Lethal inflammasome activation by a multi-drug resistant pathobiont upon antibiotic disruption of the microbiota

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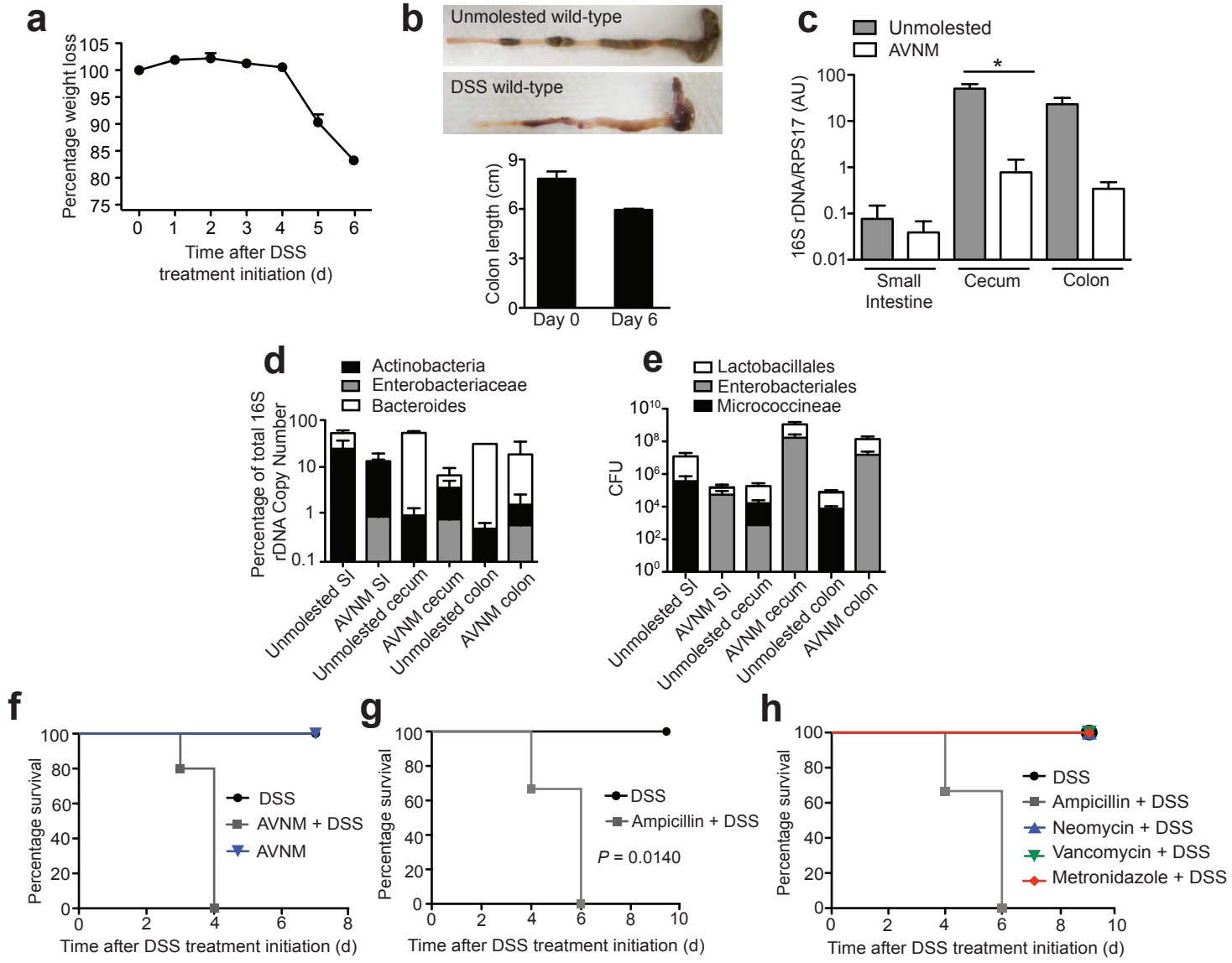


Figure S1. Wild-type mice treated with AVNM and ampicillin are hypersusceptible to DSS.
(**a** – **b**) Mice were treated with 5% DSS and (**a**) weight loss (*n* = 5) and (**b**) colon length were measured (*n* = 3). Error bars indicate standard deviation.
(**c** – **e**) Mice were treated with drinking water supplemented with AVNM or normal drinking water for 10 d. The levels and composition of the microbiota were analyzed.
(**c**) qPCR analysis of total bacterial 16S rDNA levels in the small intestine, cecum and colon of wild-type mice. Values were normalized to the levels of the mouse housekeeping gene, Rps17
(**d**) qPCR analysis of specific bacterial 16S rDNA in the small intestine (SI), cecum and colon of wild-type mice. Values were normalized to the levels of the mouse housekeeping gene, Rps17
(**e**) Composition analysis of culturable microbes in the intestinal tract of AVNM treated and water-only treated control mice.
(**f**) Survival of wild-type mice treated with water supplemented with AVNM or regular drinking water for 10 d. AVNM treated mice were then given water supplemented with AVNM/5% DSS or continued on AVNM-only water. Water-treated control mice were given 5% DSS and survival was monitored.
(**g** – **h**) Mice were given regular drinking water or water supplemented with ampicillin, neomycin, vancomycin or metronidazole for 10 d. Mice were then given water supplemented with the same antibiotic plus 5% DSS and survival was monitored.

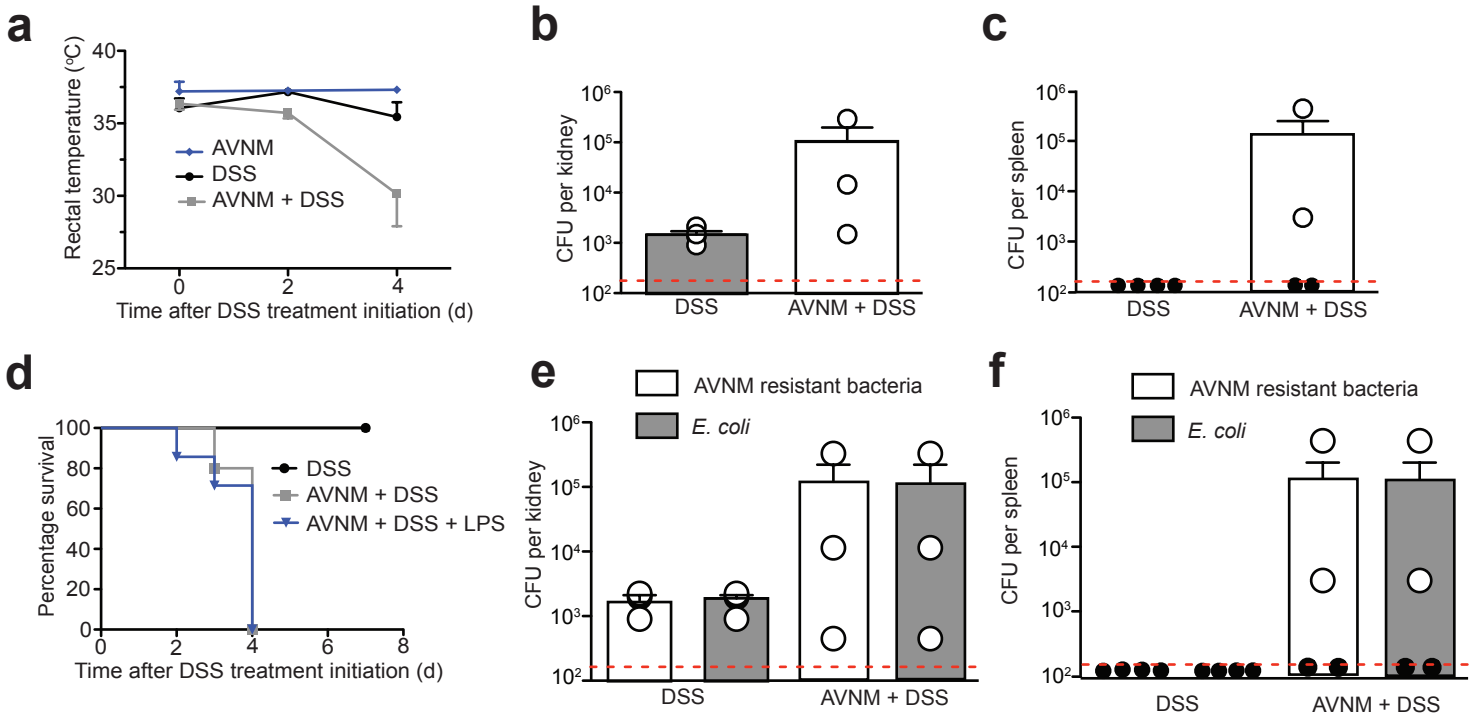


Figure S2. Antibiotic treatment plus intestinal injury triggers extraintestinal spread of bacteria in wild-type mice.
(**a**) Wild-type mice were treated with normal drinking water or water supplemented with AVNM for 10 d. Mice that received normal water were then given drinking water with 5% DSS-only. Mice that received AVNM were then given drinking water supplemented with AVNM-5% DSS or continued on AVNM-only. Rectal temperature was monitored.
(**b** – **c**) Wild-type mice were treated with normal drinking water or water supplemented with AVNM for 10 d. Mice that received normal water were then given drinking water with 5% DSS-only. Mice that received AVNM were then given drinking water supplemented with AVNM-5% DSS and culturable bacterial colonization levels of (**b**) kidney and (**c**) spleen were measured. Red dashed lines indicate limit of detection and black circles indicate individual mice below the limit of detection.
(**d**) Wild-type mice were treated with normal drinking water or water supplemented with AVNM for 10 d. Mice that received normal water were then given drinking water with 5% DSS-only and mice that received AVNM were then given drinking water supplemented with AVNM-5% DSS or 10 $\mu g ml^{-1}$ LPS O55:B5 (Sigma).
(**e** – **f**) Wild-type mice were treated with normal drinking water or water supplemented with AVNM for 10 d. Mice that received normal water were then given drinking water with 5% DSS-only. Mice that received AVNM were then given drinking water supplemented with AVNM-5% DSS and colonization levels of total AVNM resistant and AVNM resistant *E. coli* levels of the (**e**) kidney and (**f**) spleen were measured. Red dashed lines indicate limit of detection and black circles indicate individual mice below the limit of detection.

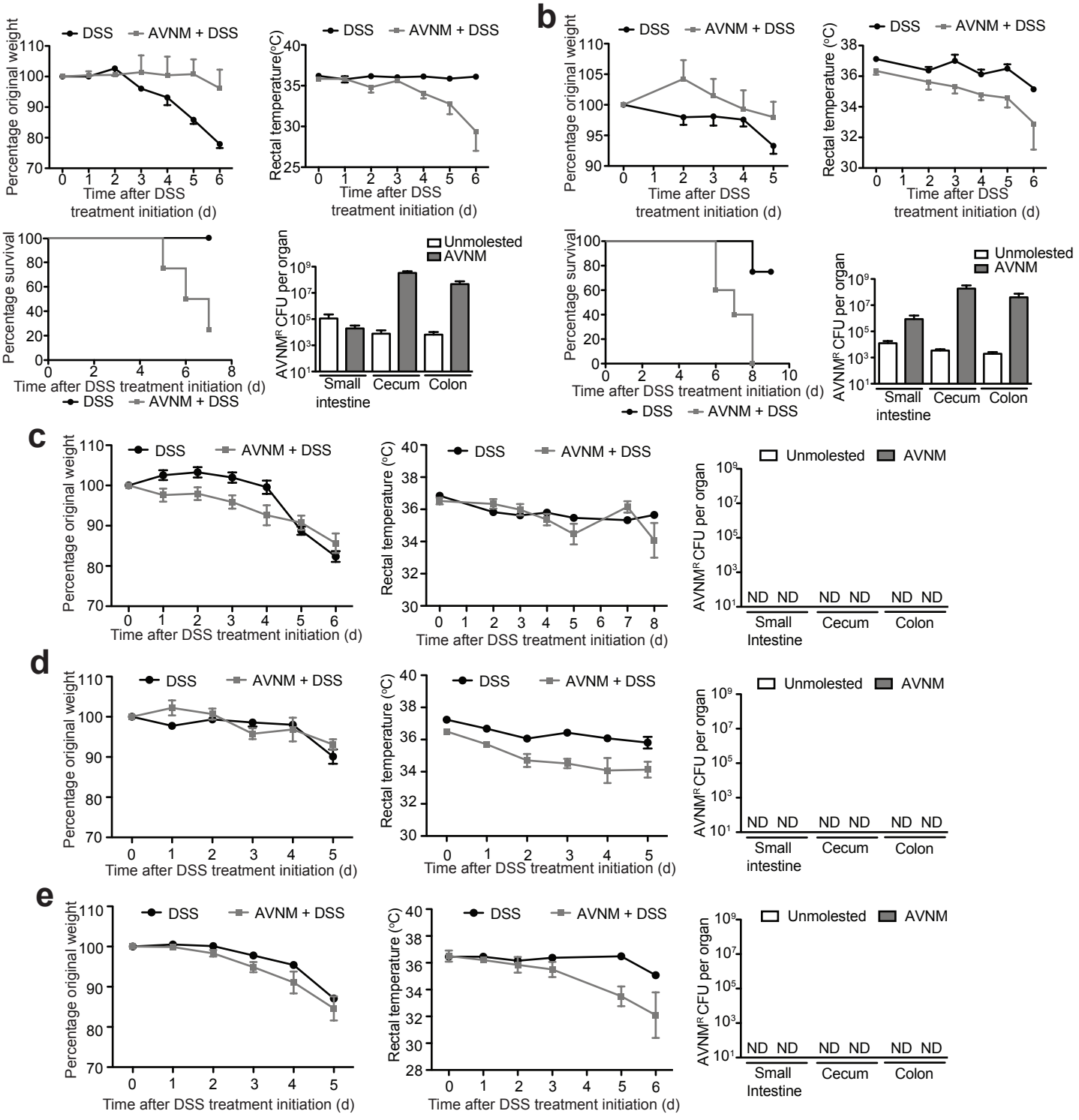


Figure S3. AVNM-DSS treatment of SPF wild-type mice from Taconic Farms and Jackson Laboratories.
SPF male C57BL/6 mice were obtained from (**a** – **b**) Taconic Farms and (**c** – **e**) Jackson Laboratories, kept in isolator cages and treated with AVNM for 7 d and then given drinking water supplemented with AVNM-5% DSS and health (weight, temperature and survival) was monitored. Immediately on receipt and after a 7 d course of AVNM treatment, the intestinal tract was subjected to CFU analysis to determine the levels of AVNM resistant *E. coli.* Each panel (**a** – **e**) represents different experiments with separate shipments of mice.

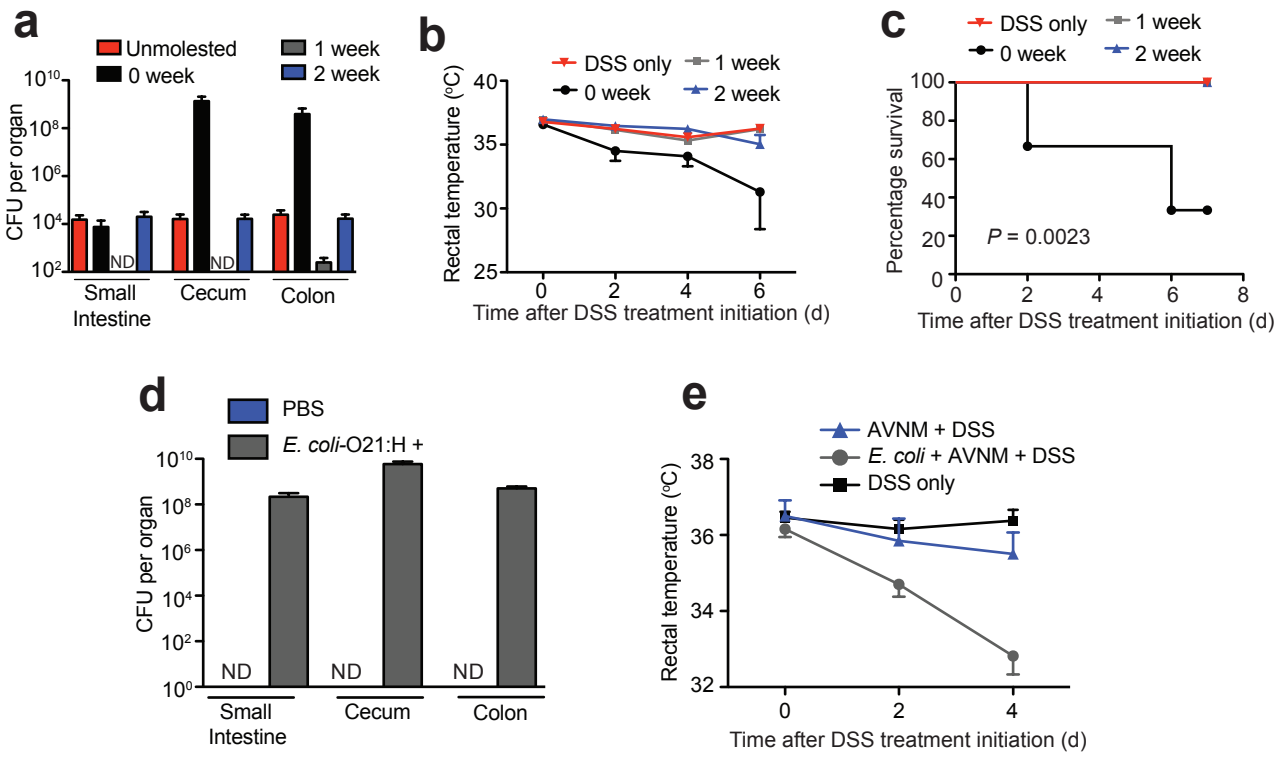
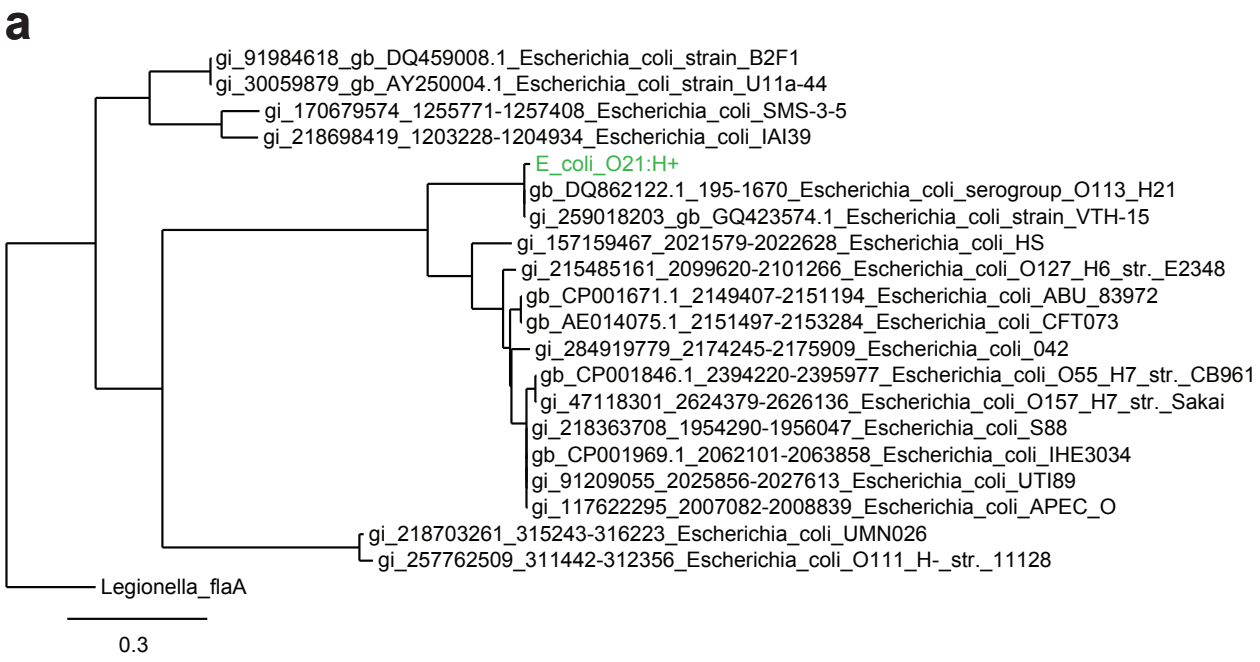


Figure S4. *E. coli*-O21:H+ colonization is associated with sepsis in AVNM-DSS treated wild-type mice.
(**a** – **c**) Suppression of MDR *E. coli* overgrowth. 5 week old, male wild-type mice were given drinking water supplemented with AVNM. After 7 d of treatment, mice were immediately given drinking water supplemented with AVNM-5% DSS (0 weeks) or normal drinking water for one (1 week) or two weeks (2 week) prior to starting AVNM-5% DSS treatment.
(**a**) levels of AVNM-resistant *E. coli* intestinal colonization after 7 d of AVNM treatment (**b**) rectal temperature (**c**) and survival were measured.
(**d** – **e**) *E. coli*-O21:H+ oral infection. 5 week old wild-type Jax male mice were treated with AVNM *ad libitum* for 7 d. On 4, 5 and 6 d, mice were oral infected with $5x10^8$ *E. coli*-O21:H+ by oral gavage. On 7 d, (**d**) intestinal organs were harvested to determine the level of *E. coli*-O21:H+ colonization or (**e**) mice were given drinking water supplemented with AVNM-5% DSS and health (rectal temperature) was monitored. AVNM-DSS-treated mice that received oral challenges of PBS and DSS-only treated mice were used as controls.



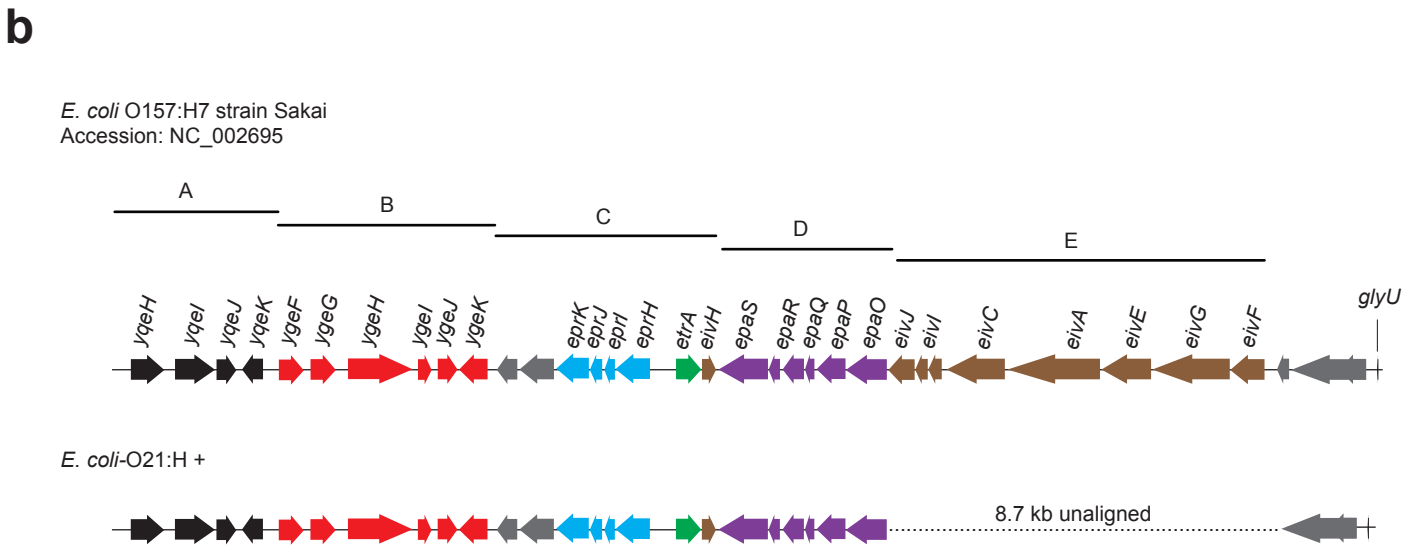


Figure S5. *E. coli*-O21:H+ harbors virulence factors.
(**a**) *fliC* phylogenetic analysis. The web based program Phylogeny.fr was used for phylogenetic analysis of the *fliC* gene sequence from *E. coli*-O21:H+ compared to publicly available *fliC* gene sequences from pathogenic and non-pathogenic *E. coli* strains. *E. coli*-O21:H+ is highlighted in green.
(**b**) A schematic of the ETT2 gene cluster identified in *E. coli* O157:H7 strain Sakai (adapted from Ren et al.) compared to the ETT2 gene content in *E. coli*-O21:H+ identified from whole genome sequencing and mapping analysis.

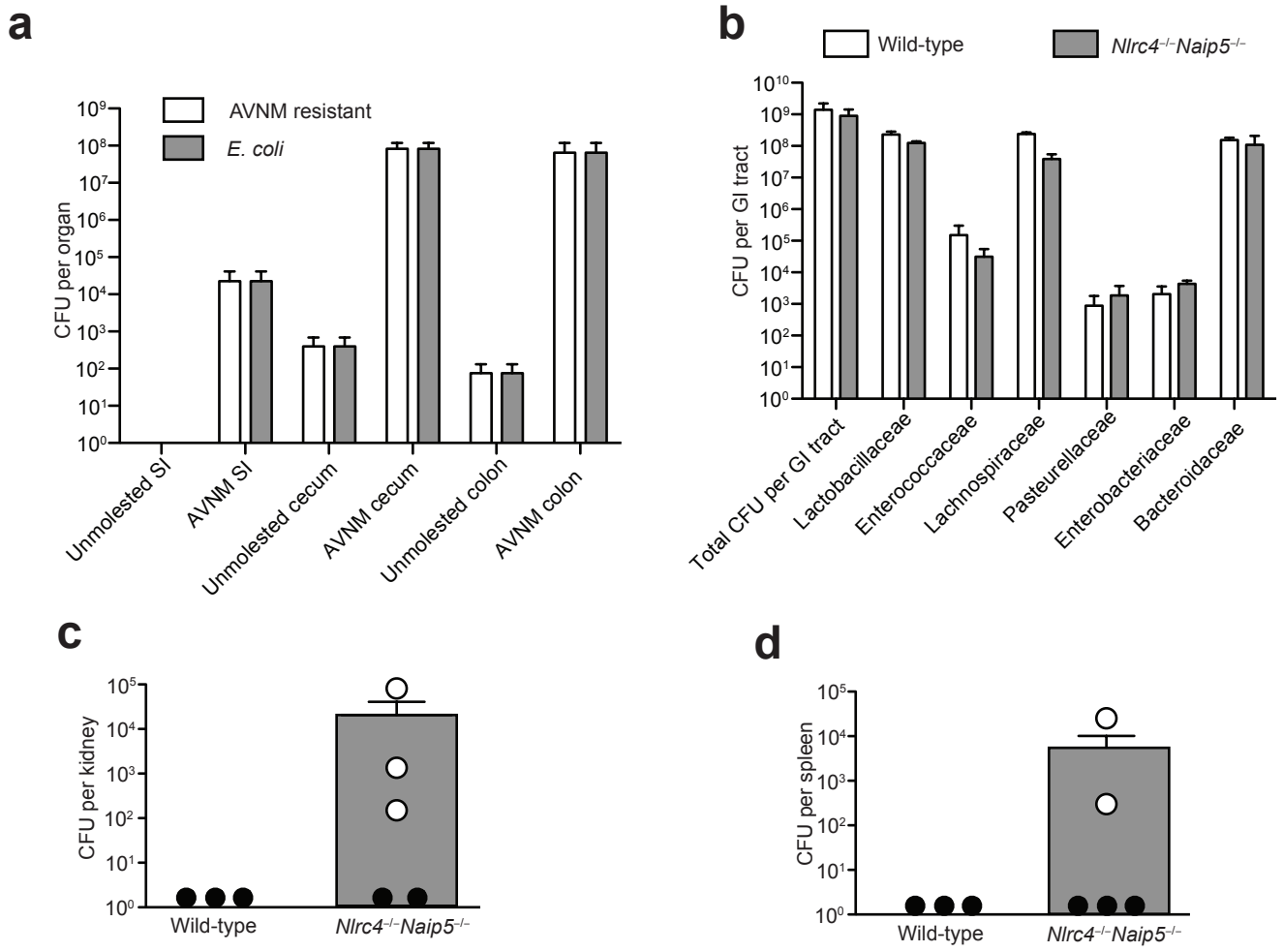


Figure S6. Microbiota analysis of inflammasome mutant and wild-type littermates.
(**a**) *Nlrc4*$^{-/-}$*Naip5*$^{-/-}$ mutant littermates were treated with AVNM in their drinking water for 7 d, after which tissues were homogenized and cultured for total AVNM resistant bacteria and AVNM resistant *E. coli* and compared to mutant littermates that received a water control.
(**b**) Microbiota compositional analysis of unmolested colons from wild-type and *Nlrc4*$^{-/-}$*Naip5*$^{-/-}$ mutant littermates using direct culturing methods and 16S rDNA sequencing analysis of colonies.
(**c** – **d**) Wild-type and *Nlrc4*$^{-/-}$*Naip5*$^{-/-}$ fostermates were treated with AVNM for 7 d and then given drinking water supplemented with AVNM-5% DSS. The levels of AVNM resistant *E. coli* colonization in the (**c**) kidney and (**d**) spleen 3 d post DSS treatment initiation are shown.

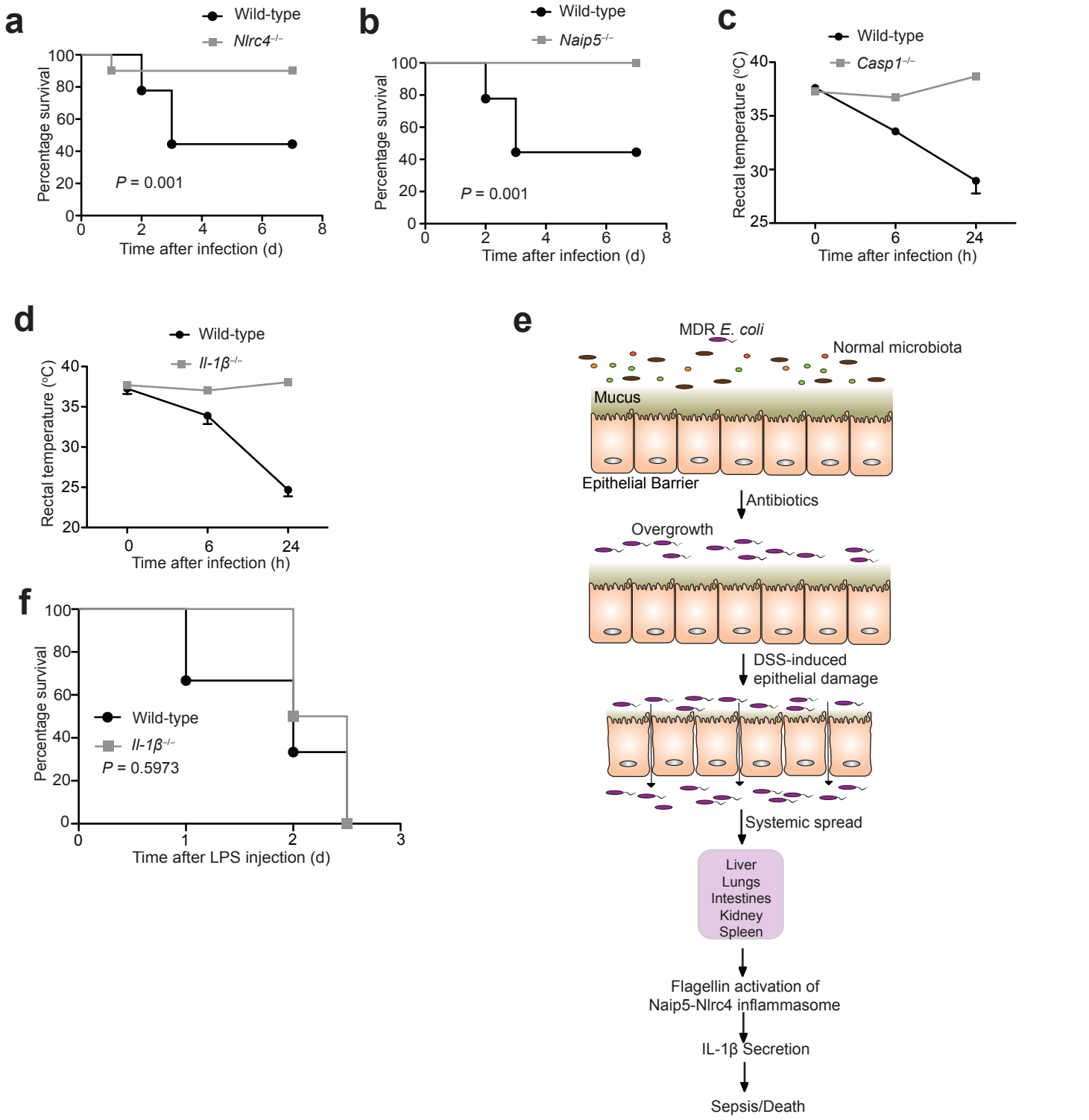


Figure S7. The NAIP5/NLRC4 inflammasome mediates the pathogenesis of a MDR *E. coli* infection.
(**a** – **d**) Wild-type and mutant mice were infected with 5x10^8 live *E. coli*-O21:H+ and monitored for (**a** – **b**) survival and (**c** – **d**) body temperature. (**a**) Survival of *Nlrc4*$^{-/-}$ mutant mice compared to wild-type mice (**b**) Survival of *Naip5*$^{-/-}$ mutant mice compared to wild-type mice (**c**) rectal temperature of *Caspase-1*$^{-/-}$ mutant mice compared to wild-type mice and (**d**) rectal temperature of *Il-1β*$^{-/-}$ mutant mice compared to wild-type mice. Data in (**a**) and (**b**) wild-type (n = 9), *Nlrc4*$^{-/-}$ (n = 10) and *Naip5*$^{-/-}$ (n = 10). Kaplan Meier plot were analyzed by log rank analysis. Data in (**c**) and (**d**) wild-type (n = 9), *Caspase-1*$^{-/-}$ (n = 6) and *Il-1β*$^{-/-}$ (n = 12).
(**e**) Inflammasome activation and sepsis-like syndrome induced by a specific pathobiont of the microbiota. In mice with a dysbiotic flora, antibiotic induced overgrowth and extraintestinal spread of an MDR E. coli pathobiont induces a lethal sepsis-like disease in a Naip5-Nlrc4 inflammasome and IL-1β dependent manner.
(**f**) *Il-1β*$^{-/-}$ mice are sensitive to a LPS challenge. Wild-type and mutant mice were injected intravenously with 5 mgkg^{-1} LPS O55:B5 (Sigma) and survival was monitored.

	Total reference length	% GC	Total consensus length	Fraction of reference covered
E. coli HS CP000802	4,643,538	50.82	4,208,019	0.91
E. coli E24377A ETEC NC_009801	4,979,619	50.62	4,392,917	0.88
E. coli 53638 EIEC AAKB00000000	5,071,018	51.1	4,310,572	0.85
E. coli 042 EAEC FN554766	5,241,977	50.56	4,116,381	0.79
E. coli O127:H6 EPEC NC_011601	4,965,553	50.57	3,918,479	0.79
E. coli IAI39 ExPEC NC_011750	5,132,068	50.63	4,070,193	0.79
E. coli S88 ExPEC NC_011742	5,032,268	50.68	3,916,798	0.78
E. coli O157:H7 EHEC NC_002695	5,498,450	50.54	4,224,923	0.77
E. coli APEC O1 CP000468	5,082,025	50.55	3,909,305	0.77
E. coli UTI89 ExPEC CP000243	5,065,741	50.6	3,910,771	0.77
E. coli CFTO73 ExPEC AE014075	5,231,428	50.47	3,951,007	0.76

Table S1. Genomic alignments of *E. coli*-O21:H+.
The *E. coli*-O21:H+ genome was sequenced using Illumina deep sequencing and aligned to eleven publicly available genomes.