Cell Reports, Volume *22*

Supplemental Information

Mechanisms to Evade the Phagocyte Respiratory Burst Arose by Convergent Evolution in Typhoidal *Salmonella* Serovars

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SUPPLEMENTARY DATA

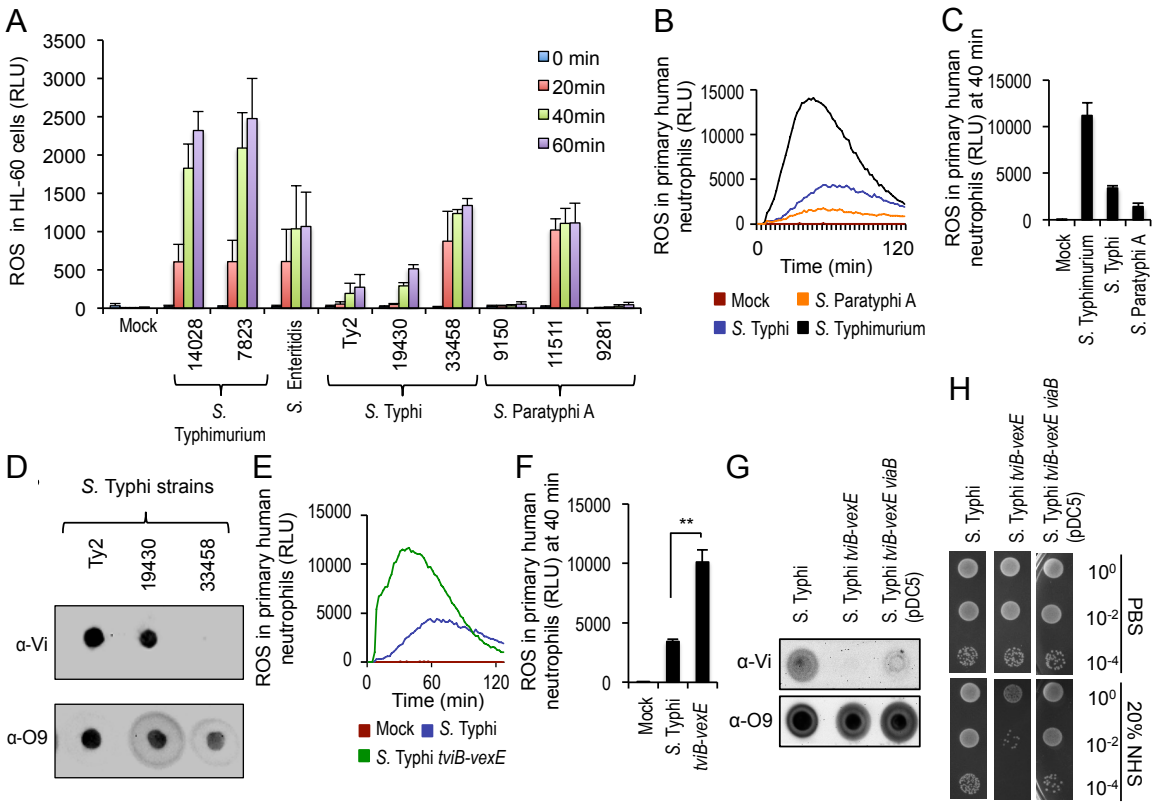

Figure S1: Activation of the phagocyte respiratory burst by typhoidal and non-typhoidal *Salmonella* serovars, related to Figure 1.

Human neutrophil-like (HL60) cells (A) or human primary neutrophils (B, C, E and F) were infected with the indicated opsonized bacterial strains and ROS production measured at the indicated time points using chemiluminescence (N = 3). (B and E) Representative experiment showing generation of chemiluminescence over time. (C and F) Quantification of chemiluminescence at the indicated time point after infection. (D and G) The indicated bacterial strains were transferred onto a dot blot to detect production of the Vi-antigen (α-Vi; top panel) or production of the O9-antigen (α-O9; bottom panel). (H) Viability of the indicated bacterial strains incubated in PBS or 20% NHS was determined by spotting serial dilutions on agar plates. **, $P < 0.01$.

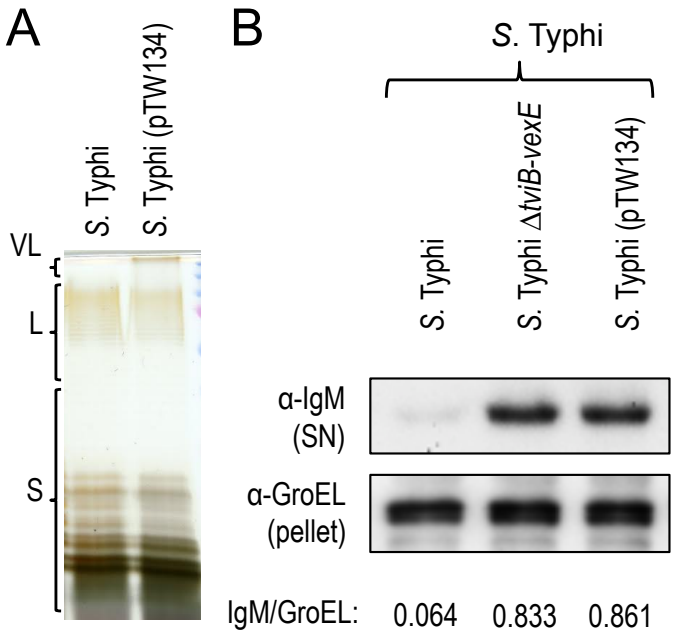

Figure S2: Very-long O-antigen chains overcome capsule-mediated inhibition of IgM binding to the surface of *S.* Typhi, related to Figure 2.

(A) LPS purified from the indicated bacterial strains was separated by SDS-PAGE and silver stained. VL, very-long O-antigen chains; L, long O-antigen chains; S, short O-antigen chains. (B) The indicated bacterial strains were incubated in human serum and surface bound proteins eluted with glycine. IgM eluted from the bacterial surface was detected by Western blot (α-IgM; top panel). The bacterial protein GroEL was detected in the bacterial pellet (α-GroEL; bottom panel) and used as a loading control by measuring the IgM/GroEL using densitometry.

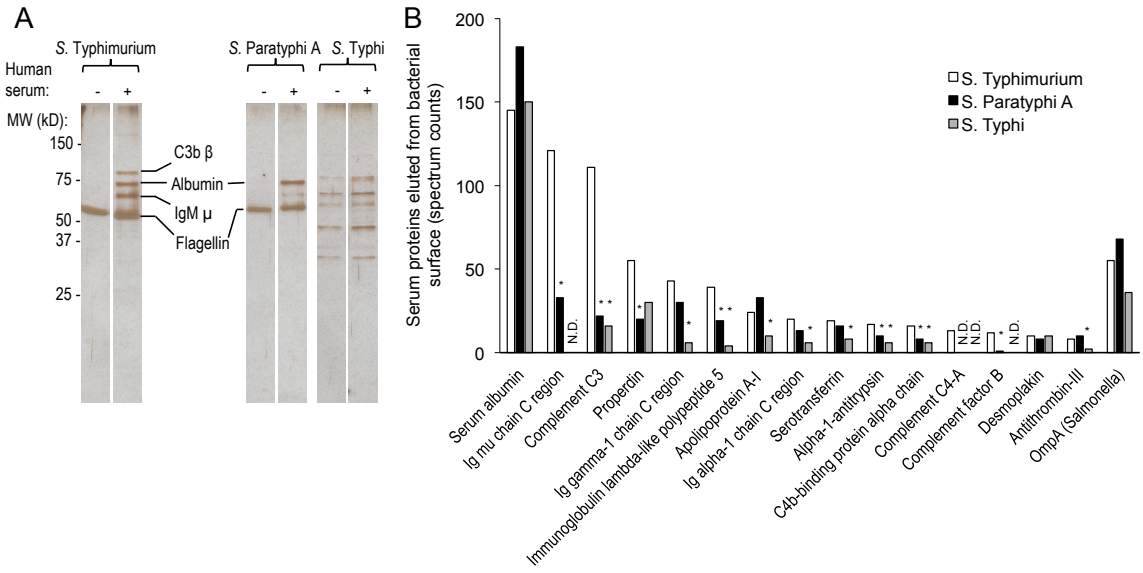

Figure S3: Binding of serum proteins to the surface of *S.* Typhimurium and *S.* Paratyphi A, related to Figure 3.

The indicated bacterial strains were incubated in human serum and surface bound proteins eluted with glycine. (A) Proteins eluted from the bacterial surface were separated by SDS-PAGE and silver stained. Molecular weight markers (MW) are shown on the left. (B) Proteins eluted from the bacterial surface were identified by MS analysis. *, eluted proteins exhibiting a more than 2-fold change in abundance compared to *S.* Typhimurium; N.D., not detected.

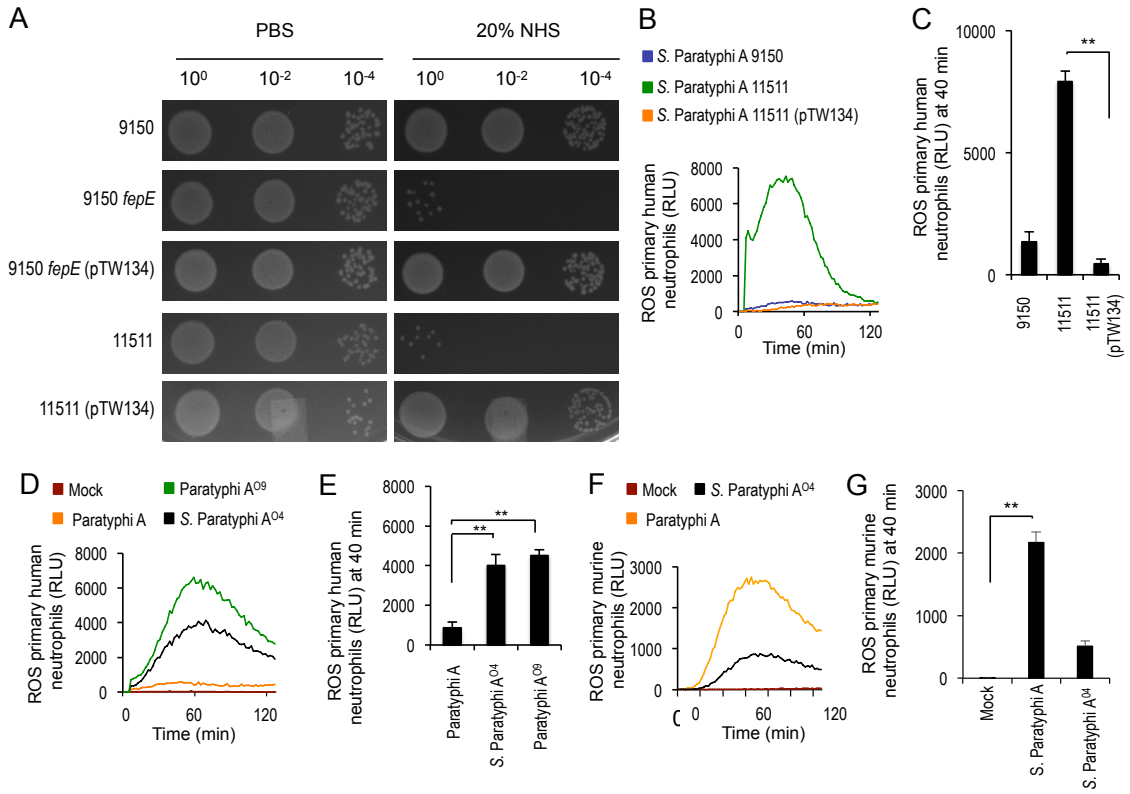

Figure S4: Very-long O-antigen chains mediate serum resistance in *S.* Paratyphi A, related to Figures 3 and 5.

(A) Viability of the indicated *S.* Paratyphi A strains was determined after incubation in buffer (PBS) or 20% normal human serum (NHS) by spotting serial dilutions on agar plates. Human primary neutrophils (B, C, D and E) or murine primary neutrophils (F and G) were infected with the indicated bacterial strains opsonized with human serum (B, C, D and E) or serum from germ-free mice (F and G) and ROS production measured at the indicated time points using chemiluminescence (N = 3). (B, D and F) Representative experiment showing generation of chemiluminescence over time. (C, E and G) Quantification of chemiluminescence at the indicated time point after infection. **, $P < 0.01$.

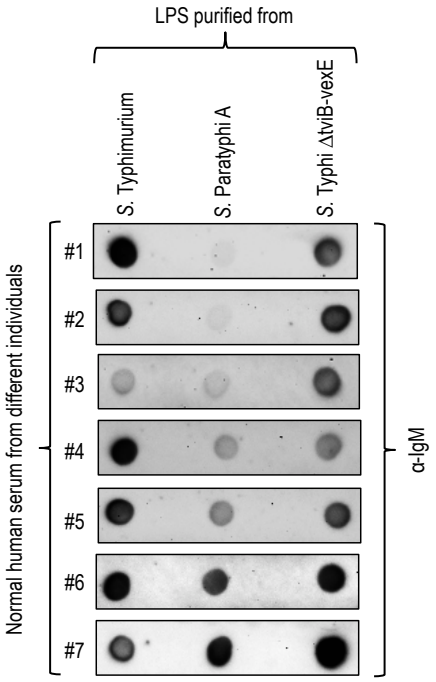

Figure S5: LPS from *S.* Paratyphi A prevents IgM binding in a subset of normal human serum samples, related to Figure 2.

LPS purified from the indicated bacterial strains was loaded on a dot blot to detect binding of IgM present in normal human serum (α-IgM) from seven individuals (#1 - #7).

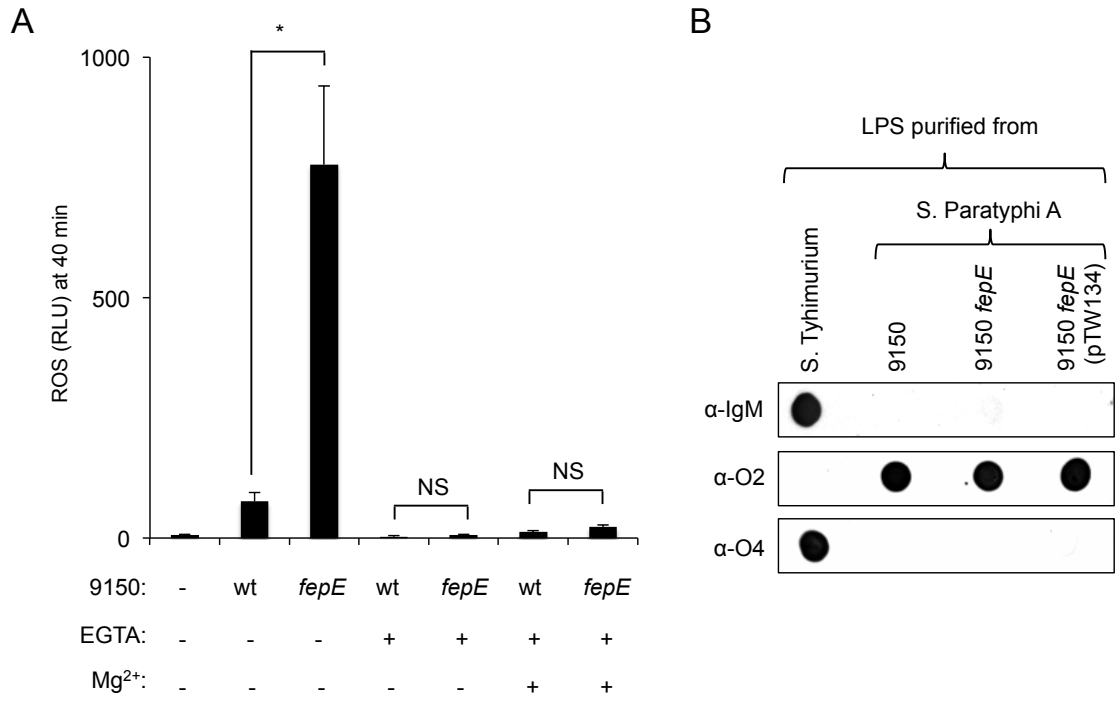

Figure S6: Lack of very-long O-antigen chains in *S.* Paratyphi A activates the respiratory burst in neutrophil-like cells through antibody-mediated complement activation, related to Figure 6.

(A) Human neutrophil-like (HL60) cells were infected with the indicated opsonized *S.* Paratyphi A strains and ROS production monitored over time using chemiluminescence. Chemiluminescence was quantified from three independent experiments at the indicated time point after infection. (B) LPS purified from the indicated bacterial strains was loaded on a dot blot to detect binding of IgM present in normal human serum (α-IgM). *, $P < 0.05$; NS, not statistically significantly different.

Supplementary table 1: Bacterial strains and plasmids used in this study

Strain designation	Relevant characterstics/Genotype	Source/Reference
S. Paratyphi A		
ATCC9150	Wild type	American type culture collection (ATCC)
ATCC11511	var. Durazzo wild type	(Kauffmann and Silberstein, 1934)
ATCC9281	Wild type	ATCC
TWS110	9150 *ΔrfbSE::rfbJ*^{STM}	This study
TWS159	9150 *ΔfepE*::Km^{R}	This study
HH121	9150*ΔrfbE*::Km^{R}	This study
HH122	9150*ΔrfbE::rfbE*^{STY}	This study
S. Typhi		
Ty2	wild-type strain, Vi+	ATCC
ATCC19430	wild-type strain, Vi+	ATCC
ATCC33458	wild-type strain, Vi-	ATCC
SW74	Ty2 *ΔtviB-vexE*::Cm^{R}	(Winter et al., 2008)
S. Enteritidis		
AJB72	CDC SSU7998 Nal^{R}, wild type	(Norris and Baumler, 1999)
S. Typhimurium		
IR715	ATCC14028 Nal^{R}, wild type	(Stojiljkovic et al., 1995)
ATCC7823	Wild type	ATCC
E. coli		
S17*λpir*	C600::RP4 2-(Tet::Mu) (Km::Tn7) *λpir*	(Simon et al., 1983)

Plasmid		
pTW76	pRDH10::*rfbHJ*^{STM}:*rfbX*^{SPA}	This study
pTW93	Up-/downstream region of *rfbE* from SPA 9150 in pRDH10	This study
pTW117	KSAC cassette flanked by Up-/downstream region of *fepE* from SPA9150 in pRDH10; Cm^R Km^R	This study
pTW126	Up-/downstream region of *rfbE* from SPA 9150 in pRDH10	This study
pTW128	*fepE* $^{SPA\ 11511}$ in PCR2.1 $Carb^R$	This study
pTW131	KSAC cassette flanked by Up-/downstream region of *rfbE* from SPA9150 in pRDH10; Cm^R Km^R	This study
pTW134	*fepE*^{SPA9150} in pWSK29; $Carb^R$	This study
pRDH10	ori(R6K) *mobRP4 sacRB* Tet^R CmR	(Kingsley et al., 1999)
pWSK29	ori(pSC101) $Carb^R$	(Wang and Kushner, 1991)
pSW172	ori(R101) *repA*101ts $Carb^R$	(Winter et al., 2013)
pDC5	ori(pSC101) $Carb^R$, carrying cloned *viaB* locus	(Raffatellu et al., 2007)

Supplementary Table 2: Primers

Designation	Purpose	Sequence (5'-3')
83	Amplification of *S.* Typhimurium *rfbHJ*	GCATAAGGGAGAGCGATGACAGCAAATAACCTGCGTG
84		GCAGAAACATCATAACCGTTTCAGTAGTTCTTCAATTC
85	Amplification of *S.* Paratyphi A 9150 *rfbX*	CGGTTATGATGTTTCTGCCCGCGAAAG
86		TCTCAAGGGCATCGGTTATGCTAGCCTTTTACTCTTATACATATAATACACTG
87	Amplification of *S.* Paratyphi A 9150 *rfbSEX* in combination with primer 86	GCATAAGGGAGAGCGATGAAAATTCTAATAATGGGAGCGTTTGG
133	Amplification of *S.* Paratyphi A 9150 *fepE* flanking region 1 (upstream)	CACACCCGTCCTGTGTGGCCGCGCAGCTAAGCC
134		CGGGTAAAGGATCCGCCGTATGACCTGAAAACTTTATCCAATG
135	Amplification of *S.* Paratyphi A 9150 *fepE* flanking region 2 (downstream)	CATACGGCGGATCCTTTACCCGAAAAGCCGGATAGCG
136		GCGTCCGGCGTAGAGCCAGTCGGCGCTGTGCGG
137	Amplification of *S.* Paratyphi A 11511 *fepE*	GCCTTATTTACCGCCATTGA
138		GCGCCGAAAGAGATTGTTAC
171	Amplification of *S.* Paratyphi A 9150 *rfbE* flanking region 1 (upstream)	GCGACCACACCCGTCCTGTGATGAAAATTCTAATAATGGGAGC
172		GCAGAAACATGGATCCTCATTTCCCTTCCTCTTC
173	Amplification of *S.* Paratyphi A 9150 *rfbE* flanking region 2 (downstream)	AGGGAAATGAGGATCCATGTTTCTGCCCGCGAAAG
174		ACGATGCGTCCGGCGTAGAGTTATGCTAGCCTTTTACTCTTATACATATAATAC
179	Amplification of *S.* Paratyphi A 9150 *fepE*	GGCCCCCCCTCGAGGGGATCCCACTGGCGCGTAAAGATTG
180		CGCTCTAGAACTAGTGGTCGACTCAGACTAACCGTTCATCTATCG

References for supplementary tables

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