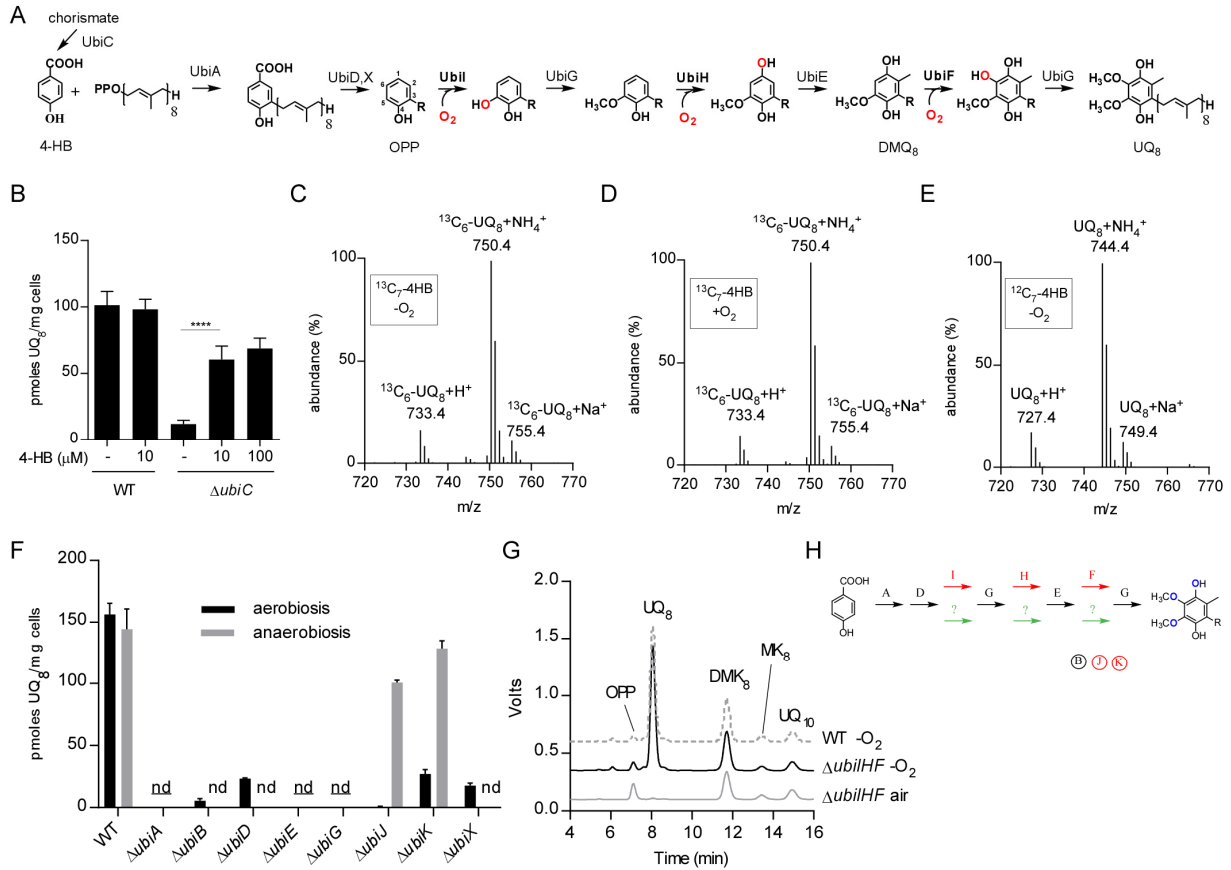


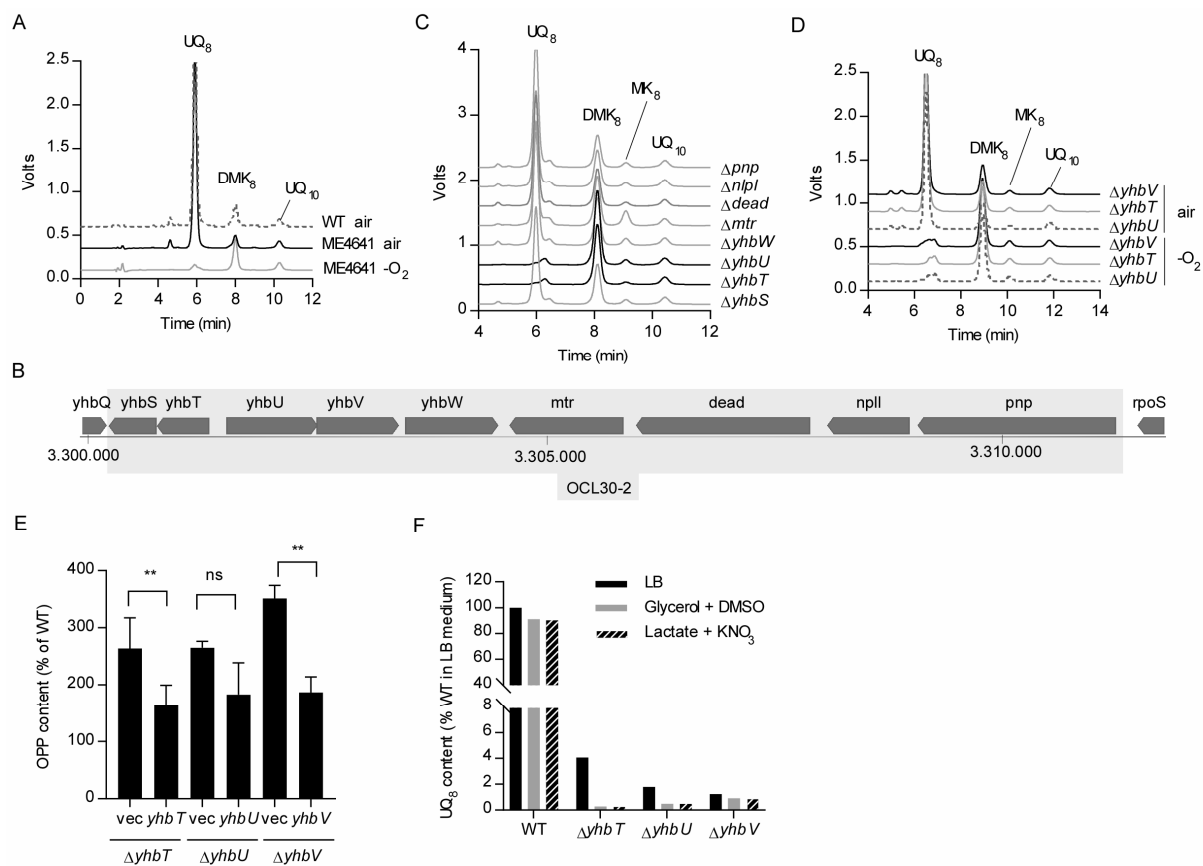
819 MAIN FIGURES

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822 **Figure 1: The aerobic and anaerobic UQ biosynthetic pathways differ only in the hydroxylation steps.** A)  
 823 O<sub>2</sub>-dependent UQ biosynthesis pathway in *E. coli*. The octaprenyl tail is represented by R on the  
 824 biosynthetic intermediates and the numbering of the aromatic carbon atoms is shown on OPP.  
 825 Abbreviations used are 4-HB for 4-hydroxybenzoic acid, OPP for octaprenylphenol, DMQ<sub>8</sub> for C6-  
 826 demethoxy-ubiquinone 8 and UQ<sub>8</sub> for ubiquinone 8. B) UQ<sub>8</sub> quantification of WT and  $\Delta ubiC$  cells grown  
 827 anaerobically in glycerol-nitrate medium supplemented or not with the indicated concentrations of 4-HB  
 828 (n=3-6), \*\*\*\*:  $p < 0.0001$ , unpaired Student's t test. C-E) Mass spectra of UQ<sub>8</sub> obtained by HPLC-MS analysis  
 829 of lipid extracts from cells grown with <sup>13</sup>C<sub>7</sub>-4HB either anaerobically (C) or aerobically (D), or anaerobically  
 830 with unlabeled 4HB (E). F) UQ<sub>8</sub> quantification from WT and  $\Delta ubi$  cells grown anaerobically in SMGN  
 831 medium overnight or aerobically in LB medium until OD 0.8. nd = not detected in aerobic and anaerobic  
 832 conditions, nd = not detected in either aerobic or anaerobic conditions, n=3-4. G) HPLC-ECD analyses  
 833 (mobile phase 1) of lipid extracts from 1 mg of WT or  $\Delta ubiIHF$  cells grown in LB medium in air or anaerobic  
 834 conditions (-O<sub>2</sub>). Chromatograms are representative of n=3 independent experiments (UQ<sub>10</sub> used as  
 835 standard). H) UQ biosynthesis represented with Ubi enzymes specific to the O<sub>2</sub>-dependent pathway (red),  
 836 to the O<sub>2</sub>-independent pathway (green), or common to both pathways (black). The same color code applies  
 837 to the accessory factors (circled).

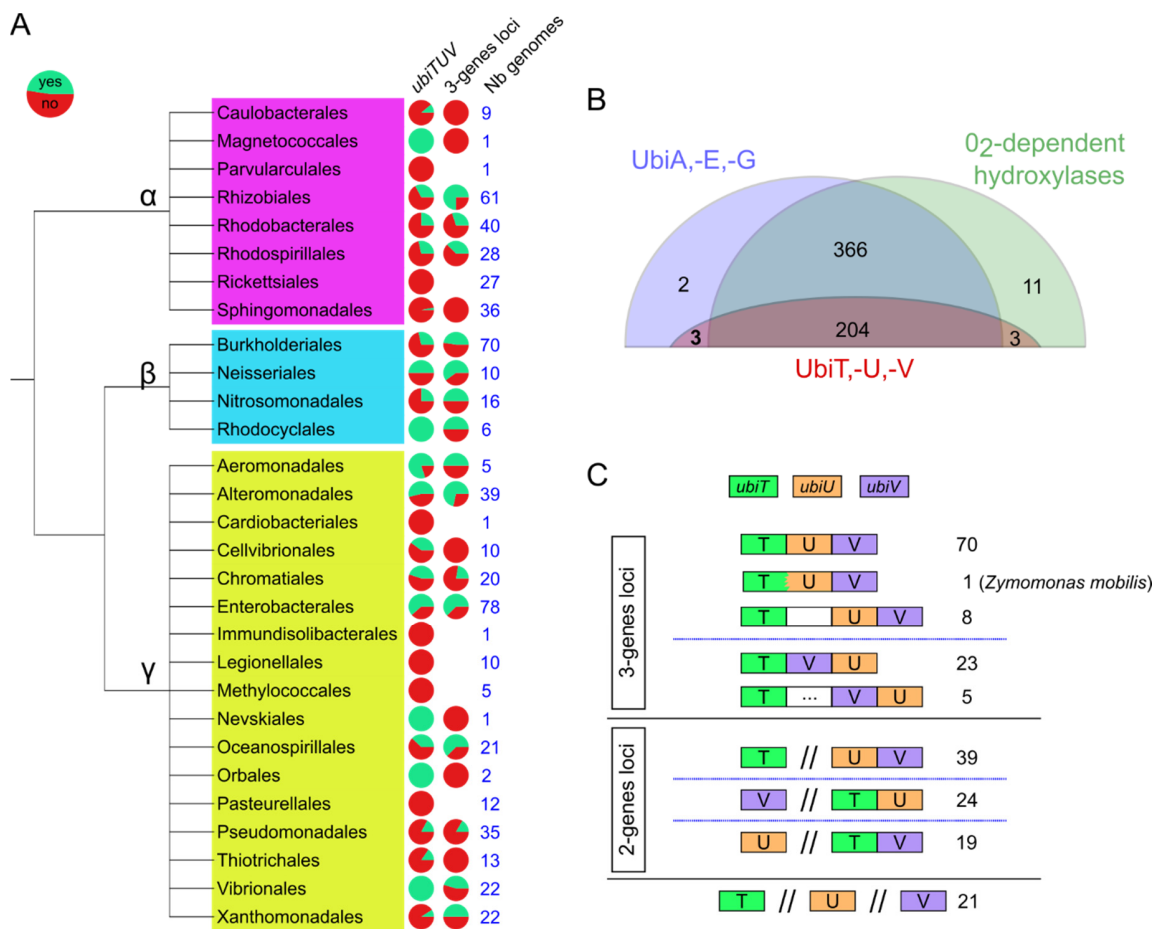


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840 **Figure 2: *yhbT*, *yhbU* and *yhbV* are essential to the anaerobic biosynthesis of UQ.** A) HPLC-ECD analysis  
 841 of lipid extracts from ME4641 strain grown in SMGN either aerobically or anaerobically (-O<sub>2</sub>). B) Genomic  
 842 region covered by the OCL30-2 deletion in the ME4641 strain. C) HPLC-ECD analysis of lipid extracts from  
 843 knock-out strains of the individual genes covered by the OCL30-2 deletion grown in SMGN anaerobically.  
 844 D) HPLC-ECD analysis of lipid extracts from  $\Delta yhbT$ ,  $\Delta yhbU$  and  $\Delta yhbV$  strains constructed in the MG1655  
 845 background and grown in glycerol nitrate medium either aerobically or anaerobically. HPLC-ECD analyses  
 846 with mobile phase 2 (A, C, D). E) OPP content (as % of WT, mass detection M+NH<sub>4</sub><sup>+</sup>) in cells from table 2.  
 847 The  $\Delta yhb$  strains contain either an empty plasmid or a plasmid carrying the indicated gene and were  
 848 cultured anaerobically in glycerol nitrate medium containing 0.02% arabinose. mean  $\pm$ SD (n=3-5), \*\*: p<  
 849 0.01, unpaired Student's t test. F) UQ<sub>8</sub> content (as % of WT grown in LB medium) of cells cultured  
 850 anaerobically in SM containing the indicated carbon sources and electron acceptors.

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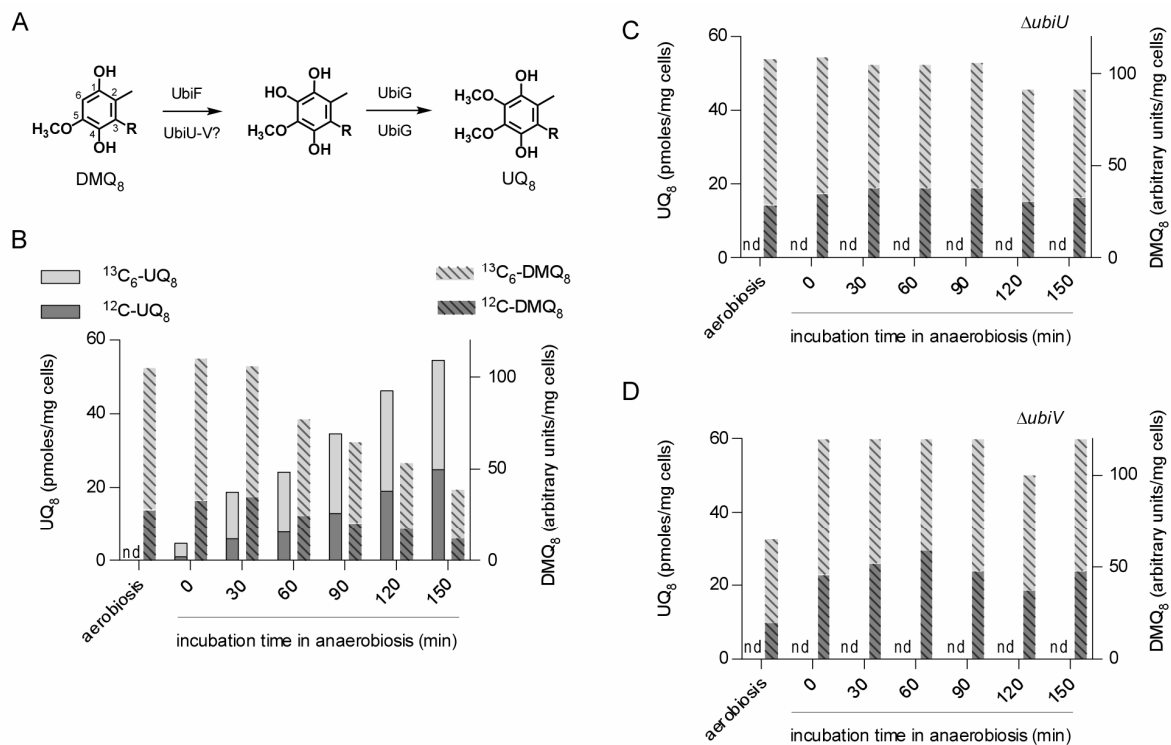
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854 **Figure 3: *ubiT*, -U and -V occurrence and genetic architecture in proteobacterial genomes.**

855 A) The proportion of genomes with (green) and without (red) all three genes "*ubiTUV*" (left column) is  
 856 indicated for each proteobacterial order known to synthesize UQ. The middle column "3-genes loci"  
 857 displays the proportion of genomes with the three genes either at a single locus (green) or at different loci  
 858 (red). The number of genomes analyzed for each order is given in the right column ("Nb Genomes"). B)  
 859 Occurrence in the reference proteobacterial genomes of the marker proteins (UbiA, -E, -G), of the O<sub>2</sub>-  
 860 dependent hydroxylases and of the UbiT, -U, -V proteins. The number in bold represents the 3 genomes (*P.*  
 861 *fulvum*, *M. marinus* and *O. formigenes*) containing exclusively the O<sub>2</sub>-independent pathway. C) The distinct  
 862 genetic architectures found for *ubiT*, -U and -V in genomes where the three genes were present are  
 863 displayed as boxes with different colors. Number of cases corresponding to each depicted architecture are  
 864 given on the right. A white box corresponds to a gene found between the genes of interest, and a white  
 865 box with dots corresponds to two to five genes between the genes of interest.

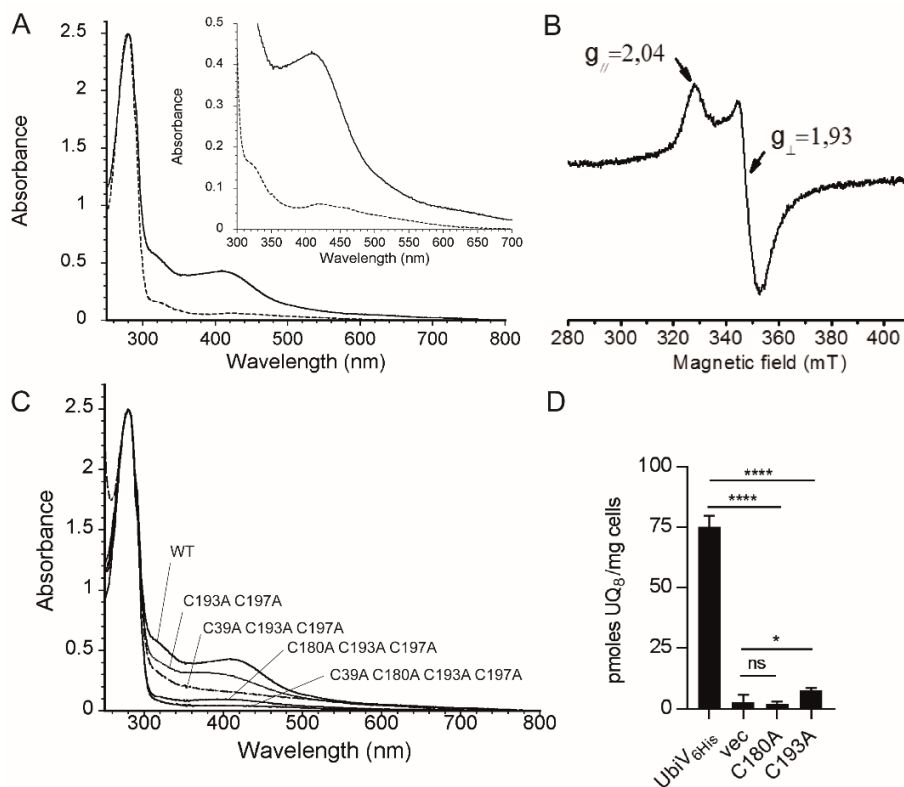
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867  
 868 **Figure 4: UbiU and UbiV are necessary for the anaerobic conversion of DMQ<sub>8</sub> into UQ<sub>8</sub>.** A) Conversion of  
 869 DMQ<sub>8</sub> to UQ<sub>8</sub> with enzymes of the O<sub>2</sub>-dependent and the O<sub>2</sub>-independent pathways, indicated respectively  
 870 above and below arrows (numbering of carbon atoms shown on DMQ<sub>8</sub> and polyprenyl tail represented by  
 871 R). B) Unlabeled (<sup>12</sup>C) and labeled (<sup>13</sup>C<sub>6</sub>) -DMQ<sub>8</sub> and -UQ<sub>8</sub> contents in  $\Delta ubiC \Delta ubiF$  cells after aerobic growth  
 872 and transition to anaerobiosis. C-D) Same as in B with  $\Delta ubiC \Delta ubiF \Delta ubiU$  cells (C) and  $\Delta ubiC \Delta ubiF \Delta ubiV$   
 873 cells (D). nd, not detected, results representative of two independent experiments (B-D).

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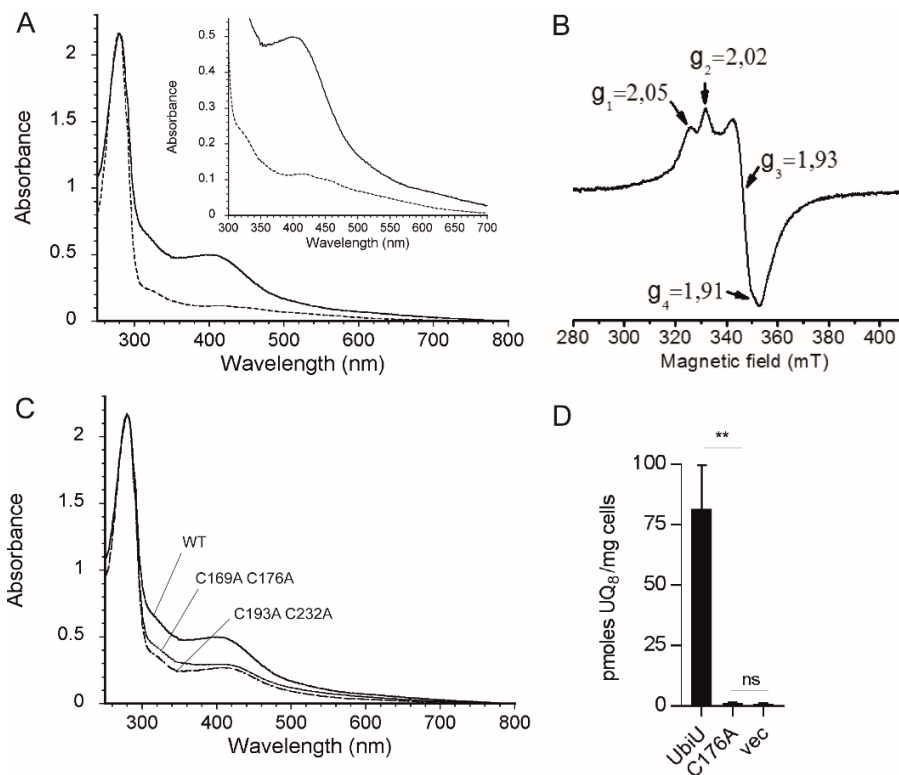


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877 **Figure 5: UbiV binds a [4Fe-4S] cluster.** A) UV-visible absorption spectra of as-purified UbiV (dotted line,  
 878 47  $\mu\text{M}$ ) and reconstituted holo-UbiV (solid line, 41  $\mu\text{M}$ ); Inset: enlargement of the 300-700 nm region. The  
 879 molar extinction coefficient  $\epsilon_{410\text{nm}}$  was determined to be  $10.8 \pm 0.4 \text{ mM}^{-1} \text{ cm}^{-1}$  for holo-UbiV. B) X-band EPR  
 880 spectrum of 785  $\mu\text{M}$  dithionite-reduced holo-UbiV. Recording conditions: temperature, 10K; microwave  
 881 power, 10 mW; modulation amplitude, 0.6 mT. C) Comparative UV-visible absorption spectra of WT and  
 882 different Cys-to-Ala mutants of UbiV after metal cluster reconstitution, with the following concentrations:  
 883 41  $\mu\text{M}$  WT, 44  $\mu\text{M}$  C193A C197A, 46  $\mu\text{M}$  C39A C193A C197A, 47  $\mu\text{M}$  C180A C193A C197A, and 54  $\mu\text{M}$  C39A  
 884 C180A C193A C197A. Proteins were in 50 mM Tris-HCl, pH 8.5, 25 mM NaCl, 15% glycerol, 1m M DTT (A-  
 885 C). D) UQ<sub>8</sub> quantification of  $\Delta\text{ubiV}$  cells transformed with pBAD-UbiV<sub>6His</sub>, pBAD-UbiV<sub>6His</sub> C180A, pBAD-  
 886 UbiV<sub>6His</sub> C193A or empty pBAD and grown overnight in anaerobic MSGN (n=4-5). Mean  $\pm$ SD, \*: p < 0.05,  
 887 \*\*\*\*: p < 0.0001, unpaired Student's t test.

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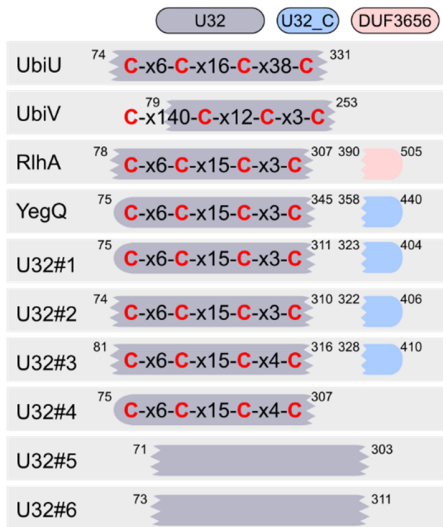


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892 **Figure 6: The UbiU-V complex binds two [4Fe-4S] clusters.** A) UV-visible absorption spectra of as-purified  
 893 UbiU-UbiV (dotted line, 17  $\mu\text{M}$ ) and reconstituted holo-UbiU-UbiV (solid line, 15.5  $\mu\text{M}$ ); Inset: enlargement  
 894 of the 300-700 nm region. B) X-band EPR spectrum of 339  $\mu\text{M}$  dithionite-reduced holo-UbiU-UbiV;  
 895 Recording conditions: temperature, 10K; microwave power, 2 mW; modulation amplitude, 0.6 mT. C)  
 896 Comparative UV-visible absorption spectra of Cys-to-Ala mutants of UbiU in the UbiU-UbiV complex after  
 897 metal cluster reconstitution with the following concentrations: 15.5  $\mu\text{M}$  WT, 16.0  $\mu\text{M}$  UbiU-C169A C176A  
 898 and 16.0  $\mu\text{M}$  UbiU-C193A C232A. Proteins were in 50mM Tris-HCl, pH 8.5, 150mM NaCl, 15% glycerol and  
 899 1mM DTT (A-C). D) UQ<sub>8</sub> quantification of  $\Delta\text{ubiU}$  cells transformed with pBAD-UbiU (n=4), pBAD-UbiU  
 900 C176A (n=2) or pBAD empty vector (n=3) and grown overnight in anaerobic MSGN. mean  $\pm$ SD, \*\*: p< 0.01,  
 901 ns: not significant, unpaired Student's t test.

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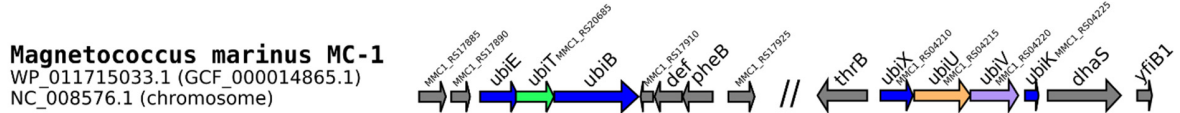
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904 **Figure 7: Conserved four cysteines motifs in the U32 protease family.**

905 The conserved 4-cysteines motifs and PFAM domains (colored boxes) found in each U32 protease family  
 906 are displayed for a set of reference sequences. These motifs were obtained by aligning the sequences  
 907 listed by Kimura *et al.* [42]. Conserved cysteines are in red, "x6" indicates that 6 residues were found in  
 908 between two conserved cysteines. Positions of the domains are displayed on the outside of the boxes for  
 909 the reference sequences. Scrambled extremities show interrupted match for the PFAM domain. No  
 910 conserved cysteines were found for U32#5 and U32#6 (see main text). Reference sequences were from *E.*  
 911 *coli* for UbiU, UbiV, YegQ and RlhA (YHBU\_ECOLI, YHBV\_ECOLI, YEGQ\_ECOLI, YDCP\_ECOLI for RlhA). For  
 912 the rest of the families, the sequence accession numbers were: R7JPV1\_9FIRM for U32#1, R6XKQ3\_9CLOT  
 913 for U32#2, S1NZZ5\_9ENTE for U32#3, H1YXA1\_9EURY for U32#4, H3NJ45\_9LACT for U32#5,  
 914 D5MIQ1\_9BACT for U32#6.

915 SUPPLEMENTARY FIGURES

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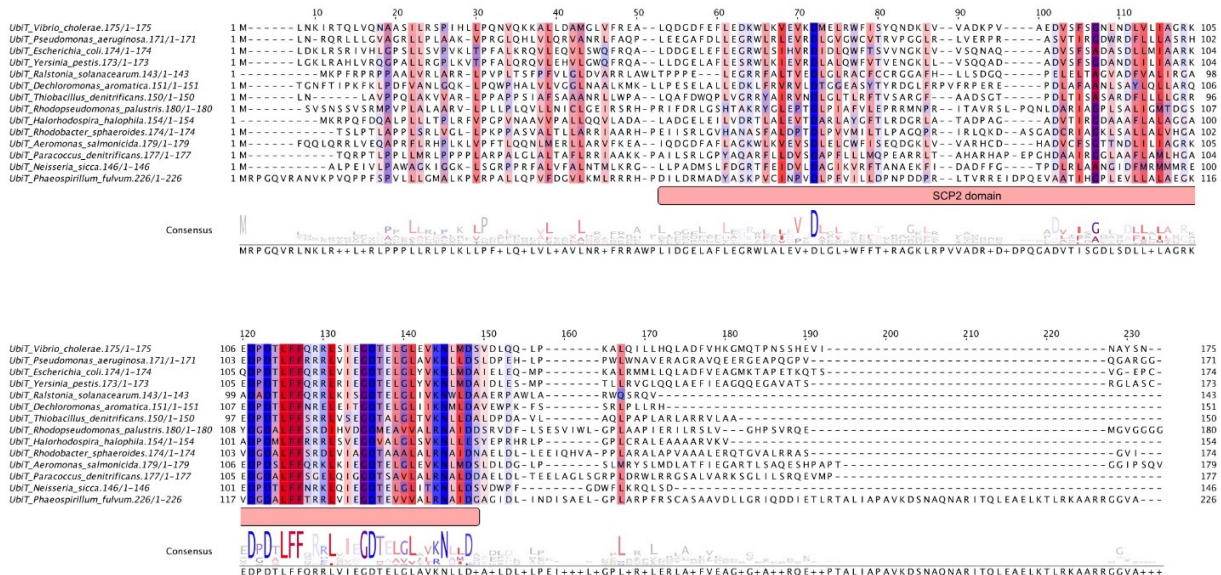
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918 **Figure S1:** Genetic context of the *ubiT,U,V* genes in *M. marinus*. The scheme was drawn from a figure  
919 obtained using the GeneSpy program [77].

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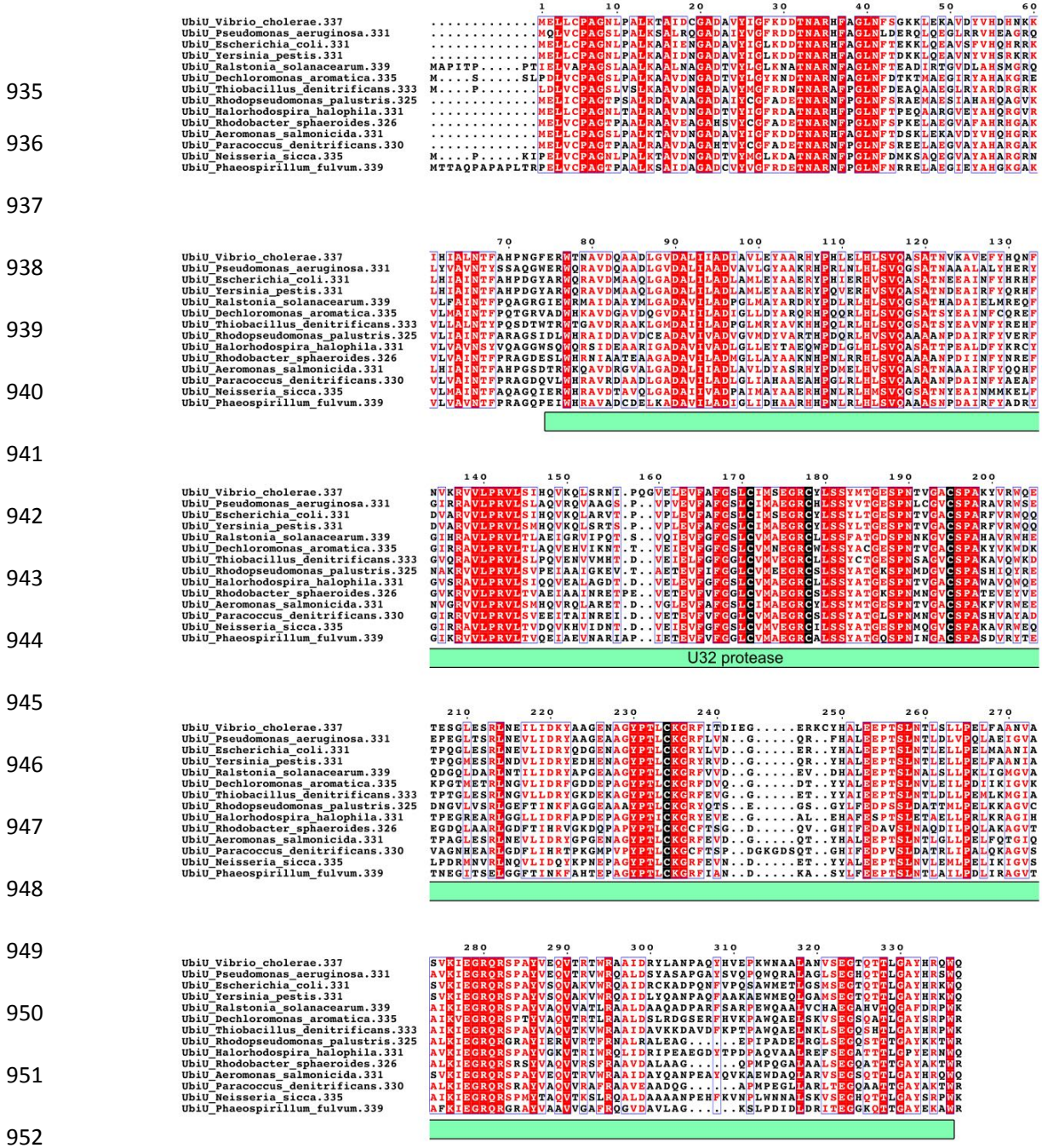


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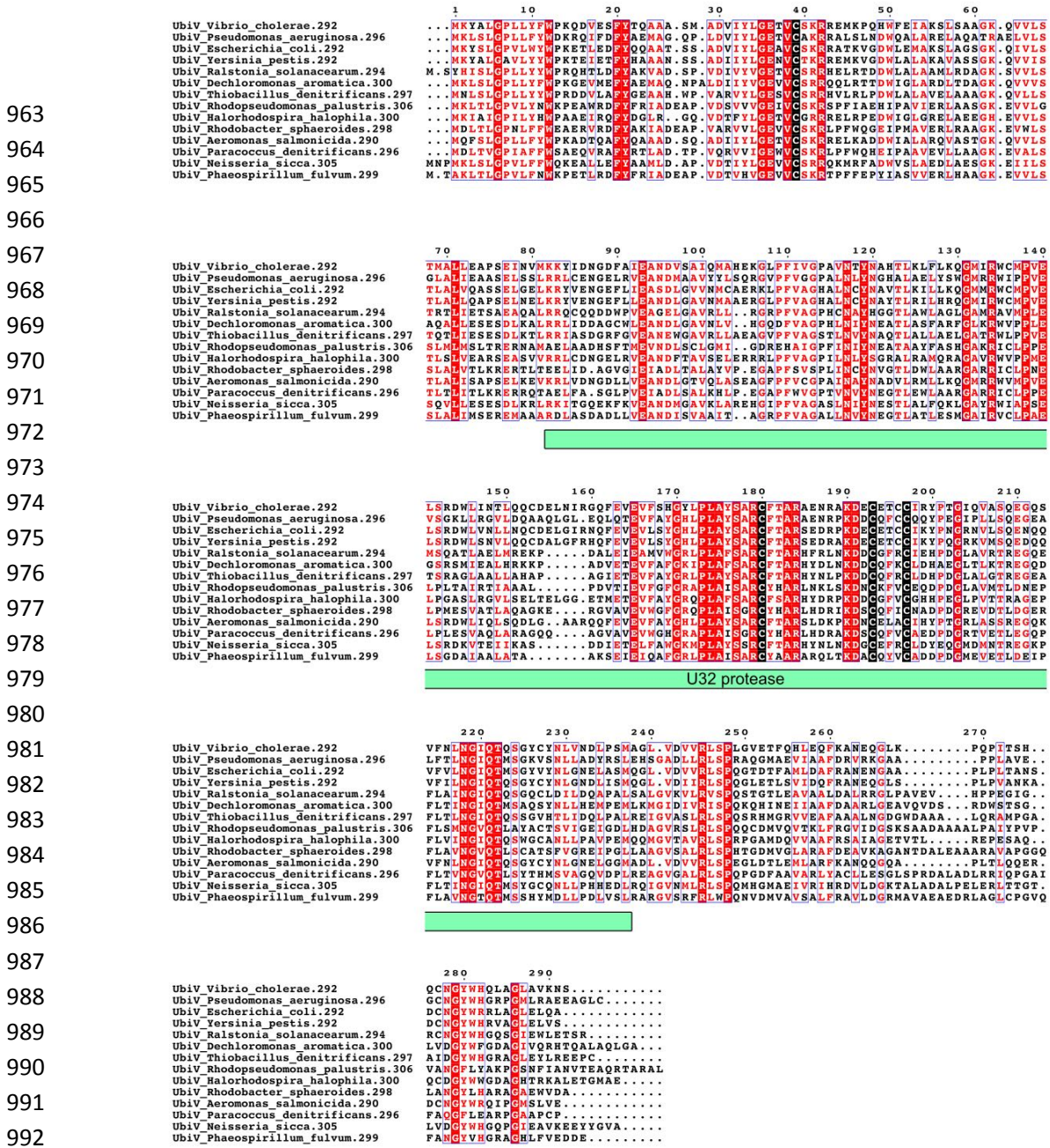
924 **Figure S2:** Multiple sequence alignment of UbiT from representative proteobacteria. Sequences were  
925 aligned using Mafft (linsi), and the output generated using Jalview and Inkscape. Hydrophobic residues are  
926 colored in blue. The position of the SCP2 domain (PF02036) is indicated by a red box. Genbank accession  
927 numbers: *Vibrio cholerae*, NP\_230303.1, *Pseudomonas aeruginosa*, NP\_252600.1, *Escherichia coli*,  
928 NP\_312065.1, *Yersinia pestis*, YP\_002348366.1, *Ralstonia solanacearum*, WP\_011004260.1,  
929 *Dechloromonas aromatica*, WP\_011285876.1, *Thiobacillus denitrificans*, WP\_011312695.1,  
930 *Rhodopseudomonas palustris*, WP\_011666115.1, *Halorhodospira halophila*, WP\_011813831.1,  
931 *Rhodobacter sphaeroides*, WP\_011909347.1, *Aeromonas salmonicida*, WP\_005310396.1, *Paracoccus*  
932 *denitrificans*, WP\_011750456.1, *Neisseria sicca*, WP\_080614297.1, *Phaeospirillum fulvum*,  
933 WP\_074767212.1.

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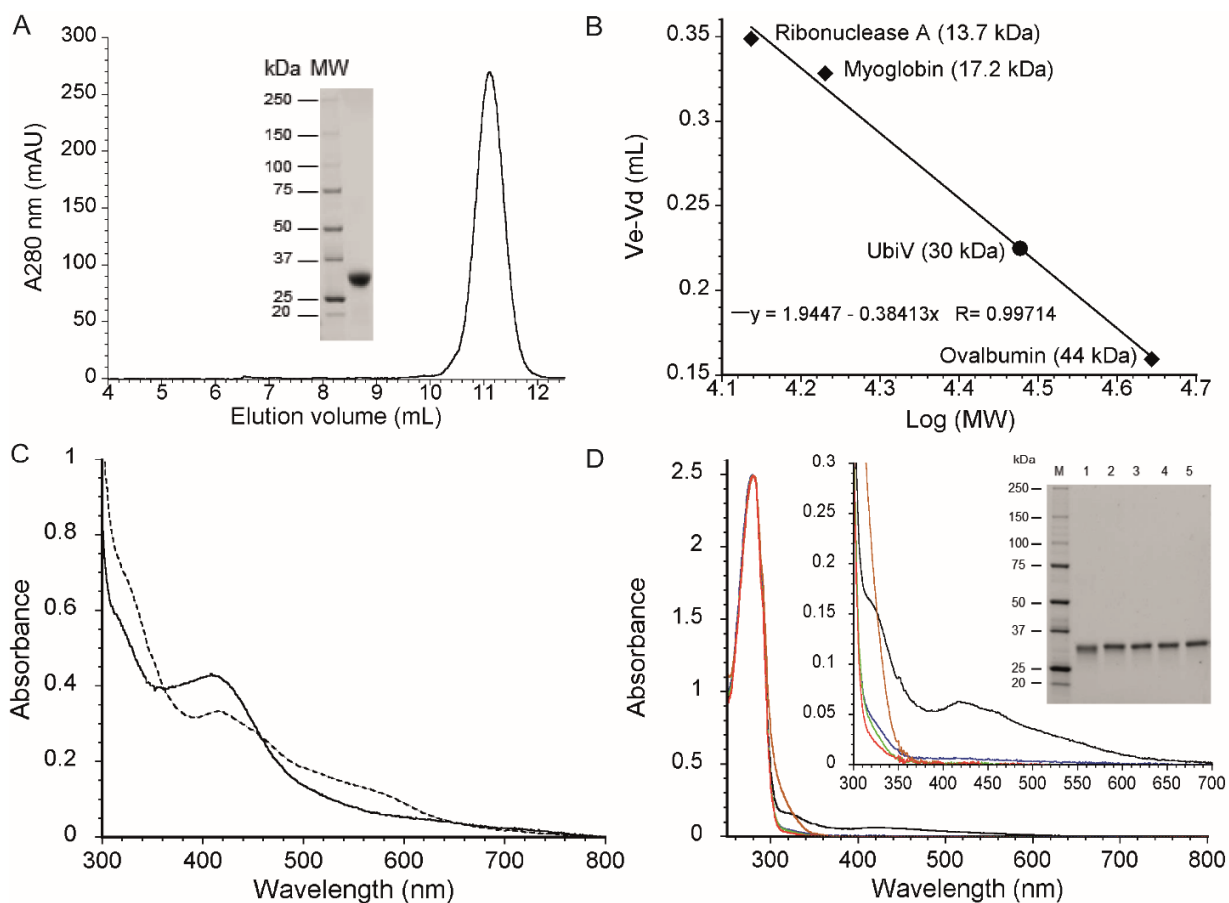




953 **Figure S3:** Multiple sequence alignment of UbiU from representative proteobacteria. Sequences were  
 954 aligned using Mafft (linzi), and the output generated using ESPrnt and Inkscape. The four conserved  
 955 cysteines (C169, C176, C193 and C232) involved in iron-sulfur binding are indicated by black columns. The  
 956 position of the domain U32 protease PF01136 is indicated by a green box. GenBank accession numbers:  
 957 *Vibrio cholerae*, NP\_230301.1, *Pseudomonas aeruginosa*, NP\_252602.1, *Escherichia coli*, NP\_312066.1,  
 958 *Yersinia pestis*, YP\_002348368.1, *Ralstonia solanacearum*, WP\_011004262.1, *Dechloromonas aromatica*,  
 959 WP\_011285878.1, *Thiobacillus denitrificans*, WP\_011312698.1, *Rhodospseudomonas palustris*,  
 960 WP\_011666114.1, *Halorhodospira halophila*, WP\_011813833.1, *Rhodobacter sphaeroides*,  
 961 WP\_011909346.1, *Aeromonas salmonicida*, WP\_005310394.1, *Paracoccus denitrificans*,  
 962 WP\_041530457.1, *Neisseria sicca*, WP\_049226489.1, *Phaeospirillum fulvum*, WP\_074767209.1.



**Figure S4:** Multiple sequence alignment of UbiV from representative proteobacteria. Sequences were aligned using Mafft (Ilnsi), and the output generated using ESript and Inkscape. The four conserved cysteines (C39, C180, C193 and C197 - positions in *E. coli*) involved in iron-sulfur binding are indicated by black columns. The position of the domain U32 protease PF01136 is indicated by a green box. GenBank accession numbers: *Vibrio cholerae*, NP\_230300.2, *Pseudomonas aeruginosa*, NP\_252601.1, *Escherichia coli*, NP\_312067.2, *Yersinia pestis*, YP\_002348369.1, *Ralstonia solanacearum*, WP\_011004261.1, *Dechloromonas aromatica*, WP\_011285877.1, *Thiobacillus denitrificans*, WP\_011312697.1, *Rhodopseudomonas palustris*, WP\_011666113.1, *Halorhodospira halophila*, WP\_011813832.1, *Rhodobacter sphaeroides*, WP\_011909345.1, *Aeromonas salmonicida*, WP\_005310392.1, *Paracoccus denitrificans*, WP\_011750458.1, *Neisseria sicca*, WP\_080614296.1, *Phaeospirillum fulvum*, WP\_074767477.1.



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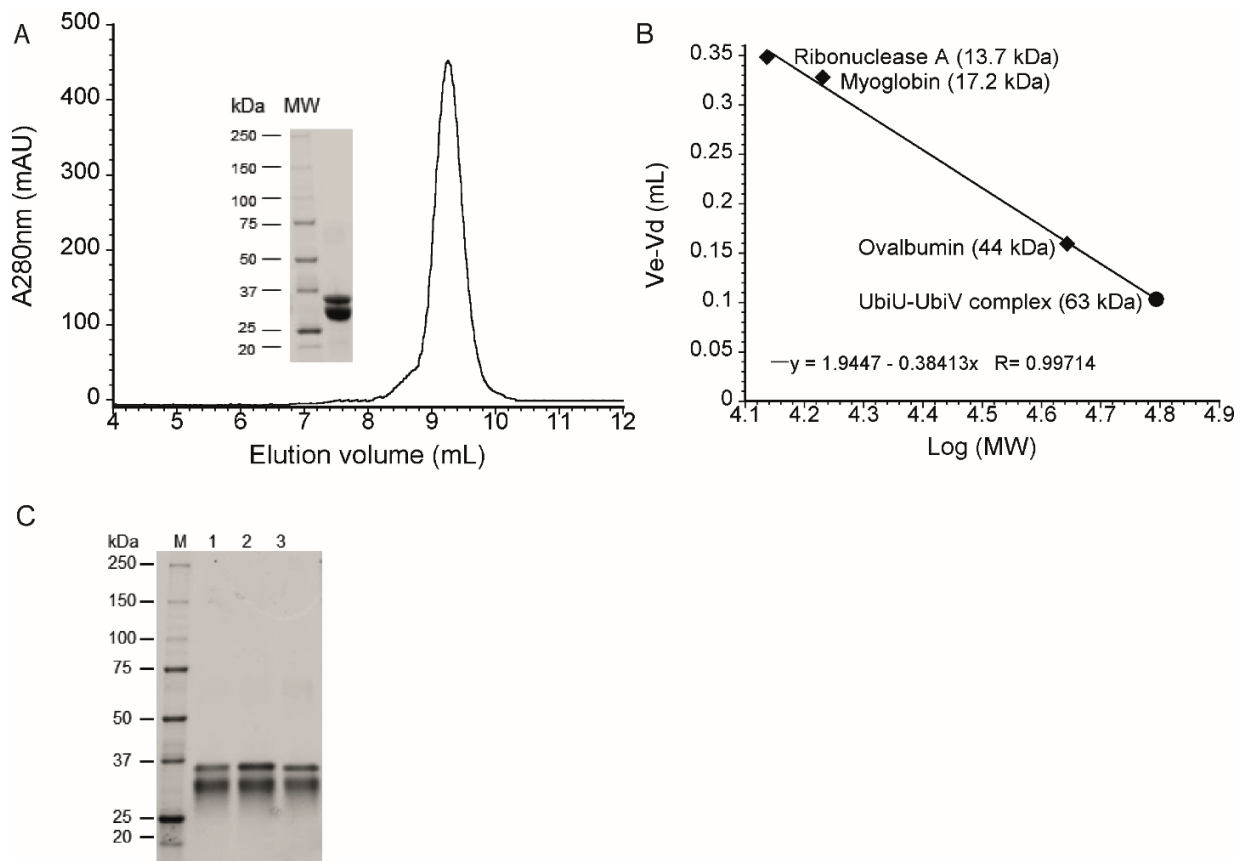
1007 **Figure S5:** A) Gel filtration chromatogram of aerobically purified UbiV on a Superdex 75 Increase 10/300  
 1008 GL column; Inset: Coomassie staining SDS-PAGE of aerobically purified UbiV. B) Calibration curve, standard  
 1009 proteins in rhombi, UbiV in circle. C) UV-visible absorption spectra of 41  $\mu$ M holo-UbiV under anaerobiosis  
 1010 (solid line) and after 20 minutes exposure to air (dotted line). D) Comparative UV-visible absorption spectra  
 1011 of aerobically purified wild-type (black) and different Cys-to-Ala mutants of UbiV (C193A C197A in blue;  
 1012 C39A C193A C197A in green; C180A C193A C197A in pink; C39A C180A C193A C197A in brown). Inset:  
 1013 Coomassie staining SDS-PAGE shows the purifications of UbiV and variants. Lanes are: M, molecular mass  
 1014 marker; 1, wild-type UbiV; 2, UbiV C193A C197A; 3, UbiV C39A C193A C197A; 4, UbiV C180A C193A C197A;  
 1015 5, UbiV C39A C180A C193A C197. All proteins were in 50 mM Tris-HCl, pH 8.5, 25 mM NaCl, 15% glycerol,  
 1016 1 mM DTT (A-D).

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1023 **Figure S6:** A) Gel filtration chromatogram of aerobically purified UbiU-UbiV on a Superdex 75 Increase  
 1024 10/300 GL column; Inset: Coomassie staining SDS-PAGE of aerobically purified UbiU-UbiV complex. B)  
 1025 Calibration curve, standard proteins in rhombi, UbiU-UbiV in circle. C) Coomassie staining SDS-PAGE  
 1026 shows the purifications of UbiU-UbiV wild-type and variants proteins: Lanes are: M, molecular mass  
 1027 marker; 1, wild-type UbiU-UbiV complex; 2, UbiU C169A C176A - UbiV wild-type; 3, UbiU C193A C232A -  
 1028 UbiV wild-type. All proteins were in buffer 50 mM Tris-HCl, pH 8.5, 150 mM NaCl, 15% glycerol, 1 mM  
 1029 DTT (A-D).

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1033 **Table S1:** UQ levels in strains from the medium- and large- deletion collections

1034 **Table S2:** Occurrence of genes from the UQ pathways in bacterial genomes.

1035 **Table S3:** List of oligonucleotides and strains used in this study

1036 **Table S4:** References of the protein sequences used to build the HMM profiles.

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