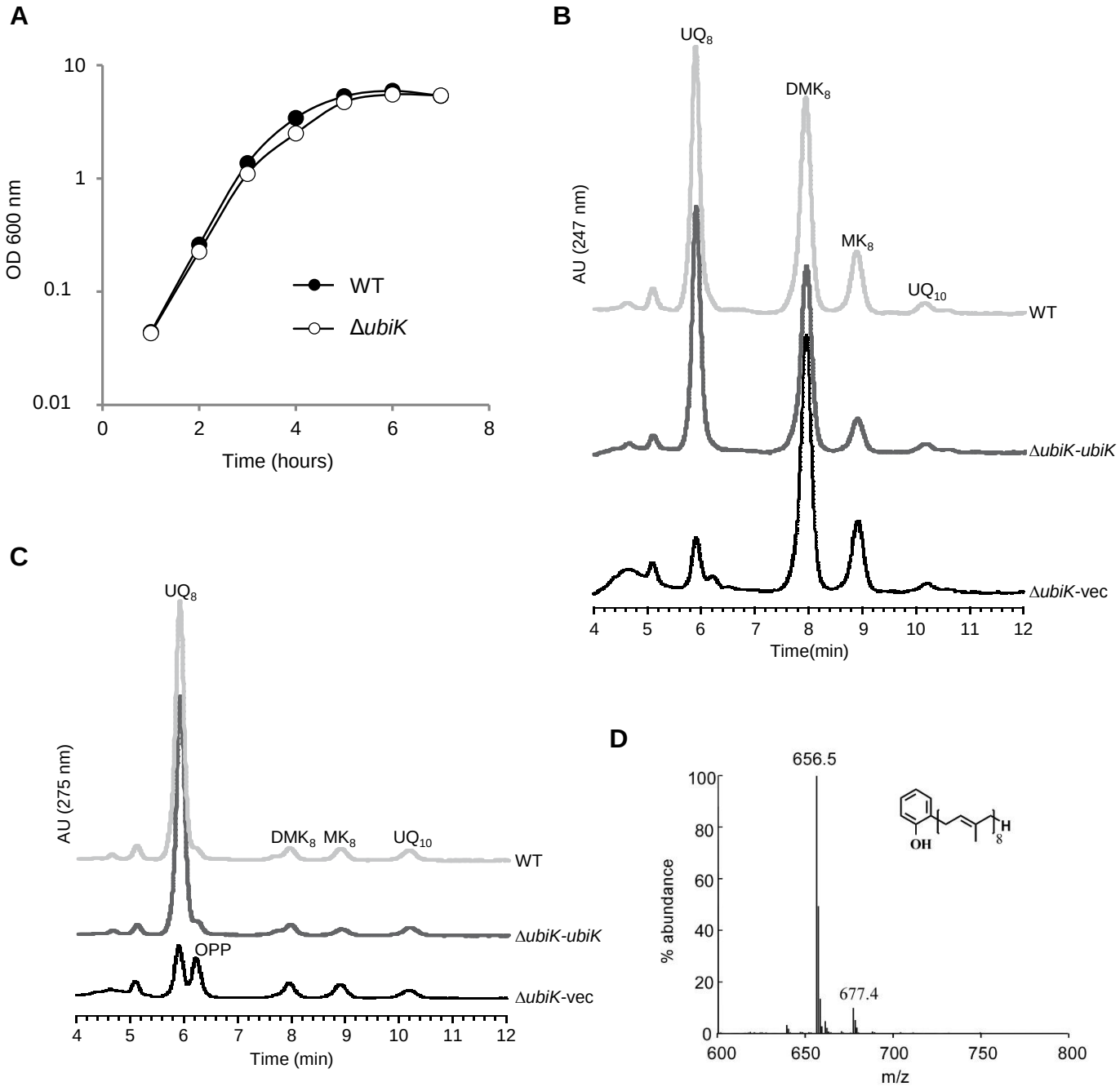


FIGURE S1. A) Wild-type (filled circles) and Δ*ubiK* (empty circles) strains were grown overnight and then diluted at an $OD_{600\ nm}$ of 0.05 in LB medium at 37°C under aerobic conditions. Growth was monitored at 600 nm. **B**) Chromatograms at 247 nm of the same extracts as in Fig. 1A, absorbance in arbitrary units. **C**) Chromatograms at 275 nm of the same extracts as in Fig. 1A, absorbance in arbitrary units. **D**) MS spectrum of OPP eluting at 6.4 min in the HPLC analysis of lipid extracts from the *ubiK* mutant grown in LB medium in aerobic conditions.

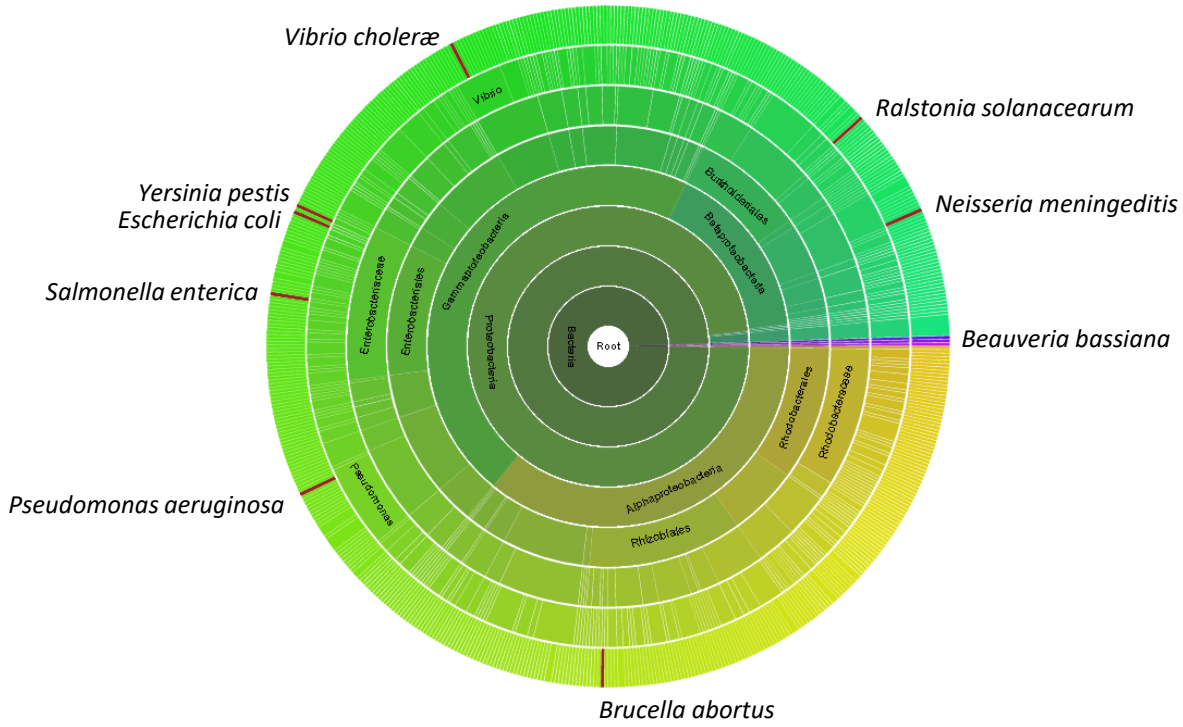


FIGURE S2. Sunburst representation of the distribution of *ubiK* sequences (BMFP family) across species. This Pfam EMBL-EBI tree (http://pfam.xfam.org/family/PF04380#tabview=tab7) shows the occurrence of this BMFP family (PF04380) across different species. This family is represented in 1104 species. Almost exclusively all UbiK sequences are found in the Proteobacteria phylum (green and yellow segments). Only 3 UbiK sequences are found in Eukaryotes (purple segment).

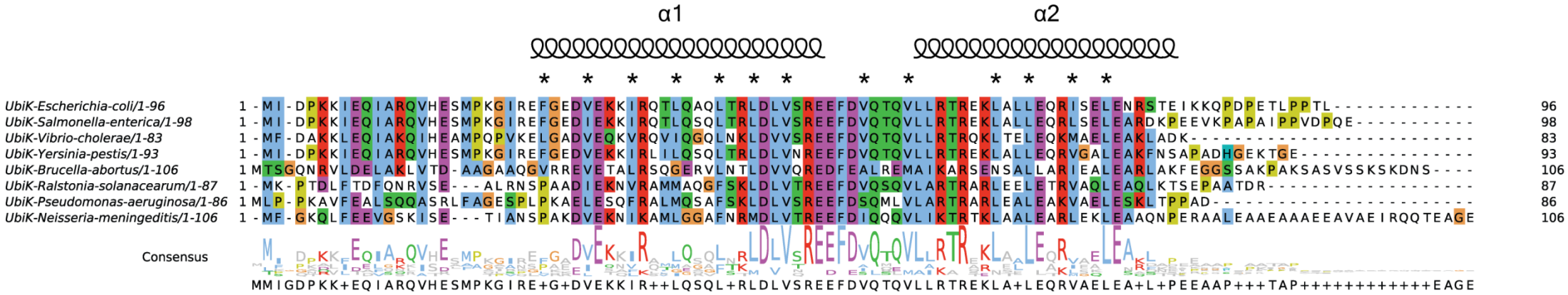


FIGURE S3. Sequence alignment of UbiK from diverse Proteobacteria. UbiK from *E.coli* (Q46868); UbiK from *Salmonella enterica* (A0A0U0WRC8); UbiK from *Vibrio cholerae* (Q9KVI3); UbiK from *Yersinia pestis* (Q7CGG8); UbiK from *Brucella abortus* (Q2YLY4); UbiK from *Ralstonia solanacearum* (Q8Y2J5); UbiK from *Pseudomonas aeruginosa* (Q9HTR5); UbiK from *Neisseria meningeditis* (Q9K0Z7). Asterisks mark the position of hydrophobic amino acids that could form the core of the trimer bundle. The predicted secondary structures are shown above the sequence.

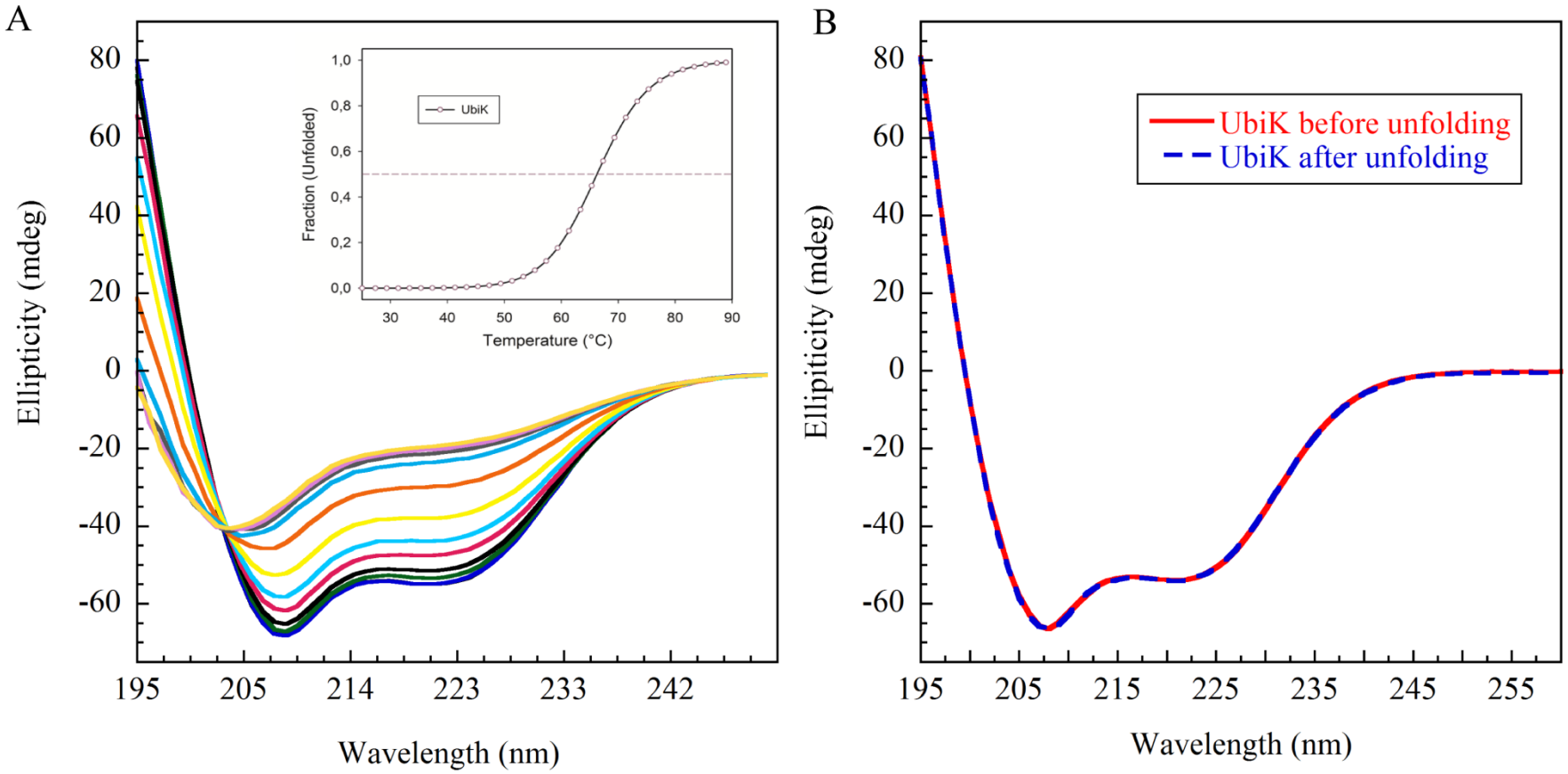


FIGURE S4. Thermal stability of *E.coli* UbiK. A) Dichroïc signal of *E.coli* UbiK secondary structures as a function of temperature (UbiK 0.07 mg/mL, 5 mM NaH_2PO_4/Na_2HPO_4 pH 8.0, L = 0.4 cm). Inset, Sigmoïdal van't Hoff transition curve, fractional extent of unfolded UbiK (222 nm) versus T (°C). B) CD spectra of UbiK before thermal unfolding (red) and after unfolding (blue).

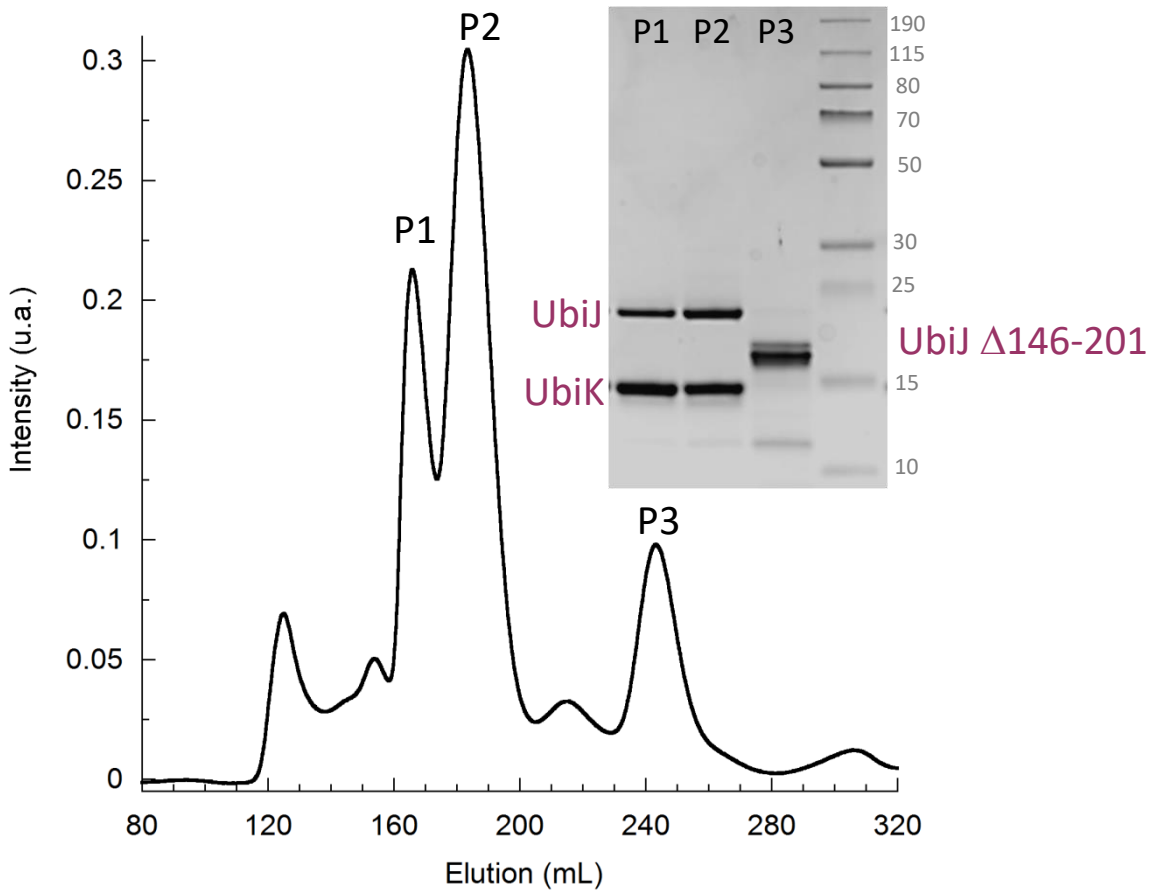


FIGURE S5. Gel filtration analysis of the UbiJ-UbiK complexes. HiLoad 16/600 Superdex 200 pg, 50 mM Tris-HCl, 150 mM NaCl, pH 7.5.

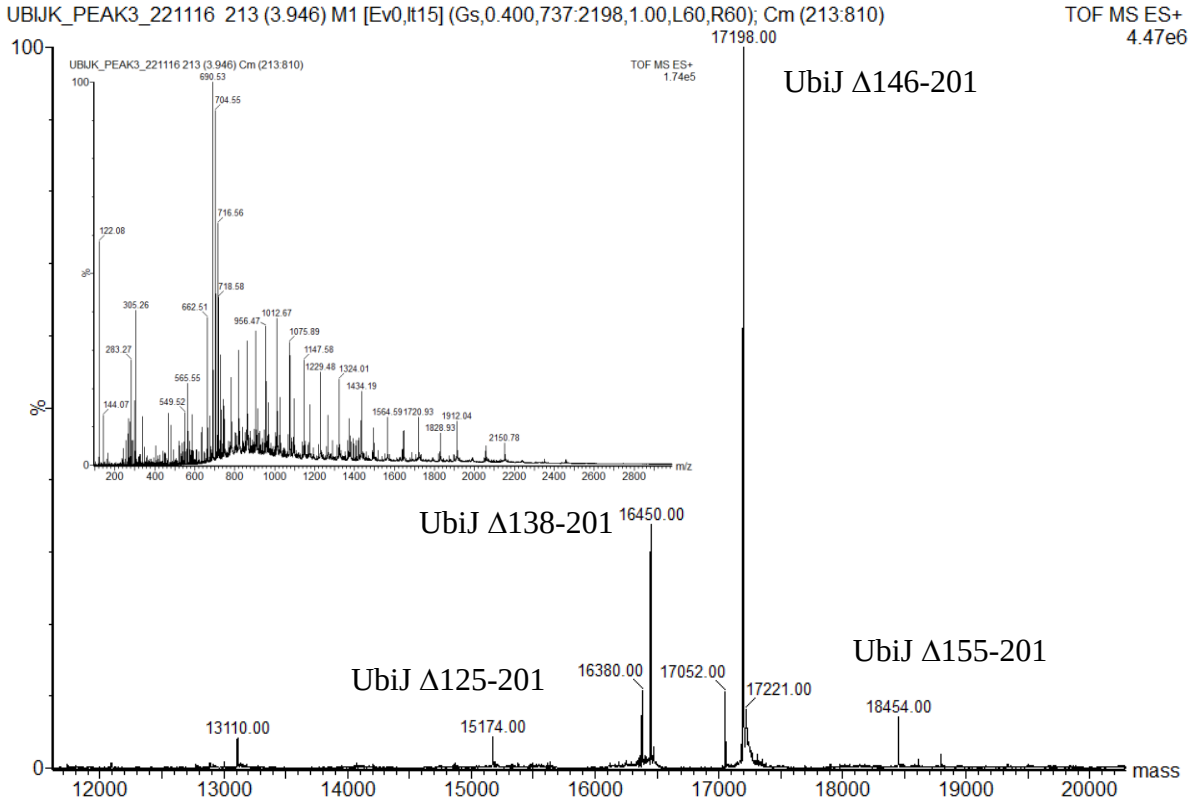


FIGURE S6. Mass spectrometry analysis of the peak 3 (P3) UbiJ fragment. ESI-MS deconvoluted spectrum (TOF MS ES+). Inset, electrospray spectrum of P3.

TOF MS ES+ Peak at 17198.00 (UbiJ Δ146-201)

MELHHHHHHEF MPFKPLVTAG IESLLNTFLY RSPALKTARS RLLGKVLRVE VKGFSTSLIL VFSERQVDVL GEWAGDADCT VIAYASVLPK LRDRQQLTAL IRSGELEVQG DIQVVQNFVA LADLAEFDPA ELLAPYTGDI AAEGISKAMR GGAKF

Molecular weight: 17 198

TOF MS ES+ Peak at 16450.00 (UbiJ Δ138-201)

MELHHHHHHEF MPFKPLVTAG IESLLNTFLY RSPALKTARS RLLGKVLRVE VKGFSTSLIL VFSERQVDVL GEWAGDADCT VIAYASVLPK LRDRQQLTAL IRSGELEVQG DIQVVQNFVA LADLAEFDPA ELLAPYTGDI AAEGISKA

Molecular weight: 16 450

UbiJ 6-His full length

MELHHHHHHEF MPFKPLVTAG IESLLNTFLY RSPALKTARS RLLGKVLRVE VKGFSTSLIL VFSERQVDVL GEWAGDADCT VIAYASVLPK LRDRQQLTAL IRSGELEVQG DIQVVQNFVA LADLAEFDPA ELLAPYTGDI AAEGISKAMR GGAKFLHHGI KRQQRYVAEA ITEEWRMAPG PLEVAWFAEE TAAVERAVDA LTKRLEKLEA K

Molecular weight: 23 625

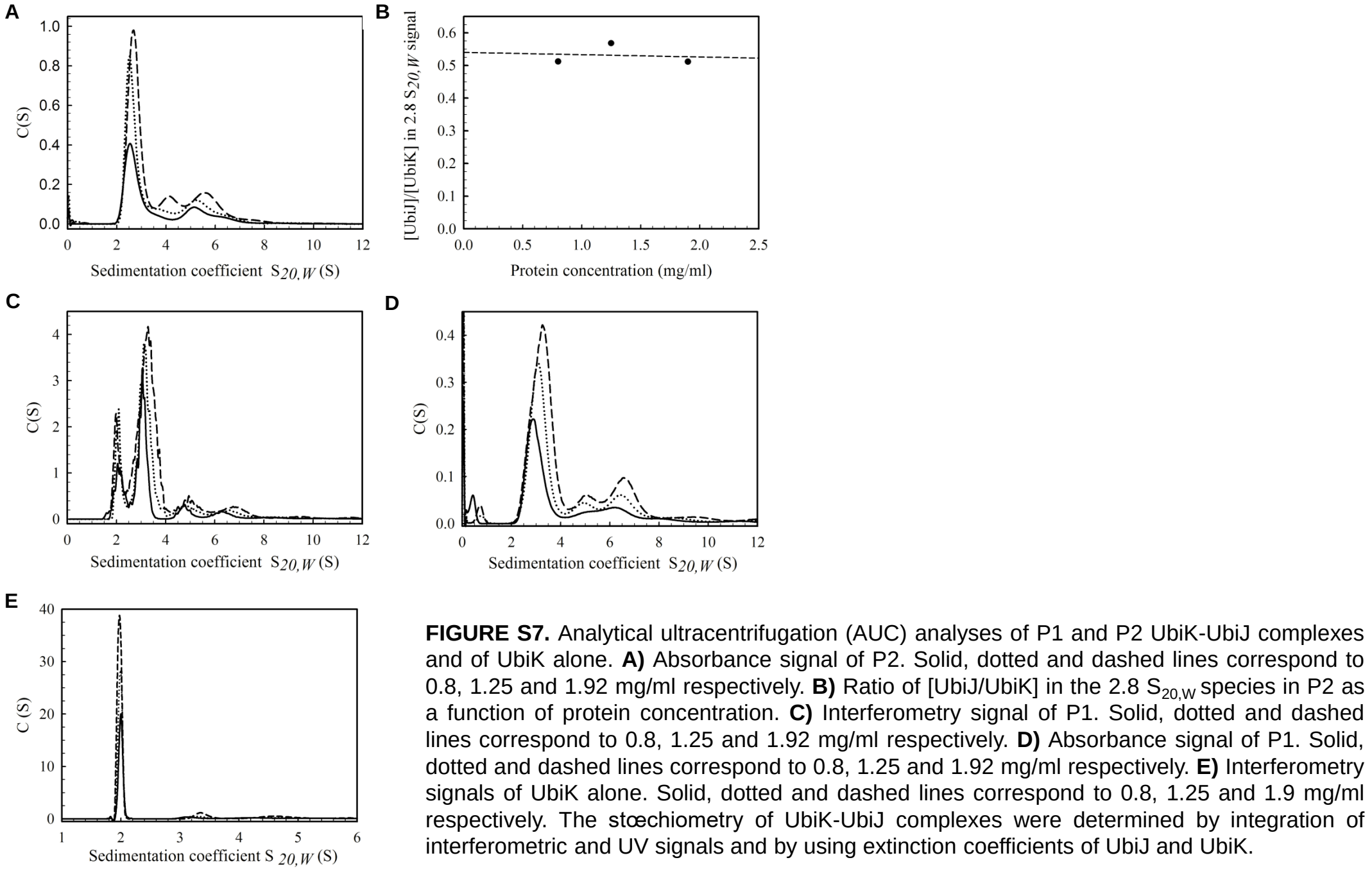


FIGURE S7. Analytical ultracentrifugation (AUC) analyses of P1 and P2 UbiK-UbiJ complexes and of UbiK alone. **A)** Absorbance signal of P2. Solid, dotted and dashed lines correspond to 0.8, 1.25 and 1.92 mg/ml respectively. **B)** Ratio of [UbiJ/UbiK] in the 2.8 $S_{20,W}$ species in P2 as a function of protein concentration. **C)** Interferometry signal of P1. Solid, dotted and dashed lines correspond to 0.8, 1.25 and 1.92 mg/ml respectively. **D)** Absorbance signal of P1. Solid, dotted and dashed lines correspond to 0.8, 1.25 and 1.92 mg/ml respectively. **E)** Interferometry signals of UbiK alone. Solid, dotted and dashed lines correspond to 0.8, 1.25 and 1.9 mg/ml respectively. The stœchiometry of UbiK-UbiJ complexes were determined by integration of interferometric and UV signals and by using extinction coefficients of UbiJ and UbiK.

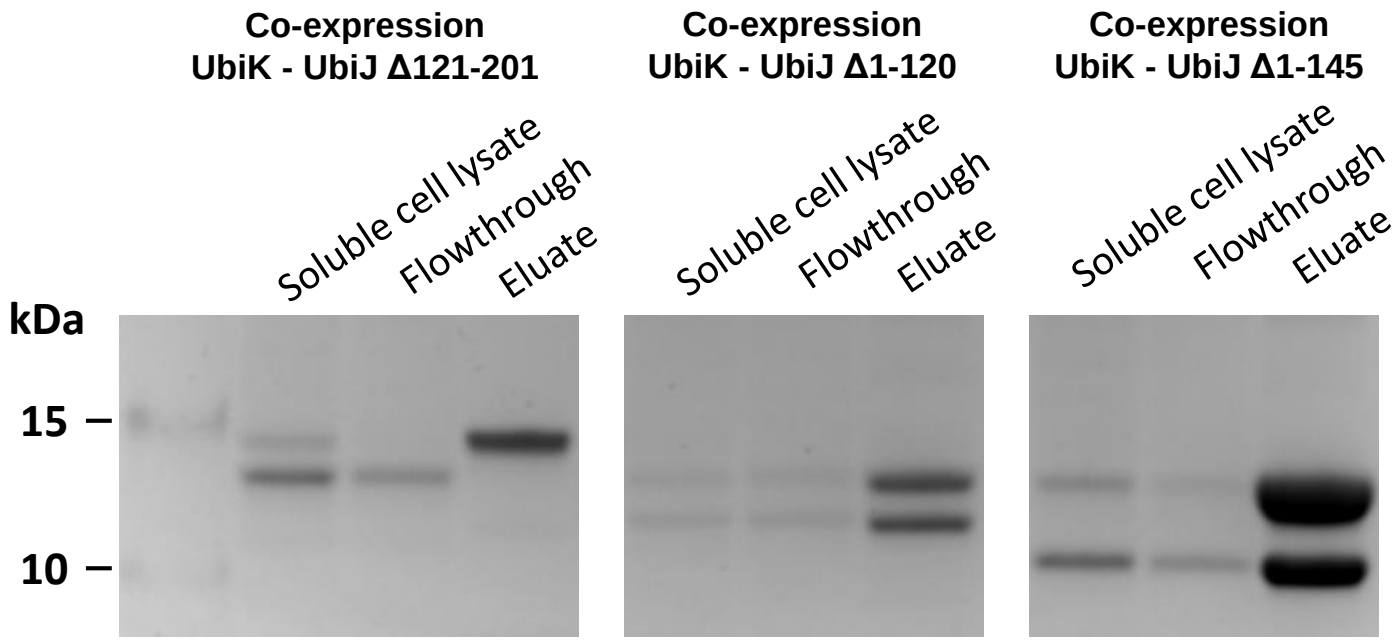
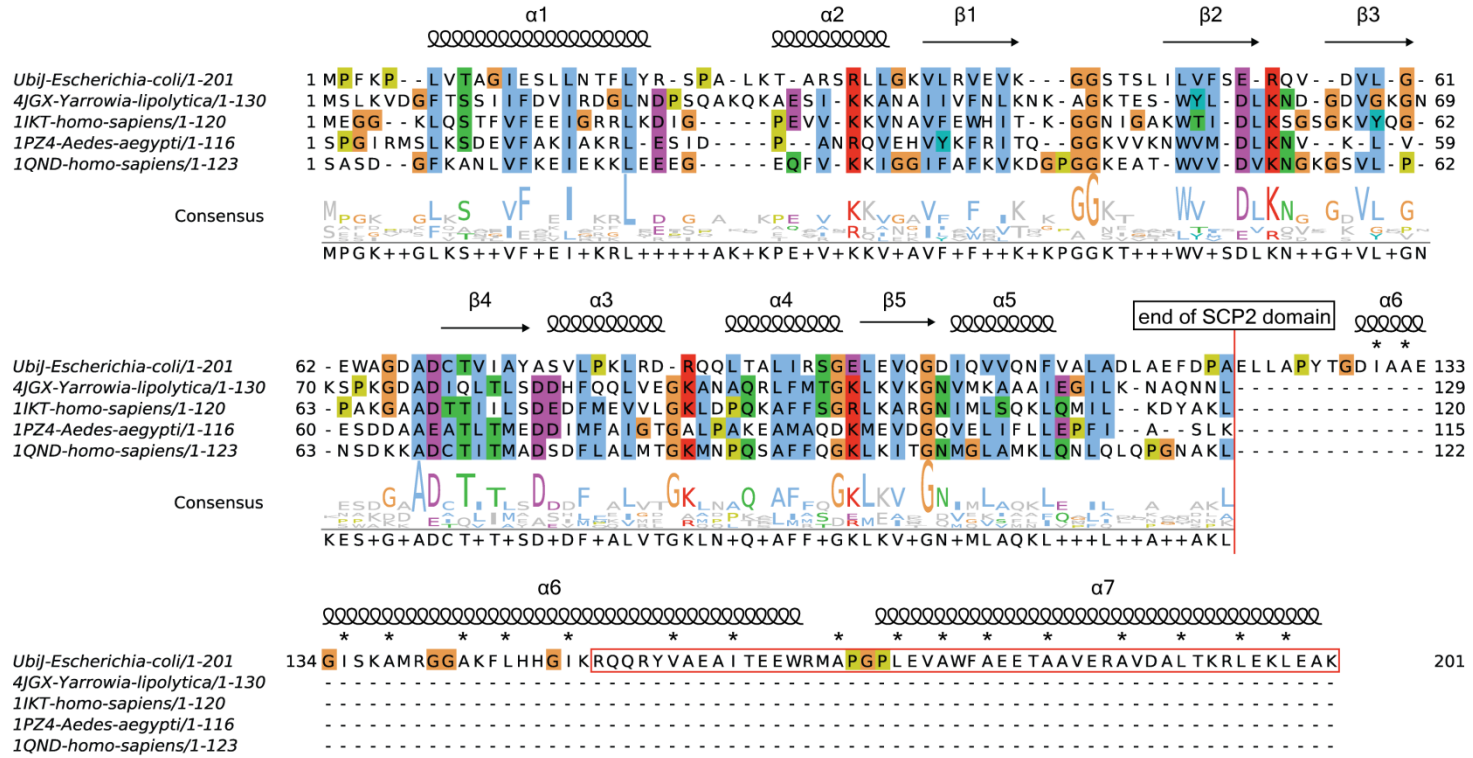


FIGURE S8. SDS-PAGE of the nickel affinity purification of truncated UbiJ coexpressed with UbiK. The coexpression of full length UbiK with no tag (11.3 kDa) was carried out with UbiJ Δ121-201 (14 kDa, left panel), UbiJ Δ1-120 (10 kDa, middle panel) and UbiJ Δ1-145 (8 kDa, right panel).

A



B

UbiJ	100 %				
4JGX	19.6 % (31.1)	100 %			
1IKT	15.8 % (29.6)	35.0 % (46.7)	100 %		
1PZ4	14.8 % (31.8)	20.9 % (28.9)	26.1 % (40.7)	100 %	
1QND	15.8 % (28.1)	29.5 % (37.0)	35.8 % (48.9)	28.7 % (40)	100 %
	UbiJ	4JGX	1IKT	1PZ4	1QND

FIGURE S9. A) Sequence alignment of the SCP-2 domain of *E.coli* UbiJ along with crystallized SCP-2 proteins with closest similarity. The following sequences, SCP-2 domain of UbiJ (1-120) from *E.coli* (P0ADP7), SCP-2 from *Yarrowia lipolytica* (PDB 4JGX), human SCP-2 domain from peroxisomal multifunction enzyme type 2 (PDB 1IKT), SCP-2 from *Aedes Aegypti* (1PZ4) and human SCP-2 (1QND) were aligned with ClustalO. The α-helices and β-sheets of the SCP-2 domain of UbiJ are predicted by Swiss Model, with 4JGX as template. The secondary structures of residues 121-201 do not exist as part of other aligned SCP-2 proteins. The final 50 residues involved in interaction with UbiK are highlighted by a red box. Asterisks mark the position of hydrophobic amino acids that could form the core of the trimer bundle when UbiJ forms a heterotrimer complex with UbiK. **B) Table of SCP-2 proteins with sequence identities and similarities to UbiJ SCP-2 domain of *E.coli*.** The percentages of sequence similarity are indicated in brackets.

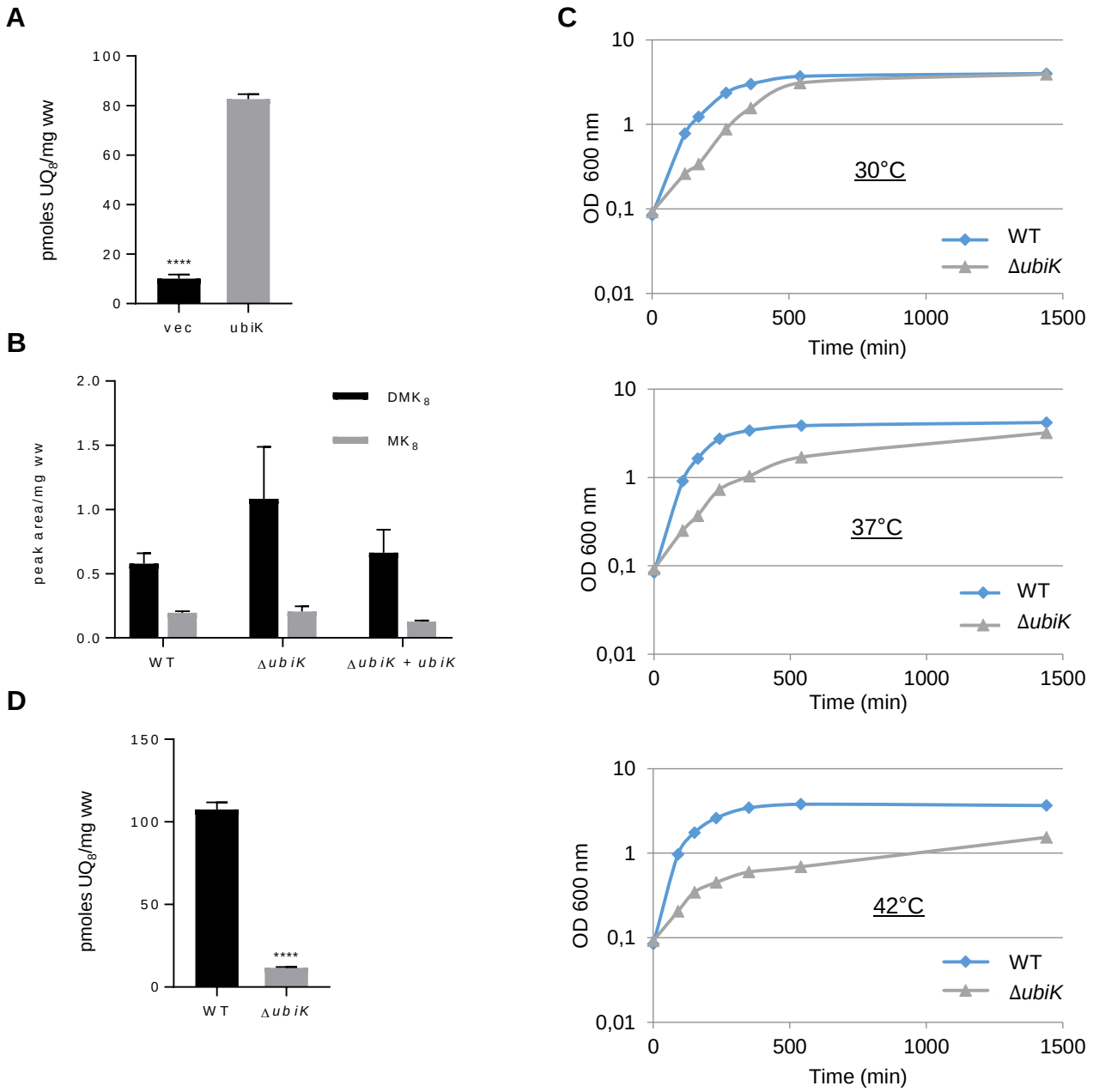


FIGURE S10. A) UQ_8 content of *S. enterica ubiK mutant* transformed with either the empty pBAD vector (vec) or the pBAD vector carrying the *S. enterica ubiK* gene grown in LB medium in aerobic conditions at 37°C. **B**) Quantification of cellular DMK_8 and MK_8 from chromatograms at 247 nm of lipid extracts from *S. enterica* WT or *ubiK mutant* cells complemented or not with pBAD-UbiK and grown as in A. **C**) Growth curves of the *S. enterica* wild-type (blue) and ubiK mutant (gray) stains in LB under aerobic conditions at 30°C, 37°C and 42°C. **D**) UQ_8 content of *S. enterica* WT or Δ*ubiK cells* after aerobic growth at 30°C in LB. A and D, Mean ± s.e.m.; n=3, ****: *P*<0.0001, unpaired two-tailed t test.