Figure S1. Mylona et al

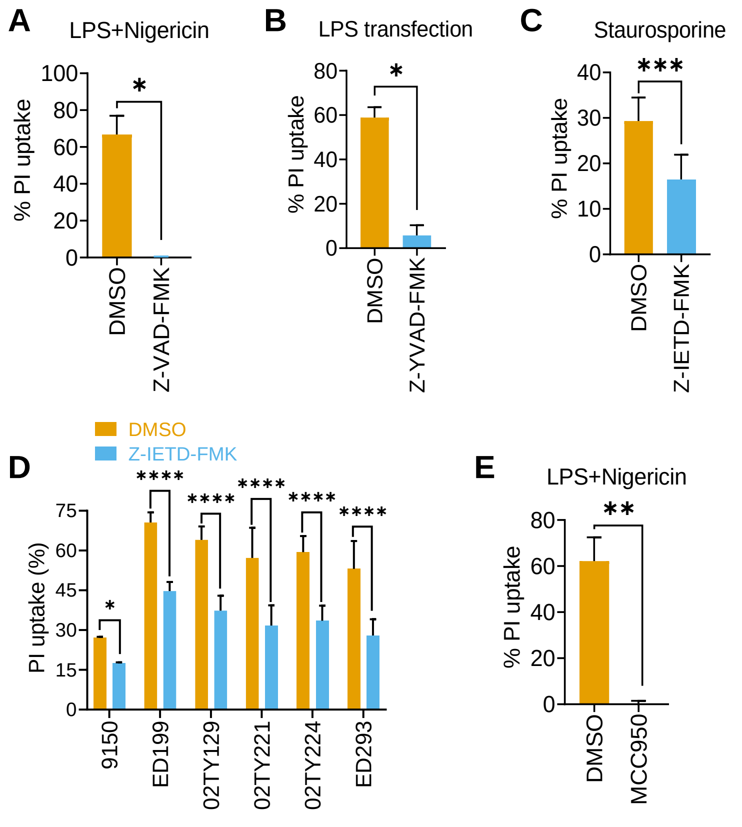

Figure S1. PI uptake into THP1 cells treated with chemical inhibitors. (A, E) THP1-cells primed with *E. coli* O111:B5 LPS were treated with either DMSO, Z-VAD-FMK **(A)** or MCC950 **(E)** and then nigericin. **(B)** THP1 cells were treated with Z-YVAD-FMK and transfected with LPS. **(C-D)** THP1 cells were treated with Z-IETD-FMK and then with Staurosporine for 5 h **(C)** or infected with the indicated SPtA strains **(D)**. Mean ± SEM from 3 **(A-D)** or 4 **(C)** independent experiments. * *P<0.05,* ** *P<0.01* by paired Student's *t*-test.

Figure S2. Mylona et al

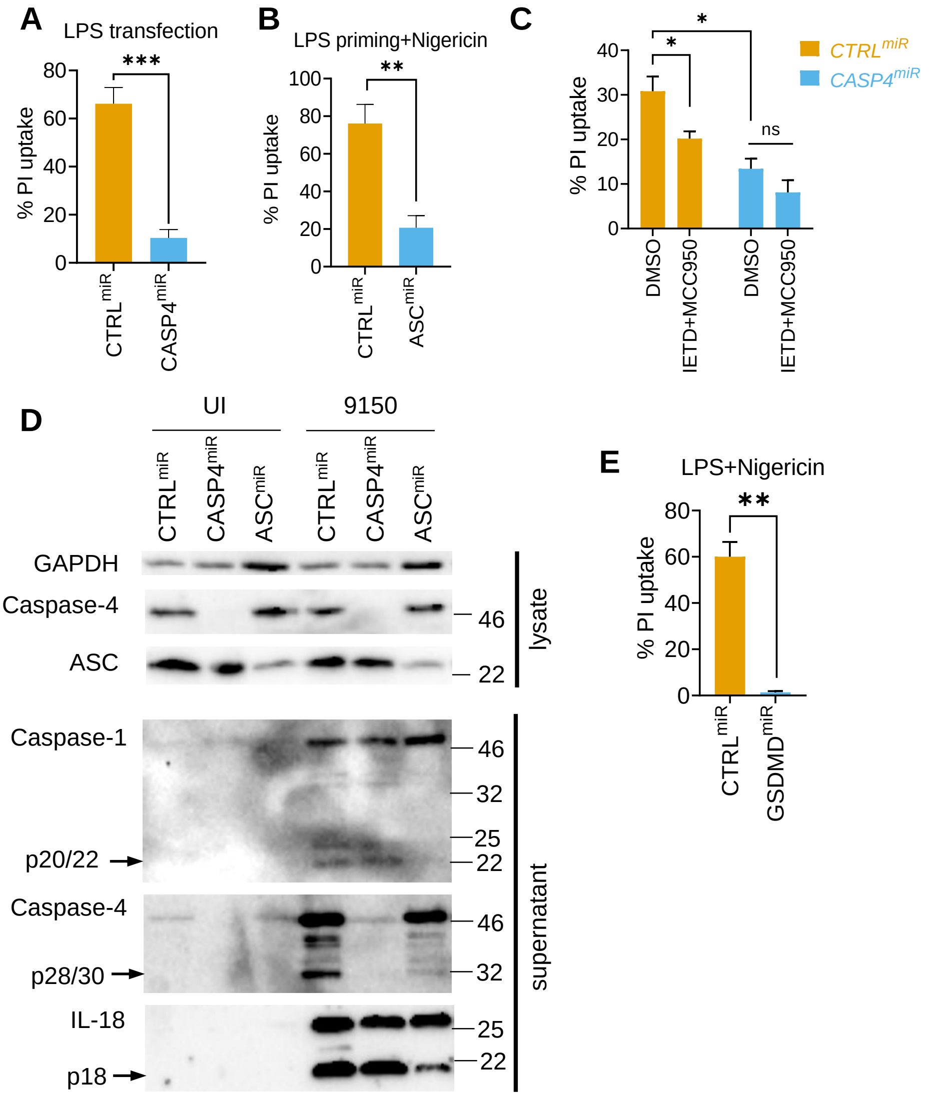

Figure S2. Functional validation of the miR THP1 cells. (A) PI uptake into CTRL^{miR} and CASP4^{miR} THP1 cells transfected with LPS. **(B)** PI uptake into CTRL^{miR} and ASC^{miR} THP1 cells primed with *E. coli* O111:B5 LPS and then treated with nigericin. **(C)** PI uptake into THP1 cells treated with DMSO or IETD plus MCC950 and infected with WT SPtA 9150. **(D)** Representative (of 2 biological repeats) immunoblots of THP1 cell lines left uninfected (UI) or infected with WT SPtA 9150 for cleaved products of caspase-1, caspase-4 and IL-18 (arrows). **(E)** PI uptake into CTRL^{miR} and GSDMD^{miR} THP1 cells primed with *E. coli* O111:B5 LPS followed by nigericin treatment. Mean ± SEM means from 6 **(A),** or 4 **(B, C, E)** independent experiments are shown. ** *P<0.01,* *** *P<0.001,* for comparisons by paired Student's *t*-test.

Figure S3. Mylona et al

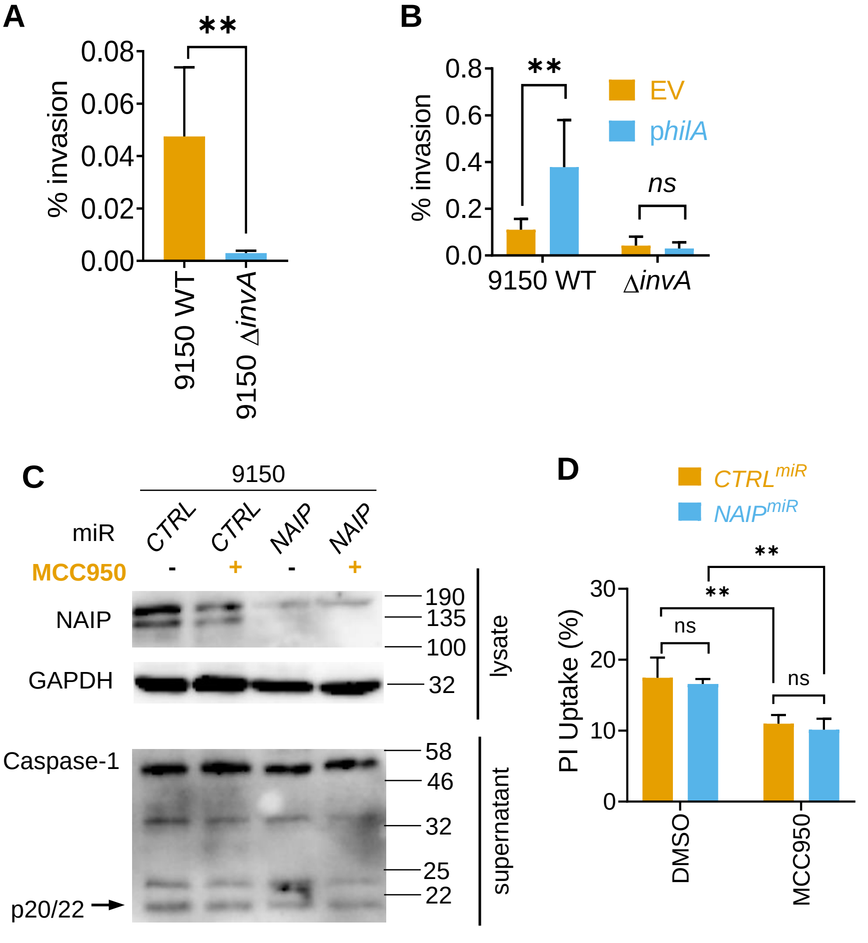

Figure S3. SPI-1 T3SS is required for HeLa cell invasion by SPtA. (A-B) Invasion of HeLa cells by the indicated SPtA 9150 strains. **(C)** Representative (of 3 independent repeats) immunoblots of THP1 cell lines left uninfected (UI) or infected with WT SPtA 9150 in the absence or presence of MCC950 for cleaved products of caspase-1 (arrow). **(D)** PI uptake assays in the indicated THP1 cells treated with DMSO or MCC950 and infected with WT SPtA 9150 for 3 h. Mean ± SEM from 4 **(A-B)** or 3 **(D)** independent experiments. ** *P<0.01* by **(A)** paired Student's *t*-test or **(B, D)** matched two-way ANOVA after correction for multiple comparisons; ns, not significant.

Figure S4. Mylona et al

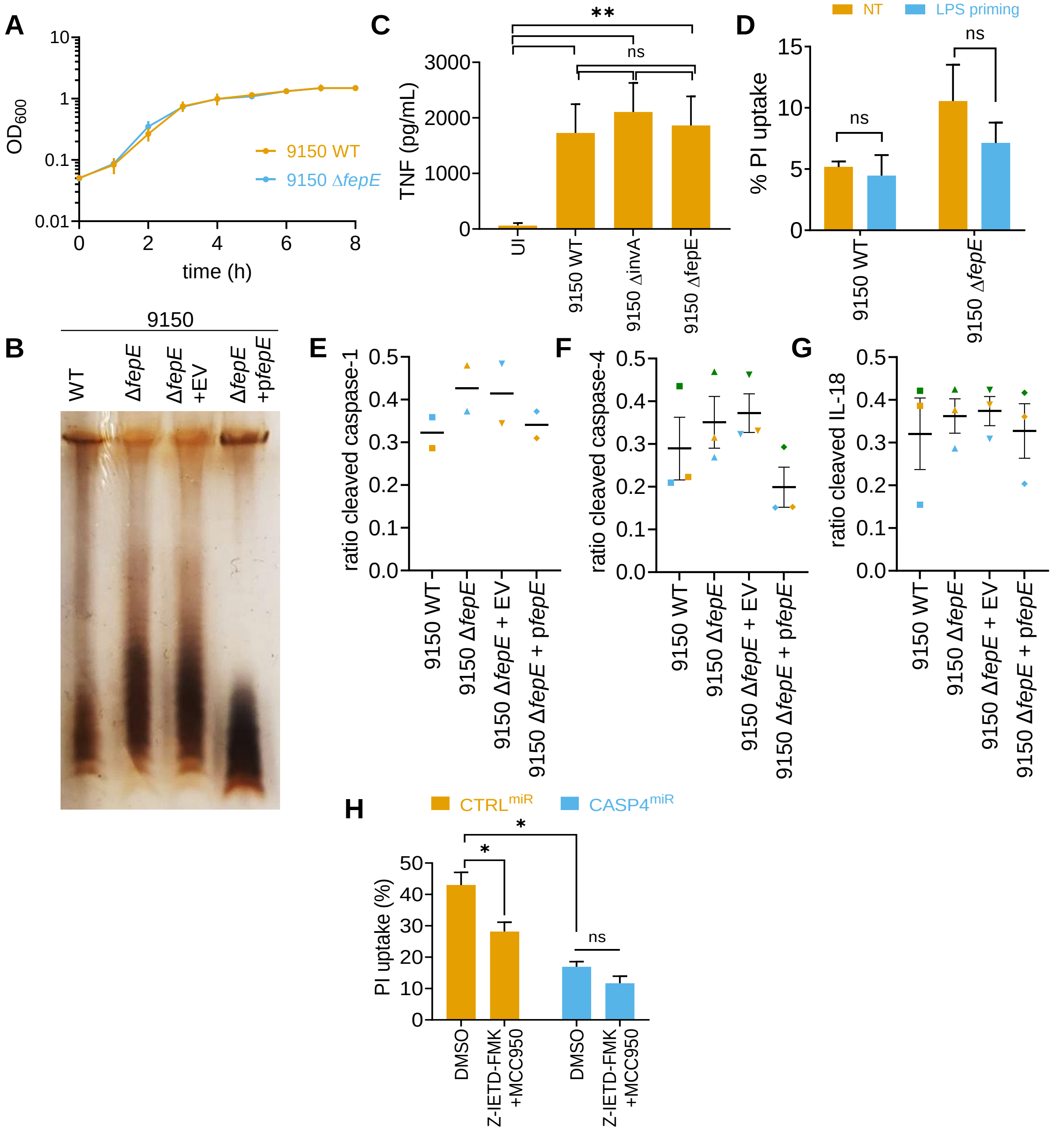

Figure S4. The effect of loss of very long LPS O-antigen chains on THP1 priming. (A) Growth curves of WT SPtA 9150 and Δ*fepE* as measured by OD_{600}. **(B)** Representative (of 2 biological repeats) image of silver-stained acrylamide gel showing electrophoretic mobility of LPS extracted from the indicated strains of SPtA. **(C)** ELISA quantification of TNF in culture supernatants of THP1 cells infected with the indicated SPtA strains. **(D)** PI uptake into unprimed THP1 cells (NT) or primed with *E. coli* O111:B5 LPS before infection with WT SPtA 9150 or Δ*fepE*. **(E-G)** Quantification of luminescent bands of western blots of caspase-1 (E), caspase-4 (F), and IL-18 (G) showing the ratio of the cleaved protein to the sum of cleaved plus pro-form of each protein in the supernatant of THP-1 cells infected with the indicated SPtA strains. **(H)** PI uptake into $CTRL^{miR}$ or $CASP4^{miR}$ THP1 cells infected with SPtA in the presence of DMSO alone or Z-IETD-FMK plus MCC950. Mean ± SEM from 2 **(A)**, 3 **(B, E-G)** or 4 **(D, H)** independent experiments. * *P<0.05*, ** *P<0.01* for indicated comparisons by matched one-way ANOVA **(C, E-G)** or two-way ANOVA **(D, H)** after correction for multiple comparisons; ns, not significant.

Figure S5. Mylona et al

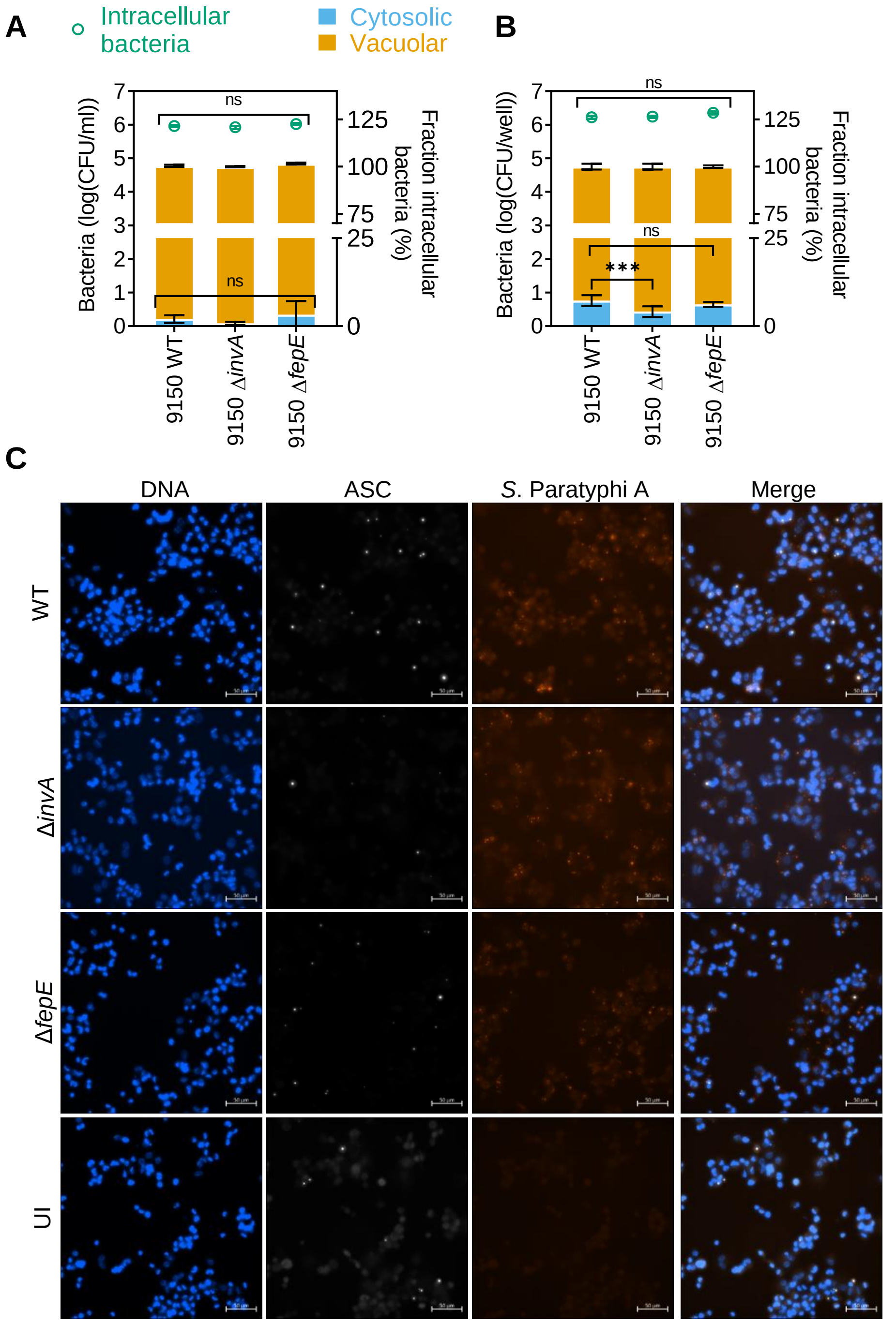

Figure S5. The role of very long O-antigen chains in vacuolar escape and ASC speck formation. (A-B) Bacterial internalisation (left-axis) or relative cytosolic escape measured by chloroquine-resistance assay (right-axis) of the indicated SPtA strains into GSDMD^{miR} THP-1 cells at 1.5 h **(A)** or 3 h **(B)**. **(C)** Representative (of 4 biological repeats) images from THP1-ASC^{mRFP} cells left uninfected (UI) or infected with the indicated SPtA strains. Scale bar 50 µm. Means ± SEM from 5 **(A)** or 3 **(B)** independent repeats are depicted. *** *P<0.001* by matched one-way ANOVA after correction for multiple comparisons; ns, not significant for comparison of WT SPtA with other strains.

Figure S6. Mylona et al

Figure S6. Protein sequence alignment of SPtA and STm *fepE*. Amino acid sequence of SPtA 9150 *fepE* and STm *fepE*; amino acid differences are depicted in red.

Table S1. Strains and plasmids used in this study

Strain or plasmid name	Source	Identifier
Strains		
Salmonella enterica serovar Paratyphi A ATCC 9150 (SPtA 9150) WT	(Gal-Mor et al., 2012)	N/A
SPtA ED199	(Dolecek et al., 2008)	N/A
SPtA 02TY129	(Arjyal et al., 2016)	N/A
SPtA 02TY221	(Arjyal et al., 2016)	N/A
SPtA 02TY224	(Arjyal et al., 2016)	N/A
SPtA ED293	(Dolecek et al., 2008)	N/A
SPtA 9150 Δ*fepE*::Kan^r	This study	ICC 1913
SPtA 9150 Δ*invA*::Kan^r	This study	ICC 1874
Salmonella enterica serovar Typhimurium 14028 (STm) WT	(Johnson et al., 2018)	ICC 797
STm Δ*fepE*::Kan^r	This study	
Plasmids		
pWS29K-Spec EV	(Johnson et al., 2017)	pICC2489
pWSK29-Spec-*hilA*	This study	pICC2767
pWSK29-Spec-*fepE*	This study	pICC2768

Table S2. Primers used in this study

Primer name	Source	Identifier
5'TCATTGGATAAAGTTTTCAGGTCATACGGCATGCCATCTCTTAATGTAAAACTGTGTAGGCTGGAGCTGCTTCG3'	This study (for generating Δ*fepE*)	N/A
5'CATATGAATATCCTCCTTAGGGATATCGCTATCCGGCTTTTCGGGTAAATCAGACTAACCGTTCATCTA3'	This study (for generating Δ*fepE*)	N/A
5'AGGCAAGTTTAATTCCGAATATTTAGC3'	This study (for sequencing *fepE* deletion)	N/A
5'TATGGGCTGCGCTGTATGATTATC3'	This study (for sequencing *fepE* deletion)	N/A
5'ACTTAACAGTGCTCGTTTACGACCTGAATTACTGATTCTGGTACTAATGGGTGTAGGCTGGAGCTGCTTC3'	This study (for generating Δ*invA*)	(Johnson et al., 2017)
5'GCTATCTGCTATCTCACCGAAAGATAAAACCTCCAGATCCGGAAAACGACCCATATGAATATCCTCCTTAG3'	This study (for generating Δ*invA*)	(Johnson et al., 2017)
5'GTTACGTAGTATGACCGATATTGC3'	This study (for sequencing *invA* deletion)	(Johnson et al., 2017)
5'CCTCCAATTAATGAAGGATCGC3'	This study (for sequencing *invA* deletion)	(Johnson et al., 2017)
5'GCTCCACCGCGGTGGCGGCCATGCCACATTTTAATCCTG3'	This study (for generating pWSK29-Spec-*hilA*)	N/A
5'GGATCCACTAGTTCTAGAGCTTACCGTAATTTAATCAAGCG3'	This study (for generating pWSK29-Spec-*hilA*)	N/A
5'GCTCTAGAACTAGTGGATC3'	This study (for generating pWSK29-Spec-*hilA*)	N/A
5'GGCCGCCACCGCGGTGGA3'	This study (for generating pWSK29-Spec-*hilA*)	N/A
5'CTCCACCGCGATGCCATCTCTTAATGTAAAAC3'	This study (for generating pWSK29-Spec-*fepE*)	N/A
5'TACCGGGCCCTCAGACTAACCGTTCATC3'	This study (for generating pWSK29-Spec-*fepE*)	N/A
5'GTTAGTCTGAGGGCCCGGTACCCAATTC3'	This study (for generating pWSK29-Spec-*fepE*)	N/A
5'GAGATGGCATCGCGGTGGAGCTCCAGCT3'	This study (for generating pWSK29-Spec-*fepE*)	N/A
5'GGGTTGCTTCTGTCCTTTCTG 3'	This study (RT-qPCR for *fepE*)	N/A

$^{5'}$TTACCTCCATATCCAACACGC$^{3'}$	This study (RT-qPCR for *fepE*)	N/A
$^{5'}$TGTAGCGGTGAAATGCGTAG$^{3'}$	This study (RT-qPCR for *16S*)	N/A
$^{5'}$CAAGGGCACAACCTCCAAG$^{3'}$	This study (RT-qPCR for *16S*)	N/A

Table S3. Cell lines (A) and oligopeptides (B) used in this study

(A) Cell line name	**Source**	**Identifier**
THP-1	John MacMicking laboratory (Shenoy et al., 2012)	N/A
HeLa	ATCC	ATCC CCL-2
(B) Oligopeptide name	**Source**	**Identifier**
pMX-CMV-YFP-LacZ^{miR} 5'-TCACGACGTTGT AATACGACGT-3'	(Eldridge et al., 2017)	N/A
pMX-mAsc-mRFP	(Goddard et al., 2019)	N/A
pMX-CMV-YFP-GSDMD^{miR} 5'-TACACATTCATTGAGGTGCTGG-3'	(Eldridge et al., 2017)	N/A
pMX-CMV-YFP-CASP4^{miR} 5'-ATATCTTGTCATGGACAGTCGT-3'	(Goddard et al., 2019; Pallett et al., 2016)	N/A
pMX-CMV-YFP-ASC^{miR} 5'-CAGCTCTTCAGTTTCACACCAG-3'	(Goddard et al., 2019)	N/A
pMX-CMV-YFP-NAIP^{miR} 5'-ATTCACAAAGTTCACCACGGCT-3'	This study	N/A

Table S4. Chemicals, reagents and antibodies used in this study

Chemical/Reagent name	Source	Identifier
Mouse anti-human Caspase-4 (4B9) antibody	Santa Cruz Biotechnology	Cat# sc-56056, RRID:AB_781828
anti-Caspase-1 (p20) (human) mAb (Bally-1) antibody	AdipoGen	Cat# AG-20B-0048, RRID:AB_2490257
Human IL-18 Polyclonal Antibody	MBL International	Cat# PM014, RRID:AB_592017
anti-Asc pAb (AL177) antibody	AdipoGen	Cat# AG-25B-0006, RRID:AB_2490440
Mouse Anti-Human GSDMDC1 Monoclonal Antibody, Unconjugated, Clone 64-Y	Santa Cruz Biotechnology	Cat# sc-81868, RRID:AB_2263768
Mouse anti-human NAIP	R&D Systems	Cat# MAB829
Rabbit anti-GAPDH	Abcam	Cat# ab9485
DnaK (E. coli), mAb (8E2/2) antibody	Enzo Life Sciences	Cat# ADI-SPA-880, RRID:AB_10619012
Rabbit anti-SipA (Salmonella)	V Koronakis Lab (Johnson et al., 2017)	N/A
Rabbit anti-SipC (Salmonella)	V Koronakis Lab (Johnson et al., 2017)	N/A
Rabbit anti-SipD (Salmonella)	V Koronakis Lab (Johnson et al., 2017)	N/A
Goat anti-CSA-1	Insight Biotechnology	Cat# 01-91-99
Peroxidase AffiniPure Goat Anti-Mouse IgG, Fcγ fragment specific	Jackson Immunoresearch	Cat# 115-035-008 RRID: AB_2313585
Peroxidase AffiniPure Goat Anti-Rabbit IgG, Fc fragment specific	Jackson Immunoresearch	Cat# 111-035-008 RRID: AB_2337937
Donkey anti-goat immunoglobulin-Cy2	Jackson ImmunoResearch	Cat# 705-225-147 RRID: AB_2307341
Hoechst 33258	Sigma-Aldrich	Cat# 94403
Phalloidin Alexa647	Startech	Cat# 23127-AAT
LPS-EB (LPS from E. coli O111:B4)	Invivogen	Cat# tlrl-3pelps
Chloroquine	Sigma-Aldrich	Cat# C6628
Nigericin	Sigma-Aldrich	Cat# N7143
Kanamycin	Sigma-Aldrich	Cat# 60615
Spectinomycin dihydrochloride pentahydrate	Sigma-Aldrich	Cat# S9007
Puromycin dihydrochloride from Streptomyces alboniger	Sigma-Aldrich	Cat# P8833

Gentamicin	Sigma-Aldrich	Cat # G1272
MCC950	Tocris Bioscience	Cat # 5479
Lipofectamine 2000 Transfection Reagent	Life Technologies	Cat# 11668027
Pierce Protease Inhibitor Mini Tablets, EDTA-free	Thermo Fisher Scientific	Cat# A32955
ECL Prime Western Blotting Detection Reagent	GE-Healthcare	Cat# RPN2236
Propidium iodide (PI)	Sigma-Aldrich	Cat # P4864
ProLong Gold Antifade Mountant	Thermo Fisher Scientific	Cat# P36930
Phenylmethanesulfonyl fluoride	Sigma-Aldrich	Cat# P7626
Phorbol myristate acetate (PMA)	Sigma-Aldrich	Cat# P8139
HEPES solution	Sigma-Aldrich	Cat# H0887
Trypsin	Sigma-Aldrich	Cat# T4674
EDTA 0.2% cell culture	Sigma-Aldrich	Cat# E8008
Dulbecco's minimal Eagle media High Glucose (4500mg/L)	Sigma-Aldrich	Cat# D5796
RPMI 1640	Sigma-Aldrich	Cat# R8758
RPMI 1640 – Phenol Red Free	Gibco	Cat# 11835030
Fetal Bovine Serum	Gibco	Cat# 10270-106
Sodium pyruvate	Sigma-Aldrich	Cat# S8636
Penicillin-Streptomycin	Sigma-Aldrich	Cat# 31985062
GlutaMAX supplement	ThermiFisher Scientific	Cat# 35050061
Gibson assembly master mix	NEB	Cat# E2611
Z-VAD-FMK	R&D Systems	Cat# FMK001
Z-YVAD-FMK	R&D Systems	Cat# FMK001
Z-IETD-FMK	R&D Systems	Cat# FMK007
Staurosporine	Sigma-Aldrich	Cat# S5921
Paraformaldehyde (PFA)	ThermoFisher Scientific	Cat# 28908
TNF alpha Human Uncoated ELISA Kit	ThermoFisher Scientific	Cat# 88-7346-22
Power Up SYBR Green	Applied Biosystems	Cat# 15350929
MycoAlert mycoplasma detection kit	Lonza	Cat# LT07-518
IL-1beta Human Uncoated ELISA Kit	ThermoFisher Scientific	Cat# 88-7261-22
RNAprotect	Qiagen	Cat# 76526
RNeasy Mini Kit	Qiagen	Cat# 74106
MMLV reverse trancriptase	Promega	Cat# M1706

DNase	Promega	Cat# M6101
Lysozyme	Sigma	Cat# L6876

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