

Nitrate Utilization Promotes Systemic Infection of *Salmonella* Typhimurium in Mice

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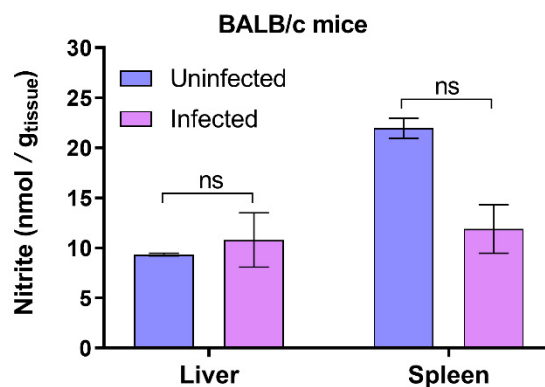


Figure S1. Nitrite levels are not significantly altered during *S. Typhimurium* systemic infection. Nitrite levels were tested in the livers and spleens of BALB/c mice that were infected or mock-infected with *S. Typhimurium* WT strain after 5 days post-infection. Data were generated from three independent experiments and are presented as the mean \pm SD. *p*-values were determined using unpaired Student's *t*-test (ns, not significant).

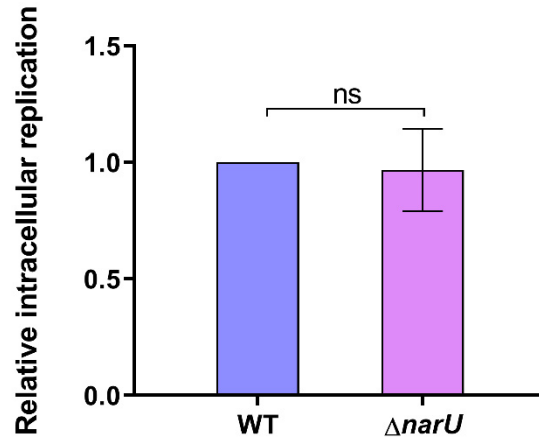


Figure S2. The mutation of *narU* did not significantly influence the replication ability of *S. Typhimurium* in macrophages. Replication of *S. Typhimurium* WT and $\Delta narU$ in RAW264.7 cells. The bacterial replication ability was determined according to the ratio of the number of intracellular bacteria at 20 h post-infection to the number of bacteria at 2 h post-infection. Data were generated from three independent experiments and are presented as mean \pm SD. *p*-value was determined using unpaired Student's *t*-test (ns, not significant).

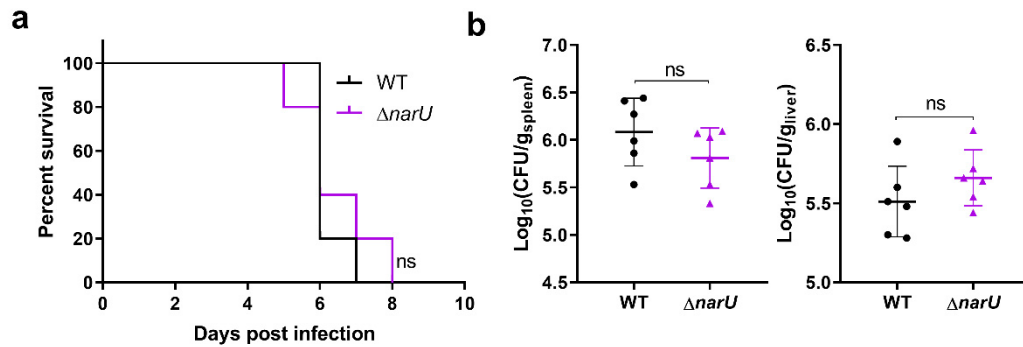


Figure S3. The mutation of *narU* did not significantly influence the systemic virulence of *S. Typhimurium*. (a) Survival curves for mice infected i.p. with the *S. Typhimurium* WT or $\Delta narU$, *n* = 5 mice/group. (b) Liver and spleen bacterial burdens in mice infected with the *S. Typhimurium* WT or $\Delta narU$ at day 5 post-infection. *n* = 6 mice/group. (a and b) Data were combined from two independent experiments and are presented as mean \pm SD. *p* values were determined using log-rank Mantel–Cox test (a) or Mann–Whitney U test (b) (ns, not significant).

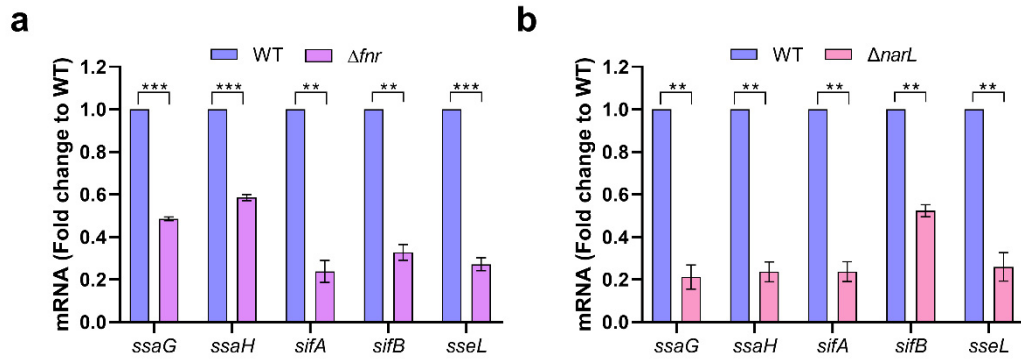


Figure S4. Mutation of *fnr* or *narL* decreased the transcription of T3SS2-related genes. (a and b) qRT-qPCR analysis of the mRNA levels of 5 T3SS2-related genes in *S. Typhimurium* WT, Δfnr mutant (a), or $\Delta narL$ mutant (b). Bacteria were grown in N-minimal medium for 6 h prior to collection. Data were generated from three independent experiments and are presented as mean \pm SD. *p*-values were determined using unpaired Student's *t*-test (***p* < 0.01; ****p* < 0.001).

Table S1. Primers used in this study

Targets	Primer sequences (5'-3')
Primers for qRT-PCR analysis	
16S rRNA	ACTGGCAGGCTTGAGTCTTGTAGA
<i>sifA</i>	GGCACAACCTCCAAGTAGACATCG AGGACATTTAGATGGGTGGAAAGCG TATGTGGGTATGCGGTGGTGGTAT
<i>sifB</i>	GTTGCTTGTTCCCTGAGCGGTTA GTCAATAGCTGTTACACCTGCCTGG
<i>ssaG</i>	ATATGCTCTCCACATGGCGCACCA GCGCTTTAATCATCGATTCTGGGTTGAGCA
<i>ssaH</i>	CGGGCGTTAACCATAGCCTGATTT TGGTGCAGGAAATAACAGACGCAG
<i>sseL</i>	ACAGGAGATCACTGGCTTCTCTGTT CCACAGCCGTTGGGTACATTGTT
<i>narK</i>	ATCCTCATCGTGCCCTGCGTAT TATTGCCAGACCACCGTTCAGA
<i>narG</i>	CGTTCTAACCTGCTTGGGTCTTCC CATTGTCACGCCACTCCACTTCTT
<i>napF</i>	CATCGTTGCCAGGACAGTTGTGA GCATGGTGGTTCTCCGCTTTGAT
Primers for the establishment of mutant strains	
$\Delta narK$	ATATCAACTTACCTTCGGCAGTAAACCCTAATGTGGCAGACATCAAATC AAGAATCAGAGGTGTCTGTGTAGGCTGGAGCTGCTTC

	GTATGTTGTAATAAATACAGATAAAAAAAGCGCGGTCTAACGCCGC
	GCAAAGGATAATCAAAAGCATATGAATATCCTCCTTAG
<i>ΔhmpA</i>	TGCAAGGGTATTTTTATAAGATGCATTTGATATACATCATTAGATTTTCA
	CATAAAGGAAGCACGTGTGTAGGCTGGAGCTGCTTC
	GAACGGGGAGGAAAACGGGCGTTCGCCTTAACGATAACGCCCGTTTTT
	TCAGAGGATTTGTTGCAACATATGAATATCCTCCTTAG
<i>ΔnarU</i>	GAGCGCGTGACGCTCTGTCTCTTTTTTGTGTTTTTCTTCAAATGATATGCG
	CATGTGAGGGGTAAAGTGTAGGCTGGAGCTGCTTC
	TTCCGCCATCCTGTGCGATTTGGCGTTAAACCATTACCGGATGGCCGTCCT
	ACAGGTGCGTATGTTGCATATGAATATCCTCCTTAG
<i>Δfnr</i>	AGACTTACGCGCTACCAAAAAGATGTTAAAATTGACAAATATCAATT
	ACGGCTTGAGCAGACCTGTGTAGGCTGGAGCTGCTTC
	CCAGATCAATAAATGAGAAAAATTTAACGATATGGCAGAAGATAACAT
	CAATGGTTTAGCTGACGCATATGAATATCCTCCTTAG
<i>ΔnarX</i>	GCCGATTGACGCCCTCTTTTTTGCTACGTTTTTTCGGCGACATTACCCC
	GAAGAAAGAAGGTAACGTGTAGGCTGGAGCTGCTTC
	GCTGCTTTACACCCGTGCGTAGCATCGGATGGTCATCGATTAACAGGAT
	GGTTGCCGGTTCCTGACATATGAATATCCTCCTTAG
<i>ΔnarL</i>	CGGAACAGAGGTCACTGTTACTTTTATTCCAGAAACAACTTCACAGA
	AACCCAGGGAGATACCCGTGTAGGCTGGAGCTGCTTC
	TGACTGAACCGTTTATCACGATGCCGGACGATAATCCGCATTGGCAACC
	GTTCCAGGAGCAATAACATATGAATATCCTCCTTAG
Primers for the pET-28a- <i>narL</i> plasmid construction	
	CCATGGGCATGTTTAAACGGTGTTTCTCTCCTCTTACG
	CTCGAGAAAGATGCGTTCCTGATGTACCCAG
Primers for the amplification of DNA sequences used in EMSA experiments	
<i>narK</i>	AGCAAAAAGAGGGGCGTCA
<i>promoter</i>	CGACACGATGGCCGCG
<i>narG</i>	CGGCAGGGCTAACCACG
<i>promoter</i>	CCTCCCAGTCCCGGTTTGT
16S	AAATTGAAGAGTTTGATCATGGCTC
rDNA	GCATGGCTGCATCAGGCTT
