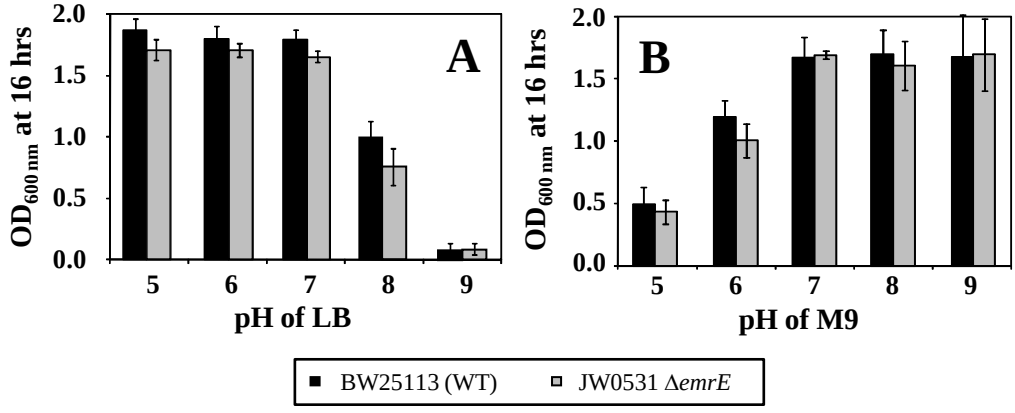
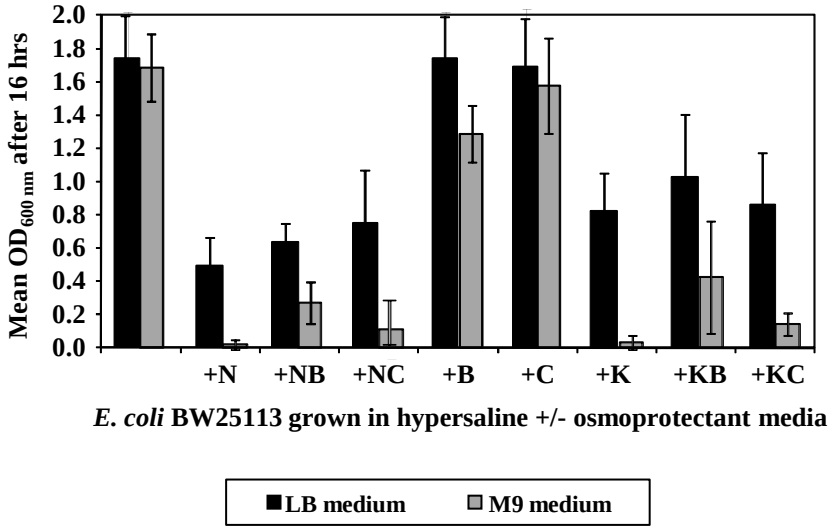
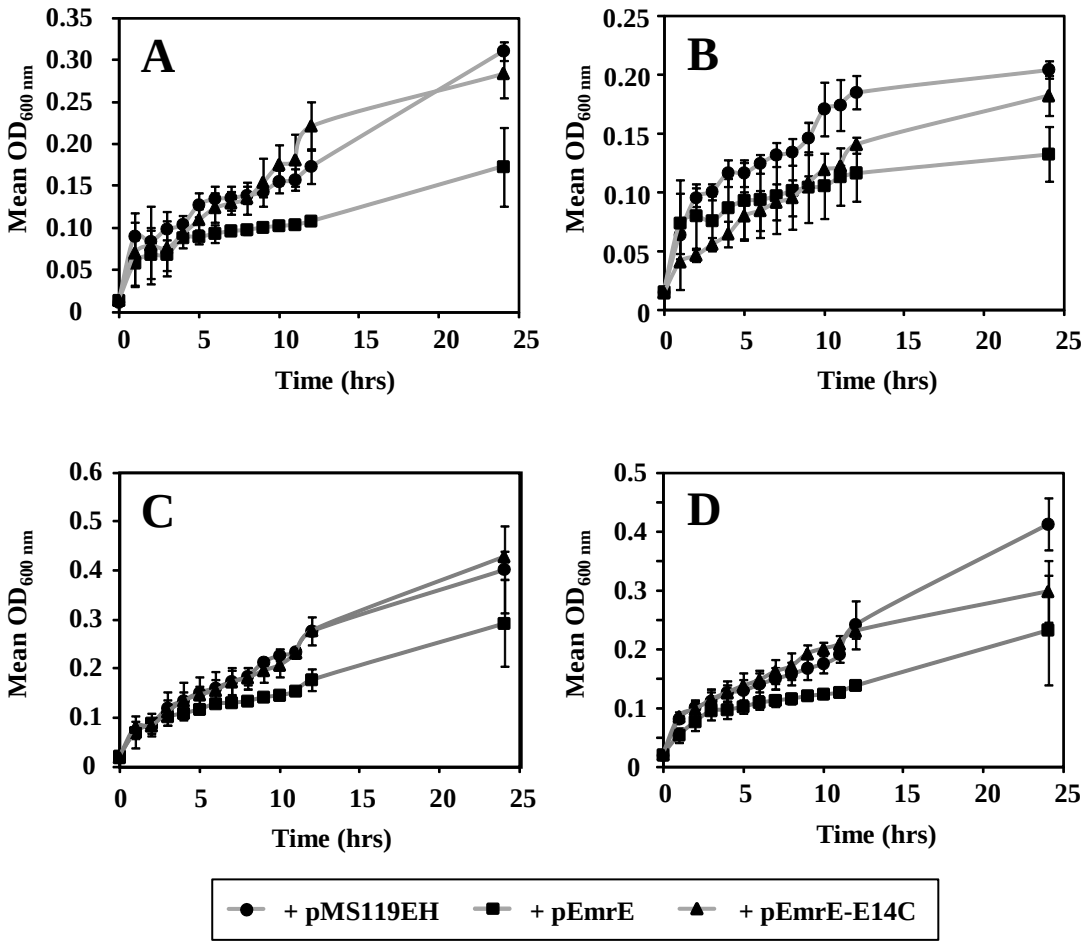


Supplementary Figure 1. pH susceptibility of *E. coli* BW25113 and JW0531 strains. Mean $OD_{600\,nm}$ values after 16 hrs of growth are presented for each *E. coli* strain cultured in either LB (A) or M9 (B) media at pH values of 5-9. In both panels *E. coli* strains BW25113 (wildtype; black) and JW0531 (Δ*emrE*; grey) are shown in bar chart format.



Supplementary Figure 2. Hypersaline screening assay of untransformed *E. coli* BW25113 control strain grown the presence of osmoprotectants betaine and choline. The mean $OD_{600\ nm}$ (y-axis) of BW25113 strains grown in either LB (black) or M9 media (grey) for 16 hrs in the presence and absence of 1.0 M NaCl (N) or KCl (K) and 10 mM osmoprotectants, betaine (B) or choline (C).



Supplementary Figure 3. Hypersaline growth susceptibility curves of *emrE* plasmid transformed *E. coli* BW25113. Mean $OD_{600\,nm}$ over 24 hrs of growth in hypersaline M9 media at 1.0 M NaCl (A-B) or KCl (C-D) in the presence of 10 mM osmoprotectants betaine (A-B) or choline (C-D). In all panels, $OD_{600\,nm}$ values are shown for *E. coli* BW25113 transformed with pMS119EH (black circle), pEmrE (light grey square) or pEmrE-E14C (dark grey triangle).

Supplementary Table 1. A summary of SMR plasmids designed and the primers used for gene cloning and site-directed mutagenesis in this study.

Vector	SMR gene cloned	SMR gene mutation	NCBI gene locus tag	Primers used for SMR gene amplification or mutagenesis
pMS119EH	—	—	—	—
pEmrE	*emrE*	—	b0531	Forward 5'ATATTCTAGAAGGAGAAATAATATGAACCCTTATATTTATCTTGG 3' Reverse 5'TATAAAGCTTTTAATGTGGTGTGCTTCGTGAC 3'
pEmrE-E14C	*emrE*	E14C	b0531	Forward 5'CTTGGTGGTGCAATACTTGCATGTGTCATTGGTACAACC 3' Reverse 5'GGTTGTACCAATGACACATGCAAGTATTGCACCACCAAG 3'
pEmrE-*myc*-His_6	*emrE*	C-terminal *myc* epitope and His_6 tag	b0531	Forward 5'ATATTCTAGAAGGAGAAATAATATGAACCCTTATATTTATCTTGG 3' Reverse-1 5'TATAAAGCTTTTAATAAGCCTCGAACTCGAGATGTGGTGTGCTTCGTGAC 3' Reverse-2 5'TATAAAGCTTTTATAAGTCCTCTTCGCTAATTAACTTCTGCTCATAAGCCTCGAACTCGAGATG 3' Reverse-3 5'ATATAAGCTTTTAGTGATGGTGATGGTGATGGTCGACAGCGCTATTTAAGTCCTCTTCGCTAATTAAC 3'

Supplementary Table 2. *E. coli* K12 strains used for this study.

E. coli* strain	**SMR gene deletion**	**Genotype**	**Resistance**	**Reference**
BW25113	—	F-, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), λ-, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	—	(1)
JW0531	*emrE*	F-, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), Δ*emrE*750::kan, &λ-, *rph*-1, Δ(rhaD-rhaB)568, *hsdR*514	kanamycin	(1)
JW0303	*betA*	F-, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), λ-, *rph*-1, Δ(*rhaD-rhaB*)568, *ΔbetA753::kan*, *hsdR*514	kanamycin	(1)
JW0304	*betB*	F-, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), λ-, *rph*-1, Δ(*rhaD-rhaB*)568, *ΔbetB754::kan*, *hsdR*514	kanamycin	(1)

*All strains used in this study were provided by the Keio Collection of single-gene knockouts constructed through a collaboration of the Institute of Advanced Bioscience at Keio University, the Nara Institute of Science and Technology in Japan.

Supplementary Table 3. Average whole cell accumulation of EmrE-*myc*-His_6 protein (μg)/ $OD_{600\ nm}$ unit after 16 hrs growth at 37°C determined from Western dot blots of *E. coli* strains BW25113 and JW0531 grown under varying pH and hypersaline conditions.

***E. coli* Strain**	**Culture Medium**	**pH**	**Salt (M)**	**Average EmrE-myc-His_6 protein (μg)/ $OD_{600\ nm}$ unit**
BW25113	M9	5	—	0.031 +/- 0.021
		6	—	0.022 +/- 0.008
		7	—	0.018 +/- 0.003
		7	0.5 NaCl	0.011 +/- 0.015
		7	0.5 KCl	0.014 +/- 0.019
		7	1.0 NaCl	ND
		7	1.0 KCl	ND
		8	—	0.020 +/- 0.003
		9	—	0.005 +/- 0.004
BW25113	LB	5	—	0.059 +/- 0.028
		6	—	0.037 +/- 0.016
		7	—	0.026 +/- 0.040
		7	0.5 NaCl	0.019 +/- 0.015
		7	0.5 KCl	0.018 +/- 0.002
		7	1.0 NaCl	0.021 +/- 0.016
		7	1.0 KCl	0.021 +/- 0.014
		8	—	0.017 +/- 0.010
JW0531	M9	5	—	0.013 +/- 0.004
		6	—	0.015 +/- 0.003
		7	—	0.015 +/- 0.005
		7	0.5 NaCl	0.011 +/- 0.004
		7	0.5 KCl	0.012 +/- 0.018
		7	1.0 NaCl	ND
		8	1.0 KCl	ND
		9	—	0.021 +/- 0.001
JW0531	LB	5	—	0.037 +/- 0.017
		6	—	0.036 +/- 0.024
		7	—	0.029 +/- 0.020
		7	0.5 NaCl	0.007 +/- 0.004
		7	0.5 KCl	0.026 +/- 0.016
		7	1.0 NaCl	0.007 +/- 0.004
		7	1.0 KCl	0.028 +/- 0.003
		8	—	0.027 +/- 0.011

*ND, not determined due to insufficient cell growth under the defined condition.