Supporting Information

Native alanine substitution in the glycine hinge modulates conformational flexibility of Heme Nitric oxide/Oxygen (H-NOX) sensing proteins

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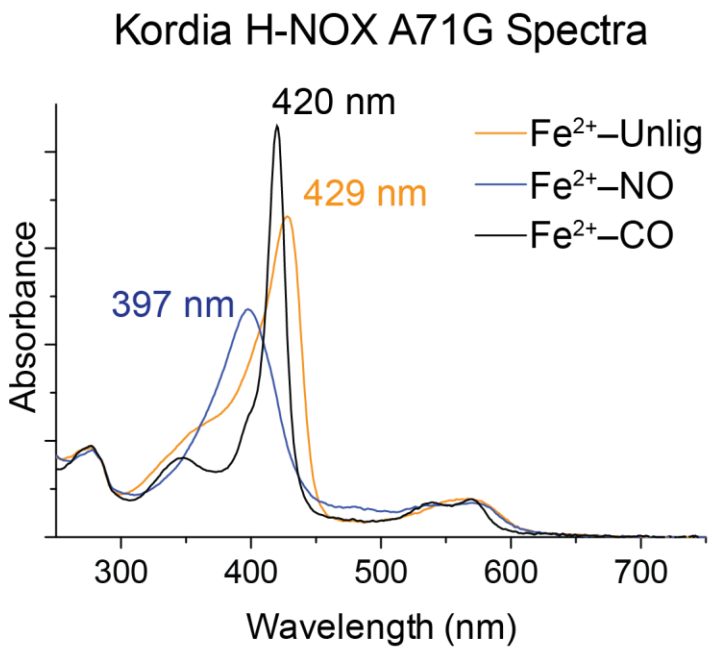
A



B

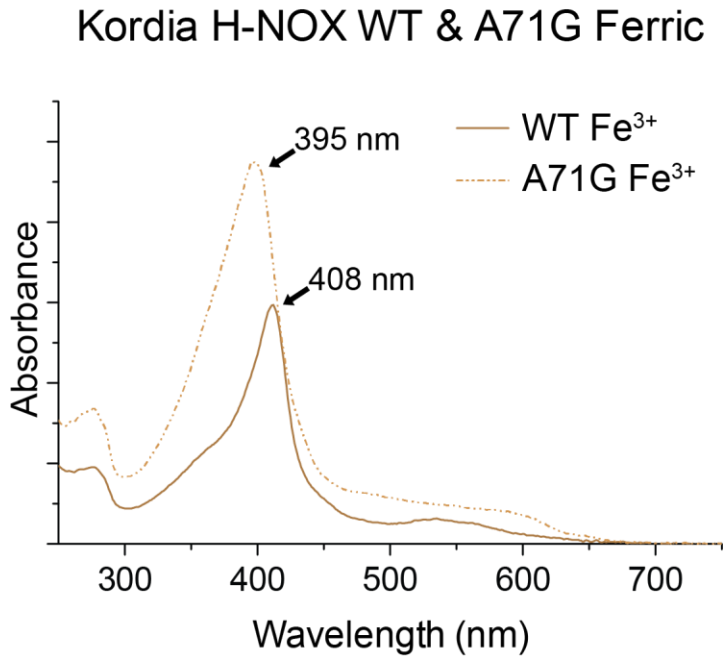


Fig. S1. (*A)* Ligand spectra of A71G *Ka* H-NOX. (*B*) Ferric spectra of WT and A71G *Ka* H-NOX.

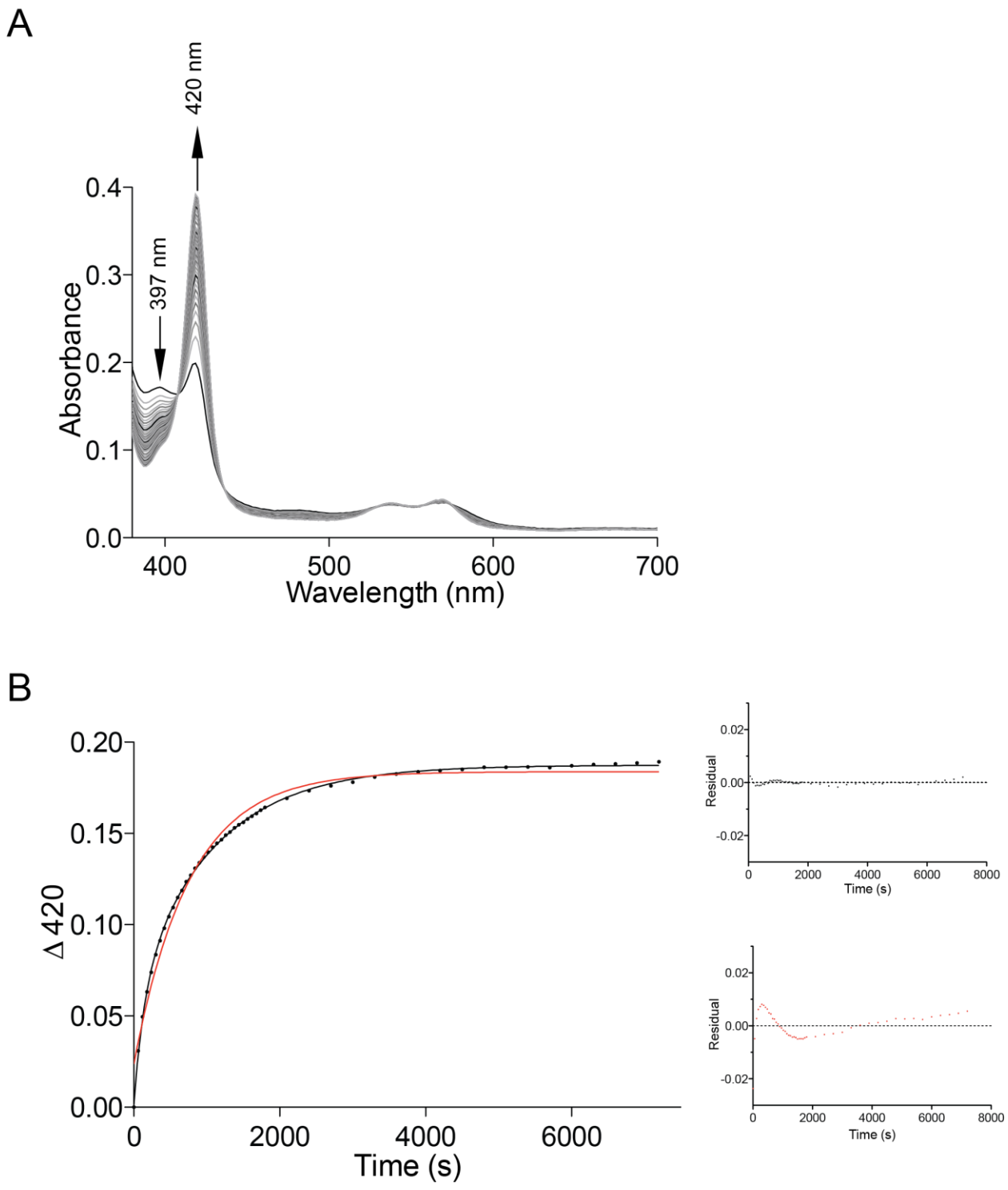


Fig. S2. (*A*) Example NO dissociation measurement spectra of WT *Ka* H-NOX. NO dissociation was measured using a CO/dithionite trap. The increase of the Soret peak at 420 nm was used to measure the k_{off} for NO. (*B*) Plot of Δ420 nm versus time. Data was fit to one (red) or two (black) phase exponential decay. On the right panel, residuals indicate that data better fits a two-phase decay model.

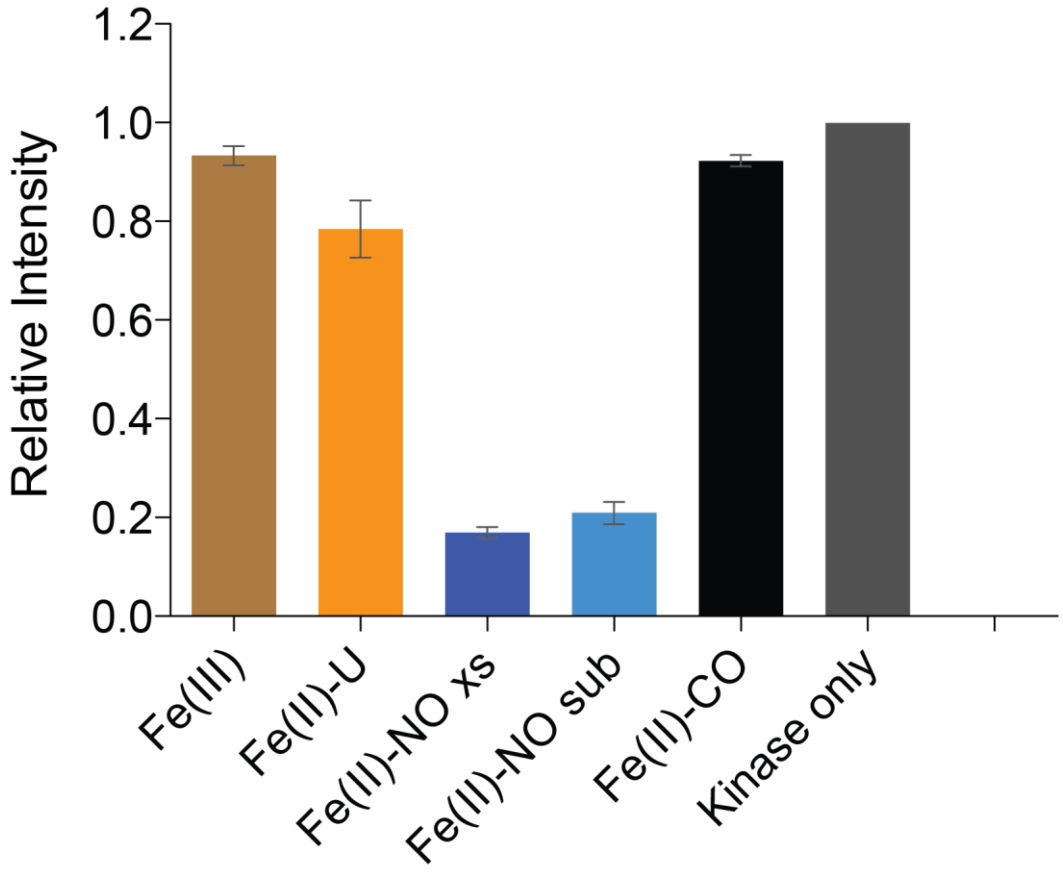


Fig. S3. HnoK (2 µM) autophosphorylation assay with 10 µM H-NOX A71G at different heme ligation or oxidation states. Kinase autophosphorylation measured with [^{32}P]-γ-ATP.

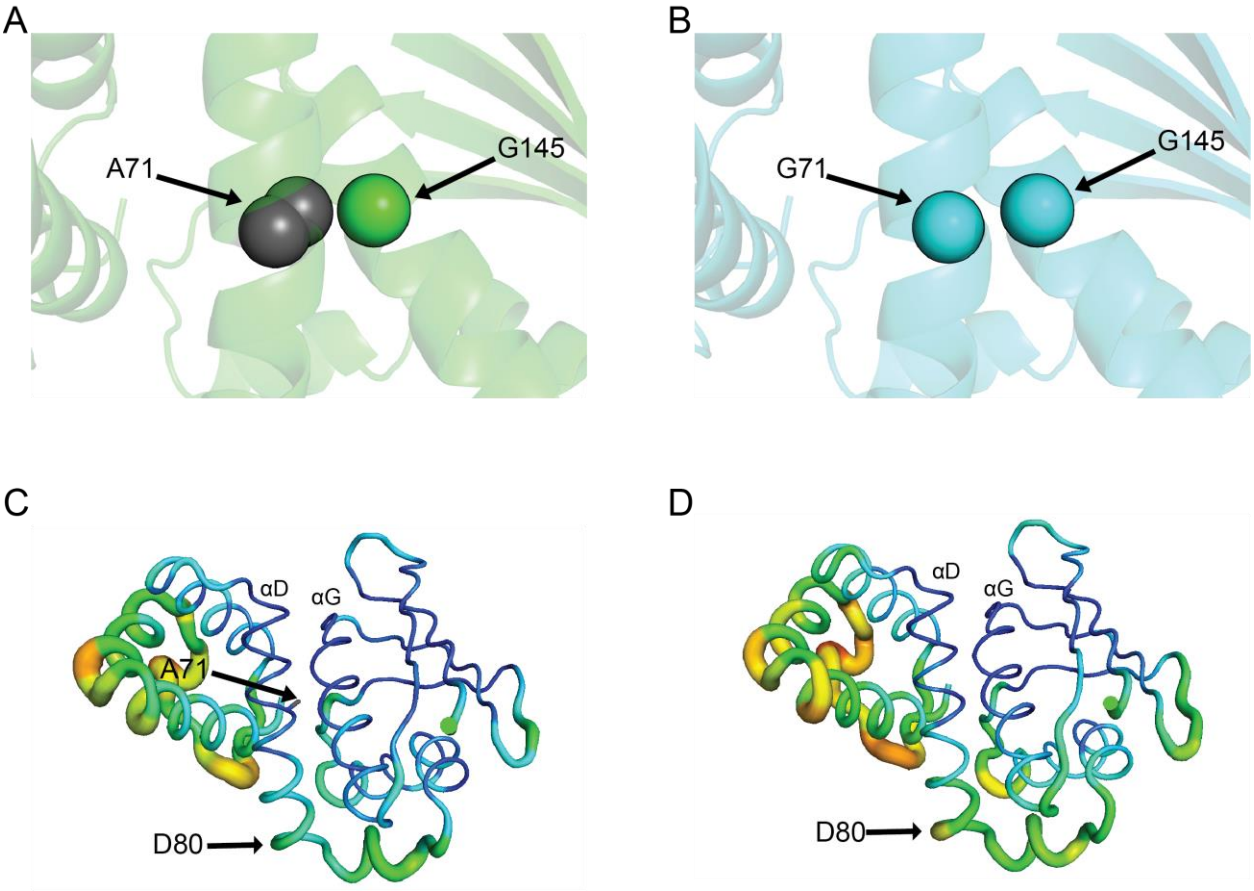


Fig. S4. (Panels *A* & *B*) Close-up views of the hinge residues in WT (*A*) and A71G (*B*) structures. The hinge residues are shown as spheres to emphasize close proximity of residues. A71 is highlighted in grey. (Panels *C* & *D*) B-factor putty of αD and αG interface. A71 is noted in grey for reference. Asp80 at the end of helix αD has higher flexibility.

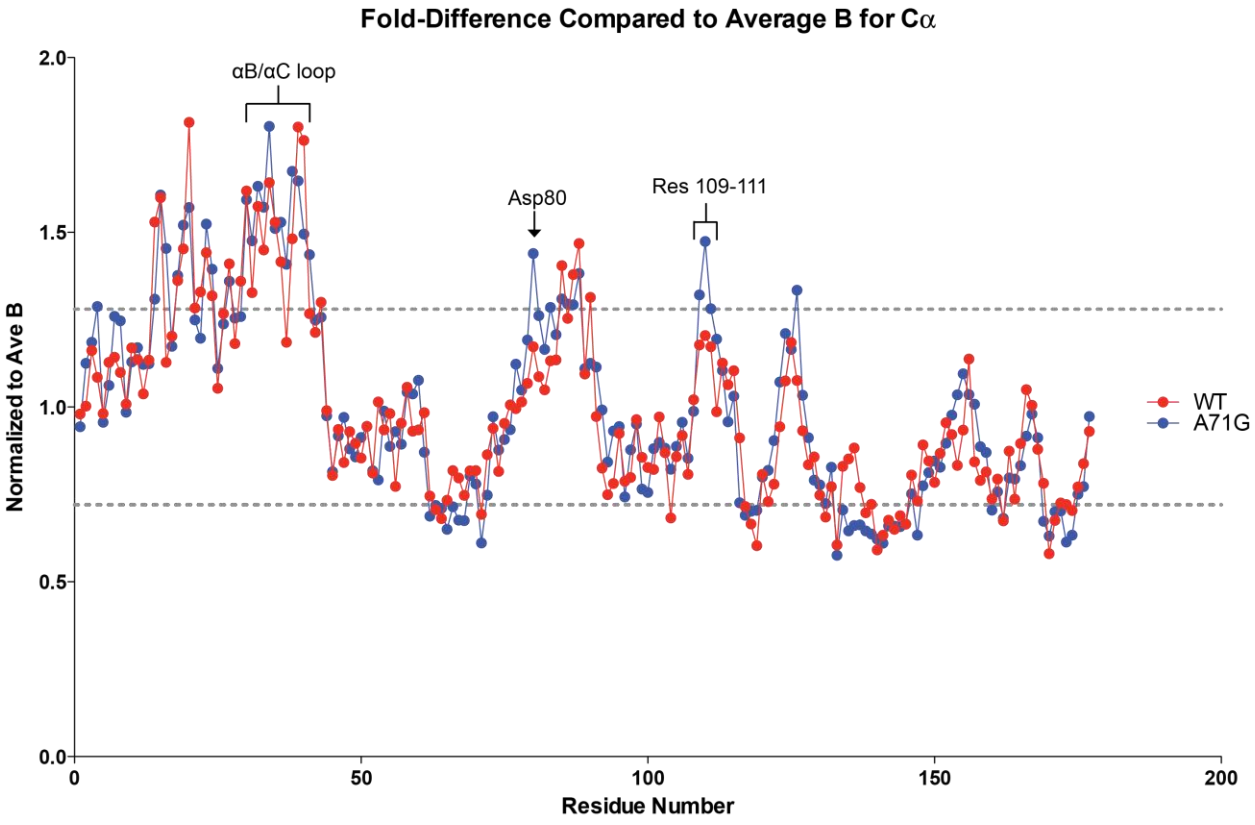
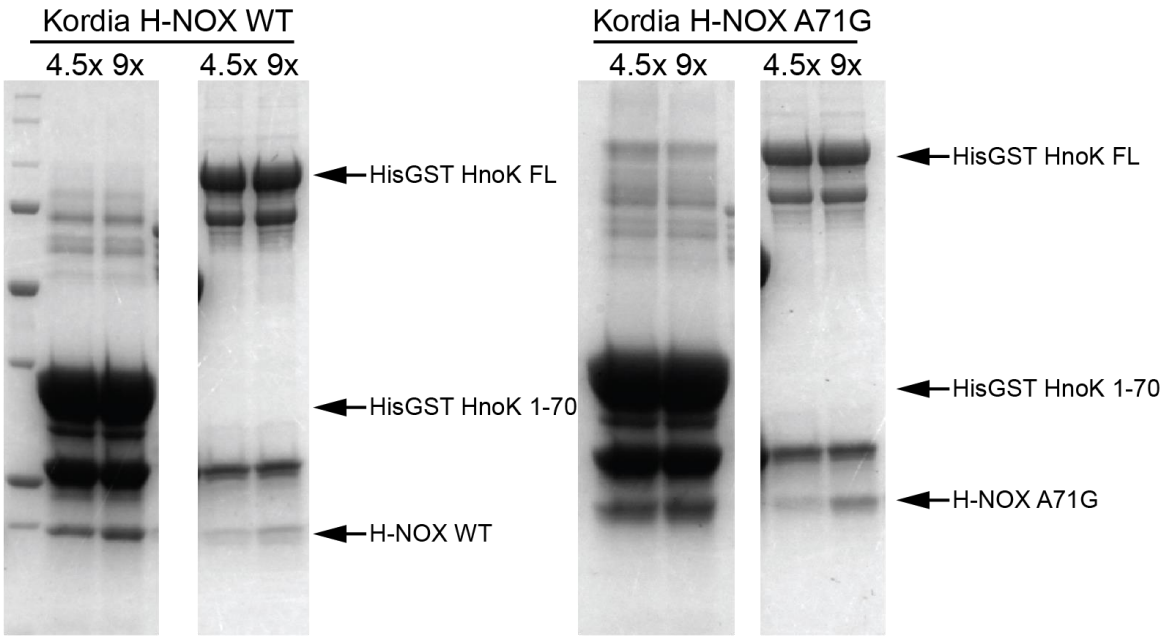


Fig. S5. B-factor differences after normalization of Cα. B-factors were normalized to the average b-factor of each structure. Grey dotted line represents standard deviation of b-factor differences in WT *Ka* H-NOX. The last six residues with higher than average b-factor values are part of a C-terminal TEV cleavage scar, not part of the native protein sequence, and, therefore, were not included in the b-factor averages.



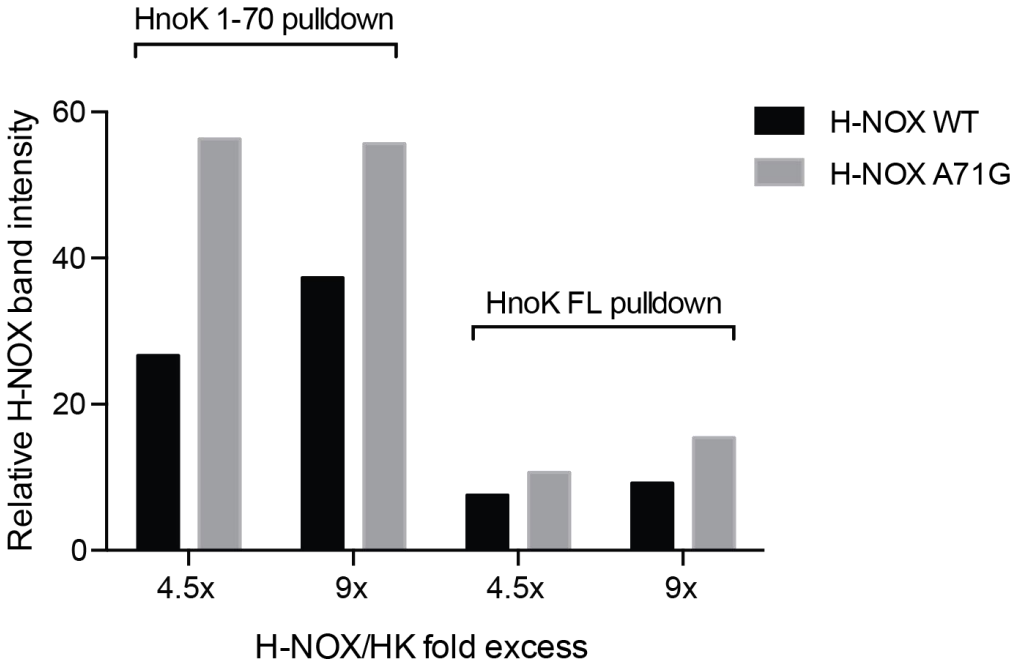


Fig. S6. Pull down assay of HisGST-tagged HnoK full-length of residues 1-70. H-NOX band intensity was normalized to eluted HisGST HnoK band. Quantification of bands indicates that H-NOX A71G has a higher affinity for full-length and residues 1-70 of HnoK compared to WT H-NOX.

Table S1. *Kordia algicida* H-NOX crystal statistics

	WT	A71G
PDB Code	6BDD	6BDE
Crystallization	0.1 M Tris-HCl pH 8.5 0.3 M LiCl 34% PEG 6000	0.1 M Tris-HCl pH 8.5 0.3 M LiCl 34% PEG 6000
Crystallographic Data		
Beamline	ALS 5.0.1	ALS 5.0.1
Wavelength (Å)	0.977	0.977
Space Group	$p2_12_12_1$	$p2_12_12_1$
Cell Dimensions (a, b, c) (Å)	48.97, 57.61, 68.80	48.79, 56.73, 68.75
	α=β=γ=90 °	α=β=γ=90 °
Resolution (Å)	39.89-1.90	37.0-1.64
No. of observations	94510	288820
No. of unique observations	15750	23652
Completeness, %	99.0 (91.4)	98.5 (88.2)
I/σ(I)	9.78 (2.41)	15.60 (3.75)
Rmerge, %	0.11 (0.67)	0.081 (0.530)
Redundancy	6	12.2
CC1/2	0.616	0.95
Refinement		
Resolution (Å)	39.89-1.90 (1.97-1.90)	37.0-1.64 (1.70-1.64)
No. of protein atoms	1496	1495
No. of ligand atoms	43	43
No. of water atoms	129	126
Rfree, %	24.2	23.6
Rcrys, %	19.3	21
Geometry		
RMS bonds (Å)	0.004	0.024
RMS angles (°)	0.65	2.04
Ramachandran Favored (%)	97.3	98.4
Ramachandran Allowed (%)	2.7	1.1
Ramachandran Disallowed (%)	0	0.6
Average B-factors (Å^2)		
Protein	42.7	47.5
Water	47.6	52.1
Ligands	31.9	36