

## **Molybdenum-independent [4Fe-4S]-Catalyzed Sulfate Assimilation Sustains *Salmonella* Virulence**

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## Supplementary Tables

**Table S1. Identity and similarity of DmsA (STM14\_1089) paralogs.**

	Identity	Similarity
STM14_1809	66%	80%
STM14_1811	68%	80%
STM14_3103	40%	58%
STM14_5178	49%	64%

	20	40	60	80	100
DmsA	-----MKTKPDAVLAAGVSRGLVKTATIGLAMASSAATILFFTR-IAAAEATSPAKTGEKVVSACTVNCGRSRLRMHVVDGEEKVETDNTGND				: 94
1809	-----MKITNPEALMAASLSRSILVKTSATIGSLALASSAATILFFSR-IAHAAADLASGNVAERAVWSCTVNCGRSRLRLHVKDDTVYVWESDTTCNDE				: 94
1811	-----VPEGEKQGTGVSRRTLVKSAALGSLALAAGGVSLFEGMRTAAAVQQAMNNEEDKIVWGACSVNCGSRCLRLHVKNRNVWVVEDNTDTCV				: 91
3103	MGFYLTSTRINKNIKSGNGEQPATSRRHFCASSALIALPFVSSPATAQAARVTATENRPAEKVVQCCSFDCGCKCDIRAHVSGIVTRHSR-PINAL				: 99
5178	-----MEKKWLNITTITRDAIAAAKVAAVTLSCAIIILFFATTAQAQDTPIPKDANGCTVRHSAATLNCGRSRLPLKIVVNRDRVVRPEPADKDA				: 93
	r 6v 6g 6pf a ek v c vICGS4C 64 hv 1 6 6e 1d				

	120	140	160	180	200
DmsA	YDGLHGVRAACLRGRSRRRVVNPDRILYPMKRVGARGEGKFERISWDEAYDIIATNMQRILKPYGNESVIYNGTITLGGTITRSWPPGKTLVARLMNCC				: 194
1809	GGVLSRSGSSTACTSAAMSVMFC-GNIGNSPDLIAATKLIVVMFGNNEATRMSGGGVYIYVEQARERSNARNIVIDPRYNDTAAGREDWLPTRPCTDG				: 188
1811	YG-NHGVRAACLRGRSRRRINHPDRILYPMKRVGARGEGKFERISWCEALDTISASLAKTVETVYGNBAVYIHSSIVSGNIRSSPAA-SPVKRLMNCY				: 189
3103	DAQMPVMRAACVRGAYRRHVYEPDRILYPMKRVGARGEGKFERISWDEATTLIDNLSSTERYGPASRVHVGTAVSGTFSGDKMAR-----LLNLT				: 194
5178	VFGHGIIRPCLGRSSRRVVSFDRILYPMKRVGARGEGKFERISWDEATAHIAETTRVSERYGREATIYNIQSAYYHTQGRPAWIR-----LLNLT				: 187
	hq6kAc6GRs R r6 PDR6kYPMKRVG RGEgKFeRISWdEA I 6 YG e y y 3g gg rL6N				

	220	240	260	280	300
DmsA	GGYLNHVGDISSAQIAAGLNYTYGWAIDGNSPSLIENSKLVVLFQNNGETRMSGGGVYIYVEQARCKSNARNIIIDPRYIDTGAGREDWIPTRPCTDA				: 294
1809	GGVLSRSGSSTACTSAAMSVMFC-GNIGNSPDLIAATKLIVVMFGNNEATRMSGGGVYIYVEQARERSNARNIVIDPRYNDTAAGREDWLPTRPCTDG				: 287
1811	GGSTINQSGSSTACTSCAMMYTYG-SNIGNSTSLIENSKLVVLMFGNNEATRMSGGGVYIYVEQARCKSNARNIVIDPRYIDTAAGREDWIPTRPCTDA				: 288
3103	ALNDAMMYVLIISNTIDRAETARYAIGFDEDSMEGVEGANESLVAYLIG-ARDGVKSPWEAKITHEVPAQTIRCLARDYANTKPAITICQGWGPQRNCG				: 291
5178	GGYLNHNTYSTAQIATTEYVHC-DYVGSHTTCLARSLVVLFGNLSLETMRMSGGGVVEELRRRLSTSKARNIIIDPRYIDSVITEHAEWLPTRPCTDA				: 286
	GGyL y yS aqi a Yt G G s 6 3kLV665G Np ETrmsggg t qa s aR I66DPRY D3 d2W6P6rP TD				

	320	340	360	380	400
DmsA	ALVNGLIAYVLITENMVDQPLDKYVGVYDEHTLEASAPRNCHYKAYILICQEDGIAKTPPEWAACITCIPADRIIRKLAREIGSAKPAYICQGWGPQRHNG				: 394
1809	ALAAAIWVLLITELIDKPELDKYIGYDEHTLEASAPRNCHYKAYILICQEDGIAKTPPEWAACITCIPADRIIRKLAREIGSAKPAYICQGWGPQRHNG				: 387
1811	ALVAGIAWVLLITENMVDQPLDKYIGYDEHTLEADAPPNGHAKAYILICQEDGIAKTPPEWAACITCIPADRIIRKLAREIGSAKPAYICQGWGPQRHNG				: 388
3103	ALNDAMMYVLIISNTIDRAETARYAIGFDEDSMEGVEGANESLVAYLIG-ARDGVKSPWEAKITHEVPAQTIRCLARDYANTKPAITICQGWGPQRNCG				: 390
5178	ALVAGIHTHTLITELIDALVNRVYVGVYRSTLEDDTAPNASTKYVYVGTEDGIAKTPPEWAACITCIPADRIIRKLAREIGSAKPAYICQGWGPQRHNG				: 386
	AL 6a v6I E 6 D f6 Yc6G5De 3EP ap N ykaY61G g DG6aK3P2WA IT 6PA I LARei s 4pay6cQGWGPQRh ng				

	420	440	460	480	500
DmsA	BIAIRAISMTAHLTGNGVNGNSCAREGSYALPFFERMFL-BNPVETSSISEMWTDAITERGPBMTLRDGVRCRDKLVEIKMWNVAGNCLINCHSET				: 493
1809	BQIARAIAISVLTGNGVNGNSCVREGTWDLGVWFSL-BNPVKICISVETWTDAIDHGAEMTTRDGVRCRDKLVEIKFLWCYASNTLINCHGDI				: 486
1811	BQIARAIAIPHTGNGVNGNSCARESTYTITIERLFL-BNPVKIATSCSWTIALARGPBMTLRDGVRCRDKLVEIKFLWNNVAGNCLINCHSDI				: 487
3103	BRTARGSTLIATITGANGVKGCSWAAGYGGCANRKAAG-BPNDNPVKAKISVMNVQADDAASKVTPDMG-LKDADKLSNRITLFSIAGNYLQNCNPLI				: 489
5178	BQIVRAIQTLPATITCFHFCFNNWNPYGTYPVPLLVG-RNPITISIPCLTWTATQHPEMVTITTMVEKADRIKTGIRKLRFNCAGNTLINCHGET				: 485
	E t Rai L 6TGrvG1 Ggn g p NP6 t Is Wtdai 6Ta g64g D4Ld I4 5 AgN L Nqh				

	520	540	560	580	600
DmsA	NRTHEILCDKKCEMVVVDCHMTSSAKYADILLFDCTASEQMFALDASCG-NMSYVIFTDQAIKFEFEKFTIYEMTSELAKRLG--VEGCFTEGRICE				: 590
1809	AHTEVLCDDSKCEMVVGEHEMTSSAKYCDILLFDLMTPEQDDIISHESAG-NMGYVILGCPATSEKFERKPIWWTLESAKRLGPDVYCTFTEGRICH				: 585
1811	NKTEHEILCDKCEMVVVDNEMTSSAKYADILLFDLMTVEQDDIIPNDYAG-NMGYLIFICPATTEKFERKPIWVWVLEIARRLGDDVYCTFTEGRICA				: 586
3103	HQAVRVLEDSLCICFVVASLDLMTSSAKYADLLLPETSFNERNWGETWGTA---SYLLSEKLIBEFERRSDMDLREVAALGL--IENEFSQGRDEK				: 584
5178	NRTHEILADESLTETTVVLENMTSSAKYADLLLPETSYLEADIVDSSYAGSHNYMTAICKTVREEMWVRSYVDICALLAGHAG--LRQCYTEGRTCA				: 583
	t 6L D kc2 I6 i MT SAKYad6LLP E 1 Y6I P 5E 4 Y e6A LG 6 532GrT2				

	620	640	660	680	700
DmsA	BWRHLYEQSRKSIENLTSEEFKQGIKORDPEG-HVYAKDFREDPOANPITPSGKIEIYSQALAHITATWELPEGDVIDELIYHFGFENYN-EP				: 688
1809	IRORYPLQLGFFYKARTSSSGNIDIIQQCCPQCIWINPIDACARGICQGITVRVENQNGEMLIIPAKVTIRILPGVTAICGGAWLNADMFGDKVVRGSS				: 683
1811	KRSTFPLQLGFFYKRSSTSSSGNIDIVQACRCQEVWINPIDACARGANGDKVVENDRGELPAKVTRIRILPGVSAMCGGAWHNADMAGDRIHGAC				: 684
3103	AWIETHIEQIRLAMPDE-NLPDFATLQKTRCHLFKSAPRIAREDNIRDPENHFFITPSGKIEIYFSKRIYIMQH-----PPIPALSHYVBAHGGPE-IA				: 675
5178	QWAEHMQQIREKRYLEBWSVAKEMVIDORATEQQSLABADFRADAEANPITPSGKIEIYSQALAHITATWELPEGDVIDELIYHFGFENYN-EP				: 683
	w P p g 6A5 fr Dp anPl TPSGKIEISS La 6a tw l I Lp 5 p e d				

	720	740	760	780	800
DmsA	ITDRFPLQLGFFYKARVSTSGNVDIVKACRCQEMWINPMDACRGICNNGDKVRIENDRGEMHIEAKVTIRMMMPGVVALGEGAWYDEIAK--RVGCGG				: 786
1809	IRORYPLQLGFFYKARTSSSGNIDIIQQCCPQCIWINPIDACARGICQGITVRVENQNGEMLIIPAKVTIRILPGVTAICGGAWLNADMFGDKVVRGSS				: 783
1811	KRSTFPLQLGFFYKRSSTSSSGNIDIVQACRCQEVWINPIDACARGANGDKVVENDRGELPAKVTRIRILPGVSAMCGGAWHNADMAGDRIHGAC				: 784
3103	IAKFFPLQLITWKGKFNASTGYANPWTIEVQQCTLWINQCDACRGRTDHGDMVVENSRGICETPEVETRIIPGVVAMQAGQWQDENG--VIRGGC				: 773
5178	ITARYPLQLGFFYKHTHTSTSNVLMHEVPDEVWINEIDASARCLKSGERFVVENDRGVNPLCKVTCRILPGVAMPAGAWTRILGNS--VIVGCG				: 781
	l 5PLQL g5h K r hS3y n L a qe6WINP DAq Rg6 GD Vr6fN rG 6pakVTpR66PGV A6 GAW D g 6D Ggc				

	820
DmsA	INVLTTORPSFLAKGNPSTNLVQVEKA : 814
1809	INVLTSRRPSFLAKGNPSSNLVQVEKA : 811
1811	INVLTTREPSFLAKGNPSTNLVLEIKI : 812
3103	ANVLSSARITLAKGNPSTNLVLEIAKA : 801
5178	INVLTSRRPSFLAKGNPSTNLVLEIKI : 809
	iN L33 rp3pLAKGNp h3nLV26 4a

**Table S2. Bacterial strains used in this study.**

Strain	Relevant characteristics	Reference
<b><i>Salmonella</i></b>		
14028s	wild-type of <i>S. Typhimurium</i>	ATCC
AV20164	WT, pHluorin	<sup>1</sup>
AV24157	WT, pWSK29::STM_1811-1807 [WT pPSR3]	This study
AV24051	WT, pWSK29::STM_3103-3101 [WT pPSR1]	This study
AV24041	WT, pWSK29::STM_5178-5180 [WT pPSR2]	This study
AV23012	WT, pWSK29::iscS [WT pISCS]	<sup>1</sup>
AV13165	$\Delta$ cysJ::Cm [ $\Delta$ cysJ]	lab stock
AV22025	$\Delta$ dmsABC::Km [ $\Delta$ dmsABC]	<sup>1</sup>
AV0785	$\Delta$ iscS::Km [ $\Delta$ iscS]	<sup>1</sup>
AV08158	$\Delta$ menA::FRT [ $\Delta$ menA]	lab stock
AV21016	$\Delta$ moaABCDE::Km [ $\Delta$ moa]	<sup>1</sup>
AV22175	$\Delta$ mstA::Km [ $\Delta$ mstA]	This study
AV22195	$\Delta$ phsABC::Cm [ $\Delta$ phsABC]	lab stock
AV24143	$\Delta$ pspE::Km [ $\Delta$ pspE]	This study
AV10074	$\Delta$ ubiC::Cm [ $\Delta$ ubiC]	lab stock
AV24048	$\Delta$ STM_1811::Cm [ $\Delta$ 1811]	This study
AV24001	$\Delta$ STM_1811-1807::Cm [ $\Delta$ 1809 or $\Delta$ psr3]	This study
AV24055	$\Delta$ STM_1811-1807::Cm, pWSK29::iscS [ $\Delta$ psr3 pISCS]	This study
AV24035	$\Delta$ STM_1811-1807::Cm, pWSK29::STM_1811-1807 [ $\Delta$ 1809 p1809]	This study
AV24158	$\Delta$ STM_1811-1807::Cm, pWSK29::STM_3103-3101 [ $\Delta$ 1809 p3103]	This study
AV24159	$\Delta$ STM_1811-1807::Cm, pWSK29::STM_5178-5180 [ $\Delta$ 1809 p5178]	This study
AV24002	$\Delta$ STM_3103-3101::Cm [ $\Delta$ 3103 or $\Delta$ psr1]	This study
AV24053	$\Delta$ STM_3103-3101::Cm, pWSK29::iscS [ $\Delta$ psr1 pISCS]	This study
AV24160	$\Delta$ STM_3103-3101::Cm, pWSK29::STM_1811-1807 [ $\Delta$ 3103 p1809]	This study
AV24052	$\Delta$ STM_3103-3101::Cm, pWSK29::STM_3103-3101 [ $\Delta$ 3103 p3103]	This study
AV24161	$\Delta$ STM_3103-3101::Cm, pWSK29::STM_5178-5180 [ $\Delta$ 3103 p5178]	This study
AV24003	$\Delta$ STM_5178-5180::Cm [ $\Delta$ 5178 or $\Delta$ psr2]	This study
AV24042	$\Delta$ STM_5178-5180::Cm, pWSK29::iscS [ $\Delta$ psr2 pISCS]	This study
AV24162	$\Delta$ STM_5178-5180::Cm, pWSK29::STM_1811-1807 [ $\Delta$ 5178 p1809]	This study
AV24163	$\Delta$ STM_5178-5180::Cm, pWSK29::STM_3103-3101 [ $\Delta$ 5178 p3103]	This study
AV24003	$\Delta$ STM_5178-5180::Cm, pWSK29::STM_5178-5180 [ $\Delta$ 5178 p5178]	This study
AV24069	$\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT [1809+]	This study
AV24059	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_5178-5180::Cm [3103+]	This study
AV24068	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm [5178+ or $\Delta$ psr2+]	This study
AV24070	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT [4 $\Delta$ ]	This study
AV24144	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, pHluorin	This study
	$\Delta$ STM_5178-5180::FRT, pWSK29::iscS [4 $\Delta$ pSCIS]	This study
AV24152	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, $\Delta$ dmsABC::Km [5 $\Delta$ ]	This study
AV24087	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, $\Delta$ moaABCDE::Km [4 $\Delta$ $\Delta$ moa]	This study
AV24200	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, $\Delta$ dmsABC::Km, pWSK29::STM_1809-1807 [psr3+ or $\Delta$ psr3ABC+]	This study
AV25112	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, $\Delta$ dmsABC::Km, pWSK29::STM_1809-1807 $\Delta$ N66 [psr3 $\Delta$ N66+]	This study
AV25113	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, $\Delta$ dmsABC::Km, pWSK29::STM_1809-1807 R105A [psr3 R105A+]	This study
AV24201	$\Delta$ STM_1811-1807::FRT, $\Delta$ STM_3103-3101::Cm, $\Delta$ STM_5178-5180::FRT, $\Delta$ dmsABC::Km,	

AV25021	pWSK29::STM_1809-1807 S199G [psr3 S199G+] ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔmoaABCDE::Km	This study
AV24196	pWSK29:: pWSK29::STM_1809-1807 [psr3+ Δmoa] ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::dmsABC [dmsA+]	This study
AV25117	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::dmsABC ΔN66 [dmsA ΔN66+]	This study
AV25118	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::dmsABC R106A [dmsA R106A+]	This study
AV24197	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::dmsABC S205G [dmsA S205G+]	This study
AV25020	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔmoaABCDE::Km pWSK29::dmsABC [dmsA+ Δmoa]	This study
AV25046	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::STM_3103~3101 [psr1+ ]	This study
AV25047	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::STM_3103~3101 S205G [psr1 S205G+]	This study
AV25048	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::STM_5178~5180 [psr2+]	This study
AV25049	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::STM_5178~5180 S198G [psr2 S198G+]	This study
AV25065	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::STM_1809 & 1808 [psr3AB+]	This study
AV25091	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::STM_1809 [psr3A+]	This study
AV25073	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::p1809::STM_1808 [psr3B+]	This study
AV25074	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::p1809::STM_1808 & 1807 [psr3BC+]	This study
AV25066	ΔSTM_1811-1807::FRT, ΔSTM_3103-3101::Cm, ΔSTM_5178-5180::FRT, ΔdmsABC::Km, pWSK29::p1809::STM_1807 [psr3C+]	This study

## ***E.coli***

DH5α	supE44 ΔlacU169 (φ80 lacZ ΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1	2
AV21115	DH5α (pWSK29-dmsABC)	1
AV25102	DH5α (pWSK29-dmsABC ΔN66)	This study
AV25106	DH5α (pWSK29-dmsABC R106A)	This study
AV24193	DH5α (pWSK29-dmsABC S205G)	This study
AV23019	DH5α (pWSK29-iscS) [pISCS]	1
AV24044	DH5α (pWSK29::STM_1811-1807)	This study
AV24076	DH5α (pWSK29::STM_1809-1807)	This study
AV25101	DH5α (pWSK29::STM_1809-1807 ΔN66)	This study
AV25105	DH5α (pWSK29::STM_1809-1807 R105A)	This study

AV24166	DH5α (pWSK29::STM_1809-1807 S199G)	This study
AV25063	DH5α (pWSK29::STM_1809 & 1808)	This study
AV25075	DH5α (pWSK29::STM_1809)	This study
AV25080	DH5α (pWSK29::p1809::STM_1808)	This study
AV25079	DH5α (pWSK29::p1809::STM_1808 & 1807)	This study
AV25064	DH5α (pWSK29::p1809::STM_1807)	This study
AV24046	DH5α (pWSK29::STM_3103-3101)	This study
AV25032	DH5α (pWSK29::STM_3103-3101 S205G)	This study
AV24040	DH5α (pWSK29::STM_5178-5180)	This study
AV25057	DH5α (pWSK29::STM_5178-5180 S198G)	This study

**Table S3. Plasmids used in this study.**

Plasmid	Relevant characteristics	Reference
pWSK29	low copy plasmid, <i>lacZα</i> , Pn <sup>r</sup>	3
pKD13	template vector for FRT-flanked Km <sup>r</sup> cassette, Km <sup>r</sup> Pn <sup>r</sup>	4
pKD3	template vector for FRT-flanked Cm <sup>r</sup> cassette, Cm <sup>r</sup> Pn <sup>r</sup>	4
pHluorin	pH-sensitive green fluorescent proteins inpGEMEX-2, Pn <sup>r</sup>	5
pISCS	pWSK29 + 1.60-kb DNA containing <i>piscS::iscS</i> , Pn <sup>r</sup>	1
pDMS	pWSK29 + 4.22-kb DNA containing <i>pdmsA::dmsABC</i> , Pn <sup>r</sup>	1
pDMS ΔN66	pWSK29 + 4.22-kb DNA containing <i>pdmsA::dmsABC</i> , <i>dmsA</i> N66 deletion, Pn <sup>r</sup>	This study
pDMS R106A	pWSK29 + 4.22-kb DNA containing <i>pdmsA::dmsABC</i> , <i>dmsA</i> R106A mutation, Pn <sup>r</sup>	This study
pDMS S205G	pWSK29 + 4.22-kb DNA containing <i>pdmsA::dmsABC</i> , <i>dmsA</i> S205G mutation, Pn <sup>r</sup>	This study
p1811~1807	pWSK29 + 6.8-kb DNA containing <i>p1811::1811</i> and <i>p1809::1809,1808, and1807</i> , Pn <sup>r</sup>	This study
p1809~1807	pWSK29 + 4.2-kb DNA containing <i>p1809::1809,1808, and1807</i> , Pn <sup>r</sup>	This study
p1809~1807 ΔN66	pWSK29 + 4.2-kb DNA containing <i>p1809::1809,1808, and1807</i> , <i>1809</i> N66 deletion, Pn <sup>r</sup>	This study
p1809~1807 R105A	pWSK29 + 4.2-kb DNA containing <i>p1809::1809,1808, and1807</i> , <i>1809</i> R105A mutation, Pn <sup>r</sup>	This study
p1809~1807 S199G	pWSK29 + 4.2-kb DNA containing <i>p1809::1809,1808, and1807</i> , <i>1809</i> S199G mutation, Pn <sup>r</sup>	This study
p1809 & 1808	pWSK29 + 3.3-kb DNA containing <i>p1809::1809 and 1808</i> , Pn <sup>r</sup>	This study
p1809	pWSK29 + 2.7-kb DNA containing <i>p1809::1809</i> , Pn <sup>r</sup>	This study
p1808	pWSK29 + 0.21-kb <i>p1809</i> DNA + 0.62-kb <i>1808</i> gene containing <i>p1809::1808</i> , Pn <sup>r</sup>	This study
p1808 & 1807	pWSK29 + 0.21-kb <i>p1809</i> DNA + 1.5-kb <i>1808&amp;1807</i> genes containing <i>p1809::1808 and 1807</i> , Pn <sup>r</sup>	This study
p1807	pWSK29 + 0.21-kb <i>p1809</i> DNA + 0.88-kb <i>1807</i> gene containing	This study
p3103~3101	pWSK29 + 4.1-kb DNA containing <i>p3103::3103,3102, and 3101</i> , Pn <sup>r</sup>	This study
p3103~3101 S205G	pWSK29 + 4.1-kb DNA containing <i>p3103::3103,3102, and 3101</i> , <i>3103</i> S205G mutation, Pn <sup>r</sup>	This study
p5178~5180	pWSK29 + 4.1-kb DNA containing <i>p5178::5178,5179, and 5180</i> , Pn <sup>r</sup>	This study
p5178~5180 S198G	pWSK29 + 4.1-kb DNA containing <i>p5178::5178,5179, and 5180</i> , <i>5178</i> S198G mutation, Pn <sup>r</sup>	This study

**Table S4. Oligonucleotides used in this study.**

<b>Strains</b>	<b>Primer Sequence (5' → 3')</b>
$\Delta dmsABC::Km$	<b>F:</b> GCATTTACCCTGCCATTTACCCGCATCGCTAACGCCGCAGTGTAGGC TGGAGCTGCTTCG <b>R:</b> CGCCAGCATCAGAATGAAGGCGACAGAGAGCAACGGTACCGATTCCG GG GATCCGTCGAC
$\Delta moaABCDE::Km$	<b>F:</b> TACTTGCGTTTGTCTGATTACCGATGTGTGTAACCTTCGTGTGTAGGCTG GAGCTGCTTCG <b>R:</b> AACGGCGCACGGGTTTTTCAGATAATCCATAATGAACTGACCATTCCGG GGATCCGTCGAC
$\Delta mstA::Km$	<b>F:</b> GACGCCCGTATGGCGCCGCCAGGACAGGAACATCGTGATGTGTAGG CTGGAGCTGCTTCG <b>R:</b> TCCAGCGTGGCGAGCGCCAGCACACGACCGCGGCCGTCACATTCC GGGGATCCGTCGAC
$\Delta pspE::Km$	<b>F:</b> GGAATATTTGCGTTAGCGTTATTCATAGCCATGCCGCTTGTGTAGGCT GGAGCTGCTTCG <b>R:</b> TTTTTTTACTTTTCGGCATATCAAGACGACTGATACCGCCCAATTCCGG GGATCCGTCGAC
$\Delta 1809::Cm$ [ $\Delta psr3$ ]	<b>F:</b> GGAGAAAAGCAACAAACAGGCGTTAGCCGCAGGACGTTAGTGTAGGC TGGAGCTGCTTCG <b>R:</b> ACCTGCGACGGCCATACCTACGGTCATATGCAGGCCATAACATATGAA TATCCTCCTTAG
$\Delta 3103::Cm$ [ $\Delta psr1$ ]	<b>F:</b> CGGATCATGAAAAATATAAAAAGCCAGGGAAATGGCGAAGTGTAGGC TGGAGCTGCTTCG <b>R:</b> AGCCAATACTAAAGAAGACGTAGCGCAGCATAATCTCGCCCATATGA ATATCCTCCTTAG
$\Delta 5178::Cm$ [ $\Delta psr2$ ]	<b>F:</b> AAATGGCTGAACACAACCATAACCCGACGTGATGCTATTGTGTAGGC TGGAGCTGCTTCG <b>R:</b> TTGGCAGCGTCCACAGATTGTAAAACGCAATCCTACCCGCCATATGA ATATCCTCCTTAG
$\Delta 1811::Cm$	<b>F:</b> GGAGAAAAGCAACAAACAGGCGTTAGCCGCAGGACGTTAGTGTAGGC TGGAGCTGCTTCG <b>R:</b> TGACTTTGGCGGGTAGACGCACCTCTCCACGATCGTTAAACATATGAA TATCCTCCTTAG
<b>Plasmids</b>	
p1811~1807	<b>F:</b> ATCGAAGCTTGCGCGAACGACACGCAT <b>R:</b> ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1809~1807	<b>F:</b> ATCGAAGCTTGACCACGGCGCCTGCAT <b>R:</b> ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1809~1808	<b>F:</b> ATCGAAGCTTGACCACGGCGCCTGCAT <b>R:</b> ATCGCTCGAGCATGCCATCCACTTCCCA
p1809	<b>F:</b> ATCGAAGCTTGACCACGGCGCCTGCAT <b>R:</b> ATCGCTCGAGCCATACTGGGTTGTCATC
p1808	Promoter 1809 part <b>F:</b> ATCGAAGCTTGACCACGGCGCCTGCAT <b>R:</b> CACTTCCCATAACAGCTCACTATGCTC 1808 part <b>F:</b> TAGTGAGCTGTTATGGGAAGTGGATGGCA <b>R:</b> ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1808 & 1807	Promoter 1809 part <b>F:</b> ATCGAAGCTTGACCACGGCGCCTGCAT <b>R:</b> CACTTCCCATAACAGCTCACTATGCTC 1808 & 1807 part

	F: GTGAGCTGTTATGACAACCCAGTATGGA R: ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1807	Promoter 1809 part F: ATCGAAGCTTGACCACGGCGCCTGCAT R: CACTTCCCATAACAGCTCACTATGCTC 1807 part F: TAGTGAGCTGTTATGGGAAGTGGATGGCA R: ATCGCTCGAGTGCGGGTCGCCCCGCACG
p3103~3101	F: ATCGAAGCTTAAATGGCGAACCTGTTAATGGC R: ATCGCTCGAGCCGGATCGATCGTCAGGTT
p5178~5180	F: ATCGAAGCTTGCCTTGCAAGGGCGCGCT R: ATCGCTCGAGCATAAACCGGGCAAAAA

### Real-time qRT-PCR

<i>cysJ</i>	F: TTCGCAAACCGGCAATGCGC R: TCGCCGAGGCTAAACACGGC Probe: 6-FAM- ATTCAAACAGATTGCCAGTGA-3BHQ-1
<i>cysK</i>	F: TATTGCGCTGGCGTATGTCTG R: CCGGAGATAAACACATCCACC Probe: 6-FAM- TTCAGAAAGCCGAAGAAATTGT-3BHQ-1
<i>cysM</i>	F: CGGTGAAAGATCGCGCGGCGC R: CAGACATAGCGAGCGCTAAATC Probe: 6-FAM- AAGGTTATCGCATGAAGCTGTTGA-3BHQ-1
<i>iscS</i>	F: TTGCCGAGAAAATGATGCAGTT R: TGTGCTTGCCTTTTTTCTGAT Probe: 6-FAM-TTGGGAACCCGGCGTCTCGT-3BHQ-1
<i>mstA</i>	F: CGAATATCGCGCCGGACATATTC R: CATTCTGTCGCGCTGCCAGCC Probe: 6-FAM- AAACACCTGGTCATATACGATGA-3BHQ-1
<i>phsA</i>	F: ACGCGCTATCTGGTTTCGTTT R: AACGCGGATCGAAGCTCAC Probe: 6-FAM- CCTGCGCGGTCATTAACCTCATGGG-3BHQ-1
<i>pspE</i>	F: CGTTAGCGTTATTCATAGCC R: ACCGCCCATATTCATCGCGTG Probe: 6-FAM- ATATTCAGGGCGCGATTAATA-3BHQ-1
<i>rpoD</i>	F: GTGGCTTGCAATTCCTTGAT R: AGCATCTGGCGAGAAATA Probe: 6-FAM-ATAAGTTCGAATACCGTCGCG-3BHQ-1
STM_1811	F: TTCGAGCGCAAGCCCATC R: ATGCCGGATCTCTTGCCT Probe: 6-FAM- ATTGCGCCTGTGTACGGCCTT-3BHQ-1
STM_3103	F: GCACGGCAAGCTACCTGA R: GCTTTCTCATCGCGCCCCTG Probe: 6-FAM- ATGCCTAATTTGCGCCGCCACC-3BHQ-1
STM_5178	F: TGCCATTGTTGCCTGTCG R: CTTTCACACCCATTGTCGT Probe: 6-FAM-CTGAATGGCGTCCGTCCACA-3BHQ-1

### Site-directed point mutations

<i>dmsA</i> ΔN66	F: TGGAGCGCCTGTACGGTA*TGTGGCAGCCGTTGTCCG R: CGGACAACGGCTGCCACA*TACCGTACAGGCGCTCCA
<i>dmsA</i> R106A	F: GTTCGCGCCTGCCTG <b>G</b> CCGGTCGTTCTATGCGC R: GCGCATAGAACGACC <b>G</b> GCCAGGCAGGCGCGAAC

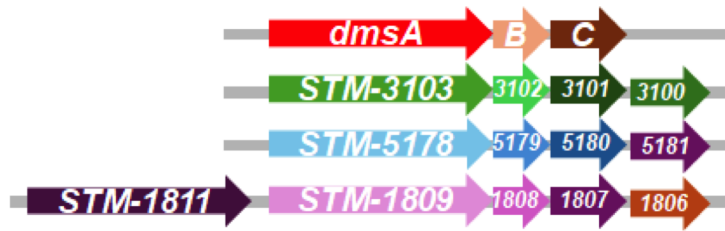
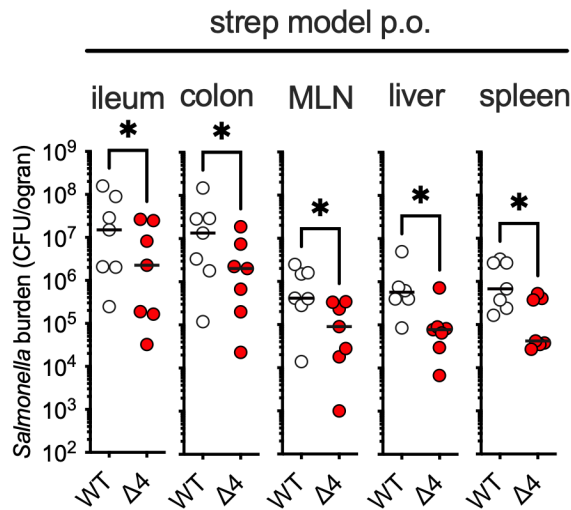
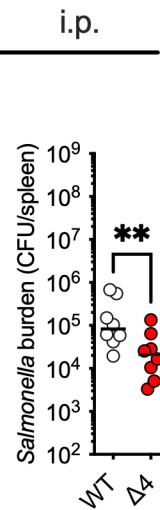
<i>dmsA</i> S205G	F: TACGGTGATTAC <b><i>GGT</i></b> TCCGCGCAAATCGCA R: TGCGATTTGCGCGGA <b><i>ACCGTA</i></b> ATCACCGTA
1809 ΔN66	F: TGGAGTTCATGTACCGTT* <b><i>TG</i></b> CGGCAGCCGCTGCCTG R: CAGGCAGCGGCTGCCGCA* <b><i>AACGGT</i></b> ACATGAACTCCA
1809 R105A	F: CAGGTACGCGCCTGCCTG <b><i>GCCG</i></b> GCCGTTCTATTTCGT R: ACGAATAGAACGGCC <b><i>GGCC</i></b> CAGGCAGGCGCGTACCTG
1809 S199G	F: TACGGTAGCTAC <b><i>GGC</i></b> ACCGCCCAAATC R: GATTTGGGCGGT <b><i>GCCG</i></b> TAGCTACCGTA
3103 S205G	F: TACCATTCTGTG <b><i>GGC</i></b> ATGGGCAACACGGCG R: CGCCGTGTTGCCCAT <b><i>GCCC</i></b> ACAGAATGGTA
5178 S198G	F: CACAACACCTAT <b><i>GGC</i></b> ACGGCGCAAATCGCC R: GGCGATTTGCGCCGT <b><i>GCC</i></b> ATAGGTGTTGTG

\* Restriction enzyme sites are underlined.

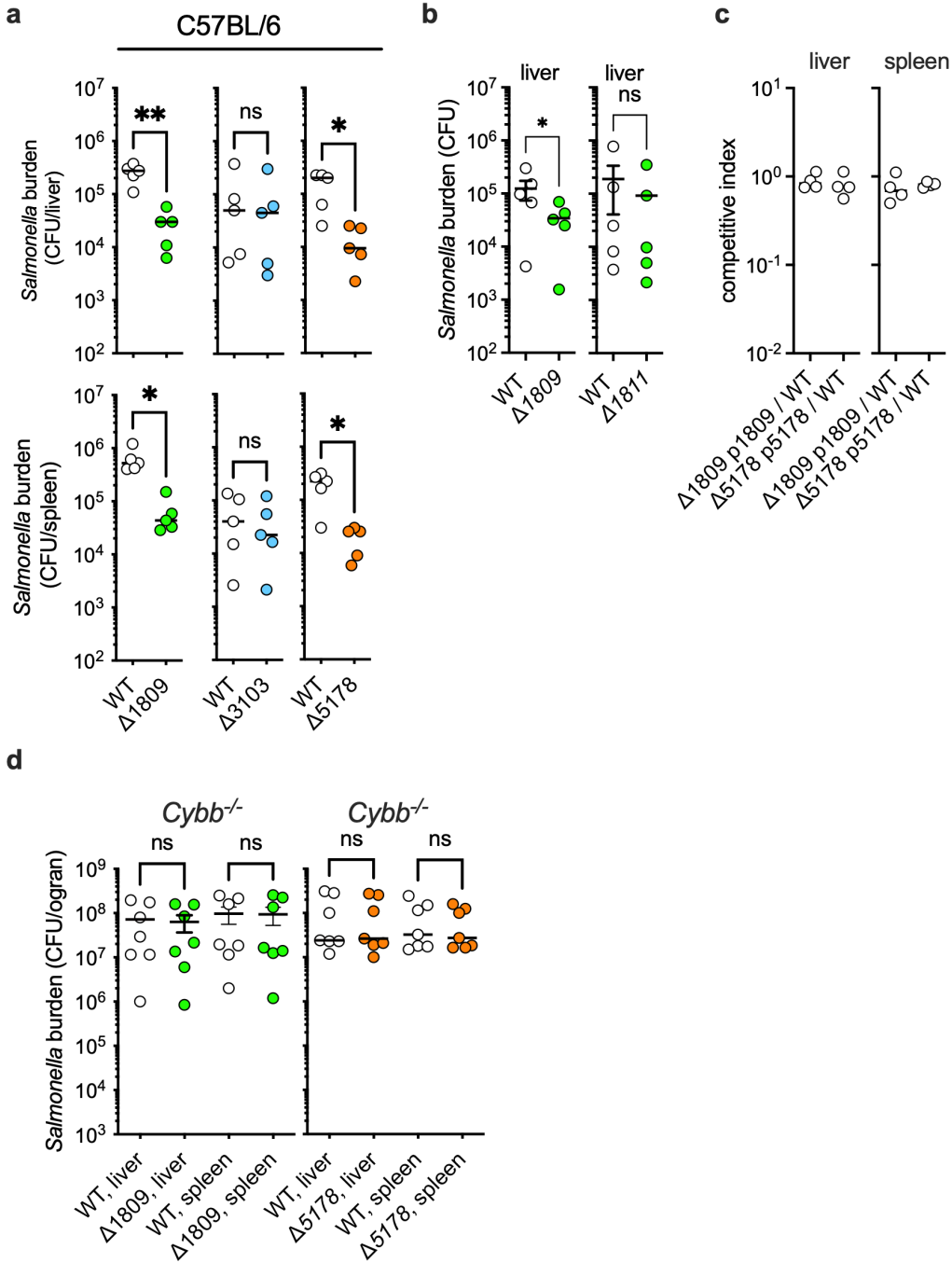
\*\* Point mutation sites are indicated in bold and italic.

\*\*\* Deleted sites are marked with an asterisk.

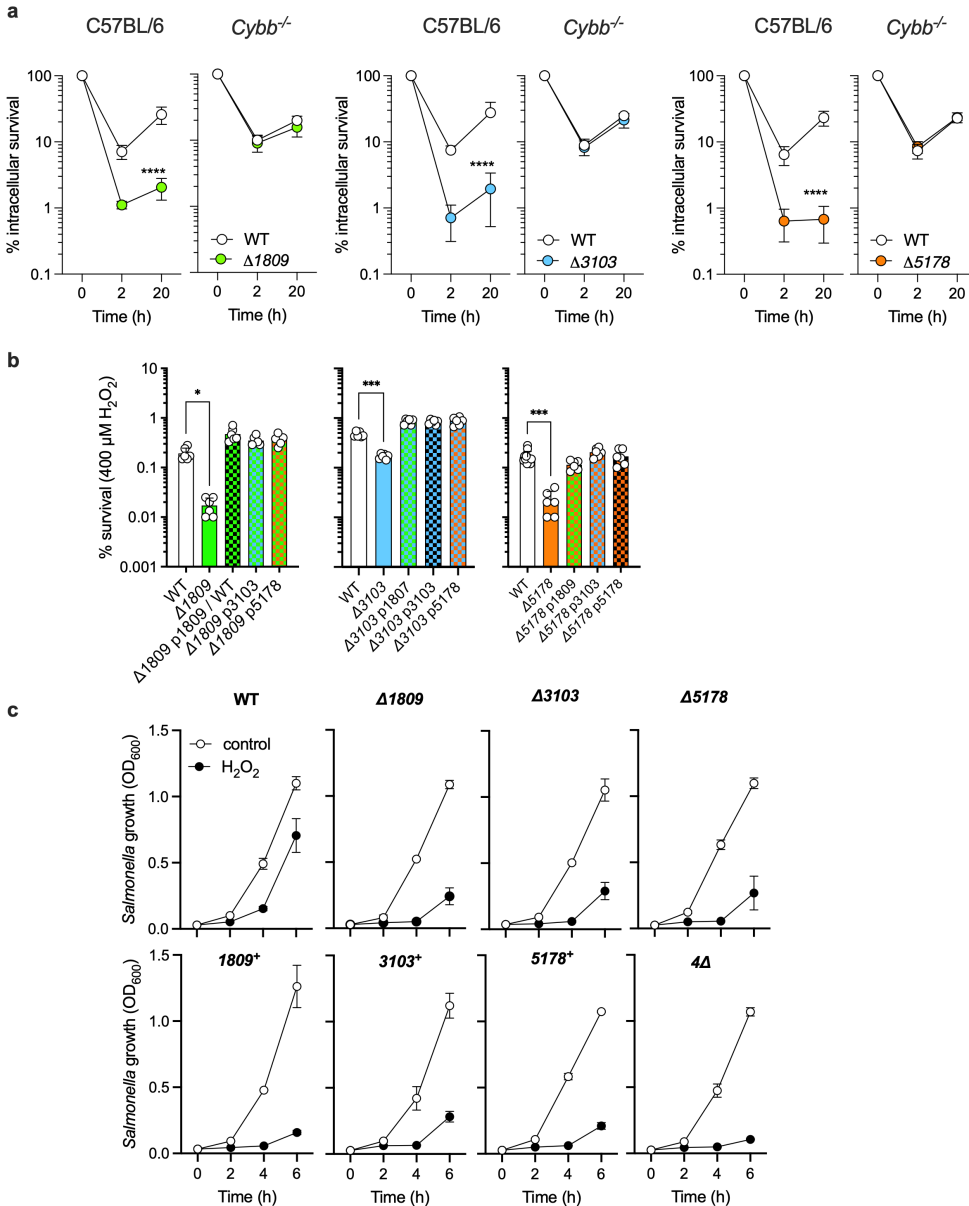


**a****b****c**

**Figure S1. Operon organization of DmsA paralogs and their contribution to *Salmonella* fitness.** **a.** Scheme of *dmsA* paralog operons. **b.** Competition of  $\Delta 4$  (quadruple mutant in all four *dmsA* paralogs) and wild-type *Salmonella* in the ileum, colon, MLN, liver and spleen of C57BL/6 mice in a streptomycin-treated model of oral infection. The bacterial burden was assessed by calculating the CFUs 4 days post-infection after p.o. inoculation. The data are mean  $\pm$  SD from 7 mice in 2 independent experiments. **c.** Virulence of  $\Delta 4$  *Salmonella* was assessed by counting bacterial burden in the spleens of C57BL/6 3 days after i.p. inoculation of  $\sim 500$  CFU mixture of equal numbers of wild-type and mutant *Salmonella*. The data are presented as mean  $\pm$  SD from 8 mice in 2 individual experiments. \*, \*\*,  $p < 0.1, 0.01$ , respectively, as estimated by paired *t*-test (**b, c**).

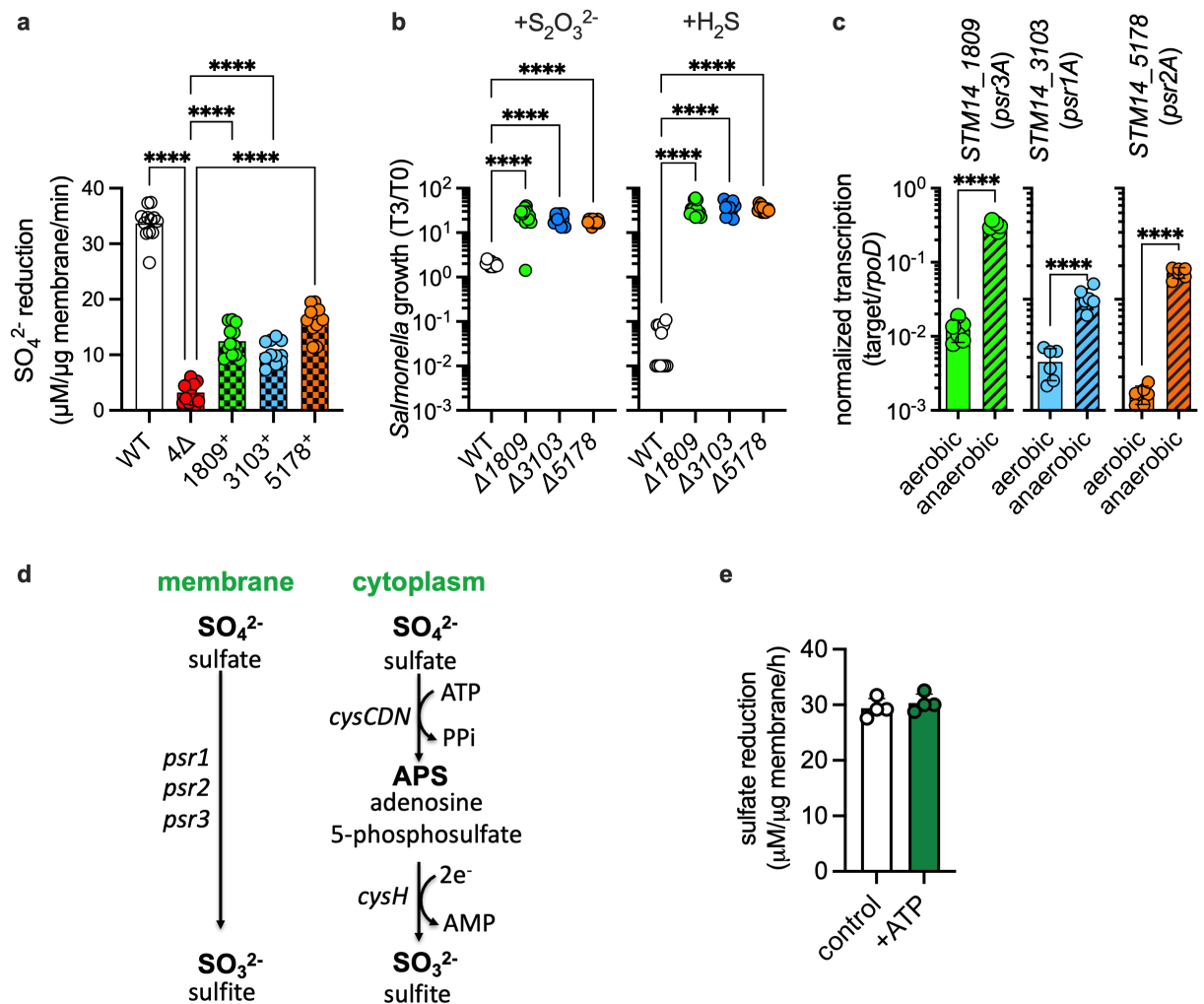


**Figure S2. DmsA paralogs enhance *Salmonella* resistance to phagocyte NADPH oxidase-driven oxidative stress.** **a, b, c, d.** The bacterial burden was quantified in the spleens and livers of C57BL/6 mice 3 days after i.p. inoculation with ~300 CFU of a mixture containing equal numbers of wild-type *Salmonella* and isogenic mutants (**a, b, d**). Competitive index (**c**) was calculated 3 days after i.p. infection. Data represent mean  $\pm$  SD from 2 independent experiments (**a** liver and spleen  $n=5$  mice; **b**  $n=4$  mice; **c**  $n=5$  mice; **d** liver and spleen  $n=7$  mice). \*, \*\*,  $p < 0.1$ , 0.01 as estimated by paired  $t$ -test (**a, b, c**) or one-way ANOVA (**d**).



**Figure S3. Susceptibility of *dmsA* paralog mutants to oxidative stress.** **a.** The percent survival of single *dmsA* paralog deletion mutants was assessed in periodate-elicited macrophages from C57BL/6 and *Cybb*<sup>-/-</sup> mice. CFU were enumerated at 0, 2, and 20 h post-infection to determine bacterial replication. The data are mean  $\pm$  SD from 2 independent experiments (C57BL/6, n=7; *Cybb*<sup>-/-</sup>, n=10). **b.** Single *dmsA* paralog deletion mutants were cultured aerobically in LB broth at 37°C and challenged with 400  $\mu$ M H<sub>2</sub>O<sub>2</sub> in PBS for 2 h. Bacterial survival was determined by CFU enumeration. Cross-complementation was performed using the *dmsA* paralog cloned into the low-copy plasmid pWSK29. The data are mean  $\pm$  SD; n=6 (right panel WT n=12) from 3-4 individual experiments. \*, \*\*\*, \*\*\*\*  $p < 0.1$ , 0.001, 0.0001, respectively, were calculated by two-way (a) or one-way ANOVA (b). **c.** Growth, as measured by OD<sub>600</sub>, of *dmsA* paralog mutants in MOPS-GLC-sulfate minimal medium supplemented with 400  $\mu$ M H<sub>2</sub>O<sub>2</sub>. The strains carried single (e.g.,  $\Delta 1809$ ) or quadruple (i.e.,  $\Delta 4$ ) mutations in *dmsA* paralogs. Strains expressing single *dmsA* paralogs (e.g., 1809<sup>+</sup>) were constructed by complementing the  $\Delta 4$  strain

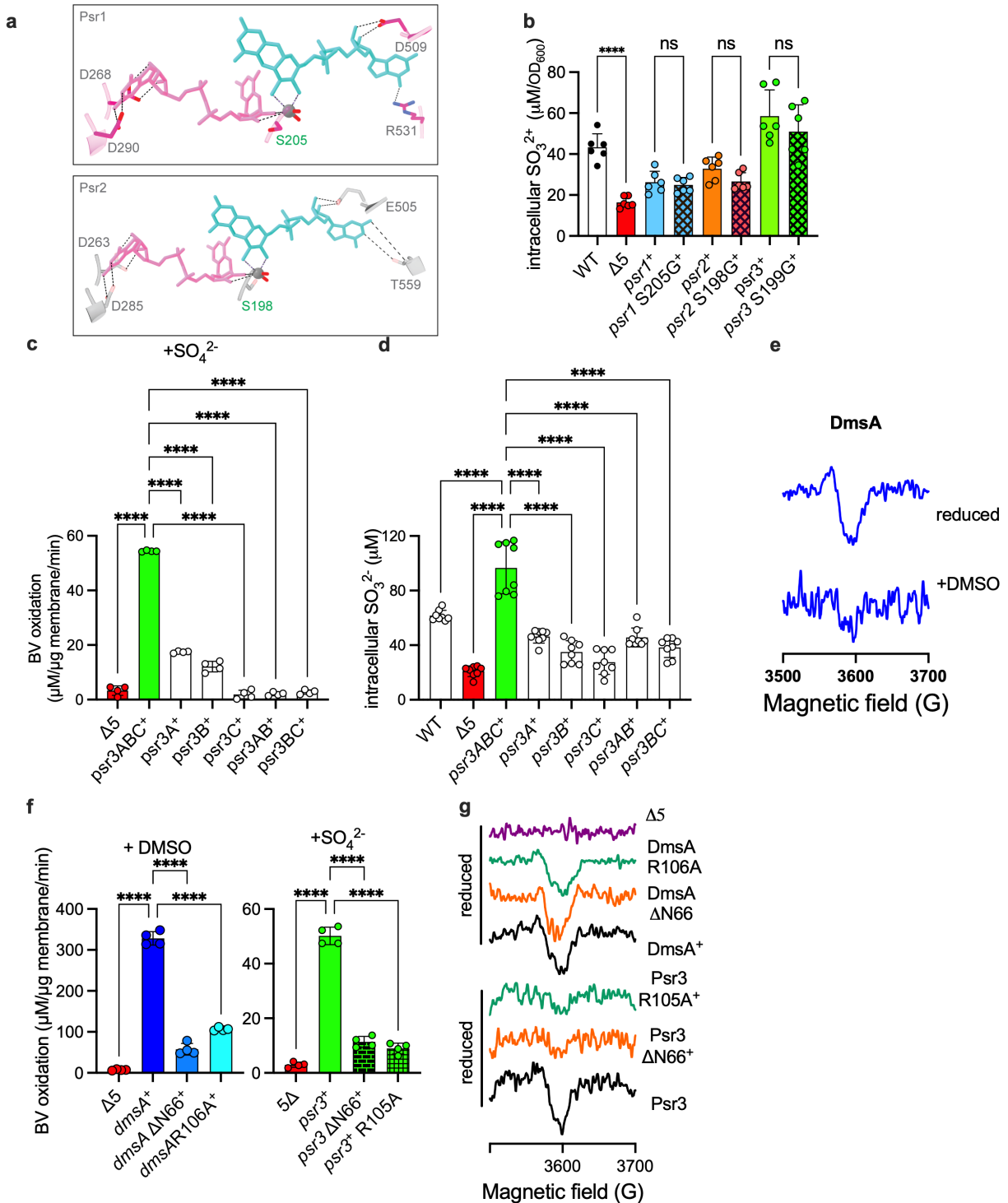
with the pWSK29 plasmid bearing *psr1*, *psr2*, or *psr3* operons. Data are represented as mean  $\pm$  SD from 2 biological replicates in 2 independent experiments.



**Figure S4. DmsA paralogs function as membrane-associated sulfate reductases, indirectly facilitating sulfur acquisition to support the growth of *Salmonella*.** **a.** Sulfate reductase activities were measured in membranes isolated from the indicated strains. The strains carried quadruple (i.e., Δ4) mutations in *dmsA* paralogs. Strains expressing single *dmsA* paralogs (e.g., 1809<sup>+</sup>) were constructed by complementing the Δ4 strain with the pWSK29 plasmid bearing specific operons. Sulfate reductase activity was monitored by following the oxidation of benzyl viologen. The data are mean  $\pm$  SD; n=12 from 4 independent experiments. **b.** Growth rescue of *dmsA* paralog mutants by exogenous sulfur compounds. Growth defects observed in single *dmsA* paralog deletion mutants were rescued by supplementation with 100 μM sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>), or sodium sulfide (Na<sub>2</sub>S). Mutants were cultured in MOPS-GLC-minimal medium containing thiosulfate or sulfide for 3 h at 37°C under hypoxic conditions. Bacterial viability was assessed by CFU enumeration. The data are mean  $\pm$  SD; n=12 from 3 independent experiments. **c.** Transcripts of *dmsA* paralog genes were quantified by real-time qRT-PCR using RNA isolated from *Salmonella* cultured aerobically or anaerobically in MOPS-GLC-minimal medium supplemented with 250 μM sulfate. The data are mean  $\pm$  SD; n=6 from 2 independent experiments. **d.** Pathway of sulfate reduction in the cell membrane and cytoplasm of *Salmonella*. **e.** Evaluation of ATP-dependent sulfate reduction. Sulfate utilization was measured in isolated

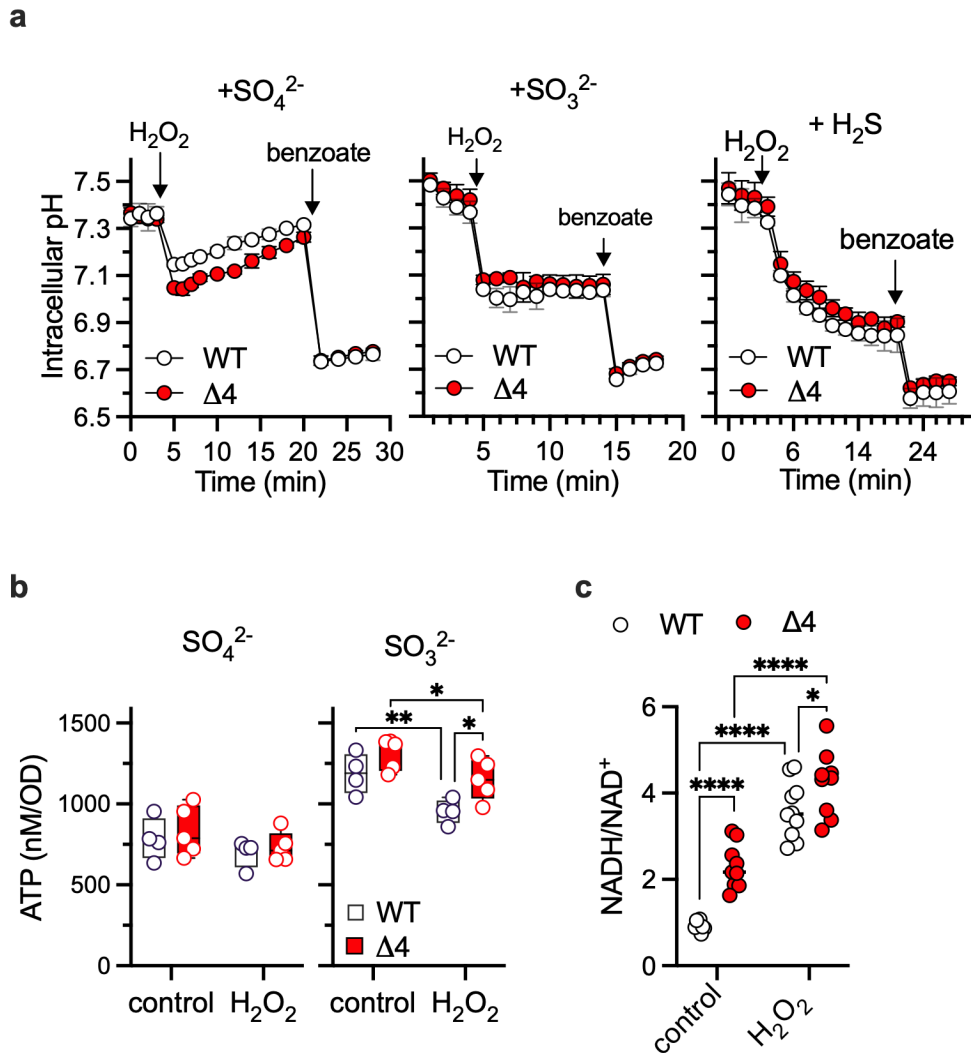
membrane fractions of *Salmonella* in the presence of 0.2 mM ATP. Data represent mean  $\pm$  SD; n=4 from 2 individual experiments. \*\*\*\*  $p < 0.0001$  as estimated by one-way ANOVA (**a**, **b**) or unpaired  $t$  test (**c**).

**Figure S5. See attached Excel file.**



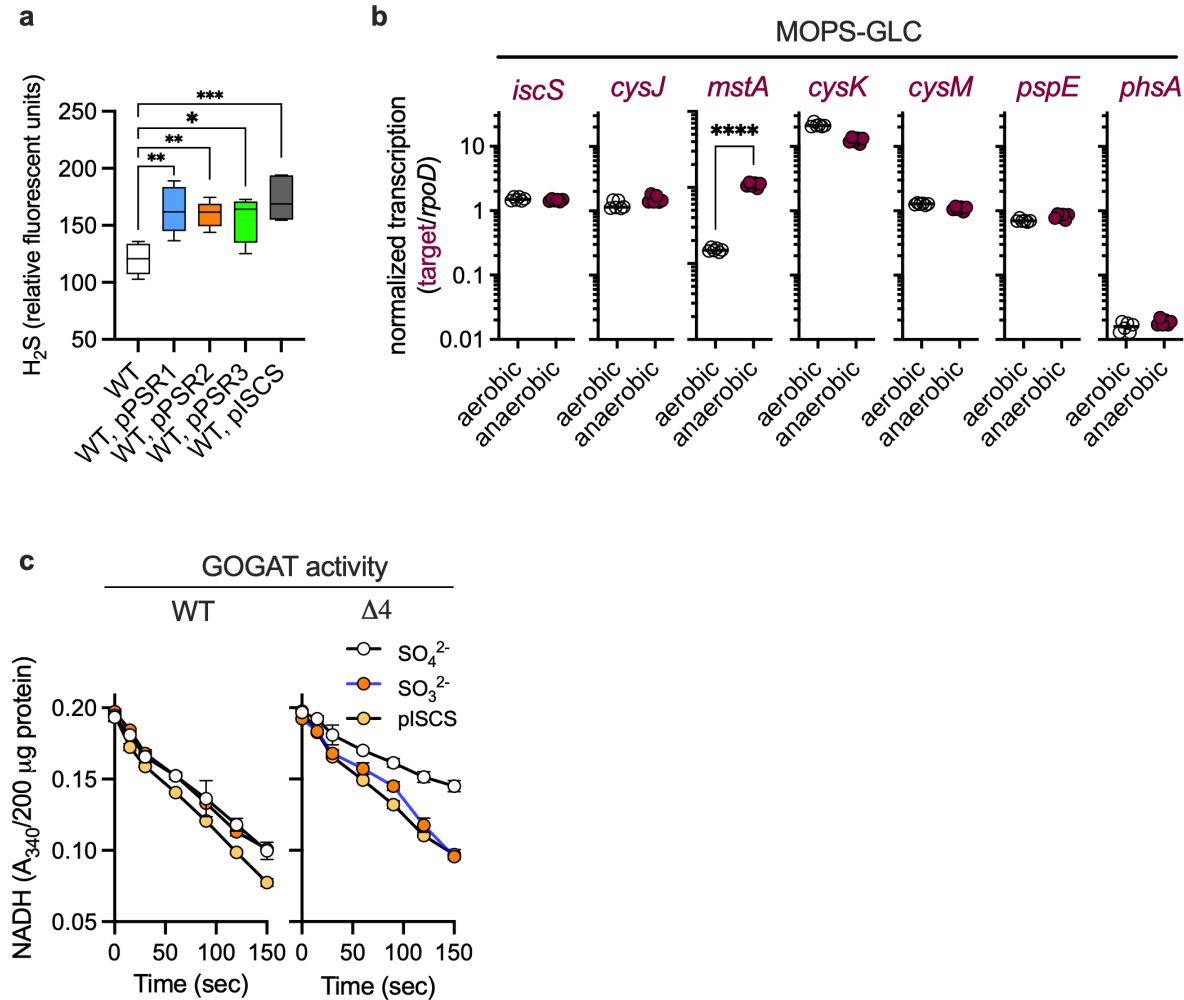
**Figure S6. The function of periplasmic sulfate reductase in *Salmonella* is dependent on the FS0 [4Fe-4S], but not molybdenum, cofactor.** **a.** The predicted Mo-bis PGD in Psr1A and Psr2A as determined by AlphaFold<sub>3</sub>. **b, d.** Intracellular sulfite concentrations were measured in wild-type *Salmonella*, the  $\Delta 5$  mutant deficient in *dmsA* and all 4 *dmsA* paralogs, and various complemented strains after overnight growth under hypoxic conditions in MOPS-GLC minimal medium supplemented with sulfate. The data are presented as mean  $\pm$  SD from 2 individual tests (**b**,  $n=6$ ; **d**,  $n=8$ ). **c.** Sulfate reductase activities were assessed by monitoring benzyl viologen oxidation in

membranes isolated from the  $\Delta 5$  mutant and its complemented strains expressing individual genes, gene pairs, or the entire *psr3ABC* operon. Data presents mean  $\pm$  SD from 2 independent experiments (n=4). **e.** EPR spectra of membranes isolated from *Salmonella* were recorded after reduction with 2 mM dithionite, either alone (reduced) or in the presence of 50 mM DMSO (**e**, +DMSO). **f.** The membranes used for EPR spectroscopy were evaluated for DMSO and sulfate reductase activities by measuring the oxidation of benzyl viologen in the presence of DMSO (left, +DMSO) or sulfate (right, +SO<sub>4</sub><sup>2-</sup>). The data are presented as mean  $\pm$  SD from 2 independent experiments (n=4). \*\*, \*\*\*, \*\*\*\*  $p < 0.01, 0.001, 0.0001$ , respectively, as estimated by one-way ANOVA (**b, c, d, f**).



**Figure S7. Effect of periplasmic sulfate reductases in the sulfate assimilatory pathway. a.** Intracellular pH of exponential-phase *Salmonella* cultured in MOPS-GLC minimal medium either 250  $\mu$ M sulfate, 250  $\mu$ M sulfite, or 100  $\mu$ M sulfide was measured using ratiometric pH-sensitive reporter pHluorin. Where indicated, the specimens were treated with 400  $\mu$ M  $H_2O_2$  or 40 mM of the protonophore benzoate. The data are mean  $\pm$  SD;  $n=3$  from 3 independent experiments. **b.** Intracellular ATP levels were measured using a luciferase-based assay from *Salmonella* grown in MOPS-GLC minimal medium supplemented with either sulfate or sulfite. Data represent the mean  $\pm$  SD from 2 individual experiments (WT  $n=4$ ,  $\Delta 4$   $n=5$ ). **c.** Intracellular NADH / NAD<sup>+</sup> ratios were quantified in *Salmonella* grown aerobically in MOPS-GLC minimal medium supplemented with sulfate. The data are the mean  $\pm$  SD from 2 individual experiments (WT control  $n=8$ ; WT  $H_2O_2$   $n=10$ ;  $\Delta 4$   $n=9$ ). \*, \*\*, \*\*\*\*  $p < 0.1$ , 0.01, 0.0001, respectively, as estimated by one-way ANOVA (b, c).





**Figure S8. Periplasmic sulfate reductases are involved in the assimilation of sulfur.** **a**, Hydrogen sulfide (H<sub>2</sub>S) production was evaluated in wild-type *Salmonella* harboring each individual periplasmic sulfate reductase operon (*psr1*, *psr2* and *psr3*) or the *iscS* gene in pWSK29. Cells were cultured in MOPS-GLC minimal medium containing sulfate. H<sub>2</sub>S was detected using the WSP5 fluorescent probe. The data are the mean  $\pm$  SD from 2-3 independent experiments (WT, n=6; pPSR3, n=4). **b**, Expression of indicated genes was quantified by real-time qRT-PCR using RNA isolated from wild-type *Salmonella* cultured aerobically or anaerobically in MOPS-GLC-minimal medium supplemented with sulfate. The data are mean  $\pm$  SD; n=6 from 2 individual experiments. \*, \*\*, \*\*\*, \*\*\*\*  $p < 0.1$ , 0.01, 0.001, 0.0001, respectively, as estimated by one-way ANOVA (**a**), or unpaired *t*-test (**b**). **c**, Glutamate synthase (GOGAT) activity was assessed kinetically by measuring the oxidation of NADH. Specimens were prepared from *Salmonella* grown in MOPS-GLC minimal medium containing either sulfate or sulfite. Selected strains harbored the *iscS* gene from the pWSK29 plasmid. The data are mean  $\pm$  SD; n=3 from 2 independent experiments.

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