Supporting Information for

The role of serine coordination in the structural and functional protection of the nitrogenase P-cluster

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-Supporting Figures S1 – S5
-Supporting Tables S1 – S3

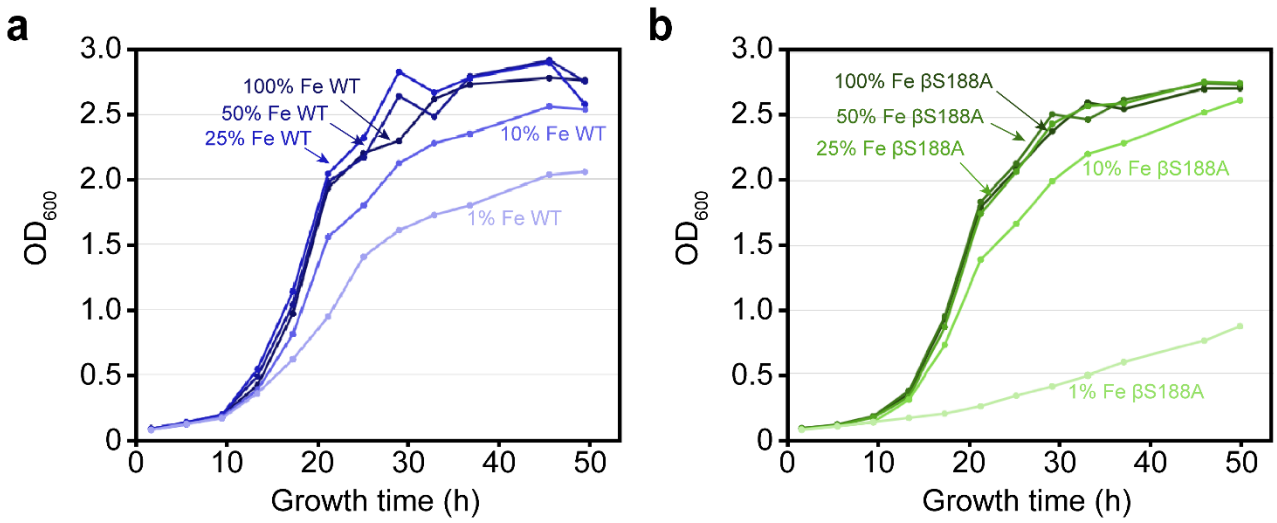

Figure S1. Diazotrophic growth curves of *Av* cells under varying Fe concentrations (100%, 50%, 25%, 10%, and 1% Fe). 100% Fe corresponds to 35 μM Fe. (a) wt *Av* growth curves had doubling times of 3.9, 4.0, 4.0, 4.2, and 6.3 h at 100%, 50%, 25%, 10%, and 1% Fe, respectively. (b) βS188A *Av* growth curves had doubling times of 3.9, 3.9, 3.8, 4.1, and 12.7 h at 100%, 50%, 25%, 10%, and 1% Fe, respectively.

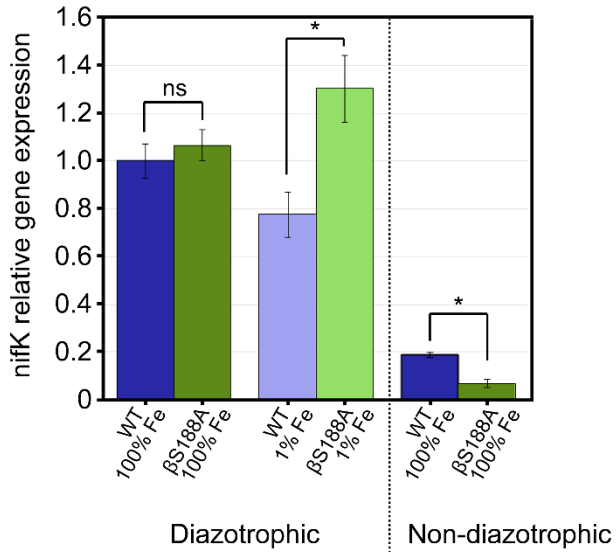

Figure S2. RT-qPCR of *nifK* gene expression of wt and βS188A *Av* cells. Left side: diazotrophic growth under varying Fe concentrations (100% and 1%). Right side: non-diazotrophic growth (100% Fe). Expression levels were normalized to the expression level of reference gene *rho*, and the presented data is relative to wt *Av* cells grown diazotrophically with 100% Fe. Experiment was carried out in technical triplicate of biological triplicate

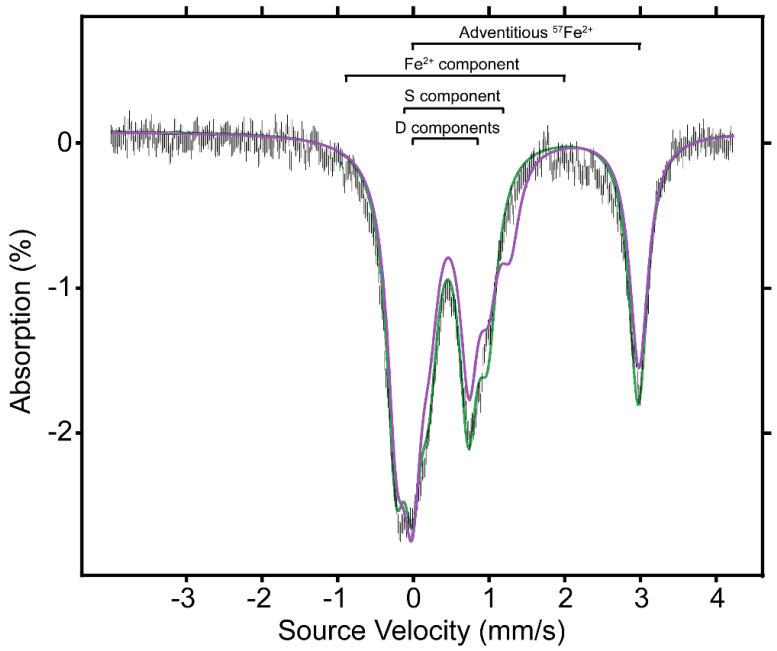

Figure S3. Mössbauer spectrum of *Av* βS188A MoFeP reconstituted with ^{57}Fe with multiple simulations. Spectrum (black) was recorded at 80 K and 54 mT. Oxidized βS188A was reconstituted with $^{57}Fe^{2+}$. The simulated fit reported in the main text is depicted in green. Simulation including the S component reported by McLean et al. (*J. Biol. Chem.* 1987, *262*, 12900), depicted in purple, did not fit the data as well.

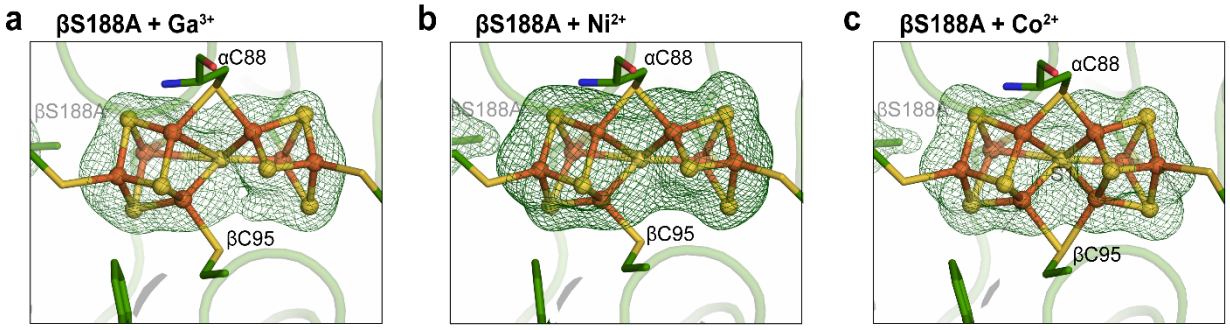

Figure S4. $2F_o–F_c$ omit maps of (a) Ga^{3+}, (b) Ni^{2+}, and (c) Co^{2+} reconstituted βS188A MoFeP contoured at 5σ corresponding to residue βSer188Ala, the P-cluster, and in (a) and (b) the location lacking anomalous density at position M1 with a tentatively modelled Na (not shown).

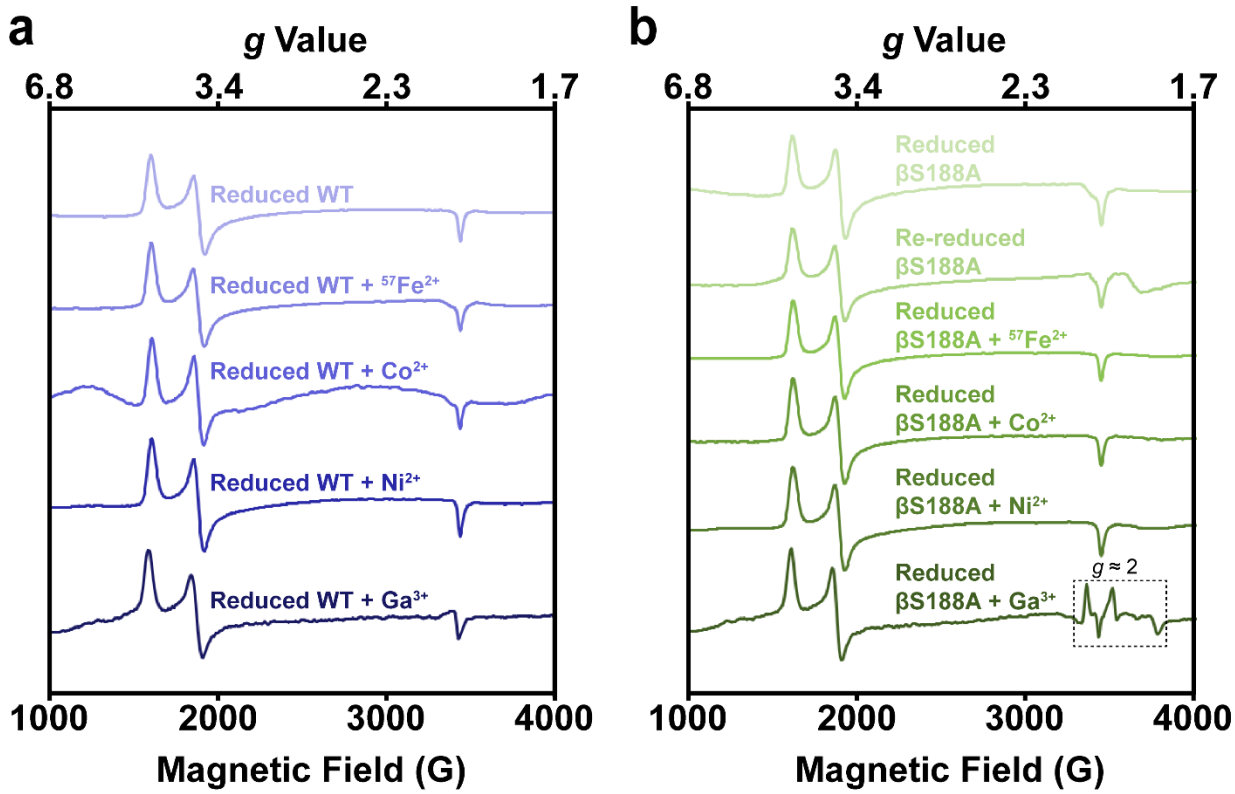

Figure S5. X-band EPR spectra collected at 4 K of DT-reduced wt and βS188A MoFeP incubated with heterometals. (a) DT-reduced wt MoFeP (top) soaked with $^{57}Fe^{2+}$, Co^{2+}, Ni^{2+}, and Ga^{3+} (top to bottom). (b) DT-reduced βS188A MoFeP (top) soaked with $^{57}Fe^{2+}$, Co^{2+}, Ni^{2+}, and Ga^{3+} (top to bottom). The Ga^{3+} spectrum has features in the $g \approx 2$ region that are similar to the features in the oxidized βS188A MoFeP spectrum that does not have any metals added.

Table S1. Mössbauer best fit resolution of βS188A after reconstitution of the isotopically $^{57}Fe^{2+}$ labeled MoFeP with natural abundance Fe^{2+}.

Feature	**ΔE_Q (mm/s)**	**δ (mm/s)**	**Relative area (%)**
D_1	0.777	0.616	22.7
D_2	0.763	0.362	39.7
Adventitious $^{57}Fe^{2+}$	3.210	1.373	37.7

Table S2. X-ray data collection and refinement statistics. Numbers in parentheses correspond to the highest resolution shell.

	Ox βS188A + Ga^{3+}	**Ox βS188A + Ni^{2+}**	**Ox βS188A + Co^{2+}**
PDB ID	8E3T	8E3U	8E3V
Data collection			
Beamline	SSRL 9-2	ALS 8.3.1	SSRL 9-2
Wavelength (Å)	1.19453, 1.19707	1.48484, 1.50832	1.60388, 1.61223
Space group	$P2_1$	$P2_1$	$P2_1$
Cell dimensions (Å)	76.61 127.97 107.27	77.26 129.01 107.76	77.13 130.04 107.76
Cell angles (°)	90.00 108.85 90.00	90.00 109.22 90.00	90.00 109.12 90.00
Resolution (Å)	54.13 - 2.20	79.89 - 1.99	54.80 - 2.00
No. unique reflections	87128	136546	127562
R_{merge}	0.190 (0.910)	0.501 (8.645)	0.134 (0.635)
Multiplicity	5.8 (5.2)	5.6 (5.2)	6.3 (6.0)
CC ½	0.986 (0.549)	0.939 (0.003)	0.993 (0.725)
$\langle I/\sigma(I) \rangle$	6.3 (1.9)	3.4 (0.5)	8.9 (2.6)
Completeness (%)	88.1 (88.8)	99.8 (99.9)	94.4 (93.0)
Refinement			
R_{work}/R_{free}	0.223 / 0.242	0.205 / 0.255	0.174 / 0.203
No. atoms	32075	31928	32582
Protein	30895	30811	30969
Ligand/ion	108	108	108
Solvent	1072	1009	1505
B-factors ($Å^2$)			
Protein	28.09	36.16	26.05
Ligand/ion	28.21	34.88	21.42
Solvent	29.76	37.24	30.58
R.m.s. deviations			
Bond lengths (Å)	0.034	0.037	0.034
Bond angles (°)	0.97	1.6	0.94
Clashscore	6.59	8.21	5.37
Ramachandran plot (%)			
Favored	95.51	96.05	96.61
Outliers	0.25	0.3	0.25
Rotamer outliers (%)	2.92	2.95	2.26

Table S3. ICP-MS analysis of MoFeP reconstituted with $^{57}Fe^{2+}$, Co^{2+}, Ni^{2+}, and Ga^{3+}. The listed values are averages of five replicate measurements of a single sample.

	WT MoFeP		**βSer188Ala MoFeP**		**Theoretical value if one metal-center is inserted per P-cluster***	**Theoretical value if two metal-centers are inserted per P-cluster***
	DT-reduced	IDS-oxidized	DT-reduced	IDS-oxidized		
^{56}Fe:^{57}Fe	48.0	39.0	34.0	4.8	14.6	7.0
Fe:Co	310.9	209.0	69.3	19.2	14	6.5
Fe:Ni	339.3	286.3	82.6	22.2	14	6.5
Fe:Ga	62.4	39.0	5.6	4.9	14	6.5

n/d represents that the reconstitution metal ($^{57}Fe^{2+}$, Co^{2+}, Ni^{2+}, or Ga^{3+}) was not detected/ below the detection limit

**Calculations determined using 30 Fe atoms per non-reconstituted MoFeP, and 2% natural abundance ^{57}Fe.*