

**Supplement Table S1. Bacterial strains and plasmids used in this study**

Strains	Relevant characteristics	References
ST1120	Wild-type <i>S. Typhimurium</i> isolated in Korea	[1]
ST2165	$\Delta fruR$	[2]
ST2166	$\Delta ssrAB$	[2]
ST2167	$\Delta hfq$	[2]
ST14028	ATCC14028	ATCC
ST2173	ST14028 with plasmid pBBR1-MCS4 (AM <sup>R</sup> )	[3]
Plasmids	Characteristics	
pCP20	FLP recombinase, temperature-sensitive replication (AM <sup>R</sup> )	[4]
pBBR1-MCS4	Broad-host-range cloning vector (AM <sup>R</sup> )	[5]

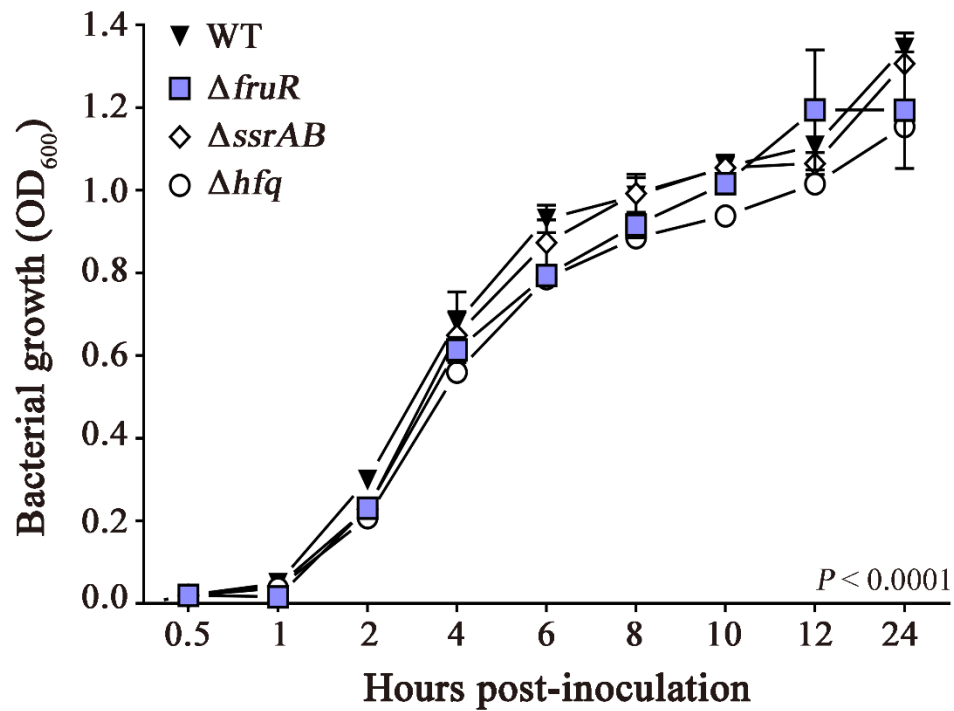
**Supplement Table S2. Primers used in this study**

<b>Primer</b>	<b>Sequence (5' to 3')</b>
<i>fruR</i> _DF	CATCGGTGGGCTTTTTTTTG
<i>fruR</i> _DR	TGTCAAGCAAGTCACAACG
<i>ssrAB</i> -DF	TTGAAGCATCGTCTCCTG
<i>ssrAB</i> _DR	GGCGATTCTATCATTCGG
<i>hfq</i> _DF	CAGGTTTTGAAGCAGAAGTC
<i>hfq</i> _DR	TTCAACTGCCTTACCTTCAC

**Supplement Table S3. Analysis of the biochemical phenotypes of the *S. Typhimurium*  $\Delta fruR$** **mutant strain**

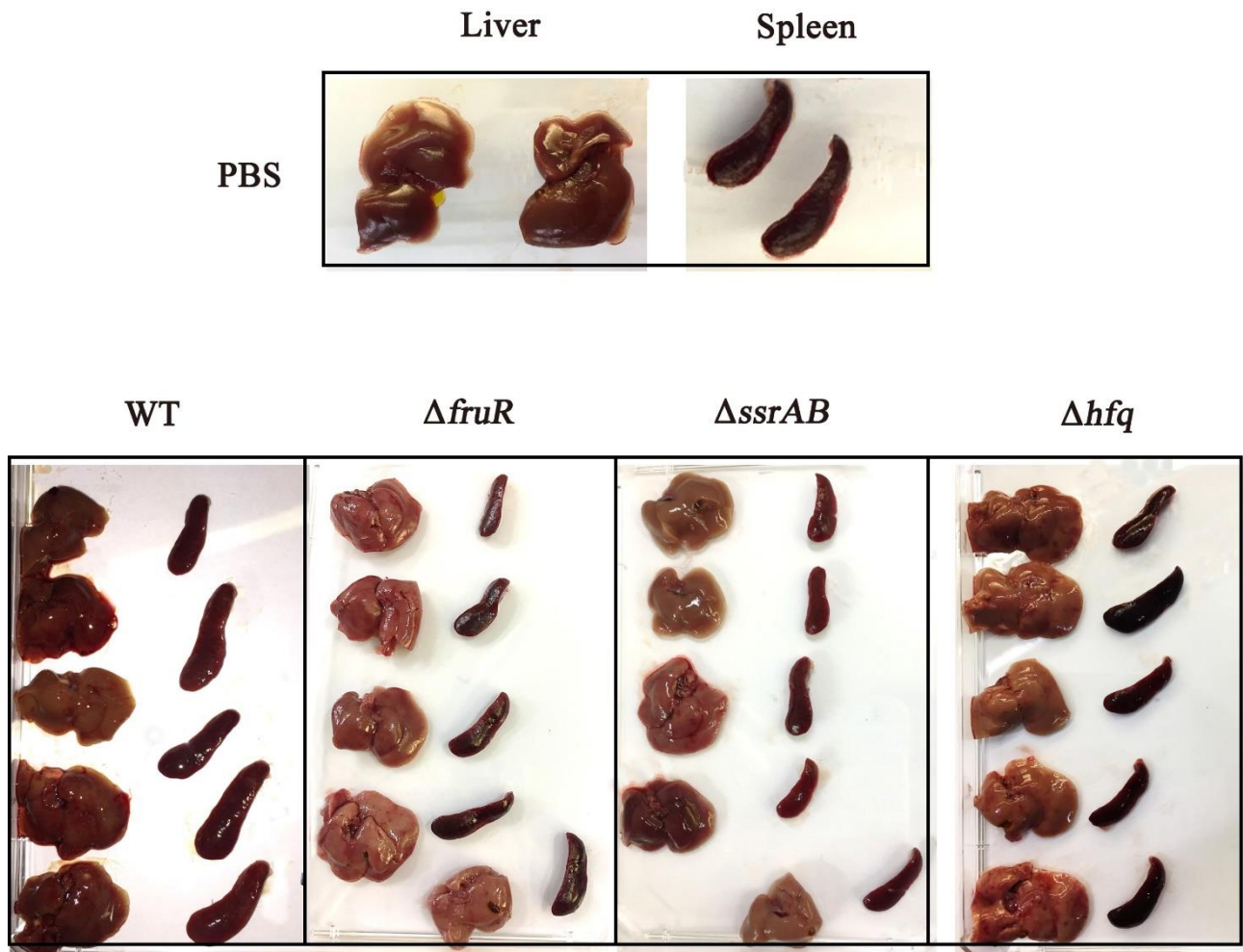
<b>Biochemical phenotypes</b>	<b>Strain characteristics</b>			
	<b>Wild-type</b>	<b><math>\Delta fruR</math></b>	<b><math>\Delta ssrAB</math></b>	<b><math>\Delta hfq</math></b>
Acetoin production	–	–	–	–
<u>Amygdalin fermentation</u>	–	+	+	+
Arabinose fermentation	+	+	+	+
<u>Arginine dihydrolase</u>	+	–	–	–
<u>Citrate utilization</u>	+	–	–	–
$\beta$ -Galactosidase	–	–	–	–
Gelatinase	–	–	–	–
Glucose fermentation	+	+	+	+
H <sub>2</sub> S production	+	+	+	+
Indole production	–	–	–	–
Inositol fermentation	+	+	+	+
<u>Lysine decarboxylase</u>	+	–	–	–
Mannitol fermentation	+	+	+	+
Melibiose fermentation	+	+	+	+
Ornithine decarboxylase	+	+	+	+
Rhamnose fermentation	+	+	+	+
Sorbitol fermentation	+	+	+	+
<u>Sucrose fermentation</u>	–	+	+	+
Tryptophan deaminase	–	–	–	–
Urease	–	–	–	–

Supplement Figure S1.



**Supplementary Figure S1.** Measurement of bacterial growth in the  $\Delta fruR$  mutant in LB broth. Growth curve of ST mutant strains ( $\Delta fruR$ ,  $\Delta ssrAB$ , and  $\Delta hfq$ ) and WT strains that were cultured in LB broth. The OD<sub>600</sub> values indicated at each time point represent the average of three replicates.

**Supplement Figure S2.**



**Supplementary Figure S2.** The enlarged livers and spleens of mice immunized with each strain after challenge. The mice that were immunized with ST mutant strains ( $\Delta fruR$ ,  $\Delta ssrAB$ , or  $\Delta hfq$ ) or WT strains were sacrificed and their organs (liver and spleen) were collected and observed. The PBS negative (N) control was not challenged.

## References

1. Kim, S.; Kim, E.; Park, S.; Hahn, T. W.; Yoon, H. Genomic approaches for understanding the characteristics of *Salmonella enterica* subsp. *enterica* serovar Typhimurium ST1120, isolated from swine feces in Korea. *J. Microbiol. Biotechnol.* **2017**, *27*, 1983–1993.
2. Yoon, H.; McDermott, J. E.; Porwollik, S.; McClelland, M.; Heffron, F. Coordinated regulation of virulence during systemic infection of *Salmonella enterica* serovar Typhimurium. *PLoS Pathog.* **2009**, *5*, e1000306.
3. Park, S.; Jung, B.; Kim, E.; Hong, S. T.; Yoon, H.; Hahn, T. W. *Salmonella* Typhimurium lacking YjeK as a candidate live attenuated vaccine against invasive *Salmonella* infection. *Front Immunol.* **2020**, *11*, 1277.
4. Datsenko, K.A.; Wanner, B.L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U S A.* **2000**, *97*, 6640–6645.
5. Truong, Q.L.; Cho, Y.; Park, S.; Park, B. K.; Hahn, T. W. *Brucella abortus* mutants lacking ATP-binding cassette transporter proteins are highly attenuated in virulence and confer protective immunity against virulent *B. abortus* challenge in BALB/c mice. *Microb. Pathog.* **2016**, *95*, 175–185.