1 Supplementary Materials

Number	Primer name	Sequence (5′ – 3′)
#1	Up-Fwd	ACCATCAGCACCACGATCTTGCCCAGCGG
#2	Up-Rev	CTGGTATGCCAAGGCCGGTTACATGCTCCC
#3	Down-Fwd	ACTCCGAGGGTACCCACGTTGGGATCTTCG
#4	Down-Rev	TCGTCGCCTTTCACCTCGACAATGGTCGGC
#5	Km ^r -Fwd	CGAAGATCCCAACGTGGGTACCCTCGGAGTGGATGAATGTCAGC TAC
#6	Km ^r -Rev	GGGAGCATGTAACCGGCCTTGGCATACCAGAGAAGGCGGCGGT GGAATCG
#7	Del_check_Fwd	CTTGACGGCATCGCCTACTACAACTCCAAC
#8	Del_check_Rev	CGATGCAGGAGCTGGAATCATACATCTTTG
#9	GSU2939_F	TCGATCCCGAGAAGGATTAC
#10	GSU2939_R	TGTCCACATTGGTGGATACG
#11	GSU2937_F	GAGAAAAACGGCTGGTATGC
#12	GSU2937_R	TGATGAACGTGGTGAAGTCC
#13	16S RNA_F	TGAGACACGGTCCAGACTCCTAC
#14	16S RNA_R	TCATTTCTTCCCTCCCGACA
#15	GSU2935_F	AATGCTACGGCTGTCATACGAAATA
#16	GSU2935_R	TTTCCCCTTGAAGGTAGAGACGTAG
#17	GSU2934_F	CCAGTTCATCCTTTACCATTCGGATTT
#18	GSU2934_R	GGTCCCAGAAGGAATCGAGAGAAAG

2 Supplementary Table S1. Primers used in this study





Supplementary Figure S1. Construction of an *extI*-deletion mutant (Δ*extI*). (A) Homologous
recombination scheme for gene disruption. The *extI* gene was disrupted with a kanamycin resistance gene
(*Km^r*) in wild-type *G. sulfurreducens* as described in the *Materials and Methods*. (B) The knockout
mutation in the genome was confirmed by PCR (upper panel) using the genomic DNA and primers #7 and
#8 shown in Supplementary Table S1 and Western blotting (lower panel) using an ExtI-specific antibody.
(C) The mRNA levels of *extI*, *extKL*, *extM*, *extN*, and *16S rRNA* in the wild-type and the Δ*extI* strains were
analyzed by semi-quantitative RT-PCR using each primer set shown in Supplementary Table S1.



Supplementary Figure S2. Effect of extl deficiency on cell growth. Wild-type (circle and solid line) and Δextl (triangle and dotted line) were anaerobically cultured in (A) NBAFYE medium containing 40 mM fumarate and (B) FWA medium containing 20 mM fumarate. Growth was measured by determining the optical density at 600 nm (OD₆₀₀).

 $\mathbf{24}$



26Supplementary Figure S3. Antibiotic susceptibility and permeability assay. Wild-type and $\Delta extI$ were27anaerobically cultured in NBAFYE medium, and cells were diluted with saline and spotted onto the28NBAFYE agar plate containing 1.25 µg/mL of (A) penicillin and (B) ampicillin and then cultured for 329days.

1*										
PaeOprP PaeOprO ExtI	MIRRHSCKGV MIRKHSLG MMNMRKIS *:. :.	GSSVAWSLLG FVASALA TISG : .	LAISAQSLAG LAVSAQAFAG VALGTALLAG :*:.: :**	TVTTDGADIV TVTTDGADIV TAFAGPRI *. :. :	IKTKGGLEVA IKTKGGLEVA TFGPEDQGAL	TTDKEFSFKL TTDKEFSFKL QIDYKGQFQM * : .*::	60 55 50			
PaeOprP PaeOprO ExtI	GGRLQADYGR GGRLQADYSR SIRDNGSGAN . * : 94*	FDGYYTNNGN FDGFYTKNGN GDDTTTNFN- *. *: .	TADAAYFRRA TADAAYFRRA FRRN ***	YLEFGGTAYR FIELGGTAYK RLAFMGKYGD : : *.	DWKYQINYDL DWKYQINFDL MLSLYVQTEF . :: :::	SRNVGNDSAG SHNTGSSDNG TEDPNVGTLG . : *	120 115 103			
PaeOprP PaeOprO ExtI	YFDEASVTYT YFDEASVTYT VGDNSADTEF *::: *	GFNPVNLKFG GFNPVNLKFG QLLDAVMRFK : . ::*	RFY-TDFGLE RFD-PDFGLE FHDGFRVNVG	KATSSKWVTA KATSSKWVTA KFKHNLTREN	LERNLTYDIA PERNAAYELA LEACEMPLTL *	DWVNDNVG-T DWINTHQDGM DRSLFIRAPY *	178 174 163			
PaeOprP PaeOprO ExtI	GIQASSVVGG GAQVNSTLAD VSTRDVGVAV . :.	MAFLSGSVFS MAYLSAGVSA WGNLFNDVFQ . * .*	ENNNDTDGDS KDADDSDGDS YRLDAMEGRK : :*.	VKRYNLRGVF VKQFNFRGVF AGDRDANGYS *	APLHEPGNVV APMHEAGNVL SPDSNFR :* *.	HLGLQYAYRD HVGVNYAYRD YTARAHVT : *:	238 234 218			
PaeOprP PaeOprO ExtI	LEDSAVDTRI LDDTAFDSRI LLDPEKDYGY * *. *	RPRMGMRGVS RPRLGMRGIA KGTYMGEKQV :	TNGGNDAGSN TSGGNDAGDN LTVGAGVQYE . * :	GNRGLFGGSS GNRATFGGVS PNVAYGNAAT * :	-AVEGLWKDD NSPAGSYKDD QSDSKDY **	SVWGLEGAWA SVWGLEGAWA TAWTVDGYFE	297 294 275			
PaeOprP PaeOprO ExtI	LGAFSAQAEY MGPFSAQAEY Y-PVEGFGTI	LRRTVKAER- LARKLKADDN TASAAYADFS *:	DREDLKASGY AYKDIKAKGY MDDAYTVSTN	YAQLAYTLTG YAQLAYTLTG VDSGAIGLNG . * *.*	EPRLYKLDGA ESRQYKLEGA EKNGWYAKAG * . :	KFDTIKPENK KFDSVKPENK YMLPNFP : . *	356 354 331			
PaeOprP PaeOprO ExtI	EIGAWELFYR EIGAWEVFYR LQIFGR ::* *	YDSIKVEDDN YDNIKVEDDN YERWSFASLN *:*	IVVDSATREV VVADTATREV NVVDQDINWY *.*	GDAKGKTHTL GDTKAKAHNL GVGAN *	GVNWYANEAV GVNWYVNDAV YYIWGQNLKL * * :	KVSANYVKAK KISAAYVKAK TAEISKTDFD	416 414 382			
PaeOprP PaeOprO ExtI	TDKISNANGD TDKITNNNGD KEGTYNGVKS .: * .	DSGDGLVMRL DDGDGFVTRL EDFTTFITQL :. : : :*	QYVF QYVF QLLF * :*				440 438 406			

32Supplementary Figure S4. Comparison of the amino acid sequence of ExtI with those of the phosphate-33selective porins. The multiple alignment of *G. sulfurreducens* ExtI, *P. aeruginosa* OprP, and *P. aeruginosa*34OprO was constructed using a ClustalW program. The first amino acid (G, 1*) of the mature proteins, which35are assumed to be formed after cleavage of the potential signal peptides, are highlighted in black, and the36conserved D (94*) and K (121*) are highlighted in green and blue, respectively. Amino acid sequences37were obtained from the KEGG databases (PaeOprP, PA3279; PaeOprO, PA3280).