SUPPLEMENTARY DATA

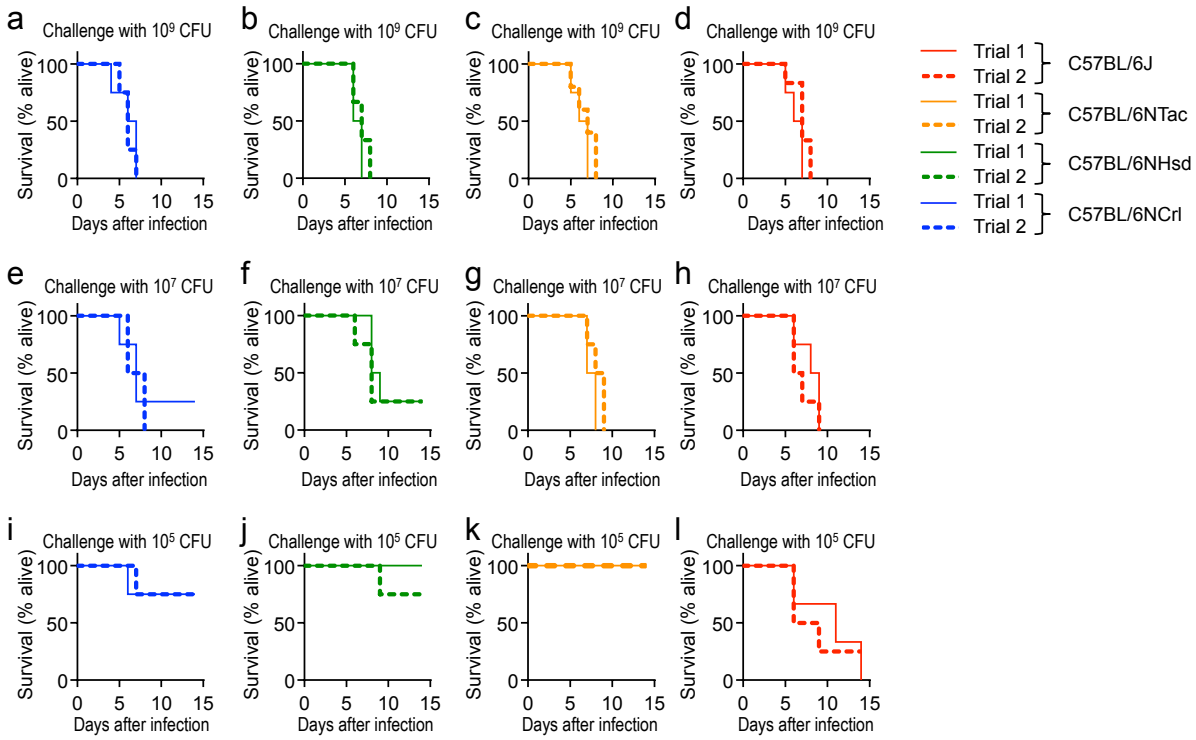
Supplementary Table 2: Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Nalidixic acid resistant derivative of *S.* Typhimurium ATCC14028	[32]	IR715
S. Typhimurium IR715 phoN::Cm	[33]	SW284
E. cloacae isolated from C57BL/6NHsd	This study	Hsd146
K. oxytoca isolated from C57BL/6NTac	This study	Tac148
P. vulgaris isolated from C57BL/6NTac	This study	Tac149
P. mirabilis isolated from C57BL/6NCrl	This study	Crl143
E. coli #1 isolated from C57BL/6NCrl	This study	Crl141
E. coli #2 isolated from C57BL/6NCrl	This study	Crl142
E. coli isolated from C57BL/6NHsd	This study	Hsd145
E. coli Nissle 1917 wild-type strain (O6:K5:H1)	[34]	EcN
E. coli Nissle 1917 *cydAB* mutant	[19]	YL219
E. coli zxx::RP4 2-(Tet^r::Mu) (Kan^r::Tn7) λpir	[35]	S17-1 λpir
E. coli K-12 strain TOP10; F$^-$ *mcrA* Δ(*mrr hsdRMS mcrBC*) Φ80*lacZ*ΔM15 *lacX*74 *recA*1 *araD*139 Δ(*ara - leu*)7697 *galU galK rpsL endA1 nupG*	Invitrogen	One Shot® TOP10 Chemically Competent *E. coli* (Cat# C404010)
Chemicals, Peptides, and Recombinant Proteins		
PCR SuperMix High Fidelity	Invitrogen	Cat # 10790020
Critical Commercial Assays		
PowerSoil DNA Isolation	MoBio	Cat# 12888-50
QIAquick PCR Purification Kit	Qiagen	Cat# 28106
Gibson Assembly Master Mix	NEB	Cat #E2611L
Zymoclean Gel DNA Recovery kit	Zymogen	Cat #D4001
Qubit dsDNA HS Assay Kit	Invitrogen	Cat# Q32854
TOPO cloning kit	Invitrogen	Cat# K456001
Deposited Data		
Raw and analyzed 16S rDNA Illumina sequencing data	This study	SRA accession number: SRP148888
Raw and analyzed 16S rDNA Sanger sequencing data	This study	Genbank accession number: MH759762-MH759768
Experimental Models: Organisms/Strains		
Female wild-type VAF B6 mice	Charles River Laboratories	Strain code: 027; C57BL/6Crl
Female wild-type SPF B6 mice	Envigo	C57BL/6Hsd
Female wild-type MPF B6 mice	Taconic Biosciences	C57BL/6NTac
Female wild-type SPF B6 mice	The Jackson Laboratories	Stock # 000664, C57BL/6J

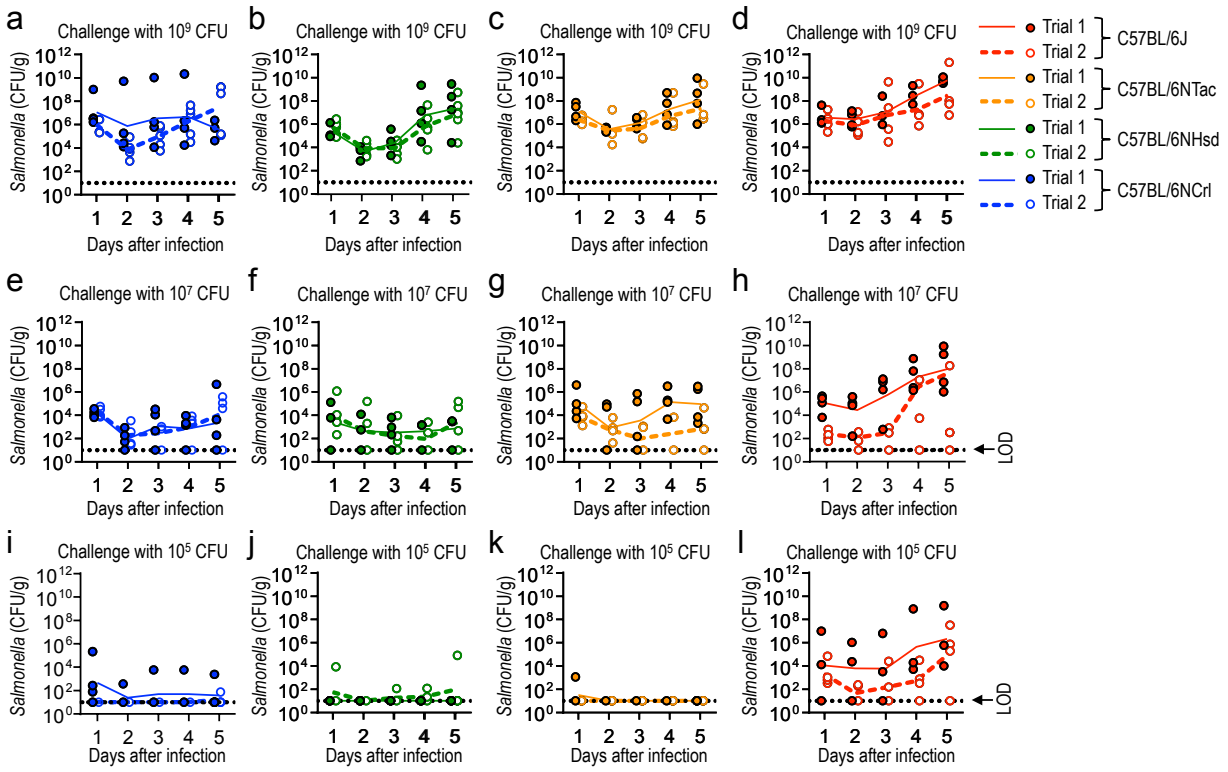
Female wild-type SPF B6 mice	The Jackson Laboratories	Stock # 005304, C57BL/6NJ
Germ-free Swiss Webster mice	Baumler Lab, University of California, Davis, USA	Tac:SW
Oligonucleotides		
Illumina 16S rRNA sequencing primer forward: AATGATACGGCGACCACCGAGATCTACAC NNNNNNNNTATGGTAATTGT GTGCCAGCMGCCGCGGTAA	[36]	515F
Illumina 16S rRNA sequencing primer reverse: CAAGCAGAAGACGGCATACGAGAT NNNNNNNNAGTCAGTCAGCC GGACTACHVGGGTWTCTAAT	[36]	806R
Full-length 16S rRNA forward sequencing primer; 5′-CAG GCC TAA CAC ATG CAA GTC-3′	[37]	63F
Full-length 16S rRNA reverse sequencing primer; 5′-GGG CGG WGT GTA CAA GGC-3	[37]	1387R
Primer for cloning *E. coli cydAB* genes (5′-ACGGTATCGATAAGCTTGATCGATAAAGCGGC TCTGTTAAATAAAATAC-3′)	This study	EcNcomp Fwd
Primer for cloning *E. coli cydAB* genes (5′-CCGGGCTGCAGGAATTCGATTTAGTACAGAGA GTGGGTGTTAC -3′)	This study	EcNcomp Rev
Recombinant DNA		
pWSK129::Ω cassette	[38]	pCAL61
pWSK29::Ω cassette	[38]	pCAL62
pCR2.1-TOPO	Invitrogen	Cat# K4500-01
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Hsd146	This study	pEV176
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Tac148	This study	pEV178
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Tac149	This study	pEV179
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Crl143	This study	pEV173
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Crl141	This study	pEV171
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Crl142	This study	pEV172
pCR2.1-TOPO containing 16S gene insert from *E. cloacae* Hsd145	This study	pEV176
pCR2.1-TOPO containing 16S gene insert from *E. coli* Nissle 1917	This study	pEV216
pCR2.1-TOPO containing 16S gene insert from *S.* Typhimurium IR715	This study	pEV218
pWSK129 containing *E. coli* Nissle 1917 *cydAB* genes	This study	pBMM17

Software and Algorithms		
Excel for Mac 2011	Microsoft	www.microsoft.com/Buy/Excel
Excel for Windows 2010	Microsoft	www.microsoft.com/Buy/Excel
Prism V7.0	Graph Pad	https://www.graphpad.com/scientific-software/prism/
Snapgene 4.1.5 for macOS	Snapgene	http://www.snapgene.com/products/snapgene/about_snapgene/
NIH nucleotide BLAST	National Center for Biotechnology Information	https://blast.ncbi.nlm.nih.gov/Blast.cgi
MEGA (version 7 for macOS)	Kumar S, 2016 (https://academic.oup.com/mbe/article/33/7/1870/2579089)	http://www.megasoftware.net/
R version 3.4.3	R Core Team (2013)	http://cran.us.r-project.org/
DADA2 R Package (1.6)	[39]	https://benjjneb.github.io/dada2/index.html
QIIME V1.91	[40]	http://qiime.org/install/install.html
QIIME V2.0	[40]	https://qiime2.org/
Greengenes Database version 13_8	[41]	http://greengenes.secondgenome.com/
Ribosomal Database Project Release 11	[42]	https://rdp.cme.msu.edu/
Linear discriminant analysis (LDA) effect size (LEfSe)	[43]	http://huttenhower.sph.harvard.edu/galaxy

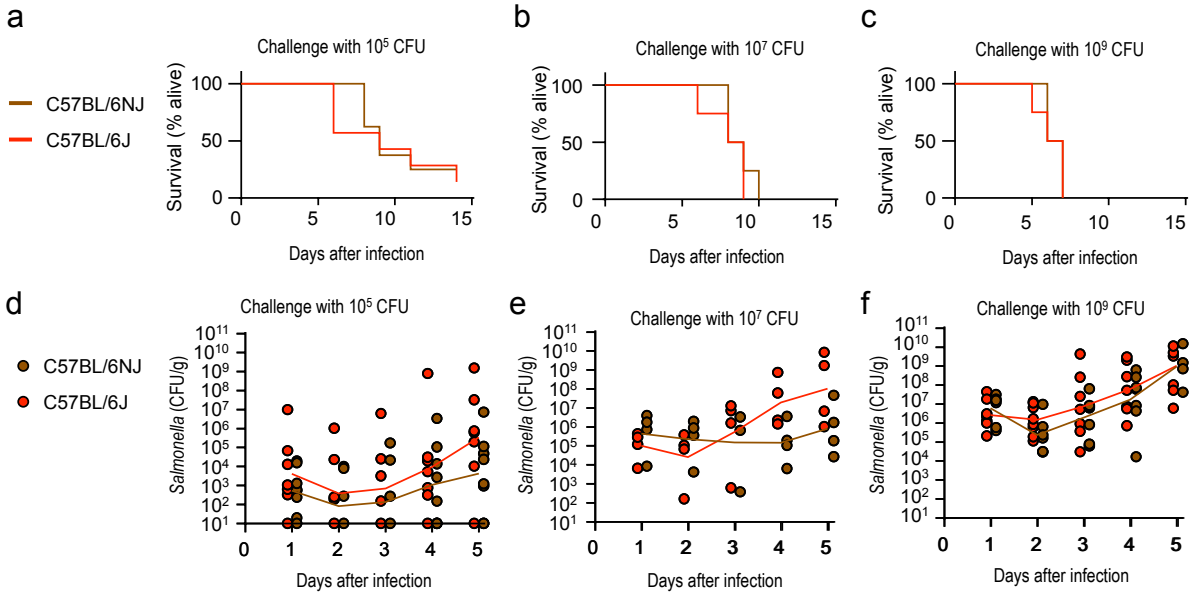
Supplementary Figures



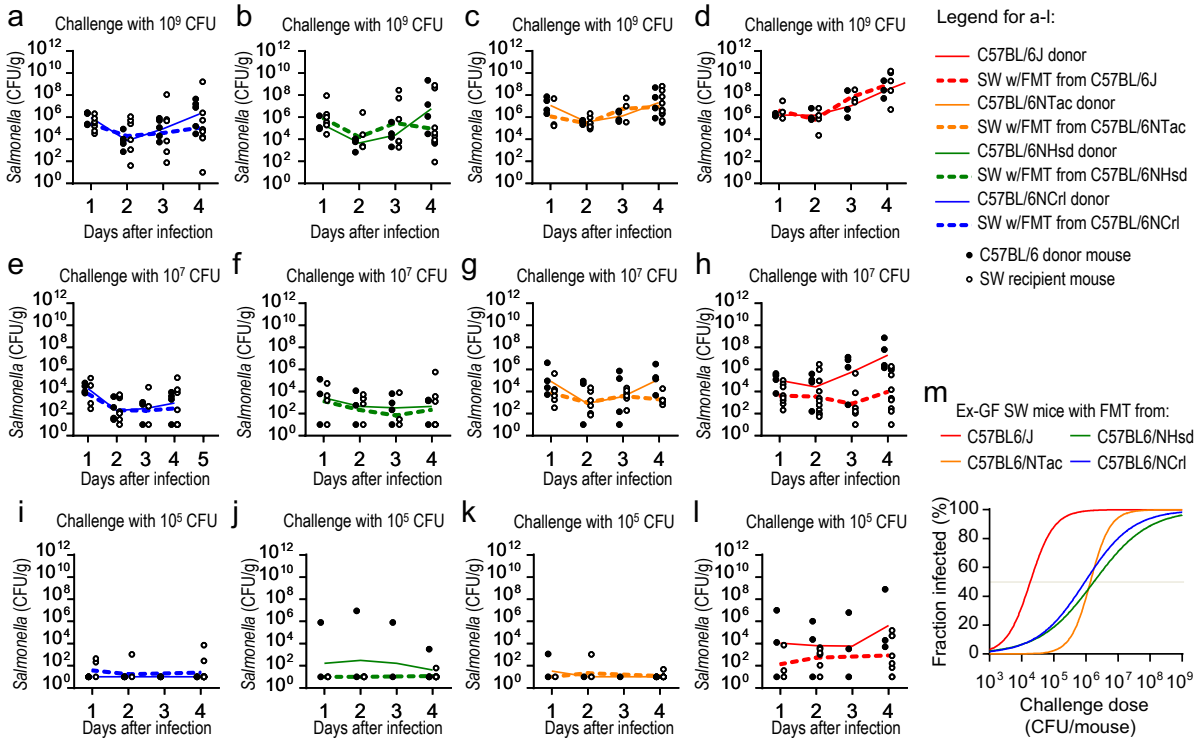
Supplementary Figure 1: Vendors are a dominant source of variation in lethal morbidity during *Salmonella* infection. Related to Figure panels 1a-c. (a-l) Mice from Charles River Laboratories (C57BL/6NCrl) (a, e and i), Harlan (C57BL/6NHsd) (b, f and j), Taconic Farms (C57BL/6NTac) (c, g and k) or Jackson Laboratories (C57BL/6J) (d, h, and l) were challenged with 10^9 (a-d), 10^7 (e-h) or 10^5 CFU of *S.* Typhimurium per animal (i-l). Lethal morbidity was monitored at the indicated time points after challenge. The experiment was repeated one year apart (*n* is given in Supplementary Figure 2) and results from both trials (trial 1, solid lines; trial 2, dashed lines) are included in the same figure panel for comparison. Results of a statistical analysis are provided in **Supplementary Table 1**.



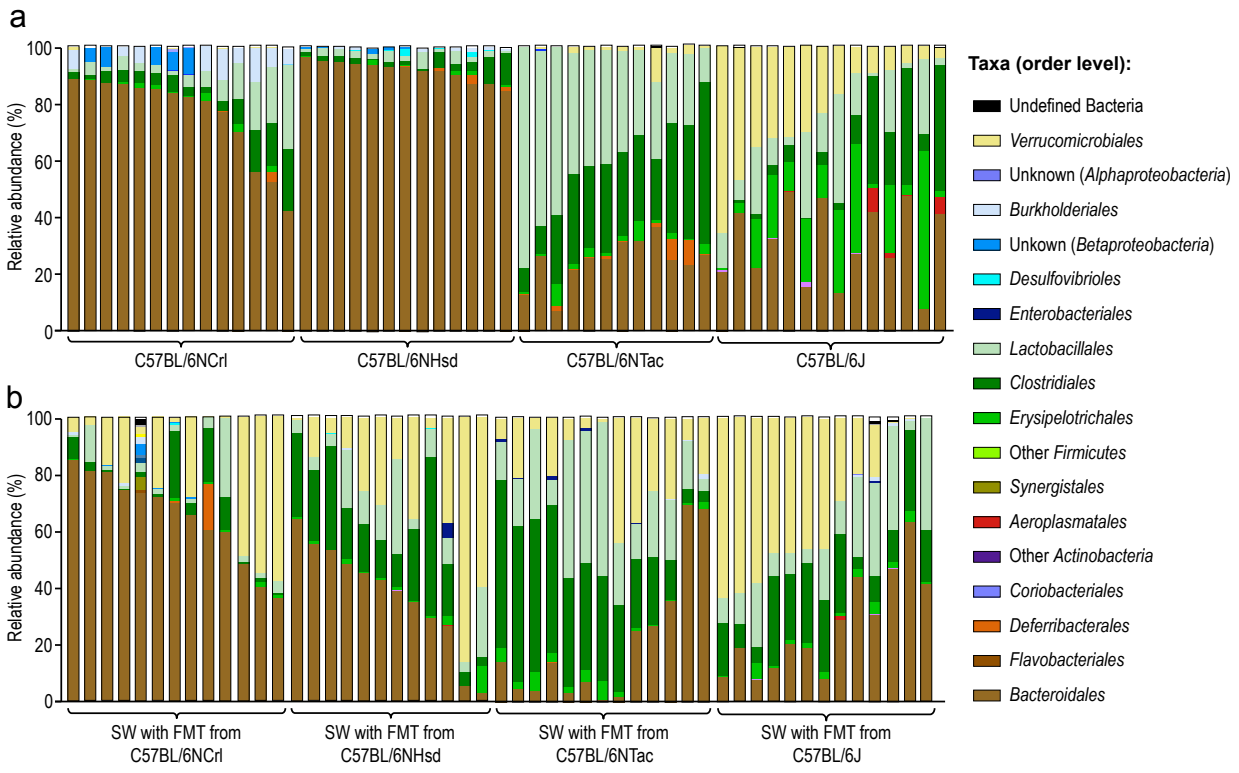
Supplementary Figure 2: Vendors are a dominant source of variation in intestinal colonization during *Salmonella* infection. Related to Figure panels 1d-f. (a-l) Mice from Charles River Laboratories (C57BL/6NCrl) (a, e and i), Harlan (C57BL/6NHsd) (b, f and j), Taconic Farms (C57BL/6NTac) (c, g and k) or Jackson Laboratories (C57BL/6J) (d, h, and l) were challenged with 10^9 (a-d), 10^7 (e-h) or 10^5 CFU of *S.* Typhimurium per animal (i-l). Fecal shedding of *S.* Typhimurium was quantified at the indicated time points after challenge. The experiment was repeated one year apart and results from both trials (trial 1, closed circles and solid lines; trial 2, open circles and dashed lines) are included in the same figure panel for comparison. Trend lines represent geometric means and each dot represents data from one animal (*n*). LOD, limit of detection. Results of a statistical analysis are provided in **Supplementary Table 1**.



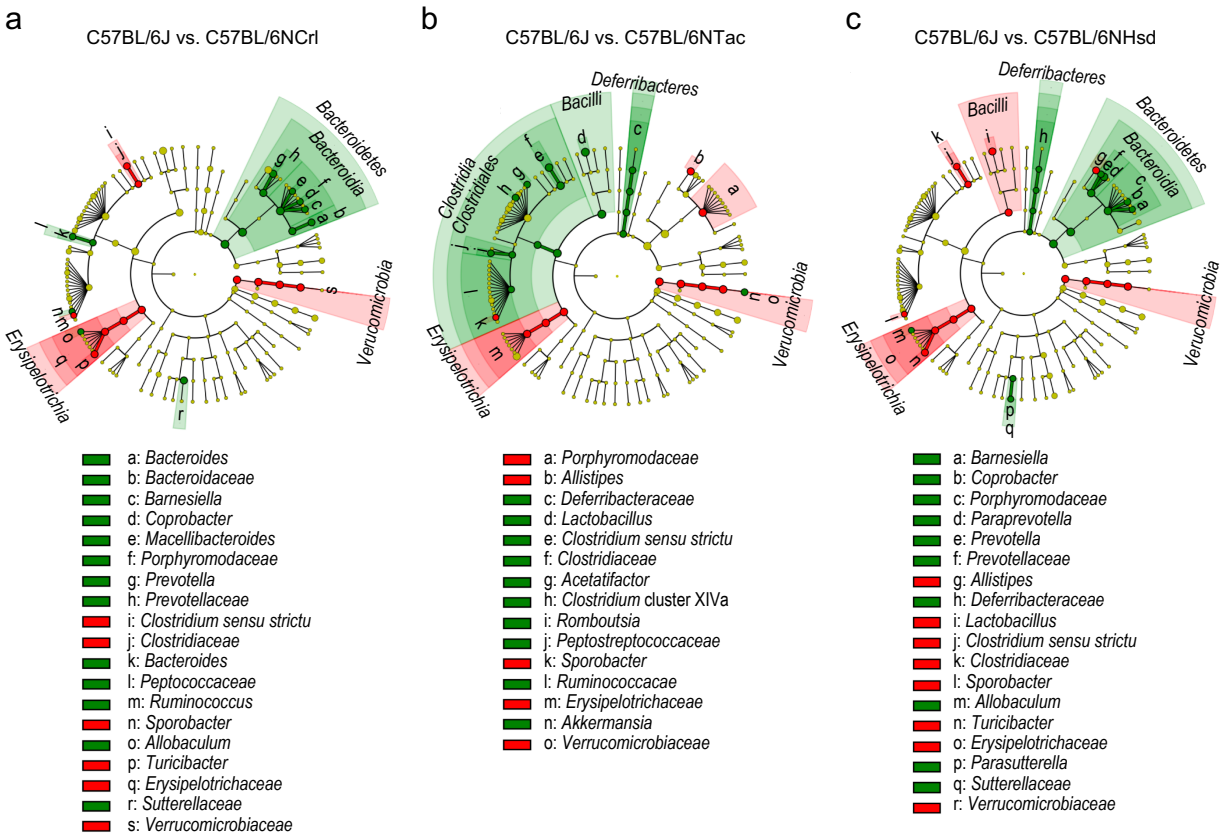
Supplementary Figure 3: Genetic variation between C57BL/6 substrains is not a dominant source of phenotypic variation during *Salmonella* infection. Related to Figure panel 1h. (a-f) Two C57BL/6 substrains (C57BL/6J and C57BL/6NJ) from Jackson Laboratories were challenged with 10^5 (a and d), 10^7 (b and e) or 10^9 CFU (c and f) of *S.* Typhimurium per animal. (a-c) Development of lethal morbidity over time was recorded. (e-f) Fecal shedding of *S.* Typhimurium was quantified at the indicated time points after challenge. Trend lines represent geometric means and each dot represents data from one animal (*n*). Results of a statistical analysis are provided in **Supplementary Table 1**.



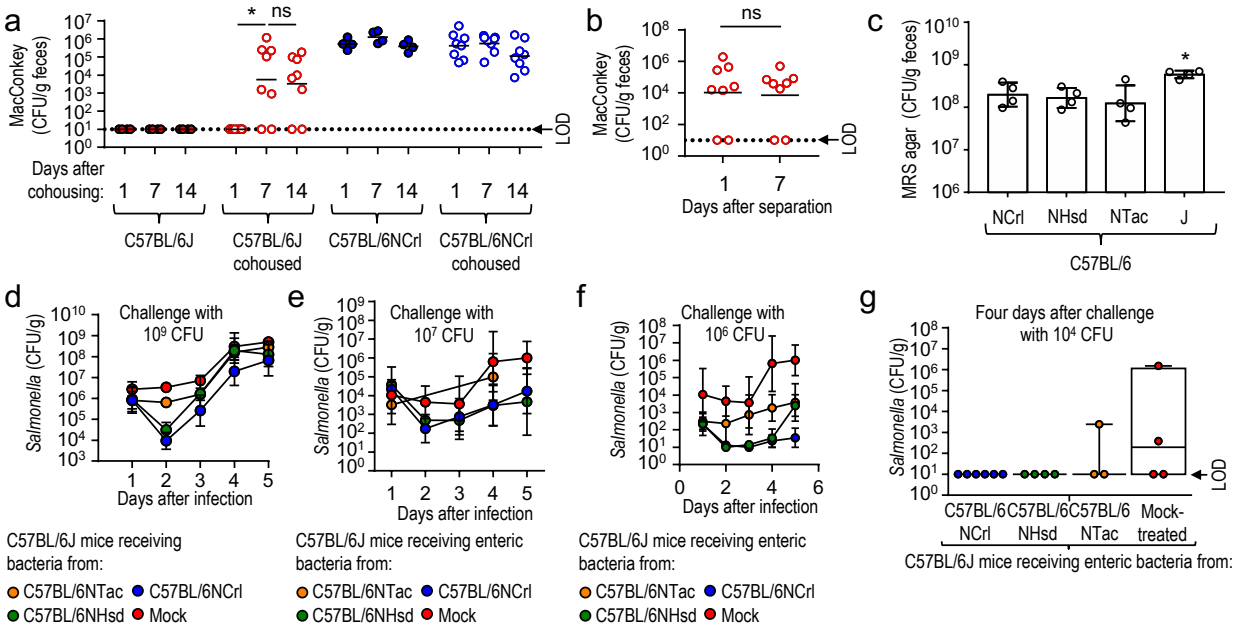
Supplementary Figure 4: Germ-free mice receiving a fecal microbiota transplant phenocopy the variation in *S.* Typhimurium intestinal colonization observed in donor mice. Related to Figure panels 2a-e. (a-l) Germ-free Swiss Webster mice received fecal microbiota transplants from Charles River mice (C57BL/6NCrl) (a, e and i), Harlan mice (C57BL/6NHsd) (b, f and j), Taconic mice (C57BL/6NTac) (c, g and k) or Jackson mice (C57BL/6J) (d, h, and l). Mice were then challenged with 10^9 (a-d), 10^7 (e-h) or 10^5 CFU of *S.* Typhimurium per animal (i-l). Fecal shedding of *S.* Typhimurium was quantified at the indicated time points after challenge (open circles and dotted lines). For comparison, fecal shedding of the respective donor mice challenged with the same dose of *S.* Typhimurium (closed circles and solid lines) from the experiment shown in Extended Data Figure 3 were included in each figure panel. Trend lines represent geometric means and each dot represents data from one animal. (m) Fraction of ex-germ-free SW mice developing intestinal carriage at different *S.* Typhimurium challenge doses. Results of a statistical analysis are provided in **Supplementary Table 1**.



Supplementary Figure 5: The relative abundance of *Clostridiales* is not a source of phenotypic variation in *Salmonella* susceptibility. Related to Figure panel 2g. (a) Fecal pellets were collected from Charles River mice (C57BL/6NCrl), Harlan mice (C57BL/6NHsd), Taconic mice (C57BL/6NTac) or Jackson mice (C57BL/6J) and subjected to 16S profiling. (b) Germ-free Swiss Webster (SW) mice received a pooled fecal microbiota transplant (FMT) from Charles River mice (C57BL/6NCrl), Harlan mice (C57BL/6NHsd), Taconic mice (C57BL/6NTac) or Jackson mice (C57BL/6J) and fecal pellets were collected five days later from each mouse for 16S profiling. (a and b) Graphs show relative taxa abundance at the order level. Each bar represents data from one animal (*n*). Results of a statistical analysis are provided in **Supplementary Table 1**.

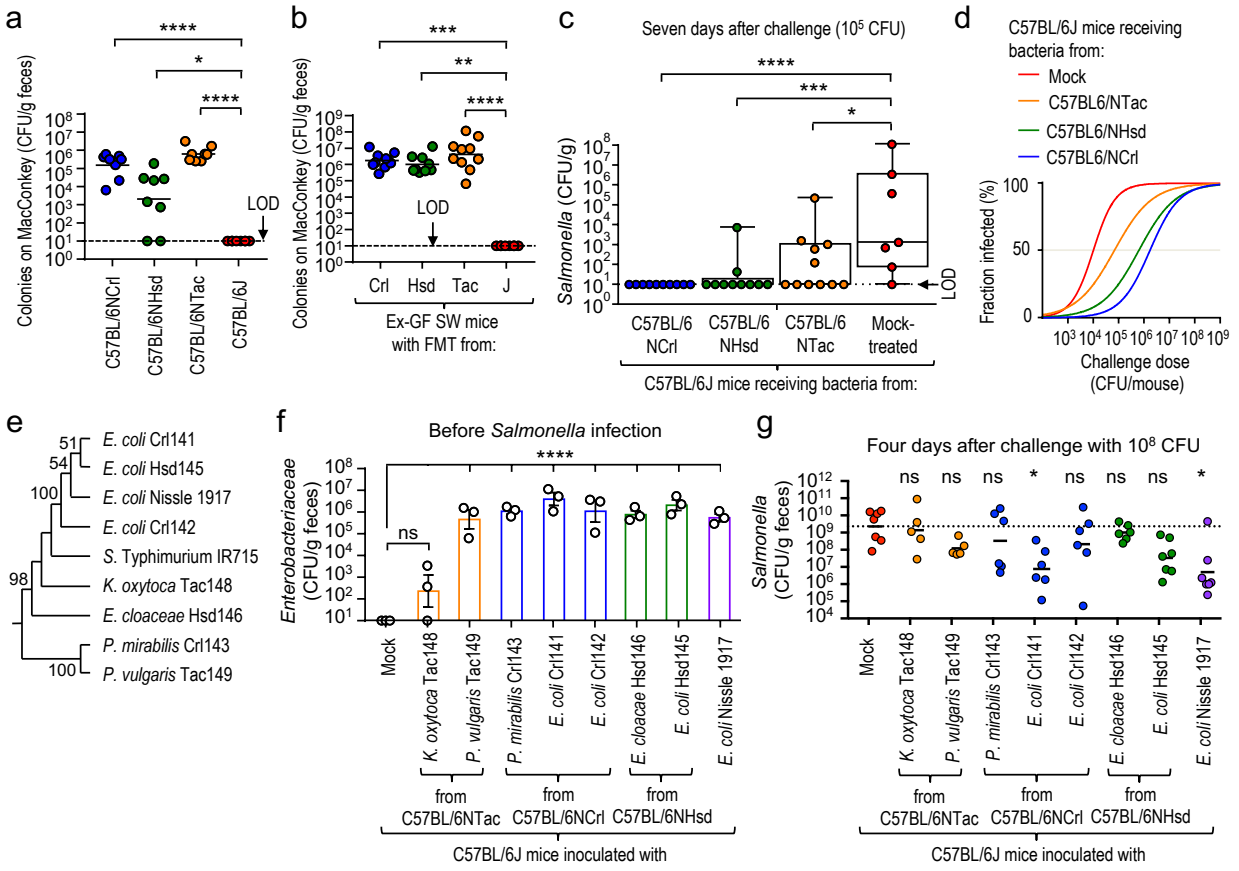


Supplementary Figure 6: Detection of differences in the microbiota composition by linear discriminant analysis. Related to Figure panel 1i. (a-c) Cladograms show differences in taxa composition between Jackson mice (C57BL/6J) and Charles River mice (C57BL/6NCrl) (a), between Jackson mice (C57BL/6J) and Taconic mice (C57BL/6NTac) (b), or between Jackson mice (C57BL/6J) and Harlan mice (C57BL/6NHsd) (c). Taxa that are more abundant in resistant mice (C57BL/6NCrl, C57BL/6NTac, or C57BL/6NHsd) compared to susceptible mice (C57BL/6J) are shown in green. Taxa less abundant in resistant mice (C57BL/6NCrl, C57BL/6NTac, or C57BL/6NHsd) compared to susceptible mice (C57BL/6J) are shown in red.



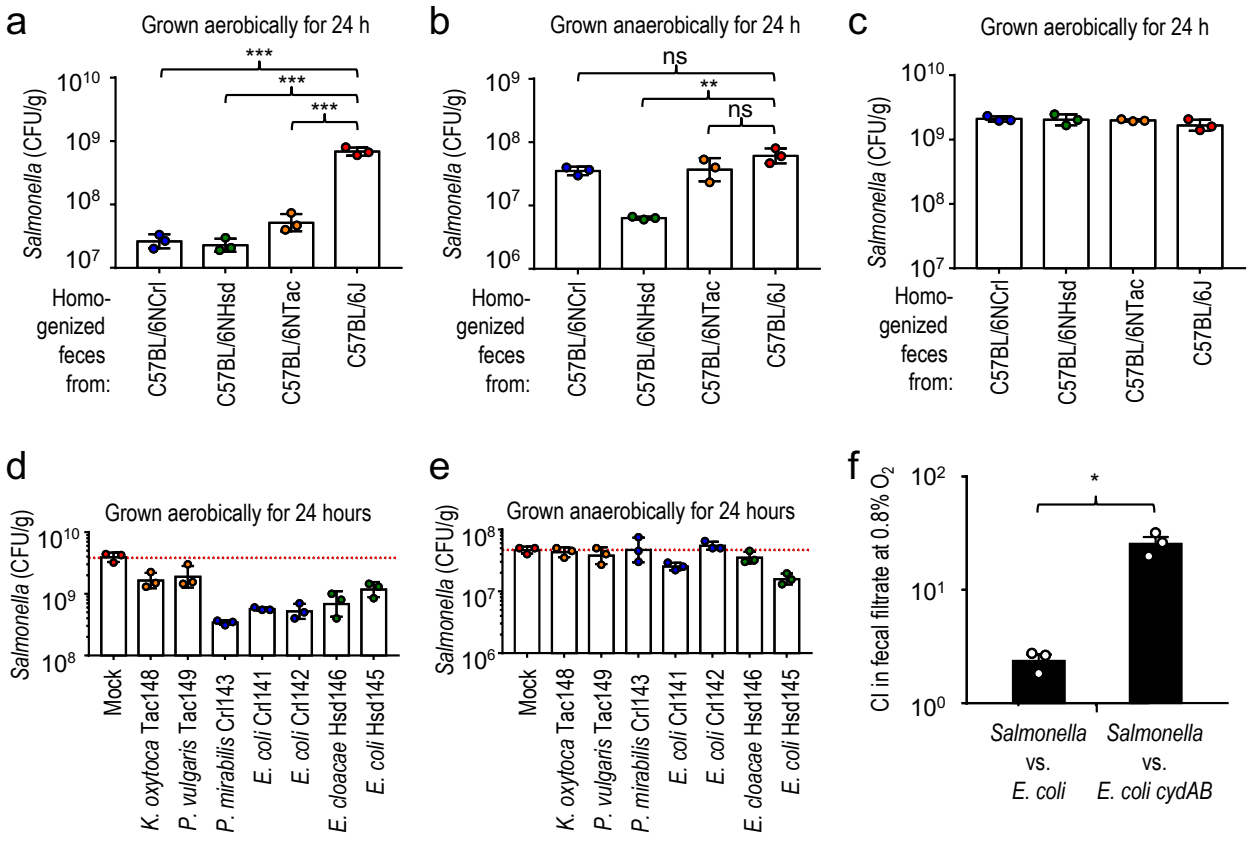
Supplementary Figure 7: *Enterobacteriaceae* are keystone species responsible for phenotypic variation. Related to Figure 3. (a) Mice from Jackson Laboratories (C57BL/6J) (red circles) or Charles River (C57BL/6NCrl) (blue circles) were housed normally (closed circles) or were cohoused (open circles). The presence of enteric bacteria in feces was determined by spreading dilutions of fecal homogenates on MacConkey agar plates at the indicated time points. Lines indicate the geometric mean of CFU recovered from feces. (b) Cohoused C57BL/6J mice from panel a were separated from C57BL/6NCrl mice and the presence of *Enterobacteriaceae* in feces was determined at the indicated time points after separation. Lines indicate the geometric mean of CFU recovered from feces. (c) The presence of *Lactobacilli* in feces of uninfected mice from the indicated vendors was determined by spreading dilutions of fecal homogenates on MRS agar plates. Bars represent the geometric mean ± geomean standard deviation. (d-g) Mice from Jackson Laboratories (C57BL/6J) were mock-treated or received enteric bacteria from the indicated donor mice. Mice were challenged with 10^9 (n = 4) (d), 10^7 (n = 3-4) (e), 10^6 (n = 3-4) (f) or 10^4 (each dot represents data from one animal) (g) CFU of *S.* Typhimurium per animal and *S.* Typhimurium shedding with the feces was monitored at the indicated time points after challenge

(d, e, and f) or at four days after challenge (g). (a, b and g) Each dot represents data from one animal (*n*). (d-f) Trend lines represent geometric means ± geomean standard error. (g) Whiskers represent minimum to maximum points and the box extends from the 25th to 75th percentile with median plotted by a line. Bars represent geometric means ± geomean standard deviation. Each dot represents data from a biological repeat (*n*). Results of a statistical analysis are provided in **Supplementary Table 1**. LOD, limit of detection; ns, $P > 0.05$; *, $P \leq 0.05$.



Supplementary Figure 8: Endogenous *Enterobacteriaceae* are keystone species responsible for phenotypic variation. Related to Figure 3. (a) The presence of *Enterobacteriaceae* in feces of uninfected C57BL/6 mice from different vendors was determined by spreading dilutions on MacConkey agar plates. (b) Germ-free Swiss Webster (SW) mice received fecal microbiota transplants(ex-germ-free SW mice) from the indicated donor mice and the presence of *Enterobacteriaceae* in feces was determined by spreading dilutions on MacConkey agar plates. (a-b) A line indicated the geometric mean. (c-d) Mice from Jackson Laboratories (C57BL/6J) were mock-treated or received *Enterobacteriaceae* from the indicated donor mice. (c) *S.* Typhimurium shedding with the feces was monitored at the indicated time points after challenge with 10^5 CFU per animal. Whiskers represent minimum to maximum points and the box extends from the 25th to 75th percentile with median plotted by a line. (d) Fraction of mice developing intestinal carriage after challenge with different *S.* Typhimurium doses (*n* = 4-11

per dose). (e) 16S rRNA gene sequences of *S.* Typhimurium strain IR715, *E. coli strain* Nissle 1917 and *Enterobacteriaceae* isolated from mice (C57BL/6NCrl, C57BL/6NHsd or C57BL/6NTac) were compared using a maximum likelihood model. (f and g) Mice from Jackson Laboratories (C57BL/6J) were inoculated with the indicated *Enterobacteriaceae*. (f) The presence of *Enterobacteriaceae* in feces prior to challenge was determined by spreading dilutions on MacConkey agar plates (n = 3). Bars represent the geometric mean ± geomean standard deviation. (g) Mice were challenged with *S.* Typhimurium and shedding of the pathogen with the feces was determined four days later. Lines indicate the geometric mean. (a, b, c, f and g) Each dot represents data from one animal, thus indicating the number (n) of repeats. LOD, limit of detection; ns, $P > 0.05$; *, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$.



Supplementary Figure 9: *Enterobacteriaceae* attenuate growth of *S.* Typhimurium in feces under aerobic conditions *in vitro*. Related to Figure 3. (a-c) Fecal pellets collected from Charles River mice (C57BL/6NCrl), Harlan mice (C57BL/6NHsd), Taconic mice (C57BL/6NTac) or Jackson mice (C57BL/6J) were homogenized. Homogenates were inoculated with *S.* Typhimurium and pathogen growth determined after aerobic incubation (a) or anaerobic incubation (b) for 24 hours (n = 3 per replicate). (c) Homogenates were sterile filtered and pathogen growth determined after aerobic incubation for 24 hours (n = 3 per replicate). (d and e) Fecal pellets collected from Jackson mice (C57BL/6J) were homogenized and mock-treated or inoculated with the indicated *Enterobacteriaceae* isolates. Each homogenate was then infected with *S.* Typhimurium and pathogen growth determined after aerobic incubation for 24 hours (d) or after anaerobic incubation for 24 hours (n = 3 per replicate) (e). (d and e) A red dotted line indicates pathogen growth in mock-treated fecal homogenates. (f) Fecal pellets collected from

Jackson mice (C57BL/6J) were homogenized, sterile filtered and inoculated with the indicated mixtures of *S.* Typhimurium and *E. coli* Nissle 1917 strains. The competitive index (CI) was calculated after growth for 24 hour in a hypoxia chamber with 0.8% oxygen. (a-f) Data represent geometric means ± geomean standard deviation. Each dot represents data from a biological repeat (*n*). Results of a statistical analysis are provided in **Supplementary Table 1**. LOD, limit of detection; ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.