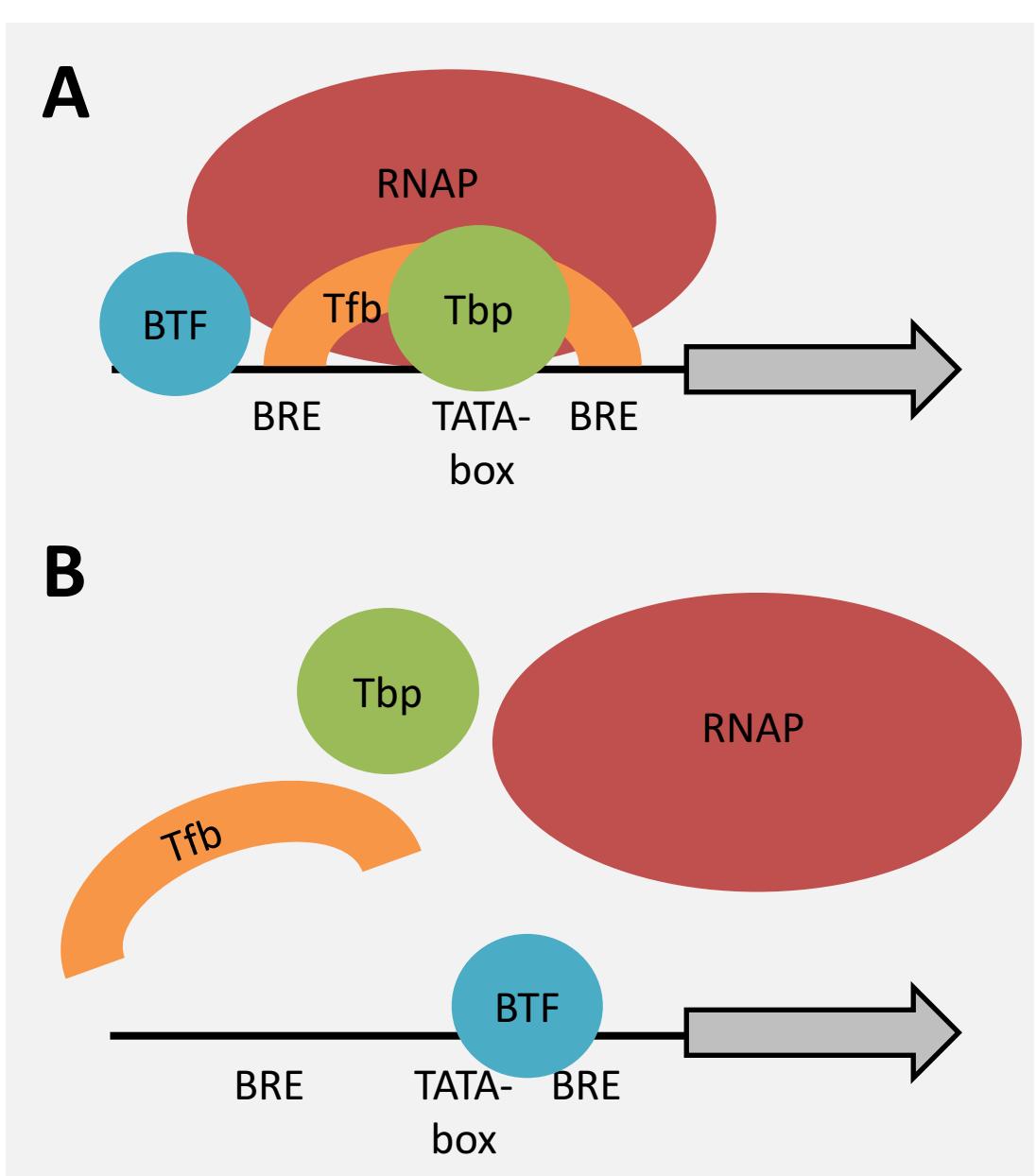
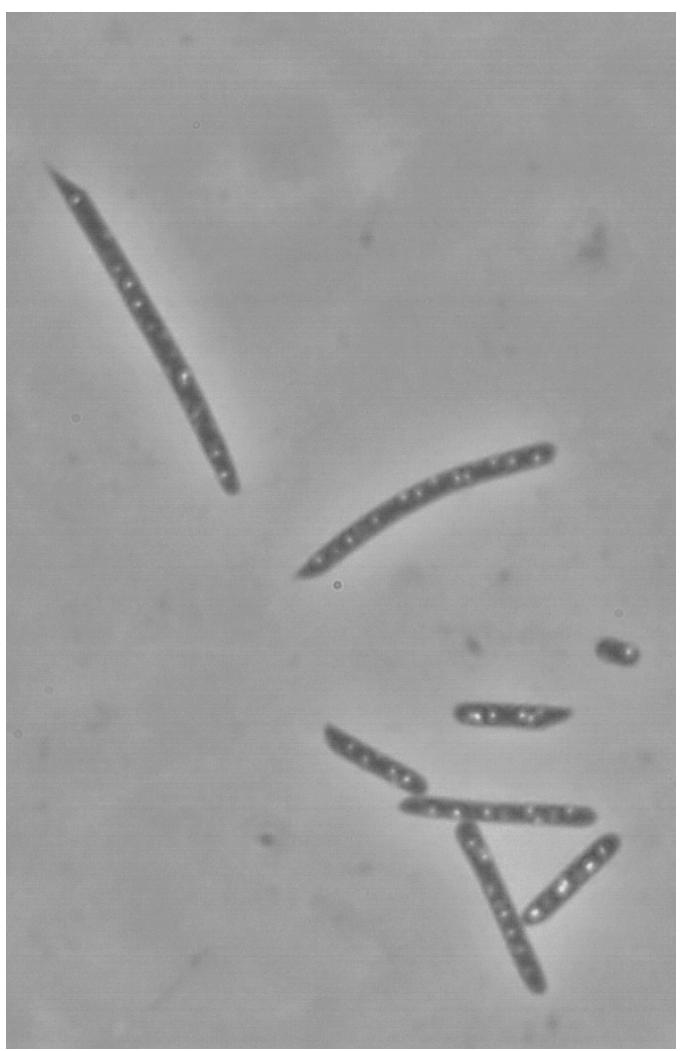




Abstract

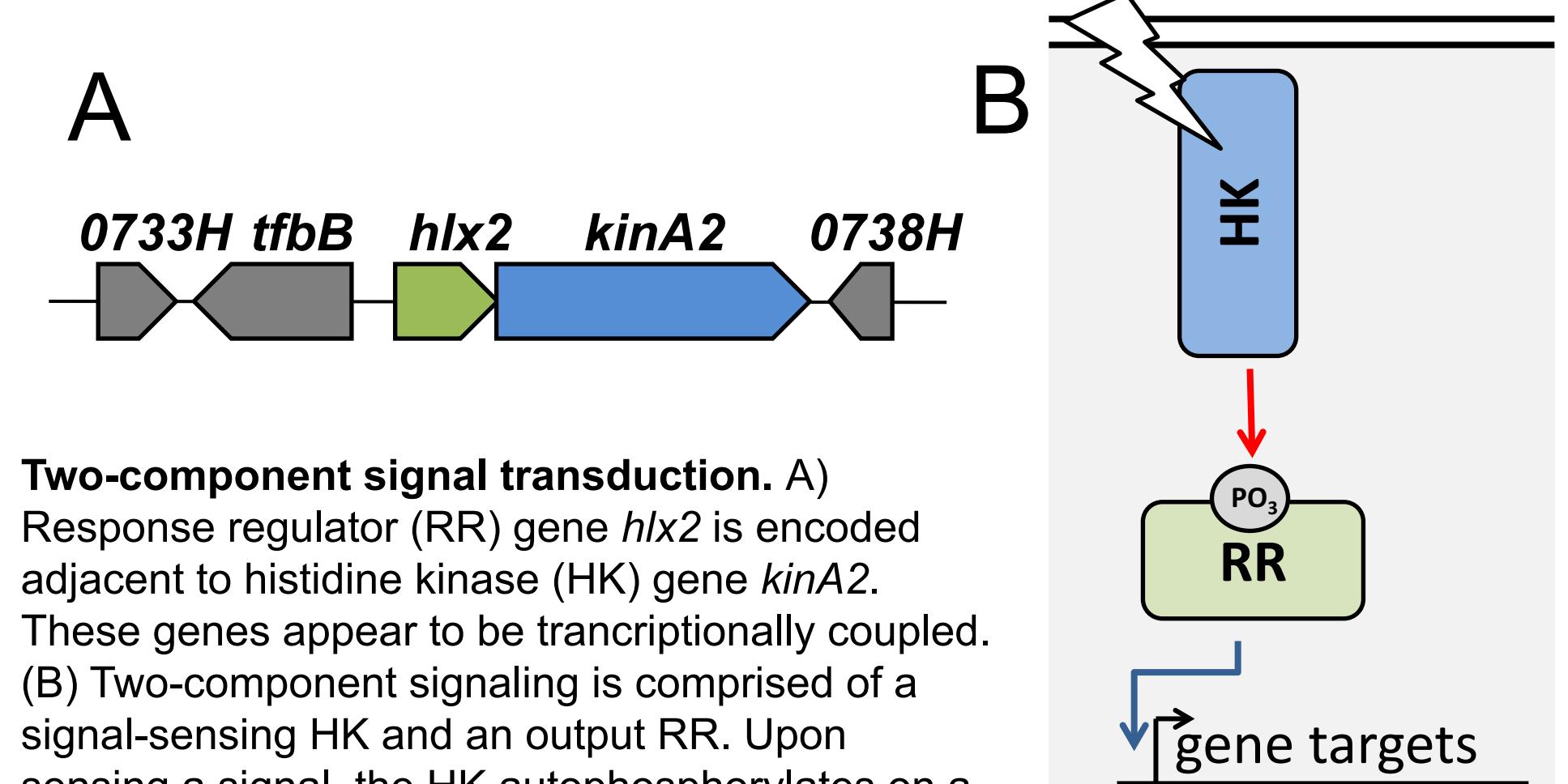
Halobacterium salinarum NRC-1 is a model Euryarchaeon, surviving the extreme environment of the salt lake. In addition to the almost saturating conditions (>5M NaCl), intense radiation and temperature fluctuations generate environmental stresses. A systems biology approach revealed the role of global regulator RosR during oxidative stress. RosR was also shown to regulate a host of transcription factors. One of these, *hix2*, encodes a DNA-binding response regulator (RR). Due to transcriptional coordination, we predict histidine kinase (HK) KinA2 and RR Hix2 make up a cognate two-component system pair. Mutations in either HK or RR gene lead to an inability to resist reactive oxygen species (ROS) such as H_2O_2 . Based on specificity residues in the HKs, we predict multiple HKs feed into this system. Indeed, HK PhoR was found to play a role during oxidative stress. Furthermore, the $\Delta hix2$ mutant was found to have a pigmentation phenotype. Targets of Hix2 revealed by RNA-seq include *perA* catalase and *bop* bacteriorhodopsin. Integration with the RosR network reveal shared and unique targets.

Halobacterium salinarum and transcription



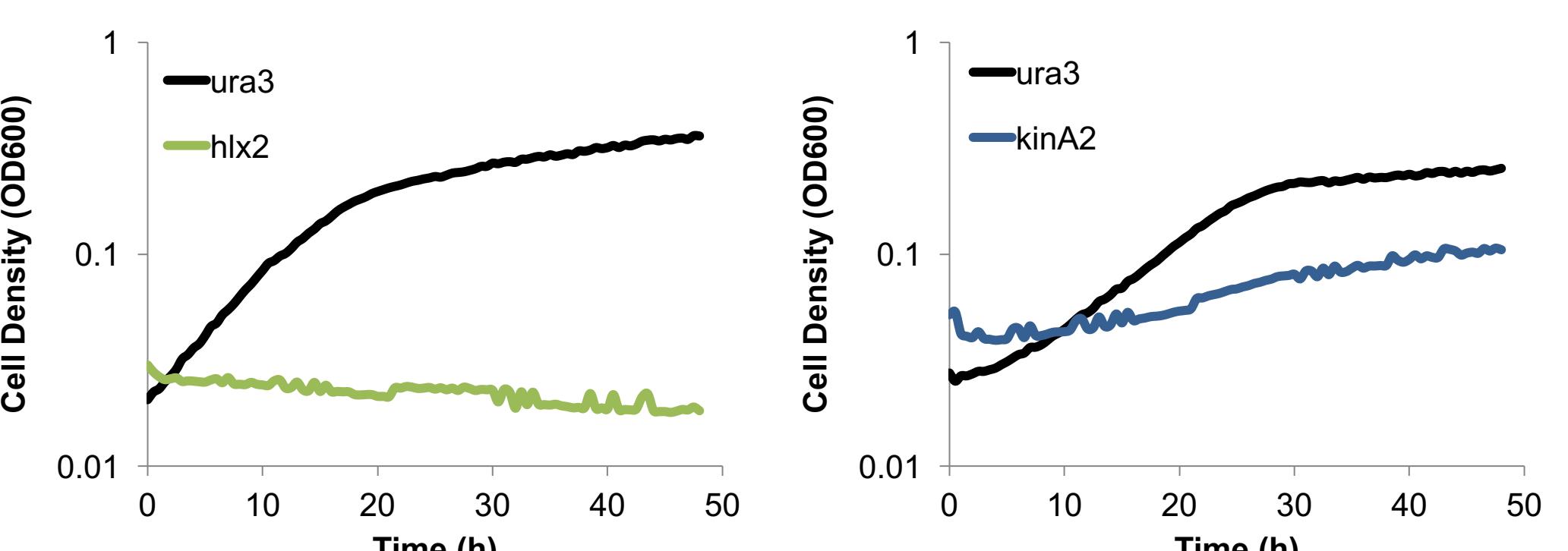
Halobacterium salinarum NRC-1 is a model archaeon. The rod-shaped cells thrive under high salinity conditions (4.2M NaCl) and *H. salinarum* is exceptional at resisting stresses such as oxidative stress. A coordinated network of transcription factors contributes to this robust resistance.

KinA2-Hix2 and Two-component systems

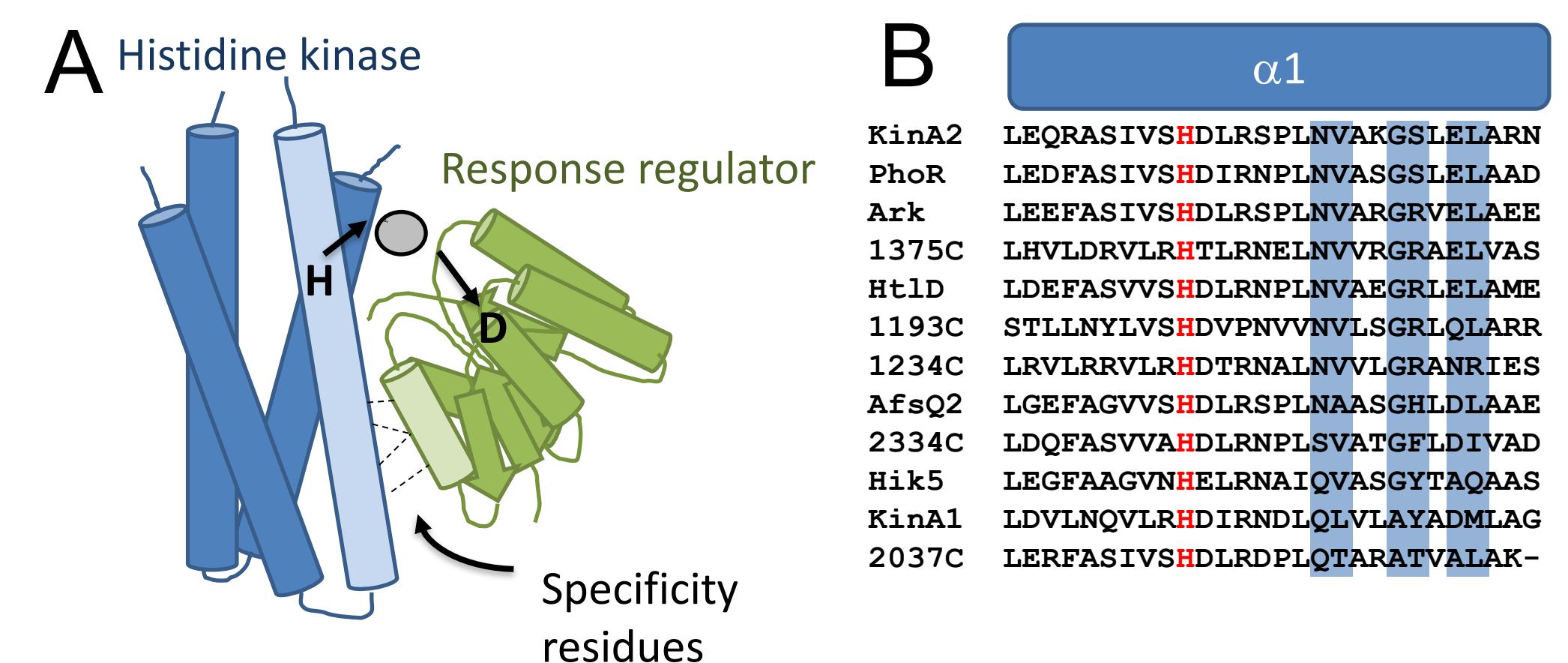


Two-component signal transduction. A) Response regulator (RR) gene *hix2* is encoded adjacent to histidine kinase (HK) gene *kinA2*. These genes appear to be transcriptionally coupled. (B) Two-component signaling is comprised of a signal-sensing HK and an output RR. Upon sensing a signal, the HK autophosphorylates on a conserved His and passes the PO_3 group to the RR. This results in a conformational change in the RR, activating or repressing its activity, such as DNA-binding.

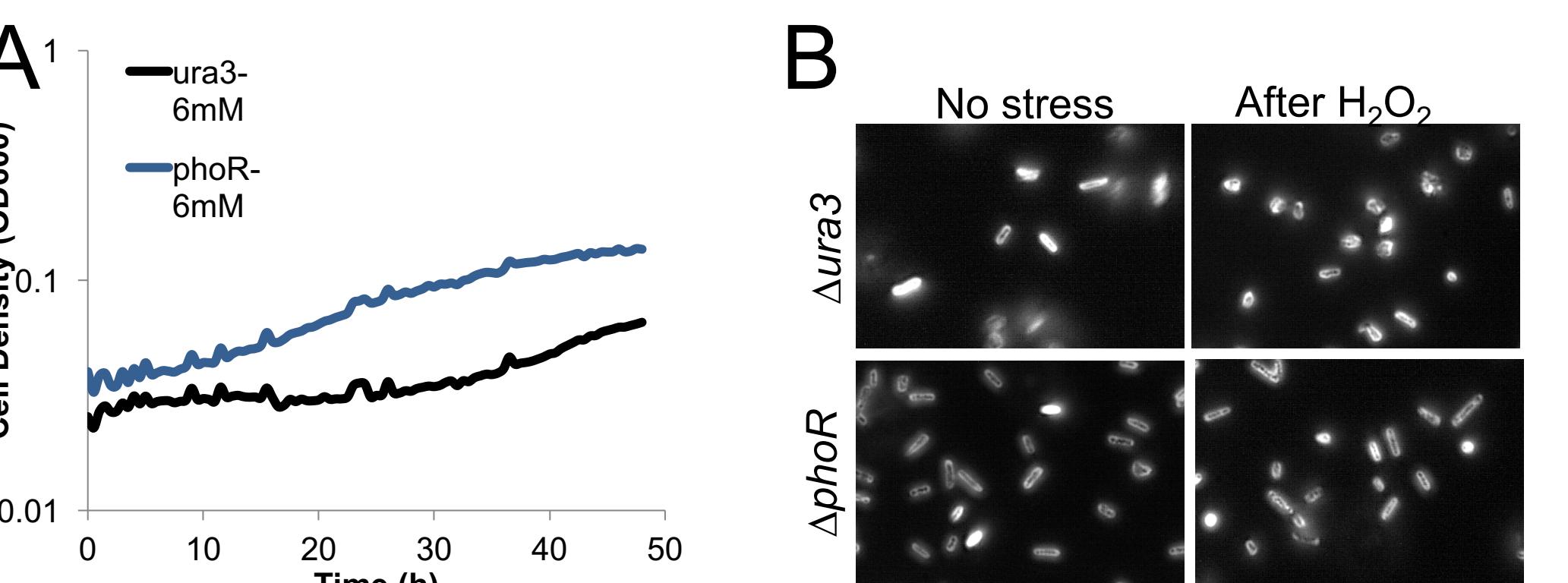
Hix2 system is required for resistance to ROS



$\Delta hix2$ and $\Delta kinA2$ are impaired for growth during oxidative stress. Growth was assayed in the absence (data not shown) or presence of 5 mM H_2O_2 in quadruplicate. Under these conditions, the strains grew poorly ($\Delta kinA2$) or not at all ($\Delta hix2$) compared to the parental $\Delta ura3$ strain, indicating a role for KinA2 and Hix2 in protecting against ROS.

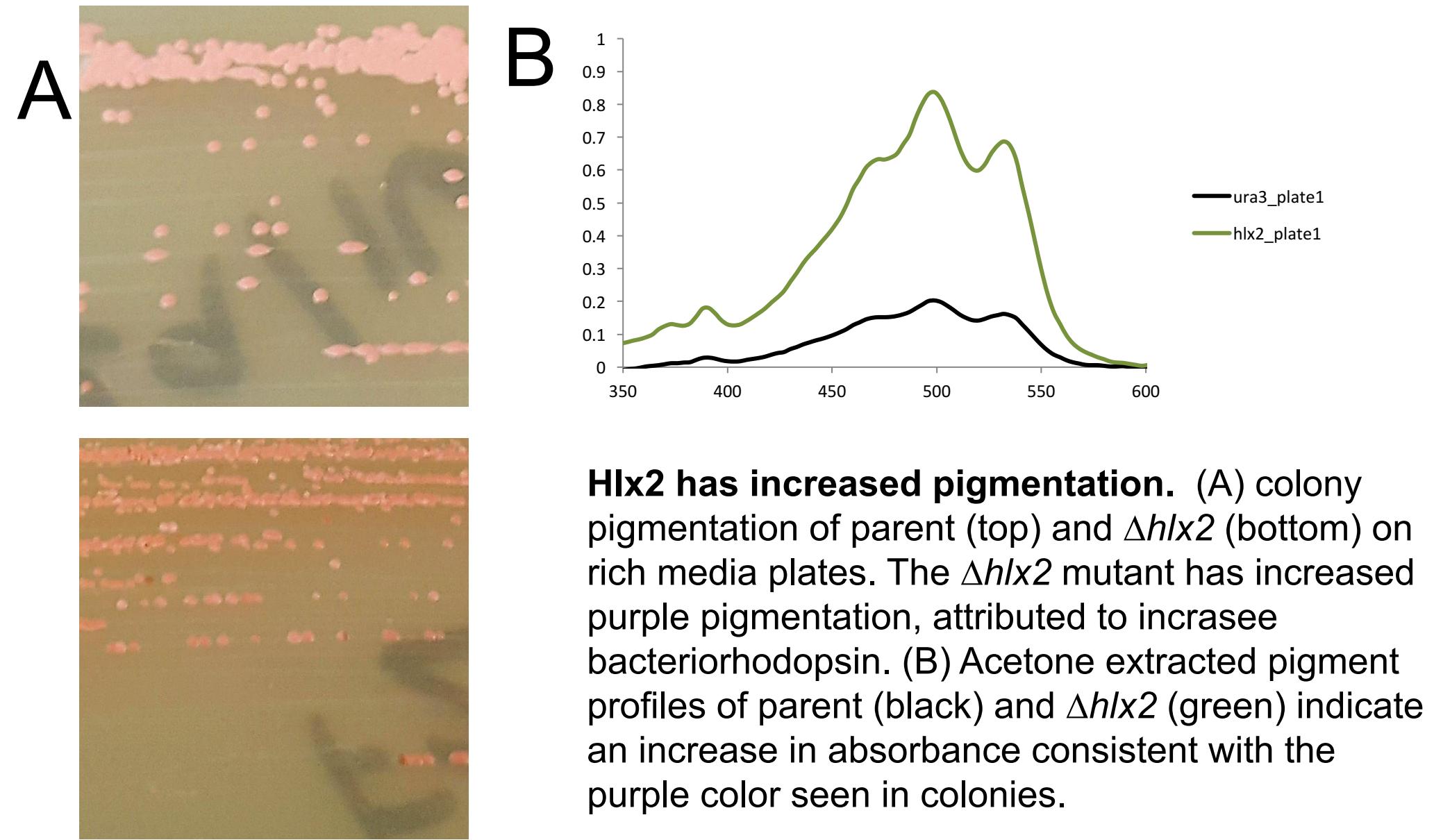


Specificity residues suggest multiple inputs to Hix2. (A) Residues co-evolved between HKs and RRs that map to the interaction region are known as specificity residues, ensuring docking of the proper RR and preventing paralogs from interaction. (B) The specificity residues in KinA2 and PhoR are identical, suggesting they may both interact with Hix2. Furthermore, other HKs have similar residues, opening the possibility of crosstalk within the global two-component network.

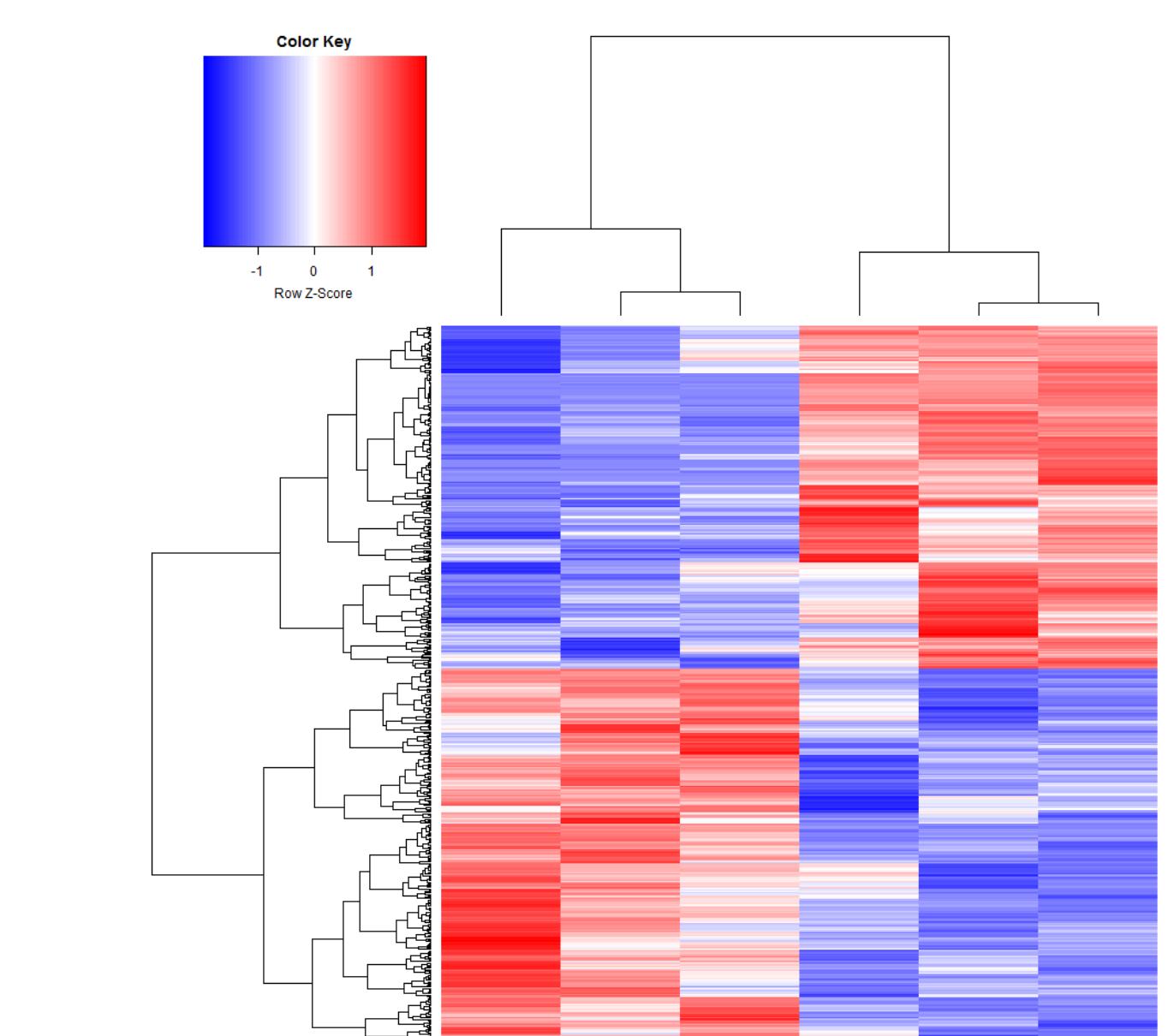


PhoR is more resistant to ROS. (A) Under the non-permissive concentration of 6 mM H_2O_2 , the parent strain fails to grow appreciably. In contrast, the $\Delta phoR$ strain is able to grow, albeit to a low level. (B) Micrographs of parent and $\Delta phoR$ cells before and after H_2O_2 treatment. These results show $\Delta phoR$ cells have increased integrity of cell shape, with more rods remaining after H_2O_2 exposure.

Δhix2 has altered pigmentation



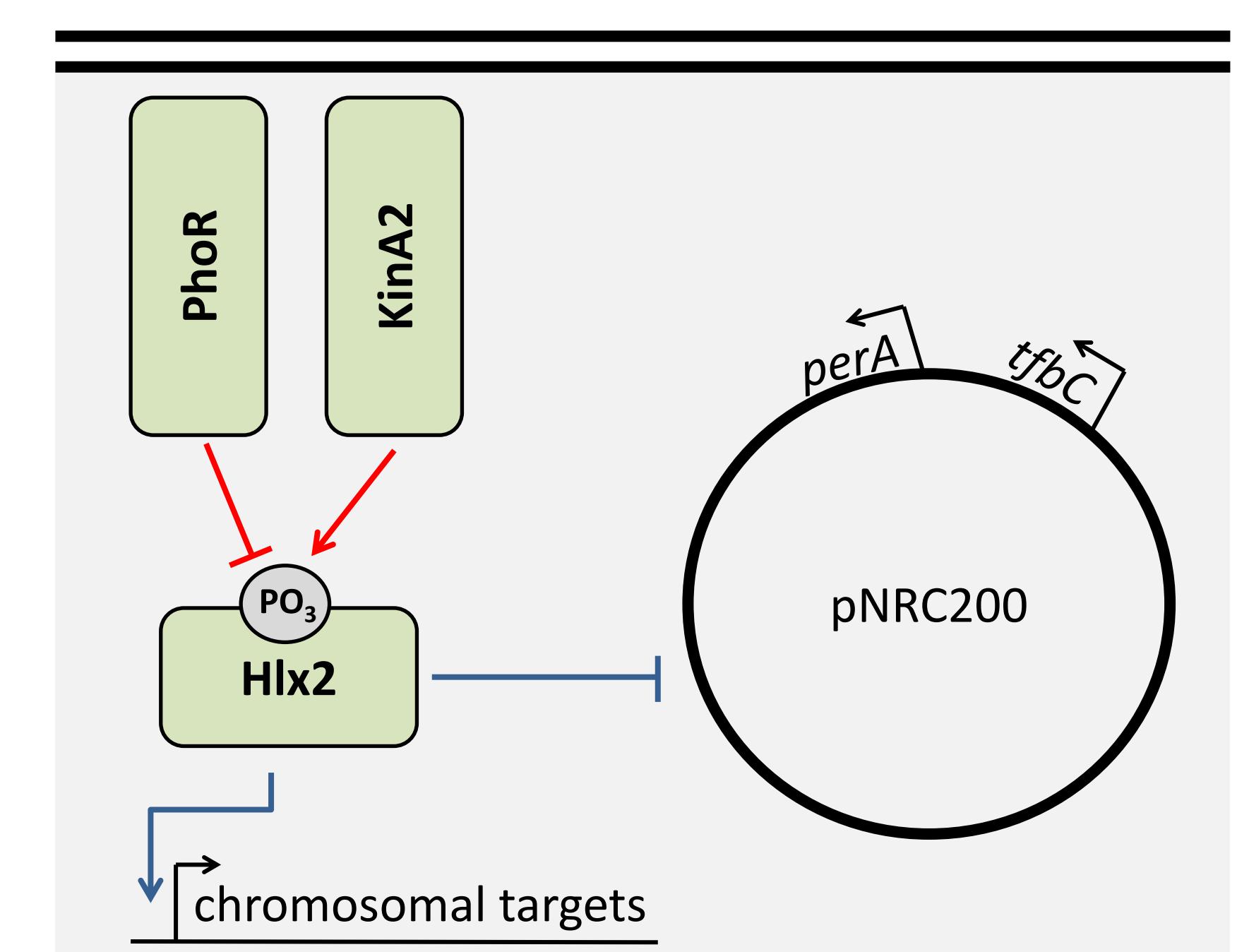
RNA-seq on $\Delta hix2$

A**B**

Locus	Gene Name	Log2 Fold Change	p-value	Protein
VNG_RS11230	<i>VNG6189H</i>	1.43	4.14E-22	hypothetical
VNG_RS03880	<i>acs2</i>	1.21	1.47E-20	acetyl-CoA synthetase
VNG_RS03640	<i>noxC</i>	1.10	1.79E-17	NADH oxidase
VNG_RS03655	<i>gufA</i>	1.06	2.79E-11	zinc transporter
VNG_RS03635	<i>yqiM</i>	1.01	2.47E-22	oxidoreductase
VNG_RS03620	<i>yvbT</i>	0.82	3.19E-09	alkane monooxygenase
VNG_RS05140	<i>VNG1315H</i>	0.76	1.26E-09	hypothetical
VNG_RS00205	<i>VNG6052H</i>	0.75	4.01E-04	transposase
VNG_RS02190	<i>VNG0557H</i>	0.74	2.61E-08	DI-1/Hfq family protein
VNG_RS03880	<i>VNG1380H</i>	0.72	1.42E-05	hypothetical
VNG_RS03310	<i>VNG0485C</i>	0.67	7.15E-07	methyltransferase type 11
VNG_RS03185	<i>VNG0214C</i>	0.67	1.07E-07	NAD dependent epimerase/dehydratase
VNG_RS05715	<i>bop</i>	0.66	5.00E-05	rhodopsin
VNG_RS11555	<i>perA</i>	-0.59	5.16E-14	catalase/hydroperoxidase HPI(I)
VNG_RS06320	<i>cbiQ</i>	-0.63	3.13E-04	cobalt transporter
VNG_RS06325	<i>cbiQ2</i>	-0.65	3.33E-04	ABC transporter ATP-binding protein
VNG_RS01065		-0.80	2.24E-04	hypothetical
VNG_RS03840		-2.78	2.63E-36	hypothetical
VNG_RS03845	<i>VNG0986H</i>	-2.79	2.59E-36	hypothetical
VNG_RS03865		-3.22	1.29E-48	hypothetical
VNG_RS03850	<i>VNG0993H</i>	-3.93	1.97E-80	integrase
VNG_RS03860	<i>VNG0992H</i>	-5.36	1.18E-172	hypothetical
VNG_RS03855	<i>VNG0991H</i>	-5.88	1.93E-222	hypothetical
VNG_RS11040	<i>tfbC</i>	-5.76	2.03E-199	TFB
71 pNRC200 genes		-7.84 -0.59	-	various

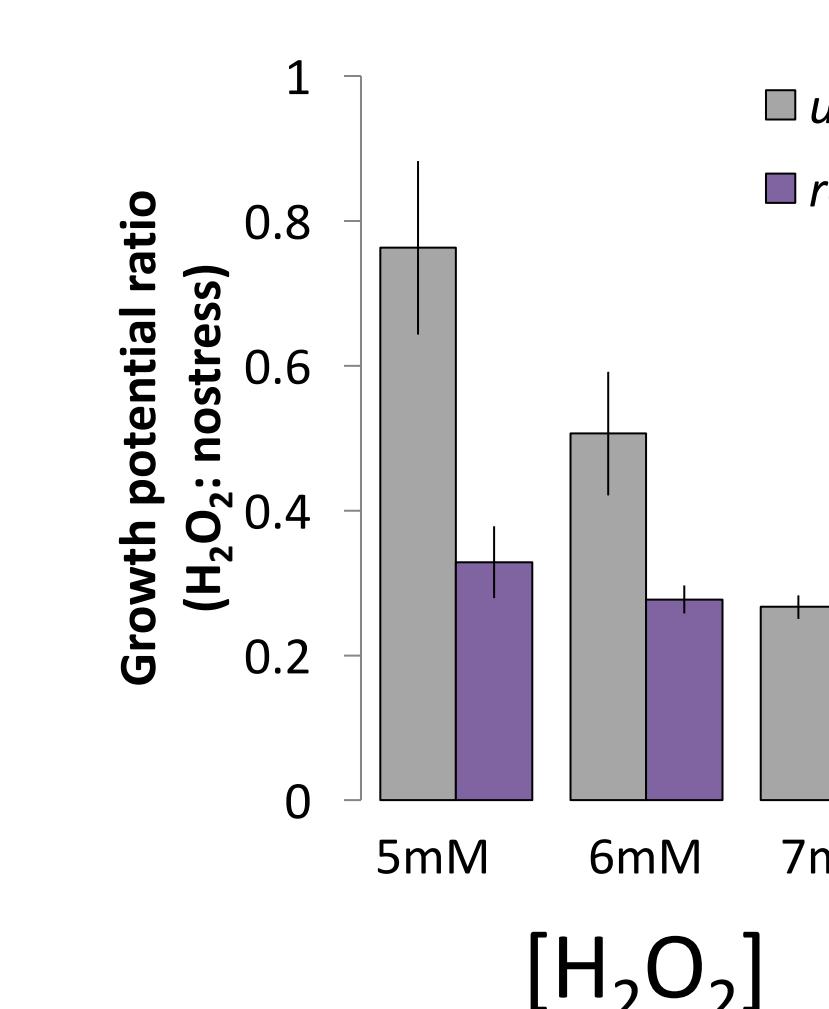
RNA-seq results from parent and $\Delta hix2$ strains. (A) Heat map depicting significantly regulated genes ($p < 0.05$) in biological triplicate. RNA was harvested from log phase cells ($OD_{600} \sim 0.4$) under aerobic growth at 42°C. Illumina Hi-seq sequencing on the resultant libraries was performed and reads were trimmed (Trim Galore!) and mapped to the genome (Bowtie2). Statistical analysis and normalization was done through the DESeq2 R package. (B) List of interesting genes with log2fold changes include *bop* bacteriorhodopsin, *perA* catalase, TFB homolog *tfbC*, and 71 other pNRC200-encoded genes.

Model of regulation

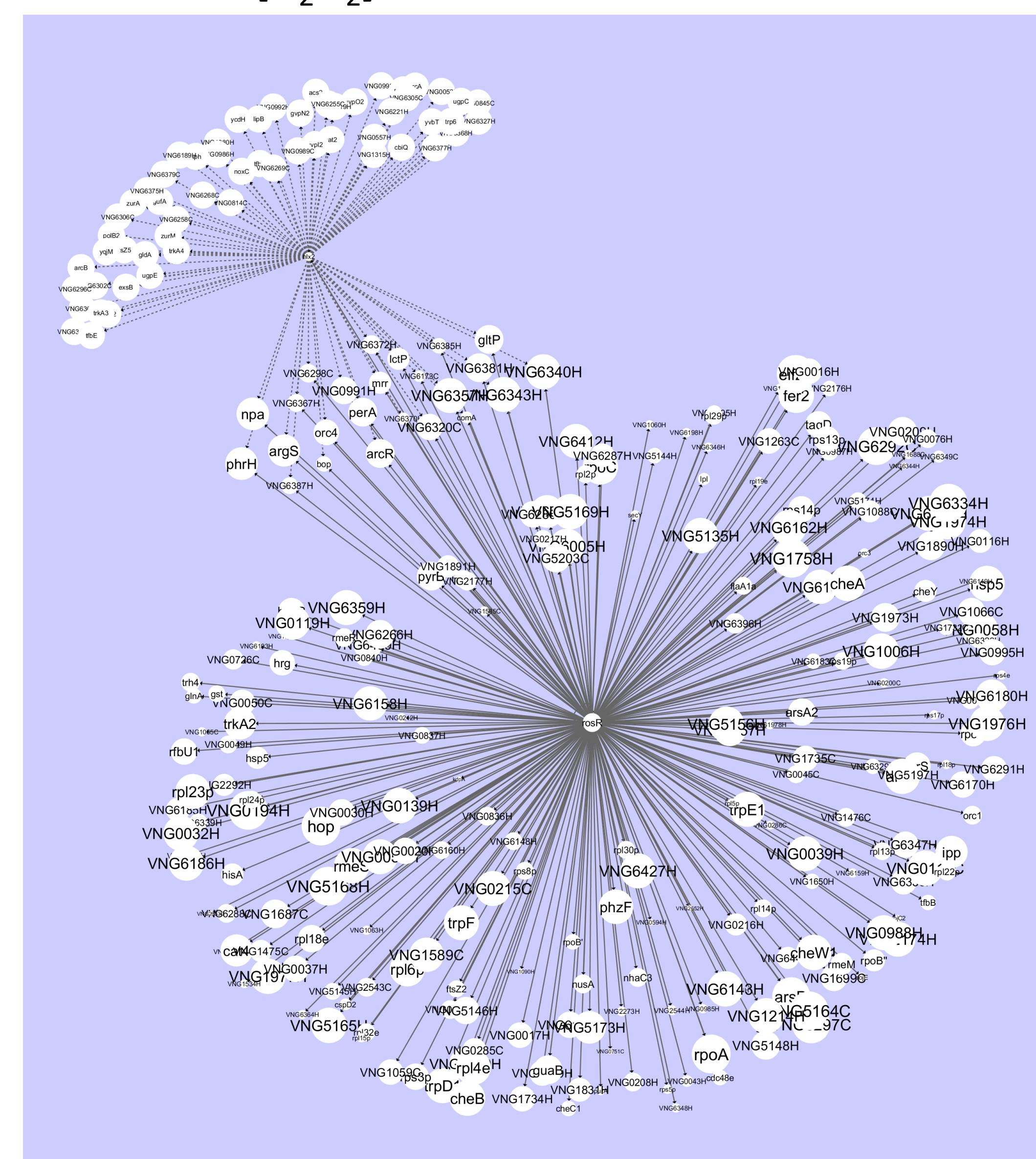


Model for Hix2 regulation. Multiple input HKs KinA2 and PhoR maintain the phosphorylation state of Hix2 through positive (kinase) and negative (phosphatase) activity. This results in the control of Hix2 DNA-binding activity. Targets of Hix2 (whether direct or indirect) include chromosomal genes and genes encoded on pNRC200. We hypothesize that Hix2 regulates a general TF, such as TfbC, to alter pNRC200 expression.

RosR and oxidative stress



The global regulator RosR is required for oxidative stress resistance. Growth assays were performed with and without H_2O_2 in quadruplicate. The area under the log transformed curve (AUC) was calculated to determine growth potential. Reported is the ratio of AUC with stress: AUC no stress. The $\Delta rosR$ strain is significantly sensitive to H_2O_2 , as previously described [1].



Hix2 influences expression of RosR targets. The network visualizing RosR and Hix2 regulons is built on gene expression (RosR, Hix2) [1] and chromatin-immunoprecipitation (RosR) data [2]. RosR regulates a host of bacterial-like transcription factors, including RR Hix2. Hix2 influences expression of some RosR targets, including *perA* catalase, as well as unique targets. Font and node size indicate gene expression to ChIP correlation for RosR targets. Solid lines indicate direct regulation; dashed lines maybe direct or indirect.

Acknowledgements and References

- [1] Sharma K, Gillum, N, Boyd JL, and A Schmid. 2012. The RosR transcription factor is required for gene expression dynamics in response to extreme oxidative stress in a hypersaline-adapted archaeon. *BMC Genomics*. 13:351
- [2] Tonner PD, Pittman AMC, Gulli JG, Sharma K, and AK Schmid. 2015. A regulatory hierarchy controls the dynamic transcriptional response to extreme oxidative stress in archaea. *PLOS Genetics*. 11:1