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Missense substitutions reflecting regulatory control of transmitter phosphatase activity in two-component signaling

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Supplementary Material

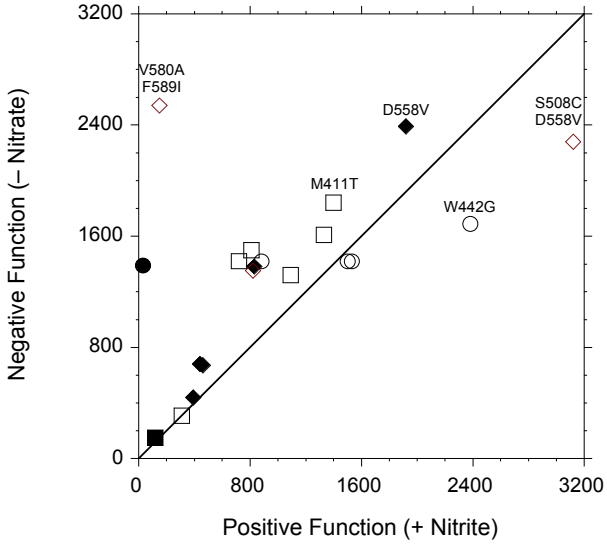

Fig. S1. Correlation between nitrite hypersensitivity and P^- phenotypes. Data from **Table 2** are plotted, with the X axis showing values for the positive function assay (cultures grown with nitrite), and tye Y axid showing values for the negative function assay (cultures grown in the absence of nitrate). Corresponding data for the D558V mutant are from **Table S1**. The diagonal line shows the position of 1:1 correspondence, and is *not* a statistical construct drawn from the data. Symbols are: ■, *narX*$^+$; ●, *narX* null; □, DHp domain helix α1 mutants; ○, DHp domain helix α2 mutants; ◆, CA domain single substitution mutants; ◇, CA domain double substitution mutants.

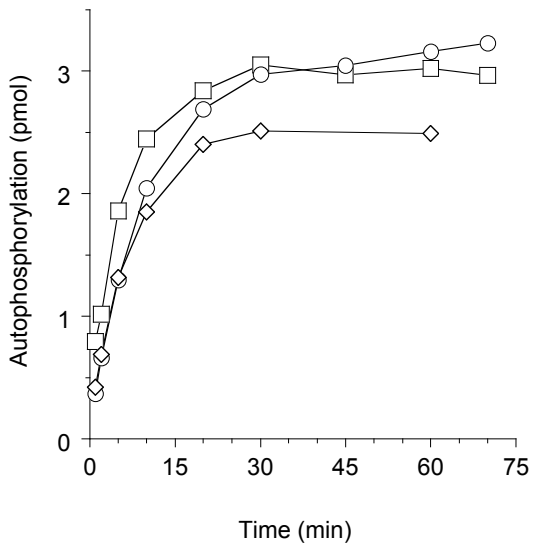

Fig. S2. Autophosphorylation by C415R and Y551F mutant versions of MBP-NarX$_{227}$ in vitro. □, NarX$^+$; ○, C415R; ◇, Y551F; ●, His$_6$-NarL.

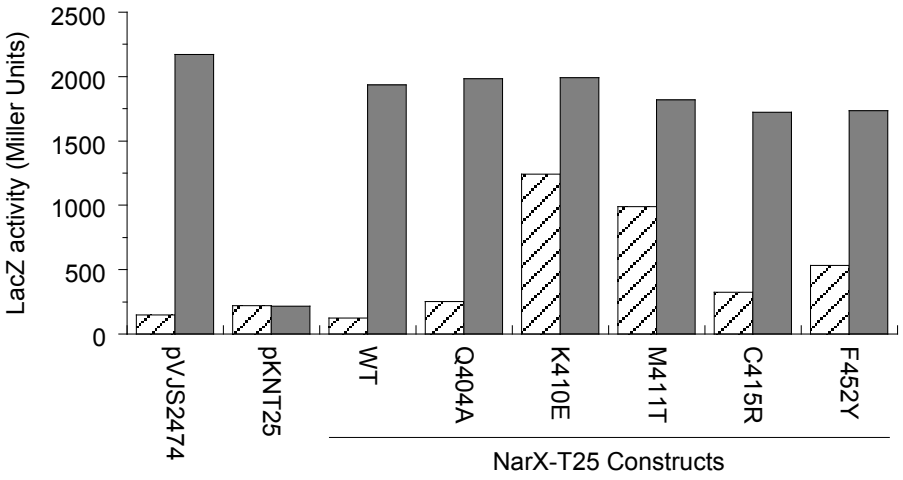

Fig. S3. In vivo functionality of NarX-T25 fusion proteins. LacZ synthesis results from expression of the chromosomal Φ(*narG-lacZ*) reporter in the *narX narQ* double null strain VJS3041. Striped and filled bars show data from cultures grown in the absence and presence of nitrate, respectively. Plasmid pVJS2474 encodes wild-type NarX. Plasmid pKNT25 is the empty T25 fusion vector.

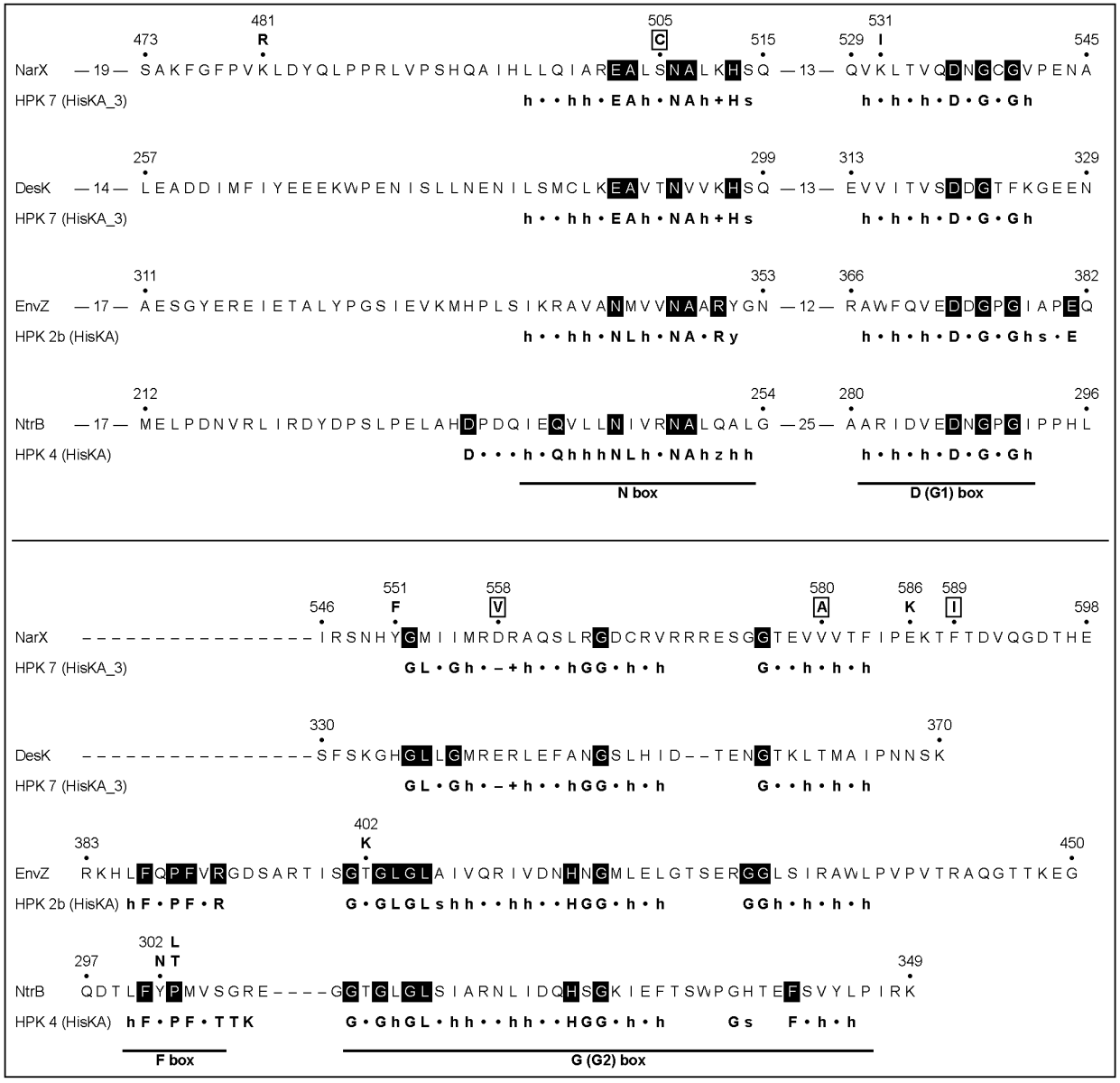

Fig. S4. CA domain sequences. Conserved motifs are as described (Grebe and Stock, 1999). Residues that match the motif sequence are enclosed in black boxes. Substitutions resulting in the K^+ P^- phenotype are shown above the sequence. For NarX, double substitutions are enclosed in boxes.

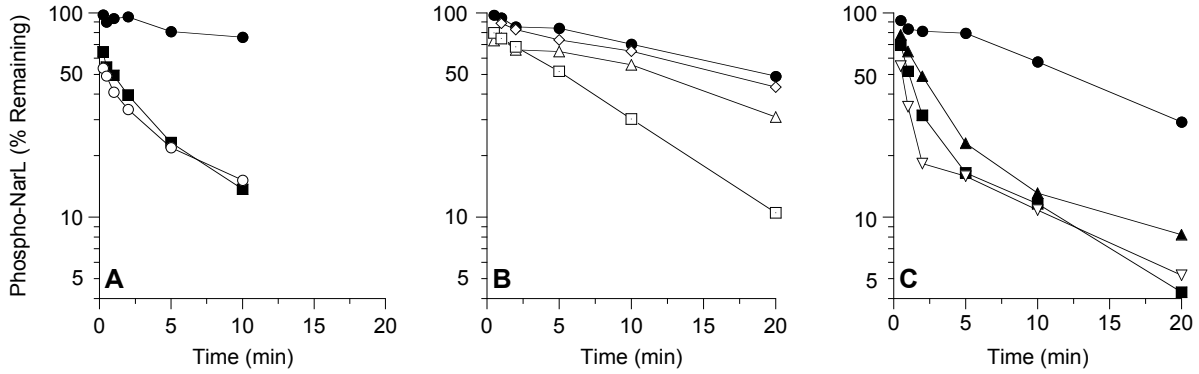

Fig. S5. Concentration dependence of MBP-NarX$_{227}$ transmitter phosphatase activity in vitro. [^{32}P]-Phospho-NarL was prepared, added to different concentrations of NarX dimers, and sampled for analysis as described in Experimental Procedures.

A. ●, no sensor; ■, 0.5 μM μM NarX^{+} (dimers; standard concentration for all other experiments); ○, 5.0 μM.

B. ●, no sensor; ◇, 0.01 μM NarX^{+} (dimers); △, 0.05 μM; □, 0.1 μM.

C. ●, no sensor; ▲, 0.2 μM NarX^{+} (dimers); ■, 0.5 μM (standard concentration for all other experiments); ▽, 1.0 μM.

Table S1. Effects of *narX* missense substitutions on Φ(*narG-lacZ*) expression.

		β-Galactosidase [a]					
		Negative Function [d]			Positive Function [e]		
Allele [b]	Region [c]	None	Nitrate	Ratio	None	Nitrate	Nitrite
pHG165	—	1,170	1,100	0.9	11 [f]	10 [f]	17 [f]
narX‡	—	330	1,310	4.3	17	1860	240
Q412R	DHp helix α1	370	1,210	3.3	60 [f]	2,090 [f]	220 [f]
V413M	DHp helix α1	3,200	1,040	0.3	120 [f]	2,330 [f]	1,250 [f]
Q412R+V413M	DHp helix α1	1,710	1,070	0.6	100 [f]	1,530 [f]	1,300 [f]
D558V	CA	2,390	1,020	0.4	130	1,710	1,920
S508C + D558V	CA	2,380	1,070	0.4	2,470	2,120	2,250

[a] Activity was measured as described in Materials and Methods, and is expressed in Miller units.

[b] Amino-acyl residue substitutions. *narX* alleles located on plasmid pVJS1241 or pVJS2474 derivatives.

[c] Location within NarX protein structure; see text for details.

[d] Negative function assayed in strain VJS4033 [Φ(*narG-lacZ*) *narL505* (V88A) *narP*$^+$ Δ*narX narQ*::Tn*10 pcnB*$^+$].

[e] Positive function assayed in strain VJS5054 [Φ(*narG-lacZ*) *narL*$^+$ *narP*$^+$ Δ*narX narQ*::Tn*10 pcnB1*].

[f] Positive function data from reference (Noriega *et al.,* 2010).

Table S2. Strains and plasmids.

Strain	Genotype	Source
***E. coli* K-12 strains:**		
VJS2197	F^- λ^- prototroph Δ(*argF-lac*)*U169* $\lambda\Phi$(*narG-lacZ*)*250*	(Rabin and Stewart, 1993)
VJS10304	F^- λ^- prototroph Δ*cya-854* Δ*narX* Δ*narQ*	This study
Derivatives of VJS2197:		
VJS4033	Δ*narX242 narL505*(V88A) *ychO2084*::Ω-Cm *narQ251*::Tn*10*d(Tc)	(Rabin and Stewart, 1993)
VJS5054	Δ*narX242 narQ251*::Tn*10*d(Tc) *pcnB1 zad-981*::Tn*10*d(Km)	(Williams and Stewart, 1997b)
VJS5720	Δ*narX242 narL505*(V88A) *ychO2084*::Ω-Cm *narQ251*::Tn*10*d(Tc) *pcnB1 zad-981*::Tn*10*d(Km)	(Williams and Stewart, 1997b)
Plasmids:		
pHG165	Ap^r; *ori* pMB1; *lacZ*α pUC8 polylinker	(Stewart *et al.*, 1986)
pUT18C	Ap^r; T18 fragment (amino acids 225 to 399 of CyaA)	(Karimova *et al.*, 1998)
pKNT25	Km^r; T25 fragment (the first 224 amino acids of CyaA)	(Karimova *et al.*, 1998)
pVJS1241	As pHG165 but *narX*†	(Williams and Stewart, 1997b)
pVJS2474	As pHG165 but *narX*‡	(Appleman and Stewart, 2003)
pVJS5321	As pKNT25 but *narX*$^{+}$	This study
pVJS5753	As pUT18C but *narL'* (REC domain)	This study