Supporting Information

Cultivation at high osmotic pressure confers ubiquinone 8-independent protection of respiration on *Escherichia coli*

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Table S1. Deletion of *ubiG* impairs ProP and LacY activity in *E. coli.*

Table S2. Osmolality response parameters of His_6-ProP in proteoliposomes.

Table S3. Oligonucleotide primers.

Figure S1. Representative growth curves

Figure S2. The growth of wild type *E. coli* and of *E. coli* BW25113 under osmotic stress is similarly impaired by a *ubiG* defect.

Figure S3. Expression of *ubiG* from pLT1 does not require arabinose induction.

Figure S4. CL is not required for osmotolerance or respiratory protection.

Figure S5. TLC analysis of intracellular trehalose

Table S1
Deletion of *ubiG* impairs ProP and LacY activity in *E. coli*[1]

Strain	Relevant Genotype	Replicate Experiment	Osmolality Response Parameter: ProP			Osmolality Dependence: LacY	
			A_{max} nmol/min/mg protein	$\Pi_{1/2}/RT$ mol/kg	B mol/kg	Slope (m) (nmol/min/mg protein)/(mol/kg)	Intercept (b) nmol/min/mg protein
WG709	*ubiG*$^+$	1	103 ± 4	0.241 ± 0.005	0.046 ± 0.005	4.7 ± 0.5	6.6 ± 1.8
		2	102 ± 3	0.236 ± 0.005	0.044 ± 0.004	9.3 ± 1.7	2.1 ± 0.5
WG1542	*ΔubiG785*::*kan*	1	11 ± 1	0.249 ± 0.016	0.066 ± 0.011	-0.3 ± 0.9	1.1 ± 0.3
		2	16 ± 1	0.247 ± 0.012	0.080 ± 0.008	-0.8 ± 0.8	1.3 ± 0.2

[1]Bacteria were cultivated, initial rates of proline or lactose uptake were measured, and data were analyzed as described in Experimental Procedures and illustrated in Figure 3.

Table S2
Osmolality response parameters of ProP-His$_6$ in proteoliposomes

Supplementary Q8	Replicate	Osmolality Response Parameter		
		A_{max}	$\Pi_{1/2}/RT$	B
		nmol/min/mg protein	mol/kg	mol/kg
None	1	893 ± 66	0.47 ± 0.02	0.09 ±0.02
	2	860 ± 35	0.44 ± 0.01	0.07 ± 0.01
1 % (w/w)	1	1009 ± 73	0.47 ± 0.02	0.10 ± 0.02
	2	838 ± 67	0.44 ± 0.03	0.10 ± 0.02

[1]PRLs were prepared without or with Q8 supplementation, initial rates of proline uptake were measured, and data were analyzed as described in Experimental Procedures and illustrated in Figure 6.

Table S3.
Oligonucleotide primers

Primer Pair	Primer Sequence (5' to 3')	Application
80-01 80-02	GGAAGCTTCCGGGGCCAATGCTCGAGGAAATCTT GGAAGCTTCTCCGTTAACCTGGAGGAGAGTATGC	PCR: presence or absence of *proP*
ProW2 ProW3	GGTGATAATGCCCGGCGTCAG GGACCAACAGTATGTCGGCATC	PCR: presence or absence of *proW*
betT3 betT5	GTGGCGTCGATCAGTTGTC CGCCATATGCAGACATGGC	PCR: presence or absence of *betT*
cls-For cls-Rev	CGCTGACCAGTGCTTTTCC GCTGGATCGAGATTGTCGG	PCR: presence or absence of *clsA*
otsA-1 otsA-2 otsA-3	GGCACAGGTGCAACTCAGG GCCTACGGTGAGTTAAGCG CGC AAT TTG CGG GAG CGG	PCR: presence or absence of *otsA*
KmF ubiG-rev	GATCTCCTGTCATCT CAC TGT CTA GTC GGC ACG G	PCR: presence of *ΔubiG785::kan*
ubiG-for ubiG-int ubiG-rev	GCT ATC CCT CTA CTG TAT CC GGT GGG ATC TGG AAG GTG CAC TGT CTA GTC GGC ACG G	PCR: presence or absence of *ubiG*
ubiGus ubiGds	GAT**CCATGG**ATGCCGAAAAATCG CCC**AAGCTT**TCACTTATTCTGCG	PCR amplification of *ubiG*, adding *Nco*I and *Hind*III sites (**bold**).
pBADfor pBADrev	CCA TAA GAT TAG CGG ATC CTA CC CTG AGC CTT TCG TTT TAT TTG ATG CC	Sequencing to verify insertion of *ubiG* into pBAD24

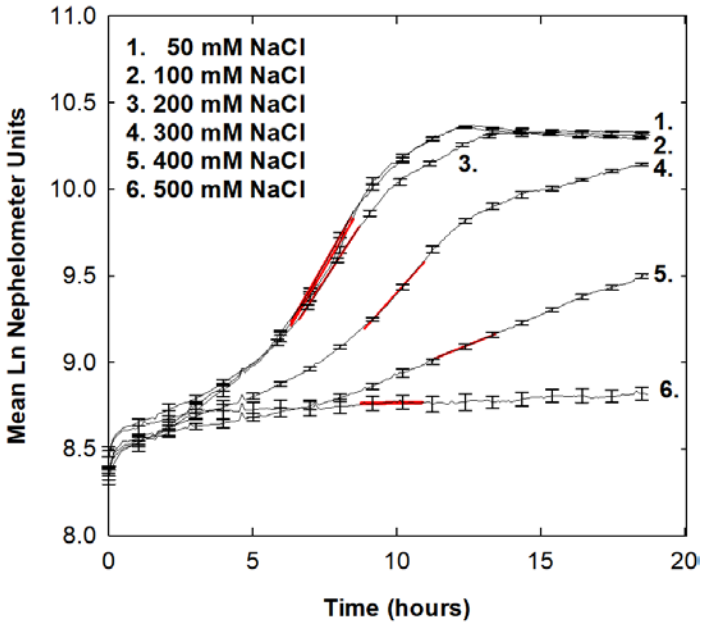

Figure S1.
Representative growth curves.
Escherichia coli strain WG1230 was cultivated in MOPS medium containing NaCl at the indicated concentrations, growth was monitored with a Nephelostar microplate nephelometer (BMG Labtech, Ortenberg, Germany) and growth rates were determined as described in Methods. The resulting, red regression lines yielded the growth rates shown in Figure 2 (Panel A, strain WG1230 (*ubiG*$^{+}$) without glycine betaine (GB)).

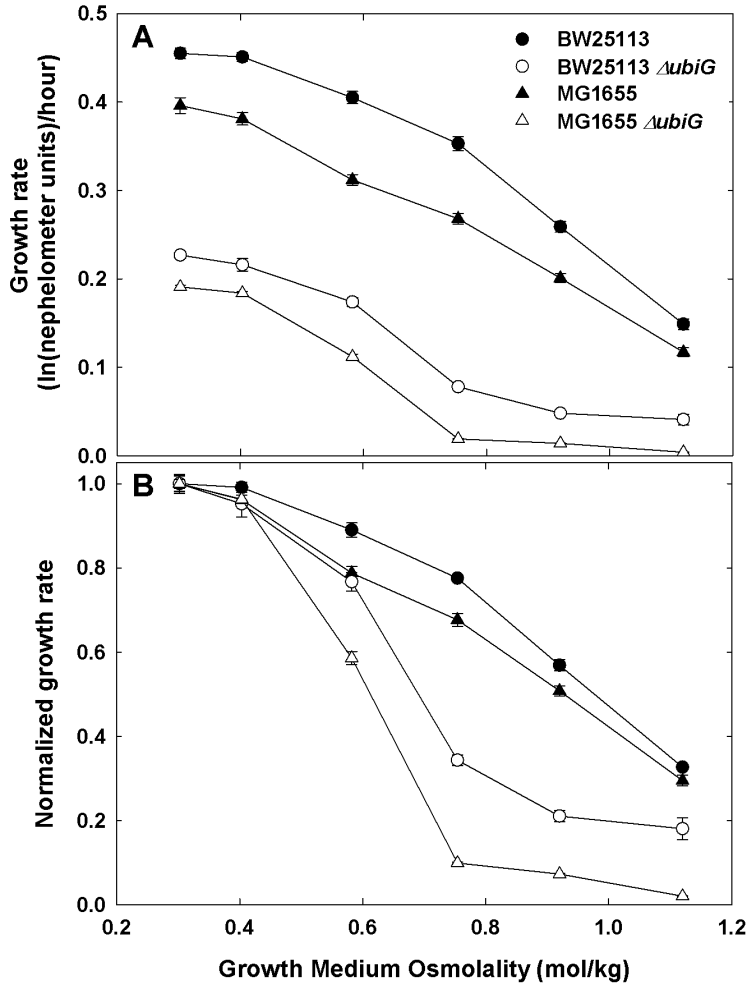

Figure S2.
Impact of a *ubiG* defect on the growth of wild type *E. coli* and *E. coli* BW25113.
Growth rates of *E. coli* strains belonging to the Keio collection (BW25113 (*ubiG*$^+$) and JW2226 (*ΔubiG785*::*kan*), circles) and those based on wild type *E. coli* (MG1655 (*ubiG*$^+$) and WG1533 (*ΔubiG785*::*kan*), triangles) were cultivated in M9 medium under osmotic stress, growth rates were determined and data were analyzed as described in the legend for Figure 1. Panel A: Solid symbols illustrate the growth rates of *ubiG*$^+$ strains (BW25113 and MG1655), while open symbols illustrate the growth rates of *ΔubiG785*::*kan* strains (JW2226 and WG1533). The osmolalities of the media were adjusted by adding NaCl (50 mM - 500 mM). Means ± standard error of four replicate measurements from one of two replicate experiments are shown. In panel B, each growth rate is normalized with respect to the mean growth rate observed at the lowest osmolality.

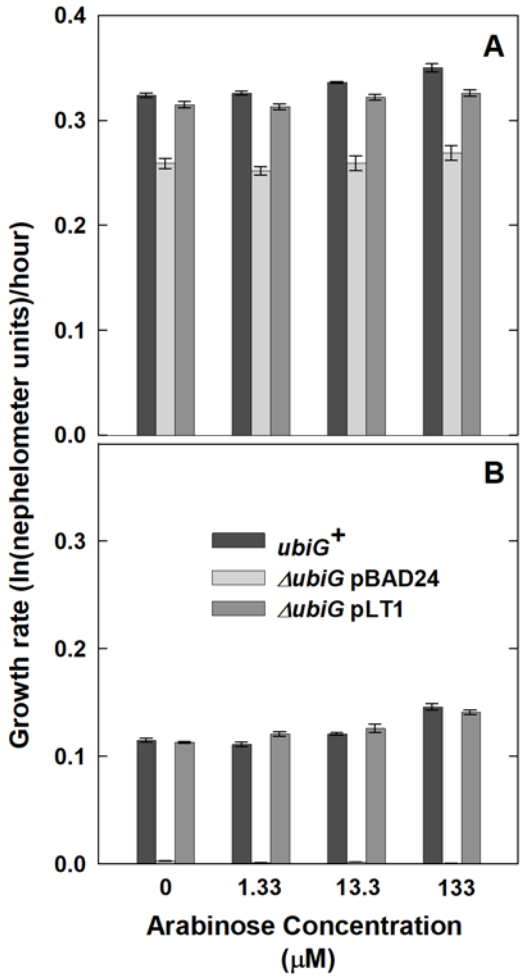

Figure S3.
Expression of *ubiG* from pLT1 does not require arabinose induction.
The growth rates of *E. coli* strains MG1655 (*ubiG*$^+$, black bars), WG1590 (MG1655 *ΔubiG785*::*kan* pBAD24, light grey bars) and WG1591 (MG1655 *ΔubiG785*::*kan* pLT1, dark grey bars) in NaCl-free MOPS media without or with arabinose (1.33 μM, 13.3 μM, and 133 μM) and with 50 mM NaCl (the low osmolality medium, panel A) or 500 mM NaCl (the high osmolality medium, panel B) were determined and the data were analyzed as described in the legend to Figure 1.

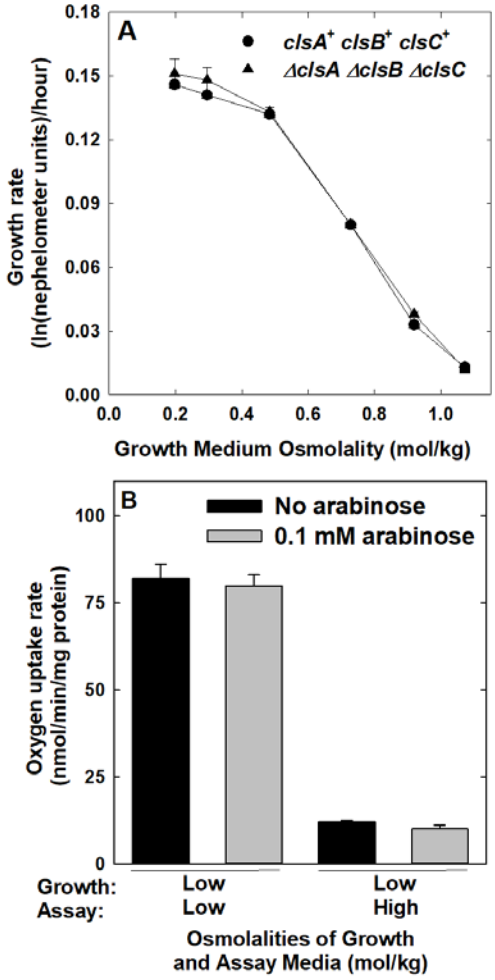

Figure S4.
CL is not required for osmotolerance or respiratory protection
Panel A: The growth rates of *E. coli* strains W3110 (wild type, circles) and BKT12 (W3110 Δ*clsA856::FRT* Δ*clsB861::FRT* Δ*clsC788*::*kan*, triangles) in NaCl-supplemented MOPS media of the indicated osmolalities were determined and the data were analyzed as described in the legend to Figure 1. ***Panel B***: *E. coli* strain WG1610 (MG1655 pDC347) was cultivated in low osmolality MOPS medium, without (black bars) or with (grey bars) arabinose (0.1 mM), and oxygen uptake was measured in low and high osmolality MOPS media as described in the legend to Figure 4.

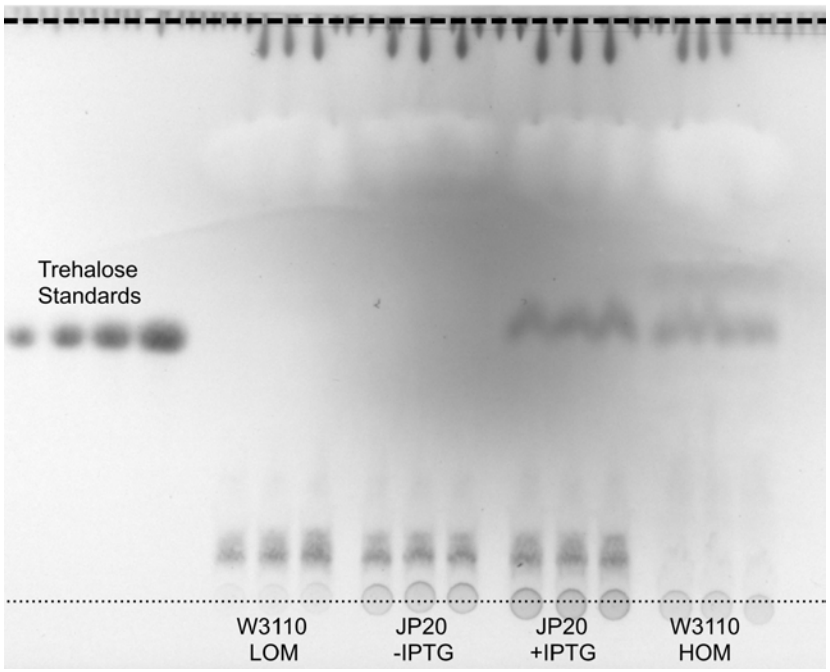

Figure S5
TLC analysis of intracellular trehalose
Cell extracts from *Escherichia coli* strain W3110 (wild type) grown in low (LOM) or high (HOM) osmolality NaCl-supplemented MOPS medium, and strain JP20 (W3110 *ΔotsBA::FRT ΔtreA::FRT ΔtreC::FRT ΔtreF::FRT FampH::lacI-Ptac-otsBA::FRT*) grown in LOM with or without IPTG (0.1 mM) were prepared, and the TLC was performed as described in Experimental Procedures. Triplicate measurements from one of two experiments are shown. Authentic trehalose standards (5 nmol, 10 nmol, 15 nmol, and 20 nmol) were used for the identification and quantification of trehalose. The plate origin and solvent front are indicated by the dotted and dashed line, respectively. The R_f value for trehalose was 0.41.