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 O2-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, DMQ₃ for C6-826 demethoxy-ubiquinone 8 and UQ₈ for ubiquinone 8. B) UQ₈ 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 UQ₈ obtained by HPLC-MS analysis 829 of lipid extracts from cells grown with ¹³C₇-4HB either anaerobically (C) or aerobically (D), or anaerobically 830 with unlabeled 4HB (E). F) UQ₈ quantification from WT and Δubi cells grown anaerobically in SMGN 831 medium overnight or aerobically in LB medium until OD 0.8. nd = not detected in aerobic and anaerobic conditions, nd= not detected in either aerobic or anaerobic conditions, n=3-4. G) HPLC-ECD analyses 832 833 (mobile phase 1) of lipid extracts from 1 mg of WT or △ubilHF cells grown in LB medium in air or anaerobic conditions ($-O_2$). Chromatograms are representative of n=3 independent experiments (UQ₁₀ used as 834 835 standard). H) UQ biosynthesis represented with Ubi enzymes specific to the O₂-dependent pathway (red), 836 to the O₂-independent pathway (green), or common to both pathways (black). The same color code applies 837 to the accessory factors (circled).





Figure 2: yhbT, yhbU and yhbV are essential to the anaerobic biosynthesis of UQ. A) HPLC-ECD analysis 840 of lipid extracts from ME4641 strain grown in SMGN either aerobically or anaerobically (-O₂). B) Genomic 841 842 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. D) HPLC-ECD analysis of lipid extracts from $\Delta yhbT$, $\Delta yhbU$ and $\Delta yhbV$ strains constructed in the MG1655 844 background and grown in glycerol nitrate medium either aerobically or anaerobically. HPLC-ECD analyses 845 with mobile phase 2 (A, C, D). E) OPP content (as % of WT, mass detection M+NH₄⁺) in cells from table 2. 846 847 The Δyhb strains contain either an empty plasmid or a plasmid carrying the indicated gene and were cultured anaerobically in glycerol nitrate medium containing 0.02% arabinose. mean ±SD (n=3-5), **: p< 848 849 0.01, unpaired Student's t test. F) UQ₈ content (as % of WT grown in LB medium) of cells cultured 850 anaerobically in SM containing the indicated carbon sources and electron acceptors.



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" displays the proportion of genomes with the three genes either at a single locus (green) or at different loci 857 858 (red). The number of genomes analyzed for each order is given in the right column ("Nb Genomes"). B) 859 Occurence in the reference proteobacterial genomes of the marker proteins (UbiA, -E, -G), of the O₂dependent hydroxylases and of the UbiT,-U, -V proteins. The number in bold represents the 3 genomes (P. 860 861 fulvum, M. marinus and O. formigenes) containing exclusively the O₂-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.



Figure 4: UbiU and UbiV are necessary for the anaerobic conversion of DMQ₈ into UQ₈. A) Conversion of DMQ₈ to UQ₈ with enzymes of the O₂-dependent and the O₂-independent pathways, indicated respectively above and below arrows (numbering of carbon atoms shown on DMQ₈ and polyprenyl tail represented by R). B) Unlabeled (¹²C) and labeled (¹³C₆) -DMQ₈ and -UQ₈ contents in $\Delta ubiC \Delta ubiF$ cells after aerobic growth and transition to anaerobiosis. C-D) Same as in B with $\Delta ubiC \Delta ubiF \Delta ubiU$ cells (C) and $\Delta ubiC \Delta ubiF \Delta ubiV$ cells (D). nd, not detected, results representative of two independent experiments (B-D).

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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 μM) and reconstituted holo-UbiV (solid line, 41 μM); Inset: enlargement of the 300-700 nm region. The molar extinction coefficient ε_{410nm} was determined to be 10.8 ± 0.4 mM⁻¹ cm⁻¹ for holo-UbiV. B) X-band EPR 879 spectrum of 785 µM dithionite-reduced holo-UbiV. Recording conditions: temperature, 10K; microwave 880 power, 10 mW; modulation amplitude, 0.6 mT. C) Comparative UV-visible absorption spectra of WT and 881 different Cys-to-Ala mutants of UbiV after metal cluster reconstitution, with the following concentrations: 882 $41\,\mu\text{M}$ WT, $44\,\mu\text{M}$ C193A C197A, $46\,\mu\text{M}$ C39A C193A C197A, $47\,\mu\text{M}$ C180A C193A C197A, and 54 μM C39A 883 884 C180A C193A C197A. Proteins were in 50 mM Tris-HCl, pH 8.5, 25 mM NaCl, 15% glycerol, 1m M DTT (A-C). D) UQ₈ quantification of $\Delta ubiV$ cells transformed with pBAD-UbiV_{6His}, pBAD-UbiV_{6His} C180A, pBAD-885 UbiV_{6His} C193A or empty pBAD and grown overnight in anaerobic MSGN (n=4-5). Mean ±SD, *: p< 0.05, 886 ****: p< 0.0001, unpaired Student's t test. 887

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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 μ M) and reconstituted holo-UbiU-UbiV (solid line, 15.5 μ M); Inset: enlargement 894 of the 300-700 nm region. B) X-band EPR spectrum of 339 µM 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 µM WT, 16.0 µM UbiU-C169A C176A 898 and 16.0 µM UbiU-C193A C232A. Proteins were in 50mM Tris-HCl, pH 8.5, 150mM NaCl, 15% glycerol and 899 1mM DTT (A-C). D) UQ₈ quantification of $\Delta ubiU$ cells transformed with pBAD-UbiU (n=4), pBAD-UbiU C176A (n=2) or pBAD empty vector (n=3) and grown overnight in anaerobic MSGN. mean ±SD, **: p< 0.01, 900 901 ns: not significant, unpaired Student's t test.



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 conserved cysteines were found for U32#5 and U32#6 (see main text). Reference sequences were from E. 910 coli for UbiU, UbiV, YegQ and RhIA (YHBU ECOLI, YHBV ECOLI, YEGQ ECOLI, YDCP ECOLI for RIhA). For 911 the rest of the families, the sequence accession numbers were: R7JPV1_9FIRM for U32#1, R6XKQ3_9CLOT 912 913 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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Figure S1: Genetic context of the *ubiT,U,V* genes in *M. marinus*. The scheme was drawn from a figureobtained using the GeneSpy program [77].

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924 Figure S2: Multiple sequence alignment of UbiT from representative proteobacteria. Sequences were aligned using Mafft (linsi), and the output generated using Jalview and Inkscape. Hydrophobic residues are 925 926 colored in blue. The position of the SCP2 domain (PF02036) is indicated by a red box. Genbank accession numbers: Vibrio cholerae, NP_230303.1, Pseudomonas aeruginosa, NP_252600.1, Escherichia coli, 927 928 NP_312065.1, Yersinia pestis, YP_002348366.1, Ralstonia solanacearum, WP_011004260.1, 929 Dechloromonas aromatica, WP 011285876.1, Thiobacillus denitrificans, WP 011312695.1, WP_011666115.1, Halorhodospira halophila, 930 Rhodopseudomonas palustris, WP 011813831.1, Rhodobacter sphaeroides, WP_011909347.1, Aeromonas salmonicida, WP_005310396.1, Paracoccus 931 932 denitrificans, WP 011750456.1, Neisseria sicca, WP 080614297.1, Phaeospirillum fulvum, WP_074767212.1. 933

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935	Ubiu Yibrio_cholerae.337 UbiU_Pseudomonas_aeruginosa.331 UbiU Zerseinia_coli.331 UbiU Yersinia_pestis.331 UbiU Tenstonia_solanacearum.339 UbiU Pechloromonas_aromatica.335 UbiU_Thiobacillus_denitrificans.333 UbiU_Thiobacillus_denitrificans.333		L C P A G NI P ALKT / C P A G SI P ALKA L C P A G NI P ALKA / A P A G SI A ALKA / C P A G SI A ALKA / C P A G SI V SI KA I C P A G T P S ALKA	AIDCGADAVYI ALRQGADAIYY AIENGADAVYI AIDNGADAVYI AIDNGADVYI AVDNGADTVYI AVDNGADTVYI AVDNGADAVYM AVAAGADAIYC	G F K D D T N A R H G F R D D T N A R H G L K D D T N A R H G L K D D T N A R H G L K N A T N A R N G F R D N T N A R A G F A D E T N A R N	F A GLN F S GK F A GLN L D E R F A GLN F T E K F A GLN F T D K F A GLN F T E A F A GLN F D T K F P GLN F D E A F P GLN F S R A	KLEKAVDY QLQEGLRR KLQEAVSF KLQEAVSF DIRTGVDL TMAEGIRY QAAEGLRY EMAESIAH	VHDHNKK VHEAGRQ VHQHRRK VHSRKRK AHSMGRQ AHAKGRE ARDRGRK AHQAGVK
936	UbiU_Halorhodospira_nalophila.331 UbiU_Rhodobacter_sphaeroides.326 UbiU_Aeromonas_salmonicida.331 UbiU_Paracoccus_denitrificans.330 UbiU_Paisseria_sicca.335 UbiU_Phaeospirillum_fulvum.339		LCPAGNLTALRA /CPAGTPAALRA LCPAGSLPALKT /CPAGTPAALRA /CPAGNLPALKT /CPAGTPAALKS	AVDNGADTVYI AVEAGAHSVYC AVDNGADAVYI AVDAGAHTVYC AVDNGADTVYM AIDAGADCVYV	GFRDATNARH GFADETNARN GFADETNARH GFADETNARN GLKDATNARN GFRDETNARN	F P GLNFT P E F P GLN F S P K F A GLN F T D S F P GLN F S R E F P GLN F D M K F P GLN F N R R	QAARGVEY ELAEGVAF KLEKAVDY ELAEGVAY SAQEGVAY ELAEGIEY	AHQRGVR AHRHGAK VHQHGRK AHARGAK AHARGRN AHGKGAK
937								
938	UbiU_Vibrio_cholerae.337 UbiU_Pseudomonas_aeruginosa.331 UbiU_Escherichia_coli.331 UbiU_Yersinia_pestis.331	70 IHIALNTFAHPNGFER LYVAVNTYSSAQGWER LHIAINTFAHPDGYAR LHIAINTFAHPDGYAR	80 TNAVDQAADLGV QRAVDQAADLGV QRAVDMAAQLGV QRAVDMAAQLGV	90 10 VDALIIADIAV VDALIAADVAV ADALILADLAM ADALILADLAM	0 11 LEYAARHYPH LGYAAKRHPR LEYAAERYPH LEYAAERYPO	O 1 LELHLSVQA LNLHLSVQG IERHVSVQA VERHVSVQA	2 O SATNVKAV SATNAAAL SATNEEAI SATNDEAI	130 EFYHQNF ALYHERY NFYHRHF RFYQRHF
939	UbiU Dechlormonas_aromatica.35 UbiU Thiobacillus_denitrificans.333 UbiU Thiobacillus_denitrificans.333 UbiU Rhodopseudomonas_palustris.325 UbiU Ralorhodospira_halophila.331 UbiU_Rhodobacter_sphaeroides.336 UbiU_Aeromonas_salmonicida.331	VLMAINTFPQTGRVAD VLLALNTYPQSDTWTR VLIAINTFPARAGSIDI VLVAVNSYVQAGGWSQ VLVAINTFPRAGDESL LHIAINTFAHPGSDTR	HKAVDGAVDQGV TGAVDRAAKLGN HRAIDDAVDCEJ QRSIDEAARIGA HRNIAATEAAGJ KQAVDRGVALGJ	ADAVILADFGL VDAIILADFGL ADAVIVADVGV ADAVIVADVGV ADAVIVADLGL ADAVILADMGL ADALIIADLAV	L DYARQRHPQ MRYAVKHHPQ MDYVARTHPD LEYTAEQWPD LAYAAKNHPN LDYASRHYPD	CRLHLSVQG QRLHLSVQG QRLHVSVQA LGLHLSVQA LRRHLSVQA MELHVSVQA	SATHADAI SATSYEAI SATSYEAV AAANPDAI SATTPEAL AAANPDII SATNAAAI	NFCQREF NFYREHF RFYVERF DFYKRCY NFYNREF RFYQQHF
940	UbiU_Paracoccus_denitrificans.330 UbiU_Peisseria_sicca.335 UbiU_Phaeospirillum_fulvum.339	VLVAINTFPRAGDQVL VLMAINTFAQAGQIER VLVAVNTFPRAGQPEI	HRAVRDAADLGJ HRAVDTAVQLGJ HRAVADCDELKJ	ADAVILADIGI ADAIIVADPAI ADAVILADIGI	I A H A A E A H P G MA Y A A E R H P N I D H A A R H H P N	LRLHLSVQA LRLHMSVQG LRLHLSVQA	AAAN PDAI S <mark>ATNYEAI</mark> A <mark>ASNPDAI</mark>	NFYAEAF NMMKELF RFYADRY
941		140 150	0 160	170	180	190	2	00
942	UbiU_Vibrio_cholerae.337 UbiU_Pseudomonas_aeruginosa.331 UbiU_Escherichia_coli.331 UbiU_Versinia_pestis.331 UbiU_Ralstonia_solanacearum.339 UbiU_Dechloromonas_aromatica.335	NVKRVVLPRVLSIHQVI GIRRAVLPRVLSIHQVI DVARVVLPRVLSIHQVI DVARVVLPRVLSIHQVI GIRRAVLPRVLTLAEI GIRRAVLPRVLTLAEV	XQLSRNI.PQGV RQVAAGS.P.VI XQLARVT.P.VI XQLSRTS.P.VI SRTS.P.VI SRTS.P.VI SRVIPQT.S.VI SHVIKNT.T.VI	ELEVFAFGSLC PVEVFAFGSLC PLEVFAFGSLC IEVFAFGSLC IEVFGFGSLC SIEVFGFGSLC	IMSEGRCYLS IMAEGRCHLS IMSEGRCYLS IMAEGRCYLS VMAEGRCLLS VMNEGRCWLS	SYMTGESPN SYVTGESPN SYLTGESPN SYLTGESPN SFATGDSPN SYACGESPN	T V GA C S PA L C G V C S PA T V GA C S PA T V GA C S PA N K G V C S PA T V GA C S PA	KYVRWQE RAVRWSE RFVRWQQ RFVRWQQ HAVRWHE KYVKWDK
943	UbiU_Thiobacillus_denitrificans.333 UbiU_Rhodopseudomonas_palustris.325 UbiU_Rhodobacter_sphaeroides.326 UbiU_Aeromonas_salmonicida.331 UbiU_Paracoccus_denitrificans.330 UbiU_Parasoccus_denitrificans.330	GVQRAVLPRVLSLPQVI NAKRVVLPRVLSVPEIJ GVSRAVLPRVLSIQQVI GVKRVVLPRVLSVAEIJ NVGRVVLPRVLSMHQVI GIRRVVLPRVLSVEEI GIRRAVLPRVLSVEEI	AAIGKEV. T A AAIGKEV. T A AAIAGDT.D VI AAINRETPE VI RQLARET.D VI CAINREI.D VI KHVIDNT.D VI	EIELFGFGGLC EIEVFGFGSLC EIEVFGFGSLC EIEVFFFGGLC SLEVFAFGSLC EIEVFVFGGLC EIEVFGFGSLC	VMVEGRCLLS VMEEGRCSLS VMAEGRCSLS VMAEGRCSLS VMAEGRCSLS VMAEGRCSLS VMVEGRCILS	SYCTGESPN SYATGKSPN SYATGESPN SYATGKSPN SYMTGESPN SYATGLSPN SYATGESPN	SAGVC SPA MDGVC SPA TVGAC SPA MNGVC SPA TVGAC SPA MNGVC SPA MQGVC SPA	KAVQWKD SHIQYRE WAVQWQE TEVEYVE KFVRWEE SHVAYAD KAVRWEQ
944	UbiU_Phaeospirillum_fulvum.339	GIK <mark>RVVLPRVLTV</mark> QEI	AEVNARIAPI	U32 prote	VMAEGRCALS CASE	SYATGOSPN	IN <mark>GACSPA</mark>	SDVRYTE
945	Whill Wilmin shalawar 207	210 220	230	240	25	0 2	60	270
946	UbiU_Pseudomonas_aeruginosa.331 UbiU_Escherichia_coli.331 UbiU_Tersinia_pestis.331 UbiU_Ralstonia_solanacearum.339 UbiU_Dechloromonas_aromatica.335 UbiU_Diobacillus_denitrificans.333	TPOGLESRINEVLIDR TPOGLESRINEVLIDR TPOGMESRINDVLIDR QDGQLDARINTILIDR KPGTMETRINGVLIDR TPTGLESRINGVLIDR	A A GE N A GY PT L (A A GE N A GY PT L (E D H E N A GY PT L (A P GE A A GY PT L (G D D E P A GY PT L (G K D E K A GY PT L	KGRFIVNG CKGRYLVDG CKGRYRVDG CKGRFVVDG CKGRFVVDG CKGRFVVDG CKGRFVVDG CKGRFDVQG	QR.F QR.Y QR.Y QR.Y EV.D DT.Y	HALEEPTSL HALEEPTSL HALEEPTSL HALEEPTSL YALEEPTSL YALEEPTSL	NTLSLLP NTLDLVPQ NTLELLPE NTLELLPE NALSLLPK NVLEILPD NTLDLLPE	LAEIGVA LMAANIA LFAANIA LIGMGVA IIKIGVK MLKMGIA
947	UbiU_Rhodopseudomonas_palustris.325 UbiU_Halorhodospira_halophila.331 UbiU_Rhodobacter_sphaeroides.326 UbiU_Aeromonas_salmonicida.331 UbiU_Paracoccus_denitrificans.330 UbiU_Pisseria_sicca.335	DNGVLVSRLGEFTINK TPEGREARLGGLIDRI EGDQLAARLGDFTINR TPAGLESRLNEVLIDR VAGNHEARLGDFLINR LPDRMNVRLNOVLIDO	FAGGEAAAYPTL FAPDEPAGYPTI GRDQPAPYPTL GPGENAGYPTL FPKGMPVPYPTL KPNEPAGYPTL	KGRYQTSE KGRYEVEG KGCFTSGD KGRFEVDG KGCFTSPD KGRFEVND	GSG QVG QVG QTY GKGDSQTG ETY	YLFEDPSSL HAFESPTSL HIFEDAVSL HALEEPTSL HIFEDPVSL YALEEPTSL	DATTMLPE ETAELLPR NAQDILPQ NTLGLLPE DATRLIPA NVLEMLPE	LKKAGVC LKRAGIH LAKAGVT LFQTGIQ LQKAGVS LIKIGVS
948	UbiU_Phaeospirillum_fulvum.339	TNEG <mark>ITSE</mark> G <u>GFTINK</u> I	FAHTEP <mark>AGYPT</mark> LO	KGRFIAND	KAS	Y L F <mark>E E P T S L</mark>	NTLAILPD	LIR <mark>AGV</mark> T
949	UbiU_Vibrio_cholerae.337 UbiU_Pseudomonas_aeruginosa.331 UbiU_Ecoborichiacoli 221	280 290 SVKIEGRORSPATVEO AVKIEGRORSPATVEO	TRTWRAAIDRYI TRTWRAAIDRYI TRVWRQALDSYJ	310 LANPAQYHVEP ASAPGAYSVOP	320 KWNAALANVS QWQRALAGLS	330 EGTQTTLGA EGHQTTLGA	YHRQWQ YHRSWQ	
950	Ubil_Versinia_pestis.331 Ubil_Ralstonia_solanacearum.339 Ubil_Dechloromonas_aromatica.335 Ubil_Thiobacillus_denitrificans.333	S VKIEGRORSPAYVSO AIKIEGRORSPAYVAO AIKIEGRORSPAYVAO AIKIEGRORSPAYVAO	AKVWRQAIDLY(VATLRAALDAA(TRTLRAALDAAL TKVWRAAIDAV	ANPAQFAAKA QADPARFSARP RDGSERFHVKP KKDAVDFKPTP	EWMEQLGAMS EWQAALVCHA AWQAELSKVS AWQAELNKLS	EGTQTTLGA EGAHVTQGA EGSQATLGA EGQSHTLGA	YHRKWQ FDRPWK YSRPWR YHRPWK	
951	UbiU Halorhodospira halophila. 31 UbiU Rhodobacter_sphaeroides.326 UbiU Paracoccus_denitrificans.331 UbiU Paracoccus_denitrificans.330 UbiU Paracoccus_denitrificans.330 UbiU Phaeospirillum_fulvum.339	A VI IEGRORS PAYVGK AL KIEGRORS PAYVGK SVKIEGRORS RSYVAQ AL KIEGRORS PAYVEQ AL KIEGRORS RAYVAQ AI KIEGRORS RAYVAQ AFKIEGRORGRAYVAQ	VATF MALRAL VTRIWRQLIDRI VVRSFRAVDAL VTRVWRAAIDAYQ VVRAFRAAVEAAI VTKSLRQALDAA VGAFRQGVDAVI	PEAG EP PEAGD YTPDP PAG PAG QANPEAYQVKA PAG QANPEHYQVKA QANPEHYKVNP AANPEHYKVNP AAG	ADETRGLS AQVAALREFS MPQGALAALS EWDAQLARVS MPEGLLARLT LWNNALSKVS LPDIDLDRIT	E GOSTITICA E GATTIL GP E GOATITGA E GSOTILGA E GQAATIGA E GHQITLGA E GGKQITGA	Y E K A WR Y E R N WQ Y A K T WR Y H R Q WQ Y A K T WR Y S R P W K Y E K A W R	

953 Figure S3: Multiple sequence alignment of UbiU from representative proteobacteria. Sequences were 954 aligned using Mafft (linsi), and the output generated using ESPript and Inkscape. The four conserved cysteines (C169, C176, C193 and C232) involved in iron-sulfur binding are indicated by black columns. The 955 position of the domain U32 protease PF01136 is indicated by a green box. GenBank accession numbers: 956 Vibrio cholerae, NP_230301.1, Pseudomonas aeruginosa, NP_252602.1, Escherichia coli, NP_312066.1, 957 Yersinia pestis, YP_002348368.1, Ralstonia solanacearum, WP_011004262.1, Dechloromonas aromatica, 958 959 WP_011285878.1, Thiobacillus denitrificans, WP_011312698.1, Rhodopseudomonas palustris, 960 WP_011666114.1, Halorhodospira halophila, WP_011813833.1, Rhodobacter sphaeroides, 961 WP_011909346.1, Aeromonas salmonicida, WP_005310394.1, Paracoccus denitrificans, WP_041530457.1, Neisseria sicca, WP_049226489.1, Phaeospirillum fulvum, WP_074767209.1. 962

963 964 965	UbiV_Vibrio_cholerae.292 UbiV_Pseudomonas_aeruginosa.296 UbiV gescherichia_coli.292 UbiV Versinia_pestis.292 UbiV Versinia_pestis.292 UbiV Pachioromonas_aromatica.300 UbiV_Thiobacillus_denitrificans.297 UbiV_Thiobacillus_denitrificans.200 UbiV_Thiobacilus_phaeroides.298 UbiV Aeromonas_salmonicida.290 UbiV_Aeromonas_salmonicida.296 UbiV_Neisseria_sicca.305 UbiV_Phaeospirillum_fulvum.299	INKILGI PLLYN PRODU'R STUTOAAA SM. ADVI'ILGET VG SKRREMKYGDWLARASKA OVVLS MKLSLGPLLYN DKRO'IPDF'AEMAG OP LDVI'LGET VG KKREMLSUNDGLARELAASK. OVVLS MKLSLGPLLYN PKETLEDT OGAAT SS. ADVI'LGET VG KKRALSUNDGLARELAASKA (OVULS MKYALGAVLYN PKTETET HAAAN SS. ADVI'LGET VG KKREMKYGDWLARKLAASKA (OVULS MKYALGAVLYN PKTETET HAAAN SS. ADVI'LGET VG SKREMKYGDWLARKLAASKA (OVULS MKYALGAVLYN PKTETET HAAAN SS. ADVI'LGET VG SKREMKYGDWLARKAASGK (OVULS MSSHI SLOULTN PKGEVNEFT AEMAG (NARVAD SP. (NARVAGSKEN OVULS MKSLED ULLTN PKGEVNEFT AEMAG (NARVAD SP. (NARVAGSKEN OVULS MKSLED ULLTN PKGEVNEFT AEMAG (NARVAD SP. (NARVAGSKEN OVULS MKSLED ULLTN PKGEVNEFT AEMAG (NARVAGSKEN OVUS MKSLED ULLTN PKGEVNEFT AEMAG (NARVAD SP. (NARVAGSKEN OVUS MKSLED ULLTN PKGEVNEFT AEMAG (NARVAGSKEN OVUS MKSLED ULLTN PKAPTGAT (NARVAGSKEN OVUS MKSLED ULLTN PKAPTGAT (NARVAGSKEN OVUS SKEN OVUS KKEN OVULS MNPKKLSLGPVLFFT OKEALLEFT AAMLD AP (NDRVIGV VG SKEN PFPEPTIASVUSLARGK) SVALS MNPKKLSLGPVLFFT OKEALLEFT AAMLD AP (NDRVIVLGT VG SKEN FPFFEPTIASVUSLARGK) EVALS MNPKKLSLGPVLFFT OKEALLEFT AAMLD AP (NDRVIVLGT VG SKEN FPFFEPTIASVUSLARGK) EVALS				
966						
967	Ubiv Vibrio cholerae,292	70 80 90 100 110 120 130 140 TMANURAPSETINUMKKYI DNGPENTSANDUSATOMANEKGLEPTUGPAUSTOMANTTIKLET.KOGMISUCMDUS				
968	Ubiv Pseudomonas acruginosa.296 Ubiv Sscherichia_coli.392 Ubiv Stonia solianacaarum.294 Ubiv Acraina postis.292 Ubiv Ralstonia solianacearum.294 Ubiv Pachloromonas aromatica.300 Ubiv Rhodopseudomonas palustris.306 Ubiv Rhodopseudomonas palustris.300 Ubiv Rhodopacter spheroides.298 Ubiv Rhodobacter spheroides.290 Ubiv Paracoccus denitrificans.290 Ubiv Paracoccus denitrificans.296	GLAL TEAASELSSL RRICENGELRVEANDMAAVYYLSOROVPFVGOPAL HLYNGHALAELYSNGMREW IP TLALVQASSELGELKRYVENGEFLIEASDLGVNMCAERKLPFVGOPAL HLYNGHALAELYSNGMREW IP TLALVQASSELSELKRYVENGEFLIEASDLGVNMCAERKLPFVGOPAL HLYNGHALAELYSNGMREW IP				
969		TRT TETS A BAQAL RROC QODD WEVE A GELGAVILL RGRPFVA GPHCNATH GGTLAWLAG LGAMRAVM LE A QAALLESESDLKAL RRLIDDAGC MLEANDLGAVINLV HGODFVA GPHLNT TNEATLISFAR FGLKRWVPLC TOTTESSDLKAL RRIDS AGEFCUCAR WAAVLIJE A GUPPA GSTLNVNA OT LAILASFAR FGLKRWVPLC				
970		SLM MSLTRERNAMAE LAADHSFTMEVNDLSCLGMIGDREHAIGPFINITNEATAAYFASHGAKRICL PE TLSUVEARSEASVVRCLCDNGELRVEANDFTAVSELERRRLPFVAGFILNUSGRALAAMGRAGAVWVPMG SLADVILKREFTLFEID.agvGILIADLFALAVVP.GGAPFAVSPLINCUNVGTLDNILAAMGRAGAV				
971		TLADISAPSELKEVKRLVDNGDLLVEANDLGTVQLASEAGFPFVCGPAINATNADVLRMLLKOGMRRMVMDVE TLTDITLKRERROTAELFA.SGLFVEIADLSALKHLF.EGAPFVVGFVNVNNGGLBWLARRGRRTCLPPE SQVDLBSESDLKRLRKITGQEFFVCEANDMGAVKLAREHGIPFVGGSLUFUNSSTLALFGRLGAVRMIALSE				
972	UbiV_Phaeospirillum_fulvum.299	<u>SLAT</u> IMSER <u>EM</u> AA <u>ARDI</u> ASDADLL <u>VEANDI</u> S <u>VA</u> AITI A <u>GRPPVAG</u> AL <mark>LNVUN</mark> EGTIATIESMEAIEVCLEAE				
973						
974	Ubiv_Vibrio_cholerae.292 Ubiv_Pseudomonas_aeruginosa.296 Ubiv_Escherichia_coli.292 Ubiv_Escrinia_pestis.292 Ubiv_Ralstonia_solanacearum.294 Ubiv_Dechloromonas_aromatica.300 Ubiv_Thiobacillus_denitrificans.297	150 160 170 180 190 200 210 LSRDWLINTLQQCDELNIRGQFEVEVFSHCVL9LAYSARGPTARAENRARDEGETCCIRYPTCIQVASQEGQS				
975		VSGKLLRGVLDQAAQLG.BQLQYBYFAYGHLPLAYSARGTTARSENRPKDDGQFCGQYPEGPLLSQBCBA LSRDWLVNLLNQCDELGINQFEVYVLSYGHLPLAYSARGTTARSEDRPKDECETCCIKYPNGRNVLSQENQQ LSRDWLNNLLQQCDALGPRNGFEVYVLSYGHLPLAYSARGTTARSEDRAKDECETCCIKYPNGRKVKSQEDQQ				
976		MSQATLAELMREKPDUELGAMYWGRLPLAFSARGTTAEHFRLNKDDGOFRGLEHPDGLAVRTREGOD GSRSMIEALHRKKPADVERGVTAFGKIPLAFSARGTTAEHYNLDGOFRGLEHPDGLAUGTREGOD TSRAGLALLAHAFAGIET <mark>EVFAYGRLPLAYSARGTTAE</mark> HYNLP <mark>KDDC</mark> OFRGLDHPDGLALGTREGEA				
977	UbiV_Rhodopseudomonas_palustris.306 UbiV_Halorhodospira_halophila.300 UbiV_Rhodobacter_sphaeroides.298	LPITAIRTIAALPUTIEVFOFORAPLAISARCHARLNKUKUSKUNGRVCEGOPDGLAVMTLDREP LPGASLRGVLSELTELGE.TYMETEVFAVGOPUTATSACFSARUSPRHODCGVCGHHPGGLPVTRAGEP LPMESVATLAQAGKERGVAVEVWGFGROPLAISGRCYHARLHDRIKDSCOFICWAPDGREVDTLDGER				
978	Ubiv Paracoccus denitrificans.296 Ubiv Neisseria sicca.305	LSRWHLDLSUDE . AARQUEEV VIAIGHLE ATANG TASLDAFKING LORDAN AND AND AND AND AND AND AND AND AND				
979	UDIV_Phaeospiriiium_ruivum.299	U32 protease				
980						
981	Ubiv_Vibrio_cholerae.292 Ubiv_Pseudomonas_aeruginosa.296 Ubiv_Escherichia_coli.292 Ubiv_Tschia_postis.292 Ubiv_Ralstonia_solanacearum.294 Ubiv_Dchloromonas_aromatica.300 Ubiv_Thiobacillus_denitrificans.297 Ubiv_Rhodopseudomonas_palustris.306 Ubiv_Balorhodospira_halophila.300 Ubiv_Rhodobacter_sphaeroides.298 Ubiv_Aeromonas_salmonicida.290 Ubiv_Aerocccus_denitrificans.296 Ubiv_Meisseria_sicca.305 Ubiv_Phoespirillum_fulvum.299	220 230 240 250 260 270 VFNLMCIONGGVCYNLWNDLPSMAGL VDWVLSDLGVETFOHLEOFKAMEGGLK				
982		VFVLNGIGTMSGYVYNLGNELASMOGL. VDVVRLSPOGTDTFAMLDAFRANENGAA				
983		FLTINGIOTMSAQSYNLLHEMPEMLKMGIDIVRISPOKOHINEIIAAFDAARLGEAVQVDSRDWSTSG. FLTINGIOTOSSGVHTLIDDIPALREIGVASLRSPOSRHMGRVVEFRAALLNGGDVDAAALORAMPGA FLSMNGVOTLAYACTSVIGEIGDIU DAGVRSLRSPOCOMVVTKLFRGVU DGSKSAADAAALPALYPYP.				
984		FLVINGIOTOSMGCANLLPAVVEMQQMGVTAVELSPRFGANDQVVAAPRSAIAGEVTUREPESAQ FLAVNGVOTLSCATSFVGREIPGLLAAGVSALRLSPHTGDAVGLARAFDEAVKAGANTDALEAARAVAPGGQ VFNINGIOTOSGYCYNLGNELIGGMADL.VDVVRLSPEGLDTLENLARFKAMQQGQPLTLQQER.				
985		FLTWNGVOTLSYTHMSVAGQUOPLIKESGVGALRLSPQFGDFAAVARLVACLLESGLSPRDALADLRRLVPGAL FltinglotmsvgcQnllphhedlrqigvinnlrlspQnhgmaeivrinkrdvldgktaladalpelerlttgt. FlavngfotmsshyndllpdivsirargvsrflwpQnvdvvavsalfravjdgknavaerdblagicpgvg				
986						
987		280 290				
988	UbiV_Vibrio_cholerae.292 UbiV_Pseudomonas_aeruginosa.296	QCNCYWHQLACLAVKNS				
989	Ubiv Yersinia_pestis.292 Ubiv Ralstonia_solanacearum.294 Ubiv_Ralstonia_solanacearum.294	DCNGYWRALADLEUGA				
990	Ubiv_Thiobacillus_denitrificans.297 Ubiv_Rhodopseudomonas_palustris.306	LVDGTWFGJAGLVQRHTQALAQLGA AIDGTWFGAGLEVINEEPC VANGELYARPGSNFIANVTEAQRTARAL				
991	Ubiv_Rhodobacter_sphaeroides.298 Ubiv_Aeromonas_salmonicida.290	LANGYLHARGAEWVDA DCNGYWRGIPGMSLVE FAGGYLHARTGAEPCA				
992	UbiV_Neisseria_sicca.305 UbiV_Phaeospirillum_fulvum.299	LVD <mark>C</mark> YNHGOPCTEAVKEEYYGVA Fa <mark>ncyvh</mark> grachlfvedde				

994 Figure S4: Multiple sequence alignment of UbiV from representative proteobacteria. Sequences were 995 aligned using Mafft (linsi), and the output generated using ESPript and Inkscape. The four conserved 996 cysteines (C39, C180, C193 and C197 - positions in *E. coli*) involved in iron-sulfur binding are indicated by 997 black columns. The position of the domain U32 protease PF01136 is indicated by a green box. GenBank 998 accession numbers: Vibrio cholerae, NP_230300.2, Pseudomonas aeruginosa, NP_252601.1, Escherichia 999 coli, NP_312067.2, Yersinia pestis, YP_002348369.1, Ralstonia solanacearum, WP_011004261.1, WP_011285877.1, Thiobacillus 1000 Dechloromonas aromatica, denitrificans, WP_011312697.1, 1001 Rhodopseudomonas palustris, WP_011666113.1, Halorhodospira halophila, WP_011813832.1, 1002 Rhodobacter sphaeroides, WP_011909345.1, Aeromonas salmonicida, WP_005310392.1, Paracoccus WP_011750458.1, Neisseria sicca, WP_080614296.1, Phaeospirillum fulvum, 1003 denitrificans, WP_074767477.1. 1004



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



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

- **Table S1:** UQ levels in strains from the medium- and large- deletion collections
- **Table S2:** Occurrence of genes from the UQ pathways in bacterial genomes.
- **Table S3:** List of oligonucleotides and strains used in this study
- **Table S4:** References of the protein sequences used to build the HMM profiles.