

## Supporting Information

Interaction of the *Streptomyces* Wbl protein WhiD with the principal sigma factor  $\sigma^{\text{HrdB}}$  depends on the WhiD [4Fe-4S] cluster

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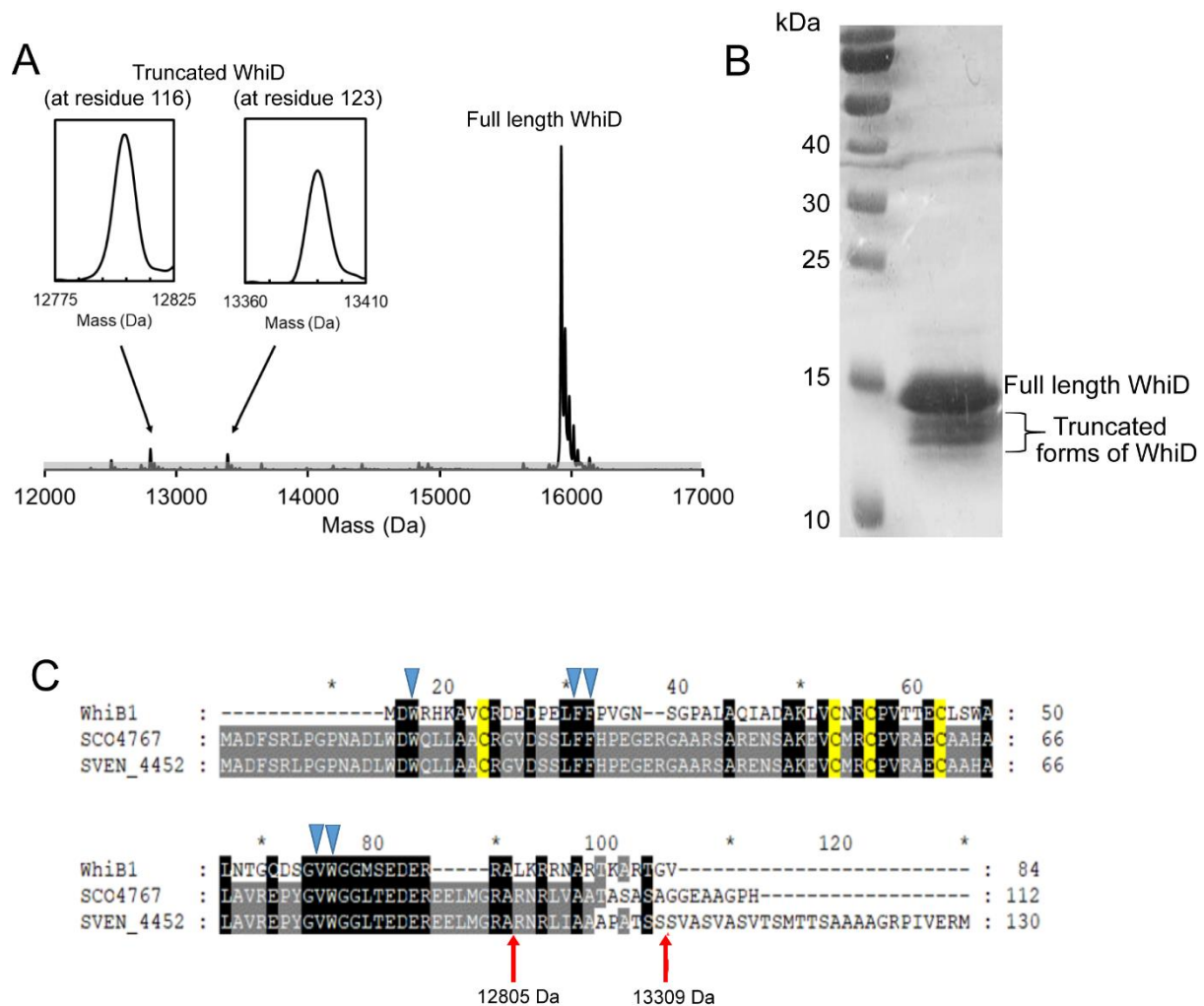
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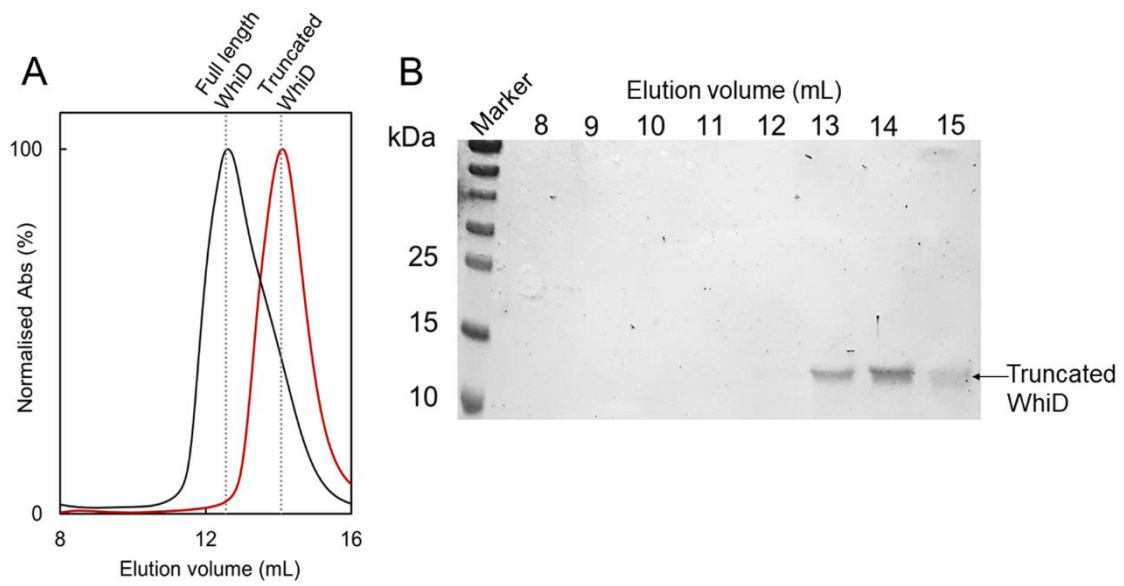
**Supporting references**

**Table S1.** Strains, plasmids and oligonucleotides.

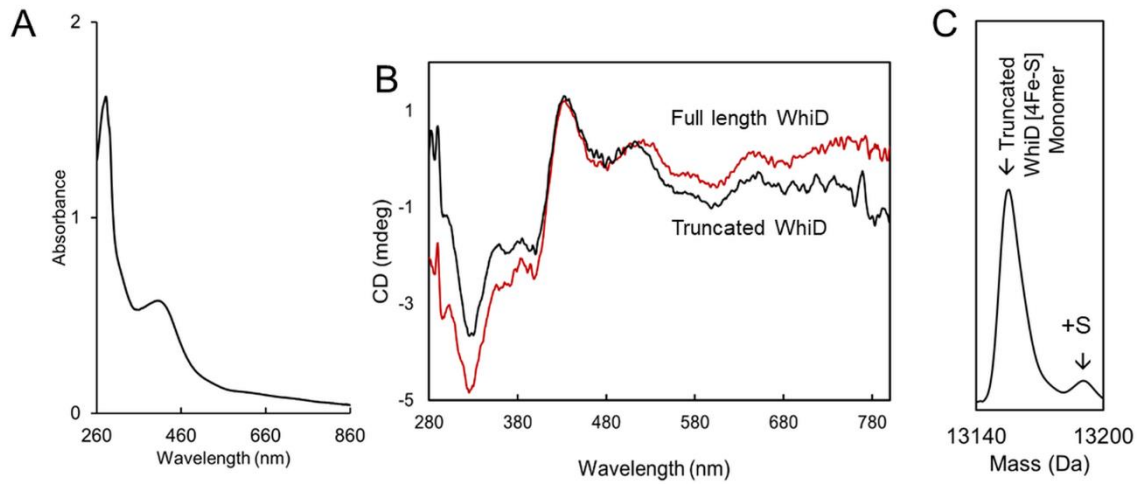
	<b>Description</b>	<b>Reference</b>
<b>Strains</b> BTH101	<i>F</i> – <i>cya-99 araD139 galE15 galK16 rpsL1 (Strr) hsdR2 mcrA1 mcrB1</i>	30
<b>Plasmids</b> pKT25 pUT18C pIJ10921 pIJ10922 pIJ10923 pIJ10925 pIJ10926 pET28a  pET15b  pMSW1 pMSW2 pMSW3	  <i>whiD</i> cloned into pKT25 <i>hrdB</i> cloned into pUT18C <i>hrdD</i> cloned into pUT18C <i>hrdB4</i> cloned into pUT18C <i>hrdBΔ4</i> cloned into pUT18C Expression vector with N-terminal hexahistidine affinity tag Expression vector with N-terminal hexahistidine affinity tag  <i>whiD</i> cloned into pET28a <i>hrdB</i> (encoding domain 4) cloned into pET15b <i>hrdD</i> (encoding domain 4) cloned into pET15b	30 30 This work This work This work This work This work Novagen  Novagen  This work This work This work
<b>Oligonucleotides</b> whiD_BACTH_F whiD_BACTH_R hrdB_BACTH_F hrdB_BACTH_R hrdD_BACTH_F hrdD_BACTH_R hrdB4_BACTH_F hrdB4_BACTH_R hrdBd4_BACTH_F hrdBd4_BACTH_R pUT18C_F	GCTCTAGAGATGGCAGATTTCTCCCGCCTT CGCGGATCCTCCATGCGCTCCACAATCGGAC GCTCTAGAGGTGACGGCCGAGGAAGGAATA CCGGTACCCGGTCGAGGTAGTCGCGCAG GCTCTAGAGATGGCAACCCGTGCCGTCG CCGGTACCCGGGCCACCGCGTCTGAAGCC GCTCTAGAGATGCCGGCCGACGCGGTGAGC CCGGTACCCGGTCGAGGTAGTCGCGCAGCACCTGC GCTCTAGAGATGACGGCCGAGGAAGGAATACAGCA CCGGTACCCGAACGACCGCCTCGGAGTCCTC GTGCCGAGCGGACGTTCTGA	



**Figure S1. Mass analysis and sequence alignment of *Sν*WhiD.** (A) Deconvoluted LC-MS spectrum of as isolated *Sν*WhiD, revealing the major species as *Sν*WhiD lacking its N-terminal Met residue. Minor components of two truncated forms are apparent. Inset are spectra of the truncated form plotted on an expanded mass scale. *Sν*WhiD (10  $\mu$ M) was in an aqueous mixture of 2% (v/v) acetonitrile, 0.1% (v/v) formic acid. (B) SDS-PAGE analysis of as isolated *Sν*WhiD, revealing full length and truncated forms. (C) Amino acid sequence alignment of *Sν*WhiD (SVEN\_4452) with *S. coelicolor* WhiD (SCO4767) and *M. tuberculosis* WhiB1. Conserved Cys residues are highlighted in yellow. Residues shown to be important for the interaction of WhiB1 with domain 4 of SigA are indicated by blue triangles. Positions of truncation for smaller versions of *Sν*WhiD are indicated.



**Figure S2. Gel filtration analysis of truncated *Sν*WhiD.** (A) Gel filtration elution profiles ( $A_{280\text{ nm}}$ ) of truncated [4Fe-4S] WhiD (red line). The elution profile for full length [4Fe-4S] WhiD is shown for comparison (black line). (B) SDS-PAGE analysis of elution fractions for truncated *Sν*WhiD, spanning the elution volume 8-15 mL. Truncated *Sν*WhiD (32  $\mu\text{M}$ ) was in 50 mM Tris, 300 mM NaCl, pH 8.



**Figure S3. Gel filtration analysis of truncated *S $\nu$ WhiD*.** (A) UV-visible absorbance and (B) CD spectroscopic characterisation of truncated WhiD. In (B) spectra of truncated and full length *S $\nu$ WhiD* are shown in black and red, respectively. (C) Deconvoluted non-denaturing ESI-MS. The spectrum features a major peak due to truncated [4Fe-4S] WhiD. A possible sulfur adduct is indicated. For spectroscopy experiments, truncated *S $\nu$ WhiD* (32  $\mu$ M) was in 50 mM Tris, 300 mM NaCl, pH 7.2. For non-denaturing ESI-MS, *S $\nu$ WhiD* (15  $\mu$ M) was in 250 mM ammonium acetate, pH 7.2.

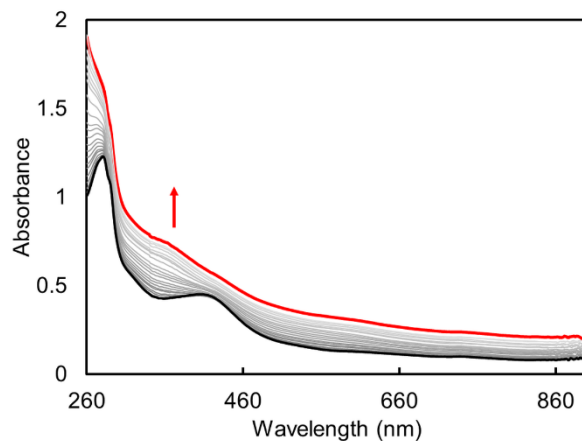
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      *           20           *           40           *           60           *           80
Sv WhiD : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. flavo : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. cb2009 : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. aureus : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. wac1280 : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. griseo : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. ss : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
S. exfoli : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
Sc WhiD : MADFSRLPGPNADLWDWQLLAACRGVDSSLFFHPEGERGAARSARENSAKEVCMRCPVRAECAAHALAVREPYGVWGGLTE : 81
MtbWhiB1 : -----MDWRHKAVCRDELPQLFFPVGNS--GPAIAQIADAKIVCNRCPTTECLSWALNTGQDSGVWGGMSE : 65

      (i)           (ii)
      *           *           *           *
Sv WhiD : DEREELMGRARNRLITAAEATS--SSVASVASVMSMTTSAAA-GRPIVERM : 130
S. flavo : DEREELMGRARNRLITAAETAS---VASTASVTSMTTSASAA-GRPIAERM : 129
S. cb2009 : DEREELMGRARNRLITAAETT---PSVASAASVTSMTTASAA-GRPIVERM : 129
S. aureus : DEREELMGRARNRLITAAETAS---VASTSATSMTTSASAT-GRPIVERM : 129
S. wac1280 : DEREELMGRARNRLITAAETAAP---VTIVTSVTSMTTSASAA-GRPIVERT : 129
S. griseo : DEREELMGRARNRLITAAETASSAPSVA-SMTS-VTSMTTSASAA-GRPSVERT : 132
S. ss : DEREELMGRARNRLISAAATAPTAGTAS-MTPMTPMTTSAAAT-GRPIVERM : 132
S. exfoli : DEREELMGRARNRLITAAETAAP---VTIVTSVTSMTTSASAAA-GRPIVERM : 130
Sc WhiD : DEREELMGRARNRLVATASASAGGEAAGPH----- : 112
Mtb WhiB1 : DERRALK-RRNARTKARTGV----- : 84

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**Figure S4. The C-terminal extension of SvWhiD is found in WhiD homologues from other *Streptomyces* species.** Alignment of SvWhiD with putative WhiD sequences from *Streptomyces flavochromogenes*, *Streptomyces* sp. CB02009, *Streptomyces aureus*, *Streptomyces* sp. WAC01280, *Streptomyces griseoluteus*, *Streptomyces* sp. SS, and *Streptomyces exfoliates*. *Streptomyces coelicolor* WhiD (*ScWhiD*) and *Mycobacterium tuberculosis* WhiB1 (*Mtb WhiB1*) are shown for comparison. Conserved Cys residues are highlighted in yellow. C-terminally truncated forms of SvWhiD as indicated by arrows correspond to i) 12,805 Da and ii) 13,309 Da. Putative dimerization helix is boxed in red.



**Figure S5. Reaction of SνWhiD with NO.** UV-visible absorbance titration of [4Fe-4S] SνWhiD with up to 20 NO per cluster. Black and red spectra correspond to the first and final spectra, respectively, with intervening spectra in grey. The arrow indicates the direction of change due to a combination of absorbance changes and scattering due to mild precipitation of the protein. WhiD (21  $\mu$ M) was in 50 mM Tris, 300 mM NaCl, pH 7.2.

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CLONE 1 -----
CLONE 2 390 -----IRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLGREPTPEELAKELDMTPEKVI 450
CLONE 3 390 -----IRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLGREPTPEELAKELDMTPEKVI 450
CLONE 4 368 -----IRAVEKFDYTKGYKFSTYATWWIRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLGREPTPEELAKELDMTPEKVI 450
CLONE 5 368 -----IRAVEKFDYTKGYKFSTYATWWIRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLGREPTPEELAKELDMTPEKVI 450
HrdB 361 QEGNGLIRAVEKFDYTKGYKFSTYATWWIRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLGREPTPEELAKELDMTPEKVI 450
          *                               *

CLONE 1 488 -----DAVSFTLLQEQLHSVLDLTLEREAGVSMRFGLTDGQPKTLDEIGKVYGVTR 540
CLONE 2 451 EVQKYGREPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDLTLEREAGVSMRFGLTDGQPKTLDEIGKVYGVTR 540
CLONE 3 451 EVQKYGREPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDLTLEREAGVSMRFGLTDGQPKTLDEIGKVYGVTR 540
CLONE 4 451 EVQKYGREPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDLTLEREAGVSMRFGLTDGQPKTLDEIGKVYGVTR 540
CLONE 5 451 EVQKYGREPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDLTLEREAGVSMRFGLTDGQPKTLDEIGKVYGVTR 540
HrdB 451 EVQKYGREPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDLTLEREAGVSMRFGLTDGQPKTLDEIGKVYGVTR 540
                                     *

CLONE 1 541 RIRQIESKTMSKLRHPSRSQVLRDYL 567
CLONE 2 541 RIRQIESKTMSKLRHPSRSQVLRDYL 567
CLONE 3 541 RIRQIESKTMSKLRHPSRSQVLRDYL 567
CLONE 4 541 RIRQIESKTMSKLRHPSRSQVLRDYL 567
CLONE 5 541 RIRQIESKTMSKLRHPSRSQVLRDYL 567
HrdB 541 RIRQIESKTMSKLRHPSRSQVLRDYL 567

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**Figure S6. T18C- $\sigma^{\text{HrdB}}$  fusion start points for the five positive  $\sigma^{\text{HrdB}}$  clones identified from a shotgun *S. venezuelae* sonicated DNA genomic library using T25-WhiD as bait. The sequences of the five clones are aligned against the C-terminal protein sequence of  $\sigma^{\text{HrdB}}$  with asterisks indicating the fusion start points. Domain 4 ( $\sigma^{\text{HrdB}}_4$ ) is underlined.**



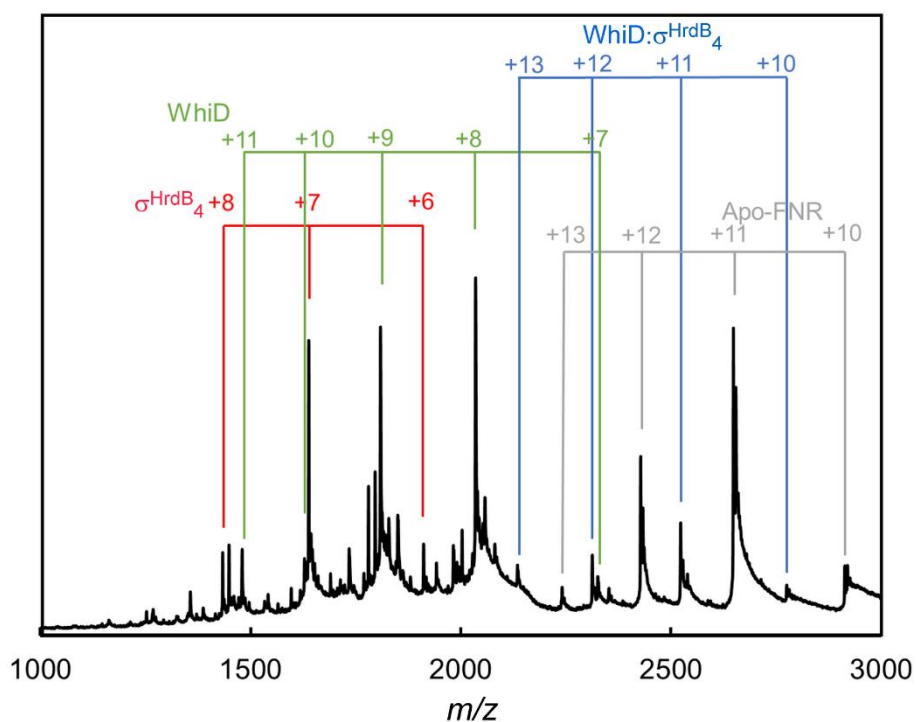
$\sigma^{\text{HrdB}}_4$ :

MGSSHHHHHSSGLVPRGSHMPADAVSFTLLQEQLHSVLDTLSEREAGVSMRFGLTDGQPKTLDEIG  
KVYGVTRERIRQIESKTMSKLRHPSRSQVLRDYLD

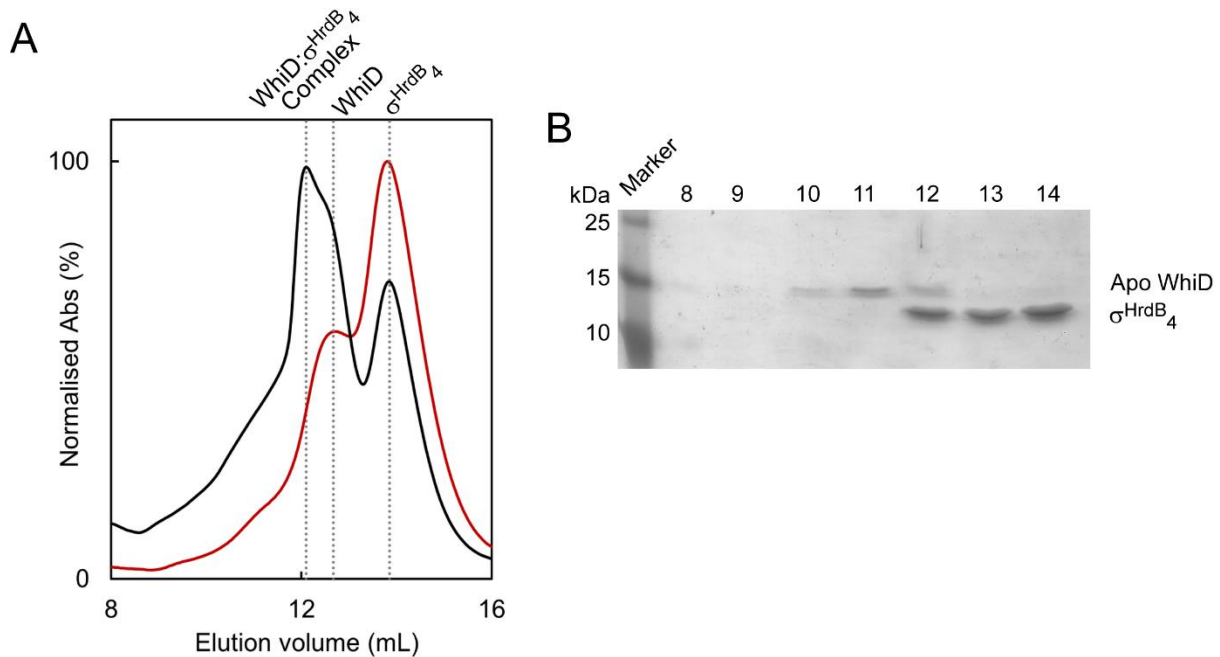
$\sigma^{\text{HrdD}}_4$ :

MGSSHHHHHSSGLVPRGSHMPEQSVLTLLRSEELDDLIDKLDHRTASIIIRMRYGIEDGRERTLTEVG  
KEHGLTRERIRQIEKHALLELKKMAHDTGFDAVA

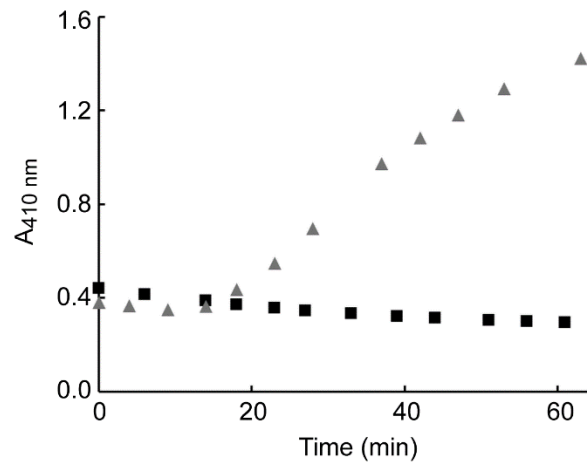
**Figure S7. Amino acid sequence of  $\sigma^{\text{HrdB}}_4$  and  $\sigma^{\text{HrdD}}_4$  used in studies of interactions with *SvWhiD*.**  
The tag sequence is indicated in green. For both proteins, the N terminal methionine was cleaved during over-expression.



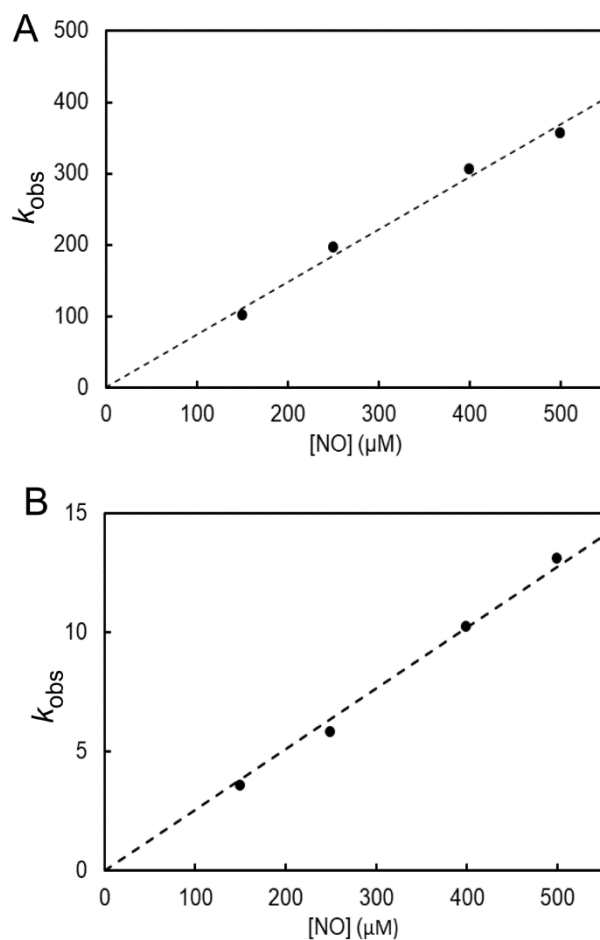
**Figure S8. Non-denaturing mass spectrometry of mixtures containing [4Fe-4S] *SνWhiD* and  $\sigma^{\text{HrdB}}_4$ .** *m/z* spectra of a solution containing *SνWhiD* and  $\sigma^{\text{HrdB}}_4$  (present in 4-fold excess). Ions due to unbound proteins, and the *SνWhiD*- $\sigma^{\text{HrdB}}_4$  complex are as indicated. Ions due to apo- I151A FNR are also present, as this species was used as an internal intensity standard for measuring changes in the concentration of the complex. *SνWhiD* (16  $\mu\text{M}$  in cluster) and  $\sigma^{\text{HrdB}}_4$  were in 250 mM ammonium acetate, pH 7.2.



**Figure S9. Apo-*SvWhiD* does not bind to domain 4 of  $\sigma^{\text{HrdB}}$ , the principal sigma factor of *S. venezuelae*.** (A) Gel filtration elution profiles ( $A_{280 \text{ nm}}$ ) of a mixture of apo-*SvWhiD* and  $\sigma^{\text{HrdB}}_4$  in a 1:5 ratio (red line). The elution profile of a mixture of cluster-containing *SvWhiD* and  $\sigma^{\text{HrdB}}_4$  in a 1:5 ratio is shown for comparison (black line). (B) Fractions spanning the elution volume 8-14 mL were analysed by SDS-PAGE and silver stained. Apo-*SvWhiD* (48  $\mu\text{M}$ ) and  $\sigma^{\text{HrdB}}_4$  (240  $\mu\text{M}$ ) were in 50 mM Tris, 300 mM NaCl pH 7.2. Note that the decrease in absorbance at 280 nm for *SvWhiD* is due to the loss of the [4Fe-4S] cluster, which contributes significantly to  $A_{280 \text{ nm}}$ .



**Figure S10. Sensitivity of *SνWhiD* to O<sub>2</sub> is modulated by complex formation with  $\sigma^{\text{HrdB}}_4$ .** Plot of A<sub>410 nm</sub> as a function of time following exposure of *SνWhiD* to O<sub>2</sub> in the absence (grey triangles) and presence (black squares) of  $\sigma^{\text{HrdB}}_4$  (two-fold excess). *SνWhiD* (25  $\mu\text{M}$ ) was in 50 mM Tris, 300 mM NaCl, pH 7.2 buffer.



**Figure. S11. Stopped-flow kinetic studies of the nitrosylation of the [4Fe-4S] cluster of *SvWhiD*.** Plots of observed rate constants for the first (A) and second (B) phases of  $\Delta A_{360\text{ nm}}$  following addition of varying concentrations of NO to *SvWhiD* (Fig. 10 of the main paper). For both, a first order dependence was observed, indicating that the rate-limiting step of these reactions involves NO. Data are similar to those previously reported for nitrosylation reactions of other Wbl proteins (see main text). Experiments were performed with *SvWhiD* (10  $\mu\text{M}$  in [4Fe-4S]) in 50 mM Tris, 300 mM NaCl pH 7.2 at 25  $^{\circ}\text{C}$ .

### Supporting references

30. Karimova, G., Pidoux, J., Ullmann, A., and Ladant, D. (1998) A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5752-5756.