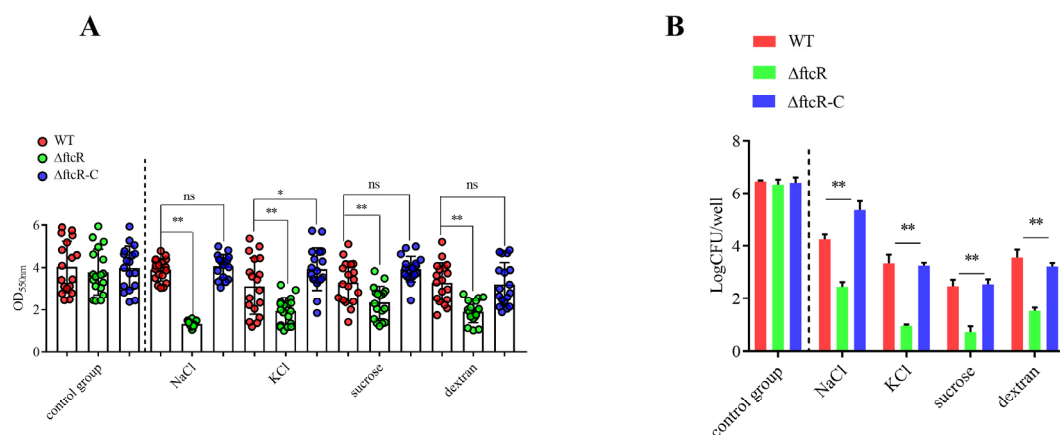
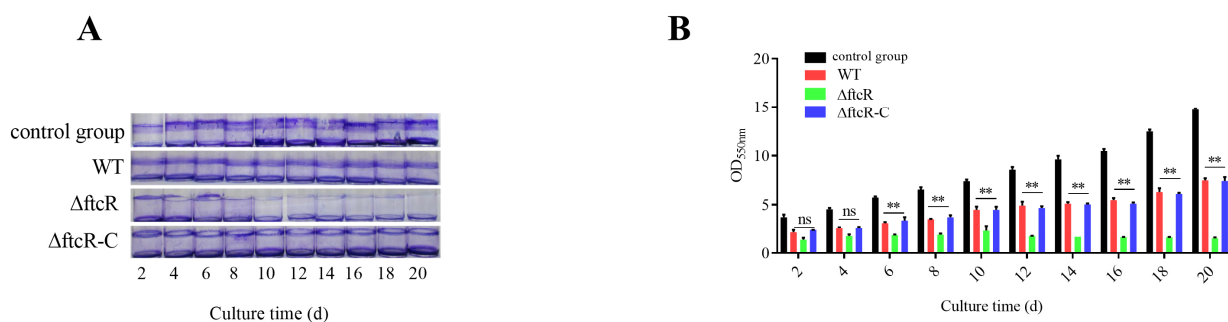


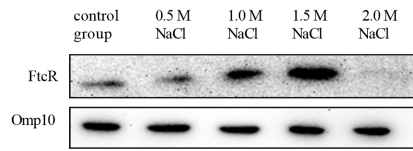
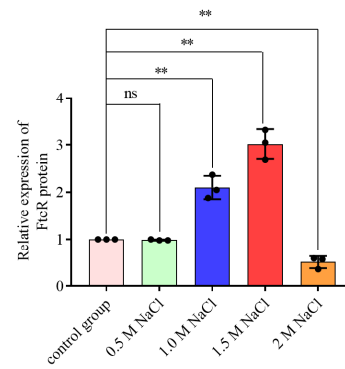
## Supplementary material



**Supplementary Figure S1.** Biofilm resistance of  $\Delta ftcR$  in response to different osmotic solutes. (A) The biofilms of WT,  $\Delta ftcR$ , and  $\Delta ftcR-C$  were treated with different osmotic solutes for 20 d and their biomass was quantified using a crystal violet (CV) assay. Each point represents an independent well. (B) Survival of WT and  $\Delta ftcR$  cells in biofilms grown under different osmotic solutes. Error bars represent standard error ( $n \geq 3$ ). \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , unpaired Student's  $t$ -test. ns, not significant.

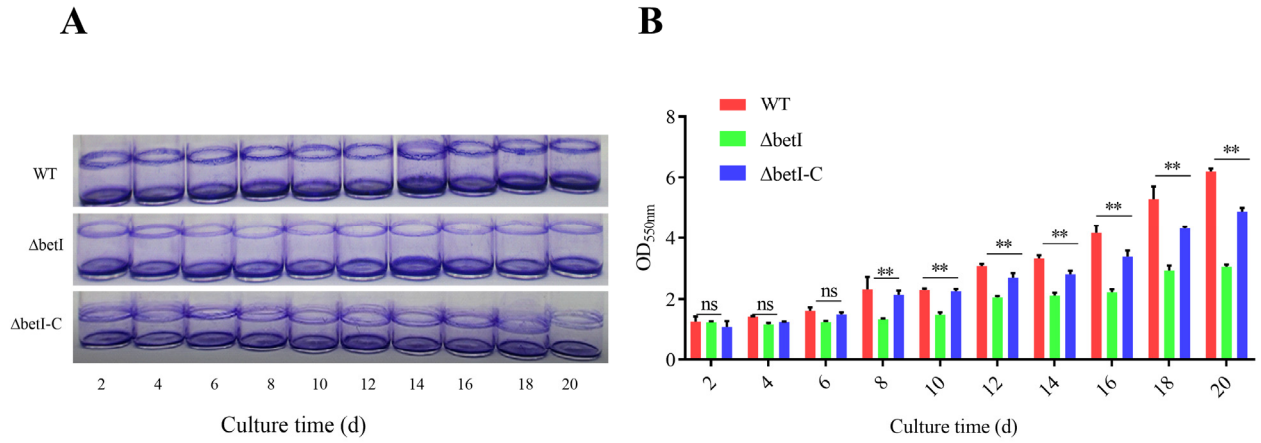


**Supplementary Figure S2.** Quantitative analysis of the biofilm using CV staining and bacterial plate count. (A) The biofilm of the WT,  $\Delta ftcR$ , and  $\Delta ftcR-C$  strains under hyperosmotic stress and quantified with 0.1% CV. (B) Survival of WT,  $\Delta ftcR$ , and  $\Delta ftcR-C$  strains in biofilms grown under hyperosmotic stress. Error bars represent standard error ( $n \geq 3$ ). \*\*  $p \leq 0.01$ , unpaired Student's  $t$ -test. ns, not significant.

**A****B**

**Supplementary Figure S3.** FtcR protein expression in biofilm under hyperosmotic stress conditions. **(A)** Expression of FtcR in biofilm cells after NaCl treatment was detected by western blotting. **(B)** Protein expression was evaluated by measuring the mean gray values of western blots using ImageJ. Error bars represent standard error ( $n \geq 3$ ).  $**p \leq 0.01$ , unpaired Student's *t*-test. ns, not significant.





**Figure S5.** Quantitative analysis of the biofilm using CV staining and bacterial plate count. **(A)** The biofilm of WT,  $\Delta betI$ , and  $\Delta betI-C$  strains under hyperosmotic stress and quantified with 0.1% CV. **(B)** Survival of WT,  $\Delta betI$ , and  $\Delta betI-C$  strains in biofilms grown under hyperosmotic stress. Error bars represent standard error ( $n \geq 3$ ).  $**p \leq 0.01$ , unpaired Student's  $t$ -test. ns, not significant.

**Table S1 FtcR binding sites identified by ChIP-Seq and RNA-Seq.**

Chromosom <sup>a</sup>	Peak start (bp)	Peak stop <sup>a</sup> (bp)	ChIP fold enrichment	Downstream gene <sup>b</sup>			Biofilm transcript log2
			(IP/Input)				fold Change ( $\Delta$ ftcR/WT)
				ID <sup>c</sup>	Name	Product annotation	
Transport and binding proteins							
NC_003317.1	159,616	162,822	20.41	BME_RS00790	amtB	ammonium transporter	-1.95
NC_003317.1	1,431,703	1,432,149	4.74	BME_RS06945	betI	transcriptional regulator BetI	-2.29
NC_003317.1	165,608	169,298	1.70	BME_RS00805	BME_RS00805	DMT family transporter	-1.3
NC_003317.1	1,462,803	1,463,632	1.48	BME_RS07095	BME_RS07095	ABC transporter permease	1.82
NC_003317.1	1,405,154	1,405,417	1.45	BME_RS06800	BME_RS06800	penicillin-binding protein 1A	-1.52
NC_003317.1	1,418,321	1,418,648	1.37	BME_RS06870	BME_RS06870	S9 family peptidase	1.91
NC_003317.1	1,989,144	1,989,754	1.40	BME_RS09570	BME_RS09570	extracellular solute-binding protein	1.19
NC_003317.1	466,058	466,440	1.32	BME_RS02235	BME_RS02235	Lrp/AsnC ligand binding domain-containing protein	1.41
NC_003318.1	950,346	950,574	1.30	BME_RS14635	BME_RS14635	amino acid permease	1.97
NC_003317.1	1,122,809	1,123,018	1.14	BME_RS05400	BME_RS05400	ATP-binding protein	1.26
NC_003318.1	563,019	563,278	1.27	BME_RS12810	BME_RS12810	ABC transporter ATP-binding protein	-1.15
NC_003317.1	1,598,343	1,598,611	1.25	BME_RS07740	sbmA	peptide antibiotic transporter SbmA	1.18
NC_003317.1	278,153	278,501	1.21	BME_RS01300	BME_RS01300	MFS transporter	1.11
Regulatory functions							
NC_003317.1	170,084	170,669	3.17	BME_RS10945	ftcR	response regulator transcription factor	-1.80
NC_003317.1	2,030,864	2,031,583	1.53	BME_RS09760	BME_RS09760	helix-turn-helix domain-containing protein	1.13
NC_003317.1	1,275,020	1,275,763	1.52	BME_RS06170	BME_RS06170	helix-turn-helix domain-containing protein	1.13
NC_003318.1	840,458	840,828	1.29	BME_RS14130	BME_RS14130	CerR family C-terminal domain-containing protein	-2.09
NC_003318.1	676,928	677,262	1.25	BME_RS13320	BME_RS13320	IclR family transcriptional regulator	-1.58
NC_003318.1	439,149	439,520	1.38	BME_RS12245	BME_RS12245	IMPACT family protein	-1.22
NC_003317.1	1,731,455	1,731,741	1.21	BME_RS08395	BME_RS08395	SANT/Myb-like DNA-binding domain-containing protein	-1.07
Cell envelope							

NC_003317.1	976,229	977,214	1.80	BME_RS04695	mscS	Small-conductance mechanosensitive channel	1.45
NC_003318.1	161,916	162,299	1.42	BME_RS10900	BME_RS10900	autotransporter outer membrane beta-barrel domain-containing protein	-1.32
NC_003317.1	609,170	609,467	1.30	BME_RS02920	bamD	outer membrane protein assembly factor	1.21
Translation							
NC_003317.1	1235	2280	1.63	BME_RS00020	rhO	transcription termination factor	1.38
NC_003317.1	796,452	796,668	1.25	BME_RS03875	rplO	50s ribosomal protein L15	1.61
Hypothetical proteins							
NC_003317.1	562,645	562,929	1.27	BME_RS02710	BME_RS02710	hypothetical protein	1.35
NC_003317.1	1,464,965	1,465,199	1.25	BME_RS07105	BME_RS07105	hypothetical protein	1.57
NC_003317.1	736,280	736,631	1.62	BME_RS03530	BME_RS03530	hypothetical protein	-1.39
NC_003317.1	1,014,836	1,015,571	1.29	BME_RS04850	BME_RS04850	DUF2059 domain-containing protein	1.40
NC_003317.1	1,263,340	1,263,620	1.50	BME_RS06115	BME_RS06115	SH3 domain-containing protein	-1.07
NC_003318.1	309,516	309,817	1.39	BME_RS11645	BME_RS11645	hypothetical protein	-1.23
NC_003317.1	540,668	540,910	1.36	BME_RS16045	BME_RS16045	hypothetical protein	2.08
NC_003317.1	1,359,006	1,359,221	1.23	BME_RS06590	BME_RS06590	DUF423 domain-containing protein	-2.08
NC_003318.1	782,398	782,682	1.31	BME_RS03750	BME_RS03750	hypothetical protein	1.36
Fatty acid and phospholipid metabolism							
NC_003317.1	929,833	930,378	1.56	BME_RS04465	BME_RS04465	acyl-CoA dehydrogenase	1.17
NC_003318.1	849,100	849,440	1.52	BME_RS14185	BME_RS14185	acyl-CoA synthetase	-1.26
NC_003317.1	826,002	826,710	1.29	BME_RS03995	BME_RS03995	acyl-CoA carboxylase subunit beta	1.02
NC_003318.1	126,309	126,561	1.24	BME_RS10785	BME_RS10785	amidohydrolase	-1.62
Cellular processes							
NC_003317.1	1,121,712	1,122,020	1.29	BME_RS05390	secDF	protein translocase subunit SecDF	1.36
NC_003318.1	363,874	364,095	1.26	BME_RS11895	BME_RS11895	D-aminopeptidase	-1.8
NC_003317.1	521,102	521,437	1.44	BME_RS02510	BME_RS02510	lytic transglycosylase domain-containing protein	-1.13
NC_003317.1	1,034,504	1,034,944	1.30	BME_RS04940	BME_RS04940	septal ring lytic transglycosylase RlpA family protein	1.15
NC_003317.1	1,093,915	1,094,349	1.32	BME_RS05270	prfB	peptide chain release factor 2	1.80
NC_003318.1	29,861	30,171	1.24	BME_RS10340	BME_RS10340	type IV secretion system protein	-2.51

NC_003317.1	2,033,625	2,033,923	1.23	BME_RS09770	BME_RS09770	hemolysin family protein	1.17
NC_003317.1	1,674,958	1,675,197	1.25	BME_RS08095	BME_RS08095	glycosyltransferase	-1.27
NC_003317.1	1,462,130	1,462,520	1.25	BME_RS07090	BME_RS07090	DegT/DnrJ/EryC1/StrS aminotransferase family protein	1.78
NC_003317.1	1,009,588	1,010,451	1.24	BME_RS04825	gltX	glutamate-tRNA ligase	1.06
NC_003317.1	1,027,230	1,027,614	1.23	BME_RS04910	BME_RS04910	MBL fold metallo-hydrolase	1.30
NC_003318.1	716,427	716,639	1.31	BME_RS13530	BME_RS13530	BA14K family protein	-1.09
NC_003317.1	555,294	557,788	1.82	BME_RS02665	BME_RS02665	YcgN family cysteine cluster protein	1.49
NC_003317.1	1,345,304	1,345,620	1.43	BME_RS06525	BME_RS06525	NYN domain-containing protein	1.28
Energy metabolism							
NC_003317.1	1,451,282	1,451,487	1.14	BME_RS07020	BME_RS07020	mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase	1.26
NC_003318.1	367,936	368,158	1.29	BME_RS11925	BME_RS11925	Gfo/Idh/MocA family oxidoreductase	-1.62

<sup>a</sup>ChIP-Seq peaks of FtcR distributed across the *Brucella melitensis* 16M genome in chromosomes I and II.

<sup>b</sup>Lists the gene located downstream from the target site and whether the gene is changed in the transcriptome.

<sup>c</sup>ID, identifier.

**Table S2. Primers of mutants and complementation used for this work.**

Primer	Sequence
ftcR-N-F	GCCGAGCCTGAATGTGGAC
ftcR-N-R	GACATTCATCCCAGGTGGCCGGCCCCGCCTTCC
ftcR-C-F	TCTGGGGTTCGAAATGACCGTATTGATGTTTTCCCGCAAGTC
ftcR-C-R	GGGCAGGACAAGCGAGG
kan-F	GCCACCTGGGATGAATGTC
kan-R	CGGTCATTTTGAACCCAGCA
fliF-N-F	CTGACACTGATGCGAAGA
fliF-N-R	GACATTCATCCCAGGTGGCCCTGTTGAACGCAAGCTA
fliF-C-F	TCTGGGGTTCGAAATGACCGGAATGAGCGCGCTTT
fliF-C-R	ATGGGCCTGCAATGC
betI-N-F	CTTTGGGCAAGCTTTACC
betI-N-R	GACATTCATCCCAGGTGGCGATATTCATCCGGTTGTCTG
betI-C-F	TCTGGGGTTCGAAATGACCGCATGGAAGCAGATTTTCG
betI-C-R	GGCATGGAACAGCGGATTG
ftcR-F-C	AAGCTTGGATGATTGTTGTCGT
ftcR-R-C	GAATTCGGTCACTCGATATTGA
betI-F-C	CGTCGACTGCCCAAGATCGGGA
betI-R-C	TGAATTCTCAATTCTCCGCCGAA
p2c2Xamont	ATATCTAGATTCAGCCGCGGCGGGCT
p2c2Baval	ATTGGATCCCCTTCGCGACCGAACCA
Biotin-ftcR-F	GACGGCAGCGGGCAAAAGCC
Biotin-ftcR-R	TTCTGCTGCCGCCTTATCAA
Mut-ftcR-F	GACGGCTGTCCTTGAAAGCC
Mut-ftcR-R	TTCTGCTGCCGCCTTATCAA
Biotin-betI-F	TGCCTGCCGCTTTGGGCAAG
Biotin-betI-R	TGTTTTTTCGCTGGGCGACATAGAA
Mut-betI-F	TGCCTGCCGCTGTCCTTGAG
Mut-betI-R	TGTTTTTTCGCTGGGCGACATAGAA



**Table S3. Primers of qRT-PCR used for this work.**

Primer	Sequence
16S rRNA-F	ACTAAGGGCGAGGGTTGC
16S rRNA-R	CACTGGACCATTACTGACGC
amtB-F	AGCCTGCCCCGACTTT
amtB-R	TCTTGCGCACCATGCC
betI-F	TGCCATCCGCACCATC
betI-R	GTTTGATCGCCGCGTT
BME_RS00805-F	GGGTGCTGATCTTTTCCGGG
BME_RS00805-R	CGACAACCAGTGAAACAGCA
BME_RS07095-F	TGGAAGGTACGCCACTTC
BME_RS07095-R	ATATAGGGGGCATATGCA
BME_RS06800-F	GGCAGAAAAAGATCGCGG
BME_RS06800-R	GCCGGGAGAAAATCGTGA
BME_RS06870-F	ACCATGGCCGACCTTGAA
BME_RS06870-R	CCCACACCGAGGAAAAAG
BME_RS09570-F	GGTGGCCATGCTTGCAAG
BME_RS09570-R	TTGAGGTGTGCGAAAGTGCC
BME_RS02235-F	TCGCAGACGGCCACC
BME_RS02235-R	GGCGTCGTGCGGTCA
BME_RS14635-F	ATGACAACACCAACCGCA
BME_RS14635-R	GAAAGAAAACGGCTGCAA
BME_RS05400-F	TGGACCGTTTGATAGCCG
BME_RS05400-R	CCAAATCTACGCCACGGA
BME_RS12810-F	CCTGCATGATGTCTCGCT
BME_RS12810-R	GCATTGCCCAGAAGCACT
sbmA-F	CCCCGTCCGAAACTG
sbmA-R	GAACACATGCGCGCC
BME_RS01300-F	GATATGGCGCCTGCG
BME_RS01300-R	AGCGAACCGAGACCG