Figure S1

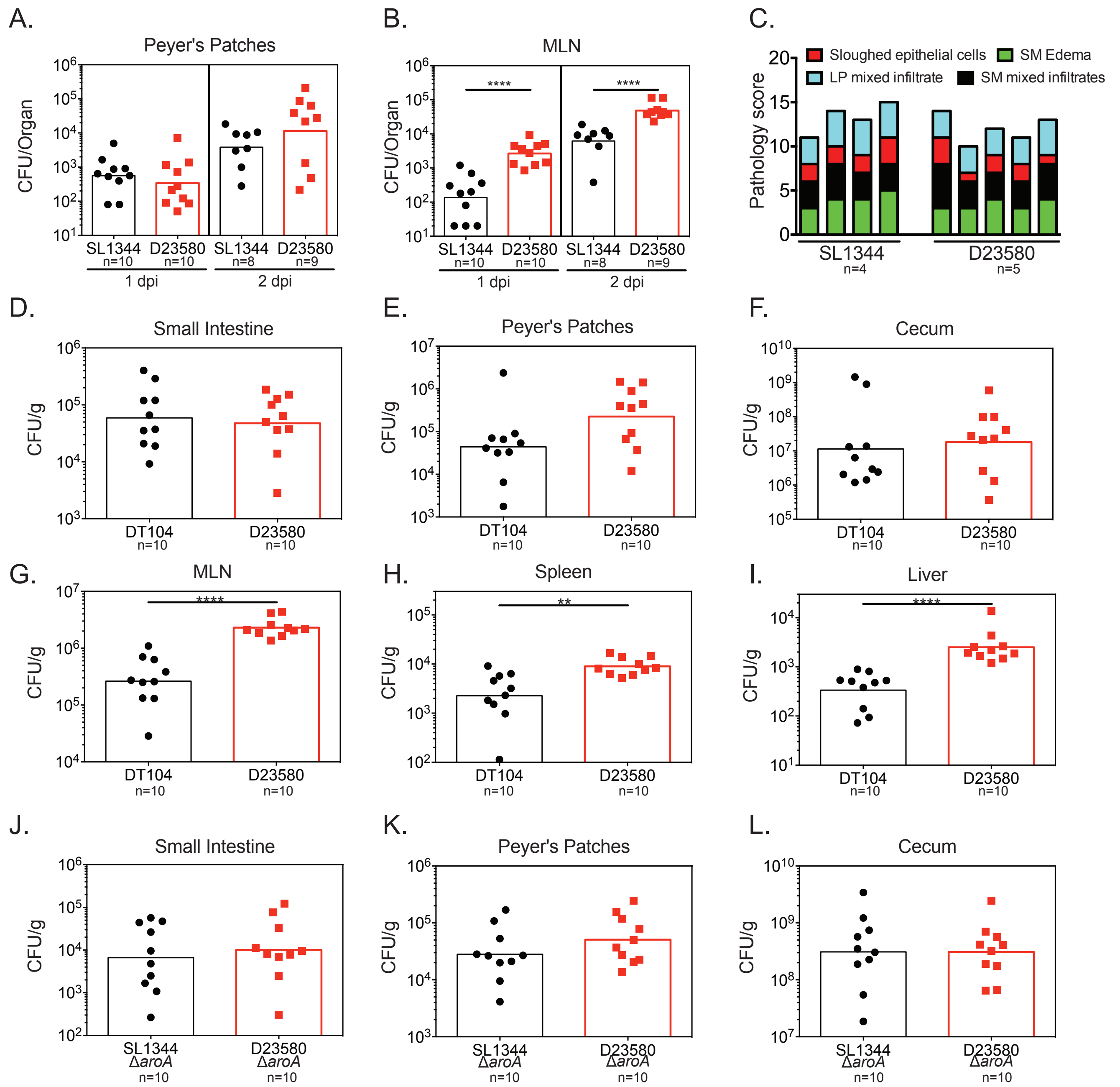


Figure S2

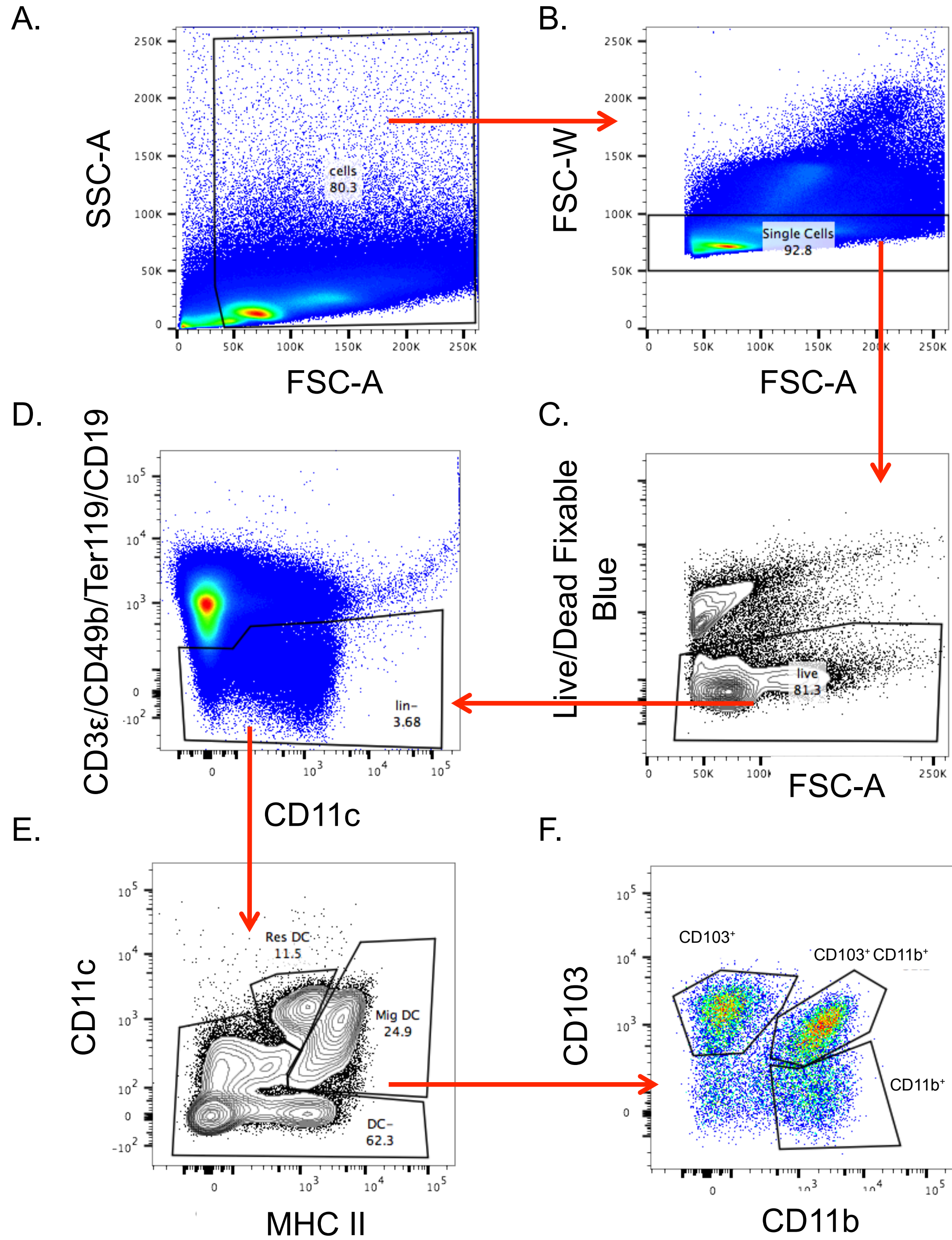


Figure S3

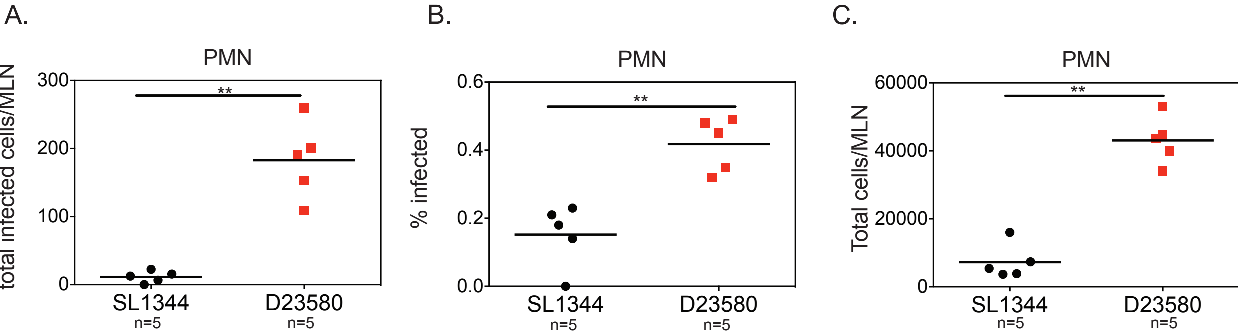


Figure S4

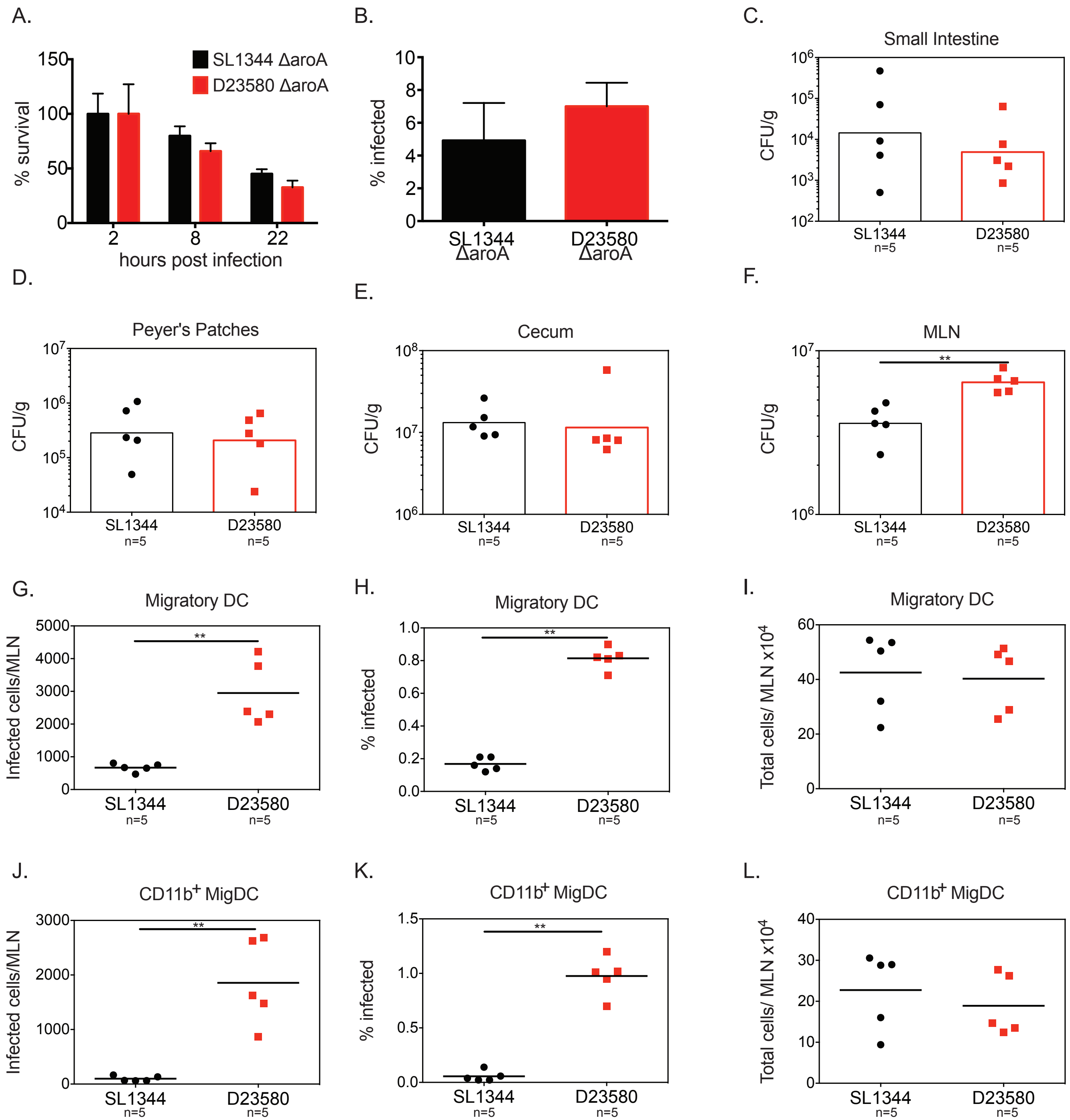


Figure S5

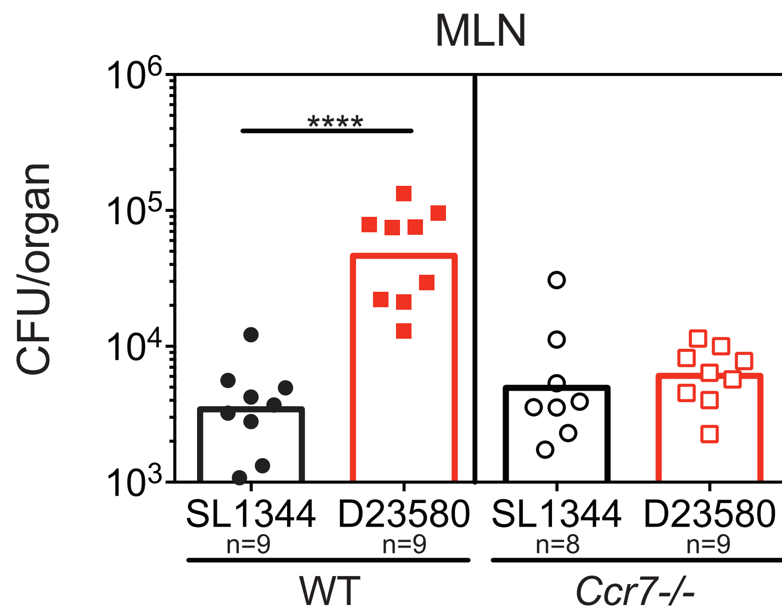
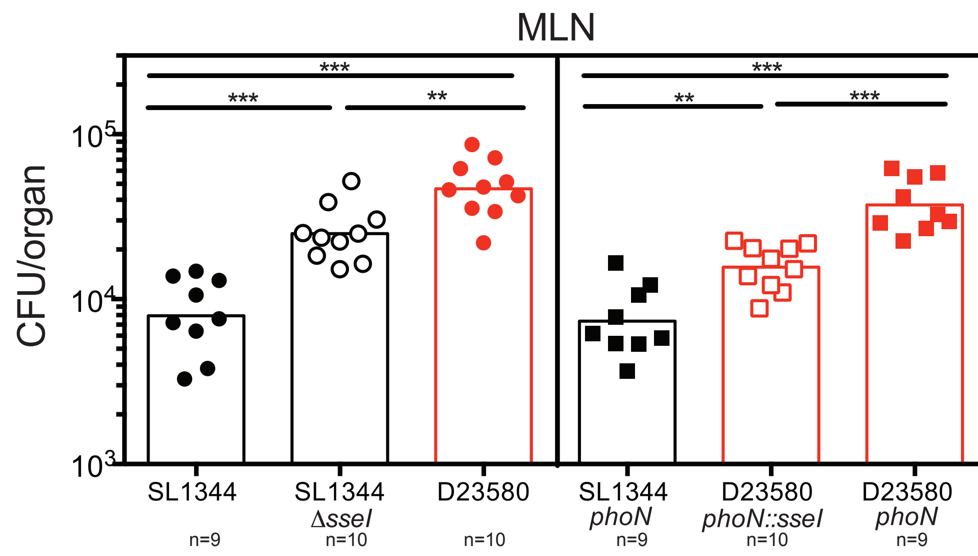


Figure S6



Supplemental Figure Legends

Figure S1, related to Figure 1: D23580 hyperdisseminates to systemic sites compared to gastroenteritis outbreak isolate DT104

(A-B) WT C57BL/6 mice were given 20 mg of streptomycin orogastrically (o.g) at –d1. The next day mice were infected with 10^8 CFU of either ST19 SL1344 or ST313 D23580 o.g. Mice were sacrificed at 1 and 2 days post infection (dpi), and *Salmonella* burden was enumerated by plating. The CFU/organ for Peyer's patches (A) and MLN (B) are presented. Data is the combination of two independent experiments with 4-5 mice per group. Geometric mean is presented for each group. **(C)** Mice were infected as in **A** and sacrificed at 2 dpi. The cecal tip was removed and prepared for histopathological analysis. Hematoxylin and eosin stained sections were evaluated by a blinded pathologist and the cumulative pathology score is presented. Data from a representative experiment is presented. Each bar represents a single mouse. Two independent experiments were performed with 4-5 mice per group. **(D-I)** WT C57BL/6 mice were given 20 mg of streptomycin o.g at –d1. The next day mice were infected with 10^8 CFU of either ST19 DT104 or ST313 D23580 o.g. Mice were sacrificed at 2 dpi and *Salmonella* burden was enumerated by plating. CFU/g for gut tissues (D-F) and systemic sites (G-I) are presented. Data is the combination of two independent experiments with 4-5 mice per group. Geometric mean is presented for each group. **(J-L)** WT C57BL/6 mice were given 20 mg of streptomycin o.g at –d1. The next day mice were infected with 10^9 CFU of either ST19 SL1344 Δ*aroA* or ST313 D23580 Δ*aroA* o.g. Mice were sacrificed at 2 dpi, and *Salmonella* burden was enumerated by plating. CFU/g for gut tissues are

24 presented (J-L). Data is the combination of two independent experiments with 4-5 mice

25 per group. Geometric mean is presented for each group. **(A-L)** Statistical significance

26 was determined using Mann-Whitney. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ****

27 $P \leq 0.0001$.

28

29 **Figure S2, related to Figure 2: Gating strategy for MLN migratory dendritic cells**

30 Cells were first gated based on size (A), then for single cells (B) Next live cells were

31 selected by gating on those cells negative for Live/Dead Fixable Blue, a stain marking

32 dead cells (C). Then B cells, T cells, NK cells, and red blood cells were excluded (D).

33 Migratory dendritic cells were then identified by variable CD11c expression and high

34 MHC II expression (E). Migratory dendritic cell subsets were then identified on the basis

35 of their expression of the integrins CD103 and CD11b (F).

36

37 **Figure S3, related to Figure 2: D23580 infects more PMN in the MLN**

38 **(A-C)** WT C57BL/6 were given 20 mg of streptomycin o.g at –d1. The next day mice

39 were infected with 10^8 CFU of each respective *Salmonella* isolate o.g. Mice were

40 sacrificed at 2 days post infection. MLN were removed and digested to form single cell

41 suspensions which were stained for cell surface markers and Salmonella and analyzed by

42 flow cytometry. Total infected PMN per MLN (A), Percent infected PMN (B) and total

43 number of PMN per MLN (C) are presented. Data shown is from representative

44 experiment with 5 mice per group out of 2 independent experiments. The line indicates

45 the mean and statistical significance was determined using Mann-Whitney. * $P \leq 0.05$, **

46 $P \leq 0.01$.

47 **Figure S4, related to Figures 2&3: Differences in *Salmonella* survival or**
48 **inflammasome activation do not mediate higher *Salmonella* burden in the MLN for**
49 **D23580**

50 **(A)** Bacterial survival was quantified over time. Differentiated bone marrow-derived
51 DCs (BMDCs) were seeded overnight at 2.5×10^5 cells/well in 24 well plates. The
52 indicated *Salmonella* strains were centrifuged onto the BMDCs at a multiplicity of
53 infection of 10 and allowed to infect for 30 min. Gentamicin was then added to BMDC
54 media to a final concentration of 100 μg/ml for 1.5 hrs. At 2 hours post infection (hpi)
55 either intracellular bacteria was enumerated or BMDCs were washed, and given BMDC
56 media containing 10 μg/ml gentamicin. Intracellular CFU was determined at 2, 8 and 22
57 hpi. To evaluate intracellular CFU, BMDCs were washed, lysed, and bacteria were
58 enumerated by plating. The percent survival is normalized to the amount of intracellular
59 bacteria at 2 hpi. **(B)** BMDCs were infected and the number of intracellular bacteria was
60 determined at 2 hpi as in **A**. The percent invasion, quantified as the percent of
61 intracellular bacteria over the initial input, is presented in **B. (A-B)** Bars represent the
62 mean and standard deviation for each isolate. Experiments were repeated at least 5 times,
63 and data shown is from a representative experiment with at least 3 biological replicates
64 per time point. Statistical significance was determined using an unpaired t test with
65 Welch's correction. * $P \leq 0.05$. **(C-L)** Streptomycin-pretreated *Casp1/11*$^{-/-}$ mice were
66 orally infected with each *Salmonella* isolate. Mice were sacrificed at 2 dpi, and
67 *Salmonella* burden was enumerated by plating (C-F). The CFU/g for gut tissues (C-E)
68 and MLN (F) are presented. **(G-L)** Single cell suspensions of the MLN were stained for
69 cell surface markers and intracellular *Salmonella*. The total amount of infected cells per

70 MLN (G,J), percent infected (H,K) and total number of cells recruited to the MLN (I, L)
71 are shown for all migDC (G-I) and the CD11b+ migDC subset (J-L), respectively. **(C-L)**
72 Data presented are from a representative experiment. Two independent experiments with
73 3-5 mice per group were performed. The geometric mean for each group is shown and
74 statistical significance was determined by the Mann-Whitney test. * P≤0.05, ** P≤0.01.
75
76 **Figure S5, related to Figure 5: CCR7-dependent cell migration is necessary for**
77 **hyperdissemination by ST313 *S.* Typhimurium to the MLN**
78 Streptomycin-pretreated WT C57BL/6 or *Ccr7-/-* mice were orally infected with each
79 *Salmonella* isolate. Mice were sacrificed at 2 dpi and *Salmonella* burden was enumerated
80 by plating. The CFU/organ for the MLN is presented. Data presented are the combination
81 of 2 independent experiments with 4-5 mice per group. The geometric mean is shown and
82 statistical significance was determined by the Mann-Whitney test. * P≤0.05, ** P≤0.01.
83
84 **Figure S6, related to Figure 6: The presence on an intact *sseI* gene lowers the total**
85 **burden in the MLN**
86 Streptomycin-pretreated C57BL/6 mice were orally infected with each respective
87 *Salmonella* strain. To genetically dissect the effect of pseudogenization of *sseI*, a clean
88 deletion of *sseI* was made in ST19 background and D23580 was complemented with a
89 functional copy of SseI. Mice were sacrificed 2 dpi, and *Salmonella* burden was
90 enumerated by plating. CFU/organ for the MLN of each strain is shown. Data presented
91 are the combination of 2 independent experiments with 4-5 mice per group. The

92 geometric mean for each group is shown and statistical significance was determined by

93 the Mann-Whitney test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

94

95

96

97

98

99

100

101

102
103
104

105
106

Table S1: Summary of studies examining relative pathogenesis of ST19 and ST313 *S.* Typhimurium isolates in animal models (related to Figure 1)

Animal	Infection Model	Results at Systemic sites	Authors
Balb/C mice	Single infections with ST19 SL1344 or ST313 D23580	ST313 D23580 had a lower LD50 than that reported for ST19 isolates. ST313 D23580 had higher levels in the spleen at 3 and 5 dpi	Yang et al., 2015
CBA/J mice	Streptomycin pre-treated mice were coinfected with a 1:1 mixture of ST19 isolate IR715 and ST313 isolate D23580 by oral gavage	ST313 D23580 outcompeted ST19 IR715 in the MLN at 1 dpi and in the spleen and liver at 4 dpi	Singletary et al., 2016
C57BL/6 mice	Mice were singly infected with ST19 isolates SL1344 and various ST313 isolates by oral gavage	Trend towards most ST313 isolates having a higher load in the liver at 4 dpi than ST19 isolate SL1344	Okoro et al., 2015
Chicken	2-week old chicks were infected orally with single isolates of ST19 and ST313	One ST313 isolate, D23580, had higher levels in the spleen than two ST19 isolates at 3 and 7 dpi	Parsons et al., 2013

Table S2: *S.* Typhimurium and *E. coli* stains and plasmids used in this study (related to *Bacterial strains and plasmids* section in *Experimental Procedures*)

***S.* Typhimurium stains used in this study**		
Strain Name	Genotype	Reference
SL1344	ST19, common laboratory *S.* Typhimurium strain	(Hoiseth and Stocker, 1981)
D23580	ST313, isolated from patient's blood in 2004, in Malawi	(Kingsley et al., 2009)
DT104	ST19, epidemic gastroenteritis strain,	ATCC 700408
SL1344 Δ*aroA*	ST19, SL1344 Δ*aroA::kan*	This study
D23580 Δ*aroA*	ST313, D23580 Δ*aroA::kan*	This study
SL1344 ΔsseI	ST19, SL1344 *ΔsseI::kan*	(McLaughlin et al., 2009)
SL1344 *phoN::kan*	ST19, SL1344 *ΔphoN::kan*	This study
D23580 *phoN::kan*	ST313, D23580 *ΔphoN::kan*	This study
D23580 *phoN::sseI*	ST313, D23580 *ΔphoN::sseI-kan*	This study
***E. coli* strains used in this study**		
Strain Name	Genotype	Reference
GTW230	*E. coli* S17-1 λ*pir* with plasmid pGW6, which has an Amp^R cassette. The plasmid also contains *sseI* and a Kan^R cassette between flanking regions of the *phoN* gene.	This study
GTW234	*E. coli* S17-1 λ*pir* with plasmid pGW10 which has an Amp^R cassette as well as a Kan^R cassette between flanking regions of the *phoN* gene	This study
Plasmids used in this study		
Plasmid Name	Plasmid Description	Reference
p*sseI*	*sseI* from *S.* Typhimurium SL1344 cloned into p*ACYC184*	(McLaughlin et al., 2009)
pUC4-KSAC	Plasmid containing kanamycin cassette KSAC	(Barany, 1985)
pSW85	*phoN* flanking regions cloned into pGP704	(Haneda et al., 2009)
pGW1	pSW85 with sseI_460bp upstream inserted between phoN flanking regions I+II(flr I+II), using SalI and SacI restriction sites	This study
pGW6	Kan^R cassette from pUC4-KSAC inserted into SacI site between sseI and flr II on pGW1	This study
pGW10	Kan^R cassette from pUC4-KSAC inserted into SacI site between flr I and flr II on pSW85	This study

Table S3: Primers used in this study (related to *Bacterial strains and plasmids* section in *Experimental Procedures)*

Name	Primer Sequence 5’ to 3’
AroA deletion	5’-TGACGTTACAACCCATCGCGCGGGTCGATGGCGCCATTAAGTGTAGGCTGGAGCTGCTTC 5’-CCTTTCGCAAACAGCGCCGTGGTGGCAATCGTCATCGCCGCATATGAATATCCTCCTTAG
AroA test	5’-ATCAGCAAAAAGCGGAGTTG 5’-GAGTATACGCGAACGCAACC
PhoN	5’-CGCACCACTATTCAAAGCCG 5’-GCGTAAAACCATGCAGACCC
sseI region on p*sseI*	5’-CTAGAACTAGTAGATCTCCCG 5’-GAAGACAGTCATAAGTGCG

Table S4: Antibodies used in this study (related to *Flow Cytometry* section in *Experimental Procedures)*

Antibodies used for flow cytometry					
Antibody Target	Clone	Fluorophore	Dilution	Specificity	Vendor
CD103	2E7	PerCP/Cy5.5	1:100	αE integrin	Biolegend
CSA-1	polyclonal	FitC	1:100	Common surface antigens of heat-killed Salmonella	KPL
CD11b	M1/70	BV 785	1:200	αMβ2 integrin	Biolegend
CD11c	HL3	PE-Cy7	1:200	αxβ2 integrin	BD
CD45	30-F11	BV 510	1:400	Pan-CD45	Biolegend
CD64	X54-5/7.1	PE	1:200	FcγRI	Biolegend
Ly6-C	HK1.4	efluor®450	1:400	Ly-6C	eBiosciences
MHC II I-A/I-E	M5/114.15.2	Alexa Fluor® 700	1:200	MHC II	eBiosciences
CD3ε	145-2C11	APC-eFluor® 780	1:100	T cell lineage	eBiosciences
CD19	1D3	APC-eFluor® 780	1:100	B cells	eBiosciences
CD49b	DX5	APC-eFluor® 780	1:100	Pan NK cells	eBiosciences
TER-119	TER-119	APC-eFluor® 780	1:100	Erythrocytes	eBiosciences
Ly-6G	1A8	BUV395	1:100	Ly-6G/ granulocytes	BD
Mer	108928	APC	1:10	Mer (also referred to as MerTK)	R&D biosystems
Antibodies used for microscopy					
Antibody Target	Clone	Fluorophore	Dilution	Specificity	Vendor
Salmonella	polyclonal	none	1:1000	Custom made against fixed *Salmonella*	Aves (custom made)
Chicken IgY	polyclonal	Alexa Fluor® 594	1:400	Chicken IgY	Thermo Fisher Scientific

1 **Supplemental Experimental Procedures:**

2

3 **Bacterial culture conditions:**

4 *S.* Typhimurium or *E. Coli* isolates were grown aerobically in LB broth (Fisher
5 Scientific) or on LB agar plates (Fisher Scientific) at 37° C overnight. Overnight cultures
6 of *Salmonella* isolates were resuspended in sterile PBS to use for mouse infections.
7 Antibiotics were used at the following concentrations when needed: streptomycin (200
8 μg/ml, Sigma), carbencillin (100 μg/ml, Invitrogen), and kanamycin (40 μg/ml, Sigma).

9

10 **Construction of plasmids:**

11 To construct pGW6, the SL1344 *sseI* gene and 460 bp upstream sequence was amplified
12 by PCR from the plasmid p*sseI* (McLaughlin et al., 2009) using oligonucleotides listed in
13 Table S3, and the 1.5 kb amplified DNA fragment was inserted into pCR2.1 using the
14 TOPO TA cloning kit (Invitrogen) and sequenced prior to subcloning. The resulting
15 plasmid was digested with the restriction enzymes SalI and SacI (New England BioLabs),
16 and the extracted *sseI*-containing fragment was cloned into the SalI and SacI restriction
17 sites between the *phoN* flanking regions on pSW85 (Haneda et al., 2009), giving rise to
18 pGW1. The kanamycin-resistance (Kan^R) cassette (KSAC) from pUC4-KSAC (Barany,
19 1985) was then excised using SacI digestion and ligated using T4 DNA ligase into the
20 compatible SacI site on pGW1, between the *sseI* region and the *phoN* flanking region II,
21 generating pGW6. The SacI-digested Kan^R cassette was also inserted directly into the
22 SacI site on pSW85, between the *phoN* flanking regions, and ligated with T4 DNA ligase
23 to produce pGW10.

24 **Construction of *Salmonella* mutants**

25 Clean deletion mutants were created via lambda-red recombination and transferred to the
26 either ST313 *S.* Typhimurium isolate D23580 or ST19 *S.* Typhimurium isolate SL1344
27 using P22 phage transduction as previously described (Datsenko and Wanner, 2000).
28 Mutants were purified from contaminating phage using green plates and cross-struck
29 against P22 H5 to confirm absence of lysogens. Complemented strains were made via
30 allelic exchange. The complemented genes or antibiotic cassette control were inserted
31 into the PhoN locus (Winter et al., 2009). Suicide plasmids were introduced into the
32 relevant *S.* Typhimurium isolates by conjugation with a S17-1 *E. coli* λ*pir* donor strain.
33 Ex-conjugants were selected on plates containing streptomycin, kanamycin and BCIP (40
34 μg/ml, Sigma) to screen for insertion into the *phoN* locus in the recipient strain.
35 Complemented strains were sequenced to verify the insertion and tested for loss of the
36 suicide plasmid.
37

38 ***Salmonella* infections:**

39 Food was removed for 4 hours prior to giving the mice a single dose of 20 mg of
40 streptomycin (Sigma) in 100 μl of sterile PBS orograstrically. Afterwards, mice were
41 again supplied with food *ad libitum.* At 16 hours after streptomycin treatment, food was
42 again removed for 4 hours prior to either orogastric or intraperitoneal inoculation with *S.*
43 Typhimurium isolates. For all oral infections except those with Δ*aroA* mutants, mice
44 were given 10^8 CFU of *S.* Typhimurium in 100 μl of sterile PBS orogastrically (Barthel et
45 al., 2003). In experiments utilizing Δ*aroA* mutants, mice were instead given 10^9 CFU of
46 the Δ*aroA Salmonella* strains orogastrically. Uninfected mice used as a control in flow

47 cytometry experiments were given 100 μl of sterile PBS orogastrically in place of
48 *Salmonella.* For intraperitoneal infection experiments, mice were injected
49 intraperitoneally with 10^3 CFU of *S.* Typhimurium in 100 μl of sterile PBS.
50
51 Mice were euthanized at the indicated time points post infection by CO_2 asphyxiation and
52 either cervical dislocation or cardiac puncture as the secondary method of euthanasia.
53 Organs were collected, weighed, and then either homogenized in PBS for CFU
54 enumeration, prepared for histopathological examination, or used to make single cell
55 suspensions. For certain experiments, the single cell suspensions of the MLN were used
56 for both flow cytometry analysis and CFU enumeration. For CFU enumeration,
57 *Salmonella* were plated on LB agar plates with either streptomycin or kanamycin as
58 needed.
59
60 **Preparation of single cell suspensions:**
61 MLN, Peyer's patches, and small intestines were removed aseptically, cleaned of
62 mesentery and placed into RPMI (Gibco) 5% fetal bovine serum (FBS, Gibco) until ready
63 for processing.
64
65 To create a single cell suspension, MLN were teased apart 23G1 needle and incubated in
66 2 ml of Hank's Balanced Salt Solution (HBSS) with calcium and magnesium (Gibco)
67 containing 25 μg/ml Liberase TL (Sigma) and 50 μg/ml DNase I (Life Technologies) for
68 22-25 minutes at 37° C without agitation. The digestion was stopped by adding 5 mM
69 EDTA (Promega). Then cells were filtered through a 70 μm cell strainer and washed in

70 RPMI 5% FBS. Cells were resuspended in RPMI 5% FBS until they were stained for
71 flow cytometry.
72
73 Peyer's patches were digested in RPMI 5% FBS with 12.5 μg/ml Liberase TL and 50
74 μg/mL DNase I for 15 minutes at 37° C without agitation. The digestion was stopped by
75 adding 5 mM EDTA. Then cells were filtered through 100 μm cell strainer, washed in
76 RPMI 5% FBS, and then filtered through a 70 μm cell strainer. Samples were kept in
77 RPMI 5% FBS until they were stained for flow cytometry.
78
79 Single cell suspensions of the small intestine were prepared as described previously
80 (Geem et al., 2012).The small intestines were cut open longitudinally and washed three
81 times by gently moving the tissue around using tweezers in PBS to remove the contents.
82 Next, the small intestines were cut into ~0.5 cm pieces and incubated twice in 30 ml of
83 Ca^{2+}, Mg^{2+} free HBSS (Gibco) with 5% FBS and 2 mm EDTA (Promega) for 20 minutes
84 at 37° C with shaking at 200 rpm. Tissues were rinsed in PBS and then minced. The
85 minced tissue was incubated in 20 ml of Ca^{2+}, Mg^{2+} free HBSS with 5% FBS, 1.5 mg/ml
86 Collagenase Type VIII (Sigma), and 250 μg/ml Dnase I (Life Technologies) for 12
87 minutes at 37° C with shaking at 200 rpm. Samples were immediately filtered through a
88 100 μm cell strainer into a tube containing 25 ml of RPMI 5% FBS to stop the digestion.
89 The cells were pelleted by centrifugation washed in RPMI 5% FBS and filtered through
90 both 70 μm and 40 um cell strainers. Samples were kept in RPMI 5% FBS until staining.
91
92

93 **Cell Staining and Flow cytometry:**

94 Single cell suspensions were blocked in FACS buffer (PBS, 2% FBS, 2 mM EDTA) with
95 10 μg/ml TruStain fcX (Biolegend) for 10-15 minutes on ice. Samples were washed
96 twice in PBS and stained for 20 minutes on ice for viability (Live/Dead fixable Blue
97 Viability dye, Invitrogen) and surface antigens. Cells were washed twice with FACS
98 buffer and then fixed and permeabilized for 12 minutes on ice using BD
99 Cytofix/Cytoperm solution (BD). After fixation, samples were washed twice in BD
100 Perm/Wash buffer (BD) and stained for intracellular *Salmonella* for 30 minutes on ice.
101 After intracellular staining, cells were washed twice Perm/Wash buffer and once in
102 FACS buffer. Finally, samples were resuspended in FACS buffer for flow cytometry
103 analysis. Multiparameter analysis of single cell suspensions was performed using BD
104 Biosciencs Fortessa (Becton Dickinson) and analyzed using FlowJo (TreeStar).

105

106 Cell populations of interest were gated as follows. All cell populations of interest were
107 gated as Live/Dead Fixable Blue^{-}, lineage^{-} (CD3ε^{-}, CD19^{-}, CD49b^{-}, Ter-119^{-}), and single
108 cells, so these characteristics will be omitted for brevity. MLN inflammatory monocytes
109 were gated as Ly6C^{Hi}CD11b^{+}Ly6G^{-}. Patrolling monocytes in the MLN were gated as
110 Ly6C^{lo}CD11b^{+}Ly6G^{-}. In the MLN, neutrophils were gated as Ly6C^{int}CD11b^{Hi}Ly6G^{+}.
111 MLN Macrophages were gated as CD64^{+}MerTK^{+}CD11b^{+}Ly6G^{-}Ly6C^{-}. Migratory DC in
112 the MLN were gated as MHC II^{Hi}CD11c^{var}. Figure S2 visually shows the gating scheme
113 for MLN migratory DC. In the MLN, migratory DC were further gated into three subsets:
114 MHC II^{Hi}CD11c^{var}CD103^{+}CD11b^{-}, MHC II^{Hi}CD11c^{var}CD103^{+}CD11b^{+}, MHC
115 II^{Hi}CD11c^{var}CD103^{-}CD11b^{+} (Figure 4a)$^{.}$ In the small intestine and Peyer's patches, DC

116 were gated as $CD45^{+}CD11c^{+}MHC\ II^{+}CD64^{-}$. $CSA\text{-}1^{+}$ (anti-*Salmonella* common surface

117 antigen) cells of all cell types were considered infected. The $CSA\text{-}1^{+}$ gate was drawn to

118 minimize the autofluorescent and nonspecific signal that fell within the gate in uninfected

119 control samples.

120

121 The total amount of cells of a cell type of interest per MLN was calculated as follows.

122 The frequency of live cells of the population of interest from FlowJo was multiplied by

123 the number of Trypan $Blue^{-}$ cells per MLN. The amount of Trypan $Blue^{-}$ cells per MLN

124 was determined using a hemocytometer.

125

126 **Fluorescent Immunohistochemistry:**

127 MLN were harvested, frozen in OCT compound (Fisher Scientific), and frozen sections

128 10-12 μm in thickness were placed on SuperFrost Plus cryosection slides (Fisher

129 Scientific). Sections were fixed in ice-cold acetone at 4° C for 10 minutes and then

130 allowed to dry. A boundary was drawn around each individual section using a pap pen

131 (Fisher Scientific). Sections were washed with PBS and then blocked with 10% normal

132 goat serum in staining buffer (PBS with 3% bovine serum albumin, 1% saponin, 0.1 %

133 triton and 0.02% sodium azide) for 1 hour at room temperature. After blocking, sections

134 were stained with the primary anti-*Salmonella* antibody in staining buffer overnight at 4°

135 C. The next day, sections were washed and then stained for 2 hours at room temperature

136 with anti-chicken IgY antibody and phalloidin Alexa Fluor 488 (Life technologies).

137 Slides were washed in PBS and then mounted using ProLong Diamond (Life

138 Technologies). Images were acquired on a Zeiss LSM 880 confocal microscope with the

139 ZEN 2010 software (Zeiss). Samples were imaged with using the tile-scan function and a
140 63x oil-immersion objective. Images were acquired at a frame size of 2048x2048 with
141 16-bit depth and processed using FIJI (Schindelin et al., 2012). Scale bars are set at
142 50µm.
143

144 **Histology**

145 Tissues were fixed in 10% neutral buffered formalin. After fixation, tissues were
146 routinely processed for paraffin embedding and stained with hematoxylin and eosin. A
147 semiquantitative scoring system of 0 to +5 was used (0 = no significant lesion, +1 =
148 minimal, +2 = mild, +3 = moderate, +4 = marked, +5 = severe) to evaluate the ceca of
149 infected mice. A blinded pathologist evaluated the location and severity of any pathology.
150 The pathology score was the numeric total for all lesions for each mouse, and the
151 pathology score was compared between groups.
152

153 **Cell culture and *in vitro* infections**

154 Bone marrow-derived dendritic cells (BMDCs) were generated from mouse bone marrow
155 following the standard procedures described in Lutz et al., 1999. In brief, bone marrow
156 from femurs and tibias was flushed with DMEM, washed once with PBS, then plated in
157 BMDC media (DMEM High Glucose (Gibco) with 10% Fetal Bovine Serum (Gibco), 10
158 mM Hepes (Gibco), 40 mM b-mercaptoethanol (Sigma), and 20 ng/ml recombinant
159 murine Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, Peprotech). Cells
160 were harvested and seeded at day 10, when routinely greater than 90% of cells were
161 positive for the DC marker CD11c.

162 In 24 well plates, 2.5 x10^5 cells were seeded per well over night. *Salmonella* cultures
163 were grown overnight. The next day, plates were centrifuged for 5 minutes at 1000 rpm,
164 before the addition of the respective *Salmonella* strains at a multiplicity of infection of
165 10. The infection was synchronized by again centrifuging BMDCs for 5 minutes at 1000
166 rpm. The *Salmonella* strains were allowed to infect for 30 minutes. After 30 min,
167 BMDCs were centrifuged again for 5 min at 1000 rpm and then gentamicin-containing
168 media was added to a final concentration of 100 μg/ml for 1.5 hours. At 2 hours post
169 infection (hpi) BMDCs were centrifuged for 5 min. at 1000 rpm, and then were washed 3
170 times in PBS with centrifugation for 5 minutes at 1000 rpm before each new wash step to
171 minimize loss of BMDCs. After washing, intracellular CFU was enumerated or the media
172 was replaced with BMDC media containing 10 μg/ml gentamicin for the duration of the
173 experiment. Intracellular CFU was measured at 2, 8 and 22 hpi. To measure intracellular
174 CFU, BMDCs were lysed using 1% triton-x for 5 minutes at room temperature and then
175 CFU was enumerated by plating. The percent invasion was quantified as the intracellular
176 CFU recovered at 2 hpi over the initial input. The percent survival over time was
177 normalized to the intracellular CFU recovered at 2 hpi.

178
179 **Supplemental References:**
180
181 Barany, F., 1985. Two-codon insertion mutagenesis of plasmid genes by using single-
182 stranded hexameric oligonucleotides. Proc. Natl. Acad. Sci. U.S.A. 82, 4202–4206.
183 Datsenko, K.A., Wanner, B.L., 2000. One-step inactivation of chromosomal genes in
184 Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. U.S.A. 97, 6640.
185 Haneda, T., Winter, S.E., Butler, B.P., Wilson, R.P., Tukel, C., Winter, M.G., Godinez,
186 I., Tsolis, R.M., Bäumler, A.J., 2009. The Capsule-Encoding viaB Locus Reduces
187 Intestinal Inflammation by a Salmonella Pathogenicity Island 1-Independent
188 Mechanism. Infect. Immun. 77, 2932–2942. doi:10.1128/IAI.00172-09
189 Lutz, M.B., Kukutsch, N., Ogilvie, A.L.J., Rößner, S., Koch, F., Romani, N., Schuler, G.,
190 1999. An advanced culture method for generating large quantities of highly pure
191 dendritic cells from mouse bone marrow. J Immunol. Meth. 223, 77–92.

192 doi:10.1016/S0022-1759(98)00204-X
193
194
195
196
197