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

Ju-Sim Kim[1], Siva Uppalapati[1], Alyssa Margolis[1], Michael McClelland[2], Hanan Elajaili[3], Eva Nozik[3], Lin Liu[1], and Andres Vazquez-Torres[1,4*]

[1]University of Colorado School of Medicine, Department of Immunology and Microbiology, Aurora, Colorado-80045, USA
[2]University of California Irvine School of Medicine, Department of Microbiology and Molecular Genetics, Irvine, CA, USA
[3]Cardiovascular Pulmonary Research Laboratories and Pediatric Critical Care Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado-80045, USA
[4]Veterans Affairs, Eastern Colorado Health Care System, Aurora, Colorado-80045, USA

Lead contact: Andres Vazquez-Torres
Department of Immunology and Microbiology
University of Colorado School of Medicine
12800 East 19th Avenue MS 8333
RC1 North room P18-9131
Aurora, Colorado 80045, USA
Email: andres.vazquez-torres@cuanschutz.edu

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%

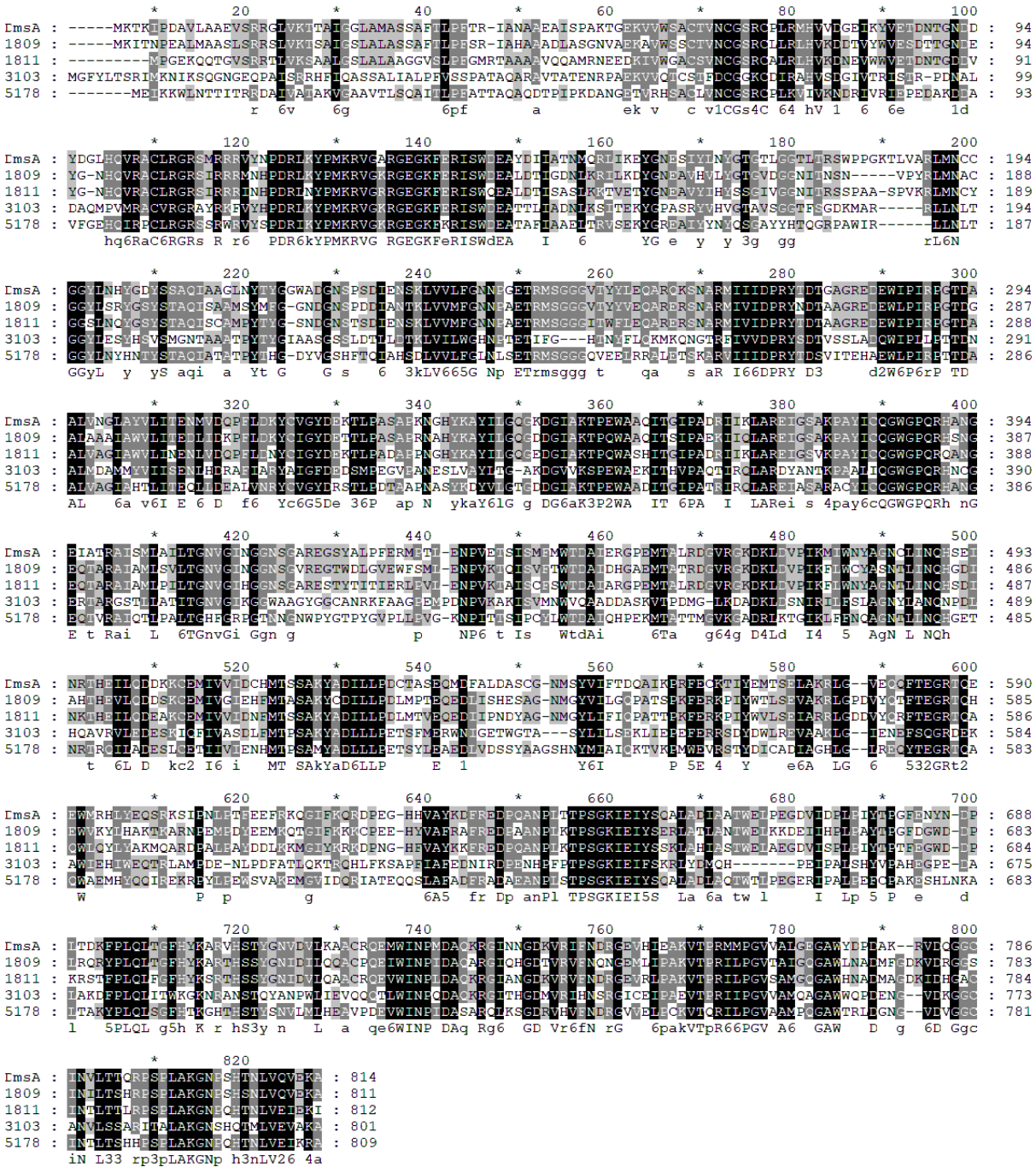

Table S2. Bacterial strains used in this study.

Strain	Relevant characteristics	Reference
Salmonella		
14028s	wild-type of *S. Typhimurium*	ATCC
AV20164	WT, pHluorin	[1]
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]	[1]
AV13165	Δ*cysIJ*::Cm [Δ*cysIJ*]	lab stock
AV22025	Δ*dmsABC*::Km [Δ*dmsABC*]	[1]
AV0785	Δ*iscS*::Km [Δ*iscS*]	[1]
AV08158	Δ*menA*::FRT [Δ*menA*]	lab stock
AV21016	Δ*moaABCDE*::Km [Δ*moa*]	[1]
AV22175	Δ*mstA*::Km [Δ*mstA*]	This study
AV22195	Δ*phsABC*::Cm [Δ*phsABC*]	lab stock
AV24143	Δ*pspE*::Km [Δ*pspE*]	This study
AV10074	Δ*ubiC*::Cm [Δ*ubiC*]	lab stock
AV24048	ΔSTM_1811::Cm [Δ1811]	This study
AV24001	Δ*STM_1811-1807::Cm* [*Δ1809* or *Δpsr3*]	This study
AV24055	Δ*STM_1811-1807::Cm*, pWSK29::*iscS* [Δ*psr3* pISCS]	This study
AV24035	Δ*STM_1811-1807::Cm*, pWSK29::*STM_1811-1807* [Δ1809 p1809]	This study
AV24158	Δ*STM_1811-1807::Cm*, pWSK29::*STM_3103-3101*[Δ1809 p3103]	This study
AV24159	Δ*STM_1811-1807::Cm*, pWSK29::*STM_5178-5180*[Δ1809 p5178]	This study
AV24002	Δ*STM_3103-3101::Cm* [Δ3103 or *Δpsr1*]	This study
AV24053	Δ*STM_3103-3101::Cm*, pWSK29::*iscS* [Δ*psr1* pISCS]	This study
AV24160	Δ*STM_3103-3101::Cm*, pWSK29::*STM_1811-1807* [Δ3103 p1809]	This study
AV24052	Δ*STM_3103-3101::Cm*, pWSK29::*STM_3103-3101* [Δ3103 p3103]	This study
AV24161	Δ*STM_3103-3101::Cm*, pWSK29::*STM_5178-5180* [Δ3103 p5178]	This study
AV24003	Δ*STM_5178-5180::Cm* [Δ5178 or *Δpsr2*]	This study
AV24042	Δ*STM_5178-5180::Cm*, pWSK29::*iscS* [Δ*psr2* pISCS]	This study
AV24162	Δ*STM_5178-5180::Cm*, pWSK29::*STM_1811-1807* [Δ5178 p1809]	This study
AV24163	Δ*STM_5178-5180::Cm*, pWSK29::*STM_3103-3101* [Δ5178 p3103]	This study
AV24003	Δ*STM_5178-5180::Cm*, pWSK29:: *STM_5178-5180* [Δ5178 p5178]	This study
AV24069	Δ*STM_3103-3101::Cm*, Δ*STM_5178-5180::FRT* [1809+]	This study
AV24059	Δ*STM_1811-1807::FRT,* Δ*STM_5178-5180::Cm* [3103+]	This study
AV24068	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm* [5178+ or *psr2*+]	This study
AV24070	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT* [4Δ]	This study
AV24144	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT*, pHluorin	This study
	Δ*STM_5178-5180::FRT,* pWSK29::*iscS* [4Δ pSCIS]	This study
AV24152	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km [5Δ]	This study
AV24087	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* ΔmoaABCDE::Km [4Δ Δmoa]	This study
AV24200	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km, pWSK29::*STM_1809-1807* [*psr3*+ or *psr3ABC*+]	This study
AV25112	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km, pWSK29::*STM_1809-1807* ΔN66 [*psr3* ΔN66+]	This study
AV25113	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km, pWSK29::*STM_1809-1807* R105A [*psr3* R105A+]	This study
AV24201	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	

	pWSK29::*STM_1809-1807* S199G [*psr3* S199G+]	This study
AV25021	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* ΔmoaABCDE::Km	
	pWSK29:: *pWSK29::STM_1809-1807* [*psr3*+ Δmoa]	This study
AV24196	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*dmsABC* [*dmsA*+]	This study
AV25117	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*dmsABC* ΔN66 [*dmsA* ΔN66+]	This study
AV25118	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*dmsABC* R106A [*dmsA* R106A+]	This study
AV24197	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*dmsABC* S205G [dmsA S205G+]	This study
AV25020	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* ΔmoaABCDE::Km	
	pWSK29::*dmsABC* [*dmsA*+ Δmoa]	This study
AV25046	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*STM_3103~3101* [*psr1*+]	This study
AV25047	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*STM_3103~3101* S205G [*psr1* S205G+]	This study
AV25048	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*STM_5178~5180* [*psr2*+]	This study
AV25049	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*STM_5178~5180* S198G [*psr2* S198G+]	This study
AV25065	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*STM_1809 & 1808* [*psr3AB*+]	This study
AV25091	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*STM_1809* [*psr3A*+]	This study
AV25073	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*p1809::STM_1808* [*psr3B*+]	This study
AV25074	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*p1809::STM_1808 & 1807* [*psr3BC*+]	This study
AV25066	Δ*STM_1811-1807::FRT,* Δ*STM_3103-3101::Cm,* Δ*STM_5178-5180::FRT,* Δ*dmsABC*::Km,	
	pWSK29::*p1809::STM_1807* [*psr3C*+]	This study

E.coli

DH5α	*supE*44 Δ*lacU*169 (ϕ80 *lacZ* ΔM15) *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	[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, *lacZ*α, Pn^r	[3]
pKD13	template vector for FRT-flanked Km^r cassette, Km^r Pn^r	[4]
pKD3	template vector for FRT-flanked Cm^r cassette, Cm^r Pn^r	[4]
pHluorin	pH-sensitive green fluorescent proteins inpGEMEX-2, Pn^r	[5]
pISCS	pWSK29 + 1.60-kb DNA containing p*iscS::iscS*, Pn^r	[1]
pDMS	pWSK29 + 4.22-kb DNA containing p*dmsA::dmsABC*, Pn^r	[1]
pDMS ΔN66	pWSK29 + 4.22-kb DNA containing p*dmsA::dmsABC*, *dmsA* N66 deletion, Pn^r	This study
pDMS R106A	pWSK29 + 4.22-kb DNA containing p*dmsA::dmsABC*, *dmsA* R106A mutation, Pn^r	This study
pDMS S205G	pWSK29 + 4.22-kb DNA containing p*dmsA::dmsABC*, *dmsA* S205G mutation, Pn^r	This study
p1811~1807	pWSK29 + 6.8-kb DNA containing p*1811::1811* and p*1809::1809,1808, and1807,* Pn^r	This study
p1809~1807	pWSK29 + 4.2-kb DNA containing p*1809::1809,1808, and1807,* Pn^r	This study
p1809~1807 ΔN66	pWSK29 + 4.2-kb DNA containing p*1809::1809,1808, and1807, 1809* N66 deletion, Pn^r	This study
p1809~1807 R105A	pWSK29 + 4.2-kb DNA containing p*1809::1809,1808, and1807, 1809* R105A mutation, Pn^r	This study
p1809~1807 S199G	pWSK29 + 4.2-kb DNA containing p*1809::1809,1808, and1807, 1809* S199G mutation, Pn^r	This study
p1809 &1808	pWSK29 + 3.3-kb DNA containing p*1809::1809 and 1808,* Pn^r	This study
p1809	pWSK29 + 2.7-kb DNA containing p*1809::1809,* Pn^r	This study
p1808	pWSK29 + 0.21-kb p1809 DNA + 0.62-kb 1808 gene containing p*1809::1808,* Pn^r	This study
p1808 & 1807	pWSK29 + 0.21-kb p1809 DNA + 1.5-kb *1808&1807* genes containing p*1809::1808 and 1807,* Pn^r	This study
p1807	pWSK29 + 0.21-kb p1809 DNA + 0.88-kb *1807* gene containing	This study
p3103~3101	pWSK29 + 4.1-kb DNA containing p*3103::3103,3102, and 3101,* Pn^r	This study
p3103~3101 S205G	pWSK29 + 4.1-kb DNA containing p*3103::3103,3102, and 3101, 3103* S205G mutation, Pn^r	This study
p5178~5180	pWSK29 + 4.1-kb DNA containing p*5178::5178,5179, and 5180,* Pn^r	This study
p5178~5180 S198G	pWSK29 + 4.1-kb DNA containing p*5178::5178,5179, and 5180, 5178* S198G mutation, Pn^r	This study

Table S4. Oligonucleotides used in this study.

Strains	**Primer Sequence (5' → 3')**
Δ*dmsABC*::Km	**F:** GCATTTACCCTGCCATTTACCCGCATCGCTAACGCCGCAGTGTAGGC TGGAGCTGCTTCG
	R: CGCCAGCATCAGAATGAAGGCGACAGAGAGCAACGGTACCGATTCCG GG GATCCGTCGAC
Δ*moaABCDE*::Km	**F:** TACTTGCGTTTGTCGATTACCGATGTGTGTAACTTTCGTGTGTAGGCTG GAGCTGCTTCG
	R: AACGGCGCACGGGTTTTCAGATAATCCATAATGAACTGACCATTCCGG GGATCCGTCGAC
Δ*mstA*::Km	**F:** GACGCCCGTATGGCGCCGCCAGGACAGGAACATCGTGATGTGTAGG CTGGAGCTGCTTCG
	R: TCCAGCGTGGCGAGCGCCAGCACCACGACCGCGGCCGTCACATTCC GGGGATCCGTCGAC
Δ*pspE*::Km	**F:** GGAATATTTGCGTTAGCGTTATTCATAGCCATGCCGCTTGTGTAGGCT GGAGCTGCTTCG
	R: TTTTTTTACTTTCGGCATATCAAGACGACTGATACCGCCCAATTCCGG GGATCCGTCGAC
Δ1809::Cm [Δ*psr3*]	**F:** GGAGAAAAGCAACAAACAGGCGTTAGCCGCAGGACGTTAGTGTAGGC TGGAGCTGCTTCG
	R: ACCTGCGACGGCCATACCTACGGTCATATGCAGGCCATAACATATGAA TATCCTCCTTAG
Δ3103::Cm [Δ*psr1*]	**F:** CGGATCATGAAAAATATAAAAAGCCAGGGAAATGGCGAAGTGTAGGC TGGAGCTGCTTCG
	R: AGCCAATACTAAAGAAGACGTAGCGCAGCATAATCTCGCCCATATGA ATATCCTCCTTAG
Δ5178::Cm [Δ*psr2*]	**F:** AAATGGCTGAACACAACCATAACCCGACGTGATGCTATTGTGTAGGC TGGAGCTGCTTCG
	R: TTGGCAGCGTCCACAGATTGTAAAACGCAATCCTACCCGCCATATGA ATATCCTCCTTAG
Δ1811::Cm	**F:** GGAGAAAAGCAACAAACAGGCGTTAGCCGCAGGACGTTAGTGTAGGC TGGAGCTGCTTCG
	R: TGACTTTGGCGGGTAGACGCACCTCTCCACGATCGTTAAACATATGAA TATCCTCCTTAG
Plasmids	
p1811~1807	**F:** ATCGAAGCTTGCGCGAACGACACGCAT **R:** ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1809~1807	**F:** ATCGAAGCTTGACCACGGCGCCTGCAT **R:** ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1809~1808	**F:** ATCGAAGCTTGACCACGGCGCCTGCAT **R:** ATCGCTCGAGCATGCCATCCACTTCCCA
p1809	**F:** ATCGAAGCTTGACCACGGCGCCTGCAT **R:** ATCGCTCGAGCCATACTGGGTTGTCATC
p1808	Promoter 1809 part **F:** ATCGAAGCTTGACCACGGCGCCTGCAT **R:** CACTTCCCATAACAGCTCACTATGCTC 1808 part **F:** TAGTGAGCTGTTATGGGAAGTGGATGGCA **R:** ATCGCTCGAGTGCGGGTCGCCCCGCACG
p1808 & 1807	Promoter 1809 part **F:** ATCGAAGCTTGACCACGGCGCCTGCAT **R:** 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:** ATCGAAGCTTGCCTTGCAGGGCGCGCT R: ATCGCTCGAGCATAAACCGGGCAAAAA

Real-time qRT-PCR	
cysJ	**F:** TTCGCAAACCGGCAATGCGC **R:** TCGCCGAGGCTAAACACGGC **Probe:** 6-FAM- ATTCAAACAGATTGCCAGTGA-3BHQ-1
cysK	**F:** TATTGCGCTGGCGTATGTCG **R:** CCGGAGATAAACACATCCACC **Probe:** 6-FAM- TTCAGAAAGCCGAAGAAATTGT-3BHQ-1
cysM	**F:** CGGTGAAAGATCGCGCGGCGC **R:** CAGACATAGCGAGCGCTAAATC **Probe:** 6-FAM- AAGGTTATCGCATGAAGCTGTTGA-3BHQ-1
iscS	**F:** TTGCCGAGAAAATGATGCAGTT **R:** TGTGCTTGCCTTTTTTCTGAT **Probe:** 6-FAM-TTGGGAACCCGGCGTCTCGT-3BHQ-1
mstA	**F:** CGAATATCGCGCCGGACATATTC R: CATTCGTCGCGCTGCCAGCC **Probe:** 6-FAM- AAACACCTGGTCATATACGATGA-3BHQ-1
phsA	**F:** ACGCGCTATCTGGTTTCGTTT **R**: AACGCGGATCGAAGCTCAC **Probe:** 6-FAM- CCTGCGCGGTCATTAACTCATGGG-3BHQ-1
pspE	**F:** CGTTAGCGTTATTCATAGCC **R**: ACCGCCCATATTCATCGCGTG **Probe:** 6-FAM- ATATTCAGGGCGCGATTAATA-3BHQ-1
rpoD	**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- ATGCCTAATTTCGCCGCCACC-3BHQ-1
STM_5178	**F:** TGCCATTGTTGCCTGTCG **R:** CTTTCACACCCATTGTCGT **Probe:** 6-FAM-CTGAATGGCGTCCGTCCACA-3BHQ-1

Site-directed point mutations	
dmsA ΔN66	**F:** TGGAGCGCCTGTACGGTA*TGTGGCAGCCGTTGTCCG **R:** CGGACAACGGCTGCCACA*TACCGTACAGGCGCTCCA
dmsA R106A	**F:** GTTCGCGCCTGCCTG***GCC***GGTCGTTCTATGCGC **R:** GCGCATAGAACGACC***GGC***CAGGCAGGCGCGAAC

dmsA S205G	**F:** TACGGTGATTAC***GGT***TCCGCGCAAATCGCA **R:** TGCGATTTGCGCGGA***ACC***GTAATCACCGTA
1809 ΔN66	**F:** TGGAGTTCATGTACCGTT*TGCGGCAGCCGCTGCCTG **R:** CAGGCAGCGGCTGCCGCA*AACGGTACATGAACTCCA
1809 R105A	**F:** CAGGTACGCGCCTGCCTG***GCC***GGCCGTTCTATTCGT **R:** ACGAATAGAACGGCC***GGC***CAGGCAGGCGCGTACCTG
1809 S199G	**F:** TACGGTAGCTAC***GGC***ACCGCCCAAATC **R:** GATTTGGGCGGT***GCC***GTAGCTACCGTA
3103 S205G	**F:** TACCATTCTGTG***GGC***ATGGGCAACACGGCG **R:** CGCCGTGTTGCCCAT***GCC***CACAGAATGGTA
5178 S198G	**F:** CACAACACCTAT***GGC***ACGGCGCAAATCGCC **R:** GGCGATTTGCGCCGT***GCC***ATAGGTGTTGTG

* Restriction enzyme sites are underlined.

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

*** Deleted sites are marked with an asterisk.

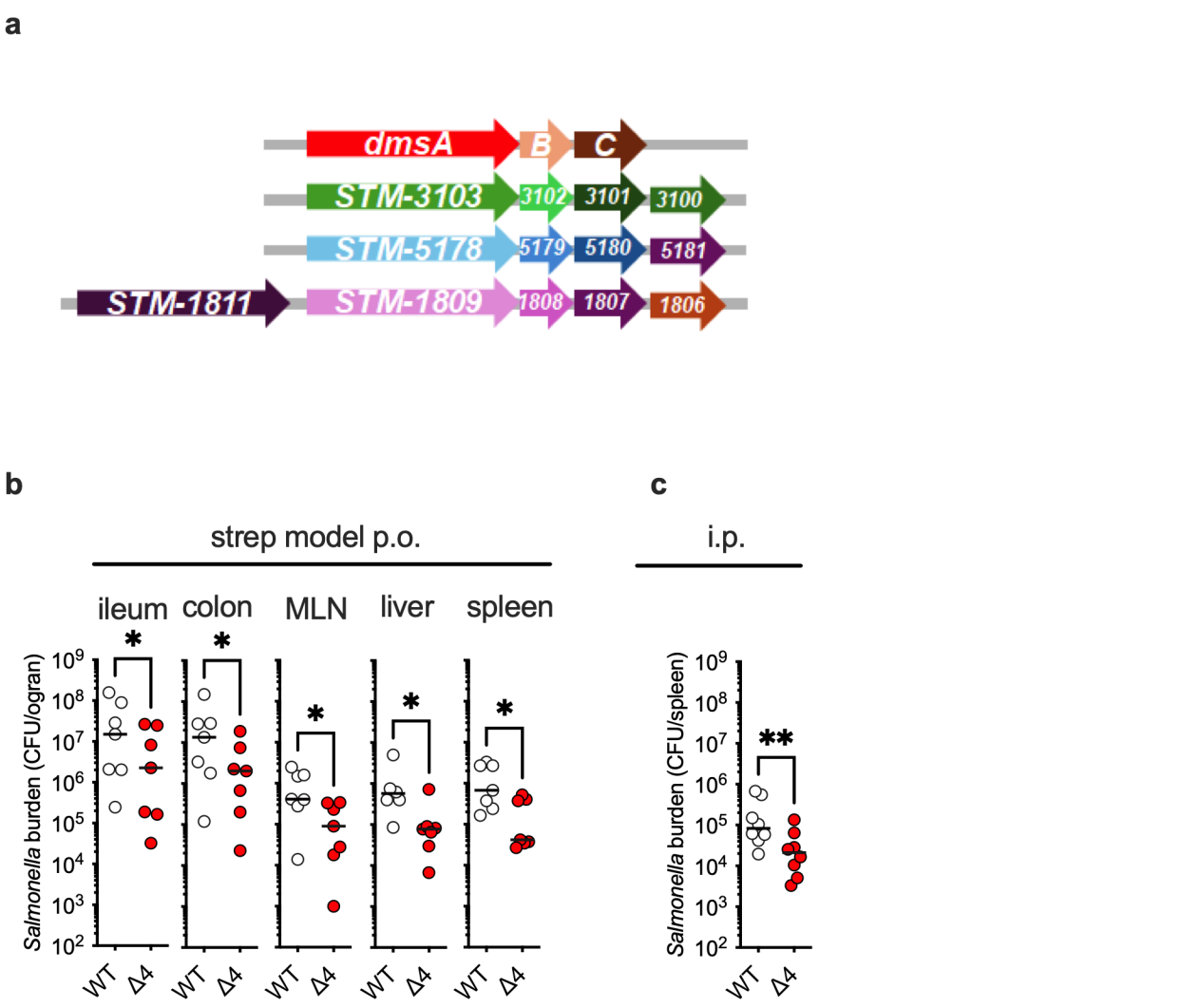

Figure S1. Operon organization of DmsA paralogs and their contribution to *Salmonella* fitness. a. Scheme of *dmsA* paralog operons. **b.** Competition of Δ4 (quadruple mutant in all four dmsA paralogs) and wild-type *Salmonella* in the ileum, colon, MLN, liver and spleen of C57BL/6 mice in a streptomycin-treated 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 ± SD from 7 mice in 2 independent experiments. **c.** Virulence of Δ4 *Salmonella* was assessed by counting bacterial burden in the spleens of C57BL/6 3 days after i.p. inoculation of ~500 CFU mixture of equal numbers of wild-type and mutant *Salmonella*. The data are presented as mean ± SD from 8 mice in 2 individual experiments. *, **, $p < 0.1, 0.01$, respectively, as estimated by paired *t*-test (**b, c**).

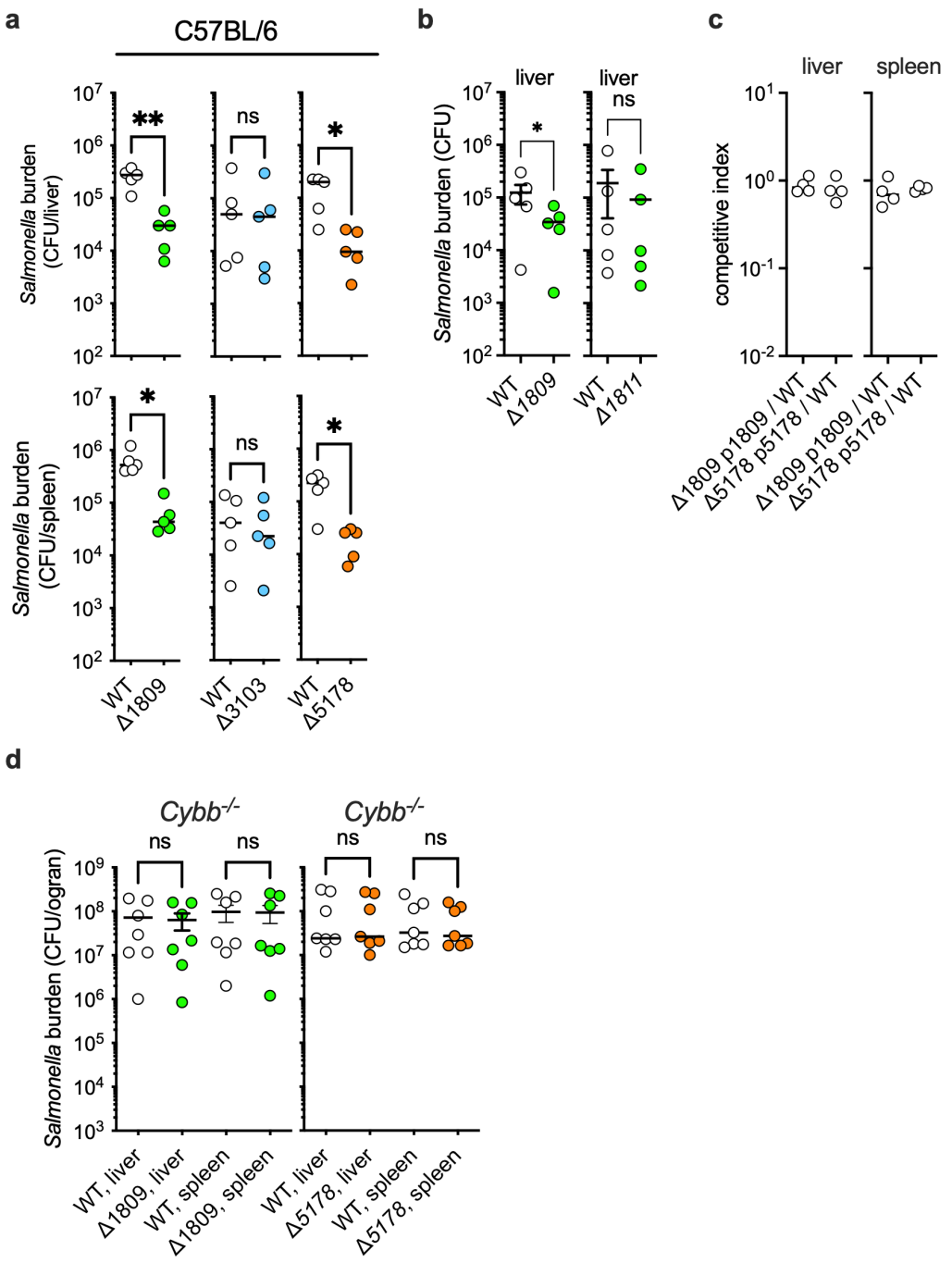

Figure S2. DmsA paralogs enhance *Salmonella* resistance to phagocyte NADPH oxidase-driven oxidative stress. a, b, c, d. The bacterial burden was quantified in the spleens and livers of C57BL/6 mice 3 days after i.p. inoculation with ~300 CFU of a mixture containing equal numbers of wild-type *Salmonella* and isogenic mutants (**a, b, d**). Competitive index (**c**) was calculated 3 days after i.p. infection. Data represent mean ± 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**).

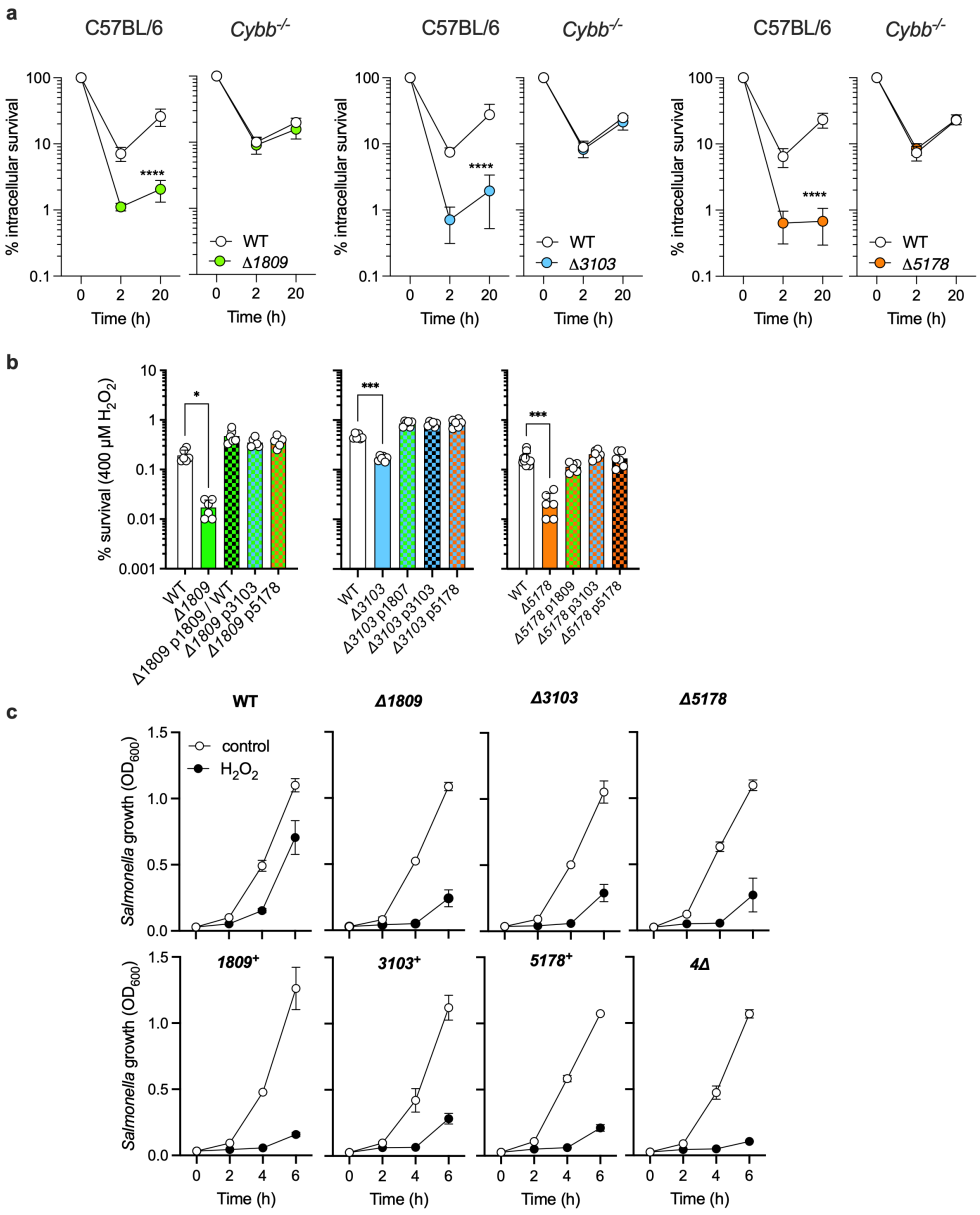

Figure S3. Susceptibility of *dmsA* paralog mutants to oxidative stress. a. The percent survival of single *dmsA* paralog deletion mutants was assessed in periodate-elicited macrophages from C57BL/6 and *Cybb*$^{-/-}$ mice. CFU were enumerated at 0, 2, and 20 h post-infection to determine bacterial replication. The data are mean ± SD from 2 independent experiments (C57BL/6, n=7; *Cybb*$^{-/-}$, n=10). **b.** Single *dmsA* paralog deletion mutants were cultured aerobically in LB broth at 37°C and challenged with 400 μM H_2O_2 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 ± 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_{600}, of *dmsA* paralog mutants in MOPS-GLC-sulfate minimal medium supplemented with 400 μM H_2O_2. The strains carried single (e.g., Δ1809) or quadruple (i.e., Δ4) mutations in *dmsA* paralogs. Strains expressing single *dmsA* paralogs (e.g., 1809$^+$) were constructed by complementing the Δ4 strain

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

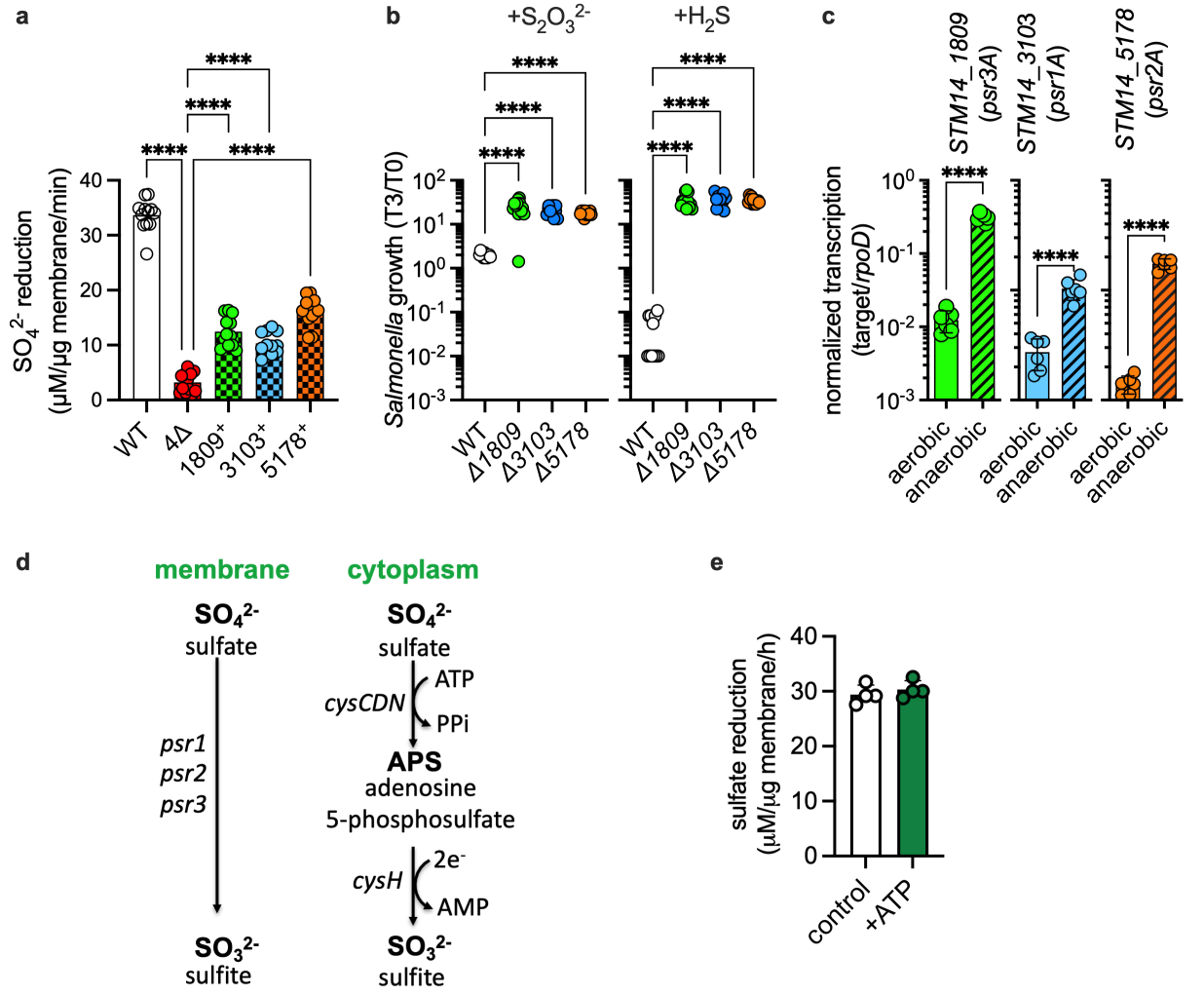

Figure S4. DmsA paralogs function as membrane-associated sulfate reductases, indirectly facilitating sulfur acquisition to support the growth of *Salmonella*. a. Sulfate reductase activities were measured in membranes isolated from the indicated strains. The strains carrried quadruple (i.e., Δ4) mutations in *dmsA* paralogs. Strains expressing single *dmsA* paralogs (e.g., 1809^{+}) 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 ± 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 ($Na_2S_2O_3$), or sodium sulfide (Na_2S). 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 ± 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 ± 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 ± 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.

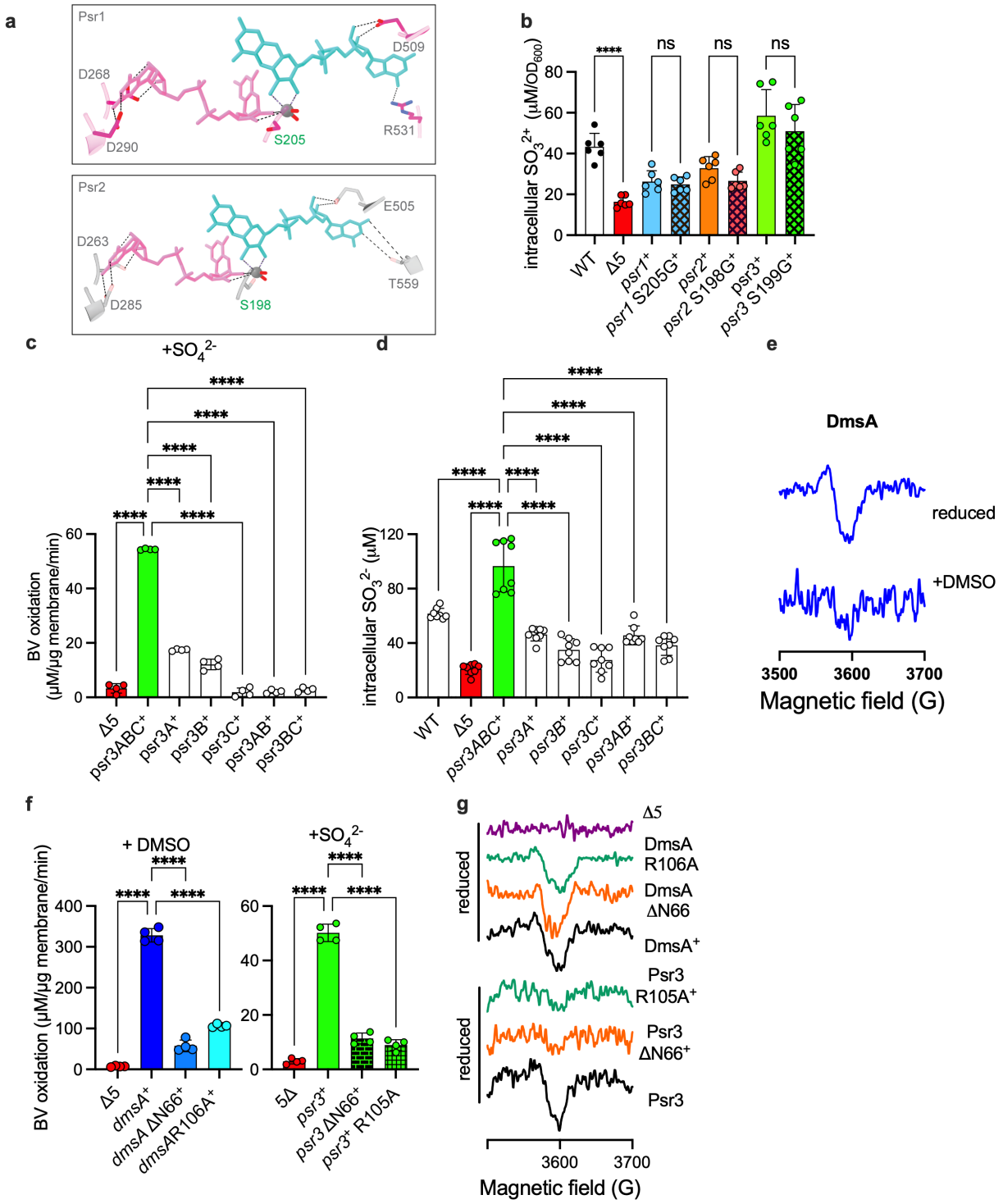

Figure S6. The function of periplasmic sulfate reductase in *Salmonella* is dependent on the FS0 [4Fe-4S], but not molybdenum, cofactor. a. The predicted Mo-bis PGD in Psr1A and Psr2A as determined by AlphaFold3. **b, d.** Intracellular sulfite concentrations were measured in wild-type *Salmonella*, the 5Δ 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 ± 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 Δ5 mutant and its complemented strains expressing individual genes, gene pairs, or the entire *psr3ABC* operon. Data presents mean ± 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, $+SO_4^{2-}$). The data are presented as mean ± 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**).

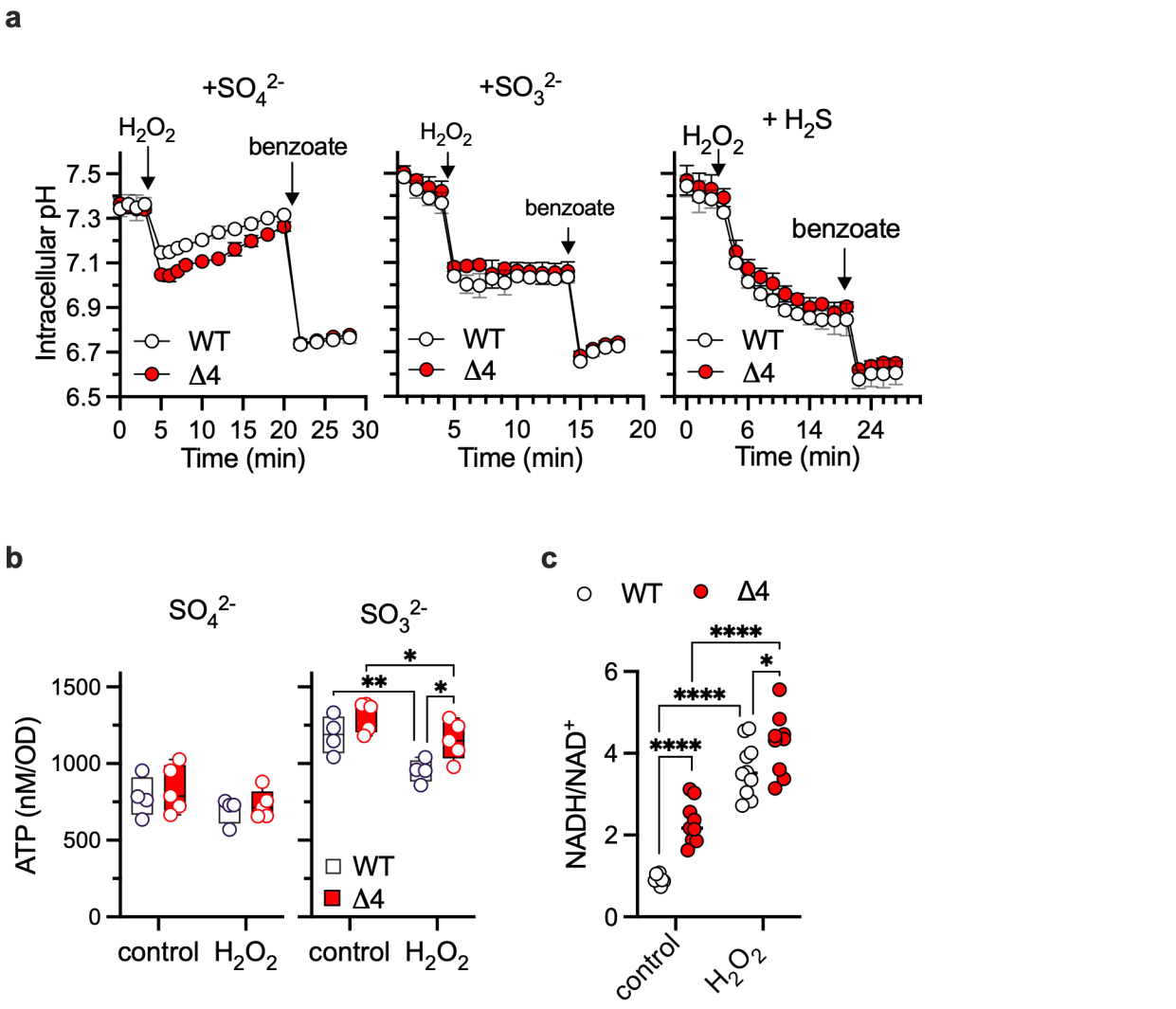

Figure S7. Effect of periplasmic sulfate reductases in the sulfate assimilatory pathway. a. Intracellular pH of exponential-phase *Salmonella* cultured in MOPS-GLC minimal medium either 250 μM sulfate, 250 μM sulfite, or 100 μM sulfide was measured using ratiometric pH-sensitive reporter pHluorin. Where indicated, the specimens were treated with 400 μM H_2O_2 or 40 mM of the protonophore benzoate. The data are mean ± 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 ± SD from 2 individual experiments (WT n=4, Δ4 n=5). **c.** Intracellular NADH / NAD^+ ratios were quantified in *Salmonella* grown aerobically in MOPS-GLC minimal medium supplemented with sulfate. The data are the mean ± SD from 2 individual experiments (WT control n=8; WT H_2O_2 n=10; Δ4 n=9). *, **, **** p <0.1, 0.01, 0.0001, respectively, as estimated by one-way ANOVA (**b, c**).

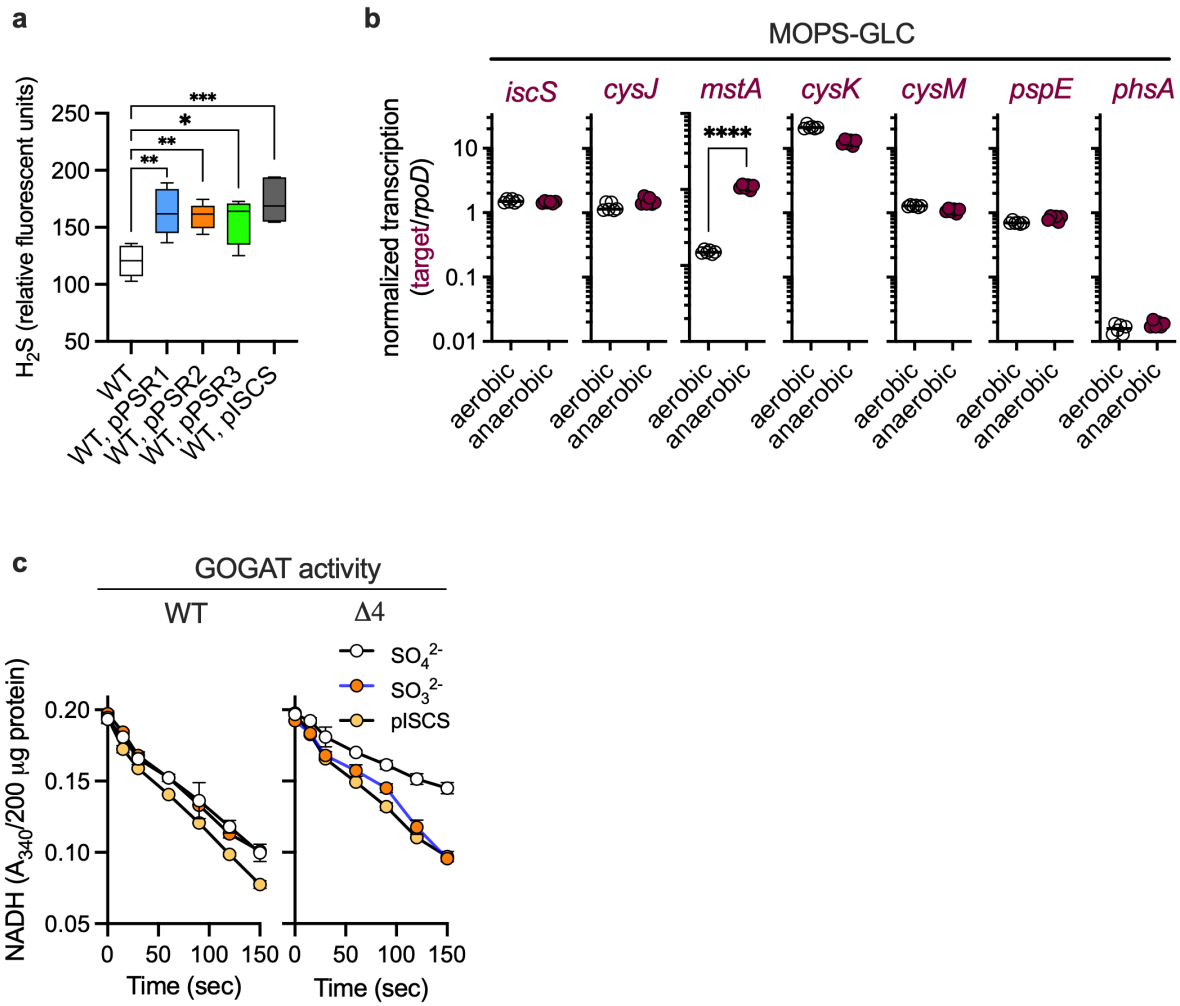

Figure S8. Periplasmic sulfate reductases are involved in the assimilation of sulfur. a, Hydrogen sulfide (H_2S) production was evaluated in wild-type *Salmonella* harboring each individual periplasmic sulfate reductase operon (*psr1*, *psr2* and *psr3*) or the *iscS* gene in pWSK29. Cells were cultured in MOPS-GLC minimal medium containing sulfate. H_2S was detected using the WSP5 fluorescent probe. The data are the mean ± 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 ± 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 ± SD; n=3 from 2 independent experiments.

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