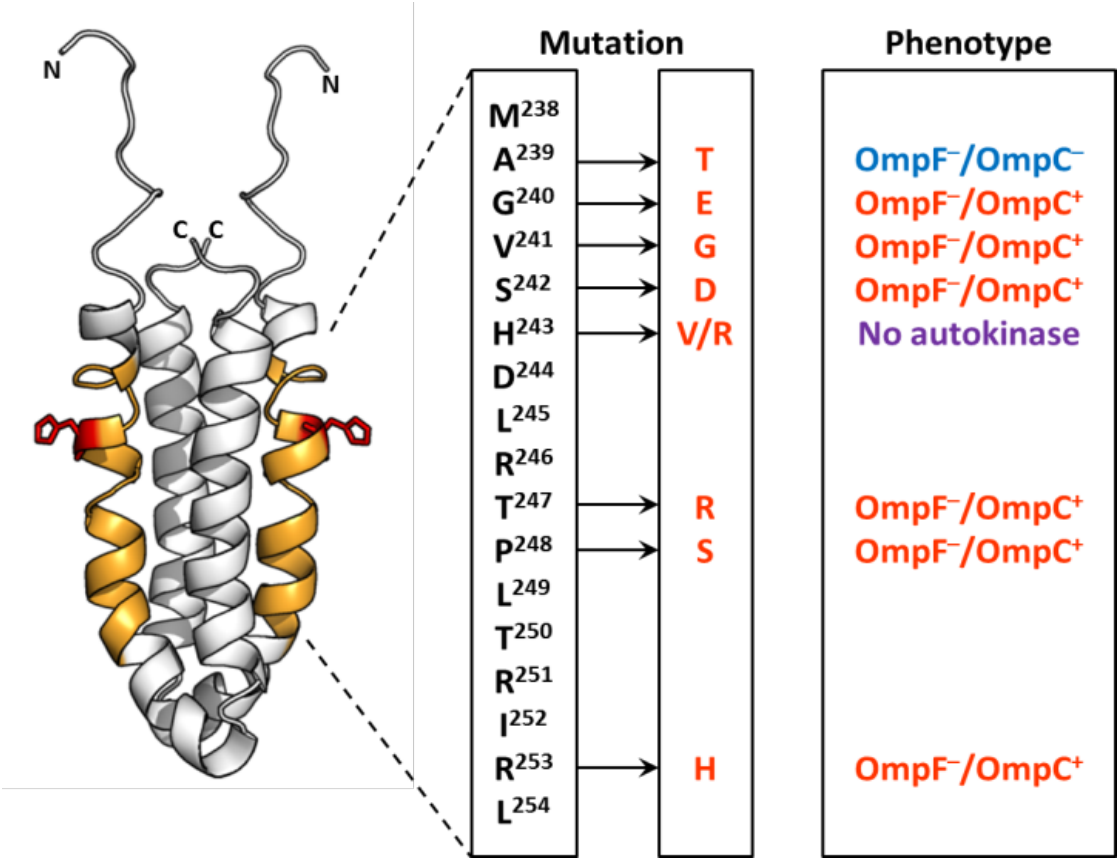


Figure S1: List of mutations and their phenotypes at the phosphorylation locus of EnvZ. (Related to Fig. 1)

Substitution of residues flanking His^{243} causes impairment in outer membrane porin (Omp) protein expression independent of the osmolality of the solution ~~(for review, see)~~. All amino acid substitutions listed resulted in constitutive OmpC expression ($OmpF^{-}/OmpC^{+}$) except for A239T, which showed negative expression of either porins ($OmpF^{-}/OmpC^{-}$) profile relative to the wild-type.

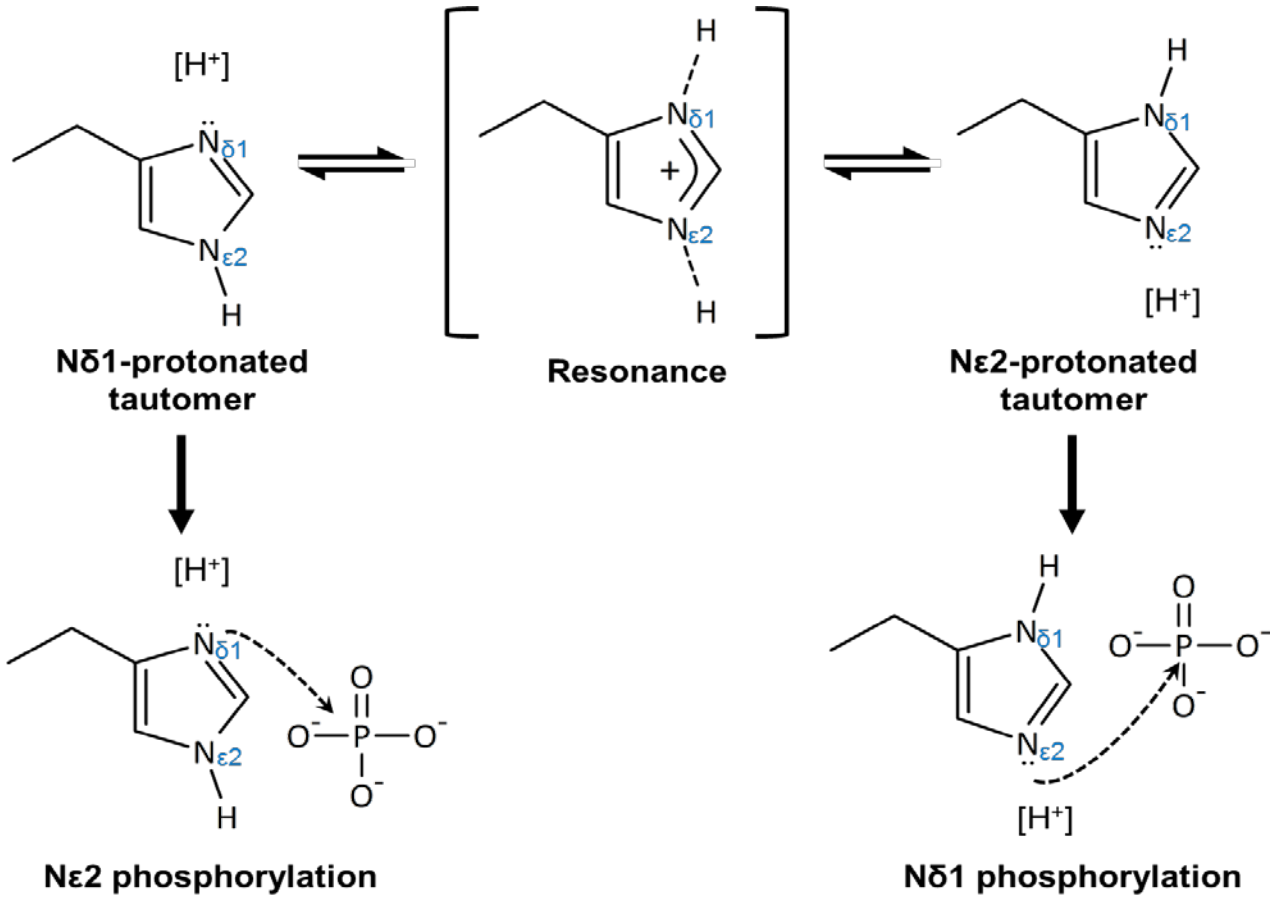


Figure S2: Histidine tautomerisation and phosphorylation. (Related to Figures 1, 6, 7)

Imidazole side chain can assume alternate tautomers (Nδ1-protonated or Nε2-protonated). Phosphorylation occurs on the nitrogen atom that is not covalently bound to a hydrogen , where the lone pair acts as a nucleophile that attacks the high-energy phosphate presented by a high energy phosphodonor such as ATP.

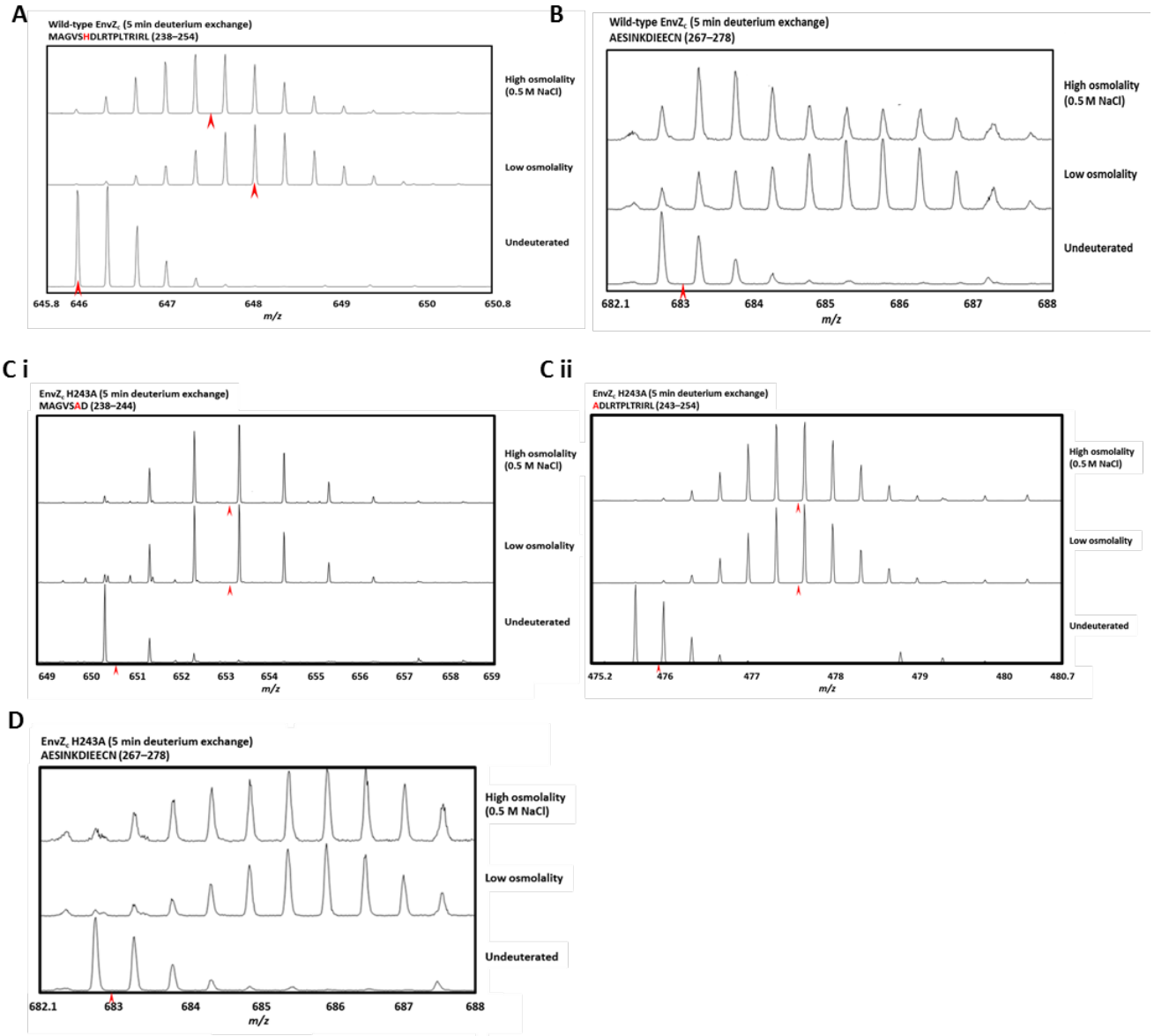


Figure S3: Amide hydrogen/deuterium exchange MS profiles of the four-helix bundle subdomain in wild-type (WT) EnvZc and EnvZc H243A. (Related to Figure 5)

(**A**) ESI-Q-TOF mass spectra for the peptide spanning residues 238–254 and containing the phosphorylatable His^{243} (highlighted in red) in WT EnvZc following 5 min of deuterium exchange at high (15.5 mM Tris-HCl, pH 7.6, 0.5 M NaCl) and low osmolality (15.5 mM Tris-HCl, pH 7.6). Red arrowheads indicate the centroid of each mass envelope. Subtraction of the centroid of the undeuterated control from the centroid of the deuterated spectra yields the average number of deuterons exchanged under each condition. The lower centroid observed when EnvZc was exposed to high osmolality reflects decreased deuterium exchange.

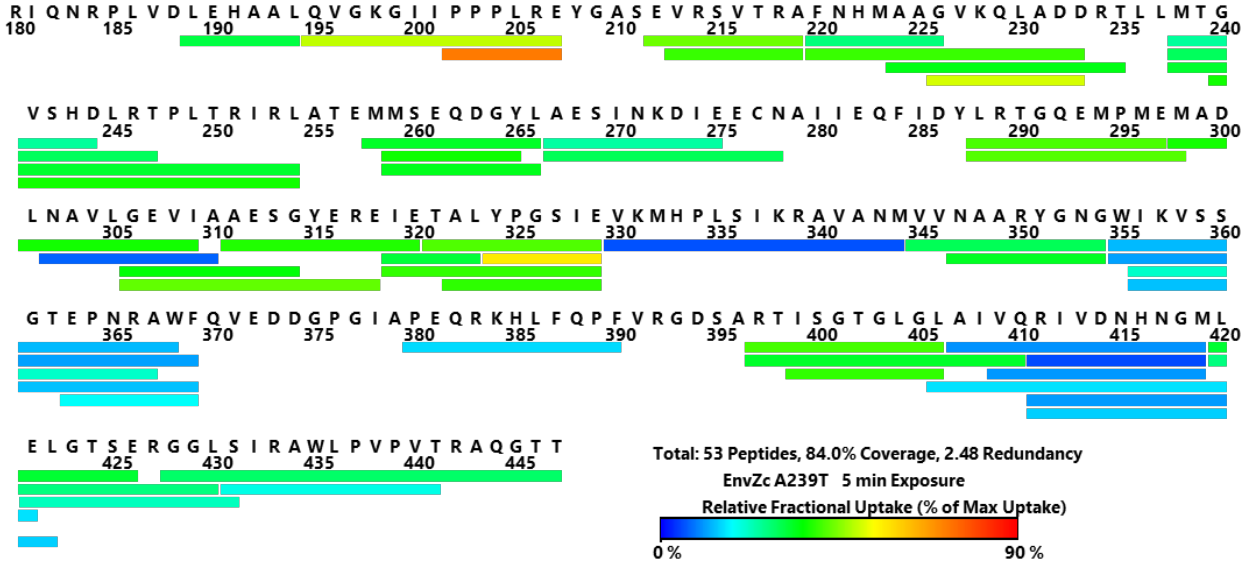
(**B**) ESI-Q-TOF mass spectra of the putative OmpR-binding site in EnvZc (residues 267–278) following 5 min deuterium exchange in the absence and presence of osmolytes (0.5 M NaCl). A characteristic bimodal behavior indicative of local unfolding in the region was observed in this peptide. The higher-

exchanging population for WT EnvZc in low osmolality condition was diminished under high osmolality, indicating greater stabilization of the helix when the protein was exposed to high osmolality.

(**C**) ESI-Q-TOF mass spectra for the region spanning residues 238–254 with the phosphorylatable His^{243} substituted to Ala (marked red in sequence) in EnvZc H243A. Details are as described in **A**. Two overlapping peptides are shown (residues 238–244 and residues 243–254) where the shift in the deuteration profile observed previously for WT EnvZc under low and high osmolality (Supplementary Figure 3A) were no longer apparent in both peptides after His^{243} was substituted to Ala in the mutant. This indicated that His^{243} has an important role in integrating osmosensory signal with enzymatic function.

(**D**) ESI-Q-TOF mass spectra of the putative OmpR-binding site in EnvZc H243A (residues 267–278). The bimodal behavior was greatly diminished in the mutant whereby the higher-exchanging population was favored in high osmolality. This indicated that the mutation also affected the OmpR-binding site. All figures were reproduced from a previous study with permission from Wiley and Sons, Inc.

S4 A



S4 B

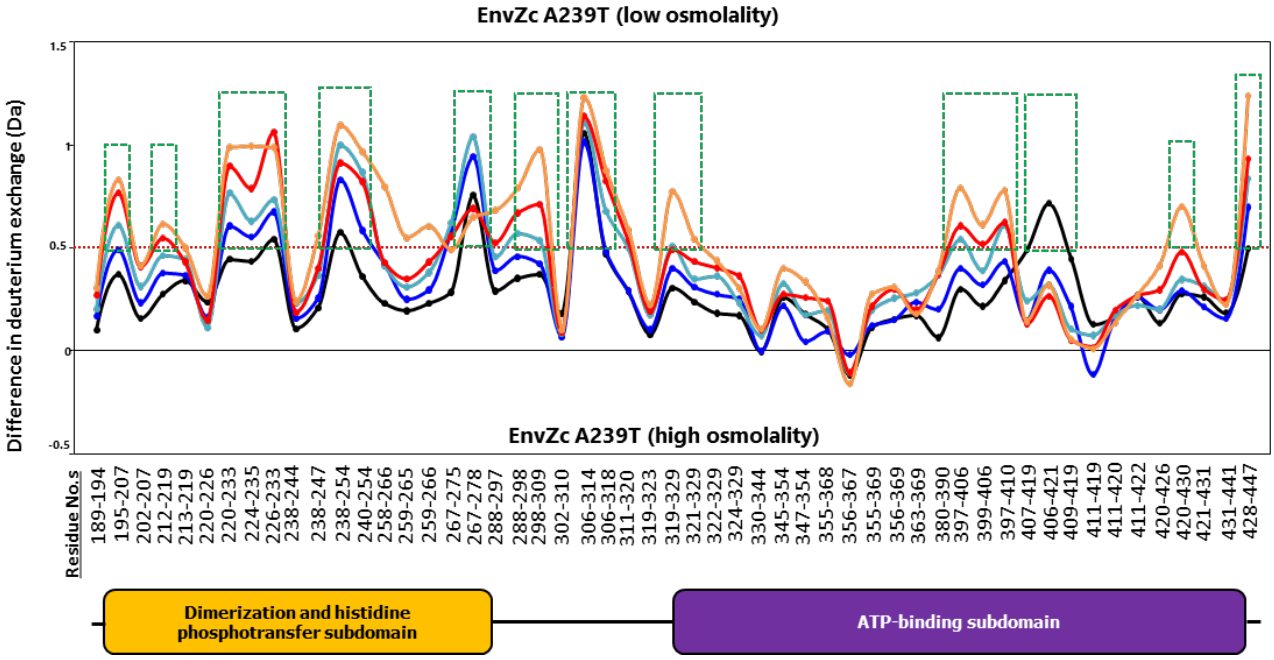
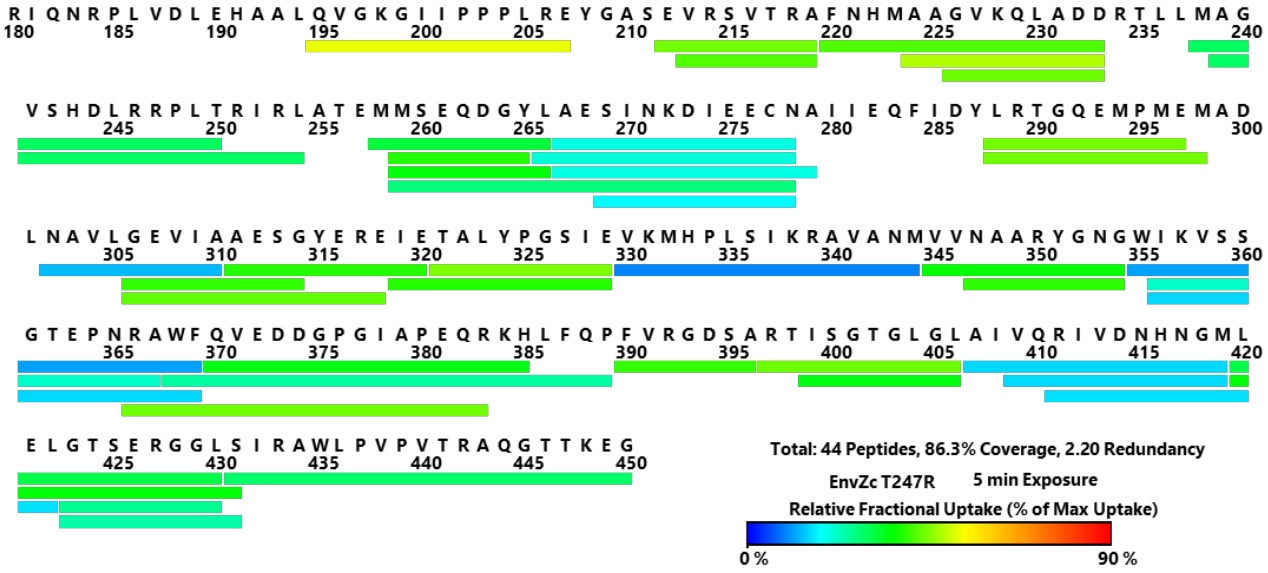


Figure S4: Protein-wide deuterium exchange profile of ~~mutants of~~ EnvZc A239T. (Related to STAR Methods)

~~(i)~~ Sequence coverage deuterium exchange heat maps (A) and difference plots(B) ~~(ii)~~ of the deuterium exchange ~~(A)~~ EnvZc A239T~~; (B) EnvZc T247R; and (C) EnvZc A239T/T247R~~ at low and high osmolality following 2, 5, 10, and 30 min deuterium exchange (represented as solid orange, red, blue, and black lines, respectively) is shown. ~~(D) 5 min deuterium exchange (orange) at low and high osmolality for EnvZc D244A.~~

Each dot on the plot represents a EnvZc pepsin fragment peptide listed from the N to C-terminus, indicated on the *x*-axis. The peptides are arranged from N- to C-terminus and the diagram below the residue numbers indicates the corresponding domain organization of EnvZc. Positive and negative deuterium exchange differences denote decreases or increases in deuterium exchange at high osmolality (*i.e.*, positive value indicates that the protein exchanges more deuterium under low osmolality while negative value indicates that the protein exchanges more deuterium at high osmolality). Values reported are not corrected for deuterium back exchange in the experiment. Changes in deuterium exchange > ±0.5 Da (green dashed line) are considered significant for comparative analysis. Green boxes indicate the regions showing differences in exchange between low and high osmolality.

S5 A



S5 B

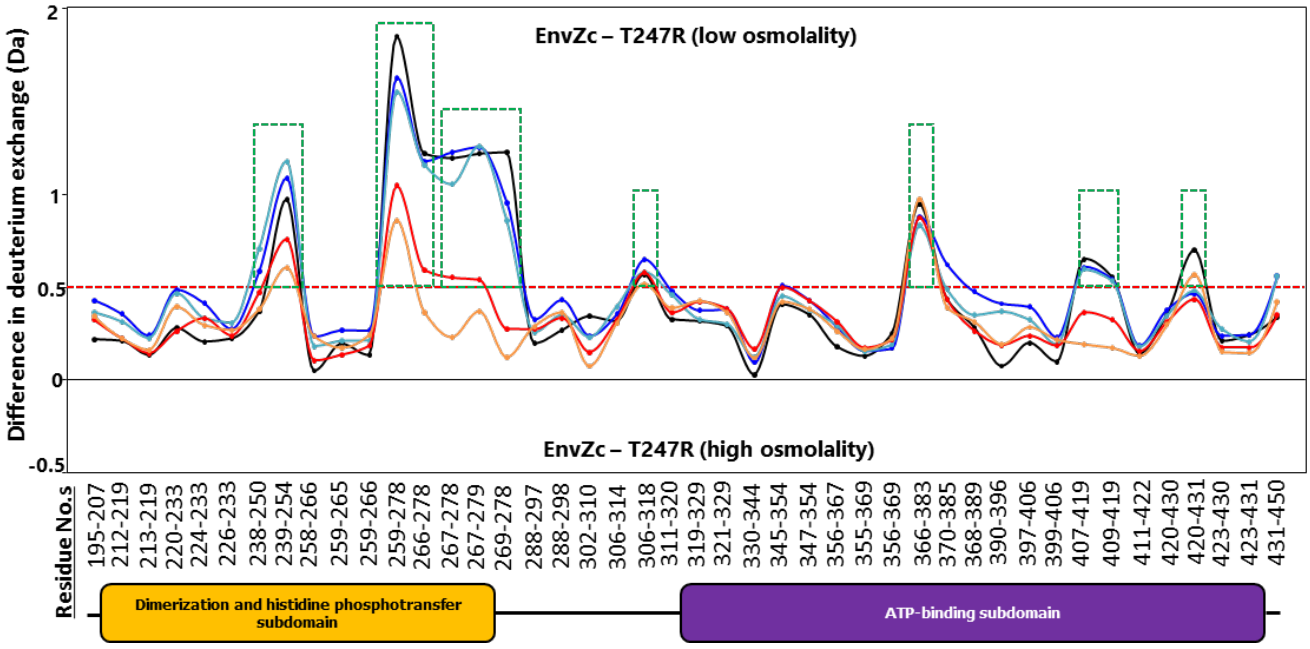
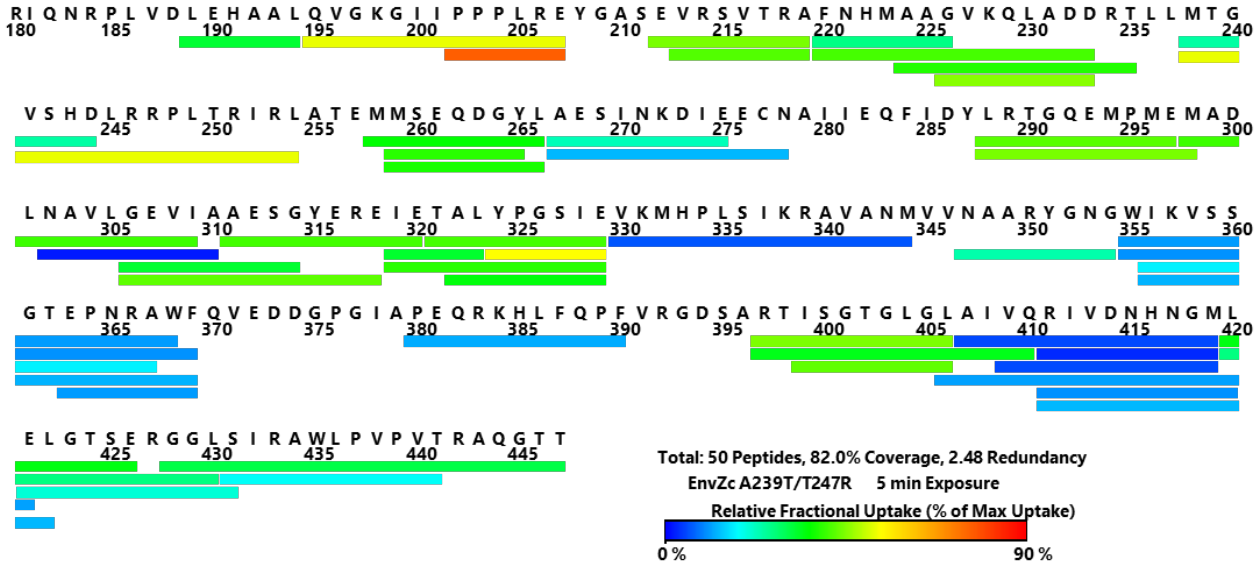


Figure S5: Protein-wide deuterium exchange profile of EnvZc T247R. (Related to STAR Methods) Sequence coverage deuterium exchange heat maps (A) and difference plots (B) of the deuterium exchange EnvZc T247R at low and high osmolality following 2, 5, 10, and 30 min deuterium exchange (represented as solid orange, red, blue, and black lines, respectively) is shown. Each dot on the plot represents a EnvZc pepsin fragment peptide listed from the N to C-terminus, indicated on the *x*-axis. The peptides are arranged from N- to C-terminus and the diagram below the residue numbers indicates the

corresponding domain organization of EnvZc. Positive and negative deuterium exchange differences denote decreases or increases in deuterium exchange at high osmolality (*i.e.*, positive value indicates that the protein exchanges more deuterium under low osmolality while negative value indicates that the protein exchanges more deuterium at high osmolality). Values reported are not corrected for deuterium back exchange in the experiment. Changes in deuterium exchange > ± 0.5 Da (green dashed line) are considered significant for comparative analysis. Green boxes indicate the regions showing differences in exchange between low and high osmolality.

S6 A



S6 B

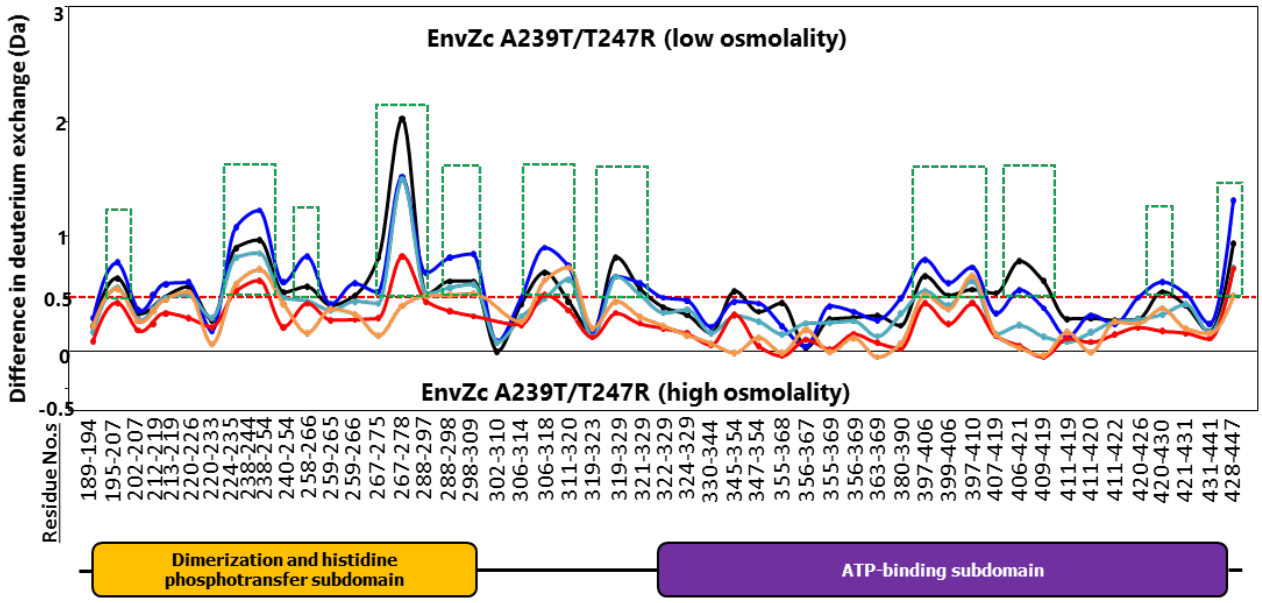
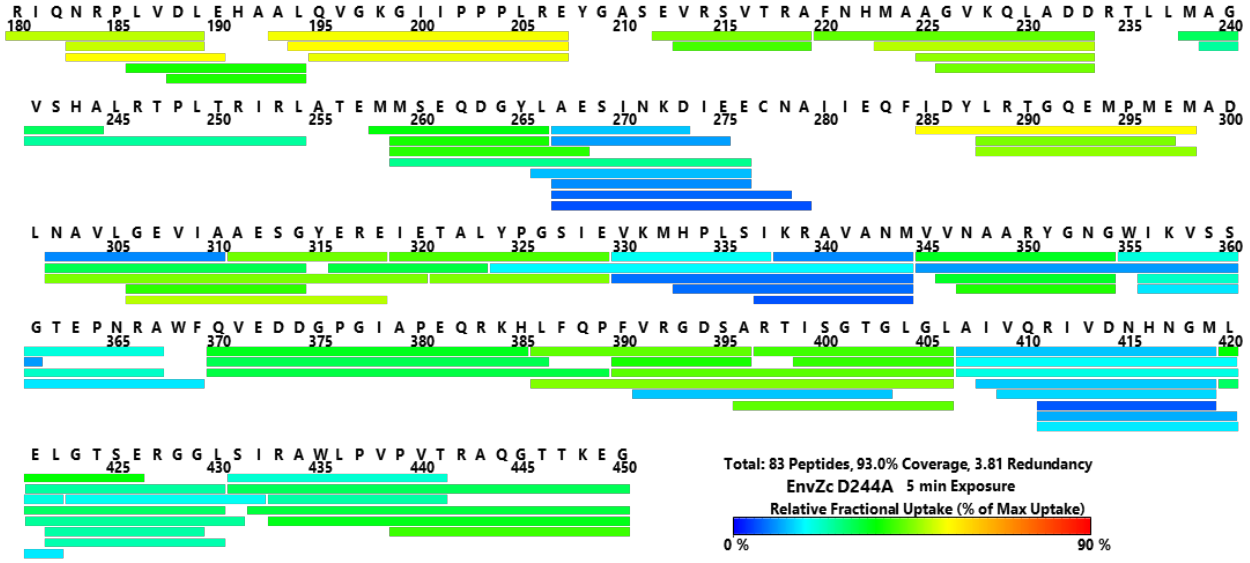


Figure S6: Protein-wide deuterium exchange profile of EnvZc A239T/T247R. (Related to STAR Methods)

Sequence coverage deuterium exchange heat maps (A) and difference plots (B) of the deuterium exchange EnvZc A239T/T247R at low and high osmolality following 2, 5, 10, and 30 min deuterium exchange (represented as solid orange, red, blue, and black lines, respectively) is shown. Each dot on the plot represents a EnvZc pepsin fragment peptide listed from the N to C-terminus, indicated on the *x*-axis. The peptides are arranged from N- to C-terminus and the diagram below the residue numbers indicates the corresponding domain organization of EnvZc. Positive and negative deuterium exchange differences denote decreases or increases in deuterium exchange at high osmolality (*i.e.*, positive value indicates that the protein exchanges more deuterium under low osmolality while negative value indicates that the protein exchanges more deuterium at high osmolality). Values reported are not corrected for deuterium back exchange in the experiment. Changes in deuterium exchange > ±0.5 Da (green dashed line) are considered significant for comparative analysis. Green boxes indicate the regions showing differences in exchange between low and high osmolality.

S7 A



S7 B

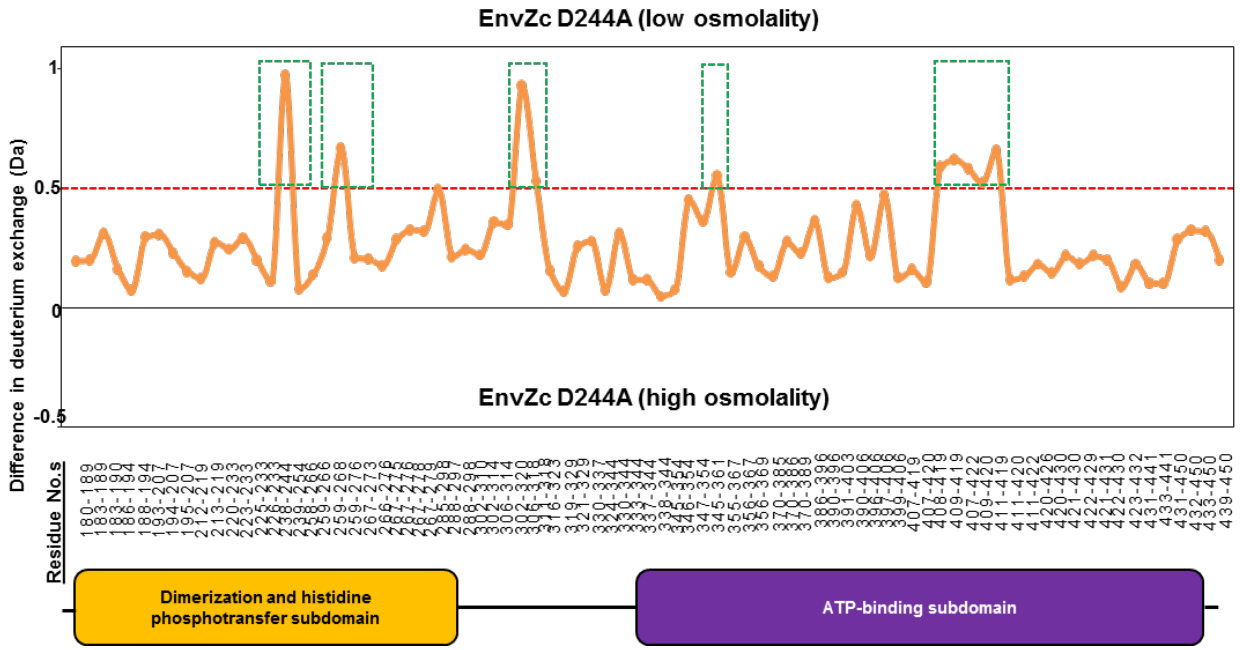


Figure S~~7~~4: Protein-wide deuterium exchange profile of ~~mutants of~~ EnvZc D244A. (Related to STAR Methods)

~~(i)~~ Sequence coverage deuterium exchange heat map (A)~~s~~ and difference plot~~s~~ (B)~~(ii)~~ of the deuterium exchange ~~(A) EnvZc A239T; (B) EnvZc T247R; and (C) EnvZc A239T/T247R at low and high osmolality following 2, 5, 10, and 30 min deuterium exchange (represented as solid orange, red, blue, and black lines, respectively) is shown. (D)~~at 5 min deuterium exchange (orange) at low and high osmolality for EnvZc D244A.

Each dot on the plot represents a EnvZc pepsin fragment peptide listed from the N to C-terminus, indicated on the *x*-axis. The peptides are arranged from N- to C-terminus and the diagram below the residue numbers indicates the corresponding domain organization of EnvZc. Positive and negative deuterium exchange differences denote decreases or increases in deuterium exchange at high osmolality (*i.e.*, positive value indicates that the protein exchanges more deuterium under low osmolality while negative value indicates that the protein exchanges more deuterium at high osmolality). Values reported are not corrected for deuterium back exchange in the experiment. Changes in deuterium exchange > ±0.5 Da (green dashed line) are considered significant for comparative analysis. Green boxes indicate the regions showing differences in exchange between low and high osmolality.

Table S1: **List of common peptides across the four EnvZc mutants in this study**. **(Related to STAR Methods)**

A total of 31 peptides (excluding the His^{243} locus, residues 238-254) were common for each EnvZc mutant, spanning a total sequence coverage of 70%.

S.No.	Start Residue	Sequence
1	195-207	QVGKGIIPPPLRE
2	212-219	EVRSVTRA
3	220-233	FNHMAAGVKQLADD
4	224-233	AAGVKQLADD
5	226-233	GVKQLADD
6	258-266	MMSEQDGYL
7	259-266	MSEQDGYL
8	266-278	LAESINKDIEECN
9	267-278	AESINKDIEECN
10	288-297	LRTGQEMPME
11	288-298	LRTGQEMPMEM
12	302-310	NAVLGEVIA
13	306-314	GEVIAAESG
14	306-318	GEVIAAESGYERE
15	311-320	AESGYEREIE
16	319-329	IETALYPGSIE
17	321-329	TALYPGSIE

18	330-344	VKMHPLSIKRAVANM
19	345-354	VVNAARYGNG
20	347-354	NAARYGNG
21	355-369	WIKVSSGTEPNRAWF
22	356-367	IKVSSGTEPNRA
23	356-369	IKVSSGTEPNRAWF
24	366-383	RAWFQVEDDGPGIAPEQR
25	397-406	RTISGTGLGL
26	399-406	ISGTGLGL
27	407-419	AIVQRIVDNHNGM
28	409-419	VQRIVDNHNGM
29	411-422	RIVDNHNGMLEL
30	420-430	LELGTSERGGL
31	423-431	GTSERGGLS

~~REFERENCES~~