A single outer-sphere amino-acid substitution turns on the NO reactivity of a hemerythrin-like protein

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Supporting Information

Table S1. Oligonucleotide primers used for site-directed mutagenesis (mutated codons are in bold).

Mutation	Primers (forward and reverse)
T47F	5'-GGAATTGACGATCCAT**TTT**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCGA**AAA**TGGATCGTCAATTCC-3'
T47Y	5'-GGAATTGACGATCCAT**TAT**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCG**ATA**ATGGATCGTCAATTCC-3'
T47W	5'-GGAATTGACGATCCAT**TGG**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCG**CCA**ATGGATCGTCAATTCC-3'
T47V	5'-GGAATTGACGATCCAT**GTG**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCG**CAC**ATGGATCGTCAATTCC-3'
T47L	5'-GGAATTGACGATCCAT**TTG**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCG**CAA**ATGGATCGTCAATTCC-3'
T47S	5'-GGAATTGACGATCCAT**AGC**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCG**GCT**ATGGATCGTCAATTCC-3'
T47A	5'-GGAATTGACGATCCAT**GCC**CGGCTCGAGGAGG-3' 5'-CCTCCTCGAGCCG**GGC**ATGGATCGTCAATTCC-3'

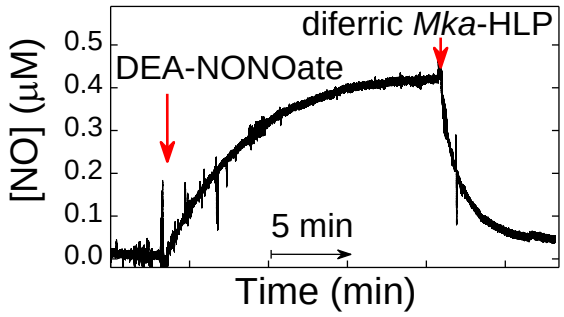

Figure S1. Room-temperature measurement of NO binding after addition of 4 μM diferric *Mka*-HLP using an NO sensing probe (WPI ISO-NP, World Precision Instruments) demonstrating sub-micromolar binding affinity.

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                    11 14                                  42 45  49    54
Mka-HLP   VNAYEVLKEHHVVIKGLGRKISEAP-VNSEERHALFDELLIELDIHFRIEDDLYYPALSA  59
Avi-HLP   MNAIELLKHDHETLKQVFERLGKTTERGVKTRGELMRHLHEELTIHTRLEEEIFYPAFKA  60
          :** *:**..* .:* : ..:.::     : *  *: .*  ** ** *:*:::***: *

                          71 74                                105 109
Mka-HLP   ATK-----LIAVAHAEHRQVIDQ-LSVLLRTPQSEPGYEDEWNSFKTVLEAHADEEERDM  113
Avi-HLP   ASGKEGDILFHEATEEHRAVEALILPDLEKTEPSSVEFAGRAKVLKDMVGHHIEEEEQEM  120
          *       *   *  *** *    *  * :*  *   : .. : :* ::  * :*** :*


Mka-HLP   IPAPPEVKITDAELEELGEKMAARMEQYRGSALYKLRTKGRAALVRSL      161
Avi-HLP   LPKAGEL-LGEERLEELGAQMETRRKELRRQ------LSGERAA----      157
          :*   *: :    *****  *  * :: *          *  *
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Figure S2. Sequence alignment of *Mka*-HLP and *Avi*-HLP (WP_012698725.1). The two paralogs share an identity of approximately 30% in their amino acid sequence. Underlined sections of *Mka*-HLP's sequence correspond to α-helical portions of the folded protein as seen in the crystal structure. Residues coordinating the iron centers are in red, second coordination sphere residues are in green, and residues peripheral to the diiron center are in green.

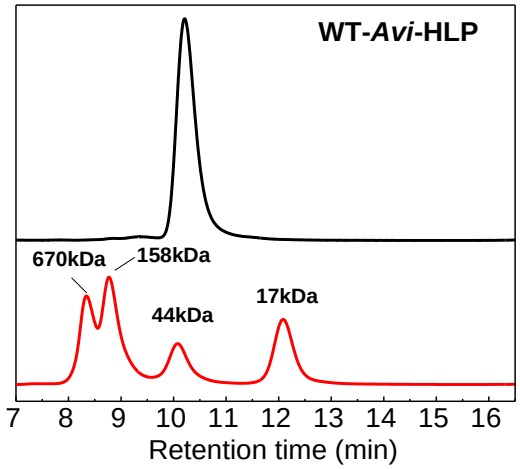

Figure S3. Size exclusion chromatography elution profile monitored at 280 nm for diferric *Avi*-HLP in 100 mM phosphate buffer, pH 7.5 (black trace) and for molecular standards (red trace, thyroglobin, γ-globulin, ovalbumin, myoglobin, and vitamin B_{12}).

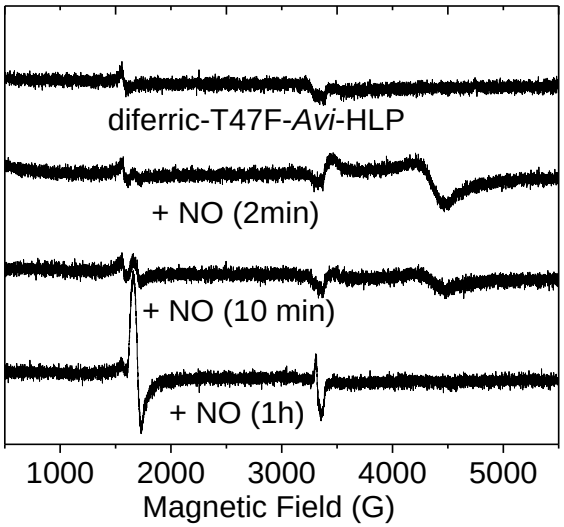

Figure S4. EPR spectra of diferric T47F-*Avi*-HLP before (top trace) and after exposure to 1 atm NO. Experimental conditions: protein concentration, 100 μM; temperature, 11 K: microwave power, 30 dB; microwave frequency, 9.45 GHz; modulation frequency, 100 kHz; modulation amplitude, 10 G.

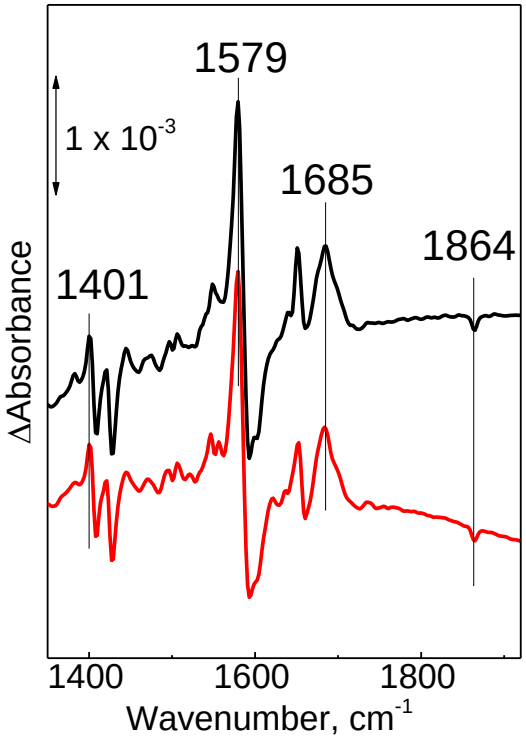

Figure S5. 30-K photolysis FTIR difference spectra of the NO adduct of diferric T47F-*Avi*-HLP (first difference spectrum, black; second difference spectrum obtained after annealing the samples above 40 K, red).

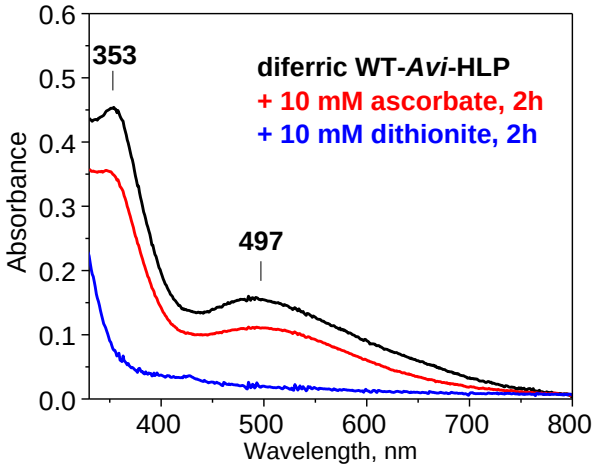

Figure S6. Room-temperature UV-vis spectra of diferric WT-*Avi*-HLP (black) and after a 2-h exposure to 10 mM ascorbate (red) or 10 mM dithionite (blue).

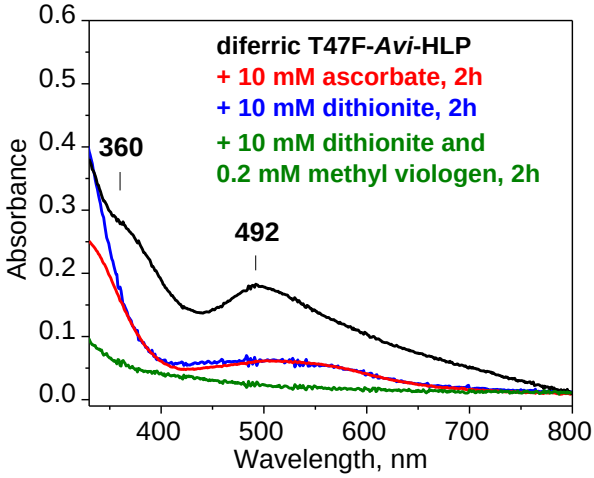

Figure S7. Room-temperature UV-vis spectra of diferric T47F-*Avi*-HLP (black) and after a 2-h exposure to 10 mM ascorbate (red), 10 mM dithionite (blue), or 10 mM dithionite and 0.2 mM methyl viologen (green).

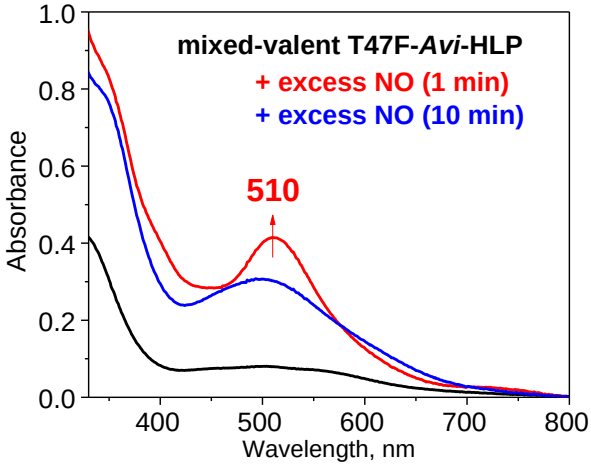

Figure S8. Room-temperature UV-vis spectra of mixed-valent T47F-*Avi*-HLP (black) immediately after exposure to 1 atm NO (red) and after 10-min incubation (blue).

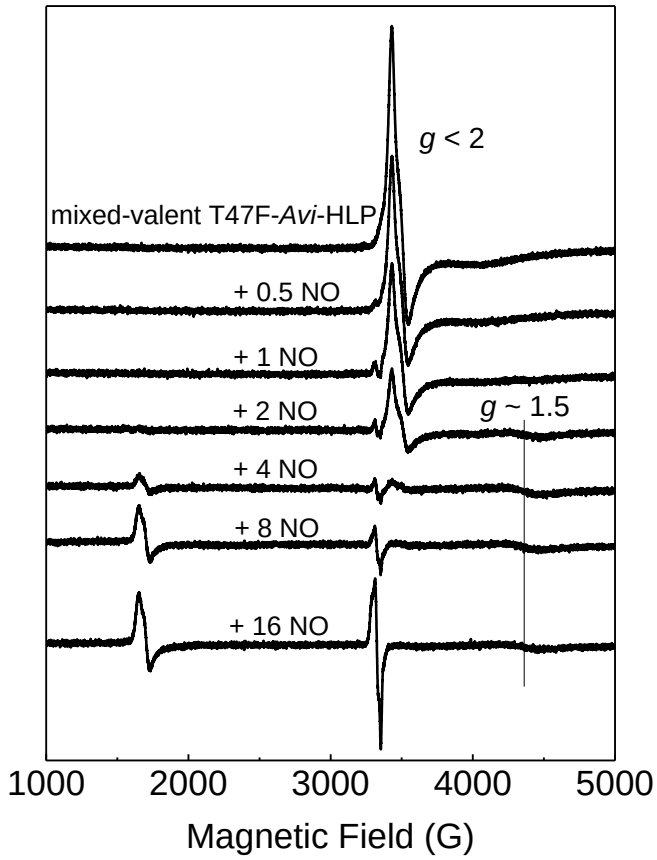

Figure S9. EPR spectra of mixed-valent T47F-*Avi*-HLP before (top trace) and after exposure to increasing NO concentrations. Experimental conditions: protein concentration, 50 μM; temperature, 13 K: microwave power, 30 dB; microwave frequency, 9.45 GHz; modulation frequency, 100 kHz; modulation amplitude, 10 G.

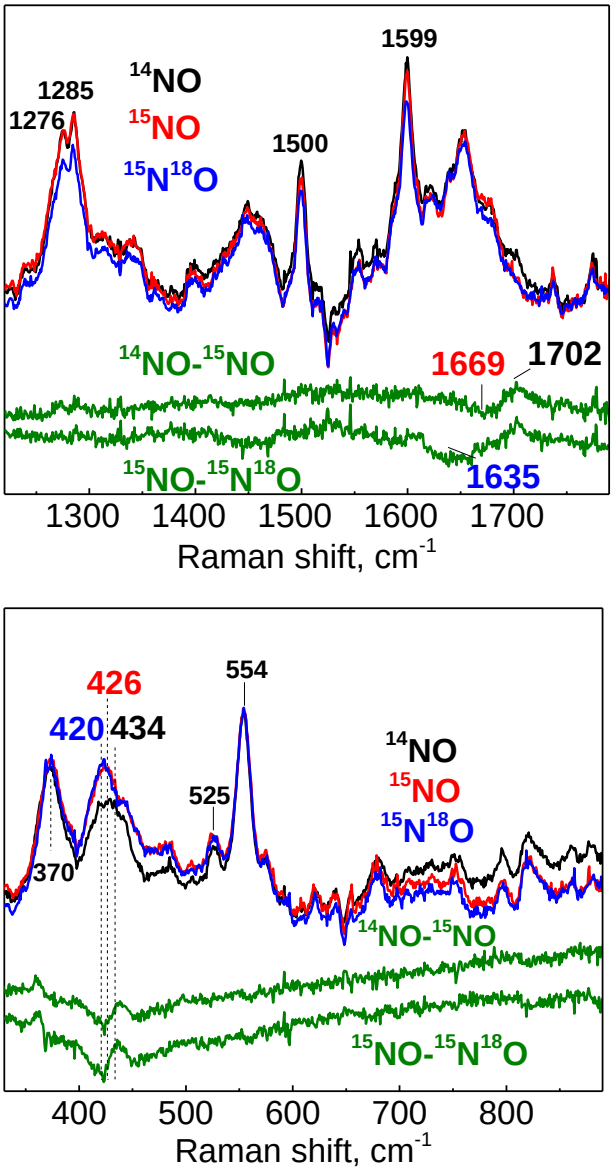

Figure S10. Room-temperature RR spectra of the product of the reaction of mixed-valent T47F-*Avi*-HLP with NO. The excitation wavelength used was 457 nm and the protein concentration 3 mM.

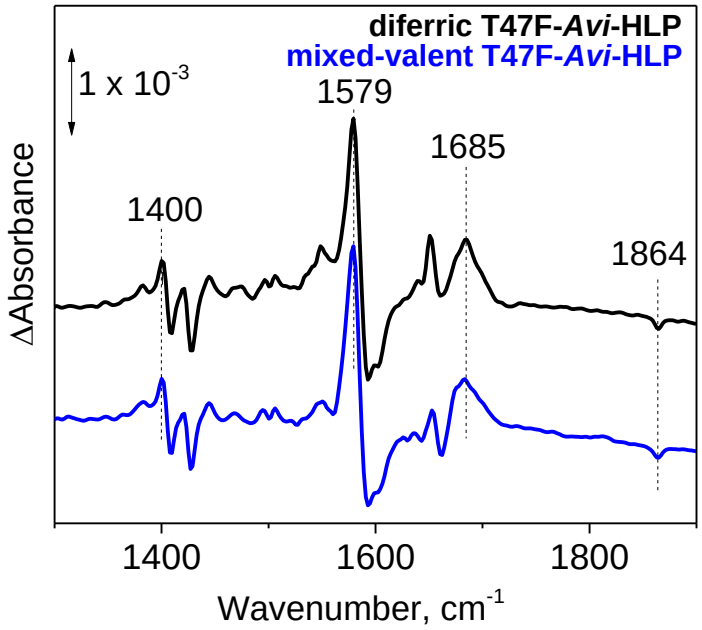

Figure S11. 30-K photolysis FTIR difference spectra of the NO adducts formed with diferric (black) and mixed-alent (blue) T47F-*Avi*-HLP.

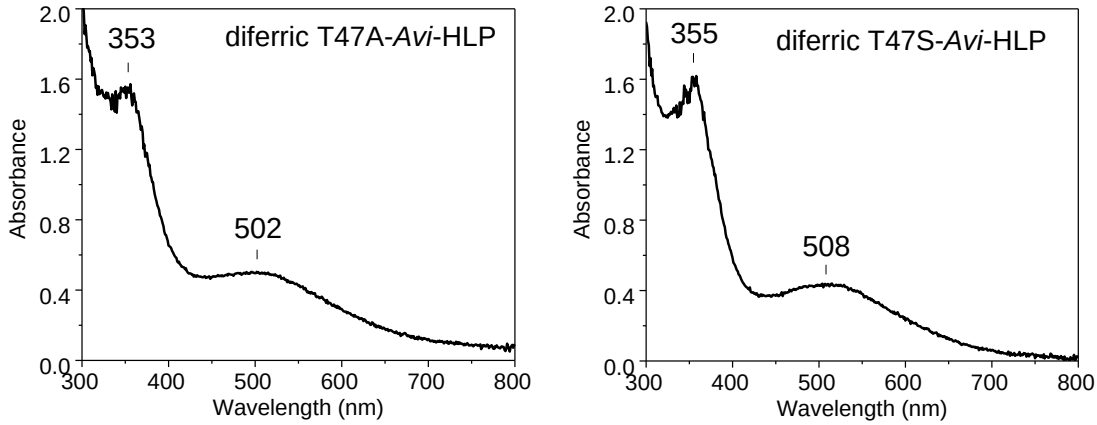

Figure S12. Room-temperature UV-vis spectra of diferric T47A- and T47S-*Avi*-HLP variants.

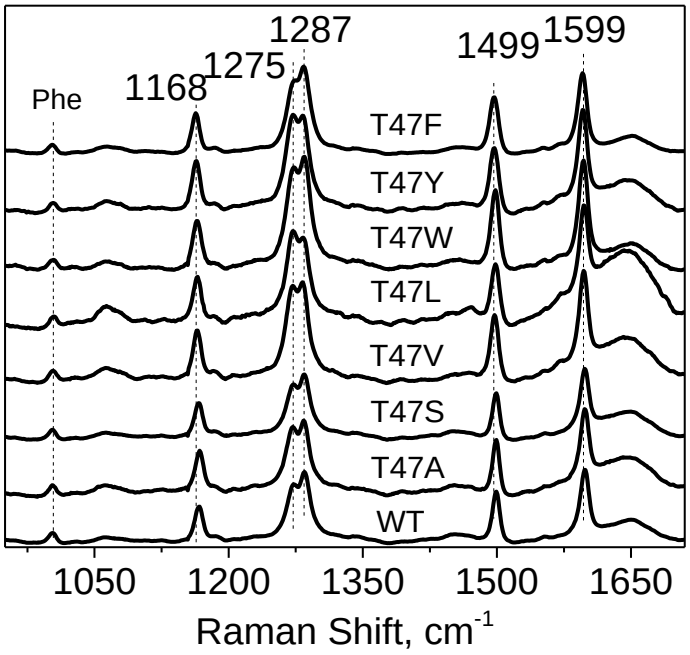

Figure S13. Room-temperature RR spectra of diferric WT-Avi-HLP and T47 variants obtained with a 647-nm excitation wavelength. All spectra are normalized on the non-resonant Phe band at 1004 cm^{-1}.

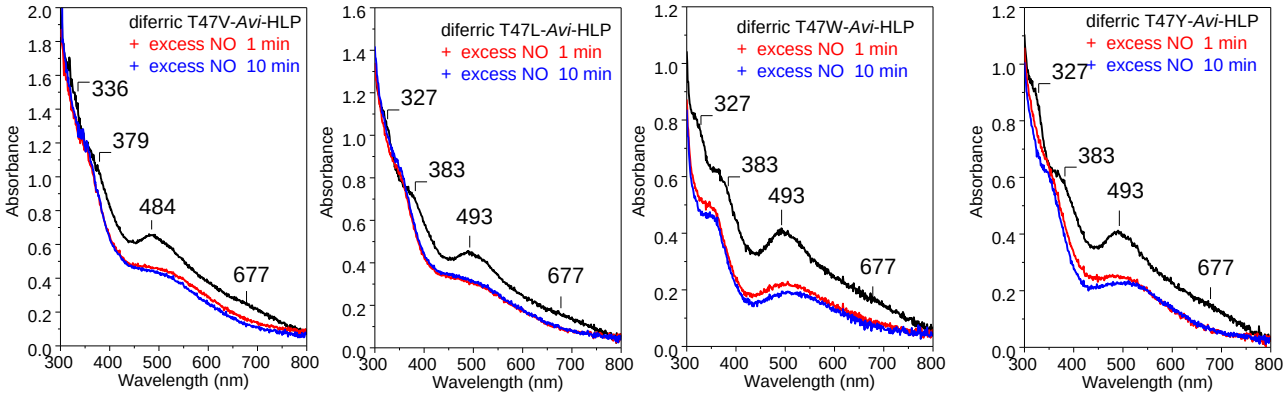

Figure S14. Room-temperature UV-vis spectra of diferric T47 variants before (black) and immediately after exposure to 1 atm NO (red) and after 10-min incubation (blue).

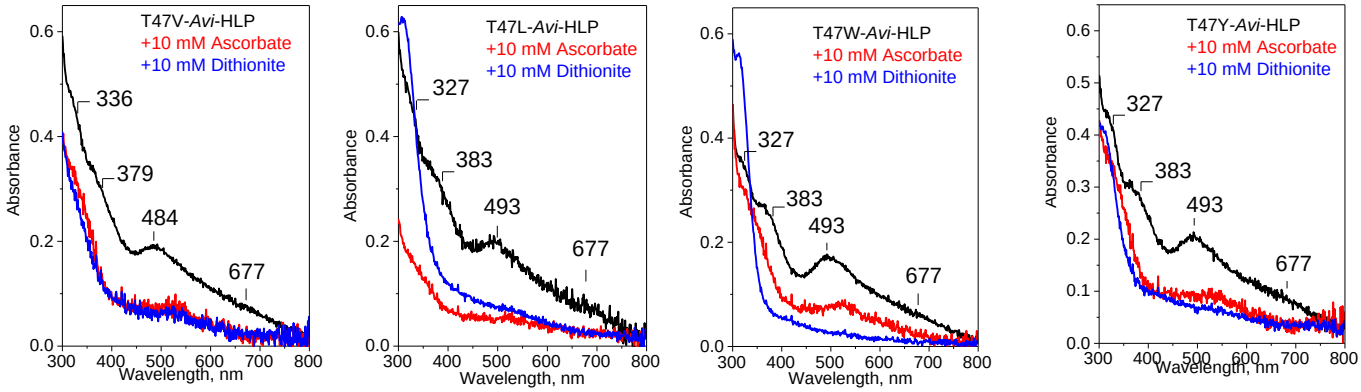

Figure S15. Room-temperature UV-vis spectra of diferric T47 variants before (black) and after a 2-h exposure to 10 mM ascorbate (red) and 10 mM dithionite (blue).