Supplementary Figure Legends

Supplementary Figure 1. TLR11 is expressed in small intestinal epithelium and limits *S. typhimurium* dissemination.

A, TLR11 expression by Q-PCR from cDNA prepared from the indicated tissues. All data is normalized to beta-actin and set as fold expression compared with expression in heart. **B**, In situ hybridization for TLR11 was performed using a specific probe for TLR11. As a control for the specificity of staining, small intestinal sections from TLR11 KO mice were probed under identical conditions. **C,** Bacterial dissemination examined by Colony Forming Units (CFU) 5 days post oral inoculation of *S. typhimurium* (10^8 CFU per animal) in wild type C57/BL6 and *tlr11*$^{-/-}$ mice. Homogenized tissue from mediastinal lymphnodes (MLN); liver; spleen, and kidney, were plated on LB plates containing streptomycin. After 16 hours of growth at 37^0C, bacterial colonies were counted and plotted as a CFU per gram tissue.

Supplementary Figure 2. TLR11 recognizes flagellin from both uropathogenic *E. coli* 8NU and *S. Typhimurium.*

A, NF-κB luciferase activity in specific fractions after treatment with DNAse, RNAse, or Proteinase-K. Induction of NF-κB was measured using fractions 8 and 14 obtained from a linear NaCl gradient performed on a Mono-Q anion exchange column. Fractions 8 and 14 were dialyzed and subjected to treatment with DNAse, RNAse, Proteinase-K, or were passed over a polymyxin-B column and then used to stimulate peritoneal macrophages

obtained from wild type and *tlr11*$^{-/-}$ mice and analyzed for IL6 from culture supernatants or following 24 hours of stimulation. **B,** Mascot search analysis result obtained using the highly purified fraction 14, excised following SDS-PAGE, cut and subjected to LC-MS/MS to identify TLR11-ligand peptides. **C**, 8NU lysates were fractionated on a Mono-Q anion exchange column using a linear NaCl gradient (50-500mM NaCl). Fractions were used to stimulate RAW-NF-κB luciferase cells. **D, 8NU Fraction 7 from** either untreated or after treatment with DNAse, RNAse, or Proteinase-K, were used to stimulate RAW-NF-κB luciferase cells. **E,** Fractions from (C) were subjected to SDS-PAGE and an approximately 60kDa protein band visualized by Coomassie blue staining, and co-eluting with the stimulatory activity, was subjected to Mass spectrometry analysis. The predominant peptide species corresponded to *E coli* flagellin.

Supplementary Figure 3. Role of TLR11 and TLR5 in the response to *S. typhimurium*.

A. TLR11 can form homodimers and heterodimerize with TLR5. HEK293 cells were transfected with plasmids encoding TLR11-Flag, TLR11-HA or TLR5-V5 as indicated. Cells were lysed and subjected to SDS-PAGE followed by Western blotting as indicated (upper panel) or immunoprecipitation with anti-flag M2 beads followed by SDS-PAGE and Western blotting as indicated (lower panel). **B, C. TLR11 expression is increased in response to *S. typhimurium* flagellin and this response is augmented in TLR5 KO mice.** **B,** Wild type macrophages were infected with S. typhimurium or were stimulated with *S. typhimurium* FliC (100ng/ml) *in vitro* for 6 hours, total RNA was extracted, cDNA was prepared and TLR11 expression was determined by Q PCR. **C,** Wild type and TLR5 KO

mice were orally infected with *S. typhimurium* and lamina propria Macropahges (LPMf) were isolated by FACS sorting, and TLR11 expression was examined by QPCR. **D. Loss of enhanced resistance of TLR5 deficient mice upon TLR11 deletion.** Wild type (WT) and *tlr11*$^{-/-}$, *tlr5*$^{-/-}$, *tlr11*$^{-/-}$ /*tlr5*$^{-/-}$ mice were challenged with *S. typhimurium* and were sacrificed on day 5 post-infection. CFU determined as in Supplementary Figure 1C.

Supplementary Figure 4. Characterization of the *tlr11*$^{-/-}$ *S. typhi* infection model.

A, The *S. typhi* virulence gene for Vi+ antigen was analyzed by PCR, to confirm the strain used in our study is a Vi Antigen positive strain**. Development of fever during low dose *S. typhi* infection. B,** body temperature was examined daily following *S. typhi* infection orally at low dose (10^6 CFU per mouse) in wild type and *tlr11*$^{-/-}$ mice. **Development of hypothermia and gastrointestinal bleeding during lethal high dose infection: C,** temperature was monitored in wild type and *tlr11*$^{-/-}$ mice infected orally with high dose ($5x10^8$ CFU per animal) *S.typhi*. **D,** A stool blood clinical score was calculated at day 10 of high dose S. typhi infection based on the following scoring system: Hemoccult negative, formed pellets (0); Hemoccult positive, formed pellets (1); Gross Blood, formed pellets (2); Gross Blood, loose stool (Bloody Diarrhea; 3).

Supplementary Figure 5. *S. typhi* dissemination and end organ histology in the *tlr11*$^{-/-}$ mouse model.

Intestinal histology following *S. typhi* infection of *tlr11*$^{-/-}$ mice. A, tissue destruction in the ileum after *S. typhi* infection was determined by H & E staining of sections of WT

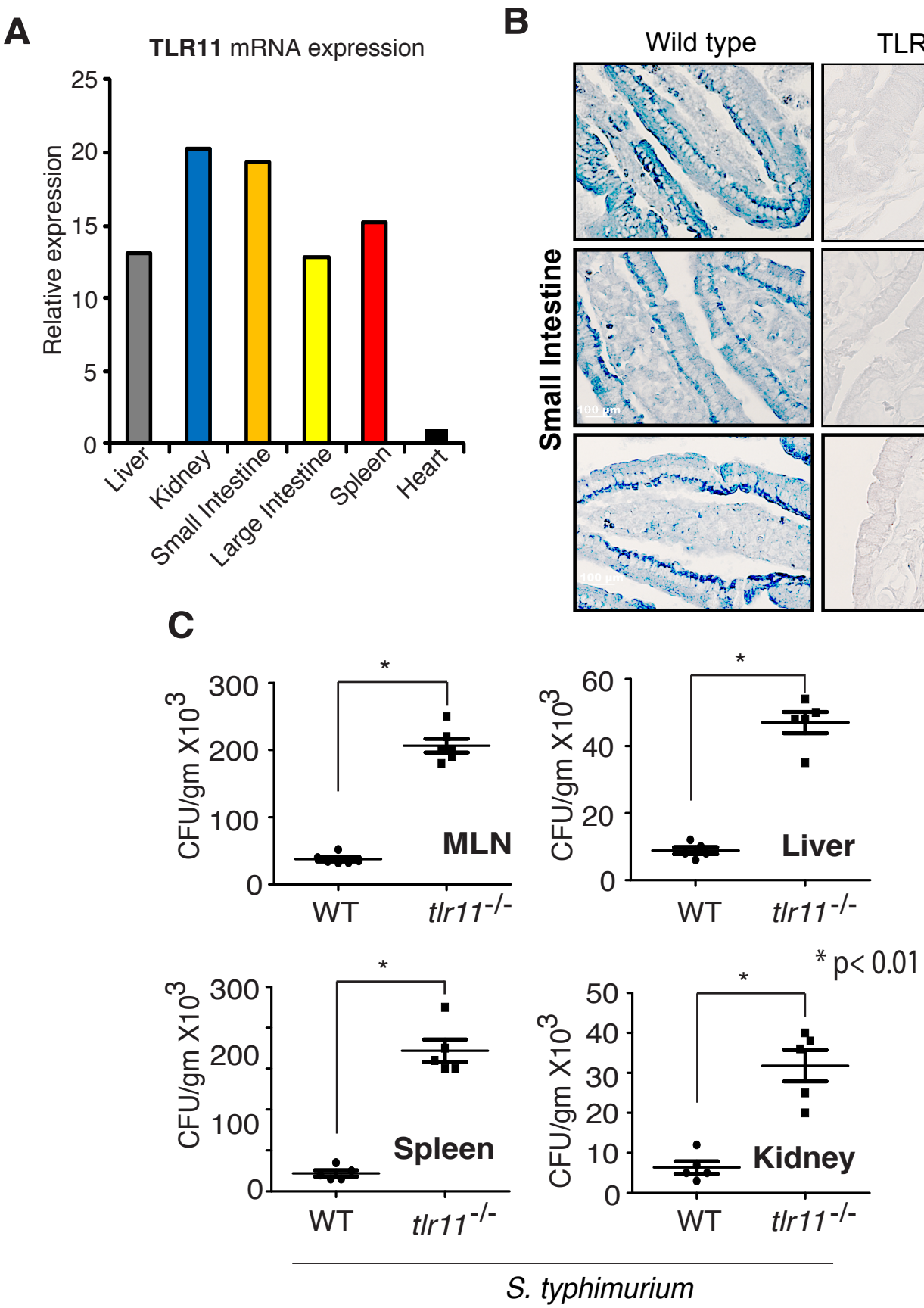
and *tlr11*$^{-/-}$ mice at day-10 post oral infection with 5x10^{8} CFU *S. typhi*. Perforations and loss of architecture was observed in the *tlr11*$^{-/-}$ mice as indicated by arrows. **B,** cecum and colon were examined as in A. Necrosis, loss of architecture, and large lymph aggregates (middle panel) was observed in the *tlr11*$^{-/-}$ mice. **End organ histology following *S. typhi* infection of *tlr11*$^{-/-}$ mice.** H & E stained sections of Spleen, **C,** and Lung and Kidney, **D,** of WT and *tlr11*$^{-/-}$ mice at day-10 post oral infection with 5x10^{8} CFU *S. typhi*. Spleen demonstrated characteristic typhoid nodules, diminished red zone, and areas of necrotic cell death**.** ***S. typhi* disseminates to the gall bladder after oral infection of *tlr11*$^{-/-}$ mice.** WT and *tlr11*$^{-/-}$ mice at day-10 post oral infection with 5x10^{8} CFU *S. typhi* were sacrificed. Gall bladder was collected and **E,** used for paraffin sections and H&E staining, or stained with anti-*Salmonella* FITC conjugated antibody. Representative bacteria stained by the a-Salmonella antibody are indicated by arrows. **F,** alternatively gall bladders was homogenized and bacterial CFU's detected by plating on LB-agar plate.

Supplementary Figure 6. *S. typhi* infection of *tlr11*$^{-/-}$ macrophages *in vitro*.

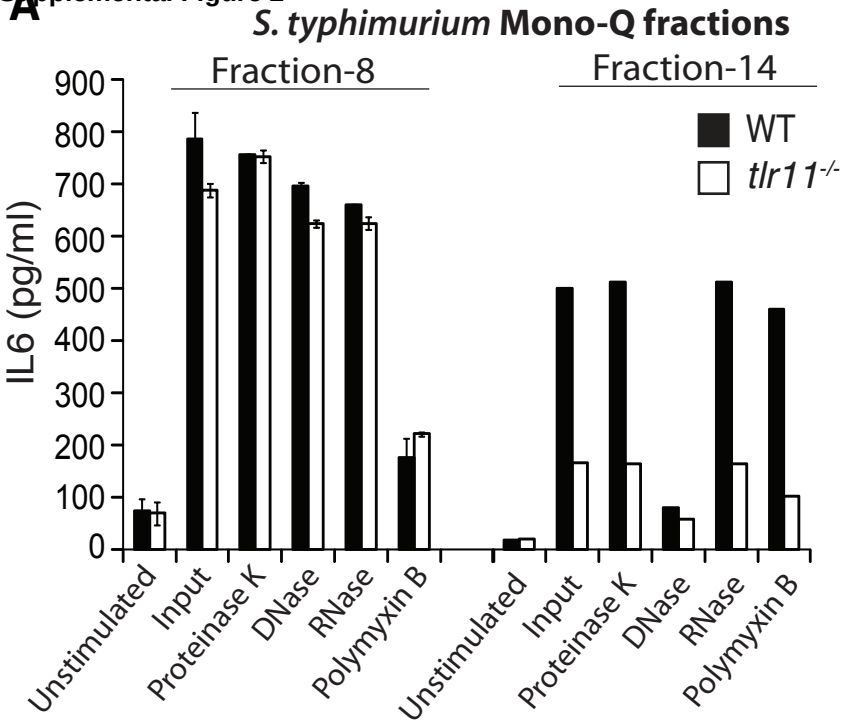
WT and *tlr11*$^{-/-}$ peritoneal macrophages were infected *in vitro* with live Cy5 labeled *S.typhi* (MOI=1:10). *S.typhi* infection was determined by, **A,** visualizing labeled *S.typhi* by microscopy and, **B,** by gentamycin assays. **Mice deficient for TLR11 are susceptible to low dose intraperitoneal *S. typhi* infection.** WT and *tlr11*$^{-/-}$ mice were infected by intraperitoneal injection of 10^{4} CFU *S.typhi.* **C,** survival kinetics for WT and *tlr11*$^{-/-}$ mice

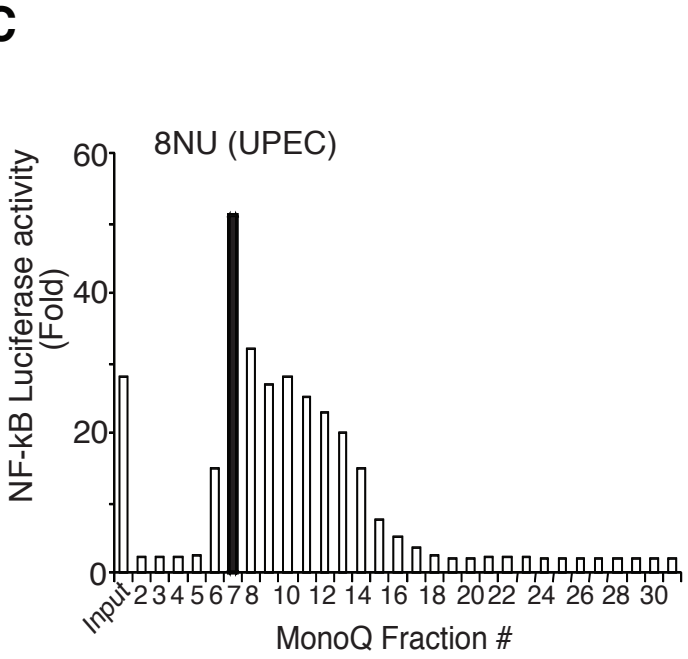
(n=5). **D**, CFU in various organs was assessed at day-2 after intraperitoneal injection of 10^4 CFU *S.typhi.*

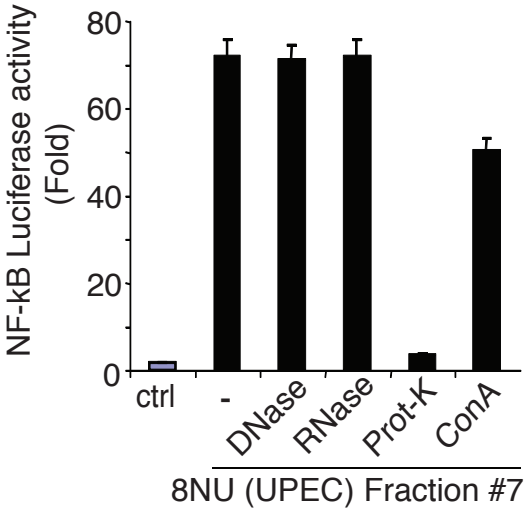
Supplementary Figures


A
TLR11 mRNA expression
Relative expression
25
20
15
10
5
0
Liver
Kidney
Small Intestine
Large Intestine
Spleen
Heart
B
Wild type
TLR11 KO
Small Intestine
100 µm
C
CFU/gm X10^3
MLN
Liver
Spleen
Kidney
WT
tlr11-/-
* p< 0.01
S. typhimurium

Supplemental Figure 2







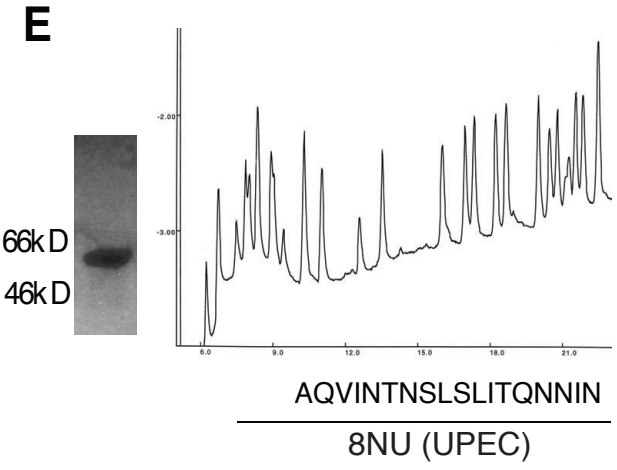
B

Mascot Search Results

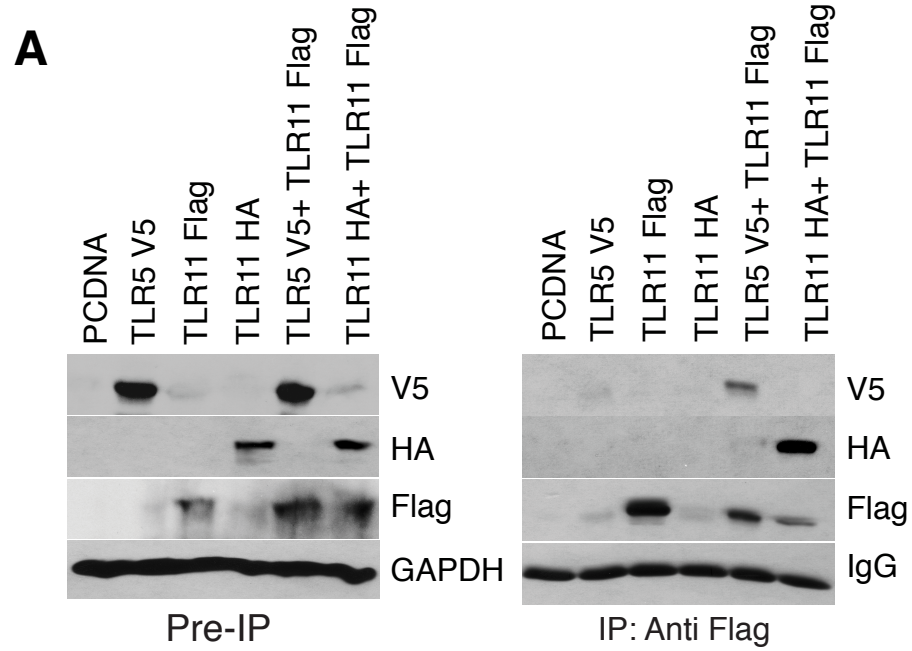
Protein View
Match to: FLIC_SALTY
Score: 883
Flagellin OS=Salmonella typhimurium GN=fliC PE=1 SV=4
Nominal mass (M_r): 51581;
Calculated pI value: 4.79
NCBI BLAST search of FLIC_SALTY
Taxonomy: Salmonella enterica subsp. enterica serovar Typhimurium
Fixed modifications: Carbamidomethyl (C) Variable modifications: Acetyl (Protein N-term), Oxidation (M) Cleavage by Trypsin: cuts C-term side of KR unless next residue is P Sequence Coverage: 29%

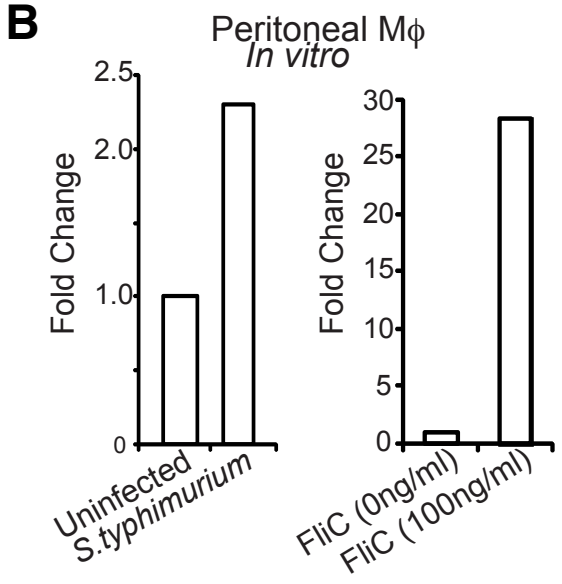
Matched peptides shown in **Bold**:
1 MAQVINTNSL SLLTQNNLNK **SQSALGTAIE RLSSGLRINS AKDDAAGQAI 51 ANR**FTANIKG LTQASRNAND GISIAQTTEG ALNEINNNLQ RVRELAVQSA **101** NSTNSQSDLD SIQAEITQRL **NEIDRVSGQT QFNGVK**VLAQ DNTLTIQVGA **151** NDGETIDIDL **KQINSQTLGL DTLNVQQK**YK VSDTAATVTG YADTTIALDN **201** STFKASATGL GGTDQKIDGD LKFDDTTGKY YAKVTVTGGT GKDGYYEVSV **251** DKTNGEVTLA GGATSPLTGG LPATATEDVK NVQVANADLT EAKAALTAAG **301** VTGTASVVKM SYTDNNGKTI DGGLAVK**VGD DYYSATQNK**D GSISINTTKY **351** TADDGTSKTA LNK**LGGADGK TEVVSIGGK**T YAASKAEGHN FKAQPDLAEA **401** AATTTENPLQ K**IDAALAQVD TLRSDLGAVQ NRFNSAITNL GNTVNNLTSA 451 RSRIEDSDYA TEVSNMSR**AQ ILQQAGTSVL AQANQVPQNV LSLLR

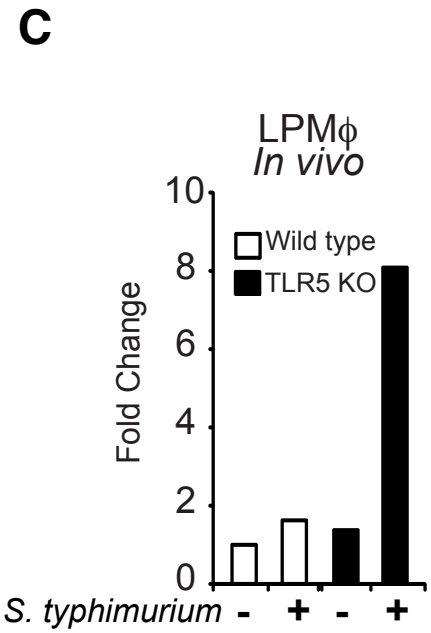
Start -	End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
21 - 31	566.7866	1131.5586	1131.5884	-0.0297	0		K.SQSALGTAIER.L (Ions score 61)
38 - 53	807.8915	1613.7684	1613.8121	-0.0437	1		R.INSAKDDAAGQAIANR.F (Ions score 47)
43 - 53	551.2425	1100.4704	1100.5210	-0.0506	0		K.DDAAGQAIANR.F (Ions score 61)
120 - 125	380.1982	758.3818	758.3922	-0.0104	0		R.LNEIDR.V (Ions score 50)
126 - 136	583.2954	1164.5762	1163.5935	0.9827	0		R.VSGQTQFNGVK.V (Ions score 74)
162 - 178	633.9872	1898.9398	1899.0061	-0.0664	0		K.QINSQTLGLDTLNVQQK.Y (Ions score 28)
162 - 178	950.5069	1898.9992	1899.0061	-0.0069	0		K.QINSQTLGLDTLNVQQK.Y (Ions score 44)
328 - 339	680.7949	1359.5752	1359.5943	-0.0190	0		K.VGDDYYSATQNK.D (Ions score 66)
364 - 379	496.5987	1486.7743	1486.7991	-0.0249	1		K.LGGADGKTEVVSIGGK.T (Ions score 133)
364 - 379	744.4009	1486.7872	1486.7991	-0.0119	1		K.LGGADGKTEVVSIGGK.T (Ions score 92)
371 - 379	445.2386	888.4626	888.4917	-0.0290	0		K.TEVVSIGGK.T (Ions score 70)
412 - 423	643.3501	1284.6856	1284.7038	-0.0181	0		K.IDAALAQVDTLR.S (Ions score 68)
424 - 432	480.2466	958.4786	958.4832	-0.0046	0		R.SDLGAVQNR.F (Ions score 54)
433 - 451	1004.0101	2006.0056	2006.0181	-0.0124	0		R.FNSAITNLGNTVNNLTSAR.S (Ions score 38)
452 - 468	653.9334	1958.7784	1958.8640	-0.0856	1		R.SRIEDSDYATEVSNMSR.A (Ions score 35)
452 - 468	980.4268	1958.8390	1958.8640	-0.0249	1		R.SRIEDSDYATEVSNMSR.A (Ions score 43)
452 - 468	659.2704	1974.7894	1974.8589	-0.0695	1		R.SRIEDSDYATEVSNMSR.A Oxidation (M) (Ions score 37)
452 - 468	988.4035	1974.7924	1974.8589	-0.0664	1		R.SRIEDSDYATEVSNMSR.A Oxidation (M) (Ions score 34)
454 - 468	858.8293	1715.6440	1715.7308	-0.0868	0		R.IEDSDYATEVSNMSR.A (Ions score 78)
454 - 468	866.8666	1731.7186	1731.7257	-0.0071	0		R.IEDSDYATEVSNMSR.A Oxidation (M) (Ions score 55)

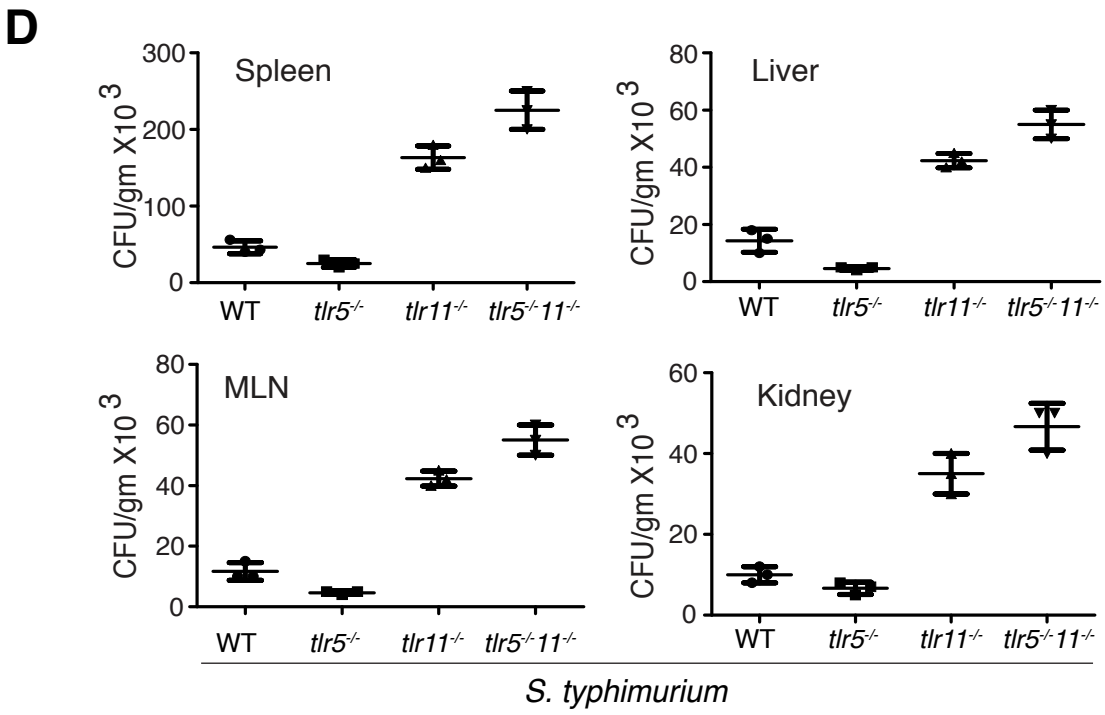


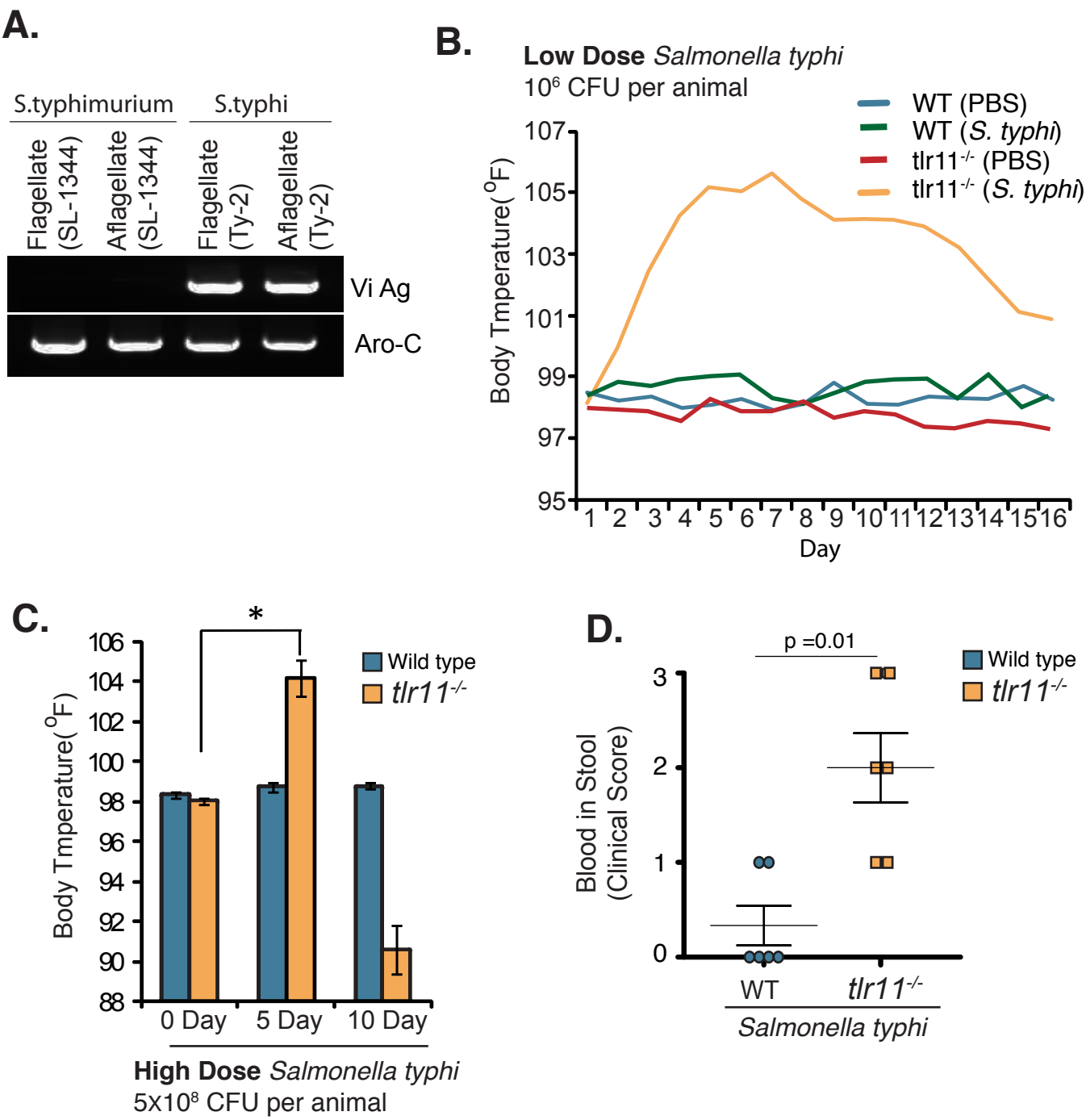
Mathur *et al.* Supplementary Figure 2


A
PCDNA
TLR5 V5
TLR11 Flag
TLR11 HA
TLR5 V5+ TLR11 Flag
TLR11 HA+ TLR11 Flag
V5
HA
Flag
GAPDH
Pre-IP
IgG
IP: Anti Flag

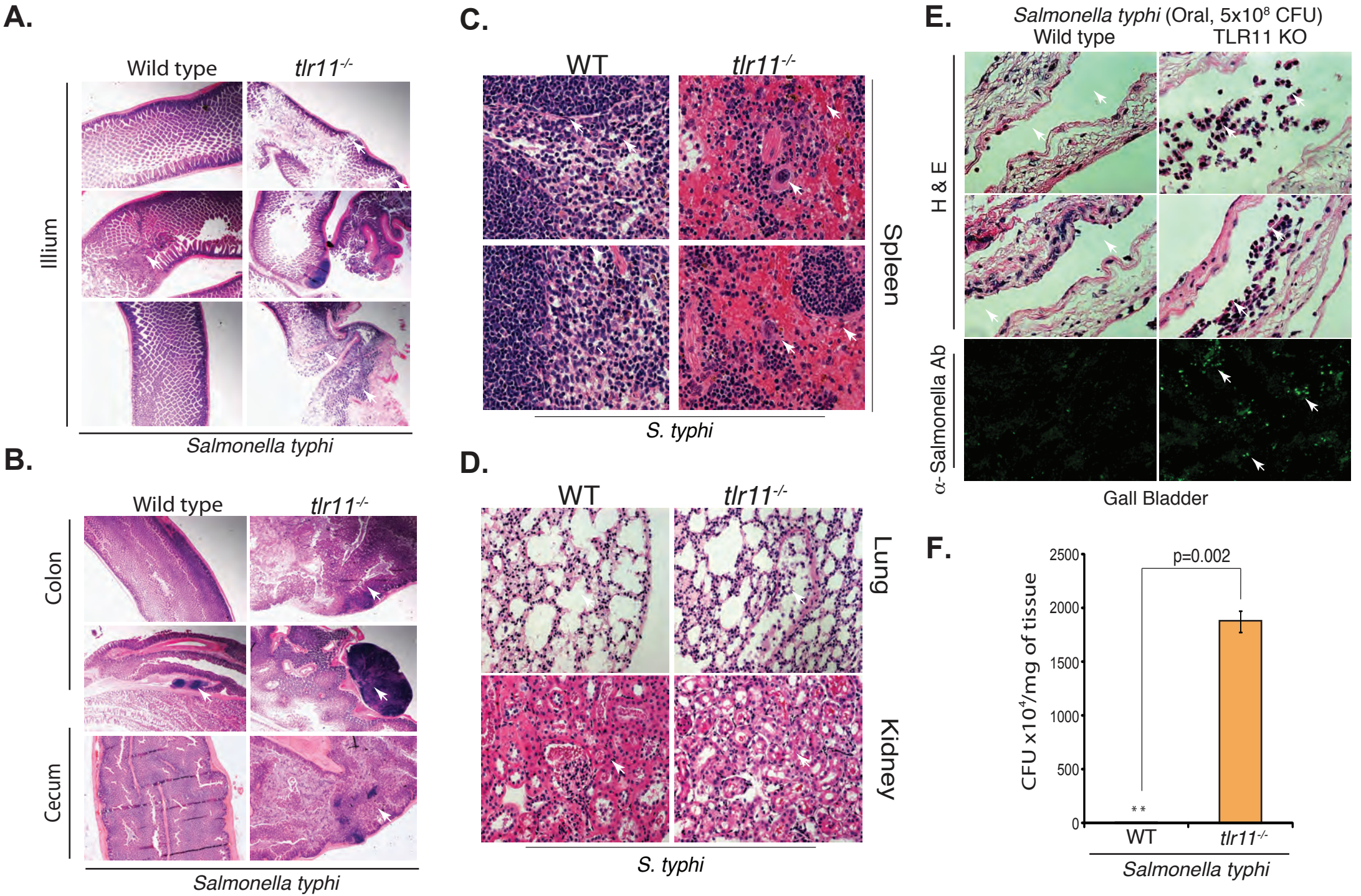

B
Peritoneal Mϕ
In vitro
Fold Change
Uninfected
S.typhimurium
FliC (0ng/ml)
FliC (100ng/ml)


C
LPMϕ
In vivo
Fold Change
Wild type
TLR5 KO
S. typhimurium

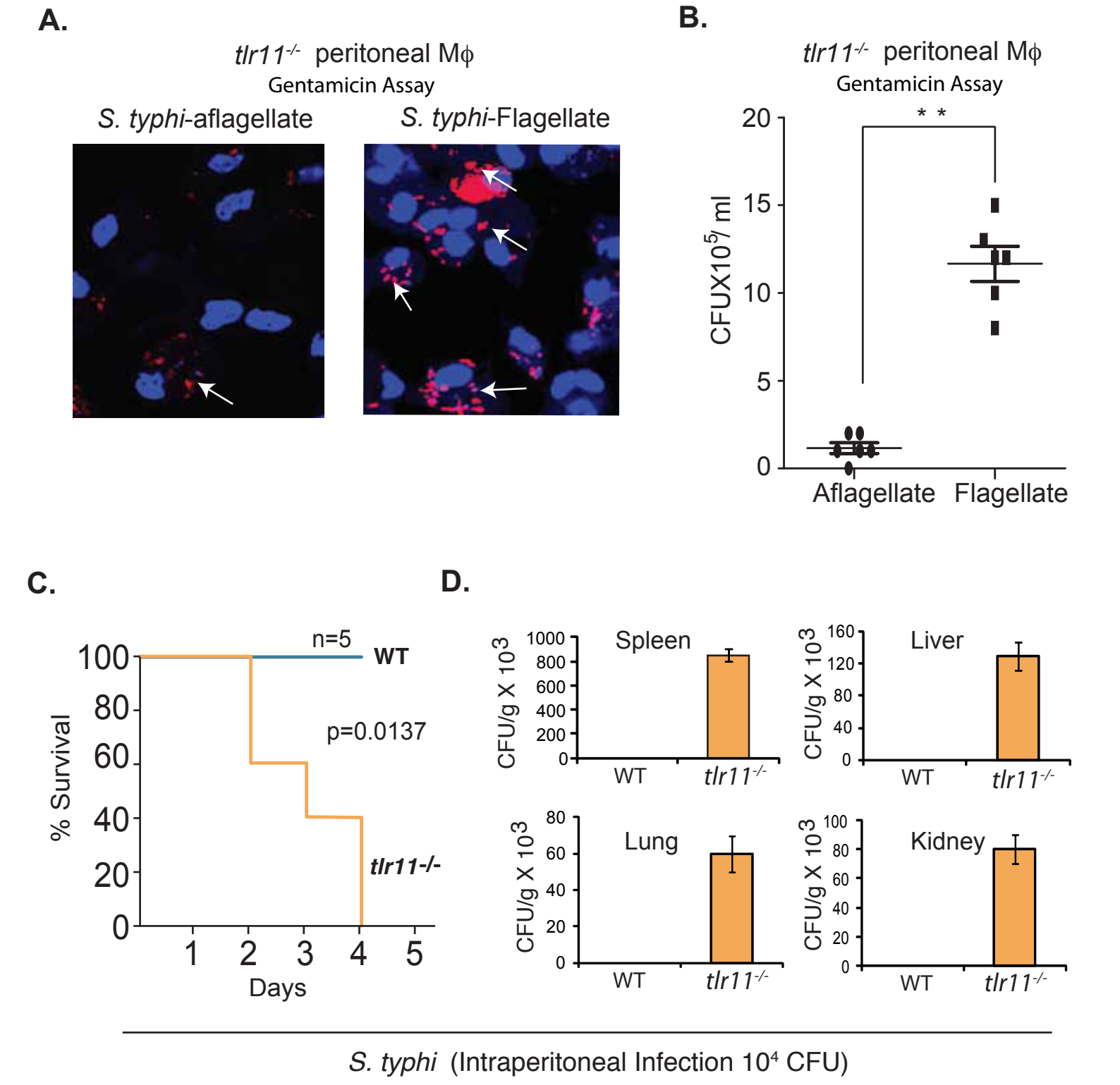

D
Spleen
Liver
MLN
Kidney
CFU/gm X10 3
WT
tlr5-/-
tlr11-/-
tlr5-/-11-/-
S. typhimurium


A.
S.typhimurium
S.typhi
Flagellate (SL-1344)
Aflagellate (SL-1344)
Flagellate (Ty-2)
Aflagellate (Ty-2)
Vi Ag
Aro-C
B.
Low Dose Salmonella typhi
10⁶ CFU per animal
WT (PBS)
WT (S. typhi)
tlr11-/- (PBS)
tlr11-/- (S. typhi)
Body Tmperature(°F)
Day
C.
Wild type
tlr11-/-
Body Tmperature(°F)
0 Day
5 Day
10 Day
High Dose Salmonella typhi
5x10⁸ CFU per animal
D.
p =0.01
Wild type
tlr11-/-
Blood in Stool (Clinical Score)
WT
tlr11-/-
Salmonella typhi

Supplemental Figure 5



Mathur *et al.* Supplementary Figure 5


A.
tlr11-/- peritoneal Mϕ
Gentamicin Assay
S. typhi-aflagellate
S. typhi-Flagellate
B.
tlr11-/- peritoneal Mϕ
Gentamicin Assay
* *
CFUX10^5/ ml
20
15
10
5
0
Aflagellate
Flagellate
C.
n=5
WT
p=0.0137
tlr11-/-
% Survival
100
80
60
40
20
0
1
2
3
4
5
Days
D.
Spleen
Liver
Lung
Kidney
CFU/g X 10^3
WT
tlr11-/-
S. typhi (Intraperitoneal Infection 10^4 CFU)