SUPPLEMENTARY INFORMATION

THE AER2 RECEPTOR FROM *VIBRIO CHOLERAE* IS A DUAL PAS-HEME OXYGEN SENSOR

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RUNNING TITLE: *V. cholerae* Aer2 is a Dual PAS-Heme O_2 Receptor

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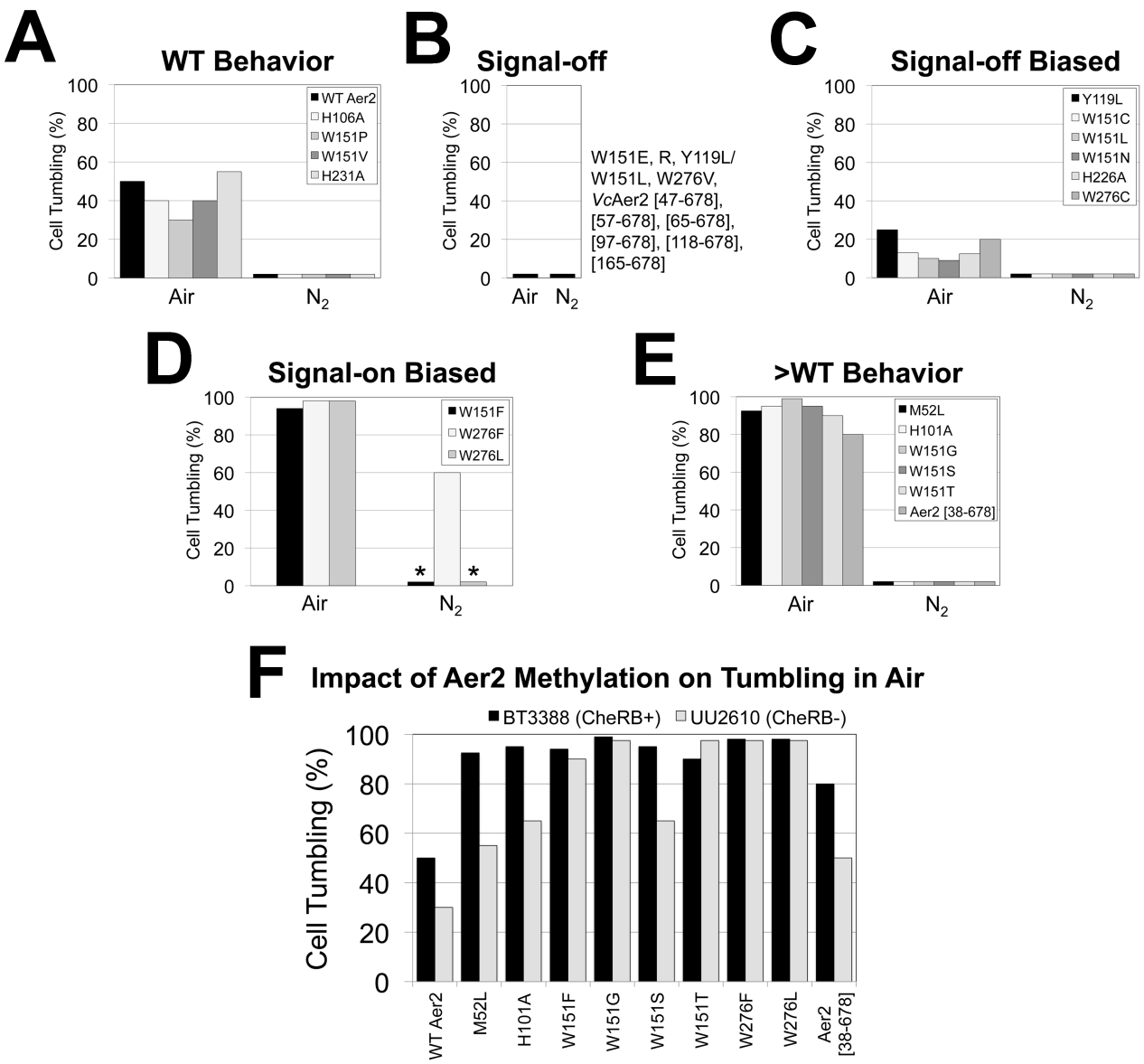

Fig. S1. Average percentage of *E. coli* BT3388 (A-F) and *E. coli* UU2610 (F) cells tumbling in the presence of full-length and N-terminally-truncated *Vc*Aer2 proteins. All cultures contained 25 μg ml^{-1} ALA and were induced for 2 h with 200 μM IPTG. Percentages represent steady-state tumbling in air (A-F) and in N_2 (A-E). All signal-off receptors in (B) are represented by the one set of bar graphs. Compared with WT *Vc*Aer2, the signal-on biased receptors containing W151F and W276L (D) both had 20-50 sec smooth-swimming delays in N_2 that are represented by an asterisk.

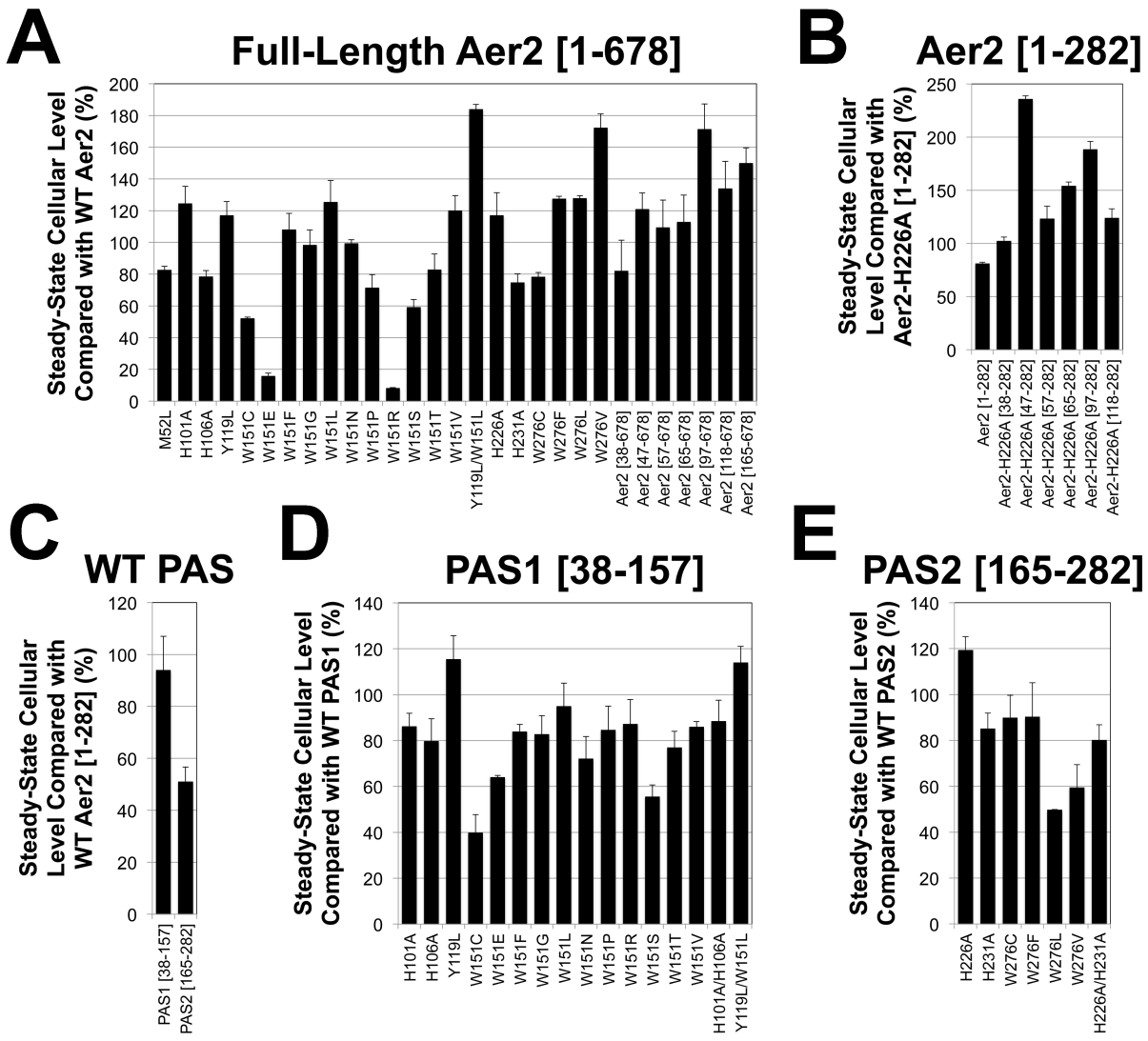

Fig. S2. Average steady-state cellular levels of full-length *Vc*Aer2 proteins and peptides in *E. coli* BT3388. Protein levels were determined from Western blots. Error bars represent standard deviations from two to five experiments.

A. Steady-state levels of full-length *Vc*Aer2 proteins and N-terminal truncation mutants compared with full-length WT *Vc*Aer2 after inducing with 50 μM IPTG. *Vc*Aer2-W151E and *Vc*Aer2-W151R were instead induced with 200 μM IPTG.

B. Steady-state levels of the N-terminally truncated PAS1-2 peptides containing H226A compared with *Vc*Aer2-H226A [1-282] after inducing with 50 μM IPTG.

C. Steady-state levels of WT PAS1 [38-157] and PAS2 [165-282] peptides compared with WT *Vc*Aer2 [1-282] after inducing with 100 μM IPTG.

D. Steady-state levels of PAS1 peptides compared with WT PAS1 [38-157] after inducing expression with 100 μM IPTG. PAS1-W151E, PAS1-W151N, and PAS1-W151R were instead induced with 200 μM IPTG.

E. Steady-state levels of PAS2 peptides compared with WT PAS2 [165-282] after inducing expression with 100 μM IPTG.

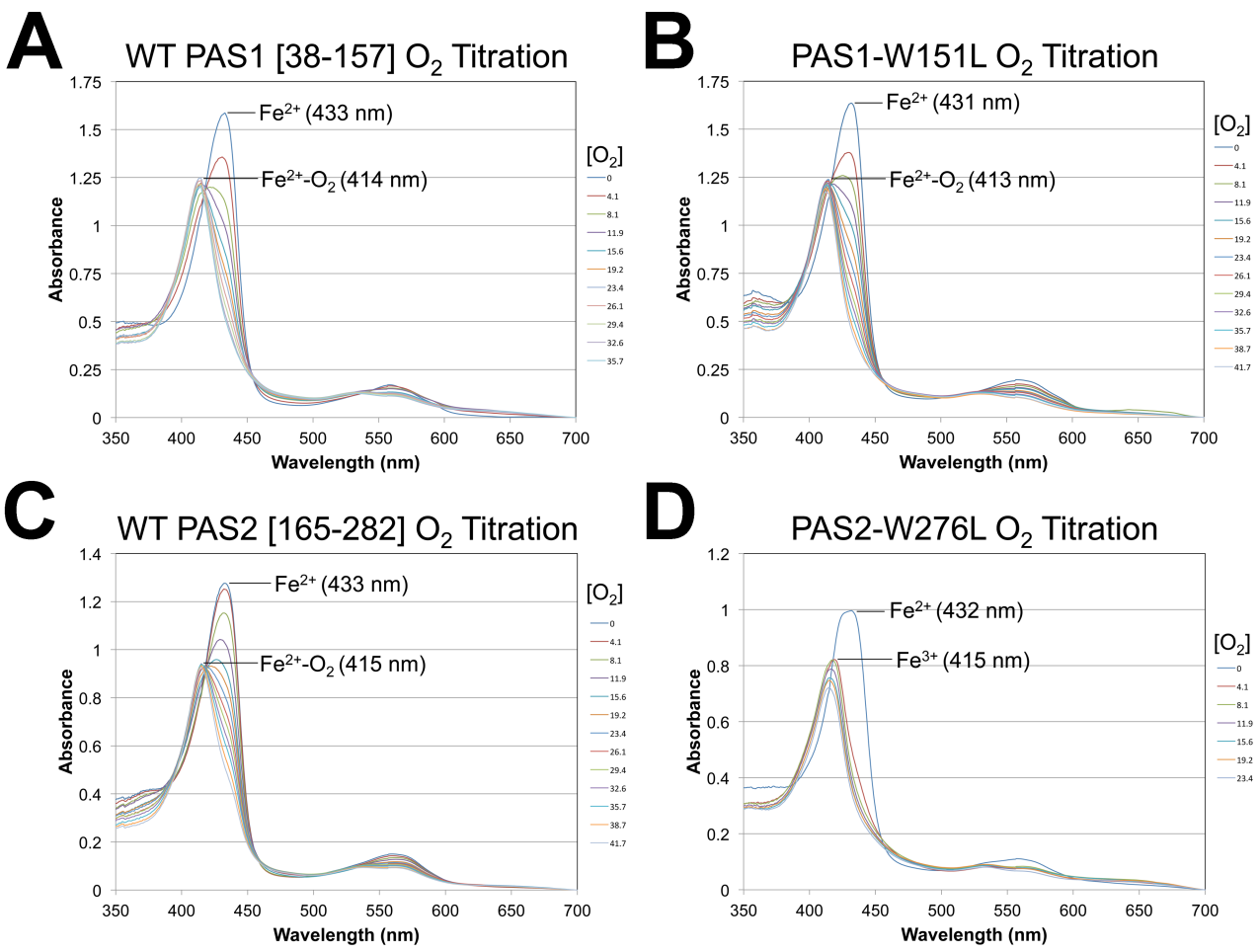

Fig. S3. Example gas titrations using 10 μM purified PAS1 [38-157] or PAS2 [165-282] peptides.

A-B. WT deoxy-PAS1 (A) and deoxy-PAS1-W151L (B) peptides titrated with 5 μl aliquots of air-saturated buffer. WT PAS1 and PAS1-W151L both bind O_2.

C-D. WT deoxy-PAS2 (C) and deoxy-PAS2-W276L (D) peptides titrated with 5 μl aliquots of air-saturated buffer. PAS2-W276L shows rapid oxidation to met-heme (with a soret maximum of 415 nm). The designation of met-heme instead of oxy-heme, was verified spectrophotometrically after oxidizing PAS peptide with potassium ferricyanide and comparing the spectra.

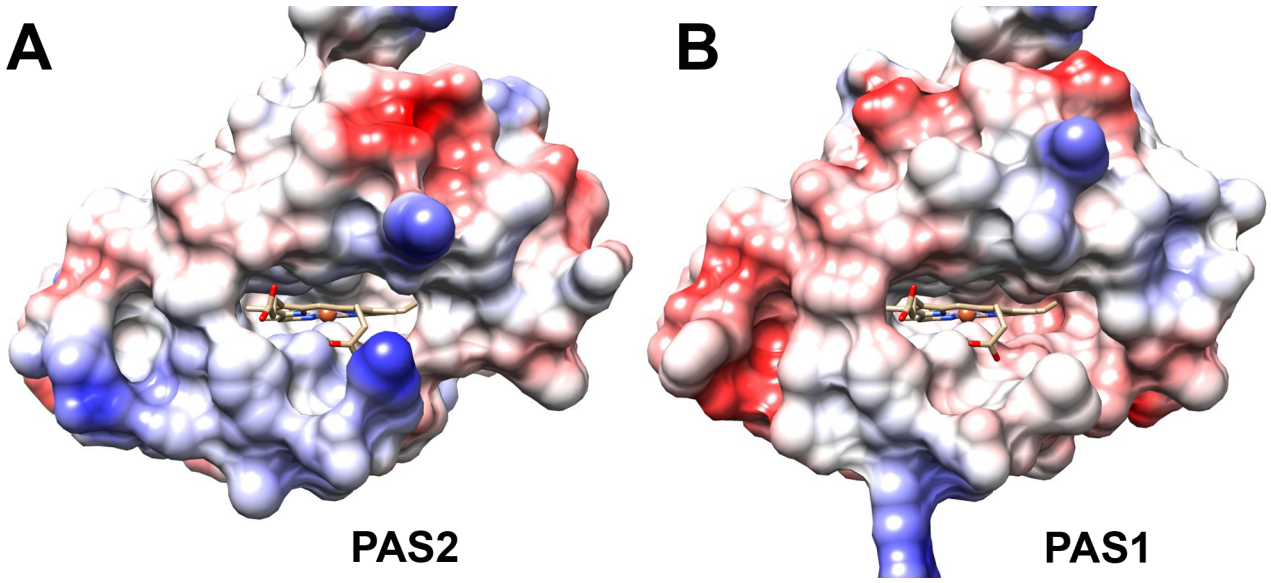

Fig. S4. Homology molecular surface models of the PAS1 and PAS2 domains of *Vc*Aer2, showing differences in electrostatic potential surrounding the heme pocket (red, negative charge; blue, positive charge). PAS1 (B) has a more negative potential within the heme pocket than PAS2 (A).

Table S1. Data collection and refinement statistics for *Vc*Aer2 PAS2-W276L

Wavelength (Å)	0.62790
Resolution range	27.16 - 1.67 (1.73 - 1.67)
Space group	P 3_2 2 1
Unit cell dimensions	
a, b, c (Å)	62.51, 62.51, 157.02
α, β, γ (°)	90, 90, 120
Unique reflections	42171 (4,146)
Completeness (%)	99.60 (98.15)
Wilson B-factor	24.16
R-work	0.2070 (0.3376)
R-free	0.2411 (0.3648)
Number of non-hydrogen atoms	3022
Macromolecules	2569
Ligands	129
Protein residues	336
RMS(bonds)	0.012
RMS(angles)	1.09
Ramachandran favored (%)	99.39
Ramachandran allowed (%)	0.61
Ramachandran outliers (%)	0
Rotamer outliers (%)	0.36
Clashscore	8.69
Average B-factor	30.25
Macromolecules	29.44
Ligands	24.11
Solvent	39.04